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Research Article

A FACILE SYNTHESIS OF FLAVONE DERIVATIVES USED AS POTENT ANTI-INFLAMMATORY AGENTS

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ABSTRACT

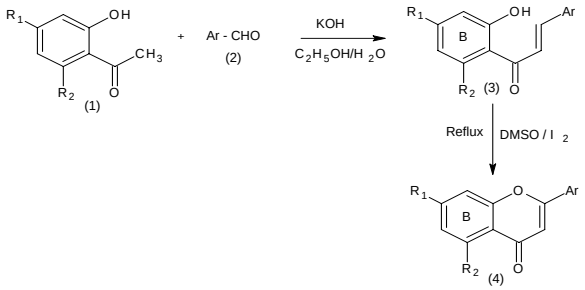
In the present study, a series of flavone derivatives [4a-4h] were synthesized by condensation of acetophenone derivatives and aldehyde derivatives in ethanolic solution of potassium hydroxide followed by cyclization. Several flavones have been synthesized and their biological activity (Anti-inflammatory) investigated using the model of carrageenan induced rat paw edema. The results showed that compound (4)f shows the higher anti-inflammatory activity. The synthesized compounds have been characterized by using Mass, IR and ^{1}H NMR Spectral data together with elemental analysis.

Keywords: Chalcone, Flavone derivatives, Anti- inflammatory activity.

INTRODUCTION

Flavones occupy a special place in the realm of natural and synthetic organic chemistry owing to their useful biological activities such as anti-oxidant [1-4], Anxiolytic [5], anti cancer [6], analgesic [7-8], and anti microbial [9]. During the fast few years various methods have been reported for the synthesis of flavones [10-15]. Recently flavones are synthesized by using EAN ionic liquids [16] under microwave irradiation and BF_4 ionic liquid at 100^{o}C temperature [17]. Drugs having pyrone ring system are well known for their anti-inflammatory activity [18, 19]. In view of above facts, it was contemplated to design and synthesis of some flavone (contains pyrone ring) derivatives from the corresponding chalcone by using DMSO/I_2 as an oxidizing agent, the synthesized compounds were evaluated for *in vivo* anti-inflammatory activity using against carrageenan induced rat paw edema method and the results were compared with standard drug (Diclofenac sodium). The structure of flavone derivatives were confirmed by melting point, TLC and spectral data.

Scheme 1:



MATERIALS AND METHODS

All the chemicals were obtained from commercial sources and used without further purification. Melting point was measured in open capillaries on a perfit electro thermal melting point apparatus and was uncorrected. ^{1}H NMR spectral was recorded at room temperature on a 300 MHz Varian Inova spectrometer in $CDCl_3$ using TMS as internal standard. The reactions were monitored by TLC using pre coated plates (Merck). Column chromatography was performed using Acme silica gel (60-120 mesh). All solvents used in chromatography were distilled before use.

General procedure for synthesis of flavone derivatives

A mixture of acetophenone derivatives (1) and aldehyde derivatives (2) in ethanolic solution of KOH (5%, 15mL) was kept at room temperature about 84 hours. The reaction mixture was diluted with ice cold water, acidified with cold dilute HCl and extracted with ether. The ether layer was washed with water, dried over anhydrous Na_2SO_4 and the solvent was evaporated. The reaction mixture was purified by preparative TLC over silica gel using n-hexane and acetone (7:1) as developing solvent and light orange crystals were obtained.

A mixture of chalcone derivatives (3) and DMSO/I_2 as a catalyst was kept in reflux. The completion of reaction was monitored by TLC; the mixture was cooled, diluted with ethyl acetate and filtered. The filtrate was washed with dilute sodium thiosulphate to remove I_2 and subsequently washed with water. After evaporation of ethyl acetate, the crude mixture was purified by column chromatography using hexane: ethyl acetate (9:1) eluent to afforded corresponding flavones. The products obtained were characterized by melting point, ^{1}H NMR, IR and Mass Spectroscopy.

Spectral data

2-(4-chlorophenyl)-4*H*-chromen-4-one (4a):

Molecular formula: $C_{15}H_9ClO_2$, Yield: 72%, M.P:187-188^{o}C, IR (KBr): 3103 (Ar-CH), 1643 (C=C), 1621 (>C=O), 1136 (-C-O-C), 758 (-C-Cl) cm^{-1}; ^{1}H NMR (DMSO-d_6,300 MHz): δ 6.79 (s, 1H, pyrone ring), 7.05 (dd, 2H, J=2.6 and 7.6 Hz, Ar-CH), 7.34 (m, 4H, Ar-CH), 7.54 (dd, 2H, J=7.6and 2.6 Hz, Ar-CH); **Mass** (LCMS): *m/z* 257 (M$^+$+H).

2-(4-chlorophenyl)-5, 7-dihydroxy-4*H*-chromen-4-one (4b):

Molecular formula: $C_{15}H_9ClO_4$, Yield: 66%, M.P:263-265 ^{o}C, IR (KBr): 3431 (-OH), 3198 (Ar-CH), 1704 (C=C), 1667 (>C=O), 1226 (-C-O-C), 787 (-C-Cl) cm^{-1}; ^{1}H NMR (DMSO-d_6,300 MHz): δ 6.67 (s, 1H, pyrone ring), 7.09 (s, 1H, Ar-CH), 7.54 (m, 4H, Ar-CH), 7.67 (s, 1H, Ar-CH), 11.77 (s, 1H, OH), 13.75 (S, 1H, OH); Mass (LCMS): *m/z* 289 (M$^+$+H).

2-(4-methylphenyl)-4*H*-chromen-4-one (4c):

Molecular formula: $C_{16}H_{12}O_2$, Yield: 68%, M.P:78-80 ^{o}C, IR (KBr): 3234 (Ar-CH), 2923 (-CH_3),1687 (C=C), 1587 (>C=O), 1213 (-C-O-C) cm^{-1}; ^{1}H NMR (DMSO-d_6,300 MHz): δ 2.41 (S, 3H, -CH_3), 6.61 (s, 1H, pyrone ring), 6.95 (dd, 2H, J=2.6 and 7.6 Hz, Ar-CH), 7.17 (m, 4H, Ar-CH), 7.43 (dd, 2H, J=7.6and 2.6 Hz, Ar-CH); Mass (LCMS): *m/z* 237 (M$^+$+H).

5, 7-dihydroxy-2-(4-methylphenyl)-4*H*-chromen-4-one (4d):

Molecular formula: $C_{16}H_{12}O_4$, Yield: 62%, M.P:163-165 ^{o}C, IR (KBr): 3296 (-OH), 3210 (Ar-CH), 2734 (-CH_3),1737 (C=C), 1546 (>C=O), 1276 (-C-O-C) cm^{-1}; ^{1}H NMR (DMSO-d_6,300 MHz): δ 2.64 (S, 3H, -CH_3), 6.67 (s, 1H, pyrone ring), 7.17 (s, 1H, Ar-CH), 7.27 (m, 4H, Ar-CH), 7.52 (s, 1H, Ar-CH), 12.17 (s, 1H, OH), 12.75 (S, 1H, OH); Mass (LCMS): *m/z* 269 (M$^+$+H).

2-(4-nitrophenyl)-4*H*-chromen-4-one (4e):

Molecular formula: $C_{15}H_9NO_4$, Yield: 52%, M.P:276-278 °C, IR (KBr): 3313 (Ar-CH), 1593 (C=C), 1576 (>C=O), 1472 (-NO_2),1098 (-C-O-C) cm^{-1}; ^1H NMR (DMSO-d_6,300 MHz): δ 6.25 (s, 1H, pyrone ring), 7.23 (dd, 2H, J=2.6 and 7.6 Hz, Ar-CH), 7.67 (m, 4H, Ar-CH), 7.94 (dd, 2H, J=7.6and 2.6 Hz, Ar-CH); Mass (LCMS): *m/z* 268 (M^++H).

5,7-dihydroxy-2-(4-nitrophenyl)-4*H*-chromen-4-one (4f):

Molecular formula: $C_{15}H_9NO_6$, Yield: 48%, M.P:285-287 °C, IR (KBr): 3410 (-OH), 3283 (Ar-CH), 1603 (C=C), 1591 (>C=O), 1532 (-NO_2),1286 (-C-O-C) cm^{-1}; ^1H NMR (DMSO-d_6,300 MHz): δ 6.17 (s, 1H, pyrone ring), 6.29 (s, 1H, Ar-CH), 6.94 (m, 4H, Ar-CH), 7.17 (s, 1H, Ar-CH), 12.41 (s, 1H, OH); Mass (LCMS): *m/z* 300 (M^++H).

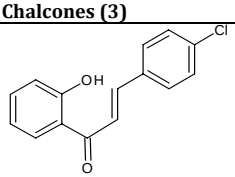
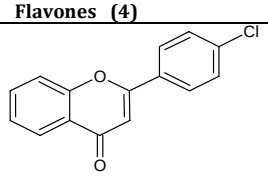
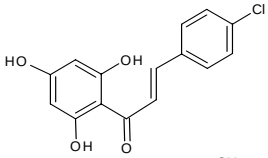
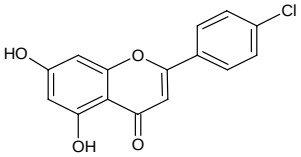
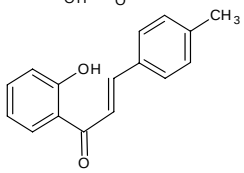
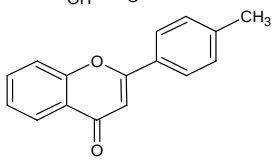
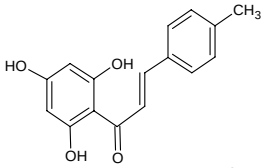
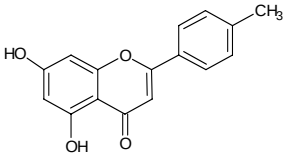
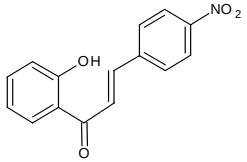
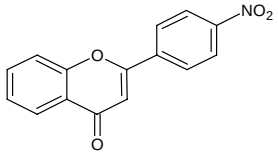
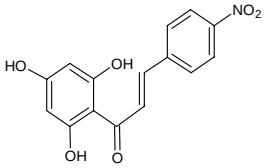
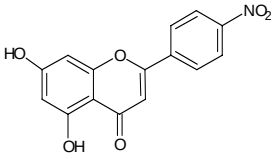
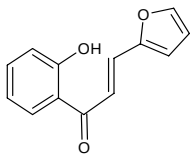
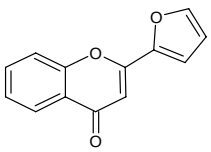
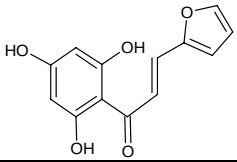
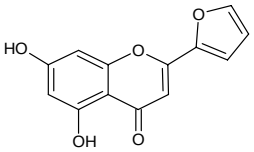
2-(furan-2-yl)-4H-chromen-4-one (4g):

Molecular formula: $C_{13}H_8O_3$, Yield: 64%, M.P:130-132 °C, IR (KBr): 3260 (Ar-CH), 1731 (C=C), 1683 (>C=O), 1086 (-C-O-C) cm^{-1}; ^1H NMR (DMSO-d_6,300 MHz): δ 6.79 (s, 1H, pyrone ring), 7.05 (dd, 2H, J=2.6 and 7.6 Hz, Ar-CH), 7.15(dd, 2H, Ar-CH), 7.47 (m, 1H, Ar-CH), 7.84 (dd, 2H, J=7.6and 2.6 Hz, Ar-CH); Mass (LCMS): m/z 213 (M^++H).

2-(furan-2-yl)-5, 7-dihydroxy-4H-chromen-4-one (4h):

Molecular formula: $C_{13}H_8O_5$, Yield: 58%, M.P:210-212 °C, IR (KBr): 3513 (-OH), 3253 (Ar-CH), 1687 (C=C), 1561 (>C=O), 1101 (-C-O-C) cm^{-1}; ^1H NMR (DMSO-d_6,300 MHz): δ 6.07 (s, 1H, pyrone ring), 7.22 (s, 1H, Ar-CH), 7.42 (dd, 2H, Ar-CH), 7.81 (m, 1H, Ar-CH), 7.97 (s, 1H, Ar-CH), 11.91 (s, 1H, OH), 12.75 (S, 1H, OH); Mass (LCMS): m/z 245 (M^++H).

Table 1: Structure, formula and physical constants of compounds 4a-h

SNo.	R_1	R_2	Ar	Chalcones (3)	Flavones (4)	Yield (%)[a]	M.p (°c)
A	H	H	4-CL			72	187-188
B	OH	OH	4-CL			66	263-265
C	H	H	4-CH_3			68	78-80
D	OH	OH	4-CH_3			62	163-165
E	H	H	4-NO_2			52	276-278
F	OH	OH	4-NO_2			48	285-287
G	H	H	FURAN			64	130-132
H	OH	OH	FURAN			58	210-212

[a] Isolated and unoptimized yields.

Anti-inflammatory activity

Carrageenan induced rat paw edema method [20]

Male albino rats weighing in between 100-200g were housed individually and provided with adequate food and water. They were divided into 10 groups. These animals were used for anti-inflammatory activity. The toxicity studies were performed and no visible toxic symptoms were observed for the first two hours and no death was reported after 24 hours up to the dose 200 mg/kg body weight in a graded manner. The 1/10 th of 200 mg/kg body weight i.e. 20 mg/kg body weight was fixed as the dose for acute anti-inflammatory screening. The animals were divided into 10 groups each six animals. One group served as a standard (Diclofenac sodium 20 mg/Kg body weight) and another group served as control (1% SCMC) and rest of the 8 groups were treated with the test compounds (4a to 4h). Food was withdrawn overnight with adequate water before the experiment.

After one hour of the drug administration to respective groups, a sub plantar injection of 0.05 ml of 1% carrageenan was administered. The volume of the injected paw was measured with plethysmograph immediately. The paw volume was again measured after 3 hours. The average paw volume in treatment group was compared with that of a group with vehicle (control group) and the percentage inhibition of paw edema was calculated by using the formula [21].

$$\% \text{ Inhibition} = 1-(V_t/V_c) \times 100$$

Where V_t and V_c represents edema volumes in test group and control group, respectively. The results are showed in Table 2.

Statistics: The results were expressed as the mean± SEM per group and the data was statistically analyzed by one way analysis of variance (ANOVA) followed by Dunnett's test. $P < 0.05$ was considered as statically significant.

Table 2: Anti-inflammatory screening of flavone derivatives

Compounds	Dose (mg/kg)	Mean paw edema volume ± SEM	% Inhibition ± SEM
Control	5 ml/kg	0.44 ±0.019	-
Diclofenac Sodium	20	0.12 ± 0.008***	72.31± 1.89
4a	20	0.37 ± 0.007**	13.96±1.60
4b	20	0.30 ± 0.010***	30.25 ± 2.46
4c	20	0.39 ± 0.013ns	10.56 ± 2.72
4d	20	0.31 ±0.009***	27.98 ± 2.16
4e	20	0.37 ± 0.007**	15.1 ± 1.62
4f	20	0.24 ± 0.016***	44.28 ± 3.83
4g	20	0.43 ± 0.009ns	3.36 ±1.51
4h	20	0.34 ±0.012***	22.68 ± 2.81

*p<0.05, **p<0.01, ***p<0.001 when compared with control (n=6). ns= Not significant where P>0.05

Values were expressed as Mean ± SEM; one way ANOVA followed by Dunnet's test

RESULTS AND DISCUSSION

Alkaline condensation of acetophenone derivatives with various aromatic aldehydes gave the corresponding chalcone in moderate yield. It was purified by preparative TLC using n-hexane-acetone (7:1) as developing solvent. The UV-Spectrum of prepared chalcone in methanol (252 and 326 nm) suggested a chalcone structure and IR absorption spectrum band at 3421 cm^{-1} indicated that the presence of hydroxyl group. A positive ferric chloride test also indicated that the compound has a free hydroxyl group and a band at 1620 cm^{-1} showed the presence of a conjugated carbonyl group (>C=O). The proton NMR Spectra of chalcone explained the presence of typical two doublets for two proton each respectively of B-ring at δ6.91 (C_3-H and C_5-H; j=2.5 and 7.6 Hz).A characteristic singlet at δ13.18 indicates the presence of chelated phenolic proton at $C_{2'}$-OH integrating for one proton.

Cyclization of chalcone into corresponding flavones was carried out by using DMSO/I_2 as catalyst. It was purified by column chromatography by using hexane: ethyle acetate and light brown needles were obtained. The formation of flavones been supported by spectral data and elemental analysis. The UV- absorption spectrum of the flavones in methanol with the λ_{max} of 260 and 335 nm suggested that the presence of flavone nucleus. The IR absorption at 1647 cm^{-1} showed the presence of carbonyl group (>C=O) and the absence of a hydroxyl group band confirmed the oxidation of chalcone into flavones. ^{1}H NMR also supported the flavones derivatives showed in spectral data section. The synthesized compounds with functional groups such as 4-Cl, 4-NO_2 with hydroxyl groups were found to have moderate anti-inflammatory activity. Compounds (4)c, (4)g exhibits insignificant activity, where as compound (4)f was found to posses better activity with 44.28% inhibition with respect to control group.

Summary

The yield of all flavone derivatives were found to be in the range of 48-72%. The purity of compounds was ascertained by melting point and TLC. The assigned structure was further established by ^{1}H NMR and Mass spectral studies. The *in vivo* anti-inflammatory activity of the synthesized compounds was screened using carrageenan induced paw edema method in rats. The synthesized compounds (4)a, (4)e exhibits less activity, compounds (4)b, (4)d, (4)h showed moderate activity, when compared with the standard drug diclofenac sodium, but the compound (4)f exhibited the highest anti-inflammatory activity with a percentage inhibition of 44.28%.

CONCLUSION

In conclusion, the synthesized flavone derivatives can be potentially useful for anti-inflammatory agents that can prompt future researchers to synthesis a series of flavone derivatives contains wide variety of substituents with the aim of obtain novel heterocyclic compounds with enhanced activity.

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