Electronic Supplementary Information

Concise Total Syntheses of Phelligridins A, C, and D

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Experimental Procedures and Spectral Data for All New Compounds.

General methods.

All moisture-sensitive reactions were performed under an atmosphere of argon or nitrogen, and the starting materials were azeotropically dried with benzene before use. Anhydrous MeOH, CH₂Cl₂, THF, toluene, DMSO, and pyridine were purchased from Kanto Chemical Co., Inc., or Wako Pure Chemical Industries Ltd., and used without further drying. TLC analyses were conducted on E. Merck precoated silica gel 60 F₂₅₄ (0.25 mm layer thickness). Fuji Silysia silica gel BW-820MH (75–200 μm) and FL-60D (45–75 μm) were used for column chromatography. Optical rotations were measured with a JASCO DIP-370 polarimeter. Infrared (IR) spectra were recorded on a JASCO FT/IR-4100 instrument, and only selected peaks are reported in wavenumbers (cm⁻¹). ¹H and ¹³C NMR spectra were recorded on a Bruker AVANCE 400 or a Bruker DPX 400 spectrometer. The ¹H and ¹³C chemical shifts (δ) were referenced with CDCl₃ (δ_H = 7.26 and δ_C = 77.0) or DMSO-d₆ (δ_H = 2.50 and δ_C = 39.5). J values are given in Hz. The following abbreviations are used for spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad. High resolution ESI/TOF mass spectra were recorded on a JEOL AccuTOFCS JMS-T100CS spectrometer.

Bromopyrone 8.

To a stirred solution of 4-hydroxy-6-methyl-2-pyrone 9 (1.01 g, 7.99 mmol) in CH₂Cl₂ (80.0 mL) was added Br₂ (0.75 mL, 14.5 mmol) at room temperature. After being stirred at the same temperature for 5.5 h in the dark, the reaction mixture was concentrated. The crude bromide 9a (1.63 g, 7.97 mmol) was used for the next reaction without further purification.

To a stirred solution of the crude bromide 9a (1.63 g, 7.97 mmol) in CH₂Cl₂ (40.0 mL) were added ¹Pr₂NEt (4.20 mL, 24.1 mmol) and MOMCl (0.91 mL, 12.0 mmol) at 0 °C. The mixture was stirred at the room temperature for 13 h, diluted with saturated aqueous NH₄Cl (50 mL), and extracted with CH₂Cl₂ (3×50 mL). The combined extracts were dried (Na₂SO₄) and concentrated. The residual solid was purified by column chromatography on silica gel (50 g, hexane–EtOAc 1:1) to give bromopyrone 8 (1.63 g, 82% in 2 steps) as a colorless solid: m.p. 76.5–78.2 °C; IR (CHCl₃) 3017, 2956, 2832, 1708, 1566, 1269, 1212, 1153, 1088, 1003, 922, 798, 719 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 6.18 (s, 1H), 5.27 (s, 2H), 3.51 (s, 3H), 2.25 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 165.2, 162.1, 160.9, 96.9, 94.7, 90.3, 57.1, 20.1; HRMS (ESI) m/z 270.9576 calcd for C₈H₆BrNaO₄ [M+Na]⁺ 270.9582.
Coupling compound 10.

To the mixture of bromopyrone 8 (280 mg, 1.13 mmol), boronate ester 7 (435 mg, 1.35 mmol), Pd(OAc)₂ (27.9 mg, 0.124 mmol), SPhos (95.2 mg, 0.232 mmol), and Na₂CO₃ (369 mg, 3.48 mmol) was added degassed H₂O–dioxane (6:1) (11.1 mL). The mixture was stirred at the room temperature for 4 h, diluted with saturated aqueous NH₄Cl (12 mL), and extracted with EtOAc (3×20 mL). The combined extracts were dried (Na₂SO₄) and concentrated. The residual oil was purified by column chromatography on silica gel (25 g, hexane–EtOAc 1:1) to give coupling compound 10 (257 mg, 63%) as a colorless solid: m.p. 165.9–167.8 °C; IR (CHCl₃) 3018, 1709, 1566, 1272, 1152, 1084, 1002, 924, 786 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.55 (s, 1H), 6.75 (s, 1H), 6.24 (q, J = 0.6 Hz, 1H), 5.07 (d, J = 7.0 Hz, 1H), 5.04 (d, J = 7.0 Hz, 1H), 3.93 (s, 3H), 3.89 (s, 3H), 3.75 (s, 3H), 3.36 (s, 3H), 2.31 (brs, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 166.5, 164.1, 163.3, 162.0, 151.8, 148.0, 126.9, 122.7, 114.3, 113.2, 107.6, 97.0, 93.9, 56.6, 56.0, 55.9, 51.8, 20.3; HRMS (ESI) 387.1075 m/z, calcd for C₁₈H₂₀NaO₈ [M+Na]⁺ 387.1056.

Dimethylphelligridin A (6).

To a stirred solution of coupling compound 10 (115 mg, 0.316 mmol) in dioxane (1.0 mL) was added 6 M HCl (1.0 mL, 6.0 mmol) at room temperatur. After being stirred at the same temperature for 12 h, the reaction mixture was concentrated to give dimethylphelligridin A (6) (86.2 mg, 95%) as a colorless solid: m.p. 247.7–249.6 °C; IR (CHCl₃) 3018, 1718, 1589, 1509, 1404, 1283, 1209, 1177, 1026, 822, 765, 731 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 8.55 (s, 1H), 7.68 (s, 1H), 7.68 (s, 1H), 7.07 (d, J = 7.0 Hz, 1H), 7.04 (d, J = 7.0 Hz, 1H), 4.06 (s, 3H), 3.99 (s, 3H), 2.38 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 162.6, 161.4, 160.8, 159.4, 155.8, 149.9, 128.8, 112.9, 110.0, 106.7, 99.6, 99.0, 56.5, 56.2, 20.2; HRMS (ESI) 311.0561 m/z, calcd for C₁₅H₁₂NaO₆ [M+Na]⁺ 311.0532.
Compound 13.
To a stirred solution of coupling compound 10 (208 mg, 0.57 mmol) in THF (4.3 mL) was added LHMDS (1.0 M solution in THF, 1.8 mL, 1.8 mmol) at −50 °C. After stirring at same temperature for 1 h, a solution of aldehyde 5a (111 mg, 0.67 mmol) in THF (0.70 mL) was added to the reaction mixture at −50 °C, and the mixture was stirred at same temperature for 3 h. The mixture was diluted with MeOH (5 ml) and saturated aqueous NH₄Cl (10 mL) and extracted with CH₂Cl₂ (3×10 mL). The combined extracts were dried (Na₂SO₄), filtered, and concentrated. The crude aldol 12 (296 mg) was used for the next reaction without further purification.

To a stirred solution of the crude aldol 12 (296 mg) in CH₂Cl₂ (2.8 mL) were added Et₃N (0.55 mL, 4.0 mmol) and MsCl (0.26 mL, 3.4 mmol) at 0 °C. After stirring for 1.5 h at same temperature, DBU (0.59 mL, 4.0 mmol) was added to the reaction mixture at 0 °C, and the mixture was stirred at same temperature for 1.5 h. The mixture was diluted with saturated aqueous NH₄Cl (10 mL) and extracted with CH₂Cl₂ (3×10 mL). The combined extracts were dried (Na₂SO₄), filtered, and concentrated. The residual solid was purified by column chromatography on silica gel (4 g, hexane–EtOAc 1:1) to give compound 13 (143 mg, 49% in 2 steps) as a yellow solid: m.p. 68.1–69.8 ºC; IR (CHCl₃) 3018, 1708, 1566, 1356, 1271, 1214, 1152, 1002, 788, 774, 721, 671 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.56 (s, 1H), 7.51 (d, J = 15.8 Hz, 1H), 7.09 (d, J = 8.3 Hz, 1H), 7.04 (s, 1H), 6.87 (d, J = 8.3 Hz, 1H), 6.80 (s, 1H), 6.55 (d, J = 15.8 Hz, 1H), 6.38 (s, 1H), 5.12 (d, J = 7.0 Hz, 1H), 5.08 (d, J = 7.0 Hz, 1H), 3.94 (s, 3H), 3.92 (s, 3H), 3.91 (s, 3H), 3.90 (s, 3H), 3.76 (s, 3H), 3.39 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 166.7, 163.3, 163.2, 158.8, 151.7, 150.5, 149.2, 148.1, 135.7, 128.3, 126.9, 122.8, 121.6, 116.9, 114.3, 113.2, 111.2, 109.3, 108.8, 97.4, 94.1, 56.6, 55.99, 55.96, 55.9, 55.8, 51.8; HRMS (ESI) 535.1606 m/z, calcd for C₂₇H₂₈NaO₁₀ [M+Na]⁺ 535.1580.

Tetramethylphelligridin D (14).
To a stirred solution of compound 13 (181 mg, 0.353 mmol) in MeOH (8.5 mL) was added 12 M HCl (2.0 mL, 24.0 mmol) at room temperature. After being stirred at reflux for 2 h in the dark, the reaction mixture was concentrated. The residual solid was washed with Et₂O to give tetramethylphelligridin D (14) (117 mg, 76%) as a yellow solid: m.p. 275.4–276.9 ºC; IR (KBr) 3180, 1715, 1514, 1369, 1285, 1225, 1156, 1034, 825, 637
Phelligridin D (3).

The mixture of tetramethylphelligridin D (14) (11.9 mg, 27.3 µmol) and HCl•py (476 mg, 4.12 mmol) was stirred at the 200 ºC for 3 h in the dark. The mixture was diluted with H2O (10 mL) and extracted with CH2Cl2 (3×10 mL). The water layer was acidified with 12 M HCl (5 ml) and extracted with EtOAc (3×15 mL). The combined extracts were dried (Na2SO4), filtered, and concentrated to give phelligridin D (3) (4.6 mg, 44%) as a brown amorphous powder: IR (KBr) 3429, 3130, 1693, 1549, 1403, 1288, 1131, 781, 727, 683 cm⁻¹; ¹H NMR (400 MHz, CDCl3) δ 10.75 (s, 1H), 10.10 (s, 1H), 9.58 (s, 1H), 9.17 (s, 1H), 8.33 (s, 1H), 7.51 (s, 1H), 7.28 (d, J = 15.3 Hz, 1H), 7.08 (s, 1H), 6.99 (d, J = 8.3 Hz, 1H), 6.78 (d, J = 15.3 Hz, 1H), 6.79 (d, J = 8.3 z, 1H), 6.71 (s, 1H); ¹³C NMR (100 MHz, CDCl3) δ 160.8, 159.5, 158.7, 158.4, 153.6, 147.9, 147.0, 145.7, 135.9, 127.0, 126.7, 120.9, 115.9, 115.5, 114.5, 114.1, 111.4, 110.5, 99.0, 98.8; HRMS (ESI) 379.0475 m/z, calcd for C20H11O8 [M–H]– 379.0454.

Boronate ester 15.

The combined extracts were dried (Na2SO4), filtered, and concentrated. To the mixture of bromide 15a (546 mg, 1.63 mmol), (pinB)2 (680 mg, 2.68 mmol), Pd(dppf)•CH2Cl2 (87.1 mg, 0.107 mmol), and KOAc (664 mg, 6.77 mmol) was added degassed 1,4-dioxane (4.0 mL). The mixture was stirred at 80 ºC for 11 h, diluted with saturated aqueous NH4Cl (15 mL), and extracted with EtOAc (3×15 mL). The combined extracts were dried (Na2SO4), and concentrated. The residual oil was purified by column chromatography on silica gel (50 g, hexane–EtOAc 7:1) to give boronate ester 15 (553 mg, 89%) as a colorless solid: m.p. 114.6–116.3 ºC; IR (CHCl3) 3026, 3012, 1710, 1546, 1515, 1270, 1223, 1151, 1024, 787, 643, cm⁻¹; ¹H NMR (400 MHz,
CDCl$_3$ $\delta$ 7.71 (s, 1H), 7.19 (s, 1H), 5.28 (s, 2H), 5.25 (s, 2H), 3.88 (s, 3H), 3.50 (s, 3H), 3.49 (s, 3H), 1.40 (s, 12H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 167.8, 150.5, 147.1, 127.7 (2C), 118.9, 117.1, 95.3, 95.1, 84.0 (2C), 56.4, 56.2, 52.2, 24.9 (4C); HRMS (ESI) 405.1710 m/z, calcd for C$_{18}$H$_{27}$BNaO$_8$ [M+Na]$^+$ 405.1697.

**Coupling compound 16.**

To the mixture of bromopyrone 8 (203 mg, 0.82 mmol), boronate ester 15 (473 mg, 1.24 mmol), Pd(OAc)$_2$ (41.8 mg, 0.19 mmol), SPhos (40.1 mg, 0.098 mmol), and Na$_2$CO$_3$ (422.8 mg, 3.99 mmol) was added degassed H$_2$O–dioxane (1:6) (17.5 mL). The mixture was stirred at room temperature for 5 h, diluted with saturated aqueous NH$_4$Cl (20 mL), and extracted with EtOAc (3×30 mL). The combined extracts were dried (Na$_2$SO$_4$), and concentrated. The residual oil was purified by column chromatography on silica gel (50 g, hexane–EtOAc 1:1) to give coupling compound 16 (228 mg, 66%) as a colorless solid: m.p. 95.2–96.8 ºC; IR (CHCl$_3$) 3019, 2961, 1711, 1546, 1509, 1269, 1153, 1070, 984, 925, 765 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.81 (s, 1H), 7.07 (s, 1H), 6.23 (s, 1H), 5.30 (d, $J$ = 6.8 Hz, 1H), 5.28 (s, 2H), 5.21 (d, $J$ = 6.8 Hz, 1H), 5.10 (d, $J$ = 7.0 Hz, 1H), 5.02 (d, $J$ = 7.0 Hz, 1H), 3.75 (s, 3H), 3.53 (s, 3H), 3.48 (s, 3H), 3.39 (s, 3H), 2.30 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 166.4, 164.0, 163.2, 161.9, 150.1, 146.0, 128.1, 124.3, 119.1, 118.4, 107.4, 97.0, 95.3, 95.1, 93.9, 56.6, 56.3 (2C), 51.9, 20.3; HRMS (ESI) 447.1266 m/z, calcd for C$_{20}$H$_{24}$NaO$_{10}$ [M+Na]$^+$ 447.1267.

**Compound 18.**

To a stirred solution of coupling compound 16 (334 mg, 0.79 mmol) in THF (3.2 mL) was added LHMDS (1.0 M solution in THF, 1.60 mL, 1.60 mmol) at −78 ºC. After stirring for 4 h at −78 ºC, a solution of aldehyde 17 (363 mg, 1.61 mmol) in THF (1.6 mL) was added to the reaction mixture at −78 ºC, and the mixture was stirred at same temperature for 3.5 h. The mixture was diluted with MeOH (5 ml) and saturated aqueous NH$_4$Cl (10 mL) and extracted with CH$_2$Cl$_2$ (3×20 mL). The combined extracts were dried (Na$_2$SO$_4$), filtered, and concentrated. The crude aldol 16a (783 mg) was used for the next reaction without further purification.
To a stirred solution of the crude aldol 16a (783 mg) in CH₂Cl₂ (18 mL) were added Et₃N (1.3 mL, 9.33 mmol) and MsCl (0.49 mL, 6.32 mmol) at 0 °C. After stirring for 1.5 h at room temperature, DBU (1.4 mL, 9.38 mmol) was added to the reaction mixture at 0 °C, and the mixture was stirred at room temperature for 2.5 h. The mixture was diluted with saturated aqueous NH₄Cl (20 mL) and extracted with CH₂Cl₂ (3×40 mL). The combined extracts were dried (Na₂SO₄), filtered, and concentrated. The residual oil was purified by column chromatography on silica gel (50 g, hexane–EtOAc 1:1) to give compound 18 (444 mg, 89%) as a yellow oil:

**IR (CHCl₃)** 3016, 1708, 1566, 1355, 1272, 1190, 1173, 1152, 795, 778, 770 cm⁻¹; **¹H NMR (400 MHz, CDCl₃)** δ 7.83 (s, 1H), 7.49 (d, J = 15.9 Hz, 1H), 7.37 (d, J = 15.9 Hz, 1H), 7.17 (d, J = 8.3 Hz, 1H), 7.13 (s, 1H), 6.55 (d, J = 15.9 Hz, 1H), 6.39 (s, 1H), 5.32 (d, J = 6.7 Hz, 1H), 5.30 (s, 2H), 5.27 (s, 2H), 5.23 (d, J = 6.7 Hz, 1H), 5.16 (d, J = 6.7 Hz, 1H), 5.08 (d, J = 7.0 Hz, 1H), 3.77 (s, 3H), 3.56 (s, 3H), 3.55 (s, 3H), 3.52 (s, 3H), 3.50 (s, 3H), 3.43 (s, 3H); **¹³C NMR (100 MHz, CDCl₃)** δ 166.5, 163.1, 158.6, 150.0, 148.5, 147.5, 146.1, 135.3, 130.0, 128.0, 124.5, 122.9 (2C), 119.1, 118.3, 117.6, 116.4, 114.9, 108.8, 97.7, 95.5, 95.3, 95.2, 95.1, 94.0, 56.6, 56.3, 56.3, 56.2 (2C), 51.9; **HRMS (ESI)** 655.2012 m/z, calcd for C₃₁H₃₆NaO₁₄ [M+Na]+ 655.2003.

**Phelligridin D (3).**

To a stirred solution of compound 18 (444 mg, 0.70 mmol) in MeOH (150 mL) was added 12 M HCl (15.0 mL, 180 mmol) at room temperature. After being stirred at reflux for 5 h in the dark, the reaction mixture was concentrated. The residual solid was washed with Et₂O to give phelligridin D (3) (175 mg, 66%) as a brown amorphous powder. The spectra data were in full agreement with described above.

**Phelligridin A (1).**

To a stirred solution of compound 16 (8.9 mg, 21 μmol) in MeOH (1.0 mL) was added 12 M HCl (0.10 mL, 1.2 mmol) at room temperature. After being stirred at room temperature for 24 h, the reaction mixture was concentrated. The residual solid was washed with Et₂O to give phelligridin A (1) (2.1 mg, 40%) as a colorless solid: m.p. >300 °C; **IR (KBr)** 3410, 3139, 1677, 1590, 1411, 1289, 1109, 1007, 899, 816, 756, 721
cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆) δ 10.7 (s, 1H), 10.1 (s, 1H), 8.29 (s, 1H), 7.51 (s, 1H), 6.57 (s, 1H), 2.31 (s, 3H); ¹³C NMR (100 MHz, DMSO-d₆) δ 162.3, 160.7, 160.2, 158.5, 153.5, 146.8, 126.6, 114.3, 111.2, 110.3, 99.2, 97.9, 19.3; HRMS (ESI) 259.0250 m/z, calcd for C₁₃H₇O₆ [M−H]− 259.0243.

**Compound 20.**

To a stirred solution of coupling compound 16 (88.3 mg, 0.21 mmol) in THF (1.0 mL) was added LHMDS (1.0 M solution in THF, 0.42 mL, 0.42 mmol) at −78 °C. After stirring for 1 h at same temperature, a solution of aldehyde 17 (96.2 mg, 0.43 mmol) in THF (0.40 mL) was added to the reaction mixture at −78 °C, and the mixture was stirred at same temperature for 4 h. The mixture was diluted with MeOH (2 ml) and saturated aqueous NH₄Cl (10 mL) and extracted with CH₂Cl₂ (3×20 mL). The combined extracts were dried (Na₂SO₄), filtered, and concentrated. The crude aldol 16b (220 mg) was used for the next reaction without further purification.

To a stirred solution of the crude aldol 16b (220 mg) in CH₂Cl₂ (4.2 mL) were added Et₃N (0.36 mL, 2.6 mmol) and MsCl (0.14 mL, 1.81 mmol) at 0 °C. After stirring for 1.5 h at room temperature, DBU (0.40 mL, 2.68 mmol) was added to the reaction mixture at 0 °C, and the mixture was stirred at room temperature for 7 h. The mixture was diluted with saturated aqueous NH₄Cl (10 mL) and extracted with CH₂Cl₂ (3×10 mL). The combined extracts were dried (Na₂SO₄), filtered, and concentrated. The residual oil was purified by column chromatography on silica gel (20 g, hexane–EtOAc 1:1) to give compound 20 (69.8 mg, 58% in 2 steps) as a yellow oil: IR (CHCl₃) 3018, 1707, 1600, 1570, 1439, 1346, 1274, 1142, 1085, 978, 931, 857, 643 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.83 (s, 1H), 7.52 (d, J = 15.9 Hz, 1H), 7.46 (d, J = 8.7 Hz, 2H), 7.12 (s, 1H), 7.05 (d, J = 8.7 Hz, 2H), 6.55 (d, J = 15.9 Hz, 1H), 6.37 (s, 1H), 5.31 (d, J = 6.8 Hz, 1H), 5.30 (s, 2H), 5.23 (d, J = 6.8 Hz, 1H), 5.20 (s, 2H), 5.16 (d, J = 7.0 Hz, 1H), 5.07 (d, J = 7.0 Hz, 1H), 3.77 (s, 3H), 3.54 (s, 3H), 3.49 (s, 3H), 3.49 (s, 3H), 3.42 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 166.5, 163.2, 158.8, 158.3, 150.0, 146.1, 135.4, 129.3, 128.9 (2C), 128.1, 124.5, 119.1, 118.3, 117.2, 116.6 (2C), 108.7, 97.5 (2C), 95.4, 95.1, 94.3, 94.0, 56.6, 56.3 (2C), 56.1, 51.9; HRMS (ESI) 595.1773 m/z, calcd for C₂₉H₂₃NaO₁₂ [M+Na]⁺ 595.1791.
Phelligridin C (2).

To a stirred solution of compound 20 (69.8 mg, 0.12 mmol) in MeOH (26 mL) was added 12 M HCl (2.6 mL, 31 mmol) at room temperature. After being stirred at reflux for 5 h in the dark, the reaction mixture was concentrated. The residual solid was washed with Et₂O to give phelligridin C (2) (16.1 mg, 37%) as a brown amorphous powder: IR (KBr) 3432, 3282, 1668, 1555, 1405, 1280, 1137, 761, 721, 684 cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆) δ 10.7 (s, 1H), 10.1 (s, 1H), 9.97 (s, 1H), 8.29 (s, 1H), 7.49 (d, J = 8.2 Hz, 2H), 7.48 (s, 1H), 7.32 (d, J = 16.0 Hz, 1H), 6.84 (d, J = 16.0 Hz, 1H), 6.78 (d, J = 8.2 Hz, 2H), 6.63 (s, 1H); ¹³C NMR (100 MHz, DMSO-d₆) δ 160.7, 159.5, 159.3, 158.7, 158.4, 153.6, 147.0, 135.5, 129.6, 127.0 (2C), 126.2, 115.9 (2C), 115.6, 114.5, 111.4, 110.5, 99.0, 98.8; HRMS (ESI) 363.0493 m/z, calcd for C₂₀H₁₁O₇ [M–H]⁻ 363.0505.

Cytotoxic activity

Stock cultures of cancer cells (HeLa S3, A549 cells: A549 cells were kindly provided by Prof. Hiroshi Nakagawa at the Department of Applied Biological Chemistry in Chubu University) were maintained in Dulbecco’s Modified Eagle Medium containing 10% fetal bovine serum and 100 µg/mL of penicillin, and 100 µg/mL streptomycin at 37 °C under 5% CO₂. For the purpose of the experiment, 5×10³ cells suspended in 100 µL of medium per well were plated in 96-well plate, and incubated at 37 °C under 5% CO₂. After incubation for 24 h, a solution of compound in DMSO (1 µL, concentration: 0.001, 0.01, 0.1, 1, 10 mM, respectively) was added to the above-mentioned well, resulting in various concentrations of the compound (0.01, 0.1, 1, 10, 100 µM) or solvent control (1% DMSO). After incubation for 96 h under the same conditions, 5 µL of WST-8 reagent solution was added to the cell culture, and the cell culture was further incubated for 2 h. Colorimetric determination of WST-8 was conducted at 450 nm with an optical reference wavelength at 595 nm using a microplate reader. The absorbance obtained upon the addition of the vehicle was considered as 100%. Data are expressed from the dose-response curve at three independent experiments. The cytotoxic effects of each compound were obtained as IC₃₀ values calculated by probit analysis using the PriProbit 1.63 software.