

Synthetic flavonols and flavones: A future perspective as anticancer agents

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Abstract: A series of flavonoid derivatives, flavones (F1-F3) and flavonols (OF1-OF3) were synthesized. Their structures were confirmed through various spectroscopic techniques and elemental analysis. These were then tested for cytotoxic activity against mouse fibroblast (NIH 3T3), human endothelial cervical (HeLa) and breast (MCF7) cell lines *in vitro* by MTT assay. The flavonol series showed prominent potentials than the flavones. The compound OF2 in flavonols exhibited greater potentials MCF7 cell with IC₅₀ value of 0.96μM and OF3 has 1.04μM. In contrast, the OF3 exhibited higher activity against HeLa cell with IC₅₀ value of 0.51μM and OF2 has 1.06μM. The compounds OF2 and OF3 exhibited activity against mouse fibroblast (NIH 3T3) cell with IC₅₀ values 2.48 and 1.24μM. The OF1 was found to be moderate to inactive against all cells. Cytotoxic screening of the tested flavones, F1 to F3 were also active against all cells but the activity was less in comparison to flavonol series of compounds suggestion the possible involvement of hydroxyl (OH) at position 3 in case of flavonols. These results indicated a cheering scaffold that may lead to innovation of potent anti-breast cancer activity.

Keywords: Flavonoid derivatives, flavones, flavonols, synthesis, anticancer activity.

INTRODUCTION

Cancer is among the major health problem in many parts of the world (Rebecca *et al.*, 2015). In 2008, almost 12.7 million case reports and 7.6 million of deaths due to cancer have been reported throughout the (Ahmedin *et al.*, 2011). In women; breast and cervical cancer is one of the most widespread cancers among women worldwide. It is most widespread and its incidence in Pakistan is reported highest among South-Central Asian countries (Mohammad *et al.*, 2016; Yamin *et al.*, 2012). Currently, surgery, chemotherapy and radiotherapy, which are the main and the major therapies, are still unavailable to the populations of many third world and underdeveloped countries (De-Shen *et al.*, 2014).

Several anticancer agents of natural origin or synthetic nature are commercially available and several are under clinical trials (Arun *et al.*, 2013). Apart from natural anticancer agents the synthetic anticancer agents has shown to posses significant anticancer potentials (Puneet *et al.*, 2013; Lin *et al.*, 2005; Gurdal *et al.*, 2015).

Flavonoids belong to polyphenolic class of compounds and are reported to help in prevention of chronic diseases such as cardiovascular (CVS) and cancer (Ling *et al.*, 2010). Baicalein, a flavonoid from *Scutellaria baicalensis* root, extensively used in Chinese herbal system of medicine and traditionally, used for the treatment of hepatitis, jaundice, respiratory tract infection, cancer and

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diarrhea (Chidambara *et al.*, 2012). Polymethoxyflavones (PMFs) are distinctive flavonoids that exist utterly in citrus peels and possess a broad range of biological potentials like anti-inflammatory, anti-tumor and anti-carcinogenic. The natural flavonoid fisetin, found in vegetables/ fruits, possess anticancer properties in several types of tumors and found to reveal a strong anticancer property in caspase-3-deficient MCF7 cells (Pei-Ming *et al.*, 2012). It is reported that six flavonoids (pinocembrine, chrysin, scutellarin, cynaroside, apigenin-7-O-glucoside and apigenin) of *Scutellaria immaculata* and *S. ramosissima* (Lamiaceae) caused the increased inhibition of HepG-2, HeLa and MCF-7 cells (Nilufar *et al.*, 2011).

Considering the existing treatment strategies of cancer, chemotherapy has been the most preferred choice for management and curing cancer (Harrison *et al.*, 2009). Paclitaxel and Docitaxel, derived from natural products, are widely used for the treatment of different types of cancers due to enhanced clinical outcomes (Carlson, 2008).

Search for anticancer drugs having greater therapeutic efficacy and minimum side effects especially against immune system has got much scientific attention. Owing to the hazardous effects associated with currently used anticancer drugs and continuation of our work on biologically active heterocycles, we report the synthesis of some flavonoid derivatives and their screening for anticancer activity against mouse fibroblast (NIH 3T3),

human endothelial cervical (HeLa) and breast cancer (MCF-7) cell lines by MTT assay.

MATERIALS AND METHODS

Chemicals and equipments

Ketone and benzaldehyde derivatives were of Sigma Aldrich Chemical Company. TLC plates were of Merck 60 F₂₅₄, Darmstadt Germany. Solvents and chemicals like ethanol, n-hexane, ethyl acetate used were of extra pure analytical grade were purchased from E. Merck.

¹H and ¹³C NMR spectra were recorded in deuterated chloroform (CDCl₃) on Bruker SF spectrometers operating at 300 and 75 megahertz (MHz) frequencies respectively. Chemical shifts values are expressed in δ (ppm) downfield relative to TMS which was used as an internal standard. Infrared (IR) spectra were recorded on Thermo scientific USA (Nicolet 6700), Infrared spectrometer on KBr disk method. All melting points are uncorrected and were taken in open capillary tubes using Electrothermal 9100 apparatus (Barnstead UK). Reaction extents and final products purities were checked on TLC plates (Merck 60 F₂₅₄, Darmstadt Germany) and spots were visualized under UV Lamp (180-365 nm) and with subsequent staining with iodine vapours.

Synthesis and characterization of flavone derivatives

General procedure. To an ethanolic solution of 2-hydroxyacetophenone (5 mmol), sodium hydroxide (10 mL, 40% ethanolic) was added dropwise at room temperature. Then corresponding benzaldehyde derivatives (5mmol) were added drop wise to this mixture and stirred for 18 to 24 hours at room temperature. TLC was used was for monitoring the reaction and after reaction completion, the contents was poured into the crushed ice and neutralized with 1N HCl solution resulting in yellow precipitates of corresponding 2'-hydroxychalcones (C1-C3).The chalcones were filtered, washed with water to remove the impurities and dried.

In the next step, the respective 2'-hydroxychalcones were cyclized to flavone derivatives in 15mL DMSO in the presence of iodine (375mg) at 140°C for 1 hour separately. Upon completion of reactions, the mixtures were cooled at room temperature and poured into water followed by ethyl acetate extraction (25mL × 3), treated with sodium thiosulfate solution (20%) and brine solution, and dried over sodium sulfate. The final products (mixture of flavone and chalcone) were subjected to column chromatography using n-hexane: ethyl acetate (9 : 1) to purify flavones derivatives (fig. 1) (Mohammad *et al.*, 2015).

Synthesis and characterization of 3-hydroxyflavone derivatives

The 2'-hydroxychalcones (C1-C3) was suspended in ethanol, and addition of NaOH (5M, 2 eq) and H₂O₂

(30%, 2.2 eq) was carried out at 4°C. The mixture was stirred at room temperature for 3-6 hours, afterwards acidified with HCl (2M) and poured into water (250 ml). Upon filtration, the precipitate was collected and recrystallized from methanol for obtaining pure product (Robert *et al.*, 2012).

2-phenyl-4H-chromen-4-one (F1)

Creamy white solid (R_f 0.58, 68%). Mp: 96-98°C; ¹H NMR (300 MHz, Chloroform-*d*) δ 8.22 (dd, *J*=8.0, 1.7 Hz, 1H), 7.75-7.53 (m, 5H), 7.50-7.37 (m, 4H), 6.85 (s, 1H). ¹³C NMR (75 MHz, CDCl₃) δ 178.48, 163.42, 156.26, 133.79, 131.77, 131.61, 129.04, 126.66, 125.71, 125.24, 123.95, 118.09, 107.58 IR (KBr), *v*, cm⁻¹, 1635.4, 1463.3, 1372.4, 766.0. Found, %: C 81.07; H 4.54. C₁₅H₁₀O₂. Calculated, %: C 81.19; H 4.60 (Donghee *et al.*, 2011; Jie *et al.*, 2011).

2-(*p*-Tolyl)-4H-chromen-4-one (F2)

Yellowish white solid (R_f 0.72, 75%). Mp: 103-107°C; ¹H NMR (300 MHz, Chloroform-*d*) δ 8.52 (dd, 2H), 7.94 (d, 2H), 7.37 (d, 2H), 7.27 (d, 2H), 6.85 (s, 1H), 2.47 (s, 3H). ¹³C NMR (75 MHz, CDCl₃) δ 178.1, 162.7, 156.2, 142.5, 134.1, 129.6, 128.4, 126.5, 125.6, 125.2, 123.5, 118.2, 106.5, 104.1, 21.5. IR (KBr) *v*, cm⁻¹, 1640, 1465, 817 (Donghee *et al.*, 2011; Jie *et al.*, 2011).

2-(4-Chlorophenyl)-4H-chromen-4-one (F3)

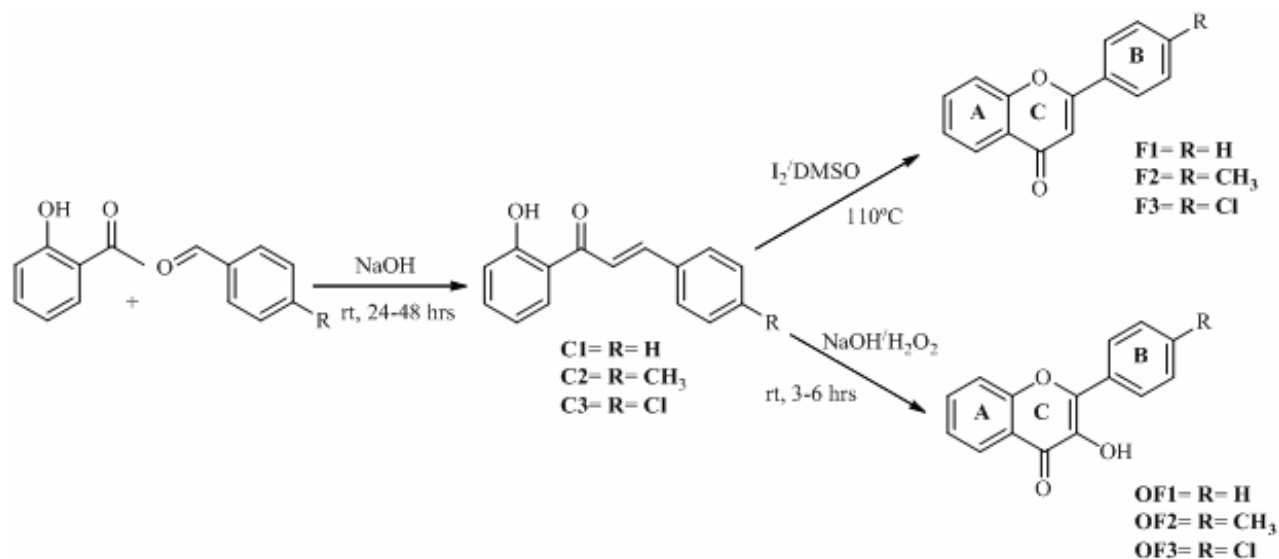
White crystals (R_f 0.69, 84%). Mp: 178-181°C; ¹H NMR (300 MHz, Chloroform-*d*) δ 8.24 (dd, 1H), 7.83 (d, 2H), 7.72 (td, 1H), 7.53 (d, 1H), 7.47 (d, 2H), 7.40 (t, 1H), 6.75 (s, 1H). ¹³C NMR (75 MHz, CDCl₃) δ 178.2, 162.1, 156.1, 137.8, 133.9, 130.2, 129.3, 127.5, 125.7, 125.3, 123.9, 118.0, 107.6. IR (KBr) *v*, cm⁻¹, 1662, 1374, 1092, 827, 754. Anal. calcd for C₁₅H₉ClO₂: C 70.19, H 3.53, found: C, 70.38, H, 3.30 (Donghee *et al.*, 2011; Jie *et al.*, 2011; Shankar *et al.*, 1983; Zhu *et al.*, 2009).

3-Hydroxy-2-phenyl-4H-chromen-4(1H)-one (OF1)

White crystals (R_f 0.63, 71%). Mp: 163-167°C; ¹H NMR (300 MHz, Chloroform-*d*) δ 8.24 (d, 2H), 8.14 (1H), 7.83-7.81 (m, 1H), 7.79 (d, 1H), 7.59 (dd, 2H), 7.49-7.54 (m, 2H), 7.51 (br s, 1H, OH). ¹³C NMR (75 MHz, CDCl₃) δ 118.9, 121.8, 125.1, 125.3, 128.1, 129.0, 130.4, 131.8, 134.2, 139.6, 145.7, 155.1, 173.5. Anal. calcd for C₁₅H₁₀O₃: C 75.62, H 4.23%; found: C 75.62, H 4.09% (Andrea *et al.*, 2012; Dajun *et al.*, 2012).

3-Hydroxy-2-(4-Tolylphenyl)-4H-chromen-4(1H)-one (OF2)

Yellow crystals (R_f 0.70, 71%). Mp: 193-197°C; ¹H NMR (300 MHz, Chloroform-*d*) δ 8.16 (d, 2H), 8.14 (1H), 7.79-7.82 (m, 1H), 7.79 (d, 1H), 7.47-7.50 (m, 1H), 7.40 (d, 2H), 7.56 (br s, 1H, OH), 2.41 (s, 3H, CH₃). ¹³C NMR (75 MHz, CDCl₃) 173.4, 155.1, 145.9, 140.3, 139.4, 134.1, 129.6, 129.1, 128.1, 125.3, 125.0, 121.7, 118.9, 21.5. Anal. calcd for C₁₆H₁₂O₃: C 75.37, H 4.86%; found: C 75.38, H 4.47% (Andrea *et al.*, 2012).



Scheme 1: Synthetic route of the title compounds (F1-F3) and (OF1-OF3).

2-(4-Chlorophenyl)-3-hydroxy-4H-chromen-4-one (OF3)

White crystals (R_f 0.68, 74%). Mp: 198-201°C; ^1H NMR (300 MHz, Chloroform-*d*) δ 8.33-8.19 (m, 2H), 7.75 (ddd, 1H), 7.67-7.40 (m, 3H), 7.35-7.05 (m, 2H), 7.44 (br s, 1H, OH). ^{13}C NMR (75 MHz, CDCl_3) δ 173.43, 155.36, 136.20, 133.85, 131.65, 129.56, 129.02, 128.91, 127.66, 125.52, 124.68, 120.56, 118.27. FT-IR (KBr): 3271.65 (OH-Aromatic), 1604.02 (C=O) cm^{-1} . Anal. calcd for $\text{C}_{15}\text{H}_9\text{ClO}_3$: C 66.07, H 3.33%; found: C 66.28, H 3.41% (Dajun *et al.*, 2012; Tatiana *et al.*, 2013).

Anticancer activity

Cytotoxic potentials of the synthesized compounds were investigated following MTT assay using 96-well micro plate reader. Three different cell lines, HeLa, MCF7 and NIH 3T3 were cultured in Minimum Essential Medium Eagle (MEM), supplemented with fetal bovine serum (FBS, 5%) antibiotics (penicillin and streptomycin each 100 IU/ml) in flasks, and incubated at 37°C in humidified CO_2 atmosphere (5%). The cells were harvested, counted with haemocytometer and diluted with medium. Cultures of cell at densities of 6×10^4 , 5×10^4 and 5×10^4 cells/ml for HeLa, MCF7 and NIH 3T3 respectively were prepared, introduced 100 μl to each well into 96-well plates and incubated for 24h. After incubation, old medium was replaced fresh medium (200 μl) containing various concentrations (0.25-5 μM) of test samples. Each well was added MTT solution (200 μl , 0.5mg/ml) after 48h and further incubated for 4 hrs. Next, each well was added DMSO (100 μl). The level of MTT reduction to formazan in the cells was quantified by measuring at 570 nm using a micro plate reader having Soft- Max Pro software (Spectra Max plus, Molecular Devices, CA, USA). Cytotoxicity of the test samples was noted, %growth inhibition and (IC_{50}) was calculated (Robert *et al.*, 2012).

STATISTICAL ANALYSIS

GraphPad Software, version 5.01 (GraphPad Software, San Diego, CA, USA) was used for statistical analysis and data are presented as mean \pm SEM.

RESULTS

Chemistry

The physical parameters (color, appearance, R_f value, yield and mp) of the synthesized flavones and flavonols are given in table 1. The route of the title compounds is given in Scheme 1.

The compound 2-phenyl-4H-chromen-4-one (F1) has already been reported and by comparing our spectroscopic data with the published literature we have strong confirmation regarding its structure. In the ^1H NMR spectrum of flavone F1, the alpha hydrogen appeared as distinct singlet at chemical shift of 6.85. Normally, the alkenes protons are supposed to appear at 4.5 to 6.5 ppm. However, a slight shift to the downfield area in this case is due to the presence of carbonyl group on one side and an aromatic attachment to other side of the alkene moiety. Moreover, we noticed a total of nine (9) protons in different splitting pattern in the aromatic region, i.e. 7.37-8.22 ppm. These nine protons are attributed to a monosubstituted and disubstituted benzene rings on F1. In ^{13}C NMR, a further confirmation of the flavone F1 is the presence of a strong signal at 178.48 ppm which represents the carbonyl carbon. An even more confirmation of carbonyl group in F1 is evident from the IR spectrum which has a strong band at 1635.4 cm^{-1} . The compound F1 has already been reported and by comparing our spectroscopic data with the published literature we have strong confirmation regarding its

Table 1: Physical parameters of the title compounds.

Compounds	R	Yeild	Appearance	R _f	mp (°C)
F1	-H	68%	Creamy white solid	0.58	96-98
F2	-CH ₃	75%	Yellowish white solid	0.72	103-107
F3	-Cl	84%	White crystals	0.69	178-181
OF1	-H	71%	White crystals	0.63	163-167
OF2	-CH ₃	71%	Yellow crystals	0.70	193-197
OF3	-Cl	74%	White crystals	0.68	198-201

Table 2: Percentage inhibition and IC₅₀ values of flavones (F1-F3).

Sample	Conc (μM)	Cell lines					
		3T3		HeLa		MCF7	
		%	IC ₅₀ (μM)	%	IC ₅₀ (μM)	%	IC ₅₀ (μM)
F1	5	39.76±1.10	>5	57.52±2.04	2.92	72.11±1.66	1.31
	2.5	32.78±1.36		47.07±1.37		58.29±2.24	
	1.25	21.23±1.45		31.14±1.40		47.09±2.56	
	1	20.41±1.41		24.71 ±1.21		41.24±1.67	
	0.75	19.86±1.87		22.60 ±1.35		38.11±1.62	
	0.5	18.58±1.50		21.03±2.85		34.18±2.05	
	0.25	9.86±0.97		17.22±2.19		27.06±1.81	
	0.125	7.35±1.12		14.13±2.32		16.88±1.67	
F2	5	62.88±1.50	2.50	72.10±2.74	1.23	70.11±1.81	1.18
	2.5	50.06±1.56		60.41±1.61		58.18±2.09	
	1.25	37.17±0.88		51.91±1.38		54.82±1.88	
	1	32.44±0.71		46.28±2.09		47.07±2.16	
	0.75	26.81±1.05		39.85±1.05		44.24±1.89	
	0.5	26.12±1.45		36.42±1.81		42.35±1.78	
	0.25	24.01±1.68		35.80 ±1.44		40.08±2.06	
	0.125	20.64±1.62		33.95±2.34		39.16±1.64	
F3	5	70.13±2.17	1.30	80.41±2.39	0.71	74.16±2.87	1.12
	2.5	62.06±2.03		71.31±1.86		59.27±1.56	
	1.25	48.61±1.49		62.34±1.45		52.06±2.07	
	1	45.02±2.15		54.07±2.41		48.15±2.25	
	0.75	39.16±1.92		50.61±2.25		40.13±1.35	
	0.5	32.25±1.87		46.16±2.07		36.21±1.71	
	0.25	31.57±1.69		44.29±1.58		34.68±1.58	
	0.125	26.45 ±1.31		36.01±1.62		30.02±1.88	

structure (Donghee *et al.*, 2011, Jie *et al.*, 2011). In the ¹H NMR spectrum of 2-(p-Tolyl)-4H-chromen-4-one (F2), a total of nine (9) protons were observed in the aromatic region (6.85-8.52 ppm) which represents the two disubstituted benzene rings on the flavone moiety. A singlet of integration value of 1H at chemical shift of 6.85 represents the alpha hydrogen. Moreover, the singlet of 3H is observed at 2.47 ppm confirming the presence of methyl group. The compound F2 has already been reported and by comparing our spectroscopic data with the published literature we have strong confirmation regarding its structure (Donghee *et al.*, 2011; Jie *et al.*, 2011). In the ¹H NMR spectrum of 2-(4-chlorophenyl)-4H-chromen-4-one (F3), a total of nine (9) protons were observed in the aromatic region (7.40-8.24 ppm) which represents the two disubstituted benzene rings on the flavone moiety. A singlet of integration value of 1H at

chemical shift of 6.75 represents the alpha hydrogen. The compound F3 has already been reported and by comparing our spectroscopic data with the published literature we have strong confirmation regarding its structure (Donghee *et al.*, 2011; Jie *et al.*, 2011; Shankar *et al.*, 1983; Zhu *et al.*, 2009).

Similar type of integrations is also achieved in case of flavonols. In case of 3-Hydroxy-2-phenyl-4H-chromen-4(1H)-one OF1, we noticed a total of nine (9) protons in different splitting pattern in the aromatic region, i.e. 7.54-8.24 ppm. The hydrogen of OH group at position 3 appeared as distinct singlet at chemical shift of 7.51.

In case of 3-Hydroxy-2-(4-Tolylphenyl)-4H-chromen-4(1H)-one (OF2), we noticed a total of nine (9) protons in different splitting pattern in the aromatic region, i.e. 7.40-

Table 3: Percentage inhibition and IC₅₀ values of flavonols (OF1-OF3).

Sample	Conc (μM)	Cell lines					
		3T3		HeLa		MCF7	
		%	IC ₅₀ (μM)	%	IC ₅₀ (μM)	%	IC ₅₀ (μM)
OF1	5	41.56±1.00	>5	65.59±1.34	2.68	73.06±1.89	1.27
	2.5	35.78±1.30		48.27±1.67		59.71±2.31	
	1.25	26.63±1.39		35.44±1.20		48.59±2.74	
	1	22.44±1.66		28.77±1.00		41.34±1.56	
	0.75	20.86±2.01		24.60±1.50		38.75±1.59	
	0.5	18.45±0.50		21.43±3.20		36.68±1.65	
	0.25	10.56±0.61		17.55±2.89		29.42±1.88	
	0.125	7.73±1.88		16.03±2.66		18.54±1.76	
OF2	5	64.34±1.50	2.48	78.50±2.34	1.06	83.56±1.71	0.96
	2.5	51.66±1.56		64.94±1.76		71.78±2.30	
	1.25	38.77±0.88		55.71±1.78		66.89±1.93	
	1	32.34±0.71		48.08±1.99		50.67±2.33	
	0.75	28.75±1.05		41.55±2.05		46.54±1.56	
	0.5	26.22±1.45		39.42±1.58		44.95±1.68	
	0.25	24.66±1.68		36.60±1.24		43.08±2.01	
	0.125	21.80±1.62		35.75±2.65		41.87±1.74	
OF3	5	71.65±2.67	1.24	88.61±2.79	0.51	79.66±3.05	1.04
	2.5	64.76±2.23		76.81±1.66		61.67±1.45	
	1.25	50.61±1.34		68.94±1.87		57.56±2.47	
	1	46.22±2.11		59.87±2.30		51.35±1.25	
	0.75	40.96±1.72		52.61±1.25		43.93±0.65	
	0.5	33.65±1.69		49.86±1.37		38.81±1.91	
	0.25	31.57±0.69		47.09±0.58		36.52±1.80	
	0.125	26.45±0.71		38.41±0.72		30.69±2.08	

8.16 ppm. The hydrogen of OH group at position 3 appeared as distinct singlet at chemical shift of 7.56. Moreover, the singlet of 3H is observed at 2.41 ppm confirming the presence of methyl group.

In case of 2-(4-Chlorophenyl)-3-hydroxy-4H-chromen-4-one (OF3), we noticed a total of nine (9) protons in different splitting pattern in the aromatic region, i.e. 7.05-8.33 ppm. The hydrogen of OH group at position 3. The synthesized flavonols OF1 to appeared as distinct singlet at chemical shift of 7.44. OF3 has already been reported and by comparing our spectroscopic data with the published literature we have strong confirmation regarding its structure (Andrea *et al.*, 2012; Dajun *et al.*, 2012; Tatiana *et al.*, 2013).

Anticancer activity

All the synthesized flavonoid derivatives were tested for *in-vitro* cytotoxic potentials against mouse fibroblast (NIH 3T3), human endothelial cervical (HeLa) and breast cancer (MCF7) cell lines *in-vitro* by MTT assay. The cytotoxic screening of the tested flavones, F1 to F3 against all cells lines are given in table 2.

By comparing the activity among flavones, it was found that halogen containing flavone (F3) was found to be the

most active against all tested cell lines having IC₅₀ values of 1.30μM, 0.71μM and 1.12μM respectively. Moreover the methyl containing flavone (F2) was found to be the second active molecule against all tested cell lines having IC₅₀ values of 2.50μM, 1.23μM and 1.18μM respectively. While the simple flavone (F1) was found to be moderate to inactive against cell lines with IC₅₀ values of >5μM, 2.92μM and 1.31μM respectively for mouse fibroblast (NIH 3T3), human endothelial cervical (HeLa) and breast cancer (MCF7) cell lines. These results suggest that the introduction of chlorine and methyl group to the flavones may increase the anticancer activities.

The flavonol series of compounds exhibited higher activity than the flavones. The cytotoxic screening of the tested flavonols, OF1 to OF3 against all cells lines are given in table 3. By comparing the activity among flavonols, it was found that halogen containing flavonol (OF3) was found to be the most active against cell lines having IC₅₀ values of 1.24μM and 0.51μM for mouse fibroblast (NIH 3T3), human endothelial cervical (HeLa) respectively and 1.04 μM for breast cancer (MCF7) cells. Moreover the methyl containing flavonol (OF2) was found to be active against mouse fibroblast (NIH 3T3), human endothelial cervical cancer (HeLa) having IC₅₀ values of 2.48μM and 1.06μM respectively and 0.96μM

for breast cancer (MCF7) cells. It's worth mentioning that OF2 showed promising results (0.96 μ M) against MCF7 cells in comparison to OF3 (1.04 μ M). In contrast, the OF3 exhibited higher activity against HeLa cell having IC₅₀ value of 0.51 μ M and OF2 has 1.06 μ M. While the simple flavonol (OF1) was found to be moderate to inactive against cell lines having IC₅₀ values of >5 μ M, 2.68 μ M and 1.27 μ M respectively for mouse fibroblast (NIH 3T3), human endothelial cervical cancer (HeLa) and breast cancer (MCF7) cell lines.

The cytotoxic screening of the tested flavones, F1 to F3 were also active against all cell lines tested but the activity was less in comparison to flavonol series of compounds suggesting the possible involvement of hydroxyl (OH) group at position 3. These results offered an encouraging framework that may lead to the discovery of activity specific flavonols against specific cancer cell lines with potent anticancer activity.

DISCUSSION

In West, the prime cause of death is breast cancer in women and prostate cancer in men. In Japan, China and Asian countries, the mortality ratio because of cancer is relatively rare which is due to comparatively high concentrations of soy isoflavones in diets (Parkin *et al.*, 1990). Almost one-third of diagnosed breast cancers is due to hormonal abnormality (Santen *et al.*, 1990). More than 50% of the flavonoids considerably subdued aromatase property, with maximum property being showed with apigenin, chrysin and hesperetin. This flavonoid-mediated inhibition of aromatase property is of prime importance in cancer chemoprevention (Hyeh *et al.*, 1999).

Although the current anticancer flavonoids have demonstrated great benefits in cancer treatment (Priva *et al.*, 2013), we still need to establish better anticancer agents that provide much more hope to mankind. We are principally interested in this work to develop potential anticancer agents against cell lines.

Despite the significant efforts, cancer is still a violent killer in the world. Furthermore, in the last decade, the novel chemotherapeutic agents of synthetic origin presently in clinical use have not as much succeeded in satisfying the desired expectations in spite of the extensive charges for their development.

The different types of food and diets contain flavonoids which are of prime importance in human diet. According to estimation, per day intake of flavonoids is 1g (Kuhhau, 1976). These compounds possess a broad range of vital biological properties along with the regulation of process which have become abnormal due to the development of cancer. Also they have antioxidant, antiallergic, anticarcinogenic, anti-inflammatory, antimutagenic and

enzyme modulation properties (Middleton *et al.*, 2000; Galati *et al.*, 2000; Yang *et al.*, 2001). Owing to their multi-dimensional pharmacological activities, they can be effective chemo-therapeutic agents against different types of cancers (Birt *et al.*, 2001).

Cognitive dysfunction is a frequent outcome for many patients of cancer and persists for long. Many cancer patients rejoin their service or other responsibilities that may be affected by cognitive functioning after cancer treatment. It is critical to explore the mechanisms underlying these cognitive effects associated with cancer (Sanne and Jeffery, 2013).

Comparing the activity among flavones, it was found that halogen containing flavones and flavonol (F3 and OF3) were found to be the most active against all tested cell lines having. For F3, the IC₅₀ values of 1.30 μ M, 0.71 μ M and 1.12 μ M respectively. The halogen containing flavonol (OF3) was found to be the most active against cell lines having IC₅₀ values of 1.24 μ M and 0.51 μ M for mouse fibroblast (NIH 3T3), human endothelial cervical (HeLa) respectively and 1.04 μ M for breast cancer (MCF7) cells.

Moreover the methyl containing flavone (F2) was found to be the second active molecule against all tested cell lines having IC₅₀ values of 2.50 μ M, 1.23 μ M and 1.18 μ M respectively. Moreover the methyl containing flavonol (OF2) was found to be active against mouse fibroblast (NIH 3T3), human endothelial cervical cancer (HeLa) having IC₅₀ values of 2.48 μ M and 1.06 μ M respectively and 0.96 μ M for breast cancer (MCF7) cells. It's worth mentioning that OF2 showed promising results (0.96 μ M) against MCF7 cells in comparison to OF3 (1.04 μ M).

Flavonoids present in the leaf of Broccolini have anticancer activity against four human cancer cells (HepG2, SW480, A549 and Hela) in a concentration dependant model. This vegetable is considered beneficial in the treatment of these four human cancers (Bingfang and Xuewu 2012).

Flavonoids significantly inhibit carcinogenesis in *in-vitro* as well as in *in-vivo* (kamei *et al.*, 1996; Caltagirone *et al.*, 2000). Cellular level *in-vivo* models animal studies showed that flavonoids efficiently inhibited tumor initiation and its progression. Experimentally, it has been confirmed that soy can avert breast cancer in animal model (Barnes 1998; Messina *et al.*, 1994). Also mammary tumorigenesis has been successfully inhibited through fermented soy milk that is rich in daidzein and genistein in rats with age of 7 weeks (Ohta *et al.*, 2000). The i.p. administration of apigenin and quercetin in syngenetic mice successfully subdued growth and metastatic property of melanoma cell (Caltagirone *et al.*, 2000).

Nobiletin (hexamethoxy-flavone), is found in the citrus peels and has been reported to decrease histological changes and high grade lesions in the prostate lobes of transgenic rats (Ching-Shu *et al.*, 2013). Another novel flavonoid derivative, flavone 8-acetic acid (FAA) is undergoing clinical trials as an anticancer drug (Maheep *et al.*, 2011).

These results from our study suggest that the introduction of chlorine and methyl group to the flavones may increase the anticancer activities. The cytotoxic screening of the tested flavones, F1 to F3 were also active against all cell lines tested but the activity was less in comparison to flavonol series of compounds suggesting the possible involvement of hydroxyl (OH) group at position 3. These results offered an encouraging framework that may lead to the discovery of activity specific flavonols against specific cancer cell lines with potent anticancer activity.

CONCLUSION

The present work involves the simple and convenient synthesis of flavonols in reasonably good yields. This strategy offered a very straightforward and efficient method to access the synthesis of these molecules. The flavonols after being characterized by IR, ¹H NMR, ¹³C NMR, MS and elemental analysis, were screened for anticancer study against cell lines. The compound OF2 in flavonols exhibited greater potentials MCF7 cell with IC₅₀ value of 0.96 μM and OF3 has 1.04 μM. In contrast, the OF3 exhibited higher activity against HeLa cell with IC₅₀ value of 0.51 μM and OF2 has 1.06 μM.

This study also supports the previous literature that flavonoids may serve as potential candidate for development of new anticancer agents with enhanced activity (Maheep *et al.*, 2011). In conclusion, the present study attested that synthesized flavones and flavonols can be used as template for modification and derivatization to design other potent and selective anticancer agents.

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