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Synthesis and antimicrobial activities of some new 1,2,4-triazole derivatives

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The synthesis of ethyl [3-(cyanomethyl)-5-alkyl-4*H*-1,2,4-triazol-4-yl]carbamates (**2a-d**) was performed starting from ethyl 2-[ethoxy(4-(aryl)methylene]hydrazinecarboxylates (**1a, 1b**). The treatment of **2a** with thiosemicarbazide afforded ethyl [3-[(5-amino-1,3,4-thiadiazol-2-yl)methyl]-5-(4-nitrophenyl)-4*H*-1,2,4-triazol-4-yl]carbamates (**3a**), whereas compound **2b** produced 5-{[4-amino-5-(4-methylphenyl)-4*H*-1,2,4-triazol-3-yl]methyl}-1,3,4-thiadiazol-2-amine (**3b**) in the same reaction conditions. The treatment of *tert*-butyl 2-[2-(4-chlorophenyl)-1-ethoxyethylidene]hydrazinecarboxylate (**5**) with malonohydrazide or cyanoacethydrazide gave the corresponding 1,2,4-triazol-ylcarbamate derivatives (**6** or **9**); then the hydrolysis of these compounds resulted in the formation of 3-{[4-amino-5-(4-chlorobenzyl)-4*H*-1,2,4-triazol-3-yl]methyl}-5-(4-chlorobenzyl)-4*H*-1,2,4-triazol-4-amine (**7**) and [4-amino-5-(4-chlorobenzyl)-4*H*-1,2,4-triazole-3-yl]acetonitrile (**10**), respectively. The synthesis of the Schiff base derivatives 3-(4-chlorobenzyl)-5-{[5-(4-chlorobenzyl)- 4-[(2-hydroxyphenyl-methylene)amino]-4*H*-1,2,4-triazol-3-yl]methyl}-*N*-(2-hydroxyphenylmethylene)-4*H*-1,2,4-triazol-4-amine (**8**), and (5-(4-chlorobenzyl)-4-{[(2,6-dichlorophenyl)methylene]amino}-4*H*-1,2,4-triazol-3-yl)acetonitrile (**12**) was performed from the reaction of compounds **7** and **10** with salicyl aldehyde (for **8**) or 2,6-dichlorobenzaldehyde (for **12**), respectively. The treatment of compounds **5** or **10** with thiosemicarbazide gave 5-{[4-amino-5-(4-chlorobenzyl)-4*H*-1,2,4-triazol-3-yl]methyl}-1,3,4-thiadiazol-2-amine (**11**).

All the newly synthesized compounds were screened for their antimicrobial activities and were found to possess good or moderate antimicrobial activity.

Key Words: 1,3,4-Thiadiazole, 1,2,4-triazole, carbamate, antimicrobial activity

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Introduction

Diseases caused by pathogenic bacteria still attract significant attention from medicinal chemists and biologists because of growing antibacterial resistance. For example, *Staphylococcus aureus*, which is one of the major causes of hospital- and community-acquired infections worldwide like wound infections, bacteremia, and sepsis, is associated with a high mortality rate.[1] Another infection, tuberculosis (TB) causes the death of approximately three million patients in the world every year. These numbers make TB one of the leading infectious causes of death, eclipsed only by AIDS. Synthetic drugs for treating TB have been available for over half a century, but incidences of the disease continue to rise worldwide. The causative organism, *Mycobacterium tuberculosis*, is a tremendously successful colonizer of the human host and is estimated to have latently infected approximately one-third of humanity.[2−5] A growing number of immuno-compromised patients are as a result of cancer chemotherapy, organ transplantation, and HIV infection, which are the major factors contributing to this increase. Therefore, it is necessary to search for and synthesize new classes of antimicrobial compounds that are effective against pathogenic microorganisms that have developed resistance to the antibiotics. In addition, the new agents should have low side effects on the human body, because, in some cases, especially in patients with impaired liver or kidney functions, the use of antimicrobial drugs to treat infections causes several problems.[6−9] Moreover, from the pharmacoeconomic cost-efficiency viewpoint, and seeking better patient compliance, antimicrobial agents with high therapeutic effect, high safety, and minimum adverse effects are considerably desirable.[10,11] Therefore, there is an urgent need to develop novel antimicrobial and antitubercular chemotherapeutic agents.

A literature survey revealed triazole derivatives belonging to an important group of heterocyclic compounds that have been the subject of extensive study in the recent past. Diverse biological activities, such as antibacterial, antifungal, anti-inflammatory, antihypertensive, and antiviral, have been associated with 1,2,4-triazole derivatives.[12−22]

Keeping this observation in view and in continuation of our study on the synthesis of biologically active nitrogen and sulfur containing heterocycles,[15,18,22−29] this paper presents the synthesis of a series of some new heterocyclic carbamates, conversion to Schiff bases, and the study of their antimicrobial activities.

Experimental

Chemistry

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 FTIR spectrometer. Combustion analysis was performed on a Costech Elemental Combustion System CHNS-O elemental analyzer. Mass spectra were obtained for compounds **2a, 3a, 3b, 6, 7, 9, 10,** and **11** on a Quattro LC-MS (70 eV) Instrument. All the chemicals were obtained from Fluka Chemie AG Buchs (Switzerland). Compounds **1a-d, 4,** and **5** were synthesized as reported previously.[30,31]

General Method for the Synthesis of Compound [3-(cyanomethyl)-5-alkyl-4*H*-1,2,4-triazol-4-yl]carbamates (2a-d).

A mixture of corresponding compound **1** and 2-cyanoacetohydrazide was refluxed at 95-100 °C for 1 h. After the residue was cooled to room temperature, n-butyl acetate-petroleum ether (1:2) was added and the mixture was kept in the cold overnight. The resulting solid separated was collected by filtration and recrystallized from an appropriate solvent to afford the desired compound.

Ethyl [3-(cyanomethyl)-5-(4-nitrophenyl)-4*H*-1,2,4-triazol-4-yl]carbamate (2a). Recrystallized from ethyl acetate. Yield 77%, mp 135-136 °C. IR (KBr, υ, cm^{-1}): 3112 (NH), 2257 (CN), 1744 (C=O), 1554 and 1525 (2C=N); Anal. Calcd. (%) for $C_{13}H_{12}O_4N_6$: C, 49.37; H, 3.82; N, 26.57. Found: C, 49.96; H, 3.88; N, 26.72; ^{1}H-NMR (DMSO-d_6, δ ppm): 1.18 (3H, t, CH_3, J= 7.7 Hz), 3.55 (2H, s, CH_2), 4.10 (2H, q, CH_2, J= 6.1 Hz), 7.51 (2H, d, arH, J= 8.4 Hz), 8.20 (2H, d, arH, J= 8.4 Hz), 11.07 (1H, br, NH); ^{13}C-NMR (DMSO-d_6, δ ppm): 17.49 (CH_3), 24.41 (CH_3), 38.08-40.58 (DMSO-d_6+CH_2), 116.13 (CN), arC: [127.45 (CH), 127.97 (CH), 129.31 (CH), 129.57 (CH), 141.13 (C), 164.45 (C)], 152.27 (triazole C-3), 156.74 (triazole C-5), 184.45 (C=O).

EI MS m/z (%): 115 (16), 123 (28), 138 (16), 229 (13), 259 (13), 271 ([M-OEt]$^+$, 24), 285 (19), 303 (25), 332 (22), 353 (75), 390 (27).

Ethyl [3-(cyanomethyl)-5-(4-methylphenyl)-4*H*-1,2,4-triazol-4-yl]carbamate (2b). Recrystallized from acetone. Yield 30%, mp 127-128 °C. IR (KBr, υ, cm^{-1}): 3188 (NH), 2260 (CN), 1690 (C=O), 1492 and 1453 (2C=N); Anal. Calcd. (%) for $C_{14}H_{15}O_2N_5$: C, 58.94; H, 5.30; N, 24.55. Found: C, 59.23; H, 5.36; N, 24.41; ^{1}H-NMR (DMSO-d_6, δ ppm): 1.20-1.39 (3H, m, CH_3), 2.45 (2H, s, CH_2), 3.40 (3H, s, CH_3), 3.93-4.15 (2H, q, CH_2, J= 7.1 Hz), 7.22-7.38 (2H, d, arH, J= 6.4 Hz), 7.40-7.54 (2H, t, arH, J= 7.7 Hz), 11.07 (1H, s, NH); ^{13}C-NMR (DMSO-d_6, δ ppm): 14.79 (CH_3), 20.88 (CH_3), 24.52 (CH_2), 66.60 (CH_2), 116.18 (CN), arC: [127.59 (CH), 127.95 (CH), 129.05 (CH), 129.20 (CH), 140.25 (C), 149.68 (C)], 152.66 (triazole C-3), 158.72 (triazole C-5), 164.27 (C=O).

Ethyl [3-(cyanomethyl)-5-phenyl-4*H*-1,2,4-triazol-4-yl]carbamate (2c). Recrystallized from dimethyl sulfoxide. Yield 30%, mp 110-111 °C. IR (KBr, υ, cm^{-1}): 2262 (CN), 1743 (C=O), 1622 and 1589 (2C=N); Anal. Calcd. (%) for $C_{13}H_{13}O_2N_5$: C, 57.56; H, 4.83; N, 25.82. Found: C, 57.51; H, 4.79; N, 25.85; ^{1}H-NMR (DMSO-d_6, δ ppm): 1.25 (3H, t, CH_3, J= 6.8 Hz), 3.88-4.15 (2H, m, CH_2), 4.24 (2H, s, CH_2), 7.51-7.63 (5H, m, arH), 10.97 (1H, s, NH).

Ethyl [3-(4-chlorophenyl)-5-(cyanomethyl)-4*H*-1,2,4-triazol-4-yl]carbamate (2d). Recrystallized from dimethyl sulfoxide. Yield 25%, mp 130-131 °C. IR (KBr, υ, cm^{-1}): 3193 (NH), 2260 (CN), 1689 (C=O); Anal. Calcd. (%) for $C_{13}H_{12}O_2N_5Cl$: C, 50.74; H, 4.59; N, 22.76. Found: C, 51.18; H, 3.96; N, 22.85; ^{1}H-NMR (DMSO-d_6, δ ppm): 1.26 (3H, t, CH_3, J= 7.0 Hz), 3.90-4.17 (2H, m, CH_2), 4.21 (2H, s, CH_2), 7.54-7.67 (4H, m, arH), 11.01 (1H, s, NH).

General Method for the Synthesis of Compounds 3a and 3b

To a solution of compound **2** (10 mmol) in trifluoroacetic acid, 1 equiv. of thiosemicarbazide was added and the mixture was heated in an oil bath for 10 h at 60 °C. Then the reaction mixture was neutralized with ammonia. The formed solid was filtered off and recrystallized from ethyl acetate to afford the desired compound.

Ethyl [3-[(5-amino-1,3,4-thiadiazol-2-yl)methyl]-5-(4-nitrophenyl)-4*H*-1,2,4-triazol-4-yl]carbamate (3a). Yield 37%, mp 175-176 °C. IR (KBr, υ, cm^{-1}): 3118 (NH_2+NH), 1740 (C=O), 1658, 1583 and 1457 (4C=N), 1514 and 1399 (NO_2), 1109 (C-O); Anal. Calcd. (%) for $C_{14}H_{14}O_4N_8S$: C, 43.07; H, 3.61; N, 28.70. Found: C, 43.12; H, 3.78; N, 28.56; ^{1}H-NMR (DMSO-d_6, δ ppm): 1.19 (3H, t, CH_3, J= 7.1 Hz), 3.43 (2H, br, CH_2), 4.24 (2H, q, CH_2, J= 7.4 Hz), 7.17 (2H, s, NH_2, exch. D_2O), 7.52 (2H, d, arH, J= 8.7 Hz), 8.21 (2H, d, arH, J= 8.9 Hz), 11.00 (1H, s, NH); ^{13}C-NMR (DMSO-d_6, δ ppm): 14.76 (CH_3), 29.96 (CH_2), 63.01 (CH_2), arC: [124.20 (2CH), 130.85 (2CH), 143.82 (C), 143.86 (C)], 147.14 (triazole C-3), 151.95 (triazole C-5 ve thiadiazole C-5), 153.70 (thiadiazole C-3), 170.20 (C=O); EI MS m/z (%): 215 (25), 390 ($[M]^+$, 100), 391 ($[M+1]^+$, 391).

5-{[4-amino-5-(4-methylphenyl)-4*H*-1,2,4-triazol-3-yl]methyl}-1,3,4-thiadiazol-2-amine (3b). Recrystallized from ethanol. Yield 32%, mp 204-205 °C. IR (KBr, υ, cm^{-1}): 3282 and 3092 (NH+NH_2), 1740 (C=O), 1634, 1583, 1514 and 1470 (4C=N); Anal. Calcd. (%) for $C_{12}H_{13}N_7S$: C, 50.16; H, 4.56; N, 34.12. Found: C, 50.08; H, 4.51; N, 34.07; ^{1}H-NMR (DMSO-d_6, δ ppm): 2.37 (3H, s, CH_3), 2.52 (2H, s, CH_2), 7.33 (2H, d, arH, J= 8.0 Hz), 7.66 (2H, d, arH, J= 7.8 Hz), 8.38 (4H, br, $2NH_2$); ^{13}C-NMR (DMSO-d_6, δ ppm): 21.42 (CH_3), 38.07-40.59 (DMSO-d_6+ CH_2), arC: [126.66 (C), 126.99 (2CH), 130.45 (2CH), 141.89 (C)], 157.03 (triazole C-5 and thiadiazole C-3), 169.24 (triazole C-3 and thiadiazole C-5). MS m/z (%): 373 (11), 117 (44), 133 (25), 150 (88), 193 (19).

***Tert*-butyl 3-({4-[(tert-butoxycarbonyl)amino]-5-(4-chlorobenzyl)-4*H*-1,2,4-triazol-3-yl}methyl)-5-(4-chlorobenzyl)-4H-1,2,4-triazol-4-ylcarbamate (6).** A mixture of *tert*-butyl 2-[2-(4-chlorophenyl)-1-ethoxyethylidene]hydrazinecarboxylate (**5**) (20 mmol) and malonohydrazide (10 mmol) was heated in an oil bath at 95 °C for 2 h. After the reaction mixture was cooled to room temperature, a few drops of ethyl acetate was added and was kept in the cold overnight. The solid formed was filtered and recrystallized from acetone to obtain compound **6.** Yield 78%, mp 174-175 °C. IR (KBr, υ, cm^{-1}): 2979 and 2936 (2NH), 1739 (2C=O); Anal. Calcd. (%) for $C_{29}H_{34}O_4N_8Cl_2$: C, 55.33; H, 5.54; N, 17.80. Found: C, 55.28; H, 5.50; N, 17.77; ^{1}H-NMR (DMSO-d_6, δ ppm): 1.35 (18H, s, $6CH_3$), 3.89 (2H, s, CH_2), 4.00 (4H, s, $2CH_2$), 7.24 (4H, d, arH, J = 8.3 Hz), 7.38 (4H, d, arH, J = 8.3 Hz), 10.75 (2H, br, 2NH); ^{13}C-NMR (DMSO-d_6, δ ppm): 27.47 ($6CH_3$), 28.75 ($3CH_2$), 81.64 (2C), arC: [128.24 (4CH), 130.44 (4CH), 131.35 (2C), 134.30 (2C)], 149.73 (triazole 2C-3), 153.12 (triazole 2C-5), 179.80 (2C=O); EI MS m/z (%): 121(25), 146 (28), 149 (18), 155 (13), 188 (16), 210 (14), 229 (100), 230 (23), 246 (18), 273 (38), 279 (14), 313 (30), 335 (41), 357 (26), 365 (28), 367 (29), 368 (14), 399 (24), 429 (88), 431 (64), 432 (19), 451 (46), 453 (31), 473 (73), 475 (50), 529 (49), 531 (31), 532 (13), 551 (31), 553 (26), 574 (13), 629 ($[M]^+$, 64), 631 (41), 652 ($[M+Na]^+$, 19).

3-{[4-Amino-5-(4-chlorobenzyl)-4H-1,2,4-triazol-3-yl]methyl}-5-(4-chlorobenzyl)-4*H*-1,2,4-triazol-4-amine (7). A mixture of compound **6** (10 mmol) and 6N HCl (5.45 mL) in tetrahydrofuran was refluxed in a water bath for 15 min. After the solvent was evaporated under reduced pressure a white solid appeared. The crude product was added to a potassium carbonate solution (10 mmol) slowly and the obtained mixture was stirred at room temperature for 30 min. The obtained solid was filtered and recrystallized from ethanol-water (1:1). Yield 81%, mp 214-215 °C. IR (KBr, υ, cm^{-1}): 3252 and 3197 ($2NH_2$+ArCH), 1650, 1606, 1521 and 1493 (4C=N); Anal. Calcd. (%) for $C_{19}H_{18}N_8Cl_2$: C, 53.16; H, 4.23; N, 26.10. Found: C, 53.08; H, 4.17; N, 26.08; ^{1}H-NMR (DMSO-d_6, δ ppm): 4.09 (4H, br, $2CH_2$), 4.19 (2H, br, CH_2), 5.97 (4H, br, 2NH), 7.21-7.40 (8H, m, arH); ^{13}C-NMR (DMSO-d_6, δ ppm): 21.08 (CH_2), 29.56 ($2CH_2$), arC: [129.00

(4CH), 131.38 (4CH), 131.87 (2C), 136.49 (2C)], 151.54 (triazole 2C-3), 154.48 (triazole 2C-5); EI MS m/z (%):116.65 (71), 134.69 (42), 148.65 (46), 163.49 (42), 179.90 (36), 180.72 (27), 196.63 (23), 208.76 (46), 229.07 (100), 244.66 (50), 245.10 (29), 273.27 (20), 287.92 (14), 313.07 (17), 357.09 (54), 367.21 (39), 367.34 (19), 371.05 (15), 399.21 (13), 412.92 (39), 415.31 (21), 429.21 ($[M]^+$, 88), 431.09 (71), 451.15 ($[M-1+Na]^+$, 71), 453.04 ($[M+1+Na]^+$, 41).

3-(4-Chlorobenzyl)-5-{[5-(4-chlorobenzyl)-4-[(2-hydroxyphenylmethylene)amino]-4*H*-1,2,4-triazol-3-yl]methyl}-N-(2-hydroxyphenylmethylene)-4H-1,2,4-triazol-4-amine (8). A mixture of compound **7** (10 mmol) and salicyl aldehyde (20 mmol) was stirred in glacial acetic acid for 15 min at room temperature. Then the mixture was refluxed for 4 h. After the solvent was evaporated under reduced pressure a white solid appeared and was recrystallized from ethanol to give the desired product **8**. Yield 87 %, mp 207-208 °C. IR (KBr, υ, cm^{-1}): 3413 (2OH), 3043 (Ar CH), 1621, 1596, 1491 and 1452 (4C=N); Anal. Calcd. (%) for $C_{33}H_{26}N_8O_2Cl_2$: C, 62.17; H, 4.11; N, 17.58. Found: C, 62.15; H, 4.08; N, 17.55; ^{1}H-NMR (DMSO-d_6, δ ppm): 4.13 (4H, s, $2CH_2$), 4.44 (2H, s, CH_2), 6.83-6.96 (4H, m, arH), 7.16 (4H, d, arH, J = 8.2 Hz), 7.38 (4H, d, arH, J = 8.2 Hz), 7.42 (2H, t, arH, J = 7.4 Hz), 7.62 (2H, d, arH, J = 8.2 Hz), 8.78 (2H, s, 2CH), 10.37 (2H, s, 2OH); ^{13}C-NMR (DMSO-d_6, δ ppm): 29.57 ($2CH_2$), 38.45-40.17 (DMSO-d_6+CH_2), 116.47 (2CH), arC:[117.70 (2C), 119.33 (2CH), 127.90 (2CH), 128.24 (4CH), 130.27 (4CH), 131.22 (2C), 134.47 (2CH), 134.79 (2C), 146.44 (2C)], 149.25 (triazole 2C-3), 158.42 (triazole 2C-5), 163.31 (2CH). EI MS m/z (%): 104.98 (41), 134.92 (54), 152.99 (13), 169.00 (13), 209.95 (18), 349.18 (34), 351.18 (14), 399.07 (14), 518.17 (29), 540.17 (23), 542.17 (14), 637.21 ($[M]^+$, 59), 659.15 ($[M+1+Na]^+$, 81), 675.15 (100), 677.16 ($[M+1+K]^+$, 77).

***Tert*-butyl [3-(4-chlorobenzyl)-5-(cyanomethyl)-4*H*-1,2,4-triazol-4-yl]carbamate (9).** A mixture of compound **5** (10 mmol) and cyanoacethydrazide (10 mmol) was heated in an oil bath at 100 °C for 1.5 h. After the residue was cooled to room temperature, 2-3 mL of ethyl acetate-petroleum ether (1:2) was added and the mixture was kept overnight in the cold. The resulting solid separated was collected by filtration and recrystallized from ethyl acetate-petroleum ether (1:2) to yield compound **9**. Yield 72%, mp 105-106 °C. IR (KBr, υ, cm^{-1}): 3141 (NH), 2261 (CN), 1728 (C=O); Anal. Calcd. (%) for $C_{16}H_{18}N_5O_2Cl$: C, 55.25; H, 5.22; N, 20.14. Found: C, 55.23; H, 5.22; N, 20.11; ^{1}H-NMR (DMSO-d_6, δ ppm): 1.38 (9H, s, $3CH_3$), 4.01 (2H, s, CH_2), 4.19 (2H, s, CH_2), 7.20 (2H, d, arH, J =8.3 Hz), 7.39 (2H, d, arH, J = 8.3 Hz), 10.79 (1H, br, NH); ^{13}C-NMR (DMSO-d_6, δ ppm): 29.51 ($3CH_2$), 36.34 (CH_2), 38.55-41.07 (DMSO-d_6+ CH_2), 62.98 (CN), 74.98 (C), arC:[129.05 (2CH), 131.29 (2CH), 132.27 (C), 134.78 (C)], 151.30 (triazole C-3), 154.42 (triazole C-5), 170.43 (C); EI MS m/z (%): 153 (16), 229 (22), 248 (25), 294 (31), 314 (36), 316 (11), 348 ($[M]^+$, 19), 370 ($[M+Na]^+$).

[4-Amino-5-(4-chlorobenzyl)-4*H*-1,2,4-triazole-3-yl]acetonitrile (10). To a solution of compound **9** (10 mmol) in tetrahydrofuran was added 6N HCl (21.49 mL) and the mixture was refluxed in a water bath for 15 min. After the solvent was evaporated under reduced pressure a white solid appeared. The solid was added to a potassium carbonate solution (10 mmol) slowly and was stirred at room temperature for 30 min. The mixture was kept overnight in the cold, and the resulting solid separated was collected by filtration and recrystallized from ethanol-water (1:1). Yield 80%, mp 130-131 °C. IR (KBr, υ, cm^{-1}): 3235 and 3116 (NH_2), 2261 (CN); Anal. Calcd. (%) for $C_{11}H_{10}N_5Cl$: C, 53.34; H, 4.07; N, 28.26. Found: C, 53.29; H, 4.03; N, 28.24; ^{1}H-NMR (DMSO-d_6, δ ppm): 4.09 (2H, s, CH_2), 4.18 (2H, s, CH_2), 5.94 (2H, s, NH_2), 7.28-7.41 (4H, q, arH, J= 8.4 Hz); ^{13}C-NMR (DMSO-d_6, δ ppm): 14.56 (CH_2), 29.12 (CH_2), 116.19 (CN), arC: [128.77

(2CH), 131.11 (2CH), 131.80 (C), 135.46 (C)], 147.27 (triazole C-3), 155.18 (triazole C-5); EI MS m/z (%): 153 (44), 214 (41), 259 (17), 280 (34).

5-{[4-Amino-5-(4-chlorobenzyl)-4*H*-1,2,4-triazol-3-yl]methyl}-1,3,4-thiadiazol-2-amine (11).

Method 1: The mixture of compound **10** (10 mmol) and thiosemicarbazide (10 mmol) in tetrahydrofuran was refluxed in an oil bath at 80 °C for 9 h. After cooling to room temperature, the reaction mixture was neutralized with ammonia, and kept in cold overnight. The resulting solid separated was collected by filtration and recrystallized from acetone to afford the desired product.

Method 2: The mixture of compound **9** (10 mmol) and thiosemicarbazide (10 mmol) in tetrahydrofuran was refluxed in an oil bath at 80 °C for 9 h. After cooling to room temperature, the reaction mixture was neutralized with ammonia, and kept in the cold overnight. The solid formed was filtered and recrystallized from acetone to afford the desired product. Yield 79%, mp 247-248 °C. IR (KBr, υ, cm^{-1}): 3337 and 3261 (NH_2), 1631, 1607, 1518 and 1491 (4C=N); Anal. Calcd. (%) for $C_{12}H_{12}N_7SCl$: C, 44.79; H, 3.76; N, 30.47. Found: C, 44.75; H, 3.76; N, 30.46; ^{1}H-NMR (DMSO-d_6, δ ppm): 4.08 (2H, s, CH_2), 4.31 (2H, s, CH_2), 5.90 (2H, s, NH_2), 7.14 (2H, s, NH_2), 7.27-7.40 (4H, q, arH, J= 8.4 Hz); ^{13}C-NMR (DMSO-d_6, δ ppm): 25.34 (CH_2), 28.77 (CH_2), arC: [128.17 (2CH), 130.59 (2CH), 131.14 (C), 135.52 (C)], 151.24 (triazole C-3), 152.48 (thiadiazole C-2), 153.63 (triazole C-5), 169.21 (thiadiazole C-5); EI MS m/z (%): 306 (17), 322 ($[M]^+$, 100), 324 (41), 344 (31), 346 (11).

5-(4-Chlorobenzyl)-4-{[(2,6-dichlorophenyl)methylene]amino}-4*H*-1,2,4-triazol-3-yl)acetonitrile (12). To the solution of compound **10** (10 mmol) in glacial acetic acid was added 2,6-dichloro benzaldehyde (10 mmol) and the mixture was allowed to reflux for 3 h. The reaction solvent was evaporated under reduced pressure. Then 2-3 mL of ethanol was added to the obtained oily product and kept overnight in the cold. The resulting solid that separated was collected by filtration and recrystallized from acetone to yield the title compounds. Yield 83%, mp 209-210 °C. IR (KBr, υ, cm^{-1}): 2235 (CN); Anal. Calcd. (%) for $C_{18}H_{12}N_5Cl_3$: C, 53.42; H, 2.99; N, 17.31. Found: C, 53.41; H, 2.96; N, 17.34; ^{1}H-NMR (DMSO-d_6, δ ppm): 4.19 (2H, s, CH_2), 6.32 (2H, s, CH_2), 7.37 (4H, d, arH, J= 8.2 Hz), 7.41-7.59 (1H, t, arH, J= 7.9 Hz), 7.70 (2H, d, arH, J= 8.0 Hz), 8.56 (1H, s, CH); ^{12}C-NMR (DMSO-d_6, δ ppm): 29.23 (CH_2), 37.23-40.14 (DMSO-d_6+CH_2), 108.28 (CN), 113.91 (CH), arC:[126.84 (CH), 128.53 (2CH), 128.61 (2CH), 129.00 (2CH), 131.27 (CH), 132.033 (C), 133.16 (C), 135.46 (C), 143.16 (C)], 146.81 (triazol C-3), 157.22 (triazole C-5).

Antimicrobial activity

Antimicrobial activity assessment

All bacterial and yeast strains were obtained from the Re?k Saydam Hıfzıssıhha Institute (Ankara, Turkey) and were as follows: *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Yersinia pseudotuberculosis* ATCC 911, *Enterobacter aeruginosa* ATCC 13048, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* 702 Roma, *Mycobacterium smegmatis* ATCC607, *Candida albicans* ATCC 60193, and *Candida tropicalis* ATCC 13803. All the newly synthesized compounds were dissolved in dimethyl sulfoxide (DMSO) and ethanol to prepare chemicals of stock solution of 10 mg/1 mL.

Minimal inhibition concentration method

The antimicrobial effects of compounds **3a, 3b,** and **9-11** were tested quantitatively in respective broth media by using double dilution and the minimal inhibition concentration (MIC) values (μg/mL) were determined.[32] 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, Detroit, MI, USA) 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 drug, respectively. Dimethyl sulfoxide with dilution of 1:10 was used as solvent control. The results are shown in the Table.

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

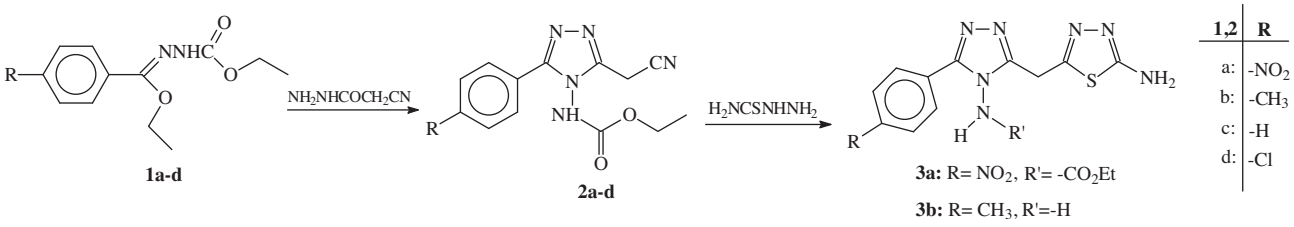
Comp. No.	Microorganisms and Minimal Inhibition Concentration Values										
	Ec	Ea	Yp	Kp	Pa	Sa	Ef	Bc	Ms	Ca	Ct
2a	> 500	> 500	> 500	125	> 500	> 500	> 500	> 500	> 500	> 500	> 500
2b	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500
2c	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500
3a	< 0.12	1.95	0.49	0.24	1.95	15.6	125	31.3	> 500	> 500	> 500
6	> 500	250	250	> 500	> 500	250	> 500	250	250	> 500	> 500
7	250	> 500	> 500	250	> 500	> 500	> 500	> 500	> 500	> 500	> 500
8	> 500	> 500	> 500	> 500	250	> 500	250	250	> 500	> 500	> 500
9	< 0.12	0.24	< 0.12	< 0.12	< 0.12	0.24	0.24	0.24	> 500	> 500	> 500
10	< 0.12	0.49	0.49	0.24	0.98	1.95	1.95	0.49	> 500	> 500	> 500
11	< 0.12	0.98	< 0.12	< 0.12	0.24	0.24	0.49	0.49	> 500	> 500	> 500
12	> 500	> 500	> 500	> 500	250	250	> 500	250	250	> 500	> 500
Amp.	10	10	> 18	> 18	> 18	35	10	15			
Strep.									35		
Flu.										25	25

Ec: *Escherichia coli* ATCC 25922, Yp: *Yersinia pseudotuberculosis* ATCC 911, Ea: *Enterobacter aeruginosa* ATCC 13048, Kp: *Klebsiella pneumoniae* ATCC 13883, 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, *Candida tropicalis* ATCC 13803, Amp.: Ampicillin, Flu.: Fluconazole, Strep.: Streptomycin.

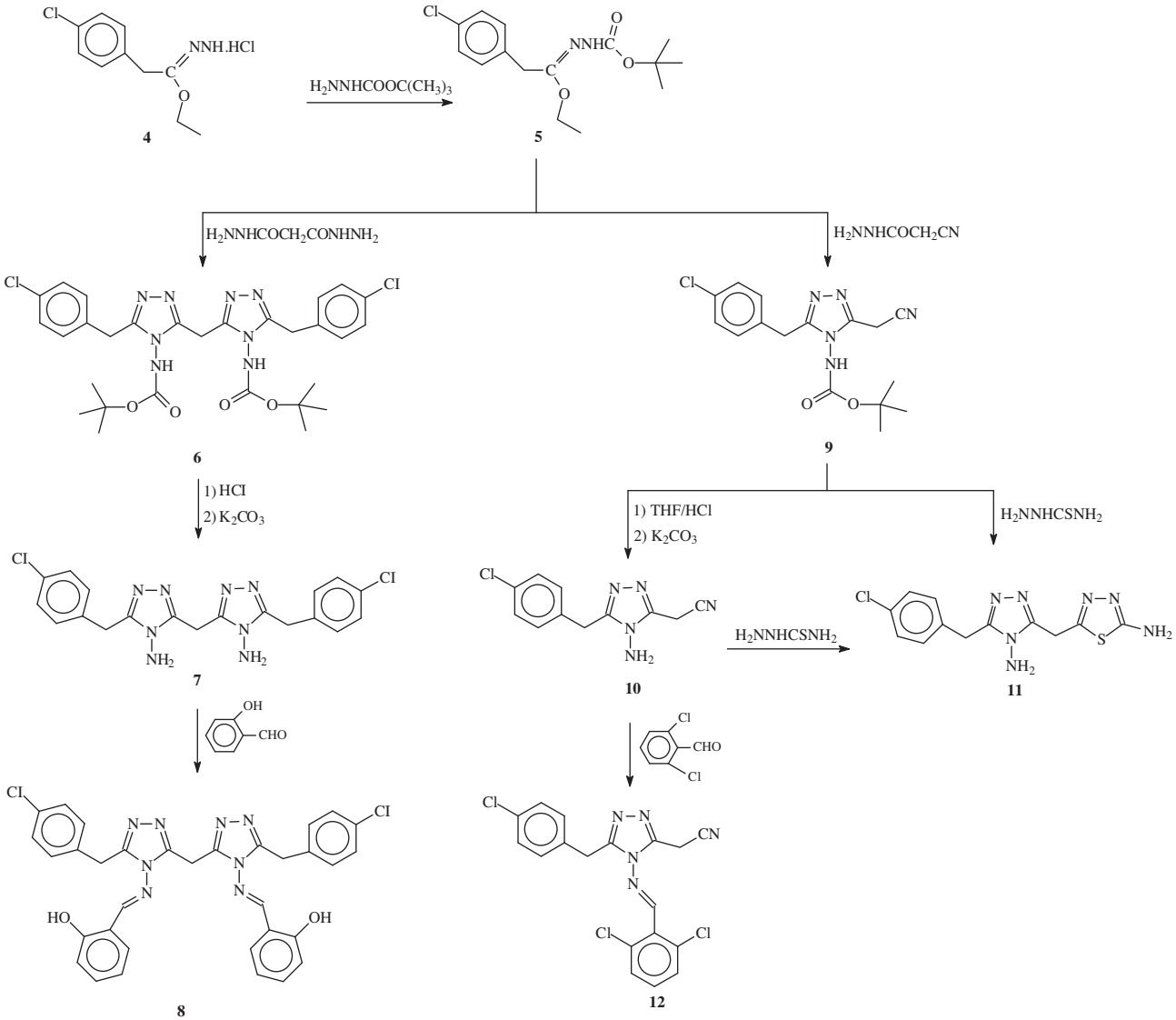
Results and discussion

The synthetic routes to obtain the designed compounds and their intermediates are described in Schemes 1 and 2. Compounds **1a-d** and **5** underwent nucleophilic attack by 2-cyanoacetohydrazide to give **2a-d** and **9**. The IR spectra of **2a-d, 9, 10,** and **12** exhibited a sharp peak between 2235 and 2262 cm^{-1} due to cyano absorption. In the ^{13}C-NMR spectra of these compounds, the cyano group was observed between 62.98 and 116.19 ppm.

Due to slight solubility in any NMR solvent of compounds **2c** and **2d**, satisfactory ^{13}C-NMR spectra of these compounds could not be obtained.



Scheme 1. Synthetic pathway for the preparation of compounds **2a-d** and **3a, 3b**.



Scheme 2. Synthetic pathway for the preparation of compounds **5-12**.

The cyclocondensation of compounds **2a, 2b,** and **9** with thiosemicarbazide was carried out by an analog way reported earlier.[33] This method containing the heating of the reaction mixture in trifluoroacetic acid at 60 °C for 10 h yielded compounds **3a, 3b,** and **11**, whereas the reactions of compounds **2c** and **2d** in the same conditions resulted in decomposition. Another way for the synthesis of compound **11** involved the reaction of compound **10** with thiosemicarbazide. In the ^{1}H-NMR spectra of compounds **3a** and **3b,** the signal was observed at 7.17 ppm (for **3a**) and 8.38 ppm (for **3b**), attributed to the amino group on position 5 of the 1,3,4-thiadiazole ring. The ^{1}H-NMR spectrum of compound **11**, 2 signals were recorded at 5.90 ppm and 7.14 ppm, which were checked by changing with D_2O. The one that appeared at the downfield region represents the $-NH_2$ group on the 1,3,4-thiadiazole ring, while the upfield one points to the amino group on the 1,2,4-triazole ring. In addition, the IR spectra of compounds **3a, 3b,** and **11** displayed no signal originating from the cyano group. Moreover, in the ^{13}C-NMR spectra of these compounds the signal derived from the cyano group disappeared, while additional signals belonging to thiadiazole C-2 and C-5 were recorded at 158.3 and 147.0-147.7 ppm, respectively. Furthermore, compounds **3a, 3b,** and **11** gave molecular ion peak and elemental analysis data consistent with the assigned structures.

Our efforts towards the synthesis of *tert*-butyl 3-({4-[(tert-butoxycarbonyl)amino]-5-(4-chlorobenzyl)-4*H*-1,2,4-triazol-3-yl}methyl)-5-(4-chlorobenzyl)-4H-1,2,4-triazol-4-ylcarbamate **(6)** involved the reaction of *tert*-butyl 2-[2-(4-chlorophenyl)-1-ethoxyethylidene]hydrazinecarboxylate (**5**) with malonohydrazide according to the method developed by us earlier.[34] In the ^{1}H-NMR spectrum of compound **6**, the signals derived from the *tert*-butoxy group were recorded at 1.35 ppm. This group resonated at 27.47 ppm in the ^{13}C-NMR spectrum. Moreover, the ^{1}H-NMR spectrum of compound **6** displayed a singlet at 10.75 ppm integrating for 2 protons, which points to the presence of 2 exocyclic -NH- groups (controlled with changing by D_2O) in the structure. Furthermore, the mass spectrum of **6,** as a typical example, contained a peak at m/z = 629.54 that agreed with the molecular weight of the molecular formula $C_{29}H_{34}O_4N_8Cl_2$ of the assigned structure. Moreover, compound **6** gave satisfactory elemental analysis data.

Compound **7** was prepared starting from compound **6** in 2 steps. In the first step, the hydrolysis of 2 *tert*-butoxy groups was achieved by the treatment of compound **6** with 6N HCl. However, the reaction produced the desired compound (**7**) as its hydrochloride, which was not isolated in the present study. In the second step, the treatment of the intermediate forming in the first step, with aqueous K_2CO_3, yielded the target amino compound, 3-{[4-amino-5-(4-chlorobenzyl)-4*H*-1,2,4-triazol-3-yl]methyl}-5-(4-chlorobenzyl)-4*H*-1,2,4-triazol-4-amine (**7**). The IR spectrum of **7** contained 2 absorption bands originated from 2 $-NH_2$ groups at 3252 and 3197 cm^{-1}. In the ^{1}H-NMR spectrum, a singlet signal belonging to the amino groups appeared at 5.97 ppm integrating for 4 protons (controlled with changing by D_2O), while no signal representing *tert*-butoxy- and exocyclic –NH- groups was present. In addition, mass spectrum containing a peak at m/z= 429 and elemental analysis data of compound **7** corresponded to the molecular weight of the molecular formula $C_{19}H_{18}N_8Cl_2$ of the assigned structure.

Compound **7** was treated with salicyl aldehyde in glacial acetic acid at room temperature to yield the corresponding Schiff base 3-(4-chlorobenzyl)-5-{[5-(4-chlorobenzyl)-4-[(2-hydroxyphenylmethylene)amino]-4*H*-1,2,4-triazol-3-yl]methyl}-*N*-(2-hydroxyphenylmethylene)-4*H*-1,2,4-triazol-4-amine (**8**). The structure of **8** was proven by IR, ^{1}H- and ^{13}C-NMR spectroscopic techniques and elemental analysis. The IR spectrum of compound **8** contained no peak corresponding to amino groups. Similarly, no signal was present in the ^{1}H-NMR

spectrum of compound **8** derived from the $-NH_2$ group. Instead, new signals due to 2-hydroxyphenylmethylene group were recorded at the related chemical shift values.

The treatment of compound **9** or **10**, prepared by an analogue way with compound **7**, with thiosemicarbazide produced compound **11**. Similar to compounds **3a** and **3b**, the ^{1}H-NMR spectrum of compound **11** exhibited 2 NH_2 signals at 5.90 ppm and 7.14 ppm (controlled by changing with D_2O) belonging to 1,2,4-triazole-NH_2 and 1,3,4-thiadiazole-NH_2, respectively. Furthermore, compound **11** displayed a mass spectrum containing a molecular ion peak and elemental analysis data consistent with the assigned structure.

The synthesis of compound **12** was performed by refluxing of **10** with 2,6-dichlorobenzaldehyde in ethanol. Compound **12** exhibited IR, ^{1}H-NMR, ^{13}C-NMR spectral data, and elemental analysis consistent with the proposed structure.

It was reported that the compounds having an arylidene-amino structure may exist as E/Z geometrical isomers about the –N=CH- double bond.[26,27,35,36] The literature survey revealed that compounds containing an imine bond may be present in higher percentages in dimethyl-d_6 sulfoxide solution in the form of geometrical E isomer about the –N=CH- double bond.[34] The Z isomer can be stabilized in less polar solvents by an intramolecular hydrogen bond. However, the ^{1}H- and ^{13}C-NMR spectra of compounds **8** and **12** showed the existence of only one isomer. It can be concluded that compounds **8** and **12** exist as their E geometrical isomers considering bulky arylidene substituent, which is in agreement with the literature.[37–43]

Antimicrobial activities of the newly synthesized compounds were determined by the minimal inhibition concentration method. Among ethyl [3-(cyanomethyl)-4H-1,2,4-triazol-4-yl]carbamates (**2a-d**), compound **2a** containing a 4-nitrophenyl group at the position 5 of the 1,2,4-triazole ring exhibited slight activity against *K. pneumonia* with the MIC value 125 μg/mL. The conversion of the cyano group into a 5-amino-1,3,4-thiadiazole ring (for compounds **3a, 3b**) greatly increased the antibacterial activity, while no antimicobacterial and antifungal activity was observed for these compounds.

Compound **6**, which is a carbamate derivative containing two 1,2,4-triazole rings linked to each other via a methylene linkage, was found to possess activity on *E. aeroginosa*, *Y. pseudotuberculosis*, *S. aureus*, *B. cereus*, and *M. smegmatis*. On the other hand, the replacement of the carbamate group by an amino group caused activity against only *E. coli* and *K. pneumonia*. In contrast to other newly synthesized compounds, compounds **6** and **12** were found to be active towards *M. smegmatis*. Nonpigmented rapidly growing mycobacteria (*M. smegmatis* is one of them) constitute a group of species of the genus *Mycobacterium* that share some characteristics. Due to differences between strains, these organisms require individualized treatment, which needs to be selected on the basis of results obtained from in vitro susceptibility tests. Compounds **9-11** exhibited good antibacterial activities, but no antimycobacterial and antifungal activity was observed for these compounds. In compound **12,** the conversion of amino function into 2,6-dichlorophenylmethylenamino moiety greatly decreased the antimicrobial effect; on the other hand, this conversion caused slight activity for compound **12** towards *M. smegmatis.*

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