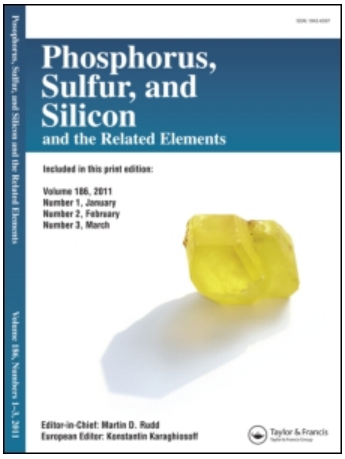

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Synthesis and In-Vitro Antimicrobial Activity of Some Heterocyclic Compounds via 7*H*-1,2,4-triazolo[1,5-*d*]tetrazol-6-ylsulfanyl Acetic Acid Hydrazide

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Synthesis and In-Vitro Antimicrobial Activity of Some Heterocyclic Compounds via 7*H*-1,2,4-triazolo[1,5-*d*]tetrazol-6-ylsulfanyl Acetic Acid Hydrazide

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*{7*H*-1,2,4-triazolo[1,5-*d*]tetrazol-6-ylsulfanyl} acetic acid hydrazide was utilized by different reagent, namely isothiocyanates, formic acid, triethyl orthoformate, and carbon disulfide, to yield the corresponding compounds, which were cyclized to construct 1,2,4-triazoles, 1,3,4-oxadiazoles, 1,3,4-thiadiazoles, and 1,2,4-triazolo[3,4-b]-1,3,4-thiadiazoles. The structure of the products was deduced through physicochemical as well as spectral data (IR, ^{1}H NMR, and MS). Representative members of the prepared compounds were tested for antimicrobial activity.*

Keywords {7*H*-1,2,4-triazolo[1,5-*d*]tetrazol-6-ylsulfanyl} acetic acid hydrazide; antimicrobial activity; structure elucidation; synthesis; various heterocyclic systems

In pursuance of our work on the synthesis[1–3] of some heterocyclic systems with isolated 1,2,4-triazolo[1,5-*d*]tetrazole nucleus, we proposed the synthesis of some 1,2,4-triazoles, 1,3,4-oxadiazoles, 1,3,4-thiadiazoles, and 1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazoles starting from readily available {7*H*-1,2,4-triazolo[1,5-*d*]tetrazol-6-ylsulfanyl} acetic acid hydrazide (**1**) through different reagents. In view of the potential biological activity of members of previous heterocyclic moieties,[4–8] it was of interest to us to prepare new compounds to test their antimicrobial activity.

The synthetic routes followed for preparation of the designed compounds are depicted in Scheme 1. Reaction of {7*H*-1,2,4-triazolo[1,5-*d*]tetrazol-6-ylsulfanyl} acetic acid hydrazide[3] (**1**) with

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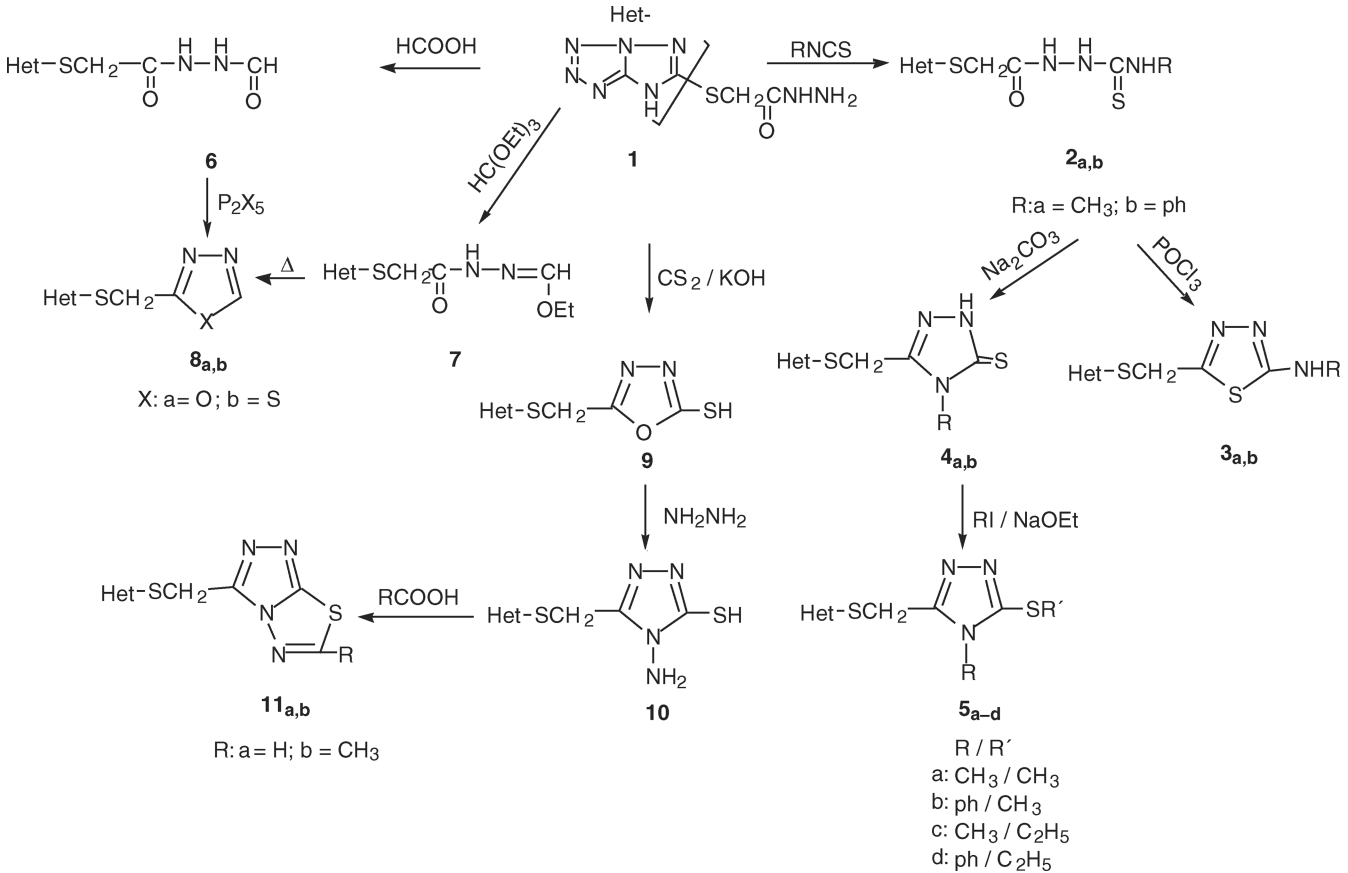

SCHEME 1

methyl or phenyl isothiocyanate in ethanol at ambient temperature[9] afforded 4-methyl(phenyl)-1-{7*H*-1,2,4-triazolo[1,5-*d*]-tetrazol-6-ylsulfanylacetyl}thiosemicarbazide (**2**$_{a,b}$). 1-acyl-4-arylthiosemicarbazides are known to undergo dehydrocyclization in acidic medium to yield 2-alkyl-5-arylamino-1,3,4-thiadiazoles.[10,11] Applying such dehydrocyclization to **2**$_{a,b}$ with phosphoryl chloride gave the corresponding 5-methyl(phenyl)amino-2-methylthio {7*H*-1,2,4-triazolo[1,5-*d*]tetrazol-6-yl}-1,3,4-thiadiazole (**3**$_{a,b}$). The IR spectra of (**3**$_{a,b}$) showed the disappearance of the amide absorption bands present in the spectra of the parent thiosemicarbazides (**2**$_{a,b}$).

1-acyl-4-arylthiosemicarbazides were reported to undergo dehydrocyclization in basic medium to obtain 3-alkyl-4-aryl-5-thioxo-1,2,4-triazoloes.[11,12] Cyclization of thiosemicarbazides (**2**$_{a,b}$) by treatment with aqueous sodium carbonate solution afforded 3-methylthio {7*H*-1,2,4-triazolo[1,5-*d*]tetrazol-6-yl}-4-methyl(phenyl)-1,4-dihydro-5*H*-1,-2,4-triazole-5-thione (**4**$_{a,b}$). ^{1}H NMR spectra of compounds (**4**$_{a,b}$) revealed two exchangeable iminoproton signals of triazolo tetrazole and triazole rings. Further support of the structure of 1,2,4-triazoles (**4**$_{a,b}$) was obtained from their mass spectra (cf. Experimental section). Furthermore, the alkylation of structures (**4**$_{a,b}$) with methyl (ethyl) iodide led to the direct formation of S-alkylated derivatives (**5**$_{a-d}$). The ^{1}H NMR spectra of those products revealed signals characteristic of the 5-methyl (ethyl) thio at 2.65 (4.29, 1.92) ppm. This assignment is in harmony with the reported results.[13]

On the other hand, the reaction of hydrazide (**1**) with formic acid resulted 1-formyl-2-{7*H*-1,2,4-triazolo[1,5-*d*]tetrazol-6-ylsulfanyl} acetylhydrazine (**6**). Ring closure of the latter compound by refluxing with phosphorus pentoxide in toluene yielded the 1,3,4-oxadiazole structure (**8**$_a$). In an alternative route compound (**8**$_a$) was obtained by reaction of (**1**) with triethyl orthoformate, which afforded ethoxyformaldehyde hydrazone structure (**7**) followed by thermal cyclization.

Similarly, 1,3,4-thiadiazole **8**$_b$ was also directly obtained by refluxing compound **6** with phosphorus pentasulfide in toluene.

In addition, the 1,3,4-oxadiazole moiety was also synthesized[14] by the requisite starting hydrazide (**1**) and carbon disulfide in a base catalyzed to produce 1,3,4-oxadiazole ring system (**9**). Hydrazinolysis[15] of the latter compound yielded 4-amino-4*H*-1,2,4-triazole-3-thiol structure (**10**). The ^{1}H NMR spectrum of the latter compound revealed the existance of SH and NH_2 proton signals. Moreover, we found that 1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazole derivatives (**11**$_{a,b}$) can easily be obtained directly from aminothiol (**10**) by adding carboxylic acid derivatives. These structures were inferred on the basis of ^{1}H NMR spectra of (**11**$_{\mathbf{a,b}}$), which

TABLE I Antimicrobial Activity of the Prepared Compounds

	S. aureus		*E. coli*		*C. albicans*	
Compound No.	I.Z.	MIC	I.Z.	MIC	I.Z.	MIC
3b	14	>200	20	>200	19	50
4b	18	50	19	50	13	25
5a	14	100	23	>200	22	100
5d	17	>200	14	>200	12	>200
8a	15	50	21	50	22	50
10	19	100	17	100	20	100
11b	14	>200	15	100	14	100
Ampicillin	40	12.5	36	25	—	—
Clotrimazole	—	—	—	—	38	12.5

IZ = inhibition zone.

revealed the disappearance of the SH and NH_2 signals present in the spectrum of parent compound (**10**).

The antimicrobial activity (determined in Extension Laboratory, Faculty of Agriculture, Alexandria University, Alexandra, Egypt) of prepared compounds $\mathbf{3_b}$, $\mathbf{4_b}$, $\mathbf{5_{a,d}}$, $\mathbf{8_a}$, **10**, and **11b** (Table I) was evaluated against *Staphylococcus aureus, Escherichia coli*, and *Candida albicans* using the cup diffusion technique.[16,17] The results showed that compounds **4b** and **8a** were 25% comparable to the activity of ampicillin against *S. aureus*. The activity of **4b** and **8a** was 50% of the ampicillin against *E. coli*. Moreover, compounds **4b** and (**3b, 8a**) were 50% and 25% against *C. albicans* comparable to clotrimazole, respectively. The rest of the compounds showed lower activity than the reference standards (ampicillin and clotrimazole) against the test organisms.

In conclusion, this investigation demonstrated the utility of {7*H*-1,2,4-triazolo[1,5-*a*]tetrazol-6-ylsulfanyl}acetic acid hydrazide (**1**) as a synthon for the construction of 1,2,4-triazoles, 1,3,4-oxadiazoles, 1,3,4-thiadiazoles, and 1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazoles by choosing the proper cyclizing reagents. The antibacterial and antifungal activities of the prepared compounds were comparable to ampicillin and clotrimazole.

EXPERIMENTAL

Melting points were determined in capillary tubes in a MEL-TEMP II melting apparatus and are uncorrected. The infrared spectra (IR) were recorded on a Perkin-Elmer FT Paragon 1000 and Pye-Unicam SP-300 spectrometers. ^{1}H NMR spectra were scanned on a Varian Mercury

VXR-3000 spectrometer using tetramethyl silane (TMS) as an internal standard. MS were recorded on a Shimadzu GCMS-Q 1000 EX mass spectrometer at 70ev. Microanalyses were performed by the Microanalytical Unit, Cairo University, Giza, Egypt.

4-Methyl-1-{7*H*-1,2,4-triazolo[1,5-*d*]tetrazol-6-ylsulfanylacetyl}thiosemicarbazide (2a)

To a solution of compound **1** (1 g, 4.67 mmoles) in ethanol (10 mL), methyl isothiocyanate (0.34 g, 4.67 mmoles) was added and the mixture was stirred for 6 h at ambient temperature. The precipitate was filtered and crystallized from ethanol-water to give (60%) of **2a**, m.p. 170°C; IR (KBr, ν cm^{-1}): 3340, 3250, 3055 (NH), 1670 (CON), 1630 (C=N); ^{1}H NMR (DMSO-d_6, δ ppm): 12. 41, 11.52, 11.04, 11.00 (s, 1 H each, exchangeable, 4 NH), 4.15 (s, 2 H, CH_2), 2.91 (s, 3 H, NCH_3). Found: C, 25.5; H, 3.5; N, 44.3%. $C_6H_9N_9OS_2$ (287) required: C, 25.1; H, 3.1; N, 43.9%.

4-Phenyl-1-{7*H*-1,2,4-triazolo[1,5-*d*]tetrazol-6-ylsulfanylacetyl}thiosemicarbazide (2b)

To a solution of compound **1** (1 g, 4.67 mmoles) and phenyl isothiocyanate (0.63 g, 4.67 mmoles) as previously described for the procedure of (**2a**) in 55% yield, m.p. 190°C (ethanol-water); ^{1}H NMR (DMSO-d_6, δ ppm): 13.01, 12.42, 11.41, 11.22 (s, 1 H each, exchangeable, 4 NH), 8.24–7.52 (m, 5 H, aromatic H), 4.25 (s, 2 H, CH_2). Found: C, 38.2; H, 3.1; N, 35.9%. $C_{11}H_{11}N_9OS_2$ (349) required: C, 37.8; H, 3.2; N, 36.1%.

5-Methylamino-2-methylthio{7*H*-1,2,4-triazolo[1,5-*d*]tetrazol-6-yl}-1,3,4-thiadiazole (3a)

A mixture of **2a** (0.6 g, 2.09 mmoles) and phosphoryl chloride (15 mL) was heated under reflux for 1 h. The excess of phosphoryl chloride was removed under reduced pressure, the residue added crushed ice, and the mixture was stirred at r.t. for 1 h. During this time the solution was gradually neutralized with a cold saturated solution of sodium bicarbonate, and the product that separated was filtered, washed with water, dried, and crystallized from ethanol to give (54%) of **3a**, m.p. 200°C; IR (KBr, ν cm^{-1}): 3300, 3040 (NH), 1600 (C=N); MS: m/z (%) 270 (M^++1,10). Found: C, 27.1; H, 2.3; N, 46.5%. $C_6H_7N_9S_2$ (269) required: C, 26.8; H, 2.6; N, 46.8%

2-Methylthio{7*H*-1,2,4-triazolo[1,5-*d*]tetrazol-6-yl}-5-phenylamino-1,3,4-thiadiazole (3b)

A mixture of **2b** (0.6 g, 1.72 mmoles) and phosphoryl chloride (15 mL) as previously described for the preparation of (**3a**) in 53% yield, m.p. 215°C (ethanol-water), ^{1}H NMR (DMSO-d_6 δ ppm): 13.10, 12.01 (s, 1 H each, exchangeable, 2 NH), 8.24–7.50 (m, 5H, aromatic H), 4.15 (s, 2H, CH_2). Found: C, 39.6; H, 3.1; N, 37.9%. $C_{11}H_9N_9S_2$ (331) required: C, 39.9; H, 2.7; N, 38.1%.

4-Methyl-3-methylthio{7*H*-1,2,4-triazolo[1,5-*d*]tetrazol-6-yl}-1,4-dihydro-5*H*-1,2,4-triazole-5-thione (4a)

A stirring mixture of compound **2a** (0.6 g, 2.09 mmoles) and 5% aqueous sodium carbonate solution (15 mL) was refluxed for 5 h. After cooling, the resulting solution was acidified with hydrochloric acid, and the precipitate was filtered and crystallized from ethanol to give (71%) of **4a**, m.p. 190°C; IR (KBr, ν cm^{-1}): 3250, 3045 (NH), 1600 (C=N); ^{1}H NMR (DMSO-d_6 δ ppm): 12.15, 11.56 (s, 1H each exchangeable, 2NH), 4.20 (s, 2 H, CH_2), 3.09 (s, 3 H, NCH_3); MS: m/z (%) 269 (M^+, 25), 255 (15), 227 (23), 213 (100). Found: C, 26.5; H, 3.0; N, 46.4%. $C_6H_7N_9S_2$ (269) required: C, 26.8; H, 2.6; N, 46.8%.

3-Methylthio{7*H*-1,2,4-triazolo[1,5-*d*]tetrazol-6-yl}-4-phenyl-1,4-dihydro-5*H*-1,2,4-triazole-5-thione (4b)

A mixture of **2b** (0.6 g, 1.72 mmoles) and 5% aqueous sodium carbonate (15 mL) as previously deacribed for method of **4a** in 61% yield, m.p. 230°C (ethanol); ^{1}H NMR (DMSO-d_6 δ ppm): 12.20, 11.70 (s, 1 H each, exchangeable, 2NH), 8.50–7.60 (m, 5 H, aromatic H), 4.20 (s, 2 H, CH_2); MS: *m*/*z*(%) 331 (M^+, 56), 317 (25), 289 (100). Found: C, 40.2; H, 2.5; N, 38.4%. $C_{11}H_9N_9S_2$ (331) required: C, 39.9; H, 2.7; N, 38.1%.

General procedure for the preparation of 3-methylthio{7*H*-1,2,4-triazolo [1,5-*d*]tetrazol-6-yl}-4-substituted-5-alkylthio-4*H*-1,2,4-triazoles (5_{a-d})

To a solution of compound $\mathbf{4_{a,b}}$ (0.6 g, 2.21 mmoles) in sodium exthoxide (10 mL) was added, and the solution was refluxed for 20 min. The appropriate alkyl iodide (2.21 mmoles) was then added, and refluxing was continued for an additional 1 hour. The reaction mixture was then cooled and poured onto cold water, whereby the solid that formed was filtered off, dried, and crystallized from ethanol.

The following compounds were prepared.

3-Methylthio{7*H*-1,2,4-triazolo[1,5-*d*]tetrazol-6-yl}-4-methyl-5-methylthio-4*H*-1,2,4-triazole (5a)

Yield: 56%, m.p. 145°C; IR (KBr, ν cm^{-1}): 3040 (NH), 1600 (C=N); ^1H NMR (DMSO-d_6 δ ppm): 12.07 (s, 1 H, NH), 4.25 (s, 2 H, CH_2), 3.27 (s, 3 H, NCH_3), 2.65 (s, 3 H, SCH_3). Found: C, 30.0; H, 2.9; N, 44.9%. $C_7H_9N_9S_2$ (283) required: C, 29.7; H, 3.2; N, 44.5%.

3-Methylthio{7*H*-1,2,4-triazolo[1,5-*d*]tetrazol-6-yl}-4-phenyl-5-methylthio-4*H*-1,2,4-triazole (5b)

Yield: 48%, m.p. 170°C; MS: m/z (%) 346 (M^+ + 1, 20). Found: C, 41.3; H, 3.6; N, 36.9%. $C_{12}H_{11}N_9S_2$ (345) required: C, 41.7; H, 3.2; N, 36.5%.

3-Methylthio{7*H*-1,2,4-triazolo[1,5-*d*]tetrazol-6-yl}-4-methyl-5-ethylthio-4*H*-1,2,4-triazole (5c)

Yield: 61%, m.p. 160°C; ^1H NMR (DMSO-d_6 δ ppm): 12.91 (s, 1 H, NH), 4.29 (q, 2 H, SCH_2CH_3), 4.15 (s, 2 H, CH_2), 2.88 (s, 3 H, NCH_3), 1.92 (t, 3 H, SCH_2CH_3). Found: C, 31.9; H, 3.8; N, 42.5%. $C_8H_{11}N_9S_2$ (297) required: C, 32.3; H, 3.7; N, 42.4%.

3-Methylthio{7*H*-1,2,4-triazolo[1,5-*d*]tetrazol-6-yl}-4-phenyl-5-ethylthio-4*H*-1,2,4-triazole (5d)

Yield: 46%, m.p. 190°C; MS: m/z (%) 361 (M^+ + 2, 40). Found: C, 43.0; H, 3.2; N, 35.6%. $C_{13}H_{13}N_9S_2$ (359) required: C, 43.5; H, 3.6; N, 35.1%.

1-Formyl-2-{7*H*-1,2,4-triazolo[1,5-*d*]tetrazol-6-ylsulfanylacetyl}hydrazine (6)

A solution of compound **1** (1 g, 4.67 mmoles) in formic acid (15 mL) was refluxed for 1 h. The solvent was evaporated and the residue was crystallized from ethanol to afford (47%) of **6**, m.p. 180°C; IR (KBr, ν cm^{-1}): 3320, 3240, 3020 (NH), 1690, 1660 (CON), ^1H NMR (DMSO-d_6, δ ppm): 12.42, 11.50, 10.99 (s, 1 H each, exchangeable, 3 NH), 8.40 (s, 1 H, formyl H), 4.30 (s, 2 H, CH_2). Found: C, 25.2; H, 2.8; N, 45.9%. $C_5H_6N_8O_2S$ (242) required: C, 24.8; H, 2.5; N, 46.3%.

Ethoxyformaldehyde{7*H*-1,2,4-triazolo[1,5-*d*]tetrazol-6-ylsulfanylacetyl} hydrazone (7)

The title compound was prepared from **1** (1 g, 4.67 mmoles), and triethyl orthoformate (10 mL) was heated at reflux for 5 h and then evaporated under reduced pressure. The obtained residue was crystallized from ethanol to the result (56%) of **7**, m.p. 175°c; IR (KBr, ν cm^{-1}): 3340, 3120 (NH), 1670 (CON), 1630 (C=N); ^{1}H NMR (DMSO-d_6, δ ppm): 13.01, 12.12 (s, 1 H each, 2 NH), 7.01 (s, 1 H, HC=N), 4.31 (q, 2 H, CH_2CH_3), 4.15 (s, 2 H, CH_2), 1.85 (t, 3 H, CH_2CH_3). Found: C, 31.5; H, 3.5; N, 41.2%. $C_7H_{10}N_8O_2S$ (270) required: C, 31.1; H, 3.7; N, 41.5%.

2-Methylthio{7*H*-1,2,4-triazolo[1,5-*d*]tetrazol-6-yl}-1,3,4-oxadiazole (8a)

Method A

To a solution of compound **6** (1 g, 4.13 mmoles) in toluene (20 mL), phosphorus pentoxide (4.13 mmoles) was added. The mixture was refluxed for 2 h. The solvent was evaporated, and water (5 mL) was added and extracted with chloroform. The solvent was evaporated and the residue was crystallized from ethanol to yield (44%) of **8a**, m.p. 130°C; IR (KBr, ν cm^{-1}): 3160 (NH); ^{1}H NMR (DMSO-d_6, δ ppm): 12.31 (s, 1 H, NH), 8.35 (s, 1 H, oxadiazole H), 4.25 (s, 2 H, CH_2); MS: m/z (%) 224 (M^+, 28). Found: C, 27.1; H, 2.3; N, 50.3%. $C_5H_4N_8OS$ (224) required: C, 26.8; H, 1.8; N, 50.0%.

Method B

Compound **7** (1 g, 3.70 mmoles) was heated at 10°C above its melting point for 20 min in an oil bath. The mass obtained after cooling was crystallized from ethanol to give (56%) of **8a**.

2-Methylthio{7*H*-1,2,4-triazolo[1,5-*d*]tetrazol-6-yl}-1,3,4-thiadiazole (8b)

A solution of compound **6** (1 g, 4.13 mmoles) in toluene (20 mL) was treated with phosphorus pentasulfide (0.004 mole) and heated under reflux for 1 h. The solvent was evaporated, water (5 mL) was added and the obtained product was crystallized from ethanol to give (61%) of **8b**, m.p. 150°C; MS: m/z (%) 242 (M^++ 2, 43). Found: C, 24.6; H, 2.1; N, 47.1%. $C_5H_4N_8S_2$ (240) required: C, 25.0; H, 1.7; N, 46.7%.

2-Methylthio{7*H*-1,2,4-triazolo[1,5-*d*]tetrazol-6-yl}-1,3,4-oxadiazole-5-thiol (9)

Carbon disulfide (4.67 mmoles) was added to a mixture of **1** (1 g, 4.67 mmoles) and potassium hydroxide (4.67 mmoles) in ethanol (30 mL). The reaction mixture was refluxed for 5 h and then, poured onto water followed by the addition of hydrochloric acid untill the solution became slightly acidic. The formed solid was filtered, dried, and crystallized from ethanol to yield (59%) of **9**, m.p. 180°C: ^{1}H NMR (DMSO-d_6, δ ppm): 12.01 (s, 1 H, NH), 4.30 (s, 2 H, CH_2), 3.82 (s, 1 H, SH). Found: C, 23.7; H, 2.1; N, 43.3%. $C_5H_4N_8OS_2$ (256) required: C, 23.4; H, 1.6; N, 43.8%.

4-Amino-4*H*-5-methylthio{7*H*-1,2,4-triazolo[1,5-*d*]tetrazol-6-yl}-1,2,4-triazole-3-thiol (10)

A solution of compound **9** (0.5 g, 1.95 mmoles), in ethanol (10 mL), was treated with 95% hydrazine (5 mL) was refluxed for 3 h, diluted with cold water, and acidified by hydrochloric acid. The solid mass was filtered, washed with water, and crystallized from ethanol to give (48%) of **10**, m.p. 160°C; IR (KBr, ν cm^{-1}): 3230 (NH_2), 3130 (NH); ^{1}H NMR (DMSO-d_6, δ ppm): 12.70 (s, 1 H, NH), 5.72 (s, 2 H, NH_2), 4.25 (s, 2 H, CH_2), 3.80 (s, 1 H, SH). Found: C, 22.5; H, 2.6; N, 52.3%. $C_5H_6N_{10}S_2$ (270) required: C, 22.2; H, 2.2; N, 51.9%.

3-Methylthio{7*H*-1,2,4-triazolo[1,5-*d*]tetrazol-6-yl}-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazole (11a)

A mixture of aminothiol **10** (0.5 g, 1.85 mmoles) and formic acid (10 mL) was refluxed for 2 h. The mixture was evaporated under reduced pressure, and the obtained residue was crystallized from ethanol to give (65%) of **11a**, m.p. 125°C; IR (KBr, ν cm^{-1}): 3090 (NH); ^{1}H NMR (DMSO-d_6, δ ppm): 12.33 (s, 1 H, NH), 8.23 (s, 1 H, CH), 4.15 (s, 2 H, CH_2), MS: m/z (%) 281 ($M^+ + 1$, 56). Found: C, 26.1; H, 1.9; N, 50.4%. $C_6H_4N_{10}S_2$ (280) required: C, 25.7; H, 1.4; N, 50.0%.

6-Methyl-3-methylthio{7*H*-1,2,4-triazolo[1,5-*d*]tetrazol-6-yl}-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazole (11b)

Compound **11b** was prepared from **10** (0.5 g, 1.85 mmoles) and acetic acid (10 mL) as previously described for the preparation of **11a**. It was crystallized from ethanol, yield (62%), m.p. 140°C, ^{1}H NMR (DMSO-d_6, δ ppm): 12.01 (s, 1 H, NH), 4.20 (s, 2 H, CH_2), 1.94 (s, 3 H, CH_3). Found:

C, 29.1; H, 2.4; N, 48.1%. $C_7H_6N_{10}S_2$ (294) required: C, 28.6; H, 2.0; N, 47.6%.

Antimicrobial Screening

The products were in vitro screened for activity against a variety of Gram-positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli*) and yeast-like fungi (*Candida albicans*), using the cup diffusion technique[16] to determine the inhibition zones (IZ, in mm). Furthermore, the microdilution suceptibility test in Müller-Hinton Broth (Oxoid) and Sabouraud Liquid Medium (Oxoid) were used for the determination of antibacterial and antifungal activities in the form of the MIC (in μg).[17] Ampicillin trihydrate and clotrimazole were used as standard antibacterial and antifungal agents, respectively.

Inhibition Zone Measurement

The compounds were dissolved in propylene glycol at a concentration of 1 mg/mL. Sterile nutrient agar (Oxoid) was incubated with the tested organism, so as each 100 mL of the medium received 1 mL of a 24-h boroth culture, 3 drops of the tested compounds were placed separately in cups (8 mm diameter), cut in the agar. The plates were incubated at 37°C for 24 h, and the resultant IZs were measured in mm. Propylene glycol alone showed no inhibition to any of the tested organisms. Ampicillin trihydrate and clotrimazole at a concentration of 0.1% solution in propylene glycol were used as standards.

Minimal Inhibitory Concentration Measurement

Solutions of the test compounds, ampicillin trihydrate and clotrimazole, were prepared in DMSO at a concentration of 1600 μg/mL. The twofold dilution of the compounds were prepared (800, 400, ... 6.25 μg/mL). The microorganism suspensions at 10^6 CFU/mL (Colony Forming Unit/mL) concentration were inoculated to the corresponding wells. Plates were incubated at 36°C for 24 h to 48 h. The incubation chamber was kept sufficiently humid. At the end of the incubation period, the MICs were determined. Controls for the DMSO microorganisms and media microorganisms were also done.

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