Supporting Information for

Mononuclear Nonheme Iron(III) Complexes that Show Superoxide Dismutase-like Activity and Antioxidant Effects against Menadione-Mediated Oxidative Stress

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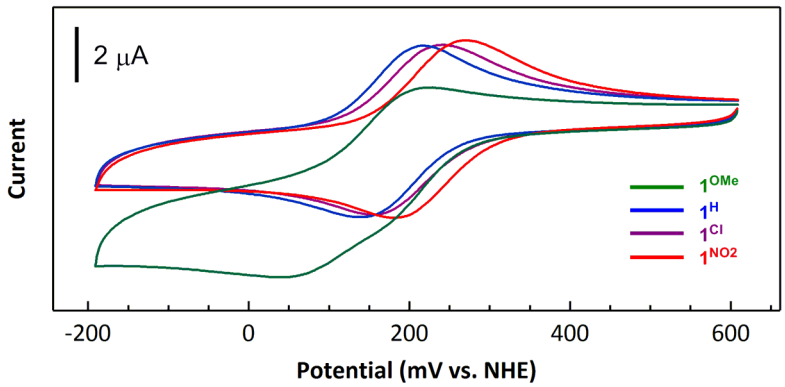

Fig. S1 Cyclic voltamogram of $\mathbf{1}^{\mathbf{R}}$ (R = OMe, H, Cl and NO_2) in deaerated PIPES buffer (10 mM, pH 7.5) containing 1M NaCl at 25 °C; working electrode: GC, counter electrode: Pt, reference electrode: Ag/AgCl (3M NaCl aq.), scan rate: 20 mV s^{-1}.

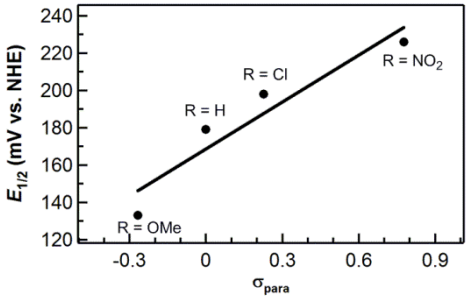

Fig. S2 Hammett plot of the redox potentials of $\mathbf{1}^{\mathbf{R}}$ (R = OMe, H, Cl and NO_2).

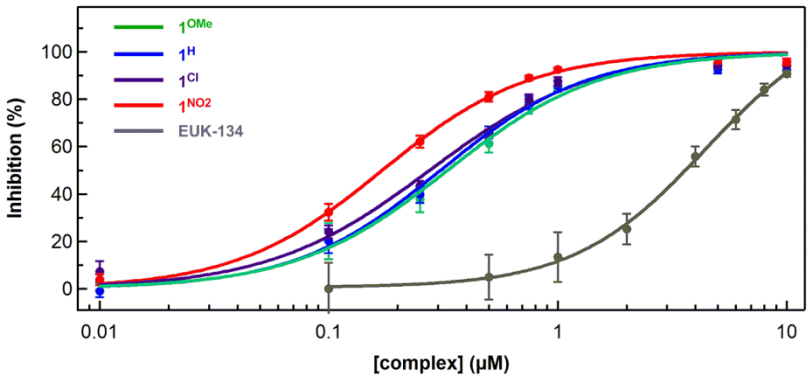

Fig. S3 Inhibition of the reaction of WST-1 with $O_2^{\bullet-}$ in the presence of varied concentration of $\mathbf{1}^{\mathbf{R}}$ (R = OMe, H, Cl and NO_2) and EUK-134.

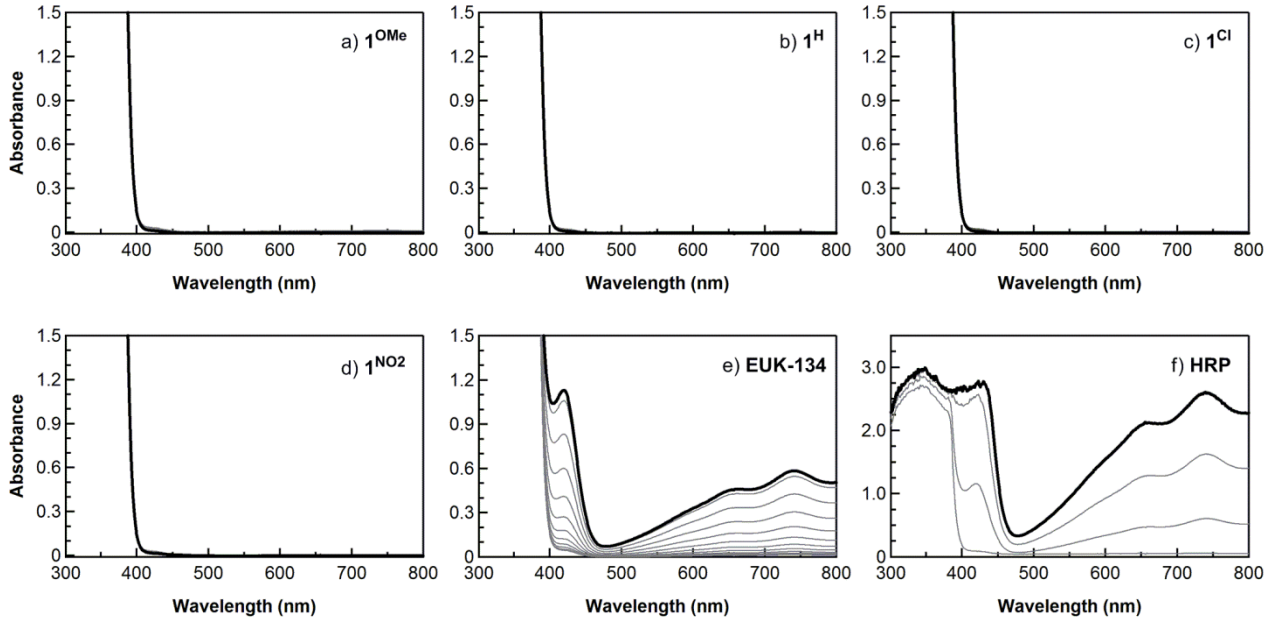

Fig. S4 Electronic spectral changes for the reaction of ABTS (1 mM) with H_2O_2 (100 μM) in the presence of $\mathbf{1}^{\mathbf{R}}$ (1 μM) (R = OMe (a), H (b), Cl (c) and NO_2 (d)), (e) EUK-134 (1 μM) and (f) HRP (0.5 units/mL) in PIPES buffer (10 mM, pH 7.5) at 37 °C.

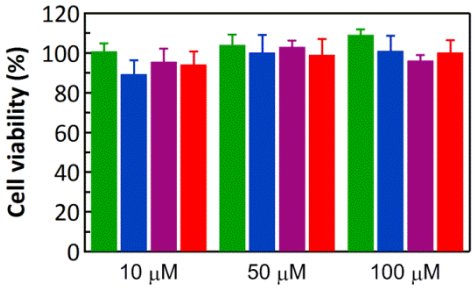

Fig. S5 Cytotoxicity of $\mathbf{1}^{\mathbf{R}}$ (R = OMe (green), H (blue), Cl (purple) and NO_2 (red)) for HeLa cell.

General. All chemicals used in this study were commercial products of the highest available purity and were further purified by the standard methods. Menadione and hydroethidium were purchased from Abcam Inc. and Polyscience, Inc., respectively. The ligand H-dpaq^{R} was prepared according to the reported procedure.[S1,S2] EUK-134 was prepared according to the literature.[S3] Infrared (IR) spectra were recorded on a Shimadzu IRAffinity-1S Fourier transform infrared spectrophotometer equipped with MIRacle-10 ZnSe single reflection ATR plate. ESI-MS (electrospray ionization mass spectra) measurements were performed on a JEOL JMS-T100CS spectrometer. Elemental analyses were recorded with a Perkin-Elmer Elemental Analyzer 2400 II. Microplate assays were carried out with OPTImax and FilterMax F5 (Molecular Devices).

Represented synthesis of **1^{R}**:
A solution of H-dpaq^{R} (0.78 mmol) in CH_3OH (5 mL) containing Et_3N (109 μL, 0.78 mmol) was added into a solution of $FeCl_3{\cdot}6H_2O$ (127 mg, 0.78 mmol) in CH_3OH (2 mL) at the room temperature, and the mixture was stirred for 12 h. The precipitate was collected, washed with diethyl ether, and dried in vacuo.

[Fe(dpaq^{H})Cl]Cl (**1^{H}**): The pure product was obtained by recrystallization from CH_2Cl_2/hexane. Green solid. yield: 61%. Anal. Calcd for [Fe(dpaq^{H})Cl]Cl$(H_2O)_{2.5}(CH_2Cl_2)_{0.25}$: C, 48.53; H, 4.47; N, 12.17. Found: C, 48.34; H, 4.93; N, 11.77. Selected IR frequencies (cm^{-1}, FT-ATR): 1633 (C=O). Electronic absorption spectrum in H_2O (pH 7.5) (nm (M^{-1} cm^{-1})): 349 (5300), 570 (580). ESI-MS, positive mode: *m*/*z* 469.2 {[Fe(dpaq^{H})(OMe)]}$^{+}$, 473.2 {[Fe(dpaq^{H})Cl]}$^{+}$.

[Fe(dpaq^{OMe})Cl]Cl (**1^{OMe}**): The pure product was obtained by recrystallization from CH_2Cl_2/hexane. Brown solid. yield: 78%. Anal. Calcd for [Fe(dpaq^{OMe})Cl]Cl$(H_2O)_3(CH_2Cl_2)_{1.5}$: C, 42.50 ;H, 4.34; N, 9.72. Found: C, 42.74; H, 4.61; N, 9.40. Selected IR frequencies (cm^{-1}, FT-ATR): 1603 (C=O). Electronic absorption spectrum in H_2O (pH 7.5) (nm (M^{-1} cm^{-1})): 375 (4000), 656 (490). ESI-MS, positive mode: *m*/*z* 499.2 {[Fe(dpaq^{OMe})(OMe)]}$^{+}$, 503.2 {[Fe(dpaq^{OMe})Cl]}$^{+}$.

[Fe(dpaq^{Cl})Cl]Cl (**1^{Cl}**): The pure product was obtained by recrystallization from ethanol/diethyl ether. Dark green solid. yield: 78%. Anal. Calcd for [Fe(dpaq^{Cl})Cl]Cl$(H_2O)(EtOH)_2$: C, 49.60; H, 5.09; N, 10.71. Found: C, 49.19; H, 5.53; N, 11.09. Selected IR frequencies (cm^{-1}, FT-ATR): 1644 (C=O). Electronic absorption spectrum in H_2O (pH 7.5) (nm (M^{-1} cm^{-1})): 367 (5600), 590 (580). ESI-MS, positive mode: *m*/*z* 503.2 {[Fe(dpaq^{Cl})(OMe)]}$^{+}$, 507.2 {[Fe(dpaq^{Cl})Cl]}$^{+}$.

[Fe(dpaq^{NO2})Cl]Cl (**1^{NO2}**): The pure product was obtained by recrystallization from ethanol/diethyl ether. Green solid. yield: 84%. Anal. Calcd for [Fe(dpaq^{NO2})Cl]Cl(H_2O): C, 48.28; H, 3.70; N, 14.69. Found: C, 48.42; H, 3.89; N, 14.34. Selected IR frequencies (cm^{-1}, FT-ATR): 1650 (C=O). Electronic absorption spectrum in H_2O (pH 7.5) (nm (M^{-1} cm^{-1})): 385 (10000), 580 (520). ESI-MS, positive mode: *m*/*z* 514.3 {[Fe(dpaq^{NO2})(OMe)]}$^{+}$, 518.2 {[Fe(dpaq^{NO2})Cl]}$^{+}$.

Electrochemical Measurements: Cyclic voltammetric measurements were performed on a BAS CV-50W electrochemical analyzer in deaerated 10 mM PIPES buffer (pH 7.5) containing 1 M NaCl as a supporting electrolyte and 0.5 mM [Fe(dpaq^{R})Cl]Cl. The glassy carbon working electrode (BAS) was polished with BAS polishing alumina suspension and rinsed with Milli-Q water before use. The counter electrode was a platinum wire and the reference electrode was an aqueous Ag/AgCl electrode containing 3M NaCl.

SOD-like activity: SOD-like activity of **1^{R}** and EUK-134 was assayed by following the inhibition of the reduction of a water soluble tetrazolium salt (WST-1) in the presence of xanthine/xanthine oxidase, a superoxide-generating system as described by McCord and Fridovich,[S4] with some modifications. Reactions were carried out in 50 mM PIPES buffers (pH 7.5) at 37 °C in a 96-well microplate whose each well contains **1^{R}** or EUK-134, together with WST-1, xanthine, and catalase. After incubation 37 °C for 10 min, the reactions were initiated by adding xanthine oxidase. The reduced form of WST-1 was monitored at 450 nm. The final volume is 300 μL, and the final concentrations are as follow; 50 μM WST-1, 50 μM xanthine, 7.4 units/mL catalase, 56 units/mL xanthine oxidase, and 0 to 10 μM Fe complex. Under the conditions, these complexes did not inhibit the reaction of xanthine oxidase with xanthine, which was separately examined by monitoring the formation of uric acid at 296 nm. The SOD-like activity of each compound are given as IC_{50} values, which is the concentration at which of Fe complex the reduction of WST-1 was inhibited at 50%. The formation rate constant of formazan from WST-1 was $k_{WST\text{-}1}$ = 4.6×10^{4} M^{-1} s^{-1} in the absence of the iron complex. The rate constants of the reaction between **1^{R}** and $O_2^{\bullet-}$, k_2 (M^{-1} s^{-1}) were recalculated by using the following equation: $k_2 = k_{WST\text{-}1}\times[\text{WST-1}]/IC_{50}$.[S5]

Peroxidase-like activity: Peroxidase-like activity of **1^{R}** and EUK-134 was evaluated by monitoring the formation of the radical of ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) oxidation at 420 nm (ε = 36 $mM^{-1}cm^{-1}$).[S6] Reaction mixtures consisted of 5 mM ABTS, 100 μM H_2O_2, and 1 μM **1^{R}** or EUK-134 in PIPES buffer (10 mM, pH 7.5) at 37 °C.

Catalase-like activity: Reactions were carried out in a 96-well microplate whose each well contains **1^{R}** or EUK-134 (10 μM) with H_2O_2 (100 μM) in PIPES buffer (pH 7.5, 10 mM) at 37 °C for 30 or 60 min. After H_2O_2 disproportionation reaction, HRP (0.5 units/mL) and ABTS (1 mM) solutions were added into the reaction mixture to measure residual H_2O_2 concentration at 420 nm.

Cell culture: HeLa cells were 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.

MTT assay: HeLa cells were seeded at a density of 1×10^{5} cells/mL in each wells of a 96-well plate (Iwaki, 3860-096) and allowed to grow to ~80% confluence for 24 h. Stock solutions of **1^{R}** or EUK-134 in DMEM with serum were added to obtain from 10 to 100 μM. The final volume was 100 μL in each well. After incubation at 37 °C for 3 h, the cells were washed three times in DMEM with serum, and then, 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.

Quantification of $O_2^{\bullet-}$ inside cells by hydroethidium: HeLa cells were seeded at a density of 1×10^5 cells/mL in each wells of a sold-black 96-well plate (Iwaki, 3860-096) and allowed to grow to ~80% confluence for 24 h. After washing cells three times in DMEM with serum, stock solutions of $\mathbf{1^R}$ or EUK-134 in DMEM with serum were added to obtain from 10 μM. The final volume was 100 μL in each well. After incubation at 37 °C for 3 h, the cells were washed three times in DMEM with serum, and then a stock solution of hydroethidium in 5%DMSO-H_2O was added to obtain 10 μM. After incubation at 37 °C for 1 h, the cells were washed three times in DMEM with serum, a stock solution of menadione in 5%DMSO-H_2O was added to obtain 10 μM. After incubation at 37 °C for 24 h, the fluorescence intensities at 620 nm were measured using a plate reader with an excitation wavelength of 535 nm.

References

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