Photophysical and cellular uptake properties of novel phosphorescent cyclometalated iridium(III) bipyridine D-fructose complexes

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Electronic Supplementary Information

Materials. All solvents were of analytical grade and purified according to standard procedures.[1] D-Glucose, SeO_2, cisplatin, *N*,*N*'-dicyclohexylcarbodiimide (DCC), *N*-hydroxysuccinimide (NHS), triethylamine, KPF_6, $AgNO_3$, Na_2CO_3, $MgSO_4$ and $Na_2S_2O_5$ were obtained from Acros. MTT was purchased from Sigma. *n*-Butylamine, dibenzylamine, glacial acetic acid, Pd/C, Hppy, Hpq, bpy, $IrCl_3 \cdot 3H_2O$ and DHN were supplied by Aldrich. Autoclaved Milli-Q water was used for the preparation for the aqueous solutions. MCF-7 and HEK293T cells were obtained from American Type Culture Collection. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), phosphate-buffered saline (PBS) at pH 7.2, trypsin-EDTA, penicillin/streptomycin and MitoTracker Deep Red FM were purchased from Invitrogen. The growth medium for cell culture contained DMEM with 10% FBS and 1% penicillin/streptomycin.

Bpy-fructose. Fru-NH_2-AcOH[2] (617 mg, 2.20 mmol) was dissolved in a minimum amount of warm water and the solution was added dropwise to a DMF (40 mL) solution of bpy-NHS[3] (797 mg, 2.56 mmol) at room temperature under an inert atmosphere of nitrogen. Triethylamine (5 mL) was added to the reaction mixture. This yellow solution was stirred at room temperature under an inert atmosphere of nitrogen in the dark for 24 h. The solvent was evaporated to dryness and the crude product was purified by column chromatography on silica gel. The desired product was eluted with CH_2Cl_2/MeOH/$NH_3 \cdot H_2O$ (3:1:0.1, *v*/*v*/*v*) and eventually isolated as a white solid. Yield: 554 mg (67%); ^{1}H NMR (400 MHz, CD_3OD, 298 K, TMS): δ 8.76 (d, 1H, J = 4.8 Hz, H6 of bpy), 8.65 (s, 1H, H3 of bpy), 8.52 (d, 1H, J = 4.8 Hz, H6' of bpy), 8.19 (s, 1H, H3' of bpy), 7.79 – 7.77 (m, 1H, H5 of bpy), 7.30 (d, 1H, J = 4.8 Hz, H5' of bpy), 4.03 – 3.44 (m, 7H, H on fructosyl ring), 2.47 (s, 3H, CH_3 on bpy); MS (ESI): *m*/*z* 376 $[M + H]^+$.

Bpy-butyl. A mixture of *n*-butylamine (2 mL, 20.15 mmol) and triethylamine (5 mL) was added to bpy-NHS (863 mg, 2.77 mmol) in CH_2Cl_2 (40 mL). The solution was stirred at room temperature for 48 h. The solvent was removed by rotary evaporation. The remaining pale yellow solid was purified by column chromatography on silica gel, and the desired product was eluted with *n*-hexane/EtOAc/$NH_3 \cdot H_2O$ (2:1:0.1, *v*/*v*/*v*). The solvent was removed by rotary evaporation to yield bpy-butyl as a pale yellow solid. Yield: 387 mg (52%); ^{1}H NMR (400 MHz, $CDCl_3$ 298 K, TMS): δ 8.57 – 8.54 (m, 2H, H3 and H6 of bpy), 8.30 (d, 1H, J = 5.2 Hz, H6' of bpy), 8.05 (s, 1H, H3' of bpy) 7.60 – 7.59 (m, 1H, H5 of bpy), 7.44 (br, 1H, CONH), 6.97 (d, 1H, J = 5.2 Hz, H5' of bpy), 3.31 – 3.26 (m, 2H, CONHCH_2), 2.26 (s, 3H, CH_3 on bpy), 1.47 – 1.39 (m, 2H, $NHCH_2CH_2$), 1.25 – 1.19 (m, 2H, $NHCH_2CH_2CH_2$), 0.76 (t, 3H, J = 7.6 Hz, $NHCH_2CH_2CH_2CH_3$); MS (ESI): *m*/z 270 $[M + H]^+$.

[Ir(ppy)$_2$(bpy-fructose)](PF$_6$) (1a). A mixture of $[Ir_2(ppy)_4Cl_2]$ (154 mg, 0.14 mmol) and bpy-fructose (99 mg, 0.262 mmol) in CH_2Cl_2 (30 mL) was stirred at room temperature under an inert atmosphere of nitrogen in the dark for 24 h. KPF_6 (97 mg, 0.53 mmol) was added and the mixture was stirred for 1 h. The solvent was removed by rotary evaporation. Subsequent recrystallization of the product from acetone/diethyl ether afforded the complex as orange crystals. Yield: 221 mg (82%); ^{1}H NMR (400 MHz, CD_3OD, 298 K, TMS): δ 9.05 (s, 1H, H3 of bpy), 8.69 (s, 1H, H3' of bpy), 8.15 – 8.12 (m, 3H, H6 of bpy and H6 of pyridyl ring of ppy), 7.90 – 7.84 (m, 6H, H6' and H5 of bpy, H3 of phenyl ring of ppy, and H3 of pyridyl ring of ppy), 7.67 – 7.63 (m, 2H, H5 of pyridyl ring of ppy), 7.44 (d, 1H, J = 5.2 Hz, H5' of bpy), 7.08 – 7.03 (m, 4H, H5 of phenyl ring of ppy and H4 of pyridyl ring of ppy), 6.93 – 6.88 (m, 2H, H4 of phenyl ring

of ppy), 6.31 (t, 2H, J = 8.0 Hz, H6 of phenyl ring of ppy), 4.04 – 3.38 (m, 7H, H on fructosyl ring), 2.62 (s, 3H, CH_3 on bpy); IR (KBr) ν/cm^{-1}: 845 (s, PF_6^-); MS (ESI): m/z 877 [M – PF_6^-]$^+$; elemental analysis calcd (%) for $IrC_{40}H_{37}N_5O_6PF_6 \cdot CH_3OH \cdot H_2O$: C, 46.62; H, 4.05; N, 6.55; found: C, 46.36; H, 4.30; N, 6.51.

[Ir(ppy)$_2$(bpy-butyl)](PF$_6$) (1b). A mixture of [$Ir_2(ppy)_4Cl_2$] (207 mg, 0.19 mmol) and bpy-butyl (95 mg, 0.35 mmol) in CH_2Cl_2/MeOH (30 mL, 1:1, v/v) was heated to reflux under an inert atmosphere of nitrogen in the dark for 24 h. The reaction mixture was then cooled to room temperature, and KPF_6 (129 mg, 0.70 mmol) was added and the mixture was stirred for 1 h. The solvent was removed by rotary evaporation. The orange-yellow residual oil was dissolved in CH_2Cl_2 and purified by column chromatography on silica gel. The desired product was eluted with CH_2Cl_2/MeOH (40:1, v/v). Subsequent recrystallization of the product from CH_2Cl_2/diethyl ether afforded the complex as orange crystals. Yield: 135 mg (42%); ^{1}H NMR (400 MHz, $(CD_3)_2CO$, 298 K, TMS): δ 9.06 (s, 1H, H3 of bpy), 8.83 (s, 1H, H3’ of bpy), 8.26 – 8.18 (m, 4H, CONH, H6 of bpy and H3 of pyridyl ring of ppy), 8.00 – 7.89 (m, 7H, H6’ of bpy, H3 of phenyl ring, H4 and H6 of pyridyl ring of ppy), 7.84 (d, 1H, J = 5.8 Hz, H5 of bpy), 7.58 (d, 1H, J = 5.6 Hz, H5’ of bpy), 7.18 – 7.16 (m, 2H, H5 of pyridyl ring of ppy), 7.06 – 7.02 (m, 2H, H4 of phenyl ring of ppy), 6.95 – 6.91 (m, 2H, H5 of phenyl ring of ppy), 6.37 – 6.30 (m, 2H, H6 of phenyl ring of ppy), 3.44 – 3.40 (m, 2H, CONHCH_2), 2.64 (s, 3H, CH_3 on bpy), 1.63 – 1.56 (m, 2H, $NHCH_2CH_2$), 1.44 – 1.35 (m, 2H, $NHCH_2CH_2CH_2$), 0.93 (t, 3H, J = 7.2 Hz, $NHCH_2CH_2CH_2CH_3$); IR (KBr) ν/cm^{-1}: 845 (s, PF_6^-); MS (ESI): m/z 771 [M – PF_6^-]$^+$; elemental analysis calcd (%) for $IrC_{38}H_{35}N_5OPF_6 \cdot CH_3OH$: C, 49.47; H, 4.15; N, 7.40; found: C, 49.38; H, 4.03; N, 7.39.

[Ir(pq)$_2$(bpy-fructose)](PF$_6$) (2a). The synthetic procedure was similar to that of complex **1a** except that [Ir$_2$(pq)$_4$Cl$_2$] (165 mg, 0.13 mmol) was used instead of [Ir$_2$(ppy)$_4$Cl$_2$]. Yield: 95 mg (36%); ^{1}H NMR (400 MHz, CD$_3$OD, 298 K, TMS): δ 8.65 (s, 1H, H3 of bpy), 8.44 – 8.37 (m, 5H, H3 of phenyl ring of pq, H3 of quinoline of pq and H6 of bpy), 8.28 (s, 1H, H3' of bpy), 8.20 (d, 2H, J = 6.0 Hz, H4 of quinoline of pq), 8.12 (d, 1H, J = 5.6 Hz, H5 of bpy), 7.88 – 7.82 (m, 3H, H6' of bpy and H8 of quinoline of pq), 7.44 (d, 1H, J = 5.6 Hz, H5' of bpy), 7.41 – 7.35 (m, 4H, H5 and H7 of quinoline of pq), 7.20 – 7.17 (m, 2H, H6 of quinoline of pq), 7.07 – 7.03 (m, 2H, H4 of phenyl ring of pq), 6.82 (q, 2H, J = 6.8 Hz, H5 of phenyl ring of pq), 6.54 – 6.49 (m, 2H, H6 of phenyl ring of pq), 4.02 – 3.47 (m, 7H, H on fructosyl ring), 2.48 (s, 3H, CH$_3$ on bpy); IR (KBr) ν/cm^{-1}: 846 (s, PF$_6^-$); MS (ESI): m/z 977 [M – PF$_6^-$]$^+$; elemental analysis calcd (%) for IrC$_{48}$H$_{41}$N$_5$O$_6$PF$_6$·CH$_2$Cl$_2$: C, 48.80; H, 3.59; N, 5.81; found: C, 48.92; H, 3.60; N, 5.62.

[Ir(pq)$_2$(bpy-butyl)](PF$_6$) (2b). The synthetic procedure was similar to that of complex **1b** except that [Ir$_2$(pq)$_4$Cl$_2$] (225 mg, 0.18 mmol) was used instead of [Ir$_2$(ppy)$_4$Cl$_2$]. Yield: 181 mg (99%); ^{1}H NMR (400 MHz, (CD$_3$)$_2$CO, 298 K, TMS): δ 8.69 (s, 1H, H3 of bpy), 8.54 (d, 4H, J = 6.0 Hz, H3 of phenyl ring of pq and H3 of quinoline of pq), 8.46 (d, 1H, J = 5.6 Hz, H6 of bpy), 8.41 (s, 1H, H3' of bpy), 8.26 (d, 2H, J = 8.0 Hz, H4 of quinoline of pq), 8.21 (d, 1H, J = 5.6 Hz, H6' of bpy), 8.13 (s, 1H, CONH), 8.02 – 8.00 (m, 1H, H5 of bpy), 7.97 – 7.92 (m, 2H, H8 of quinoline of pq), 7.59 (d, 1H, J = 5.2 Hz, H5' of bpy), 7.49 – 7.42 (m, 4H, H5 and H7 of quinoline of pq), 7.20 – 7.13 (m, 4H, H4 of phenyl ring of pq and H6 of quinoline of pq), 6.84 (td, 2H, J =

7.4, 3.6 Hz, H5 of phenyl ring of pq), 6.56 (t, 2H, J = 7.2 Hz, H6 of phenyl ring of pq), 3.34 – 3.42 (m, 2H, CONHCH_2), 2.51 (s, 3H, CH_3 on bpy), 1.56 – 1.50 (m, 2H, $NHCH_2CH_2$), 1.37 – 1.31 (m, 2H, $NHCH_2CH_2CH_2CH_2$), 0.89 (t, 3H, J = 7.6 Hz, $NHCH_2CH_2CH_2CH_3$); IR (KBr) ν/cm^{-1}: 848 (s, PF_6^-); MS (ESI): m/z 871 [M – PF_6^-]$^+$; elemental analysis calcd (%) for $IrC_{46}H_{39}N_5OPF_6 \cdot 0.5CH_2Cl_2$: C, 52.81; H, 3.81; N, 6.62; found: C, 52.61; H, 3.93; N, 6.76.

Physical measurements and instrumentation. The instrumentation for physical measurements has been described previously.[4] Electronic absorption and steady-state spectra were recorded on a Hewlett-Packard 8453 diode array spectrophotometer and a SPEX FluoroLog 3-TCSPC spectrophotometer, respectively. Emission lifetimes were measured in the Fast MCS or TCSPC mode with a NanoLED N-375 as the excitation source. Unless specified, all the 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 the optically dilute method[5] with an aerated aqueous solution of $[Ru(bpy)_3]Cl_2$ (Φ_{em} = 0.028) as the standard solution.[6] The lipophilicity of the complexes was presented as the log $P_{o/w}$ values, which were determined by using the shake-flask method.[7]

Cell cultures. MCF-7 cells were cultured in high glucose DMEM supplemented with 10% FBS and 1% penicillin-streptomycin in a humidified chamber at 37 °C under a 5% CO_2 atmosphere. They were subcultured every 2 to 3 days.

ICP-MS. Cells grown in a 60-mm tissue culture dish were incubated with the iridium(III) complexes (50 μM) in fructose-free medium/DMSO (99:1 *v*/*v*) at 37 °C under a 5% CO_2 atmosphere for 15 min. The medium was removed and the cell layer was washed gently with PBS (1 mL × 3). The cells were then trypsinized and harvested with PBS (500 μL × 4) before being digested with 65% HNO_3 (2 mL) at 70°C for 2 h. The digested solution was analyzed using an Elan 6100 DRC-ICP-MS (PerkinElmer SCIEX Instruments, USA).

MTT assays. Cells were seeded in a 96-well flat-bottomed microplate (*ca*. 10,000 cells per well) in growth medium (100 μL) and incubated at 37 °C under a 5% CO_2 atmosphere for 24 h. The iridium(III) complexes and cisplatin (positive control), respectively, in different concentrations, were added to the wells in a mixture of growth medium/DMSO (99:1, *v*/*v*). After the microplate was incubated for 48 h, MTT in PBS (5 mg mL^{-1}, 10 μL) was added to each well. The microplate was then incubated for another 4 h. The medium was removed carefully and isopropanol (200 μL) was added to each well. The microplate was further incubated for 5 min. The absorbance of the solutions at 570 nm was measured with a SPECTRAmax 340 microplate reader (Molecular Devices Corp., Sunnyvale, CA). The IC_{50} values of the complexes were determined from dose dependence of surviving cells after exposure to the iridium(III) complexes and cisplatin.

Live-cell confocal imaging. Cells in growth medium were seeded on a sterilized coverslip in a 60-mm tissue culture dish and grown at 37 °C under a 5% CO_2 atmosphere for 48 h. The growth medium was removed and replaced with fructose-free medium/DMSO (99:1, *v*/*v*) containing the iridium(III) complexes (50 μM). After incubation for 15 min, the medium was removed and the cell layer was washed gently

with PBS (1 mL × 3). The coverslip was mounted onto a sterilized glass slide and then imaged using a Leica TCS SPE confocal microscope with an oil immersion 40× or 63× objective and an excitation wavelength at 405 nm. The emission was measured using a long-pass filter at 532 nm. In the colocalization experiments, after treated with complexes, the MCF-7 cells were incubated with MitoTracker Deep Red FM (100 nM) in the FBS-free medium for 20 min and then washed with PBS (1 mL × 3). The excitation wavelength for MitoTracker Deep Red FM was 633 nm. The Pearson's colocalization coefficient was determined by the program Image J (Version 1.4.3.67).

Photocytotoxicity experiments. MCF-7 cells incubated with the iridium(III) complexes for 2 h were irradiated with the long wave (> 365 nm) of a UV lamp (Spectronics ENF-260C/FE) for 30 min and then incubated in the dark for further 45.5 h before being analyzed by the MTT assay.

Single-oxygen sensitizing measurements. The iridium(III) complex (7.5 μM) and DHN (75 μM) in air-saturated acetonitrile/2-propanol (4:1, *v*/*v*) were irradiated at 350 nm using a SPEX FluoroLog 3-TCSPC spectrophotometer equipped with a xenon lamp for 4 h. The electronic absorption spectra were recorded at an interval of 30 min on an Agilent 8453 diode array spectrophotometer. The production yield of photooxidation of DHN to Juglone was calculated using the extinction coefficient of Juglone at 427 nm (ε = 3811 M^{-1} cm^{-1}).

Table 1. Electronic absorption spectral data of the iridium(III) complexes at 298 K

Complex	Solvent	λ_{abs}/nm (ε/dm^3 mol^{-1} cm^{-1})
1a	CH_2Cl_2	257 (69,705), 269 sh (64,625), 323 sh (22,190), 339 sh (13,670), 360 sh (11,750), 384 sh (10,800), 413 sh (5,515), 467 (1,405)
	CH_3CN	254 (61,420), 270 sh (53,995), 320 sh (20,425), 362 sh (10,055), 383 sh (8,640), 413 sh (4,230), 467 sh (1,250)
1b	CH_2Cl_2	256 (62,650), 271 sh (57270), 320 sh (22050), 339 sh (12,465), 360 sh (10,495), 382 sh (9,610), 416 sh (4,590), 471 sh (1,230)
	CH_3CN	254 (52,585), 268 sh (48,170), 319 sh (18,080), 380 sh (7,560), 416 sh (3,340), 472 sh (1,010)
2a	CH_2Cl_2	259 sh (44,210), 283 (44,995), 320 sh (22,040), 349 sh (19,330), 395 sh (5,605), 437 (4,880)
	CH_3CN	258 (48,370), 281 sh (47,125), 317 sh (22,895), 348 sh (19,465), 394 sh (5,450), 434 (4,865)
2b	CH_2Cl_2	261 sh (61,210), 281 (63,495), 316 sh (29,670), 349 sh (26,290), 394 sh (7,055), 438 (6,335), 528 sh (615)
	CH_3CN	261 (53,375), 283 sh (49,485), 314 sh (24,905), 338 (22,650), 434 (5,170)

Fig. S1 Laser-scanning confocal microscopy images of MCF-7 cells incubated with complexes **1a**, **b** and **2a**, **b** (50 μM) at 37 °C for 15 min.

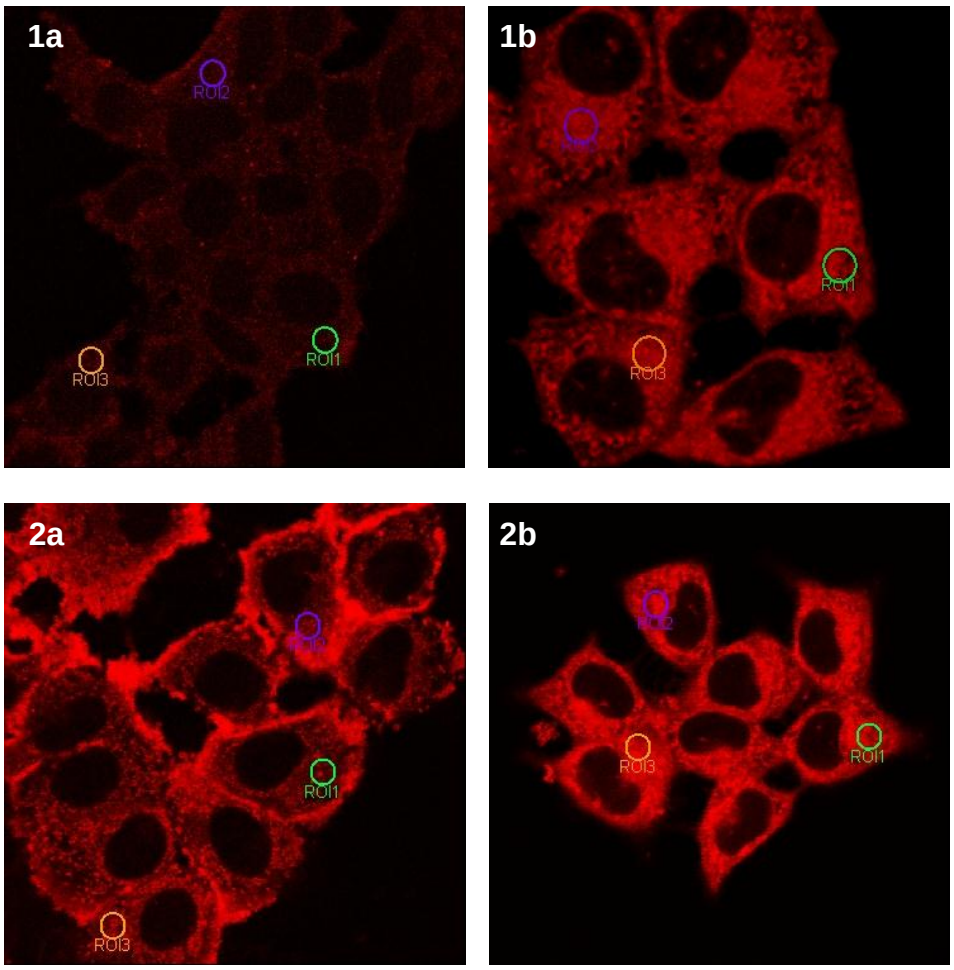


Fig. S2 Laser-scanning confocal microscopy images of MCF-7 cells incubated with complexes **1a**, **b** and **2a**, **b** (50 μM) at 4 °C for 15 min.

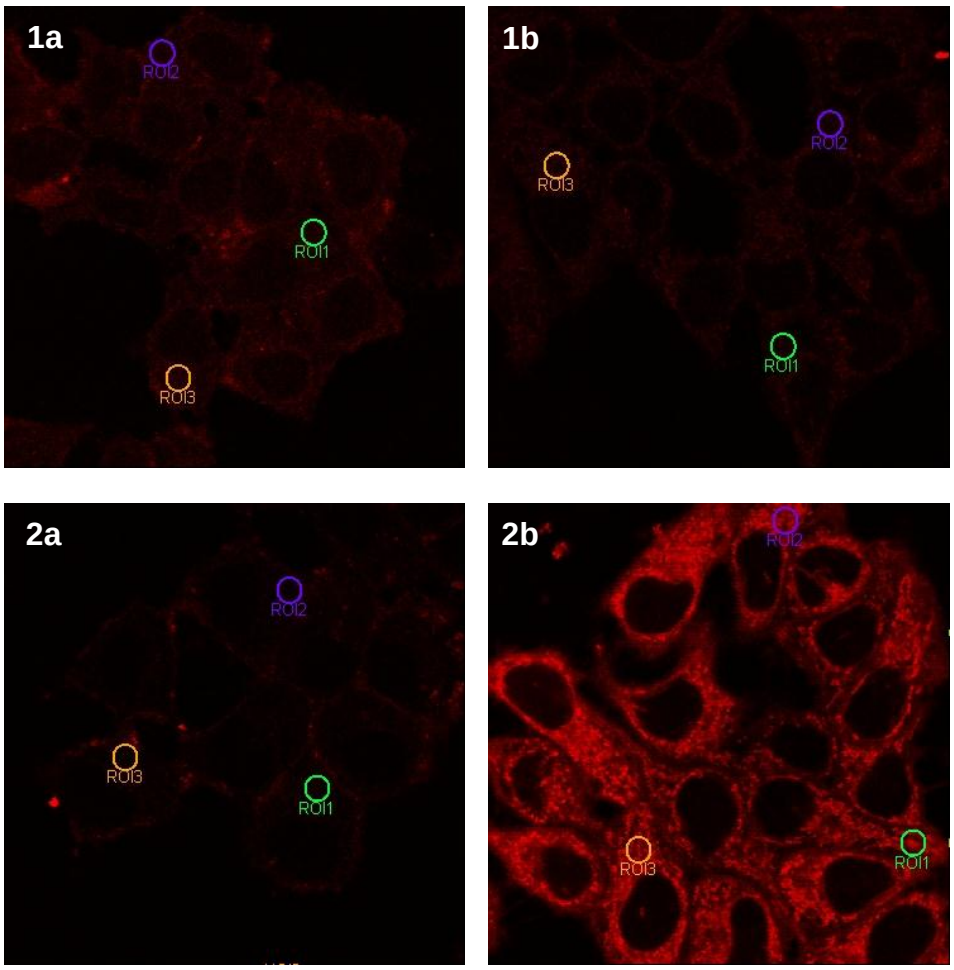


Fig. S3 Electronic absorption spectral traces of a solution of complex **2a** (7.5 μM) and DHN (75 μM) in air-saturated acetonitrile/2-propanol (4:1, *v*/*v*) upon irradiation at 350 nm.

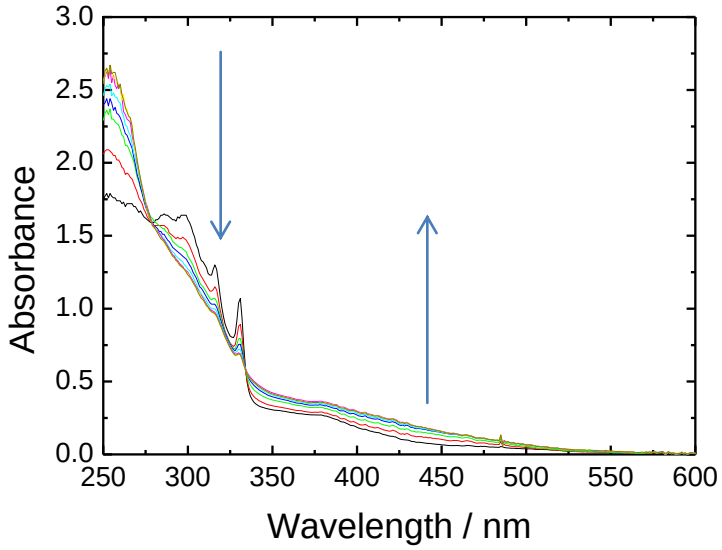
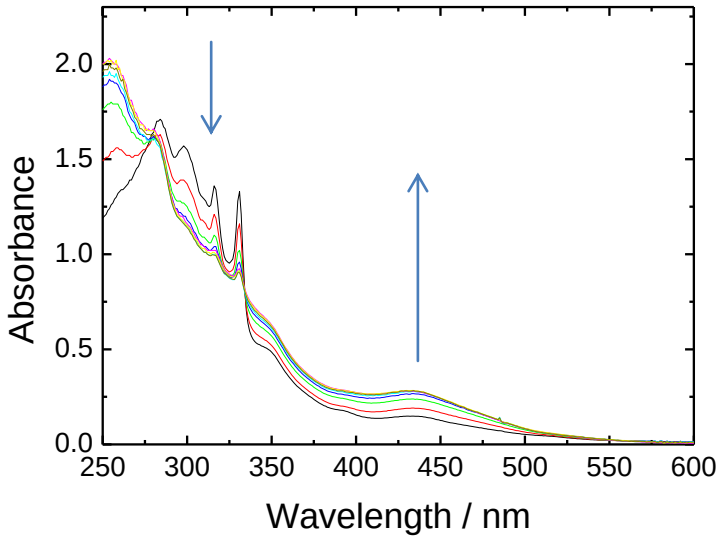


Fig. S4 Electronic absorption spectral traces of a solution of complex **2b** (7.5 μM) and DHN (75 μM) in air-saturated acetonitrile/2-propanol (4:1, *v*/*v*) upon irradiation at 350 nm.



References

1 D. D. Perrin and W. L. F. Armarego, *Purification of Laboratory Chemicals*, Elsevier, Oxford, 6th edn., 2009.

2 Y. Hou, X. Wu, W. Xie, P. G. Braunschweiger and P. G. Wang, *Tetrahedron Lett*., 2001, **42**, 825.

3 B. M. Peek, G. T. Ross, S. W. Edwards, G. J. Meyer, T. J. Meyer and B. W. Erickson, *Int. J. Pept. Prot. Res.*, 1991, **38**, 114.

4 K. Y. Zhang and K. K.-W. Lo, *Inorg*. *Chem*., 2009, **48**, 6011.

5 J. N. Demas and G. A. Crosby, *J*. *Phys*. *Chem*., 1971, **75**, 991.

6 K. Nakamura, *Bull*. *Chem*. *Soc*. *Jpn*., 1982, **55**, 2697.

7 M. J. McKeage, S. J. Berners-Price, P. Galettis, R. J. Bowen, W. Brouwer, L. Ding, L. Zhuang and B. C. Baguley, *Cancer Chemother. Pharmacol*., 2000, **46**, 343.