

Supplementary Figure legends

Supplementary Fig. 1. 4'-Demethylnobiletin enhances phosphorylation of PKA

substrates in cultured rat hippocampal neurons. Hippocampal neuronal cells were treated with vehicle or 30 μ M of 4'-demethylnobiletin for 5 min. Western blot analyses were performed using anti-phospho-(Ser/Thr) PKA substrate antibody followed by reprobing with anti-PKA α antibody. Shown are representative results from three independent experiments.

Supplementary Fig. 2. Illustration of the synthetic scheme of 4'-demethylnobiletin.

Synthetic procedures of 4'-demethylnobiletin

All reactions (Supplementary Fig. 2) were carried out under an atmosphere of argon unless otherwise specified. All solvents were dried and distilled by standard procedures. Yields refer to chromatographically and spectroscopically (^1H NMR) homogeneous materials unless otherwise stated. Reagents were purchased at the highest commercial quality and used without further purification unless otherwise stated.

Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm Merck silica gel plates (60F-254) using UV light as visualizing agent and *p*-anisaldehyde in ethanol/aqueous $\text{H}_2\text{SO}_4/\text{CH}_3\text{CO}_2\text{H}$ for staining. Column chromatography was performed using silica gel 60 particle size 0.063-0.210 mm. The eluents employed are reported as volume : volume percentages.

Proton nuclear magnetic resonance (^1H NMR) spectra were recorded using a 400 MHz spectrometers. Chemical shift (δ) is reported in parts per million (ppm) downfield relative to tetramethylsilane (TMS). Coupling constants (J) are reported in Hz. Multiplicities are reported using the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad; app, Apparent. Carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra were recorded using a 100 MHz spectrometers. Chemical shift is reported in ppm relative to the center of CDCl_3 .

Melting points were determined using Yazawa BY-2 melting point apparatus and are reported uncorrected. Infrared spectra were obtained on a JASCO FT-IR-410 at 4.0 cm^{-1}

resolution and are reported in wavenumbers. High resolution mass spectra (HRMS) were recorded on a JMS-AX500 or JMS-700 using electron impact (EI). Low resolution mass spectra (MS) were recorded on JEOL JMS-DX303. Elemental analyses were performed using Yanaco CHN CORDER MT-6.

3,4,5-Trimethoxyphenol

To a stirred solution of 3,4,5-trimethoxybenzaldehyde (2.0 g, 10.2 mmol) and sulfuric acid (0.54 ml, 10.2 mmol) in MeOH (20.4 ml, 0.5 M) at 0 °C, 30% aq. hydrogen peroxide (3.5 ml, 30.6 mmol) was slowly added. The mixture was allowed to warm to room temperature, and stirred for an additional 15 min. The reaction mixture was then quenched with 10% aq. NaOH and sodium sulfite (solid). The layers were separated and the aqueous phase was extracted with AcOEt. The organic layers were combined, washed with brine, dried over MgSO₄ and concentrated under reduced pressure. The crude mixture was purified by silica gel column chromatography (AcOEt:Hexane, 5:6) to give 3,4,5-trimethoxyphenol (1.08 g, 7.0 mmol, 58%) as a white solid.

Colorless plate (AcOEt/Hexane) : mp 145-147 °C. lit. 149-150 °C. IR (CHCl₃) : 3303, 1612, 1129 cm⁻¹. ¹H-NMR (400 MHz, CDCl₃) δ : 6.09 (2H, s), 5.90 (1H, s), 3.79 (3H, s), 3.76 (6H, s). ¹³C-NMR (100 MHz, CDCl₃) δ : 153.6, 152.7, 131.4, 93.0, 61.0, 55.9. MS m/z : 184 (M⁺). HRMS Calcd. for C₉H₁₂O₄ : 184.0736. Found : 184.0715.

1,2,3,4,5-Pentamethoxybenzene

To a solution of 3,4,5-trimethoxyphenol (0.10 g, 0.54 mmol) in DMF (3.6 ml, 0.15 M) was added IBX (160 mg, 5.7 mmol) at room temperature. After stirring for 30 min, 10% Pd/C (10 mg) was added to the mixture and stirring was continuing for 1 day under atmospheric pressure of H₂. After addition of K₂CO₃ (220 mg, 1.6 mmol) and Me₂SO₄ (0.13 ml, 1.4 mmol), the mixture was stirred for 3 h under atmosphere of Ar and subsequently filtered through celite pad eluting with Et₂O and the filtrate was concentrated under reduced pressure. The residue was diluted with Et₂O and washed successively with H₂O and washed with brine, dried over MgSO₄ and concentrated under reduced pressure. The crude mixture was purified by silica gel column chromatography (AcOEt:Hexane, 5:7) to give 1,2,3,4,5-pentamethoxybenzene (42 mg, 0.18 mmol, 34%) as a white solid.

Colorless needle (Hexane) : mp 59-61 °C. lit. 55.5-57 °C. IR (CHCl₃) : 1108 cm⁻¹. ¹H-NMR (400 MHz, CDCl₃) δ : 6.30 (1H, s), 3.95 (3H, s), 3.85 (6H, s), 3.83 (6H, s). ¹³C-NMR (100 MHz, CDCl₃) δ : 149.1, 147.9, 136.7, 93.7, 61.4, 61.3, 56.5. MS m/z : 228 (M⁺). HRMS Calcd. for C₁₁H₁₆O₅ : 228.0998. Found : 228.0983. Anal. Calcd. for C₁₁H₁₆O₅ : C, 57.88. H, 7.07. Found : C, 57.77. H, 7.00.

1,2,3,5-Tetramethoxybenzene

To a suspension of Raney Ni (100-300 mesh, 50% Ni, 3.5 g) in MeOH (100 ml, 0.59 M) was added 2,6-dimethoxy[1,4]benzoquinone (10 g, 58.8 mmol) and stirred for 6 h under atmospheric pressure of H₂. The mixture was filtered through celite pad eluting with Et₂O and the filtrate was concentrated under reduced pressure to give the crude 2,6-dimethoxybenzene-1,4-diol.

The crude 2,6-dimethoxybenzene-1,4-diol was dissolved in acetone (100 ml, <0.59 M), and K₂CO₃ (41 g, 297 mmol) and Me₂SO₄ (14 ml, 147 mmol) were added to the mixture, was refluxed for 3 h. The mixture was added H₂O and extracted with AcOEt and the combined organic layers were washed with brine, dried over MgSO₄ and concentrated under reduced pressure. The crude mixture was purified by silica gel column chromatography (AcOEt:Hexane, 1:2) to give 1,2,3,5-tetramethoxybenzene (11.5 g, 58.1 mmol, 99%) as a colorless oil.

Colorless oil. IR (CHCl₃) : 2931, 1594, 1506, 1129 cm⁻¹. ¹H-NMR (400 MHz, CDCl₃) δ : 6.15 (2H, s), 3.85 (6H, s), 3.79 (3H, s), 3.79 (3H, s). ¹³C-NMR (100 MHz, CDCl₃) δ : 156.2, 153.7, 132.4, 91.7, 61.0, 56.1, 55.5. MS m/z : 198 (M⁺). HRMS Calcd. for C₁₀H₁₄O₄ : 198.0892. Found : 198.0885.

2'-Hydroxy-3',4',6'-trimethoxy acetophenone

To a solution of 1,2,3,5-tetramethoxybenzene (11.5 g, 58.1 mmol) in Et₂O (200 ml, 0.29 M) was added AlCl₃ (26 g, 192 mmol) at 0 °C. Then to the mixture was slowly added AcCl (5.4 ml, 75.5 mmol). After the mixture was stirred for 2 h at room temperature, H₂O was slowly added until the solid completely dissolved at 0 °C. The layers were separated and the aqueous phase was extracted with Et₂O. The organic

layers were combined, washed with brine, dried over MgSO_4 and concentrated under reduced pressure. The crude mixture was purified by silica gel column chromatography (AcOEt:Hexane, 1:1) to give 2'-hydroxy-3',4',6'-trimethoxyacetophenone (11.1 g, 49.1 mmol, 85%) as a yellow solid.

Yellow needle (AcOEt) : mp 114-116 °C. lit. 111-113 °C. IR (CHCl_3) : 1626, 1587, 1126 cm^{-1} . $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ : 13.80 (1H, s), 5.97 (1H, s), 3.94 (3H, s), 3.90 (3H, s), 3.82 (3H, s), 2.62 (3H, s). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) δ : 203.7, 159.0, 158.8, 158.4, 130.5, 106.3, 86.4, 60.7, 55.9, 55.5, 33.2. MS m/z : 226 (M^+). HRMS Calcd. for $\text{C}_{11}\text{H}_{14}\text{O}_5$: 226.0841. Found : 226.0829. Anal. Calcd. for $\text{C}_{11}\text{H}_{14}\text{O}_5$: C, 58.40. H, 6.24. Found : C, 58.37. H, 6.18.

2'-Hydroxy-3',4',5',6'-tetramethoxy acetophenone

To a solution of 2'-hydroxy-3',4',6'-trimethoxyacetophenone (10.8 g, 47.7 mmol) in MeOH (130 ml, 0.37 M) was added 10% aq. NaOH (40 ml, 48 mmol). Then, to the mixture was slowly added 6% aq. hydrogen peroxide (82 ml, 143 mmol) at 0 °C. After stirring for 30 min at room temperature, the reaction mixture was quenched with 10% aq. HCl and sodium sulfite (solid). The layers were separated and the aqueous phase was extracted with AcOEt. The combined organic layers were washed with brine, dried over MgSO_4 and concentrated under reduced pressure to give the crude 3,4,6-trimethoxybenzene-1,2-diol as colorless solid.

To a solution of the crude 3,4,6-trimethoxybenzene-1,2-diol in acetone (150 ml) was added K_2CO_3 (170 g, 1.2 mol) and Me_2SO_4 (22 ml, 239 mmol). After the mixture was refluxed for 3 h at room temperature, H_2O was added. The layers were separated and the aqueous phase was extracted with AcOEt. The combined organic layers were washed with brine, dried over MgSO_4 and concentrated under reduced pressure. The crude mixture was purified by silica gel column chromatography (AcOEt:Hexane, 5:7) to give the crude 1,2,3,4,5-pentamethoxybenzene (11.5 g, 158.1 mmol, 99%) as a mixture with Me_2SO_4 .

To a solution of the crude 1,2,3,4,5-pentamethoxybenzene in Et_2O (300 ml) at 0 °C was added AlCl_3 (42 g, 313 mmol) followed by AcCl (14 ml, 187.5 mmol). After stirring for 2 h at room temperature, H_2O was slowly added until the solid completely dissolved

at 0 °C. The layers were separated and the aqueous phase was extracted with Et₂O. The organic layers were combined, washed with brine, dried over MgSO₄ and concentrated under reduced pressure. The crude mixture was purified by silica gel column chromatography (AcOEt:Hexane, 1:3) to give 2'-hydroxy-3',4',5',6'-tetramethoxyacetophenone (4.5 g, 17.6 mmol, 37%) as a yellow oil.

Yellow oil. IR (CHCl₃) : 2985, 1621, 1409, 1064 cm⁻¹. ¹H-NMR (400 MHz, CDCl₃) δ : 13.12 (1H, s), 4.08 (3H, s), 3.96 (3H, s), 3.86 (3H, s), 3.81 (3H, s), 2.68 (3H, s). ¹³C-NMR (100 MHz, CDCl₃) δ : 204.2, 154.6, 153.7, 151.4, 138.0, 136.8, 110.4, 61.3, 61.2, 61.1, 61.0, 32.2. MS m/z : 256 (M⁺). HRMS Calcd. for C₁₂H₁₆O₆ : 256.0947. Found : 256.0963.

3-(4-Benzyloxy-3-methoxyphenyl)-1-(2-hydroxy-3,4,5,6-tetramethoxyphenyl)-propenone

To a solution of 4-benzyloxy-3-methoxybenzaldehyde (340 mg, 1.41 mmol) and 2'-hydroxy-3',4',5',6'-tetramethoxyacetophenone (240 mg, 0.94 mmol) in EtOH (5.0 ml, 0.19 M) was added 50% aq. KOH (0.37 ml, 3.3 mmol). After stirring for 1 day at room temperature, the reaction mixture was then quenched with 10% aq. HCl. The layers were separated and the aqueous phase was extracted with AcOEt. The organic layers were combined, washed with brine, dried over MgSO₄ and concentrated under reduced pressure. The crude mixture was purified by silica gel column chromatography (AcOEt:Hexane, 1:2) to give 3-(4-benzyloxy-3-methoxyphenyl)-1-(2-hydroxy-3,4,5,6-tetramethoxyphenyl)-propenone (0.32 g, 0.66 mmol, 71%) as a yellow solid.

Yellow needle (AcOEt/Hexane) : mp 99-100 °C. IR (CHCl₃) : 1626, 1509, 1408, 1259, 1055 cm⁻¹. ¹H-NMR (400 MHz, CDCl₃) δ : 13.26 (1H, s), 7.82 (1H, d, *J* = 15.6 Hz), 7.78 (1H, d, *J* = 15.6 Hz), 7.45-7.31 (5H, m), 7.17 (1H, d, *J* = 8.4 Hz), 7.16 (1H, s), 6.90 (1H, d, *J* = 8.4 Hz), 5.21 (2H, s), 4.09 (3H, s), 3.95 (3H, s), 3.89 (3H, s), 3.87 (3H, s), 3.86 (3H, s). ¹³C-NMR (100 MHz, CDCl₃) δ : 193.3, 154.8, 153.2, 150.7, 150.4, 149.6, 144.0, 138.3, 137.1, 136.4, 128.5, 128.4, 127.9, 127.1, 124.2, 122.8, 113.4, 111.0, 110.9, 70.8, 62.2, 61.6, 61.3, 61.0, 56.0. Anal. Calcd. for C₂₂H₂₈O₈ : C, 67.49. H, 5.87. Found : C, 67.28. H, 5.83.

2-(4-Benzyloxy-3-methoxyphenyl)-5,6,7,8-tetramethoxychromen-4-one

A mixture of 3-(4-benzyloxy-3-methoxyphenyl)-1-(2-hydroxy-3,4,5,6-tetramethoxyphenyl)-propenone (95 mg, 0.20 mmol) and I₂ (3 mg, 0.01 mmol) in DMSO (1.5 ml, 0.13 M) was heated for 1.5 h at 150 °C. After cooling, the reaction mixture was quenched with H₂O and sodium sulfite (solid). The mixture was extracted with Et₂O. The organic extract was washed with brine, dried over MgSO₄ and concentrated under reduced pressure. The crude mixture was purified by silica gel column chromatography (AcOEt:Hexane, 3:1) to give 2-(4-benzyloxy-3-methoxyphenyl)-5,6,7,8-tetramethoxychromen-4-one (79 mg, 0.16 mmol, 83%) as a yellow solid.

Pale yellow plate (AcOEt/Hexane) : mp 109-111 °C. lit. 154-155 °C. IR (CHCl₃) : 1641, 1514, 1361 cm⁻¹. ¹H-NMR (400 MHz, CDCl₃) δ : 7.49-7.32 (7H, m), 6.99 (1H, d, *J* = 8.5 Hz), 6.60 (1H, s), 5.24 (2H, s), 4.10 (3H, s), 4.01 (3H, s), 3.98 (3H, s), 3.95 (6H, s). ¹³C-NMR (100 MHz, CDCl₃) δ : 177.2, 160.8, 151.3, 150.9, 149.7, 148.3, 147.6, 144.0, 137.9, 136.2, 128.6, 128.0, 127.1, 124.2, 119.4, 114.8, 113.4, 108.9, 106.9, 70.9, 62.3, 62.0, 61.8, 61.7, 56.1. Anal. Calcd. for C₂₇H₂₆O₈ : C, 67.77. H, 5.48. Found : C, 67.55. H, 5.58.

2-(4-Hydroxy-3-methoxyphenyl)-5,6,7,8-tetramethoxychromen-4-one

To a solution of 2-(4-benzyloxy-3-methoxyphenyl)-5,6,7,8-tetramethoxychromen-4-one (79 mg, 0.17 mmol) in EtOH (2 ml, 0.08 M) was added Pd/C (8 mg) and stirred for 2 h under atmospheric pressure of H₂. The mixture was filtered through celite pad eluting with AcOEt and the filtrate was concentrated under reduced pressure. The crude mixture was purified by silica gel column chromatography AcOEt to give 2-(4-Hydroxy-3-methoxyphenyl)-5,6,7,8-tetramethoxychromen-4-one, a4'-demethylated derivative of nobiletin (64 mg, 0.17 mmol, 99%) as a yellow solid.

Colorless prism (AcOEt/Hexane) : mp 155-156 °C. lit. 140-142 °C. IR (CHCl₃) : 3201, 1634, 1515, 1355, 1076 cm⁻¹. ¹H-NMR (400 MHz, CDCl₃) δ : 7.53 (1H, d, *J* = 8.3 Hz), 7.40 (1H, s), 7.05 (1H, d, *J* = 8.3 Hz), 6.60 (1H, s), 6.13 (1H, m), 4.09 (3H, s), 4.04 (3H, s), 3.97 (3H, s), 3.93 (6H, s). ¹³C-NMR (100 MHz, CDCl₃) δ : 177.4, 161.2, 151.4, 149.1,

148.3, 147.6, 147.0, 144.0, 138.0, 123.4, 120.2, 115.1, 114.7, 108.2, 106.5, 62.2, 61.9, 61.7, 61.6, 56.0. Anal. Calcd. for $C_{20}H_{20}O_8$: C, 61.85. H, 5.19. Found : C, 61.72. H, 5.29.