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-a]quinoxaline derivatives. 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; Tetrazolotriazoloquinoxaline; 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], antidepresant [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 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 anelating 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,4'-c]quinoxalines and tetrazolo[1,5-a]-1,2,4-triazolo[3,4-c]quinolinaxin (formula R.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 Scientific Stone, Staffordshire, UK and were uncorrected. IR spectra were recorded for potassium bromide discs, ν (cm⁻¹) on Perkin Elmer 1430 spectrophotometer. 1H-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 (s, singlet; d, doublet; t, triplet and m, multiplet). 13C-NMR spectra were determined on Jeol (125 MHz), Faculty of Science, Alexandria University, or on Jeol (500 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 (s, singlet; d, doublet; t, triplet and m, multiplet).

Yield 88%, M.P. 254-6°C (ethanol); IR (KBr, cm⁻¹): 3264 (NH); 1632 (C=N); 1570 (6NH); 1520,1494 (C=O)-NMR (300 MHz,DMSO-d₆); δ (ppm): 3.02 (s, 2H, CH₂); 4.68 (s, 2H, NH); 6.07 (6, J = 7.2 Hz, 1H, triazoloquinox.C-H); 6.50 (d, J = 7.2 Hz, 1H, triazoloquinox.C-H); 8.10 (d, J = 8.4 Hz, 1H, triazoloquinox.C-H); 9.38 (s, 1H, NH); DMSO-d₆ exchangeable).EI-MS m/z (relative abundance %): 215 (16.01) [M⁺+1], 214 (100) [M⁺], 199 (13), 185 (74), 158 (11), 144 (51), 129 (13), 118 (10), 90 (57), 27 (26), 63 (22), 57 (15), 51 (29). Anal. Calc. for C₁₁H₈N₂ (C₆H₆, N₂): C, 56.18; H, 4.68; N, 39.50.

Figure 2: The proposed design of the newly synthesized compounds.
1-Methyl-5-(4-p-tolylcarbonylmethyl)-1,2,4-triazolo[4,3-a]quinazoline-4(5H)-ones (5b): Yield 69%, M.P. >300ºC (DMF); IR (KBr, cm\(^{-1}\)) : 1618 (C=O); 1604 (C=O); 1503 (s, 3H, CH\(_2\)); 5.93 (s, 2H, CH\(_2\)); 7.38-7.48 (m, 5H, triazoloquinazolyl C\(-\text{H}\) and p-tolyl C\(-\text{H}\)); 8.04 (d, J = 7.8 Hz, 2H, p-tolyl C\(-\text{H}\)); 8.18 (d, J = 3.3 Hz, 1H, triazoloquinazolyl C\(-\text{H}\)). Anal. Calcd. for C\(_{117}H\(_{67}\)N\(_{4}\): C, 68.83; H, 4.52; N, 26.89. Found: C, 68.83; H, 4.52; N, 26.89.

3-Methyl-1,2,4-triazolo[4,3-a:3',4'-c']quinazoline (6): A mixture of 4-hydrazinyl-1-methyl-1,2,4-triazolo[4,3-a]quinazoline 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. >300ºC (DMF/ethanol); IR (KBr, cm\(^{-1}\)) : 1620 (C=O); 1600,1550 (C=O); 1500 (C=O); IR (300 MHz, DMSO-d\(_6\)); δ (ppm): 2.90-3.10 (2H, CH\(_2\)); 7.67-7.71 (2H, bis-triazoloquinazolyl C\(-\text{H}\)); 8.3 (dd, J = 6.3, 3.3 Hz, 1H, bis-triazoloquinazolyl C\(-\text{H}\)); 9.97 (s, 1H, bis-triazoloquinazolyl C\(-\text{H}\)). EI-MS (relative abundance %): 225 (20.1) [M\(+1\)], 224 (18.5) ([M\(+1\)] -1); 222 (18.3) ([M\(+1\)] -2); 220 (18.2) ([M\(+1\)] -3). Anal. Calcd. for C\(_{117}H\(_{67}\)N\(_{4}\): C, 68.78; H, 4.49; N, 26.74. Found: C, 68.83; H, 4.52; N, 26.89.

3-Alkyl-1,2,4-triazolo[4,3-a:3',4'-c']quinazoline (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. >300ºC (GAA); IR (KBr, cm\(^{-1}\)) : 1615 (C=O); 1579,1490 (C=O). IR (100), Anal. Calcd. for C\(_{209}H\(_{137}\)N\(_{7}\): C, 76.00; H, 4.67; N, 19.33. Found: C, 75.82; H, 4.64; N, 19.25.

3-(10-Methylbis-1,2,4-triazolo-[4,3-a:3',4'-c]quinoxalin-3-yl)benzoic 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 washed, dried and recrystallized from water.

2-(10-Methyl-1,2,4-triazolo-[4,3-a:3',4'-c]quinoxalin-3-yl)benzoic acid (9a): Yield 75%, M.P. >300ºC (ethanol); IR (KBr, cm\(^{-1}\)) : 3500-3400 (broad OH); 1739 (acidic C=O); 1600 (C=O); 1575,1547,1500(C=O); IR (300 MHz, DMSO-d\(_6\)); δ (ppm): 3.32 (s, 3H, CH\(_3\)); 7.36-7.43 (3H, bis-triazoloquinazolyl C\(-\text{H}\)); 7.90-8.03 (4H, aromatic CH\(_\text{aromatic}^\text{1}\)); 8.15 (d, J = 7.6 Hz, 1H, bis-triazoloquinazolyl C\(-\text{H}\)); 10.97 (s, 1H, OH, D\(_\text{O exchangeable}\)). MS, m/z (relative abundance %): 345 [M\(+1\)] (14.5), 344 [M\(+1\)] (62.56), 299 (100), 259 (10.43); 238 (10.40); 220 (10.96); 203 (7.97); 183 (0.21); 169 (7.89); 157 (3.52); 143 (14.55); 128 (16.29); 116 (14.25); 104 (69.47); 90 (24.56); 76 (78.38); 64 (12.23). Anal. Calcd. for C\(_{209}H\(_{137}\)N\(_{7}\): C, 76.29; H, 3.51; N, 24.41. Found: C, 76.86; H, 3.54; N, 24.33.

3-(10-Methyl-1,2,4-triazolo-[4,3-a:3',4'-c]quinoxalin-3-yl)propanoic acid (9b): Yield 85%, M.P. >300ºC (ethanol); IR (KBr, cm\(^{-1}\)) : 3400-3200 (broad OH); 1730 (C=O); 1618 (C=O); 1574,1545,1500(C=O). IR (300 MHz, DMSO-d\(_6\)); δ (ppm): 2.72-2.92 (2H, 4H, 2 CH\(_2\)); 3.06 (s, 3H, CH\(_3\)); 7.43 (t, J = 7.2 Hz, 1H, bis-triazoloquinazolyl C\(-\text{H}\)); 7.48 (t, J = 6.9 Hz, 1H, bis-triazoloquinazolyl C\(-\text{H}\)); 7.59 (d, J = 7.2 Hz, 1H, bis-triazoloquinazolyl C\(-\text{H}\)); 8.16 (d, J = 8.1 Hz, 1H, bis-triazoloquinazolyl C\(-\text{H}\)); 10.80 (s, 1H, OH, D\(_\text{O exchangeable}\)). MS, m/z (relative abundance %): 297 [M\(+1\)] (18.6), 296 [M\(+1\)] (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\(_{209}H\(_{137}\)N\(_{7}\)O\(_2\): C, 75.97; H, 4.08; N, 28.36. Found: C, 75.89; H, 4.14; N, 28.49.

3-(2-Arylidenehydrazinyl)-1-methyl-1,2,4-triazolo[4,3-a]quinazolines (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 6 h. The reaction mixture was left to cool to room temperature, poured onto crushed ice and neutralized with concentrated ammonia to pH 7 during which precipitation of product occurred. The precipitated solid was filtered, washed with water, dried and crystallized from ethanol.
precipitation of the product was filtered. 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]quinazoline (10a): Yield 82%, M.P. 250-1°C (ethanol) δ (IR (KBr, cm⁻¹)): 3300 (NH); 1600 (C=N); 1567 (δN=H); 1500 (C=C); 1495 (C=C); 1259; 1023 (C-O-C-H); 950 (NH-C-N); δ (ppm): 2.90, 3.02 (two s, 3H, CH₃, CH₂, CH₂, N) 7.71, 7.14, 7.31-7.34 (two m, 1H, 2H, triazolquinox.) 7.45 (d, J = 8.4 Hz, 2H, p-chlorophenylCH); 7.48 (d, J = 8.4 Hz, 2H, p-chlorophenylCH); 7.7, 8.08 (two d, J = 7.6 Hz, 1H, ½H triazolquinox., H, E & Z isomers); 7.87, 8.01 (two d, J = 8.4 Hz, ½H, 1H, triazolquinox., H, Z & E isomers); 8.51, 8.54 (two s, ½H, 1H, N=CH₂, Z & E isomers); 10.71, 12.01 (two s, 1H, NH, D,O exchangeable, E & Z isomers). Anal. Calcld. for C₂₉H₂₂N₄O₂ (636.39): C, 76.97; H, 5.01; N, 23.37.

10-Aryl-3-methyl-1,2,4-triazolo-[4,3-a:3',4'-c']quinazolines (11a-d): To a stirred mixture of 10a-d (1 mmol) and anhydrous sodium carbonate (0.25 g, 3 mmol) in methylene chloride (10 ml), bromine (0.33 ml, 7.1 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-phenylbis-1,2,4-triazolo[4,3-a:3',4'-c']quinazoline (11a): Yield 70%, M.P. 214-5°C (ethanol) δ (IR (KBr, cm⁻¹)): 1590 (C=C); 1587.1 (C=C); δ (ppm): 3.07 (s, 3H, CH₃); 7.33 (t, J = 8.4, 1.3 Hz, H, phenylCH); 7.38 (td, J = 8.4, 1.3 Hz, 1H, phenylCH₂); 7.60 (td, J = 7.8, 1.8 Hz, 1H, phenylCH₂); 7.66-7.76 (m, 5H, 5H, phenylCH₂, H-bis-triazolquinox.); 8.32 (d, J = 8.7 Hz, 1H, H-bis-triazolquinox.); MS, m/z (relative abundance %): 301 [M+1] (36.25), 300 [M] (52.53), 263 (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. Calcld. for C₂₉H₂₃N₄O₂ (300.32): C, 67.99; H, 4.03; N, 27.89. Found: C, 68.13; H, 4.07; N, 28.17.

10-(4-Chlorophenyl)-3-methyl-1,2,4-triazolo[4,3-a:3',4'-c']quinazoline (11b): Yield 68%, M.P. > 300°C (ethanol) δ (IR (KBr, cm⁻¹)): 1629 (C=C); 1600, 1577, 1493 (C=C), 829 (C=C); δ (ppm): 3.03 (s, 3H, CH₃); 7.32 (d, J = 8.4 Hz, 1H, bis-triazolquinox.); 7.40 (t, J = 7.6 Hz, 1H, bis-triazolquinox.); 7.58 (t, J = 7.6 Hz, 1H, bis-triazolquinox.); 7.72-7.78 (m, 4H, p-chlorophenylCH₃, CO₂H); 8.82 (d, J = 8.4 Hz, 1H, bis-triazolquinox.). Anal. Calcld. for C₂₉H₂₂N₄O₂ (336.76): C, 60.99; H, 3.31; N, 25.10. Found: C, 61.14; H, 3.37; N, 25.22.

10-(3,4-Dimethoxyphenyl)-3-methyl-1,2,4-triazolo[4,3-a:3',4'-c']quinazoline (11c): Yield 90%; M.P. > 300°C (ethanol) δ (IR (KBr, cm⁻¹)): 1580 (C=C); 1490 (C=C); δ (ppm): 2.9, 2.95 (two s, each 3H, 2 OCH₃); 7.19-7.76 (m, 3H, 3,4-dimethoxyphenylCH₂, H-bis-triazolquinox.); 7.32-7.44 (m, 2H, bis-triazolquinox.); 7.56 (s, 1H, 3,4-dimethoxyphenylCH₂); 8.26 (d, J = 7.65 Hz, 1H, bis-triazolquinox.). Anal. Calcld. for C₂₉H₂₂N₄O₂ (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-methyl-1,2,4-triazolo[4,3-a:3',4'-c']quinazoline (11d): Yield 89%, M.P. 292-3°C (dimethylformamide) δ (IR (KBr, cm⁻¹)): 1626 (C=C); 1578, 1468 (C=C), 1237, 1033 (C-O-C); δ (ppm): 2.97 (s, 3H, CH₃); 6.12 (s, 2H, benzodioxolylCH₂); 7.13-7.14 (m, 2H, benzodioxolylCH₂); 7.17 (s, 1H, benzodioxolylCH); 7.33 (d, J = 8.4 Hz, 1H, bis-triazolquinox.); 7.36 (t, J = 8.4 Hz, 1H, bis-triazolquinox.); 7.54 (t, J = 8.4 Hz, 1H, bis-triazolquinox.); 8.20 (d, J = 8.4 Hz, 1H, bis-triazolquinox.). Anal. Calcld. for C₂₉H₂₂N₄O₂ (344.33): C, 62.79; H, 3.51; N, 24.41. Found: C, 62.87; H, 3.48; N, 24.57.

6-Methyltriazolo[1,5-a']1,2,4-triazolo[3,4-a']quinazoline (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); δ (IR (KBr, cm⁻¹)): 1637 (C=C); 1575, 1484 (C-C); δ (ppm): 3.13 (s, 3H, CH₃); 7.81-7.90 (m, 2H, tetrazolotriazoloquinox.); 8.46 (dd, J = 7.2, 2.1 Hz, 1H, tetrazolotriazoloquinox.); 8.60 (dd, J = 6.7, 1.8 Hz, 1H, tetrazolotriazoloquinox.). El-MS m/z (relative abundance
Table 1: The inhibition zones (IZ) in mm diameter of the synthesized compounds 3-12.

| Cpd No | S. aureus | B. subtilis | P. aeruginosa | 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          | 16            | 18      | 16          |
| 10b    | 18        | 14          | 16            | 16      | 18          |
| 10c    | 18        | 16          | 14            | 16      | 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          |
| A*     | 9         | 12          | 7             | 10      | -           |
| C*     | -         | -           | -             | -       | -           |

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

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 inactivated with the test organisms; each 5 mL received 0.1 mL of the 1 mg/mL solution in DMSO. The test organisms were grown in their suitable broth: 24 h for bacteria and 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 bacterial concentration (MBC) measurement: MIC tests were always extended to measure the MBC as follows: A loopful 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(H)-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 phenyl 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',4'-c'] quinoxaline derivatives 6 and 7 respectively. Treatment of 3 with p-nitrobenzoic or phenyl acetic acid in phosphorus 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-
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. 1H-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 CH₃ groups of the two isomers and two deshielded D₂O exchangeable singlets corresponding to the NH groups, in addition to the aromatic signals integrated to the double number of triazoloquinoxaline protons.

The 1H-NMR spectrum of 10c characterized by the existence of two upfield singlets assigned for the protons of the OCH₃ 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 1H-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. Compounds 10a-d underwent oxidative cyclization by bromine in presence of anhydrous sodium carbonate to the corresponding bis-triazoloquinoxalines 11a-d. 1H-NMR spectra for 11a-d revealed the disappearance of the two singlets corresponding to N=CH and NH protons present in their precursors. 1H-NMR spectra for 11a-c lacked the paired signals for CH₃ 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 tetrazo compounds 12. The structures of the newly synthesized compounds were substantiated by elemental analyses, IR, MS, 1H-NMR and 13C-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 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. 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 3a, 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-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-nitrophénol 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]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-methylbis-1,2,4-triazolo[4,3-a]quinazolines (6, 7, 8a,b, 9a,b and 11a-d) and 6-methyl tetratolo[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, 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.

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