

Synthesis and antimicrobial evaluation of some 4-quinolinylazo-*N*-pyrimidinyl benzenesulfonamide derivatives

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ABSTRACT

Ten new compounds of quinoline clubbed with sulfonamide moiety were synthesized to be used as antimicrobial agents. Therefore, the diazotized *N*-(pyrimidin-2-yl)-benzenesulfonamide was diazocoupled with 8-hydroxyquinoline to furnish 4-(8-hydroxyquinolin-5-yl)-*N*-(pyrimidin-2-yl)benzenesulfonamide (3) which underwent chloroacetylation by chloroacetyl chloride to give the corresponding *O*-chloroacetylated product 6. The reactions of quinolinyl 2-chloroacetate derivative 6 with different nucleophiles (ethyl 2-mercaptoacetate, 2-mercapto-4,6-dimethylnicotinonitrile, *o*-aminothiophenol and/or malononitrile) were studied and utilized to pick up various heterocyclic systems 7, 10, 12 and 14. The chemical structures of newly prepared quinoline-containing scaffolds have been confirmed based on their spectral data (IR, ¹H NMR and MS) and have been tested for their antimicrobial activity. The results showed that compounds 5d, 6, 7 and 14 displayed the highest activity against Gram-positive bacteria..

Keywords: Sulfonamides; 8-Hydroxyquinoline; Chloroacetyl chloride; Malononitrile; Antimicrobial activity.

1. INTRODUCTION

Sulfonamides (sulfa drugs) are considered to be an important branch of synthetic bacteriostatic antibiotics used for treatment of bacterial infections and were used as main sources for treatment against bacteria before the introduction of penicillin in 1941. Based on their absorption, sulfonamides were classified into three major groups: Oral absorbable, oral non absorbable and topical agents. Firstly, absorption of sulfonamides is started from the stomach and small intestine followed by distribution widely to tissues. Through glomerular filtration inactivated metabolites and sulfonamides are mainly expelled by the kidney [1]. In the early the 20th century, the beginning of the chemotherapy progress [2] are started as a result of the discovery of antimicrobial effect of prontosil (Prontosil rubrum) by Gerhard Domagk [3]. As shown in figure (1), under the action cellular enzymes [4] Prontosil which is considered as azoic dye compound was converted into sulfonamide and 1,2,4-triaminobenzene. Sulfonamides are the first successfully synthesized selectively toxic antimicrobial drugs [5-7]. Moreover, various other therapeutic applications, in cancer chemotherapy, diuretics, hypoglycemia and the anti-impotence agent Viagra [8] have been discovered as the wide result of spread potential value of sulfonamides. The bacteriostatic action of sulfonamides was achieved by inhibiting folic acid synthesis in bacteria [9]. Additionally, the quinoline scaffold exists in numerous natural products and synthetically prepared compounds

have great importance to humanity due to their wide spectrum of biological activities, many quinoline derivatives have been used as antibiotic [10], anticancer [11-13], antituberculosis [14], and antimalarial drugs [15]. Therefore, many synthetic routes have been investigated for the synthesis of some heterocycles based on the quinoline having different properties and enhance the antibacterial profile [16,17]. Especially, 8-hydroxyquinoline and its derivatives, because of their widespread application have been reported to act as organic inhibitors of corrosion [18-20], potential HIV-1, integrase inhibitors [21-24], neuroprotection against Alzheimer, Parkinson, and other neurodegenerative diseases [25-27]. Thus, in the present work, synthesis of compounds having a combination of 8-hydroxyquinoline and sulphonamide moiety would be described to afford less toxic and more potent drugs in the field of medicinal chemistry. In continuation of our previous work on the synthesis of biologically active compounds [28-35], the aim of the present work is to synthesize series of some substituted 8-hydroxyquinoline derivatives incorporating sulphonamide moiety. The synthesized compounds were characterized by IR, ¹H NMR and mass analysis spectroscopic tools and tested against Gram-positive and Gram-negative bacteria.

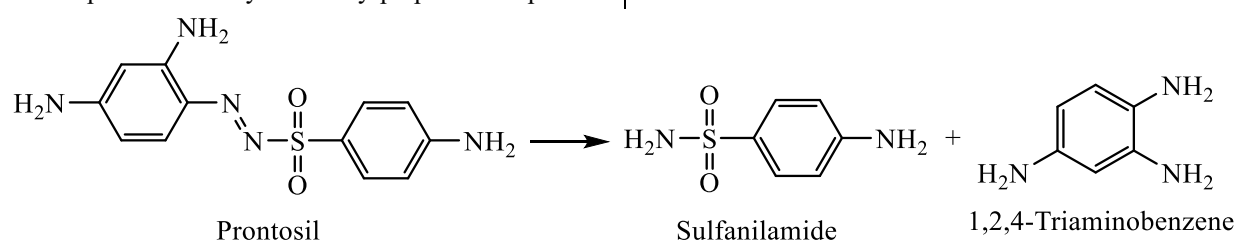


Figure 1. Conversion of Prontosil to sulfanilamide.

2. MATERIALS AND METHODS

2.1. General Methods.

Melting points (uncorrected) were measured on electrothermal Gallenkamp melting point instrument. The infrared spectra were recorded in KBr disks on a Nicolet iS10 FT-IR spectrometer (not all frequencies are reported). ¹H NMR spectra were measured on JEOL 500 MHz using DMSO-*d*₆ as a solvent, using TMS as an internal standard. Mass spectra were carried out on single quadrupole mass analyzer in (Thermo Scientific GCMS) model (ISQ LT). Elemental analyses were carried out on Perkin Elmer 2400 analyzer. Antimicrobial activities were carried out at the laboratory of the Micro-analytical Center, Cairo University, Egypt.

2.2. Chemical Synthesis.

Synthesis of 4-((8-hydroxyquinolin-5-yl)azo)-*N*-(pyrimidin-2-yl)-benzenesulfonamide (**3**).

To a cold suspension of sulfapyrimidine **1** (5.0 g, 0.02 mol) in 6 ml HCl (0-5°C), solution of sodium nitrite (1.4 g in 15 ml water) was added dropwise. The diazotized sulfapyrimidine was added to a solution of 8-hydroxyquinoline (2.9 g, 0.02 mol) in sodium hydroxide (10 mL, 10%) at 0-5°C. After complete addition of diazonium salt, the reaction mixture was stirred at 0-5°C for 1 h to obtain a homogenous material. The reaction mixture was left overnight and the separated solid material was then filtered and recrystallized from ethanol to yield compound **3**.

Yield = 71%; m.p. > 300°C. IR (ν/cm⁻¹): 3450 (O-H), 3310 (N-H), 1557 (N=N), 1316 (SO₂). ¹H NMR (δ/ppm): 6.76-8.61 (m, 12H, Ar-H), 9.13 (s, 1H, NH), 9.67 (s, 1H, OH). MS: m/z (%) = 406 (M⁺, 87.6). Anal. Calcd. for C₁₉H₁₄N₆O₃S (406): C, 56.15; H, 3.47; N, 20.68%. Found: C, 55.94; H, 3.40; N, 20.81%.

Synthesis of 2-amino-4-aryl-6-((4-(*N*-(pyrimidin-2-yl)-sulfamoyl)-phenyl)azo)-4*H*-pyrano[3,2-*h*]quinolines **5a-d**.

To a solution of **3** (0.81 g, 0.002 mol) in a mixture of DMF/ethanol (10 mL:10 mL), the arylidenemalononitriles and/or ethyl 2-arylidene cyanoacetates (0.001 mol) and few drops of morpholine were added and refluxed for 4 hrs. The reaction mixture was cooled and then poured onto ice-cold water. The solid that obtained was filtered and recrystallized from ethanol to give compounds **5a-d**.

4-((2-Amino-3-cyano-4-(4-tolyl)-4*H*-pyrano[3,2-*h*]quinolin-6-yl)-azo)-*N*-(pyrimidin-2-yl)benzenesulfonamide (**5a**).

Yield = 73%; m.p. > 300°C. IR (ν/cm⁻¹): 3424, 3371 (NH₂ and NH), 2196 (C≡N), 1547 (N=N), 1346 (SO₂). ¹H NMR (δ/ppm): 2.27 (s, 3H, CH₃), 4.47 (s, 1H, CH), 6.61 (s, 2H, NH₂), 7.12-8.44 (m, 15H, Ar-H), 9.11 (s, 1H, NH). MS: m/z (%) = 574 (M⁺, 100). Anal. Calcd. for C₃₀H₂₂N₈O₃S (574): C, 62.71; H, 3.86; N, 19.50%. Found: C, 62.53; H, 3.95; N, 19.36%.

4-((2-Amino-4-(4-chlorophenyl)-3-cyano-4*H*-pyrano[3,2-*h*]quinolin-6-yl)-azo)-*N*-(pyrimidin-2-yl)benzenesulfonamide (**5b**).

Yield = 66%; m.p. > 300°C. IR (ν/cm⁻¹): 3420 (NH₂ and NH), 2203 (C≡N), 1547 (N=N) and 1346 (SO₂). ¹H NMR (DMSO-*d*₆) δ (ppm): 4.51 (s, 1H, CH), 6.74 (s, 2H, NH₂), 7.18-8.38 (m, 15H, Ar-H), 9.13 (s, 1H, NH). MS: m/z (%) = 595 (M⁺, 58.6). Anal. Calcd. for C₂₉H₁₉ClN₈O₃S (595): C, 58.54; H, 3.22; N, 18.83%. Found: C, 58.71; H, 3.30; N, 18.68%.

Ethyl 2-amino-6-((4-(*N*-(pyrimidin-2-yl)sulfamoyl)-phenyl)azo)-4-(4-tolyl)-4*H*-pyrano[3,2-*h*]quinoline-3-carboxylate (**5c**).

Yield = 73%; m.p. > 300°C. IR (ν/cm⁻¹): 3422-3100 (NH₂ and NH), 1724 (C=O), 1546 (N=N), 1348 (SO₂). ¹H NMR (δ/ppm): 1.28 (t, 3H, CH₃), 2.33 (s, 3H, CH₃), 4.31 (q, 2H, CH₂), 4.58 (s, 1H, CH), 6.74 (s, 2H, NH₂), 7.05-8.52 (m, 15H, Ar-H), 9.01 (s, 1H, NH). MS: m/z (%) = 621 (M⁺, 84.2). Anal. Calcd. for C₃₂H₂₇N₇O₅S (621): C, 61.83; H, 4.38; N, 15.77%. Found: C, 61.65; H, 4.44; N, 15.62%.

Ethyl 2-amino-4-(4-chlorophenyl)-6-((4-(*N*-(pyrimidin-2-yl)-sulfamoyl)-phenyl)azo)-4*H*-pyrano[3,2-*h*]quinoline-3-carboxylate (**5d**).

Yield = 78%; m.p. > 300°C. IR (ν/cm⁻¹): 3422-3100 (NH₂ and NH), 1724 (C=O), 1546 (N=N) and 1348 (SO₂). ¹H NMR (δ/ppm): 1.25 (t, 3H, CH₃), 4.28 (q, 2H, CH₂), 4.65 (s, 1H, CH), 6.82 (s, 2H, NH₂), 7.14-8.46 (m, 15H, Ar-H), 9.28 (s, 1H, NH). MS: m/z (%) = 642 (M⁺, 76.8). Anal. Calcd. for C₃₁H₂₄ClN₇O₅S (642): C, 57.99; H, 3.77; N, 15.27%. Found: C, 57.82; H, 3.86; N, 15.11%.

Synthesis of 5-((4-(*N*-(pyrimidin-2-yl)sulfamoyl)phenyl)azo)-quinolin-8-yl 2-chloroacetate (**6**).

A mixture of **3** (1.62 g, 0.005 mol) and chloroacetyl chloride (0.64 mL, 0.008 mol) was stirred at room temperature in DMF (20 mL) in presence of potassium carbonate (0.5 g) for 2 hrs. The reaction mixture was poured onto ice-cold water. The separated solid material was filtered and recrystallized from ethanol-DMF mixture (1:1) to give compound **6**.

Yield = 79%; m.p. > 300°C. IR (ν/cm⁻¹): 3150 (N-H), 1737 (C=O), 1547 (N=N) and 1348 (SO₂). ¹H NMR (δ/ppm): 4.12 (s, 2H, CH₂), 7.36-8.41 (m, 12H, Ar-H), 10.24 (s, 1H, NH). MS: m/z (%) = 482 (M⁺, 36.5). Anal. Calcd. for C₂₁H₁₅ClN₆O₄S (482.5): C, 52.23; H, 3.13; N, 17.40 %. Found: C, 52.41; H, 3.21; N, 17.48%.

Synthesis of ethyl 2-((2-oxo-2-((5-((4-(*N*-(pyrimidin-2-yl)sulfamoyl)-phenyl)azo) quinolin-8-yl)oxy)ethyl)thio)acetate (**7**).

A mixture of compound **6** (0.48 g, 0.001 mol) and ethyl 2-mercaptoacetate (0.12 g, 0.001 mol) was heated on water bath in DMF (20 mL) in presence of potassium carbonate (0.14 g) for 4 hrs. The reaction mixture was allowed to cool and then poured onto ice-cold water. The separated solid material was filtered and recrystallized from ethanol/DMF mixture (1:1).

Yield = 70%; m.p. > 300°C. IR (ν/cm⁻¹): 3350 (N-H), 1727 (C=O), 1657 (N=N). ¹H NMR (δ/ppm): 1.24 (t, 3H, CH₃), 3.71 (s, 2H, CH₂), 3.84 (s, 2H, CH₂), 4.18 (q, 2H, CH₂), 7.03-8.97 (m, 12H, Ar-H), 10.65 (s, 1H, NH). MS: m/z (%) = 566 (M⁺, 62.5). Anal. Calcd. for C₂₅H₂₂N₆O₆S₂ (566): C, 53.00; H, 3.91; N, 14.83%. Found: C, 53.17; H, 3.84; N, 14.95%.

Synthesis of 5-((4-(*N*-(pyrimidin-2-yl)sulfamoyl)phenyl)azo)-quinolin-8-yl 3-amino-4,6-dimethylthieno[2,3-*b*]pyridine-2-carboxylate (**10**).

A mixture of **6** (0.48 g, 0.001 mol) and 2-mercapto-4,6-dimethylthienonitrile (**8**) (0.16 g, 0.001 mol) was refluxed in sodium ethoxide solution (sodium metal 0.05 g in 20 mL EtOH) for 4 hrs. The reaction mixture was poured onto ice-cold water and neutralized by drops of dil. HCl. The solid that formed was collected by filtration and recrystallized from ethanol/DMF mixture (1:1).

Yield = 68%; m.p. > 300°C. IR (ν/cm⁻¹): 3424, 3368 (NH₂ and NH), 1735 (C=O), 1546 (N=N) and 1348 (C-O-C). ¹H NMR (δ/ppm): 2.37 (s, 3H, CH₃), 2.43 (s, 3H, CH₃), 6.68 (s, 2H, NH₂), 7.02 (s, 1H, pyridine H5), 7.22-8.31 (m, 12H, Ar-H), 9.66 (s, 1H,

NH). MS: m/z (%) = 610 (M^+ , 100). Anal. Calcd. for $C_{29}H_{22}N_8O_4S_2$ (610): C, 57.04; H, 3.63; N, 18.35%. Found: C, 57.21; H, 3.60; N, 18.15%.

Synthesis of 4-((8-((2H-benzo[b][1,4]thiazin-3-yl)oxy)quinolin-5-yl)azo)-N-(pyrimidin-2-yl)benzenesulfonamide (12).

A mixture of compound **6** (0.48 g, 0.001 mol) and *o*-aminothiophenol (0.13 g, 0.001 mol) was heated on water bath in DMF (20 mL) in presence of potassium carbonate (0.5 g) for 4 hrs. The reaction mixture was allowed to cool and then poured onto ice-cold water. The solid that obtained was filtered and recrystallized from ethanol/DMF mixture (1:1).

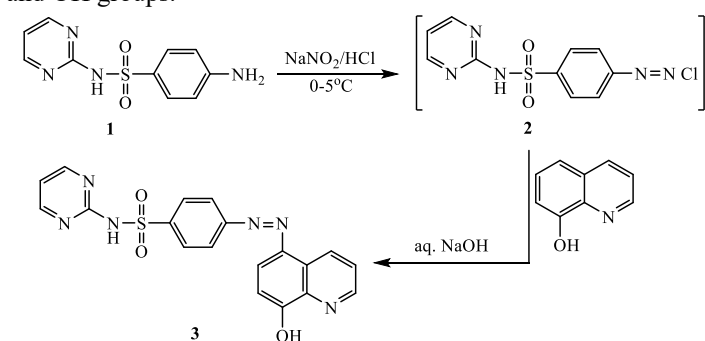
Yield = 71%; m.p. > 300°C. IR (ν/cm^{-1}): 3429 (N-H), 1583 (C=N) and 1547 (N=N). 1H NMR (δ/ppm): 3.74 (s, 2H, CH_2), 7.08-8.52 (m, 16H, Ar-H), 9.65 (s, 1H, NH). MS: m/z (%) = 525 (M^+ - N_2 , 100). Anal. Calcd. for $C_{27}H_{19}N_7O_3S$ (553): C, 58.58; H, 3.46; N, 17.71%. Found: C, 58.36; H, 3.55; N, 17.94%.

Synthesis of 4-((8-((5-amino-4-cyanofuran-2-yl)oxy)quinolin-5-yl)-azo)-N-(pyrimidin-2-yl)benzenesulfonamide (14).

3. RESULTS

3.1. Chemical Synthesis.

Sulfapyrimidine of the type **1** was diazotized by nitrous acid (prepared *in situ* from sodium nitrite and HCl) and the corresponding diazonium chloride **2** was coupled with 8-hydroxyquinoline in aqueous sodium hydroxide to afford the corresponding coupling product, 4-((8-hydroxyquinolin-5-yl)azo)-N-(pyrimidin-2-yl)-benzenesulfonamide (**3**) (Scheme 1). Compound **3** was characterized by both spectral and elemental analyses. The infrared spectrum of compound **3** displayed absorptions at 3450 and 3310 cm^{-1} attributed to the stretching frequencies of O-H and NH groups, respectively. In addition to absorption bands at 1557 and 1316 cm^{-1} due to stretching frequencies of azo group N=N and SO_2 group, respectively. 1H NMR spectrum showed two doublet signals at δ 7.76 and δ 8.51 ppm due to four aromatic protons of benzene ring (AB system), two doublets at δ 8.41 and δ 7.09 for C_6 -H and C_7 -H of quinoline ring, in addition to multiple extended in the region δ 6.76-8.61 ppm due to the additional aromatic protons. The two singlet signals at δ 9.13 and δ 9.67 were attributed to the protons of NH and OH groups.



Scheme 1. Synthesis of 4-((8-hydroxyquinolin-5-yl)azo)-N-(pyrimidin-2-yl)-benzenesulfonamide

The reaction of hydroxyquinoline derivative **3** with four different types of arylidene acrylonitriles **4a-d** was studied. Thus, compound **3** was boiled in DMF containing five drops of morpholine with arylidene malononitriles and/or ethyl arylidene cyanoacetate to furnish the corresponding 2-amino-4-aryl-6-((4-(N-(pyrimidin-2-yl)sulfamoyl)-phenyl)azo)-4H-pyrano[3,2-*h*]-quinoline **5a-d** (Scheme 2). The infrared spectrum of compounds **5a** displayed absorptions at 3424 & 3371, 2196, 1547 and 1346 cm^{-1} related to the $-NH_2$ & N-H, $-C\equiv N$, $-N=N-$ and $-SO_2$ groups,

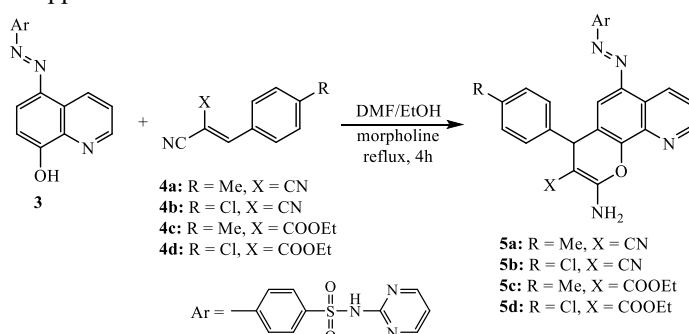
A mixture of **6** (0.48 g, 0.001 mol) and malononitrile (0.07 g, 0.001 mol) was heated on water bath in DMF (20 mL) in presence of triethylamine (0.5 mL) for 4 hrs. The reaction mixture was allowed to cool and then poured onto ice-cold water. The solid that formed was filtered and recrystallized from ethanol/DMF mixture (1:1).

Yield = 69%; m.p. > 300°C. IR (ν/cm^{-1}): 3444, 3153 (NH₂ and N-H), 2136 (C \equiv N), 1611 (C=C) and 1539 (N=N). 1H NMR (δ/ppm): 6.11 (s, 2H, NH₂), 6.57 (s, 1H, furan H-3), 7.11-8.47 (m, 12H, Ar-H), 9.77 (s, 1H, NH). MS: m/z (%) = 512 (M^+ , 62.7). Anal. Calcd. for $C_{24}H_{16}N_8O_4S$ (512): C, 56.25; H, 3.15; N, 21.86%. Found: C, 56.08; H, 3.24; N, 21.72%.

Antimicrobial evaluation and minimal inhibitory concentration (MIC) measurement:

Antimicrobial and minimal inhibitory concentration measurements (MIC) were evaluated as previously described in the reported work [37].

respectively. 1H NMR spectrum of **5a** showed singlet signal at δ 2.27 ppm attributable to CH_3 protons, singlet at δ 4.47 ppm for the proton of methin group (CH), broad singlet at δ 6.61 ppm due to the protons of NH₂, multiplet in the region δ 7.12-8.44 ppm for the aromatic protons and singlet at δ 9.11 ppm due to NH proton. Moreover, 1H NMR of **5c** displayed triplet at δ 1.28 ppm and quartet centred at δ 4.31 ppm owing to ethyl group of ester function ($-OCH_2CH_3$), singlet at δ 2.33 ppm for three protons of methyl group (CH_3), singlet at δ 4.58 ppm for the proton of methin group (CH), and singlet at δ 6.74 ppm for the protons of NH₂ group. The aromatic protons appeared as multiplet signals at δ 7.05-8.52 ppm while the NH proton appeared singlet signal at δ 9.01 ppm.

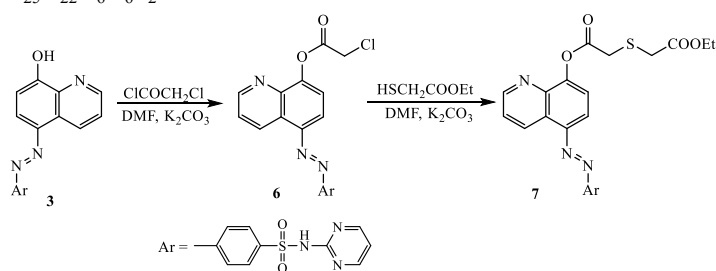


Scheme 2. Synthesis of 2-amino-4-aryl-6-((4-(N-(pyrimidin-2-yl)sulfamoyl)-phenyl)azo)-4H-pyrano[3,2-*h*]-quinoline

In addition, chloroacetylation of compound **3** has been achieved at the hydroxyl group by stirring with chloroacetyl chloride in DMF catalysed by anhydrous potassium carbonate to give the *O*-chloroacetylated product, 5-((4-(N-(pyrimidin-2-yl)sulfamoyl)-phenyl)azo)-3,7-(dihydroquinolin-8-yl)-2-chloroacetate (**6**) (Scheme 3). Compound **6** was established by both spectral and elemental analyses. Its infrared spectrum displayed absorptions at 3150, 1737, 1547 and 1348 cm^{-1} compatible with the N-H, C=O, $-N=N-$ and SO_2 functions, respectively. 1H NMR showed characteristic two singlet signals at δ 4.12 and δ 10.24 ppm due to CH_2 and NH protons. Mass spectrum displayed m/z at 482 (M^+ , 36.5%) corresponding to its correct molecular formula ($C_{21}H_{15}ClN_6O_4S$).

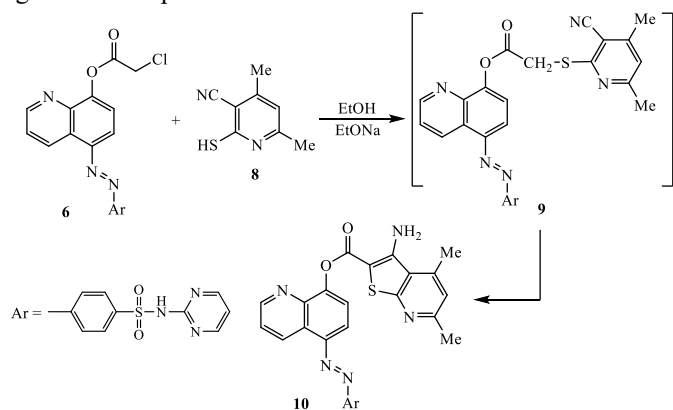
The nucleophilic substitution of the chlorine atom of compound **6** was investigated by various types of nucleophilic reagents (sulphur, nitrogen and/or carbon). Therefore, the reaction

of **6** with ethyl 2-mercaptoacetate in boiling DMF catalyzed with anhydrous potassium carbonate afforded the sulphide product, ethyl-2-((2-oxo-2-((5-((4-(*N*-pyrimidin-2-yl)sulfamoyl)-phenyl)azo)-3,7-dihydroquinolin-8-yl)oxy)ethyl)thio)acetate (**7**). Compound **7** was established by correct spectral and elemental analyses. Its infrared spectrum displayed characteristic absorptions at 3350, 1727 and 1657 cm^{-1} characteristic to the N-H, C=O (ester) and N=N functions, respectively. ^1H NMR spectrum revealed three singlet signals at δ 3.71, δ 3.84 and δ 10.65 for the protons of two methylene and NH groups, respectively. In addition to triplet at δ 1.24 ppm and quartet at δ 4.18 ppm owing to the protons of ethyl group (O-CH₂-CH₃) and the aromatic protons resonated as multiplet signals at δ 7.03-8.97 ppm. Moreover, the mass analysis gave more confirmation for the correct structure, which showed $m/z = 566$ (M^+ , 62.5%) corresponding to the formula $\text{C}_{25}\text{H}_{22}\text{N}_6\text{O}_6\text{S}_2$.



Scheme 3. Synthesis of ethyl-2-((2-oxo-2-((5-((4-(*N*-pyrimidin-2-yl)sulfamoyl)-phenyl)azo)-3,7-dihydroquinolin-8-yl)oxy)ethyl)thio)acetate

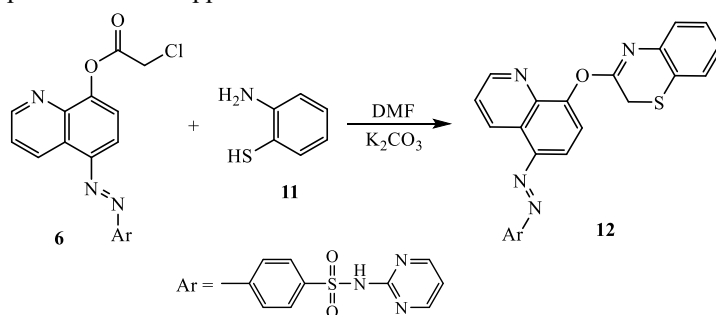
In a similar manner, when the quinolinyl 2-chloroacetate derivative **6** subjected to the reaction with 2-mercapto-4,6-dimethylnicotinonitrile (**8**) in refluxing ethanolic sodium ethoxide solution for 4 hours, the corresponding thieno[2,3-*b*]pyridine derivative **10** was picked up as the main product (Scheme 4). The reaction starts via nucleophilic substitution of the chlorine by the mercapto group of **8** to form the intermediate sulphide **9** which underwent in situ intramolecular addition of the methylene group to the nitrile function. ^1H NMR spectrum of **10** showed a characteristic four singlet signals appeared at δ 2.37, δ 2.43, broad at δ 6.68 and δ 9.66 ppm due to two methyl groups, NH₂ and NH protons respectively. The proton of pyridine C-5 resonated as singlet at δ 7.02 ppm.



Scheme 4. Synthesis of thieno[2,3-*b*]pyridine derivative

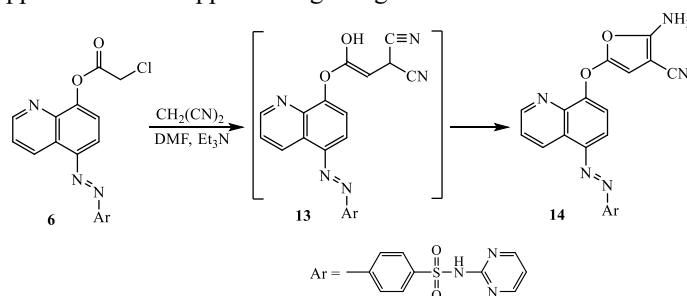
The reaction of quinolinyl 2-chloroacetate derivative **6** with *o*-aminothiophenol (**11**) proceeded in boiling DMF in presence of anhydrous potassium carbonate to afford 4-((8-((2*H*-benzo[*b*][1,4]thiazin-3-yl)oxy)quinolin-5-yl)azo)-*N*-(pyrimidin-2-yl)-benzenesulfonamide (**12**) (Scheme 5). The structure of **12** was

confirmed by spectral and elemental analyses. The infrared spectrum displayed absorptions at 3429, 1583 and 1547 cm^{-1} related to the N-H, C=N and N=N functions, respectively. ^1H NMR spectrum showed the characteristic singlet signal for -CH₂- protons at δ 3.74 ppm.



Scheme 5. Reaction of 2-chloroacetate derivative **6** with *o*-aminothiophenol

Finally, when quinolinyl 2-chloroacetate derivative **6** reacted with malononitrile (as an example of carbon nucleophile) in boiling DMF containing drops of triethylamine, the corresponding intermediate of this nucleophilic substitution reaction **13** was cyclized intramolecularly to afford 4-((8-((5-amino-4-cyanofuran-2-yl)oxy)quinolin-5-yl)-azo)-*N*-(pyrimidin-2-yl)benzenesulfonamide (**14**) (Scheme 6). The structure of **14** was established by spectral and elemental analyses. The infrared spectrum exhibited absorptions at 3444 and 3153 cm^{-1} attributed to the NH₂ and NH functions, C≡N group appeared at 2136 cm^{-1} , a characteristic band at 1611 cm^{-1} due to -C=C- group and 1539 cm^{-1} due to -N=N-. ^1H NMR spectrum displayed a characteristic singlet signal at δ 6.11 ppm due to NH₂, while the NH proton appeared at δ 9.77 ppm as singlet signal.



Scheme 6. Reaction of 2-chloroacetate derivative **6** with malononitrile

3.2. Antimicrobial activity.

The newly constructed 4-quinolinylazo-*N*-pyrimidinyl benzenesulfonamide scaffolds were tested to evaluate their antibacterial activities against Gram-positive bacteria taken *Bacillus subtilis* and *Bacillus thuringiensis* as examples and Gram-negative bacteria taken *Escherichia coli* and *Pseudomonas aeruginosa* as examples. In addition, they tested as antifungal agents (*in vitro*) against *Fusarium oxysporum* and *Botrytis fabae* fungal strains. Agar-diffusion technique [36] was applied to determine the preliminary antibacterial and antifungal activities. Chloramphenicol, cephalothin and cycloheximide were used as reference drugs. The MIC ($\mu\text{g/mL}$) and the values inhibition zone diameters are recorded in tables 1 and 2.

Results shown in Tables 1 and 2 showed that most of the tested quinolinylazo-*N*-pyrimidinyl benzenesulfonamide scaffolds have variable inhibitory effects on the growth of the Gram-

positive and Gram-negative bacteria as well as against antifungal strain. The results of structure activity relationship of the benzenesulfonamide derivatives against Gram-positive bacteria showed that compounds **5d**, **6**, **7** and **12** displayed broad-spectrum antimicrobial profile against Gram-positive bacteria. Compounds **6**, **7** and **14** were equipotent to both chloramphenicol and cephalothin in inhibiting the growth of *B. subtilis* and *B. thuringiensis* (MIC 3.125 µg/mL), while compound **5d** was equipotent to chloramphenicol against *B. subtilis* (MIC 3.125 µg/mL) and showed 25% of the activity of cephalothin against the growth of *B. thuringiensis* (MIC 12.5 µg/mL). Compounds **5c** and **10** showed 50% lower than chloramphenicol against *B. subtilis* and displayed 50% and 25% of the activity of chloramphenicol and cephalothin in inhibiting the growth of *B. subtilis* and *B. thuringiensis*, respectively. The activity of **5c** may be attributed to the high electron with drawing properties of carbethoxy, chlorine

atom and pyran moiety, while the activity of compound **10** actually related to the electron with drawing character of both pyridine and thiazole ring.

On the other hand, quinoline derivatives **5a** and **12** exhibited moderate inhibition activity against the growth of Gram-positive bacteria, their MIC values (MIC 12.5 µg/mL). The antibacterial activities of derivatives **5b** and **3** were weak against the tested Gram-positive bacteria (MIC 50.0 µg/mL). These results may be attributed to the presence of electron donating groups CH₃, OH and NH₂. While compounds **5a**, **5c**, **6** and **14**, their activity was 25% lower than of chloramphenicol and cephalothin against *E. coli*. The antibacterial activities of derivatives **3**, **5b**, **5d**, **7**, **10** and **12** were weak against the tested Gram-negative bacteria (MIC 50.0-100.0 µg/mL). Moreover, compounds **7** and **14** showed 25% lower than chloramphenicol and cephalothin against *P. aeruginosa*.

Table 1. MIC (µg/mL) and inhibition zone (mm) of the prepared 4-quinolinylazo-*N*-pyrimidinyl benzenesulfonamides as antibacterial agents.

Compound Number	MIC (µg/mL) and inhibition zone (mm)			
	Gram-positive Bacteria		Gram-negative Bacteria	
	<i>B. subtilis</i>	<i>B. thuringiensis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
3	50.0 (16)	50.0 (12)	100.0 (12)	100.0 (16)
5a	12.5 (25)	12.5 (30)	25.0 (22)	100.0 (14)
5b	50.0 (18)	50.0 (15)	100.0 (12)	100.0 (12)
5c	6.25 (32)	6.25 (30)	25.0 (20)	50.0 (19)
5d	3.125 (42)	12.5 (31)	50.0 (20)	100.0 (14)
6	3.125 (45)	3.125 (40)	25.0 (22)	50.0 (20)
7	3.125 (45)	3.125 (42)	100.0 (10)	25.0 (22)
10	6.25 (35)	12.5 (30)	100.0 (14)	50.0 (15)
12	12.5 (30)	50.0 (15)	100.0 (14)	100.0 (14)
14	3.125 (40)	3.125 (41)	25.0 (22)	25.0 (22)
Chloramphenicol	3.125 (44)	3.125 (44)	6.25 (37)	6.25 (38)
Cephalothin	6.25 (36)	6.25 (37)	6.25 (38)	6.25 (37)

MIC: Minimal inhibitory concentration value; SEM = 0.02 (the lowest concentration that inhibited the bacterial growth).

Table 2. MIC (µg/mL) and inhibition zone (mm) of the prepared 4-quinolinylazo-*N*-pyrimidinyl benzenesulfonamides as antifungal agents.

Compound Number	Fungi	
	<i>F. oxysporum</i>	<i>B. fabae</i>
3	100.0 (16)	100.0 (17)
5a	100.0 (14)	100.0 (14)
5b	100.0 (15)	100.0 (14)
5c	50.0 (19)	50.0 (21)
5d	100.0 (15)	100.0 (15)
6	25.0 (22)	25.0 (22)
7	25.0 (25)	6.25 (20)
10	50.0 (20)	25.0 (22)
12	100.0 (15)	100.0 (15)
14	25.0 (22)	25.0 (22)
Cycloheximide	3.125 (43)	3.125 (42)

MIC: Minimal inhibitory concentration value
SEM = 0.02 (the lowest concentration that inhibited the fungi growth)

The results of antifungal activities of the tested compounds showed that compound **7** was 50% lower than cycloheximide in inhibitory the growth of *B. fabae* (MIC 6.25 µg/mL). The rest of the other compounds showed moderate-weak activity. It's worth mentioning that conversion of compound **3** to pyranoquinoline derivatives **5a-d**, quinolin-8-yl 2-chloroacetate

derivative **6**, sulphide derivative **7**, thienopyridine derivative **10**, benzothiazine derivative **12** and furan-2-yl-oxy-quinoline derivative **14** promoted an excellent and potent value of antimicrobial activity which show that the presence of nitrogen and sulfur atoms improve the antimicrobial activities in comparing with chloramphenicol (drug reference).

4. CONCLUSIONS

In conclusion, in the present study some new functionalized heterocycles derived from 4-quinolinylazo-*N*-

pyrimidinylbenzenesulfonamide were synthesized and investigated as antimicrobial agents. In general, most of the tested

compounds exhibited better activity against the Gram-positive rather than the Gram-negative bacteria.

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