IJPSR (2012), Vol. 3, Issue 02 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 04 October, 2011; received in revised form 21 January, 2012; accepted 29 January, 2012

DESIGN, SYNTHESIS AND CYTOTOXIC ACTIVITY OF SOME NOVEL FLAVONES

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Keywords:
Flavones,
Cytotoxicity,
Acyl chloride,
HeLa cells

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ABSTRACT

A series of flavones **(5-15)** were synthesized from resorcinol via Baker-Venkataraman transformation, characterized and evaluated for their cytotoxicity against human cervical cell line (HeLa) by MTT assay method. The result of preliminary MTT cytotoxicity studies shown that the compounds **5**, **9** and **10** were found to be most potent against HeLa cells; IC_{50} values were of 0.22, 0.88 and 1.26 µM respectively.

INTRODUCTION: A large number of biological effects have been attributed to these flavonoids, including anticancer [1-3], antifungal [4-5], antiviral [6-7], anti-inflammatory [8], antioxidant [8-9], anti-osteoporotic [10-11] and anti-carcinogenic activities [12]. In this regard, a number of flavone derivatives have been synthesized and antitumor mechanisms have been investigated[13-14]. Many lines of evidence indicated that flavonoid compounds are able to inhibit proliferation of HeLa cells [15-16].

With this evidence, in this study we synthesized a series of new flavones derivatives with different substituents on the flavones structure and studied the structural activity relationship.

All the compounds were evaluated for *In-vitro* cytotoxicity activity in cervical cancer cell line (HeLa) by MTT assay method. The results indicated that all the tested compounds have displayed noticeable cytotoxicity activity.

MATERIALS AND METHODS:

Chemistry: All the chemicals were obtained from commercial sources and used without further purification. Melting points were determined by the open capillary tube with electro thermal melting point apparatus and are uncorrected. The completion of the reaction and purity of the compounds were monitored by TLC. Infrared (IR) spectra were recorded for the compounds on JASCO 4100 FT-IR using KBr pellet disc technique. NMR spectra were recorded on a Bruker Advance spectrometer. The elemental analysis was performed using Perkin-Elmer model 240 C analyzer.

General procedure:

1. **General procedure for the preparation of compound 2:** The starting material 1 was synthesized according to the literature report [17]. To resacetophenone (11.4 g, 0.25 M) in 15 mL of pyridine and 12 mL (0.35M) of benzoyl chloride was added and stirred the contents in

magnetic stirrer for 20 minutes. After this reaction mixture was poured with stirring into 360 mL of cold 1M hydrochloric acid. Product formed was filtered with suction and washed with 15 mL of ice cold methanol and 15 mL of water.

2. **Preparation of 1- (2, 4- dihydroxyphenyl)- 3-phenylpropane 1, 3dione (Compound 3):** 10.8 g of 2-acetyl 5-hydroxy phenyl benzoate **2** was dissolved in 36 mL of pyridine and heated the solution to 50^{o}C. 3.4g of pulverized KOH (china dish heated at 100^{o}C then KOH was added and crushed with mortar) was added to the above solution with stirring and stirring was continued for 15 min. After cooling to room temperature and acidified it with acetic acid (50 mL of 10% aqueous acetic acid), pale yellow product was collected by suction filtration.

3. **Preparation of 7-hydroxy flavones (Compound 4):** 2.7 g of compound 3 was dissolved in 15 mL of glacial acetic acid. 0.6 mL of Conc. Sulphuric acid was added with shaking to the above reaction mixture and refluxed on a boiling water bath with intermittent shaking for 1 hour. The resulting solution was then poured with stirring in to about 150 g cursed ice, and allowed the ice to melt, filtered the creamy light brown 7-hydroxy flavone **4** separated, washed it with cold water.

4. **General procedure for the preparation of compounds (5-15):** To a cooled solution (0^{0}C) of 7-hydroxy flavones (0.207 M, 2.48 g) in 7 mL of pyridine, substituted acyl chlorides (0.311 M) were added and the reaction mixture stirred at 0^{0}C for one to two hours. The reaction was stopped by diluting the reaction mixture with 50 mL of ice cold 5% dil. hydrochloric acid and after brief stirring the solution was extracted with ethyl acetate; the organic phase was washed with sodium hydroxide, dried over anhydrous sodium sulphate. Final crude product was recrystallized with rectified spirit.

Pharmacology:

MTT Assay Method: The human cervical cancer cell line (**HeLa**), was obtained from National Centre for Cell Science (NCCS), Pune. The HeLa was grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS).

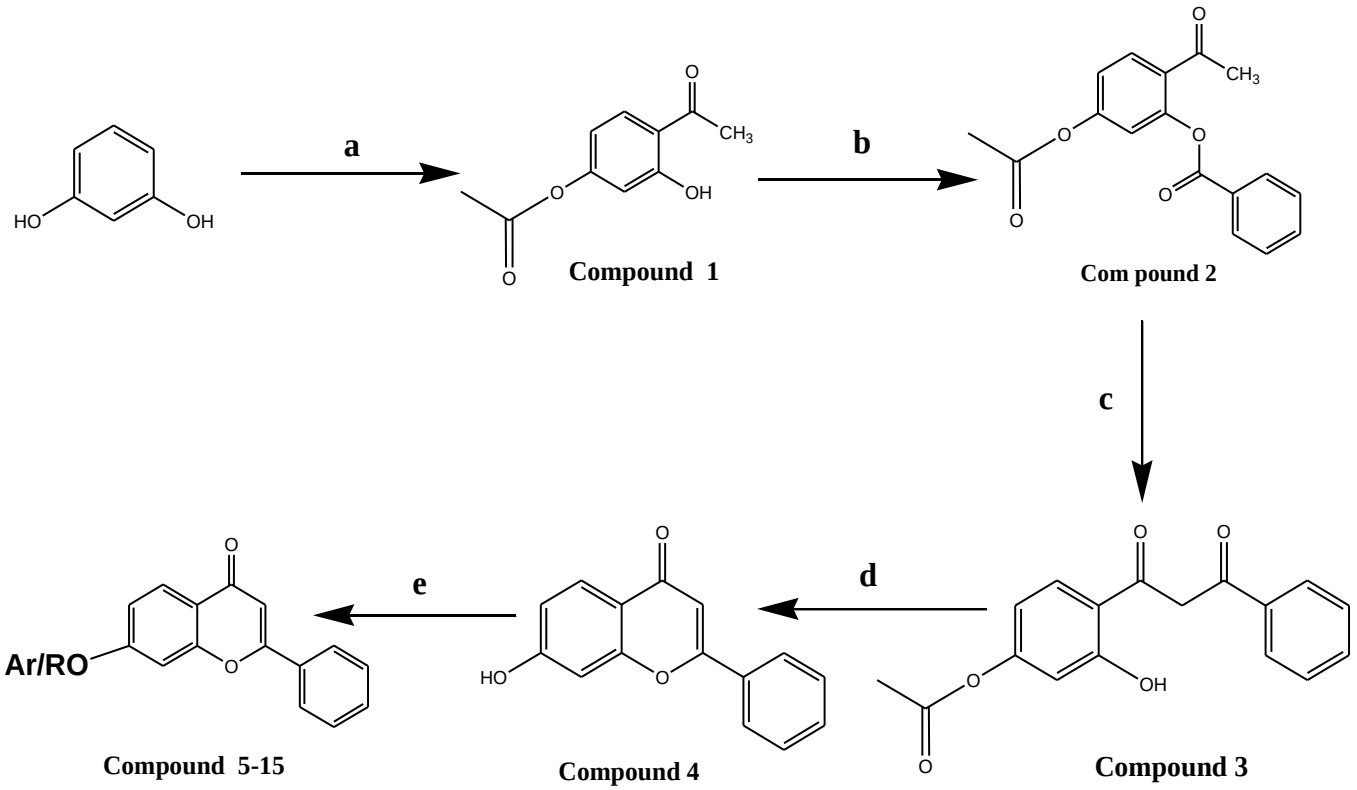
For screening experiment, the cells were seeded into 96-well plates in 100 µL of respective medium containing 10% FBS, at plating density of 10,000 cells/well and incubated at 37^{o}C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of test compounds. The compounds were solubilised in DMSO and diluted with respective serum free medium. After 24 h, 100 µl of the medium containing the compounds at various concentration (e.g., 6.25, 12.5, 25, 50 µg/mL etc) was added and incubated at 37^{o}C, 5% CO_2, 95% air and 100% relative humidity for 48h. Triplicate was maintained and the medium containing without test compounds were served as control.

After 48h, 15µl of MTT (5mg/mL) in phosphate buffered saline (PBS) was added to each well and incubated at 37^{o}C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilised in 100 µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula and graph was plotted between percentage Cell inhibition and concentration and from this IC_{50} was calculated by using graph pad prism software.

Percentage Cell Inhibition = 100 - Abs (drug)/Abs (control) x100.

RESULTS AND DISCUSSION:

Chemistry: Compounds **5-15** were synthesized using the **scheme 1**. The structures of compounds were confirmed by IR, NMR and Mass spectral data's. Resacetophenone (**Compound 1)** was synthesised by adding the resorcinol to the heating mixture of glacial acetic acid and zinc chloride kept at 120^{o}C, then temperature was raised 142^{o}C for 20 minutes. The flavones **4** was prepared from the resacetophenone **(1)** by the most common method of synthesizing flavones is known as the Baker-Venkataraman transformation. 11 aliphatic/ aryl acyl derivatives of flavones **(5-15, Scheme-1**) were prepared from corresponding acyl chlorides through shown in Scheme-I.



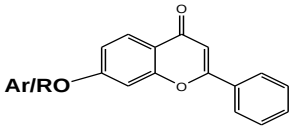
SCHEME 1: Reagents and condition (a) $ZnCl_2$ (1.2 mol), acetic acid (15.8 ml), heated upto142^oC, (b) Benzoyl chloride (0.35 mol) stirred, 20 min, (c) KOH (0.0 6mol), stirred, 15 min, (d) H_2SO_4 (0.6ml), Refluxed on water bath, 1 hr, (e) RCOCl/ ArCOCl (2.5 gm), ice bath 0^oC , stirred for 2 hrs

The **compound 1** was stirred with benzoyl chloride in pyridine afforded the 2-acetyl-5-hydroxy phenyl benzoate **2. Compound 2** was heated to 50^oC with pyridine, to which pulverised KOH was added and stirred for 20 minutes at room temperature and neutralised with acetic acid to yield 1-(2, 4 dihydroxy phenyl)-3-phenyl propane-1, 3-dione **(3)** which was further treated with acetic acid and few drops of Conc. sulphuric acid and refluxed for 1.5 hrs with occasional stirring to form 7- hydroxyl Flavones **(4).** Finally 7-hydroxyl flavones, treated with various acyl chlorides in pyridine so that 7-OH group is esterified (stirred at 0-5^oC for 1-2hrs) to corresponding acyl derivatives (**5-15)** as a final compound of first series.

Physical and spectral data of synthesized compounds (5-15) are given in **table 1 and 2**. All the structures of newly synthesized compounds were assigned on the basis of their spectroscopic data, IR, ^1H NMR and Mass. The lead compound 4 showed the absorption band at 3126 due to the presence of –OH at 7 position of flavonoid ring where as in ester derivatives **5-15** this absorption bands were absent in this region instead the absorption bands were appeared at 1749 by C=O, this slightly higher than that the normal ester C=O stretch 1740 because of the phenyl conjugation with alcohol oxygen. Peak at 1238 also indicated the presence of C-O stretch of (C (=O)-O). Compounds **10** and **11** were also shown the peaks in this region along with additional band at 1518-1462 cm^{-1} due to C=N aryl stretch. The ^1H NMR spectrum, compounds **9** and **14** showed the broad singlet signals at 3.308 ppm, which were assigned to the N–H protons of amino group. Phenolic -OH proton of compound **13** and **14** exhibited singlets at10.584 ppm. Compound **6** ethenyl proton adjacent to C=O (=CH (C=O) exhibited singlet at 12.286 ppm. Compound **15,** $-CH_3$ showed the singlet at 1.24 ppm.

The obtained molecular ion m/z values of the final flavonoid derivatives were consistent with the molecular weight of the compounds and base peak due to cleavage of $[C_8H_7]^{+\bullet}$ at m/z= 103 and another intense peaks at m/z 146 due to fragmentation from base peak with relative intensity of 58 %.

TABLE: 1 PHYSICAL AND SPECTRAL DATA OF SYNTHESIZED COMPOUNDS (5-15)



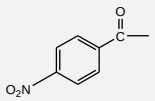
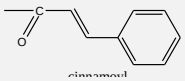
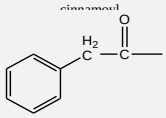
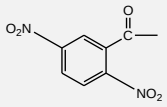
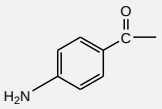
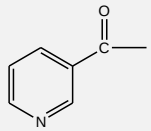
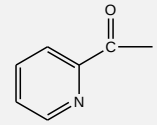
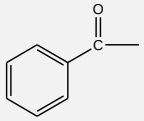
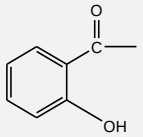
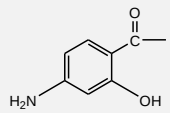
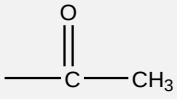
Comp. code	Structure (Ar/R)	MP range (^{0}C)	Mol. Wt	%Yield	(IR) υ_{max} (KBr/cm^{-1})	Molecular ion peak (m/z)
5	 4-nitrobenzoyl	160-165	387.34	75	1744 (C=O) 1053 (C-O-C) 1238 (COO) 1316 (C-N) 876 (di substituted aryl) 1519 (Ar-NO_2)	388.37 (M+1)
6	 cinnamoyl	86-96	368.38	90	1743 (C=O) 1052 (C-O-C) 1236 (COO)	370.69 (M+1)
7	 2-phenylacetyl	105-110	356.37	78	1745 (C=O) 1052 (C-O-C) 1238 (COO) 3123 (aryl C-H stretch)	357.45(M+1)
8	 2,5 dinitro benzoyl	145-150	432.34	62	1743 (C=O) 1052 (C-O-C) 1241 (COO) 910 (tri substituted aryl)	433.69 (M+1)
9	 4-aminobenzoyl	115-120	357.36	91	1744 (C=O) 1052 (C-O-C) 1238 (COO) 910 (di substituted aryl) 2880 (N-H Stretch) 1648 (Ar-NH_2)	358.39 (M+1)
10	 nicotinoyl	120-125	343.33	80	1743 (C=O) 1053 (C-O-C) 1250 (COO) 1314 (C-N stretch)	344.59 (M+1)
11	 isonicotinyl	85-90	343.33	60	1741 (C=O) 1052 (C-O-C) 1238 (COO) 1368 (C-N aryl)	344.45 (M+1)
12	 benzoyl	90-95	342.34	62	1686 (C=O) 1058 (C-O-C) 1178 (COO) 706 (mono Substituted aryl)	343.89 (M+1)
13	 2-hydroxybenzoyl	80-85	358.08	65	1742 (C=O) 1053 (C-O-C) 1241 (COO) 3114 (- OH Stretch), 849 (di substituted aryl)	359.11 (M+1)
14	 4-amino-2-hydroxybenzoyl	85-90	374.35	52	1742 (C=O) 1064 (C-O-C) 1166 (COO) 3130 (-OH Stretch) 1240 (-OH bend), 877 (tri substituted aryl)	375.67 (M+1)
15		90-95	280.27	55	1744 (C=O) 1051 (C-O-C) 1143 (COO) 1020 (-CH_3)	281.77 (M+1)

TABLE 2: ^{1}H NMR DATA OF COMPOUNDS 5-15

Compound	^{1}H NMR (300 MHz, $CDCl_3$): δ
5	8.56 (s,1H), 8.03-8.19 (m,1H), 7.73-7.8 (m,1H),7.55-7.66(m,1H),7.48-7.5(d,2H, J= 15Hz),7.080 (s,2H), 6.99-7.0(d,2H, J= 13Hz), 6.84-6.933 (m,1H).
6	12.39(s, 1H), 8.10-8.19(m, 2H), 7.87(m, 2H), 7.55-7.68(m, 2H), 7.39-7.41(m, 1H), 7.08 (s, 1H), 6.49-6.53 (m, 5H).
7	8.33(s,1H), 8.0-8.09(m,1H), 7.63-7.70(m,1H), 7.25-7.36 (m,1H), 7.18-7.20(d,2H,J= 9Hz), 7.98(s,2H), 6.59-7.0(d,2H,J= 8Hz), 6.44-6.53(m,1H).
8	8.99(s,1H), 8.53-8.59(m,1H), 7.89-7.93(m,1H), 7.76-7.82(m,1H), 7.68-7.72(d,2H,J= 9Hz), 7.46(s,2H), 6.99-7.32(d,2H,J= 9Hz), 6.91-6.98(m,1H).
9	3.3(s, 2H), 7.87 -7.91(m, 3H), 7.71-7.75(d, 1H, J= 7Hz), 7.88-7.98(d, 2H, J= 8Hz), 6.51-6.60(d, 2H, J= 7Hz), 6.23-6.23(d, 2H, J= 8Hz).
10	8.18(s,1H), 8.03-8.19(m,1H), 7.13-7.20(m,1H), 7.25-7.36(m,1H), 7.08-7.10(d,2H,J= 7Hz), 7.08(s,2H), 6.51-6.60(d,2H,J= 9Hz), 6.84-6.93(m,1H).
11	8.184(s, 1H), 8.03-8.19(m, 1H), 7.13-7.20 (m, 1H), 7.25-7.36(m, 1H), 7.25-7.300 (d, 2H, J= 8 Hz), 7.08(s, 2H), 6.51-6.60(d, 2H), 6.84-6.93(m, 1H).
12	8.33(s, 1H), 7.90-7.95(s, 1H), 7.63-7.70(m, 1H), 7.25-7.36(m, 1H), 7.18-7.20(d, 2H, J= 7Hz), 7.98(s, 2H), 6.59-7.00 (d, 2H), 6.44-6.53(m, 1H).
13	8.77(s, 1H) 7.92-7.98(s, 1H), 7.63-7.78(m, 1H), 7.25-7.36 (m, 1H), 7.88-7.99(d, 2H, J= 8Hz), 7.97(s, 2H) 6.58-7.00(d, 2H, J= 7Hz), 6.72-6.73(m, 1H).
14	3.30(s, 2H), 7.87 -7.91(m, 3H), 7.21-7.23(d, 1H, J= 7Hz), 7.88-7.98, (d, 2H, J= 7Hz), 6.33-6.38 (d, 2H, J= 7Hz), 6.23(d, 2H, J= 8Hz).
15	8.67-8.69 (m, 3H), 3.23-3.26(s, 3H), 7.08(s, 2H), 6.99(d, 2H, J= 7Hz).

In-vitro **cytotoxicity:** The synthesized flavone derivatives **5-15**, were evaluated for anti-proliferative activities against human cancer cell line HeLa by screening by MTT assay. The results were summarized in **Table 3**. All the compounds showed anti-proliferative activities against the Hela cell line with the half maximal inhibitory concentration (IC_{50}) values of 0.22–1.81 µM. Structure–activity relationship (SAR) analysis indicated that compounds bearing p-nitrobenzoyl, p-aminobenzoyl and nicotinoyl at 7th position of flavone ring played an important role for the activity of the compounds **5, 9** and **10 with** the IC_{50} values of 0.22, 0.88 and 1.26 µM respectively. It is clear from the results that the anti cancer potential of compounds associated with the nature substituent at the 7th position of flavones ring.

TABLE 3: CYTOTOXIC ACTIVITY OF COMPOUNDS 5-15

Compounds	5	6	7	8	9	10	11	12	13	14	15
Inhibition % (3µM)	96.60	88.90	90.32	91.26	96.60	96.18	97.22	93.68	94.86	97.28	90.40
IC_{50}	0.51	0.22	0.21	0.26	0.58	0.78	0.25	0.23	0.22	0.28	0.21

In future there is wide scope of modifications are possible for flavone , chemical modification and SAR studies can lead to more potent and highly active compounds.

ACKNOWLEDGEMENT: The authors are thankful to Chairman Dr. Nalla G. Palanisamy & Dr. Thavamani D. Palanisamy, Kovai Medical Centre Research and Educational trust, Coimbatore, for providing facilities and laboratories.

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