BioChem

MDPI

Supplementary data

Fluorinated Derivatives of Digalloyl-Flavan-3-ol Induce Autophagic Cell Death by Forming Granular Aggregates Containing Mitochondria

Ryo Doge *, Yuki Nishino and Akiko Saito *

Graduate School of Engineering, Osaka Electro-Communication University (OECU), 18-8 Hatsu-cho, Neyagawa, Osaka 572-8530, Japan

* Correspondence: de20a001@oecu.jp (R.D.); a-saito@osakac.ac.jp (A.S.); Tel.: +81-72-824-1131(A.S.)

*Supplementary data 1. Synthesis fluorinated compounds **3** and **4***

Compounds **3** and **4** were synthesized and secured as shown in Scheme S1 and used in cell assays. The purity of the compound was confirmed to be 95% or more using ^{1}H-NMR. The syntheses of compounds **1** and **2** have already been reported in the paper [10]. Since the syntheses of compounds **7** to **10** have already been reported in Scheme 1S, the synthetic methods and compound data of compounds **11**, **12**, **3** and **4** are shown here.

Supplementary data 1-1. Reagents for synthesis

Special grade solvents and reagents were used for the synthesis without further purification. Synthetic reactions were tracked by thin-layer chromatography 60F$_{254}$Art 5715 (Merch, Darmstadt, Germany) and compounds were purified by silica gel column chromatography (SilicaGel 60N, spherical, neutral, 63-210 μm, KANTO Co. Tokyo, Japan). ^{1}H-NMR spectra (DD2-400 MHz, Agilent, CA, USA) were used to confirm the structure and purity of the each compound.

*Supplementary data 1-2. Synthesis of compound **11***

Under an argon atmosphere, compound **9** (50 mg, 0.079 mmol) was dissolved in dehydrated dichloromethane (20 ml), and 3,4,5-trifluorobenzoic acid (57 mg, 0.32 mmol) and EDC (46 mg, 0.24 mmol) and a catalytic amount of DMAP (1 mg, 8.1 μmol) were added and stirred. Then, water was added and the mixture was extracted with chloroform and dried over anhydrous magnesium sulfate. After filtration and concentration under reduced pressure, purification was performed by silica gel chromatography (*n*-hexane/EtOAc, 50/1 45/1 40/1 35/1 30/1 25/1 10/1 5/1 1/1) to give compound **11** (69 mg, 0.073 mmol, 92%).

$[\alpha]_D^{24}$ –25.3 (c 2.8, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) 7.82 (2H, t, *J* = 6.7 Hz), 7.48 (2H, t, *J* = 6.8 Hz), 6.897 (1H, dd, *J* = 2.0, 8.8 Hz), 6.896 (1H, d, *J* = 2.0 Hz), 6.79 (1H, d, *J* = 8.8 Hz), 6.52 (1H, d, *J* = 2.3Hz), 6.37 (1H, d, *J* = 2.3 Hz), 5.60-5.55 (1H, m), 5.14 (1H, br s), 3.09 (1H, dd, *J* = 4.5, 17.4 Hz), 2.89 (1H, d, *J* = 17.4 Hz), 0.99 (9H, s) 0.94 (18H, s), 0.25 (3H, s), 0.25 (3H, s), 0.154 (3H, s), 0.145 (3H, s), 0.12 (3H, s), 0.11 (3H, s); ^{13}C-NMR (100 MHz, $CDCl_3$) 163.0, 161.8, 155.7, 155.6, 152.6-152.2(m), 150.1-149.7 (m), 149.8, 1 147.1, 147.0, 130.2, 125.8-125.9(m), 125.1-125.9(m), 125.1-125.0 (m), 121.2, 119.3, 119.2, 115.2-114.9(m), 114.6-114.3(m), 107.5, 106.7, 104.9, 77.1, 69.2, 26.2, 25.98, 25.96, 25.8, 18.6, 18.5, 18.3, –4.01, –4.03, –4.07,–4.11, –4.29, –4.33.

*Supplementary data 1-3. Synthesis of compound **3***

Compound **11** (22 mg, 0.023 mmol) was dissolved in dehydrated THF (20 ml) under argon atmosphere, and AcOH (7 μl, 0.12 mmol) and TBAF (24 μl, 0.083 mmol) were added dropwise at 0 °C. and agitate. After that, AcOH (14 μl, 0.24 mmol) was added dropwise, and after concentration under reduced pressure, silica gel chromatography (*n*-hexane/EtOAc, 10,1 9/1 8/1 7/1 6/1 5/1 4/1 3 /1 2/ 1/1) to give compound **3** (14 mg, 0.023 mmol, 100%).

$[\alpha]_D^{25}$ –34.8 (c = 1.1, CH_3OH); ^{1}H-NMR (400 MHz, CD_3OD) 7.93 (2H, t, *J* = 7.0 Hz), 7.54 (2H, t, *J* = 7.0 Hz), 6.93 (1H, d, *J* = 2.0 Hz), 6.78 (1H, dd, *J* = 2.0, 8.2 Hz), 6.70 (2H, d, *J* = 8.2 Hz), 6.44 (1H, d, *J* = 2.3 Hz), 6.33 (1H, d, *J* = 2.3 Hz), 5.57 (1H, dd, *J* = 1.9, 4.3 Hz), 5.17 (1H, br s), 3.11 (1H, dd, *J* = 4.3, 17.3 Hz), 2.84 (1H, dd, *J* = 1.9, 17.3 Hz); ^{13}C-NMR (100 MHz, CD_3OD) 164.3, 163.2, 158.5, 157.2, 151.5, 146.24, 146.23, 130.5, 118.8, 116.2, 114.6, 116.4-115.1 (m), 104.3, 103.7, 102.4, 78.5, 71.0, 24.

*Supplementary data 1-4. Synthesis of compound **12***

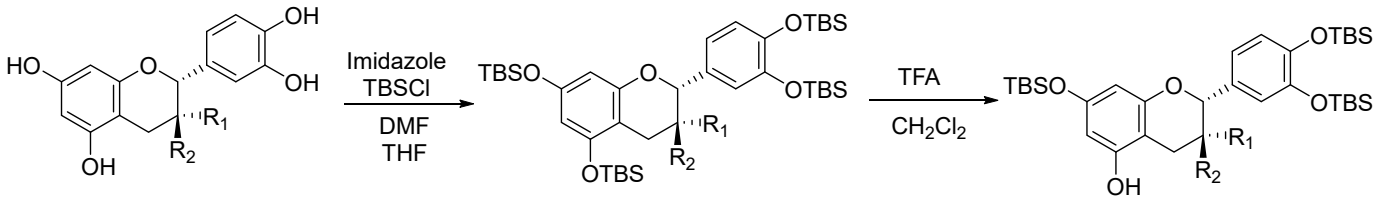
Under an argon atmosphere, compound **10** (173 mg, 00.27 mmol) was dissolved in dehydrated dichloromethane (20 ml), and 3,4,5-trifluorobenzoic acid (190 mg, 1.10 mmol) and EDC (190 mg, 1.08 mmol) and a catalytic amount of DMAP were added and stirred. Then, water was added and the mixture was extracted with chloroform and dried over anhydrous magnesium sulfate. After filtration and concentration under reduced pressure, purification was performed by silica gel chromatography (*n*-hexane/EtOAc, 50/1 45/1 40/1 35/1 30/1 25/1 10/1 5/1 1/1) to give compound **12** (252 mg, 0.27 mmol, 97%).

$[\alpha]_D^{24}$ –10.8 (c 0.19, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) 7.80 (2H, t, *J* = 6.6 Hz), 7.50 (2H, t, *J* = 6.7 Hz), 6.86-6.77 (3H, m), 6.47 (1H, d, *J* = 1.9 Hz), 6.37 (1H, d, *J* = 1.9 Hz), 5.45 (1H, ddd, *J* = 5.0, 6.4, 6.7 Hz), 5.13 (1H, d, *J* = 6.4 Hz), 2.93 (1H, dd, *J* = 5.0, 16.4 Hz), 2.72 (1H, dd, *J* = 6.7, 16.4 Hz), 0.98 (9H, s), 0.94 (9H, s), 0.93 (9H, s), 0.23 (3H, s), 0.16 (3H, s), 0.15 (3H, s), 0.12 (3H, s), 0.10 (3H, s), ^{13}C-NMR (100 MHz, $CDCl_3$) 162.9, 161.7, 155.8, 155.2, 149.5, 147.4, 147.3, 130.2, 121.4, 119.7, 119.3, 115.1-114.3 (m), 107.5, 106.4, 105.4, 78.3, 70.4, 25.997, 25.980, 25.7, 24.3, 18.6, 18.5, 18.3, 0.38, –4.00, –4.08, –4.14, –4.30, –4.32; , –4.1 (d), –4.3.

*Supplementary data 1-5. Synthesis of compound **4***

Compound **12** (75 mg, 0.079 mmol) was dissolved in dehydrated THF (20 ml) under argon atmosphere, and AcOH (23 μl, 0.40 mmol) and TBAF (82 μl, 0.28 mmol) were added dropwise at 0 °C. and agitate. After that, AcOH (23 μl, 0.40 mmol) was added dropwise, and after concentration under reduced pressure, silica gel chromatography (*n*-hexane/EtOAc, 10,1 9/1 8/1 7/1 6/1 5/1 4/1 3 /1 2/ 1/1) to give compound **4** (43 mg, 0.071 mmol, 90%).

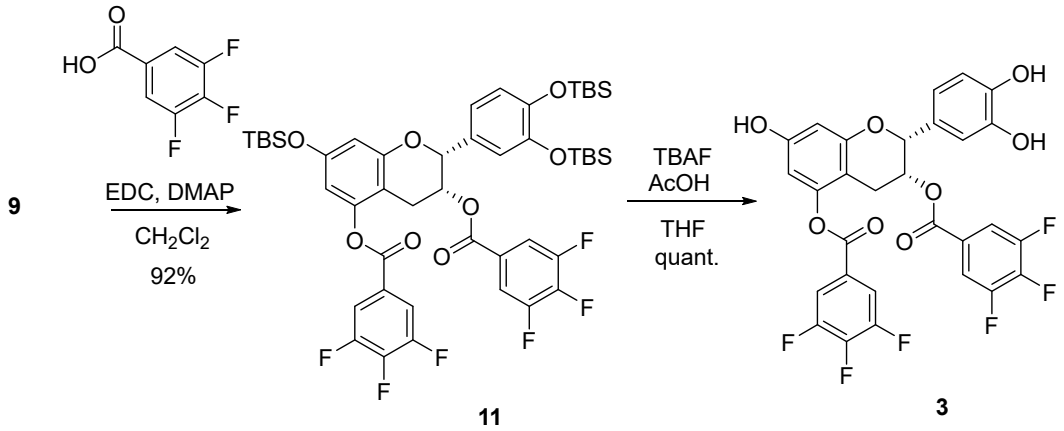
$[\alpha]_D^{22}$ +44.3 (c 1.8, CH_3OH); ^{1}H-NMR (400 MHz, CD_3OD) 7.90 (2H, dd, *J* = 6.7, 7.9 Hz), 7.58 (2H, dd, *J* = 6.8, 8.0 Hz), 6.83 (1H, br s), 6.72 (1H, br s), 6.71 (1H, br s), 6.38 (1H, d, *J* = 2.3 Hz), 6.32 (1H, d, *J* = 2.3 Hz), 5.41 (1H, ddd, *J* = 5.1, 6.4, 6.6 Hz), 5.15 (1H, d, *J* = 6.4 Hz), 2.86 (1H, dd, *J* = 5.1, 16.3 Hz), 2.74 (1H, dd, *J* = 6.6, 16.3 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) 164.02-164.0 (m), 163.2-163.1 (m), 158.7, 156.7, 153.7-150.9 (Cx2, m), 151.2, 146.6, 146.5, 146.2-140.9 (Cx2, m), 130.4, 127.6-126.7 (Cx2, m), 119.2, 116.3, 116.0-115.1 (Cx2, m), 114.4, 104.8, 104.0, 102.3, 79.5, 72.0, 24.7.

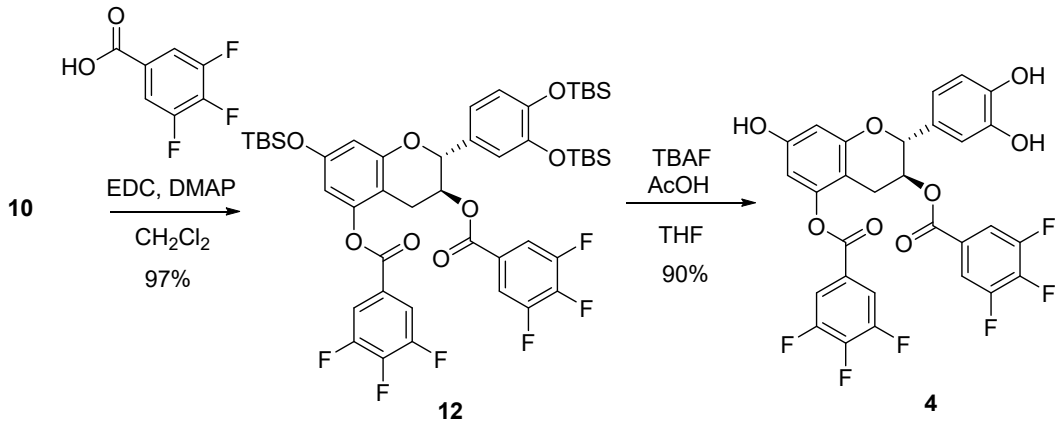


R_1 = OH, R_2 = H: (–)-epicatechin (**5**)
R_1 = H, R_2 = OH: (–)-catechin (**6**)

7: R_1 = OH, R_2 = H (51%)
8: R_1 = H, R_2 = OH (46%)

9: R_1 = OH, R_2 = H (31%)
10: R_1 = H, R_2 = OH (51%)





Scheme S1. Synthesis of fluorinated compounds **3** and **4**.

Table S1. ANOVA statistical processing results for cell viability in Figure 3.

vs. DMSO	*p* Value	
	HeLa cells	A549 cells
DMSO+BAF	***p<0.001	***p<0.001
1	***p<0.001	***p<0.001
1+BAF	***p<0.001	***p<0.001
2	0.0083*	***p<0.001
2+BAF	***p<0.001	***p<0.001
3	***p<0.001	***p<0.001
3+BAF	***p<0.001	***p<0.001
4	***p<0.001	***p<0.001
4+BAF	***p<0.001	***p<0.001

*Supplementary data 3. Simple measurement of the effects of compounds **1**-**4** on the cell cycle*

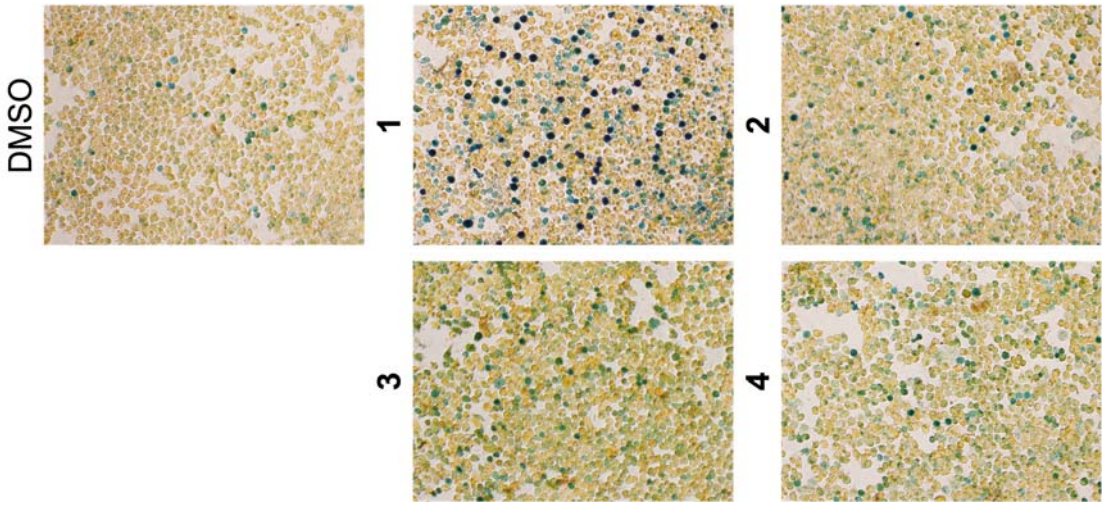


Figure S1. Simple evaluation of effects of compounds **1** to **4** on cell cycle using Cell-Clock™. HeLa S3 cells treated with 25 μM synthetic flavan-3-ol derivatives for 24 h. The four major phases of the cell cycle stained Cell-Clock™ for 15 minutes and observed. Stained yellow cells represent the G0/G1 phase cells. Stained green cells represent the S phase cells. Stained dark blue cells represent the G2/M phase cells.

*Supplementary data 4. Compound **4** forms granular aggregates at high concentrations*

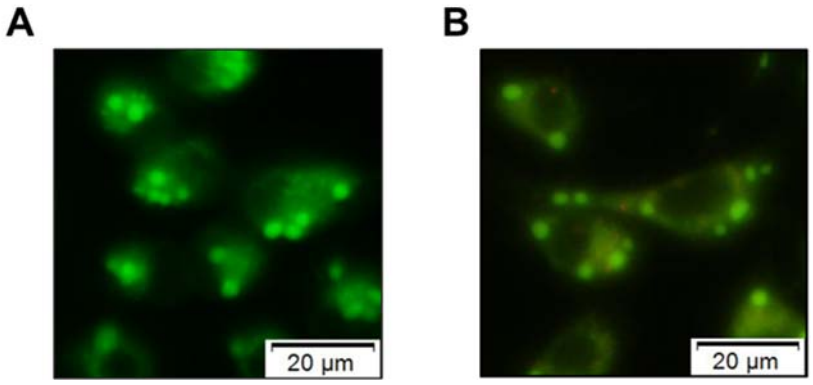


Figure S2. Compound **4** forms granular aggregates at high concentrations. HeLa(A) and A549(B) cells treated with 60 μM synthetic flavan-3-ol derivatives **4** for 1 h. The mitochondrial membrane potential stained JC-1 for 15 minutes and observed fluorescence microscope.