Electronic Supplementary Information

Development and Cellular Application of Visible-Light-Controllable HNO Releasers Based on Caged Piloty's Acid

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General methods

Decomposition points were determined using a Yanagimoto micro melting point apparatus or a Büchi 545 melting point apparatus and were left uncorrected. Proton nuclear magnetic resonance spectra (^{1}H NMR) and carbon nuclear magnetic resonance spectra (^{13}C NMR) were recorded on a JEOL JNM-LA500, JEOL JNM-A500, Varian VNMRS 500 or Bruker AVANCE600 spectrometer in the indicated solvent. Chemical shifts (δ) are reported in parts per million relative to the internal standard, tetramethylsilane. Elemental analysis was performed with a Yanaco CHN CORDER NT-5 analyzer, and all values were within ±0.4% of the calculated values. High-resolution mass spectra (HRMS) and fast atom bombardment (FAB) mass spectra were recorded on a JEOL JMS-SX102A mass spectrometer. GC-MS analyses were performed on a Shimadzu GCMS-QP2010. Confocal fluorescence images were taken with an IX-71 (Olympus) equipped with a disc scanning unit. Reagents and solvents were purchased from Aldrich, Tokyo Kasei Kogyo, Wako Pure Chemical Industries, Kanto Kagaku, Junsei Kagaku, Dojindo, and Invitrogen, and were used without purification. Flash column chromatography was performed using Silica Gel 60 supplied by Taiko-Shoji.

<Synthesis>

Synthesis of 7-(diethylamino)-2-oxo-2H-chromene-4-carbaldehyde (2)

To a solution of 7-diethylamino-4-methylcoumarin (**1**) (5033 mg, 21.8 mmol) in *p*-xylene (120 mL) was added SeO_2 (4030 mg, 36.3 mmol, 1.7 equiv.), and the mixture was heated at reflux with vigorous stirring. After 24 hours, the mixture was filtered through Celite and the Celite pad was washed with

AcOEt. The filtrate was concentrated under reduced pressure to give crude **2** as a dark-brown residual oil.

Synthesis of 7-(diethylamino)-4-(hydroxymethyl)-2*H*-chromen-2-one (3)

Crude **2** was dissolved in EtOH (120 mL), then $NaBH_4$ (417 mg, 11.0 mmol, 0.5 equiv.) was added, and the mixture was stirred overnight at room temperature under an N_2 atmosphere. Thereafter, the suspension was carefully hydrolyzed with 1 N HCl, neutralized with sat. $NaHCO_3$, diluted with H_2O, partially concentrated under reduced pressure, and extracted with AcOEt. The organic solution was washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel flash column chromatography (CH_2Cl_2/acetone = 8/1) to give 1907 mg (7.71 mmol, 35% in two steps) of **3** as a dark orange solid: ^{1}H NMR (DMSO-d_6, 500 MHz, δ; ppm) 7.42 (1H, d, J = 9.0 Hz), 7.28 (1H, dd, J_1 = 9.0 Hz, J_2 = 2.6 Hz), 6.52 (1H, d, J = 2.5 Hz), 6.07 (1H, s), 5.58 (1H, t, J = 5.6 Hz), 4.67 (2H, dd, J_1 = 5.7 Hz, J_2 = 1.3 Hz), 3.42 (4H, q, J = 7.0 Hz), 1.16 (6H, t, J = 7.1 Hz).

Synthesis of (7-(diethylamino)-2-oxo-2H-chromen-4-yl)methylmethanesulfonate (4)

To a stirred suspension of **3** (786 mg, 3.18 mmol) in CH_2Cl_2 (20 mL) were added NEt_3 (887 μL, 6.36 mmol, 2.0 equiv.) and methanesulfonyl chloride (369 μL, 4.77 mmol, 1.5 equiv.) at 0 °C. The reaction mixture was stirred for 30 minutes at 0 °C, then washed with sat. $NaHCO_3$ and water, dried over Na_2SO_4, filtered and concentrated to give crude **4**.

Synthesis of 4-(bromomethyl)-7-(diethylamino)-2*H*-chromen-2-one (5)

To a solution of crude **4** in CH_2Cl_2 (10 mL) was added LiBr (1104 mg, 12.7 mmol, 4.0 equiv.). The mixture was stirred for 2 hours, and extracted with CH_2Cl_2. The organic solution was washed with H_2O and brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel flash column chromatography (*n*-hexane/AcOEt = 3/1) to give 837 mg (2.70 mmol, 85 % in two steps) of **5** as an orange solid: ^{1}H NMR (DMSO-d_6, 500 MHz, δ; ppm) 7.62 (1H, d, J = 9.1 Hz), 7.28 (1H, d, J = 9.1 Hz), 6.54 (1H, s), 6.24 (1H, s), 4.77 (2H, s), 3.42 (4H, m), 1.13 (6H, t, J = 6.9 Hz).

Synthesis of 4-{(aminooxy)methyl}-7-(diethylamino)-2*H*-chromen-2-one (6)

To an ice-cold solution of *N*-Boc-hydroxylamine (582 mg, 3.90 mmol, 1.5 equiv.) in anhydrous THF (10 mL) was added sodium hydride (60 % in mineral oil; 93.5 mg, 3.90 mmol, 1.5 equiv.) under an N_2 atmosphere. The mixture was allowed to warm to room temperature, stirred for 2 hours, and then cooled to 0 °C again. A solution of **5** (806 mg, 2.60 mmol) and TBAI (1152 mg, 3.12 mmol, 1.2 equiv.) in anhydrous THF (10 mL) was added dropwise to the resultant suspension at 0 °C. The reaction mixture was stirred at 0 °C overnight and then poured into ice-cold water. The mixture was extracted with CH_2Cl_2 and the combined extracts were dried over Na_2SO_4, filtered and concentrated. The residue

was purified by flash column chromatography (*n*-hexane/AcOEt = 3/1) to give 892 mg (2.46 mmol, 95%) of **6** as an orange solid: ^{1}H NMR (DMSO-d_6, 500 MHz, δ; ppm) 10.28 (1H, s), 7.62 (1H, d, J = 9.1 Hz), 6.68 (1H, dd, J_1 = 9.1 Hz, J_2 = 2.5 Hz), 6.53 (1H, d, J = 2.5 Hz), 6.10 (1H, s), 4.89 (2H, s), 3.42 (4H, q, J = 7.0 Hz), 1.41 (9H, s), 1.13 (6H, t, J = 7.0 Hz).

Synthesis of 4-{(aminooxy)methyl}-7-(diethylamino)-2*H*-chromen-2-one (7)

To a solution of **6** (378 mg, 1.04 mmol) in CH_2Cl_2 (5 mL) was added 4 N HCl/AcOEt (3 mL). The reaction mixture was stirred for 30 minutes at room temperature, and then concentrated by evaporation *in vacuo* to give **7** (crude).

Synthesis of *N*-{(7-(diethylamino)-2-oxo-2*H*-chromen-4-yl)methoxy)-2-nitrobenzenesulfonamide (compound 8)

To a solution of crude **7** and DMAP (176 mg, 1.44 mmol, 1.4 equiv.) in pyridine (30 mL) was added 2-nitrobenzenesulfonyl chloride (266 mg, 1.20 mmol, 1.2 equiv.) in portions on an ice-bath. The reaction flask was removed from the ice-bath and the mixture was stirred for about 10 minutes at room temperature in the dark. The resulting suspension was poured into 1 N HCl aq., and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel flash column chromatography (*n*-hexane/AcOEt = 1/1) and recrystallized from AcOEt/$CHCl_3$/*n*-hexane) to give 145 mg (22% in 2 steps) of **compound 8** as an orange solid: decomp. point 197.2–198.0 °C; ^{1}H NMR (DMSO-d_6, 500 MHz, δ; ppm) 11.43 (1H, s), 8.09–8.06 (2H, m), 7.96–7.94 (2H, m), 7.42 (1H, d, J = 9.2 Hz), 6.66 (1H, dd, J_1 = 9.0 Hz, J_2 = 2.5 Hz), 6.53 (1H, d, J = 2.5 Hz), 6.04 (1H, s), 4.89 (2H, s), 3.42 (4H, q, J = 7.1 Hz), 1.15 (6H, t, J = 7.0 Hz); ^{13}C NMR (DMSO-d_6, 125 MHz, δ; ppm) 160.67, 155.93, 150.42, 149.77, 148.70, 135.38, 132.73, 130.05, 129.20, 125.81, 124.69, 108.72, 106.96, 105.62, 96.84, 74.28, 44.03, 12.33; MS (ESI$^+$) m/z: 470 ([M+Na]$^+$); Anal. Calcd. for $C_{20}H_{21}N_3O_7S$ ・ 1/5H_2O: C, 53.19; H, 4.87; N, 9.29, Found: C, 53.25; H, 4.78; N, 9.32.

Synthesis of *N*-{(7-(diethylamino)-2-oxo-2*H*-chromen-4-yl)methoxy)-2-bromobenzenesulfonamide (compound 9)

To a solution of crude **7** and DMAP (127 mg, 1.04 mmol, 1.2 equiv.) in pyridine (30 mL) was added 2-nitrobenzenesulfonyl chloride (221 mg, 0.860 mmol) in portions on an ice-bath. The reaction flask was removed from the ice-bath and the mixture was stirred for about 10 minutes at room temperature in the dark. The resulting suspension was poured into 1 N HCl aq., and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel flash column chromatography (*n*-hexane/AcOEt = 1/1) and recrystallized from AcOEt/$CHCl_3$/*n*-hexane) to give 111 mg (28% in 2 steps) of **compound 9** as an orange solid: ^{1}H NMR

(DMSO-d_6, 500 MHz, δ; ppm) 11.08 (1H, s), 8.04–8.01 (1H, m), 7.90–7.88 (1H, m), 7.63–7.59 (2H, m), 7.41 (1H, d, J = 9.1 Hz), 6.67 (1H, dd, J_1 = 9.1 Hz, J_2 = 2.5 Hz), 6.51 (1H, d, J = 2.5 Hz), 6.03 (1H, s), 5.05 (2H, s), 3.43 (4H, q, J = 7.0 Hz), 1.17 (6H, t, J = 7.0 Hz); ^{13}C NMR (DMSO-d_6, 125 MHz, δ; ppm) 160.68, 155.91, 150.39, 149.79, 136.52, 135.63, 135.09, 131.70, 128.27, 125.86, 119.92, 108.72, 107.16, 105.68, 96.83, 74.14, 44.03, 12.34; MS (ESI$^+$) m/z: 503 ([M+Na]$^+$) 505 ([M+2+Na]$^+$) ; Anal. Calcd. for $C_{20}H_{21}BrN_2O_5S$ ・ $1/10H_2O$: C, 49.90; H, 4.40; N, 5.82, Found: C, 49.72; H, 4.42; N, 5.80.

Synthesis of 2-nitrobenzenesulfinic acid (compound 11)

To a solution of 2-nitrobenzenesulfonyl chloride (**10**) (500 mg, 2.26 mmol) in THF (10 mL) was added $NaBH_4$ (428 mg, 11.3 mmol, 5.0 equiv.) in portions at 0 °C. The mixture was stirred for 3 hours, then H_2O (10 mL) was added, and the mixture was acidified with 2 N HCl to pH < 2 on an ice-bath. The resulting mixture was extracted with CH_2Cl_2, and the organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was recrystallized from $CHCl_3$ and washed with hexane to give 115 mg (27 %) of **compound 11** as pale yellow crystals: decomp. point 110.9–111.3 °C; ^{1}H NMR (DMSO-d_6, 500 MHz, δ; ppm) 8.22 (1H, dd, J_1 = 8.0 Hz, J_2 = 1.1 Hz), 8.12 (1H, dd, J_1 = 7.8 Hz, J_2 = 1.4 Hz), 8.01 (1H, dt, J_1 = 7.5 Hz, J_2 = 1.1 Hz), 7.83 (1H, dt, J_1 = 8.1 Hz, J_2 = 1.5 Hz) ; ^{13}C NMR (DMSO-d_6, 125 MHz, δ ; ppm) 145.89. 144.77, 134.93, 132.48, 125.34, 124.98; MS (FAB) m/z: 188 ([M+1]$^+$); Anal. Calcd. for $C_6H_5NO_4S$: C, 38.50; H, 2.69; N, 7.48, Found: C, 38.37; 2.83; N, 7.35.

Synthesis of 7-(diethylamino)-2-oxo-2H-chromene-4-carbaldehyde oxime (compound 12)

To a solution of **2** (100 mg, 0.408 mmol) in MeOH (10 mL) were added potassium carbonate (68 mg, 0.49 mmol, 1.2 equiv.) and 50 % hydroxylamine (33 μL, 0.490 mmol, 1.2 equiv.). The mixture was stirred at room temperature for 2 hours and further potassium carbonate (68 mg, 0.490 mmol, 1.2 equiv.) was added, together with 50 % hydroxylamine (33 μL, 0.490 mmol, 1.2 equiv.). The mixture was stirred for 1 hour and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, filtered, stirred overnight and evaporated to dryness. The crude product was washed with CH_2Cl_2 to give 11 mg (0.0422 mmol, 10 %) of **compound 12** as an orange solid; ^{1}H NMR (DMSO-d_6, 500 MHz, δ ; ppm) 13.23 (1H, s), 8.38 (1H, s), 8.10 (1H, d, J = 9.2 Hz), 6.70 (1H, d, J = 9.2 Hz), 6.55 (1H,s), 6.23 (1H, s), 3.44 (4H, q, J = 7.1 Hz), 1.13 (6H, t, J = 7.0 Hz).

<Assays>

Measurement of absorbance

A solution of **compound 8** or **9** (10 mM in DMSO, 1 μL) in DMSO (9 μL) was diluted with Milli Q

(990 μL) and transferred into a cuvette. As a blank, 1 mL of 1 % DMSO in Milli Q was also transferred into a cuvette. UV/vis spectra were recorded by using an Agilent 8453 spectrometer at room temperature.

HPLC analysis – detection of decomposed compounds, benzenesulfinic acid

Solutions of **compound 3**, **8**, **11**, **12** (10 mM in DMSO, 10 μL) were diluted with DMSO (90 μL) and Tris buffer (50 mM, pH 7.4, 900 μL) to prepare sample solutions. The sample solutions were photoirradiated (400–430 nm, 30 mW/cm^2) at room temperature for 10 minutes. An aliquot of each solution (20 μL) was loaded onto an Inertsil ODS-3 column (5 mm; 150 × 4.6 mm) fitted on a Shimadzu HPLC system. The eluate was monitored with a photodiode array detector. (A) Milli-Q water containing 0.1% TFA and (B) MeCN containing 0.1% TFA were used as developing solvents. Gradient conditions were as follows: 0 min, A 70% and B 30% → 20 min, A 20% and B 80% → 30 min, A 20% and B 80% →40 min, A 70% and B 30%.

HPLC analysis – quantification of compound 3 upon photoirradiation of compound 8 for estimation of *path I* fraction

A solution of **compound 3** (0.3, 1, 3 mM in DMSO, 1 μL) were diluted with Tris buffer (50 mM, pH 7.4, 99 μL) to prepare sample solutions. An aliquot of each solution (20 μL) was loaded onto an Inertsil ODS-3 column (5 mm; 150 × 4.6 mm) fitted on a Shimadzu HPLC system. The eluate was monitored with a photodiode array detector. (A) Milli-Q water containing 0.1% TFA and (B) MeCN containing 0.1% TFA were used as developing solvents. Gradient conditions were as follows: 0 min, A 70% and B 30% → 20 min, A 20% and B 80% → 30 min, A 20% and B 80% →40 min, A 70% and B 30%. Peak area of **compound 3** (t_R = 7.6 min) was calculated and plotted for the preparation of a standard line. This standard line was used to estimate the fraction of *path I* reaction of 100 μM **compound 8** upon photoirradiation (by using the result of the above HPLC analysis).

GC-MS analysis – Detection of N_2O release from compound 8 or 9

A solution of **compound 8** or **9** (10 mM in DMSO, 60 μL) in DMSO (240 μL) was diluted with Tris-HCl buffer (50 mM, pH 7.4, 2700 μL). The sample solution was transferred into a 4 mL cuvette, which was sealed with a rubber septum. The cuvette was shaken for 10 seconds and irradiated (400–430 nm, light intensity: 22 mW/cm^2) for 20 minutes. It was shaken again, and the reaction headspace gas (50 μL) was sampled and injected into a Shimadzu GC-2010 gas chromatograph equipped with a mass spectrometer (QP2010) and an Rt-QPLOT column (0.32 × 15 m) attached with a 15 m inactivated fused silica capillary (total 30 m). The GC injector was operated with a split ratio of 0.1 at 200 °C. The carrier gas (He) was set at a flow rate of 2.2 mL/min. The GC oven was held at 35 °C. The mass interface was set to 280 °C. The peak area of N_2O, excluding CO_2, was calculated.

GC-MS analysis – Detection of N_2O release from Angilie's Salt (AS)

A solution of **AS** (10 mM in MilliQ, 60 μL) in DMSO (300 μL) was diluted with Tris-HCl buffer (50 mM, pH 7.4, 2640 μL). The sample solution was transferred into a 4 mL cuvette, which was sealed with a rubber septum. The cuvette was shaken for 10 seconds and incubated for 20 minutes. It was shaken again, and the reaction headspace gas (50 μL) was sampled and analyzed by GC-MS.

Detection of HNO released from compound 8 or 9 with P-Rhod

A solution of **P-Rhod** (10 mM in DMSO, 1 μL) in DMSO (8 μL) was diluted with PBS buffer (200 mM, pH = 7.0, 250 μL) and Milli Q (740 μL). A solution of **compound 8** or **9** (10 mM in DMSO, 1 μL) was added to the solution and either irradiated (400–430 nm, light intensity: 22 mW/cm^2) for 10 minutes or incubated in the dark for 10 minutes at room temperature. The fluorescence spectrum (500 nm-650 nm) was measured with excitation at 491 nm.

Detection of HNO released from Angilie's Salt (AS) with P-Rhod

A solution of **P-Rhod** (10 mM in DMSO, 1 μL) in DMSO (9 μL) was diluted with PBS buffer (200 mM, pH 7.0, 250 μL) and Milli Q (740 μL). A solution of **AS** (10 mM in NaOH, 1 μL) was added to it, and the mixture was incubated for 30 minutes at room temperature. The fluorescence spectrum (500 nm-650 nm) was measured with excitation at 491 nm.

Detection of HNO released from compound 8 in the presence of 2-mercaptoethanol with P-Rhod

A solution of **P-Rhod** (10 mM in DMSO, 1 μL) in DMSO (8 μL) was diluted with PBS buffer (200 mM, pH 7.0, 250 μL) and Milli Q (739 μL). A solution of **compound 8** (10 mM in DMSO, 1 μL) and 2-mercaptoetanol (1 μL, final 14 mM) was added to it, and the mixture was irradiated (400–430 nm, light intensity: 22 mW/cm^2) for 10 minutes at room temperature. The fluorescence spectrum (500 nm-650 nm) was measured with excitation at 491 nm.

Time-dependence of HNO release from compound 8, detected with P-Rhod

A solution of **P-Rhod** (10 mM in DMSO, 1 μL) in DMSO (8 μL) was diluted with PBS buffer (200 mM, pH = 7.0, 250 μL) and Milli Q (740 μL). A solution of **compound 8** (10 mM in DMSO, 1 μL) was added to it, and the mixture was irradiated (400–430 nm, light intensity: 22 mW/cm^2) for 0, 1, 2, 5, or 10 min at room temperature. As a control, a solution of **P-Rhod** (10 μM) without **compound 8** was irradiated (400–430 nm, light intensity: 22 mW/cm^2) for 10 minutes at room temperature. The fluorescence spectrum (500 nm-650 nm) was measured with excitation at 491 nm.

LC-MS analysis for quantification of HNO formation from compound 8

For the standard curve: a solution of *N*-acetylcysteine (1 M in 200 mM PBS buffer (pH 7.0), 3 μL) was diluted with PBS buffer (50 mM, pH = 7.0, 291 μL) and 3 μL DMSO, then a solution of **AS** (0, 1.25, 2.5, 5, 10 mM in 0.1 N NaOH aq., 3 μL) was added to it, and the mixture was incubated for 2 hr at room temperature. For sample solutions: a solution of *N*-acetylcysteine (1 M in 200 mM PBS buffer (pH 7.0), 3 μL) was diluted with PBS buffer (50 mM, pH = 7.0, 294 μL), then a solution of **compound 8** (10 mM in DMSO, 3 μL) or DMSO for control was added to it, and the mixture was irradiated (400–430 nm, light intensity: 22 mW/cm^2) for 20 min at room temperature. Aliquots of the above solutions were loaded onto an Imtakt US-C18 column (5 mm; 150×2 mm) fitted on a Waters ACQUITY/Quattro Premier XE system. (A) Milli-Q water containing 0.1% FA and (B) MeCN containing 0.1% FA were used as developing solvents. Gradient conditions were as follows: 0 min, A 99% and B 1% → 5 min, A 99% and B 1% → 8 min, A 80% and B 20% →18 min, A 0% and B 100% → 21 min, A 0% and B 100% →23 min, A 99% and B 1% →30 min, A 99% and B 1%; ion mode, negative; cone voltage 30 V. Peak area of *m/z* = 323 (*N*,*N*'-diacetyl-L-cystine: t_R = 10.75 min) was calculated and plotted for the preparation of a standard curve from the results of **AS**. This standard curve was used to calculate the amount of HNO released from 100 μM **compound 8**.

Co-localization staining

HEK293T cells were placed on 3.5 cm glass-bottomed dishes coated with poly-L-lysine at 4.0×10^5 cells/dish with 2 mL of DMEM containing 5% fetal bovine serum (FBS). The cells were incubated at 37 °C in a humidified 5% (v/v) CO_2 incubator for a day, then washed with 2 mL of D-PBS twice, and placed with 1 mL of Dulbecco's PBS (D-PBS). The cells were treated with 1 μL of **compound 8** (10 mM, final 1 μM) and 1 μL of ER-Tracker Green® (1 mM, final 1 μM, Invitrogen) and incubated for 30 min under the above conditions, then washed with 1 mL of D-PBS once, and subjected to confocal microscopy.

Detection of HNO in HEK293T cells

HEK293T cells were plated on 3.5 cm glass-bottomed dishes coated with poly-L-lysine at 2.0×10^5 cells/dish with 2 mL of DMEM containing 5% FBS. The cells were incubated at 37 °C in a humidified atmosphere of 5 % (v/v) CO_2 in air for 2 days. The medium was replaced with 2 mL of D-PBS. The cells were treated with 0.2 μL of **P-Rhod** in DMSO (10 mM, final 1 μM) and incubated for 150 minutes under the above conditions. Next, the cells were treated with 4 μL of **compound 8** in DMSO (10 mM, final 20 μM), incubated for 30 minutes, washed with 2 mL of D-PBS three times, irradiated with a MAX -302 (400–430 nm, 20 mW/cm^2) for 30 minutes, and subjected to confocal microscopy.

Note: we expected that HNO release from compound 8 in cellular systems might decrease, because cells have several HNO scavengers, such as biological thiols (glutathione, cysteine, etc). In this experiment, compound 8 was photoirradiated for 30 minutes for complete degradation.

Cytotoxicity of compound 8 to HEK293T cells

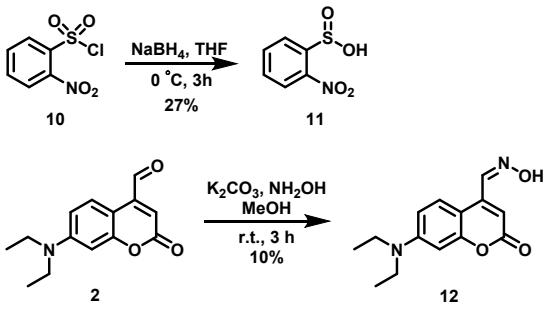
HEK293T cells were plated on a 96-well microplate coated with poly-L-lysine at 5.0×10^3 cells/well with 100 μL of DMEM containing 5% FBS. The cells were incubated at 37 °C in a humidified atmosphere of 5 % (v/v) CO_2 in air for 2 days. Various concentrations of **compound 8** (320, 160, 80, 40, 20, 10 μM in DMEM containing 5% FBS and 5% DMSO, 10 μL, final 1/10 dilution) were added, and the microplate was further incubated for 30 hours, then treated with CCK-8 reagent (10 μL/well, Dojindo) according to the manufacturer's protocol. The absorbance at 450 nm was measured.

HPLC analysis – stability of compound 8 in cell culture medium

A solution of **compound 8** (10 mM in DMSO, 2 μL, final 100 μM) was diluted with DMEM containing 5% fetal bovine serum (FBS) (198 μL) and incubated at 37 °C. At 0, 1, 4, 8 and 24 hour, an aliquot (20 μL) was taken and loaded onto an Inertsil ODS-3 column (5 mm; 150 × 4.6 mm) fitted on a Shimadzu HPLC system. The eluate was monitored with a photodiode array detector. (A) Milli-Q water containing 0.1% TFA and (B) MeCN containing 0.1% TFA were used as developing solvents. Gradient conditions were as follows: 0 min, A 70% and B 30% → 20 min, A 20% and B 80% → 30 min, A 20% and B 80% →40 min, A 70% and B 30%.

Statistical analysis

Data were expressed as mean ± S.D. (shown as error bars). Statistical significance of differences was examined by one-way ANOVA with Bonferroni correction: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.



Scheme S1. Synthesis of **compounds 11** and **12.**

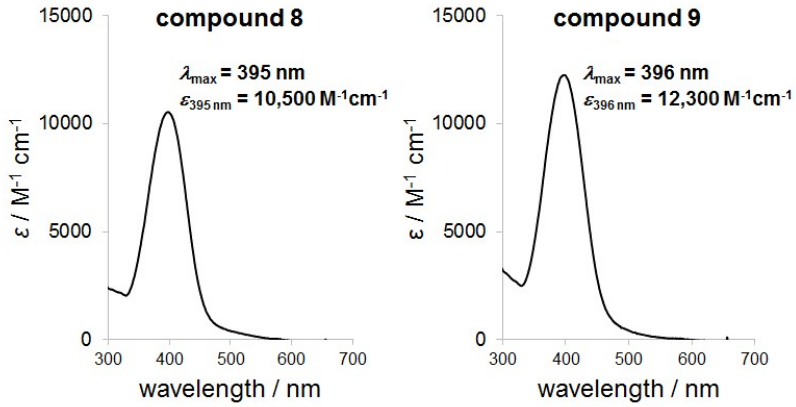


Figure S1. Absorption spectra of **compounds 8** and **9** (100 µM). Spectra were measured in MilliQ water (containing 1 % DMSO).

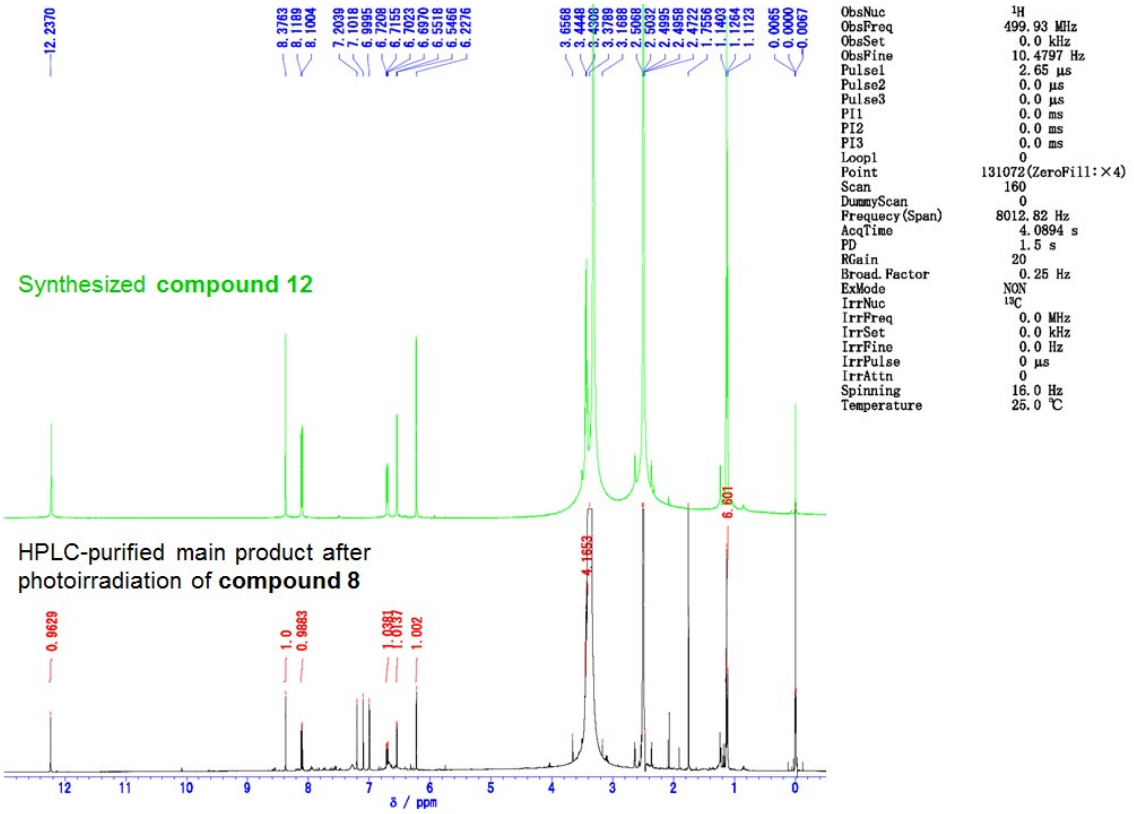


Figure S2. NMR spectra of separately synthesized **compound 12** and the purified product obtained by preparative reverse-phase HPLC after photoirradiation of **compound 8**.

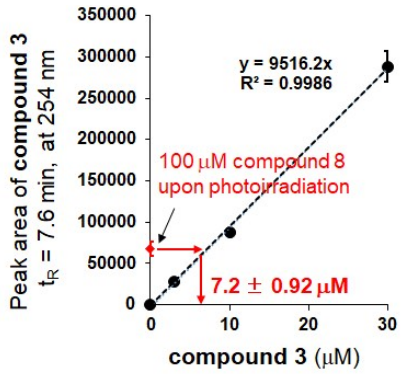


Figure S3. Estimation of a fraction of *path I* upon photoirradiation of **compound 8**. The standard line was determined based on peak areas of various concentrations of **compound 3**. From this calculation, photoirradiation (20 mW/cm^2, 10 min) of 100 μM **compound 8** underwent 7.2 ± 0.92% *path I* reaction. The results are mean ± S.D. (n = 3).

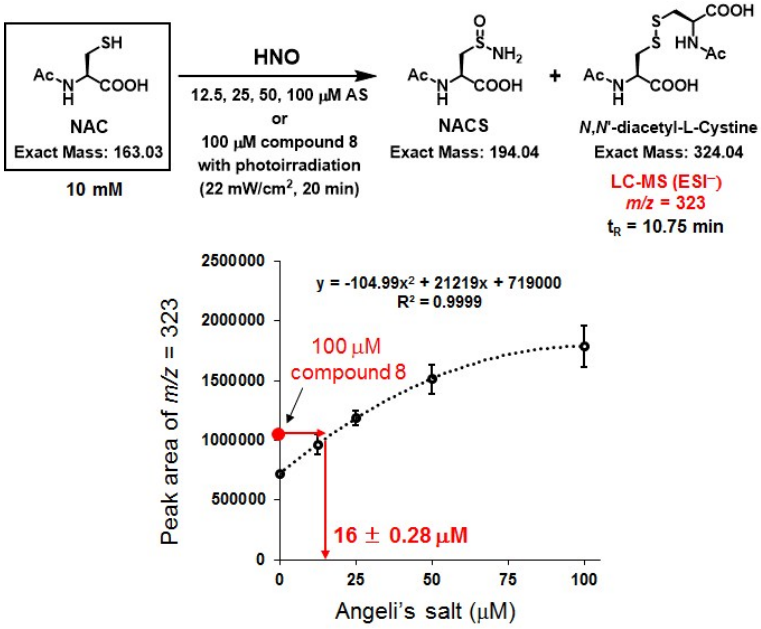


Figure S4. LC-MS analysis for quantification of HNO released from **compound 8** upon photoirradiation. The standard curve was determined based on formation of *N*,*N*'-diacetyl-L-cystine (*m/z* = 323) from *N*-acetylcysteine (NAC) through the reaction between 10 mM NAC and HNO generated via spontaneous degradation of Angeli's salt (**AS**: final 0, 12.5, 25, 50, 100 μM) at R.T. From this calculation, photoirradiation (22 mW/cm^2, 20 min) of 100 μM **compound 8** released HNO corresponding to HNO spontaneously formed from 16 ± 0.28 μM AS. The results are mean ± S.D. (n = 3).

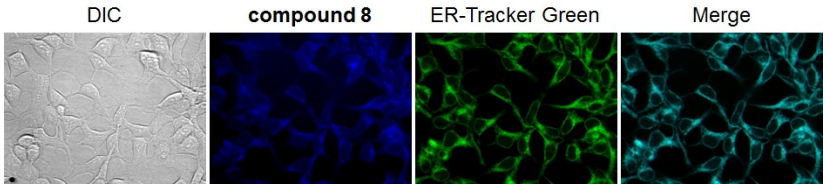


Figure S5. Co-localization fluorescence images; HEK293T cells were treated with **compound 8** (10 μM) and ER-Tracker Green® (1 μM, total 0.2% DMSO) in a humidified 5% (v/v) incubator for 30 minutes, and then subjected to confocal fluorescence microscopy.

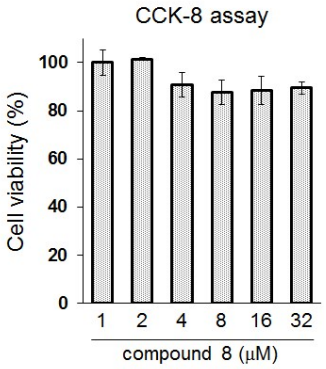


Figure S6. Cytotoxicity of **compound 8** to HEK293T cells. HEK293T cells were treated with the indicated concentrations of **compound 8** for 30 hours, and then cell viability was determined with Cell Counting Kit-8 (CCK-8) reagent. The results are mean ± S.D. (n = 3).

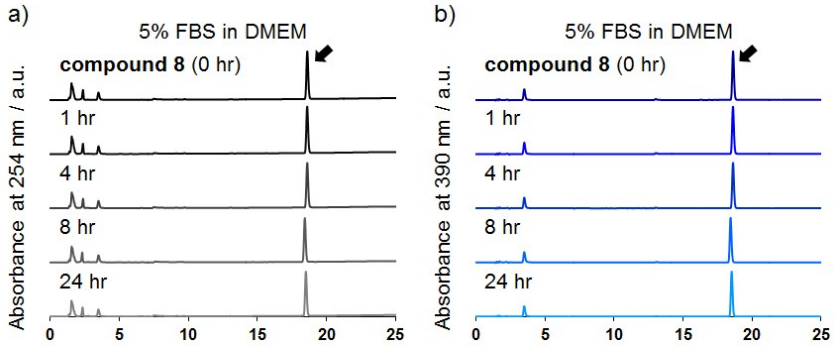


Figure S7 Stability of **compound 8** in culture medium. **Compound 8** was incubated in DMEM containing 5% FBS for 1, 4, 8, or 24 hours at 37 °C, and the mixture was analyzed by HPLC. Absorption was monitored at (a) 254 nm and (b) 390 nm.