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Design, synthesis, and antimicrobial activity of fused triheterocyclic nitrogen systems involving tetrazolo[1,5-*b*][1,2,4]triazines [1]

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Abstract Dehydrogenative cyclization of the 6-substituted 7-arylidenehydrazinotetrazolo[1,5-*b*][1,2,4]triazines derived from 6-methyl and 6-phenyl derivatives of 7-hydrazinotetrazolo[1,5-*b*][1,2,4]triazines and aromatic aldehydes gave the corresponding 6-substituted 9-aryl-[1,2,4]triazolo[4,3-*d*]tetrazolo-[1,5-*b*]-[1,2,4]triazines. The latter compounds were also obtained by an alternative route involving dehydrative cyclization of 6-methyl and 6-phenyl derivatives of 7-chlorotetrazolo[1,5-*b*][1,2,4]triazines with aromatic hydrazides through the isolable aroylhydrazino intermediates. Also, the triazolotetrazolotriazine rings were accomplished by one-pot cyclization of cyclic amidrazones with aromatic acid chlorides. The ditetrazolo[1,5-*b*:1′,5′-*d*][1,2,4]triazine systems were synthesized by cyclization of the former cyclic amidrazones with nitrous acid, or cyclic imidoyl chlorides with sodium azide. The bis-triazolotetrazolotriazine derivatives were synthesized by cyclization of two equivalents of each cyclic imidoyl chloride with acid dihydrazides through the isolable bis-hydrazide products. The antimicrobial activity of representative compounds was studied.

Keywords Cyclic amidrazones; Cyclic imidoyl chlorides; Hydrazides; Fused nitrogen triheterocycles; Antimicrobial activity.

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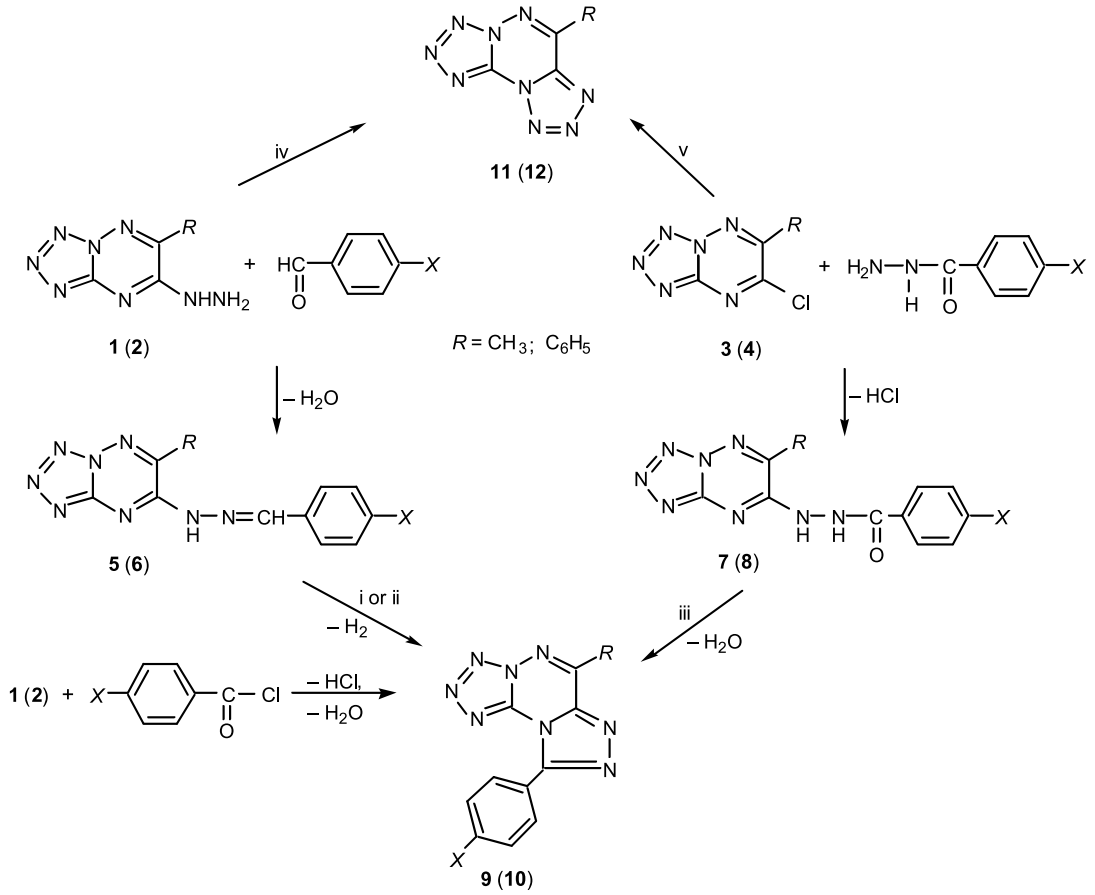
Introduction

A review of several compounds comprising the tetrazole nucleus has been published [2]. Recently, numerous biological properties have been reported for tetrazolo heterocycles, such as being useful due to their antibacterial [1, 3, 4], antiproliferation [5], anticancer [5], and anticonvulsant [6] activities. The chemistry of condensed 1,2,4-triazines has been surveyed in recent reviews [7, 8]; various members of these compounds have received considerable attention over the past years due to their therapeutic and pharmacological properties [1, 9–11]. Consequently, tetrazole and 1,2,4-triazine rings have proven to be valuable synthons for the synthesis of a wide range of biologically active compounds. Within this context, it seemed of interest to synthesize the title compounds and to evaluate their antimicrobial activities.

Results and discussion

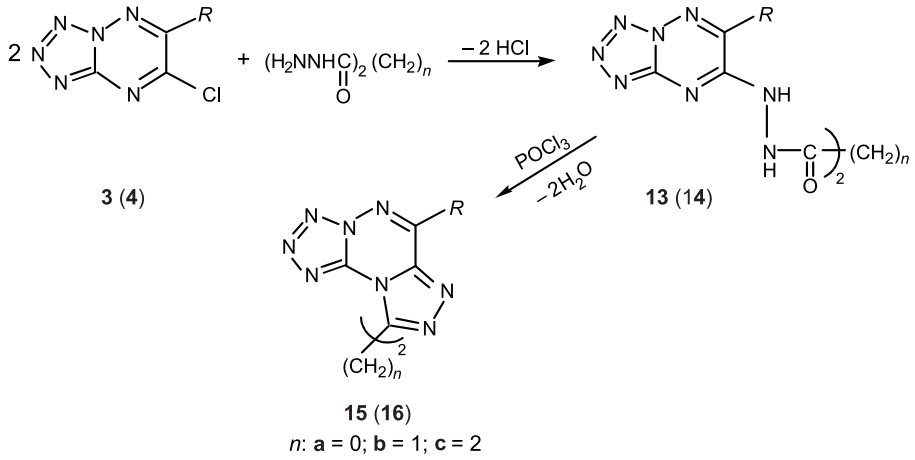
Condensation of 7-hydrazino-6-methyltetrazolo[1,5-*b*][1,2,4]triazine (**1**) [1] or 7-hydrazino-6-phenyltetrazolo[1,5-*b*][1,2,4]triazine (**2**) [1] with equimolar amounts of the appropriate aromatic aldehyde in boiling methanol afforded the corresponding 6-substituted 7-arylidenehydrazinotetrozolo[1,5-*b*][1,2,4]-triazines **5a–5d** and **6a–6d** (Scheme 1) in 80–90% overall yields, showing the expected NH in IR absorption as well as ^{1}H NMR signals characteristic



Scheme 1

of NH (D_2O-exchangeable), methylenic (–CH=N–), and aromatic protons. Their MS revealed the correct molecular ions which were supported by elemental analysis. Subjecting these hydrazone derivatives **5a**–**5d** and **6a**–**6d** to dehydrogenative cyclization [12, 13] with bromine in acetic acid in the presence of anhydrous sodium acetate or ethanolic iron(III) chloride solution yielded the corresponding 6-substituted 9-aryl-[1,2,4]triazolo-[4,3-*d*]tetrazolo[1,5-*b*][1,2,4]triazines **9a**–**9d** and **10a**–**10d**. These structures lacked the NH absorption in IR and methylenic proton signal in ^{1}H NMR. Alternatively, compounds **9a**–**9d** and **10a**–**10d** were also prepared by reaction of 7-chloro-6-methyltetrazolo[1,5-*b*][1,2,4]triazine (**3**) or 7-chloro-6-phenyltetrazolo[1,5-*b*][1,2,4]triazine (**4**) with the equimolar amounts of the corresponding aromatic hydrazides followed by dehydrative cyclization using phosphorus oxychloride. The isolable aroylhydrazino intermediates **7a**–**7d** and **8a**–**8d** were decisively assigned on the basis of their IR spectra which showed NH and CON bands, and their ^{1}H NMR which exhibited two NH (D_2O-exchangeable) and aromatic proton signals. In addition, the chemical proof for the assigned triazolotetrazolotriazine structures **9a**–**9d** and **10a**–**10d** was also obtained by one-pot cyclization of **1** or **2** with aromatic acid chlorides through the un-isolable aroylhydrazino intermediates **7a**–**7d** and **8a**–**8d** which were formed and concomitantly dehydratively cyclized to compounds **9a**–**9d** and **10a**–**10d** (Scheme 1). The aforementioned products **9a**–**9d** and **10a**–**10d** were proved to be identical in all respects (mp, mixed mp, TLC, and IR) with methods of cyclization mentioned above this article.

When cyclic amidrazones **1** or **2** were treated with sodium nitrite in hydrochloric acid at 0°C, they cyclized into the corresponding 6-methyl(phenyl)-ditetrazolo[1,5-*b*:1′,5′-*d*][1,2,4]triazines **11** or **12** (Scheme 1). The IR and ^{1}H NMR spectra showed the



Scheme 2

disappearance of NH and NH_2 groups. Alternatively, stirring the cyclic imidoyl chlorides **3** or **4** with equimolar amounts of sodium azide in a dipolar aprotic solvent like dimethylformamide resulted in better yields and purity of the desired products **11** or **12** identical with those prepared by the aforementioned route. The IR spectra of the products **11** or **12** did not show absorption bands at 2160–2120 cm^{-1} diagnostic of azido functions [14, 15], and may be considered as existing in a tautomeric equilibrium with tetrazole system [16].

Reaction of two molar equivalents of **3** or **4** with one molar equivalent of oxalic, malonic, or succinic dihydrazide in refluxing methanol caused a product in each case whose structure was deduced from spectroscopic data. Its IR spectrum showed absorptions characteristic of NH and CON as well as 4NH (D_2O-exchangeable) ^1H NMR signals. The products were assigned [17] therefore, proposed as bishydrazides **13a**–**13c** and **14a**–**14c** (Scheme 2). The latter compounds underwent cyclodehydration by phosphorus oxychloride to provide the bistriazolotetrazolotriazine structures **15a**–**15c** and **16a**–**16c** which revealed only a C=N absorption and, most importantly, lacked any NH and CON absorption bands in the IR region. Moreover, the MS of these compounds showed molecular ions in agreement with the assigned structures.

The products **5d**, **6d**, **7b**, **8b**, **9a**, **10c**, **13a**, **14b**, **15a**, and **16b** were *in vitro* evaluated [18] for their antimicrobial properties against *Gram*-positive (*Staphylococcus aureus*) and *Gram*-negative bacteria (*Escherichia coli*) in addition to a fungus (*Candida albicans*). The minimal inhibitory concentration ($MIC/\mu g \cdot cm^{-3}$) [19] is displayed in Table 1 showing that **5d**, **6d**, and **8b** exhibit an antimicrobial activity against *S. aureus* (25%). While compound **5d** possessed activity comparable to that of ampicillin against *E. coli*, but the action of **6d** and **8b** were 50%. Moreover, compound **6d** exhibited antimycotic activity against *C. albicans* comparable to that of clotrimazole, while the activity of **8b** is 50% of that clotrimazole. The rest of compounds showed lower activity than the reference standards (ampicillin and clotrimazole) against the test organisms.

Table 1 Minimal inhibitory concentration ($MIC/\mu g \cdot cm^{-3}$) of the prepared compounds

Test compound	*S. aureus*	*E. coli*	*C. albicans*
5d	50	25	>200
6d	50	50	12.5
7b	100	>200	200
8b	50	50	25
9a	>200	>200	100
10c	100	>200	100
13a	100	>200	200
14b	100	>100	100
15a	>200	>200	100
16b	>200	>100	100
Ampicillin	12.5	25	–
Clotrimazole	–	–	12.5

In conclusion, the foregoing results demonstrated the utility of 7-hydrazino(chloro)-6-methyl(phenyl)-tetrazolo[1,5-*b*][1,2,4]triazines as synthons for the construction of fused triheterocyclic nitrogen systems by different cyclization reagents. The antibacterial and antifungal activities of the synthesized compounds were even comparable to ampicillin and clotrimazole.

Experimental

Melting points were determined in capillary tubes in a Stuart Scientific SMP1 apparatus. The IR spectra were recorded (KBr) on a Satellite 1000 spectrophotometer. ^{1}H NMR spectra were obtained on a Varian Mercury VXR-3000 and Jeol ECA 500 spectrometers and chemical shifts are expressed in δ/ppm using *TMS* as an internal standard. MS were recorded on a HP model MS-5988 spectrometer at electron ionizing energy of 70 eV. Microanalyses were performed by the Microanalytical Unit, Cairo University, Egypt; their results agreed with the calculated values.

Synthesis of 6-substituted 7-arylidehydrazinotetrazolo-[1,5-b][1,2,4]triazines ***5a–5d*** and ***6a–6d***

A solution of 3 mmol **1** or **2** in 15 cm^3 methanol was added to 3 mmol appropriate aromatic aldehyde and the mixture was heated at 100°C for 10 min. The reaction mixture was kept at ambient temperature for 24 h and the product which separated was filtered off, washed with ether, dried, and crystallized from methanol.

7-Benzylidenehydrazino-6-methyltetrazolo[1,5-b][1,2,4]-triazine (**5a**, $C_{11}H_{10}N_8$)

Yield: 0.66 g (86%); pale yellow; mp 152–154°C; IR: $\bar{\nu} = 3320$ (NH), 1625 (C=N) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 11.60$ (s, NH, D_2O-exchangeable), 8.20–7.90 (m, 5 *Ar*H), 7.61 (s, 1H, methylenic H), 2.40 (s, CH_3) ppm; MS: m/z (%) = 254 (M^+, 13), 255 ($M^+ + 1$, 6).

7-p-Tolylmethylidenehydrazino-6-methyltetrazolo[1,5-b]-[1,2,4]triazine (**5b**, $C_{12}H_{12}N_8$)

Yield: 0.71 g (88%); yellow; mp 160–162°C; IR: $\bar{\nu} = 3340$ (NH), 1615 (C=N) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 10.85$ (s, NH, D_2O-exchangeable), 8.15–7.82 (m, 4 *Ar*H), 7.70 (s, 1H, methylenic H), 2.40, 2.35 (2s, 2CH_3) ppm; MS: m/z (%) = 268 (M^+, 28).

7-p-Chlorobenzylidenehydrazino-6-methyltetrazolo[1,5-b]-[1,2,4]triazine (**5c**, $C_{11}H_9ClN_8$)

Yield: 0.75 g (86%); yellow; mp 165–166°C; IR: $\bar{\nu} = 3290$ (NH), 1620 (C=N) cm^{-1}; MS: m/z (%) = 288 (M^+, 30).

7-p-Nitrobenzylidenehydrazino-6-methyltetrazolo[1,5-b]-[1,2,4]triazine (**5d**, $C_{11}H_9N_9O_2$)

Yield: 0.81 g (90%); organe; mp 181–182°C; IR: $\bar{\nu} = 3345$ (NH), 1625 (C=N) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 10.66$ (s, NH, D_2O-exchangeable), 8.20–7.61 (m, 4 *Arl*I), 7.25 (s, 1H, methylenic H), 2.45 (s, CH_3) ppm; MS: m/z (%) = 299 (M^+, 15).

7-Benzylidenehydrazino-6-phenyltetrazolo[1,5-b][1,2,4]-triazine (**6a**, $C_{16}H_{12}N_8$)

Yield: 0.78 g (80%); yellow; mp 145–146°C; IR: $\bar{\nu} = 3340$ (NH), 1630 (C=N) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 11.42$ (s, NH, D_2O-exchangeable), 8.15–7.85 (m, 10 *Ar*H + methylenic H) ppm; MS: m/z (%) = 316 (M^+, 42).

7-p-Tolylmethylidenehydrazino-6-phenyltetrazolo[1,5-b]-[1,2,4]triazine (**6b**, $C_{17}H_{14}N_8$)

Yield: 0.85 g (84%); yellow; mp 148–150°C; IR: $\bar{\nu} = 33600$ (NH), 1634 (C=N) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 11.80$ (s, NH, D_2O-exchangeable), 8.25–7.90 (m, 9 *Ar*H + methylenic H), 2.30 (s, CH_3) ppm; MS: m/z (%) = 330 (M^+, 35).

7-p-Chlorobenzylidenehydrazino-6-phenyltetrazolo[1,5-b]-[1,2,4]triazine (**6c**, $C_{16}H_{11}ClN_8$)

Yield: 0.89 g (82%); yellow; mp 160–162°C; IR: $\bar{\nu} = 3340$ (NH), 1620 (C=N) cm^{-1}; MS: m/z (%) = 350 (M^+, 12).

7-p-Nitrobenzylidenehydrazino-6-phenyltetrazolo[1,5-b]-[1,2,4]triazine (**6d**, $C_{16}H_{11}N_9O_2$)

Yield: 0.95 g (86%); red; mp 190–192°C; IR: $\bar{\nu} = 3290$ (NH), 1620 (C=N) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 12.10$ (s, NH, D_2O-exchangeable), 8.10–7.75 (m, 9 *Ar*H + methylenic H) ppm; MS: m/z (%) = 361 (M^+, 14).

Synthesis of 7-aroylhydrazino-6-substituted tetrazolo-[1,5-b][1,2,4]triazine ***7a–7d*** and ***8a–8d***

To a solution of 3 mmol **3** or **4** in 10 cm^3 methanol, a solution of 3 mmol suitable aromatic hydrazide in 10 cm^3 methanol was added, and the mixture was heated at reflux for 1 h and then allowed to cool at room temperature. The separated product was filtered off, washed several times with ether, dried, and crystallized from methanol.

7-Benzoylhydrazino-6-methyltetrazolo[1,5-b][1,2,4]triazine (**7a**, $C_{11}H_{10}N_8O$)

Yield: 0.61 g (81%); mp 180–182°C; IR: $\bar{\nu} = 3340$, 3280 (2 NH), 1680 (CON), 1630 (C=N) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 12.65$ (s, 2NH, D_2O-exchangeable), 8.35–7.20 (m, 5*ArH*), 2.47 (s, CH_3) ppm; MS: m/z (%) = 270 (M^+, 40).

7-p-Toluoylhydrazino-6-methyltetrazolo[1,5-b][1,2,4]-triazine (**7b**, $C_{12}H_{12}N_8O$)

Yield: 0.65 g (78%); mp 190–192°C; IR: $\bar{\nu} = 3330$, 3290 (2NH), 1670 (CON), 1625 (C=N) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 12.82$ (s, 2NH, D_2O-exchangeable), 8.65–7.75 (m, 4*ArH*), 2.45, 2.30 (2s, 2CH_3) ppm; MS: m/z (%) = 285 ($M^+ + 1$, 45).

7-p-Chlorobenzoylhydrazino-6-methyltetrazolo[15,b]-[1,2,4]triazine (**7c**, $C_{11}H_9ClN_8O$)

Yield: 0.72 g (81%); mp 198–200°C; IR: $\bar{\nu} = 3340$, 3325 (2 NH), 1685 (CON), 1630 (C=N) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 12.71$ (s, 2NH, D_2O-exchangeable), 8.40–7.70 (m, 4 *Ar*H), 2.40 (s, CH_3) ppm; MS: m/z (%) = 304 (M^+, 38).

7-p-Nitrobenzoylhydrazino-6-methyltetrazolo[1,5-b][1,2,4]-triazine (**7d**, $C_{11}H_9N_9O_3$)

Yield: 0.84 g (91%); mp 225–257°C; IR: $\bar{\nu} = 3350$, 3310 (2NH), 1690 (CON), 1610 (C=N) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 12.65$, 12.40 (2s, 2NH, D_2O-exchangeable), 8.60–7.35 (m, 4*ArH*), 2.40 (s, CH_3) ppm; MS: m/z (%) = 315 (M^+, 27).

7-Benzoylhydrazino-6-phenyltetrazolo[1,5-b][1,2,4]triazine (**8a**, $C_{16}H_{12}N_8O$)
Yield: 0.78 g (78%); mp 165–166°C; IR: $\bar{\nu} = 3310$, 3290 (2NH), 1675 (CON), 1620 (C=N) cm^{-1}; ^1H NMR (*DMSO*-d_6): $\delta = 12.61$, 12.22 (2 s, 2NH, D_2O-exchangeable), 8.75–7.89 (m, 10 *Ar*H) ppm; MS: m/z (%) = 333 ($M^+ + 1$, 50).

7-p-Toluoylhydrazino-6-phenyltetrazolo[1,5-b][1,2,4]-triazine (**8b**, $C_{17}H_{14}N_8O$)
Yield: 0.77 g (74%); mp 175–177°C; IR: $\bar{\nu} = 3290$, 3270 (2 NH), 1670 (CON), 1610 (C=N) cm^{-1}; ^1H NMR (*DMSO*-d_6): $\delta = 12.42$, 12.95 (2s, 2NH, D_2O-exchangeable), 8.85–7.60 (m, *9Ar*H), 2.30 (s, CH_3) ppm; MS: m/z (%) = 346 (M^+, 18).

7-p-Chlorobenzoylhydrazino-6-phenyltetrazolo[1,5-b]-[1,2,4]triazine (**8c**, $C_{16}H_{11}ClN_8O$)
Yield: 0.85 g (77%), mp 190–191°C; IR: $\bar{\nu} = 3320$, 3280 (2NH), 1660 (CON), 1618 (C=N) cm^{-1}; MS: m/z (%) = 366 (M^+, 36).

7-p-Nitrobenzoylhydrazino-6-phenyltetrazolo[1,5-b][1,2,4]-triazine (**8d**, $C_{16}H_{11}N_9O_3$)
Yield: 0.79 g (85%), mp 240–242°C; IR: $\bar{\nu} = 3400$, 3380 (2NH), 1620 (CON), 1630 (C=N) cm^{-1}; ^1H NMR (*DMSO*-d_6): $\delta = 12.70$ (s, 2NH, D_2O-exchangeable), 8.80–7.90 (m, 9 *Ar*H) ppm; MS: m/z (%) = 377 (M^+, 12).

Synthesis of 6-substituted 9-aryl[1,2,4]triazolo[4,3-d]-tetrazolo[1,5-b][1,2,4]triazines ***9a–9d*** and ***10a–10d***
Method A. To a solution of 2 mmol of each **5a–5d** and **6a–6d** in 15 cm^3 glacial acetic acid containing 2 mmol anhydrous sodium acetate, 2 mmol bromine in 10 cm^3 glacial acetic acid were added gradually with stirring. The reaction mixture was then warmed on a boiling water bath for 5 min, left to cool and then poured onto water. The precipitated solid was filtered off, washed thoroughly with water, and crystallized from methanol.

Method B. A solution of 2 mmol of the respective hydrazone **5a–5d** and **6a–6d** in 20 cm^3 ethanolic iron(III) chloride solution (10%) was boiled for 10 min, and then left at room temperature overnight. The separated product was filtered off, washed with water, dried, and crystallized from methanol.

Method C. A mixture of 2 mmol of the particular aroyl hydrazine **7a–7d** and **8a–8d**, and 10 cm^3 phosphorus oxychloride was heated under reflux for 1 h, then cooled and poured onto of 30 cm^3 cold saturated solution of sodium bicorbonate. The crude solid that precipitated was filtered off, washed with water, dried, and finally crystallized from methanol.

Method D. A 3 mmol of **1** or **2** was dissolved in 2 cm^3 pyridine, 3 mmol of freshly distilled appropriate aromatic acid chloride were added, and the mixture was heated under reflux at 100°C for 1 h. It was cooled at ambient temperature, poured onto ice-water, the crude solid that precipitated was filtered, washed with water and crystallized from methanol.

6-Methyl-9-phenyl-[1,2,4]triazolo[4,3-d]tetrazolo[1,5-b]-[1,2,4]triazine (**9a**, $C_{11}H_8N_8$)
Yield: method A 0.27 g (54%), method B 0.22 g (44%), method C 0.26 g (56%); mp 220°C, identical with authentic sample prepared from method D according to Ref. [1].

6-Methyl-9-p-tolyl-[1,2,4]triazolo[4,3-d]tetrazolo[1,5-b]-[1,2,4]triazine (**9b**, $C_{12}H_{10}N_8$)
Yield: method A 0.28 g (57%), method B 0.25 g (50%), method C 0.29 g, method D 0.61 g (76%); mp 225–227°C; IR: $\bar{\nu} = 1630$ (C=N) cm^{-1}; ^1H NMR (*DMSO*-d_6): $\delta = 8.25$–7.88 (m, 4 *Ar*H), 2.45, 2.30 (2 s, $2CH_3$) ppm; MS: m/z (%) = 266 (M^+, 25), 267 ($M^+ + 1$, 40).

9-p-Chlorophenyl-6-methyl-[1,2,4]triazolo[4,3-d]tetrazolo-[1,5-b][1,2,4]triazine (**9c**, $C_{11}H_7ClN_8$)
Yield: method A 0.31 g (62%), method B 0.27 g (54%), method C 0.33 g (70%), method D 0.66 g (77%); mp 235–236°C; IR: $\bar{\nu} = 1635$ (C=N) cm^{-1}; ^1H NMR (*DMSO*-d_6): $\delta = 8.40$–7.63 (m, 4*Ar*H), 2.50 (s, CH_3) ppm; MS: m/z (%) = 286 (M^+, 22).

6-Methyl-9-p-nitrophenyl-[1,2,4]triazolo[4,3-d]tetrazolo-[1,5-b][1,2,4]triazine (**9d**, $C_{11}H_7N_9O_2$)
Yield: method A 0.37 g (74%), method B 0.26 g (52%), method C 0.38 g (81%), method D 0.70 g (78%); mp 250°C; IR: $\bar{\nu} = 1620$ (C=N) cm^{-1}; ^1H NMR (*DMSO*-d_6): $\delta = 8.45$–7.88 (m, *4Ar*H), 2.40 (s, CH_3) ppm; MS: m/z (%) = 298 ($M^+ + 1$, 15).

6,9-Diphenyl-[1,2,4]triazolo[4,3-d]tetrazolo[1,5-b][1,2,4]-triazine (**10a**, $C_{16}H_{10}N_8$)
Yield: method A 0.35 g (50%), method B 0.29 g (42%), method C 0.39 g (59%); mp 240–241°C, identical with authentic sample prepared from method D according to Ref. [1].

6-Phenyl-9-p-tolyl-[1,2,4][triazolo[4,3-d]tetrazolo[1,5-b]-[1,2,4]triazine (**10b**, $C_{17}H_{12}N_8$)
Yield: method A, 0.38 g (55%), method B 0.35 g (50%), method C 0.39 g (59%), method D, 0.61 g (60%); mp 210–212°C; IR: $\bar{\nu} = 1640$ (C=N) cm^{-1}; ^1H NMR (*DMSO*-d_6): $\delta = 8.60$–7.90 (m, 9 *Ar*H), 2.25 (s, CH_3) ppm; MS: m/z (%) = 328 (M^+, 35).

9-p-Chlorophenyl-6-phenyl-[1,2,4]triazolo[4,3-d]tetrazolo-[1,5-b][1,2,4]triazine (**10c**, $C_{16}H_9ClN_8$)
Yield: method A 0.41 g (59%), method B 0.36 g (52%); method C 0.46 g (69%); method D 0.80 g (75%); mp 218–220°C; IR: $\bar{\nu} = 1625$ (C = N) cm^{-1}; ^1H NMR (*DMSO*-d_6). $\delta = 8.42$–7.80 (m, *9Ar*H) ppm; MS: m/z (%) = 348 (M^+, 35).

9-p-Nitrophenyl-6-phenyl-[1,2,4]triazolo[4,3-d]tetrazolo-[1,5-b][1,2,4]triazine (**10d**, $C_{16}H_9N_9O_2$)
Yield: method A 0.51 g (73%), method B 0.34 g (49%), method C 0.53 g (80%); method D 0.85 g (77%); mp 270–272°C; IR: $\bar{\nu} = 1620$ (C=N) cm^{-1}; ^1H NMR (*DMSO*-d_6): $\delta = 8.60$–7.45 (m, *9Ar*H) ppm; MS: m/z (%) = 359 (M^+, 45).

*Synthesis of **11** and **12***

Method A. A 3 mmol of **1** or **2** were dissolved in 10 cm^3 hydrochloric acid (2 *N*) and the solution was cooled to 0°C. An aqueous solution of (0.2 g) sodium nitrite 3 mmol in 10 cm^3 water was added portionwise to the above cooled solution, with stirring. The solid which separated, it was filtered off after 1 h of stirring, washed with cold water, dried, and crystallized from a mixture of water and methanol.

Method B. A mixture of 3 mmol **3** or **4** and (0.2 g) sodium azide 3 mmol in 15 cm^3 dry dimethylformamide was stirred at room temperature for 30 min, when a clear solution resulted. The reaction mixture was thereafter heated on a water-bath for 1 h, then poured onto ice-water whereby the solid product which precipitated was filtered off, dried, and crystallized water/methanol.

6-Methylditetrazolo[1,5-b:1′,5′-d][1,2,4]triazine
(**11**, $C_4H_3N_9$)
Yield: method A 0.37 g (69%), method B 0.43 g (81%); mp 240–242°C; IR: $\bar{\nu} = 1635$ (C=N) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 2.45$ (s, CH_3) ppm; MS: m/z (%) = 178 ($M^+ + 1$, 55).

6-Phenylditetrazolo[1,5-b: 1′,5′-d][1,2,4]triazine
(**12**, $C_9H_5N_9$)
Yield: method A 0.48 g (67%), method B 0.57 g (79%); mp 255–257°C; IR: $\bar{\nu} = 1630$ (C=N) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 8.60$–7.95 (m, 5 *Ar*H) ppm; MS: m/z (%) = 239 (M^+, 48).

*Synthesis of **13a–13c** and **14a–14c***
To a solution of 2 mmol **3** or **4** in 15 cm^3 methanol, a solution of 2 mmol acid dihydrazide in 20 cm^3 methanol was gradually added, and the mixture was heated under reflux at 100°C for 1 h. The product which separated upon cooling was filtered off, washed with methanol, and crystallized from a mixture of water and methanol.

Oxalyl bis{(6-methyltetrazolo[1,5-b][1,2,4]triazin-7-yl)-hydrazide} (**13a**, $C_{10}H_{10}N_{16}O_2$)
Yield: 0.63 g (80%); mp 215–217°C; IR: $\bar{\nu} = 3290$, 3265 (2 NH), 1665 (CON), 1625 (C=N) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 12.20$, 12.05 (2s, 4NH, D_2O-exchangeable), 2.45, 2.40 (2s, CH_3 each) ppm; MS: m/z (%) = 386 (M^+, 40).

Malonyl bis{(6-methyltetrazolo[1,5-b][1,2,4]triazin-7-yl)-hydrazide} (**13b**, $C_{11}H_{12}N_{16}O_2$)
Yield: 0.64 g (78%); mp 190–191°C; IR: $\bar{\nu} = 3320$, 3280 (2NH), 1660 (CON), 1615 (C=N) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 11.85$, 11.40 (2s, 2NH each, D_2O-exchangeable), 4.25 (s, CH_2), 2.55 (s, $2CH_3$) ppm; MS: m/z (%) = 400 (M^+, 27).

Succinyl bis{(6-methyltetrazolo[1,5-b][1,2,4]triazin-7-yl)-hydrazide} (**13c**, $C_{12}H_{14}N_{16}O_2$)
Yield: 0.55 g (65%); mp 198–200°C; IR: $\bar{\nu} = 3330$, 3308 (2NH), 1665 (CON), 1620 (C=N) cm^{-1}; MS: m/z (%) = 415 ($M^+ + 1$, 18).

Oxalyl bis{(6-phenyltetrazolo[1,5-b][1,2,4]triazin-7-yl)-hydrazide} (**14a**, $C_{20}H_{14}N_{16}O_2$)
Yield: 0.77 g (78%); mp 190–192°C; IR: $\bar{\nu} = 3350$, 3295 (2 NH), 1670 (CON), 1625 (C=N) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 12.55$, 11.93 (2s, 2NH each, D_2O-exchangeable), 8.65–7.50 (m, 10 *Ar*H) ppm; MS: m/z (%) = 510 (M^+, 28).

Malonyl bis{(6-phenyltetrazolo[1,5-b][1,2,4]triazin-7-yl)-hydrazide} (**14b**, $C_{21}H_{16}N_{16}O_2$)
Yield: 0.72 g (71%); mp 175–177°C; IR: $\bar{\nu} = 3380$, 3295 (2NH), 1655 (CON), 1615 (C=N) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 12.50$, 12.10 (2s, 4NH, D_2O-exchangeable), 8.60–7.50 (m, 10*Ar*H), 4.20 (s, CH_2) ppm; MS: m/z (%) = 525 ($M^+ + 1$, 35).

Succinyl bis{(6-phenyltetrazolo[1,5-b][1,2,4]triazin-7-yl)-hydrazide} (**14c**, $C_{22}H_{18}N_{16}O_2$)
Yield: 0.67 g (64%); mp 180–182°C; IR: $\bar{\nu} = 3360$, 3320 (2NH), 1655 (CON), 1610 (C=N) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 12.40$, 11.94 (2s, 4NH, D_2O-exchangeable), 8.65–7.95 (m, 10*Ar*H), 4.40 (s, $2CH_2$) ppm; MS: m/z (%) = 538 (M^+, 26).

*Synthesis of **15a–15c** and **16a–16c***
The respective of 2 mmol **13a–13c** and **14a–14c** was treated with 10 cm^3 phosphorus oxychloride and heated under reflux for 1 h. After attaining ambient temperature, the mixture was poured onto a cold saturated solution of sodium bicarbonate and the crude solid which separated was filtered off, washed with water, dried, and crystallized from a mixture of water and methanol.

Bis{6-methyl-[1,2,4]triazolo[4,3-d]tetrazolo[1,5-b][1,2,4]-triazin-9-yl} (**15a**, $C_{10}H_6N_{16}$)
Yield: 0.49 g (77%); mp 240–241°C, IR: $\bar{\nu} = 1620$ (C=N) cm^{-1}; MS: m/z (%) = 350 (M^+, 38); ^{1}H NMR (*DMSO*-d_6): $\delta = 2.50$, 2.45 (2s, $2CH_3$) ppm.

Bis{6-methyl-[1,2,4]triazolo[4,3-d]tetrazolo[1,5-b][1,2,4]-triazin-9-yl}methane (**15b**, $C_{11}H_8N_{16}$)
Yield: 0.43 g (67%), mp 210–212°C; IR: $\bar{\nu} = 1625$ (C=N) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 4.15$ (s, CH_2), 2.55, 2.40 (2s, $2CH_3$) ppm; MS: m/z (%) = 364 (M^+, 42).

Bis{6-methyl-[1,2,4]triazolo[4,3-d]tetrazolo[1,5-b][1,2,4]-triazin-9-yl}ethane (**15c**, $C_{12}H_{10}N_{16}$)
Yield: 0.33 g (52%), mp 220–222°C; IR: $\bar{\nu} = 1620$, (C=N) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 4.45$ (s, $2CH_2$), 2.50, 2.40 (2s, CH_3) ppm.

Bis{6-phenyl-[1,2,4]triazolo[4,3-d]tetrazolo[1,5-b][1,2,4]-triazin-9-yl} (**16a**, $C_{20}H_{10}N_{16}$)
Yield: 0.63 g (75%), mp 215–217°C; IR: $\bar{\nu} = 1625$ (C=N) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 8.25$–7.92 (m, 10*Ar*H) ppm; MS: m/z (%) = 474 (M^+, 48).

Bis{6-phenyl-[1,2,4]triazolo[4,3-d]tetrazolo[1,5-b][1,2,4]-triazin-9-yl}methane (**16b**, $C_{21}H_{12}N_{16}$)
Yield: 0.52 g (62%), mp 205–207°C; IR: $\bar{\nu} = 1625$ (C=N) cm^{-1}; ^1H NMR (*DMSO*-d_6): $\delta = 8.70$–7.90 (m, 10*Ar*H), 4.10 (s, CH_2) ppm.

Bis{6-phenyl-[1,2,4]triazolo[4,3-d]tetrazolo[1,5-b][1,2,4]-triazin-9-yl}ethane (**16c**, $C_{22}H_{14}N_{16}$)
Yield: 0.43 g (51%), mp 230–232°C; IR: $\bar{\nu} = 1618$ (C=N) cm^{-1}; ^1H NMR (*DMSO*-d_6): $\delta = 8.80$–7.20 (m, 10*Ar*H), 4.35 (s, $2CH_2$) ppm; MS: m/z (%) = 502 (M^+, 37).

Antimicrobial screening
Solutions of the test compounds, ampicillin trihydrate and clotrimazole were prepared in *DMSO* at a concentration of 1600 $\mu g/cm^3$ and twofold dilutions of the compounds were prepared (800, 400, ... 6.25 $\mu g/cm^3$). The microorganism suspensions at 10^6 Colony Forming Unit/cm^3 (*CFU*/cm^3) concentration were inoculated to the corrosponding wells. Plates were incubated at 36°C for 24 to 48 h. The incubation chamber was kept sufficiently humid. At the end of the incubation period, the minimial inhibitory concentrations (*MIC*) were determined. Controls with *DMSO* and uninoculated media were also investigated.

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