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Original article

# Synthesis, *in vitro* and *in vivo* antitumor activity of scopoletin-cinnamic acid hybrids

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## ABSTRACT

A series of hybrids of scopoletin and substituted cinnamic acid were designed, synthesized and evaluated *in vitro* and *in vivo* against five human tumor cell lines [MCF-7, MDA-MB-231, A549, HCT-116, and HeLa] with doxorubicin as the positive control. Compounds **17a**, **17b**, **17c** and **17g** exhibited potent cytotoxic activity. Especially, compound **17b** displayed broad spectrum activity with $IC_{50}$ values ranging from 0.249 μM to 0.684 μM. Moreover, in a preliminary pharmacological study, **17b** not only remarkably induced cellular apoptosis, but also clearly induced A549 cells cycle arrest at S phase. *In vivo* study showed that **17b** significantly suppressed tumor growth in a dose-dependent manner without causing the loss of the mean body weight of mice, which was superior to doxorubicin. These preliminary results indicate that **17b** is an optimal anti-cancer leading compound and merit further structural modification.

## 1. Introduction

Coumarins widely present in higher plants such as Rutaceae, Apiaceae, Leguminosae, Thymelaeaceae, as well as occur as animal and microbial metabolite, playing an important role in the agricultural and pharmaceutical industries [1]. Most of them and their derivatives are found to possess versatile biological activities, including anticancer [2–7], enzyme inhibition [8], antioxidant [9,10], anti-inflammatory [11], and anti-HIV [12].

Scopoletin (6-methoxy-7-hydroxy-coumarin) is a phenolic coumarin (Fig. 1) and has been isolated from many medicinal plants, such as *Erycibe obtusifolia*, *Aster tataricus*, and *Foeniculum vulgare*, which is commonly used in traditional Chinese medicine (TCM) for treating various rheumatoid diseases with a long history [13]. Reports have indicated that scopoletin exhibits significant anti-tumor [14–16] and anti-angiogenesis activity [17]. These studies demonstrated that scopoletin could induce cell cycle arrest and increase apoptosis in human prostate tumor cells and human leukemia cell line via activation of caspase-3 and blocking angiogenesis by inhibiting the endothelial cell growth. While its antitumor activity is not potent enough to be used directly in clinic. Therefore, scopoletin is considered to be an ideal lead compound for anti-tumor agents. Many derivatives have been designed and synthesized to increase its anti-tumor activity [18–21].

Cinnamic acid (Fig. 1) is a naturally occurring aromatic fatty acid composed of a phenyl ring substituted with an acrylic acid group, commonly in the trans-geometry and with low toxicity in human exposure [22]. Cinnamic acid and hydroxyl cinnamic acid derivatives, such as caffeic acid, sinapinic acid and ferulic acid (Fig. 1), were found in coffee, apples, citric fruits, vegetable oils, propolis and wine [23]. In recent years, trans-cinnamic acid derivatives have attracted many attentions due to their anticancer, antioxidant, and antimicrobial properties [24]. Mechanism study indicated that cinnamic acid could induce apoptotic cell death and cytoskeleton disruption in human melanoma cells in 2013 [25]. P. De et al. provides a comprehensive review concerning cinnamic acid derivatives in anticancer research [26]. In this review, we found that many natural and synthetic compounds would have an increasing anticancer activity when cinnamoyl residues were introduced in the form of amides or esters. In addition, cinnamic acid possessing α, β-unsaturated ketones carbonyl moiety are often applied in the design of antitumor drugs [27,28]. These findings prompted us to design and synthesize the hybrids of cinnamic acid and scopoletin

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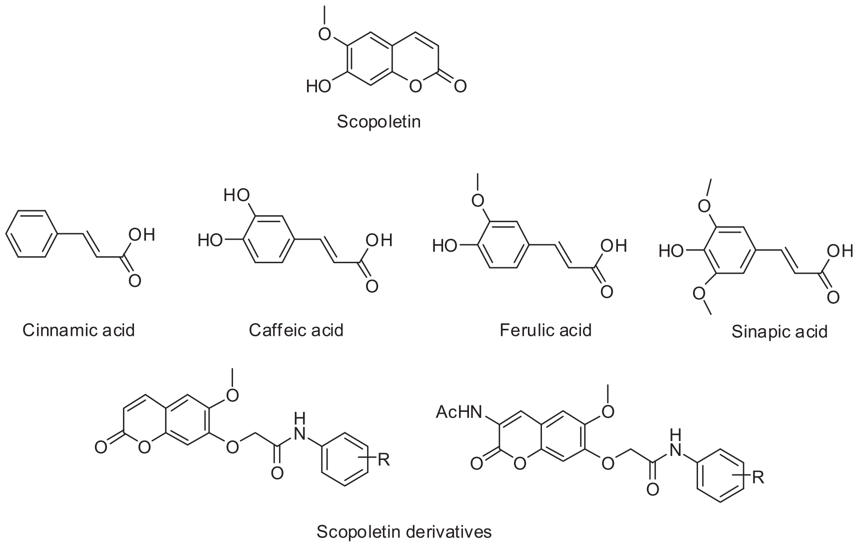

**Fig. 1.** Structures of some natural and synthetic cinnamic and scopoletin derivatives.

as anti-tumor agents with high potency and low toxicity.

Herein, a series of scopoletin-cinnamic hybrids (Fig. 2) were synthesized and screened for cytotoxicity against five human tumor cell line using doxorubicin as a potent antitumor reference. Then, the most potent cytotoxic compounds **17a**, **17b**, **17c** and **17g** were assayed for their anti-proliferative activity indicated by $IC_{50}$ values. **17b** was selected for further pharmacological studies in A549 human lung cancer cell line to more fully elucidate the compound' mechanisms of action and anti-tumor activity in mice with lung cancer.

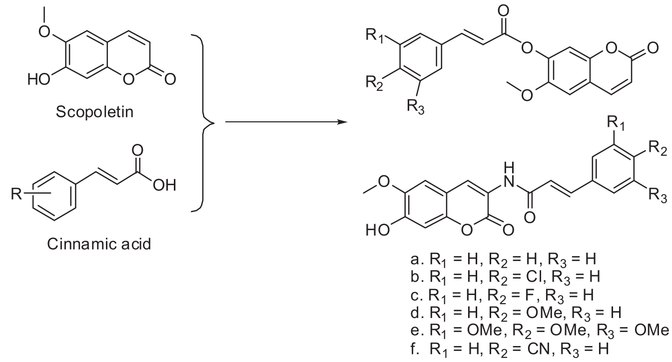

**Fig. 2.** Design of scopoletin-substituted cinnamic acid hybrids.

## 2. Results and discussion

### 2.1. Chemistry

The synthetic route of target compounds **7a**–**f** is outlined in Scheme 1. The synthesis of scopoletin **6** was reported previously [18,19]. Compound **4** was synthesized in a one-step reaction from commercially 2, 4, 5-trimethoxybenzaldehyde **3** by treatment with aluminum (III) chloride in dry dichloromethane and followed acid hydrolysis. Compound **4** was treated with malonic acid in pyridine at room temperature (rt) using phenylamine as catalysts affording scopoletin-3-carboxylic acid **5**, which was then decarboxylated by refluxing in pyridine/ethylene glycol mixture to give scopoletin **6**. Cinnamic acid analogs **1a**–**f** were converted to acid chlorides **2a**–**f** in the present of thionyl chloride using DMF as catalyst. Finally, intermediates **2a**–**f** were treated with scopoletin in pyridine to obtain target hybrids **7a**–**f**.

In the synthesis of **7g** and **7h**, a benzyl protected group was used as shown in Scheme 2 to avoid side reactions of the phenolic hydroxyl group of ferulic acid **1h** [29]. Benzyl *O*-benzylferulate **9** was obtained through the alkylation of **1h** with benzylbromide and potassium carbonate in DMF. The protected *O*-benzylferulic acid **1g** was afforded by the hydrolysis of ester **9** with potassium hydroxide in methanol-water (9:1). The combination of **2g** with scopoletin was the same as that of **7a**–**f**. Removal of the benzyl group was achieved by hydrogenation in chloroform at room temperature using Pd/C. But the double bond was reduced simultaneously and **7h** was obtained unexpectedly.

The synthetic pathway of target compounds **17a**–**g** as shown in Scheme 3 was also started from **4**, which was condensed with glycine in acetic anhydride using sodium acetate as base. Burk and Allen's one-pot protocol was employed to convert the acetamide **12** to Boc-protected 3-amino-scopoletin **14** [30]. The key intermediate **15** was afforded after removing the Boc-group using 15% $TFA/CHCl_3$. The next step involved double acylation of hydroxyl group and amine group to afford **16a**–**g** without purification, which were selectively hydrolyzed by $K_2CO_3$ in methanol to give the target compounds **17a**–**g**.

The chemical structures of all the new compounds were established on the basis of analytical and spectral data. The mass spectra showed molecular ion peaks $[M+H]^+$ for **7a**–**h** and $[M-H]^+$ for **17a**–**g**. In the IR spectra, the target compounds exhibited the characteristic peak of cinnamic acid double bond at 1680–1620 $cm^{-1}$. The two absorption bands in the region 1750–1650 $cm^{-1}$ revealed the presence of C=O from coumarin and cinnamic acid. In $^1H$ NMR spectra, aromatic protons resonated at $\delta$ 6.4–7.9 ppm for **7a**–**h**, and $\delta$ 6.8–8.9 ppm for **17a**–**g**. The presence of –CONH– group and Ar-OH group of **17a**–**g** was confirmed by two proton singlet between $\delta$ 9.6–9.9 ppm and $\delta$ 10.0–10.7 ppm respectively. The conjugated double bond of cinnamic acid showed two doublet around $\delta$ 7.8 and 6.6 ppm, and the *E*-isomeric form was confirmed and characterized on the basis of coupling constant values ($J$ = 13.8–17.1 Hz). *Cis* coupling ($J$ = 9.5–9.6 Hz) was observed between H3 and H4 of **7a**–**h** coumarin skeleton. The coupling was disappeared in $^1H$ NMR spectra of **17a**–**g** due to the substitution of H3 with –NH–, and the chemical shift of H4 moved