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Research Article

Synthesis and Antimicrobial Activity of Some [1,2,4]-Triazole Derivatives

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A series of novel [1,2,4]-triazolo piperidine (**8**), [1,2,4]-triazolo piperazine (**9a-c**), [1,2,4]-triazolo phenylether (**10a-e**), and [1,2,4]-triazolo aniline (**11a-c**) derivatives have been synthesized. The chemical structures of the newly synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, and LCMS. The newly synthesized compounds were screened for antimicrobial activity. Among all the compounds tested, **11b** (R_4 = 4-MeO–) showed the highest activity against *Staphylococcus aureus* and *Escherichia coli*, and **9a** (R_1 and R_2 = Cl) showed the highest activity against *Pseudomonas aeruginosa*.

1. Introduction

The antimicrobial agents available now have various drawbacks such as toxicity, drug resistance to microbes, and narrow spectrum of activity. Hence the design of new compounds to deal with the above problems has become one of the most challenging targets in antibacterial and antifungal research today.

1,2,4-Triazole derivatives are known to exhibit a wide range of biological activities, such as antibacterial [1–4] and antifungal [1–5], analgesic [6], anticancer [7, 8], antiviral [9], antitubercular [10, 11], anti-inflammatory [12], anticonvulsant [13, 14], antidepressant [15], and central nervous system (CNS) [16]. A number of 1,2,4-triazole-3-one and its heterocyclic derivatives represent an interesting class of compounds possessing a broad spectrum of biological activities like antimicrobial [17, 18], anticonvulsant [19, 20], and 5-HT2 antagonists [21]. Furthermore, 1,2,4-triazoles bearing piperazine substituents show biological activities, such as antibacterial [22, 23], antifungal [5, 22, 23], and anticancer [7]. Also, some halo phenylethers incorporating the 1,2,4-triazole nucleus possess good anticancer [24] and analgesic [25] activities.

1,2,4-Triazole and 1,2,4-triazol-3-one bearing piperazine substituent find application in the treatment of several diseases (Figure 1). Itraconazole and posaconazole are important antifungal drugs [26]. Also several compounds containing the piperazine nucleus are well known as drugs. For example Prazosin, Lidoflazine, and Urapidil are used as cardiovascular agents [27–29], in addition to Norfloxacin, Ciprofloxacin, and Ofloxacin, which are effective drugs against respiratory, urinary, gastrointestinal tracts, skin, and soft tissue infections caused by either Gram-positive or Gram-negative bacteria [30, 31].

In view of all these facts, the aim of the present study is to synthesize 1,2,4-triazole-3-one containing piperidine, piperazine, phenylether, and aniline with the hope that new antimicrobial agents will be developed.

1.1. Chemistry. The synthetic route for key intermediate methanesulfonic acid 2-[1-(4-methoxy-phenyl])-3-methyl-5-oxo-1,5-dihydro-[1, 2, 4]-triazol-4-yl]-ethylester (**7**) was depicted in Scheme 1. This intermediate was prepared from 4-methoxy aniline through the sydnone by two ring transformation reaction following the literature method [32].

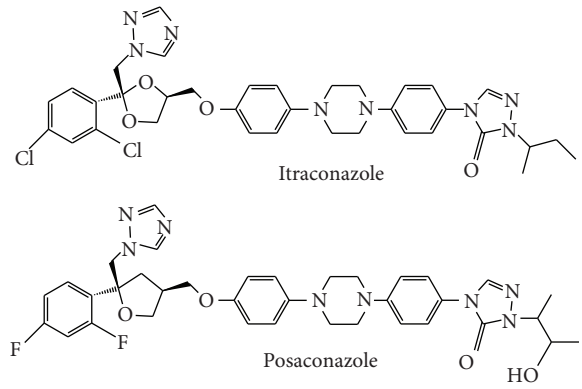
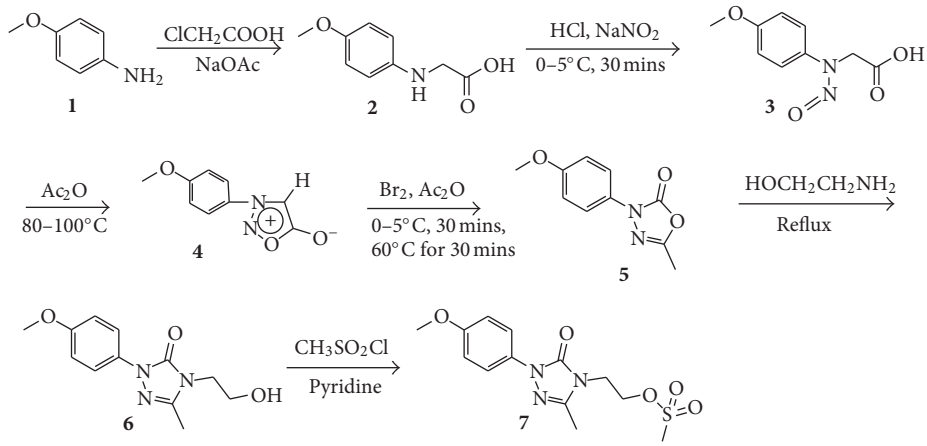


Figure 1: Drugs containing 1,2,4-triazole, 1,2,4-triazole-3-one, piperazine, and 2,4-dihalo phenyl moieties.



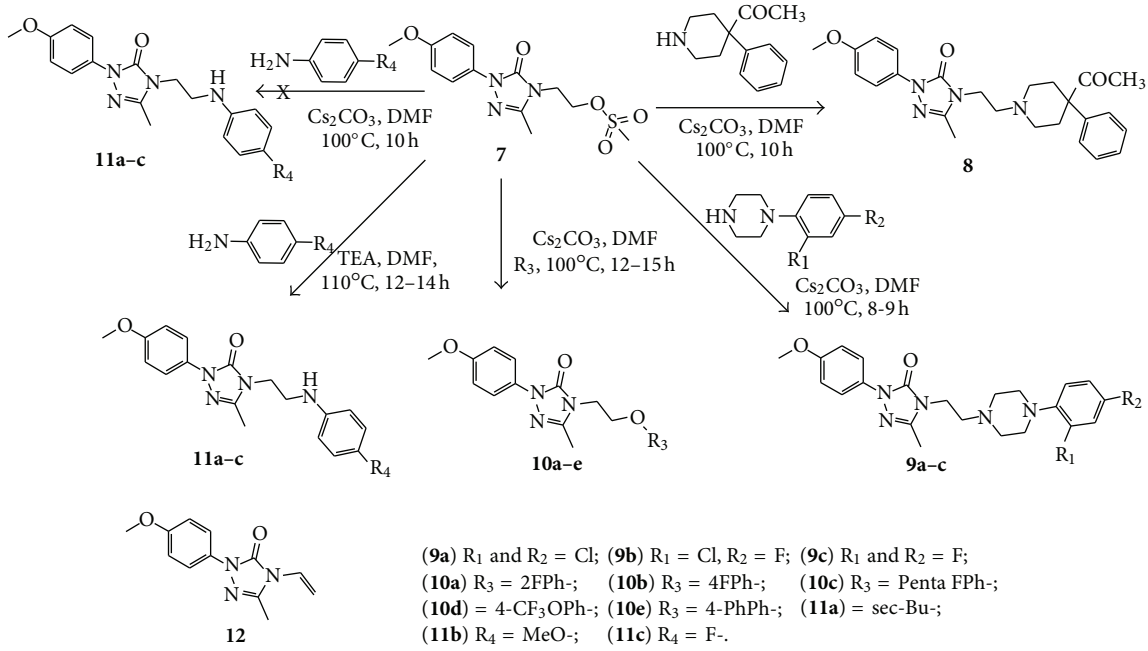
Scheme 1: Synthetic route for the key intermediate.

The synthetic route followed for the preparation of the new compounds is outlined in Scheme 2. The reaction of methanesulfonic acid 2-[1-(4-methoxy-phenyl])-3-methyl-5-oxo-1,5-dihydro-[1, 2, 4]-triazol-4-yl]-ethylester (**7**) with substituted piperidine, piperazines, and phenols in the presence of Cs_2CO_3 gave [1, 2, 4]-triazolo piperidine (**8**), [1, 2, 4]-triazolo piperazines (**9a-c**) and [1, 2, 4]-triazolo phenylethers (**10a-e**) in moderate to good yields. We have tried similar conditions for anilines and instead of the desired products **11a-c**, we obtained alkene (**12**), the structure of which was confirmed by ^{1}H NMR and LCMS. This is possible due to the fact that anilines are less reactive towards nucleophilic substitution. Hence in the presence of Cs_2CO_3 dehydromethylsulphonation is prefered over substitution reaction. Triethylamine first reacts with **7** and forms more reactive quaternary salt, which intern reacts with less nucleophilic anilines. We have obtained the desired products **11a-c** after changing the base from Cs_2CO_3 to triethyl amine.

2. Experimental Section

Melting points were determined on a Buchi B-545 melting point apparatus and are uncorrected. ^{1}H NMR spectra of the samples in $CDCl_3$ and DMSO-d_6 were recorded on a Bruker Avance (300 or 400 MHz) spectrometer using tetramethylsilane (TMS) as an internal standard. ^{13}C NMR spectra were recorded in DMSO-d_6 at 100 MHz with TMS as an internal reference. IR spectra were recorded on a Nicolet 6700 FT-IR spectrometry using ATR-technique (ATR = Attenuated Total Reflectance). C, H, N, and S analyses were carried out on an Elementor (Vario microcube). Mass spectra were recorded on a LC-MS-Agilent 1200 spectrometer.

2.1. General Procedure for the Synthesis of Piperidine (8) and Piperazine (9a-c) Derivatives. To a solution of compound (**7**) (4 mmol) in DMF (8 mL) was added substituted piperidine/piperazines (4.4 mmol), Cs_2CO_3 (6 mmol), and



Scheme 2: Synthesis of [1, 2, 4]-triazolo piperidine, piperazine, phenylether, and aniline derivatives.

the mixture was stirred at 100°C for 14–15 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was poured on to ice water. The product was extracted with ethyl acetate (2 × 50 mL). The combined organic phases were washed with water (2 × 40 mL) and brine (40 mL). The organic phase was dried over sodium sulfate and the solvent was evaporated under reduced pressure to afford [1, 2, 4]-triazolo piperidine (**8**) and [1, 2, 4]-triazolo piperazine derivatives (**9a-c**) in moderate to good yields.

4-[2-(4-Acetyl-4-phenylpiperidin-1-yl)-ethyl]-2-(4-methoxyphenyl)-5-methyl-2,4-dihydro[1, 2, 4]triazol-3-one (**8**).

Purified by column chromatography (Petroleum ether/Ethyl acetate, 50/50); white solid, yield: 70%, m.p. 102–106°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.74 (d, 2H, J = 9.14 Hz), 7.38–7.31 (m, 4H, Ar–H), 7.28–7.24 (m, 1H, Ar–H), 6.99 (d, 2H, J = 9.14 Hz, Ar–H), 3.75 (s, 3H, O–CH_3), 3.72–3.69 (m, 2H, triazole-N–CH_2–), 2.68–2.62 (m, 2H, N–CH_2–), 2.50–2.49 (m, 2H, N–CH_2–), 2.38–2.34 (m, 2H, N–CH_2–), 2.28 (s, 3H, Ar–CH_3), 2.26–2.21 (m, 2H, C–CH_2–), 1.93–1.91 (m, 2H, C–CH_2–), 1.86 (s, 3H, CO–CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 210.0, 156.8, 151.9, 145.5, 141.8, 131.7, 129.3, 127.5, 126.9, 120, 114.7, 56.5, 55.7, 54.4, 51.0, 39.1, 32.7, 25.8, 11.9. MS: m/z 435 [m+1]; Anal. Calculated for $C_{25}H_{30}N_4O_3$: C. 69.10, H. 6.96, N. 12.89. Found C. 68.93, H. 6.97, N. 12.92%.

4-{2-[4-(2,4-Dichlorophenyl)piperazin-1-yl]ethyl}-2-(4-methoxyphenyl)-5-methyl-2,4-dihydro[1, 2, 4]triazol-3-one (**9a**).

Purified by column chromatography (Petroleum ether/Ethyl acetate, 50/50); off-white solid, yield: 60%, m.p. 108–109°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.75 (d, 2H, J = 9.13 Hz, Ar–H), 7.53 (s, 1H, Ar–H), 7.35 (d, 1H, J = 8.6 Hz, Ar–H), 6.99 (d, 2H, J = 9.13 Hz, Ar–H), 3.79–3.75 (m, 2H, triazole-N–CH_2–), 3.75 (s, 3H, O–CH_3), 2.98–2.94 (m, 4H, N–CH_2–), 2.63–2.60 (m, 6H, N–CH_2–), 2.33 (s, 3H, triazole-CH_3). MS: m/z 462 [m+1]; Anal. Calculated for $C_{22}H_{25}Cl_2N_5O_2$: C. 57.15, H. 5.45, N. 15.15. Found C. 57.01, H. 5.47, N. 15.13.

4-{2-[4-(2-Chloro-4-fluorophenyl)piperazin-1-yl]ethyl}-2-(4-methoxyphenyl)-5-methyl-2,4-dihydro[1, 2, 4]triazol-3-one (**9b**).

Purified by column chromatography (Petroleum ether/Ethyl acetate, 50/50); off-white solid, yield: 59%, m.p. 115.8–117°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.75 (d, 2H, J = 8.80 Hz, Ar–H), 7.20 (t, 1H, J = 9.08 Hz, Ar–H), 7.08–7.06 (m, 1H, A–H), 6.99 (d, 2H, J = 8.80 Hz, Ar–H), 6.95–6.91 (m, 1H, Ar–H), 3.80–3.74 (m, 2H, triazole-N–CH_2–), 3.76 (s, 3H, O–CH_3), 3.12–3.00 (m, 4H, N–CH_2–), 2.57–2.59 (m, 6H, N–CH_2–), 2.33 (s, 3H, triazole-CH_3). MS: m/z 446.2 [m+1]; Anal. Calculated for $C_{22}H_{25}ClFN_5O_2$: C. 59.26, H. 5.65, N. 15.71. Found C. 59.40, H. 5.62, N. 15.70%.

4-{2-[4-(2,4-Difluorophenyl)piperazin-1-yl]ethyl}-2-(4-methoxyphenyl)-5-methyl-2,4-dihydro[1, 2, 4]triazol-3-one (**9c**).

Purified by column chromatography (Petroleum ether/Ethyl acetate, 50/50); off-white solid; yield: 62%, m.p. 101–102.4°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.75 (d, 2H, J = 9.13 Hz), 7.20–7.14 (m, 1H, Ar–H), 7.07–7.03 (m, 1H, Ar–H), 6.99 (d, 2H, J = 9.14 Hz, Ar–H), 6.98–6.95 (m, 1H, Ar–H), 3.77–3.74 (m, 2H, triazole-N–CH_2–), 3.75 (s, 3H, O–CH_2–), 2.93 (m, 4H, N–CH_2–), 2.61–2.57 (m, 6H, N–CH_2–), 2.32 (s, 3H, N–CH_2–); ^{13}C NMR (100 MHz,

DMSO-d_6): δ 157.3, 156.8, 155.1, 151.9, 137.1, 131.6, 120.5, 119.9, 114.6, 111.4, 115.1, 56.3, 55.7, 53.3, 50.7, 38.6, 12.0. MS: m/z 430.2 [m+1]; Anal. Calculated for $C_{22}H_{25}F_2N_5O_2$: C. 61.53, H. 5.87, N. 16.31. Found C. 61.48, H. 5.89, N. 16.29%.

2.2. General Procedure for the Synthesis of Phenol Derivatives (10a-e). A mixture of compound (**7**) (4 mmol), the appropriate substituted phenols (4.4 mmol), Cs_2CO_3 (6 mmol), and CuI (0.4 mmol) in DMF (8 mL) was stirred at 100°C for 13–15 h. After completion of the reaction, the mixture was poured on to ice water. The product was extracted with ethyl acetate (2 × 50 mL). The combined organic phases were washed with water (2 × 50 mL) and brine (50 mL). The organic phase was dried over sodium sulfate and the solvent was evaporated under reduced pressure to give phenylethers (**10a-e**) in good yields.

4-[2-(2-Fluorophenoxy)ethyl]-2-(4-methoxyphenyl)-5-methyl-2,4-dihydro[1, 2, 4]triazol-3-one (**10a**).

Purified by column chromatography (Petroleum ether/Ethyl acetate, 70/30); white solid; yield: 95%, m.p. 135–136°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.74 (d, 2H, J = 9.08 Hz, Ar–H), 7.12–7.08 (m, 2H, Ar–H), 7.00–6.94 (m, 4H, Ar–H), 4.17 (t, 2H, J = 5.14 Hz, O–CH_2–), 4.02 (t, 2H, J = 5.14 Hz, triazole-N–CH_2), 3.75 (s, 3H, O–CH_3), 2.35 (s, 3H, Ar–CH_3). MS: m/z 344 [m+1]; Anal. Calculated for $C_{18}H_{18}FN_3O_3$: C. 62.97, H. 5.28, N. 12.24. Found C. 62.94, H. 5.29, N. 12.26%.

4-[2-(4-Fluorophenoxy)ethyl]-2-(4-methoxyphenyl)-5-methyl-2,4-dihydro[1, 2, 4]triazol-3-one (**10b**).

Purified by column chromatography (Petroleum ether/Ethyl acetate, 70/30); white solid; yield: 94%, mp 160–161°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.75 (d, 2H, J = 9.10 Hz, Ar–H), 7.23–7.16 (m, 2H, Ar–H), 7.13–7.08 (m, 1H, Ar–H), 6.98 (d, 2H, J = 9.10 Hz, Ar–H), 6.96–6.93 (m, 1H, Ar–H), 4.25 (t, 2H, J = 5.12 Hz, O–CH_2–), 4.07 (t, 2H, J = 5.12 Hz, N–CH_2–), 3.76 (s, 3H, O–CH_3), 2.37 (s, 3H, triazole-CH_3). MS: m/z 343 [m+1]; Anal. Calculated for: $C_{18}H_{18}FN_3O_3$: C, 62.97, H, 5.28, N, 12.24, Found: C, 62.98, H, 5.28, N, 12.27.

4-[2-(Pentafluorophenoxy)ethyl]-2-(4-methoxyphenyl)-5-methyl-2,4-dihydro[1, 2, 4]triazol-3-one (**10c**).

Purified by column chromatography (Petroleum ether/Ethyl acetate, 65/35); off-white solid; yield: 80%, m.p. 94–95°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.73 (d, 2H, J = 9.08 Hz, Ar–H), 7.01 (d, 2H, J = 9.08 Hz, Ar–H), 4.43 (t, 2H, J = 5.04 Hz, O–CH_2–), 4.06 (t, 2H, J = 5.04 Hz, N–CH_2–), 3.75 (s, 3H, O–CH_3), 2.32 (s, 3H, triazole-CH_2–). MS: m/z 416 [m+1]; Anal. Calculated for $C_{18}H_{14}F_5N_3O_3$: C. 52.06, H. 3.40, N. 10.12 Found: C. 52.10, H. 3.42, N. 10.11%.

4-[2-(4-Trifluoromethoxyphenoxy)ethyl]-2-(4-methoxyphenyl)-5-methyl-2,4-dihydro[1, 2, 4]triazol-3-one (**10d**).

Purified by column chromatography (Petroleum ether/Ethyl acetate, 70/30); white solid; yield: 92%, m.p. 152–153°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.73 (d, 2H, J = 9.09 Hz, Ar–H), 7.25–7.04 (m, 4H, Ar–H), 6.98 (d, 2H, J = 9.09 Hz, Ar–H), 4.24 (t, 2H, J = 5.10 Hz, O–CH_2–), 4.06 (t, 2H, J = 5.10 Hz, N–CH_2–), 3.74 (s, 3H, O–CH_3), 2.36 (s, 3H, triazole–CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 157.2, 156.9, 151.7, 145.6, 142.5, 131.5, 123.0, 121.9, 116.2, 114.7, 66.1, 55.7, 41.1, 12.0. MS: m/z 410 [m+1]; Anal. Calculated for $C_{19}H_{18}F_3N_3O_4$: C. 55.75, H. 4.43, N. 10.26, Found C. 55.70, H. 4.42, N. 10.24%.

4-[2-(4-Phenylphenoxy)ethyl]-2-(4-methoxyphenyl)-5-methyl-2,4-dihydro[1, 2, 4]triazol-3-one (**10e**).

Purified by column chromatography (Petroleum ether/Ethyl acetate, 70/30); white solid; yield: 88%, m.p. 133–134°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.75 (d, 2H, J = 9.00 Hz, Ar–H), 7.60–7.58 (m, 4H, Ar–H), 7.44–7.40 (m, 2H, Ar–H), 7.32–7.30 (m, 1H, Ar–H), 7.05–6.99 (m, 4H, Ar–H), 4.26 (t, 2H, J = 5.12 Hz, O–CH_2–), 4.07 (t, 2H, J = 5.12 Hz, N–CH_2–), 3.76 (s, 3H, O–CH_3), 2.38 (s, 3H, triazole-CH_3). MS: m/z 402 [m+1]; Anal. Calculated for $C_{24}H_{23}N_3O_3$: C. 71.80, H. 5.77, N. 10.47 Found C. 71.91, H. 10.50, N. 10.45%.

2.3. General Procedure for the Synthesis of Aniline Derivatives (11a-c). A mixture of compound (**7**) (2 mmol), the appropriate substituted anilines (2.5 mmol), and triethylamine (3 mmol) in DMF (4 mL) was stirred at 115°C for 15–16 h. The completion of the reaction was monitored by TLC. The reaction mixture was diluted with water and the product was extracted with ethyl acetate (2 × 25 mL). The combined organic phases were washed with water (2 × 20 mL) and brine (20 mL). The organic phase was dried over sodium sulfate and solvent evaporated under reduced pressure to yield the [1, 2, 4]-triazolo aniline derivatives (**11a-c**) in moderate yields.

4-[2-(4-sec-Butylphenylamino)ethyl]-2-(4-methoxyphenyl)-5-methyl-2,4-dihydro[1, 2, 4]triazol-3-one (**11a**).

Purified by column chromatography (Petroleum ether/Ethyl acetate, 70/30); off-white solid; yield: 52%, m.p. 85–86°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.74 (d, 2H, J = 9.14 Hz, Ar–H), 6.98 (d, 2H, J = 9.14 Hz, Ar–H), 6.89 (d, 2H, J = 8.48 Hz, Ar–H), 6.53 (d, 2H, J = 8.48 Hz, Ar–H), 5.65 (t, 1H, J = 6.28 Hz, Ar–NH–), 3.75 (s, 3H, –O–CH_3), 3.75–3.72 (m, 2H, triazole-NCH_2–) 3.33 (m, 2H, –NHCH_2–), 2.40 (m, 1H, Ar–CH–), 2.15 (s, 3H, triazole-CH_3), 1.46–1.42 (m, 2H, CH_3–CH_2–), 1.22 (d, 3H, J = 6.96 Hz, CH_3CH–), 0.72 (t, 2H, CH_3–CH_2–). MS: m/z 381 [m+1]; Anal. Calculated for $C_{22}H_{28}N_4O_2$: C. 69.45, H. 7.42, N. 14.72. Found C. 69.40, H. 7.41, N. 14.75%.

4-(4-Methoxyphenyl)-4-[2-(4-methoxyphenylamino)ethyl]-5-methyl-2,4-dihydro[1, 2, 4]triazol-3-one (**11b**).

Purified by column chromatography (Petroleum ether/Ethyl acetate, 70/30); brownish liquid; yield: 45%, ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.75 (d, 2H, J = 9.14 Hz, Ar–H), 6.99 (d, 2H, J = 9.14 Hz, Ar–H), 6.70 (m, 2H, J = 8.90 Hz, Ar–H), 6.55 (d, 2H, J = 8.90 Hz, Ar–H), 5.46 (t, 1H, Ar–NH–), 3.75 (s, 3H, O–CH_3), 3.74–3.72 (m, 2H, triazole-N–CH_2–), 3.61 (s, 3H, O–CH_3), 3.32–3.29 (m, 2H, NH–CH_2–), 2.14 (s, 3H, triazole-CH_3). MS: m/z 355.2 [m+1]; Anal. Calculated for $C_{19}H_{22}N_4O_3$: C. 64.39, H. 6.26, N. 15.81. Found C. 64.19, H. 6.24, N. 15.80%.

4-[2-(4-Fluorophenylamino)ethyl]-2-(4-methoxyphenyl)-5-methyl-2,4-dihydro[1, 2, 4]triazolo-3-one (**11c**).

Table 1: Antibacterial activity of the compounds (**8**), (**9a–c**), (**10a–e**), and (**11a–c**).

Compounds	MIC of the compounds in μg/mL				
	Gram-positive organisms			Gram-negative organisms	
	B. subtilis	*S. aureus*	*S. epidermidis*	*E. coli*	*P. aeruginosa*
8	18.75	18.75	18.75	18.75	18.75
9a	18.75	18.75	18.75	18.75	**9.75**
9b	37.5	37.5	37.5	37.5	18.75
9c	37.5	37.5	37.5	37.5	18.75
10a	150	150	75	150	75
10b	150	75	75	150	75
10c	150	75	75	150	75
10d	150	75	150	150	37.5
10e	37.5	37.5	37.5	37.5	18.75
11a	37.5	18.75	18.75	18.75	18.75
11b	18.75	**9.75**	18.75	**9.75**	37.5
11c	150	37.5	75	150	37.5
Streptomycin	**6.25**	**1.56**	**1.562**	**3.125**	**3.125**
Pencillin	**1.526**	**6.25**	**3.125**	**6.25**	**12.5**

Table 2: Antifungal activity of the compounds (**8**), (**9a–c**), (**10a–e**), and (**11a–c**).

Compound	MIC of the compounds in μg/mL				
	R. oryzae	*A. niger*	*A. flavus*	*C. albicans*	*S. cerevisiae*
8	18.75	18.75	150	150	150
9a	37.5	150	75	150	150
9b	150	150	150	37.5	150
9c	18.75	150	150	75	150
10a	150	150	150	150	150
10b	75	75	75	150	150
10c	75	37.5	75	150	150
10d	37.5	37.5	150	150	150
10e	18.75	150	37.5	150	150
11a	37.5	75	75	150	150
11b	75	37.5	75	75	150
11c	37.5	150	150	150	150
Amphotericin-B	**1.562**	**1.56**	**6.25**	**6.25**	**6.25**

MIC, μg/mL: minimum inhibitory concentration.

Purified by column chromatography (Petroleum ether/Ethyl acetate, 70/30); off-white solid; yield: 50%, m.p. 88–89°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.75 (d, 2H, J = 9.14 Hz, Ar–H), 6.99 (d, 2H, J = 9.14 Hz, Ar–H), 6.91 (m, 2H, Ar–H), 6.60-6.57 (m, 2H, Ar–H), 5.80 (t, 1H, J = 6.28 Hz, Ar–NH–), 3.75 (s, 3H, O–CH_3), 3.74–3.72 (m, 2H, triazole-N–CH_2–), 3.32–3.30 (m, 2H, NH–CH_2–), 2.14 (s, 3H, triazole-CH_3). MS: m/z 342.8 [m+1]; Anal. Calculated for $C_{18}H_{19}FN_4O_2$: C. 63.15, H. 5.59, N. 16.36. Found C. 63.01, H. 5.60, N. 16.35%.

3. Biology

3.1. Antimicrobial Activity. The *in vitro* antimicrobial activity was carried by using the disc diffusion method [33, 34]. All the newly synthesized compounds were evaluated for their antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli,* and *Pseudomonas aeruginosa*. The antifungal activity was evaluated against *R. oryzae*, *A. niger*, *A. flavus*, *C. albicans,* and *S. cerevisiae*. The minimum inhibition concentration (MIC) was determined by using the twofold serial dilution method with 64-well microtest plates. The test compounds were dissolved in dimethylformamide (DMF). Further dilutions were made at the required concentrations of 300, 150, 75, 37.5, 18.75, 9.75, 6.25, 3.125, and 1.56 μg/mL, respectively. Streptomycin and Penicillin were used as reference standards for antibacterial activity and Amphotericin-B as reference standard for antifungal activity. The standard drugs were selected based on our previous work [1] in order to compare the activity. The results of antibacterial and antifungal activity are given in Tables 1 and 2, respectively.

4. Results and Discussion

From the experimental results, we observed that the Cs_2CO_3works better for compounds (**8**), (**9a-c**), and (**10a-c**) while, for less reactive anilines organic base, triethyl amine works better compared to Cs_2CO_3. Hence, triethylamine is used as excellent base for these reactions which in turn increases safety and cost effectiveness.

All the new compounds were characterized by their spectral data. The IR spectra of 1,2,4-triazolo piperidine (**8**), 1,2,4-triazolo piperazine (**9a-c**), 1,2,4-triazolo phenylether (**10a-e**), and 1,2,4-triazolo aniline (**11a-c**) derivatives show bands in the range 1680–1720 cm^{-1} corresponding to C=O of triazolinone and 1520–1560 cm^{-1} for –C=N–. The ^1H and ^{13}C NMR spectrum of compound **8** confirmed the conversion from **7.** The -$COCH_3$ proton signals of compound **8** were observed as a singlet at 1.86 ppm and in ^{13}C NMR the acetyl C=O signals were observed at 210 ppm. The piperazine proton signals of compounds (**9a-c**) appeared in the ranges of 3.10-2.90 and 2.60–2.50 ppm. The signals at 6.93 and 7.79 ppm are assigned to the aromatic protons. The compounds **8** and **9a-c** gave the stable M+1 ion peaks in mass spectra.

The formation of compounds **10a-e** and **11a-c** from **7** was confirmed by ^1H NMR spectrum. The methylene protons of **10a-e** Ar-OCH_2- and triazole-N-CH_2- were observed in the ranges of 4.45–4.16 and 4.16–4.00 ppm, respectively. The signals in the range 7.84 to 6.74 ppm are due to the aromatic protons. In addition, the two distorted triplets appeared in the ranges 3.80–3.73 and 3.35–3.25 ppm are assigned to the methylene protons. The triplet for single proton of Ar–NH– was observed in the range 5.85–5.4 ppm. The peaks in the range 6.78 to 6.50 ppm are assigned to the aromatic protons. The compounds **10a-e** and **11a-c** were further confirmed by their stable M+1 ion peaks in mass spectra.

From the antibacterial activity results it was observed that compounds **11b** (R_4 = 4-MeO–) showed the highest activity against *Staphylococcus aureus* and *Escherichia coli.* The compound (**9a**) (R_1 and R_2 = Cl) showed the highest activity against *Pseudomonas aeruginosa.* However, relatively lesser activity was noticed in compounds **9b** and **9c** due to the substitution of one or both chlorine atoms in **9a** by fluorine. This difference in activity may be attributed to the presence of bulkier as well as more lipophilic chlorine atoms in **9a**. Almost all the newly synthesized compounds have poor activity against all types of fungal strains tested.

5. Conclusion

A series of novel [1, 2, 4]-triazolo piperidine (**8**),[1, 2, 4]-triazolo piperazine **(9a-c**), [1, 2, 4]-triazolo phenylether (**10a-e**), and [1, 2, 4]-triazolo aniline (**11a-c**) derivatives were synthesized in appreciable yields and characterized by NMR, mass spectrometry and IR studies. The compounds **11a-c** were synthesized using simple and nonhazardous triethylamine base in moderate yields. The newly synthesized compounds were screened for antibacterial and antifungal activity. It was concluded that among all the [1, 2, 4]-triazole derivatives, **11b** (R = 4-MeO–) showed highest activity against *Staphylococcus aureus* and *Escherichia coli.* Also, **9a** (R_1 and R_2 = Cl) showed the highest activity against *Pseudomonas aeruginosa.*

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