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Synthesis and biological activities of some 3,5-disubstituted-Δ²-pyrazoline derivatives

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ABSTRACT

Synthesis and biological activities (antimicrobial, anti-inflammatory & analgesic) of 1*H*-3,5-disubstituted-Δ²-pyrazolines (IIa-e) and 1-acetyl-3,5-disubstituted-Δ²-pyrazolines (IIIa-c) are described. The structure of synthesized compounds have been established on the basis of IR, ¹H NMR, Mass and elemental analysis. All the tested compounds showed significant antibacterial and antifungal activity. Some of the synthesized compounds also showed moderate to good anti-inflammatory and analgesic activity.

Key words: Chalcones, pyrazoline, antimicrobial, anti-inflammatory, analgesic and spectral studies.

INTRODUCTION

Pyrazole containing heterocyclic compound plays an important role in medicinal chemistry. Since a very long time the usefulness and great therapeutic value of pyrazole nucleus has been recognized and the wide range of biological activities[1,2] of this nucleus evaluated. Cox-2 inhibitory activity of pyrazole are well proved and many compounds containing pyrazole nucleus like celecoxib, sulphenazole, sulphinepyrazole & analgin are the well established in the market.

In the present study we have synthesized some 1*H*-3,5-disubstituted-Δ²-pyrazolines (IIa-e) by the cyclisation of different chalcones (Ia-e) in the presence of hydrazine hydrate. The required chalcones (Ia-e) were prepared by the condensation of appropriate aromatic aldehyde & acetophenones.1*H*-3,5-disubstituted-"²-pyrazolines (IIa-c) were further acetylated to 1-acetyl-3,5-disubstituted-"²-pyrazolines (IIIa-c) with the help of acetic acid (Scheme I). These compounds were also evaluated for their antimicrobial, anti-inflammatory and analgesic activities.

MATERIAL AND METHODS

The melting points were determined by open capillary method and are uncorrected.IR (KBr) spectra were recorded on a Shimadzu 8201PC infrared spectrophotometer. The ¹H NMR spectra were recorded on a Bruker DRX-300 spectrophotometer in DMSO using TMS as internal standard (Chemical shift are expressed in ppm). Mass spectra were recorded on Jeol-SX-102 (FAB) spectrometer. The purity of the compounds was checked by thin layer chromatography (TLC) on silica gel G coated plates and the spots were visualized by exposure to iodine vapors.

4-Substituted phenyl-4'-substituted chalcones (Ia-e)

To appropriate acetophenone (0.01mol) in ethanol (50ml) was added 4-substituted benzaldehyde (0.01 mol). The mixture was heated to boiling and hot solution of aqueous NaOH (40%) was added with continuous stirring during heating. After some time, a coloured solid was obtained, which was allowed to stand overnight. Then, it was poured into ice-cold water and neutralized with

hydrochloric acid (10%). The crystallized product was filtered, washed with cold water, dried and recrystallised from ethanol.

1*H*-3,5-Disubstituted-Δ²-pyrazoline (IIa-e) General method

To 4-substituted phenyl-substituted chalcones (Ia-e) (0.01 mol) in ethanol (25 ml) hydrazine hydrate (0.01 mol) was added. The reaction mixture was refluxed for 2 hr, concentrated and allowed to cool. The crystallized product was filtered, dried and recrystallised from ethanol.

1*H*-3-(*p*-Chlorophenyl)-5-anisyl-Δ²-pyrazoline (IIa)

I.R. (KBr): 3319 (N-H), 1514 (C=N), 1260 (C-O-C), 830 (C-Cl); ^{1}H NMR (DMSO) δ: 6.97-7.89 (d,8H+1H,ArH+NH), 5.10-5.40 (dd,1H,H_A), 3.76 (m, 3H+1H, OCH_3 + H_M), 3.49-3.60 (dd,1H,H_X).

1*H*-3-Phenyl-5-phenyl-Δ²-pyrazoline (IIb)

I.R. (KBr): 3455 (N-H), 1569 (C=N); ^{1}H NMR (DMSO) δ: 7.33-7.83 (m,10H+1H, ArH & NH), 5.24-5.28 (dd,1H,H_A), 3.87-3.94 (dd,1H,H_M), 3.57-3.63 (dd,1H,H_X); MS: m/z 223 (M^++1), 222(M^+).

1*H*-3-(*p*-Chlorophenyl)-5-phenyl-Δ²-pyrazoline (IIc)

I.R. (KBr): 3364 (N-H), 1580 (C=N), 830 (C-Cl); ^{1}H NMR (DMSO) δ: 7.10-7.77 (m,10H +1H, ArH & NH), 5.82-5.87 (dd,1H,H_A), 3.83-3.91 (dd,1H,H_M), 3.21-3.27 (dd,1H,H_X).

1*H*-3-(*p*-Chlorophenyl)-5-(*p*-chlorophenyl)-Δ²-pyrazoline (IId)

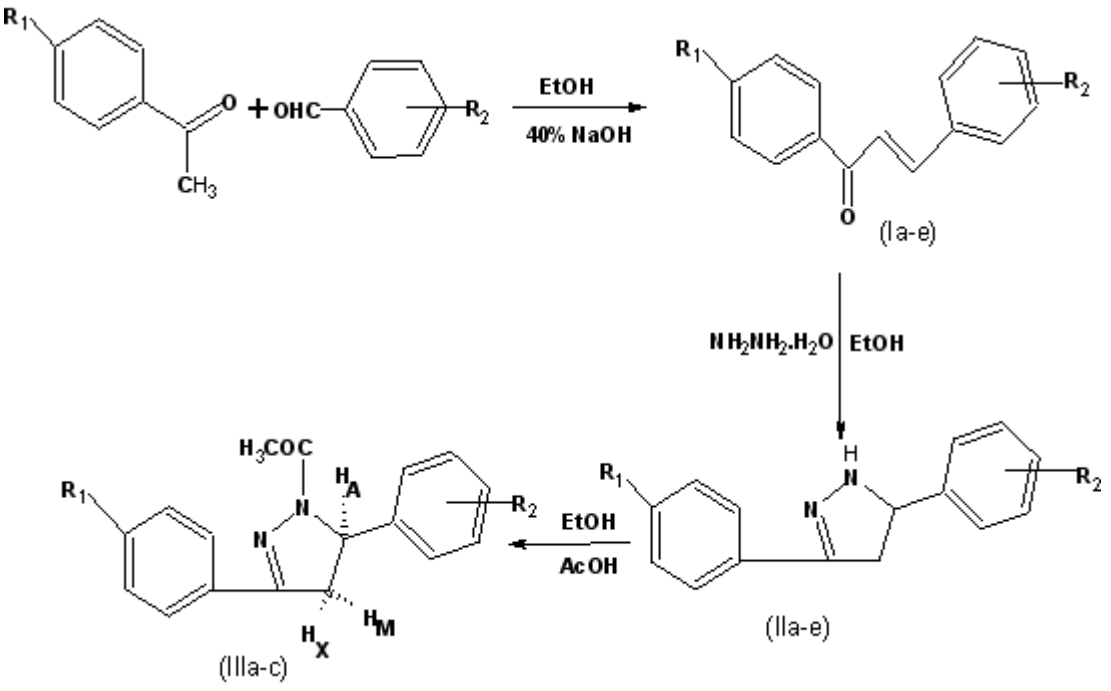
I.R. (KBr) : 1559 (C=N), 3428 (N-H), 825 (C-Cl); ^{1}H NMR (DMSO) δ: 7.28-7.87 (m,8H, ArH&1NH), 5.15-5.21 (dd,1H,H_A), 3.79-3.88 (dd,1H,H_M), 3.38-3.57 (dd,1H,H_X).

1*H*-3-(*p*-Chlorophenyl)-5-(*o*-hydroxyphenyl) -Δ²-pyrazoline (IIe)

I.R.(KBr): 3390 (OH), 3370(N-H), 2917(C-H,Ali), 851(C-Cl).

1-Acetyl-3,5-disubstituted–Δ²-pyrazoline(IIIa-c) General method

1*H*-3,5-disubstituted-Δ²-pyrazoline (IIa-c) was dissolved in glacial acetic acid (10 ml).The



Scheme 1

solution was refluxed for 2hr, concentrated and allowed to cool. The crystallized product was filtered, dried and recrystallised from ethanol.

1-Acetyl-3-(*p*-chlorophenyl)-5-anisyl– Δ²-pyrazoline (IIIa)

I.R. (KBr): 1657 (C=O), 1512 (C=N), 1248 (C-OC), 821 (C-Cl); ^{1}H NMR (DMSO) δ: 6.86-7.80 (d,8H,ArH), 5.48-5.52 (dd,1H,H_A pyrazoline), 3.72 (m,3H+1H,OCH_3+H_M pyrazoline), 3.13-3.15 (dd, 1H,H_X pyrazoline), 2.28 (s,3H,$COCH_3$).

1-Acetyl-3-phenyl-5-phenyl-Δ²-pyrazoline (IIIb)

I.R. (KBr): 3054 (CH, Ar), 2980 (CH, Ali), 1570 (C=N); ^{1}H NMR (DMSO) δ: 7.34-7.92 (m,10H,ArH), 5.20-5.26 (dd,1H,H_A), 3.89-3.98 (dd,1H,H_M), 3.63-3.72 (dd,1H,H_X), 2.50 (s, 3H, $COCH_3$).

1-Acetyl-3-(*p*-chlorophenyl)-5-phenyl-Δ²-pyrazoline (IIIc)

I.R.(KBr): 3052 (C-H,Ar), 2962 (C-H,Ali), 1666 (C=O), 1588 (C=N), 821 (C-Cl); ^{1}H NMR (DMSO) δ: 7.16-7.68 (m,9H,ArH), 5.58-5.62 (dd,1H,H_A), 3.69-3.76 (dd,1H,H_M), 3.10-3.16 (dd,1H,H_X), 2.41 (s, 3H,$COCH_3$); MS: m/z 300 (M^++2), 299 (M^++1), 298 (M^+).

Biological evaluation

Antimicrobial activity

All the synthesized compounds(IIa-e,IIIa-c) were screened for their in vitro antibacterial activity against *E.coli* (gram-negative) and *S.aureus* (gram-positive) and antifungal activity against *A. niger*, *A. flavus* and *P. citrinum* using cup plate method[3] at 200,100 and 50 µg/ml concentration in DMSO. Ciprofloxacin and ketoconazole were used as standard drugs for antibacterial and antifungal activity respectively at 50 µg/ml concentration in DMSO (Table 2).

Anti-inflammatory activity

Selected synthesized compound (IIa, IIb, IIe, IIIa, IIIc) were subjected for their anti-inflammatory activity by carrageenan induced paw edema method of winter *et al*[4] at an oral dose of 10 mg/kg. Indomethacin was used as standard drug at same oral dose of 10 mg/kg (Table 3).

Table 1: Physical characterization data of synthesized compounds

Compd	R_1	R_2	m.p.°C	Yield%	Mol. formula
Ia	Cl	*p*-OCH_3	150	90	$C_{16}H_{13}ClO_2$
Ib	H	H	160	90	$C_{15}H_{12}O$
Ic	Cl	H	200	85	$C_{15}H_{11}ClO$
Id	Cl	*p*-Cl	210	85	$C_{15}H_{10}Cl_2O$
Ie	Cl	*o*-OH	180	90	$C_{15}H_{11}ClO_2$
IIa	Cl	*p*-OCH_3	186	80	$C_{16}H_{15}N_2OCl$
IIb	H	H	185	85	$C_{15}H_{14}N_2$
IIc	Cl	H	176	75	$C_{15}H_{13}N_2Cl$
IId	Cl	*p*-Cl	190	80	$C_{15}H_{12}N_2Cl_2$
IIe	Cl	*o*-OH	140	80	$C_{15}H_{13}N_2ClO$
IIIa	Cl	*p*-OCH_3	136	75	$C_{18}H_{17}N_2O_2Cl$
IIIb	H	H	180	70	$C_{17}H_{16}N_2O$
IIIc	Cl	H	122	70	$C_{17}H_{15}N_2OCl$

All compounds showed satisfactory elemental analysis

% inhibition of edema is measured according to the following method:-

$$= \frac{(\text{Final foot volume of control} - \text{Final foot volume of standard/test})}{\text{Final foot volume of control}} \times 100$$

Analgesic activity

The compound which were tested for their anti-inflammatory activity were further tested for their analgesic activity at an oral dose of 10 mg/kg. The Eddy & Leimbach et al hot plate method[5] was used to evaluate the analgesic activity. Indomethacin was used as standard drug at same oral dose (Table 3).

Table 2: Antimicrobial activity of synthesized compounds

Compd	Concentration (µg/ml)	Zone of inhibition (in mm)				
		Antibacterial		Antifungal		
		E.coli	*S.aureus*	*A.niger*	*A.flavus*	*P.citrinum*
IIa	200	-	14	15	19	-
	100	-	13	15	19	-
	50	-	11	9	14	-
IIb	200	10	14	19	17	23
	100	-	12	15	15	18
	50	-	10	15	15	18
IIc	200	10	12	17	21	21
	100	-	10	16	21	20
	50	-	8	16	17	18
IId	200	14	-	18	19	-
	100	13	-	16	17	-
	50	11	-	12	17	-
IIe	200	8	12	20	16	-
	100	-	10	18	15	-
	50	-	10	17	14	-
IIIa	200	10	8	12	17	15
	100	8	7	11	15	14
	50	8	7	11	15	14
IIIb	200	8	12	17	17	16
	100	8	10	15	16	15
	50	-	-	12	15	14
IIIc	200	16	-	20	20	19
	100	12	-	17	18	18
	50	10	-	15	14	18
Ciprofloxacin	50	19	22	××	××	××
Ketoconazole	50	××	××	20	20	22

(-) no zone of inhibition; (××) not tested

Table 3: Anti-inflammatory and analgesic activity of compounds

Compd.	Anti-inflammatory activity #	Analgesic activity ##		
	% inhibition of edema after 4hr Mean ± SEM	Pre-treatment (sec) (0 min.)	Post treatment (sec) (After 4 hrs)	% Analgesia
Indomethacin	80.85 ± 1.875*	8.05 ± 0.31	8.68 ± 0.31+	81.63
IIa	51.10 ± 1.125*	4.56 ± 0.36	6.18 ± 0.38++	53.00
IIb	46.22 ± 2.874*	5.13 ± 0.24	6.41 ± 0.33+++	53.38
IIe	39.99 ± 2.036*	7.75 ± 0.36	8.52 ± 0.33+	77.36
IIIa	61.66 ± 2.632*	5.48 ± 0.34	6.59 ± 0.27++	67.74
IIIc	51.99 ± 3.443*	5.06 ± 0.37	6.08 ± 0.36+	70.23

Data of test compounds was compared w.r.t. std *P< 0.0001; Data were analyzed by unpaired student 't' test for n=6 ## Data was relative to pre-treatment and analyzed by paired student 't' test for n=6 +P< 0.0001; ++P< 0.001; +++P< 0.01

RESULTS AND DISCUSSION

The target cpmpounds (Ia-e,IIa-e,IIIa-c)were synthesized through the route depicted in the scheme 1.The structure of the synthesized compounds was confirmed on the basis of IR,^{1}H-NMR, Mass spectral data and elemental analysis. The investigation of antibacterial screening data revealed that all the tested compounds (IIa-e, IIIa-c) showed noticeable degree of bacterial inhibition. Among the synthesized compounds IIIc & IId showed highest activity against *E.coli* at 200 µg/ml, whereas compound IIb & IIa showed highest zone of inhibition against *S.aureus* at 200 µg/ml.

The investigation of antifungal activity data revealed that all the synthesized compounds(IIa-e,IIIa-c) exhibited considerabe inhibitory action .All the tested compounds except IIa, IId & IIe showed antifungal activity against all the fungal strain's used at all the concentration. Compound IIa, IId, IIe showed antifungal activity against *A.niger* & *A.flavus* at 200 µg/ml.Compound IIIc showed comparable antifungal activity to that standard drug ketoconazole (50 µg/ml) against all the strains used at 200 µg/ml. Compound IIb showed more zone of inhibition at 200 µg/ml than standard drug against *P.citrinum*, whereas compound IIc showed more zone of inhibition against *A.flavus* & comparable activity against *P.citrinum* at 200 & 100 µg/ml than that of standard.

Some of the synthesized pyrazoline (IIa,IIb,IIe,IIIa,IIIc) have been evaluated for anti-inflammatory activity. The synthesized compounds showed anti-inflammatory activity in the range of 39.99-61.66% whereas standard drug showed 80.85% inhibition in paw edema.

Some of the synthesized pyrazoline (IIa,IIb,IIe,IIIa,IIIc) have also been evaluated for analgesic activity. Compound IIe showed the highest activity (77.36%) comparable to standard drug (81.63%). Rest of the compounds showed moderate to good analgesic activity (53.00-70.23%).

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