Full Paper

Synthesis of Novel 1-Pyrazolylpyridin-2-ones as Potential Anti-Inflammatory and Analgesic Agents

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A new series of 4-alkyl/aryl-2-oxo-1-pyrazolyl-1,2-dihydropyridine-3-carbonitriles, pyrazolo[3,4-*b*]pyridine-5-carbonitriles and pyrido[2,3-*d*]pyrimidine-6-carbonitriles have been synthesized and tested for their anti-inflammatory and analgesic activities. Among the tested compounds, **3e** and **8b** exhibited comparable anti-inflammatory activity to the standard (indomethacin). Compounds **5**, **7a**, and **8b** displayed potent analgesic activity. Detailed syntheses, spectroscopic and biological data are reported.

Keywords: Analgesic activities / Anti-inflammatory / Condensed pyridone / 1-Pyrazolylpyridin-2-one

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Introduction

Antipyrine and its 4-amino derivative (amidopyrine) were shown to exert antinociceptive and anti-inflammatory activities in various test models. The 4-substitution of antipyrine and derivatives of 4-aminoantipyrine proved to be more active than the parent compounds [1]. On the other hand, 3-cyano-4,6-diarylpyridin-2-ones exhibited marked anti-inflammatory and/or analgesic activities [2]. Furthermore, many fused pyridine derivatives such as pyrazolo[3,4-*b*]pyridines induced reduction of pro-inflammatory cytokines and were found to possess anti-inflammatory and analgesic activities [3, 4]. Several reports revealed that pyrido[2,3-*d*]pyrimidine exerted promising anti-inflammatory and analgesic activities [5, 6]. Herein, we report the preparation and evaluation of anti-inflammatory and analgesic activities of a new series of 1-pyrazolyl-2-pyridone-3-carbonitriles and fused analogs, with the objective of determining the influence of different 4-, 5-, and 6-substituents of the pyridine-2-one nucleus on the biological activity.

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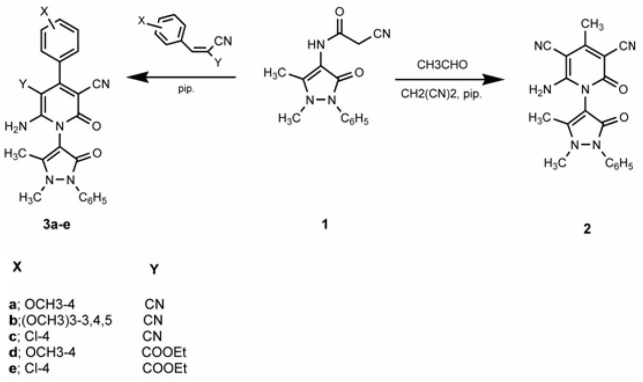
Results and discussion

Chemistry

The designed target compounds are depicted in Schemes 1–3. The 4-alkyl-2-oxo-1,2-dihydropyridine-3-carbonitrile **2** was obtained through a one-pot reaction of cyanoacetanilide **1** with acetaldehyde and malononitrile in ethanol / piperidine [7–9]. On the other hand, reaction of **1** with different arylidenes in presence of piperidine as catalyst [10] afforded substituted 4-aryl-2-oxo-1,2-dihydropyridine-3-carbonitriles **3a–e**. Cyclocondensation of **1** with acetyl acetone or benzoyl acetone in ethanol using catalytic amount of piperidine [8, 9] furnished 1-pyrazolyl-4-methyl-2-oxo-6-substituted-1,2-dihydropyridine-3-carbonitriles **4a, b**. It is important to emphasize that the presence of the nitrile group at the 3-position of the pyridone ring activates the methyl group at position 4. Condensation of the 4-methyl group of compound **4a** with dimethylformamide-dimethylacetal (DMF-DMA) in xylene [8, 9, 11] produced the 4-enamine derivative **5**. Treatment of **1** with 4-anisaldehyde afforded the aryli-



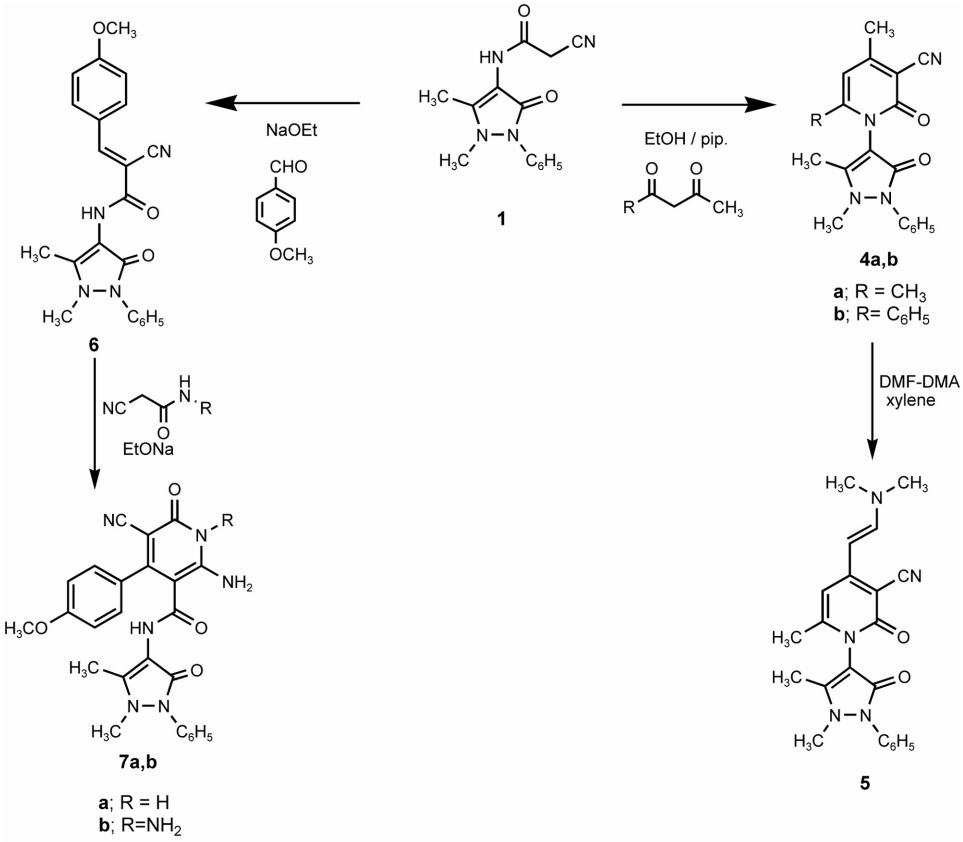
Scheme 1. Synthesis of compounds **1 – 3**.

dine **6** which was allowed to react in a Michael – addition fashion with cyanoacetamide or cyano-acetic acid hydrazide [12, 13] to afford the desired compounds **7a, b**. Cyclocondensation of **3a, b** with $N_2H_4 \times H_2O$ [9, 13] under reflux condition led to the additional fused pyridones, pyrazolo[3,4-*b*]pyridines **8a, b**. Acetylation of **3a, b** using acetic anhydride under stirring, furnished the 6-acetamido derivatives **9a, b**. However, reflux of **3a, b** with Ac_2O for 6 h gave the intermediate 4-imino-pyrido[2,3-*d*]oxazinone which rearranged directly to the pyrido[2,3-*d*]pyrimidine derivatives **10a, b** [9]. Pharmacological evaluation: The new pyridone derivatives **2**, **3b**,**c**,**e**, **4a**,**b**, **5**, **7a**, **8b**, **9b**, **10b** were screened for anti-inflammatory and analgesic activities.

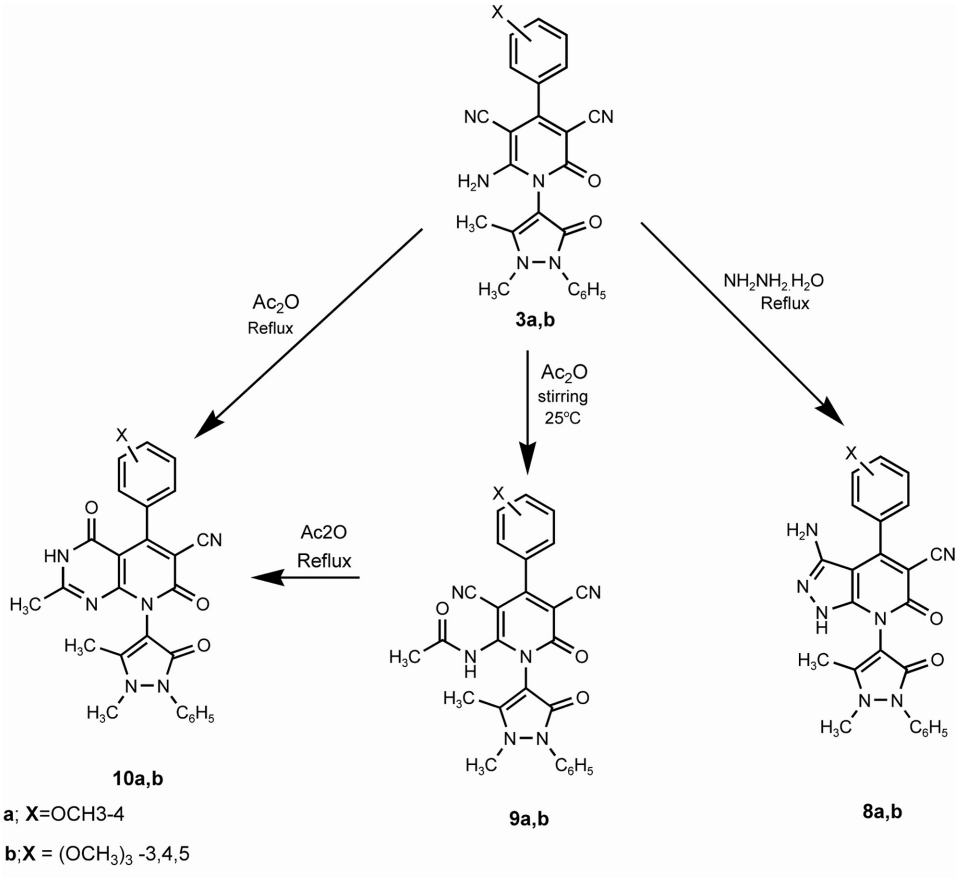
Pharmacology

Anti-inflammatory activity

The anti-inflammatory activity of the tested compounds was evaluated against the reference drug (indomethacin) at doses of 50 mg and 5 mg, respectively. Then, the efficacy of the tested compounds was determined after 6 h of administration, when the percentage of inhibition reaches the maximum value (Table 1). Compounds **3e** and **8b** exhibited strong anti-inflammatory activity comparable to the reference drug. Compounds **2**, **3b**,**c**, and **5** showed moderate activities. Mild to weak effects were exerted by the other compounds. Compounds **2**, **3b**, and **3c** showed a relative efficacy of RE = 0.7 compared to indomethacin. The electronic effect of the substituents on the 4-phenyl group seems not to influence the activity; compound **3b** bearing an electron-releasing group (3,4,5-trimethoxyphenyl) has the same RE as **3c** having an electron-withdrawing group (4-chlorophenyl). Acetylation of



Scheme 2. Synthesis route of compounds **1 – 7**.



Scheme 3. Synthesis route of compounds **3**–**10.**

the 6-amino group of **3b** abruptly reduced the anti-inflammatory activity (**9b**, RE = 0.1), while its cyclocondensation with Ac_2O yielded **10b** with slightly enhanced activity. It is worth mentioning that conversion of **4a** to the 4-enamine derivative **5** enhanced the anti-inflammatory activity (RE = 0.6).

On the other hand, incorporation of the antipyrine nucleus to pyridine at position 5 via a carboxamide linker **7a** unfortunately produced weak anti-inflammatory activity, while cyclocondensation of **3b** with hydrazine hydrate afforded **8b** which possesses a potent anti-inflammatory activity (RE = 0.9).

Analgesic activity

The recorded results in Table 2 show equipotent analgesic effects (RE = 0.97) as compared with the reference drug, displayed by 4-enamine derivative **5**. In addition, 5-pyrazolylcarbamoylpyridine **7a** displayed a strong analgesic activity (RE = 0.84). Pyrazolo[3,4-*b*]pyridine **8b** as well elicited potent analgesia (RE = 0.87), while moderate to mild activities were exhibited by the other compounds.

Conclusion

The ester function at position 5 of pyridine enhanced the anti-inflammatory activity and acetylation of 6-amino-pyridine reduced the anti-inflammatory activity. The 4-enamine group imparted high analgesic activity approximately equal to that of indomethacin. Incorporation of antipyrine to the 2-pyridone nucleus at position 5 via a carboxamide linker produced a highly analgesic agent. Pyrazolo[3,4-*b*]pyridine exerted both potent analgesic and anti-inflammatory activities.

Experimental

Elemental analyses (C, H, N) were performed on an Perkin-Elmer 2400 analyzer (Perkin-Elmer, Norwalk, CT, USA) at the Microanalytical Unit of Cairo University. All compounds were within ± 0.4% of the theoretical values. Melting points were determined in open capillaries on an Electrothermal LA 9000 Series (Electrothermal Engineering Ltd., Essex, UK) and are uncorrected. TLC chromatography was performed on precoated silica gel ^{60}F 254 plates (Merck Co., Sofia, Bulgaria). Infrared spectra were

Table 1. The anti-inflammatory activities of test compounds (50 mg/kg) and indomethacin (5 mg/kg).

Compound	Paw oedema thickness (mm)					
	1 h X ± S.E.	% Oedema inhibition	2 h X ± S.E.	% Oedema inhibition	3 h X ± S.E.	% Oedema inhibition
Control	0.174 ± 0.0044	–	0.190 ± 0.0040	–	0.250 ± 0.0052	–
2	0.078 ± 0.0028*	55.2	0.131 ± 0.0035*	34.2	0.192 ± 0.0048*	23.2
3b	0.077 ± 0.0026*	55.7	0.125 ± 0.0033*	34.2	0.191 ± 0.0033*	23.6
3c	0.09 ± 0.0025*	54.59	0.109 ± 0.0034*	4.6	0.192 ± 0.0035*	23.2
3e	0.042 ± 0.0019*	75.8	0.105 ± 0.0019*	44.7	0.156 ± 0.0120*	37.6
4a	0.145 ± 0.0039*	16.6	0.174 ± 0.0040*	8.4	0.233 ± 0.0050*	6.8
4b	0.13 ± 0.0034*	24.2	0.161 ± 0.0041*	15.2	0.240 ± 0.0056*	4
5	0.087 ± 0.0028*	50	0.138 ± 0.0030*	27.3	0.205 ± 0.0049*	18
Indometh.	0.040 ± 0.0018*	77	0.090 ± 0.0026*	5.6	0.140 ± 0.0048*	44

Compound	Paw oedema thickness (mm)						
	4 h X ± S.E.	% Oedema inhibition	5 h X ± S.E.	% Oedema inhibition	6 h X ± S.E.	% Oedema inhibition	Efficacy
Control	0.160 ± 0.0015	-	0.125 ± 0.0013	-	0.105 ± 0.0010	-	-
2	0.150 ± 0.0026*	6.3	0.100 ± 0.0015*	26.4	0.089 ± 0.0011*	17.2	0.7
3b	0.150 ± 0.0100*	6.3	0.092 ± 0.0028*	26.4	0.087 ± 0.0026*	17.2	0.7
3c	0.149 ± 0.0020*	6.8	0.100 ± 0.0018*	0	0.089 ± 0.0016*	15.3	0.7
3e	0.132 ± 0.0029*	17.5	0.089 ± 0.0019*	28.8	0.059 ± 0.0018*	43.8	0.9
4a	0.140 ± 0.0033*	12.5	0.125 ± 0.0029*	-	0.098 ± 0.0022*	6.6	0.2
4b	0.152 ± 0.0053*	5	0.121 ± 0.0032*	3.2	0.091 ± 0.0031*	13.3	0.3
5	0.151 ± 0.0029*	5.6	0.121 ± 0.0100*	3.2	0.090 ± 0.0028*	14.2	0.6
Indomethacin	0.114 ± 0.0035*	28.7	0.088 ± 0.0015*	29.6	0.052 ± 0.0013*	50	1

Compound	Paw oedema thickness (mm)					
	1 h X ± S.E.	% Oedema inhibition	2 h X ± S.E.	% Oedema inhibition	3 h X ± S.E.	% Oedema inhibition
Control	0.174 ± 0.0044	–	0.190 ± 0.0040	–	0.250 ± 0.0052	–
7a	0.137 ± 0.0035*	21.3	0.149 ± 0.0032*	21.5	0.9 ± 0.0048*	8.4
8b	0.051 ± 0.0020*	70.6	0.105 ± 0.0024*	44.7	0.183 ± 0.0050*	26.8
9b	0.160 ± 0.0043*	8.1	0.178 ± 0.0046*	6.3	0.249 ± 0.0053*	0.4
10b	0.138 ± 0.0035*	44.3	0.159 ± 0.0041*	16.3	0.239 ± 0.0050*	4.4
Indomethacin	0.040 ± 0.0018*	77	0.090 ± 0.0026*	52.6	0.140 ± 0.0048*	44

Compound	Paw oedema thickness (mm)						
	4 h X ± S.E.	% Oedema inhibition	5 h X ± S.E.	% Oedema inhibition	6 h X ± S.E.	% Oedema inhibition	Efficacy
Control	0.160 ± 0.0015	–	0.15 ± 0.0013	–	0.105 ± 0.0010	–	–
7a	0.160 ± 0.0020*	–	0.122 ± 0.0017*	2.4	0.096 ± 0.0011*	8.5	0.3
8b	0.140 ± 0.0034*	20.7	0.091 ± 0.0019*	27.2	0.068 ± 0.017*	35.3	0.9
9b	0.159 ± 0.0022*	0.6	0.125 ± 0.0019*	–	0.100 ± 0.0015*	4.7	0.1
10b	0.157 ± 0.0023*	1.8	0.123 ± 0.0018*	1.6	0.097 ± 0.0014*	7.6	0.5
Indometh.	0.130 ± 0.0035*	28.7	0.088 ± 0.0015*	29.6	0.052 ± 0.0013*	50	1

Significant difference from the control value at $P < 0.05$; S.E. Standard error; Compounds **3a**, **3d**, **7b**, **8a**, **9a**, and **10a** were not evaluated due to low yields on preparation.

Table 2. The analgesic activity of the tested compounds (50 mg/kg) and indomethacin (5 mg/kg).

Compound	Reaction time (s)						
	0 X ± S.E.	30 min. X ± S.E.	Efficacy	1 h X ± S.E.	Efficacy	2 h X ± S.E.	Efficacy
2	5.6 ± 0.2*	6.5 ± 0.4* (15.2)	0.37	6.5 ± 0.4* (16)	0.37	6.5 ± 0.4* (25.4)	0.20
3b	5.9 ± 0.4*	6.8 ± 0.4* (15.2)	0.35	6.8 ± 0.4* (15.2)	0.19	7.4 ± 0.1* (25.4)	0.31
3c	6.5 ± 0.3*	7.1 ± 0.2* (9.2)	0.21	8.2 ± 0.2* (26)	0.33	8.2 ± 0.3* (26)	0.32
3e	6.4 ± 0.4*	8.4 ± 0.5* (31.2)	0.72	10.4 ± 0.5* (62.5)	0.80	8.6 ± 0.5* (34.3)	0.43
7a	7.3 ± 0.4*	9.3 ± 0.7* (27.3)	0.63	11.1 ± 0.3* (52)	0.67	12.2 ± 0.4* (67.1)	0.84
4a	5.4 ± 0.3*	7.2 ± 0.5* (33)	0.76	7.4 ± 0.4* (37)	0.47	6.5 ± 0.8* (20.3)	0.25
4b	6.8 ± 0.4	8.7 ± .4 (27.9)	0.64	9.4 ± 0.5 (38.2)	0.49	8.9 ± 0.4 (30.8)	0.38
5	7.2 ± 1.0*	9.0 ± 0.5* (25)	0.58	11.9 ± 1.0* (65.2)	0.84	12.8 ± 0.5* (77.7)	0.97
9b	5.6 ± 0.2*	6.6 ± 0.9* (17.8)	0.41	7.8 ± 0.8*- (39.2)	0.50	7.4 ± 0.5* (32.1)	0.40
10b	7.3 ± 0.3	7.7 ± 0.6* (5.4)	0.12	8.0 ± 0.7 (9.5)	0.12	8.2 ± 0.1* (12.3)	0.15
8b	6.2 ± 0.6*	8.0 ± 0.4* (29)	0.67	9.6 ± 0.5* (54)	0.69	10.6 ± 0.5* (70)	0.87
Indomethacin	7.9 ± 0.4*	11.3 ± 0.4* (43)	1.0	14 ± 0.6 (77.2)	1.0	14.2 ± 0.4 (79.7)	1.0

Values between parentheses represent the increase of reaction time compared to zero time. Compounds **3a**, **3d**, **7b**, **8a**, **9a**, and **10a** are not evaluated due to low yields on preparation. * Significant difference from the control value at $P < 0.05$; S.E. Standard error.

Table 3. Physicochemical data of synthesized compounds.

Formulae	Mp (°C)	Yield (%)	Solvent	Compound
$C_{19}H_{16}N_6O_2$	178–180	65	EtOH	**2**
$C_{27}H_{24}N_6O_5$	177–180	82	EtOH	**3b**
$C_{24}H_{17}ClN_6O_2$	317–320	47	Dioxan	**3c**
$C_{27}H_{25}N_5O_5$	159–161	40	MeOH	**3d**
$C_{26}H_{22}ClN_5O_4$	232–234	39	EtOH	**3e**
$C_{19}H_{18}N_4O_2$	217–219	63	EtOH	**4a**
$C_{24}H_{20}N_4O_2$	237–240	32	EtOH	**4b**
$C_{22}H_{23}N_5O_2$	143–145	43	MeOH	**5**
$C_{22}H_{20}N_4O_2$	137–139	59	MeOH	**6**
$C_{25}H_{22}N_6O_4$	157–160	32	EtOH	**7a**
$C_{25}H_{23}N_7O_4$	159–162	33	EtOH	**7b**
$C_{25}H_{21}N_7O_3$	223–225	29	Dioxan	**8a**
$C_{27}H_{25}N_7O_5$	308–310	30	Dioxan	**8b**
$C_{27}H_{22}N_6O_4$	258–260	24	Dioxan	**9a**
$C_{29}H_{26}N_6O_6$	192–195	63	EtOH	**9b**
$C_{27}H_{22}N_6O_4$	237–240	33	Dioxan	**10a**
$C_{29}H_{26}N_6O_6$	205–207	31	EtOH	**10b**

recorded on Pye Unicam SP 1000 IR spectrophotometer (Thermoelectron, Egelsbach, Germany). ^{1}H-NMR spectra were recorded on Varian Gemini EM-300 MHz NMR spectrophotometer (Varian, Fort Collins, CO, USA). DMSO-d_6 was used as solvent, TMS was used as internal standard, and chemical shifts were measured in d ppm. Mass spectra were recorded on Varian MAT 311-A 70 eV (Varian). 2-(Arylidene) malononitrile [14] **3a** was prepared according to the reported method [10]. MF: CHNO; mp. 307–310°C; yield: 55.5%.

Synthesis

6-Amino-3,5-dicyano-1-(1,5-dimethyl-2-phenyl-3(1H)-pyrazolon-4-yl)-4-methyl-2(1H)-pyridone ***2***

A mixture of equimolar amounts (0.01 mol) of **1** (2.7 g), acetaldehyde (0.4 g) and malononitrile (0.6 g) in ethanol (20 mL) containing few drops of piperidine was refluxed for 3 h. The reaction mixture was allowed to cool and the separated solid was filtered and crystallized (Table 3).

2: IR (cm^{-1}): 3336 (broad, NH_2), 2210 (CN), 1688, 1660 (C=O). ^{1}H-NMR (DMSO-d_6): δ 2.09 (s, 3H, CH_3-pyrazole), 2.41 (s, 3H, CH_3-pyridine), 3.20 (s, 3H, NCH_3), 7.37–7.58 (m, 5H, PhH), 8.34 (bs, 2H, NH_2, D_2O exchangeable).

6-Amino-3,5-dicyano-1-(1,5-dimethyl-2-phenyl-3(1H)-pyrazolon-4-yl)-4-substitutedphenyl-2(1H)-pyridone 3b,c; and 6-amino-3-cyano-1-(1,5-dimethyl-2-phenyl-3(1H)-pyrazolon-4-yl)-5-ethoxycarbonyl-4-substitutedphenyl-2(1H)-pyridone ***3d, e***

A mixture of equimolar amounts (0.01 mol) of compound **1** (2.7 g) and the appropriate arylidene derivatives in ethanol (20 mL) containing few drops of piperidine was refluxed for 3 h. The reaction mixture was allowed to cool, and the solid so obtained was filtered and crystallized from the appropriate solvent (Table 3).

3b: IR (cm^{-1}): 3496 broad (NH_2), 2216 (CN), 1664, 1642 (C=O), ^{1}H-NMR (DMSO-d_6): δ 2.14 (s, 3H, CH3), 3.24 (s, 3H, NCH_3) 3.75 (s, 3H, OCH_3), 3.82 (s, 6H, 2 × OCH_3), 6.93 (s, 2H, ArH), 7.39–7.58 (m, 5H, Ph-H), 8.49 (s, 2H, NH_2, D_2O exchangeable), MS (m/z,%): 512 [M$^+$] (21.0), 122 (100) 405 (10.2), 364 (9.07), 107 (5.52).

3c: IR (cm^{-1}): 3445, 3277 (NH_2), 2213 (CN), 1680, 1664 (C=O), MS (m/z,%): 456 [M$^+$] (26.08), 458 [M+2] (68.75), 56 (100), 336 (30.70), 302 (27.51), 199 (11.70).

3d: IR (cm^{-1}): 3333, 3269 (NH_2), 2254 (CN), 1636 (CO-N), 1719 (COO), ^{1}H-NMR (DMSO-d_6): δ 1.05 (t, 3H, $COOCH_2CH_3$, J = 6.9 Hz), 2.15 (s, 3H, CH_3), 3.28 (s, 3H, NCH_3), 3.78 (q, 2H, $COOCH_2CH_3$, J = 6.9 Hz), 3.81 (s, 3H, OCH_3), 7.00–7.25 (2d, 4H, ArH, AB system J = 9 Hz), 7.37–7.58 (m, 5H, Ph-H), 8.48 (s, 2H, NH_2, D_2O exchangeable).

3e: IR (cm^{-1}): 3448, 3261 (NH_2), 2210 (CN), 1670 (CO-N), 1700 (COO), ^{1}H-NMR (DMSO-d_6): δ 1.51 (t, 3H, $COOCH_2CH_3$, J = 7.0 Hz), 2.31 (s, 3H, CH_3), 3.19 (s, 3H, NCH_3), 4.11 (q, 2H, $COOCH_2CH_3$, J = 7.0 Hz), 7.29 (d, 2H, AB system J = 9 Hz), 7.36 (d, 2H, AB system J = 9 Hz), 7.39–7.56 (m, 5H, Ph-H), 9.36 (s, 2H, NH_2, D_2O exchangeable).

1-(1,5-Dimethyl-2-phenyl-3-(1H)-pyrazolon-4-yl)-3-cyano-4-methyl-6-substituted-2(1H)-pyridones ***4a, b***

A mixture of equimolar amounts (0.01 mol) of compound **2** (2.7 g) and acetyl acetone (1 mL) or benzoyl acetone (1.6 g) in ethanol (20 mL) containing a few drops of piperidine was refluxed for 3 h. The reaction mixture was cooled and the solid so obtained was filtered and crystallized (Table 3).

4a: IR (cm^{-1}): 2218 (CN), 1660 (C=O), ^{1}H-NMR (DMSO-d_6): δ 2.10 (s, 3H, CH_3-pyrazole), 2.30 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 3.10 (s, 3H, NCH_3), 6.40 (s, 1H, H-5), 7.30 – 7.60 (m, 5H, Ph-H). **4b**: IR (cm^{-1}): 2211 (CN), 1665 (C=O), MS (m/z,%): 396 [M^+] (15), 56 (100), 275 (9.30), 140 (16.34), 77 (37.33).

3-Cyano-1-(1,5-dimethyl-2-phenyl-3(1H)-pyrazolon-4yl)-4-[(2-di-methylamino)-vinyl]-6-methyl-2(1H)-pyridone ***5***

A mixture of equimolar amounts (0.01 mol) of **4a** (3.3 g) and DMF-DMA (1.2 g) in xylene (20 mL) was refluxed for 4 h. The reaction mixture was cooled, washed with diethylether, and the separated solid was crystallized (Table 3).

5: IR (cm^{-1}): 1661 (C=O), 2200 (CN), ^{1}H-NMR (DMSO-d_6): δ 2.10 (s, 3H, CH_3-pyrazole), 2.25 (s, 3H, CH_3), 3.20 (s, 3H, NCH_3-pyrazole), 3.24, 3.39 (2s, 6H, 2 × NCH_3), 5.10 (d, 1H, N-CH=), 6.44 (s, 1H, H-5), 7.35 – 7.90 (m, 6H, 5 Ph-H & 1H CH=C), MS (m/z,%): 389 [M^+] (5.17), 56 (100), 334 (16.20), 93 (13.15), 77 (33.51).

1,5-Dimethyl-4-[2-cyano-3-(4-methoxyphenyl)-acrylamido]-2-phenyl-3(1H)-**pyrazolone** ***6***

A mixture of equimolar amounts (0.01 mol) of compound **1** (2.7 g), *p*-anisaldehyde (1.3 g) and sodium ethoxide (0.7 g) in ethanol (10 mL) was refluxed for 1 h. The reaction mixture was cooled and the solid so obtained was filtered and crystallized (Table 3).

6: IR (cm^{-1}): 3195 (NH), 2213 (CN), 1657 (C=O), ^{1}H-NMR (DMSO-d_6): δ 2.17 (s, 3H, CH_3), 3.14 (s, 3H, NCH_3) 3.86 (s, 3H, OCH_3), 7.14 – 8.00 (m, 9H, ArH), 8.22 (s, 1H, CH=), 9.51 (s, 1H, NH, D_2O exchangeable).

6-Amino-5-[(1,5-dimethyl-2-phenyl-3(1H)-pyrazolon-4-yl)-aminocarbonyl]-3-cyano-4-(4-methoxyphenyl)-2(1H)-pyridone ***7a****; and 1,6-diamino-5-[(1,5-dimethyl-2-phenyl-3(1H)-pyrazolon-4-yl)-aminocarbonyl]-3-cyano-4-(4-methoxyphenyl)-2(1H)-pyridone* ***7b***

A mixture of equimolar amounts (0.01 mol) of compound **6** (3.8 g) and cyanoacetamide (0.86 g) or cyanoacetic acid hydrazide (0.9 g) and sodium ethoxide (0.7 g) in ethanol (20 mL) was refluxed for 3 h, then cooled and poured into cold water. A few drops of HCl were added and the separated solid was filtered and crystallized (Table 3).

7a: IR (cm^{-1}): 3448, 3306, 3171, (NH_2, NH), 2208 (CN), 1698 (C=O).

7b: IR (cm^{-1}): 3332, 3203 (NH_2, NH), 2208 (CN), 1690 (C=O), ^{1}H-NMR (DMSO-d_6): δ 2.36 (s, 3H, CH_3), 3.10 (s, 3H, NCH_3), 3.85 (s, 3H, OCH_3), 6.92 – 7.53 (m, 11H, 9ArH & NH_2), 11.48 (s, 1H, NHCO), 11.81 (s, 2H, NH_2, D_2O exchangeable).

3-Amino-5-cyano-7-(1,5-dimethyl-2-phenyl-3(1H)-pyrazolon-4-yl)-4-substituted-phenyl-1H,7H-pyrazolo[3,4-b]pyridin-6-ones ***8a, b***

A mixture of the appropriate **3a**, **b** (0.01 mol) and hydrazine hydrate (0.5 mL, 90%) in ethanol (20 mL) was refluxed for 3 h. The reaction mixture was cooled and the solid so obtained was filtered and crystallized (Table 3).

8a: ^{1}H-NMR (DMSO-d_6): δ 2.15 (s, 3H, CH_3), 3.22 (s, 3H, NCH_3) 3.86 (s, 3H, OCH_3), 5.32 (s, 2H, NH_2, D_2O exchangeable), 7.15 – 7.52 (m, 10H, 9ArH & NH).

8b: IR (cm^{-1}): 3378, 3211, 3138 (NH, NH_2), 2212 (CN), 1660, 1623 (C=O), ^{1}H-NMR (DMSO-d_6): δ 2.15 (s, 3H, CH_3), 3.22 (s, 3H, NCH_3) 3.76, 3.83 (2s, 9H, 3 × OCH_3) 5.55 (bs, 2H, NH_2, D_2O exchangeable), 6.92 (s, 2H, ArH), 7.37–7.51 (m, 6H, 5 Ph-H & NH), MS (m/z,%): 527 [M^+] (100), 528 [M+1] (38.2), 526 [M-1] (78.3), 392 (43.10), 356 (63.70).

6-Acetamido-[3,5-dicyano-1-(1,5-dimethyl-2-phenyl-3(1H)pyrazolon-4-yl)-4-substitutedphenyl-2(1H)-pyridones ***9a, b***

A mixture of **3a** or **3b** (0.01 mol) and acetic anhydride (15 mL) was stirred for 2 h at room temperature. The solid so obtained was filtered and crystallized (Table 3).

9a: IR (cm^{-1}): 3456 (NH), 2222 (CN), 1690, 1670 (C=O).

9b: IR (cm^{-1}): 3436 (NH), 2215 (CN), 1679, 1623 (C=O). ^{1}H-NMR (DMSO-d_6): δ 1.90 (s, 3H, CH_3), 2.14 (s, 3H, $COCH_3$), 3.26 (s, 3H, NCH_3), 3.75 (s, 3H, OCH_3), 3.83 (s, 6H, 2 × OCH_3), 6.93 (s, 2H, ArH), 7.39 – 7.55 (m, 5H, Ph-H), 8.50 (s, 1H, NH, D_2O-exchangeable).

6-Cyano-8-(1,5-dimethyl-2-phenyl-3(1H)pyrazolon-4-yl)-2-methyl-5-substituted phenyl-3H,8H-pyrido[2,3-d]pyrimidine-4,7diones ***10a, b***

A mixture of the appropriate **3a** or **3b** (0.01 mol) and acetic anhydride (15 mL) was refluxed for 6 h, cooled, and then poured into cold water. Then, a few drops of HCl were added and the solid so obtained was filtered and crystallized (Table 3).

Alternative procedure: A mixture of the appropriate **9a** or **9b** (0.01 mol) and acetic anhydride (15 mL) was refluxed for 5 h and then proceeded as before.

10a: IR (cm^{-1}): 3424 (NH), 2217 (CN), 1660 (C=O), ^{1}H-NMR (DMSO-d_6): δ 1.90, 2.04 (2s, 6H, 2 × CH_3), 3.29 (s, 3H, NCH_3), 3.85 (s, 3H, OCH_3), 7.12 – 7.49 (m, 10H, 9ArH & NH, D_2O exchangeable).

10b: IR (cm^{-1}): 3446 (NH), 2215 (CN), 1677 (C=O), ^{1}H-NMR (DMSO-d_6): δ 1.90, 1.98 (2s, 6H, 2 × CH_3), 3.03 (s, 3H, NCH_3), 3.76 (s, 3H, OCH_3), 3.85 (s, 6H, 2 × OCH_3), 6.93 (s, 2H, ArH), 7.07-7.59 (m, 6H, 5 Ph-H & NH, D_2O exchangeable), MS (m/z,%): 552 [M-2] (7.75), 89 (100), 524 (8.11), 368 (25.70), 236 (23.50), 313 (34.83).

Pharmacological evaluation

Materials: Eighty adult albino rats of both sexes weighing (120 – 150 g) and eighty mice weighing (20 – 25 g) were obtained from animal house lab, Nile company, Cairo, Egypt and acclimatized for one week in the animal facility that has a 12 h light/dark cycles with controlled temperature (21 – 23°C). Normal rat chow and water were made available, carrageenan sodium (1%, Sigma), tween 80 (2%), saline, distilled water, and indomethacin cap (lot No 0.30687, MUB, Egypt). Dial micrometer; Baty and Co. Ltd, Sussex, England.

Preparation of samples

The tested compounds and the reference standard were prepared as suspension in tween 80 (2%). The administered oral dose of the tested compound (50 mg/kg body weight) was calculated according to the literature [2] and the negative control group received 1 mL of water suspended in tween 80.

Anti-inflammatory studies

The anti-inflammatory study was screened according to the method described by Winter *et al*. [15]. Method: Rats were divided into 13 groups, each consisting of six animals. One group is receiving the reference standard, 11 groups the test compounds, and one group as control. The reference drug, indomethacin, and the tested compounds were given by oral route at doses of 5 mg/kg and 50 mg/kg body weight, respectively. One hour later, 0.05 mL of 1% carrageenan sodium was subplantary injected in the right hind paw. The thickness of the paw was measured after administration of the compounds at time intervals 1, 2, 3, 4, 5, and 6 h, by using a dial micrometer. The results were expressed as the percentage inhibition of oedema thickness at each time interval versus that of the standard drug.

Analgesic activity

The analgesic activity was evaluated according to the reported method of Janssen *et al*. [16]. Method: Mice were divided into 13 groups, each consisting of six animals. The reference drug, indomethacin, and the test compounds were given orally at doses of 5 mg/kg and 50 mg/kg body weight, respectively. The comparison parameter is the reaction time from introducing the animal into the hot cylinder till it licked its feet or jumped out of the glass jar. The time taken was recorded after administration of the compounds at intervals 0.5, 1, and 2 h.

Statistical analysis

Student's t-test was used for analysis of the biochemical parameters. The data were expressed as mean ± standard error. Statistical analysis was done according to Snedecor and Cochron [17].

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