Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2018, 10(10): 120-132

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Synthesis and Biological Evaluation of Coumarin Derivatives as Antiproliferative Agents

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ABSTRACT

The present research work involves the synthesis of a total of ten coumarin derivatives and their preliminary in vitro biological evaluation as anticancer agents. The newly synthesized compounds were characterized on the basis of various spectral analytical techniques like IR and 1H-NMR. In vitro testing was done using SRB assay protocol, each derivative was tested at 4 dose levels i.e. 10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml. All the selected synthesized compounds were found to possess good to moderate anticancer activity. The order for the % control growth inhibition of SK-OV-3 cell line at 80 µg/ml was found to be 6j>6i>6d>6c>6e>6b>6a>6g.

Keywords: Coumarin; Anticancer; SKVO-3 cell lines

INTRODUCTION

Cancer results from a series of molecular events that fundamentally alter the normal properties of cells. In cancer cells, the normal control systems that prevent cell overgrowth and the invasion of other tissues are disabled. These altered cells divide and grow in the presence of signals that normally inhibit cell growth; therefore, they no longer require special signals to induce cell growth and division. As these cells grow they develop new characteristics, including changes in cell structure, decreased cell adhesion, and production of new enzymes. These heritable changes allow the cell and its progeny to divide and grow, even in the presence of normal cells that typically inhibit the growth of nearby cells. Such changes allow the cancer cells to spread and invade other tissues. Cancer is probably the biggest epidemic of the 21st century. It is leading cause of death in developed countries, while second leading cause of death in developing countries [1].

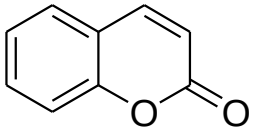
It is the most common disease occurring between the 40-60 years age and may also occur at younger age [2]. It can be treated if detected in early stage. Cancer and neoplasm is the appearance of a tumor, an abnormal mass of tissue.

Anticancer agents are the agents which either kill the cancer cells or modify their growth. The development of new anticancer therapeutic agents is one of the fundamental goals in medicinal chemistry. The available anticancer drugs have distinct mechanism of action which may vary in their effects on different type of normal and cancer cells [3]. A single cure for cancer has proved difficult to describe, since there is not a single type of cancer but as many as 100

different types of cancer. The development of anticancer drug is more difficult than discovering cures for bacterial infection. The reason is that, there are very few biochemical differences between cancerous cell and normal cells. For this reason the effectiveness of many anticancer drugs is limited by their toxicity to the normal rapidly growing cells in the intestinal and bone marrow areas [4]. A final problem is that cancerous cells which are initially suppressed by a specific drug may develop a resistance to that drug. For this reason cancer chemotherapy may consist of using several drugs in combination for varying lengths of time.

Chemotherapy drugs are sometimes feared because of its toxic effects. Their role is to slow and hopefully halt the growth spread of a cancer. There are three goals associated with the use of the most commonly used anticancer agents [5]. The chemotherapeutic drugs work by impairing mitosis, (targeting fast dividing cells). As these drugs cause damage to cells, they are known as cytotoxic. They prevent mitosis by various mechanisms including damaging DNA of the affected cancer cells and Inhibit the synthesis of DNA strands involved in the cell division [6].

In the past few years, molecularly targeted therapeutics and immunotherapeutic become part of research for several types of cancer. The resistance towards available drug is rapidly becoming a major worldwide problem. The need to design new compounds to deal with resistance has become one of the most important areas of research today [7]. The hybridization of two or more bioactive drug fragments with complementary functions or different mechanisms of action into a single molecule is a novel approach that often results in synergistic activity and enhanced drug efficacy. Solomon *et al*. [8] was the first to report this dual action/hybrid pharmacophore strategy using isatin.
Coumarin (1) is the most abundant naturally occurring secondary metabolite, found in several plant families, essential oil, microorganism and a few animal species. Coumarin is effective not only for the treatment of cancer, but also to treat the side effect caused by radiotherapy.



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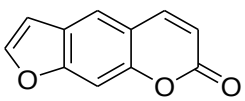
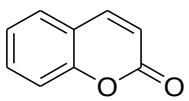
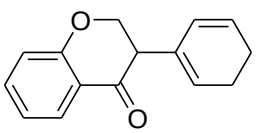
Coumarin had exhibited certain biochemical properties which exert certain biological effects in the cellular environment [9]. Coumarin have been found good maintenance therapy in case of melanoma and also found to inhibit the spread of tumor and possess a wide range of biological activites, anti-viral, anti-tumor, anti-HIV, antimicrobial, and antioxidant. Coumarin is very significant in the treatment of prostate cancer, renal cell carcinoma and leukaemia [9]. Coumarin derivatives like furano coumarin, pyrano coumarin, isoflavones and benzopyrones have a significant role in the treatment of different cancer conditions.



Furano Coumarin



Pyrano Coumarin

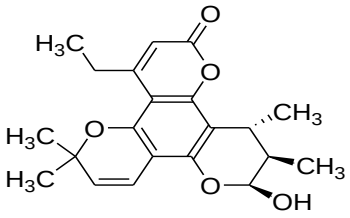


Isoflavones

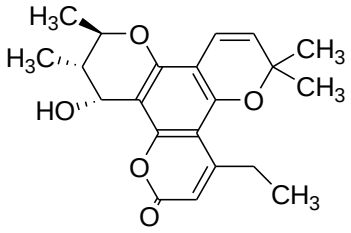
They are important as photo chemotherapeutic agents that are used to treat a variety of skin diseases. Action of coumarins on tumor cells is being exhibited *via* different mechanisms and some of them have been reported to possess high selectivity towards the cancer cell lines.

Coumarins can act on various tumour cells by different mechanisms *viz.* inhibition of the tolemerase enzyme, inhibition of protein kinases and down regulation of oncogens expression or induce the caspase-9-mediated apoptosis which suppress cancer cell proliferation by arresting cell cycle in G0/G1 phase, G2 /M phase and affecting the cancer cells [10].

The coumarin derivatives such as Costatolide and Calanolide B have been marketed and utilized in the treatment of breast cancer [11-18].



Costatolide



Calanolide B

MATERIALS AND METHODS

Melting points were recorded on melting point apparatus by capillary method and were uncorrected. All the IR spectra of the synthesized compounds were recorded on Bruker alpha-E FTIR-ATR. 1HNMR spectra were recorded on Bruker Avance II (400MHz) spectrometer using $CDCl_3$ and DMSO as solvent at IIT, Ropar. TMS was taken as standard and chemical shift data were reported in parts per million (ppm) where s, d, t and m are designated as singlet, doublet, triplet and multiplet respectively. The reaction is monitored by thin layer chromatography on pre coated (Merck 60F254) and self-prepared silica gel coated plates. The solvent system used for developing the chromatogram was chloroform: methanol in variable ratios. UV chambers were used for visualization of TLC spots [19-25].

Preparation of 7hydroxy-4-methyl-2H-chromene-2-one (3)

Added resorcinol (3.7g) to ethyl acetoacetate (4.5g), stirred the reaction mixture until a complete solution is obtained. Now, added this solution slowly to the sulphuric acid so that the temperature of the mixture did not rise above 0°C, and then continued the stirring for 30 minutes. Poured the mixture on to crushed ice, the solid 7-hydroxy-4-methyl-coumarin separates [12]. Filtered off the coumarin at the pump. For purification, first dissolved the coumarin, on cold 10% aqueous sodium hydroxide solution and reprecipitated it by the addition of dilute hydrochloric acid and then recrystallized it from ethanol or methylated spirit [25-28].

Preparation of 3-acetyl-7hydroxy-4-methyl-2H-chromene-2-one (4)

7-hydroxy-4-methyl-2H-chromene-2-one (3g) was dissolved in acetic acid (16ml) and phosphorus oxychloride (5.6 ml) was added. The mixture was heated to reflux for 30 min [13]. After cooling, the precipitate were collected and recrystallized from ethanol.

Preparation of 7-hydroxy-4-methyl-3-(3-hydroxyphenyl-acryloyl)-2H-chromene-2-one (6)

Placed a solution of 3- acetyl-7-hydroxy-4-methyl-2H-chromene-2-one (0.003 mol) and the appropriate substitution aromatic aldehyde (0.003 mol) was dissolved in chloroform. The catalytic amount of piperidine (0.002 mol) was added and the reaction mixture was refluxed for 5 hrs [14-]. The chloroform was distilled out and the residue was washed with methanol (Figure 1).

Reaction scheme

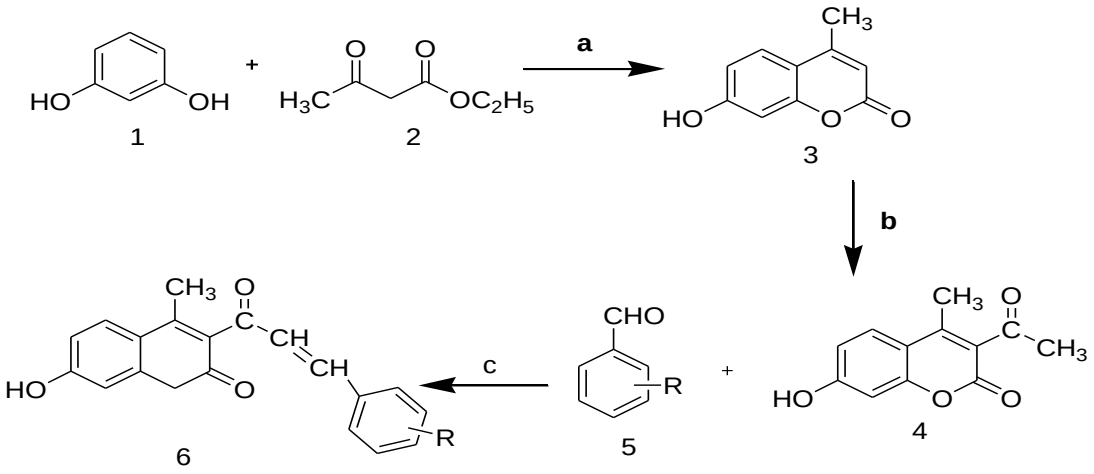


Figure 1. Proposed reaction scheme

RESULTS AND DISCUSSION

As per the proposed reaction scheme, In the initial step, 7-hydroxy-4methyl-2H-chromene -2-one was synthesized via the base-catalysed claisen-Schmidt condensation of 3-acetyl-7-hydroxy-4-methyl-2H-chromene-2-one. The ring closure reaction of the 7- hydroxy-4-methyl-2H-chromene-2-one with sulphuric acid and sodium hydroxide afforded 3-acetyl-7-hydroxy-4-methyl-2H-chromene-2-one derivatives 6(a-j). The reaction was monitored by thin layer chromatography using solvent system Chloroform: Methanol (7:3) (Table 1).

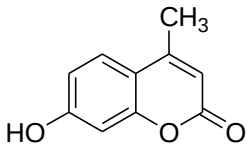
Table 1. Physical characteristics of synthesized compounds

Comp. No.	Molecular Formula	Colour	Molecular Weight (g)	Melting point (ºC)	R_f^*	Yield (%)
6a	$C_{19}H_{16}O_4$	Yellow	308.33	160-170	0.7	33.43
6b	$C_{19}H_{15}NO_6$	Brown	353.33	85-95	0.7	37.54
6c	$C_{19}H_{15}NO_6$	Brown	353.33	100-107	0.7	29.71
6d	$C_{19}H_{13}ClO_4$	Yellow	340.76	170-175	0.7	64.08

6e	$C_{19}H_{13}BrO_4$	Dark Brown	385.21	155-160	0.6	59.31
6f	$C_{21}H_{18}O_6$	Brown	366.36	80-90	0.6	38.26
6g	$C_{19}H_{16}O_5$	Light Brown	324.33	110-120	0.7	34
6h	$C_{20}H_{18}O_5$	Yellow	338.35	120-125	0.7	45.9
6i	$C_{20}H_{13}NO_4$	Brown	331.08	157-167	0.6	33.66
6j	$C_{19}H_{16}NO_6$	Pale Yellow	353.33	80-85	0.7	37.56

Spectral Characterization of the Synthesized Compounds

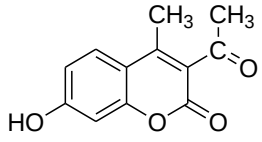
7-Hydroxy-4-methyl-2H-Chromene-2-one (3):



IR (KBr, cm^{-1}): 3257 cm^{-1} (Ar-C-H stretch), 1745 cm^{-1} (C=O stretch), 1446 cm^{-1} (C=C stretch), 2990 cm^{-1} (Ar-H stretch), 3435 cm^{-1} (O-H stretch), 1266 cm-1 (C-O-C), 1664 cm^{-1} (C-C stretch).

1HNMR (DMSO, 400MHz, δ ppm): 7.48(d, J=9.16 Hz, 1H, Ar-H), 6.96 (d, J=2.44 Hz, 1H, Ar-H), 6.83(t, J=10.96 Hz, 1H, Ar-H), 6.13(d, J=1.24 Hz, 1H, Ar-H), 2.4 (d, J=1.2 Hz, 2H, Ar-H,O-H).

3-Acetyl-7-Hydroxy-4-methyl-2H-Chromene-2-one (4)

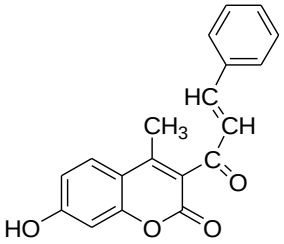


(4)

IR (KBr, cm^{-1}): 1758 cm^{-1} (C=O stretch), 1446 cm^{-1} (CH3 stretch), 1662 cm^{-1} (C=C stretch), 3369 cm-1 (O-H stretch).

1HNMR (DMSO, 400MHz, δ ppm): 1.66 (s, 3H, CH3), 2.39 (s, 3H, CH3), 6.13 (s, 1H, Ar-H), 6.81 (s, 1H, Ar-H), 7.49 (s, 1H, Ar-H).

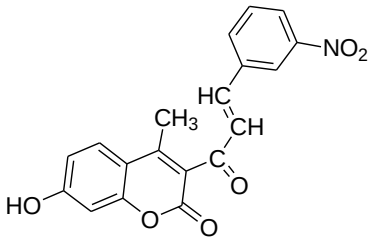
7-Hydroxy-4-methyl-3-(3-phenyl acryloyl)-2H- Chromene-2-one (6a)



IR (KBr, cm^{-1}): 1495 cm^{-1} (Ar-C=C stretch), 1720 cm^{-1} (C=O stretch), 1083 cm^{-1} (C-O-C stretch), 3327 cm^{-1} (O-H), 2953 cm^{-1} (CH3 stretch).

1HNMR (DMSO, 400MHz, δ ppm): 2.39-2.40 (d, 3H, J=1.24 Hz, CH3), 6.13 (d, 1H, J=1.36 Hz, C-H), 6.82-6.94 (m, 7H, Ar-H), 7.47 (s, 1H, C-H), 7.49 (s, 1H, Ar-H), 8.30 (s, 1H, O-H).

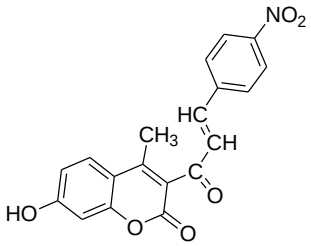
7-Hydroxy-4-methyl-3(3-nitrophenyl) acryloyl)-2H- Chromene-2-one (6b)



IR (KBr, cm^{-1}): 1669 cm^{-1} (C=O stretch), 1083 cm^{-1} (C-O-C stretch), 1379 cm^{-1} (N-O stretch), 1589 cm^{-1} (C=C stretch), 3117 cm^{-1} (O-H stretch).

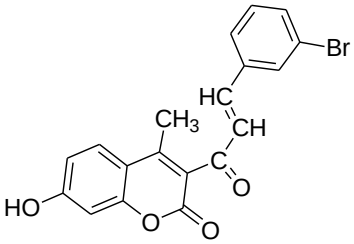
1HNMR (DMSO, 400MHz, δ ppm): 2.39 (d, 3H, J=1.24 Hz, CH3), 3.15 (s, 1H, CH), 6.13 (s, 1H, C-H), 6.82-7.48 (m, 7H, Ar-H), 9.09 (s, 1H, O-H).

7-Hydroxy-4-mmethyl-3(4-nitrophenyl) acryloyl)-2H- Chromene-2-one (6c)



IR (KBr, cm^{-1}): 1382 cm^{-1} (N=O stretch), 1447 cm^{-1} (C=O stretch), 3487 cm^{-1} (O-H stretch), 1668 cm^{-1} (C=C stretch), 1063 cm^{-1} (C-O-C stretch).

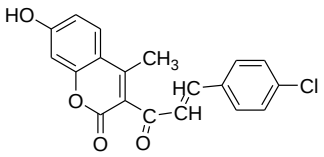
7-Hydroxy-4-methyl-3-(3-(3-Bromobenzaldehyde acryloyl)-2H Chromene-2-one (6d)



IR (KBr, cm^{-1}): 3119 cm^{-1} (O-H stretch), 1444 cm^{-1} (C=C stretch), 1671 cm^{-1} (C=O stretch), 1119 cm^{-1} (C-O-C stretch), 2802 cm^{-1} (CH_3 stretch), 633cm^{-1} (C-Br stretch).

^{1}HNMR (DMSO, 400MHz, δ ppm): 2.39 (d, 1H, J=1.24 Hz, CH_3), 6.13 (s, 2H, C-H), 6.81-6.85 (m, 3H, Ar-H), 7.46-7.48 (m, 3H, Ar-H), 7.48-8.00 (d, 2H, J=8.76 Hz, Ar-H, O-H).

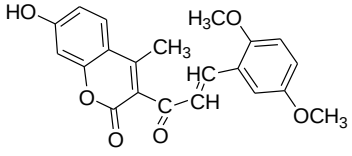
7-Hydroxy-4-methyl-3(3-(4-chlorophenyl) acryloyl)-2H- Chromene-2-one (6e)



IR (KBr, cm^{-1}): 3428 cm^{-1} (O-H stretch), 1446 cm^{-1} (C=C stretch), 1673 cm^{-1} (C=O stretch), 1059 cm^{-1} (C-O-C stretch), 2950 cm^{-1} (CH_3 stretch), 740 cm^{-1} (C-Cl stretch).

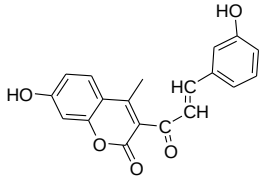
^{1}HNMR (DMSO, 400MHz, δ ppm): 2.39 (d, J=1.24 Hz, 3H, CH_3), 6.13-6.14 (d, J=1.2 Hz, 1H, CH), 6.80-6.88 (m, 4H, Ar-H), 6.98 (s, 1H, CH), 7.24-7.50 (m, 3H, Ar-H), 8.20 (s, 1H, O-H).

7-Hydroxy-4-methyl-3(3-2, 5-Dimethoxy phenyl) acryloyl)-2H- Chromene-2-one (6f)



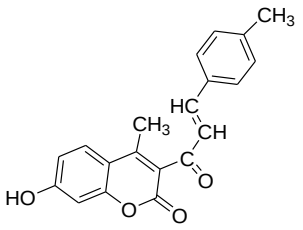
IR (KBr, cm^{-1}): 2911cm^{-1} (Ar-C-H stretch), 1658 cm^{-1} (C=O stretch), 703 cm^{-1} (Ar-C-Cl stretch), 1011 cm^{-1} (C-S-C stretch), 1483 cm^{-1} (Ar-C=C stretch), 1589 cm^{-1} (C=N stretch).

7-Hydroxy-4-methyl-3(3-(3-hydroxy phenyl) acryloyl)-2H- Chromene-2-one (6g)



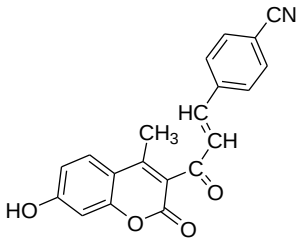
IR (KBr, cm^{-1}): 3140cm^{-1} (Ar-C-H stretch), 1675 cm^{-1} (C=O stretch), 1451 cm^{-1} (C=C stretch), 3422 cm^{-1} (O-H stretch), 1333cm^{-1} (CH_3 stretch), 1040 cm^{-1} (C-O-C stretch).

7-Hydroxy-4-methyl-3(3-(4-methoxy phenyl) acryloyl)-2H-Chromene-2-one (6h)



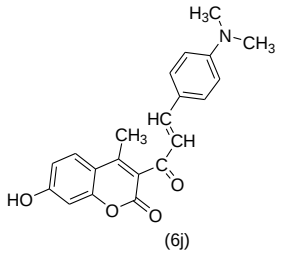
IR (KBr, cm^{-1}): 3491cm^{-1} (Ar-C-H stretch), 1664cm^{-1} (C=O stretch), 1448 cm^{-1} (C=C stretch), 3438 cm^{-1} (O-H stretch), 1344cm^{-1} (CH_3 stretch), 1065 cm^{-1} (C-O-C stretch).

7-Hydroxy-4-methyl-3(3-(oxoprop)-lenyl) benzonitrite)-2H-Chromene-2-one (6i)



IR (KBr, cm^{-1}): 3123 cm^{-1} (Ar-C-H stretch), 1587 cm^{-1} (C=C stretch), 1672 cm^{-1} (C=O stretch), 1057 cm^{-1} (C-O-C stretch), 1083 cm^{-1} (CN), 2804 cm^{-1} (CH_3).

7-Hydroxy-4-methyl-3(3-(4-dimethylamino phenyl) acryloyl-2H-Chromene-2-one (6j)



(6j)

IR (KBr, cm^{-1}): 1664 cm^{-1} (C=O stretch), 1067 cm^{-1} (C-O-C stretch), 3440 cm^{-1} (O-H stretch), 2629 cm^{-1} (CH_3 stretch).

Anticancer Drug Screening

All the synthesized compounds were screened against SK-OV-3 cell line to determine their growth inhibitory effect. *In vitro* testing was done using SRB assay protocol; each derivative was tested at 4 dose levels (10 µg/mL, 20 µg/mL, 40 µg/mL, 80 µg/mL).

The order for the % control growth inhibition of SK-OV-3 cell line at 80 µg/mL was found to be 6j>6i>6d>6c>6e>6b>6a>6g and have been presented in the Tables 2-6.

Table 2. *In vitro* percentage control growth of SK-OV-3 cell line at different concentrations of compounds (Experiment 1)

C. No.	% Control Growth			
	Drug Concentrations (µg/ml)			
	10	**20**	**40**	**80**
6a	30.7	19.7	19.2	11.2
6b	35.3	34.8	22.9	15.2
6c	39.9	39.1	25.7	19.9
6d	41.9	36.1	25.9	16.5
6e	37.1	24.7	18.8	9.2
6f	14.1	11.9	11.3	10.5
6g	11.8	10.0	8.0	5.8
6h	26.1	15.6	10.9	7.1
6i	42.9	34.8	24.2	14.7
6j	45.1	37.6	26.2	16.4
ADR	-30.3	-34.6	-33.2	-24.9

Table 3. *In vitro* percentage control growth of SK-OV-3 cell line at different concentrations of compounds (Experiment 2)

C. No.	% Control Growth			
	Drug Concentrations (µg/ml)			
	10	**20**	**40**	**80**
6a	36.5	35.6	31.9	21.7
6b	39.3	35.3	34.5	22.2
6c	41.3	37.1	33.6	22.1
6d	40.3	40.3	39.1	22.7
6e	39.4	38.8	36.4	21.9
6f	42.4	40.0	28.6	27.4
6g	19.1	12.0	11.2	7.7
6h	19.9	18.2	9.6	6.4
6i	39.8	35.9	28.9	17.5
6j	50.2	42.2	36.8	18.6
ADR	-38.3	-36.3	-46.7	-26.2

Table 4. *In vitro* percentage control growth of SK-OV-3 cell line at different concentrations of compounds (Experiment 3)

C. No.	% Control Growth			
	Drug Concentrations (µg/ml)			
	10	**20**	**40**	**80**

6a	7.6	6.6	6.5	4.8
6b	9.2	8.3	6.9	6.1
6c	11.0	9.8	9.4	7.5
6d	10.6	10.2	10.1	8.7
6e	9.1	8.3	8.2	6.3
6f	9.4	9.2	8.2	7.3
6g	17.6	10.7	8.7	7.9
6h	35.2	19.2	8.2	6.5
6i	30.6	28.2	25.3	18.6
6j	45.4	33.9	29.9	19.1
ADR	-32.4	-39.8	-48.6	-24.2

Table 5. Average values of percentage control growth of SK-OV-3 cell line at different drugs concentrations

C. No.	% Control Growth			
	Drug Concentrations (µg/ml)			
	10	**20**	**40**	**80**
6a	4.8	24.9	20.6	19.2
6b	6.1	27.9	26.1	21.4
6c	7.5	30.7	28.6	22.9
6d	8.7	30.9	28.9	25.0
6e	6.3	28.5	23.9	21.2
6f	7.3	21.9	20.3	16.1
6g	7.9	16.2	10.9	9.3
6h	6.5	27.1	17.7	9.6
6i	18.6	37.8	33.0	26.1
6j	19.1	46.9	37.9	31.0
ADR	-24.2	-33.7	-36.9	-42.8

Table 6. Synthesized compound 6a-6j concentrations (µg/ml) as GI_{50} calculated for SK-OV-3 cell line

SK-OV-3	LC50	TGI	GI50*
6a	NE	NE	>80
6b	NE	NE	>80
6c	NE	NE	>80
6d	NE	NE	>80
6e	NE	NE	>80
6f	NE	NE	>80
6g	NE	NE	>80
6h	NE	NE	<10
6i	NE	NE	>80
6j	NE	NE	>80
ADR	34.2	<10	<10

Out of the ten synthesized derivatives, the compound 6h at concentration 80 µg/ml possess best activity. However, in the coumarin Derivatives the compound 6d was found to be the second most active compound. The parameter like $LC_{50,}$ GI_{50} and TGI_{50} were calculated using the graph obtained by plotting drug concentration and % control inhibition (Figures 2 and 3).

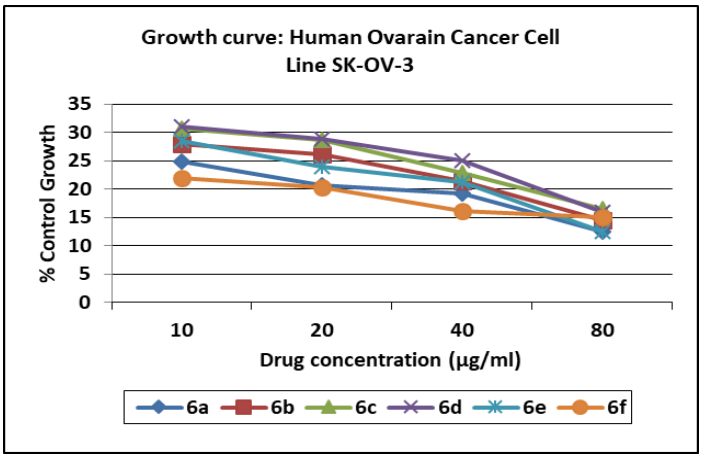


Figure 2. A graph of % control growth versus drug conc. of compound 6a-6f

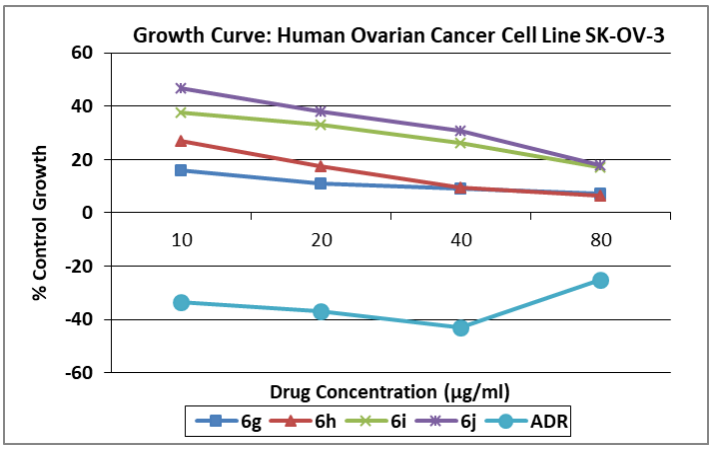


Figure 3. A graph of % control growth versus drug conc. of compound 6g-6h

The LC_{50} which is the concentration of drug acting as lethal for 50% of cells could not be observed for all the compounds. Total growth inhibitions (TGI) define as the concentration of drug required for the complete inhibition of the growth of cells [28-32].

For 50% growth inhibition of cells, the parameter calculated is $GI_{50.}$ For getting the idea of the activity of the compounds the GI_{50} value of $\leq 10^{-6}$ molar or $\leq$ 10 µg/ml has been considered to demonstrate activity in case of pure compounds. It has been clearly implied that all the compound 6h have GI_{50} value of <10 µg/ml demonstrating their potential activity.

CONCLUSION

All the synthesized compounds were screened against SK-OV-3 cell line to determine their growth inhibitory effect. In vitro testing was done using SRB assay protocol; each derivative was tested at 4 dose levels (10 μg/ml, 20 μg/ml, 40 μg/ml, 80 μg/ml).

The order for the % control growth inhibition of SK-OV-3 cell line at 80 μg/ml was found to be 6j>6i>6d>6c>6e>6b>6a>6g.

However, in the coumarin derivatives the compound 6d was found to be the second most active compound. For 50% growth inhibition of cells, the parameter calculated is GI50. For getting the idea of the activity of the compounds the GI50 value of ≤ 10-6 molar or ≤ 10 μg/ml has been considered to demonstrate activity in case of pure compounds. It has been clearly implied that all the compound 6h have GI50 value of <10 μg/ml demonstrating their potential activity.

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