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Synthesis and Biological Evaluation of 3-Amino Pyrazolones

GALLA RAJITHA*, K.V.S.R.G. PRASAD and KOGANTI BHARATHI

Institute of Pharmaceutical Technology, Sri Padmavathi Mahila Visvavidyalayam (Women's University), Tirupati-517 502, India

*Corresponding author: Tel: +91 877 2243018, E-mail: rajitha_galla@yahoo.com

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A series of 3-amino-4-(substituted benzylidene)-1*H*-pyrazol-5(4*H*)-ones were synthesized by condensation of ethyl-2-cyano-3-(substituted) phenylacrylates with hydrazine hydrate. The chemical structures of synthesized compounds were confirmed by means of IR, ^{1}H NMR, mass spectral and elemental analysis. All the compounds were screened for their antiinflammatory, analgesic and antioxidant activities. Out of these compounds, 4-hydroxy-3,5-dimethoxy (**24**) and 4-dimethylamino (**17**) derivatives showed good antiinflammatory activity. 4-dimethylamino (**17**) and 5-bromo-4-hydroxy-3-methoxy (**23**) derivatives showed moderate analgesic activity.

Key Words: Pyrazolones, Antiinflammatory, Analgesic, Antioxidant.

INTRODUCTION

Pyrazolones are associated with the broad spectrum of biological activities including antimicrobial[1,2], anticancer[3], antiinflammatory[4-8], *etc.* These findings prompted us to synthesize compounds containing pyrazolone moiety and to evaluate for antiinflammatory activity. A series of 3-amino-4-substituted benzylidene-1*H*-pyrazol -5(4*H*)-ones were synthesized by condensation of ethyl-2-cyano-3-(substituted) phenylacrylates with hydrazine hydrate in the presence of methanol. Ethyl-2-cyano-3-(substituted) phenylacrylates were obtained by stirring benzaldehydes and ethylcyanoacetate in the presence of piperidine and rectified spirit. The chemical structures of synthesized compounds were confirmed by means of IR, ^{1}H NMR, mass spectral and elemental analysis.

EXPERIMENTAL

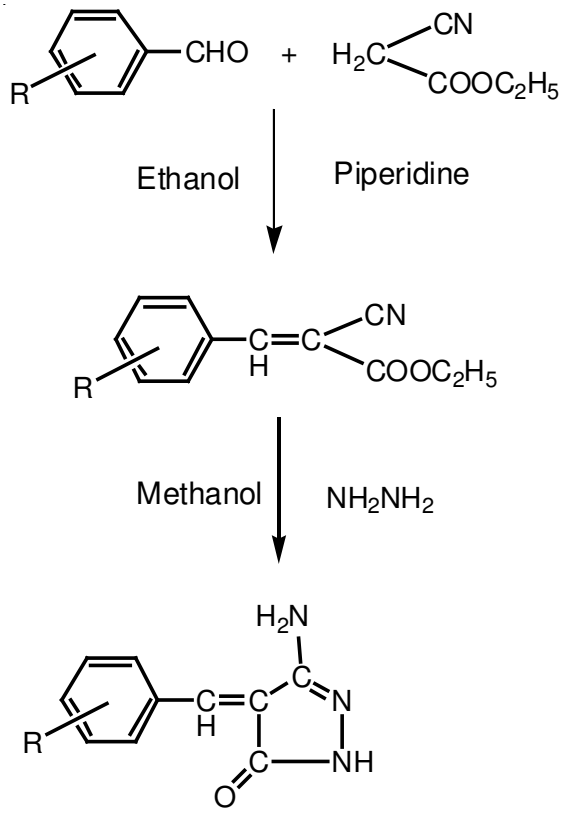
All the melting points reported in this series were determined in open capillaries using Thermonik precision melting point cum boiling point apparatus model C-PMB-2 and are uncorrected. Purity of the compounds was checked by using precoated TLC plates (E. Merck Kieselgel 60 F_{254}). The IR spectra were recorded using KBr Pellets on a Perkin-Elmer 1760 spectrophotometer (cm^{-1}). ^{1}H NMR spectra were recorded on GE Omega 400 MHz spectrometer or Bruker Avance (300 MHz) spectrometer using TMS as an internal standard (chemical shift in δ ppm). Mass spectra were recorded on a JEOL-JMS-D-300 spectrometer.

General method of synthesis of ethyl-2-cyano-3-(substituted)phenylacrylates (1-12): Synthesis of ethyl-2-cyano-3-phenylacrylate (1): Benzaldehyde (0.005 mol) and ethylcyanoacetate (0.005 mol) and two drops of piperidine were added in 10 mL of rectified spirit. The mixture was stirred for 4-6 h at room temperature. The resulting yellow coloured liquid was added to ice water. The separated solid was filtered washed with water and crystallized from ethanol. The various ethyl-2-cyano-3-(substituted)phenylacrylates **2-12** were prepared by a similar procedure.

General method of synthesis of 3-amino-4-[substituted benzylidene]-1*H*-pyrazol-5(4*H*)-ones (13-25): Synthesis of 3-amino-4-[(phenyl)methylene]-1*H*-pyrazol-5(4*H*)-one (13): A mixture of ethyl-2-cyano-3-phenylacrylate (0.01 mol) and hydrazine hydrate 99 % (0.02 mol) in methanol (6 mL) was refluxed on a water bath for 3 h. The reaction mixture was concentrated *in vacuo* and added to crushed ice. Crystalline masses, that deposited from the solution during ice cooling were purified by recrystallization from ethanol. The compounds **14-24** were prepared by a similar procedure. The physical data of the synthesized compounds is given in Table-1.

3-Amino-4-[(phenyl)methylene]-1*H*-pyrazol-5(4*H*)-one (13): IR (KBr, ν_{max}, cm^{-1}): 3480 (N-H), 2933 (=CH), 1673 (C=O), 1624 (C=C). ^{1}H NMR ($CDCl_3$) δ: 6.9-7.7 (m, 5H, Ar-H), 8.5 (s, H,CH=C), 10.0 (br s, 1H, -CONH-), m.f. $C_{10}H_9N_3O$.

3-Amino-4-[(4-chlorophenyl)methylene]-1*H*-pyrazol-5(4*H*)-one (14): IR (KBr, ν_{max}, cm^{-1}): 3223 (N-H), 2942 (=CH), 1624 (C=O), 1606 (C=C). ^{1}H NMR ($CDCl_3$) δ: 6.8-7.6 (m,



Scheme-I

TABLE-1
PHYSICAL DATA AND BIOLOGICAL ACTIVITIES COMPOUNDS **13-24**

Compd.	R	m.p. (ºC)	Yield (%)	Inhibition (%)	
				Edema[d]	Writhing
13	H	108-110	70	23	–
14	4-Cl	96-98	70	61.5*	5.7
15	4-NO_2	122-124	70	12.8	–
16	4-CH_3	124-126	75	30.95	–
17	4-$N(CH_3)_2$	130-132	80	69*	45.13*
18	4-OCH_3	98-100	68	38.5*	–
19	3,4-$(OCH_3)_2$	148-150	70	46.1*	–
20	3,4,5-$(OCH_3)_3$	148-150	60	33.3	–
21	4-OH	164-166	65	46.1*	–
22	4-OH, 3,-OCH_3	120-122	70	50*	4.2
23	5-Br, 4-OH, 3-OCH_3	202-204	75	64.2*	48.93*
24	4-OH, 3,5-$(OCH_3)_2$	176-178	80	71.4*	45*
	Phenylbutazone	–	–	74*	–
	Aspirin	–	–	–	64*

(a) At 100 mg/kg (P.O) per cent Edema inhibition calculated by comparing Edema volume with that of respective vehicle treated control animals. (b) Per cent Writhing inhibition was calculated comparing number of writhes with that for the respective vehicle treated control animals *Statistically significant ($p < 0.05$ Mann-Whitney). ($p < 0.05$, Student's 't' test).

4H, Ar-H), 8.6 (s, H,CH=C), 10.1 (br s, 1H, -CONH-), m.f. $C_{10}H_8N_3OCl$.

3-Amino-4-[(4-nitrophenyl)methylene]-1*H*-pyrazol-5(4*H*)-one (15): IR (KBr, ν_{max}, cm^{-1}): 3320 (N-H), 3010 (=CH), 1660 (C=O), 1603 (C=C). ^1H NMR ($CDCl_3$) δ: 6.8-7.6 (m, 4H, Ar-H), 8.6 (s, H, CH=C), 10.0 (br s, 1H, -CONH-), m.f. $C_{10}H_8N_4O_3$.

3-Amino-4-[(4-methylphenyl)methylene]-1*H*-pyrazol-5(4*H*)-one (16): IR (KBr, ν_{max}, cm^{-1}): 3320 (NH_2), 2989 (=CH), 1665 (C=O), 1606 (C=C). ^1H NMR (δ, ppm): 3.0 (s, 3H, $-CH_3$), 6.7-7.7 (m, 4H, Ar-H), 8.4 (s, 1H, =CH), m.f. $C_{11}H_{11}N_3O$.

3-Amino-4-[(4-dimethylphenyl)methylene]-1*H*-pyrazol-5(4*H*)-one (17): IR (KBr, ν_{max}, cm^{-1}): 3421 (NH_2), 2911 (=CH), 1666 (C=O) 1602 (C=N), 3421 (NH). ^1H NMR ($CDCl_3$) δ: 3.0 (s, 6H, $-CH_3$), 6.7-7.7 (m, 4H, Ar-H), 8.4 (s, 1H, =CH), m.f. $C_{12}H_{14}N_4O$.

3-Amino-4-[(4-methoxyphenyl)methylene]-1*H*-pyrazol-5(4*H*)-one (18): IR (KBr, ν_{max}, cm^{-1}): 3338 (N-H), 2920 (=CH), 1642 (C=O), 1604 (C=C). ^1H NMR ($CDCl_3$) δ: 3.9 (s, 3H, $-OCH_3$), 6.8-7.4 (m, 4H, Ar-H) 8.4 (s, 1H, CH=C-), m.f. $C_{11}H_{11}N_3O_2$.

3-Amino-4-[(3,4,dimethoxyphenyl)methylene]-1*H*-pyrazol-5(4*H*)-one (19): IR (KBr, ν_{max}, cm^{-1}): 3434 (N-H), 2948 (=CH), 1624 (C=O), 1602 (C=C). ^1H NMR ($CDCl_3$) δ: 3.9 (s, 6H, $-OCH_3)_2$), 6.8-7.4 (m, 3H, Ar-H) 8.4 (s, 1H, CH=C-), m.f. $C_{12}H_{13}N_3O_3$.

3-Amino-4-[(3,4,5-trimethoxyphenyl)methylene]-1*H*-pyrazol-5(4*H*)-one (20): IR (KBr, ν_{max}, cm^{-1}): 3339 (N-H), 2910 (=CH), 1660 (C=O), 1602 (C=C). 3339 (N-H), 2910 (=CH), 1660 (C=O), 1602 (C=C). ^1H NMR($CDCl_3$) δ: 3.9-4.0 (s, 9H, $(OCH_3)_3$), 6.9-7.4 (m, 2H, Ar-H), 8.5 (s,1H,CH=C-), m.f. $C_{13}H_{15}N_3O_4$.

3-Amino-4-[(4-hydroxyphenyl)methylene]-1*H*-pyrazol-5(4*H*)-one (21): IR (KBr, ν_{max}, cm^{-1}): 3332 (N-H), 2939 (=CH), 1655 (C=O) 1606 (C=C), m.f. $C_{10}H_9N_3O_2$.

3-Amino-4-[(4-hydroxy,3-methoxyphenyl)methylene]-1*H*-pyrazol-5(4*H*)-one (22): IR (KBr, ν_{max}, cm^{-1}): 3478 (N-H), 2923 (=CH), 1625 (C=O), 1602 (C=N). ^1H NMR ($CDCl_3$) δ: 3.9 (s, 3H,-OCH_3), 6.8-7.4 (m, 3H, ArH), 8.6 (s, 1H, -CH=C), 9.6 (br s, 1H, -CONH-), m.f. $C_{11}H_{11}N_3O_3$.

3-Amino-4-[(5-bromo-4-hydroxy-3-methoxyphenyl)-methylene]-1*H*-pyrazol-5(4*H*)-one (23): IR (KBr, ν_{max}, cm^{-1}): 3348 (N-H), 3144 (O-H), 2919 (=CH), 1649 (C=O), 1634 (C=C), m.f. $C_{11}H_{10}N_3O_3Br$.

3-Amino-4-[(3,5-dimethoxy,4-hydroxyphenyl)methylene]-1*H*-pyrazol-5(4*H*)-one (24): IR (KBr, ν_{max}, cm^{-1}): 3557 (OH), 3475 (NH), 2936 (C=H), 1690 (C=O), 1634 (C=N), 1600 (C=C), 1249 (C-O-C). ^1H NMR ($CDCl_3$) δ: 3.9 (s, 3H, $-OCH_3)_2$), 6.9-7.2 (m, 2H, ArH), 8.5 (s, 1H, -CH=C), 9.6 (br s, 1H, -CONH-), m.f. $C_{12}H_{13}N_3O_4$.

Antiinflammatory activity: The method of Winter *et al.*[9] was followed.

Analgesic activity: This method was based on acetic acid induced writhings in mice[10].

Gastric ulcerogenicity: The ulcerogenic liability was determined in albino rats of either sex (60-100 g) following the method of Saxena *et al.*[11].

Statistical analysis: All values are expressed as mean ± standard deviation. Tests of significance were analyzed by the Mann - Whitney method or Student's 't' - test significant.

Inhibition of lipid peroxidation in rat brain homogenate: The inhibition of lipid peroxidation was determined in albino rats of either sex (180-200 g) following the method of Sreejayan and Rao[12].

Assay of nitric oxide (NO) scavenging activity: The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and on subsequent coupling with N-naphthylethylene diamine was read[13] at 546 nm.

Interaction with stable free radical DPPH: DPPH assay was performed as described earlier[14]. Solutions of various drugs at 100 μM concentration were added to 100 μM DPPH in 95 % ethanol and tubes were kept at an ambient temperature for 20 min and absorbance was measured at 517 nm. Ethanol was used as blank and DPPH solution in ethanol served as the control.

RESULTS AND DISCUSSION

Antiinflammatory activity: Antiinflammatory activity of the title compounds was evaluated by the carrageenan induced edema model in rats. The data are given in Table-1. Among the phenolic compounds in the series, 5-bromovanillinyl (**23**) and 4-hydroxy-3,5-dimethoxy (**24**) derivatives showed good activity where as vanillinyl (**22**) and 4-hydroxy (**21**) derivatives exhibited moderate activity. This shows the importance of steric hindrance on the phenolic hydroxyl group. Substitution by 4-chloro (**14**) and 4-dimethylamino (**17**) also showed increased activity. 4-Methoxy (**18**) and 3,4-dimethoxy (**19**) derivatives exhibited moderate activity. The good antiinflammatory activity of these compounds may be due to the presence of pyrazolone moiety and the nature of substitution on the phenyl ring of the benzylidene moiety.

Analgesic activity: Compounds **14**, **17**, **22**, **23** and **24** which exhibited good antiinflammatory activity were screened for analgesic activity in acetic acid induced writhing test (Table-1). Although 4-dimethylamino (**17**), 5-bromovanillinyl (**23**) and 4-hydroxy-3,5-dimethoxy (**24**) derivatives showed statistically significant activity none of these compounds exhibited activity comparable to aspirin.

Inhibition of lipid peroxidation: Data is given in Table-2. Many phenolic compounds showed potent inhibition of lipid peroxidation. 4-Hydroxy-3,5-dimethoxy (**24**) and 5-bromovanillinyl (**23**) derivatives showed good inhibition of lipid peroxidation higher than α-tocopherol. 4-Hydroxy (**21**) and vanillinyl (**22**) analogs displayed activity comparable to α-tocopherol. The activity of these might be due to inductive effect of the methoxy group ortho to the phenolic group. Electron donating groups such as 3,4-dimethoxy (**19**) and 3,4,5-trimethoxy (**20**) showed significant inhibition of lipid peroxidation.

Nitric oxide scavenging activity: All the compounds were tested for their ability to scavenge nitric oxide data is given in Table-2. It is interesting to note that all the phenolic compounds were found to be active in scavenging nitric oxide. 4-Hydroxy-3,5-dimethoxy (**24**) derivative exhibited good scavenging of nitric oxide. Other phenolic compounds exhibited significant inhibition of nitric oxide. Dimethylamino (**17**) derivative exhibited good scavenging of nitric oxide.

TABLE-2
ANTIOXIDANT ACTIVITIES OF THE SYNTHESIZED COMPOUNDS **13-24** AT 100 μM

Compd.	R	% Inhibition of lipid peroxidation at 100 μM	% Scavenging of nitric oxide at 100 μM	% Reduction of DPPH at 100 μM
13	H	30	30	62.5
14	4-Cl	36	38	35
15	4-NO_2	28	26	25
16	4-CH_3	26	32	15
17	4-$N(CH_3)_2$	33	68	12
18	4-OCH_3	20	30	30
19	3,4-$(OCH_3)_2$	45	20	26
20	3,4,5-$(OCH_3)_3$	47	16	30
21	4-OH	50	55	80
22	4-OH, 3,-OCH_3	50	60	85
23	5-Br, 4-OH, 3-OCH_3	75	50	86
24	4-OH, 3,5-$(OCH_3)_2$	77	72	92
	α-Tocopherol	51.6	–	–

Reduction of stable free radical DPPH: Table-2 shows the reactivity's of test compounds with DPPH at 100 μM concentration. Among the phenolic compounds 4-hydroxy-3,5-dimethoxy (**24**), 5-bromovanillinyl (**23**) vanillinyl (**22**) and 4-hydroxy (**21**) analogs showed superior activity than α-tocopherol. These results agree with earlier work indicating that the antioxidant efficiency of monophenols is a strongly enhanced by the introduction of one or two methoxy substitutions in position ortho to the phenolic group[15]. Other compounds were found to be inactive.

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