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Total synthesis and cytotoxicity evaluation of syrinenin-4-*O*-farnesylether and its analogues

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First synthesis of natural product, syrinenin-4-*O*-farnesylether (**1**), was carried out via two different paths. Four of its derivatives (**9**–**12**) were also prepared. Cytotoxicity screening of the selected compounds were performed on six tumour cell lines. Compound **12** exhibited prominent IC_{50} values of 1.9 μM and 0.8 μM on CNE and PC-3 cells, respectively.

Keywords: Syrinenin derivative; Syrinenin-4-*O*-farnesylether; Biological activity; Cytotoxicity

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1. Introduction

The family Compositae, widely distributed in China, is abundant with syrinenin derivatives [1–3]. One example is syrinenin-4-*O*-farnesylether (**1**), which was isolated from the aerial part of *Gypothamnium pinifolium* [2]. Although there was no report on biological assays available for this natural product, Zhao et al. reported that the structural similar syrinenin derivatives **2**–**7** (figure 1) from *Ligularia nelumbifolia*, a traditional herbal medicine belong to Senecioneae at Yunnan Province, were cytotoxic to KB cells with IC_{50} values at the micromolar scale [3]. The authors further indicated that when the 4-*O* side chain of the aromatic ring was prolonged, the corresponding cytotoxicity became stronger, e.g. from 4-*O*-isopentyl derivative **2** to 4-*O*-geranyl derivative **3**, their values to KB cells decreased from 7.8 to 3.0 μM. It was also found that the geranyloxy sinapyl aldehyde **4** possesses nearly equal cytotoxicity against KB cells ($IC_{50} = 2.6$ μM) with geranyloxy sinapyl alcohol **3** [3].

To further investigate the structure-activity relationship (SAR) of this type of compound, especially the SAR characters of **1**, the allylic acid derivative of **1** (**11**), the ethyl ester (**10**), the aldehyde analogue of **1** (**12**), as well as the benzaldehyde intermediate (**9**) were also synthesized and subjected to the screenings of six tumour cell lines, including PC-3, CNE, KB, A549, BEL-7404 and HeLa. The synthetic procedure and the cytotoxic results of compound **1** and related analogues are reported herein.

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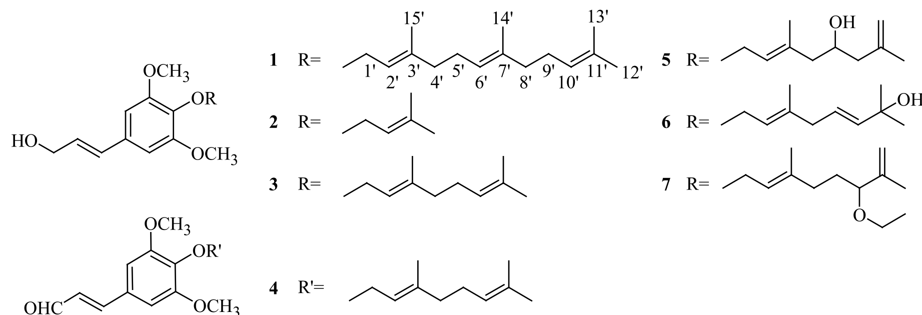

Figure 1. Structures of the related syrinenin derivatives **1–7.**

2. Results and discussion

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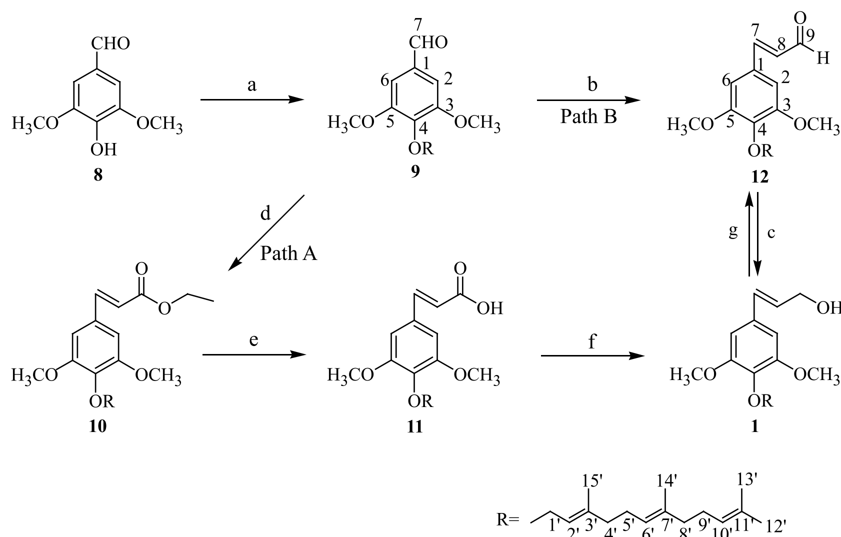
Two synthetic paths (Path A and Path B) [4–10] utilized to prepare compound **1** (scheme 1) were both starting from the same material, 4-hydroxy-3,5-dimethoxy benzaldehyde (**8**), which was derived from naturally abundant gallic acid via four steps [7,10–12]. In path A, **8** was subjected to an alkylation on 4-OH by farnesyl bromide at the presence of potassium carbonate under reflux [4]. The resulting benzaldehyde (**9**) was further condensed with (carbethoxymethylene)-triphenylphosphorane by Wittig reaction [5] to give an allylic acid ethyl ester (**10**) (69.5%), which was hydrolysed to afford equivalently an allylic acid **11** [6]. Compound **11** was further reduced by lithium aluminium hydride in ether to afford the target molecule **1** in 78% yield [7]. The total yield of **1** in path A is 17%.

By another synthetic process (path B), the alkylated aldehyde **9** was condensed directly with 40% acetaldehyde in EtOH at room temperature to give an allylic aldehyde **12** (65%) [8], which was then reduced by $NaBH_4$ to afford **1** in 85% yield [9]. The total yield of **1** in path B is 17.5%. Moreover, oxidation of **1** by pyridinium chlorochromate–aluminium oxide



Scheme 1. Synthetic paths of **1** and its derivatives. Reagents and conditions: (a) RBr, K_2CO_3, acetone, reflux, 3 h; (b) 40% CH_3CHO, EtOH, room temperature, 24 h; (c) $NaBH_4$, MeOH, 0°C, 1 h; (d) $Ph_3PCH = CO_2Et$, benzene, reflux, 2 h; (e) KOH, EtOH, H_2O, reflux, 3 h; (f) $AlLiH_4$, Et_2O, 10°C, 30 min; (g) PCC, CH_2Cl_2, room temperature, 6 h.

complex (PCC–Al_2O_3) gave the allylic aldehyde **12** in 70% yield [10]. The structures of synthetic compounds were confirmed by ^{1}H NMR, ^{13}C NMR and MS spectral data.

The cytotoxicity of the synthetic compounds (**1**, **9–12**) was determined by the colorimeter assay MTT [13–15] using Cisplatin (DDP) as a positive control (see Section 3). By a scrutiny on the MTT assay results (table 1), it was found that compared to previously reported data on KB and A-549 cell lines [3], the 15 carbon 4-*O*-side chain of **1** lost its cytotoxicity against KB cells ($IC_{50} > 100\,\mu g/ml$), while the 5-carbon 4-*O* side chain compound **2** and the 10-carbon 4-*O* side chain analogue **3** exhibited IC_{50} values on KB cells at 7.8 and 3.0 μM, respectively [3]. The 10-carbon 4-*O* side chain compound **3** also showed cytotoxicity on A-549 cells with an IC_{50} value of 34 μM. Furthermore, compound **12**, the allylic aldehyde derivative of the 15 carbon 4-*O* side chain compound **1**, also exhibited less cytotoxic on KB and A-549 cell lines (IC_{50}: 140.2 μM and 67.0 μM, respectively) (table I) when comparing with the shorter 4-*O*-side chain derivative **4** (IC_{50} values on KB and A549 cell lines: 2.6 μM and 22.0 μM, respectively) [3]. This suggested that the prolongation of the 4-*O* side chain (longer than 10 atoms) might be related to the reduction of their bioactivities on human KB and A549 tumour cell lines.

It was also observed that the oxidation of the allylic alcohol (**1**) to an allylic aldehyde **12** promoted the cytotoxicity on all of the six tumour cell lines (table 1). However, further oxidation of the allylic aldehyde **12** to an allylic acid **11** failed to make the same effect, in which the cytotoxicities on PC-3, CNE, and BEL-7407cell lines were instead weakened; nevertheless the cytotoxic IC_{50} value on HeLa cells remained almost the same. Interestingly, this oxidation (from **12** to **11**) obviously enhanced the cytotoxicity against KB and A-549 cell lines, especially substantial for the former (table 1). Moreover, when the allylic acid is esterified (e.g. compound **10**), the pertinent bioactivities became undetectable (i.e. IC_{50} values $> 100\,\mu g/ml$).

Compared to the allylic aldehyde **12**, the benzaldehyde **9** also showed similarly remarkable cytotoxicity against HeLa cells as well as moderate bioactivities to KB and A-549 cell lines. Meanwhile, **9** seemed to be less cytotoxic on BEL-7407 cells and much less cytotoxic on PC-3 and CNE cell lines (table 1). This suggested that the allylic aldehyde derivative **12** is a relatively broad-spectrum cytotoxic agent, and this might be related to the characteristic of moderate electron-poor allylic substituent conjugated to the benzene ring.

It should be noticed that the allylic aldehyde **12** exhibited significant cytotoxic IC_{50} values on PC-3 and on CNE cell lines at 1.9 and 0.8 μM, respectively. The cytotoxicity is comparable to DDP, a marketed anti-tumour agent, which suggested that this type of

Table 1. Inhibitory results of syrinenin-4-*O*-farnesylether and its derivatives.

	IC_{50} (μM) of tumour cell lines					
Compound	*PC-3*	*CNE*	*KB*	*A549*	*BEL-7404*	*HeLa*
9	165.8	39.0	140.8	68.8	104.7	29.7
10	–	–	–	–	–	–
1	27.3	75.7	–	–	31.3	48.9
11	43.0	83.5	79.8	55.2	50.4	49.4
12	1.9	0.8	140.2	67.0	21.4	36.9
DDP	0.69	0.45	0.04	0.83	0.34	0.20

"–" implies that the corresponding IC_{50} value is larger than 100 μg/ml.

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syrinenin derivative is valuable for further detailed investigation, especially for finding a candidate for the treatment of nasopharyngeal carcinoma and human prostate cancer.

The serial preparation and relative investigations of the action mechanism of these cytotoxic agents on the cell cycle are in progress.

3. Experimental

3.1 General experimental procedures

^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian INOVA 400 spectrometer with TMS as internal standard and $CDCl_3$ as solvent. ESI-MS data were recorded on a Bruker Esquire 3000 + spectrometer. TLC was performed on silica gel (GF_{254}). Column chromatography was carried out on silica gel H (10–40 μm). Silica gel GF_{254} and silica gel H were purchased from Qingdao Haiyang Chemical Co. Ltd, China.

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3.2 4-O-Farnesyl-3,5-dimethoxy-benzaldehyde (9)

A solution of compound **8** (0.2 g, 1.08 mmol) in 3 ml of acetone and farnesyl bromide (399 mg, 1.4 mmol) was added to a suspension of K_2CO_3 (298 mg, 2.16 mmol) and acetone (7 ml) in a dry flask. The mixture was refluxed for 3 h and cooled to room temperature. The solvent was removed and the concentrate was diluted with water, acidified with 1 M HCl to pH 9, extracted with Et_2O (30 ml × 3), dried over Na_2SO_4 and evaporated to give a yellow gum. Column chromatography of the crude product (petroleum ether/EtOAc = 8:1) afforded **9** as a colourless oil (261 mg, 62.5%). R_f 0.64 (petroleum ether/EtOAc = 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.87 (1H, s, H-7), 7.12 (2H, s, H-2, H-6), 3.93 (6H, s, OCH_3-3, OCH_3-5), 4.65 (2H, d, $J = 7.2$ Hz, H-1′), 5.55 (1H, brt, $J = 7.2$ Hz, H-2′), 2.03 (8H, m, H-4′, 5′, 8′, 9′), 5.09 (2H, m, H-6′, H-10′), 1.66 (3H, brs, H-12′), 1.58 (3H, brs, H-13′), 1.60 (3H, brs, H-14′, 1.68 (3H, brs, H-15′); ^{13}C NMR (100 MHz, $CDCl_3$): see table 2.

3.3 4-O-Farnesyl-sinapic acid ethyl ester (10)

A mixture of compound **9** (1.31 g, 3.39 mmol) in anhydrous benzene (25 ml) and (carbethoxymethylene)-triphenylphosphorane (1.90 g, 5.42 mmol) was refluxed for 2 h. The solvent was removed and the concentrate was purified by column chromatography (petroleum ether/EtOAc = 6:1) to give **10** as colourless oil (1.07 g, 69.5%). R_f 0.65 (petroleum ether/EtOAc = 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.74 (2H, s, H-2, H-6), 3.87 (6H, s, OCH_3-3, OCH_3-5), 7.60 (1H, d, $J = 16.0$ Hz, H-7), 6.35 (1H, d, $J = 16.0$ Hz, H-8), 4.26 (2H, q, H-10), 1.34 (3H, t, H-11), 4.55 (2H, d, $J = 7.2$ Hz, H-1′), 5.57 (1H, brt, $J = 7.2$ Hz, H-2′), 2.02 (8H, m, H-4′, 5′, 8′, 9′), 5.09 (2H, m, H-6′, H-10′), 1.66 (3H, brs, H-12′), 1.59 (3H, brs, H-13′), 1.61(3H, brs, H-14′), 1.68 (3H, brs, H-15′); ^{13}C NMR (100 MHz, $CDCl_3$): see table 2; ESIMS *m/z* $[M + Na]^{+}$479.

3.4 4-O-Farnesyl-sinapic acid (11)

A KOH solution (48 mg, 0.87 mmol in 3 ml of H_2O) was added to the solution of compound **10** (132 mg, 0.29 mmol) in EtOH (5 ml). The mixture was refluxed for 3 h and cooled to room

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Table 2. ^{13}C NMR data of compounds **1**, **9**–**12**.

No.	*Compound **1***	*Compound **9***	*Compound **10***	*Compound **11***	*Compound **12***
1	136.4 s	142.4 s	129.8 s	129.4 s	129.4 s
2	103.5 d	106.5 d	105.0 d	105.3 d	105.6 d
3	153.7 s	154.1 s	153.6 s	153.9 s	154.0 s
4	141.3 s	135.4 s	141.7 s	142.0 s	142.1 s
5	153.7 s	154.1 s	153.6 s	153.9 s	154.0 s
6	103.5 d	106.5 d	105.0 d	105.3 d	105.6 d
7	131.2 d	191.2 s	144.6 d	147.2 d	153.0 d
8	127.7 d	–	117.2 d	116.3 d	107.2 d
9	63.6 t	–	166.9 s	172.5 s	193.5 s
10	–	–	60.4 t	–	–
11	–	–	32.0 q	–	–
OCH_3	56.0 q	56.2 q	56.0 q	56.1 q	56.1 q
1′	69.4 t	69.5 t	69.4 t	69.4 t	69.4 t
2′	120.2 d	119.7 d	120.0 d	119.8 d	119.8 d
3′	135.3 s	135.3 s	135.3 s	135.3 s	135.3 s
4′	39.8 t	39.9 t	39.8 t	39.9 t	39.9 t
5′	26.5 t	26.5 t	26.3 t	26.6 t	26.3 t
6′	123.9 d	123.8 d	123.8 d	123.8 d	123.8 d
7′	132.2 s	131.7 s	131.5 s	131.6 s	131.6 s
8′	39.6 t	39.6 t	39.6 t	39.6 t	39.6 t
9′	26.7 t	26.7 t	26.6 t	26.7 t	26.7 t
10′	124.7 d	124.5 d	124.6 d	124.6 d	124.6 d
11′	131.5 s	131.3 s	131.2 s	131.4 s	131.3 s
12′	25.6 q	25.7 q	25.6 q	25.7 q	25.7 q
13′	17.6 q	17.7 q	17.6 q	17.7 q	17.7 q
14′	16.3 q	16.4 q	16.3 q	16.4 q	16.4 q
15′	15.9 q	16.0 q	15.9 q	16.0 q	16.0 q

temperature. The organic solvent was removed, the residue was diluted with water (2 ml) and acidified by 1 M HCl to pH 5 to afford a white suspension. The suspension was extracted by Et_2O (20 ml × 3), washed with brine (5 ml × 3) and dried over Na_2SO_4. Removal of Et_2O afforded a white solid (124 mg, 100%). R_f 0.14 (petroleum ether/EtOAc/HCOOH = 4:1:0.05); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.77 (2H, s, H-2, H-6), 3.89 (6H, s, OCH_3-3, OCH_3-5), 7.71 (1H, d, J = 16.0 Hz, H-7), 6.36 (1H, d, J = 16.0 Hz, H-8), 4.56 (2H, d, J = 7.2 Hz, H-1′), 5.56 (1H, brt, J = 7.2 Hz, H-2′), 2.03 (8H, m, H-4′, 5′, 8′, 9′), 5.09 (2H, m, H-6′, H-10′), 1.66 (3H, brs, H-12′), 1.59 (3H, brs, H-13′), 1.61(3H, brs, H-14′), 1.68 (3H, brs, H-15′); ^{13}C NMR (100 MHz, $CDCl_3$):see table 2; ESIMS *m/z* 451 $[M + Na]^+$.

3.5 4-O-Farnesyl-3,5-dimethoxy-cinnamic aldehyde (12)

3.5.1 From 9. A 40% acetaldehyde solution (14 μl, 0.13 mmol) was added dropwise to compound **9** (73 mg, 0.19 mmol) in 1.5 ml of EtOH. After 1 h, another 14 μl of the 40% acetaldehyde solution was added and the solution was stirred at room temperature for 24 h. The reaction was quenched by adding an excess of NaCl. The mixture was extracted by Et_2O and the organic layer was dried over Na_2SO_4. The target compound **12** (51 mg, 65%) was obtained through column chromatography (silica gel H, petroleum ether/EtOAc = 3:1). R_f 0.51 (petroleum ether/EtOAc = 3:1).

3.5.2 From 1. To a solution of compound **1** (100 mg, 0.24 mmol) in CH_2Cl_2 (10 ml), PCC–Al_2O_3 complex (750 mg, 0.38 mmol) was added and the reaction mixture was stirred at room

temperature for 3 h. The mixture was filtered and evaporated. Column chromatography (silica gel H, petroleum ether/EtOAc = 6:1) of the crude product afforded **12** as a pale yellow oil (69 mg, 70%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.79 (2H, s, H-2, H-6), 3.90 (6H, s, OCH_3-3, OCH_3-5), 7.40 (1H, d, J = 16.0 Hz, H-7), 6.64 (1H, dd, J = 16.0, 7.6 Hz, H-8), 9.68 (1H, d, J = 7.6 Hz, H-9), 4.58 (2H, d, J = 7.2 Hz, H-1′), 5.56 (1H, brt, J = 6.8 Hz, H-2′), 2.02 (8H, m, H-4′, 5′, 8′, 9′), 5.09 (2H, m, H-6′, H-10′), 1.67 (3H, brs, H-12′), 1.59 (3H, brs, H-13′), 1.60(3H, brs, H-14′), 1.69 (3H, brs, H-15′); ^{13}C NMR (100 MHz, $CDCl_3$): see table 2; ESIMS *m/z* 451 $[M + K]^+$.

3.6 4-O-Farnesyl-sinapyl alcohol (1) (syrinetinin-4-O-farnesylether)

3.6.1 From 12. Compound **12** (412 mg, 1.0 mmol) was dissolved in dry MeOH (15 ml) under argon. $NaBH_4$ (95 mg, 2.5 mmol) was slowly added at 0°C and the mixture was stirred for 1 h. Cold water was carefully added and the solution was acidified to pH 4 with 5% aqueous HCl. The MeOH was removed *in vacuo* and the mixture was extracted with CH_2Cl_2 (25 ml × 3), washed with brine (10 ml × 3), dried over $MgSO_4$ and evaporated to give an yellow oil. The crude product was purified through column chromatography (silica gel H, petroleum ether/EtOAc = 4:1) to give **1** (352 mg, 85%). R_f 0.19 (petroleum ether/EtOAc = 3:1).

3.6.2 From 11. $LiAlH_4$ (28 mg, 0.725 mmol) was added to anhydrous ether (8 ml) with vigorous stirring in an ice-salt bath until no gas appeared. Compound **11** (124 mg, 0.29 mmol) in ether (5 ml) was added dropwise to the mixture at − 10°C in 2 min and the solution was stirred below 0°C for 30 min. Water (2 ml) was added to destroy the excessive $LiAlH_4$, and the solution was acidified to pH 5. The aqueous layer was extracted with Et_2O (15 ml × 3), and the combined organic layer was washed with brine and dried over Na_2SO_4 for 10 h. Compound **1** (94 mg, 78.1%) was purified through column chromatography (silica gel H, petroleum ether/EtOAc = 4:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.60 (2H, s, H-2, H-6), 3.86 (6H, s, OCH_3-3, OCH_3-5), 6.54 (1H, d, J = 15.6 Hz, H-7), 6.35 (1H, dt, J = 15.6, 6.0 Hz, H-8), 4.53 (2H, d, J = 7.2 Hz, H-1′), 5.57 (1H, brt, J = 7.2 Hz, H-2′), 2.02 (8H, m, H-4′, 5′, 8′, 9′), 5.09 (2H, m, H-6′, H-10′), 1.65 (3H, brs, H-12′), 1.59 (3H, brs, H-13′), 1.60(3H, brs, H-14′), 1.68 (3H, brs, H-15′); ^{13}C NMR (100 MHz, $CDCl_3$): see table 2; ESIMS *m/z* 437 $[M + Na]^+$.

3.7 Pharmacological investigation

The tumour cells were cultivated in RPMI 1640 medium supplemented with 10% foetal bovine serum (FBS), 2 mM glutamine and 100 U/ml penicillin–streptomycin, at 37°C in a 5% CO_2 and 95% air atmosphere.

Cytotoxicity of tested compounds against tumour cells were measured by the colorimetric assay MTT [9,10]. Exponentially growing cells were seeded in quadruplicate into 96-well flat-bottomed plates at a concentration of 5×10^3 cells per well. After 24 h incubation, the compounds studied were added to the wells. After 72 h, 10 μl of MTT solution (5 mg /ml in phosphate buffered solution) were added to the culture medium and incubated at 37°C for a further 4 h. After removing unconverted MTT, 200 μl of DMSO was added to each well and the plates were shaken to dissolve the reduced MTT crystals (formazan); the optical density was measured on a microplate reader at a wavelength of 570 nm. The average 50% inhibitory

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concentration (IC_{50}) was determined graphically from the dose–response curves. The results are recorded in table 2.

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