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Structure-activity Relationship of Sintenin and its Analogues on Six Human Tumor Cell Lines

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Abstract: The synthesis of a cytotoxic natural ester sintenin **1** and thirty-three of its analogues **2-34** were carried out. The cytotoxicities of the synthetic compounds have been screened for human tumor cell lines such as PC-3, Hela, A549, BEL7404, CNE, and KB. The results showed that phenolic derivatives exhibited strongest cytoxicity, and the unsaturated esters were more cytotoxic than their saturated analogues.

Keywords: Synthesis, natural products, sintenin, cytotoxicity, SAR.

Cytotoxic compounds are crucial in the course of finding new anti-tumor leading compounds. Chen and his co-workers recently reported the isolation of sintenin **1** which possessed selective cytotoxicity against P-388 cells with an ED_{50} value of 0.21 μg/mL[1]. As to our knowledge, this kind of esters should have broader-spectrum of biological activity[2,3]. Furthermore, the structure of **1** is relatively similar to nelumol B-D, the sinapyl alcohol derivatives isolated from *Ligularia nelumbifolia*, which was reported to be cytotoxic to A549, HL-60 and KB cell lines[4,5]. We have therefore designed and prepared this natural ester **1** and its derivatives **2-34** (**Table 1**) and tested their cytotoxicities on KB, Hela, PC-3, CNE, A549 and BEL7404 cell lines.

Scheme 1 described the procedure of preparing **1** and **18**. The synthesis started from 3,4-dimethoxybenzaldehyde, which was subjected to a Knoevenagel condensation to furnish **35**[6,7]. The allylic alcohol **36** was achieved by reduction of **35** with $LiAlH_4$[8~10]. **35** was treated with carbonyldiimidazole (CDI) and DBU to afford **18** which was further hydrogenated to give **1**[11,12]. **19-25** (**Table 1**) were obtained by the same path while different aromatic substituents were introduced to the starting materials benzaldehydes. Hydrogenation of these unsaturated esters by palladium-charcoal catalysis afforded a series of saturated esters **2-7** (**Table 1**).

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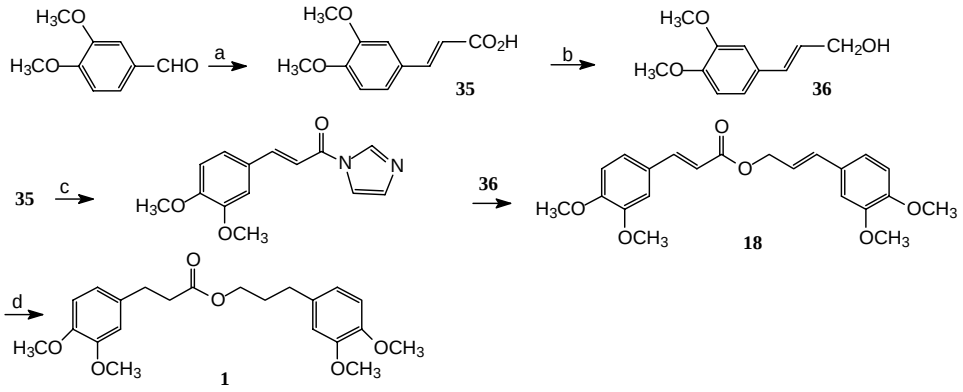
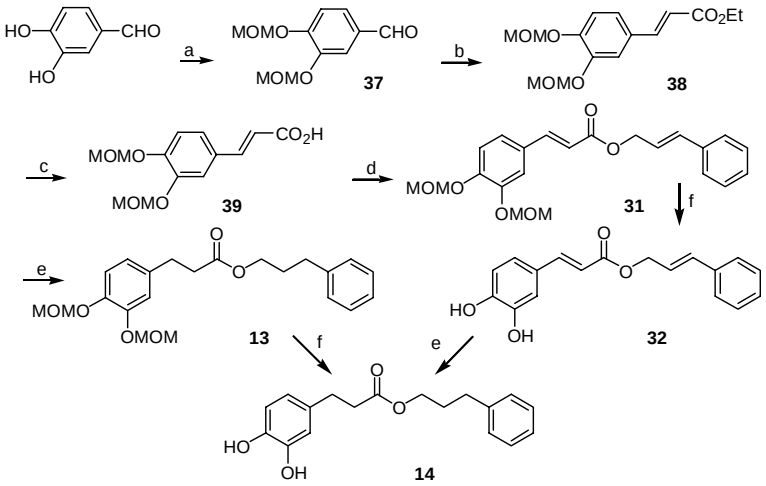
Scheme 1



Reagents and conditions: a) malonic acid, pyridine, piperidine, reflux, 1.5 h; HCl, r.t., 1 h; b) LAH, THF, r.t., 4 h; c) CDI, DBU, THF, 45°, 1 d; d) H_2, Pd/C, AcOEt, r.t., 12 h.

Scheme 2

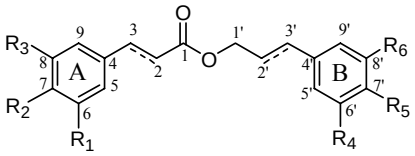


Reagents and conditions: a) MOMCl, K_2CO_3, acetone, reflux, 4 h, 70%; b) $Ph_3P{=}CHCO_2Et$, benzene, reflux, 6 h, 89%; c) KOH/H_2O, EtOH, r.t. 4 h, 80%; d) cinnamic alcohol, DCC, DMAP, CH_2Cl_2, r.t. 24 h, 63%; e) H_2, Pd/C, AcOEt, r.t. 12 h, 93% of **13** and 82% of **14**; f) 10% HCl, MeOH, reflux, 0.5 h, 34% of **32** and 46% of **14**.

Scheme 2 demontrated the general synthetic method of compounds **8**-**17** as well as compounds **26**-**34**. 3,4-Dihydroxybenzaldehyde reacted with chloromethyl methyl ether to afford the aldehyde **37**[13]. This aldehyde was subjected to a Wittig reaction to afford a substituted sinapic acid ethyl ester **38**[14], which was then hydrolyzed by KOH to give **39**. Acid **39** was further condensed with cinnamic alcohol under the catalysis of DCC and DMAP to yield the unsaturated ester **31**[15]. Ester **31** was hydrogenated under 10% Pd-C to afford **13**. Treatment of **31** and **13** with 10% HCl gave **32** and **14**, respectively, which contained free phenolic hydroxyls in the aromatic ring[11,16].

All of the synthetic compounds **1-34** were subjected to *in vitro* cytotoxicity screenings on six human tumor cell lines with the marketed agent cisplatine (DDP) as a standard reference. Compounds **1-7** did not exhibit significant cytotoxicities on the selected six cell lines, while **18-24** exhibited more visible cytotoxicities compared to their saturated analogues **1-7**. The compounds containing OCH_2OCH_3 groups in the molecules did not exhibit satisfactory cytotoxicities. However, after the phenolic hydroxyls were revealed, the cytotoxicity increased remarkably. In terms of the broadness of cytotoxic spectrum, ester **34** exhibited widest cytotoxicity with the IC_{50} values ranging from 4.0 to 19.3×10^{-5} mol/L for the measured six cell lines. Meanwhile, the unsaturated ester **32**, which contained two free phenolic hydroxyls, possessed more selective cytotoxicity against KB, BEL 7404 and A549 cells with the corresponding IC_{50} values of 5.4, 9.1 and 7.1 at 10^{-5} mol/L scale, respectively[17].

Table 1



Compounds	Substituted Group					
	R_1	R_2	R_3	R_4	R_5	R_6
1[a]**/18**[b]	OCH_3	OCH_3	H	OCH_3	OCH_3	H
2[a]**/19**[b]	H	OCH_3	H	OCH_3	OCH_3	H
3[a]**/20**[b]	H	H	H	H	H	H
4[a]**/21**[b]	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	H
5[a]**/22**[b]	H	OCH_3	H	H	OCH_3	H
6[a]**/23**[b]	OCH_3	OCH_3	OCH_3	H	OCH_3	H
7[a]	H	NH_2	H	OCH_3	OCH_3	H
24[b]	H	NO_2	H	OCH_3	OCH_3	H
8[a]**/29**[b]	H	OCH_2OCH_3	H	H	H	H
25[b]	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3
9[a]**/30**[b]	H	OH	H	H	H	H
10[a]	H	CH_3	H	H	CH_3	H
11[a]**/27**[b]	H	OCH_2OCH_3	H	H	OCH_2OCH_3	H
12[a]**/28**[b]	OCH_2OCH_3	OCH_2OCH_3	H	H	OCH_2OCH_3	H
13[a]**/31**[b]	H	OCH_2OCH_3	OCH_2OCH_3	H	H	H
14[a]**/32**[b]	H	OH	OH	H	H	H
15[a]**/33**[b]	H	OCH_2OCH_3	H	H	OCH_3	H
16[a]**/34**[b]	H	OH	H	H	OCH_3	H
17[a]**/26**[b]	H	OCH_2OCH_3	H	OCH_2OCH_3	OCH_2OCH_3	H

[a]) 2,3 and 2′,3′ are saturated ethylenes; [b]) 2,3-double bond; 2′,3′-double bond.

The cytotoxic evaluation suggested that the esters with one or more free phenol groups are more toxic than those possessing alkyloxyl, alkyl, nitro or amine substituents in the aromatic rings. The cytotoxicity would be improved by adding the number of methoxy substituents in the B ring. However, the prolongation of OCH_3 group by OCH_2OCH_3 did not enhance the cytotoxicity. In general, unsaturated esters showed stronger cytotoxicity than the saturated analogues, which might be due to the presence of a large π–π conjugative system in the unsaturated molecules. These results might serve as fundamental information for further SAR investigation on this type of cytotoxic esters.

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17. The data of the IC_{50} values of the synthetic compounds against the selected six human tumor cell lines were submitted to the editorial office of CCL.

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