# Supporting Information

## Cellular imaging properties of phosphorescent iridium(III) complexes substituted with ester or amide groups

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**Materials and measurements.** All solvents were of analytical grade and purified according to standard procedures. All chemical reagents used were purchased from Energy Chemical and Shanghai Bide Pharmatech Co., Ltd. without further purification. HeLa cells were obtained from Jiangsu KeyGEN BioTECH Co., Ltd. $^{1}$H NMR and $^{13}$C NMR spectra were recorded on Bruker ACF400 spectrometer at 298 K using deuterated solvents. Chemical shifts ($\delta$, ppm) were reported relative to tetramethylsilane (TMS). Mass spectra were obtained on Bruker autoflex matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometer (MS). UV-Vis absorption spectra were recorded on UV-2600 Shimadzu UV-Vis spectrophotometer. Photoluminescence spectra and lifetime decay curves were recorded on Edinburgh FL 980 spectrophotometer. Luminescence imaging was carried out on an Olympus IX81 laser-scanning confocal microscope. The PLIM setup is integrated with the same Olympus IX81 laser-scanning confocal microscope and the lifetime values were calculated with professional software provided by PicoQuant Company.

**Cytotoxicity assay.** The cytotoxicity of the complexes toward HeLa cells was determined using the methyl thiazolyltetrazolium (MTT) assay. Cells were seeded into a 96-well cell culture plate at $10^4$/well and were cultured at 37 °C with 5% $CO_2$ for 24 h. Different concentrations of complexes **1** – **4** (0, 1, 2, 5, 10 and 20 μM, in DMEM supplemented with 10% FBS (fetal bovine serum), 0.08 mg/mL streptomycin and 80 U/mL penicillin) were then added into the wells. The cells were subsequently incubated for 6 h at 37 °C under 5% $CO_2$. Then, MTT (20 μL/well, 5 mg/mL) was added to each well and the plate was incubated for an additional 4 h at 37 °C under 5% $CO_2$. The

medium was then replaced with 150 μL dimethyl sulfoxide (DMSO) per well and the cells was further incubated for 15 min. The absorbance of the solution at 492 nm was monitored by a Microplate Spectrophotometer (TECAN SUNRISE).

**Colocalization.** Cells were seeded onto confocal petri dish, and incubated for 24 h at 37 °C with 5% $CO_2$. Then the cells were incubated with complexes **1** – **4** (5 μM) for 0.5 h at 37 °C, respectively. After that, the cells were washed with PBS and then incubated with Hoechst 33342 (5 μg/mL, 20 min, 37 °C) or MitoTracker Deep Red FM (200 nM, 5 min, 37 °C). Imaging was performed with an excitation wavelength at 405 nm for complexes and Hoechst and 635 nm for MitoTracker. The emission was measured using a band-pass filter at 460 ± 20 nm for Hoechst, 580 ± 20 nm for complexes **2** and **4**, 660 ± 20 nm for complexes **1** and **3**, and 675 ± 25 nm for MitoTracker. The colocalization coefficient was determined by ImageJ.

**Cellular uptake.** HeLa cells were planted on confocal petri dish and allowed to adhere for 24 h at 37 °C with 5% $CO_2$. Then the cells were treated under different conditions. 4 °C: the cells were preincubated at 4 °C for 0.5 h, then the cells were incubated with complex **1** (5 μM) at 4 °C for 0.5 h. $NH_4Cl$: the cells were pretreated with $NH_4Cl$ (50 mM) at 37 °C for 1 h, the medium was removed and the cells were washed with PBS (1 mL × 3). Then the cells were incubated with complex **1** (5 μM) at 37 °C for 0.5 h. Chlorpromazine: the cells were pretreated with chlorpromazine (10 μg/mL) at 37 °C for 1 h, the medium was removed and the cells were washed with PBS (1 mL × 3). Then the cells were incubated with complex **1** (5 μM) at 37 °C for 0.5 h. Nocodazole: the cells were pretreated with nocodazole (10 μM) at 37 °C for 1 h, the medium was

removed and the cells were washed with PBS (1 mL × 3). Then the cells were incubated with complex **1** (5 μM) at 37 °C for 0.5 h. Imaging was performed with an excitation wavelength at 405 nm. The emission was measured using a band-pass filter at 660 ± 20 nm.

**Cell cycle imaging.** The cells were seeded in confocal petri dish and allowed to adhere for 24 h at 37 °C with 5% $CO_2$. Luminescence confocal microscopy images of HeLa cells synchronized at the M phase via treatment with colchicine (0.5 μg/mL, 4 h, 37 °C) and then treated with complex **1** and **3** (5 μM, 0.5 h, 37 °C) and Hoechst 33342 (5 ug/mL, 20 min, 37 °C). For interphases imaging, the cells were treated with colchicine (0.5 μg/mL) for 4 h at 37 °C. The dish was then replaced with DMEM supplemented with 10% FBS (fetal bovine serum), 0.08 mg/mL streptomycin and 80 U/mL penicillin. After 5.5 h, 16.5 h and 21 h, the cells were incubated with complex **1** (5 μM) for 0.5 h at 37 °C to obtain G1, S, G2 phases. Imaging was performed with an excitation wavelength at 405 nm. The emission was measured using a band-pass filter at 460 ± 20 nm for Hoechst and 660 ± 20 nm for the complexes.

**Lifetime imaging.** Cells were seeded onto confocal petri dish, and incubated for 24 h at 37 °C with 5% $CO_2$. Then the cells were incubated with complexes **1** – **4** (5 μM) for 0.5 h at 37 °C, respectively. Imaging was performed with an excitation wavelength at 405 nm. The luminescence signal was collected at 580 ± 20 nm for complexes **2** and **4** and 660 ± 20 nm for complexes **1** and **3** by charge coupled device module and the lifetime signal was handled by time-correlated single photon counting module. For hypoxia imaging, after incubation with complex **1** (5 μM, 0.5 h, 37 °C), the cells were

washed with PBS and incubated under an atmosphere containing 5% $O_2$ for 0.5 h and then imaging was performed.

## Synthesis and characterization

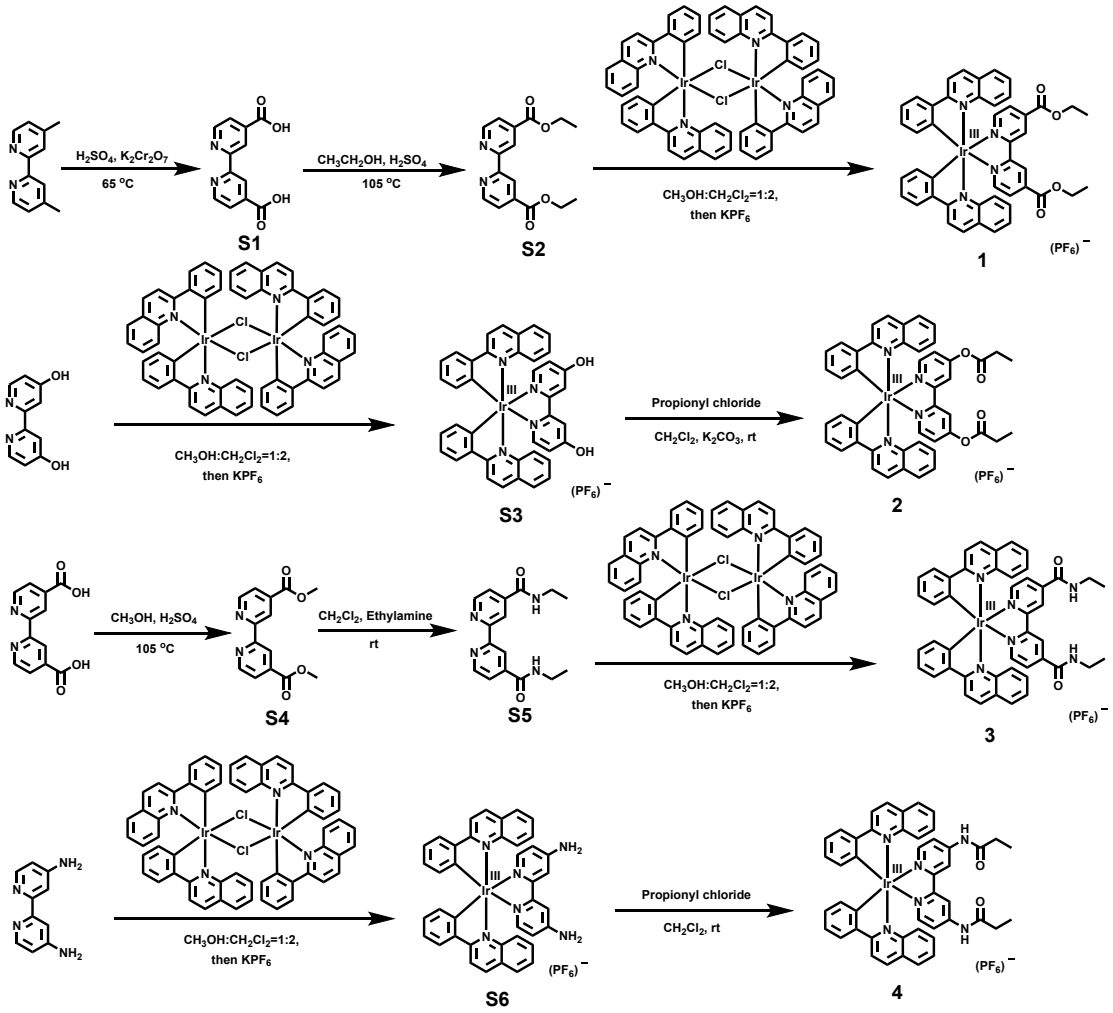


**Fig. S1** The synthetic route of complexes **1** – **4**.

Complexes **S3** and **S6** were synthesized according to previous reports (*Organomet. Chem.*, 2015, **791**, 175–182).

**Synthesis of 4,4′-bis(ethoxycarbonyl)-2,2′-bipyridine (S2).** A round-bottomed flask was charged with 4,4′-bis(methyl)-2,2′-bipyridyl (1050 mg, 5.71 mmol). The flask cooled to 0 °C in an ice/water bath. $H_2SO_4$ (20 mL) was added dropwise to the flask via syringe. Next, $K_2Cr_2O_7$ (3381 mg, 11.5 mmol) was added to the reaction mixture. The reaction flask was placed in an oil bath and stirred at 65 °C for 15 h. The solution was cooled to room temperature and $H_2O$ was added to the mixture, the white precipitate was collected by filtration and dried in vacuo, yielding 4,4′-bis(carboxylic acid)-2,2′-bipyridine (**S1**) as a white solid. After, the compound **S1** (500 mg, 2.05 mmol) was dissolved in $CH_3CH_2OH$ (70 mL), $H_2SO_4$ (15 mL) was added to the flask. The reaction mixture was stirred at 105 °C for 40 h. Then, $H_2O$ was added to the mixture and neutralised with NaOH to pH 8.0. The precipitate formed was filtered off and dried to give compound **S2** of white solid (yield: 73%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 8.94 – 8.93 (m, 2H), 8.82 (s, 2H), 7.93 – 7.92 (m, 2H), 4.42 – 4.37 (m, 4H), 1.37 – 1.35 (m, 6H).

**Synthesis of [Ir(pq)$_2$(bpy-COOC$_2$H$_5$)](PF$_6$) (1).** A mixture of dichloride bridged iridium(III) bis-phenylquinoline dimer (650 mg, 0.51 mmol) and compound **S2** (320 mg, 1.07 mmol) were placed in a 50 mL round bottom-flask under nitrogen atmosphere, then $CH_3OH/CH_2Cl_2$ (24 mL, 1:2, v/v) was added. The solution was refluxed and stirred for 6 h. Following, the resulting mixture was cooled to room temperature and $KPF_6$ (282 mg, 1.53 mmol) was introduced. After stirring at room temperature for 4 h, the solution was evaporated and the crude product was purified by column chromatography on silica gel using $CH_2Cl_2/CH_3OH$ (20:1, v/v) as an eluent to afford complex **1** (yield:

72%). $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta$: 8.57 (d, $J$ = 1.2 Hz, 2H), 8.42 – 8.41 (m, 2H), 8.29 – 8.23 (m, 4H), 8.07 – 8.03 (m, 4H), 7.74 – 7.71 (m, 2H), 7.36 – 7.32 (m, 2H), 7.20 – 7.14 (m, 4H), 7.02 – 6.98 (m, 2H), 6.85 – 6.81 (m, 2H), 6.52 – 6.50 (m, 2H), 4.43 (q, $J$ = 7.2 Hz, 4H), 1.40 (t, $J$ = 6.8 Hz, 6H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 169.7, 162.9, 155.7, 149.5, 148.9, 147.2, 145.2, 140.5, 140.3, 134.6, 131.4, 131.2, 129.5, 127.7, 127.6, 127.4, 127.0, 124.3, 123.7, 123.2, 117.9, 63.3, 14.1. MALDI-TOF-MS (m/z): 901.6 $[M–PF_6^-]^+$.

**Synthesis of $[Ir(pq)_2(bpy\text{-}OCOC_2H_5)](PF_6)$ (2).** The $[Ir(pq)_2(bpy\text{-}OH)](PF_6)$ (**S3**) (280 mg, 0.30 mmol) was dissolved in $CH_2Cl_2$ (10 mL) and propionyl chloride (1.5 mL) and $K_2CO_3$ were added to the solution. After stirring at room temperature for 3 h. After that, the mixture was poured to water and extracted with $CH_2Cl_2$ (100 mL × 3), and the concentrated organic phase was dried with $MgSO_4$. The crude product was purified by column chromatography on silica gel using $CH_2Cl_2/CH_3OH$ (20:1, v/v) as an eluent to afford complex **2** (yield: 39%). $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta$: 8.20 (q, $J$ = 8.8 Hz, 4H), 8.10 (d, $J$ = 6.0 Hz, 2H), 8.01 – 7.97 (m, 4H), 7.73 (d, $J$ = 8.0 Hz, 2H), 7.38 (t, $J$ = 7.2 Hz, 2H), 7.32 (d, $J$ = 6.0 Hz, 2H), 7.22 (d, $J$ = 8.8 Hz, 2H), 7.15 (t, $J$ = 7.6 Hz, 2H), 7.09 – 7.05 (m, 2H), 6.81 (t, $J$ = 7.6 Hz, 2H), 6.54 (d, $J$ = 7.6 Hz, 2H), 2.64 (q, $J$ = 7.2 Hz, 4H), 1.19 (t, $J$ = 7.6 Hz, 6H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 171.3, 169.7, 159.9, 157.1, 150.3, 148.9, 147.5, 145.4, 140.1, 134.7, 131.5, 130.9, 129.2, 127.6, 127.1, 127.0, 124.5, 123.1, 121.0, 117.9, 117.3, 27.7, 8.5. MALDI-TOF-MS (m/z): 901.3 $[M–PF_6^-]^+$.

**Synthesis of 4,4'-bis(methoxycarbonyl)-2,2'-bipyridine (S4).** The compound **S1** (400 mg, 1.64 mmol) was dissolved in $CH_3OH$ (50 mL) and $H_2SO_4$ (10 mL) was added to the solution. After stirring at 105 °C for 40 h. After cooling, the reaction mixture was poured into water and neutralised with NaOH to pH 8.0. The precipitate formed was filtered off and dried to give compound **S4** of white solid (yield: 81%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 8.97 – 8.96 (m, 2H), 8.87 (dd, $J$ = 5.0, 0.8 Hz, 2H), 7.91 (dd, $J$ = 5.0, 1.6 Hz, 2H), 4.00 (s, 6H).

**Synthesis of 4,4′-bis(ethylaminocarbonyl)-2,2′-bipyridine (S5).** The compound **S4** (320 mg, 1.18 mmol) was dissolved in $CH_2Cl_2$ (10 mL) and ethylamine (10 mL) was added to the solution. After stirring at room temperature for 48 h. The solvent was evaporated and the solid residue was recrystallised to afford compound **S5** (yield: 65%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 8.96 – 8.94 (m, 2H), 8.84 (d, $J$ = 5.2 Hz, 2H), 8.77 (s, 2H), 7.84 – 7.82 (m, 2H), 3.35 – 3.29 (m, 4H), 1.14 (t, $J$ = 7.2 Hz, 6H).

**Synthesis of [Ir(pq)$_2$(bpy-CONHC$_2$H$_5$)](PF$_6$) (3).** A mixture of dichloride bridged iridium(III) bis-phenylquinoline dimer (500 mg, 0.39 mmol) and compound **S5** (250 mg, 0.84 mmol) were placed in a 50 mL round bottom-flask under nitrogen atmosphere, then $CH_3OH/CH_2Cl_2$ (24 mL, 1:2, v/v) was added. The solution was refluxed and stirred for 6 h. Following, the resulting mixture was cooled to room temperature and $KPF_6$ (215 mg, 1.17 mmol) was introduced. After stirring at room temperature for 4 h, the solution was evaporated and the crude product was purified by column chromatography on silica gel using $CH_2Cl_2/CH_3OH$ (20:1, v/v) as an eluent to afford complex **3** (yield: 41%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 8.38 (s, 2H), 8.24 (d, $J$ = 6.0 Hz, 2H), 8.23 – 8.15

(m, 4H), 8.01 (d, $J$ = 7.6 Hz, 2H), 7.91 – 7.89 (m, 2H), 7.72 – 7.69 (m, 2H), 7.49 – 7.42 (m, 2H), 7.40 – 7.36 (m, 2H), 7.34 – 7.32 (m, 2H), 7.20 – 7.16 (m, 2H), 7.04 – 7.00 (m, 2H), 6.86 – 6.82 (m, 2H), 6.51 (d, $J$ = 7.6 Hz, 2H), 3.49 – 3.42 (m, 4H), 1.24 – 1.21 (m, 6H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 169.7, 162.9, 155.8, 150.2, 148.0, 147.3, 145.3, 144.9, 140.2, 134.6, 131.7, 131.1, 129.2, 127.5, 127.3, 127.0, 124.3, 123.3, 121.0, 117.3, 35.6, 29.7. MALDI-TOF-MS (m/z): 898.8 $[M–PF_6^-]^+$.

**Synthesis of $[Ir(pq)_2(bpy\text{-}NHCOC_2H_5)](PF_6)$ (4).** The $[Ir(pq)_2(bpy\text{-}NH_2)](PF_6)$ (**S6**) (300 mg, 0.32 mmol) was dissolved in $CH_2Cl_2$ (15 mL) and propionyl chloride (1.5 mL) was added to the solution. After stirring at room temperature for 12 h. After that, the mixture was poured to water and extracted with $CH_2Cl_2$ (100 mL × 3), and the concentrated organic phase was dried with $MgSO_4$. The crude product was purified by column chromatography on silica gel using $CH_2Cl_2/CH_3OH$ (1:1, v/v) as an eluent to afford complex **4** (yield: 34%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 10.72 (s, 2H), 8.31 (dd, $J$ = 6.4, 2.0 Hz, 2H), 8.25(d, $J$ = 1.6 Hz, 2H), 8.17 – 8.11 (m, 4H), 7.96 (d, $J$ = 7.2 Hz, 2H), 7.82 (d, $J$ = 6.4 Hz, 2H), 7.69 (dd, $J$ = 8.4, 1.2 Hz, 2H), 7.39 – 7.34 (m, 4H), 7.12 (t, $J$ = 7.6 Hz, 2H), 7.04 – 7.00 (m, 2H), 6.80 – 6.76 (m, 2H), 6.55 (d, $J$ = 7.6 Hz, 2H), 2.42 (q, $J$ = 7.6 Hz, 4H), 1.06 (t, $J$ = 7.4 Hz, 6H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$: 170.1, 163.0, 155.7, 150.7, 148.6, 147.1, 146.2, 144.7, 141.1, 134.2, 131.7, 131.2, 130.1, 128.3, 127.9, 127.3, 126.9, 124.4, 123.4, 122.4, 118.8, 34.9, 14.8. MALDI-TOF-MS (m/z): 899.6 $[M–PF_6^-]^+$.

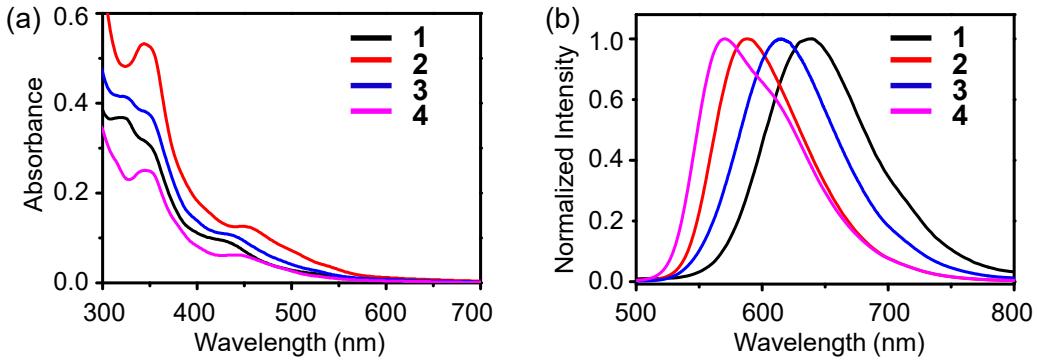


**Fig. S2** Electronic absorption (a) and photoluminescence (b) spectra of complexes of **1** – **4** ($10^{-5}$ M) in DMSO/PBS (1:99, v/v, pH = 7.4) at 298 K.

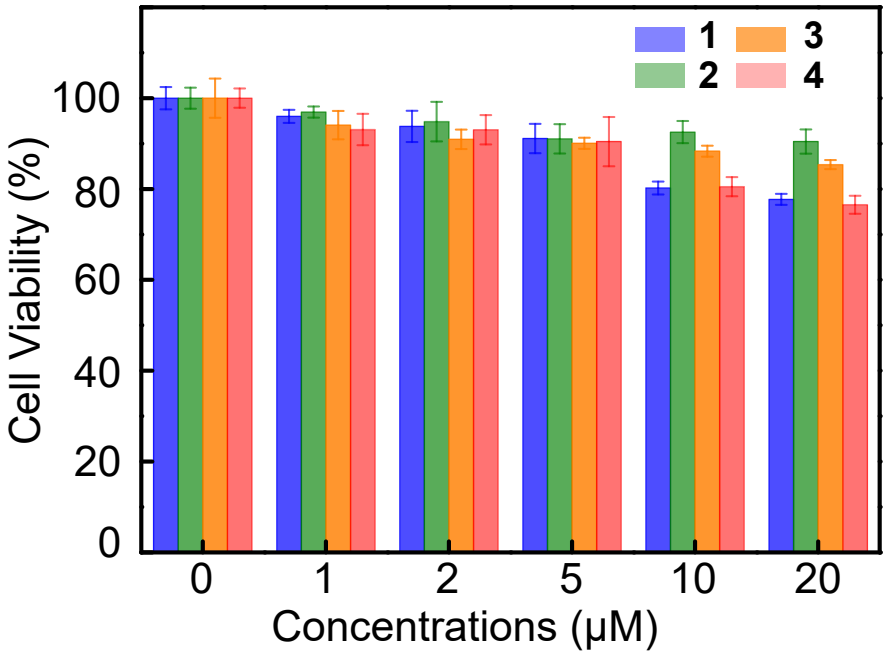


**Fig. S3** Cell viability of HeLa cells incubated with complexes **1** – **4** at different concentrations at 37 °C for 6 h.

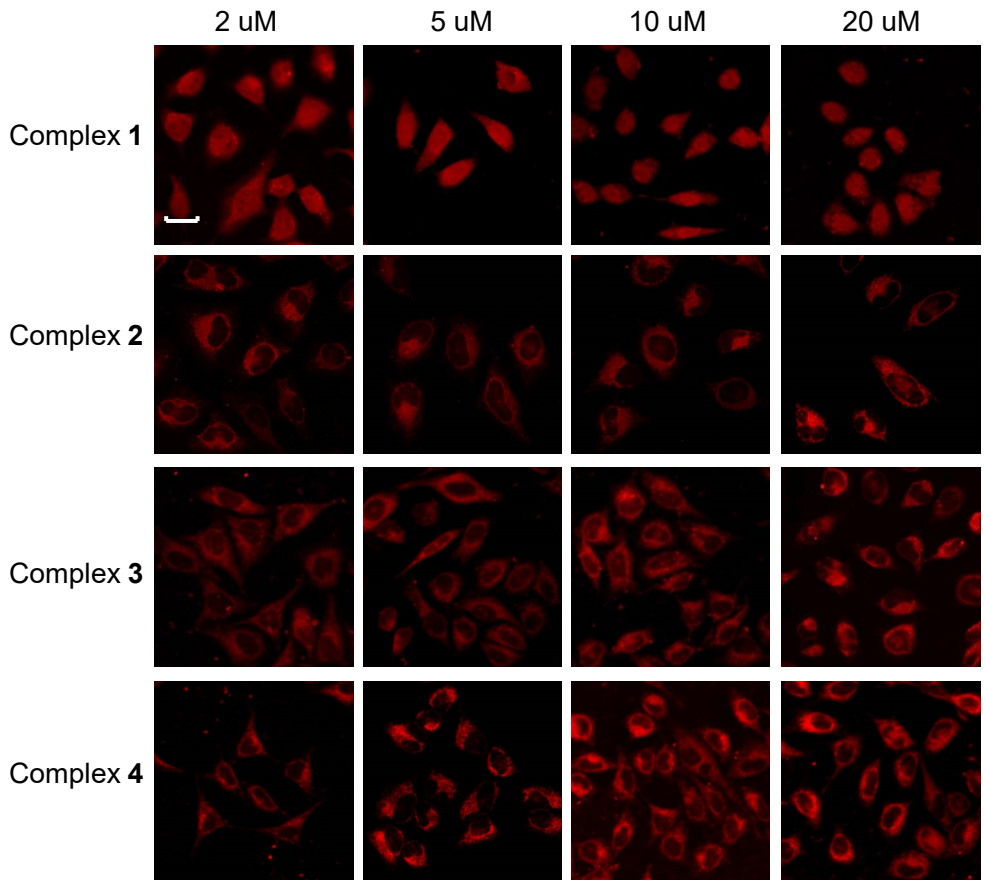


**Fig. S4** Luminescence confocal microscopy images of HeLa cells treated with complexes **1 – 4** at a concentration of 2 – 20 μM (0.5 h, 37 °C). $\lambda_{ex}$ = 405 nm. $\lambda_{em}$ = 580 ± 20 nm for complexes **2** and **4**, and 660 ± 20 nm for complexes **1** and **3**. Scale bar: 20 μm.

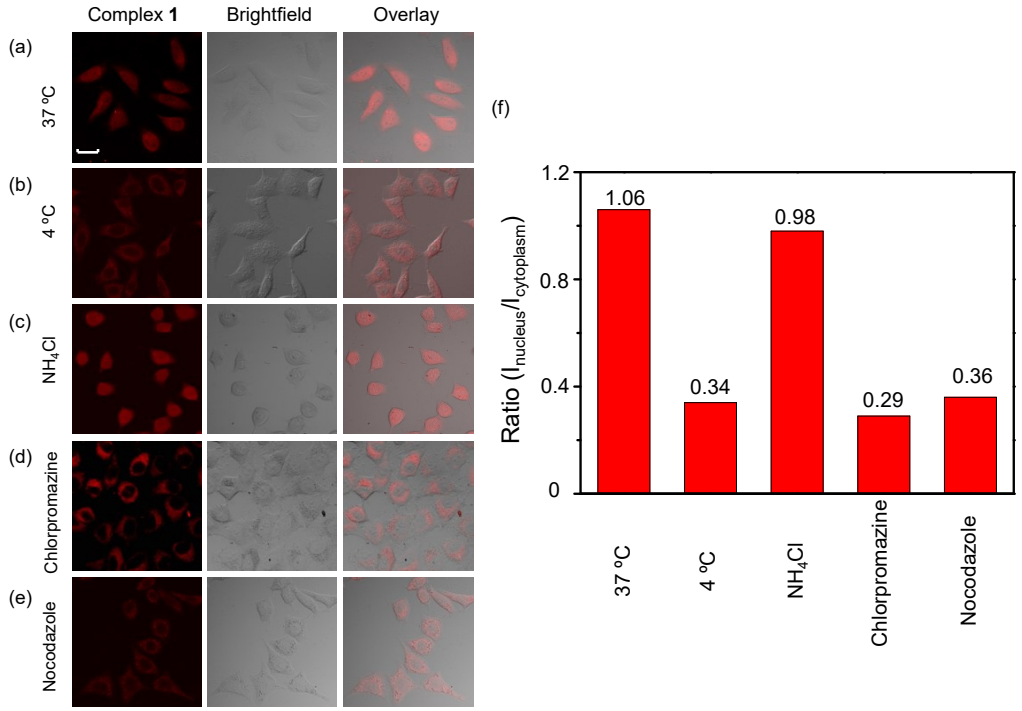


**Fig. S5** Luminescence confocal microscopy images of HeLa cells preincubated at 4 °C for 0.5 h, then treated with complex **1** (5 μM, 0.5 h) at (a) 4 °C, the cells were preincubated with (b) $NH_4Cl$ (50 mM), (c) chlorpromazine (10 μg/mL), and (d) nocodazole (10 μM) at 37 °C for 1 h and then incubated with complex **1** (5 μM) at 37 °C for 0.5 h. $\lambda_{ex}$ = 405 nm. $\lambda_{em}$ = 660 ± 20 nm. Scale bar: 20 μm. (f) The intensity ratios of complex **1** in the nuclei over that in the cytoplasm in (a) – (e).

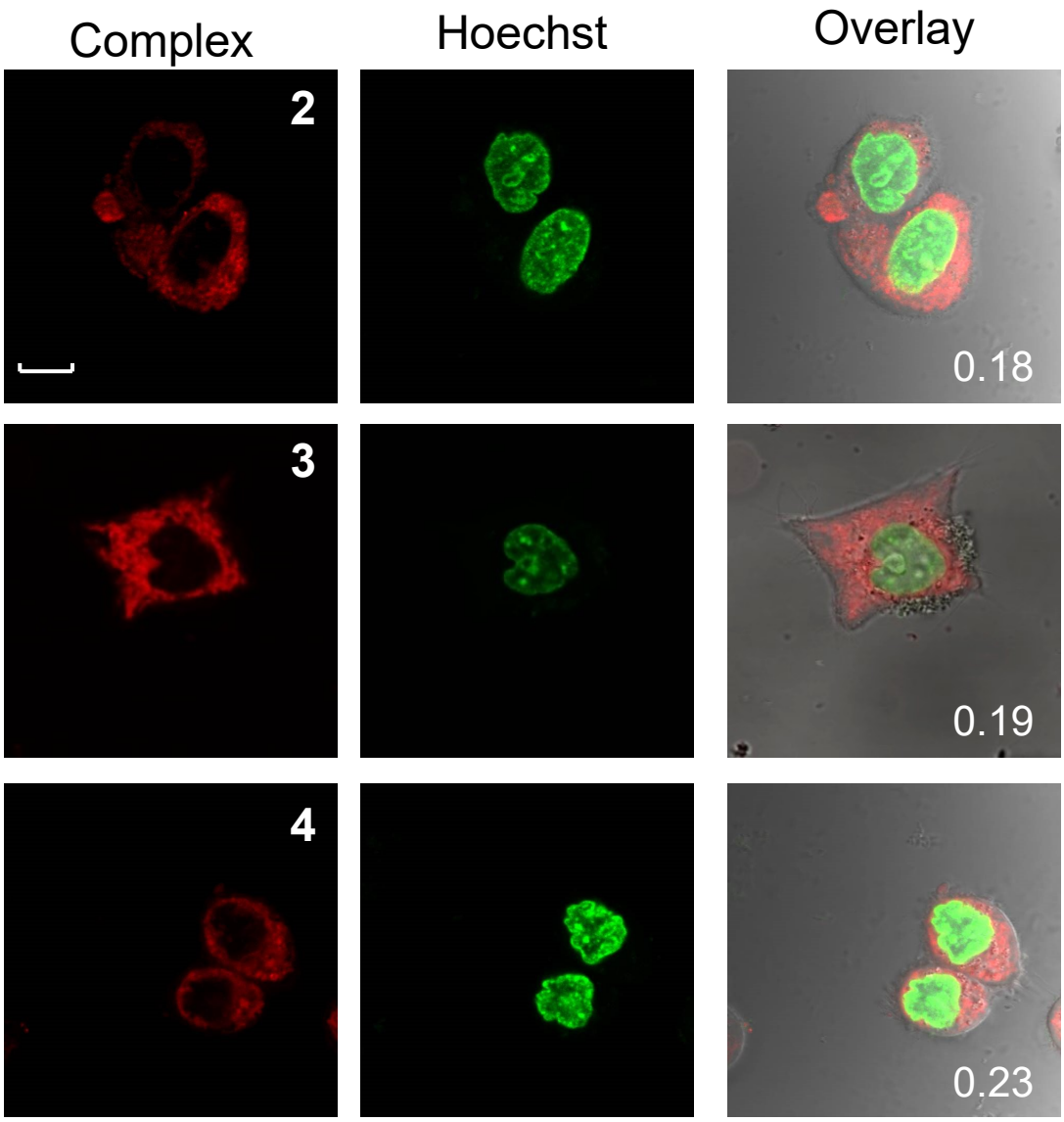


**Fig. S6** Luminescence confocal microscopy images of HeLa cells synchronized at the M phase via treatment with colchicine (0.5 μg/mL, 4 h, 37 °C) and then treated with complex **2 – 4** (5 μM, 0.5 h, 37 °C) and Hoechst 33342 (5 ug/mL, 20 min, 37 °C). $\lambda_{ex}$ = 405 nm. $\lambda_{em}$ = 460 ± 20 nm for Hoechst, 580 ± 20 nm for complexes **2** and **4**, and 660 ± 20 nm for complex **3**. Scale bar: 20 μm.

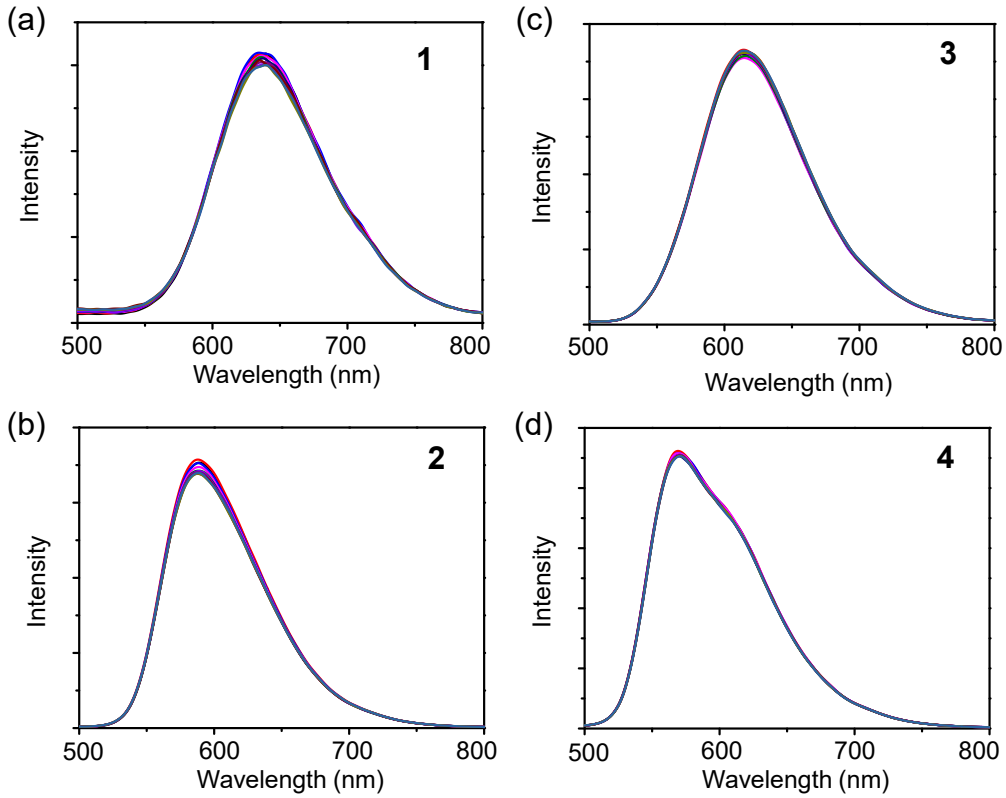


**Fig. S7** Emission spectra of complexes **1** – **4** (10 μM) in Tris-Cl buffer (50 mM, pH 7.4)/DMSO (99:1, v/v) in different concentration of calf thymus DNA (0 – 20 μM) at 298 K.

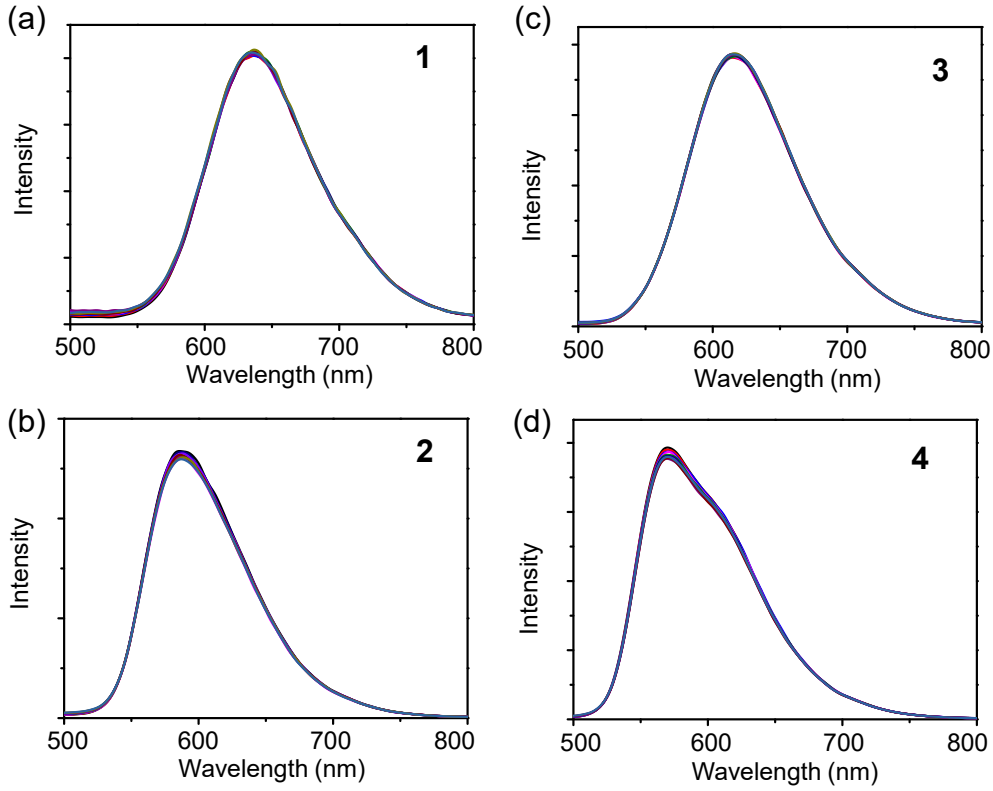


**Fig. S8** Emission spectra of complexes **1** – **4** (10 μM) in Tris-Cl buffer (50 mM, pH 7.4)/DMSO (99:1, v/v) in different concentration of yeast RNA (0 – 20 μg/mL) at 298 K.

**Table S1** Photophysical data of complexes **1** – **4** in deaerated DMSO/PBS (1:99, v/v, pH = 7.4) at 298 K.

| Complex | $\lambda_{em}$/nm | $\tau_0$/ns | $\Phi_{em}$/%[a] |
|---|---|---|---|
| **1** | 640 | 91.7 | 0.55 |
| **2** | 588 | 321.2 | 4.5 |
| **3** | 614 | 268.7 | 2.7 |
| **4** | 570, 610 sh | 702.6 | 6.4 |

[a]The emission quantum yields were determined using $[Ru(bpy)_3]Cl_2$ ($\Phi_{em}$ = 0.028 in aerated $H_2O$) as a reference.