

Synthesis and antimicrobial activities of some new [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles and [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines

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Abstract

Acetic acid hydrazides containing 1,2,4-triazol-3-one ring (**4a-d**) were synthesized by the reaction of compounds **2a-k** with hydrazine hydrate. (5-Mercapto-1,3,4-oxadiazol-2-yl)methyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one derivatives (**5a-d**) were obtained from the reaction of compounds **4a-d** with carbon disulfide in the presence of KOH. The treatment of compounds **5a-d** with hydrazine hydrate led to the conversion of 1,3,4-oxadiazole ring to amino-1,2,4-triazole ring in compounds **6a-d**. A second method involving the reaction of compounds **7a-d** with thiocarbohydrazide also gave the same products, **6a-d**. The condensation of compounds **6a-d** with acetic acid produced 5-alkyl-4-amino-2-[(6-methyl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)methyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-ones (**8a-d**) while its condensation with carbon disulphide or formic acid afforded 5-alkyl-4-amino-2-[(6-mercapto[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)methyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-ones (**9a-d**) and 5-alkyl-4-amino-2-{[(1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)methyl}-2,4-dihydro-3*H*-1,2,4-triazol-3-ones (**10a-d**), respectively. On the other hand, the synthesis of 5-alkyl-4-amino-2-[(6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)methyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-ones (**11a-d**) were performed by the treatment of compounds **6a-d** with α -bromo acetophenone.

Keywords: 1,2,4-Triazol-3-one, [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole, [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine, antimicrobial activity

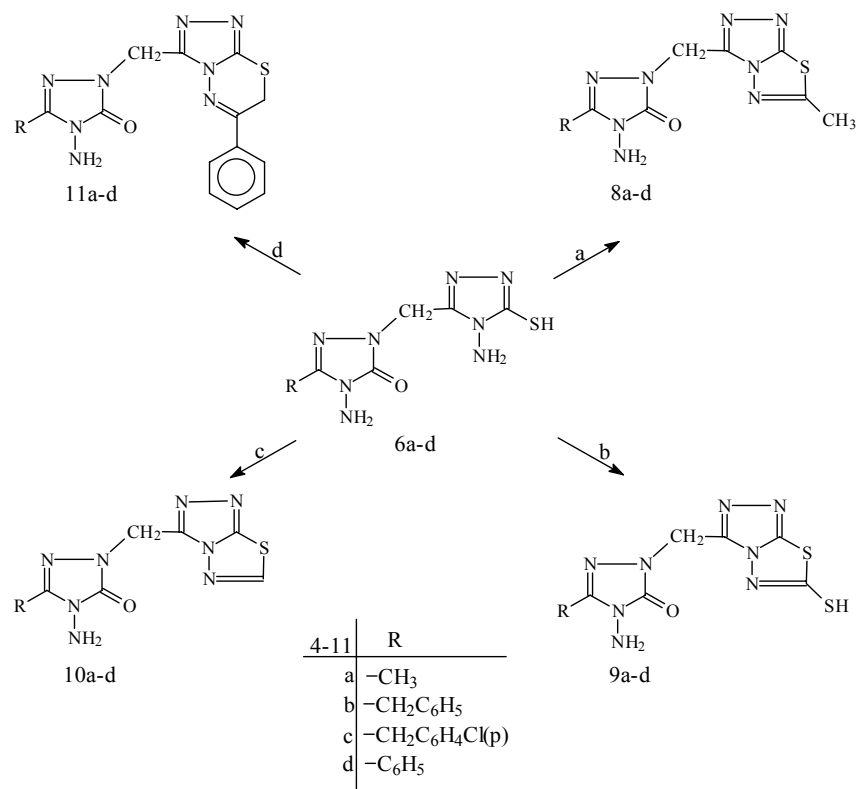
Introduction

The therapeutic effects of 1,2,4-triazole and 1,2,4-triazol-3-one containing compounds have been well studied for a number of pathological conditions including inflammation, cancer, pain, tuberculosis or hypertension.¹⁻⁹ In addition, it was reported that 1,3,4-thiadiazoles exhibit various biological activities possibly due to the presence of the =N-C-S moiety.¹⁰ Moreover, synthesis of

triazoles fused to another heterocyclic ring has attracted wide spread attention due to their diverse applications as antibacterial-, antidepressant-, antiviral-, antitumoral- and anti-inflammatory agents, pesticides, herbicides dyes, lubricant and analytical reagents.^{10,11} Among these, the commonly known systems are generally triazoles fused to pyridines, pyridazines, pyrimidines, pyrazines and triazines. Although there are not many triazoles fused to thiadiazines or thiadiazoles, a number of them are incorporated into a wide variety of therapeutically important compounds possessing a broad spectrum of biological activities.¹¹⁻¹⁵ In this connection, some biheterocyclic compounds containing two 1,2,4-triazol-3-one rings or both 1,2,4-triazol-3-one and 1,3,4-thiadiazole rings have been synthesized in our laboratory as antimicrobial compounds.¹⁶ In addition, we have obtained some Schiff Base derivatives of the 1,2,4-triazol-3-one ring as antitumor agents, recently.^{7,16-18}

4-Amino-1,2,4-triazol-3-thiones can be considered as useful tools in fusing to triazolothiadiazoles or triazolothiadiazines. The amino and mercapto groups are ready-made nucleophilic centers for the synthesis of condensed heterocyclic rigs.^{19,20}

In the present study, prompted by these observations, the synthesis of a series of new [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles (**8**, **9**, **10**) and [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines (**11**) were performed (Scheme 1) and their structures were characterized by ¹H NMR, ¹³C NMR, IR spectroscopy and elemental analysis.



Scheme 1. Synthetic pathway for the preparation of target compounds (**8**, **9**, **10** and **11**). Reagents: (a) CH₃COOH/POCl₃. (b) CS₂/KOH. (c) HCOOH, H₂SO₄. (d) BrCH₂COC₆H₅.

The possible antimicrobial activities of compounds **8a-d**, **9a-d**, **10a-d** and **11a-d** were investigated to ten standard organisms including bacterial and fungal strains. The obtained results were presented in Table 1.

Table 1. Antimicrobial activities of compounds **8-11** ($\mu\text{g/ml}$)

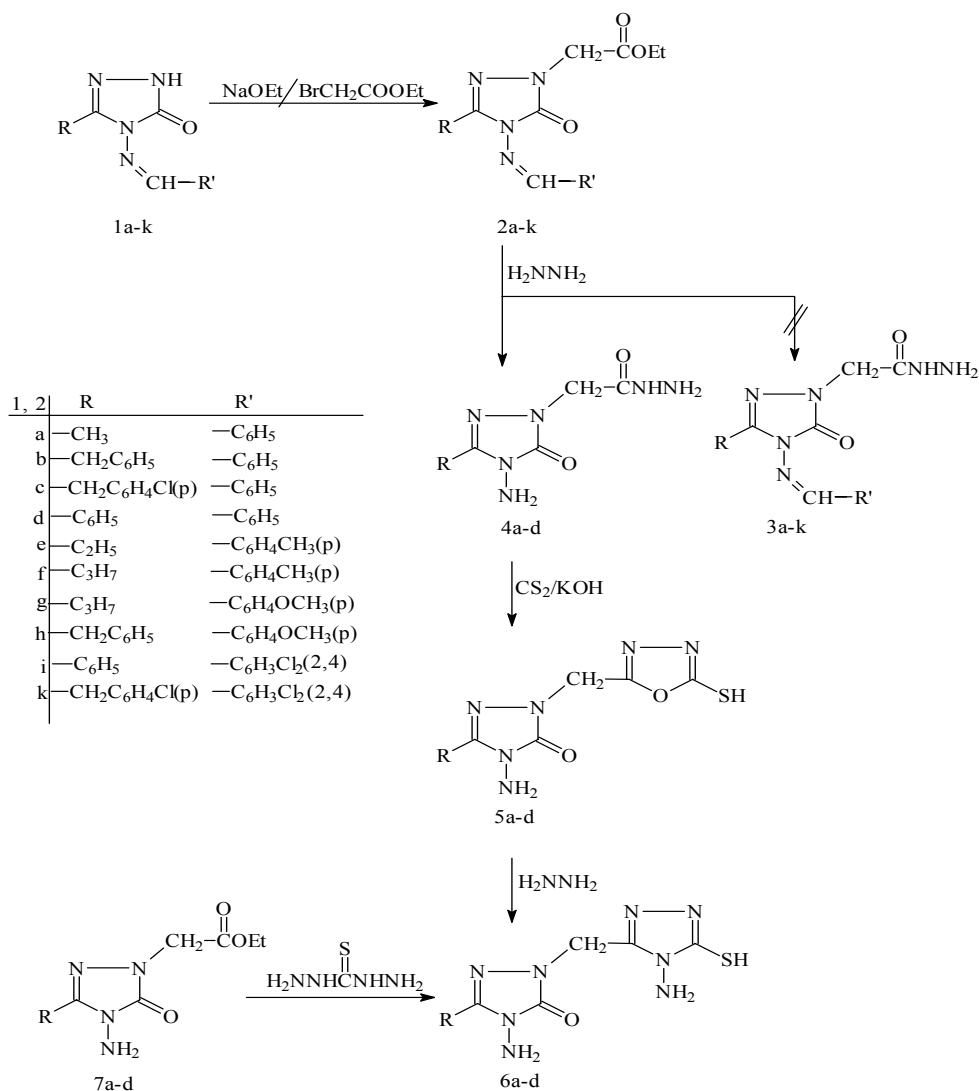
Comp.	<i>Ec</i>	<i>Kp</i>	<i>Yp</i>	<i>Pa</i>	<i>Ef</i>	<i>Sa</i>	<i>Bs</i>	<i>Ca</i>	<i>Ct</i>	<i>Cg</i>
No	MIC	MIC	MIC	MIC	MIC	MIC	MIC	MIC	MIC	MIC
8a	78	78	1250	312	19	19	9	-	-	-
8b	39	39	312	625	9	9	4	-	-	-
8c	39	78	625	312	19	19	9	-	-	-
8d	39	19	625	312	9	9	9	-	-	-
9a	78	9	625	312	9	9	9	-	-	-
9b	39	39	1250	156	9	9	19	-	-	-
9c	39	9	625	625	9	9	9	-	-	-
9d	78	78	>1250	312	39	19	19	-	-	-
10a	>1250	78	625	>1250	78	2	2	-	-	-
10b	>1250	39	1250	>1250	78	4	4	-	-	-
10c	>1250	156	-	>1250	39	19	9	-	-	-
10d	39	78	625	>1250	9	9	9	-	-	-
11a	>1250	39	>1250	>1250	156	19	9	-	-	-
11b	>1250	78	1250	>1250	39	9	4	-	-	-
11c	>1250	156	>1250	>1250	78	39	9	-	-	-
11d	>1250	78	1250	>1250	9	9	4	-	-	-
Amp.	8	32	32	>128	2	2	<1			
Flu.								<1	8	64

Escherichia coli (*Ec*) ATCC 25922, *Klebsiella pneumoniae* (*Kp*) ATCC 13883, *Yersinia pseudotuberculosis* (*Yp*) ATCC 911, *Pseudomonas aeruginosa* (*Pa*) ATCC 10145, *Enterococcus faecalis* (*Ef*) ATCC 29212, *Staphylococcus aureus* (*Sa*) ATCC 25923, *Bacillus subtilis* (*Bs*) ATCC 6633, *Candida albicans* (*Ca*) ATCC 60193, *Candida tropicalis* (*Ct*) ATCC 13803, *Candida glabrata* (*Cg*) ATCC 66032. Amp.: Ampicillin, Flu.: Fluconazole, (—), no activity (5mg/ml).

Results and Discussion

The NH proton at position 1 of 2,4-dihydro-3*H*-1,2,4-triazol-3-one ring is adequately acidic for further reactions and various compounds having biological activity were synthesized via this NH substitution in our laboratories.^{2,4,5,7,21} In the first part of this study, some new ethyl [3-alkyl-4-(ethylidenamino)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl] acetates (**2a-k**) were obtained from

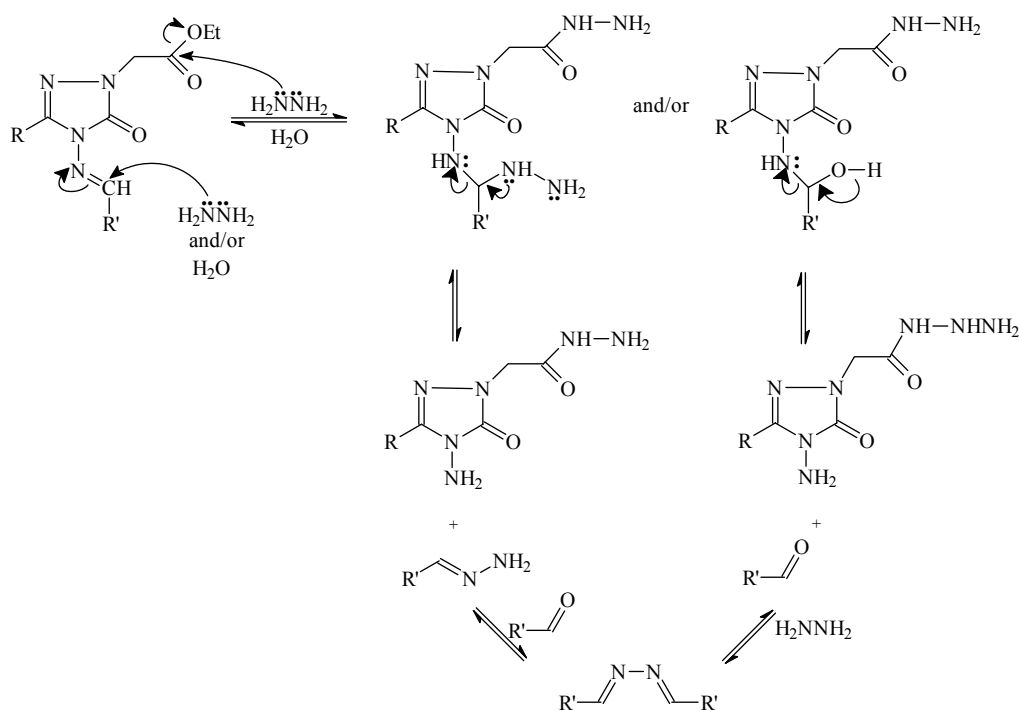
the reaction of 5-alkyl-4-amino-2,4-dihydro-3*H*-1,2,4-triazol-3-ones (**1a-k**) with ethyl bromoacetate in reasonable good yields (Scheme 2).



Scheme 2. Synthetic Pathway for the preparation of title compounds (**6a-d**).

The IR spectra of compounds **2a-k** showed two sharp absorption bands, one of which appearing at 1705-1720 cm⁻¹ was attributed to carbonyl function of 1,2,4-triazol-3-one ring and the other observed at 1744-1761 cm⁻¹ was assigned to -C=O stretching frequency corresponding to acyl carbonyl. In the ¹H NMR spectra, the proton signals due to ester group were recorded between 1.18-1.30 ppm (-OCH₂CH₃) integrating for three protons and 4.08-4.25 ppm (-OCH₂CH₃) integrating for two protons. The ¹³C signals of the same group appeared around 13.86-14.16 ppm (-OCH₂CH₃) and 61.14-62.00 ppm (-OCH₂CH₃). In the NMR spectra of each of compound **2a-k**, there exist signals belonging to corresponding arylidene group at aromatic region.

The treatment of compounds **2a-k** with hydrazine hydrate did not produce expected compounds **3a-k**; instead, compounds **4a-d** formed as reaction products. In the initial stage of this reaction, while hydrazine hydrate should attack both azomethyne group and ester carbonyl, at the same time one molecule H₂O attacks azomethyne group selectively, followed by the elimination of one molecule RCHO or R-CH=NNH₂, and as a result compounds **4a-d** are obtained (Scheme 3).



Scheme 3. The mechanism for the formation of compounds **4**.

The fact that same products were obtained by using four different types of arylidenamino compounds such as benzylidenamino-, *p*-methyl benzylidenamino-, 2,4-dichlorobenzylidenamino- and *p*-methoxybenzylidenamino- supports this mechanism. Compounds **4a-d** were obtained by the first time by a similar way using different type of [1,2,4]triazol-3-one-esters in our laboratory.¹⁶ The spectral data, melting points and elemental analysis of compounds **4a-d** are consistent with those of compounds obtained previously by us.

Acid hydrazides can be considered as useful intermediates leading to the formation of some heterocyclic rings such as 1,2,4-triazol-3-thione, 1,3,4-thiadiazole and 1,3,4-oxadiazole.^{22,23} In the third part of this study, compounds **5a-d** including both 5-mercapto-1,3,4-oxadiazole and 1,2,4-triazol-3-one rings linked to each other via a methylene group were synthesized from the reaction of compounds **4a-d** with carbon disulfide in the presence of potassium hydroxide (Scheme 2). In the IR spectra of compounds **5a-d**, no signal derived from exocyclic carbonyl function was observed, while the signal belonging to carbonyl function of 1,2,4-triazol-3-one

ring was present. Moreover, -NHNH₂ signals disappeared in the ¹H NMR and IR spectra of compounds **5a-d**.

It has been reported that 5-mercapto-1,3,4-oxadiazoles undergo recyclisation with the formation of 4-amino-5-mercapto-1,2,4-triazoles in the reaction with hydrazine hydrate.^{22,23} Continuing in our study, compounds **5a-d** were converted to 5-alkyl-4-amino-2-[4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl)methyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-ones (**6a-d**) by the reported way (Method A).²² The formation of compounds of type **6** was also proven by an alternative method containing the reaction of ethyl (3-alkyl-4-amino-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl) acetates (**7a-d**) with thiosemicarbazide (Method B and Method C) (Scheme 2).

The IR spectra of compounds **5a-d** and **6a-d** displayed the -SH absorption at 2512-2601 cm⁻¹ beside the -C=S absorption at 1268-1388 cm⁻¹. The -NH and -SH protons derived from tautomeric equilibrium resonated between 13.71-14.64 ppm as a broad singlet integrating for one proton. As different from those of **5a-d**, the IR spectra of compounds **6a-d** contained additional -NH₂ absorption bands representing the conversion of oxadiazole structure to aminotriazole structure. Moreover, while proton signals due to -NH₂ group of compounds **5a-d** resonated at 5.23-5.62 ppm integrating for two protons, the same signals resonated about the same ppm integrating for four protons in case of compounds **6a-d**.

The synthesis of 5-alkyl-4-amino-2-[(6-methyl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)methyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-ones (**8a-d**) were performed by the reaction of compounds **6a-d** with acetic acid in the presence of phosphorus oxychloride. The condensation of the same intermediates (**6a-d**) with carbon disulphide in basic media produced a series of 5-alkyl-4-amino-2-[(6-mercapto[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)methyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-ones (**9a-d**), while their condensation with formic acid afforded 5-alkyl-4-amino-2-{[(1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-ylmethyl)}-2,4-dihydro-3*H*-1,2,4-triazol-3-ones (**10a-d**). On the other hand, the reaction of compounds **6a-d** with α -bromoacetophenone resulted in the formation of 5-alkyl-4-amino-2-[(6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)methyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-ones (**11a-d**). The structures of compounds **8a-d**, **9a-d**, **10a-d** and **11a-d** were confirmed by ¹H NMR, ¹³C NMR, IR spectral data and elemental analysis. In the IR spectra of compounds **8**, **9**, **10** and **11**, the absence of absorption bands due to -SH (-C=S) and -NH₂ stretching frequencies of parent compounds (**6a-e**) clearly indicated the fusing between compounds **6a-e** and acetic acid, formic acid carbon disulphide or α -bromoacetophenone. The ¹H NMR spectra of compounds **8a-d** showed a singlet at 2.55-2.64 ppm integrating for three protons of the methyl group at position-6 of triazolothiadiazole ring, while the ¹H NMR spectra of compounds **9a-d** displayed a singlet at 12.97-13.50 ppm integrating one proton due to -SH group. In the ¹³C NMR spectra of compounds **8a-d**, the signal belonging to the methyl carbon at position-6 of triazolothiadiazole ring appeared between 13.55-13.58 ppm. In the ¹H NMR spectra of compounds **11a-d**, the signal observed at 3.27-3.48 ppm integrating for two protons was assigned to -SCH₂ group on triazolothiadiazine ring. The peak belonging to the same group was observed at 33.17-34.16 ppm in the ¹³C NMR spectra. As reported earlier,^{5,16,23,24} the ¹³C signals of triazole-C-3 and triazole-

C-5 in newly synthesized compounds were observed around 143-147 ppm and 149-153 ppm, respectively, while ^{13}C signals derived from C-6 of triazolothiadizole ring of compounds **10a-d** were recorded at 164.47-164.56 ppm. The proton signals of the latter group appeared at 8.25-8.82 ppm as expected. The other signals were recorded at the expected chemical shifts and integral values. Moreover, elemental analyses are consistent with the structures proposed for compounds **8**, **9**, **10** and **11** (Table 2).

Table 2. Experimental data for new compounds

Comp No.	M.P.(°C), Yield (%)	Formula, (M.W. g/mol)	Elemental Analysis, Calculated/Found		
			C	H	N
2a	101-102, 69.20	$\text{C}_{14}\text{H}_{16}\text{N}_4\text{O}_3$ (288.31)	58.32/58.91	5.59/5.59	19.43/19.17
2b	117-118, 98.47	$\text{C}_{20}\text{H}_{20}\text{N}_4\text{O}_3$ (364.40)	65.92/66.18	5.53/5.67	15.38/15.93
2c	127-128, 99.45	$\text{C}_{20}\text{H}_{19}\text{ClN}_4\text{O}_3$ (398.85)	60.23/60.12	4.80/4.83	14.05/14.12
2d	108-109, 64.89	$\text{C}_{19}\text{H}_{18}\text{N}_4\text{O}_3$ (350.38)	65.13/65.42	5.18/5.19	15.99/15.23
2e	110-111, 72.65	$\text{C}_{16}\text{H}_{20}\text{N}_4\text{O}_3$ (316.36)	60.75/61.32	6.37/6.41	17.71/17.67
2f	110-111, 68.91	$\text{C}_{17}\text{H}_{22}\text{N}_4\text{O}_3$ (330.38)	61.80/62.04	6.71/6.87	16.96/16.73
2g	108-109, 66.13	$\text{C}_{17}\text{H}_{22}\text{N}_4\text{O}_4$ (346.38)	58.95/59.19	6.40/6.52	16.18/16.10
2h	117-118, 74.36	$\text{C}_{21}\text{H}_{22}\text{N}_4\text{O}_4$ (394.43)	63.95/63.79	5.62/5.89	14.20/14.13
2i	124-125, 78.13	$\text{C}_{19}\text{H}_{16}\text{Cl}_2\text{N}_4\text{O}_3$ (419.27)	54.43/55.18	3.85/3.94	13.36/13.21
2k	141-142, 76.72	$\text{C}_{20}\text{H}_{17}\text{Cl}_3\text{N}_4\text{O}_3$ (467.74)	51.36/52.02	3.66/3.70	11.98/12.37
5a	236-237, 78.92	$\text{C}_6\text{H}_8\text{O}_2\text{N}_6\text{S}$ (228.23)	31.58/32.08	3.53/3.78	36.82/36.56
5b	194-195, 76.11	$\text{C}_{12}\text{H}_{12}\text{N}_6\text{O}_2\text{S}$ (304.33)	47.36/47.67	3.97/3.98	27.61/28.12
5c	233-234, 77.34	$\text{C}_{12}\text{H}_{11}\text{ClN}_6\text{O}_2\text{S}$ (338.77)	42.55/43.12	3.27/3.31	24.81/24.62
5d	228-229, 72.47	$\text{C}_{11}\text{H}_{10}\text{N}_6\text{O}_2\text{S}$ (290.30)	45.51/45.69	3.47/3.45	28.95/28.63
6a	234-235, 42.19 (A), 23.14(B), 41.35 (C)	$\text{C}_6\text{H}_{10}\text{ON}_8\text{S}$ (242.26)	29.75/29.91	4.16/4.23	46.25/46.10
6b	217-218, 45.63 (A), 22.18 (B), 39.42 (C)	$\text{C}_{12}\text{H}_{14}\text{N}_8\text{OS}$ (318.36)	45.27/46.11	4.43/4.67	35.20/35.53
6c	254-255, 42.39 (A), 18.09 (B), 35.92 (C)	$\text{C}_{12}\text{H}_{13}\text{ClN}_8\text{OS}$ (352.80)	40.85/41.29	3.71/3.77	31.76/31.84
6d	232-233, 42.23 (A), 20.17 (B), 38.91 (C)	$\text{C}_{11}\text{H}_{12}\text{N}_8\text{OS}$ (304.33)	43.41/43.75	3.97/4.07	36.82/36.15
8a	255-256, 53.11	$\text{C}_8\text{H}_{10}\text{N}_8\text{OS}$ (266.28)	36.08/35.68	3.79/3.38	42.08/42.51
8b	196-197, 44.02	$\text{C}_{14}\text{H}_{14}\text{N}_8\text{OS}$ (342.38)	49.11/48.87	4.12/4.33	32.73/32.51
8c	288-289, 51.63	$\text{C}_{14}\text{H}_{13}\text{ClN}_8\text{OS}$ (376.83)	44.62/44.97	3.48/3.55	29.74/29.67
8d	291-292, 50.36	$\text{C}_{13}\text{H}_{12}\text{N}_8\text{OS}$ (328.35)	47.55/47.79	3.68/3.72	34.12/33.99
9a	171-172, 51.27	$\text{C}_7\text{H}_8\text{N}_8\text{OS}_2$ (284.32)	29.57/30.04	2.84/2.86	39.41/38.75
9b	165-166, 49.65	$\text{C}_{13}\text{H}_{12}\text{N}_8\text{OS}_2$ (360.42)	43.32/43.87	3.36/3.39	31.09/30.78

Table 2. Continued

9c	169-170, 52.36	C ₁₃ H ₁₁ ClN ₈ OS ₂ (394.86)	39.54/39.65	2.81/2.90	28.38/28.11
9d	188-189, 51.38	C ₁₂ H ₁₀ N ₈ OS ₂ (346.39)	41.61/41.89	2.91/3.06	32.35/32.24
10a	276-277, 63.70	C ₇ H ₈ N ₈ OS (252.26)	33.33/33.21	3.20/3.05	44.42/44.48
10b	264-265, 65.12	C ₁₃ H ₁₂ N ₈ OS (328.35)	47.55/48.16	3.68/3.72	34.13/33.88
10c	255-256, 58.25	C ₁₃ H ₁₁ ClN ₈ OS (362.80)	43.04/43.65	3.06/3.11	30.89/30.47
10d	289-290, 61.13	C ₁₂ H ₁₀ N ₈ OS (314.33)	45.85/46.13	3.21/3.37	35.65/35.24
11a	278-279, 51.02	C ₁₄ H ₁₄ N ₈ OS (342.80)	49.11/49.33	4.12/4.18	32.73/32.19
11b	210-212, 44.36	C ₂₀ H ₁₈ N ₈ OS (418.48)	57.40/57.75	4.34/4.37	26.78/26.12
11c	267-268, 40.13	C ₂₀ H ₁₇ ClN ₈ OS (452.92)	53.04/53.47	3.78/3.89	24.74/24.51
11d	296-297, 41.35	C ₁₉ H ₁₆ N ₈ OS (404.45)	56.42/55.63	3.99/4.08	27.71/28.26

As seen in Table 1, all newly synthesized compounds exhibited promising activities against *Enterococcus faecalis* (*Ef*), *Staphylococcus aureus* (*Sa*) and *Bacillus subtilis* (*Bs*). The highest activity was observed for compound **8a** with the MIC value of 2 µg/mL. Marginal activities were observed against *Escherichia coli* (*Ec*), *Klebsiella pneumoniae* (*Kp*), *Yersinia pseudotuberculosis* (*Yp*) and *Pseudomonas aeruginosa* (*Pa*). When the MIC's values in Table 1 were compared, it could be seen that all the tested compounds exhibited relatively better activities against Gram positive bacteria than those of Gram negative bacteria, but no antifungal activity was observed against yeast-like fungi.

Experimental Section

General Procedures. Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. ¹H NMR and ¹³C 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 Carlo Erba 1106 elemental analyzer. All the chemicals were obtained from Fluka Chemie AG Buchs (Switzerland). Compounds **1a-k** and **7a-d** were synthesized by published methods.^{5,9,16}

General method for the synthesis ethyl [3-alkyl-4-(ethylidenamino)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl] acetates (2). The corresponding 5-alkyl-4-arylidenamino-2,4-dihydro-3H-1,2,4-triazol-3-one (**1**) (0.01 mol) was refluxed with an equivalent amount of sodium in absolute ethanol for 2 hours. Then, ethyl bromoacetate (0.01 mol) was added and refluxed for an additional 5 hours. Having evaporated it at 35-40°C under reduced pressure, a solid appeared. This was recrystallised from ethanol/water (1:1) to afford the desired compound. **2a.** IR (KBr) (ν, cm⁻¹) 1747 (ester-C=O), 1707 (triazole-C=O), 1606 (-C=N), 1597 (-C=N), 1226 (C-O); ¹H NMR (DMSO-d₆) δ (ppm) 1.22 (t, -OCH₂CH₃, *J* = 7.0 Hz), 2.31 (s, -CH₃), 4.19 (q, -OCH₂CH₃, *J* = 7.0 Hz), 4.61 (s, -NCH₂), 7.42-7.60 (m, 3H, arH), 7.84-7.95 (m, 2H, arH), 9.67 (s, -N=CH);

^{13}C NMR (DMSO- d_6) δ (ppm) 10.82 (-CH₃), 13.89 (-OCH₂CH₃), 46.14 (-NCH₂), 61.15 (-OCH₂CH₃), ar-C: [127.70 (2CH), 128.87 (2CH), 131.54 (CH), 133.06 (C)], 143.65 (triazole-C-3), 149.23 (triazole-C-5), 154.23 (-N=CH), 167.59 (ester-C=O); (EI MS, 70 eV) m/z (%) 289.08 (M^+ ,41), 265.07 (13), 250.09 (13), 249.09 (100), 227.11 (13).

2b. IR (KBr) (ν , cm^{-1}) 1746 (ester-C=O), 1708 (triazole-C=O), 1604 (-C=N), 1574 (-C=N), 1249 (C-O); ^1H NMR (DMSO- d_6) δ (ppm) 1.20 (t, -OCH₂CH₃, J = 7.0 Hz), 4.21-4.32 (m, -OCH₂CH₃ + benzylic -CH₂), 4.65 (s, -NCH₂), 7.15-7.22 (m, 5H, arH), 7.30-7.48 (m, 3H, arH), 7.53-7.80 (m, 2H, arH), 9.63 (s, -N=CH); ^{13}C NMR (DMSO- d_6) δ (ppm) 13.87 (-OCH₂CH₃), 30.71 (benzylic CH₂), 46.28 (-NCH₂), 61.17 (-OCH₂CH₃), ar-C: [126.74 (CH), 127.73 (2CH), 128.39 (2CH), 128.67 (2CH), 128.91 (2CH), 131.58 (CH), 133.05 (C), 135.27 (C)], 145.49 (triazole-C-3), 149.64 (triazole-C-5), 154.09 (-N=CH), 167.55 (ester-C=O).

2c. IR (KBr) (ν , cm^{-1}) 1744 (ester-C=O), 1720 (triazole-C=O), 1603 (-C=N), 1582 (-C=N), 1217 (C-O); ^1H NMR (DMSO- d_6) δ (ppm) 1.20 (t, -OCH₂CH₃, J = 7.0 Hz), 4.13-4.18 (m, -OCH₂CH₃ + benzylic-CH₂), 4.64 (s, -NCH₂), 7.37 (bs, 4H, arH), 7.53 (bs, 3H, arH), 7.79 (bs, 2H, arH), 9.64 (s, -N=CH); ^{13}C NMR (DMSO- d_6) δ (ppm) 13.86 (-OCH₂CH₃), 30.21 (benzylic CH₂), 46.27 (-NCH₂), 61.14 (-OCH₂CH₃), ar-C: [127.73 (2CH), 128.31 (2CH), 128.89 (2CH), 130.60 (2CH), 131.42 (C), 131.59 (CH), 132.97 (C), 134.21 (C)], 145.17 (triazole-C-3), 149.59 (triazole-C-5), 154.15 (-N=CH), 167.49 (ester-C=O); (EI MS, 70 eV) m/z (%) 398.90 (M^+ ,33), 249.97 (14), 248.97 (100), 245.95 (12), 100.05 (14), 60.16 (18).

2d. IR (KBr) (ν , cm^{-1}) 1746 (ester-C=O), 1709 (triazole-C=O), 1611 (-C=N), 1589 (-C=N), 1228 (C-O); ^1H NMR (DMSO- d_6) δ (ppm) 1.25 (t, -OCH₂CH₃, J = 7.0 Hz), 4.20-4.25 (m, -OCH₂CH₃, J = 7.0 Hz), 4.79 (s, -NCH₂), 7.40-7.60 (m, 6H, arH), 7.82-7.98 (m, 4H, arH), 9.64 (s, -N=CH); ^{13}C NMR (DMSO- d_6) δ (ppm) 13.92 (-OCH₂CH₃), 46.67 (-NCH₂), 61.28 (-OCH₂CH₃), ar-C: [125.73 (C), 127.94 (4CH), 128.58 (2CH), 129.04 (2CH), 130.43 (CH), 131.58 (CH), 132.88 (C)], 143.70 (triazole-C-3), 149.76 (triazole-C-5), 157.40 (-N=CH), 167.49 (ester-C=O).

2e. IR (KBr) (ν , cm^{-1}) 1761 (ester-C=O), 1713 (triazole-C=O), 1608 (-C=N), 1590 (-C=N), 1210 (C-O); ^1H NMR (DMSO- d_6) δ (ppm) 1.18-1.27 (m, -OCH₂CH₃+ -CH₂CH₃), 2.37 (s, -CH₃), 2.78 (q, -CH₂CH₃, J = 7.2 Hz), 4.22 (q, -OCH₂CH₃, J = 7.0 Hz), 4.64 (s, -NCH₂), 7.34 (d, 2H, arH, J = 8 Hz), 7.75 (d, 2H, arH, J = 8 Hz), 9.63 (s, -N=CH); ^{13}C NMR (DMSO- d_6) δ (ppm) 9.78 (-CH₂CH₃), 13.93 (-OCH₂CH₃), 18.37 (-CH₂CH₃), 21.00 (-CH₃), 46.28 (-NCH₂), 61.16 (-OCH₂CH₃), ar-C: [127.72 (2CH), 129.55 (2CH), 130.48 (C), 141.73 (C)], 147.35 (triazole-C-3), 149.85 (triazole-C-5), 154.48 (-N=CH), 167.67 (ester-C=O); (EI MS, 70 eV) m/z (%) 317.13 (M^+ ,100), 249.13 (16), 243.07 (15), 126.08 (16).

2f. IR (KBr) (ν , cm^{-1}) 1752 (ester-C=O), 1709 (triazole-C=O), 1601 (-C=N), 1592 (-C=N), 1226 (C-O); ^1H NMR (CDCl₃) δ (ppm) 1.03-1.26 (m, -CH₂CH₂CH₃, J =7.4 Hz), 1.30 (t, -OCH₂CH₃, J = 7.0 Hz), 2.18 (m, -CH₂CH₂CH₃), 2.40 (s, -CH₃), 2.75 (t, -CH₂CH₂CH₃, J = 7.4 Hz), 4.25 (q, -OCH₂CH₃, J = 7.0 Hz), 4.62 (s, -NCH₂), 7.35 (d, 2H, arH, J = 8.0 Hz), 7.90 (d, 2H, arH, J = 8.0 Hz), 9.67 (s, -N=CH); ^{13}C NMR (CDCl₃) δ (ppm) 9.73 (-CH₂CH₂CH₃), 13.87 (-OCH₂CH₃), 18.97 (-CH₂CH₂CH₃), 21.00 (-CH₃), 26.85 (-CH₂CH₂CH₃), 46.20 (-NCH₂), 61.10 (-OCH₂CH₃),

ar-C: [127.68 (2CH), 129.84 (2CH), 131.13 (C), 141.85 (C)], 146.96 (triazole-C-3), 149.82 (triazole-C-5), 154.27 (-N=CH), 167.36 (ester-C=O).

2g. IR (KBr) (ν , cm^{-1}) 1746 (ester-C=O), 1702 (triazole-C=O), 1604 (-C=N), 1574 (-C=N), 1249 (C-O); ^1H NMR (CDCl_3) δ (ppm) 1.02 (bs, $-\text{CH}_2\text{CH}_2\text{CH}_3$), 1.25 (t, $-\text{OCH}_2\text{CH}_3$, $J=7.0$ Hz), 1.61-1.77 (m, $-\text{CH}_2\text{CH}_2\text{CH}_3$), 1.81-2.07 (t, $-\text{CH}_2\text{CH}_2\text{CH}_3$, $J=7.3$ Hz), 3.86 (s, $-\text{OCH}_3$), 4.22 (q, $-\text{OCH}_2\text{CH}_3$, $J=7.0$ Hz), 4.55 (s, $-\text{NCH}_2$), 6.93 (d, 2H, arH, $J=8.0$ Hz), 7.75 (d, 2H, arH, $J=8.0$ Hz), 9.72 (s, $-\text{N}=\text{CH}$); ^{13}C NMR (CDCl_3) δ (ppm) 9.88 ($-\text{CH}_2\text{CH}_2\text{CH}_3$), 13.87 ($-\text{OCH}_2\text{CH}_3$), 19.51 ($-\text{CH}_2\text{CH}_2\text{CH}_3$), 27.45 ($-\text{CH}_2\text{CH}_2\text{CH}_3$), 46.50 ($-\text{NCH}_2$), 55.46 ($-\text{OCH}_3$), 61.83 ($-\text{OCH}_2\text{CH}_3$), ar-C: [114.27 (2CH), 129.63 (2CH), 134.00 (C), 133.17 (C)], 143.62 (triazole-C-3), 151.27 (triazole-C-5), 153.88 (-N=CH), 167.50 (ester-C=O).

2h. IR (KBr) (ν , cm^{-1}) 1746 (ester-C=O), 1709 (triazole-C=O), 1603 (-C=N), 1571 (-C=N), 1210 (C-O); ^1H NMR ($\text{DMSO-}d_6$) δ (ppm) 1.21 (t, $-\text{OCH}_2\text{CH}_3$, $J=7.0$ Hz), 4.08-4.20 (m, $-\text{OCH}_2\text{CH}_3$ + benzylic $-\text{CH}_2$), 4.64 (s, $-\text{NCH}_2$), 7.06 (d, 2H, ar H, $J=7.6$ Hz), 7.13-7.35 (m, 5H, arH), 7.77 (d, 2H, arH, $J=7.6$ Hz), 9.52 (s, $-\text{N}=\text{CH}$); ^{13}C NMR ($\text{DMSO-}d_6$) δ (ppm) 13.89 ($-\text{OCH}_2\text{CH}_3$), 30.76 (benzylic CH_2), 46.29 ($-\text{NCH}_2$), 55.31 ($-\text{OCH}_3$), 61.17 ($-\text{OCH}_2\text{CH}_3$), ar-C: [114.41 (2CH), 125.49 (C), 126.71 (CH), 128.39 (2CH), 128.67 (2CH), 129.58 (2CH), 135.33 (C), 161.95 (C)], 145.43 (triazole-C-3), 149.73 (triazole-C-5), 154.28 (-N=CH), 167.58 (ester-C=O); (EI MS, 70 eV) m/z (%) 395.00 (M^+ , 100), 284.18 (23), 264.94 (28), 248.97 (81), 228.03 (21), 227.02 (49), 212.14 (56), 202.00 (70), 114.99 (20), 123.81 (26), 121.92 (71), 117.96 (92), 116.02 (75), 102.07 (44), 94.09 (23), 86.11 (69), 79.07 (19).

2i. IR (KBr) (ν , cm^{-1}) 1752 (ester-C=O), 1710 (triazole-C=O), 1606 (-C=N), 1587 (-C=N), 1234 (C-O); ^1H NMR ($\text{DMSO-}d_6$) δ (ppm) 1.25 (t, $-\text{OCH}_2\text{CH}_3$, $J=7.0$ Hz), 4.24 (q, $-\text{OCH}_2\text{CH}_3$, $J=7.0$ Hz), 4.79 (s, $-\text{NCH}_2$), 7.40-7.70 (m, 4H, arH), 7.80-8.02 (m, 4H, ar H), 10.07 (s, $-\text{N}=\text{CH}$); ^{13}C NMR ($\text{DMSO-}d_6$) δ (ppm) 13.92 ($-\text{OCH}_2\text{CH}_3$), 46.61 ($-\text{NCH}_2$), 61.35 ($-\text{OCH}_2\text{CH}_3$), ar-C: [125.46 (C), 128.14 (3CH), 128.57 (3CH), 129.40 (C), 129.62 (CH), 130.53 (CH), 135.25 (C), 136.91 (C)], 143.90 (triazole-C-3), 148.80 (triazole-C-5), 151.60 (-N=CH), 167.42 (ester-C=O).

2k. IR (KBr) (ν , cm^{-1}) 1743 (ester-C=O), 1705 (triazole-C=O), 1608 (-C=N), 1581 (-C=N), 1213 (C-O); ^1H NMR (CDCl_3) δ (ppm) 1.29 (t, $-\text{OCH}_2\text{CH}_3$, $J=7.0$ Hz), 4.07 (s, benzylic $-\text{CH}_2$), 4.25 (q, $-\text{OCH}_2\text{CH}_3$, $J=7.0$ Hz), 4.57 (s, $-\text{NCH}_2$), 7.20-7.35 (m, 5H, arH), 7.44 (s, 1H, arH), 7.80 (d, 1H, arH, $J=8.6$ Hz), 10.16 (s, $-\text{N}=\text{CH}$); ^{13}C NMR (CDCl_3) δ (ppm) 14.16 ($-\text{OCH}_2\text{CH}_3$), 31.23 (benzylic CH_2), 46.56 ($-\text{NCH}_2$), 62.00 ($-\text{OCH}_2\text{CH}_3$), ar-C: [127.66 (2CH), 128.88 (2CH), 130.17 (3CH), 133.29 (2C), 136.42 (2C), 137.80 (C)], 145.71 (triazole-C-3), 149.97 (-N=CH), 151.10 (triazole-C-5), 167.34 (ester-C=O).

General method for the synthesis of 5-alkyl-4-amino-2-[(5-mercapto-1,3,4-oxadiazol-2-yl)methyl]-2,4-dihydro-3H-1,2,4-triazol-3-ones (5)

Corresponding compound **4** (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 H_2O and 50 mL ethanol. The reaction mixture was refluxed for 3 hours. After evaporating it in reduced pressure to dryness, a solid obtained. This was

dissolved in 300 mL H₂O and acidified with conc. HCl. The precipitate was filtered off, washed with H₂O and recrystallised from ethanol to afford the desired compound.

5a. IR (KBr) (v, cm⁻¹) 3315 and 3269 (-NH₂), 2551 (-SH), 1674 (-C=O), 1594, 1507 and 1451 (3C=N), 1388 (-C=S); ¹H NMR (DMSO-d₆) δ (ppm) 2.16 (s, -CH₃), 4.99 (s, -NCH₂), 5.23 (s, -NH₂), 14.47 (bs, -NH+SH); ¹³C NMR (DMSO-d₆) δ (ppm) 10.57 (-CH₃), 39.48 (-NCH₂), 146.32 (triazole-C-3), 152.62 (triazole-C-5), 159.09 (Oxadiazole-C-2), 177.85 (Oxadiazole-C-5).

5b. IR (KBr) (v, cm⁻¹) 3308 and 3204 (-NH₂), 2594 (-SH), 1676 (-C=O), 1577, 1523 and 1495 (3C=N), 1330 (-C=S); ¹H NMR (DMSO-d₆) δ (ppm) 2.16 (s, -CH₃), 3.68 (s, benzylic CH₂), 4.92 (s, -NCH₂), 5.58 (s, -NH₂), 7.49 (bs, arH, 3H), 7.86 (bs, 2H, arH), 14.62 (bs, -NH+SH); ¹³C NMR (DMSO-d₆) δ (ppm) 33.17 (benzylic CH₂), 39.37 (-NCH₂), arC: [126.13 (C), 127.72 (2CH), 128.12 (2CH), 131.07 (CH)], 145.18 (triazole-C-3), 152.99 (triazole-C-5), 158.67 (Oxadiazole-C-2), 175.86 (Oxadiazole-C-5).

5c. IR (KBr) (v, cm⁻¹) 3308 and 3205 (-NH₂), 2512 (-SH), 1677 (-C=O), 1582, 1510 and 1488 (3C=N), 1330 (-C=S); ¹H NMR (DMSO-d₆) δ (ppm) 3.62 (s, benzylic CH₂), 5.36 (s, -NCH₂), 5.51 (s, -NH₂), 7.35 (s, 2H, arH, J= 8.2 Hz), 7.56 (s, 2H, arH, J= 8.2 Hz), 14.64 (bs, -NH+SH); ¹³C NMR (DMSO-d₆) δ (ppm) 33.21 (benzylic CH₂), 39.42 (-NCH₂), arC: [126.08 (C), 127.66 (2CH), 128.39 (2CH), 131.88 (C)], 145.77 (triazole-C-3), 152.86 (triazole-C-5), 158.17 (Oxadiazole-C-2), 175.68 (Oxadiazole-C-5).

5d. IR (KBr) (v, cm⁻¹) 3313 and 3202 (-NH₂), 2548 (-SH), 1670 (-C=O), 1536, 1506 and 1450 (3C=N), 1319 (-C=S); ¹H NMR (DMSO-d₆) δ (ppm) 5.21 (s, -NCH₂), 5.62 (s, -NH₂), 7.51 (bs, 3H, arH), 7.87 (bs, 2H, arH), 14.60 (bs, -NH+SH); ¹³C NMR (DMSO-d₆) δ (ppm) 39.37 (-NCH₂), arC: [126.07 (C), 127.62 (2CH), 128.30 (2CH), 130.16 (CH)], 145.75 (triazole-C-3), 153.10 (triazole-C-5), 158.89 (Oxadiazole-C-2), 175.92 (Oxadiazole-C-5).

General method for the synthesis of 5-alkyl-4-amino-2-[4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)methyl]-2,4-dihydro-3H-1,2,4-triazol-3-ones (6)

Method A. To a solution of corresponding compounds **5** (0.01 mol) in 50 mL *n*-butanol hydrazine hydrate (1.46 mL 0.03 mol) was added. Having refluxed this mixture for 4 hours, KOH (0.84 g, 0.015 mol) was added to reaction media and the precipitate formed was filtered off. The solid obtained was acidified with conc. HCl to pH ≈ 3, filtered off, washed with H₂O and recrystallised from dimethyl sulfoxide/water (1:1) solvent.

Method B. The corresponding compound **7** (0.01 mol) and thiocarbohydrazide (0.106 g, 0.01 mol) were heated at 160°C in an oil bath for 2 hours. After cooling it to room temperature, a solid appeared. This was recrystallised from dimethylsulfoxide/water (1:1) to afford the desired product.

Method C. A solution of corresponding compounds **7** (0.01 mol) in 25 mL sodium ethoxide and 25 mL ethanol was refluxed for 30 minutes. Then, thiocarbohydrazide (0.106 g, 0.01 mol) was added and the reaction mixture was refluxed for 10 hours. The reaction mixture was evaporated under reduced pressure to dryness and a solid residue was obtained. This residue was dissolved

in 20 mL H₂O, filtered off and the filtrate was acidified with conc. HCl. The precipitate was filtered off, washed with water and recrystallised from dimethylsulfoxide/water (1:1).

6a. IR (KBr) (ν , cm⁻¹) 3313, 3235 and 3184 (2NH₂), 2596 (-SH), 1704 (-C=O), 1598, 1512 and 1488 (3C=N), 1314 (-C=S); ¹H NMR (DMSO-d₆) δ (ppm) 2.12 (s, -CH₃), 5.05 (s, -NCH₂), 5.58 (bs, 2NH₂), 14.16 (bs, -NH+SH); ¹³C NMR (DMSO-d₆) δ (ppm) 10.45 (-CH₃), 39.66 (-NCH₂), 147.51 (triazole-C-3), 148.69 (triazole-C-5), 153.57 (triazole-C-3'), 160.66 (triazole-C-5').

6b. IR (KBr) (ν , cm⁻¹) 3312, 3237 and 3184 (2NH₂), 2601 (-SH), 1712 (-C=O), 1612, 1591 and 1498 (3C=N), 1311 (-C=S); ¹H NMR (DMSO-d₆) δ (ppm) 3.32 (s, benzylic CH₂), 5.03 (s, -NCH₂), 5.52 (bs, 2NH₂), 7.32 (bs, 3H, arH), 7.85 (bs, 2H, arH), 13.95 (bs, -NH+SH); ¹³C NMR (DMSO-d₆) δ (ppm) 33.15 (benzylic CH₂), 39.12 (-NCH₂), ar-C: [126.12 (C), 127.50 (2CH), 128.12 (2CH), 129.63 (CH)], 147.51 (triazole-C-3), 148.66 (triazole-C-5), 152.99 (triazole-C-3'), 160.67 (triazole-C-5').

6c. IR (KBr) (ν , cm⁻¹) 3325, 3236 and 3164 (2NH₂), 2612 (-SH), 1701 (-C=O), 1615, 1592 and 1467 (3C=N), 1268 (-C=S); ¹H NMR (DMSO-d₆) δ (ppm) 3.11 (s, benzylic CH₂), 4.97 (s, -NCH₂), 5.58 (bs, 2NH₂), 7.37 (d, 2H, arH, *J* = 8.2 Hz), 7.48 (d, 2H, arH, *J* = 8.2 Hz), 13.92 (bs, -NH+SH); ¹³C NMR (DMSO-d₆) δ (ppm) 33.15 (benzylic CH₂), 39.43 (-NCH₂), ar-C: [126.69 (C), 126.95 (2CH), 128.11 (2CH), 129.96 (C)], 147.49 (triazole-C-3), 148.62 (triazole-C-5), 154.12 (triazole-C-3'), 160.24 (triazole-C-5').

6d. IR (KBr) (ν , cm⁻¹) 3312, 3234 and 3185 (2NH₂), 2594 (-SH), 1703 (-C=O), 1614, 1589 and 1482 (3C=N), 1269 (-C=S); ¹H NMR (DMSO-d₆) δ (ppm) 5.05 (s, -NCH₂), 5.62 (bs, 2NH₂), 7.49 (bs, 2H, arH), 7.98 (bs, 2H, arH), 13.71 (bs, -NH+SH); ¹³C NMR (DMSO-d₆) δ (ppm) 39.47 (-NCH₂), ar-C: [127.45 (C), 127.55 (2CH), 128.24 (2CH), 129.97 (CH)], 147.51 (triazole-C-3), 147.68 (triazole-C-5), 153.51 (triazole-C-3'), 160.68 (triazole-C-5').

General method for the synthesis of 5-alkyl-4-amino-2-[(6-methyl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)methyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-ones (8)

To a mixture of corresponding compound **6** (0.01 mol) and acetic acid (0.52 mL, 0.01 mol), phosphorus oxychloride (10 mL) was added and the reaction contents were refluxed for 2 hours on a water bath. After removing the excess of phosphorus oxychloride under reduced pressure, ice water was added to residue with vigorous stirring. The precipitate was filtered off and washed with 20% sodium bicarbonate solution and water. This white solid was recrystallised from dimethylsulfoxide/water (1:1).

8a. IR (KBr) (ν , cm⁻¹) 3315 and 3269 (-NH₂), 1701 (-C=O), 1603, 1595, 1557 and 1446 (4C=N); ¹H NMR (DMSO-d₆) δ (ppm) 2.14 (s, -CH₃), 2.55 (s, -CH₃), 4.82 (s, -NCH₂), 5.55 (s, -NH₂); ¹³C NMR (DMSO-d₆) δ (ppm) 13.55 (-CH₃), 14.80 (-CH₃), 44.47 (-NCH₂), 146.66 (triazole-C-3), 148.18 (triazole-C-5), 154.66 (triazolothiadiazole-C-3), 160.08 (triazolothiadiazole-C-6), 167.86 (triazolothiadiazole-C-8).

8b. IR (KBr) (ν , cm⁻¹) 3313 and 3232 (-NH₂), 1704 (-C=O), 1602, 1594, 1507 and 1485 (4C=N); ¹H NMR (DMSO-d₆) δ (ppm) 2.61 (s, -CH₃), 3.72 (s, benzylic CH₂), 5.42 (s, -NCH₂), 5.57 (s, -NH₂), 7.39 (bs, arH, 3H), 7.46 (bs, 2H, arH); ¹³C NMR (DMSO-d₆) δ (ppm) 13.58 (-CH₃), 33.17

(benzylic CH₂), 44.47 (-NCH₂), ar-C: [125.50 (CH), 129.23 (2CH), 128.10 (2CH), 137.15 (C)], 146.03 (triazole-C-3), 148.54 (triazole-C-5), 153.47 (triazolothiadiazole-C-3), 160.48 (triazolothiadiazole-C-6), 167.11 (triazolothiadiazole-C-8).

8c. IR (KBr) (v, cm⁻¹) 3311 and 3256 (-NH₂), 1704 (-C=O), 1612, 1594, 1516 and 1452 (4C=N); ¹H NMR (DMSO-d₆) δ (ppm) 2.64 (s, -CH₃), 3.76 (s, benzylic CH₂), 4.45 (s, -NCH₂), 5.63 (s, -NH₂), 7.35 (d, arH, 2H, *J* = 8.1 Hz), 7.48 (d, 2H, arH, *J* = 8.1 Hz); ¹³C NMR (DMSO-d₆) δ (ppm) 13.56 (-CH₃), 33.25 (benzylic CH₂), 44.56 (-NCH₂), ar-C: [125.68 (CH), 128.93 (2CH), 128.52 (CH), 137.15 (C), 137.76 (C)], 145.03 (triazole-C-3), 147.44 (triazole-C-5), 153.03 (triazolothiadiazole-C-3), 160.49 (triazolothiadiazole-C-6), 167.56 (triazolothiadiazole-C-8).

8d. IR (KBr) (v, cm⁻¹) 3309 and 3245 (-NH₂), 1703 (-C=O), 1606, 1596, 1509 and 1450 (4C=N); ¹H NMR (DMSO-d₆) δ (ppm) 2.63 (s, -CH₃), 4.52 (s, -NCH₂), 5.63 (s, -NH₂), 7.75 (bs, 3H, arH), 7.86 (bs, 2H, arH); ¹³C NMR (DMSO-d₆) δ (ppm) 13.58 (-CH₃), 44.56 (-NCH₂), ar-C: [125.92 (CH), 128.61 (2CH), 129.90 (2CH), 132.20 (C)], 145.02 (triazole-C-3), 148.49 (triazole-C-5), 154.01 (triazolothiadiazole-C-3), 161.88 (triazolothiadiazole-C-6), 167.48 (triazolothiadiazole-C-8).

General method for the synthesis of 5-alkyl-4-amino-2-[(6-mercapto[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)methyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-ones (9)

To a solution of corresponding compound **6** (0.01 mol) in ethanol, KOH (1 g) and CS₂ (2 mL) were added and the reaction content was allowed to reflux for 2 hours. The solvent removed under reduced pressure, then, ice-water was added onto reaction content while stirring. The obtained solid was washed with water and recrystallised from ethanol.

9a. IR (KBr) (v, cm⁻¹) 3317 and 3264 (-NH₂), 2598 (-SH), 1703 (-C=O), 1608, 1587, 1543 and 1432 (4C=N); ¹H NMR (DMSO-d₆) δ (ppm) 2.17 (s, -CH₃), 4.82 (s, -NCH₂), 5.39 (s, -NH₂), 13.50 (s, -SH); ¹³C NMR (DMSO-d₆) δ (ppm) 13.57 (-CH₃), 46.39 (-NCH₂), 145.63 (triazole-C-3), 149.12 (triazole-C-5), 154.61 (triazolothiadiazole-C-3), 160.24 (triazolothiadiazole-C-6), 168.06 (triazolothiadiazole-C-8).

9b. IR (KBr) (v, cm⁻¹) 3313 and 3232 (-NH₂), 2595 (-SH), 1714 (-C=O), 1602, 1591, 1507 and 1497 (4C=N); ¹H NMR (DMSO-d₆) δ (ppm) 3.56 (s, benzylic CH₂), 5.48 (s, -NCH₂), 5.37 (s, -NH₂), 7.41 (bs, arH, 3H), 7.48 (bs, 2H, arH), 13.48 (-SH); ¹³C NMR (DMSO-d₆) δ (ppm) 33.67 (benzylic CH₂), 44.53 (-NCH₂), ar-C: [119.57 (CH), 119.83 (2CH), 123.72 (2CH), 125.18 (C)], 144.53 (triazole-C-3), 148.92 (triazole-C-5), 153.11 (triazolothiadiazole-C-3), 160.52 (triazolothiadiazole-C-6), 167.35 (triazolothiadiazole-C-8).

9c. IR (KBr) (v, cm⁻¹) 3338 and 3224 (-NH₂), 2557 (-SH), 1718 (-C=O), 1620, 1594, 1517 and 1463 (4C=N), ; ¹H NMR (DMSO-d₆) δ (ppm) 3.76 (s, benzylic CH₂), 4.54 (s, -NCH₂), 5.33 (s, -NH₂), 7.35 (d, arH, 2H, *J* = 8.1 Hz), 7.53 (d, 2H, arH, *J* = 8.1 Hz), 13.42 (-SH); ¹³C NMR (DMSO-d₆) δ (ppm) 33.27 (benzylic CH₂), 44.86 (-NCH₂), ar-C: [121.14 (CH), 121.33 (2CH), 122.49 (CH), 126.15 (C), 127.76 (C)], 145.03 (triazole-C-3), 148.42 (triazole-C-5), 153.82 (triazolothiadiazole-C-3), 161.07 (triazolothiadiazole-C-6), 167.48 (triazolothiadiazole-C-8).

9d. IR (KBr) (ν , cm^{-1}) 3339 and 3223 (-NH₂), 2583 (-SH), 1719 (-C=O), 1604, 1582, 1512 and 1468 (4C=N); ¹H NMR (DMSO-d₆) δ (ppm) 4.52 (s, -NCH₂), 5.33 (s, -NH₂), 7.67 (bs, 3H, arH), 7.73 (bs, 2H, arH), 12.97 (-SH); ¹³C NMR (DMSO-d₆) δ (ppm) 44.41 (-NCH₂), ar-C: [120.23 (CH), 120.64 (2CH), 121.45 (2CH), 130.22 (C)], 144.62 (triazole-C-3), 148.49 (triazole-C-5), 154.00 (triazolothiadiazole-C-3), 161.26 (triazolothiadiazole-C-6), 167.49 (triazolothiadiazole-C-8).

General method for the synthesis of 5-alkyl-4-amino-2-{([1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-ylmethyl)}-2,4-dihydro-3H-1,2,4-triazol-3-ones (10)

A mixture of corresponding compound **6** (0.01 mol) and formic acid (1 mL) in benzene (20 mL) was refluxed for 1 hour. After evaporating the reaction mixture under reduced pressure an oily product was obtained. The crude product was crystallized from ethanol. The obtained product (0.01 mol) was treated with cold conc. H₂SO₄ (15 mL) and the formed oily mass was poured into water. The precipitate was recrystallised from dimethylsulfoxide/water (1:1).

10a. IR (KBr) (ν , cm^{-1}) 3321 and 3266 (-NH₂), 1709 (-C=O), 1606, 1582, 1551 and 1432 (4C=N); ¹H NMR (DMSO-d₆) δ (ppm) 2.19 (s, -CH₃), 4.96 (s, -NCH₂), 5.46 (s, -NH₂), 8.27 (s, -N=CH-S); ¹³C NMR (DMSO-d₆) δ (ppm) 13.68 (-CH₃), 46.35 (-NCH₂), 146.04 (triazole-C-3), 149.12 (triazole-C-5), 154.68 (triazolothiadiazole-C-3), 160.63 (triazolothiadiazole-C-6), 164.48 (-N=CH-S), 168.17 (triazolothiadiazole-C-8).

10b. IR (KBr) (ν , cm^{-1}) 3336 and 3245 (-NH₂), 1707 (-C=O), 1613, 1594, 1511 and 1496 (4C=N); ¹H NMR (DMSO-d₆) δ (ppm) 3.63 (s, benzylic CH₂), 4.78 (s, -NCH₂), 5.42 (s, -NH₂), 7.46 (bs, arH, 3H), 7.53 (bs, 2H, arH), 8.25 (s, -N=CH-S); ¹³C NMR (DMSO-d₆) δ (ppm) 33.58 (benzylic CH₂), 45.12 (-NCH₂), ar-C: [121.31 (CH), 122.23 (2CH), 123.02 (2CH), 124.20 (C)], 144.62 (triazole-C-3), 147.72 (triazole-C-5), 153.34 (triazolothiadiazole-C-3), 160.37 (triazolothiadiazole-C-6), 164.56 (-N=CH-S), 167.03 (triazolothiadiazole-C-8).

10c. IR (KBr) (ν , cm^{-1}) 3335 and 3219 (-NH₂), 1712 (-C=O), 1622, 1531, 1517 and 1484 (4C=N); ¹H NMR (DMSO-d₆) δ (ppm) 3.77 (s, benzylic CH₂), 4.54 (s, -NCH₂), 5.39 (s, -NH₂), 7.33 (d, arH, 2H, J = 8.1 Hz), 7.48 (d, 2H, arH, J = 8.1 Hz), 8.82 (s, -N=CH-S); ¹³C NMR (DMSO-d₆) δ (ppm) 33.45 (benzylic CH₂), 44.57 (-NCH₂), ar-C: [120.33 (CH), 120.86 (CH), 122.48 (2CH), 125.18 (C), 126.48 (C)], 145.24 (triazole-C-3), 147.62 (triazole-C-5), 154.12 (triazolothiadiazole-C-3), 160.47 (triazolothiadiazole-C-6), 164.43 (-N=CH-S), 167.47 (triazolothiadiazole-C-8).

10d. IR (KBr) (ν , cm^{-1}) 3325 and 3229 (-NH₂), 1705 (-C=O), 1612, 1576, 1512 and 1473 (4C=N); ¹H NMR (DMSO-d₆) δ (ppm) 4.85 (s, -NCH₂), 5.42 (s, -NH₂), 7.68 (bs, 3H, arH), 7.83 (bs, 2H, arH), 8.56 (s, -N=CH-S); ¹³C NMR (DMSO-d₆) δ (ppm) 45.21 (-NCH₂), ar-C: [121.41 (2CH), 121.45 (2CH), 121.64 (CH), 130.11 (C)], 144.35 (triazole-C-3), 147.49 (triazole-C-5), 154.93 (triazolothiadiazole-C-3), 160.03 (triazolothiadiazole-C-6), 164.50 (-N=CH-S), 167.38 (triazolothiadiazole-C-8).

General method for the synthesis of 5-alkyl-4-amino-2-[(6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)methyl]-2,4-dihydro-3H-1,2,4-triazol-3-ones (11)

To a suspension of corresponding compound **6** (0.01 mol) in absolute ethanol α -bromoacetophenone (1.99 g, 0.01 mol) was added and the reaction mixture was refluxed for 2 hours, cooled to room temperature and then neutralized with ammonia. The precipitate filtered off and washed with water. The solid obtained recrystallised from dimethylsulfoxide/water (1:1).

11a. IR (KBr) (ν , cm^{-1}) 3318 and 3269 (-NH₂), 1677 (-C=O), 1613, 1592, 1537 and 1462 (4C=N); ¹H NMR (DMSO-d₆) δ (ppm) 2.48 (s, -CH₃), 3.42 (s, -SCH₂), 4.59 (s, -NCH₂), 5.69 (s, -NH₂), 7.38-7.56 (m, 5H, arH); ¹³C NMR (DMSO-d₆) δ (ppm) 13.95 (-CH₃), 33.17 (triazolothiadiazine-C-7), 46.84 (-NCH₂), ar-C: [128.63 (2CH), 129.04 (2CH), 130.82 (CH), 131.25 (C)], 144.83 (triazole-C-3), 146.38 (-triazole-C-5), 154.26 (triazolothiadiazine-C-3), 163.86 (triazolothiadiazine-C-6), 166.59 (triazolothiadiazine-C-9).

11b. IR (KBr) (ν , cm^{-1}) 3317 and 3266 (-NH₂), 1677 (-C=O), 1613, 1594, 1537 and 1462 (4C=N); ¹H NMR (DMSO-d₆) δ (ppm) 3.27 (s, -SCH₂), 3.86 (s, benzylic CH₂), 4.56 (s, -NCH₂), 5.57 (s, -NH₂), 7.28-7.36 (m, 5H, arH), 7.49-7.53 (m, 3H, arH), 7.74-7.86 (m, 2H, arH); ¹³C NMR (DMSO-d₆) δ (ppm) 32.76 (benzylic CH₂), 33.58 (triazolothiadiazole-C-7), 46.70 (-NCH₂), ar-C: [125.08 (CH), 126.53 (2CH), 128.67 (2CH), 129.18 (2CH), 129.66 (2CH), 130.99 (CH), 132.14 (C), 134.23 (C)], 143.62 (triazole-C-3), 145.76 (triazole-C-5), 154.25 (triazolothiadiazine-C-3), 163.83 (triazolothiadiazine-C-6), 166.66 (triazolothiadiazine-C-9).

11c. IR (KBr) (ν , cm^{-1}) 3318 and 3255 (-NH₂), 1677 (-C=O), 1612, 1598, 1529 and 1484 (4C=N); ¹H NMR (DMSO-d₆) δ (ppm) 3.87 (s, benzylic CH₂), 3.37 (s, -SCH₂), 4.69 (s, -NCH₂), 5.63 (s, -NH₂), 7.38-7.46 (m, 5H, arH), 7.58 (d, 2H, arH, J = 8.0 Hz), 7.62 (d, 2H, arH, J = 8.0 Hz); ¹³C NMR (DMSO-d₆) δ (ppm) 32.76 (benzylic CH₂), 34.08 (triazolothiadiazole-C-7), 46.83 (-NCH₂), ar-C: [126.18 (CH), 126.68 (2CH), 127.57 (2CH), 129.64 (2CH), 129.79 (2CH), 131.99 (C), 133.21 (C), 136.23 (C)], 143.95 (triazole-C-3), 146.52 (triazole-C-5), 154.13 (triazolothiadiazine-C-3), 163.85 (triazolothiadiazine-C-6), 166.91 (triazolothiadiazine-C-9).

11d. IR (KBr) (ν , cm^{-1}) 3322 and 3258 (-NH₂), 1679 (-C=O), 1610, 1586 1523 and 1493 (4C=N); ¹H NMR (DMSO-d₆) δ (ppm) 3.48 (s, -SCH₂), 4.72 (s, -NCH₂), 5.67 (s, -NH₂), 7.36-7.42 (m, 3H, arH), 7.43-7.56 (m, 5H, arH), 7.59-7.74(m, 2H, arH); ¹³C NMR (DMSO-d₆) δ (ppm) 34.16 (triazolothiadiazole-C-7), 46.88 (-NCH₂), ar-C: [128.63 (4CH), 129.07 (2CH), 129.91 (CH), 127.61 (2CH), 130.82 (CH), 132.96 (C), 134.62 (C)], 142.92 (5-oxo-triazole-C-3), 144.78 (5-oxo-triazole-C-5), 154.16 (triazolothiadiazine-C-3), 163.86 (triazolothiadiazine-C-6), 167.97 (triazolothiadiazine-C-9).

Antimicrobial activity assessment

All test microorganisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Yersinia pseudotuberculosis* ATCC 911, *Pseudomonas aeruginosa* ATCC 10145, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 60193, *Candida tropicalis* ATCC 13803, *Candida*

glabrata ATCC 66032. All the newly synthesized compounds were weighed and dissolved in dimethylsulphoxide (DMSO) to prepare extract stock solution of 100 mg/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 ($\mu\text{g/ml}$) were determined.²⁵ The antibacterial and antifungal assays were performed in Mueller-Hinton broth (MH) (Difco, Detroit, MI) at pH.7.3 and buffered Yeast Nitrogen Base (Difco, Detroit, MI) at pH 7.0, respectively. The MIC was defined as the lowest concentration that showed no growth. Ampicillin and fluconazole were used as standard antibacterial and antifungal drugs, respectively. Dimethylsulphoxide (DMSO) with dilution of 1:10 was used as solvent control. The results are shown in Table 1.

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