Indian Journal of Chemistry
Vol. 49B, December 2010, pp. 1671-1674

Note

Synthesis and bioactivity evaluation of pyrazolone derivatives

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Received 9 November 2009; accepted (revised) 12 October 2010

3-Methyl-4-substituted benzylidene-pyrazol-5-ones **1-10** are synthesized by the condensation of 3-methyl-pyrazol-5-one with substituted aliphatic and aromatic aldehydes. Their structures have been elucidated from UV-Vis, IR, ^{1}H NMR and mass spectral data. Among the synthesized derivatives **5, 6, 7** and **10** are found to have a potent anti-inflammatory response whereas compounds **1, 4, 5, 8** and **10** have an effective analgesic response. There is no remarkable difference in bioactivity of pyrazolones derived from aliphatic and aromatic aldehydes. All the experimental data are statistically significant at $p<0.05$.

Keywords: Pyrazolone, analgesic, anti-inflammatory, protein kinase C inhibitor

Pyrazolone is a key structure in numerous compounds of therapeutic importance. Pyrazolone derivatives have been used as analgesics[1], antimicrobial agents[2], fungicides[3], and hypoglycemic agents[4]. Some of them have been tested as potential cardiovascular drugs[5]. They inhibit the production of TNF-α and decrease levels of pro-inflammatory cytokines and thereby reduce inflammation and prevent further tissue destruction in diseases such as Rheumatoid Arthritis (RA), Osteoarthritis (OA), and Crohns disease[6-8]. Researchers from Merck proved that pyrazolones are inhibitors of p38 kinase[9]. Recently their new derivative, named TELIN, was chemically synthesized and identified as a potent inhibitor of human telomerase. Another effective pyrazolone derivative, Nafazatrom, has dual arachidonate enzyme inhibition property. It exhibits antithrombotic and thrombolytic action. It also reduces the myocardial infarct size after experimental coronary artery occlusion and reperfusion. The above mentioned literature is worthwhile to prompt synthesis of some novel molecules by taking pyrazolone as heterocyclic key pharmacophore.

Results and Discussion

The present study reports the synthesis (**Scheme I**), analgesic and anti-inflammatory activity of pyrazolone derivatives. Perusal of the results on their analgesic activity by tail-flick method revealed that almost all of them to exert significant activity. Among them, compounds **1, 4, 5, 8** and **10** were found to have an effective analgesic response at $P < 0.01$ (**Table I**). All the synthesized compounds were screened for anti-inflammatory activity against Carrageenan-induced paw edema in rats. When compared with the control, all the compounds showed reduction in edema volume with prominent percentage inhibition to the inflammatory response ranging from 44% to 65% at 4th hour of observation. Compounds **5, 6, 7** and **10** were found to have a potent anti-inflammatory response at $p < 0.01$ level (**Table II**).

Experimental Section

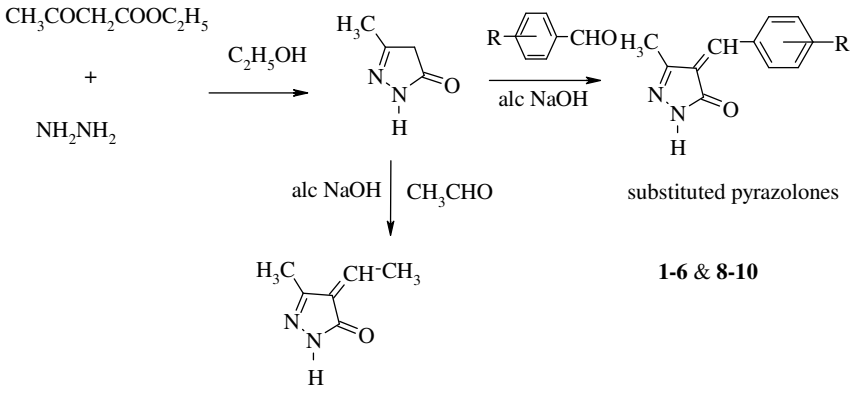
Melting points were determined in open capillary tubes and are uncorrected. The lambda max of the compounds were measured by UV-Vis spectrophotometer (UV–Pharma Spec 1700 Shimadzu). The IR spectra were recorded on Perkin-Elmer IR spectrometer 8400s using KBr disc. The ^{1}H NMR spectra were obtained on a Bruker DRX-600 MHz spectrometer in $CDCl_3$ using TMS as internal standard and chemical shifts are expressed in δ scale. The mass spectra were recorded on a Jeol SX-102 (FAB) Spectrometer.

Synthesis of 3-methyl pyrazol-5-one[10]

65 g (0.5 mole*)* of ethyl acetoacetate was taken in a 250 mL conical flask and stirred magnetically during slow drop wise addition of a solution of 25 g (0.5 mole) of hydrazine hydrate in 40 mL absolute ethanol. The temperature of the reaction mixture increased during reaction so that temperature was regulated at 60°C. A crystalline deposit was separated after stirring for 1 hr at 60°C. The reaction mixture was cooled in an ice bath to complete the crystallization. After standing for some time for completion of crystallization, it was filtered and the solid was washed with cold alcohol, dried and used for further step.

Synthesis of 3-methyl pyrazol-5-one derivatives 1-10

Pyrazolone (0.01 mole) was taken in a 100 mL round bottom flask, and then 50 mL of freshly prepared 20% sodium hydroxide alcoholic solution



Compd	R	Compd	R
1	—$N(CH_3)_2$	**6**	H
2	C_6H_5CH=CH-	**8**	4 —OMe
3	—OCH_3(2,3)	**9**	3 · OCH_3
4	3 · NO_2	**10**	OH
5	3 – Cl		

Scheme I — Synthesis of pyrazolone derivatives

Table I — Analgesic activity of pyrazolone derivatives in rat by tail flick method

Compd Dose (100 mg/kg p.o)	Reaction time in sec (Mean ±SEM)			
	After 1 hr	After 2 hr	After 3 hr	After 4 hr
Control	2.97±0.160	2.97±0.165	2.97±0.165	2.98±0.148
Diclofenac (10 mg/kg p.o)	6.93±0.139**	7.65±0.166**	8.25±0.042**	9.03±0.108**
1	4.91±0.127**	5.82±0.143**	6.4±0.050**	7.03±0.108**
2	4.19±0.034*	5.07±0.110*	5.9±0.022*	6.22±0.028*
3	4.19±0.036*	5.06±0.110*	5.79±0.140*	6.91±0.129*
4	4.72±0.128**	5.30±0.132**	6.17±0.027**	7.03±0.104**
5	4.29±0.026**	5.23±0.028**	5.96±0.136**	6.76±0.181**
6	5.43 ±0.024*	5.31±0.030*	6.21±0.019*	7.16±0.017*
7	4.43±0.019*	5.17±0.017*	6.12 ±0.025*	7.07±0.026*
8	4.07±0.021**	5.81±0.136**	6.52±0.023**	7.60±0.098**
9	4.30±0.030*	5.12±0.119*	5.93 ±0.137*	6.89±0.126*
10	4.49±0.029**	5.47±0.030**	6.40±0.029**	7.21±0.024**

**P<0.01vs Control, *p<0.05vs Control (n=6)

was poured into it. The mixture was stirred with magnetic stirrer for 30 min. After that substituted aromatic and aliphatic aldehyde (0.01 mole) was added to the reaction mixture and kept under stirring for 8 hr. The reaction mixture was transferred into crushed ice and neutralized with dilute hydrochloric acid to precipitate the product. It was filtered, dried and purified by recrystallization from ethanol. Similarly, other compounds were prepared with some change in reflux time and reaction work up. The physiochemical data of the compounds are given in **Table III**. The spectral and analytical data are given in **Table IV**.

Acute Toxicity Studies

Toxicological studies of the test compounds (as suspension in 0.5% w/v carboxy methylcellulose)

Table II — Anti-inflammatory activity of pyrazolone compounds on carrageenan-induced paw oedema in rats

Compd 100 mg/po	Paw volume in mL, Mean ± SEM (% inhibition of paw edema) After 1 hr	After 2 hr	After 3 hr	After 4 hr
Control	0.97 ± 0.016	0.93 ± 0.017	0.91 ± 0.013	0.89 ± 0.013
Aspirin	0.61±0.008(37.11)**	0.53±0.011(42.85)**	0.41±0.008(54.5)**	0.3±0.006(89.99) **
1	0.84±0.007(13.79) *	0.65± 0.007 (30) *	0.55±0.004 (39.23)*	0.42±0.003(52.59) *
2	0.83±0.005(14.82) *	0.67±0.008(28.21)*	0.63±0.005 (41.78)*	0.43±0.003 (51.29) *
3	0.88±0.009 (9.36) *	0.68±0.009(26.78)*	0.50 ±0.023(44.01)*	0.411±0.004(54.32)*
4	0.88±0.009 (9.19) *	0.68±0.008(27.21)*	0.51 ±0.007(43.79)*	0.40±0.004 (54.81)*
5	0.65±0.009(33.22)**	0.55±0.005(40.35)**	0.4 ±0.006(54.56)**	0.30±0.003(65.74)**
6	0.69±0.007(29.13)**	0.56±0.006(39.35)**	0.46±0.007(49.08)**	0.31±0.004(64.99)**
7	0.65±0.006(33.56)**	0.56±0.008(39.28)**	0.46±0.003(48.89)**	0.38 ± 0.003(57.4)**
8	0.73 ± 0.010 (25) *	0.61±0.011 (34.5) *	0.51 ± 0.004(44.01)*	0.46±0.005 (48.18) *
9	0.75±0.006 (23.33) *	0.66±0.010 (28.57) *	0.53 ± 0.005(45.25)*	0.5 ± 0.006 (44.44)*
10	0.63±0.010(35.26)**	0.56±0.015(39.28)**	0.43±0.006(52.55)**	0.36±0.004(59.25)**

**P<0.01*vs* Control, * p<0.05*vs* Control (n=6)

Table III — Physical characterization data of compounds **1-10**

Compd	Mol formula	m.p. (°C)	Yield (%)
1	$C_{13}H_{15}N_3O$	236-38	65
2	$C_{13}H_{12}N_2O$	168-70	72
3	$C_{13}H_{14}N_2O_3$	208-10	68
4	$C_{11}H_9N_3O_3$	180-82	56
5	$C_{11}H_9N_2OCl$	216-18	84
6	$C_{11}H_{10}N_2O$	240-42	61
7	$C_6H_8N_2O$	258-60	75
8	$C_{12}H_{12}N_2O_2$	250-52	66
9	$C_{12}H_{12}N_2O_2$	170-72	57
10	$C_{11}H_{10}N_2O_2$	212-14	79

were carried out by standard method in oral dose of 100 to 1000 mg/kg body weight in albino mice. The mice were continuously observed for 8 hr for any signs of acute toxicity such as increased-decreased motor activity, ataxia, tremors, convulsions, sedation, lacrimation, *etc*. After 24 hr the mice were sacrificed, stomach, intestine, and liver were inspected under the magnifying lenses for any ulcer-haemorrhagic spots. The acute toxicity and gross behavior studies revealed that the entire compounds in the present investigation were nontoxic upto 1000 mg/kg body weight. All the animal experiments were performed by the approval of Institutional Animal Ethics Committee, Himalayan Pharmacy Institute.

Analgesic Activity

It was measured by D'Amour and Smith method[11]. The tips of tail of animals were individually placed on radiant heat source at constant temperature of 55±1°C and the reaction of the animals, like flicking of the tail was noted. Male albino rats of 12 groups (6 no's in each) were taken for study. First group was kept as control, second as standard and rest as test groups for different synthesized compounds. Test compounds **1-10** at the dose of 100 mg/kg p.o were administered. Diclofenac 10 mg/kg p.o was used as standard drug. The tail-flick latency was assessed by analgesiometer (Techno, India). Basal reaction time to radiant heat was taken for rat, the rat which responds within 2-3 sec only considered for studies. After administration, the reaction time was noted at 1 hr, 2 hr, 3 hr and 4 hr time interval of the above mentioned groups. The cut-off reaction time was fixed at 10 sec to avoid tissue damage. The observations were made and data obtained were statistically analyzed.

Anti-inflammatory Activity

It was studied by inducing paw edema according to Winter's method[12]. Male albino rats of 12 groups (6 no's in each) were taken for study. Group one was kept as control, group two was treated with standard drug aspirin 100 mg/kg p.o and the remaining groups were administered with test compounds **1-10** at the dose of 100 mg/kg p.o. A mark was made on left paws just beyond tibio-tarsal junction (knee joint) of each animal of all groups, so that each time the paw was dipped in the water column of digital paw edema meter (520-R,

Table IV — Spectral characterization data of compounds **1- 10**

Compd	Physical state	UV-Vis (nm)	^{1}H NMR (δ, ppm)	MS (*m/z*)
1	Dark brick red powder	482	δ8.25(s,1H,NH),6.80-8.61(m,4H,Ar-H),6.11 (s,1H,=CH-Ar),3.11(s, 6H,N(CH_3)$_2$),1.93(s,3H, CH_3)	229, 230, 231
2	Dark yellow powder	457	8.25 (s, 1H, NH), 7.31-7.66 (m, 4H, Ar-H), 6.11 (s, 1H, =CH-Ar), 3.29 (s, 6H, N(CH_3)$_2$), 1.93 (s, 3H, CH_3)	212, 213
3	White crystalline powder	397	8.25 (s, 1H, NH), 7.31-7.66 (m, 4H, Ar-H), 6.11 (s, 1H, =CH-Ar), 3.29 (s, 6H, N(CH_3)$_2$), 1.93 (s, 3H, CH_3)	246 ,247
4	Pale yellow powder	406	8.25 (s, 1H, NH), 7.31-7.66 (m, 4H, Ar-H), 6.11 (s, 1H, =CH-Ar), 3.29 (s, 6H, N(CH_3)$_2$), 1.93 (s, 3H, CH_3)	231 ,232
5	Pale pinkish powder	428	8.25 (s, 1H, NH), 7.31-7.66 (m, 4H, Ar-H), 6.11 (s, 1H, =CH-Ar), 3.29 (s, 6H, N(CH_3)$_2$), 1.93 (s, 3H, CH_3)	221
6	Pale yellow powder	360	8.25 (s, 1H, NH), 7.31-7.66 (m, 4H, Ar-H), 6.11 (s, 1H, =CH-Ar), 3.29 (s, 6H, N(CH_3)$_2$), 1.93 (s, 3H, CH_3)	186,187,188
7	White powder	370	8.25 (s, 1H, NH), 7.31-7.66 (m, 4H, Ar-H), 6.11 (s, 1H, =CH-Ar), 3.29 (s, 6H, N(CH_3)$_2$), 1.93 (s, 3H, CH_3)	124,125,126
8	Yellow powder	437	8.25 (s, 1H, NH), 7.31-7.66 (m, 4H, Ar-H), 6.11 (s, 1H, =CH-Ar), 3.29 (s, 6H, N(CH_3)$_2$), 1.93 (s, 3H, CH_3)	216, 217
9	Pale orange powder	455	8.25 (s, 1H, NH), 7.31-7.66 (m, 4H, Ar-H), 6.11 (s, 1H, =CH-Ar), 3.29 (s, 6H, N(CH_3)$_2$), 1.93 (s, 3H, CH_3)	216,217,218.
10	Orange powder	465	8.25 (s, 1H, NH), 7.31-7.66 (m, 4H, Ar-H), 6.11 (s, 1H, =CH-Ar), 3.29 (s, 6H, N(CH_3)$_2$), 1.93 (s, 3H, CH_3)	202,203,204

s-singlet, d-doublet, t-triplet, m-multiplet

IITC Life Science, USA) up to the fixed mark made on left paws to ensure constant paw volume. Carrageenan (1%, 0.1 mL) (Sigma-Aldrich, Milan, Italy) was injected subcutaneously into the plantar surface of the rat hind paw 1 hr after the oral administration of the test compound. After the administration of carrageenan solution, the paw volume of control, standard and test groups were noted at 1 hr, 2 hr, 3 hr and 4 hr time interval. The percentage of inhibition was calculated by applying New bould formula[13].

Acknowledgements

Authors are grateful to the Director Dr H. P. Chhetri, Himalayan Pharmacy Institute, Majhitar, East Sikkim for providing laboratory facilities and to the Director, IICB Kolkata India for providing spectral data. The financial support from AICTE, New Delhi for sanctioning the project on RPS (Ref No. 8023/BOR/RID/RPS-47/2008-09) is gratefully acknowledged.

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