Electronic Supplementary Information (ESI)

Lysine-targeting inhibition of amyloid β oligomerization by a green perilla-derived metastable chalcone in vitro and in vivo

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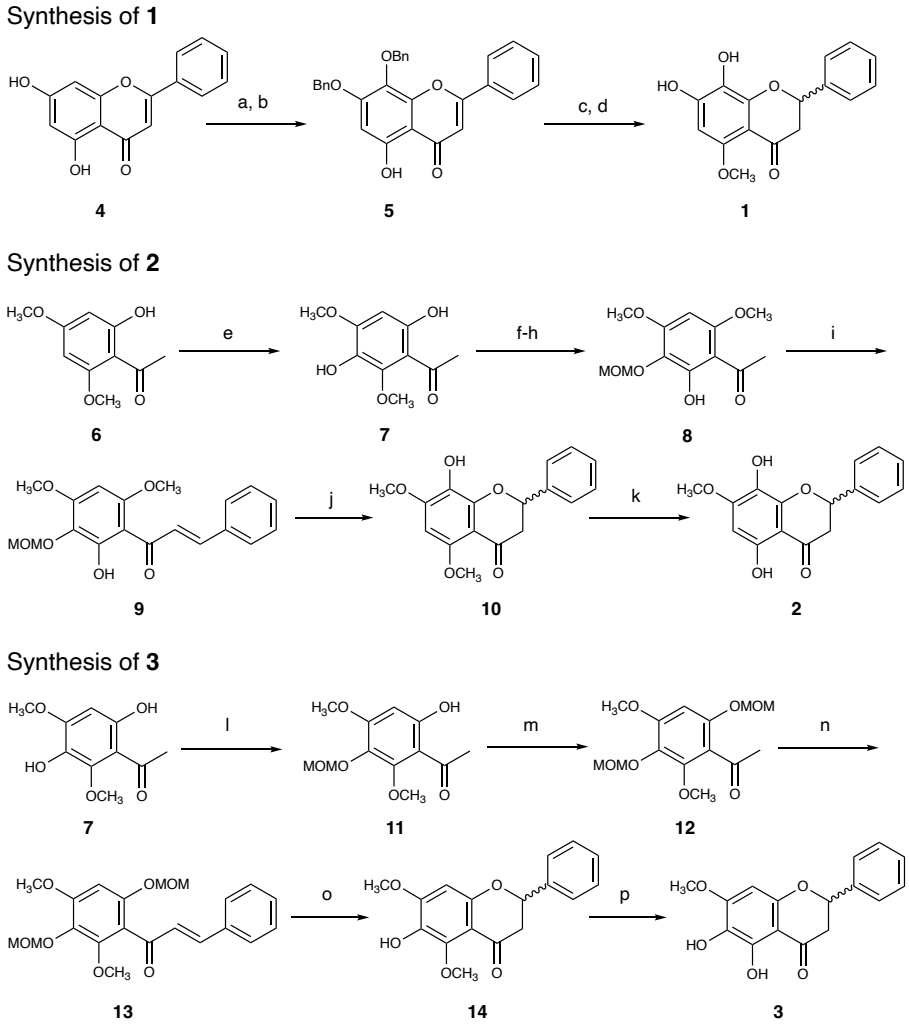
- Experimental procedures for synthesis of **1**–**3**
- ^{1}H and ^{13}C NMR spectra of **1**–**3**
- Figure S1–S6

Synthetic schemes of 1–3



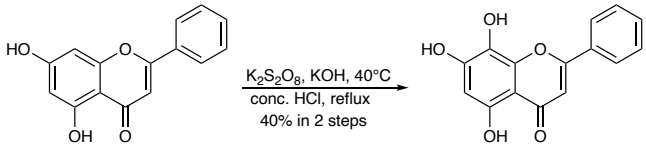
(a) (1) $K_2S_2O_8$, KOH, 40 °C, 2.5 h; (2) conc. HCl, 103 °C, 4 h; 40% in 2 steps. (b) BnBr, K_2CO_3, DMF, 100 °C, 2 h, 77%. (c) CH_3I, K_2CO_3, acetone/DMF, 59 °C, 17.5 h, 76%. (d) H_2, Pd/C (4 atm), THF, 100 °C, 4.5 h, 11%. (e) (1) $K_2S_2O_8$, KOH, 40 °C, 1.5 h; (2) conc. HCl, 103 °C, 3.5 h; 22% in 2 steps. (f) BBr_3, CH_2Cl_2, rt, 51 °C, 7.5 h. (g) MOMCl, (*i*-Pr)$_2$EtN, CH_2Cl_2, 0 °C, 19 h, 29%. (h) CH_3I, K_2CO_3, acetone, reflux, 4 h, 35%. (i) benzaldehyde, NaOH, EtOH, rt, 44 h, quant. (j) AcOH, reflux, 20.5 h, 25%. (k) $AlCl_3$, CH_3CN, reflux, 2 h, 49%. (l) MOMCl, (*i*-Pr)$_2$EtN, $CHCl_3$, 50 °C, 2 h, 83%. (m) MOMCl, NaOH, Bu_4NBr, CH_2Cl_2, rt, 18 h, 84%. (n) benzaldehyde, NaOH, EtOH, rt, 123 h, 76%. (o) AcOH, reflux, 5.5 h, 72%. (p) $AlCl_3$, CH_3CN, reflux, 2 h, 36%.

Experimental procedures for synthesis of 1–3

General remarks

The following spectroscopic and analytical instruments were used: Optical rotation, P-2000 Digital Polarimeter (Jasco, Tokyo, Japan); FT/IR, FT/IR-470 Plus (Jasco); ^{1}H and ^{13}C NMR, AVANCE III 400 and AVANCE III 500 (Reference: $Si(Me)_4$, Bruker, Billerica, MA, USA); HPLC, Model 600E with a Model 2487 UV detector (Waters, Milford, MA); HR-FAB-MS, JMS-MS700V (JEOL, Tokyo, Japan). HPLC was carried out on a YMC-Pack ODS-A AA12S05-1520WT (YMC Co., Ltd., Kyoto, Japan). Silica gel column chromatography was performed with Wakogel C-200 (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) or Kieselgel 60 (Merck, Darmstadt, Germany). Flash column chromatography was performed with a Model pump 800E with Model prep UV-10 UV detector (Yamazen, Osaka, Japan) using Wakogel C-200 as the stationary phase. Analytical thin-layer chromatography was performed with TLC Silica gel 60 F254 or TLC Silica gel 60 RP-18 F254 (Merck). All other chemicals and reagents were purchased from FUJIFILM Wako Pure Chemical Industries, NACALAI TESQUE, INC. (Kyoto, Japan) or Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan), and used without further purification.

*Synthesis of **1***

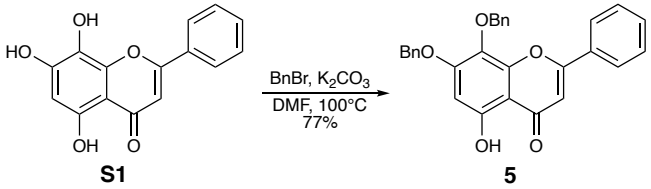


Compound S1: To a solution of chrysin (**4**) (934 mg, 3.67 mmol) in 10% potassium hydroxide (w/v) (7.05 mL, 14.0 mmol, 3.8 equiv.) was added a solution of potassium persulfate (2.08 g, 7.71 mmol, 2.1 equiv.) in water (30 mL) dropwise at 0 °C. After stirring for 2.5 h at 40 °C, the mixture was washed with EtOAc (40 mL x 3). The resultant aqueous layers were added sodium hydrogen sulfite (1.80 g, 17.3 mmol, 4.7 equiv.), and acidified to pH 1 with con.HCl. The reaction mixture was refluxed for 4 h and extracted with EtOAc (50 mL x 3). The combined organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexane: EtOAc : AcOH = 80 : 20 : 0.5) to afford **S1** (394 mg, 5.50 mmol, 40%).

R_f (silica, EtOAc/hexane = 1:1) = 0.60.

^{1}H NMR (500 MHz, DMSO-d_6, 0.037 M, 297 K): δ 6.30 (s, 1H), 6.94 (s, 1H), 7.57–7.62 (m, 3H), 8.15–8.17 (m, 2H), 8.84 (s, 1H), 10.55 (s, 1H), 12.26 (s, 1H) ppm.

HR-ESI-MS: *m/z*, 269.0456 ($[M–H]^-$, calcd for $C_{15}H_9O_5$ 269.0450).

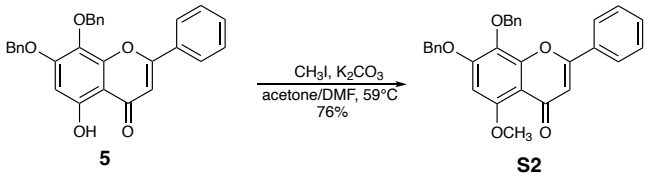


Compound 5: To a solution of **S1** (180 mg, 0.67 mmol) in DMF (2.20 mL) was added K_2CO_3 (193 mg, 1.40 mmol, 2.1 equiv.). The mixture was stirred for 10 min at room temperature and added BnBr (166 μL, 1.40 mmol, 2.1 equiv.). After stirring for 2 h at 100 °C under Ar, the reaction was quenched with water (10 mL) and K_2CO_3 (193 mg) in MeOH (2.2 mL). The mixture was filtered to afford **5** (230 mg, 0.51 mmol, 77%).

$\mathbf{R}_f$ (silica, EtOAc/hexane = 1:1) = 0.78.

^{1}H NMR (500 MHz, $CDCl_3$, 0.044 M, 297 K): δ 5.10 (s, 2H), 5.22 (s, 2H), 6.51 (s, 1H), 6.65 (s, 1H), 7.28–7.30 (m, 3H), 7.38–7.54 (m, 10H), 7.81–7.84 (m, 2H), 12.56 (s, 1H, 5-OH) ppm.

HR-ESI-MS: *m/z,* 449.1395 ([M–H]$^-$, calcd for $C_{29}H_{21}O_5$ 449.1389).

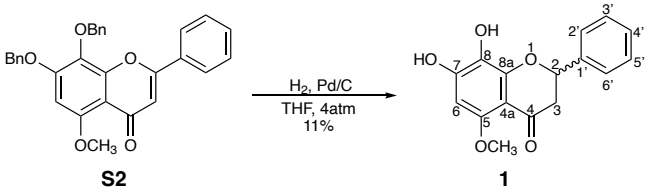


Compound S2: To a solution of **5** (154 mg, 0.34 mmol) in acetone (2.0 mL) and DMF (2.0 mL) was added potassium carbonate (235 mg, 1.70 mmol, 5.0 equiv.). The reaction mixture was stirred for 10 min at room temperature and iodomethane (106 μL, 1.70 mmol, 5.0 equiv.) was added. After stirring for 17.5 h at 59 °C under Ar atmosphere. The reaction was quenched with water (15 mL) and extracted with $CHCl_3$ (20 mL x 3). The combined organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexane : EtOAc = 75 : 25) to afford **S2** (121 mg, 0.26 mmol, 76%).

$\mathbf{R}_f$ (silica, EtOAc/hexane = 1:1) = 0.19.

^{1}H NMR (500 MHz, $CDCl_3$, 0.044 M, 297 K): δ 3.91 (3H, s), 5.12 (2H, s), 5.27 (2H, s), 6.49 (1H, s), 6.68 (1H, s), 7.30–7.33 (3H, m), 7.37–7.51 (10H, m), 7.81–7.84 (2H, m) ppm.

HR-ESI-MS: *m/z*, 465.1691 ([M+H]$^+$, calcd for $C_{30}H_{25}O_5$ 465.1702).



Compound 1: Compound **S2** (54.8 mg, 0.12 mmol) was dissolved in THF (2.4 mL), and Pd/C (38 mg, 0.30 equiv.) was added. The mixture was stirred under hydrogen at the pressure of 4 atm for 4.5 h at room temperature. The reaction mixture was filtered thorough Sep-Pak Silica and concentrated *in* vacuo. The residue was purified by HPLC [column, YMC Pack ODS-A (20 mm

i.d. x 150 mm; YMC); solvent, 30% MeCN/H_2O (0.1% TFA), flow rate, 8.0 mL/min; UV detector, 254 nm] to afford **1** (3.6 mg, 12.6 μmol, 11%).

R_f (silica, EtOAc/hexane = 2:1) = 0.39.

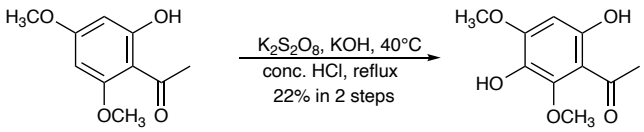
^{1}H NMR (500 MHz, CD_3OD, 0.025 M, 297 K): δ 2.78 (1H, dd, *J* = 3.0, 16.7 Hz, 3-H), 3.04 (1H, dd, *J* = 12.0, 16.7 Hz, 3-H), 3.78 (3H, s, 5-OCH_3), 5.50 (1H, dd, *J* = 2.8, 11.9 Hz, 2-H), 6.17 (1H, s, 6-H), 7.34–7.37 (1H, m, 4'-H), 7.39–7.42 (2H, m, 3'-H, 5'-H), 7.54–7.55 (2H, m, 2'-H, 6'-H) ppm.

^{13}C NMR (125 MHz, CD_3OD, 0.025 M, 297 K): δ 46.5 (C-3), 56.3 (5-O*C*H_3), 80.8 (C-2), 94.3 (C-6), 106.0 (C-4a), 127.7 (C-2', C-6'), 128.0 (C-8), 129.7 (C-3', C-4', C-5',), 140.6 (C-1'), 153.2 (C-8a), 155.3 (C-7), 156.8 (C-5), 192.3 (C-4) ppm.

HR-ESI-MS: *m/z*, 285.0771 ([M–H]$^-$, calcd for $C_{16}H_{13}O_5$ 285.0763).

IR (KBr): 3215, 2970, 1666, 1598, 1516, 1455, 1357, 1281, 1201, 1095, 925, 883, 820 cm^{-1}.

*Synthesis of **2***



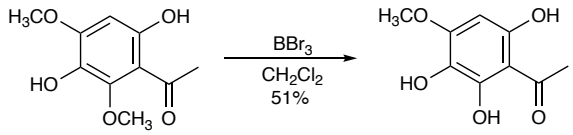
6 **7**

Compound 7: To a solution of 4',6'-dimethoxy-2'-hydroxyacetophenone (**6**) (4.98 g, 25.4 mmol) in 10% potassium hydroxide (w/v) (4.36 g, 77.7 mmol, 3.1 equiv.) was added a solution of potassium persulfate (14.4 g, 53.3 mmol, 2.1 equiv.) in water (30 mL) dropwise at room temperature. After stirring for 1.5 h at 40 °C, the mixture was washed with EtOAc (90 mL x 3). The resultant aqueous layer was added sodium hydrogen sulfite (12.4 g, 119 mmol, 4.7 equiv.), and acidified to pH 1 with HCl. The reaction mixture was refluxed for 3.5 h and extracted with EtOAc (100 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexane: EtOAc : AcOH = 85 : 15 : 0.5) to afford **7** (1.17 g, 5.50 mmol, 22%) as a yellow solid.

R_f (silica, EtOAc/hexane = 1:1) = 0.38.

^{1}H NMR (500 MHz, $CDCl_3$, 0.098 M, 296 K): δ 2.68 (3H, s), 3.92 (3H, s), 3.96 (3H, s), 5.10 (1H, s), 6.26 (1H, s), 13.19 (1H, s) ppm.

HR-FAB-MS (matrix: *m*-nitrobenzyl alcohol): *m/z*, 212.0684 (M^+, calcd for $C_{10}H_{12}O_5$ 212.0685).



7 **S3**

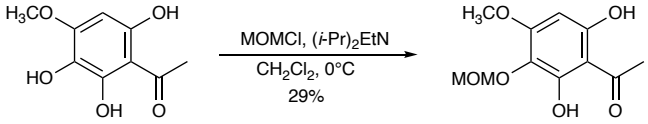
Compound S3: To a solution of **7** (504 mg, 2.38 mmol) in CH_2Cl_2 (50 mL) was added boron tribromide (5.04 mL, 5.04 mmol, 2.1 equiv.) dropwise at –78 °C under Ar atmosphere. After

stirring for 10 min at –78 °C, the reaction mixture was warmed to room temperature and stirred for 7.5 h. The reaction was quenched with water (50 mL) and concentrated *in vacuo*. The mixture was acidified to pH 1 with 1 M HCl, and extracted with EtOAc (50 mL x 3). The combined organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexane : EtOAc : AcOH = 85 : 15 : 0.5) to afford **S3** (241 mg, 1.21 mmol, 51%).

R_f (silica, EtOAc/hexane = 1:1) = 0.26.

^{1}H NMR (400 MHz, $CDCl_3$, 0.030 M, 298 K): δ 2.67 (3H, s), 3.86 (3H, s), 6.33 (1H, s), 12.91 (1H, s) ppm.

HR-ESI-MS: *m/z*, 197.0455 ([M–H]$^-$, calcd for $C_9H_9O_5$ 197.0450).



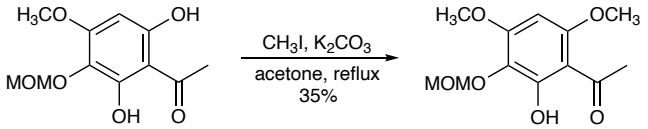
S3 **S4**

Compound S4: To a solution of **S3** (241 mg, 1.21 mmol) in CH_2Cl_2 (23.7 mL) was added DIPEA (0.83 mL, 4.85 mmol, 4.0 equiv.) at 0 °C. The resulting mixture was stirred for 10 min at 0 °C and methoxymethyl chloride (96.8 μL, 1.27 mmol, 1.1 equiv.) was added. After stirring for 19 h at 0 °C, the reaction was quenched with 1 M NH_4Cl (25 mL) and extracted with CH_2Cl_2 (30 mL x 3). The combined organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexane : EtOAc : AcOH = 85 : 15 : 0.3) to afford **S4** (84.0 mg, 0.35 mmol, 29%).

R_f (silica, EtOAc/hexane = 1:3) = 0.19.

^{1}H NMR (400 MHz, $CDCl_3$, 0.083 M, 296 K): δ 2.68 (3H, s), 3.51 (3H, s), 3.97 (3H, s), 5.27 (2H, s), 6.50 (1H, s), 12.96 (1H, s) ppm.

HR-FAB-MS (matrix: *m*-nitrobenzyl alcohol): *m/z,* 243.0866 ([M+H]$^+$, calcd for $C_{11}H_{15}O_6$ 243.0869).



S4 **8**

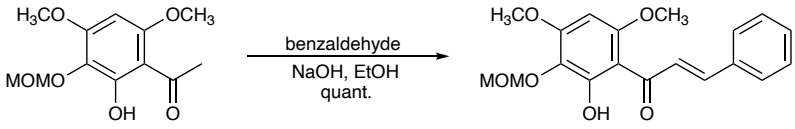
Compound 8: To a solution of **S4** (124 mg, 0.51 mmol) in acetone (0.86 mL) was added potassium carbonate (353 mg, 2.56 mmol, 5.0 equiv.). The reaction mixture was stirred for 10 min at 0 °C and iodomethane (33.4 μL, 0.54 mmol, 1.1 equiv.) was added. After stirring for 10 min at room temperature, the mixture was refluxed for 4 h under Ar atmosphere. The reaction was quenched with water (15 mL) and adjusted to pH 8 with 1 M HCl and extracted with EtOAc (30 mL x 3). The combined organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexane : EtOAc : AcOH = 95 :

5 : 0.3) to afford **8** (46.4 mg, 0.18 mmol, 35%).

R_f (silica, EtOAc/hexane = 1:3) = 0.40.

^{1}H NMR (500 MHz, $CDCl_3$, 0.078 M, 297 K): δ 2.66 (3H, s), 3.51 (3H, s), 3.80 (3H, s), 4.00 (3H, s), 5.26 (2H, s), 6.47 (1H, s), 13.23 (1H, s) ppm.

HR-FAB-MS (matrix: *m*-nitrobenzyl alcohol): *m/z*, 256.0942 (M^+, calcd for $C_{12}H_{16}O_6$ 256.0947).



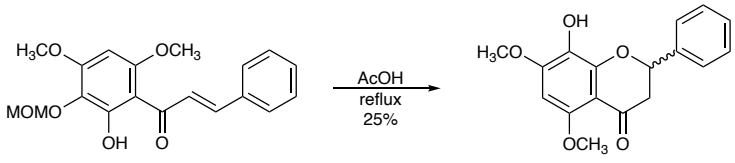
8 **9**

Compound 9: To a solution of **8** (46.4 mg, 0.18 mmol) in EtOH (2.9 mL) was added sodium hydroxide (72.4 mg, 1.81 mmol, 10 equiv.). After stirring for 10 min at room temperature, to the reaction mixture was added benzaldehyde (24.0 μL, 0.24 mmol, 1.3 equiv.) in EtOH (76.0 μL). The mixture was stirred for 44 h at room temperature and quenched with water (10 mL) and extracted with EtOAc (15 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexane : EtOAc = 95 : 5) to afford **9** (61.6 mg, 0.18 mmol, 99%) as yellow oil.

R_f (silica, EtOAc/hexane = 1:3) = 0.29.

^{1}H NMR (400 MHz, $CDCl_3$, 0.066 M, 298 K): δ 3.53 (3H, s), 3.86 (3H, s), 3.94 (3H, s), 5.28 (2H, s), 6.54 (1H, s), 7.41–7.43 (3H, m), 7.64–7.66 (2H, m), 7.83 (1H, d, *J* = 15.7 Hz, α-H) ,7.94 (1H, d, *J* = 15.7 Hz, β-H) ppm, 13.39 (s, 1H, 2'-OH) ppm.

HR-FAB-MS (matrix: *m*-nitrobenzyl alcohol): *m/z*, 344.1253 (M^+, calcd for $C_{19}H_{20}O_6$ 344.1260).



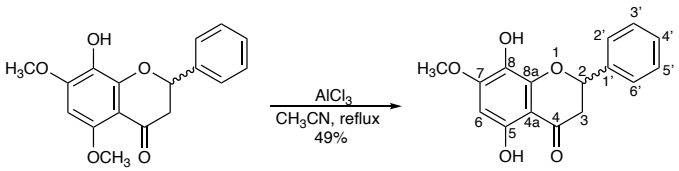
9 **10**

Compound 10: A solution of **9** (61.6 mg, 0.18 mmol) in AcOH (1.5 mL) was refluxed for 20.5 h under Ar atmosphere. The reaction mixture was azeotroped with toluene to remove AcOH. The residue was purified by column chromatography (silica gel, hexane : EtOAc = 85 : 15) to afford **10** (13.3 mg, 0.044 mmol, 25%).

R_f (silica, EtOAc/hexane = 1:1) = 0.46.

^{1}H NMR (500 MHz, $CDCl_3$, 0.067 M, 297 K): δ 2.81 (1H, dd, *J* = 2.9, 16.8 Hz, 3-H), 3.01 (1H, dd, *J* = 13.2, 16.8 Hz, 3-H), 3.93 (3H, s, 7-OC*H*$_3$), 3.94 (3H, s, 5-OC*H*$_3$), 5.39 (1H, dd, *J* = 2.9, 13.2 Hz, 2-H), 6.41 (1H, s, 6-H), 7.38–7.46 (5H, m, 2'-H, 3'-H, 4'-H, 5'-H, 6'-H) ppm.

HR-FAB-MS (matrix: *m*-nitrobenzyl alcohol): *m/z*, 323.0897 ($[M+Na]^+$, calcd for $C_{17}H_{16}O_5Na$ 323.0895).



10 **2**

Compound 2: To a solution of **10** (13.3 mg, 0.044 mmol) in MeCN (0.88 mL) was added aluminium chloride (23.6 mg, 0.177 mmol, 4.0 equiv.). The mixture was refluxed for 2 h under Ar atmosphere. The reaction mixture was quenched with 1 M HCl (10 mL) and extracted with EtOAc (15 mL x 3). The combined organic layer was dried over Na_2SO_4, filtered, and purified by HPLC [column, YMC-Pack ODS-A (20 mm i.d. x 150 mm; YMC); solvent, 70% MeOH/H_2O (0.05% TFA), flow rate, 8.0 mL/min; UV detector, 254 nm] to afford **2** (6.3 mg, 0.022 mmol, 49%).

R_f (silica, EtOAc/hexane = 1:1) = 0.65.

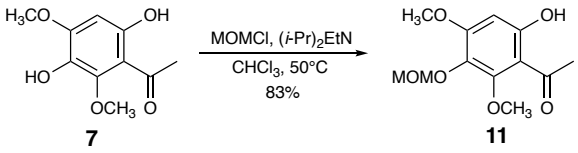
^{1}H NMR (500 MHz, $CDCl_3$, 0.035 M, 297 K): δ 2.83 (1H, dd, *J* = 2.9, 17.2 Hz, 3-H), 3.08 (1H, dd, *J* = 13.0, 17.1 Hz, 3-H), 3.95 (3H, s, 7-OC*H*$_3$), 5.40 (1H, dd, *J* = 2.8, 13.0 Hz, 2-H), 6.14 (1H, s, 6-H), 7.38–7.45 (5H, m, 2'-H, 3'-H, 4'-H, 5'-H, 6'-H), 12.18 (s, 1H, 5-OH) ppm.

^{13}C NMR (125 MHz, $CDCl_3$, 0.035 M, 298 K): δ 43.4 (C-3), 61.0 (7-O*C*H$_3$), 79.3 (C-2), 94.6 (C-6), 103.1 (C-4a), 126.1 (C-2', C-6'), 128.4 (C-8), 128.9 (C-3', C-4', C-5'), 138.4 (C-1'), 154.4 (C-8a), 157.5 (C-7), 158.6 (C-5), 196.6 (C-4) ppm.

HR-FAB-MS (matrix: *m*-nitrobenzyl alcohol): *m/z*, 287.0919 ([M+H]$^+$, calcd for $C_{16}H_{15}O_5$ 287.0919).

IR (KBr): 3107, 3026, 2946, 2796, 1637, 1589, 1457, 1312, 1180, 1086, 1010, 899, 842 cm^{-1}.

*Synthesis of **3***



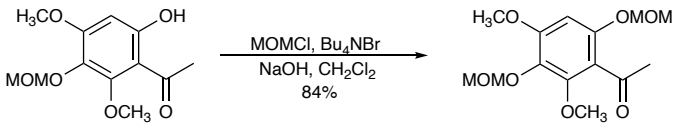
7 **11**

Compound 11: To a solution of **7** (56.1 mg, 0.26 mmol) in $CHCl_3$ (0.90 mL) was added DIPEA (121 μL, 0.70 mmol, 2.6 equiv.) and methoxymethyl chloride (59 μL, 0.75 mmol, 2.8 equiv.). After stirring for 2 h at 50 °C, the reaction was quenched with water (10 mL) and extracted with EtOAc (15 mL x 3). The combined organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexane : EtOAc : AcOH = 80 : 20) to afford **11** (56.5 mg, 0.22 mmol, 83%).

R_f (silica, EtOAc/hexane = 1:1) = 0.68.

^{1}H NMR (500 MHz, $CDCl_3$, 0.014 M, 295 K): δ 2.66 (3H, s), 3.61 (3H, s), 3.88 (3H, s), 3.96 (3H, s) 5.02 (2H, s), 6.26 (1H, s), 13.43 (1H, s) ppm.

HR-ESI-MS, *m/z* 257.1029 ([M+H]$^+$, calcd. for $C_{12}H_{17}O_6$ 257.1025).



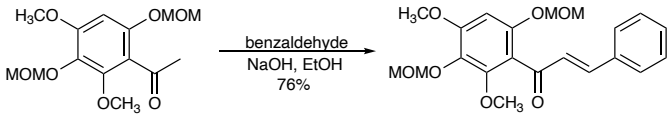
11 **12**

Compound 12: To a solution of sodium hydroxide (647 mg, 16.2 mmol, 6.8 equiv.) and Bu_4NBr (161.1 mg, 0.50 mmol, 0.21 equiv.) in water (10.8 mL) was added **11** (610 mg, 2.38 mmol) in CH_2Cl_2 (10.8 mL). After stirring for 15 min at room temperature, to the reaction mixture was added methoxymethyl chloride (0.97 mL, 12 mmol, 5.2 equiv.) in CH_2Cl_2 (3.6 mL) at 0 °C. The mixture was stirred for 18 h at room temperature and the reaction was diluted with water (20 mL) and extracted with EtOAc (40 mL x 3). The combined organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexane : EtOAc = 90 : 10) to afford **12** (602 mg, 2.01 mmol, 84%).

R_f (silica, EtOAc/hexane = 1:1) = 0.49.

^{1}H NMR (500 MHz, $CDCl_3$, 0.047 M, 296 K): δ 2.50 (3H, s), 3.48 (3H, s), 3.60 (3H, s), 3.85 (3H, s), 3.87 (3H, s), 5.06 (2H, s), 5.13 (2H, s), 6.55 (1H, s) ppm.

HR-ESI-MS, *m/z* 301.1264 ($[M+H]^+$, calcd. for $C_{14}H_{21}O_7$ 301.1287).



12 **13**

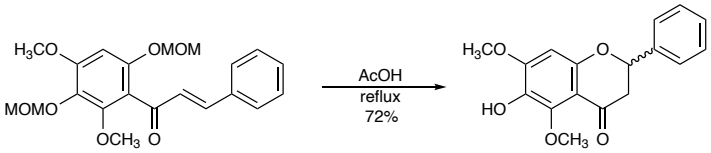
Compound 13: To a solution of **12** (215 mg, 0.717 mmol) was added sodium hydroxide (287 mg, 7.17 mmol, 10.0 equiv.) in EtOH (6.3 mL). After stirring for 10 min at room temperature, to the reaction mixture was added benzaldehyde (93.2 μL, 0.93 mmol, 1.3 equiv.) in EtOH (2.4 mL). The resulting mixture was stirred for 123 h at room temperature, and quenched with water (10 mL) and extracted with EtOAc (15 mL x 3). The combined organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexane : EtOAc = 85 : 15) to afford **13** (212.3 mg, 0.55 mmol, 76%).

R_f (silica, EtOAc/hexane = 1:1) = 0.47.

^{1}H NMR (500 MHz, $CDCl_3$, 0.054 M, 298 K): δ 3.41 (3H, s), 3.61 (3H, s), 3.84 (3H, s), 3.89 (3H, s), 5.10 (2H, s), 5.10 (2H, s), 6.60 (1H, s), 6.99 (1H, d, J = 16 Hz), 7.37 (1H, d, J = 16 Hz), 7.37–7.39 (3H, m), 7.52–7.54 (2H, m) ppm.

^{13}C NMR (125 MHz, $CDCl_3$, 0.054 M, 298 K) δ 56.2, 56.3, 57.3, 62.1, 95.4, 96.3, 98.7, 118.1, 127.3, 128.4 (C2), 128.9 (C2), 130.5, 133.9, 134.8, 145.1, 151.1, 151.9, 155.0, 193.6 ppm.

HR-ESI-MS, *m/z* 389.1588 ($[M+H]^+$, calcd. for $C_{21}H_{25}O_7$ 389.1600).



13 **14**

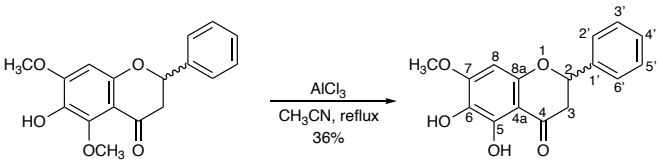
Compound 14: To a solution of **13** (91.1 mg, 0.18 mmol) in AcOH (2.4 mL) was refluxed for 5.5 h under Ar atomosphere. The reaction mixture was azeotroped with toluene to remove AcOH. The residue was purified by column chromatography (silica gel, hexane : EtOAc : AcOH = 75 : 25 : 0.3) to afford **14** (50.4 mg, 0.168 mmol, 72%).

$\mathbf{R}_f$ (silica, EtOAc/hexane = 1:1) = 0.36.

^{1}H NMR (500 MHz, $CDCl_3$, 0.064 M, 296 K): δ 2.80 (1H, dd, *J* = 2.9, 16.8 Hz), 3.03 (1H, dd, *J* = 13.5, 16.8 Hz), 3.92 (3H, s), 3.96 (3H, s), 5.40 (1H, dd, *J* = 2.8, 13.5 Hz), 5.50 (1H, s), 6.39 (1H, s), 7.39–7.48 (5H, m) ppm.

^{13}C NMR (125 MHz, $CDCl_3$, 0.064 M, 298 K) δ 45.5, 56.3, 61.7, 79.5, 96.3, 108.6, 126.1 (C2), 128.7, 128.8 (C2), 133.9, 138.8, 146.1, 153.9, 157.2, 189.4 ppm.

HR-ESI -MS: *m/z,* 301.1087 ($[M+H]^+$, calcd. for $C_{17}H_{17}O_5$ 301.1076).



14 **3**

Compound 3: To a solution of **14** (50.4 mg, 0.168 mmol) in MeCN (3.33 mL) was added aluminium chloride (89.5 mg, 0.671 mmol, 4.0 equiv.). The mixture was refluxed for 2 h under Ar atmosphere. The reaction mixture was quenched with 1 M HCl (10 mL) and extracted with EtOAc (15 mL x 3). The combined organic layer was dried over Na_2SO_4, filtered, and purified by HPLC [column, YMC-Pack ODS-A (20 mm i.d. x 150 mm); solvent, 40–70% MeCN/H_2O (0.1% TFA) under the linear gradient for 40 min, flow rate, 8.0 mL/min; UV detector, 254 nm] to afford **3** (17.5 mg, 0.061 mmol, 36%).

$\mathbf{R}_f$ (silica, EtOAc/hexane = 1:1) = 0.49.

^{1}H NMR (500 MHz, $CDCl_3$, 0.027 M, 296 K): δ 2.83 (1H, dd, *J* = 3.0, 17.2 Hz, 3-H), 3.10 (1H, dd, *J* = 13.3, 17.2 Hz, 3-H), 3.91 (3H, s, 7-OCH_3), 5.05 (1H, s, 6-OH), 5.41 (1H, dd, *J* = 2.9, 13.3 Hz, 2-H), 6.16 (1H, s, 8-H), 7.38–7.44 (5H, m, 2'–6'-H), 11.7 (1H, s, 5-OH) ppm.

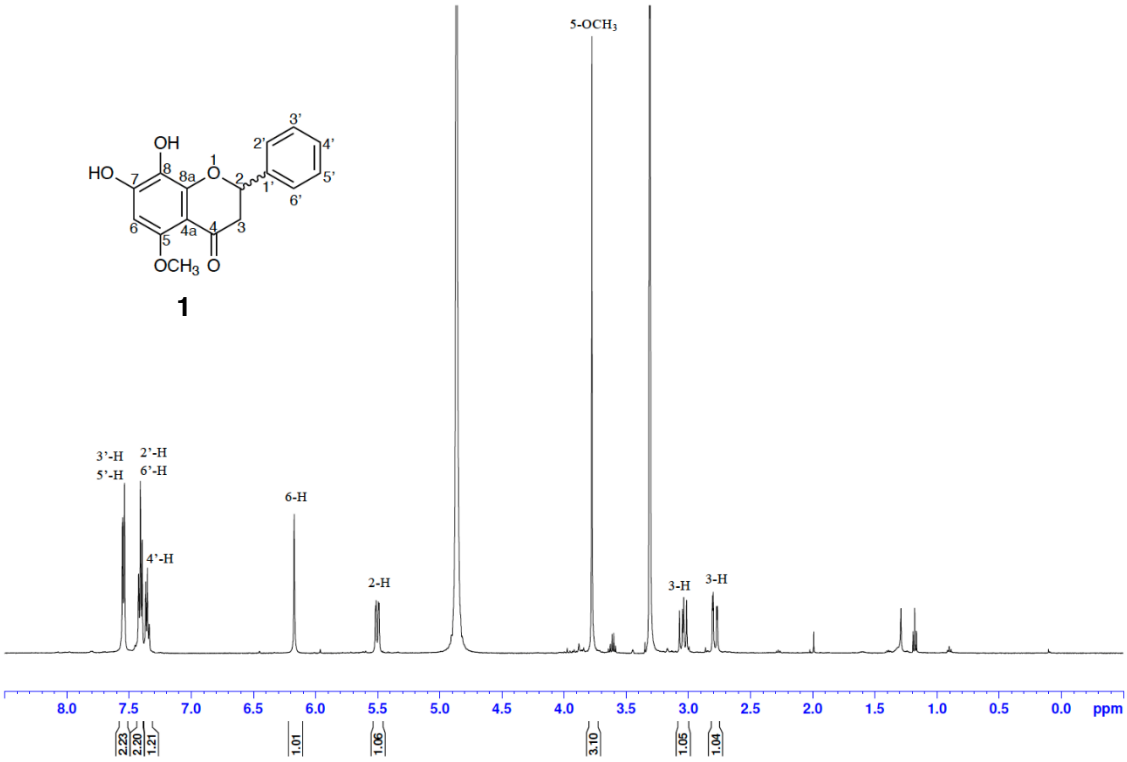
^{13}C NMR (125 MHz, $CDCl_3$, 0.027 M, 296 K): δ 43.6 (C-3), 56.4 (7-OCH_3), 79.7 (C-2), 91.5 (C-8), 103.0 (C-4a), 126.2 (C-2', C-6'), 127.5 (C-6), 128.9 (C-3', C-4', C-5'), 138.5 (C-1'), 148.0 (C-5), 154.8 (C-7), 155.9 (C-8a), 196.6 (C-4) ppm.

HR-ESI -MS: *m/z*, 285.0757 ($[M–H]^-$, calcd. for $C_{16}H_{13}O_5$ 285.0763).

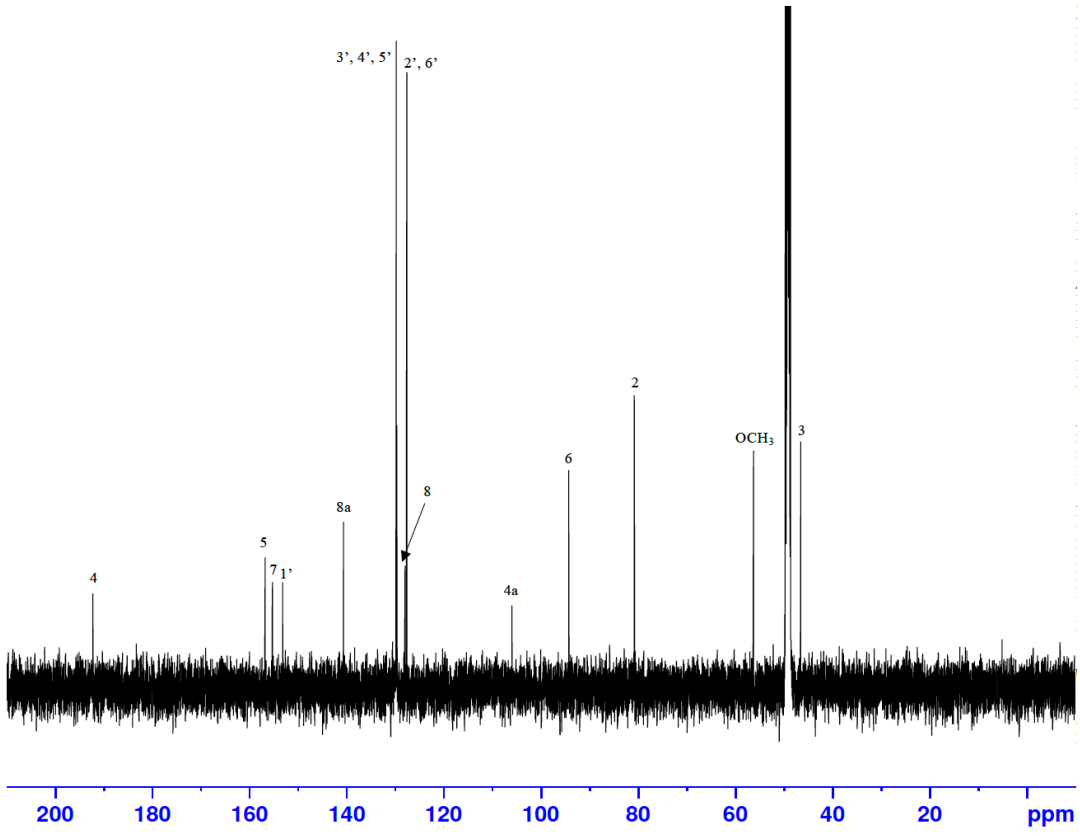
IR (KBr): 3228, 3066, 2967, 1649, 1581, 1504, 1454, 1369, 1297, 1246, 1208, 1166, 1096 cm^{-1}.

^{1}H and ^{13}C NMR spectra of 1–3

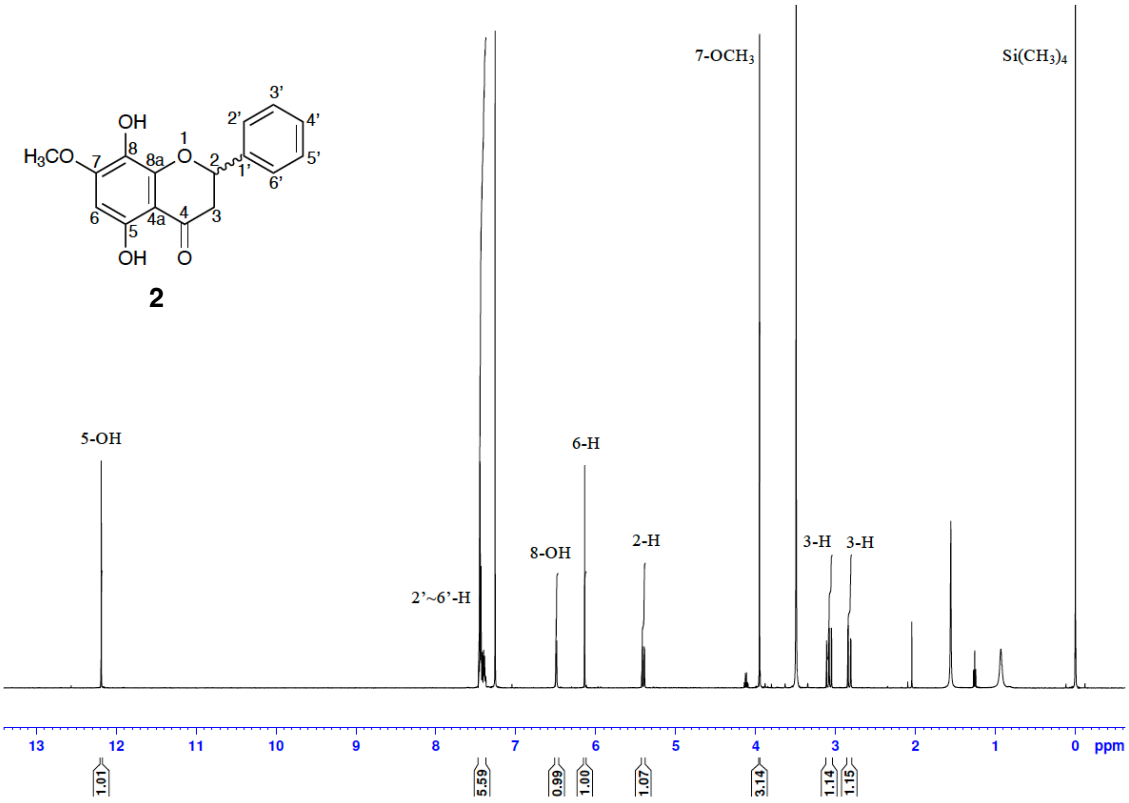
^{1}H NMR spectrum of **1** (500 MHz, 297 K, MeOD-d_4, 0.025 M)



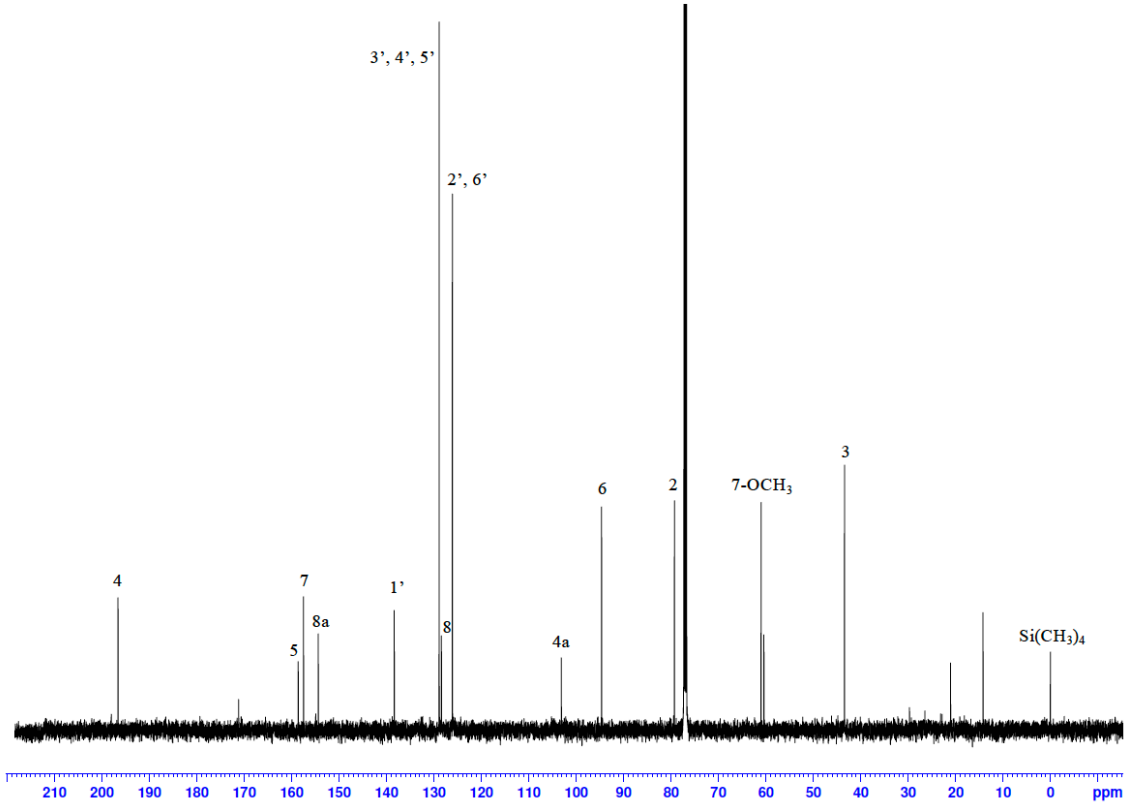
^{13}C NMR spectrum of **1** (125 MHz, 297 K, MeOD-d_4, 0.025 M)



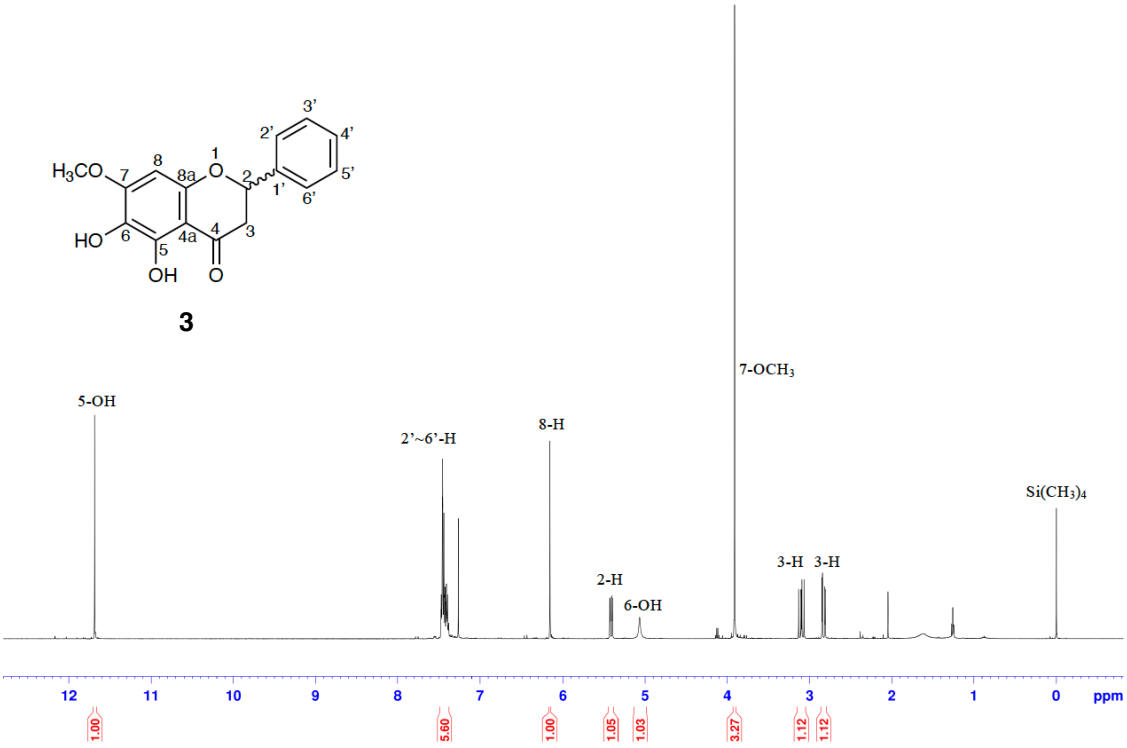
^{1}H NMR spectrum of **2** (500 MHz, 297 K, $CDCl_3$, 0.007 M)



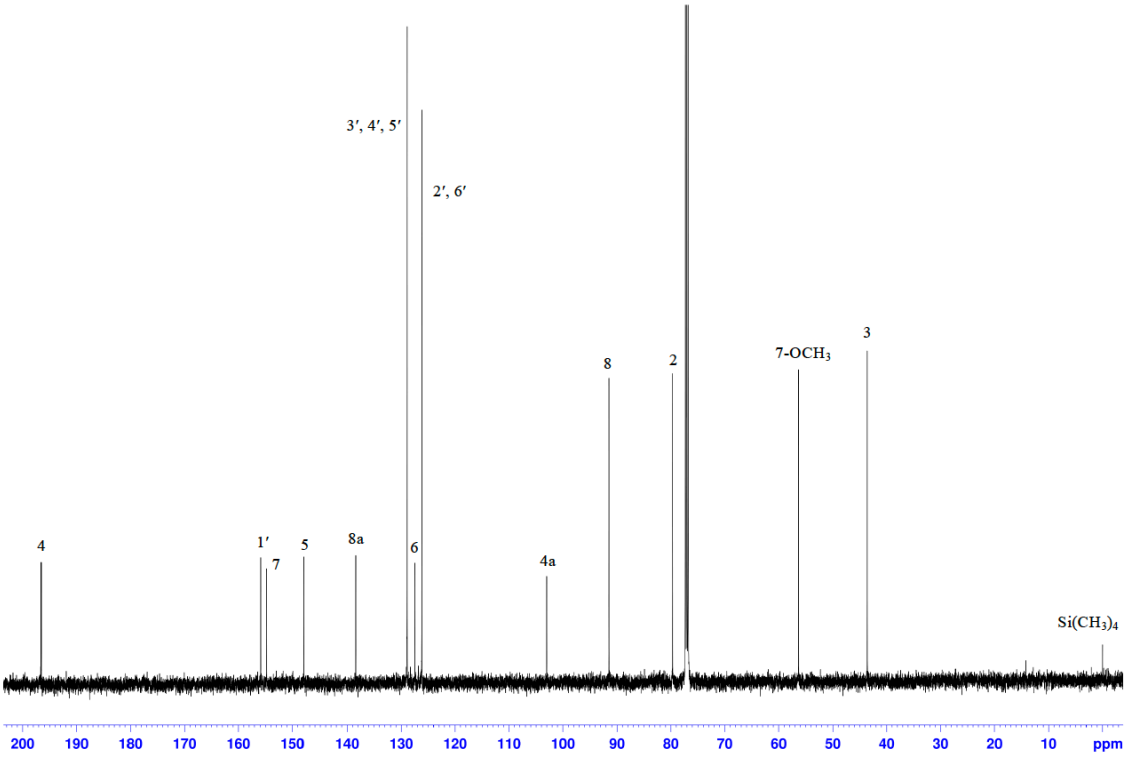
^{13}C NMR spectrum of **2** (125 MHz, 298 K, $CDCl_3$, 0.035 M)



^{1}H NMR spectrum of **3** (500 MHz, 296 K, $CDCl_3$, 0.027 M)



^{13}C NMR spectrum of **3** (125 MHz, 298 K, $CDCl_3$, 0.027 M)



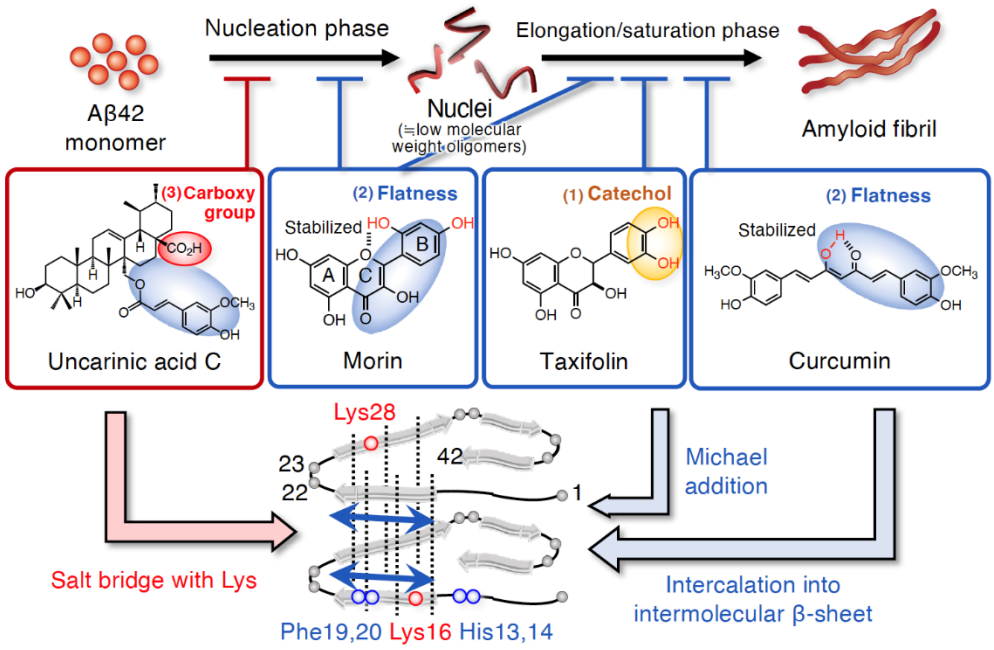


Fig. S1. Nucleation-dependent polymerization model of Aβ aggregation *in vitro* and inhibitory mode of action by natural products. After the nucleation phase, the subsequent elongation and saturation phases occur. The inhibitory mechanism of Aβ aggregation by flavonoids, triterpenoids, and curcuminoids based on the following structural features: [1] catechol structure, [2] planarity structure due to α,β-unsaturated carbonyl groups conjugated with aromatic structure, and [3] carboxy acid group.

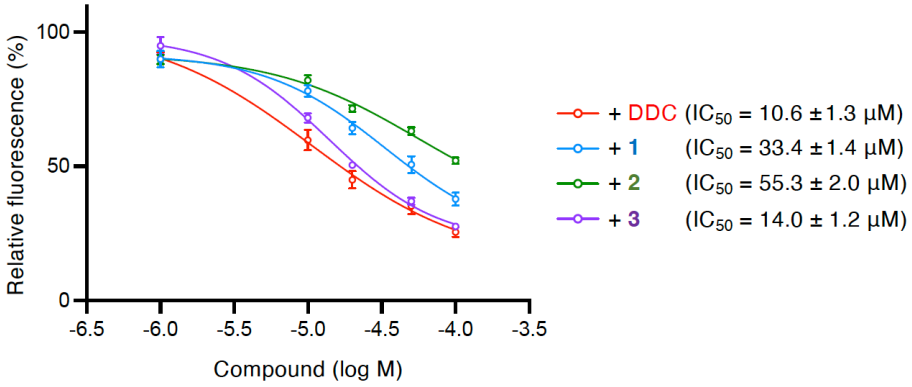


Fig. S2. Th-T fluorescence curves showing inhibitory activities of DDC and 1–3 against Aβ42 aggregation. The IC_{50} values were calculated from nonlinear regression based on the inhibitory rate (%) of each compound on aggregation of Aβ42 (25 μM) after 24 h of incubation at 37 °C in Th-T fluorescence assay. Data are presented as the mean ± s.d. (n = 4).

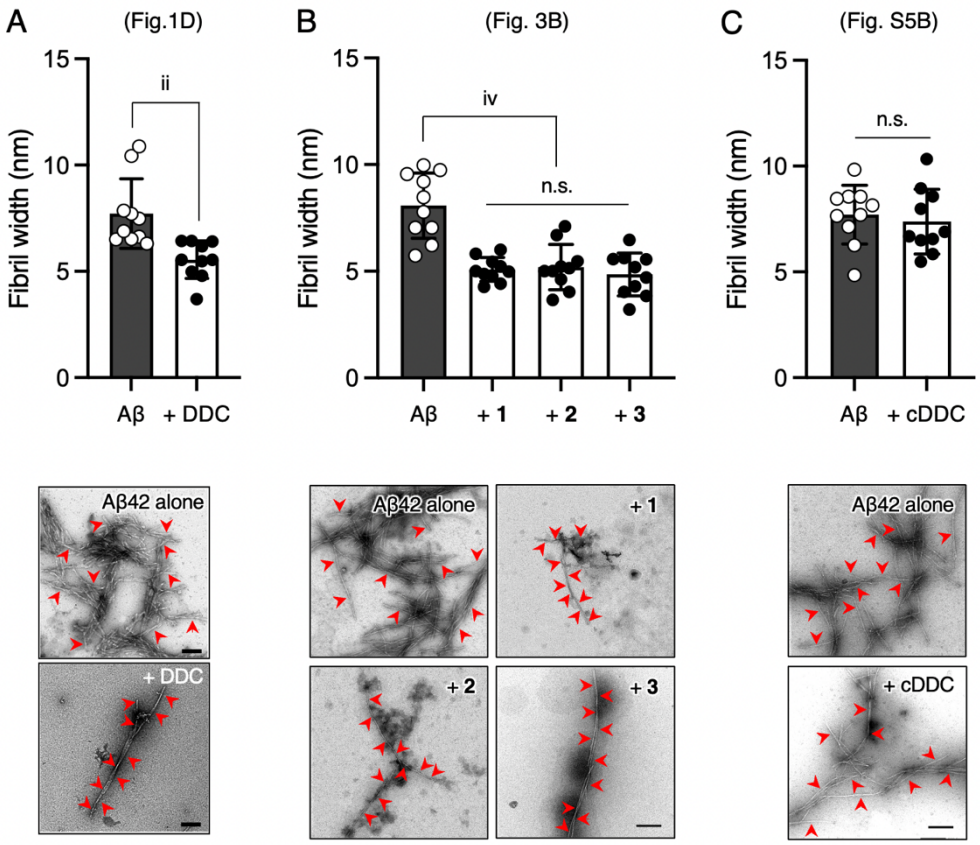


Fig. S3. Quantitative analysis of transmission electron microscopy performed in this study. Width of fibrils marked with red arrowheads from (A) Fig. 1D, (B) Fig. 3B, and (C) Fig. S5B were measured by Image J 1.53k software (Wayne Rasband, NIH, MD, USA). Data are presented as the mean ± s.d. (n = 10). ii, $p < 0.01$; iv, $p < 0.0001$. n.s., not significant.

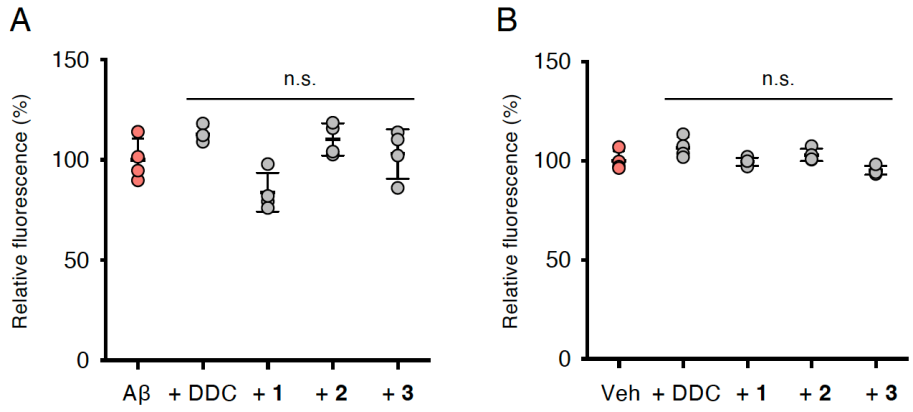


Fig. S4. Effects of DDC and 1–3 on Th-T interference. Measurement of (A) Th-T fluorescence of Aβ42 aggregates in the presence of each compound and (B) Th-T fluorescence of each compound itself. Data are presented as the mean ± s.d. (n = 4). n.s., not significant (versus Aβ42 alone or vehicle). Veh, vehicle.

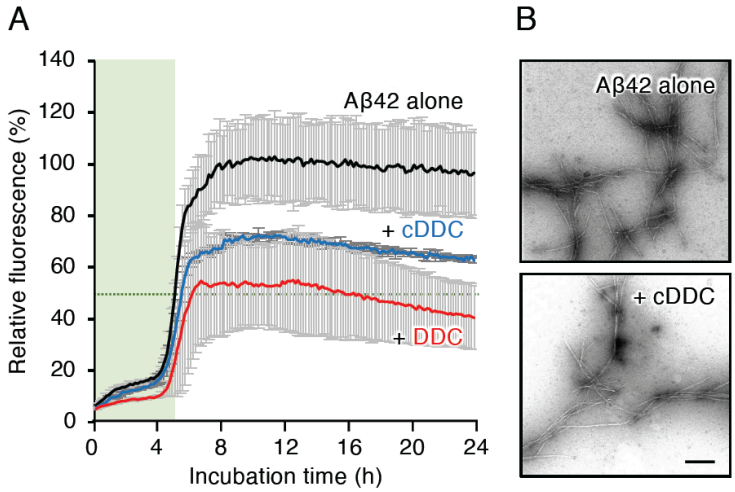


Fig. S5. Effects of cDDC on the aggregation of Aβ42. (A) Th-T test of DDC and cDDC against Aβ42 aggregation. Time response curves of aggregation of Aβ42 (10 μM) during incubation for 24 h at 25 °C in the presence of each compound (50 μM) are indicated. Data are presented as the mean ± s.d. (n = 3). Green shadows indicate the nucleation phase, where the relative aggregation of Aβ42 is 50%. (B) TEM analysis of Aβ42 aggregates incubated with DDC and cDDC after Th-T test. Scale bar = 200 nm.

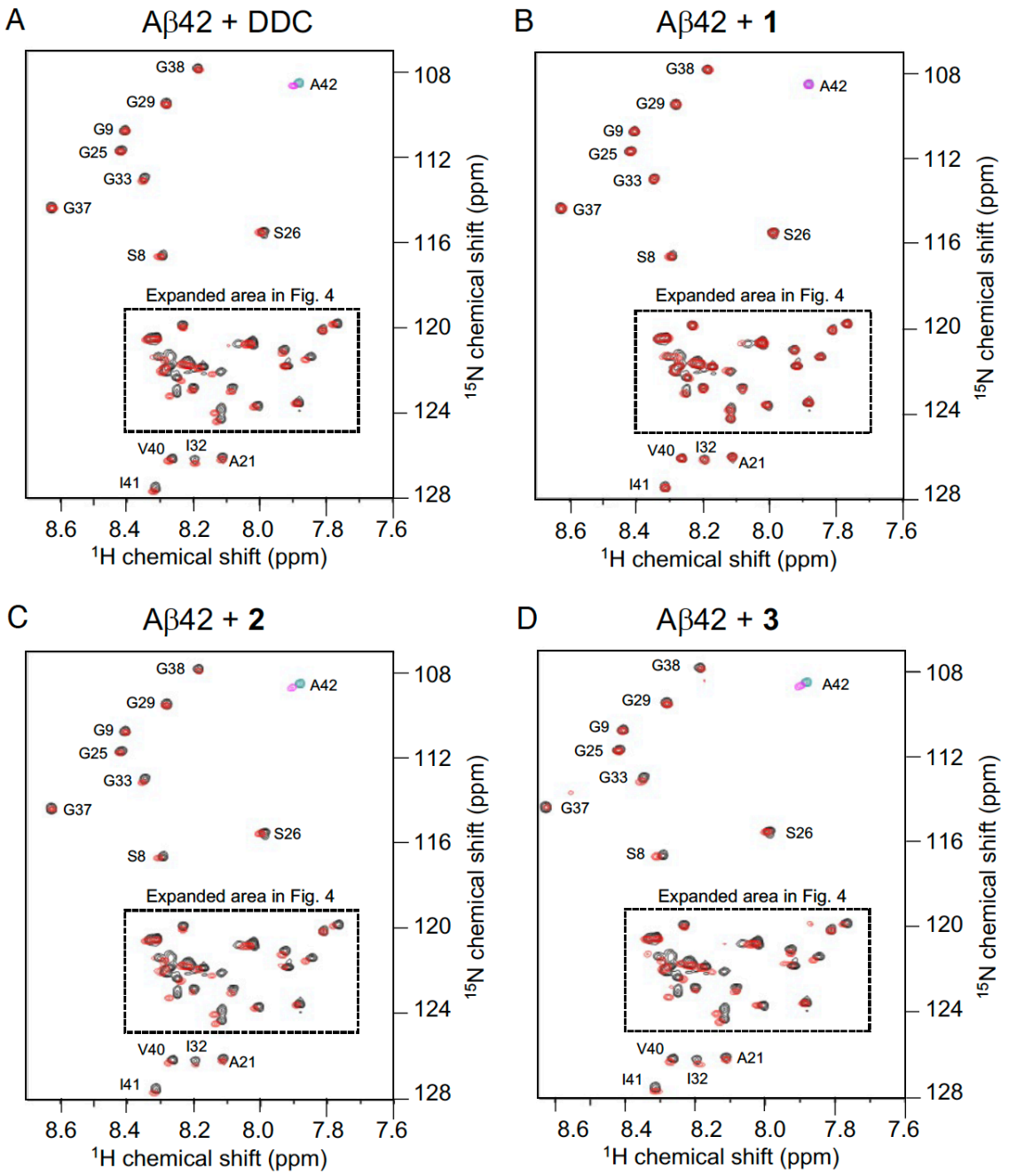


Fig. S6. Full spectra of ^{1}H-^{15}N SOFAST-HMQC NMR of Aβ42 in the presence of DDC and 1–3. The expanded version of which is shown in Fig. 4 in the main text. Black cross peaks, Aβ42 alone; red cross peaks, Aβ42 treated with each compound.