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SYNTHESIS AND BIOLOGICAL EVALUATION OF SULFONAMIDE DERIVATIVES AS ANTIMICROBIAL AGENTS

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Abstract: The present study investigates, the synthesis of new derivatives of benzenesulfonamide nucleus hybridized with various substituted pyrazole **4**, **8** and thiazole ring **6** using 4-amino-*N*-butylbenzenesulfonamide **1** as the key starting compound. Furthermore, 3,5-diaminopyrazole derivative **10** was allowed to react with different reagents such as an active methylene compounds, ketone dithioacetal, ethoxymethylene malononitrile and cyanoguanidine for a preparation of new benzenesulfonamide derivatives **11**-**18** conjugated with different substituted hetero-bicyclic ring systems. *In vitro*-antimicrobial evaluation was performed for most of the newly synthesized compounds using ciprofloxacin and Fluconazole as antibacterial and antifungal standard drugs, respectively The most promising dual antibacterial and antifungal potency was gained by the sulfamoylphenyl butenoic acid derivative **7**, followed by the sulfamoylphenyl-2-chloroacetamide **5** and its heterocyclic pyrazolopyrimidine derivative 16. Further development and structural optimization will be carried out to get new more potent and safer antimicrobials.

Keywords: sulfonamides, pyrazole, pyrazolopyrimidine, antimicrobial activity

Infectious diseases caused by bacteria and fungi are one of the most important leading causes of morbidity and mortality over the world (1-4). The evolution of antibacterial resistance in bacterial strains against the currently available antibiotics is an increasing concern in recent years (5). Consequently, there is an urgent necessity to widen new and improved antimicrobial agents, which have a broad spectrum of activity against the resistant microorganisms. Numerous efforts have been carried out to overcome the widespread multidrug resistance in bacteria and fungi (6-9).

Sulfonamides form the basis for the first drugs largely employed and systematically used as preventive and chemotherapeutic agents against various diseases (10). Furthermore, clinically used sulfa drugs produce numerous pharmacological activities including, antibacterial (11), antifungal (12), antihypertensive (such as bosentan) (13), antimalarial (14) and antiprotozoal (15) activities. Also, others are considered as non-peptidic vasopressin receptor antagonists (16) and translation initiation inhibitors (17). Sulfonamides are still modified by their incorporation with various heterocycles to found derivatives with more convenient antimicrobial properties (18, 19) (Fig. 1).

Based on the above considerations and as an extension of our previous studies for designing and synthesis of new effective antimicrobial agents (20, 21), in this work, we synthesized new derivatives bearing pyrazole, pyrazole-pyrimidine conjugate, and different side chains tagged with *N*-butyl-benzenesulfonamide backbone. The newly synthesized compounds were *in vitro* evaluated for their antimicrobial activities against a number of human pathogenic microbes.

EXPERIMENTA

Chemistry

All melting points are uncorrected and were taken in open capillary tubes using electrothermal apparatus 9100. Elemental microanalyses were carried out at Microanalytical Unit, Central Services

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Laboratory, National Research Centre, Dokki, Cairo, Egypt, using Vario Elemental Analyzer and were found within ± 0.4% of the theoretical values. Infrared spectra were recorded on a Jasco FT/IR-6100, Fourier transform, Infrared spectrometer at cm^{-1} scale using KBr disc technique at Central Services Laboratory, National Research Centre, Dokki, Cairo, Egypt. ^1H NMR spectra were determined by using a JEOL AS-500 NMR spectrometer at Central Services Laboratory, National Research Centre, Dokki, Cairo, Egypt, chemical shifts are expressed in δ (ppm) downfield from TMS as an internal standard. The mass spectra were measured with a GC MS-Qp1000EX Shimadzu, Cairo University, Cairo, Egypt. Follow up of the reactions and checking the purity of the compounds were made by TLC on silica gel-precoated aluminium sheets (Type 60, F 254, Merck, Darmstadt, Germany) using chloroform/ methanol (10 : 1, v/v) and the spots were detected by exposure to UV lamp at λ_{254} nanometer for few seconds and by iodine vapor.

The chemical names given for the prepared compounds are according to the IUPAC system. *N*-butylsulfanilamide was obtained from commercial sources and was used without further purification.

_N_-Butyl-4-(2-(2,4-dioxopentan-3-ylidene)hydrazinyl)benzenesulfonamide (3)

A solution of sodium nitrite (0.9 g, 0.013 M) in distilled water (5 mL) was added portionwise to an ice cold solution of *N*-butylsulfanilamide **1** (2.28 g, 0.01 M in concentrated hydrochloric acid (2.5 mL) and distilled water (5 mL). This solution was added portionwise to a well-stirred cold solution of acetyl acetone (1.0 g, 0.01 M) in ethanol (30 mL) containing sodium acetate (0.9 g, 0.011 M). The reaction mixture was kept in an ice bath for 2 h and the formed precipitate was filtered, washed several times with water, dried and recrystallized from ethanol to give the title compound **3**.

Yield 45%; m.p. 135-137°C; IR (KBr, cm^{-1}): 3243, 3209 (2NH), 2943, 2851 (CH-aliph.), 1651 (2CO), 1329, 1165 (SO_2); ^1H NMR (DMSO-d_6, δ, ppm): 0.82 (t, 3H, CH_3), 1.24 (m, 2H, CH_2), 1.35 (m, 2H, CH_2), 2.45, 2.49 (2s, 6H, $2CH_3$), 2.74 (t, 2H, N-CH_2), 7.74 (d, 2H, Ar-H), 7.80 (d, 2H, Ar-H), 7.51, 13.60 (2br. s, 2H, 2NH, D_2O exchangeable); ^{13}C

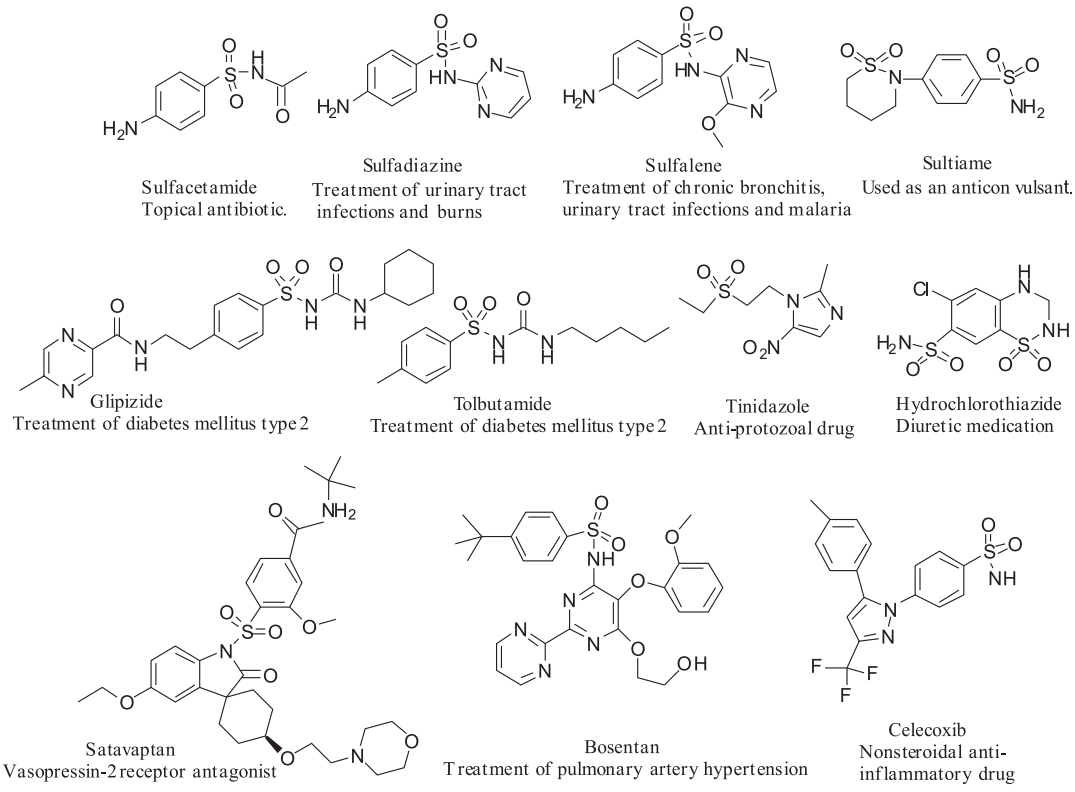


Figure 1. Examples of sulfa drugs producing various pharmacological activities

NMR (DMSO-d_6, δ, ppm): 13.41, 26.27 ($3CH_3$), 19.40, 31.17 ($2CH_2$), 42.16 (N-CH_2), 116.17-147.76 (6 Ar-C + C=N), 196.4, 197.9 (2C=O); MS, m/z (%): 339 [M^+] (11), 76 [C_6H_4] (100); Analysis: calcd. for $C_{15}H_{21}N_3O_4S$ (339.13): C, 53.08; H, 6.24; N, 12.38; S, 9.45%; found: C, 52.98; H, 6.45; N, 12.52; S, 9.23%.

_N_-Butyl-4-[(3,5-dimethyl-1_H_-pyrazol-4-yl)diazenyl]benzenesulfonamide (4)

A mixture of compound **3** (3.39 g, 0.01 M) and hydrazine hydrate (98%, 2.0 mL, 0.02 M) in absolute ethanol (20 mL) was refluxed for 2 h. On cooling the formed precipitate was filtered, dried and recrystallized from ethanol to give the title compound **4**.

Yield 52%; m.p. 102-104°C; IR (KBr, cm^{-1}): 3294, 3178 (2NH), 2929, 2869 (CH-aliph.), 1328, 1160 (SO_2); ^{1}H NMR (DMSO-d_6, δ, ppm): 0.81 (t, 3H, CH_3), 1.23 (m, 2H, CH_2), 1.33 (m, 2H, CH_2), 2.02 (s, 6H, $2CH_3$), 2.64 (t, 2H, N-CH_2), 6.62 (d, 2H, Ar-H), 7.41 (d, 2H, Ar-H), 5.88, 7.02 (2 br. s, 2H, 2NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, δ, ppm): 9.71, 13.49 ($3CH_3$), 19.27, 30.97 ($2CH_2$), 42.10 (N-*C*H_2), 112.23-148.76 (8 Ar-C), 152.30 (C=N); MS, m/z (%): 335 [M^+] (23), 96 [$C_5H_8N_2$] (100); Analysis: calcd. for $C_{15}H_{21}N_5O_2S$ (335.42): C, 53.71; H, 6.31; N, 20.88; S, 9.56%; found: C, 53.91; H, 6.25; N, 21.02; S, 9.39%.

_N_-[4-(_N_-Butylsulfamoyl)phenyl]-2-chloroacetamide (5)

A suspension of compound **1** (2.28 g, 0.01 M) in dry benzene (30 mL) was treated with chloroacetyl chloride (1.24 g, 0.011M) gradually with stirring. The reaction mixture was refluxed for 1 h on a water bath and the solvent was then evaporated to dryness under reduced pressure. The crude product was washed several times with CH_2Cl_2 and finally recrystallized from acetone to give the title compound **5**.

Yield 67%; m.p. 151-153°C; IR (KBr, cm^{-1}): 3213, 3170 (2NH), 2930, 2869 (CH-aliph.), 1692 (C=O), 1360, 1153 (SO_2); ^{1}H NMR (DMSO-d_6, δ, ppm): 0.79 (t, 3H, CH_3), 1.25 (m, 2H, CH_2), 1.33 (m, 2H, CH_2), 2.72 (t, 2H, N-CH_2), 4.30 (s, 2H, CH_2), 7.75 (d, 2H, Ar-H), 7.79 (d, 2H, Ar-H), 7.45, 10.66 (2 br. s, 2H, 2NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, δ, ppm): 13.40 (CH_3), 19.17, 31.00 ($2CH_2$), 42.14 (N-CH_2), 43.50 (CH_2), 119.12-141.75 (6 Ar-C), 165.21 (C=O); MS, m/z (%): 303, 305 [M^+] (10, 4),77 [C_6H_5] (100); Analysis: calcd. for $C_{12}H_{17}ClN_2O_3S$ (304.79): C, 47.29; H, 5.62; N, 9.19; S, 10.52%; found: C, 47.03; H, 5.51; N, 8.92; S, 10.39%.

4-(2-Aminothiazol-4-ylamino)-_N_-butylbenzenesulfonamide (6)

To a solution of compound **5** (3.04 g, 0.01 M) in ethanol (50 mL), thiourea (0.76 g, 0.01 M) was added. This reaction mixture was heated under reflux for 4 h , then poured into ice/old water. The formed precipitate was filtered,washed several times with sodium carbonate solution, dried and recrystallized from absolute ethanol to give compound **6**.

Yield 37%; m.p. 94-99°C; IR (KBr, cm^{-1}): 3420, 3294, 3213 (NH, NH_2), 2938, 2858 (CH-aliph.), 1352, 1146 (SO_2); ^{1}H NMR (DMSO-d_6, δ, ppm): 0.80 (t, 3H, CH_3), 1.22 (m, 2H, CH_2), 1.32 (m, 2H, CH_2), 2.65 (t, 2H, N-CH_2), 5.87 (s, 1H, CH-thiazole), 6.61 (d, 2H, Ar-H), 7.40 (d, 2H, Ar-H), 7.45, 10.66 (2 br. s, 2H, 2NH, D_2O exchangeable), 8.54 (br. s, 2H, NH_2, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, δ, ppm): 13.98 (CH_3), 19.43, 32.00 ($2CH_2$), 42.18 (N-CH_2), 105.24 (CH-thiazole), 117.34-138.56 (7 Ar-C), 157.56 (C=N); MS, m/z (%): 326 [M^+] (14), 116 [$C_3H_6N_3S$] (100); Analysis: calcd. for $C_{13}H_{18}N_4O_2S_2$ (326.43): C, 47.83; H, 5.56; N, 17.16; S, 19.64%; found: C, 47.97; H, 5.41; N, 17.35; S, 19.73%.

4-{[4-(_N_-Butylsulfamoyl)phenyl]amino}-4-oxobut-2-enoic acid (7)

A mixture of compound **1** (2.28 g, 0.01 M) and maleic anhydride (0.98 g, 0.01 M) was refluxed in dry toluene (30 mL) for 6 h. The formed solid was collected by vacuum filteration, dried, and recrystallized from ethanol to give the title compound **7**.

Yield 67%; m.p. 164-166°C; IR (KBr, cm^{-1}): 3356-3213 (OH, NH), 2925, 2863 (CH-aliph.),1715 (C=O carboxylic), 1662 (C=O amide), 1350, 1141 (SO_2); ^{1}H NMR (DMSO-d_6, δ, ppm): 0.80 (t, 3H, CH_3), 1.23 (m, 2H, CH_2), 1.34 (m, 2H, CH_2), 2.72 (t, 2H, N-CH_2), 6.33 (d, 1H, J = 12.2 Hz, CO-CH=CH), 6.50 (d, 1H, J = 12.2 Hz, CH=CHCOOH), 7.43, 10.04 (2 br. s, 2H, 2NH, D_2O exchangeable), 7.75 (d, 2H, Ar-H), 7.78 (d, 2H, Ar-H), 13.42 (br. s, 1H, OH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, δ, ppm): 13.42 (CH_3), 19.18, 31.02 ($2CH_2$), 42.15 (N-CH_2), 119.13-142.01 (6 Ar-C, CH=CH), 165.69, 166.81 (2C=O); MS, m/z (%): 326 [M^+] (7), 76 [C_6H_4] (100); Analysis: calcd. for $C_{14}H_{18}N_2O_5S$ (326.37): C, 51.52; H, 5.56; N, 8.58; S, 9.82%; found: C, 51.37; H, 5.49; N, 8.31; S, 10.01%.

5-{[4-(_N_-Butylsulfamoyl)phenyl]amino}-1_H_-pyrazole-3-carboxylic acid (8)

A mixture of compound **7** (3.08 g, 0.01 M) and hydrazine hydrate 99% (3 mL) in absolute ethanol (30 mL) was refluxed for 3 h. After cooling, the

reaction mixture was poured into cold water; the formed solid was filtered, dried and recrystallized from ethanol to give compound **8**.

Yield 46%; m.p. 89-90°C; IR (KBr, cm^{-1}): 3445, 3398, 3285, 3274 (OH, NH), 2918, 2862 (CH-aliph.), 1358, 1138 (SO_2); ^{1}H NMR (DMSO-d_6, δ, ppm): 0.80 (t, 3H, CH_3), 1.23 (m, 2H, CH_2), 1.33 (m, 2H, CH_2), 2.65 (t, 2H, N-CH_2), 6.61 (s, 1H, CH-pyrazole), 7.01, 9.98, 11.02 (3 br. s, 3H, 3NH, D_2O exchangeable), 7.40 (d, 2H, Ar-H), 7.42 (d, 2H, Ar-H), 13.45 (br. s, 1H, OH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, δ, ppm): 13.45 (CH_3), 19.26, 30.97 ($2CH_2$), 42.10 (N-*C*H_2), 104.83 (CH-pyrazole), 119.62-128.34 (7 Ar-C), 152.30 (C=N), 167.42 (C=O); MS, m/z (%): 338 [M^+] (9), 54 [C_4H_6] (100); Analysis: calcd. for $C_{14}H_{18}N_4O_4S$ (338.38): C, 49.69; H, 5.36; N, 16.56; S, 9.47%; found: C, 49.47; H, 5.49; N, 16.21; S, 9.39%.

General procedure for the synthesis of *N*-butyl-4-[(substituted benzylidene)amino]benzenesulfonamide 9a-c

A mixture of compound **1** (2.28 g, 0.01 M), an appropriate aromatic aldehyde namely 4-chlorobenzaldehyde, 2,4-dimethoxybenzaldehyde and/or 3,4,5-trimethoxybenzaldehyde (0.01 M) in ethanol (20 mL) containing 3 drops of acetic acid was refluxed for 6–8 h. The precipitate formed after cooling was filtered, washed, dried and recrystallized from ethanol to give compounds **9a-c**, respectively.

***N*-Butyl-4-[(4-chlorobenzylidene)amino]benzenesulfonamide (9a)**

Yield 75%; m.p. 123-125°C; IR (KBr, cm^{-1}): 3256 (NH), 2942, 2864 (CH-aliph.), 1331, 1164 (SO_2); ^{1}H NMR (DMSO-d_6, δ, ppm): 0.73 (t, 3H, CH_3), 1.18 (m, 2H, CH_2), 1.28 (m, 2H, CH_2), 2.64 (t, 2H, N-CH_2), 7.23 (br. s, 1H, NH, D_2O exchangeable), 7.28 (d, 2H, Ar-H), 7.40 (d, 2H, Ar-H), 7.42 (d, 2H, Ar-H), 7.46 (d, 2H, Ar-H), 8.63 (s, 1H, CH=N (azomethine protone)); ^{13}C NMR (DMSO-d_6, δ, ppm): 13.24 (CH_3), 19.89, 31.13 ($2CH_2$), 42.54 (N-CH_2), 115.98-137.45 (12 Ar-C), 158.24 (CH=N); MS, m/z (%): 350, 352 [M^+] (6, 4), 115 [$C_6H_{11}S$ (100); Analysis: calcd. for $C_{17}H_{19}ClN_2O_2S$ (350.86): C, 58.20; H, 5.46; N, 7.98; S, 9.14%; found: C, 58.03; H, 5.76; N, 8.12; S, 9.01%.

***N*-Butyl-4-[(2,4-dimethoxybenzylidene)amino]benzenesulfonamide (9b)**

Yield 58%; m.p. 130-131°C; IR (KBr, cm^{-1}): 3235 (NH), 2932, 2845 (CH-aliph.), 1335, 1165 (SO_2); ^{1}H NMR (DMSO-d_6, δ, ppm): 0.76 (t, 3H, CH_3), 1.16 (m, 2H, CH_2), 1.30 (m, 2H, CH_2), 2.67 (t, 2H, N-CH_2), 3.30, 3.33 (2s, 6H, $2OCH_3$), 7.19 (br. s, 1H, NH, D_2O exchangeable), 7.40 (d, 2H, Ar-H), 7.42 (d, 2H, Ar-H), 7.74 (m, 3H, Ar-H), 8.61 (s, 1H, CH=N (azomethine protone)); ^{13}C NMR (DMSO-d_6, δ, ppm): 13.19 (CH_3), 19.73, 31.24 ($2CH_2$), 41.95 (N-CH_2), 53.67, 55.43 ($2OCH_3$), 117.56-134.42 (12 Ar-C), 157.98 (CH=N); MS, m/z (%): 376 [M^+] (6), 160 [$C_6H_{10}NO_2S$] (100); Analysis: calcd. for $C_{19}H_{24}N_2O_4S$ (376.47): C, 60.62; H, 6.43; N, 7.44; S, 8.52%; found: C, 60.54; H, 6.61; N, 7.23; S, 8.31%.

***N*-Butyl-4-[(3,4,5-trimethoxybenzylidene)amino]benzenesulfonamide (9c)**

Yield 79%; m.p. 120-121°C; IR (KBr, cm^{-1}): 3242 (NH), 2993, 2853 (CH-aliph.), 1339, 1159 (SO_2); ^{1}H NMR (DMSO-d_6, δ, ppm): 0.74 (t, 3H, CH_3), 1.15 (m, 2H, CH_2), 1.28 (m, 2H, CH_2), 2.66 (t, 2H, N-CH_2), 3.39, 3.41 (2s, 9H, $3OCH_3$), 7.43 (br. s, 1H, NH, D_2O exchangeable), 7.44 (d, 2H, Ar-H), 7.47 (d, 2H, Ar-H), 7.65 (d, 2H, Ar-H), 8.64 (s, 1H, CH=N (azomethine protone)); ^{13}C NMR (DMSO-d_6, δ, ppm): 13.28 (CH_3), 18.89, 33.46 ($2CH_2$), 42.32 (N-*C*H_2), 53.67, 58.36 ($3OCH_3$), 116.90-138.05 (12 Ar-C), 158.14 (CH=N); MS, m/z (%): 406[M^+] (5), 198 [$C_{10}H_{16}NO_3$] (100); Analysis: calcd. for $C_{20}H_{26}N_2O_5S$ (406.50): C, 59.10; H, 6.45; N, 6.89; S, 7.89%; found: C, 58.93; H, 6.23; N, 7.01; S, 8.02%.

General procedure for the synthesis of 4-[(2-amino-5,7-dioxo-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidin-3-yl)diazenyl]-*N*-butylbenzenesulfonamide (11), 4-[(2-amino-5,7-dimethylpyrazolo[1,5-*a*]pyrimidin-3-yl)diazenyl]-*N*-butylbenzenesulfonamide (12), *N*-butyl-4-[(2,5-diamino-7-oxo-6,7-dihydropyrazolo[1,5-*a*]pyrimidin-3-yl)diazenyl]benzenesulfonamide (13) and 4-[(2-amino-5-methyl-7-oxo-6,7-dihydropyrazolo[1,5-*a*]pyrimidin-3-yl)diazenyl]-*N*-butylbenzenesulfonamide (14)

A mixture of compound **10** (3.37 g, 0.01 M) and an active methylene compounds, namly dimethyl malonate, acetyl acetone, ethyl cyanacetate and/or ethyl acetoacetate (0.01 M) in acetic acid (20 mL) was refluxed for 6 h. The solid that separated after cooling was filtered, dried and recrystallized from ethanol to give the title compounds **11**-**14**, respectively.

4-[(2-Amino-5,7-dioxo-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidin-3-yl)diazenyl]-*N*-butylbenzenesulfonamide (11)

Yield 72%; m.p. 221-223°C; IR (KBr, cm^{-1}): 3388, 3239, 3205 (NH_2, NH), 2923, 2845 (CH-aliph.), 1703 (C=O), 1356, 1123 (SO_2); ^{1}H NMR

(DMSO-d_6, δ, ppm): 0.79 (t, 3H, CH_3), 1.23 (m, 2H, CH_2), 1.34 (m, 2H, CH_2), 2.74 (t, 2H, N-CH_2), 3.83 (s, 2H, CH_2), 7.05, 11.15 (2br. s, 2H, 2NH, D_2O exchangeable), 7.32 (br. s, 2H, NH_2, D_2O exchangeable), 7.73 (d, 2H, Ar-H), 7.84 (d, 2H, Ar-H); ^{13}C NMR (DMSO-d_6, δ, ppm): 13.86 (CH_3), 19.54, 31.31 ($2CH_2$), 35.62 (CH_2), 42.78 (N-*C*H_2), 114.10-147.53 (8 Ar-C), 153.45 (C=N), 168.98, 169,34 (2C=O); MS, m/z (%): 405 [M^+] (23), 389 [M^+-NH_2] (100); Analysis: calcd. for $C_{16}H_{19}N_7O_4S$ (405.43): C, 47.40; H, 4.72; N, 24.18; S, 7.91%; found: C, 47.24; H, 4.69; N, 24.31; S, 8.09%.

4-[(2-Amino-5,7-dimethylpyrazolo[1,5-*a*]pyrimidin-3-yl)diazenyl]-*N*-butylbenzenesulfonamide (12)

Yield 64%; m.p. 249-250°C; IR (KBr, cm^{-1}): 3354, 3275, 3189 (NH_2, NH), 2925, 2847 (CH-aliph.), 1351, 1127 (SO_2); ^1H NMR (DMSO-d_6, δ, ppm): 0.79 (t, 3H, CH_3), 1.23 (m, 2H, CH_2), 1.35 (m, 2H, CH_2), 2.57, 2.60 (2s, 6H, $2CH_3$), 2.78 (t, 2H, N-CH_2), 7.01 (s, 1H, CH-pyrimidine), 7.34 (br. s, 2H, NH_2, D_2O exchangeable), 7.57 (br. s, 1H, NH, D_2O exchangeable), 7.86 (d, 2H, Ar-H), 7.94 (d, 2H, Ar-H); ^{13}C NMR (DMSO-d_6, δ, ppm): 13.92, 16.91, 24.50 ($3CH_3$), 19.68, 31.51 ($2CH_2$), 42.69 (N-CH_2), 111.11-147.27 (10 Ar-C), 152.14, 155.76 (2C=N); MS, m/z (%): 401 [M^+] (100), 371 [M^+ - $2CH_3$] (67); Analysis: calcd. for $C_{18}H_{23}N_7O_2S$ (401.49): C, 53.85; H, 5.77; N, 24.42; S, 7.99%; found: C, 53.71; H, 5.89; N, 24.61; S, 8.29%.

***N*-Butyl-4-[(2,5-diamino-7-oxo-6,7-dihydropyrazolo[1,5-*a*]pyrimidin-3-yl)diazenyl]benzenesulfonamide (13)**

Yield 78%; m.p. 172-173°C; IR (KBr, cm^{-1}): 3354, 3297, 3285, 3180 (NH_2, NH), 2922, 2867 (CH-aliph.), 1702 (C=O), 1341, 1126 (SO_2); ^1H NMR (DMSO-d_6, δ, ppm): 0.79 (t, 3H, CH_3), 1.23 (m, 2H, CH_2), 1.35 (m, 2H, CH_2), 2.77 (t, 2H, N-CH_2), 3.42 (s, 2H, CH_2), 6.76 (br. s, 1H, NH, D_2O exchangeable), 7.56, 7.66 (2br. s, 4H, $2NH_2$, D_2O exchangeable), 7.91 (d, 2H, Ar-H), 8.03 (d, 2H, Ar-H); ^{13}C NMR (DMSO-d_6, δ, ppm): 13.41 (CH_3), 19.67, 31.50 ($2CH_2$), 36.46 (CH_2), 42.68 (N-*C*H_2), 121.72-149.89 (8 Ar-C), 154.86, 155.79 (2C=N), 169.59 (C=O); MS, m/z (%): 404 [M^+] (68), 240 [M^+ - $C_5H_6N_5O$] (100); Analysis: calcd. for $C_{16}H_{20}N_8O_3S$ (404.45): C, 47.52; H, 4.98; N, 27.71; S, 7.93%; found: C, 47.34; H, 5.19; N, 27.69; S, 8.11%.

4-[(2-Amino-5-methyl-7-oxo-6,7-dihydropyrazolo[1,5-*a*]pyrimidin-3-yl)diazenyl]-*N*-butylbenzenesulfonamide (14)

Yield 54%; m.p. 243-244°C; IR (KBr, cm^{-1}): 3323, 3278, 3234, 3156 (NH_2, NH), 2923, 2847 (CH-aliph.), 1723 (C=O), 1343, 1123 (SO_2); ^1H NMR (DMSO-d_6, δ, ppm): 0.80 (t, 3H, CH_3), 1.25 (m, 2H, CH_2), 1.36 (m, 2H, CH_2), 2.38 (s, 3H, CH_3), 2.78 (t, 2H, N-CH_2), 3.44 (s, 2H, CH_2), 6.75 (br. s, 2H, NH_2, D_2O exchangeable), 7.58 (br. s, 1H, NH, D_2O exchangeable), 7.88 (d, 2H, Ar-H), 8.01 (d, 2H, Ar-H); ^{13}C NMR (DMSO-d_6, δ, ppm): 13.40, 18.78 ($2CH_3$), 19.16, 31.03 ($2CH_2$), 37.31 (CH_2), 42.19 (N-*C*H_2), 100.87-149.92 (8 Ar-C), 151.54, 154.58 (2C=N), 168.24 (C=O); MS, m/z (%): 403 [M^+] (37), 388 [M^+ - CH_3] (100); Analysis: calcd. for $C_{17}H_{21}N_7O_3S$ (403.46): C, 50.61; H, 5.25; N, 24.30; S, 7.95%; found: C, 50.39; H, 5.11; N, 24.59; S, 8.01%.

***N*-Butyl-4-[(4,7-diamino-3-cyanopyrazolo[5,1-*c*][1,2,4]triazin-8-yl)diazenyl]benzene sulfonamide (15)**

A solution of sodium nitrite (0.9 g, 0.013 M) in distilled water (5 mL) was added portionwise to an ice cold solution of compound **10** (3.37 g, 0.01 M) in concentrated hydrochloric acid (2.5 mL) and distilled water (5 mL). This solution was added portion wisely to a well-stirred cold solution of malononitrile (0.66 g, 0.01 M) in ethanol (30 mL) containing sodium acetate (0.9 g, 0.011 M). The reaction mixture was kept in an ice bath for 2 h and the formed precipitate was filtered, washed several times with water, dried and recrystallized from ethanol to give the title compounds **15**.

Yield 43%; m.p. 259-261°C; IR (KBr, cm^{-1}): 3432, 3388, 3347, 3286, 3256, 3198 (NH_2, NH), 2920, 2861 (CH-aliph.), 2225 (CN), 1357, 1134 (SO_2); ^1H NMR (DMSO-d_6, δ, ppm): 0.80 (t, 3H, CH_3), 1.24 (m, 2H, CH_2), 1.35 (m, 2H, CH_2), 2.73 (t, 2H, N-CH_2), 6.99 (br. s, 1H, NH, D_2O exchangeable), 7.47 (br. s, 2H, NH_2, D_2O exchangeable), 7.80 (d, 2H, Ar-H), 7.95 (d, 2H, Ar-H), 9.25 (br. s, 2H, NH_2, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, δ, ppm): 13.92 (CH_3), 19.66, 31.55 ($2CH_2$), 42.70 (N-*C*H_2), 118.62 (CN), 122.05-144.30 (10 Ar-C), 152.56 (C=N); MS, m/z (%): 414 [M^+] (24), 147 [M^+ - $C_{10}H_{14}N_3O_2S$] (100); Analysis: calcd. for $C_{16}H_{18}N_{10}O_2S$ (414.44): C, 46.37; H, 4.38; N, 33.80; S, 7.74%; found: C, 46.14; H, 4.19; N, 33.91; S, 7.69%.

***N*-Butyl-4-[(2,5-diamino-6-cyano-7-(methylthio) pyrazolo[1,5-*a*]pyrimidin-3-yl)diazenyl] benzenesulfonamide (16)**

To a suspension of compound **10** (3.37 g, 0.01 M) and 2-(bis(methylthio)methylene)-mal-

ononitrile (1.70 g, 0.01 M) in dimethylformamide (20 mL), (0.5 mL) of trimethylamine was added. The mixture was refluxed for 5 h. and then allowed to cool. The formed precipitate was filtered, washed several times with water, dried and recrystallized from ethanol to give the title compound **16**.

Yield 27%; m.p. 174-175°C; IR (KBr, cm^{-1}): 3434, 3375, 3327, 3286, 3191 (NH_2, NH), 2918, 2853 (CH-aliph.), 2223 (CN), 1367, 1131 (SO_2); ^1H NMR (DMSO-d_6, δ, ppm): 0.81 (t, 3H, CH_3), 1.24 (m, 2H, CH_2), 1.35 (m, 2H, CH_2), 2.47 (s, 3H, SCH_3), 2.75 (t, 2H, N-CH_2), 7.37 (br. s, 1H, NH, D_2O exchangeable), 7.57 (br. s, 4H, $2NH_2$, D_2O exchangeable), 7.87 (d, 2H, Ar-H), 8.02 (d, 2H, Ar-H); ^{13}C NMR (DMSO-d_6, δ, ppm): 13.41, 17.70 ($2CH_3$), 19.17, 31.02 ($2CH_2$), 42.18 (N-*C*H_2), 115.81-139.63 (10 Ar-C + CN), 151.03 (2C=N); MS, m/z (%): 443 [M^+ - NH_2] (54), 276 [M^+ -$C_4H_{10}NO_2S$] (100); Analysis: calcd. for $C_{18}H_{21}N_9O_2S_2$ (459.13): C, 47.04; H, 4.61; N, 27.43; S, 13.96%; found: C, 47.19; H, 4.54; N, 27.38; S, 13.75%.

_N_-Butyl-4-[(2,7-diamino-6-cyanopyrazolo[1,5-*a*]pyrimidin-3-yl)diazenyl] benzenesulfonamide (17)

To a suspension of compound **10** (3.37 g, 0.01 M) and ethoxymethylene malononitrile (1.22 g, 0.01 M) in ethanol (30 mL), (0.5 mL) of trimethylamine was added. The mixture was refluxed for 6 h. and then allowed to cool. The formed precipitate was filtered, dried and recrystallized from dioxane to give the title compound **17**.

Yield 45%; m.p. 131-133°C; IR (KBr, cm^{-1}): 3456, 3345, 3314, 3234, 3159 (NH_2, NH), 2922, 2845 (CH-aliph.), 2226 (CN), 1356, 1134 (SO_2); ^1H NMR (DMSO-d_6, δ, ppm): 0.80 (t, 3H, CH_3), 1.22 (m, 2H, CH_2), 1.33 (m, 2H, CH_2), 2.78 (t, 2H, N-CH_2), 7.38 (br. s, 1H, NH, D_2O exchangeable), 7.67 (br. s, 4H, $2NH_2$, D_2O exchangeable), 7.97 (d, 2H, Ar-H), 8.01 (d, 2H, Ar-H), 8.54 (s, 1H, C*H*-pyrimidine); ^{13}C NMR (DMSO-d_6, δ, ppm): 13.19 (CH_3), 19.14, 31.45 ($2CH_2$), 42.78 (N-*C*H_2), 115.32-147.78 (10 Ar-C + CN), 152.34, 153.43 (2C=N); MS, m/z (%): 397 [M^+-NH_2] (12), 240 [M^+ - $C_7H_3N_5$] (100); Analysis: calcd. for $C_{17}H_{19}N_9O_2S$ (413.46): C, 49.38; H, 4.63; N, 30.49; S, 7.76%; found: C, 49.29; H, 4.84; N, 30.31; S, 7.90%.

_N_-Butyl-4-[(2,4,7-triamino-4-cyanamido-3,4-dihydropyrazolo[1,5-*a*][1,3,5]triazin-8-yl) diazenyl] benzenesulfonamide (18)

A mixture of compound **10** (3.37 g, 0.01 M) and cyanoguanidine (0.84 g, 0.01 M) in absolute ethanol (30 mL) was refluxed for 6 h. After cooling, the reaction mixture was poured into cold water; the formed solid was filtered, dried and recrystallized from dioxane to give compound **18**.

Yield 23%; m.p. 258-260°C; IR (KBr, cm^{-1}): 3474, 3412, 3378, 3334, 3276, 3181 (NH_2, NH), 2923, 2856 (CH-aliph.), 2226 (CN), 1364, 1136 (SO_2); ^1H NMR (DMSO-d_6, δ, ppm): 0.77 (t, 3H, CH_3), 1.21 (m, 2H, CH_2), 1.34 (m, 2H, CH_2), 2.74 (t, 2H, N-CH_2), 2.88 (br. s, 2H, NH_2, D_2O exchangeable), 6.95 (br. s, 1H, NH, D_2O exchangeable), 7.52, 7.63 (2br. s, 4H, $2NH_2$, D_2O exchangeable), 7.79 (d, 2H, Ar-H), 7.94 (d, 2H, Ar-H), 8.23, 8.56 (2br. s, 2H, 2NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, δ, ppm): 13.91 (CH_3), 19.67, 31.26 ($2CH_2$), 42.68 (N-CH_2), 116.33-150.02 (9 Ar-C + CN), 152.71, 159.62 (2C=N); MS, m/z (%): 445 [M^+-1] (10), 405 [M^+ - NHCN] (100); Analysis: calcd. for $C_{16}H_{22}N_{12}O_2S$ (446.49): C, 43.04; H, 4.97; N, 37.64; S, 7.18%; found: C, 44.24; H, 4.80; N, 37.78; S, 6.99%.

_N_-Butyl-4-[(2,5-diamino-6-cyano-7-(naphthalen-1-yl)pyrazolo[1,5-*a*]pyrimidin-3-yl)diazenyl]benzenesulfonamide (19)

A mixture of compound **10** (3.37 g, 0.01 M) and 2-(naphthalen-2-ylmethylene)malononitrile (2.04 g, 0.01 M) in absolute ethanol (30 mL) was refluxed for 6 h. After cooling, the reaction mixture was poured into cold water; the formed solid was filtered, dried and recrystallized from methanol to give compound **19**.

Yield 56%; m.p. 181-182°C; IR (KBr, cm^{-1}): 3474, 3376, 3345, 3245, 3186 (NH_2, NH), 2927, 2851 (CH-aliph.), 2221 (CN), 1363, 1135 (SO_2); ^1H NMR (DMSO-d_6, δ, ppm): 0.77 (t, 3H, CH_3), 1.21 (m, 2H, CH_2), 1.32 (m, 2H, CH_2), 2.73 (t, 2H, N-CH_2), 7.27 (br. s, 2H, NH_2, D_2O exchangeable), 7.54-7.69 (m, 7H, Ar-H), 7.81 (d, 2H, Ar-H), 7.91 (d, 2H, Ar-H), 8.13 (br. s, 2H, NH_2, D_2O exchangeable), 8.75 (br. s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, δ, ppm): 13.89 (CH_3), 19.64, 31.48 ($2CH_2$), 42.65 (N-*C*H_2), 80.01, 115.72-148.93 (20 Ar-C + CN), 152.42, 155.38 (2C=N); MS, m/z (%): 412 [M^+ - $C_{10}H_7$] (100); Analysis: calcd. for $C_{27}H_{25}N_9O_2S$ (539.61): C, 60.10; H, 4.67; N, 23.36; S, 5.94%; found: C, 59.98; H, 4.78; N, 23.28; S, 6.19%.

4,4'-(2,5,7-Triaminopyrazolo[1,5-*a*]pyrimidine-3,6-diyl)bis(diazene-2,1-diyl)bis(*N*-butylbenzenesulfonamide) (21)

Method A: To a solution of hydrazone **20** (6.10 g, 0.02 M) and pyridine 1 mL in 30 mL ethanol was

added hydrazine hydrate 99% (3 mL). The reaction mixture was heated under reflux for 3 h, then cooled to room temperature. The separated solid was filtered, washed with hot ethanol, dried and recrystallized from DMF to give **21**.

Method B: To a solution of diaminopyrazole **10** (3.37 g, 0.01 M) and pyridine 0.5 mL in 30 mL ethanol was added compound **20** (3.05 g, 0.01 M). The reaction mixture was heated under reflux for 4 h, then cooled to room temperature. The separated solid was filtered, washed with hot ethanol, dried and recrystallized from DMF to give **21**.

Yield 54%; m.p. over 300°C; IR (KBr, cm^{-1}): 3467, 3343, 3342, 3278, 3242, 3198 (NH_2, NH), 2922, 2851 (CH-aliph.), 1365, 1138 (SO_2); ^1H NMR (DMSO-d_6, δ, ppm): 0.79 (t, 6H, $2CH_3$), 1.23 (m, 4H, $2CH_2$), 1.35 (m, 4H, $2CH_2$), 2.77 (t, 4H, 2N-CH_2), 7.08 (br. s, 2H, 2NH, D_2O exchangeable), 7.57, 7.67 (2br. s, 4H, $2NH_2$, D_2O exchangeable), 7.84 (d, 4H, Ar-H), 8.22 (d, 4H, Ar-H), 9.51 (br. s, 2H, NH_2, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, δ, ppm): 13.92 ($2CH_3$), 19.69, 31.51, 33.53 ($4CH_2$), 41.06, 42.69 (2N-*C*H_2), 109.57-148.53 (16 Ar-C), 154.91, 156.02 (2C=N); MS, m/z (%): 642 [M^+] (8), 402 [M^+ - $C_{10}H_{14}N_3O_2S$] (100); Analysis: calcd. for $C_{26}H_{34}N_{12}O_4S_2$ (642.76): C, 48.58; H, 5.33; N, 26.15; S, 9.98%; found: C, 48.67; H, 5.23; N, 26.02; S, 10.12%.

_N_-Butyl-4-[(2,5,7-triamino-6-((4-(_N_-cyclohexylsulfamoyl)phenyl)diazenyl)pyrazolo[1,5-_a_]pyrimidin-3-yl)diazenyl]benzenesulfonamide (23)

To a solution of diaminopyrazole 10 (3.37 g, 0.01 M) and pyridine 0.5 mL in 30 mL ethanol was added compound hydrazone **22** (3.31 g, 0.01 M). The reaction mixture was heated under reflux for 4 h, then cooled to room temperature. The separated solid was filtered, washed with hot ethanol, dried and recrystallized from DMF to give **23**.

Yield 67%; m.p. over 300°C; IR (KBr, cm^{-1}): 3472, 3356, 3315, 3256, 3209, 3178 (NH_2, NH), 2924, 2853 (CH-aliph.), 1361, 1145 (SO_2); ^1H NMR (DMSO-d_6, δ, ppm): 0.81 (t, 3H, CH_3), 1.12-1.16 (m, 6H, 3(CH_2)-cyclohexyl protons), 1.26 (m, 2H, CH_2), 1.37 (m, 2H, CH_2), 1.58-1.60 (m, 4H, 2(CH_2)-cyclohexyl protons), 2.79 (t, 2H, N-CH_2), 2.87-2.98 (m, 1H, -NCH-cyclohexyl proton), 7.06 (br. s, 2H, 2NH, D_2O exchangeable), 7.53, 7.66 (2br. s, 4H, $2NH_2$, D_2O exchangeable), 7.68 (d, 2H, Ar-H), 7.78 (br. s, 2H, NH_2, D_2O exchangeable), 7.84 (d, 2H, Ar-H), 7.88 (d, 2H, Ar-H), 8.20 (d, 2H, Ar-H); ^{13}C NMR (DMSO-d_6, δ, ppm): 13.42 (CH_3), 19.18, 22.04, 24.33, 24.83, 28.48, 31.06, 33.21 ($7CH_2$), 42.20 (N-*C*H_2), 52.11 (N-*C*H), 116.75-147.63 (16 Ar-C), 154.28 (2C=N); MS, m/z (%): 666 [M^+ - 2] (10), 402 [M^+ - $C_{12}H_{16}N_3O_2S$] (100); Analysis: calcd. for $C_{28}H_{36}N_{12}O_4S_2$ (668.79): C, 50.28; H, 5.43; N, 25.13; S, 9.59%; found: C, 50.12; H, 5.37; N, 25.29; S, 9.78%.

Biological screening

Antimicrobial assay

The antibacterial activities of the synthesized compounds were tested against *Escherichia coli* NRRL B-210 and *Pseudomonas* NRRL B-23 (Gram -ve bacteria), *Bacillus subtilis* NRRL B-543 and *Staphylococcus aureus* NRRL B-313 (Gram +ve bacteria) using nutrient agar medium. The antifungal activity of these compounds was also tested against *Candida albicans* NRRL Y-477 using Sabouraud dextrose agar medium.

Agar diffusion medium

The synthesized compounds were screened *in vitro* for their antimicrobial activity against, by agar diffusion method (22). 0.5 mL suspension of each of the aforementioned microorganisms was added to sterile nutrient agar media at 45°C and the mixture was transferred to sterile Petri dishes and allowed to solidify. Holes of 9 mm in diameter were made using a cork borer. Amounts of 0.1 mL of the synthesized compounds were poured inside the holes. A hole filled with DMSO was also used as a control. The plates were left for 1 hour at room temperature as a period of pre-incubation diffusion to minimize the effects of variation in time between the applications of the different solutions. The diameters of the inhibition zone of were measured and compared with that of the standard and the values were tabulated. The same method was carried out using Sabouraud dextrose agar medium on using *Candida albicans* NRRL Y-477. The plates were then incubated at 30°C for 24 hours and observed for antibacterial activity. The diameters of inhibition zone were measured and compared with that of the standard, the values were tabulated. Ciprofloxacin (10 mg/mL) and fluconazole (10 mg/mL) were used as a standard for antibacterial and antifungal activity, respectively. The observed zone of inhibition is presented in Table 1.

Minimum inhibitory concentration (MIC)

MIC was evaluated by turbidity method against the previously mentioned standard strains using nutrient broth and sabouraud dextrose broth for antibacterial and antifungal assay, respectively. A loop full from each strain suspension was inocu-

lated in 5 mL of sterilized broth media in a test-tubes and incubated at 30°C for 24 h. The test compounds were prepared by weighting and dissolving in a minimal volume of DMSO and were serially diluted in sterile nutrient media broth at concentrations in the range of 0.1 – 20 mg/mL. Fifty μL from the 24 h strains cultures were added to each tube containing serially diluted synthesized compounds and incubated at 30°C for 24 h. A control sample was tubes without organic compounds addition. The growth of the bacteria was determined by measuring the turbidity after 24 h. Thus, the MIC was generally read as the smallest concentration of organic compounds in the series that prevents the development of visible growth of test organism. We tested ciprofloxacin and fluconazole as antimicrobial agents for quality control of the method. All the experiments were done in duplicate (Table 2).

RESULTS AND DISCUSSION

Chemistry

Continuing in our search (23), the synthesis of the designed target compounds were achieved as outlined in Schemes 1-3. The synthetic route started with diazotization of 4-amino-*N*-butylbenzenesulfonamide **1** followed by coupling with acetylacetone in a basic medium using one equivalent of sodium acetate to yield the corresponding hydrazone derivative **3**.

It was reported that various β-enaminonitriles reacted with different hydrazines to afford the corresponding pyrazole derivatives. Thus, the treatment of compounds **3** with hydrazine hydrate in ethanol furnished the corresponding 3,5-dimethylpyrazole derivatives **4**. Furthermore, condensation of the aminosulfonamide **1** with chloroacetyl chloride in

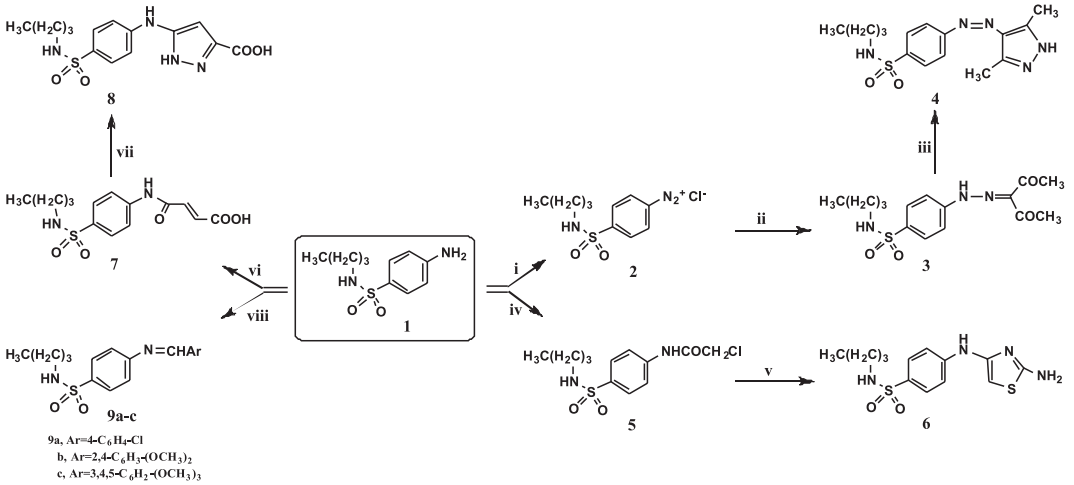
Table 1. *In vitro* antimicrobial activity by agar diffusion method of tested compounds.

Compound No.	Microorganism inhibition zone diameter (mm)				
	Gram positive bacteria		Gram negative bacteria		Fungi
	Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*
3	-ve	-ve	-ve	-ve	-ve
4	11.5	11	10	12	10
5	16	10	11.5	12	-ve
6	12	10	11	12	10
7	17	16	21	20	15
8	-ve	-ve	-ve	-ve	-ve
9a	15	13	14.5	15	12
9b	10	9.5	10	10	-ve
9c	12	10	12	11	10
11	-ve	-ve	-ve	-ve	-ve
12	11	9	10	11	-ve
13	15	10	11	12	9.5
14	-ve	-ve	-ve	-ve	-ve
15	15	13	16	14	12
16	15	13	15	15	12
17	11	10	11	10	-ve
18	12	10	11.5	13	9.5
19	11	10	10	11	-ve
21	16	15	15	20	14
23	10	9.5	11	10	-ve
Ciprofloxacin	23	24	23	22	-
Fluconazole	-	-	-	-	25

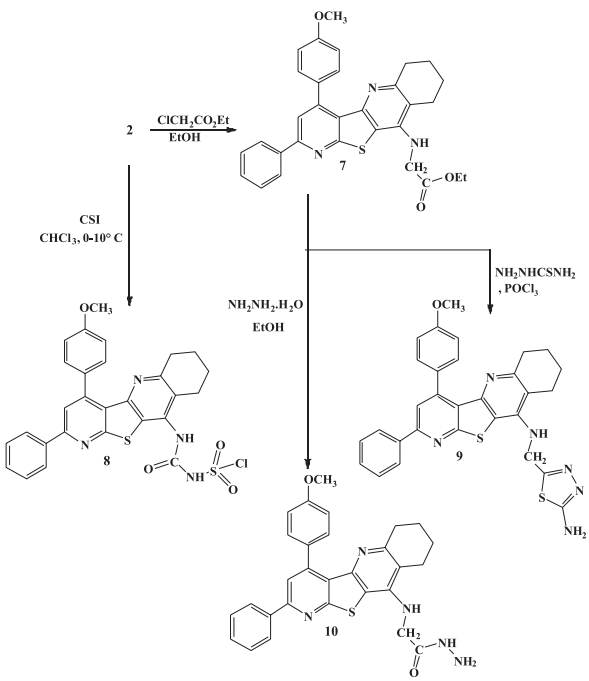
Highly active = (inhibition zone ≥ 19 mm); Moderately active = (inhibition zone 14 - 18 mm); Slightly active = (inhibition zone 9.5 - 13 mm); Inactive (-ve) = no inhibition zone.

dry benzene yielded the corresponding chloroacetamide derivative **5**. The later compound converted into 2-aminothiazole derivative **6** when reacted with thiourea.

Also, 4-{[4-(*N*-butylsulfamoyl)phenyl]amino}-4-oxobut-2-enoic acid **7** was proceeded *via* the condensation of the aminosulfonamide **1** with phthalic anhydride. Cyclization of compound **7** with hydrazine hydrate in ethanol afforded the final pyrazole-3-carboxylic acid derivative **8**. In addition, compound **1** was condensed with aromatic aldehydes (namely, 4-chloro, 2,4-dimethoxy and/or 3,4,5-



Scheme 1. Synthesis of the target compounds **2**-**9**. Reagents and conditions: **i**) sodium nitrite/HCl, stirring 0°C; **ii**) acetyl acetone//EtOH, stirring 0°C; **iii**) hydrazine hydrate/EtOH, reflux; **iv**) chloroacetyl chloride/dry benzene, reflux; **v**) thioureal/EtOH, reflux; **vi**) maleic anhydride/dry toluene, reflux; **vii**) hydrazine hydrate/EtOH, reflux; **viii**) 4-chlorobenzaldehyde, 2,4-dimethoxybenzaldehyde and/or 3,4,5-trimethoxybenzaldehyde/EtOH/AcOH, reflux



Scheme 2. Synthesis of the target compounds **11**-**19**. Reagents and conditions: **i**) dimethyl malonate/AcOH, reflux; **ii**) acetyl acetone/AcOH, reflux; **iii**) ethyl cyanoacetate/AcOH, reflux; **iv**) ethylacetoacetate/AcOH,reflux; **v**) sodium nitrite/HCl/malononitrile/EtOH, stirring 0°C; **vi**) 2-(bis(methylthio)methylene)-malononitrile/DMF/TEA, reflux; **vii**) ethoxymethylene malononitrile/EtOH/TEA, reflux; **viii**) cyanoguanidine/EtOH, reflux; **ix**) 2-(naphthalen-2-ylmethylene) malononitrile/EtOH, reflux

trimethoxybenzaldehydes) in ethanol under reflux afforded the corresponding Schiff compounds **9a-c**, respectively (Scheme 1).

3,5-Diaminopyrazole **10** was prepared in good yield as described in the literature (23) and it was employed as a key intermediate for the further synthesis of the other target compounds. Pyrazolo[1,5-*a*]pyrimidines were considerable of pharmacological important as purine analogues (24-26). The reactivity of 3,5-diaminopyrazole **10** towards an active methylene compounds was investigation for obtaining pyrazolo[1,5-*a*]pyrimidines containing sulfonyl group in their structure. Thus, condensation of compound **10** with an active methylene compounds, namely dimethyl malonate, acetyl acetone, ethyl cyanoacetate and/or ethyl acetoacetate in acetic acid effected cyclization to afford the corresponding pyrazolo[1,5-*a*]pyrimidines **11-14**, respectively. Also, the pyrazolo[5,1-*c*][1,2,4]triazine derivative **15** was synthesized *via* diazotized of 3,5-diaminopyrazole **10** followed by coupling with malononitrile in a basic medium.

The study was extended to include the behavior of 3,5-diaminopyrazole **10** with ketone dithioacetal and ethoxymethylene malononitrile. Thus, treatment of compound **10** with [bis(methylsulfanyl) methylidene]malononitrile in DMF, yielded the corresponding pyrazolo[1,5-*a*]pyrimidine **16**. Furthermore, 3,5-diaminopyrazole **10** was reacted with ethoxymethylene malononitrile in ethanol to afford 2,7-diamino pyrazolo[1,5-*a*]pyrimidine **17**.

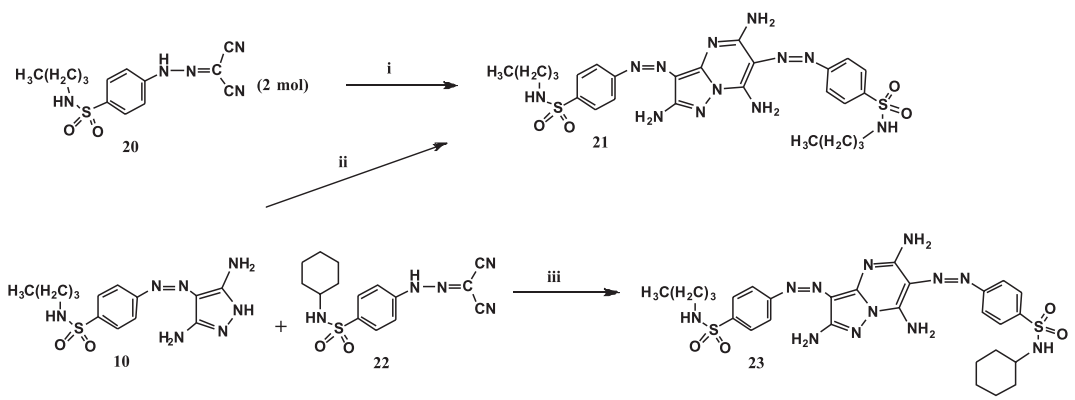
In this investigation, the reaction of compound **10** with cyanoguanidine yielded pyrazolo[1,5-*a*][1,3,5]triazine derivative **18**. The proposed structure was assumed to proceed *via* the addition of compound **10** on two moles of cyanoguanidine followed by NH_3 and NH_2CN elimination then cyclized to give the corresponding derivative **18**. Also, condensation of compound **10** with 2-(naphthalen-2-ylmethylene)malononitrile in refluxing ethanol was attempted to form *N*-Butyl-4-[(2,5-diamino-6-cyano-7-(naphthalen-1-yl)pyrazolo[1,5-*a*]pyrimidin-3-yl)diazenyl]benzenesulfonamide (**19**). (Scheme 2)

Finally, symmetrical 4,4'-(2,5,7-triaminopyrazolo[1,5-*a*]pyrimidine-3,6-diyl)bis(diazene-2,1-diyl)bis(*N*-butylbenzenesulfonamide) (**21**) was synthesized by the cyclization, involving the reaction of hydrazone **20** (23) with hydrazine hydrate in the molar ratio 2:1 in ethanol under reflux for 3 h (Method A), or by the cyclization, involving the reaction of 3,5-diaminopyrazole **10** with equimolar of hydrazone **20** under the same procedure (Method B). Asymmetrical heterocyclic compound **23** were synthesized by the cyclization of diaminopyrazole 10 with different hydrazone derivative **22** in ethanol under reflux for 4 h (Scheme 3).

Pharmacological screening

Antimicrobial assay

Most of the synthesized compounds were evaluated as antibacterial and antifungal agents. (Table 1) depicts the resultant data of the *in vitro* antimicrobial activity screening of the tested derivatives using agar diffusion method. The diameters of inhibition zones were measured in (mm) and compared with those obtained by ciprofloxacin and fluconazole which were used as antibacterial and antifungal standard drugs, respectively. The minimum inhibitory concentrations (MIC) were evaluated for the compounds that exhibited the highest growth inhibitory potency as described in Table 2. It has been observed that the sulfamoylphenyl butenoic acid derivative 7 exhibited the most promising potency of MIC; 0.16, 0.18 mg/mL



Scheme 3. Synthesis of the target compounds **20**-**23**. Reagents and conditions: **i**) hydrazone **20**/pyridine/EtOH/hydrazine hydrate, reflux; **ii**) diaminopyrazole **10**/pyridine/EtOH, reflux; **iii**) diaminopyrazole **10**/pyridine/hydrazone **22**, reflux

Table 2. MIC in mg/mL of the newly synthesized compounds against microorganisms.

Compound No.	Microorganism inhibition zone diameter (mm)				
	Gram positive bacteria		Gram negative bacteria		Fungi
	Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*
7	0.16	0.18	0.12	0.13	0.20
9a	0.80	1.20	0.90	0.80	1.50
15	4.0	4.50	3.90	4.60	4.80
16	0.80	0.90	0.85	0.80	1.10
21	4.0	4.20	4.30	3.50	4.50
Ciprofloxacin	0.062	0.06	0.063	0.065	-
Fluconazole	-	-	-	-	0. 057

against the tested Gram positive bacteria, 0.12, 0.13 mg/mL against the tested Gram negative bacteria and 0.20 mg/mL against the fungal *Candida albicans* strain comparing to MIC of ciprofloxacin 0.062 mg/mL and MIC of fluconazole 0.05 mg/mL. A recorded reduction in the activity was observed by the replacement of the 4-oxobut-2-enoic acid side chain of compound **7** with acetylchloride moiety as compound **5** or its cyclization to the corresponding pyrazolopyrimidine heterocyclic ring system as compound **16** showing MIC range 0.80-0.1.22 mg/mL against the tested Gram positive and Gram negative bacteria and 1.5, 1.10 mg/mL against the fungal strain. Further decrease in the antimicrobial activity was detected upon conjugation of diazenybenzenesulfonamide side chain to the pyrazolopyrimidine ring as compound **21** or due to its conversion to the isosteric pyrazolotriazine ring system as compound **15**. Their MIC ranged from 4.0-4.50 mg/mL against the tested Gram positive bacteria, 3.90-4.60 mg/mL against the tested Gram negative bacteria and 4.50, 4.80 mg/mL against the tested fungal strain.

More structural modification, optimization and substitution of the above mentioned sulfonamide derivatives are required in the future to improve their antimicrobial profile.

CONCLUSIONS

This work reported the synthetic procedures of two new series of benzenesulfonamide derivatives. Starting with 4-amino-N-butylbenzenesulfonamide **1**, which was allowed to react with different reagents led to the formation of the corresponding derivatives of the first benzenesulfonamide series incorporated with different substituted pyrazole and thiazole rings **4**, **6**, **8**. The previously prepared 3,5-diaminopyrazole derivative **10** was used as another key intermediate in this study to generate the second benzenesulfonamide series conjugated with the bicyclic pyrazolo[1,5-*a*]pyrimidine ring. Accordingly, compound **10** was treated with different active methylene compounds, diazotized and coupled with various malononitrile derivatives.

Most of the newly synthesized compounds were assessed as antimicrobial agents against a number of Gram positive, Gram negative and fungal strains, using ciprofloxacin and fluconazole as antibacterial and antifungal reference drugs. The obtained data reflected that the most potent dual antibacterial and antifungal activities were gained by the butenoic acid derivative **7**, followed by the chloroacetamide **5** and its heterocyclic pyrazolopyrimidine derivative **16**.

Aiming to get new antimicrobials of more potent activity to overcome the microbial resistance obstacle, further benzenesulfonamide derivatization is required in the future studies.

REFERENCES

1. Pfaller M.A., Diekema D.J.: Clin. Microbiol. Rev. 20, 133 (2007).
2. Mukherjee P.K., Sheehan D.J., Hitchcock C.A., Ghannoum M.A.: Clin. Microbiol. Rev. 18, 163, (2005).
3. Shapiro R.S., Robbins N., Cowen L.E.: Microbiol. Mol. Biol. Rev. 75, 213 (2011).
4. Altıntop M.D., Kaplancıklı Z.A., Çiftçi G.A., Demirel R.: Eur. J. Med. Chem. 74, 264 (2014).
5. Soni J.N., Soman S.S.: Eur. J. Med. Chem. 75, 77 (2014).
6. Kamal A., Swapna P., Shetti R., Shaik A.B.,Narasimha Rao M.P. et al.: Eur. J. Med. Chem. 62, 661 (2013).

7. Phillips O.A., Udo E.E., Ali A.A.M., Samuel S.M.: Eur. J. Med. Chem., 42, 214 (2007).
8. Phillips O.A., Udo E.E., Abdel-Hamid M.E., Varghese R.: Eur. J. Med. Chem. 44, 3217 (2009).
9. Kim S.-Y., Park H.B., Cho J.-H., Yoo K.H., Oh C.-H.: Bioorg. Med. Chem. 19, 2558 (2009).
10. Bendjeddou A., Abbaz T., Ayari A., Benahmed M., Gouasmia A. et al..: Orient. J. Chem. 32, 799 (2016).
11. Stokes S.S., Albert R., Buurman Ed.T., Andrews B., Shapiro A.B. et al.: Bioorg. Med. Chem. Lett. 22, 7019 (2012).
12. Rahavi Ezabadi I., Camoutsis C., Zoumpoulakis P., Geronikaki A., Soković M. et al.: Bioorg. Med. Chem. 16, 1150 (2008).
13. Kanda Y., Kawanishi Y., Oda K., Sakata T., Mihara S. et al.: Bioorg. Med. Chem. 9, 897 (2001).
14. Boechat N., Pinheiro L.C.S., Santos-Filho O.A., Silva I.C.: Molecules 16, 8083 (2011).
15. Chibale K., Haupt H., Kendrick H., Yardley V., Saravanamuthu A. et al.: Bioorg. Med. Chem. Lett., 11, 2655 (2001).
16. Serradeil-Le Gal C.: Cardiovascular Drug Rev. 19, 201 (2001).
17. Natarajan A., Guo Y., Harbinski F., Fan Y.-H., Chen H. et al.: J. Med. Chem. 47, 4979 (2004).
18. Krátký M., Vinsová J., Volková M., Buchta V., Trejtnar F. et al.: Eur. J. Med. Chem. 50, 433 (2012).
19. Tommasino J.-B., Renaud F.N.R., Luneau D., Pilet G.: Polyhedron 30, 1663 (2011).
20. Mohamed N.A., El-Serwy W.S., Abd El-Karim S.S., Awad G.E. A., Elseginy S.A.: Res. Chem. Intermed. 42, 1363 (2016).
21. Gouhar R.S., Abd El-Karim S.S., Haiba M.E., El-Zahar M.I., Awad G.E.A.: Acta Pol. Pharm. 72, 475 (2015).
22. Khalil A.El-G.M., Berghot M.A., Gouda M.A.: J. Serb. Chem. Soc. 76, 329 (2011).
23. Abbas H.-A.S., Abd El-Karim S.S., Ahmed E.M., Eweas A.F., El-Awdan S.A.: Acta Pol. Pharm. 73, 1163 (2016)
24. Hassan A.S., Hafez T.S., Osman S.A.M., Ali M.M.: Turk. J. Chem. 39, 1102 (2015).
25. El-Gaby M.S.A., Atalla A.A., Gaber A.M., Abd Al-Wahab K.A.: Farmaco. 55, 596 (2000).
26. Compton D.R., Sheng S., Carlson K.E., Rebacz N.A., Lee I.Y. et al.: J. Med. Chem., 47, 5872 (2004).

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