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# Quinoxaline derivatives part II: Synthesis and antimicrobial testing of 1,2,4-triazolo[4,3-a]quinoxalines, 1,2,4-triazino[4,3-a]quinoxalines and 2-pyrazolylquinoxalines

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Three main classes of quinoxaline derivatives have been synthesized. The first class comprises the synthesis of three novel series of 1,2,4-triazolo[4,3-*a*]quinoxalines; namely 1-substituted-1,2,4-triazolo[4,3-*a*]quinoxalines **3a**–**f**, 1-substituted aminomethyl-1,2,4-triazolo[4,3-a]quinoxalines **14a**–**d** and 1-cyano or ethoxycarbonylmethyl-1,2,4-triazolo[4,3-*a*]quinoxalines **6, 12**. The second class involves the synthesis of 2-substituted-1*H*-1,2,4-triazino[4,3-*a*]quinoxalines **4a**–**d**. The third class deals with the synthesis of a variety of 2-pyrazolylquinoxalines, namely 2-(5-amino-3-arylpyrazol-1-yl)-3-phenylquinoxalines **5a**–**d**, 2-[5-hydroxy-3-phenyl-4-(4-substituted sulfamoylphenyl)azopyrazol-1-yl]-3-phenylquinoxalines **15a**, **b**, and 2-(5-hydroxy-4-nitroso-3-phenylpyrazol-1-yl)-3-phenylquinoxaline (**16**). The prepared compounds were tested *in vitro* for their antimicrobial activity. Compounds **13** and **14b** exhibited promising antifungal activity against *C. albicans* (MIC 25, 50 μ/ml respectively). Compound **13** was as active as the antibiotic nystatin.

## 1. Introduction

It has been recently reported that some quinoxaline derivatives display anti-cancer and anti-HIV activities [1–3]. In addition, it is well documented that properly substituted quinoxalines possess antimicrobial [4], antifungal [5], antimycobacterial [6, 7] and antiviral [8] activities. On the other hand, pyrazoles [9, 10] and triazoles [11] possess antimicrobial and antifungal activities.

In a previous paper we reported the synthesis and antimicrobial testing of thiazolinyl, thiazolidinonylquinoxalines and 1-substituted amino-1,2,4-triazolo[4,3-*a*]quinoxalines [12]. Interesting antimicrobial activity was observed for 1-butylamino-1,2,4-triazolo[4,3-*a*]quinoxaline which showed a MIC $< 25$ μg/ml against *Staphylococcus aureus*.

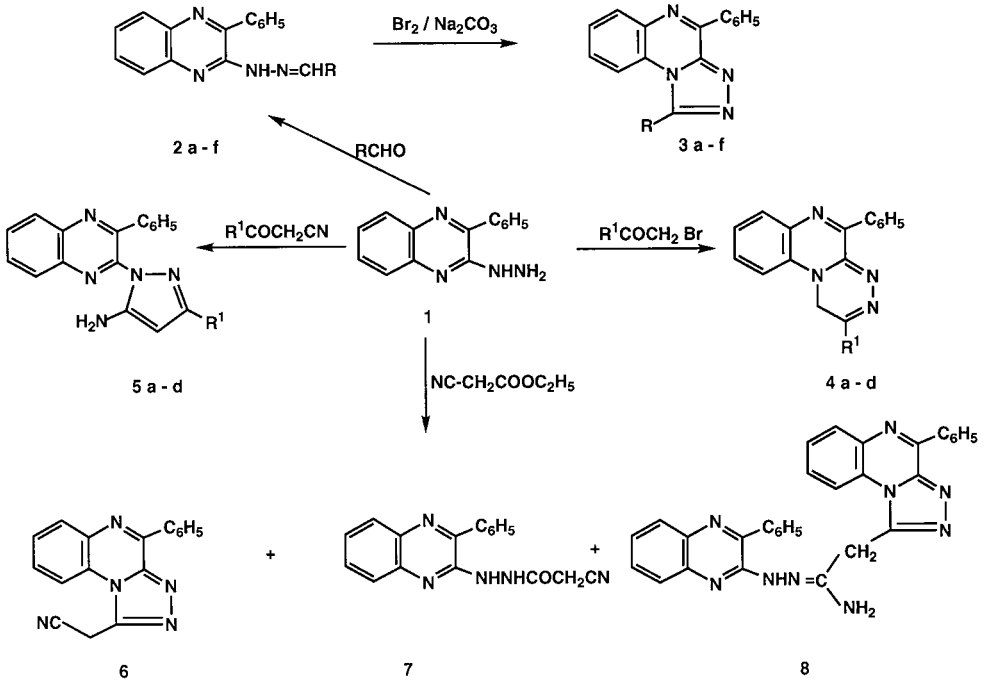
On the basis of these findings and in persuing our research in this field, the present investigation deals with the synthesis of three novel series of 1,2,4-triazolo[4,3-*a*]quinoxalines. Moreover, in trials to find other new series of quinoxaline derivatives with antimicrobial activities we also synthesized 1,2,4-triazino[4,3-*a*]quinoxalines and 2-pyrazolylquinoxalines.

## 2. Investigations, results and discussion

### *2.1. Synthesis and characterisation*

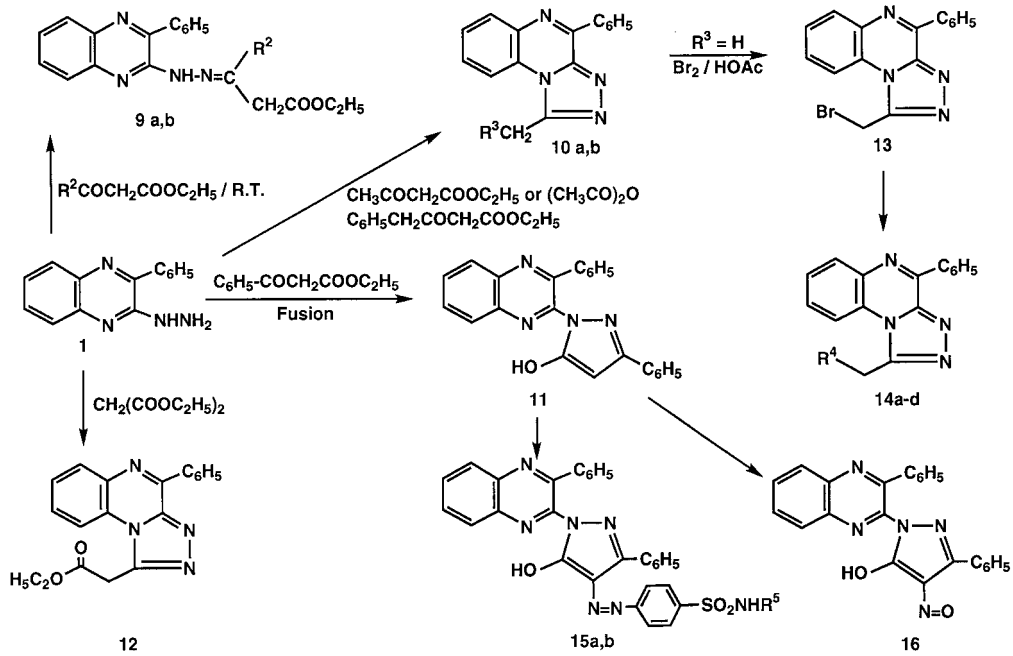
For the synthesis of the target compounds the reactions outlined in Schemes 1 and 2 were applied. Thus, 2-hydrazino-3-phenylquinoxaline (**1**) was reacted with different

**Scheme 1**




For R,R[1] See Tables 2,3,4,5.

**Scheme 2**




aldehydes to produce the 2-arylidenehydrazino-3-phenylquinoxalines **2a**–**f**. Oxidative cyclization of compounds **2a**–**f** using bromine and sodium carbonate afforded the required 1-substituted-4-phenyl-1,2,4-triazolo[4,3-*a*]quinoxalines **3a**–**f**. Their $^1$H NMR revealed the abscence of =CH and NH protons present in their precursors. Compounds **3a** and **3c** have been previously prepared by fusion of **1** with the corresponding aldehydes [13]. However, no spectral data were published.

Reaction of **1** with phenacyl bromides gave 2-aryl-5-phenyl-1 *H*-1,2,4-triazino[4,3-*a*]quinoxalines **4a**–**d** which showed in their $^1$H NMR a singlet at $\delta = 5.1-5.6$ ppm due to triazinoquinoxaline $-C_1-H_2$.

Reaction of **1** with ω-cyanoacetophenones afforded 2-(5-amino-3-arylpyrazol-1-yl)-3-phenylquinoxalines **5a**–**d**, their IR showed two NH stretching bands at 3453–3450 and 3358–3334 $cm^{-1}$. The $^1$H NMR of **5c** showed a singlet at $\delta = 5.6$ ppm due to pyrazolyl $-C_4-H$ and a $D_2O$ exchangeable singlet at $\delta = 5.9$ ppm due to the $NH_2$.

Three products were isolated from the reaction of the hydrazino derivative **1** with ethyl cyanoacetate, namely 1-cyanomethyl-4-phenyl-1,2,4-triazolo[4,3-*a*]quinoxaline (**6**), 2-cyanomethylcarbonylhydrazino-3-phenylquinoxaline (**7**), and 1-[(3-phenylquinoxalin-2-yl)hydrazono-2-aminoethylidene]-4-phenyl-1,2,4-triazolo[4,3-*a*]quinoxaline (**8**). Compound **7** could be obtained by reaction of **1** with the ester group of ethyl cyanoacetate forming an acid hydrazide which was cyclized under the experimental condition to yield **6**, some of the unreacted **1** reacted with **6** to produce the condensation product **8**. It is worth mentioning here that, under milder reaction conditions for example room temperature, in refluxing ethanol or dioxan no reaction occured. These compounds were identified by studying their IR; $^1$H-NMR and MS spectra. Compound **6** showed in its IR the stretching vibration band of C≡N at 2258 $cm^{-1}$ and in its $^1$H NMR, a singlet at $\delta = 4.65$ ppm due to $CH_2C\equiv N$. While compound **7** showed in its IR bands due to NH, C=O, C≡N stretching vibrations, its $^1$H NMR showed a singlet at $\delta = 3.6$, $D_2O$ exchangeable due to the two NH and a singlet at $\delta = 4.7$ due to $CH_2$. The condensation product **8** showed in its IR only bands due to NH, C=N and C=C. The MS of compounds **6**, **7**, **8** showed molecular ion peaks at m/z = 285, 303, 521 respectively.

The reaction of 1 with different β-ketoesters is described in Scheme 2. Upon treatment of **1** with different β-ketoesters at room temperature only ethyl acetoacetate and ethyl benzoylacetate afforded the open-chain hydrazone intermediate **9a**, **b**. Fusion of **1** with ethyl acetoacetate or ethyl γ-phenyl-acetoacetate yielded the 1-substituted-1,2,4-triazolo[4,3-*a*]quinoxalines **10a**, **b**, whereas fusion of **1** with ethyl benzoylacetate resulted in the formation of 2-(5-hydroxy-3-phenylpyrazol-1-yl)-3-phenylquinoxaline **11** which have been previously reported [13]. However, no spectral data were published and there was a difference in the melting point. It was found to exist in both keto and enol forms as indicated from its IR and $^1$H NMR (Experimental section).

In the present investigation, compounds **10a** und **11** were also obtained by heating the corresponding open-chain intermediate **9a**, **b** in an oil bath at 160 – 170 °C. Compound **10a** was also prepared by refluxing **1** in acetic anhydride.

Fusion of **1** with diethyl malonate produced 1-ethoxycarbonylmethyl-1,2,4-triazolo[4,3-*a*]quinoxaline (**12**) which showed in its $^1$H NMR a triplet at $\delta = 1.3$ ppm and a quartet at $\delta = 4.2$ ppm due to the ethyl group of the ester moiety. It also showed a singlet at $\delta = 4.6$ due to $CH_2$.

Bromination of **10a** using bromine in glacial acetic acid afforded 1-bromomethyl-4-phenyl-1,2,4-triazolo[4,3-*a*]quinoxaline (**13**) which was reacted with different amines to yield the target 1-substituted aminomethyl-1,2,4-triazolo[4,3-a]quinoxalines **14a**–**d** which were identified by their IR and $^1$H NMR (Experimental part).

**Table 1: Antimicrobial activity of the synthesized compounds**

| Compd. | *C. albicans* | | *S. aureus* | | *E. coli* | | Compd. | *C. albicans* | | *S. aureus* | | *E. coli* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | IZ | MIC | IZ | MIC | IZ | MIC | | IZ | MIC | IZ | MIC | IZ | MIC |
| **2a** | 21.5 | – | – | – | 26 | >100 | **6** | 19 | – | 17 | – | 21 | – |
| **2b** | 23 | >100 | 15 | – | 27 | >100 | **7** | 21 | – | 19 | – | 22 | >100 |
| **2c** | 20 | – | 20 | >100 | 25 | >100 | **9a** | 21.5 | – | 20 | – | 21 | – |
| **2d** | 19 | – | 20 | >100 | 24 | >100 | **9b** | 22 | 100 | 18 | – | 23 | >100 |
| **2e** | 20 | – | 19 | – | 21.5 | – | **10a** | 27 | 100 | – | – | 25 | >100 |
| **2f** | 21 | – | 20 | >100 | 20 | – | **10b** | 24 | >100 | 19 | – | 21 | – |
| **3a** | 21 | – | – | – | 25 | >100 | **11** | 21 | – | 19 | – | 21 | – |
| **3b** | 20 | – | 19 | – | 22 | >100 | **12** | 19 | – | 18 | – | 22 | >100 |
| **3c** | 21 | – | 18 | – | 22 | >100 | **13** | 26 | 25 | 16 | – | 19 | – |
| **3d** | 20.5 | – | 19 | – | 21 | – | **14a** | 18 | – | 16 | – | 19 | – |
| **3e** | 20 | – | 20 | >100 | 21 | – | **14b** | 24 | 50 | 11 | – | 20 | >100 |
| **3f** | 19 | – | 19.5 | – | 21 | – | **14c** | 18 | – | 15 | – | 17 | – |
| **4a** | 27 | 100 | – | – | 21 | – | **14d** | 18 | – | 15 | – | 20 | 100 |
| **4b** | 22 | >100 | 18 | – | 21 | – | **15a** | 19 | – | 18 | – | 22 | >100 |
| **4c** | 22 | 100 | 17 | – | 22 | >100 | **15b** | 19 | – | 19 | – | 22.5 | >100 |
| **4d** | 19 | – | 17 | – | 22 | >100 | **16** | 20 | – | 25 | >100 | 21 | – |
| **5a** | 20 | – | 14 | – | 20 | >100 | DMF | 16 | – | – | – | 17 | – |
| **5b** | – | – | 16 | – | 17 | – | Nystatin | – | 25 | – | – | – | – |
| **5c** | 17 | – | 17 | – | 17 | – | Cefotaxim | – | – | – | 2 | – | 4 |
| **5d** | 18 | – | 11 | – | 19 | – | | | | | | | |

IZ: Inhibition zones (mm); MIC: minimal inhibitory concentration (μg/ml)

**Table 2: 2-Arylidenehydrazino-3-phenylquinoxalines 2a–f**

| Compd. | R | Yield (%) | M.p. (°C) Cryst. solv. | Mol. Formula Mol. Wt. |
|---|---|---|---|---|
| **2a** | $C_6H_5$ | 91.78 | 87–88[a] EtOH | $C_{21}H_{16}N_4$ 324.4 |
| **2b** | 2-Cl-$C_6H_4$ | 98.77 | 151–152 EtOH | $C_{21}H_{15}ClN_4$ 358.8 |
| **2c** | 4-$NO_2$-$C_6H_4$ | 79.96 | 199–200 dioxane-$H_2O$ | $C_{21}H_{15}N_5O_2$ 369.4 |
| **2d** | 3-$CH_3O$-$C_6H_4$ | 65.48 | 114–115 $CHCl_3$/pet. ether | $C_{22}H_{18}N_4O$ 354.4 |
| **2e** | 3,4-$CH_2O_2C_6H_3$ | 91.39 | 146–147 EtOH | $C_{22}H_{16}N_4O_2$ 368.4 |
| **2f** | 2-Furyl | 50.11 | 138–140 EtOH/$H_2O$ | $C_{19}H_{14}N_4O$ 314.4 |

[a] a reported (118 C) [13]

**Table 3: 1-Substituted 4-phenyl-1,2,4-triazolo [4,3-*a*]quinoxalines 3a–f**

| Compd. | R | Yield (%) | M.p. (°C) Cryst. solv. | Mol. Formula Mol. Wt. |
|---|---|---|---|---|
| **3a** | $C_6H_5$ | 80.50 | 233–234[a] EtOH | $C_{21}H_{14}N_4$ 322.4 |
| **3b** | 2-Cl-$C_6H_4$ | 78.44 | 186–187 EtOH | $C_{21}H_{13}ClN_4$ 356.8 |
| **3c** | 4-$NO_2$-$C_6H_4$ | 91.64 | 252–253[b] Dioxane/$H_2O$ | $C_{21}H_{13}N_5O_2$ 367.4 |
| **3d** | 3-$CH_3O$-$C_6H_4$ | 87.43 | 164–165 EtOH | $C_{22}H_{16}N_4O$ 352.4 |
| **3e** | 3,4-$CH_2O_2C_6H_3$ | 99.00 | 254–255 EtOH | $C_{22}H_{14}N_4O_2$ 366.4 |
| **3f** | 2-Furyl | 96.97 | 149–150 EtOH | $C_{19}H_{12}N_4O$ 312.3 |

[a] reported (235 °C) [13]; [b] reported (252 °C) [13]

Coupling of diazotized sulphonamides with **11** produced the target azodyes **15a**, **b**.

Compound **11** reacted with nitrous acid to produce 2-(5-hydroxy-4-nitroso-3-phenyl-pyrazol-1-yl)-3-phenylquinoxaline (16).

### *2.2. Antimicrobial results*

The results of the antimicrobial studies presented in Table 1 revealed that most of the newly synthesized compounds showed antimicrobial activity against the fungus

**Table 4: 2-Aryl-5-phenyl-1*H*-1,2,4-triazino [4,3-*a*]quinoxalines 4a–f**

| Compd. | $R^1$ | Yield (%) | M.p. (°C) | Mol. Formula Mol. Wt. |
|---|---|---|---|---|
| **4a** | $C_6H_5$ | 81.85 | 242–243 | $C_{22}H_{16}N_4.HBr$ 417.3 |
| **4b** | 4-Cl–$C_6H_4$ | 86.46 | 234–235 | $C_{22}H_{15}ClN_4.HBr.H_2O$ 469.8 |
| **4c** | 4-$CH_3$–$C_6H_4$ | 79.47 | 221–222 | $C_{23}H_{18}N_4.HBr$ 431.3 |
| **4d** | 4-$CH_3O$–$C_6H_4$ | 55.39 | 200–201 | $C_{23}H_{18}N_4O.HBr$ 447.3 |

**Table 5: 2-(5-Amino-3-arylpyrazol-1-yl)-3-phenylquinoxalines 5a–d**

| | $R^1$ | Yield (%) | M.p. (°C) | Mol. Formula Mol. Wt. |
|---|---|---|---|---|
| **5a** | $C_6H_5$ | 86.69 | 241–242 | $C_{23}H_{17}N_5$ 363.4 |
| **5b** | 4-Cl–$C_6H_4$ | 79.18 | 211–212 | $C_{23}H_{16}ClN_5$ 397.9 |
| **5c** | 4-$CH_3$–$C_6H_4$ | 67.82 | 186–187 | $C_{24}H_{19}N_5$ 377.5 |
| **5d** | 4-$CH_3O$–$C_6H_4$ | 72.06 | 205–206 | $C_{24}H_{19}N_5O$ 393.5 |

**Table 6: 1-Substituted aminomethyl-4-phenyl-1,2,4-triazolo-[4,3-*a*]quinoxalines 14a–d**

| | $R^4$ | Yield (%) | M.p. (°C) | Mol. Formula Mol. Wt. |
|---|---|---|---|---|
| **14a** | 4-Cl–$C_6H_4$–NH | 68.55 | 272–273 | $C_{22}H_{16}ClN_5$ 385.9 |
| **14b** | 4-$CH_3$–$C_6H_4$–NH | 83.54 | 263–264 | $C_{23}H_{19}N_5$ 365.4 |
| **14c** | 1-Morpholinyl | 72.00 | 205–206 | $C_{20}H_{19}N_5O$ 345.4 |
| **14d** | 1-Piperidinyl | 65.84 | 220–221 | $C_{21}H_{21}N_5$ 343.4 |

*Candida albicans* and the gram negative bacteria *Escherichia coli* (IZ 17–27 mm). However, they were less active against the gram positive bacteria *Staphylococcus aureus*. The most active compounds against *C. albicans* were the 1,2,4-triazino[4,3-*a*]quinoxalines **4a**, **c**, ethyl 3-[(3-phenylquinoxaline-2-yl)hydrazono]-3-phenylpropanoate (**9b**) and the 1,2,4-triazolo[4,3-*a*]quinoxalines **10a**, **13**, **14b**. Their MIC were 100, 100, 100, 100, 25, 50 μg/ml, respectively. Comparing the results with that of nystatin it was found that compounds **13** and **14b** exhibit promising antifungal activity against *C. albicans* (MIC 25, 50 μg/ml respectively). Compound 13 was as active as nystatin. The most active compound against E. coli was 1-(1-piperidinylmethyl)-1,2,4-triazolo[4,3-a]-3-phenyl quinoxaline (**14d**) (MIC 100 μg/ml).

## 3. Experimental

M.p.'s were uncorrected and determined is open glass capillaries. IR spectra were measured in KBr discs on a Perkin Elmer 1430 spectrophotometer. $^1$HNMR were recorded on a Varian Gemini 200 at 200 MHz in DMSO-$d_6$, or $CDCl_3$ using TMS as internal standard, the chemical shifts are given in δ (ppm) values and the exchangeable protons were confirmed by $D_2O$. The MS were run on a Finnigan mass spectrometer model SSQ/7000 (70 ev.). The microanalyses were performed at the mincroanalytical unit, Faculty of Science, Cairo University, and the data were within ± 0.4% of the theoretical values.

### *3.1. Synthesis of the compounds*

*3.1.1. 2-Arylidenehydrazino-3-phenylquinoxalines* **2a–f**

To a solution of 1 (0.48 g, 2 mmol) in EtOH (15 ml) the appropriate aromatic aldehyde (2.2 mmol) was added. The reaction mixture was heated under reflux for 5 min, then cooled to RT and the separated crystalline hydrazone (from yellow to orange color) was filtered, dried and crystallized from the proper solvent (Table 2).
IR (KBr, cm$^{-1}$): 3349–3339 (NH); 1663, 1646 (C=N); 1607–1602, 1523–1499 (C=C); 1588–1560 (δ NH) and in the case of **2c** 1547, 1344 ($NO_2$). In case of **2d**, **2e** 1262 and 1030 (C–O–C).
$^1$HNMR (**2d**), (DMSO-$d_6$, δ, ppm); 3.9 (s, 3 H, $OCH_3$); 6.8–8.2 (m, 11 H, Ar–H); 8.5 (s, 1 H, =CH) 8.7 (dd, 2 H, quinoxaline $C_{5,8}$–H); 11.1 (s, 1 H, NH $D_2O$ exchangeable).

*3.1.2. 1-Substituted 4-phenyl-1,2,4-triazolo[4,3-a]quinoxalines* **3a–f**

To a stirred mixture of **2** (1 mmol) and anh. $NaHCO_3$ (0.15 g) in $CHCl_3$ (10 ml) was added bromine (0.15 ml). The mixture was stirred at RT for 5 h, left to stand overnight, evaporated under vaccum. The residue was triturated with ice-cold $H_2O$, the product was filtered, washed with $H_2O$, dried and crystallized from the proper solvent (Table 3). IR (KBr, cm$^{-1}$): 1663, 1645–1643 (C=N); 1628–1600; 1533–1516 (C=C) and in the case of **3c** 1553, 1344 ($NO_2$), in the case of **3d**, **e** 1258 and 1031 (C–O–C). $^1$H NMR (**3d**) (DMSO-$d_6$, δ, ppm): 3.9 (s, 3 H, $OCH_3$); 7–8.2 (m, 11 H, Ar–H); 8.7–8.9 (m, 2 H, quinoxaline $C_{5,8}$–H).

*3.1.3. 2-Aryl-5-phenyl-1 H-1,2,4-triazolo[4,3-a]quinoxalines* **4a–d**

To the solution of **1** (0.48 g, 2 mmol) in dry dioxane (10 ml) the appropriate phenacyl bromide (2 mmol) was added. The reaction mixture was heated under reflux; a reddish precipitate separated out during the first 5 min. The reflux was continued for 1 h, cooled, whereupon a crystalline product separated out, this was filtered, dried and crystallized from EtOH (Table 4). IR (KBr, cm$^{-1}$): 1637–1630 (C=N); 1615–1599; 1540–1537 (C=C) and the case of **4d** 1260, 1184, 1031 (C–O–C). $^1$HNMR (**4c**) (DMSO-$d_6$, δ, ppm): 2.3 (s, 3 H, $CH_3$); 5.1 (s, 2 H, triazinoquinoxaline $C_1$–$H_2$); 7.4, 8 (two d, J = 8 Hz, each 2 H, $C_6H_4$–$CH_3$); 7.5–8.4 (m, 9 H, Ar–H). $^1$H NMR (**4d**) (DMSO-$d_6$, δ, ppm): 3.8 (s, 3 H, $OCH_3$); 5.6 (s, 2 H, triazinoquinoxaline $C_1$–$H_2$); 7–8.5 (m, 13 H, Ar–H).

*3.1.4. 2-(5-Amino-3-arylpyrazol-1-yl)-3-phenylquinoxalines* **5a–d**

To a solution of **1** (0.48 g, 2 mmol) in EtOH (8 ml) containing acetic acid (2 ml) the appropriate ω-cyanoacerophenone (2 mmol) was added. The reaction mixture was heated under reflux for 2 h, cooled, the separated crystalline product was filtered, dried and crystallized from EtOH (Table 5). IR (KBr, cm$^{-1}$): 3453–3450, 3358–3334 (NH); 1665, 1642–1640 (C=N); 1609, 1533–1522 (C=C); 1572–1555 (δ NH). $^1$HNMR (**5c**) (DMSO-$d_6$, δ, ppm): 2.3 (s, 3 H, $CH_3$); 5.6 (s, 1 H, pyrazol–$C_4$–H); 5.9 (s, 2 H, $NH_2$, $D_2O$ exchangeable); 7–8 (m, 11 H, Ar–H); 8.1–8.3 (m, 2 H, quinoxaline $C_{5,8}$–H). MS of **5c** m/z (%): 379 (8) ($M^{+\cdot}$ + 2), 378 (45) ($M^{+\cdot}$ + 1); 377 (100) ($M^{+\cdot}$; 376 (98); 362 (18); 361 (65); 337 (10); 286 (5); 235 (10); 220 (16); 219 (10); 205 (14); 188.5 (13); 142 (5); 115 (14); 91 (5); 90 (5); 77 (5); 65 (3).

*3.1.5. 1-Cyanomethyl-4-phenyl-1,2,4-triazolo[4,3-a]quinoxaline* (**6**)*, 2-cyanomethylcarbonylhydrazino-3-phenylquinoxaline* (**7**)*, 1-[(3-phenylquinoxalin-2-yl)hydrazino-2-aminoethylidene]4-phenyl-1,2,4-triazolo[4,3-a]quinoxaline* (**8**)

A mixture of **1** (0.95 g, 4 mmol) and ethyl cyanoacetate (0.5 g, 0.47 ml, 4.4 mmol) was heated in an oil bath at 160–170 °C for 1 h, cooled and the residue was triturated with EtOH, filtered and dried. Yield: 0.8 g of pinkish

white material; TLC indicated the presence of three products which were separated by fractional crystallization from EtOH. The pinkish white crystalline material separated from EtOH by crystallization from EtOH. The pinkish white crystalline material separated from EtOH by crystallization (6, 0.25 g, 21.79% of pure product, m.p. 194–195 °C). The mother liquor was concentrated to yield a white crystalline material, (**7**, 0.09 g, 7.39% m.p. 168–169 °C). The EtOH insoluble material was purified by boiling several times with EtOH, filtered, dried to yield 0.2 g (9.54%) of compound **8**, m.p. 290–292 °C.

IR (**6**) (KBr, $cm^{-1}$): 2258 (C≡N); 1663, 1645 (C=N); 1609; 1535 (C=C).$^1$H-NMR ($CDCl_3$, δ, ppm): 4.65 (s, 2 H, $CH_2$); 7.5–8.3 (m, 7 H, Ar–H); 8.7 (dist d, 2 H, triazoloquinoxaline $C_{6,9}$–H). MS m/z (%): 287 (2.7) ($M^{+\cdot}+2$); 286 (18) ($M^{+\cdot}+1$); 285 (67) ($M^{+\cdot}$); 259 (2); 220 (15), 219 (100); 218 (23); 193 (6); 192 (5); 142.5 (3); 109.7 (5); 90 (3); 77 (3).
$C_{17}H_{11}N_5$ (285.3)

IR (**7**) (KBr, $cm^{-1}$): 3313 (NH); 2262 (C≡N); 1689 (C=O), 1663, 1645 (C=N); 1628, 1519 (C=C); 1537 (δ NH).$^1$H-NMR ($CDCl_3$, δ, ppm): 3.6 (s, 2 H, 2 NH, $D_2O$ exchangeable); 4.7 (s, 2 H, $CH_2$); 7.5–8.3 (m, 7 H, Ar–H); 8.8 (dd, 2 H, quinoxaline- $C_{5,8}$–H). MS m/z (%): 305 (1.8) ($M^{+\cdot}+2$); 304 (19) ($M^{+\cdot}+1$); 303 (29) ($M^{+\cdot}$); 285 (3); 264 (18), 263 (100); 259 (23); 235 (10); 220 (28); 219 (32); 218 (18); 205 (12); 129 (5); 90 (3); 77 (3); 68 (3).
$C_{17}H_{13}N_5O$ (303.3)

IR (**8**) (KBr, $cm^{-1}$): 3443 (NH); 1663, 1646 (C=N); 1628, 1519 (C=C); 1552 (δ NH); MS m/z (%): 521 (100) ($M^{+\cdot}$); 506 (23); 505 (19); 504 (33); 441 (23); 440 (81); 363 (3); 336 (19); 286 (10); 285 (63); 260 (10); 246 (9); 220 (35); 219 (100); 218 (61); 205 (8); 194 (35); 129 (5); 90 (7); 77 (3).
$C_{31}H_{23}N_9$ (521.6)

### *3.1.6. Ethyl 3-[(3-phenylquinoxalin-2-yl)hydrozono]butyrate* (**9a**)

To the solution of **1** (0.48 g, 2 mmol) in MeOH/$CHCl_3$ (1 : 1) (10 ml) ethyl acetoacetate (0.29 g, 0.28 ml, 2.2 mmol) was added. The reaction mixture was left at RT for 24 h. The organic solvent was evaporated under vaccum at RT, the residue was triturated with pet. ether, 40–60 °C, filtered, dried and crystallized from $CHCl_3$/pet. ether (40–60 °C), yield 0.65 g (92.85%), m.p. 75–76 °C. IR (KBr, $cm^{-1}$): 3565 (OH enolic); 3444 (NH); 1712 (C=O); 1646, 1629 (C=N); 1610, 1589, 1502 (C=C); 1556 (δ NH); 1278, 1089 (C–O–C). $^1$HNMR ($CDCl_3$, δ, ppm): 1.3, 1.4 (two t, each 3 H, $CH_2\underline{CH_3}$ keto and enol); 1.7, 2.3 (two s, each 3 H, N=C–$CH_3$) keto and enol); 3.4 (s, 2 H, $CH_2$); 3.6 (s, 1 H, =CH enol); 4.2, 4.5 (two q, each 2 H, $\underline{CH_2}$ $CH_3$, keto and enol); 6.9–8.5 (m, 20 H, Ar–H; and NH of keto and enol); 9.5 (s, 1 H, OH enol $D_2O$ exchangeable).
$C_{20}H_{20}N_4O_2$ (348.4)

### *3.1.7. Ethyl 3-[(3-phenylquinoxalin-2-yl)hydrozono]3-phenylpropanoate* (**9b**)

This compound was prepared following the same procedure as described for **9a** from **1** (2 mmol) and ethyl benzoylacetate (0.42 g, 3.8 ml, 2.2 mmol) in MeOH/$CHCl_3$ (1 : 1) (10 ml) after 24 h, a yellow crystalline solid was separated out, filtered, washed with EtOH, dried and crystallized from EtOH, yield 0.68 g (82.92%), m.p. 143–144 °C. IR (KBr, $cm^{-1}$): 3547 (enolic OH); 3310 (NH); 1721 (C=O); 1646, 1628 (C=N); 1603, 1520 (C=C); 1574 (δ NH); 1208, 1025 (C–O–C).
$C_{25}H_{22}N_4O_2$ (410.5)

### *3.1.8. 1-Methyl-4-phenyl-1,2,4-triazolo[4,3-a]quinoxaline* (**10a**)

Method A: A mixture of **1** (0.48 g, 2 mmol) and ethyl acetoacetate (0.29 g, 0.28 ml, 2.2 mmol) was heated in an oil bath at 160–170 °C for 1 h, cooled, triturated with pet. ether (60–80 °C), filtered, dried and crystallized from EtOH, yield 0.34 g (65.38%), m.p. 223–224 °C; reported m.p. 220 °C [13].

Method B: The title compound was also prepared by heating **9a** in an oil bath at 160–170 °C for 1 h and treated as described in method A. Yield (72.40%).

Method C: **10a** was also prepared by heating 1 (0.48 g, 2 mmol) in acetic anhydride (5 ml) under reflux for 1 h, then cooled. White crystalline needles were filtered, washed with $H_2O$, dried, yield 0.51 g (98.1%). The products of method A, B and C have the same TLC, m.p., IR, $^1$HNMR. Mixed m.p. showed no depressions. IR (KBr, $cm^{-1}$): 1646 (C=N); 1629, 1610, 1518 (C=C). $^1$H-NMR (MDSO-$d_6$, δ, ppm): 2.6 (s, 3 H, $CH_3$); 7.4–8.5 (m. 7 H, Ar–H); 7.8 (dd, 2 H, triazoloquinoxaline $C_{6,9}$–H).
$C_{16}H_{12}N_4$ (260.3)

### *3.1.9. 1-Benzyl-4-phenyl-1,2,4-triazolo[4,3-a]quinoxaline* (**10b**)

This compound was similarly prepared as described for **10a** from **1** and ethyl γ-phenylacetoacetate and crystallized from EtOH. Yield 0.45 g (67.16%), m.p. 193–195 °C. IR (KBr, $cm^{-1}$): 1645 (C=N); 1628, 1608, 1518 (C=C). $^1$HNMR (DMSO-$d_6$, δ, ppm): 3.1 (s, 2 H, $CH_2$); 7.5–8.4 (m. 12 H, Ar–H); 8.7–9.0 (m, 2 H, triazoloquinoxaline $C_{6,9}$–H).
$C_{22}H_{16}N_4$ (336.4)

### *3.1.10. 2-(5-Hydroxy-3-phenylpyrazol-1-yl)-3-phenylquinoxaline* (**11**)

Method A: A mixture of **1** (0.48 g, 2 mmol) and ethyl benzoylacetate (0.42 g, 0.38 ml, 2.2 mmol) was heated in an oil bath at 160–170 °C for 1 h, then the reaction mixture was treated as described for **10a**. It was crystallized from EtOH, yield 0.55 g (76.38%), m.p. 192–193 °C, reported 257 °C [13].

Method B: The title compound was also prepared by heating **9b** at 160 to 170 °C for 1 h and then treated as described in method A, yield (87.5%). The product had the same m.p., TLC, IR and $^1$HNMR as that prepared by method A.

IR (KBr, $cm^{-1}$): 3442 (br. OH enolic); 1681 (C=O); 1664, 1646 (C=N), 1560, 1513 (C=C). $^1$HNMR ($CDCl_3$, δ, ppm): 3.7 (s, 2 H, pyrazolone $C_4$–$H_2$); 6.0 (s. ½ H,= CH, enol); 7–8.3 (m, 14 H, Ar–H); 8.8 (br. s, ½ H, OH enol $D_2O$ exchangeable).
$C_{23}H_{16}N_4O$ (364.4)

### *3.1.11. 1-Ethoxycarbonylmethyl-4-phenyl-1,2,4-triazolo[4,3-a]quinoxaline* (**12**)

A mixture of **1** (0.71 g, 3 mmol) and diethylmalonate (0.53 g, 0.5 ml, 3.3 mmol) was heated in an oil bath at 160–170 °C for 1 h, cooled, triturated with pet. ether (60–80 °C), filtered, dried and crystallized from EtOH, yield 0.85 g (85.26%), m.p. 200–201 °C;. IR (KBr, $cm^{-1}$): 1728 (C=O); 1662, 1630 (C=N), 1587, 1501 (C=C); 1205, 1022 (C–O–C). $^1$HNMR ($CDCl_3$, δ, ppm): 1.3 (t, J = 6.5 Hz, 3 H, $CH_2\underline{CH_3}$); 4.2 (q, J = 6.5 Hz, 2 H, $\underline{CH_2}CH_3$); 4.6 (s, 2 H, $CH_2$); 7.5–8.3 (m, 7 H, Ar–H); 8.7 (dd, 2 H, triazoloquinoxaline $C_{6,9}$–H).
$C_{19}H_{16}N_4O_2$ (332.4)

### *3.1.12. 1-Bromomethyl-4-phenyl-1,2,4-triazolo[4,3-a]quinoxaline hydrobromide* (**13**)

To a solution of **10a** (1.3 g, 5 mmol) in glacial acetic acid (20 ml) bromine (0.8 g, 10 mmol) was added. The mixture was stirred at RT for 1 h, then at 70 °C for 5 h. The reaction mixture was left to stand at RT overnight, the separated yellow crystalline product was filtered washed with acetic acid, dried and crystallized from acetic acid, yield 2 g (94.79%), m.p. 286 to 287 °C. IR (KBr, $cm^{-1}$): 2012 (NH); 1646 (C=N); 1616, 1594, 1515 (C=C).
$C_{16}H_{11}BrN_4HBr$ (420.1)

### *3.1.13. 1-Substituted aminomethyl-4-phenyl-1,2,4-triazolo[4,3-a]quinoxalines* **14a–d**

To a solution of **13** (0.42 g, 1 mmol) in acetonitrile (10 ml) the appropriate amine (3 mmol) was added. The reaction mixture was heated under reflux for 1 h, and cooled to RT. The produced crystalline product was filtered, dried and crystallized from acetonitrile (Table 6). IR **14a** (KBr, $cm^{-1}$): 3443 (NH); 1663, 1646 (C=N); 1616, 1502 (C=C); 1527 (δ NH); 767 (C–Cl). $^1$HNMR (**14c**) ($CDCl_3$, δ, ppm): 3.2 (s, 2 H, $CH_2$–N); 3.7, 4 (two t, each 4 H, morpholine $C_{3,5}$ and $C_{2,6}$–H respectively); 7.5–7.8 (m, 7 H, Ar–H); 8.2–8.4 (m, 2 H, triazoloquinoxazole-$C_{6,9}$-H).

### *3.1.14. 2-[5-Hydroxy-3-phenyl-4-(4-substituted sulfamoylphenyl)azopyrazol-1-yl]-3-phenylquinoxaline* **15a, b**

To an ice cooled solution of **11** (0.36 g, 1 mmol) in EtOH (15 ml) containing NaOH (1 g) and sodium acetate (2 g) the appropriate ice-cooled diazonium salt (prepared from 1 mmol sulfonamide in 1 ml HCl and $NaNO_2$ 0.1 g, 1.5 mmol in the least amount of $H_2O$) was added. The cooling was continued for 1 h with stirring, left to stand in a refregirator overnight then neutralized carefully by dropwise addition of conc. HCl. The formed yellow to orange precipitate was filtered, dried. **15a**, $R^5$=H, crystallized from EtOH, yield 59.87%, m.p. 168–170 °C. IR (KBr, $cm^{-1}$): 3522–3501 (OH); 3440–3119 (NH); 1666, 1643 (C=N); 1590, 1513 (C=C and δ NH); 1548 (N=N); 1355, 1161 ($SO_2$).
$C_{29}H_{21}N_7O_3S$ (547.6)

**15b**, $R^5$ = 2-pyrimidinyl, crystallized from DMF/EtOH, yield (87.37%) m.p. 302–303 °C. IR (KBr, $cm^{-1}$): 3565–3546 (OH); 3443, 3368 (NH); 1676, 1646 (C=N); 1628, 1520 (C=C); 1583, (δ NH); 1552 (N=N); 1355, 1169 ($SO_2$).
$C_{33}H_{23}N_9O_3S$ (625.7)

### *3.1.15. 2-(5-Hydroxy-4-nitroso-3-phenylpyrazol-1-yl)-3-phenyl-quinoxaline* **16**

To an ice cooled solution of **11** (0.36 g, 1 mmol) in EtOH (10 ml) containing acetic acid (3 ml) a cold solution of $NaNO_2$ (0.1 g, 1.5 mmol) in $H_2O$ (1 ml) was added. The mixture was left at RT overnight. The obtained orange precipitate was filtered, washed with $H_2O$, dried, and crystallized from EtOH/$H_2O$, yield 0.3 g (76.34%), m.p. 123–124 °C. IR (KBr, $cm^{-1}$): 3440 (OH enolic); 1710 (C=O); 1662, 1645 (C=N); 1625, 1519 (C=C); 1552 (=N–OH); 1533 (N=O).
$C_{23}H_{15}N_5O_2$ (393.4)

### *3.2. Antimicrobial testing*

The tested compounds were evaluated by the agar diffusion technique [14] using a 2 mg/l ml solution in DMF. The test organisms were *Candida albicans* (ATCC 10231), *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (NCTC 10418). DMF showed no inhibition zone against *S. aureus* while, against *C. albicans* and *E. coli* it showed inhibition zones (16, 17 mm respectively). The minimal inhibitory concentration (MIC) of the most active compounds was measured using the two fold serial broth dilution method [15]. Reference antibiotics were hystatin for *C. albicans* and cefotaxime for both *S. aureus* and *E. coli*.

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