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 1H-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 of this nucleus evaluated. Cox-2 inhibitory activity of pyrazole are well proved and many compounds containing pyrazole nucleus like celecoxib, sulphenazoal, sulphinepyrazole & analgin are the well established in the market.

In the present study we have synthesized some 1H-3,5-disubstituted-Δ²-pyrazolines (IIa-e) by the cyclisation of different chalcones (Ia-e) in the presence of hydrazine hydrate. The required chalcones (la-e) were prepared by the condensation of appropriate aromatic aldehyde & acetophenones. 1H-3,5-disubstituted-Δ²-pyrazolines (IIa-c) were further acetylated to 1-acetyl-3,5-disubstituted-Δ²-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.

1H-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.

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

I.R. (KBr): 3319 (N-H), 1514 (C=N), 1260 (C-O-C), 830 (C-Cl); 1H NMR (DMSO) δ: 6.97-7.89 (d, 8H+1H, ArH+NH), 5.10-5.40 (dd, 1H, HA), 3.76 (m, 3H+1H, OCH₃ + HM), 3.49-3.60 (dd, 1H, HX).

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

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

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

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

1H-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**

1H-3,5-disubstituted-Δ2-pyrazoline (IIa-c) was dissolved in glacial acetic acid (10 ml). The
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); °H NMR (DMSO) δ: 6.86-7.80 (d,8H, ArH), 5.48-5.52 (dd,1H,H₆ pyrazoline), 3.72 (m,3H+1H,OCH₃+HM pyrazoline), 3.13-3.15 (dd, 1H,H₇ pyrazoline), 2.28 (s,3H,COCH₃)

1-Acetyl-3-phenyl-5-phenyl-Δ²-pyrazoline (IIlb)
I.R. (KBr): 3054 (C-H, Ar), 2980 (C-H, Ali), 1570 (C=N); °H NMR (DMSO) δ: 7.34-7.92 (m,10H,ArH), 5.20-5.26 (dd,1H,H₆), 3.89-3.98 (dd,1H,H₇), 3.63-3.72 (dd,1H,H₈), 2.50 (s, 3H, COCH₃).

1-Acetyl-3-(p-chlorophenyl)-5-phenyl-Δ²-pyrazoline (IIlc)
I.R.(KBr): 3052 (C-H,Ar), 2962 (C-H,Ali), 1666 (C=O), 1588 (C=N), 821 (C-Cl); °H NMR (DMSO) δ: 7.16-7.68 (m,9H,ArH), 5.58-5.62 (dd,1H,H₆), 3.69-3.76 (dd,1H,H₇), 3.10-3.16 (dd,1H,H₈), 2.41 (s,3H,COCH₃); 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 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, IIla, IIlc) were subjected for their anti-inflammatory activity by carrageenan induced paw edema method of winter et al 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>
<thead>
<tr>
<th>Compd</th>
<th>R₁</th>
<th>R₂</th>
<th>m.p.°C</th>
<th>Yield%</th>
<th>Mol. formula</th>
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<tr>
<td>Ia</td>
<td>Cl</td>
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<td>90</td>
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<tr>
<td>Ib</td>
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<td>H</td>
<td>160</td>
<td>90</td>
<td>C₁₅H₁₂O</td>
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<tr>
<td>Ic</td>
<td>Cl</td>
<td>H</td>
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<tr>
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<td>85</td>
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<td>le</td>
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<td>o-OH</td>
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<td>90</td>
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<td>80</td>
<td>C₁₆H₁₃N₂OCl</td>
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<tr>
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<td>H</td>
<td>H</td>
<td>185</td>
<td>85</td>
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<td>C₁₆H₁₃N₂Cl</td>
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<tr>
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<td>180</td>
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<td>C₁₇H₁₆N₂O</td>
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<tr>
<td>IIlc</td>
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<td>H</td>
<td>122</td>
<td>70</td>
<td>C₁₇H₁₄N₂OCl</td>
</tr>
</tbody>
</table>

All compounds showed satisfactory elemental analysis.
% inhibition of edema is measured according to the following method:

\[
\% \text{ inhibition} = \frac{\text{Final foot volume of control} - \text{Final foot volume of standard}}{\text{Final foot volume of control}} \times 100
\]

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 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

<table>
<thead>
<tr>
<th>Compd</th>
<th>Concentration (µg/ml)</th>
<th>Antibacterial</th>
<th>Zone of inhibition (in mm)</th>
<th>Antifungal</th>
<th>P.citrinum</th>
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<tr>
<td></td>
<td></td>
<td>E.coli</td>
<td>S.aureus</td>
<td>A.niger</td>
<td>A.flavus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.00</td>
<td>10.00</td>
<td>8.00</td>
<td>6.00</td>
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<td>9.00</td>
<td>7.00</td>
<td>5.00</td>
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<td>7.00</td>
<td>5.00</td>
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<td>6.00</td>
<td>5.00</td>
<td>3.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

(-) no zone of inhibition; (××) not tested
RESULTS AND DISCUSSION

The target compounds (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 considerable 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, Ile 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 Ile 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 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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REFERENCES