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

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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-α]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.

Keywords: Synthesis; Triazoloquinoxalines; Bistriazoloquinoxalines; Tetratrazolotriazoloquinoxaline; 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 antalgicama 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 which 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-α]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-α]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
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-1,2,4-triazolo[4,3-a,4′-c]quinazolines and tetrazolo[1,5-a:1,2,4-triazolo[3,4-c]quinolinin (formula B, 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 melting points apparatus (Stuart Scientific; Staffordshire, UK) and are uncorrected. IR spectra were recorded, for potassium bromide discs, (cm⁻¹), on Perkin Elmer 1430 spectrophotometer. 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 6 ppm values (s, singlet; d, doublet; t, triplet and m, multiplet). 13C-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]quinolinin-(4H)-one with acetic anhydride. Heating 1 with phosphorous oxychloride afforded compound 2 according to a previously reported reaction conditions [20-22].

4-Hydrazinyl-1-methyl-1,2,4-triazolo[4,3-a]quinolinin (3): A mixture of 2 (0.42 gm, 1 mmol) and hydrazine hydrate 99% (0.2 ml, 0.3 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=O); 1570 (NH); 1520,1494 (C=O), 1340, 1293, 784, 520 (C=O). EI-MS m/z (relative abundance %): 215 (16.01) [M⁺], 214 (100) [M⁺], 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. Calc. for C₈H₈N₂ (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]quinolinin-(5H)-yl)acetate (4): A mixture of 1 (0.2 gm, 1 mmol), ethyl bromoacetate (0.22 ml, 0.33 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 recrystallized 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). 1H-NMR (500 MHz, DMSO-d₆): δ (ppm): 1.25 (t, J=7.6 Hz, 3H, CH2CH3); 2.93 (s, 3H, CH3); 4.13 (q, J=7.6 Hz, 2H, CH2CH3); 5.04 (s, 2H, CH2CO); 7.38 (t, J=7.6 Hz, 1H, triazoloquinox. C=H); 7.41 (d, J=8.0 Hz, 1H, triazoloquinox. C=H); 7.49 (t, J=7.6 Hz, 1H, triazoloquinox. C=H); 8.07 (d, J=7.6 Hz, 1H, triazoloquinox. C=H); 8.10 (d, J=8.4 Hz, 1H, triazoloquinox. C=H); 9.38 (s, 1H, NH); 117.37 (CH2CO); 61.38 (CH2CO); 116.46 (triazoloquinox. C=H); 117.37 (triazoloquinox. C=H); 121.43 (triazoloquinox. C=H); 123.94 (triazoloquinox. C=H); 127.76 (triazoloquinox. C=H); 129.34 (triazoloquinox. C=H); 143.07 (triazoloquinox. C=H); 149.48 (triazoloquinox. C=H); 151.62 (triazoloquinox. C=H); 167.60 (CH2CO). Anal. Calc. for C15H15N2O2 (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]quinolinin-(4H)-ones (5a): 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]quinolinin-(4H)-ones (5a): Yield 42%, M.P. >300°C(DMF); IR (KBr, cm⁻¹): 1679 (C=O); 1594 (C=N); 1507 (C=O); 1H-NMR (500 MHz, DMSO-
1-Methyl-5-(4-p-tolylcarboxymethyl)-1,2,4-triazolo[4,3-a]quinoline-4(5H)-ones (5b): Yield 69%. M.P. >300°C (ethanol); IR (KBr, cm⁻¹): 1618 (C=O); 1604 (C=O); 1509 (C=C). 1H-NMR (300 MHz, DMSO-d₆): δ (ppm): 2.50 (3H, CH₃, p-tolyl); 3.05 (3H, CH₃); 5.93 (3H, 2H, CH₂, CO); 7.38-7.48 (m, 5H, triazoloquinolino-C₆-H and p-tolyl-C₆-H); 8.04 (d, J = 7.8 Hz, 2H, p-tolyl-C₆-H); 8.18 (d, J = 6.3 Hz, 1H, triazoloquinolino-C₆-H). Anal. Calcd. For C₁₇H₁₴N₃O (332.36): C, 86.83; H, 4.85; N, 17.06. Found: C, 86.83; H, 4.52; N, 26.89.

3-Methyl-1,2,4-triazolo[4,3-a,3'-c',4'-c']quinazaline (6): A mixture of 4-hydrazinyl-1-methyl-1,2,4-triazolo[4,3-a]quinazoline 3 (0.21 g, 1 mmol) and formic acid (2.5 ml) was heated under reflux for 4 h. The reaction mixture was concentrated by evaporation 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=O); 1600,1550 (C=C). 1H-NMR (300 MHz, DMSO-d₆): δ (ppm): 3.04 (3H, CH₃); 7.67-7.71 (m, 2H, bis-triazoloquinolino-C₆-H); 8.3 (dd, J = 6.6, 3.3 Hz, 1H, bis-triazoloquinolino-C₆-H); 8.42 (dd, J = 6.3, 3.3 Hz, 1H, bis-triazoloquinolino-C₆-H); 9.97 (s, 1H, bis-triazoloquinolino-C₆-H). EI-MS (relative abundance %): 225 (20.01) [M⁺ + 1], 224 [M⁻] (1.88); 222 (7.39); 208 (6.63); 189 (9.51); 180 (5.76); 166 (3.75); 152 (17.65); 129 (18.23); 123 (25.76); 111 (38.97); 97 (66.34); 83 (75.56); 69 (100). Anal. Calcd. For C₁₇H₁₄N₃O (345.31): C, 59.13; H, 3.21; N, 28.39. Found: C, 59.19; H, 3.24; N, 28.58.

2-(10-Methyl-1,2,4-triazolo-[4,3-a,3'-c',4'-c']quinazolin-3-yl)benzoic acid and 3-(10-Methyl-1,2,4-triazolo-[4,3-a,3'-c',4'-c']quinazolin-3-yl)propanoic acid (9a,9b): A mixture of 3 (0.21 g, 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 formed product was washed, dried and recrystallized from water.

2-(10-Methyl-1,2,4-triazolo-[4,3-a,3'-c',4'-c']quinazolin-3-yl)benzooic acid (9a): Yield 75%. M.P. >300°C (ethanol); IR (KBr, cm⁻¹): 3500-3400 (broad OH); 1739 (acidic C=O); 1600 (C=O); 1575,1547,1500 (C=C). 1H-NMR (500 MHz, DMSO-d₆): δ (ppm): 3.32 (3H, CH₃); 7.36-7.43 (m, 3H, bis-triazoloquinolino-C₆-H); 7.30-7.00 (4H, bis-triazoloquinolino-C₆-H); 10.97 (s, 1H, OH, D₂O exchangeable). MS, m/z (relative abundance %): 345 [M⁺ + 1] (25.0), 344 [M⁻] (62.56), 299 (100); 252 (7.38); 238 (10.43); 220 (10.96); 203 (7.97); 183 (0.21); 169 (7.89); 157 (5.32); 143 (15.45); 128 (16.29); 116 (14.25); 104 (69.47); 90 (24.56); 78 (76.38); 64 (12.23). Anal. Calcd. For C₁₇H₁₄N₃O (344.33): C, 62.79; H, 3.51; N, 24.41. Found: C, 62.86; H, 3.54; N, 24.53.

3-(10-Methyl-1,2,4-triazolo-[4,3-a,3'-c',4'-c']quinazolin-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=O); 1574,1545,1500 (C=C). 1H-NMR (300 MHz, DMSO-d₆): δ (ppm): 2.72-2.92 (2H, 4H, 2CH₃); 3.06 (3H, 3H, CH₃); 7.43 (t, J = 7.2 Hz, 1H, bis-triazoloquinolino-C₆-H); 7.58 (t, J = 7.2 Hz, 1H, bis-triazoloquinolino-C₆-H); 8.16 (d, J = 8.1 Hz, 1H, bis-triazoloquinolino-C₆-H). 10.80 (s, 1H, OH, D₂O exchangeable). MS, m/z (relative abundance %): 284 [M⁺ + 1] (13.72), 282 (65.46); 252 (73.32); 251 (73.32); 241 (33.85); 224 (18.26); 353 (39.32); 345 (3.45); 218 (18.37); 145 (3.17); 129 (9.12); 116 (24.91); 105 (26.27); 90 (40.41); 78 (15.69); 56 (17.49); 55 (42.49). Anal. Calcd. For C₁₇H₁₄N₃O (296.28): C, 59.75; H, 4.08; N, 28.36. Found: C, 59.89; H, 4.14; N, 28.54.

4-(2-Arylidenecyano)-1-methyl-1,2,4-triazolo[4,3-a]quinolines (10a-d): To a solution of 3 (0.21 g, 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 83%, M.P. 283-4°C (ethanol); IR (KBr, cm⁻¹): 3186 (NH); 1604 (C=N); 1567 (δNH); 1500 (C=C); 1H-NMR (500 MHz, DMSO-d₆); δ (ppm): 2.91, 3.01 (two s, 3H, CH₃); 7.20, 7.43 (two m, 2H, 2H, CH₂ and E & Z isomers); 7.31, 7.34 (two m, 1H, 2H, triazoloquinoc.).

3-Methyl-10-phenylbis-1,2,4-triazolo[4,3-a]quinoxaline (10c): Yield 83%, M.P. 283-4°C (ethanol); IR (KBr, cm⁻¹); 1637 (C=N); 1575, 1464 (C=C); 1327, 1033 (C-O); 1H-NMR (500 MHz, DMSO-d₆); δ (ppm): 3.02 (s, 3H, CH₃); 7.32, 7.38, 3.85 (four s, 1H, 2H, 3H, 1H, 2OCH₂ and E & Z isomers); 6.99, 7.13 (two t, J = 8.4 Hz, 2H, 2H, 2H, triazoloquinoc.-H and E & Z isomers); 7.17, 7.89 (two d, J = 8.0 Hz, 2H, 1H, triazoloquinoc.-H, Z and E isomers); 7.33, 7.46 (two t, J = 7.6 Hz, 4H, 1H, 1H, triazoloquinoc.-H, E & Z isomers); 7.36 (d, J = 8.4 Hz, 2H, 3,4-dimethoxyphenyl-H); 7.44 (d, J = 8.4 Hz, 1H, 3,4-dimethoxyphenyl-H); 6.87, 8.1 (two d, J = 7.6 Hz, 2H, 2H, triazoloquinoc.-H, E & Z isomers); 7.73 (s, 1H, 3,4-dimethoxyphenyl-H); 8.4 (s, 1H, 1H, N=CH₂ and E & Z isomers); 10.7, 11.84 (2s, 1H, 1H, NH, D,O exchangeable, E & Z isomers). Anal. Calcd. for C₂₆H₂₆N₄O₄: C, 60.63; H, 3.89; N, 24.41. Found: C, 60.78; H, 3.91; N, 25.13.

10-(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); 1629 (C=N); 1605, 1577, 1493 (C=C); 829 (C=C); 1H-NMR (500 MHz, DMSO-d₆); δ (ppm): 3.03 (s, 3H, CH₃); 7.32 (d, J = 8.4 Hz, 1H, bis-triazoloquinoc.-H); 7.40 (t, J = 7.6 Hz, 1H, bis-triazoloquinoc.-H); 7.58 (t, J = 7.6 Hz, 1H, bis-triazoloquinoc.-H); 7.72-7.78 (m, 4H, p-chlorophenyl-H); 8.82 (d, J = 8.4 Hz, 1H, bis-triazoloquinoc.-H). Anal. Calcd. for C₂₆H₂₆ClN₄O₄: C, 67.99; H, 4.03; N, 27.98. Found: C, 67.31; H, 4.48; N, 25.38.

10-(3,4-Dimethoxyphenyl)-1-methyl-1,2,4-triazolo[4,3-a]quinoxaline (10d): Yield 89%, M.P. > 300°C (ethanol); IR (KBr, cm⁻¹); 1637 (C=N); 1575, 1481 (C=C); 1327 (C-O); 1H-NMR (500 MHz, DMSO-d₆); δ (ppm): 3.02 (s, 3H, CH₃); 3.71, 3.86 (two s, each 3H, 2, 2OCH₂); 7.19-7.26 (m, 3H, 3,4-dimethoxyphenyl-H, bis-triazoloquinoc.-H); 7.32-7.44 (m, 2H, bis-triazoloquinoc.-H); 7.56 (s, 1H, 3,4-dimethoxyphenyl-H); 8.26 (d, J = 7.65 Hz, 1H, bis-triazoloquinoc.-H). Anal. Calcd. for C₂₆H₂₆N₄O₆: C, 63.62; H, 4.48; N, 23.32. Found: C, 63.75; H, 4.54; N, 23.58.

10-(4-Chlorobenzylidene)hydrazinyl)-1-methyl-1,2,4-triazolo[4,3-a]quinoxaline (10e): Yield 89%, M.P. 283-4°C (ethanol); IR (KBr, cm⁻¹); 1612 (C=N); 1589, 1505 (C=C); 1258, 1201 (C-O); 1H-NMR (500 MHz, DMSO-d₆); δ (ppm): 3.01 (s, 3H, CH₃); 3.71, 3.85 (two s, each 3H, 1H, 2OCH₂); 7.20, 7.44 (two m, 1H, 3H, 1H, 3,4-dimethoxyphenyl-H); 7.37, 7.65 (two d, J = 7.6 Hz, 1H, bis-triazoloquinoc.-H); 7.67 (s, 1H, 3,4-dimethoxyphenyl-H); 8.4 (s, 1H, 1H, N=CH₂ and E & Z isomers); 10.7, 11.84 (2s, 1H, 1H, NH, D,O exchangeable, E & Z isomers). Anal. Calcd. for C₂₆H₂₆ClN₄O₆: C, 60.63; H, 3.89; N, 24.41. Found: C, 60.78; H, 3.91; N, 25.13.

4-(2-Benzoyl-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); 1H-NMR (500 MHz, DMSO-d₆); δ (ppm): 2.91 (s, 3H, CH₃); 6.07 (s, 2H, benzoidiazoc-l-C=CH₂); 6.98 (d, J = 7.6 Hz, 1H, benzoidiazoc-l-C=CH₂); 7.13 (t, J = 7.6 Hz, 1H, triazoloquinoc.-C=CH₂); 7.34 (t, J = 8.4 Hz, 1H, triazoloquinoc.-C=CH₂); 7.38 (d, J = 7.6 Hz, 1H, benzoidiazoc-l-C=CH₂); 7.72 (d, J = 7.6 Hz, 1H, triazoloquinoc.-C=CH₂); 7.87 (s, 1H, benzoidiazoc-l-C=CH₂); 7.91 (d, J = 8.4 Hz, 1H, triazoloquinoc.-C=CH₂); 8.47 (s, 1H, N=CH₂) and 10.60 (s, 1H, NH, D,O exchangeable). Anal. Calcd. for C₂₆H₂₆N₄O₆: C, 62.42; H, 4.07; N, 24.27. Found: C, 62.49; H, 4.11; N, 24.34.


Antimicrobial screening

Inhibition-zone measurements: All the synthesised 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.

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<td>11b</td>
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<td>11c</td>
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<td>11d</td>
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<tr>
<td>A+</td>
<td>14</td>
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<td>12</td>
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<td>C+</td>
<td>9</td>
<td>12</td>
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</table>

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

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 1 and 2. The key intermediate 1-methyl-1,2,4-triazolo[4,3-a]quinoxalin-4(3H)-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-hyrazino 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 phenyl bromide resulted in the formation of 5-(arylmethoxycarbonyl)methyltriazoloquinazolines 5a,b.

Scheme 2 illustrates the cyclocondensations of 4-hyrazinotriazoloquinazoline 3. Refluxing 3 with formic acid or acetic anhydride afforded the corresponding bis-1,2,4-triazolo[4,3-a']-quinoxalin-4(3H)-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-hyrazino 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 phenyl bromide resulted in the formation of 5-(arylmethoxycarbonyl)methyltriazoloquinazolines 5a,b.

While treatment of 3 with phthalic or succinic anhydride in refluxing glacial acetic afforded the expected bis-1,2,4-
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-triazoloquinazolines (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-triazoloquinoxalines 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 bistriazoloquinoxalines 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 reported 1-substituted-4-phenyl 1,2,4-triazolo[4,3-a]quinazolines motivated us to report herein the synthesis of some 5-substituted 1,2,4-triazolo[4,3-a] quinazolines 4 and 5a,b in order to achieve further knowledge of structure activity relationship. In addition, some new 10-substituted-3,4-methylbis-1,2,4-triazolo[4,3-a]quinazolines (6, 7, 8a,b, 9a,b and 11a-d) and 6-methyl tetrazolo[1,5-a]-1,2,4-triazolo[3,4-c]quinazoline 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, 11c 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**