Constituents of *Boronia pinnata*[1]

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A novel quinolone, pinolinone (**1**); seven new phenylpropanoids, boropinols A (**2**), B (**3**), C (**4**), boropinals A (**5**), B (**6**), C (**7**), and boropinic acid (**8**); and a new lignan, boropinan (**9**), were isolated from the roots of *Boronia pinnata*, and their structures were elucidated by NMR and MS analyses. In a search for novel cancer chemopreventive agents (antitumor-promoters), we screened 10 compounds isolated from the plant for their inhibitory effects on Epstein−Barr virus early antigen (EBV-EA) activation induced by 12-*O*-tetradecanoylphorbol-13-acetate in Raji cells. Boropinic acid (**8**) and 4′-hydroxy-3′-prenylcinnamaldehyde were observed to significantly inhibit the EBV-EA activation.

Boronia pinnata Sm. (Rutaceae) is a shrub found only in New South Wales, Australia.[2] Terpenes,[3,4] acetophenones,[5] flavanones,[5] chalcones,[6] quinolone alkaloids,[7] acridone alkaloids,[7] and furoquinoline alkaloids[7] have been reported as constituents of the plant genus *Boronia*.[8] In our search for cancer chemopreventive agents (antitumor-promoters) from medicinal plants, an extract of the roots of *B. pinnata* was found to have an inhibitory activity for tumor-promotion. Previously, we reported that several phenylpropanoids isolated from this plant exhibited significant inhibitory effects on the Epstein−Barr virus early antigen (EBV-EA) activation. [9]

This paper describes the isolation and structure elucidation of a novel quinolone, pinolinone (**1**); seven new phenylpropanoids, boropinols A (**2**), B (**3**), C (**4**), boropinals A (**5**), B (**6**), C (**7**), and boropinic acid (**8**); and a new lignan, boropinan (**9**), from the roots of *B. pinnata*. We also report the results of an assay on inhibitory effects of 10 compounds isolated from the plant on EBV-EA activation.

Results and Discussion

Dried roots of *B. pinnata* were extracted with acetone at room temperature. The acetone extract was fractionated by a combination of Si gel column chromatography and preparative TLC to give nine new compounds (**1**−**9**) along with several known compounds.

Pinolinone (**1**) was obtained as a colorless oil. Its molecular formula was derived as $C_{15}H_{19}NO_3$ by HRMS. The UV spectrum indicated the presence of an aromatic ring in **1**. The IR spectrum showed hydroxyl and amide carbonyl absorption. In the ^{1}H NMR spectrum, a four-spin proton signal was assigned to four contiguous aromatic protons (H-5, H-7, H-6, and H-8). An *N*-methyl signal appeared at δ 3.39. The presence of a prenyl moiety was indicated by ^{1}H NMR and by EIMS [*m*/*z* 192, $M^+ - \cdot CH_2CH{=}C(CH_3)_2$]. Two singlets at δ 3.93 and 2.70 disappeared on addition of D_2O. The ^{1}H NMR spectrum in DMSO-d_6 showed a 1H doublet (δ 4.54, J = 4.8 Hz) coupled with an OH, and a 1H broad triplet (δ 5.04) due to an olefinic proton on the prenyl moiety. The structure, *N*-methyl-2-quinolone with a prenyl moiety at C-3, was confirmed by HMBC correlations from C-2 to the N−CH_3, H-1′, and OH-3, and also from C-3 to H-2′ and OH-3, and by the NOE between N−CH_3 and H-8. Further, correlations from C-4 to 3-OH and H-5 suggested that the two hydroxyl groups were at C-3 and C-4 on the 2-quinolone nucleus. *trans*-Orientation of the hydroxyl groups at C-3 and C-4 was established by NOE between OH-3 and H-4 in the ^{1}H NMR spectrum in DMSO-d_6. From the aforementioned results, together with HMBC results (Figure 1), the structure of pinolinone was concluded to be **1**, except for the absolute stereochemistry.

Boropinols A (**2**), B (**3**), and C (**4**) each showed sharp UV absorption bands at λ_{max} 209−222 nm and at λ_{max} 263−268 nm with strong and medium intensities, respectively. Additionally, in consideration of the chemical shifts, multiplicities, and J values of the ^{1}H NMR signals, the phenylpropanoid (C-6−C-3) structure with (*E*)-CH=CH−CH_2−O− side chain as the C-3 unit was indicated as a common structural feature for the boropinols.

Boropinol A (**2**), $C_{15}H_{20}O_3$, was obtained as a colorless oil. The presence of a prenyloxy moiety [−$OCH_2CH{=}C(CH_3)_2$] and a methoxy group in the molecule was suggested by the characteristic ^{1}H NMR signals and a significant mass fragment ion at *m*/*z* 180 arising from loss of C_5H_8 from the molecular ion in EIMS. Locations of a methoxy and a prenyloxy moiety at C-3′ and C-4′, respectively, were deduced by an ABC-type signal on the aromatic ring in the ^{1}H NMR spectrum and by NOE between H-2′ and OCH_3, H-2, and H-3, and between H-5′ and H-1″. Thus, the structure of boropinol A was concluded to be **2**.

The ^{1}H NMR spectrum of boropinol B (**3**) ($C_{13}H_{18}O_4$) showed signals of one aliphatic and three aromatic methoxyls along with an allylic methylene group (H-1). The location of three methoxyls at C-3′, -4′, -5′ was indicated by HMBC correlations from H-3 to C-2′ (C-6′). The structure of the C-3 side chain was also deduced from two significant mass fragments at *m*/*z* 207 [$M^+ - \cdot OCH_3$] and 167 [$M^+ - \cdot CH{=}CH{-}CH_2OCH_3$] in the EIMS accompanied by the ^{1}H NMR signals. These results confirmed the structure of boropinol B to be **3**.

Boropinol C (**4**), $C_{16}H_{22}O_4$, was isolated as an amorphous solid. The signal pattern in the ^{1}H NMR spectrum was similar to that of **3**, except for signals due to the prenyloxy moiety and an OH group, instead of two methoxy singlets. The presence of a hydroxymethyl group on the C-3 side

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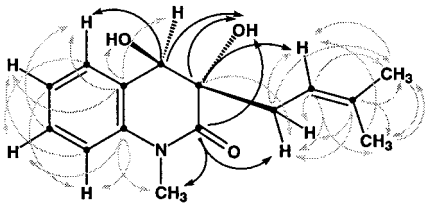
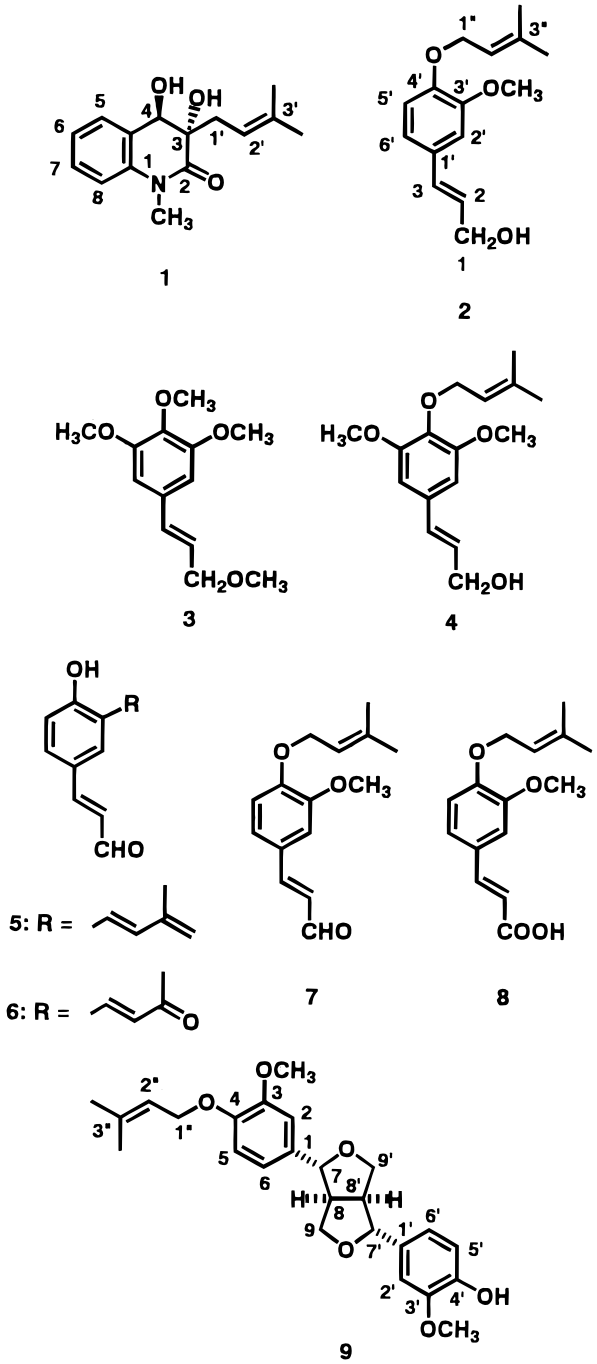


Figure 1. C−H long-range correlations in the HMBC spectrum of pinolinone (**1**) in $CDCl_3$. Bold line: more significant correlations in the structure determinations.



chain was proposed by ^{1}H NMR signals of an OH group coupled with methylene protons. The prenyloxy group at C-4′ was confirmed by NOE from 2H-singlet (H-2′, H-6′) to an OCH_3 6H-singlet and to the doublet (H-3). Based on these results, we assigned structure **4** to boropinol C.

Boropinals A (**5**) and B (**6**) showed a medium absorption band at λ_{max} 204 nm and a strong band at λ_{max} 296 and 293 nm, respectively, with a shoulder at λ_{max} 320 nm. In boropinal C (**7**), a strong band at λ_{max} 335 nm with a shoulder at λ_{max} 302 nm was observed. Red shifts of the UV bands of boropinals (**5**−**7**) compared with those of boropinols (**2**−**4**) suggested the presence of an additional conjugated system in those molecules. The common structure [(*E*)-CH═CH−CHO] in these compounds was indicated by ^{1}H NMR signals of a lower-field doublet at δ 9.66−9.61 (J = 7.7 Hz) coupled with olefinic protons [δ 6.68−6.61 (dd, J = 15.8, 7.7 Hz) and 7.57−7.41 (d, J = 15.8 Hz)], the same as compounds **2**−**4**, together with a conjugated carbonyl band at υ_{max} 1670−1672 cm^{-1} in the IR spectra.

The IR spectrum of boropinal A (**5**) ($C_{14}H_{14}O_2$) showed absorption bands due to OH and aldehyde carbonyl groups. The presence of an additional C-5 side chain [−CH═CH−$C(CH_3)$═CH_2] in the molecule was shown by the ^{1}H NMR signals of a vinyl methyl singlet, (*E*)-olefinic doublets, and broad *exo*-methylene singlets. Also, the appearance of an EIMS fragment at m/z 147 [$M^+ - \cdot C_5H_7$] was consistent with this partial structure. Placement of substituents on the aromatic ring was proposed by observation of ABC-type signals (H-2′, H-6′, and H-5′) and by NOE from H-3 to H-2′ and H-1 and from H-2′ to H-1″ and H-3. Thus, structure **5** was indicated for boropinal A.

Boropinal B (**6**), $C_{13}H_{12}O_3$, was obtained as a yellow oil. The ^{1}H NMR spectrum, except for the lack of the *exo*-methylene signals of **5**, confirmed that this compound was closely related to **5**. The structure of the C-4 substituent [(*E*)-CH═CH−$C(CH_3)$═O] was proposed by appearances of a methyl singlet and a pair of olefinic doublets having a large coupling constant (J = 16.5 Hz) in the ^{1}H NMR spectrum and an IR band at 1672 cm^{-1}. The location of the C-4 substituent at C-3′ with an OH group at the remaining site (C-4′) was confirmed by NOE from H-2′ to H-2, H-3, H-2″, and H-1″. These results suggested the structure of boropinal B to be **6**.

The ^{1}H NMR spectrum of boropinal C (**7**) ($C_{15}H_{18}O_3$) showed the presence of a methoxy group, a prenyloxy, and a three-spin proton system (H-2′, H-6′, and H-5′). The substitution pattern on the aromatic ring was deduced by differential NOE from H-2′ to 3′-OCH_3, H-2, and H-3 and from H-1″ to H-5′. Thus, boropinal C was assigned structure **7**.

The presence of an α,β-unsaturated acid moiety in boropinic acid (**8**) ($C_{15}H_{18}O_4$) was indicated by the IR bands at υ_{max} 3518, 3201 (br), 1685 cm^{-1} and (*E*)-oriented α,β proton signals in the ^{1}H NMR spectrum. Further, the presence of a prenyloxy moiety was revealed by the ^{1}H NMR and EIMS fragment peak at m/z 194 [$M^+ - \cdot CH_2$-CH═$C(CH_3)_2 + \cdot H$]. Additionally, ABC-type proton signals (H-2′, H-5′, and H-6′) and a methoxy signal were observed. These spectral data, coupled with the results of NOE, indicated boropinic acid to be **8**.

The molecular formula of boropinan (**9**) was determined as $C_{25}H_{30}O_6$ by HRMS. The ^{1}H NMR spectrum in acetone-d_6 showed two 1,3,4-trisubstituted aromatic, two methoxy, one hydroxy, and one prenyloxy proton signals, along with two sets of $-CH_2-CH-CH-$ protons. The ^{1}H and ^{13}C NMR spectra in $CDCl_3$, except for signals due to a prenyloxy moiety in **9**, showed close similarities to those of (−)-pinoresinol,[10] which has a 2,6-diaryl-3,7-dioxabicyclo[3.3.0]-octane nucleus, although only half the number of proton signals appeared due to its symmetrical structure, suggesting a diarylfurofuran lignoid structure for this compound. The locations of the prenyloxy moiety and two methoxyls at C-4, and C-3, C-3′, respectively, were assigned based on NOE (Figure 2, in acetone-d_6) between H-1″ and

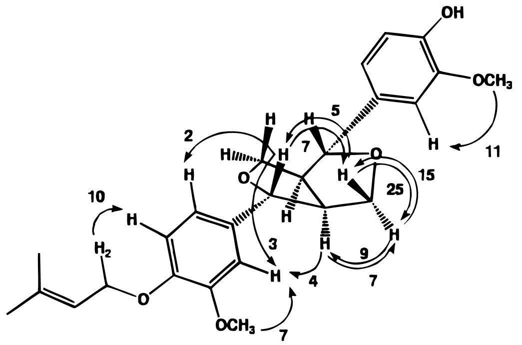


Figure 2. NOE (%) of boropinan (**9**) in acetone-d_6.

H-5, and between 3- and 3′-methoxyls and an overlapped 2H signal (H-2, -2′). The HMBC results also supported structure **9**. Diequatorial conformation of the two aryl groups, including the absolute stereochemistry of **9**, was proposed by NOE between the angular proton signal (overlapped H-8 and -8′) and an aromatic proton signal (overlapped H-2 and -2′), and between H-7, H-7′ and H-9, H-9′, together with the chemical shift of two benzylic protons (H-7, -7′) and the negative $[\alpha]_D$ value of this compound.[11] Recently, isolation of the (+)-isomer of **9** from *Zanthoxylum integrifoliolum* was reported.[12]

Other compounds isolated from this plant material were fully characterized as elemicin,[13,24] 3-(3′-methoxy-4′-prenyloxy)phenyl-1-propene,[14] dictamine,[15] (*E*)-3′,4′-dimethoxycinnamaldehyde,[16] 4′-hydroxy-3′-prenylcinnamaldehyde,[17] (*E*)-3′,4′,5′-trimethoxycinnamaldehyde,[18,24] evolitrine,[19] preskimmianine,[20] braylin,[21] (*E*)-3′,4′-(methylenedioxy)cinnamyl alcohol,[22] (*E*)-3′,4′,5′-trimethoxycinnamyl alcohol,[23,24] (*E*)-3′,4′-dimethoxycinnamyl alcohol,[23] and folimine,[25] by comparison of the ^{1}H NMR and IR spectra with those of authentic samples or with spectroscopic data reported in the literature.[13−25] Methyleugenol, syringaldehyde, and *p*-hydroxycinnamic acid were spectroscopically identical with authentic samples of commercially available material from the Tokyo Kasei Kogyo Co., Ltd., Japan.

The primary screening test of the 10 compounds was carried out utilizing a short-term in vitro synergistic assay on EBV-EA activation. Their inhibitory effects on EBV-EA activation induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) on the viability of Raji cells are shown in Table 1. All the compounds tested showed inhibitory effects on EBV-EA activation without cytotoxicity toward the Raji cells. At a concentration of 1×10^2 mol ratio/TPA, all the compounds showed inhibitory effects (12.8−37.8%). However, at 1×10 mol ratio/TPA, quinolones (preskimmianine and folimine), furoquinolines (dictamine and evolitrine), and braylin showed no effect on EBV-EA activation. Among the phenylpropanoids, compounds (*E*)-3′,4′-(methylenedioxy)cinnamyl alcohol and *p*-hydroxycinnamic acid, lacking a prenyl moiety in the molecules, showed no inhibitory effect at 1×10 mol ratio/TPA. Boropinic acid (**8**) and 4′-hydroxy-3′-prenylcinnamaldehyde, with a prenyl moiety, showed significant inhibitory effects (11.5−16.0%) even at 1×10 mol ratio/TPA. At concentration of 1×10^3 mol ratio/TPA, boropinic acid (**8**) and 4′-hydroxy-3′-prenylcinnamaldehyde fully blocked the TPA-induced EBV-EA activation. Syringaldehyde also showed a moderate inhibitory effect at 1×10^3 mol ratio/TPA. We have reported that the presence of a prenyl moiety in a phenylpropanoid molecule is essential for inhibitory effects on EBV-EA induction.[9] Our present study also suggests that the prenyl group in phenylpropanoids plays a role in the tumor inhibitory effects of *B. pinnata*.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR, NOE, HMQC, and HMBC (J = 8 Hz) spectra were recorded on an A-400 or A-600 (JEOL) spectrometer. Chemical shifts are shown in δ (ppm) with tetramethylsilane (TMS) as an internal reference. All mass spectra were obtained under electron impact (EI) conditions, unless otherwise stated, using an M-80 (Hitachi) spectrometer with a direct inlet system. UV spectra were recorded on a UVIDEC-610C double-beam spectrophotometer (JASCO) in MeOH; IR spectra, on an IR-230 (JASCO) in $CHCl_3$; and optical rotations, on a DIP-370 (JASCO) in $CHCl_3$ at 25 °C. Preparative TLC was performed on Kieselgel 60 F_{254} (Merck).

Plant Material. The dried roots of *Boronia pinnata* cultivated at Hayakawa Garden (Aichi) were collected in May 1995. A voucher specimen (MUY0101) has been deposited in the Faculty of Pharmacy, Meijo University.

Extraction and Isolation. The dried roots (333 g) of *B. pinnata* were extracted with acetone at room temperature. The acetone extract was subjected to Si gel column chromatography eluting successively with hexane, hexane−acetone (19:1, 9:1, 17:3, 4:1, 3:1, 13:7, 11:9, 2:3), acetone, CH_2Cl_2−MeOH (3:1), and MeOH to give 12 fractions. Each fraction was further subjected to Si gel column chromatography and preparative TLC with appropriate combinations of hexane, CH_2Cl_2, *iso*-Pr_2O, benzene, $CHCl_3$, EtOAc, acetone, and MeOH as developing solvents. Obtained from the hexane−acetone (19:1) eluate were elemicin (18.9 mg), methyleugenol (9.0 mg), and 3-(3′-methoxy-4′-prenyloxy)phenyl-1-propene (60.1 mg). From the hexane−acetone (17:3) eluate: boropinol B (**3**) (140.5 mg), boropinal C (**7**) (3.0 mg), and dictamine (4.5 mg). From the hexane−acetone (4:1) eluate: pinolinone (**1**) (1.0 mg), boropinal A (**5**) (8.4 mg), (*E*)-3′,4′-dimethoxycinnamaldehyde (4.7 mg), 4′-hydroxy-3′-prenylcinnamaldehyde (0.8 mg), (*E*)-3′,4′,5′-trimethoxycinnamaldehyde (2.0 mg), evolitrine (32.1 mg), preskimmianine (15.2 mg), and braylin (9.0 mg). From the hexane−acetone (3:1) eluate: boropinol A (**2**) (2.8 mg) and (*E*)-3′,4′-(methylenedioxy)cinnamyl alcohol (0.6 mg). From the hexane−acetone (13:7) eluate: boropinol C (**4**) (2.2 mg), boropinic acid (**8**) (1.2 mg), boropinan (**9**) (1.8 mg), and (*E*)-3′,4′,5′-trimethoxycinnamyl alcohol (2.7 mg). From the hexane−acetone (11:9) eluate: boropinal B (**6**) (1.2 mg), (*E*)-3′,4′-dimethoxycinnamyl alcohol (8.8 mg), folimine (1.0 mg), and syringaldehyde (2.5 mg). From the hexane−acetone (2:3) eluate: *p*-hydroxycinnamic acid (1.1 mg). Known components were fully characterized by comparison of the ^{1}H NMR and IR data with those reported in the literature.

Pinolinone (1): colorless oil; $[\alpha]_D$ −9.4° (c 0.128, $CHCl_3$); UV (MeOH) λ_{max} 211, 258 nm; IR ($CHCl_3$) υ_{max} 3595, 3495 (br), 1668, 1606 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.61 (1H, d, J = 7.7 Hz, H-5), 7.34 (1H, t, J = 7.7 Hz, H-7), 7.19 (1H, t, J = 7.7 Hz, H-6), 6.99 (1H, d, J = 7.7 Hz, H-8), 5.03 (1H, overlapped with H-4, H-2′), 5.01 (1H, overlapped with H-2′, H-4), 3.93 (1H, br s, 3-OH), 3.39 (3H, s, N−CH_3), 2.70 (1H, br s, 4-OH), 2.47 (1H, dd, J = 14.7, 8.1 Hz, H-1′), 1.98 (1H, dd, J = 14.7, 7.3 Hz, H-1′), 1.64 (3H, s, 3′-CH_3), 1.41 (3H, s, 3′-CH_3); ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.38 (1H, d, J = 7.8 Hz, H-5), 7.28 (1H, t, J = 7.8 Hz, H-7), 7.06 (1H, d, J = 7.8 Hz, H-8), 7.05 (1H, t, J = 7.8 Hz, H-6), 5.65 (1H, d, J = 4.8 Hz, 4-OH), 5.15 (1H, br s, 3-OH), 5.04 (1H, br t, J = 7.7 Hz, H-2′), 4.54 (1H, d, J = 4.8 Hz, H-4), 3.30 (3H, s, N−CH_3), 2.30 (1H, dd, J = 15.0, 7.7 Hz, H-1′), 2.15 (1H, dd, J = 15.0, 7.7 Hz, H-1′), 1.55 (3H, s, 3′-CH_3), 1.37 (3H, s, 3′-CH_3); differential NOE (DMSO-d_6, 600 MHz), irradiation of N−CH_3 (δ 3.30) gave 23% NOE at H-8 (δ 7.06); irradiation of H-4 (δ 4.54) gave 16, 4, and 7% NOE at 4-OH (δ 5.65), 3-OH (δ 5.15), and H-5 (δ 7.38), respectively; ^{13}C NMR ($CDCl_3$, 150 MHz) δ 172.2 (s, C-2), 137.2 (s, C-8a), 136.2 (s, C-3′), 127.3 (s, C-4a), 128.5 (d, C-7), 125.0 (d, C-5), 124.2 (d, C-6), 116.9 (d, C-2′), 114.2 (d, C-8), 75.9 (s, C-3), 72.7 (d, C-4), 30.2 (q, N−CH_3), 28.7 (t, C-1′), 25.9 (q, 3′-CH_3), 17.6 (q, 3′-CH_3); EIMS, m/z 261 $[M]^+$ (5), 243 (7), 226 (31), 192 (12), 175 (55), 146 (81), 91 (52), 69 (100); HRMS, m/z 261.1374 (calcd for $C_{15}H_{19}NO_3$, 261.1364).

Table 1. Inhibitory Effects of Chemical Constituents from *Boronia pinnata* on TPA-induced EBV-EA Activation[a]

	EBV-EA-positive cells (% viability)			
	compound concentration (mol ratio/32 pmol TPA)			
	1000	500	100	10
preskimmianine[20]	12.3 ± 1.0 (60)	43.1 ± 2.1 (>80)	70.0 ± 1.1 (>80)	100.0 ± 0.4 (>80)
folimine[25]	16.8 ± 1.3 (60)	45.5 ± 2.4 (>80)	74.2 ± 1.6 (>80)	100.0 ± 0.2 (>80)
dictamine[15]	20.5 ± 1.0 (60)	49.1 ± 2.2 (>80)	76.0 ± 0.8 (>80)	100.0 ± 0.2 (>80)
evolitrine[19]	18.7 ± 1.2 (60)	44.1 ± 2.0 (>80)	75.0 ± 1.5 (>80)	100.0 ± 0.5 (>80)
braylin[21]	19.4 ± 1.3 (60)	52.2 ± 2.4 (>80)	87.2 ± 1.6 (>80)	100.0 ± 0.2 (>80)
(*E*)-3′,4′-(methylenedioxy)cinnamyl alcohol[22]	20.3 ± 1.1 (70)	39.6 ± 1.7 (>80)	79.2 ± 1.5 (>80)	100.0 ± 0.2 (>80)
p-hydroxycinnamic acid	18.8 ± 1.2 (60)	36.4 ± 1.2 (>80)	72.7 ± 2.2 (>80)	100.0 ± 0.3 (>80)
boropinic acid (**8**)	0.0 ± 0.4 (70)	23.1 ± 1.1 (>80)	62.2 ± 1.5 (>80)	84.0 ± 0.3 (>80)
4′-hydroxy-3′-prenylcinnamaldehyde	0.0 ± 0.5 (60)	27.2 ± 1.8 (>80)	65.5 ± 1.0 (>80)	88.5 ± 0.4 (>80)
syringaldehyde	4.1 ± 0.3 (60)	39.2 ± 2.2 (>80)	77.2 ± 1.2 (>80)	94.7 ± 1.5 (>80)

[a] Mole 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 (%) ± s.d. in the presence of the test compound relative to the positive control (100%). Values in parentheses represent the viability % of Raji cells measured through Trypan Blue staining. At least 60% viability of Raji cells 2 days after treatment with the compounds is required for an accurate result.

Boropinol A (2): colorless oil; UV (MeOH) λ_{max} 209, 263, 300 (sh) nm; IR ($CHCl_3$) υ_{max} 3608, 1509 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 6.92 (1H, d, J = 1.8 Hz, H-2′), 6.88 (1H, dd, J = 8.4, 1.8 Hz, H-6′), 6.80 (1H, d, J = 8.4 Hz, H-5′), 6.53 (1H, d, J = 15.8 Hz, H-3), 6.22 (1H, dt, J = 15.8, 5.9 Hz, H-2), 5.49 (1H, m, H-2″), 4.56 (2H, J = 6.6 Hz, H-1″), 4.29 (2H, d, J = 5.9 Hz, H-1), 3.86 (3H, s, 3′-OCH_3), 1.75 (3H, s, 3″-CH_3), 1.71 (3H, s, 3″-CH_3); differential NOE ($CDCl_3$, 600 MHz), irradiation of H-2′ (δ 6.92) gave 11, 8, and 2% NOE at 3′-OCH_3 (δ 3.86), H-2 (δ 6.22), and H-3 (δ 6.53), respectively; irradiation of H-1″(δ 4.56) gave 19% NOE at H-5′(δ 6.80); irradiation of 3′-OCH_3 (δ 3.86) gave 11% NOE at H-2′ (δ 6.92); EIMS, *m*/*z* 248 $[M]^+$ (3), 180 (43, $M^+ - \cdot C_5H_8$), 137 (38), 124 (48), 69 (100); HRMS, *m*/*z* 248.1405 (calcd for $C_{15}H_{20}O_3$, 248.1411).

Boropinol B (3): yellow oil; UV (MeOH) λ_{max} 222, 268 nm; IR ($CHCl_3$) υ_{max} 1583, 1506 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 6.62 (2H, s, H-2′, -6′), 6.53 (1H, d, J = 15.8 Hz, H-3), 6.20 (1H, dt, J = 15.8, 5.9 Hz, H-2), 4.08 (2H, d, J = 5.9 Hz, H-1), 3.86 (6H, s, 3′-OCH_3, 5′-OCH_3), 3.84 (3H, s, 4′-OCH_3), 3.39 (3H, s, 1-OCH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 153.1 (s × 2, C-3′, C-5′), 137.7 (s, C-4′), 132.3 (s, C-1′), 132.1 (d, C-3), 125.3 (d, C-2), 103.4 (d × 2, C-2′, C-6′), 72.8 (t, C-1), 60.7 (q, 4′-OCH_3), 57.8 (q, 1-OCH_3), 55.9 (q × 2, 3′-OCH_3, 5′-OCH_3); HMBC C−H correlations ($CDCl_3$, 600 MHz), C-2 → H-1; C-3 → H-1; C-1′ → H-3; C-2′ → H-3, H-6′; C-3′ → H-2′, 3′-OCH_3; C-4′ → H-2′, H-6′, 4′-OCH_3; C-5′ → H-6′, 5′-OCH_3; C-6′ → H-3, H-2′; EIMS, *m*/*z* 238 $[M]^+$ (100), 223 (19, $M^+ - \cdot CH_3$), 207 (45, $M^+ - \cdot OCH_3$), 191 (14), 176 (31), 167 (2, $M^+ - \cdot CH{=}CH{-}CH_2OCH_3$), 163 (16), 149 (25); HRMS, *m*/*z* 238.1197 (calcd for $C_{13}H_{18}O_4$, 238.1204).

Boropinol C (4): amorphous solid; UV (MeOH) λ_{max} 220, 268 nm; IR ($CHCl_3$) υ_{max} 3604, 1583, 1504 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 6.54 (2H, s, H-2′, -6′), 6.47 (1H, d, J = 15.8 Hz, H-3), 6.22 (1H, dt, J = 15.8, 5.9 Hz, H-2), 5.49 (1H, m, H-2″), 4.42 (2H, d, J = 7.0 Hz, H-1″), 4.25 (2H, d, J = 5.9 Hz, H-1), 3.79 (6H, s, 3′-OCH_3, 5′-OCH_3), 1.67 (3H, s, 3″-CH_3), 1.60 (3H, s, 3″-CH_3); ^1H NMR (DMSO-d_6, 600 MHz) δ 6.69 (2H, s, H-2′, H-6′), 6.45 (1H, d, J = 16.1 Hz, H-3), 6.32 (1H, dt, J = 15.8, 5.1 Hz, H-2), 5.39 (1H, m, H-2″), 4.83 (1H, t, J = 5.1 Hz, 1-OH), 4.32 (2H, d, J = 7.3 Hz, H-1″), 4.09 (2H, t, J = 5.1 Hz, H-1), 3.76 (6H, s, 3′-OCH_3, 5′-OCH_3), 1.68 (3H, s, 3″-CH_3), 1.58 (3H, s, 3″-CH_3); differential NOE ($CDCl_3$, 600 MHz), irradiation of 3′-OCH_3, 5′-OCH_3 (δ 3.79) gave 11% NOE at H-2′, H-6′ (δ 6.54); irradiation of H-3 (δ 6.47) gave 9% NOE at H-2′, H-6′ (δ 6.54); irradiation of H-2′, H-6′ (δ 6.54) gave 9, 7, and 8% NOE at 3′-OCH_3, 5′-OCH_3 (δ 3.79), H-2 (δ 6.22), and H-3 (δ 6.47), respectively; EIMS, *m*/*z* 278 $[M]^+$ (2), 210 (100, $M^+ - \cdot C_5H_8$), 182 (42), 167 (44), 154 (33), 149 (24); HRMS, *m*/*z* 278.1513 (calcd for $C_{16}H_{22}O_4$, 278.1516).

Boropinal A (5): yellow oil; UV (MeOH) λ_{max} 204, 296, 320 (sh), 356 (sh) nm; IR ($CHCl_3$) υ_{max} 3587, 1670, 1622, 1597 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 9.65 (1H, d, J = 7.7 Hz, H-1), 7.65 (1H, d, J = 2.0 Hz, H-2′), 7.44 (1H, d, J = 15.8 Hz, H-3), 7.35 (1H, dd, J = 8.4, 2.0 Hz, H-6′), 6.92 (1H, d, J = 16.1 Hz, H-1″), 6.87 (1H, d, J = 8.4 Hz, H-5′), 6.75 (1H, d, J = 16.1 Hz, H-2″), 6.64 (1H, dd, J = 15.8, 7.7 Hz, H-2), 6.27 (1H, br s, 4′-OH), 5.16 (1H, br s, H-4″), 5.13 (1H, br s, H-4″), 2.00 (3H, s, 3″-CH_3); differential NOE ($CDCl_3$, 400 MHz), irradiation of H-3 (δ 7.44) gave 17% NOE at H-1 (δ 9.65) and 6% NOE at H-2′ (δ 7.65); irradiation of H-1″ (δ 6.92) gave 7% NOE at H-2′ (δ 7.65) and 8% NOE at H-4″ (δ 5.16); irradiation of H-2′ (δ 7.65) gave 7, 4, 10, and 8% NOE at H-2 (δ 6.64), H-2″ (δ 6.75), H-1″ (δ 6.92) and H-3 (δ 7.44), respectively; EIMS, *m*/*z* 214 $[M]^+$ (18), 199 (100), 171 (23), 147 (20), 138 (32), 115 (35), 91 (34); HRMS, *m*/*z* 214.0991 (calcd for $C_{14}H_{14}O_2$, 214.0992).

Boropinal B (6): yellow oil; UV (MeOH) λ_{max} 204, 217 (sh), 293, 320 (sh) nm; IR ($CHCl_3$) υ_{max} 3587, 3300 (br), 1672, 1601 cm^{-1}; ^1H NMR (acetone-d_6, 600 MHz) δ 9.61 (1H, d, J = 7.7 Hz, H-1), 8.02 (1H, d, J = 2.4 Hz, H-2′), 7.84 (1H, d, J = 16.5 Hz, H-1″), 7.61 (1H, dd, J = 8.7, 2.4 Hz, H-6′), 7.57 (1H, d, J = 15.8 Hz, H-3), 7.04 (1H, d, J = 8.7 Hz, H-5′), 6.94 (1H, d, J = 16.5 Hz, H-2″), 6.68 (1H, dd, J = 15.8, 7.7 Hz, H-2), 2.28 (3H, s, 3″-CH_3); differential NOE ($CDCl_3$, 600 MHz), irradiation of H-2′ (δ 8.02) gave 3, 6, 4 and 5% NOE at H-2 (δ 6.68), H-2″ (δ 6.94), H-3 (δ 7.57), and H-1″ (δ 7.84), respectively; EIMS, *m*/*z* 216 $[M]^+$ (17), 201 (23), 173 (24), 147 (15), 145 (20), 115 (100), 91 (75); HRMS, *m*/*z* 216.0812 (calcd for $C_{13}H_{12}O_3$, 216.0786).

Boropinal C (7): amorphous solid; UV (MeOH) λ_{max} 204, 219, 238, 246 (sh), 302, 335 nm; IR ($CHCl_3$) υ_{max} 1670, 1622, 1597, 1508 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 9.66 (1H, d, J = 7.7 Hz, H-1), 7.41 (1H, d, J = 15.8 Hz, H-3), 7.14 (1H, br d, J = 8.4 Hz, H-6′), 7.08 (1H, br s, H-2′), 6.90 (1H, d, J = 8.4 Hz, H-5′), 6.61 (1H, dd, J = 15.8, 7.7 Hz, H-2), 5.51 (1H, m, H-2″), 4.64 (2H, d, J = 6.6 Hz, H-1″), 3.91 (3H, s, 3′-OCH_3), 1.79 (3H, s, 3″-CH_3), 1.75 (3H, s, 3″-CH_3); differential NOE ($CDCl_3$, 600 MHz), irradiation of H-1″ (δ 4.64) gave 11% NOE at H-5′ (δ 6.90); irradiation of H-2′ (δ 7.08) gave 9, 12, and 3% NOE at 3′-OCH_3 (δ 6.54), H-2 (δ 6.61), and H-3 (δ 7.41), respectively; EIMS, *m*/*z* 246 $[M]^+$ (2), 226 (2), 178 (23), 161 (3), 147 (10), 118 (7), 91 (19), 69 (100); HRMS, *m*/*z* 246.1247 (calcd for $C_{15}H_{18}O_3$, 246.1254).

Boropinic acid (8): colorless oil; UV (MeOH) λ_{max} 203, 216, 232, 286, 311 nm; IR ($CHCl_3$) υ_{max} 3518, 3201 (br), 1685, 1630, 1599 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.73 (1H, d, J = 15.7 Hz, H-3), 7.11 (1H, dd, J = 8.4, 1.8 Hz, H-6′), 7.07 (1H, d, J = 1.8 Hz, H-2′), 6.31 (1H, d, J = 15.7 Hz, H-2), 6.88 (1H, d, J = 8.4 Hz, H-5′), 5.51 (1H, m, H-2″), 4.63 (2H, d, J = 6.6 Hz, H-1″), 3.91 (3H, s, 3′-OCH_3), 1.78 (3H, s, 3″-CH_3), 1.75 (3H, s, 3″-CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.3 (s, C-1), 150.9 (s, C-4′), 149.6 (s, C-3′), 147.1 (d, C-3), 138.3 (s, C-3″), 126.9 (s, C-1′), 123.0 (d, C-6′), 119.3 (d, C-2″), 114.6 (d, C-2), 112.5 (d, C-5′), 110.0 (d, C-2′), 65.8 (t, C-1″), 55.9 (q, 3′-OCH_3), 25.8 (q, 3″-CH_3), 18.3 (q, 3″-CH_3); HMBC C−H correlations ($CDCl_3$, 400 MHz), C-1 → H-3; C-3 → H-2′, H-6′; C-1′ → H-2, H-5′; C-2′ → H-3, H-6′; C-3′ → H-5′, 3′-OCH_3; C-4′ → H-2′, H-6′, H-1″; C-6′ → H-3, H-2′; C-2″ → H-1″, 3″-CH_3; C-3″ → H-1″, 3″-CH_3. differential NOE ($CDCl_3$, 600 MHz), irradiation of H-1″ (δ 4.63) gave 6% NOE at H-5′ (δ 6.88); irradiation of H-2′ (δ 7.07) gave

7% NOE at 3′-OCH_3 (δ 3.91) and 2% NOE at H-3 (δ 7.73); irradiation of 3′-OCH_3 (δ 3.91) gave 10% NOE at H-2′ (δ 7.07); irradiation of H-3 (δ 7.73) gave 3% NOE at H-2′ (δ 7.07) and 2% NOE at H-6′ (δ 7.11); EIMS, *m/z* 194 (59), 149 (5), 133 (5), 105 (9), 69 (100). FABMS, *m/z* 263 $[M + H]^+$; HRFABMS, *m/z* 263.1273 (calcd for $C_{15}H_{19}O_4$, 263.1283).

Boropinan (9): colorless oil; $[\alpha]_D$ −54° (*c* 0.125, $CHCl_3$); UV (MeOH) λ_{max} 206, 231, 280 nm; IR ($CHCl_3$) υ_{max} 3545 (br), 1606, 1514 cm^{-1}; ^1H NMR (acetone-d_6, 600 MHz) δ 7.51 (1H, br s, 4′-OH), 6.98 (2H, br s, H-2, H-2′), 6.90 (1H, d, *J* = 8.1 Hz, H-5), 6.88 (1H, dd, *J* = 8.1, 1.8 Hz, H-6), 6.83 (1H, dd, *J* = 8.1, 1.8 Hz, H-6′), 6.78 (1H, d, *J* = 8.1 Hz, H-5′), 5.46 (1H, m, H-2″), 4.68 (1H, d, *J* = 4.0 Hz, H-7 or H-7′), 4.66 (1H, d, *J* = 4.0 Hz, H-7′ or H-7), 4.52 (2H, d, *J* = 7.0 Hz, H-1″), 4.20 (2H, dd, *J* = 7.0, 8.8 Hz, H-9, H-9′), 3.83 (3H, s, 3′-OCH_3), 3.81 (2H, overlapped with OCH_3, H-9′, H-9), 3.80 (3H, s, 3-OCH_3), 3.08 (2H, m, H-8, H-8′), 1.75 (3H, s, 3″-CH_3), 1.71 (3H, s, 3″-CH_3); ^1H NMR ($CDCl_3$, 600 MHz) δ 6.90−6.83 (6H, overlapped, H-2, -2′, -5, -5′, -6, -6′), 5.58 (1H, br s, 4′-OH), 5.51 (1H, m, H-2″), 4.75 (1H, d, *J* = 4.4 Hz, H-7 or H-7′), 4.74 (1H, d, *J* = 4.4 Hz, H-7′ or H-7), 4.58 (2H, d, *J* = 6.6 Hz, H-1″), 4.25 (2H, t, *J* = 7.0 Hz, H-9, H-9′), 3.91 (3H, s, 3-OCH_3 or 3′-OCH_3), 3.89 (2H, overlapped with OCH_3, H-9′, H-9), 3.88 (3H, s, 3′-OCH_3 or 3-OCH_3), 3.11 (2H, m, H-8, H-8′), 1.77 (3H, s, 3″-CH_3), 1.73 (3H, s, 3″-CH_3); ^{13}C NMR (acetone-d_6, 150 MHz) δ 150.8 (s, C-3), 148.8 (s, C-4), 148.3 (s, C-3′), 146.8 (s, C-4′), 137.5 (s, C-3″), 135.5 (s, C-1), 134.1 (s, C-1′), 121.4 (d, C-2″), 119.6 (d, C-6′), 119.0 (d, C-6), 115.5 (d, C-5′), 114.3 (d, C-5), 111.0 (d, C-2), 110.5 (d, C-2′), 86.6 (d, C-7 or C-7′), 86.5 (d, C-7′ or C-7), 72.23 (t, C-9 or C-9′), 72.19 (t, C-9′ or C-9), 66.2 (t, C-1″), 56.2 (q, 3-OCH_3 or 3′-OCH_3), 56.0 (q, 3′-OCH_3 or 3-OCH_3), 55.22 (d, C-8 or C-8′), 55.21 (d, C-8′ or C-8), 25.8 (q, 3″-CH_3), 18.1 (q, 3″-CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 149.7 (s), 147.9 (s), 146.7 (s), 145.3 (s), 137.6 (s), 133.6 (s), 133.0 (s), 120.0 (d), 119.0 (d), 118.2 (d), 114.3 (d), 113.0 (d), 109.5 (d), 108.6 (d), 85.92 (d), 85.85 (d), 71.8 (t), 71.7 (t), 65.9 (t), 56.0 (q × 2), 54.23 (d), 54.15 (d), 25.8 (q), 18.2 (q); HMBC C−H three-bond correlations (acetone-d_6, 600 MHz), C-1 → H-5; C-2 → H-6; C-3 → H-5, 3- OCH_3; C-4 → H-2, H-6, H-1″; C-6 → H-2, H-7; C-7 → H-2, H-6, H-9; C-9 → H-7; C-1′ → H-5′; C-2′ → H-6′; C-3′ → H-5′, 3′-OCH_3, 4′-OH; C-4′ → H-2′, H-6′; C-5′ → 4′-OH; C-6′ → H-2′, H-7′; C-7′ → H-2′, H-6′, H-9′; C-9′ → H-7′; C-2″ → 3″-CH_3; C-3″ → H-1″; 3″-CH_3 → H-2″; EIMS, *m/z* 426 $[M]^+$ (3), 358 (16, $M^+ - \cdot C_5H_9 + \cdot H$), 205 (4), 163 (13), 151 (30), 137 (22), 131 (9); HRMS, *m/z* 426.2033 (calcd for $C_{25}H_{30}O_6$, 426.2040).

In Vitro EBV-EA Activation Experiments. The inhibitory effects of EBV-EA activation were assayed using the same method described previously.[9]

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