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Synthesis, anti-HIV and anti-oxidant activities of caffeoyl 5,6-anhydroquinic acid derivatives

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ABSTRACT

In our continued research on chlorogenic acid analogues and derivatives with improved bioactivity, we have synthesized some caffeoyl 5,6-anhydroquinic acid derivatives. The 1,7 acetonides of chlorogenic acid (**15**), and of the mono-caffeoyl 5,6-anhydroquinic acids (**7**–**8**) showed appreciable anti-HIV activity. The 3,4-dicaffeoyl 5,6-anhydroquinic acid (**12**) exhibited an anti-HIV activity twice as that of 3,5-dicaffeoylquinic acid (**22**). The caffeoyl 5,6-anhydroquinic acid derivatives displayed potent anti-oxidant activities. The mono-caffeoyl 5,6-anhydroquinic acids (**10**–**11**) were more than twice stronger than chlorogenic acid (**21**) on SOD-like activity.

1. Introduction

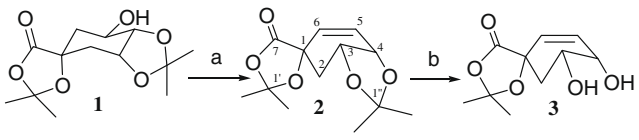
Caffeoylquinic acids with the most well known compound being chlorogenic acid (5-caffeoylquinic acid), are a group of natural products found in many medicinal and dietary plants. They are potent antioxidants and might contribute to the prevention of Type 2 diabetes mellitus, cardiovascular disease and certain aging related diseases.[1,2] Dicaffeoylquinic acids have been reported to demonstrate a strong hepatoprotective activity in experimental liver injury models.[3] More interest in these compounds has come from the report that dicaffeoylquinic acids showed anti-HIV activity by inhibiting the HIV integrase.[4]

Because of its wide availability and multifunctional groups in the structure of chlorogenic acid, this compound is an ideal starting material for the synthesis of derivatives with more potent or new bioactivities. Chlorogenic acid derivatives with a lipophilic chain at position 1 were reported to have anti-diabetic activity by inhibiting hepatic flucose-6-phosphate translocase.[5] In our effort of synthesis and bioactivity evaluation of chlorogenic acid derivatives and analogues, we have reported that addition of a lipophilic chain to position 7 of chlorogenic acid through amide bonds led to compounds of potent anti-fungal activity;[6] Addition of lipophilic chains through acetal/ketal bonds to chlorogenic acid produced potent α-glucosidase inhibitors which may be useful for developing anti-diabetic agents.[7] The current paper reports the synthesis, anti-oxidant, and anti-HIV activities of chlorogenic acid analogues with modification at the quinic acid moiety, that is, new caffeoyl 5,6-anhydroquinic acids.

2. Results and discussion

2.1. Chemistry

Quinic acid bisacetonide (**1**) was synthesized using the procedure reported by Sefkow.[8] Compound **2** was prepared by treating **1** with sulfuryl chloride in pyridine in the presence of DMAP. Deprotection of **2** in 0.4 N HCl afforded **3** (Scheme 1). Condensation of **3** with acetylcaffeoic acid chloride followed by deprotection resulted in a mixture of **4**, **5** and **6**, which upon separation by column chromatography afforded pure individual compounds. When large excess amount of acetylcaffeoic acid chloride is used to react with **3**, compound **6** was obtained almost quantitatively. Treatment of **4**–**6** with 0.8–1 N HCl at rt followed by column chromatography



Scheme 1. Chemical structures and synthesis of 5,6-anhydroquinic acid derivatives. (a) SO_2Cl_2, DMAP, pyridine; (b) 0.4 N HCl, rt, 1h.

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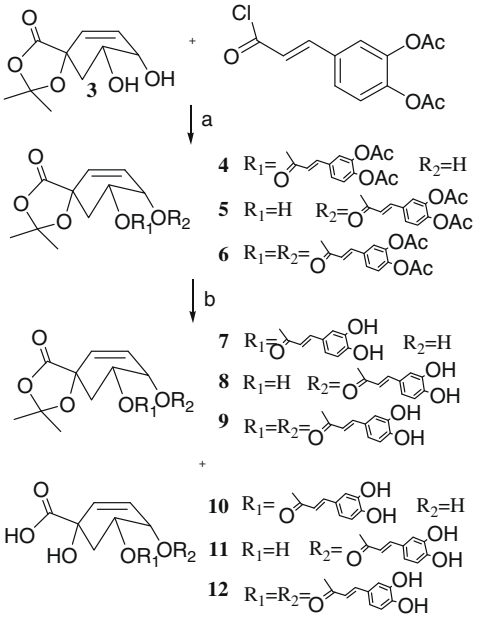


Scheme 2. Chemical structures and synthesis of caffeoyl 5,6-anhydroquinic acids. (a) DMAP, pyridine; (b) 0.8 N HCl, rt, 24 h.

afforded **7–12** (Scheme 2). The ketal derivatives (**15–20**) were prepared as described in previous paper.[7] Using the same method compounds **13** and **14** were prepared (Scheme 3).

2.2. Biological activity

The anti-HIV activity of the compounds synthesized was screened using MT-4 cells infected with HIV-1 (LAV-1) according to the procedure described in a previous paper.[9] The results are shown in Figure 1. The 1,7 acetonides of chlorogenic acid (**15**), 3-caffeoyl 5,6-anhydroquinic acids (**7**) and 4-caffeoyl 5,6-anhydroquinic acid (**8**) showed appreciable anti-HIV activity, with IC_{100} being 25, 50 and 50 μg/ml, respectively. The anti-HIV activity retained even when the phenolic groups were acetylated as demonstrated by the activity of **4** (IC_{100} = 50 μg/ml). However, the anti-HIV activity was found to have diminished after the acetonide groups were removed or only the carboxyl group was masked by a methyl group since compounds **10**, **11** and **16** did not exhibit anti-HIV activity. The findings that the introduction of an acetonide group in chlorogenic acid or its analogues led to anti-HIV activity warranted further investigation on derivatives with longer or different orientations of ketal/acetal chains. For this purpose, compounds **13** and **14** were synthesized and tested together with the previously synthesized compounds **17–20**[7] for their anti-HIV activity. Like compound **15**, compounds **13** and **14** have an alkyl group

Table 1
Anti-oxidant activities of caffeoyl 5,6-anhydroquinic and chlorogenic acid derivatives

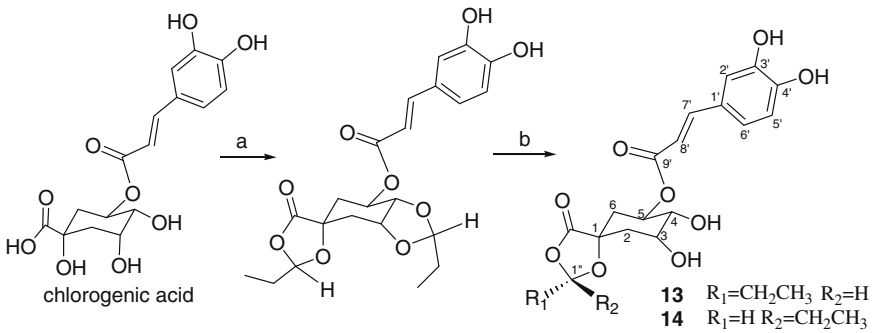
#	IC_{50}	EC_{50}	#	IC_{50}	EC_{50}
1	>100.0	>50	2	90.0	>50
3	>100.0	>50	4	1.8	>50
5	2.9	>50	6	6.3	>50
7	1.5	20	8	1.3	18
9	2.0	20	10	0.5	16
11	0.7	15	12	1.9	11
13	1.1	20	14	1.0	19
15	1.6	8	16	1.4	20
17	2.0	29	18	2.0	30
19	2.0	22	20	2.9	28
21	1.5	17	22	2.3	16

IC_{50}: concentration (μg/ml) of SOD-like compound that inhibited the formation of WST-1 formazan by 50%.
EC_{50}: compound concentration (μg/ml) to produce 50% reduction of the DPPH.

of 3 carbons linked with C-1 and C-7 of quinic acid through oxygen atoms (acetal/ketal) with the only difference in the stereochemistry of C-1″. Compounds **13** and **14** with asymmetric alkyl groups were found to be inactive against HIV. Though all possessing symmetric alkyl-groups, compounds **17–20** with alkyl-chains longer than that of compound **15** did not show anti-HIV activity.

In the case of the dicaffeoyl compounds (**9** and **12**), the one with free hydroxyl and carboxyl groups (3,4-dicaffeoyl 5,6-anhydroquinic acid, **12**) showed anti-HIV activity with IC_{100} of 6.25 μg/ml that is twice as potent as 3,5-dicaffeoylquinic acid[4] (**22**, IC_{100} of 12.5 μg/ml, in the same experiment), a known anti-HIV agent. These results suggest that the hydroxyl group(s) (at least the one at position 5) of the quinic acid part is (are) not essential for the anti-HIV activity of dicaffeoylquinic acids. Contrary to the anti-HIV activity of the acetonides of mono-caffeoyl compounds (**7**, **8** and **4**), the acetonide of the dicaffeoyl derivative (**9**) showed no anti-HIV activity (see Fig. 1).

Since caffeoylquinic acid derivatives are well known to demonstrate strong anti-oxidant activities, the newly synthesized caffeoyl 5,6-anhydroquinic acids were tested for their anti-oxidant activities, SOD-like activity using a SOD Assay Kit-WST and radical scavenging activity against DPPH (2,2-dipheny1-1-picrylhydrazy1). In the SOD-like activity assay, WST-1 produces a water-soluble formazan upon reduction with superoxide anion. The reduction rate is linearly related to the xanthine oxidase (XO) activity, and is inhibited by SOD or other antioxidants. The inhibitory activity of the compounds was expressed as IC_{50} representing the concentration that inhibited the formation of WST-1 formazan by 50%. As shown in Table 1, all the caffeoyl quinic/5,6-anhydroquinic acid derivatives showed SOD-like activity. Compound **2**, a 5,6-anhydroquinic acid derivative whose 3,4-hydroxyls are masked by an acetal group showed appreciable SOD-like activity even though there is no aromatic moiety in its structure. Despite the lack of free phenolic hydroxyl in their structures, compounds **4–6** showed



Scheme 3. Chemical structures and synthesis of the 1,7-acetal compounds. (a) propionaldehyde, TMSOTf; (b) 0.4 N HCl, rt, 1h.

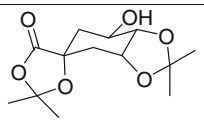
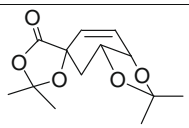
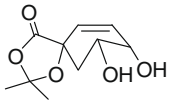
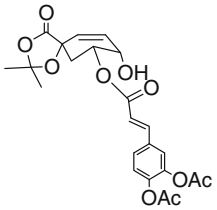
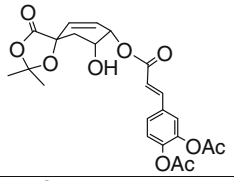
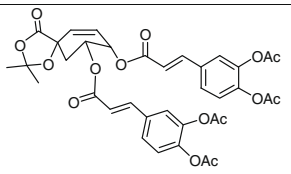
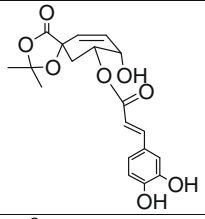
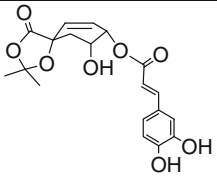
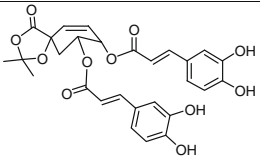
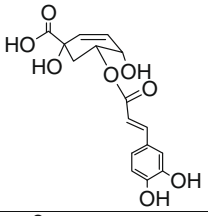
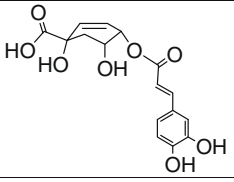
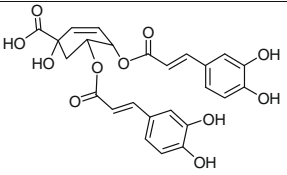
Code and activity	Structure	Code and activity	Structure
1 CC >100 IC:NE		**2** CC >100 IC:NE	
3 CC >100 IC:NE		**4** CC >100 IC>50	
5 Not tested		**6** CC >6.25 IC>NE	
7 CC >100 IC>50		**8** CC >100 IC>50	
9 CC >25 IC>NE		**10** CC >25 IC:NE	
11 CC >25 IC:NE		**12** CC >25 IC>6.25	

Figure 1. Structures and anti-HIV activities of caffeoyl 5,6-anhydroquinic and chlorogenic acid derivatives.

SOD-like activity of the potency similar to that of compounds **7–9** in which the hydroxyl groups are not protected. The most potent SOD-like activity was observed with the two caffeoyl anhydroquinic acid derivatives (**10–11**), which showed more than twice stronger SOD-like activity than chlorogenic acid did. On the other hand, radical scavenging activities against DPPH were strictly limited to those with free phenolic groups in their structures. Apparently, the phenolic groups are the essential in serving as hydrogen-donors to the hydrogen abstracting DPPH radical, which caused a decrease in absorption around 517 nm.[10]

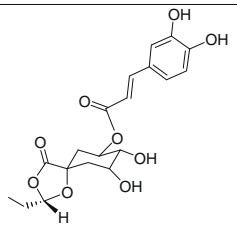
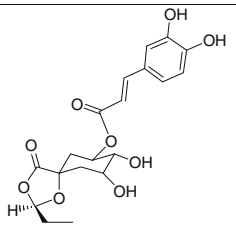
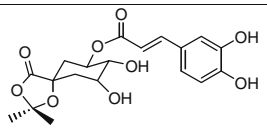
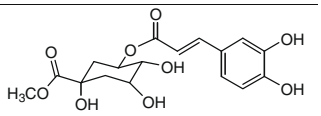
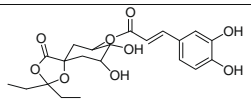
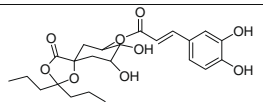
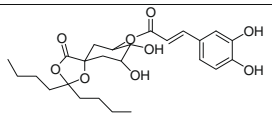
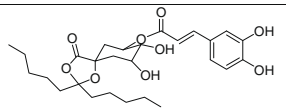
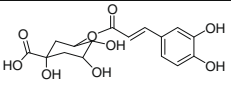
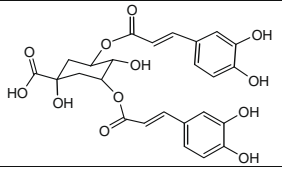
3. Conclusions

The current study described for the first time the synthesis of caffeoyl 5,6-anhydroquinic acids. This method could be applied to the synthesis of other acyl 5,6-anhydroquinic acids, such as *p*-coumaroyl, feruloyl or galloyl 5,6-anhydroquinic acids. Generally, acyl quinic acids are important antioxidants ingredients of coffee, many fruits, and vegetables. Our results revealed that caffeoyl 5,6-anhydroquinic acids possessed equal or stronger anti-oxidant activity compared to those of the known caffeoylquinic acid derivatives. Due to the multi-hydroxyl groups in the quinic acid moiety, the acyl quinic acids are very hydrophilic with mi Log *P* for chlorogenic acid being −0.453 calculated using Molinspiration.[11] The 5,6-anhydroquinic acid derivatives with one less hydroxyl group in their structures are less hydrophilic. The mi Log *P* for caffeoyl 5,6-anhydroquinic acid was calculated to be 0.223. The favorable mi Log *P* values for the acyl 5,6-anhydroquinic acids may suggest that these compounds would have better pharmacokinetic profiles than the acyl quinic acid counterparts.

4. Experimental

4.1. Chemistry

HPLC grade solvents were purchased from Wako Pure Chemical Industries, Ltd. Other chemical reagents were purchased from Sigma–Aldrich, Inc. Column chromatography was carried out on

13 CC >12.5 IC: NE		**14** CC >12.5 IC: NE	
15 CC >50 IC>25		**16** CC >25 IC:NE	
17 CC>25 IC: NE		**18** CC>25 IC: NE	
19 CC>25 IC: NE		**20** CC>12.5 IC: NE	
21* Not tested		**22**** CC >50 IC>12.5	

CC: Cytotoxic concentration (μg/ml) at which reduced viability of MT-4 cells was visualized.

IC: The inhibitory concentration (μg/ml) of tested compound required to completely prevent the HIV-1-induced cytopathic effect.

*Chlorogenic acid was reported to have no anti-HIV acitivty.[4]

Compound **22 is a known anti-HIV compound[4] and was used as a positive control in this experiment.

Fig. 1 (*continued*)

Wakogel 50C18 (38–63 μm, Wako Pure Chemical Industries, Ltd). Preparative HPLC was performed on a Tosoh CCPM-II system (Tosoh Co., Tokyo) with a UV 8020 detector. NMR spectra were measured with a Varian Unity 500 (^{1}H, 500 MHz; ^{13}C, 125 MHz) or a Varian Gemini 300 (^{13}C, 75 MHz) NMR spectrometer. TMS was used as an internal standard and *J* values were reported in hertz. Electrospray ionization mass (ESI-MS) spectra were obtained on an Esquire 3000^{plus} spectrometer (Bruker Daltonik GmbH, Bremen, Germany). High-resolution-FAB-MS was measured on a Jeol JMS-700 with a resolution of 5000 using *m*-nitrobenzyl alcohol as the matrix. The purities of synthesized compounds were verified with ^{1}H NMR, ^{13}C NMR and HPLC.

4.2. Synthesis of the 5,6-anhydroquinic acid derivatives

To a pyridine solution (270 ml) of **1** (8 g, 29 mmol) was added DMAP (672 mg, 5.5 mmol) and sulfuryl chloride (13.44 ml, 166 mmol) at 0 °C. After being stirred at rt for 21 h, the mixture was evaporated to dryness. The residue was partitioned in chloroform and water. The chloroform layer was evaporated to dryness and chromatographed on silica gel column (6 × 40 cm) eluted with hexane–ethylacetate. The hexane–ethylacetate (8:2) eluted part was re-chromatographed on ODS column (4.5 × 16 cm) to obtain **2** (4 g) from 60% methanol eluted part.

Compound ***2***: (yield: 54%) amorphous powder; $[\alpha]_D^{25}$ +112.1 (*c* 0.56, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.39 (3H, s) and 1.49 (3H, s) and 1.63 (6H, s) (4 × CH_3), 2.00 (1H, dd, *J* = 9.5, 13.0 Hz, H-2a), 2.30 (1H, dd, *J* = 13.5, 5.0 Hz, H-2b), 4.61 (1H, m, H-4), 4.70 (1H, dt, *J* = 14.0, 4.5 Hz, H-3), 5.82 (1H, d, *J* = 10.0 Hz, H-6), 6.07 (1H, dd, *J* = 3.5, 10.0 Hz, H-5). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 25.8 and 28.0 and 28.5 and 28.7 (4 × CH_3), 36.1 (C-2), 69.9 (C-4), 70.5 (C-3), 78.3 (C-1), 110.4 (C-1′), 110.9 (C-1″), 129.5 (C-6), 130.5 (C-5), 172.8 (C-7). HR-EI-MS *m*/*z* 254.1176 (calcd For $C_{13}H_{18}O_5$, requires 254.1154).

Compound **2** (3.4 g) was treated with 4 ml 1 N HCl in 6 ml acetone at rt for 1 h. To the mixture was added 1 N Na_2CO_3 till pH 6. The acetone was evaporated and the water solution was passed through ODS column (3 × 8 cm) to obtain **3** (1.3 g) from 30% to 40% MeOH eluted part and recovered some **2** (0.61 g).

Compound ***3***: (yield: 45%) amorphous powder; $[\alpha]_D^{25}$ +220.3 (*c* 0.2, MeOH); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.63 (3H, s) and 1.64 (3H, s) (2 × CH_3), 2.26 (2H, m, H-2a and H-2b), 4.19 (1H, m, H-4), 4.24 (1H, *m*, H-3), 5.70 (1H, d, *J* = 10.0 Hz, H-6), 6.06 (1H, dd, *J* = 3.5, 10.0 Hz, H-5). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 28.5 and 28.7 (2 × CH_3), 36.6 (C-2), 65.5 (C-3), 66.0 (C-4), 77.0 (C-1), 110.8 (C-1′), 127.1 (C-6), 133.7 (C-5), 172.9 (C-7). HR-EI-MS *m*/*z* 214.0828 (calcd For $C_{10}H_{14}O_5$, requires 214.0841).

4.3. Synthesis of the caffeoyl 5,6-anhydroquinic acid derivatives

To a solution of **3** (1 g, 4.7 mmol) and DMAP (180 mg, 1.5 mmol) in CH_2Cl_2 (50 ml) were added pyridine (15 ml) and di-*O*-acety-

lcaffeoyl chloride (5 g, 17. 7 mmol, 3.8 equiv.) at room temperature. The reaction mixture was stirred at rt for 5 h and acidified with 1 N HCl to pH 3. The aqueous phase was re-extracted with CH_2Cl_2. The combined organic extracts were concentrated and purified by SiO_2 column chromatography (6 × 40 cm) eluted with hexane–ethylacetate. Compound **6** (750 mg) was obtained from hexane–ethylacetate 9:1–8:2 eluted part. The hexane–ethylacetate (2:3) eluted part was applied to ODS column (3 × 8 cm) eluted with large amount of 40% MeOH to remove di-*O*-acetylcaffeic acid. Compound **4** (450 mg) and a mixture of **4** and **5** were eluted out by 40% MeOH after di-*O*-acetylcaffeic acid. The mixture of **4** and **5** was further chromatographed on SiO_2 column (2.5 × 30 cm) to obtain compound **4** (265 mg) and **5** (440 mg) from hexane–ethylacetate (2:3) eluted part.

Repeated the above procedure with **3** (100 mg, 0.47 mmol), DMAP (36 mg, 0.30 mmol), pyridine (3 ml), di-*O*-acetylcaffeoyl chloride (1 g, 3.5 mmol, 7.6 equiv) and CH_2Cl_2 (10 ml) to obtain 300 mg of **6**.

Compound ***4***: (yield: 33%) amorphous powder; $[\alpha]_D^{24}$ +103.5 (*c* 1.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.56 (3H, s) and 1.60 (3H, s) (2 × CH_3), 2.27 (1H, dd, *J* = 3.0, 13.5 Hz, H-2a), 2.31 (6H, s, 2 × $COCH_3$), 2.56 (1H, dd, *J* = 14.0, 8.0 Hz, H-2b), 4.44 (1H, dd, *J* = 4.5, 2.0 Hz, H-4), 5.41 (1H, *m*, H-3), 5.73 (1H, d, *J* = 10.0 Hz, H-6), 6.09 (1H, dd, *J* = 3.5, 10.0 Hz, H-5), 6.42 (1H, d, *J* = 15.5 Hz, H-8′), 7.22 (1H, d, *J* = 8.0 Hz, H-5′), 7.37 (1H, d, *J* = 2.0 Hz, H-2′), 7.40 (1H, dd, *J* = 2.0, 8.0 Hz, H-6′), 7.65 (1H, d, *J* = 15.5 Hz, H-7′). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 20.6 (2 × $COCH_3$), 28.4 and 28.7(2 × CH_3), 33.6 (C-2), 64.8 (C-4), 68.7 (C-3), 76.9 (C-1), 110.7 (C-1″), 118.6 (C- 8′), 122.7 (C-1′), 122.8 (C-2′), 124.0 (C-5′), 126.5 (C-6′), 127.9 (C-6), 132.9 (C-5), 143.7 (C-7′), 143.0 (C-3′), 147.9 (C-4′), 165.9 (C-9′), 168.1 (2 × $COCH_3$), 172.7 (C-7). ESI-MS (positive): *m*/*z* 461.0 ($[M+H]^+$, 478.1 ($[M+NH_4]^+$.

Compound ***5***: (yield: 20%) amorphous powder; $[\alpha]_D^{24}$ +161.6 (*c* 1.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.64 (3H, s) and 1.65 (3H, s) (2 × CH_3), 2.31 (6H, s, 2 × $COCH_3$), 2.32 (2H, m, H-2), 4.48 (1H, dt, *J* = 4.0, 9.0 Hz, H-3), 5.48 (1H, ddd, *J* = 4.0, 4.0, 1.0 Hz, H-4), 5.82 (1H, d, *J* = 10.0 Hz, H-6), 6.10 (1H, dd, *J* = 4.0, 10.0 Hz, H-5), 6.42 (1H, d, *J* = 15.5 Hz, H-8′), 7.23 (1H, d, *J* = 8.5 Hz, H-5′), 7.36 (1H, d, *J* = 2.0 Hz, H-2′), 7.40 (1H, dd, *J* = 2.0, 8.5 Hz, H-6′), 7.66 (1H, d, *J* = 15.5 Hz, H-7′). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 20.6 (2 × $COCH_3$), 28.5 and 28.7(2 × CH_3), 36.9 (C-2), 64.1 (C-3), 68.3 (C-4), 77.4 (C-1), 110.8 (C-1″), 118.4 (C-8′), 122.9 (C-2′), 124.0 (C-5′), 126.5 (C-6′), 129.3 (C-5), 130.2 (C-6), 132.9 (C-1′), 142.4 (C-3′), 143.7 (C-7′), 144.0 (C-4′), 166.1 (C-9′), 168.0 (2 × $COCH_3$), 172.7 (C-7). ESI-MS (positive): *m*/*z* 483.0 ($[M+Na]^+$.

Compound ***6***: (yield: 23% and 91% when 3.8 and 7.6 equiv of acid chloride used, respectively) amorphous powder; $[\alpha]_D^{24}$ +125.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.61 (3H, s) and 1.64 (3H, s) (2 × CH_3), 2.29 (6H, s, 2 × $COCH_3$), 2.30 (6H, s, 2 × $COCH_3$), 2.38 (1H, dd, *J* = 2.5, 14.0 Hz, H-2a), 2.54 (1H, dd, *J* = 10.0, 14.0 Hz, H-2b), 5.65 (1H, dt, *J* = 4.0, 9.0 Hz, H-3), 5.71 (1H, ddd, *J* = 4.0, 4.0, 1.0 Hz, H-4), 5.86 (1H, d, *J* = 10.0 Hz, H-6), 6.13 (1H, dd, *J* = 4.0, 10.0 Hz, H-5), 6.36 (1H, d, *J* = 15.5 Hz) and 6.40 (1H, d, *J* = 15.5 Hz) (H-8′, 8″),7.19 (1H, d, *J* = 8.5 Hz) and 7.21 (1H, d, *J* = 8.5 Hz) (H-5′,5″), 7.38 (4H, m, *J* = 2.0 Hz, H-2′,2″,6′,6″), 7.60 (1H, d, *J* = 15.5 Hz) and 7.64 (1H, d, *J* = 15.5 Hz) (H-7′,7″). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 20.7 (4 × $COCH_3$), 28.6 and 28.8(2 × CH_3), 34.2 (C-2), 65.6 (C-3), 66.0 (C-4), 77.3 (C-1), 110.7 (C-1‴), 118.4 and 118.5 (C-8′,8″), 122.6 and 122.7 (C-2′,2″), 123.8 and 123.9 (C-5′,5″), 126.5 (C-6′,6″), 129.2 (C-5), 130.2 (C-6), 132.9 and 133.0 (C-1′,1″), 142.4 (C-3′,3″), 143.4 and 143.5 and 143.7 (C-7′,7″,4′,4″), 165.2 and 165.4 (C-9′,9″), 167.8 and 167.9 (4 × $COCH_3$), 172.2 (C-7). ESI-MS (positive): *m*/*z* 724.9 ($[M+NH_4]^+$.

Compound **4** (100 mg) was treated with 2 ml THF and 8 ml 1 N HCl at rt for 24 h then 1 N Na_2CO_3 was added to adjust the solution to pH6. THF was evaporated and the suspension was passed through ODS to get **10** (14 mg), **7** (12 mg) and recovered **4** (43 mg) from 10% to 30% and 40% MeOH eluted part, respectively.

Compound **5** (100 mg) was treated in the same way as for **4** to obtain **11** (16 mg), **8** (13 mg) and recovered **5** (40 mg) from 10% to 30% and 40% MeOH eluted part of an ODS column, respectively.

Compound ***7***: (yield: 26% calculated from the reacted part of **4**) amorphous powder; $[\alpha]_D^{24}$ +317.0 (*c* 0.2, MeOH); δ 1.57 (3H, s) and 1.61 (3H, s) (2 × CH_3), 2.22 (1H, dd, *J* = 3.0, 13.5 Hz, H-2a), 2.46 (1H, dd, *J* = 14.0, 8.0 Hz, H-2b), 4.38 (1H, t, *J* = 3.5 Hz, H-4), 5.35 (1H, *m*, H-3), 5.78 (1H, d, *J* = 10.0 Hz, H-6), 6.09 (1H, dd, *J* = 3.5, 10.0 Hz, H-5), 6.30 (1H, d, *J* = 15.5 Hz, H-8′), 6.78 (1H, d, *J* = 8.0 Hz, H-5′), 6.94 (1H, dd, *J* = 2.0, 8.0 Hz, H-6′), 7.06 (1H, d, *J* = 2.0 Hz, H-2′), 7.60 (1H, d, *J* = 15.5 Hz, H-7′). ^{13}C NMR (CD_3OD, 125 MHz), 28.6 and 28.8 (2 × CH_3), 34.7 (C-2), 64.8 (C-4), 69.6 (C-3), 79.2 (C-1), 111.9 (C-1″), 115.1 (C- 2′), 115.2 (C-5′), 116.5 (C-8′), 122.9 (C-1′), 127.8 (C-6), 129.4 (C-6′), 134.0 (C-5), 146.8 (C-7′), 147.2 (C-4′), 149.6 (C-3′),168.7 (C-9′),174.6 (C-7). ESI-MS (negative): *m*/*z* 375.0 $[M-H]^-$. HR-FAB-MS $[M-H]^-$ *m*/*z* 375.1106 (calcd For $C_{19}H_{19}O_8$, requires 375.1080).

Compound ***8***: (yield: 27% calculated from the reacted part of **5**) amorphous powder; $[\alpha]_D^{24}$ +223.7 (*c* 0.2, MeOH); ^{1}H NMR (CD_3OD, 500 MHz) δ 1.32 (3H, s) and 1.33 (3H, s) (2 × CH_3), 2.26 (2H, m, H-2), 4.39 (1H, m, H-3), 5.40 (1H, *m*, H-4), 5.88 (1H, d, *J* = 10.0 Hz, H-6), 6.16 (1H, dd, *J* = 3.5, 10.0 Hz, H-5), 6.33 (1H, d, *J* = 15.5 Hz, H-8′), 6.78 (1H, d, *J* = 8.0 Hz, H-5′), 6.95 (1H, dd, *J* = 2.0, 8.0 Hz, H-6′), 7.07 (1H, d, *J* = 2.0 Hz, H-2′), 7.60 (1H, d, *J* = 15.5 Hz, H-7′). ^{13}C NMR (CD_3OD, 125 MHz), 28.7(2 × CH_3), 38.1 (C-2), 64.8 (C-3), 68.4 (C-4), 79.8 (C-1), 112.0 (C-1″), 114.9 (C- 2′), 115.1 (C-5′), 116.5 (C-8′), 123.1 (C-1′), 127.8 (C-6), 130.4 (C-6′), 132.4 (C-5), 146.8 (C-7′), 147.4 (C-4′), 149.7 (C-3′), 168.8 (C-9′), 174.4 (C-7). ESI-MS (negative): *m*/*z* 375.0 $[M-H]^-$. HR-FAB-MS $[M-H]^-$ *m*/*z* 375.1124 (calcd For $C_{19}H_{19}O_8$, requires 375.1080).

Compound ***10***: (yield: 34% calculated from the reacted part of **4**) amorphous powder; $[\alpha]_D^{24}$ +191.6 (1.1, MeOH); ^{1}H NMR (CD_3OD, 500 MHz) δ 2.27 (2H, m, H-2), 4.34 (1H, t, *J* = 4.0 Hz, H-4), 5.29 (1H, m, H-3), 5.83 (1H, d, *J* = 10.0 Hz, H-6), 5.98 (1H, dd, *J* = 4.5, 10.0 Hz, H-5), 6.32 (1H, d, *J* = 15.5 Hz, H-8′), 6.77 (1H, d, *J* = 8.0 Hz, H-5′), 6.94 (1H, dd, *J* = 1.5, 8.0 Hz, H-6′), 7.05 (1H, *J* = 2.0 Hz, H-2′), 7.59 (1H, d, *J* = 15.5 Hz, H-7′). ^{13}C NMR (CD_3OD, 125 MHz) δ 34.8 (C-2), 64.6 (C-4), 71.0 (C-3), 74.4 (C-1), 115.0 (C-2′), 115.2 (C- 8′), 116.4 (C-5′), 122.9 (C-6′), 127.8 (C-1′), 130.8 (C-5),133.0 (C-6), 147.0 (C-7′), 146.8 (C-3′), 149.6 (C-4′), 168.7 (C-9′), 176.9 (C-7). ESI-MS (negative): *m*/*z* 335.0 $[M-H]^-$. HR-FAB-MS $[M-H]^-$ *m*/*z* 335.0748 (calcd For $C_{16}H_{15}O_8$, requires 335.0767)

Compound ***11***: (yield: 37% calculated from the reacted part of **5**) amorphous powder; $[\alpha]_D^{24}$ +249.2 (*c* 2.0, MeOH); ^{1}H NMR (CD_3OD, 500 MHz) δ 2.14 (1H, t, *J* = 12.0 Hz, H-2a), 2.32 (1H, d, *J* = 12.0 Hz, H-2b), 4.26 (1H, dt, *J* = 12.0, 4.0 Hz, H-3), 5.35 (1H, t, *J* = 4.0 Hz, H-4), 5.91 (1H, d, *J* = 10.0 Hz, H-6), 6.00 (1H, dd, *J* = 4.0, 10.0 Hz, H-5), 6.31 (1H, d, *J* = 15.5 Hz, H-8′), 6.78 (1H, d, *J* = 8.0 Hz, H-5′), 6.94 (1H, dd, *J* = 1.5, 8.0 Hz, H-6′), 7.05 (1H, *J* = 2.0 Hz, H-2′), 7.60 (1H, d, *J* = 15.5 Hz, H-7′). ^{13}C NMR (CD_3OD, 125 MHz) δ 38.2 (C-2), 66.5 (C-3), 69.5 (C-4), 74.7 (C-1), 115.0 (C-2′), 115.2 (C- 8′), 116.5 (C-5′), 123.0 (C-6′), 127.8 (C-1′), 127.0 (C-5),135.2 (C-6), 146.8 (C-3′),147.1 (C-7′), 149.6 (C-4′), 168.9 (C-9′), 176.9 (C-7). ESI-MS (negative): *m*/*z* 335.0 $[M-H]^-$. HR-FAB-MS $[M-H]^-$ *m*/*z* 335.0760 (calcd For $C_{16}H_{15}O_8$, requires 335.0767).

Compound **6** (300 mg) was treated in 12 ml THF and 8 ml 2 N HCl at rt for 48 h. To the solution was added 10 ml H_2O and Na_2CO_3 powder till pH was around 5. The THF was evaporated and the water suspension was passed through ODS column (2 × 8 cm) eluted with 0.1% TFA H_2O–MeOH. The 20–50% MeOH eluted part was purified by preparative HPLC (TSKgel ODS 80Ts G001, 0.1% TFA H_2O–MeOH 20–100% 100 min at flow rate of 5 ml/min) to obtain **12** (20 mg) from 65 to 67.5 min. Compound **12** was further

purified on Sephadex LH 20 column (1.5 × 20 cm) to obtain pure **12** in 80% MeOH eluted part. The 50–100% MeOH eluted part from the above ODS column was separated on preparative HPLC of the same condition as above to afford **9** (90 mg) at 84–86 min, and recover **6** (70 mg) after 90 min.

Compound **9** *(yield: 39.4%)*: amorphous powder; $[\alpha]_D^{24}$ +248.1 (*c* 1.9, CH_3OH); ^1H NMR ($CDCl_3$, 500 MHz) δ 1.61 (3H, s) and 1.64 (3H, s) (2 × CH_3), 2.38 (1H, dd, *J* = 3.0, 14.0 Hz, H-2a), 2.47 (1H, dd, *J* = 10.0, 13.5 Hz, H-2b), 5.62 (1H, dt, *J* = 4.0, 10.0 Hz, H-3), 5.69 (1H, t, *J* = 4.0 Hz, H-4), 5.95 (1H, d, *J* = 10.0 Hz, H-6), 6.12 (1H, dd, *J* = 4.0, 10.0 Hz, H-5), 6.19 (1H, d, *J* = 16.0 Hz) and 6.30 (1H, d, *J* = 15.5 Hz) (H-8′,8″), 6.72 (1H, d, *J* = 8.5 Hz) and 6.76 (1H, d, *J* = 8.5 Hz) (H-5′,5″), 6.84 (1H, dd, *J* = 2.0, 8.0 Hz) and 6.92 (1H, dd, *J* = 2.0, 8.0 Hz) (H-6′,6″), 7.00 (1H, d, *J* = 2.0 Hz) and 7.05 (1H, d, *J* = 2.0 Hz) (H-2′,2″), 7.52 (1H, d, *J* = 16.0 Hz) and 7.56 (1H, d, *J* = 15.5 Hz) (H-7′,7″). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 28.6 and 28.7(2 × CH_3), 35.3 (C-2), 66.3 (C-4), 67.1 (C-3), 79.1 (C-1), 112.2 (C-1′), 114.4, 114.5 (C-8′,8″), 114.9 and 115.1 (C-2′,2″), 116.4 and 116.5 (C-5′,5″), 123.3 (C-6′,6″), 127.6 (C-1′,1″), 130.1 (C-5), 132.2 (C-6), 146.8 (C-3′,3″), 147.6 and 147.8 (C-7′,7″), 149.8 (C-4′,4″), 168.0 and 168.2 (C-9′,9″), 174.1 (C-7). ESI-MS (negative): *m*/*z* 537.1 $[M-H]^-$. HR-FAB-MS $[M-H]^-$ *m*/*z* 537.1415 (calcd For $C_{28}H_{25}O_{11}$, requires 537.1397).

Compound ***12***: (yield: 9.5%) amorphous powder; $[\alpha]_D^{24}$ +406.4 (*c* 0.39, CH_3OH); ^1H NMR ($CDCl_3$, 500 MHz) δ 2.29 (1H, t, *J* = 12.0 Hz, H-2a), 2.39 (1H, dd, *J* = 3.0, 12.0 Hz, H-2b), 5.54 (1H, dt, *J* = 4.0, 11.4 Hz, H-3), 5.67 (1H, m, H-4), 5.95 (2H, br s, H-5, 6), 6.19 (1H, d, *J* = 16.0 Hz) and 6.30 (1H, d, *J* = 16.0 Hz) (H-8′ and H-8″), 6.70 (1H, d, *J* = 8.5 Hz) and 6.77 (1H, d, *J* = 8.5 Hz) (H-5′,5″), 6.83 (1H, dd, *J* = 2.0, 8.0 Hz) and 6.93 (1H, dd, *J* = 2.0, 8.0 Hz) (H-6′,6″), 7.00 (1H, d, *J* = 2.0 Hz) and 7.05 (1H, d, *J* = 2.0 Hz) (H-2′,2″), 7.49 (1H, d, *J* = 16.0 Hz) and 7.56 (1H, d, *J* = 15.5 Hz) (H-7′,7″). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 35.3 (C-2), 66.3 (C-4), 68.7 (C-3), 74.4 (C-1), 114.7 and 114.8 and 114.9 and 115.1 (C-8′,8″,2′,2″), 116.4 and 116.5 (C-5′,5″), 123.3 and 123.3 (C-6′,6″), 126.8 (C-1′,1″), 127.6 (C-5), 136.0 (C-6), 146.8 (C-3′,3″), 147.6 and 147.8 (C-7′,7″), 149.7 and 149.8 (4′,C-4″), 168.0 and 168.5 (C-9′,9″), 176.5 (C-7). ESI-MS (negative): *m*/*z* 497.1 $[M-H]^-$. HR-FAB-MS $[M-H]^-$ *m*/*z* 497.1080 (calcd For $C_{25}H_{21}O_{11}$, requires 497.1084).

4.4. Synthesis of acetal chlorogenic acid derivatives 13 and 14

To the solution of chlorogenic acid (789 mg) and propionaldehyde (0.6 ml) in tetrahydrofuran (5 ml) in an ice bath, trimethylsilyl trifluoromethanesulfomate (TMSOTf, 0.5 ml) was slowly added. The mixture was stirred at rt overnight. After adding 10 ml ice and 20 drops of 1 N NaOH, the mixture was evaporated under vacuum to remove THF and TMSOTf and then the water suspension was applied to an ODS column (2 × 8 cm) eluted with gradient MeOH–H_2O to obtain the diacetal compound (238 mg, yield 25%) from 60% to 70% MeOH eluted part. The diacetal compound (188 mg) was deprotected with 0.4 N HCl in a MeOH–H_2O (8:2) solution at rt for 1 h. The mixture was neutralized with 1 N NaOH to pH 6 and was subjected to evaporation to remove MeOH. The residue was purified using an ODS column (2 × 7 cm) to obtain a mixture of **13** and **14** (60 mg) from 50% to 60% MeOH eluted part. A part of the mixture of **13** and **14** (30 mg) was applied to preparative HPLC (20 × 200 mm 5C18-AR-II Waters HPLC column) with a flow rate of 5 ml/min and the mobile phase of 40–70% MeOH in 60 min, 70–100% MeOH in 20 min to obtain **14** (10 mg) at 30–37 min and **13** (10 mg) at 41–43 min.

Compound ***13***: white solid (yield: 12%). $[\alpha]_D^{24}$ −3.2 (*c* 0.4, CH_3OH). ^1H NMR (CD_3OD, 500 MHz), δ 1.02 (3H, t, *J* = 7.5 Hz, H-3″), 1.86 (3H, m, H-2a, 2″), 1.96 (1H, dd, *J* = 6.5, 13.0 Hz, H-6a), 2.23 (1H, m, H-6b), 2.27 (1H, dd, *J* = 3.5, 14.0 Hz, H-2b), 3.73 (1H, dd, *J* = 3.5, 9.0 Hz, H-4), 4.21 (1H, br s, H-3), 5.35 (1H, m, H-5), 5.67 (1H, t, *J* = 4.5 Hz, H-1″), 6.29 (1H, d, *J* = 15.5 Hz, H-8′), 6.79 (1H, d, *J* = 8.5 Hz, H-5′), 6.94 (1H, dd, *J* = 2.0, 8.5 Hz, H-6′), 7.05 (1H, d, *J* = 2.0 Hz, H-2′), 7.59 (1H, d, *J* = 15.5 Hz, H-7′). ^{13}C NMR (CD_3OD, 125 MHz), δ 6.9 (C-3″), 28.2 (C-2″), 34.1 (C-2), 38.3 (C-6), 70.5 (C-3), 70.8 (C-4), 73.8 (C-5), 80.7 (C-1), 105.2 (C-1″), 115.1 and 115.2 (C-2′ and 8′), 116.5 (C-5′), 123.0 (C-6′), 127.8 (C-1′), 146.8 (C-4′), 147.2 (C-7′), 149.6 (C-3′), 168.9 (C-9′), 175.7 (C-7). Important NOE correlation: H-1″ and H-2. ESI-MS (negative): *m*/*z* 393.0 ($[M-H]^-$, 100%).

Compound ***14***: white solid (yield: 12%). $[\alpha]_D^{24}$ −36.8 (*c* 0.20, CH_3OH). ^1H NMR (CD_3OD, 500 MHz), δ 1.02 (3H, t, *J* = 7.5 Hz, H-3″), 1.77 (1H, dd, *J* = 11.5, 13.5 Hz, H-6a), 1.86 (2H, m, H-2″), 2.06 (1H, m, H-2a), 2.13 (1H, dd, *J* = 3.5, 14.5 Hz, H-2b), 2.41 (1H, m, H-6b), 3.75 (1H, dd, *J* = 3.5, 9.0 Hz, H-4), 4.26 (1H, br s, H-3), 5.35 (1H, m, H-5), 5.69 (1H, t, *J* = 4.5 Hz, H-1″), 6.28 (1H, d, *J* = 15.5 Hz, H-8′), 6.77 (1H, d, *J* = 8.5 Hz, H-5′), 6.94 (1H, dd, *J* = 2.0, 8.5 Hz, H-6′), 7.04 (1H, d, *J* = 2.0 Hz, H-2′), 7.59 (1H, d, *J* = 15.5 Hz, H-7′). ^{13}C NMR (CD_3OD, 125 MHz), δ 6.9 (C-3″), 28.1 (C-2″), 33.6 (C-2), 38.0 (C-6), 69.8 (C-3), 70.9 (C-4), 73.3 (C-5), 80.5 (C-1), 105.1 (C-1″), 115.1 (C-2′ and 8′), 116.5 (C-5′), 123.0 (C-6′), 127.8 (C-1′), 146.8 (C-4′), 147.3 (C-7′), 149.6 (C-3′), 168.9 (C-9′), 175.7 (C-7). Important NOE correlation: H-1″ and H-6. ESI-MS (negative): *m*/*z* 393.0 $[M-H]^-$.

4.5. Screening for anti-HIV-1 activity[9]

MT-4 cells were infected for 1 h with HIV-1 (HTLV-IIIB) at a 50% tissue culture infected dose ($TCID_{50}$) of 0.001/cell. The cells were then resuspended in RPMI-1640 medium at 1 × 10^5 cells/ml. The cell suspension was (200 μl/well) cultured for 5 days in 96-well plates containing various concentrations of the test compounds. Control assays were performed in the absence of test compound in HIV-1-infected and -uninfected cells. The inhibitory concentration (IC) at which the test compound completely inhibited the HIV-1- induced cytopathic effect was determined on day 5 through an optical microscope and the cell growth was visualized to give a cytotoxic concentration (CC) that reduced the viability of MT-4 cells.

4.6. Test for superoxide dismutase (SOD)-like activity

Superoxide dismutase (SOD)-like activity of the synthesized compounds was evaluated in 96-well plates using a SOD Assay Kit-WST (Dojindo Chemical, Kumamoto, Japan). To each well was added 10 μl of test compound solution (DMSO) and 100 μl of WST working solution. The reaction was initiated by the addition of 10 μl of xanthine oxidase solution. After incubating at 37 °C for 20 min, the absorbance at 450 nm was measured with an InterMed ImmunoReader (Nippon InterMed K.K. Tokyo, Japan). The SOD-like activity was calculated as: inhibition rate % = $\{[(A_{\text{blank 1}} - A_{\text{blank 3}}) - (A_{\text{compound}} - A_{\text{blank 2}})]/(A_{\text{blank 1}} - A_{\text{blank 3}})\} \times 100$, where blank 1 contained DMSO in place of compound solution; blank 2 contained buffer in place of enzyme; blank 3 contained DMSO and buffer only. Each compound was tested at 4 concentrations and the IC_{50} values were calculated by plotting the inhibition rate% against concentrations.

4.7. Test for radical scavenging activity against DPPH

Radical scavenging activity of the newly synthesized compounds was evaluated on 96-well plates. Each well contained 10 μl of compound solution (DMSO) and 190 μl of (0.1 mM) DPPH ethanol solution. The mixture was incubated for 20 min at rt and the absorbance at 540 nm was measured using a plate reader. The radical scavenging activity was calculated as (effective rate %) = $100 \times (A_{\text{control}} - A_{\text{compound}})/A_{\text{control}}$, where control contained DMSO in place of compound solution. Compounds were tested at 4 concentrations and the EC_{50} values were calculated by plotting the effective rate % against compound concentrations.

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Supplementary data

Supplementary data (NMR spectra of the synthesized new compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.11.043.

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