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SYNTHESIS AND BIOLOGICAL ACTIVITY OF SOME NOVEL PYRAZOLINES

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Abstract: Chalcones were prepared from substituted acetophenones and substituted benzaldehydes and condensed with hydrazine hydrate in ethanol to get the corresponding pyrazolines (**Pdc1** to **Pdc 14**). The compounds were synthesized and characterizes by TLC, melting points, IR, and ^{1}H-NMR spectra. All the synthesized compounds were screened for their antibacterial and anti-inflammatory activities. The *in vitro* antibacterial activity was checked against two Gram positive microorganisms (*S. aureus* and *B. subtilis*) and two Gram negative microorganisms (*E. coli* and *P. aureginosa*). Some of tested compounds exhibited promising antibacterial and anti-inflammatory activities.

Keywords: pyrazolines, chalcones, antibacterial, anti-inflammatory, biological activity

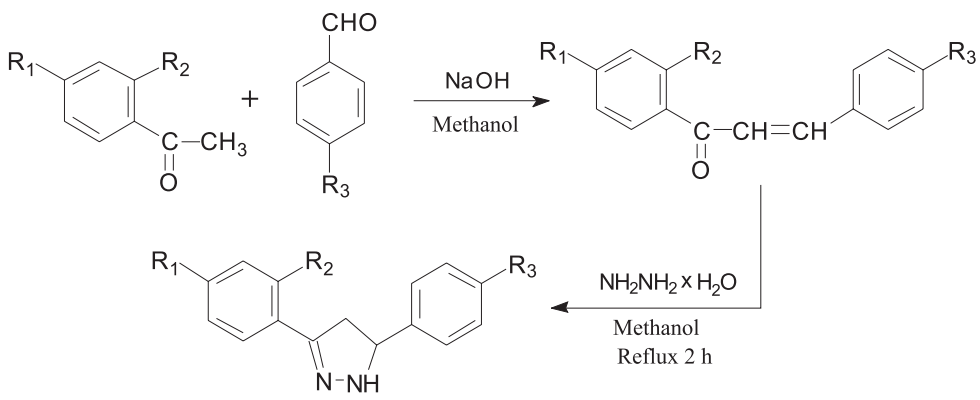
The review of the literature shows that the pyrazoline nucleus is quite stable and has inspired chemists to utilize these stable fragments in bioactive moieties to synthesize new compounds possessing biological activities (1–3). The past studies of substituted pyrazolines revealed that they exhibit antibacterial (4), analgesic (5), anti-inflammatory (5, 6), antiviral (7), antifungal (8), antiarthritic (9), cerebroprotective effect (10) and antidepressant (11) properties. There are several substituted pyrazolines having bleaching property or act as luminescent and fluorescent agents (12). They are also useful as biodegradable agrochemicals (13).

Encouraged by these facts and in continuation with the wok related to the synthesis, spectral studies and biological properties of pyrazolines, herein is reported the synthesis of some novel pyrazolines, then their antibacterial and anti-inflammatory activities were investigated.

EXPERIMENTAL

Chemistry

Melting points of the synthesized compounds were determined by open capillary tube method. The structures of the compounds were assigned on



Scheme 1. Synthesis of title compounds (**Pdc 1–14**)

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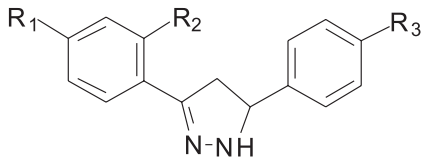
Table 1. New substituted derivatives of pyrazolines.



Compound code	**R_1**	**R_2**	**R_3**
Pdc-1	H	2-OH	4-NO_2
Pdc-2	H	2-OH	4-OCH_3
Pdc-3	H	2-OH	4-$N(CH_3)_2$
Pdc-4	H	2-OH	4-Cl
Pdc-5	4-OCH_3	H	4-NO_2
Pdc-6	4-OCH_3	H	4-Cl
Pdc-7	4-OCH_3	H	4-$N(CH_3)_2$
Pdc-8	4-CH_3	H	4-NO_2
Pdc-9	4-CH_3	H	4-Cl
Pdc-10	4-CH_3	H	4-$N(CH_3)_2$
Pdc-11	4-CH_3	H	4-OCH_3
Pdc-12	4-OH	2-OH	4-NO_2
Pdc-13	4-OH	2-OH	4-Cl
Pdc-14	4-OH	2-OH	4-$N(CH_3)_2$

Table 2. Physical data of the synthesized compounds.

Code	Molecular formula	Molecular weight	Melting point [^oC]	Yield %	R_f
Pdc-1	$C_{15}H_{13}O_3N_3$	283.280	220–223	57	0.56
Pdc-2	$C_{16}H_{16}O_2N_2$	268.310	134–136	36	0.57
Pdc-3	$C_{17}H_{19}ON_3$	281.536	245–247	33	0.49
Pdc-4	$C_{15}H_{13}ON_2Cl$	272.720	131–134	42	0.61
Pdc-5	$C_{16}H_{15}O_3N_3$	297.491	150–153	44	0.59
Pdc-6	$C_{16}H_{15}ON_2Cl$	286.879	152–155	23	0.77
Pdc-7	$C_{18}H_{21}ON_3$	295.563	235–237	21	0.67
Pdc-8	$C_{16}H_{15}O_2N_3$	281.492	133–135	54	0.73
Pdc-9	$C_{16}H_{15}N_2Cl$	270.88	138–140	13	0.55
Pdc-10	$C_{18}H_{21}N_3$	279.564	258–261	17	0.59
Pdc-11	$C_{17}H_{18}N_2O$	266.462	140–142	25	0.38
Pdc-12	$C_{15}H_{13}O_4N_3$	300.471	278–281	49	0.47
Pdc-13	$C_{15}H_{13}O_2N_2Cl$	288.720	254–256	20	0.36
Pdc-14	$C_{17}H_{15}O_2N_3$	293.320	250–253	54	0.61

the basis of IR and ^{1}H-NMR spectral data. ^{1}H-NMR spectra were recorded on a Bruker AM 300 MHz spectrometer using $CDCl_3$ as a solvent and tetramethylsilane as an internal standard. The chemical shifts are expressed in δ (ppm). FT IR spectrometer of Perkin Elmer was used for study. Thin layer chromatography (TLC) was done with pre-coated silica gel plates (GF_{254} Merck) using benzene : ethyl

acetate (9.5 : 0.5, v/v) as the mobile phase. Syntheses of substituted chalcones from substituted acetophenones and substituted benzaldehydes were done according to (14). Substituted acetophenone (0.01 mol) was dissolved in sodium hydroxide solution (0.02 mol). Then, substituted benzaldehyde (0.01 mol) dissolved in methanol, was added with stirring and kept overnight. The reaction mixture was poured in ice cold water, neutralized with hydrochloric acid and filtered. Then, the mixture of chalcone (0.01 mol) and hydrazine hydrate (0.02 mol) in 50 mL ethanol was refluxed for 2 h. An excess of ethanol was distilled off and the resulting residue was kept overnight. Crystalline solid

Table 3. Antibacterial activities of the synthesized compounds. Zones of inhibition.

Compound	Gram positive organism (mm)		Gram negative organism (mm)	
	S. aureus (MTCC-496)	*B. subtilis* (MTCC-619)	*E. coli* (MTCC 443)	*P. aeruginosa* (MTCC-424)
Standard (ofloxacin)	18	16	20	17
Pdc-1	8	6	-	2
Pdc-2	10	11	10	9
Pdc-3	10	8	12	11
Pdc-4	-	3	-	-
Pdc-5	8	6	14	12
Pdc-6	-	-	7	8
Pdc-7	9	7	-	-
Pdc-8	6	5	-	4
Pdc-9	10	8	-	-
Pdc-10	12	10	6	7
Pdc-11	6	4	6	6
Pdc-12	10	11	10	11
Pdc-13	14	11	14	12
Pdc-14	8	7	-	3

Table 4. Anti-inflammatory activity.

Group	Treatment	Doses (mg/kg)	Paw volume (mean ± SEM)			
			0 min	0 min	120 min	240 min
I	Control	DMSO 0.5 mL	0.728 ± 0.033	0.720 ± 0.021	0.712 ± 0.018	0.702 ± 0.022
II	Standard	Diclofenac sodium (4 mg/kg)	0.732 ± 0.010	0.651 ± 0.022	0.580 ± 0.040	0.532 ± 0.021
III	**Pdc-2**	50	0.736 ± 0.018	0.690 ± 0.032	0.580 ± 0.040	0.541 ± 0.02**
IV	**Pdc-3**	50	0.738 ± 0.012	0.652 ± 0.021	0.582 ± 0.036	0.542 ± 0.038**
V	**Pdc-4**	50	0.712 ± 0.018	0.636 ± 0.033	0.618 ± 0.012	0.596 ± 0.032
VI	**Pdc-6**	50	0.739 ± 0.048	0.650 ± 0.026	0.576 ± 0.036	0.540 ± 0.014**
VII	**Pdc-9**	50	0.722 ± 0.022	0.690 ± 0.041	0.682 ± 0.042	0.676 ± 0.019
VIII	**Pdc-10**	50	0.702 ± 0.029	0.650 ± 0.018	0.580 ± 0.028	0.543 ± 0.041**
IX	**Pdc-13**	50	0.714 ± 0.018	0.676 ± 0.018	0.620 ± 0.019	0.610 ± 0.032
X	**Pdc-14**	50	0.718 ± 0.026	0.651 ± 0.031	0.588 ± 0.021	0.536 ± 0.042

The values are expressed as the mean ± SEM. Values were find out by ANOVA, followed by Newman Keul's multiple range test. ** values are significantly different from control ($p < 0.01$)

Table 5. Spectral data of the synthesized compounds.

Compound	IUPAC NAME	IR [cm^{-1}]	^1H NMR (δ, ppm), (*J* Hz)
Pdc-1	3-(2-hydroxyphenyl)-5-(4-nitrophenyl)-pyrazoline	3400 (N-H), 2924 (C-H), 1603 (-C=C-), 1518 (N-O), 1345 (C-N)	2.01–2.03 (d, *J* = 6, 2H, -CH_2), 5.30 (s, 1H, Ar-OH), 6.89–6.91 (d, *J* = 9, 1H, -NH), 7.38–7.41 (d, *J* = 9, 4H, Ar-H,), 7.71–7.75 (d, *J* = 12, 4H, Ar-H)
Pdc-2	3-(2-hydroxyphenyl)-5-(4-methoxyphenyl)-pyrazoline	3434 (N-H), 1031 (C-N), 1251 (C-O), 1421 (C-H), 2369 (C-H)	2.03–2.05 (d, *J* = 6, 2H, -CH_2), 3.71 (s, 3H, CH_3), 5.14 (s, 1H, Ar-OH), 6.74–6.76 (d, *J* = 6, 4H, Ar-H), 6.94–6.97 (d, *J* = 9, 1H, -NH), 7.26–7.29 (d, *J* = 9, 4H, Ar-H)
Pdc-3	3-(2-hydroxyphenyl)-5-(4-dimethylaminophenyl)-pyrazoline	3448 (N-H), 2912, 2366 (C-H), 1601 (C=C), 1426 (C-H), 1363 (C-N)	2.01–2.03 (d, *J* = 6, 2H, -CH_2), 2.74 (s, 3H, N-CH_3), 5.14 (s, 1H, Ar-OH), 6.56–6.58 (d, *J* = 6, 4H, Ar-H), 6.90–6.92 (d, *J* = 6, 1H, -NH), 7.33–7.36 (d, *J* = 9, 4H, Ar-H)
Pdc-4	3-(2-hydroxyphenyl) 5-(4-chlorophenyl)-pyrazoline	3433 (N-H), 2922 (C-H) 1459 (C-H), 1016 (C-N), 669 (C-Cl)	2.05–2.07 (d, *J* = 6, 2H, -CH_2), 5.14 (s, 1H, Ar-OH), 7.02–7.04 (d, 4H, Ar-H, *J* = 6), 6.97–6.99(d, *J* = 6, 1H, -NH), 7.28–7.30 (d, *J* = 6, 4H, Ar-H)
Pdc-	**5** 3-(4-methoxyphenyl) 5- (4-nitrophenyl)-pyrazoline	3447 (N-H), 1603 (C=C), 1510 (N-H), 1254 (C-O), 1343 (C-N), 2363 (C-H)	2.05–2.07 (d, *J* = 6, 2H, -CH_2), 3.69 (s, 1H, OCH_3) 6.72–6.75 (d, *J* = 9, 4H, Ar-H), 6.96–6.98 (d, *J* = 6, 1H, -NH), 7.96–7.99 (d, *J* = 9, 4H, Ar-H)
Pdc-6	3-(4-methoxyphenyl)-5- (4-chlorophenyl)-pyrazoline	3399 (N-H), 1260 (C-O), 1595 (C=C), 1543 (N-H), 780 (C-Cl), 1310 (C-N)	2.01–2.03 (d, *J* = 6, 2H, -CH_2), 3.71 (s, 1H, OCH_3), 7.41–7.44 (d, *J* = 9, 4H, Ar-H), 6.96–6.98 (d, *J* = 6, 1H, -NH), 7.12–7.15 (d, *J* = 9, 4H, Ar-H)
Pdc -7 -	3-(4-methoxyphenyl)5 (4-dimethylaminophenyl)-pyrazoline	3446 (N-H), 2919, 2851 (C-H), 1227 (C-O), 1604 (C=C), 1521 (N-H), 1428 (C-N)	2.05–2.07 (d, *J* = 6, 2H, -CH_2), 2.81 (s, 3H, N-CH_3), 3.71 (s, 1H, OCH_3), 6.60–6.63 (d, *J* = 9, 4H, Ar-H), 6.99–7.02 (d, *J* = 9, 1H, -NH), 7.14–7.17 (d, *J* = 9, 4H, Ar-H)
Pdc -8	3-(4-methylphenyl)-5- (4-nitrophenyl)-pyrazoline	3437 (N-H), 1605 (C=C), 1519 (N-H), 1345 (C-H), 1110 (C-N)	2.04–2.06 (d, *J* = 6, 2H, -CH_2), 2.21 (s, 3H, Ar-CH_3), 6.69–6.72 (d, *J* = 9, 4H, Ar-H,), 6.90–6.93 (d, *J* = 9, 1H, -NH), 7.86–7.89 (d, *J* = 9, 4H, Ar-H)
Pdc-9	3-(4-methylphenyl)-5-(4-chlorophenyl)-pyrazoline	3448 (N-H), 2922, 2516 (C-H), 1422 (C-H), 713 (C-Cl)	2.01–2.03 (d, *J* = 6, 2H, -CH_2), 2.28 (s, 3H, Ar-CH_3), 6.99–7.02 (d, *J* = 9, 1H, -NH), 7.14–7.17 (d, *J* = 9, 4H, Ar-H), 7.43–7.46 (d, *J* = 9, 4H, Ar-H)
Pdc-10	3-(4-methylphenyl)-5-(4-dimethylaminophenyl)-pyrazoline	3449 (N-H), 2371 (C-H), 1423 (C-H), 1054 (C-N)	1.89–1.91 (d, *J* = 6, 2H, -CH_2), 3.1 (s, 3H, Ar-CH_3), 2.76 (s, 3H, N-CH_3), 6.78–6.81 (d, *J* = 9, 1H, -NH), 6.74–6.77 (d, *J* = 9, 4H, Ar-H), 7.21–7.24 (d, *J* = 9, 4H, Ar-H)
Pdc-11	3-(4-methylphenyl)-5-(4-methoxyphenyl)-pyrazoline	3447 (N-H), 2371 (C-H), 1423 (C-H), 1637 (C=C), 1112 (C-N)	1.91–1.93 (d, *J* = 6, 2H, -CH_2), 2.28. (s, 3H, Ar-CH_3), 3.69 (s, 3H, OCH_3), 6.78–6.81 (d, *J* = 9, 1H, -NH), 6.89–6.92 (d, *J* = 9, 4H, Ar-H), 7.29–7.32 (d, *J* = 9, 4H, Ar-H)
Pdc-12	3-(2,4-dihydroxyphenyl) 5-(4-nitrophenyl)-pyrazoline	3393 (N-H), 2371 (C-H), 1601 (C=C), 1438 (C-H), 1110 (C-N)	1.85–1.87 (d, *J* = 6, 2H, -CH_2), 4.92 (s, 1H, Ar-OH), 6.91–6.94 (d, *J* = 9, 1H, -NH), 6.74–6.77 (d, *J* = 9, 4H, Ar-H), 7.93–7.96 (d, *J* = 9, 4H, Ar-H)
Pdc-13	3-(2,4-dihydroxyphenyl)5-(4-chlorophenyl)-pyrazoline	3386 (N-H), 1606 (C=C), 2369, 2343 (C-H), 1089 (C-N), 1544 (N-H).	1.91–1.93 (d, *J* = 6, 2H, -CH_2), 4.98 (s, 1H, Ar-OH), 6.91–6.94 (d, *J* = 9, 1H, -NH), 6.74–6.77 (d, *J* = 9, 4H, Ar-H), 7.12–7.15 (d, *J* = 9, 4H, Ar-H)
Pdc-14	3-(2,4-dihydroxyphenyl)5-(4-dimethylaminophenyl)-pyrazoline	3403 (N-H), 2913 (C-H), 1603 (C=C), 1429 (C-H), 1227 (C-N)	1.95–1.97 (d, *J* = 6, 2H, -CH_2), 2.82 (s, 3H, N-CH_3), 4.91 (s, 1H, Ar-OH), 6.91–6.94 (d, *J* = 9, 1H, -NH), 6.51–6.54 (d, *J* = 9, 4H, Ar-H), 6.78–6.81 (d, *J* = 9, 4H, Ar-H)

obtained was filtered and recrystallized from ethanol. Similarly, all the substituted pyrazoline derivatives were prepared. The structures of different compounds are given in Table 1 and the physical data of the synthesized compounds are given in Table 2.

BIOLOGICAL ACTIVITY

Antibacterial activity

All the synthesized compounds were screened *in vitro* for antibacterial activity against *S. aureus* (MTCC 96), *B. subtilis* (MTCC 619), *E. coli* (MTCC 722) and *P. aeruginosa* (MTCC 424) at the concentrations 10 µg/mL each, by cup-plate agar diffusion method (15). The concentrations used in screening were chosen after determining the MICs of each compound. The solvent used was dimethyl sulfoxide (DMSO) further diluted with water. Muller Hinton agar was used as the growth medium for the bacterial species. DMSO was used as a control. The control showed no activity against the strains of microorganisms used. Antimicrobial activity was measured as a function of diameter of zone of inhibition (mm). The results were compared with standard drug ofloxacin for antibacterial activity by measuring the zone of inhibition in mm at 10 µg/mL. Both the organisms (one Gram positive and one Gram negative strain) showed maximum zone of inhibition that were 18 mm and 20 mm, respectively. Other two strains were also tested against the standard drug. The results are given in Table 3.

Anti-inflammatory activity (16)

Carrageenan-induced rat hind paw edema method was used for testing anti-inflammatory activity. Male albino rats (150–200 g) were used for the experiment. They were procured from the animal house of KM college of Pharmacy, Madurai. The animals were dosed according to OECD guidelines and 50 mg/kg body weight was fixed as the dose for acute anti-inflammatory screening.

The rats were divided into 10 groups and each of 6 animals. A mark was made on the hind paw just behind tibio-tarsal junction, so that the paw was dipped in the mercury column up to the fixed mark to ensure constant paw volume. The paw volume of each animal was measured before the administration of the drug. One group served as a standard (diclofenac), another group served as a control (0.5 mL DMSO) and other groups were used for the test drugs. The rats were given the synthesized compounds orally at 50 mg/kg body weight. Diclofenac was given at a dose of 4 mg/kg body weight. Test compounds and diclofenac were suspended in DMSO which was used as a vehicle for the control group. The drugs were given orally. A solution of 1% carrageenan was used to induce inflammation. One hour after this treatment, edema was induced by injecting 0.1 mL of 1% w/v suspension of carrageenan in subplantar region of the hind paw. The paw volume was measured immediately with a plethysmograph. The paw volume was again measured after 60, 120 and 240 min. The average paw volume in a group of drug treated rats were compared with that of a group with vehicle (control group) and the percentage inhibition of edema was calculated using the formula: percent inhibition = (1 – Vt/Vc) × 100, where Vt is the mean paw volume of the test drug, Vc is the mean paw volume of the control. The results are given in Table 4.

The values showed that synthetic compounds **Pdc-2**, **Pdc-3**, **Pdc- 6**, **Pdc-10** and **Pdc-14** significantly reduced the inflammation when compared to control, but other compounds (**Pdc-4**, **Pdc-9** and **Pdc-13**) do not possess significant anti-inflammatory activity.

RESULTS

The results generated in this study lead to the following conclusions. Test compounds **Pdc-2**, **Pdc-10**, **Pdc-12** and **Pdc-13** were found to possess good antibacterial activity against Gram positive organism, while compounds **Pdc-3**, **Pdc-5**, **Pdc-12** and **Pdc-13** were found to possess good antibacterial activity against Gram negative organism. Test compounds **Pdc-2**, **Pdc-3**, **Pdc-6**, **Pdc-10** and **Pdc-14** were found to exhibit potent anti-inflammatory activity.

DISCUSSION AND CONCLUSION

Fourteen novel pyrazoline derivatives were synthesized and characterized by spectral analysis. The melting points of the synthesized compounds were measured by open capillary tube method and the results were uncorrected. The purity of the compounds was checked by TLC using silica Gel G as an adsorbent. Benzene and ethyl acetate (9.5 : 0.5, v/v) mixture was used as the mobile phase. The spots were visualized by iodine vapor. The structures of synthesized compounds were characterized by their IR and ^{1}H-NMR spectral analysis (Table 5). The antibacterial activity of the compounds was evaluated against two Gram positive microorganisms (*S. aureus* and *B. subtilis*) and two Gram negative microorganisms (*E. coli* and *P. aureginosa*). The

zone of inhibition was measured as the parameter of activity. The compounds **Pdc-2**, **Pdc-10**, **Pdc-12** and **Pdc-13** exhibited more than 55% of activity against Gram positive organisms. The compounds **Pdc-3**, **Pdc-5**, **Pdc-12** and **Pdc-13** exhibited more than 55% of activity against Gram negative organisms. It was concluded from the data obtained that compounds **Pdc-12** and **Pdc-13** showed wide spectrum of activity. It was shown that synthetic compounds **Pdc-2**, **Pdc-3**, **Pdc-6**, **Pdc-10** and **Pdc-14** significantly reduce the inflammation when compared with the control.

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