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Research Article

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

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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

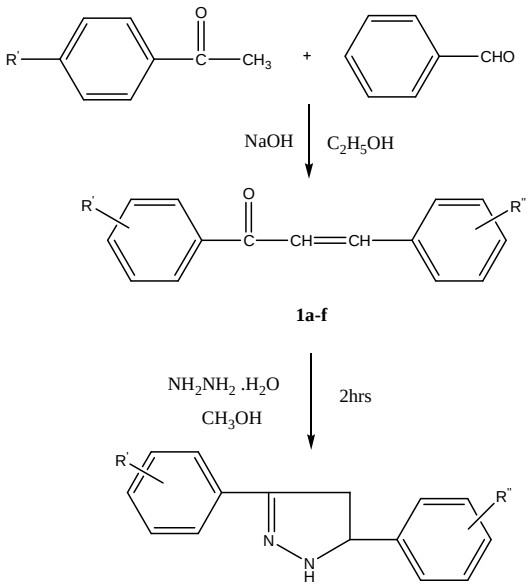
The pyrazoline is a dihydroproduct of pyrazole. It have been reported that the pyrazoline nucleus it exhibits a wide range of biological activities such as antifungal, antibacterial, antidepressant, anticonvulsant, anti-inflammatory, antitumor, antidiabetic, anesthetic, analgesic, ulcerogenic, lipid peroxidation and immunosuppressive properties[1-4]. Several substituted pyrazolines are found as effective bleaching agents, luminescent and fluorescents[5]. It was reported that triaryl pyrazoline inhibits flavivirus RNA replication[6]. Survey of literature in the past reveals that some pyrazoline derivatives also possess cerebroprotective effect[7]. 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 R_f 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. ^{1}H 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 analytical 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.



1a-f

2a-f

Scheme 1: R'=2, 6-dihydroxy, p-Cl, 2-Br, p-F. R"=3-ethoxy-4-hydroxy, 2-OH, p-$(CH_3)_2NH_2$

General procedure for synthesis of compounds (2a-2f)

A mixture of chalcone derivative and hydrazine hydrate was added in methanol and reflux for 2 hrs, excess methanol was distilled and the resulting solution was kept overnight. The solid product was filtered and dried. This is a cyclisation reaction.

2.3a 2-(5-(3-ethoxy-4-hydroxyphenyl)-4, 5-dihydro-1H-pyrazol-3yl) benzene-1, 3-diol:

Yield-75%, m.p-200-215°c,

IR (KBr)- 3092,2970,2930,2878,1927,1630,1600 $cm^{-1.}$ ^1H NMR (DMSO): δ ppm, 7.0 (s,1H $C_3H_6N_2$), 6.9 (s, 1H $C_6H_6O_2$), 6.46-6.57 (T, 3H $C_8H_{10}O_2$), 6.3 (s, 2H $C_6H_6O_2$), 5.0 (s, 2H $C_6H_6O_2$, 1H of OH of $C_8H_{10}O_2$), 3.90 (s, 1H $C_3H_6N_2$), 3.98 (d, 1H OC_2H_5), 2.0 (s, 1H $C_3H_6N_2$), 1.33 (s, 3H OC_2H_5). m/e: (M^+)314.13), (M^++1) 315.13, (M^++2) 316.13

2.3b 2-(5-(2-hydroxyphenol)-4, 5-dihydro-1H-pyrazol-3-yl) benzene-1, 3-diol:

Yield-79%, m.p-150-156°c,

IR(KBr)3341,3079,2893,1782,1676,1655,1609,1579,1550,1521,1351,1100,980,864,850,758cm

^1H NMR (DMSO): δ ppm, 7.0 (s, 1H, NH of $C_3H_6N_2$), 6.91-6.95(d, 2H C_6H_60), 6.9 (s, IH $C_6H_6O_2$), 6.68-6.77 (D, 2H C_6H_6O), 6.3(d, 2H $C_6H_6O_2$), 5.0 (s, 2H $C_6H_6O_2$, 1H OH of C_6H_6O), 3.9 (s, 1H $C_3H_6N_2$), 2.0 (s, 1H, $C_3H_6N_2$). m /e= (M^+) 270.10, (M^++1) 271.10,(M^++2) 272.11

2.3c 4-(3-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)N, Ndimethylbenzenamine

Yield- 72%, m.p- 140-150°c

IR(KBr)3340,3078,2894,2846,2809,2646,2617,2564,1925,1826,1766,1736,1676,1656,1513,1440,1349,1217,824,807,758,730,606 cm^{-1}

^1H NMR (DMSO): δ ppm, 7.3-7.6(d, 4H C_6H_5Cl), 7.0 (D, 1H NH of $C_3H_6N_2$), 6.54-6.94 (d, 4H $C_8H_{11}N$), 3.9(s, 1H $C_3H_6N_2$), 2.85 (s, 6H $(CH_3)_2N$), 2.0 (s, 1H $C_3H_6N_2$). m/e: (M^+) 299.19, (M^++1) 301.116,(M^++2) 300.122

2.3d 2-(3-2-bromophenyl)-4, 5-dihydro-1H-pyrazol-5-yl) phenol:

Yield-82%, m.p- 170-176°c,

IR(KBr):3322,2870,2732,2599,2508,1835,1805,1730,1671,1508,1397,1375,1345,1103,1043,983,897,872,752,602,563,535,524 cm^{-1}

^1H NMR (DMSO): δ ppm, 7.2-7.5 (d, 4H C_6H_5Br), 7.0 (IH of NH, $C_3H_6N_2$), 6.68-6.95 (d, 4H C_6H_6O), 3.9 (T, 1H $C_3H_6N_2$), 2.0 (m, 1H $C_3H_6N_2$). m/e: ($M^{+)}$ 299.19, (M^++1) 301.116, (M^++2) 300.122

2.3e 4-(3-(4-fluorophenyl)-4, 5-dihydro-1H-pyrazol-5-yl)-N, N-dimethylbenzamine:

Yield-74%, m.p-102-110°c,

IR(KBr):3345,3339,3043,2852,1762,1655,1524,1509,1410,1345,1301,1281,1221,1187,1166,1060,1007,965,823,731 cm^{-1}

^1H NMR (DMSO): δ ppm, 7.0-7.6 (d, 4H C_6H_5F), 6.54-6.94 (d, 4H $C_8H_{11}N$), 7.0 (1H of NH $C_3H_6N_2$), 3.9 (d, 1H $C_3H_6N_2$), 2.85 (s, 6H $(CH_3)_2N$, 2.0 (s, IH $C_3H_6N_2$). ($M^{+)}$ 283.14, (M^++1) 284.15, (M^++2) 285.15

2.3f 2-(5-(4-(dimethylamino) phenyl)-4,5-dihydro-1H-pyrazol-3-yl)benzene-1,3-diol:

Yield-85%, 130-139°c,

IR (KBr)- 2912,2803,1602,1551,1365,1124,1063,743,636 cm^{-1}

^1H NMR (DMSO): δ ppm, 6.9 (s, 1H $C_6H_6O_2$), 7.0 (s, IH of NH $C_3H_6N_2$), 6.94 (s, 2H $C_8H_{11}N$), 6.54 (d, 2H $C_8H_{11}N$), 6.3 (d, 2H $C_6H_6O_2$), 5.0 (s, 2H OH of $C_6H_6O_2$), 3.9 (m, 1H $C_3H_6N_2$), 2.85 (s, 6H $(CH_3)_2$), 2.0 (s, 1H $C_3H_6N_2$). m/e: ($M^{+)}$ 297.15, (M^++1) 298.15, (M^++2) 299.15

Analgesic activity

Tail-flick method[9]: Wistar albino mice (35-40g) in the groups of three animals each were selected by random sampling technique. Analgin (25mg/kg) was administered as a standard drug for comparison. The test compounds were administered orally at a dose of 50mg/kg. 1% Tween 80 was used as a vehicle. The reaction time was recorded at 0, 30, 60 and 90 mins respectively. The cut off time of 10mins was considered to avoid the tail damage. The percentage analgesic activity was calculated.

Acetic-acid induced writhing method[10]: 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 and compared. 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-disustituted 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, 2 e were administered orally in doses up to 500 mg/kg by suspending in 1% C.M.C 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 3092cm^{-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 1630cm^{-1}. The peak at 1600 cm^{-1} represents N-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-1,3-diol

The IR spectrum of the compound 2b shows a peak at 3341 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 δ7.53 shows 1H (NH of pyrazoline), δ3.34 shows 4H (pyrazoline), δ2.86 shows 6H, multiplet at δ2.83 shows 1H (CH of pyrazoline), doublet at δ6.7 shows 2H (CH of benzamine).The mass spectrum of the compound 2b shows the M^+ peak at 269.58 indicating the molecular mass is 297.1 which is in agreement with the calculated molecular mass 270.28 of the proposed structure.

4-(3-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)-N,N-dimehtylbenzamine

The IR spectrum of the compound 2c shows a peak at 3340cm^{-1} which shows N-H stretching in the pyrazoline nucleus. The value at 2617 shows the peak that shows O-H stretching. The peak at 1656 cm^{-1} is assigned to alkenes. The peak obtained at 1826 cm^{-1} represents 5-membered ring. The value 824cm^{-1} corresponds to two adjacent hydrogen atoms. The ^1H NMR spectrum of the compound 2c shows the following observations. Singlets at δ7.53 show 1H (NH of pyrazoline), multiplet at δ6.7-6.68 shows 1H (CH of benzene), δ2.86 shows 5H (CH of dimethylamine), doublet at δ7.63 shows 2H (CH of benzenamine), and δ7.43 shows 1H (CH of chlorophenyl). The mass spectrum of the compound 2c shows the M^+ peak at 299.43 indicating the molecular mass is 297.1 which is in agreement with the calculated molecular mass 299.8 of the proposed structure.

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 2599 represent the O-H stretching. The peak at 1508 cm^{-1} represents aromatic ring. The peak at 1375 shows O-H bending. The peak at 1043 cm^{-1} represents C-N vibrations. The value 535 cm^{-1} corresponds to C-Br bending.

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 3043cm^{-1} is assigned to O-H stretching. The peak obtained at 1410 cm^{-1} represents C-N vibrations. The value 1345cm^{-1} corresponds to O-H bending. 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 δ7.41 show 1H (NH of pyrazoline), δ2.86 show 6H (CH of dimethylamine), multiplet at δ7.67 shows 2H (CH of fluorophenyl), δ4.75 show 1H (CH of pyrazoline), δ3.36 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-3yl)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 1602cm^{-1} is assigned to N-H stretching. The peaks at 1551 cm-1 represent O-H stretching. The peak obtained at 1063 represents the aromatic compound. The peak at 743 and 636 shows the halogen may be present in the structure.

Table 1: Analgesic activity of by tail-flick method

Compound	Dose	Average (±SE) reaction time (sec)			
		0mins	30mins	60mins	90mins
Control (1% Tween 80)		3.89±0.014	4.035±0.0104	4.058±0.011	4.008±0.007
Standard (analgin)	100mg/kg	4.78±0.007	5.85±0.009**	7.27±0.585**	8.79±0.007**
2b	100mg/kg	4.38±0.0103*	5.17±0.01**	6.851±0.007**	7.365±0.01**
2c	100mg/kg	3.83±0.011	4.023±0.01**	5.17±0.008**	5.41±0.114**
2e	100mg/kg	3.636±0.012*	4.978±0.007**	5.59±0.012**	6.41±0.008**

Significance levels *p<0.05, **p<0.01 compared with respective control (ANOVA followed by Dunnett's test). Each value represents ±SEM (n=3)

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

Compound	Dose	Analgesic activity (mean±SEM) Number of writhing	% protection
Control	2% CMC	34.83±0.87	---
Standard	5mg/kg	7.50±0.42	78
2b	50mg/kg	14.15±0.018	60
2c	50mg/kg	18.16±0.021	48
2e	50mg/kg	13.50±0.016	62

Data are analyzed by one way ANOVA followed by Dunnett'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.

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