Reduction, Mannich reaction, and antimicrobial activity evaluation of some new 1,2,4-triazol-3-one derivatives

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Ethyl[4-arylmethyleneamino-3-(4-metylphenyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazole-1-yl]acetates (3a-e and 10a-d) were obtained starting from 4-amino-2,4-dihydro-3H-1,2,4-triazol-3-ones (1 and 9) in 2 steps. The treatment of 3a-e with NaBH₄ resulted in the formation of 3 kinds of product incorporating carboxilic acid (4a-c) or alcohol (5a-e) functionality. [4-[(4-Methoxyphenyl)methylene]amino]-and [4-[(pyridin-4-yl)methylene]amino]-3-(4-methylphenyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazole-1-yl] acetic acids (6a, 6e) were obtained by the hydrolysis of the corresponding esters (5a-5e). The treatment of 6a with NaBH₄ caused the reduction of only the imine bond; the carboxyl group remained unchanged. Then this carboxylic acid was converted to the corresponding hydrazide (8) in 2 steps by reaction with ethanol and hydrazine hydrate, respectively. The synthesis of Mannich bases was performed by the reaction of the corresponding 1,2,4-triazoles containing an imine group (10a-d) with several primary or secondary amines including morpholine or piperazine nucleus.

All newly synthesized compounds were screened for their antimicrobial activity. The antimicrobial activity study revealed that the Mannich bases (11-16) showed good activity against the test microorganisms as compared with ampicillin.

Key Words: 1,2,4-Triazole-3-one, reduction, hydrolysis, Mannich base, antimicrobial activity

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Introduction

The synthesis of high nitrogen-containing heterocyclic systems has been attracting increasing interest over the past decade due to their utility in various applications, such as propellants, explosives, pyrotechnics, and especially chemotherapy. In medicinal chemistry, azoles are a widely used and studied class of antimicrobial agent due to their safety profile and high therapeutic index.\(^1-^7\)

The 1,2,4-triazole nucleus has been incorporated into a wide variety of therapeutically important agents. Ribavirin (antiviral), rizatriptan (antimigraine), alprazolam (anxiolytic), vorozole, letrozole, and anastrozole (antitumoral) are some examples of drugs containing 1,2,4-triazole moiety.\(^8-^12\) Conazoles constitute another class ofazole-based drugs such as itraconazole, fluconazole, voriconazole, and ravuconazole.\(^2-^5\) Some of the other major applications of conazoles are for crop protection. As pharmaceuticals, they have been used for the treatment of local and systemic fungal infections, which are important problems in phytopathology and especially in medicine.

Substituted piperazines are important pharmacophores that constitutes a part of some important drugs such as crixivan (an HIV protease inhibitor) and siprofloxacin (a potent antibacterial agent).\(^13,^14\) The drugs prazosin, lidoflazine, and urapidil, which contain a piperazine nucleus in their structures, have been used as cardiovascular agents.\(^15,^16\)

Several compounds that contain a piperazine or morpholine nucleus possessing antimicrobial activity have been synthesized, some of which contains anazole ring as well.\(^17-^22\) For example, while eperezolid, which is an oxazolidinone class antibiotic, consists of morpholine and oxazolidinone rings linked to each other via a fluorophenylene linkage; another antibiotic, linezolid, contains a piperazine ring instead of morpholine.\(^23,^24\) On the other hand, itraconazole, posaconazole, and ketoconazole contain a piperazine and one or more azole rings in their structures.\(^25\)

Multi-component reactions (MCRs) have been considered an important method in organic synthesis with the advantages ranging from lower reaction times, increased reaction rates to higher yields, and reproducibility.\(^26\) The Mannich reaction is a 3-component condensation reaction involving an active hydrogen containing compound, formaldehyde, and a primary or secondary amine.\(^26\) The aminoalkylation of aromatic substrates by Mannich reaction constitutes a major strategy for the preparation of several modifications of biologically active compounds.\(^27\) Mannich bases are physiologically reactive due to the basic function making the molecule soluble in aqueous solvents when it is transformed into aminium salt.\(^15\) Mannich bases have been reported as potential biological agents. They find application as antitubercular,\(^28\) antimalarial,\(^29\) vasorelaxing,\(^30\) anticancer,\(^31\) and analgesic drugs.\(^32\) They have also found several applications in the polymer industry as paints and surface active reagents.\(^33\)

Mannich bases derived from 1,2,4-triazole compounds carrying N-methyl piperazine moiety have been reported to possess protozocidal and antibacterial activity. The drugs prazosin, lidoflazine, and urapidil, which contain a piperazine nucleus in their structures, have been used as cardiovascular agents.\(^15,^16,^34\)

Imine compounds constitute another intensively synthesized and studied class of organic molecules due to their different applications, such as biological activities,\(^35-^39\) investigation of their ability to make a coordination complex with transition metal cations, and improvement of their properties for analytical applications.\(^40-^42\) Some Schiff base derivatives of 1,2,4-triazoles have been synthesized as antimicrobial and/or antitumoral agents in our laboratories.\(^36,^43-^50\)
Over 10 years our interest has focused on the synthesis of novel heterocyclic systems that have antimicrobial activity. In the present study, as a part of our ongoing study, we synthesized new 1,2,4-triazole derivatives as possible antimicrobial agents, some of which contain different heterocyclic rings such as piperazine and morpholine moieties.

**Experimental**

All the chemicals were purchased from Fluka Chemie AG Buchs (Switzerland) and were used without further purification. Melting points of the synthesized compounds were determined in open capillaries on a Büchi B-540 melting point apparatus and are uncorrected. The reactions were monitored by thin-layer chromatography (TLC) on silica gel 60 F254 aluminum sheets. The mobile phase was ethanol and ethyl acetate (1:1) and detection was performed using UV light. IR spectra were recorded as potassium bromide pellets using a PerkinElmer 1600 series FTIR spectrometer. $^1$H-NMR and $^{13}$C-NMR spectra were recorded on a BRUKER AVANCE II 400 MHz NMR Spectrometer (chemical shift in ppm downfield from TMS as an internal reference). The elemental analysis was performed on a Costech Elemental Combustion System CHNS-O elemental analyzer. All the compounds gave C, H and N analysis within ±0.4% of the theoretical values. The mass spectra were obtained on a Quattro LC-MS (70 eV) instrument.

**General method for the preparation of compounds 2a-e and 10a-d**

The corresponding compound 1 or 9 (10 mmol) was heated in an oil bath with a suitable aromatic aldehyde (10 mmol) at 130-140 °C for 2 h. On cooling the mixture to room temperature, a solid was obtained. This crude product was recrystallized from ethanol solvent to obtain the desired compound.

4-{{[(4-Methoxyphenyl)methylene]amino}-5-(4-methylphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-one (2a).

Yield: 2.52 g, 81%; mp 200-202 °C; IR (KBr, cm$^{-1}$): 3214 (NH), 1697 (C=O), 1605 (C=N); $^1$H-NMR (DMSO-d$_6$ δ ppm): 2.32 (s, 3H, CH$_3$), 3.38 (s, 3H, OCH$_3$), [ar-H: 7.02 (dd, 2H, $J = 8.8$ Hz), 7.28 (d, 2H, $J = 8.2$ Hz), 7.69-7.76 (m, 6H), 9.46 (s, 1H, CH), 12.26 (s, 1H, NH); $^{13}$C-NMR (DMSO-d$_6$ δ ppm): 156.86 (N=CH), 151.74 (triazole C5), 144.84 (triazole C3), ar-C: [114.81 (2CH), 124.25 (C), 126.11 (C), 128.04 (2CH), 129.34 (2CH), 129 (2CH), 140.06 (C), 162.56 (C)], 55.67 (OCH$_3$), 21.24 (CH$_3$); Anal. calcd (%) for C$_{17}$H$_{16}$N$_4$O$_2$: C, 66.22; H, 5.23; N, 18.17. Found: C, 66.28; H, 5.21; N, 18.10.

4-{{[2-Furylmethylene]amino}-5-(4-methylphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-one (2b).

Yield: 1.81 g, 67%; mp 211-213 °C; IR (KBr cm$^{-1}$): 3162 (NH), 1698 (C=O), 1609 (C=N); $^1$H-NMR (DMSO-d$_6$ δ ppm): 2.37 (s, 3H, CH$_3$), [ar-H: 6.74 (dd, 1H, $J_1 = 4.0$ Hz, $J_2 = 1.8$ Hz), 7.20 (d, 1H, $J = 3.4$ Hz), 7.33 (d, 2H, $J = 8.2$ Hz), 7.77 (d, 2H, $J = 8.4$ Hz), 9.54 (s, 1H, N=CH), 12.35 (s, 1H, NH); $^{13}$C-NMR (DMSO-d$_6$ δ ppm): 146.77 (N=CH), 151.28 (triazole C5), 144.40 (triazole C3), ar-C: [112.53 (CH), 118.00 (CH), 123.68 (C), 127.69 (2CH), 128.93 (2CH), 139.75 (C), 145.03 (CH), 148.18 (C)], 20.85 (CH$_3$); LC-MS: m/z (% [M]+ (%) 291 (100) [M+Na]$^+$, 269 (46) [M+1]$^+$; 254 (16), 176 (35), 146 (22), 138 (16); Anal. calcd (%) for C$_{14}$H$_{16}$N$_4$O$_2$: C, 62.68; H, 4.51; N, 20.88. Found: C, 62.77; H, 4.63; N, 20.71.

5-(4-Methylphenyl)-4-{{[phenylmethylene]amino}-2,4-dihydro-3H-1,2,4-triazole-3-one (2c).

Yield: 0.82 g, 68%; mp 195-196 °C; IR (KBr cm$^{-1}$): 3176 (NH), 1695 (C=O), 1612 (C=N); $^1$H-NMR (DMSO-d$_6$ δ ppm): 2.37 (s, 3H, CH$_3$), [ar-H: 7.36 (d, 2H, $J = 8.4$ Hz), 7.46 (m, 3H), 7.70-7.85 (m, 4H)], 9.58 (s,
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1H, N=CH), 12.31 (s, 1H, NH); 13C-NMR (DMSO-d$_6$ $\delta$ ppm): 156.23 (N=CH), 151.24 (triazole C5), 144.56 (triazole C3), ar-C: [123.71 (C), 127.70 (4CH), 129.00 (4CH), 131.55 (CH), 133.27 (C), 139.78 (C)], 20.88 (CH$_3$); Anal. calcd (%) for C$_{16}$H$_{14}$N$_4$O$_2$: C, 69.05; H, 5.07; N, 20.13. Found: C, 69.12; H, 5.18; N, 20.07.

5-(4-Methylphenyl)-4-[(pyridin-2-ylmethylene)amino]-2,4-dihydro-3H-1,2,4-triazol-3-one (2d). Yield: 1.81 g, 67%; mp 209-210 °C; IR (KBr, cm$^{-1}$): 3165 (NH), 1719 (C=O), 1584 (C=N); $^1$H-NMR (DMSO-d$_6$ $\delta$ ppm): 2.39 (s, 3H, CH$_3$), [ar-H: 7.36 (d, 2H, $J$ = 8.0 Hz), 7.73-7.80 (m, 4H), 8.73 (d, 2H, $J$ = 5.0 Hz)], 9.79 (s, 1H, N=CH), 12.44 (s, 1H, NH); $^{13}$C-NMR (DMSO-d$_6$ $\delta$ ppm): 152.72 (N=CH), 151.07 (triazole C5), 144.63 (triazole C3), ar-C: [121.33 (2CH), 123.41 (C), 127.94 (2CH), 129.04 (2CH), 139.46 (C), 140.54 (C), 150.47 (2CH)], 20.89 (CH$_3$); LC-MS: $m/z$ (%): [M]+ 302 (66) [M+Na]+, 280 (100) [M+1]+; 299 (12), 291 (9), 281 (17), 105 (17); Anal. calcd (%) for C$_{15}$H$_{13}$N$_5$O: C, 64.51; H, 4.69; N, 25.07. Found: C, 64.73; H, 4.85; N, 24.86.

4-[(2-Furylmethylene)amino]-5-methyl-2,4-dihydro-3H-1,2,4-triazol-3-one (10a). Yield: 1.63 g, 72%; mp 209-210 °C; IR (KBr, cm$^{-1}$): 3182 (NH), 1701 (C=O), 1613 (C=N); $^1$H-NMR (DMSO-d$_6$ $\delta$ ppm): 2.28 (s, 3H, CH$_3$), [ar-H: 6.72 (d, 1H, $J$ = 4.0 Hz), 7.02 (d, 1H, $J$ = 4.0 Hz), 7.90 (d, 1H, $J$ = 1.2 Hz)], 9.40 (s, 1H, N=CH), 12.18 (s, 1H, NH); $^{13}$C-NMR (DMSO-d$_6$ $\delta$ ppm): 148.63 (N=CH), 151.62 (triazole C5), 143.88 (triazole C3), ar-C: [112.23 (CH), 118.68 (CH), 145.90 (CH), 149.42 (C)], 20.12 (CH$_3$); Anal. calcd (%) for C$_8$H$_8$N$_4$O$_2$: C, 50.00; H, 4.20; N, 29.15. Found: C, 50.12; H, 4.58; N, 29.22.

5-Methyl-4-[(phenylmethylene)amino]-2,4-dihydro-3H-1,2,4-triazol-3-one (10b). Yield: 1.12 g, 75%; mp 201-202 °C; IR (KBr, cm$^{-1}$): 3198 (NH), 1710 (C=O), 1616 (C=N); $^1$H-NMR (DMSO-d$_6$ $\delta$ ppm): 2.22 (s, 3H, CH$_3$), [ar-H: 7.12-7.46 (m, 3H), 7.74-7.8846 (m, 2H)], 9.45 (s, 1H, N=CH), 12.28 (s, 1H, NH); $^{13}$C-NMR (DMSO-d$_6$ $\delta$ ppm): 155.56 (N=CH), 150.65 (triazole C5), 144.28 (triazole C3), ar-C: [128.68 (2CH), 129.84 (2CH), 132.68 (C), 135.72 (C)], 21.42 (CH$_3$); Anal. calcd (%) for C$_{10}$H$_{10}$N$_4$O: C, 59.40; H, 4.98; N, 27.71. Found: C, 59.35; H, 4.88; N, 27.65.

4-[(2-Methoxyphenyl)methylene]amino]-5-methyl-2,4-dihydro-3H-1,2,4-triazol-3-one (10c). Yield: 1.96 g, 80%; mp 189-190 °C; IR (KBr, cm$^{-1}$): 3222 (NH), 1615 (C=N); $^1$H-NMR (DMSO-d$_6$ $\delta$ ppm): 2.20 (s, 3H, CH$_3$), 3.52 (s, 3H, OCH$_3$), [ar-H: 7.04 (d, 2H, $J$ = 8.4 Hz), 7.78 (d, 2H, $J$ = 8.4 Hz)], 9.32 (s, 1H, CH), 12.32 (s, 1H, NH); $^{13}$C-NMR (DMSO-d$_6$ $\delta$ ppm): 152.46 (N=CH), 150.74 (triazole C5), 143.18 (triazole C3), ar-C: [114.38 (2CH), 122.46 (C), 127.26 (2CH), 160.34 (C)], 55.88 (OCH$_3$), 22.12 (CH$_3$); Anal. calcd (%) for C$_{11}$H$_{12}$N$_4$O$_2$: C, 56.89; H, 5.21; N, 24.12. Found: C, 56.75; H, 5.32; N, 24.22.

4-[(4-Hydroxyphenyl)methylene]amino]-5-methyl-2,4-dihydro-3H-1,2,4-triazol-3-one (10d). Yield: 2.31 g, 82%; mp 211-212 °C; IR (KBr, cm$^{-1}$): 3232 (NH), 1705 (C=O), 1618 (C=N); $^1$H-NMR (DMSO-
General method for the synthesis of compounds 3a-e

The solution of the corresponding compound (10 mmol) in absolute ethanol was stirred under reflux with an equivalent amount of sodium for 2 h. Then ethyl bromoacetate (10 mmol) was added and the mixture was refluxed for an additional 8 h. After evaporating the solvent under reduced pressure, a solid appeared. This was recrystallized from ethanol/water (1:2) to afford the desired compound.

Ethyl [4-[(4-methoxyphenyl)methylene]amino]-3-(4-methylphenyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-ylacetate (3a). Yield: 2.52 g, 81%; mp 115-116°C; IR (KBr, cm$^{-1}$): 1704, 1751 (C=O), 1606 (C=N); $^1$H-NMR (DMSO-$d_6$): 1.24 (s, 3H, CH$_3$), 3.79 (s, 3H, CH$_3$), 3.85 (s, 3H, OCH$_3$), 4.19 (q, 2H, OCH$_2$, J = 7.0 Hz), 4.76 (s, 2H, N=CH$_2$), 6.94 (d, 2H, 9Hz), 7.27 (d, 2H, 7.8 Hz), 7.72 (d, 2H, 8.6 Hz), 7.85 (d, 2H, 7.8 Hz), 9.68 (s, 1H, N=CH); $^{13}$C-NMR (DMSO-$d_6$): 168.18 (C=O), 156.07 (N=CH), 151.04 (triazole C5), 145.43 (triazole C3), ar-C: [114.81 (2CH), 124.25 (C), 126.11 (C), 128.04 (2CH), 137.02 (CH), 142.60 (CH), 159.48 (C)], 23.56 (CH). Anal. calcd (%) for C$_{19}$H$_{19}$N$_4$O$_2$: C, 55.04; H, 4.62; N, 25.68. Found: C, 55.12; H, 4.58; N, 25.75.

Ethyl (3-(4-methylphenyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methacrylate (3b). Yield: 3.3 g, 96.7%; mp 124-125°C; IR (KBr, cm$^{-1}$): 1705, 1752 (C=O), 1609 (C=N); $^1$H-NMR (DMSO-$d_6$): 1.29 (t, 3H, CH$_3$), 2.38 (s, 3H, CH$_3$), 4.26 (q, 2H, OCH$_2$, J = 6.6 Hz), 4.66 (s, 2H, N=CH$_2$), 6.53 (dd, 1H, J$_1$ = 1.0 Hz, J$_2$ = 1.2 Hz), 6.90 (d, 1H, J = 3.4 Hz), 7.27 (d, 2H, J = 8.4 Hz), 7.59 (s, 1H, J = 8.0 Hz)], 9.69 (s, 1H, N=CH); $^{13}$C-NMR (DMSO-$d_6$): 167.37 (C=O), 145.86 (N=CH), 150.72 (triazole C5), 144.46 (triazole C3), ar-C: [112.22 (CH), 116.82 (CH), 123.22 (C), 128.40 (2CH), 129.15 (2CH), 140.69 (C), 145.01 (CH), 148.92 (C)], 61.92 (OCH$_2$), 46.83 (NCH$_2$), 21.51 (CH$_3$), 14.16 (CH$_3$); LC-MS: m/z (%M+Na$^+$): 377 (100) [M+Na$^+$], 355 (70) [M+1$^+$]; 356 (28), 333 (12), 281 (30), 144 (47), 146 (28); Anal. calcd (%) for C$_{18}$H$_{19}$N$_4$O$_4$: C, 63.95; H, 5.62; N, 14.20. Found: C, 64.18; H, 5.77; N, 14.26.

Ethyl (3-(4-methylphenyl)-5-oxo-4-{[2-furylmethylene]amino}-4,5-dihydro-1H-1,2,4-triazol-1-yl) acetate (3c). Yield: 2.05 g, 81%; mp 110-111°C; IR (KBr, cm$^{-1}$): 1704, 1748 (C=O), 1508 (C=N); $^1$H-NMR (DMSO-$d_6$): 1.24 (t, 3H, CH$_3$), 2.39 (s, 3H, CH$_3$), 4.20 (q, 2H, OCH$_2$, J = 7.2 Hz), 4.77 (s, 2H, N=CH$_2$), 7.37 (d, 1H, J = 8.0 Hz), 7.44 (dd, 1H, J$_1$ = 7.0 Hz, J$_2$ = 4.4 Hz), 7.81 (d, 2H, J = 8.0 Hz), 7.96 (d, 2H, J = 3.6 Hz), 8.72 (d, 1H, J = 4.6 Hz)], 7.90 (s, 1H, NH); $^{13}$C-NMR (DMSO-$d_6$): 167.46 (C=O), 149.88 (N=CH), 151.91 (triazole C5), 143.82 (triazole C3), ar-C: [120.56 (CH), 122.69 (C), 125.73 (CH), 128.0 (2CH), 129.13 (2CH), 137.22 (CH), 140.37 (C), 149.66 (CH), 155.58 (CH), 61.29 (OCH$_2$), 46.58 (NCH$_2$), 20.88 (CH$_3$), 13.89 (CH$_3$)]; LC-MS: m/z (%M+Na$^+$): 388 (100) [M+Na$^+$], 366 (50) [M+1$^+$]; 367 (10), 153 (25), 105 (17); Anal. calcd (%) for C$_{19}$H$_{19}$N$_5$O$_3$: C, 62.46; H, 5.24; N, 19.17. Found: C, 62.63; H, 5.48; N, 19.19.
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**triazol-1-yl) acetate (3e)**. Yield: 2.5 g, 69%; mp 157-160 °C; IR (KBr cm⁻¹): 1709, 1740 (C=O), 1508 (C=N); ¹H-NMR (DMSO-d₆ δ ppm): 1.32 (t, 3H, CH₃, J = 7.2 Hz), 2.44 (q, 4H, OCH₂, J = 7.2 Hz), 4.67 (s, 2H, NCH₂), [ar-H: 7.31 (d, 2H, J = 7.8 Hz), 7.63 (d, 2H, J = 4.6 Hz), 7.82 (d, 2H, J = 8.4 Hz), 8.73 (d, 2H, J = 5.0 Hz)], 9.93 (s, 1H, NH); ¹³C-NMR (DMSO-d₆ δ ppm): 167.20 (C=O), 149.98 (N=CH), 150.50 (triazole C₅), 145.17 (triazole C₃), ar-C: [123.36 (C), 127.43 (2CH), 127.56 (2CH), 140.26 (C), 143.40 (CH), 150.81 (C)], 47.48 (NCH₂), 21.33 (CH₃). LC-MS: m/z (%) [M⁺] 388 (100) [M+Na⁺], 366 (19) [M+1⁺]; 283 (20), 105 (51); Anal. calcd (%) for C₁₉H₁₆N₅O₃: C, 61.95; H, 5.47; N, 15.21. Found: C, 61.95; H, 5.47; N, 15.20.

**General method for the synthesis of compounds 4a-c and 5a-e**

The solution of NaBH₄ (10 mmol) in absolute ethanol was added to the solution of the corresponding compound 3 (10 mmol) in absolute ethanol, and the reaction mixture was allowed to reflux for 3 h. Then 50 mL of water was added and the separated solid was collected by filtration and recrystallized from ethanol/water to afford compound 5a-e. After neutralization of the filtrate by HCl, a solid appeared. This was filtered off, washed with water, and recrystallized from (0.9 g, 24%) ethanol/water (1:2) to give compounds 4a-c.

**[4-[(4-Methoxybenzyl)amino]-3-(4-methylphenyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl] acetic acid (4a)**. Yield: 0.9 g, 24%; mp 197-198 °C; IR (KBr, cm⁻¹): 3285 (NH), 1666, 1763 (C=O), 1613 (C=N); ¹H-NMR (DMSO-d₆ δ ppm): 2.40 (s, 3H, CH₃), 3.75 (s, 3H, OCH₃), 4.11 (d, 2H, NHCH₂, J = 4.8 Hz), 4.56 (s, 2H, NCH₂), 5.57 (t, 1H, NH, J = 4.8 Hz), [ar-H: 6.70 (d, 2H, J = 8.8 Hz), 7.11 (d, 2H, J = 8.8 Hz), 7.16 (d, 2H, J = 8.4 Hz), 7.73 (d, 2H, J = 8.4 Hz)], 11.24 (s, 1H, OH); ¹³C-NMR (DMSO-d₆ δ ppm): 169.10 (C=O), 153.26 (triazole C₅), 149.98 (N=CH), 150.50 (triazole C₃), ar-C: [113.48 (2CH), 122.90 (C), 128.58 (2CH), 130.46 (2CH), 139.86 (C), 158.95 (C)], 55.02 (OCH₂), 46.45 (NHCH₂), 21.33 (CH₃); LC-MS: m/z (%) 391 (100) [M+Na⁺], 369 [M+1⁺]; 377 (25), 369 (30), 229 (98), 191 (13); Anal. calcd (%) for C₁₉H₂₀N₄O₄: C, 61.95; H, 5.47; N, 15.21. Found: C, 62.17; H, 5.53; N, 15.20.

**[4-[(2-Furylmethyl)amino]-3-(4-methylphenyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl] acetic acid (4b)**. Yield: 1.26 g, 37%; mp 138-141 °C; IR (KBr, cm⁻¹): 3285 (NH), 1666, 1737 (C=O), 1613 (C=N); ¹H-NMR (DMSO-d₆ δ ppm): 2.31 (s, 3H, CH₃), 4.53 (s, 2H, NHCH₂), 4.18 (d, 2H, NHCH₂, J = 3.6 Hz), 6.76 (t, 1H, J = 3.8 Hz), [ar-H: 6.13 (d, 1H, J = 3.2 Hz), 6.23 (dd, 1H, J₁ = 4H, J₂ = 2.2 Hz), 7.19 (d, 2H, J = 8.2 Hz), 7.40 (d, 1H, J = 1.8 Hz), 7.65 (d, 2H, J = 8.0 Hz)], 10.95 (s, 1H, OH); ¹³C-NMR (DMSO-d₆ δ ppm): 169.90 (C=O), 153.26 (triazole C₅), 149.98 (N=CH), 150.50 (triazole C₃), ar-C: [113.48 (2CH), 122.90 (C), 128.74 (2CH), 129.39 (2CH), 140.26 (C), 143.40 (CH), 150.81 (C)], 47.48 (NCH₂), 45.38 (NHCH₂), 21.64 (CH₃); LC-MS: m/z (%) 351 (100) [M+Na⁺], 329 (87) [M+1⁺]; M/Z 316 (9), 315 (59), 220 (13), 176 (20), 153 (32), 152 (36), 146 (53), 138 (38), 123 (32); Anal. calcd (%) for C₁₆H₁₆N₄O₄: C, 58.53; H, 4.91; N, 17.06. Found: C, 58.57; H, 5.14; N, 17.09.

**[4-Benzylamino]-3-(4-methylphenyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl] acetic acid (4c)**. Yield: 1.1 g, 31%; mp 185-186 °C; IR (KBr cm⁻¹): 3265 (NH), 1677, 1737 (C=O), 1503 (C=N); ¹H-NMR (DMSO-d₆ δ ppm): 2.40 (s, 3H, CH₃), 4.20 (d, 2H, NHCH₂, J = 4.8 Hz), 4.56 (s, 2H, NCH₂), 5.77 (t, 1H, NH, J = 4.8 Hz), [ar-H: 7.17-7.23 (m, 7H), 7.75 (d, 2H, J = 8.0 Hz)], 10.98 (s, 1H, OH); ¹³C-NMR (DMSO-d₆ δ ppm): 169.18 (C=O), 153.24 (triazole C₅), 144.90 (triazole C₃), ar-C: [123.36 (C), 127.43 (2CH), 127.56
(CH), 128.14 (2CH), 128.66 (2CH), 129.20 (2CH), 135.61 (C), 139.85 (C)], 53.74 (NCH2), 46.82 (NCH2H), 21.30 (CH3); LC-MS: \( m/z \) (\%) 361 (100) [M+Na]+, 339 (90) [M+1]+; 340 (19), 149 (18), 106 (15); Anal. calec (%) for \( C_{18}H_{18}N_4O_3 \); C, 63.90; H, 5.36; N, 16.56. Found: C, 64.16; H, 5.41; N, 16.54.

2-(2-Hydroxyethyl)-4-[[4-methoxybenzyl]amino]-5-(4-methylphenyl)-2,4-dihydro-3H-1,2,4-triazol-3-one (5a). Yield: 2.5 g, 66%; mp 104-105 °C; IR (KBr, cm\(^{-1}\)) : 3263 (NH), 1699 (C=O), 1613 (C=N); \(^1\)H-NMR (DMSO-\( d_6 \) \( \delta \) ppm): 2.40 (s, 3H, CH3), 3.48 (brs, 1H, OH), 3.77 (s, 3H, OCH3), 4.09 (d, 2H, NHCH2, \( J = 5.4 \) Hz), 3.93-4.10 (m, 4H, NCH2OCH2), 4.98 (t, 1H, NH, \( J = 5.6 \) Hz), [ar-H: \( 7.75 (d, 2H, J = 7.6 \) Hz), 7.10-7.25 (m, 4H), 7.57 (d, 2H, \( J = 7.5 \) Hz)]; \(^1^3\)C-NMR (DMSO-\( d_6 \) \( \delta \) ppm): 133.57 (triazole C5), 144.71 (triazole C3), ar-C: [113.77 (2CH), 123.23 (C), 127.29 (C), 127.33 (2CH), 128.87 (2CH), 130.61 (2CH), 140.39 (C), 159.31 (C)], 61.24 (CH2OH), 55.20 (OCH3), 54.00 (NCH2), 48.92 (NHCH2), 21.47 (CH3); LC-MS: \( m/z \) (%) 378 (100) [M+Na]+, 355 (9) [M+1]+; 355 (13), 229 (13); Anal. calec (%) for \( C_{19}H_{22}N_4O_4 \); C, 64.39; H, 6.26; N, 15.81. Found: C, 64.56; H, 6.41; N, 15.77.

4-[[2-Furylmethyl]amino]-2-(2-hydroxyethyl)-5-(4-methylphenyl)-2,4-dihydro-3H-1,2,4-triazol-3-one (5b). Yield: 2.3 g, 71%; mp 70-74 °C; IR (KBr, cm\(^{-1}\)) : 3292 (NH), 1697 (C=O), 1507 (C=N); \(^1\)H-NMR (DMSO-\( d_6 \) \( \delta \) ppm): 2.39 (s, 3H, CH3), 3.76 (brs, 1H, OH), 4.01-4.06 (m, 4H, NCH2OCH2), 4.22 (d, 2H, NHCH2, \( J = 4.8 \) Hz), 5.09 (t, 1H, NH, \( J = 4.8 \) Hz), [ar-H: 6.15-6.22 (m, 2H), 7.19 (s, 1H), 7.24 (d, 2H, \( J = 7.2 \) Hz), 7.79 (d, 2H, \( J = 8.4 \) Hz)]; \(^1^3\)C-NMR (DMSO-\( d_6 \) \( \delta \) ppm): 153.76 (triazole C5), 145.03 (triazole C3), ar-C: [110.08 (CH), 110.53 (CH), 123.23 (C), 127.52 (2CH), 129.23 (2CH), 140.62 (C), 143.00 (CH), 149.50 (C)], 61.10 (CH2OH), 49.13 (NCH2), 46.88 (NHCH2), 21.69 (CH3); LC-MS: \( m/z \) (%) 337 (100) [M+Na]+, 315 (8) [M+1]+; 338 (19), 315 (8); Anal. calec (%) for \( C_{16}H_{18}N_4O_3 \); C, 61.14; H, 5.77; N, 17.82. Found: C, 61.33; H, 5.92; N, 17.67.

2-(2-Hydroxyethyl)-4-benzylamino-5-(4-methylphenyl)-2,4-dihydro-3H-1,2,4-triazol-3-one (5c). Yield: 2.03 g, 62%; mp 128-129 °C; IR (KBr, cm\(^{-1}\)) : 3236, 3285 (NH), 1703 (C=O), 1528 (C=N); \(^1\)H-NMR (DMSO-\( d_6 \) \( \delta \) ppm): 2.40 (s, 3H, CH3), 4.17 (d, 2H, NHCH2, \( J = 5.6 \) Hz), 3.95-4.10 (m, 4H, NCH2OCH2), 4.97 (t, 1H, NH, \( J = 5.8 \) Hz), [ar-H: 7.20-7.30 (m, 7H), 7.75 (d, 2H, \( J = 8.0 \) Hz)], 3.45 (brs., OH); \(^1^3\)C-NMR (DMSO-\( d_6 \) \( \delta \) ppm): 153.24 (triazole C5), 144.90 (triazole C3), ar-C: [123.36 (C), 127.43 (2CH), 127.56 (CH), 128.14 (2CH), 128.66 (2CH), 129.20 (2CH), 135.61 (C), 139.85 (C)], 60.93 (CH2OH), 53.74 (NCH2), 46.82 (NHCH2), 21.30 (CH3); LC-MS: \( m/z \) (%) 347 (100) [M+Na]+, 325 (58) [M+1]+; 149 (9), 119 (7); Anal. calec (%) for \( C_{18}H_{20}N_4O_2 \); C, 66.65; H, 6.21; N, 17.27. Found: C, 66.78; H, 6.27; N, 17.19.

2-(2-Hydroxyethyl)-5-(4-methylphenyl)-4-[[pyridin-2-ylmethy]amino]-2,4-dihydro-3H-1,2,4-triazol-3-one (5d). Yield: 1.93 g, 62%; mp 112-114 °C; IR (KBr, cm\(^{-1}\)) : 3269 (NH), 1699 (C=O), 1596 (C=N); \(^1\)H-NMR (DMSO-\( d_6 \) \( \delta \) ppm): 2.33 (s, 3H, CH3), 4.29 (d, 2H, NHCH2, \( J = 5.5 \) Hz), 3.62-3.83 (m, 4H, NCH2OCH2), 6.83 (t, 1H, \( J = 5.2 \) Hz, NH), [ar-H: 7.17-7.27 (m, 4H), 7.61-7.75 (m, 1H), 7.728 (d, 2H, \( J = 8.4 \) Hz), 8.42 (d, 1H, \( J = 4.6 \) Hz)], 4.86 (brs., OH); \(^1^3\)C-NMR (DMSO-\( d_6 \) \( \delta \) ppm): 152.65 (triazole C5), 143.79 (triazole C3), ar-C: [122.30 (CH), 122.79 (CH), 123.54 (C), 127.05 (2CH), 128.56 (2CH), 136.19 (CH), 139.23 (C), 148.61 (CH), 156.24 (C)], 47.50 (CH2OH), 58.29 (NCH2), 54.12 (NHCH2), 20.74 (CH3); LC-MS: \( m/z \) (%) 327 (9), 326 (38), 229 (13), 345 (6); Anal. calec (%) for \( C_{17}H_{19}N_5O_2 \); C, 62.76; H, 5.89; N, 21.52. Found: C, 62.91; H, 5.95; N, 21.44.

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2-(2-Hydroxymethyl)-5-(4-methylphenyl)-2,4-dihydro-3H-1,2,4-triazol-3-one (5e). Yield: 1.39 g, 63%; mp 243-245 °C; IR (KBr, cm\(^{-1}\)): 3383 (NH), 1694 (C=O), 1513 (C=N); \(^1\)H-NMR (DMSO-\(d_6\) δ ppm): 2.34 (s, 3H, CH\(_3\)), 3.66-3.74 (m, 4H, NCH\(_2\)CH\(_2\)), 12.11 (s, 1H, NH), [ar-H: 7.67 (d, 2H, 8.2 Hz), 7.29 (d, 2H, 7.3 Hz)], 3.42 (brs., OH); \(^13\)C-NMR (DMSO-\(d_6\) δ ppm): 154.46 (triazole C5), 143.31 (triazole C3), ar-C: [123.89 (C), 124.56 (2CH), 129.40 (2CH), 139.56 (C)], 46.68 (CH\(_2\)OH), 58.50 (NCH\(_2\)), 20.84 (CH\(_3\)); LC-MS: \(m/z\) (%) 342 (41), 328 (38), 230 (24); Anal. calcld (%) for C\(_{11}\)H\(_{13}\)N\(_3\)O\(_2\): C, 60.26; H, 5.98; N, 19.17. Found: C, 60.32; H, 5.86; N, 19.22.

General method for the synthesis of compounds 6a, 6e

The suspension of compound 2a (10 mmol, 3.94 g) in 3 M HCl (50 mL) was refluxed for 3 h. On cooling the reaction mixture to room temperature, a white solid appeared. This was recrystallized from ethanol to give the pure compound.

[4-[[[(4-Methoxyphenyl)methylene]amino]-3-(4-methylphenyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl] acetic acid (6a). Yield: 2.96 g, 80%; mp 218-222 °C; IR (KBr, cm\(^{-1}\)): 1691, 1758 (C=O), 1573 (C=N); \(^1\)H-NMR (DMSO-\(d_6\) δ ppm): 2.37 (s, 3H, CH\(_3\)), 3.49 (brs, OH), 3.83 (s, 3H, OCH\(_3\)), 4.63 (s, 2H, NCH\(_2\)CH\(_2\)), [ar-H: 7.07 (d, 2H, \(J = 8.6\) Hz), 7.33 (d, 2H, \(J = 8.2\) Hz), 7.76-7.81 (m, 4H)], 9.49 (s, 1H, N=CH); \(^13\)C-NMR (DMSO-\(d_6\) δ ppm): 169.01 (C=O), 162.11 (triazole C5), 143.44 (triazole C3), ar-C: [114.48 (2CH), 123.15 (C), 125.42 (C), 127.73 (2CH), 129.76 (2CH), 130.56 (2CH), 140.10 (C), 149.91 (C), 157.19 (N=CH), 46.68 (NCH\(_2\)), 55.32 (OCH\(_3\)), 20.87 (CH\(_3\))); LC-MS: \(m/z\) (%) 389 (9) [M+Na]\(^+\), 367 (100) M+1\(^+\); 133 (30), 187 (61), 233 (70), 321 (13), 368 (25); Anal. calcld (%) for C\(_{19}\)H\(_{18}\)N\(_4\)O\(_4\): C, 62.29; H, 4.95; N, 15.29. Found: C, 62.43; H, 5.16; N, 15.12.

(3-(4-Methylphenyl)-5-oxo-4-[[pyridin-4-ylmethylene]amino]-4,5-dihydro-1H-1,2,4-triazol-1-yl] acetic acid (6e). Yield: 3 g, 90%; mp > 290 °C; IR (KBr, cm\(^{-1}\)): 1707 (C=O), 1617 (C=N); \(^1\)H-NMR (DMSO-\(d_6\) δ ppm): 2.39 (s, 3H, CH\(_3\)), 3.43 (brs, OH), 4.65 (s, 2H, NCH\(_2\)), [ar-H: 7.37 (d, 2H, \(J = 7.8\) Hz), 7.74-7.81 (m, 4H), 8.74 (d, 2H, \(J = 6.0\) Hz)], 9.75 (s, 1H, N=CH); \(^13\)C-NMR (DMSO-\(d_6\) δ ppm): 168.86 (C=O), 149.56 (triazole C5), 147.76 (triazole C3), ar-C: [121.45 (2CH), 122.69 (C), 128.00 (2CH), 129.16 (2CH), 130.76 (2CH), 139.21 (C), 140.39 (C), 150.42 (2CH)], 153.53 (N=CH), 46.67 (NCH\(_2\)), 20.88 (CH\(_3\))); LC-MS: \(m/z\) (%) 360 (8) [M+Na]\(^+\), 338 (66) [M+1]\(^+\); 152 (15), 187 (9), 273 (31), 339 (14); Anal. calcld (%) for C\(_{17}\)H\(_{15}\)N\(_5\)O\(_3\): C, 60.53; H, 4.48; N, 20.76. Found: C, 60.58; H, 4.57; N, 20.62.

4-[((4-Methoxybenzyl)amino)-3-(4-methylphenyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl] acetate (7). The solution of compound 4a in absolute ethanol was stirred under reflux conditions in the presence of a catalytic amount of conc. sulfuric acid for 4 h. On cooling the reaction mixture to room temperature a white solid was obtained. This crude product was collected by filtration, washed with water, and recrystallized from ethanol to give the pure compound. Yield: 3.25 g, 79%; mp 119-120 °C; IR (KBr, cm\(^{-1}\)): 3306, 3293 (NH), 1704, 1751 (C=O), 1617 (C=N); \(^1\)H-NMR (DMSO-\(d_6\) δ ppm): 1.30 (t, 3H, \(J = 7.6\) Hz, CH\(_3\)), 2.40 (s, 3H, CH\(_3\)), 3.77 (s, 3H, CH\(_3\)), 4.12 (s, 2H, NHCH\(_2\)), 4.25 (q, 2H, OCH\(_2\), \(J = 7.0\) Hz), 4.63 (s, 2H, NCH\(_2\)), [ar-H: 6.75 (m, 3H, \(J = 8.6\) Hz + NH), 7.15-7.26 (m, 4H), 7.78 (d, 2H, \(J = 8.2\) Hz)]; \(^13\)C-NMR (DMSO-\(d_6\) δ ppm): 167.51 (C=O), 153.38 (triazole C5), 145.23 (triazole C3), ar-C: [113.82 (2CH), 123.33 (C), 127.31 (2CH), 127.58 (C), 128.95 (2CH), 130.77 (2CH), 140.43 (C), 159.38 (C)], 61.85 (OCH\(_2\)), 55.26 (OCH\(_3\)), 574
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54.19 (NCH$_2$), 47.10 (NHCH$_2$), 21.53 (CH$_3$), 20.12 (CH$_3$); Anal. calcd (%) for C$_{21}$H$_{24}$N$_4$O$_4$: C, 63.62; H, 6.10; N, 14.13. Found: C, 63.70; H, 6.18, N, 14.11.

Synthesis of 2-[4-[(4-methoxybenzyl)amino]-3-(4-methylphenyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl]acetohydrazide (8). Hydrazine hydrate (25 mmol) was added to the solution of compound 7 in ethanol and the reaction mixture was allowed to reflux for 3 h. Then the resulting solution was kept overnight in cold conditions. The separated solid was collected by filtration and recrystallized from ethanol to yield the target compounds. Yield: 3.2 g, 87%; mp 173-174°C; IR (KBr, cm$^{-1}$): 3264, 3318 (NH), 1691, 1710, 1746 (C=O), 1611 (C=N); $^1$H-NMR (DMSO-d$_6$ δ ppm): 2.34 (s, 3H, CH$_3$), 3.68 (s, 3H, OCH$_3$), 4.08 (d, 2H, J = 4.0 Hz, NHCH$_2$), 6.74 (t, 3H, NH+NH$_2$), 9.30 (s, NH); $^13$C-NMR (DMSO-d$_6$ δ ppm): 16.570 (C=O), 151.92 (triazole C5), 145.03 (triazole C3), ar-C: [113.38 (2CH), 119.50 (C), 127.13 (2CH), 128.17 (C), 128.57 (2CH), 130.19 (2CH), 135.70 (C)], 54.86 (OCH$_3$), 51.84 (NCH$_2$), 41.82 (NHCH$_2$), 20.85 (CH$_3$); Anal. calcd (%) for C$_{19}$H$_{22}$N$_6$O$_3$: C, 59.67; H, 5.80; N, 21.98. Found: C, 59.81; H, 5.81; N, 21.91.

General method for the synthesis of compounds 11-16

The suitable amine (10 mmol) was added to the solution of the corresponding compound 10 (10 mmol) in water and the reaction content was stirred at room temperature in the presence of formaldehyde solution (20 mmol) for 5 h. Then, the separated solid was filtered off and recrystallized from benzene-petroleum ether (1:2) (for 11-14) or acetone (for 15 and 16) to yield the target compound.

2-{{[4-(4-Fluorophenyl)piperazin-1-yl]methyl}-4-[[2-furylmethylene]amino]-5-methyl-2,4-dihydro-3H-1,2,4-triazol-3-one (11). Yield: 3.92 g, 85%; mp 150°C; IR (KBr, cm$^{-1}$): 1712 (C=O), 1508 (C=N), 1316 (C-N); $^1$H-NMR (DMSO-d$_6$ δ ppm): 2.48 (s, 3H, CH$_3$), 2.86 (s, 4H, 2CH$_2$), 3.14 (s, 2H, CH$_2$), [ar-H: 6.72 (s, 2H), 6.82-7.12 (m, 2H), 7.34-7.46 (m, 3H), 7.53-7.74 (m, 2H)], 9.62 (s, 1H, N=CH); $^13$C-NMR (DMSO-d$_6$ δ ppm): 20.47 (CH$_3$), 48.20 (2CH$_2$), 51.56 (2CH$_2$), 68.47 (CH$_2$), ar-C: [112.18 (CH), 115.58 (2CH), 120.85 (CH), 123.76 (C), 125.78 (CH), 130.60 (2CH), 143.56 (C), 145.92 (CH), 147.34 (CH), 148.67 (C)], 151.16 (triazole C5), 154.40 (N=CH), 159.09 (triazole C3); MS (ESI): m/z (%) 100.78 (100), 116.77 (100), 180.91 (78), 399.22 (13); Anal. calcd (%) for C$_{19}$H$_{22}$FN$_6$O$_2$: C, 59.37; H, 5.51; N, 21.86. Found: C, 59.48; H, 5.63; N, 21.74.

2-[4-(4-Fluorophenyl)piperazin-1-yl]methyl]-4-[(phenylmethylene)amino]-5-Methyl-2,4-dihydro-3H-1,2,4-triazol-3-one (12). Yield: 4.34 g, 90%; mp 136°C; IR (KBr, cm$^{-1}$): 1702 (C=O), 1508 (C=N), 1316 (C-N); $^1$H-NMR (DMSO-d$_6$ δ ppm): 2.48 (s, 3H, CH$_3$), 2.86 (s, 4H, 2CH$_2$), 3.14 (s, 2H, CH$_2$), [ar-H: 6.72 (s, 2H), 6.82-7.12 (m, 2H), 7.34-7.46 (m, 3H), 7.53-7.74 (m, 2H)], 9.62 (s, 1H, N=CH); $^13$C-NMR (DMSO-d$_6$ δ ppm): 20.47 (CH$_3$), 48.20 (2CH$_2$), 51.56 (2CH$_2$), 68.47 (CH$_2$), ar-C: [112.18 (CH), 115.58 (2CH), 120.85 (CH), 123.76 (C), 125.78 (CH), 130.60 (2CH), 143.56 (C), 145.92 (CH), 147.34 (CH), 148.67 (C)], 151.16 (triazole C5), 155.64 (N=CH), 159.09 (triazole C3); MS (ESI): m/z (%) 100.78 (100), 116.77 (78), 399.22 (13); Anal. calcd (%) for C$_{19}$H$_{22}$FN$_6$O$_2$: C, 59.37; H, 5.51; N, 21.86. Found: C, 59.48; H, 5.63; N, 21.74.

2-[[4-(4-Methoxyphenyl)methylene]amino]-5-methyl-2-(morpholin-4-ylmethyl)-2,4-dihydro-3
H-1,2,4-triazol-3-one (13). Yield: 2.28 g, 68%; mp 152 °C; IR (KBr cm⁻¹): 1701 (C=O), 1438 (C=N), 1116 (C-O); ¹H-NMR (DMSO-d₆ δ ppm): 2.28 (s, 3H, CH₃), 2.56 (brs, 4H, 2CH₂), 3.27 (brs, 4H, 2CH₂), 3.81 (s, 3H, OCH₃), 4.54 (s, 2H, CH₂), [ar-H: 7.02-7.06 (m, 2H), 7.72-7.75 (m, 2H)], 9.53-9.54 (s, 1H, N=CH); ¹³C-NMR (DMSO-d₆ δ ppm): 20.21 (CH₃), 50.61 (2CH₂), 56.10 (OCH₃), 62.36 (CH₂), 66.69 (2CH₂), arC: [116.42 (CH), 128.56 (CH), 129.14 (CH), 130.33 (CH), 132.38 (C), 145.23 (C)], 151.01 (triazole C5), 155.04 (N=CH), 162.70 (triazole C3); MS (ESI): m/z (%) 100.09 (56), 127.80 (100), 128.80 (28), 148.81 (20), 383.18 (35), 399.20 (59); Anal. calcd (%) for C₁₆H₂₁N₅O₃: C, 57.99; H, 6.39; N, 21.13. Found: C, 57.82; H, 6.43; N, 21.18.

4-[(2-Hydroxyphenyl)methylene]amino]-5-methyl-2-(morpholin-4-ylmethyl)-2,4-dihydro-3H-1,2,4-triazol-3-one (14). Yield: 1.16 g, 38%; mp 170 °C IR (KBr, cm⁻¹): 1702 (C=O), 1419 (C=N), 1112 (C-O); ¹H-NMR (DMSO-d₆ δ ppm): 2.42 (s, 3H, CH₃), 2.51 (brs, 4H, 2CH₂), 3.48 (brs, 4H, 2CH₂), 4.46 (s, 2H, CH₂), 4.73 (s, 1H, OH), [ar-H: 6.88 (m, 2H) 7.26 (brs, 1H), 7.77 (brs, 1H)], 9.86 (m, 1H, N=CH); ¹³C-NMR (DMSO-d₆ δ ppm): 11.63 (CH₃), 50.22 (2CH₂), 52.20 (CH₂), 66.48 (2CH₂), arC: [116.92 (CH), 120.22 (CH), 127.03 (CH), 133.71 (CH), 143.80 (C), 150.94 (C)], 152.11 (triazole C5), 158.08 (N=CH), 163.26 (triazole C3); LC-MS: m/z (%) 127.73 (28), 131.80 (18), 155.82 (23), 178.84 (100), 187.84 (52), 227.87 (91), 228.94 (18), 318.01 ([M+1]⁺, 21), 356.04 ([M+K]⁺, 12); Anal. calcd (%) for C₁₅H₁₉N₅O₃: C, 56.77; H, 6.03; N, 22.07. Found: C, 56.62; H 6.17; N, 22.12.

4-[(2-Hydroxyphenyl)methylene]amino]-5-methyl-2-[(2-morpholin-4-ylmethyl)methylene]-2,4-dihydro-3H-1,2,4-triazol-3-one (15). Yield: 1.28 g, 25%; mp 131 °C; IR (KBr, cm⁻¹): 1707 (C=O), 1419 (C=N), 1114 (C-O); ¹H-NMR (DMSO-d₆ δ ppm): 2.12 (s, 3H, CH₃), 2.16 (brs, 4H, 2CH₂), 2.44 (brs, 2H, CH₂), 1.92 (s, 4H, 2CH₂), 3.45 (brs, 2H, CH₂), 4.74 (s, 2H, NCH₂N), 4.94 (s, 1H, OH), [ar-H: 6.87 (brs, 2H), 7.29 (brs, 1H), 7.75 (brs, 1H)], 9.87 (s, 1H, N=CH), 10.27 (s, 1H, OH); ¹³C-NMR (DMSO-d₆ δ ppm): 11.77 (CH₃), 53.71 (2CH₂), 57.71 (CH₂), 66.62 (2CH₂), 67.70 (2CH₂), arC: [117.08 (CH), 120.05 (C), 127.03 (CH), 130.44 (CH), 133.80 (CH), 143.90 (C)], 151.74 (triazole C5), 152.15 (N=CH), 158.18 (triazole C-3); MS (ESI): m/z (%) 100.71 (76), 113.72 (75), 130.80 (100), 154.82 (25), 170.83 (21), 186.84 (40), 373.05 (40); Anal. calcd (%) for C₁₅H₁₉N₅O₃: C, 56.65; H, 6.71; N, 23.32. Found: C, 56.63; H, 6.48; N, 23.47.

Antimicrobial activity

All test microorganisms were obtained from the Refik Saydam Hygiene Institute (Ankara, Turkey) and were as follows: E. coli ATCC35218, E. aerogenes ATCC13048, Y. pseudotuberculosis ATCC911, P. aeruginosa
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ATCC43288, S. aureus ATCC25923, E. faecalis ATCC29212, B. cereus 709 Roma, C. albicans ATCC60193, C. tropicalis ATCC 13803, A. niger RSKK 4017, and S. cerevisiae RSKK 251. All the newly synthesized compounds were weighed and dissolved in dimethylsulphoxide to prepare extract stock solution of 5.000 mg/mL. A screening test using agar-well diffusion as adapted earlier was used for all newly synthesized compounds. Each microorganism was suspended in Mueller Hinton (MH) (Difco, Detroit, MI, USA) broth and diluted approximately to \(10^6\) colony forming units (cfu)/mL. They were flood-inoculated onto the surface of MH agar and Sabouraud Dextrose Agar (SDA) (Difco) and then dried. For C. albicans and C. tropicalis, SDA was used. Five millimeter diameter wells were cut from the agar using a sterile cork-borer, and 50 mL of the extract substances was delivered into the wells. The plates were incubated for 18 h at 35 °C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. Ampicillin (10 mg) and fluconazole (5 mg) were the standard drugs. Dimethyl sulfoxide was used as solvent control. The antimicrobial activity results are summarized in the Table.

<table>
<thead>
<tr>
<th>Compound no.</th>
<th>Microorganisms and inhibition zone (mm)</th>
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<tr>
<td></td>
<td>Ec</td>
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<td>16</td>
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<tr>
<td>Amp.</td>
<td>10</td>
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<td>Flu</td>
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</table>


Result and discussion

Synthesis of the intermediate and target compounds was performed according to the reactions outlined in Schemes 1-3. The starting compounds 1, 2c, 3c, and 9 were prepared following a previously reported literature procedure. Ethoxycarbonylmethylation of 2,4-dihydro-3H-1,2,4-triazole-3-one derivatives (2a-e) with ethyl bromoacetate by refluxing in absolute ethanol in the presence of sodium ethoxide afforded the ethyl acetate derivatives (3a-e) in good yields. The \(^1\)H- and \(^{13}\)C-NMR spectra of compounds 3a-e exhibited additional signals derived from the \(-\text{CH}_2\text{CO}_2\text{Et}\) group at the related chemical shift values. Moreover, compounds 3a and 3b gave a stable M+1 ion peak.
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Scheme 1. Synthesis of compounds 2-5.

Scheme 2. Synthesis of compounds 6-8.
The treatment of compounds 3a-d with NaBH₄ resulted in the formation of 2 kinds of product: one of which was formed from the reduction of the ester groups of compounds 3a-d to alcohol functionality while the other corresponded to the hydrolyzed derivatives of esters to carboxylic acids.

The ¹H- and ¹³C-NMR spectra of 4 and 5 type compounds displayed no signals belonging to the -OCH₂CH₃ group; instead, new signals derived from the carboxylic acid functionality or ethyl alcohol moiety appeared at 3.62-4.10 ppm (-NCH₂-NCH₂) and 3.45-4.86 ppm (-OH), respectively. Moreover, spectroscopic data showed that NaBH₄ reduced imine functionality of compounds 3a-d to the corresponding arylmethylamino substituent (controlled by changing with D₂O). Furthermore, compounds 4a and 4b gave a relatively stable M+1 ion peak and all compounds 4 gave reasonable elemental analysis data.

On the other hand, under the same reaction conditions, compound 3e produced 2-(2-hydroxyethyl)-5-(4-methylphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-one (5e), in which the pyridine-4-ylmethylamino moiety is removed from the structure.

The synthesis of carboxylic acids (6a and 6b) was performed from the treatment of 2a and 2b with 3 M HCl or 3 M NaOH without any reduction in the imine group. The treatment of 6a with NaBH₄ afforded the
corresponding compound 4a, as expected. [4-[(4-Methoxybenzyl)amino]-3-(4-methylphenyl)-5-oxo-4,5-dihydro-1\(H\)-1,2,4-triazole-1-yl]acetate (7) was obtained by Fisher esterification of 4a; then the ester was converted to the corresponding hydrazide, 2-[4-[(4-methoxybenzyl)amino]-3-(4-methylphenyl)-5-oxo-4,5-dihydro-1\(H\)-1,2,4-triazole-1-yl]acetohydrazide (8) by treatment with hydrazine hydrate. The structures of compounds 7 and 8 were confirmed on the basis of spectroscopic methods.

The aminoalkylation of imine compounds, 2a-c, 2f was carried out by Mannich reaction using several amines containing a morpholine or piperazine nucleus. The obtained 2-alkyltriazole derivatives (11-16) exhibited spectroscopic and elemental analysis data consistent with the proposed structures.

All the newly synthesized compounds were tested for their antimicrobial activities and only the positive results are presented in the Table. Among them, compounds 11-16, which possess a Mannich base structure and carry a morpholine or piperazine nucleus in the position 2 of the 1,2,4-triazole scaffold, demonstrated good antimicrobial activities against gram-positive and gram-negative bacterial strains, while they exhibited no activity against the yeast-like fungi Candida albicans and Saccharomyces cerevisiae.

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