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Synthesis and structure–activity relationship studies of cytotoxic cinnamic alcohol derivatives

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Three series of di- and trisubstituted derivatives of cinnamic alcohol and its conjugated dienol analogues were designed and synthesised. The derivatives were screened for cytotoxicity against nine tumour cell lines: KB, A549, Hela, CNE, PC-3, BEL-7404, HL-60, BGC823 and P388D1. Most of the cinnamic alcohol derivatives showed cytotoxic activity. The compound 7-(4′,5′-dichlorobenzyloxy)-6,8-dihydroxycinnamic alcohol (**55**) exhibited significant cytotoxicity to seven human tumour cell lines on a micromolar range, especially with regard to the KB and P388D1 cell lines, showing IC_{50} values of 0.4 and 0.5 μM, respectively. The structure–activity relationships of the derivatives are discussed.

Keywords: cinnamic alcohol derivatives; cytotoxicity; structure–activity relationship

1. Introduction

Cinnamic alcohols are a widespread group of natural phenylpropanoids and a number of those originating from higher plants have been shown to be highly bioactive, possessing significant cytotoxic activity (Choi et al., 2004; Cis, Nowak, Horoszkiewicz-Hassan, & Kisiel, 2003; Ito et al., 2000; Zhao et al., 2002). Despite their structural simplicity and interesting cytotoxic properties, their structure–cytotoxicity relationship has rarely been described (Zou et al., 2006). Our previously reported studies have shown that the 6,8-dimethoxy-7-(5′-ethoxybenzyloxy)-cinnamic alcohol (**1**, Figure 1), a derivative of the natural geranyloxysinapyl alcohol isolated from *Ligularia nelumbifolia*, shows potent cytotoxicity to several human tumour cell lines, and is metabolically stable due to substitution of the arylalkyloxy substituent instead of the geranyloxy unit (Zou et al., 2006). Comparison of the previously reported cinnamic alcohol derivative (**2**) with its methylated derivatives indicates that the phenolic groups, instead of the methoxy group, on C6 and C8 may enhance the cytotoxic effect on a number of selected human tumour cell lines

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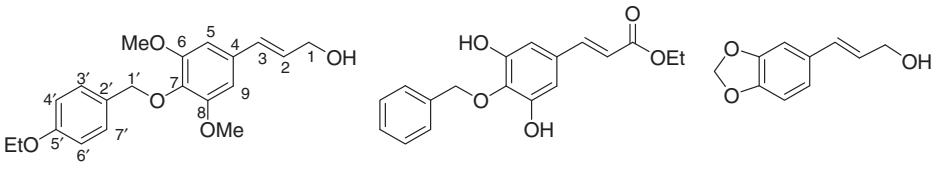


Figure 1. Cinnamic alcohol derivatives.

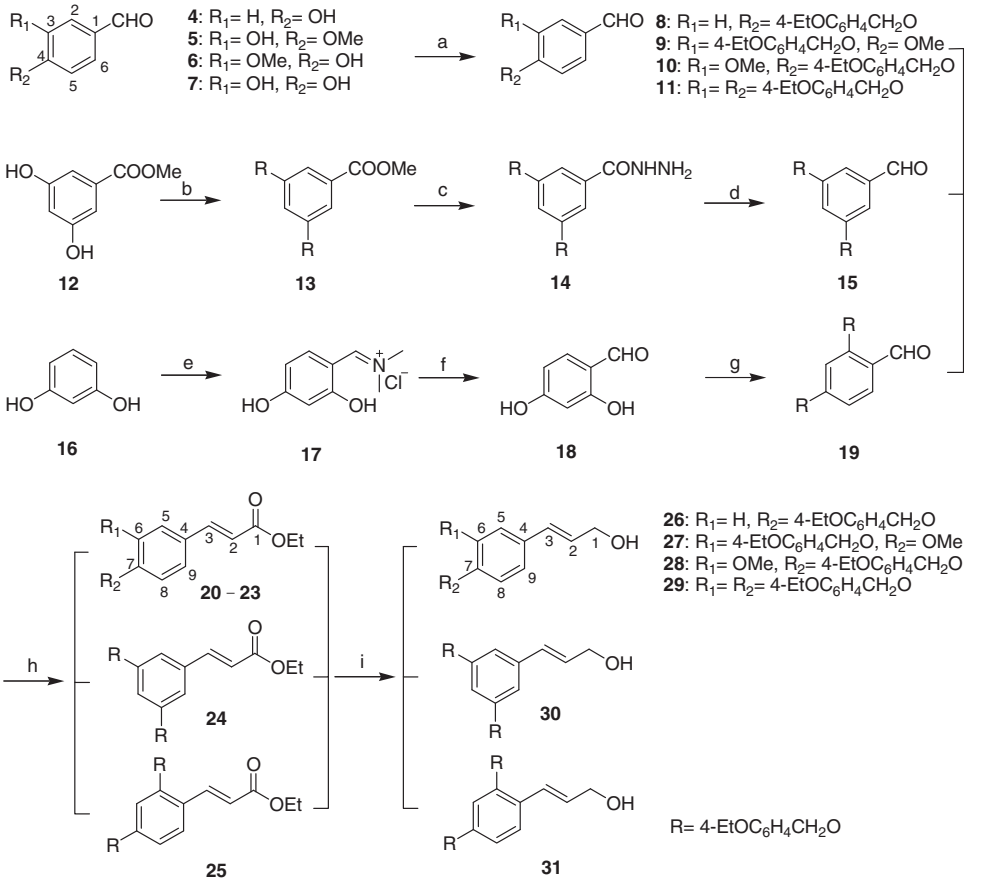
(Zou et al., 2006). Previous 3D-QSAR studies of the sinapyl alcohol derivatives lead to the conclusion that the bulky negative charged substituents at the C1 of cinnamic alcohol confer increased cytotoxicity (Zou et al., 2006). In 2000, Ito et al. reported that 6,7-(methylenedioxy)-cinnamic alcohol (**3**), isolated from the root of *Boronia pinnata*, inhibits Epstein-Barr virus early antigen (EBV-EA) activation (Ito et al., 2000), which is considered to be one of the causative agents of nasopharyngeal carcinoma. This suggests that the trisubstituted unit of cinnamic alcohol is not necessary for its cytotoxic activity.

In order to further investigate the structure–cytotoxicity relationship of cinnamic alcohol derivatives, disubstituted derivatives with different substitution positions (Scheme 1), trisubstituted derivatives with variant substituents (Scheme 2) and their conjugated dienols (Scheme 3) were synthesised resulting in three series of 20 compounds based on the structures of compounds **1**–**3** (Figure 1). The synthetic procedures are reported herein. The synthesised cinnamic alcohol derivatives and some of the intermediates were then used to screen nine tumour cell lines. The results showed that the compounds possess cytotoxic potential. Moreover, the structure–activity relationship discussion on the cytotoxicity effect of structural variations in substituents, substitution position and charge properties are also discussed as the basis for further systematic research in this field.

2. Results and discussion

2.1. *Synthesis of cinnamic alcohol derivatives*

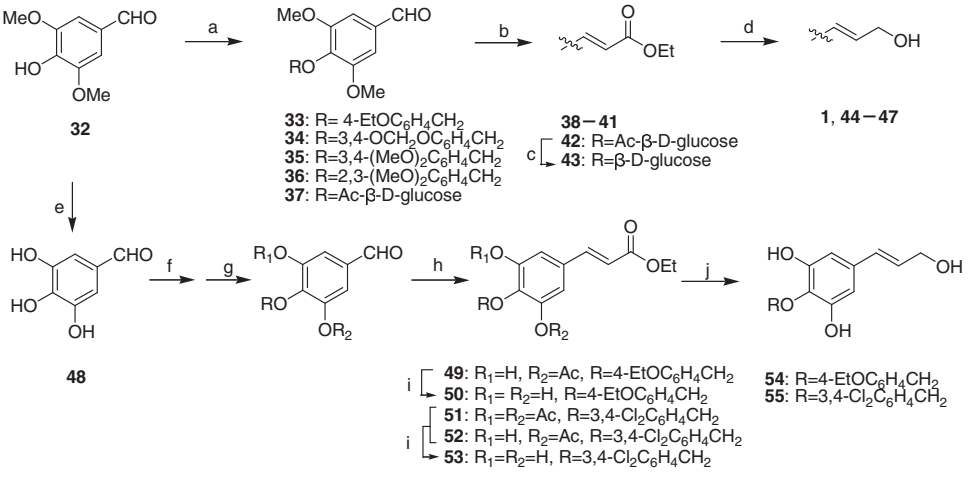
Three series of sinapyl alcohol derivatives were synthesised (Schemes 1–3) and some of them were included in a Chinese Patent (Zhao, Wang, Zeng, Wu, & Bai, 2008a; Zhao et al., 2008b). The synthetic route to double substituted cinnamic alcohol derivatives (series 1) is illustrated in Scheme 1. Compounds **4**–**7** were subjected to an alkylation of the corresponding hydroxy group by 4-ethoxy-benzyl bromide in the presence of potassium carbonate, to provide the 3,4-disubstituted aldehydes **8**–**11** (K. Park, Han, & J. Park, 2001). The 3,5-disubstituted aldehyde **15** was prepared via three steps from starting material **12**, including alkylation with 4-ethoxy-benzyl bromide to obtain **13** (Park et al., 2001). Treatment of **13** with hydrazine afforded **14** (Macaev et al., 2005) and an oxidation with potassium ferrocyanide gave the aldehyde **15** (Chida, Vani, Chandrasekharam, Srinivasan, & Singh, 2001). 2,4-Dihydroxybenzalaldehyde (**18**) was obtained by Vilsmeier–Haack reaction (Mendelson & Hayden, 1996) from resorcinol (**16**) and a further alkylation of **18** with 4-ethoxy-benzyl bromide afforded **19** (Park et al., 2001). Further condensation of **8**–**11**, **15** and **19** directly with monoethyl malonate afforded compounds **20**–**25**



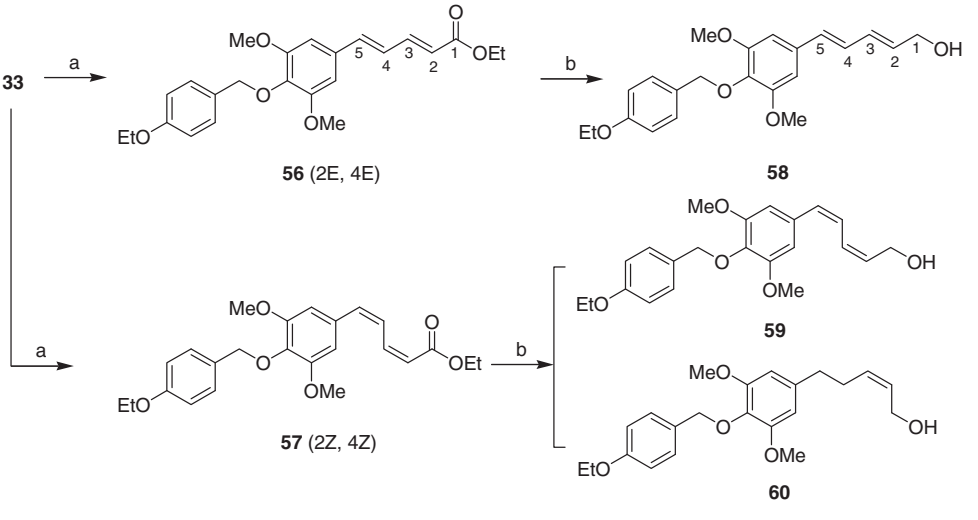
Scheme 1. Reagents and conditions: (a) RBr, K_2CO_3, acetone, reflux, 4 h; (b) same as (a), 96%; (c) 85% $NH_2NH_2 \cdot H_2O$, EtOH, reflux, 3 h, 63%; (d) $K_3Fe(CN)_6$, Bu_4NBr, $NH_3 \cdot H_2O$, benzene, H_2O, room temperature, 30 min, 66%; (e) DMF, $(COCl)_2$, MeCN, −20–25°C, 3.5 h; (f) NaS_2O_3, H_2O, 52°C, 53% for (e) and (f); (g) same as (a), 68%; (h) monoethyl malonate, pyridine, piperidine, reflux, 4 h; (i) $LiAlH_4$, Et_2O, 10°C, 2 h.

(List, Doehring, Hechavarria Fonseca, Job, & Rios Torres, 2005), which were reduced with lithium aluminumhydride in ether to give the corresponding disubstituted cinnamic alcohol derivatives **26**–**31** (Ghera & Ben-David, 1988).

The trisubstituted cinnamic alcohol derivatives **1** and **44**–**47** (series 2) were synthesised from the starting material syringaldehyde (**32**), which was alkylated with corresponding bromides (Park et al., 2001) to afford aldehydes **33**–**37** (Scheme 2). The aldehydes were subjected to Knoevengel reaction (List et al., 2005) to afford cinnamic acid ethyl esters **38**–**42**, of which **42** was further hydrolysed (Oyama & Kondo, 2004) to get **43** with a free *β*-D-glucopyranosyl substituent. Reduction of **38**–**41** and **43** with lithium aluminumhydride (Ghera & Ben-David, 1988) afforded the cinnamic alcohols **1** and **44**–**47**. The 7-alkyloxy-6,8-dihydroxy substituted cinnamic alcohol derivatives **54**–**55** were obtained by reduction (Ghera & Ben-David, 1988) of corresponding esters (**50**, **53**) which were synthesised from syringaldehyde via five steps, as previously reported (Zou et al., 2006).



Scheme 2. Reagents and conditions: (a) RBr, K_2CO_3, acetone, reflux, 4 h; (b) monoethyl malonate, pyridine, piperidine, reflux, 4 h; (c) NaOMe, MeOH, room temperature, 1 min; (d) $LiAlH_4$, Et_2O, 0–10°C, 2 h; (e) $AlCl_3$, CH_2Cl_2, room temperature, 48 h, 31%; (f) Ac_2O, Et_3N, 0°C then room temperature, 4 h, 48%; (g) RBr, K_2CO_3, DMF, 45°C, 12 h; (h) $Ph_3P{=}CHCO_2Et$, benzene, reflux, 2 h; (i) K_2CO_3, MeOH, room temperature, 1.5 h; (j) $LiAlH_4$–$AlCl_3$, Et_2O, 10°C, 2 h.



Scheme 3. Reagents and conditions: (a) $(Ph_3P^+{=}CHCH{=}CHCO_2Et)Br^-$, Na, EtOH, THF, reflux, 4 h; (b) $LiAlH_4$–$AlCl_3$, Et_2O, 0°C, 1.5 h.

Wittig reaction of 3,5-dimethoxy-4-(5′-ethoxybenzyloxy)benzaldehyde (**33**) and the phosphorus ylide, prepared from 4-bromocrotonic acid ethyl ester and triphenylphosphine (Mazur, Hope-Ross, Kadla, Sederoff, & Chang, 2007), afforded two dienoic acid ethyl esters **56** (2E, 4E) and **57** (2Z, 4Z) (Scheme 3). Further reduction of **56** and **57** with lithium aluminumhydride and aluminum chloride

complex afforded two dienols **58** (2E, 4E), **59** (2Z, 4Z) and one enol **60** (2Z) (series 3). For all these compounds, TLC on silica gel (GF_{254}) was used for analysis and column chromatography was carried out on silica gel H (10–40 μm).

2.2. *Structure–activity relationship of synthesised cytotoxic cinnamic alcohol derivatives*

The targeted cinnamic alcohol derivatives and their intermediates (**8**–**60**) were evaluated against nine tumour cell lines by the colourimeter assay MTT (Horowitz & King, 2000; Putnam, Bombick, & Doolittle, 2002). Although the binding sites of both the anticancer drug cisplatin (DDP) and the series of described cinnamic alcohol derivatives might not be identical, DDP was used as a reference compound because of its well-established clinical application in cancer treatment. 7-(5′-Ethoxybenzyloxy)-6,8-dimethoxycinnamic alcohol (**1**) was also used as a control. Results showing the concentrations required to inhibit cell growth by 50% (IC_{50} values) are presented in Table 1.

Analysis of the results of the MTT assay indicated that most of the analogues of series 1 showed moderate cytotoxicity to the nine tumour cell lines. They also suggest that analogues of series 2 with two free hydroxyl groups (**53**–**55**) were generally more potent than those from series 1 and 3. Compound **55** displayed significant cytotoxicity to eight of the tumour cell lines, especially to the KB and P388D1 cell lines, with IC_{50} values of 0.4 and 0.5 μM, which was 50 and 30 times higher than that observed for the control compound **1** and DDP values, respectively. The bioassay results also suggest that the cytotoxic activity of the dienols from series 3 is tightly linked to the configuration of their double bond.

By thorough analysis of analogues **8**–**31** (series 1), the 6,8-disubstituted cinnamic alcohol **30** and the 5,7-disubstituted one (**31**) showed intensive cytotoxicity to most of the nine tumour cell lines tested, especially **31** to P388D1, with an IC_{50} value of 1.2 μM. In contrast, the 6,7-disubstituted cinnamic alcohol derivatives (**27**–**29**), cinnamic acid esters (**21**–**23**) and their intermediate aldehydes (**9**–**11**) showed weak cytotoxicity to the nine tumour cell lines, which suggests that for the cytotoxicity of disubsitituted cinnamic alcohols, positions C-6 and C-7 are not favoured for substitution by an alkyloxy group. The mono-subsitituted cinnamic alcohol **26** alone showed potent cytotoxic activity to seven of the selected tumour cell lines, in particular to KB and HL-60 cell lines. Interestingly, the intermediate hydrazide **14** and aldehyde **15** exhibited efficient cytotoxicity in the micromolar range on some tumour cell lines, especially for Hela and CNE cell lines.

In series 2, the 6,8-dimethoxy-7-substituted cinnamic alcohols **44**–**47** were found to have no cytotoxic activity towards all the tested tumour cell lines, while compound 6,8-dimethoxy-7-(5′-ethoxybenzyloxy)cinnamic alcohol (**1**) was selectively cytotoxic against a number of tumour cell lines. These results might indicate that the small substituent around the aromatic cycle of C-7 confers the series 2 compound cytotoxicity. Comparing compounds **54**, **55**, **1** and 6,8-dimethoxy-7-(4′,5′-dichlorobenzyloxy)cinnamic alcohol, which we have previously reported (Zou et al., 2006), it can also be deduced that the two free hydroxy groups at C-6 and C-8 are crucial for activity of series 2: compounds **54** and **55** showed significant cytotoxicity to most of the tumour cell lines,

Table 1. Inhibitory results of synthesised cinnamic alcohol derivatives to nine tumour cell lines.[a]

Compounds	IC_{50} (μM)								
	KB	A549	Hela	CNE	PC-3	BEL-7404	HL-60	BGC823	P388D1
8	154 ± 9.3	–	–	–	–	129 ± 8.6	–	–	–
9	–	–	–	195 ± 12.6	–	–	187 ± 11.1	158 ± 8.4	–
10	–	–	–	–	–	–	–	–	–
11	70.4 ± 3.5	–	–	–	–	–	111 ± 6.6	–	57.9 ± 3.1
14	66.5 ± 2.4	20.0 ± 1.1	8.6 ± 0.4	8.4 ± 0.5	55.6 ± 1.8	–	–	22.9 ± 1.2	–
15	113 ± 4.8	24.1 ± 1.3	4.9 ± 0.2	4.3 ± 0.3	–	–	–	70.4 ± 3.0	–
19	10.3 ± 0.6	82.8 ± 4.7	24.3 ± 1.2	53.4 ± 2.3	–	–	135 ± 5.2	54.9 ± 1.6	16.3 ± 1.0
20	–	61.3 ± 2.4	55.1 ± 2.1	54.5 ± 3.0	122 ± 4.9	–	–	–	–
21	–	–	–	–	–	–	–	–	–
22	–	70.2 ± 4.3	64.6 ± 3.0	92.6 ± 3.6	98.3 ± 2.9	–	–	–	–
23	–	42.0 ± 2.0	44.1 ± 1.8	54.6 ± 2.7	79.8 ± 2.4	–	–	–	–
24 & **25**	–	–	–	–	–	–	–	–	–
26	7.0 ± 0.2	59.9 ± 2.3	19.4 ± 1.1	40.5 ± 1.8	60.6 ± 2.3	29.9 ± 1.1	4.5 ± 0.2	–	–
27	108 ± 4.5	–	–	–	–	–	32.7 ± 1.1	–	–
28	–	–	162 ± 7.0	158 ± 4.6	–	–	98.9 ± 4.5	–	–
29	–	–	–	–	–	–	–	–	–
30	47.5 ± 2.0	110 ± 3.8	48.4 ± 2.1	96.9 ± 3.9	–	111 ± 5.0	111 ± 4.6	–	74.4 ± 3.6
31	39.5 ± 1.8	74.8 ± 3.5	114 ± 5.0	117 ± 3.8	113 ± 4.4	24.6 ± 1.1	131 ± 4.2	–	1.2 ± 0.02
34	118 ± 5.4	–	190 ± 10.0	195 ± 11.4	–	183 ± 9.9	154 ± 8.5	–	197 ± 8.1
33, **35**–**39**	–	–	–	–	–	–	–	–	–
40	56.2 ± 1.9	–	36.4 ± 1.6	46.8 ± 2.0	–	131 ± 5.5	25.5 ± 1.2	–	–
41–**47**	–	–	–	–	–	–	–	–	–

49	111 ± 4.7	–	117 ± 4.1	–	–	–	–	140 ± 5.7	–
50	97.6 ± 3.7	–	–	93.0 ± 3.7	–	107 ± 2.6	182 ± 6.2	57.1 ± 3.2	–
51	45.2 ± 1.5	94.0 ± 4.1	38.8 ± 1.6	36.6 ± 1.0	–	79.4 ± 2.5	80.5 ± 3.8	85.6 ± 3.3	–
52	38.7 ± 1.4	139 ± 6.3	86.0 ± 3.1	–	–	43.1 ± 2.0	122 ± 5.7	183 ± 5.9	–
53	0.9 ± 0.1	97.0 ± 4.2	11.7 ± 0.5	5.6 ± 0.4	9.9 ± 0.8	111 ± 5.4	3.6 ± 0.4	17 ± 1.3	1.3 ± 0.1
54	15 ± 1.2	156 ± 4.9	2.9 ± 0.2	9.0 ± 0.7	85 ± 3.5	–	7.3 ± 0.6	87 ± 6.0	16.2 ± 1.5
55	0.4 ± 0.04	15.0 ± 1.3	3.6 ± 0.3	1.7 ± 0.2	3.8 ± 0.4	130 ± 9.7	1.5 ± 0.1	8.1 ± 0.5	0.5 ± 0.04
56	–	–	–	–	–	–	–	–	–
57	60.9 ± 2.5	–	119 ± 5.5	104 ± 5.0	120 ± 4.8	96.1 ± 3.2	36.6 ± 1.6	–	1.6 ± 0.04
58	33.3 ± 1.6	–	176 ± 6.3	147 ± 4.8	–	–	165 ± 7.2	–	–
59	34.3 ± 2.5	62.0 ± 2.6	151 ± 8.1	103 ± 4.6	127 ± 5.3	67.2 ± 3.0	22.1 ± 1.7	130 ± 4.8	75.1 ± 2.6
60	81.5 ± 3.0	–	–	–	151 ± 4.9	148 ± 6.3	117 ± 5.0	134 ± 5.1	85.0 ± 4.6
1	73.1. ± 4.5	–	29.6 ± 1.2	62.4 ± 2.1	–	–	53 ± 2.1	–	40.1 ± 1.2
DDP	6.7 ± 0.2	17.9 ± 1.1	12.0 ± 1.0	12.8 ± 0.4	17.8 ± 0.9	14.6 ± 0.9	10.9 ± 0.5	2.4 ± 0.06	19.6 ± 1.4

Notes: '–' indicates that the IC_{50} values greater than 200 μM were considered as inactive and omitted here. [a]Key to cell lines: KB, human oral epithelial cell line; A549, human lung adenocarcinoma cell line; Hela, human cervical carcinoma cell line; CNE, nasopharyngeal carcinoma cell line; PC-3, human prostate cancer cell line; BEL7404, human hepatocellular carcinoma line; HL-60, human romyelocytic leukaemia cell line; BGC-823, human gastric cancer cell line; P388D1, mouse macrophage-like lymphoma cell line.

particularly **55** with respect to KB and P388D1 cell lines, with IC_{50} values of 0.4 and 0.5 μM, respectively.

The bioassay results of the cinnamic dienols **58** and **59** of series 3 demonstrated that compound **59**, possessing two conjugated double bonds (*Z*-configuration), showed relatively high cytotoxicity to all nine tumour cell lines. By comparison to the dienoic acid ester derivatives **56** and **57** we found that ester **56** with *E*-configuration revealed no cytotoxic activity to any tested tumour cell lines, while the ester **57** with *Z*-configuration exhibited potent cytoxicity to seven of the selected tumour cell lines, in particular to P388D1, with an IC_{50} value of 1.6 μM. These results further indicate that the two conjugated double bonds with *Z*-configuration are necessary for the cytotoxicity of dienol derivatives (series 3). Compared to **59**, compound **60**, which has one of the conjugated double bonds reduced, lost cytotoxicity against A549, Hela and CNE cell lines and showed weaker activity to the remaining cell lines. This result suggests that a negatively charged unit in this region will lead to an increase of bioactivity.

3. Experimental

3.1. *Materials*

3.1.1. *Materials for synthesis*

All of the synthetic reagents used in this research work are commercially available. The silica gel GF_{254} and silica gel H were purchased from Qingdao Marine Chemical Factory, Qingdao, China.

3.1.2. *Tumour cell lines*

The nine tumour cell lines were obtained from the Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. The 96-well cell culture plates (Falcon) for the biological screening were bought from BD Company, Bedford, USA.

3.2. *Methods*

3.2.1. *Synthetic approaches*

Three series of sinapyl alcohol derivatives have been synthesised as outlined in Schemes 1–3, which were described in Section 2.1.

4-(5′-Ethoxybenzyloxy)benzaldehyde (8): 4-Hydroxybenzaldehyde (131 mg, 1.08 mmol) and 4-ethoxybenzyl bromide (13.0 mmol), which was formed by bromination of 4-ethoxybenzyl alcohol under HBr and conc. H_2SO_4 catalysis, were dissolved in 3 mL of acetone. This was added to a suspension of K_2CO_3 (290 mg, 21.6 mmol) and acetone (10 mL) in a dry flask. The mixture was refluxed for 3 h and cooled to room temperature. The reaction was monitored by TLC using petro ether : EtOAc (3 : 1) as the mobile phase. The solvent was removed and the residue 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 (petro ether : EtOAc 4 : 1) to afford **7**. Yield: 86.3%; white crystalline solid; m.p. = 80–82°C;

R_f (hexane : EtOAc 3 : 1) 0.26; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.89 (1H, s, C*H*O), 7.84 (2H, d, $J = 8.8$ Hz, H-2,6), 7.35 (2H, d, $J = 8.8$ Hz, H-3′, H-7′), 7.08 (2H, d, $J = 8.8$ Hz, H-4′, H-6′), 6.93 (2H, d, $J = 8.8$ Hz, H-3, 5), 5.07 (2H, s, H-1′), 4.05 (2H, q, $J = 7.2$ Hz, OCH_2CH$_3$), 1.43 (3H, t, $J = 7.2$ Hz, OCH_2CH$_3$); ESI-MS m/z $[M + H]^+$ 257.

3-(5′-Ethoxybenzyloxy)-4-methoxybenzaldehyde (9): This aldehyde was prepared from 3-hydroxy-4-methoxybenzaldehyde in a similar way as described for the synthesis of compound **8**. Yield: 80.8%; white crystalline solid; m.p. = 71–72°C; R_f (hexane : EtOAc 1 : 1) 0.70; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.82 (1H, s, C*H*O), 7.47 (1H, d, $J = 1.6$ Hz, H-2), 7.46 (1H, dd, $J = 8.4$,1.6 Hz, H-6), 7.38 (2H, d, $J = 8.4$ Hz, H-3′, H-7′), 6.99 (1H, d, $J = 8.4$ Hz, H-5), 6.90 (2H, d, $J = 8.4$ Hz, H-4′, H-6′), 5.12 (2H, s, H-1′), 4.04 (2H, q, $J = 7.2$ Hz, OCH_2CH$_3$), 3.95 (3H, s, OCH_3), 1.42 (3H, t, $J = 7.2$ Hz, OCH$_2$CH_3); ESI-MS m/z $[M + Na]^+$ 309.

4-(5′-Ethoxybenzyloxy)-3-methoxybenzaldehyde (10): This compound was prepared from 4-hydroxy-3-methoxybenzaldehyde in a similar way as described for the synthesis of compound **8**. Yield: 83.6%; white crystalline solid; m.p. = 100–101°C; R_f (hexane : EtOAc 1 : 1) 0.70; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.84 (1H, s, C*H*O), 7.42 (1H, d, $J = 2.0$ Hz, H-2), 7.40 (1H, dd, $J = 8.0$,2.0 Hz, H-6), 7.36 (2H, d, $J = 8.4$ Hz, H-3′, H-7′), 7.01 (1H, d, $J = 8.0$ Hz, H-5), 6.90 (2H, d, $J = 8.4$ Hz, H-4′, H-6′), 5.17 (2H, s, H-1′), 4.04 (2H, q, $J = 7.2$ Hz, OCH_2CH$_3$), 3.94 (3H, s, OCH_3), 1.42 (3H, t, $J = 7.2$ Hz, OCH$_2$CH_3); ESI-MS m/z $[M-H]^-$ 285.

3,4-Di-(5′-ethoxybenzyloxy)benzaldehyde (11): Compound **11** was prepared in a similar way as described for the synthesis of compound **8** from 3,4-dihydroxybenzaldehyde and a double amount of 4-ethoxybenzyl bromide. Yield: 73.2%; white crystalline solid; m.p. = 100–102°C; R_f (hexane : EtOAc 1 : 1) 0.73; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.84 (1H, s, C*H*O), 7.48 (1H, d, $J = 1.6$ Hz, H-2), 7.41 (1H, dd, $J = 8.4$,1.6 Hz, H-6), 7.36 (2H, d, $J = 8.4$ Hz, H-3″, H-7″), 7.34 (2H, d, $J = 8.4$ Hz, H-3′, H-7′), 7.02 (1H, d, $J = 8.4$ Hz, H-5), 6.89 (2H, d, $J = 8.4$ Hz, H-4″, H-6″), 6.88 (2H, d, $J = 8.4$ Hz, H-4′, H-6′), 5.17 (2H, s, H-1″), 5.12 (2H, s, H-1′), 4.04 (2H, q, $J = 6.8$ Hz, 5″-OCH_2CH$_3$), 4.02 (2H, q, $J = 6.8$ Hz, 5′-OCH_2CH$_3$), 1.43 (6H, m, OCH$_2$CH_3-5′, 5″); ESI-MS m/z $[M-H]^-$ 405.

3,5-Di-(5′-ethoxybenzyloxy)benzoic acid hydrazide (14): A suspension of 3,5-dihydroxy-benzoic acid methyl ester (1.0 g, 6.0 mmol), 4-ethoxybenzyl bromide (4.5 g, 18.0 mmol) and K_2CO_3 (2.48 g, 18.0 mmol) were refluxed in acetone (100 mL) for 10 h followed by concentration to remove acetone. The concentrate was purified by column chromatography (hexane : EtOAc 8 : 1) to afford 3,5-di-(5′-ethoxybenzyloxy)benzoic acid methyl ester (**13**) (2.4 g, 5.5 mmol, 93.3%) and was dissolved in 20 mL ethanol and refluxed with 85% hydrazine hydrate (1.76 g, 30.3 mmol) for 3 h. The mixture was evaporated and the residue was extracted by ethyl acetate (50 mL × 3). The extract was dried overnight by Na_2SO_4 and the filtrate was concentrated to get crude solid followed by crystallisation with hexane and ethyl acetate to give **14** (1.5 g). Yield: 63.2%; m.p. 151–153°C; R_f ($CHCl_3$: MeOH 10 : 1) 0.59; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.67 (1H, s, NHNH$_2$), 7.34 (4H, d, $J = 8.4$ Hz, H-3′, 7′, 3″, 7″), 7.05 (2H, d, $J = 2.0$ Hz, H-2,6), 6.92 (4H, d, $J = 8.4$ Hz, H-4′, 6′, 4″, 6″), 6.75 (1H, dd, $J = 2.4$,2.0 Hz, H-4), 5.02 (4H, s, H-1′, 1″), 4.45 (2H, d,

$J = 9.6$ Hz, NHNH_2-7), 4.03 (4H, q, $J = 7.2$ Hz, OCH_2CH$_3$-5′, 5″), 1.32 (6H, t, $J = 7.2$ Hz, OCH$_2$CH_3-5′, 5″); ESI-MS m/z [M−H]$^-$ 435.

3,5-Di-(5′-ethoxybenzyloxy)benzaldehyde (15): 3,5-Di-(5′-ethoxybenzyloxy)-benzoic acid hydrazide (1.05 g, 2.4 mmol) and tetrabutyl ammonium bromide (776 mg, 2.4 mmol) were suspended in 25% ammonia (2.5 mL), water (1.25 mL) and benzene (20 mL). $K_3Fe(CN)_6$ (1.66 g, 5.06 mmol) in 4 mL water was added to this suspension under stirring for 30 min and the mixture was filtered. The filtrate was separated and the aqueous layer was extracted by benzene (10 mL × 2). The organic layer was combined, washed with water (5 mL × 2), brine (10 mL × 2) and dried over Na_2SO_4 overnight. Removal of benzene afforded a crude product which was further purified by column chromatography (hexane : EtOAc 7 : 1) to get **15** (750 mg). Yield: 66.2%; white crystalline solid; m.p. = 97–99°C; R_f (hexane : EtOAc 5 : 1) 0.40; ^{1}H NMR (400 MHz, CDCl$_3$): δ 9.89 (1H, s, CHO), 7.34 (4H, d, $J = 8.4$ Hz, H-3′, 7′, 3″, 7″), 7.09 (2H, d, $J = 2.0$ Hz, H-2,6), 6.92 (4H, d, $J = 8.4$ Hz, H-4′, 6′, 4″, 6″), 6.84 (1H, dd, $J = 1.6$,2.0 Hz, H-4), 5.01 (4H, s, H-1′, 1″), 4.05 (4H, q, $J = 7.2$ Hz, OCH_2 CH$_3$-5′, 5″), 1.42 (6H, t, $J = 7.2$ Hz, OCH$_2$CH_3-5′, 5″); ESI-MS m/z [M − H]$^-$405.

2,4-Di-(5′-ethoxybenzyloxy)benzaldehyde (19): The product was prepared in the same way as described for the synthesis of compound **11** from 2,4-dihydroxybenzaldehyde, which was prepared from resorcinol by Vilsmeier−Haack reaction (Mendelson & Hayden, 1996). Yield: 68.0%; m.p. = 116–118°C; R_f (hexane : EtOAc 5 : 1) 0.28; ^{1}H NMR (400 MHz, CDCl$_3$): δ 10.34 (1H, s, CHO), 7.82 (1H, d, $J = 8.8$ Hz, H-6), 7.32 (4H, d, $J = 8.8$ Hz, H-3′, 7′, 3″, 7″), 6.92 (4H, d, $J = 8.4$ Hz, H-4′, 6′, 4″, 6″), 6.63 (1H, dd, $J = 8.8$,1.6 Hz, H-5), 6.59 (1H, d, $J = 1.6$ Hz, H-3), 5.05−5.03 (4H, s, H-1′, 1″), 4.05 (4H, q, $J = 7.2$ Hz, OCH_2CH$_3$-5′, 5″), 1.42 (6H, t, $J = 7.2$ Hz, OCH$_2$CH_3-5′, 5″); ESI−MS m/z [M−H]$^-$ 405.

7-(5′-Ethoxybenzyloxy)cinnamic acid ethyl ester (20): Monoethyl malonate (121 mg, 0.92 mmol) and piperidine (0.1 mL) were added to 4-(5′-ethoxybenzyloxy)-benzaldehyde (**8**) (117 mg, 0.46 mmol) in 5 mL pyridine. The mixture was refluxed for 6 h. After cooling to room temperature, diluted HCl (1 M) was added to adjust pH 5 followed by extraction with EtOAc. The extract was concentrated to give a residue, which was purified by column chromatography to afford **20** (133 mg). Yield: 89.2%; m.p. = 103–104°C; R_f (hexane : EtOAc 3 : 1) 0.30; ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.65 (1H, d, $J = 16.0$ Hz, H-3), 7.48 (2H, d, $J = 8.8$ Hz, H-5,9), 7.35 (2H, d, $J = 8.8$ Hz, H-3′, 7′), 6.97 (2H, d, $J = 8.8$ Hz, H-6,8), 6.92 (2H, d, $J = 8.8$ Hz, H-4′, 6′), 6.32 (1H, d, $J = 16.0$ Hz, H-2), 5.02 (2H, s, H-1′), 4.26 (2H, q, $J = 7.2$ Hz, OCH_2CH$_3$-1), 4.05 (2H, q, $J = 7.2$ Hz, OCH_2CH$_3$-5′), 1.43 (3H, t, $J = 7.2$ Hz, OCH$_2$CH_3-5′), 1.34 (3H, t, $J = 7.2$ Hz, OCH$_2$CH_3-1); ESI-MS m/z [M + H]$^+$ 327.

Compounds **21**–**25** and **38**–**42** were synthesised by treatment of corresponding aldehydes **9**–**11**, **15**, **19** and **33**–**37** with monoethyl malonate according to the procedure performed for **20**.

6-(5′-Ethoxybenzyloxy)-7-methoxycinnamic acid ethyl ester (21): Yield: 81.7%; white crystalline solid; m.p. = 82–83°C; R_f (hexane : EtOAc 1 : 1) 0.77; ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.58 (1H, d, $J = 16.0$ Hz, H-3), 7.36 (2H, d, $J = 8.8$ Hz, H-3′, 7′), 7.12 (1H, d, $J = 2.0$ Hz, H-5), 7.10 (1H, dd, $J = 8.8$,2.0 Hz, H-9), 6.90 (2H, d, $J = 8.8$ Hz, H-4′, 6′), 6.88 (1H, d, $J = 8.8$ Hz, H-8), 6.24 (1H, d, $J = 16.0$ Hz, H-2),

5.09 (2H, s, H-1′), 4.25 (2H, q, $J = 7.2$ Hz, OCH_2CH_3-1), 4.04 (2H, q, $J = 6.8$ Hz, OCH_2CH_3-5′), 3.90 (3H, s, OCH_3), 1.42 (3H, t, $J = 6.8$ Hz, OCH_2CH_3-5′), 1.34 (3H, t, $J = 6.8$ Hz, OCH_2CH_3-1); ESI-MS m/z $[M + H]^+$ 357.

7-(5′-Ethoxybenzyloxy)-6-methoxycinnamic acid ethyl ester (22): Yield: 83.1%; white crystals; m.p. = 100–102°C; R_f (hexane : EtOAc 1 : 1) 0.81; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61 (1H, d, $J = 16.0$ Hz, H-3), 7.35 (2H, d, $J = 8.4$ Hz, H-3′, 7′), 7.06 (1H, d, $J = 2.0$ Hz, H-5), 7.04 (1H, dd, $J = 8.4$,2.0 Hz, H-9), 6.89 (2H, d, $J = 8.4$ Hz, H-4′, 6′), 6.88 (1H, d, $J = 8.4$ Hz, H-8), 6.30 (1H, d, $J = 16.0$ Hz, H-2), 5.31 (2H, s, H-1′), 4.26 (2H, q, $J = 7.2$ Hz, OCH_2CH_3-1), 4.03 (2H, q, $J = 6.8$ Hz, OCH_2CH_3-5′), 1.41 (3H, t, $J = 6.8$ Hz, OCH_2CH_3-5′), 1.34 (3H, t, $J = 7.2$ Hz, OCH_2CH_3-1); ESI-MS m/z $[M + H]^+$ 357.

6,7-Di-(5′-ethoxybenzyloxy)cinnamic acid ethyl ester (23): Yield: 88.6%; white crystals; m.p. = 96–98°C; R_f (hexane : EtOAc 5 : 1) 0.36; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.57 (1H, d, $J = 15.6$ Hz, H-3), 7.33 (4H, dd, $J = 8.4$,2.0 Hz, H-3′, 7′, 3″, 7″), 7.11 (1H, d, $J = 1.6$ Hz, H-5), 7.06 (1H, dd, $J = 8.4$,1.6 Hz, H-9), 7.05 (1H, d, $J = 8.4$ Hz, H-8), 6.91 (2H, d, $J = 8.4$,2.0 Hz, H-4″, 6″), 6.88 (2H, d, $J = 8.4$,2.0 Hz, H-4′, 6′), 6.24 (1H, d, $J = 15.6$ Hz, H-2), 5.11 (2H, s, H-1″), 5.08 (2H, s, H-1′), 4.25 (2H, q, $J = 7.2$ Hz, OCH_2CH_3-1), 4.04 (2H, q, $J = 7.2$ Hz, OCH_2CH_3-5″), 4.03 (2H, q, $J = 7.2$ Hz, OCH_2CH_3-5′), 1.43 (6H, m, $J = 7.2$ Hz, OCH_2CH_3-5′, 5″), 1.33 (3H, t, $J = 7.2$ Hz, OCH_2CH_3-1); ESI-MS m/z $[M + H]^+$ 477.

6,8-Di-(5′-ethoxybenzyloxy)cinnamic acid ethyl ester (24): Yield: 93.1%; white crystals; m.p. = 99–100°C; R_f (hexane : EtOAc 5 : 1) 0.34; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.58 (1H, d, $J = 16.0$ Hz, H-3), 7.33 (4H, d, $J = 8.4$ Hz, H-3′, 7′, 3″, 7″), 6.91 (4H, d, $J = 8.4$ Hz, H-4′, 6′, 4″, 6″), 6.75 (2H, d, $J = 2.4$ Hz, H-5,9), 6.63 (1H, dd, $J = 2.4$,2.0 Hz, H-7), 6.38 (1H, d, $J = 16.0$ Hz, H-2), 4.97 (4H, s, H-1′, 1″), 4.26 (2H, q, $J = 7.2$ Hz, OCH_2CH_3-1), 4.04 (4H, q, $J = 7.2$ Hz, OCH_2CH_3-5′, 5″), 1.43 (6H, t, $J = 7.2$ Hz, OCH_2CH_3-5′, 5″), 1.34 (3H, t, $J = 7.2$ Hz, OCH_2CH_3-1); ESI-MS m/z $[M + H]^+$ 477.

5,7-Di-(5′-ethoxybenzyloxy)cinnamic acid ethyl ester (25): Yield: 90.6%; white crystalline solid; m.p. = 88–91°C; R_f (hexane : EtOAc 5 : 1) 0.32; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.97 (1H, d, $J = 16.0$ Hz, H-3), 7.46 (1H, dd, $J = 8.4$,2.4 Hz, H-9), 7.33 (4H, dd, $J = 8.4$,2.0 Hz, H-3′, 7′, 3″, 7″), 6.90 (4H, d, $J = 8.4$,2.0 Hz, H-4′, 6′, 4″, 6″), 6.57 (2H, m, H-6,8), 6.42 (1H, d, $J = 16.0$ Hz, H-2), 5.04 (2H, s, H-1′), 4.97 (2H, s, H-1″), 4.24 (2H, q, $J = 7.2$ Hz, OCH_2CH_3-1), 4.04 (4H, q, $J = 7.2$ Hz, OCH_2CH_3-5′, 5″), 1.42 (6H, t, $J = 7.2$ Hz, OCH_2CH_3-5′, 5″), 1.31 (3H, t, $J = 7.2$ Hz, OCH_2CH_3-1); ESI-MS m/z $[M + H]^+$ 477.

7-(5′-Ethoxybenzyloxy)cinnamic alcohol (26): $LiAlH_4$ (28 mg, 0.72 mmol) was added to anhydrous ether (8 mL) with vigorous stirring in an ice-salt bath. Compound **20** (94 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 30–60 min. About 2 mL 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. After removal of the solvent, the residue was purified through column chromatography with hexane : EtOAc (10 : 3) to give **26** (53 mg). Yield: 64.8%; white crystals;

m.p. = 132–134°C; R_f (hexane : EtOAc 5 : 2) 0.34; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.48 (2H, d, J = 8.8 Hz, H-5,9), 7.35 (2H, d, J = 8.0 Hz, H-3′, H-7′), 6.96 (2H, d, J = 8.8 Hz, H-6,8), 6.91 (2H, d, J = 8.0 Hz, H-4′, 6′), 6.56 (1H, d, J = 16.0 Hz, H-3), 6.25 (1H, dt, J = 16.0,6.0 Hz, H-2), 4.99 (2H, s, H-1′), 4.31 (2H, t, J = 6.0 Hz, H-1), 4.04 (2H, q, J = 7.2 Hz, OCH_2CH_3-5′), 1.44 (3H, t, J = 7.2 Hz, OCH_2CH_3-5′); ESI-MS m/z $[M+H]^+$ 285. Compounds **27**–**31** and **44**–**47** were synthesised by treatment of corresponding cinnamic acid esters **21**–**25**, **39**–**41** and **43** with $LiAlH_4$ according to the procedure used for **26**.

6-(5′-Ethoxybenzyloxy)-7-methoxycinnamic alcohol (27): Yield: 60.1%; white crystalline solid; m.p. = 89–91°C; R_f (hexane : EtOAc 1 : 1) 0.48; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.37 (2H, d, J = 8.4 Hz, H-3′, 7′), 6.99 (1H, d, J = 2.0 Hz, H-5), 6.93 (1H, d, J = 8.4 Hz, H-9), 6.91 (2H, d, J = 8.4 Hz, H-4′, 6′), 6.84 (1H, d, J = 8.0 Hz, H-8), 6.50 (1H, d, J = 15.6 Hz, H-3), 6.18 (1H, dt, J = 15.6,6.0 Hz, H-2), 5.08 (2H, s, H-1′), 4.29 (2H, d, J = 6.0 Hz, H-1), 4.04 (2H, q, J = 7.2 Hz, OCH_2CH_3-5′), 3.89 (3H, s, OCH_3), 1.42 (3H, t, J = 7.2 Hz, OCH_2CH_3-5′); ESI-MS m/z $[M+H]^+$ 315.

7-(5′-Ethoxybenzyloxy)-6-methoxycinnamic alcohol (28): Yield: 57.7%; white crystalline solid; m.p. = 76–78°C; R_f (hexane : EtOAc 1 : 1) 0.42; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35 (2H, d, J = 8.4 Hz, H-3′, 7′), 6.96 (1H, d, J = 1.6 Hz, H-5), 6.88 (2H, d, J = 8.4 Hz, H-4′, 6′), 6.86 (1H, dd, J = 8.4,1.6 Hz, H-9), 6.84 (1H, d, J = 8.4 Hz, H-8), 6.54 (1H, d, J = 16.0 Hz, H-3), 6.24 (1H, dt, J = 16.0,6.0 Hz, H-2), 5.08 (2H, s, H-1′), 4.31 (2H, d, J = 6.0 Hz, H-1), 4.03 (2H, q, J = 7.2 Hz, OCH_2CH_3-5′), 3.90 (3H, s, OCH_3), 1.41 (3H, t, J = 7.2 Hz, OCH_2CH_3-5′); ESI-MS m/z $[M+H]^+$ 315.

6,7-Di-(5′-ethoxybenzyloxy)cinnamic alcohol (29): Yield: 67.4%; white crystalline solid; m.p. = 60–62°C; R_f (hexane : EtOAc 5 : 2) 0.22; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34 (4H, d, J = 8.4 Hz, H-3′, 7′, 3″, 7″), 7.00 (1H, s, H-5), 6.88 (1H, d, J = 8.4 Hz, H-9), 6.87 (1H, d, J = 8.4 Hz, H-8), 6.87 (4H, d, J = 8.4 Hz, H-4′, 6′, 4″, 6″), 6.50 (1H, d, J = 16.0 Hz, H-3), 6.17 (1H, dt, J = 16.0,6.0 Hz, H-2), 5.07 (4H, s, H-1′, 1″), 4.29 (2H, dd, J = 6.0,1.2 Hz, H-1), 4.10 (4H, m, OCH_2CH_3-5′, 5″), 1.42 (6H, m, OCH_2CH_3-5′, 5″); ESI-MS m/z $[M+H]^+$ 435.

6,8-Di-(5′-ethoxybenzyloxy)cinnamic alcohol (30): Yield: 64.8%; white crystalline solid; m.p. = 65–67°C; R_f (hexane : EtOAc 3 : 1) 0.13; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.33 (4H, d, J = 8.4 Hz, H-3′, 7′, 3″, 7″), 6.91 (4H, d, J = 8.4 Hz, H-4′, 6′, 4″, 6″), 6.63 (2H, s, H-5,9), 6.53 (1H, d, J = 16.0 Hz, H-3), 6.52 (1H, s, H-7), 6.33 (1H, dd, J = 16.0,5.6 Hz, H-2), 4.95 (4H, s, H-1′, 1″), 4.32 (2H, d, J = 5.6 Hz, H-1), 4.05 (4H, q, J = 6.8 Hz, OCH_2CH_3-5′, 5″), 1.42 (6H, t, J = 6.8 Hz, OCH_2CH_3-5′, 5″); ESI-MS m/z $[M+H]^+$ 435.

5,7-Di-(5′-ethoxybenzyloxy)cinnamic alcohol (31): Yield: 65.8%; white crystalline solid; m.p. = 67–86°C; R_f (hexane : EtOAc 5 : 2) 0.25; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.46 (1H, dd, J = 8.4,2.4 Hz, H-9), 7.31 (4H, d, J = 8.4 Hz, H-3′, 7′, 3″, 7″), 6.90 (4H, d, J = 8.4 Hz, H-4′, 6′, 4″, 6″), 6.59 (2H, m, H-6, 8), 6.53 (1H, d, J = 16.0 Hz, H-3), 6.33 (1H, dd, J = 16.0,5.6 Hz, H-2), 4.95 (4H, s, H-1′, 1″), 4.32 (2H, d, J = 5.6 Hz, H-1), 4.05 (4H, q, J = 6.8 Hz, OCH_2CH_3-5′, 5″), 1.42 (6H, t, J = 6.8 Hz, OCH_2CH_3-5′, 5″); ESI-MS m/z $[M+H]^+$ 435.

3,5-Dimethoxy-4-(5′-ethoxybenzyloxy)benzaldehyde (33): This compound was synthesised by treatment of 3,4-methylenedioxybenzyl bromide and syringaldehyde (**32**) according to the method previously reported in Zou et al. (2006).

3,5-Dimethoxy-4-(4′,5′-methylenedioxybenzyloxy)benzaldehyde (34): Compound **34** was obtained by treatment of 3,4-methylenedioxybenzyl bromide and **32** similar to the method described for **8**. Yield: 73.7%; white crystalline solid; m.p. = 86–88°C; R_f (hexane : EtOAc 5 : 2) 0.42; δ 9.87 (1H, s, CHO), 7.12 (2H, s, H-2,6), 7.03 (1H, d, J = 1.6 Hz, H-3′), 6.90 (1H, dd, J = 8.0,1.6 Hz, H-7′), 6.76 (1H, d, J = 8.0 Hz, H-6′), 5.96 (2H, s, OCH_2O), 5.03 (2H, s, H-1′), 3.92 (6H, s, OCH_3-3,5); ESI-MS m/z $[M + Na + H]^+$ 339.

3,5-Dimethoxy-4-(4′,5′-dimethoxybenzyloxy)benzaldehyde (35): This compound was prepared by treatment of 3,4-dimethoxybenzyl bromide and **32** similar to the method described for **8**. Yield: 65.8%; white crystalline solid; m.p. = 81–83°C; R_f (hexane : EtOAc 5 : 2) 0.49; δ 9.87 (1H, s, CHO), 7.17 (1H, dd, J = 8.0,1.6 Hz, H-7′), 7.13 (2H, s, H-2,6), 7.03 (1H, d, J = 1.6 Hz, H-3′), 6.97 (1H, d, J = 8.0 Hz, H-6′), 5.18 (2H, s, H-1′), 3.89 (3H, s, OCH_3-4′), 3.88 (6H, s, OCH_3-3,5), 3.87 (3H, s, OCH_3-5′); ESI-MS m/z $[M + Na]^+$ 355.

3,5-Dimethoxy-4-(3′,4′-dimethoxybenzyloxy)benzaldehyde (36): Compound **36** was synthesised by treatment of 3,4-dimethoxybenzyl bromide and **32** similar to the method described for **8**. Yield: 67.7%; white crystalline solid; m.p. = 86–88°C; R_f (hexane : EtOAc 5 : 2) 0.43; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.87 (1H, s, CHO), 7.17 (1H, dd, J = 8.0,1.6 Hz, H-7′), 7.13 (2H, s, H-2,6), 7.06 (1H, dd, J = 8.0,8.0 Hz, H-6′), 6.89 (1H, dd, J = 8.0,1.6 Hz, H-5′), 5.18 (2H, s, H-1′), 3.89 (3H, s, OCH_3-3′), 3.88 (6H, s, OCH_3-3, 5), 3.87 (3H,s,OCH_3-4′); ESI-MS m/z $[M + Na]^+$ 355.

3,5-Dimethoxy-4-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosyloxyl)benzaldehyde (37): This compound (Delay & Delmotte, 1990) was synthesised by treatment of 2′,3′,4′, 6′-tetra-*O*-acetyl-*β*-D-glucopyranosyloxyl bromide and **32** similar to the method used for **8**.

6,8-Dimethoxy-7-(5′-ethoxybenzyloxy)cinnamic acid ethyl ester (38): Compound **38** was identical to that we have previously reported (Zou et al., 2006).

6,8-Dimethoxy-7-(4′,5′-methylenedioxybenzyloxy)cinnamic acid ethyl ester (39): Yield: 77.2%; white crystalline solid; m.p. = 104–106°C; R_f (hexane : EtOAc 5 : 1) 0.26; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60 (1H, d, J = 15.6 Hz, H-3), 7.05 (1H, d, J = 1.6 Hz, H-3′), 6.90 (1H, dd, J = 8.0,1.6 Hz, H-7′), 6.76 (1H, d, J = 8.0 Hz, H-6′), 6.74 (2H, s, H-5,9), 6.35 (1H, d, J = 15.6 Hz, H-2), 5.95 (2H, s, OCH_2O), 4.94 (2H, s, H-1′), 4.27 (2H, q, J = 7.2 Hz, OCH_2CH$_3$-1), 3.87 (6H, s, OCH_3-6,8), 1.34 (3H, t, J = 7.2 Hz, OCH$_2$CH_3-1); ESI-MS m/z $[M + Na]^+$ 409.

6,8-Dimethoxy-7-(4′,5′-dimethoxybenzyloxy)cinnamic acid ethyl ester (40): Yield: 76.7%; white crystalline solid; m.p. = 81–83°C; R_f (hexane : EtOAc 5 : 1) 0.10; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60 (1H, d, J = 16.0 Hz, H-3), 7.11 (1H, d, J = 1.6 Hz, H-3′), 6.94 (1H, dd, J = 8.0,1.6 Hz, H-7′), 6.81 (1H, d, J = 8.0 Hz, H-6′), 6.74 (2H, s, H-5,9), 6.35 (1H, d, J = 16.0 Hz, H-2), 5.01 (2H, s, H-1′), 4.24 (2H, q, J = 7.2 Hz, OCH_2CH$_3$-1), 3.90 (3H, s, OCH_3-5′), 3.87 (6H, s, OCH_3-6,8), 3.86 (3H, s, OCH$_3$-4′), 1.34 (2H, t, J = 7.2 Hz, OCH$_2$CH_3-1); ESI-MS $[M + Na]^+$ 425.

6,8-Dimethoxy-7-(3′,4′-dimethoxybenzyloxy)cinnamic acid ethyl ester (41): Yield: 84.2%; white crystalline solid; m.p. = 6–78°C; *Rf* (hexane : EtOAc 5 : 1) 0.21; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61 (1H, d, J = 16.0 Hz, H-3), 7.19 (1H, dd, J = 8.0,1.2 Hz, H-7′), 7.07 (1H, dd, J = 8.0,8.0 Hz, H-6′), 6.89 (1H, dd, J = 8.0,1.2 Hz, H-5′), 6.87 (2H, s, H-5,9), 6.36 (1H, d, J = 16.0 Hz, H-2), 5.10 (2H, s, H-1′), 4.28 (3H, t, J = 7.2 Hz, OCH_2CH_3-1), 3.87 (6H, s, OCH_3-6,8), 3.85 (3H, s, OCH_3-3′), 3.84 (3H, s, OCH_3-4′), 1.35 (3H, t, J = 7.2 Hz, OCH_2CH_3-1); ESI-MS m/z $[M + Na]^+$ 425.

6,8-Dimethoxy-7-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosyloxyl)cinnamic acid ethyl ester (42): Yield: 68.7%; white crystalline solid; m.p. = 153–156°C; R_f (hexane : EtOAc 4 : 5) 0.62; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.58 (1H, d, J = 15.6 Hz, H-3), 6.74 (2H, s, H-5,9), 6.35 (1H, d, J = 15.6 Hz, H-2), 5.24−5.33 (3H, m, H-1′, 2′, 4′), 5.12 (1H, d, J = 6.8 Hz, H-3′), 4.25 (3H, m, OCH_2CH_3-1, H-6′α), 4.12 (1H, dd, J = 12.0,2.4 Hz, H-6′β), 3.85 (6H, s, OCH_3-6, 8), 3.71 (1H, m, H-5′), 2.03 (12H, s, CH_3CO), 1.34 (3H, t, J = 6.8 Hz, OCH_2CH_3-1). ESI-MS m/z $[M + Na]^+$ 605.

6,8-Dimethoxy-7-β-D-glucopyranosyloxylcinnamic acid ethyl ester (43): This compound was synthesised by treatment of **42** with sodium methoxide as previously reported by Mazur, Hope-Ross, Kadla, Sederoff and Chang (2007). Yield: 63.4%; white crystalline solid; m.p. 180–182°C; R_f ($CHCl_3$: MeOH 4 : 1) 0.67; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88 (1H, d, J = 16.0 Hz, H-3), 7.03 (2H, s, H-5,9), 6.71 (1H, d, J = 16.0 Hz, H-2), 5.98 (1H, d, J = 6.8 Hz, H-1′), 4.44 (1H, d, J = 10.8 Hz, H-6′α), 4.38−4.25 (6H, m, H-2′, 3′, 4′, 6′β, OCH_2CH_3-1), 4.01 (1H, m, H-5′), 3.80 (6H, s, OCH_3-6, 8), 1.23 (3H, t, J = 7.2 Hz, OCH_2CH_3-1); ESI-MS m/z $[M + Na]^+$ 437.

6,8-Dimethoxy-7-(4′,5′-methylenedioxybenzyloxy)cinnamic alcohol (44): Yield: 50.4%; white crystalline solid; m.p. = 89–90°C; R_f (hexane : EtOAc 5 : 2) 0.13; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.05 (1H, d, J = 1.6 Hz, H-3%), 6.91 (1H, dd, J = 8.0, 1.6 Hz, H-7%), 6.76 (1H, d, J = 8.0 Hz, H-6%), 6.59 (5.94 (2H, s, H-5, 9), 6.52 (1H, d, J = 15.6 Hz, H-3), 6.27 (1H, dt, J = 15.6,6.0 Hz, H-2), 5.93 (2H, s, OCH_2O), 4.88 (2H, s, H-1%), 4.30 (2H, dd, J = 5.6,0.8 Hz, H-1), 3.84 (6H, s, OCH_3-6,8); ESI-MS m/z $[M + H]^+$ 345.

6,8-Dimethoxy-7-(4′,5′-dimethoxybenzyloxy)cinnamic alcohol (45): Yield: 49.3%; white crystalline solid; m.p. = 99–100°C; R_f (hexane : EtOAc 5 : 2) 0.14; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.12 (1H, d, J = 1.6 Hz, H-3′), 6.95 (1H, dd, J = 8.0,2.0 Hz, H-7′), 6.81 (1H, d, J = 8.0 Hz, H-6′), 6.60 (2H, s, H-5,9), 6.54 (1H, d, J = 15.6 Hz, H-3), 6.29 (1H, dt, J = 15.6,6.0 Hz, H-2), 4.97 (2H, s, H-1′), 4.32 (2H, dd, J = 5.6,0.9 Hz, H-1), 3.90 (3H, s, OCH_3-5′), 3.87 (3H, s, OCH_3-4′), 3.84 (6H, s, OCH_3-6,8); ESI-MS m/z $[M + H]^+$ 361.

6,8-Dimethoxy-7-(3′,4′-dimethoxybenzyloxy)cinnamic alcohol (46): Yield: 51.7%; white crystalline solid; m.p. = 66–71°C; R_f (hexane : EtOAc 5 : 2) 0.12; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.22 (1H, d, J = 7.6 Hz, H-7′), 7.07 (1H, dd, J = 8.0,7.6 Hz, H-6′), 6.89 (1H, d, J = 8.0 Hz, H-5′), 6.62 (2H, s, H-5, 9), 6.54 (1H, d, J = 16.0 Hz, H-3), 6.30 (1H, dt, J = 16.0,6.0 Hz, H-2), 5.08 (2H, s, H-1′), 4.32 (2H, dd, J = 5.6,0.6 Hz, H-1), 3.88 (3H, s, OCH_3-3′), 3.87 (3H, s, OCH_3-4′), 3.82 (6H, s, OCH_3-6, 8); ESI-MS m/z $[M + H]^+$ 361.

6,8-Dimethoxy-7-β-D-glucopyranosyloxylcinnamic alcohol (47): Yield: 30.4%; ESI-MS m/z $[M+Na]^+$ 395; R_f ($CHCl_3$: MeOH 4 : 1) 0.44; this compound was identical to that reported by Delay and Delmotte (1990).

6-Acetoxy-8-hydroxy-7-(5′-ethoxybenzyloxy)cinnamic acid ethyl ester (49): This compound was prepared according to the procedure previously reported in Zou et al. (2006) by three steps from the starting material 4-ethoxybenzyl bromide and 3,4,5-trihydroxybenzaldehyde (**48**). Yield 70%; white crystalline solid; m.p. = 73–75°C; R_f (hexane : EtOAc 3 : 1) 0.29; ^{1}H NMR (400 MHz, $(CD_3)_2CO$): δ 8.74 (1H, brs, O*H*-8), 7.52 (1H, d, J = 16.0 Hz, H-3), 7.37 (2H, d, J = 8.4 Hz, H-3′, 7′), 7.11 (1H, d, J = 2.4 Hz, H-9), 6.98 (1H, d, J = 2.4 Hz, H-5), 6.91 (2H, dd, J = 8.4,2.0 Hz, H-4′, 6′), 6.39 (1H, d, J = 16.0 Hz, H-2), 5.01 (2H, s, H-1′), 4.17 (2H, q, J = 7.2 Hz, OCH_2CH_3-1), 4.05 (2H, q, J = 5.6 Hz, OCH_2CH_3-5′), 2.22 (3H, s, CH_3CO-6), 1.36 (3H, t, J = 7.2 Hz, OCH_2CH_3-5′), 1.27 (3H, t, J = 7.2 Hz, OCH_2CH_3-1); ESI-MS m/z $[M]^+$ 400.

6,8-Dihydroxy-7-(5′-ethoxybenzyloxy)cinnamic acid ethyl ester (50): This compound was synthesised by treatment of **49** with potassium carbonate (Zou et al., 2006). Yield: 70.4%; white crystalline solid; m.p. = 90–92°C; R_f (hexane : EtOAc 3 : 1) 0.21; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52 (1H, d, J = 16.0 Hz, H-3), 7.30 (2H, d, J = 8.4 Hz, H-3′, 7′), 6.89 (2H, d, J = 8.4 Hz, H-4′, 6′), 6.84 (2H, s, H-5, 9), 6.29 (1H, d, J = 16.0 Hz, H-2), 5.59 (2H, brs, O*H*-6,8), 5.00 (2H, s, H-1′), 4.26 (2H, q, J = 7.2 Hz, OCH_2CH_3-1), 4.03 (2H, q, J = 7.2 Hz, OCH_2CH_3-5′), 1.42 (3H, t, J = 7.2 Hz, OCH_2CH_3-5′), 1.34 (3H, t, J = 7.2 Hz, OCH_2CH_3-1); ESI-MS m/z $[M]^+$ 358.

6,8-Diacetoxy-7-(4′,5′-dichlorobenzyloxy)cinnamic acid ethyl ester (51): This compound (69%), accompanied by **52** (31%), were synthesised as previously reported in Zou et al. (2006) by three steps from the starting material 3,4-dichlorobenzyl bromide and 3,4,5-trihydroxybenzaldehyde (**48**). Yield: 68.2%; white crystalline solid; R_f (hexane : EtOAc 3 : 1) 0.44; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.55 (1H, d, J = 16.0 Hz, H-3), 7.51 (1H, d, J = 2.0 Hz, H-3′), 7.44 (1H, dd, J = 8.0,2.0 Hz, H-7′), 7.41 (1H, d, J = 8.0 Hz, H-6′), 7.16 (2H, s, H-5, 9), 6.77 (1 H, d, J = 12.4 Hz, H-3), 6.33 (1H, d, J = 16.0 Hz, H-2), 5.95 (0.25 H, d, J = 12.4 Hz, H-2), 4.96 (2H, s, H-1′), 4.25 (2H, q, J = 7.2 Hz, OCH_2CH_3-1), 2.24 (6H, s, CH_3CO-6, 8), 1.33 (3H, t, J = 7.2 Hz, OCH_2CH_3-1); ESI-MS m/z $[M]^+$ 466.

6-Acetoxy-8-hydroxy-7-(4′,5′-dichlorobenzyloxy)cinnamic acid ethyl ester (52): Yield: 74.2%; white crystalline solid; m.p. 150–152°C; R_f (hexane : EtOAc 3 : 1) 0.33; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52 (1H, d, J = 16.0 Hz, H-3), 7.50 (1H, d, J = 2.0 Hz, H-3′), 7.46 (1H, d, J = 8.0 Hz, H-6′), 7.20 (1H, dd, J = 8.0,2.0 Hz, H-7′), 7.00 (1H, d, J = 2.4 Hz, H-5), 6.83 (1H, d, J = 2.4 Hz, H-9), 6.31 (1H, d, J = 16.0 Hz, H-2), 4.96 (2H, s, H-1′), 4.25 (2H, q, J = 7.2 Hz, OCH_2CH_3-1), 2.26 (3H, s, CH_3CO-6), 1.33 (3H, t, J = 7.2 Hz, OCH_2CH_3-1); ESI-MS m/z $[M]^+$ 424.

6,8-Dihydroxy-7-(4′, 5′-dichlorobenzyloxy)cinnamic acid ethyl ester (53): Compound **53** was obtained by treatment of **51** and **52** with potassium carbonate (Zou et al., 2006). Yield: 74.2%; white crystalline solid; m.p. = 150–152°C; R_f (hexane : EtOAc 3 : 1) 0.33; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52 (1H, d, J = 16.0 Hz, H-3), 7.50 (1H, d, J = 2.0 Hz, H-3′), 7.46 (1H, d, J = 8.0 Hz, H-6′), 7.20 (1H, dd, J = 8.0,2.0 Hz, H-7′), 6.85 (2H, s, J = 2.4 Hz, H-5,9), 6.31 (1H, d, J = 16.0 Hz, H-2), 4.95 (2H, s, H-1′), 4.26

(2H, q, $J = 7.2$ Hz, OCH_2CH$_3$-1), 1.34 (3H, t, $J = 7.2$ Hz, OCH$_2$CH_3-1); ESI-MS m/z $[M]^+$ 424.

7-(5′-Ethoxybenzyloxy)-6,8-dihydroxycinnamic alcohol (54): Yield: 44.6%; white crystalline solid; m.p. = 102–104°C; R_f (hexane : EtOAc 2 : 3) 0.46; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39–7.36 (5H, m, H-3′–7′), 6.50 (2H, s, H-2,6), 6.38 (1H, d, $J = 16.0$ Hz, H-3), 6.19 (1H, dt, $J = 16.0$,6.0 Hz, H-2), 5.83 (2H, brs, OH-6, 8), 5.01 (2H, s, H-1′), 4.26 (2H, d, $J = 6.0$ Hz, H-1); ESI-MS m/z $[M]^+$ 272.

7-(4′, 5′-Dichlorobenzyloxy)-6,8-dihydroxycinnamic alcohol (55): Yield: 44.6%; white crystalline solid; m.p. = 102–104°C; R_f (hexane : EtOAc 2 : 3) 0.46; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39−7.36 (5H, m, H-3′−7′), 6.50 (2H, s, H–2,6), 6.38 (1H, d, $J = 16.0$ Hz, H–3), 6.19 (1H, dt, $J = 16.0$,6.0 Hz, H-2), 5.83 (2H, brs, OH-6, 8), 5.01 (2H, s, H-1′), 4.26 (2H, d, $J = 6.0$ Hz, H-1); ESI-MS m/z $[M]^+$ 272.

(2E, 4E) 5-[3′,5′-Dimethoxy-4′-(5″-ethoxybenzyloxy)phenyl]-2,4-pentadienoic acid ethyl ester (56): Sodium (85 mg, 3.67 mmol) was added to absolute ethanol (2 mL) and stirred to an even mixture. After cooling to room temperature an additional 10 mL of tetrahydrofuran and [(2E)-4-ethoxy-4-oxo-2-buten-1-yl]triphenyl-phosphonium bromide (1.84 g, 4.04 mmol) were added to give a yellow suspension. Compound **33** (1.16 g, 3.67 mmol) was added and the resulting mixture was stirred for 2 h. Addition of small portions of water quenched the reaction and 1 M HCl was used to adjust to pH 7. The solvent was evaporated and the residue was purified by chromatography to give two isomers **56** (main product) and **57**. Data for **56:** Yield: 68.8%; white crystalline solid; m.p. = 112–114°C; R_f (hexane : EtOAc 5 : 2) 0.25; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.49 (1H, dd, $J = 15.6$,9.2 Hz, H-3), 7.39 (2H, d, $J = 8.4$ Hz, H-3′, 7′), 6.85 (2H, d, $J = 8.4$ Hz, H-4′, 6′), 6.76 (2H, m, H-4,5), 6.66 (2H, s, H-7,11), 5.98 (1H, d, $J = 15.6$ Hz, H-2), 4.97 (2H, s, H-1′), 4.22 (2H, q, $J = 7.2$ Hz, OCH_2CH$_3$-1), 4.03 (2H, q, $J = 7.2$ Hz, OCH_2CH$_3$-5′), 3.85 (6H, s, OCH$_3$-8,10), 1.43 (3H, t, $J = 7.2$ Hz, OCH$_2$CH_3-5′), 1.32 (3H, t, $J = 7.2$ Hz, OCH$_2$CH_3-1); ESI-MS m/z $[M + H]^+$ 413.

(2Z, 4Z) 5-[3′,5′-Dimethoxy-4′-(5″-ethoxybenzyloxy)phenyl]-2,4-pentadienoic acid ethyl ester (57): Yield: 12.1%; white crystalline solid; m.p. = 75–77°C; R_f (hexane : EtOAc 5 : 2) 0.31; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.46 (1H, t, $J = 11.6$ Hz, H-3), 7.39 (2H, d, $J = 8.4$ Hz, H-3′, 7′), 7.07 (1H, t, $J = 11.6$ Hz, H-5), 6.86 (2H, d, $J = 8.4$ Hz, H-4′, 6′), 6.83 (1H, t, $J = 11.6$ Hz, H-4), 6.50 (2H, s, H-7,11), 5.78 (1H, d, $J = 11.6$ Hz, H-2), 4.98 (2H, s, H-1′), 4.22 (2H, q, $J = 7.2$ Hz, OCH_2 CH$_3$-1), 4.04 (2H, q, $J = 7.2$ Hz, OCH_2CH$_3$-5′), 3.82 (6H, s, OCH$_3$-8,10), 1.43 (3H, t, $J = 7.2$ Hz, OCH$_2$CH_3-5′), 1.33 (3H, t, $J = 7.2$ Hz, OCH$_2$CH_3-1); ESI-MS m/z $[M + H]^+$ 413.

(2E, 4E) 5-[3′, 5′-Dimethoxy-4′-(5″-ethoxybenzyloxy)phenyl]-2,4-pentadien-1-ol (58): Compound **58** was synthesised by treatment of **56** with $LiAlH_4$–$AlCl_3$ complex similar to the preparation of **26**. Yield: 52.4%; white crystalline solid; m.p. = 108–109°C; R_f (hexane : EtOAc 5 : 2) 0.15; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.37 (2H, d, $J = 8.4$ Hz, H-3′, 7′), 6.84 (2H, d, $J = 8.4$ Hz, H-4′, 6′), 6.68 (1H, t, $J = 15.6$ Hz, H-3), 6.59 (2H, s, H-7,11), 6.46 (1H d, $J = 15.6$ Hz, H-5), 6.46 (1H d, $J = 15.6$ Hz, H-4), 5.95 (1H, dt, $J = 15.6$,5.2 Hz, H-2), 4.93 (2H, s, H-1′), 4.25 (2H, d, $J = 5.2$ Hz, H-1), 4.02

(2H, q, $J = 7.2$ Hz, OCH_2CH_3-5′), 3.85 (6H, s, OCH_3-8,10), 1.23 (3H, t, $J = 7.2$ Hz, OCH_2CH_3-5′); ESI-MS m/z $[M + H]^+$ 371.

(2Z, 4Z) 5-[3′, 5′-Dimethoxy-4′-(5″-ethoxybenzyloxy)phenyl]-2,4-pentadien-1-ol (59): Compound **59** was synthesised by treatment of **57** with $LiAlH_4$–$AlCl_3$ complex similar to the method used for **26**. Over-reduction of **57** also afforded compound **60** with only one double bond. Yield: 43.4%; white crystalline solid; m.p. = 66–68°C; R_f (hexane : EtOAc 5 : 2) 0.20; ^{1}H NMR [400 MHz, $(CD_3)_2CO$]: δ 7.38 (2H, d, $J = 8.4$ Hz, H-3′, 7′), 6.83 (2H, d, $J = 8.4$ Hz, H-4′, 6′), 6.67 (2H, s, H-7,11), 6.53 (1H, d, $J = 10.8$ Hz, H-5), 6.43 (1H, dd, $J = 12.0$,11.2 Hz, H-3), 5.63 (1H, dt, $J = 12.0$, 5.2 Hz, H-2), 4.84 (1H, dd, $J = 11.2$,10.8 Hz, H-4), 4.83 (2H, s, H-1′), 4.16 (2H, d, $J = 5.2$ Hz, H-1), 4.03 (2H, q, $J = 7.2$ Hz, OCH_2CH_3-5′), 3.83 (6H, s, OCH_3-8,10), 1.36 (3H, t, $J = 7.2$ Hz, OCH_2CH_3-5′); ESI-MS m/z $[M + H]^+$ 371.

(2Z) 5-[3′, 5′-Dimethoxy-4′-(5″-ethoxybenzyloxy)phenyl]-2-penten-1-ol (60): Yield: 13.7%; white crystalline solid; m.p. = 60–62°C; R_f (hexane : EtOAc 5 : 2) 0.22; ^{1}H NMR [400 MHz, $(CD_3)_2CO$]: δ 7.40 (2H, d, $J = 8.4$ Hz, H-3′, 7′), 6.88 (2H, d, $J = 8.4$ Hz, H-4′, 6′), 6.54 (2H, s, H-7,11), 6.53 (1H, dt, $J = 11.2$,6.8 Hz, H-3), 5.59 (1H, m, H-2), 4.83 (2H, s, H-1′), 4.24 (2H, d, $J = 5.2$ Hz, H-1), 4.03 (2H, q, $J = 6.8$ Hz, OCH_2CH_3-5′), 3.80 (6H, s, OCH_3-8, 10), 2.41 (1H, t, $J = 7.6$ Hz, H-5), 2.22 (1H, m, H-4), 1.36 (3H, t, $J = 6.8$ Hz, OCH_2CH_3-5′); ESI-MS m/z $[M + H]^+$ 373.

3.2.2. *Structure elucidation*

The structures of these compounds were confirmed by both mass spectrometry and ^{1}H NMR spectral data. Melting points were measured on a Perkin-Taike X-4 apparatus and then corrected. ^{1}H NMR spectra were recorded on a Varian INOVA 400 spectrometer with TMS as the internal standard and $CDCl_3$ as the solvent. ESI-MS data were recorded on a Bruker Esquire 3000+ spectrometer and EI-MS was performed on a Varian MAT-95 MS instrument.

3.2.3. *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^{-1} in phosphate buffered solution) as added to the culture medium and incubated at 37°C for further 4 h. Following removal of 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 concentration (IC_{50}) was determined graphically from the dose–response curves.

4. Conclusions

Three series of di- and tri-substituted derivatives of cinnamic alcohol and its conjugated dienol analogues were synthesised, and their cytotoxicity was evaluated on nine tumour cell lines. Most of the cinnamic alcohol derivatives showed cytotoxic activity. Most of the synthesised disubstituted cinnamic alcohols exhibited moderate

cytotoxicity to the tested tumour cell lines, while the trisubstituted derivatives with two free hydroxy groups showed potential cytotoxic activity. In particular, compound **55**, with a 4′,5′-dichlorobenzyloxy group at C-7, exhibited significant cytotoxicity to seven human tumour cell lines in the micromolar range, especially to KB and P388D1 cell lines, with IC_{50} values of 0.4 and 0.5 μM, respectively, which was 20 times higher than the control DDP. The finding that the configuration of the cinnamic dienol analogues is also crucial for its cytotoxicity, indicating that the both *Z*-configurated conjugated double bonds will enhance the bioactivity, is also to be noted. The structure relationship of these three series was discussed. This extensive structure–activity relationship analysis will guide us in designing optimised cytotoxic cinnamic derivatives, and compound **55** will serve as the lead compound for the synthesis of compounds with better and more selective cytotoxicity to human tumour cell lines.

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