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Synthesis and antimicrobial activities of some new biheterocyclic compounds containing 1,2,4-triazol-3-one and 1,3,4-thiadiazole moieties

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2-(4-Amino-3-(4-chlorophenyl)-5-oxo-4,5–dihydro-1*H*-1,2,4-triazol-1-yl)-*N*′-[(2,6-dihalogenophenyl)-methylene]acetohydrazides (**3a,b**) was obtained via the formation of 2-(4-amino-3-(4-chlorophenyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl)acetohydrazide (**2**), which was obtained starting from 4-amino-5-(4-chlorophenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**1**) in 2 steps. 2-{[4-amino-3-(4-chlorophenyl)-5-oxo-4,5–dihydro-1*H*-1,2,4-triazol-1-yl]acetyl}-*N*-phenylhydra-zine carbothioamide (**4**), which was prepared starting from **2**, was converted to the corresponding 1,3,4-thiadiazole derivative (**5**) in acidic media. Moreover, the basic treatment of **4** resulted in the formation of 4-amino-5-(4-chlorophenyl)-2-[(4-phenyl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)methyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**7**). The reactions of compounds **5** and **7** with methyl iodide in the presence of sodium ethoxide afforded the corresponding N-methyl (**6**) and S-methyl (**8**) derivatives, respectively. The synthesis of Mannich bases (**10a** and **10b**) was performed from the reaction of **7** with morpholine or piperazine in the presence of formaldehyde.

All the newly synthesized compounds were screened for their antimicrobial activity. The antimicrobial activity study revealed that compounds **3a, 3b**, and **5** showed good antimicrobial activities against the test microorganisms as compared with ampicillin.

Key Words: 1,2,4-triazol-3-one, 5-thioxo-1,2,4-triazole, 1,3,4-thiadiazole, Mannich base, antimicrobial activity

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Introduction

The synthesis of new compounds that are used to treat infections effectively and that lack the side effects associated with current therapies remains a major challenge in medicinal chemistry. In some cases, especially in patients with impaired liver or kidney functions, the use of antimicrobial drugs to treat infections causes several problems. Moreover, from the pharmacoeconomic cost-efficiency point and seeking better patient compliance, antimicrobial agents with high therapeutic effect, high safety, and minimum adverse effects are considerably desirable.[1,2]

The Mannich reaction is a 3-component condensation reaction involving an active hydrogen containing compound, formaldehyde, and a secondary amine. The aminomethylation of aromatic substrates by the Mannich reaction is of considerable importance for the synthesis and modification of biologically active compounds.[3] In recent years, Mannich bases have gained importance due to their application in pharmaceutical chemistry. They have been found to possess antibacterial, antifungal, anticancer, antitubercular, analgesic, and antiinflammatory properties.[3]

1,2,4-Triazoles are an important class of heterocycles, and have been the subject of great interest due to their pharmacological properties.[4–12] Very promising therapeutic applications have been obtained using the 1,2,4-triazole system. There are a number of drugs containing 1,2,4-triazole nucleus, such as itraconazole, fluconazole, and voriconazole (antifungal), that have been used for the treatment of fungal infections.[13–16] Some other dugs including this heterocycle are ribavirin (antiviral), rizatriptan (antimigraine), alprazolam (anxiolytic), vorozole (antitumoral), letrozole (antitumoral), and anastrozole (antitumoral).[17–21]

Another azole ring, 1,3,4-thiadiazole, is incorporated into various biologically active compounds.[22–24] Among these, acetazolamide is a carbonic anhydrase inhibitor and has been used for the treatment of glaucoma, epilepsy, fluid retention, and altitude sickness.[25] The other 1,3,4-thiadiazole containing drug, cefazolin, is a first-generation cephalosporin antibiotic, and has been used in the treatment of a variety of infections caused by gram-positive bacteria such as pharyngitis, uncomplicated skin infections, and endocarditis. It can also be used for the treatment of several bacterial infections involving the lung, bone, joint, stomach, blood, heart valve, and urinary tract.[26–28]

In view of the above facts and in continuation of our interest in the synthesis of 1,2,4-triazole derivatives as antimicrobial agents,[4,8,10–12,29–33] herein we report the synthesis and antimicrobial activities of new 1,2,4-triazole derivatives.

Experimental

Melting points were determined on a Büchi B-540 melting point apparatus and are uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian-Mercury 200 MHz spectrometer. The IR spectra were measured as potassium bromide pellets using a Perkin-Elmer 1600 series FT-IR spectrometer. Mass spectra were run on a Quattro LC-MS (ESI, 70 eV) Instrument. Combustion analysis was performed on a Costech Elemental Combustion System CHNS-O elemental analyzer. All the chemicals were obtained from Fluka Chemie AG Buchs (Switzerland).

Synthesis of 2-(4-amino-3-(4-chlorophenyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl)acetohydrazide (2). Compound **1** (10 mmol) was refluxed with an equivalent amount of sodium in absolute ethanol for 2 h. Then ethyl bromoacetate (10 mmol) was added and the mixture refluxed for an additional 8 h. After evaporating the solvent under reduced pressure, a solid appeared. This product was refluxed with hydrazine hydrate (25 mmol) in *n*-butanol for 4 h. After cooling to room temperature, a white solid appeared. This crude product was recrystallized from ethanol to afford the desired product. Yield 98%, mp = 215-218 °C; Anal. Calcd. (%) for: $C_{10}H_{11}N_6ClO_2$: C, 42.49, H, 3.92, N, 29.73. Found: C, 42.50, H, 3.90, N, 29.65; IR (KBr, ν, cm^{-1}): 3302-3213 (NH+2NH_2), 1717 (C=O), 1668 (C=O), 1577 (C=N); ^{1}H-NMR (DMSO-d_6)δ (ppm): 4.34 (s, 2H, NCH_2), 4.46 (s, 2H, NH<u>NH_2</u>), 5.55 (s, 2H, NH_2), 7.52-7.65 (m, 2H, ar-H), 7.82-8.05 (m, 2H, ar-H), 9.27 (s, <u>NH</u>NH_2); ^{13}C-NMR (DMSO-d_6)δ (ppm): 48.69 (CH_2), arC: [126.82 (C), 127.38 (C), 133.82 (C), 134.93 (C), 138.47(C), 138.97 (C)], 151.79 (triazole C-3), 158.55 (triazole C-5), 167.45 (C=O); MS (ESI): m/z (%) 283.69 (M+1, 32), 305 (100), 273 (34), 229 (48).

General Method for the synthesis of compounds 3

A solution of compound **2** (0.01 mol) in ethanol was refluxed with 2,6-dichlorobenzaldehyde (for **3a**) or 2-chloro-6-fluorobenzaldehyde (for **3b**) (0.01 mol) for 3 h. On cooling the reaction mixture to room temperature, a white solid appeared. This crude product was recrystallized from dimethyl sulfoxide–water (1:2) to afford the desired product.

2-(4-Amino-3-(4-chlorophenyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl)-*N*'-[(2,6-dichlorophenyl)-methylene]acetohydrazide (3a). Yield 85%, mp 258-259 °C; Anal. Calcd. (%) for: $C_{17}H_{13}N_6O_2Cl_3$: C, 46.44, H, 2.98, N, 19.11, Found; C, 46.42, H, 2.95, N, 19.15; IR (KBr, ν, cm^{-1}): 3206, 3067 (NH+NH_2), 1714, 1687 (C=O), 1585 (C=N); ^{1}H-NMR (DMSO-d_6) δ (ppm): 4.91 and 5.00 (s, 2H, benzyl-CH_2, *cis/trans* amide conformers), 5.62 (s, 2H, NH_2), 7.39-7.66 (m, 4H, ar-H), 7.82-8.03 (m, 2H, ar-H), 8.28-8.30 (m, 1H, ar-H), 9.66 (s, 1H, N=CH), 12.01 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6)δ (ppm): 46.87 (CH_2), arC: [115.56 (C), 118.68 (C), 124.70 (C), 125.96 (C), 127.88 (C), 128.23 (C), 131.45 (C), 132.88 (C), 136.56 (C), 137.82 (C), 143.34 (C), 151.11 (C)], 154.65 (N=CH), 156.24 (triazole C-3), 162.86 (triazole C-5), 167.48 (C=O); MS (ESI): m/z (%) 439 (M, 20), 396 (52), 328 (68), 229 (100), 215 (50).

2-(4-Amino-3-(4-chlorophenyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl)-*N*'-[(2-chloro-6- fluorophenyl)methylene]acetohydrazide (3b). Yield 92%, mp 267-268 °C; Anal. Calcd. (%) for: $C_{17}H_{13}N_6O_2Cl_2$: C, 48.24, H, 3.10, N, 19.86, Found; C, 48.20, H, 3.12, N, 19.82; IR (KBr, ν, cm^{-1}): 3208, 3131 and 3070 (NH+NH_2), 1716, 1693 (C=O), 1566 (C=N); ^{1}H-NMR (DMSO-d_6)δ (ppm): 4.91 and 5.00 (s, 2H, benzyl-CH_2, *cis/trans* amide conformers), 5.65 (s, 2H, NH_2), 7.31-7.66 (m, 4H, ar-H), 7.84-8.08 (m, 2H, ar-H), 8.28 (s, 1H, ar-H), 9.66 (s, 1H, N=CH), 12.07 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6)δ (ppm): 46.52 (CH_2), arC: [115.27 (C), 119.78 (C), 124.70 (C), 125.96 (C), 127.98 (C), 128.74 (C), 131.45 (C), 132.88 (C), 135.09 (C), 137.06 (C), 142.46 (C), 150.09 (C)], 153.81 (N=CH), 156.88 (triazole C-3), 162.87 (triazole C-5), 167.63 (C=O); MS (ESI): m/z (%) 423 (M, 8), 445 (40), 229 (100).

2-{[4-Amino-3-(4-chlorophenyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl]acetyl}-*N*-phenylhydrazinecarbothioamide (4). A mixture of acid hydrazide (**2**) (0.01 mol) and phenyl isothiocyanate (0.015 mol) was allowed to reflux in ethanol for 2 h. Then the solution was cooled to room temperature and a white

solid appeared. This product was filtered and recrystallized from dimethyl sulfoxide–water (1: 2) to yield the target product.

Yield 91%, mp 192-193 °C; Anal. Calcd. (%) for: $C_{17}H_{16}ClN_7O_2S$: C, 48.86, H, 3.86, N, 23.46, S, 7.67, Found; C, 48.85, H, 3.83, N, 23.47, S, 7.64; IR (KBr, ν, cm^{-1}): 3269 ($3NH+NH_2$), 1714 (triazole-C=O), 1689 (exocyclic-C=O), 1598 (C=N), 1352 (C=S); ^{1}H-NMR (DMSO-d_6)δ (ppm): 4.66 (s, 2H, NCH_2), 5.63 (s, NH_2), 7.18-7.31 (m, 1H, ar-H), 7.35-7.45 (m, 3H, ar-H), 7.53-7.67 (m, 3H, ar-H), 7.82-8.05 (m, 2H, ar-H), 9.64 (s, NH), 9.78 (s, NH), 10.41 (s, NH); ^{13}C-NMR (DMSO-d_6)δ (ppm): 51.12 (CH_2), arC: [128.78 (C), 128.86 (2C), 129.29 (2C), 129.75 (2C), 130.48 (2C), 133.55 (C), 135.51 (C), 139.55 (C)], 150.78 (triazole C-3), 154.51 (triazole C-5), 171.88 (C=O), 183.12 (C=S).

4-Amino-2-[(5-anilino-1,3,4-thiadiazol-2-yl)methyl]-5-(4-chlorophenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (5). A mixture of the carbothioamide (**4**) (0.01 mol) in cold concentrated sulfuric acid (28 mL) was stirred for 10 min. Then the mixture was allowed to reach room temperature. After stirring for an additional 30 min, the resulting solution was poured into ice-cold water and made alkaline to pH 8 with ammonia. The precipitated product was filtered and recrystallized from ethanol to obtain the desired product. Yield 92%, mp 206-208 °C; Anal. Calcd. (%) for: $C_{17}H_{14}ClN_7OS$: C, 51.06, H, 3.53, N, 24.52, S, 8.02, Found; C, 51.02, H, 3.50, N, 24.55, S, 8.08; IR (KBr, ν, cm^{-1}): 3324-3115 ($NH+NH_2$), 1711 (C=O), 1601 (C=N); ^{1}H-NMR (DMSO-d_6)δ (ppm): 5.31 (s, 2H, NCH_2), 5.60 (s, NH_2), 6.94-7.02 (m, 1H, ar-H), 7.28-7.36 (m, 2H, ar-H), 7.51-7.64 (m, 4H, ar-H), 7.81-8.05 (m, 2H, ar-H), 10.36 (s, NH); ^{13}C-NMR (DMSO-d_6)δ (ppm): 45.93 (CH_2), arC: [119.37 (2C), 123.97 (C), 129.91 (2C), 130.35 (2C), 130.95 (2C), 131.99 (C), 137.20 (C), 148.32 (C)], 154.97 (triazole C-3), 155.35 (triazole C-5), 159.42 (thiadiazole C-2), 167.42 (thiadiazole C-5); MS (ESI): m/z (%) 400 (M, 75), 422 (92), 229 (38), 190 (100).

General method for the synthesis of compounds 6 and 8

To a solution of compound **5** (for **6**) or compound **7** (for **8**) in absolute ethanol was added an equivalent amount of sodium and the mixture was stirred at room temperature for 30 min. Then methyl iodide (0.01 mol) was added and refluxed for an additional 4 h. After evaporating the solvent under reduced pressure a solid appeared. This crude product was recrystallized from ethanol to obtain the target compound.

4-Amino-5-(4-chlorophenyl)-2-({5-[methyl(phenyl)amino]-4,5-dihydro-1,3,4-thiadiazol-2-yl} methyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (6). Yield 74%, mp 187-188 °C; Anal. Calcd. (%) for: $C_{18}H_{16}ClN_7OS$: C, 52.24, H, 3.90, N, 23.69, S, 7.75, Found; C, 51.84, H, 3.45, N, 24.22, S, 7.84; IR (KBr, ν, cm^{-1}): 3233 (NH_2), 1725 (C=O), 1610 (C=N); ^{1}H-NMR (DMSO-d_6)δ (ppm): 3.24 (s, 3H, CH_3), 5.38 (s, 2H, NCH_2), 5.58 (s, 2H, NH_2), 6.90-7.18 (m, 1H, ar-H), 7.26-7.46 (m, 2H, ar-H), 7.58-7.72 (m, 4H, ar-H), 7.80-8.15 (m, 2H, ar-H); ^{13}C-NMR (DMSO-d_6)δ (ppm): 32.14 (CH_3), 42.65 (CH_2), arC: [118.26 (2C), 122.92 (C), 128.98 (2C), 130.36 (2C), 130.88 (2C), 132.39 (C), 136.68 (C), 149.56 (C)], 154.96 (triazole C-3), 156.68 (triazole C-5), 159.65 (thiadiazole C-2), 167.48 (thiadiazole C-5); MS (ESI): m/z (%) 414 (M, 70), 229 (65), 190 (100).

4-Amino-5-(4-chlorophenyl)-2-{[5-(methylthio)-4-phenyl-4*H*-1,2,4-triazol-3-yl]methyl}-2,4-dihydro-3*H*-1,2,4-triazol-3-one (8). Yield 67%, mp 148-149 °C; Anal. Calcd. (%) for: $C_{18}H_{16}ClN_7OS$: C, 52.24, H, 3.90, N, 23.69, S, 7.75, Found; C, 52.25, H, 3.92, N, 23.65, S, 7.70; IR (KBr, ν, cm^{-1}): 3297

(NH_2), 1709 (C=O), 1499 (C=N); ^{1}H-NMR (DMSO-d_6)δ (ppm): 3.37 (s, 3H, CH_3), 5.06 (s,2H, NCH_2), 5.37 (s, 2H, NH_2), 7.36-7.49 (m, 7H, ar-H), 7.86-7.89 (d, 2H, ar-H, J =7.2 Hz); ^{13}C-NMR (DMSO-d_6)δ (ppm): 14.18 (CH_3), 38.09-40.59 (DMSO-d_6+ NCH_2), arC: [124.97 (C), 126.73 (2C), 128.39 (2C), 129.12 (2C), 129.57 (2C), 129.95 (C), 132.12 (C), 134.65 (C)], 143.72 (triazole C-3), 143.75 (triazole C-3'), 150.88 (triazole C-5), 152.52 (triazole C-5'); MS (ESI): m/z (%) 414 (M,100), 416 (M+2, 42), 271 (22), 204 (28), 153 (82).

4-Amino-5-(4-chlorophenyl)-2-[(4-phenyl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)methyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-one (7). A solution of carbothioamide (**4**) (0.01 mol) in 2N NaOH was refluxed for 3 h. Then the resulting solution was cooled to room temperature and acidified to pH 3-4 with 37% HCl. The precipitate formed was filtered, washed with water, and recrystallized from ethanol–water (1:1) to afford the desired compound. Yield 93%, mp 177-178 °C; Anal. Calcd. (%) for: $C_{17}H_{14}ClN_7OS$: C, 51.06, H, 3.53, N, 24.52, S, 8.02, Found; C, 51.00, H, 3.57, N, 24.52, S, 8.06; IR (KBr, ν, cm^{-1}): 3307-3098 (NH_2), 2667 (SH), 1698 (C=O), 1492 (C=N), 1342 (C=S); ^{1}H-NMR (DMSO-d_6)δ (ppm): 4.97 (s, 2H, NCH_2), 5.38 (s, NH_2), 7.35-7.65 (m, 7H, ar-H), 7.00-7.91 (m, 2H, ar-H), 14.00 (s, SH); ^{13}C-NMR (DMSO-d_6)δ (ppm): 38.07-40.83 (DMSO-d_6 + CH_2), arC: [124.89 (C), 126.53 (C), 127.47 (C), 127.96 (C), 128.43 (C), 129.18 (C), 129.46 (C), 129.78 (C), 132.80 (C), 134.76(C), 143.99 (C), 147.21 (C)], 148.50 (triazole C-3), 152.54 (triazole C-3'), 156.79 (triazole C-5), 168.31 (triazole C-5'); MS (ESI): m/z (%) 400 (M, 75), 402 (M+2, 32), 423 (M+Na, 92), 229 (95), 211 (40).

5-(4-Chlorophenyl)-4-{[(4-methoxyphenyl)methylene]amino}-2-[(4-phenyl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)methyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-one (9). A mixture of compound **7** (0.01 mol) with anisaldehyde (0.01 mol) was heated in an oil bath at 140 °C for 2 h. On cooling it to room temperature, a solid appeared. The crude product recrystallized from ethanol to afford the desired compound. Yield 83%, mp 138-140 °C; Anal. Calcd. (%) for: $C_{25}H_{20}ClN_7O_2S$: C, 57.97, H, 3.89, N, 18.93, S, 6.19, Found; C, 57.92, H, 3.85, N, 18.95, S, 6.21; IR (KBr, ν, cm^{-1}): 3155 (NH), 2726 (SH), 1712 (C=O), 1490 (C=N), 1204 (C=S); ^{1}H-NMR (DMSO-d_6)δ (ppm): 3.84 (s, 3H, OCH_3), 5.09 (s, 2H, NCH_2), 7.05-7.09 (d, 2H, ar-H, J=8.6 Hz), 7.33-7.48 (m, 5H, ar-H), 7.57- 7.61 (d, 2H, ar-H, J =8.6 Hz), 7.72-7.81 (m, 4H, ar-H), 9.18 (s, N=CH), 14.08 (s, SH); ^{13}C-NMR (DMSO-d_6)δ (ppm): 43.50 (CH_2), 55.37 (OCH_3), arC: [110.17 (C), 111.48 (C), 114.40 (C), 117.38 (C), 121.14 (C), 123.27 (C), 124.42 (C), 126.83 (C), 127.52 (C), 128.39 (C), 129.67 (C), 131.61 (C), 132.91 (C), 133.53 (C), 135.22 (C), 140.43 (C), 142.9(C), 157.02 (C)], 147.28 (CH), 148.64 (triazole C-3), 152.99 (triazole C-3'), 162.23 (triazole C-5), 168.49 (triazole C-5'); MS (ESI): m/z (%) 518 (M, 100), 520 (M+2, 60), 486 (34), 365 (42), 313 (44).

General method for the synthesis of compounds 10a and 10b

To a solution of compound **8** (0.01 mol) in dichloromethane was added morpholine (for **9a**) or piperazine (for **9b**) in the presence of formaldehyde (40%, 1.5 mL) and the mixture was stirred at room temperature for 2 h. Then water was added and kept overnight in cold conditions. The solid separated was collected by filtration and recrystallized from ethylacetate–petroleum ether (1:2) to yield the target compounds.

5-(4-Chlorophenyl)-4-{[(4-methoxyphenyl)methylene]amino}-2-{[4-phenyl-1-(morpholin-4-ylmethyl)-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl]methyl}-2,4-dihydro- 3*H*-1,2,4-triazol-3-one (10a). Yield 68%, mp 133-135 °C; Anal. Calcd. (%) for: $C_{30}H_{29}ClN_8O_3S$: C, 58.39, H, 5.74, N, 18.16, S,

5.20, Found; C, 58.35, H, 5.72, N, 18.13, S, 5.15; IR (KBr, ν, cm^{-1}): 1712 (C=O), 1492 (C=N), 1313 (C=S); ^{1}H-NMR (DMSO-d_6)δ (ppm): 2.74 (t, 2H, CH_2, J=2.2 Hz), 3.60 (t, 2H, CH_2, J=2.4 Hz), 3.85 (s, 3H, OCH_3), 5.12 (s, 4H, CH_2), 5.49 (brs, 2H, NCH_2), 5.52 (brs, 2H, NCH_2), 7.08-7.11 (m, 2H, ar-H), 7.35-7.50 (m, 4H, ar-H), 7.54-7.63 (m, 4H, ar-H), 7.74-7.78 (m, 3H, ar-H), 9.19 (s, N=CH); ^{13}C-NMR (DMSO-d_6)δ (ppm): 45.54 (CH_2), 57.30 (OCH_3), 58.12 ($2CH_2$), 59.45 ($2CH_2$), 65.23 (CH_2), arC: [116.45 (C), 125.15 (C), 127.05 (2C), 129.38 (2C), 130.57 (2C), 131.14 (2C), 131.38 (2C), 131.56 (C), 131.76 (C), 135.12 (2C), 137.10 (C), 157.02 (C)], 146.73 (CH), 148.64 (triazole C-3), 151.34 (triazole C-5), 164.56 (triazole C-3'), 167.49 (triazole C-5'); MS (ESI): m/z (%) 618 (M+1, 18), 606 (28), 518 (46), 229 (100).

5-(4-Chlorophenyl)-4-{[(4-methoxyphenyl)methylene]amino}-2-{[4-phenyl-1-(piperazine-1-ylmethyl)-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl]methyl}-2,4-dihydro- 3*H*-1,2,4-triazol-3-one (10b). Yield 78%, mp 169-170 °C; Anal. Calcd. (%) for: $C_{31}H_{32}ClN_9O_2S$: C, 59.09, H, 5.12, N, 20.00, S, 5.09, Found; C, 59.02, H, 5.10, N, 19.90, S, 5.13; IR (KBr, ν, cm^{-1}): 1716 (C=O), 1489 (C=N), 1296 (C=S); ^{1}H-NMR (DMSO-d_6)δ (ppm): 2.44 (s, 3H, NCH_3), 2.74 (t, 2H, CH_2, J= 2.4 Hz), 3.56 (brs, 2H, CH_2), 3.84 (s, 3H, OCH_3), 4.97 (s, 4H, CH_2), 5.21 (brs, 2H, NCH_2), 5.42 (brs, 2H, NCH_2), 7.06-7.10 (m, 2H, ar-H), 7.59-7.63 (m, 4H, ar-H), 7.77-7.82 (m, 4H, ar-H), 7.90-7.95 (m, 3H, ar-H), 9.49 (s, N=CH);^{13}C-NMR (DMSO-d_6)δ (ppm): 43.55 (CH_3), 45.36 (CH_2), 56.48 (OCH_3), 58.72 ($2CH_2$), 59.83 ($2CH_2$), 66.31 (CH_2), arC: [115.35 (C), 124.15 (C), 126.05 (2C), 129.49 (2C), 130.89 (2C), 131.14 (2C), 131.38 (2C), 131.92 (C), 131.77 (C), 134.73 (2C), 137.10 (C), 157.65 (C)], 146.71 (CH), 148.45 (triazole C-3), 151.55 (triazole C-5), 164.66 (triazole C-3'), 167.54 (triazole C-5'); MS (ESI): m/z (%) 631 (M+1, 56), 606 (34), 518 (46), 229 (100).

Antimicrobial activity

All test microorganisms were obtained from the Refik Saydam Hıfzıssıhha Institute (Ankara, Turkey) and were as follows: *Escherichia coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 13883, *Yersinia pseudotuberculosis* ATCC 911, *Enterobacter aerogenes* ATCC 13048, *Pseudomonas aeruginosa* ATCC 10145, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* 709 Roma, *Candida tropicalis* ATCC 13803, *Candida glabrata* 66032, and *Candida albicans* ATCC 60193. All the newly synthesized compounds were weighed and dissolved in dimethylsulphoxide to prepare extract stock solution of 10,000 μg/mL.

The antimicrobial effects of the substances were tested quantitatively in respective broth media by using double dilution and the minimal inhibition concentration (MIC) values (μg/mL) were determined.[34] The antibacterial and antifungal assays were performed in Mueller-Hinton broth (MH) (Difco, Detroit, MI, USA) at pH 7.3 and buffered Yeast Nitrogen Base (Difco) at pH 7.0, respectively. The MIC was defined as the lowest concentration that showed no growth. Ampicillin (10 μg) was used as standard antibacterial and antifungal drugs. Dimethylsulphoxide at a dilution of 1:10 was used as solvent control.

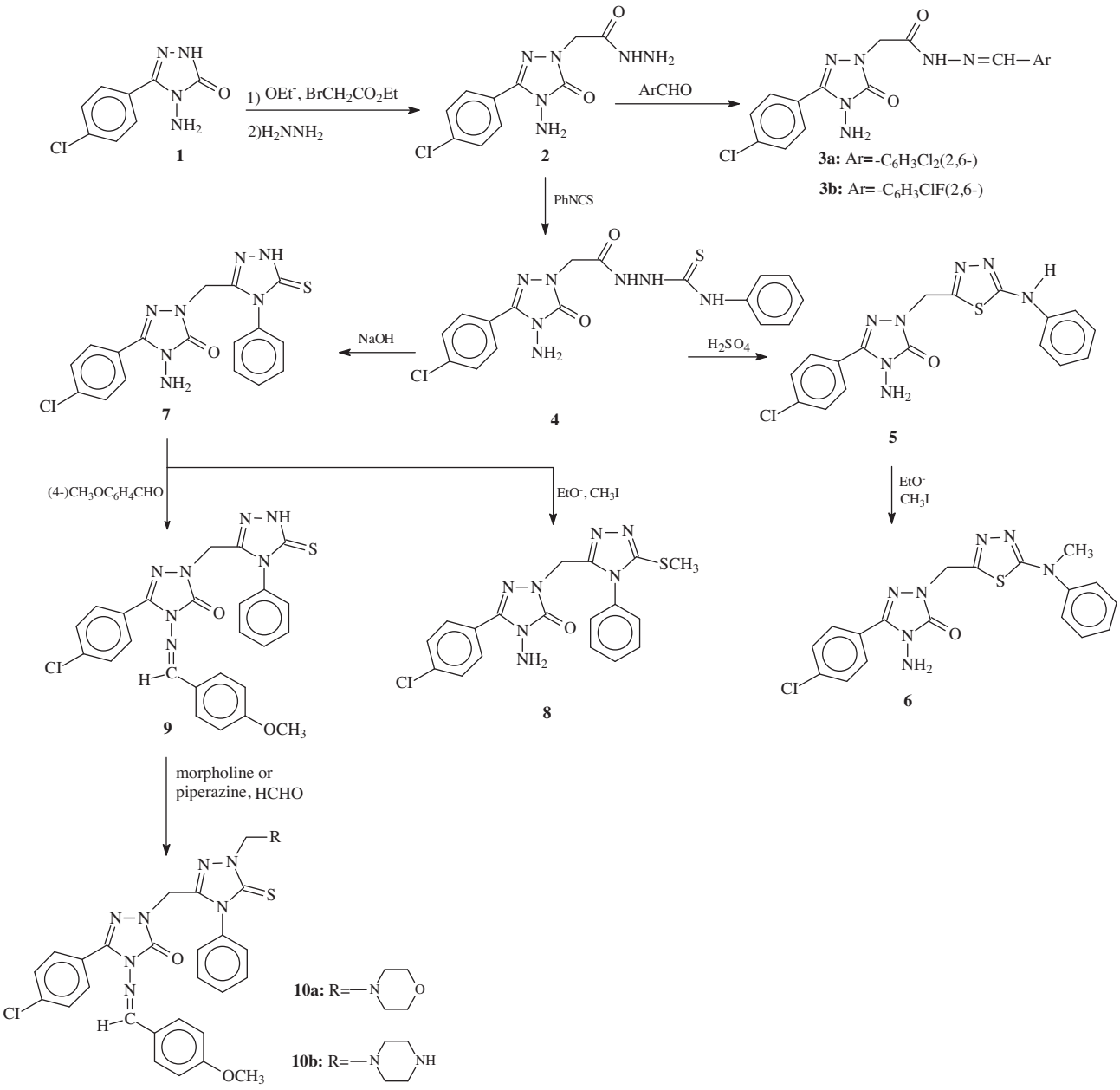
Results and discussion

The synthesis of the intermediate and target compounds was performed according to the reactions outlined in the Scheme. The starting compound, 4-amino-5-(4-chlorophenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**1**), was prepared following a previously reported literature procedure.[35] The reaction of compound **1** with hydrazine

hydrate followed by the treatment of ethyl bromoacetate in basic media led to the formation of compound **2** in a good yield. The ^{1}H-NMR spectrum of compound **2** exhibited 3 different signals integrating one proton that are exchangeable with D_2O. The first one, which was observed at 4.46 ppm, belongs to the amino group of the hydrazide structure (-NH$\underline{NH}_2$); the second one, which was recorded at 5.55 ppm, represents the amino group at position 4 of the 1,2,4-triazole ring; and the third one, which was observed at 9.29 ppm, points to hydrazide -$\underline{NH}NH_2$. The condensation of compound **2** with some aromatic aldehydes, namely 2,6-dichlorobenzaldehyde and 2-chloro-6-fluorobenzaldehyde, in absolute ethanol yielded the corresponding 2-(4-amino-3-(4-chlorophenyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl)-*N*′-(2,6-dihalogenophenylmethylene)-acetohydrazides (**3a, 3b**). The evidence for the formation of arylidenamino derivatives (**3a, 3b**) can be achieved by ^{1}H-NMR, ^{13}C-NMR, IR, and mass spectroscopic methods and elemental analysis. In the ^{1}H-NMR spectra of compounds **3a** and **3b,** additional signals that originated from arylidene moiety was observed in the aromatic region, and an additional signal was recorded at 9.66 ppm derived from the –N=CH- bond, whereas the signal due to the –NH$\underline{NH}_2$ group amino group disappeared. The presence of a peak at 5.62 (for **3a**) and 5.65 (for **3b**) ppm in the ^{1}H-NMR spectra of compounds **3a** and **3b** indicated that only hydrazide-NH_2 condensed with aromatic aldehydes in the applied reaction conditions and the other amino group at position 4 of the 1,2,4-triazole ring remained unchanged. In the ^{13}C-NMR spectra, a new signal belonging to the –N=CH group appeared at 154.65 (**3a**) ppm or 153.81 (**3b**) ppm. Moreover, compounds **3a** and **3b** exhibited mass spectra and elemental analysis data consistent with the assigned structures. The compounds having an arylidene-hydrazide structure may exist as *E*/*Z* geometrical isomers about a –C=N double bond and *cis*/*trans* amide conformers.[36–38] According to the literature, the compounds containing an imine bond are present in higher percentages in dimethyl-d_6 sulfoxide solution in the form of geometrical *E* isomer about a –C=N double bond.[36–38] The *Z* isomer can be stabilized in less polar solvents by an intramolecular hydrogen bond. In the present study, the spectral data were obtained in dimethyl-d_6 sulfoxide solution and no signal belonging to *Z* isomer was observed. On the other hand, the *cis-trans* conformers of *E* isomer were present in the dimethyl-d_6 sulfoxide solution of compounds **3a** and **3b**. In the ^{1}H-NMR spectra of compounds **3a,b**, 2 sets of signals each belonging to the benzylic CH_2 group of *cis*- and *trans*- conformers were observed at 4.91 ppm and 5.00 ppm, respectively. The upfield lines of benzylic-CH_2 protons were assigned to *cis*-conformer of the amide structure and downfield lines of the protons of the same group to *trans*-conformer of the amide structure.

2-{[4-amino-3-(4-chlorophenyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl]acetyl}-*N*-phenylhydrazinecarbothioamide (**4**) was obtained in high yield and purity by the nucleophilic addition of hydrazide-NH_2 of compound **3** to phenylisothiocyanate. The reaction was carried out at reflux temperature in ethanol and afforded the desired cabothioamide derivative (**4**), which was the starting material for further cyclizations. The IR spectrum of compound **4** displayed a broad signal at 3269 cm^{-1} due to 3 NH and 1 NH_2 absorptions. In the ^{1}H-NMR spectra of compound **4**, these groups were observed at 9.64, 9.78, 10.41, and 5.63 ppm, respectively.

The acidic treatment of compound **4** in cold conditions and then at room temperature resulted in the formation of 4-amino-2-[(5-anilino-1,3,4-thiadiazol-2-yl)methyl]-5-(4-chlorophenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**5**) in good yield. According to the literature, it is expected that the treatment of carbothioamides with aqueous NaOH resulted in a cyclization leading to the formation of a 5-thioxo-1,2,4-triazole ring.[38–42] Beside this method, in another study performed in our laboratory,[8] type **7** compounds were prepared by the heating of the corresponding carbohioamides to 130 °C in an oil bath for 2 h or refluxing type **4** compounds in ethanol



Scheme. Synthetic pathway for the preparation of compounds **2-10**.

for 8 h. The yields of these 3 methods were very close to each other. In the ^{1}H-NMR spectra of compounds **5** and **7** and **8a,c,d,f** the presence of only 1 signal for the –NH group integrating for 1 proton (exch. with D_2O) confirmed the cyclization at the side chain of the triazole ring in compound **4**. In the ^{13}C-NMR spectra of compound **5**, no signal due to the –C=S group was observed, while the signal derived from this group was resonated at 168.31 ppm (triazole C-5) in the spectrum of compound **7**. Furthermore, the ^{1}H- NMR spectrum of compound **7** exhibited a singlet peak at 14.00 representing the presence of a –SH proton, while the ^{1}H-NMR

spectrum of compound **5** displayed a signal at 10.36 ppm pointing to an exocyclic –NH- group. These values are consistent with the literature.[4,19,32,38,42,43] In addition, elemental analyses were consistent with the assigned structures for compounds **5** and **7,** and the mass spectra of the cyclization products (**5**, **7**) showed molecular ion peaks consistent with their molecular formulae.

It is interesting to note that compounds **7** are present in their thioxo-mercapto tautomeric forms in solids as indicated by their IR spectra (presence of an absorption in the region of 2667 cm^{-1} for -SH stretching and presence of two absorption maxima at 1342 cm^{-1} characteristic of -C=S group in these type of compounds.[4,20,33,44] These tautomers were present also in dimethyl sulfoxide as suggested by NMR spectral data.

The newly synthesized compounds **2-10** were evaluated in vitro for their antimicrobial activities. The results are presented in the Table. The compounds having a substituted phenylmethylene-acetohydrazide structure, **3a** and **3b,** and **5**, which contains a 1,3,4-thiadiazole nucleus, showed the most potent antimicrobial activities against the test microorganisms. The replacement of the –NH- group in compound **5** by a hydrophobic methyl group leading to compound **6** diminished the antimicrobial activity. Compound **7** demonstrated slight activity against *Enterobacter aerogenes* (En), *Staphylococcus aureus* (Sa), *Enterococcus faecalis* (Ef), and *Bacillus careus* (Bc). When compound **7** was converted into S-methyl derivative (**8**) no change was observed in antimicrobial activity. Similarly, for compound **9,** the conversion of amino group into 4-methoxyphenylmethyleneamino group caused no change in the antimicrobial activity. Comparison of the antimicrobial activity of **10a,b** with compound **9** indicates that the replacement of triazole-NH- proton by bulky morpholine or piperazine ring resulted in no change in antimicrobial activity except for *Bacillus careus* (Bc).

Table. Antimicrobial activity of the compounds (μg/mL).

Compounds	Microorganisms and Minimal Inhibition Concentration							
No	Ec.	Kp.	Yp.	En.	Pa.	Sa.	Ef.	Bc.
2	> 500	> 500	> 500	> 250	> 500	250	250	> 250
3a	< 1.95	< 1.95	< 1.95	< 1.95	< 1.95	< 1.95	< 1.95	< 1.95
3b	< 1.95	< 1.95	< 1.95	< 1.95	< 1.95	< 1.95	< 1.95	< 1.95
4	> 500	> 500	> 500	62.5	> 500	125	125	125
5	< 1.95	< 1.95	< 1.95	3.90	< 1.95	1.95	7.81	3.90
6	> 500	500	250	7.81	500	15.63	125	1.95
7	> 500	> 500	> 500	125	> 500	125	125	125
8	> 500	> 500	> 500	125	> 500	125	125	125
9	> 500	> 500	> 500	125	> 500	125	125	62.5
10a	> 500	> 500	> 500	250	> 500	500	500	125
10b	> 500	> 500	> 500	250	> 500	500	500	125
Amp.	10	> 128	18	> 128	18	35	10	15

Ec.: *Escherichia coli* ATCC 35218, Kp.: *Klebsiella pneumoniae* ATCC 13883, Yp.: *Yersinia pseudotuberculosis* ATCC 911, En.: *Enterobacter aerogenes* ATCC 13048, Pa.: *Pseudomonas aeruginosa* ATCC 10145, Sa.: *Staphylococcus aureus* ATCC 25923, Ef.: *Enterococcus faecalis* ATCC 29212, Bc.: *Bacillus cereus 709 Roma,* Amp.: Ampicillin.

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