Turk J Chem
34 (2010) , 347 – 358.

doi:10.3906/kim-0911-10

Synthesis and antimicrobial activities of 2-(5-mercapto)-1,3-oxadiazol-2-ylmethyl-1,2,4-triazol-3-one derivatives

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Received 04.11.2009

The synthesis of 4-amino-2-[(5-mercapto-1,3,4-oxadiazol-2-yl)methyl]-5-(4-methylphenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**2**) and 4-amino-2-[(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl)methyl]-5-(4-methylphenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**5**) was performed starting from 2-[4-amino-3-(4-methylphenyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl]acetohydrazide (**1**). The treatment of **2** and **5** with 4-fluorobenzaldehyde (for **8**) or salicylaldehyde (for **6**) afforded the corresponding Schiff bases (**6** and **8**). The condensation of **5** with phenacyl bromide produced 4-amino-5-(4-methylphenyl)-2-[(6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)methyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**7**). The alkylation of **2** and **8** was carried out by using methyl iodide in basic media.

Compounds **3** and **10a,b** were prepared by aminoalkylation of **2** and **8** with 4-fluorophenyl piperazine, morpholine, or 4-methyl piperazine in the presence of formaldehyde.

The synthesized compounds were screened for their antimicrobial activities and, with the exception of **2, 4, 7**, and **9**, were found to possess good or moderate activities against the screened bacterial strains except *Candida tropicalis* and *Candida albicans*.

Key Words: 1,2,4-Triazole, 1,3,4-oxadiazole, Mannich base, antimicrobial activity.

Introduction

The treatment of infectious diseases remains an important and challenging problem due to a combination of factors including emerging infectious diseases and the increasing number of multidrug resistant microbial

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pathogens. In spite of the wide range of antimicrobial drugs with different mechanisms of action used to treat microbial infections, either alone or in combination, and the existence many compounds used in different phases of clinic trials, microbial infections are becoming a worldwide problem. There is already evidence that antimicrobial resistance is associated with an increase in mortality. The problem with clinically used drugs is not only the increasing microbial resistance, but also that they are accompanied by toxic side effects that are often dose limiting.[1−5] Due to these reasons, it is important to search for and synthesize new classes of compounds that preferably consist of chemical characteristics that clearly differ from those of existing agents.

Many compounds consisting of 5-membered heterocyclic rings represent important building blocks in organic and medicinal chemistry. In addition, they are interesting in their own right, due to their pharmacological properties. Therefore, the synthesis of new derivatives of triazoles, oxadiazoles, and thiadiazoles has been attracting considerable attention for the past few decades.[6−19] Among the heterocycles, the 1,2,4-triazole nucleus has been incorporated into a wide variety of therapeutically important agents. Ribavirin, Rizatriptan, and Alprazolam are the best examples of drugs containing 1,2,4-triazole moiety (Chart 1).[11−13]Some of the other drugs being used for the treatment of fungal disease and including a 1,2,4-triazole ring in their structures are Ravuconazole, Fluconazole, Posaconazole, Voriconazole, and Itraconazole (Chart 1).[14−17] Furthermore, Mannich bases of 1,2,4-triazole derivatives have been reported to possess protozocidal and antibacterial activity. Drugs such as Prazosin, Lidoflazin, and Urapidil carrying a piperazine nucleus have been used as good cardiovascular agents (Chart 2).[17] Furthermore, compounds containing a 1,3,4-oxadiazole ring have also been reported to possess a broad spectrum of biological activities including insecticidal, antibacterial, anticancer, and anti-inflammatory activities.[18−20]

In addition, several compounds that contain a piperazine or morpholine nucleus with antimicrobial activity have been synthesized; some of which contain an azole ring as well. For instance, while Eperezolide, which is a member of the oxazolidinone class of antibiotics, consists of morpholine and oxazolidinone rings linked each other via a fluorophenylene linkage, another antibiotic, Linezolide, contains a piperazine ring instead of morpholine.[21] Some Mannich bases derived from 1,2,4-triazole derivatives carrying a methyl piperazine or morpholine ring have been described as possessing protozocidal, antimicrobial, or anticancer activities.[17−22]

As part of our continuing study on the synthesis of antitumoral and antimicrobial compounds, we reported a series of Schiff base derivatives containing 1,2,4-triazol-5-one ring as antitumor agents.[10,23−27] In the library screening of our new compounds as antimicrobial assay system, several biheterocyclic compounds consisting of 1,2,4-triazol-5-one and 1,3,4-thiadiazole rings provided inhibitory activity on tested microorganisms.[10] Moreover, we have reported some compounds consisting of a triazolothiadiazine or triazolothiadiazole ring as antimicrobial agents.[28] According to these observations, in the design of new bioactive compounds, the development of hybrid molecules through the combination of different pharmacophores in one frame may lead to compounds with interesting biological profiles.

In the present study, prompted by these observations, the synthesis and antimicrobial screening of new 1,2,4-triazole derivatives incorporating different pharmacophores as hybrid molecules possessing antimicrobial activity were aimed.

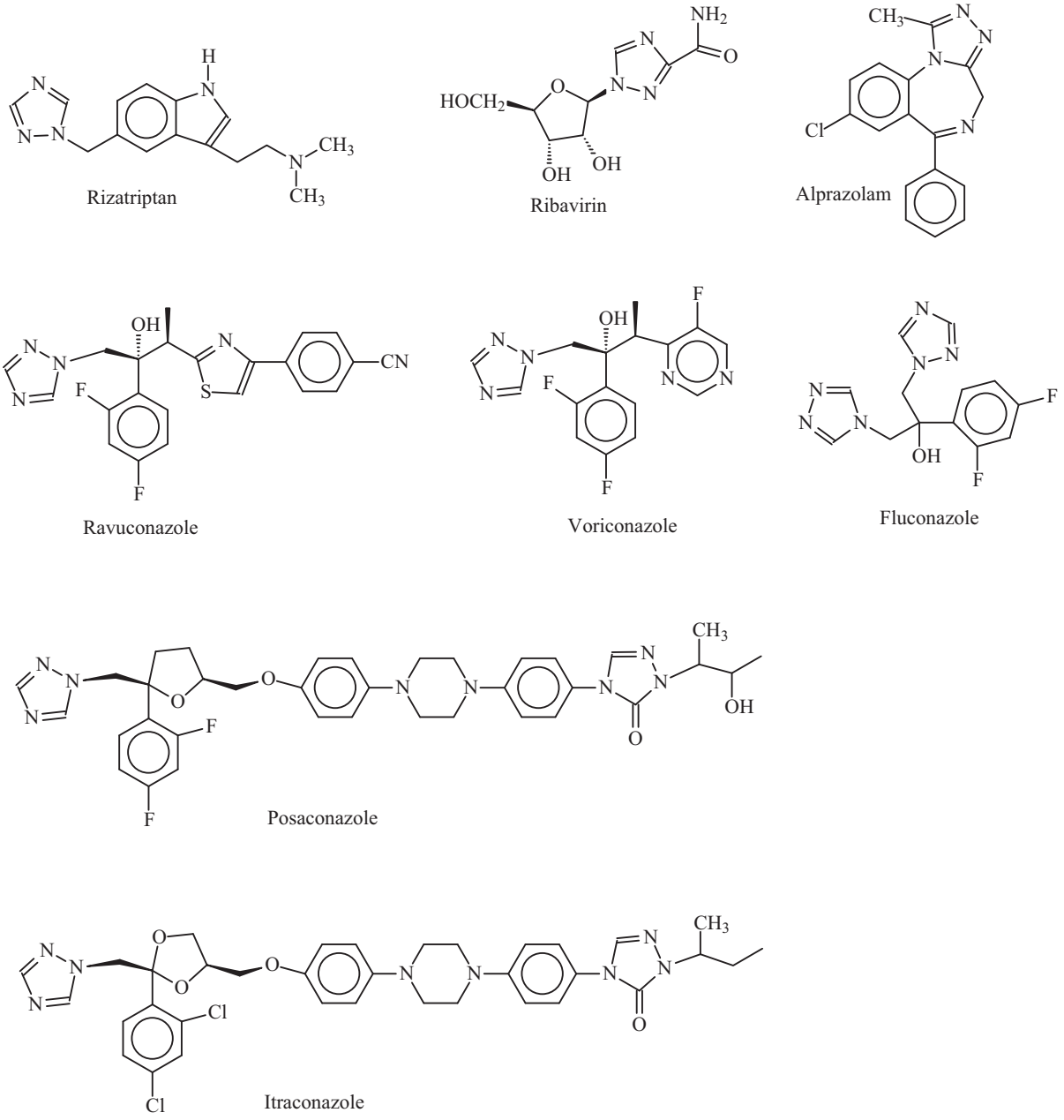
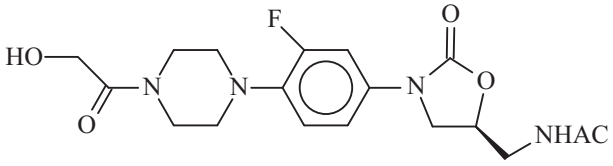


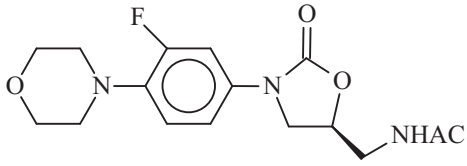
Chart 1.

Experimental

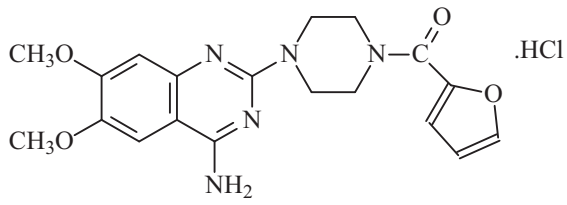
Melting points were determined on a Büchi B-540 melting point apparatus and are uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian-Mercury 200 MHz spectrometer. The IR spectra were measured on potassium bromide pellets using a Perkin-Elmer 1600 series FTIR spectrometer. Combustion analysis was performed on a Costech Elemental Combustion System CHNS-O elemental analyzer. All the chemicals were



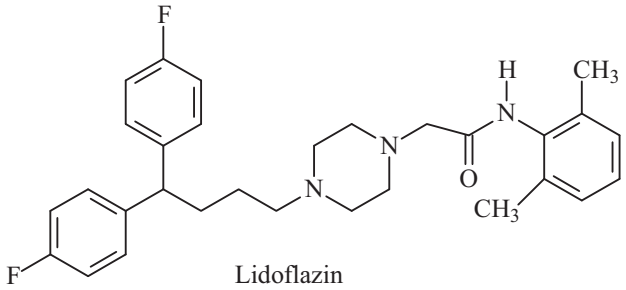
Eperezolid



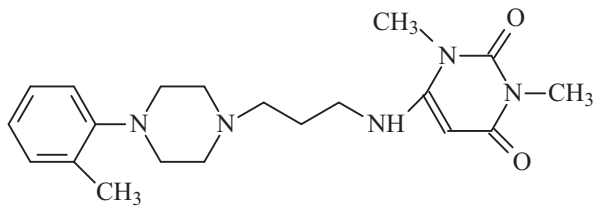
Linezolid



Prazosin



Lidoflazin



Urapidil

Chart 2.

obtained from Fluka Chemie AG Buchs (Switzerland). Compound **1** was obtained in the way reported earlier.[10]

General Method for the Synthesis of 4-Amino-2-[(5-mercapto-1,3,4-oxadiazol-2-yl)methyl]-5-(4-methyl phenyl)-2,4-dihydro-3H-1,2,4-triazol-3-one (2). 2-[4-amino-3-(4-methylphenyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl]acetohydrazide (**1**) (0.01 mol) and CS_2 (0.60 mL, 0.01 mol) were added to a solution of KOH (0.56 g, 0.01 mol) in 50 mL of H_2O and 50 mL of ethanol. The reaction mixture was refluxed for 3 h. After evaporating the solvents under reduced pressure to dryness, a solid was obtained. It was dissolved in 300 mL of H_2O and acidified with conc. HCl. The precipitate was filtered off, washed with H_2O, and recrystallized from ethanol to afford the desired compound. Yield 72%, mp 225-227 °C; IR (KBr, υ, cm^{-1}): 3323 and 3213 (NH_2), 2750 (SH), 1676 (C=O), 1519, 1505 and 1601 (C=N); *Anal.* Calcd (%) for $C_{12}H_{12}N_6O_2S$: C, 47.36; H, 3.97; N, 27.62. Found; C, 47.56; H, 4.02; N, 27.43; ^{1}H-NMR (DMSO-$d_6\delta$ ppm): 2.35 (3H, s, CH_3), 5.12 (2H, s, CH_2), 5.56 (2H, bs, NH_2), 7.30 (2H, d, j=8.0 Hz, arH), 7.88 (2H, d, j=8.0 Hz, arH), 14.65 (H, bs, SH); ^{13}C-NMR (DMSO-$d_6\delta$ ppm): 20.81 (CH_3), 40.58-38.48 (CH_2 + DMSO d_6), arC:

[123.23 (C), 127.45 (2CH), 128.78 (2CH), 139.88 (C)], 145.73 (C-3, triazole), 153.06 (C-5, triazole), 158.85 (C-2, oxadiazole), 177.86 (C-5, oxadiazole).

General method for the synthesis of compounds 6 and 8

Salicyl aldehyde (for **6**) or 4-fluorobenzaldehyde (for **8**) (10 mmol) was added to the solution of the corresponding compound **2** or **5** (10 mmol) in absolute ethanol, and the mixture was heated until a clear solution was obtained. Then a few drops of concentrated sulfuric acid were added as a catalyst and the solution was allowed to reflux for 3-4 h. On cooling of the reaction mixture to room temperature, a solid appeared. The product was filtered off and recrystallized from dimethylsulfoxide-water (1:1) to obtain the desired compound.

4-{[(2-Hydroxyphenyl)methylene]amino}-2-[(4-{[(2-hydroxyphenyl)methylene] amino}-5-mercapto-4*H*-1,2,4-triazol-3-yl)methyl]-5-(4-methylphenyl)-2,4-dihydro-3H-1,2,4-triazol-3-one (6). Yield: 94%; mp 238-239 °C; IR (KBr , υ, cm^{-1}): 3414 (2OH), 2712 (SH), 1713 (C=O), 1620, 1603 and 1582 (3C=N), *Anal.* Calcd (%) for $C_{26}H_{22}N_8O_3S$: C, 59.30; H, 4.21, N, 21.28. Found: C, 59.46; H, 4.28; N, 21.06; ^{1}H-NMR (DMSO-d_6 δ ppm): 2.36 (3H, s, CH_3), 4.41 (2H, s, NCH_2), 6.72-6.99 (4H, m, arH), 7.29-7.42 (3H, m, arH), 7.63-7.68 (3H, m, arH), 7.87 (2H, m, arH), 9.84 (1H, s, N=CH), 10.08 (1H, s, N=CH), 10.38 (2H, s, 2OH), 14.07 (1H, s, SH).

4-{[(4-Fluorophenyl)methylene]amino}-2-[(5-mercapto-1,3,4-oxadiazol-2-yl)methyl]-5-(4-methylphenyl)-2,4-dihydro-3H-1,2,4-triazol-3-one (8). Yield: 87%; mp 224-225 °C; IR (KBr, υ, cm^{-1}): 2723 (SH), 1696 (C=O), 1613, 1601 and 1508 (3C=N); *Anal.* Calcd (%) for $C_{19}H_{15}FN_6O_2S$: C, 55.60; H, 3.68, N, 20.48. Found: C, 55.76; H, 3.73; N, 20.46; ^{1}H-NMR (DMSO-d_6 δ ppm): 2.12 (3H, s, CH_3), 4.72 (2H, s, NCH_2), 7.30-7.38 (4H, m, arH), 7.58 (2H, d, j=8.0 Hz, arH), 8.49 (2H, d, j=8.0 Hz), 9.03 (1H, s, N=CH), 13.28 (1H, bs, SH); ^{13}C-NMR (DMSO-d_6 δ ppm): 20.45 (CH_3), 40.53-38.66 (CH_2 + DMSO-d_6), arC: [114.93 and 114.24 (2<u>CH</u>-C-F), 158.15 and 162.47 (<u>C</u>-F), 124.17 (2CH), 125.27 (2CH), 133.02 (2CH), 128.98 (C), 139.84 (C), 145.12 (C)], 136.42 (N=CH), 143.65 (C-3, triazole), 155.83 (C-5, triazole), 159.43 (C-2, oxadiazole), 178.17 (C-5, oxadiazole).

General method for the synthesis of compounds 3 and 10a,b

Into a solution of the corresponding compound **2** or **8** (10 mmol) in dimethyl formamide were added 4-(4-fluorophenylpiperazine (for **3**), morpholine (for **10a**) or methylpiperazine (for **10b**) (10 mmol), and formaldehyde (40%, 1.5 mL) and the mixture was stirred at room temperature for 2 h. Then distilled water was added and the resulting solution was kept overnight in the cold. The separated solid was collected by filtration and recrystallized from ethanol to yield the target compounds.

4-Amino-5-(4-methyphenyl)-2-[(4-{[4-(4-fluorophenyl)piperazin-1-yl]methyl}-5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl}methyl)-2,4-dihydro-3H-1,2,4-triazol-3-one (3). Yield: 64%; mp 142-144 °C (from butyl acetate–petroleum ether 1:1); IR (KBr, υ, cm^{-1}): 3413 and 3230 (NH_2), 1710 (C=O), 1509 and 1617 (C=N), 1162 (C=S); *Anal.* Calcd (%) for $C_{23}H_{25}FN_8O_2S$: C, 55.63; H, 5.07, N, 22.57. Found: C, 55.81; H, 5.13; N, 22.44; ^{1}H-NMR (DMSO-d_6 δ ppm): 2.07 (3H, s, CH_3), 2.45-2.58 (4H, m, morpholine-2CH_2), 3.71-3,86 (4H, m, morpholine-2CH_2), 4.72 (2H, s, NCH_2), 5.28 (2H, N<u>CH_2</u>NH), 5.37 (2H, s, NH_2), 6.79 (2H, d, arH, j= 8.8 Hz), 6.94 (2H, d, arH, j= 8.8 Hz), 7.19 (2H, d, arH, j= 8.0 Hz), 8.64 (2H, d,

arH, j=8.0 Hz); ^{13}C-NMR (DMSO-$d_6\delta$ ppm): 21.07 (CH_3), 44.63 (NCH_2), 48.24 (morpholine $2CH_2$), 55.76 (morpholine $2CH_2$), 66.26 (NCH_2N), arC: [114.97 and 114.24 (2CH-C-F), 158.13 and 162.46 (C-F), 124.14 (2CH), 124.73 (2CH), 125.64 (C), 128.26 (CH), 136.79 (CH), 140.13 (C), 145.12 (C)], 144.16 (C-3, triazole), 152.61 (C-5, triazole), 160.07 (C-2, oxadiazole), 177.38 (C-5, oxadiazole).

4-{[(4-Fluorophenyl)methylene]amino}-5-(4-methylphenyl)-2-{[4-(morpholin-4-ylmethyl)-5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl]methyl}-2,4-dihydro-3H-1,2,4-triazol-3-one (10a). Yield: 78%, mp 217-220 °C (from ethanol); IR (KBr, υ, cm^{-1}): 1713 (C=O), 1600 and 1508 (2C=N), 1328 (C=S); *Anal.* Calcd (%) for $C_{24}H_{24}FN_7O_3S$: C, 56.57; H, 4.75, N, 19.24. Found: C, 56.58; H, 4.70; N, 19.22; ^{1}H-NMR (DMSO-d_6)δ ppm: 2.39 (3H, s, CH_3), 3.21-3.58 (8H, m, morpholine-$4CH_2$), 4.96 (2H, s, CH_2), 5.22 (2H, s, CH_2), 7.26 (2H, d, arH, j=9.4 Hz), 7.75-7.91 (6H, m, arH), 9.65 (1H, s, N=CH); ^{13}C-NMR (DMSO-d_6)δ ppm: 20.18 (CH_3), 44.23 (NCH_2), 48.24 (morpholine $2CH_2$), 55.76 (morpholine $2CH_2$), 65.68 (NCH_2N), arC: [115.04 and 115.24 (2CH-C-F), 158.10 and 162.73 (C-F), 123.18 (2CH), 125.33 (2CH), 125.62 (C), 129.37 (CH), 137.41 (CH), 139.82 (C), 145.18 (C)], 129.71 (N=CH), 145.06 (C-3, triazole), 153.15 (C-5, triazole), 163.36 (C-2, oxadiazole), 178.13 (C-5, oxadiazole).

4-{[(4-Fluorophenyl)methylene]amino}-5-(4-methylphenyl)-2-({4-[(4-methyl piperazin-1-yl) methyl]-5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl]methyl}-2,4-dihydro-3H-1,2,4-triazol-3-one (10b). Yield: 72%, mp 157-159 °C (from ethanol); IR (KBr, υ, cm^{-1}): 1717 (C=O), 1624, 1601 and 1585 (C=N), 1223 (C=S); *Anal.* Calcd (%) for $C_{25}H_{27}FN_8O_2S$: C, 57.46; H, 5.21, N, 21.44. Found: C, 57.61; H, 5.26; N, 21.37; ^{1}H-NMR (DMSO-$d_6\delta$ ppm): 2.23 (3H, s, CH_3), 2.41 (8H, bs, morpholine-$4CH_2$), 2.75 (3H, s, CH_3), 4.96 (2H, s, CH_2), 5.23 (2H, s, CH_2), 7.30-7.36 (4H, m, arH), 7.78 (2H, d, j= 8.4 Hz, arH), 7.91 (2H, t, arH, j= 5.2 Hz), 9.64 (1H, s, N=CH); ^{13}C-NMR (DMSO-$d_6\delta$ ppm): 21.34 (CH_3), 23.20 (N-CH_3) 46.28 (NCH_2), 51.97 (morpholine $2CH_2$), 54.42 (morpholine $2CH_2$), 67.48 (NCH_2N), arC: [158.65 and 165.24 (C-F), 115.44 and 116.28 (2CH-C-F), 129.10 (CH), 129.70 (CH), 128.58 (C), 124.48 (2CH), 125.64 (C), 136.50 (2CH), 141.37 (C)], 136.71 (N=CH), 142.12 (C-3, triazole), 157.18 (C-5, triazole), 163.96 (C-2, oxadiazole), 180.17 (C-5, oxadiazole).

General method for the synthesis of compounds 4 and 9

Into a solution of the corresponding compound **2** or **8** (10 mmol) in absolute ethanol was added an equivalent amount of methyl iodide, and the reaction mixture was refluxed in the presence of sodium ethoxide (10 mmol) for 3-4 h (the completion of the reaction was checked with TLC). After the removal of the reaction solvent under reduced pressure, a solid was obtained. It was recrystallized from ethyl acetate-petroleum ether (comp. **4**) or ethanol (comp. **9**) to obtain the desired compound.

4-Amino-5-(4-methylphenyl)-2-{[5-(methylthio)-1,3,4-oxadiazol-2-yl]methyl}-2,4-dihydro-3H-1,2,4-triazol-3-one (4). Yield: 74%, mp 143-145 °C, IR (KBr, υ, cm^{-1}): 3326 and 3202 (NH_2), 1721 (C=O), 1629, 1584 and 1507 (C=N), *Anal.* Calcd (%) for $C_{13}H_{14}N_6O_2S$: C, 49.05; H, 4.43, N, 26.40. Found; C, 49.21; H, 4.48; N, 26.24; ^{1}H-NMR (DMSO-$d_6\delta$ ppm): 2.02 (3H, s, CH_3), 2.52 (3H, s, CH_3), 4.70 (2H, s, NCH_2), 5.33 (2H, s, NH_2), 7.23 (2H, d, j= 8.0 Hz, arH), 7.98 (2H, d, j=8.0 Hz, arH); ^{13}C-NMR (DMSO-$d_6\delta$ ppm): 20.45 (CH_3), 22.18 (CH_3), 44.27 (NCH_2 + DMSO-d_6), arC: [125.16 (2CH), 127.66 (C), 134.40 (2CH), 139.84 (C)], 142.94 (C-3, triazole), 154.73 (C-5, triazole), 162.43 (C-2, oxadiazole), 181.05 (C-5, oxadiazole).

4-{[(4-Fluorophenyl)methylene]amino}-5-(4-methylphenyl)-2-{[5-(methylthio)-1,3,4-oxa-

diazol-2-yl]methyl}-2,4-dihydro-3H-1,2,4-triazol-3-one (9). Yield: 71%, mp 175-177 °C, IR (KBr, υ, cm^{-1}): 1715 (C=O), 1601, 1518 and 1509 (3C=N); *Anal.* Calcd (%) for $C_{20}H_{17}FN_6O_2S$: C, 56.59; H, 4.04; N, 19.80. Found; C, 56.71; H, 4.12; N, 19.74; ^{1}H-NMR (DMSO-$d_6\delta$ ppm): 2.41 (3H, s, CH_3), 2.73 (3H, s, CH_3), 5.31 (2H, s, CH_2), 7.21-7.33 (4H, m, arH), 7.76-7.91 (4H, m, arH), 9.67 (1H, s, N=CH); ^{13}C-NMR (DMSO-$d_6\delta$ ppm): 20.22 (CH_3), 24.76(S -CH_3) 46.67 (NCH_2), arC: [115.12 and 115.47 (2CH-C-F), 157.12 and 162.34 (C-F), 123.43 (2CH), 125.33 (2CH), 125.65 (C), 129.23 (CH), 138.73 (CH), 138.57 (C), 145.36 (C)], 130.53 (N=CH), 144.25 (C-3, triazole), 154.57 (C-5, triazole), 163.67 (C-2, oxadiazole), 178.69 (C-5, oxadiazole).

Synthesis of 4-Amino-2-[(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)methyl]-5-(4-methylphenyl)-2,4-dihydro-3H-1,2,4-triazol-3-one (5). Method 1: To a solution of the compound **2** (10 mmol) in 1-butanol was added hydrazine hydrate and the reaction mixture was allowed to reflux for 5 h (completion of the reaction was checked with TLC). After cooling of the reaction mixture in the cold, a solid appeared. This was recrystallized from ethanol to obtain the desired compound.

Method 2: A solution of compound **1** (10 mmol) was treated with a solution of potassium hydroxide (15 mmol) dissolved in absolute alcohol at 0-5 °C by stirring. Then 15 mmol of carbon disulfide was added slowly and the reaction mixture was stirred overnight at room temperature. The separated solid product was filtered, washed with cold absolute ethanol, and finally dried. It was directly used for the next step without purification. The solid product was mixed with a mixture of water (80 mL) and hydrazine hydrate (20 mmol) and refluxed for 5 h. During the progress of the reaction, the reaction mixture turned green with evolution of hydrogen sulfide and finally it became homogeneous. It was diluted with a small amount of cold water and then treated with concentrated hydrochloric acid. The white precipitate was filtered, washed with cold water, and recrystallized from dimethyl sulfoxide-water (1/1). Yield: 77%, mp 227-228 °C, IR (KBr, υ, cm^{-1}): 3301-3170 ($2NH_2$), 2747 (SH), 1700 (C=O), 1614, 1577 and 1535 (3C=N); *Anal.* Calcd (%) for $C_{12}H_{14}N_8OS$: C, 45.27; H, 4.43; N, 35.20. Found; C, 45.41; H, 4.52; N, 35.14; ^{1}H-NMR (DMSO-$d_6\delta$ ppm): 2.40 (3H, s, CH_3), 4.87 (2H, s, NCH_2), 5.33 (4H, brs, $2NH_2$), 7.33 (2H, d, arH, j=8.0 Hz), 7.82 (2H, d, arH, j=8.0 Hz), 13.70 (1H, s, SH); ^{13}C-NMR (DMSO-$d_6\delta$ ppm): 20.27 (CH_3), 45.64 (NCH_2), arC: [125.47 (2CH), 126.96 (C), 135.12 (2CH), 139.67 (C)], 142.94 (C-3, triazole), 154.73 (C-5, triazole), 154.76 (C-2, triazole), 155.07 (C-5, triazole).

4-Amino-5-(4-methylphenyl)-2-[(6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadia-zin-3-yl) methyl]-2,4-dihydro-3H-1,2,4-triazol-3-one (7). A mixture of compound **5** (10 mmol) and phenacyl bromide (12 mmol) in absolute ethanol was refluxed for 6 h. The reaction mixture was slowly quenched with crushed ice by stirring and was neutralized with solid K_2CO_3. The solid that separated after standing overnight was filtered, washed with cold water, dried and recrystallized from dimethyl sulfoxide-water (1:1) to afford the desired compound. Yield 98%, mp 262-263 °C; IR (KBr, υ, cm^{-1}): 3277-3200 (NH_2), 1708 (C=O), 1644, 1506 and 1463 (4C=N); *Anal.* Calcd (%) for $C_{20}H_{18}N_8OS$: C, 57.40; H, 4.34; N, 26.78. Found; C, 57.41; H, 4.42; N, 26.54; ^{1}H-NMR (DMSO-$d_6\delta$ ppm): 2.34 (3H, s, CH_3), 4.42 (2H, s, CH_2), 5.33 (2H, s, NH_2), 5.58 (2H, bs,SCH_2), 7.27 (2H, d, j=7.8 Hz, arH), 7.54-7.61 (3H, m, arH), 7.87 (2H, j=7.8 Hz, arH), 8.06 (2H, d, j=7.4 Hz, arH); ^{13}C-NMR (DMSO-$d_6\delta$ ppm): 21.73 (CH_3), 33.39 (CH_2, thiadiazine) 47.43 (NCH_2), arC: [125.69 (2CH), 129.79 (2CH), 130.16 (C), 131.37 (3CH), 133.22 (2CH), 140.34 (C), 141.07 (C)], 148.63 (C-8a, triazolothiadiazine), 152.78 (C-3, triazole), 154.49 (C-5, triazole), 159.24 (C-3, triazolothiadiazine), 161.17 (C-6, triazolothiadiazine).

Microbiology

Antimicrobial activity assessment

All test microorganisms, gram negative bacteria: *Escherichia coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 13883, *Yersinia pseudotuberculosis* ATCC 911, *Enterobacter aerogenes* ATCC 13048, *Pseudomonas aeruginosa* ATCC 10145; gram positive bacteria: *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* 709 Roma, and yeast-like fungi: *Candida tropicalis* ATCC 13803, *Candida glabrata* 66032, and *Candida albicans* ATCC 60193) were obtained from the Refik Saydam Hifzissihha Institute (Ankara, Turkey). All 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.[30] 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) and fluconazole (5 μg) were used as standard antibacterial and antifungal drugs, respectively. Dimethylsulphoxide at a dilution of 1:10 was used as solvent control. The results are shown in the Table.

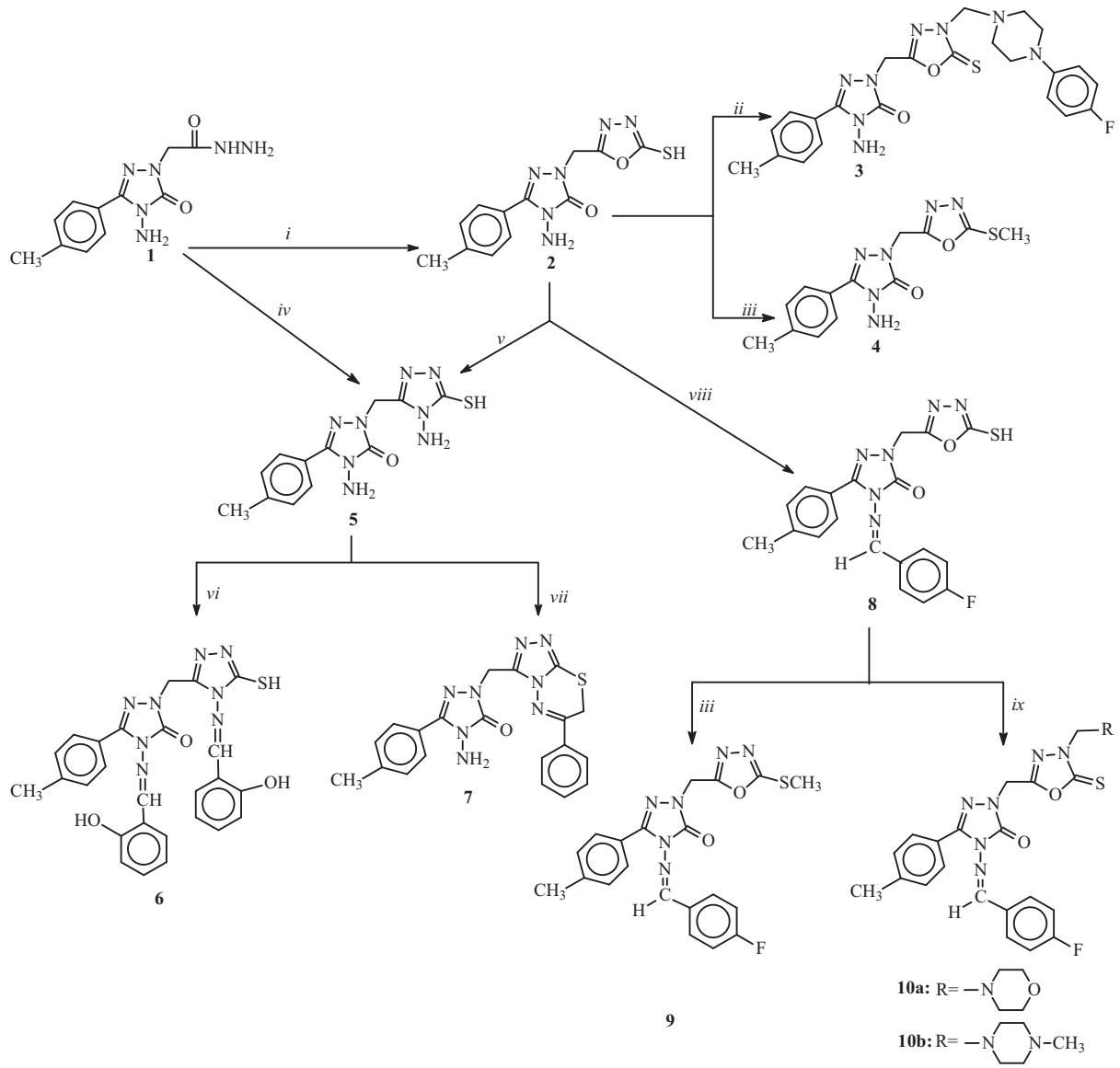
Table. Antimicrobial activities of the newly synthesized compounds (μg/mL).

Comp.	Microorganisms and Minimal Inhibition Concentration Values (μg/mL)									
No.	Ec	Kp	Yp	En	Pa	Sa	Ef	Bc	Ca	Ct
2[a]	> 500	> 500	> 500	> 500	> 500	> 500	> 500	500	> 500	> 500
3[a]	< 0.12	< 0.12	0.24	0.49	0.98	0.49	0.98	0.49	> 500	> 500
4[a]	> 500	> 500	> 500	125	> 500	125	125	> 500	> 500	> 500
5[a]	< 0.12	0.24	< 0.12	0.49	0.98	1.95	1.95	1.95	> 500	> 500
6[a]	< 0.12	< 0.12	< 0.12	0.24	0.98	0.49	0.98	0.49	> 500	> 500
7[a]	> 500	> 500	> 500	125	> 500	125	125	> 500	> 500	> 500
8[b]	125	125	125	125	125	31.3	31.3	3.9	> 500	> 500
9[a]	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500
10a[a]	125	125	125	125	125	62.5	62.5	15.6	> 500	> 500
10b[a]	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	> 500	> 500
Amp.	10	> 128	18	> 128	18	35	10	15		
Flu.									< 1	8

Ec: *Escherichia coli* ATCC 25922, Yp: *Yersinia pseudotuberculosis* ATCC 911, Pa: *Pseudomonas aeruginosa* ATCC 27853, Ef: *Enterococcus faecalis* ATCC 29212, Sa: *Staphylococcus aureus* ATCC 25923, Kp: *Klebsiella pneumoniae* ATCC 13883, En: *Enterobacter aerogenes* ATCC 13048; Bc: *Bacillus cereus* 702 Roma, Ct: *Candida tropicalis* ATCC 13803, Ca: *Candida albicans* ATCC 60193, [a]: solvent is ethanol, [b]: solvent is dimethyl sulfoxide, Amp: Ampicillin, Flu: Fluconazole .

Results and discussion

In the present study, a series of 10 compounds was synthesized. The Scheme illustrates the method used for the preparation of target compounds. The structures of all the newly synthesized compounds were elucidated by IR, ^{1}H-NMR, and ^{13}C-NMR spectroscopic methods and elemental analysis. In the IR spectra of all compounds C=N bands were observed at about 1629-1463 cm^{-1}. According to the IR spectroscopic data of compounds **2**, **5**, **6**, and **8**, which have a mercapto structure, the observation of -SH function at 2750-2723 and the absence of an absorption at about 1300-1100 cm^{-1} region cited for the C=S stretching band showed that these compounds were in mercapto form.



Scheme. *i*= CS_2/KOH, *ii*= HCHO and 4-aminomorpholine or 1-(4-fluorophenyl)piperazyne, *iii*= 1) CS_2/KOH, room temp. 2) H_2NNH_2, *v*= H_2NNH_2 (reflux), *vi*= salicyl aldehyde, *vii*= phenacylbromide, *viii*= $CHO_2H_4F(p\text{-})$, *ix*= HCHO and morpholine or 4-methyl piperazyne.

The treatment of hydrazides with CS_2 in the presence of KOH is a general method leading to the formation of 5-thioxo-1,3,4-oxadiazole derivatives.[28,29] As the starting material 2-[4-amino-3-(4-methylphenyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl]aceto-hydrazide (**1**) was used to produce synthesis of 4-amino-2-[(5-mercapto-1,3,4-oxadiazol-2-yl)methyl]-5-(4-methyl phenyl)-2,4-dihydro-3H-1,2,4-triazol-3-one (**2**) (Scheme). In contrast to compound **1**, the ^{1}H-NMR spectrum of compound **2** displayed a signal at 1.65 ppm belonging to the –SH group, while the -$NHNH_2$ signals disappeared. Compound **5** was synthesized starting from compound **1** or **2**. The IR spectra of compound **5** showed 2 sharp absorption bands at 3301 and 3170 cm^{-1} representing 2 –NH_2 groups. The signal integrating 4 protons seen at 5.33 ppm in the ^{1}H-NMR spectrum of compound **5** were attributed to the 2 –NH_2 groups (exch. with D_2O). Furthermore, the ^{1}H-NMR spectra of **5** displayed an additional singlet due to the –SH group at 13.70 ppm integrating 1 proton, while no signal representing a hydrazide structure appeared.

The synthesis of the corresponding Schiff base derivatives (**6** and **8**) of compounds **2** and **5** was performed by the reaction of compounds **2** and **5** with 4-fluorophenylbenzaldehyde (for **8**) or salicylaldehyde (for **6**) in the presence of the catalytic amount of H_2SO_4. In the ^{1}H-NMR spectra of the compounds **6** and **8**, no signal representing the presence of an amino group in the structure appeared; instead, additional signals due to a 4-fluorophenyl or salicyl nucleus were recorded at the aromatic region. Due to slight solubility in dimethyl sulfoxide-d_6 solution, a satisfactory ^{13}C-NMR spectrum was not recorded for compound **6**. The absorption bands due to the 2 –OH groups of 2-hydroxyphenyl moiety were recorded at 3414 cm^{-1} in the IR spectra of **6**. Moreover, the signal recorded at 10.38 ppm in the ^{1}H-NMR spectra of **6** was assigned to the 2 –OH groups.

Both compounds **2** and **8** were converted to their corresponding S-methyl derivatives (**4** and **9**) by the reaction with methyl iodide in the presence of sodium ethoxide. The absence of the signal corresponding to the –SH group in the IR and ^{1}H-NMR spectra of compounds **4** and **9**, and the appearance of an additional singlet signal at 2.52 (for **4**) and 2.73 (for **9**) ppm in the ^{1}H-NMR spectra indicated the S-methyl derivatives of compounds **2** and **8**. In the ^{13}C-NMR spectra, these methyl groups resonated at 22.18 and 24.76 ppm, for **4** and **9**, respectively.

The aminoalkylation of compounds **2** and **8** was performed in the presence of 4-fluorophenyl piperazine (for **3**), morpholine (for **10a**) or 4-methyl piperazine (for **10b**), and formaldehyde. In the ^{1}H- and ^{13}C-NMR spectra of compounds **3**, **10a**, and **10b**, the presence of additional signals due to morpholine, 4-fluorophenyl morpholine, or methyl piperazine moiety confirmed the conversion of compounds **2** and **8** into the corresponding Mannich bases.

The treatment of compound **5** with phenacyl bromide facilitated the conversion of 4-amino-5-mercapto-1,2,4-triazole ring **5** into [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine system; thus, compound **7** was obtained. In contrast to compound **5**, the ^{1}H-NMR spectra of compound **7** exhibited no signal due to the –SH group, whereas signals that originated from thiadiazine C-6 and phenyl group at position 5 of the thiadiazine ring were present. The disappearance of the signal at 13.70 ppm, corresponding to the –SH proton of the parent compound **5**, in the ^{1}H-NMR spectrum of product **7** confirmed the involvement of the –SH proton in the condensation reaction.

All the synthesized compounds gave elemental analysis data consistent with the assigned structures.

All the compounds were tested for their antimicrobial activities. MICs were recorded as the minimum concentrations of compounds that inhibit the growth of tested microorganisms. Based on the obtained results,

it can be concluded that the conversion of a 1,3,4-oxadiazole ring (compound **2**) into an amino-1,2,4-triazole ring (compound **5**) afforded good antimicrobial activity with MIC values in the range of 0.12-1.95 μg/mL. Moreover, the 2-hydroxyphenyl methylenamino derivative (**6**) of compound **5** was found to be active against the test microorganisms (except *Candida tropicalis* and *Candida albicans*), with MIC values between 0.12 and 0.98 μg/mL. The other compound having a Schiff base structure, **8**, displayed moderate antimicrobial activity against the gram negative bacterial strain and good activity against the gram positive bacteria used in the study. On the other hand, the S-methyl derivative (**9**) of compound **8** was found to possess no activity. Similarly, the other S-methyl compound, **4**, was found to be inactive toward the test microorganisms. Among the Mannich bases (**3**, **10**, and **10b**) synthesized in the study, the most active compound was **3**, with MIC values in the range of 0.12-0.98 μg/mL. Compound **10a** was found to be active against the microbial strains used in the study (except *Candida tropicalis* and *Candida albicans*), with the MIC value 62.5 μg/mL. On the other hand, compound **10b** exhibited activity with the MIC value of 125 μg/mL against *Escherichia coli*, *Klebsiella pneumoniae*, *Yersinia pseudotuberculosis*, *Enterobacter aerogenes*, and *Pseudomonas aeruginosa*, while it displayed good activity with the MIC value of 62.5 μg/mL towards *Staphylococcus aureus, Enterococcus faecalis*, and *Bacillus cereus.*

Acknowledgements

The support provided by the Scientific and Technological Research Council of Turkey (TÜBİTAK, Project no: 107T333) and Karadeniz Technical University, BAP, Turkey (Ref. No. 2005.111.002.4), is gratefully acknowledged.

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