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Synthesis and Antimicrobial Activities of Some Novel Quinoxaline Derivatives

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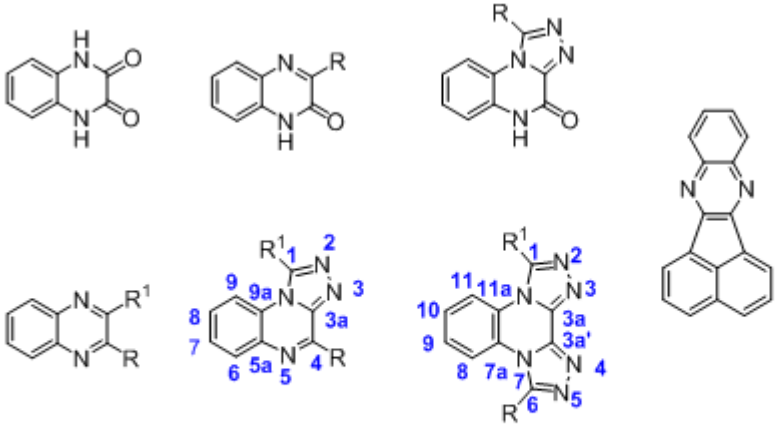
Abstract: *Literature review highlighted and presented the importance of the heterocyclic compounds that contain quinoxaline skeleton. Therefore, the aim of this project was to synthesis some novel of 2,3-substituted-quinoxaline. Thus, a series of 18 such derivatives have been synthesized, characterized on the basis of spectroscopic method and were tested for their antimicrobial activity against Staphylococcus aureus (Staph. aureus) (Gram +ve bacteria), Escherichia coli (E. coli) (Gram –ve bacteria) and one pathogenic fungi Candida albicans(C. albicans). Although none of the synthesized compounds were active against E. coli ATCC 25922, however, most of them showed considerable activity against Staph. Aureus ATCC 25923 (zone of inhibition 12-18 mm), whereas all the synthesized compounds showed activity against Candida albicans ATCC 10231(zone of inhibition 13-18.5 mm).*

Keywords: *Quinoxaline, heterocycle, antibacterial activity, antifungal activity.*

1. INTRODUCTION

Quinoxalines are 1,4-benzodiazine derivatives which represent an important category among heterocyclic of medical, biological and industrial interests [1-4]. According to several previous studies, the variation of the substituent on the quinoxaline core, could improve the biological activity, also some quinoxalines fused with other moieties such as triazole, ditriazolo, or acenaphtho have been proved as antimicrobial agents[5-8].



R: -$NHNH_2$,-NH-N=CH-(sub) Ph.,-(sub) Ph,-Cl, 3,5-dimethyl pyrazolyl, -$CH_2COOCH_2CH_3$, carboxyl-2,3,4,5-tetra chloro-Ph.
R^1: -Cl, -$NHNH_2$, NH-N=CH-(sub) Ph.,-(sub) Ph,

Fig1. *Quinoxaline derivatives available for this project*

On the light of these findings, the present work focuses on the synthesis of certain 1,4-substituted-[1,2,4]triazolo[4,3-a]quinoxalin, 1,6-substituted-[1,2,4]ditriazolo[4,3-a:3',4'-c]quinoxaline and acenaphtho[1,2-b]quinoxaline derivatives as potential antimicrobial agents. Moreover, it was reported that the nature of the substituent at position-4 of triazolo quinoxaline could affect the biological activity of the compound [9]. Hence, a further investigation was adopted to predict the effect of changing the oxo group with hydrazine, or with chlorine for possible antimicrobial activity. Likewise a series of 1,6-substituted-[1,2,4]ditriazolo[4,3-a:3',4'-c]quinoxaline were prepared for evaluation of possible antimicrobial activity. The synthetic approaches utilized for the preparation of the target compounds are summarized in Figure 1.

2. EXPERIMENTAL

The melting points (m.p.) were determined using Gallenkamp melting point apparatus. The IR spectra were recorded in KBr discs on a Perkin Elmer 1000 FT-IR spectrophotometer (υmax in cm^{-1}). The ^{1}H NMR and ^{13}C NMR spectra were collected in DMSO-*d*6 or ($CDCl_3$) using a JEOL-ECP-400. The chemical shifts were reported as parts per million (d ppm) and the coupling constants (*J*) are given in Hz, tetramethyl silane (TMS) was used as an internal standard. The mass spectra (m/z, %) were obtained on electron impact using an AEI MS902 mass spectrometer. The purity of all compounds was checked by TLC using glass plates coated with silica gel and chloroform/methanol (9:1) as a solvent system. Spectral data (IR, NMR, and mass spectra) confirmed the structures of the synthesized compounds.

Synthesis of 1,4-Dihydro-quinoxaline-2,3-dione (1)

1,4-Dihydro-quinoxaline-2,3-dione (1) was prepared; by heating a solution of oxalic acid dihydrate (0.238 mole, 30 g) in H_2O (100 ml), followed this step by addition of conc.HCl 4.5 ml, then *o*- phenylendiamine (0.204 mole, 22 g) was added. The reaction was heated under reflux for 20 min, and then was cooled by addition of ice. The solid was filtered, washed with water, purified by recrystallization from ethanol until 1,4-Dihydro-quinoxaline-2,3-dione (1) was isolated as white crystals [10] (32 g, 98%), m.p. >300c˚(lit. >300c˚, [11]. (δH (DMSO-d6) 11.92 (2H, s, NH), 7.11 (2H, d,^{3}J 10.28, 5-H & 8-H), 7.08 (2H, d,^{3}J 10.28, 6-H & 7H); δC (DMSO-d6) 155.72 (C-2 & C-3), 126.14 (C-4a & C-8a), 115.67 (C-5 & C-8), 123.54 (C-7), 123.45 (C6); υmax /cm^{-1} (KBr) 3445 (N-H), 3049 (C-H, *sp2*), 1681 (C=O). MS (EI): m/z 162 (M+., 100%), 134 (M-CO, 54.4%), 134 (M-2CO, 62.8%).

Synthesis of 3-Hydrazino-1*H*-quinoxalin-2-one (2)

According to Cheeseman *et al.* (1971) [12], mixture of 1,4-dihydro-quinoxaline-2,3-dione (1) (0.062 mole, 10.04 g), hydrazine hydrate 99.9% (1 mole, 50ml,) and water (50 ml) was heated under reflux for 2hrs, then cooled to room temperature, the precipitate was filtered, washed with water and crystallized from butanol until 3-hydrazino-1*H*-quinoxalin-2-one (2) obtained as yellow needles (9.28 g,84%), m.p. >300c˚(lit. >360c˚,[13]. (δH (DMSO-d6) 12.08 (2H, s, NH), 8.70 (1H, s, NH-NH_2), 4.52 (2H, s, NH_2), 7.36 (1H, dd,^{3}J 7.32, ^{4}J 1.44 8-H), 7.12 (1H, dd,^{3}J 9.16, ^{4}J 1.48, 5-H), 7.12 (2H, dd,^{3}J 9.16, ^{4}J 2.2, 6-H & 7-H); δC (DMSO-d6) 151.46 (C-3),150.53 (C-2), 133.86 (C-4a), 128.38 (C-8a), 124.7 (C-7) 123.81 (C-6), 123.55 (C-5), 115.47 (C-8); υmax /cm^{-1} (KBr) 3415 (N-H, NH_2), 3051 (C-H, *sp2*), 1682 (C=O). MS (EI): m/z 176 (M+., 100%).

Synthesis of 3-[*N*'-(substituted-benzylidene)-hydrazino]-1*H*-quinoxalin-2-one (3,4)

3-[*N*'-(substituted-benzylidene)-hydrazino]-1*H*-quinoxalin-2-one (3,4) have been prepared [14, 15]**,** by refluxing a mixture of 3-hydrazino-1*H*-quinoxalin-2-one (2) and the corresponding aromatic aldehyde (0.01 mole of each) in ethanol (20 ml) for 5hrs. After cooling the mixtures; the precipitates were obtained, filtered, dried and crystallized from ethanol;

Compound (3) collected as a greenish solid (3.2 g, 96%), m.p 285c˚. (δH (DMSO-d6) 12.4 (1H, s, NHCO), 11.70 (1H, s, NH), 8.95 (1H, s, CH), 8.12 (1H, d,^{3}J 8.04, 6'-H), 7.53 (1H, d,^{3}J 7.68, 5'-H), 7.69 (1H, d,^{4}J 1.48, 3'-H), 7.21 (1H, d,^{3}J 6.60, 8-H), 7.19 (2H, d,^{3}J 6.60, 6-H & 7-H), 7.12 (1H, d,^{3}J 6.60, 5-H); δC (DMSO-d6) 146.7 (C-2),135.11 (C-3), 134.11 (C=N-NH), 131.87 (C-4a), 131.84 (C-4') 129.72 (C-2'), 128.72 (C-8a), 125.38 (C-1' & C-3'), 124.06 (C-5'), 115.64 (C-5,C-6,C-7,C-8); υmax /cm^{-1} (KBr) 3415 (N-H), 3199 (C-H, *sp2*), 1671 (C=O), 1618 and 1468 (C=N). MS (EI): m/z 332 (M+., 10.4%),M+2 at m/z 334(6.91%), M+4 at m/z 336 (1.22%).

Whereas compound (4), collected as yellow solid (2.9 g, 89%), m.p 270c˚. (δH (DMSO-d6) 12.39 (1H, s, NHCO), 11.11 (1H, s, NH), 8.49 (1H, s, CH), 7.34 (1H, s, 2'-H), 7.20 (1H, d,^3J 6.24, 5'-H), 7.18 (1H, d,^3J 6.24, 6'-H), 7.05 (2H, d,^3J 8.43, 5-H & H-8), 7.02 (2H, d,^3J 8.4, 6-H & 7-H), 3.38 (2H, s, OCH_3); δC (DMSO-d6) 151.43 (C-2),150.90 (C-3), 149.50 (C=N-NH), 146.57 (C-4a), 147.6 (C-4') 112.13 (C-2' & C-5'), 133.48 (C-8a), 146.57 (C-3'),125.95 (C-1'), 129.18 (C-7), 128.06 (C-6), 115.57 (C-5 & C-8), 124.28 (C-6'), 56.11 (C-4',OCH_3), 56.06 (C-3',OCH_3); υmax /cm^{-1} (KBr) 3415 (N-H), 3248 (C-H, *sp2*), 1681 (C=O), 1615 and 1461 (C=N). MS (EI): MS (EI): m/z 324 (M+., 19.7%).

Synthesis of 1-(substituted-benzyl)-5*H*-[1,2,4]triazolo[4,3-a]quinoxalin-4-one (5,6)

1-(substituted-benzyl)-5*H*-[1,2,4]triazolo[4,3-a]quinoxalin-4-one (5,6) have been prepared according to Cheeseman *et al*. (1962) [16]**,** mixture of hydrazone derivatives (3,4) (0.02 mole) and anhydrous sodium acetate (0.04 mole, 3.3g) were stirred in glacial acetic acid (100ml), then 10% v/v solution of bromine in glacial acetic acid (10ml) was added in a small portion. The reaction mixtures were stirred at 25˚C for 1hr, and then poured onto ice water (200ml). The precipitates were filtered washed with water, followed by aqueous $NaHSO_3$ solution (10%) (2×50ml). Finally, recrystallization the crud products from ethanol to obtained the pure desired compounds.

Compound (5) collected as a light brown solid (5.47 g, 83%), m.p >300c˚. (δH (DMSO-d6) 12.0 (1H, s, NHCO), 8.04 (1H, s, 3'-H), 7.81 (1H, dd,^3J 10.06, ^4J 5.49, 5'-H), 7.62 (1H, d, ^3J 8.79, 6'-H), 7.39 (1H, dd,^3J 8.3, ^4J 3.69, 9-H), 7.23 (1H, d,^3J 6.13,6-H), 7.07 (1H, dd,^3J 8.43, ^4J 4.04, 8-H), 6.87 (1H, m,^3J 7.68, ^3J 8.43 7-H),; δC (DMSO-d6) 155.53 (C-4), 155.42 (C-3a), 155.3 (C-1), 152.2 (C-5a), 152.07 (C-1'), 134.45 (C-2' & C-4'),130.44 (C-3'), 129.21 (C-6'), 127.2 (C-7), 126 (C-9), 119.53 (C-5'), 117.79 (C-8), 117.64 (C-6), 116.73 (C-9a); υmax /cm^{-1} (KBr) 3414 (N-H), 3189 (C-H, *sp2*), 1692 (C=O), 1638 and 1488 (C=N). MS (EI): m/z 3302 (M+., 100%),M+2 at m/z 332 (64.59%), M+4 at m/z 334 (10.48%).

Whereas compound (6), was obtained as yellow solid (5.53 g, 86%), m.p >300c˚. (δH (DMSO-d6) 12.13 (1H, s, NHCO), 7.34 (1H, s, 2'-H), 7.28 (1H, d,^3J 6.60, 5'-H), 7.24 (1H, d, ^3J 6.24, 6'-H), 7.17 (2H, dd,^3J 10.62, ^4J 4.38, 9-H & 6-H), 7.07 (1H, m,^3J 6.31, ^4J 3.3, 7-H & 8-H), 3.89 (1H, s, OCH_3, 3'-H), 3.75 (1H, s, OCH_3, 4'-H); δC (DMSO-d6) 152.5 (C-4), 151.27 (C-3a), 149.45 (C-1), 144.63 (C-5a), 129.95 (C-2' & C-4'), 128.25 (C-7 & C-9), 123.29 (C-1' & C-3'), 123.21 (C-8), 117.66 (C-6'), 116.88 (C-6),113.55 (C-5'), 112.46 (C-9a), 56.19 (OCH3, C4'), 56.3 (OCH3, C3'); υmax /cm^{-1} (KBr) 3508 (N-H), 3190 (C-H, *sp2*), 1693 (C=O), 1638 and 1489 (C=N). MS (EI): MS (EI): m/z 322 (M+., 100%).

Synthesis of 2,3-Dichloro-quinoxaline (7)

2,3-Dichloro-quinoxaline (7) has been prepared by refluxing a mixture of 1,4-dihydro-quinoxaline-2,3-dione (1)(0.05 mole, 8.1 g), $POCl_3$ (0.08 mole, 7.35 ml) and pyridine (0.05 mole, 9.65 ml,) for 5 hrs. The mixture was allowed to cool, and then poured onto crushed ice, the precipitate was filtered and washed with water[16]**.** 2,3-Dichloro-quinoxaline (7) was recrystallized from ethanol until grayish crystals obtained (9.5, 96%), m.p. 150C˚. (δH (DMSO-d6) 8.06 (2H, dd,^3J 8.25, ^4J 3.3, 5-H & 8-H), 7.95 (1H, dd,^3J 6.24, ^4J 3.66, 6-H & 7-H); δC (DMSO-d6) 145.17 (C-4a & C-8a), 140.57 (C-2 & C-3), 132.33 (C-5 & C-8), 128.46 (C-6 & C-7); υmax /cm^{-1} (KBr) 3041 (C-H, *sp2*), 1645 and 1457 (C=N). MS (EI): MS (EI): m/z 198 (M+., 100%), M+2 at m/z 200 (63.4%), M+4 at m/z 202 (1.60%).

Synthesis of (3-chloro-quinoxalin-2-yl)-hydrazine (8)

(3-Chloro-quinoxalin-2-yl)-hydrazine (8) has been prepared according to Sarges *et al*. (1990) [17]**,** a mixture of 2,3-dichloro-quinoxaline (7) (0.05 mole, 9.95 g) and hydrazine hydrate 99.9% (0.1 mole, 5 ml) was stirred in absolute ethanol (200 ml) for 2 hrs, at 25˚C. The precipitate was filtered, washed with ethanol and crystallized from toluene to obtained the pure product as yellow solid (8.9, 92%), m.p. 165C˚. (δH (DMSO-d6) 7.62 (1H, s, NH), 7.37 (1H, dd,^3J 7.10, ^4J 1.83, 8-H), 7.58 (2H, dd,^3J 7.40, ^4J 1.83, 6-H & H-7), 7.72 (1H, d,^3J 8.43, 5-H); δC (DMSO-d6) 140.85 (C-2), 135.95 (C-3), 130.84 (C-4a & C-8a), 128.04 (C-5 & C-8), 125.17 (C-7), 124.95 (C-6);

υmax /cm^{-1} (KBr) 3436 (NH), 3246 (C-H, *sp2*), 1631 and 1460 (C=N). MS (EI): MS (EI): m/z 194 (M+., 100%), M+2 at m/z 196 (33.3%).

Synthesis of *N*-(4-chloro-benzylidene)-*N*'-(3-chloro-quinoxalin-2-yl)-hydrazine (9)

N-(4-chloro-benzylidene)-*N*'-(3-chloro-quinoxalin-2-yl)-hydrazine (9) prepared by mixing (3-Chloro-quinoxalin-2-yl)-hydrazine (8) (0.005 mole, 0.97 g) and *p*-chlorobenzaldehyde (0.005 mole, 0.7025 g) in absolute ethanol (30 ml), then allowed the mixture to stirred at room temperature for 2 hrs [16]. The product was filtered, washed with ethanol and crystallized from aqueous DMF. Finally, orange powder of *N*-(4-chloro-benzylidene)-*N*'-(3-chloro-quinoxalin-2-yl)-hydrazine (9)**,** was obtained (1.29 g, 82%), m.p:159 C°. (δH (DMSO-d6) 11.18 (1H, s, NH), 8.62 (1H, s, CH=N), 8.09 (1H, dd,^{3}J 6.24, ^{4}J 3.2, 6'-H), 7.93 (2H, d,^{3}J 6.24, 5-H & 8-H), 7.82 (2H, d,^{3}J 8.43, 6-H & 7-H), 7.56 (3H, m, ^{3}J 8.79, ^{4}J 2.2, 2'-H, 3'-H & 5'-H); δC (DMSO-d6) 161.2 (C-2), 156.7 (CH=N),132.3 (C-3), 131.22 (C-8a), 130.7 (C-4a), 130.6 (C-4'), 129.5 (C-1'), 129.6 (C-2'& C-6'), 129.1 (C-3'& C-5'), 128.49 (C-7), 126.9 (C-5 & C-8), 115.6 (C-6); υmax /cm^{-1} (KBr) 3436 (NH), 3062 (C-H, *sp2*), 1614 and 1486 (C=N). MS (EI): m/z 316 (M+., 15.05%), M+2 at m/z 318 (10.05%).

Synthesis of 4-Chloro-1-(4-chloro-phenyl)-[1,2,4]triazolo[4,3-a]quinoxaline (10)

4-Chloro-1-(4-chloro-phenyl)-[1,2,4]triazolo[4,3-*a*]quinoxaline (10) was synthesized by addition the solution of bromine (0.01 mole,1.6 g) in glacial acetic acid (4.5 ml) to the hydrazine derivative *N*-(4-chloro-benzylidene)-*N*'-(3-chloro-quinoxalin-2-yl)-hydrazine (9) (0.01 mole, 3.17 g) and anhydrous sodium acetate (0.02 mole,1.64 g) in glacial acetic acid (20ml). Then the reaction was stirred at room temperature for 1 hrs, and then poured into 0.5 N NaOH solution (100ml) [16]. The solid was filtered, washed with water and crystallized from aqueous DMF to give compound (10) as greenish solid (1.98, 63%), m.p:> 300 C°. (δH (DMSO-d6) 7.83 (2H, d,^{3}J 7.23, 6'-H & 5'-H), 7.74 (2H, d,^{3}J 6.6, 5-H & 8-H), 7.63 (2H, d,^{3}J 8.43, 6-H & 7-H), 7.43 (2H, d, ^{3}J 8.43, 2'-H & 3'-H);δC (DMSO-d6) 145.3 (C-4), 136.4 (C=N),133.1 (C-1), 130.2 (C-3a), 125.3 (C-5a & C-9a), 121.9 (C-6 & C-9), 115.6 (C-7 & C-8), 129.1 (C-1'), 129.4 (C-4'), 128.5 (C-2', C-3', C-5', & C-6'), υmax /cm^{-1} (KBr) 1634 and 1493 (C=N).

Synthesis of [1-(4-Chloro-phenyl)-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-yl]-hydrazine (11)

Compound[1-(4-Chloro-phenyl)-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-yl]-hydrazine was synthesized according Cheeseman *et al*. (1971) [12], a mixture of 4-Chloro-1-(4-chloro-phenyl)-[1,2,4]triazolo[4,3-*a*]quinoxaline (10) (0.05 mole 15.75 g) and hydrazine hydrate 99.9% (0.1 mole, 5 ml) in absolute ethanol (200 ml) was stirred for 2 hrs at 25˚C. The precipitate was filtered, washed with ethanol and crystallized from DMF to give an orange solid (10 g, 65%), m.p: 270 C°. (δH (DMSO-d6) 9.61 (1H, s, NH), 4.74 (1H, s, NH_2), 7.81 (2H, d,^{3}J 8.43, 2'-H & 6'-H), 7.74 (2H, d,^{3}J 8.79, 3'-H & 5'-H), 7.62 (1H, d,^{3}J 8.43, 9-H), 7.39 (2H, dd, ^{3}J 8.07, ^{4}J 2.48 6-H), 7.17 (1H, d,^{3}J 7.71, 8-H), 7.07 (1H, dd,^{3}J 8.43, ^{4}J 2.2, 7-H); δC (DMSO-d6) 149.4 (C-4), 147 (C-1), 140 (C-5a), 136.5 (C-3a), 132.5 (C-9a), 129.8 (C-1'), 127.9 (C-4'), 126.9 (C-3' & C-5'), 123.6 (C-7 & C-9), 123.1 (C-2' & C-6'), 115.9 (C-6 & C-8); υmax /cm^{-1} (KBr) 3311 & 3242 (NH & NH_2) 1631 and 1460 (C=N); MS (EI): m/z 310 (M+., 100%), M+2 at m/z 312 (32.95%).

Synthesis of 1-(4-choloro phenyl)-[1,2,4]ditriazolo[4,3-*a*:3',4'-*c*]quinoxaline (12).

1-(4-Choloro phenyl)-[1,2,4]ditriazolo[4,3-*a*:3',4'-*c*]quinoxaline was synthesized [18] by heating the mixture of [1-(4-chloro-phenyl)-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-yl]-hydrazine (11) (0.003 mole, 0.9315 g) and triethylorthoformate (15ml) under reflux with stirring for 10 hrs. After cooling, the precipitate was filtered, washed with ethanol and crystallized from DMF to obtain brownish orange solid for compound (12) (0.88 g, 92%), m.p: >300C°. (δH (DMSO-d6) 10.05 (1H, s, 6-H), 8.44 (1H, d,^{3}J 8.43, 10-H), 7.78 (4H, br.s, 2'-H, 3'-H, 5'-H& 6'-H), 7.64 (1H, d,^{3}J 8.4, 9-H), 7.44 (2H, d, ^{3}J 8, 8-H), 7.30 (1H, d,^{3}J 8.43, 7-H); υmax /cm^{-1} (KBr) 3053 (C-H, *sp2*),1631 and 1462 (C=N).

Synthesis of 1-ethoxy carbonyl methyl-6-(4-chloro phenyl)-[1,2,4]ditriazolo[4,3-*a*:3',4'-*c*]quinoxaline (13)

1-ethoxy carbonyl methyl-6-(4-chloro phenyl)-[1,2,4]ditriazolo[4,3-*a*:3',4'-*c*]quinoxaline (13) has been synthesized [15] from a mixture of [1-(4-chloro-phenyl)-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-

yl]-hydrazine (11) (0.003 mole, 0.9315 g) and diethylmalonate (10 ml), which was heated under reflux for 2hrs. After cooling, petroleum ether (30 ml) was added and the mixture was stirred overnight. Finally, the precipitate was filtered, washed with petroleum ether and recrystallized from ethanol. Compound (13) was isolated as a brown powder (1.11 g, 91%), m.p: 250C°. (δH (DMSO-d6) 8.02 (1H, d,^3J 8.4, 3'-H), 7.75 (4H, m, 7-H, 8-H, 9-H& 10-H), 7.57 (1H, br.s, 5'-H), 7.42 (1H, br.s, 2'-H), 7.35 (1H, br.s, 6'-H), 4.38 (2H, br.s, OCH_2), 4.11 (2H, br.s, CH_2CO), 1.15 (3H, t, ^3J 6.24, ^3J 9.87 CH_3); δC (DMSO-d6) 168.3 (C=O), 150.07 (C-1), 136.4 (C-6), 132.3 (C-3a & C-3a'), 128.2 (C-7a & C-11a), 128.04 (C-4'), 127.5 (C-8 & C-11), 124.3 (C-10), 124.1 (C-9), 123.6 (C-1'), 118.6 (C-3' & C-5'), 118.3 (C-2' & C-6'), 62 ($O\underline{CH_2}$), 33.5 ($CO\underline{CH_2}$), 14.5 (CH_3); υmax /cm^{-1} (KBr) 2977 (C-H, *sp2*),1641 and 1474 (C=N), 1736 (C=O); MS (EI): m/z 406 (M+., 100%), M+2 at m/z 408 (38%).

Synthesis of 2,3,4,5-Tetrachloro-6-(1-(4-choloro phenyl)- [1,2,4] ditriazolo [4,3-*a*:3',4'-*c*]quinoxalin)-1-yl-benzoic acid (14)

2,3,4,5-Tetrachloro-6-(1-(4-choloro phenyl)- [1,2,4] ditriazolo [4,3-*a*:3',4'-*c*]quinoxalin)-1-yl-benzoic acid (14) was synthesized [15] by mixing an equimolar amount of [1-(4-chloro-phenyl)-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-yl]-hydrazine (11) and tetrachlorophethalic anhydride (0.003 mole of each) in glacial acetic acid (20 ml), then the mixture was heated under reflux for 5 hrs. At the end of the reaction toluene was added, and then the resulting solid was filtered, washed with ether and lastly recrystallized from ethanol. A beige powder was obtained (1.26 g, 73%), m.p: >300C°. (δH (DMSO-d6) 11.46 (1H, s, OH), 7.86 (2H, d,^3J 7.6, 2'-H & 6'-H), 7.76 (2H, d,^3J 8.04, 3'-H & 5'-H), 7.54 (1H, d,^3J 7.32, 7-H), 7.39 (1H, d,^3J 6, 10-H), 7.25 (1H, br.s, 8-H & 9-H); υmax /cm^{-1} (KBr) 3500 (OH), 1639 and 1457 (C=N), 1845 (C=O); MS (EI): m/z 578.5 (M+, 100%), M+1 at m/z 579.5 (36.4%), M+2 at m/z 580.5 (81.2%), M+3 at m/z 581.5 (28.4%).

Synthesis of 1-(4-Chloro-phenyl)-4-(3,5-dimethyl-pyrazol-1-yl)-[1,2,4]triazolo[4,3-*a*] quinoxaline(15)

[1-(4-chloro-phenyl)-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-yl]-hydrazine **(11)** (0.003 mole, 0.9315 g) and acetyl acetone (0.0045 mole, 0.45 ml) were dissolved in absolute ethanol (25ml) and then heated under reflux for 4 hrs [15]. At the end of the reaction, the precipitate was filtered, washed with ether and recrystallized from ethanol to give compound (15) as a brown solid (0.52 g, 47%), m.p: 220C°.υmax /cm^{-1} (KBr) 3073 (CH,sp^2),1644 and 1470 (C=N); MS (EI): m/z 374 (M+, 50.69%), M+2 at m/z 376 (18.23%).

Synthesis of 2,3-Bis-(4-substituted-phenyl)-quinoxaline (16,17)

A mixture of *o*- phenylenediamine and 1,2-dicarbonyl derivatives (0.01 mole of each), heated under reflux in ethanol for 12 hrs. At the end of the reaction, the precipitate was filtered, washed with ethanol, and then the crud products have recrystallized from ethanol.

2,3-Diphenyl-quinoxaline (16)

(2.79 g, 99%), m.p: 282C°. (δH (DMSO-d6) 8.15 (2H, dd,^3J 8.02, ^4J 3.66, 5-H & 8-H), 7.89 (2H, dd,^3J 8.7, ^4J 4.4, 6-H & 7-H), 7.47 (4H, d,^3J 6.96, 2'-H, 6'-H, 2"-H& 6"-H), 7.37 (6H, d,^3J 6.96, 3'-H, 4'-H, 5'-H, 3"-H, 4"-H & 5"-H); (δC (DMSO-d6) 153.6 (C-2 & C-3), 140.9 (C-4a & C-8a), 139.3 (C-1' & C-1"), 131.9 (C-5 & C-8), 130.3 (C-6, C-7, C-3',C-5', C-3", &C-5"), 129.4 (C-4' & C-4"), 128.6 (C-2', C-6', C-2", & C-6"); υmax /cm^{-1} (KBr) 3056 (C-H, *sp2*), 1636 and 1478 (C=N); MS (EI): m/z.282 (M+., 100%).

2,3-Bis-(4-bromo-phenyl)-quinoxaline (17)

(3.38 g, 77%), m.p: 180C° ,(δH (DMSO-d6) 8.16 (2H, dd,^3J 9.8, ^4J 4.7, 5-H & 8-H), 7.9 (2H, dd,^3J 6.24, ^4J 3.66, 6-H & 7-H), 7.60 (4H, d,^3J 8.43, 3'-H, 5'-H, 3"-H, & 5"-H),7.43 (4H, d,^3J 8.43, 2'-H, 6'-H, 2"-H& 6"-H); (δC (DMSO-d6) 141 (C-2 & C-3), 152.4 (C-4a & C-8a), 138.3 (C-1' & C-1"), 132.3 (C-3',C-5', C-3", &C-5"), 131.8 (C-5 & C-8), 131.3 (C-2', C-6', C-2", & C-6"), 129.4 (C-6 &C-7), 123.2 (C-4' & C-4"); υmax /cm^{-1} (KBr) 3059 (C-H, *sp2*),1630 and 1477 (C=N); MS (EI): m/z 438 (53.3%), M+2 at m/z 440 (100%), M+4 at m/z 442 (51.37%).

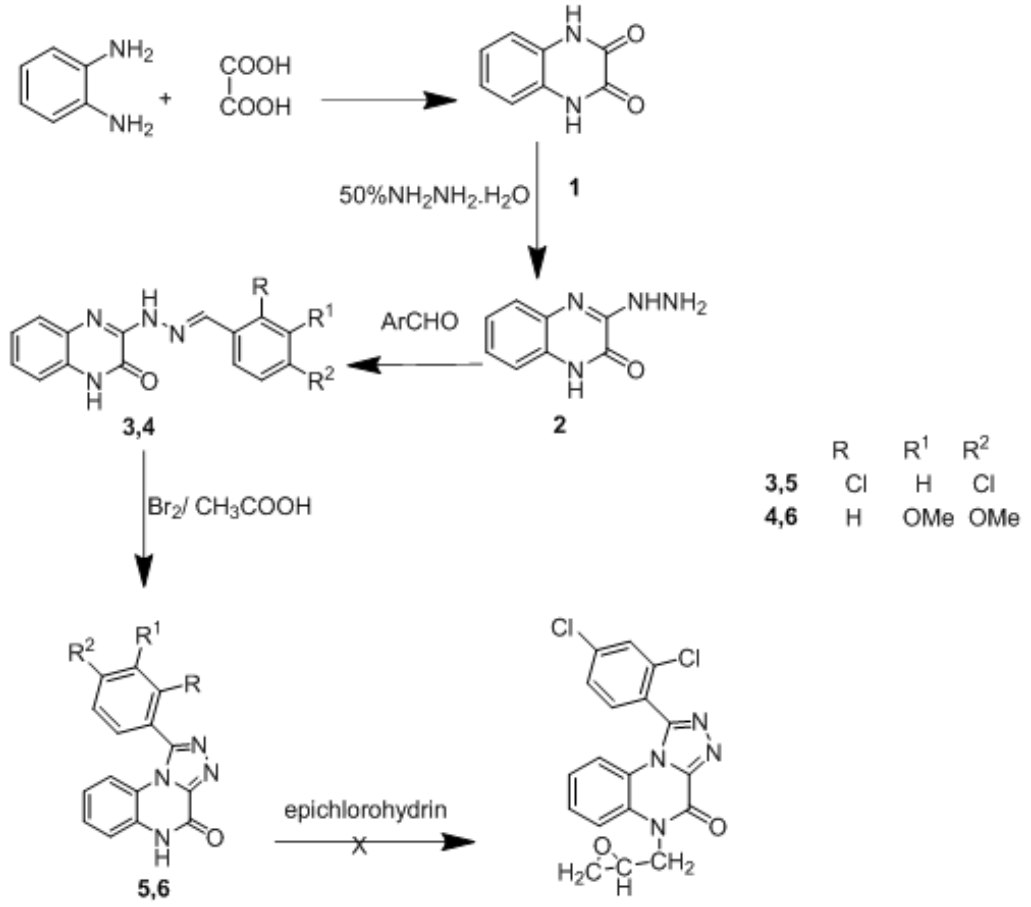
Synthesis of Acenaphtho[1,2-*b*]quinoxaline (18)

Acenaphtho[1,2-*b*]quinoxaline (18) has been prepared by stirred a mixture of equimolar amounts of an acenaphthaquinone and *o*-phenelynediamine (10 mmole of each), then iodine (10 mole %) in DMSO (10ml) was added,. The mixture was stirred at room temperature for 30 min. After completion of the reaction (monitored by TLC), the reaction mixture was poured onto crushed ice and stirred for 15 min., the solid was filtered, washed with aqueous sodium thiosulfate solution to remove iodine and subsequently with water, and then recrystallized from ethanol to afford pure quinoxaline (18) [19]. Product obtained in excellent yield (2.51g, 99%) with m.p. >300C°. υmax /cm^{-1} (KBr) 3056 (C-H, *sp2*), 1637 and 1477 (C=N); MS (EI): m/z.254 (M+., 100%).

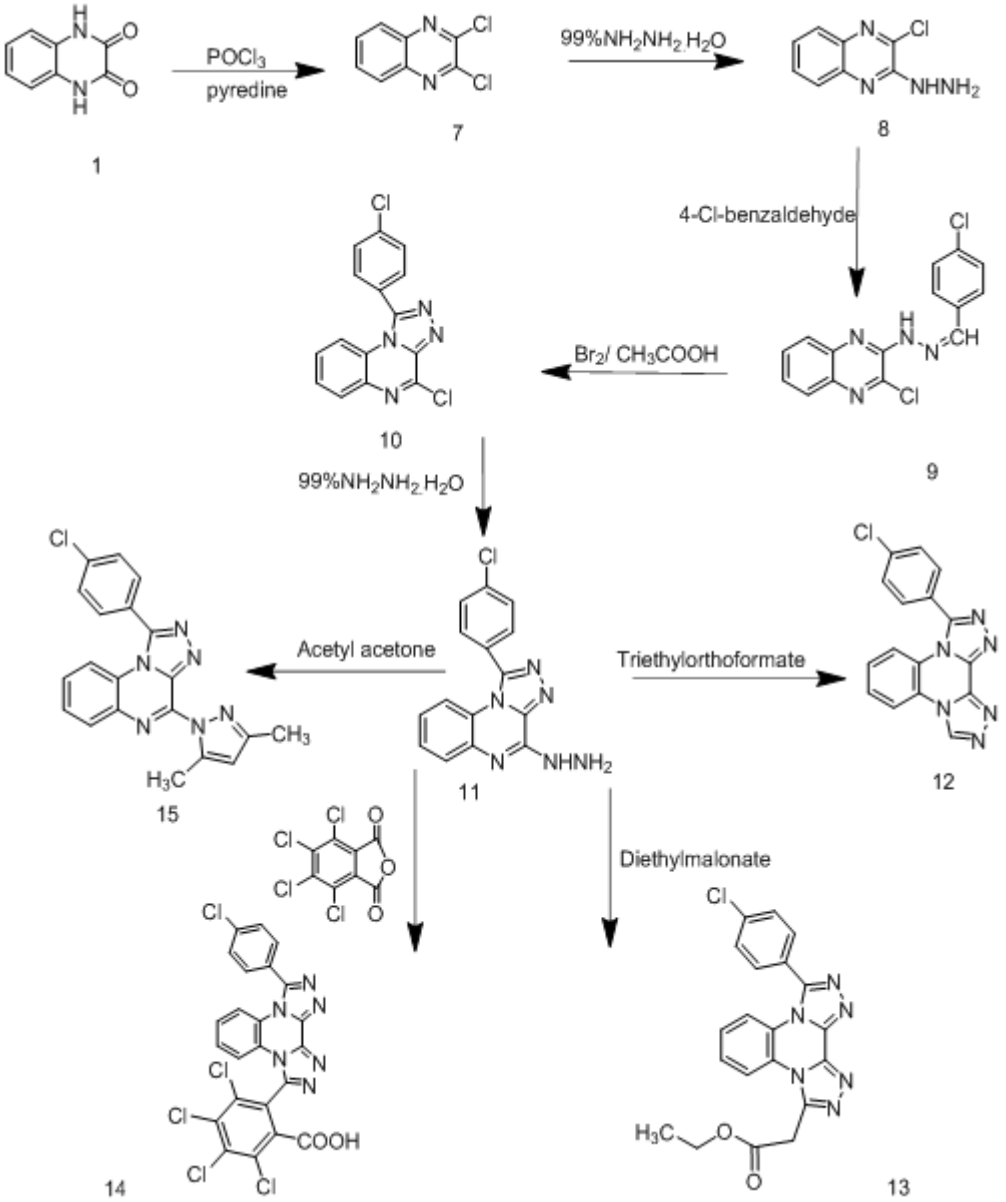
2.1. Screening for Antimicrobial Activity by the Well Diffusion Method

The strains were obtained from school of Pharmacy, King Saud University, Riyadh, Saudi Arabia. Antimicrobial activity was determined by the well diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS) methodology (National Committee for Clinical Laboratory Standards, 1993), to assess if the compounds had any appreciable antimicrobial activities. The broad spectrum antibiotic ciprofloxacin and amphotericin B were included as comparators. Petri dishes containing 20 ml of Agar (Malt Extract Agar (Oxoid) for fungi strain, and Iso-sensitest agar (Oxoid) for the bacterial strains) were seeded with cultures (2-3 day of fungal inoculum and overnight agar cultures for bacterial strains) in sterile distilled water, and adjusted to a 0.5 McFarland turbidity standard (Remel). Wells (6 mm in diameter) were cut into the agar with a sterile cork borer, and 50 µl of compound diluted in DMSO was added at a concentration of 5 mg/ml (250 µg per well). Plates were incubated at 37°C for 1-3 days depending on growth rate of each strain. Antimicrobial activity was determined based on measurement of the diameter of the inhibition zone formed around each well.

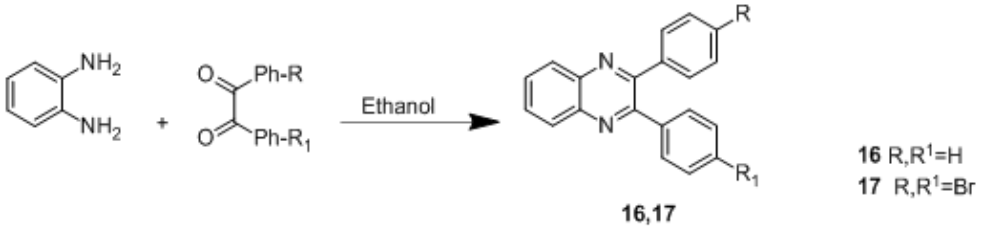
3. RESULTS AND DISCUSSION

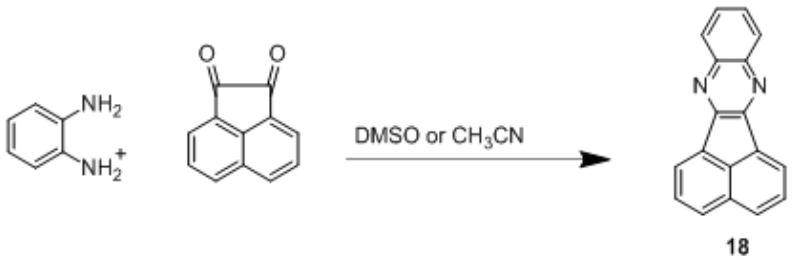


Scheme1.*Group 1 target; 2,3-substituted-quinoxaline and 1,4-substituted-[1,2,4]triazolo[4,3-a]quinoxalin derivatives*



Scheme2.*Group 2 target: 2,3-substituted-quinoxaline, 1,4-substituted-[1,2,4]triazolo[4,3-a]quinoxalin, and 1,6-substituted-[1,2,4]ditriazolo[4,3-a:3',4'-c]quinoxaline derivatives*





Scheme3. *Group 3 target; 2,3-substituted-quinoxaline and acenaphtho[1,2-b]quinoxaline derivatives*

3.1. Chemistry

The problem of multidrug resistance in bacteria is a serious health concern without adequate treatment options [4]. In the US, it was estimated that about two million people became infected with antibiotic resistant strain every year [2]. Accordingly, it is clear that there is a critical need for new effective chemotherapeutic drugs. From the chemistry point of view, there are several routes for the synthesis of quinoxaline ring, depending on the starting nuclei, to give either substituted or fused quinoxaline with other heterocyclic moieties such as, traizole, ditriazolo, or acenaphtho.

The experimental study conclude synthesized 1,4-dihydro-quinoxaline-2,3-dione 1 *via* the reaction between phenylenediamine and oxalic acid [10], then the later compound was used to prepare 3-hydrazino-1*H*-quinoxalin-2-one 2 after reacted with hydrazine hydrate [12], which, in turn, refluxed with different of aromatic benzaldehyde to afford 3-[*N*'-(substituted-benzylidene)-hydrazino]-1*H*-quinoxalin-2-one 3,4 [14, 15]. Cyclization the later compounds in exist of bromine in acetic acid, gave 1-(substituted-benzyl)-5*H*-[1,2,4]triazolo[4,3-a]quinoxalin-4-ones 5,6 (Scheme 1)[16].

Moreover, compound 1,4-dihydro-quinoxaline-2,3-dione 1 (Scheme 2)was also used to prepare 2,3-dichloro-quinoxaline 7 [16], following the reaction with hydrazine hydrate gave (3-chloro-quinoxalin-2-yl)-hydrazine 8 [17]. Subsequently react the later product with 4-chlorobenzaldehyde yielded *N*-(4-chloro-benzylidene)-*N*'-(3-chloro-quinoxalin-2-yl)-hydrazine 9 [16]. When compound 9 was cyclized by reacting with bromine in acetic acid, 4-chloro-1-(4-chloro-phenyl)-[1,2,4]triazolo[4,3-*a*]quinoxaline 10 afforded [16]. Treated the later compound 10 with hydrazine hydrate gave a [1-(4-chloro-phenyl)-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-yl]-hydrazine 11 [12]. Later compound 11 were submitted for several reactions to yield ditriazolo quinoxaline derivatives. First reaction[18] was treated compound 11 with triethyl orthoformate, to give 1-(4-choloro phenyl)-[1,2,4]ditriazolo[4,3-*a*:3',4'-*c*] quinoxaline 12. Then compound 11 was reacted with diethylmalonate to yield 1-ethoxy carbonyl methyl-6-(4-chloro phenyl)-[1,2,4]ditriazolo[4,3-*a*:3',4'-*c*]quinoxaline 13. Moreover, compound 11 was also reacted with tetrachlorophthalic anhydride to afford 2,3,4,5-tetrachloro-6-(1-(4-choloro phenyl)- [1,2,4] ditriazolo [4,3-*a*:3',4'-*c*]quinoxalin)-1-yl-benzoic acid 14 [15]. Lastly, *via* reaction with acetyl acetone the key intermediate 11, 1-(4-chloro-phenyl)-4-(3,5-dimethyl-pyrazol-1-yl)-[1,2,4]triazolo[4,3-a]quinoxaline 15 (Scheme 2)obtained [15].

By heating the reaction mixture of *o*-phenylene diamine and 1,2-dicarbonyl derivatives under reflux, compounds 2,3-diphenyl-quinoxaline 16 and 2,3-bis-(4-bromo-phenyl)-quinoxaline 17 (Scheme 3), were formed. Finally, the reaction of *o*-phenylenediamine and 1,2-acenaphthenequinone with iodine as catalyst agent, using DMSO as solvent [19], acenaphtho[1,2-*b*]quinoxaline 18 was obtained.

It is worth to mention that compound 2 was used specifically to synthesize compound [1-(4-chloro-phenyl)-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-yl]-hydrazine 11, by reaction with different derivatives of benzoic acid in presence of $POCl_3$, then addition of hydrazine hydrate in one step, but no product was collected. Also trails were made to prepare 1-(2,4-dichloro-phenyl)-5-oxiranylmethyl-5*H*-[1,2,4]triazolo[4,3-a]quinoxalin-4-one, *via* the reaction between 1-(2,4-dichloro-benzyl)-5*H*-[1,2,4]triazolo[4,3-a]quinoxalin-4-one 5 and epichlorohydrin, but, unfortunately, it was unsuccessful.

3.2. Antimicrobial Activity

The antimicrobial activities of the synthesized compounds were determined by the agar diffusion technique. The organisms tested were *Staph. Aureus* ATCC 25923, *E. coli* ATCC 25922, *Candida albicans* ATCC 10231. The agar media were inoculated with test organisms and a solution of the test compounds in DMSO (250µg/ml) was placed separately in wells (6mm diameter) in the agar medium. The inhibition zones were measured after the incubation period. The results of antimicrobial activity tests are summarized in Table 1 [20].

In case of the antibacterial assay, none of the synthesized compounds were active against *E. coli* ATCC 25922. However, all synthesized compounds except **2**, **10**, **11**, **12**, **13**, and **17**; showed activity against *Staph. aureus* ATCC 25923 . In terms of the SAR, the presence of $NHNH_2$ at position **3** for compound **2**, greatly reduced the activity in comparison to the activity of its analogue compound **1**. After the cyclization of compound **9** to form the traizolo ring, the activity against the selected strain was totally lost.

Table1. *Antimicrobial activity of the synthesized compounds*

Test organisms Compd.	***Staph.aureus* ATCC 25923 mm**	***E. coli* ATCC 25922 mm**	***C.albicans* ATCC 10231 mm**
1	**12.5**	0.0	**15.3**
2	0.0	0.0	**17**
3	**12.5**	0.0	**15.6**
4	**12.6**	0.0	**18**
5	**20**	0.0	**17.6**
6	**13**	0.0	**15**
7	**15.5**	0.0	**13.5**
8	**13.5**	0.0	**15.5**
9	**12**	0.0	**13**
10	0.0	0.0	**16**
11	0.0	0.0	**17**
12	0.0	0.0	**17**
13	0.0	0.0	**13**
14	**12**	0.0	**14**
15	**18**	0.0	**13.3**
16	**13**	0.0	**15.5**
17	0.0	0.0	**18.5**
18	**13.5**	0.0	**15**
Ciprofloxacin	**33**	**33**	**-**
Amphotericin B	**-**	**-**	**22**

Moreover, replacing the chlorine atom at position 2 of compound (**10**), showed also loss of the activity against the selected strain for compound **11,** which this could support the hypothesis that there is a direct relationship between biological activity and the electron withdrawing effect [9]. Similarly, cyclization of compound **11** formed ditriazolo quinoxaline derivative **12**, which also lost the activity as well as the former compound (**11**). Although, the lack of activity remain even after replacing the hydrogen at position one of the traizolo ring (**12)** with the substituent of ethoxy carbonyl methyl (**13)**. Finally, the presence of bromine atom at to position 4 of the phenyl substituents lack the activity of compound **17** comparing to compound **16**.

In addition, as in case of the antifungal assay, all the compounds showed activity against *Candida albicans* ATCC 10231, and according to the SAR study, the key factor is the nature of the quinoxaline and triazolo ring.

4. CONCLUSION

The aim of this work was to synthesize quinoxaline derivatives and to evaluate their *in vitro* activity against selected bacterial and fungal strains. Consequently, 18 compounds were synthesized based on the 2, 3-substituted quinoxaline, 1, 4-substituted traizoloquinoxaline, and 1, 6-substituted ditraizoloquinoxaline derivatives. According to their antifungal activity, all the synthesized compounds showed activity against *Candida albicans* ATCC 10231. However, most of the synthesized compounds showed antibacterial activity against *Staphylococcus aureus* ATCC 25923, but none of them were active against *Escherichia coli* ATCC 25922.

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