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Original article

Synthesis of some new 1,2,4-triazoles, their Mannich and Schiff bases and evaluation of their antimicrobial activities

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Abstract

4-Phenyl-5-pyridin-4-yl-4*H*-1,2,4-triazole-3-thiol (**3**) was obtained in basic media via the formation of 2-isonicotinoyl-*N*-phenylhydrazinecarbothioamide (**2**), and converted to some alkylated derivatives (**4a**,**b**) and Mannich base derivatives (**5a**−**c**). 2-[(4-Phenyl-5-pyridin-4-yl-4*H*-1,2,4-triazol-3-yl)thio]acetohydrazide (**7**) that was obtained by using compound **3** as precursor in two steps was converted to thiosemicarbazide derivative (**8**), Schiff base derivatives (**9**) and 5-{[(4-phenyl-5-pyridin-4-yl-4*H*-1,2,4-triazol-3-yl)thio]methyl}-1,3,4-oxadiazole-2-thiol (**10**). Moreover, 5-{[(4-phenyl-5-pyridin-4-yl-4*H*-1,2,4-triazol-3-yl)thio]methyl}-3-{[(2-morpholin-4-ylethyl)amino]methyl}-1,3,4-oxadiazole-2(3*H*)-thione (**11**) was synthesized via reaction of compound **10** with 2-(4-morpholino)ethylamine. The treatment of compound **8** with NaOH gave 4-(4-methylphenyl)-5-{[(4-phenyl-5-pyridine-4-yl-4*H*-1,2,4-triazol-3-yl)thio]methyl}-4*H*-1,2,4-triazole-3-thiol (**12**), while the acidic treatment of compound **8** afforded 5-{[(4-phenyl-5-pyridin-4-yl-4*H*-1,2,4-triazol-3-yl)thio]methyl}-2(4-methylphenyl)-amino-1,3,4-thiadiazole (**14**). *N*-Methyl derivative of compound **14** and a Mannich base derivative of compound **12** were synthesized from the reactions of these precursors with methyl iodide and methyl piperazine, respectively.

All newly synthesized compounds were screened for their antimicrobial activities. The antimicrobial activity study revealed that all the compounds screened showed good or moderate activity except compounds **3**, **5c**, **7**, **9c**, **9e**, **9g**, **9h**, **11**, and **13**.

Keywords: Isonicotinic acid hydrazide; 1,2,4-Triazole; 1,3,4-Oxadiazole; 1,3,4-Thiadiazole; Antimicrobial activity

1. Introduction

In the past 25 years, the incidence of microbial infection has increased on alarming levels over the world as a result of antimicrobial resistance. 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. The health problem demands to search and synthesize a new class of antimicrobial compounds effective against pathogenic microorganisms that developed resistance to the antibiotics used in the current regimen [1−4].

The therapeutic importance of 1,2,4-triazoles is well documented. Among these, there are simple molecules including 1,2,4-triazole ring, besides bi- and polyheterocyclic compounds that contain triazole ring or triazole-fused compounds [5−13]. For instance, some bi-heterocyclic compounds, consisting of 1,2,4-triazole and 1,3,4-thiadiazole rings or two 1,2,4-triazole rings, have been synthesized by us as antimicrobial compounds [14].

Literature survey reveals that piperazine or morpholine ring is important for antimicrobial activity [15−18]. For instance, Linezolid, Eperezolid, AZD2563 and Itraconazole, which are currently important antibiotics used for the treatment of microbial infections, contain a piperazine or morpholine ring in their structures. They also contain an azole ring such as oxazolidinone or 1,2,4-triazole ring [19,20]. Other important chemotherapeutics, such as Vorozole, Letrozole and Anastrozole that consist of substituted 1,2,4-triazole ring, are currently being used for the treatment of breast cancer[21].

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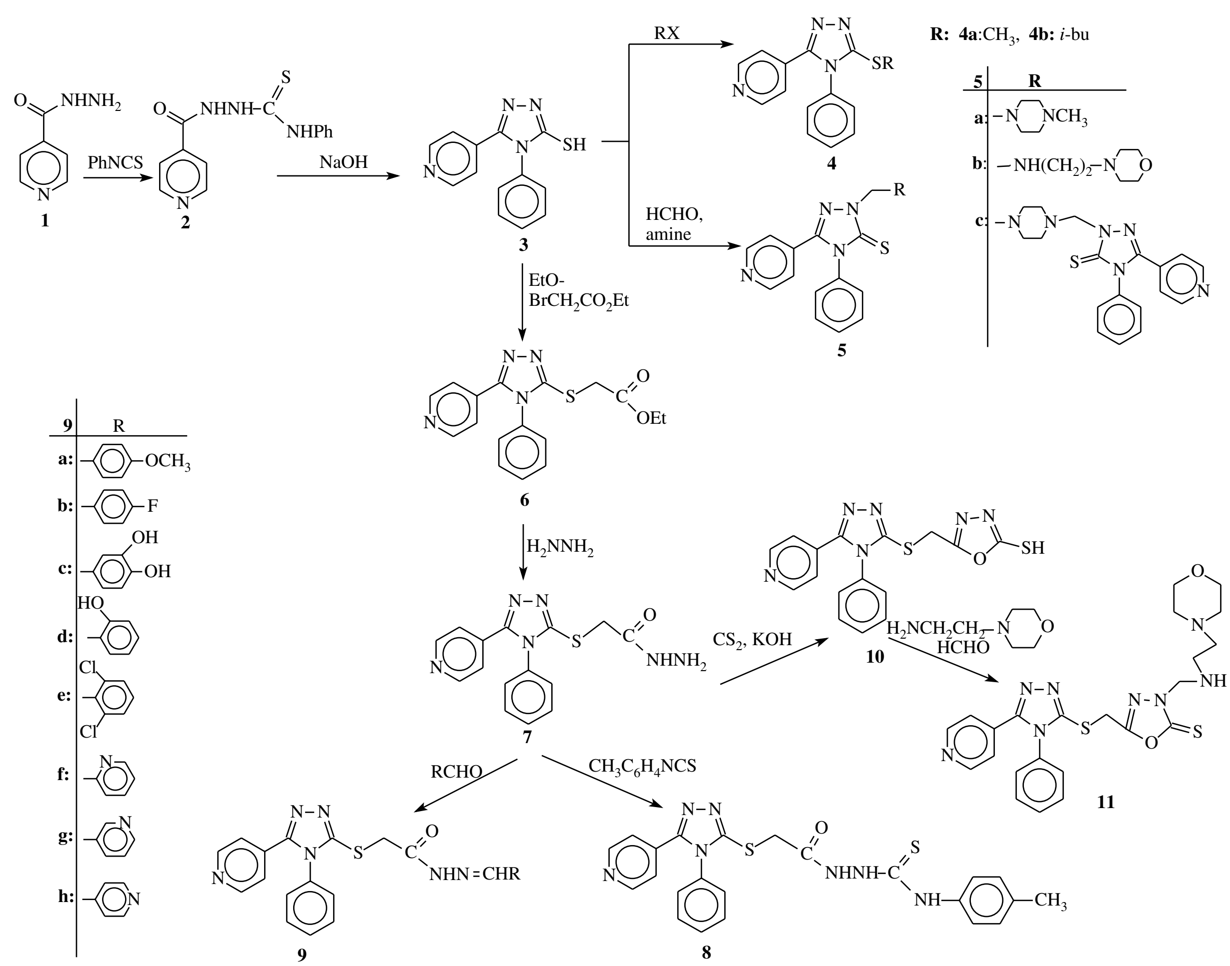
Our efforts for the synthesis of compounds possessing antitumor activity led us to the discovery of several Schiff base derivatives of 1,2,4-triazol-5-ones [14,22–24]. Among these, some Schiff base derivatives of acetic acid hydrazides containing 1,2,4-triazol-5-one ring have displayed antitumoral activity only against breast cancer, while 2-phenyl ethylidenamino and 2-phenyl ethylamino derivatives of 4-amino-1,2,4-triazol-5-ones have been found to be effective towards non-small cell lung cancer, CNC and breast cancer [5,22,23].

In view of these facts, the aim of the present study is to obtain 1,2,4-triazole derivatives incorporating Schiff base and Mannich base structures (Schemes 1 and 2) as antibacterial agents.

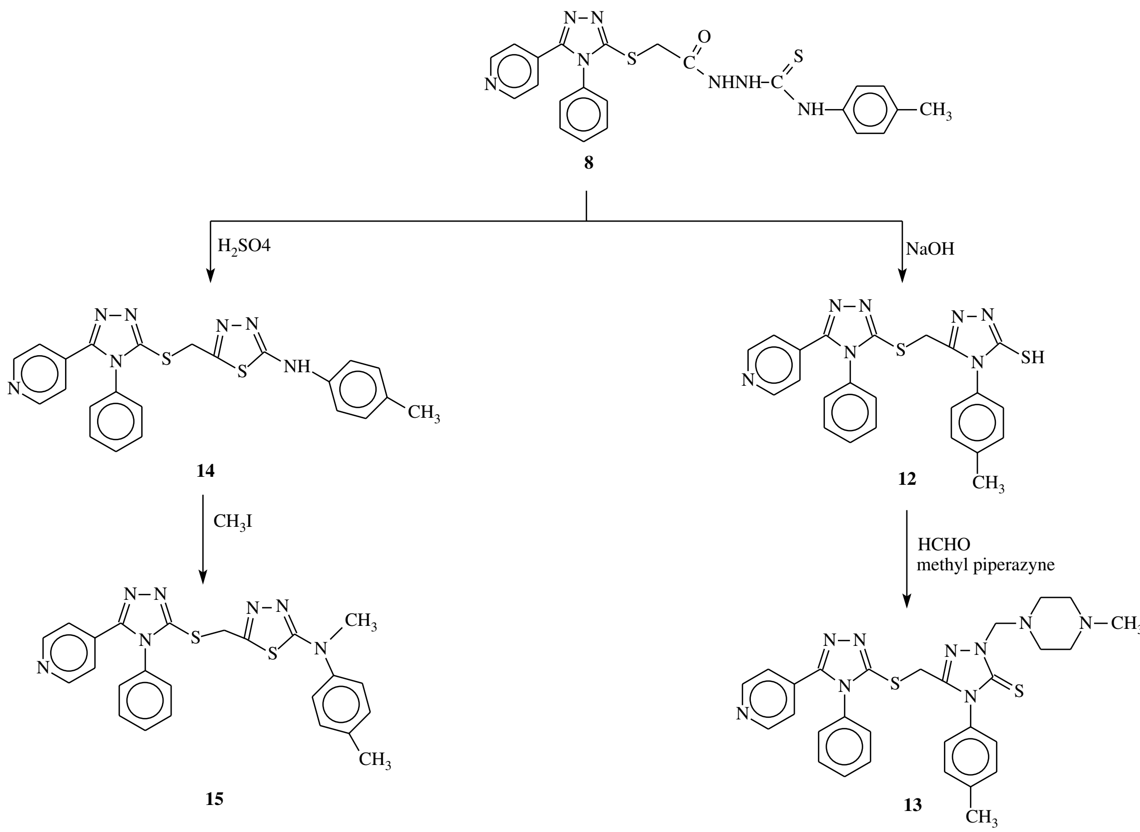
2. Chemistry

1-Isonicotinoyl-4-phenyl thiosemicarbazide (**2**) was obtained by the reaction of isonicotinic acid hydrazide with phenylisothiocyanate. The cyclization of compound **2** in the presence of 2 N NaOH resulted in the formation of 4-phenyl-5-pyridin-4-yl-4*H*-1,2,4-triazole-3-thiol (**3**). The alkylation of 1,2,4-triazole-3-thiol (**3**) was performed by the reaction with methyl iodide or isobutyl bromide in basic media, thus compounds **4a** and **4b** were obtained. On the other hand, the reactions of the same 1,2,4-triazole-3-thiol (**3**) with several amines in the presence of formaldehyde solution afforded the corresponding Mannich base derivatives (**5a–c**) incorporating piperazine or morpholine ring. The reaction of 4-phenyl-5-pyridin-4-yl-4*H*-1,2,4-triazole-3-thiol (**3**) with ethyl bromoacetate in the presence of sodium ethoxide or triethylamine produced ethyl [(4-phenyl-5-pyridin-4-yl-4*H*-1,2,4-triazol-3-yl)thio]acetate (**6**). Then, this ester (**6**) was converted to the corresponding hydrazide derivative, 2-[(4-phenyl-5-pyridin-4-yl-4*H*-1,2,4-triazol-3-yl)thio]acetohydrazide (**7**) via the reaction with hydrazine hydrate. The treatment of **7** with CS_2 in the presence of KOH resulted in the formation of 5-{[(4-phenyl-5-pyridin-4-yl-4*H*-1,2,4-triazol-3-yl)thio]methyl}-1,3,4-oxadiazole-2-thiol (**10**), which was converted to the corresponding Mannich base derivative (**11**) by using 2-(4-morpholino)ethylamine in the presence of formaldehyde solution.

The treatment of acetohydrazide derivative (**7**) with several aldehydes gave *N*′-arylmethylene-2-[(4-phenyl-5-pyridin-4-yl-4*H*-1,2,4-triazol-3-yl)thio]acetohydrazides (**9a–h**). The compounds having arylidene–hydrazide structure may exist as *E*/*Z* geometrical isomers about –C=N double bond and as *cis*/*trans* amide conformers (Scheme 3) [25,26]. According



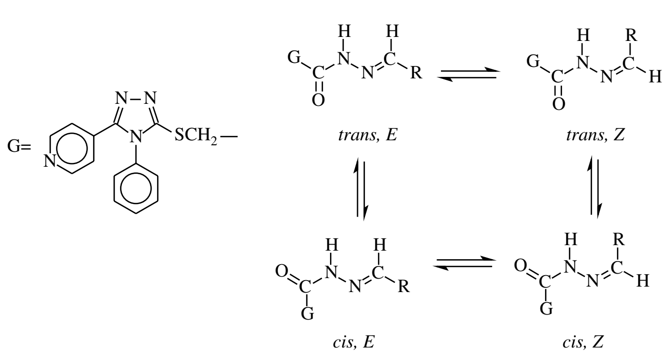
Scheme 1. Synthetic pathway for the preparation of compounds **2–11**.



Scheme 2. Synthetic pathway for the preparation of compounds **12–15**.

to the literature [26], the compounds containing imine bond are present in higher percentage in dimethyl-d_6 sulfoxide solution in the form of geometrical *E* isomer about −C=N double bond. 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 **9a–h**.

The reaction of compound **7** with 4-methylphenylisothiocyanate produced *N*-(4-methylphenyl)-2-{[(4-phenyl-5-pyridin-4-yl-4*H*-1,2,4-triazol-3-yl)thio]acetyl} hydrazinecarbothioamide (**8**). The treatment of compound **8** with sulfuric acid caused the conversion of carbothioamide structure into 1,3,4-thiadiazole ring; thus, 5-{[(4-phenyl-5-pyridin-4-yl-4*H*-1,2,4-triazol-3-yl)thio]methyl}-2(4-methylphenyl)-amino-1,3,4-thiadiazole (**14**) was obtained. On the other hand, the cyclization of the same compound (**8**) in the presence of 2 N NaOH resulted in the formation of 4-(4-methylphenyl)-5-{[(4-phenyl-5-pyridine-4-yl-4*H*-1,2,4-triazol-3-yl)thio]methyl}-4*H*-1,2,4-triazole-3-thiol (**12**), which was converted to 5-{[(4-phenyl-5-pyridine-4-yl-4*H*-1,2,4-triazol-3-yl)thio]methyl}-4-(4-methylphenyl)-2-[(4-methylpiperazin-1-yl)methyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**13**) by Mannich reaction. Compound **14** was condensed with methyl iodide in the presence of sodium ethoxide, thus, 5-{[(4-phenyl-5-pyridin-4-yl-4*H*-1,2,4-triazol-3-yl)thio]methyl}-2(4-methylphenyl)-2-methylamino-1,3,4-thiadiazole (**15**) was obtained.



Scheme 3. *E/Z* Geometrical isomers and *cis/trans* conformers of compounds **9a–h**.

3. Results and discussion

In the ^{1}H NMR spectra of compound **2**, additional −NH− signals (controlled with D_2O) derived from thiosemicarbazide structure were observed at 9.65, 9.75 and 10.30 ppm, while the signal due to $-NH_2$ group of hydrazide structure did not appear. Additional signals belonging to phenyl ring were observed in the aromatic region in the ^{1}H and ^{13}C NMR spectra of compound **2**. Moreover, −C=S group resonated at 180.55 ppm in the ^{13}C NMR spectra of compound **2**. When compound **2** was converted to 4-phenyl-5-pyridin-4-yl-4*H*-1,2,4-triazole-3-thiol (**3**) in basic media, −NH− peaks disappeared, while new signal due to −SH group was observed at

14.36 ppm (controlled with D_2O) in the ^{1}H NMR spectra of compound **3**. It is interesting to note that thiocarbonyl compounds are present in their thion–thiol tautomeric forms in solution as indicated by their IR and ^{1}H NMR spectra [14]. These tautomeric forms are also present in dimethyl sulfoxide as suggested by NMR spectral data. When compound **3** was converted to its alkylated derivatives (**4a** and **4b**), the –SH signal disappeared, instead, new signals that originated from methyl or isobutyl groups were observed in the ^{1}H and ^{13}C NMR spectra of compounds **4a** and **4b**. The ^{1}H NMR spectra of compounds **5a–c** displayed additional signals due to $-CH_2-$ group at 4.68–5.22 ppm integrating two protons besides the peaks belonging to methyl piperazine, piperazine or 2-(4-morpholino)ethylamine moiety, which are used as amine components in these Mannich reactions; these groups resonated between 2.42 and 5.70 ppm. The carbon signals of these groups were recorded between 46.55 and 53.70 ppm. Due to less solubility of compounds **5a** and **5c** in DMSO-d_6, ^{13}C NMR spectrum could not be recorded.

The –SH proton is acidic enough and some substitution reaction could be achieved on this group in the presence of a base [27,28]. In the present study, compound **6**, acetic acid ester derivative of compound **3**, was obtained by two methods in good yields. In the ^{1}H NMR spectrum of compound **6**, additional signals derived from ester group were observed at 1.29 ($-OCH_2CH_3$), 4.13 ($-SCH_2$) and 4.22 ($-OCH_2CH_3$) ppm integrating for three protons, two protons and two protons, respectively. In the ^{13}C NMR spectrum of the compound, the signals belonging to the same groups were recorded at 14.05, 34.43 and 62.13 ppm, respectively.

The ^{1}H NMR spectrum of compound **7** displayed no signals belonging to $-OCH_2CH_3$ group; instead, new signals derived from hydrazide structure appeared at 4.32 ($-NH$*NH_2*) and 9.31 (–*NH*NH_2) ppm integrating for two protons and one proton, respectively (controlled by changing D_2O).

The IR spectra of compounds **6** and **7** showed a peak at 1735 and 1673 cm^{-1} due to carbonyl function derived from ester or hydrazide structure, respectively.

The synthesis of eight different Schiff base derivatives of compound **7** was carried out by the reaction of compound **7** with several aromatic aldehydes in ethanol in the presence of catalytic amount of H_2SO_4. The ^{1}H and ^{13}C NMR spectra of compounds **9a–h** displayed additional signals due to the aromatic ring derived from aldehyde moiety at aromatic region, while the signal belonging to $-NH_2$ group of hydrazide structure did not appear.

In the ^{1}H NMR spectra of compounds **9a–h** two sets of signals each belonging to the $-SCH_2$ group, –N=CH group and –NH group of *cis*- and *trans*-conformers were observed between 4.11 and 4.60, 7.98 and 8.44 and 11.68 and 12.14 ppm, respectively. The upfield lines of $-SCH_2$, –N=CH, and –NH 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 [29]. In the ^{13}C NMR spectra of compounds **9a–h**, the –N=CH signals belonging to individual cis/trans conformers were observed as two sets between 112.21 and 119.85 ppm.

In addition, $-OCH_3$ group of compound **9a** resonated at 3.84 ppm integrating three protons as a singlet in the ^{1}H NMR spectrum, while this group was observed at 54.84 ppm in the ^{13}C NMR spectrum. Moreover, the signals derived from one –OH group in compound **9c** and two –OH groups in compound **9d** were recorded at 8.65 ppm integrating two protons and 10.91 and 10.58 ppm integrating one proton, respectively (controlled by changing D_2O). The –OH group of compound **9d** was seen as two different singlets due to the existence of *cis*/*trans*-amide conformers.

Compound **8** was obtained by a way analogous to the synthesis of compound **2** in 85% yield, and its structure was confirmed on the basis of IR, NMR and mass spectroscopic methods and elemental analysis. The treatment of compound **8** with sulfuric acid and sodium hydroxide produced 4-(4-methylphenyl)-5-{[(4-phenyl-5-pyridine-4-yl-4*H*-1,2,4-triazol-3-yl)thio]methyl}-4*H*-1,2,4-triazole-3-thiol (**12**) and 5-{[(4-phenyl-5-pyridin-4-yl-4*H*-1,2,4-triazol-3-yl)thio]methyl}-2(4-methylphenyl)-amino-1,3,4-thiadiazole (**14**), respectively. These compounds displayed IR, ^{1}H and ^{13}C NMR spectra and elemental analyses consistent with the assigned structures. In the ^{1}H NMR spectrum of compound **12**, additional signal due to –SH group appeared at 13.89 ppm (controlled by changing D_2O), while the –NH– signals disappeared. Moreover, the IR spectrum of compound **12** displayed an –SH stretching band at 2732 cm^{-1}. The –NH– group in compound **14** resonated at 10.30 ppm in the ^{1}H NMR spectrum. The –NH– stretching band was observed at 3187 cm^{-1} in the IR spectra. When compound **14** was converted to be an alkylated derivative (**15**), the signal disappeared; instead, new signal belonging to methyl protons appeared at 2.83 ppm as singlet in the ^{1}H NMR spectrum of compound **15**. The elemental analysis of compound **15** is consistent with the assigned structure.

The synthesis of compound **13** was carried out by a Mannich reaction containing compound **12** as a carbonyl component, methyl piperazine as an amine component and formaldehyde. In the ^{1}H NMR spectrum of compound **13**, the signal derived from –SH group disappeared, instead, new signals due to methyl piperazine moiety were detected in the ^{1}H and ^{13}C NMR spectrum of compound **13**.

4-Phenyl-5-pyridin-4-yl-4*H*-1,2,4-triazole-3-thiol (**3**) displayed no antimicrobial activity, whereas its alkylated derivatives and Mannich bases except **5c**, displayed good activities against all tested microorganisms except *Candida tropicalis* and *Candida albicans*. Ethyl [(4-phenyl-5-pyridin-4-yl-4*H*-1,2,4-triazol-3-yl)thio]acetate (**6**) showed moderate antimicrobial activities towards *C. tropicalis* and *C. albicans*, while 2-[(4-phenyl-5-pyridin-4-yl-4*H*-1,2,4-triazol-3-yl)thio]acetohydrazide (**7**) showed no activity against all bacterial and yeast strains. *N*-(4-Methylphenyl)-2-{[(4-phenyl-5-pyridin-4-yl-4*H*-1,2,4-triazol-3-yl)thio]acetyl} hydrazinecarbothioamide (**8**) indicated slight activity against *C. tropicalis* (*Ct.*) and *C. albicans* (*Ca.*). The conversion of hydrazide structure in compound **7** to 1,3,4-oxadiazole ring of 5-{[(4-phenyl-5-pyridin-4-yl-4*H*-1,2,4-triazol-3-yl)thio]-methyl}-1,3,4-oxadiazole-2-thiol (**10**) caused slight antimicrobial activities against *Staphylococcus aureus*, *Bacillus cereus*, *Ct.* and *Ca*. 5-{[(4-Phenyl-5-pyridin-4-yl-4*H*-1,2,4-triazol-3-yl) thio]methyl}-3-{[(2-morpholin-4-ylethyl) amino]methyl}-1, 3,4-oxadiazole-2(3*H*)-thione (**11**), which is the Mannich base derivative of compound **10**, demonstrated slight activity only towards *Escherichia coli*. The conversion of compound **8** to compound **12** caused no important antimicrobial activity. Similarly, 5-{[(4-phenyl-5-pyridine-4-yl-4*H*-1,2,4-triazol-3-yl)thio]methyl}-4-(4-methylphenyl)-2-[(4-methylpi perazin-1-yl)methyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**13**) displayed no activity. On the contrary to the activity of compound **12**, compound **14**, which was obtained from the reaction of compound **8** with H_2SO_4, indicated good activities towards all bacterial strains except *C. tropicalis* and *C. albicans*. The methylated derivative of compound **14**, 5-{[(4-phenyl-5-pyridin-4-yl-4*H*-1,2,4-triazol-3-yl)thio]methyl}-2(4-methylphenyl)-2-methylamino-1,3,4-thiadiazole (**15**), also showed good activity except against *Ct.* and *Ca.* Among *N'*-(arylmethylene)-2-[(4-phenyl-5-pyridin-4-yl-4*H*-1,2,4-triazol-3-yl)thio] acetohydrazides (**9a–h**), which are the Schiff bases of compound **7**, compounds **9a**, **9b**, **9d** and **9f** displayed good activities against the tested microorganisms except *Ct.* and *Ca.*, while **9c**, **9e**, **9g** and **9h** showed no activity.

4. Conclusions

This study reports the synthesis of some heterocyclic compounds containing 1,2,4-triazole, 1,3,4-oxadiazole, 1,3,4-thiadiazole and/or morpholine, piperazine rings. The antimicrobial activity study revealed that all the compounds screened showed good and moderate antimicrobial activities, except compounds **3**, **5c**, **7**, **9c**, **9e**, **9g**, **9h**, **11**, and **13**.

Among compounds **9f–h**, the compound which contains a pyridinyl ring in their structures displayed diverse antimicrobial activities due to the position of nitrogen atom in the heterocyclic ring.

5. Experimental

5.1. Chemistry

Melting points were determined on a Büchi B-540 melting point apparatus and are uncorrected. ^{1}H 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. All the chemicals were obtained from Fluka Chemie AG Buchs (Switzerland).

5.1.1. General method for the synthesis of compounds 2 and 8

A mixture of corresponding compound **1** or **7** (10 mmol) and phenylisothiocyanate (for compound **2**) or 4-methylphenylisothiocyanate (for compound **8**) (15 mmol) was refluxed in ethanol for 4 h. The solution was cooled and a white solid appeared. This was filtered and recrystallized from ethanol (compound **2**) or dimethyl sulfoxide/water (1:2) to afford the desired product.

*5.1.1.1. 2-Isonicotinoyl-N-phenylhydrazinecarbothioamide (**2**).* Yield 90%, m.p. 120 °C. IR (KBr, ν, cm^{-1}): 3375 and 3298 (3NH), 1717 (C=O), 1309 (C=S); Anal. Calcd (%) for $C_{13}H_{12}N_4OS$: C, 57.34; H, 4.44; N, 20.57. Found: C, 57.37; H, 4.48; N, 20.52; ^{1}H NMR (DMSO-d_6, δ ppm): 7.17–7.22 (2H, m, arH), 7.30–7.44 (5H, m, arH), 8.43–8.79 (2H, m, arH), 9.65 (1H, s, NH), 9.75 (1H, s, NH), 10.30 (1H, s, NH); ^{13}C NMR (DMSO-d_6, δ ppm): arC: [125.25 (CH), 128.04 (4CH), 127.89 (2CH), 129.46 (2CH), 133.84 (C), 138.62 (C)], 166.56 (C=O), 180.55 (C=S).

*5.1.1.2. N-(4-Methylphenyl)-2-{[(4-phenyl-5-pyridin-4-yl-4H-1,2,4-triazol-3-yl)thio]acetyl} hydrazinecarbothioamide (**8**).* Yield 85%, m.p. 122 °C. IR (KBr, ν, cm^{-1}): 3216 and 3156 (3NH), 1720 (C=O), 1602 and 1551 (2C=N), 1187 (C=S); Anal. Calcd (%) for $C_{23}H_{21}N_7OS_2$: C, 58.08; H, 4.45; N, 20.62. Found: C, 58.11; H, 4.48; N, 20.64; ^{1}H NMR (DMSO-d_6, δ ppm): 2.14 (3H, s, CH_3), 3.92 (2H, s, S–CH_2), 7.26–7.35 (2H, dd, $j = 6$ Hz, arH), 7.42–7.46 (2H, m, arH), 7.69–7.72 (5H, m, arH), 8.54–8.62 (4H, d, $j = 5$ Hz, arH), 9.31 (1H, s, NH), 9.56 (1H, s, NH), 10.67 (1H, s, NH); ^{13}C NMR (DMSO-d_6, δ ppm): 21.44 (CH_3), 34.00 (S–CH_2), arC: [121.12 (2CH), 121.33 (2CH), 127.83 (2CH), 130.22 (2CH), 131.53 (2CH), 132.92 (C), 134.41 (C), 135.15 (C), 138.67 (C), 147.17 (3CH)], 151.41 (triazole C-3), 154.73 (triazole C-5), 166.38 (C=O), 180.79 (C=S); MS: *m/z* (%) 118.68 (22), 148.76 (27), 254.93 (13), 302.94 (13), 316.97 (13), 334.65 (81), 476.13 (M + 1, 11), 530.10 (11).

5.1.2. General method for the synthesis of compounds 3 and 12

A solution of corresponding carbothioamide **2** (for compound **3**) or **8** (for compound **12**) (10 mmol) in 2 N NaOH was refluxed for 3 h. 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 compounds.

*5.1.2.1. Synthesis of 4-phenyl-5-pyridin-4-yl-4H-1,2,4-triazole-3-thiol (**3**).* Yield 83%, m.p. 195–200 °C. IR (KBr, ν, cm^{-1}): 2652 (SH), 1326 (C=S), 1608 and 1569 (2C=N); Anal. Calcd (%) for $C_{13}H_{10}N_4S$: C, 61.40; H, 3.96; N, 22.03. Found: C, 61.49; H, 4.01; N, 21.98; ^{1}H NMR (DMSO-d_6, δ ppm): 7.8–7.21 (2H, m, arH), 7.39–7.42 (2H, m, arH), 7.49–7.52 (3H, m, arH), 8.45–8.60 (2H, m, arH), 14.36 (1H, s, SH); ^{13}C NMR (DMSO-d_6, δ ppm): arC: [121.74 (2CH), 128.49 (2CH), 129.36 (2CH), 129.63 (CH), 133.00 (C), 133.97 (C), 149.90 (2CH)], 148.24 (triazole C-3), 169.02 (triazole C-5).

*5.1.2.2. 4-(4-Methylphenyl)-5-{[(4-phenyl-5-pyridine-4-yl-4H-1,2,4-triazol-3-yl)thio]methyl}-4H-1,2,4-triazole-3-thiol (**12**).* Yield 48%, m.p. 276 °C. IR (KBr, ν, cm^{-1}): 2732 (SH), 1607, 1516, 1495, 1439 (4C=N), 1237 (C=S); Anal. Calcd (%) for $C_{23}H_{19}N_7S_2$: C, 60.37; H, 4.19; N, 21.43. Found: C,

60.44; H, 4.26; N, 21.32; ^{1}H NMR (DMSO-d_6, δ ppm): 2.36 (3H, s, CH_3), 4.20 (2H, s, CH_2), 7.16–7.52 (7H, m, arH), 7.49–7.53 (2H, m, arH), 8.02–8.09 (2H, m, arH), 8.57–8.62 (2H, m, arH), 13.89 (1H, s, SH); ^{13}C NMR (DMSO-d_6, δ ppm): 26.84 (CH_3), 34.99 (CH_2), arC: [121.72 (2CH), 126.95 (2CH), 127.91 (2CH), 128.32 (2CH), 129.35 (2CH), 132.52 (CH), 134.69 (C), 136.89 (C), 137.91 (C), 139.56 (C), 151.84 (2CH)], 149.07 (triazole C-3), 149.12 (triazole C-3), 151.46 (triazole C-5, C–SH), 159.38 (triazole C-5).

5.1.3. General method for the synthesis of compounds **4** and **15**

To a solution of compound **3** (for compounds **4a** and **4b**) or **14** (for compound **15**) (10 mmol) in absolute ethanol, 1 equiv. of sodium was added and the mixture was stirred at room temperature for 30 min. Then, methyl iodide (for compounds **4a** and **15**) or isobutyl bromide (for compound **4b**) (20 mmol) was added and refluxed for 4 h. After evaporating the solvent under reduced pressure a solid appeared. The solid was recyrstallized from ethanol/water (1:1) to obtain target compound.

*5.1.3.1. 3-Methylthio-4-phenyl-5-pyridin-4-yl-4H-1,2,4-triazole (**4a**).* Yield 46%, m.p. 168–170 °C. IR (KBr, ν, cm^{-1}): 1655 and 1600 (2C=N); Anal. Calcd (%) for $C_{14}H_{12}N_4S$: C, 62.66; H, 4.51; N, 20.88. Found: C, 62.72; H, 4.57; N, 20.81; ^{1}H NMR (DMSO-d_6, δ ppm): 2.74 (3H, s, CH_3), 7.05–7.40 (4H, m, arH), 7.50–7.62 (3H, m, arH), 8.44–8.61 (2H, m, arH); ^{13}C NMR (DMSO-d_6, δ ppm): 14.22 (CH_3), arC: [121.38 (2CH), 127.03 (2CH), 127.24 (CH), 130.45 (2CH), 133.95 (C), 133.99 (C), 150.12 (2CH)], 152.1 (triazole C-3), 171.0 (triazole C-5).

*5.1.3.2. 3-Isobutylthio-4-phenyl-5-pyridin-4-yl-4H-1,2,4-triazole (**4b**).* Yield 54%, m.p. 182–184 °C. IR (KBr, ν, cm^{-1}): 1600 and 1498 (2C=N); Anal. Calcd (%) for $C_{17}H_{18}N_4S$: C, 65.78; H, 5.84; N, 18.05. Found: C, 65.81; H, 5.89; N, 17.99; ^{1}H NMR (DMSO-d_6, δ ppm): 0.88–1.08 (6H, d, $j = 6$ Hz, $2CH_3$), 1.80–2.19 (1H, m, CH), 3.00–3.22 (2H, d, $j = 7$ Hz, CH_2), 7.28–7.34 (2H, m, arH), 7.43–7.58 (2H, m, arH), 7.61–7.74 (3H, m, arH), 8.57–8.62 (2H, d, $j = 4$ Hz, arH); ^{13}C NMR (DMSO-d_6, δ ppm): 21.82 ($2CH_3$), 27.90 (CH), 40.05 (CH_2), arC: [121.82 (2CH), 127.86 (2CH), 130.08 (2CH), 130.15 (CH), 150.03 (2CH), 133.88 (C), 134.05 (C)], 151.99 (triazole C-3), 153.34 (triazole C-5).

*5.1.3.3. 5-{[(4-Phenyl-5-pyridin-4-yl-4H-1,2,4-triazol-3-yl)thio]methyl}-2(4-methylphenyl)-2-methylamino-1,3,4-thiadiazole (**15**).* Yield 54%, m.p. 231 °C. IR (KBr, ν, cm^{-1}): 3183 (NH), 1688 and 1600 (4C=N); Anal. Calcd (%) for $C_{24}H_{21}N_7S_2$: C, 61.12; H, 4.49; N, 20.79. Found: C, 61.14; H, 4.53; N, 20.88; ^{1}H NMR (DMSO-d_6, δ ppm): 2.18 (3H, s, CH_3), 3.88 (3H, s, CH_3), 4.71 (2H, s, S–CH_2), 7.12–7.17 (2H, d, $j = 8$ Hz, arH), 7.30 (2H, br s, arH), 7.46–7.55 (5H, m, arH), 7.55–7.62 (4H, m, arH); ^{13}C NMR (DMSO-d_6, δ ppm): 20.25 (CH_3), 32.45 (S–CH_2), 34.91 (N–CH_3), arC: [121.13 (2CH), 123.76 (2CH), 125.46 (3CH), 128.15 (2CH), 131.48 (2CH), 136.13 (C), 136.61 (C), 137.19 (C), 142.44 (C), 147.21 (2CH)], 152.38 (thiadiazole C-2), 156.44 (triazole C-3 and thiadiazole C-5), 166.02 (triazole C-5).

5.1.4. General method for the synthesis of compounds **5**, **11** and **13**

To a solution of corresponding compound **3**, **10** or **12** (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 h. 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 dimethyl sulfoxide/water (1:2) to yield the title compounds.

*5.1.4.1. 2-[(4-Methylpiperazin-1-yl)aminomethyl]-4-phenyl-5-pyridin-4-yl-2,4-dihydro-3H-1,2,4-triazole-3-thione (**5a**).* Yield 93%, m.p. 170–174 °C. IR (KBr, ν, cm^{-1}): 1602 (C=N), 1326 (C=S); Anal. Calcd (%) for $C_{19}H_{22}N_6S$: C, 62.27; H, 6.05; N, 22.93. Found: C, 62.31; H, 6.11; N, 22.99; ^{1}H NMR (DMSO-d_6, δ ppm): 2.16 (3H, s, CH_3), 2.47–2.58 (8H, t, $j = 6$ Hz, $4CH_2$), 5.25 (2H, s, CH_2), 7.26 (2H, br s, arH), 7.38–7.70 (5H, m, arH), 8.59 (2H, br s, arH).

*5.1.4.2. 4-Phenyl-2-{[(2-morpholin-4-ylethyl)amino]methyl}-5-pyridin-4-yl-2,4-dihydro-3H-1,2,4-triazole-3-thione (**5b**).* Yield 89%, m.p. 191–193 °C. IR (KBr, ν, cm^{-1}): 3051 (NH), 1680 (C=N), 1279 (C=S); Anal. Calcd (%) for $C_{20}H_{24}N_6OS$: C, 62.11; H, 6.10; N, 21.20. Found: C, 62.05; H, 6.17; N, 21.23; ^{1}H NMR (DMSO-d_6, δ ppm): 2.42 (4H, br s, $2CH_2$), 3.38 (4H, br s, $2CH_2$), 3.92 (2H, br s, CH_2), 4.68 (2H, s, CH_2), 5.43–5.70 (2H, m, CH_2), 7.25–7.38 (3H, m, arH), 7.43–7.60 (4H, m, arH), 8.52–8.62 (2H, m, arH), 14.37 (1H, br s, NH).

*5.1.4.3. 4-Phenyl-5-pyridin-4-yl-2-{[(4-{[(4-phenyl-5-pyridine-4-yl-3-thioxo-2,4-dihydro-3H-1,2,4-triazol-2-yl)methyl]amino}piperazin-1-yl)amino]methyl}-2,4-dihydro-3H-1,2,4-triazole-3-thione (**5c**).* Yield 85%, m.p. 245–247 °C. IR (KBr, ν, cm^{-1}): 1600, 1498 (2C=N), 1325 (C=S); Anal. Calcd (%) for $C_{32}H_{30}N_{10}S_2$: C, 62.11; H, 4.89; N, 22.64. Found: C, 62.07; H, 4.95; N, 22.59; ^{1}H NMR (DMSO-d_6, δ ppm): 2.54 (8H, br s, $4CH_2$), 5.21 (4H, br s, $2CH_2$), 7.14 (4H, d, $j = 5$ Hz, arH), 7.38–7.79 (10H, m, arH), 8.72 (4H, d, $j = 5$ Hz, arH).

*5.1.4.4. 5-{[(4-Phenyl-5-pyridin-4-yl-4H-1,2,4-triazol-3-yl)thio]methyl}-3-{[(2-morpholin-4-ylethyl)amino]methyl}-1,3,4-oxadiazole-2(3H)-thione (**11**).* Yield 45%, m.p. 288 °C. IR (KBr, ν, cm^{-1}): 3211 (NH), 1603, 1506 (2C=N), 1265 (C=S); Anal. Calcd (%) for $C_{23}H_{26}N_8O_2S_2$: C, 54.10; H, 5.13; N, 21.94. Found: C, 54.19; H, 5.13; N, 21.56; ^{1}H NMR (DMSO-d_6, δ ppm): 2.52 (4H, br s, $2CH_2$), 2.78 (2H, br s, CH_2), 2.85 (2H, br s, CH_2), 3.41 (4H, br s, $2CH_2$), 4.18 (2H, s, CH_2), 7.20 (2H, s, N*CH*$_2$NH), 7.38–7.63 (7H, m, arH), 8.60 (2H, br s, arH), 14.40 (1H, br s, NH); ^{13}C NMR (DMSO-d_6, δ ppm): 31.61 (CH_2), 49.67 (2NH–*CH*$_2$), 52.77 (2N–*CH*$_2$), 54.67 (N–CH_2), 63.00 (N–*CH*$_2$–N), 67.56 (O–

CH$_2$), arC: [121.72 (2CH), 129.09 (2CH), 129.78 (2CH), 129.92 (CH), 133.54 (C), 133.89 (C), 150.23 (2CH)], 149.19 (triazole C-3), 152.90 (oxadiazole C-2), 166.65 (triazole C-5), 172.77 (oxadiazole C-5).

*5.1.4.5. 5-{[(4-Phenyl-5-pyridine-4-yl-4H-1,2,4-triazol-3-yl)thio]methyl}-4-(4-methyl phenyl)-2-[(4-methylpiperazin-1-yl)methyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione (**13**).* Yield 66%, m.p. 283 °C. IR (KBr, ν, cm^{-1}): 2735 (SH), 1609, 1518, 1498 and 1442 (4C=N); 1609, 1518 and 1494 (3C=N), 1235 (C=S); Anal. Calcd (%) for $C_{29}H_{31}N_9S_2$: C, 61.13; H, 5.48; N, 22.13. Found: C, 61.27; H, 5.50; N, 22.29; ^{1}H NMR (DMSO-d_6, δ ppm): 2.39 (3H, s, CH$_3$), 2.42 (3H, s, CH$_3$), 2.55–2.63 (8H, t, $j = 6$ Hz, 4CH$_2$), 4.23 (2H, s, CH$_2$), 4.58 (2H, s, CH$_2$), 7.18–7.58 (7H, m, arH), 8.63–8.67 (2H, m, arH), 7.55–7.59 (2H, m, arH), 8.11–8.14 (2H, m, arH); ^{13}C NMR (DMSO-d_6, δ ppm): 26.15 (CH$_3$), 35.09 (CH$_2$), 45.62 (CH$_3$), 52.14 (2N–*CH*$_2$), 53.20 (2N–*CH*$_2$), 66.03 (CH$_2$), arC: [121.66 (2CH), 127.09 (2CH), 127.94 (2CH), 128.67 (2CH), 129.42 (2CH), 132.60 (CH), 134.78 (C), 136.98 (C), 137.96 (C), 139.51 (C), 151.71 (2CH)], 149.23 (triazole C-3, second ring), 149.32 (triazole C-3), 151.51 (triazole C-5, C–SH), 159.51 (triazole C-5).

*5.1.5. Ethyl [(4-phenyl-5-pyridin-4-yl-4H-1,2,4-triazol-3-yl) thio]acetate (**6**)*

Method 1: the corresponding compound **3** (10 mmol) was refluxed with 1 equiv. of sodium in absolute ethanol for 2 h. Then, ethyl bromoacetate (10 mmol) was added and refluxed for an additional 3 h. After evaporating the solvent under reduced pressure, a solid appeared. The solid was recrystallized from ethanol/water (1:2) to afford compound **6**. Yield 75%, m.p. 119–121 °C.

Method 2: to a solution of compound **3** (10 mmol) in ethanol, ethyl bromoacetate (10 mmol) was added and the mixture was stirred at room temperature for 1 h and refluxed for 4 h in the presence of triethylamine (10 mmol). Then, the solvent was removed under reduced pressure and a solid obtained. The solid was recrystallized from ethanol/water (1:2) to afford compound **6**. Yield 71%, m.p. 119–121 °C. IR (KBr, ν, cm^{-1}): 1735 (C=O), 1599 and 1499 (2C=N), 1182 (C–O); Anal. Calcd (%) for $C_{17}H_{16}N_4O_2S$: C, 59.98; H, 4.74; N, 16.46. Found: C, 60.02; H, 4.79; N, 16.50; ^{1}H NMR (DMSO-d_6, δ ppm): 1.29 (3H, s, $j = 7$ Hz, CH$_3$), 4.13 (2H, s, S–CH$_2$), 4.22 (2H, q, $j = 7$ Hz, CH$_2$), 7.26–7.35 (5H, m, arH), 7.54–7.63 (4H, m, arH); ^{13}C NMR (DMSO-d_6, δ ppm): 14.05 (CH$_3$), 34.43 (S–CH$_2$), 62.13 (O–CH$_2$), arC: [126.30 (C), 127.08 (3CH), 130.38 (3CH), 130.61 (3CH), 150.12 (C)], 152.2 (triazole C-3), 161.80 (triazole C-5), 169.17 (C=O).

5.1.6. 2-[(4-Phenyl-5-pyridin-4-yl-4H-1,2,4-triazol-3-yl)thio]acetohydrazide (7)

A solution of the corresponding compound **6** (10 mmol) in *n*-butanol was refluxed with hydrazine hydrate (25 mmol) for 4 h. After cooling it to room temperature, a white solid appeared. The solid was recrystallized from dimethyl sulfoxide/water (1:1) to afford the desired product. Yield 98%, m.p. 239–241 °C. IR (KBr, ν, cm^{-1}): 1673 (C=O), 1619 and 1600 (2C=N), 3321 and 3241 (NH–NH$_2$); Anal. Calcd (%) for $C_{15}H_{14}N_6OS$: C, 55.20; H, 4.32; N, 25.75. Found: C, 55.28; H, 4.28; N, 25.69; ^{1}H NMR (DMSO-d_6, δ ppm): 3.90 (2H, s, S–CH$_2$), 4.32 (2H, s, NH$_2$), 7.23–7.38 (2H, dd, $j = 6$ Hz, arH), 7.44–7.48 (2H, m, arH), 7.68–7.74 (3H, m, arH), 8.54–8.62 (2H, d, $j = 5$ Hz, arH), 9.31 (1H, s, NH); ^{13}C NMR (DMSO-d_6, δ ppm): 34.00 (CH$_2$), arC: [122.02 (CH), 127.86 (2CH), 130.05 (2CH), 130.57 (2CH), 133.02 (C), 138.09 (C), 150.06 (2CH)], 152.10 (triazole C-3), 153.30 (triazole C-5), 165.84 (C=O).

*5.1.7. General method for the synthesis of compound **9a**–**9h***

A solution of the corresponding compound **7** (10 mmol) in absolute ethanol was refluxed with appropriate aldehyde (10 mmol) for 3 h. After cooling the mixture to room temperature, a white solid appeared. This crude product was recrystallized from dimethyl sulfoxide/water (1:2) to afford the desired product.

*5.1.7.1. N′-[(4-Methoxyphenyl)methylene]-2-[(4-phenyl-5-pyridin-4-yl-4H-1,2,4-triazol-3-yl)thio]acetohydrazide (**9a**).* Yield 97%, m.p. 224–226 °C. IR (KBr, ν, cm^{-1}): 3189 (NH), 1689 (C=O), 1618 and 1604 (2C=N); Anal. Calcd (%) for $C_{23}H_{20}N_6O_2S$: C, 62.15; H, 4.54; N, 18.91. Found: C, 62.18; H, 4.50; N, 19.01; ^{1}H NMR (DMSO-d_6, δ ppm): 3.84 (3H, s, CH$_3$), 4.58 and 4.18 (2H, s, S–CH$_2$, *trans/cis* conformers), 6.93–7.02 (2H, d, $j = 8$ Hz, arH), 7.29–7.31 (2H, m, arH), 7.42–7.78 (7H, m, arH), 8.19 and 7.98 (1H, s, N=CH, *trans/cis* conformers), 8.57–8.62 (2H, d, $j = 5$ Hz, arH), 11.72 and 11.68 (1H, s, NH, *trans/cis* conformers); ^{13}C NMR (DMSO-d_6, δ ppm): 34.48 (CH$_2$), 54.81 (CH$_3$), 113.82 and 113.36 (N=CH, *trans/cis* conformers), arC: [120.99 (2CH), 122.34 (C), 125.23 (C), 127.06 (3CH), 127.97 (2CH), 129.71 (3CH), 133.34 (C), 143.26 (CH), 149.59 (2CH), 160.24 (C)], 147.67 (triazole C-3), 152.48 (triazole C-5), 167.64 (C=O).

*5.1.7.2. N′-[(4-Fluorophenyl)methylene]-2-[(4-phenyl-5-pyridin-4-yl-4H-1,2,4-triazol-3-yl)thio]-acetohydrazide (**9b**).* Yield 94%, m.p. 231–232 °C. IR (KBr, ν, cm^{-1}): 3186 (NH), 1681 (C=O), 1616 and 1600 (2C=N); Anal. Calcd (%) for $C_{22}H_{17}FN_6OS$: C, 61.10; H, 3.96; N, 19.43. Found: C, 61.16; H, 4.00; N, 19.46; ^{1}H NMR (DMSO-d_6, δ ppm): 4.49 and 4.18 (2H, s, S–CH$_2$, *trans/cis* conformers), 7.22–7.38 (4H, m, arH), 7.51–7.79 (7H, m, arH), 8.22 and 8.12 (1H, s, N=CH, *trans/cis* conformers), 8.63 (2H, br s, arH), 11.97 and 11.75 (1H, s, NH, *trans/cis* conformers); ^{13}C NMR (DMSO-d_6, δ ppm): 34.44 (CH$_2$), 115.67 and 115.24 (N=CH, *trans/cis* conformers), arC: [121.13 (2CH), 127.10 (3CH), 128.67 (2CH), 129.75 (2CH), 130.05 (CH), 132.90 (C), 133.34 (C), 142.26 (CH), 149.63 (2CH), 163.27 (C), 164.89 (C)], 149.86 (triazole C-3), 151.71 (triazole C-5), 167.92 (C=O).

*5.1.7.3. N′-[(3,4-Dihydroxyphenyl)methylene]-2-[(4-phenyl-5-pyridin-4-yl-4H-1,2,4-triazol-3-yl)-thio]acetohydrazide (**9c**).* Yield 89%, m.p. 243–245 °C. IR (KBr, ν, cm^{-1}): 3193 (NH), 1688 (C=O), 1622 and 1609 (2C=N); Anal. Calcd

(%) for $C_{22}H_{18}N_6O_3S$: C, 59.18; H, 4.06; N, 18.82. Found: C, 59.12; H, 4.09; N, 18.79; ^{1}H NMR (DMSO-d_6, δ ppm): 4.54 and 4.11 (2H, s, S–CH_2, *trans*/*cis* conformers), 6.65–6.88 (2H, m, arH), 6.81–6.98 (2H, m, arH), 7.18–7.38 (3H, m, arH), 7.42–7.63 (3H, m, arH), 8.12 and 7.84 (s, N=CH *trans*/*cis* conformers), 8.65 (2H, br s, 2OH), 9.37–9.58 (2H, m, arH), 11.59 and 11.50 (1H, s, NH, *trans*/*cis* conformers); ^{13}C NMR (DMSO-d_6, δ ppm): 35.05 (CH_2), 112.42 and 112.21 (N=CH, *trans*/*cis* conformers), arC: [111.31 (CH), 120.17 (2CH), 120.48 (2CH), 121.37 (CH), 125.08 (C), 125.20 (C), 127.32 (2CH), 130.04 (CH), 130.32 (CH), 133.07 (C), 133.16 (C), 133.89 (C), 144.53 (CH), 149.60 (CH)], 152.82 (triazole C-3), 162.44 (triazole C-5), 167.59 (C=O).

5.1.7.4. N′-[(2-Hydroxyphenyl)methylene]-2-[(4-phenyl-5-pyridin-4-yl-4H-1,2,4-triazol-3-yl)-thio]acetohydrazide (***9d***). Yield 95%, m.p. 235–237 °C. IR (KBr, ν, cm^{-1}): 3189 (NH), 1686 (C=O), 1620 and 1606 (2C=N); Anal. Calcd (%) for $C_{22}H_{18}N_6O_2S$: C, 61.38; H, 4.21; N, 19.52. Found: C, 61.42; H, 4.25; N, 19.60; ^{1}H NMR (DMSO-d_6, δ ppm): 4.65 and 4.22 (2H, s, CH_2, *trans*/*cis* conformers), 6.87–7.05 (3H, m, arH), 7.28–7.44 (4H, m, arH), 7.52–7.71 (6H, m, arH), 8.44 and 8.33 (1H, s, N=CH, *trans*/*cis* conformers), 10.91 and 10.58 (1H, s, ar-OH, *trans*/*cis* conformers), 12.32 and 11.61 (1H, s, C–NH, *trans*/*cis* conformers); ^{13}C NMR (DMSO-d_6, δ ppm): 34.89 and 34.44 (CH_2, *trans*/*cis* conformers), 116.22 and 116.03 (N=CH *trans*/*cis* conformers), arC: [119.29 (2CH), 126.04 (CH), 127.40 (2CH), 128.97 (CH), 130.14 (CH), 130.42 (CH), 131.20 (CH), 131.42 (CH), 141.15 (CH), 146.98 (CH), 149.98 (CH), 152.71 (C), 156.30 (C), 157.13 (C), 157.13 (C)], 152.07 (triazole C-3), 163.01 (triazole C-5), 167.95 (C=O).

5.1.7.5. N′-[(2,6-Dichlorophenyl)methylene]-2-[(4-phenyl-5-pyridin-4-yl-4H-1,2,4-triazol-3-yl)-thio]acetohydrazide (***9e***). Yield 98%, m.p. 265–267 °C. IR (KBr, ν, cm^{-1}): 3186 (NH), 1686 (C=O), 1618 and 1600 (2C=N); Anal. Calcd (%) for $C_{22}H_{16}Cl_2N_6OS$: C, 54.66; H, 3.34; N, 17.39. Found: C, 54.68; H, 3.37; N, 17.41; ^{1}H NMR (DMSO-d_6, δ ppm): 4.50 and 4.19 (2H, s, S–CH_2, *trans*/*cis* conformers), 7.21–7.37 (2H, m, arH), 7.39–7.70 (8H, m, arH), 8.41 and 8.22 (1H, s, N=CH, *trans*/*cis* conformers), 8.57 (2H, br s, arH), 12.14 and 11.99 (1H, s, NH, *trans*/*cis* conformers); ^{13}C NMR (DMSO-d_6, δ ppm): 34.52 (CH_2), 115.92 and 115.68 (N=CH, *trans*/*cis* conformers), arC: [121.52 (2CH), 127.17 (3CH), 128.90 (2CH), 130.12 (2CH), 133.19 (C), 133.22 (C), 142.51 (CH), 149.72 (2CH), 150.23 (C), 165.37 (2C)], 150.49 (triazole C-3), 152.68 (triazole C-5), 167.79 (C=O).

5.1.7.6. 2-[(4-Phenyl-5-pyridin-4-yl-4H-1,2,4-triazol-3-yl)thio]-N′-[pyridin-2-ylmethylene]-acetohydrazide (***9f***). Yield 89%, m.p. 180–183 °C. IR (KBr, ν, cm^{-1}): 3187 (NH), 1691 (C=O), 1622 and 1606 (2C=N); Anal. Calcd (%) for $C_{21}H_{17}N_7OS$: C, 60.71; H, 4.12; N, 23.60. Found: C, 60.68; H, 4.15; N, 23.64; ^{1}H NMR (DMSO-d_6, δ ppm): 4.58 and 4.27 (2H, s, S–CH_2, *trans*/*cis* conformers), 7.21–7.32 (1H, m, arH), 7.39–7.72 (9H, m, arH), 7.84–7.98 (1H, m, arH), 8.22 and 8.07 (1H, s, N=CH, *trans*/*cis* conformers), 8.57–8.70 (2H, m, arH), 12.07 and 11.92 (1H, s, NH, *trans*/*cis* conformers); ^{13}C NMR (DMSO-d_6, δ ppm): 34.65 (CH_2), 119.87 and 119.69 (N=CH, *trans*/*cis* conformers), arC: [120.13 (3CH), 123.46 (CH), 127.76 (CH), 131.69 (2CH), 132.35 (CH), 133.16 (C), 134.70 (C), 136.04 (CH), 144.13 (CH), 147.42 (CH), 148.85 (CH), 149.96 (CH), 152.12 (C)], 153.35 (triazole C-3), 164.31 (triazole C-5), 168.51 (C=O).

5.1.7.7. 2-[(4-Phenyl-5-pyridin-4-yl-4H-1,2,4-triazol-3-yl)thio]-N′-[pyridin-3-ylmethylene]-acetohydrazide (***9g***). Yield 91%, m.p. 186–188 °C. IR (KBr, ν, cm^{-1}): 3184 (NH), 1689 (C=O), 1618 and 1602 (2C=N); Anal. Calcd (%) for $C_{21}H_{17}N_7OS$: C, 60.71; H, 4.12; N, 23.60. Found: C, 60.68; H, 4.15; N, 23.64; ^{1}H NMR (DMSO-d_6, δ ppm): 4.62 and 4.42 (2H, s, S–CH_2, *trans*/*cis* conformers), 7.21–7.29 (4H, m, arH), 7.42–7.69 (6H, m, arH), 8.21 and 8.07 (1H, s, N=CH, *trans*/*cis* conformers), 8.53–8.68 (3H, m, arH), 12.11 and 11.91 (1H, s, NH, *trans*/*cis* conformers); ^{13}C NMR (DMSO-d_6, δ ppm): 34.61 (CH_2), 119.85 and 119.68 (N=CH, *trans*/*cis* conformers), arC: [121.36 (CH), 124.31 (CH), 127.44 (3CH), 130.11 (2CH), 130.34 (CH), 132.68 (C), 133.83 (C), 137.23 (CH), 143.77 (CH), 147.11 (CH), 149.16 (CH), 149.91 (CH), 152.21 (C)], 152.75 (triazole C-3), 164.03 (triazole C-5), 168.55 (C=O).

5.1.7.8. 2-[(4-Phenyl-5-pyridin-4-yl-4H-1,2,4-triazol-3-yl)thio]-N′-[pyridin-4-ylmethylene]-acetohydrazide (***9h***). Yield 87%, m.p. 205–207 °C. IR (KBr, ν, cm^{-1}): 3189 (NH), 1687 (C=O), 1616 and 1600 (2C=N); Anal. Calcd (%) for $C_{21}H_{17}N_7OS$: C, 60.71; H, 4.12; N, 23.60. Found: C, 60.68; H, 4.15; N, 23.64; ^{1}H NMR (DMSO-d_6, δ ppm): 4.60 and 4.41 (2H, s, S–CH_2, *trans*/*cis* conformers), 7.24–7.31 (4H, m, arH), 7.42–7.69 (5H, m, arH), 8.21 and 8.12 (1H, s, N=CH, *trans*/*cis* conformers), 8.51–8.59 (4H, m, arH), 12.10 and 11.90 (1H, s, NH, *trans*/*cis* conformers); ^{13}C NMR (DMSO-d_6, δ ppm): 33.91 (CH_2), 119.82 and 119.67 (N=CH, *trans*/*cis* conformers), arC: [120.39 (2CH), 122.71 (CH), 125.83 (2CH), 129.17 (2CH), 130.41 (CH), 133.11 (C), 133.71 (C), 136.81 (CH), 144.13 (CH), 147.16 (CH), 148.31 (CH), 149.96 (CH), 150.21 (C)], 153.04 (triazole C-3), 164.78 (triazole C-5), 168.49 (C=O).

5.1.8. 5-{[(4-Phenyl-5-pyridin-4-yl-4H-1,2,4-triazol-3-yl)thio]methyl}-1,3,4-oxadiazole-2-thiol (***10***)

Corresponding compound **7** (10 mmol) and CS_2 (0.60 mL, 10 mmol) were added to a solution of KOH (0.56 g, 10 mol) in 50 mL H_2O and 50 mL ethanol. The reaction mixture was refluxed for 3 h. Then, the reaction content was acidified with conc. HCl. The precipitate was filtered off, washed with H_2O and recrystallized from ethanol to afford the desired compound. Yield 57%, m.p. 293 °C. IR (KBr, ν, cm^{-1}): 2565 (SH), 1617 and 1596 (2C=N); Anal. Calcd (%) for $C_{16}H_{12}N_6OS_2$: C, 52.16; H, 3.28; N, 22.81. Found: C, 52.20; H, 3.31; N, 22.78; ^{1}H NMR (DMSO-d_6, δ ppm): 4.60 (2H, s, CH_2), 7.25–7.35 (2H, m, arH), 7.48–7.72 (5H, m, arH), 8.60 (2H, br s, arH), 14.60 (1H, br s, SH); ^{13}C NMR (DMSO-d_6, δ ppm): 26.00 (CH_2), arC: [121.99 (2CH), 127.34 (2CH), 130.04 (2CH), 130.44 (CH), 132.93 (C), 133.59 (C), 149.80 (2CH)], 150.99 (triazole C-3), 152.48 (oxadiazole C-2), 160.02 (triazole C-5), 172.36 (oxadiazole C-5).

*5.1.9. 5-{[(4-Phenyl-5-pyridin-4-yl-4H-1,2,4-triazol-3-yl)thio]methyl}-2(4-methylphenyl)-amino-1,3,4-thiadiazole (**14**)*

A mixture of the corresponding carbothioamide (**8**) (10 mmol) in cold concentrated sulfuric acid (28 mL) was stirred for 10 min. Then, the mixture was allowed reach to cool to 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 afford the desired product. Yield 63%, m.p. 211–214 °C. IR (KBr, ν, cm^{-1}): 3187 (NH), 1696 and 1604 (4C=N); Anal. Calcd (%) for $C_{23}H_{19}N_7S_2$: C, 60.37; H, 4.19; N, 21.43. Found: C, 60.41; H, 4.21; N, 21.39; ^{1}H NMR (DMSO-d_6, δ ppm): 2.22 (3H, s, CH_3), 4.72 (2H, s, CH_2), 10.30 (1H, s, NH), 7.16–7.19 (2H, d, $j = 8$ Hz, arH), 7.34 (2H, br s, arH), 7.48–7.53 (5H, m, arH), 7.58–7.69 (4H, m, arH); ^{13}C NMR (DMSO-d_6, δ ppm): 20.26 (CH_3), 30.55 (CH_2), arC: [117.41 (2CH), 127.43 (2CH), 129.40 (2CH), 130.13 (2CH), 130.51 (3CH), 130.78 (CH), 133.10 (C), 133.59 (C), 138.02 (C), 150.06 (CH), 151.99 (C)], 152.54 (thiadiazole C-2), 155.37 (triazole C-3 and thiadiazole C-5), 165.47 (triazole C-5).

Table 1
Screening for antimicrobial activity of compounds (mm)

Compound no.	Microorganisms and inhibition zone (mm)							
	Ec.	*Yp.*	*Pa.*	*Ef.*	*Sa.*	*Bc.*	*Ct.*	*Ca.*
3[a]	–	–	–	–	–	–	–	–
4a[a]	30	30	30	30	25	30	9	9
4b[a]	25	30	30	30	25	30	8	8
5a[a]	24	24	30	28	24	23	–	–
5b[a]	20	22	14	16	21	15	–	–
5c[a]	–	–	–	–	–	–	–	–
6[a]	–	–	–	–	–	–	13	13
7[a]	–	–	–	–	–	–	–	–
8[a]	–	–	–	–	–	–	7	7
9a[a]	24	30	30	28	28	25	–	–
9b[a]	30	25	38	30	30	23	–	–
9c[a]	–	–	–	–	–	–	–	–
9d[a]	30	30	30	30	25	25	–	–
9e[a]	–	–	–	–	–	–	–	–
9f[a]	42	30	40	20	24	20	–	–
9g[a]	8	7	–	–	–	–	–	–
9h[a]	–	–	–	–	–	–	–	–
10[b]	–	–	–	–	8	6	6	6
11[b]	6	–	–	–	–	–	–	–
12[b]	–	–	–	15	–	6	–	–
13[b]	–	–	–	–	–	–	–	–
14[b]	31	22	35	20	25	20	–	–
15[b]	28	16	15	7	22	17	–	–
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.

5.2. Antimicrobial activity

5.2.1. 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, *S. aureus* ATCC 25923, *B. cereus* 709 ROMA, *C. tropicalis* ATCC 13803 and *C. albicans* ATCC 60193. All the newly synthesized compounds were dissolved in dimethyl sulfoxide (DMSO) and ethanol to prepare chemicals of stock solution of 10 mg/1 mL.

5.2.1.1. Agar-well diffusion method. Simple susceptibility screening test using agar-well diffusion method [30] as adapted earlier [31] was used. Each microorganism was suspended in Mueller Hinton (MH) (Difco, Detroit, MI) broth and diluted approximately to 10^6 colony forming unit (cfu)/mL. They were ‘‘flood-inoculated’’ onto the surface of MH agar and Sabouraud Dextrose Agar (SDA) (Difco, Detriot, MI) and then dried. For *C. albicans* and *C. tropicalis*, SDA were used. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 50 µL of the extract substances was delivered into the wells. The plates were incubated for 18 h at 35 °C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. Ampicillin (10 µg) and Fluconazole (5 µg) were used as standard drugs. Dimethyl sulfoxide and ethanol were used as solvent controls. The antimicrobial activity results are summarized in Table 1.

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