Supplementary Material

Synthesis, Antioxidant Activity, and Density Functional Theory Study of Catechin Derivatives†

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1. Materials and instruments

(-)-epicatechin (EC), (-)-epicatechin-3-gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epigallocatechin-3-gallate (EGCG) were purchased from Shanghai Yuanye Biological Technology Co., Ltd (Shanghai, China). All other reagents and solvents involved in the synthesis were of analytical grade and were used as received without further purification. Column chromatography (CC) was performed on flash silica gel (200–300 mesh; Qingdao Makall Group Co., Ltd; Qingdao; China). All reactions were monitored using thin-layer chromatography (TLC) on silica gel plates. The structure of all synthesized compounds was confirmed by ^{1}H-NMR and ^{13}C-NMR spectra, recorded on a Bruker AV-400 or DRX-500 (Bruker BioSpin GmbH, Rheinstetten, Germany) instrument, using tetramethylsilane as an internal standard. MS data were obtained in the electrospray ionization (ESI) mode on an API QStar Pulsar instrument. High-resolution MS data were obtained in the ESI mode on an LCMS-IT-TOF instrument (Shimadzu, Kyoto, Japan).

2. Chemical Experimental Section

2.1. General procedure for the synthesis of permethylated epicatechin derivatives (**5**–**8**)[1]

A mixture of epicatechins **1**–**4** (1.0 mm), Me_2SO_4 (10.0 mmol) and anhydrous K_2CO_3 (10.0 mmol) in dry acetone (10 mL) was refluxed for 2 h with stirring until TLC showed the reaction to be complete. After removal of inorganic salts by filtration, the filtrate was concentrated and the residue was purified by CC (etroleum ether/ethyl acetate = 4:1→2:1) to afford the products **5**–**8**.

*2.1.1. (2R,3R)-2-(3,4-dimethoxyphenyl)-5,7-dimethoxychroman-3-ol (**5**)*

Yield: 98%; ^{1}H-NMR (CD_3OD, 500 MHz) δ 7.06 (d, 1H, J = 1.9 Hz, $C^{2'}$-H), 7.00–7.03 (m, 1H, $C^{6'}$-H), 6.94 (s, 1H, $C^{5'}$-H), 4.90 (s, 1H, C^{2}-H), 4.19–4.21 (m, 1H, C^{3}-H), 3.84 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 2.89 (dd, 1H, J = 4.6 Hz, 17.3 Hz, C^{4}-H_a), 2.80–2.83 (m, 1H, C^{4}-H_b); ^{13}C-NMR (CD_3OD, 125 MHz) δ 160.9 (C-7), 160.5 (C-5), 157.0 (C-9), 150.1 (C-3′), 150.0 (C-4′), 133.5 (C-1′), 120.3 (C-6′), 112.4 (C-5′), 111.9 (C-2′), 102.0 (C-10), 79.9 (C-2), 67.3 (C-3), 56.5 (OCH_3), 56.4 (OCH_3), 55.9 (OCH_3), 55.7 (OCH_3), 29.4 (C-4); ESIMS: *m/z* 369 $[M + Na]^+$.

*2.1.2. (2R,3R)-2-(3,4-dimethoxyphenyl)-5,7-dimethoxychroman-3-yl 3,4,5-trimethoxybenzoate (**6**)*

Yield: 96%; ^{1}H-NMR (CD_3OD, 500 MHz) δ 7.16 (s, 2H, $C^{2''}$-H, $C^{6''}$-H), 7.00 (s, 1H, $C^{2'}$-H), 6.81 (d, 2H, J = 8.4 Hz, $C^{5'}$-H, $C^{6'}$-H), 6.23 (d, 1H, J = 2.3 Hz), 6.10 (d, 1H, J = 2.3 Hz), 5.61–5.62 (m, 1H, C^{2}-H), 5.11 (s, 1H, C^{3}-H), 3.76–3.85 (m, 21H, 7 × OCH_3), 3.03–3.04 (m, 2H, C^{4}-CH_2); ^{13}C-NMR (CD_3OD, 100 MHz) δ 165.1 (C=O), 159.6 (C-7), 158.8 (C-5), 155.5 (C-9), 152.7 (C-3″, C-5″), 148.7 (C-3′), 148.7 (C-4′), 142.0 (C-4″), 130.3 (C-1′), 125.0 (C-1″), 118.9 (C-6′), 110.7 (C-5′), 109.7 (C-2′), 107.0 (C-2″, C-6″), 100.0 (C-10), 93.1 (C-8), 91.7 (C-6), 77.4 (C-2), 68.8 (C-3), 60.8 (OCH_3), 56.1 (OCH_3), 56.1 (OCH_3), 55.7 (OCH_3), 55.6 (OCH_3), 55.3 (OCH_3), 55.3 (OCH_3), 25.7 (C-4); ESIMS: *m/z* 563 $[M + Na]^+$.

*2.1.3. (2R, 3R)-5,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-ol (**7**)*

Yield: 98%; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 6.73 (s, 2H, $C^{2'}$-H, $C^{6'}$-H), 6.19 (d, 1H, J = 2.3 Hz), 6.10 (d, 1H, J = 2.3 Hz), 4.92 (s, 1H, C^{2}-H), 4.27–4.28 (m, 1H, C^{3}-H), 3.74–3.88 (m, 15H, 5 × OCH_3), 2.85–2.97 (m, 2H, C^{4}-CH_2); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 159.6 (C-7), 159.1 (C-5), 154.9 (C-9), 153.3 (C-3′, C-5′), 137.5 (C-4′), 133.9 (C-1′), 103.1 (C-2′, C-6′), 100.2 (C-10), 93.2 (C-8), 92.1 (C-6), 78.6 (C-2), 66.3 (C-3), 60.7 (OCH_3), 56.1 (OCH_3), 56.1 (OCH_3), 55.4 (OCH_3), 55.3 (OCH_3), 28.0 (OCH_3); ESIMS: *m/z* 399 $[M + Na]^+$.

*2.1.4. (2R,3R)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl) chroman-3-yl-3,4, 5-trimethoxybenzoate (**8**)*

Yield: 98%; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.17 (s, 2H, $C^{2''}$-H, $C^{6''}$-H), 6.70 (s, 2H, $C^{2'}$-H, $C^{6'}$-H), 6.24 (d, 1H, J = 2.3 Hz), 6.12 (d, 1H, J = 2.3 Hz), 5.66 (brs, 1H, C^{2}-H), 5.08 (s, 1H, C^{3}-H), 3.71–3.85 (m, 24H, 8 × OCH_3), 3.04–3.05 (m, 2H,

C^4-CH_2); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 165.2 (C=O), 159.7 (C-7), 158.9 (C-5), 155.5 (C-9), 153.2 (C-3'', C-5''), 152.8 (C-3', C-5'), 142.5 (C-4''), 137.8 (C-4'), 133.4 (C-1'), 125.1 (C-1''), 107.2 (C-2'', C-6''), 103.9 (C-2', C-6'), 100.2 (C-10), 93.2 (C-8), 91.9 (C-6), 77.8 (C-2), 68.7 (C-3), 60.9 (OCH_3), 60.8 (OCH_3), 56.3 (OCH_3), 56.2 (OCH_3), 56.0 (OCH_3), 55.9 (OCH_3), 55.8 (OCH_3), 55.4 (OCH_3), 26.0 (C-4); ESIMS: *m/z* 593 $[M + Na]^+$.

*2.2. Synthesis of (2R,3R)-5, 7-bis(benzyloxy)-2-(3,4,5-tris(benzyloxy)phenyl)chroman-3-yl 3,4,5-tris(benzyloxy)benzoate (**9**)*[2]

To a solution of EGCG (458 mg, 1.0 mmol) in dry dimethylformamide (DMF, 10.0 mL) under nitrogen at –60 ºC, NaH (60% dispersion in mineral oil, 200 mg, 5.0 mmol) was added. After 2 h, BnBr (0.9 mL, 8.0 mmol) was added via a syringe. The mixture was stirred at –60 ºC for an additional 30 min and then at room temperature for 24 h. The reaction was quenched by adding HCl (1 N, 2.0 mL) and water (15.0 mL). The aqueous layer was extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with water and brine, then dried over Na_2SO_4 and evaporated *in vacuo*. The residue was purified by CC (petroleum ether/ethyl acetate = 5:1) to afford the product **9** (250.0 mg, 25%). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.20–7.34 (m, 42H, Ac-H), 6.73 (s, 2H), 6.39 (s, 1H, *J* = 2.3 Hz), 6.34 (s, 1H, *J* = 2.3 Hz), 5.66 (s, 1H), 5.05–4.90 (m, 13H, Ac-CH_2), 4.79 (d, 1H, *J* = 11.0 Hz), 4.67 (d, 1H, *J* = 11.0 Hz), 3.12 (dd, 1H, *J* = 4.6 Hz, 17.5 Hz, C^4-H_a), 3.06 (dd, 1H, *J* = 2.5 Hz, 17.5 Hz, C^4-H_b); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 164.8 (C=O), 158.8 (C-5), 158.0 (C-9), 155.7 (C-7), 152.9 (C-3'', C-5''), 152.4 (C-3', C-5'), 142.7 (C-4''), 137.8 (C-4'), 137.5, 137.4, 136.9, 136.8, 136.8, 136.8, 136.4, 136.4, 133.2 (C-1'), 128.6, 128.5, 128.4, 128.4, 128.3, 128.2, 128.1, 128.1, 128.0, 127.9, 127.8, 127.8, 127.8, 127.7, 127.5, 127.5, 127.4, 127.3, 125.0 (C-1''), 109.1 (C-2'', C-6''), 106.7 (C-2', C-6'), 101.0 (C-10), 94.6 (C-8), 94.0 (C-6), 78.0 (C-2), 75.1, 75.0, 71.3, 71.2, 71.2, 71.1, 70.2, 70.0, 68.3 (C-3), 25.6 (C-4); ESIMS: *m/z* 1201 $[M + Na]^+$.

*2.3. Synthesis of (2R,3R)-5,7-bis(benzyloxy)-2-(3,4,5-tris(benzyloxy)phenyl)chroman-3-ol (**10**)*[2]

To a solution of compound **9** (756.0 mg, 1.0 mmol) in MeOH (25.0 mL), NaOH (40 mg, 1.0 mmol) was added. The mixture was stirred at room temperature for 4 h, then it was concentrated, water was added, and the mixture was extracted with EtOAc (3 × 20.0 mL). The combined organic layers were dried over Na_2SO_4, and evaporated *in vacuo*. The residue was purified by CC (petroleum ether/ethyl acetate = 4:1) to afford the product **10** (680.4 mg, 90%). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.17–7.44 (m, 27H, Ac-H), 6.72 (s, 2H), 6.28 (s, 2H), 5.12 (s, 1H), 4.85–5.04 (m, 11H, Ac-CH_2), 3.12 (dd, 1H, *J* = 4.6 Hz, 17.5 Hz, C^4-H_a), 3.06 (dd, 1H, *J* = 2.5 Hz, 17.5 Hz, C^4-H_b); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 156.3 (C-5), 156.1 (C-9), 153.0 (C-3', C-5'), 152.8 (C-7), 142.1 (C-4''), 137.9 (C-4'), 137.3, 137.3, 137.2, 137.1, 137.0, 134.0 (C-1'), 125 (C-1''), 105.6 (C-2', C-6'), 101.1 (C-10), 91.6 (C-8, C-6), 78.2 (C-2), 75.2, 71.1, 71.0, 70.6, 70.1, 66.3 (C-3), 28.2 (C-4); ESIMS: *m/z* 483 $[M + Na]^+$.

*2.4. Synthesis of (2R,3R)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl 3,4,5-trimethoxybenzoate (**11**)*

To a solution of compound **10** (1.0 mmol), 4-Dimethylaminopyridine (DMAP, 0.1 mmol), and *N*,*N*'-dicyclohexylcarbodiimide (DCC, 0.3 mmol) in CH_2Cl_2 was added with 3,4,5-trimethoxybenzoic acid (0.2 mmol). The solution was stirred at room temperature for 12 h and the insoluble urea was filtered. The filtrate was concentrated under reduced pressure to provide a crude product, which was directly used for the next step. A solution of the above crude product (1.0 mmol) in MeOH (2.0 mL) was hydrogenated over 5% Pd/C (20 mg) for 4 h at room temperature. The mixture was filtered and the filtration residue was washed with MeOH (10 mL). The combined filtrates were evaporated *in vacuo*. The residue was purified by CC (petroleum ether/ethyl acetate = 1:1) to afford the product **11** (260.0 mg, 52%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 6.62 (s, 2H), 5.91–5.95 (m, 3H), 4.84 (s, 1H), 3.86–3.93 (m, 9H, 3 × OCH_3), 2.77–2.86 (m, 2H, C^4-CH_2); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 166.1 (C=O), 158.0 (C-7),

157.7 (C-5), 157.6 (C-9), 154.4 (C-3″, C-5″), 151.1 (C-3′, C-5′), 143.8 (C-4″), 139.2 (C-4′), 131.8 (C-1′), 126.0 (C-1″), 108.7 (C-2″, C-6″), 107.1 (C-2′, C-6′), 100.0 (C-10), 96.4 (C-8), 95.9 (C-6), 79.6 (C-2), 67.5 (C-3), 61.2 (OCH_3), 56.7 (OCH_3), 56.6 (OCH_3), 29.0 (C-4); ESIMS: *m/z* 499 $[M - H]^-$.

*2.5. Synthesis of (2R,3R)-5,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)chroman-3-yl 3,4,5-trihydroxybenzoate (**12**)*

To a solution of compound **7** (1.0 mmol), DMAP (0.1 mmol), and DCC (0.3 mmol) in CH_2Cl_2, gallic acid (0.2 mmol) was added. The solution was stirred at room temperature for 12 h, and the insoluble urea was filtered. The filtrate was concentrated under reduced pressure. The residue was purified by CC (petroleum ether/ethyl acetate = 1:1) to afford the product **12** (179.5 mg, 34%). ^{1}H-NMR (CD_3OD, 500 MHz) δ 7.39 (d, 2H, *J* = 1.8 Hz, $C^{2''}$-H, $C^{6''}$-H), 7.25 (d, 2H, *J* = 1.8 Hz, $C^{2'}$-H, $C^{6'}$-H), 7.20 (s, 2H, C^6-H, C^8-H), 5.18 (s, 1H, C^2-H), 4.58 (s, 1H, C^3-H), 3.68–3.80 (m, 15H, 5 × OCH_3), 2.85–2.98 (m, 2H, C^4-CH_2); ^{13}C-NMR (CD_3OD, 125 MHz) δ 166.6 (C=O), 159.7 (C-7), 158.9 (C-5), 151.5 (C-9), 147.4 (C-3″, C-5″), 146.5 (C-3′, C-5′), 143.9 (C-4″), 140.5 (C-4′), 140.1 (C-1″, C-1′), 110.0 (C-10), 93.2 (C-8), 92.1 (C-6), 56.3 (OCH_3), 56.3 (OCH_3), 52.5 (OCH_3), 52.5 (OCH_3), 51.6 (OCH_3), 21.1 (C-4); ESIMS: *m/z* 551 $[M + Na]^+$.

3. Computational Studies

All computational studies were carried out using the Gaussian 09 software package.[3,4] The structure of each prepared compound was fully optimized using the B3LYP/6-311++G (d, p)[5-7] computational level. A starting structure for the DFT calculations was prepared using the GaussView 5.0.8 software.[8]

4. Procedure for Antioxidant Activity Study

The antioxidant activity of the catechins and their derivatives was measured by DPPH assay. DPPH free radical scavenging capability was tested by the method described by Hung.[9] First, DPPH (10 mg) was mixed with 200 mL of ethanol. After storage at room temperature for 30 min, the absorbance of the reaction mixture was recorded at 519 nm against a blank.

The percentage of inhibition of DPPH radical scavenging activity (*I*%) was calculated according to the following equation:

$$\text{Scavenging activity (\%)} = [(A_{blank} - A_{sample})/A_{blank}] \times 100 \quad (1)$$

Here, A_{sample} is the absorbance of a sample solution, and A_{blank} is the absorbance of the blank solution (containing all reagents except the test sample). The IC_{50} value is the effective concentration that could scavenge 50% of the DPPH radicals.

References and notes

1. F. Hashimoto, G. Nonaka, I. Nishioka. *Chem. Pharm. Bull.* 1989, **37**, 77.
2. K. Osanai, C. Huo, K. R. Landis-Piwowar, Q. P. Doub, T. H. Chan, *Tetrahedron* 2007, **63**, 7565.
3. M. Frisch, G. Trucks, H. Schlegel, G. Scuseria, M. Robb, J. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. Petersson. Fox Gaussian 09, Revision C. 01; Gaussian Inc.: Wallingford, CT, USA, 2013.
4. M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, €O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, D.J. Fox. Gaussian 09, Revision B.01, Gaussian, Inc., Wallingford CT, 2009.
5. A. D. Becke. *Phys. Rev. A* 1988, **38**, 3098.
6. T. Yanai, D.P. Tew, N.C. Handy. *Chem. Phys. Lett.* 2004, **393**, 51.
7. P. J. Stephens, F. J. Devlin, C. F. Chabalowski, M. J. Frisch. *J. Phys. Chem.* 1994, **98**, 11623.

8. D. Roy, K. Todd, M. John. Gauss View; Version 5; Semichem, Inc.: Shawnee Mission, KS, USA, 2009.
9. P. V. Hung, T. Maeda, K. Miyatake, N. Morita. *Food Res. Int.* 2009, **42**, 185.