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Design, synthesis, and SAR analysis of cytotoxic sinapyl alcohol derivatives

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Abstract—Five series totalling 51 of sinapyl alcohol derivatives were designed and synthesized. Their cytotoxicity analyses were performed on six human tumor cell lines such as PC-3, CNE, KB, A549, BEL-7404, and HeLa. Certain sinapyl alcohol derivatives showed significant cytotoxic activities. Compound **14d** exhibited especially potent cytotoxicity against the BEL-7404 cell line with an IC_{50} value of 0.7 μM, which showed more cytotoxic activity than the positive control, cisplatin. The structure–cytotoxicity relationships were discussed and the CoMFA analysis was performed using the cytotoxic data against HeLa cells as a template.

1. Introduction

The family Compositae, widely distributed in China, is abundant with sinapyl alcohol derivatives.[1–5] However, the knowledge base on the biological activities of this kind of compounds still calls for continuing enrichment. In addition to our previous report on the cytotoxicity of several sinapyl alcohol derivatives **1**–**4** (Fig. 1) isolated from *Ligularia nelumbifolia*,[5] only some anti-inflammatory and antinociceptive effects were evaluated by Choi et al. in 2004.[6] Among the four isolated natural products, geranyloxysinapyl alcohol (**1**) was found to possess notable cytotoxicity with the IC_{50} value of 3.0 μM against KB cells. This suggested further development for compound **1** in the direction of potential cytotoxic agents.[5] To efficiently discover more potent leading compounds, further investigation on the structure–cytotoxicity relationship of this kind of sinapyl alcohol derivatives is requisite. Therefore, the derivatives of **1**, consisting of series of ethyl esters (**11**), acids (**12**), alcohols (**13**), aldehydes (**14**), benzaldehyde intermediates (**10**), and the demethyl sinapyl alcohol derivative (**19**), were designed and synthesized. The total 51 synthetic compounds were subjected to a wide-spectrum cytotoxic screenings of six cultured human tumor cell lines including PC-3, CNE, KB, A549, BEL-7404, and HeLa. The CoMFA analysis[7,8] was performed using the cytotoxic data against HeLa cells as a template to accomplish the SAR study on these compounds. The synthetic procedures and the biological assay results are also provided herein.

2. Results and discussion

2.1. Synthetic approach

Two synthetic routes (Schemes 1 and 2) were utilized to prepare the five series of sinapyl alcohol derivatives. Both of them are constructed with the same starting

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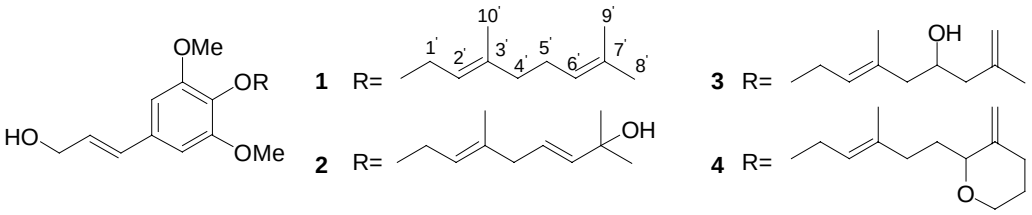
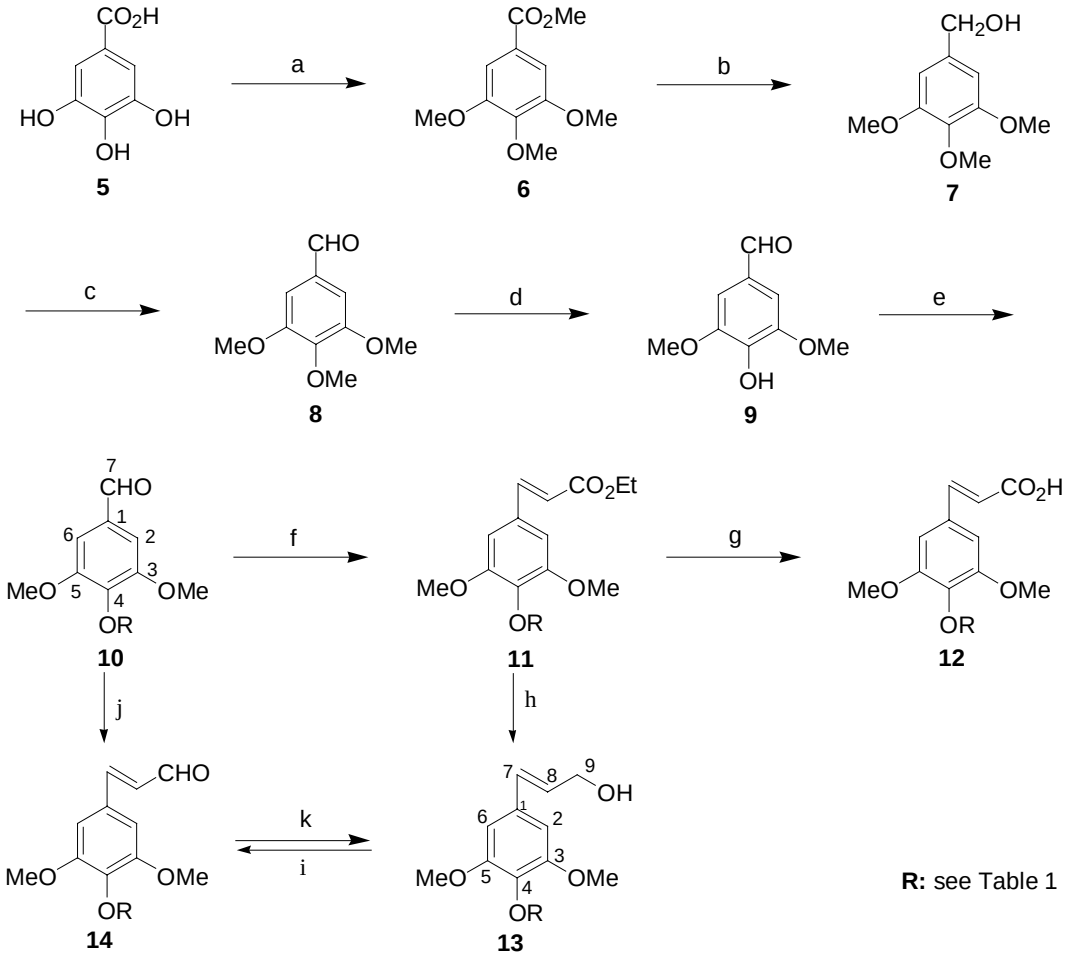
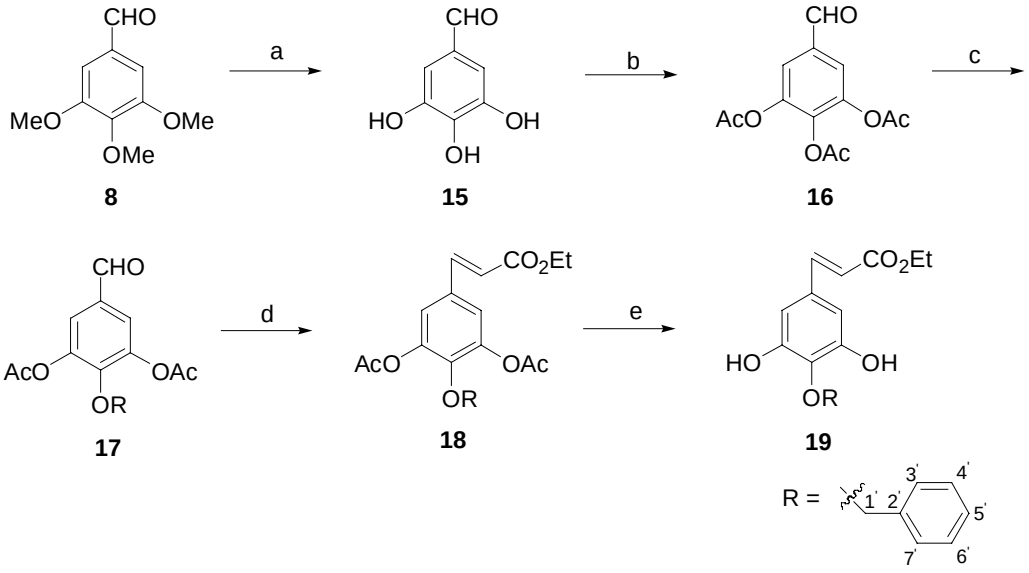


Figure 1. Sinapyl alcohol derivatives from *Ligularia nelumbifolia*.



Scheme 1. Reagents and conditions: (a) NaOH, Me_2SO_4, reflux (70%); (b) $LiAlH_4$, THF, reflux (72%); (c) PCC, CH_2Cl_2, rt (85%); (d) NaH, PhSH, HMPT, toluene, reflux (92%); (e) RBr, K_2CO_3, acetone, reflux, 3 h; (f) $Ph_3PCH{=}CO_2Et$, benzene, reflux, 2 h; (g) KOH, EtOH, H_2O, reflux, 3 h; (h) $LiAlH_4$, Et_2O, 10 °C, 30 min; (i) PCC–Al_2O_3, CH_2Cl_2, rt, 6 h; (j) 40% CH_3CHO, EtOH, rt, 24 h; (k) $NaBH_4$, MeOH, 0 °C, 1 h.

material, 3,4,5-trimethoxy-benzaldehyde (**8**), prepared from natural abundant gallic acid (**5**) via four steps (Scheme 1) with a total yield of 39%.[9–11] Compound **9** was obtained by selective demethylation[12] of **8** and was subjected to an alkylation of 4-OH by the corresponding allyl bromides in the presence of potassium carbonate[13] to provide the aldehydes **10** in the yields of 53–95%. Condensation with (carbethoxymethylene)-triphenylphosphorane[14] afforded the target ethyl esters **11** in the yields of 60–95%. Reduction of esters **11** with lithium aluminum hydride in ether[10] gave the allylic alcohols **13** in the yields of 60–70%. Moreover, compound **13** could also be prepared from a reduction[15] of allylic aldehydes **14**, which could be synthesized by condensation of **10** with 40% acetaldehyde in EtOH at room temperature in the yields of 40–70%.[16] Additionally, the allylic aldehydes **14** could also be easily obtained by oxidation of **13** with pyridinium chlorochromate–aluminum oxide complex (PCC–Al_2O_3).[11] Furthermore, hydrolysis of the ethyl esters **11** afforded the allylic acids **12** in the yields of 65–96%.[17] Scheme 2 demonstrates the synthetic procedure of 3,5-demethyl sinapic acid ethyl ester **19**. The aldehyde **8** was subjected successfully to demethylation and acetylation to afford an acetylated aldehyde **16** in a 75% yield, which was selectively substituted by benzyl bromide at 4-*O* position to give **17** in a 60% yield. Condensation of the aldehyde **17** with (carbethoxymethylene)-triphenylphosphorane afforded the sinapic acid ethyl ester **18** all in an *E* form with a yield of 80%.[16,18,19] Hydrolysis of **18** with potassium carbon-



Scheme 2. Reagents and conditions: (a) $AlCl_3$, CH_2Cl_2, rt (30%); (b) Ac_2O, Py, rt (50%); (c) RBr, K_2CO_3, DMF, 42 °C (95%); (d) $Ph_3PCH{=}CO_2Et$, benzene, reflux (70%); (e) K_2CO_3, MeOH, 50 °C (75%).

ate provided the deacetylated phenol **19** in a 70% yield without influence of the ester group[18] (Table 1).

2.2. Biological activity tests

The cytotoxicities of the compounds were evaluated against six cultured human tumor cell lines by the colorimetric assay MTT.[20–22] Although the binding sites of both, the anticancer drug cisplatin (DDP) and the series of described sinapyl alcohol derivatives, might not be identical, DDP was used as a reference compound because of its well-established clinical application in cancer treatment. The results are presented in Table 2 which illustrates the concentrations required to inhibit cell growth by 50% (IC_{50} values). By a scrutiny of the MTT assay results, it could be found that the oxidation of the allylic alcohols (**13a**, **13d**) to allylic aldehydes (**14a**, **14d**) promoted the cytotoxicities on almost all the six tumor cell lines. However, further oxidation of the allylic aldehydes (**14a**, **14d**) to allylic acids (**12a**, **12d**) reduced the cytotoxicity especially to PC-3 cell lines (**12a**–**12n**, $IC_{50} > 200$ μM). Moreover, when the allylic acids were esterified (**11a**–**11i**), their cytotoxicities were dramatically diminished ($IC_{50} > 200$ μM). Interestingly, the intermediate benzaldehydes **10a**–**10l** exhibited somewhat efficient cytotoxicity on some tumor cell lines, except for A549 and BEL-7404 cell lines.

Furthermore, the sinapyl alcohol derivatives (**12d**, **13d**) with a geranyl substituent showed efficient cytotoxicity on almost all of the six tumor cell lines, while compounds **12j** and **13j** with the hexadecyl substituents lose their cytotoxicity. This suggested that a long unbranched saturated 4-*O*-side chain might reduce the cytotoxic potency. Moreover, comparison of the cytotoxicities of demethyl-4-benzyl-sinapic acid ethyl ester **19** and 4-benzyl-sinapic acid ethyl ester **11b** led to an impression that the phenolic groups, instead of the methoxy group on C3 and C5 (refer to Scheme 1 for numbering), may enhance the cytotoxicity on select tumor cell lines.

In addition, it should be noticed that the allylic aldehyde **14d** showed potent cytotoxic activities on all six cultured human tumor cell lines, for example, an IC_{50} value of 9.0 μM to a PC-3 cell line. More impressively, this aldehyde exhibited a significant cytotoxic IC_{50} value of 7.0×10^{-7} M to BEL-7404 cells. This value indicates that **14d** possesses a much higher cytotoxicity when compared to that of the well-known frontline anti-cancer drug cisplatin and therefore suggests that these kind of compounds are worthy of further investigation.

2.3. CoMFA analysis

In this study, 31 compounds were employed for the CoMFA analysis. For 3D-QSAR analyses, 26 compounds (unasterisked molecules in Table 3) were selected as training set for model construction, and the remaining 5 compounds (asterisked molecules in Table 3) as testing set for model validation.

Structures of entire sets of sinapyl alcohol derivatives were built using SYBYL 6.91 molecular modeling software. The structural energy minimization was performed using standard TRIPOS force field and Gasteiger–Hückel charge with an energy gradient convergence criterion of 0.001 kcal/mol and a distance-dependent dielectric constant. Systematic conformational searches were carried out to find the lowest energy structures. It was noticed that all the 26 molecules in the training set have the same aryloxy skeleton. Therefore, the 3,4,5-trioxyaromatic structure of **13a** (the most active substituted sinapyl alcohol) was chosen as template for the structural alignment of the 31 molecules.

The CoMFA result is summarized in Table 4. The cross-validated value, q^2, is 0.610, with an optimum number of

Table 1. Benzaldehydes and sinapyl alcohol derivatives synthesized

Compound	R
Benzaldehydes	
10a	5′-$EtOC_6H_4CH_2$
10b	$C_6H_5CH_2$
10c	3′-Cl-5′-$FC_6H_3CH_2$
10d	Geranyl
10e	3′-$BrC_6H_4CH_2$
10f	5′-$BrC_6H_4CH_2$
10g	4′,5′-$Cl_2C_6H_3CH_2$
10h	5′-Br-3′-$FC_6H_3CH_2$
10i	3′-Phenylallyl
10j	Hexadecyl
10k	4′-$BrC_6H_4CH_2$
10l	Propargyl
Sinapic acid ethyl esters	
11a	5′-$EtOC_6H_4CH_2$
11b	$C_6H_5CH_2$
11c	3′-Cl-5′-$FC_6H_3CH_2$
11d	Geranyl
11e	3′-$BrC_6H_4CH_2$
11f	5′-$BrC_6H_4CH_2$
11g	4′,5′-$Cl_2C_6H_3CH_2$
11h	5′-Br-3′-$FC_6H_3CH_2$
11i	3′-Phenylallyl
11j	Hexadecyl
11k	4′-$BrC_6H_4CH_2$
11l	Propargyl
Sinapic acids	
12a	5′-$EtOC_6H_4CH_2$
12b	$C_6H_5CH_2$
12c	3′-Cl-5′-$FC_6H_3CH_2$
12d	Geranyl
12e	3′-$BrC_6H_4CH_2$
12f	5′-$BrC_6H_4CH_2$
12g	4′,5′-$Cl_2C_6H_3CH_2$
12h	5′-Br-3′-$FC_6H_3CH_2$
12i	3′-Phenylallyl
12j	Hexadecyl
12k	4′-$BrC_6H_4CH_2$
12m	4′-$FC_6H_4CH_2$
12n	Isopentenyl
Sinapyl alcohols	
13a	5′-$EtOC_6H_4CH_2$
13b	$C_6H_5CH_2$
13c	3′-Cl-5′-$FC_6H_3CH_2$
13d	Geranyl
13e	3′-$BrC_6H_4CH_2$
13f	5′-$BrC_6H_4CH_2$
13g	4′,5′-$Cl_2C_6H_3CH_2$
13h	5′-Br-3′-$FC_6H_3CH_2$
13j	Hexadecyl
13k	4′-$BrC_6H_4CH_2$
Sinapyl aldehydes	
14a	5′-$EtOC_6H_4CH_2$
14d	Geranyl
14o	Me

components of 5. The non-cross-validated partial least-squares (PLS) analysis produced a r^2 of 0.976. The estimated F value is 168, and standard error is 0.005. These statistical indexes are reasonably high, indicating that the new CoMFA model has a strong predictive ability. Figure 2 depicts the correlation between experimental results and predicted values of the 27 studied compounds. Table 4 and Figure 2 demonstrate that the predicted values using the newly constructed CoMFA model are in good agreement with experimental data, suggesting that the new CoMFA model is reliable.

The data in Table 4 also show that the CoMFA steric field descriptor explains 51.5% of the variance, while the electrostatic descriptor explains the remaining 48.5%. These steric and electrostatic fields are presented as contour plots in Figures 3A and B, respectively. A huge sterically favorable region (Fig. 3A, green contours) is located near the atoms C7, C8, and C9 of the allyl part, suggesting that bulky groups in this area would increase the cytotoxicity. The yellow polyhedra indicates that the bulky substituent near the region of allyl part is not favorable to molecular bioactivity. CoMFA electrostatic contour map is shown in Figure 3B. There is a main red polyhedron very close to the atom C9, indicating that the negatively charged substituents in this region lead to an increase of bioactivity. A large blue polyhedron near the atoms C7 and C8 suggested that more positive charges in these areas should play a favorable role in improving cytotoxicity.

3. Conclusions

Five series of sinapyl alcohol derivatives were synthesized and subjected to the screenings of six cultured human tumor cell lines. Some of them especially the allylic alcohols (series **13**) and the allylic aldehydes (series **14**) showed significant cytotoxicity on the selected tumor cell lines. However, the ethyl ester substituent on C9 is unfavorable to the cytotoxicity of series **11**. Compound **19** also showed significant cytotoxicity which indicates that the methyl group on O3 and O5 might not be necessary for the cytotoxicities. Compounds**14a** and **14d** exhibited the potency to be lead compounds for future synthetic strategies due to their potential cytotoxicities. Further investigation on the SAR utilized a CoMFA analysis to HeLa cell line was carried out. Further design, synthesis, and cytotoxic check of derivatives with various substituents at different positions are under consideration.

4. Experimental

4.1. Materials

Melting points were measured on a Perkin-Taike X-4 apparatus and have been corrected. ^1H 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 and EI-MS was performed on a Varian MAT-95 MS instrument. All of the CoMFA calculations were performed on a SGI O2 workstation using the Sybyl 6.91 program. The optical density was measured spectrophotometrically at 570 nm on an enzyme-linked immunosorbent assay reader (Bio-Tek Synergy HT, Bio-Tek Instruments Inc, Winooski, VT, USA). Thin-layer chromatography was performed on

Table 2. Inhibitory results of the synthesized compounds to six tumor cell lines[a]

Compound	IC_{50} (μM)					
	PC-3	CNE	KB	A549	BEL-7404	HeLa
10a	82 ± 7.8	81 ± 12.0	38 ± 4.3	—[b]	—	72 ± 5.4
10b	—	—	—	—	—	199 ± 12.7
10c	142 ± 11.3	94 ± 6.5	—	—	—	134 ± 10.8
10d	—	—	28 ± 1.9	—	—	—
10e	—	—	192 ± 2.1	—	—	—
10f	39 ± 6.2	—	—	199 ± 14.2	—	86 ± 11.3
10g	—	—	—	—	—	—
10h	145 ± 16.5	—	16 ± 2.0	—	—	158 ± 9.8
10i	54 ± 4.1	74 ± 5.4	—	—	120 ± 7.8	47 ± 2.5
10j	—	—	—	—	—	—
10k	63 ± 4.3	65 ± 3.8	—	—	139 ± 12.4	123 ± 11.3
10l	—	179 ± 14.9	—	—	—	100 ± 7.5
11a–i	—	—	—	—	—	—
11j	—	—	30 ± 2.7	—	—	—
11k	—	—	—	—	—	126 ± 9.6
11l	—	52 ± 6.3	—	—	—	21 ± 3.1
12a	—	—	55 ± 9.8	—	216 ± 15.7	—
12b	—	—	46 ± 3.2	—	—	—
12c	—	101 ± 9.3	—	181 ± 7.9	—	135 ± 15.4
12d	—	147 ± 11.7	138 ± 12.4	54 ± 4.8	44 ± 3.6	56 ± 4.0
12e	—	—	38 ± 6.1	—	—	182 ± 12.3
12f	—	—	36 ± 5.3	174 ± 14.0	180 ± 15.6	109 ± 12.2
12g	—	—	14 ± 2.9	74 ± 4.8	35 ± 5.2	158 ± 11.6
12h	—	—	25 ± 1.7	59 ± 6.1	—	—
12i	—	131 ± 11.6	96 ± 10.5	46 ± 5.3	59 ± 4.4	95 ± 7.8
12j	—	—	—	—	42 ± 6.2	—
12k	—	—	—	67 ± 4.3	32 ± 4.1	—
12m	—	—	30 ± 5.3	—	—	—
12n	—	—	—	—	—	56 ± 5.3
13a	—	60 ± 6.2	78 ± 10.4	—	295 ± 5.4	32 ± 2.3
13b	—	—	192 ± 14.8	—	—	100 ± 9.4
13c	—	—	41 ± 6.2	—	—	105 ± 11.7
13d	174 ± 11.9	61 ± 9.4	71 ± 7.3	99 ± 8.4	192 ± 16.3	33 ± 4.2
13e	97 ± 6.5	58 ± 3.0	—	—	—	81 ± 7.8
13f	—	76 ± 6.4	43 ± 5.6	186 ± 17.2	143 ± 11.8	91 ± 6.3
13g	71 ± 6.5	123 ± 11.6	105 ± 12.3	114 ± 13.0	42 ± 5.0	76 ± 6.8
13h	89 ± 10.3	—	10 ± 2.1	109 ± 13.4	—	109 ± 14.3
13j	—	—	—	—	—	—
13k	90 ± 6.8	—	12 ± 1.6	61 ± 7.0	57 ± 6.4	70 ± 6.1
14a	9.0 ± 0.1	32 ± 2.5	70 ± 9.3	—	80 ± 7.1	45 ± 5.4
14d	34 ± 4.3	9.0 ± 1.2	2.0 ± 0.3	28 ± 1.3	0.7 ± 0.1	7 ± 0.6
14o	61 ± 5.9	40 ± 5.1	19 ± 3.2	32 ± 2.1	40 ± 5.3	31 ± 4.1
19	38 ± 5.3	69 ± 9.7	8.0 ± 1.3	—	—	91 ± 8.0
DDP	6.9 ± 0.7	4.5 ± 0.3	0.4 ± 0.02	8.3 ± 1.3	3.4 ± 0.3	2.0 ± 0.1

[a] Key to cell lines: PC-3, human prostate cancer cell line; CNE, nasopharyngeal carcinoma cell line; KB, human oral epithelial cell line; A549, human lung adenocarcinoma cell line; BEL7404, human hepatocellular carcinoma line; HeLa, human cervical carcinoma cell line.
[b] IC_{50} values greater than 200 μM were considered as inactive and omitted here.

silica gel GF_{254}. Column chromatography was carried out on silica gel H (10–40 μm). All of the silica gel GF_{254} and silica gel H were purchased from Qingdao Marine Chemical Factory, China.

4.2. Synthesis

4.2.1. General procedure for the preparation of 4-*O*-substituted-3,5-dimethoxy-benzaldehyde 10a–10l. A solution of compound **9** (0.2 g, 1.08 mmol) in 3 mL acetone and allyl bromide (1.5 mmol) were added to a suspension of K_2CO_3 (2.16 mmol) and acetone (7 mL) in a dry flask. The mixture was refluxed for 3–5 h and cooled to room temperature. The reaction was monitored by TLC using pet. ether/EtOAc (3:1) as the mobile phase. The solvent was removed and the concentrate was diluted with water, adjusted with 1 M HCl to pH 9, extracted with Et_2O (3× 30 mL), and dried over anhydrous Na_2SO_4. After removal of the solvent under reduced pressure, the residue was purified by column chromatography with pet. ether/EtOAc (7:1 to 4:1) to afford **10** (53–95%).

4.2.1.1. 3,5-Dimethoxy-4-(5′-ethoxybenzyloxy)-benzaldehyde (10a). Yield: 86%; mp 50–52 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.85 (1H, s, H-7), 7.35 (2H, d, J = 6.8 Hz, H-3′, 7′), 7.10 (2H, s, H-2, H-6), 6.85 (2H, d, J = 6.8 Hz, H-4′, 6′), 5.06 (2H, s, H-1′), 4.04 (2H, q,

Table 3. Predicted activities (PA) from CoMFA models compared with the experimental activities (EA) and the residues (δ)

Compound	EA	COMFA	
		PA	δ
10a*	4.14	4.20	−0.06
10b	3.70	3.80	−0.10
10c	3.87	3.82	0.05
10f	4.07	4.15	−0.08
10h	3.80	3.80	0.00
10i	4.33	4.26	0.07
10k	3.91	3.76	0.15
10l	4.00	4.06	−0.06
11h	3.81	3.80	0.01
11k	3.90	3.88	0.02
11l	4.67	4.71	−0.04
12c	3.87	3.85	0.02
12d*	4.25	4.42	−0.17
12e	3.74	3.74	0.00
12f	3.96	3.93	0.03
12g	3.80	3.84	−0.04
12i	4.02	4.06	−0.04
12n*	4.25	4.47	−0.22
13a	4.50	4.49	0.01
13b	4.00	4.05	−0.05
13c	3.98	4.00	−0.02
13d	4.48	4.48	0.00
13e	4.09	4.10	−0.01
13f	4.04	4.00	0.04
13g	4.12	4.14	−0.02
13h	3.96	3.98	−0.02
13k*	4.15	4.33	−0.18
14a*	4.35	4.15	0.18
14d	5.08	5.05	0.03
14o	4.51	4.48	0.03
19	4.04	4.01	0.03

* Compounds of the testing set.

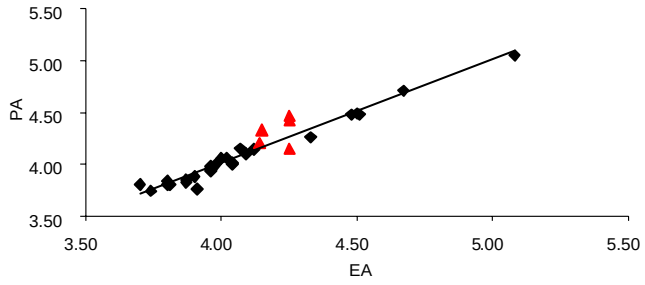


Figure 2. Predicted activities (PA) by CoMFA models versus experimental activities (EA) of sinapyl alcohol derivatives. ♦, compounds of the training set; ▲, compounds of the testing set.

J = 6.8 Hz, OCH_2CH$_3$-5′), 3.89 (6H, s, OCH_3-3, OCH_3-5), 1.40 (3H, t, J = 6.8 Hz, OCH$_2$CH_3-5′); ESI-MS m/z [M−H]$^-$ 351.

4.2.1.2. 4-Benzyloxy-3,5-dimethoxy-benzaldehyde (10b). Yield: 85%; This compound was identical to that reported by Battersby et al.[23]

4.2.1.3. 4-(3′-Chloro-5′-fluorobenzyloxy)-3,5-dimethoxy-benzaldehyde (10c). Yield: 60%; mp 99–101 °C; ^{1}H NMR (400 MHz, CDCl$_3$): δ 9.88 (1H, s, H-7), 7.67 (1H, dd, J = 8.4, 5.2 Hz, H-7′), 7.14 (2H, s, H-2, H-6), 7.12 (1H, dd, J = 8.4, 2.4 Hz, H-4′), 7.03 (1H, dt, J = 8.4, 2.4 Hz, H-6′), 5.20 (2H, s, H-1′), 3.89 (6H, s, OCH_3-3, OCH_3-5); ESI-MS m/z [M]$^+$ 324.

4.2.1.4. 4-(9′,10′-Dimethyl-octa-2′,6′-dienyloxy)-3,5-dimethoxy-benzaldehyde (10d). Yield: 65%; this compound was identical to that reported by Zhao et al.[5]

4.2.1.5. 4-(3′-Bromo-benzyloxy)-3,5-dimethoxy-benzaldehyde (10e). Yield: 55%; mp 104–106 °C; ^{1}H NMR (400 MHz, CDCl$_3$): δ 9.86 (1H, s, H-7), 7.75 (1H, dd, J = 8.4, 1.6 Hz, H-4′), 7.52 (1H, dd, J = 8.4, 1.6 Hz, H-7′), 7.32 (1H, ddd, J = 8.4, 8.4, 1.6 Hz, H-5′), 7.15 (1H, ddd, J = 8.4, 8.4, 1.6 Hz, H-6′), 7.11 (2H, s, H-2, H-6), 5.00 (2H, s, H-1′), 3.90 (6H, s, OCH_3-3, OCH_3-5); ESI-MS m/z [M]$^+$ 351.

4.2.1.6. 4-(5′-Bromo-benzyloxy)-3,5-dimethoxy-benzaldehyde (10f). Yield: 55%; mp 102–104 °C; ^{1}H NMR (400 MHz, CDCl$_3$): δ 9.87 (1H, s, H-7), 7.47 (2H, d, J = 8.4 Hz, H-3′, 7′), 7.35 (2H, d, J = 8.4 Hz, H-4′, 6′), 7.12 (2H, s, H-2, H-6), 5.08 (2H, s, H-1′), 3.90 (6H, s, OCH_3-3, OCH_3-5); ^{13}C NMR (100 MHz, CDCl$_3$): δ 191.1 (C-7), 141.9 (C-1), 153.8 (C-3, 5), 136.2 (C-4), 131.9 (C-2′), 131.3 (C-4′, 6′), 130.0 (C-3′, 7′), 122.0 (C-5′), 106.5 (C-2, 6), 74.1 (C-1′), 56.2 (OCH$_3$-3, OCH$_3$-5); ESI-MS m/z [M]$^+$ 351.

4.2.1.7. 4-(4′,5′-Dichloro-benzyloxy)-3,5-dimethoxy-benzaldehyde (10g). Yield: 65%; mp 72–76 °C; ^{1}H NMR (400 MHz, CDCl$_3$): δ 9.87 (1H, s, H-7), 7.64 (1H, s, H-3′), 7.41 (1H, d, J = 8.4 Hz, H-6′), 7.28 (1H, d, J = 8.4 Hz, H-7′), 7.12 (2H, s, H-2, H-6), 5.07 (2H, s, H-1′), 3.92 (6H, s, OCH_3-3, OCH_3-5); ESI-MS m/z [M]$^+$ 341.

4.2.1.8. 4-(5′-Bromo-3′-fluoro-benzyloxy)-3,5-dimethoxy-benzaldehyde (10h). Yield: 75%; mp 101–103 °C; ^{1}H NMR (400 MHz, CDCl$_3$): δ 9.87 (1H, s, H-7), 7.47 (1H, t, J = 8.0 Hz, H-7′), 7.29 (1H, dd, J = 8.0, 2.0 Hz, H-6′); 7.23 (1H, dd, J = 9.2, 2.0 Hz, H-4′), 7.11 (2H, s, H-2, H-6), 5.15 (2H, s, H-1′), 3.89 (6H, s, OCH_3-3, OCH_3-5); ESI-MS m/z [M]$^+$ 369.

4.2.1.9. 3,5-Dimethoxy-4-(3′-phenyl-allyloxy)-benzaldehyde (10i). Yield: 60%; mp 76–77 °C; ^{1}H NMR (400 MHz, CDCl$_3$): δ 9.87 (1H, s, H-7), 7.37 (2H, dd, J = 7.2, 1.6 Hz, H-5′, H-9′), 7.31 (1H, ddd, J = 7.2, 7.2, 1.6 Hz, H-7′); 7.25 (2H, ddd, J = 7.2, 7.2, 1.6 Hz, H-6′, 8′), 7.13 (2H, s, H-2, H-6), 6.63 (1H, d, J = 16.0 Hz, H-3′), 6.46 (1H, dd, J = 16.0, 6.4 Hz, H-2′), 4.78 (2H, s, H-1′), 3.89 (6H, s, OCH_3-3, OCH_3-5); ESI-MS m/z [M+H]$^+$ 299.

4.2.1.10. 3,5-Dimethoxy-4-hexadecyloxy-benzaldehyde (10j). Yield: 53%; mp 71–74 °C; ^{1}H NMR (400 MHz, CDCl$_3$): δ 9.86 (1H, s, H-7), 7.12 (2H, s, H-2, H-6), 4.07 (2H, t, J = 6.8 Hz, H-1′), 3.91 (6H, s, OCH_3-3, OCH_3-5), 1.57 (2H, m, H-2′), 1.30 (2H, m, H-4′); 1.26 (24H, m, H-5′–15′), 0.88 (3H, t, J = 6.8 Hz, H-16′); ESI-MS m/z [M+H]$^+$ 407.

4.2.1.11. 4-(4′-Bromo-benzyloxy)-3,5-dimethoxy-benzaldehyde (10k). Yield: 60%; mp 72–76 °C; ^{1}H NMR (400 MHz, CDCl$_3$): δ 9.87 (1H, s, H-7), 7.70 (1H, s,

Table 4. Statistical indexes of CoMFA model based on 26 compounds

	Cross-validated		Conventional		Field distribution (%)	
	q^2	Optimal comp	r^2	F	Steric	Electrostatic
CoMFA	0.610	5	0.976	168	51.5	48.5

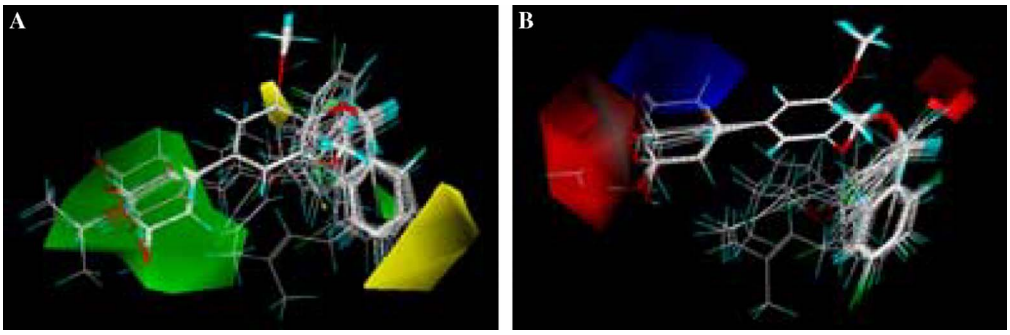


Figure 3. Steric and electrostatic CoMFA maps of the compounds (Table 3) showing contributions to the inhibitory activities on the HeLa cell line. The color code is as follows: (A) For the steric map, yellow denotes regions where steric bulk is detrimental to the bioactivity and green denotes regions where steric bulk enhances the activity. (B) For the electrostatic map, red denotes regions where positive charge is detrimental to the bioactivity and blue denotes regions where positive charge enhances the bioactivity.

H-3′), 7.43 (1H, d, J = 7.6 Hz, H-5′), 7.37 (1H, d, J = 7.6 Hz, H-7′), 7.22 (1H, dd, J = 7.6, 7.6 Hz H-6′), 7.12 (2H, s, H-2, H-6), 5.09 (2H, s, H-1′), 3.93 (6H, s, OCH_3-3, OCH_3-5); ESI-MS m/z $[M]^+$ 351.

4.2.1.12. 3,5-Dimethoxy-4-propargyl-benzaldehyde (10l). Yield: 60%; mp 108–109 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.88 (1H, s, H-1), 7.26 (2H, s, H-2, H-6), 4.84 (2H, s, H-1′), 3.93 (6H, s, OCH_3-3, OCH_3-5), 2.44 (1H, s, H-3′); ESI-MS m/z $[M+K]^+$ 259.

4.2.2. General procedure for the preparation of 4-*O*-substituted-sinapic acid ethyl ester 11a–11l. A mixture of compound **10** (3.39 mmol) in anhydrous benzene (25 mL) and (carbethoxymethylene)-triphenylphosphorane (1.90 g, 5.42 mmol) were refluxed for 2–3 h. The reaction was monitored by TLC using pet. ether/EtOAc (5:1) as the mobile phase. The solvent was removed and the concentrate was purified by column chromatography with pet. ether/EtOAc (9:1 to 4:1) to give **11** (60–95%).

4.2.2.1. 4-(5′-Ethoxybenzyl) sinapic acid ethyl ester (11a). Yield: 95%; mp 98–100 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.59 (1H, d, J = 16.0 Hz, H-7), 7.36 (2H, br d, J = 8.4 Hz, H-3′, 7′), 6.85 (2H, br d, J = 8.4 Hz, H-4′, 6′), 6.73 (2H, s, H-2, H-6), 6.34 (1H, d, J = 16.0 Hz, H-8), 4.98 (2H, s, H-1′), 4.27 (2H, q, J = 7.2 Hz, OCH_2CH$_3$-9), 4.03 (2H, q, J = 7.2 Hz, OCH_2CH$_3$-5′), 3.84 (6H, s, OCH_3-3, OCH_3-5), 1.40 (3H, t, J = 7.2 Hz, O CH$_2$CH_3-5′), 1.34 (3H, t, J = 7.2 Hz, OCH$_2$CH_3-9); ESI-MS m/z $[M-H]^-$ 385.

4.2.2.2. 4-Benzyl sinapic acid ethyl ester (11b). Yield: 70%; mp 81–82 °C; This compound was identical to that reported by Ren et al.[24]

4.2.2.3. 4-(3′-Chloro-5′-fluorobenzyl) sinapic acid ethyl ester (11c). Yield: 95%; mp 114–116 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69 (1H, dd, J = 8.4, 5.2 Hz, H-7′), 7.61 (1H, d, J = 16.0 Hz, H-7), 7.12 (1H, dd, J = 8.4, 2.4 Hz, H-4′), 7.01 (1H, dt, J = 8.4, 2.4 Hz, H-6′), 6.76 (2H, s, H-2, H-6), 6.36 (1H, d, J = 16.0 Hz, H-8), 5.13 (2H, s, H-1′), 4.28 (2H, q, J = 7.2 Hz, OCH_2CH$_3$-9), 3.85 (6H, s, OCH_3-3, OCH_3-5), 1.35 (3H, t, J = 7.2 Hz, OCH$_2$CH_3-9); ESI-MS m/z $[M]^+$ 394.

4.2.2.4. 4-Geranyl sinapic acid ethyl ester (11d). Yield: 75%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60 (1H, d, J = 16.0 Hz, H-7), 6.74 (2H, s, H-2, H-6), 6.35 (1H, d, J = 16.0 Hz, H-8), 5.55 (1H, br t, J = 7.2 Hz, H-2′), 5.07 (1H, br t, J = 6.8 Hz, H-6′), 4.59 (2H, br d, J = 7.2 Hz, H-1′), 4.26 (2H, q, J = 7.2 Hz, OCH_2CH$_3$-9), 3.88 (6H, s, OCH_3-3, OCH_3-5), 1.99 (4H, m, H-4′, 5′), 1.67 (3H, s, H-8′), 1.65 (3H, s, H-9′), 1.59 (3H, s, H-10′), 1.34 (3H, t, J = 7.2 Hz, OCH$_2$CH_3-9); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.0 (C-9), 153.9 (C-3, 5), 144.7 (C-7), 141.7 (C-1), 138.9 (C-4), 131.6 (C-3′), 129.9 (C-7′), 124.0 (C-6′), 120.1 (C-2′), 117.3 (C-8), 105.1 (C-2, 6), 69.5 (C-1′), 60.4 (OCH$_2$CH$_3$-9), 39.6 (C-4′), 56.1 (OCH$_3$-3, 5), 26.4 (C-5′), 25.7 (C-8′), 17.6 (C-9′), 16.3 (C-10′), 13.4 (OCH$_2$$CH_3$-9); ESI-MS m/z $[M+1]^+$ 389.

4.2.2.5. 4-(3′-Bromobenzyl) sinapic acid ethyl ester (11e). Yield: 60%; mp 89–91 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (1H, dd, J = 7.6, 1.6 Hz, H-4′), 7.59 (1H, d, J = 16.0 Hz, H-7), 7.52 (1H, dd, J = 7.2, 1.6 Hz, H-7′), 7.32 (1H, ddd, J = 7.2, 7.2, 1.6 Hz, H-6′), 7.15 (1H, ddd, J = 7.2, 7.6, 1.6 Hz, H-5′), 6.74 (2H, s, H-2, H-6), 6.35 (1H, d, J = 16.0 Hz, H-8), 5.00 (2H, s, H-1′), 4.27 (2H, q, J = 7.2 Hz, OCH_2CH$_3$-9), 3.89 (6H, s, OCH_3-3, OCH_3-5), 1.34 (3H, t, J = 7.2 Hz, OCH$_2$CH_3-9); ESI-MS m/z $[M]^+$ 421.

4.2.2.6. 4-(5′-Bromobenzyl) sinapic acid ethyl ester (11f). Yield: 60%; mp 92–94 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.59 (1H, d, J = 16.0 Hz, H-7), 7.46 (2H, d, J = 8.4 Hz, H-3′, 7′), 7.35 (2H, d, J = 8.4 Hz, H-4′, 6′),

6.74 (2H, s, H-2, H-6), 6.35 (1H, d, J = 16.0 Hz, H-8), 5.00 (2H, s, H-1′), 4.27 (2H, q, J = 7.2 Hz, OCH_2CH$_3$-9), 3.85 (6H, s, OCH_3-3, OCH_3-5), 1.34 (3H, t, J = 7.2 Hz, OCH$_2$CH_3-9); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.8 (C-9), 153.5 (C-3, 5), 144.4 (C-7), 138.5 (C-1), 131.2 (C-4′, 6′), 130.2 (C-2′), 130.0 (C-3′, 7′), 121.8 (C-5′), 117.6 (C-8), 105.0 (C-2, 6), 74.1 (C-1′), 60.4 (OCH$_2$CH$_3$-9), 56.1 (OCH$_3$-3, 5), 14.3 (OCH$_2$$CH_3$-9); HREI-MS calcd for $C_{20}H_{21}BrO_5$ 420.0572, found 420.0583.

4.2.2.7. 4-(4′,5′-Dichlorobenzyl) sinapic acid ethyl ester (11g). Yield: 70%; mp 98–100 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.64 (1H, d, J = 1.6 Hz, H-3′), 7.60 (1H, d, J = 16.0 Hz, H-7), 7.40 (1H, d, J = 8.4 Hz, H-6′), 7.29 (1H, dd, J = 8.4, 1.6 Hz, H-7′), 6.74 (2H, s, H-2, H-6), 6.35 (1H, d, J = 16.0 Hz, H-8), 5.00 (2H, s, H-1′), 4.27 (2H, q, J = 7.2 Hz, OCH_2CH$_3$-9), 3.86 (6H, s, OCH_3-3, OCH_3-5), 1.34 (3H, t, J = 7.2 Hz, OCH$_2$CH_3-9); ESI-MS m/z [M]$^+$ 411.

4.2.2.8. 4-(5′-Bromo-3′-fluoro-benzyl) sinapic acid ethyl ester (11h). Yield: 70%; mp 109–111 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60 (1H, d, J = 16.0 Hz, H-7), 7.48 (1H, t, J = 8.0 Hz, H-7′), 7.29 (1H, dd, J = 8.0, 1.6 Hz, H-6′), 7.22 (1H, dd, J = 9.2, 1.6 Hz, H-4′), 6.73 (2H, s, H-2, H-6), 6.35 (1H, d, J = 16.0 Hz, H-8), 5.08 (2H, s, H-1′), 4.27 (2H, q, J = 7.2 Hz, OCH_2CH$_3$-9), 3.83 (6H, s, OCH_3-3, OCH_3-5), 1.34 (3H, t, J = 7.2 Hz, OCH$_2$CH_3-9); ESI-MS m/z [M]$^+$ 439.

4.2.2.9. 4-(3′-Phenyl-allyl) sinapic acid ethyl ester (11i). Yield: 75%; mp 85–87 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60 (1H, d, J = 16.0 Hz, H-7), 7.37 (2H, dd, J = 7.2, 1.6 Hz, H-5′, H-9′), 7.31 (1H, ddd, J = 7.2, 7.2, 1.6 Hz, H-7′); 7.25 (2H, ddd, J = 7.2, 7.2, 1.6 Hz, H-6′, 8′), 6.75 (2H, s, H-2, H-6), 6.62 (1H, d, J = 16.0 Hz, H-3′), 6.47 (1H, dd, J = 16.0, 6.4 Hz, H-2′), 6.35 (1H, d, J = 16.0 Hz, H-3′), 4.71 (2H, d, J = 6.4 Hz, H-1′), 4.26 (2H, q, J = 7.2 Hz, OCH_2CH$_3$-9), 3.88 (6H, s, OCH_3-3, OCH_3-5), 1.34 (3H, t, J = 7.2 Hz, OCH$_2$CH_3-9); ESI-MS m/z [M+H]$^+$ 369.

4.2.2.10. 4-Hexadecyl sinapic acid ethyl ester (11j). Yield: 77%; mp 64–65 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61 (1H, d, J = 16.0 Hz, H-7), 6.76 (2H, s, H-2, H-6), 6.35 (1H, d, J = 16.0 Hz, H-8), 4.28 (2H, q, J = 7.2 Hz, OCH_2CH$_3$-9), 4.01 (2H, t, J = 6.8 Hz, H-1′), 3.88 (6H, s, OCH_3-3, OCH_3-5), 1.76 (2H, m, H-2′), 1.44 (2H, m, H-3′), 1.35 (3H, t, J = 7.2 Hz, OCH$_2$CH_3-9), 1.34 (2H, m, H-4′), 1.27 (22H, m, H-5′–15′), 0.89 (3H, t, J = 7.2 Hz, H-16′); ESI-MS m/z [M+H]$^+$ 477.

4.2.2.11. 4-(4′-Bromobenzyl) sinapic acid ethyl ester (11k). Yield: 70%; mp 80–81 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (1H, s, H-3′), 7.60 (1H, d, J = 16.0 Hz, H-7), 7.43 (1H, d, J = 7.6 Hz, H-5′), 7.37 (1H, d, J = 7.6 Hz, H-7′), 7.20 (1H, dd, J = 7.6, 7.6 Hz, H-6′), 6.74 (2H, s, H-2, H-6), 6.35 (1H, d, J = 16.0 Hz, H-8), 5.01 (2H, s, H-1′), 4.27 (2H, q, J = 7.2 Hz, OCH_2CH$_3$-9), 3.86 (6H, s, OCH_3-3, OCH_3-5), 1.35 (3H, t, J = 7.2 Hz, OCH$_2$CH_3-9); ESI-MS m/z [M]$^+$ 421.

4.2.2.12. 4-Propargyl sinapic acid ethyl ester (11l). Yield: 70%; mp 89–91 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60 (1H, d, J = 16.0 Hz, H-7), 6.75 (2H, s, H-2, H-6), 6.35 (1H, d, J = 16.0 Hz, H-8), 4.75 (2H, s, H-1′), 4.27 (2H, q, J = 7.2 Hz, OCH_2CH$_3$-9), 3.87 (6H, s, OCH_3-3, OCH_3-5), 2.44 (1H, s, H-3′), 1.34 (3H, t, J = 7.2 Hz, OCH$_2$CH_3-9); ESI-MS m/z [M+H]$^+$ 291.

4.2.3. General procedure for the preparation of 4-*O*-substituted sinapic acid 12a–12n. A KOH solution (48 mg, 0.87 mmol in 3 mL H_2O) was added to the solution of compound **11** (0.29 mmol) in EtOH (5 mL). The mixture was refluxed for 3 h and cooled to room temperature. The reaction was monitored by TLC using pet. ether/EtOAc/formic acid (4:1:0.05) as the mobile phase. 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 ether (3× 20 mL) three times, washed with brine, and dried over anhydrous Na_2SO_4. Removal of ether afforded **12** (65–96%).

4.2.3.1. 4-(5′-Ethoxy-benzyl) sinapic acid (12a). Yield: 96%; mp 135–137 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (1H, d, J = 15.6 Hz, H-7), 7.38 (2H, br d, J = 8.4, 6.8 Hz, H-3′, 7′), 6.85 (2H, br d, J = 8.4, 6.8 Hz, H-4′, 6′), 6.76 (2H, s, H-2, H-6), 6.35 (1H, d, J = 15.6 Hz, H-8), 5.00 (2H, s, H-1′), 4.03 (2H, q, J = 7.2 Hz, OCH_2CH$_3$-5′), 3.86 (6H, s, OCH_3-3, OCH_3-5), 1.40 (3H, t, J = 7.2 Hz, OCH$_2$CH_3-5′); ESI-MS m/z [M − H]$^-$ 357.

4.2.3.2. 4-Benzyl sinapic acid (12b). Yield: 96%; This compound was identical to that reported by Kametani et al.[25]

4.2.3.3. 4-(3′-Chloro-5′-fluoro-benzyl) sinapic acid (12c). Yield: 85%; mp 192–194 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69 (1H, d, J = 16.0 Hz, H-7), 7.68 (1H, dd, J = 8.4, 5.2 Hz, H-7′), 7.10 (1H, dd, J = 8.4, 2.4 Hz, H-4′), 6.98 (1H, dt, J = 8.4, 2.4 Hz, H-6′), 6.76 (2H, s, H-2, H-6), 6.36 (1H, d, J = 16.0 Hz, H-8), 5.08 (2H, s, H-1′), 3.85 (6H, s, OCH_3-3, OCH_3-5); ESI-MS m/z [M − H]$^-$ 365.

4.2.3.4. 4-Geranyl sinapic acid (12d). Yield: 85%; This compound was identical to that reported by Zhao et al.[5]

4.2.3.5. 4-(3′-Bromobenzyl) sinapic acid (12e). Yield: 75%; mp 169–171 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72 (1H, d, J = 16.0 Hz, H-7), 7.72 (1H, dd, J = 8.4, 1.6 Hz, H-4′), 7.54 (1H, dd, J = 8.4, 1.6 Hz, H-7′), 7.33 (1H, ddd, J = 8.4, 8.4, 1.6 Hz, H-6′), 7.16 (1H, ddd, J = 8.4, 8.4, 1.6 Hz, H-5′), 6.78 (2H, s, H-2, H-6), 6.37 (1H, d, J = 16.0 Hz, H-8), 5.15 (2H, s, H-1′), 3.86 (6H, s, OCH_3-3, OCH_3-5); ESI-MS m/z [M]$^-$ 393.

4.2.3.6. 4-(5′-Bromobenzyl) sinapic acid (12f). Yield: 75%; mp 169–171 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (1H, d, J = 16.0 Hz, H-7), 7.47 (2H, d, J = 8.4 Hz, H-3′, 7′), 7.35 (2H, d, J = 8.4 Hz, H-4′, H-6′), 6.76 (2H, s, H-2, H-6), 6.36 (1H, d, J = 16.0 Hz, H-8), 5.01

(2H, s, H-1′), 3.86 (6H, s, OCH_3-3, OCH_3-5); ESI-MS *m*/*z* $[M]^-$ 393.

4.2.3.7. 4-(4′,5′-Dichlorobenzyl) sinapic acid (12g). Yield: 70%; mp 193–195 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (1H, d, J = 16.0 Hz, H-7), 7.64 (1H, s, H-3′), 7.41 (1H, d, J = 8.0 Hz, H-6′), 7.30 (1H, d, J = 8.0 Hz, H-7′), 6.77 (2H, s, H-2, H-6), 6.36 (1H, d, J = 16.0 Hz, H-8), 5.01 (2H, s, H-1′), 3.88 (6H, s, OCH_3-3, OCH_3-5); ESI-MS *m*/*z* $[M]^-$ 383.

4.2.3.8. 4-(5′-Bromo-3′-fluorobenzyl) sinapic acid (12h). Yield: 70%; mp 187–189 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69 (1H, d, J = 16.0 Hz, H-7), 7.48 (1H, t, J = 8.0 Hz, H-7′), 7.28 (1H, dd, J = 8.0, 1.6 Hz, H-6′), 7.23 (1H, dd, J = 9.2, 1.6 Hz, H-4′), 6.76 (2H, s, H-2, H-6), 6.36 (1H, d, J = 16.0 Hz, H-8), 5.09 (2H, s, H-1′), 3.85 (6H, s, OCH_3-3, OCH_3-5); ESI-MS *m*/*z* $[M]^-$ 411.

4.2.3.9. 4-(3′-Phenyl-allyl) sinapic acid (12i). Yield: 75%; mp 123–124 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.68 (1H, d, J = 16.0 Hz, H-7), 7.38 (2H, d, J = 7.2 Hz, H-5′, H-9′), 7.31 (1H, ddd, J = 7.2, 7.2, 1.6 Hz, H-7′); 7.24 (2H, ddd, J = 7.2, 7.2, 1.6 Hz, H-6′, 8′), 6.80 (2H, s, H-2, H-6), 6.63 (1H, d, J = 16.0 Hz, H-3′), 6.47 (1H, dd, J = 16.0, 6.4 Hz, H-2′), 6.35 (1H, d, J = 16.0 Hz, H-8), 4.73 (2H, d, J = 6.4 Hz, H-1′), 3.88 (6H, s, OCH_3-3, OCH_3-5); ESI-MS *m*/*z* $[M - H]^-$ 339.

4.2.3.10. 4-Hexadecyl sinapic acid (12j). Yield: 70%; mp 75–76 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (1H, d, J = 16.0 Hz, H-7), 6.77 (2H, s, H-2, H-6), 6.35 (1H, d, J = 16.0 Hz, H-8), 4.01 (2H, t, J = 6.8 Hz, H-1′), 3.88 (6H, s, OCH_3-3, OCH_3-5), 1.75 (1H, m, H-2′), 1.44 (2H, m, H-3′), 1.20–1.28 (24H, m, H-4′–15′), 0.88 (3H, t, J = 7.2 Hz, H-16′); ESI-MS *m*/*z* $[M - H]^-$ 447.

4.2.3.11. 4-(4′-Bromobenzyl) sinapic acid (12k). Yield: 65%; mp 116–117 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71 (1H, s, H-3′), 7.70 (1H, d, J = 15.6 Hz, H-7), 7.43 (1H, d, J = 7.6 Hz, H-5′), 7.37 (1H, d, J = 6.8 Hz, H-7′), 7.21 (1H, dd, J = 7.6, 6.8 Hz, H-6′), 6.76 (2H, s, H-2, H-6), 6.36 (1H, d, J = 15.6 Hz, H-8), 5.02 (2H, s, H-1′), 3.87 (6H, s, OCH_3-3, OCH_3-5); ESI-MS *m*/*z* $[M]^-$ 393.

4.2.3.12. 4-(4′-Fluorobenzyl) sinapic acid (12m). Yield: 65%; mp 134–136 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61 (1H, d, J = 16.0 Hz, H-7), 7.28–7.42 (3H, m, H-3′, H-6′, H-7′), 7.08 (1H, dt, J = 8.4, 2.0 Hz, H-5′),7.06 (2H, s, H-2, H-6), 6.50 (1H, d, J = 16.0 Hz, H-8), 5.05 (2H, s, H-1′), 3.91 (6H, s, OCH_3-3, OCH_3-5); ESI-MS *m*/*z* $[M - H]^-$ 331.

4.2.3.13. 4-(3′-Isopentenyl) sinapic acid (12n). Yield: 65%; mp 99–101 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72 (1H, d, J = 16.0 Hz, H-7), 6.78 (2H, s, H-2, H-6), 6.36 (1H, d, J = 16.0 Hz, H-8), 5.56 (1H, br t, J = 7.6 Hz, H-4′), 4.55 (2H, br d, J = 7.6 Hz, H-1′), 3.89 (6H, s, OCH_3-3, OCH_3-5), 1.68 (3H, s, H-5′); ESI-MS *m*/*z* $[M - H]^-$ 291.

4.2.4. General procedure for the preparation of 4-*O*-substituted sinapyl alcohol (13a–13k)

4.2.4.1. Method A (compound 13a, 13d). Compound **14** (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 1–2 h. The reaction was monitored by TLC using pet. ether/EtOAc (3:1) as the mobile phase. 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 (3× 25 mL), washed with brine (3× 10 mL), dried over anhydrous $MgSO_4$, and evaporated to give the residue. The crude product was purified through column chromatography (silica gel H) with pet. ether/EtOAc (5:1 to 3:1) to give **13** (60–70%).

4.2.4.2. Method B. $LiAlH_4$ (28 mg, 0.72 mmol) was added to anhydrous ether (8 mL) with vigorous stirring in an ice-salt bath. Compound **11** (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 30–60 min. The reaction was monitored by TLC using pet. ether/EtOAc (3:1) as the mobile phase. Two milliliters of water was added to destroy the excessive $LiAlH_4$, and the solution was acidified to pH 5. The aqueous layer was extracted with ether (3× 15 mL) and the combined organic layer was washed with brine and dried over anhydrous Na_2SO_4 for 10 h. Removal of the solvent and the residue was purified through column chromatography (silica gel H) with pet. ether/EtOAc (6:1 to 3:1) to give **13** (40–70%).

4.2.4.3. 4-(5′-Ethoxybenzyl) sinapyl alcohol (13a). Yield: 70%; mp 116–118 °C; 1H NMR (400 MHz, $CDCl_3$): δ 7.38 (2H, dd, J = 6.8, 2.0 Hz, H-3′, 7′), 6.84 (2H, dd, J = 6.8, 2.0 Hz, H-4′, 6′), 6.59 (2H, s, H-2, H-6), 6.55 (1H, d, J = 16.0 Hz, H-7), 6.28 (1H, dt, J = 4.8, 16.0 Hz, H-8), 4.94 (2H, s, H-1′), 4.32 (2H, br d, J = 4.8 Hz, H-9), 4.01 (2H, q, J = 6.8 Hz, OCH_2CH$_3$-5′), 3.83 (6H, s, OCH_3-3, OCH_3-5), 1.41 (1H, t, J = 6.8 Hz, OCH$_2$CH_3-5′); ESI-MS *m*/*z* $[M+H]^+$ 345.

4.2.4.4. 4-Benzyl sinapyl alcohol (13b). Yield: 50%; this compound was identical to that reported by Zhao et al.[5]

4.2.4.5. 4-(3′-Chloro-5′-fluorobenzyl) sinapyl alcohol (13c). Yield: 45%; mp 98–100 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.68 (1H, d, J = 8.4, 5.2 Hz, H-7′), 7.10 (1H, dd, J = 8.4, 2.4 Hz, H-4′), 6.98 (1H, dt, J = 8.4, 2.4 Hz, H-6′), 6.54 (1H, d, J = 15.6 Hz, H-7), 6.31 (2H, s, H-2, H-6), 6.31 (1H, dt, J = 15.6, 5.2 Hz, H-8), 5.07 (2H, s, H-1′), 4.32 (2H, br d, J = 5.2 Hz, H-9), 3.82 (6H, s, OCH_3-3, OCH_3-5); ESI-MS *m*/*z* $[M+H]^+$ 353.

4.2.4.6. 4-Geranyl sinapyl alcohol (13d). Yield: 60%; ESI-MS *m*/*z* $[M+K]^+$ 385. This compound was identical to that reported by Zhao et al.[5]

4.2.4.7. 4-(3′-Bromobenzyl) sinapyl alcohol (13e). Yield: 50%; mp 65–67 °C; ^{1}H NMR (400 MHz, $CDCl_3$):

δ 7.75 (1H, d, J = 7.6 Hz, H-4′), 7.52 (1H, dd, J = 7.6 Hz, H-7′), 7.32 (1H, dd, J = 7.6, 7.6 Hz, H-6′), 7.15 (1H, dd, J = 7.6, 7.6 Hz, H-5′), 6.62 (2H, s, H-2, H-6), 6.55 (1H, d, J = 15.6 Hz, H-7), 6.30 (1H, dt, J = 5.6, 15.6 Hz, H-8), 5.09 (2H, s, H-1′), 4.33 (2H, br d, J = 5.6 Hz, H-9), 3.83 (6H, s, OCH_3-3, OCH_3-5); ESI-MS m/z $[M]^+$ 379.

4.2.4.8. 4-(5′-Bromobenzyl) sinapyl alcohol (13f). Yield: 45%; mp 106–108 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.47 (1H, d, J = 8.0 Hz, H-3′, H-7′), 7.37 (1H, dd, J = 7.6 Hz, H-4′, H-6′), 6.52 (1H, d, J = 12.0 Hz, H-7), 6.46 (2H, s, H-2, H-6), 5.85 (1H, dt, J = 12.0, 6.4 Hz, H-8), 4.95 (2H, s, H-1′), 4.45 (2H, d, J = 6.4 Hz, H-9), 3.83 (6H, s, OCH_3-3, OCH_3-5); ESI-MS m/z $[M]^+$ 379.

4.2.4.9. 4-(4′,5′-Dichlorobenzyl) sinapyl alcohol (13g). Yield: 55%; mp 102–104 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.64 (1H, s, H-3′), 7.60 (1H, d, J = 16.0 Hz, H-7), 7.40 (1H, d, J = 8.0 Hz, H-6′), 7.29 (1H, d, J = 8.0 Hz, H-7′), 6.74 (2H, s, H-2, H-6), 6.35 (1H, d, J = 12.0, 6.4 Hz, H-8), 5.00 (2H, s, H-1′), 4.45 (2H, br d, J = 6.4 Hz, H-9), 3.86 (6H, s, OCH_3-3, OCH_3-5); ESI-MS m/z $[M]^+$ 369.

4.2.4.10. 4-(5′-Bromo-3′-fluorobenzyl) sinapyl alcohol (13h). Yield: 50%; mp 79–81 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.59 (1H, d, J = 16.0 Hz, H-7), 7.48 (1H, t, J = 8.0 Hz, H-7′), 7.27 (1H, dd, J = 8.0, 1.6 Hz, H-6′), 7.22 (1H, dd, J = 9.2, 2.0 Hz, H-4′), 6.73 (2H, s, H-2, H-6), 6.35 (1H, dt, J = 7.2, 16.0 Hz, H-8), 5.08 (2H, s, H-1′), 4.27 (2H, br d, J = 7.2 Hz, H-9), 3.83 (6H, s, OCH_3-3, OCH_3-5); ESI-MS m/z $[M]^+$ 397.

4.2.4.11. 4-Hexadecyl sinapyl alcohol (13j). Yield: 40%; mp 57–58 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.61 (2H, s, H-2, H-6), 6.54 (1H, d, J = 15.6 Hz, H-7), 6.30 (1H, dt, J = 4.8, 15.6 Hz, H-8), 4.32 (2H, br d, J = 4.8 Hz, H-9), 3.95 (2H, t, J = 6.8 Hz, H-1′), 3.85 (6H, s, OCH_3-3, OCH_3-5), 1.74 (2H, m, H-2′), 1.43 (2H, m, H-3′), 1.20–1.28 (24H, m, H-4′–15′), 0.87 (3H, t, J = 6.8 Hz, H-16′); ESI-MS m/z $[M+H]^+$ 435.

4.2.4.12. 4-(4′-Bromobenzyl) sinapyl alcohol (13k). Yield: 50%; mp 88–90 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (1H, s, H-3′), 7.62 (1H, d, J = 16.0 Hz, H-7), 7.43 (1H, d, J = 7.6 Hz, H-5′), 7.35 (1H, dd, J = 8.4, 1.6 Hz, H-7′), 7.21 (1H, dd, J = 8.4, 7.6 Hz, H-6′), 6.72 (2H, s, H-2, H-6), 6.36 (1H, dt, J = 16.0, 6.8 Hz, H-8), 5.00 (2H, s, H-1′), 4.23 (2H, br d, J = 6.8 Hz, H-9), 3.87 (6H, s, OCH_3-3, OCH_3-5); ESI-MS m/z $[M]^+$ 379.

4.2.5. General procedure for the preparation of 4-*O*-substituted sinapyl aldehyde 14a, 14d, 14o

4.2.5.1. Method A (compound 14a). A 40% acetaldehyde solution (14 μL), 0.13 mmol) was added to compound **10a** (0.19 mmol) in 1.5 mL 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 monitored by TLC using pet. ether/EtOAc (3:1) as the mobile phase. Then it was quenched by adding excess of NaCl solution. The mixture was extracted by ether and the organic layer was dried over anhydrous Na_2SO_4. The target compound **14a** was purified through column chromatography (silica gel H, pet. ether/EtOAc 5:1) to give **14a** in a yield of 50%.

4.2.5.2. Method B. To a solution of compound **13** (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 3–4 h. The reaction was monitored by TLC using pet. ether/EtOAc (3:1) as the mobile phase. The residue was obtained by filtration and evaporation which was purified by column chromatography (silica gel H) with pet. ether/EtOAc (6:1 to 4:1) to give **14** in the yields of 50–70%.

4.2.5.3. 4-(5′-Ethoxybenzyl) sinapyl aldehyde (14a). Yield: 50%; mp 123–125 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.68 (1H, d, J = 7.6 Hz, H-9), 7.38 (1H, d, J = 15.6 Hz, H-7), 7.36 (2H, d, J = 8.4 Hz, H-3′, H-7′), 6.85 (2H, d, J = 8.4 Hz, H-4′, 6′), 6.77 (2H, s, H-2, H-6), 6.63 (1H, dd, J = 15.6, 7.6 Hz, H-8), 5.01 (2H, s, H-1′), 4.04 (2H, q, J = 6.8 Hz, OCH_2CH$_3$-5′), 3.86 (6H, s, OCH_3-3, OCH_3-5), 1.40 (3H, t, J = 6.8 Hz, OCH$_2$CH_3-5′); ESI-MS m/z $[M-H]^-$ 357.

4.2.5.4. 4-Geranyl sinapyl aldehyde (14d). Yield: 50%; ESI-MS m/z $[M+Na]^+$ 367; This compound was identical to that reported by Zhao et al.[26]

4.2.5.5. 4-Methyl sinapyl aldehyde (14o). Yield: 70%; This compound was identical to that reported by Olstein and Stephenson.[27]

4.2.6. Procedure for the preparation of 3,5-demethyl-4-*O*-substituted sinapic acid ethyl ester

4.2.6.1. 3,4,5-Trihydroxy-benzaldehyde (15). Anhydrous aluminum chloride (1.89 g, 14.3 mmol) was suspended in 10 mL CH_2Cl_2 followed by adding the solution of **8** in CH_2Cl_2 (10 mL). Then the reaction mixture was stirred at room temperature for 12 h. The reaction was cooled in an ice-salt bath and quenched with 1 M HCl. The mixture was evaporated to remove CH_2Cl_2 and extracted by ethyl acetate (3× 20 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated to give the residue. The crude product was purified through column chromatography with $CHCl_3$/MeOH (4:1) to give **15** (100 mg, 30%). This compound was identical to that reported by Freudenberg and Hübner.[28]

4.2.6.2. 3,4,5-Triacetoxy-benzaldehyde (16). Triethyl amine (0.22 mL, 1.55 mmol) was slowly added to 3,4,5-trihydroxy-benzaldehyde (40 mg, 0.26 mmol) in acetic anhydride (1.0 mL) at 0 °C. Then it was stirred at room temperature for 2 h. The reaction was monitored by TLC using pet. ether/EtOAc (3:1) as the mobile phase. The excess of acetic anhydride was destroyed by careful addition of EtOH (0.5 mL) at 0 °C. The mixture was diluted with water and extracted with ether acetate. The organic layer was dried over anhydrous Na_2SO_4 followed by filtration and concentration to give a residue, which was subjected to column chromatography (silica gel H, pet. ether/EtOAc 5:2) to afford a pure pale yellow oil **17** (54 mg). Yield: 75%; ^{1}H NMR (400 MHz,

$CDCl_3$): δ 9.98 (1H, s, H-7), 7.67 (2H, s, H-2, H-6), 2.33 (CH_3COO-3, CH_3COO-4, CH_3COO-5, s, 9H); ESI-MS *m/z* $[M+H]^+$ 281.

4.2.6.3. 4-Benzyloxy-3,5-diacetoxy-benzaldehyde (17). Benzyl bromide (54 mg, 0.32 mmol) was addded to a mixture of **16** (50 mg, 0.16 mmol) and K_2CO_3 (66 mg, 0.48 mmol) in 4 mL DMF. The resulting mixture was stirred at 40 °C for 3 h. The reaction was monitored by TLC using pet. ether/EtOAc (3:1) as the mobile phase. K_2CO_3 was filtered and the mixture was diluted with ethyl acetate (30 mL) and washed by water. The organic phase was dried over anhydrous $NaSO_4$ overnight. Filtration, concentration gave a residue, which was further purified by column chromatography (silica gel H, pet. ether/EtOAc 9:1) to afford a pure pale yellow oil **17** (35 mg). Yield: 60%; ^1H NMR (400 MHz, $CDCl_3$): δ 9.88 (1H, s, H-7), 7.56 (2H, s, H-2, H-6), 7.40 (5H, m, H-3′, 4′, 5′, 6′, 7′), 5.05 (2H, s, H-1′), 2.26 (CH_3COO-3, CH_3COO-5, s, 6H); ESIMS *m/z* $[M+H]^+$ 329.

4.2.6.4. 4-Benzyloxy-3,5-diacetoxy-sinapic acid ethyl ester (18). A mixture of compound **17** (40 mg, 0.12 mmol) in anhydrous benzene (5 mL) and (carbethoxymethylene)-triphenylphosphorane (57 mg, 0.16 mmol) was refluxed for 2–3 h. The reaction was monitored by TLC using pet. ether/EtOAc (5:1) as the mobile phase. The solvent was removed and the concentrate was purified by column chromatography (pet. ether/EtOAc 7:1) to give **18** (39 mg). Yield: 80%; ^1H NMR (400 MHz, $CDCl_3$): δ 7.53 (1H, d, J = 16.0 Hz, H-7), 7.40 (5H, m, H-3′, 4′, 5′, 6′, 7′), 6.70 (2H, s, H-2, H-6), 6.30 (1H, d, J = 16.0 Hz, H-8), 5.09 (2H, s, H-1′), 4.28 (2H, q, J = 7.2 Hz, OCH_2CH_3-9), 2.27 (CH_3COO-3, CH_3COO-5, s, 6H), 1.34 (3H, t, J = 7.2 Hz, OCH_2CH_3-9); ESI-MS *m/z* $[M+H]^+$ 399.

4.2.6.5. 4-Benzyloxy-3,5-dihydroxy-sinapic acid ethyl ester (19). K_2CO_3 (61 mg, 0.44 mmol) was added to the solution of **18** (30 mg) in MeOH (2 mL) and water (0.5 mL). The mixture was stirred at room temperature for 20 min. The reaction was monitored by TLC using pet. ether/EtOAc (3:1) as the mobile phase. Evaporation removed the MeOH and the residue, was acidified with 1 M HCl to pH 5 followed by extraction with ethyl acetate. Then the organic layer was washed with brine and dried over anhydrous $NaSO_4$ overnight. Filtration and concentration afforded the residue which was purified by column chromatography (silica gel H, pet. ether/EtOAc 9:1) to afford **19** (16 mg). Yield: 70%; mp 102–104 °C; ^1H NMR (400 MHz, $CDCl_3$): δ 7.53 (1H, d, J = 16.0 Hz, H-7), 7.41 (5H, m, H-3′, 4′, 5′, 6′, 7′), 6.70 (2H, s, H-2, H-6), 6.30 (1H, d, J = 16.0 Hz, H-8), 5.09 (2H, s, H-1′), 4.28 (2H, q, J = 7.2 Hz, OCH_2CH_3-9), 1.34 (3H, t, J = 7.2 Hz, OCH_2CH_3-9); ESI-MS *m/z* $[M+H]^+$ 315.

4.3. Cell culture

Various human tumor cell lines were cultured in minimum essential medium (MEM), supplemented with 10% fetal calf serum (Gibco Laboratories, Grand Island, NY), 100 U/mL penicillin, and 100 mg/mL streptomycin in a humidified atmosphere in 5% CO_2 at 37 °C. Cell culture media were renewed every three days, up to the confluence of the monolayer. Cell culture was passaged when they had formed confluent cultures, using trypsin–EDTA to detach the cells from their culture flasks or dishes. Test compounds were stored at −70 °C and solubilized in 100% DMSO.

4.4. Cytotoxicity evaluation

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) was added to the culture medium and incubated at 37 °C for 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 measured on a microplate reader at a wavelength of 570 nm. The average 50% inhibitory concentration (IC_{50}) was determined graphically from the dose–response curves.

4.5. CoMFA

Steric and electrostatic interactions were calculated using a sp^3 carbon atom as steric probe and a +1 charge as electrostatic probe with Tripos force field. The CoMFA grid spacing is 2.0 Å in the x, y, and z directions. The minimum *r* (column filtering) was set to 2.0 kcal/mol to improve the signal-to-noise ratio by omitting those lattice points whose energy variation was below this threshold. A cutoff of 30 kcal/mol was adopted, and the regression analysis was carried out using the full cross-validated partial least-squares (PLS) method (leave-one-out) with CoMFA standard options for scaling of variables. The final model (non-cross-validated conventional analysis) was developed with the optimum number of components equal to that yielding the highest q^2.

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Supplementary data

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