

Antimicrobial Evaluation of Novel Pyrrole, Pyrazole, Pyrimidine and Pyrrolo [2, 3-d]-Pyrimidine Derivatives Bearing Sulfonamide Moiety

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Abstract: Novel pyrrole **5** and **6**, pyrrolopyrimidine **7-10**, pyrazole **14** and **15** or pyrimidine **16** and **17** derivatives bearing biologically active sulfonamide moiety were synthesized and tested for their antimicrobial activity. The synthesized compounds possessed antibacterial and antifungal activities with MIC ranging from 4–256 µg/mL. The most resistant species was *Aspergillus flavus*, while the most sensitive were *Aspergillus fumigatus* and *Penicillium chrysogenum*. The results of the antimicrobial screening showed that all the tested compounds possess significant activity and some were found to be more active than the reference drugs used (ciprofloxacin and ciclopiroxolamine).

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1. Introduction:

Nitrogen heterocycles are of special interest as they constitute an important class of natural and non-natural products, many of which exhibit useful biological activities. Pyrazoles and their derivatives exhibit a broad spectrum of biological activities such as antimicrobial [1], anti-inflammatory [2] and antitumor [3] activities. With growing application on their synthesis and bioactivity, chemists and biologists in recent years have directed considerable attention on the research of pyrazole derivatives. Pyrimidines are of chemical and pharmacological interest and compounds containing the pyrimidine ring system have been shown to possess antibacterial [4], antifungal [5], antimalarial [6], anticonvulsant [7] and antitumor [8] activities. Furthermore, pyrrole and pyrrolopyrimidines containing compounds were found to possess several biological activities including antimicrobial activity [9-13]. On the other hand, sulfonamides and their different derivatives are extensively used in medicine due to their pharmacological properties such as antibacterial activity [14, 15]. They interfere with the use of p-aminobenzoic acid (PABA) in the biosynthesis of tetrahydrofolic acid, which is important in both human and bacterial cells, as it is an enzyme cofactor that provides one carbon unit for the synthesis of the pyrimidine nucleic acid bases required for DNA synthesis [16]. In the light of these facts, this paper deals with the synthesis of novel pyrrole, pyrazole, pyrimidine and pyrrolo [2, 3-d] pyrimidine

derivatives bearing sulfonamide moiety, where the nitrogen of the sulfonamide group is substituted by thiazole, pyrimidine or quinoxaline moieties due to the well-documented antimicrobial activity of these biologically active moieties [17-20]. The newly synthesized compounds were evaluated as antimicrobial agents against gram positive and gram negative bacteria and fungi.

2. Material and Methods

Experimental Design:

Chemistry

Melting points are uncorrected and were determined on a Stuart melting point apparatus (Stuart Scientific, Redhill, UK). Elemental analysis (C, H, N) were performed on Perkin-Elmer 2400 analyser (Perkin-Elmer, Norwalk, CT, USA) at the microanalytical laboratories of the Faculty of Science, Cairo University. All compounds were within ±0.4% of the theoretical values. The IR spectra (KBr) were measured on Shimadzu IR 110 spectrophotometer (Shimadzu, Koyoto, Japan), ¹H-NMR spectra were obtained on a Bruker proton NMR-Avance 300 (300 MHz) (Bruker, Munich, Germany), in DMSO-d₆ as a solvent, using tetramethylsilane (TMS) as internal standard. Mass spectra were run on HP Model MS-5988 (Hewlett Pack-ard, Palo, Alto, California, USA). All reactions were monitored by thin layer chromatograph (TLC) using precoated Aluminium sheets Silica gel Merck

60 F254 and were visualized by UV lamp (Merck, Darmstadt, Germany).

4-(2-Oxo-2-phenylethylamino)-N-(substituted-2-yl) benzene-sulfonamide (3, 4).

A mixture of sulfadiazine or sulfaquinoxaline (0.01 mol) and phenacyl bromide (0.01 mol) was refluxed in ethanol for 3 h. the solid obtained was filtered and crystallized from dioxane to give 3 and 4, respectively.

4-(2-Oxo-2-phenylethylamino)-N-(pyrimidin-2-yl) benzene-sulfonamide (3).

Yield%: 95, m.p.: 203-205°C. IR (KBr, cm^{-1}): 3450, 3392 (NH), 3096 (CH arom.), 2965, 2834 (CH aliph.), 1688 (C=O), 1360, 1144 (SO_2). MS (m/z, RI%): 368 (M^+ , 4.2%), 309 (30.8%), 268 (51%), 226 (25.8%), 105 (100%), 77 (26%). $^1\text{H-NMR}$ DMSO- d_6 (ppm): 4.0[s, 1H, NH, exchangeable with D_2O], 3.3[s, 2H, CH_2], 6.5[s, 1H, CH pyrimidine], 7.1-7.8[m, 5H, Ar-H], 7.9, 8.1[2d, 2H, Ar-H AB system], 8.38[d, 2H, 2CH pyrimidin], 8.9[s, 1H, SO_2NH , exchangeable with D_2O]. Analysis Calc. for $\text{C}_{18}\text{H}_{16}\text{N}_4\text{O}_3\text{S}$: C, 58.68; H, 4.38; N, 15.21. Found: C, 58.00; H, 4.43; N, 15.61.

4-(2-oxo-2-phenylethylamino)-N-(quinoxalin-2-yl) benzene-sulfonamide (4).

Yield%: 94, m.p.: 197-198°C. IR (KBr, cm^{-1}): 3372, 3288 (NH), 3060 (CH arom.), 2975, 2883 (CH aliph.), 1684 (C=O), 1328, 1148 (SO_2). MS (m/z): 418 (M^+ , 3.8%), 303 (26.5%), 198 (100%), 105 (76%), 77 (39.1%), 51 (20.6%). $^1\text{H-NMR}$ DMSO- d_6 (ppm): 4.2[s, 1H, NH, exchangeable with D_2O], 3.6[s, 2H, CH_2], 7.2-7.6 [m, 5H, Ar-H], 7.5, 7.7 [2d, 4H, Ar-H AB system], 8.05[s, 1H, CH quinoxaline], 8.2-8.4[m, 4H, 4CH quinoxaline], 8.7[s, 1H, SO_2NH , exchangeable with D_2O]. Analysis Calc. for $\text{C}_{22}\text{H}_{18}\text{N}_4\text{O}_3\text{S}$: C, 63.14; H, 4.34; N, 13.39. Found: C, 63.32; H, 4.01; N, 13.00.

4-(2-Amino-3-cyano-4-phenyl-1H-pyrrol-1-yl)-N-(substituted-2-yl) benzenesulfonamide (5, 6).

A mixture of 3 or 4 (0.01 mol) and malononitrile (0.01 mol) in ethanol (30 ml) containing sodium ethoxide (0,5 g) was refluxed for 3 h, the reaction mixture was cooled, poured into ice water and acidified with dil.HCl. The solid obtained was recrystallized from dioxane to give 5 and 6, respectively.

4-(2-Amino-3-cyano-4-phenyl-1H-pyrrol-1-yl)-N-(pyrimidin-2-yl) benzenesulfonamide (5).

Yield %: 88, m.p.: 188-190°C. IR (KBr, cm^{-1}): 3450, 3338, 3216 (NH, NH_2), 3090 (CH arom.), 2204 (CN), 1380, 1142 (SO_2). MS (m/z): 416 (M^+ ,

100%), 259 (26.5%), 258 (66.1%), 256 (37.3%), 155 (31.1%), 101 (34.1%). $^1\text{H-NMR}$ DMSO- d_6 (ppm): 4.3[s, 2H, NH_2 , exchangeable with D_2O], 6.3[s, 1H, CH-pyrrole], 6.9-7.2[m, 5H, Ar-H], 7.5, 7.9[2d, 2H, Ar-H AB system], 8.3[d, 2H, 2CH pyrimidin], 8.9[s, 1H, SO_2NH , exchangeable with D_2O]. Analysis Calc. for $\text{C}_{21}\text{H}_{16}\text{N}_6\text{O}_2\text{S}$: C, 60.56; H, 3.87; N, 20.18. Found: C, 60.20; H, 3.00; N, 19.01.

4-(2-amino-3-cyano-4-phenyl-1H-pyrrol-1-yl)-N-(quinoxalin-2-yl) benzenesulfonamide (6).

Yield%: 88, m.p.: 183-185°C. IR (KBr, cm^{-1}): 3450, 3334, 3224 (NH, NH_2), 3056 (CH arom.), 2220 (CN), 1378, 1140 (SO_2). MS (m/z, RI%): 466 (M^+ , 40%), 364 (19%), 184 (100%), 108 (33%), 92 (45%), 65 (34%). $^1\text{H-NMR}$ DMSO- d_6 (ppm): 4.5[s, 2H, NH_2 , exchangeable with D_2O], 6.9[s, 1H, CH-pyrrole], 7.2-7.5[m, 5H, Ar-H], 7.7, 7.9[2d, 2H, Ar-H AB system], 8.1[s, 1H, CH quinoxaline], 8.3[m, 4H, 4CH quinoxaline], 8.6[s, 1H, SO_2NH , exchangeable with D_2O]. Analysis calc. for $\text{C}_{25}\text{H}_{18}\text{N}_6\text{O}_2\text{S}$: C, 64.36; H, 3.89; N, 18.01. Found: C, 64.91; H, 3.34; N, 18.20.

4-(4-Oxo-5-phenyl-3H-pyrrolo [2, 3-d] pyrimidin-7(4H)-yl)-N-(substituted-2-yl) benzenesulfonamide (7, 8).

A solution of 5 or 6 (0.01 mol) in formic acid (30 ml) was refluxed for 5 h. The reaction mixture was cooled and then poured onto ice water. The precipitated solid was crystallized from ethanol to give 7 and 8, respectively.

4-(4-Oxo-5-phenyl-3H-pyrrolo [2, 3-d] pyrimidin-7(4H)-yl)-N-(pyrimidin-2-yl) benzenesulfonamide (7).

Yield%: 87, m.p. 185-187°C. IR (KBr, cm^{-1}): 3450, 3392 (NH), 3094 (CH arom.), 1668 (C=O), 1324, 1142 (SO_2). MS (m/z, RI%): 444 (M^+ , 13.6%), 368 (15%), 287 (100%), 267 (17.1%), 152 (35.7%), 64 (32.9%). $^1\text{H-NMR}$ DMSO- d_6 (ppm): 4.0[s, 1H, NH, exchangeable with D_2O], 6.5[s, 1H, CH pyrimidine], 6.9[s, 1H, CH-pyrrole], 7.1-7.3[m, 5H, Ar-H], 7.5, 7.9[2d, 2H, Ar-H AB system], 8.38[d, 2H, 2CH pyrimidin], 8.9[s, 1H, SO_2NH]. Analysis calc. for $\text{C}_{22}\text{H}_{16}\text{N}_6\text{O}_3\text{S}$: C, 59.45; H, 3.63; N, 18.91. Found: C, 59.51; H, 3.90; N, 18.83.

4-(4-oxo-5-phenyl-3H-pyrrolo [2, 3-d] pyrimidin-7(4H)-yl)-N-(quinoxalin-2-yl) benzenesulfonamide (8).

Yield%: 87, m.p.: 274-276°C. IR (KBr, cm^{-1}): 3447, 3392 (NH), 3094 (CH arom.), 1675 (C=O), 1329, 1145 (SO_2). MS (m/z, RI%): 494 (M^+ , 19.3%), 379 (52.6%), 378 (100%), 286 (33.3%), 189 (33.3%), 76 (33.3%). $^1\text{H-NMR}$ DMSO- d_6 (ppm): 4.4[s, 1H,

NH exchangeable with D₂O], 6.9[s, 1H, CH-pyrrole], 7.2-7.5[m, 5H, Ar-H], 7.6, 7.9[2d, 2H, Ar-H AB system], 8.06[s, 1H, CH quinoxaline], 8.1-8.3[m, 4H, 4CH quinoxaline], 8.6[s, 1H, SO₂NH, exchangeable with D₂O]. Analysis calc. for C₂₆H₁₈N₆O₃S: C, 63.15; H, 3.67; N, 16.99. Found: C, 63.56; H, 3.12; N, 16.40.

4-(4-Amino-5-phenyl-7H-pyrrolo [2, 3-d] pyrimidin-7-yl)-N-(substituted-2-yl) benzenesulfonamide (9, 10).

A solution of 5 or 6 (0.01 mol) in formamide (30 ml) was refluxed for 5 h. The reaction mixture was cooled and then poured onto ice water. The precipitated solid was crystallized from dioxane to give 9 and 10, respectively.

4-(4-Amino-5-phenyl-7H-pyrrolo [2, 3-d] pyrimidin-7-yl)-N-(pyrimidin-2-yl) benzenesulfonamide (9).

Yield%: 81, m.p.: >300°C. IR (KBr, cm⁻¹): 3450, 3361, 3224 (NH, NH₂), 3094 (CH arom.), 1336, 1141 (SO₂). ¹H-NMR DMSO-d₆ (ppm): 3.5[s, 2H, NH₂, exchangeable with D₂O], 6.5[s, 1H, CH-pyrimidin], 6.9[s, 1H, CH pyrrole], 7.1-7.3[m, 5H, Ar-H], 7.5, 7.9[2d, 4H, Ar-H AB system], 8.2[d, 2H, 2CH pyrimidine], 8.4[s, 1H, CH pyrimidine], 8.9[s, 1H, SO₂NH, exchangeable with D₂O]. Analysis calc. for C₂₂H₁₇N₇O₂S: C, 59.58; H, 3.86; N, 22.11. Found: C, 59.66; H, 3.09; N, 21.80.

4-(4-amino-5-phenyl-7H-pyrrolo [2, 3-d] pyrimidin-7-yl)-N-(quinoxalin-2-yl) benzenesulfonamide (10).

Yield%: 80, m.p.: 256-258°C. IR (KBr, cm⁻¹): 3450, 3355, 3223 (NH, NH₂), 3080 (CH arom.), 1326, 1147 (SO₂). MS (m/z, RI%): 493 (M⁺, 33.9%), 303 (52.7%), 299 (37.6%), 105 (100%), 78 (30.6%), 64 (40%). ¹H-NMR DMSO-d₆ (ppm): 4.4[s, 2H, NH₂, exchangeable with D₂O], 6.9[s, 1H, CH-pyrrole], 7.2-7.5[m, 5H, Ar-H], 7.6, 7.9[2d, 2H, Ar-H AB system], 8.06[s, 1H, CH quinoxaline], 8.1-8.3[m, 4H, 4CH quinoxaline], 8.4[s, 1H, CH pyrimidine], 8.6[s, 1H, SO₂NH, exchangeable with D₂O]. Analysis calc. for C₂₆H₁₉N₇O₂S: C, 63.27; H, 3.88; N, 19.87. Found: C, 63.89; H, 3.42; N, 19.23.

4-(2,2-Dicyanovinylamino)-N-(substituted)-benzenesulfonamides (12, 13).

A mixture of sulfathiazole or sulfadiazine (0.01 mol), malononitrile (0.01 mol), triethylorthoformate (0.01 mol) and acetic acid (1 ml) in methanol (30 ml) was refluxed for 5 h, the reaction mixture was filtered and the filtered solid was crystallized from ethanol to give 12, 13, respectively.

4-(2,2-Dicyanovinylamino)-N-(thiazol-2-yl) benzenesulfonamide (12)

Yield%: 75, m.p.=189-190, IR, cm⁻¹: 3412, 3308 (NH), 3038 (CH arom.), 2955, 2867 (CH aliph.), 2216 (CN), 1333, 1146 (SO₂). ¹H DMSO d₆ (ppm): 4.9[s, 1H, NH, exchangeable with D₂O], 6, 5, 7, 5[2d, 4H, Ar-H AB system], 6.8, 7.6[2d, 2H, 2CH-thiazol], 7.7[s, 1H, CH], 8.9[s, 1H, SO₂NH, exchangeable with D₂O]. Anal. Calcd. For C₁₃H₉N₅O₂S₂: C, 47.12; H, 2.74; N, 21.13. Found: C, 47.10; H, 2.80; N, 21.20.

4-(2,2-Dicyanovinylamino)-N-(pyrimidin-2-yl)benzene-sulfonamide (13)

Yield%: 80, m.p.=214-216^o. IR, cm⁻¹: 3336, 3238 (NH), 3090 (CH arom.), 2977, 2834 (CH aliph.), 2218 (CN), 1310, 1158 (SO₂). ¹H DMSO d₆ (ppm): 4.0[s, 1H, NH, exchangeable with D₂O], 6.5[s, 1H, CH pyrimidine], 6.6, 7.6[2d, 4H, Ar-H AB system], 7.7[s, 1H, CH], 8.2[d, 2H, 2CH pyrimidine], 8.5[s, 1H, SO₂NH, exchangeable with D₂O]. Anal. Calcd. For C₁₄H₁₀N₆O₂S: C, 51.53; H, 3.09; N, 25.75. Found: C, 51.80; H, 3.70; N, 25.50.

4-((3, 5-Diamino-1H-pyrazol-4-ylidene)methylamino)-N-(substituted)-benzene-sulfonamide (14, 15).

Compounds 12 or 13 (0.01 mol) were mixed with hydrazine hydrate (0.01 mol) in dioxane (20 ml) and refluxed for 5 h, the reaction mixture was cooled, poured onto ice water. The precipitated solid products were filtered and crystallized from methanol to give compounds 14 and 15, respectively.

4-((3, 5-Diamino-1H-pyrazol-4-ylidene)methylamino)-N-(thiazol-2-yl)benzenesulfonamide (14)

Yield%: 75, m.p.: 112-114^o, IR, cm⁻¹: 3424, 3373, 3254 (NH, NH₂), 3022 (CH arom.), 2989, 2845 (CH aliph.), 1315, 1143 (SO₂). MS (m/z, RI%): 363 (M⁺, 10%), 360 (60%), 230 (77%), 92 (86%), 90 (100%). ¹H DMSO d₆ (ppm): 4.0[s, 1H, NH, exchangeable with D₂O], 4.9[s, 4H, 2NH₂, exchangeable with D₂O], 6.9, 7.3[2d, 4H, Ar-H AB system], 7.4, 7.6[2d, 2H, 2CH-thiazol], 7.7[s, 1H, CH], 8.9[s, 1H, SO₂NH, exchangeable with D₂O]. Anal. Calcd. For C₁₄H₁₄N₈O₂S: C, 46.92; H, 3.94; N, 31.27. Found: C, 46.82; H, 3.67; N, 31.68.

4-((3, 5-Diamino-1H-pyrazol-4-ylidene)methylamino)-N-(pyrimidin-2-yl)-benzenesulfonamide (15)

Yield%: 70, m.p.: 72-74^o. IR, cm⁻¹: 3267, 3232, 3167 (NH, NH₂), 3120 (CH arom.), 2987, 2864 (CH aliph.), 1310, 1146 (SO₂). MS (m/z, RI%): 358 (M⁺, 10.2%), 321 (67%), 145 (78%), 123(100%). ¹H DMSO d₆ (ppm): 4.0[s, 1H, NH, exchangeable with D₂O], 4.4[s, 4H, 2NH₂, exchangeable with D₂O],

6.5[s, 1H, CH pyrimidine], 6,9, 7,2[2d, 4H, Ar-H AB system], 7.7[s, 1H, CH],8.3[d, 2H, 2CH pyrimidine], 8.9[s, 1H, SO₂NH, exchangeable with D₂O]. Anal. Calcd. For C₁₄H₁₄N₈O₂S: C, 46.92; H, 3.94; N, 31.27. Found: C, 46.34; H, 3.56; N, 31.67.

4-(5-Cyano-4-oxo-3-phenyl-2-thioxo-3,4-dihydropyrimidin-1(2H)-yl)-N-(substituted)-benzenesulfonamide (16, 17).

A mixture of 12 or 13 (0.01 mol), phenyl isothiocyanate (0.01 mol) and sodium hydroxide (0.01 mol) in ethanol (20 ml) was refluxed for 3 h. The reaction mixture was cooled, poured onto ice water, acidified with dil.HCl, and then the solid product was filtered and crystallized from dioxane to give 16 or 17, respectively.

4-(5-Cyano-4-oxo-3-phenyl-2-thioxo-3,4-dihydropyrimidin-1(2H)-yl)-N-(thiazol-2-yl)-benzenesulfonamide (16)

Yield%: 75, m.p.:110-112°, IR, cm⁻¹: 3364 (NH), 3032 (CH arom.), 2220 (CN), 1730 (C=O), 1312, 1146 (SO₂), 1270 (C=S). MS (m/z, RI%): 467(M⁺, 25.5%), 226(100%), 184(53.6%), 134(31.9%), 65(29.6%). ¹H DMSO d₆ (ppm): 6.7, 7.6[2d, 4H, Ar-H AB system], 7.0-7.5[m, 5H, Ar-H], 7.7[s, 1H, CH pyrimidine], 6.5, 7.8[2d, 2H, 2CH thiazol], 8.9[s, 1H, SO₂NH, exchangeable with D₂O]. Anal. Calcd. For C₂₀H₁₃N₅O₃S₃: C, 51.38; H, 2.80; N, 14.98. Found: C, 51.66; H, 2.30; N, 14.45.

4-(5-Cyano-4-oxo-3-phenyl-2-thioxo-3,4-dihydropyrimidin-1(2H)-yl)-N-(pyrimidin-2-yl)benzenesulfonamide (17)

Yield%: 80, m.p.:100-102°, IR, cm⁻¹: 3320 (NH), 3030 (CH arom.), 2218 (CN), 1690 (C=O), 1314, 1148 (SO₂), 1270 (C=S). MS (m/z, RI%): 462 (M⁺, 5%), 310 (100%), 277 (31.7%), 152 (29.5%), 90 (29.3%). ¹H DMSO d₆ (ppm): 6.4[s, 1H, CH pyrimidine], 6.7, 7.6 [2d, 4H, Ar-H AB system], 7.0-7.5[m, 5H, Ar-H], 7.7[s, 1H, CH pyrimidine], 8.4[d, 2H, 2CH pyrimidine], 8.9 [s, 1H, SO₂NH, exchangeable with D₂O]. Anal. Calcd. For C₂₁H₁₄N₆O₃S₂: C, 54.53; H, 3.05; N, 18.17. Found: C, 54.76; H, 3.88; N, 18.34.

Antimicrobial evaluation

In order to show the antimicrobial activity of 13 newly synthesized compounds obtained in pure dry powder form, different clinical specimens (clinical isolate) of fungi and bacteria were used. The fungal isolates included *Aspergillus fumigatus*, *Aspergillus flavus*, *Penicillium chrysogenum* and a yeast-like fungus *Candida albicans*.

The mould isolates were maintained in sterile water and were subcultured on antimicrobial

agent-free potato dextrose agar to ensure viability and purity. The bacterial isolates included Gram-positive bacteria: *Staphylococcus aureus* and Gram-negative bacteria: *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*.

The organisms were obtained from the Microbiology Department, National Center for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt.

The tested compounds were dissolved in dimethyl formamide (DMF) and DMF had no inhibitory effect on the microorganism in the concentrations studied, and kept at 4°C. Nutrient broth (NB; Difco), and nutrient agar (NA) for bacteria and potato dextrose agar and Sabouraud liquid medium for fungi.

The plates used were 96-well microtiter plates with U-shaped wells. The plates were arranged to give 12 rows by eight lanes and these were filled with 0.1 ml amounts of medium. The stock suspensions of drugs were diluted in DMF (pH 6.8) and eight serial dilutions for each drug were prepared and 0.1 ml volumes were dispensed into plates. Plates were stored at -25°C until use.

Antibacterial activity

Microdilution test.

The following bacteria, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*, were used in order to investigate the antibacterial activity of 13 sulfonamides drugs.

The bacterial suspension was adjusted with sterile saline to an optical OD (optical density) of 0.2-0.3. The inocula were daily prepared and stored at 4°C until use. Dilutions of the inocula were cultured on solid medium to verify the absence of contamination and to check the validity of the inoculums [22].

Micro dilution technique was used to obtain the minimum inhibitory concentrations (MICs) was determined using 96-well microtiter plates. The bacterial suspension was adjusted with sterile saline to an OD of 0.2-0.5. Compounds to be investigated were dissolved in DMF to achieve the wanted concentrations (4.0-1024 µg/mL). Each well contained 100 µl of the investigated compound with 100 µl bacterial inoculum. The micro plates were incubated for 18 hours at 37°C. The lowest concentrations without visible growth (under the binocular microscope) were defined as concentrations that completely inhibited bacterial growth (MICs). The minimum bactericidal concentrations (MBCs) were determined by serial subcultivation of a 2µl, from the microplate wells without visible growth, into microtiter plates containing 100µl of broth per well and further incubation for 48hrs at 25°C. The

lowest concentration with no visible growth was defined as the MBC, indicating=99.5% killing of the original inoculum.

The commercial antibiotic Ciprofloxacin were used as standard drugs powders for bacteria (4.0-1024 μ g/mL) was used as a positive control and the solvent (DMF) was used as a negative control. Two replicates were done for each compound and experiment was repeated two times.

Minimal inhibitory concentrations (MIC) were determined by microdilution broth method following the procedures recommended by the National Committee for Clinical Laboratory Standards.(14,15 and 16) MIC's were defined as the lowest concentrations of the antimicrobial agents that inhibited visible growth of the microorganism [23-25].

Antifungal activity

In order to investigate the antifungal activity of compounds we used the following fungi: *Aspergillus fumigatus*, *Aspergillus flavus*, *Penicillium chrysogenum* a yeast-like fungus such as *Candida albicans*.

The micromycetes were maintained on malt agar (MA) and the cultures were stored at 4°C and subcultured once a month. In order to investigate the antifungal activity of compounds the modified microdilution technique was used [26].

The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (v/v). The spore suspension was adjusted with sterile saline to an OD 0.5 in a final volume of 100 μ l per well. The inocula were stored at 4°C for further use. Dilutions of the inocula were cultured on solid MA to verify the absence of contamination and to check the validity of the inoculum.

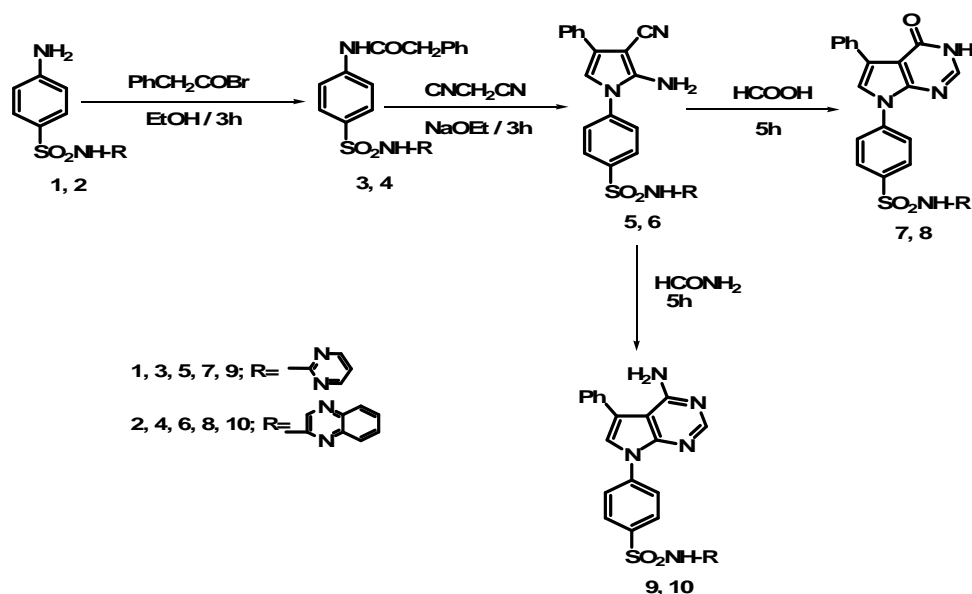
Minimum inhibitory concentration (MIC) determination performed by a serial dilution technique using 96-well microtiter plates. Compounds investigated were dissolved in DMF and in concentrations of 4.0-1024 μ g/mL added in broth malt medium with fungal inoculum. The microtiter plates were incubated for 72 hrs at 28°C. The lowest concentration without visible growth (under the binocular microscope) was defined as MIC. The minimum fungicidal concentrations (MFCs) were determined by serial subcultivation of a 2 μ l, from the micro plate wells without visible growth, into micro plates containing 100 μ l of broth per well and further incubation for 72hrs at 28°C. The lowest

concentration with no visible growth was defined as the MFC, indicating = 99.5% killing of the original inoculum. The commercial fungicidal Ciclopiroxolamine was used as a positive control (4.0-250.0 μ g/mL).The zone of inhibition and minimum inhibitory concentrations (MICs) were determined in comparison with the standard drug Ciclopiroxolamine.

3. Results and discussion

Chemistry

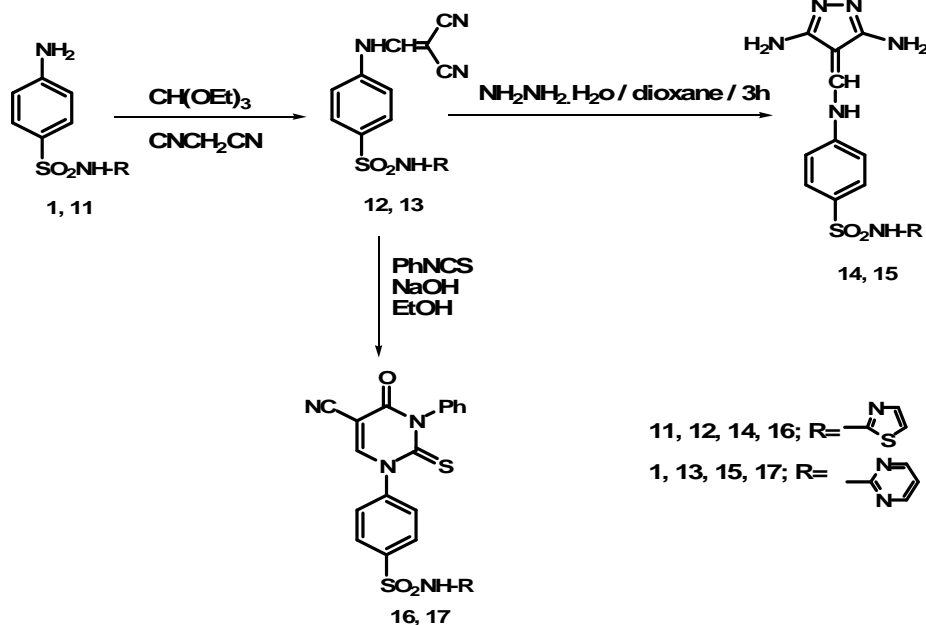
The synthesis of 4- (2-amino- 3 -cyano -4 - phenyl- pyrrol -1- yl) -benzenesulfonamide derivatives 5 and 6 is described in scheme 1, where, the reaction of sulfadiazine 1 or sulfaquinoxaline 2, with phenacyl bromide furnished 4-(2-oxo-2-phenylethylamino)-benzenesulfonamide derivatives 3, 4, the reaction proceeded via a simple nucleophilic substitution reaction with elimination of one molecule of HBr. The structure of compounds 3 and 4 was confirmed by microanalytical and spectral data, their IR spectra showed the presence of bands at 1688, 1684 cm^{-1} , respectively, characteristic for the carbonyl groups. Their mass spectra showed a molecular ion peaks at m/z 368 and 418, respectively. While, their $^1\text{H-NMR}$ spectra showed singlet at 3.3, 3.6 ppm, respectively, for CH_2 group and multiplet at 7.1-7.8, 7.2-7.6 ppm, respectively, for the aromatic protons. Upon reaction of compounds 3 or 4 with malononitrile in sodium ethoxide gave pyrrole derivatives 5, 6. (Scheme 1). The structures of compounds 5 and 6 were confirmed by microanalytical and spectral data, where, their IR spectra showed the disappearance of carbonyl bands and the presence of new bands at 2204 and 2220 cm^{-1} , respectively, due to carbonitrile groups in addition to the forked bands for NH_2 groups. While, their mass spectra revealed a molecular ion peaks at m/z 416 and 466, respectively. $^1\text{H-NMR}$ spectra of compounds 5 and 6 revealed the presence of singlet at 4.3 and 4.5 ppm, respectively, which is exchangeable with D_2O corresponding to NH_2 group, in addition to a singlet at 6.3 and 6.9 ppm, respectively, for the CH-pyrrole. On the other hand, pyrrolo [2, 3-*d*] pyrimidine-4-ones 7, 8 and 4-amino- pyrrolo [2, 3-*d*] pyrimidines 9, 10 were obtained via condensation of the pyrroles 5 and 6 with formic acid or formamide, respectively. Their IR spectra revealed the absence of the band corresponding to the cyano group. Additionally, mass spectra of compounds 7, 8 and 10 revealed molecular ion peaks at m/z 444, 494 and 493, respectively.



Scheme 1.

Interaction of sulfathiazole and sulfadiazine with malononitrile in the presence of triethylorthoformate yielded sulfonamide derivatives 12, 13, respectively; their structures were identified by elemental and spectral data. Their IR spectra showed the presence of characteristic CN bands at 2216 and 2218 cm^{-1} , respectively. Due to the biological importance of pyrazole and pyrimidine rings as anticancer agents, sulfonamide derivatives 12, 13 were reacted with different nucleophiles in order to obtain biologically active pyrazole and pyrimidine derivatives bearing biologically active sulfonamide moieties. Thus, interaction of

sulfonamide derivatives 12, 13 with hydrazine hydrate yielded the corresponding pyrazole derivatives 14, 15. Their IR spectra showed disappearance of CN bands and presence of forked bands characteristic for NH_2 , while their mass spectra revealed molecular ion peaks at 363 and 358, respectively. In addition, interaction of compounds 12, 13 with phenyl isothiocyanate in NaOH / ethanol gave the corresponding pyrimidine derivatives 16 or 17, respectively, their IR spectra showed the presence of $\text{C}=\text{O}$ and $\text{C}=\text{S}$ bands, while their mass spectra showed molecular ion peaks at 467 and 462, respectively (scheme 2).



Scheme 2.

Antimicrobial evaluation

The new compounds were subjected to *in vitro* antibacterial and antifungal studies by microdilution method against both Gram-positive bacteria: *Staphylococcus aureus* and Gram-negative bacteria: *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*, and antifungal activity was determined against *Aspergillus fumigatus*, *Aspergillus flavus*, *Penicillium chrysogenum* and *Candida albicans* by broth microdilution method.

The cultures were obtained from nutrient broth (Difco) for all the bacterial strains after 24 hours of incubation at 37 °C. The yeast was maintained in malt broth (Difco) after incubation for 24 hours at 25 °C, and fungal spores suspensions were collected from 7 days actively growth fungi at 25 °C with tween 80. The final inoculum optical Density (OD) were 0.2-0.3 and 0.5 for bacteria and fungi, respectively. The stock solutions were prepared in dimethylformamide (DMF) which has no effect on the microorganism in the concentrations studied.

The doubling concentrations used for both of bacteria and fungi were 1024–4 µg/ml. Ciprofloxacin and Ciclopiroxolamine were used as standard drugs powders for bacteria and fungi, respectively. The antibacterial activity was measured after 18 hours of incubation at 37 °C and after incubation at 25 °C for 48 hours for the antifungal assay.

Antibacterial activity

The reference drug used in this study was ciprofloxacin which is a broad spectrum antibacterial and its MIC was found to be 10 µg/ml against the bacterial species. The inhibition zones were 19-29 mm for *S. aureus*, 25-23mm for *P. aeruginosa*, 20-25 mm for *K. pneumoniae*, and 18-26mm for *E. coli*, respectively.

From table 1, the antibacterial results evidently showed that the tested compounds were active against all the tested bacteria with MIC ranging from 8-32 µg/mL.

Considering each bacterial species, for *S. aureus*, the most active was the pyrrolopyrimidine 7 (MIC= 4 µg/mL) which was found to be more active than the reference drug and also more active than the pyrrole starting materials 5, 6 (MIC= 8 µg/mL) while, the pyrazole and pyrimidine derivatives 14-17 were less active with MIC ranging from 16-32 µg/mL. for *P. aeruginosa*, the pyrrole derivatives 5, 6 (MIC= 8 µg/mL) and the pyrazole derivatives 14, 15 (MIC= 8 µg/mL) was found to be more active than

the reference drug. On the other hand, cyclization of the pyrroles 5, 6 to the corresponding pyrrolopyrimidines 7-10 resulted in a decrease in the activity with MIC ranging from 16-32 µg/mL, while, the pyrimidine derivatives 16, 17 showed MIC= 16 µg/mL. For *E. coli*, the most active were found to be the pyrrolopyrimidines, pyrazoles and pyrimidines 10-17 (MIC= 8 µg/mL), while for *K. pneumoniae*, the most active compounds were the pyrroles 5, 6 and their cyclized pyrrolopyrimidine derivatives 7-10 (MIC= 8 µg/mL). Considering the activity on all the tested bacteria when using thiazole, pyrimidine or quinoxaline as substituents on the sulfonamide group, the activity doesn't change significantly which indicate that these sulfonamides may be equipotent on all bacterial species.

It is known that *S. aureus* (Gram positive bacterium), and *E. coli* (Gram negative bacterium) have different cell wall constitution. *E. coli* has an outer lipidic membrane layer while *S. aureus* does not have one. Probably, the antimicrobial result is due to the fact that the penetration into the cell was less difficult in microorganisms with a less lipophilic cell wall. This is probably due to the lipophilic alkyl chain that helps the molecule to penetrate through the lipid cell membrane of Gram-negative bacteria. From the results obtained, it comes out that the antibacterial activity decreases as the length of the carbon chain increases. This could be due to bulkiness of the carbon chain, which renders the molecule unable to penetrate through the cell wall of the bacteria [21].

Antifungal activity

The reference drug used in this study was ciclopiroxolamine with MIC 10 µg/ml against the tested fungal species, the inhibition zones were 18-26 mm for *Aspergillus flavus*, 22-30 mm for *Aspergillus fumigatus*, 20-25 mm for *Penicillium chrysogenum*, and 20-25 mm for *Candida albicans*, respectively.

From table 2, all compounds tested, showed high fungicidal potential with MIC of 4 µg/mL against *Aspergillus fumigatus* and *Penicillium chrysogenum*, which is more potent than the reference drug. On the other hand, their activity was decreased to 16 µg/mL against *Candida albicans*. All tested compounds showed fungicidal effect at 256 µg/ml against *Aspergillus flavus* which was found to be the most resistant species.

It is interesting that all the compounds exhibited the best antifungal activity against *Aspergillus fumigatus* and *Penicillium chrysogenum*. It can be concluded that there is a connection between antifungal activity and chemical structure of these compounds.

Table 1: Antibacterial activity data in MIC ($\mu\text{g/mL}$)

Compound no	Antibacterial activity data			
	MIC ($\mu\text{g/mL}$)			
	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>
3	16	16	16	16
4	8	16	16	16
5	8	8	16	8
6	8	8	16	8
7	4	16	16	8
8	16	32	16	8
9	16	16	16	8
10	8	16	8	8
12	16	16	8	16
13	16	16	8	16
14	16	8	8	16
15	32	8	8	16
16	32	16	8	16
17	32	16	8	16
Ciprofloxacin	10	10	10	10

Table 2: Antifungal activity data in MIC ($\mu\text{g/mL}$)

Compound no	Antifungal activity data MIC ($\mu\text{g/mL}$)			
	<i>Aspergillus flavus</i>	<i>Aspergillus fumigatus</i>	<i>Penicillium chrysogenum</i>	<i>Candida albicans</i>
3	256	4	4	16
4	256	4	4	16
5	256	4	4	16
6	256	4	4	16
7	256	4	4	16
8	256	4	4	16
9	256	4	4	16
10	256	4	4	16
12	256	4	4	16
13	256	4	4	16
14	256	4	4	16
15	256	4	4	16
16	256	4	4	16
17	256	4	4	16
Ciclopiroxolamine	10	10	10	10

4. Conclusion:

From the above results we can conclude that, administration of the tested compounds against gram +ve and gram -ve bacteria showed significant antibacterial activity, some of them were found to be equipotent and others were more potent than the reference drug ciprofloxacin, concerning the fungicidal evaluation, the tested compounds showed significant activity especially against *A. fumigatus* and *P. chrysogenum*, while the most resistant species was *A. flavus*. 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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