SYNTHESIS, CHARACTERISATION AND EVALUATION OF ANALGESIC ACTIVITY OF 3, 5-DISUBSTITUTED PYRAZOLINE DERIVATIVES

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Received: 12 Oct 2011, Revised and Accepted: 26 Dec 2011

ABSTRACT

The novel reaction of substituted acetophenones with substituted aromatic aldehydes gave chalcone derivatives by Claisen-Schmidt reaction. The obtained chalcones derivatives on treatment with hydrazine hydrate resulted in 3, 5-disubstituted pyrazoline derivatives. The spectral data was obtained by IR and NMR spectroscopy for the synthesized compounds. The compounds were screened for analgesic activity by tail-flick method and acetic acid induced writhing method.

Keywords: Claisen-Schmidt reaction, Chalcones, Analgesic, Tail-flick method, Acetic-acid induced writhing method.

INTRODUCTION

The pyrazoline is a dihydropyrazole product of pyrazole. It has been reported that the pyrazoline nucleus it exhibits a wide range of biological activities such as antifungal, antibacterial, antidepressant, anticonvulsant, anti-inflammatory, antitumor, antidiabetic, anesthetic, analgesic, ukerogenic, lipid peroxidation and immunosuppressive properties. Several substituted pyrazolines are found as effective bleaching agents, luminouscent and fluorescents. It was reported that triaryl pyrazoline inhibits flavivirus RNA replication. Survey of literature in the past reveals that some pyrazoline derivatives also possess cerebroprotective effect. This work is selected with a view to explore that the present nucleus has the analgesic activity.

MATERIALS AND METHODS

All the chemicals and solvents used were Analytical Grade R quality and the drugs complies with I.P standards. The purity of compounds was determined using Thin layer chromatographic studies. The TLC plates were prepared using silica gel and the mobile phase as chloroform: petroleum ether (4:1) and the spots were detected using iodine vapour and the Rf values were calculated. The melting points were determined by open capillary tube method and it was uncorrected. IR spectra were recorded on Bruker spectrophotometer at Vels University. The samples for IR spectra were prepared using KBr discs. 1H NMR spectrum (DMSO) was recorded on 500 MHZ-Bruker spectrophotometer (Indian Institute Of Technology, Chennai, Tamil Nadu, India) using tetra methyl silane as internal standard. Mass spectra were recorded on JEOL GC mate system (sophisticated analyticial instrument facility, Indian Institute of Technology, Chennai, Tamil Nadu and India).

General procedure for synthesis of compounds (1a-1f)

An equimolar concentration of substituted acetophenone (0.01mol) is made to react with substituted aromatic aldehyde (0.01mol) in the presence of 25ml of ethyl alcohol and 40% sodium hydroxide. The mixture was stirred and kept aside for 24hrs. The reaction mixture was poured into ice water and acidified with dilute hydrochloric acid. The solid product was filtered and recrystallized from ethanol. The chalcones were synthesized by base catalyzed claisen-schmidt condensation of aldehyde and ketone followed by dehydration.

Scheme 1: R=2, 6-dihydroxy, p-Cl, 2-Br, p-F, R"=3-ethoxy-4-hydroxy, 2-OH, p-(CH3)2NH:
Analgesic activity was calculated. The 0.6% v/v solution of acetic acid was used as writhing inducing agents. The test compounds were administered orally 1 hr prior to acetic acid injection. Mice were divided into groups of three animals. The control was given to group 1. The standard drug indomethacin was given to group 2 and the other groups received test drugs. All the drugs were prepared as homogenous suspensions in 2% CMC and were administered orally to animals. Acetic acid was administered intraperitoneally. The number of writhing were counted for 20 min in control, standard and test compounds. Analgesic activity was measured as percent decrease in writhing in comparison to control. All the results are expressed as mean ± SEM.

Acute toxicity: Acute toxicity tests were performed according to the organization of economic co-operation and development (OECD) guideline for testing of chemicals. Acute toxicity of 3,5-disubstituted pyrazoline derivatives were determined in Wister albino mice. Each group of 3 animals was fasted for 24 hours prior to the administration of the test compounds. The test compounds 2b, 2c e were administered orally in doses up to 500 mg/kg by suspending in 1% CMC solution and were kept under observation for period of 24 hours.

RESULTS AND DISCUSSION

2-(5-(3-ethoxy-4-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)benzene-1,4-diol

The IR spectrum of the compound 2a shows a broad peak at 3092 cm⁻¹ which is assigned to the hydrogen bonded O-H stretching. The bands at 2970 cm⁻¹ is attributed to the stretching of C-H stretching. The presence of alkene is confirmed by the band at 1656 cm⁻¹, which shows the peak for alkenes. The value 980 cm⁻¹ corresponds to C-H bending vibrations. The peak at 1579 cm⁻¹ represents N-H stretching. The band at 1399 cm⁻¹ shows carboxylate anion stretching.

2-(5-(2-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)benzene-1,4-diol

The IR spectrum of the compound 2b shows a peak at 3340 cm⁻¹ which shows N-H stretching in the pyrazoline nucleus. The peak at 3079 cm⁻¹ is assigned to C-H stretching. The peak obtained at 1676 cm⁻¹ represents ketone stretching. The value 980 cm⁻¹ corresponds to a peak for alkenes. The 1H NMR spectrum of the compound 2b shows the following observations. Singlets at 6.77 ppm shows 1H (CH of pyrazoline), doublet at 6.52 ppm shows 2H (CH₂ of pyrazoline), triplet at 5.09 ppm shows 3H (CH₃ of benzylic), doublet at 4.47 ppm shows 2H (CH₂ of pyrazoline), singlet at 3.98 ppm shows 1H (NH of pyrazoline), multiplet at 2.85 ppm shows 6H (CH₃ of benzylic) and multiplet at 2.05 ppm shows 2H (CH₂ of benzene).
2-(3-(2-bromophenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenol

The IR spectrum of the compound 2d shows a peak at 3322 cm\(^{-1}\) which shows N-H stretching in the pyrazoline nucleus. The peak at 2870 cm\(^{-1}\) is assigned to C-H stretching. The peak obtained at 2839 represents the O-H stretching. The peak at 1508 cm\(^{-1}\) represents aromatic ring. The peak at 1375 shows O-H bending. The peak at 1345 cm\(^{-1}\) is assigned to N-H stretching in the pyrazoline nucleus. The peak at 1063 represents the aromatic compound. The peak at 743 and 636 shows the halogen may be present in the structure.

4-(3-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)-N,N-dimethylbenzamine

The IR spectrum of the compound 2e shows a peak at 3345 cm\(^{-1}\) which shows N-H stretching in the pyrazoline nucleus. The peak at 3043 cm\(^{-1}\) is assigned to O-H stretching. The peak obtained at 1410 cm\(^{-1}\) represents C-N vibrations. The value 1345 cm\(^{-1}\) corresponds to C-Br bending. The peak at 1043 cm\(^{-1}\) represents C-N vibrations. The peak at 965 shows the presence of alkene. The value at 823 shows the aromatic ring is present. The \(^1\)H NMR spectrum of the compound 2e shows the following observations. Singlets at 67.41 show 1H (NH of pyrazoline), 82.86 show 6H (CH of dimethylamine), multiplet at 67.67 shows 2H (CH of fluorophenyl), 84.75 show 1H (CH of pyrazoline), 83.36 shows 2H (CH of pyrazoline), and doublet at 6.70 shows 2H (CH of pyrazoline). The mass spectrum of the compound 2e shows the M\(^+\) peak at 283.73 indicating the molecular mass is 297.1 which is in agreement with the calculated molecular mass 283.34 of the proposed structure.

2-(5-(4-(dimethylamino) phenyl)-4,5-dihydro-1H-pyrazol-3-yl)benzene-1,3-diol

The IR spectrum of the compound 2f shows a peak at 2912 cm\(^{-1}\) which shows C-H stretching. The peak at 1602 cm\(^{-1}\) represents the aromatic compound. The peak at 1410 cm\(^{-1}\) represents C-N vibrations. The value 1322 cm\(^{-1}\) corresponds to N-H stretching. The peak obtained at 1410 cm\(^{-1}\) represents the aromatic compound. The peak at 1043 cm\(^{-1}\) represents C-N vibrations. The value 535 cm\(^{-1}\) is assigned to C-Br bending.

Table 1: Analgesic activity of by tail-flick method

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose</th>
<th>Average (±SE) reaction time (sec)</th>
<th>Number of writhing</th>
<th>% protection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0mins</td>
<td>30mins</td>
<td>60mins</td>
</tr>
<tr>
<td>Control</td>
<td>1% Tween 80</td>
<td>3.89±0.014</td>
<td>4.03±0.0104</td>
<td>4.05±0.011</td>
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<tr>
<td>Standard</td>
<td>analgin</td>
<td>4.78±0.007</td>
<td>5.85±0.009**</td>
<td>7.27±0.585**</td>
</tr>
<tr>
<td>2b</td>
<td>100mg/kg</td>
<td>4.38±0.0103</td>
<td>5.17±0.001**</td>
<td>6.85±0.007</td>
</tr>
<tr>
<td>2c</td>
<td>100mg/kg</td>
<td>3.83±0.011</td>
<td>4.02±0.01</td>
<td>5.17±0.008**</td>
</tr>
<tr>
<td>2e</td>
<td>100mg/kg</td>
<td>3.63±0.012</td>
<td>4.97±0.007**</td>
<td>5.99±0.12**</td>
</tr>
</tbody>
</table>

Table 2: Analgesic activity by acetic-acid induced writhing method

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose</th>
<th>Analgesic activity (mean±SEM)</th>
<th>% protection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number of writhing</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2% CMC</td>
<td>34.83±0.87</td>
<td>---</td>
</tr>
<tr>
<td>Standard</td>
<td>5mg/kg</td>
<td>7.50±0.42</td>
<td>78</td>
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<tr>
<td>2b</td>
<td>50mg/kg</td>
<td>14.1±0±18</td>
<td>60</td>
</tr>
<tr>
<td>2c</td>
<td>50mg/kg</td>
<td>18.1±0.21</td>
<td>48</td>
</tr>
<tr>
<td>2e</td>
<td>50mg/kg</td>
<td>13.50±0.16</td>
<td>62</td>
</tr>
</tbody>
</table>

Data are analyzed by one way ANOVA followed by Dunnet's test. Values are significant at P<0.01**. Activity results: The synthesized compounds 2b, 2c and 2e were screened for analgesic activity using tail-flick method. The results obtained from table-1 shows the compound 2b and 2e has good activity and 2c has moderate activity. The results from table-2, the acetic-acid induced writhing method shows good activity for 2b and 2e and moderate activity for 2c.

Acknowledgement

The authors are thankful to The Chancellor, Vels University, Chennai for providing the infrastructure and facilities. The authors are also thankful to Indian institution of technology for providing spectral data.

References