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Design, synthesis and cytotoxic evaluation of novel scopoletin derivatives

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Abstract: A series of scopoletin derivatives were designed and synthesized by introducing α-aminoacetamide, acrylamide and β-aminopropamide respectively to 3-position of scopoletin and their chemical structures were confirmed by ESI-MS, IR, ^{1}H NMR and ^{13}C NMR spectra. All target compounds were evaluated *in vitro* against four human cancer cell lines (MDA-MB-231, MCF-7, HepG2 and A549) by MTT method. Cytotoxic assay showed that compounds **7a**, **7b**, **7e**, **7f**, **8a** and **8e** exhibited more potent cytotoxicities compared to scopoletin. Besides, we have further evaluated the growth inhibitory activities of these selected compounds against normal tissue cell lines HFL-1. Although compound **8a** showed the strongest anti-proliferative activity *in vitro*, it exhibited strong cytotoxicity on normal cells HFL-1, which limited its further study. Compound **7a** and **7b** exhibited higher anti-proliferative activity against MDA-MB-231 and HepG2 cells and weak cytotoxicity on HFL-1, which suggested that **7a** and **7b** might be ideal anticancer candidates. The SARs showed that the introduction of the acrylamide and its analogues β-aminopropamide could significantly improve activity, while the α-aminoacetamide failed to enhance potency obviously. Therefore, the mechanism of compound **7a** and **7b** is worthy of further research and the structure of compound **8a** should be further optimized.

Key words: scopoletin, α-aminoacetamide, acrylamide, β-aminopropamide, cytotoxic activity

Coumarins, widely present in higher plants, animals and microbial metabolites, play a major role in agricultural and pharmaceutical industries (1). Most of them and their derivatives possess a variety of biological activities including anticancer, antioxidant, antidepressant and anti-inflammatory (2, 3).

Scopoletin (6-methoxy-7-hydroxycoumarin), a phenolic coumarin present in many medicinal plants, has been proven to possess versatile pharmacological properties, such as anti-tumor, anti-inflammatory and hypouricemic activities (4, 10, 11). In recent years, the mechanism of antitumor effects of scopoletin has been reported. It was able to induce cell cycle arrest and trigger apoptosis via activation of caspase-3 and inhibition of the endothelial

cell growth in human prostate tumor cells and leukemia cells (5-7). More important, scopoletin also has no cytotoxicity against normal cells. However, its low antitumor activity *in vitro* and *in vivo* and a high elimination rate *in vivo* limited its clinical application. Therefore, scopoletin is regarded as an ideal lead compound and our group have designed and synthesized a great deal of derivatives (8, 9). Some scopoletin derivatives exhibited higher antitumor activities *in vitro* and *in vivo* than scopoletin **(Fig. 1)**. For example, those scopoletin derivatives which cinnamic acid was introduced into 7-position of scopoletin or 3-position of 3-amino scopoletin were screened for *in vitro* cytotoxic activity against five human tumor cell lines (MCF-7, MDA-MB-231, A549, HCT-116 and Hela) with doxorubicin as the positive control (8). Among them, compound SC-III$_3$ **(Fig. 1)**, (E)-3-(4-chlorophenyl)-N-(7-hydroxy-6-methoxy-2-oxo-2H-chromen-3-yl)acrylamide, showed high potent anticancer activity at low concentrations and had no cytotoxicity to normal liver cells *in vitro* and significantly dose-independently suppressed tumor growth without causing the decreasing of the mean body weight of mice *in vivo* (10).

Fig. 1

The mechanism of SC-III$_3$ was that it could induce cytotoxicity in hepatocellular carcinoma through ROS mediated DNA damage and ataxiatelangiectasia-mutated nuclear protein kinase activation (10). In addition, SC-III$_3$ also led to the autophagy of human hepatoma HepG2 cells through inducing mitochondrial dysfunction, depleting ATP and activating AMPK-mTOR pathway (11). Further study of the structure-activity relationships (SARs) of these active scopoletin derivatives revealed that almost all the compounds have α, β-unsaturated carbonyl group (the Michael-acceptor), and as we mentioned above, a great number of studies reported that this group could interact with nucleophilic residues in the body which suggested it might be a pharmacophore (12-19). For example, irreversible acrylamide-based inhibitors, the Michael-acceptor compounds, could form covalent bonds with cysteine or other nucleophilic residues in the protein to get the conjugate-addition products. The irreversible acrylamide inhibitors have a lot of potential advantages including enhanced pharmacological potency and selectivity and prolonged pharmacodynamics (17-19). However, the high reactivity of the acrylamide group may cause rapid metabolism or nonspecific reaction with off targets (19). In order to solve this problem, a protection strategy for the acrylamide would be beneficial. It is reported that the β-aminoethyl ketones could be bioconverted through a β-elimination reaction to the α,β-unsaturated ketones, and then reacted with nucleophiles, such as thiols of cysteines (20-22). According to this strategy, Carml et al (20) converted the acrylamide to the β-aminopropamide which could undergo a β-elimination reaction to release the acrylamide, and then reacted with thiols of cysteine residues in the intracellular environment **(Fig. 2)**.

Fig. 2

Based on these findings and our previous researches, for one thing, in this paper we hypothesized that introduction of acrylamide and β-aminopropamide to 3-position of scopoletin could obtain novel anticancer reagents. For another, in order to compare the effects of different linker on the activity of scopoletin, the

α-aminoacetamide was also introduced to 3-position of scopoletin. Therefore, a series of scopoletin derivatives by introducing α-aminoacetamide, acrylamide and β-aminopropamide respectively to 3-position of scopoletin were synthesized and screened for cytotoxicity four tumor cell line using scopoletin as a positive control **(Fig. 3)**. Then, the most potent cytotoxic compounds **7a**, **7b**, **7e**, **7f**, **8a** and **8b** were assayed for their anti-proliferative activity indicated by IC_{50} values.

Fig. 3

Results and discussion

Chemistry

The synthetic pathway of target compounds **7a-7i** was summarized in **Scheme 1**. According to the previous reported method (23, 24), compound **2** was synthesized from 2,4,5-trimethoxybenzaldehyde **1** by treatment with dry aluminum chloride in dry DCM and followed acid hydrolysis. Compound **2** was condensed with glycine in acetic anhydride using sodium acetate as base. Burk and Allen's one-pot protocol (25) was applied to convert acetamide **3** to Boc-protected-3-amino-scopoletin **4**. Compound **5** was synthesized from **4** and acetic anhydride by treatment with DMAP and pyridine in dry DCM at room temperature. The intermediate **6** was afforded via removing the Boc-group using 20% TFA/DCM. Compound **7a** was synthesized in one-step reaction from **6** and acryloyl chloride using trimethylamine as acid-binding agent, which was hydrolyzed by potassium carbonate in methanol at reflux to get compound **7b**. The next step was that **7b** was reacted with various secondary amines in CH_3CN/THF (v/v=3:1) at reflux to get the target compounds **7c-7i.**

Scheme 1

The synthetic route of target compounds **8a-8i** was outlined in **Scheme 2**. Compound **6** was treated with chloroacetyl chloride in dry DCM at room temperature using trimethylamine as acid-binding agent to get compound **8a**, which was reacted with various secondary amines by treatment with anhydrous potassium carbonate and potassium iodide in 2-butanone, and then hydrolyzed by potassium carbonate in methanol to give the target compounds **8b-8i**.

Scheme 2

Pharmacology

All target compounds **7a-7i** and **8a-8i** were preliminarily evaluated *in vitro* for their cytotoxicity to human breast cancer cells MDA-MB-231 and MCF-7 and human liver cancer cells HepG2 respectively by using the standard MTT method adopting scopoletin as a positive control. All data were given in **Table 1**. The results showed that several compounds (the inhibition rate greater than 50%) exhibited strong growth inhibitory activities against the tested cells, such as **7a**, **7b**, **7e**, **7f**, **8a** and **8e**.

Table 1 Growth inhibition (%) obtained from the single dose (100 μM, 72h) test.

Compd.	Growth inhibition (%)		
	MDA-MB-231	HepG2	MCF-7
7a	93.55	81.61	83.75
7b	94.64	90.63	89.25
7c	2.77	16.59	23.05
7d	14.16	7.36	19.49
7e	46.03	71.15	89.91
7f	54.91	62.29	91.36
7g	NE	NE	7.35
7h	NE	NE	7.91
7i	22.78	18.12	12.85
8a	94.68	84.72	87.52
8b	24.94	5.82	19.26
8c	5.87	NE	12.92
8d	25.58	NE	8.61
8e	57.21	61.97	78.84
8f	11.96	NE	2.67
8g	8.72	19.33	12.28
8h	28.26	23.21	18.21
8i	NE	34.81	74.31
scopoletin	8.47	3.67	2.63

NE = no effect at 100 μM.

Compounds **7a**, **7b**, **7e**, **7f**, **8a** and **8e** were next assayed for their anti-proliferative activity against human breast cancer cells MDA-MB-231, human liver cancer cells HepG2 and human lung cancer cells A549 indicated by IC_{50} values, which were summarized in **Table 2**. It was found that these compounds displayed significant anti-proliferative activity compared to scopoletin. Compound **7a**, **7b** and **7f** exhibited higher anti-proliferative activity against MDA-MB-231 and HepG2 cells, while the compound **7e** and **8e** manifested lower activity against three human tumor cells. Especially, compound **8a** displayed the strongest anti-proliferative activity against most human cancer lines with IC_{50} values ranging from 2.195 to 3.961 μM. Based on these results, we have further assayed the growth inhibitory activities of six active compounds against the human normal tissue (human lung fibroblasts) cell lines and the datum are summarized in **Table 3**. The results showed that **7a** and **7b** displayed weak cytotoxicity on HFL-1, which suggested that **7a** and **7b** might be ideal anticancer candidates. Their mechanism of anti-proliferative activity is worthy of further research. Compound **8a** has a good activity *in vitro* while it exhibits strong cytotoxicity on normal cells HFL-1, which has limited its further study. Considering this reason, the structure of compound **8a** should be further optimized in the future. From the SARs we may conclude that the introduction of the acrylamide and its analogue β-aminopropamide can significantly improve cytotoxicity, while the α-aminoacetamide fails to enhance potency obviously. This preliminary result indicates that

β-aminopropamide may be converted into Michael receptor acrylamide to play biological activity. In addition, the activity of chloroacetamide at 3-position of scopoletin was superior to the α-aminoacetamide at 3-position of scopoletin. When the β-aminopropamide was introduced, the piperidine and 4-methylpiperidine analogues (**7e** and **7f**) exhibited good activity. At the same time, when the α-aminoacetamide was introduced, the piperazine analogue (**8e**) showed good activity.

Table 2 IC_{50} values of compounds **7a**, **7b**, **7e**, **7f**, **8a** and **8e** as determind based on MTT assay

Compd.	IC_{50}(μM)		
	MDA-MB-231	HepG2	A549
7a	8.882±0.93[a]	8.710±0.94	>100
7b	21.96±2.29	4.80±0.11	>100
7e	25.46±1.42	58.20±2.16	>100
7f	8.579±0.48	14.15±0.71	>100
8a	2.195±0.21	3.721±0.61	3.961±0.89
8e	71.86±2.45	30.97±1.49	41.16±1.73
scopoletin[b]	>100	>100	>100

[a] Values represent mean ± standard deviation for at least two separate experiments performed in triplicate.

[b] Positive control drug.

Table 3 Cytotoxicities (IC_{50}, μM) of six active compounds on normal cell HFL-1

Compd.	HFL-1	Compd.	HFL-1
7a	47.04±1.45[a]	**7f**	18.56±1.66
7b	42.17±0.98	**8a**	2.56±0.87
7e	166.2±2.18	**8e**	21.25±1.28

[a] Values represent mean ± standard deviation for at least two separate experiments performed in triplicate.

Conclusion

In summary, a series of scopoletin derivatives by introducing α-aminoacetamide, acrylamide and β-aminopropamide respectively to 3-position of scopoletin were synthesized and tested their anti-proliferative activity against four human cancer cell lines (MDA-MB-231, MCF-7, HepG2 and A549). Cytotoxic assay showed that compounds **7a**, **7b**, **7e**, **7f**, **8a** and **8e** exhibited high potent cytotoxicities compared to scopoletin. Among them, compound **7a** and **7b** exhibited higher anti-proliferative activity against MDA-MB-231 and HepG2 cells and weak cytotoxicity on normal cells HFL-1, which suggested that **7a** and **7b** might be ideal anticancer candidates. Compound **8a** showed the strongest anti-proliferative activity *in vitro* while it exhibited strong cytotoxicity on normal cells HFL-1, which had limited its further study. From the SARs we may conclude that the introduction of the acrylamide and its analogue β-aminopropamide can significantly improve activity, while the α-aminoacetamide fails to enhance potency obviously. Therefore, the mechanism of compound **7a** and **7b** is worthy of further research and the structure of compound **8a** should be further optimized.

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Conflict of Interest statement

The authors confirm that this article content has no conflict of interest.

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Supporting Information

Experimental section, ESI-MS and ^{1}H NMR of synthetic compounds

Figure Legends

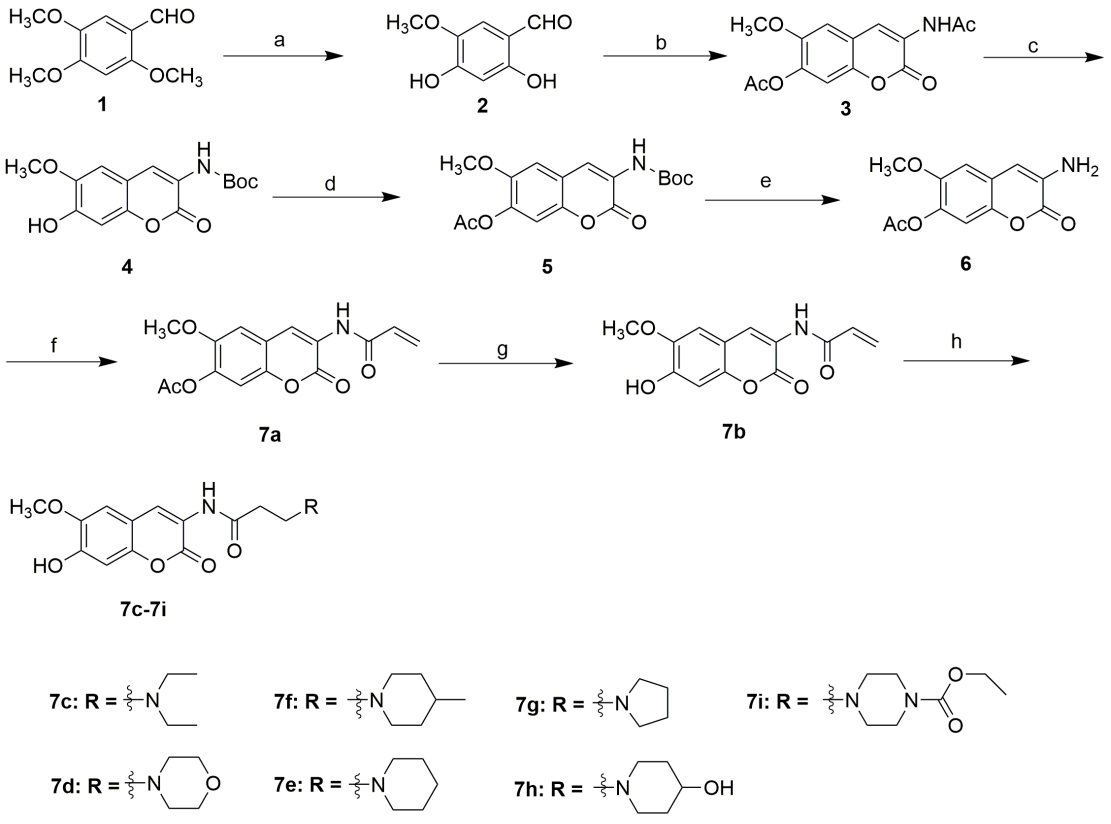
Fig. 1. Structures of scopoletin and its derivatives

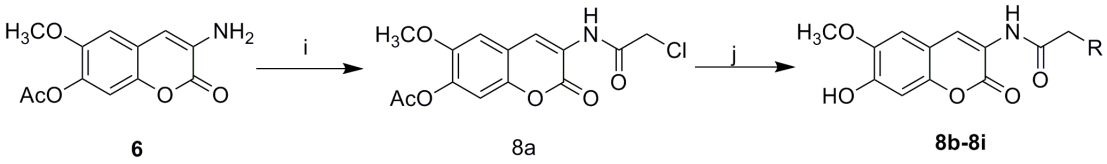
Fig. 2. The mechanism between acrylamide and β-aminopropamide

Fig. 3. Design of scopoletin-substituted analogues

Scheme 1. Synthesis of target compounds **7a-7i**. Reagents and conditions: (a) $AlCl_3$ (6eq), dry CH_2Cl_2, CTAB (0.002eq), reflux; (b) glycine (2eq), Ac_2O, AcONa (4eq), reflux, (c) Boc_2O (2eq), DMAP (0.2eq), THF, reflux; then hydrazine hydrate (0.3eq), CH_3OH, rt; (d) Ac_2O (1.3eq), DMAP (0.2eq), pyridine (2.5eq), dry CH_2Cl_2, rt; (e) trifluoroacetic acid, CH_2Cl_2, rt; (f) acryloyl chloride (1.5eq), Et_3N (2.5eq), dry CH_2Cl_2, rt; (g) K_2CO_3 (1.1eq), CH_3OH, reflux; (h) various secondary amines (2eq), CH_3CN/THF (v/v=3:1), reflux.

Scheme 2. Synthesis of target compounds **8a-8i**. Reagents and conditions: (i) Et_3N (1.5eq), chloroacetyl chloride (1.3eq), dry CH_2Cl_2, rt; (b) various secondary amines (1.3eq), K_2CO_3 (2.5eq), KI (0.1eq), 2-butanone, reflux; then K_2CO_3 (1.1eq), CH_3OH, reflux.





6 8a **8b-8i**

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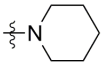
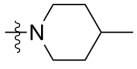
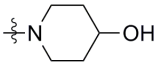
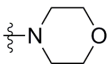
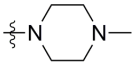
8d: R = 

8f: R = 

8h: R = 

8c: R = 

8e: R = 

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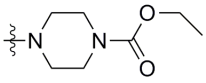
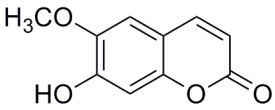
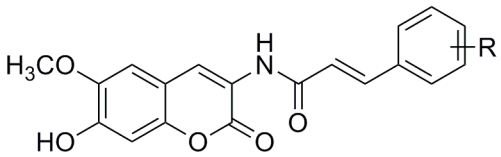
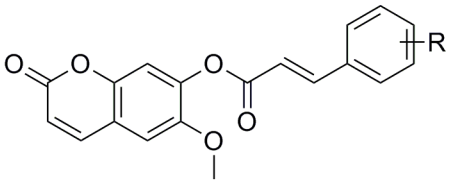
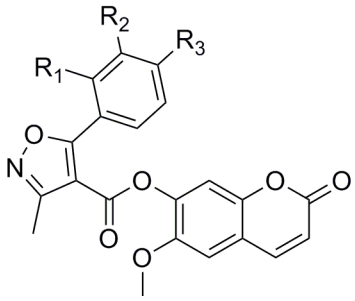
8i: R = 

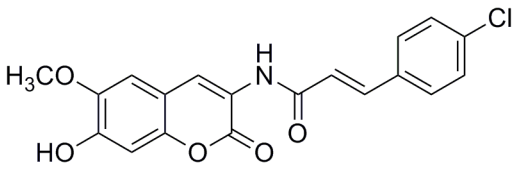


Scopoletin









SC-III3

Scopoletin derivatives