

Synthesis and Analgesic Activity of 2-(un)Substituted-3-Amino-5-Aryl-6-Phenyl Pyrazolo [3,4-*d*]pyrimidine-4-(5H)-Ones

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A series of some novel compounds 2-(un)substituted-3-amino-5-aryl-6-phenylpyrazolo[3,4-*d*]pyrimidine-4-(5H)-ones were synthesized by condensation of 2-phenyl-3-aryl-5-cyano-6-thiomethyl pyrimidine-4(3H)-ones independently with hydrazine hydrate/methyl hydrazine/phenyl hydrazine in presence of dimethyl formamide and catalytic amount of anhydrous potassium carbonate. These derivatives were characterized by their spectral data. When evaluated for analgesic activity by hot plate method, compounds 4e, 4i, 4l and 4m exhibited superior activity than Pentozocin hydrochloride at dose of 10 mg/kg. The ED₅₀ values of all thirteen synthesized compounds were determined.

The pyrazole ring system is reported in literature to form a part of potential pharmacophore for analgesic and antiinflammatory activity. Condensed pyrimidines have been reported for wide range of biological activities. The annelation of the pyrazole ring on the pyrimidine nucleus leads to pyrazolo-pyrimidines, which are also known to exhibit analgesic and antiinflammatory activity¹⁻⁶, antileukemic activity⁷, antifungal activity⁸, anticoccidial activity⁹, schistosomocidal activity¹⁰ and activity against HIV reverse transcriptase¹¹. Keeping in view this biological importance of pyrazolopyrimidines and as a part of continuation of our work on pyrazolo[3,4-*d*] pyrimidine¹²⁻¹⁴, some novel derivatives of pyrazolo[3,4-*d*]pyrimidine were envisaged for synthesis and evaluation their for analgesic activity.

MATERIALS AND METHODS

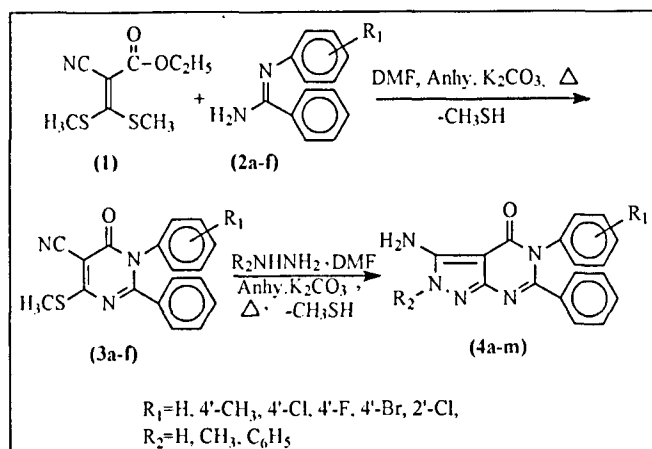
Ethyl-2-cyano-3,3-bismethylthio acrylate¹⁵ (0.005 mol) (1) was refluxed independently with 0.005 mol of N-aryl benzamide (2a-f) in the presence of dimethylformamide (DMF) and catalytic amount of anhydrous potassium carbonate to obtain the product, phenyl-3-aryl-5-cyano-6-

thiomethyl pyrimidine-4(3H)-one (3a-f). The structures of compounds 3a-f were confirmed on the basis of spectral data.

The 2,3-diphenyl-5-cyano-6-thiomethyl pyrimidine-4(3H)-one (3a) on heating with hydrazine hydrate in the presence of DMF and catalytic amount of anhydrous potassium carbonate afforded the product, which was assigned to be 2H-3-amino-5,6-diphenyl pyrazolo[3,4-*d*]pyrimidine-4-(5H)-one (4a) on the basis of elemental and spectral data. The IR spectrum of compound 4a showed the absence of bands in the region 2240-2200 cm⁻¹ due to -CN stretching, indicating the formation of cyclized product. The presence of additional functional groups, NH₂ and C=O in compound 4a was further confirmed by the presence of absorption bands at 3460 cm⁻¹, 3300 cm⁻¹ and 1700 cm⁻¹, which are amino and carbonyl stretching frequencies respectively. The PMR spectrum exhibited singlets at δ 5.3, δ 8.1 and multiplets at δ 7.4 - δ 7.9 due to NH₂, NH and aromatic protons, respectively. The mass spectrum showed the presence of molecular ion peak with m/z 303 that substantiated the molecular weight of 4a. Similarly, the other derivatives 4b-4m were prepared by reacting independently 2-phenyl-3-aryl-5-cyano-6-thiomethyl pyrimidine-4 (3H)-ones (3a-f) with hydrazine hydrate/methyl hydrazine/phenyl hydrazine

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Scheme 1: Synthesis of compound 4a-m

$R_1 = \text{H, 4'-CH}_3, 4'\text{-F, 4'-Br, 2'-Cl, } R_2 = \text{H, CH}_3, \text{C}_6\text{H}_5$

in presence of DMF and catalytic amount of anhydrous potassium carbonate (Scheme 1). The structures of 4b-4m were confirmed on the basis of elemental data, IR, NMR and Mass spectra.

MATERIAL AND METHODS

Melting points were determined in open capillaries and are uncorrected. The UV Spectra were recorded on a Shimadzu UV/Vis 160-A Spectrophotometer. IR spectra were recorded in KBr on Perkin Elmer IR Spectrophotometer 841 model, ¹H NMR spectra were recorded on Varian EM-360 L Spectrophotometer at 60 MHz using tetramethyl silane as internal standard. Mass spectra were obtained on Jeol-300 electron impact mass spectrophotometer at 70 eV ionizing beam. Elemental analysis was performed on Heraeus CHN rapid analyzer. All the

reactions were monitored by thin layer chromatography, carried out on 0.2 mm silica gel-G plate using iodine vapour for detection.

2-Phenyl-3-aryl-5-cyano-6-thiomethyl pyrimidine-4(3H)-one (3a-f):

Ethyl-2-cyano-3,3-bismethylthio acrylate (0.005mol) (1) was refluxed independently with 0.005 mol of N-phenyl benzamidine (2a), N-(4'-methyl phenyl) benzamidine (2b), N-(4'-chloro phenyl) benzamidine (2c), N-(4'-fluoro phenyl) benzamidine (2d), N-(4'-bromo phenyl) benzamidine (2e), N-(2'-chloro phenyl) benzamidine (2f) in the presence of 20-25 ml of DMF and catalytic amount of anhydrous potassium carbonate for 4 to 6 h. The reaction mixture was allowed to cool and poured in ice-cold water. The solid that separated was collected by filtration and recrystallised from chloroform-ethanol mixture to give crystalline solids of 3a-f. Physical and Spectral data of 3a-f are given in Table 1.

2-(Un)substituted-3-amino-5-aryl-6-phenyl pyrazolo[3,4-d]pyrimidine-4-(5H)-ones(4a-m):

The 0.005 mol of 2-Phenyl-3-aryl-5-cyano-6-thiomethyl pyrimidine-4(3H)-one (3a-f) was refluxed independently with hydrazine hydrate/methyl hydrazine/phenyl hydrazine (0.01 mol) in presence of 20-25 ml of DMF and catalytic amount of anhydrous potassium carbonate for 3-4 h. The reaction mixture was allowed to cool and poured on ice-cold water. The solid that precipitated was collected by filtration and recrystallised from chloroform-ethanol mixture to obtain compounds 4a-m as crystalline solids. Physical and Spectral data of compounds 4a-m are given in Table 2 and Table 3.

TABLE 1: PHYSICAL AND SPECTRAL DATA OF 2-PHENYL-3-ARYL-5-CYANO-6-THIOMETHYL PYRIMIDINE-4(3H)-ONES (3a-f)

Comp No.	R ₁	Mol. formula	Mol. wt.	mp °	Yield (%)	IR in KBr (cm ⁻¹)
3a	H	C ₁₈ H ₁₃ N ₃ OS	319	228-230	75	1680(C=O), 2240(CN)
3b	-4'-CH ₃	C ₁₉ H ₁₅ N ₃ OS	333	232-234	76	1700(C=O), 2220(CN)
3c	-4'-Cl	C ₁₈ H ₁₂ N ₃ OSCl	353	220-222	65	1690(C=O), 2230(CN)
3d	-4'-F	C ₁₈ H ₁₂ N ₃ OSF	337	190-192	78	1680(C=O), 2190(CN)
3e	-4'-Br	C ₁₈ H ₁₂ N ₃ OSBr	398	209-210	40	1695(C=O), 2225(CN)
3f	-2'-Cl	C ₁₈ H ₁₂ N ₃ OSCl	353	208-212	77	1680(C=O), 2220(CN)

All the compounds were recrystallised from ethanol-chloroform mixture.

TABLE 2: PHYSICAL DATA FOR 2-(UN) SUBSTITUTED-3-AMINO-5-ARYL-6-PHENYL PYRAZOLO[3, 4-*d*] PYRIMIDIN-4(5H)-ONES (4a-m)

Comp No	R ₁	R ₂	Mol. formula	Mol. wt.	mp °	Yield (%)	Elemental analysis (Calcd.)		
							%C	%H	%N
4a	-H	-H	C ₁₇ H ₁₃ N ₅ O	303	265	72	67.25 (67.32)	4.25 (4.29)	23.00 (23.10)
4b	-H	-CH ₃	C ₁₈ H ₁₅ N ₅ O	317	250	75	68.10 (68.13)	4.70 (4.73)	22.02 (22.08)
4c	-H	C ₆ H ₅	C ₂₃ H ₁₇ N ₅ O	379	215	73	72.50 (72.82)	4.35 (4.48)	18.40 (18.46)
4d	-4'-CH ₃	-H	C ₁₈ H ₁₅ N ₅ O	317	260	76	68.08 (68.13)	4.65 (4.70)	21.90 (22.08)
4e	-4'-CH ₃	CH ₃	C ₁₉ H ₁₇ N ₅ O	331	254	78	68.86 (68.88)	5.10 (5.13)	21.05 (21.14)
4f	-4'-CH ₃	-C ₆ H ₅	C ₂₄ H ₁₉ N ₅ O	393	233	64	73.26 (73.28)	7.70 (4.83)	17.80 (17.81)
4g	-4'-Cl	-H	C ₁₇ H ₁₂ N ₅ OCl	337	251	48	60.50 (60.53)	3.30 (3.56)	20.70 (20.77)
4h	-4'-Cl	-CH ₃	C ₁₈ H ₁₄ N ₅ OCl	351	250	66	61.25 (61.53)	3.91 (3.98)	19.92 (19.94)
4i	-4'-Cl	-C ₆ H ₅	C ₂₃ H ₁₆ N ₅ OCl	413	240	53	66.72 (66.82)	3.80 (3.86)	16.88 (16.94)
4j	-4'-F	-C ₆ H ₅	C ₂₃ H ₁₆ N ₅ OF	397	235	45	69.42 (69.52)	4.00 (4.03)	17.52 (17.63)
4k	-4'-Br	-C ₆ H ₅	C ₂₃ H ₁₆ N ₅ OBBr	457	245	43	60.26 (60.39)	3.49 (3.50)	15.25 (15.31)
4l	-2'-Cl	-CH ₃	C ₁₈ H ₁₄ N ₅ OCl	351	280	70	61.40 (61.53)	3.89 (3.98)	19.30 (14.44)
4m	-2'-Cl	-C ₆ H ₅	C ₂₃ H ₁₆ N ₅ OCl	413	258	64	66.75 (66.82)	3.82 (3.86)	16.88 (16.94)

All the compounds were recrystallised from ethanol-chloroform mixture.

Analgesic activity:

The Institutional Animal Ethics Committee, prior to experimentation, approved all the experimental protocols. The analgesic activity of newly synthesized compounds 4a-m was determined by hot plate method¹⁶ using Wistar mice (25-30 g) of either sex. The animals were selected by random sampling technique and were divided into groups of 3 animals each. The time taken by each of the animal to show the paw licking response was recorded by placing the animal on a hot plate maintained at temperature of 55±0.5° before administration of compounds. This data was used as the control reading. The test compounds at 3 dose levels of 2.5 mg/kg, 5.0 mg/kg and 10 mg/kg were administered orally in the form of suspension in sodium CMC (1%) and water, while one group received pentazocin hydrochloride as a standard at a dose of 10 mg/kg body weight. After 60 min.

interval, the time taken for the paw licking responses was recorded for all the animals and percent analgesic activity was determined. The results of analgesic activity carried out by hot plate method for three different doses were used for calculation of ED₅₀ value for each compound. The results of analgesic activity are summarized in Table 4.

RESULTS AND DISCUSSION

As can be noted from the Table 4, compounds 4l and 4m were found to be the most potent analgesic compounds with the ED₅₀ of 3.30 and 3.23 mg/kg, respectively. Interestingly, these two are the only derivatives with the ortho substitution at the aromatic ring, (R₁). The compounds with unsubstituted aromatic ring viz; 4a, 4b, 4c and the compounds with electron donating methyl substitution at para position of the aromatic ring viz; 4d, 4e, 4f were found to be moderately active, with the ED₅₀ ranging from 4.15 to 7.10

TABLE 3: SPECTRAL DATA FOR 2-(UN) SUBSTITUTED-3-AMINO-5-ARYL-6-PHENYL PYRAZOLO[3, 4-*d*] PYRIMIDIN-4(5H)-ONES (4a-m)

Comp No.	λ_{\max} UV (MeOH) nm	IR in KBr (cm ⁻¹)	¹ H-NMR (CDCl ₃) in δ ppm
4a	293	3460, 3300 3240, 1700	2.9 (s, 3H, CH ₃), 5.3 (s, 2H, NH ₂) 7.4-7.9(m, 10H, Ar-H), 8.1(s, 1H, NH)
4b	288	3460, 3300 1690	3.1 (s, 3H, CH ₃), 5.2 (s, 2H, NH ₂) 7.4-7.8(m, 10H, Ar-H)
4c	284	3400, 3360 1700	5.3 (s, 2H, NH ₂) 7.4-8.0(m, 15H, Ar-H)
4d	291	3360, 3280 3240, 1700	2.8 (s, 3H, CH ₃), 5.4 (s, 2H, NH ₂) 7.5-7.9(m, 10H, Ar-H), 8.0(s, 1H, NH)
4e	288	3300, 3260 1690	2.5 (s, 3H, CH ₃), 5.6 (s, 2H, NH ₂) 7.3-7.8(m, 9H, Ar-H)
4f	287	3450, 3320 1680	2.4 (s, 3H, Ar-CH ₃), 3.5(s, 3H, N-CH ₃), 5.3 (s, 2H, NH ₂), 7.4-7.9(m, 14H, Ar-H)
4g	290	3460, 3300 3240, 1700	5.5 (s, 2H, NH ₂) 7.5-7.8(m, 9H, Ar-H), 8.2(s, 1H, NH)
4h	287	3460, 3300 1690	3.2 (s, 3H, CH ₃), 5.2 (s, 2H, NH ₂) 7.4-7.8(m, 9H, Ar-H)
4i	287	3480, 3360 1680	5.4 (s, 2H, NH ₂) 7.4-7.9(m, 14H, Ar-H)
4j	286	3480, 3360 1680	5.3 (s, 2H, NH ₂) 7.2-7.8(m, 14H, Ar-H)
4k	285	3440, 3400 1680	5.1 (s, 2H, NH ₂) 7.3-7.9(m, 14H, Ar-H)
4l	290	3480, 3300 1700	3.4 (s, 3H, CH ₃), 5.5 (s, 2H, NH ₂) 7.2-7.7(m, 9H, Ar-H)
4m	295	3380, 3300 1700	5.4 (s, 2H, NH ₂) 7.4-7.9(m, 14H, Ar-H)

TABLE 4: ANALGESIC ACTIVITY OF 2-(UN) SUBSTITUTED-3-AMINO-5-ARYL-6-PHENYL PYRAZOLO[3, 4-*d*] PYRIMIDIN-4(5H)-ONES (4a-m) (HOT PLATE METHOD)

Comp. No.	Mean increase in latency period \pm SEM(sec)			% Analgesic activity			ED ₅₀ (mg/kg)
	2.5 mg	5.0 mg	10.0 mg	2.5 mg	5.0 mg	10.0 mg	
4a	0.8 \pm 0.06	3.6 \pm 0.21	8.8 \pm 0.72	6.95	31.30	76.52	7.10
4b	1.5 \pm 0.09	9.8 \pm 0.23	11.6 \pm 0.50	13.00	85.21	108.6	4.20
4c	2.4 \pm 0.20	9.3 \pm 0.83	10.1 \pm 0.56	20.86	80.86	87.82	4.15
4d	2.1 \pm 0.19	5.8 \pm 0.40	9.4 \pm 0.73	18.26	50.43	81.73	5.81
4e	3.1 \pm 0.21	4.7 \pm 0.75	14.1 \pm 0.65	26.95	84.34	122.6	3.50
4f	1.6 \pm 0.30	5.5 \pm 0.23	8.1 \pm 0.50	13.91	47.82	70.43	6.67
4g	1.9 \pm 0.20	2.8 \pm 0.40	6.1 \pm 0.38	16.50	24.24	53.04	9.57
4h	0.5 \pm 0.26	2.3 \pm 0.20	5.9 \pm 0.47	04.10	20.00	51.30	9.78
4l	3.2 \pm 0.17	8.0 \pm 0.83	13.2 \pm 0.62	27.82	69.56	114.7	3.98
4j	2.3 \pm 0.09	3.2 \pm 0.40	6.1 \pm 0.56	20.00	29.56	53.00	9.39
4k	0.6 \pm 0.40	2.2 \pm 0.30	5.9 \pm 0.40	05.20	19.13	51.30	9.84
4l	3.4 \pm 0.34	9.9 \pm 0.50	13.4 \pm 0.60	29.56	86.00	116.5	3.30
4m	3.7 \pm 0.18	9.6 \pm 0.10	12.8 \pm 0.66	32.10	83.47	111.3	3.23

Analgesic activity was determined using hot plate method. Standard used is pentozocin HCl at a dose of 10 mg/kg, 11.50 \pm 0.9 secs.

mg/kg. The compounds with electron withdrawing substitution -Cl at the para position of the aromatic ring were found to be less active except for the 2-Phenyl derivative, 4i, which was found to be one of the most active compounds in the series, with the ED₅₀ of 3.98 mg/kg. However, the para fluoro derivative (4j) and para bromo derivative (4k) were found to be the least active derivatives.

Compounds 4l and 4m with the ortho chloro substitution were found to be the most active derivatives amongst all. The results of analgesic activity showed that compound 4b, 4e, 4i, 4l and 4m exhibited activity in the range of 108 to 122% at dose of 10mg/kg, indicating that these compounds are superior to Pentazocin hydrochloride at the same dose level. The compounds 4c and 4d showed the significant analgesic activity. The above results of analgesic activity showed that methyl or phenyl substituents at position 2 (R₂) are favourable for analgesic activity.

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