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Synthesis and antiinflammatory activity of novel pyrazolo [3,4-*d*] pyrimidines

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ABSTRACT

*The synthesis and biological evaluation of some novel pyrazolo [3,4-d] pyrimidines was aimed at creating a new scaffold. Considering previous articles activity profile of pyrazolo [3,4-d] pyrimidines, seven new 4-(4-substituted benzoylamino) pyrazolo[3,4-d]pyrimidines have been synthesized by reacting different 4-substituted benzoylchlorides with 4-amino pyrazolo [3,4-d] pyrimidine in DMF solvent. All these compounds were characterized by physical and spectral data. These compounds were screened for anti-inflammatory activity by carrageenan induced edema model. Among the compounds tested for anti-inflammatory activity, Electron withdrawing groups such as chloro **6b** and fluoro **6d** substituted compounds showed appreciable results at 3mg/kg when compared with the standard drug indomethacin at10mg/kg respectively. Electron releasing groups such as methyl **6c** and amino **6e** substituted compounds showed inferior activity when compared to **6b** and **6d** derivatives.*

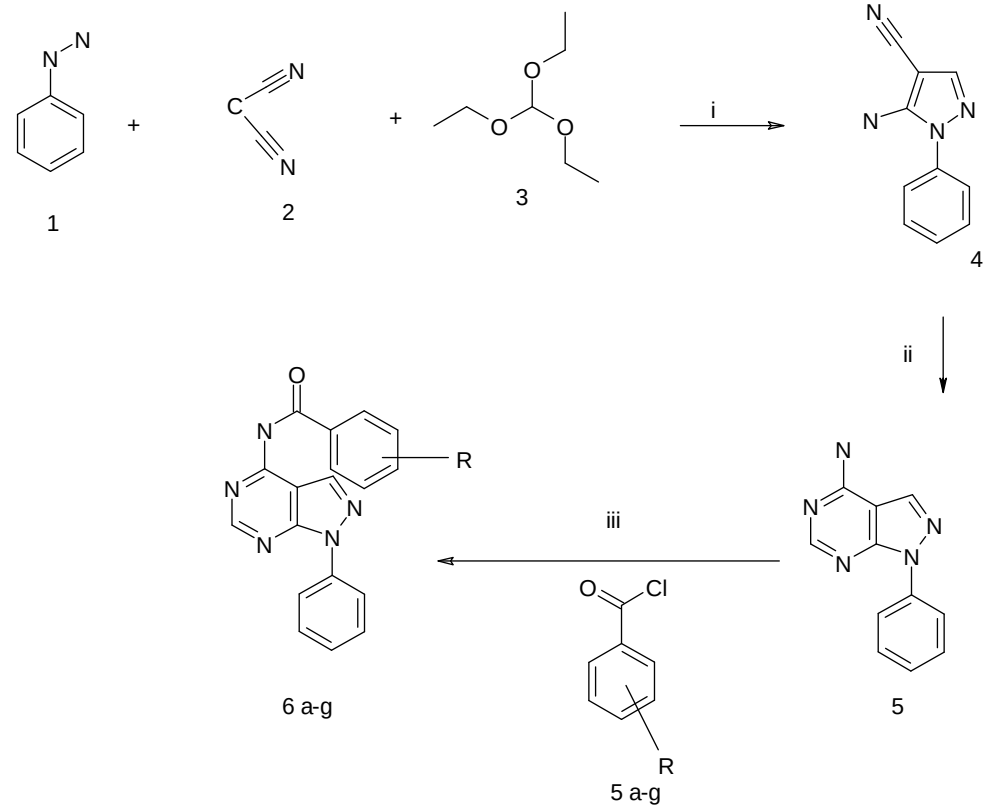
Key Words: Pyrimidines, pyrazoles, anti-inflammatory activity, carrageenan induced edema model.

INTRODUCTION

Inflammation is a complex reaction to injurious agents including microbes and involving vascular responses, such as activation and migration of leukocytes and systemic reactions [1]. The chemistry of pyrimidine and fused pyrimidine derivatives has been of increasing interest, since molecules based on the pyrazolo [3,4-d] pyrimidine ring system exhibit a multitude of interesting pharmacological properties including anticancer [2–9] and antibacterial agents [10–12]. Also, pyrimidine derivatives were found to possess several pharmacological properties,

including antiviral [13], antiallergic [14], hypoglycemic [15, 16] and diuretic [17] activities. As an extension of our studies on the synthesis of some new biologically active heterocyclic compounds [18], we now wish to report the synthesis of some new Schiff's bases of pyrazolo[3, 4-d]pyrimidine derivatives to evaluate their antiinflammatory activity by carrageenan paw edema method. In the present study reaction of melanonitrile, triethylformate and phenyl hydrazine in ethanol leads to the formation of 4-cyno 3-amino 2-phenyl pyrazole which is further refluxed with formamide gives 4-amino pyrazolo [3,4-d] pyrimidine which upon reaction with benzyl chlorides gives different substituents of pyrazolo [3,4-d] pyrimidines.

Scheme: Synthesis of pyrazolo[3,4-d]pyrimidines



i = Alcohol, RT, overnight, cool
ii = Formamide, refluxing
iii = Bezoylchlorides, DMF, DIPEA, RT

R : a = H, b = 4-Cl, c = 4-CH_3, d = 4-F, e = 4-NH_2, f = 4-CF3, g = 4-NO_2

EXPERIMENTAL SECTION

Melting points, were recorded in open glass capillaries using a **Polmon** melting point apparatus and uncorrected. Infra red spectra recorded on **Shimadzu** infrared spectrophotometer in KBr pellet. Mass spectra obtained on **VG-7070H** mass spectrometer. ^{1}H NMR spectra were recorded at 300MHz on a **Bruker Advance** NMR spectrometer in $CDCl_3$ (δ 7.26) or DMSO- d_6 (δ 2.49). The chemical are purchased from M/s Sigma Aldrich and S. D Fine chemicals. All the solvents

are purchased from M/s. Merck. Thin layer chromatography was performed on pre-coated silica gel F_{254} (Merck). Column chromatography was performed using silica gel 60-120 mesh.

Step-1: Synthesis of 4-cyno 3-amino2-phenyl pyrazole (4)

1.5 mol of malononitrile **2**, 0.693 mol of triethylformate **3** and 0.462 mol of phenyl hydrazine (1) taken in ethanol (5T) solution. In which first half of portion of mixture of malononitrile and triethylorthoformate was added drop wise to solution of alcohol and phenyl hydrazine with shaking and remaining amount was added at boiling. After complete addition the boiling was stopped and allowed for refrigeration till overnight. The reaction mixture was filtered by using vacuum pump to get solid material. Solid was washed with ether to remove the impurities. Finally to get white solid material (4).

Step-2: Synthesis of 4-amino pyrazolo[3,4-d]pyrimidine (5)

0.06 moles of **4** refluxed with 0.06 mol of formamide for a period of 1 hour 30 mins then warm solution was diluted with water and then allowed for refrigeration till over night. The excess of formamide and water was removed by vacuum under reduced pressure. The residue was formed and to it water and 2-3 drops of concentrated hydrochloric acid were added. The solution was warmed for 15min then treated the reaction mixture with charcoal and filtered. The filtrate basified with potassium hydroxide and the warm solution was chilled for overnight. The solid was formed (5) and separated by filtration.

Step-3: Reaction of 4-amino pyrazolo[3,4-d]pyrimidine with 4- substituted benzyl chlorides (6a-g)

0.007 mol of compound (5) was taken in single neck R.B flask to this added 0.0014 mol of benzoyl chlorides (4-substituted) and hunig base in dimethyl formamide and allowed for stirring. After completion of the reaction, which was monitored over TLC, the stirring was stopped. The compound formation was checked by taking the drop of compound dissolved in water and extract with ethyl acetate and checked the TLC.

Assessment of anti-inflammatory activity

Sprague Daley or Wistar rats of either sex weighing between 100-150 g are used. The animals are starved overnight with water being provided ad libitum. The test compounds and standard drugs are administered by oral or intraperitoneal route. Thirty minutes later the rats are challenged by S. C. injection of 0.05 ml of 1% solution of carrageenan on the plantar surface of the left hind paw. The paw is marked with ink at the level of lateral malleolus and immersed in the mercury column of a plethysmometer for measuring the paw volume. The volume is measured immediately after the carrageenan injection and then at 2, 3, 4 and 6 hr. Several models of plethysmometer are now commercially available. The peak effects of carrageenan usually occur at 3 hrs after the injection. The increase in paw volume at 3 hr is calculated as percentage compared with the volume measured after the injection of carrageenan for each animal. The difference of average values between treated animals and control groups is calculated for each dose of the drug. A dose response curve is plotted and used for determination of ED_{50} [8].

RESULTS AND DISCUSSION

Anti-inflammatory activity: Biological data of compounds 6a-g

Comp. No.	R	Anti inflammatory activity (**%**)				
		Dose mg/kg	30 min	1 hr	3 hr	5 hr
6a	4-H	3	18.3	16.8	12.9	16.8
6b	4-Cl	3	30.6	40.1	31.1	44.6
6c	4-CH_3	3	40.6	39.8	30.5	34.4
6d	4-F	3	34.2	36	35.6	43.4
6e	4-NH_2	3	17.4	21.2	24.2	21.6
6f	4-CF_3	3	21.8	17.4	23.8	16.3
6g	4-NO_2	3	9.2	17.4	18.7	11.1
Indomethacin		10		42.8	58.9	61.3

The synthesized pyrazolo [3,4-d] pyrimidines demonstrated anti-inflammatory activity in the range from 11.1% to 47.9% in the time interval of one hour to five hour. Acute toxicity test was not carried for the synthesized molecules hence two different doses were set for all the seven test compounds viz, 3 mg/kg, while Indomethacin used as standard was screened at 10 mg/kg dose. Compound, **6b** (R=Cl) demonstrated significant anti-inflammatory activity of 31.1% and 44.6% at 3rd and 5th hour of carrageenan challenge where as at the same period indomethacin demonstrated 58% and 61% respectively at 10 mg/kg. A slight decrease in anti-inflammatory activity was observed when chloro is substituted (**6d R=F**) by fluoro in which anti-inflammatory activity was found to be 35.6% and 43.4% at 3rd and 5th hour respectively. Electron releasing groups such as methyl **6c** and amino **6e** substituted compounds showed inferior activity when compared to chloro derivative **6b**. Among the two **6c** was found to be superior, which exhibited 40.6% at the end of first hour and 34.4% at the end of 5th hour of carrageenan exposure when administered 3 mg/kg dose. The decrease in the activity may be attributed to early metabolic degradation. The amine derivative **6e** however exhibited nearly 50% reduced activity of 6c, 17.4% anti-inflammatory activity was observed at the 1st hour and 24.4% at 3rd hour, which subsequently decreased to 21.6%. Compounds with electron withdrawing groups also demonstrated decreased anti-inflammatory activity when compared to chloro derivative **6b**.

N-(1H-pyrazolo[3,4-d]pyrimidin-4-yl)benzamide : Yield: 75 %; m.p.: 203°C; %. IR (KBr, cm–1): 3502 (N–H streching), 1714 (C=O Streching), 3061 (Ar- H Streching),1637 (C=N streching), 1543 (C=C Strching), ^{1}H NMR (CD_3OH): 7.5 (t, 2H, Ar-H),7.35 (t, 2H, Ar-H), 8.7 (s, 2H, Ar-H(pyrazole)), 8-8.2 (d, 1H, Ar-H), 8.9-9 (s,*br* 1H, NH), (M^+): 315.

4-Chloro-N-(1H-pyrazolo[3,4-d]pyrimidin-4-yl)benzamide: Yield: 70 %; m.p.: 197°C; %. IR (KBr, cm–1): 3502 (N–H streching), 1716 (C=O Streching), 3060 (Ar- H Streching),1639 (C=N streching), 1545 (C=C Streching), 720 (C-Cl Streching^{1}H NMR (CD_3OH): 4 (s, C-Cl), 7.5 (t, 2H, Ar-H),7.35 (t, 2H, Ar-H), 8.7 (s, 2H, Ar-H(pyrazole)), 8-8.2 (d, 1H, Ar-H), 8.9-9 (s,*br* 1H, NH), (M^+): 349.

4-Methyl-N-(1H-pyrazolo[3,4-d]pyrimidin-4-yl)benzamide: Yield: 55 %; m.p.: 183°C; %. IR (KBr, cm–1): 3500 (N–H streching), 1714 (C=O Streching), 3061 (Ar- H Streching),1637 (C=N streching), 1545 (C=C Strching), 1400 (C-H Streching), ^{1}H NMR (CD_3OH): 1.5-1.8 (m,

2H, CH_3), 7.5 (t, 2H, Ar-H),7.35 (t, 2H, Ar-H), 8.7 (s, 2H, Ar-H(pyrazole)), 8-8.2 (d, 1H, Ar-H), 8.9-9 (s,*br* 1H, NH), (M^+): 329.3.

4-Fluoro-N-(1H-pyrazolo[3,4-d]pyrimidin-4-yl)benzamide: Yield: 68 %; m.p.: 177°C; %. IR (KBr, cm–1): 3502 (N–H streching), 1714 (C=O Streching), 3061 (Ar- H Streching),1637 (C=N streching), 1543 (C=C Strching), 690 (C-F Streching) ^{1}H NMR (CD_3OH): 4.3 (s, C-Cl), 7.5 (t, 2H, Ar-H),7.35 (t, 2H, Ar-H), 8.7 (s, 2H, Ar-H(pyrazole)), 8-8.2 (d, 1H, Ar-H), 8.9-9 (s,*br* 1H, NH), (M^+): 333.3.

4-Amino-N-(1H-pyrazolo[3,4-d]pyrimidin-4-yl)benzamide: Yield: 80 %; m.p.: 190°C; %. IR (KBr, cm–1): 3502 (N–H streching),1714 (C=O Streching), 3061 (Ar- H Streching),1637 (C=N streching), 1543 (C=C Streching), 3300 (N–H streching),^{1}H NMR (CD_3OH): 2.9 (s,1H, NH_2), 7.5 (t, 2H, Ar-H),7.35 (t, 2H, Ar-H), 8.7 (s, 2H, Ar-H(pyrazole)), 8-8.2 (d, 1H, Ar-H), 8.9-9 (s,*br* 1H, NH), (M^+): 330.3.

N-(1H-pyrazolo[3,4-d]pyrimidin-4-yl)-4-(trifluoromethyl)benzamide: Yield: 77 %; m.p.: 211°C; %. IR (KBr, cm–1): 3502 (N–H streching), 1714 (C=O Streching), 3061 (Ar- H Streching),1637 (C=N streching), 1543 (C=C Streching), 750 (CF_3 streching), ^{1}H NMR (CD_3OH): 4.3 (s,1H, CF_3), 7.5 (t, 2H, Ar-H),7.35 (t, 2H, Ar-H), 8.7 (s, 2H, Ar-H(pyrazole)), 8-8.2 (d, 1H, Ar-H), 8.9-9 (s,*br* 1H, NH), (M^+): 383.3.

4-Nitro-N-(1H-pyrazolo[3,4-d]pyrimidin-4-yl)benzamide: Yield: 80 %; m.p.: 179°C; %. IR (KBr, cm–1): 3502 (N–H streching), 1714 (C=O Streching), 3061 (Ar- H Streching),1637 (C=N streching), 1543 (C=C Streching), 1483 (C-NO_2 Streching), ^{1}H NMR (CD_3OH): 7.5 (t, 2H, Ar-H),7.35 (t, 2H, Ar-H), 8.7 (s, 2H, Ar-H(pyrazole)), 8-8.2 (d, 1H, Ar-H), 8.9-9 (s,*br* 1H, NH), (M^+): 360.2.

CONCLUSION

Compounds of anilide derivatives of pyrazolo[3,4-d]pyrimidines speculated to possess anti-inflammatory activity. All the compounds (6a-g) were analyzed and structures are characterized by IR, NMR and MASS. The synthesized compounds were screened for anti-inflammatory activity following carregenan induced rat paw method. Among the compounds, compound bearing 4-chloro (6b) found to possess 44% protection at 3mg/kg at 5th hour of carregenan challenge.

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