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ONE POT METHOD FOR THE SYNTHESIS OF ARYLIDENE FLAVANONES AND SOME OF ITS ACTIVITIES

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OBJECTIVE:

To synthesize E-3-arylidene flavanones by one pot method and screen their analgesic , anti-oxidant and antibacterial activities.

Method : A set of three E-3Arylidene flavanones were synthesized by simple base catalysed condensation of appropriate aryl aldehydes and 2'-hydroxy 4-methoxy acetophenone.

Analgesic activity was screened by hot plate method , anti-oxidant activity by spectrophotometric method and antibacterial activity by cup-plate method.

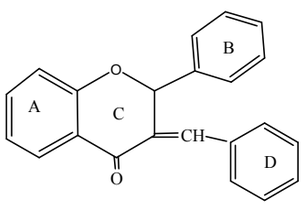
Results: A set of three E-3Arylidene flavanones were synthesized. Two were found to exhibit reliable degree of analgesic activity ,all produced anti-oxidant action and antibacterial activity .

Conclusion: Due to structural similarity with those of natural flavanones, all the synthesized compounds were expected to exhibit analgesic activity, but only two were found to exhibit analgesic action. But all showed a reliable degree of anti-oxidant activity .In antibacterial activity studies,all were active against E.coli .

Key Words: E-3Arylidene flavanone , 2'-hydroxy 4-methoxy acetophenone , One Pot Method, Analgesic activity , Anti-oxidant activity, Natural flavanone.

INTRODUCTION

Flavonoids are a group of polyphenolic compounds which are widely distributed through out the plant kingdom (1). Flavonoids can be classified into flavonols, flavones, flavanones and dihydroflavonols (2,3). Arylidene flavanones are also known as flavindogenides. Basic structure of E-3 Arylidene flavanones has four rings: Ring A, Ring B, Ring C and Ring D.



In addition to basic structure of flavanone, E-3 - arylidene flavanone 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 or this type of cmpounds.

Krishna murthy (4) suggests 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 have been utilized for alkali catalyzed synthesis of many arylidene flavanones been reported by them. The special feature of long conjugation with 4-keto group of flavanone moiety is expected to impart very significant biological activity of this type of compounds.

Seiket *et al* (2) and Sha *et al* (3) reported the formation of 3-arylidene flavanone in alkaline medium. Chawla et al[06] reported the synthesis of

seven 3- arylidene flavanone 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. Knishnamurthy *et al* (3) 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 in 1904 (07). For a long time, E-3 arylidene flavanones (E-34) were synthesized solely by the acid catalyzed condensation of flavanones (08) and aromatic aldehydes (09-013. The reaction usually has been performed in alcoholic solution saturated with anhydrous hydrochloric acid at various temperatures and for different time. Albert levai et al[16] introduced a very simple base-catalyzed condensation for the synthesis of E3-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}HNMR spectra Keane *et al* (17) explained the stereochemistry of synthetic E and Z- 3- arylidene flavanones.

So here is an attempt made to synthesise a few E-3 -arylidene flavanones by one pot method and to screen the synthesized compounds for the analgesic, anti-oxidant and antibacterial activities .

MATERIALS & METHO DS

For the synthesis of proposed compounds, 2'-hydroxy,4-methoxy acetophenone have been purchased from Sigma Aldrich chemical company Inc. U.S.A, Furfuraldehyde, P-Chloro benzal-dehyde, and p-methoxy benzaldehyde have been purchased from S.D fine chemicals, Mumbai.

INSTRUMENTS USED

U.V : Beckman 650 iu Spectrophotometer

I.R : Shimadzu – FTIR 8300

^{1H} NMR: Varian Gemini-200 MHz

SYNTHETIC PROTOCOL:

The proposed cmpounds were synthesized as per the following procedure;[18] One pot method: To a mixture of 2'-hydroxy,4-methoxy acetophenone (1 mM) and aromatic aldehyde (2.5 mM),a warm (45^{0}c) aqueous alcoholic solution of potassium hydroxide(15%) added and stirred the solution to get a uniform solution. The solution stand for four days in a stoppered condition. Methanol added dropwise to remove turbidity formed on cooling. The separated material washed with cold aqueous alcohol(50 % methanol). Then crystallized from aqueous alcohol. Each compound have been synthesized in the same manner.

Biological Experimental Protocol for Analgesic activity:

To study the analgesic activities of the synthesized compounds, albino mice of either sex were used. All mice were screened by exposure to thermal stimulus. Mice weighing between 20-25 g selected and made into six groups having six animals in each group. The first group served as control which received 2% gum acacia suspension. Second group served as standard,which received diclofenac sodium orally at a dose of 200mg/Kg body weight of animal (suspension of test compounds(20mg/ml)were prepared in 2% gum acacia). Animals were placed on perspax cylinder on heated surface and the time to exhibit discomfort reaction(licking paws or jumping) was considered as reaction time with the cut off time being 60 seconds. The first reading was taken immediately after administration of compounds and afterwards at the intervals of 30 minutes. The results were recorded.

EXPERIMENTAL PROTOCOL FOR ANTI-OXIDANT ACTIVITY:

Equimixture of 1,1-diphenyl-2-picryhydrazyl(3.9 mg in 10 ml ethanol) and test compounds (10 mg /10 ml ethanol) mixed and kept for 20 minutes at room temperature. Then absorbance measured at 517 n.m. Curcumin used as standard drug to compare the activity.

EXPERIMENTAL PROTOCOL FOR ANTI-BACTERIAL 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. Then it was sterilized for 30 minutes at 15lbs 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. As Cup-1: Standard (ciprofloxacin).Cup-2: solvent control (DMF).Cup3-: Test compound.

ASSESMENT OF SYNTHESIZED COMPOUNDS

Physical datas tested compounds are as follows. Ethanol had used as solvent to find out λ -max by U.V spectroscopy. KBr pellets used to measure I.R spectrum and $CDCl_3$ used for ^{1} HNMR spectrum .

Compond A:

λ-max- 244 n.m,350 n.m.

I.R(KBr): 1673.8c.m^{-1} (C=O), 1217.8 and 1189.6 c.m^{-1} (C-O-C), 854.1 and 812.2 c.m^{-1} (C-H def), 1474.8 and 1459.4 c.m^{-1} (C=C)

^{1}HMR ($CDCl_3$ ppm): 7.04-7.11(H-2), 6.19-6.22 (H-3', H-4'), 6.44-6.52 (H-6), 6.53-6.61 (H-4"), 7.24 ($CHCl_3$), 7.35-7.4 (H-5'), 7.52-7.59 (H-5"), 7.9-7.93 (H-5), 3.8-3.9 (OCH3-proton), 7.62-7.64 (H-β).

Compound B:

λ-max- 230 n.m,360 n.m.

I.R(KBr): 1668.12c.m^{-1} (C=O), 1361.5 and 1249.65c.m^{-1} (C-O-C), 943.02 & 844.669 c.m^{-1} (C-H def), 1637.27, 1523.49 & 1454.06(C=C)

^{1}HMR ($CDCl_3$ ppm): 6.59-6.625(H-2), 6.82-7.0 (H-6, H-8, H-3', H-5'), 7.2-7.43(H-7, H-2', H-6', H-2", H-6"), 3.7-3.9 (OCH_3 proton), 9.1-7.95 (H-5), 3.99 (OCH_3), 8.02-8.12 (H-β).

Compound C:

λ-max-258 n.m, 290 n.m.

I.R(KBr):1668.2c.m $^{-1}$(C0=O), 1250.66 and1146.4c.m $^{-1}$(C-O-C), 854.4 and 824.2 c.m $^{-1}$(C-H def), 748.2 c.m $^{-1}$(monochloro), 1604.2and 1510.4 and1472 and 1458.6c.m $^{-1}$(C=C) .

^{1}HMR($CDCl_3$ppm):6.58-6.63(H-2),6.8-7(H-6,H-8,H-3',H-5',3",H-5"),7.2-7.42(H-7,H- 2',H-6',H-2',H-6"), 3.98 (OCH_3), 8.04-8.1(H- β).

RESULTS

As per the synthetic protocol three E-3 Arylidene flavanones have been synthesized and screened their analgesic activity by hot plate method. Anti-oxidant activity tested by spectrophotometric method.Antibacterial activity by cup-plate method. Observations for analgesic activity are shown in table1 , that of anti-oxidant activity in table 2 and antibacterial activity in table-3.

Table:1 Analgesic activity of synthesized compounds.

Compound I.D	Dose(orally) Mg/Kg	Average reaction time in seconds [a] 0	30	60	90
A	200	3.05	3.10	3.10	3.10
B	200	2.50	2.6	2.6	2.55
C	200	3.00	4.00	6.00	6.08
Std.	200	3.00	5.25	8.25	8.28
Control	-----	3.00	3.00	3.00	3.00

a=Average reaction time expressed as mean (±S.D)of a group.

Table2:Anti-oxidant activity.

Compound I.D	**Absorbance at 517 n.m**	**Relative % activity considering that of standard as 100%**
Curcumin(Std.)	2.596	100%
A	2.142	82.5%
B	2.012	77.5%
C	2.482	95.6%

Table:3 Antibacterial activity.

Compound I.D	Zone of inhibition in m.m E.Coli	Pseudomonas aerugenosa	Staphylococcus aureus	Bacillus subtilis
A	12	-	9	-
B	23	20	-	20
C	18	-	16	24
Std.(Ciprofloxacin)	28	26	24	32

DISCUSSIONS

Three E-3 Arylidene flavanones have been synthesized by one pot method which reduces the usual tedious multisteps involved in the synthesis of medicinal compounds. The results of the assessment of synthesized compounds have good agreement with the datas given in the literature. Due to structural similarity with those of natural flavanones, all the synthesized compounds were expected to exhibit analgesic activity, as per the studies two were found to exhibit analgesic action. The results shows less analgesic activity for all tested compounds than the standard drug namely Diclofenac sodium. Among the three compounds compound C showed maximum analgesic activity. Compound B showed least analgesic activity. Among the three compounds exhibited analgesic activity ,the compound C showed more activity than others, probably due to the presence of halogen atom.

Considering anti-oxidant activity, compound C showed maximum activity and compound B showed least activity. More anti-oxidant activity of compound-C may be due to the presence of chlorine. In anti-bacterial studies all compounds exhibit activity against E.Coli. Only compound B showed activity against Pseudomonas.Compound C showed good activity against Staphylococcus aureus. Compound B as well as compound C showed good activity against Bacillus subtilis.

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