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Synthesis and *in vitro* evaluation of novel coumarin-chalcone hybrids as potential Anticancer agents

Koneni V. Sashidhara [a,*], Abdhesh Kumar [a], Manoj Kumar [a] Jayanta Sarkar [b] and Sudhir Sinha [b]

Medicinal and Process Chemistry Division [a], Central Drug Research Institute, (CDRI-CSIR), Lucknow 226 001, India

Drug Target Discovery and development Division [b], Central Drug Research Institute, (CDRI-CSIR), Lucknow 226 001, India

ABSTRACT

A series of coumarin-chalcone hybrids have been synthesized and evaluated for their *in vitro* cytotoxicity against a panel of four human cancer cell lines and normal fibroblasts (NIH3T3). Among 21 compounds screened, three compounds (**23**, **25** & **26**) showed IC_{50} range in between 3.59 to 17.97 µM. The most promising compound **26** showed around 30 fold more selectivity towards C33A (cervical carcinoma) cells over normal fibroblast NIH3T3 cells with an IC_{50} value of 3.59 µM.

***Keywords*:** Synthesis, Coumarin, Chalcone, *in vitro*, Cytotoxicity.

Part of this work has been filed for provisional Indian patent vide DEL No. 1843-2010.

☆ Part VIII in the series, "Advances in drug design and discovery."

*Corresponding author. Tel.: +91 9919317940; Fax: +91-522-2623405.

Email: sashidhar123@gmail.com, kv_sashidhara@cdri.res.in (K V Sashidhara)

INTRODUCTION

Cancer, a diverse group of diseases characterized by uncontrolled growth of abnormal cells, is a major worldwide problem. It is a fatal disease standing next to the cardiovascular disease in terms of morbidity and mortality. Although the cancer research has led to a number of new and effective solutions, the medicines used as treatments have clear limitations and unfortunately cancer is projected as the primary cause of death in the future.[1, 2] currently there is a huge scientific and commercial interest in the discovery of potent, safe and selective anticancer drugs.

Coumarins class of compounds, which occupy a special role in nature. They belongs to the flavonoid class of plant secondary metabolite, which have been found to exhibit a variety of biological activities, usually associated with low toxicity and have raised considerable interest because of their potential beneficial effects on human health.[3-4] They have attracted intense interest in recent years because of their diverse pharmacological properties like anti-HIV,[5] anticoagulant,[6] antibacterial,[7] antioxidant,[8] and dyslipidemic.[9] Among these properties, cytotoxic effects were most extensively examined.[10-11] Recently, Lee *et al*. reported that neo-tanshinlactone, (Figure 1) a coumarin containing compound, showed significant inhibition against two ER+ human breast cancer cell lines and was 10-fold more potent and 20-fold more selective than Tamoxifen.[12]

On the other hand, chalcones (1,3-diaryl-2-propen-1-ones) constitute an another important class of natural products belonging to the flavonoid family, which display interesting biological activities including anti-inflammatory,[13] antibacterial,[14] antioxidant,[15] antimalarial[16] and anticancer.[17] Due to their abundance in plants and ease of synthesis, this class of compounds has provoked great interest for possible therapeutic uses. They are also effective *in vivo* as cell proliferating inhibitors, anti-tumor promoting and chemopreventing agents (Figure 1). Since a number of clinically useful anticancer drugs have genotoxic effects due to interaction with the amino groups of nucleic acids, chalcones may be devoid of this important side effect.[18]

In the design of new drugs, the development of hybrid molecules through the combination of different pharmacophores may lead to compounds with interesting biological profiles. In recent years, combination chemotherapy with agents possessing different mechanisms of action is one of the methods that is being adopted to treat cancer. Therefore, a single molecule containing more than one pharmacophore, each with different mode of action could be beneficial for the treatment of cancer.[19-20] Adopting this approach, several research groups have recently reported hybrid molecules by coupling coumarins with different bioactive molecules like: resveratrol, maleimide and alpha-lipoic acid; these studies resulted in new compounds showing antiplatelet, antioxidant and anti-inflammatory activities.[21-23] Recently, F. Belluti *et al.* explored anticancer activities of stilbene-coumarin hybrid compounds.[24] Furthermore, Bombardelli *et al.* synthesized a series of coumarin-chalcone hybrids (see prototype in figure 1). These hybrids have shown significant inhibition in taxol resistant cancers.[25] Inspired by this study, we have designed and synthesized a series of novel compounds that have both coumarin and chalcones entities in one molecule and have evaluated them for their antitumor activity.

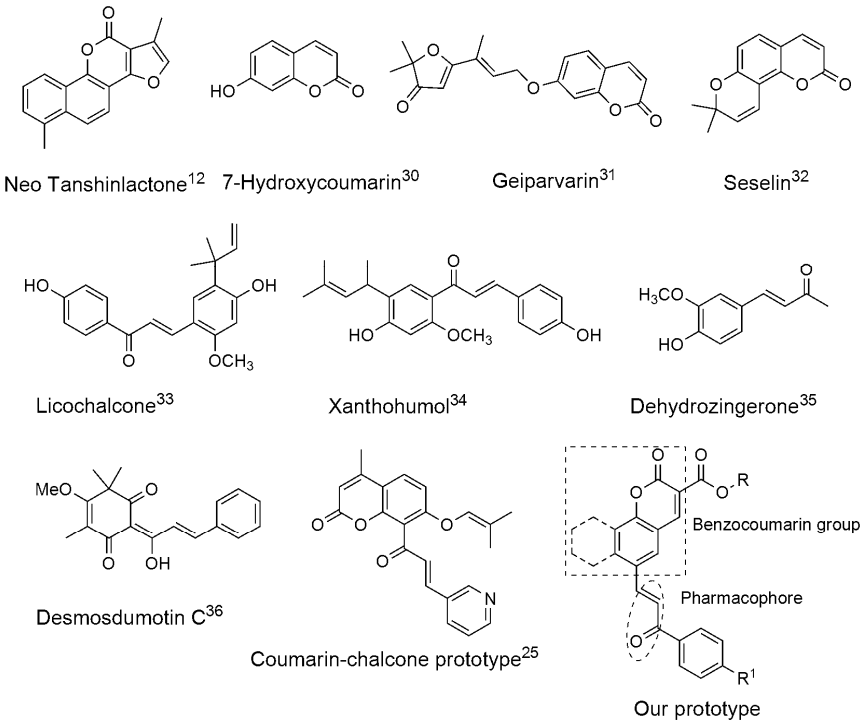
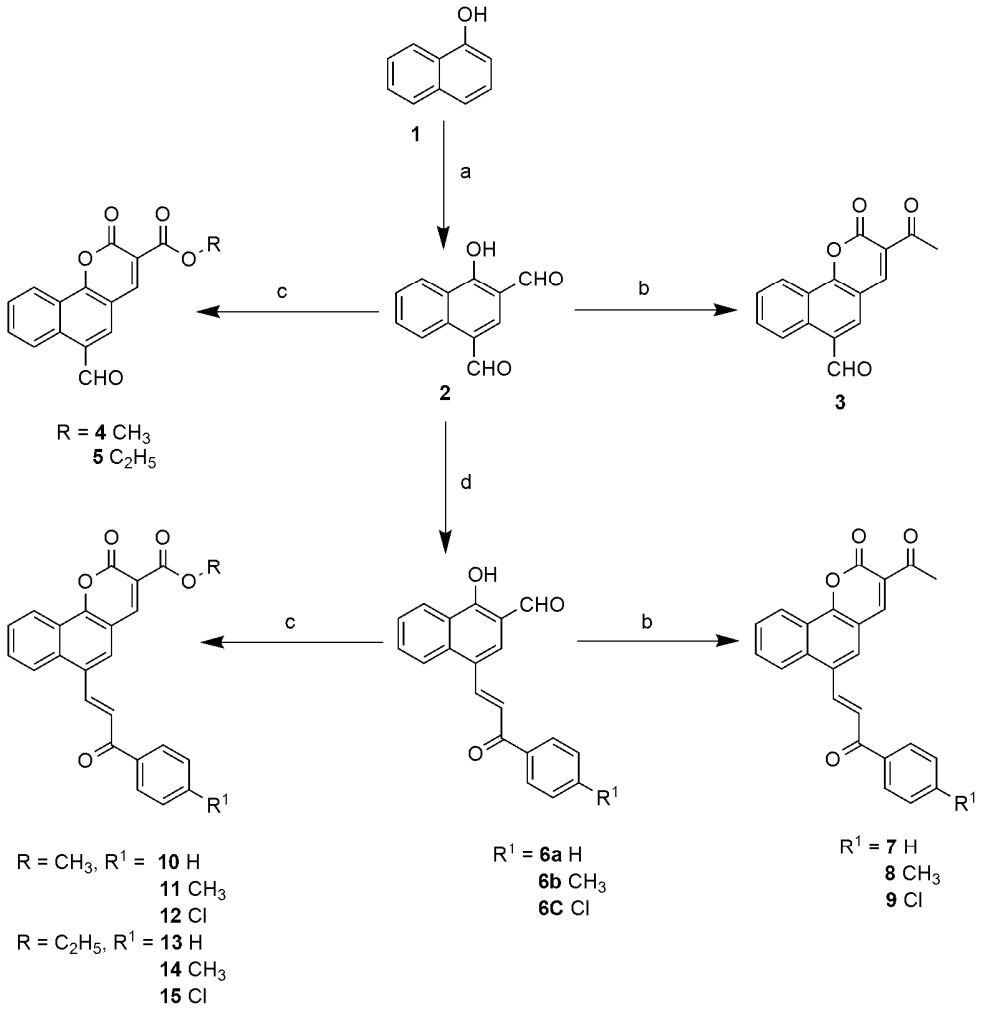


Figure 1. Naturally occuring coumarin and chalcone as anticancer compounds and general structure of synthesized coumarin-chalcone hybrids.

Figure 1- Chemical structure of some naturally occurring coumarin and chalcones with potent anticancer activity and general structure of our synthesized hybrids.

Figure 1 shows the chemical structures of some naturally occurring potent anticancer molecules that either contain a coumarin or chalcone in their molecular makeup and form the basis of our designed prototype.

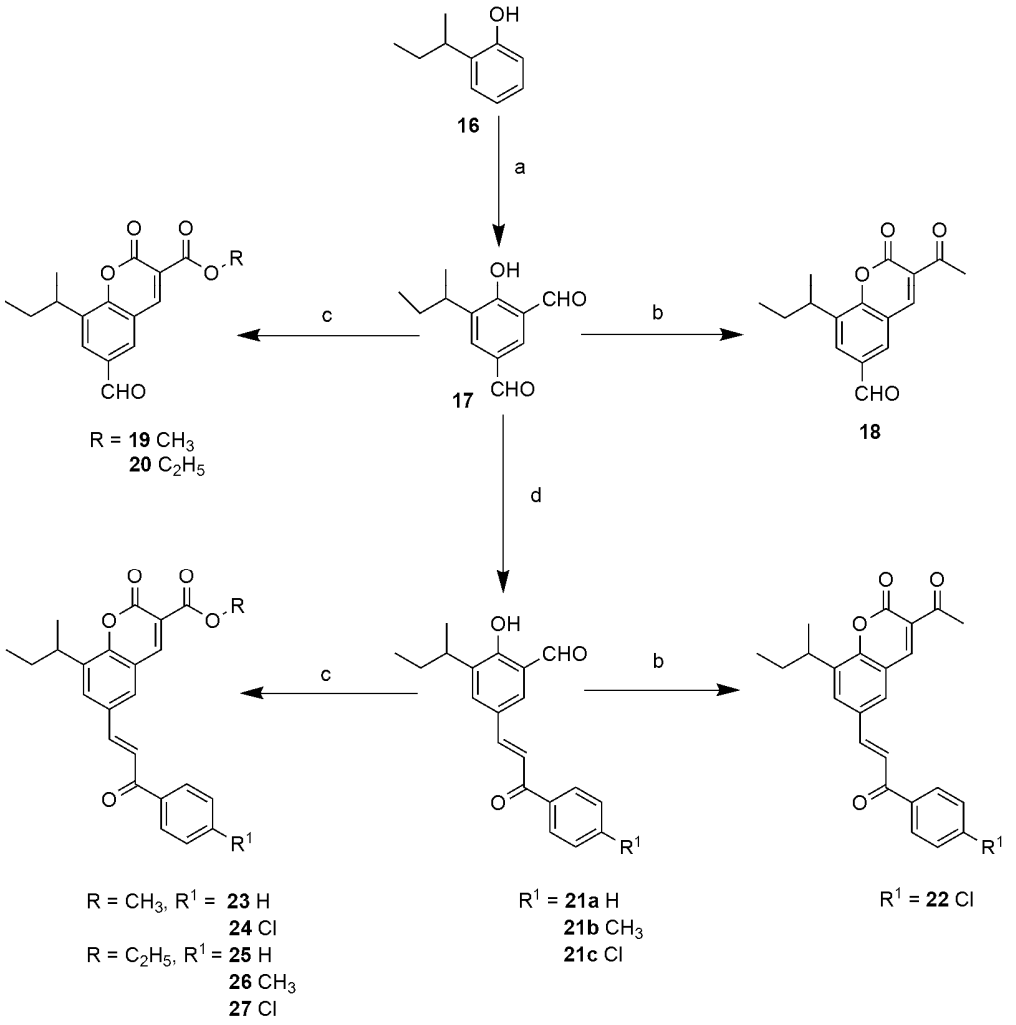
The route followed for the preparation of coumarin derivatives and coumarin–chalcone hybrids is illustrated in Scheme 1.



Scheme 1. Reagents and conditions: (a) 1. Hexamethylenetetramine / TFA, 120 °C, 3h 2.10% H_2SO_4, 90-100 °C, 2h (b) $CH_3COCH_2COOC_2H_5$, EtOH, Piperidine, reflux, 30 min. (c) $CH_2(COOR)_2$, ROH, Piperidine, reflux, 30 min. (d) Conc.HCl, *p*-$R^1C_6H_4COCH_3$, dioxane, 80-90 °C, 2.5-3.5 h.

Scheme 1- Synthesis of coumarins (**3**–**5**) and novel coumarin-chalcone hybrids (**7**–**15**).

The Duff reaction on naphthalen-1-ol **1** gave compound **2** which was engaged in a Knoevenagel-type reaction with different active methylene compounds resulted in the formation of coumarinic compounds (**3**–**5**). Alternatively, compound **2** on reaction with different acetophenone in refluxing dioxane in the presence of a catalytic amount of conc.HCl gave regioselective *para*-condensed chalcones[26] (**6a**–**6c**) in good yields. These chalcone derivatives on subsequent Knoevenagel-type condensation with different active methylene compounds furnished coumarinic-chalcone hybrids[27] (**7**–**15**) (Scheme 1). Similarly, another series of coumarinic-chalcones were prepared starting from 2-*sec*-butylphenol **16** which was subjected to same series of above mentioned transformations resulting in coumarinic compounds (**18**–**20**) and coumarinic-chalcone hybrids (**22**–**27**) (Scheme 2).

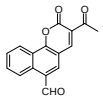
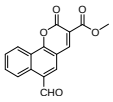
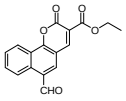
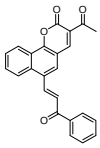
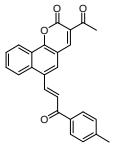
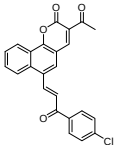
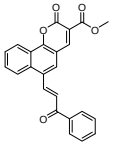
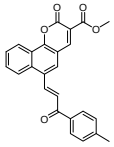
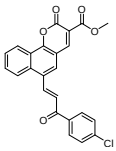
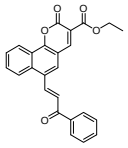
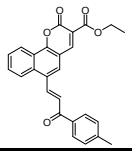


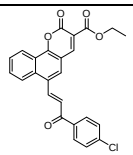
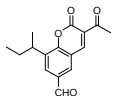
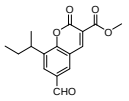
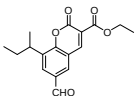
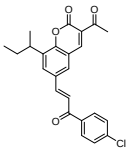
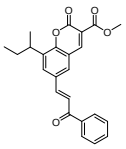
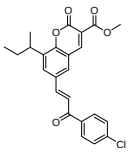
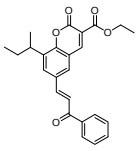
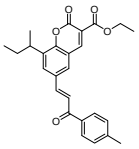
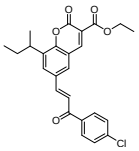
Scheme 2. Reagents and conditions: (a) 1. Hexamethylenetetramine / TFA, 120 °C, 3h 2.10% H_2SO_4, 90-100 °C, 2h (b) $CH_3COCH_2COOC_2H_5$, EtOH, Piperidine, reflux, 30 min. (c) $CH_2(COOR)_2$, ROH, Piperidine, reflux, 30 min. (d) Conc.HCl, *p*-$R^1C_6H_4COCH_3$, dioxane, 80-90 °C, 1.0-1.5 h.

Scheme 2- Synthesis of coumarins (**18**–**20**) and novel coumarin-chalcone hybrid (**22**–**27**).

In all the chalcones synthesized the *Trans* double bond (on the basis of coupling constant) was obtained exclusively. All compounds were characterized using ^{1}H NMR, ^{13}C NMR, Mass spectrometry and IR spectroscopy. The purity of these compounds was ascertained by TLC and spectral analysis.

The new compounds were evaluated for their *in vitro* anticancer activity using Sulforhodamine B assays.[28-29] The growth-inhibitory effects was undertaken in four human cancer cell lines, KB (Oral squamous cell carcinoma), C33A (cervical carcinoma), MCF-7 (Breast adenocarcinoma), A549 (lung) and one normal human NIH3T3 (Mouse embryo fibroblast) in order to determine their cyto-selective nature. The results are presented in Table1.

Compounds	Structure	KB	C33A	MCF-7	A549	NIH3T3
3		109.21	72.82	146.99	>200	>200
4		57.91	30.07	61.70	75.67	65.96
5		123.75	93.55	155.24	>200	>200
7		NA	NA	NA	NA	NA
8		NA	NA	NA	NA	NA
9		NA	NA	NA	NA	NA
10		90.76	62.40	102.73	>200	>200
11		70.38	52.04	117.79	>200	>200
12		NA	NA	NA	NA	NA
13		NA	NA	NA	NA	NA
14		NA	NA	NA	NA	NA

15		NA	NA	NA	NA	NA
18		176.36	70.04	141.40	>200	>200
19		145.73	87.99	>200	>200	>200
20		131.59	64.30	112.89	146.82	>200
22		NA	NA	NA	NA	NA
23		13.41	6.28	11.41	10.69	>200
24		28.25	5.90	10.80	16.67	42.41
25		10.47	8.12	88.09	12.87	>200
26		17.97	3.59	81.10	32.80	>200
27		14.29	4.54	11.07	12.85	38.42
	Doxorubicin	0.22	0.82	0.61	0.52	ND

NA = Not active, ND = Not determined

Table 1- Anticancer activity (IC_{50}, μg/mL) of coumarins and novel coumarin-chalcone hybrids.

IC_{50} values were based on dose response curves. Each test compound displayed a concentration-dependent cytotoxic profile in all four cell lines. Out of all the compounds evaluated, five compounds showed IC_{50} range in between 3.59 to 17.97 μM and eight

compounds exhibited IC_{50} range in between 30.07 to 176.36 μM. The remaining compounds, having IC_{50} value more than 200 μM, were considered inactive.

A closure look into the structure activity relationship indicates that out of the two series of coumarinic–chalcones hybrids synthesized (**7**–**15**) and (**22**–**27**), the former were inactive with just two exceptions (**3** & **4**) that exhibited very limited activity, while the latter were found to be more active against one or the other cell lines. Furthermore, in the second series of compounds, as far as pharmacophore 1 (coumarin core) is considered, it revealed that the substitution at position 3 play a pivotal role, it is interesting to note that the ester- containing members all posses interesting activity, while the ketone (**22**) does not. Furthermore, the ethyl esters seem to be less effective against MCF-7 cell line compared to the methyl esters (**23** & **24**). A cursory look at the second pharmacophore (chalcone core) reveals that the *para*-chloro substituent significantly diminishes selectivity (**24** &**27**) for cancer versus non-cancer cell lines.

In conclusion, we report here a series of new coumarin-chalcones hybrids (23-27) prepared by a novel method and their ability to kill tumor cells *in vitro*. Since the other series of hybrids were not really active. Though the mechanisms underlying this process remain to be fully elucidated, previous literature studies reveal that both coumarin and chalcone are known microtubule inhibitor with antimitotic activity.[37-38] detailed mechanistic studies and lead optimization of these coumarin-chalcone hybrids are under investigation. It is intended that results from these studies will assist in elucidating their precise mechanisms of action and provide an approach to develop new potent coumarin-chalcone hybrid prototypes for further optimization and development to get new leads for the treatment of cancer.

Acknowledgments
The authors wish to thank the SIP 0026 project for financial support, SAIF-CDRI for providing spectral data and to CSIR, New Delhi, India for providing fellowships (A.K. & M.K.).

Supplementary data
Supplementary data associated with this article can be found in the online version.

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27. Representive procedure for the synthesis of compound 7 ((E)-3-Acetyl-6-(3-oxo-3-phenylprop-1-enyl)-2H-benzo[h]chromen-2-one) : A solution of 1-Hydroxy-4-(3-oxo-3-phenyl-propenyl)-naphthalene-2-carbaldehyde **6a** (200 mg, 0.66 mmol) and ethylacetoacetate (85.8 mg, 0.66 mmol) in absolute ethanol (25 mL) was treated with piperidine (0.2 mL) and refluxed for 30 min. Most of the excess solvent was evaporated under reduced pressure, and the residue was neutralized with acetic acid. To this residue water (25 mL) was added and extracted 3-fold with 20 mL of $CHCl_3$. The combined organic layers were dried with anhydrous Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The crude product thus obtained was purified by washing with MeOH to furnish (285 mg, 85% yield) of pure compound 7 ((E)-3-Acetyl-6-(3-oxo-3-phenylprop-1-enyl)-2H-benzo[h]chromen-2-one) as light yellow solid.
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29. ***In Vitro* Cytotoxicity Bioassay.** The human cancer cell lines- KB (oral squamous cell carcinoma), C33A (cervical carcinoma), MCF-7 (breast adenocarcinoma), A549 (lung carcinoma) and mouse embryo fibroblast (NIH3T3) were obtained from American Type Culture Collection (ATCC), USA. These cells were grown in recommended media supplemented with 10% FBS, 50 µg/mL gentamycin and 2.5 µg/mL amphotericin B in a 5% CO_2 humidified atmosphere at 37 $^{\circ}$C. Cells below 15 passage level were used for this study. A colorimetric sulforhodamine B assay was used for the measurement of cell cytotoxicity. 1 X 10^4 cells (in 180 µL) were added to each well of 96-well plate and incubated overnight to allow for cell attachment. Cells were then treated with serial two-fold dilutions of test compounds (100 to 1.6 µg/mL) and untreated cells receiving the same volume of medium served as control. After 48 h of exposure, cells were fixed with ice-cold 50% TCA, stained with 0.4% (w/v) SRB in 1% acetic acid, washed and air dried. Bound dye was dissolved in 150 µL of 10mM tris base. The plates were read at 540 nm absorbance on plate reader (Polarstar Galaxy, BMG, Germany). The cytotoxic effects of compounds were calculated as % inhibition in cell growth as per the formula [100-(Absorbance of compound treated cells / Absorbance of untreated cells)] X 100. Determination of 50% inhibitory concentration (IC_{50}) was based on dose-response curves

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