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RESEARCH ARTICLE

Design and synthesis of some novel sulfonamide derivatives as potential antimicrobial agents

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Abstract

A series of novel of sulfonamide derivatives were synthesized from N-(4-(N-quinoxalin-2-ylsulfamoyl) phenyl) acetamide 1 and the structure of the newly compounds was confirmed on the basis of elemental analysis and spectral data. All the compounds were screened in vitro for their antibacterial and antifungal activities. Especially, 2-[1-(4-(*N*-quinoxalin-2-ylsulfamoyl) phenyl amino) ethylidene] hydrazine carbothioamide, 4-(2-aminothiazol-4-ylamino)-*N*-(quinoxalin-2-yl) benzene sulfonamide, 3-(4-hydroxy- 3-methoxy phenyl) -*N*-[4- (*N*-quinoxalin- 2yl sulfamoyl) phenyl] acrylamide, 4-[5-cyano-4-(4-hydroxy-3-methoxyphenyl)-6-oxo-1,6-dihydropyridin-2-ylamino]-*N*-(quinoxalin-2-yl) benzene sulfonamide were found to be the most potent compounds against all the tested strains except for *Candida albicans* (RCMB 05036) and *Pseudomonas aeruginoca* (RCMB 010043).

1. Introduction

Due to the resistance of pathogenic bacteria toward available antibiotics is rapidly become major worldwide problem; the design of new class of chemical moieties to deal with resistance bacteria has become one of the important areas of antibacterial research today. In addition, fungal infections continue to increase rapidly because of the increased number of immune compromised patients. As known, not only biochemical similarity of the human cell and fungi forms a handicap for selective activity, but also the easily gained resistance is the main problem noticed in developing safe and efficient antifungal. So, it is necessary to design a new kind of antimicrobial and antifungal agents. Sulfonamides have a variety of biological activities such as; antibacterial **[1]**, insulin releasing **[2],** carbonic anhydrase inhibitory **[3, 4]**, anti-inflammatory **[5]** and antitumor activities **[6-11]**. In view of these findings the present work deals with the design and syntheses of some novel heterocyclic sulfaquinoxaline derivatives by substituting the amino group by different biologically active moieties (e.g. thiazole, pyrazole, chalcone and pyridine) to evaluate their antibacterial and antifungal activities. Thus, synthesis of the pyridine ring system and its derivatives occupy an important place in the realm of synthetic organic chemistry, due to their therapeutic and pharmacological properties **[12-14]**. Also, they have emerged as integral backbones of huge number of existing drugs **[15, 16]**. In addition, they have reported to possess biological activities such as antimicrobial **[17-19]**, anti-inflammatory **[20]** and neurotropic activity **[21]**. Also, the pyrazole ring is a prominent structural motif found in numerous pharmaceutically active compounds. Due to the easy preparation and rich biological activity, pyrazole framework plays an essential role in biologically active compounds and therefore represents an interesting template for combinatorial **[22, 23]** as well as medicinal chemistry. Many of these heterocyclic compounds exhibiting an antiviral/ antitumor **[24, 25]**, antibacterial **[26-28]**, anti- inflammatory **[29]**, and anti-hyperglycemic activity **[30, 31]**.

2. Experimental

2.1. **Chemistry**

All melting points are uncorrected and were determined on a Stuart melting point apparatus. IR spectra were recorded on a Shimadzu-440 IR spectrophotometer using the KBr technique (Shimadzu, Japan).^{1}H NMR spectra were measured on a Varian Mercury VX-300 NMR spectrometer in DMSO-d_6 as a solvent and were run at 300 MHz, using tetramethylsilane (TMS) as an internal standard. The mass spectra were recorded on Shimadzu GCMS-QP1000EX mass spectrometers at 70 eV. The purity of the synthesized compounds was monitored by TLC. Elemental analyses were carried out by the Micro-analytical Research Centre, Faculty of Science - Cairo University. All compounds were within ±0.4 of the calculated values.

_N_-[4-(_N_-quinoxalin-2-ylsulfamoyl) phenyl] acetamide (1).

A solution of sulfaquinoxaline (0.01 mol) in acetic anhydride (10 ml) was refluxed for 1h. The reaction mixture leaves to overnight then ppt. dissolved in ethanol and poured into crushed ice. The solid product was collected and recrystallized from ethanol to give **1**. Yield (90%), m.p. 210-214°C.; IR (KBr, cm^{-1}): 3446 (NH), 2923 (CH-Aliph.), 1695 (C=O) and 1362, 1136 (SO_2). M/S m/z (%) 342 (17.09), 278(100).^{1}HNMR (300MHz, DMSO-d_6): 2.04 (s, 3H, CH_3), 7.57-8.04 (m, 9H, Ar-H+ CH-quinoxaline), 8.61 (s, 1H, $NHSO_2$) and 10.28 (s, 1H, NH). MS m/z (%): 342(17.09), 278 (100). Anal. Calcd. for: $C_{16}H_{14}N_4O_3S$: C, 56.13; H, 4.12; N, 16.36. Found: C, 55.73; H, 4.02; N, 16.06%.

2-[1-(4-(_N_-quinoxalin-2-ylsulfamoyl) phenylamino) ethylidene] hydrazinecarbothio amide (**2**).

Equimolar amounts of compound **1** (0.01 mol), thiosemicarbazide (0.01 mol) and a few drops of conc. HCl in ethanol (30 ml) was refluxed for 3h. The solid product which produced on heating was collected and recrystallized from acetic acid as yellow crystals to give **2**. Yield (70%); m.p. 260 ^{0}C; IR (KBr, cm^{-1}): 3438, 3358, 3167(NH_2, NH), 2966, 2829 (CH-Aliph.), 1233 (C=S) and 1312, 1151(SO_2). ^{1}HNMR (300MHz, DMSO-d_6): 2.06 (s, 3H, CH_3), 5.34(s, 2H, NH_2), 6.44-7.76 (m, 10H, Ar-H+2NH), 8.04(s, 1H, CH-quinoxaline) and 8.48 (s, 1H, $NHSO_2$). MS m/z (%): 415(15.3), 101 (100). Anal. Calcd. for : $C_{17}H_{17}N_7O_2S_2$:C, 49.14; H, 4.12; N, 23.60. Found: C, 48.96; H, 3.92; N, 23.20%.

_N_'-(4-oxo-4, 5-dihydrothiazol-2-yl)-_N_-[4-(_N_-quinoxalin-2-ylsulfamoyl)phenyl]acetohydrazonamide (3).

A mixture of compound **1** (0.01 mol), chloro acetyl chloride (0.01 mol), anhydrous sodium acetate (0.04 mol) and absolute ethanol was refluxed for 4h. The solid product was cooled and collected by filtration, washed with water, dried, and recrystallized from a mixture of EtOH-DMF (1:1) to give compound **3**.Yield (48%); m.p. 340 ^{0}C; IR (KBr, cm^{-1}): 3229 (NH), 2959, 2829 (CH-Aliph.), 1629 (C=O), 1598(C=N) and117,1304 (SO_2). ^{1}H NMR (300MHz, DMSO-d_6): 2.08 (s, 3H, CH_3), 3.7(s, 2H, CH_2-thiazol), 6.69-7.75 (m, 13H, Ar-H+NH), 8.55 (s, 1H, CH-quinoxaline) and 9.12 (s, 1H, $NHSO_2$). MS m/z: 455(12.3), 226 (100%). Anal. Calcd. for : $C_{19}H_{17}N_7O_3S_2$. : C, 50.10; H, 3.76; N, 21.52. Found: C, 49.80; H, 3.56; N, 21.42%.

4-(2-aminothiazol-4-ylamino)-_N_-(quinoxalin-2-yl) benzene sulfonamide (4).

A mixture of compound **1** (0.01 mol), thiourea (0.02 mol) and iodine (0.01 mol) was heated on a steam bath for 4 h. The hydro iodide separated, was filtered, washed with ether and dried. It was dissolved in hot water, filtered while hot and the clear solution neutralized with a strong solution of ammonia. The solid separated was filtered, washed with water and recrystallized from ethanol to give **4** as yellow crystals.Yield (65%); m.p.250-260 ^{o}C; IR (KBr,cm^{-1}): 3438, 3360 (NH_2), 3253(NH), 2838 (CH-Aliph.), 1638 (C=N) and 1188, 1312(SO_2). ^{1}H NMR (300MHz, DMSO-d_6): 6.04 (s, 2H, NH_2), 7.60- 7.93 (m, 14H, Ar-H+CH-thiazole+ CH-quinoxaline), 8.55(s, 1H, $NHSO_2$) and 11.4(s, 1H, NH). MS, m/z (%): 398 (22), 277 (100). Anal. Calcd. for : $C_{17}H_{14}N_6O_2S_2$: C, 51.24; H, 3.54; N, 21.09. Found: C, 51.04; H, 3.44; N, 21.12%.

4-[6-amino-4-(4-chlorophenyl)-5-cyanopyridine-2-ylamino]-_N_-(quinoxalin-2-yl)benzenesulfonamide(5a).4-[6-amino-5-cyano-4-(4-hydroxy-3-methoxyphenyl)pyridin-2-ylamino]-_N_-(quinoxalin-2-yl)benzenesulfonamide (5b).

To a mixture of compound **1** (0.01mol) and the appropriate arylidene, namely; 2-(4-chlorobenzylidene) malono-nitrile and 2-(4-hydroxy -3-methoxy benzylidene) malononitrile (0.01mol) in ethyl alcohol (20 ml) and amm. acetate

(0.015 mol) were added. The reaction mixture was refluxed for 4 h. The obtained solid was filtered off, washed with absolute ethyl alcohol and recrystallized from ethyl alcohol to give the desired compounds **5a, 5b** respectively.

5a- Yield (76%); m.p. 140 ^{o}C; IR (KBr, cm^{-1}): 3426, 3381(NH_2), 3110 (NH), 2225 (C≡N), 1611(C=N) and 1352,1154 (SO_2).^{1}H NMR (300MHz, DMSO-d_6): 4.96 (s, 2H, NH_2), 7.61–8.02 (m, 19H, Ar-H +CH quinoxaline), 8.55 (s, 2H, $NHSO_2$ + CH- pyridine) and 10.29 (s,1H, NH) . MS, m/z (%): 527 (9.2), 276 (100). Anal. Calcd. for : $C_{26}H_{18}N_7O_2SCl$: C, 59.15; H, 3.44; N, 18.57. Found: C, 58.75; H, 3.24; N, 18.27%.

5b- Yield (60%); m.p. 130 ^{o}C; IR (KBr, cm^{-1}): 3432 (OH, NH_2 brs.), 3118 (NH), 3073 (CH-Ar.), 2228 (C≡N) 1661(C=N) and 1354,1161 (SO_2).^{1}H NMR (300 MHz, DMSO-d_6): 3.8 (s, 3H, OCH_3), 5.5 (s, 2H, NH_2),7.49–7.93 (m, 19H, Ar-H +NH), 8.01 (s,1H, CH-quinoxaline), 8.28 (s, 2H, $NHSO_2$+ CH- pyridine) , and 10.8 (s,1H, OH) . MS, m/z (%): 539 (11.40), 271 (100). Anal. Calcd. for: $C_{27}H_{21}N_7O_4S$: C, 60.10; H, 3.92; N, 18.17. Found: C, 60.33; H, 3.82; N, 18.07%.

2-Oxo-*N*-[4-(quinoxalin-2-ylsulfamoyl) phenyl] succinamat ethylester (6).

To a stirred solution of **1** (0.01 mol) and diethyl oxalate (0.04 mol) was added drop wise a solution of sodium ethoxide (0.02 mol). The reaction mixture was refluxed for 5 h, cooled and then acidified with acetic acid (3%). The solid separated was collected and crystallized from dioxane to give **6** as red crystals. Yield, 48 %; m.p. 260 ^{0}C; IR, (KBr, cm^{-1}): 3301, 3186 (2NH), 3055 (CH-Ar.), 2862 (CH-Aliph.), 1782 (C=O ,α- keto ester), 1678 (B-diketon ester) and 1310,1153 (SO_2).^{1}H NMR (300MHz, DMSO-d_6): 1.71 (t, 3H, CH_3), 3.6 (s, 2H, -$COCH_2CO$-), 4.3 (q, 2H, CH_2-ester) , 6.9-8.01 (m, 12H, Ar-H+ CH quinoxaline), 8.54 (s, 1H, $NHSO_2$), 9.02(s, 1H, NH). MS, m/z (%): 442 (5.27), 90 (100). Anal. Calcd. for: $C_{20}H_{18}N_4O_6S$: C, 54.29; H, 4.10; N, 12.66. Found: C, 53.99; H, 4.06; N, 12.56.

2-[1-(4-(*N*-quinoxalin-2-ylsulfamoyl) phenylamino) ethylidene]-2-phenylhydrazine (8).

A mixture of **1** (0.01 mol) and phenyl hydrazine (0.01 mol) in glacial acetic acid (10 ml) was refluxed for 2 h. After cooling, the reaction mixture was poured onto water (50 ml) and the solid that formed was filtered off, washed with water, dried and recrystallized from absolute ethanol to give **8**. Yield 85 %, m.p. 250 ^{o}C. IR (KBr, cm^{-1}): 3265, 3184 (2NH), 3067(CH-Ar.), 1681 (C=N) and 1368, 1143(SO_2). MS, m/z (%): 432 (11), 72 (100). Anal. Calcd. for: $C_{22}H_{20}N_6O_2S$: C, 61.10; H, 4.66; N, 19.43. Found: C, 61.49; H, 4.56; N, 19.22%.

4-(4-formyl-1-phenyl-1*H*-pyrazole-3-ylamino)-*N*-(quinoxalin-2-yl) benzenesulfonamide (9).

To a solution of compounds **8** (0.01 mol) in dimethylformamid (15 ml), phosphorus oxychloride (0.03 mol) was added drop wise at 0^{o}C while stirring. After complete addition of $POCl_3$, the reaction mixture was left to stir for 15 h, and then poured onto ice-water (20 ml). The solid that formed was filtered off, air dried and recrystallized from absolute ethanol to give **9**. Yield 70 %, m.p. 280^{o}C. IR (KBr, cm^{-1}): 3405 (NH), 2708-2831(CH- Ald.), 1710 (C=O), 1604 (C=N) and 1363,1179 (SO_2).^{1}H NMR (300MHz, DMSO-d_6): 6.54 (s, 1H, CH-pyrazole), 7.01-7.91(m, 19H, Ar–H+NH), 8.04(s, 1H, CH-quinoxaline), 8.61(s, 1H, $NHSO_2$), 10.1 (s, 1H, CHO). MS, m/z (%): 470 (16.65), 372 (100). Anal. Calcd. for: $C_{24}H_{18}N_6O_3S$: C, 61.27; H, 3.86; N, 17.86. Found: C, 61.11; H, 3.79; N, 17.66%.

3-(4-hydroxy-3-methoxyphenyl)-*N*-[4-(*N*-quinoxalin-2ylsulfamoyl) phenyl] acrylamide (10).

A mixture of 4-hydroxy-3-methoxy benzaldehyde (0.01 mol) and N-(4-(N-quinoxalin-2-ylsulfamoyl) phenyl) acetamide **1** (0.01 mol) in ethanol (30 ml) and 10% ethanolic potassium hydroxide solution (10 ml) was refluxed for 3h. Then the hot mixture was filtered off. After cooling the filtrate was diluted with (50 ml) of water, the resulting precipitate was filtered off and recrystallized from ethanol to give **10** as yellow crystals. Yield (85%), m.p. 130 °C; IR (KBr, cm^{-1}): 3486 (OH), 3248, 3177(2NH), 3068 (CH-Ar.), 2843(CH-Aliph.), 1679 (C=O), 1591(C=C) and 1315, 1163 (SO_2) .^{1}H NMR (300 MHz, DMSO-d_6): 3.72 (s, 3H, OCH_3), 7.60-8.02 (m, 20H, Ar-H and CH=CH) , 8.33 (s, 1H, CH- quinoxaline), 8.57 (s, 1H, $NHSO_2$), 9.75(s, 1H, NH) and 10.24 (s, 1H, OH). MS, m/z (%): 476 (20), 280(100). Anal. Calcd. for : $C_{24}H_{20}N_4O_5S$: C, 60.49; H, 4.23; N, 11.76. Found: C, 60.33; H, 4.19; N, 11.65 %.

4-[5-(4-hydroxy-3-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-ylamino]-*N*-(quinoxalin-2-yl)benzenesulfonamide (11) .

A mixture of chalcone **10** (0.01 mol) hydrazine hydrate 95% (0.02 mol) in ethyl alcohol (30 ml) and few drops of piperidene was refluxed for 8h. The reaction mixture was cooled and the formed precipitate was filtered off, washed and recrystallized from ethyl alcohol to give **11**. Yield 73%, m.p. 180^{o}C; IR (KBr, cm^{-1}): 3353 (OH, brs.), 3296, 3259 (2NH), 1669 (C=N) and 1301,1095 (SO_2) .^{1}H NMR (300MHz, DMSO-d_6): 3.73 (s, 3H, OCH_3), 3.86 (dd, 1H,

C_4-H of pyrazoline), 4.40(dd,1H, C_4-H of pyrazoline), 6.4 (dd, 1H, C_5-H of pyrazoline) , 7.21-7.84(m, 18H, Ar-H), 8.33 (s, 1H, CH-quinoxaline), 8.57(s, 1H. $NHSO_2$), 10.29 (s, 1H, NH) and 11.82 (s, 1H, OH). MS, m/z (%): 490 (15.01), 277 (100). Anal. Calcd. for: $C_{24}H_{22}N_6O_4S$: C, 58.76; H, 4.52; N, 17.13. Found: C, 58.45; H, 4.48; N, 17.03%.

3-[5-(4-hydroxy-3-methoxyphenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazol-3-ylamino]-*N*-quinoxalin-2-ylbenzene sulfonamide(12).

A mixture of the chalcone **10** (0.01 mol), phenyl hydrazine (0.01 mol) in ethyl alcohol (30 ml) and anh. sod. acetate was refluxed for 4-6h. The reaction mixture was cooled and the formed precipitate was filtered off, washed and recrystallized from ethyl alcohol to give the desired compound **12**. Yield, 87%, m.p. 228–230 ^{0}C: IR (KBr, cm^{-1}): 3432 (OH, 2NH brs.), 3010 (CH-Ar), 2928 (CH-Aliph.), 1639 (C=N) and1315, 1134 (SO_2).^{1}H NMR (300MHz, DMSO-d_6): 3.72 (s, 3H, OCH_3), 4.04 (dd, 1H, C_4-H for pyrazoline), 4.84 (dd, 1H, C_4-H for pyrazoline), 6.4 (dd, 1H, C_5-H for pyrazoline), 6.66 -7.91 (m, 25H, Ar-H+NH), 8.02(s, 1H, CH-quinoxaline), 8.49 (s, 1H, $NHSO_2$), 10.05 (s, 1H, OH). MS, m/z (%): 566 (20), 94 (100). Anal. Calcd. for: $C_{30}H_{26}N_6O_4S0$: C, 63.59; H, 4.62; N, 14.83. Found: C, 63.42; H, 4.22; N, 14.63.

4-[1-acetyl-5-(4-hydroxy-3-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-ylamino]-*N*-(quinoxalin-2-yl)benzene sulfonamide (13).

A mixture of chalcone **10** (0.01 mol) and hydrazine hydrate 99% (0.02 mol) in amixture of acetic acid and glacial acetic acid (10 ml) was refluxed for 6-8 h. The reaction mixture was cooled and diluted with water; the formed precipitate was filtered off, washed and recrystallized from ethyl alcohol to give compound **14**. Yield, 80 %, m.p. 220 ^{0}C: IR (KBr, cm^{-1}): 3328 (OH, NH brs.), 3112(NH), 3011(CH-Ar.), 2941(CH-Aliph.), 1699 (C=O), 1591 (C=N) and 1322, 1143(SO_2). ^{1}H NMR (300MHz, DMSO-d_6): 2.40 (s, 3H, $COCH_3$), 3.19 (s, 3H, OCH_3), 3.8 (dd,1H, C_4-H for pyrazoline), 4.04(dd, 1H, C_4-H for pyrazoline), 6.55 (dd, 1H, C_5-H for pyrazoline), 7.57–8.04 (m,19H, Ar-H+CH-quinoxaline), 8.56 (s,1H, $NHSO_2$), 9.65 (s,1H,NH) and 10.29 (s,1H,OH). MS, m/z (%): 532 (23), 175 (100). Anal. Calcd. for: $C_{26}H_{24}N_6O_5S$: C, 58.64; H, 4.54; N, 15.78. Found: C, 58.38; H, 4.44; N, 15.87%.

5-(4-hydroxy-3-methoxyphenyl)-3-[4-(quinoxalin-2-ylsulfamoyl) phenylamino]-4,5-dihydropyrazol-1-carbo-thioamide(14).

A mixture of chalcone **10** (0.01mol) in absolute ethyl alcohol (20 ml) and thiosemicarbazide (0.01mol) in glacial acetic acid (5 ml) was refluxed for 8h. The reaction mixture was cooled and poured onto crushed ice; the formed solid was filtered off, washed and crystallized from ethyl alcohol to give the title compounds **14**. Yield, 82 %, m.p. 225–230 ^{0}C: IR (KBr, cm^{-1}): 3371 (OH, brs.), 3262, 3178 (NH_2), 1591(C=N), 1321 (C=S) and 1297, 1143(SO_2). ^{1}H NMR (300MHz, DMSO-d_6): 3.34 (dd, 1H, C_4-H for pyrazoline), 3.8 (s, 3H, OCH_3), 4.4 (dd,1H, C_4-H for pyrazoline), 6.41 (dd,1H, C_5-H for pyrazoline), 6.90–8.01 (m, 20H, Ar-H+NH_2), 8.05 (s,1H, CH-quinoxaline), 8.61-(s,1H, $NHSO_2$), 9.4 (s, 1H, NH) and 10.29 (s, 1H, OH). MS, m/z (%): 548 (20), 300 (100). Anal. Calcd. for: $C_{25}H_{23}N_7O_4S_2$: C, 54.63; H, 4.21; N, 17.83. Found: C, 54.48; H, 4.11; N, 17.62%.

***N*-Hydroxy-3-(4-hydroxy-3-methoxyphenyl)-*N*-[4-(quinoxalin-2-ylsulfamoyl) phenyl] acrylamidine (15).**

A mixture of compound **10** (0.01 mol), hydroxylamine hydrochloride (0.01 mol) and potassium hydroxide (0.01 mol) in ethanol (15 ml) was refluxed for 6-8 h, then cold and poured onto ice/water and neutralized with dilute hydrochloric acid. The precipitated was filtered and recrystallized from ethanol to give **15**.Yield, 84 %; m.p. 240 ^{0}C. IR (KBr, cm^{-1}): 3432 (OH broad), 3273 (2NH), 3015 (CH- Ar.), 2926, 2829 (CH- Aliph.) and 1318, 1142 (SO_2). ^{1}H NMR (300MHz, DMSO-d_6): 2.3(s, 1H, NOH), 3.76(s, 3H, -OCH_3), 6.43 (dd, 1H, CH=), 6.77 (dd, 1H, =CHC_6H_4), 6.93- 7.97 (m, 19H, Ar-H+NH), 8.03(s, 1H, CH- quinoxaline), 10.08 (s, 1H, $NHSO_2$) and 10.42 (s, 1H, OH). MS, m/z (%): 491 (30), 72 (100). Anal. Calcd. for : $C_{24}H_{21}N_5O_5S$: C, 58.65; H, 4.31; N, 14.25. Found: C, 58.44; H, 4.20; N, 14.05 %.

4-[5-cyano-4-(4-hydroxy-3-methoxyphenyl)-6-oxo-1,6-dihydropyridin-2-ylamino]-*N*-(quinoxalin-2-yl)benzene sulfonamide (16).

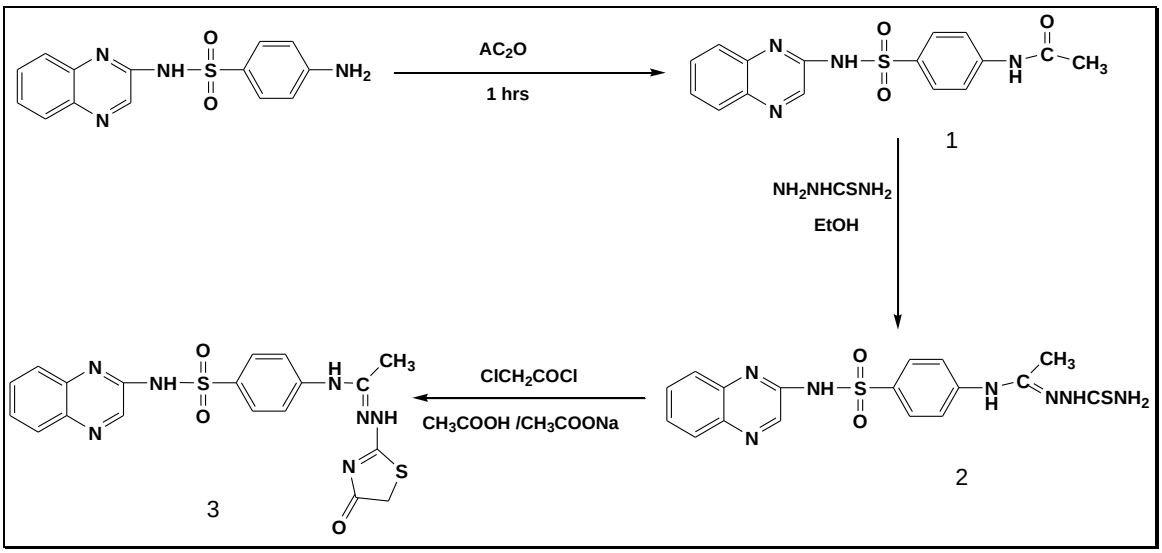
An ethanolic mixture of chalcone **10** (0.01 mol), and ethyl cyano acetate (0.01 mol) in the presence of ammonium acetate (0.02 mol) was refluxed for 5–6 h, after cooling, the obtained solid was filtered off, washed with ethyl alcohol and recrystallized from ethyl alcohol to give compound **16**. Yield, 78%; m.p. 245-250 ^{0}C; IR (KBr, cm^{-1}):

3439 (OH, brs.), 3337, 3113 (2NH), 2050 (C≡N) and 1695(C=O).^{1}H NMR (300MHz, DMSO-d_6): 3.31 (s, 3H, OCH_3), 6.89 (s, 1H, pyridine proton),7.61–8.01 (m, 13H, Ar-H+ NH-pyridine), 8.04(s,1H, CH-quinoxaline), 8.58 (s,1H, $NHSO_2$) and 10.29 (s ,1H, OH). MS, m/z (%): 540 (10), 278 (100). Anal. Calcd. for: $C_{27}H_{20}N_6O_5$ S: C, 59.99; H, 3.73; N, 15.55. Found: C, 59.71; H, 3.61; N, 15.25%.

3. Results and discussion

3.1. Chemistry

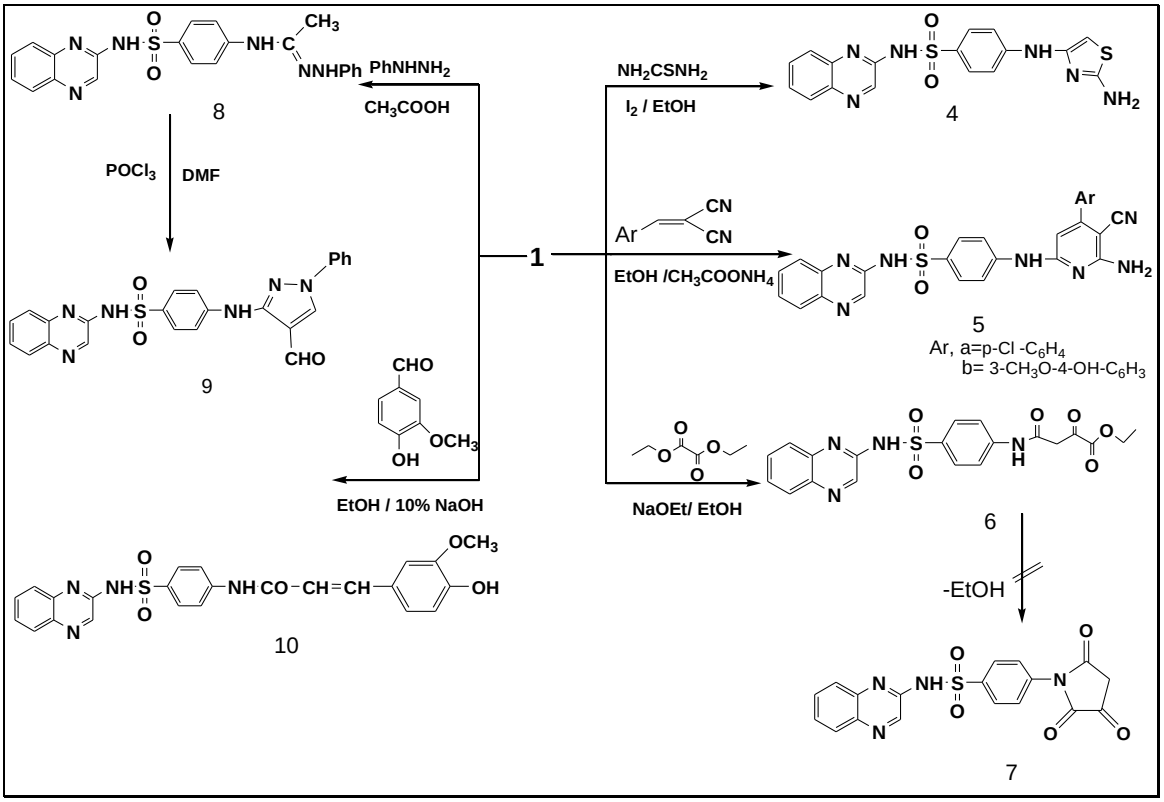
The synthetic route for the preparation of the target compounds is illustrated in (Scheme 1–4). In (Scheme 1), the starting compound N-(4-(N-quinoxalin-2-ylsulfamoyl) phenyl) acetamide **1** was prepared by acetylating of sulfaquinoxaline with acetic anhydride in excellent yield. Structure of the product **1** was confirmed on the basis of its correct elemental analysis and spectral data. IR spectra of compound **1** revealed the presence of characteristic bands for carbonyl functional group and disappearance NH_2.



(Scheme 1)

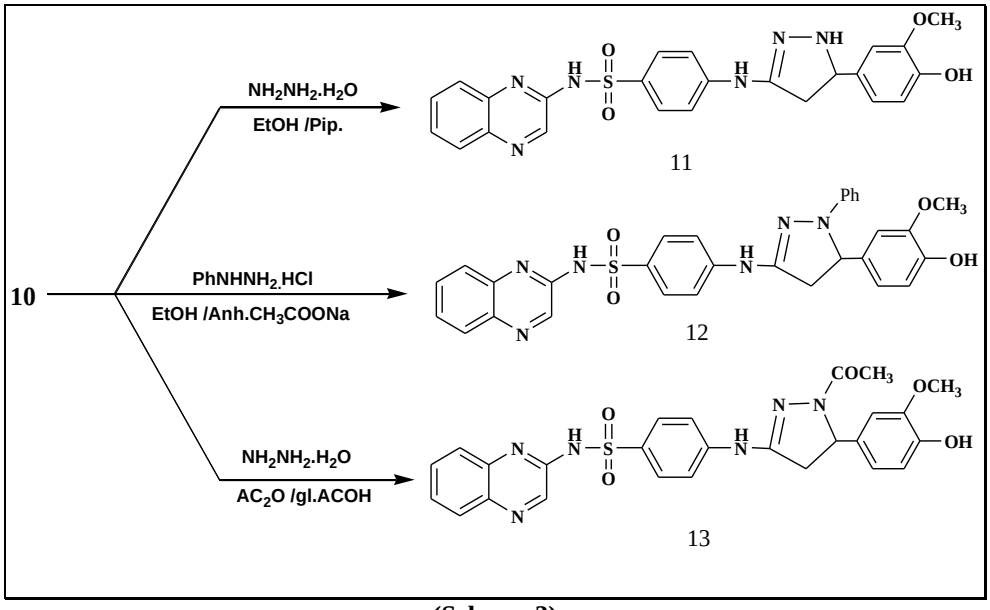
Thiosemicarbazone derivative **2** was obtained via condensation of acetyl sulfaquinoxaline **1** with thiosemicarbazide in ethanol as the solvent affording in satisfactory yield (70%). On the other hand reaction of thiosemicarbazone derivative **2** with chloro acetyle chloride afforded the corresponding 5-thiazolidinone derivative **3** (Scheme 1).

The molecular structure of compound **3** was established on the basis of its elemental analysis and spectral data. The infrared spectrum of **3** revealed the presence of characteristic bands for carbonyl functional group and disappearance the amino group. Furthermore, 4-(5-aminothiazol-2-ylamino)-N-(quinoxalin-2-yl) benzenesulfonamide **4** was obtained via the reaction of acetyl sulfaquinoxaline derivative **1** with thiourea in presence of iodine **[32]**. Compound **4** was characterized by its elemental analysis and spectral data. In contrast to the behavior of acetyl sulfaquinoxaline **1** towards arylidene malononitrile, acetyl sulfaquinoxaline **1** reacted with 2-(4-chlorobenzylidene) malononitrile and 2-(4-hydroxy-3-methoxybenzylidene) malononitrile in ethanol and amm. acetate to yield pyridine derivative **5a, 5b** respectively. Elemental analysis and spectral data are in full agreement with the proposed structure **5a, 5b** (See Experimental Section). In the present study the Claisen condensation at this stage, it was attempted to prepare the proposed pyrrolidine -2, 3, 5-trione 7 derivatives of sulfaquinoxaline by the Claisen condensation of 4-acetyl sulfaquinoxaline **1** with diethyl oxalate in molar ratio 1:1 in the presence of sodium ethoxide. Surprisingly, 2-Oxo-*N*-[4-(quinoxalin-2-ylsulfamoyl) phenyl] succinamat ethylester **6** was obtained instead. The structure of such unexpected compound **6** was substantiated on basis of their IR, Mass and ^{1}H NMR spectral data. Condensation reaction of compound **1** with phenyl hydrazine in acetic acid at 25 ^{0}C provided the corresponding hydrazones **8 [33]** (Scheme 2). Vilsmeier–Haack reaction of the latter hydrazone **8** using 2.5 equivalent moles of Vilsmeier reagent (DMF/ $POCl_3$) preformed double addition of reagent on the methyl group to afford ultimately after hydrolysis, the cyclize pyrazole-4-carboxaldehyde derivative **9** with good yield (Scheme 2).



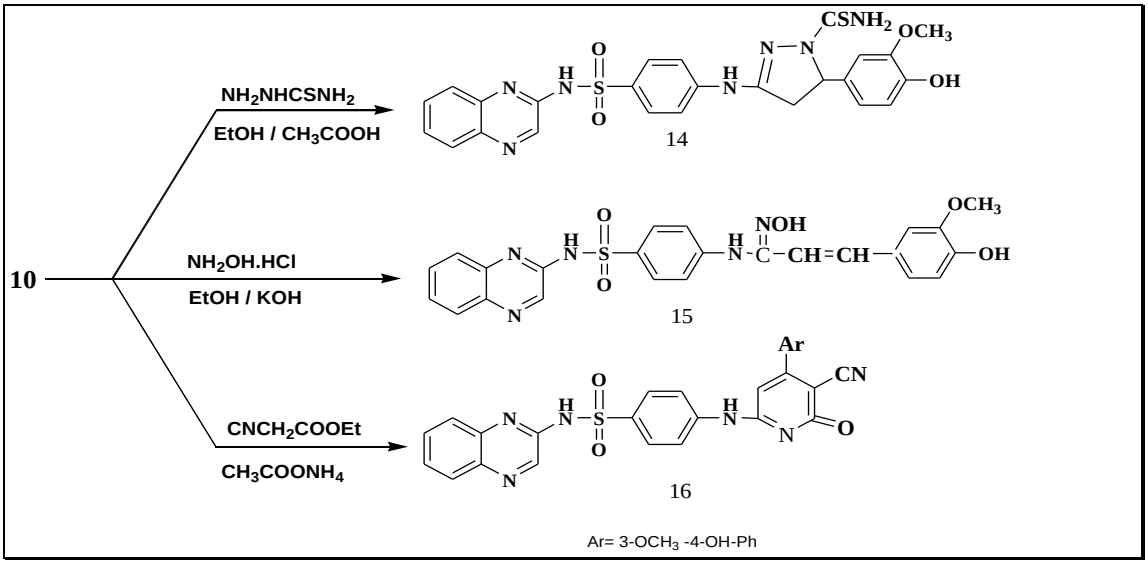
(Scheme 2)

The second pathway was adopted to design and synthesize sulfonamide chalcone for structure activity relationship (SAR) studies in antimicrobial activities. The chalcone **10** was prepared by the Claisene-Schmidt condensation between acetylsulfaquinoxaline **1** and aromatic aldehyde in the presence of potassium hydroxide in ethanol (Scheme 3). Structure of the sulfonamide chalcone **10** was confirmed on the basis of its correct elemental analysis and spectral data. In the present work, Hetero cyclization of the appropriate hydrazine's with electrophilic species such as sulfonamide chalcone afforded the corresponding pyrazoline; cyclo condensation of chalcone **10** with hydrazine hydrate in ethyl alcohol in basic medium gave pyrazoline **11**. Similarly, N-Phenylpyrazoline **12** was also prepared from chalcone **10** and phenylhydrazine (Scheme 3). Also, N-acetyl pyrazoline **13** were obtained by refluxing the key chalcone **10** with hydrazine hydrate in glacial acetic acid.



(Scheme 3)

Moreover, the compound **14** were prepared by refluxing a mixture of chalcone **10** and thiosemicarbazide in ethyl alcohol in the presence of glacial acetic acid (Scheme 4). The treatment of chalcone **10** with hydroxylamine hydrochloride in absolute ethanol containing KOH afforded the oxime derivative **15 [34-36]**. In addition, for treatment of chalcone **10** with ethyl cyanoacetate in presence of absolute ethanol containing amm. acetate afforded the pyridinone derivative **16** (Scheme 4).



(Scheme 4)

4. Antimicrobial activity:

4.1. Antifungal activity

Screening of antimicrobial activity was performed at a Microbiology Lab in Faculty of Agriculture, Al-Azhar University - Cairo, Egypt. The newly synthesized compounds were screened separately in vitro for their antifungal activity against four fungal species, namely *Aspergillus fumigatus* (RCMB 02568), *Geotrichum candidum* (RCMB 05097), *Candida albicans* (RCMB 05036) and *Syncephalastrum racemosum* (RCMB 05922) on Sabouraud dextrose agar plates. The culture of fungi was purified by single spore isolation technique. The antifungal activity was determined by agar well diffusion method **[37].** The test was performed three times for each fungus. Amphotericin B was used as reference to evaluate the potency of the tested compounds under the same conditions. Zones of inhibition were determined for **1, 2, 3, 4, 5a, 5b, 6, 9, 10, 12, 13, 14** and **16** the results were summarized in Table 1. An examination of the data in Table 1 it is revealed that most of compounds showed moderate to good inhibition zone.

Table 1: Antifungal activity data of chemical substances tested.

Compound NO.	Inhibition zone diameter			
	Aspergillus fumigatus (RCMB 02568)	Syncephalastrum racemosum (RCMB 05922)	Geotricum candidum (RCMB 05097)	Candida albicans (RCMB 05036)
1	15.5± 1.5	14.2 ± 0.25	17.3 ±1.5	NA
2	19.3± 1.2	17.2 ± 0.63	20.1 ±0.58	NA
3	NA	NA	NA	NA
4	20.3 ± 1.2	19.2 ±0.25	21.4 ±0.63	NA
5a	16.3 ± 0.63	14.6 ± 0.58	14.6 ± 0.58	NA
5b	NA	NA	NA	NA
6	15.3 ± 1.2	14.2 ± 0.25	20.4 ± 1.2	NA
9	18.2 ± 0.58	16.3 ±0.58	16.4 ±1.5	NA
10	23.5 ± 1.2	21.9 ± 1.5	17.3 ±1.5	NA
12	14.3 ± 1.5	17.1 ± 1.2	17.4 ± 1.2	NA
13	NA	NA	NA	NA
14	14.3 ± 1.5	14.2 ± 0.25	17.3 ±0.63	NA
16	23.5 ± 1.2	22.1 ±0.25	21.9 ± 1.5	NA
Amphotericin B	23.7 ± 1.2	19.7 ±0.1.5	25.4 ±1.5	25.4 ±1.5

Mean zone of inhibition in mm ± Standard deviation beyond well diameter (6 mm) produced on a range of environmental and clinically pathogenic microorganisms using (10 mg/ml) concentration of tested samples and standard using (30 µg/ml). The test was done using the diffusion agar technique, Well diameter: 6.0 mm (100 µl was tested). NA: No activity, data are expressed in the form of mean ± SD.

4.2. Antibacterial

Antibacterial activities were investigated using agar well diffusion method .The activity of tested samples was studied against the *Streptococcus pneumoniae* (RCMB 010010) and *Bacillus subtilis* (RCMB 010067) (as gram positive bacteria) while *Pseudomonas aeruginoca* (RCMB 010043) and *Escherichia coli* (RCMB 010052) (as gram negative bacteria). The test was performed three times for each bacterium culture. *Penicillin G* and *Gentamicin* were used as antibacterial standard drugs **[38].** Inhibition zones were determined for **1**, **2, 3, 4, 5a, 5b, 6, 9, 10, 12, 13, 14** and **16** the results were summarized in Table 2. An examination of the data in Table 2 it is revealed that most of compounds showed moderate to good inhibition zone.

Table 2: Antibacterial activity of chemical substances tested.

Compound No.	Inhibition zone diameter			
	Gram positive bacteria		Gram negative bacteria	
	Streptococcus pneumoniae (RCMB 010010)	Bacillis subtilis (RCMB 010067)	Pseudomonas aeruginosa (RCMB 010043)	Escherichia coli (RCMB 010052)
1	12.6±0.35	14.3±0.25	NA	10.8±0.58
2	21.3±0.44	21.9±0.67	NA	17.3±0.46
3	11.2±0.63	12.3±1.2	NA	10.2±0.37
4	21.3±1.5	22.1±0.42	NA	18.6±0.53
5a	17.2±0.63	19.3±0.53	NA	10.3±0.58
5b	NA	NA	NA	NA
6	17.4±0.58	19.2±0.63	NA	19.6±0.72
9	19.3±1.5	20.8±0.72	NA	16.5±0.58
10	24.2±0.44	28.3±1.2	NA	22.5±0.58
12	15.2±0.44	16.4±0.58	NA	15.2±0.58
13	12.6±0.34	14.3±0.25	NA	10.8±1.5
14	12.6±1.2	14.2±0.53	NA	14.9±0.72
16	25.2±0.44	28.9±1.2	NA	22.6±0.63
Ampicillin	23.8±1.2	32.4±0.72	NA	NA
Gentamicin	NA	NA	17.3±0.63	19.9±0.58

Mean zone of inhibition in mm± Standard deviation beyond well diameter (6 mm) produced on a range of environmental and clinically pathogenic microorganisms using (10 mg/ml) concentration of tested samples and standard using (30 µg/ml). The test was done using the diffusion agar technique, Well diameter: 6.0 mm (100 µl was tested). NA: No activity, data are expressed in the form of mean ±SD.

4.3. Minimum inhibition concentration

The agar plate method was used to determine the minimum inhibition concentration (MIC) of tested samples, The MIC was considered to be the lowest concentration that completely inhibits against inoculums comparing with the control, disregarding a single colony or a faint haze caused by the inoculums. Whereas, the good activity of the newly synthesized compounds especially **2, 4, 10 and 16** against antimicrobial activity, so the minimum inhibition concentration (MIC) were determined for these compounds, the results were depicted in Table 3.

Table 3: Antimicrobial Activity as MICS (µg/ml) of tested samples against tested microorganisms.

Compound No.	Minimum inhibitory concentration (µg/ml).							
	Antifungal				Antibacterial			
					Gram Positive Bacteria		Gram negative Bacteria	
	Aspergillus fumigatus (RCMB 02568)	Geotricum candidum (RCMB 05097)	Candida albicans (RCMB 05036)	Syncephalastrumracemosum (RCMB 05922)	Streptococcus pneumoniae (RCMB 010010)	Streptococcus pneumoniae (RCMB 010010)	Pseudomonas aeruginosa (RCMB010043)	Escherichia coli (RCMB 010052)
2	0.98	0.98	NA	0.98	0.49	0.24	NA	3.93
4	0.98	0.49	NA	0.98	0.49	0.24	NA	3.9
10	0.49	0.24	NA	1.95	0.49	0.24	NA	0.49
16	0.98	0.24	NA	0.98	0.24	0.24	NA	0.49

5. Conclusion

The investigation of antifungal and antibacterial screening data revealed that all the tested compounds, **1-16** showed moderate to good inhibition in DMSO. The structures of the newly synthesized compounds were confirmed by spectral data and elemental analyses. These preliminary results of biological screening of the tested compounds could offer an encouraging framework in this field that may lead to the discovery of novel antimicrobial agent.

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