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Electronic Supplementary Information

Modulation of emission and singlet oxygen photosensitisation in live cells utilising bioorthogonal phosphorogenic probes and protein tag technology

Peter Kam-Keung Leung[a] and Kenneth Kam-Wing Lo*[abc]

[a] Department of Chemistry, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, P. R. China

[b] State Key Laboratory of Terahertz and Millimeter Waves, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, P. R. China

[c] Centre of Functional Photonics, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, P. R. China

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Experimental

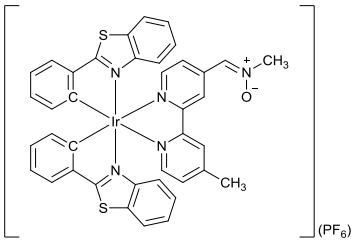
Materials and Reagents

All solvents were of analytical grade and purified according to standard procedures.[1] *N*-Methylhydroxylaminehydrochloride, 9,10-phenanthrenequinone, ethylenediamine, 1,2-diaminobenzene, potassium hexafluorophosphate (KPF_6), sodium bicarbonate ($NaHCO_3$), magnesium sulfate ($MgSO_4$), triethylamine (Et_3N), hydrogen peroxide (H_2O_2), *tert*-butyl hydroperoxide (^tBuOOH), sodium hypochlorite (NaClO), potassium superoxide (KO_2), ammonium iron(II) sulfate hexahydrate $[(NH_4)_2Fe(SO_4)_2]\cdot 6H_2O$, sodium nitrite ($NaNO_2$), sodium nitrate ($NaNO_3$), cysteine (Cys), homocysteine (Hcy), and glutathione (GSH) were purchased from Acros. 4,4'-Dimethyl-2,2'-bipyridine, selenium dioxide (SeO_2), sodium metabisulfite ($Na_2S_2O_5$), iridium(III) chloride trihydrate ($IrCl_3\cdot 3H_2O$), (1*R*,8*S*,9*s*)-bicyclo[6.1.0]non-4-yn-9-ylmethanol (**BCN-OH**), (1*R*,8*S*,9*s*)-bicyclo[6.1.0]non-4-yn-9-ylmethyl-*N*-succinimidyl carbonate (BCN-NHS), 1,3-diphenylisobenzofuran (DPBF), 9,10-Anthracenediyl-bis(methylene)dimalonic acid (ABDA) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich. 6-((4-(Aminomethyl)phenyl]methoxy)-9*H*-purin-2-amine ($BG\text{-}NH_2$) was purchased from Bide Pharmatech Ltd. All these chemicals were used without further purification. The ligands 4-((methyl(oxido)imino)methyl)-4'-methyl-2,2'-bipyridine (bpy-nitrone),[2] Hbt,[3] Hbsn,[3] Hdbq,[4] and Hdbpz[4] and all the iridium(III) dimers $[Ir_2(N\text{^}C)_4Cl_2]$[5,6] were prepared according to literature procedures. YM-10 and YM-50 microcon filters were purchased from Millipore. All buffer components were of biological grade and used as received. Autoclaved Milli-Q water was used for the preparation for the aqueous solutions. Chinese hamster ovary (CHO)-K1 cells were obtained from American Type Culture Collection. F-12 nutrient mixture, phosphate-

buffered saline (PBS), fetal bovine serum (FBS), trypsin-EDTA, and penicillin/streptomycin were purchased from Invitrogen. Lipofectamine 3000 and Opti-MEM medium were obtained from Thermo Fisher Scientific. Purified SNAP-tag protein and the $pSNAP_f$ Vector were purchased from New England Biolabs. The phage UbiC SNAP-KDEL Vector was a gift from Jeffrey Chao (Addgene plasmid #104982; http://n2t.net/addgene:104982; RRID: Addgene_104982).[7] The growth medium for cell culture contained F-12 nutrient mixture with 10% FBS and 1% penicillin/streptomycin.

Synthesis

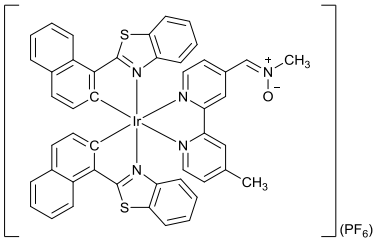
[Ir(bt)$_2$(bpy-nitrone)](PF$_6$) (***1***)



A mixture of $[Ir_2(bt)_4Cl_2]$ (100 mg, 0.08 mmol) and bpy-nitrone (35 mg, 0.16 mmol) in CH_2Cl_2/MeOH (20 mL) (1:1, *v*/*v*) was stirred under an inert atmosphere of N_2 in the dark for 18 h. The mixture was further stirred for 1 h after addition of solid KPF_6 (71 mg, 0.40 mmol). The solvent was removed under reduced pressure and the crude product was purified by column chromatography on silica gel using CH_2Cl_2/MeOH (20:1, *v*/*v*) as the eluent. The solvent was rotary evaporated to give an orange solid. Subsequent recrystallisation of the solid from CH_2Cl_2/Et_2O afforded the complex as orange crystals. Yield: 88 mg (60%). Positive-ion ESI-MS ion cluster at *m*/*z* 840 $[M - PF_6^-]^+$. ^{1}H NMR (300 MHz, CD_3COCD_3, 298 K, TMS): δ = 9.53 (s, 1H, H3 of bpy), 8.59 (s, 1H, H3’ of bpy), 8.32 (dd, *J* = 5.9 and 1.5 Hz, 1H, H6 of bpy), 8.26 (d, *J* = 5.9 Hz, 1H, H5 of bpy), 8.20 (t, *J* = 7.0 Hz, 2H, H4 of benzothiazole ring of bt), 8.11 (d, *J* = 5.3 Hz, 2H, H6’ of bpy and bpy-CH=N), 8.00 – 7.97 (m, 2H, H6 of phenyl ring of bt), 7.65 (d, *J* = 4.7 Hz, 1H, H5’ of bpy), 7.50 – 7.43 (m, 2H, H5 of benzothiozale ring of bt), 7.25 – 7.11 (m, 4H, H6 of benzothiozale ring and H5 of phenyl ring of bt), 6.93 (dt, *J* = 7.5 and 1.3 Hz, 2H, H4 of phenyl ring of bt), 6.52 (d, *J* = 8.3 Hz, 1H, H7 of benzothiozale ring of bt), 6.46 (d, *J* = 7.4 Hz, 2H, H7 of benzothiozale ring and H3 of phenyl ring of bt), 6.38 (d, *J* = 8.3 Hz, 1H, H3 of phenyl ring of bt), 4.00 (s, 3H, CH_3 of nitrone), 2.66 (s, 3H, CH_3 of bpy). IR (KBr) $\tilde{\nu}$/cm^{-1}: 1605 (C=N), 1178 (N–O),

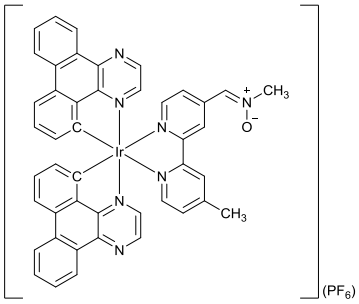
844 (PF_6^-). Anal. calcd. for $IrC_{39}H_{29}N_5OS_2PF_6 \cdot H_2O$: C 46.71, H 3.12, N 6.98, found: C 47.00, H 2.98, N 6.93%.

*[Ir(bsn)$_2$(bpy-nitrone)](PF$_6$) (**2**)*



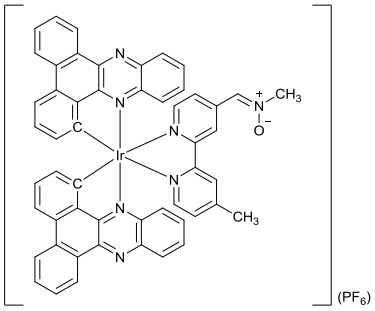
The synthetic procedure was similar to that for the preparation of complex **1**, except that $[Ir_2(bsn)_4Cl_2]$ (100 mg, 0.07 mmol) was used instead of $[Ir_2(bt)_4Cl_2]$. Subsequent recrystallisation of the solid from $CH_2Cl_2/(CH_3)_2CO/Et_2O$ afforded the complex as orange crystals. Yield: 94 mg (65%). Positive-ion ESI-MS ion cluster at *m/z* 940 $[M - PF_6^-]^+$. ^{1}H NMR (300 MHz, CD_3COCD_3, 298 K, TMS): δ = 9.54 (s, 1H, H3 of bpy), 8.73 (dd, *J* = 8.4 and 1.5 Hz, 2H, H8 of napthyl ring of bsn), 8.59 (s, 1H, H3' of bpy), 8.33 – 8.23 (m, 4H, H5 of napthyl ring of bsn and H6 and H5 of bpy), 8.13 (d, *J* = 5.6 Hz, 1H, H6' of bpy), 8.10 (s, 1H, bpy-CH=N), 7.88 (d, *J* = 8.1 Hz, 2H, H4 of benzothiozale ring of bsn), 7.82 – 7.77 (m, 2H, H7 of napthyl ring of bsn), 7.60 – 7.40 (m, 7H, H5 and H6 of benzothiozale ring and H6 of napthyl ring of bsn and H5' of bpy), 7.29 – 7.21 (m, 2H, H4 of napthyl ring of bsn), 6.72 (d, *J* = 2.0 Hz, 1H, H7 of benzothiozale ring of bsn), 6.70 – 6.65 (m, 2H, H7 of benzothiozale ring and H3 of napthyl ring of bsn), 6.53 (d, *J* = 8.5 Hz, 1H, H3 of nathyl ring of bsn), 3.99 (s, 3H, CH_3 of nitrone), 2.64 (s, 3H, CH_3 of bpy). IR (KBr) $\tilde{\nu}$/cm^{-1}: 1605 (C=N), 1179 (N–O), 845 (PF_6^-). Anal. calcd. for $IrC_{47}H_{33}N_5OS_2PF_6 \cdot (CH_3)_2CO$: C 52.53, H 3.44, N 6.13, found: C 52.59, H 3.25, N 5.90%.

*[Ir(dbq)$_2$(bpy-nitrone)](PF$_6$) (**3**)*



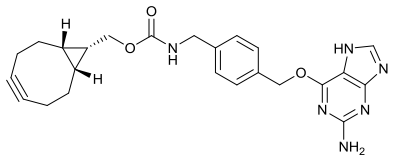
The synthetic procedure was similar to that for the preparation of complex **1**, except that [Ir$_2$(dbq)$_4$Cl$_2$] (100 mg, 0.07 mmol) was used instead of [Ir$_2$(bt)$_4$Cl$_2$]. Subsequent recrystallisation of the solid from CH_2Cl_2/Et_2O afforded the complex as red crystals. Yield: 88 mg (59%). Positive-ion ESI-MS ion cluster at *m*/*z* 878 [M – PF_6^-]$^+$. ^{1}H NMR (300 MHz, CD_3COCD_3, 298 K, TMS): δ = 9.65 (s, 1H, H3 of bpy), 9.25 (d, *J* = 8.0 Hz, 2H, dbq), 8.84 – 8.80 (m, 4H, dbq), 8.68 (s, 1H, H3’ of bpy), 8.45 (d, *J* = 3.1 Hz, 1H, dbq), 8.38 (d, *J* = 3.1 Hz, 1H, dbq), 8.33 (s, 1H, H6 of bpy), 8.31 (s, 1H, dbq), 8.19 – 8.12 (m, 3H, H5 and H6’ of bpy and bpy-CH=N), 8.03 (d, *J* = 5.6 Hz, 1H, dbq), 7.99 – 7.94 (m, 2H, dbq), 7.91 – 7.86 (m, 2H, dbq), 7.52 – 7.50 (d, *J* = 4.8 Hz, 1H, H5’ of bpy), 7.32 (t, *J* = 7.7 Hz, 2H, dbq), 6.62 (dd, *J* = 7.1 and 3.2 Hz, 2H, dbq), 4.01 (s, 3H, CH_3 of nitrone), 2.63 (s, 3H, CH_3 of bpy). IR (KBr) $\tilde{\nu}$/cm^{-1}: 1602 (C=N), 1176 (N–O), 845 (PF_6^-). Anal. calcd. for $IrC_{45}H_{31}N_7OPF_6{\cdot}CH_2Cl_2$: C 49.87, H 3.00, N 8.85, found: C 49.83, H 2.97, N 8.96%.

*[Ir(dbpz)$_2$(bpy-nitrone)](PF$_6$) (**4**)*



The synthetic procedure was similar to that for the preparation of complex **4**, except that [Ir$_2$(dbpz)$_4$Cl$_2$] (100 mg, 0.06 mmol) was used instead of [Ir$_2$(bt)$_4$Cl$_2$]. Subsequent recrystallization of the solid from CH$_2$Cl$_2$/CH$_3$OH/Et$_2$O afforded the complex as red crystals. Yield: 80 mg (80%). Positive-ion ESI-MS ion cluster at *m*/*z* 978 [M – PF$_6^-$]$^+$. ^{1}H NMR (300 MHz, CD$_3$COCD$_3$, 298 K, TMS): δ = 9.46 – 9.41 (m, 2H, dbpz), 9.37 (s, 1H, H3 of bpy), 8.79 (d, *J* = 8.2 Hz, 2H, dbpz), 8.47 – 8.42 (m, 3H, dbpz and H3’ of bpy), 8.34 (d, *J* = 8.0 Hz, 2H, dbpz), 8.14 (d, *J* = 5.9 Hz, 1H, H6 of bpy), 8.05 – 7.88 (m, 10H, dbpz and H5 and H6’ of bpy and bpy-CH=N), 7.63 (d, *J* = 8.1 Hz, 1H, dbpz), 7.54 – 7.47 (m, 2H, dbpz and H5’ bpy), 7.41 – 7.39 (m, 1H, dbpz), 7.19 (t, *J* = 7.7 Hz, 2H, dbpz), 6.72 – 6.69 (m, 2H, dbpz), 3.92 (s, 3H, CH$_3$ of nitrone), 2.52 (s, 3H, CH$_3$ of bpy). IR (KBr) $\tilde{\nu}$/cm^{-1}: 1602 (C=N), 1178 (N–O), 844 (PF$_6^-$). Anal. calcd. for IrC$_{53}$H$_{35}$N$_7$OPF$_6$·2CH$_3$OH: C 55.65, H 3.65, N 8.26, found: C 55.35, H 3.46, N 7.87%.

BCN-BG



$BG\text{-}NH_2$ (28 mg, 0.10 mmol) was dissolved in anhydrous DMSO (500 μL) with gentle heating and stirring. BCN-NHS (20 mg, 0.07 mmol) and Et_3N (48 μL, 0.34 mmol) was then added to the solution. The mixture was stirred at room temperature under an inert atmosphere of N_2 in the dark for 18 h. Distilled water (10 mL) was then added to the mixture to quench the reaction. The product was extracted with ethyl acetate (10 mL x 3) and the combined organic extract was dried over anhydrous $MgSO_4$ and filtered. The solvent was rotary evaporated under reduced pressure. The white residue was purified by column chromatography on silica gel using CH_2Cl_2/MeOH (20:1) as the eluent. The solvent was removed under reduced pressure and the product was subsequently isolated as white solid. Yield: 28 mg (92%). Positive-ion ESI-MS ion cluster at *m*/*z* 448 $[M + H]^+$. ^1H NMR (300 MHz, CD_3OD, 298 K, TMS): δ = 7.85 (s, 1H, imidazole ring of BG), 7.48 (d, *J* = 8.0 Hz, 2H, phenyl ring of BG), 7.30 (d, *J* = 8.0 Hz, 2H, phenyl ring of BG), 5.53 (s, 2H, CH_2 of BG), 4.29 (s, 2H, CH_2 of BG), 4.17 (d, *J* = 8.0 Hz, 2H, OCH_2 of BCN), 2.28 – 1.96 (m, 6H, BCN), 1.60 – 1.57 (m, 2H, BCN), 1.41 – 1.36 (m, 1H, BCN), 0.95 – 0.89 (m, 2H, BCN). ^{13}C NMR (75 MHz, methanol-d_4, 298 K, TMS): δ = 160.3, 158.0, 139.4, 135.4, 128.2, 126.9, 98.1, 67.3, 62.5, 43.8, 28.7, 20.5, 20.0, 17.6.

Physical Measurements and Instrumentation

^{1}H NMR spectra were recorded on a Bruker AVANCE III 300 MHz or 400 MHz NMR spectrometer at 298 K using deuterated solvents. Chemical shifts (δ, ppm) were reported relative to tetramethylsilane (TMS). Positive-ion electrospray ionisation (ESI) mass spectra were recorded on a SCIEX API-2000 or API-3200 Triple-Q MS/MS mass spectrometer at 298K. Infra-red (IR) spectra of samples in potassium bromide (KBr) pellets were recorded in the range of 4000 – 400 cm^{-1} using a Thermo Scientific Nicolet iS50 FTIR spectrometer. Elemental analyses were carried out on an Elementar Analysensysteme GmbH Vario MICRO elemental analyser. Electronic absorption spectra were recorded on an Agilent 8453 diode array spectrophotometer. Steady-state emission spectra were recorded on a HORIBA FluoroMax-4 spectrofluorometer. Unless specified, all solutions for photophysical studies were degassed with no fewer than four successive freeze-pump-thaw cycles and stored in a 10-cm^3 round bottomed flask equipped with a side-arm 1-cm fluorescence cuvette and sealed from the atmosphere by a Rotaflo HP6/6 quick-release Teflon stopper. Luminescence quantum yields were measured by optically dilute method[8] using an aerated aqueous solution of the $[Ru(bpy)_3]Cl_2$ (Φ = 0.028, excitation wavelength at 455 nm) as the standard solution.[9] The concentrations of the standard and sample solutions were adjusted until the absorbance at 455 nm was 0.1. Emission lifetimes were measured in the fast multi-channel scaling (MCS) lifetime mode with NanoLED N-375 as an excitation source.

Kinetics Studies

The reaction kinetics of the strain-promoted alkyne-nitrone cycloaddition (SPANC) between the iridium(III) complexes and a strained cyclooctyne **BCN-OH** was studied by electronic absorption spectroscopy. The second-order rate constants (k_2) of the complexes (10 μM) were measured under pseudo first order conditions with a 50- to 200-fold excess of **BCN-OH** (0.5, 1.0, 1.5, and 2.0 mM) in MeOH at 298 K. The reaction process was followed by monitoring the exponential decay of the absorbance at 340 nm. The data were fitted to a single-exponential equation to give the observed rate constants (k_{obs}), which were plotted against the concentrations of **BCN-OH** to obtain the k_2 from the slope of the plots.

Stability toward RONS and Biothiols

The iridium(III) complexes (10 μM) were incubated with various reactive oxygen/nitrogen species (RONS) (100 μM), cysteine (1 mM), and GSH (10 mM) in aerated PBS (50 mM, pH 7.4)/DMSO (99:1, *v*/*v*) at 298 K for 24 h. H_2O_2 was diluted from a stabilised 30% aqueous solution. Hypochlorite (ClO^-), superoxide (O_2^-), nitrite (NO_2^-), and nitrate (NO_3^-) were delivered from NaClO, KO_2, $NaNO_2$, and $NaNO_3$, respectively. Singlet oxygen ($^1O_2$) was generated by the reaction of H_2O_2 with NaClO in a 10:1 ratio, and the NaClO concentration represents $^1O_2$ concentration.[10] Hydroxyl radicals (·OH) was generated by the reaction of H_2O_2 with $[(NH_4)_2Fe(SO_4)_2]\cdot 6H_2O$ in a 10:1 ratio, and the $[(NH_4)_2Fe(SO_4)_2]\cdot 6H_2O$ concentration represents ·OH concentration.[11,12] To study the possible reactions of the iridium(III) complexes with RONS and biological thiols, the reaction mixture (1 mL) was extracted with CH_2Cl_2 (500 μL x 3) and the combined organic extract was washed with H_2O (500 μL x 3) and subjected to ESI-MS analysis.

Preparation of BCN-modified Proteins

*BCN-modified SNAP-tag (**BCN-SNAP**).* **BCN-BG** (5 nmol) in anhydrous DMSO (5 μL) and DTT (0.5 μmol) in PBS (50 mM, pH 7.4) (95μL) was added to purified SNAP-tag (2.5 nmol) in PBS (50 mM, pH 7.4) (400 μL). The mixture was incubated in the dark at 37°C for 1 h. The solution was washed with potassium phosphate buffer (50 mM, pH 7.4) with a YM-10 microcon filter. Concentrated protein was diluted to 5 μM and stored at –20 °C before use.

*BCN-modified BSA (**BCN-BSA**).* BCN-BSA was prepared according to a published procedure.[13] BCN-NHS (1.46 mg, 5 μmol) in anhydrous DMSO (100 μL) was added to the protein BSA (33 mg, 0.5 μmol) in carbonate buffer (50 mM, pH 10) (400 μL). The mixture was incubated in the dark at 298 K for 18 h. The solution was loaded onto a PD-10 size exclusion column that was preequilibrated with potassium phosphate buffer (50 mM, pH 7.4). Volume fractions between 2.5 and 5.0 mL were collected and concentrated with a YM-50 microcon filter. The protein was stored at –20 °C before use.

Labeling of BCN-modified Proteins with Complexes

The iridium(III) complexes (1.25 nmol) in MeOH (50 μL) were added to the BCN-modified proteins (1.25 nmol) in PBS (50 mM, pH 7.4) (250 μL). The mixture was diluted to 500 μL with the same buffer and stirred in the dark at 298 K for 18 h. An aliquot (10 μL) of the reaction mixture was analysed by SDS-PAGE.

Preparation of Isoxazoline Derivatives of Iridium(III) Complexes

A mixture of the iridium(III) complexes (10 μmol) and **BCN-OH** (20 μmol) in CH_2Cl_2/MeOH (1:1, *v*/*v*) (500 μL) was stirred under an inert atmosphere of N_2 at room temperature in the dark for 18 h. The crude product was purified by column chromatography on silica gel using CH_2Cl_2/MeOH as the eluent. The solution was rotary evaporated to dryness to give orange to red solids, which were washed with Et_2O and dried *in vacuo*. The formation of the isoxazoline derivatives was confirmed by ESI-MS analysis.

Measurement of Singlet Oxygen Generation Quantum Yields

1,3-Diphenylisobenzofuran (DPBF) assay.[14] An air-equilibrated DMSO solution (2 mL) containing the iridium(III) complexes and DPBF (10 μM) was introduced into a quartz cuvette of 1 cm path length and irradiated at 365 nm with a 6 W UV-A lamp (Spectroline, USA). Methylene blue was used as a reference for $^1O_2$ sensitisation (Φ_Δ = 0.52). The absorbance of the complexes and methylene blue at 365 nm was *ca.* 0.15. The absorbance of DPBF at 410 nm was monitored every 10 s. A DMSO solution of DPBF without the complexes was examined to determine its photostability under identical irradiation conditions. The Φ_Δ of the complex was determined by comparing Φ_Δ of iridium(III)-sensitised DPBF photooxidation to Φ_Δ of methylene blue-sensitised DPBF photooxidation (as reference) and calculated by the following equation:

$$\Phi_\Delta^{unk} = \Phi_\Delta^{MB} \times \frac{m^{unk} \times F^{MB}}{m^{MB} \times F^{unk}}$$

where m is the slope of a linear fit of the change in absorbance of DPBF at 410 nm against the irradiation time and F is the absorption correlation factor, which is given as $F = 1–10^{-AL}$ (A = absorbance at 365 nm and L = path length of the cuvette).

Cellular Studies

Transient Transfection of CHO-K1 Cells

Lipoplexes were prepared according to the instruction of the manufacturer with a ratio of Lipofectamine 3000:plasmid (2 μL:1 μg). Lipofectamine 3000 (3 μL) was diluted in Opti-MEM (50 μL) and mixed well. The phage UbiC SNAP-KDEL or $pSNAP_f$ (1.5 μg), which are the DNA plasmids encoding the endoplasmic reticulum (ER)-targeting and cytoplasm-enriched, respectively, SNAP-tag protein, and P3000 reagent (3 μL) were mixed in Opti-MEM (50 μL), followed by incubation at 298 K for 5 min. The two solutions were then combined, mixed gently, and incubated at 298 K for an additional 15 min to allow the formation of lipoplexes.

CHO-K1 cells were seeded in tissue culture dishes and grown to 70% confluency at 37°C under a 5% CO_2 atmosphere. The medium was replaced with Opti-MEM and incubated for 1 h. The lipoplexes were added to the medium with gently mixing. After incubation for 6 h, the medium was removed and replaced with antibiotic-free medium (F-12 nutrient mixture with 10% FBS) to incubate for 18 h. Transfected cells expressing SNAP-tag were then used for live cell imaging directly.

*Treatment of cells with **BCN-BG***

CHO-K1 cells were incubated in a mixture of complete medium/DMSO (99:1, *v*/*v*) containing **BCN-BG** (100 μM) at 37°C under a 5% CO_2 atmosphere for 1 h. The medium was removed, the cells were washed thoroughly with PBS (1 mL x 3), and then incubated in a fresh medium for 1 h.

Live Cell Confocal Imaging

CHO-K1 cells in growth medium seeded in a 35-mm tissue culture dish with a sterilised coverslip. The cells were transfected with corresponding lipoplexes (100 μL) and then treated with **BCN-BG**. The medium was replaced with a mixture of medium/DMSO (99:1, *v*/*v*) containing the iridium(III) complexes (5 μM) for 1 h. After washing with PBS (1 mL x 3), the cells were imaged using a Leica TCS SPE (inverted configuration) confocal microscope and a 63x oil-immersion objective lens. Control experiments of cells without transfection and without treatment of **BCN-BG** were also carried out.

MTT Assays

CHO-K1 cells were seeded in a 96-well flat-bottomed microplate. Cells with and without treatment of **BCN-BG** were incubated in medium/DMSO (100 μL, 99:1, *v*/*v*) containing the iridium(III) complexes with concentrations ranging from 10^{-5} to 10^{-8} M in the dark at 37°C under a 5% CO_2 atmosphere for 1 h. Wells containing untreated cells were used as blank control. For evaluation of photoinduced cytotoxicity, the culture medium was further replaced with fresh medium and the microplate was irradiated at 365 nm for 30 min with a 6W UV-A lamp (Spectroline, USA) placed at 5 cm above. After the treatments, the culture medium was replaced with fresh medium and the cell was incubated for 20 h. MTT in PBS (10 μL, 5 mg mL^{-1}) was added to each well. The microplate was incubated at 37°C under a 5% CO_2 atmosphere for another 4 h. The growth medium was then removed, and DMSO (100 μL) was added to each well. The absorbance of the solutions at 570 nm was measured with a Powerwave XS MQX200R microplate spectrophotometer (BioTek Instruments Inc., Winooski,

VT) . The procedures for transfected cells was similar to the non-transfected cells, except with prior transfection by lipoplexes of $pSNAP_f$ (10 μL) added to each well.

ICP-MS Measurements

CHO-K1 cells were grown in a 35 mm tissue culture dish and incubated at 37°C under a 5% CO_2 atmosphere for 48 h. Cells with and without pretreatment of **BCN-BG** were incubated with the iridium(III) complex (5 μM) in growth medium/DMSO (99:1, *v*/*v*) for 1 h. After the treatments, the medium was removed, and the cell layer was gently washed with PBS (1 mL x 3). The cells were trypsinised and harvested with PBS. The resultant solution (2 mL) was heated with 65% HNO_3 (2 mL) at 70°C for 2 h, cooled to room temperature, and analysed using a PE NexION 2000 ICP-MS (PerkinElmer Instruments). Cells transfected with $pSNAP_f$ were subjected to the same procedures for ICP-MS measurements.

Table S1 Photophysical data of complexes **1 – 4** in degassed solutions at 298 K.

Complex	Solvent	λ_{em}/nm	τ_o/μs	Φ_{em}
1	CH_2Cl_2	524 (max), 560, 606 sh, 669 sh	2.05	0.023
	CH_3CN	526 (max), 563, 611 sh, 670 sh	2.07	0.019
2	CH_2Cl_2	595 (max), 645, 708 sh	3.54	0.005
	CH_3CN	594 (max), 644, 708 sh	2.50	0.004
3	CH_2Cl_2	561	1.77	0.011
	CH_3CN	582	1.27	0.015
4	CH_2Cl_2	630	1.32	0.012
	CH_3CN	649	0.85	0.007

Table S2 Amount of iridium (fmol) of complexes **1 – 4** associated with an average CHO-K1 cell upon incubation with the complexes (5 μM) at 37°C for 1 h, as determined by ICP-MS.

Complex	Amount of iridium[a]	Amount of iridium[a,b]	Amount of iridium[b,c]
1	0.168 ± 0.014	0.174 ± 0.004	0.171 ± 0.019
2	0.336 ± 0.004	0.330 ± 0.002	0.324 ± 0.019
3	0.082 ± 0.001	0.076 ± 0.001	0.091 ± 0.001
4	0.139 ± 0.001	0.143 ± 0.004	0.135 ± 0.003

[a] Non-transfected cells.

[b] Treatment of **BCN-BG** (100 μM) for 1 h, followed by washing with PBS (1 mL x 3).

[c] Cells had been transfected with the $pSNAP_f$ Vector to express SNAP-tag in the cytoplasm.

Fig. S1 Pseudo first-order kinetics for the reactions of complexes **1** – **4** with **BCN-OH** at different concentrations in MeOH at 298 K. The slope corresponds to the k_2 of the reaction.

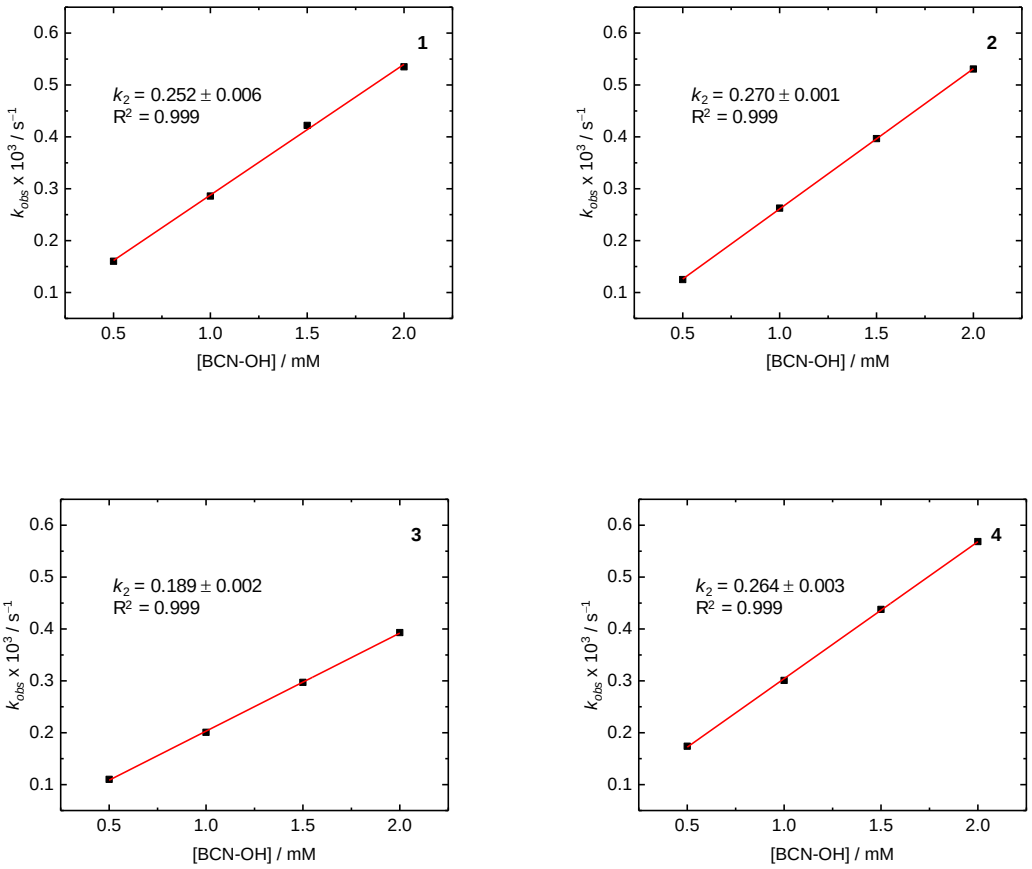


Fig. S2 Emission spectra of complexes **1 – 4** (10 μM) in the absence (black) and presence (red) of **BCN-OH** (250 μM) in aerated potassium phosphate buffer (50 mM, pH 7.4)/MeOH (9:1, *v*/*v*) at 298 K. Excitation wavelength = 350 nm.

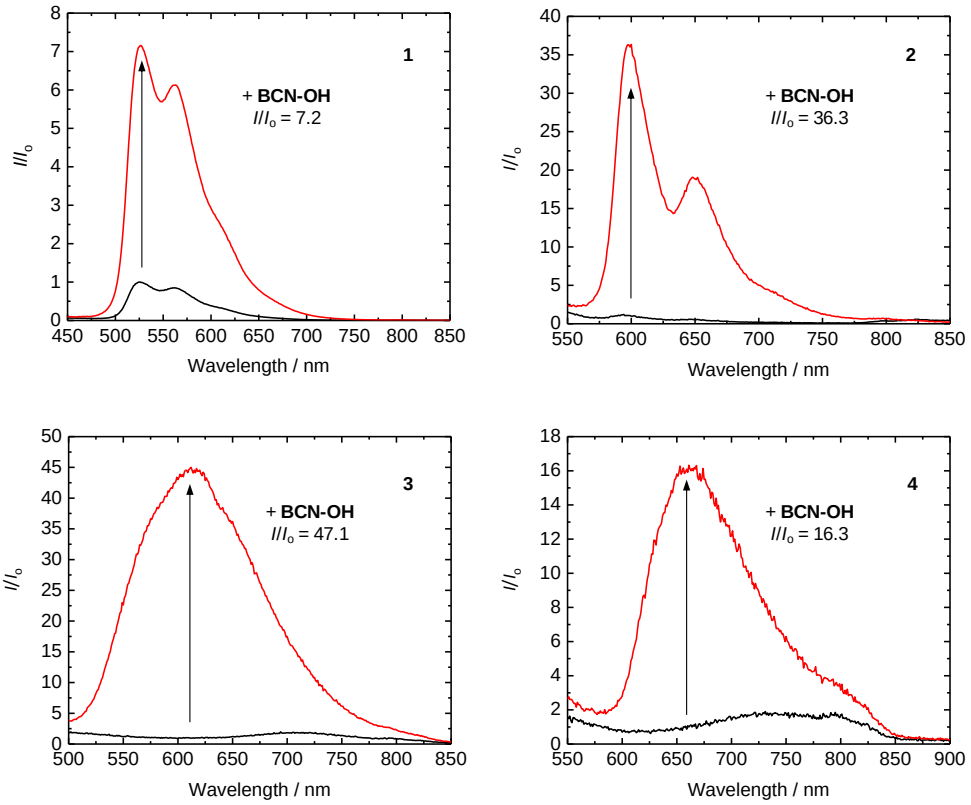


Fig. S3 Emission changes of complexes **1** – **4** (10 μM) in the presence of different RONS (100 μM), cysteine (1 mM), and GSH (10 mM) in aerated potassium phosphate buffer (50 mM, pH 7.4)/DMSO (99:1, *v*/*v*) at 298 K.

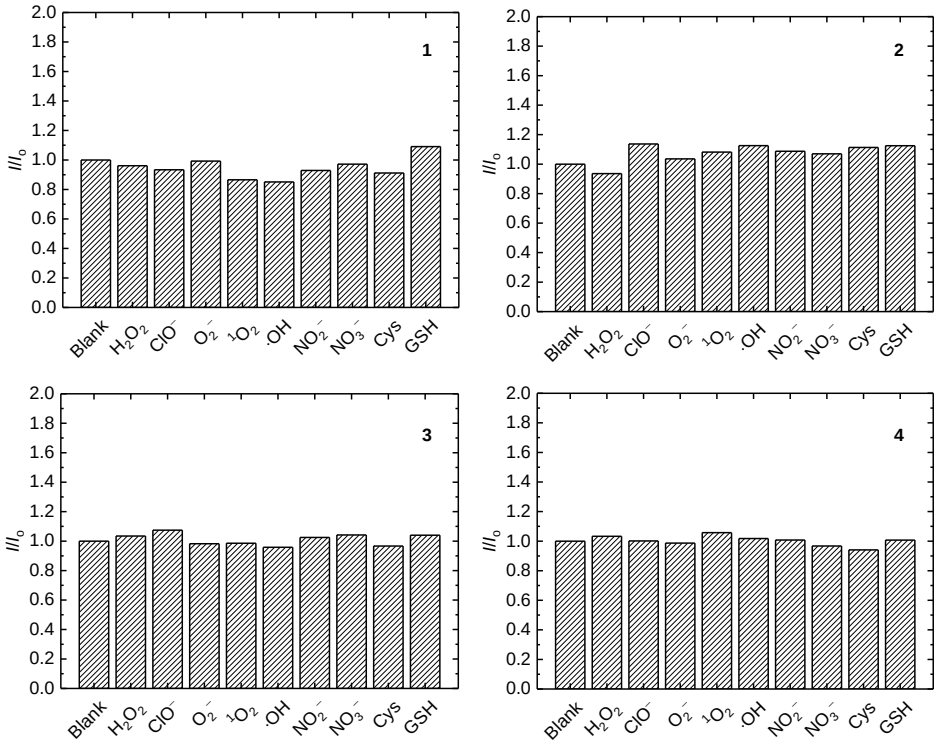


Fig. S4 SDS-PAGE analysis of the reactions of complexes **1** – **4** with **BCN-BSA**. Left: UV transillumination; right: Coomassie Blue staining. Lane 1: protein ladder; lanes 2, 4, 6, and 8: complexes **1**, **2**, **3**, and **4**, respectively, with **BCN-BSA**; lanes 3, 5, 7, and 9: complexes **1**, **2**, **3**, and **4**, respectively, with unmodified BSA.

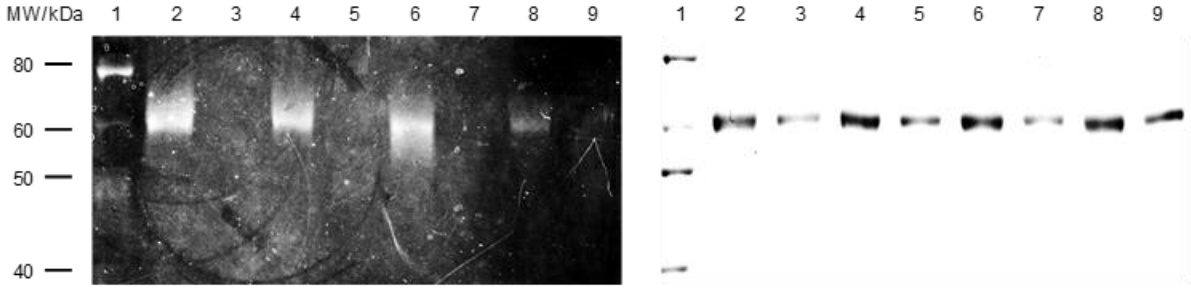


Fig. S5 SDS-PAGE analysis of the reaction of complex **1** with **BCN-SNAP**. Left: UV transillumination; right: Coomassie Blue staining. Lane 1: protein ladder; lane 2: complex **1** with **BCN-SNAP**; lane 3: complex **1** with unmodified SNAP-tag.

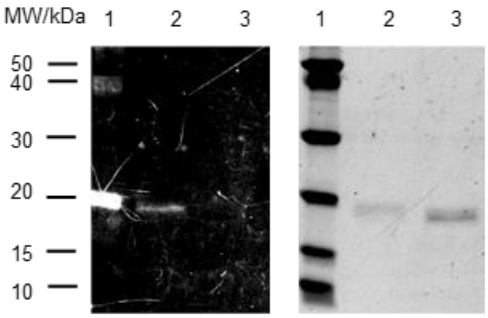
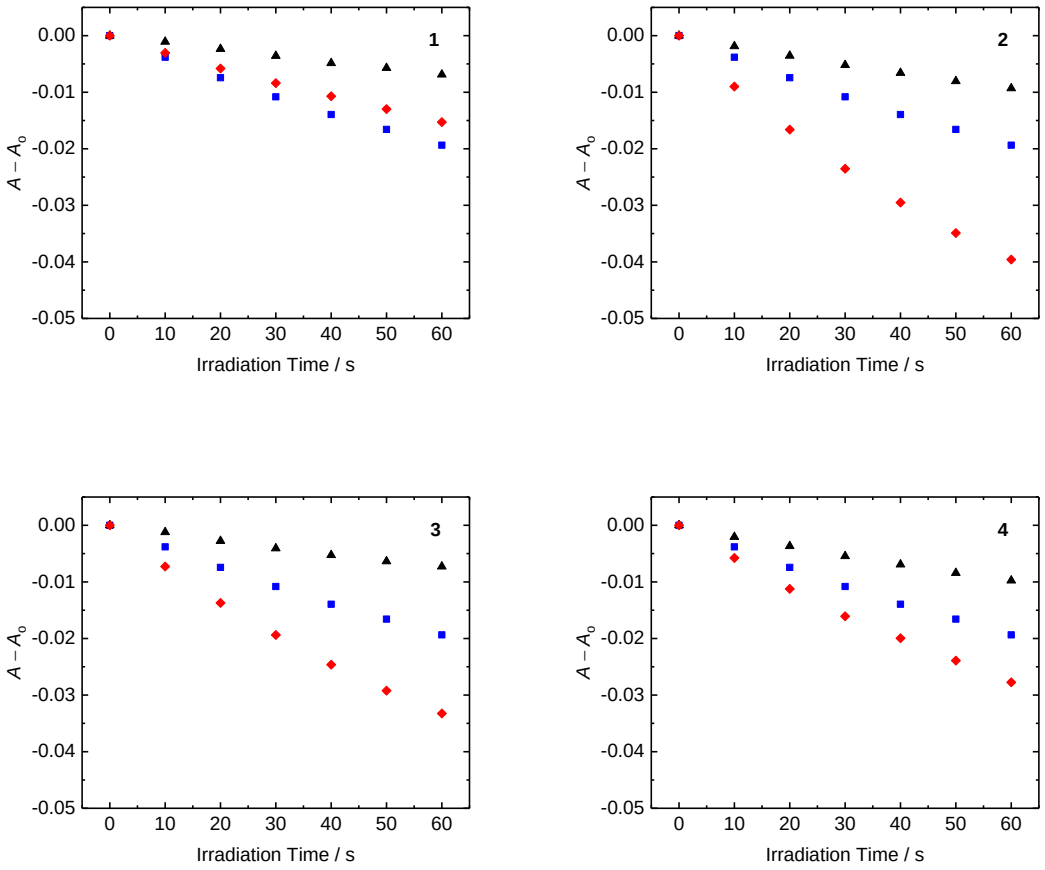


Fig. S6 Plots of rates of decay in absorbance of DPBF (10 μM) at 410 nm in aerated DMSO in the presence of complexes **1** – **4** (triangles) and their isoxazoline counterparts (diamonds), and methylene blue (squares) upon irradiation at 365 nm.



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