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Anti-inflammatory and antibacterial activity of *E*-3-arylidene flavanones synthesized by one-pot method

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Abstract

The objective of the present study was to synthesize *E*-3-arylidene flavanones by one-pot method and screen them for anti-inflammatory and antibacterial activity. A set of four *E*-3-arylidene flavanones were synthesized by simple base-catalyzed condensation of appropriate aryl aldehydes and 2'-hydroxy acetophenone. Screening for the anti-inflammatory activity was done by carrageenan-induced paw edema method. Test for antibacterial activity was carried out by cup-plate method. Due to structural similarity with natural flavanones, all the synthesized compounds were expected to exhibit some activity, however only three of the compounds exhibited anti-inflammatory and antibacterial action.

***Keywords*:** *E*-3-Arylidene flavanone; One-pot method; Anti-inflammatory activity; Antibacterial activity.

Introduction

Flavonoids are a group of polyphenolic compounds which are widely distributed throughout the plant kingdom (Kuhnau *et al.*, 1976). Flavonoids can be classified into flavanols, flavones, flavanones and dihydroflavanols (Hyun *et al*., 2002). 3-Arylidene flavanones are also known as flavindogenides. The basic structure of *E*-3-arylidene flavanones has four rings - A, B, C and D. In addition to the basic structure of flavanone, *E*-3-arylidene flavanones have an extended conjugation at C-3 with carbonyl group. The special feature of long conjugation with the keto groups of flavanone moiety is expected to impart very significant biological activity on this type of compounds.

Krishnamurthy *et al.* (1989) suggested that *E*-3-arylidene flavanones and their heterocyclic analogues have poor solubility in aqueous medium starting either from 2-hydroxy chalcone or from *o*-hydroxy acetophenone. The reactivity of condensing aldehyde is an important factor in the synthesis. Presence of alcohol enhances the formation of products. This fact has been utilized for alkali-catalyzed synthesis of many arylidene flavanones reported by them. The special feature of long conjugation with 4-keto group of flavanone moiety is expected to impart very significant biological activity on this type of compounds. Seiket *et al*. (1962) and Shah *et al*. (1964) reported the formation of 3-arylidene flavanone in alkaline medium. Chawla *et al*. (1987) reported the synthesis of

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seven 3-arylidene flavanones by condensing 2-hydroxy acetophenones with aromatic aldehydes in aqueous alkaline medium, along with corresponding chalcones. According to these authors arylidene flavanones were accessible only by a low-yielding circuitous route. Krishnamurthy *et al*. (1989) concluded that 3-arylidene flavanones are obtained by acid catalyzed condensation between flavanone and aryl halide. Their first representatives were synthesized by Katshalowsky and Kostanecky (1904). For a long time, *E*-3-arylidene flavanones (E-34) were synthesized solely by the acid catalyzed condensation of flavanones (Lornad *et al*., 1996), and aromatic aldehydes (Ryan *et al*., 1929; Algar *et al*., 1929; Diesbach *et al*., 1945; Szell *et al*., 1968; Reichel *et al*., 1966; Reichel *et al*., 1968; Dhara *et al*., 1997). The reaction usually has been performed in alcoholic solution saturated with anhydrous hydrochloric acid at various temperatures and for different time. Levai *et al*. (1978) introduced a very simple base-catalyzed condensation for the synthesis of *E*-3-arylidene flavanones. A mixture of equimolar amounts of flavanones and aromatic aldehyde and a few drops of piperidine was allowed to react at 150°C and *E*-3-arylidene flavanone was obtained in good yield without any purification. On the basis of ^{1}H NMR spectra Keane *et al*. (1970) explained the stereochemistry of synthetic *E*- and *Z*-3-arylidene flavanones. So here an attempt is made to synthesize a few *E*-3-arylidene flavanones by one-pot method and to screen the synthesized compounds for the anti-inflammatory and antibacterial activity.

Experimental

Chemicals: For the synthesis of proposed compounds, 2'-Hydroxyacetophenone was purchased from Sigma Aldrich Chemical Co. Inc., U.S.A. Benzaldehyde, furfuraldehyde, *p*-chlorobenzaldehyde and anisaldehyde were purchased from S.D fine chemicals, Mumbai. Carrageenan was obtained from Sigma Aldrich Chemical Company Inc., U.S.A.

Instruments: Beckman 650 iu UV Spectrophotometer; Shimadzu 8300 FTIR Spectrometer; Varian Gemini - 200 MHz NMR Spectrometer.

Synthetic protocol: The proposed compounds were synthesized as per the procedure by Dhara *et al*. (1996) using one-pot method. To a mixture of 2'-Hydroxy acetophenone (1 mM) and aromatic aldehyde (2.5 mM) a warm (45^{o}C) aqueous alcoholic solution of potassium hydroxide (15%) was added and stirred to get a uniform solution. The solution was allowed to stand for four days in a stoppered condition. Methanol was added dropwise to remove turbidity formed on cooling. The separated material was washed with cold aqueous alcohol (50% methanol) then crystallized from aqueous alcohol. (Each compound was synthesized in the same manner).

Anti-inflammatory activity: To study the anti-inflammatory properties of the synthesized compounds, Wistar male albino rats weighing between 150-220g were selected and carrageenan-induced paw edema model applied. The rats were divided into six groups having six animals in each group. Four groups received, orally, synthesized compounds at a dose of 100 mg/kg in a suspension of 0.5% sodium carboxymethyl cellulose. To one group, standard drug, ibuprofen, made in 0.5% sodium carboxymethyl cellulose was administered in the same manner. The remaining group was given 0.5% sodium carboxymethyl cellulose and treated as control. Edema was induced after one hour of the compounds being administered, by injecting 0.05 ml 1% carrageenan suspension subcutaneously into the subplantar tissue of the right hind foot of rats. Paw volume was measured immediately and after 3 hrs of the carrageenan injection.

Antibacterial activity: The media used in present study, nutrient agar and nutrient broth, were prepared according to Indian Pharmacopoeia .The pH of the solution was adjusted to 6.5-6.6 by using 1M sodium hydroxide and 1M hydrochloric acid. It was then sterilized for 30 minutes at 15 lbs pressure. 10mg of each test compound was dissolved in 10ml of DMF (dimethyl formamide) in serially and suitably labeled sterile test tubes, thus giving a final concentration of 100µg/0.1ml. Using sterile pipettes the standard and the sample solutions (0.1ml) of known concentrations were fed into the bored cups. Cup-1: Standard (ciprofloxacin). Cup-2: solvent control (DMF). Cup-3: Test compound.

Results

As per the synthetic protocol four *E*-3-arylidene flavanones have been synthesized and screened for their anti-inflammatory activity by carrageenan-induced paw edema method. The results are reported as mean ± S.D. The data obtained was analysed by unpaired Student's 't' test. Level of significance was fixed at $p<0.05$. The results of anti-inflammatory activity and antibacterial activity are shown below.

Spectroscopic data of active compounds

Out of four compounds synthesized three were found to have anti-inflammatory activity. Their physical data are given below. Ethanol was used as solvent in U.V spectroscopy. KBr pellets were used to measure I.R. spectra and $CDCl_3$ used for ^1H NMR spectra.

Compound A:

UV: λ -max -244 nm,

IR (KBr): 1650.77 cm^{-1} (C=O); 1286.29 and 1195.65 cm^{-1} (C-0-C); 3110.54 and 3305.39 cm^{-1} (C-H), 836.95 and 782.958 cm^{-1} (C-H def), 1596.77 and 1382.71 cm^{-1} (C=C).

^1H NMR ($CDCl_3$ ppm): 6.65-6.68 (H-2), 6.9-7 (H-6, H-8), 7.25 ($CHCl_3$), 7.9-8 (H-3), 7.2-7.4 (H).

Compound B:

UV: λ -max – 232 nm,

IR (KBr): 1608.41 cm^{-1}(C=O), 1272.79 and 1151.9 cm^{-1} (C-O-C), 2967 cm^{-1} (C-H), 763.673 and 833.098 cm^{-1} (C-H def), 1575.56 and 1533.49 and 1413.57 cm^{-1} (C=C).

^1H NMR ($CDCl_3$ ppm) 6.1-6.2 (H-3', H-4'), 6.5-6.55 (H-4"), 6.92-6.953 (H-9-8 (H-5) 8), 7-7.07 (H-6), 7-7.1(H-2), 7.25 $CHCl_3$, 7.4-7.5 (H-7), 7.5-7.6 (H-5"), 7.9-8 (H-5)

Compound C:

UV: λ-max-240 nm.

IR (KBr): 1737.53 cm^{-1} (C=O), 1248.65 and 1160.94 cm^{-1} (C-O-C), 2927.41 cm^{-1} (C-H), 914.093 cm^{-1} ($C-H_{def}$), 767.53 cm^{-1} (Cl), 1610.27 and 1419.35cm^{-1} (C=C).

^1H NMR ($CDCl_3$ ppm) 6.5-6.56 (H-2), 6.9-7 (H-6, H-8), 7.9-7.96 (H-5), 8.02-8.05 (H-B).

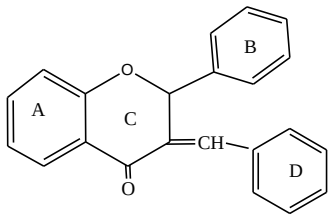

Fig. 1: General structure of *E*-3-arylidene flavanone

Table: 1 Anti-inflammatory activity

Compound I.D	Edema volume [a] (ml ± S.D)	% Edema inhibition [b]
A	0.10 ± 0.005	34.47%
B	0.12 ± 0.007	41.36%
C	0.14 ± 0.008	48.25 %
D	0.82 ± 0.48	NA
Std.	0.17 ± 0.009	58.6 %

Edema volume for control (Sodium Carboxy Methyl Cellulose alone) = 0.442 ± 0.039
a=Edema volume expressed as mean (±S.D) of a group.
b= % Edema inhibition calculated by comparing each edema volume with control.

Table: 2 Antibacterial activity.

	Zone of inhibition (mm)			
Compound I.D	*E. coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Bacillus subtilis*
A	18	-	-	16
B	14	-	-	15
C	20	-	12	15
Std.(Ciprofloxacin)	28	26	24	32

Discussion

Four *E*-3-arylidene flavanones have been synthesized by one-pot method which reduces the tedious multi-steps involved in the synthesis of medicinal compounds. The results of the assessment of synthesized compounds have good agreement with the data given in literature. Due to structural similarity with those of natural flavanones, all the synthesized compounds were expected to exhibit anti-inflammatory activity. From the studies, three were found to exhibit anti-inflammatory and antibacterial actions. The results show lower anti-inflammatory activity than the standard drug ibuprofen. Among the four compounds, compound C showed maximum anti-inflammatory activity. Compound D did not show anti-inflammatory activity. Among the three compounds that exhibited anti-inflammatory activity, compound C was most active, probably due to the presence of an electronegative atom like chlorine.

In antibacterial studies all the compounds exhibited activity against *E. coli* and *Bacillus subtilis.* Compound B showed the least activity. Compound C also showed good activity against *Staphylococcus aureus.* None of the compounds was active against *Pseudomonas aeruginosa.*

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