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Original article

Synthetic cinnamylphenol derivatives as cancer chemopreventive agents

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Abstract

Several substituted cinnamylphenol (1,3-diphenylpropene) derivatives were synthesized and tested for their inhibitory activities against *in vitro* Epstein-Barr virus early antigen activation induced by 12-*O*-tetradecanoylphorbol-13-acetate in Raji cells. The prenylated cinnamylphenols were found to show remarkably potent activity. Furthermore, prenylated cinnamylphenols (**19** and **25**) exhibited a marked inhibitory effect on mouse skin tumor promotion in an *in vivo* two-stage carcinogenesis test. These results indicate that some prenylated cinnamylphenols might be valuable as potential cancer chemopreventive agents (anti-tumor promoters).

Keywords: Cinnamylphenols; Cancer chemopreventive agents; Anti-tumor promoters; Epstein-Barr virus activation; Two-stage mouse skin carcinogenesis

1. Introduction

We prepared a series of cinnamylphenols, one of the chemical constituents of the *Dalbergia* species (Leguminosae) for evaluation as cancer chemopreventive agents (anti-tumor promoters). Cinnamylphenols possess the same chemical subunits (C6 + C3 + C6) biogenetically analogous to flavonoids [1]. We have previously reported that naturally occurring flavonoids [2,3], isoflavonoids [4–6] and cinnamylphenols [7] might be valuable as potential anti-tumor promoters. However, because of the continuing need for new cancer chemopreventive agents with novel structures and mechanisms of action, we also screened the synthetic cinnamylphenols for anti-tumor promoting activity. Several prenylated cinnamylphenols showed marked inhibitory activities against Epstein-Barr virus early antigen (EBV-EA) activation induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in Raji cells. We report herein the active structures, synthetic methods, and anti-tumor promotion results from this study of cinnamylphenol derivatives.

2. Chemistry

Schemes 1–3 show the synthetic methods and structures of target compounds **1**–**28**. Methoxyphenol, methoxycatechol, dimethoxyphenol and trimethoxybenzene were reacted with 3-phenyl-2-propen-1-ol in 90% formic acid at 60 °C for 30 min to give cinnamylphenol derivatives **1**–**12** (Scheme 1) [8]. In Scheme 2, it has been shown that vinyl quinone methide prepared from eugenol by treatment with Ag_2O in $CHCl_3$, easily reacts with 3-methoxycatechol in the presence of catalytic amounts of *p*-toluenesulfonic acid (*p*-TsOH) to give cinnamylphenol derivatives **21** and **22** [9]. Cinnamylphenol derivatives **27** and **28** were obtained by reacting coniferyl diacetate and 3-methoxycatechol in 90% formic acid at 60 °C for 7.5 h (Scheme 3). Finally, prenylated cinnamylphenol derivatives **13**–**20** and **23**–**26** were prepared from each corresponding cinnamylphenol by treatment with prenyl bromide in the

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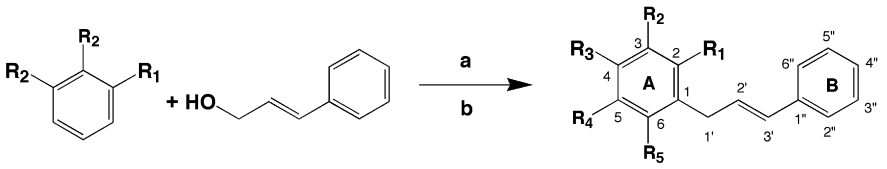
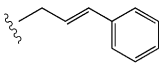


R_1	R_2	R_3		R_1	R_2	R_3	R_4	R_5
OH	H	OCH_3	1	OH	H	OCH_3	H	H
			2	OH	H	H	H	OCH_3
			3	OCH_3	cinnamyl	OH	H	H
			4	OCH_3	H	OH	H	H
OH	OH	OCH_3	5	OH	OH	OCH_3	H	H
			6	OCH_3	OH	OH	H	H
OH	OCH_3	OCH_3	7	OH	OCH_3	OCH_3	H	H
			8	OCH_3	OCH_3	OH	H	H
			9	OH	OCH_3	OCH_3	cinnamyl	H
OCH_3	OH	OCH_3	10	OCH_3	OH	OCH_3	H	H
OCH_3	OCH_3	OCH_3	11	OCH_3	OCH_3	OCH_3	H	H
			12	OCH_3	OCH_3	OCH_3	cinnamyl	H
			13	prenyloxy	H	OCH_3	H	H
			14	prenyloxy	H	H	H	OCH_3
			15	OCH_3	H	prenyloxy	H	H
			16	OH	prenyloxy	OCH_3	H	H
			17	prenyloxy	prenyloxy	OCH_3	H	H
			18	prenyloxy	OH	OCH_3	H	H
			19	OCH_3	prenyloxy	prenyloxy	H	H
			20	OCH_3	OCH_3	prenyloxy	H	H

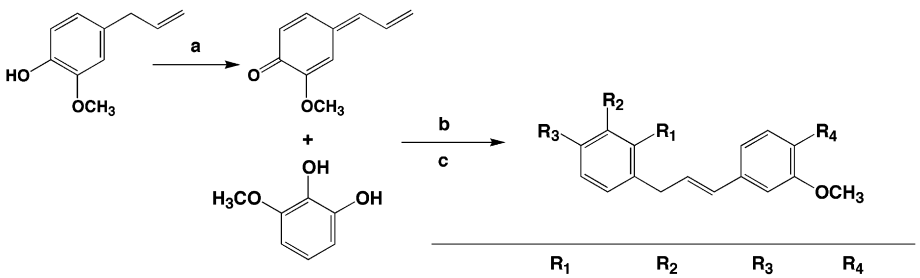
cinnamyl = 

Scheme 1. Structures of synthetic cinnamylphenol derivatives **1**–**20**. (a) 90% formic acid, 60 °C, 30 min, (b) prenyl bromide, K_2CO_3/acetone, 80 °C, 1 h.

presence of anhydrous potassium carbonate (K_2CO_3) in acetone at 80 °C for 1 h, respectively.

3. Results and discussion

The synthesized cinnamylphenol derivatives were tested for their ability to inhibit tumor-promoting activity using a short-term *in vitro* assay for TPA-induced EBV-EA activation in Raji cells. The activation of EBV-EA expression in an EBV genome-carrying human lymphoblastoid cell line has been used to detect tumor promoter or anti-tumor promoter activity. This assay system is composed of EBV-non-producer cells as the indicator, *n*-butyric acid as the trigger, TPA as the EBV-activator and the test substance. Their inhibitory effects on activation of the virus-genome, the viability of the Raji cells and the 50% inhibitory concentration (IC_{50}) values are shown in Table 1. Of the cinnamylphenol derivatives shown in Scheme 1, both phenols (**1**–**10**) and non-phenolic methyl ethers (**11** and **12**) showed weaker activity than that of curcumin ($IC_{50} = 341$) [10,11], a compound used as a reference in cancer prevention studies. Also, the inhibitory effects of phenols **21** and **22** (Scheme 2) and **27** and **28** (Scheme 3) were weaker than that of curcumin. Prenylation of one or two of the hydroxy groups in these phenols increased the inhibitory activity.



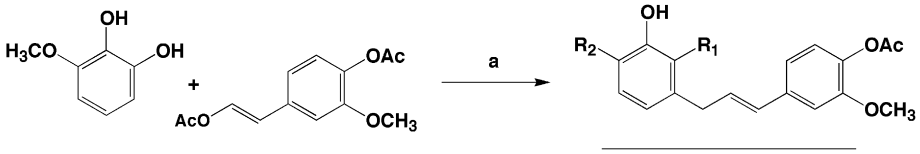
	R_1	R_2	R_3	R_4
21	OH	OH	OCH_3	OH
22	OCH_3	OH	OH	OH
23	OH	prenyloxy	OCH_3	OH
24	prenyloxy	OH	OCH_3	OH
25	OH	prenyloxy	OCH_3	prenyloxy
26	prenyloxy	prenyloxy	OCH_3	prenyloxy

Scheme 2. Structures of synthetic cinnamylphenol derivatives **21**–**26**. (a) $Ag_2O/CHCl_3$, (b) PTSA/$CHCl_3$, (c) prenyl bromide, K_2CO_3/acetone, 80 °C, 1 h.

All prenylated cinnamylphenols (**13**–**20** and **23**–**26**) inhibited EBV-EA activation even at 1×10 mol ratio/TPA and fully blocked EBV-EA activation at a high concentration (1×10^3 mol ratio/TPA) without causing a decrease in viability (>70% survival) of the Raji cells. In the cinnamylphenols with only one prenyl side chain on the A-ring, these values corresponded to an IC_{50} value of 277–300 mol ratio/TPA. The inhibitory effect of 3,3″-dimethoxy-2,4″-diprenyloxy-6-cinnamylphenol (**25**) ($IC_{50} = 277$) was present even at 1×10 mol ratio/TPA (10.9%) and the activation was fully blocked at 1×10^3 mol ratio/TPA. Furthermore, the corresponding IC_{50} values of the non-phenolic derivatives (**17**, **19**, and **26**), with two prenyl side chains on the A-ring, were 225–278 mol ratio/TPA. Prenylated non-phenolic derivatives (**19** and **26**) showed the most potent inhibitory activity ($IC_{50} = 225$ and 226, respectively). From the viewpoint of structure–activity relationships, an essential feature for the activity of the cinnamylphenols examined in the present study is the presence of two prenyl groups on the A-ring of the cinnamylphenol skeleton.

Based on the results obtained *in vitro*, we studied the inhibitory effect of two diprenylated cinnamylphenols (**19** and **25**), as representative compounds of more active non-phenolic and phenolic derivatives *in vitro*, in an *in vivo* two-stage carcinogenesis test focusing on mouse skin papillomas induced by DMBA as an initiator and TPA as a promoter. The results from the *in vivo* bioassay using these compounds are shown in Fig. 1. The control animals showed a 100% incidence of papillomas in 10 weeks after DMBA-TPA tumor promotion, while treatment with non-phenolic derivatives (**19**) with two prenyl groups in the A-ring along with the initiator and promoter remarkably reduced the percentage of tumor-bearing mice to 77% and 30% after 10 and 20 weeks, respectively (Fig. 1A). The inhibitory activity of phenolic derivative (**25**), with two prenyl groups in the A- and B-rings, resulted in a lower incidence of papilloma-bearing mice to 87% and 53% after 10 and 20 weeks, respectively (Fig. 1A). As shown in Fig. 1B, the number of papillomas per mouse was reduced by 44–48% after 20 weeks in mice treated with derivatives **19** and **25** as compared to untreated mice.

In previous studies, we reported that the presence of a prenyl moiety in the flavanones [3] and isoflavonoids [4,6] containing a C6 + C3 + C6 structural unit plays an important role in the inhibition of EBV-EA induction. In view of the present findings, a hydrophobic prenyl moiety on the cinnamylphenol nucleus might be an important factor for producing the observed chemopreventive effects against chemical-induced carcinogenesis.



	R_1	R_2
27	OH	OCH_3
28	OCH_3	OH

Scheme 3. Structures of synthetic cinnamylphenol derivatives **27** and **28**. (a) 90% formic acid, 60 °C, 7.5 h.

Table 1
Inhibitory effects of synthetic cinnamylphenol derivatives on TPA-induced EBV-EA activation[a]

Compound	EBV-EA-positive cells (% viability)				IC_{50}[b]
	Compound concentration (mol ratio/32 pmol TPA)				
	1000	500	100	10	
1	20.7 ± 1.2 (60)	45.1 ± 1.3 (>80)	73.7 ± 2.0 (>80)	100.0 ± 0.2 (>80)	486
2	18.3 ± 0.6 (60)	43.7 ± 1.8 (>80)	70.5 ± 2.1 (>80)	100.0 ± 0.3 (>80)	430
3	12.8 ± 0.5 (60)	41.5 ± 1.1 (>80)	68.2 ± 1.9 (>80)	100.0 ± 1.1 (>80)	420
4	18.1 ± 0.6 (60)	44.7 ± 1.4 (>80)	73.2 ± 1.8 (>80)	100.0 ± 0.9 (>80)	433
5	10.8 ± 0.5 (60)	42.5 ± 1.3 (>80)	71.5 ± 2.0 (>80)	100.0 ± 0.7 (>80)	439
6	10.2 ± 0.3 (60)	41.3 ± 1.7 (>80)	71.0 ± 1.5 (>80)	100.0 ± 0.4 (>80)	432
7	13.3 ± 0.4 (60)	45.5 ± 1.8 (>80)	76.2 ± 2.4 (>80)	100.0 ± 0.2 (>80)	430
8	12.5 ± 0.3 (60)	44.3 ± 1.3 (>80)	74.0 ± 2.0 (>80)	100.0 ± 0.5 (>80)	440
9	15.9 ± 0.3 (60)	46.4 ± 1.1 (>80)	75.6 ± 1.9 (>80)	100.0 ± 0.4 (>80)	438
10	14.1 ± 0.4 (60)	41.2 ± 1.1 (>80)	76.9 ± 1.9 (>80)	100.0 ± 0.5 (>80)	401
11	14.2 ± 0.5 (60)	41.5 ± 1.8 (>80)	76.6 ± 2.7 (>80)	100.0 ± 0.5 (>80)	425
12	12.6 ± 0.4 (60)	39.8 ± 1.4 (>80)	75.2 ± 2.0 (>80)	100.0 ± 0.5 (>80)	419
13	0.0 ± 0.7 (70)	29.9 ± 1.8 (>80)	67.8 ± 2.1 (>80)	91.7 ± 0.6(>80)	280
14	0.0 ± 0.3 (70)	30.8 ± 1.2 (>80)	68.9 ± 2.0 (>80)	92.7 ± 0.4 (>80)	282
15	0.0 ± 0.6 (70)	29.3 ± 1.1 (>80)	65.2 ± 1.3 (>80)	87.6 ± 0.6 (>80)	284
16	0.0 ± 0.5 (70)	30.6 ± 1.3 (>80)	65.6 ± 1.3 (>80)	90.8 ± 0.7 (>80)	291
17	0.0 ± 0.2 (70)	24.6 ± 1.2 (>80)	62.2 ± 1.2 (>80)	88.6 ± 0.3 (>80)	278
18	0.0 ± 0.4 (70)	27.6 ± 1.3 (>80)	64.5 ± 1.5 (>80)	90.6 ± 0.5 (>80)	280
19	0.0 ± 0.6 (70)	22.6 ± 1.0 (>80)	64.5 ± 2.2 (>80)	88.9 ± 1.3 (>80)	225
20	0.0 ± 0.3 (70)	29.8 ± 1.4 (>80)	68.2 ± 2.0 (>80)	91.5 ± 1.5 (>80)	282
21	8.7 ± 0.5 (60)	45.5 ± 1.9 (>80)	73.2 ± 2.0 (>80)	95.7 ± 0.3 (>80)	435
22	7.5 ± 0.5 (60)	43.7 ± 1.8 (>80)	70.2 ± 2.0 (>80)	95.7 ± 0.3 (>80)	420
23	0.0 ± 0.6 (70)	28.3 ± 1.0 (>80)	64.2 ± 1.5 (>80)	87.1 ± 0.7 (>80)	300
24	0.0 ± 0.4 (70)	27.8 ± 1.4 (>80)	63.2 ± 1.2 (>80)	88.1 ± 0.8 (>80)	294
25	0.0 ± 0.0 (70)	27.5 ± 1.5 (>80)	68.6 ± 2.1 (>80)	89.1 ± 0.5 (>80)	277
26	0.0 ± 0.3 (70)	23.6 ± 1.2 (>80)	61.5 ± 2.0 (>80)	83.3 ± 0.3 (>80)	226
27	10.0 ± 0.7 (60)	37.6 ± 1.3 (>80)	74.6 ± 2.3 (>80)	100.0 ± 0.3 (>80)	407
28	9.4 ± 0.5 (60)	36.1 ± 1.6 (>80)	74.0 ± 2.4 (>80)	100.0 ± 0.4 (>80)	404
Curcumin[c]	0.0 ± 0.5 (60)	22.8 ± 1.8 (>80)	81.7 ± 1.9 (>80)	100.0 ± 0.2 (>80)	341

[a] Mol ratio/TPA (32 pmol = 20 ng/ml), 1000 mol ratio = 32 nmol, 500 mol ratio = 16 nmol, 100 mol ratio = 3.2 nmol, and 10 mol ratio = 0.32 nmol. Values are EBV-EA activation (%) ± SD in the presence of test compound relative to the positive control (100%). Values in parentheses represent the surviving Raji cells measured by Trypan Blue staining. A minimum 60% survival rate of Raji cells two days after treatment with the compounds is required for an accurate result.
[b] IC_{50} represents the mol ratio to TPA that inhibits 50% of the positive control (100%) activated with 32 pmol of TPA.
[c] Positive control substance.

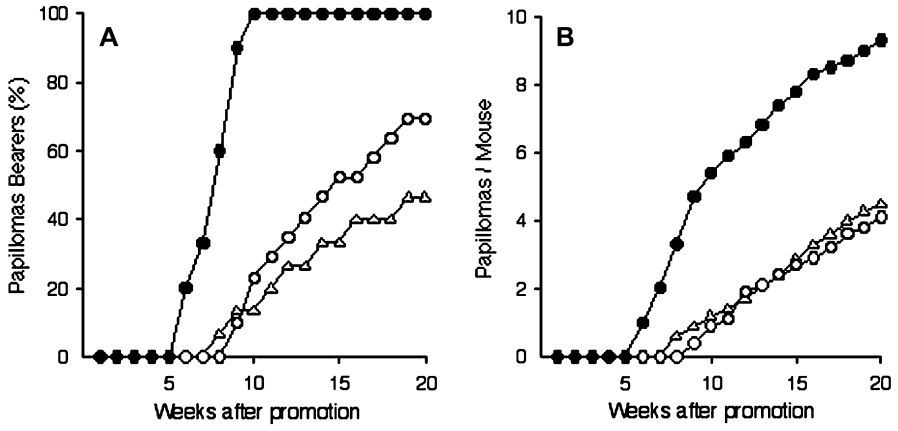


Fig. 1. Inhibitory effects of prenylated cinnamylphenols (**19** and **25**) on DMBA-TPA mouse skin carcinogenesis. Tumors were initiated in all mice with DMBA (390 nmol) and promoted with TPA (1.7 nmol) twice weekly starting one week after initiation. A: Percentage of mice with papillomas. B: Average number of papillomas per mouse. ●, control TPA alone; ○, TPA + 85 nmol of **19**; △, TPA + 85 nmol of **25**. After 20 weeks of tumor promotion, a significant difference in the number of papillomas per mouse between the groups treated with each test compound and the control group was evident ($p < 0.05$).

4. Experimental protocols

4.1. General experimental procedures

^{1}H and ^{13}C NMR, COSY, HMQC, HMBC ($J = 8$ Hz), and NOE were measured using a JNM A-400, A-600 and/or ECP-500 (JEOL) spectrometer. Chemical shifts are shown in δ (ppm) with tetramethylsilane (TMS) as an internal reference. All mass spectra were taken under EI conditions, unless otherwise stated, using an HX-110 (JEOL), and/or JMS-700 (JEOL) spectrometer with a direct inlet system. UV spectra were recorded on an UVIDEC-610C double-beam spectrophotometer (JASCO) in MeOH, IR spectra were recorded on an IR-230 (JASCO) in $CHCl_3$. Preparative TLC was done on Kieselgel 60 F_{254} (Merck).

4.2. General procedure for the synthesis of cinnamylphenol derivatives

Method I: Methoxyphenol, methoxycatechol, dimethoxyphenol and trimethoxybenzene were reacted with 3-phenyl-2-propen-1-ol in 90% formic acid at 60 °C for 30 min to give cinnamylphenols **1**–**12** [8].

Method II: Vinyl quinone methide, prepared from eugenol by treatment with Ag_2O in $CHCl_3$, easily reacts with 3-methoxycatechol in the presence of catalytic amounts of *p*-toluenesulfonic acid to give cinnamylphenols **21** and **22** [9].

Method III: Cinnamylphenols **27** and **28** were obtained by reacting coniferyl diacetate and 3-methoxycatechol in 90% formic acid at 60 °C for 7.5 h.

Method IV: Prenylated cinnamylphenols **13**–**20** and **23**–**26** were prepared from each corresponding cinnamylphenol by treatment with prenyl bromide in the presence of potassium carbonate (K_2CO_3) in acetone at 80 °C for 1 h, respectively.

All cinnamylphenols were named on the basis of cinnamylphenol. Seven of the known compounds (**1**, **4**–**8**, and **10**) were synthesized according to usual methods and fully characterized by comparison of their physical and spectroscopic data with those previously reported in the literatures [8,12–15].

4.3. Synthesis of cinnamylphenols *1–4*

Following the method I, *m*-methoxyphenol (620 mg) and 3-phenyl-2-propen-1-ol (670 mg) afford cinnamylphenols **1** (241 mg) **2** (20 mg) **3** (14.7 mg), and obtustyrene **4** (174.7 mg).

4.3.1. 3-Methoxy-2-cinnamylphenol (*2*)

Colorless oil; IR ($CHCl_3$) ν_{max} 3599, 3312 (br), 1596 cm^{-1}; ^{1}H NMR ($CDCl_3$): 7.32 (2H, d, $J = 7.7$ Hz), 7.26 (2H, t, $J = 7.7$ Hz), 7.17 (1H, t, $J = 7.7$ Hz), 7.08 (1H, t, $J = 8.0$ Hz), 6.51 (1H, d, $J = 8.4$ Hz), 6.48 (1H, d, $J = 8.4$ Hz), 6.46 (1H, d, $J = 16.1$ Hz), 6.34 (1H, dt, $J = 16.1$, 6.6 Hz), 4.99 (1H, br s, OH), 3.82 (3H, s, OCH_3), 3.60 (2H, d, $J = 6.6$ Hz); HRMS *m/z* 240.1151 (calcd for $C_{16}H_{16}O_2$: 240.1150).

4.3.2. 3-Hydroxy-2,6-dicinnamylphenol methyl ether (*3*)

Pale yellow oil; IR ($CHCl_3$) ν_{max} 3596, 3345 (br), 1599 cm^{-1}; ^{1}H NMR ($CDCl_3$): 7.35 (2H, d, $J = 7.7$ Hz), 7.33 (2H, d, $J = 7.7$ Hz), 7.28 (2H, t, $J = 7.7$ Hz), 7.27 (2H, t, $J = 7.7$ Hz), 7.19 (2H, t, $J = 7.7$ Hz), 7.03 (1H, d, $J = 8.4$ Hz), 6.62 (1H, d, $J = 8.4$ Hz), 6.50 (1H, d, $J = 15.8$ Hz), 6.44 (1H, d, $J = 15.8$ Hz), 6.40 (1H, dt, $J = 15.8$, 6.6 Hz), 6.35 (1H, dt, $J = 15.8$, 6.6 Hz), 5.00 (1H, br s, OH), 3.76 (3H, s, OCH_3), 3.65 (2H, d, $J = 6.6$ Hz), 3.53 (2H, d, $J = 6.6$ Hz); HRMS *m/z* 356.1730 (calcd for $C_{25}H_{24}O_2$: 356.1776).

4.4. Synthesis of cinnamylphenols *5* and *6*

Following the method I, 3-methoxycatechol (700 mg) and 3-phenyl-2-propen-1-ol (670 mg) afford compound **5** (196 mg) and hydroxyobtustyrene **6** (120 mg).

4.5. Synthesis of cinnamylphenols *7–9*

Following the method I, 2,3-dimethoxyphenol (700 mg) and 3-phenyl-2-propen-1-ol (609 mg) afford compound **7** (107 mg), mucronustyrene **8** (79 mg), and compound **9** (89 mg).

4.5.1. 2,3-Dimethoxy-4,6-dicinnamylphenol (*9*)

Yellow oil; IR ($CHCl_3$) ν_{max} 3527, 3293 (br), 1598 cm^{-1}; ^{1}H NMR ($CDCl_3$): 7.34 (4H, m), 7.26 (4H, t, $J = 7.7$ Hz), 7.18 (2H, t, $J = 7.7$ Hz), 6.74 (1H, s), 6.45 (1H, d, $J = 15.8$ Hz), 6.41 (1H, d, $J = 15.8$ Hz), 6.34 (2H, m), 5.76 (1H, s, OH), 3.93 (3H, s, OCH_3), 3.83 (3H, s), 3.49 (2H, d, $J = 6.6$ Hz), 3.45 (2H, d, $J = 6.6$ Hz); HRMS *m/z* 386.1839 (calcd for $C_{26}H_{26}O_3$: 386.1882).

4.6. Synthesis of cinnamylphenol *10*

Following the method I, 2,6-dimethoxyphenol (70 mg) and 3-phenyl-2-propen-1-ol (65 mg) afford isomucronustyrene **10** (11.3 mg).

4.7. Synthesis of cinnamylphenols *11* and *12*

Following the method I, trimethoxybenzene (70 mg) and 3-phenyl-2-propen-1-ol (65 mg) afford cinnamylphenols **11** (48 mg) and **12** (7 mg).

4.7.1. 2,3-Dimethoxy-6-cinnamylphenol methyl ether (*11*)

Pale yellow oil; IR ($CHCl_3$) ν_{max} 1599 cm^{-1}; ^{1}H NMR ($CDCl_3$): 7.35 (2H, d, $J = 7.7$ Hz), 7.27 (2H, t, $J = 7.7$ Hz), 7.19 (1H, t, $J = 7.7$ Hz), 6.88 (1H, d, $J = 8.4$ Hz), 6.63 (1H, d, $J = 8.4$ Hz), 6.41 (1H, d, $J = 15.8$ Hz), 6.36 (1H, dt, $J = 15.8$, 6.6 Hz), 3.88 (6H, s, OCH_3), 3.85 (3H, s, OCH_3), 3.49 (2H, d, $J = 6.6$ Hz); HRMS *m/z* 284.1407 (calcd for $C_{18}H_{20}O_3$: 284.1412).

4.7.2. 2,3-Dimethoxy-4,6-dicinnamylphenol methyl ether (*12*)

Pale yellow oil; IR ($CHCl_3$) ν_{max} 1599 cm^{-1}; ^{1}H NMR ($CDCl_3$): 7.34 (2H, d, $J = 7.7$ Hz), 7.26 (2H, t, $J = 7.7$ Hz),

7.18 (1H, t, $J = 7.7$ Hz), 6.78 (1H, s), 6.43 (1H, d, $J = 15.8$ Hz), 6.32 (1H, dt, $J = 15.8$, 6.6 Hz), 3.92 (3H, s, OCH_3), 3.87 (6H, s, OCH_3), 3.48 (2H, d, $J = 6.6$ Hz); HRMS *m/z* 400.2006 (calcd for $C_{27}H_{28}O_3$: 400.2039).

4.8. Synthesis of cinnamylphenol **13**

Following the method IV, cinnamylphenol **1** (11.3 mg) and prenyl bromide (8.4 mg) afford cinnamylphenol **13** (8.2 mg).

4.8.1. 5-Methoxy-2-cinnamylphenol prenyl ether (**13**)

Colorless oil; IR ($CHCl_3$) ν_{max} 1610 cm^{-1}; ^1H NMR ($CDCl_3$): 7.33 (2H, d, $J = 7.7$ Hz), 7.27 (2H, t, $J = 7.7$ Hz), 7.17 (1H, t, $J = 7.7$ Hz), 7.07 (1H, d, $J = 8.1$ Hz), 6.47 (1H, d, $J = 2.2$ Hz), 6.43 (1H, dd, $J = 8.1$, 2.2 Hz), 6.39 (1H, d, $J = 15.8$ Hz), 6.37 (1H, dt, $J = 15.8$, 6.6 Hz), 5.49 (1H, m), 4.51 (2H, d, $J = 6.6$ Hz), 3.79 (3H, s, OCH_3), 3.47 (2H, d, $J = 6.6$ Hz), 1.78 (3H, s), 1.72 (3H, s); HRMS *m/z* 308.1747 (calcd for $C_{21}H_{24}O_2$: 308.1777).

4.9. Synthesis of cinnamylphenol **14**

Following the method IV, cinnamylphenol **2** (10.6 mg) and prenyl bromide (7.8 mg) afford cinnamylphenol **14** (7.6 mg).

4.9.1. 3-Methoxy-2-cinnamylphenol prenyl ether (**14**)

Colorless oil; IR ($CHCl_3$) ν_{max} 1594 cm^{-1}; ^1H NMR ($CDCl_3$): 7.32 (2H, d, $J = 8.0$ Hz), 7.25 (2H, t, $J = 8.0$ Hz), 7.14 (2H, t, $J = 8.0$ Hz), 6.58 (1H, d, $J = 8.0$ Hz), 6.56 (1H, d, $J = 8.0$ Hz), 6.41 (1H, d, $J = 15.8$ Hz), 6.33 (1H, dt, $J = 15.8$, 6.6 Hz), 5.50 (1H, m), 4.54 (2H, d, $J = 6.2$ Hz), 3.84 (3H, s, OCH_3), 3.58 (2H, d, $J = 6.6$ Hz), 1.79 (3H, s), 1.72 (3H, s); HRMS *m/z* 308.1740 (calcd for $C_{21}H_{24}O_2$: 308.1776).

4.10. Synthesis of cinnamylphenol **15**

Following the method IV, cinnamylphenol **4** (13.6 mg) and prenyl bromide (8.4 mg) afford cinnamylphenol **15** (10.3 mg).

4.10.1. 5-Prenyloxy-2-cinnamylphenol methyl ether (**15**)

Colorless oil; IR ($CHCl_3$) ν_{max} 1620 cm^{-1}; ^1H NMR ($CDCl_3$): 7.34 (2H, d, $J = 7.7$ Hz), 7.27 (2H, t, $J = 7.7$ Hz), 7.18 (1H, t, $J = 7.7$ Hz), 7.06 (1H, d, $J = 8.4$ Hz), 6.49 (1H, d, $J = 2.2$ Hz), 6.45 (1H, dd, $J = 8.4$, 2.2 Hz), 6.40 (1H, d, $J = 15.8$ Hz), 6.35 (1H, dt, $J = 15.8$, 6.6 Hz), 5.50 (1H, m), 4.49 (1H, d, $J = 7.0$ Hz), 3.81 (3H, s, OCH_3), 3.46 (2H, d, $J = 6.6$ Hz), 1.80 (3H, s), 1.74 (3H, s); HRMS *m/z* 308.1770 (calcd for $C_{21}H_{24}O_2$: 308.1777).

4.11. Synthesis of cinnamylphenols **16–18**

Following the method IV, cinnamylphenol **5** (10 mg) and prenyl bromide (11.6 mg) afford cinnamylphenols **16** (8.8 mg), **17** (1.4 mg), and **18** (2.2 mg).

4.11.1. 3-Methoxy-2-prenyloxy-6-cinnamylphenol (**16**)

Colorless oil; IR ($CHCl_3$) ν_{max} 3516, 1619, 1596 cm^{-1}; ^1H NMR ($CDCl_3$): 7.51 (1H, s, OH), 7.36 (2H, d, $J = 7.7$ Hz), 7.27 (2H, t, $J = 7.7$ Hz), 7.17 (1H, t, $J = 7.7$ Hz), 6.79 (1H, d, $J = 8.5$ Hz), 6.46 (1H, d, $J = 8.5$ Hz), 6.42 (2H, m), 5.50 (1H, m), 4.52 (2H, d, $J = 7.3$ Hz), 3.81 (3H, s, OCH_3), 3.45 (2H, d, $J = 6.6$ Hz), 1.69 (3H, s), 1.61 (3H, s); HRMS *m/z* 324.1707 (calcd for $C_{21}H_{24}O_3$: 324.1725).

4.11.2. 3-Methoxy-2-prenyloxy-6-cinnamylphenol prenyl ether (**17**)

Colorless oil; IR ($CHCl_3$) ν_{max} 1598 cm^{-1}; ^1H NMR ($CDCl_3$): 7.37 (2H, d, $J = 7.7$ Hz), 7.28 (2H, t, $J = 7.7$ Hz), 7.17 (1H, t, $J = 7.7$ Hz), 6.88 (1H, d, $J = 8.4$ Hz), 6.70 (1H, d, $J = 8.4$ Hz), 6.40 (2H, m), 5.54 (1H, m), 5.53 (1H, m), 4.56 (2H, d, $J = 7.0$ Hz), 4.48 (2H, d, $J = 7.0$ Hz), 3.80 (3H, s, OCH_3), 3.47 (2H, d, $J = 6.6$ Hz), 1.73 (6H, s), 1.66 (6H, s); HRMS *m/z* 392.2336 (calcd for $C_{26}H_{32}O_3$: 392.2351).

4.11.3. 2-Hydroxy-3-methoxy-6-cinnamylphenol prenyl ether (**18**)

Colorless oil; IR ($CHCl_3$) ν_{max} 3539, 1619 cm^{-1}; ^1H NMR ($CDCl_3$): 7.37 (2H, d, $J = 7.7$ Hz), 7.27 (2H, t, $J = 7.7$ Hz), 7.17 (1H, t, $J = 7.7$ Hz), 6.65 (2H, m), 6.41 (2H, m), 5.54 (1H, m), 4.58 (2H, d, $J = 7.0$ Hz), 3.80 (3H, s, OCH_3), 3.47 (2H, d, $J = 6.6$ Hz), 1.72 (3H, s), 1.64 (3H, s); HRMS *m/z* 324.1727 (calcd for $C_{21}H_{24}O_3$: 324.1726).

4.12. Synthesis of cinnamylphenol **19**

Following the method IV, cinnamylphenol **6** (5.2 mg) and prenyl bromide (6.7 mg) afford cinnamylphenol **19** (10.7 mg).

4.12.1. 2,3-Diprenyloxy-6-cinnamylphenol methyl ether (**19**)

Pale yellow oil; IR ($CHCl_3$) ν_{max} 1599 cm^{-1}; ^1H NMR ($CDCl_3$): 7.34 (2H, d, $J = 7.7$ Hz), 7.28 (2H, t, $J = 7.7$ Hz), 7.18 (1H, t, $J = 7.7$ Hz), 6.84 (1H, d, $J = 8.4$ Hz), 6.62 (1H, d, $J = 8.4$ Hz), 6.41 (1H, d, $J = 15.8$ Hz), 6.34 (1H, dt, $J = 15.8$, 6.6 Hz), 5.59 (1H, m), 5.51 (1H, m), 4.53 (2H, d, $J = 7.0$ Hz), 4.51 (2H, d, $J = 7.0$ Hz), 3.89 (3H, s, OCH_3), 3.48 (2H, d, $J = 6.6$ Hz), 1.77 (3H, s), 1.76 (3H, s), 1.72 (3H, s), 1.70 (3H, s); HRMS *m/z* 392.2313 (calcd for $C_{26}H_{32}O_3$: 392.2351).

4.13. Synthesis of cinnamylphenol **20**

Following the method IV, cinnamylphenol **8** (9.0 mg) and prenyl bromide (6.0 mg) afford cinnamylphenol **20** (4.5 mg).

4.13.1. 2-Methoxy-3-prenyloxy-6-cinnamylphenol methyl ether (**20**)

Pale yellow oil; IR ($CHCl_3$) ν_{max} 1598 cm^{-1}; ^1H NMR ($CDCl_3$): 7.35 (2H, d, $J = 7.7$ Hz), 7.28 (2H, t, $J = 7.7$ Hz), 7.19 (1H, t, $J = 7.7$ Hz), 6.86 (1H, d, $J = 8.4$ Hz), 6.64 (1H, d, $J = 8.4$ Hz), 6.43 (1H, d, $J = 15.8$ Hz), 6.34 (1H, dt, $J = 15.8$, 6.6 Hz), 5.51 (1H, m), 4.55 (2H, d, $J = 7.0$ Hz), 3.89 (3H, s, OCH_3), 3.49 (2H, d, $J = 6.6$ Hz), 1.79 (3H, s),

1.74 (3H, s); HRMS *m/z* 338.1843 (calcd for $C_{22}H_{26}O_3$: 338.1882).

4.14. Synthesis of cinnamylphenols **21** and **22**

Following the method II, 3-methoxycatechol (1 g) and eugenol (1.17 g) afford cinnamylphenols **21** (126 mg) and **22** (34 mg).

4.14.1. 2,4″-Dihydroxy-3,3″-dimethoxy-6-cinnamylphenol (**21**)

Colorless oil; IR ($CHCl_3$) ν_{max} 3549, 1632, 1601 cm^{-1}; ^1H NMR ($CDCl_3$): 6.88 (1H, d, $J = 1.5$ Hz), 6.85 (1H, dd, $J = 8.4$, 1.5 Hz), 6.82 (1H, d, $J = 8.4$ Hz), 6.67 (1H, d, $J = 8.4$ Hz), 6.43 (1H, d, $J = 8.4$ Hz), 6.37 (1H, d, $J = 15.8$ Hz), 6.21 (1H, dt, $J = 15.8$, 6.6 Hz), 5.55 (1H, s), 5.40 (2H, s), 3.87 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 3.49 (2H, d, $J =$ 6.6 Hz); HRMS *m/z* 302.1139 (calcd for $C_{17}H_{18}O_5$: 302.1154).

4.14.2. 2,3,4″-Trihydroxy-3″-methoxy-6-cinnamylphenol methyl ether (**22**)

Colorless oil; IR ($CHCl_3$) ν_{max} 3545, 3265 (br), 1613, 1509 cm^{-1}; ^1H NMR ($CDCl_3$): 6.87 (1H, br s), 6.84 (2H, s), 6.68 (2H, s), 6.34 (1H, d, $J = 15.8$ Hz), 6.15 (1H, dt, $J = 15.8$, 6.6 Hz), 5.57 (1H, s), 5.50 (1H, s), 5.22 (1H, br s), 3.88 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 3.48 (2H, d, $J = 6.6$ Hz); HRMS *m/z* 302.1135 (calcd for $C_{17}H_{18}O_5$: 302.1154).

4.15. Synthesis of cinnamylphenols **23–25**

Following the method IV, cinnamylphenol **22** (130 mg) and prenyl bromide (70 mg) afford cinnamylphenols **23** (15.3 mg), **24** (8.5 mg) and **25** (6.0 mg).

4.15.1. 4″-Hydroxy-3,3″-dimethoxy-2-prenyloxy-6-cinnamylphenol (**23**)

Pale yellow oil; IR ($CHCl_3$) ν_{max} 3536, 1616 cm^{-1}; ^1H NMR ($CDCl_3$): 6.87 (1H, d, $J = 1.5$ Hz), 6.83 (2H, s), 6.82 (1H, d, $J = 8.4$ Hz), 6.42 (1H, d, $J = 8.4$ Hz), 6.34 (1H, d, $J = 15.8$ Hz), 6.20 (1H, dt, $J = 15.8$, 6.6 Hz), 5.94 (1H, s, OH), 5.55 (1H, s, OH), 5.52 (1H, m), 4.57 (2H, d, $J = 7.0$ Hz), 3.87 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 3.47 (2H, d, $J = 6.6$ Hz), 1.76 (3H, s), 1.67 (3H, s); HRMS *m/z* 370.1773 (calcd for $C_{22}H_{26}O_5$: 370.1780).

4.15.2. 2,4″-Dihydroxy-3,3″-dimethoxy-6-cinnamylphenol prenyl ether (**24**)

Yellow oil; IR ($CHCl_3$) ν_{max} 3540, 1612 cm^{-1}; ^1H NMR ($CDCl_3$): 6.87 (1H, d, $J = 1.5$ Hz), 6.83 (2H, s), 6.69 (1H, d, $J = 8.4$ Hz), 6.61 (1H, d, $J = 8.4$ Hz), 6.34 (1H, d, $J =$ 15.8 Hz), 6.16 (1H, dt, $J = 15.8$, 6.6 Hz), 5.58 (1H, m), 5.57 (1H, s, OH), 5.55 (1H, s, OH), 4.54 (2H, d, $J = 7.0$ Hz), 3.87 (6H, s, OCH_3), 3.50 (2H, d, $J = 6.6$ Hz), 1.78 (3H, s), 1.69 (3H, s); HRMS *m/z* 370.1780 (calcd for $C_{22}H_{26}O_5$: 370.1780).

4.15.3. 3,3″-Dimethoxy-2,4″-diprenyloxy-6-cinnamylphenol (**25**)

Pale yellow oil; IR ($CHCl_3$) ν_{max} 3517, 1602 cm^{-1}; ^1H NMR ($CDCl_3$): 6.90 (1H, d, $J = 1.5$ Hz), 6.85 (1H, dd, $J = 8.4$, 1.8 Hz), 6.80 (1H, d, $J = 8.4$ Hz), 6.78 (1H, d, $J = 8.4$ Hz), 6.42 (1H, d, $J = 8.4$ Hz), 6.35 (1H, d, $J = 15.8$ Hz), 6.22 (1H, dt, $J = 15.8$, 6.6 Hz), 5.94 (1H, s, OH), 5.52 (1H, m), 5.51 (1H, m), 4.57 (2H, d, $J = 7.0$ Hz), 4.56 (2H, d, $J = 7.0$ Hz), 3.86 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 3.47 (2H, d, $J = 6.6$ Hz), 1.76 (6H, s), 1.72 (3H, s), 1.68 (3H, s); HRMS *m/z* 438.2401 (calcd for $C_{27}H_{34}O_5$: 438.2407).

4.16. Synthesis of cinnamylphenol **26**

Following the method IV, cinnamylphenol **23** (5.5 mg) and prenyl bromide (4.9 mg) afford cinnamylphenol **26** (3.6 mg).

4.16.1. 3,3″-Dimethoxy-2,4″-diprenyloxy-6-cinnamylphenol prenyl ether (**26**)

Pale yellow oil; IR ($CHCl_3$) ν_{max} 1601 cm^{-1}; ^1H NMR ($CDCl_3$): 6.90 (1H, d, $J = 1.5$ Hz), 6.88 (1H, d, $J = 8.4$ Hz), 6.84 (1H, dd, $J = 8.4$, 1.5 Hz), 6.79 (1H, d, $J = 8.4$ Hz), 6.62 (1H, d, $J = 8.4$ Hz), 6.36 (1H, d, $J = 15.8$ Hz), 6.19 (1H, dt, $J = 15.8$, 6.6 Hz), 5.59 (1H, m), 5.57 (1H, m), 5.51 (1H, m), 4.56 (4H, d, $J = 7.0$ Hz), 4.52 (2H, d, $J = 7.0$ Hz), 3.86 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 3.49 (2H, d, $J = 6.6$ Hz), 1.77 (3H, s), 1.76 (6H, s), 1.72 (3H, s), 1.69 (3H, s), 1.68 (3H, s); HRMS *m/z* 506.3020 (calcd for $C_{32}H_{42}O_5$: 506.3032).

4.17. Synthesis of cinnamylphenols **27** and **28**

Following the method III, 3-methoxycatechol (700 mg) and coniferyl diacetate (670 mg) afford cinnamylphenols **27** (121 mg) and **28** (91 mg).

4.17.1. 4″-Acetoxy-2-hydroxy-3,3″-dimethoxy-6-cinnamylphenol (**27**)

Pale yellow oil; IR ($CHCl_3$) ν_{max} 3552, 3321 (br), 1761, 1632, 1602 cm^{-1}; ^1H NMR ($CDCl_3$): 6.94 (1H, d, $J = 1.5$ Hz), 6.93 (1H, d, $J = 8.4$ Hz), 6.91 (1H, dd, $J = 8.4$, 1.5 Hz), 6.66 (1H, d, $J = 8.4$ Hz), 6.43 (1H, d, $J = 8.4$ Hz), 6.40 (1H, d, $J = 15.8$ Hz), 6.32 (1H, dt, $J = 15.8$, 6.6 Hz), 5.42 (2H, s, OH), 3.86 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 3.50 (2H, d, $J = 6.6$ Hz), 2.30 (3H, s, $COCH_3$); HRMS *m/z* 344.1224 (calcd for $C_{19}H_{20}O_6$: 344.1260).

4.17.2. 4″-Acetoxy-2,3-dihydroxy-3″-methoxy-6-cinnamylphenol methyl ether (**28**)

Pale yellow oil; IR ($CHCl_3$) ν_{max} 3547, 3335 (br), 1761, 1603 cm^{-1}; ^1H NMR ($CDCl_3$): 6.94 (1H, d, $J = 8.4$ Hz), 6.93 (1H, d, $J = 1.5$ Hz), 6.91 (1H, dd, $J = 8.4$, 1.5 Hz), 6.67 (1H, d, $J = 8.4$ Hz), 6.65 (1H, d, $J = 8.4$ Hz), 6.37 (1H, d, $J = 15.8$ Hz), 6.27 (1H, dt, $J = 15.8$, 6.6 Hz), 5.62 (1H, s, OH), 5.36 (1H, s, OH), 3.82 (6H, s, OCH_3), 3.81 (3H, s, OCH_3), 3.49 (2H, d, $J = 6.6$ Hz), 2.30 (3H, s, $COCH_3$); HRMS *m/z* 344.1242 (calcd for $C_{19}H_{20}O_6$: 344.1260).

4.18. In vitro and in vivo biological assays

The inhibition of EBV-EA activation was assayed using Raji cells (virus non-producer type) as previously described [2–7]. The inhibition of mouse skin tumor promotion in an *in vivo* two-stage carcinogenesis test was also assayed using the same method as described previously [3,4,6].

4.18.1. Short-term in vitro bioassay for anti-tumor promoters

The inhibition of EBV-EA activation was assayed using the same method as described previously [2–7]. In brief, Raji cells were grown to a density of 10^6 cells/mL, harvested by centrifugation and resuspended in RPMI 1640 medium (Sigma Chemical Co., St. Louis, USA) with 10% FCS containing 4 mM *n*-butyric acid as inducer, 32 pmol of TPA (20 ng/mL in DMSO), and 32, 16, 3.2 or 0.32 nmol of the test compound (DMSO solutions). The cells were incubated at 37 °C for 48 h, and cell number and viability were determined after 48 h by means of a hemocytometer (Trypan Blue staining method). EBV-EA inhibitory activity of the test compounds was estimated on the basis of the percentage of positive cells compared to that observed in a control without the test product. In each assay, at least 500 cells were counted and the results were read blind. As regards the effects of acridone alkaloids on this assay, the IC_{50} values were estimated by the probit transformation technique.

4.18.2. In vivo two-stage mouse skin carcinogenesis test

Female SENCAR mice were obtained at 5–6 weeks of age from SLC Co., Ltd. (Shizuoka, Japan), and maintained in Kyoto Prefectural University of Medicine, Animal Center. Groups of animals (15 animals per group) were housed in subgroups of five in polycarbonate cages. Mice were permitted free access to an MF solid diet (Oriental yeast Co., Ltd. Chiba, Japan) and drinking water at all times during the study. The back of each mouse was shaved with surgical clippers before the first day of initiation. Tumors on the back of the mice were initiated with dimethylbenz[*a*]anthracene (DMBA; 390 nmol) in acetone (0.1 ml). One week after initiation, they were promoted twice a week by the application of TPA (1.7 nmol) in acetone (0.1 ml). The mice in the test compound treated groups were treated with the test compounds (85 nmol) in acetone (0.1 ml) 1 h before each TPA treatment. The incidence of papillomas was observed weekly for 20 weeks.

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