Research Article

Synthesis and Pharmacological Activities of Pyrano[2,3-d]pyrimidine and Pyrano[2,3-d]pyrimidine-5-one Derivatives as New Fused Heterocyclic Systems

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Various fused oxazine such as 4-(4-methoxyphenyl)-3,7-dimethyl-1,4-dihydro-5H-pyrazolo [4',3':5,6]pyrano[2,3-d][1,3]oxazin-5-one **2** has been prepared and utilized as a starting material for novel pyrazolopyranopyrimidinones **3**, **5**, **6**, and **7a-c** and pyrazolopyranopyrimidines **4**, **9**, **10**, and **11** which are expected to possess considerable chemical and pharmacological activities. The structures of the new compounds have been elucidated by spectroscopic data and elemental analysis. The antioxidant and anticancer activities of synthesized products have been evaluated.

1. Introduction

Heterocyclic compounds are one of the most important molecules in the synthesis of pharmacologically active compounds [1–4]. Enaminonitriles have been utilized as starting materials for synthesis of huge number of heterocyclic compounds containing pyranopyrimidine [5–9] and pyridopyrimidine [10–13] moieties. Oxazines [14] and quinazolines [15] are natural products which exhibit a widespread spectrum of biological activity including antitumor [16–18], anti-inflammatory [19], and antiplatelet [20] properties. In view of these reports and as a continuation of our previous work on heterocyclic chemistry [21–27], we aimed to incorporate the pyrano moiety into the 1,5-position of oxazinone and pyrimidinone ring systems to obtain new heterocyclic system which is anticipated to possess notable chemical and pharmacological activities. So we utilized enaminonitrile in the synthesis of pyrano[2,3-d][1,3]oxazin-5-one derivative and pyrano[2,3-d]pyrimidine and pyrano[2,3-d]pyrimidine-5-one derivatives in addition to studying their antioxidant activities.

2. Results and Discussion

2.1. Chemistry. Our present investigation aimed to synthesize of 6-amino-4-(4-methoxyphenyl)-3-methyl-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile **1** [28], which was used as a precursor for the synthesis of previously unreported 4-(4-methoxyphenyl)-3,7-dimethyl-1,4-dihydro-5H-pyrazolo[4',3':5,6]pyrano[2,3-d][1,3]oxazin-5-one **2** as new heterocyclic compound containing oxazine and pyranopyrazole moieties. Compound **1** has been prepared by four-component one-pot reaction [29] and three-component one-pot reaction [30] (Scheme 1).

Treatment of **1** by freshly distilled acetic anhydride afforded **2** as a sole product. The structure of **2** was elucidated by spectral analysis. The IR spectrum showed a strong absorption band at 1732 cm^{-1} corresponding to lactonic carbonyl group and the disappearance of the cyano group. Also the structure was confirmed by elemental analysis; $^1\text{H-NMR}$ spectrum showed a singlet at δ 12.52 ppm of NH, a doublet at δ 7.162–7.136 ppm and δ 6.84–6.81 ppm of aromatic protons, a singlet at δ 4.7 ppm of benzylic proton, and a singlet at δ 3.68 ppm of three protons for OCH_3 group.

SCHEME 1

Ammonolysis of **2** by fusion with ammonium acetate and/or formamide afforded 4-(4-methoxyphenyl)-3,7-dimethyl-4,6-dihydro-pyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidine-5(1H)-one **3**. The structure of **3** was confirmed by the IR spectrum which exhibited strong absorption bands at 3200, 3281, 1664, and 1610 cm⁻¹ corresponding to ν_{NH} , $\nu_{\text{C=O}}$, and $\nu_{\text{C=N}}$, respectively and lacked a band corresponding to lactonic carbonyl group and also the presence of acidic hydrogen in the ¹H-NMR spectrum at δ 12.38. The electrophilicity of the lactonic carbonyl group of compound **2** was studied by nucleophilic reaction with 1,2-diaminobenzene and thiosemicarbazide, as 1,4-dinucleophile, to afford 4-(4-methoxyphenyl)-3,7-dimethyl-4-hydrobenzimidazolo[1,2,c]pyrazolo[4'3':5,5]pyrano[2,3-d]pyrimidine **4** and N-[4-(4-methoxyphenyl)-3,7-dimethyl-5-oxo-1,4-dihydro-pyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidin-6(5H)-yl]thiourea **5**, as sole products. The pyrazolopyranopyrimidine derivative **4** was formed via ring opening of the oxazine ring by an amino group followed by ring closer and then dehydration with the other amino group. The IR spectrum of compound **4** showed strong absorption bands at 3180 and 1609 cm⁻¹ corresponding to ν_{NH} and $\nu_{\text{C=N}}$. The IR spectrum of compound **5** showed well defined absorption bands at 3251, 3158, 3121, 1665, 1619, and 1254 cm⁻¹ corresponding to ν_{NH_2} , ν_{NH} , $\nu_{\text{C=O}}$, $\nu_{\text{C=N}}$, and $\nu_{\text{C=S}}$. Compound **2** was allowed to react with primary amines, namely, hydrazine hydrate, 4-methylaniline, 4-methoxyaniline, and/or 4-aminoaniline, to afford the pyrazolopyranopyrimidine derivatives **6** and **7a-c**, respectively (Scheme 2).

4-(4-Methoxyphenyl)-3,7dimethyl-1,4,6-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidin-5(1H)-thione **8** was synthesized by sulfuration of **3** using Lawson's reagent or by reaction of thiourea with 5-chloro-4-(4-methoxyphenyl)-3,7-dimethyl-1,4-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidine **9**. Compound **9** was prepared by chlorination of **3** by POCl₃ and PCl₅ mixture. Hydrazinolysis

of **9** afforded 5-hydrazino-4-(4-methoxyphenyl)-3,7-dimethyl-1,4-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidine **10**.

IR spectrum of compound **10** showed strong absorption bands at 3215, 3156, 3161, and 1612 cm⁻¹ corresponding to ν_{NH_2} , ν_{NH} , and $\nu_{\text{C=N}}$. Also the structure was confirmed by ¹H-NMR spectrum that showed a singlet at δ 12.01 ppm of NH, multiplet at δ 7.83 ppm of aromatic protons, a singlet at δ 4.8 ppm of benzylic proton, a singlet at δ 3.7 ppm of three protons for OCH₃ group, and a singlet at δ 5.354 of NH₂.

Acetylation of **10** by refluxing with acetic anhydride gave the diacetyl derivative **11** and not the expected triazolo derivative **12**. The structure of **11** was confirmed by the spectral data such as the IR which showed the absorbance for the carbonyl group at 1732 and 1695 cm⁻¹ and the two methyl groups signals at δ 2.678 ppm for 2CH₃ in ¹H-NMR spectrum (Scheme 3).

2.2. Pharmacological Activity. Antioxidant activity using 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) inhibition. The prepared compounds were tested for antioxidant activity as reflected in their ability to inhibit oxidation in rat brain and kidney homogenates, from the result in Table 1. Compounds **1**, **4**, **7a**, and **10** exhibit a similar antioxidant activity to ascorbic acid, used as standard. Compounds **8** and **11** exhibited moderate antioxidant activity, while **2**, **3**, **7b**, **5**, and **9** showed lower activity.

Bleomycin Dependent DNA Damage. The bleomycins are a family of glycopeptides antibiotics [31] that are routinely used as antitumor agents. The bleomycin assay has been adopted for assessing the prooxidant activity of food antioxidants. The antitumor antibiotic bleomycin binds iron ions and DNA. If the samples are able to reduce bleomycin-Fe³⁺ to bleomycin-Fe, DNA degradation in the system will be stimulated, resulting in a positive test for prooxidant activity. DNA

SCHEME 2

SCHEME 3

degradation is accompanied by the formation of a product similar to malondialdehyde.

L-Ascorbic acid was used as the reducing agent to reduce Fe³⁺ to Fe²⁺. The synthesized compounds were selected for bleomycin dependent DNA damage testing (Table 1).

Results in Table 1 showed that compounds **1**, **4**, **7a**, **8**, and **10** have the ability to protect DNA from the damage induced

by bleomycin. On the other hand, the rest of the compounds exhibited weak activities.

Cytotoxic Activity of Some Compounds against Human Tumor Cells. The prepared compounds were tested for cytotoxic activity against four human tumor cells. Best results were observed for compounds **1**, **4**, **7a**, and **10** which were found

TABLE 1: The antioxidant activities of the synthesized compounds.

Compound number	Antioxidant activity		Bleomycin dependent DNA damage
	Absorbance	Inhibition %	
1	0.111	78.6	0.098
2	0.427	17.9	0.127
3	0.419	19.4	0.135
4	0.062	88.1	0.083
5	0.393	24.4	0.138
7a	0.083	84.0	0.091
7b	0.428	17.7	0.151
8	0.274	47.3	0.089
9	0.446	14.2	0.142
10	0.060	88.5	0.066
11	0.355	31.7	0.112
Ascorbic acid	0.065	89.2	0.076
ABTS	0.520	—	—

TABLE 2: The cytotoxic activity of some compounds against human tumor cells.

Compounds	In vitro cytotoxicity IC ₅₀ (μ g/ml)*			
	HePG2	HCT-116	PC3	MCF-7
5-FU	7.9 $\pm$ 0.12	5.3 $\pm$ 0.14	8.3 $\pm$ 0.25	5.4 $\pm$ 0.21
1	9.4 $\pm$ 0.27	13.5 $\pm$ 1.65	16.9 $\pm$ 1.43	26.8 $\pm$ 1.52
2	82.1 $\pm$ 5.34	95.9 $\pm$ 6.34	>100	>100
3	45.6 $\pm$ 3.21	51.1 $\pm$ 3.50	61.1 $\pm$ 4.96	37.2 $\pm$ 2.07
4	8.4 $\pm$ 0.30	9.0 $\pm$ 0.41	16.0 $\pm$ 1.30	14.6 $\pm$ 1.36
5	36.2 $\pm$ 3.22	52.2 $\pm$ 3.67	41.3 $\pm$ 2.65	40.1 $\pm$ 3.22
7a	8.7 $\pm$ 0.26	13.61 $\pm$.48	17.9 $\pm$ 1.57	19.5 $\pm$ 1.81
7b	63.5 $\pm$ 3.98	>100	85.2 $\pm$ 6.31	85.5 $\pm$ 5.62
8	16.4 $\pm$ 1.04	31.4 $\pm$ 2.64	19.9 $\pm$ 1.68	26.9 $\pm$ 1.91
9	52.0 $\pm$ 2.83	69.2 $\pm$ 4.05	47.2 $\pm$ 2.54	58.5 $\pm$ 3.54
10	8.3 $\pm$ 0.41	11.2 $\pm$ 0.86	8.9 $\pm$ 0.50	9.1 $\pm$ 0.65
11	22.1 $\pm$ 1.56	37.8 $\pm$ 1.93	26.4 $\pm$ 1.79	30.0 $\pm$ 2.80

*IC₅₀ (μ g/ml): 1–10 (very strong), 11–20 (strong), 21–50 (moderate), 51–100 (weak), and above 100 (nontoxic); 5-FU = 5-fluorouracil.

to very strong cytotoxic compounds. Compound **8** was also considered strongly cytotoxic. Finally, compounds **3**, **5**, and **11** were moderate in their cytotoxic activity and compounds **2**, **7b**, and **9** were weakly cytotoxic (Table 2).

2.3. Experimental. All melting points were measured on a Gallenkamp electric melting point apparatus and are uncorrected. The infrared spectra were recorded in potassium bromide disks on a pye Unicam SP-3-300 and Shimadzu FTIR 8101 PC infrared spectrophotometers.

The ¹H-NMR and ¹³C-NMR were recorded at a Varian Mercury VX-300 MHz and 75 MHz, respectively, using TMS as internal standard in deuterated chloroform (CDCl₃) or deuterated dimethyl sulfoxide (DMSO-d₆). Chemical shifts are measured in ppm. The mass spectra were recorded on a Shimadzu GCMS-QP-1000EX mass spectrometer at 70 eV. Elemental analyses were carried out at the Microanalytical Center of Cairo University. All the reactions and the purity

of the new compounds were followed and checked by TLC using TLC aluminum sheets silica gel F₂₅₄.

2.4. Synthesis of 6-Amino-4-(4-methoxyphenyl)-3-methyl-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (**1**)

Method 1. An equimolar mixture of anisaldehyde (10 mmol, 1.36 mL), ethyl acetoacetate (10 mmol, 1.3 mL), hydrazine hydrate (0.5 mL), and malononitrile (10 mmol, 0.66 g) was stirred for 30 min at room temperature in the presence of triethyl amine and water (5 mL). The precipitate was filtered off and recrystallized from ethanol, yield (81%).

Method 2. A mixture of 3-methyl-5-pyrazolone (10 mmol, 0.98 g) and arylidine (10 mmol, 1.1 g) was refluxed in (30 mL) ethanol and 5 drops of piperidine for 2 h. The precipitate was filtered off and recrystallized from ethanol to give solid **1**, yield (87%).

Method 3. A mixture of 3-methyl-5-pyrazolone (10 mmol, 0.98 g), anisaldehyde (10 mmol, 1.36 mL), and malononitrile (10 mmol, 0.66 g) was refluxed in (30 mL) ethanol in presence of 5 drops of piperidine for 2 h. The precipitate was filtrated off and recrystallized from ethanol to give solid 1, yield (80%)

2.5. Synthesis of 4-(4-Methoxyphenyl)-3,7-dimethyl-1,4-dihydro-5H-pyrazolo[4',3':5,6]pyrano[2,3-d][1,3]oxazin-5-one (2). A mixture of **1** (10 mmol, 2.82 g) and freshly distilled acetic anhydride (20 mL) was heated under reflux for 3 h. The solid that deposited after distilling the excess solvent was collected and recrystallized from ethanol to give (**2**).

m.p. over 300°C. Anal. Calcd. for $C_{17}H_{15}N_3O_4$ (325.32): C, 62.76; H, 4.65; N, 12.92. Found: C, 63.07; H, 4.29; N, 13.10. FTIR (KBr) (cm^{-1}): 3157 (NH), 1732 (C=O), 1624 (C=N), 1606 (C=C). 1H -NMR (DMSO- d_6): δ_H (ppm) 12.52 (s, 1H, NH, D_2O exchangeable), 7.162–7.136 (d, J = 7.8, 2H, Ar-H), 6.84–6.81 (d, J = 9, H, Ar-H), 4.7 (s, 1H, benzylic proton), 3.68 (s, 3H, OCH_3), 2, 16 (s, 3H, CH_3) and 1.831 (s, 3H, CH_3). ^{13}C -NMR (75 MHz, DMSO- d_6): δ 173.7, 165, 163.4, 161.9, 157.2, 138.6, 134.2, 130.3, 130.3, 114.4, 114.5, 112.9, 99.8, 55.5, 34.4, 15.7, 13.4. MS, m/z (%): 325 (M^{+} , 6), 281 (27), 241 (13), 218 (100), 107 (28).

2.6. Synthesis of 4-(4-Methoxyphenyl)-3,7-dimethyl-4,6-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidine-5-(1H)-one (3). A mixture of **2** (10 mmol, 3.25 g) and formamide (10 mL) or excess ammonium acetate was refluxed for 4 h. After cooling white solid was collected, washed with water, dried, and recrystallized from benzene: ethanol mixture (1:1) to give (**3**).

m.p. over 300°C. Anal. Calcd. for $C_{17}H_{16}N_4O_3$ (324.33): C, 62.95; H, 4.97; N, 17.27. Found: C, 62.07; H, 4.92; N, 17.10. FTIR (KBr) (cm^{-1}): 3200, 3281 (NH), 1664 (C=O), 1610 (C=N). 1H -NMR (DMSO- d_6): δ_H (ppm) 12.381 (s, 1H, NH, D_2O exchangeable), 12.01 (s, 1H, NH, D_2O exchangeable), 7.359–7.334 (d, J = 7.5, 2H, Ar-H), 6.790–6.762 (d, J = 8.4, 2H, Ar-H), 4.899 (s, 1H, benzylic proton), 3.668 (s, 3H, OCH_3), 2.176 (s, 3H, CH_3) and 1.791 (s, 3H, CH_3). MS, m/z (%): 324 (M^{+} , 16), 281 (19), 240 (24), 217 (100), 107 (51).

2.7. Synthesis of 4-(4-Methoxyphenyl)-3,7-dimethyl-4-hydrobenzoimidazolo[1,2-c]pyrazolo[4',3':5,5]pyrano[3,2-d]pyrimidine (4). A mixture of **2** (10 mmol, 3.25 g) and 2-amino aniline (10 mmol, 1.08 g) in n-butanol (20 mL) was heated under reflux for 4 h. The solid that separated after concentration and cooling was filtered off and recrystallized from petroleum ether at 60–80°C.

m.p. 102–104°C. Anal. Calcd. For $C_{23}H_{19}N_5O_2$ (397): C, 69.51; H, 4.82; N, 17.62. Found: C, 69.26; H, 4.78; N, 17.23. FTIR (KBr) (cm^{-1}): 3180 (NH), 1609 (C=N). 1H -NMR (DMSO- d_6): δ_H (ppm) 11.252 (s, 1H, NH, D_2O exchangeable), 8.023–7.215 (m, 8H, Ar-H), 5.099 (s, 1H, benzylic proton), 3.212 (s, 3H, OCH_3), 2.801 (s, 3H, CH_3) and 2.176 (s, 3H, CH_3). MS, m/z (%): 395 (M^{+} , 7), 317 (9), 290 (12), 267 (10), 181 (25), 80 (100).

2.8. Synthesis of N-[4-(4-Methoxyphenyl)-3,7-dimethyl-5-oxo-1,4-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidine-6(5H)-yl]thiourea (5). A mixture of **2** (10 mmol, 3.25 g) and thiosemicarbazide (10 mmol, 0.91 g) in dimethylformamide

(20 mL) was heated under reflux for 6 h. The solid that was separated after concentration and cooling was filtered off, washed with water, and recrystallized from ethanol to give (**5**).

m.p. 265–267°C. Anal. Calcd. for $C_{18}H_{18}N_6O_3S$ (398.44): C, 54.26; H, 4.55; N, 21.09; S, 8.05. Found: C, 53.70; H, 4.19; N, 20.83. FTIR (KBr) (cm^{-1}): 3251, 3158, 3121 (NH₂, NH), 1665 (CO), 1619 (C=N) and 1254 (C=S). 1H -NMR (DMSO- d_6): δ_H (ppm) 11.912 (s, 1H, NH, D_2O exchangeable), 7.923–7.751 (m, 4H, Ar-H), 8.213 (s, 1H, NH, D_2O exchangeable), 6.754 (s, 2H, NH₂, D_2O exchangeable), 5.013 (s, 1H, benzylic proton), 3.934 (s, 3H, OCH_3), 2.178 (s, 3H, CH_3) and 1.987 (s, 3H, CH_3). ^{13}C -NMR (75 MHz, DMSO- d_6): δ 182.7, 163.2, 161.9, 160.3, 158.5, 157.1, 139.8, 135.2, 130.3, 130.3, 114.6, 114.6, 113.8, 102.9, 55.9, 35.7, 22.3, 13.3. MS, m/z (%): 398 (M^{+} , 23), 323 (20), 291 (18), 107 (100), 75 (21).

2.9. Synthesis of 6-Amino-4-(4-methoxyphenyl)-3,7-dimethyl-4,6-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidin-5(1H)-one (6). To a solution of **2** (10 mmol, 3.25 g) in 20 mL of ethanol, hydrazine hydrate (0.5 mL) was added. The reaction mixture was refluxed for 4 h. The separated solid was filtered, dried, and recrystallized from ethanol to give (**6**).

m.p. 268–290°C. Anal. Calcd. for $C_{17}H_{17}N_5O_3$ (339.35): C, 60.17; H, 5.05; N, 20.64. Found: C, 60.07; H, 4.94; N, 20.53. FTIR (KBr) (cm^{-1}): 3325, 3210, 3154 (NH₂, NH), 1669 (CO), 1609 (C=N). 1H -NMR (DMSO- d_6): δ_H (ppm) 12.012 (s, 1H, NH, D_2O exchangeable), 7.632–7.115 (m, 4H, Ar-H), 6.845 (s, 2H, NH₂, D_2O exchangeable), 5.013 (s, 1H, benzylic proton), 3.798 (s, 3H, OCH_3), 2.867 (s, 3H, CH_3) and 1.872 (s, 3H, CH_3). MS, m/z (%): 322 (M^{+} -NH₃, 23), 282 (19), 240 (10), 80 (100).

2.10. General Procedure for the Synthesis of Pyrazolopyranopyrimidine (7a–c). A solution of **2** (10 mmol, 3.25 g) in dimethylformamide (20 mL) and (10 mmol) of 4-methyl aniline, 4-methoxyaniline, and/or 4-amino aniline was heated under reflux for 5 h. The solid that was separated out after concentration and cooling was recrystallized from the proper solvent to give (**7a–c**).

2.11. 4-(4-Methoxyphenyl)-3,7-dimethyl-6-(p-tolyl)-4,6-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidin-5(1H)-one (7a). Solvent: ethanol m.p. 278–280°C. Anal. Calcd. for $C_{24}H_{22}N_4O_3$ (414.46): C, 69.55; H, 5.35; N, 13.52. Found: C, 68.67; H, 4.89; N, 12.98. FTIR (KBr) (cm^{-1}): 3191 (NH), 1682 (CO), 1608 (C=N). 1H -NMR (DMSO- d_6): δ_H (ppm) 10.972 (s, 1H, NH, D_2O exchangeable), 7.963–7.785 (m, 8H, Ar-H), 4.876 (s, 1H, benzylic proton), 3.912 (s, 3H, OCH_3), 2.911 (s, 3H, CH_3) and 2.176 (s, 3H, CH_3) and 1.987 (s, 3H, CH_3). MS, m/z (%): 414 (M^{+} , 17), 386 (8), 307 (19), 107 (100).

2.12. 4,6-Bis(4-methoxyphenyl)-3,7-dimethyl-4,6-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidin-5(1H)-one (7b). Solvent: ethanol. m.p. over 300°C. Anal. Calcd. for $C_{24}H_{22}N_4O_4$ (430.46): C, 66.97; H, 5.15; N, 13.02. Found: C, 66.17; H, 4.98; N, 13.41. FTIR (KBr) (cm^{-1}): 3154 (NH), 1685 (CO), 1611 (C=N). 1H -NMR (DMSO- d_6): δ_H (ppm) 11.241

(s, 1H, NH, D₂O exchangeable), 8.054–7.815 (m, 8H, Ar-H), 5.045 (s, 1H, benzylic proton), 3.912 (s, 6H, 2 OCH₃), 2.832 (s, 3H, CH₃), 2.092 (s, 3H, CH₃) and 1.898 (s, 3H, CH₃). MS, *m/z* (%): 430 (M⁺⁺, 8), 402 (7), 295 (19), 80 (100).

2.13. 6-(4-Aminophenyl)-4-(methoxyphenyl)-3,7-dimethyl-4,6-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidin-5(1H)-one (7c). Solvent: dioxane. m.p. over 300°C Anal. Calcd. for C₂₃H₂₁N₅O₃ (415.44): C, 66.49; H, 5.09; N, 16.86. Found: C, 66.16; H, 4.92; N, 16.16. FTIR (KBr) (cm⁻¹): 3312, 3215, 3145 (NH₂, NH), 1679 (CO), 1611 (C=N). ¹H-NMR (DMSO-*d*₆): δ_H (ppm) 9.471 (s, 1H, NH, D₂O exchangeable), 7.439–7.005 (m, 8H, Ar-H), 6.450 (s, 2H, NH₂, D₂O exchangeable), 4.713 (s, 1H, benzylic proton), 3.803 (s, 3H, OCH₃), 2.367 (s, 3H, CH₃) and 1.912 (s, 3H, CH₃). MS, *m/z* (%): 415 (M⁺⁺, 10), 398 (9), 372 (20), 77 (100).

2.14. Synthesis of 4-(4-Methoxyphenyl)-3,7-dimethyl-4,6-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidine-5(1H)-thione (8). A mixture of **9** (10 mmol, 3.4 g) and thiourea (10 mmol, 0.76 g) in ethanol (20 mL) was heated under reflux for 6 h. The separated solid was filtered, dried, and recrystallized from ethanol to give **(8)**.

m.p. 218–220°C. Anal. Calcd. for C₁₇H₁₆N₄O₂S (340): C, 59.98; H, 4.74; N, 16.46. Found: C, 60.03; H, 4.91; N, 16.10. FTIR (KBr) (cm⁻¹): 3158, 3121 (NH), and 1225 (C=S). ¹H-NMR (DMSO-*d*₆): δ_H (ppm) 11.912 (s, 1H, NH, D₂O exchangeable), 10.112 (s, 1H, NH, D₂O exchangeable), 7.923–7.751 (m, 4H, Ar-H), 4.731 (s, 1H, benzylic proton), 3.876 (s, 3H, OCH₃), 2.112 (s, 3H, CH₃) and 1.831 (s, 3H, CH₃). MS, *m/z* (%): 340 (M⁺⁺, 16), 281 (19), 241 (23), 233 (100), 107 (18).

2.15. Synthesis of 5-Chloro-4-(4-methoxyphenyl)-3,7-dimethyl-1,4-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidine (9). A mixture of **3** (10 mmol, 3.24 g), phosphorus oxychloride (15 mL), and phosphorus pentachloride (150 mmol, 3.13 g) was heated in water bath at 80°C for 8 h. The reaction mixture after cooling was poured onto crushed ice and the solid obtained was filtered off and recrystallized from ethanol to give pale brown solid **(9)**.

m.p. 198–200°C. Anal. Calcd. for C₁₇H₁₅ClN₄O₂ (342): C, 59.57; H, 4.41; N, 10.34. Found: C, 58.97; H, 3.95; N, 10.10. FTIR (KBr) (cm⁻¹): 3123 (NH), 1620 (C=N). ¹H-NMR (DMSO-*d*₆): δ_H (ppm) 12.012 (s, 1H, NH, D₂O exchangeable), 7.832–7.615 (m, 4H, Ar-H), 4.832 (s, 1H, benzylic proton), 3.843 (s, 3H, OCH₃), 2.546 (s, 3H, CH₃) and 1.687 (s, 3H, CH₃). MS, *m/z* (%): 342 (M⁺⁺, 10), 344 (25), 235 (12), 237 (30), 214 (28), 201 (100), 107 (30).

2.16. Synthesis of 5-Hydrazino-4-(4-methoxyphenyl)-3,7-dimethyl-1,4-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidine (10). A mixture of **9** (10 mmol, 3.4 g) and hydrazine hydrate (0.5 mL) in (30 mL) ethanol was heated under reflux for 5 h. The solid separated after concentration and cooling was filtered off and recrystallized from ethanol to give **(10)**.

m.p. 243–245°C. Anal. Calcd. for C₁₇H₁₈N₆O₂ (338.36): C, 60.34; H, 5.36; N, 24.84. Found: C, 61.07; H, 5.23; N, 24.73. FTIR (KBr) (cm⁻¹): 3215, 3156, 3161 (NH₂, NH), 1612 (C=N)

and. ¹H-NMR (DMSO-*d*₆): δ_H (ppm) 12.012 (s, 1H, NH, D₂O exchangeable), 8.123–7.836 (m, 4H, Ar-H), 6.013 (s, 1H, NH, D₂O exchangeable), 5.354 (s, 2H, NH₂, D₂O exchangeable), 4.803 (s, 1H, benzylic proton), 3.734 (s, 3H, OCH₃), 2.576 (s, 3H, CH₃) and 2.087 (s, 3H, CH₃). MS, *m/z* (%): 338 (M⁺⁺, 12), 307 (21), 244 (11), 201 (100), 107 (18).

2.17. Synthesis of 5-(2N,N-diacetylhydrazino)-4-(4-methoxyphenyl)-3,7-dimethyl-1,4-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidine (11). A mixture of **10** (10 mmol, 3.3 g) was heated under reflux with 30 mL of fresh distilled acetic anhydride for 8 h. The excess acetic anhydride was removed by distillation and the separated product was filtered off and recrystallized from ethanol to give **(11)**.

m.p. 278–280°C. Anal. Calcd. for C₂₁H₂₂N₆O₄ (422.44): C, 59.71; H, 5.25; N, 19.81. Found: C, 60.17; H, 4.96; N, 19.23. FTIR (KBr) (cm⁻¹): 3211, 3198 (NH), 1732, 1695 (CO), and 1619 (C=N). ¹H-NMR (DMSO-*d*₆): δ_H (ppm) 12.901 (s, 1H, NH, D₂O exchangeable), 7.902–7.714 (m, 4H, Ar-H), 6.213 (s, 1H, NH, D₂O exchangeable), 5.630 (s, 1H, benzylic proton), 3.919 (s, 3H, OCH₃), 2.013 (s, 3H, CH₃), 2.678 (s, 6H, 2CH₃) and 1.987 (s, 3H, CH₃). ¹³C-NMR (75 MHz, DMSO-*d*₆): δ 176.6, 174.6, 173.8, 171.8, 163.5, 160.2, 157.2, 138.7, 130.7, 130.0, 130.0, 114.6, 114.6, 113.1, 98.2, 56.0, 39.3, 25.2, 21.4, 21.4, 12.8. MS, *m/z* (%): 422 (M⁺⁺, 13), 322 (17), 307 (100), 201 (23), 107 (28), 101 (16).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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