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Synthesis and biological evaluation of a novel class of coumarin derivatives

Hong Li [a], Xiaomin Wang [b], Guichao Xu [a], Li Zeng [a], Kai Cheng [a], Pengchao Gao [c], Qing Sun [a], Wei Liao [a], Jianwei Zhang [a,*]

[a] College of Pharmaceutical Sciences, Capital Medical University, Beijing 100069, PR China
[b] Department of Physiology, Key Laboratory for Neurodegenerative Disorders of the Ministry of Education, Capital Medical University, Beijing 100069, PR China
[c] School of Pharmaceutical Sciences, Peking University, Beijing 100191, PR China

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ABSTRACT

In this study, several novel coumarin derivatives, 7-hydroxy-2-oxo-2H-chromene-3-carboxyl-Trp-Trp-AA-OBzl compounds, were designed and synthesized as potential anticancer agents. Their in vitro cytotoxic activities were evaluated using methylthiazoltetrazolium (MTT) assay. The anti-tumor activity of the newly coumarin derivatives was determined in a S180 bearing mouse model and some of the compounds demonstrated tumor growth inhibition similar to the positive control, doxorubicin. Compared to doxorubicin, most of the compounds exhibited enhanced immunologic function suggesting a relatively minor toxic effect. The intercalation of the coumarin derivatives synthesized with calf thymus (CT) DNA was also studied.

Cancer has long been one of the serious diseases threatening human health and continues to be a major health problem worldwide. Therefore, discovering new compounds with potent anticancer activity is of utmost importance. Among current anticancer chemotherapeutic agents DNA-recognizing molecules, including intercalating agents, alkylating compounds and groove binders, are especially interesting. DNA intercalating agents are important in clinical oncology, and several representative compounds (anthracyclines, anthraquinones and acridines) are conventionally used.[1] Intercalation results in conformational alters of the double helix, and then changes the processes of DNA replication, transcription and repairing. Thus the discovery of novel DNA intercalating agents is considered as a promising approach toward anticancer drugs.

Coumarins are the most important classes of natural products with a variety of pharmacological activity. Coumarin and Coumarin-related compounds have proved for many years to have significant therapeutic potential and are a plentiful source of potential drugs candidate in relation to its safety and efficacy. The bioactivity of coumarin and more complex related derivatives appears to be based on the coumarin nucleus.[1] Biological effects observed include anti-bacterial,[2] anti-thrombotic and vasodilatory,[3] anti-mutagenic,[4] lipoxygenase and cyclooxygenase inhibition,[5,6] scavenging of reactive oxygen species, and anti-tumour.[7–13] Previous studies on a variety of synthetic coumarin derivatives have demonstrated the influence of the coumarin skeleton and substitutions at Positions 3 and 7 on antitumor activities.[14,15] Some studies demonstrated that coumarin derivatives containing a substituted hydroxy group at the Position 7 possess antibiotic and antifungal activities. The in vitro effects of coumarins on the growth of renal cell carcinoma-derived cell lines showed that coumarin and 7-hydroxycoumarin were potent cytotoxic and cytostatic agents.[16] The hydroxyl group on the coumarin ring is crucial to its anti-cancer activity. Some studies demonstrated that hydroxylated derivatives containing a substituted carboxylic group at Position 3 were capable of decreasing cancer cell viability and inhibiting DNA synthesis.

It is well proved that Trp-Trp is biologically important either as a dipeptide or as a fragment of some peptides. Trp-Trp-OBz was used as a lead, and twenty tripeptide benzyl esters, Trp-Trp-AA-OBz, were synthesized as DNA intercalators.[17] Coumarin-3-carboxamides were reported to exhibit selective cytotoxicity against mammalian cancer cell lines and as inhibitors of serine proteases, α-chymotrypsin (CT) and human leukocyte elastase (HLE).[18] In view of the importance associated with the above cited moieties in their potent anti-tumor activity, it was thought of considerable interest to synthesize derivatives of Trp-Trp-AA-OBz for the generation of new 7-hydroxy-2-oxo-2H-chromene-3-carboxyl

* Corresponding author. Tel.: +86 10 8391 1522; fax: +86 10 8391 1533.
E-mail address: jwzhang2006@163.com (J. Zhang).

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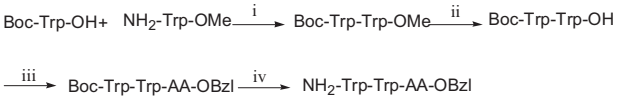
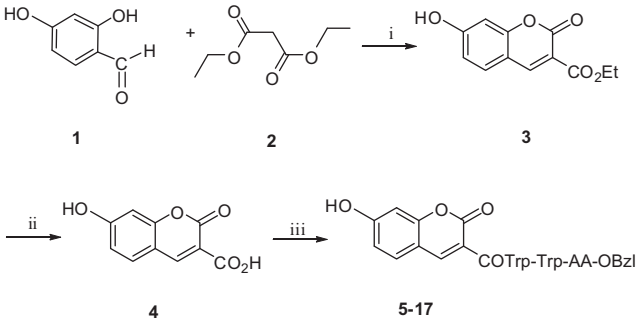
coumarins which are represented in the Table 1. In addition, the use of tripeptide in coumarin derivatives designed was based upon the concept that many amino acids with functional side chains are capable of making base-specific contacts with more than one type of DNA bases.[19] Moreover, amino acid conjugates might target the gastrointestinal transporters involved in the absorption of amino acids and small peptides resulting in improved oral bioavailability.[20,21] The various side chains of different amino acids allow the addition of amino acids to coumarin to manipulate the pharmacokinetics profiles of the compounds. In addition, various amino acids can be introduced to enhance solubility. Therefore, in order to search for better anti-tumor agents, coumarin was used as the basic molecule and a series of novel 7-hydroxycoumarin derivatives bearing Trp-Trp-AA-OBzl at Position 3 were designed and synthesized and evaluated for their anti-tumor activity in the present study.

As shown in Scheme 2, the syntheses of coumarin derivatives were carried out using a multi-step synthetic route. Firstly, ethyl 7-hydroxy-2-oxo-2H-chromene-3-carboxylate (**3**) was synthesized via the Knoevenagel condensation of 2,4-dihydroxybenzaldehyde (**1**) with diethyl malonate (**2**) in piperidine in an almost 90% yield. Subsequently, removal of the ethyl group with refluxing 4M hydrochloric acid resulted in 7-hydroxy-2-oxo-2H-chromene-3-carboxylic acid (**4**).[22] Finally, tripeptide benzyl esters, NH_2-Trp-Trp-AA-OBzl, from Scheme 1 were introduced into **4** by the DCC/HOBt/*N*-methylmorpholine (NMM) procedure to provide the respective coumarin derivatives, 7-hydroxy-2-oxo-2H-chromene-3-carboxyl-Trp-Trp-AA-OBzl (**5**–**17**), as shown in Scheme 2 (yields, 59–84%). The chemical structures of all coumarin derivatives (**3**–**17**) are provided in Table 1 and were confirmed by ^{1}H NMR, ^{13}C NMR, IR, and HR-ESIMS. The spectroscopic data are given in the 'Supporting information' Section. The ^{1}H NMR spectra of coumarin derivatives showed signals at δ 8.67–8.78 for one proton corresponding to H-4 of the coumarin skeleton. The two pairs of 1H double doublet at δ 4.72–4.87, 4.62–4.77 are in agreement with α-methine proton of tryptophan. Signals at 4.72–4.87 and 4.62–4.77 in the COSY spectrum indicated methylenes adjacent to



Scheme 1. Preparation of NH_2-Trp-Trp-AA-OBzl. Reagents and conditions: (i) NMM, DCC, HOBt; (ii) 2 M NaOH; (iii) NH_2-AA-OBzl, NMM, DCC, HOBt; (iv) hydrogen chloride in ethyl acetate (4 mol/L).

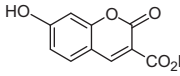
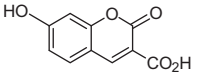
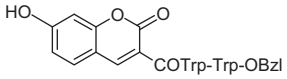
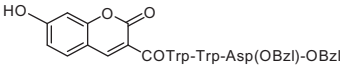
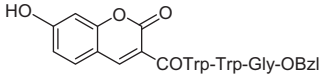
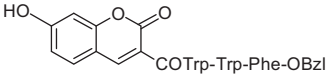
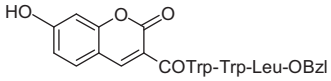
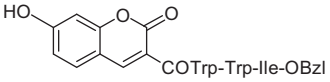
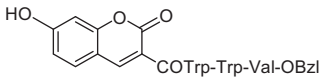
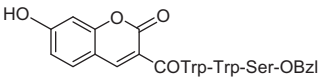
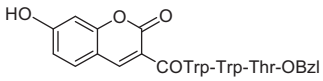
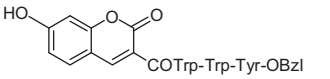
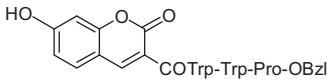
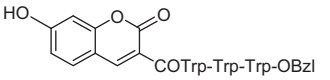


Scheme 2. Synthesis of 7-hydroxy-2-oxo-2H-chromene-3-carboxylic acid-Trp-Trp-AA-OBzl compounds. Reagents and conditions: (i) diethyl malonate and piperidine; (ii) HCl; (iii) NH_2-Trp-Trp-AA-OBzl, NMM, DCC, HOBt.

methines. ^{1}H NMR chemical shifts values at δ 3.22–2.87 suggested the presence of methylene protons of tryptophan. ^{1}H NMR spectroscopic signals at δ 4.68–3.94 are in agreement with α-methine proton of the AA in NH_2-Trp-Trp-AA-OBzl.

The in vitro cytotoxicity of the coumarin derivatives synthesized above was evaluated in human lung adenocarcinoma cells (A549), chronic myeloid leukemia cells (K562), human liver carcinoma cells (HepG2), Human Glioblastoma cells (A172), and human hepatocellular carcinoma cells (Bel-7402) using MTT assay.[23] Doxorubicin (adriamycin, ADM) was used as positive control. In brief, cells were exposed to **4**–**17** of concentrations ranging from

Table 1
Structures of coumarin derivatives synthesized

Compound No.	Chemical structure	Compound No.	Chemical structure
3		**4**	
5		**6**	
7		**8**	
9		**10**	
11		**12**	
13		**14**	
15		**16**	
17