RESEARCH PAPER

Accepted 26 October 1998
Received 11 December 1997
Indian J. Pharm. Sci., 1999, 61 (1) 25-28

Synthesis and Pharmacological Activity of Substituted Pyrazoles

Y.S.R. REDDY*, T. SOSAMMA MANI, G.V.S. RAMA SARMA AND B. SURESH
Department of Pharmaceutical Chemistry,
J.S.S. College of Pharmacy, Ootacamund - 643 001,
Tamil Nadu

Ten new title compound (2a-2j) were synthesized by the reaction of propenoates with hydrazines. All compounds were characterised and screened for analgesic and anti-inflammatory activities. A significant correlation between *in vitro* and *in vivo* anti-inflammatory activities was found.

Literature survey indicates that pyrazoles possess a wide spectrum of pharmacological activities such as anti inflammatory 1-3, analgesic[1,4], antipyretic[1,4] hypoglycemic[5] and many other activities[3,4,6,7]. Based on the pharmacological importance of the pyrazole ring, a new series of title compounds (2a-2j) have been synthesized and screened for possible analgesic and anti-inflammatory activities. Denaturation of protein is one of the causes of inflammation[8,9] and anti-inflammatory drugs are known to inhibit the denaturation of bovine serum albumin (BSA) *in vitro*[10] studies. We have studied *in vivo* and *in vitro* anti-inflammatory activity and tried to establish correlation between the observations made for the test compounds.

The required ethyl 3-methylthio (substitutedamino)-2-carbonitrilopropenoates (1a-e) were obtained by the reaction of ethyl bis(methylthio) methylene cyanoacetate with anilines in methanol. These propenoates on treatment with hydrazines gave substituted pyrazoles (2a-j). The compounds were characterized by their spectral data and elemental analysis.

EXPERIMENTAL

Purity of compounds were checked by TLC. Melting points were determined in open capillaries using VMP-1 Veego melting point apparatus and were uncorrected. IR spectra were recorded on Perkin-Elmer FTIR 1600 using KBr pellets. PMR spectra were recorded on a varian A 60-D using TMS as internal reference.

Ethyl-3-methylthio (substitutedanilino) -2-Carbonitrilopropenoates (1a-e) :

A mixture of anilines (0.02 mol) and ethyl bis-(methylthio)-methylene cyanoacetate (0.02 mol) was refluxed in 50 ml dry methanol for 2 h. The solvent was evaporated to get a residue and it was recrystallized from methanol.

Ethyl -5-amino-3-(substituted anilino)-1-substitutedpyrazole-4-carboxylate (2a-j) :

Respective mixture of propenoates (1a-f) (10 mol) and hydrazines (10 mmol) was heated at 100° for 1h. The solid product was recrystallized from appropriate solvent. (2g) spectral data, IR (KBr) cm^{-1} : 3460 (NH_2), 3310 (NH), 1690 (C=O), 1650 (C=N); PMR ($CDCl_3$) δ, ppm:1.3 (t, 3H, ester CH_3), 4.5 (q, 2H, ester CH_2), 6.28 (br, 2H, NH_2), 7.2-8.6 (m, 9H, AR-H), 11.2 (s, 1H, NH); Mass:m/z 355 (38%). Pysical and elemental analysis data of the compounds are given in Table-1.

Pharmacological Activity

All the title compounds (2a-j) were subjected to acute toxicity study using a previously reported method[11] and based on the observations recorded, a dose of 100 mg/kg body weight was chosen as test dose in the present study.

* For correspondence and reprints

Table-1: Characterisation data of various compounds (2a-2j)

Compd.	Substituents		M.P.	Yield	M.F.	Analysis Found (Calc.)		%
	R	R^1	°c	%		C	H	N
2a	P-BrC_6H_4-	H	140-143	61	$C_{12}H_{13}N_4O_2Br$	44.27 (44.31)	3.93 (4.00)	17.18 (17.23)
2b	P-ClC_6H_4-	H	108-110	57	$C_{12}H_{13}N_4O_2Cl$	51.69 (57.34)	4.69 (4.63)	17.89 (19.96)
2c	P-$NO_2C_6H_4$-	H	138-140	42	$C_{12}H_{13}N_5O_4$	49.88 (49.48)	4.53 (4.47)	24.01 (24.05)
2d	P-OHC_6H_4-	H	175-178	57	$C_{12}H_{14}N_4O_3$	54.88 (54.96)	5.28 (5.34)	21.41 (21.37)
2e	o-$OCH_3C_6H_4$-	H	120-125	58	$C_{13}H_{16}N_4O_3$	56.43 (56.52)	5.69 (5.80)	20.23 (20.29)
2f	P-BrC_6H_4-	C_6H_5	121-123	60	$C_{18}H_{17}N_4O_2Br$	53.98 (53.86)	4.17 (4.24)	13.92 (13.96)
2g	P-ClC_6H_4-	C_6H_5	98-101	58	$C_{18}H_{17}N_4O_2Cl$	60.63 (60.59)	4.81 (4.77)	15.69 (15.71)
2h	P-$NO_2C_6H_4$-	C_6H_5	119-121	55	$C_{18}H_{17}N_5O_4$	59.01 (58.86)	4.60 (4.63)	19.05 (19.07)
2i	P-OHC_6H_4-	C_6H_5	135-136	62	$C_{18}H_{18}N_4O_3$	63.91 (64.01)	5.33 (5.29)	16.57 (16.62)
2J	o-$OCH_3C_6H_4$-	C_6H_5	98-100	55	$C_{19}H_{20}N_4O_3$	64.82 (64.77)	5.65 (5.68)	15.89 (15.91)

* Recrystallization was carried out in Methanol for all the compounds, except 2d and 2e (Methanol-Banzene 70:30).

Tail Immersion Method

The analgesic activity of the synthesized compounds was carried out by the method of Ghosh[12]. Albino mice (25-32 g) of either sex were used for the study. The compounds were given by i.p. route, in 0.5 % carboxy methyl cellulose at a dose of 100 mg/kg body weight and buprenorphin (100 mg/kg body weight) is used as standard drug. Hot water at temperature of 55±5° was used and basal reaction time was observed at regular intervals of 0, 30, 60, 120, 240 and 360 minutes.

Writhing Test

All the compounds were screened using the method of Ghosh[12]. Albino mice (25-32g) of either sex were used for the study. Percentage protection exhibited by the test compounds administered at a dose of 100 mg/kg body weight in 0.5% carboxy methyl cellulose suspension intraperitoneally against the acetic acid (0.6% w/w;; 10 ml/kg body weight) induced writhing or stretching syndrome was recorded. Aspirin (100 mg/kg body weight) was employed as reference standard under similar conditions.

Inhibition of BSA Denaturation :

Bovine serum albumin (Loba-chem), Ibuprofen (Tablets India, Ltd) and all other chemicals (Sigma were of analytical grade. Inhibition of denaturation of BSA was studied according to a previously reported method[13]. The test compounds were dissolved in minimum amount of dimethyl formamide (DMF) and diluted with phosphate buffer (0.2 M, pH 7.4). Final concentration of DMF in all solutions was 2%. Test solutions (1 ml) containing different concentrations of drug were mixed with 1 ml of 1 mM BSA in phosphate buffer and incubated at 27° for 15 minutes. Denaturation was induced by keeping the reaction mixture in a water bath at 60° for 10 minutes. After cooling the turbidity was measured at 660 nm (Shimadzu 160-A). Each experiment was repeated five times and the average values were recorded. Percent inhibition of denaturation was calculated and data has shown in table-2.

Carrageenin-induced oedema in rats :

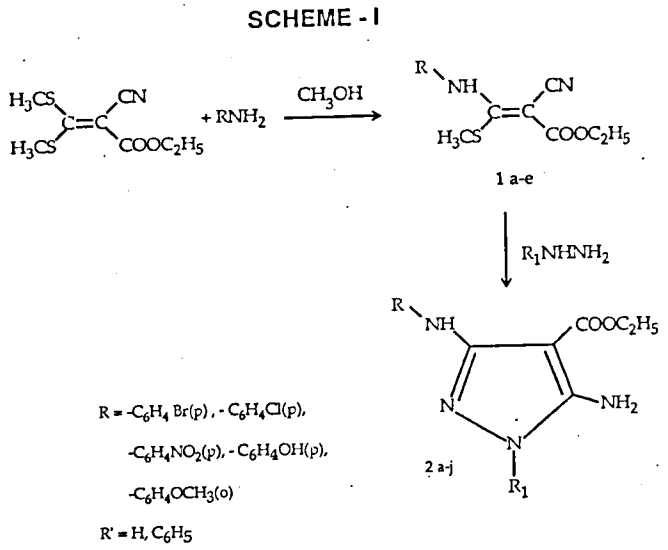
All compounds except 2a exhibited more than 25% BSA denaturation inhibition and were taken for this study. Hind paw oedema was induced in groups of 5 rats using the method of winter et al[14]. Carrageenin solution (1% w/v)

Table -2 : Inhibition of Bovine serum albumin denaturation

Compound	Control	2a	2b	2c	2d	2e	2f	2g	2h	2i	2j	Ibuprofen
Absorbance	0.0012 ±	0.014 ±	0.005 ±	0.005 ±	0.001 ±	0.003 ±	0.0046 ±	0.0067 ±	0.009 ±	0.005 ±	0.001±	0.001±
± SE	0.0007	0.0005	0.0007	0.0007	0.0007	0.0001	0.0005	0.0002	0.001	0.0005	0.0007	0.0007
% Inhibition	-	16.6	58.3	58.3	97.7	75	66.5	50	25	66.6	91.7	91.7

All the values mean ±SEM of 5 samples.

SCHEME - I



in normal saline in a volume of 0.1 ml was injected subcutaneously into the subplantor region of left hind paw 1 h after oral drug treatment. Paw volume was measured with a plethysmograph, before and after 4 h of carrageenin injection. The results are shown in table-3.

RESULTS AND DISCUSSION

Acute toxicity studies revealed that all the compounds were non-toxic upto the dose as high as 1000 mg/kg body weight. All the compounds were tested for analgesic activity by both tailflick and writhing methods to study the mode of action. In these two tests, all compounds failed to show significant activity when compared to standard drugs.

Inhibition of denaturation of BSA was studied using all the synthesized compounds and they inhibited in the range of 16.7 to 91.7%. Those compounds which showed more than 25% inhibition were taken for further study of *in vivo* anti-inflammatory activity using carrageenin-induced oedema method. In carrageenin-induced oedema, compounds showed 18.4 to 53.4 % protection. It was found that inhibition of denaturation of BSA and anti-inflammatory activity have significant degree of correlation. Compounds 2b, 2i, and 2j showed appreciable inhibitory potency against inflammatory as well as BSA denaturation.

ACKNOWLEDGEMENTS

The authors wish to place on record their heartfelt thanks to His Holiness Jagadguru Sri Shivarathri

Table - 3: Anti-inflammatory activity by Carrageenin-induced rat paw oedema

Compound	Interval	Control	2b	2c	2d	2e	2f	2g	2h	2i	2j	Ibuprofen
Volume of	0	4.7 ±	4.6 ±	4.4 ±	4.7 ±	4.5 ±	4.7 ±	5.0 ±	4.6 ±	4.2 ±	4.8 ±	4.7 ±
Rat paw		0.28	0.39	0.35	0.35	0.15	0.25	0.79	0.35	0.35	0.34	0.35
in ml	4	8.99 ±	6.7 ±	7.0 ±	7.8 ±	7.2 ±	8.2 ±	7.8 ±	7.4 ±	6.6 ±	6.8 ±	5.9 ±
		0.25	0.14**	0.66	0.05	0.15	0.15	0.22	0.34	0.43**	0.31**	0.68**
% Inhibition	-	-	51.04	32.40	27.73	37.06	18.41	34.73	34.73	44.01	53.38	73.89

All the values an Mean±SEM of 5 values ** P>0.001.

Deshikendra Mahaswamigalavaru of Sri Suttur Mutt, Mysore for providing necessary facilities and to Dr. L.V.G. Nargund, Nargund, Nargund College of Pharmacy, Bangalore for his encouragement.

REFERENCES

1. Furco, R., Bianchi, M., Bonacia, F. and Osvaldo, A., **Farmaco. Ed. Sci.**, 1968, 23, 919.
2. Bauer, J.V., Milliams, R.P.and Safir, S.R., **J. Med. Chem.**, 1971, 14, 454.
3. Robert, H.W., **J. Heterocyclic Chem.**, 1976, 13, 545.
4. Dupont, E.T., De Nemours & co., **Brit. Lit.**, 1, 015,922.; **Chem. Abst.**, 1966, 64, 12681.
5. Joshi, K.C. and Pathak, V.N., **Indian J. Chem.**, 1973, 11, 398.
6. Jakaria,, G.S. and Sharma, H.R., **J. Indian Chem. Soc.** 1974, 51, 351.
7. Alberti, C. and Tironi, C., **Farmaco. Ed. Sci.**, 1967, 22, 418.
8. Spector, W.G., and Milloughby, D.A., **Nature**, 1962, 196, 1104.
9. Opiet, E.L., **J. Exp. Med.**, 1963, 117, 425.
10. Mizushima, Y., **The Lancet,**, 1965, 2, 167.
11. Turner, B.A. **Screening methods in pharmacology**, Acad. press, London, 1965, 61.
12. Ghosh, M.N., **Fundamentals of Experimental pharmacology,** Scientific Book Agency, Calcutta, 1981, 153.
13. Nargund, L.V.G., Robin Jose and Reddy, Y.S.R., **Arzneim-Forschung**, 1994, 44, 156.
14. Winter, C.A., Risley, E.A. and Nuss, G.W., **Proc. Soc. Exp. Biol. Med.**, 1962, 111, 544.