Structure–Activity Relationship of Caffeoylquinic Acids on the Accelerating Activity on ATP Production

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Caffeoylquinic acid (CQA) is one of the phenylpropanoids which have various bioactivities such as antioxidant, antibacterial, anticancer, antihistamic, and other biological effects. We previously reported that 3,5-di-*O*-caffeoylquinic acid inhibited amyloid $\beta_{1—42}$-induced cellular toxicity on human neuroblastoma SH-SY5Y cells and increased the mRNA expression level of glycolytic enzymes and the intracellular ATP level. To investigate structure–activity relationship on the accelerating activity on ATP production, we synthesized 1,4,5-tri-*O*-caffeoylquinic acid, 4,5-di-*O*-caffeoylquinic acid, 3,4,5-tri-*O*-caffeoylquinic acid, and other derivatives. Additionally, we evaluated intracellular ATP level in SH-SY5Y treated with each CQA derivative. As a result, 3,4,5-tri-*O*-caffeoylquinic acid showed the highest accelerating activity on ATP production among tested compounds. It was suggested that caffeoyl groups bound to quinic acid are important for activity and the more caffeoyl groups are bound to quinic acid, the higher accelerating activity on ATP production exhibits.

Key words caffeoylquinic acid; SH-SY5Y; accelerating activity on ATP production

Caffeoylquinic acid (CQA) is one of the phenylpropanoids found in coffee beans, sweetpotato, propolis, and other plants.[1—3] CQA derivatives have a variety of bioactivities such as antioxidant, antibacterial, anticancer, antihistamic, and other biological effects.[4—8] In our previous study, we demonstrated that 3,5-di-*O*-caffeoylquinic acid (3,5-di-CQA) inhibits amyloid $\beta_{1—42}$-induced cellular toxicity on human neuroblastoma SH-SY5Y cells, and increases the mRNA expression level of glycolytic enzyme, phosphoglycerate kinase 1 (PGK1) and the intracellular ATP level.[9] We also indicated that 3,5-di-CQA administration induced the improvement of spatial learning and memory on senescence accelerated-prone mice 8 (SAMP8), and the overexpression of PGK1 mRNA level.[9] Moreover, we found that 3,4,5-tri-*O*-caffeoylquinic acid (3,4,5-tri-CQA) showed higher accelerating activity on ATP production than 3,5-di-CQA, suggesting that the number of caffeoyl groups is important for the activity (unpublished observation). However, structure–activity relationship of CQAs on the accelerating activity on ATP production are not clarified in detail.

CQAs are classified into various derivatives, according to the number or the position of caffeoyl groups. The syntheses of mono-CQA derivatives including one caffeoyl group were reported by Sefkow *et al.*[10,11] There were, however, few reports about synthesis of CQA derivatives with more than two caffeoyl groups. Accordingly, we planed the syntheses of the CQA derivatives with more than two caffeoyl groups. We also evaluated the intracellular ATP level in SH-SY5Y cells treated with these derivatives for structure–activity relationship on the accelerating activity on ATP production.

Results and Discussion

Synthesis of 1,4,5-tri-*O*-Caffeoylquinic Acid CQA derivatives that caffeoyl groups bind to 3-, 4-, and 5-hydroxyl groups in quinic acid are found abundantly in natural resources. On the other hand, the derivatives that caffeoyl group binds to 1-hydroxyl group were rare in them. It was also interested whether they have the accelerating activity on ATP production. So, we initially synthesized 1,4,5-tri-*O*-caffeoylquinic acid (1,4,5-tri-CQA) as shown in Chart 1. Isopropyridene quinide (**2**) was synthesized from commercially available quinic acid (**1**), according to previously reported method.[12] Di-*O*-acetylcaffeoyl chloride (**b**) was used for followed esterification reaction. Acid chloride **b** was obtained from commercially available caffeic acid (**a**) in two steps, acetylation with Ac_2O in pyridine and chlorination with $SOCl_2$.[10] Isopropyridene quinide (**2**) reacted with acid chloride **b** in pyridine and CH_2Cl_2 to afford ester **3** in 50% yield.[11] The acetonide group of **3** was selectively cleaved with 90% trifluoroacetic acid (TFA) to give diol **4** in 61% yield. Diol **4** was heated with five equivalents of **c** under reflux in benzene for 24 h to afford tri-ester **5** in 86% yield. Hydrolysis of all protecting groups was accomplished with 1 M HCl at room temperature to obtain 1,4,5-tri-CQA (**6**) in 46% yield. To our knowledge, this is the first report that archive the synthesis of 1,4,5-tri-CQA.

Synthesis of 4,5-di-*O*-Caffeoylquinic Acid Next, we planed to synthesize 4,5-di-*O*-caffeoylquinic acid (4,5-di-CQA) by the introduction of protective group to 1-hydroxyl group (Chart 2). One regioselective synthesis of 4,5-di-CQA has been reported in the literature.[13] We attempted to improve the yield by changing the protective group to trichloroethylethoxy carbamate group that was specifically cleaved with zinc and AcOH.[14] The hydroxyl group at C-1 of isopropyridene quinide **2** was protected with trichloroethylethoxy carbamate to give compound **7** in 84% yield. The C-4,5-acetonide group of **7** was selectively removed by 90% TFA to afford diol **8** in 93% yield. In these results, the yields of **7** and **8** were improved, compared with previous literature.[14] Diol **8** was heated with four equivalents of **b** under reflux in benzene for 24 h to afford di-ester **9** in 50% yield. Cleavages of acetyl groups and lactone moiety by 2 M HCl at room temperature afforded compound **10** and 4,5-di-CQA (**11**) in 56% and 29% yield, respectively. Finally, the carbamate group was removed by zinc powder and AcOH to obtain 4,5-di-CQA (**11**) in 73% yield.

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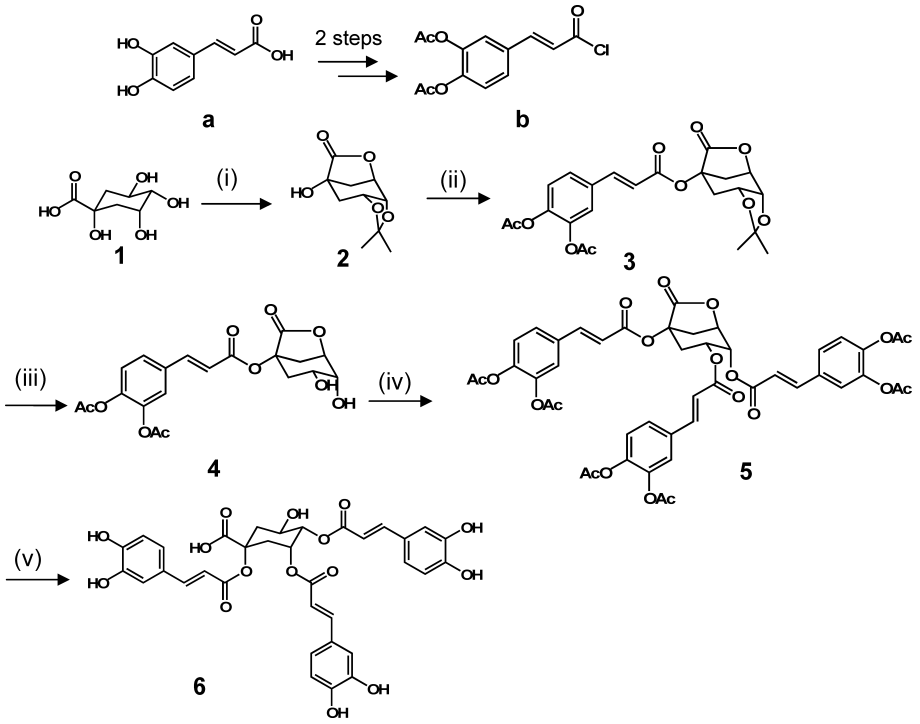
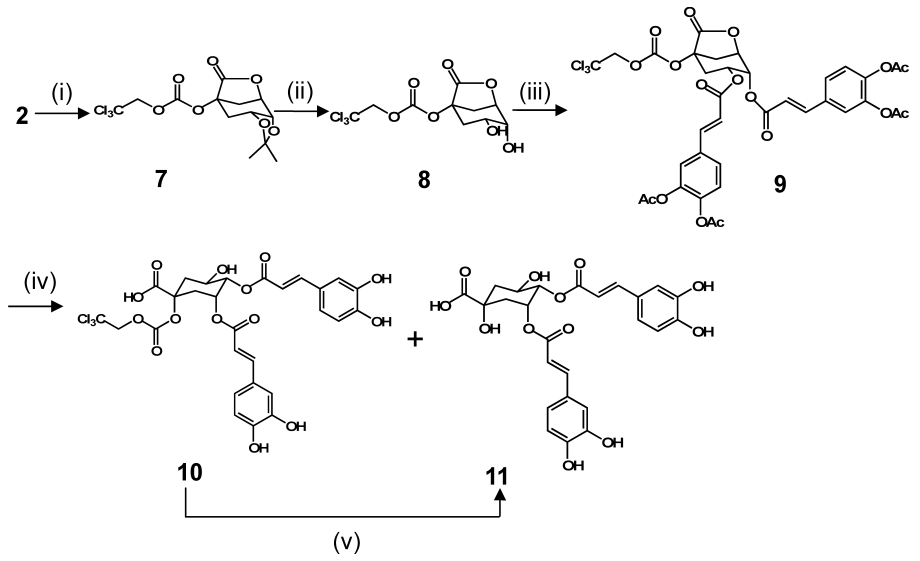


(i) ref. 12; (ii) **b**, DMAP, Pyr., CH_2Cl_2, rt, 4 h, 47%; (iii) 90% TFA, rt, 1 h, 61%; (iv) **b**, DMAP, Pyr., benzene, reflux, 24 h, 86%; (v) 1 M HCl, THF, rt, 12 d, 46%.

Chart 1



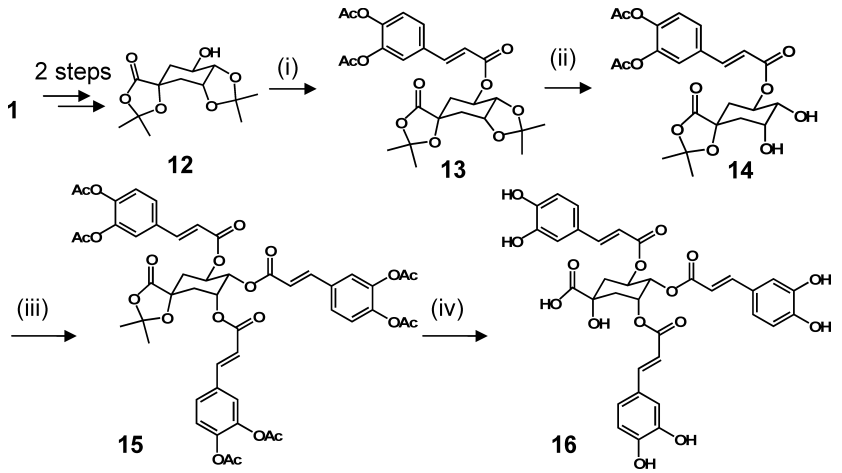
(i) trichloroethylchloroformate, Pyr., CH_2Cl_2, rt, 4 h, 84%; (ii) 90% TFA, rt, 80 min, 93%; (iii) **b**, DMAP, Pyr., benzene, reflux, 24 h, 50%; (iv) 2 M HCl, THF, rt, 14 d, 56% (**10**) and 29% (**11**); (v) Zn, AcOH, THF, rt, 26 h, 73%.

Chart 2

Synthesis of 3,4,5-tri-*O*-Caffeoylquinic Acid Then, we planed to synthesize 3,4,5-tri-CQA by introduction of caffeoyl groups to 4- and 5-hydroxyl groups subsequently to 3-hydroxyl group in quinic acid (Chart 3). Bisacetonide **12**, which was synthesized from quinic acid (**1**), was reacted with acid chloride **b** in pyridine and CH_2Cl_2 providing ester **13**, according to the previous report.[10] The 4,5-acetonide group of **13** was selectively removed with 0.4 M HCl in 80% MeOH and tetrahydrofuran (THF) to afford diol **14** in 69% yield. Diol **14** was heated with four equivalents of acid chloride **b** under reflux in benzene for 24 h to give tri-ester **15** in 40% yield. Hydrolysis of the acetonide and acetyl groups were accomplished with 2 M HCl at room temperature to obtain 3,4,5-tri-CQA (**16**) in 58% yield. To our knowledge, this is first report that archive the synthesis of 3,4,5-tri-CQA.

Syntheses of Other Chemical Derivatives Double bond moiety of chlorogenic acid was reduced by H_2 and Pd/C, providing reduced chlorogenic acid in equal quantity. 3,5-Di-CQA methyl ester and 3,4,5-tri-CQA methyl ester were synthesized according to the literature.[13]

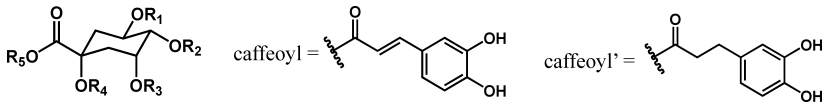
The Accelerating Activity on ATP Production Based on our previous study, CQA has a neuroprotective effect



(i) ref. 10; (ii) 0.4 M HCl, 80% MeOH, THF, 3.5 h, 69%; (iii) **b**, DMAP, Pyr., benzene, reflux, 24 h, 40%; (iv) 2 M HCl, THF, rt, 11 d, 58%.

Chart 3

Table 1. Structures of Test Compounds



Compound	R_1	R_2	R_3	R_4	R_5
Quinic acid	H	H	H	H	H
Caffeic acid	—	—	—	—	—
Chlorogenic acid	caffeoyl	H	H	H	H
Reduced chlorogenic acid	caffeoyl'	H	H	H	H
3,5-di-*O*-Caffeoylquinic acid	caffeoyl	H	caffeoyl	H	H
3,5-di-*O*-Caffeoylquinic acid methyl ester	caffeoyl	H	caffeoyl	H	CH_3
3,4,5-tri-*O*-Caffeoylquinic acid	caffeoyl	caffeoyl	caffeoyl	H	H
3,4,5-tri-*O*-Caffeoylquinic acid methyl ester	caffeoyl	caffeoyl	caffeoyl	H	CH_3
1,4,5-tri-*O*-Caffeoylquinic acid	H	caffeoyl	caffeoyl	caffeoyl	H
4,5-di-*O*-Caffeoylquinic acid	H	caffeoyl	caffeoyl	H	H

through the up-regulation of glycolytic enzyme expression and ATP production.[9] Levels of ATP in SH-SY5Y treated with each CQA derivative were evaluated for structure–activity relationship on energy production accelerating activity in neuronal cells. The structures of tested compounds are shown in Table 1. SH-SY5Y cells were treated with each compound and intracellular ATP levels were measured (Fig. 1).

ATP level in quinic acid treated cells was not significantly changed compared to control cells. In chlorogenic acid, 3,5-di-CQA, 3,4,5-tri-CQA, and 1,4,5-tri-CQA treated cells, each ATP level was significantly increased ($p<0.01$). Among these compounds, 3,4,5-tri-CQA showed the highest accelerating activity. In fact, ATP levels were significantly increased by 116±7.4% (5 μM), 124±9.2% (10 μM), and 114±6.6% (20 μM) compared to control cells ($p<0.01$). On the other hand, caffeic acid alone did not significantly increase ATP level. These results suggest that caffeoyl groups bound to quinic acid are important for activity, and the more caffeoyl groups are bound to quinic acid, the higher accelerating activity on ATP production exhibit. Reduced-chlorogenic acid did not significantly increase ATP level, so it was thought that double bond moiety in caffeoyl groups was important for the activity. In 3,5-di-CQA methyl ester and 3,4,5-tri-CQA methyl ester treated cells (5 μM), intracellular ATP levels were significantly increased ($p<0.01$), while in 20 μM-treated cells the ATP levels were decreased. Thus, it suggested that carboxyl group in quinic acid was important for the accelerating activity on ATP production.

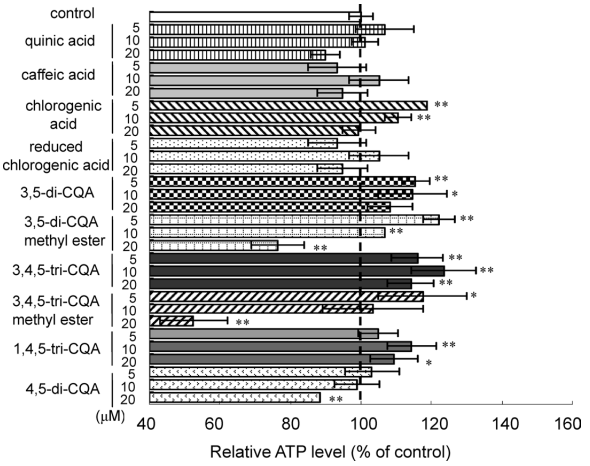


Fig. 1. Effects of CQA Derivatives on ATP Production of SH-SY5Y Cells

SH-SY5Y cells were treated with 5, 10, and 20 μM each compound for 48 h. Each bar represents the mean±S.D. ($n=12$). * Significant difference, $0.01<p<0.05$, ** Significant difference, $p<0.01$.

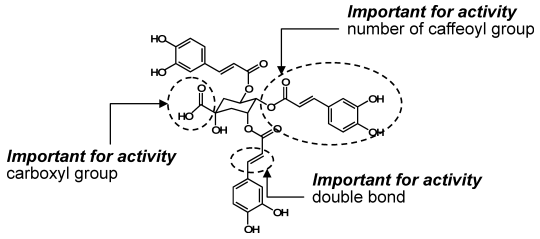


Fig. 2. Structure–Activity Relationship of Caffeoylquinic Acid on the Accelerating Activity on ATP Production

Experimental

General Experimental Procedure Optical rotations were recorded on a Jasco DIP-370. UV spectra were recorded on a HITACHI U-2000A spectrometer. IR spectra were recorded on a JASCO FT/IR-300 spectrometer. ^{1}H- and ^{13}C-NMR spectra were measured and recorded on a Bruker Avance 500 spectrometer in $CDCl_3$ and CD_3OD. The resonances of $CDCl_3$ at δ_H 7.26 and δ_C 77.0 and the resonance of CD_3OD at δ_H 3.35 and δ_C 49.8 were used as internal references for NMR spectra. High resolution-electrospray ionization-mass spectra (HR-ESI-MS) were recorded on a Waters Synapt G2 mass spectrometer.

1-*O*-Acetylcaffeoyl-1,5-quinide (4) Compound **3** (0.90 g, 1.9 mmol) was dissolved in 80% TFA (20 ml) at room temperature. The reaction mixture was stirred at room temperature for 1 h. Saturated $NaHCO_3$ aq (30 ml) was added at 0 °C, and aqueous phases were extracted with EtOAc (50 ml×2). The organic layer was washed with brine and dried over $MgSO_4$, filtered, and the solvents were removed *in vacuo*. The residue was purified by silica gel chromatography ($CHCl_3$: MeOH=98 : 2) to afford compound **4** (491 mg, 61%) as a colorless oil: ^{1}H-NMR (CD_3OD, 500 MHz) δ: 7.74 (1H, d, J=16.0 Hz), 7.58 (1H, dd, J=8.3, 2.1 Hz), 7.56 (1H, d, J=2.1 Hz), 7.31 (1H, d, J=8.3 Hz), 6.58 (1H, d, J=16.0 Hz), 4.08 (1H, m), 3.85 (1H, m), 3.10 (1H, m), 2.65 (1H, br d, J=11.1 Hz), 2.33 (3H, s), 2.31 (3H, s), 2.24 (1H, br d, J=11.1 Hz), 2.21 (2H, m). ^{13}C-NMR (CD_3OD, 125 MHz) δ: 175.5, 170.6, 170.4, 166.9, 146.5, 146.3, 144.9, 135.1, 128.6, 126.0, 125.0, 120.0, 79.4, 79.2, 67.9, 67.8, 38.5, 34.7, 21.2, 21.2. IR (KBr) cm^{-1}: 3448, 2921, 1793, 1774, 1735, 1637, 1213, 1180, 1056. UV λ_{max} (MeOH) nm (ε): 219 (18100), 283 (22700). HR-ESI-MS (positive ion) *m/z*: 443.0950 (M+Na)$^+$ (Calcd for $C_{20}H_{20}O_{10}Na$: 443.0954). $[\alpha]_D^{25}$ −19° (c=1.0, MeOH).

1,4,5-tri-*O*-Acetylcaffeoyl-quinide (5) To a solution of 1-*O*-acetylcaffeoyl-1,5-quinide (**4**, 180 mg, 0.43 mmol) and dimethyl aminopyridine (DMAP) (5 mg, 48 μmol) in dry benzene (30 ml) was added dry pyridine (1 ml) at room temperature under N_2 atmosphere. Acid chloride (**b**, 602 mg, 2.1 mmol) was added, and the reaction mixture was refluxed for 24 h. The solution was cooled to room temperature and quenched by the addition of 1 M HCl. The aqueous phase was extracted with EtOAc (20, 50 ml). The organic layer was washed with brine and dried over $MgSO_4$, filtered, and the solvent were removed *in vacuo*. The residue was purified by silica gel chromatography ($CHCl_3$: MeOH=98 : 2) to afford compound **5** (336 mg, 86%) as a colorless oil: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.69 (1H, d, J=15.9 Hz), 7.68 (1H, d, J=15.9 Hz), 7.60 (1H, d, J=15.9 Hz), 7.46—7.31 (6H, m), 7.24 (1H, d, J=8.2 Hz), 7.23 (1H, d, J=8.2 Hz), 7.13 (1H, d, J=8.2 Hz), 6.65 (1H, d, J=15.9 Hz), 6.42 (1H, d, J=15.9 Hz) 6.28 (1H, d, J=15.9 Hz), 5.71 (1H, m), 5.39 (1H, m), 5.01 (1H, m), 3.20 (1H, m), 2.77 (1H, br d, J=11.7 Hz), 2.51 (1H, br d, J=11.7 Hz), 2.48 (1H, m), 2.32 (3H, s), 2.32 (3H, s), 2.31 (3H, s), 2.31 (3H, s), 2.30 (3H, s), 2.30 (3H, s). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 171.0, 168.0, 168.0, 167.9, 164.8, 164.7, 164.6, 164.6, 164.5, 164.4, 145.0, 144.9, 144.8, 144.0, 143.9, 143.9, 143.7, 143.6, 143.5, 132.8, 132.7, 132.6, 126.8, 126.7, 126.6, 124.1, 124.0, 123.9, 123.0, 122.9, 122.8, 117.8, 117.7, 117.6, 76.4, 73.8, 66.1, 65.0, 34.2, 33.7, 20.7, 20.6, 20.5, 20.5, 20.4, 20.3. IR (KBr) cm^{-1}: 2940, 1774, 1718, 1654, 1637, 1207, 1110, 1014. UV λ_{max} (EtOAc) nm (ε): 278 (52500), 283 (52600). HR-ESI-MS (positive ion) *m/z*: 935.1994 (M+Na)$^+$ (Calcd for $C_{46}H_{40}O_{20}Na$: 935.2011). $[\alpha]_D^{25}$ +116° (c=1.0, EtOAc).

1,4,5-tri-*O*-Caffeoylquinic Acid (6) 1,4,5-tri-*O*-Acetylcaffeoyl quinide (**5**, 150 mg, 0.19 mmol) was dissolved in a mixture of THF (3 ml) and 1 M HCl (1 ml) at room temperature. The reaction mixture was stirred at room temperature, and progress was monitored by TLC. After 12 d, the solution was extracted with EtOAc (50 ml×2) and H_2O (50 ml). The combined organic phases were washed with brine and dried over $MgSO_4$, filtered, and the solvents were removed *in vacuo*. The residue was purified by Octadecylsilyl (ODS) chromatography (Cosmosil, OPN; MeOH : H_2O : AcOH= 30 : 70 : 1) to afford 1,4,5-tri-*O*-caffeoylquinic acid (**6**, 59.5 mg, 46%): ^{1}H-NMR (CD_3OD, 500 MHz) δ: 7.64 (1H, d, J=15.9 Hz), 7.57 (1H, d, J=15.9 Hz), 7.48 (1H, d, J=16.0 Hz), 7.09 (1H, br d, J=1.9 Hz), 7.04 (1H, br d, J=1.9 Hz), 6.94 (1H, m), 6.83 (2H, d, J=1.8 Hz), 6.78 (1H, m), 6.75 (1H, d, J=8.1 Hz), 6.55 (1H, br s), 6.51 (1H, d, J=8.2 Hz), 6.38 (1H, d, J=15.9 Hz), 6.27 (1H, d, J=15.9 Hz), 6.13 (1H, d, J=15.9 Hz), 5.70 (1H, br d, J=3.3 Hz), 5.03 (1H, dd, J=9.6, 3.4 Hz), 4.53 (1H, m), 2.94 (1H, m), 2.66 (1H, m), 2.54 (1H, m), 2.04 (1H, m). ^{13}C-NMR (CD_3OD, 125 MHz) δ: 175.4, 169.4, 169.1, 168.6, 150.6, 150.4, 150.2, 148.5, 148.4, 148.1, 147.7, 147.6, 147.3, 128.5, 128.3, 128.1, 124.0, 123.9, 122.6, 117.5, 117.4, 117.3, 117.2, 116.2, 116.1, 115.7, 115.6, 115.5, 81.8, 77.7, 70.8, 66.3, 42.5, 33.7. IR (KBr) cm^{-1}: 3421, 2911, 1700, 1685, 1637, 1608, 1284, 1164, 1110. UV λ_{max} (MeOH) nm (ε): 218 (38200), 244 (32500), 293 (36300), 313 (36600), 320 (36900). HR-ESI-MS (positive ion) *m/z*: 701.1497 (M+Na)$^+$ (Calcd for $C_{34}H_{30}O_{15}Na$: 701.1482). $[\alpha]_D^{25}$ −204° (c=1.0, MeOH).

1-*O*-(2,2,2-Trichloroethoxycarbonyl)-quinic Lactone (7) To a solution of compound **2** (500 mg, 2.3 mmol) in dry CH_2Cl_2 (15 ml) was added dry pyridine (1.5 ml) and 2,2,2-trichloroehylchloroformate (0.41 ml, 3.0 mmol). The reaction mixture was stirred at room temperature for 4 h. The solution was quenched by the addition of 1 M HCl, the aqueous phase was extracted with $CHCl_3$. The organic layer was washed with brine and dried over $MgSO_4$, filtered, and the solvent were removed *in vacuo*. The residue was recrystallized from MeOH to afford compound **7** (764 mg, 84%) as a colorless powder: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 4.81 (1H, d, J=11.8 Hz), 4.80 (1H, m), 4.72 (1H, d, J=11.8 Hz), 4.56 (1H, m), 4.33 (1H, ddd, J=6.5, 2.3, 1.3 Hz), 3.06 (1H, m), 2.66 (1H, br d, J=11.4 Hz), 2.56 (1H, ddd, J=14.6, 7.7, 2.4 Hz), 2.41 (1H, dd, J=14.7, 2.8 Hz), 1.56 (3H, s), 1.50 (3H, s), 1.33 (3H, s). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 172.4, 151.4, 110.0, 93.9, 78.7, 76.9, 75.3, 72.3, 71.0, 35.2, 30.1, 26.9, 24.2. IR (KBr) cm^{-1}: 2920, 2836, 1809, 1773, 1685, 1654, 1637, 1560, 1254, 1078. $[\alpha]_D^{25}$ −9° (c=1.0, EtOAc).

1-*O*-(2,2,2-Trichloroethoxycarbonyl-)-1,5-quinide (8) Compound **7** (0.70 g, 1.8 mmol) was dissolved in 90% TFA (7 ml) at room temperature. The reaction mixture was stirred at room temperature for 80 min. Saturated $NaHCO_3$ aq (30 ml) was added at 0 °C, and the aqueous phase was extracted with EtOAc (50 ml×2). The organic layer was washed with brine and dried over $MgSO_4$, filtered, and the solvents were removed *in vacuo*. The residue was purified by silica gel chromatography ($CHCl_3$: MeOH=98 : 2) to afford compound **8** (581 mg, 93%) as a colorless powder: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 4.93 (1H, dd, J=6.8, 4.8 Hz), 4.81 (1H, d, J=11.9 Hz), 4.74 (1H, d, J=11.9 Hz), 4.18 (1H, m), 4.02 (1H, td, J=11.2, 6.7 Hz), 3.05 (1H, ddd, J=11.6, 6.7, 3.2 Hz), 2.68 (1H, br d, J=11.2 Hz), 2.47 (1H, br d, J= 7 Hz), 2.38 (1H, ddd, J=11.6, 6.7, 3.2 Hz). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 171.9, 151.9, 94.3, 79.3, 77.4, 76.6, 66.2, 66.1, 36.8, 32.9. IR (KBr) cm^{-1}: 3404, 2939, 2856, 1782, 1685, 1654, 1637, 1560, 1248, 1039. $[\alpha]_D^{25}$ −13° (c=1.0, MeOH).

1-*O*-(2,2,2-Trichloroethoxycarbonyl)-4,5-di-*O*-acetylcaffeoyl Quinide (9) To a solution of compound **8** (580 mg, 1.7 mmol) in dry benzene (60 ml) was added dry pyridine (4 ml) at room temperature under N_2 atmosphere. Acid chloride (**b**, 1.88 g, 6.7 mmol) was added, and the reaction mixture was refluxed for 23 h. The solution was cooled to room temperature, and quenched by the addition of 1 M HCl. The aqueous phase was extracted with EtOAc (100, 150 ml). The organic layer was washed with brine and dried over $MgSO_4$, filtered, and the solvent were removed *in vacuo*. The residue was purified by silica gel chromatography (hexane : EtOAc=1 : 1) to afford compound **9** (770 mg, 50%) as a colorless oil: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.67 (1H, d, J=15.9 Hz), 7.59 (1H, d, J=16.0 Hz), 7.42—7.40 (2H, m), 7.33—7.30 (2H, m), 7.23 (1H, m), 7.18 (1H, d, J=8.3 Hz), 6.44 (1H, d, J=16.0 Hz), 6.28 (1H, d, J=16.0 Hz), 5.71 (1H, br d, J=4.7 Hz), 5.36 (1H, m), 5.02 (1H, m), 4.85 (1H, d, J=11.8 Hz), 4.75 (1H, d, J=11.8 Hz), 3.22 (1H, m), 2.70 (1H, br d, J=11.5 Hz), 2.55 (1H, m), 2.45 (1H, br d, J=11.8 Hz), 2.31 (3H, s), 2.31 (3H, s), 2.28 (3H, s), 2.27 (3H, s). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 170.0, 168.0, 167.9, 164.7, 164.7, 164.6, 164.6, 151.4, 145.1, 144.5, 144.0, 143.8, 142.5, 142.4, 132.8, 132.5, 126.8, 126.7, 124.1, 124.0, 122.9, 122.8, 117.6, 117.4, 93.9, 85.1, 78.6, 73.6, 65.9, 64.8, 33.7, 33.6, 20.6, 20.6, 20.5, 20.5. IR (KBr) cm^{-1}: 2923, 1810, 1774, 1718, 1654, 1637, 1241, 1207, 1180, 1035. UV λ_{max} (EtOAc) nm (ε): 278 (41600). HR-ESI-MS (positive ion) *m/z*: 863.0516 (M+Na)$^+$ (Calcd for $C_{36}H_{31}O_{17}Na^{35}Cl_3$, 863.0525). $[\alpha]_D^{25}$ +115° (c=1.0, EtOAc).

1-*O*-(2,2,2-Trichloroethoxycarbonyl-)-4,5-di-*O*-caffeoylquinic Acid (10) Compound **9** (690 mg, 0.82 mmol) was dissolved in a mixture of THF (9 ml) and 2 M HCl (6 ml) at room temperature. The reaction mixture was stirred at room temperature, and progress was monitored by TLC. After 14 d, the solution was extracted with EtOAc (100 ml×2) and H_2O (100 ml). The combined organic phases were washed with brine and dried over $MgSO_4$, fil-

tered, and the solvents were removed *in vacuo*. The residue was purified by ODS chromatography (Cosmosil, OPN; MeOH : H_2O=50 : 50) to afford compound **10** (320 mg, 56%) and 4,5-di-*O*-caffeoylquinic acid (**11**, 125 mg, 29%): ^{1}H-NMR (CD_3OD, 500 MHz) δ: 7.63 (1H, d, J=15.9 Hz), 7.58 (1H, d, J=15.9 Hz), 7.07 (1H, d, J=8.4 Hz), 7.05 (1H, d, J=8.4 Hz), 7.00 (1H, dd, J=8.2, 2.1 Hz), 6.89 (1H, dd, J=8.2, 2.1 Hz), 6.82 (1H, d, J=8.1 Hz), 6.75 (1H, d, J=8.1 Hz), 6.28 (1H, d, J=15.9 Hz), 6.27 (1H, d, J=15.9 Hz), 5.70 (1H, m), 5.03 (1H, dd, J=9.8, 3.5), 4.86 (1H, m), 4.54 (1H, d, J=12 Hz), 4.45 (1H, m), 2.92 (1H, dd, J=12.9, 3.2 Hz), 2.64—2.56 (2H, m), 2.07 (1H, dd, J=13.9, 11.3 Hz). ^{13}C-NMR (CD_3OD, 125 MHz) δ: 174.0, 169.4, 169.2, 154.5, 150.6, 150.4, 148.4, 148.2, 147.7, 147.6, 128.6, 128.5, 124.1, 124.0, 117.3, 117.2, 116.2, 116.0, 115.9, 115.8, 96.5, 85.1, 78.5, 77.4, 70.6, 66.0, 42.0, 33.6. IR (KBr) cm^{-1}: 3448, 2931, 1700, 1637, 1608, 1282, 1162, 1116. UV λ_{max} (MeOH) nm (ε): 218 (32100), 244 (23700), 298 (30700), 320 (35400). $[\alpha]_D^{25}$ −138° (c=1.0, MeOH).

4,5-di-*O*-Caffeoylquinic Acid (11) To a solution compound **10** (234 mg, 0.33 mmol) in THF (3 ml) was added zinc powder (132 mg) and AcOH (1.5 ml). The reaction mixture was stirred at room temperature for 26 h, and the solution was filtered off zinc, and evaporated. The residue was extracted with EtOAc (30 ml×3) and H_2O (30 ml). The combined organic phases were dried over $MgSO_4$, filtered and the solvents were removed *in vacuo*. The residue was purified by ODS chromatography (Cosmosil, OPN; MeOH : H_2O=30 : 70) to afford 4,5-di-*O*-caffeoylquinic acid (**11**, 122 mg, 73%): ^{1}H-NMR (CD_3OD, 500 MHz) δ: 7.61 (1H, d, J=15.8 Hz), 7.58 (1H, d, J=15.9 Hz), 7.07 (1H, d, J=10.0 Hz), 7.06 (1H, d, J=10.1 Hz), 6.97 (1H, dd, J=8.1, 1.9 Hz), 6.91 (1H, dd, J=8.1, 1.9 Hz), 6.81 (1H, d, J=8.1 Hz), 6.77 (1H, d, J=8.1 Hz), 6.34 (1H, d, J=15.8 Hz), 6.30 (1H, d, J=15.9 Hz), 5.68 (1H, m), 5.03 (1H, dd, J=8.9, 3.4 Hz), 4.41 (1H, td, J=9.4, 4.3 Hz), 2.39 (1H, dd, J=14.9, 3.7 Hz), 2.26 (1H, m), 2.19—2.10 (2H, m). ^{13}C-NMR (CD_3OD, 125 MHz) δ: 178.7, 169.4, 169.3, 150.4, 150.3, 148.2, 148.1, 147.6, 147.5, 128.6, 128.5, 124.0, 123.9, 117.3, 117.2, 116.0, 115.9, 115.8, 115.7, 77.3, 76.0, 70.9, 66.6, 42.7, 37.8. IR (KBr) cm^{-1}: 3448, 2923, 1718, 1700, 1654, 1637, 1282, 1182, 1116. UV λ_{max} (MeOH) nm (ε): 217 (20000), 244 (13900), 299 (18300), 326 (22100). HR-ESI-MS (positive ion) m/z: 539.1163 (M+Na)$^+$ (Calcd for $C_{25}H_{24}O_{12}Na$: 539.1165). $[\alpha]_D^{25}$ −111° (c=1.0, MeOH).

3-*O*-Acetylcaffeoyl-1,7-isopropyridene-quinide (14) To a solution compound **13** (750 mg, 1.5 mmol) in THF (5 ml) and 80% MeOH (15 ml) was added 0.4 M HCl (30 ml) at room temperature. The reaction mixture was stirred at room temperature for 3.5 h. The solution was extracted with EtOAc (100, 150 ml) and H_2O (150 ml). The combined organic phases were washed with brine and dried over $MgSO_4$, filtered and the solvent were removed *in vacuo*. The residue was purified by silica gel chromatography ($CHCl_3$: MeOH=98 : 2) to afford compound **14** (480 mg, 69%) as a colorless oil: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.65 (1H, d, J=15.9 Hz), 7.39 (1H, dd, J=8.4, 2.1 Hz), 7.35 (1H, d, J=2.1 Hz), 7.22 (1H, d, J=8.4 Hz), 6.40 (1H, d, J=15.9 Hz), 5.34 (1H, ddd, J=11.8, 9.8, 4.6 Hz), 4.24 (1H, dd, J=7.5, 3.4 Hz), 3.67 (1H, m), 2.87 (1H, m), 2.34 (1H, m), 2.31 (3H, s), 2.30 (3H, s), 2.22 (1H, dd, J=15.3, 3.3 Hz), 2.12 (1H, dd, J=15.3, 3.3 Hz), 1.68 (3H, s), 1.67 (3H, s). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 172.6, 168.0, 167.9, 166.6, 143.8, 143.6, 142.4, 133.0, 126.5, 124.0, 122.8, 118.7, 111.3, 79.8, 73.3, 70.1, 70.0, 38.0, 37.6, 28.6, 28.4, 20.6, 20.5. IR (KBr) cm^{-1}: 3448, 2923, 1774, 1718, 1706, 1654, 1637, 1209, 1182, 1112, 1016. UV λ_{max} (MeOH) nm (ε): 218 (18100), 278 (22300). HR-ESI-MS (positive ion) m/z: 501.1424 (M+Na)$^+$ (Calcd for $C_{23}H_{26}O_{11}Na$: 501.1373). $[\alpha]_D^{25}$ −7° (c=1.0, MeOH).

3,4,5-tri-*O*-Acetylcaffeoyl-1,7-isopropyridene-quinide (15) To a solution of compound **14** (170 mg, 0.36 mmol) and DMAP (5 mg, 40 μmol) in dry benzene (20 ml) was added dry pyridine (1 ml) at room temperature under N_2 atmosphere. Acid chloride (**b**, 410 mg, 1.4 mmol) was added and the reaction mixture was refluxed for 24 h. The solution was cooled to room temperature and quenched by the addition of 1 M HCl. The aqueous phase was extracted with EtOAc (30, 50 ml). The organic layer was washed with brine and dried over $MgSO_4$, filtered, and the solvent were removed *in vacuo*. The residue was purified by silica gel chromatography (hexane : EtOAc=2 : 3) to afford compound **15** (140 mg, 40%) as a colorless oil: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.69 (1H, d, J=15.9 Hz), 7.60 (1H, d, J=15.9 Hz), 7.59 (1H, d, J=15.9 Hz), 7.46 (1H, dd, J=8.4, 2.0 Hz), 7.43 (1H, m), 7.37 (1H, dd, J=8.4, 2.0 Hz), 7.34 (1H, d, J=2.0 Hz), 7.31 (1H, m), 7.31 (1H, m), 7.25 (1H, m), 7.20 (1H, d, J=8.3 Hz), 7.17 (1H, d, J=8.3 Hz), 6.49 (1H, d, J=15.9 Hz), 6.33 (1H, d, J=15.9 Hz), 6.32 (1H, d, J=15.9 Hz), 5.80 (1H, m), 5.56 (1H, m), 5.28 (1H, dd, J=10.1, 3.2 Hz), 2.48—2.40 (2H, m), 2.32 (3H, s), 2.31 (3H, s), 2.29 (3H, s), 2.28 (3H, s), 2.27 (3H, s), 2.26 (3H, s), 2.23—2.14 (2H, m), 1.64 (3H, s), 1.57 (3H, s), 1.56 (3H, s), 1.48 (3H, s). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 173.0, 168.1, 168.1, 168.0, 167.9, 167.8, 167.7, 165.7, 165.6, 165.5, 144.2, 144.0, 143.9, 143.8, 143.7, 143.6, 142.5, 142.4, 142.3, 133.0, 132.9, 132.8, 126.8, 126.7, 126.6, 124.0, 123.9, 123.8, 122.8, 122.7, 122.6, 110.9, 78.7, 72.1, 69.6, 67.1, 38.8, 35.6, 28.5, 28.3, 20.7, 20.6, 20.6, 20.5, 20.5, 20.4. IR (KBr) cm^{-1}: 3448, 2925, 1774, 1718, 1654, 1637, 1207, 1112, 1014. UV λ_{max} (EtOAc) nm (ε): 277 (52500). HR-ESI-MS (positive ion) m/z: 993.2419 (M+Na)$^+$ (Calcd for $C_{49}H_{46}O_{21}Na$: 993.2429). $[\alpha]_D^{25}$ −219° (c=1.0, EtOAc).

3,4,5-tri-*O*-Caffeoylquinic Acid (16) 3,4,5-tri-*O*-Acetylcaffeoyl-1,7-isopropyridene-quinide (**15**, 104 mg, 107 μmol) was dissolved in a mixture of THF (5 ml) and 2 M HCl (5 ml) at room temperature. The reaction mixture was stirred at room temperature, and progress was monitored by TLC. After 11 d, the solution was extracted with EtOAc (50 ml×2) and H_2O (50 ml). The combined organic phases were washed with brine, and dried over $MgSO_4$, filtered, and the solvents were removed *in vacuo*. The residue was purified by ODS chromatography (Cosmosil, OPN; MeOH : H_2O=50 : 50) to afford 3,4,5-tri-*O*-caffeoylquinic acid (**16**, 42 mg, 58%): ^{1}H-NMR (CD_3OD, 500 MHz) δ: 7.64 (1H, d, J=15.8 Hz), 7.58 (1H, d, J=15.7 Hz), 7.56 (1H, d, J=15.7 Hz), 7.10 (1H, m), 7.06 (1H, m), 7.03 (1H, m), 6.99 (1H, m), 6.98 (2H, m), 6.81 (1H, d, J=8.3 Hz), 6.79 (1H, d, J=8.3 Hz), 6.75 (1H, d, J=8.3 Hz), 6.36 (1H, d, J=15.8 Hz), 6.27 (1H, d, J=15.8 Hz), 6.24 (1H, d, J=15.8 Hz), 5.71 (2H, m), 5.36 (1H, dd, J=8.2, 3.0 Hz), 2.49—2.34 (4H, m). ^{13}C-NMR (CD_3OD, 125 MHz) δ: 178.1, 169.3, 169.1, 168.9, 150.5, 150.4, 150.4, 148.7, 148.5, 148.4, 147.6, 147.5, 147.2, 128.6, 128.5, 128.4, 117.3, 117.2, 117.1, 116.6, 116.0, 115.9, 115.8, 115.4, 115.1, 73.6, 73.5, 70.8, 70.0, 39.5, 37.5. IR (KBr) cm^{-1}: 3407, 2921, 1718, 1700, 1685, 1654, 1637, 1608, 1280, 1162, 1116. UV λ_{max} (MeOH) nm (ε): 218 (36800), 236 (27500), 244 (28600), 298 (34600), 323 (36900). HR-ESI-MS (positive ion) m/z: 701.1503 (M+Na)$^+$ (Calcd for $C_{34}H_{30}O_{15}Na$: 701.1482). $[\alpha]_D^{25}$ −314° (c=1.0, MeOH).

Reduced Chlorogenic Acid (18) Chlorogenic acid (100 mg, 0.28 mmol) was dissolved in EtOH (5 ml). Following to addition of Pd/C (20 mg), the reaction mixture was stirred at room temperature under a H_2 atmosphere. After 6 h, Pd/C was removed with celite and the filtrate was evaporated to afford reduced chlorogenic acid (100 mg, quant.) as a colorless oil: ^{1}H-NMR (CD_3OD, 500 MHz) δ: 6.71—6.69 (2H, m), 6.55 (1H, dd, J=8.0, 2.0 Hz), 5.29 (1H, ddd, J=9.7, 4.6 Hz), 4.15 (1H, m), 3.66 (1H, dd, J=9.0, 3.1 Hz), 2.81 (2H, br t, J=7.4 Hz), 2.63—2.60 (2H, m), 2.17—2.09 (2H, m), 2.03—1.94 (2H, m). ^{13}C-NMR (CD_3OD, 125 MHz) δ: 179.1, 175.3, 147.0, 145.4, 143.4, 121.3, 117.3, 117.2, 77.5, 74.8, 73.0, 72.7, 40.1, 39.3, 38.2, 32.2. IR (KBr) cm^{-1}: 3430, 2930, 1718, 1685, 1654, 1637, 1399, 1273, 1115. UV λ_{max} (MeOH) nm (ε): 208 (9100), 220 (5600), 283 (2700). HR-ESI-MS (positive ion) m/z: 379.1001 (M+Na)$^+$ (Calcd for $C_{16}H_{20}O_9Na$: 379.1005). $[\alpha]_D^{25}$ −53° (c=1.0, MeOH).

Cells and Cell Culture Human dopaminergic neuroblastoma SH-SY5Y cell line was obtained from American Type Culture Collection (ATCC). Culture were maintained in DMEM (Sigma, St. Louis. MO, U.S.A.) mediujm supplemented with 50% F-12 (GIBCO, Carlsbad, CA, U.S.A.), 15% fetal bovine serum (FBS) and 1% penicillin (5000 U/ml)–streptomycin (5000 μg/ml) in 100 mm fibronectin coating dish (BD, Biocoat, Franklin Lakes, NJ, U.S.A.). The cells were grown at 37 °C in 5% CO_2.

Measurement of Intracellular ATP Content Intracellular ATP level was assessed by firefly bioluminescence using the luminescence luciferase assay kit (TOYO Ink, Tokyo, Japan). SH-SY5Y cells were cultured in 100 μl test medium at a density of 2.0×10^4 cells/well in fibronectin coated 96 well micro-plate (BD, Biocoat). After 24 h incubation at 37 °C, 5% CO_2, cells were treated with each compound dissolved 100 μl of OPTI-MEM (GIBCO). After 48 h, the medium was changed to 100 μl of OPTI-MEM without test compounds. ATP content was measured according to the manufacturer's protocol.

Statistical Analysis Statistical differences between means were assessed for significance using the Student's t-test. The value of $p<0.05$ was considered significant.

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