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Cytotoxicity of phenylpropanoid esters from the stems of *Hibiscus taiwanensis*

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Abstract—The separation of an extract prepared from the stems of the previously uninvestigated *Hibiscus taiwanensis* led to the isolation of three new phenylpropanoid esters, (7*S*,8*S*)-demethylcarolignan E (**1**), hibiscuwanin A (**2**), hibiscuwanin B (**3**), in addition to eight known ones. The structures of these compounds were elucidated by spectroscopic and chemical transformation studies. In cytotoxicity evaluation of the isolates, 9,9′-*O*-feruloyl-(−)-secoisolaricinresinol (**8**) showed strong cytotoxic activity against human lung carcinoma and breast carcinoma cell lines in an in vitro cytotoxicity assay with EC_{50} values of 1.8 and 3.9 μg/mL, respectively.
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1. Introduction

Hibiscus taiwanensis Hu, indigenous to Taiwan, is a moderate shrub and widely distributed throughout Taiwan.[1] This species belongs to the Malvaceae family comprised of 75 genera of herbaceous plants and shrubs distributed throughout tropical and temperate region. Species from the genus *Hibiscus* have been used in several applications, such as antidote to poisoning with chemicals and venomous mushrooms in traditional medicine and as a source of fibre to pulp and paper industries. A literature review indicated that this genus is a source of lignanamides, naphthalenes, polyphenol compounds, carotenoids, anthocyanins, sterols and long chain fatty esters. To our knowledge, there are no previous reports of chemical and biological studies on *H. taiwanensis*. In the course of our programme to study cytotoxic constituents from plant resources, *H. taiwanensis* was chosen for detailed chemical investigation due to the promising cytotoxic activity of the crude methanolic extract against gastric and nasopharyngeal carcinoma cell lines revealed in the preliminary screen.[2] The water soluble portion of the methanolic extract from the stems of *H. taiwanensis* was defatted with hexane and then partitioned with $CHCl_3$ and EtOAc, successively. Three new phenylpropanoid esters, (7*S*,8*S*)-demethylcarolignan E (**1**), hibiscuwanin A (**2**), hibiscuwanin B (**3**) and eight known phenylpropanoids, *threo*-carolignan E (**4**),[3] *erythro*-carolignan E (**5**),[3] cleomiscosin A (**6**),[4] cleomiscosin C (**7**),[4] 9,9′,-*O*-feruloyl-(−)-secoisolariciresinol (**8**),[5] dihydrodehydrodiconifenyl alcohol (**9**),[6] boehmenan (**10**)[3] and (−)-syringaresinol (**11**),[7] have been isolated after series of chromatographic separations of $CHCl_3$ and EtOAc solubles individually. Herein, we describe the isolation, structural elucidation and cytotoxic properties of these phenylpropanoids.

2. Results and discussion

(7*S*,8*S*)-Demethylcarolignan E (**1**) was isolated as colourless amorphous powder. The HRFABMS at *m/z* 716.2464 $[M]^+$ suggested the molecular formula $C_{39}H_{40}O_{13}$. The ^1H NMR signals at δ 3.83 (3H, s, OCH_3–3), 3.99 (1H, dd, $J = 12.1$, 4.2 Hz, H-9a), 4.16 (1H, m, H-8), 4.44 (1H, dd, $J = 12.1$, 3.0 Hz, H-9b), 5.01 (1H, d, $J = 8.1$ Hz, H-7), and 6.88–6.93 containing H-2, -5, and -6 indicated the existence of guaicylglycerol, 4-hydroxy-3-methoxyphenyl-1,2,3-propanetriol moiety. A 3′,4′-dihydroxyphenylpropanol moiety was revealed by the signals at δ 1.97 (2H, tt, $J = 7.3$, 6.3 Hz, H-8′), 2.64 (2H, t, $J = 7.3$ Hz, H-7′), 4.18 (2H, t, $J = 6.3$ Hz, H-9′), 6.61 (1H, dd, $J = 7.9$, 1.6 Hz, H-6′), 6.81 (1H, d, $J = 1.6$ Hz, H-2′), 7.00 (1H, d, $J = 7.9$ Hz, H-5′). These two units were linked together by an ether linkage

Keywords: Malvaceae; *Hibiscus taiwanensis*; Lignan; 9,9′-*O*-feruloyl-(−)-secoisolaricinresinol.
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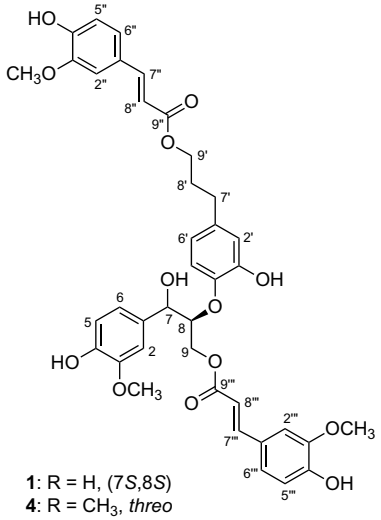
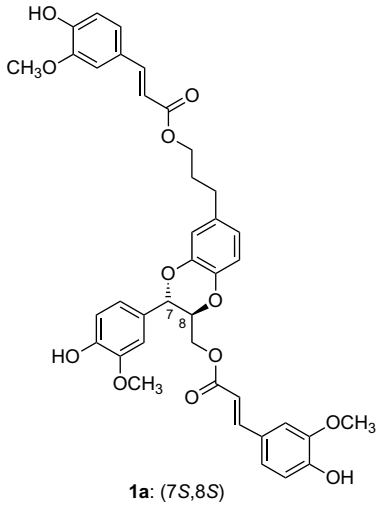
between C-8 and C-4′ as H-8 (δ 4.16) showed HMBC correlation with C-4′ (δ 144.0). Two AB sets of signals at δ 6.27 (1H, d, H-8‴), 7.57 (1H, d, H-7‴) and 6.29 (1H, d, H-8″), 7.60 (1H, d, H-7″) with coupling constant of 15.9 Hz for two *trans* vinylic systems together with overlapped signals in the range of 6.88–6.93 and 7.00–7.08 for H-2″, -2‴, -5″, -5‴, -6″, and 6‴, and a six-proton singlet at δ 3.92 (OCH_3-3″, -3‴) indicated that compound **1** has two feruloyl moieties. The HMBC correlations between H-9 (δ 3.99 and 4.44) and C-9‴ (δ 166.9) and between H-9″ (δ 4.18) and C-9″ (δ 167.4) confirmed the connections of these feruloyl moieties at C-9 and C-9′. The *threo* configuration at C-7 and C-8 was inferred by the large coupling constant of 8.1 Hz between H-7 and H-8.[3] These data were very similar to those of *threo*-carolignan E (**4**) and *erythro*-carolignan E (**5**), also isolated in this study, except the presence of hydroxyl group instead of methoxyl at C-3′. Consequently, compound **1** was identified as *threo*-demethylcarolignan E.

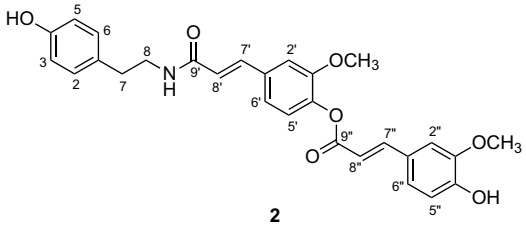
In order to confirm this structure further, a cyclization reaction was performed in acetic acid in the presence of catalytic amount of H_2SO_4 to form benzodioxane derivative **1a** having molecular formula $C_{39}H_{38}O_{12}$. A water molecule was lost during the cyclization process. The HMBC correlations between H-7 (δ 4.93) and C-3′



1: R = H, (7*S*,8*S*)
4: R = CH_3, *threo*
5: R = CH_3, *erythro*



1a: (7*S*,8*S*)



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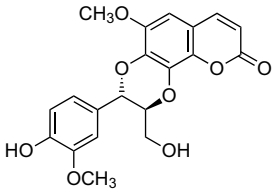

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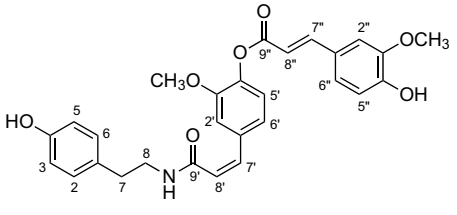

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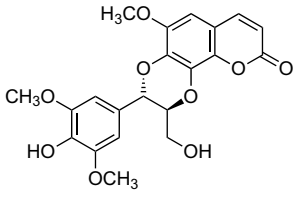

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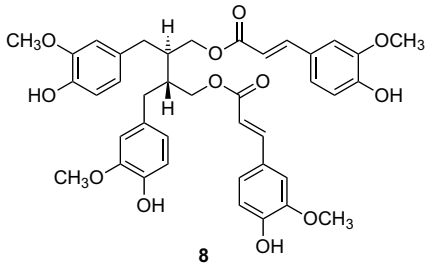

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Table 1. Comparison of the chemical shifts of C-7 and -8 for compounds **1**, **1a**, **4**, **5** in $CDCl_3$ and **6**, **7** in pyridine-d_5

Compound	Chemical shift (δ)	
	C-7	C-8
1	74.6	85.3
4	74.4	86.3
5	72.1	84.5
1a	76.6	75.7
6	77.6	79.9
7	77.9	79.9

(δ 143.4), H-8 (δ 4.25) and C-4′ (δ 141.2) ascertained the formation of benzodioxane moiety. The large coupling constant of 8.0 Hz between H-7 and H-8 showed that we isolated a *threo* benzodioxane **1a**.[3] The positive Cotton effect at 234 nm for **1a** indicated that it possessed the absolute configuration 8*S* according to the study of related benzodioxane system.[8] Therefore, *threo*-**1a** have the (7*S*,8*S*) configuration and *threo*-**1** would be (7*S*,8*S*)-demethylcarolignan E. Examining the ^{13}C spectral data of a series of 8-*O*-phenylpropanetriols **1**, **4** and **5** as well as benzodioxanes **1a**, **6** and **7**, it is worthy to mention that a phenyloxy group on C-8 led the chemical shifts of C-7 and C-8 around at δ 75 and 85, respectively. Once it cyclized to benzodioxane ring, C-8 was upfield-shifted and the chemical shifts were not higher than 80 (Table 1).

Hibiscuwanin A (**2**) had the molecular formula $C_{28}H_{27}NO_7$ as determined from HREIMS at m/z 489.1786. The presence of tyramine moiety in compound **2** was deduced from the ^1H NMR signals in CD_3OD at δ 2.74 (2H, t, $J = 7.3$ Hz, H-7), 3.45 (2H, t, $J = 7.3$ Hz, H-8), 6.71 (2H, d, $J = 8.3$ Hz, H-3 and 5) and 7.04 (2H, d, $J = 8.3$ Hz, H-2 and -6) and the HMBC correlation of H-7 (δ 2.74) with C-2, -6 (δ 130.7). Two AB sets of signals at δ 6.39 (d, $J = 15.6$ Hz, H-8′), 7.42 (d, $J = 15.6$ Hz, H-7′) as well as 6.32 (d, $J = 15.9$ Hz, H-8″), 7.46 (d, $J = 15.9$ Hz, H-7″) for two vinylic systems with *trans* configuration and proton signals at δ 3.88 (6H, s, OCH_3-3′ and -3‴), 6.78 (2H, d, $J = 8.1$ Hz, H-5′ and -5″), 7.02 (1H, dd, $J = 8.1$, 1.2 Hz, H-6′), 7.10 (1H, d, $J = 1.2$ Hz, H-2′) as well as 7.02 (1H, dd, $J = 8.1$, 1.2 Hz, H-6″), 7.14 (1H, d, $J = 1.2$ Hz, H-2″) were consistent with the presence of two *trans* feruloyl groups in the molecule. The HMBC correlation of C-9′ (δ 169.2) with H-8 (δ 3.45), H-7′ (δ 7.42) and H-8′ (δ 6.39) indicated an amide linkage between a feruloyl and tyramine moieties. The second feruloyl group connected to either feruloyl part or tyramine part could be determined by NOE experiment. An NOE cross peak between the signals at δ 6.78 and 6.32 assignable to H-5′ and H-8″, respectively, indicated that the second feruloyl group formed an ester linkage with the OH-4′ of the former feruloyl group. The structure of hibiscuwanin A (**2**), thus, concluded to be *N*-(4′-*O*-*trans*-feruloyl)-*trans*-feruloyltyramine. In deed, the hydrolysate of **2** in acidic condition yielded a compound whose ^1H NMR spectrum was identical to *N*-*trans*-feruloyltyramine.[9]

The molecular formula $C_{28}H_{27}NO_7$ assigned by HREIMS for compound **3** indicated it to be an isomer of **2**.

Table 2. Cytotoxicity of the compounds **1**–**3** and **5**–**10** from the stems of *H. taiwanensis* towards two human cancer lines A549 and MCF-7[a]

Compound	EC_{50} (μg/mL)[b]	
	A549	MCF-7
1	9.7	8.3
2	>20 (14)	>20 (36)
3	>20 (28)	>20 (40)
5	10.6	8.5
6	>20 (22)	>20 (35)
7	>20 (33)	>20 (38)
8	**1.8**	**3.9**
9	>20 (16)	>20 (48)
10	18.4	10.9

[a] A549=human lung carcinoma; MCF-7=human breast carcinoma.
[b] If inhibition >50% at 2.5 μg/mL, percent observed is the value in bracket.

Accordingly, the ^1H NMR of **3** contained signals for a tyramine unit at δ 2.68 (2H, t, $J = 7.3$ Hz, H-7), 3.38 (2H, t, $J = 7.3$ Hz, H-8), 6.68 (2H, d, $J = 8.4$ Hz, H-3 and -5) and 6.98 (2H, d, $J = 8.4$ Hz, H-2 and -6); a *cis*-feruloyl group at 3.82 (3H, s, OCH_3-3′), 5.80 (1H, d, $J = 12.6$ Hz, H-8′), 6.60 (1H, d, $J = 12.6$ Hz, H-7′), 6.73 (1H, d, $J = 8.2$ Hz, H-5′), 6.92 (1H, dd, $J = 8.2$, 1.2 Hz, H-6′) and 7.34 (1H, d, $J = 1.2$ Hz, H-2′); and a *trans*-feruloyl group at δ 3.88 (3H, s, OCH_3-3″), 6.31 (1H, d, $J = 15.9$ Hz, H-8″), 6.79 (1H, d, $J = 8.0$ Hz, H-5″), 7.03 (1H, dd, $J = 8.4$, 1.4 Hz, H-6″), 7.15 (1H, d, $J = 1.4$ Hz, H-2″) and 7.52 (1H, d, $J = 15.9$ Hz, H-7″). The HMBC correlation between H-8 (δ 3.38) and C-9′ (δ 170.3) inferred that the *cis*-feruloyl group was connected to the nitrogen atom of tyramine through an amide bond. The NOE of OCH_3-3′ (δ 3.82) with H-7″ (δ 7.52) and H-5′ (δ 6.73) with H-8″ (δ 6.31) confirmed the attachment of *trans*-feruloyl group by an ester bond with OH-4 of *cis*-feruloyl unit. Hence, the structure of hibiscuwanin B (**3**) was deduced to be *N*-(4′-*O*-*trans*-feruloyl)-*cis*-feruloyltyramine. Due to the ease of *cis* and *trans* isomerization in acidic condition, it had possibility of **2** or **3** as an artefact of **3** or **2**.

The compounds **1**–**3**, **5**–**10** were subjected to cytotoxicity and anti-HIV evaluation. Among them, 9,9′,-*O*-feruloyl-(−)-secoisolaricinresinol (**8**) showed strong cytotoxic activity against human lung carcinoma (A549) and breast carcinoma (MCF-7) cell lines with EC_{50} 1.8 and 3.9 μg/mL, respectively (Table 2) and no tested compound inhibited HIV replication in H9 lymphocyte cells.

3. Experimental section

3.1. General

Optical rotations were measured on a Jasco DIP-370 digital polarimeter. UV spectra were recorded on an Agilent 8453 spectrophotometer. IR spectra were recorded on a Nicolet Magna FT-IR spectrophotometer. NMR spectra were recorded on Bruker Avance 300 FT-NMR spectrometer; all chemical shifts were given in

ppm from tetramethylsilane as an internal standard. Mass spectra were obtained on VG 70-250S spectrometer by a direct inlet system. CD spectra were determined on a Jasco J-715 spectropolarimeter.

3.2. Plant material

The stems of *H. taiwanensis* were collected from Tainan Hsien, Taiwan, Republic of China, in February 2001. It was authenticated by Prof. C. S. Kuoh, Department of Biology, National Cheng Kung University, Tainan, Taiwan. A voucher specimen (No PLW-010001) was deposited in the Herbarium of National Cheng Kung University, Tainan, Taiwan.

3.3. Extraction and isolation

The air-dried stems of *H. taiwanensis* (20 kg) were powdered and extracted under reflux with MeOH for six times. The combined extracts were concentrated under reduced pressure to give dark brown syrup. The syrup was then suspended in H_2O and then partitioned with hexane, $CHCl_3$ and EtOAc, successively. The concentrated $CHCl_3$ layer was repeatedly chromatographed on a silica gel column by eluting with a gradient of hexane and Me_2CO (3:1 to 100% Me_2CO) to give (−)-syringaresinol (**11**) (36 mg), 9,9′,-*O*-feruloyl-(−)-secoisolaricinresinol (**8**) (113 mg), boehmenan (**10**) (15 mg), *erythro*-carolignan E (**5**) (12 mg), (7*S*,8*S*)-demethylcarolignan E (**1**) (528 mg), cleomiscosin A (**6**) (10 mg), cleomiscosin C (**7**) (38 mg) and dihydrodehydrodiconifenyl alcohol (**9**) (8 mg), successively. The concentrated EtOAc layer was subjected to column chromatography on silica gel and eluted with a gradient of $CHCl_3$ and MeOH (9:1 to 100% MeOH) to give *threo*-carolignan E (**4**) (17 mg), hibiscuwanin A (**2**) (5 mg) and hibiscuwanin B (**3**) (8 mg).

3.3.1. (7*S*,8*S*)-Demethylcarolignan E (1). Colourless amorphous powder; $[\alpha]_D$ −4.5° (*c* 0.12, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 204 (4.75), 232 (4.35), 289 (4.30), 326 (4.41) nm; IR (KBr) ν_{max} 3450, 1695, 1646, 1540 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.97 (2H, tt, *J* = 7.3, 6.3 Hz, H-8′), 2.64 (2H, t, *J* = 7.3 Hz, H-7′), 3.83 (3H, s, OCH_3-3), 3.92 (6H, s, OCH_3-3″ and -3‴), 3.99 (1H, dd, *J* = 12.1, 4.2 Hz, H-9a), 4.16 (1H, m, H-8), 4.18 (2H, t, *J* = 6.3 Hz, H-9′), 4.44 (1H, dd, *J* = 12.1, 3.0 Hz, H-9b), 5.01 (1H, d, *J* = 8.1 Hz, H-7), 6.27 (1H, d, *J* = 15.9 Hz, H-8‴), 6.29 (1H, d, *J* = 15.9 Hz, H-8″), 6.61 (1H, dd, *J* = 7.9, 1.6 Hz, H-6′), 6.81 (1H, d, *J* = 1.6 Hz, H-2′), 6.88–6.93 (5H, m, H-2, -5, -5″, -5‴, -6), 7.00 (1H, d, *J* = 7.9 Hz, H-5′), 7.00–7.08 (4H, m, H-2″, -2‴, -6″, -6‴), 7.57 (1H, d, *J* = 15.9 Hz, H-7‴), 7.60 (1H, d, *J* = 15.9 Hz, H-7″); ^{13}C NMR ($CDCl_3$) δ 30.2 (C-8′), 31.6 (C-7′), 56.0 (3×OCH_3), 62.8 (C-9), 63.7 (C-9′), 74.6 (C-7), 85.3 (C-8), 108.9 (C-2), 109.4 (C-2‴), 109.5 (C-2″), 114.4 (C-5‴), 114.6 (C-5″), 114.7 (C-5 and -8‴), 115.4 (C-8″), 115.9 (C-2′), 119.8 (C-6′), 120.1 (C-6), 120.3 (C-5′), 123.1 (C-6‴)123.2 (C-6″), 126.7 (C-1‴), 126.9 (C-1″), 131.0 (C-1), 138.1 (C-1′), 144.0 (C-4′), 144.9 (C-7″), 145.9 (C-7‴), 146.1 (C-3′), 146.8 (C-3″ and -3‴), 146.9 (C-3), 147.9 (C-4), 148.2 (C-4‴), 148.6 (C-4″), 166.9 (C-9‴), 167.4 (C-9″); FABMS *m*/*z* (rel int.) 716 (M^+, 2), 699 ($[M-H_2O+H]^+$, 4), 177 (100); HRFABMS *m*/*z* 716.2464 $[M]^+$ (calcd for $C_{39}H_{40}O_{13}$, 716.2469), 699.2437 $[M-H_2O+H]^+$ (calcd for $C_{39}H_{39}O_{12}$, 699.2436).

3.3.2. Cyclization of (7*S*,8*S*)-demethylcarolignan E (1) in HOAc.[10] (7*S*,8*S*)-Demethylcarolignan E (**1**) (100 mg, 0.14 mmol) and 5% H_2SO_4 (1.5 mL) were added to 10 mL of HOAc. The resulting mixture was heated at 60 °C with stirring for 20 min. After cooling, water was added to the solution, neutralized with $NaHCO_3$ and extracted with EtOAc. The EtOAc extract was washed with water, dried over anhydrous $MgSO_4$ and evaporated under high vacuum. The crude product was purified by column chromatography on silica gel using $CHCl_3$–MeOH (30:1) eluent to yield pure (7*S*,8*S*)-benzodioxane **1a** as colourless amorphous powder (82 mg, 84% yield): $[\alpha]_D$ +14.4° (*c* 0.41, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 232 (4.60), 289 (4.48), 326 (4.62) nm; IR (KBr) ν_{max} 3419, 1701, 1633, 1593, 1514 cm^{-1}; 1H NMR ($CDCl_3$) δ 1.98 (2H, tt, *J* = 7.5, 6.3 Hz, H-8′), 2.67 (2H, t, *J* = 7.5 Hz, H-7′), 3.86 (3H, s, OCH_3-3), 3.91 (6H, s, OCH_3-3″ and -3‴), 4.12 (1H, dd, *J* = 12.3, 4.2 Hz, H-9a), 4.21 (2H, t, *J* = 6.3 Hz, H-9′), 4.25 (1H, m, H-8), 4.35 (1H, dd, *J* = 12.3, 2.6 Hz, H-9b), 4.93 (1H, d, *J* = 8.0 Hz, H-7), 5.84 (1H, br s, OH-4), 6.05 (2H, br s, OH-4″ and -4‴), 6.29 (2H, d, *J* = 15.9 Hz, H-8″ and -8‴), 6.75 (1H, dd, *J* = 8.1, 1.5 Hz, H-6′), 6.84 (1H, d, *J* = 1.5 Hz, H-2′), 6.87 (1H, s, H-2), 6.92 (5H, m, H-5, -5′, -5″, -5‴, -6), 7.02 (2H, s, H-2″ and -2‴), 7.07 (2H, d, *J* = 8.3 Hz, H-6″ and -6‴), 7.57 and 7.59 (1H each, d, *J* = 15.9 Hz, H-7″ and -7‴); ^{13}C NMR ($CDCl_3$) δ 30.2 (C-8′), 31.4 (C-7′), 55.9 (3×OCH_3), 63.0 (C-9), 63.6 (C-9′), 75.7 (C-8), 76.6 (C-7), 109.4 (C-2, -2″ and -2‴), 114.4 and 115.4 (C-8″ and -8‴), 114.7 (C-5, -5″ and -5‴), 116.9 (C-2′ and -5′), 120.7 (C-6), 121.6 (C-6′), 123.0 and 123.2 (C-6″ and -6‴), 126.6 and 126.9 (C-1″ and -1‴), 127.7 (C-1), 134.7 (C-1′), 141.2 (C-4′), 143.4 (C-3′), 144.8 and 145.6 (C-7″ and -7‴), 146.7 (C-4), 146.8 (C-3″ and -3‴), 146.9 (C-3), 147.9 and 148.2 (C-4″ and -4‴), 166.7 (C-9‴), 167.3 (C-9″); FABMS *m*/*z* (rel int.) 698 ($[M]^+$, 11), 177 (100); HRFABMS *m*/*z* 698.2362 $[M]^+$ (calcd for $C_{39}H_{38}O_{12}$, 698.2363). CD (CH_3OH, 1.7×10^{-4} M) $[\theta]_{234}$ +357, $[\theta]_{267}$ +936, $[\theta]_{281}$ −999, $[\theta]_{284}$ +1664, $[\theta]_{288}$ −1066.

3.3.3. Hibiscuwanin A (2). Pale yellow amorphous powder; UV (CH_3OH) λ_{max} (log ε) 290 (4.23), 318 (4.30) nm; IR (KBr) ν_{max} 3305, 1698, 1634, 1575, 1523 cm^{-1}; ^1H NMR (CD_3OD) δ 2.74 (2H, t, *J* = 7.3 Hz, H-7), 3.45 (2H, t, *J* = 7.3 Hz, H-8), 3.88 (6H, s, OCH_3-3′ and -3″), 6.32 (1H, d, *J* = 15.9 Hz, H-8″), 6.39 (1H, d, *J* = 15.6 Hz, H-8′), 6.71 (2H, d, *J* = 8.3 Hz, H-3 and 5), 6.78 (2H, d, *J* = 8.1 Hz, H-5′ and 5″), 7.02 (2H, dd, *J* = 8.1, 1.2 Hz, H-6′ and -6″), 7.04 (2H, d, *J* = 8.3 Hz, H-2 and -6), 7.10 (1H, d, *J* = 1.2 Hz, H-2′), 7.14 (1H, d, *J* = 1.2 Hz, H-2″), 7.42 (1H, d, *J* = 15.6 Hz, H-7′), 7.46 (1H, d, *J* = 15.9 Hz, H-7″); ^{13}C NMR (CD_3OD) δ 35.8 (C-7), 42.5 (C-8), 56.4 (OCH_3-3′ and -3″), 111.5 (C-2′ and -2″), 116.3 (C-3 and -5),

116.5 (C-5′ and -5″), 118.7 (C-8′), 119.3 (C-8″), 123.2 (C-6′), 123.4 (C-6″), 128.3 (C-1′), 128.5 (C-1″), 130.7 (C-2 and 6), 131.3 (C-1), 142.0 (C-7′), 144.4 (C-7″), 149.3 (C-3′ and -3″), 149.8 (C-4′ and 4″), 156.9 (C-4), 169.2 (C-9′), 173.3 (C-9″); EIMS m/z (rel int.) 489 (M^+, 5), 369 (9), 192 (96), 177 (85), 145 (34), 120 (40), 107 (100), 77 (75), 51 (40); HR-EIMS m/z 489.1786 $[M]^+$ (calcd for $C_{28}H_{27}NO_7$, 489.1788).

3.3.4. Hibiscuwanin B (3). Pale yellow amorphous powder; UV (CH_3OH) λ_{max} ($\log \varepsilon$) 288 (4.19), 315 (4.22) nm; IR (KBr) ν_{max} 3345, 1694, 1633, 1593, 1515 cm^{-1}; ^1H NMR (CD_3OD) δ 2.68 (2H, $t = 7.3$ Hz, H-7), 3.38 (2H, t, $J = 7.3$ Hz, H-8), 3.82 (3H, s, OCH_3-3′), 3.88 (3H, s, OCH_3-3″), 5.80 (1H, d, $J = 12.6$ Hz, H-8′), 6.31 (1H, d, $J = 15.9$ Hz, H-8″), 6.60 (1H, d, $J = 12.6$ Hz, H-7′), 6.68 (2H, d, $J = 8.4$ Hz, H-3 and -5), 6.73 (1H, d, $J = 8.2$ Hz, H-5′), 6.79 (1H, d, $J = 8.0$ Hz, H-5″), 6.92 (1H, dd, $J = 8.2$, 1.2 Hz, H-6′), 6.98 (2H, d, $J = 8.4$ Hz, H-2 and -6), 7.03 (1H, dd, $J = 8.0$, 1.4 Hz, H-6″), 7.15 (1H, d, $J = 1.4$ Hz, H-2″), 7.34 (1H, d, $J = 1.2$ Hz, H-2′), 7.52 (1H, d, $J = 15.9$ Hz, H-7″); ^{13}C NMR (CD_3OD) δ 35.6 (C-7), 42.4 (C-8), 56.4 (OCH_3-3′ and -3″), 111.6 (C-2″), 113.9 (C-2′), 115.8 (C-5″), 116.2 (C-3 and -5), 116.4 (C-5′), 117.7 (C-8″), 121.6 (C-8′), 123.7 (C-6″), 124.8 (C-6′), 128.2 (C-1″), 128.5 (C-1′), 130.7 (C-2 and -6), 131.2 (C-1), 138.4 (C-7′), 145.6 (C-7″), 148.5 (C-4′), 149.3 (C-3′ and -4″), 150.1 (C-3″), 156.9 (C-4), 170.3 (C-9′), 172.2 (C-9″); EIMS m/z (rel int.) 489 (M^+, 12), 369 (36), 352 (25), 192 (32), 177 (53), 120 (41), 107 (100), 77 (69), 65 (23), 51 (29); HR-EIMS m/z 489.1790 $[M]^+$ (calcd for $C_{28}H_{27}NO_7$, 489.1788).

3.3.5. Cytotoxicity and anti-HIV assay. The cytotoxicity and anti-HIV assays were carried out according to the procedure described in the literature.[11,12]

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