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Synthesis and Antimicrobial Evaluation of Some New Thienopyrazoles, Pyrazolothienopyridines and Pyrazolothienopyrimidines via Gewald reaction

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ABSTRACT

*5-Amino-3-methyl-1-phenyl-1H-thieno[3,2-c]pyrazole-6-carbonitrile (**3a**) was synthesized via Gewald reaction and used as starting materials for the synthesis of new series of, pyrazolo[3',4':4,5]thieno[2,3-b]pyridine derivatives and pyrazolo[3',4':4,5]thieno[2,3-d]-pyrimidine derivatives. Also ethyl 5-amino-3-methyl-1-phenyl-1H-thieno[3,2-c]pyrazole-6-carboxylate (**3b**) was used to synthesize a new series of pyrazolo[3',4':4,5]thieno[2,3-d]-pyrimidine derivative. The structure of the newly synthesized compounds was established based on elemental analysis and IR, ^{1}H NMR, ^{13}C NMR and MS spectra. Some of the newly synthesized compounds were screened for antibacterial and antifungal activity.*

Keywords: Gewald reaction, Thienopyrazoles, Pyrazolothienpyridines, Pyrazolothienopyrimidines, Antimicrobial Activities.

1. INTRODUCTION

Recently some reviews devoted to the biological activities [1,2], and the chemistry [3], of pyrazole derivatives were reported. Pyrazoles are good synthon for thienopyrazoles which are an important class of antimicrobial [4,5], CHK1 inhibitors [6], anti-oxidants against toxicity of 4-nonylphenol in Clarias gariepinus [7], anticancer [8], kinase inhibitors [9]. Moreover, pyrazolothienopyridines were reported to possess anti-Alzheimer [10], antibacterial [11], and anticancer [11], activities. In addition, pyrazolothienopyrimidines have considerable pharmacological importance as antimicrobial [4,12], anti-parkinsonism, hypoglycemic [12], CHK1 inhibitors [6], anti-tuberculosis [13], anti-inflammatory [5,14], analgesic [14], anticancer[8], activities. In continuation of our program in the synthesis of biologically active heterocyclic compounds [15-23], we report herein the synthesis of some new thienopyrazole, pyrazolothienopyridine, pyrazolo thienopyrimidinone via Gewald reaction and the study of their antimicrobial activities.

2. MATERIALS AND METHODS

2.1 Chemistry

All chemicals and solvents used are of analytical grade and were purchased from Sigma and Aldrich Chemical Co. All melting points are uncorrected. IR spectra (KBr) were recorded on a FTIR 5300 spectrometer (ν, cm^{-1}). The ^{1}H NMR and ^{13}C-NMR spectra were recorded in DMSO-d_6 at 200, 300 MHz on a Varian Gemini NMR spectrometer (δ, ppm) using TMS as an internal standard. Mass spectra were obtained on GC Ms-QP 1000 EX mass spectrometer at 70 ev. Elemental analyses were carried out by the Micro analytical Research Center, Faculty of Science, Cairo University and Assiut University. Microbiology screening was carried out in Botany Department, Faculty of Science, Al-Azhar University, Assiut. Compounds **1** [23], and **3b** [4], were prepared according the literature procedures.

5-Amino-3-methyl-1-phenyl-1H-thieno[3,2-c]pyrazole-6-carbonitrile (3a):
A mixture of compound **1** (0.01 mole), with malononitrile **2** (0.01 mole) and elemental sulfur (0.01 mole) in absolute ethanol (30 mL) containing few drops of triethylamine was refluxed for 6 h. The reaction mixture was cooled and the solid product so formed was collected and crystallized from the proper solvent to give **3,** as brown crystals from ethanol; yield 80%; mp. 215˚C; IR (KBr) ν cm^{-1} 3415, 3312 (NH_2), 3058 (CH-arom.), 2965 (CH-aliph.), 2225 (CN); ^{1}H NMR (DMSO-d_6) δ = 2.72 (s, 3H, CH_3), 6.86 (s, 2H, NH_2), 7.35-7.64 (m, 5H, Ar-H); ^{13}C NMR (DMSO-d_6): 14.5 (CH_3), 115.3 (CN), 82.4, 108.6, 124.3, 126.5, 128.5, 129.8, 139.1, 140.8, 150.2 (Ar-C); MS: m/z = 254 [M^+]. Anal. Calc. For $C_{13}H_{10}N_4S$ (254.31): C, 61.40; H, 3.96; N, 22.03; S, 12.61%.Found: C, 61.63; H, 3.75; N, 22.24; S, 12.82%.

2.2 Preparation of Compounds 4 and 6: General Procedure
A solution of **3a** (0.01 mole) and malononitrile or cyanothioacetamide (0.01 mole) in ethanol (30 mL) containing piperidine (0.5 mL), was heated under reflux for 8 hours. The reaction mixture was cooled, poured onto ice-cooled water and acidified with dilute HCl. The solid product so formed was filtered off and crystallized from the proper solvent to give compounds **4** and **6**, respectively.

6,8-Diamino-3-methyl-1-phenyl-1H-pyrazolo [3',4':4,5]thieno[2,3-b]-pyridine-7-carbonitrile (4)
It was obtained brown crystals from ethanol / dioxane; yield 60%; m.p. 298˚C; IR (KBr): υ cm^{-1} 3407, 3332 ($2NH_2$), 3055 (CH-arom.), 2927 (CH-aliph.), 2213 (CN); ^{1}H NMR (DMSO-d_6) δ = 2.65 (s, 3H, CH_3), 6.22 (s, 2H, NH_2), 7.42-7.77 (m, 7H, Ar-H + NH_2). Anal. Calc. for $C_{16}H_{12}N_6S$ (320.37): C, 59.98; H, 3.78; N, 26.23; S, 10.01% .Found: C, 59.77; H, 3.56; N, 26.45; S, 10.23 %.

8-Amino-3-methyl-1-phenyl-6-thioxo-5,6-dihydro-1H-pyrazolo-[3',4':4,5]-thieno[2,3-b]pyridine-7-carbonitrile (6)
It was obtained as brown crystals from DMf / ethanol; yield 55%; m.p. >300˚C; IR (KBr): υ cm^{-1} 3427, 3325, 3218 (NH_2 and NH), 3076 (CH-arom.), 2947 (CH-aliph.), 2218 (C≡N); ^{1}H NMR (DMSO-d_6) δ = 2.42 (s, 3H, CH_3), 6.75 (s, 1H, NH_2), 7.38-7.60 (m, 5H, Ar-H) and 7.70 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6): 15.2 (CH_3), 115.8 (CN), 88.3, 107.7, 124.5, 125.4, 126.7, 128.2, 129.1, 130.6, 138.8, 140.5, 154.4, 158.8 (Ar-C), 188.7 (CS). MS: m/z = 337 [M^+]. Anal. Calc. for $C_{16}H_{11}N_5S_2$ (337.42): C, 56.95; H, 3.29; N, 20.76; S, 19.01% .Found: C, 56.72; H, 3.41; N, 20.97; S, 19.21%.

8-Amino-3-methyl-1,6-diphenyl-1H-pyrazolo [3',4':4,5]thieno[2,3-b]-pyridine-7-carbonitrile (7a):
A mixture of compound **3a** (0.01 mole), benzylidenemalononitrile (0.01mole) and piperidine (0.5 mL) in ethanol (50 mL) was heated under reflux for 8 h, allowed to cool, and poured into ice / H_2O and acidified with HCl. The solid product was collected and recrystallized from dioxane to give **7a** (73%) as pale yellow crystals, m.p. >300˚C; IR (KBr): υ cm^{-1} 3344, 3227 (NH_2), 3082 (CH-arom.), 2936 (CH-aliph.), 2215 (C≡N); ^{1}H NMR (DMSO-d_6) δ = 2.71 (s, 3H, CH_3), 6.27 (s, 2H, NH_2), 7.35- 8.33 (m, 10H, Ar-H). Anal. Calc. for $C_{22}H_{15}N_5S$ (381.45): C, 69.27; H, 3.96; N, 18.36; S, 8.41% .Found: C, 69.48; H, 3.74; N, 18.58; S, 8.71%.

8-Amino-3,6-dimethyl-1-phenyl-1H-pyrazolo [3',4':4,5]thieno[2,3-b]-pyridine-7-carbonitrile (7b):
A solution of equimolar amounts of acetaldehyde and malononitrile (0.01 mole) in absolute ethanol (50 mL) was added to a suspension of **3a** (0.01 mole) in absolute ethanol (20 mL) and piperidine (0.5 mL). The reaction mixture was heated under reflux for 8 h. The solvent was then evaporated under reduced pressure and the solid product was recrystallized from dioxane to give **7b,** (71%) as pale yellow crystals, m.p. 290˚C; IR (KBr): υ cm^{-1} 3376, 3215 (NH_2), 3042 (CH-arom.), 2963 (CH-aliph.), 2217 (C≡N); ^{1}H NMR (DMSO-d_6) δ = 2.45 (s, 3H, CH_3), 2.75 (s, 3H, CH_3), 6.27 (s, 2H, NH_2), 7.41- 7.64 (m, 5H, Ar-H). Anal. Calc. for $C_{17}H_{13}N_5S$ (319.38): C, 63.93; H, 4.10; N, 21.93; S, 10.04 % .Found: C, 63.72; H, 4.33; N, 21.70; S, 10.26%.

N'-(6-Cyano-3-methyl-1-phenyl-1H-thieno[3,2-c] pyrazol-5-yl)-N,N-dimethyl-methanimidamide (8):
A solution of **3a** (0.01 mol) in dry xylene (30 mL) and DMF-DMA (0.012 mol) was heated under reflux for 5 h. The reaction mixture was then cooled. The solid product thus formed was filtered off and crystallized from ethanol to give **8** as yellow crystals, yield 60%; m.p. 174˚C. IR (KBr): υ cm^{-1} 3088 (CH-arom.), 2984 (CH-aliph.), 2212 (CN); ^{1}H NMR (DMSO-d_6) δ = 2.64 (s, 3H, CH_3), 3.62 (s, 6H, $N(CH_3)_2$), 7.37- 7.62 (m, 5H, Ar-H), 7.95 (s, 1H, N=CH). Anal. Calc. for $C_{16}H_{15}N_5S$ (309.39): C, 62.11; H, 4.89; N, 22.64; S, 10.36 % .Found: C, 62.33; H, 4.68; N, 22.85; S, 10.57 %.

8-Hydrazinyl-3-methyl-1-phenyl-1H-pyrazolo [3',4':4,5]thieno[2,3-d]-pyrimidine (10):
To a solution of **8** (0.01 mol) in ethanol (30 mL), hydrazine hydrate (2 mL) was added. The reaction mixture was heated under reflux for 5 h. The solid product that formed after cooling was collected by filtration and recrystallized from dioxane to give **10** as yellow crystals, yield 51%; mp. 296˚C. IR (KBr): υ cm^{-1} 3418, 3342 and 3225 (NH_2 and NH), 3070 (CH-arom.), 2985 (CH-aliph.); ^{1}H NMR (DMSO-d_6) δ = 2.70 (s, 3H, CH_3), 5.72 (s, 2H, NH_2), 7.36-7.70 (m, 5H, Ar-H) , 7.92 (s, 1H, NH) and 8.15 (s, 1H, C_6-H). ^{13}C-NMR (DMSO-d_6): 14.6 (CH_3), 107.8, 118. 2, 124.3, 126.7, 128.5, 129.8, 138.4, 139.6, 140.5, 145.9, 147.0, 156.2 (Ar-C), MS: m/z = 296 [M^+]. Anal. Calc. for $C_{14}H_{12}N_6S$ (296.35): C, 56.74; H, 4.08; N, 28.36; S, 10.28 % .Found: C, 56.95; H, 4.27; N, 28.58; S, 10.51 %.

3-Methyl-1-phenyl-1H-pyrazolo[3',4':4,5]thieno [2,3-d]pyrimidin-8-amine (11):
A solution of compound **3** (0.01 mol) in formamide (10 mL) was heated under reflux for 6 h, then allowed to

cool and poured into cold water. The solid product was collected and crystallized from ethanol / dioxan to give **11** as green crystals, yield 63%; m.p. > 300◦C. IR (KBr): υ cm^{-1} 3410, 3338 (NH_2), 3058 (CH-arom.), 2927 (CH-aliph.); ^{1}H NMR (DMSO-d_6) δ = 2.73 (s, 3H, CH_3), 7.35-7.82 (m, 5H, Ar-H), 7.95 (s, 2H, NH_2) and 8.28 (s, 1H, C_6-H). Anal. Calc. for $C_{14}H_{11}N_5S$ (281.34): C, 59.77; H, 3.94; N, 24.89; S, 11.40 % .Found: C, 59.97; H, 3.73; N, 24.56; S, 11.62%.

5-(Benzylideneamino)-3-methyl-1-phenyl-1H-thieno[3,2-c]pyrazole-6-carbonitrile (12):
A mixture of compound **3** (0.01 mole), benzaldehyde (0.01 mole) and a few drops of piperidine in ethanol (30 mL) was refluxed for 6 h. The solid precipitate produced on hot was collected by filtration and recrystallized from dioxane to give **12** as white crystals, yield 70%; mp. 260˚C. IR (KBr): υ cm^{-1} 3077 (CH-arom.), 2924 (CH-aliph.), 2210 (CN); ^{1}H NMR (DMSO-d_6) δ = 2.65 (s, 3H, CH_3), 7.41-8.0 (m, 10H, Ar-H) and 8.72 (s, 1H, N=CH). Anal. Calc. for $C_{20}H_{14}N_4S$ (342.42): C, 70.15; H, 4.12; N, 16.36; S, 9.36 % .Found: C, 70.36; H, 4.33; N, 16.59; S, 9.57%.

5-Amino-3-methyl-1-phenyl-1H-thieno[3,2-c]pyrazole-6-carboxamide (13):
A sample of compound **3** (0.01 mole) was dissolved in conc. Sulfuric acid (10 mL) was stirred at room temperature for 15 h. The reaction mixture was diluted with ice-cold water and neutralized with ammonium hydroxide. The resulting precipitate was collect by filtration and recrystallized from ethanol to give **13** as brown crystals, yield 73%; m.p.; 233 ˚C. IR (KBr): υ cm^{-1} 3420, 3334, 3227 and 3185 ($2NH_2$), 3075 (CH-arom.), 2921 (CH-aliph.) and 1653 (CO); ^{1}H NMR (DMSO-d_6) δ = 2.62 (s, 3H, CH_3), 5.17 (s, 2H, NH_2), 6.91 (s, 2H, $CONH_2$), 7.39-7.63 (m, 5H, Ar-H). Anal. Calc. for $C_{13}H_{12}N_4OS$ (372.33): C, 57.34; H, 4.44; N, 20.57; O, 5.88; S, 11.77 % .Found: C, 57.55; H, 4.67; N, 20.78; O, 5.65; S, 11.56%.

3-Methyl-1,6-diphenyl-1,7-dihydro-8H-pyrazolo[3',4':4,5]thieno[2,3-d]pyrimidin-8-one (14):
A mixture of compound **13** (0.01 mole) and benzoyl chloride (0.01 mole) was refluxed in acetic acid (10 mL) for 12 h. The reaction mixture was allowed to cool, and then poured onto ice-cold water. The separated solid was filtered, washed with water and recrystallized from DMF/ethanol to give **14** as pale brown crystals, yield 54%; m.p.; > 300˚C. IR (KBr): υ cm^{-1} 3328 (NH), 3065 (CH-arom.), 2924 (CH-aliph.), 1680 (CO); ^{1}H NMR (DMSO-d_6) δ = 2.72 (s, 3H, CH_3), 7.38-7.85 (m, 10H, Ar-H) and 8.02 (s, 1H, NH), ^{13}C-NMR (DMSO-d_6): 15.4 (CH_3), 108.2, 124.7, 126.5, 128.2, 128.7, 129.3, 129.9, 130.7, 132.5, 134.3, 139.1, 140.6, 158.5, 159.3 (Ar-C), 165.1 (CO). Anal. Calc. for $C_{20}H_{14}N_4OS$ (358.42): C, 67.02; H, 3.94; N, 15.63; O, 4.46; S, 8.95% .Found: C, 67.24; H, 3.72; N, 15.85; O, 4.87; S, 8.73%.

Ethyl5-acetamido-3-methyl-1-phenyl-1H-thieno[3,2-c]pyrazole-6-carboxylate (15):
A mixture of compound **3b** (0.01 mol) and acetic anhydride (30 mL) was heated under reflux for 3 h. The reaction mixture was allowed to cool to room temperature and the solid formed was collected by filtration, dried, and crystallized from ethanol to give **15** as white crystals, yield 67%; m.p.; 223˚C. IR (KBr): υ cm^{-1} 3415 (NH), 3045 (CH-arom.), 2976 (CH-aliph.), 1720, 1690 (2CO); ^{1}H NMR (DMSO-d_6) δ = 1.28 (t, J = 7.2 Hz, 3H, $OCH_2\underline{CH_3}$), 2.02 (s, 3H, $COCH_3$) , 2.77 (s, 3H, CH_3) , 4.31 (q, J = 7.2 Hz, 2H, CH_2, $O\underline{CH_2}CH_3$), 7.40-7.61 (m, 5H, Ar-H), 9.15 (s, 1H, NH). Anal. Calc. for $C_{17}H_{17}N_3O_3S$ (343.40): C, 59.46; H, 4.99; N, 12.24; O, 13.98; S, 9.34% .Found: C, 59.68; H, 4.74; N, 12.45; O, 13.75; S, 9.58%.

7-Amino-3,6-dimethyl-1-phenyl-1H-pyrazolo[3',4':4,5]thieno[2,3-d]-pyrimidin-8(7H)-one (17):
A mixture of **15** (0.01 mol) and hydrazine hydrate (3 mL) in ethanol (30 mL) was heated under reflux for 5h. The reaction mixture was allowed to cool to room temperature and poured into water (100 mL). The solid formed was collected by filtration, dried and crystallized from ethanol to give **17** as yellow powder, yield 62%; m.p.; 274˚C. IR (KBr): υ cm^{-1} 3418, 3355 (NH_2), 3085 (CH-arom.), 2926 (CH-aliph.), 1685 (CO); ^{1}H NMR (DMSO-d_6) δ = 2.17 (s, 3H, CH_3) , 2.70 (s, 3H, CH_3), 7.37-7.78 (m, 7H, Ar-H+ NH_2). Anal. Calc. for $C_{15}H_{13}N_5OS$ (311.36): C, 57.86; H, 4.21; N, 22.49; O, 5.14; S, 10.30% .Found: C, 57.64; H, 4.43; N, 22.71; O, 5.35; S, 10.52%.

Ethyl 3-methyl-1-phenyl-5-thioureido-1H-thieno [3,2-c]pyrazole-6-carboxylate (18):
A mixture of **3b** (0.01mol) and 10 % HCl (10 mL) was refluxed with potassium thiocyanate (0.015 mol) for 4 h. The reaction mixture was allowed to cool to room temperature. The solid formed was collected by filtration, washed with water, dried and crystallized from dioxane to give **18** as brown powder, yield 80%; m.p.; 227˚C. IR (KBr): υ cm^{-1} 3425, 3380 (NH_2), 3260 (NH), 3043 (CH-arom.), 2966 (CH-aliph.), 1715 (CO); ^{1}H NMR (DMSO-d_6) δ = 1.29 (t, J = 7.2 Hz, 3H, $OCH_2\underline{CH_3}$), 2.62 (s, 3H, CH_3) , 4.32 (q, J = 7.2 Hz, 2H, CH_2, $O\underline{CH_2}CH_3$), 6.84 (s, 2H, NH_2), 7.42-7.64 (m, 6H, Ar-H+ NH). Anal. Calc. for $C_{16}H_{16}N_4O_2S_2$ (360.07): C, 53.31; H, 4.47; N, 15.54; O, 8.88; S, 17.79% .Found: C, 53.52; H, 4.68; N, 15.77; O, 8.64; S, 17.58%.

3-Methyl-1-phenyl-6-thioxo-6,7-dihydro-1H-pyrazolo[3',4':4,5]-thieno[2,3-d]pyrimidin-8(5H)-one (19):
A solution of **18** (0.01 mol) in ethanolic sodium ethoxide (0.01 mol of sodium atom in 30 mL ethanol) was stirred under reflux for 6 h. After cooling, the reaction mixture was neutralized with cooled 10 % HCl and the solid formed was collected by filtration, washed with water, dried and then crystallized from dimethylformamide to give **19** as pale brown powder, yield 67%; m.p.; 286˚C. IR (KBr): υ cm^{-1} 3380, 3385 (2NH), 3092 (CH-arom.), 2947 (CH-aliph.), 1676 (CO); ^{1}H NMR (DMSO-d_6) δ = 2.47 (s, 3H, CH_3), 7.39-7.58 (m,

6H, Ar-H+ NH), 8.13 (s, 1H, NH), ^{13}C-NMR (DMSO-*d*$_6$): 15.2 (CH_3), 107.9, 124.4, 126.5, 128.7, 129.5, 133.6, 138.1, 139.8, 142.5 (Ar-C), 165.2 (C=O), 175.5 (C=S). Anal. Calc. for $C_{14}H_{10}N_4OS_2$ (314.39): C, 53.49; H, 3.21; N, 17.82; O, 5.09; S, 20.40% . Found: C, 53.70; H, 3.43; N, 17.60; O, 5.32; S, 20.64%.

Ethyl 3-methyl-1-phenyl-5-(3-phenylthioureido)-1H-thieno[3,2-c]pyrazole-6-carboxylate (20):

A mixture of **3b** (0.01 mol) and phenyl isothiocyanate (0.01 mol) in absolute ethanol (20 mL) was heated under reflux for 4 h. The solid product that formed after cooling was collected by filtration and recrystallized from ethanol to give **20** as yellow crystals, yield 76%; m.p.; 205˚C. IR (KBr): υ cm^{-1} 3336, 3225 (2NH), 3065 (CH-arom.), 2974 (CH-aliph.), 1725 (CO); ^{1}H NMR (DMSO-*d*$_6$): δ = 1.24 (t, *J* = 7.2 Hz, 3H, $OCH_2\underline{CH_3}$), 2.60 (s, 3H, CH_3), 4.19 (q, *J* = 7.2 Hz, 2H, CH_2, $O\underline{CH_2}CH_3$), 7.37-7.82 (m, 11H, Ar-H+ NH), 11.07 (s, 1H, NH). Anal. Calc. for $C_{22}H_{20}N_4O_2S_2$ (436.55): C, 60.53; H, 4.62; N, 12.83; O, 7.33; S, 14.69% .Found: C, 60.74; H, 4.85; N, 12.61; O, 7.54; S, 14.92%.

3-Methyl-1,7-diphenyl-6-thioxo-6,7-dihydro-1H-pyrazolo[3',4':4,5]-thieno[2,3-d]pyrimidin-8(5H)-one (21):

Route A:

To a solution of compound **3b** (0.01 mol) in pyridine 30 mL, phenyl isothiocyanate (0.01 mol) was added. The mixture was heated under reflux for 24 h. The reaction mixture was cooled, poured onto ice cold water The solid product that formed was collected by filtration and recrystallized from dioxane to give **21** (55%) as brown crystals.

Route B:

A solution of **20** (0.01 mol.) in pyridine 30 mL was heated under reflux for 6 h. The reaction mixture was cooled, poured onto ice cold water. The solid product formed was collected by filtration and recrystallized from dioxane to give **21** (65%) as brown crystals. m.p. > 300◦C. IR (KBr): υ cm^{-1} 3370, (NH), 3085 (CH-arom.), 2927 (CH-aliph.), 1655 (CO).^{1}H NMR (DMSO-*d*$_6$): δ = 2.71 (s, 3H, CH_3), 7.20–7.75 (m, 11H, Ar-H+ NH), ^{13}C-NMR (DMSO-*d*$_6$): 15.5 (CH_3), 108.1, 124.5, 126.2, 127.6, 128.1, 128.8, 129.4,130.3, 132.5, 134.2, 139.4, 140.7, 143.9 (Ar-C), 165.7 (C=S), 179.5 (C=O). Anal. Calc. for $C_{20}H_{14}N_4OS_2$ (390.48): C, 61.52; H, 3.61; N, 14.35; O, 4.10; S, 16.42% .Found: C, 61.75; H, 3.83; N, 14.57; O, 4.32; S, 16.64%.

7-Amino-3-methyl-1-phenyl-6-(phenylamino)-1H-pyrazolo[3',4':4,5]-thieno[2,3-d]pyrimidin-8(7H)-one (23):

A mixture of **20** (0.01 mol) and excess hydrazine hydrate (10 mL) was heated under reflux for 15 h. The solid product that formed after cooling was collected by filtration and recrystallized from ethanol / dioxane to give **23** as white crystals, yield 60%; mp. 267˚C. IR (KBr): υ cm^{-1} 3380, 3320, 3215 (NH_2 and NH), 3055 (CH-arom.), 2927 (CH-aliph.), 1665 (CO); ^{1}H NMR (DMSO-*d*$_6$): δ = 2.73 (s, 3H, CH_3), 5.57 (s, 2H, NH_2), 7.25-7.94 (m, 11H, Ar-H+ NH); MS: m/z = 388 [M$^+$]. Anal. Calc. for $C_{20}H_{16}N_6OS$ (388.45): C, 61.84; H, 4.15; N, 21.63; O, 4.12; S, 8.25% .Found: C, 61.62; H, 4.36; N, 21.84; O, 4.33; S, 8.48%.

Table 1. Antimicrobial activities of some newly synthesized compounds.

Compound no.	Gram positive bacteria		Gram negative bacteria		Fungi
	Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Aspergillus Niger*
3a	+++	+	-	++	+
4	++	+	+	+	-
6	+++	++	+	++	+
7a	+	-	-	++	+
10	+++	++	+	++	-
11	++	+	++	-	-
14	-	++	+	+	-
17	+++	-	+	+	+
19	-	++	+	-	+
21	+++	+	-	-	++
DMF	-	-	-	-	-
Nystatin	-	-	-	-	+++
Ciprofloxacin	+++	+++	+++	+++	-

Inhibition Zone = 0.1 - 0.5 cm beyond control = + (slightly active); Inhibition Zone = 0.6 - 1.0 cm beyond control = + + (moderately active); Inhibition Zone = 1.1 - 1.5 cm beyond control = + + + (highly active); Inhibition Zone = 0.0 cm beyond control = – (inactive).

2.3 Antimicrobial Evaluation

Some of the newly synthesized compounds were screened for their in vitro antibacterial activities against Gram positive bacteria; *Staphylococcus aureus* and *Bacillus subtilis* (G +ve), Gram negative bacteria; *Escherichia coli* and *Pseudomonas aeruginosa* (G -ve) and for their Antifungal activity against *Aspergillus niger* by the agar diffusion technique [27], 1.0 mg / ml solution in dimethylformamide (DMF) was used. The bacteria are maintained on nutrient agar. DMF showed no inhibition zones. The agar media were inoculated with different microorganism's culture tested after 24 hours of inoculation at 37 °C for bacteria and for antifungal tested after 72 hours of inoculation at 28 °C. The diameter of inhibition zone (cm) was measured. The data obtained is summarized in table (1). The

results indicated that compounds (**3a**), (**6**), (**10**), (**17**) and (**21**) showed very high antimicrobial activity against the examined Gram positive bacteria *Staphylococcus aureus*. In addition, compound (**21**) showed moderate antifungal activity against *Aspergillus niger*. In summary, results of antimicrobial activity revealed that the synthesized compounds showed moderate and / or very high antimicrobial activity against bacteria and fungi, respectively. It could be concluded from these results that the biologically active synthesized compounds are nearly as active as the standard antibacterial Ciprofloxacin against the both tested Gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). On the other hand, the biologically active synthesized compounds are active as the standard fungicide Nystatin against the tested fungi *Aspergillus niger*.

3. RESULTS AND DISCUSSION

3.1 Chemistry

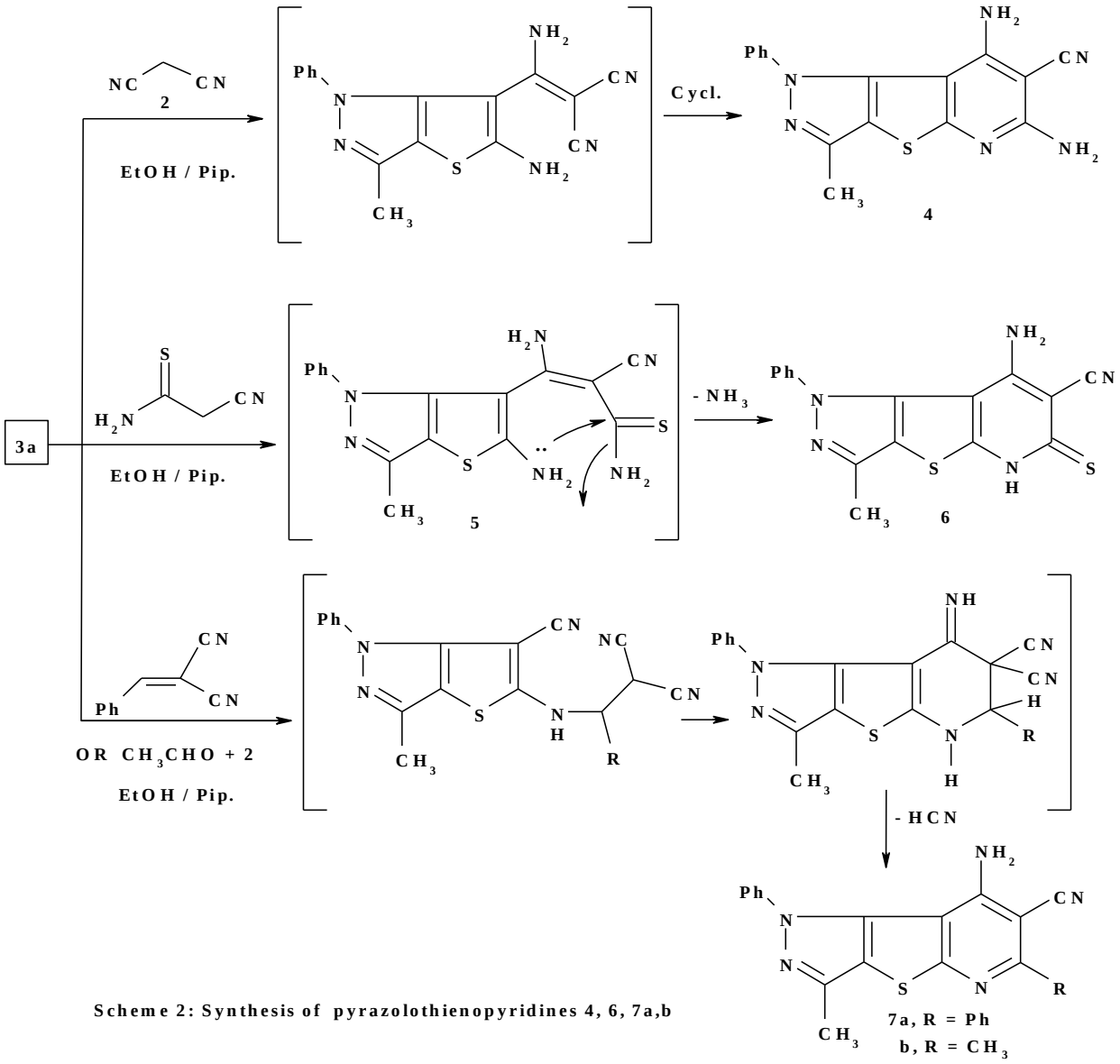
Treatment of 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one (**1**) [24], with malononitrile (**2**) and elemental sulfur in presence of a few drops of triethylamine under Gewald reaction conditions [13], furnished 5-amino-3-methyl-1-phenyl-1*H*-thieno[3,2-*c*]pyrazole-6-carbonitrile **(3a)** (Scheme 1). The IR spectrum of compound **3** exhibited the appearance of absorption band due to the NH_2 and CN group. While its ^{1}H NMR spectrum revealed the presence of three protons as singlet signal at δ = 2.72 ppm assignable to CH_3, two protons as a singlet signal at δ = 6.86 ppm assignable to the NH_2 group and multiplet in the range of δ 7.35-7.64 (aromatic protons). Further, ^{13}C NMR spectrum exhibited confirmatory signals of the methyl carbon and the cyano group around δ 14.5 and δ 115.3 ppm respectively. The mass spectrum of **3a** showed a molecular ion peak at m/z = 254 (M^+) corresponding to the molecular formula $C_{13}H_{10}N_4S$.

Scheme 1: Synthesis of thienopyrazole via Gewald reacction

The starting material **3a** was proved to be a versatile for synthesis of some novel pyrazolothienopyridine and pyrazolothienopyrimidine derivatives. So, compound **3a** was treated with malononitrile (**2**) under reflux in ethanol solution in presence of a catalytic amount of piperidine to yield 6,8-diamino-3-methyl-1-phenyl-1*H*-pyrazolo[3',4':4,5]thieno[2,3-*b*]-pyridine-7-carbonitrile **(4)**. Compound **4** was established based on its elemental analysis and spectral data. The ^{1}H NMR spectrum of compound **4** revealed the signals at δ = 2.65 (s, 3H, CH_3), 6.22 (s, 2H, NH_2), 7.42-7.77 (m, 7H, Ar-H + NH_2). Similarly, the reaction of **3** with cyanothioacetamide in ethanol piperidine solution yielded a reaction product which was formulated as 8-amino-3-methyl-1-phenyl-6-sulfanylidene-5,6-dihydro-1*H*-pyrazolo[3',4':4,5]-thieno[2,3-*b*]pyridine-7-carbonitrile (**6)** (Scheme 2).

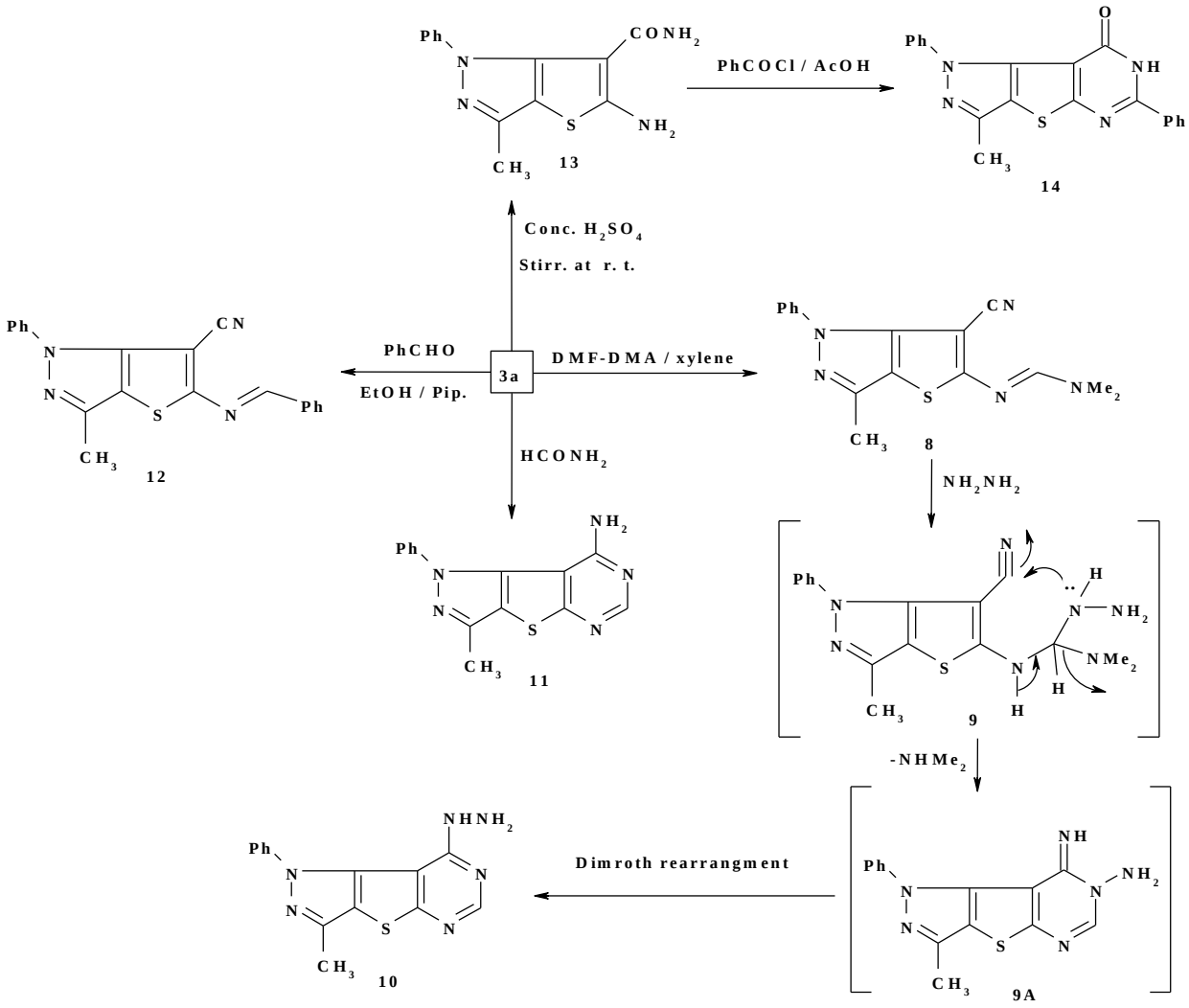
Confirmation of compound **6** was based on its compatible spectroscopic data (IR, ^{1}H NMR, MS and ^{13}C NMR). Thus, ^{1}H NMR of structure **6** revealed a singlet signal at δ 7.70 ppm assigned to NH proton in addition to all the other signals in the structure. Also, ^{13}C NMR of **6** revealed signal at δ = 188.7 ppm assigned to C=S carbon (experimental section). The mass spectrum of **6** exhibited a molecular ion peak at m/z = 337 (M^+) corresponding to molecular formula $C_{16}H_{11}N_5S_2$, with a base peak at m/z =77 corresponding to phenyl ion. Formation of **6** was proceeded through the nunisolable adduct intermediate **5** via the loss of ammonia molecule.

The behavior of **3a** towards electrophilic reagents under alkaline conditions was investigated. Thus, the reaction of **3a** with benzylidenemalononitril in refluxing ethanol containing a catalytic amount of piperidine afforded the 8-amino-3-methyl-1,6-diphenyl-1*H*-pyrazolo[3',4':4,5]thieno-[2,3-*b*]pyridine-7-carbonitrile **(7a)** which was established by spectral data (IR and ^{1}H NMR). IR spectrum of **7a** showed bands at 3344, 3227 (NH_2) and 2215 (C≡N). The ^{1}H NMR spectrum of (**7a** in DMSO-d_6) revealed signals at δ 2.71 (s, 3H, CH_3), 6.27 (s, 1H, NH_2), 7.35- 8.33 (m, 10H, Ar-H). The formation of compound **7a** can be explained on the basis of an initial Michael addition of the amino function group in compound **3a** to the double bond of benzylidinemalononitrile followed by intramolecular cyclization, with the loses of hydrogen cyanide and tautomerization to give **7a**. Similarly, compound **7b** was obtained in good yield via the reaction of **3a** with acetaldehyde and malononitrile. The analytical and spectral data of **7b** are found in good agreement with the proposed structure (Scheme 2 and Experimental part).



Scheme 2: Synthesis of pyrazolothienopyridines 4, 6, 7a,b

This work was extended to study the reactivity of the amino group in compound **3a** as nucleophile. Thus, **3a** reacted with dimethylformamide-dimethylacetal (DMF-DMA) in dry xylene to afford the corresponding *N*'-(6-Cyano-3-methyl-1-phenyl-1*H*-thieno[3,2-*c*]pyrazol-5-yl)-*N*,*N*-dimethylformimidamide (**8**). The structure of **8** was inferred via elemental analysis, spectral data, and chemical transformations. ^{1}H NMR spectrum of **8** revealed the new signals of N $(CH_3)_2$ at (δ = 3.62 ppm). The reaction of **8** with hydrazine hydrate in ethanol under reflux gave the corresponding pyrazolothienopyrimidine derivative **10**. The structure of **10** was established based on elemental analysis and spectral studies. ^{1}H NMR spectra revealed the signals of NH_2 , NH, pyrimidine protons, and absence of the signal of $N(CH_3)_2$ protons. Mass spectrum of **10** showed a molecular ion peak m/z at 296 (M^+). The formation of **10** in this reaction was proceeded via the addition of hydrazine hydrate to **8** to give the nunisolable adduct **9**, followed by cyclization with elimination of dimethylamine giving the imino derivative **9A** that underwent Dimroth rearrangement [21,25], yielding the 8-hydrazinyl-3-methyl-1-phenyl-1*H*-pyrazolo[3',4':4,5]thieno[2,3-*d*]pyrimidine **(10)**. Furthermore, treatment of compound **3** with formamide under reflux gave the expected 3-methyl-1-phenyl-1*H*-pyrazolo[3',4':4,5]thieno- [2,3-*d*]pyrimidin-8-amine (**11**). The analytical and spectral data was found in agreement with the proposed structure (See scheme 3 and the Experimental section).



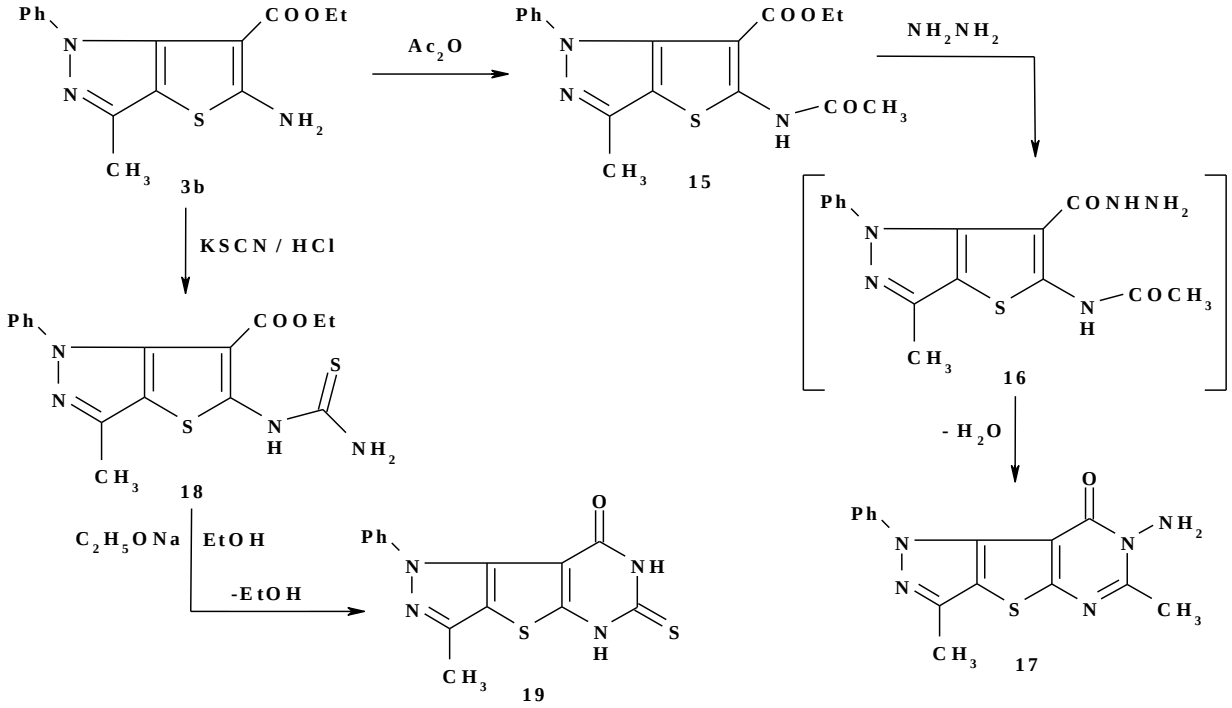
Scheme 3: Synthesis of pyrazolothienopyrimidines 10, 11 and 14

On the other hand, the Schiff base **12** was obtained from the reaction of **3a** with benzaldehyde in ethanolic piperidine solution. The IR spectrum of compound **12** exhibited the disappearance of the absorption band due to the NH_2 function group and the appearance of absorption band due to the CN functional group at υ 2210 cm^{-1}. The ^1H NMR spectrum of compound **12** revealed the presence of the protons assigned to the methyl group at δ = 2.65 ppm and the signal of N=CH at δ = 8.72 ppm and the disappearance of signal assignable to the NH_2. The aminonitrile derivative **3a** was subjected to partial hydrolysis by stirring with concentrated sulphuric acid to yield 6-carboxamide derivative **13** (Scheme 3). The IR spectrum of compound **13** exhibited the disappearance of the absorption band due to the CN function group. The ^1H NMR spectrum of compound **13** revealed the presence of singlet signals at δ = 5.17 and 6.91 ppm corresponding to NH_2 and $CONH_2$ protons. Acid-catalyzed bis-nucleophilic cyclocondensation of aminocarboxamide derivative **13** with benzoyl chloride in glacial acetic acid furnished pyrimidinone derivative **14**. The ^1H NMR spectra of compound **14** revealed the disappearance of the signals attributed to NH_2 and $CONH_2$ protons of compound **13** (at δ = 5.17 and 6.91 ppm) and appearance of a singlet signal at δ = 8.02 ppm which may be attributed to NH proton. Also, the ^{13}C NMR of compound **14** revealed a signal at 15.4 ppm (CH_3) and 165.1 ppm (C=O), in addition to the sp^2 carbon atoms as in the experimental section.

On the other hand, ethyl 5-amino-3-methyl-1-phenyl-1*H*-thieno[3,2-*c*]pyrazole-6-carboxylate (**3b**), which previously prepared [4], by the reaction of **1** with ethyl cyanoacetate and elemental sulfur, was taken as starting for the synthesis of other biologically active pyrazolothienopyrimidine derivatives, thus treatment of **3b** with acetic anhydride under reflux afforded the acetamide derivative **15**. The ^1H NMR spectrum of **15** revealed the absence of any signal may be attributed to NH_2 protons, and appearance of a signal assigned to NH proton at δ = 9.15 ppm. Moreover, when compound **15** was allowed to react with hydrazine hydrate, it yielded aminopyrazolothienopyrimidinone derivative **17**, through the acid hydrazide intermediate **16** via loss of water molecule. The structure of **17** was established based on elemental analysis and spectral studies. The IR spectrum of compound **17** exhibited the appearance of absorption band due to the NH_2 functional group at υ = 3418, 3355 cm^{-1}, and absence of absorption band due to the $COCH_3$ function group. The ^1H NMR spectrum of **17** revealed the absence of any signals may be

attributed to ($CO_2C_2H_5$) and NH protons, and appearance only the signals assigned to $2CH_3$, NH_2 and aromatic protons. Furthermore, the reaction of **3b** with potassium thiocyanate afforded the formation of 5-thioureido-1*H*-thieno-[3,2-*c*]pyrazole-6-carboxylate derivative **18**. The structure of **18** was further elucidated via elemental analysis and their cyclization into the corresponding 6-thioxo-6,7-dihydro-1*H*-pyrazolo[3',4':4,5]thieno[2,3-*d*]pyrimidin-8(5*H*)-one derivative **19** upon treatment with ethanolic sodium ethoxide under reflux. The structure of **19** was confirmed based on elemental analysis and spectral data (Scheme 4 and Experimental part).

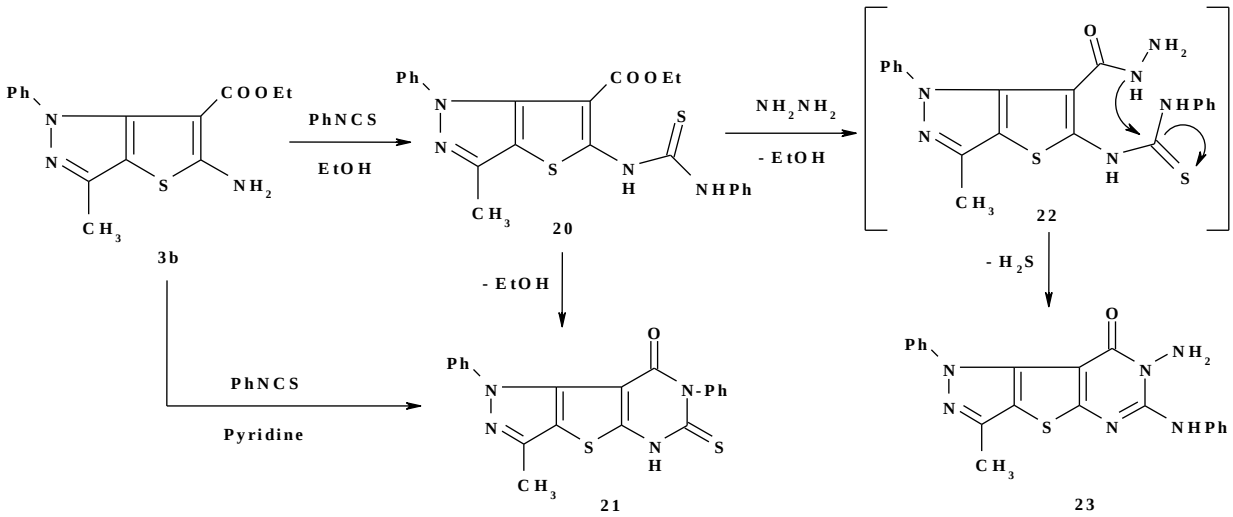
The ^{1}H NMR spectrum of compound **19** revealed signals at δ 2.47 , 8.13 ppm assigned to the CH_3 and NH protons beside the other protons in their proper positions. Also, ^{13}C NMR of the structure revealed signals at 15.2 ppm (CH_3), 165.2 ppm (C=O), 175.5 ppm (C=S), in addition to all signals assigned to carbons of sp^2 in the molecule.



Scheme 4: Synthesis of pyrazolothienopyrimidines 17 and 19

The reaction of **3b** with phenyl isothiocyanate in boiling absolute ethanol gave the corresponding thiourea derivative **20**, which underwent cyclization by refluxing in pyridine solution to give 3-methyl-1,7-diphenyl-6-sulfanylidene-1,5,6,7-tetrahydro-8*H*-pyrazolo-[3',4':4,5]thieno[2,3-*d*]pyrimidin-8-one (**21**) via loss of ethanol molecule. The ^{1}H NMR spectrum of **21** revealed the absence of any signals may be attributed to ($CO_2C_2H_5$) and NH_2 protons, and appearance of a signal assigned to NH. Also, the structure of compound **21** was supported by ^{13}C NMR spectrum. Compound **21** was also synthesized by conducting the reaction between **3b** and phenyl isothiocyanate in pyridine solution under reflux (Scheme 5 and Experimental part).

Treatment of compound **20** with hydrazine hydrate in ethanol afforded 7-amino-6-anilino-3-methyl-1-phenyl-1,7-dihydro-8*H*-pyrazolo[3',4':4,5]thieno[2,3-*d*]-pyrimidin-8-one (**23**). The ^{1}H NMR spectrum of **23** showed signals at δ 2.73 ppm for CH_3 group and δ 5.57 ppm for NH_2 protons beside the signals characteristic of aromatic and NH protons. Mass spectrum of compound **23** revealed a molecular ion peak m/z at 388 (M^+) corresponding to the molecular formula $C_{20}H_{16}N_6OS$. The formation of compound **23** was proceeded through the nunisolable hydrazide intermediate **22** via the loss of hydrogen sulfide molecule [26], (Scheme 5 and Experimental part).



Scheme 5: Synthesis of pyrazolothienopyrimidines 21 and 23

4. CONCLUSION

The achieved derivative of new thienopyrazole was synthesized via Gewald reaction and was used to prepare a new pyrazolothienopyridines and pyrazolothienopyrimidinones, that are expected to have biological activities, and their structures were confirmed by their spectral data and elemental analyses. Ten of the newly synthesized compounds were evaluated for their antimicrobial activity against gram positive and gram negative bacteria and fungous, some of the tested compounds showed highly active and other of moderate activity.

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