Cytotoxic Biflavonoids from *Selaginella delicatula*

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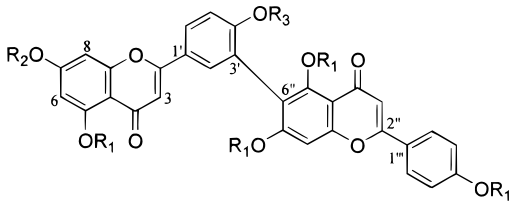
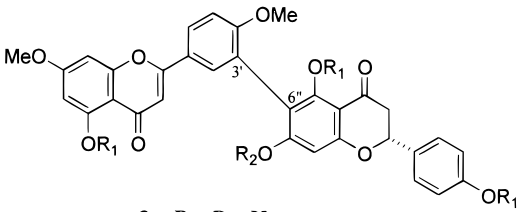
Four new biflavonoids—robustaflavone 4′-methyl ether (**1**), robustaflavone 7,4′-dimethyl ether (**2**), 2″,3″-dihydrorobustaflavone 7,4′, -dimethyl ether (**3**), and 2″,3″-dihydrorobustaflavone 7,4′, 7″-trimethyl ether (**4**)—as well as two known biflavonoids, robustaflavone and amentoflavone, and three caffeoylquinic acids, 3,5-di-*O*-caffeoylquinic acid, 3,4-di-*O*-caffeoylquinic acid, and 4,5-di-*O*-caffeoylquinic acid, were isolated from *Selaginella delicatula*. The structures of the new compounds were established by spectroscopic analysis and chemical modification. The cytotoxic activity of these compounds on various tumor cell lines was evaluated, and both compounds **1** and **3** significantly suppressed the growth of Raji and Calu-1 tumor cell lines.

The genus *Selaginella* is rich in biflavonoids,[1−6] and some of the members of this genus are used extensively in Chinese traditional medicine in the treatment of cancer,[4,7] gastritis,[8] hepatitis,[8] and cardiovascular diseases.[9] *Selaginella delicatula* Desv. Alston (Selaginellaceae) is a perennial herb growing throughout the mountain forest floors at low and medium altitudes in Taiwan.[10] Biflavonoids[11] and sterols[12] have been reported from *S. delicatula*; we have reexamined the whole plant of this species and report herein the isolation and characterization of four new biflavonoids (**1**−**4**), besides five known compounds: robustaflavone (**5**), amentoflavone (**6**), 3,5-di-*O*-caffeoylquinic acid, 3,4-di-*O*-caffeoylquinic acid, and 4,5-di-*O*-caffeoylquinic acid. These compounds were evaluated for their cytotoxicity against a small panel of human tumor cell lines.



1 R_1=H R_2=H R_3=Me
1a R_1= R_2=Ac R_3=Me
2 R_1=H R_2= R_3=Me
2a R_1=Ac R_2=R_3=Me



3 R_1=R_2=H
3a R_1=R_2=Ac
4 R_1=H R_2=Me
4a R_1=Ac R_2=Me

Results and Discussion

Compound **1** was obtained as a yellow powder. The positive FABMS of **1** gave a pseudomolecular ion at *m*/*z* 553 $[M + H]^+$, corresponding to a molecular formula of $C_{31}H_{20}O_{10}$, which was confirmed by HRFABMS (found *m*/*z* 553.11345, calcd 553.11347), consistent with the compound being a biflavonoid. The IR spectrum showed absorption bands 3400, 1653, 1627, 1515, and 1440 cm^{-1}, suggesting the presence of hydroxyl, chelated carbonyl, and aromatic ring functionalities. The ^{1}H NMR spectra of **1** and its acetate (**1a**) proved the presence of five hydroxyl groups in **1** of which two appeared downfield at δ 12.95 and 13.22, indicating the presence of chelated hydroxyls at the C-5 and C-5″ positions. The UV spectrum of **1** in methanol exhibited absorption maxima at 340 and 270 nm, and addition of sodium methoxide caused band I to shift to 394 nm, indicating the presence of a free hydroxyl group at C-4′ or C-4‴.[13] The ^{13}C NMR spectrum of **1** showed signals for all 31 carbons of the molecule, including one methoxyl group (55.8 ppm) and two carbonyl groups (181.8 and 181.7 ppm). The ^{1}H NMR spectrum of **1** showed the presence of an ABM coupling system, with signals at δ 8.08 (dd, J = 8.5, 2.0 Hz, H-6′), 7.82 (d, J = 2.0 Hz, H-2′), and 7.24 (d, J = 8.5 Hz, H-5′), indicating that C-3′ was the position of linkage of the two flavonoid units.[14] Two *meta*-coupled proton signals at H-6 and H-8 appeared at δ 6.20 and 6.50 (J = 2.0 Hz), and an A_2X_2 coupling system was established from the signals at δ 6.94 (d, J = 8.5 Hz, H-3‴, -5‴) and 7.96 (d, J = 8.5 Hz, H-2‴, -6‴). Further, three proton signals appeared at δ 6.87 (s, H-3), 6.82 (s, H-3″) and 6.64 (s, H-8″), which were confirmed by an HMBC experiment. The remaining signal at δ 3.80 (3H, s) showed a cross-peak with δ 7.24 (H-5′) in the NOESY spectrum, which confirmed that the OCH_3 group is attached to C-4′. In the HMBC spectrum, the C-6″ signal at δ 108.6 was correlated with resonance at δ 6.64 (H-8″), 7.82 (H-2′), and 13.22 (OH-5″), and the resonance at δ 122.6 (C-3′) correlated with the H-5′ signal (δ 7.24), indicating that **1** was a biflavonoid with a C-3′−C-6″ interflavonoid linkage corresponding to the robustaflavone series.[15] On comparison of the ^{13}C NMR spectrum (Table 2) with that of robustaflavone,[15] it was observed that C-1′ and C-5′ in **1** showed a downfield shift of Δ1.7 ppm and an upfield shift of Δ4.4 ppm, respectively. Therefore, compound **1** was identified as the new compound, robustaflavone 4′-methyl ether.

Compound **2** was isolated as a yellow powder. The IR spectrum and UV absorption maxima of **2** were very similar those of **1**, which suggested that both compounds possess the same flavonoid skeleton. On acetylation, **2** gave a tetraacetate. HRFABMS of **2** gave a pseudomolecular ion

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Table 1. ^{1}H NMR Data of Compounds **1**−**4**[a]

proton	1	2	3	4
3	6.87 (s)	6.96 (s)	6.94 (s)	6.94 (s)
6	6.20 (d, 2.0)	6.34 (d, 2.2)	6.35 (d, 2.0)	6.36 (br s)
8	6.50 (d, 2.0)	6.82 (d, 2.2)	6.85 (d, 2.0)	6.82 (br s)
2′	7.82 (d, 2.0)	7.87 (d, 2.0)	7.82 (d, 2.0,)	7.82 (d, 2.0)
5′	7.24 (d, 8.5)	7.25 (d, 8.5)	7.22 (d, 8.5)	7.22 (d, 8.5)
6′	8.08 (dd, 2.0, 8.5)	8.11 (dd, 2.0, 8.5)	8.11 (dd, 2.0, 8.5)	8.10 (dd, 2.0, 8.5)
2″			5.53 (dd, 3.0, 13.0)	5.53 (dd, 2.5, 13.0)
3″	6.82 (s)	6.78 (s)	2.73 (dd, 3.0, 17.5)	2.77 (dd, 2.5, 17.5)
			3.27 (dd, 13.0, 17.5)	3.29 (dd, 13.0, 17.5)
8″	6.64 (s)	6.63 (s)	6.07 (s)	6.34 (s)
2‴/6‴	7.96 (d, 8.5)	7.94 (d, 8.0)	7.35 (d, 8.0)	7.38 (d, 8.5)
3‴/5‴	6.94 (d, 8.5)	6.94 (d, 8.0)	6.81 (d, 8.0)	6.84 (d, 8.5)
OH-5	12.95 (br s)	12.93 (br s)	12.93 (br s)	12.94 (br s)
OH-5″	13.22 (br s)	13.22 (br s)	12.37 (br s)	12.18 (br s)
−OH			10.72 (br s)	9.62 (br s)
−OH			9.60 (br s)	
OMe-7		3.84 (s)	3.83 (s)	3.84 (s)
OMe-4″	3.80 (s)	3.81 (s)	3.78 (s)	3.78 (s)
OMe-7″				3.72 (s)

[a] Measured in DMSO-d_6; multiplicity and coupling constant (J in Hz) assigned in parentheses; br s, broad singlet; d, doublet; dd, double doublet; s, singlet.

Table 2. ^{13}C NMR Data of Compounds **1**−**4**[a]

carbon	1	2	3	4
2	163.4	163.7	163.7	163.6
3	103.5	103.7	103.6	103.7
4	181.8	181.8	181.9	181.9
5	161.1	161.1	161.1	161.1
6	98.8	98.2	98.2	98.1
7	164.0	165.2	165.2	165.2
8	94.1	92.7	92.7	92.7
9	157.4	157.4	157.3	157.3
10	103.8	104.8	104.8	104.7
1′	122.4	122.2	122.1	122.2
2′	130.3	130.3	130.4	130.2
3′	122.6	122.7	122.7	122.4
4′	160.6	160.8	160.9	160.8
5′	111.7	111.8	111.7	111.7
6′	127.9	128.0	127.9	128.0
2″	163.7	163.7	78.3	78.6
3″	102.9	102.8	41.9	41.9
4″	181.7	182.0	196.6	197.1
5″	158.9	158.9	160.9	159.6
6″	108.6	108.6	105.4	106.1
7″	161.4	161.2	164.3	165.0
8″	93.4	93.5	94.5	91.5
9″	156.4	156.4	161.9	162.9
10″	103.8	103.4	101.5	102.3
1‴	121.2	121.2	128.9	128.7
2‴	128.5	128.5	128.4	128.4
3‴	115.9	116.0	115.2	115.2
4‴	161.1	161.1	157.8	157.8
5‴	115.9	116.0	115.2	115.2
6‴	128.5	128.5	128.4	128.4
OMe-7		56.1	56.1	56.0
OMe-4′	55.8	55.9	55.8	55.9
OMe-7″				56.3

[a] Measured in DMSO-d_6 and based on ^{13}C DEPT, ^{1}H−^{1}H COSY, HMQC, and HMBC spectra.

at m/z 567.12852 $[M + H]^+$ (calcd 567.12912), corresponding to a molecular formula of $C_{32}H_{22}O_{10}$, indicating that this compound is a methyl derivative of **1**. The ^{1}H and ^{13}C NMR spectra of **2** closely resembled most of **1**, except for the appearance of a methoxyl-group signal at C-7, which was proved by the NOESY spectrum where cross-peaks were observed between H-6 (δ 6.34) and OCH_3-7 (δ 3.81) and between H-8 (δ 6.82) and OCH_3-7. In the ^{13}C NMR spectrum, C-8 showed a downfield shift of Δ1.4 ppm when compared with **1**,[15] which was further proof of the above assignment. Therefore, compound **2** was characterized as robustaflavone 7,4′-dimethyl ether.

The positive FABMS of **3** gave a pseudomolecular ion at m/z 569 $[M + H]^+$ corresponding to a molecular formula of $C_{32}H_{24}O_{10}$, which was confirmed by HRFABMS (found m/z 569.14243 $[M + H]^+$, calcd 569.14477). The IR spectrum showed absorption bands at 3395, 1654, 1604, 1563, 1497, and 1440 cm^{-1}, suggesting the presence of hydroxyl, chelated-carbonyl, and aromatic-ring groups. On acetylation, **3** gave a tetraacetate. The UV spectrum of **3** in methanol exhibited absorption maxima at 335, 293, and 269 nm. The ^{13}C NMR spectrum of **3** showed signals for all 32 carbons of the molecule, including two methoxyl groups (δ 55.8, 56.1) and two carbonyl groups (δ 196.6 and 181.9). The ^{1}H NMR spectrum of **3** exhibited a one-proton singlet at δ 6.94 and three double doublets at δ 5.53 (H-2″), 3.27 (H-3″α), and 2.73 (H-3″β), characteristic of a flavone and flavanone unit,[13] respectively. The ^{1}H NMR spectrum of the flavone portion (Table 1) of **3** was similar to that of **2**. Furthermore, an A_2X_2 coupling system appeared at δ 6.81 (H-3‴, -5‴) and 7.35 (H-2‴, -6‴), and a proton singlet appeared at δ 6.07 (H-8″) in the flavanone portion. The HMBC spectrum of **3** confirmed the involvement of C-3′ (δ 122.7) and C-6″ (δ 105.4) in the interflavonoid linkage as these carbons correlated with H-5′ (δ 7.22) and with H-2′ (δ 7.82), H-8″ (δ 6.07), and OH-5″ (δ 12.37), respectively. The signals at δ 160.9 (C-4′) and 165.2 (C-7) correlated with δ 3.78 and 3.83, respectively, indicating that C-4′ and C-7 were methoxylated. Thus, compound **3** was determined structurally as 2″,3″-dihydrorobustaflavone 7,4′-dimethyl ether.

The HRFABMS of **4** gave a pseudomolecular ion at m/z 583.16044 $[M + H]^+$ (calcd 583.16042) corresponding to a molecular formula of $C_{33}H_{26}O_{10}$, consistent with being a methyl derivative of **3**. The ^{1}H and ^{13}C NMR spectra of **4** closely resembled those of **3** except for the additional signal of a methoxyl group, which was proved by the NOESY spectrum where a cross-peak was observed between H-8″ (δ 6.34) and OCH_3-7″ (δ 3.72). The ^{13}C NMR spectrum of **4** showed a highfield shift of Δ3.0 ppm for C-8″ when compared to that of **3**, which was further proof of the above assignment. Therefore, compound **4** was elucidated structurally as 2″,3″-dihydrorobustaflavone 7,4′,7″-trimethyl ether.

All of the isolated biflavonoids were tested against a panel of human cancer cell lines according to established protocols.[16] With the exception of compounds **1** and **3**, no inhibitory activity on tumor cells was detected in the other

Table 3. The Inhibitory Activity of Compounds **1** and **3** on Various Tumor Cell Growth[a]

		Cell Line					
		Raji	Calu-1	K562	Vero	Wish	HeLa
compound	dose (μM)	inhibitory activity (%)					
1	100	98.4 ± 3.7	100 ± 3.5	10.8 ± 3.5	−6.6 ± 2.9	−90.0 ± 5.2	5.5 ± 3.2
	50	68.5 ± 3.2	77.9 ± 1.5	N.D.[b]	N.D.	N.D.	N.D.
	25	60.7 ± 1.9	44.6 ± 2.0	N.D.	N.D.	N.D.	N.D.
	12.5	34.2 ± 5.7	42.6 ± 2.4	N.D.	N.D.	N.D.	N.D.
3	100	96.3 ± 5.3	100 ± 5.5	18.0 ± 4.6	26.8 ± 2.2	−65.0 ± 3.2	35.0 ± 2.8
	50	24.7 ± 3.5	55.4 ± 3.2	N.D.	N.D.	N.D.	N.D.
	25	22.3 ± 3.4	29.9 ± 2.0	N.D.	N.D.	N.D.	N.D.
	12.5	17.9 ± 2.4	15.8 ± 2.1	N.D.	N.D.	N.D.	N.D.

[a] Each tumor cell line was cultured with or without various concentrations of compound **1** or compound **3** for 3 days. Then tritiated thymidine was pulsed for 16 h before harvest. Radioactivity was determined by a scintillation counter, and inhibitory activity was calculated. Each value represents the mean of three independent experiments. [b] N.D.: not done.

test compounds because these gave IC_{50} values higher than 100 μM. As shown in Table 3, both compounds **1** and **3** significantly inhibited Raji and Calu-1 cell growth in a concentration-dependent manner. By contrast, compounds **1** and **3** had no suppressory activity on K562, HeLa, Vero, and Wish tumor cell lines.

These results show that *S. delicatula* biflavonoids possess mainly the C-3′−C-6″ interflavonoid linkage instead of the C-3′−C-8″ interflavonoid linkage, which is common for most of the biflavonoids in the genus of *Selaginella*. *S. delicatula*, similar to other *Selaginella* species, contained biflavonoid substances that exhibited the cytotoxic activities against cancer cells in cultures.[5,6]

Experimental Section

General Experimental Procedures. Melting points were determined with a Yanagimoto micromelting point apparatus and are uncorrected. IR spectra were obtained as KBr pellets on a Perkin-Elmer 781 IR spectrometer. UV spectra were obtained on a Hitachi U-3200 spectrophotometer in MeOH. ^{1}H, ^{13}C, and 2D NMR spectra were measured with a Varian Inova-500 spectrometer with deuterated solvent as internal standard. FABMS and HRFABMS were recorded in the positive ion mode on a JEOL JMX-SX 102A and a JEOL JMS-HX 110 spectrometer, respectively.

Plant Material. The whole plant of *S. delicatula* was collected at Lienhuachyr, Hualien, Taiwan, in October 1997. A voucher specimen has been deposited in the herbarium of the Department of Botany of the National Taiwan University.

Extraction and Isolation. The whole plant of *S. delicatula* (6.0 kg) was extracted with 50% EtOH (40 L × 3). The solvent was evaporated in vacuo at ca. 50 °C to give 767 g of residue. This crude extract was partitioned successively between H_2O and EtOAc, followed by *n*-BuOH, yielding 200, 130, and 437 g of each residue, respectively. The EtOAc extract was subjected to Si gel column chromatography with a gradient of EtOAc in *n*-hexane, and 13 fractions were collected. Fractions 10 (20 g) and 11 (72 g) were rechromatographed individually over Sephadex LH-20, eluting with acetone. Fractions were collected in 200-mL portions and pooled according to their TLC profiles in MeOH−$CHCl_3$ (silica gel, 5%). Of these, fractions 10-1, 10-2, 11-5, and 11-7 were individually further purified by Sephadex LH-20 column chromatography (acetone) and Si gel MPLC (MeOH−$CHCl_3$, 2−5%) to give **4** (48 mg) from fraction 10-1; **3** (354 mg) from fraction 10-2; amentoflavone (**6**, 39 mg),[15,17] **1** (351 mg), and **2** (10 mg) from fraction 11-5; and robustaflavone (**5**, 40 mg)[15] from fraction 11-7. The *n*-BuOH extract was subjected to chromatography on a Sephadex LH-20 column and eluted with acetone to give fractions I−VI. Subfraction V was rechromatographed repeatedly over Sephadex LH-20, eluting with MeOH to give 3,5-di-*O*-caffeoylquinic acid (14 mg);[18] 3,4-di-*O*-caffeoylquinic acid (12 mg);[18] and 4,5-di-*O*-caffeoylquinic acid (7 mg).[18]

Robustaflavone 4′-methyl ether (1): pale yellow powder (MeOH); mp >300 °C; UV (MeOH) λ_{max} (log ϵ) 340 (4.14), 270 (4.09) nm; (+ NaOMe) 394, 276 nm; (+ $AlCl_3$) 395 (sh), 354, 302, 279 nm; (+$AlCl_3$−HCl) 391 (sh), 348, 301, 279 nm; (+ NaOAc) 343, 271 nm; (+ NaOAc−H_3BO_3) 341, 270 nm; IR (KBr) ν_{max} 3400 (OH), 1653, 1630 (C═O), 1515, 1440, 1367, 1291, 1253, 1167, 1030, 1013 cm^{-1}; ^{1}H and ^{13}C NMR (DMSO-d_6) data, see Tables 1 and 2, respectively; FABMS *m/z* 553 [M + H]$^+$; HRFABMS *m/z* 553.11345 [M + H]$^+$ (calcd 553.11347 for $C_{31}H_{20}O_{10}$).

Acetylation of 1. Compound **1** (10 mg) was dissolved in a mixture of pyridine (2 mL) and Ac_2O (2 mL). The reaction mixture was maintained at room temperature for 24 h, then poured into ice water. The product was extracted with $CHCl_3$ and purified with preparative TLC to give a pentaacetate (**1a**): ^{1}H NMR ($CDCl_3$) δ 2.05, 2.21, 2.34, 2.36, 2.48 (3H each, s, −OAc m~ 5), 3.84, (3H, s, OMe-4′), 6.61 (1H, s, H-3), 6.68 (1H, s, H-3″), 6.85 (1H, br s, H-6), 7.11 (1H, d, J = 8.5 Hz, H-5′), 7.28 (2H, d, J = 8.5 Hz, H-3‴, -5‴), 7.33 (1H, br s, H-8), 7.45 (1H, s, H-8″), 7.77 (1H, s, H-2′), 7.91 (1H, d, J = 8.5 Hz, H-6′), 7.92 (2H, d, J = 8.5 Hz, H-2‴, -6‴); APCIMS *m/z* 763 [M + H]$^+$, 721 (M$^+$ −$COCH_2$ + H), 679 (M$^+$ −$COCH_2$×2 + H), 637 (M$^+$ −$COCH_2$×3 + H), 595 (M$^+$ −$COCH_2$×4 + H), 553 (M$^+$ −$COCH_2$×5 + H).

Robustaflavone 7,4′-dimethyl ether (2): yellow powder (MeOH); mp >300 °C; UV (MeOH) λ_{max} (log ϵ) 338 (3.09), 270 (3.03) nm; (+ NaOMe) 392, 333, 274 nm; (+ $AlCl_3$) 390 (sh), 352, 301, 279 nm; (+ $AlCl_3$−HCl) 390 (sh), 349, 301, 279 nm; (+ NaOAc) 337, 270 nm; (+ NaOAc−H_3BO_3) 339, 270 nm; IR (KBr) ν_{max} 3395 (OH), 1654, 1630 (C═O), 1580, 1490, 1439, 1367, 1252, 1150, 1025, 998, 824, 766 cm^{-1}; ^{1}H and ^{13}C NMR (DMSO-d_6) data, see Tables 1 and 2, respectively; FABMS *m/z* 565 [M − H]$^-$; HRFABMS *m/z* 567.12852 [M + H]$^+$ (calcd 567.12912 for $C_{32}H_{22}O_{10}$).

Acetylation of 2. Compound **2** was acetylated in the same manner as **1** to give the tetraacetate (**2a**): ^{1}H NMR ($CDCl_3$) δ 2.03, 2.18, 2.33, 2.42 (3H each, s, −OAc×4), 3.81 (3H, s, OMe-4′), 3.88, (3H, s, OMe-7), 6.54 (1H, br s, H-3), 6.58 (1H, br s, H-6), 6.65 (1H, s, H-3″), 6.86 (1H, br s, H-8), 7.07 (1H, d, J = 9.0 Hz, H-5′), 7.26 (2H, d, J = 8.5 Hz, H-3‴, -5‴), 7.43 (1H, s, H-8″), 7.76 (1H, d, J = 2.0 Hz, H-2′), 7.86 (1H, dd, J = 8.5, 2.0 Hz, H-6′), 7.89 (2H, d, J = 8.5 Hz, H-2‴, -6‴); APCIMS *m/z* 735 [M + H]$^+$, 693 (M$^+$−$COCH_2$ + H), 651 (M$^+$−$COCH_2$×2 + H), 609 (M$^+$ −$COCH_2$×3 + H).

2″,3″-Dihydrorobustaflavone 7,4′-dimethyl ether (3): yellow granules (MeOH); mp 196−198 °C; $[\alpha]^{25}_D$ −2.9° (*c* 0.68, dioxane); UV (MeOH) λ_{max} (log ϵ) 335 (4.45), 293 (4.43), 269 (4.44), 239 (sh, 4.45) nm; (+ NaOMe) 330, 270, 242 nm; (+ $AlCl_3$) 384, 348, 292, 281 nm; (+ $AlCl_3$−HCl) 384, 346, 293, 282 nm; (+ NaOAc) 335, 292, 269 nm; (+ NaOAc−H_3BO_3) 336, 292, 270 nm; IR (KBr) ν_{max} 3395 (OH), 1654, 1630 (C═O), 1580, 1490, 1439, 1367, 1252, 1150, 1025, 998, 824, 766 cm^{-1}; ^{1}H and ^{13}C NMR (DMSO-d_6) data, see Tables 1 and 2, respectively, FABMS *m/z* 565 [M − H]$^-$; HRFABMS *m/z* 567.12852 [M + H]$^+$ (calcd 567.12912 for $C_{32}H_{22}O_{10}$).

Acetylation of 3. Compound **3** was acetylated in the same manner as **1** to give a tetraacetate (**3a**): ^{1}H NMR ($CDCl_3$) δ 1.97, 2.10, 2.31, 2.42 (3H each, s, −OAc×4), 2.77−3.07 (2H, m, H-3″), 3.81, 3.88 (3H each, s, −OMe×2), 5.53 (1H, m, H-2″), 6.52 (1H, H-3), 6.58 (1H, d, J = 2.5 Hz, H-6), 6.85 (1H, d, J = 2.5 Hz, H-8), 6.85 (1H, s, H-8″), 7.04 (1H, d, J = 8.5 Hz, H-5′),

7.16 (2H, d, J = 8.0 Hz, H-3‴, -5‴), 7.47 (2H, d, J = 8.0 Hz, H-2‴, -6‴), 7.71 (1H, d, J = 2.5 Hz, H-2′), 7.83 (1H, dd, J = 8.5, 2.5 Hz, H-6′); APCIMS m/z 737 $[M + H]^+$, 695 ($M^+ - COCH_2 + H$), 653 ($M^+ - COCH_2 \times 2 + H$), 611 ($M^+ - COCH_2 \times 3 + H$).

2″,3″-Dihydrorobustaflavone 7,4′,7″-trimethyl ether (4): light yellow powder (MeOH); mp 243−244 °C; $[\alpha]^{25}_D$ −2.3° (c 0.44, dioxane); UV (MeOH) λ_{max} (log ϵ) 337 (4.39), 289 (4.46), 270 (4.46) nm; (+ NaOMe) 404, 322, 289, 269 nm; (+ $AlCl_3$) 383, 348, 297 (sh), 281 nm; (+ $AlCl_3$−HCl) 381, 345, 297 (sh), 282 nm; (+ NaOAc) 336, 288, 270 nm; (+ NaOAc−H_3BO_3) 337, 289, 270 nm; IR (KBr) ν_{max} 3400 (OH), 1653, 1607, 1490, 1439, 1367, 1252, 1150, 1025, 998, 824, 766 cm^{-1}; ^1H and ^{13}C NMR (DMSO-d_6) data, see Tables 1 and 2, respectively, FABMS m/z 581 $[M - H]^-$; HRFABMS m/z 583.16374 $[M + H]^+$ (calcd 583.16042 for $C_{33}H_{27}O_{10}$).

Acetylation of 4. Compound **4** was acetylated in the same manner as **1** to give a triacetate (**4a**): ^1H NMR ($CDCl_3$) δ 2.08, 2.32, 2.41 (3H each, s, −OAc×3), 2.75−3.02 (2H, m, H-3″), 3.77, 3.82, 3.87 (3H each, s, −OMe×3), 5.52 (1H, m, H-2″), 6.52 (2H, H-3, -8″), 6.58 (1H, d, J = 2.5 Hz, H-6), 6.85 (1H, d, J = 2.5 Hz, H-8), 6.97 (1H, d, J = 8.5 Hz, H-5′), 7.03 (2H, d, J = 8.0 Hz, H-3‴, -5‴), 7.47 (2H, d, J = 8.0 Hz, H-2‴, -6‴), 7.67 (1H, d, J = 2.5 Hz, H-2′), 7.82 (1H, dd, J = 8.5, 2.5 Hz, H-6′); APCIMS m/z 709 $[M + H]^+$, 667 ($M^+ - COCH_2 + H$), 625 ($M^+ - COCH_2 \times 2 + H$), 583 ($M^+ - COCH_2 \times 2 + H$).

Cell Lines. The K562, Raji, Vero, Calu-1, HeLa, and Wish cell lines were utilized as target cells in the cytotoxic assay. K562 and Raji cells are erythroleukemia and EBV-transformed B cell lines, respectively [American Type Culture Collection (ATCC) Rockville, MD]. They were cultured in RPMI-1640 medium (Hyclone, Logan, UT) containing 10% fetal calf serum (FCS, Gibco, Grand Island, NY), 100 U/mL penicillin, and 100 μg/mL streptomycin. The Vero cell is a green monkey kidney tumor cell line (ATCC, Rockville, MD). The Wish cell is a transformed epithelial cell line, and the Calu-1 cell is a human lung carcinoma cell line (ATCC, Rockville, MD). The HeLa cell is a human cervical carcinoma cell line (ATCC, Rockville, MD). The Vero, Wish, Calu-1, and HeLa cell lines were cultured in MEM containing 10% FCS, 100 μg/mL streptomycin, and 100 U/mL penicillin. These cell lines were cultured at 37 °C in an atmosphere of humidified 5% CO_2.

The Growth Inhibition Assays. The method followed was described previously.[16]

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