Synthesis of Some Novel Pyridazine, Thienopyridazine, Pyrazolopyridine, Pyridopyrazolopyrimidine and Pyridopyrazolotriazine Derivatives with Their Antimicrobial Activity

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Abstract: Coupling of compound 1 with diazotized aromatic amines in ethanol afforded the arylhydrazones 3a,b. Fusion of 3a,b with active methylene derivatives 4a,b afforded the pyridazine derivatives 6a–d. Also, when compounds 6b,c were reacted with elemental sulphur afforded the thienopyridazine derivatives 7a,b. Treatment of compound 8 with hydrazine hydrate produced pyrazolopyridine derivative 9. Pyridopyrazolopyrimidines (12–19) and pyridopyrazolotriazines 21, 22a,b were achieved by the reaction of pyrazolopyridine with different reagents in basic media. The antimicrobial activities of the new compounds were also evaluated. The newly synthesized compounds were characterized by IR, $^1$H NMR and $^{13}$C-NMR spectral studies.

Keywords: Pyridazine; Thienopyridazine; Pyrazolopyridine; Pyridopyraz-Olopyrimidine, Pyridopyrazolotriazine.

1. INTRODUCTION

Nitrogen-containing heterocyclic compounds are one of the most fruitful and extensively developing fields of heterocyclic chemistry. These compounds exhibit various kinds of biological activities. During the past decades increasing interest in the synthesis and biological activities of pyridazine derivatives has been observed [1–3]. Pyridazine compounds have been reported to possess varied biological activities such as antimicrobial [4], antihypertensive [5], anticancer [6], anti-inflammatory[7] and antifungal activities [8]. These facts have prompted us to synthesize some novel pyridazine derivatives. Recently, pyridazinone nucleus has been extensively studied in the search for new and selective medicinal agents as drugs acting on the cardiovascular system [9]. Furthermore, a number of thienopyridazines have been claimed to possess interesting biological and pharmacological activities such as, anticancer [10], useful as NAD(P)H oxidase inhibitor [11], and identified as a new allosteric modulator of the adenosine A1 receptor (A1AR)$^{12}$. Also, Pyrazolo [3,4-b]pyridines comprise a very
interesting class of compounds because of their significant and versatile biological, and pharmacological activities, such as antimicrobial [13], cardiovascular [14], antiviral [15] and antileishmanial [16] activities. The pyrazolo[3,4- b]pyridine moieties represent important building blocks in both natural and synthetic bioactive compounds [17]. They show anxiolytic activity along with Xanthine oxidase inhibitors, cholesterol formation-inhibitor, and Anti-Alzheimer [18]. They also act as potent and selective inhibitors of glycogen synthase kinase-3 (GSK-3) [19]. Moreover, fused heterocyclic containing pyrazolopyridine systems have been described associated with several biological and medicinal activities [20–23]. In connection with our efforts to synthesize fused heterocycles [24-27] from readily available starting materials, we report here on the synthesis of some novel pyridazine, thienopyridazine, pyrazolopyridine, pyridopyrazolopyrimidine and pyridopyrazolotriazine derivatives in order to investigate the antimicrobial activity.

2. EXPERIMENTAL

All melting points are uncorrected. IR spectra (KBr) were recorded on a FTIR 5300 spectrometer (ν, cm⁻¹). The ¹H NMR and ¹³C-NMR spectra were recorded in DMSO-d₆ at 200, 300 MHz on a Varian Gemini NMR spectrometer (δ, ppm) using TMS as an internal standard. Elemental analyses were carried out at the Microanalytical Center, Faculty of Science, Cairo University and Assiut University. Microbiology screening was carried out in Botany Department, Faculty of Science, Al-Azhar University, Assiut.

2.1 Preparation of Compounds 3a, b: General Procedure

A solution of 1 (0.01 mole) in ethanol (30 mL) containing sodium acetate (2gm) was cooled to 0°C, stirred and treated gradually with cooled solution of aryl diazonium chloride (prepared from 0.01 mole of amine and the appropriate quantities of HCl and NaNO₂). The solid product formed on standing was collected and recrystallized from the proper solvent to give 3a, b.

N-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-3-oxo-2-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)hydrazono)butanamide (3a)

It was obtained as yellow crystals from ethanol; yield 86%; m.p. 158°C; IR (KBr) ν cm⁻¹ 3368, 3242, 3182 (3NH), 3057 (CH-arom.), 2946 (CH-aliph.), 2936, 1675, 1663 (3C=O); ¹H NMR (DMSO-d₆) δ = 1.82 (s, 3H, CH₃), 2.26 (s, 3H, CH₃), 3.02 (s, 3H, NCH₃), 6.89–8.05 (m, 11H, Ar-H), 10.43 (s, 1H, NH), 11.24 (s, 1H, NH), 13.52 (s, 1H, NH). Anal. Calc. For C₂₃H₂₃N₇O₅S (553.61): C, 52.07; H, 4.19; N, 17.71; S, 11.58%. Found: C, 52.22; H, 4.41; N, 17.92; S, 11.79%.

N-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-2-(2-(4-(N-(5-dimethoxazol-2-yl)sulfamoyl)phenyl)hydrazono)oxobutanamide (3b)

It was obtained as brown crystals from ethanol; yield 82%; m.p. 174°C; IR (KBr): ν cm⁻¹ 3384, 3267, 3126 (3NH), 3072 (CH-arom.), 2965 (CH-aliph.), 1695, 1664, 1645 (3CO); ¹H NMR (DMSO-d₆): δ = 1.72 (s, 3H, CH₃), 2.12 (s, 3H, CH₃), 2.28 (s, 3H, CH₃), 2.46 (s, 3H, CH₃), 3.17 (s, 3H, NCH₃), 6.90–7.98 (m, 9H, Ar-H), 9.93 (s, 1H, NH), 11.66 (s, 1H, NH), 13.43 (s, 1H, NH). Anal. Calc. For C₂₃H₂₇N₇O₅S (565.60): C, 55.21; H, 4.81; N, 17.33; S, 5.67%. Found: C, 55.41; H, 4.59; N, 17.54; S, 5.88%.

2.2 Preparation of Compounds 6a-d: General Procedure

An equimolar amount of either 3a (0.01 mole) or 3b (0.01 mole) in ammonium acetate (2 gm) was added to either malononitrile (4a) (0.01 mole) or ethyl cyanoacetate (4b) (0.01 mole). The reaction
mixture, in each case, was fused for 1 h. Then left to stand at room temperature and triturated with ethanol. The solid product so formed, in each case, was collected by filtration and recrystallized from the proper solvent to give 6a-d.

5-Cyano-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-6-imino-4-methyl-1-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)-1,6-dihydropyridine-3-carboxamide (6a)

It was obtained as brown crystals from DMF/ethanol; yield 68%; m.p. 267°C; IR (KBr) ν cm⁻¹ 3375, 3332, 3254 (3NH), 3057 (CH-arom.), 2945 (CH-aliph.), 2205 (CN), 1662, 1647 (2CO); ¹H NMR (DMSO-d6) δ = 1.85 (s, 3H, CH₃), 2.47 (s, 3H, CH₃), 3.11 (s, 3H, NCH₃), 7.25–8.12 (m, 12H, Ar-H+NH), 10.15 (s, 1H, NH), 11.82 (s, 1H, NH). ¹³C NMR (DMSO-d₆) δ C = 10.54, 13.15, 34.61 (CH₂), 116.12 (CN), 102.92, 104.42, 112.38, 117.45, 123.23, 125.65, 130.23, 131.44, 133.10, 136.42, 137.31, 138.46, 143.11, 150.26, 155.15, 160.56 (Ar-C), 167.21, 169.75 (2CO); Anal. Calc. For C₂₇H₂₂N₅O₅S₂ (601.66): C, 56.76; H, 4.43; N, 18.23; S, 5.22%. Found: C, 56.88; H, 4.47; N, 18.45; S, 5.43%.

5-Cyano-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-4-methyl-6-oxo-1-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)-1,6-dihydropyridine-3-carboxamide (6b)

It was obtained as brown crystals from DMF/ethanol; yield 66%; m.p. 254°C; IR (KBr) ν cm⁻¹ 3349, 3207 (2NH), 3068 (CH-arom.), 2935 (CH-aliph.), 2198 (CN), 1668, 1670, 1653 (3C=O); ¹H NMR (DMSO-d₆) δ = 1.57 (s, 3H, CH₃), 2.23 (s, 3H, CH₃), 3.02 (s, 3H, NCH₃), 7.22–8.08 (m, 12H, Ar-H+NH), 10.85 (s, 1H, NH). Anal. Calc. For C₂₇H₂₉N₅O₅S₂ (602.64): C, 53.81; H, 3.68; N, 18.59; S, 10.64%. Found: C, 53.58; H, 3.89; N, 18.82; S, 10.86%.

5-Cyano-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-1-(4-(N-(4,5-dimethyloxazol-2-yl)sulfamoyl)phenyl)-6-imino-4-methyl-1,6-dihydropyridine-3-carboxamide (6c)

It was obtained as green crystals from DMF/ethanol; yield 66%; m.p. 273°C; IR (KBr): υ cm⁻¹ 3348, 3215, 3192 (3NH), 3060 (CH-arom.), 2934 (CH-aliph.), 2193 (CN), 1668, 1647 (2CO); ¹H NMR (DMSO-d₆): δ = 1.46 (s, 3H, CH₃), 2.15 (s, 3H, CH₃), 2.26 (s, 3H, CH₃), 2.48 (s, 3H, CH₃), 3.05 (s, 3H, NCH₃), 7.18–7.98 (m, 11H, Ar-H+2NH), 11.32 (s, 1H, NH). Anal. Calc. For C₂₉H₂₇N₅O₅S (613.65): C, 56.76; H, 4.43; N, 20.54; S, 5.23%. Found: C, 56.95; H, 4.64; N, 20.76; S, 5.44%.

5-Cyano-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-1-(4-(N-(4,5-dimethyloxazol-2-yl)sulfamoylphenyl)-4-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide (6d)

It was obtained as green crystals from DMF/ethanol; yield 67%; m.p. 270°C; IR (KBr): ν cm⁻¹ 3363, 3181 (2NH), 3053 (CH-arom.), 2933 (CH-aliph.), 2203 (CN), 1686, 1670, 1658 (3C=O); ¹H NMR (DMSO-d₆): δ = 1.27 (s, 3H, CH₃), 2.24 (s, 3H, CH₃), 2.38 (s, 3H, CH₃), 2.47 (s, 3H, CH₃), 3.11 (s, 3H, NCH₃), 7.27–8.22 (m, 10H, Ar-H+NH), 11.21 (s, 1H, NH). Anal. Calc. For C₂₉H₂₅N₅O₅S (614.63): C, 56.67; H, 4.26; N, 18.23; S, 5.22%. Found: C, 56.88; H, 4.47; N, 18.45; S, 5.43%.

2.3 Preparation of Compounds 7a, b: General Procedure

A mixture of compound 6b or 6c (0.01 mole), elemental sulphur (0.01 mole) in ethanol (30 ml) and dimethylformamide (5 mL) containing triethylamine (0.5 mL) was heated under reflux for 7 h. Then cooled, poured into crushed ice and acidified with HCl. The solid product, formed in each case, was filtered off and recrystallized from the proper solvent to give 7a, b.
5-Amino-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-4-oxo-3-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)-3,4-dihydrothieno[3,4-d]pyrimidine-1-carboxamide (7a)

It was obtained as dark brown crystals from dioxane; yield 58%; m.p. 297°C; IR (KBr) ν cm\(^{-1}\) 3425, 3348 (NH\(_2\)), 3256, 3188 (2NH), 3045 (CH-arom.), 2928 (CH-aliph.), 1672, 1665, 1644 (3C=O); \(^1\)H NMR (DMSO-\(d_6\)) δ = 2.26 (s, 3H, CH\(_3\)), 3.15 (s, 3H, NCH\(_3\)), 5.16 (s, 1H, NH\(_2\)), 6.24 (s, 1H, CH-thiophene), 7.11-8.15 (m, 12H, Ar-H+ NH), 11.38 (s, 1H, NH). \(^13\)C NMR (DMSO-\(d_6\)) δ C = 12.21, 34.42 (2CH\(_3\)), 103.92, 112.12, 115.18, 117.84, 121.57, 124.45, 125.88, 129.63, 130.14, 131.18, 132.84, 133.35, 136.68, 138.68, 146.62, 150.36, 154.45, 160.41 (Ar-C), 165.47, 168.36, 175.52 (3CO); Anal. Calc. For C\(_{27}\)H\(_{25}\)N\(_5\)O\(_3\)S (634.71): C, 51.09; H, 3.49; N, 17.65; S, 15.16%. Found: C, 51.30; H, 3.69; N, 17.86; S, 15.37%.

5-Amino-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-3-(4-(N-(4,5-dimethylpyrazol-2-yl)sulfamoyl)phenyl)-4-imino-3,4-dihydrothieno[3,4-d]pyrimidine-1-carboxamide (7b)

It was obtained as green crystals from DMF/ethanol; yield 55%; m.p. 284°C; IR (KBr): ν cm\(^{-1}\) 3412, 3377 (NH\(_2\)), 3252, 3175 (NH), 3075 (CH-arom.), 2947 (CH-aliph.), 1668, 1652 (2CO); \(^1\)H NMR (DMSO-\(d_6\)): δ = 1.72 (s, 3H, CH\(_3\)), 2.13 (s, 3H, CH\(_3\)), 2.36 (s, 3H, CH\(_3\)), 3.02 (s, 3H, NCH\(_3\)), 5.20 (s, 1H, NH\(_2\)), 7.21-8.12 (m, 12H, Ar-H+ 2NH + CH-thiophene), 11.17 (s, 1H, NH). Anal. Calc. For C\(_{29}\)H\(_{23}\)N\(_5\)O\(_3\)S\(_2\) (645.71): C, 53.94; H, 4.21; N, 19.52; S, 9.93%. Found: C, 53.73; H, 4.42; N, 19.75; S, 9.70%.

3-Amino-4-(4-chlorophenyl)-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-6-methyl-1H-pyrazolo[3,4-b]pyridazine-5-carboxamide (9)

A mixture of pyridinethione 8 (0.01 mole) and excess of hydrazine hydrate (3 mL) was heated under reflux for 1/2 h, then ethanol (20 mL) was added and complete reflux for 14 h. The solid product that formed was collected by filtration and recrystallized from dioxane to give 9 as yellow crystals, yield 69%; m.p. 305 °C. IR (KBr): ν cm\(^{-1}\) 3427, 3358 (NH\(_2\)), 3274, 3212 (2NH), 3060 (CH-arom.), 2954 (CH-aliph.), 1673, 1650 (2CO). \(^1\)H NMR (DMSO-\(d_6\)): δ = 1.88 (s, 3H, CH\(_3\)), 2.16 (s, 3H, CH\(_3\)), 3.01 (s, 3H, NCH\(_3\)), 4.32 (s, 2H, NH\(_2\)), 6.88 –7.75 (m, 9H, Ar-H), 9.44 (s, 1H, NH), 12.25 (s, 1H, NH). \(^13\)C NMR (DMSO-\(d_6\)) δ C = 11.64, 22.48, 36.12 (3CH\(_3\)), 91.52, 104.33, 122.64, 123.24, 125.65, 127.47, 129.34, 130.51, 132.34, 133.28, 135.75, 136.32, 149.22, 150.74, 152.55, 162.17 (Ar-C), 166.37, 168.16 (2CO); Anal. Calc. for C\(_{23}\)H\(_{17}\)ClN\(_4\)O\(_2\) (487.94): C, 61.54; H, 4.54; Cl, 7.27; N, 20.09%. Found: C, 61.76; H, 4.75; Cl, 7.50; N, 20.30%.

10-(4-Chlorophenyl)-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-8-methyl-4-phenyl-2-(p-tolyl)pyrido[2′,3′:4,5]pyrazolo[1,5-a]pyrimidine-9-carboxamide (12)

A mixture of compound 9 (0.01 mole) and 3-phenyl-1-(p-tolyl)prop-2-en-1-one (0.01 mole) in ethanol (30 mL) containing a few drops of piperidine was heated under reflux for 8 h. The solid product formed after cooling was collected by filtration and recrystallized from ethanol to give (12; 70%) as yellow crystals; m.p. 195°C; IR (KBr) ν cm\(^{-1}\) 3255 (NH), 3070 (CH-arom.), 2943 (CH-aliph.), 1692, 1657 (2C=O); \(^1\)H NMR (DMSO-\(d_6\)) δ = 1.78 (s, 3H, CH\(_3\)), 2.28 (s, 3H, CH\(_3\)), 2.32 (s, 3H, CH\(_3\)), 3.12 (s, 3H, NCH\(_3\)), 6.87–7.96 (m, 19H, Ar-H+ CH-pyrimidine), 9.87 (s, 1H, NH). Anal. Calc. for C\(_{41}\)H\(_{32}\)ClN\(_5\)O\(_2\) (690.19): C, 71.35; H, 4.67; Cl, 5.14; N, 14.21%. Found: C, 71.56; H, 4.89; Cl, 5.36; N, 14.43%.
2-Amino-10-(4-chlorophenyl)-3-cyano-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-8-methyl-4-phenylpyrido[2',3':3,4]pyrazolo[1,5-alpyrimidine-9-carboxamide (14)

A mixture of compound 9 (0.01 mole) and benzylidinemalononitrile (0.01 mole) in ethanol (30 mL) containing a few drops of piperidine was heated under reflux for 8 h. The solid product formed after cooling was collected by filtration and recrystallized from ethanol to give 14 as pale yellow crystals from ethanol; yield 50%; m.p. 208°C; IR (KBr) ν cm⁻¹ 3420, 3375 (NH), 3225 (NH), 3050 (CH-arom.), 2926 (CH-ariph.), 2215 (CN), 1680, 1645 (2CO); ¹H NMR (DMSO-d₆) δ = 1.92 (s, 3H, CH₃), 2.18 (s, 3H, CH₃), 2.04 (s, 3H, NCH₃), 6.98-7.94 (m, 16H, Ar-C), 10.12 (s, 1H, NH). ¹³C NMR (DMSO-d₆) δ C = 11.89, 13.44, 34.36 (3CH₂), 115.96 (CN), 88.67, 104.48, 106.31, 122.28, 123.13, 126.65, 127.21, 128.43, 128.85, 129.46, 130.10, 130.23, 130.74, 131.44, 132.77, 133.10, 136.38, 137.43, 139.29, 148.16, 158.82, 159.25, 161.21 (Ar-C), 166.94, 169.25 (2CO); Anal. Calc. for C₃₅H₃₈ClN₉O₂ (640.09): C, 65.67; H, 4.09; Cl, 5.54; N, 19.69%. Found: C, 65.87; H, 4.32; Cl, 5.76; N, 19.81%.

2.4 Preparation of Compounds 16-19: General Procedure

To a solution of 9 (0.01 mole) in ethanol (25 mL) containing piperidine (0.5 mL), either acetyl acetone (0.01 mol), ethyl acetacetate (0.01 mol), ethyl cyanoacetate (0.01 mol) or malononitrile (0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 7 h, then cooled, poured into crushed ice and acidified with HCl. The solid product, formed in each case, was filtered off and recrystallized from dioxane to afford 16-19.

10-(4-Chlorophenyl)-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-2,4,8-trimethylpyrido[2',3':3,4]pyrazolo[1,5-alpyrimidine-9-carboxamide (16)

It was obtained as brown crystals, yield 60%; m.p. 225°C; IR (KBr): ν cm⁻¹ 3227 (NH), 3052 (CH-arom.), 2943 (CH-ariph.), 1672, 1654 (2CO); ¹H NMR (DMSO-d₆): δ = 1.35 (s, 6H, 2CH₃), 2.42 (s, 3H, CH₃), 2.33 (s, 3H, CH₃), 3.14 (s, 3H, NCH₃), 7.03-7.99 (m, 10H, Ar-H + CH-pyrimidine), 9.68 (s, 1H, NH). ¹³C NMR (DMSO-d₆) δ C = 12.46, 16.41, 21.74, 24.33, 36.18 (5CH₂), 102.69, 104.86, 105.58, 122.43, 123.45, 125.22, 128.68, 128.39, 129.57, 132.12, 133.49, 134.13, 136.35, 138.78, 145.47, 149.46, 154.51, 157.62, 160.35, (Ar-C), 165.26, 168.28 (2CO); Anal. Calc. for C₃₀H₂₈ClN₇O₂ (552.03): C, 65.27; H, 4.75; Cl, 6.42; N, 17.76%. Found: C, 65.48; H, 4.96; Cl, 6.63; N, 17.96%.

10-(4-Chlorophenyl)-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-2,4-hydroxy-8-dimethylpyrido[2',3':3,4]pyrazolo[1,5-alpyrimidine-9-carboxamide (17)

It was obtained as buff crystals, yield 51%; m.p. 222°C; IR (KBr): ν cm⁻¹ 3500 (OH), 3249 (NH), 3065 (CH-arom.), 2928 (CH-ariph.), 1660, 1647 (2CO); ¹H NMR (DMSO-d₆): δ = 1.38 (s, 3H, CH₃), 2.30 (s, 3H, CH₃), 2.41 (s, 3H, CH₃), 3.11 (s, 3H, NCH₃), 7.12-7.89 (m, 10H, Ar-H + CH-pyrimidine), 9.27 (s, 1H, NH), 11.53 (s, 1H, OH). Anal. Calc. for C₃₀H₂₉ClN₇O₃ (554.00): C, 62.87; H, 4.37; Cl, 6.40; N, 17.70%. Found: C, 62.65; H, 4.58; Cl, 6.62; N, 17.91%.

2-Amino-10-(4-chlorophenyl)-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-4-hydroxy-8-methylpyrido[2',3':3,4]pyrazolo[1,5-alpyrimidine-9-carboxamide (18)

It was obtained as pale yellow crystals, yield 52%; m.p. 237°C; IR (KBr): ν cm⁻¹ 3500 (OH), 3420, 3375 (NH₂), 3200 (NH), 3042 (CH-arom.), 2928 (CH-ariph.), 1664, 1652 (2CO); ¹H NMR (DMSO-d₆): δ = 1.86 (s, 3H, CH₃), 2.43 (s, 3H, CH₃), 3.05 (s, 3H, NCH₃), 7.16-7.95 (m, 12H, Ar-H + NH₃+ CH-pyrimidine), 9.62 (s, 1H, NH), 11.25 (s, 1H, OH). Anal. Calc. for C₃₅H₃₈ClN₉O₃ (554.99): C, 60.60; H, 4.18; Cl, 6.39; N, 20.19%. Found: C, 60.82; H, 4.39; Cl, 6.60; N, 20.42%.
2,4-Diamino-10-(4-chlorophenyl)-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-8-methylpyrido[2’,3’:3,4]pyrazolo[1,5-a]pyrimidine-9-carboxamide (19)

It was obtained as pale yellow crystals, yield 68%; m.p. 226ºC; IR (KBr): ν cm⁻¹ 3430, 3382 (2NH₂), 3237 (NH), 3066 (CH-arom.), 2945 (CH-aliph.), 1667, 1648 (2CO); ¹H NMR (DMSO-d₆): δ = 1.26 (s, 3H, CH₃), 2.25 (s, 3H, CH₃), 3.15 (s, 3H, NCH₃), 6.86 (s, 2H, NH₂), 7.24-7.98 (m, 12H, Ar-H + NH₂+ CH-pyrimidine), 10.38 (s, 1H, NH). Anal. Calc. for C₂₈H₂₅ClN₅O₂ (554.00): C, 60.70; H, 4.37; Cl, 6.40; N, 22.75%. Found: C, 60.92; H, 4.58; Cl, 6.63; N, 22.56%.

2.5 Preparation of Compounds 21, 22a, b: General Procedure

To a cold solution (0-5ºC) of either ethyl acetoacetate (0.01 mole), malononitrile (0.01 mole) or ethyl cyanoacetate (0.01 mole) in ethanol (30 mL) containing sodium acetate (2 g), solution of diazonium chloride 20 (prepared by adding a cold solution of sodium nitrite (0.01 mol) to a solution of aminopyrazole 9 (0.01 mole) in HCl (3 mL) with continuous stirring) was added. The reaction mixture, in each case, was left at room temperature for 2 h with stirring. The solid produ- ct, formed in each case, was filtered off and recrystallized from the proper solvent to yield 21, 22 a,b.

3-Acetyl-10-(4-chlorophenyl)-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-4-hydroxy-8-methylpyrido[2’,3’:3,4]pyrazolo[5,1-c][1,2,4]triazine-9-carboxamide (21)

It was obtained as brown crystals from DMF / ethanol, yield 61%; m.p. > 300 ºC: IR (KBr): ν cm⁻¹ 3500 (OH), 3216 (NH), 3055 (CH-arom.), 2932 (CH-aliph.) 1715, 1678, 1654 (3CO); ¹H NMR (DMSO-d₆): δ = 1.22 (s, 3H, CH₃), 2.12 (s, 3H, CH₃), 3.45 (s, 3H, COCH₃), 3.02 (s, 3H, NCH₃), 6.92-7.83 (m, 10H, Ar-H); ¹³C NMR (DMSO-d₆) δ C = 30.31, 22.65, 27.83, 34.25 (4CH₃), 101.81, 105.65, 122.82, 125.14, 126.32, 129.39, 129.77, 132.51, 133.47, 134.72, 135.15, 136.23, 148.36, 151.41, 154.55, 158.64, 159.15, 160.11, (Ar-C), 167.42, 169.22, 190.39 (3CO); Anal. Calc. for C₂₉H₂₅ClN₅O₄ (583.00): C, 59.74; H, 3.98; Cl, 6.08; N, 19.22%. Found: C, 59.95; H, 3.75; Cl, 6.29; N, 19.43%.

4-Amino-10-(4-chlorophenyl)-3-cyano-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-8-methylpyrido[2’,3’:3,4]pyrazolo[5,1-c][1,2,4]triazine-9-carboxamide (22a)

It was obtained as brown crystals from dioxane; yield 65%; m.p. > 300 ºC; IR (KBr) ν cm⁻¹ 3380, 3364 (NH₂), 3228 (NH), 3054 (CH-arom.), 2928 (CH-aliph.), 2217 (CN), 1669, 1648 (2C=O); ¹H NMR (DMSO-d₆) δ = 1.38 (s, 3H, CH₃), 2.23 (s, 3H, CH₃), 3.03 (s, 3H, NCH₃), 6.55 (s, 2H, NH₂), 6.88-7.74 (m, 9H, Ar-H), 9.17 (s, 1H, NH), Anal. Calc. for C₂₈H₂₅ClN₁₀O₂ (564.99): C, 59.52; H, 3.75; Cl, 6.28; N, 24.79%. Found: C, 59.73; H, 3.96; Cl, 6.49; N, 24.58%.

Ethyl-4-amino-10-(4-chlorophenyl)-9-((1,5-dimethyl-3-oxo-2-phenyl-2,3- dihydro-1H-pyrazol-4-yl)carbamoyl)-8-methylpyrido[2’,3’:3,4]pyrazolo[5,1-c][1,2,4]triazine-3-carboxylate (22b)

It was obtained as buff crystals from dioxane; yield 57%; m.p. 275 ºC; IR (KBr) ν cm⁻¹ 3346, 3320 (NH₂), 3218 (NH), 3058 (CH-arom.), 2925 (CH-aliph.), 1720, 1682, 1656 (3C=O); ¹H NMR (DMSO-d₆) δ = 1.34 (t, 3H, CH₃), 2.45 (s, 3H, CH₃), 2.77 (s, 3H, CH₃), 3.14 (s, 3H, NCH₃), 4.07 (q, 2H, CH₂), 6.72 (s, 2H, NH₂), 7.32-7.87 (m, 9H, Ar-H), 9.76 (s, 1H, NH), Anal. Calc. for C₉H₂₅ClN₁₀O₄ (612.04): C, 58.87; H, 4.28; Cl, 5.79; N, 20.60%. Found: C, 58.65; H, 4.50; Cl, 5.48; N, 20.82%.

3. RESULTS AND DISCUSSION

Treatment of N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-3-oxobutanamide
with diazonium salt has been reported. So, we observed that coupling of compound 1 with diazotized aromatic amines in ethanol buffered with sodium acetate at 0 ºC afforded the aryl hydrazones [24,29] 3a,b based on their spectral data. For example, the 1H NMR spectrum of 3a showed the presence of a multiple signal at δ = 6.89- 8.05 ppm corresponding to aromatic protons and three signals at δ = 10.43, 11.24 and 13.52 ppm corresponding to 3NH groups. It should be emphasized here that the signal corresponding to NH function of hydrazo group appears at downfield at δ = 13.52 ppm due to the intramolecular hydrogen bonding with carbonyl group. The IR spectrum of the same product further supports the hydrozo structure. So, the obtained arylhydrazones have been utilized as starting materials for preparing the targeted pyridazine ring system. Fusion of aryl hydrazones 3a,b with either malononitrile (4a) or ethyl cyanoacetate (4b) in the presence of ammonium acetate over melting point for one hour afforded the pyridazine derivatives 6a-d. The structures of 6a-d were confirmed based on elemental analysis and spectral data. Thus, the IR spectrum of compound 6a, for example, indicated the presence of the absorption band of the CN functional group at υ 2205 cm⁻¹. The 1H NMR spectrum of compound 6a revealed the presence of a multiplet signal at δ = 7.25- 8.12 ppm corresponding to aromatic protons, NH group and singlet signals at δ 10.15, 11.82 ppm assigned to 2NH groups beside the signals assigned to CH₃ protons in the molecule. Also, 13C NMR of the structure revealed signals at δ 10.54, 13.15, 34.61 ppm (3CH₃), 116.12 ppm (CN), 167.21, 169.75 ppm (2C=O), in addition to the sp² carbon atoms as in the experimental section. (Scheme 1 and Experimental part).

Scheme 1: Synthesis of pyridazines
The formation of 6 from 3 is assumed to proceed via intermediate of non isolable 5. The pyridazine derivatives 6b,c were used as starting material to obtain some fused azines. Thus, the dihydropyridazines 6b,c were reacted with elemental sulphur in ethanol containing little amount of triethylamine to afford the thienopyridazine derivatives 7a,b. The structures of 7a,b were confirmed based on elemental analysis and spectral data. The IR spectrum of compound 7a, for example, exhibited the disappearance of the absorption band due to the CN function group and the appearance of absorption band due to the NH2 functional group at \(\nu = 3425\), \(3348\) cm\(^{-1}\). \(^1\)H NMR spectrum of compound 7a revealed a singlet signal at \(\delta = 5.16\) ppm assigned to NH2, singlet signal at \(\delta = 6.24\) ppm assigned to CH-thiophene and a multiplet signals at \(\delta = 7.11-8.15\) ppm assigned to aromatic protons. Also, \(^1^3\)C NMR of the structure revealed signals at 12.21, 34.42 ppm (2CH3), 165.47, 168.36, 175.52 ppm (3C=O), and absence of CN carbon atom, in addition to the \(sp^2\) carbon atoms as in the experimental section (Scheme 2 and Experimental part).

\[
6 \xrightarrow{S} \text{EtOH / T. E. A} 7\text{ a,b}
\]

\[
7\text{ a, Ar= C}_6\text{H}_4\text{-SO}_2\text{NH-thiazol-2-yl} \quad \text{X=O} \\
7\text{ b, Ar= C}_6\text{H}_4\text{-SO}_2\text{NH-(4,5-dimethyloxazol-2-yl)} \quad \text{X= NH}
\]

Scheme 2: synthesis of thienopyridazines

This work was extended to study the reactivity of 8 towards hydrazine hydrate as binucleophile. Thus, treatment of 8 with hydrazine hydrate in boiling ethanol afforded a sulfur free reaction product 9. The IR spectrum of this reaction product showed the presence of absorption peaks at \(\nu = 3427\), \(3358\) (NH2), 3274, 3212 (2NH) and the absence of any absorption peak in the range of 2190-2250 cm\(^{-1}\) due to CN group. The \(^1\)H NMR spectrum of 9 revealed the presence of two protons as a singlet at \(\delta = 4.32\) ppm assignable to the NH2 group and signals at \(\delta = 9.44, 12.25\) ppm assigned to 2NH beside the other protons in their proper positions (Scheme 3).

\[
8 \xrightarrow{\text{EtOH reflux}} \text{NH}_2\text{NNH}_2 9
\]

Scheme 3: synthesis of pyrazolopyridine
Aim to prepare polyfunctionally substituted polycondensed pyridines, we report synthesis of several new pyridopyrazolopyrimidines via reaction of aminopyrazolopyridine 9 with non-symmetrical double bond system and with different active methylene reagents. Thus, the aminopyrazolopyridine 9 reacted with arylidineacetophenone to give pyridopyrazolopyrimidine derivative 12. The analytical and spectral data are in agreement with the proposed structure (Scheme 4 and Experimental part). Formation of 12 is assumed to proceed via an initial Michael addition of the endocylic NH in 9 with arylidineacetophenone followed by intramolecular cyclodehydration and spontaneous auto-oxidation under the reaction conditions. It should be pointed out that the reaction of 9 with arylidineacetophenone might involve the exocyclic amino group. However, the involvement of the endocyclic pyrazole-NH was considered based on the literature \cite{31, 32} reported that the ring NH in aminopyrazole is the most reactive center in the molecule. Similarly, the reaction of 9 with benzylidinemalononitrile in ethanolic piperidine afforded the pyridopyrazolo-pyrimidine derivative 14. The IR spectrum of 14 exhibited the presence of the absorption band due to the CN function group. The formation of compound 14 is assumed to proceed via an initial Michael addition of the endocyclic NH in 9 to the active double bond in benzylidinemalononitrile to yield the intermediate 13 followed by intramolecular cyclization and aromatized by a loss of hydrogen molecule to the final product 14 (Scheme 4).

Our investigation was extended to include the reactivity of the aminopyrazole 9 towards active methylene compounds, namely, acetyl acetone, ethyl acetoacetate, ethyl cyanoacetate in ethanolic piperidine to yield pyridopyrazolopyrimidine derivatives 16-18 \cite{33}, and with malononitrile to yield the diaminopyridopyrazolopyrimidine derivative 19. The structures of 16–19 were confirmed based on elemental analysis and spectral data (cf. Scheme 5 and Experimental part).
The IR spectrum of 16, for example, indicated the absence of the absorption band due to the NH₂ group. The ¹H NMR spectrum of the same product revealed the absence of any signals may be attributed to NH₂ and pyrazole-NH protons. Furthermore, the structure of compound 16 was supported by ¹³C NMR spectrum (Scheme 5 and Experimental part).

The formation of compound 16 is assumed to proceed via the condensation of the endocylic NH in 9 with acetyl acetone followed by intramolecular cyclization through dehydration (Scheme 5). Moreover, the diazotization of 9 and its reactions with active methylene reagents have been studied to develop a synthetic approach to polyfunctionality substituted fused heterocycles. Thus, coupling of different active methylene reagents such as ethyl acetoacetate, malononitrile and ethyl cyanoacetate with diazotized amino group in compound 9 afforded the pyridopyrazolotriazine derivatives 21 and 22a,b. ¹H NMR spectra of 21 revealed the disappearance of the signal corresponding to NH₂ group. Also, the ¹³C NMR of compound 21 revealed a signal at high downfield at 190.39 ppm assigned to acetyl carbonyl group (cf. Scheme 6 and Experimental part).
3.1 Antimicrobial Activity

Thirteen compounds were screened in vitro for their antimicrobial activities against two strains of bacteria (Gram positive bacteria; Bacillus cereus (G + ve), and Gram negative bacteria; Klebsiella pneumonia (G − ve) and one fungal specie (Aspergillus flavus) using the filter paper disc method [34]. The filter paper disc method was performed in nutrient agar for bacteria and Dox agar for fungi. These agar media were inoculated with 0.5 mL of the 24 h. liquid cultures. Filter paper discs (5mm diameter) saturated with each compound solution (10 mg/mL of DMSO) was placed on the indicated agar media. The incubation time was (48 h at 37 °C for bacteria and 72 h at 28 °C for fungi). Discs saturated with DMSO were used as control. Ciprofloxacin flucoral was used as a reference substance. The diameter of inhibition zones (mm) were measured and recorded. The results revealed that all the tested compounds were found to possess various antimicrobial activities towards the entire microorganisms used (Table 1). Compound 7a exhibited the highest inhibitory activity against Bacillus cereus (G +ve), compound 12 was found to be very active against Klebsiella pneumonia (G − ve). In addition, compounds 6a, 12, 21 revealed a moderate activity against Bacillus cereus (G +ve). Compounds 6, 16 showed a moderate activity against Klebsiella pneumonia (G -ve), compounds 9, 19 showed a moderate activity against Aspergillus flavus. Moreover, compounds 3a, 6b, 7b, 14, 16 and 22a showed the lowest inhibitory activity against Bacillus cereus (G +ve), compounds 3a, 6a, 7a, 17, 19 and 22a are less active towards Klebsiella pneumonia (G -ve). Whereas compounds 6a, 7a, 12, 17 exhibited the lowest inhibitory activity towards Aspergillus flavus. The results indicated that most of the synthesized compounds exhibited...
noticeable antimicrobial activity, and that the bacterial isolates were less active to the synthesized compounds than the fungal specie.

Table 1. Antimicrobial activities of some newly synthesized compounds.

<table>
<thead>
<tr>
<th>Compd. No.</th>
<th>Gram positive bacteria</th>
<th>Gram negative bacteria</th>
<th>Fungi</th>
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<tr>
<td></td>
<td>Bacillus cereus</td>
<td>Klebsiella pneumonia</td>
<td>Aspergillus flavus</td>
</tr>
<tr>
<td>3a</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6a</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6b</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7a</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7b</td>
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<td>++</td>
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</tr>
<tr>
<td>12</td>
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</tr>
<tr>
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<tr>
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<td>++++</td>
</tr>
<tr>
<td>Flucoral</td>
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</tr>
</tbody>
</table>

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).

4. CONCLUSION

The achieved derivatives of new hydrazo, pyridazine, thienopyridazine, pyrazolopyridine, pyridopyrazolopyrimidine, and pyridopyrazolotriazine that are expected to have biological activities, have been synthesized and their structures confirmed by their spectral data, elemental analyses, and with some chemical reactions.

REFERENCES


The authors declare no conflict of interest

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