Research Article

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Design and microwave-assisted synthesis of a novel Mannich base and conazole derivatives and their biological assessment

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Abstract: 4-Amino-5-methyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**1**) was converted to the corresponding Schiff base (**2**) by treatment with salicylaldehyde. 1,2,4-Triazoles were then converted to the corresponding Mannich bases containing fluroquinolone core using a one-pot three-component procedure. Moreover, the synthesis of six compounds, which can be considered as conazole analogues, was performed starting from 1,2,4-triazole-3-one compounds via three steps by either conventional or microwave-mediated conditions. All the newly synthesized compounds were screened for their antimicrobial activities. Most exhibited good to moderate antibacterial and/or antifungal activity. The structural assignments of the new compounds were based on elemental analysis and spectral (IR, ^{1}H NMR, ^{13}C NMR, and LC-MS) data.

Keywords: 1,2,4-triazole, fluoroquinolone, conazole, antimicrobial activity

1 Introduction

Heterocyclic compounds are common structural units in marketed drugs and also in medicinal chemistry targets in the drug discovery process. The main reason behind this is the high prevalence of oxygen, sulfur, and especially nitrogen-containing rings in drug molecules [1]. In the last few decades, the chemistry of *N*-heterocycles derived from 1,2,4-triazole and their fused heterocyclic derivatives have received much attention owing to their synthetic and effective medical applications. These *N*-bridged heterocyclic compounds are known to possess significant activity, such as antibacterial [2], anti-inflammatory [3], anticancer [4], anti-allergic [5], antimicrobial [6], antitubercular [7], antiviral [8], antitumor [9], antioxidant [10], anthelmintic [11], anticonvulsant [12,13], antifungal [14], analgesic [15], and antiparasitic [16] properties.

Triazole Schiff base derivatives have many important applications in industry, agriculture, and medicine [16,17]. They can be used as fungicides, anticancer drugs, pharmaceutical intermediates, antioxidants of polymers, and ultraviolet absorbers [18,19]. Triazole Schiff base derivatives, as five-membered heterocyclic compounds, contain the basic structural skeleton of a Schiff base in their molecular structure; therefore, they can also be used as ligands to chelate some trace metal ions in organisms and thus have a wide range of biological activities and play an important role in pharmacodynamics [20,21]. These compounds have good bioactivities and are widely used in medicine, materials, and other fields. They can also be used as antibacterial agents, insecticides, and plant growth regulators in medicine and agriculture [22,23].

Of the classes of antimycotics, the most useful in the treatment of fungal infections are compounds with an azole moiety within the structure (conazoles) [24,25].

Investigations of the first generation of conazoles, e.g., fluconazole and itraconazole, that involved broadening of the activity spectrum and improvement of the therapeutic index, resulted in the development of new drugs with posaconazole being one of the most promising antifungals [26–28] (Figure 1).

Posaconazole, a structural analogue of itraconazole, was approved in the E. U. (2005) as well as in the USA (2006) for treatment of aspergillosis, candidiasis, and other invasive fungal infections in immunocompromised patients older than 13 years. At present, there are three approved formulations of posaconazole that include oral suspension, intravenous injections, and delayed-release tablets [27–29].

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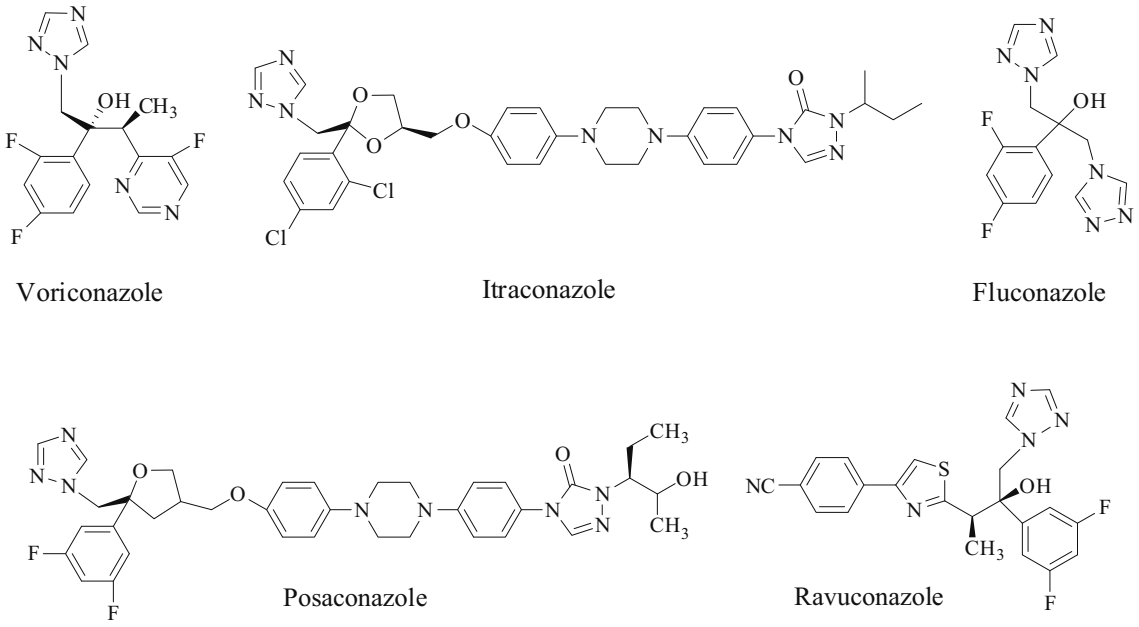


Figure 1: Some known azole class antifungals.

Compared to the previous conazoles, posaconazole has an extended spectrum of antimycotic activity including most yeasts, filamentous fungi, and *Candida* spp., as well as these resistant to fluconazole like *C. glabata*, *C. krusei*, *C. guilliermondii*, *C. dubliniensis*, *C. parapsilosis*, and *C. tropicalis* [30,31].

This study focused on the design, eco-friendly synthesis, and antimicrobial assessment of new azole class antifungals (Figure 2).

2 Results and discussion

2.1 Chemistry

In this study, we aim to synthesize new triazole-fluoroquinolone hybrids as possible drug candidates with antibacterial activity. On the basis of ^{1}H, ^{13}C NMR, FT IR, and EI-MS data, the structure of the target products was established. The MICs against clinically important

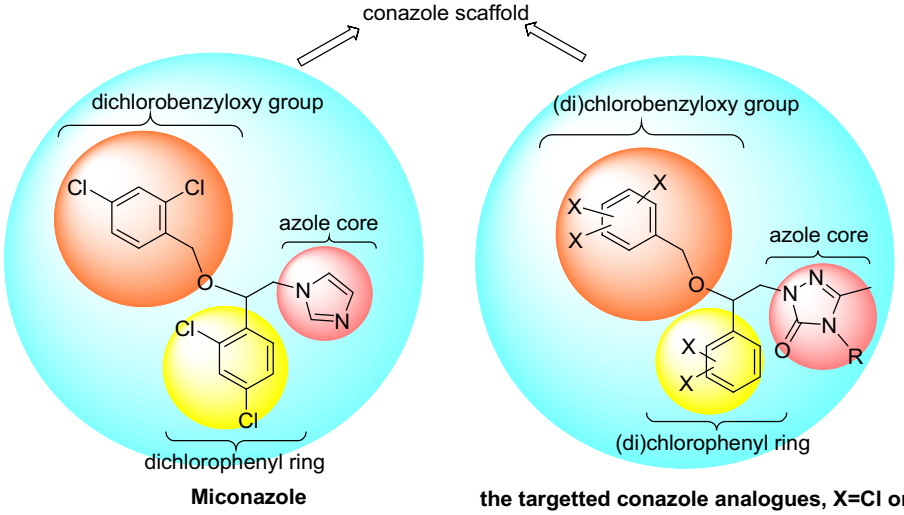
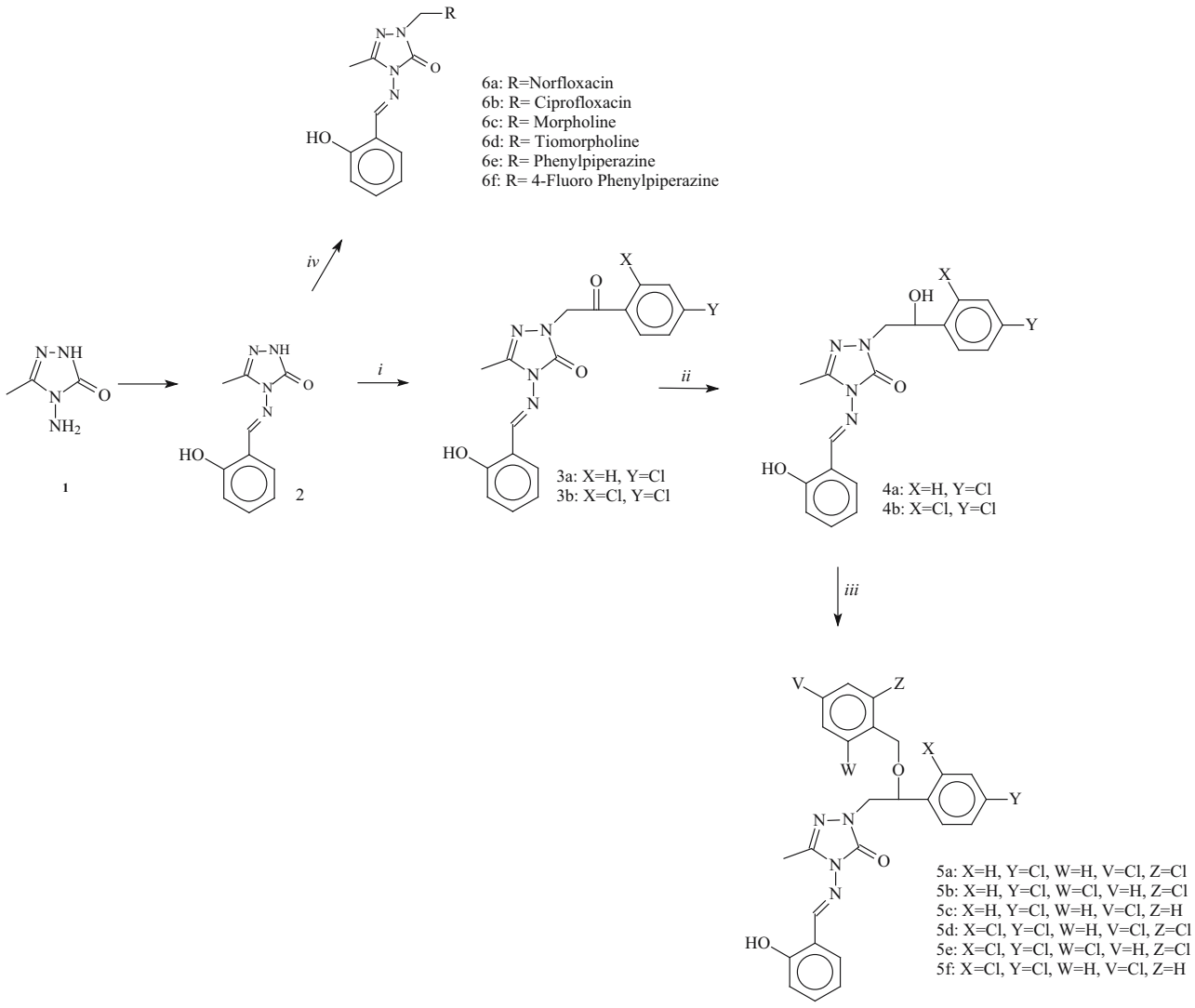


Figure 2: Schematic representation of the relationship between the structures of miconazole and the synthesized conazole analogues.



Scheme 1: (i) 2-Bromo-1-(4-chlorophenyl)ethanone or 2-chloro-1-(2,4-dichlorophenyl)ethanone, NaOEt, reflux, or 175 W MW; (ii) $NaBH_4$, EtOH, reflux; (iii) 2,6-dichlorobenzylchloride, 2,4-dichlorobenzylchloride, or 4-chlorobenzylchloride, THF, NaH, reflux, or 200 W MW, (iv) Ciprofloxacine or norfloxacine, DMF, and HCHO.

Gram-negative and Gram-positive pathogens were determined as well. The synthetic methodologies adopted to obtain the target compounds are depicted in Scheme 1.

The synthesis of 4-{[(1*E*)-(2-hydroxyphenyl)methylidene]amino}-5-methyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (2) was performed by the treatment of aromatic aldehyde. The process via MW irradiation ensured the more helpful road with developed synthesis yields and shorter synthesis times (Table 1) [32]. Green Chemistry has the advantages of the high yield of synthesis processes, the use of less toxic solvents, and the decrease in phases of synthetic schemes [33].

Alkylation of product **3** via 2-bromo-1-(4-chlorophenyl)-ethanone and 2-bromo-1-(2,4-dichlorophenyl)ethanone in

Table 1: Yield difference between the conventional method and the MW irradiation method

	MW irradiation method	Conventional method
3a	95	80
3b	98	81
4a	95	72
4b	99	71
5a	83	65
5b	85	63
5c	81	62
5d	79	59
5e	80	60
5f	84	64

ethanol yielded compound **3**. The NH protons attached to the triazole group disappeared for compound **3** in the ^{1}H NMR data. New aromatic protons were resonated in the region 6.92–7.94 ppm. In the ^{13}C NMR data of molecules, the carbon atom (C═O) was observed at 192.62 and 194.51 ppm for the newly added carbonyl group. Considering the EI MS spectra of the product, the existence of [M + Na] ion signals confirmed its molecular mass. Compound **4** was obtained with a reduction of the carbonyl structure of product **3** with sodium borohydride using MW irradiation. Considering compound **4**, the carbonyl group peak evanesced at the ^{1}H NMR and ^{13}C NMR data, and the OH peak resonated at 3.99 and 4.51 ppm in the ^{1}H NMR spectra. The spreading band for the OH group appeared at 3,236 and 3,286 cm^{-1}, in the FT-IR data of molecules. In the ^{1}H NMR and ^{13}C NMR data of molecules, extra signals from the substituted benzyl group were observed at the concerned chemical ranges. Compounds **5a–f** were synthesized using the MW synthesis method at 100°C and 150 W for 17 minutes using molecule **4a–b** and benzyl chlorides such as 4-chloro-, 2,4-dichloro-, and 2,6-dichlorobenzyl chlorides with NaH. In the ^{13}C NMR spectra, triazole C-3 and C-5 of compounds **5a–f** resonated at 155.21–158.17 (triazole C-3) and 157.39–158.48 (triazole C-5), respectively, consistent with the literature findings [34–36]. Moreover, [M + K] and [M + Na] ion signals appeared at the concerned m/z ranges in addition to the obtained structures of molecules **5a–f**.

Furthermore, several Mannich bases of triazole derivatives, including piperazine, thiomorpholine, or morpholine moiety, were synthesized as antimicrobial agents in our laboratory [37,38]. Moreover, it is well known that the presence of fluorinated units in organic compounds may dramatically modify the physicochemical profile of organic molecules. Thus, the heterocyclic compounds containing fluorine atom have been attracting much interest due to their potent biological activities and their role in the development of new drug candidates [39]. Considering these facts in this research, the aminoalkylation of structure **2** with different amines, such as norfloxacin (for **6a**), ciprofloxacin (for **6b**), morpholine (for **6c**), thiomorpholine (**6d**), 4-phenylpiperazine (for **6e**) and 4-fluorphenylpiperazine (for **6f**) in an ambiance with formaldehyde was performed using the MW-assisted Mannich synthesis reactions.

In the ^{1}H NMR and ^{13}C NMR spectra of molecules, additional signals arising from amine moieties were seen at the attended chemical ranges. These molecules exhibition spectral

Table 2: Optimization of the model reaction conditions for compounds **3b–5b**

Entry	Time (min)	Power (W)	Yield (%)	Temperature (°C)	Solvent
Comp **3b**					
1	15	200	97	175	EtOH
2	10	100	98	125	EtOH
3	20	150	96	125	EtOH
4	16	125	97	100	EtOH
5	10	100	90	100	EtOH
6	10	100	92	125	EtOH
Comp **4b**					
1	6	200	97	150	EtOH
2	8	150	99	125	EtOH
3	10	200	96	150	THF
4	4	150	93	100	THF
5	10	200	74	150	MeCN
6	10	200	81	200	MeCN
Comp **5b**					
1	25	100	65	100	EtOH
2	25	75	85	75	THF
3	25	100	60	100	MeCN
4	27	100	50	100	DCM
5	15	150	71	100	EtOH
6	17	150	77	100	THF
7	16	150	69	100	MeCN
8	18	150	68	100	DCM
9	10	200	65	100	EtOH
10	10	200	70	100	THF
11	10	200	73	100	MeCN
12	8	200	40	100	DCM

Table 3: Screening for the activity of newly synthesized compounds

Comp no.	Microorganisms and minimal inhibitory concentrations (μg mL^{-1})								
	Ec	**Yp**	**Pa**	**Sa**	**Ef**	**Bc**	**Ms**	**Ca**	**Sc**
2	—	—	—	250	—	—	31.25	—	—
3a	—	—	—	—	—	—	—	—	—
3b	—	—	—	—	—	—	—	—	—
4a	0.24	—	—	—	—	—	—	125	125
4b	—	—	—	—	—	—	—	—	—
5a	0.24	—	—	—	—	—	—	125	125
5b	—	—	—	—	—	—	—	—	—
5c	0.24	—	—	—	—	—	—	125	125
5d	0.24	—	—	—	—	—	—	125	500
5e	0.24	—	—	—	—	—	—	—	—
5f	0.24	—	—	—	—	—	—	125	125
6a	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	—	500	125
6b	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	—	500	—
6c	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	—	500	125
6d	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	—	500	—
6e	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	—	500	500
6f	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	—	125	500
Amp.	10	18	>128	10	35	15			
Strep.							4		
Flu.								<8	<8

Ec: *Escherichia coli* ATCC 25922, Yp: *Yersinia pseudotuberculosis* ATCC 911, Pa: *Pseudomonas aeruginosa* ATCC 43288, Sa: *Staphylococcus aureus* ATCC 25923, Ef: *Enterococcus faecalis* ATCC 29212, Bc: *Bacillus cereus* 702 Roma, Ms: *Mycobacterium smegmatis* ATCC607, Ca: *Candida albicans* ATCC 60193, Sc: *Saccharomyces cerevisiae* RSKK 251, Amp.: ampicillin, Str.: streptomycin (—): Flu.: fluconazole, (—): no activity.

datum and elemental analysis records fair with their structures. MW-mediated methods were used in the literature to introduce 1,2,4-triazole nuclei into the piperazine skeleton and biologically active compounds were obtained [40]. Mannich reactions were made without solvent in an occasion with Lewis and Bronsted acid catalysts such as HCl. Solvent-free handles are particularly dependent on organic reactions for Green Chemistry situations. The use of the microwave (MW) irradiation method consequences in very influential and clean results with notable developments compared to classical processes.

For MW-mediated reactions leading to the formation of compounds **5a–f**, the production of compound **5b** was selected as a model and the effects of various reaction parameters, including solvent, temperature, time, and MW power were examined on the model reaction, and the results are summarized in Table 2.

In order to improve the MW conditions, the reaction leading to the formation of **5b** was selected as a model reaction and the effects of several parameters including time, power, and solvent were examined. The best conditions were obtained in 25 min of MW irradiation at 75 W in THF. After optimization of the conditions for the preparation of **5b**, the synthesis of the remaining compounds **5** was carried out. By comparison of the two methods, conventional and MW-irradiated procedures showed that the use of MW irradiation provided a more efficient and green way for the synthesis of compounds **5a–f** with better reaction yields and much shorter reaction times. In the NMR spectra of compounds **5a–f**, the number of signals and their chemical shifts are in accordance with the assigned structures.

2.2 Antimicrobial activity

Most of the compounds synthesized in the present study exhibited activity on the test compounds (Table 3). Among them, **6a–d**, which contain a fluoroquinolone nucleus in their structures, demonstrated excellent activities on Gram-positive and Gram-negative bacteria of the test microorganisms with the mic values <0.24 μg mL^{-1}. The carboamides, **2a**, **2b**, and triazoles, **3a**, **3b**, which were obtained from intramolecular cyclization of **2a**, **2b**, displayed selective activity on a Gram-positive coccal bacterium, *Staphylococcus aureus* (Sa), and *Mycobacterium smegmatis* (Ms), atypical tuberculosis factor leading to morbidity and mortality. A remarkable antifungal activity was observed for **5a–f** and **6a–f** with the MIC values.

3 Experimental

3.1 General

All the chemicals were purchased from Fluka Chemie AG Buchs (Switzerland) and used without further purification. The melting points of the synthesized compounds were determined in open capillaries on a Büchi B-540 melting point apparatus and are uncorrected. Reactions were monitored by thin-layer chromatography (TLC) on silica gel 60 F254 aluminum sheets. The mobile phase was ethyl acetate/ethyl ether (1:1), and detection was made using UV light. MW-irradiated syntheses were carried out using monomode CEM-Discover MW apparatus. FT-IR spectra were recorded using a Perkin Elmer 1600 series FTIR spectrometer. ^{1}H NMR and ^{13}C NMR spectra were registered in DMSO-d_6 on a BRUKER AVENE II 400 MHz NMR Spectrometer (400.13 MHz for 1H and 100.62 MHz for 13C). The chemical shifts are given in ppm relative to Me4Si as an internal reference, and *J* values are given in Hz. The mass spectra were obtained on a Quattro EI-MS (70 eV) Instrument.

3.1.1 4-Amino-5-methyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (1)

Hydrazine hydrate (0.025 mol) in 3% water solution was added to the ester ethoxycarbonylhydrazone compound (0.01 mol) contained in a round bottom flask and the reaction was boiled under a reflux system for 8 h. The white solid formed after the flask was left in the freezer overnight was filtered off and purified by crystallization from ethanol.

Yield: 70%, m.p.: 210–212°C. FT-IR (υ_{max}, cm^{-1}): 3,295 and 3,207 (NH_2), 3,218 (NH), 1,683 (C=O), 1,588 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.07 (3H, s, CH_3), 5.13 (2H, s, NH_2), 11.22 (1H, s NH). ^{13}C NMR (DMSO-d_6, δ ppm): 11.23 (CH_3), 146.21 (triazole C-3), 154.97 (triazole C-5). EI MS *m/z* (%): 113.15 (100), 113.40 (90), 154.06 ([M + K + 1]$^+$, 13), 135.18 (12).

3.1.2 4-{[(1*E*)-(2-Hydroxyphenyl)methylidene]amino}-5-methyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (2)

A solution of the corresponding compound **1** (10 mmol) in absolute ethanol was refluxed with salicylaldehyde (10 mmol) for 3 h. On cooling the mixture to room temperature, a white solid appeared. This crude product was recrystallized from ethanol to afford the desired product.

Yield: 75%, m.p.: 245–247°C. FT-IR (υ_{max}, cm^{-1}): 3,170 (OH), 3,047 (aromatic CH), 1,697 (C=O), 1,595 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.26 (3H, s, CH_3), 6.89–6.97 (2H, m, arH), 7.33 (1H, d, *J* = 8.0 Hz, arH), 7.79 (1H, t, *J* = 8.0 Hz, arH), 9.96 (1H, s, CH), 10.33 (1H, s, NH), 11.80 (1H, d, *J* = 8.0 Hz, OH). ^{13}C NMR (DMSO-d_6, δ ppm): 11.58 (CH_3), 116.88 (CH), arC: [119.93 (CH), 119.9 (C), 127.00 (CH), 133.26 (CH), 144.67 (C), 151.70 (CH)], 151.75 (triazole C-3), 157.99 (triazole C-5). EI MS *m/z* (%): 241.05 ([M + Na]$^+$, 100), 242.18 ([M + Na + 1]$^+$, 21), 219.09 ([M + 1]$^+$, 16), 257.20 ([M + K]$^+$, 12).

3.1.3 General method for the synthesis of compounds 3a–b

The solution of compounds **2** (10 mmol) and sodium ethoxide (10 mmol) in ethanol (10 mL) was irradiated in closed vessels at 100°C, 125 W, for 10 min (the progress of the reaction was monitored by TLC). Then, 2-bromo-1-(4-chlorophenyl)ethanone (for **3a**) or 2-chloro-1-(2,4-dichlorophenyl)ethanone (10 mmol) (for **3b**) was added into it and irradiated for additional 15 min. The mixture was poured into ice-water and a solid was obtained. This crude product was collected by filtration and recrystallized from an appropriate solvent to afford the desired product.

3.1.3.1 2-[2-(4-Chlorophenyl)-2-oxoethyl]-4-{[(1*E*)-(2-hydroxyphenyl)methylidene]amino}-5-methyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (3a)

Yield: 95% m.p.: 160–162°C. FT-IR (υ_{max}, cm^{-1}): 3,372 (OH), 3,063 (aromatic CH), 1,704 (C=O), 1,693 (C=O), 1,589 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.33 (3H, s, CH_3), 5.39 (2H, s, CH_2), 6.93–6.97 (2H, m, arH), 7.67 (3H, d, *J* = 8.0 Hz, arH), 8.06 (3H, d, *J* = 8.0 Hz, arH), 9.93 (1H, s, CH), 11.79 (1H, s, OH). ^{13}C NMR (DMSO-d_6, δ ppm): 11.48 (CH_3), 52.14 (CH_2), arC: [112.77 (CH), 116.93 (CH), 119.87 (C), 120.00 (CH), 124.38 (CH), 124.69 (CH), 126.86 (CH), 129.35 (CH), 130.56 (CH), 133.56 (C), 139.52 (C), 144.20 (C)], 152.07 (CH), 150.70 (triazole C-3), 158.11 (triazole C-5), 192.62 (C=O). EI MS *m/z* (%): 393.26 ([M + Na]$^+$, 100), 146.05 (84), 320.30 (78), 233.08 (62), 371.30 ([M + 1]$^+$, 46), 395.20 (31), 425.29 (28), 276.13 (26).

3.1.3.2 2-[2-(2,4-Dichlorophenyl)-2-oxoethyl]-4-{[(1*E*)-(2-hydroxyphenyl)methylidene]amino}-5-methyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (3b)

Yield: 98%. e.n.: 167–169°C. FT-IR (υ_{max}, cm^{-1}): 3,174 (OH), 3,066 (aromatic CH), 1,704 (C=O), 1,666 (C=O), 1,597 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.33 (3H, s, CH_3), 5.25 (2H, s, CH_2), 6.92–6.98 (2H, m, arH), 7.36–7.63

(1H, m, arH), 7.80–7.84 (1H, m, arH), 7.92–7.94 (2H, m, arH), 10.32 (1H, s, CH), 11.78 (1H, s, OH). ^{13}C NMR (DMSO-d_6, δ ppm): 11.60 (CH_3), 54.10 (CH_2), arC: [126.83 (CH), 126.96 (CH), 128.15 (CH), 130.94 (CH), 131.86 (CH), 132.46 (CH), 133.56 (C), 134.21 (C), 137.79 (C), 144.35 (C), 151.75 (C)], 150.52 (CH), 152.06 (triazole C-3), 158.13 (triazole C-5), 194.51 (C=O). EI MS *m/z* (%): 273.13 (100), 360.60 (62), 447.57 (42), 428.42 ([M + Na]$^+$, 10).

3.1.4 General method for the synthesis of compounds 4a–b

The solution of the corresponding compound **3** (10 mmol) in ethanol was irradiated with MW energy at 150°C, 125 W in the presence of $NaBH_4$ (30 mmol) with pressure control (the progress of the reaction was monitored by TLC). Then, the solvent was removed under reduced pressure and the solid appeared. This crude product was washed with water and recrystallized from acetone/water (1:3).

3.1.4.1 2-[2-(4-Chlorophenyl)-2-hydroxyethyl]-4-{[(1*Z*)-(2-hydroxyphenyl) methyl ene]amino}-5-methyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (4a)

Yield: 95%. FT-IR (υ_{max}, cm^{-1}): 3,236 (OH), 1,673 (C=O), 1,596 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 1.70 (3H, s, CH_3), 3.79 (2H, d, J = 8.0 Hz, CH), 3.99 (1H, d, J = 4.0 Hz, OH), 4.88–4.91 (2H, m, CH_2), 6.67–6.70 (2H, m, arH), 6.69 (2H, d, J = 8.0 Hz, arH), 6.80 (2H, d, J = 8.0 Hz, arH), 6.91 (2H, d, J = 8.0 Hz, arH), 9.81 (1H, s, CH), 10.20 (1H, s, NH), 11.80 (1H, s, OH). ^{13}C NMR (DMSO-d_6, δ ppm): 10.55 (CH_3), 52.27 (CH_2), 70.08 (CH), arC: [103.55 (CH), 115.64 (CH), 118.95 (CH), 123.72 (C), 128.52 (CH), 128.53 (CH), 128.96 (CH), 129.13 (CH), 131.30 (2CH), 141.51 (C), 142.23 (C), 144.88 (C)], 152.86 (triazole C-3), 156.57 (triazole C-5). EI MS *m/z* (%): 397.20 ([M + Na + 2]$^+$, 100), 399.14 (30), 323.18 (29), 291.02 (25), 327.31 (18).

3.1.4.2 2-[2-(2,4-Dichlorophenyl)-2-hydroxyethyl]-4-{[(1*E*)-(2hydroxyphenyl) methylene]amino} -5-methyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (4b)

Yield: 99%. FT-IR (υ_{max}, cm^{-1}): 3,286 (OH), 1,686 (C=O), 1,589 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 1.08–1.15 (3H, m, CH_3), 3.78 (2H, s, CH_2), 4.40 (1H, s, CH), 4.51 (1H, s, OH), 7.28–7.50 (7H, m, arH), 9.53 (1H, d, J = 8.0 Hz, CH), 11.81 (1H, s, OH). ^{13}C NMR (DMSO-d_6, δ ppm): 10.52 (CH_3), 48.23 (CH_2), 78.19 (CH), arC: [111.57 (CH), 115.61 (CH), 121.72 (CH), 123.35 (CH), 123.75 (CH), 124.81 (CH), 128.03 (CH), 128.11 (C), 128.79 (C), 129.07 (C), 129.15 (C), 129.47 (C)], 144.92 (CH), 152.98 (triazole C-3), 157.99 (triazole C-5). EI MS *m/z* (%): 431.25 ([M + Na + 1]$^+$, 100), 134.98 (68), 433.25 (60), 325.19 (34).

3.1.5 General method for the synthesis of compounds 5a–f

3.1.5.1 Method 1

NaH (10 mmol) was added to the solution of the corresponding compound **4** (10 mmol) in THF and the mixture was refluxed for 6 h. Then, the corresponding benzyl chloride was added to it and the mixture was refluxed for an additional 14 h. After evaporating the solvent under reduced pressure, an oily mass formed. This was extracted with 15 mL of ethyl acetate three times in the presence of K_2CO_3 and the organic layer was dried on Na_2SO_4. After the removal of solvents at reduced pressure, a solid was obtained, which was recrystallized from acetone.

3.1.5.2 Method 2

NaH (1 mmol) was added to the solution of the corresponding compound **4** (1 mmol) in THF (10 mL) and the mixture was irradiated at 75°C, 75 W for 10 min. Then, the corresponding substituted benzylchloride (3 mmol) was added to it and irradiation was continued for 45 min (for 5a–f) at 125°C, 150 W. The solvent was evaporated under reduced pressure, and the obtained oily product was extracted with 15 mL of ethyl acetate three times in the presence of K_2CO_3. The organic layer was dried on Na_2SO_4. After the removal of solvents at reduced pressure, an oily product was formed, which was purified by column chromatography (*n*-hexane/ethyl acetate) on silica gel.

3.1.5.1 2-{2-(4-Chlorophenyl)-2-[(2,4-dichlorobenzyl) oxy]ethyl}-4-{[(1*Z*)-(2hydroxyphenyl)methylene] amino}-5-methyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (5a)

Yield: 83%. FT-IR (υ_{max}, cm^{-1}): 3,275 (OH), 1,587 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 1.10–1.13 (3H, m, CH_3), 3.87 (2H, s, CH_2), 3.95 (2H, s, CH_2), 4.38 (1H, s, CH), 7.10–7.35 (7H, m, arH), 7.40–7.55 (4H, m, arH), 9.10 (1H, d, J = 8.0 Hz, CH), 11.10 (1H, s, OH). ^{13}C NMR (DMSO-d_6, δ ppm): 11.78 (CH_3), 47.85 (CH_2), 50.12 (CH_2), 76.12 (CH), arC: [110.17 (CH), 110.98 (CH), 111.85 (CH), 112.85 (CH), 113.20 (CH), 114.78 (CH), 115.69 (CH), 117.33 (CH), 118.87

(CH), 121.91 (CH), 122.53 (CH), 123.98 (C), 130.58 (C), 132.10 (C), 133.83 (C), 139.17 (C), 140.23 (C), 141.37 (C)], 145.21 (CH), 156.89 (triazole C-3), 158.41 (triazole C-5). EI MS *m/z* (%): 570.83 ([M + K]$^+$, 100), 312.85 (85), 187.12 (51).

3.1.5.2 2-{2-(4-Chlorophenyl)-2-[(2,6-dichlorobenzyl) oxy]ethyl}-4-{[(1*Z*)-(2-hydroxyphenyl) methylene]amino}-5-methyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (5b)

Yield: 85%. FT-IR (υ_{max}, cm^{-1}): 3,288 (OH), 3,083 (aromatic CH), 1,685 (C═O), 1,582 (C═N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.31 (3H, s, CH_3), 3.36 (1H, s, CH), 5.17 (2H, s, CH_2), 5.50 (2H, s, CH_2), 7.17–7.48 (5H, m, arH), 7.91–8.30 (6H, m, arH), 9.74 (1H, s, CH), 11.69 (1H, s, OH). ^{13}C NMR (DMSO-d_6, δ ppm): 19.30 (CH_3), 55.87 (CH_2), 663.28 (CH_2), 75.23 (CH), arC: [111.94 (CH), 112.87 (CH), 114.61 (CH), 115.52 (CH), 118.78 (CH), 119.65 (CH), 120.21 (CH), 121.84 (CH), 123.59 (CH), 126.74 (CH), 128.95 (CH), 131.10 (C), 132.41 (C), 133.73 (C), 135.74 (C), 136.74 (C), 137.20 (C), 138.36 (C)], 147.10 (CH), 155.21 (triazole C-3), 158.20 (triazole C-5). EI MS *m/z* (%): 555.14 ([M + K]$^+$, 100), 557.16 ([M + K + 2]$^+$, 98), 559.11 (38), 397.20 (28).

3.1.5.3 2-[2-[(4-Chlorobenzyl)oxy]-2-(4-chlorophenyl) ethyl]-4-{[(1*Z*)-(2-hydroxyphenyl)methyl ene] amino}-5-methyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (5c)

Yield: 81%. FT-IR (υ_{max}, cm^{-1}): 3,312 (OH), 3,075 (aromatic CH), 1,695 (C═O), 1,573 (C═N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.08 (3H, s, CH_3), 3.09 (1H, s, CH), 3.22 (2H, s, CH_2), 3.37 (2H, s, CH_2), 6.97–7.09 (5H, m, arH), 7.26–7.46 (7H, m, arH), 8.69 (1H, s, CH), 8.72 (1H, s, OH). ^{13}C NMR (DMSO-d_6, δ ppm): 18.74 (CH_3), 50.78 (CH_2), 58.37 (CH_2), 77.21 (CH), arC: [118.10 (2CH), 119.10 (2CH), 120.64 (2CH), 123.54 (2CH), 124.21 (CH), 125.71 (CH), 126.30 (CH), 127.41 (CH), 128.63 (CH), 130.61 (C), 131.31 (C), 133.10 (C), 134.87 (C), 136.44 (C), 140.69 (C)], 148.21 (CH), 157.60 (triazole C-3), 158.37 (triazole C-5). EI MS *m/z* (%): 536.38 ([M + K]$^+$, 100), 387.64 (77), 134.21 (41).

3.1.5.4 2-[2-[(2,4-Dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-4-{[(1*E*)-(2-hydroxyphenyl) methylene]amino}-5-methyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (5d)

Yield: 79%. FT-IR (υ_{max}, cm^{-1}): 3,289 (OH), 3,095 (aromatic CH), 1,692 (C═O), 1,588 (C═N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.07 (3H, s, CH_3), 4.55 (2H, s, CH_2), 4.78 (2H, s, CH_2), 4.86 (1H, s, CH), 7.39–7.41 (5H, m, arH), 7.57–7.61 (5H, m, arH), 10.45 (1H, s, CH), 11.64 (1H, s, OH). ^{13}C NMR (DMSO-d_6, δ ppm): 14.20 (CH_3), 50.28 (CH_2), 57.71 (CH_2), 77.21 (CH), arC: [114.45 (CH), 115.21 (CH), 116.20 (CH), 117.80 (CH), 118.34 (CH), 119.49 (CH), 120.21 (CH), 121.61 (CH), 123.66 (CH), 125.52 (CH), 130.57 (C), 131.10 (C), 132.47 (C), 135.45 (C), 136.27 (C), 137.88 (C)], 146.21 (CH), 158.17 (triazole C-3), 159. 37 (triazole C-5). EI MS *m/z* (%): 605.27 ([M + K]$^+$, 100), 398.27 (58), 117.21 (33).

3.1.5.5 2-[2-[(2,6-Dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-4-{[(1*E*)-(2-hydroxyphenyl) methylene]amino}-5-methyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (5e)

Yield: 80%. FT-IR (υ_{max}, cm^{-1}): 3,067 (aromatic CH), 1,576 (C═N). ^{1}H NMR (DMSO-d_6, δ ppm): 2,11 (3H, s, CH_3), 3,86 (2H, s, CH_2), 4,28 (2H, s, CH_2), 4,97 (1H, s, CH), 7,12–7,20 (6H, m, arH), 7.33–7.47 (4H, m, arH), 10.36 (1H, s, CH). ^{13}C NMR (DMSO-d_6, δ ppm): 15.23 (CH_3), 52.21 (CH_2), 53.85 (CH_2), 78.10 (CH), arC: [110.52 (CH), 111.74 (CH), 112.30 (CH), 113.10 (CH), 114.74 (CH), 115.30 (CH), 116.17 (CH), 120.19 (CH), 121.38 (CH), 122.41 (CH), 125.33 (C), 129.76 (C), 130,30 (C), 131.11 (C), 132.88 (C), 133.28 (C), 134.74 (C), 138.29 (C)], 147.20 (CH), 156.71 (triazole C-3), 157.39 (triazole C-5). EI MS *m/z* (%): 589.27 ([M + Na]$^+$, 100), 371.30 (60), 122.21 (37).

3.1.5.6 2-[2-[(4-Chlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-4-{[(1*E*)-(2-hydroxyphenyl) methylene]amino}-5-methyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (5f)

Yield: 84%. FT-IR (υ_{max}, cm^{-1}): 3,291 (OH), 3,095 (aromatic CH), 1,692 (C═O), 1,589 (C═N). ^{1}H NMR (DMSO-d_6, δ ppm): 1.71 (3H, s, CH_3), 3.37 (2H, s, CH_2), 4.77 (2H, s, CH_2), 5.11 (1H, s, CH), 7.43–7.48 (11H, m, arH), 9.46 (1H, s, CH), 11.78 (1H, s, OH). ^{13}C NMR (DMSO-d_6, δ ppm): 10.56 (CH_3), 45.63 (CH_2), 62.55 (CH_2), arC: [104.41 (CH), 111.55 (CH), 115.56 (CH), 119.14 (CH), 121.70 (CH), 123.33 (CH), 123.73 (CH), 124.73 (CH), 124.78 (CH), 130.11(CH), 130.41 (CH), 131.17 (CH), 131. 31 (CH), 132.57 (CH), 133.04 (C), 133.42 (C), 137.18 (C), 139.75 (C), 142.03 (C), 144.91 (C), 152.86 (C)], 156.89 (triazole C-3), 158.48 (triazole C-5). EI MS *m/z* (%): 310.25 (100), 532.85 ([M + 1]$^+$, 75), 432.52 (61).

3.1.6 General method for the synthesis of compounds 6a–f

To the solution of corresponding compound **2** (10 mmol) in dimethylformamide, suitable primary or secondary

amine (10 mmol) was added and the mixture was stirred at room temperature in the presence of formaldehyde (37 %, 3.72 mL, 5 mmol) for 24 h (the progress of the reaction was monitored by TLC). The solid that precipitated was collected by filtration and recrystallized from dimethylsulfoxide/water (1:1) to give the desired compound.

3.1.6.1 1-Ethyl-6-fluoro-7-{4-[(4-{[(1*Z*)-(2-hydroxyphenyl)methylene]amino}-3-methyl-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl)methyl]piperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6a)

Yield: 85%. FT-IR (υ_{max}, cm^{-1}): 3,057 (aromatic CH), 1,727 (C═O), 1,705 (C═O), 1,518 (C═N). ^{1}H NMR (DMSO-d_6, δ ppm): 1.41 (3H, s, CH_3), 2.28 (3H, d, J = 16.0 Hz, CH_3), 2.74 (2H, s, CH_2), 2.83 (2H, s, CH_2), 2.89 (2H, s, CH_2), 4.56 (4H, d, J = 8.0 Hz, $2CH_2$), 4.64 (2H, s, CH_2), 6.91–6.97 (2H, m, arH), 7.15 (1H, d, J = 8.0 Hz, arH), 7.35 (1H, s, arH), 7.78–7.88 (2H, m, arH), 8.90 (1H, s, CH), 9.93 (1H, s, CH), 10.21 (1H, s, OH), 15.26 (1H, s, OH). ^{13}C NMR (DMSO-d_6, δ ppm): 11.50 (CH_3), 14.75 (CH_3), 49.49 (CH_2), 49.73 (CH_2), 49.86 (CH_2), 49.90 (CH_2), 55.39 (CH_2), 66.07 (CH_2), 106.38 (CH), 107.51 (C), arC: [111.47 (CH), 111.70 (CH), 116.89 (CH), 119.62 and 119.69 (C, d, J = 7.0 Hz), 119.93 (CH), 126.79 (CH), 133.26 and 133.44 (CH, d, J = 18.0 Hz), 137.59 (CH), 143.58 (C), 145.78 and 145.88 (C, d, J = 10.0 Hz), 152.03 (C)], 148.86 (CH), 154.51 (triazole C-3), 158.09 (triazole C-5), 166.55 (C═O), 176.58 (C═O). EI MS m/z (%): 542.31 (100), 550.74 ([M + 1]$^+$, 75), 572.07 ([M + Na]$^+$, 70), 619.39 (69), 516.70 (61), 512.23 (58), 607.31 (52), 589.38 ([M + K + 1]$^+$, 28).

3.1.6.2 1-Cyclopropyl-6-fluoro-7-{4-[(4-{[(1*Z*)-(2-hydroxyphenyl)methylene]amino}-3-methyl-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl)methyl]piperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6b)

Yield: 88%. FT-IR (υ_{max}, cm^{-1}): 3,516 (OH), 3,351 (OH), 3,052 (aromatic CH), 1,729 (C═O), 1,706 (C═O), 1,538 (C═N). ^{1}H NMR (DMSO-d_6, δ ppm): 1.15 (2H, s, CH_2), 1.30 (2H, d, J = 4.0 Hz, CH_2), 2.30 (3H, s, CH_3), 2.83 (2H, s, CH_2), 3.32 (2H, s, CH_2), 3.78 (2H, s, CH_2), 4.64 (4H, s, $2CH_2$), 6.89–6.95 (2H, m, arH), 7.33 (1H, d, J = 8.0 Hz, arH), 7.51 (1H, d, J = 4.0 Hz, arH), 7.78–7.82 (2H, m, arH), 8.60 (2H, s, 2CH), 9.94 (1H, s, CH), 10.29 (1H, s, OH), 15.16 (1H, s, OH). ^{13}C NMR (DMSO-d_6, δ ppm): 8.01 (CH_2), 11.52 (CH_3), 36.25 (CH_2), 49.09 (CH_2), 49.77 (CH_2), 49.81 (CH_2), 66.06 ($2CH_2$), 106.85 (CH), 107.16 (C), arC: [111.20 and 111.43 (CH, d, J = 23.0 Hz), 116.88 (CH), 118.95 and 119.02 (C, d, J = 7.0 Hz), 119.87 (C), 119.92 (CH), 126.75 (CH), 131.30 (CH), 133.44 (CH), 139.53 (C), 143.60 (C), 145.46 and 145.56 (C, d, J = 10.0 Hz), 150.75 (C), 152.15 (C), 154.63 (C)], 148.30 (CH), 151.81 (CH), 158.08 (triazole C-3), 159.10 (triazole C-5), 166.34 (C═O), 176.71 (C═O). EI MS m/z (%): 584.22 ([M + Na]$^+$, 100), 585.10 ([M + Na + 1]$^+$, 49), 150.97 (21), 134.92 (18).

3.1.6.3 4-[(*Z*)-2-(2-Hydroxyphenyl)vinyl]-5-methyl-2-(morpholin-4-ylmethyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (6c)

Yield: 86%. FT-IR (υ_{max}, cm^{-1}): 3,059 (aromatic CH), 1,698 (C═O), 1,595 (C═N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.30 (3H, s, CH_3), 2.57 (4H, t, J = 4.0 Hz, $2CH_2$), 3.54–3.57 (4H, m, $2CH_2$), 4.52 (2H, s, CH_2), 6.89–6.96 (2H, m, arH), 7.33–7.37 (1H, m, arH), 7.80 -7.83 (1H, m, arH), 9.94 (1H, s, CH), 10.31 (1H, s, OH). ^{13}C NMR (DMSO-d_6, δ ppm): 11.49 (CH_3), 50.42 (CH_2), 66.32 ($2CH_2$), 66.49 ($2CH_2$), arC: [116.91 (CH), 118.66 (CH), 119.90 (C), 126.81 (CH), 131.30 (CH), 143.53 (C)], 151.91 (CH), 150.76 (triazole C-3), 158.09 (triazole C-5). EI MS m/z (%): 217.17 (100), 113.02 (56), 155.03 (49), 318.36 ([M + 1]$^+$, 47), 175.41 (35), 340.19 ([M + Na]$^+$, 26).

3.1.6.4 4-[(*Z*)-2-(2-Hydroxyphenyl)vinyl]-5-methyl-2-(thiomorpholin-4-ylmethyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (6d)

Yield: 89%. FT-IR (υ_{max}, cm^{-1}): 3,062 (aromatic CH), 1,708 (C═O), 1,594 (C═N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.30 (3H, s, CH_3), 2.58–2.61 (4H, m, $2CH_2$), 2.84 (4H, t, J = 4.0 Hz, $2CH_2$), 4.54 (2H, s, CH_2), 6.90–7.00 (2H, m, arH), 7.33–7.37 (1H, m, arH), 7.81 (1H, d, J = 4.0 Hz, arH), 9.94 (1H, s, CH), 10.30 (1H, s, OH). ^{13}C NMR (DMSO-d_6, δ ppm): 11.50 (CH_3), 27.61 ($2CH_2$), 52.44 ($2CH_2$), 67.68 (CH_2), arC: [116.91 (CH), 119.90 (C), 119.96 (CH), 126.83 (CH), 133.48 (CH), 143.50 (C)], 151.93 (CH), 150.71 (triazole C-3), 158.10 (triazole C-5). EI MS m/z (%): 334.40 ([M + 1]$^+$, 100), 340.41 (78), 178.96 (62), 134.74 (55).

3.1.6.5 4-[(*Z*)-2-(2-Hydroxyphenyl)vinyl]-5-methyl-2-[(4-phenylpiperazin-1-yl)methyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-one (6e)

Yield: 90%. FT-IR (υ_{max}, cm^{-1}): 3,305 (OH), 3,059 (aromatic CH), 1,700 (C═O), 1,601 (C═O). ^{1}H NMR (DMSO-d_6,

δ ppm): 2.09 (3H, s, CH_3), 2.30 (2H, s, CH_2), 2.73 (2H, m, CH_2), 3.31 (2H, s, CH_2), 4.61 (2H, s, CH_2), 5.01 (2H, d, J = 8.0 Hz, CH_2), 6.76 (1H, s, arH), 6.90 (1H, d, J = 8.0 Hz, arH), 6.96 (3H, d, J = 8.0 Hz, arH), 7.18 (2H, d, J = 8.0 Hz, arH), 7.34 (1H, d, J = 8.0 Hz, arH), 7.80 (1H, d, J = 4.0 Hz, arH), 9.94 (1H, d, J = 12.0 Hz, CH), 10.30 (1H, d, J = 12.0 Hz, OH). ^{13}C NMR (DMSO-d_6, δ ppm): 11.50 (CH_3), 48.73 (CH_2), 50.04 (CH_2), 66.13 (CH_2), 67.54 ($2CH_2$), arC: [116.07 (2CH), 116.91 (2CH), 119.37 (CH), 119.91 (C), 119.95 (CH), 126.82 (CH), 129.34 (CH), 133.46 (CH), 143.50 (C), 143.88 (C)], 151.90 (CH), 151.53 (triazole C-3), 158.10 (triazole C-5). EI MS m/z (%): 120.08 (100), 175.14 (18), 393.26 ([M + 1]$^+$, 10).

3.1.6.6 2-{[4-(2-Fluorophenyl)piperazin-1-yl]methyl}-4-[(*Z*)-2-(2-hydroxyphenyl) vinyl]-5-methyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (6f)

Yield: 91%. FT-IR (υ_{max}, cm^{-1}): 3,239 (OH), 3,059 (aromatic CH), 1,708 (C═O), 1,686 (C═O), 1,595 (C═N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.30 (3H, s, CH_3), 2.73 (2H, s, CH_2), 3.06 (2H, m, CH_2), 3.35 (2H, s, CH_2), 4.60 (2H, s, CH_2), 5.01 (2H, d, J = 8.0 Hz, CH_2), 6.89–7.05 (6H, m, arH), 7.35 (1H, s, arH), 7.82 (2H, d, J = 4.0 Hz, arH), 10.31 (1H, s, CH), 11.13 (1H, s, OH). ^{13}C NMR (DMSO-d_6, δ ppm): 11.50 (CH_3), 49.52 (CH_2), 50.03 (CH_2), 66.09 (CH_2), 67.54 ($2CH_2$), arC: [115.57 (CH), 115.79 (CH), 116.91 (CH), 117.80 (CH), 117.88 (CH), 126.81 (CH), 133.47 (CH), 148.43 (C), 149.74 (C), 150.76 (C), 151.89 (CH), 155.34 (C)], 157.69 (triazole C-3), 158.10 (triazole C-5). EI MS m/z (%): 411.48 ([M + 1]$^+$, 100), 412.23 (50), 433.44 ([M + K]$^+$, 49), 459.28 (46).

3.2 Antimicrobial activity assessment

The test microorganisms were obtained from the Hifzissihha Institute of RefikSaydam (Ankara, Turkey) and were as follows: *Escherichia coli* (*E. coli*) ATCC35218, *Yersinia pseudotuberculosis* (*Y. pseudotuberculosis*) ATCC911, *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC43288, *Enterococcus faecalis* (*E. faecalis*) ATCC29212, *Staphylococcus aureus* (*S. aureus*) ATCC25923, *Bacillus cereus* (*B. cereus*) 709 Roma, *Mycobacterium smegmatis* (*M. smegmatis*) ATCC607, *Candida albicans* (*C. albicans*)ATCC60193, and *Saccharomyces cerevisiae* (*S. cerevisia*) RSKK 251. All the newly synthesized compounds were weighed and dissolved in hexane to prepare the extract stock solution of 20.000 µg mL^{-1}.

The antimicrobial effects of the substances were tested quantitatively in respective broth media by using double microdilution and the minimal inhibition concentration (MIC) values (µg mL^{-1}) were determined. The antibacterial and antifungal assays were performed in Mueller–Hinton broth (MH) (Difco, Detroit, MI) at pH.7.3 and buffered Yeast Nitrogen Base (Difco, Detroit, MI) at pH 7.0, respectively. The micro dilution test plates were incubated for 18–24 h at 35°C. Brain heart infusion broth (BHI) (Difco, Detriot, MI) was used for *M. smegmatis* and incubated for 48–72 h at 35°C [41]. Ampicillin (10 µg) and fluconazole (5 µg) were used as standard antibacterial and antifungal drugs, respectively. Dimethyl sulfoxide with a dilution of 1:10 was used as solvent control. The results obtained are presented in Table 2.

4 Conclusion

In this research, the successful synthesis of some new 4-{[(1*E*)-(2-hydroxyphenyl)methylidene]amino}-5-methyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one and conversion of some of them into the corresponding Mannich bases and conazole derivatives as well as the antimicrobial screening studieswere carried out . 1,2,4-Triazole nucleus is one of the effective unit currents in many standard drugs and it is known to enhance the pharmacological activity of the molecules. The presence of *N*-methylpiperazine, morpholine, norfloxacin, and ciprofloxacin moiety is also instrumental in contributing to the net biological activity of a system. Also, we already reported antimicrobial activities of some biheterocyclic compounds incorporating 1,2,4-triazole ring, in addition to some alkylated derivatives of 1,2,4-triazole compounds. Hence, herein, we combined all these two potential units, namely 1,2,4-triazole methyl piperazine, morpholine, norfloxacin, and ciprofloxacin rings. The antimicrobial screening suggests that among the newly synthesized compounds, **2**, **3a**–**b**, **4a**–**b**, **5a**–**f** and **6a**–**f** exhibited moderate activity against all the tested microorganisms except *M. smegmatis* and *C. albicans*. On the contrary to what was expected, the structure of compounds **5a–f** by Conazole derivatives did not exhibit antimicrobial activity.

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References

[1] Gomtsyan A. Heterocycles in drugs and drug discovery. Chem Heterocycl Compd. 2012;48(1):12–5.

[2] Foroumadi A, Mansouri S, Kiani Z, Kiani A. Synthesis and in vitro antibacterial evaluation of *N*-[5-(5-nitro-2-thienyl)-1,3,4-thiadiazole-2-yl] piperazinyl quinolones. Eur J Med Chem. 2003;38:851–4.

[3] Amir M, Kumar H, Khan SA. Synthesis and pharmacological evaluation of pyrazoline derivatives as new anti-inflammatory and analgesic agents. Bioorg Med Chem Lett. 2003;18(3):918–22.

[4] Ruddarraju RR, Murugulla AC, Kotla R, Tirumalasetty MB, Wudayagiri R, Donthabakthuni S, et al. Design, synthesis, anticancer activity and docking studies of theophylline containing 1,2,3-triazoles with variant amide derivatives. Med Chem Comm. 2017;8:176–83.

[5] Celik F, Unver Y, Barut B, Ozel A, Sancak K. Synthesis, characterization and biological activities of new symmetric bis-1,2,3-triazoles with click chemistry. Med Chem. 2018;14(3):230–41.

[6] Sahu JK, Ganguly S, Kaushik A. Synthesis of some novel heterocyclic 1, 2, 4-triazolo [3, 4-b][1, 3, 4] thiadiazole derivatives as possible antimicrobial agents. J Appl Pharm Aci. 2014;4(2):081–6.

[7] Shaikh MH, Subhedar DD, Nawale L, Sarkar D, Khan FAK, Sangshettic JN, et al. 1,2,3-Triazole derivatives as antitubercular agents: synthesis, biological evaluation and molecular docking study. Med Chem Commun. 2015;6:1104–16.

[8] Kritsanida M, Mouroutsou A, Marakos P, Pouli N, Papakonstantinou-Garoufalias S, Pannecouque C, et al. Synthesis and antiviral activity evaluation of some new 6-substituted 3-(1-adamantyl)-1,2,4-triazolo[3,4-b][1,3,4]thiadiazoles. Il Farmaco. 2002;57:253–7.

[9] Bhat KS, Poojary B, Prasad JD, Naik P, Holla BS. Synthesis and antitumor activity studies of some new fused 1,2,4-triazole derivatives carrying 2,4-dichloro-5-fluorophenyl moiety. Eur J Med Chem. 2009;44(12):5066–70.

[10] Saraei M, Ghasemi Z, Dehghan G, Hormati M, Ojaghi K. Synthesis of some novel 1,2,3-triazole derivatives containing kojic acid moiety and evaluation for their antioxidant activity. Monatsh Chem. 2017;148:917–23.

[11] Gupta JK, Mishra P. Antimicrobial and anthelmintic activities of some newly synthesized triazoles. Asian J Pharm Clin Res. 2017;10(6):139–45.

[12] Kamboj VK, Verma PK, Dhanda A, Ranjan S. 1,2,4-triazole derivatives as potential scaffold for anticonvulsant activity. Cent Nerv Syst Agents Med Chem. 2015;15:17–22.

[13] Denga X, Song M, Gong G, Wang S, Quan Z. Synthesis and anticonvulsant evaluation of some new 6-(substituted-phenyl) thiazolo [3, 2-b][1, 2, 4] triazole derivatives in mice. Iran J Pharm Res. 2014;13(2):459–69.

[14] Shalini K, Kumar N, Drabu S, Sharma PK. Advances in synthetic approach to and antifungal activity of triazoles. Beilstein J Org Chem. 2011;7:668–77.

[15] Khanage SG, Raju A, Mohite PB, Pandhare RB. Analgesic activity of some 1, 2, 4-triazole heterocycles clubbed with pyrazole, tetrazole, isoxazole and pyrimidine. Adv Pharm Bull. 2013;3(1):13–8.

[16] Asif M. Antiviral and antiparasitic activities of various substituted triazole derivatives: a mini. Chem Int. 2015;1(2):71–80.

[17] Süleymanoğlu N, Ustabaş R, Direkel Ş, Alpaslan YB, Ünver Y. 1,2,4-Triazole derivative with Schiff base; thiol-thione tautomerism, DFT study and antileishmanial activity. J Mol Struct. 2017;1150:82–7.

[18] Karrouchi K, Chemlal L, Taoufik J, Cherrah Y, Radi S, El Abbes Faouzi M, et al. Synthesis, antioxidant and analgesic activities of Schiff bases of 4-amino-1,2,4-triazole derivatives containing a pyrazole moiety. Ann Pharm Fr. 2016;74:431–8.

[19] Liu XL, Zhao ZG, Shi ZC, et al. Microwave-prompted synthesis and bioactivity of novel substituted bis-triazole Schiff bases containing pyridine rings. J Southwest Minzu Univ (Nat Sci Ed). 2017;43:469–73.

[20] Wang BL, Zhang LY, Zhan YZ, Zhang Y, Zhang X, Wang LZ, et al. Synthesis and biological activities of novel 1,2,4-triazole thiones and bis(1,2,4-triazole thiones)containing phenylpyrazole and piperazine moieties. J Fluor Chem. 2016;184:36–44.

[21] Zhang SH, Wang JM, Zhang HY, Fan YP, Xiao Y. Highly efficient electrochemiluminescence based on 4-amino-1,2,4-triazole Schiff base two-dimensional Zn/Cd coordination polymers. Dalton Trans. 2017;46:410–9.

[22] Wajda-Hermanowicz K, Pieniążczak D, Wróbel R, Zatajska A, Ciunik Z, Berski S. A study on the condensation reaction of aryl substituted 4-amine-1,2,4-triazole with benzaldehydes: structures and spectroscopic properties of schiff bases and stable hemiaminals. J Mol Struct. 2016;1114:108–22.

[23] Sun Q, Zheng F, Sun X, Wang W. Construction of a dinuclear silver (I) coordination complex with a Schiff base containing 4-amino1,2,4-triazole ligands. Acta Crystallogr Sect E Struct Rep Online. 2009;65:m283–4.

[24] Heeres J, Meerpoel L, Lewi P. Conazoles. Molecules. 2010;15:4129e4188. doi: 10.3390/molecules15064129.

[25] Andes DR, Dismukes WE, Kauffman CA, Pappas PG, Sobel JD. Azoles. In: Dismukes WE, editor. Essentials Clin. Mycol. 7, Second edn. New York: Springer-Verlag New York; 2011. p. 61e93. doi: 10.1007/978-1-4419-6640.

[26] Leung S, Poulakos M, Machin J. Posaconazole, an update of its clinical use. Pharmacy. 2015;3:210e268. doi: 10.3390/pharmacy3040210.

[27] Moore JN, Healy JR, Kraft WK. HHS public access. Expert Rev Clin Pharmacol. 2015;8:321e334. doi: 10.7205/MILMED-D-14-00168. (Long-chain).

[28] Kauffman CA, Malani AN, Easley C, Kirkpatrick P. Posaconazole. Nat Rev Drug Discov. 2007;6:183e184. doi: 10.1038/nrd2270.

[29] Farowski F, Vehreschild JJ, Cornely OA. Posaconazole: a next-generation triazole antifungal. Future Microbiol. 2007;2:231e243. doi: 10.2217/17460913.2.3.231.

[30] Peyton LR, Gallagher S, Hashemzadeh M. Triazole antifungals: a review. Drugs Today. 2015;51:705e718. doi: 10.1358/dot.2015.51.12.2421058.

[31] Rhoden E, Nix WA, Weldon WC, Selvarangan R. Antifungal azoles itraconazole and posaconazole exhibit potent in vitro antiviral activity against clinical isolates of parechovirus A3 (Picornaviridae). Antivir Res. 2018;149:75e77. doi: 10.1016/j.antiviral.2017.11.011.

[32] Loupy A. Solvent-free microwave organic synthesis as an efficient procedure for green chemistry. C R Chim. 2004;7:103–12. doi: 10.1016/j.crci.2003.10.015.

[33] Grewal AS, Kumar K, Redhu S, Bhardwaj S. Microwave assisted synthesis: a green chemistry approach. Int Res J Pharm App Sci. 2013;3:278-2851066.
[34] Bayrak H, Demirbas A, Karaoglu SA, Demirbas N. Synthesis of some new 1,2,4-triazoles, their Mannich and Schiff bases and evaluation of their antimicrobial activities. Eur J Med Chem. 2009;44(3):1057–66. doi: 10.1016/j.ejmech.2008.06.019. PMID: 18676062.
[35] Bayrak H, Demirbas A, Demirbas N, Karaoglu SA. Synthesis of some new 1,2,4-triazoles starting from isonicotinic acid hydrazide and evaluation of their antimicrobial activities. Eur J Med Chem. 2009;44(11):4362–6. doi: 10.1016/j.ejmech.2009.05.022. PMID: 19647352.
[36] Demirci S, Basoglu S, Bozdereci A, Demirbas N. Preparation and antimicrobial activity evaluation of some new bi- and tri-heterocyclic azoles. Med Chem Res. 2013;22:4930–45. doi: 10.1007/s00044-013-0498-3
[37] Ozyanık M, Demirci S, Bektas H, Demirbas N, Demirbas A, Alpay-Karaoglu S. Preparation and antimicrobial activity evaluation of some quinoline derivatives containing an azole nucleus. Turk J Chem. 2012;36:233–46.
[38] Fandaklı S, Basoglu S, Bektas H, Yolal M, Demirbas A, Alpay-Karaoglu S. Reduction, Mannich reaction, and antimicrobial activity evaluation of some new 1,2,4-triazol-3-one derivatives. Turk J Chem. 2012;36:567–82.
[39] Althagafi II, Shaaban MR. Microwave assisted regioselective synthesis of novel pyrazoles and pyrazolopyridazines via fluorine containing building blocks. J Mol Struct. 2017;1142:122–9. doi: 10.1016/j.molstruc.2017.04.047.
[40] Ozdemir SB, Demirbas N, Demirbas A, Ayaz FA, Çolak N. Microwave assisted synthesis, antioxidant, and antimicrobial evaluation of piperazine azole fluoroquinolone based 1,2,4 triazole derivatives. J Heterocycl Chem. 2018;55:2744. doi: 10.1002/jhet.3336.
[41] Willanova M. National Committee for Clinical Laboratory Standard Methods for determining bactericidal activity of antibacterial agents. App Guid NCCLS. 1999;19:18–9.