Supporting Information for

Uncaging a Catalytic Hydrogen Peroxide Generator through the Photo-Induced Release of Nitric Oxide from a $\{MnNO\}^6$ Complex

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Contents

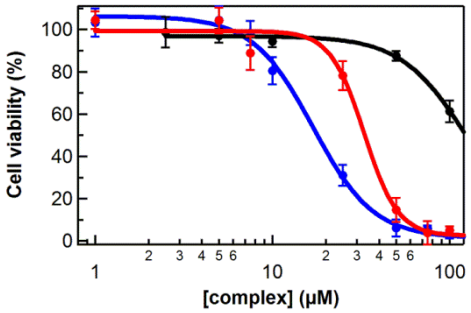


Fig. S1 Cell viability of HeLa cells treated with different concentrations of UG1NO in the dark (black), and after white-light exposure for 10 sec (blue) and after light irradiation at 650 nm for 10 sec. The cells were incubated at 37 °C for 24 h after the light exposure.

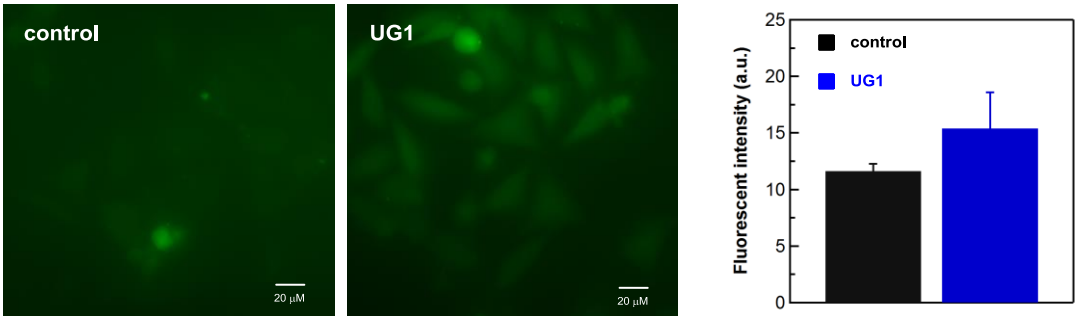


Fig. S2 Fluorescence images of HeLa cells with 2',7'-dichlorodihydrofluorescin diacetate (DCFH-DA) and UG1NO before and after light exposure (left and middle, Scale bars: 20 μm), and relative fluorescence intensity values (right).

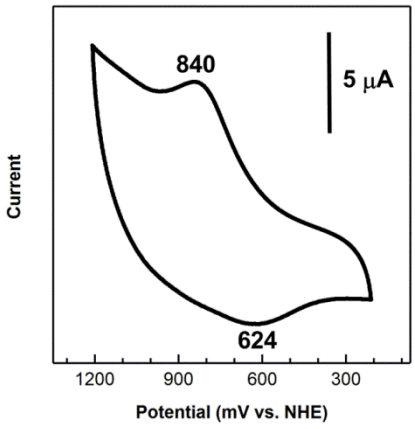


Fig. S3 Cyclic voltammogram obtained at glassy carbon electrode in 5% DMSO-H_2O containing 0.5 mM of UG1 and 50 mM NaCl. Experimental conditions: scan rate of 20 mV s^{-1}. Counter electrode:

Pt, Reference electrode: Ag/AgCl (3M NaCl).

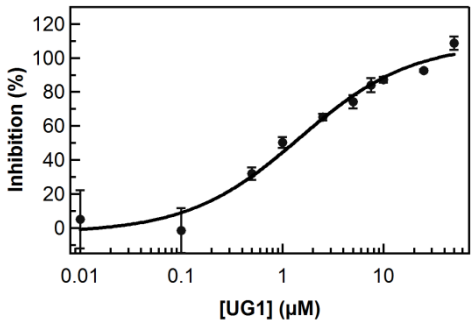


Fig. S4 Inhibition of the reaction of WST-1 with $O_2^{\bullet-}$ in the presence of varied concentration of UG1. IC_{50} = 1.5 ± 0.5 μM, log k_{cat} = 6.2 ± 0.2 M^{-1} s^{-1}.

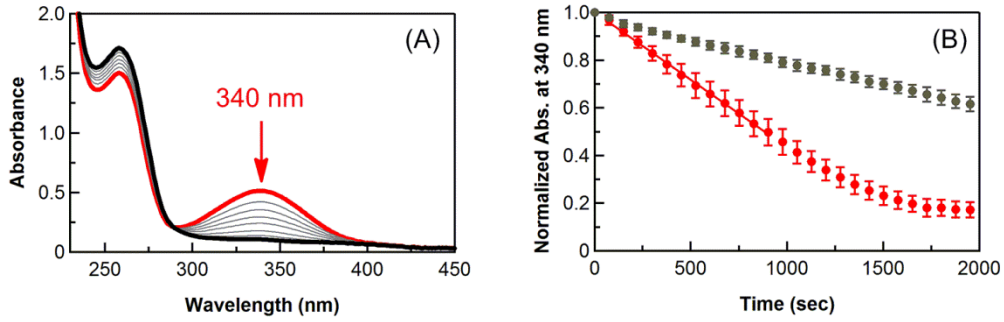


Fig. S5 Oxidation of GSH to GSSH catalyzed by UG1 under aerobic conditions, monitored by the glutathione reductase/NADPH coupled method. (A) Spectral change of the reaction mixture containing 10 μM UG1, 100 μM NADPH, 5 mM GSH, and 1 unit/mL of glutathione reductase in 20 mM sodium phosphate (pH 7.5, 5% DMSO) at 37 °C under air. The arrow indicates the consumption of NADPH. (B) Time courses of the absorption change monitored at 340 nm in the absence (black) and presence (red) of UG1. The turnover number of UG1 was four at 2000 sec.

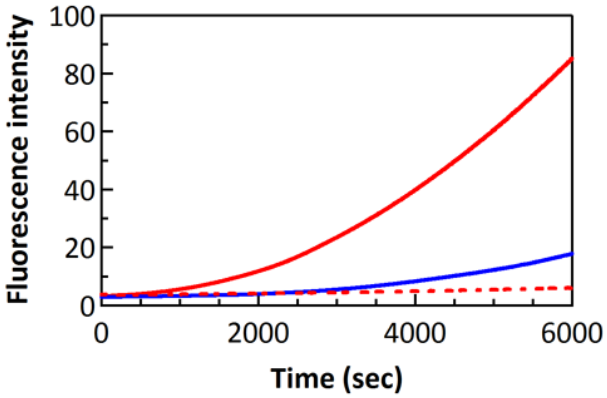


Fig. S6 H_2O_2 generation in air-saturated solutions containing 20 mM sodium phosphate buffer (pH 7.5) monitored by PF1 (10 μM) at 37 °C. Red line: 25 μM UG1 and 1 mM GSH. Blue line: 25 μM UG1 without reductants. Red dotted line: 1 mM GSH without UG1.

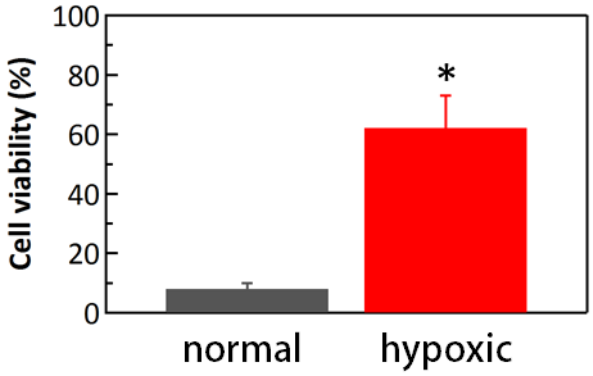


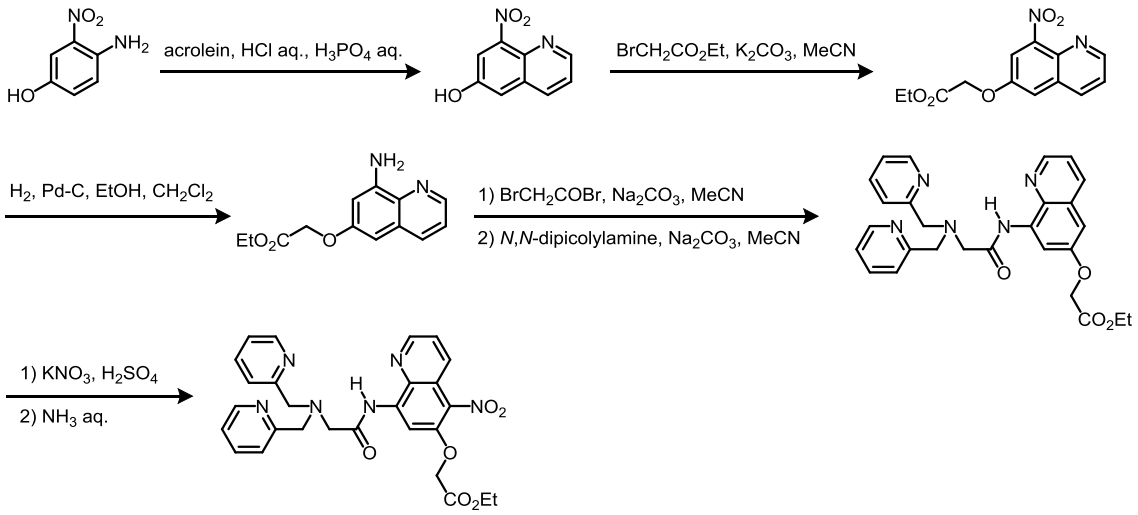
Fig. S7 Cell viability of HeLa cells incubated with 25 μM of UG1 under the normal conditions (grey) and under the hypoxic conditions (red, 2% O_2). *P < 0.005 compared with the control.

EXPERIMENTAL SECTION

Materials. The reagents and the solvents used in this study, except the ligand and the iron complex, were commercial products of the highest available purity and were further purified by the standard methods, if necessary. NOC-7 and SIN-1 were purchased from Dojindo Laboratories. (Kumamoto, Japan). DAF-2DA, DCFH-DA, and cisplatin were obtained from Sekisui Medical, Co. Ltd., SIGMA-Aldrich, and WAKO, respectively. PF1 were synthesized according to the literature.[S1] HeLa cells were purchased from the RIKEN cell bank.

Methods. FT-IR spectra were recorded on a Shimadzu IRAffinity-1 spectrometer with a Pike MIRacle10 ATR system (ZnSe). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a JMN-A 500 spectrometer. Electrospray ionization mass spectroscopy (ESI-MS) was performed on a JEOL

JMS-T100CS spectrometer. UV–visible spectra were taken on an Agilent 8543 UV–visible spectrometer. Fluorescence spectra were taken on a Hitachi F-7000 spectrophotometer. Elemental analyses were recorded on a Perkin-Elmer Elemental Analyzer (2400 Series II). Microplate assays were carried out with an OPTImax (Molecular Devices). Fluorescence images of cells were taken by an Olympus IX81 fluorescence microscope. The light was irradiated from the top of 96-well plates cell using a 300 W xenon lamp (Asahi Spectra Co. Ltd.) directly or through a band-pass filter transmitting λ = 650 nm (FWHW: 6 nm). The intensity was measured at the sample plane by a photoradiometer (Delta OHM, S.r.L., HD 2303.0) equipped with an irradiance measurement probe (Delta OHM, S.r.L., LP471RAD). The light powers were 0.1 and mW/cm^2 and 5×10^{-3} mW/cm^2 for white light (385 to 740 nm) and 650-nm light, respectively. The quantum yield of NO release from UG1NO was determined according to the previously reported procedure.[S2] Hypoxic cell culturing was performed using a hypoxic culture kit (BIONIX-1, Sugiyamagen Corp., Tokyo, Japan), which is composed of an oxygen absorber (AneroPack-Anero, Mitsubishi Gas Chemical Company, Inc., Tokyo, Japan), an oxygen monitor (OXY-1, JIKCO, Tokyo, Japan), pouches and plastic clips for sealing the pouch.



Scheme S1. Synthetic Scheme of H-L.

Synthesis of the ligand

6-hydroxy-8-nitroquinoline

A solution of 4-amino-3-nitrophenol (5.0 g, 33 mmol) in conc. HCl aq. (40 mL) and conc. H_3PO_4 (15 g) was heated at 80 ˚C and slowly added acrolein (6.5 mL, 97 mmol) for 1 h. The reaction mixture was stirred at 95 °C. After 3 hour, the reaction mixture was cooled at 0 ˚C and neutralized with NH_3 solution. The resulting powder was filtered out and dissolved in acetone. Evaporation of the solvent

gave a red powder. Yield 4.2 g (69%). ^{1}H NMR (500 MHz, DMSO-d_6): δ7.48 (s, Qu5, 1H), 7.58 (dd, *J* = 8.3, 4.0 Hz, Qu3, 1H), 7.82 (s, Qu7, 1H), 8.34 (d, *J* = 8.3 Hz, Qu4, 1H), 8.78 (d, *J* = 4.0 Hz, Qu2, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): 112.9 (Qu5), 116.0 (Qu7), 123.7 (Qu3), 130.3 (Qu9), 134.0 (Qu10), 135.3 (Qu4), 149.1 (Qu6), 149.8 (Qu2), 154.9 (Qu8). FT-IR (ATR): 783 cm^{-1} (C-N).

Ethyl[(8-nitroquinolin-6-yloxy)acetate]

To a dry acetone (60 mL) solution of 6-hydroxy-8-nitroquinoline (4.0 g, 21 mmol) and K_2CO_3 (5.8 g, 42 mmol) was slowly added ethyl bromoacetate (5.3 g, 32 mmol) under nitrogen and resulting mixture was stirred over night at room temperature. After removal of K_2CO_3 by Celite filtration, the filtrate solvent was evaporated in vacuo and purified by a column chromatography (alumina, hexane/EtOAc (1 : 1)). Yield 1.8 g (30%). ^{1}H NMR (500 MHz, $CDCl_3$): δ1.32 (t, *J* = 7.2 Hz, -CH_2CH_3, 3H), 4.31 (q, *J* = 7.2 Hz, -CH_2CH_3, 2H), 4.80 (s, -OCH_2, 2H), 7.24 (d, *J* = 2.3 Hz, Qu5, 1H), 7.50 (dd, *J* = 8.3, 4.3 Hz, Qu3, 1H), 7.79 (d, *J* = 2.3 Hz, Qu7, 1H), 8.12 (dd, *J* = 8.3, 1.4 Hz, Qu4, 1H), 8.92 (dd, *J* = 4.3, 1.4 Hz, Qu2, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): 14.4 (-CH_2CH_3), 62.1 (-CH_2CH_3), 66.1 (-CH_2-), 111.0 (Qu5), 116.9 (Qu7), 123.4 (Qu3), 130.0 (Qu9), 135.2 (Qu4), 136.1 (Qu10), 149.2 (Qu8), 150.8 (Qu2), 154.6 (Qu6), 167.9 (*C*=O). FT-IR (ATR): 1746 cm^{-1} (C=O), 1165 cm^{-1} (C-O).

Ethyl[(8-aminoquinolin-6-yloxy)acetate]

Ethyl[(8-nitroquinoline-6-yloxy)acetate] (0.96 g, 3.5 mmol) was dissolved in CH_2Cl_2 (40 mL) and EtOH (80 mL), 10% Pd/C (0.096 g) was added, and then the mixture was stirred under hydrogen for 6 h. After removal of the catalyst by filtration, the solvent was evaporated under reduced pressure. The residue was dried in vacuo. Yield 0.84 g (98%). ^{1}H NMR (500 MHz, $CDCl_3$): δ1.31 (t, *J* = 7.2 Hz, -CH_2CH_3, 3H), 4.29 (q, *J* = 7.2 Hz, -CH_2CH_3, 2H), 4.70 (s, -OCH_2, 2H), 5.03 (NH_2, 2H), 6.39 (d, *J* = 2.3 Hz, Qu5, 1H), 6.69 (d, *J* = 2.3 Hz, Qu5, 1H), 7.33 (dd, *J* = 8.3, 4.3 Hz, Qu3, 1H), 7.93 (d, *J* = 8.3 Hz, Qu4, 1H), 8.62 (dd, *J* = 4.3 Hz, Qu2, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): 14.4 (-CH_2CH_3), 61.6 (-CH_2CH_3), 65.5 (-CH_2-), 95.5 (Qu5), 101.7 (Qu7), 122.1 (Qu3), 129.7 (Qu9), 135.1 (Qu4), 135.8 (Qu10), 145.6 (Qu8), 145.7 (Qu2), 157.2 (Qu6), 169.1 (*C*=O). FT-IR (ATR): 3460 cm^{-1} (-NH_2), 1748 cm^{-1} (C=O), 1171 cm^{-1} (C-O).

Ethyl 2-[(8-(2-bis(pyridine-2-ylmethyl)amino)acetamido)quinolin-6-yloxy)acetate]

Bromoacetyl bromide (0.34 mL 3.9 mmol) was slowly added by means of a syringe to a stirred solution of ethyl[8-aminoquino-6-yloxy)acetate] (0.8 g, 3.6 mmol) and Na_2CO_3 (0.41 g, 3.9 mmol) in anhydrous MeCN (50 mL) under a nitrogen atmosphere at 0 °C, and stirring was continued for 20 min at 0°C. The mixture was filtered through a Celite pad and the solid was washed twice with CH_2Cl_2 (2 mL). The combined filtrates were concentrated to give an orange powder. The powder

was dissolved in anhydrous MeCN (50 mL), and Na_2CO_3 (0.41 g, 3.9 mmol) and *N*,*N*-dipicolylamine (0.78 mg, 3.9 mmol) were added to the solution under nitrogen at 0 °C. The mixture was stirred overnight at 0 °C. The mixture was filtered through a Celite pad and the solid was washed twice with CH_2Cl_2 (2 mL). The combined filtrates were evaporated in vacuo and a red powder was obtained, which was purified by a column chromatography (alumina, hexane/AcOEt (1/1) to ethyl acetate as eluent). Yield 0.95 g (60%). ^{1}H NMR (500 MHz, $CDCl_3$): δ1.31 (t, *J* = 7.2 Hz, -CH_2CH_3, 3H), 3.53 (s, -CH_2CO-, 2H), 4.01 (s, -CH_2Py-, 4H), 4.28 (q, *J* = 7.2 Hz, -CH_2CH_3, 2H), 4.75 (s, -OCH_2, 2H), 6.80 (d, *J* = 2.9 Hz, Qu5, 1H), 7.16 (dd, *J* = 7.5, 5.2 Hz, Py5, 2H), 7.47 (dd, *J* = 8.6, 4.3 Hz, Qu3, 1H), 7.65 (dd, *J* = 7.5, 7.5 Hz, Py4, 2H), 7.97 (d, *J* = 7.5 Hz, Py3, 2H), 8.06 (d, *J* = 8.6 Hz, Qu4, 1H), 8.52 (d, *J* = 5.2 Hz, Py6, 2H), 8.59 (d, *J* = 2.9 Hz, Qu5, 1H), 8.80 (d, *J* = 4.3 Hz, Qu2, 1H), 11.59 (s, -NHCO-, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): 14.4 (-CH_2CH_3), 59.5 (-CH_2CO-), 61.3 (-CH_2Py-), 65.8 (-CH2-), 101.7 (Qu7), 108.8 (Qu5), 122.4 (Qu3), 122.6 (Py5), 123.6 (Py3), 129.0 (Qu9), 135.4 (Qu4), 135.8 (Qu10), 135.9 (Qu8), 136.8 (Py4), 146.2 (Qu2), 149.4 (Py6), 156.8 (Qu6), 158.4 (Py2), 168.9 (*C*=O). FT-IR (ATR): 3273 cm^{-1} (amide N-H), 1751 cm^{-1} (C=O), 1680 cm^{-1} (amide C=O), 1165 cm^{-1} (C-O).

Ethyl 2-((8-(2-bis(pyridine-2-ylmethyl)amino)acetamidoquinolin-6-yl)oxy)acetate] (H-L)

KNO_3 (0.47 g, 4.7 mmol) was slowly added to a stirred solution of Ethyl 2-[(8-(2-bis(pyridine-2-ylmethyl)amino)acetamido)quinolin-6-yloxy)acetate] (0.8 g, 3.9 mmol) in conc. H_2SO_4 (10 mL), and stirring was continued for 5 h at room temperature. The reaction mixture was slowly added to cold NH_3 solution, and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, filtered and evaporated. The resulting yellow powder was dried in vacuo. Yield 0.58 g (66%). ^{1}H NMR (500 MHz, $CDCl_3$): δ1.29 (t, *J* = 7.1 Hz, -CH_2CH_3, 3H), 3.58 (s, -CH_2CO-, 2H), 4.03 (s, -CH_2Py-, 4H), 4.27 (q, *J* = 7.1 Hz, -CH_2CH_3, 2H), 4.89 (s, -OCH_2, 2H), 7.16 (dd, *J* = 7.5, 4.9 Hz, Py5, 2H), 7.64 (m, Py4 Qu3, 3H), 7.81 (d, *J* = 7.8 Hz, Py3, 2H), 8.27 (d, *J* = 8.7 Hz, Qu4, 1H), 8.52 (d, *J* = 4.9 Hz, Py6, 2H), 8.69 (s, Qu7,1H), 8.89 (d, *J* = 4.3 Hz, Qu2, 1H), 11.77 (s, -NHCO-, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): 14.1 (-CH_2CH_3), 59.2 (-CH_2CO-), 61.1 (-CH_2Py-), 61.8 (-CH_2CH_3-), 66.3 (-CH_2-), 103.5 (Qu7), 121.9 (Qu5), 122.6 (Py5), 123.4 (Py3), 124.3 (Qu3), 129.8 (Qu9), 130.5 (Qu4), 133.4 (Qu10), 136.7 (Py4), 138.7 (Qu8), 147.3 (Qu2), 149.2 (Py6), 149.9 (Qu6), 157.7 (Py2), 167.4 (*C*=O), 170.7 (*C*=O). FT-IR (ATR): 3280 cm^{-1} (amide N-H), 1687 cm^{-1} (amide C=O), 1514 cm^{-1} (nitro NO_2).

Synthesis of UG1.

Caution: Perchlorate salts of metal complexes are potentially explosive and should be handled in small quantities with care.

A solution of **H-L** (0.20 g, 0.40 mmol) in EtOH (8 mL) containing Et_3N (60 μL, 0.48 mmol) was

added to a solution of $Mn(ClO_4)_2 \cdot 6H_2O$ (0.17 g, 0.48 mmol) in EtOH (2 mL) at room temperature. The mixture was stirred for 30 min. The light yellow precipitates were collected, washed with diethyl ether, and dried in vacuo. Yield 0.23 g (85%). Anal. Calcd for $[Mn(L)](ClO_4)(H_2O)_{1.5}$: C, 45.61; H, 3.97; N, 11.82. Found: C, 45.37; H, 3.63; N, 11.56. FT-IR (ATR): 1733 cm^{-1} (C=O), 1606 cm^{-1} (amide C=O), 1542 cm^{-1} (nitro NO_2). UV-vis (CH_3CN): 403 (10000), 335 (4400), 261 (33000). ESI-MS, positive mode: *m*/*z* 584.05 $\{Mn^{II}(L)\}^+$.

Synthesis of UG1NO.

All procedures were performed in the dark. A solution of $[Mn(L)]ClO_4$ (0.35 g, 0.55 mmol) in CH_3CN : MeOH = 1 : 1 (50 mL) was degassed and bubbled with purified NO gas (50 mL × 5) with vigorous stirring. Dark red precipitates were filtered, washed with Et_2O, and dried in vacuo. Yield 0.350 g (94 %). Anal. Calcd for $[Mn(NO)(L)](ClO_4)$: C, 45.42; H, 3.53; N, 13.73. Found: C, 45.31; H, 3.44; N, 13.6. ^{1}H NMR (500 MHz, CD_3CN): δ1.18 (t, *J* = 7.3 Hz, -CH_2CH_3, 3H), 4.04 (s, -CH_2CO-, 2H), 4.36, 4.57 (d, *J* = 15.5, -CH_2Py-, 2H), 4.19 (q, *J* = 7.2 Hz, -CH_2CH_3, 2H), 4.91 (s, -OCH_2, 2H), 6.43 (d, *J* = 5.4 Hz, Py3, 2H), 7.08 (t, *J* = 6.8 Hz, Py5, 2H), 7.49 (d, *J* = 7.9 Hz, Py6, 2H), 7.73 (dd, *J* = 4.8, 8.7 Hz, Qu3, 1H), 7.86 (t, *J* = 7.9 Hz, Py4, 2H), 8.32 (d, *J* = 8.7 Hz, Qu4, 1H), 8.84 (s, Qu7, 1H), 9.21 (d, *J* = 4.8 Hz, Qu2, 1H). FT-IR (ATR): 1625 cm^{-1} (amido C=O), 1746 cm^{-1} (N-O). Electronic absorption spectrum in H_2O (nm (M^{-1} cm^{-1})): 494 (2700), 385 (8300). ESI-MS, positive mode: *m*/*z* 614.31 $\{Mn^{II}(L)(NO)\}^+$.

Cell culture Conditions.

HeLa cells was purchased from Riken Cell Bank (RCB0007) was maintained in Dulbecco's modified Eagle's medium (DMEM, Wako 048-30275) supplemented with 10% (v/v) fetal bovine serum (FBS, Biowest, S05831S1820), penicillin (100 units/mL), and streptomycin (100 μg/mL) in a humidified incubator under 5% CO_2 in 95% air.

Cytotoxicity in the dark

HeLa cells were seeded at a density of 1 × 10^5 cells/mL in each wells of 96-well plate (Iwaki, 3860-096) and allowed to grow to ~80% confluence for 24 h. A stock solution of UG1NO or UG1 was prepared in 5%DMSO-H_2O, and added to obtain from 1 to 100 μM. The final volume was 100 μL in each well. After incubation at 37 °C for 24 h, cytotoxicity was analyzed by the MTT assay. For this purpose, 20 μL of the MTT reagent solution (2.5 mg/mL Dojindo) was added to each well. The plate was incubated at 37 °C for 3 h, and the absorbance intensities at 570 nm were measured using a plate reader.

Cytotoxicity upon light irradiation

HeLa cells were seeded at a density of 1×10^5 cells/mL in each wells of 96-well plate (Iwaki, 3860-096) and allowed to grow to ~80% confluence for 24 h. A stock solution of UG1NO or UG1 was prepared in 5%DMSO-H_2O, and added to obtain from 1 to 100 μM. The final volume was 100 μL in each well. After incubation at 37 °C for 3 h, 650-nm light (FWHW: 6 nm) or white light (385 to 740 nm) was exposed to the 96-well plate for 1 h or 10 sec, respectively, by using 300 W Xe lamp as a light source (MAX-303, Asahi Spectra Co. Ltd). After the cells were incubated at 37 °C for 24 h, cytotoxicity was analyzed by the MTT assay.

Intracellular NO detection

HeLa cells were seeded at a density of 1×10^5 cells/mL in glass-bottomed dishes (IWAKI, 3910-035), and incubated at 37 °C for 24 h. After the cells were washed thrice with DMEM, a stock solution of DAF2-DA in DMSO was added to medium (final 10 μM concentration). After incubation for 30 min, the cells were washed thrice with DMEM, and a stock solution of UG1NO in 5%DMSO in H_2O was added (final 100 μM concentration). After incubation for 30 min, 650-nm light (FWHW: 6 nm) was exposed to the cells for 30 min. Fluorescence images were taken with an Olympus IX81 fluorescence microscope (λ_{ex} = 495 nm, λ_{em} = 515 nm).

Intracellular H_2O_2 detection

HeLa cells were seeded at a density of 1×10^5 cells/mL in glass-bottomed dishes (IWAKI, 3910-035), and incubated at 37 °C for 24 h. After the cells were washed thrice with DMEM, a stock solution of PF1 in DMSO was added to medium (final 10 μM concentration). After incubation for 30 min, the cells were washed thrice with DMEM, and a stock solution of UG1 in 5%DMSO in H_2O was added (final 10 μM concentration). After incubation for 24 h, fluorescence images were taken.

Intracellular ROS detection

HeLa cells were seeded at a density of 1×10^5 cells/mL in glass-bottomed dishes (IWAKI, 3910-035), and incubated at 37 °C for 24 h. After the cells were washed thrice with DMEM, a stock solution of DCFH-DA in DMSO was added to medium (final 100 μM concentration). After incubation for 1 h, the cells were washed thrice with DMEM, and a stock solution of UG1 in 5%DMSO in H_2O was added (final 100 μM concentration). After incubation for 3 h, fluorescence images were taken.

Catalytic dioxygen consumption

Oxygen consumption was determined using a 5300A Clark electrode oxygen monitor (YSI Inc., Yellow Springs, OH, USA) connected a sealed vial containing 16 mL of sodium phosphate buffer (pH 7.5) containing 1 mM ascorbate at 30 °C. The reaction was started by adding UG1 in 5%DMSO

in H_2O (final 100 μM concentration) to the vessel.

Glutathione oxidation

The glutathione oxidation catalyzed by UG1 was determined spectrophotometrically at 340 nm, using the glutathione reductase/NADPH-coupled assay, according to the litterature.[S3] The reaction was started by adding UG1 in 5%DMSO-H_2O (final 10 μM concentration) to a solution containing 20 mM sodium phosphate (pH 7.5), 5 mM GSH, 0.1 mM NADPH, 1 unit/mL of glutathione reductase at 37 °C under air.

References

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