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Original article

Synthesis and antimicrobial activity of some new heterocycles incorporating antipyrine moiety

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Abstract

2-Cyano-*N*-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)acetamide (**1**) was utilized as key intermediate for the synthesis of some new coumarin **2**, pyridine **3**, pyrrole **4**, thiazole **7**, pyrido[2′,3′:3,4][pyrazolo[5,1-*c*]triazine and aminopyrazole **9**. Treatment of aminopyrazole **9** with nitrous acid, 1,3-dicarbonyl compounds, enaminone, and DMF–DMA led to the formation of pyrazolo[3,4-*d*]triazine **10**, pyrazolo[1,5-*a*]pyrimidines **11**, **12**, **14**, and pyrazolo[3,4-*d*]pyrimidine **13**, respectively. Condensation of **9** with isatin afforded Schiff base **16**. The newly synthesized compounds were characterized by IR, ^{1}H NMR and mass spectral studies. Representative compounds of the synthesized products were tested and evaluated as antimicrobial agents.

Keywords: Antipyrine; Pyrazole; Pyrazolo[1,5-*a*]pyrimidine; Pyrazolo[3,4-*d*]triazine; Pyridine; Antimicrobial activity

1. Introduction

Antipyrine (2,3-dimethyl-1-phenyl-3-pyrazolin-5-one) was the first pyrazolone derivative used in the management of pain and inflammation, and their derivatives have attracted the attention of several research groups due to their potential activities [1–5]. In this context, broad spectra of bioactive antipyrine derivatives have been investigated and diversities of bioactivities such as analgesic [6,7], anti-inflammatory [8], antimicrobial [9–11], and anticancer activity [12] have been reported. The antibacterial activity caught our attention because antimicrobial resistance developed by important pathogens has increased in the last decade [13]. Besides, emerging and re-emerging bacterial infectious diseases still cause death and disability worldwide [14].

In view of the above mentioned findings and as continuation of our effort [15–20] to identify new candidates that may be of value in designing new, potent, selective and less toxic antimicrobial agents, we report herein the synthesis of some new heterocycles incorporating antipyrine moiety starting from *N*-antipyrinyl cyanoacetamide in order to investigate their antimicrobial activity.

2. Results and discussion

2.1. Chemistry

The synthetic procedures adopted to obtain the target compounds are depicted in Schemes 1 and 2. The starting compound, 2-cyano-*N*-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)acetamide (**1**) was prepared according to the previously reported procedure [21]. Thus, cyclocondensation of compound **1** with salicylaldehyde in boiling ethanol containing a catalytic amount of piperidine afforded the coumarin derivative **2**. The reaction of **1** with 1,3-dicarbonyl compound was studied in the aim of formation of pyridine derivatives with potential biological activities [22,23]. Thus, it reacted with acetylacetone to give the pyridine derivative **3**. Structure of the latter product was based on analytical and spectral data. The IR spectrum showed three absorption bands at 2217, 1662 and 1645 cm^{-1} due to CN and two CO groups.

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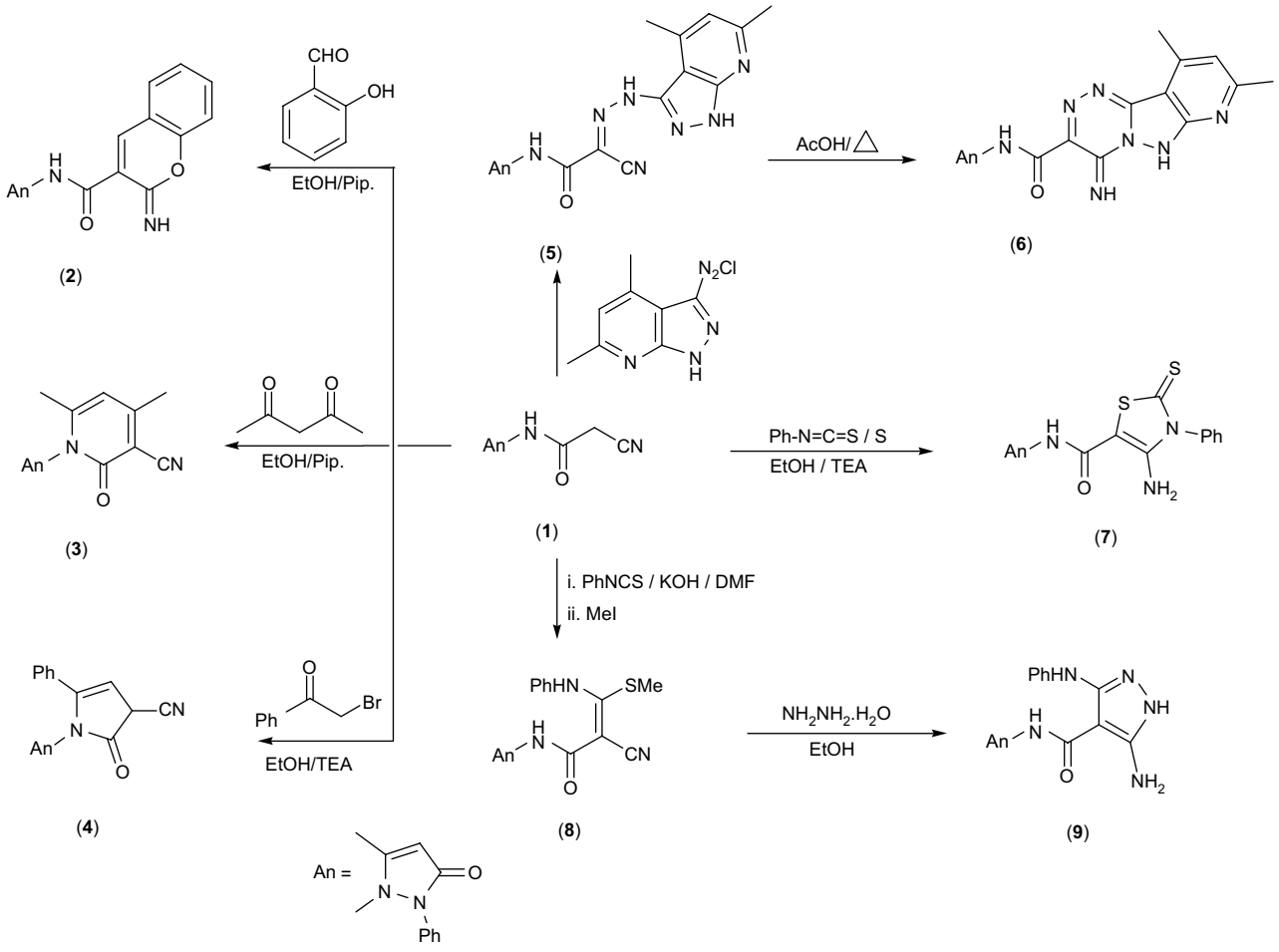


Scheme 1.

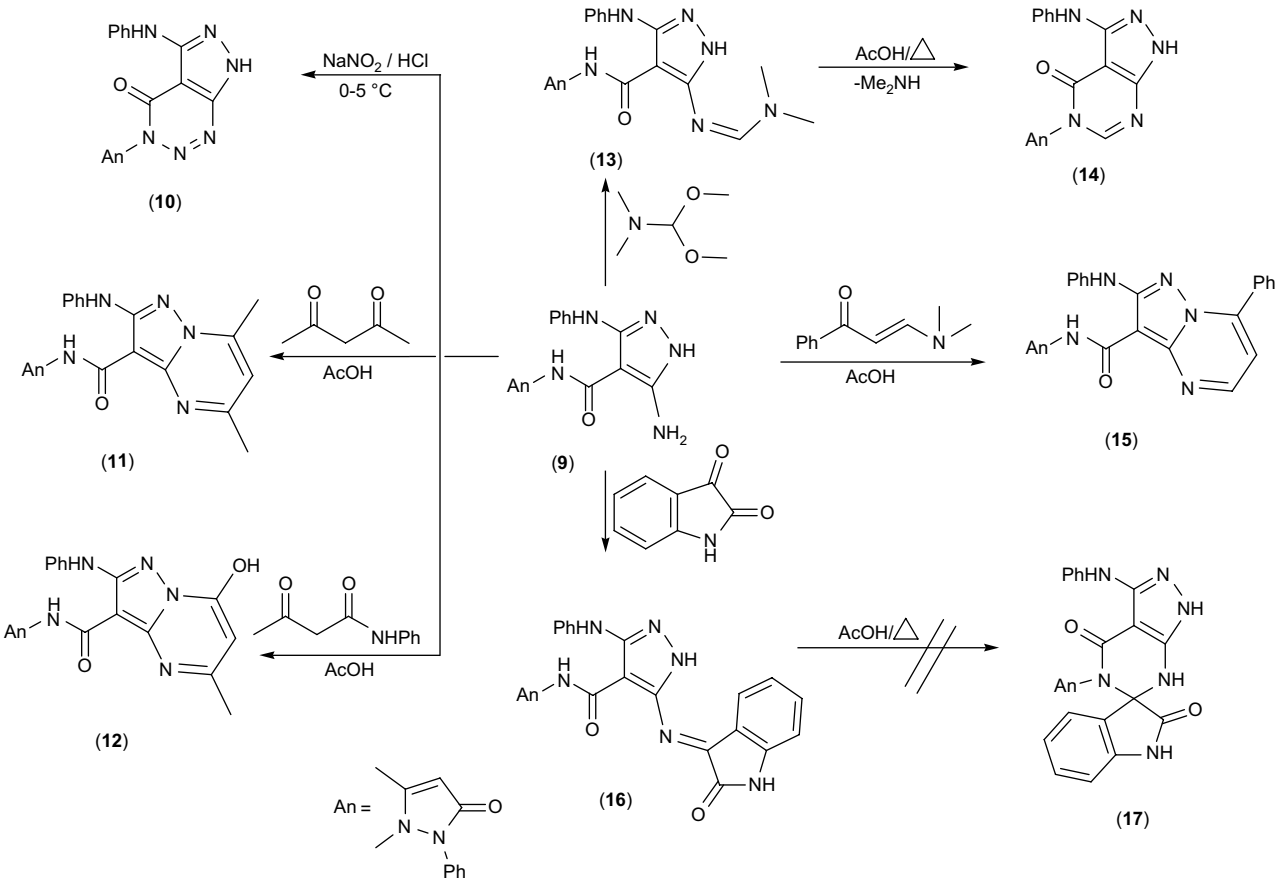
Its ^{1}H NMR spectrum revealed the appearance of three new singlets at δ 2.25, 2.36, and 6.15 ppm assigned to two methyl protons and the pyridinone H-5.

Recently, we have reported the reaction of cyanoacetamide moiety with α-halocarbonyl compounds which represents a new, simple and efficient synthetic route for the synthesis of pyrrole derivatives [24]. Therefore, it was interesting to study the reaction of **1** with phenacyl bromide. Cyclocondensation of **1** with phenacyl bromide in boiling ethanol using triethylamine as a basic catalyst furnished the pyrrole derivative **4**. The analytical and spectral data are in agreement with the proposed structure. Thus, ^{1}H NMR spectrum of the reaction product showed two doublet signals at δ 4.54 and 5.65 ppm corresponding to the two vicinal pyrroline protons H-3 and H-4, respectively, two singlets at δ 2.24, 3.24 ppm corresponding to CH_3, NCH_3, and a multiplet at δ 7.15–7.67 ppm corresponding to aromatic protons. The IR spectrum showed the presence of two C=O groups stretching at 1690, 1645 cm^{-1} and CN group at 2195 cm^{-1}.

In continuation of our interest in the synthesis of bridgedhead nitrogen heterocyclic systems [25], we have found that diazotized heterocyclic amine is an excellent building block for the synthesis of the target compound. Thus, coupling of compound **1** with 4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-diazonium chloride [26] in pyridine at 0–5 °C afforded the corresponding hydrazono compound **5**. When compound **5** is refluxed in acetic acid, it can be cyclized to *N*-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-4-imino-4,6-dihydropyrido[2′,3′:3,4]pyrazolo[5,1-*c*][1,2,4]triazine-3-carboxamide (**6**). The formation of **6** may be interpreted through the nucleophilic attack of ring nitrogen on cyano group. The IR spectrum of compound **6** showed three absorption bands at 3406, 3288, and 3228 cm^{-1} due to three NH groups besides two carbonyl absorption bands at 1672 and 1641 cm^{-1}. Its ^{1}H NMR spectrum revealed three D_2O-exchangeable singlets at δ 9.12, 9.33, and 10.24 ppm due to three NH protons, in addition to four singlets at δ 2.21, 3.18, 2.73, and 3.07 ppm assignable for two methyl protons of the pyridine ring and two methyl protons of the antipyrine ring, respectively.

The reaction of compound **1** with both elemental sulfur and phenyl isothiocyanate in warming ethanol containing a catalytic amount of triethylamine gave the thiazole derivative **7**. Elemental analysis, IR, ^{1}H NMR and ^{13}C NMR are in agreement with the proposed structure.

Treatment of compound **1** with phenyl isothiocyanate in DMF, and in the presence of potassium hydroxide, at room



Scheme 2.

temperature, followed by treatment with methyl iodide afforded the novel ketene *N*,*S*-acetal **8**. The structure of **8** was established on the basis of its elemental analysis and spectral data. Its IR spectrum showed absorption bands at 3350, 3268, and 2190 cm^{-1} due to two NH groups and nitrile functions, respectively. The mass spectrum showed a molecular ion peak at *m*/*z* 419 (M^+) which agree with its molecular formula $C_{22}H_{21}N_5O_2S$. Reaction of **8** with hydrazine in refluxing ethanol gave the corresponding pyrazole derivative **9**. The chemical structure of **9** was established on the basis of its elemental analysis and spectral data. Its ^1H NMR spectrum displayed a broad signal at δ 6.05 ppm corresponding to the NH_2 group, a multiplet signal at 6.92–7.45 ppm related to the aromatic protons, and another three singlets at δ 7.97, 8.92, and 11.35 ppm assignable to the three NH protons. The ^{13}C NMR spectrum was characterized by signals at 11.7, 31.2 ppm assigned to the two methyl carbons, and signals at 123.8–135.4 ppm assigned to aromatic carbons, and signals at 162.5, 164.2 ppm assigned to the two amidic carbonyl carbon atoms.

5-Aminopyrazoles are versatile reagents and have been extensively used as synthetic intermediates for the synthesis of polysubstituted fused pyrazoles of potential biological activity [27–30]. It was thus of interest to study the reactivity of 5-aminopyrazole derivative **9** towards a variety of chemical reagents. Diazotization of compound **9** with sodium nitrite and hydrochloric acid at 0–5 °C led to the formation of pyrazolo[3,4-*d*]triazine derivative **10** in excellent yield. The structure of compound **10** was established on the basis of analytical and spectral data. The IR spectrum showed the disappearance of the amino group and presence of a sharp intense absorption band at 2182 cm^{-1} indicating the cyclic diazo group. The formation of compound **10** may be rationalized *via* intramolecular attack of the electrophilic nitrogen of a diazo group on a carboxamide function [31,32].

Next, we moved on to investigate the applicability and synthetic potency of **9** to develop a facile and convenient route to polyfunctionally substituted pyrazolopyrimidine derivatives of an expected wide spectrum of bioresponses [33–35]. Thus, cyclocondensation reactions of compound **9** with some 1,3-dicarbonyl compounds were also studied. The reaction of **9** with either acetylacetone or acetoacetanilide in boiling acetic acid produced in each case a single product, as evidenced by TLC. The reaction products can be formulated as pyrazolo[1,5-*a*]pyrimidine derivatives **11** and **12**, evidence for the assigned structures being provided by analytical and spectroscopic data.

Although one may argue that the reaction of **9** with acetoacetanilide may lead to the other regioisomer, the regioselectivity of such reactions is well established [36]. The formation of compounds **11** and **12** is, therefore, assumed to proceed *via* initial attack of the exocyclic amino group of **9** on the keto group of 1,3-dicarbonyls followed by intramolecular cyclization *via* elimination of water and aniline, respectively.

The reaction of **9** with dimethylformamide–dimethylacetal (DMF–DMA) in dry dioxane afforded *N*,*N*-dimethylaminomethyleneamino pyrazole derivative **13**. Structure **13** was confirmed on the basis of elemental analysis and spectral studies. The mass spectrum revealed a molecular ion peak at *m*/*z* 458 corresponding to $C_{24}H_{26}N_8O_2$. Its ^{1}H NMR spectrum showed in addition to the expected signals three singlets at δ 3.04, 3.11, 8.21 ppm assigned to dimethylamino group ($N(CH_3)_2$) and azomethine proton (CH=N), respectively. Compound **13** could be transformed to pyrazolo[3,4-*d*]pyrimidine derivative **14** upon heating in glacial acetic acid. The IR and mass spectra were consistent with the proposed structure.

Furthermore, we also investigated the reactivity of aminopyrazole **9** towards enaminone with the aim of preparing pyrazolo[1,5-*a*]pyrimidine. Thus, reaction of **9** with 3-(dimethylamino)-1-phenylprop-2-en-1-one [37] in glacial acetic acid at reflux afforded pyrazolo[1,5-*a*]pyrimidine derivative **15**. Structure of the latter product was confirmed on the basis of its correct elemental analysis and spectral data. The mass spectrum showed a molecular ion peak at *m*/*z* 515 (M^+) corresponding to a molecular formula $C_{30}H_{25}N_7O_2$. Its ^{1}H NMR spectrum showed in addition to the expected signals two doublet signals at δ 8.23 and 8.34 ppm with coupling constant ($J = 4.5$ Hz) assigned to two vicinal protons of the pyrimidine ring H-4 and H-5, respectively. The formation of compound **15** is assumed to take place *via* an initial Michael addition of the exocyclic amino group in **9** to the activated double bond in enaminone followed by cyclization and aromatization *via* loss of both dimethylamine and water molecules. Although the endocyclic imino group in compound **9** is the most nucleophilic center [38–40], nevertheless, it is the most sterically hindered site [41].

Condensation of **9** with isatin in boiling ethanol furnished Schiff's base **16** in excellent yield. The chemical structure of **16** was elucidated on the basis of elemental analysis and spectral data. The IR spectrum indicated the presence of three strong absorption bands at 1744, 1703, and 1640 cm^{-1} due to three carbonyl groups. In contrast to the behavior of compound **13** towards acetic acid, compound **16** failed to give the spiro derivative **17**. This may be attributed to steric hindrance in compound **16** which restricted cyclization process.

3. Pharmacology

3.1. Antimicrobial evaluation

Twelve compounds were screened *in vitro* for their antimicrobial activities against two strains of bacteria *Bacillus thuringiensis*, *Klebsiella pneumoniae*, and two strains of fungi *Botrytis fabae* and *Fusarium oxysporum* by the agar diffusion technique [42]. A 0.15 mg/mL solution in DMSO was used. The bacteria and fungi were maintained on nutrient agar and Czapek's-Dox agar media, respectively. DMSO showed no inhibition zones. The agar media were incubated with different microorganism cultures tested. After 24 h of incubation at 30 °C for bacteria and 72 h of incubation at 28 °C for fungi, the diameter of inhibition zone (mm) was measured (Table 1). Ampicillin, Chloroamphenicol, and Fluconazole were purchased from Egyptian market and used in a concentration of 25 μg/mL as references for antibacterial and antifungal activities. The results for antibacterial activities depicted in Table 1 revealed that compounds **2**, **13** and **14** exhibited good activities. On the other hand, most of the prepared compounds **2**, **10–14,** exhibited interesting high antifungal activities against the reference chemotherapeutics. It is worth mentioning that incorporation of antipyrine to the coumarin nucleus at position 3 *via* a carboxamide linker **2** produced a high antimicrobial activity. Conversion of 5-aminopyrazole derivative **9** to pyrazolo[3,4-*d*]pyrimidine derivative **14** enhanced also the antimicrobial activity. On other hand, incorporation of the antipyrine nucleus to pyridine at position 1 in **3** unfortunately produced weak antimicrobial activity.

Table 1
Inhibition zone (mean diameter of inhibition in mm) as a criterion of antibacterial and antifungal activities of the newly synthesized compounds

Compound no.	Inhibition zone in mm			
	Bacteria		Fungi	
	Gram positive bacteria	Gram negative bacteria	*B. fabae*	*F. oxysporum*
	B. thuringiensis	*K. pneumoniae*		
1	12	13	10	12
2	24	22	18	18
3	10	13	12	12
5	16	19	16	16
6	13	14	13	14
7	12	12	13	13
9	15	12	16	12
10	22	22	26	22
11	21	28	24	25
12	20	24	27	25
13	27	24	25	21
14	28	29	27	25
Reference drugs				
Ampicillin	18	19	17	15
Chloramphenicol	23	20	16	15
Fluconazole	–	–	22	16

In conclusion, we reported herein a simple and convenient route for the synthesis of some new heterocycles based on antipyrine for antimicrobial evaluation.

4. Experimental

Melting points were measured with a Gallenkamp apparatus and are uncorrected. IR spectra were recorded for KBr disc on a Mattson 5000 FTIR spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker AC 300 (300 MHz) in $CDCl_3$ or DMSO-d_6 as solvent, using TMS as an internal standard, and chemical shifts are expressed as δ_{ppm}. Mass spectra were determined on Finnigan Incos 500 (70 eV). Elemental analyses were carried out at the Microanalytical Center of Cairo University. All reactions were followed by TLC (Silica gel, aluminum sheets 60 F_{254}, Merck). 2-Cyano-*N*-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)acetamide **1** [21], 4,6-dimethyl-1*H*-pyrazolo [3,4-*b*]pyridin-3-diazonium chloride [26], and 3-(dimethylamino)-1-phenylprop-2-en-1-one [37] were prepared according to the procedures reported in the literature.

4.1. Synthesis of N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-2-imino-2H-chromene-3-carboxamide (2)

To a solution of compound **1** (0.54 g, 2 mmol) in absolute ethanol (20 mL) containing piperidine (0.5 mL), salicylaldehyde (0.3 g, 2 mmol) was added. The reaction mixture was heated under reflux for 3 h, and then allowed to cool. The precipitate that formed was filtered off, washed with ethanol, dried and recrystallized from ethanol to afford **2**.

Pale yellow crystal; yield 90%; mp 225–227 °C; IR (KBr): $\nu/cm^{-1} = 3223$, 3431 (2NH), 1685, 1620 (2C=O). ^1H NMR ($CDCl_3$): $\delta_{ppm} = 2.33$ (s, 3H, CH_3), 3.06 (s, 3H, NCH_3), 7.03–7.48 (m, 9H, ArH), 7.77 (s, 1H, CH=), 8.43 (s, 1H, NH=), 11.79 (s, 1H, NHCO). MS *m/z* (%): 375 ($M^+ + 1$, 2.45), 374 (M^+, 6.89), 307 (13.25), 270 (2.95), 255 (4.8), 230 (6.15), 203 (11.58), 56 (100). Anal. for $C_{21}H_{18}N_4O_3$ (374.14): calcd.: C 67.37, H 4.85, N 14.96%; found: C 67.28, H 4.81, N 15.00%.

4.2. Synthesis of 1-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-4,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (3)

A mixture of compound **1** (0.54 g, 2 mmol) and acetylacetone (0.21 mL, 2 mmol) in ethanol (15 mL) containing a few drops of piperidine (3 drops) was refluxed for 10 h. The reaction mixture was cooled and the solid so obtained was filtered off and recrystallized from ethanol to give **3**.

Colorless crystals; yield 85%; mp 235–237 °C; IR (KBr): $\nu/cm^{-1} = 2217$ (CN), 1662, 1645 (2C=O). ^1H NMR ($CDCl_3$): $\delta_{ppm} = 2.16$ (s, 3H, CH_3-pyrazole), 2.25 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 3.23 (s, 3H, NCH_3), 6.15 (s, 1H, pyridine H-5), 7.25–7.59 (m, 5H, ArH). ^{13}C NMR ($CDCl_3$): $\delta_{ppm} = 11.5$ (q, CH_3), 20.9 (q, CH_3), 21.3 (q, CH_3), 35.5 (q, NCH_3), 101.4, 106.2, 109.2, 115.3 (CN), 125.1, 127.7, 129.4, 134.0, 152.3, 152.8, 159.5 (C=O), 160.0 (C=O). Anal. for $C_{19}H_{18}N_4O_2$ (334.14): calcd.: C 68.25, H 5.43, N 16.76%; found: C 68.32, H 5.31, N 16.56%.

4.3. Synthesis of 1-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-2-oxo-5-phenyl-2,3-dihydro-1H-pyrrole-3-carbonitrile (4)

A mixture of compound **1** (0.54 g, 2 mmol) and phenacyl bromide (0.389 g, 2 mmol) in ethanol (30 mL) containing triethylamine (0.5 mL) was refluxed for 3 h. The formed solid product was filtered off, dried and recrystallized from ethanol to give compound **4**.

Pale yellow powder; yield 45%; mp 140–142 °C; IR (KBr): $\nu/cm^{-1} = 2195$ (CN), 1690, 1645 (2C=O). ^1H NMR ($CDCl_3$): $\delta_{ppm} = 2.24$ (s, 3H, CH_3), 3.24 (s, 3H, NCH_3), 4.54 (d, $J = 2.5$ Hz, 1H, pyrrole H-3), 5.65 (d, $J = 2.5$ Hz, 1H, pyrrole H-4), 7.15–7.67 (m, 10H, ArH). MS *m/z* (%): 371 ($M^+ + 1$, 3.3), 370 (M^+, 6.9), 229 (18.1), 142 (2.6), 119 (10.1), 56 (100). Anal. for $C_{22}H_{18}N_4O_2$ (370.40): calcd.: C 71. 34, H 4.90, N 15.13%; found: C 71.22, H 4.86, N 15.09%.

4.4. Synthesis of 2-cyano-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-2-[(4,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-3-yl) hydrazono]acetamide (5)

To a cold (0–5 °C) solution of compound **1** (0.54 g, 2 mmol) in pyridine (25 mL) was added the 4,6-dimethyl-2*H*-pyrazolo[3,4-*b*]pyridine-3-yl diazonium salt [which was prepared by dissolving sodium nitrite (0.14 g, 2 mmol) in water (2 mL) and adding to a cold solution of 3-amino-4,6-dimethyl-2*H*-pyrazolo[3,4-*b*]pyridine (0.32 g, 2 mmol) containing the appropriate amount of hydrochloric acid with continuous stirring] portionwise over a period of 30 min. The reaction mixture was kept in an ice box overnight and then diluted with water. The solid that precipitated was filtered off, washed with water and dried, and recrystallized from a mixture of ethanol and DMF (2:1) to give compound **5**.

Red powder; yield 75%; mp 198–200 °C; IR (KBr): $\nu/cm^{-1} = 3392$ (NH), 1660, 1645 (2C=O), 2197 (CN). ^1H NMR (DMSO-d_6): $\delta_{ppm} = 2.23$ (s, 3H, CH_3), 2.71 (s, 3H, CH_3), 3.03 (s, 3H, CH_3), 3.16 (s, 3H, NCH_3), 7.11 (s, 1H, pyridine H-5), 7.41–7.61 (m, 5H, ArH), 9.10 (s, 1H, NH), 9.31 (s, 1H, NH), 10.19 (s, H, NH). MS *m/z* (%): 443 (M^+, 19.38), 413 (25), 375 (3.13), 291 (3.13), 269 (7.8), 202 (13.75), 56 (100). Anal. for $C_{22}H_{21}N_9O_2$ (443.46): calcd.: C 59.58, H 4.77, N 28.43%; found: C 59.42, H 4.61, N 28.56%.

4.5. Synthesis of N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-4-imino-8,10-dimethyl-4,6-dihydropyrido[2′,3′:3,4]pyrazolo[5,1-c][1,2,4]-triazine-3-carboxamide (6)

A solution of **5** (0.89 g, 2 mmol) in glacial acetic acid (20 mL) was refluxed for 3 h, and then allowed to cool. The precipitate that formed was filtered off, washed with ethanol and recrystallized from a mixture of ethanol and DMF (1:1) to give compound **6**.

White powder; yield 80%; mp >300 °C; IR (KBr): $\nu/cm^{-1} =$ 3406, 3288, 3228 (3NH), 1672, 1641 (2C=O). ^1H NMR (DMSO-d_6): $\delta_{ppm} = 2.21$ (s, 3H, CH_3-pyrazole), 2.73 (s, 3H, CH_3), 3.07 (s, 3H, CH_3), 3.18 (s, 3H, NCH_3), 6.98 (s, 1H, pyridine H-5), 7.36–7.58 (m, 5H, ArH), 9.12 (s, 1H, NH), 9.33 (s, 1H, NH), 10.24 (s, H, NH). Anal. for $C_{22}H_{21}N_9O_2$ (443.46): calcd.: C 59.58, H 4.77, N 28.43%; found: C 59.46, H 4.41, N 28.36%.

4.6. Synthesis of 4-amino-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-3-phenyl-2-thioxo-2,3-dihydro-1,3-thiazole-5-carboxamide (7)

To a solution of compound **1** (0.54 g, 2 mmol) in ethanol (20 mL) containing triethylamine (0.5 mL), elemental sulfur (0.064 g, 2 mmol) and phenyl isothiocyanate (0.27 g, 2 mmol) were added. The reaction mixture was heated at 60 °C for 2 h with continuous stirring and then poured into a beaker containing an ice/water mixture containing few drops of hydrochloric

acid. The formed solid product was collected by filtration, dried, and recrystallized from DMF and ethanol (3:1) to give compound **7**.

White powder; yield 65%; mp 236–238 °C; IR (KBr): $\nu/cm^{-1} = 3420$ (NH_2), 3345 (NH), 1693, 1670 (2C=O), 1210 (C=S). ^1H NMR (DMSO-d_6): $\delta_{ppm} = 2.13$ (s, 3H, CH_3), 3.06 (s, 3H, NCH_3), 6.82 (s, 2H, NH_2), 7.21–7.63 (m, 10 H, ArH), 8.99 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): $\delta_{ppm} = 11.3$, 36.3, 124.1, 126.8, 129.3, 129.5, 130.5, 135.3, 135.4, 153.9, 162.6, 186.1. Anal. for $C_{21}H_{19}N_5O_2S_2$ (437.54): calcd.: C 57.65, H 4.38, N 16.01%; found: C 57.52, H 4.41, N 16.06%.

4.7. Synthesis of 3-anilino-2-cyano-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-3-(methylthio)acrylamide (8)

To a stirred solution of potassium hydroxide (0.11 g, 2 mmol) in dimethylformamide (20 mL) was added compound **1** (0.544 g, 2 mmol). After stirring for 30 min, phenyl isothiocyanate (0.27 g, 2 mmol) was added to the resulting mixture. Stirring was continued for 6 h, and then methyl iodide (0.28 g, 2 mmol) was added. Stirring was continued for additional 3 h. Then, the reaction mixture was poured onto ice water. The solid product that formed was filtered off, dried and recrystallized from ethanol to afford **8**.

Yellow powder; yield 85%; mp 85–87 °C; IR (KBr): $\nu/cm^{-1} = 3350$, 3268 (2NH), 2190 (CN), 1660, 1645 (2C=O), 1623 (C=C). ^1H NMR (DMSO-d_6): $\delta_{ppm} = 2.17$ (s, 3H, CH_3-pyrazole), 2.28 (s, 3H, SCH_3), 3.18 (s, 3H, NCH_3), 7.07–7.64 (m, 10H, ArH), 9.12 (s, 1H, NH), 11.15 (s, 1H, NH). MS *m/z* (%): 419 (M^+, 3.4), 372 ($M^+ - SMe$, 8.9), 230 (10.6), 229 (20.8), 203 (10.6), 189 (2.3), 171 (5.5), 169 (6.8), 119 (2.7), 102 (1.9), 56 (100). Anal. for $C_{22}H_{21}N_5O_2S$ (419.50): calcd.: C 62.99, H 5.05, N 16.69%; found: C 62.86, H 4.98, N 16.61%.

4.8. Synthesis of 5-amino-3-anilino-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-5,7-dimethylpyrazolo[1,5-a]pyrimidine-3-carboxamide (9)

A mixture of compound **8** (0.9 g, 2 mmol) and hydrazine hydrate 98% (0.5 mL, 5 mmol) was heated on a steam bath for 1 h and then left to cool. The reaction mixture was triturated with ethanol and the resulting solid was filtered off and recrystallized from ethanol to give compound **9**.

Colorless crystals; yield 85%; mp 243–245 °C; IR (KBr): $\nu/cm^{-1} = 1628$ (C=O), 3240, 3355 (2NH), 3400 (NH_2). ^1H NMR (DMSO-d_6): $\delta_{ppm} = 2.21$ (s, 3H, CH_3), 3.23 (s, 3H, NCH_3), 6.05 (s, 2H, NH_2), 6.92–7.45 (m, 10H, ArH), 7.97 (s, 1H, NH), 8.92 (s, 1H, NH), 11.35 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): $\delta_{ppm} = 11.7$, 31.2, 107.9, 116.2, 119.3, 123.8, 126.5, 126.1, 129.1, 135.4, 143.3, 148.3, 151.2, 153, 162.5, 164.2. Anal. for $C_{21}H_{21}N_7O_2$ (403.44): calcd.: C 62.52, H 5.25, N 24.30%; found: C 62.61, H 5.16, N 24.41%.

4.9. Synthesis of 5-anilino-3-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-3,7-dihydro-4H-pyrazolo[3,4-d][1,2,3]triazin-4-one (10)

A well-stirred solution of compound **9** (0.403 g, 10 mmol) in 3 mL concentrated hydrochloric acid and 3 mL water was cooled in an ice-bath (0–5 °C), then a cooled solution of sodium nitrite (0.7 g) in 5 mL water was added dropwise to the above solution. The reaction mixture was stirred for 2 h and the separated solid was filtered off, dried well and recrystallized from ethanol to give compound **10**.

Pale yellow crystals; yield 90%; mp 198–200 °C; IR (KBr): $\nu/cm^{-1} = 3423$ (NH), 2182 (N=N), 1706, 1643 (2C=O). ^1H NMR ($CDCl_3$): $\delta_{ppm} = 2.42$ (s, 3H, CH_3), 3.31 (s, 3H, NCH_3), 6.88–7.65 (m, 10H, ArH), 8.39 (s, 1H, NH), 14.22 (s, H, NH). MS *m/z* (%): 415 ($M^+ + 1$, 2.2), 414 (M^+, 6.7), 399 (0.75), 386 (9.47), 321 (0.75), 229 (100), 187 (5.13). Anal. for $C_{21}H_{18}N_8O_2$ (414.42): calcd.: C 60.86, H 4.38, N 27.04%; found: C 60.76, H 4.26, N 27.21%.

4.10. General procedure for the reaction of 5-aminopyrazole 9 with 1,3-dicarbonyl compounds

A mixture of compound **9** (0.81 g, 2 mmol) and an equimolar amount of the appropriate 1,3-dicarbonyl compound (acetylacetone or acetoacetanilide) in glacial acetic acid (20 mL) was refluxed for 3 h, then the reaction mixture was poured into crushed ice, and the separated solid was filtered off, dried well and recrystallized from a suitable solvent to give compounds **11** and **12**.

4.10.1. 2-Anilino-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-5,7-dimethylpyrazolo[1,5-a]pyrimidine-3-carboxamide (11)

White crystals (EtOH:DMF); yield 85%; mp 255–257 °C; IR (KBr): $\nu/cm^{-1} = 3432$, 3280 (2NH), 1670, 1650 (2C=O). ^1H NMR ($CDCl_3$): $\delta_{ppm} = 1.93$ (s, 3H, CH_3-pyrazole), 2.62 (s, 3H, CH_3), 2.83 (s, 3H, CH_3), 3.37 (s, 3H, NMe), 6.93 (s, 1H, pyrimidine H-6), 7.26–8.19 (m, 10H, ArH), 9.46 (s, 1H, NH), 9. 86 (s, 1H, NH). MS *m/z* (%): 467 (M^+, 17), 413 (6.0), 265 (100), 238 (23.3), 202 (11.2), 119 (5.4), 56 (42.0). Anal. for $C_{26}H_{25}N_7O_2$ (467.52): calcd.: C 66.79, H 5.39, N 20.97%; found: C 66.81, H 5.46, N 20.89%.

4.10.2. 2-Anilino-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-7-hydroxy-5-methylpyrazolo[1,5-a]pyrimidine-3-carboxamide (12)

White crystals (EtOH:$CHCl_3$); yield 82%; mp 285–287 °C; IR (KBr): $\nu/cm^{-1} = 3455$ (OH), 3430, 3304 (2NH), 1695, 1629 (2CO). ^1H NMR (DMSO-d_6): $\delta_{ppm} = 2.23$ (s, 3H, CH_3-pyrazole), 2.39 (s, 3H, CH_3), 3.14 (s, 3H, NMe), 5.84 (s, 1H, pyrimidine H-6), 6.91–7.72 (m, 10H, ArH), 8.87 (s, 1H, NH), 9.34 (s, 1H, NH), 11.98 (br s, 1H, OH). MS *m/z* (%): 469 (M^+, 10.6), 267 (25.8), 240 (100), 203 (40.6), 119 (36.5), 56 (73.6). Anal. for $C_{25}H_{23}N_7O_3$ (469.50): calcd.: C 63.96, H 4.94, N 20.88%; found: C 63.81, H 4.86, N 20.71%.

*4.11. Synthesis of 3-anilino-5-{[1-(dimethylamino)methylene]amino}-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-1H-pyrazole-4-carboxamide (**13**)*

A mixture of compound **9** (0.81 g, 2 mmol) and dimethylformamide–dimethylacetal (DMF–DMA) (0.24 g, 2 mmol) in dry dioxane (30 mL) was refluxed for 4 h, then allowed to cool. The precipitated product that formed was filtered off, washed with petroleum ether (60–80 °C), dried, and recrystallized from a mixture of ethanol and DMF (2:1) to give compound **13**.

White crystals; yield 85%; mp 238–240 °C; IR (KBr): $\nu/cm^{-1} = 3405$, 3325, 3233 (3NH), 1697, 1645 (2C=O), 1630 (C=N). ^{1}H NMR (DMSO-d_6): $\delta_{ppm} = 2.29$ (s, 3H, CH_3), 3.02 (s, 3H, NCH_3), 3.04 (s, 3H, NCH_3), 3.11 (s, 3H, NCH_3), 8.21 (s, 1H, CH=N), 8.86 (s, 1H, NH), 9.41 (s, H, NH), 11.83 (s, 1H, NH). MS *m/z* (%): 458 (M^+, 13.7), 414 (25.4), 413 (100), 254 (6.9), 211 (29.0), 156 (18.9), 109 (80.4), 56 (90.4). Anal. for $C_{24}H_{26}N_8O_2$ (458.52): calcd.: C 62.87, H 5.72, N 24.44%; found: C 62.81, H 5.66, N 24.31%.

*4.12. Synthesis of 3-anilino-5-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (**14**)*

A solution of compound **13** (0.92 g, 2 mmol) in glacial acetic acid (30 mL) was refluxed for 3 h, and then allowed to cool. The precipitate that formed was filtered off, washed with ethanol, dried and recrystallized from a mixture of ethanol and DMF (2:1) to give compound **14**.

White crystals; yield 78%; mp 260–262 °C; IR (KBr): $\nu/cm^{-1} = 1678$, 1645 (2C=O), 1629 (CH=N). ^{1}H NMR ($CDCl_3$): $\delta_{ppm} = 2.02$ (s, 3H, CH_3), 3.01 (s, 3H, NCH_3), 6.63–7.32 (m, 10H, ArH), 7.37 (s, 1H, NH), 7.68 (s, 1H, CH=N). Anal. for $C_{22}H_{19}N_7O_2$ (413.43): calcd.: C 63.91, H 4.63, N 23.72%; found: C 63.81, H 4.76, N 23.61%.

*4.13. Synthesis of 2-anilino-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-7-phenylpyrazolo[1,5-a]pyrimidine-3-carboxamide (**15**)*

A solution of compound **9** (0.4 g, 1 mmol) in glacial acetic acid (15 mL) which was treated with 3-(dimethylamino)-1-prop-2-en-1-one (0.175 g, 1 mmol) was heated under reflux for 2 h. The solvent was then evaporated under *vacuo* and triturated with ethanol. The solid deposited was collected by filtration and recrystallized from ethanol to give compound **15**.

Yellow crystals, yield 89%; mp 246–248 °C; IR (KBr): $\nu/cm^{-1} = 3278$ (NH), 1665, 1645 (2CO), 1615 (C=N). ^{1}H NMR ($CDCl_3$): $\delta_{ppm} = 2.43$ (s, 3H, CH_3), 3.14 (s, 3H, NCH_3), 6.97–7.70 (m, 15H, ArH), 8.18 (d, $J = 4.5$ Hz, 1H, pyrimidine H-5), 8.51 (d, $J = 4.5$ Hz, 1H, pyrimidine H-4), 9.32 (s, 1H, NH), 9.49 (s, 1H, NH). MS *m/z* (%): 517 ($M^+ + 2$, 5.3), 516 ($M^+ + 1$, 8.6), 515 (M^+, 12.5), 314 (14.3), 313 (100), 312 (11.9), 285 (37.6), 103 (5.8), 77 (8.5), 56 (65.2). Anal. for $C_{30}H_{25}N_7O_2$ (515.21): calcd.: C 69.89, H 4.89, N 19.02%; found: C 69.86, H 4.79, N 19.11%.

*4.14. Synthesis of 3-anilino-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-5-{[2-oxo-1,2-dihydro-3H-indol-3-ylidene]amino}-1H-pyrazole-4-carboxamide (**16**)*

A mixture of compound **9** (0.8 g, 2 mmol) and isatin (0.147 g, 1 mmol) in ethanol (30 mL) containing piperidine (3 drops) was refluxed for 2 h. The precipitate obtained was filtered off, dried well and recrystallized from a mixture of ethanol and DMF (1:2) to give compound **16**.

Reddish brown powder; yield 85%; mp 261–263 °C; IR (KBr): $\nu/cm^{-1} = 3418$, 3336, 3275 (3NH), 1744, 1703, 1640 (3C=O). ^{1}H NMR (DMSO-d_6): $\delta_{ppm} = 2.29$ (s, 3H, CH_3), 3.06 (s, 3H, NMe), 6.82–7.83 (m, 14H, ArH), 9.00 (s, 1H, NH), 9.13 (s, 1H, NH), 10.99 (s, 1H, NH), 13.19 (s, 1H, NH). Anal. for $C_{29}H_{24}N_8O_3$ (532.55): calcd.: C 65.40, H 4.54, N 21.04%; found: C 65.51, H 4.46, N 21.11%.

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