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Original article

Synthesis of some new 1,2,4-triazoles starting from isonicotinic acid hydrazide and evaluation of their antimicrobial activities

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ABSTRACT

5-Pyridin-4-yl-1,3,4-oxadiazole-2-thiol (**2**) was obtained from the reaction of isonicotinic acid hydrazide with carbon disulfide in basic media and converted into 4-amino-5-pyridin-4-yl-4*H*-1,2,4-triazole-3-thiol (**5**) by the treatment with hydrazine hydrate. The synthesis of **3** and **6** was performed from the reaction of **2** and **5** with ethyl bromide. The treatment of **5** with 4-fluorobenzaldehyde or indol-3-carbaldehyde resulted in the formation of 4-[(arylmethylene)amino]-5-pyridin-4-yl-4*H*-1,2,4-triazole-3-thiols (**7a** and **7b**). The reactions of **2**, **5** and **7a** with some primary and secondary amines in the presence of formaldehyde afforded the corresponding Mannich bases, **4a**, **4b**, **9a**–**9c** and **8**.

All newly synthesized compounds were screened for their antimicrobial activity. The antimicrobial activity study revealed that all the compounds screened showed good or moderate activity except compounds **2**, **7a**, **7b**, **8** and **9b**.

1. Introduction

During the past decades, the human population affected with life-treating infectious diseases caused by multidrug-resistant Gram-positive and Gram-negative pathogen bacteria increased an alarming level around the world. Due to this reason, new classes of antibacterial agents with novel mechanisms are crucial need to combat with the multidrug-resistant infections. In the past years, some azole derivatives were developed as new antimicrobial agents, for instance, Linezolid and Eperezolid (Chart 1) are currently used for the treatment of multidrug-resistant Gram-positive infections [1–3]. There are a number of antimicrobial compounds containing a 1,2,4-triazole ring in their structures such as Fluconazole, Itraconazole, Voriconazole, Ravuconazole and Posaconazole that are important antifungal drugs (Chart 1) [4].

Heterocycles containing a 1,2,4-triazole or 1,3,4-thiadiazole moiety, and the compounds consisting of 1,2,4-triazole and 1,3,4-thiadiazole condensed nucleus systems constitute a class of compounds possessing a wide spectrum of biological activities such as anti-inflammatory, antiviral and antimicrobial and antitumoral properties [5–8]. Furthermore, Schiff bases derived from various heterocycles were reported to possess some biological activities [9]. Among these, some 1,2,4-triazole derivatives containing also a Schiff base structures were synthesized in our laboratories as antitumor agents [10–13].

Mannich bases of 1,2,4-triazoles containing *N*-methylpiperazine moiety are known as antimicrobial agents. More recently, some Mannich bases possessing antimicrobial activity were synthesized by using 1,2,4-triazole-Schiff bases and methyl piperazine or morpholine [14]. The drugs, Prazosin, Lidoflazine and Urapidil, containing piperazine nucleus are used as cardiovascular agents (Chart 2) [15–17]. Moreover, Norfloxacin, Ciprofloxacin and Ofloxacin (Chart 2), that are effective drugs against respiratory, urinary, gastrointestinal tracts, skin and soft tissue infections caused by either Gram-positive or Gram-negative bacteria, contain a methyl piperazine or piperazine ring in their structures [18,19], as well as Itraconazol and Posaconazol (Chart 1).

Heterocycles including amino and mercapto groups together can be considered as useful intermediates in organic synthesis. Some triazolothiadiazoles or triazolothiadiazines were obtained in our laboratories starting from 4-amino-5-mercapto-1,2,4-triazoles. The amino and mercapto groups are ready-made nucleophilic centers for the synthesis of condensed heterocyclic rings [20–22]. Moreover, these groups can react with electrophiles. For instance, some alkylation and Mannich reactions that take place at S- or N-atom were reported [23–25].

In view of these facts, the aim of this present study is to obtain 1,3,4-oxadiazole and 1,2,4-triazole derivatives incorporating also Schiff Base and Mannich Base structures (Scheme 1) as antimicrobial agents.

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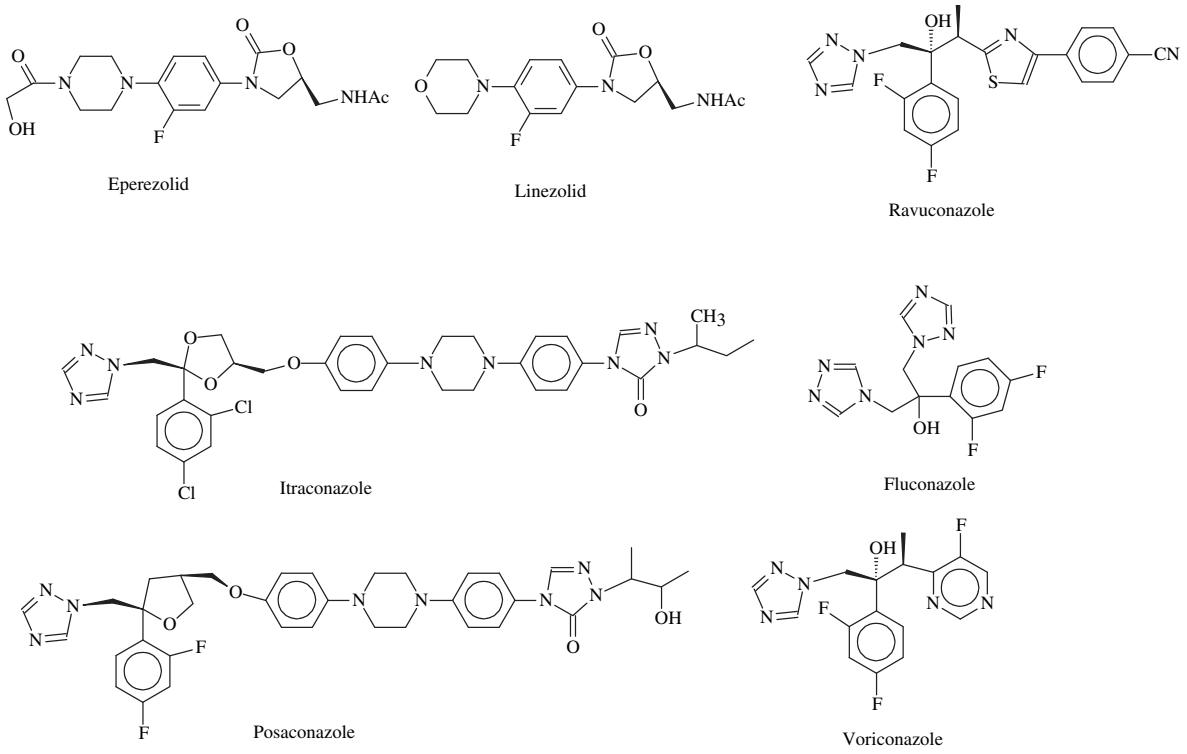


Chart 1.

2. Chemistry

Compound **2** was obtained from the reaction of isonicotinic acid hydrazide with CS_2 in basic media in good yield. The treatment of **2** with hydrazine hydrate produced compound **5**. Both compounds **2** and **5** are known [5,26]. Reaction of 5-pyridin-4-yl-1,3,4-oxadiazole-2-thiol and 4-amino-5-pyridin-4-yl-4*H*-1,2,4-triazole-3-thiol (**2** and **5**) with ethyl bromide in basic media afforded compounds **3** and **6**, respectively. The synthesis of 4-{[(4-fluorophenyl)- or 4-{[1*H*-indol-3-ylmethylene]amino}-5-pyridin-4-yl-4*H*-1,2,4-triazole-3-thiols (**7a** and **7b**) was performed by the treatment of compound **5** with the corresponding aromatic aldehydes. On the other hand, the reactions of compounds **2**, **5** and **7a** with several amines in the presence of formaldehyde solution afforded the corresponding Mannich base derivatives (**4a**, **4b**, **9a**–**9c** and **8**) incorporating piperazine or morpholine ring.

3. Results and discussion

The appearance of the signals approximately at 1.44 ppm (CH_3) and 3.19–4.12 ppm (CH_2) integrating three and two protons, respectively, in the ^{1}H NMR spectra of compounds **3** and **6** confirmed the conversion of **2** and **5** into the corresponding S-ethylated derivatives (**3** and **6**). Aromatic protons of compounds **3** and **6** were observed at 7.93 and 8.94 ppm as doublets. These groups were observed at 121.43–153.65 ppm in the ^{13}C NMR spectra. Compound **6** did not give molecular ion peak in the mass spectrum. Compounds **4a**, **4b**, **9a**–**9c** and **8** which are Mannich base derivatives of compounds **2**, **5** and **7a** gave a singlet at 5.12 ppm and 5.80 ppm in the ^{1}H NMR spectra integrating two protons. Also these individual $-CH_2$ groups were observed at 66.43 ppm and 67.76 ppm in the ^{13}C NMR spectra. In the mass spectrum of compound **8**, a stable molecular ion peak was not observed whereas a peak due

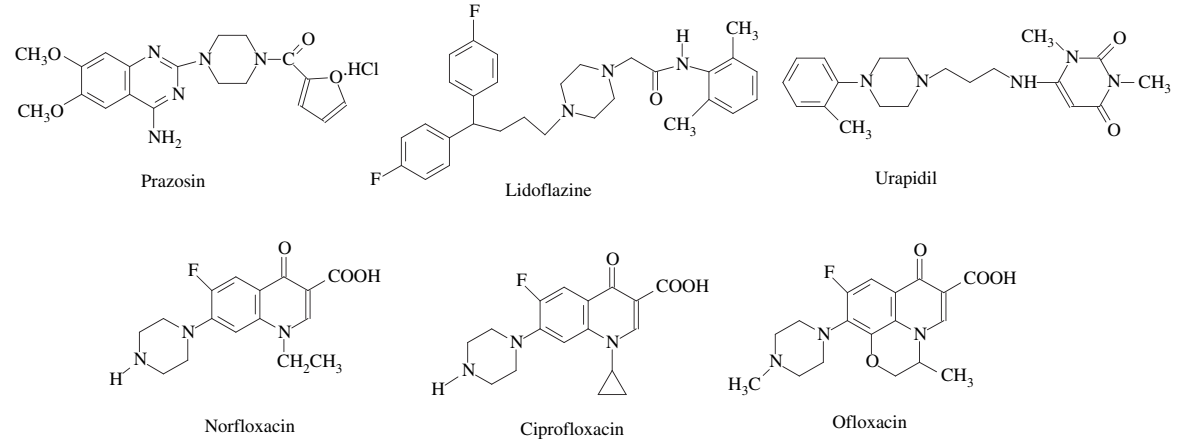
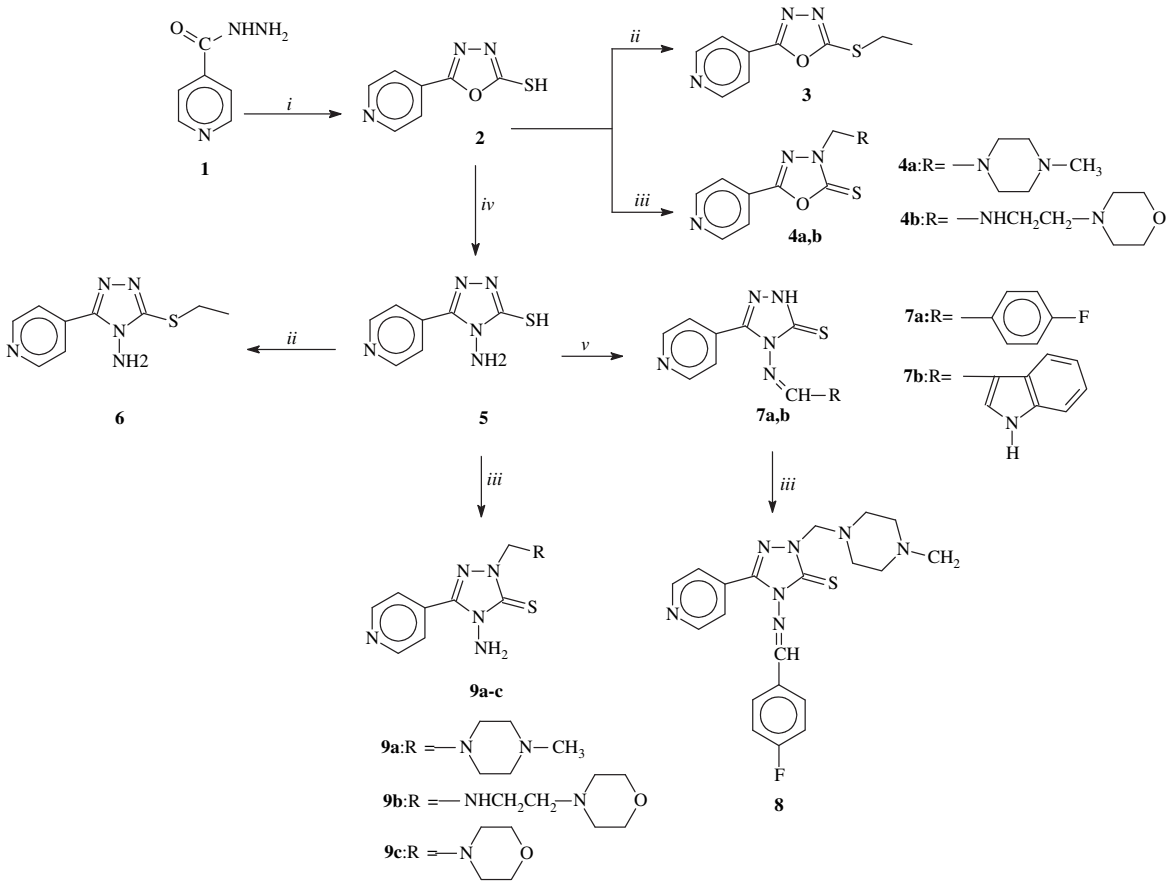


Chart 2.



Scheme 1. Synthetic pathway for the preparation of compounds **2**–**9**.

to M + Na was present. On the other hand **9a** displayed a stable molecular ion peak. When compound **5** was converted into the corresponding Schiff bases (**7a** and **7b**), additional one proton at 8.18 and 8.23 ppm was observed in the ^{1}H NMR spectra as a singlet. **7a** and **7b** gave stable M + 1 or M^+ ion peaks in the mass spectra, respectively. All the newly synthesized compounds gave elemental analysis data consistent to assigned structures.

Among the tested compounds, the S-ethylated compounds (**3** and **6**), displayed good antimicrobial activity against the test microorganisms except *Candida tropicalis* (Ct.) and *Candida albicans* (Ca.). The corresponding Mannich bases (**4a**, **4b**) of **2** showed moderate activity towards the test microorganisms. Among the corresponding Mannich bases (**9a**–**9c**) **9a** was active against all test microorganisms except *C. tropicalis* (Ct.) and *C. albicans* (Ca.); **9c** was active only against *Escherichia coli* (Ec.) and *Yersinia pseudotuberculosis* (Yp.), while **9b** displayed no activity. The Schiff bases (**7a** and **7b**) and the Mannich base (**8**) of compound **7a** showed no activity against the test microorganisms.

4. Experimental

4.1. 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. Mass spectra were obtained for compounds **6**, **8**, **9a**, **7a** and **7b** at a Quattro LC–MS (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). Compound **1** is available as commercial.

4.1.1. General method for the synthesis of compounds *3* and *6*

To a solution of compound **2** (for **3**) or **5** (for **6**) (10 mmol) in absolute ethanol equivalent amount of sodium was added and the mixture was stirred at room temperature for 30 min. Then, ethyl bromide (10 mmol) was added and refluxed for 4 hours. After evaporating the solvent in reduce pressure a solid appeared. This was recrystallized from ethanol to obtain target compound.

*4.1.1.1. 4-[5-(Ethylthio)-1,3,4-oxadiazol-2-yl]pyridine (**3**).* Yield 77.8%, m.p. 75–78 °C. IR (KBr, cm^{-1}): 1606 and 1539 (2C=N); Anal. Calcd (%) for $C_9H_9N_3OS$: C, 52.16; H, 4.38; N, 20.28. Found: C, 52.14; H, 4.33; N, 20.32; ^{1}H NMR (DMSO-d_6, δ ppm): 1.44 (3H, t, CH_3), 3.36 (2H, q, CH_2), 7.81–7.98 (2H, m, arH), 8.81 (2H, br s, arH); ^{13}C NMR (DMSO-d_6, δ ppm): 14.55 (CH_3), 27.67 (CH_2), arC: [121.43 (pyridine C-2 and C-6), 140.44 (C), 153.65 (pyridine C-3 and C-5)], 165.14 (oxadiazole, C-2), 166.32 (oxadiazole,C-5).

*4.1.1.2. 3-(Ethylthio)-5-pyridin-4-yl-4H-1,2,4-triazol-4-amine (**6**).* Yield 74.3%, m.p. 125–127 °C. IR (KBr, cm^{-1}): 3072 and 2987 (NH_2), 1606 and 1581 (2C=N); Anal. Calcd (%) for $C_9H_{11}N_5S$: C, 48.85; H, 5.01; N, 31.65. Found: C, 48.89; H, 5.03; N, 31.69; ^{1}H NMR (DMSO-d_6, δ ppm): 1.43 (3H, t, CH_3), 3.19–4.12 (2H, m, CH_2), 7.93–8.02 (2H, m, arH), 8.94 (2H, br s, arH), 11.66 (2H, s, NH_2); ^{13}C NMR (DMSO-d_6, δ ppm): 13.66 (CH_3), 27.43 (CH_2), arC: [121.47 (pyridine C-2 and C-

6), 139.87 (C), 153.45 (pyridine C-3 and C-5)], 165.03 (oxadiazole, C-2), 166.21 (oxadiazole,C-5); MS: *m*/*z* (%) 121 (47), 123 (22), 130 (22).

*4.1.2. General method for the synthesis of compounds **4a**, **4b**, **8** and **9a–9c***

To a solution of corresponding compound **2**, **5** or **7a** (10 mmol) in dimethyl formamide, methyl piperazine (for **4a** and **9a**) (10 mmol), 2-(4-morpholinoethylamine) (for **4b** and **9b**) (10 mmol) or morpholine (for **9c**) was added in the presence of formaldehyde (40%, 1.5 mL) and the mixture was stirred at room temperature for 2 hours. Then, water was added and kept overnight in cold. The solid separated was collected by filtration and recrystallized from dimethyl sulfoxide to yield the target compounds.

*4.1.2.1. 3-[(4-Methylpiperazin-1-yl)methyl]-5-pyridin-4-yl-1,3,4-oxadiazole-2(*3H*)-thione (**4a**).* Yield 88%, m.p. 240–241 °C. IR (KBr, cm^{-1}): 1315 (C=S); Anal. Calcd (%) for $C_{13}H_{17}N_5OS$: C, 53.59; H, 5.88; N, 24.04. Found; C, 53.64; H, 5.92; N, 24.00; ^{1}H NMR (DMSO-d_6, δ ppm): 2.19 (3H, s, CH_3), 2.38–2.43 (4H, m, $2CH_2$), 2.78–2.85 (4H, m, $2CH_2$), 5.12 (2H, s, CH_2), 7.81–7.83 (2H, d, arH), 7.87–7.89 (2H, d, arH); ^{13}C NMR (DMSO-d_6, δ ppm): 42.87 (CH_3), 51. 94 (piperazine C-2 and C-6), 54.44 (piperazine C-3 and C-5), 67.73 (CH_2), arC: [119.28 (pyridine C-2 and C-6), 141.09 (C), 150.96 (pyridine C-3 and C-5)], 162.60 (oxadiazole C-3), 175.47 (oxadiazole C-5).

*4.1.2.2. 3-{[(2-Morpholin-4-ylethyl)amino]methyl}-5-pyridin-4-yl-1,3,4-oxadiazole-2(*3H*)-thione (**4b**).* Yield 86%, m.p.169–171 °C. IR (KBr, cm^{-1}): 1314 (C=S); Anal. Calcd (%) for $C_{14}H_{19}N_5O_2S$: C, 52.32; H, 5.96; N, 21.79. Found: C, 52.29; H, 5.99; N, 21.82; ^{1}H NMR (DMSO-d_6, δ ppm): 2.31 (2H, t, CH_2), 2.42 (4H, t, CH_2), 2.70 (2H, t, CH_2), 3.35–3.39 (4H, m, $2CH_2$), 5.43 (2H, s, CH_2), 7.80–7.82 (2H, d, arH), 7.86–7.88 (2H, d, arH), 10.56 (1H, br s, NH); ^{13}C NMR (DMSO-d_6, δ ppm): 46.51 (CH_2), 51.58 (CH_2), 53.67 (morpholine C-2 and C-6), 61.60 (morpholine C-3 and C-5), 66.43 (CH_2), arC: [119.16 (pyridine C-2 and C-6), 140.98 (C), 150.93 (pyridine C-3 and C-5)], 161.54 (oxadiazole C-3), 172.02 (oxadiazole C-5).

*4.1.2.3. 4-{[(4-Fluorophenyl)methylene]amino}-5-pyridin-4-yl-2-[(4-methylpiperazin-1-yl)methyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**8**).* Yield 73%, m.p. 133–135 °C. IR (KBr, cm^{-1}): 1315 (C=S); Anal. Calcd (%) for $C_{20}H_{22}N_7SF$: C, 58.38; H, 5.39; N, 23.83. Found; C, 58.42; H, 5.33; N, 23.88; ^{1}H NMR (DMSO-d_6, δ ppm): 2.19 (3H, s, CH_3), 2.42–2.53 (4H, m, $2CH_2$), 2.62–2.71 (4H, m, $2CH_2$), 5.40 (2H, s, CH_2), 7.22–7.34 (2H, m, arH), 7.45–7.48 (2H, m, arH), 8.42–8.44 (2H, dd, arH), 8.72–8.73 (2H, dd, arH), 11.02 (1H, s, SH); ^{13}C NMR (DMSO-d_6, δ ppm): 42.78 (CH_3), 51. 96 (piperazine C-2 and C-6), 54.45 (piperazine C-3 and C-5), 66.67 (CH_2), arC: [115.03 (pyridine C-2 and C-6), 123.89 (phenyl C-2 and C-6), 131.34 (C), 132.44 (phenyl C-3 and C-5), 132.77 (C), 150.11 (pyridine C-3 and C-5), 165.78 (C)], 135.45 (CH), 151.41 (triazole C3), 157.47 (triazole C5); MS: *m*/*z* (%) 124 (46), 139 (48), 149 (11), 162 (25), 179 (34), 301 (11), 423 (M + Na, 50), 424 (14).

*4.1.3. General method for the synthesis of compounds **9a–9c***

To the solution of corresponding compound **5** (10 mmol) in dimethyl formamide, formaldehyde (37%, 1.55 mL) and amine (10 mmol) were added and the mixture was stirred at room temperature for 2.5 hours. Then, excess amount of pure water was added to this solution and the mixture was kept overnight in cold. The resulting solid separated was collected by filtration, washed with water, recrystallized from dimethylsulfoxide/water (1:2) to yield the title compounds.

*4.1.3.1. 4-Amino-2-[(4-methylpiperazin-1-yl)methyl]-5-pyridin-4-yl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**9a**).* Yield 71%, m.p. 231–233 °C. IR (KBr, cm^{-1}): 3276 and 3167 (NH_2), 1609 and 1572 (2C=N), 1314 (C=S); Anal. Calcd (%) for $C_{13}H_{19}N_7S$: C, 51.13; H, 6.27; N, 32.10. Found: C, 51.16; H, 6.30; N, 32.14; ^{1}H NMR (DMSO-d_6, δ ppm): 2.41 (3H, s, CH_3), 2.82 (4H, t, $2CH_2$), 3.02 (4H, t, $2CH_2$), 5.80 (2H, s, CH_2), 7.62–8.23 (2H, m, arH), 8.41–7.00 (2H, m, arH), 13.84 (2H, br s, NH_2); ^{13}C NMR (DMSO-d_6, δ ppm): 42.88 (CH_3), 51.95 (piperazine C-2 and C-6), 54.46 (piperazine C-3 and C-5), 67.76 (CH_2), arC: [120.10 (pyridine C-2 and C-6), 139.90 (C), 151.02 (pyridine C-3 and C-5)], 148.99 (triazole C-3), 176.65 (triazole C-5); MS: *m*/*z* (%) 119 (69), 184 (47), 305 (69), 305 (84), 305 (M^+, 97), 306 (25), 337 (11), 343 (11).

*4.1.3.2. 4-Amino-2-{[(2-morpholin-4-ylethyl)amino]methyl}-5-pyridin-4-yl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**9b**).* Yield 76%, m.p. 238–241 °C. IR (KBr, cm^{-1}): 3273 and 3163 (NH_2), 1607 and 1570 (2C=N), 1314 (C=S); Anal. Calcd (%) for $C_{14}H_{21}N_7OS$: C, 50.13; H, 6.31; N, 29.23. Found: C, 50.09; H, 6.32; N, 29.18; ^{1}H NMR (DMSO-d_6, δ ppm): 2.29–2.32 (4H, m, $2CH_2$), 2.37–2.42 (4H, m, $2CH_2$), 3.38–3.44 (4H, m, $2CH_2$), 5.53 (2H, s, CH_2), 7.78–7.80 (2H, dd, arH), 8.12 (2H, br s, NH_2), 8.02–8.50 (2H, dd, arH), 11.07 (1H, s, NH); ^{13}C NMR (DMSO-d_6, δ ppm): 46.51 (CH_2), 51.60 (CH_2), 53.67 (morpholine C-2 and C-6), 61.54 (CH_2), 66.47 (morpholine C-3 and C-5), arC: [119.78 (pyridine C-2 and C-6), 135.67 (C), 151.12 (pyridine C-3 and C-5)], 147.85 (triazole C-3), 175.66 (triazole C-5).

*4.1.3.3. 4-Amino-2-(morpholin-4-ylmethyl)-5-pyridin-4-yl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**9c**).* Yield 65%, m.p. 234–236 °C. IR (KBr, cm^{-1}): 3274 and 3163 (NH_2), 1607 and 1571 (2C=N), 1314 (C=S); Anal. Calcd (%) for $C_{12}H_{16}N_6OS$: C, 49.30; H, 5.52; N, 28.75. Found: C, 49.27; H, 5.56; N, 28.79; ^{1}H NMR (DMSO-d_6, δ ppm): 2.55–2.62 (4H, m, $2CH_2$), 3.34–3.41 (4H, m, $2CH_2$), 5.69 (2H, s, CH_2), 7.54–7.58 (2H, d, arH), 7.78–7.83 (2H, d, arH), 8.15 (2H, br s, NH_2); ^{13}C NMR (DMSO-d_6, δ ppm): 59.32 (morpholine C-2 and C-6), 60.44 (morpholine C-3 and C-5), 67.51 (CH_2), arC: [121.45(pyridine C-2 and C-6), 139.83 (C), 153.38 (pyridine C-3 and C-5)], 148.07 (triazole, C-3), 175.10 (triazole,C-5).

*4.1.4. General method for the synthesis of compounds **7a** and **7b***

A solution of the corresponding compound **5** (10 mmol) in absolute ethanol was refluxed with appropriate aldehyde (10 mmol) for 3 hours. After cooling the mixture to room temperature, a white solid appeared. This crude product was recrystallized from dimethylsulfoxide/water (1:2) or ethanol to afford the desired product.

*4.1.4.1. 4-{[(4-Fluorophenyl)methylene]amino}-5-pyridin-4-yl-4*H*-1,2,4-triazole-3-thione (**7a**).* Yield 87%, m.p. 245–247 °C. IR (KBr, cm^{-1}): 1317 (C=S), 3156 (NH); Anal. Calcd (%) for $C_{14}H_{10}N_5SF$: C, 56.18; H, 3.37; N, 23.40. Found: C, 56.22; H, 3.33; N, 23.43; ^{1}H NMR (DMSO-d_6, δ ppm): 7.41–7.43 (2H, m, arH), 7.45–7.57 (2H, m, arH), 8.18 (1H, s, CH), 8.45–8.48 (2H, dd, arH), 8.51–8.54 (2H, dd, arH), 11.07 (1H, s, SH); ^{13}C NMR (DMSO-d_6, δ ppm): arC: [115.09 (pyridine C-2 and C-6), 122.85 (phenyl C-2 and C-6), 130.89 (C), 131.42 (C), 133.32 (phenyl C-3 and C-5), 151.43 (pyridine C-3 and C-5), 165.77 (C)], 148.54 (triazole C-3), 153.71 (triazole C-5), 155.17 (CH); MS: *m*/*z* (%) 109 (46), 120 (38), 124 (34), 139 (29), 149 (34), 161 (31), 162 (22), 164 (14), 180 (16), 199 (15), 301 (M + 2, 24).

*4.1.4.2. 4-{[1*H*-Indol-3-ylmethylene]amino}-5-pyridin-4-yl-4*H*-1,2,4-triazole-3-thione (**7b**).* Yield 89%, m.p. 279.281 °C. IR (KBr, cm^{-1}): 1317 (C=S), 3167 (NH); Anal. Calcd (%) for $C_{16}H_{12}N_6S$: C, 59.96; H, 3.78; N, 26.23. Found: C, 59.99; H, 3.74; N, 26.17; ^{1}H NMR (DMSO-d_6, δ ppm): 7.14–7.16 (1H, dd, arH), 7.41–7.43 (1H, m, arH),

Table 1
Screening for antimicrobial activity of compounds (mm).

Compound no	Microorganisms and inhibition zone (mm)							
	Ec.	Yp.	Pa.	Ef.	Sa.	Bc.	Ct.	Ca.
2[a]	–	–	–	–	10	–	30	25
3[a]	30	22	25	25	25	18	7	7
4a[a]	23	15	28	17	17	17	–	–
4b[a]	13	13	13	15	14	14	–	–
5[a]	34	30	42	24	34	20	–	–
6[a]	30	24	25	25	25	20	8	8
7a[b]	–	–	–	–	–	7	8	8
7b[b]	–	–	–	–	–	–	–	–
8[b]	–	–	–	–	–	–	–	–
9a[b]	30	24	35	25	28	22	–	–
9b[b]	–	–	–	–	–	–	–	–
9c[b]	20	13	–	–	–	–	–	–
Ethanol	–	–	–	–	–	–	11	11
DMSO	–	–	–	–	–	–	–	–
Amp.	10	18	18	10	35	15		
Flu.							25	25

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, Bc: *Bacillus cereus* 702 Roma, Ct: *Candida tropicalis* ATCC 13803, Ca: *Candida albicans* ATCC 60193. Amp.: Ampicillin, Flu.: Fluconazole, (–): no activity.
[a] Solvent is DMSO.
[b] Solvent is ethanol.

7.45–7.47 (1H, m, arH), 7.70–7.73 (1H, m, arH), 7.76–7.77 (1H, m, arH), 8.23 (1H, s, CH), 8.54–8.56 (2H, d, arH), 8.66 (1H, br s, NH), 9.03–9.06 (2H, dd, arH), 11.06 (1H, s, SH); ^{13}C NMR (DMSO-d_6, δ ppm): arC: [118.84 (CH), 119.24 (pyridine C-2 and C-6), 119.89 (C), 120.48 (CH), 121.43 (CH), 121.57 (CH), 128.18 (CH), 131.07 (C), 134.23 (C), 136.44 (C), 152.88 (pyridine C-3 and C-5)], 143.99 (C), 148.66 (triazole C-5), 153.98 (triazole C-3); MS: *m*/*z* (%) 105 (47), 118 (72), 119 (61), 120 (23), 121 (14), 135 (25), 143 (23), 150 (34), 180 (23), 194 (16), 254 (16), 321 (M^+, 46), 322 (10).

4.2. Antimicrobial activity

4.2.1. Antimicrobial activity assessment

All bacterial and yeast strains were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: *E. coli* ATCC 25922, *Y. pseudotuberculosis* ATCC 911, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* 709 ROMA, *C. tropicalis* ATCC 13803 and *C. albicans* ATCC 60193. All the newly synthesized compounds were dissolved in Dimethylsulphoxide (DMSO) or ethanol to prepare chemicals stock solution of 10 mg/1 mL.

4.2.1.1. Agar-well diffusion method. Simple susceptibility screening test using agar-well diffusion method [27] as adapted earlier [28] was used. Each microorganism was suspended in Mueller Hinton (MH) (Difco, Detroit, MI) broth and diluted approximately 10^6 colony forming unit (cfu) per mL. They were "flood-inoculated" onto the surface of MH Agar and Sabouraud Dextrose Agar (SDA) (Difco, Detroit, MI) 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 μL of the extract substances were delivered into the wells. The plates were incubated for 18 hours at 35 °C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. Ampicillin (10 μg) and Fluconazole (5 μg) were standard drugs. Dimethylsulphoxide and ethanol were used as solvent control. The antimicrobial activity results are summarized in Table 1.

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