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Original article

Synthesis and tumor inhibitory activity of novel coumarin analogs targeting angiogenesis and apoptosis

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ABSTRACT

A sequence of coumarin analogs **5a**–**j** was obtained by multi step synthesis from hydroxy benzophenones (**1a**–**j**). The *in vitro* antiproliferative effect of the title compounds was tested against Ehrlich ascites carcinoma (EAC) and Daltons lymphoma ascites (DLA) cell lines. Among the series, compound **5c** with bromo group in the benzophenone moiety was endowed with excellent antiproliferative potency with significant IC_{50} value. Further, *in vivo* antitumor effect of compound **5c** against murine EAC and solid DL tumor model system was evident by the extended survivality. The tumor inhibitory mechanism of compound **5c** was due to the antiangiogenesis and promotion of apoptosis. These results suggest possible applications of compound **5c** which could be developed as a potent anticancer drug in the near future.

1. Introduction

Most, if not all, human cancers share six acquired capabilities that enable malignant growth as proposed by Hanahan and Weinberg. Promotion of angiogenesis and resistant to apoptosis are the two important hallmarks of cancer [1]. Tumor growth and expansion requires an ability not only to proliferate, but also to down-modulate cell death and activate angiogenesis to produce a tumor neovasculature. Thus, the promotion of apoptosis and anti-angiogenesis targeting strategies is one of the important focus in current cancer therapy [2]. The development of such novel, effective and less or no toxic compounds with multiple mode of action for targeted cancer therapy has become an innovative approach and efforts have been directed toward discovering such anticancer agents endowed with cytotoxic action [3,4].

Coumarins are an old class of compounds obtained from both natural products and synthetic methods. The pharmacological and biochemical properties and therapeutic applications of coumarins depend upon the pattern of substitution and have attracted intense interest in recent years because of their diverse pharmacological properties [5]. Among these properties, their cytotoxic effects were the most extensively examined, this reflects in anticancer activity. Studies have revealed the mechanism behind the anticancer effect of coumarin analogs which include antiangiogenesis and induction of apoptosis independently [6–12]. The current strategies in cancer drug development shifted toward the multiple mechanistic approach and several drugs have been validated and developed. Such validation of structure–system–activity–relationship of coumarins with special respect to angiogenesis and apoptosis leads to cancer-preventing activities should be continued [13]. The vast majority of coumarins, completely innocuous, may be beneficial in a variety of human cancer, in spite of some ongoing controversy [14]. Hence it is very essential to synthesize and develop novel coumarin analogs with multiple targets. In the present study efforts have been made to synthesize novel derivatives of coumarin analogs with antiangiogenic and proapoptotic activity leading to inhibition of tumor growth in mouse model systems.

2. Results and discussion

2.1. Chemistry

The synthesis of the title compounds **5a**–**j** is as outlined in Scheme 1. A series of *N*-[2-(2-aroyl-4-methylphenoxy)-acetyl]-hydrazide methanone coumarins **5a**–**j** were obtained starting from

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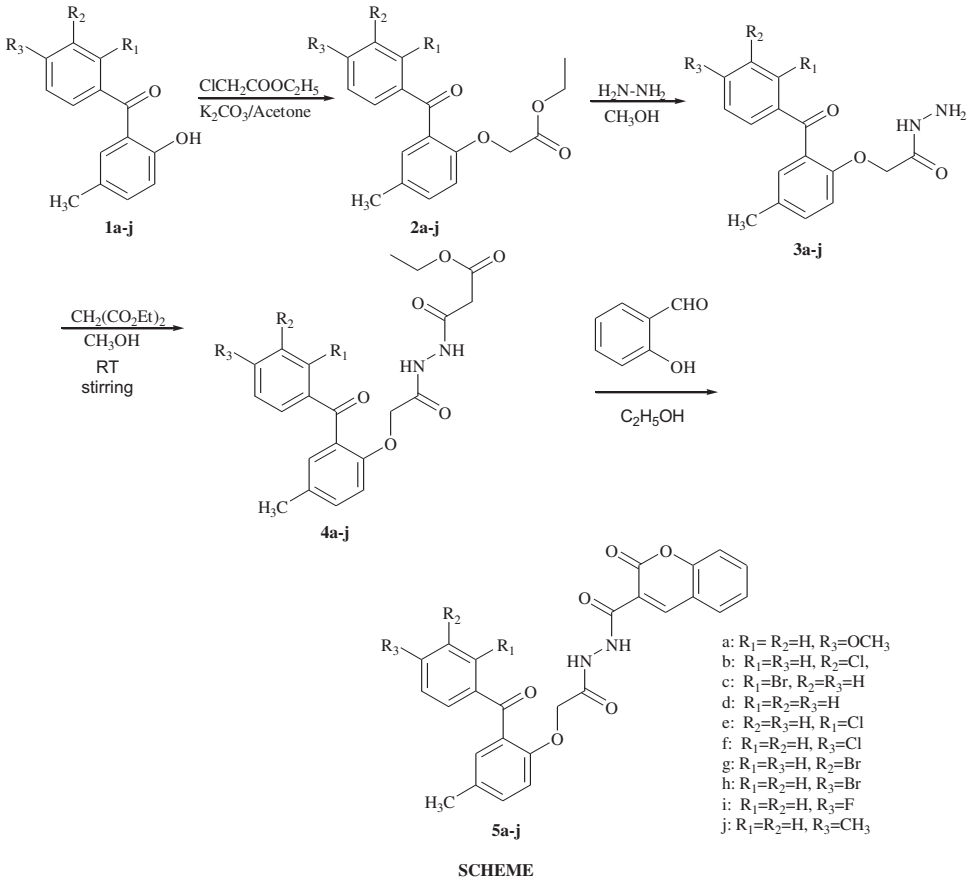

SCHEME

Scheme 1. Synthesis of coumarin conjugated benzophenone analogs.

hydroxyl benzophenones **1a**–**j**. Compounds **1a**–**j** on reaction with ethyl chloroacetate afford ethyl 2-aroyl-4-methylphenoxy acetates **2a**–**j** [19], which on treatment with hydrazine hydrate in the presence of ethanol yields 2-aroyl-4-methylphenoxy acetohydrazides **3a**–**j** [22]. Condensation of **3a**–**j** with diethyl malonate in the presence of methanol at room temperature affords {*N*-[2-(2-aroyl-4-methyl-phenoxy)-acetyl]-hydrazinocarbonyl}-acetic acid ethyl ester **4a**–**j**. Finally the title compounds **5a**–**j** were achieved by intramolecular cyclization of **4a**–**j** with *o*-hydroxy benzaldehyde in the presence of alcohol. The structures of the compounds were confirmed by IR, NMR and mass spectroscopy. In IR spectra the disappearance of O–C stretching band of ester group and appearance of amide C=O and ring C=O stretching bands were observed. Besides, the compounds were confirmed by disappearance of $COCH_2$, CH_2 and CH_3 protons and enhancement in the number of aromatic protons in ^1H NMR spectra and also by mass spectra and CHN analysis.

2.2. Pharmacology

2.2.1. *5c is the lead compound*

The synthesized coumarin analogs were initially tested for their cytotoxic and antiproliferative activity in EAC and DLA cells *in vitro* (Table 1). Among the series of compounds **5a**–**j**, the

Table 1
IC_{50} values of compounds **5a**–**j** calculating based upon trypan blue, MTT at 48 h in EAC and DLA cells.

	EAC cells			DLA cells		
	Trypan blue assay IC_{50} values (μM)	MTT assay IC_{50} values (μM)	LDH release assay IC_{50} values (μM)	Trypan blue assay IC_{50} values (μM)	MTT assay IC_{50} values (μM)	LDH release assay IC_{50} value (μM)
Control	–	–	–	–	–	–
5a	41.2	38.4	41.2	43.5	42.0	43.4
5b	67.3	64.9	65.4	68.6	65.5	67.4
5c	**9.0**	**8.0**	**9.4**	**10.0**	**10.0**	**10.6**
5d	86.5	78.4	81.1	89.1	86.4	87.0
5e	48.4	45.2	47.6	54.8	53.8	54.4
5f	57.8	56.6	58.0	61.6	59.1	60.4
5g	68.5	66.4	68.1	71.9	68.5	69.8
5h	91.1	87.3	89.0	95.5	92.2	95.3
5i	43.4	39.7	40.8	46.1	43.4	45.7
5j	82.6	78.1	81.4	87.3	84.6	87.2
5-Fluorouracil (standard)	16.4	14.3	16.3	14.3	15.7	15.8

The bold values signify potent compound.