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Supplementary information

Hypocrellin B-based activatable photosensitizer for specific photodynamic effects against high H_2O_2-expressing cancer cells

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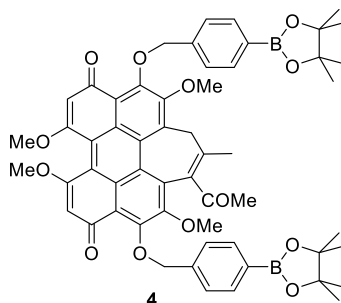
General methods for chemical synthesis

NMR spectra were recorded on a JEOL ECA-500 (500 MHz for ^{1}H, 125 MHz for ^{13}C) spectrometer. ^{1}H NMR data are reported as follows; chemical shift in parts per million (ppm) downfield or upfield from tetramethylsilane (δ 0.00) or $CDCl_3$ (δ 7.26), integration, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, and m = multiplet) and coupling constants (Hz). ^{13}C chemical shifts are reported in ppm downfield or upfield from $CDCl_3$ (δ 77.0). ESI-TOF Mass spectrum was measured on a Waters LCT premier XE. Melting point was determined on a micro hot-stage (Yanako MP-S3) and was uncorrected. Silica gel TLC and preparative TLC were performed using Merck TLC 60F-254 (0.25 mm) and Merck PLC 60F-254 (0.5 mm), respectively. UV/Vis spectra were recorded on a JASCO V-550 spectrometer. Air- and moisture-sensitive reaction was carried out under an argon atmosphere using oven-dried glassware.

Synthesis of H_2O_2-activatable photosensitizer 4



To a solution of hypocrellin B (**3**) (5.7 mg, 10.8 μmol) in dry DMF (285 μL) were added 4-(bromomethyl)phenylboronic acid pinacol ester (12.8 mg, 43.1 μmol) and K_2CO_3 (6.0 mg, 43.4 μmol) at room temperature. After being stirred at the same temperature for 24 h, the reaction mixture was concentrated in *vacuo*. The residue was purified by preparative TLC (1/1 *n*-hexane/acetone) to give **4** (3.9 mg, 4.06 μmol, 38% yield). Orange solid; R_f 0.61 (1/1 *n*-hexane/acetone); m.p. >300 ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 (2H, d, J = 8.0 Hz), 7.83 (2H, d, J = 8.0 Hz), 7.65 (4H, d, J = 8.0 Hz), 6.16 (2H, s), 5.34 and 5.16 (2H, ABq, J = 11.0 Hz), 5.29 and 5.19 (2H, ABq, J = 10.5 Hz), 4.01 (1H, d, J = 11.5 Hz), 3.98 (3H, s), 3.95 (3H, s), 3.94 (3H, s), 3.89 (3H, s), 2.92 (1H, d, J = 11.5 Hz), 2.36 (3H, s), 1.74 (3H, s), 1.36 (24H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 199.8, 184.6, 184.5, 166.2, 165.7, 154.9, 153.1, 152.9, 150.7, 146.3, 140.0, 139.9, 134.9×3, 134.2, 133.5, 128.1, 128.0, 126.6, 126.3, 126.1, 125.7, 125.0×2, 120.1, 118.5, 106.5, 106.2, 83.8, 76.1, 76.0, 62.1, 61.7, 56.1×2, 34.4, 29.5, 24.9, 20.8; HRMS (ESI-TOF) *m/z* 961.4144 (961.4142 calcd. for $C_{56}H_{59}B_2O_{13}$, $[M+H]^+$).

Materials for biological assay

Bovine serum albumin (BSA) was purchased from Sigma-Aldrich Co. LLC. 1,3-Diphenylisobenzofuran (DPBF) was purchased from Merck Schuchardt OHG. Catalase (from bovine liver) was purchased from FUJIFILM Wako Pure Chemical Corporation. The mouse melanoma cell line, B16F10 (RCB2630), was provided by the RIKEN BRC through the National Bio-Resource Project of the MEXT, Japan. The normal human lung fibroblast cell line, WI-38 (IFO50075), was purchased from the Japanese Collection of Research Bioresources (JCRB) Cell Bank (Osaka, Japan).

Photodegradation of proteins

The protein photodegradation experiments were performed with BSA (1.5 μM) in a volume of 10 μL in 1% DMSO-PBS (pH 7.4, 10 mM) containing indicated concentrations of each compound at 37 ºC for 2 h with or without irradiation using a LED (660 nm, 3 W, 33 mW/cm^2, EX-660, OPTCODE Corporation) placed 10 cm from the sample. After photo-irradiation, 2.00 μL of electrophoresis buffer consisted of Tris (0.35 M), SDS (10%, wt/vol), glycerol (36%, wt/vol), 2-mercaptoethanol (5%, wt/vol), and bromophenol blue (0.012%, wt/vol) was added to the samples. The photodegradation products were separated by SDS-PAGE in 8% polyacrylamide gels. The gels were run by applying 30 mA for 90 min, stained with SYPRO Ruby Protein Gel Stain (Bio-Rad Lab. Inc.) for 14 h, destained in acetic acid (7%, vol/vol) and methanol (10%, vol/vol) for 0.5 h, and washed with deionized water. The gels were scanned with a ChemiDoc Touch MP Imaging System (Bio-Rad Lab. Inc.). Molecular weight markers were used in each gel for calibration purpose.

Singlet oxygen productivity assay

A solution of **3** or **4** (5 μM) and DPBF (500 μM) in a volume of 120 μL in 80% DMSO-PBS (pH 7.4, 10 mM) was incubated at room temperature for 0-120 s under irradiation with a LED (660 nm, 3 W, 10 mW/cm^2) placed 20 cm from the sample in a dark room. After each resultant solution was moved to a 96-well plate, UV/Vis spectra of them were measured using SpectraMax i3 (Molecular Devices) micro plate reader.

EPR spectrometry

EPR experiments were carried out with a Bruker Biospin EMX EPR, and EPR spectra were recorded under the following conditions: temperature 296 K, microwave frequency 9.394 GHz, microwave power 16 mW, field modulation 0.1 mT at 100 kHz, scan time 3 min. For detection of singlet oxygen, 2,2,6,6-tetramethyl-4-piperidone (4-oxo-TEMP)

was used as a spin-trapping agent.[1] **3** or **4** (5 μM) and 4-oxo-TEMP (200 mM) were incubated in 1% DMSO-PBS (pH 7.4, 10 mM) containing 1 mM DETAPAC under irradiation with a LED (660 nm, 3 W, 10 mW/cm^2) placed 20 cm from a flat cell.

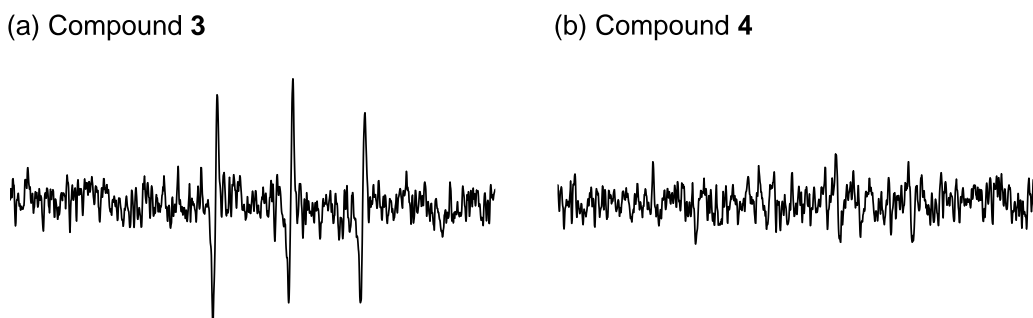


Fig. S1 EPR spectra obtained during photo-irradiation of (a) **3** and (b) **4** in the presence of 4-oxo-TEMP. Each compound (5 μM) and 4-oxo-TEMP (200 mM) were incubated in 1% DMSO-PBS (pH 7.4, 10 mM) containing 1 mM DETAPAC under irradiation with a LED (660 nm, 3 W, and 10 mW/cm^2) placed 20 cm from a flat cell for 3 min. DETAPAC = diethylenetriaminepentaacetic acid.

HPLC analysis

Analytical HPLC was performed on JASCO apparatus with a COSMOSIL 5C$_{18}$-AR-II column (4.6 × 250 mm, nacalai tesque, Inc.). Detection of products was made by UV detector (JASCO, UV-2077 Plus). A solution of **4** (20 μM) and H_2O_2 (0-1000 μM) in 5% DMSO-PBS (pH 7.4, 10 mM) was incubated at 37 ºC for 0-180 min, and analyzed by HPLC (0.01 M TFA-acetonitrile:0.01 M TFA aq. = 65:35 (0 to 14 min), 65:35 to 100:0 (14 to 15 min), 100:0 (15 to 25 min); flow rate 1.0 mL/min; 30 ºC; detection by UV (450 nm)).

ROS generating system

Various ROS (300 μM) were administered to **4** as follows. Hydrogen peroxide (H_2O_2), *tert*-butyl hydroperoxide (TBHP), and hypochlorite ($^-$OCl) were delivered from 30%, 70%, and 5% aqueous solutions, respectively. The concentration of H_2O_2 was determined from the absorption at 240 nm (ε = 43.6 M^{-1} cm^{-1}).[2] The concentration of $^-$OCl was determined from the absorption at 292 nm (ε = 350 M^{-1} cm^{-1}).[2] Superoxide ($O_2^{-\bullet}$) was added as solid KO_2 in the presence of catalase (5 units). Hydroxyl radical ($^\bullet$OH) and *tert*-butoxy radical ($^\bullet$O^{t}Bu) were generated by reaction of 1 mM Fe^{2+} with 300 μM H_2O_2 or 300 μM TBHP, respectively. A solution of **4** (20 μM) and various ROS (300 μM) in 5%

DMSO-PBS (pH 7.4, 10 mM) was incubated at 37 ºC for 120 min, and analyzed by HPLC (COSMOSIL $5C_{18}$-AR-II, 4.6 × 250 mm; 0.01 M TFA-acetonitrile:0.01 M TFA aq. = 65:35 (0 to 14 min), 65:35 to 100:0 (14 to 15 min), 100:0 (15 to 25 min); flow rate 1.0 mL/min; 30 ºC; detection by UV (450 nm)).

Singlet oxygen productivity assay in the presence of H_2O_2

A solution of **4** (0 or 25 μM) and H_2O_2 (1 mM) in 5% DMSO-PBS (pH 7.4, 10 mM) was incubated at 37 ºC for 2 h. The product (24 μL) was then added to 96 μL of DPBF (625 μM, final conc. of DPBF was 500 μM) in 98.75% DMSO-PBS (final conc. of DMSO was 80%), and incubated at room temperature for 0-120 s under irradiation with a LED (660 nm, 3 W, 10 mW/cm^2) placed 20 cm from the sample in a dark room. After each resultant solution was moved to a 96-well plate, UV/Vis spectra of them were measured using a micro plate reader.

Cell culture

<B16F10>

The B16F10 cell line was routinely grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% (v/v) Fetal bovine serum, 0.5% (v/v) penicillin and kanamycin. The cells were maintained at 37 ºC in a humidified atmosphere containing 5% CO_2.

<WI-38>

The WI-38 cell line was routinely grown in Eagle's minimum essential medium (MEM) supplemented with 10% (v/v) Fetal bovine serum, 0.5% (v/v) penicillin and kanamycin. The cells were maintained at 37 ºC in a humidified atmosphere containing 5% CO_2.

MTT assay

The cells were seeded into 96-well plates (90.0 μL, 4.0×10^3 cells). After 24 h, cells were treated with 10.0 μL of **3** or **4** (0, 1, 3, 10 or 30 μM) in 10% DMSO-medium (final conc. of DMSO was 1%) and incubated for 3 h at 37 ºC. And then, cells were incubated for 30 min with or without photo-irradiation using a LED (660 nm, 3 W, 17 mW/cm^2) placed 15 cm from the sample. The samples were further incubated for 24 h at 37 ºC. Cell viability was evaluated using the MTT assay. 10.0 μL of 5.00 mg/mL MTT dissolved in PBS was added to each well. After incubation for 3 h at 37 °C, medium was aspirated and 100 μL of DMSO was added to each well. The absorbance of the mixture was measured using a micro plate reader at 540 nm.

References

1) J. Moan and E. Wold, *Nature*, 1979, **279**, 450.
2) M. Abo, Y. Urano, K. Hanaoka, T. Terai, T. Komatsu and T. Nagano, *J. Am. Chem. Soc.*, 2011, **133**, 10629.

^{1}H and ^{13}C NMR spectrum charts

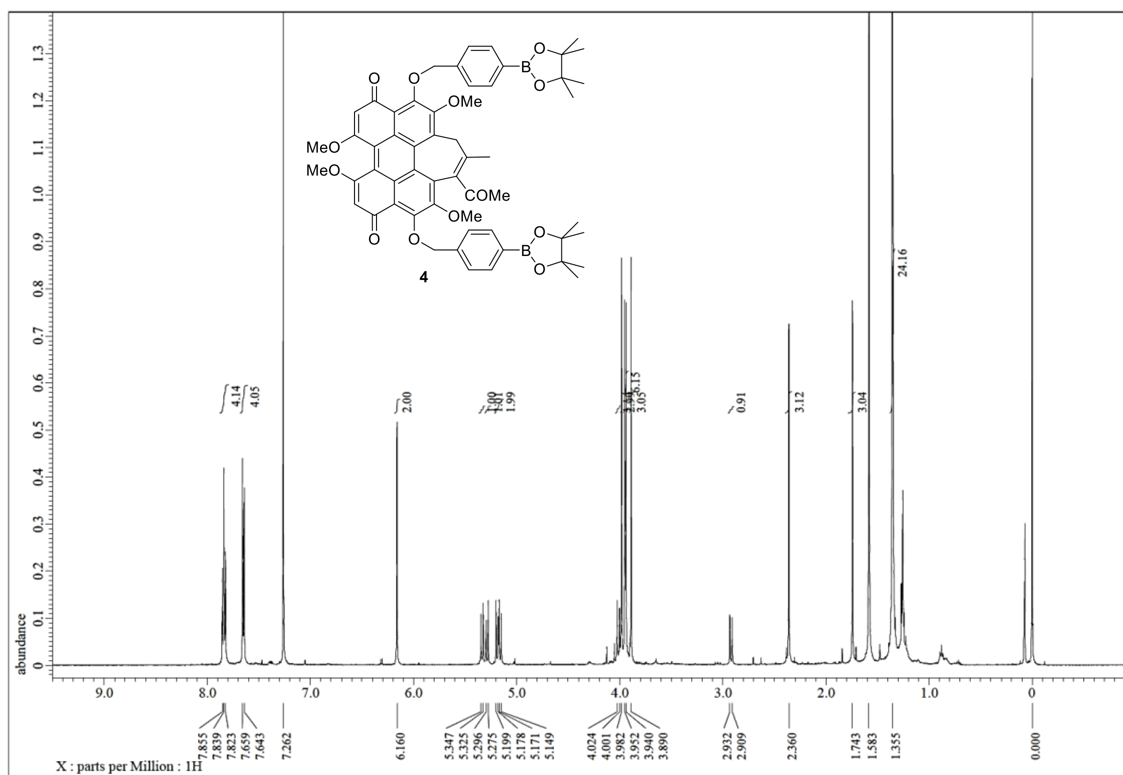


Fig. S2 ^{1}H NMR spectrum of **4**

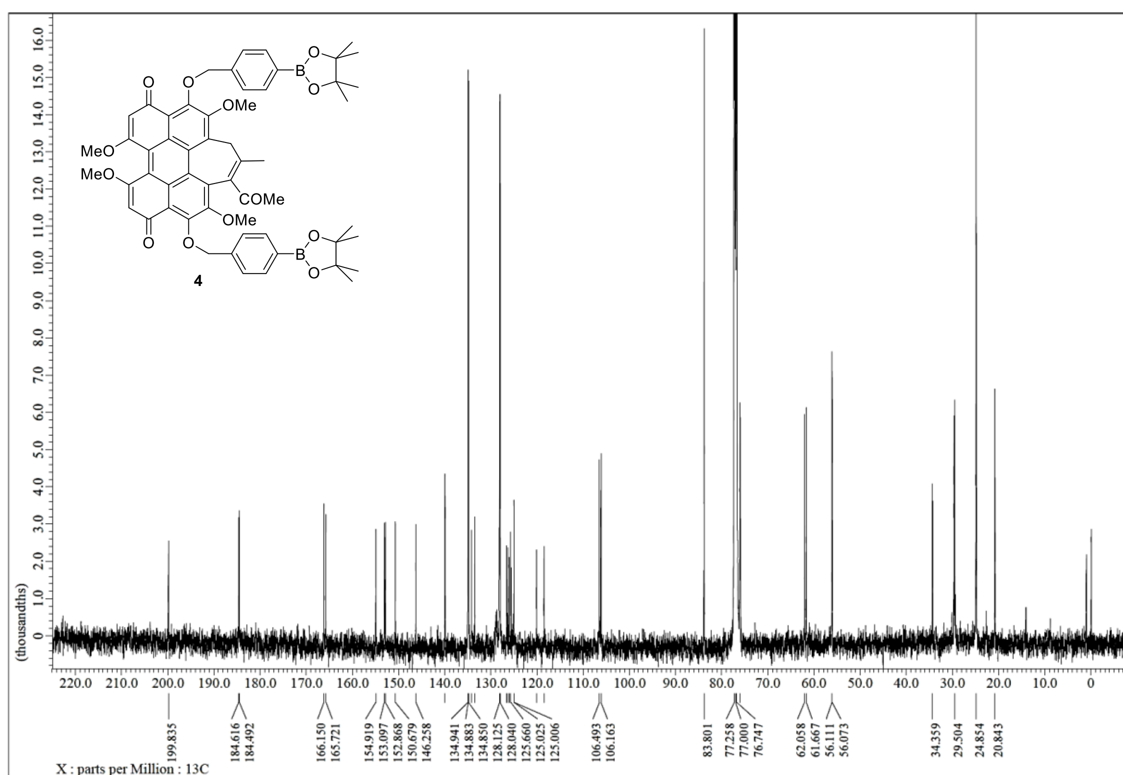


Fig. S3 ^{13}C NMR spectrum of **4**