New Regioselective Synthesis and Biological Activity of Substituted 1H-[1,2,4]Triazolo[3,4-c][1,2,4]triazoles

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Two regioselective synthetic approaches for the title compounds **7** *via* reaction of hydrazonoyl halides **1** with 3-methylthio-5-phenyl-1,2,4-triazole **3** and base-catalyzed cyclization of N-phenyl-N-(5-phenyl-s-triazol-3-yl)thiohydrazides **6** are described. The mechanisms of the reactions studied and the biological activity of the isolated products **6** and **7** are pointed out.

Keywords: Hydrazonoyl halides; Nitrilimines; s-Triazolo[3,4-c]-s-triazoles; Antimicrobial activity.

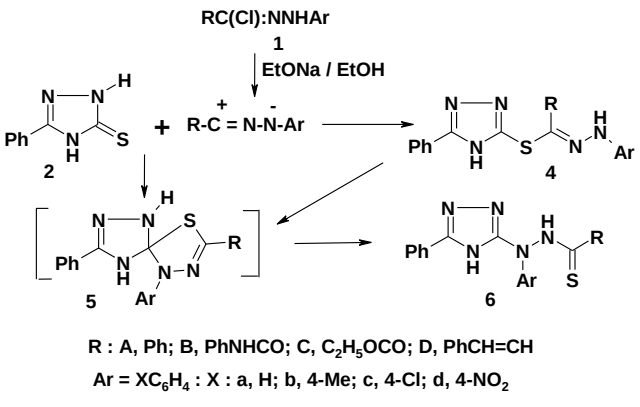
INTRODUCTION

A literature search revealed that many derivatives of 1,2,4-triazoles and annelated 1,2,4-triazoles have received considerable attention among medicinal chemists because such compounds have been found to display a wide range of antihypertensive, antifungal, and antibacterial activities as well as being potential agents in agricultural chemistry.[1,2] In the light of these considerations and in continuation of our interest in developing new syntheses of functionally substituted heterocycles utilizing hydrazonoyl halides **1**,[3-6] we investigated the reactivity of the latter halides with 5-phenyl-1,2,4-triazole-3(2H)-thione **2** and its 3-methylthio derivative **3** with the following objectives in mind. Firstly, we thought it interesting to explore the utility of the halides **1** as synthons for new derivatives of 1,2,4-triazole and fused 1,2,4-triazolo-1,2,4-triazole ring systems. The second objective of the present study was to shed some light on the regiochemistry of the target reactions. This is because both of **2** and **3** have more than one reactive site so that their reactions with **1** can lead to isomeric products depending on which nitrogen (N2 or N4) of the 1,2,4-triazole ring will participate. For example, condensation of **1** with **3** can lead to the formation of [1,2,4]triazolo[3,4-c][1,2,4]triazole derivatives **7** and/or [1,2,4]triazolo[4,3-b][1,2,4]triazole **8** (Scheme IV). Furthermore, it was thought worthwhile to explore the biological activity of the products that will be isolated from the reactions to be studied.

RESULTS AND DISCUSSION

Treatment of the hydrazonoyl halides **1** with 5-phenyl-1,2,4-triazole-3(2H)-thione **2** in ethanol in the presence of sodium ethoxide at room temperature afforded, in each case, only one isolable product as evidenced by tlc analysis of the crude products. Both microanalysis and mass spectral data were consistent with either the thiohydrazonate structure **4** or the thiohydrazide structure **6** (Scheme I). A comparison of the mass spectra of the isolated products with those of typical thiohydrazonate esters immediately ruled out the thiohydrazonate type structure **4**. This is because the mass spectra of both aryl and heteroaryl thiohydrazonates were reported to be characterized by elimination of the elements of the corresponding arenethiol and heteroaryl thiol from their molecular ions, respectively.[7] Such peaks were absent in the mass spectra of the isolated products. Instead, the mass spectra of **6** exhibited a characteristic peak at *m/z* corresponding to (M^+ - RCSN) species. This finding indicates that the isolated products have the thiohydrazide structure **6**. Further evidence in support of the assigned structure **6** for the isolated products was derived from their ^{13}C NMR spectra which revealed the

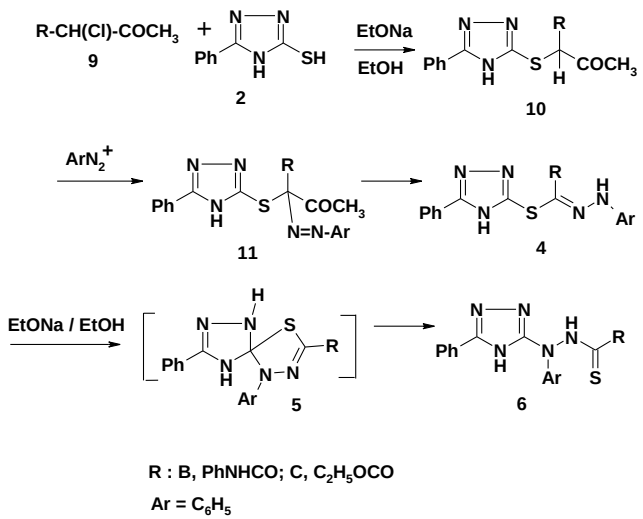
Scheme I



presence of a thiohydrazide carbon signal near δ 155-156 and the absence of the signal near δ 141-142 due to the carbon atom of the -S-C=N-NH- group.[8]

The structure of the thiohydrazides **6** was further supported by their alternate synthesis. Thus, treatment of 5-phenyl-1,2,4-triazole-3(4H)-thione **2** with the appropriate active chloromethylene compounds **9B**,**C** in ethanol in the presence of sodium ethoxide at room temperature afforded the respective (1,2,4-triazol-3-yl)thiomethylene compounds **10B**,**C**, respectively (Scheme II). The formation of the latter products **10** from **2** and **9** is analogous to the S-alkylation exhibited by 1,2,4-triazole-3(2H)-thione derivatives when treated with α-halo derivatives of ketones, acids or esters.[9] The structures of the products **10** isolated were established by their microanalyses and their mass, ^{1}H NMR and IR spectra which showed all the expected signals (see Experimental).

Scheme II

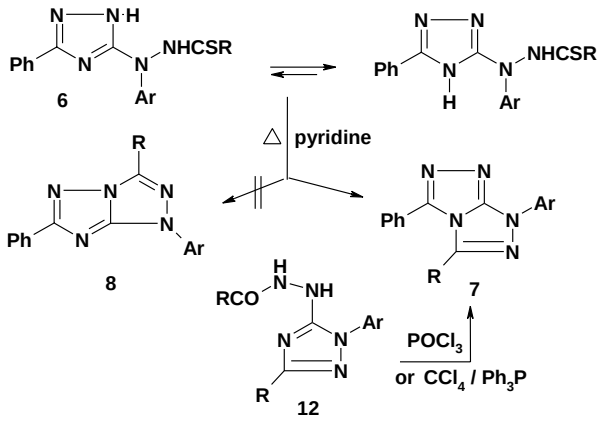


When **10B**,**C** were treated with benzenediazonium chloride in aqueous ethanol containing sodium acetate, they afforded the azo products **11B**,**C**, respectively (Scheme II). In no case the expected Japp-Klingemann product, namely the thiohydrazonate ester **4**, was produced. Structural assignments for the isolated products **11** were made on the basis of their mass, ^{1}H NMR and IR data (see Experimental).

Treatment of the azo products **11** with sodium ethoxide in ethanol at room temperature, to effect the Japp-Klingemann cleavage of the acetyl group, was found to afford products that proved identical in all respects with the products **6** obtained above from reaction of **1** with the thione **2**. This finding indicates that both reactions namely [**1** + thione **2** + EtO^- → **6**] and [**11** + EtO^- → **6**] involve the formation of the thiohydrazonates **4** as intermediates which undergo *in situ* Smiles rearrangement[7,10] as soon as they are formed to yield the thiohydrazides **6** as end products (Schemes I and II).

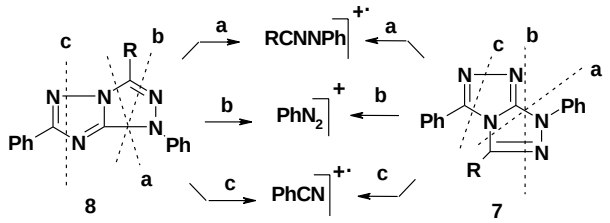
Next, cyclization of the thiohydrazides **6** was examined. Refluxing each of the thiohydrazides **6** in pyridine for 24 h resulted in elimination of hydrogen sulfide and the formation of a single product, in each case as evidenced by tlc analysis of the crude product. Elemental analysis and the mass spectral data were compatible with each of the two isomeric structures namely 1,3,5-trisubstituted 1H-[1,2,4]triazolo[3,4-c][1,2,4]triazoles **7** and 1,3,6-trisubstituted 1H-[1,2,4]triazolo[4,3-b][1,2,4]triazoles **8** (Scheme III). For example, the mass spectra of the latter products were characterized by molecular ion, base peaks of the spectra, abundant [RCN], [RCNNPh] and [PhN_2] ions (Chart I). The [RCNNPh] ions confirm the integrity of the original 1,2,4-triazole ring. Thus, on the basis of these data together with the IR data, it was not possible to distinguish between these two regioisomeric structures **7** or **8**. Also, the ^{1}H NMR spectra of the isolated products were not of much help to distinguish between the two structures **7** and **8**. The structural assignment of the isolated products was, however, secured as **7** on the basis of the identity (mp, m.mp, IR and ^{1}H NMR spectra) of the product, isolated from cyclization of **6Aa**, with an authentic sample of 1,3,5-triphenyl[1,2,4]triazolo[3,4-c][1,2,4]triazole **7Aa** prepared by an unequivocal method *via* cyclization of 3-benzoylhydrazino-2,5-diphenyl[1,2,4]triazole **12Aa** with $POCl_3$ as previously described[11] (Scheme III).

Scheme III



In an attempt to develop a new one-step strategy for synthesis of **7**, reactions of 3-methylthio-5-phenyl-1,2,4-triazole **3** with each of N-aryl hydrazonoyl halides **1A-D**

Chart I

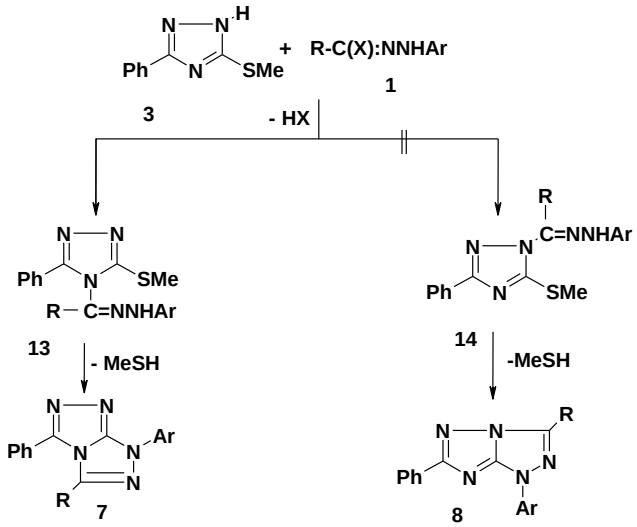


were examined in ethanol in the presence of sodium ethoxide at reflux (Scheme IV). In our hands, each of such reactions was found to give a single product as evidenced by tlc analysis of the crude product. The isolated products were assigned structure **7** as they proved to be identical in all respects with those obtained above from cyclization of the respective thiohydrazides **6** (Scheme III). This finding indicates that reaction of **1** with **3** is also regioselective and it proceeds *via* the formation of the amidrazones **13** as intermediates rather than **14** which cyclize *in situ* through elimination of methanethiol as soon as they are formed to give **7** as end products (Scheme IV). The regioselectivity of the reaction of **1** with **3** seems consistent with literature reports which indicate that N-4 is the site of preference for cyclization of N-(5-phenyl-1,2,4-triazol-3-yl) arenecarbohydrazonoyl bromides leading to 3-aryl-5-phenyltriazolo[3,4-c][1,2,4]triazoles.[12]

In conclusion, the foregoing results indicate that reactions of hydrazonoyl halides **1** with either thione **2** or its methylthio derivative **3** provide facile and novel regioselective routes for synthesis of 1H-[1,2,4]triazolo[3,4-c][1,2,4]-triazoles.

The compounds **6A-C** and **7A-C** were tested for their antimicrobial activities using four bacteria species namely Escherichia coli **Ec**, Bacillus subtilis **Bs**, Pseudomonas

Scheme IV



R : A, Ph; B, PhNHCO; C, EtOCO; D, PhCH=CH

Ar = XC_6H_4; X : a, H; b, 4-Me; c, 4-Cl; d, 4-NO_2

aeruginosa **Pa** and Staphylococcus aureus **Sa**. Also, five fungi species namely Candida spp. **Cs**, Syncephalastrum racemosum **Sr**, Penicillium marneffei **Pm**, Aspergillus fumigatus **Af** and Alternaria alternata **Aa** were tested. The organisms were tested against the activity of solutions of concentration of 1.0 μg/mL of each compound and using inhibition zone diameter in cm (IZD) as criterion for the antimicrobial activity. The fungicide Terbinafine and the bactericide Chloramphenicol were used as references to evaluate the potency of the tested compounds under the same conditions. The results are depicted in Table 1. They revealed that compound **6Aa** exhibited the highest degree of inhibition against all tested organisms. Furthermore, while the data

Table 1. Antimicrobial Activity of the Products **6** and **7**

	Micro-organism / IZD (cm)*								
Compd. no.	**Ec**	**Bs**	**Pa**	**Sa**	**Cs**	**Sr**	**Pm**	**Af**	**Aa**
6Aa	+	++	++	++	++	++	-	-	-
6Ba	+	+	+	+	++	+	-	-	-
6Ca	-	-	+	-	+	+	-	-	-
7Aa	++	+	+	+	-	-	-	-	-
7Ba	+	+	+	+	+	+	-	-	-
7Ca	-	+	+	-	+	+	-	-	-
CA[a]	2.6	2.6	2.8	2.4					
TE[b]					4.7	5.0	3.6	3.0	3.6

f 50 mL of solution whose concentration 1.0 μg/mL was tested.
[a] Chloramphenicol; [b] Terbinafine
* IZD beyond control / (sign): 1.1-1.5 cm / (+++); 0.6-1.0 cm / (++); 0.1-0.5 cm / (+); 0 cm / (-).

showed that all other compounds exhibited lower activity against the tested microorganisms, they revealed that all compounds exhibited no inhibition of the three fungi species **Pm**, **Af** and **Aa**.

EXPERIMENTAL SECTION

Melting points were determined on a Gallenkamp apparatus and are uncorrected. IR spectra were recorded in potassium bromide using Fourier Transform Infrared and Pye Unicam SP300 Infrared spectrophotometers. ^{1}H- and ^{13}C-NMR spectra were recorded using a Varian Mercury VXR-300 NMR spectrometer (300 MHz) in $CDCl_3$ or DMSO-d_6. Chemical shifts were related to that of the solvent used. Mass spectra were recorded on a GCMS-QP 1000 EX Varian MAT 711 and SSQ 7000 spectrometers. Elemental analyses were carried out at the Microanalytical Laboratory of Cairo University, Giza, Egypt.

The biological evaluation of the studied compounds **6** and **7** was carried out at the Regional Center for Mycology and Biotechnology at Alazhar University, Cairo, Egypt.

The hydrazonoyl chlorides **1A-D**,[13-17] 5-phenyl-1,2,4-triazole-3(2H)thione **2**,[18] 3-methylthio-5-phenyl-1,2,4-triazole **3**[18] and the active chloromethylene compounds **9A-C**[19,20] were prepared as previously described.

Synthesis of the thiohydrazides (6)

Method A

To a stirred ethanolic sodium ethoxide solution, prepared by dissolving sodium metal (0.23 g, 10 mg atom) in absolute ethanol (20 mL), was added 5-phenyl-1,2,4-triazole-3(4H)-thione **2** (1.77 g, 10 mmoles). After 15 min, the appropriate hydrazonoyl chloride **1** (10 mmoles) was added, and the resulting mixture was stirred at room temperature for 12 h. The crude product that precipitated was collected by filtration, washed with water, dried and finally crystallized from the appropriate solvent to give the respective **6**.

Method B

To a stirred ethanolic sodium ethoxide solution, prepared by dissolving sodium metal (0.23 g, 10 mg atom) in absolute ethanol (20 mL) was added the appropriate azo derivative **11**, and the mixture was stirred at room temperature for 12 h. During this period the reactant **11** dissolved and the crude product **6** precipitated. The latter was filtered, washed with water, dried and finally crystallized from the appropriate solvent. The products **6**, prepared by this method proved identical in all respects with those obtained by method A above. The physical constants of the products **6A-D** are given below.

Thiobenzoic acid N′-phenyl-N′-[5-phenyl-4H-1,2,4]-triazol-3-yl])hydrazide (6Aa)

M.p. 139 °C (MeOH), Yield 60%; ν (cm^{-1}) 3126; ^{1}H NMR δ 6.86-7.99 (m, 16H), 9.25 (s, 1H). Anal. calcd. for $C_{21}H_{17}N_5S$ (371): C, 67.90; H, 4.61; N, 18.85. Found: C, 67.50; H, 4.50; N, 18.9%.

Thiobenzoic acid N′-(4-nitrophenyl)-N′-(5-phenyl-4H-1,2,4-triazol-3-yl)hydrazide (6Ad)

M.p. 182 °C (EtOH-dioxane), Yield 75%; ν (cm^{-1}) 3421; ^{1}H NMR δ 7.26-8.25 (m, 15H), 9.77 (s, 1H). Anal. calcd. for $C_{21}H_{16}N_6O_2S$ (416): C, 60.57; H, 3.84; N, 20.19. Found: C, 60.48; H, 3.81; N, 20.14%.

N-Phenyl-2-[N-phenyl-N′-(5-phenyl-4H-1,2,4-triazol-3-yl)-hydrazino]-2-thioxoacetamide (6Ba)

M.p. 208-9 °C (MeOH), Yield 70%; ν (cm^{-1}) 3155, 3215, 3386, 1654, 1640; ^{1}H NMR δ 6.86-7.86 (m, 15H), 8.89 (s, 1H), 9.80 (s, 1H). MS *m/z* (%) 415 (M^{+}+1, 5.4), 414 (5.8), 236 (100), 119 (25), 91 (46), 77 (98). Anal. calcd. for $C_{22}H_{18}N_6OS$ (414): C, 63.78; H, 4.38; N, 20.28. Found: C, 63.69; H, 4.30; N, 20.25%.

2-[N′-(4-methylphenyl)-N′-(5-phenyl)-4H-1,2,4-triazol-3-yl)-hydrazino]-N-phenyl-2-thioxoacetamide (6Bb)

M.p. 174 °C (AcOH-water), Yield 70%; ν (cm^{-1}) 3352, 1674; ^{1}H NMR δ 2.32 (s, 3H), 7.25 - 8.01 (m, 14H), 10.06 (s, 1H), 10.51 (s, 1H), 13.26 (s, 1H). MS *m/z* (%) 429 (M^{+}+1, 2), 428 (3), 336 (13), 250 (97), 146 (31), 118 (29), 91 (100), 77 (59). Anal. calcd. for $C_{23}H_{20}N_6OS$ (428): C, 64.48; H, 4.67; N, 19.62. Found: C, 64.39; H, 4.55; N, 19.54%.

Ethyl 2-[N′-phenyl-N′-(5-phenyl-4H-1,2,4-triazol-3-yl)-hydrazino](thioxo)acetate (6Ca)

M.p. 172 °C (EtOH), Yield 60%; ν(cm^{-1}) 3122, 1703, 1667; ^{1}H NMR δ 1.35 (t, *J* = 7 Hz, 3H), 4.45 (q, *J* = 7 Hz, 2H), 7.0-7.45 (m, 10H), 8.3 (s, 1H), 9.9 (s, 1H). Anal. calcd. for $C_{18}H_{17}N_5O_2S$ (367): C, 58.84; H, 4.66; N, 19.06. Found: C, 58.70; H, 4.70; N, 19.0%.

3-Phenylthioacrylic acid N′-phenyl-N′-(5-phenyl-4H-1,2,4-triazol-3-yl)hydrazide (6Da)

M.p. 186 °C (aq. dioxane), Yield 70%; ν (cm^{-1}) 3275;

^{1}H NMR δ 6.88 (d, *J* = 17 Hz, 1H), 7.15 (d, *J* = 17 Hz, 1H), 7.24-7.93 (m, 16H), 10.09 (s, 1H). Anal. calcd. for $C_{23}H_{19}N_5S$ (397): C, 69.52; H, 4.78; N, 17.63. Found: C, 69.35; H, 4.66; N, 17.53%.

Synthesis of 1,2,4-Triazolo[3,4-c][1,2,4]triazoles (7)
Method A

To a stirred ethanolic sodium ethoxide solution, prepared from sodium metal (0.23 g, 10 mg atom) and absolute ethanol (20 mL), was added 5-phenyl-3-methylthio-1,2,4-triazole **3** (1.91 g, 10 mmoles). To the resulting solution was added the appropriate hydrazonoyl chloride **1** (10 mmoles) portionwise while stirring the mixture. After the addition was complete, the reaction mixture was refluxed till methanethiol ceased to evolve (18 h), then cooled. The solid that precipitated was filtered off, washed with water, air dried and finally crystallized from the appropriate solvent to give the respective triazolotriazole **7** in 62% yield. The latter products proved identical in all respects with those obtained above.

Method B

A solution of the appropriate thiohydrazide **6** (1 g) in pyridine (5 mL) was refluxed till all hydrogen sulfide ceased to evolve (30 h) while being stirred. The mixture was then cooled and acidified with hydrochloric acid. The solid that precipitated was filtered off, washed with water and air dried. Crystallization from the appropriate solvent gave the respective product **7** in 55-65% yield.

1,3,5-Triphenyl[1,2,4]triazolo[3,4-c][1,2,4]triazole (7Aa)

M.p. 246 °C (EtOH/DMF) (Lit.[11] m.p. 240-245 °C); Yield 62%.

3,5-Diphenyl-1-(4-nitrophenyl)-[1,2,4]triazolo[3,4-c][1,2,4]-triazole (7Ad)

M.p. 260 °C (Dioxane), Yield 60%; ν (cm^{-1}) 1593; ^{1}H NMR δ 7.25 (d, *J* = 11 Hz, 2H), 7.90 (d, *J* = 11 Hz, 2H), 8.31-8.54 (m, 10H). Anal. calcd. for $C_{21}H_{14}N_6O_2$ (382): C, 65.96; H, 3.66; N, 21.98. Found: C, 65.77; H, 3.54; N, 21.80%.

1,5-Diphenyl-3-phenylaminocarbonyl[1,2,4]triazolo[3,4-c]-[1,2,4]triazole (7Ba)

M.p. 238 °C (aq. AcOH), Yield, 60%, ν (cm^{-1}) 3323, 1676; ^{1}H NMR δ 7.22-8.97 (m, 15H), 9.32 (s, 1H). MS *m/z* (%): 381 (M^++1, 67.8), 380 (74.1), 260 (74.3), 144 (30.6), 103 (100), 77 (82.9). Anal. calcd. for $C_{22}H_{16}N_6O$ (380): C, 69.46; H, 4.24; N, 22.09. Found: C, 69.31; H, 4.23; N, 21.93%.

1-(4-Methylphenyl)-5-phenyl-3-phenylaminocarbonyl-[1,2,4]triazolo[3,4-c][1,2,4]triazole (7Bb)

M.p. 220 °C (AcOH), Yield 65%; (cm^{-1}) 3348, 1697; ^{1}H NMR δ 2.42 (s, 3H), 7.16 - 8.33 (m, 14H), 9.22 (s, 1H). MS *m/z* (%) 395 (M^++1, 27.6), 394 (100), 274 (58), 144 (18), 117 (31), 91 (33), 77 (32). Anal. calcd. for $C_{23}H_{18}N_6O$ (394): C, 70.00; H, 4.56; N, 21.31. Found: C, 69.80; H, 4.4o; N, 21.18%.

Ethyl 1,5-diphenyl-[1,2,4]triazolo[3,4-c][1,2,4]triazole-3-carboxylate (7Ca)

M.p. 200 °C (EtOH/DMF), Yield, 55%, ν (cm^{-1}) 3070, 1720; ^{1}H NMR δ 1.53 (t, *J* = 7 Hz, 3H), 4.63 (q, *J* = 7 Hz, 2H), 7.26-8.34 (m, 10H). MS *m/z* (%) 334 (M^++1, 16), 333 (30), 260 (19), 103 (100), 91 (7), 77 (29). Anal. calcd. for $C_{18}H_{15}N_5O_2$ (333): C, 64.86; H, 4.54; N, 21.01. Found: C, 65.00; H, 4.60; N, 21.2%.

Ethyl 1-(4-chlorophenyl)-5-phenyl-[1,2,4]triazolo[3,4-c]-[1,2,4]triazole-3-carboxylate (7Cc)

M.p. 166 °C (DMF-EtOH), Yield 60%; ν (cm^{-1}) 1740, 1597; ^{1}H NMR δ 1.53 (t, *J* = 7 Hz, 3H), 4.65 (q, *J* = 7 Hz, 2H), 7.26-8.31 (m, 9H). MS *m/z* (%) 368 (M^++1, 14), 367 (72), 294 (18), 137 (100), 102 (24), 77 (14). Anal. calcd. for $C_{18}H_{14}ClN_5O_2$ (367): C, 58.77; H, 3.80; N, 19.04. Found: C, 58.70; H, 3.67; N, 19.12%.

1,5-Diphenyl-3-(2-phenylethenyl)-[1,2,4]triazolo[3,4-c]-[1,2,4]triazole (7Da)

M.p. 165 °C (DMF-EtOH), Yield 70%; ν (cm^{-1}) 1600, 1597; ^{1}H NMR δ 7.16-8.34 (m). MS *m/z* (%) 364 (M^++1, 2), 363 (6), 286 (2), 260 (1), 235 (9), 155 (7), 103 (100), 77 (13). Anal. calcd. for $C_{23}H_{17}N_5$ (363): C, 76.03; H, 4.68; N, 19.28. Found: C, 75.90; H, 4.48; N, 19.15%.

Synthesis of 3-Oxo-2-[(5-phenyl-1,2,4-triazol-3-yl)thio]-butanoic acid derivatives (10B,C)
General Procedure

To a stirred ethanolic sodium ethoxide solution, prepared from sodium metal (0.23 g sodium metal, 10 mg atom) and absolute ethanol (20 mL), was added equimolar quantities of 5-phenyl-1,2,4-triazole-3(4H)thione **2** and the appropriate chloromethylene compound **9** (10 mmoles, each). The resulting mixture was stirred for 12 h at room temperature. During this period the reactants dissolved and a new solid

precipitated. The latter was filtered, washed with water, dried and finally crystallized from the appropriate solvent to give the respective product **10**. The physical constants of the products **10Ba** and **10Ca** are given below.

N-Phenyl-2-[(5-phenyl-1,2,4-triazol-3-yl)thio]-3-oxobutanamide (10Ba)

M.p. 134 °C (MeOH), yield 65%, ν (cm^{-1}) 3356, 3371, 1687, 1658; ^{1}H NMR δ 2.43 (s, 3H), 6.05 (s, 1H), 7.04-7.91 (m, 10H), 8.9 (s, 1H), 9.8 (s, 1H). Anal. calcd. for $C_{18}H_{16}N_4O_2S$ (352): C, 61.35; H, 4.58; N, 15.90. Found: C, 61.30; H, 4.20; N, 15.80%.

Ethyl 2-[(5-phenyl-1,2,4-triazol-3-yl)thio]-3-oxobutanoate (10Ca)

M.p. 108 °C (Acetone/H_2O); Yield 60%; ν (cm^{-1}) 3130, 1741, 1666; ^{1}H NMR δ 1.24 (t, *J* = 7 Hz, 3H), 2.42 (s, 3H), 4.27 (q, *J* = 7 Hz, 2H), 7.26 (s, 1H), 7.42-8.0 (m, 5H), 13.83 (s, 1H). Anal. calcd. for $C_{14}H_{15}N_3O_3S$ (305): C, 55.07; H, 4.95; N, 13.76. Found: C, 55.03; H, 4.90; N, 13.66%.

Synthesis of 2-Arylazo-3-Oxo-2-[(5-phenyl-s-triazol-3-yl)thio]-butanoic acid derivatives (11Ba and 11Ca) General Procedure

To a solution of the appropriate **10** (10 mmoles) in ethanol (40 mL) was added sodium acetate trihydrate (3 g), and the mixture was cooled in an ice bath to 0-5 °C while being stirred. To the resulting cold solution was added portionwise a cold solution of benzenediazonium chloride, prepared as usual by diazotizing aniline (10 mmoles) in hydrochloric acid (6 mL, 6 M) with sodium nitrite (0.7 g, 10 mmoles) in water (10 mL). After all diazonium salt solution was added, the reaction mixture was stirred for a further 30 min while cooling in an ice bath and left overnight in a refrigerator. The solid that precipitated was filtered, washed with water, air dried and finally crystallized from the appropriate solvent to give the respective arylazo derivative **11**. The products **11Ba** and **11Ca** prepared together with their physical constants are listed below.

N-Phenyl-2-phenylazo-2-[(5-phenyl-1,2,4-triazol-3-yl)thio]-3-oxobutanamide (11Ba)

M.p. 210-2 °C (MeOH), Yield 75%; ν (cm^{-1}) 3238, 1732, 1654; ^{1}H NMR δ 2.6 (s, 3H), 6.41-7.82 (m, 15H), 8.81 (s, 1H), 9.82 (s, 1H). Anal. calcd. for $C_{24}H_{20}N_6O_2S$ (456.5): C, 63.14; H, 4.42; N, 18.46. Found: C, 62.95; H, 4.51; N, 18.32%.

Ethyl 2-phenylazo-2-[(5-phenyl-1,2,4-triazol-3-yl)thio]-3-oxobutanoate (11Ca)

M.p. 180-2 °C (EtOH), Yield 69%; ν (cm^{-1}) 3201, 1745, 1693; ^{1}H NMR δ 1.34 (t, *J* = 7 Hz, 3H), 2.78 (s, 3H), 4.35 (q, *J* = 7 Hz, 2H), 7.05-7.50 (m, 10H), 9.12 (s, 1H). Anal. calcd. for $C_{20}H_{19}N_5O_3S$ (409): C, 58.67; H, 4.68; N, 17.11. Found: C, 58.55; H, 4.55; N, 16.92%.

Antimicrobial assay

Cultures of five fungi species namely Candida spp. **Cs**, Syncephalastrum racemosum **Sr**, Penicillium marneffei **Pm**, Aspergillus fumigatus **Af** and Alternaria alternata **Aa** were tested as well as four bacteria species namely Escherichia coli **Ec**, Bacillus subtilis **Bs**, Pseudomonas aeruginosa **Pa** and Staphylococcus aureus **Sa**. were used to investigate the antimicrobial activity of the compounds **6** and **7**. The antimicrobial activity was assayed biologically using diffusion plate technique. The latter technique was carried out by pouring a spore suspension of the fungal species (one ml of sterile water contains approximately 10^8 conidia) or spreading bacterial suspension over a solidified malt agar medium. The layer is allowed to set for 30 min. A solution of the test compound **6** or **7** (1.0 μg/mL) was placed onto sterile 5 mm filter paper discs and allowed to dry, then the discs were placed on the centre of the malt agar plate and incubated at optimum incubation temperature 28 ± 2 °C. Test organism growth may be affected by the inhibitory action of the test compound, so a clear zone around the disc appears as an indication of the inhibition of test organism growth. The size of the clearing zone is proportional to the inhibitory action of the compound. The fungicide Terbinafine and the bactericide Chloramphenicol were used as standards under the same conditions. Measurements were considered after 72 h for fungi and 24 h for bacteria. The results are summarized in Table 1.

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