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Design and Synthesis of Some New 1,2,4-Triazolo[4,3-*a*]Quinoxaline Derivatives as Potential Antimicrobialagents

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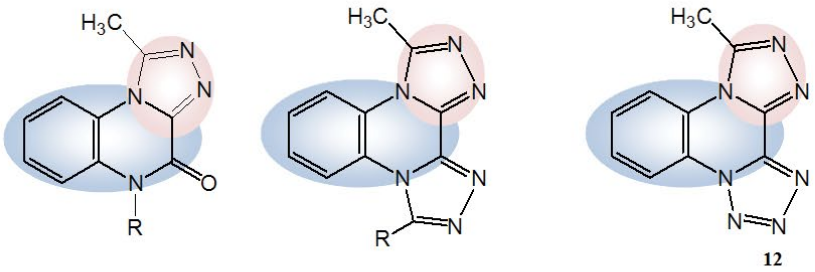
Abstract

As a part of an ongoing research program to achieve new chemical entities suitable for development as new class of antimicrobial agents, the present work describes the design and synthesis of a new series of substituted-1-methyl-1,2,4-triazolo[4,3-*a*]quinoxalines, The newly synthesized compounds were screened for their *in vitro* antimicrobial activity. The results revealed that the compounds demonstrated significant activity against Gram negative bacteria. Compounds **3** and **11b** exhibited twice the activity of ampicillin against *Pseudomonas aeruginosa*, while compounds **4, 5b, 7, 9a, 10d, 11a, 11c** and **12** were equipotent to ampicillin. On the other hand, the tested compounds demonstrated mild antifungal activity. Compound **11d** exhibited nearly one-half the activity of clotrimazole against *Candida albicans*.

Synthesis and antimicrobial evaluation of some novel 1,2,4-triazolo[4,3-*a*] quinoxalines. The results revealed that some of the synthesized compounds exhibited significant antibacterialactivity.



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4.R= $CH_2COOC_2H_5$, 5b. R= 4-$CH_3C_6H_4$-$COCH_2$

7. R = CH_3, 9a. R= 2-C_6H_4COOH, 11a. R= C_6H_5, 11c. R= 3,4-OCH_3-C_6H_3

Keywords: Synthesis; Triazoloquinoxalines; Bistriazoloquinoxalines; Tetrazolotriazoloquioxaline; Antibacterial activity; Antifungal activity.

Introduction

Resistance of pathogenic bacteria towards the clinically used antibiotics makes it harder to eliminate infections from the body as existing drugs become less effective creating a challenging problem worldwide. As a result, discovery and development of new class of antimicrobial drugs are urgently needed to combat the growing threat of drug-resistant microbes [1].

Literature survey revealed that quinoxaline and fused quinoxaline ring systems are attractive candidates in medicinal chemistry as they constitute the building blocks of wide range of many pharmacologically active compounds having anticancer [2], antimicrobial [3,4], anti-inflammatory [5], antidepressant [6], antiviral [7], antidiabetic[8], antihypertensive [9], antihistaminic [10] and antiglaucoma activities [11]. In addition, it has been reported that quinoxaline moiety constitutes the basic skeleton for many natural and synthetic pharmacologically active compounds [12]. For example, quinoxaline ring is a part of the naturally occurring antibiotics, triostin A and echinomycin that are known to inhibit the growth of Gram positive bacteria and they are active against various transplantable tumors [13-15]

Moreover, in a previous publication, the synthesis and antimicrobial evaluation of a series of substituted 1,2,4-triazolo[4,3-*a*]quinoxalines have been reported [16,17]. The screening results revealed that compounds A and B (Figure 1) exhibited significant activity against *Staphylococcus aureus* and *Candida albicans* respectively.

In view of the above mentioned results and as a continuation of our research on quinoxaline derivatives in an attempt to identify new lead compounds that might be of value for future development as new class of antimicrobial agents, we report herein the synthesis and antimicrobial evaluation of a new series of 5- substituted 1,2,4-triazolo[4,3-*a*]quinoxaline derivatives (formula A, Figure 2) in order to achieve further knowledge of the structure-activity relationship.

Furthermore, it has been reported that annelating the 1,2- bond of the quinoxaline ring with an additional "electron rich" ring might extend the planarity of the hetero ring and modulate either the lipophilicity or

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hydrogen bond accepting property. In other words, this might increase selectivity and affinity of a pharmacophore element towards different receptor binding site [18]. Accordingly, some new 3,10-disubstituted-bis-1,2,4-triazolo[4,3-*a*:3',4'-c]quinoxalines and tetrazolo[1,5-*a*]-1,2,4-triazolo[3,4-*c*]quinoxalin (formula B,C. Figure 2) were also designed so as to further extend the planarity of the heterocyclic ring system hoping to add some synergistic biological significance to the target molecules. The substitution pattern of target compounds was carefully selected so as to impart various electronic and lipophilic properties to the target molecules that might contribute to the enhancement of antimicrobial activity.

Experimental

Chemistry

All reagents and solvents were purchased from commercial suppliers and were dried and purified when necessary by standard techniques. Melting points were determined in open glass capillaries using Stuart capillary melting point apparatus (Stuart Scientific Stone, Staffordshire, UK) and are uncorrected. IR spectra were recorded, for potassium bromide discs, ύ (cm^{-1}), on Perkin Elmer 1430 spectrophotometer. ^{1}H-NMR spectra were determined either on a Bruker Avance spectrometer (300 MHz) at the microanalytical unit, Faculty of Science, Cairo University, or on Jeol (500 MHz) at the microanalytical unit, Faculty of Science, Alexandria University, using DMSO-d6 as a solvent and TMS as internal standard. The chemical shifts are given in δ ppm values (s, singlet; d, doublet; t, triplet and m, multiplet). ^{13}C-NMR spectra were determined on Jeol (125 MHz), Faculty of Science, Alexandria University, using TMS as internal standard. Mass spectra were run on a Finnigan mass spectrometer model SSQ/7000 (70 eV), Faculty of Science, Cairo University. Microanalyses were performed at the microanalytical unit, Faculty of Science, Cairo University. The results of the microanalyses were within ± 0.4 % of the calculated values. Follow-up of the reactions and checking the homogeneity of the compounds were made by ascending TLC run on silica gel G (Merck 60) coated glass plates. The spots were visualized by exposure to iodine vapor or UV lamp at k 254 nm for few seconds. Compound **1** was prepared according to the reported method [19] by refluxing 1-methyl-1,2,4-triazolo[4,3-*a*]quinoxalin-4(*5H*)-one with acetic anhydride . Heating **1** with phosphorous oxychloride afforded compound **2** according to a previously reported reaction conditions [20-22].

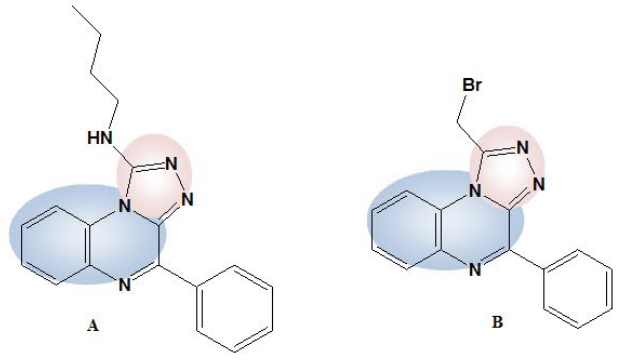


Figure 1: Structures of reported 1-sustituted-4-phenyl-1,2,4-triazolo[4,3-*a*] quinoxalines as antimicrobial leads.

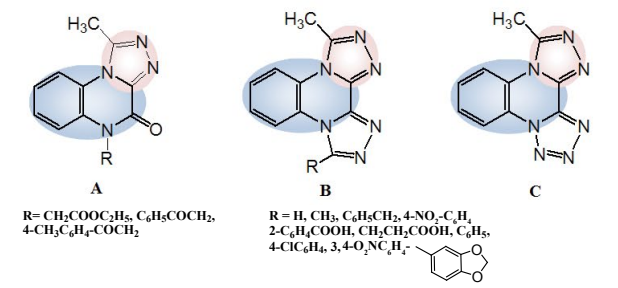


Figure 2: The proposed design of the newly synthesized compounds.

4-Hydrazinyl-1-methyl-1,2,4-triazolo[4,3-*a*]quinoxaline (3): A mixture of **2** (0.42 gm, 1 mmol) and hydrazine hydrate 99% (0.29 ml, 0.3 gm, 6 mmol) in absolute ethanol (3 ml) was heated under reflux for 1 h during which precipitation of yellow crystals occurred. The reaction mixture was concentrated, left to cool to room temperature and the formed crystals was filtered, washed with water, dried and recrystallized from ethanol.

Yield 88%, M.P. 254-6 C (ethanol) ; IR (KBr, cm^{-1}): 3264 (NH); 1632 (C=N); 1570 (δNH); 1520,1494 (C=C).^{1}H-NMR (300 MHz,DMSO-d_6); δ (ppm): 3.02 (s, 3H, CH_3); 4.68 (s, 2H, $NHNH_2$, D_2O exchangeable), 7.31 (t, *J* = 7.2 Hz, 1H, triazoloquinox.C_8-H); 7.46 (t, *J* = 7.2 Hz, 1H, triazoloquinox.C_7-H); 7.64 (d, *J* = 8.1 Hz, 1H, triazoloquinox.C_9-H); 8.10 (d, *J* = 8.4 Hz, 1H, triazoloquinox.C_6-H); 9.38 (s, 1H, $NHNH_2$, D_2O exchangeable). EI-MS m/z (relative abundance %): 215 (16.01) [$M^{+\cdot}$+1], 214 (100) [$M^{+\cdot}$], 199 (13.87), 185 (74.27), 158 (11.02), 144 (51.78), 129 (13.87), 118 (31.89), 105 (22.42), 102 (16.98), 90 (57.81), 77 (26.05), 63 (22.55), 57 (15.10), 51 (29.16).Anal. Calcd. for $C_{10}H_{10}N_6$ (214.23): C, 56.07; H, 4.71; N, 39.23. Found: C, 56.18; H, 4.68; N, 39.50.

Ethyl 2-(1-methyl-4-oxo-1,2,4-triazolo[4,3-*a*]quinoxalin-5(4*H*)-yl)acetate (4): A mixture of **1** (0.2 gm, 1 mmol) , ethyl bromoacetate (0.22 ml, 0.33 gm, 2 mmol) and anhydrous potassium carbonate (0.27 gm, 2 mmol) in dry acetone (5 ml) was heated under reflux with stirring for 4 h. The reaction mixture was left to cool to room temperature and the precipitated white product was filtered, washed with water, dried and crystallized from ethanol.

Yield 71%, M.P. 265-6 °C (ethanol); IR (KBr, cm^{-1}): 1737 (ester C=O); 1680 (C=O); 1509 (C=C); 1226, 1016 (C-O-C). ^{1}H-NMR (500 MHz,DMSO-d_6); δ (ppm): 1.52 (t, *J*=7.6 Hz, 3H, CH_2CH_3); 2.93 (s, 3H, CH_3); 4.13 (q, *J*=7.6 Hz, 2H, CH_2CH_3); 5.04 (s, 2H, CH_2CO_2); 7.38 (t, *J* = 7.6 Hz, 1H, triazoloquinox.C_8-H); 7.41 (d, *J* = 8.0 Hz, 1H, triazoloquinox. C_9-H); 7.49 (t, *J* = 7.6 Hz, 1H, triazoloquinox.C_7-H); 8.07 (d, *J* = 7.6 Hz, 1H, triazoloquinox.C_6-H). ^{13}C-NMR (75 MHz,DMSO-d_6) δ (ppm): 13.98 (CH_2CH_3); 14.77 (CH_3); 43.41 (NCH_2CO); 61.38 (CH_2CH_3); 116.46 (triazoloquinox.C_9); 117.37 (triazoloquinox.C_6); 121.43 (triazoloquinox.C_8); 123.94 (triazoloquinox.C_7); 127.76 (triazoloquinox. C_{9a}); 129.34 (triazoloquinox.C_{5a}); 143.07 (triazoloquinox.C_1); 149.48 (triazoloquinox.C_{3a}); 151.62 (triazoloquinox.C_4); 167.60 (CH_2CO). Anal. Calcd. for $C_{14}H_{14}N_4O_3$ (286.29): C, 58.73; H, 4.93; N, 19.57. Found: C, 58.85; H, 4.96; N, 19.73.

1-Methyl-5-(4-substituted-phenylcarbonylmethyl)-1,2,4-triazolo[4,3-*a*]quinoxalin-4(5H)-ones (5a,b): A mixture of **1** (0.2 gm, 1 mmol) , appropriate phenacyl bromide (2 mmol) and anhydrous potassium carbonate (0.27 gm, 2 mmol) in dry dimethyl formamide (5 ml) was heated under reflux for 6 h during which precipitation of white crystals occurred. The reaction mixture was left to cool to room temperature and the crystalline product was filtered, washed with water, dried and recrystallized from dimethylformamide.

1-Methyl-5-(4-phenylcarbonylmethyl)-1,2,4-triazolo[4,3-*a*]quinoxalin-4(5H)-ones (5a): Yield 42%, M.P. >300°C(DMF); IR (KBr, cm^{-1}): 1679 (C=O); 1594 (C=N); 1507 (C=C).^{1}H-NMR (500 MHz,DMSO-

d_6); δ (ppm): 2.94 (s, 3H, CH_3); 5.94 (s, 2H, CH_2CO); 7.37 (t, *J* = 6.8 Hz, 1H, phenyl C_4-H); 7.43 (t, *J* = 7.6 Hz, 1H, triazoloquinox.C_8-H); 7.46 (t, *J* = 6.8 Hz, 1H, triazoloquinox.C_7-H); 7.59 (t, *J* = 7.6 Hz, 2H, phenyl $C_{3,5}$-H); 7.7 (d, *J* = 7.6 Hz, 1H, triazoloquinox.C_9-H); 8.14 (d, *J* = 7.6 Hz, 2H, phenyl $C_{2,6}$-H); 8.15 (d, *J* = 8.0 Hz, 1H, triazoloquinox.C_6-H). ^{13}C-NMR (125 MHz,DMSO-d_6) δ (ppm): 15.37(CH_3); 40.37(NCH_2CO); 117.38(triazoloquinox.C_9); 117.87(triazoloquinox.C_6); 122.00 (triazoloquinox.C_8); 122.40(triazoloquinox.C_7); 128.32(phenyl C_4); 128.90(phenyl $C_{3,5}$); 129.50(phenyl $C_{2,6}$); 130.20(triazoloquinox.C_{9a}); 133.90(phenyl C_1); 134.00(triazoloquinox.C_{5a}); 143.70(triazoloquinox.C_1); 150.00(triazoloquinox.C_{3a}); 152.20(triazoloquinox.C_4); 193.04(CH_2CO).Anal. Calcd. for $C_{18}H_{14}N_4O_2$ (318.33):C, 67.91; H, 4.43; N, 17.60. Found: C, 68.04; H, 4.49; N, 17.82.

1-Methyl-5-(4-p-tolylcarbonylmethyl)-1,2,4-triazolo[4,3-*a*] quinoxalin-4(5H)-ones (5b): Yield 69%, M.P. >300°C(DMF); IR (KBr, cm^{-1}): 1681 (C=O); 1604 (C=N); 1509 (C=C).^{1}H-NMR (300 MHz,DMSO-d_6); δ (ppm): 2.50 (s, 3H, CH_3, p-tolyl); 3.05 (s, 3H, CH_3); 5.93 (s, 2H, CH_2CO); 7.38-7.48 (m, 5H, triazoloquinox.$C_{7,8,9}$-H and p-tolyl $C_{3,5}$-H); 8.04 (d, *J* = 7.8 Hz, 2H, p-tolyl $C_{2,6}$-H); 8.18 (d, *J* = 8.7 Hz, 1H, triazoloquinox.C_6-H). Anal. Calcd. For $C_{19}H_{16}N_4O_2$ (332.36): C, 68.66; H, 4.85; N, 16.86. Found: C, 68.79; H, 4.91; N, 17.02.

3-Methylbis-1,2,4-triazolo[4,3-*a*:3',4'-*c*]quinoxaline(6): A mixture of 4-hydrazinyl-1-methyl-1,2,4-triazolo[4,3-*a*]quinoxaline **3** (0.21 gm, 1 mmol) and formic acid (2.5 ml) was heated under reflux for 2 h. The reaction mixture was concentrated by evaporating under vacuum, cooled and then poured onto crushed ice during which precipitation of yellow product occurred. The formed product was filtered, washed with water, dried and crystallized from dimethylformamide/ethanol.

Yield 95%, M.P. >300°C(DMF/ethanol); IR (KBr, cm^{-1}): 1620 (C=N); 1600,1500 (C=C).^{1}H-NMR (300 MHz,DMSO-d_6); δ (ppm): 3.04 (s, 3H, CH_3); 7.63-7.71 (m, , 2H, bis-triazoloquinox.$C_{6,7}$-H); 8.3 (dd, *J* = 6.4, 3.3Hz, 1H, bis-triazoloquinox.C_5-H); 8.42 (dd, *J* = 6.3, 3.3 Hz, 1H, bis-triazoloquinox.C_8-H); 9.97 (s, 1H, bis-triazoloquinox.C_{10}-H). EI-MS (relative abundance %): 225 (2.01) [$M^{+\cdot}$ +1], 224 [$M^{+\cdot}$] (1.88); 222 (7.39); 208 (6.63); 189 (9.51); 180 (5.76); 166 (7.35); 152 (17.65); 129 (18.23); 123 (25.76); 111 (38.9); 97 (66.34); 83 (75.56); 69 (100). Anal. Calcd. for $C_{11}H_8N_6$ (224.22): C, 58.92; H, 3.60; N, 37.48. Found: C, 58.97; H, 3.64; N, 37.63.

3,10-Dimethylbis-1,2,4-triazolo[4,3-*a*:3',4'-*c*]quinoxaline(7)

A mixture of **3** (0.21 gm, 1 mmol) and acetic anhydride (5 ml) was heated under reflux for 3 h. The reaction mixture was concentrated by evaporating under vacuum, diluted with cold water and left in refrigerator overnight during which separation of white crystals occurred. The formed crystalline product was filtered, washed with water, dried and recrystallized from glacial acetic acid.

Yield 72%, M.P. >300°C (GAA); IR (KBr, cm^{-1}): 1615 (C=N); 1579,1490 (C=C). ^{1}H-NMR (300 MHz,DMSO-d_6); δ (ppm): 3.05 (s, 6H, 2 CH_3); 7.62-7.79 (m, 2H, bis-triazoloquinox.$C_{6,7}$-H); 8.29-8.4 (m, 2H, bis-triazoloquinox.$C_{5,8}$-H). EI-MS m/z (relative abundance %): 239 [$M^{+\cdot}$ +1] (3.45), 238 [$M^{+\cdot}$] (2.34); 236 (21.27); 222 (5.95); 208 (6.47); 194 (7.91); 180 (5.44); 166 (7.33); 152 (17.25); 129 (17.77); 123 (24.42); 111 (37.65); 97 (64.85); 83 (75.01); 69 (100); 55 (89.15). Anal. Calcd. for $C_{12}H_{10}N_6$ (238.25): C, 60.50; H, 4.23; N, 35.27. Found: C, 60.63; H, 4.30; N, 35.41.

10-Substituted 3-methylbis-1,2,4-triazolo[4,3-*a*:3',4'-*c*]quinoxalines (8a,b)

A mixture of **3** (0.21 gm, 1 mmol), appropriate carboxylic acid (1 mmol) and phosphorus oxychloride (3 ml) was heated under reflux for 6 h. The reaction mixture was left to cool to room temperature, poured onto crushed ice and neutralized with concentrated ammonia to pH7 during which precipitation of product occurred. The precipitated solid was filtered, washed with water, dried and crystallized from ethanol.

10-Benzyl-3-methylbis-1,2,4-triazolo[4,3-*a*:3',4'-*c*]quinoxaline (8a): Yield 32%, M.P. >300°C(ethanol); IR (KBr, cm^{-1}): 1626 (C=N); 1580, 1551, 1500 (C=C).^{1}H-NMR (500 MHz,DMSO-d_6); δ (ppm): 2.94 (s, 3H, CH_3); 2.99 (s, 2H, CH_2); 7.24 (t, *J* = 7.6 Hz, 3H, phenyl$C_{3,4,5}$-H); 7.34-7.44 (m, 2H, phenyl$C_{2,6}$-H); 7.48 (t, *J* = 7.6 Hz, 2H, bis-triazoloquinox.$C_{6,7}$-H); 7.98 (d, *J* = 7.6 Hz, 2H, bis-triazoloquinox.$C_{5,8}$-H). Anal. Calcd. for $C_{18}H_{14}N_6$ (314.34): C, 68.78; H, 4.49; N, 26.74. Found: C, 68.83; H, 4.52; N, 26.89.

3-Methyl-10-(4-nitrophenyl)bis-1,2,4-triazolo[4,3-*a*:3',4'-*c*] quinoxaline (8b): Yield 38%, M.P. >300°C(ethanol); IR (KBr, cm^{-1}): 1603 (C=N); 1550,1345 (NO_2); 1500 (C=C).^{1}H-NMR (500 MHz,DMSO-d_6); δ (ppm): 3.04 (s, 3H, CH_3); 7.30 (d, *J* = 8.4 Hz, 1H, bis-triazoloquinox.C_5-H); 7.37 (t, *J* = 8.4 Hz, 1H, bis-triazoloquinox.C_6-H); 7.60 (t, *J* = 8.4 Hz, 1H, bis-triazoloquinox.C_7-H); 8.02 (d, *J* = 8.4 Hz,, 2H, p-nitrophenyl$C_{2,6}$-H); 8.30 (d, *J* = 8.4 Hz, 1H, bis-triazoloquinox.C_8-H); 8.48 (d, *J* = 8.4 Hz, 2H, p-nitrophenyl$C_{3,5}$-H).Anal. Calcd. for $C_{17}H_{11}N_7O_2$ (345.31): C, 59.13; H, 3.21; N, 28.39. Found: C, 59.19; H, 3.24; N, 28.58.

2-(10-Methylbis-1,2,4-triazolo-[4,3-a:3',4'-c]quinoxalin-3-yl) benzoic acid and **3-(10-Methylbis-1,2,4-triazolo-[4,3-a:3',4'-c] quinoxalin-3-yl) propanoic acid (9a,b):** A mixture of **3** (0.21 gm, 1 mmol) and the appropriate acid anhydride (1 mmol) in glacial acetic acid (5 ml) was heated under reflux for 6 h during which yellow crystalline product was separated. The reaction mixture was left to cool to room temperature and the crystalline product was filtered, washed with water, dried and recrystallized from ethanol.

2-(10-Methylbis-1,2,4-triazolo-[4,3-*a*:3',4'-*c*]quinoxalin-3-yl) benzoic acid (9a): Yield 75%, M.P. >300°C(ethanol); IR (KBr, cm^{-1}): 3500-3400 (broad OH)**;** 1739 (acidic C=O); 1600 (C=N); 1575,1547,1500 (C=C).^{1}H-NMR (500 MHz,DMSO-d_6); δ (ppm): 3.32 (s, 3H, CH_3); 7.36-7.43 (m, 3H, bis-triazoloquinox.$C_{6,7,8}$-H); 7.90-8.02 (m, 4H, benzoic.$C_{3,4,5,6}$-H); 8.15 (d, *J* = 7.6 Hz, 1H, bis-triazoloquinox.C_5-H); 10.97 (s, 1H, OH, D_2O exchangeable). MS, m/z (relative abundance %):345 [$M^{+\cdot}$ +1] (14.5), 344 [$M^{+\cdot}$] (62.56); 299 (100); 259 (10.43);238 (10.44); 220 (10.96); 203 (7.97); 183 (0.21); 169 (7.89); 157 (3.52); 143 (15.45); 128 (16.29); 116 (14.25); 104 (69.47); 90 (24.56); 76 (78.38); 64 (12.23). Anal. Calcd. for $C_{18}H_{12}N_6O_2$ (344.33): C, 62.79; H, 3.51; N, 24.41. Found: C, 62.86; H, 3.54; N, 24.53.

3-(10-Methylbis-1,2,4-triazolo-[4,3-*a*:3',4'-*c*]quinoxalin-3-yl) propanoic acid (9b): Yield 85%, M.P. >300°C(ethanol); IR (KBr, cm^{-1}): 3400-3200 (broad OH)**;** 1730 (C=O); 1618 (C=N); 1574,1545,1500 (C=C). ^{1}H-NMR (300 MHz,DMSO-d_6); δ (ppm): 2.72-2.92 (m, 4H, 2 CH_2); 3.06 (s, 3H, CH_3); 7.43 (t, *J* = 7.2 Hz, 1H, bis-triazoloquinox.C_7-H); 7.48 (t, *J* = 6.9 Hz, 1H, bis-triazoloquinox.C_6-H); 7.59 (d, *J* = 7.2 Hz, 1H, bis-triazoloquinox.C_8-H); 8.16 (d, *J* = 8.1 Hz, 1H, bis-triazoloquinox.C_5-H); 10.80 (s, 1H, OH, D_2O exchangeable).MS, m/z (relative abundance %): 297 [$M^{+\cdot}$+1] (18.6), 296 [$M^{+\cdot}$] (100); 252 (7.38); 251 (33.72); 241 (33.85); 224 (18.26); 199 (48.05); 185 (39.32); 170 (4.03); 158 (18.89); 143 (30.17); 129 (9.13); 116 (24.91); 105 (26.27); 90 (0.41); 78 (15.69); 56 (17.49); 55 (42.49).Anal. Calcd. for $C_{14}H_{12}N_6O_2$ (296.28): C, 59.75; H, 4.08; N, 28.36. Found: C, 59.89; H, 4.14; N, 28.54.

4-(2-Arylidenehydrazinyl)-1-methyl-1,2,4-triazolo[4,3-*a*] quinoxalines (10a-d): To a solution of **3** (0.21 gm, 1 mmol) in absolute ethanol (5 ml), the appropriate aldehyde (1.1 mmol) was added and the reaction mixture was heated under reflux for 4-5 h during which

precipitation of the product was formed. The reaction mixture was left to cool to room temperature, filtered, washed with water, dried and crystallized from the proper solvent.

4-(2-Benzylidenehydrazinyl)-1-methyl-1,2,4-triazolo[4,3-*a*] quinoxaline (10a): Yield 82%, M.P. 250-1 °C (ethanol) ;IR (KBr, cm^{-1}): 3300 (NH); 1624 (C=N); 1580 (δNH); 1560,1500 (C=C).^{1}H-NMR (300 MHz,DMSO-d_6); δ (ppm): 2.51, 2.96 (two s, 3H, 1½H, CH_3, E & Z isomers); 7.2 (t, *J* = 6.0 Hz, 1H, phenylC_4-H); 7.38, 7.55 (two d, *J* = 7.2 Hz, 1H, ½H triazoloquinox.C_9-H, E & Z isomers); 7.49 (t, *J* = 7.8 Hz, 2H, phenyl$C_{3,5}$ -H); 7.78 (t, *J* = 7.3 Hz, 3H, triazoloquinox.$C_{7,8}$-H, E & Z isomers); 7.97, 8.18 (two d, *J* = 8.1 Hz, 7.2 Hz, 1H, ½H, triazoloquinox. C_6-H, E & Z isomers); 8.06-8.09 (m, 2H, phenyl$C_{2,6}$-H); 8.46, 8.54 (two s, ½H, 1H, N=CH, Z & E isomers); 10.71, 11.96 (two s, 1H, ½H, NH, D_2O exchangeable, E & Z isomers). MS, m/z (relative abundance %): 301 [M-1] (5.19), 290 (3.95); 275 (10.39); 257 (27.14); 241 (17.41); 236 (38.72); 199 (21.21); 153 (26.55); 139 (34.97); 124 (33.87); 98 (37.97); 84 (36.06); 83 (100); 57 (90.05); 56 (20.63).Anal. Calcd. for $C_{17}H_{14}N_6$ (302.33): C, 67.54; H, 4.67; N, 27.80. Found: C, 67.71; H, 4.71; N, 27.98.

4-(2-(4-Chlorobenzylidene)hydrazinyl)-1-methyl-[1,2,4] triazolo[4,3-*a*]quinoxaline (10b): Yield 83%, M.P. 283-4 °C (ethanol); IR (KBr, cm^{-1}): 3186 (NH); 1604 (C=N); 1567 (δNH); 1500 (C=C). ^{1}H-NMR (500 MHz,DMSO-d_6); δ (ppm): 2.9, 3.01 (two s, 3H, 1½H, CH_3, E & Z isomers); 7.11-7.14, 7.31-7.34 (two m, 1H, 2H, triazoloquinox. $C_{7,8}$-H, Z & E isomers); 7.45 (d, *J* = 8.4 Hz, 2H, p-chlorophenyl$C_{2,6}$-H); 7.48 (d, *J* = 8.4 Hz, 2H, p-chlorophenyl$C_{3,5}$-H); 7.7, 8.08 (two d, *J* = 7.6 Hz, 1H, ½H triazoloquinox.C_9-H, E & Z isomers); 7.87, 8.01 (two d, *J* = 8.4 Hz, ½H, 1H, triazoloquinox.C_6-H, Z & E isomers); 8.51, 8.54 (two s, ½H, 1H, N=CH, Z & E isomers); 10.71, 12.01 (two s, 1H, ½H, NH, D_2O exchangeable, E & Z isomers).Anal. Calcd. for $C_{17}H_{13}ClN_6$ (336.78): C, 60.63; H, 3.89; N, 24.95. Found: C, 60.78; H, 3.91; N, 25.13.

4-(2-(3,4-Dimethoxybenzylidene)hydrazinyl)-1-methyl-1,2,4-triazolo[4,3-*a*]quinoxaline (10c): Yield 85% , M.P. 267-9 °C (ethanol); IR (KBr, cm^{-1}): 3200 (NH); 1625 (C=N); 1598 (δNH); 1508 (C=C); 1259,1023 (C-O-C).^{1}H-NMR (500 MHz,DMSO-d_6); δ (ppm): 2.90, 3.02 (two s, 3H, 1½H, CH_3, E & Z isomers); 3.77, 3.78, 3.81, 3.85 (four s, 1½H, 3H, 1½H, 3H, $2OCH_3$, Z & E isomers); 6.99, 7.13 (two t, *J* = 8.4 Hz, 7.6 Hz, 1H, ½H, triazoloquinox.C_8-H, E & Z isomers); 7.17, 7.89 (two d, *J* = 8.0 Hz, ½H, 1H, triazoloquinox.C_9-H, Z & E isomers); 7.33, 7.46 (two t, *J* = 7.6 Hz, 1H, ½H, triazoloquinox.C_7-H, E & Z isomers); 7.36 (d, *J* = 8.4 Hz, 1H, 3,4-dimethoxyphenylC_5-H); 7.44 (d, *J* = 8.4 Hz, 1H, 3,4-dimethoxyphenylC_6-H); 7.68, 8.1 (two d, J = 7.6 Hz, 1H, ½H, triazoloquinox.C_6-H, E & Z isomers); 7.73 (s, 1H, 3,4-dimethoxyphenylC_2-H); 8.4 (s, 1½ H, N=CH, E & Z isomers); 10.7, 11.84 (2s, 1H, ½H, NH, D_2O exchangeable, E & Z isomers).Anal. Calcd. for $C_{19}H_{18}N_6O_2$ (362.39): C, 62.97; H, 5.01; N, 23.19. Found: 63.12; H, 5.08; N, 23.37.

4-[2-(Benzo[d][1,3]dioxol-5-ylmethylene)hydrazinyl]-1-methyl-[1,2,4]triazolo[4,3-*a*] quinoxaline (10d)

Yield 99%, M.P. 293-4°C(dimethylformamide); IR (KBr, cm^{-1}): 3300 (NH); 1600 (C=N); 1567 (δNH); 1495 (C=C); 1259,1038 (C-O-C).^{1}H-NMR (500 MHz,DMSO-d_6); δ (ppm): 2.91 (s, 3H, CH_3); 6.07 (S, 2H, benzodioxoleC_2-H); 6.98 (d, *J* = 7.6 Hz, 1H, benzodioxoleC_7-H); 7.13 (t, *J* = 7.6 Hz, 1H, triazoloquinox.C_8-H); 7.34 (t, *J* = 8.4 Hz, 1H, triazoloquinox.C_7-H); 7.38 (d, *J* = 7.6 Hz, 1H, benzodioxoleC_6-H); 7.72 (d, *J* = 7.6 Hz, 1H, triazoloquinox.C_9-H); 7.87 (s, 1H, benzodioxoleC_4-H); 7.91 (d, *J* = 8.4 Hz, 1H, triazoloquinox.C_6-H); 8.47 (s, 1H, N=CH); 10.60 (s, 1H, NH, D_2O exchangeable).Anal. Calcd. for $C_{18}H_{14}N_6O_2$ (346.34): C, 62.42; H, 4.07; N, 24.27. Found: C, 62.49; H, 4.11; N, 24.34.

10-Aryl-3-methylbis-1,2,4-triazolo[4,3-*a*:3',4'-*c*]quinoxalines (11 a-d): To a stirred mixture of **10 a-d** (1 mmol) and anhydrous sodium carbonate (0.25 gm, 3 mmol) in methylene chloride (10 ml), bromine (0.33 ml, 7 drops) was added. The reaction mixture was stirred at room temperature overnight and the solvent was evaporated under vacuum to dryness. The residue was triturated with ice-cold water, filtered, washed with water, dried and crystallized from the proper solvent.

3-Methyl-10-phenylbis-1,2,4-triazolo[4,3-*a*:3',4'-*c*]quinoxaline (11a): Yield 70% , M.P. 214-5°C (ethanol); IR (KBr, cm^{-1}): 1590 (C=N); 1587,1492 (C=C).^{1}H-NMR (300 MHz,DMSO-d_6); δ (ppm): 3.07 (s, 3H, CH_3); 7.33 (td, *J* = 8.4, 1.3 Hz, 1H, phenylC_4-H); 7.38 (td, *J* = 8.4, 1.3 Hz, 1H, phenylC_3-H); 7.60 (td, *J* = 7.8, 1.8 Hz, 1H, phenylC_5-H); 7.66-7.76 (m, 5H, phenyl$C_{2,6}$-H, bis-triazoloquinox.$C_{5,6,7}$-H); 8.32 (d, *J* = 8.7 Hz , 1H, bis-triazoloquinox.C_8-H). MS, m/z (relative abundance %):301 [$M^{+\cdot}$ + 1] (36.25), 300 [$M^{+\cdot}$] (52.53); 263 (56.74); 239 (8.99); 231 (76.4); 219 (31.74); 202 (32.58); 194 (36.24); 176 (31.99); 158 (36.52); 146 (40.73); 128 (47.47); 103 (100); 95 (33.15); 76 (50.84); 66 (57.87); 51 (35.11). Anal. Calcd. for $C_{17}H_{12}N_6$ (300.32): C, 67.99; H, 4.03; N, 27.98. Found: C, 68.13; H, 4.07; N, 28.17.

10-(4-Chlorophenyl)-3-methylbis-1,2,4-triazolo[4,3-*a*:3',4'-*c*] quinoxaline (11b): Yield 68%, M.P. > 300°C (ethanol); IR (KBr, cm^{-1}): 1629 (C=N); 1600, 1577, 1493 (C=C), 829 (C-Cl).^{1}H-NMR (500 MHz,DMSO-d_6); δ (ppm): 3.03 (s, 3H, CH_3); 7.32 (d, *J* = 8.4 Hz, 1H, bis-triazoloquinox.C_5-H); 7.40 (t, *J* = 7.6 Hz, 1H, bis-triazoloquinox.C_6-H); 7.58 (t, *J* = 7.6 Hz, 1H, bis-triazoloquinox.C_7-H); 7.72-7.78 (m, 4H, p-chlorophenyl$C_{2,3,5,6}$-H); 8.82 (d, *J* = 8.4 Hz , 1H, bis-triazoloquinox. C_8-H).Anal. Calcd. for $C_{17}H_{11}ClN_6$ (334.76): C, 60.99; H, 3.31; N, 25.10. Found: C, 61.14; H, 3.37; N, 25.22.

10-(3,4-Dimethoxyphenyl)-3-methylbis-1,2,4-triazolo[4,3-*a*:3',4'-*c*]quinoxaline (11c): Yield 90%, M.P. > 300°C (ethanol);IR (KBr, cm^{-1}): 1606 (C=N); 1580,1507 (C=C); 1261, 1021 (C-O-C). ^{1}H-NMR (500 MHz,DMSO-d_6); δ (ppm): 3.02 (s, 3H, CH_3); 3.71, 3.86 (two s, each 3H, 2 OCH_3); 7.19-7.26 (m, 3H, 3,4-dimethoxyphenyl$C_{5,6}$-H, bis-triazoloquinox.C_5-H); 7.32-7.44 (m, 2H, bis-triazoloquinox.$C_{6,7}$-H); 7.56 (s, 1H, 3,4-dimethoxyphenylC_2-H); 8.26 (d, *J* = 7.65 Hz , 1H, bis-triazoloquinox.C_8-H).Anal. Calcd. for $C_{19}H_{16}N_6O_2$ (360.37): C, 63.32; H, 4.48; N, 23.32. Found: C, 63.57; H, 4.54; N, 23.58.

10-(Benzo[d][1,3]dioxol-5-yl)-3-methylbis-1,2,4-triazolo[4,3-*a*:3',4'-*c*]quinoxaline (11d): Yield 89%, M.P.292-3°C (dimethylformamide); IR (KBr, cm^{-1}): 1626 (C=N); 1578,1468 (C=C); 1237,1033 (C-O-C).^{1}H-NMR (500 MHz,DMSO-d_6); δ (ppm): 2.97 (s, 3H, CH_3); 6.12 (S, 2H, benzodioxoleC_2-H); 7.13-7.14 (m, 2H, benzodioxole$C_{6,7}$-H); 7.17 (s,1H, benzodioxoleC_4-H); 7.33 (d, *J* = 8.4 Hz, 1H, bis-triazoloquinox. C_5-H); 7.36 (t, *J* = 8.4 Hz, 1H, bistriazoloquinox.C_6-H); 7.54 (t, *J* = 8.4 Hz, 1H, bis-triazoloquinox.C_7-H); 8.20 (d, *J* = 8.4 Hz, 1H, bis-triazoloquinox.C_8-H).Anal. Calcd. for $C_{18}H_{12}N_6O_2$ (344.33): C, 62.79; H, 3.51; N, 24.41. Found: C, 62.87; H, 3.48; N, 24.57.

6-Methyltetrazolo[1,5-*a*]-1,2,4-triazolo[3,4-*c*]quinoxaline (12): An ice-cold solution of sodium nitrite (0.07 gm, 1 mmol) in water (2 ml) was added dropwise to a stirred solution of **3** (0.21 gm, 1 mmol) in hydrochloric acid (1-2 ml). The reaction mixture was stirred at room temperature for 3 h during which precipitation of white product occurred. The obtained product was filtered, washed with water, dried and crystallized from dimethylformamide.

Yield 54%, M.P. 273-5 C(DMF); IR (KBr, cm^{-1}): 1637 (C=N); 1575, 1484 (C=C). ^{1}H-NMR (300 MHz, DMSO-d_6); δ (ppm): 3.13 (s, 3H, CH_3); 7.81-7.90 (m, 2H, tetrazolotriazoloquinox.$C_{9,10}$-H); 8.46 (dd, *J* = 7.2, 2.1 Hz, 1H, tetrazolotriazoloquinox.C_8-H); 8.60 (dd, *J* = 6.7, 1.8 Hz, 1H, tetrazolotriazoloquinox.C_{11}-H). EI-MS m/z (relative abundance

%): 226 [$M^{+\cdot}$ + 1] (11.33), 225 [$M^{+\cdot}$] (14.51); 210 (12.92); 199 (13.52); 191 (14.71); 185 (12.92); 163 (14.12); 149 (19.88); 129 (20.28); 123 (25.84); 97 (47.51); 83 (51.09); 69 (93.84); 55 (100). Anal. Calcd. for $C_{10}H_7N_7$ (225.21): C, 53.33; H, 3.13; N, 43.54. Found: C, 53.51; H, 3.21; N, 43.69.

Antimicrobial screening

Inhibition-zone measurements: All the synthesized compounds were evaluated by the agar cup diffusion technique [23] using a 1 mg/mL solution in DMSO. The test organisms were *Staphylococcus aureus* ((DSM 1104)) and *Bacillus subtilis* (ATCC 6633) as Gram-positive bacteria; *Escherichia coli* (ATCC 11775) and *Pseudomonas aeruginosa* (ATCC 10145) as Gram-negative bacteria. *Candida albicans* (DSM 70014) was also used as a representative for fungi. Each 100 mL of sterile molten agar (at 45°C) received 1 mL of 6 h-broth culture and then the seeded agar was poured into sterile Petri dishes. Cups (8 mm in diameter) were cut in the agar. Each cup received 0.1 mL of the 1 mg/mL solution of the test compounds. The plates were then incubated at 37°C for 24 h or, in case of *C. albicans*, for 48 h. A control using DMSO without the test compound was included for each organism. Ampicillin was used as standard antibacterial, while clotrimazole was used as antifungal reference. The resulting inhibition zones are recorded in table 1.

Minimal inhibitory concentration (MIC) measurement: The minimal inhibitory concentrations (MIC) of the compounds were measured using the two fold serial broth dilution method [24]. The test organisms were grown in their suitable broth: 24 h for bacteria and 48 h for fungi at 37°C. Two fold serial dilutions of solutions of the test compounds were prepared using 200, 100, 50, 25, and 12.5 µg/mL. The tubes were then inoculated with the test organisms; each 5 mL received 0.1 mL of the above inoculum and were incubated at 37°C for 48 h. Then, the tubes were observed for the presence or absence of microbial growth. The MIC values of the prepared compounds are listed in table 2 .

Table 1: The inhibition zones (IZ) in mm diameter of the synthesized compounds 3-12.

Cpd No	*S. aureus*	*B. subtilis*	*P. aeroginosa*	*E. coli*	*C. albicans*
3	16	16	12	15	14
4	18	16	14	15	16
5a	16	18	14	16	18
5b	12	14	16	16	18
6	12	18	16	15	14
7	14	15	13	14	14
8a	17	14	16	16	17
8b	15	14	14	18	14
9a	15	14	18	16	14
9b	12	16	16	18	15
10a	16	13	18	16	16
10b	18	14	16	16	16
10c	18	16	14	14	16
10d	16	16	17	14	14
11a	16	16	18	15	15
11b	14	16	18	15	16
11c	18	15	16	16	14
11d	14	16	18	15	18
12	14	18	12	18	16
A[a]	9	12	7	10	-
C[b]	-[c]	-	-	-	10

a: **A**=Ampicillin trihydrate (Standard broad spectrum antibiotic); b: **C**=Clotrimazole (Standard broad spectrum antifungal agent); c: Totally inactive.

Table 2: Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of the synthesized compounds 3-12 in µg/mL.

Cpd No	*S. aureus*		*B. subtilis*		*P. aeroginosa*		*E. coli*		*C. albicans*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
3	100	200	100	100	25	50	25	50	100	100
4	100	200	100	100	50	100	25	50	50	100
5a	25	50	50	50	100	100	50	50	25	50
5b	100	100	100	200	50	100	25	50	50	100
6	25	50	100	100	100	100	100	200	25	50
7	100	100	100	200	50	100	100	100	100	200
8a	100	100	50	50	100	100	25	25	100	200
8b	100	100	25	50	100	100	100	200	25	50
9a	100	100	100	200	50	100	25	25	50	100
9b	100	100	100	100	100	200	100	200	100	100
10a	50	100	100	100	100	100	25	50	25	50
10b	25	50	100	100	100	200	50	100	50	100
10c	50	50	100	100	100	200	100	100	100	100
10d	50	100	100	100	50	50	100	200	100	100
11a	50	100	25	50	50	100	50	50	100	100
11b	50	100	100	200	25	50	50	100	100	100
11c	50	100	50	100	50	50	100	200	100	100
11d	100	200	100	200	100	100	50	50	12,5	25
12	100	200	25	50	50	100	25	50	100	200
A[a]	5		12.2		50		9.8		-	
C[b]	-[c]		-		-		-		5	

a: **A**=Ampicillin trihydrate (Standard broad spectrum antibiotic); b:**C**=Clotrimazole (Standard broad spectrum antifungal agent); c: Totally inactive (MIC >200 µg/mL).

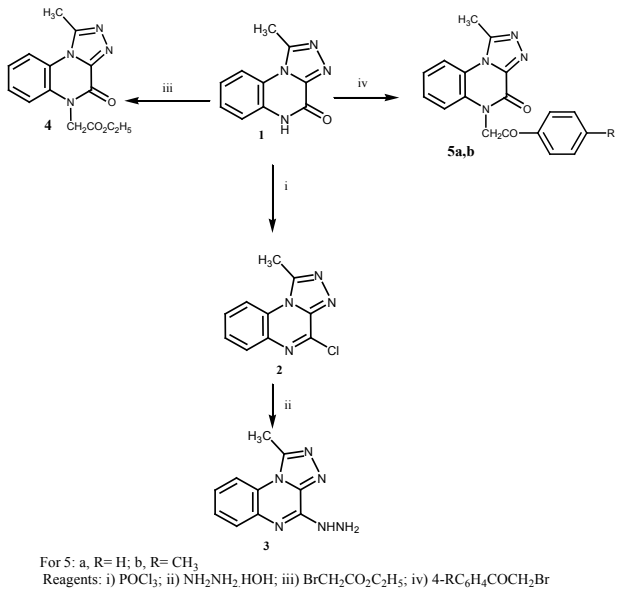
Minimal bactericidal concentration (MBC) measurement: MIC tests were always extended to measure the MBC as follows: A loop-full from the tube that did not show visible growth (MIC) was spread over a quarter of Müller–Hinton agar plate. After 18 h of incubation, the plates were examined for growth. Again, the tube containing the lowest concentration of the test compound that failed to yield growth on subculture plates was judged to contain the MBC of that compound for the respective test organism (Table 2).

Results and Discussion

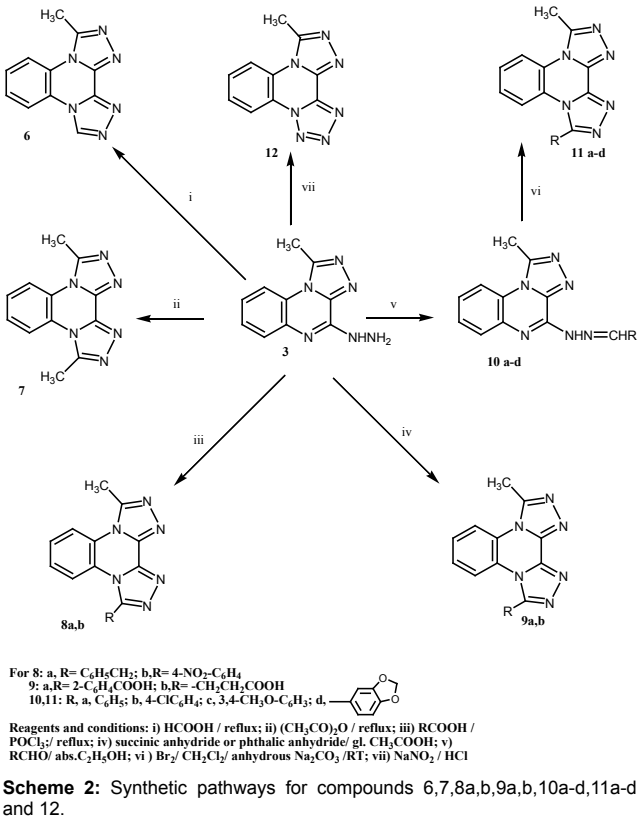
Chemistry

The synthetic procedures adopted to obtain the target compounds are illustrated in Schemes 1and 2. The key intermediate 1-methyl-1,2,4-triazolo[4,3-*a*]quinoxalin-4(*5H*)-one **1** was prepared according to a previously reported procedure [19]. Reaction of **1** with excess phosphorus oxychloride afforded the corresponding 4-chloro-1-methyl-1,2,4-triazolo[4,3-*a*]quinoxaline **2** [20-22]. Refluxing a mixture of **2** and hydrazine hydrate in absolute ethanol yielded the required 4-hydrazino derivative **3**. Reacting **1** with ethyl bromoacetate in dry acetone containing anhydrous potassium carbonate yielded the respective 4-ethyl acetate derivative **4**. Analogously, reaction of **2** with the appropriate phenacyl bromide resulted in the formation of 5-(arylcarbonylmethyl)triazoloquinoxalines **5a,b**.

Scheme 2 illustrates the cyclocondensations of 4-hydrazinotriazoloquinoxaline **3**. Refluxing **3** with formic acid or acetic anhydride afforded the corresponding bis-1,2,4-triazolo[4,3-*a*:3',4'-*c*] quinoxaline derivatives **6** and **7** respectively. Treatment of **3** with *p*-nitrobenzoic or phenyl acetic acid in phosphorous oxychloride furnished the corresponding 10-substituted-1-methylbis-1,2,4-triazolo[4,3-*a*:3',4'-*c*]quinoxalines **8a,b**. While treatment of **3** with phthalic or succinic anhydride in refluxing glacial acetic afforded the expected bis-1,2,4-



Scheme 1: Synthetic pathways for the key intermediate 3 and compounds 4 and 5a,b.



Scheme 2: Synthetic pathways for compounds 6,7,8a,b,9a,b,10a-d,11a-d and 12.

triazolo[4,3-*a*:3',4'-*c*] quinoxaline **9a,b**. On the other hand, condensation of **3** with the appropriate aromatic aldehyde in boiling ethanol afforded the corresponding hydrazones **10a-d**. ^{1}H-NMR data confirmed the existence of the two geometrical isomers E and Z of compounds **10a-c** as it revealed the existence of two upfield singlets assigned to two CH_3 groups of the two isomers and two deshielded D_2O exchangeable singlets corresponding to the NH groups, in addition to the aromatic signals integrated to the double number of triazoloquinoxaline protons. The ^{1}H-NMR spectrum of **10c** characterized by the existence of two upfield singlets assigned for the protons of the OCH_3 groups. It is worthy to mention that the ratio of the paired signals corresponding to the two geometric isomers is 2:1. On the other hand, the ^{1}H-NMR spectrum for **10d** did not show paired signals for any protons which could be explained by steric hindrance of the benzodioxole moiety that force the molecule to exist in the most stable isomer. Compouds **10a-d** underwent oxidative cyclization by bromine in presence of anhydrous sodium carbonate to the corresponding bis-triazoloquinoxalines **11a-d**. ^{1}H-NMR spectra for **11a-d** revealed the disappearance of the two singlets corresponding to N=CH and NH protons present in their precursors. ^{1}H-NMR spectra for **11a-c** lacked the paired signals for CH_3 and triazoloquinoxaline protons which confirms the disappearance of the E and Z geometrical isomers by cyclization. Moreover, reacting **3** with sodium nitrite solution in hydrochloric acid at 5°C gave the target tetrazolo derivative **12**. The structures of the newly synthesized compounds were substantiated by elemental analyses, IR, MS, ^{1}H-NMR and ^{13}C-NMR spectral data (experimental section).

Biological evaluation

Antimicrobial screening: All the newly synthesized compounds were preliminary evaluated for their *in-vitro* antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* as Gram-positive bacteria, *Escherichia coli* and *Pseudomonas aeruginosa* as Gram-negative bacteria. They were also tested for their *in-vitro* antifungal potential against *Candida albicans*. Their inhibition zones using the cup-diffusion technique [23] were measured and further evaluation was carried out to determine their minimal inhibitory concentration (MIC) and minimum bacterial concentration (MBC) using the twofold serial dilution method [24]. Ampicillin was used as standard antibacterial while clotrimazole was used as antifungal reference. Dimethylsulfoxide (DMSO) was used as blank and showed no antimicrobial activity.

As revealed from tables 1 and 2, the tested compounds displayed promising inhibitory effects on the growth of the tested organisms. In general, the compounds were highly effective against Gram-negative bacteria than Gram-positive and fungi. Compounds **3** and **11b** proved to be two times as active as ampicillin (MIC = 25 µg/mL) against *P. aeruginosa*. Whereas, compounds **4, 5b, 7, 10d, 11a, 11c** and **12** were as active as the reference. While, compounds **3, 4, 5b, 8a, 9a, 10a** and **12** (MIC = 25 µg /mL) showed nearly half the activity of ampicillin against *E. coli*.

Concerning the antibacterial potency against *S. aureus*, compounds **5a, 6** and **10b** displayed considerable activity (MIC = 25 µg/mL). In addition, compounds **8b, 11a** and **12** showed one-half the activity of ampicillin in inhibiting the growth of *B. subtilis* (MIC = 25 µg/mL).

On the other hand, the results revealed that the tested compounds displayed notable antifungal activity. Compound **11d** exhibited nearly one-half the activity of clotrimazole against *C. albicans*. (MIC = 12.5 µg/mL).

According to the MIC and MBC limits derived from the latest National Committee on Clinical Laboratory Standards (NCCLS), it can be determined whether the test compound is bactericidal or bacteriostatic to the test organism. Accordingly, and as revealed from table 2, only compounds **10d** and **11c** were bactericidal against *P. aeruginosa* while the remaining compounds were bacteriostatic against the test organisms.

Structural- activity correlation of the tested compounds indicated that 5-substituted-1-methyl-1,2,4-triazoloquinoxalines (**4** and **5b**)

demonstrated promising activity against *P. aeruginosa*, being as active as ampicillin. Moreover, they displayed notable activity against *E. coli*. While, the 4-hydrazinyl-1-methyl-1,2,4-triazoloquinoxaline **3** showed enhanced activity towards *P. aeruginosa*, being two time as active as the reference which might be due to the presence of 4-hydrazino group which increased the possibility of hydrogen bonding. Conversion of **3** into the corresponding Schiff's bases **10a-c** resulted in remarkable decrease in activity against *P. aeruginosa* being one-half as active as the reference. While, derivative **10d** was found to be as active as reference against *P. aeruginosa*. Such activity might be due to the presence of the 1,3-dioxole moiety.

Furthermore, cyclization of compounds **10a-c** into 10-aryl-3-methylbis-1,2,4-triazoloquinoxalines **11a-c** resulted in an increase of activity towards *P. aeruginosa*. The presence of Cl atom at position-4 of the phenyl ring in **11b** enhanced the activity against *P. aeruginosa* to be twice the activity of the reference. On the other hand, cyclization of compound **10d** into the corresponding bistriazolo derivative **11d** led to decrease in antibacterial activity against *P. aeruginosa*.

Cyclocondensation of **3** into the lipophilic tetracyclic bis-triazoloquinoxalines **7** and **8a,b** decreased the antibacterial activity towards the Gram negative *P. aeruginosa* which could be explained by the increase of lipophilicity of the cyclic compounds. While cyclocondensation of **3** into the hydrophilic 10-carboxy bistriazolo analog **9a** exhibited activity as the reference against *P. aeruginosa.* It is worthy to mention that the *p*-nitrophenyl ring in compound **8b** might be the reason for increasing the antibacterial activity towards *B. subtilis*. As well, the enhanced activity of compound **12** towards *B.subtilis* and *E. coli* might be attributed to the tetrazole moiety.

Conclusion

The significant antimicrobial results of our previously reported1-substituted-4-phenyl 1,2,4-triazolo[4,3-*a*]quinoxalines motivated us to report herein the synthesis of some 5-substituted 1,2,4-triazolo[4,3-*a*] quinoxalines **4** and **5a,b** in order to achieve further knowledge of structure activity relationship. In addition, some new 10-substituted-3-methylbis-1,2,4-triazolo[4,3-*a*:3',4'-*c*]quinoxalines (**6, 7, 8a,b, 9a,b** and **11a-d**) and 6-methyl tetrazolo[1,5-*a*]-1,2,4-triazolo[3,4-*c*]quinoxaline **12** were designed so as to extend the planarity of the heterocyclic ring system and modulate either the lipophilicity or hydrogen bond accepting properties towards different receptor binding sites aiming to add some synergetic biological significance to the target molecules. Moreover, various substitutions of the target molecules were designed to confer various electronic and lipophilic environments to the target molecule in order to investigate the effect of such structural modification on the expected biological effects.

Antimicrobial screening results indicated that the target compounds were highly effective against G-negative bacteria than G-positive bacteria and fungi. Compounds **3** and **11b** displayed twice the activity of that of the reference ampicillin. Whereas compounds **4, 5b, 7, 9a, 10d, 11a, c** and **12** were as active as the reference against *P. aeruginosa*. Consequently, such series of compounds could be considered as structural leads that deserve further structural modification and investigation to optimize their antimicrobial efficacy aiming at finding out a new class of antimicrobial agents.

References

1. Brooks BD, Brooks AE (2014) Therapeutic strategies to combat antibiotic resistance. Adv Drug Deliv Rev 78: 14-27.
2. Noolvi MN, Patel HM, Bhardwaj V, Chauhan A (2011) Synthesis and in vitro antitumor activity of substituted quinazoline and quinoxaline derivatives: search for anticancer agent. Eur J Med Chem 46: 2327-2346.
3. Suresh M, Lavanya P, Sudhakar D, Vasu K, Venkata Rao C (2010) Synthesis and biological activity of 8-chloro-[1,2,4]triazolo[4,3-a]quinoxalines. J Chem Pharm Res 2: 497-504.
4. Refaat HM, Moneer AA, Khalil OM (2004) Synthesis and antimicrobial activity of certain novel quinoxalines. Arch Pharm Res 27: 1093-1098.
5. Mahaney PE, Webb MB, Ye F, Sabatucci JP, Steffan RJ, et al. (2006) Synthesis and activity of a new class of pathway-selective estrogen receptor ligands: hydroxybenzoyl-3,4-dihydroquinoxalin-2(1H)-ones. Bioorg Med Chem 14: 3455-3466.
6. Sarges R, Howard HR, Browne RG, Lebel LA, Seymour PA, et al. (1990) 4-Amino[,2,4]triazolo[4,3-a]quinoxalines. A novel class of potent adenosine receptor antagonists and potential rapid-onset antidepressants. J Med Chem 33: 2240-2254.
7. Patel N, Bergman J, Gräslund A (1991) ^{1}H-NMR studies of the interaction between a self-complementary deoxyoligonucleotide duplex and indolo[2,3-b] quinoxaline derivatives active against herpes virus. Eur J Biochem 197: 597-604.
8. Hair SRJ (2005) Drug Discov Today 10: 417-424.
9. Ishida Y, Ozaki H, Shibata S (1980) Vasorelaxant action of caroverine fumarate (a quinoxaline derivative), a calcium-blocking agent. Br J Pharmac 71: 343-348.
10. Sridevi CH, Balaji K, Naidu A, Sudhakaran R (2010) E-Journal of Chemistry 7: 234-238.
11. Li JJ (1999) Synthesis of Novel 3-Substituted Pyrrolo[2,3-b]quinoxalines via an Intramolecular Heck Reaction on an Aminoquinoxaline Scaffold. J Org Chem 64: 8425-8427.
12. Patinar AK, Jeyakandan M, Mobiya AK, Selvam G (2011) Int J Pharm Tech Res 3: 386-392.
13. Dell A, Williams DH, Morris HR, Smith GA, Feeney J, et al. (1975) Structure revision of the antibiotic echinomycin. J Am Chem Soc 97: 2497-2502.
14. Heravi MM, Bakhtiari K, Tehrani MH, Javadi NM, Oskooie HA (2006) Arkivoc (xvi) 16-22.
15. Raw SA, Wilfred CD, Taylor RJ (2003) Preparation of quinoxalines, dihydropyrazines, pyrazines and piperazines using tandem oxidation processes. Chem Commun (Camb): 2286-2287.
16. Habib NS, El-Hawash SA (1997) Synthesis and antimicrobial testing of thiazolinyl-, thiazolidinonyl-quinoxalines and ,2,4-triazolo[4,3-a]quinoxalines. Pharmazie 52: 594-598.
17. El-Hawash SA, Habib NS, Fanaki NH (1999) Quinoxaline derivatives. Part II: Synthesis and antimicrobial testing of ,2,4-triazolo[4,3-a]quinoxalines, ,2,4-triazino[4,3-a]quinoxalines and 2-pyrazolylquinoxalines. Pharmazie 54: 808-813.
18. Corona P, Vitale G, Loriga M, Paglietti G, La Colla P, et al. (2006) 4-Substituted anilino imidazo[,2-a] and triazolo[4,3-a]quinoxalines. Synthesis and evaluation of in vitro biological activity. Eur J Med Chem 41: 1102-1107.
19. Rashed N, El Massry AM, El Ashry ESH, Amer A, Zimmer H (1990) J Heterocycl Chem 27: 691-694.
20. Ramalingam PGS, Rao CV (2008) Acta Ciencia Indica Chemistry 34: 609-612.
21. McLaughlin KC, Organic Syntheses 30: 89.
22. Essassi E, Ahoya C, Bouhfid R, Daouda B, Hançali A, et al. (2011) Arkivoc ii 217-226.
23. Jain SR, Kar A (1971) The antibacterial activity of some essential oils and their combinations. Planta Med 20: 118-123.
24. Collee JG, Duguid JP, Fraser AG, Marmion BP, Scott AC (1989) In Mackie and McCarteny Practical Medical Microbiology. Vol. 2. Churchill Livingstone, New York. pp: 161-181.