Vanillic Acid Glycoside and Quinic Acid Derivatives from Gardeniae Fructus

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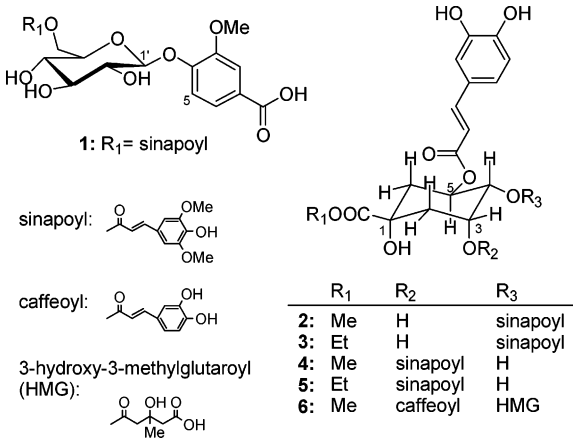
Bioassay-directed chromatographic fractionation of an ethyl acetate extract of *Gardenia jasminoides* (Gardeniae Fructus) afforded a new vanillic acid 4-*O*-*β*-D-(6′-sinapoyl)glucopyranoside (**1**) and five new quinic acid derivatives, methyl 5-*O*-caffeoyl-3-*O*-sinapoylquinate (**2**), ethyl 5-*O*-caffeoyl-3-*O*-sinapoylquinate (**3**), methyl 5-*O*-caffeoyl-4-*O*-sinapoylquinate (**4**), ethyl 5-*O*-caffeoyl-4-*O*-sinapoylquinate (**5**), and methyl 3,5-di-*O*-caffeoyl-4-*O*-(3-hydroxy-3-methyl)-glutaroylquinate (**6**), together with three known quinic acid derivatives, two flavonoids, two iridoids, and two phenolic compounds. The structures of new compounds were elucidated by the aid of spectroscopic methods. These compounds were assessed for antioxidant activity using three different cell-free bioassay systems and for HIV-1 integrase inhibitory activity. Five new quinic acid derivatives showed potent DPPH radical scavenging, superoxide anion scavenging, and lipid peroxidation inhibition activities. These new quinic acid derivatives also exhibited HIV-1 integrase inhibitory activity.

Most free radical reactions involve the reduction of molecular oxygen, leading to the formation of reactive oxygen species including superoxide anion and hydroxyl radicals. The reactive oxygen species can cause oxidative damage to cell components and may, therefore, play an important role in various pathological conditions. They attack biological molecules such as lipids, proteins, enzymes, DNA, and RNA, leading to cell or tissue injury associated with degenerative diseases.[1] Excessive free radical production and lipid peroxidation are also known to cause pathological conditions including atherosclerosis, aging, nephrites, diabetes mellitus, rheumatic disease, cardiac and cerebral ischemia, cancer, and adult respiratory distress syndrome.[2] Therefore, antioxidants could have considerable relevance as prophylactic and therapeutic agents for diseases in which oxidants or free radicals are implicated.[3]

HIV (human immunodeficiency virus) requires three key enzymes—reverse transcriptase, protease, and integrase—for viral replication inside a host cell. Inhibitors of the first two enzymes are targets, which currently provide the basis for most AIDS therapies. To augment these approaches for AIDS therapies, inhibitors directed at new enzyme targets are needed. HIV integrase catalyzes the integration of the HIV DNA copy into the host cell DNA, and this step is essential for the production of progeny viruses. Therefore, a therapeutic agent that can interrupt this process should be an effective anti-HIV agent.[4] Despite extensive efforts directed at developing potent integrase inhibitors, no clinically useful inhibitors are available yet.

In our continued search for biologically active compounds from traditional medicine, we have found that the ethyl acetate fraction of Gardeniae Fructus showed potent antioxidant activities in our assay systems. Gardeniae Fructus has been reported to exhibit sedative, antipyretic, diuretic,[5] cholagogic, and antiinflammatory activities and inhibitory effects on 5-lipoxygenase.[6,7] Gardeniae Fructus is a crude drug made of ripe fruits of *Gardenia jasminoides* Ellis (Rubiaceae) and has also been used as a yellow dye. Gardeniae Fructus is known to contain crocin and crocetin as yellow pigments,[8] iridoids such as geniposide, gardenoside,[9] geniposidic acid, shanzhiside,[10] gardoside (8,10-dehydrologanic acid), scandoside methyl ester,[11] and genipin-1-*β*-gentiobioside,[12] and quinic acid derivatives such as chlorogenic acid, 3,4-di-*O*-caffeoylquinic acid, 3-*O*-caffeoyl-4-*O*-sinapoylquinic acid, and 3,5-di-*O*-caffeoyl-4-*O*-(3-hydroxy-3-methyl)glutaroylquinic acid.[7]

In this paper, we describe the isolation of vanillic acid 4-*O*-*β*-D-(6′-sinapoyl)glucopyranoside (**1**) and five new quinic acid derivatives, methyl 5-*O*-caffeoyl-3-*O*-sinapoylquinate (**2**), ethyl 5-*O*-caffeoyl-3-*O*-sinapoylquinate (**3**), methyl 5-*O*-caffeoyl-4-*O*-sinapoylquinate (**4**), ethyl 5-*O*-caffeoyl-4-*O*-sinapoylquinate (**5**), and methyl 3,5-di-*O*-caffeoyl-4-*O*-(3-hydroxy-3-methyl)glutaroylquinate (**6**) together with nine known compounds, ethyl 5-*O*-caffeoylquinate (**7**),[13] 3,5-dicaffeoylquinic acid (**8**),[14] 4,5-dicaffeoylquinic acid (**9**),[15] quercetin (**10**), quercetin 3-*O*-glucopyranoside (**11**),[16] geniposide (**12**),[9] geniposidic acid (**13**),[10] caffeic acid (**14**), and 3,4-dihydroxybenzoic acid (**15**). The structures of new compounds were elucidated using spectroscopic methods.



	R_1	R_2	R_3
2:	Me	H	sinapoyl
3:	Et	H	sinapoyl
4:	Me	sinapoyl	H
5:	Et	sinapoyl	H
6:	Me	caffeoyl	HMG

Results and Discussion

The ^{1}H and ^{13}C NMR spectra of **1** were similar to those of vanillic acid 4-*O*-*β*-D-(6′-*O*-galloyl)glucopyranoside isolated from *Terminalia macroptera*, except for the presence of a sinapoyl group instead of a galloyl group.[17] The ^{1}H NMR spectrum showed two vinyl proton signals at δ_H 6.41 (1H, d, J = 15.9 Hz, H-2″) and 7.62 (1H, d, J = 15.8 Hz, H-3″), two protons of the sinapoyl group at δ_H 6.90 (2H, s, H-5″, 9″), three aromatic protons characteristic of an ABX spin system at δ_H 7.16 (1H, d, J = 8.2 Hz, H-5), 7.55 (1H, dd, J = 1.91, 8.44 Hz, H-6), and 7.60 (1H, dd, J = 1.89 Hz,

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H-2), two methoxy signals at δ_H 3.89 (s, 6H) and 3.90 (s, 3H), and proton signals typical of a sugar residue. The chemical shifts of the six carbons at δ 102.0 (C-1′), 74.8 (C-2′), 77.8 (C-3′), 71.7 (C-4′), 75.7 (C-5′), and 64.7 (C-6′) in the ^{13}C NMR spectrum indicated the sugar to be 1′,6′-disubstituted glucose. In the HMBC spectrum of **1**, the H-6′ sugar proton at δ_H 4.35 (1H, dd, $J = 6.83$, 12.0 Hz) and two vinyl proton signals at δ_H 6.41 (H-2″) and 7.62 (H-3″) correlated with the carbonyl carbon signal (δ 168.8) of the sinapoyl group, and the signal of anomeric proton at δ_H 4.90 (H-1′) showed a ^{1}H−^{13}C long-range correlation with a signal of the vanillic acid moiety at δ_C 151.5 (C-4), indicating the presence of vanillic acid and sinapoyl moieties at the C-1′ and 6′ positions of glucose, respectively. Thus, compound **1** was established as vanillic acid 4-*O*-*β*-D-(6′-sinapoyl)glucopyranoside.

The ^{1}H and ^{13}C NMR spectra of **2** were similar to those of 3-*O*-caffeoyl-4-*O*-synapoylquinic acid except for the quinic acid moiety and methyl ester signal at δ_H 3.65 (s, 3H).[7] The signals of H-5 at δ_H 5.48 (1H, m) and H-4 at δ_H 5.06 (dd, $J = 3.03$, 8.26 Hz) were shifted significantly downfield compared with those of quinic acid, indicating the presence of caffeoyl and sinapoyl groups at C-5 and C-4. The HMBC spectrum of **2** indicated that the carbonyl carbon signals at δ_C 168.3 (C-1′) of the caffeoyl group and at δ_C 168.8 (C-1″) of the sinapoyl moiety showed a ^{1}H−^{13}C long-range correlation with the signals at δ_H 5.48 (H-5) and 5.06 (H-4), respectively. The methyl ester signal at δ_H 3.65 was correlated with a carbonyl signal at δ_C 175.6 of C-1. These data indicated that the methoxy, caffeoyl, and sinapoyl groups were located at C-1, C-5, and C-4 of the quinic acid, respectively. On the basis of these data, compound **2** was established as methyl 5-*O*-caffeoyl-4-*O*-sinapoylquinate.

The ^{1}H and ^{13}C NMR spectra of **3** were similar to those of compound **2** except for the ethyl ester moiety. The ^{13}C NMR spectrum of **3** exhibited signals of an ethyl ester group (δ_C 62.7, 14.3) of C-1. Signals at δ_H 5.08 (H-4) and 5.49 (H-5) showed a ^{1}H−^{13}C long-range correlation (HMBC) with the carbonyl carbon signals at δ_C 168.4 (C-1″) of the sinapoyl moiety and δ_C 168.0 (C-1′) of the caffeoyl group, respectively. The methylene signals of the ethyl ester were correlated with the carbonyl signal at δ_C 174.7 of C-1. These data indicated that the ethoxy, caffeoyl, and sinapoyl groups were located at C-1, C-5, and C-4 of the quinic acid, respectively. Thus, compound **3** was established as ethyl 5-*O*-caffeoyl-4-*O*-sinapoylquinate.

The ^{1}H and ^{13}C NMR spectra of **4** were similar to those of **2** except for the quinic acid moiety. The HMBC spectrum of **4** showed that the H-3 signal (δ_H 5.38) was correlated with the carbonyl carbon signal at δ_C 168.6 (C-1″) of the sinapoyl moiety. Thus, compound **4** was established as methyl 5-*O*-caffeoyl-3-*O*-sinapoylquinate.

The ^{1}H and ^{13}C NMR spectra of **5** were similar to those of **4** except for the ethyl ester group at δ_H 1.21 (t, $J = 7.1$ Hz, 3H) and 4.11 (m, 2H). The ^{13}C NMR spectrum of **5** exhibited signals of ethyl ester (δ_C 62.6, 14.3) and methoxy groups (δ_C 56.8) of a sinapoyl moiety. In the HMBC spectrum of **5**, the H-3 signal at δ_H 5.30 was correlated with the carbonyl carbon signal at δ_C 168.6 (C-1″) of the sinapoyl moiety, and the methylene signal at δ_H 4.11 of the ethyl ester was correlated with the carbonyl carbon signal at δ_C 175.2 (C-1). Thus, compound **5** was established as ethyl 5-*O*-caffeoyl-3-*O*-sinapoylquinate.

In previous reports, ethyl esters of 3,4- and 4,5-di-*O*-caffeoylquinic acids were synthesized as artifacts by refluxing a plant extract containing 3,4- and 4,5-di-*O*-caffeoylquinic acids with 1 N HCl in EtOH/H_2O.[18] On the other hand, Bouchet *et al.* identified 3,4,5-tri-*O*-galloylquinic acid ethyl ester as a newly isolated compound.[19] To test whether compounds **3** and **5** are artifacts, we used 3,5-*O*-dicaffeoylquinic acid since we could not isolate 5-*O*-caffeoyl-4-*O*-sinapoylquinic acid and 5-*O*-caffeoyl-3-*O*-sinapoylquinic acid. When 3,5-*O*-dicaffeoylquinic acids in EtOH were kept at room temperature for 3 days in the presence of Sephadex LH-20, a condition similar to the isolation procedure, the ethyl ester of 3,5-*O*-dicaffeoylquinic acid was not obtained, indicating that compounds **3** and **5** were not artifacts formed during the isolation process.

Table 1. ^{13}C NMR Data of Compounds **1**−**6** (75 MHz, CD_3OD)[a]

C	1	2	3	4	5	6
1	127.3	76.2	75.8	74.7	74.6	74.4
2	114.4	38.8	38.2	35.7	35.6	36.3
3	150.3	69.1	68.7	72.1	71.8	69.2
4	151.5	75.4	75.0	69.8	69.8	70.8
5	116.5	69.5	69.1	72.2	72.1	69.3
6	124.5	39.0	38.6	36.8	36.8	37.3
1′	102.0	168.3	168.0	168.0	168.0	168.3
2′	74.8	114.9	114.2	114.8	114.8	115.0
3′	77.8	148.1	147.7	147.4	147.3	147.6
4′	71.7	127.9	127.4	127.6	127.6	127.8
5′	75.7	115.6	115.1	115.1	115.1	115.1
6′	64.7	147.2	146.9	146.9	146.9	146.9
7′		150.2	150.1	149.8	149.8	149.9
8′		116.9	116.5	116.5	116.5	116.4
9′		123.5	123.1	123.1	123.0	123.2
1″	168.8	168.8	168.4	168.6	168.6	167.5
2″	115.7	116.0	115.5	116.3	116.3	114.4
3″	147.3	148.2	147.8	147.2	147.2	147.9
4″	126.5	127.0	126.5	126.8	126.8	127.5
5″	106.9	107.3	106.9	106.9	106.9	114.9
6″	149.5	149.8	149.4	149.5	149.5	146.8
7″	139.6	140.0	139.7	139.5	139.5	149.7
8″	149.5	149.8	149.4	149.5	149.5	116.5
9″	106.9	107.3	106.9	106.9	106.9	123.3
1‴						171.4
2‴						47.2
3‴						71.1
4‴						47.5
5‴						179.4
CO	170.1	175.6	174.7	175.6	175.2	175.7
OMe	56.7	53.5		53.0		53.1
2×OMe	56.9	57.2	56.8	56.8	56.8	
OCH_2			62.7		62.6	
CH_3			14.3		14.3	27.6

[a] The assignment was based upon COSY, HMQC, and HMBC experiments.

The ^{1}H and ^{13}C NMR spectra of **6** were similar to those of 3,5-di-*O*-caffeoyl-4-*O*-(3-hydroxy-3-methyl)glutaroylquinic acid,[7] except that it contained a methoxy group located on a quinic acid moiety. The ^{1}H NMR spectrum of **6** showed the signals of a methoxy peak at δ_H 3.74 in addition to those assignable to 3,5-di-*O*-caffeoyl-4-*O*-(3-hydroxy-3-methyl)glutaroylquinic acid. In the HMBC spectrum of **6**, the methoxy peak at δ_H 3.74 correlated with the carbonyl carbon signal at δ_C 175.5 (C-1), and the signals at δ_H 5.29 (H-4) and 2.65 (H-2‴) correlated with the carbonyl peak of the glutaric acid at δ_C 171.4 (C-1‴), which placed the methoxy group at C-1 and the glutaroyl group at C-4. Thus, compound **6** was established as methyl 3,5-di-*O*-caffeoyl-4-*O*-(3-hydroxy-3-methyl)glutaroylquinate.

All of the isolates were assessed for antioxidant activities in three different bioassay systems (Table 2). For comparisons, ascorbic acid, vitamin E, and resveratrol were included as positive controls. The new quinic acid derivatives (**2**−**6**) showed potent DPPH radical and superoxide anion radical scavenging activities and showed significant inhibition of lipid peroxidation by ferric thiocyanate assay using AAPH [2,2′-azobis(2-amidinopropane)dihydrochloride]. It has been reported that antioxidant activity is mainly due to the catechol moiety in similar structures.[20] On the other hand, vanillic acid 4-*O*-*β*-D-(6′-sinapoyl)glucopyranoside (**1**) showed moderate antioxidant activity in DPPH and superoxide anion radical scavenging assay systems.

All of the isolates were also tested for HIV-1 integrase inhibitory activity (Table 3). The activity data of L-chicoric acid and curcumin were included as positive controls. Among the new compounds,

Table 2. Antioxidant Activities of Compounds **1**−**15** from Gardeniae Fructus

compound	IC_{50} (μg/mL) on DPPH[a]	IC_{50} (μg/mL) on superoxide anion[a]	inhibitory effect on linoleic acid oxidation (%) at 3.125 μg/mL
1	22.7 ± 0.4	46.3 ± 3.5	38.4
2	8.3 ± 0.2	1.8 ± 0.4	56.0
3	6.1 ± 0.1	2.0 ± 0.3	54.9
4	8.5 ± 0.4	2.2 ± 0.3	54.7
5	9.5 ± 0.3	2.7 ± 0.1	53.8
6	4.4 ± 0.1	2.3 ± 0.2	52.0
7	7.1 ± 0.4	1.0 ± 0.1	51.1
8	5.6 ± 0.1	2.9 ± 0.1	51.5
9	5.9 ± 0.2	3.3 ± 0.4	51.7
10	5.9 ± 0.7	>50	54.2
11	16.6 ±1.0	3.4 ± 0.3	49.9
12	>50	>50	2.0
13	>50	>50	4.8
14	3.2 ± 0.1	0.5 ± 0.1	54.1
15	6.9 ± 1.1	13.0 ± 3.1	38.1
ascorbic acid	5.5 ± 0.1	>50	44.1
vitamin E	9.4 ± 0.3	>50	61.2
resveratrol	17.1 ± 1.1	37.9 ± 3.11	39.1

[a] All values are averages of at least three runs.

Table 3. HIV-1 Integrase Inhibitory Activities of Compounds from Gardeniae Fructus

compound	IC_{50} (μg/mL)[a]	compound	IC_{50} (μg/mL)
1	>100	**10**	−[c]
2	19.4 ± 2.1	**11**	−
3	43.5 ± 3.9	**12**	>100
4	23.6 ± 6.8	**13**	>100
5	47.4 ± 4.5	**14**	−
6	20.1 ± 5.1	**15**	−
7	44.5 ± 7.1	L-chicoric acid[b]	7.4 ± 3.3
8	5.9 ± 2.1	curcumin	51.3 ± 3.5
9	5.4 ± 2.6		

[a] All values are averages of at least three runs. [b] L-Chicoric acid was prepared by a known method.[25] [c] Not tested.

the methyl quinate compounds **2**, **4**, and **6** showed more potent HIV-1 integrase inhibition ($IC_{50} \approx 20$ μg/mL) than ethyl quinate compounds **3** and **5** ($IC_{50} \approx 45$ μg/mL) and curcumin ($IC_{50} = 51.3 \pm 3.5$ μg/mL). 3,5-Dicaffeoylquinic acid (DCQA) (**8**) and 4,5-DCQA (**9**) showed the most potent HIV-1 integrase inhibition, with IC_{50} values of 5.9 ± 2.1 and 5.4 ± 2.6 μg/mL, respectively, and these activities were comparable to that of L-chicoric acid ($IC_{50} = 7.4 \pm 3.3$ μg/mL). The HIV-1 integrase inhibitory activity in the quinic acid derivatives decreased in the order of DCQAs, methyl quinates, and ethyl quinates, respectively. The location of the sinapoyl moiety in the quinic acid was not important for inhibition of HIV-1 integrase. On the basis of the above results, DCQA derivatives exhibit the dominant antioxidant effects and HIV-1 integrase inhibitory effects of Gardeniae Fructus.

Experimental Section

General Experimental Procedures. Optical rotations were determined on an Autopol III automatic polarimeter (Rudolph Research Flanders, NJ). ^{1}H and ^{13}C NMR spectra were recorded in CD_3OD on a Bruker 300 MHz spectrometer for ^{1}H and 75.43 MHz for ^{13}C. ^{1}H−^{1}H COSY, HMBC, and HMQC spectra were obtained with the usual pulse sequences, and data processing was performed using standard software.

Materials. Xanthine, nitroblue tetrazolium (NBT), xanthine oxidase, 1,1-diphenyl-2-picrylhydrazyl (DPPH), AAPH [2,2′-azobis(2-amidinopropane)dihydrochloride], linoleic acid, ascorbic acid, resveratrol, vitamin E, and other chemical reagents were purchased from Sigma Chemical Co. (St. Louis, MO).

Plant Material. Gardeniae Fructus was purchased from Kyoungdong herbal drug market (Seoul, Korea) in June 2000 and identified by Dr. Jae Ock Hwang at Hwang's Oriental Medical Center. Voucher specimens (895-15) have been deposited in our laboratory at KIST.

Extraction and Isolation. Gardeniae Fructus (3.0 kg) was extracted three times with MeOH (3.8 L). The dried extract residue (500.4 g) was suspended in H_2O (3.0 L) and then partitioned in turn with CH_2Cl_2 (2.5 L × 3), ethyl acetate (2.5 L × 3), and *n*-butanol (2.5 L × 3). The ethyl acetate extract was evaporated under reduced pressure to yield 25.9 g of residue. A part of this residue (22.8 g) was separated into nine fractions (EA−EI) by column chromatography (CC) using Sephadex LH-20 (5 × 40 cm) and MeOH (2.5 L) as an eluent. Fraction ED (19.0 g) was again applied to Sephadex LH-20 CC (5 × 40 cm) using MeOH (800 mL) as eluent to give 11 subfractions (ED1−ED10). Subfraction ED9 (1.86 g) was further purified by CC over Sephadex LH-20 (3 × 30 cm) using EtOH (500 mL) as eluting solvent. Fraction ED9e (779.4 mg) was chromatographed over silica gel (2.5 × 35 cm, CH_2Cl_2/MeOH/H_2O = 6:1:0.1 → 3:1:0.2, each 400 mL) followed by preparative RP-18 TLC (20 × 20 cm, 0.1 mm, Merck) developed with 64% MeOH to afford 6.0 mg of **4**. Compound **6** was obtained from ED9g (290.4 mg) by CC over silica gel (1.5 × 30 cm, CH_2Cl_2/MeOH/H_2O = 4:1:0.1, 400 mL). ED9e4 (577.5 mg) was further purified by CC over silica gel (3.0 × 30 cm, CH_2Cl_2/MeOH/H_2O = 7:1:0.1 → 3:1:0.2, each 500 mL) to give **2** (90.1 mg), **1** (4.3 mg), **14** (35.0 mg), and **15** (46.1 mg), respectively. ED10 (3.04 g) was purified by column chromatography over Sephadex LH-20 (3.5 × 35 cm) using EtOH (800 mL) as eluting solvent, and ED10d (123.4 mg) was purified by CC over silica gel (1.5 × 35 cm, CH_2Cl_2/MeOH/H_2O = 8:1:0 → 4:1:0.1, each 250 mL). The subfraction ED10d2 (51.1 mg) was further purified by preparative RP-18 TLC (20 × 20 cm, 0.1 mm, Merck) developed with 64% MeOH to afford **5** (6.4 mg), **3** (5.7 mg), and **7** (4.3 mg). Fraction ED5 (356.8 mg) was purified by CC over Sephadex LH-20 (2.5 × 35 cm) using MeOH (400 mL) as eluent to give eight subfractions (ED5a−ED5f). Subfraction ED5b (185.9 mg) was purified by CC over silica gel (1.5 × 35 cm, CH_2Cl_2/MeOH/H_2O = 6:1:0.1 → 3:1:0.1, each 300 mL), and ED5b8 (30.6 mg) by preparative HPLC (LiChrosorb 250-10, 7 μm, RP-18, Merck) eluted with CH_3CN/H_2O, 10:90, 3.4 mL/min, to afford 14.1 mg of compound **12** (t_R 14.8 min). ED5b16 (18.5 mg) was further purified by preparative RP-18 TLC developed with 23% MeOH to afford **13** (6.0 mg). Fraction EF (800.5 mg) was chromatographed on LiChroprep RP-18 (3.5 × 35 cm) using mixtures of MeOH/H_2O (40:60, 50:50, and 70:30, each 500 mL) to give nine fractions (EE1−EE9). Fraction EE3 (129.9 mg) was purified by CC over LiChroprep RP-18 (1.5 × 30 cm) using mixtures of MeOH/H_2O (35:65, 200 mL) to yield compound **8** (4.8 mg). Fraction EF4 (268.0 mg) was purified by CC (2.5 × 27.5 cm) on RP-18 (40% MeOH, 250 mL) and Toyopearl HW-40C (1 × 10.5 cm, MeOH, 50 mL) to afford compounds **9** (16.8 mg), **10** (5.4 mg), and **11** (5.2 mg).

Scavenging of DPPH Radicals. The potential antioxidant activity of plant extracts and pure compounds was assessed on the basis of scavenging activity of the DPPH free radical. Reaction mixtures containing test samples (in EtOH) and 100 μM DPPH ethanolic solution in 96-well microtiter plates were incubated at 37 °C for 30 min. Absorbances were measured at 515 nm. Percent inhibition was determined by comparison with an ethanol-treated control. IC_{50} values denote the concentration of samples required to scavenge 50% of the DPPH free radicals.[21]

Scavenging of Superoxide Anion Radicals by Xanthine Oxidase. The reaction mixture consisted of 40 mM sodium carbonate buffer (pH 10.2) containing 0.1 mM xanthine, 0.1 mM EDTA, 50 μg protein/mL bovine serum albumin, 25 mM NBT, and 1.4×10^{-8} units of xanthine oxidase (EC 1.2.3.2) in a final volume of 200 μL. After incubation at 25 °C for 20 min, the reaction was terminated by the addition of 6.6 μL of 6 mM $CuCl_2$. The absorbance of formazan produced was determined at 560 nm, and IC_{50} values denote the concentration of samples required to scavenge 50% of the superoxide anion radicals.[22]

Inhibition of Lipid Peroxidation by Ferric Thiocyanate. Antioxidative activity was evaluated by using 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH)-induced lipid peroxidation of a Tween-emulsified linoleic acid system and measured by the ferric thiocyanate assay.[23]

HIV-1 Integrase Inhibition Assay. A standard reaction assay of endonucleolytic activity was carried out as described previously.[24]

Vanillic acid 4-*O*-*β*-D-(6′-sinapoyl)glucopyranoside (1): mp (uncorrected) 147−149 °C; $[\alpha]^{21}_D$ −34.9 (*c* 0.22, MeOH); UV (MeOH) λ_{max} (log ϵ) 243 (4.69), 292 (sh), 328 (4.00); IR (KBr) ν_{max} 3448, 1702, 1641, 1604, 1518, 1476, 1434, 1347, 1266, 1277, 1187, 1157, 1122, 1087, 1033, 833 cm^{-1}; ^{1}H NMR (CD_3OD, 300 MHz) δ 3.43 (1H, t, J = 8.9 Hz, H-4′), 3.49 (1H, t, J = 8.86 Hz, H-3′), 3.54 (1H, dd, J =

7.4, 9.0 Hz, H-2′), 3.74 (1H, m, H-5′), 3.89 (s, 6H, OMe), 3.90 (s, 3H, OMe), 4.35 (1H, dd, J = 6.83, 12.0 Hz, H-6′), 4.54 (1H, dd, J = 2.07, 12.0 Hz, H-6′), 4.90 (1H, d, J = 7.3 Hz, H-1′), 6.41 (1H, d, J = 15.9 Hz, H-2″), 6.90 (2H, s, H-5″, 9″), 7.16 (1H, d, J = 8.2 Hz, H-5), 7.55 (1H, dd, J = 1.91, 8.44 Hz, H-6), 7.60 (1H, dd, J = 1.89 Hz, H-2), 7.62 (1H, d, J = 15.8 Hz, H-3″); ^{13}C NMR (CD_3OD, 75 MHz), see Table 1; HRFABMS (negative-ion mode) m/z 535.1431 (calcd for $C_{25}H_{27}O_{13}$, 535.1452).

Methyl 5-*O*-caffeoyl-4-*O*-sinapoylquinate (2): mp (uncorrected) 130−132 °C; $[\alpha]^{19}_D$ −238.7 (c 1.45, MeOH); UV (MeOH) λ_{max} (log ϵ) 239 (1.74), 330 (4.32); IR (KBr) ν_{max} 3422, 1702, 1641, 1604, 1514, 1458, 1431, 1282, 1167, 1122, 1072, 983 cm^{-1}; ^{1}H NMR (CD_3OD, 300 MHz) δ 2.03 (1H, dd, J = 6.13, 14.0 Hz, H-2), 2.23 (2H, m, H-6, overlapped with H-2), 2.24 (1H, dd, J = 3.27, 14.0 Hz, H-2), 3.65 (3H, s, OMe), 3.75 (6H, s, 2 × OMe), 4.29 (1H, m, H-3), 5.06 (1H, dd, J = 3.03, 8.26 Hz, H-4), 5.48 (1H, m, H-5), 6.09 (1H, d, J = 15.9 Hz, H-2′), 6.33 (1H, d, J = 15.9 Hz, H-2″), 6.67 (2H, d, J = 8.14 Hz, H-8′), 6.77 (2H, s, H-5″, H-9″), 6.83 (1H, dd, J = 1.86, 8.25 Hz, H-9′), 6.93 (1H, d, J = 1.86 Hz, H-5′) 7.43 (1H, d, J = 15.9 Hz, H-3′), 7.56 (1H, d, J = 15.8 Hz, H-3″); ^{13}C NMR (CD_3OD, 75 MHz), see Table 1; HRFABMS (positive-ion mode) m/z 597.1597 (calcd for $C_{28}H_{30}O_{13}$-Na, 597.1584).

Ethyl 5-*O*-caffeoyl-4-*O*-sinapoylquinate (3): mp (uncorrected) 129−130 °C (dec); $[\alpha]^{20}_D$ −225.9 (c 0.29, MeOH); UV (MeOH) λ_{max} (log ϵ) 242 (2.36), 331 (4.34); IR (KBr) ν_{max} 3422, 1702, 1641, 1604, 1518, 1476, 1426, 1292, 1260, 1167, 1122, 988 cm^{-1}; ^{1}H NMR (CD_3-OD, 300 MHz) δ 1.19 (3H, t, J = 7.13 Hz, CH_3), 2.05 (1H, dd, J = 6.06, 14.0 Hz, H-2), 2.22 (2H, m, H-6, overlapped with H-2), 2.27 (1H, dd, J = 3.26, 14.1 Hz, H-2), 3.76 (6H, s, 2 × OMe), 4.09 (2H, m, CH_2), 4.31 (1H, m, H-3), 5.08 (1H, dd, J = 3.02, 8.31 Hz, H-4), 5.49 (1H, m, H-5), 6.11 (1H, d, J = 15.9 Hz, H-2′), 6.35 (1H, d, J = 15.8 Hz, H-2″), 6.68 (1H, d, J = 8.14 Hz, H-8′), 6.77 (2H, s, H-5″, H-9″), 6.84 (1H, dd, J = 1.90, 8.27 Hz, H-9′), 6.94 (1H, d, J = 1.85 Hz, H-5′) 7.44 (1H, d, J = 15.9 Hz, H-3′), 7.57 (1H, d, J = 15.8 Hz, H-3″); ^{13}C NMR (CD_3OD, 75 MHz), see Table 1; HRFABMS (positive-ion mode) m/z 611.1738 (calcd for $C_{29}H_{32}O_{13}Na$, 611.1741).

Methyl 5-*O*-caffeoyl-3-*O*-sinapoylquinate (4): mp (uncorrected) 140−144 °C (dec); $[\alpha]^{21}_D$ −148.0 (c 0.30, MeOH); UV (MeOH) λ_{max} (log ϵ) 242 (2.68), 330 (4.78); IR (KBr) ν_{max} 3422, 1702, 1636, 1604, 1514, 1458, 1431, 1297, 1260, 1162, 1122, 988 cm^{-1}; ^{1}H NMR (CD_3-OD, 300 MHz) δ 2.10−2.34 (4H, m, H-2, H-6), 3.67 (3H, s, OMe), 3.86 (6H, s, 2×OMe), 3.95 (1H, dd, J = 3.08, 6.61 Hz, H-4), 5.29 (1H, m, H-5), 5.38 (1H, m, H-3), 6.20 (1H, d, J = 15.9 Hz, H-2′), 6.44 (1H, d, J = 15.9 Hz, H-2″), 6.75 (1H, d, J = 8.15 Hz, H-8′), 6.90 (2H, s, H-5″, H-9″), 6.94 (1H, dd, J = 1.89, 8.18 Hz, H-9′), 7.02 (1H, d, J = 1.91 Hz, H-5′), 7.52 (1H, d, J = 15.8 Hz, H-3′), 7.65 (1H, d, J = 15.9 Hz, H-3″); ^{13}C NMR (CD_3OD, 75 MHz), see Table 1; HRFABMS (positive-ion mode) m/z 597.1581 (calcd for $C_{28}H_{30}O_{13}$-Na, 597.1584).

Ethyl 5-*O*-caffeoyl-3-*O*-sinapoylquinate (5): mp (uncorrected) 140−142 °C (dec); $[\alpha]^{21}_D$ −133.7 (c 0.32, MeOH); UV (MeOH) λ_{max} (log ϵ) 238 (2.06), 331 (4.70); IR (KBr) ν_{max} 3448, 1702, 1641, 1604, 1518, 1458, 1292, 1260, 1157, 1122, 1043, 978 cm^{-1}; ^{1}H NMR (CD_3-OD, 300 MHz) δ 1.21 (3H, t, J = 7.08 Hz, CH_3), 2.13−2.30 (4H, m, H-2, 6), 3.86 (6H, s, 2 × OMe), 3.95 (1H, dd, J = 2.93, 6.35 Hz, H-4), 4.11 (2H, m, CH_2), 5.29 (1H, m, H-5), 5.36 (1H, m, H-3), 6.20 (1H, d, J = 15.9 Hz, H-2′), 6.43 (1H, d, J = 15.9 Hz, H-2″), 6.75 (1H, d, J = 8.15 Hz, H-8′), 6.89 (2H, s, H-5″, H-9″), 6.91 (1H, dd, J = 1.71, 8.3 Hz, H-9′), 6.99 (1H, d, J = 1.75 Hz, H-5′) 7.50 (1H, d, J = 15.9 Hz, H-3′), 7.63 (1H, d, J = 15.9 Hz, H-3″); ^{13}C NMR (CD_3OD, 75 MHz), see Table 1; HRFABMS (positive-ion mode) m/z 611.1752 (calcd for $C_{29}H_{32}O_{13}Na$, 611.741).

Methyl 3,5-di-*O*-caffeoyl-4-*O*-(3-hydroxy-3-methyl)glutaroylquinate (6): mp (uncorrected) 165−168 °C (dec); $[\alpha]^{20}_D$ −129.6 (c 0.30, MeOH); UV (MeOH) λ_{max} (log ϵ) 243 (1.95), 296 (sh), 332 (4.42); IR (KBr) ν_{max} 3422, 1745, 1702, 1604, 1522, 1456, 1406, 1262, 1167, 1127, 1038, 988 cm^{-1}; ^{1}H NMR (CD_3OD, 300 MHz) δ 1.35 (3H, s, CH_3), 2.16 (1H, dd, J = 9.0, 14.4 Hz, H-2), 2.22 (1H, m, H-6), 2.39 (1H, d, J = 14.7 Hz, H-4‴), 2.47 (1H, m, H-6, overlapped with H-2), 2.48 (1H, dd, J = 4.2, 13.5 Hz, H-2), 2.62 (1H, d, J = 12.6 Hz, H-4‴), 2.65 (2H, s, H-2‴), 3.74 (3H, s, OMe), 5.29 (1H, dd, J = 3.3, 6.6 Hz, H-4), 5.43 (1H, m, H-5), 5.62 (1H, m, H-3), 6.23 (1H, d, J = 15.9 Hz, H-2″), 6.33 (1H, d, J = 15.9 Hz, H-2′), 6.80 (1H, d, J = 8.1 Hz, H-8′), 6.82 (1H, d, J = 8.4 Hz, H-8″), 6.98 (1H, dd, J = 2.1, 8.1 Hz, H-9′), 7.00 (1H, dd, J = 2.1, 8.1 Hz, H-9″), 7.09 (1H, d, J = 2.1 Hz, H-5′), 7.12 (1H, d, J = 1.5 Hz, H-5″), 7.56 (1H, d, J = 15.9 Hz, H-3″), 7.62 (1H, d, J = 15.9 Hz, H-3′); ^{13}C NMR (CD_3OD, 75 MHz), see Table 1; HRFABMS (positive-ion mode) m/z 675.1915 (calcd for $C_{32}H_{35}O_{16}$, 675.1925).

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Supporting Information Available: Additional experimental details of the bioassays and ^{1}H and ^{13}C NMR spectra of compounds **1**−**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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