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# Synthesis and Biological Activity of Some Novel Heterocyclic Sulfonamides

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## ABSTRACT

*The utility of 4-isothiocyanato phenylsulfaonamide in the synthesis of some novel thiosemicarbazide, 1,3,4-thiadiazole, pyrazole, pyrazolo[3,4-b]pyridine, 2-oxopyridine and chromene derivatives is reported. All these compounds were characterized by infrared spectroscopy, $^1$H NMR, mass spectra and elemental analysis. These compounds were screened for their antimicrobial activity.*

**Keywords:** 4-isothiocyanato phenylsulfaonamide, 1,3,4-thiadiazole, Pyrazolo[3,4-b]pyridine, Chromene

## INTRODUCTION

Sulfonamides are extensively used in therapy due to their pharmacological properties [1]. Over 30 drugs containing this functionality are in clinical use, including antihypertensive agent bosentan [2], antibacterial [3], antiprotozoal [4], antifungal [5], anti-inflammatory [6], non-peptidic vasopressin receptor antagonists [7] and translation initiation inhibitors [8]. Some important sulfonamide derivatives used as carbonic anhydrase inhibitors of commercial importance [9]. They are also effective for the treatment of urinary, intestine, and ophthalmic infections, scalds, ulcerative colitis [10], rheumatoid arthritis [11], male erectile dysfunction as the phosphodiesterase-5 inhibitor sildenafil–better known under its commercial name, Viagra [12] and obesity [13]. More recently, sulfonamides have been used as an anticancer agent [14], as the antiviral HIV protease inhibitor amprenavir [15] and in Alzheimer's disease [16]. Prompted by the above facts and in continuation of our efforts in developing novel antimicrobial agents [17-20], we hereby report the synthesis and antimicrobial evaluation of some novel thiosemicarbazide, 1,3,4-thiadiazole, pyrazole, pyrazolo[3,4-*b*]pyridine, bearing sulfonamide moiety from readily available starting material.

## MATERIALS AND METHODS

All melting points were determined on a Gallenkamp apparatus and uncorrected. The purity of the compounds was checked by TLC. FT-IR spectra were recorded in a Pye-Unicam SP300 instrument in potassium bromide discs. $^1$H NMR spectra were recorded in a Varian Mercury VXR-300 spectrometer (300 MHz for $^1$H NMR) in DMSO-$d_6$ and the Chemical shifts were related to that of the solvent. Elemental analyses were carried out in the microanalytical Laboratory of Cairo University, Giza, Egypt. 3-(dimethylamino)-1-(aryl) prop-2-en-1-one 10a,b [26] and arylidenemalononitriles 13a,b [21] and 1,6-diamino-4-(4-aryl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (17a,b) [22] were synthesized using methods previously published. Antimicrobial activities were carried out at the Department of Botany and Microbiology Faculty of Science Assiut, Al-Azhar University, Egypt.

### Chemistry

#### *Synthesis of 1-(2-cyanoacetyl)-N-(4-sulfamoyl phenyl) thiosemicarbazide (4)*

To a solution of cyanoacetic acid hydrazide (0.1 mol), in 10 mL of dry 1,4- dioxane and the appropriate sulfonamide isothiocyanate 2 (0.1 mol) was added. The mixture was refluxed on a water bath for 15 min. The separated solid was filtered, washed with ethanol and crystallized from ethanol. Color: Creamy white crystals. Yield: 87%. mp: 195-197ºC. FT-IR (KBr, ν, cm$^{-1}$): 3318, 3282, 3169 (NH/NH$_2$), 2266 (C≡N), 1698 (C=O). $^1$H NMR (300 MHz, DMSO-$d_6$, δ, ppm): 3.74 (s, 2H, CH$_2$), 7.31 (s, 2H, NH$_2$ exchangable with D$_2$O), 7.69-7.80 (m, 4H, Ar-H), 9.80, 9.90, 10.40 (s, 3H, 3NH exchangable with D$_2$O); m/z 313 (M$^+$, 0.9%), 279(0.21%), 214(8.78%), 172(15%), 134(PhNCS-H; 100%), 122(19.41%), 76(23%). Anal. Calcd for $C_{10}H_{11}N_5O_3S_2$: C, 38.33; H, 3.54; N, 22.35; S, 20.47. Found: C, 38.40; H, 3.60; N, 22.40; S, 20.50%.

#### *Synthesis of 4-(3-(cyanomethyl)-5-thioxo-1H-1,2,4-triazol-4(5H)-yl)benzenesulfonamide (5)*

A mixture of 4 (0.1 mol) was dissolved in 20 mL ethanol (95%) in the presence of potassium hydroxide (5%). The reaction mixture was left to stand for 30 minutes. Yellow precipitate that formed after poured onto acidified cold water. The solid obtained was filtered and crystallized from *N*,*N*-dimethylformamide to give compound **5**. Color: Yellowish crystals. Yield: 55%. mp: 210-212ºC. FT-IR (KBr, ν, cm$^{-1}$): 3470, 3315, 3140 (NH$_2$/ NH), 2942 (CH aliph.), 2214 (C≡N), 1625 (C=N). $^1$H NMR (300 MHz, DMSO-$d_6$, δ, ppm): 4.55 (s, 2H, CH$_2$), 7.20 (s, 2H, NH$_2$ exchangable with D$_2$O), 7.69-7.79 (m, 4H, Ar-H), 11.21 (s, H, NH exchangable with D$_2$O): MS: 295 (M$^+$, 0.6%), 134 (PhNCS-H ;100%). Anal. Calcd for $C_{10}H_9N_5O_2S_2$ (295.02): C, 40.67; H, 3.07; N, 23.71; S, 21.71. Found: C, 40.70; H, 3.20; N, 23.80; S, 21.90%.

***Synthesis of 2-(5-((4-sulfamoylphenyl)amino)-1,3,4-thiadiazol-2-yl)acetamide (7)***

Thiosemicarbazide **4** (0.01, mol) was gradually added to cold concentrated sulphuric acid (3 mL, 0ºC), and the mixture was stirred at room temperature for 30 min, then the reaction mixture was poured into ice-water, filtrated after the ice melting and a few drops of ammonia were added to the filtrate till pH=8. The precipitated product was filtered, washed with cold water, dried well, and crystallized from ethanol. Color: white crystals. Yield: 60%. mp: 240-241C. FT-IR (KBr, ν, $cm^{-1}$): 3439, 3324, 3254($NH/NH_2$), 1667 (C=O): $^1$H NMR (300 MHz, DMSO-$d_6$, δ, ppm): 3.87(s, 2H, $CH_2$), 4.76, 7.18 (2s, 4H, $2NH_2$ exchangable with $D_2O$), 7.73-7.94 (m, 4H, Ar-H), 10.60 (br, H, NH exchangable with $D_2O$).MS: 313($M^+$, 1.28%), 228(42.29%), 149 (65.49%), 76 (54.0%), 64($SO_2$;100%). Anal. Calcd for $C_{10}H_{11}N_5O_3S_2$ C, 38.33; H, 3.54; N, 22.35; S, 20.47. Found: C, 38.45; H, 3.60; N, 22.56; S, 20.50%.

***4-cyano-N-(4-(N-((dimethylamino)methylene)sulfamoyl)phenyl)-3-oxo-2,3-dihydro-1H-pyrazole-1-carbothioamide (9)***

A mixture of 4 (0.01 mol), *N*,*N*-dimethylformamide-dimethylacetal (DMF-DMA) (0.015, mol) in dry 1,4-dioxane (20 mL) were refluxed for 3 h. The separated solid product obtained on standing at room temperature was collected by filtration, washed with ethanol and crystallized from 1,4-dioxane to give compound **9**. Color: Pale yellow crystals. Yield: 55%. mp: 180-181ºC. FT-IR (KBr, ν, $cm^{-1}$): 3306 (NH), 2200 (C≡N), 1625 (C=O). $^1$H NMR (300 MHz, DMSO-$d_6$, δ, ppm): 3.29 (s, 6H, $N(CH_3)_2$), 6.67 (s, H, pyrazole- H), 7.21 (s, 1H, NH exchangable with $D_2O$), 7.46, 7.67(2d, 4H, Ar-H), 8.19 (s, 1H, olefinic -H) and 9.25(s, 1H, NH exchangable with $D_2O$). MS: 378 ($M^+$, 0.34%), 269 (11.75%), 134 (44.73%), 90 (38.52%), 71 (100%). Anal. Calcd for $C_{14}H_{14}N_6O_3S_2$: (378.43). C, 44.43; H, 3.73; N, 22.21; S, 16.95. Found: C, 44.s50; H, 3.82; N, 22.30; S, 16.85%.

***General procedure for preparation of pyrazolo[3,4-b]pyridine compound 13a,b***

To a solution of thiosemcarbazide **4** (0.01, mol) and enaminone **10** (0.01, mol) in AcOH (20 mL) containing anhydrous sodium acetate (1.5 g) were refluxed for 4 h. Then, the reaction mixture was cooled to room temperature and poured onto ice cold water. The crude product was collected by filtration, washed with water and crystallized from the appropriate solvent to give 13a,b.

***3-oxo-6-phenyl-N-(4-sulfamoylphenyl)-2,3-dihydro-1H-pyrazolo [3,4-b]pyridine-1-carbothioamide (13a)***

Color: Brawn crystals. Yield: 60%. mp: 260-261ºC. FT-IR (KBr, ν, cm-1): 3430, 3350, 3268 ($NH/NH_2$), 1705 (C=O); $^1$H NMR (300 MHz, DMSO-$d_6$, δ, ppm): 5.94, 6.97 (2s, 2H, pyridine-H), 7.17 (s, 2H, $NH_2$ exchangable with $D_2O$), 7.59-7.98 (m, 9H, Ar-H+NH), 10.40 (br, 1H, NH exchangable with $D_2O$). MS:425 ($M^+$, 0.23%), 211 (10.76%), 156 (52.81%), 134 (21.75%), 108 (62.69%), 65 (100%). Anal. Calcd for $C_{19}H_{15}N_5O_3S_2$ (425.48): C, 53.63; H, 3.55; N, 16.46; S, 15.07. Found: C, 53.70; H, 3.65; N, 16.50; S, 15.20%.

***3-oxo-N-(4-sulfamoylphenyl)-6-(p-tolyl)-2,3-dihydro-1H-pyrazolo [3,4-b]pyridine-1-carbothioamide* (13b)**

Color: Pale yellow crystals. Yield 65%. m.p.: 245-246ºC. FT-IR (KBr, ν, $cm^{-1}$):3421, 3307, 3152 ($NH/NH_2$), 1660 (C=O); $^1$H NMR (300 MHz, DMSO-$d_6$, δ, ppm): 2.41 (s, 3H, $CH_3$), 5.22, 6.96 (2s, 2H, pyridine- H), 7.29 (s, 2H, $NH_2$ exchangable with $D_2O$), 7.32-7.95 (m, 8H, Ar-H), 10.90 (hump, H, NH exchangable with $D_2O$), 12.10 (br, H, NH exchangable with $D_2O$). Anal. Calcd for $C_{20}H_{17}N_5O_3S_2$ (439.51): C, 54.65; H, 3.90; N, 15.93; S, 14.59. Found: C, 54.80; H, 3.79; N, 15.85; S, 14.70%.

***General procedure for the synthesis of compounds (16a,b)***

*Method A*: A mixtures of thiosemcarbazide 4 (0.01, mol) and the appropriate arylidenmalononitrile 14 (0.01, mol) in ethanol (30 mL) containing few drops of piperidine (5 drops) was refluxed for 4 h. Then, the reaction mixture was cooled to room temperature. The solid which formed was collected by filtration, washed with hot ethanol and crystallized from ethanol to afford 16a,b.
*Method B*: Equimolar amounts of 2 (0.01, mol) and the appropriate *N*-Amino-2-pyridone derivatives 17a,b (0.01, mol) in 1,4-dioxane (30 mL) was treated with triethylamine (0.5 mL) and the reaction mixture was heated under reflux for 3 h. After cooling, the precipitate was filtered off, washed with ethanol and then recrystallized from the proper solvent to give 16a,b.

***4-(3-(6-amino-3,5-dicyano-2-oxo-4-phenylpyridin-1(2H)-yl)thioureido)- benzenesulfonamide (16a)***

Color: Yellow crystals. Yield 72%. mp: 165-166ºC. FT-IR (KBr, ν, $cm^{-1}$): 3441, 3334, 3220 ($NH/NH_2$), 2214 (C≡N), 1624 (C=O); $^1$H NMR (300 MHz,DMSO-$d_6$, δ, ppm): 3.86, 7.12 (2s, 4H, $2NH_2$ exchangable with $D_2O$), 7.22-7.98 (m, 9H, Ar-H), 10.11, 11.32 (2s, 2H, 2NH exchangable with $D_2O$). Anal. Calcd for $C_{20}H_{15}N_7O_3S_2$ (465.51): C, 51.60; H, 3.25; N, 21.06; S, 13.78. Found: C, 51.52; H, 3.30; N, 21.30; S, 13.84%.

***4-(3-(6-amino-4-(4-chlorophenyl)-3,5-dicyano-2-oxopyridin-1(2H)-yl)thioureido)benzenesulfonamide (16b)***

Color: Pale yellow crystals. Yield 68%. M.p.: 170-171ºC. FT-IR (KBr,ν, cm-1): 3328, 3243, 3126 ($NH/NH_2$), 2215 (C≡N), 1625 (C=O); $^1$H NMR (300 MHz, DMSO-$d_6$, δ, ppm): 3.86, 7.12 (2s, 4H, $2NH_2$ exchangable with $D_2O$), 7.22-7.81 (m, 8H, Ar-H), 9.40 (s, 1H, NH exchangable with $D_2O$), 10.20 (s, H, NH exchangable with $D_2O$). MS: 501(0.12%), 500 (0.17%), 499(0.11%), 294 (1.88%), 253 (11.85%), 214 (19.94%), 156 (60.47%), 92 (87.10%), 65 (100%). Anal. Calcd for $C_{20}H_{14}ClN_7O_3S_2$ (499.95): C, 48.05; H, 2.82; Cl, 7.09; N, 19.61; S, 12.83. Found: C, 48.20; H, 2.92; Cl, 7.25; N, 19.72; S, 12.90%.

***2-(2-Oxo-2H-chromene-3-carbonyl)-N-(4-sulfamoylphenyl)hydrazine- carboxamide (18)***

A mixture of thiosemcarbazide 4 (0.01, mol) and salicylaldehyde (0.01, mol) was refluxed in ethanol (25 mL) in the presence of piperidine (5 drops) for 3 h. Then, the reaction mixture was allowed to cool to room temperature, the formed crude product was collected by filtration washed with ethanol and recrystallized from dimethylformamide to give compound **18**. Color: Pale yellow crystals, yield 68% and mp:180-182ºC. FT-IR (KBr,ν, $cm^{-1}$): 3320, 3240, 3140 ($NH/NH_2$), 1706, 1652 (2C=O); $^1$H NMR (300 MHz, DMSO-$d_6$, δ, ppm): 3.88 (s, 1H,NH exchangable with $D_2O$), 7.22 (s, 2H,$NH_2$ exchangable with $D_2O$), 7.42-7.71 (m, 8H, Ar-H), 8.65 (s, 1H, chromene H-4) 9.42, 10.45 (2s, 2H, 2NH exchangable with $D_2O$). MS:403 ($M^+$-NH;3.45%), 214 (31.56%), 150 (16.59%), 135 (8.50%), 77 (12.03%), 57 (100%). Anal.Calcd for $C_{17}H_{14}N_4O_5S_2$ (418.04): C, 48.80; H, 3.37; N, 13.39; S, 15.33. Found: C, 48.90; H, 3.40; N, 13.50; S, 15.20%.

***General procedure for the synthesis of compounds (19a,b)***

A cold solution of aryl diazonium chloride (0.01, mol) was prepared by adding a solution of sodium nitrite (1.4 g dissolved in 10 mL water) to cold solution of aryl amine hydrochloride (0.01 mol of amine in 10 mL, 6M HCl) with stirring. The resulting solution of aryl diazonium chloride was then added to a cold solution of cyanoacety 4 (0.01, mol) in ethanol (50 mL) in the presence of sodium acetate trihydrate (0.015, mol). The

reaction mixture was stirred at room temperature for 1 h. The solid product was collected directly from the reaction mixture by filtration and washed with water then ethanol. Finally the obtained products were recrystallized from ethanol to afford 19a,b respectively.

***N'-(4-chlorophenyl)-2-oxo-2-(2-((4-sulfamoylphenyl) carbamothioyl)hydrazinyl)acetohydrazonoylcyanide (19a)***

Color: Orange crystals. Yield 75%. mp: 235-236ºC. FT-IR (KBr,ν, cm-1): 3330, 3240, 3254, 3180 (NH/$NH_2$), 2227 (CN), 1693 (C=O); $^1$H NMR (300 MHz, DMSO-$d_6$, δ, ppm): 5.83 (s, 2H, $NH_2$ exchangable with $D_2O$), 7.40-7.83 (m, 9H, Ar-H+NH), 10.52 (s, 1H, NH exchangable with $D_2O$) 12.60 (hump, 1H, NH exchangable with $D_2O$), 12.80 (br, H, NH exchangable with $D_2O$). MS: 454 (0.27%), 453 (0.42%), 452 (0.29%), 214 (55.79%), 156 (8.39%), 134 (100%). Anal. Calcd for $C_{16}H_{14}ClN_7O_3S_2$ (451.91): C, 42.52; H, 3.12; Cl, 7.85; N, 21.70; S, 14.19. Found: C, 42.65; H, 3.30; Cl, 7.95; N, 21.80; S, 14.25%.

***N'-(4-methoxyphenyl)-2-oxo-2-(2-((4-sulfamoyl phenyl) carbamothioyl) hydrazinyl) acetohydrazonoyl cyanide(19b)***

Color: Orange crystals. Yield 80%. M.p.: 250-252ºC. FT-IR (KBr,ν, $cm^{-1}$): 3418, 3365, 3240, 3150 (NH/$NH_2$), 2227 (CN), 1693 (C=O); $^1$H NMR (300 MHz, DMSO-$d_6$, δ, ppm): 3.57 (s, 3H, $OCH_3$), 5.74 (s, 2H, $NH_2$ exchangable with $D_2O$), 7.18-7.79 (m, 9H, Ar-H+NH), 10.48 (s, 1H, NH exchangable with $D_2O$) 12.53 (hump, 1H, NH,exchangable with $D_2O$), 13.10 (br, 1H, NH exchangable with $D_2O$). Anal. Calcd for $C_{17}H_{17}N_7O_4S_2$ (447.49): C, 45.63; H, 3.83; N, 21.91; S, 14.33. Found: C, 45.75; H, 3.90; N, 21.85; S, 14.40%.

***4-((5-(3,5-diamino-4-cyanothiophen-2-yl)-1,3,4-oxadiazol-2-yl) amino)benzenesulfonamide (21)***

Equimolar amounts of **4** (0.01, mol), malononitrile (0.01 mol) and elemental sulfur (0.01 mol) in dimethylformamide (20 mL) were treated with little amount of triethylamine (0.5 mL) and refluxed for 4 h. The solid that formed after cooling was collected and recrystallized from 1,4-dioxane/ethanol to give **21**. Color: Brawn crystals.Yield 62%. mp: >300ºC. FT-IR (KBr, ν, $cm^{-1}$): 3301, 3220, 3191 (NH/$NH_2$), 2212 (C≡N), $^1$H NMR (300 MHz, DMSO-$d_6$, δ, ppm): 3.30 (br, 2H, $NH_2$ exchangable with $D_2O$), 7.18 (s, 2H, $NH_2$ exchangable with $D_2O$), 7.70-7.71 (m, 6H, Ar-H+$NH_2$), 10.42 (s, 1H, NH exchangable with $D_2O$). Anal. Calcd for $C_{13}H_{11}N_7O_3S_2$ (377.04): C, 41.37; H, 2.94; N, 25.98; S, 16.99. Found: C, 41.45; H, 2.80; N, 25.75; S, 16.80%.

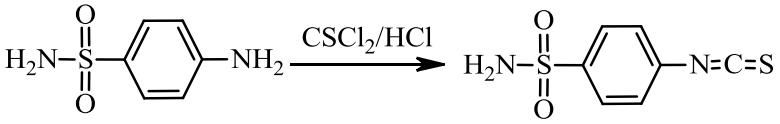
**Biological activity**

The *in vitro* antimicrobial activities of all the synthesized compounds were evaluated for five Gram-positive bacteria viz. *Bacillus Cereus, Micrococcus luteus, Enterococcus faecium, Staphylococcus aureus, Streptococcus pneumoniae* and five Gram-negative organisms' *viz. Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Salmonella enteritidis, Serattia marcescens*. Bacterial cultures were maintained in nutrient agar slants and kept at 4ºC. Each bacterial strain was reactivated prior to susceptibility testing by transferring them into a separate test tube containing nutrient broth media and incubated overnight at 37ºC. For Standardization of bacterial suspensions, Approximately 1 mL of tested bacterial culture was transferred to 9 mL of broth media of beef extract 3 g, peptone 5 g, and NaCl 5 g. The ingredients were mixed and boiled in 1000 ml distillate water, then autoclaved at 121ºC for 15 min. and incubated at 37ºC for 24 h. Antibacterial activity of tested substance was determined by saturated disks method using nutrient agar media of beef extract 3 g, peptone 5 g, NaCl 5 g and agar 20 g. The ingredients were mixed and boiled in 1000 mL distillate water, then autoclaved at 121ºC for 15 min. For each of the tested strain 200 µL of standardized bacterial stock suspensions colony were injected in 20 mL of semisolid modified nutrient agar media by sterilized tips, then this media were poured in agar plate and let to solidify. Saturated disks with the subjected chemical compound were placed on the surface of each plate then the plates incubated at 37ºC for 24 h. After incubation, the diameters of the inhibition zones for each well were measured in millimeters (mm) in three replicates.

## RESULTS AND DISCUSSION

**Chemistry**

4-Isothiocyanato phenyl sulfonamide 2 was synthesized *via* thiophosgenation of sulfanilamide 1 at room temperature in the presence of dilute hydrochloric acid (Scheme 1) [23].




1 2

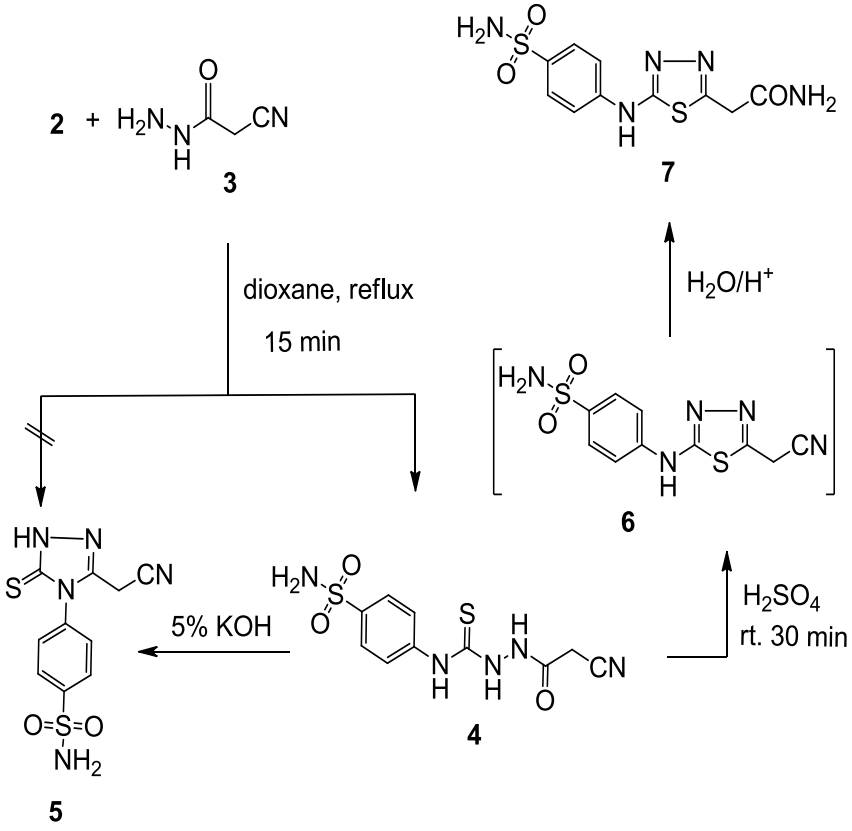
**Scheme 1: Synthesis of 4-isothicyanteo phenyl sulfonamide 2**

Treatment of isothiocyanate derivative 2 with cyanoacetic acid hydrazide 3 at reflux temperature furnished the novel thiosemicarbazide derivative 4 in high yield (87%) not 1,2,4-triazol-3-thione 5 (Scheme 2). The structure of compound 4 was established on the basis of microanalysis and spectral data. The infrared spectrum of compound 4 showed absorption bands at 3318, 3282, 3169 $cm^{-1}$ assigned to NH/$NH_2$ groups, at 2968 $cm^{-1}$ assigned to CH-aliph, at 2266 $cm^{-1}$ assigned to C≡N group and 1698 $cm^{-1}$ assigned to C=O group. In addition, the structure of compound 4 was supported by its $^1$H NMR spectrum (DMSO-$d_6$) which showed singlet at δ 3.74 ppm assigned to methylene protons, a broad singled at 7.31 ppm assigned to the amino protons and two doublets at δ 7.69, 7.80 ppm assigned to the aromatic protons and the presence of three signals at δ 9.80, 9.90, 10.4 ppm assigned to the 3NH protons. The mass spectrum of compound 4 showed a molecular ion peak at m/z=313 corresponding to a molecular formula $C_{10}H_{11}N_5O_3S_2$ and the base peak was found in the spectrum at m/z=134 (PhNCS -H, 100%). The later compound 5 was obtained by the treatment of cyanoacetyl thiosemicarbazide 4 with 5% potassium hydroxide solution in ethanol at room temperature. The structure of compound 5 is confirmed by its infrared spectrum which showed the disappearance of carbonyl group. Also, the mass spectrum exhibited a molecular ion peak at m/z=295 which corresponding to the molecular formula ($C_{10}H_9N_5O_2S_2$). Treatment of thiosemicarbazide derivative 4 with concentrated sulfuric acid afforded 2-(5-(4-sulfamoylphenyl) amino)-1,3,4-thiadiazol-2-yl) acetamide 7.

The other possible structure 6 was excluded on the basis of spectral data. The infrared spectrum of compound 7 revealed the lack of absorption band corresponding to a nitrile function and showed bands at 3439, 3324, 3254 $cm^{-1}$ assigned to the $NH/NH_2$ groups. The $^1H$ NMR spectrum (DMSO-$d_6$) of the reaction product which showed a singlet at δ 3.87 ppm assigned to the methylene protons, a singlet at δ 4.51 assigned to the amine protons, abroad singlet at δ 7.18 ppm assigned to the sulfamoyl protons, a multiplete at δ 7.73-7.94 ppm assigned to the aromatic protons and downfield singlet at δ 10.60 ppm assigned to NH proton. The mass spectrum of compound 7 was compatible with the molecular formula $C_{10}H_{11}N_5O_3S_2$. The base peak was found in the spectrum at m/z=64 ($SO_2$, 100%) (Chart I). The formation of compound 7 is assumed to take place via a ring closure reaction and hydrolysis [24] of the nitrile function (Scheme 2).

Treatment of isothiocyanate derivative 2 with cyanoacetic acid hydrazide 3 at reflux temperature furnished the novel thiosemicarbazide derivative 4 in high yield (87%) not 1,2,4-triazol-3-thione 5 (Scheme 2). The structure of compound 4 was established on the basis of microanalysis and spectral data. The infrared spectrum of compound 4 showed absorption bands at 3318, 3282, 3169 $cm^{-1}$ assigned to $NH/NH_2$ groups, at 2968 $cm^{-1}$ assigned to CH-aliph, at 2266 $cm^{-1}$ assigned to C≡N group and 1698 $cm^{-1}$ assigned to C=O group. In addition, the structure of compound 4 was supported by its $^1H$ NMR spectrum (DMSO-$d_6$) which showed singlet at δ 3.74 ppm assigned to methylene protons, a broad singled at 7.31 ppm assigned to the amino protons and two doublets at δ 7.69, 7.80 ppm assigned to the aromatic protons and the presence of three signals at δ 9.80, 9.90, 10.4 ppm assigned to the 3NH protons. The mass spectrum of compound 4 showed a molecular ion peak at m/z=313 corresponding to a molecular formula $C_{10}H_{11}N_5O_3S_2$, and the base peak was found in the spectrum at m/z=134 (PhNCS-H, 100%). The later compound 5 was obtained by the treatment of cyanoacetyl thiosemicarbazide 4 with 5% potassium hydroxide solution in ethanol at room temperature.

The structure of compound 5 is confirmed by its infrared spectrum which showed the disappearance of carbonyl group. Also, the mass spectrum exhibited a molecular ion peak at m/z=295 which corresponding to the molecular formula ($C_{10}H_9N_5O_2S_2$). Treatment of thiosemicarbazide derivative 4 with concentrated sulfuric acid afforded 2-(5-(4-sulfamoylphenyl)amino)-1,3,4-thiadiazol-2-yl)acetamide 7. The other possible structure 6 was excluded on the basis of spectral data. The infrared spectrum of compound 7 revealed the lack of absorption band corresponding to a nitrile function and showed bands at 3439, 3324, 3254 $cm^{-1}$ assigned to the $NH/NH_2$ groups. The $^1H$ NMR spectrum (DMSO-$d_6$) of the reaction product which showed a singlet at δ 3.87 ppm assigned to the methylene protons, a singlet at δ 4.51 assigned to the amine protons, abroad singlet at δ 7.18 ppm assigned to the sulfamoyl protons, a multiplete at δ 7.73-7.94 ppm assigned to the aromatic protons and downfield singlet at δ 10.60 ppm assigned to NH proton. The mass spectrum of compound 7 was compatible with the molecular formula $C_{10}H_{11}N_5O_3S_2$. The base peak was found in the spectrum at m/z=64 ($SO_2$, 100%) (Chart I). The formation of compound 7 is assumed to take place via a ring closure reaction and hydrolysis [24] of the nitrile function (Scheme 2).




**Scheme 2: Synthesis of thiosemicarbazide 4, 1,2,4-triazol-3-thione 5 and 1,3,4-thiadiazol-2-yl 7 derivatives**

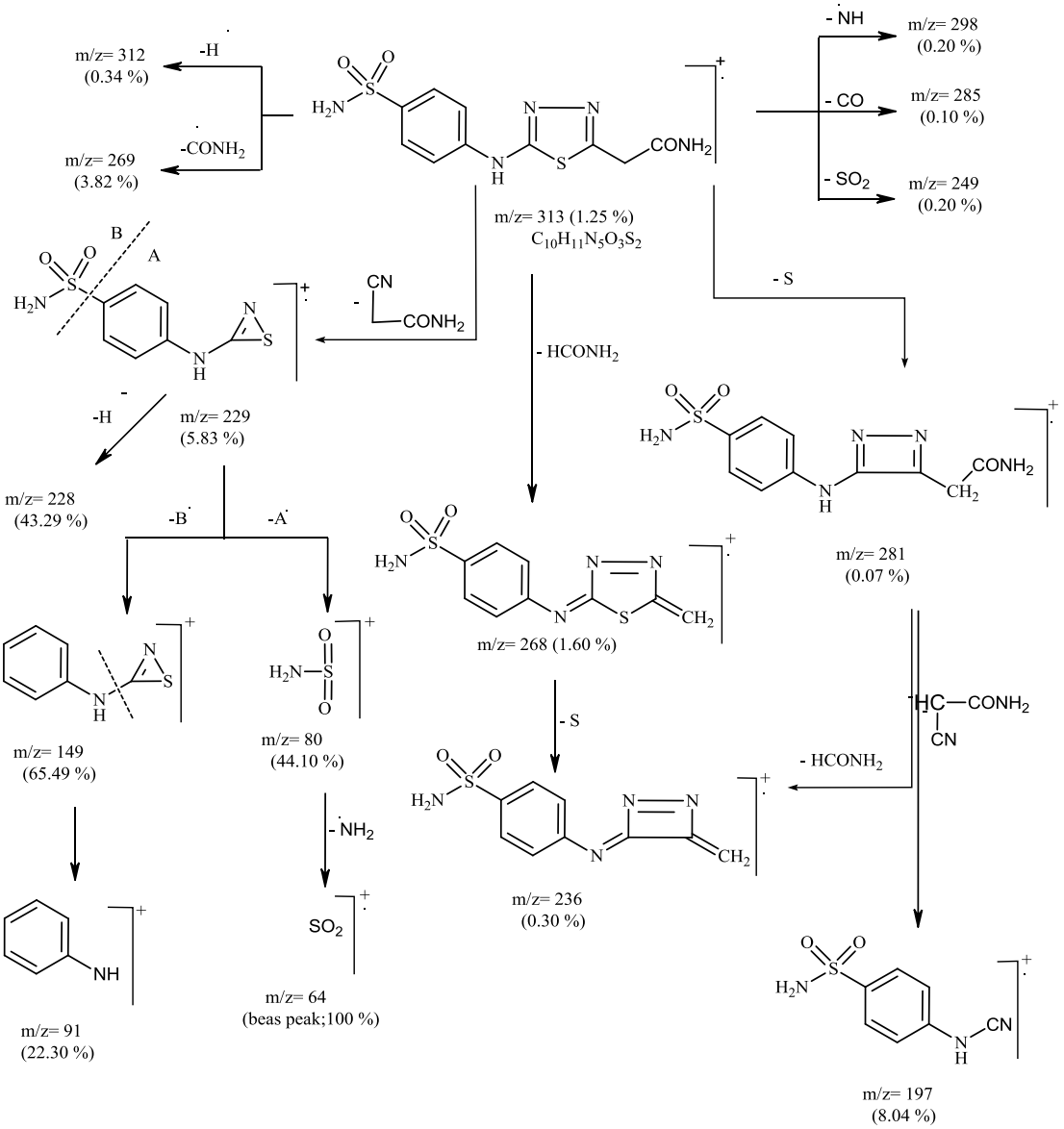
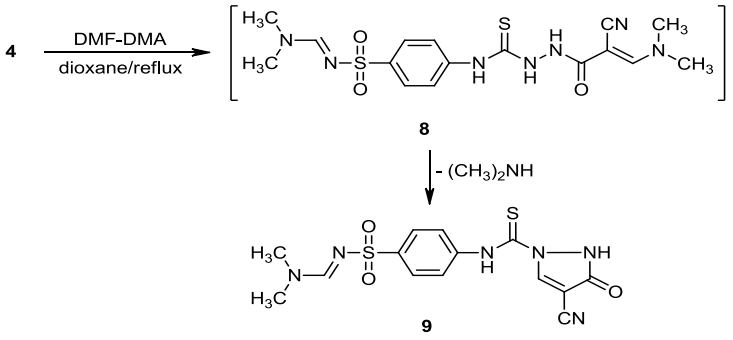



**Chart I: Fragmentation pattern of compound (7).**

The reactivity of the cyanoacetyl derivative 4 towards some electrophiles was investigated. Thus, treatment of cyanoacetyl 4 with dimethylformamide-dimethylacetal (DMF-DMA) in dry 1,4-dioxane under reflux furnished the novel pyrazole derivative 9 (Scheme 3). The structure of the isolated product was established on basis of its analytical and spectral data. The infrared spectrum of the reaction product is characterized by the presence of the NH, C≡N and C=O groups. The $^1$H NMR spectrum (DMSO-$d_6$) exhibited the absence of methylene protons and showed a singlet at δ 3.29 ppm assigned to the $N(CH_3)_2$ protons, a singlet at δ 6.67 ppm assigned to the pyrazole proton, a singlet at δ 7.21 ppm assigned to the NH proton, two doublets at δ 7.46, 7.67 ppm assigned to the aromatic protons and two downfield signals at δ 8.19, 9.25 ppm assigned to the methine and NH protons, respectively. The mass spectrum showed a molecular ion peak at *m/z*=378 compatible with the molecular formula $C_{14}H_{14}N_6O_3S_2$ with base peak at m/z=71 (Chart II).

The formation of 9 is assumed to proceed through the intermediate 8 followed by intramolecular cyclization *via* dimethyl amine elimination (Scheme 3) [25].




**Scheme 3: 4-cyano-3-oxo-2,3-dihydro-1H-pyrazole-1-carbothioamide derivative 9**

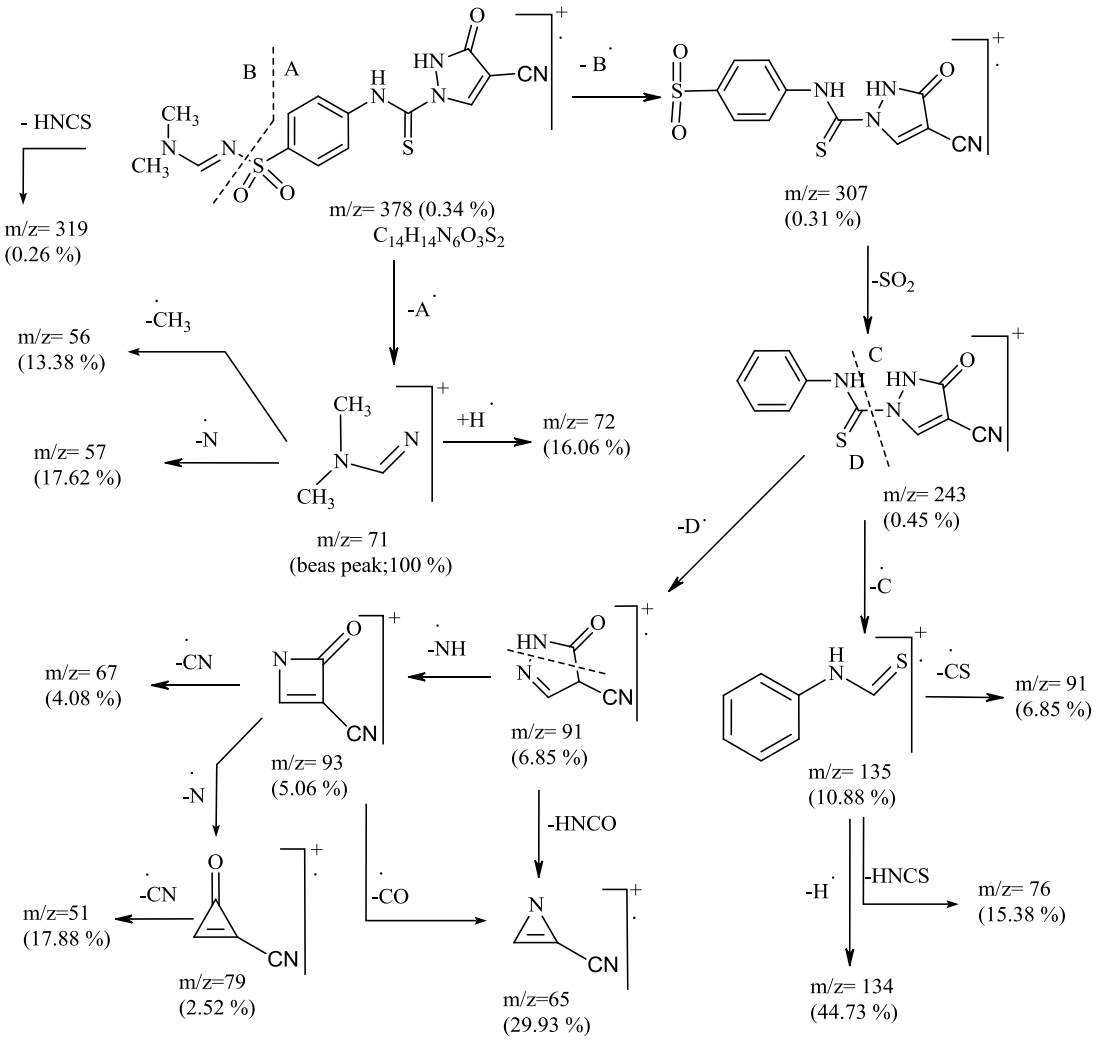
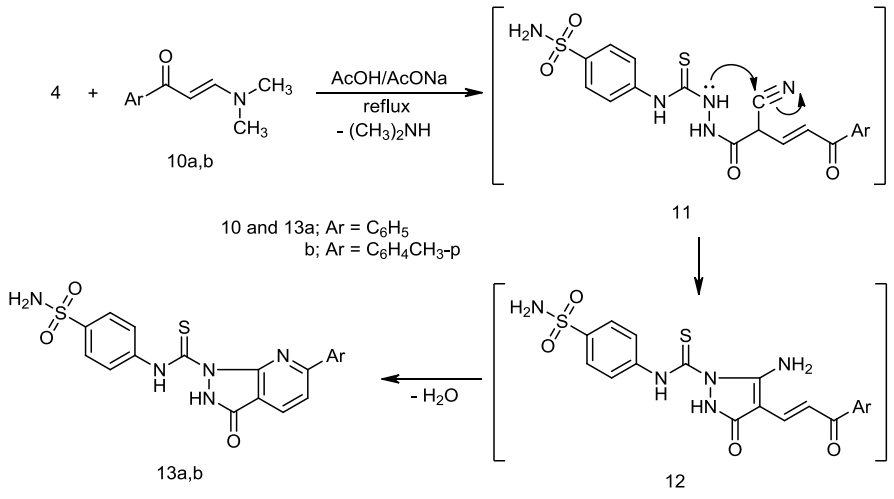


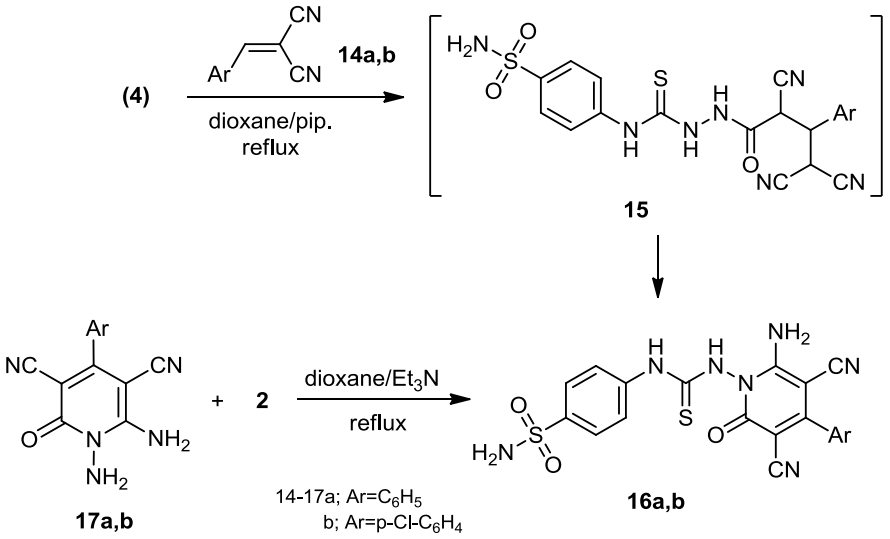

**Chart II: Fragmentation pattern of compound (9)**

The cyanoacetyl derivative 4 was reacted with enaminones 10 a,b at reflux temperature in the presence of acetic acid and fused sodium acetate to give the novel pyrazolo[3,4-*b*]pyridine derivatives 13 a,b (Scheme 4). The structures of compounds 13 a,b were established on the basis of their elemental analysis and spectral data. The infrared spectra of compounds 13 a,b were free of nitrile function and showed absorption bands for $NH_2$ and C=O groups. The $^1$H NMR spectrum (DMSO-$d_6$) of compound 13 a revealed two singlets at δ 5.94, 6.97 ppm which were readily assigned to the hydrogen atoms attached at C-3 and C-4 of the pyridine ring, respectively. Moreover, the mass spectrum for the pyrazolopyridine derivative 13 a showed a molecular ion peak at m/z=425 corresponding to the molecular formula $C_{19}H_{15}N_5O_3S_2$. The formation 13 is assumed to be formed via the addition of the active methylene group of 4 to the activated double bond in 10 followed by elimination of dimethyl amine molecule affording 11 which underwent an intramolecular cyclization to give the pyrazolo[3,4–*b*]pyridine derivative 13 (Scheme 4).



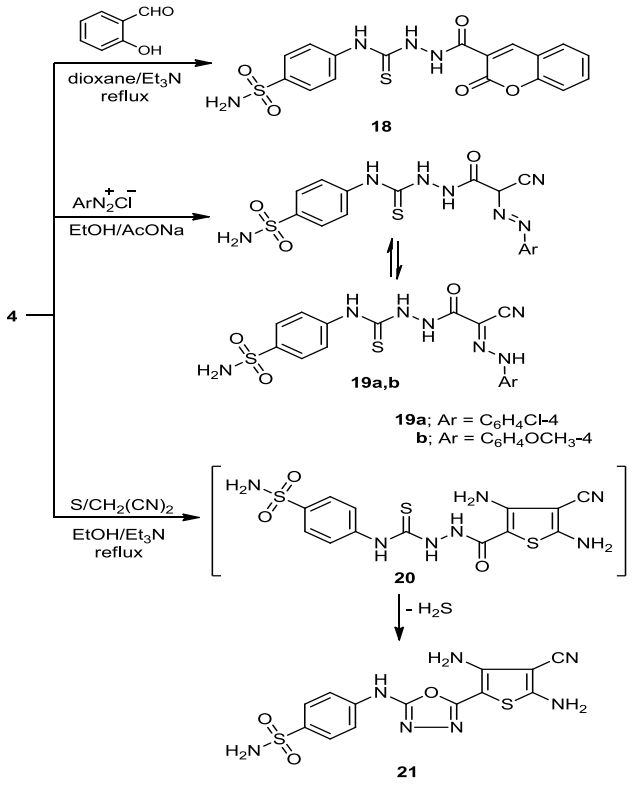

**Scheme 4: pyrazolo[3,4-*b*]pyridine derivatives 13a,b**

The reaction of cyanoacetyl derivative 4 with benzylidene malononitriles 14 in refluxing ethanol in the presence of piperidine gave the 6-amino-3,5-dicyano-2-oxopyridine derivatives 16 a,b (Scheme 4). The structure of the isolated product was established on basis of its analytical and spectral data. The mass spectrum of compound 16 b showed a molecular ion peak at *m/z*=499 compatible with the molecular formula $C_{20}H_{14}ClN_7O_3S_2$. Furthermore, the structure of compound 16 was supported chemically by the reaction of *N*-aminopyridine derivatives 17 a,b with 4-isothiocyanato phenyl sulfonamide 2 in boiling 1,4-dioxane in the presence of catalytic amount of triethylamine. The formation of pyridine 16 can be explained on the basis of an initial *Michael* addition of the active methylene in 4 to the activated double bond in 14 followed by intramolecular cyclization (Scheme 5) [26].




**Scheme 5: Synthesis of 6-amino-3,5-dicyano-2-oxopyridine derivatives 16 a,b**

Chromene derivative 18 was achieved by cyclocondensation of salicylaldehyde with cyanoacetyl derivative 4 in refluxing ethanol and piperidine. The infrared spectrum of compound 18 indicated the absence of nitrile group. The *N'*-(4-aryl)-2-oxo-2-(2-((4-sulfamoylphenyl) carbamothioyl)hydrazinyl)acetohydrazonoylcyanide 19 a,b were obtained by treatment of cyanoacetyl derivative 4 with aromatic diazonium salts on the basis of spectral data. *Gewald* reaction [27] of cyanoacetyl derivative 4 with elemental sulfur and malononitrile at reflux temperature in 1,4-dioxane in the presence of catalytic amount of triethylamine afforded the novel 3,5-diamino-4-cyanothiophene derivative 21. The formation of 21 is assumed to proceed *via* intermediate 20 followed by elimination of hydrogen sulfide molecule (Scheme 6).




**Scheme 6: Synthesis of chromene 18, hydrazone 19 and 3,5-diaminothiophene 21 derivatives**

***In vitro* antimicrobial activity**

The *in vitro* antimicrobial activities of all the synthesized compounds were evaluated for five Gram-positive bacteria viz. *B. Cereus, M. luteus, E. faecium, S. aureus, S. pneumoniae* and five Gram-negative organisms' viz. *E. coli, K. pneumoniae, P. mirabilis, S. enteritidis, S. marcescens* by a modified twofold serial dilution method [28,29]. All target compounds were evaluated at the concentrations 250 μg $mL^{-1}$ and scored for minimum inhibitory concentrations (MICs, μg $mL^{-1}$) that defined as the lowest concentrations of the compound at which microbial growth was inhibited. Ampicillin 25 μg $mL^{-1}$ was assayed as positive control against tested bacteria. The results of antimicrobial screening data revealed that most of the synthesized compounds showed varying degrees of inhibition against the Gram-positive bacteria rather than the Gram-negative bacteria. All compounds Showed weak antibacterial activity against *S. pneumonia, S. aureus, S. marcescens, S. enteritidis*. In addition, all test compounds were found to be inactive against *E. faecium, E. coli, K. pneumonia* and *P. mirabilis.* The antibacterial was depicted in Table 1.

**Table 1: Antimicrobial activity of some the newly synthesized compounds**

| Comp. no. | Gram positive bacteria | | | | | Gram negative bacteria | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Bacillus cereus* | *Micrococcus luteus* | *Streptococcus pneumonia* | *Staphylococcus aureus* | *Enterococcus faecium* | *Escherichia coli* | *Serattia marcescens* | *Klebsiella pneumonia* | *Proteus mirabilis* | *Salmonella enteritidis* |
| 4 | 13 | 12 | 5 | 11 | - | - | 2 | - | - | - |
| 5 | 9 | 10 | **5** | 10 | - | - | 2 | - | - | - |
| 7 | 7 | 11 | - | 14 | - | - | 4 | - | - | 3 |
| 9 | 7 | 13 | - | 11 | - | - | 3 | - | - | 5 |
| 13a | 5 | 12 | - | 12 | - | - | 3 | - | - | 6 |
| 13b | 13 | 11 | 8 | 11 | - | - | 4 | - | - | 4 |
| 16a | 11 | 11 | - | 13 | - | - | 4 | - | - | 5 |
| 16b | 12 | 12 | 6 | 13 | - | - | 5 | - | - | - |
| 18 | 6 | 13 | 7 | 10 | - | - | 3 | - | - | - |
| 19a | 8 | 14 | 5 | 10 | - | - | 3 | - | - | - |
| 19b | 8 | 12 | 5 | - | - | - | 5 | - | - | - |
| 21 | - | 13 | 7 | 12 | - | - | 5 | - | - | - |
| Amp | 22 | 22 | 13 | 30 | 19 | 12 | 10 | 13 | 14 | 12 |

Nill=(-), Less active (02-06 mm), moderately active (7-13 mm), highly active (14-20 mm), and very highly activity (over 21 mm). Standard: For G +ve and G-ve bacteria: Ampicillin 25 μg/mL-1.

## CONCLUSION

The reactivity of 1-(2-cyanoacetyl)-N-(4-sulfamoyl phenyl) thiosemicarbazide (4) was investigated as a versatile and readily accessible building block for the synthesis of new heterocyclic compounds such as 1,2,4-triazole,1,3,4-thiadiazole, pyrazole,and pyrazolo[3,4-*b*]pyridine derivatives incorporating a sulfamoyl moiety of antibacterial importance.

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