# Electronic Supporting Information

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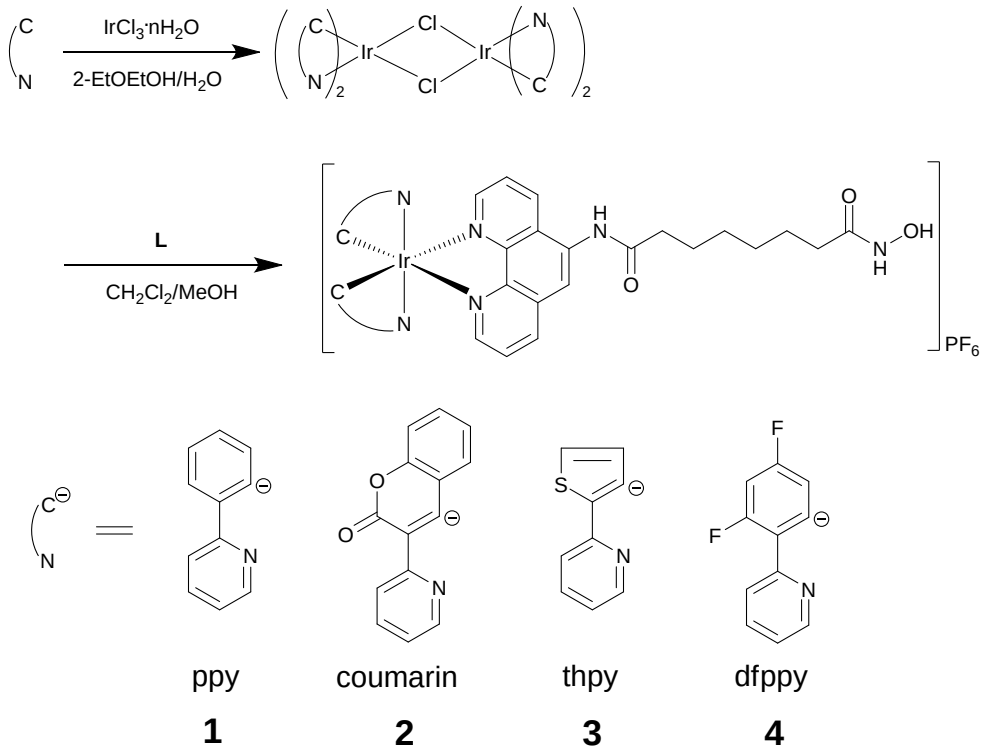
### Materials

Iridium chloride hydrate (Alfa Aesar), ppy (Sigma Aldrich), thpy (Sigma Aldrich), dfppy (Sigma Aldrich), cisplatin (Sigma Aldrich), methyl 8-chloro-8-oxooctanoate (Sigma Aldrich), $NH_2OH$ (50% in $H_2O$) (J&K Scientific Ltd.), DMAP (4-dimethylaminopyridine, J&K Scientific Ltd.), $NH_4PF_6$ (Alfa Aesar), DPBF (1,3-diphenylisobenzofuran, Sigma Aldrich), MB (methylene blue, Sigma Aldrich)， DMSO (Sigma Aldrich), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma Aldrich), propidium iodide (Sigma Aldrich), Hoechst 33342 (2'-(4-ethoxyphenyl)-5-(4-methyl-1-piperazinyl)-1H,3'H-2,5'-bibenzimidazole, Sigma Aldrich), JC-1 (Sigma Aldrich), $H_2$DCFDA (2',7'-dichlorodihydrofluorescein diacetate, Sigma Aldrich), PBS (Sigma Aldrich) and SAHA (Sigma Aldrich) were used as received. The fluorescent HDACs activity assay kit was purchased from Millipore (USA). Caspase-3/7 activity assay kit was purchased from Promega (USA). All the tested compounds were dissolved in DMSO just before the experiments, and the concentration of DMSO was 1% (v/v). The solutions of **1**–**4** in PBS were proved to be stable for at least 48 h at room temperature as monitored by UV/Vis spectroscopy and Electrospray mass spectra (ESI-MS). NMR spectra were recorded on a Bruker Avance 400 spectrometer (Germany). Shifts were referenced relative to the internal solvent signals. ESI-MS were recorded on a Thermo Finnigan LCQ DECA XP spectrometer (USA). The quoted m/z values represented the major peaks in the isotopic distribution. Microanalysis (C, H, and N) was carried out using an Elemental Vario EL CHNS analyzer (Germany). UV/Vis spectra were recorded on a Varian Cary 300 spectrophotometer (USA). Emission measurements were conducted on an FLS 920 combined fluorescence lifetime and steady state spectrometer (Japan). Quantum yields of luminescence at room temperature were calculated according to literature procedures using $[Ru(bpy)_3](PF_6)_2$ as the reference.[1]

**Synthetic protocols and characterizations**




The ligand coumarin[2] and **L**[3], the cyclometalated Ir(III) chloro-bridged dimers $[Ir(ppy)_2Cl]_2$,[4] $[Ir(coumarin)_2Cl]_2$,[5] $[Ir(thpy)_2Cl]_2$,[6] and $[Ir(dfppy)_2Cl]_2$[7] were prepared according to literature methods.

**Synthetic procedure of 1−4:** Ligand **L** (0.25 mmol, 2 equiv) and the appropriate iridium(III) chloro-bridged dimer (0.125 mmol, 1 equiv) were suspended in $CH_2Cl_2/CH_3OH$ (2:1, v/v). The reaction mixture was heated to reflux for 4 h under an inert atmosphere of nitrogen in the dark. After being cooled to room temperature, a 6-fold excess of $NH_4PF_6$ was added and the mixture was stirred for another 1 h. The desired product was obtained by evaporation under vacuum, and purified by column chromatography on silica gel using $CH_2Cl_2$/MeOH/$NH_3H_2O$ (10:1:0.1, v/v/v) as the eluent. Then, the iridium complexes were further recrystallized from $CH_2Cl_2$/diethyl ether.

**[Ir(ppy)$_2$(L)](PF$_6$) (1):** Complex **1** was synthesized according to the synthetic procedure described above, giving the product as a bright yellow powder. Yield: 0.200 g (79%). $^1$H NMR (400 MHz, [D$_6$]DMSO): $\delta$ 10.63 (s, 1H; hydroxamic OH), 9.09 (d, $J$ = 8.4 Hz, 1H; CH-13), 8.82 (d, $J$ = 7.6 Hz, 1H; CH-10), 8.64 (s, 1H; amide NH), 8.22 (dd, $J$ = 12.4, 6.6 Hz, 3H; CH-8 and CH-21×2), 8.07 (d, $J$ = 4.9 Hz, 2H; CH-17×2), 8.03–7.75 (m, 6H; CH-11, CH-16×2, CH-19×2 and hydroxamic NH ), 7.44 (t, $J$ = 6.5 Hz, 2H; CH-9 and CH-12), 6.99 (m, 7H; CH-7, CH-14×2, CH-15×2 and CH-18×2), 6.26 (t, $J$ = 7.0 Hz, 2H; CH-20×2), 2.61 (t, $J$ = 7.3 Hz, 2H; CH$_2$-6), 1.95 (t, $J$ = 7.3 Hz, 2H; CH$_2$-1), 1.74–1.62 (m, 2H; CH$_2$-5), 1.57–1.46 (m, 2H; CH$_2$-2), 1.41–1.27 (m, 4H; CH$_2$-3,4). ESI-MS (CH$_2$Cl$_2$): *m/z* 867.3 [M−PF$_6$]$^+$. Elemental analysis: calcd (%) for C$_{42}$H$_{38}$F$_6$N$_6$O$_3$PIr•2H$_2$O: C, 48.13; H, 4.04; N, 8.02; found: C, 47.91; H, 4.16; N, 7.96.

**[Ir(coumarin)$_2$(L)](PF$_6$) (2):** Complex **2** was synthesized according to the synthetic procedure described above, giving the product as a bright yellow powder. Yield: 0.232 g (81%). $^1$H NMR (400 MHz, [D$_6$]DMSO): $\delta$ 10.11 (s, 1H; hydroxamic OH), 9.09 (d, $J$ = 3.7 Hz, 1H; CH-13), 9.01 (d, $J$ = 3.4 Hz, 1H; CH-10), 8.92 (d, $J$ = 8.3 Hz, 2H; CH-21×2), 8.59 (d, $J$ = 8.3 Hz, 1H; CH-8), 8.45 (dd, $J$ = 12.7, 6.8 Hz, 2H; CH-14×2), 8.28–7.94 (m, 5H; amide NH, CH-11, CH-16×2 and hydroxamic NH), 7.76 (m, 2H; CH-18×2), 7.43–7.16 (m, 7H; CH-7, CH-9, CH-12, CH-15×2 and CH-17×2), 6.67 (t, $J$ = 7.5 Hz, 2H; CH-19×2), 6.02–5.95 (m, 2H; CH-20×2), 2.07 (t, $J$ = 7.3 Hz, 2H; CH$_2$-6), 1.55 (t, $J$ = 7.2 Hz, 2H; CH$_2$-1), 1.22 (m, 8H; CH$_2$-5, 2, 3, 4). ESI-MS (CH$_2$Cl$_2$): *m/z* 1003.3 [M−PF$_6$]$^+$. Elemental analysis: calcd (%) for C$_{48}$H$_{38}$F$_6$N$_6$O$_7$PIr: C, 50.22; H, 3.34; N, 7.32; found: C, 50.13; H, 3.38; N, 7.26.

**[Ir(thpy)$_2$(L)](PF$_6$) (3):** Complex **3** was synthesized according to the synthetic procedure described above, giving the product as a brown powder. Yield: 0.205 g (80%). $^1$H NMR (400 MHz, [D$_6$]DMSO): $\delta$ 10.40 (s, 1H; hydroxamic OH), 10.32 (s, 1H; amide NH), 8.99 (d, $J$ = 8.5 Hz, 1H;

CH-13), 8.83 (d, *J* = 7.1 Hz, 1H; CH-10), 8.64 (s, 2H; CH-7 and hydroxamic NH), 8.19–7.97 (m, 4H; CH-8, CH-11 and CH-19×2), 7.80–7.65 (m, 6H; CH-9, CH-12, CH-14×2 and CH-16×2), 7.37 (t, *J* = 7.4 Hz, 2H; CH-17×2), 6.79 (dd, *J* = 9.9, 4.6 Hz, 2H; CH-15×2), 6.24 (t, *J* = 5.0 Hz, 2H; CH-18×2), 2.58 (t, *J* = 7.3 Hz, 2H; $CH_2$-6), 1.95 (t, *J* = 7.2 Hz, 2H; $CH_2$-1), 1.74 – 1.64 (m, 2H; $CH_2$-5), 1.55–1.48 (m, 2H; $CH_2$-2), 1.41–1.29 (m, 4H; $CH_2$-3,4). ESI-MS ($CH_2Cl_2$): *m/z* 879.3 $[M-PF_6]^+$. Elemental analysis: calcd (%) for $C_{38}H_{34}F_6N_6O_3PS_2Ir$: C, 44.57; H, 3.35; N, 8.21; found: C, 44.74; H, 3.39; N, 8.10.

**[Ir(dfppy)$_2$(L)](PF$_6$) (4):** Complex **4** was synthesized according to the synthetic procedure described above, giving the product as a light yellow powder. Yield: 0.230 g (85%). $^1$H NMR (400 MHz, [$D_6$]DMSO): $\delta$ 10.43 (s, 1H; hydroxamic OH), 10.32 (s, 1H; amide NH), 9.02 (d, *J* = 8.4 Hz, 1H; CH-13), 8.87 (d, *J* = 7.5 Hz, 1H; CH-10), 8.66 (d, *J* = 4.9 Hz, 2H; CH-19×2), 8.28 (m, 3H; CH-9, CH-12 and hydroxamic NH), 8.17 (d, *J* = 4.1 Hz, 1H; CH-8), 8.06 (dd, *J* = 8.5, 5.1 Hz, 1H; CH-11), 7.99–7.92 (m, 3H; CH-7 and CH-16×2), 7.51 (t, *J* = 5.5 Hz, 2H; CH-17×2), 7.08–6.96 (m, 4H; CH-14×2 and CH-15×2), 5.72–5.65 (m, 2H; CH-18×2), 2.58 (t, *J* = 7.3 Hz, 2H; $CH_2$-6), 1.95 (t, *J* = 7.3 Hz, 2H; $CH_2$-1), 1.74–1.64 (m, 2H; $CH_2$-5), 1.56–1.46 (m, 2H; $CH_2$-2), 1.41–1.27 (m, 4H; $CH_2$-3,4). ESI-MS ($CH_2Cl_2$): *m/z* 939.3 $[M-PF_6]^+$. Elemental analysis: calcd (%) for $C_{42}H_{34}F_{10}N_6O_3PIr\cdot 1H_2O$: C, 45.78; H, 3.29; N, 7.63; found: C, 45.79; H, 3.35; N, 7.66.

## Quantification of singlet oxygen generation

The $^1O_2$ quantum yields ($\Phi_\Delta$) of Ir(III) complexes **1**−**4** were detected according to the literature procedure with slight modifications.[8] The DMSO solutions containing **1**−**4** and DPBF (50 μM) were aerated for 10 min, then photoirradiated at 365 nm (20 mW·cm$^{-2}$) or 425 nm (40 mW·cm$^{-2}$). The absorbance of DPBF at 418 nm was recorded every 2 s. MB was used as the reference ($\Phi_\Delta$ = 0.52).

The absorbance at 365 nm or 425 nm of Ir(III) complexes and MB was kept at 0.15. The $^1O_2$ quantum yields of Ir complexes were calculated according the following equation.

$$\Phi_{\Delta}^{Ir(III)} = \Phi_{\Delta}^{MB} \times (s^{Ir(III)} \times F^{MB})/(s^{MB} \times F^{Ir(III)})$$

where s is the slope of a linear fit of the change of absorbance at 418 nm against the irradiation time (s) and F is the absorption correction factor, which is given by $F = 1 - 10^{-OD}$ (OD is the optical density at the irradiation wavelength).

**Cell lines and culture conditions**

HeLa, A549, A549R and LO2 cells were obtained from Experimental Animal Center of Sun Yat-sen University (Guangzhou, China). The Cells were maintained in DMEM (Dulbecco's modified Eagle's medium, Gibco BRL) or RPMI 1640 (Roswell Park Memorial Institute 1640, Gibco BRL) medium containing 10% FBS (fetal bovine serum, Gibco BRL), 100 μg/mL streptomycin, and 100 U/mL penicillin (Gibco BRL). The cells were cultured in a humidified incubator at 37 °C under 5% $CO_2$. A549R cells were cultured in a medium containing increasing concentrations of cisplatin to maintain the resistance. In each experiment, cells treated with vehicle only DMSO (1%, v/v) were used as the reference group.

**Cellular uptake studies**

HeLa cells were seeded into 35 mm dishes (Corning) for confocal microscopy. The cells were incubated with **1**−**4** for 5 h and washed twice with cold PBS, and then viewed immediately under a confocal laser-scanning microscope (LSM 710, Carl Zeiss, Göttingen, Germany) by excitation at 405 nm. The emission filters used are 600 ± 20 nm, 530 ± 20 nm, 630 ± 20 nm and 530 ± 20 nm for **1**, **2**, **3** and **4**, respectively.

**Cytotoxicity Assay**

Growth inhibition effect of the tested compounds was determined by MTT assay as previously described.[3]

**Photocytotoxicity assay**

The cells were seeded in 96-well tissue culture plates (Corning) at a density of 10, 000 cells per well. After incubation for 24 h, the cells were treated with different concentration of the tested compounds in DMEM solutions for 12 h at 37 °C and then irradiated at 365 nm (20 $mW \cdot cm^{-2}$) or 425 nm (40 $mW \cdot cm^{-2}$) for 3 min. After illumination, the cells were incubated at 37 °C under 5% $CO_2$ atmosphere in the dark for 32 h. 20 μL MTT (5 mg/mL) was added to each well. The cells were incubated for another 4 h. At the end of the incubation period, the medium was removed and the formazan product was dissolved in DMSO (150 μL per well). Cell viability was evaluated by measurement of the absorbance at 595 nm (Infinite F200, Tecan, Switzerland). $IC_{50}$ values were calculated by non-linear regression analysis.

**HDACs enzymatic assay**

The ability of **1**−**4**, SAHA and **L** to inhibit HDACs was investigated in triplicate using a fluorescent HDACs activity assay kit (Millipore, USA) according to the manufacturer's instructions. Briefly, HeLa nuclear extracts, used as a source of HDACs, were incubated with the tested compounds. HDACs reaction was initiated by addition of the Fluor-de-Lys substrate. Samples were incubated for 30 min at 37 °C, followed by the addition of the developer solution to stop the reaction, and the mixture was incubated for another 10 min at 25 °C. The fluorescence was detected on a microplate reader (Infinite M200 Pro, Tecan, Switzerland) at an excitation wavelength of 360 nm and an emission wavelength of 460 nm. $IC_{50}$ values (the drug concentration required to inhibit HDACs activity by 50%) were calculated according to a regression analysis of the concentration-dependent inhibition data.

**Western blot analysis**

HeLa cells were seeded into 60 mm tissue culture dishes (Corning) and incubated for 24 h, and then treated with complex **1** for 24 h. Photoirradition (365 nm, 20 mW·cm$^{-2}$, 3 min) was performed after the cells were treated with complex **1** for 12 h. Cell lysates were prepared in radioimmunoprecipitation assay (RIPA) buffer supplemented with inhibitors of proteases (Roche Diagnostics GmbH, Germany) and inhibitor of phosphatases sodium orthovanadate (Sigma Aldrich). Protein concentrations were quantified by a BCA protein assay reagent kit (Novagen Inc, USA). Equal amounts of protein were loaded and separated on SDS-polyacrylamide gel electrophoresis and then transferred onto polyvinylidene difluoride membranes (Millipore, USA). The membranes were blocked with 5% non-fat dry milk in TBST (20 mM Tris/HCl (pH 7.2)/150 mM NaCl/0.05% Tween-20) at room temperature for 2 h, and subsequently incubated with primary antibodies specific to β-actin (Cell Signaling Technology, USA), anti-acetyl histone H3 (Millipore, USA) and PARP (Cell Signaling Technology, USA) in TBST containing 5% nonfat milk at 4 °C. After washing with TBST for three times the membranes were incubated with HRP-conjugated secondary antibody for 1 h at room temperature. The signals were detected using the enhanced chemiluminescence (ECL) kit (Amersham Inc, USA). Images were captured on FluorChem M (ProteinSimple, Santa Clara, CA).

**Detection of apoptosis**

**Hoechst staining**. HeLa cells were seeded into 35 mm dishes (Corning) and incubated for 24 h and then treated with complex **1** for 24 h. Photoirradition (365 nm, 20 mW·cm$^{-2}$, 3 min) was performed after the cells were treated with complex **1** for 12 h. The cells were then washed once with cold PBS and fixed with 4% paraformaldehyde at room temperature for 10 min. After washed with cold PBS, cells were labelled with Hoechst 33342 (5 μg/mL in PBS) for 5 min. The cells were analyzed immediately with a confocal laser-scanning microscope (LSM 710, Carl Zeiss, Germany).

**Annexin V-FITC staining**.

**Flow cytometry**. HeLa cells were cultured in 6-well tissue culture plates for 24 h and then treated with complex **1** (10 μM and 20 μM) for 24 h. PDT treatment was carried out as described in Hoechst Staining. The cells were harvested and stained using an annexin V-FITC apoptosis detection kit (Sigma Aldrich) according to the manufacturer's instructions. Data were collected by a flow cytometer (FACSCaliburTM, Becton Dickinson, Franklin Lakes, NJ, USA) and analyzed with FlowJo 7.6 software (Tree Star, OR, USA).

**Confocal microscopy**. HeLa cells were seeded into 35 mm dishes (Corning) and incubated for 24 h and then treated with complex **1** (10 μM) for 24 h. PDT treatment was carried out as described in Hoechst Staining. The cells were washed twice with ice-cold PBS, and stained with FITC-labelled annexin at 37 °C for 15 min in the dark, then visualised immediately by confocal microscopy (LSM 710, Carl Zeiss, Göttingen, Germany).

## Caspase-3/7 activity assay

Caspase-3/7 activity was measured using the Caspase-Glo® Assay kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. Briefly, cells were cultured in 48-well plates and treated with different concentration of complex **1** for 3 h, and then irradiated at 365 nm (20 $mW \cdot cm^{-2}$) for 3 min. After illumination, the cells were incubated at 37 °C under 5% $CO_2$ atmosphere in the dark for another 9 h. After treatment with complex **1** for 12 h, 50 μL cell lysate was added to each well, followed by the addition of 50 μL Caspase-Glo® 3/7 reagent. The mixture was incubated at room temperature for 30 min and then the luminescence was measured using a microplate reader (Infinite M200 Pro, Tecan, Männedorf, Switzerland).

## Cell cycle analysis

HeLa cells were cultured in 6-well tissue culture plates (Corning) and incubated with complex **1** for 24 h. Photoirradition (365 nm, 20 $mW \cdot cm^{-2}$, 3 min) was performed after the cells were treated with

complex **1** for 12 h. After treatment, the cells were collected and fixed in 2 mL of 70% ethanol. After storage at −20 °C overnight, cells were centrifuged and washed twice with cold PBS, and then resuspended in 500 μL PBS containing PI (50 μg/mL) and DNase-free RNase (100 μg/mL). Data were collected by a flow cytometer (FACSCaliburTM, Becton Dickinson, Franklin Lakes, NJ, USA) and analyzed by ModFit LT 2.0 software (Variety Software House, Inc., Topsham, ME, USA).

**Measurement of intracellular ROS**

**Flow cytometry**. HeLa cells were seeded into 6-well tissue culture plates (Corning) and incubated for 24 h and then treated with complex **1** (20 μM and 40 μM) for 6 h. Photoirradition (365 nm, 20 $mW \cdot cm^{-2}$, 3 min) was performed after the cells were treated with complex **1** for 3 h. After the treatment, the cells were harvested and incubated with 10 μM $H_2$DCFDA in serum-free DMEM for 15 min at 37 °C in the dark. After washed twice with serum-free DMEM, the fluorescence intensity of cells was measured immediately by flow cytometry (FACSCaliburTM, Becton Dickinson, Franklin Lakes, NJ, USA) with excitation at 488 nm and emission at 530 nm. Green mean fluorescence intensities were analyzed using FlowJo 7.6 software (Tree Star, OR, USA).

**Confocal microscopy**. HeLa cells were seeded into 35 mm dishes (Corning) for confocal microscopy and incubated for 24 h. After the treatment described above, the cells were stained with 10 μM $H_2$DCFDA in serum-free DMEM. After washed twice with serum-free DMEM, cells were examined under a confocal microscope (LSM 710, Carl Zeiss, Göttingen, Germany) with excitation at 488 nm and emission at 530 nm.

**Analysis of MMP**

**Flow cytometry**. HeLa cells were cultured in 6-well tissue culture plates (Corning) for 24 h and then treated with complex **1** (20 μM and 40 μM) for 6 h. Photoirradition (365 nm, 20 $mW \cdot cm^{-2}$, 3 min) was performed after the cells were treated with complex **1** for 3 h. After the treatment, the cells were

collected and resuspended at $1 \times 10^6$/mL in pre-warmed PBS containing 5 μg/mL JC-1 and incubated for 20 min at 37 °C. Subsequently, the cells were washed twice with PBS and analyzed immediately in a flow cytometer (FACSCaliburTM, Becton Dickinson, Franklin Lakes, NJ, USA). Red and green mean fluorescence intensities were analyzed using FlowJo 7.6 software (Tree Star, OR, USA). 10, 000 events were acquired for each sample.

**Confocal microscopy**. HeLa cells were seeded into 35 mm dishes (Corning) for confocal microscopy and incubated for 24 h. After the treatment described above, cells were incubated for 20 min in PBS containing JC-1 (5 μg/mL). After washed twice with PBS, the stained cells were visualized under a confocal laser scanning microscope (LSM 710, Carl Zeiss, Göttingen, Germany). JC-1 fluorescence was measured with single excitation (488 nm) and dual emission (shift from green 530 nm to red 590 nm).

## Statistical analysis

All biological experiments were performed at least twice with triplicates in each experiment. Representative results were depicted in this report and data were presented as means ± standard deviations.

## Supporting Figures and Tables

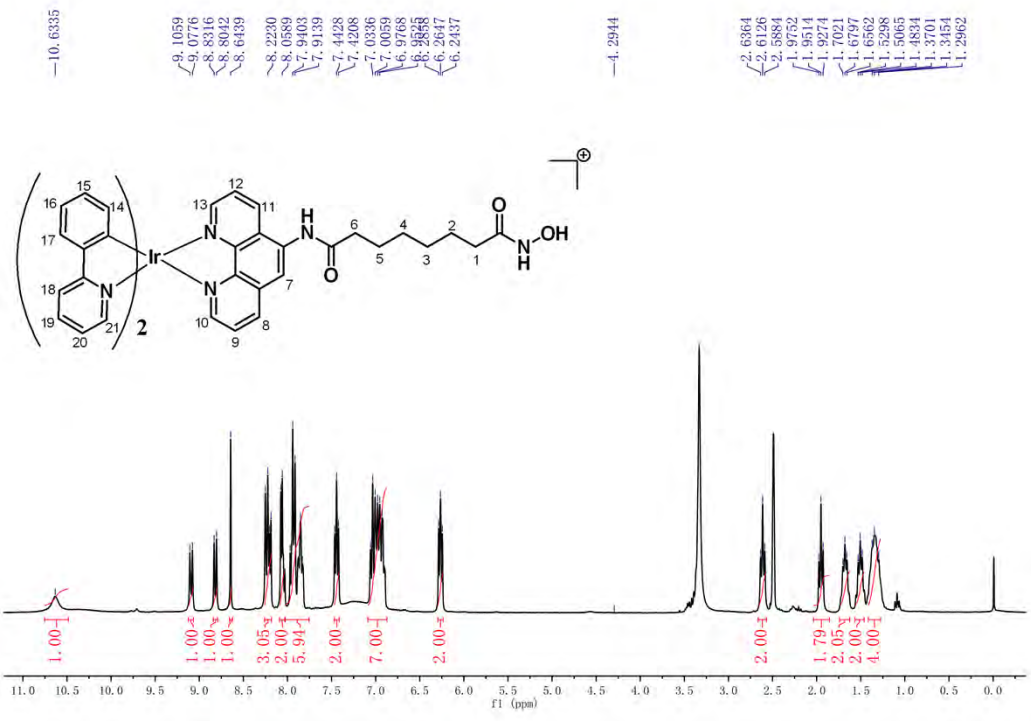


**Fig. S1** $^{1}$H NMR spectrum of **1**.

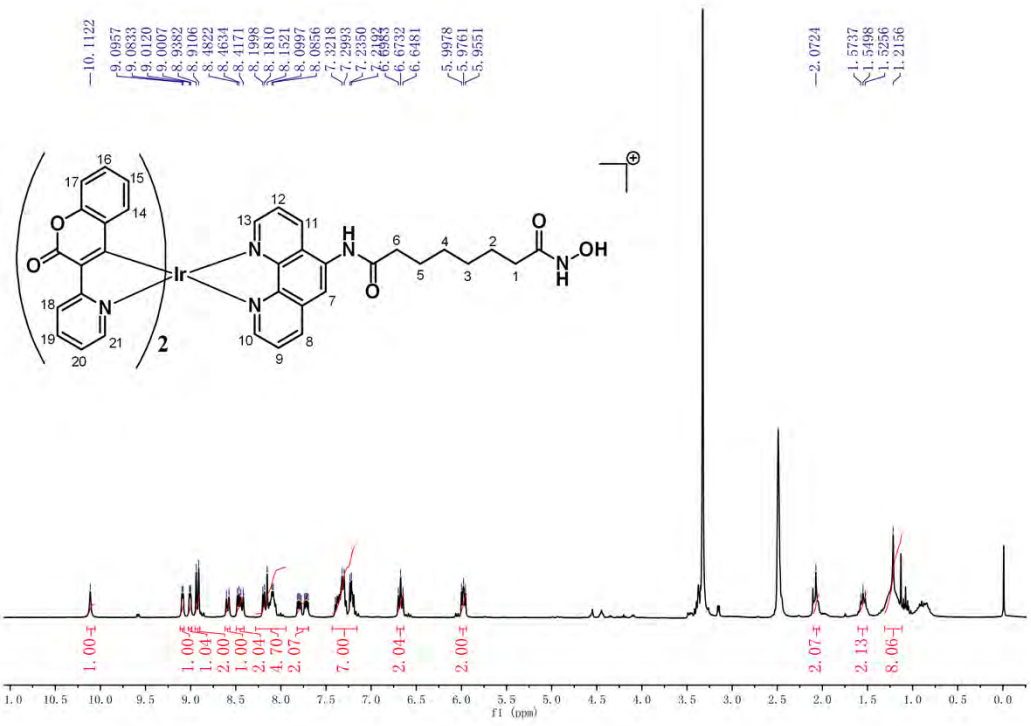


**Fig. S2** $^{1}$H NMR spectrum of **2**.

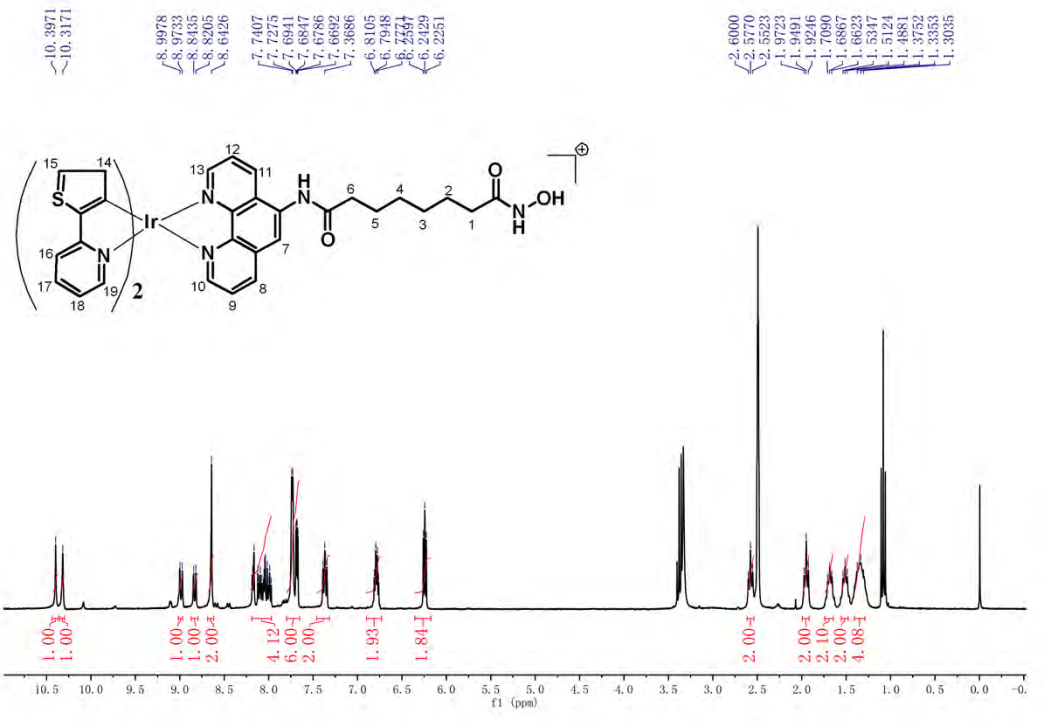


**Fig. S3** $^{1}$H NMR spectrum of **3**.

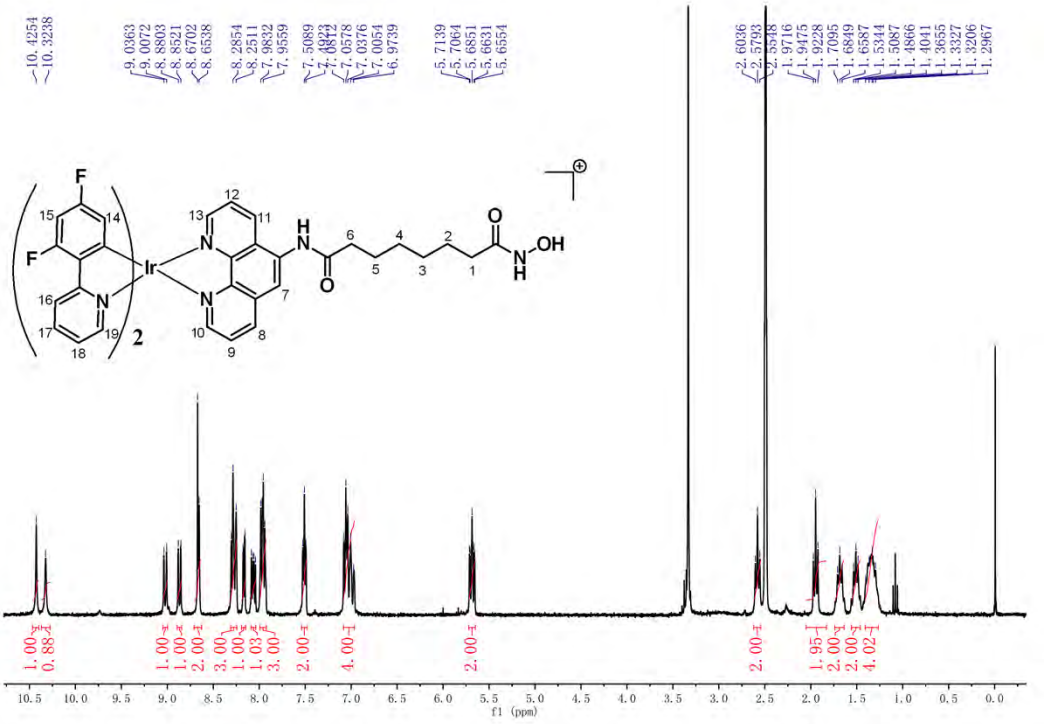


**Fig. S4** $^{1}$H NMR spectrum of **4**.

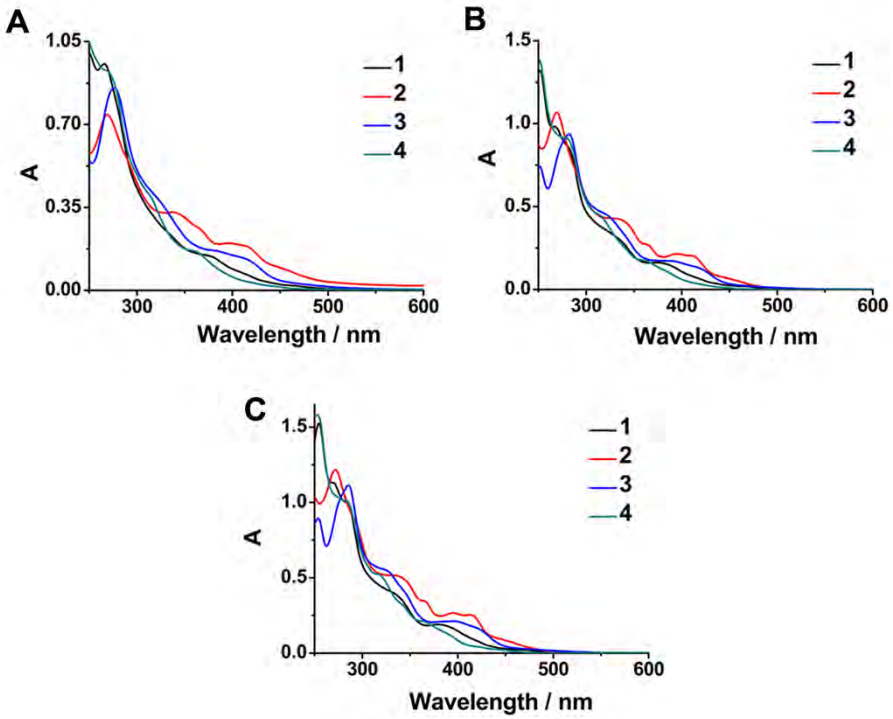



**Fig. S5** UV/Vis spectra of complexes **1**–**4** ($2 \times 10^{-5}$ M) measured in (A) PBS, (B) $CH_3CN$ and (C) $CH_2Cl_2$ at 298 K.

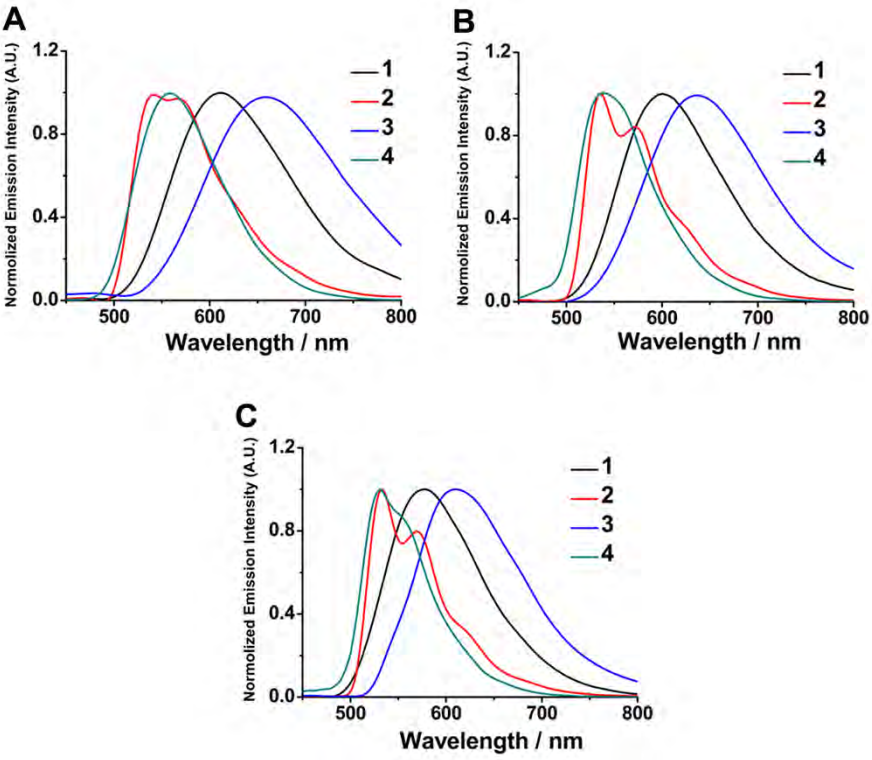



**Fig. S6** Emission spectra of complexes **1**–**4** ($2 \times 10^{-5}$ M) measured in (A) PBS, (B) $CH_3CN$ and (C) $CH_2Cl_2$ at 298 K.

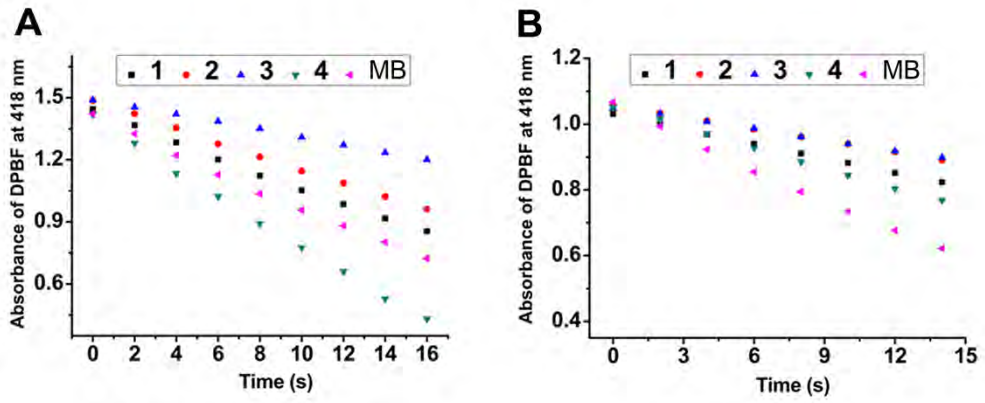



**Fig. S7** Photooxidation of DPBF by **1**–**4** under light irradiation. Changes in the absorption spectra of DPBF at 418 nm upon irradiation at (A) 365 nm and (B) 425 nm in the presence of **1**–**4** in aerated DMSO were monitored, and MB was used as a standard.

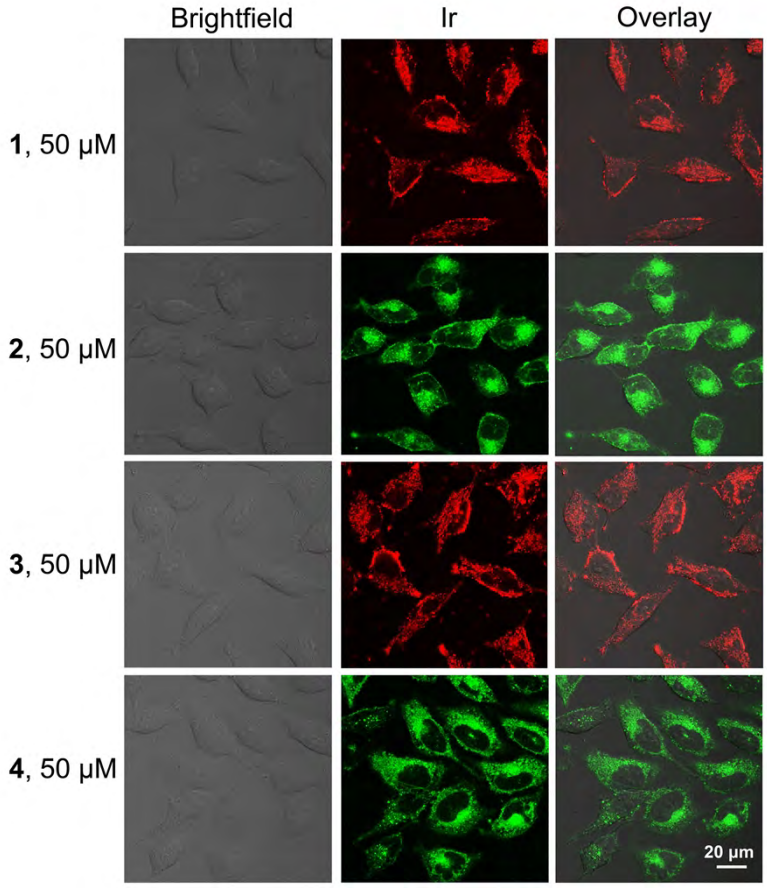



**Fig. S8** Intracellular localization and uptake of complexes **1**–**4** measured by confocal microscopy after HeLa cells were incubated with Ir(III) (50 μM) at 37 °C for 5 h. Excitation wavelength: 405 nm; Emission filters: 600 ± 20 nm (**1**), 530 ± 20 nm (**2**), 630 ± 20 nm (**3**), and 530 ± 20 nm (**4**).

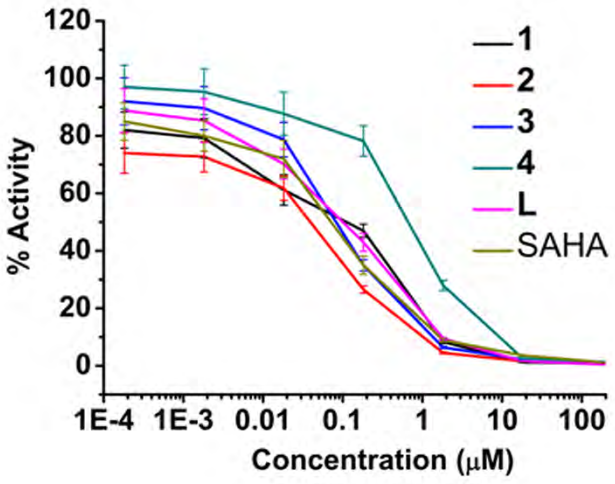



**Fig. S9** Inhibition of HDACs activity by complexes **1**–**4**, **L** and SAHA. Data were expressed as the mean of three independent experiments.

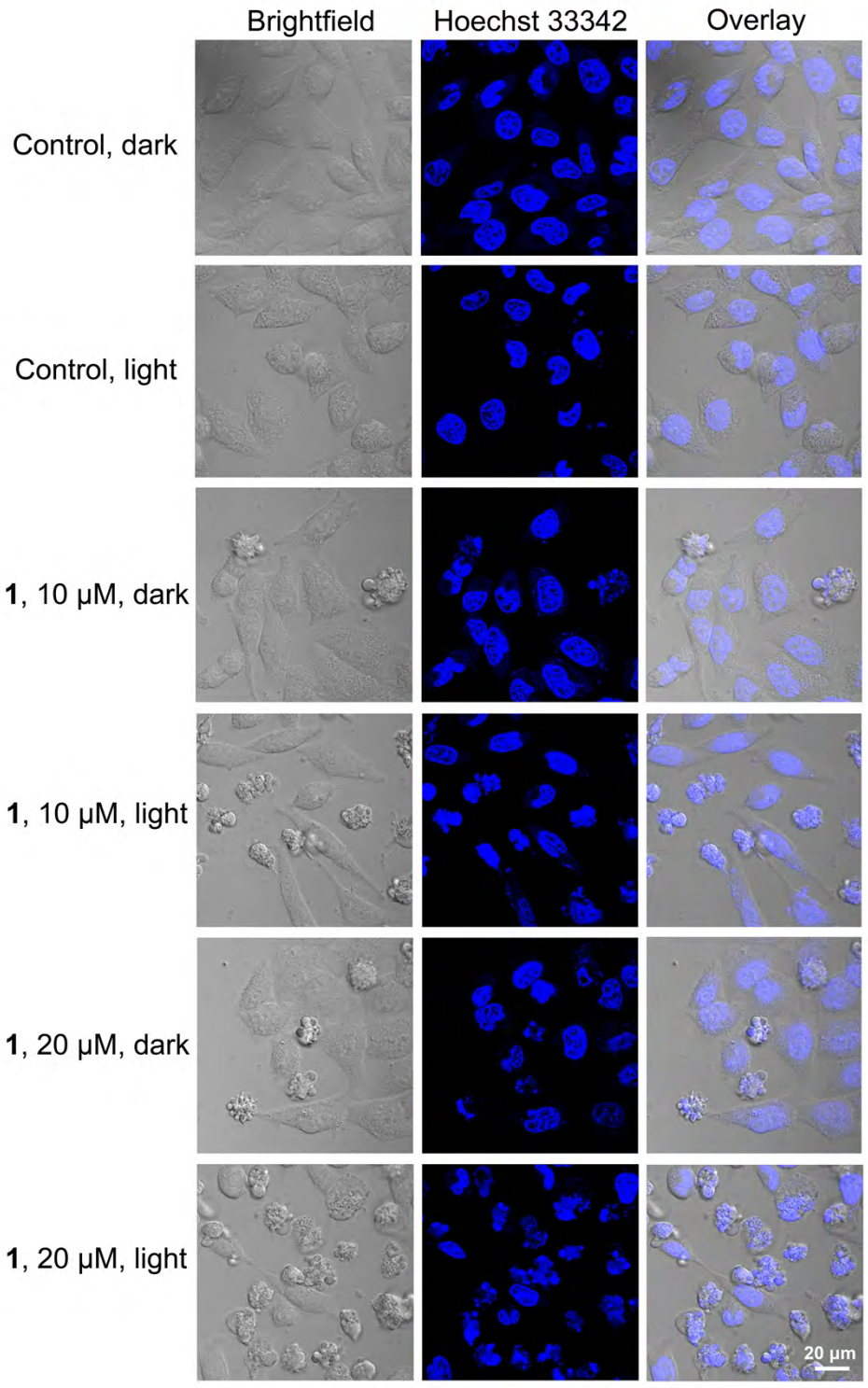



**Fig. S10** Characterization of apoptosis induced by complex **1** using Hoechst 33342 staining. **Dark**: Cells were incubated with complex **1** for 24 h. **Light**: Cells were incubated with complex **1** for 12 h in the dark, then irradiated at 365 nm (20 $mW \cdot cm^{-2}$, 3 min), and further incubated for 12 h in the dark.

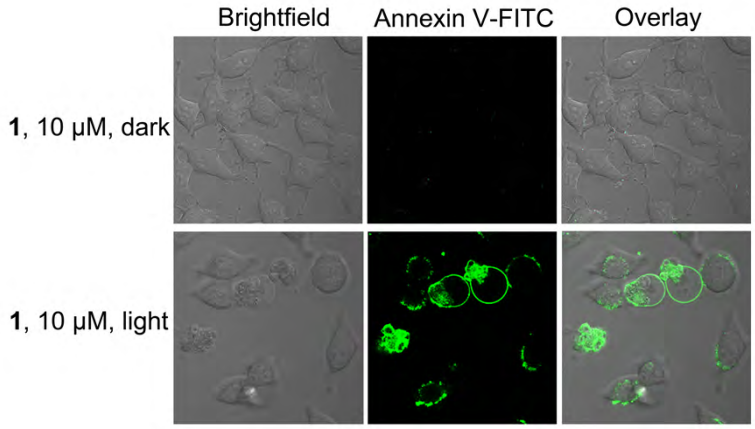



**Fig. S11** Representative confocal images of HeLa cells stained with annexin V-FITC (excitation at 488 nm and emission at 500 ± 15 nm). **Top**: cells were treated with **1** (10 μM) in the dark for 24 h; **Bottom**: cells were treated with **1** (10 μM) in the dark for 12 h, then irradiated at 365 nm (20 $mW \cdot cm^{-2}$) for 3 min, and further incubated for 12 h in the dark.

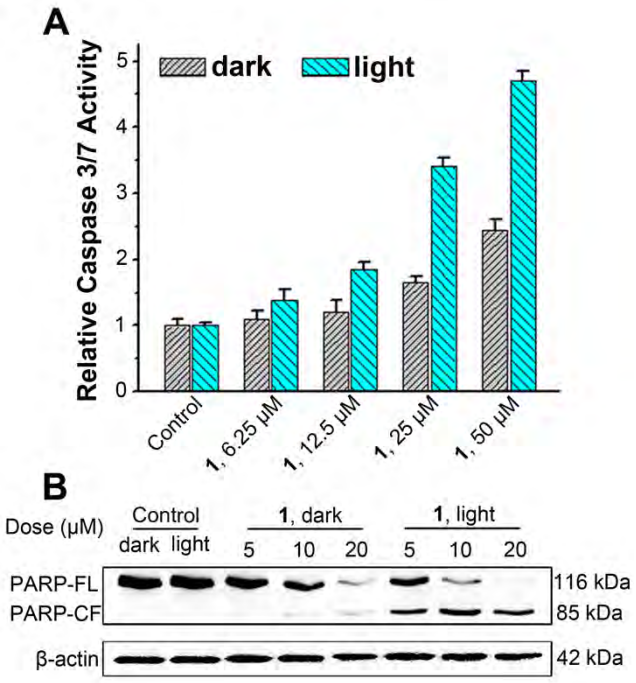



**Fig. S12** (A) Detection of caspase-3/7 activity after complex **1** treatment for 12 h at the indicated concentrations. **Dark**: Cells were incubated with complex **1** at the indicated concentrations for 12 h. **Light**: Cells were incubated with complex **1** at the indicated concentrations for 3 h in the dark and then irradiated with light at 365 nm (20 $mW \cdot cm^{-2}$, 3 min) and further incubated for 9 h in the dark. (B) Effect of complex **1** on PARP cleavage. HeLa cells were treated with vehicle or **1** at different concentrations for 24 h. For the light-treated samples, PDT treatment was performed after the cells were incubated with complex **1** for 12 h.

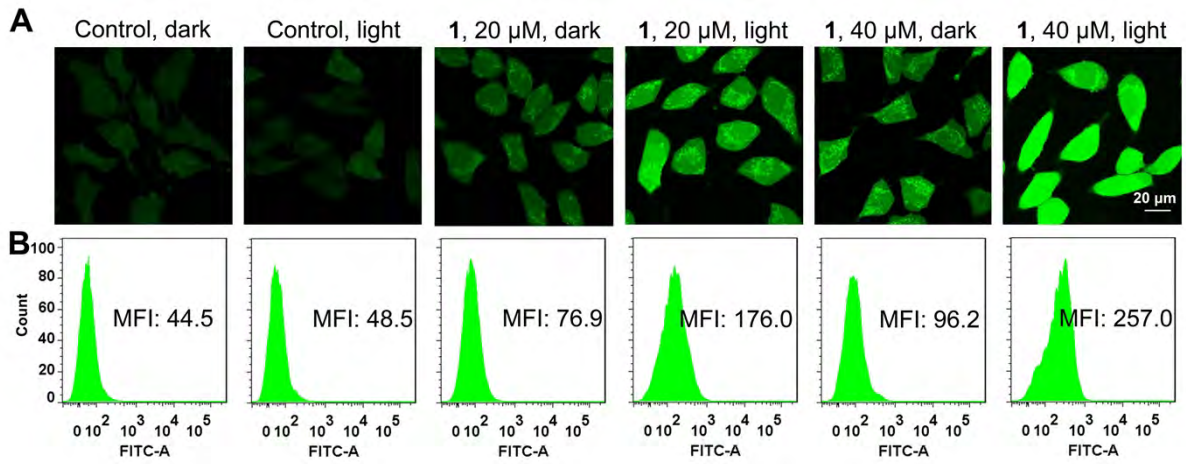



**Fig. S13** Effects of **1** on ROS generation. HeLa cells incubated with **1** at 37 °C for 6 h, after which they were labeled with $H_2$DCFDA and analyzed by (A) confocal microscopy and (B) flow cytometry. **Dark**: Cells were incubated with **1** for 6 h. **Light**: Cells were incubated with **1** for 3 h in the dark, then irradiated at 365 nm (20 mW·cm$^{-2}$, 3 min), and further incubated for 3 h in the dark. MFI: mean fluorescence intensity.

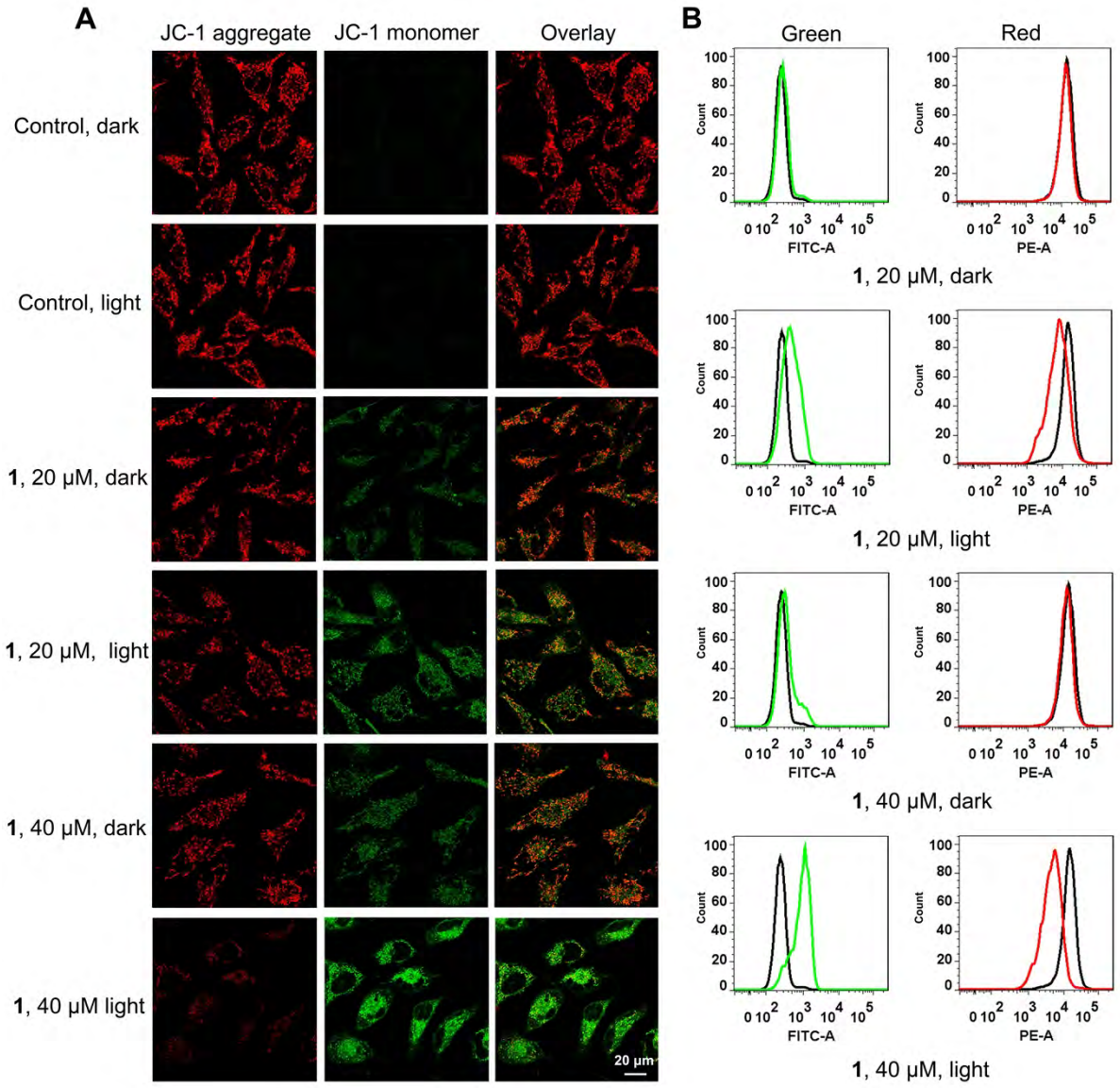



**Fig. S14** Effect of complex **1** on mitochondrial integrity. (A) Fluorescence imaging of JC-1 labeled cells taken by confocal microscopy. (B) Representative histogram of flow cytometry analysis of JC-1 stained HeLa cells. Black curve: cells treated with vehicle (1% DMSO); red and green curves: cells treated with **1**. **Dark**: Cells were incubated with **1** for 6 h. **Light**: Cells were incubated with **1** for 3 h in the dark, then irradiated with light at 365 nm (20 mW·cm$^{-2}$, 3 min), and further incubated for 3 h in the dark.

**Table S1** Photophysical data of complexes **1–4**[a]

| Compounds | Medium | $\lambda_{abs,\ max}$ (nm) | $\lambda_{em,\ max}$ (nm) | $\Phi_{em}$ [b] | $\tau_1$ [c] (ns) | $B_1$ (%) | $\tau_2$ [c] (ns) | $B_2$ (%) | $\tau_3$ [c] (ns) | $B_3$ (%) | $\tau_{av}$ [d] (ns) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | PBS | 378 | 610 | 0.021 | 36.26 | 3.21 | 69.40 | 96.79 | - | - | 68.83 |
| | $CH_3CN$ | 387 | 600 | 0.10 | 41.12 | 4.36 | 73.83 | 95.64 | - | - | 73.02 |
| | $CH_2Cl_2$ | 391 | 578 | 0.14 | 154.25 | 5.90 | 221.85 | 84.10 | - | - | 218.70 |
| **2** | PBS | 418 | 537 | 0.0068 | 7.91 | 9.91 | 25.65 | 52.60 | 172.34 | 37.48 | 145.61 |
| | $CH_3CN$ | 414 | 535 | 0.042 | 26.25 | 4.65 | 123.97 | 95.35 | - | - | 122.97 |
| | $CH_2Cl_2$ | 415 | 533 | 0.051 | 24.45 | 2.17 | 302.94 | 97.83 | - | - | 302.44 |
| **3** | PBS | 418 | 658 | 0.0016 | 45.23 | 1.33 | 100.69 | 98.67 | - | - | 100.35 |
| | $CH_3CN$ | 417 | 633 | 0.032 | 57.70 | 1.68 | 213.36 | 98.32 | - | - | 212.64 |
| | $CH_2Cl_2$ | 421 | 610 | 0.046 | 124.89 | 15.76 | 205.50 | 84.24 | - | - | 197.26 |
| **4** | PBS | 362 | 558 | 0.061 | 189.65 | 11.23 | 396.58 | 88.77 | - | - | 384.78 |
| | $CH_3CN$ | 363 | 532 | 0.017 | 201.78 | 13.05 | 450.48 | 86.95 | - | - | 434.82 |
| | $CH_2Cl_2$ | 367 | 531 | 0.028 | 201.45 | 1.55 | 1148.99 | 98.45 | - | - | 1146.38 |

[a] All emission decays were obtained on freshly prepared samples placed in quartz cuvettes. Samples were $2 \times 10^{-5}$ M in concentration. [b] Solutions of $[Ru(bpy)_3](PF_6)_2$ were used as the standard, PBS ($\Phi_{em}$ = 0.042)[9], $CH_3CN$ ($\Phi_{em}$ = 0.062)[10] and $CH_2Cl_2$ ($\Phi_{em}$ = 0.059)[11]. [c] Decay curves of compounds were recorded by an Edinburgh FLS 920 Spectrometer. All curves were fitted into a two exponential formula $F(t) = A + B_1 \exp(-t/\tau_1) + B_2 \exp(-t/\tau_2) + B_3 \exp(-t/\tau_3)$. [d] $\tau_{ay} = \frac{B_1\tau_1^2 + B_2\tau_2^2 + B_3\tau_3^2}{B_1\tau_1 + B_2\tau_2 + B_3\tau_3}$

**Table S2** The $^1O_2$ quantum yields ($\Phi_\Delta$) of **1–4** in aerated DMSO

| Compounds | $\Phi_\Delta$ [a] | |
|---|---|---|
| | 365 nm | 425 nm |
| **1** | 0.45 | 0.24 |
| **2** | 0.38 | 0.17 |
| **3** | 0.21 | 0.14 |
| **4** | 0.75 | 0.32 |

[a] MB was used as the reference in the $\Phi_\Delta$ measurements (0.52 in aerated DMSO).

**Table S3** Cytotoxicity ($IC_{50}$/μM) of the compounds in the absence and presence of 365 nm UV light towards A549, A549R and LO2 cell lines[a]

| Compounds | A549 dark[b] (light[c]) | PI[d] | A549R dark (light) | PI | LO2 dark (light) | PI |
|---|---|---|---|---|---|---|
| **1** | > 100 (3.8 ± 0.3) | > 26.3 | > 100 (4.5 ± 0.3) | > 22.2 | > 100 (55.5 ± 4.1) | > 1.8 |
| **2** | > 100 (5.8 ± 0.5) | > 17.2 | > 100 (9.3 ± 0.6) | > 10.8 | > 100 (69.2 ± 3.2) | > 1.4 |
| **3** | > 100 (21.9 ± 1.2) | > 4.6 | > 100 (13.2 ± 0.8) | > 7.6 | 87.1 ± 7.5 (53.9 ± 3.1) | 1.6 |
| **4** | 89.1 ± 5.2 (11.0 ± 0.9) | 8.1 | 49.0 ± 3.2 (4.6 ± 0.1) | 10.7 | 42.7 ± 2.6 (29.1 ± 1.3) | 1.5 |
| **L** | > 100 (> 100) | - | 56.3 ± 4.5 (52.3 ± 3.3) | 1.1 | > 100 (> 100) | - |
| SAHA | 4.3 ± 0.3 (3.9 ± 0.1) | 1.1 | 3.0 ± 0.1 (2.9 ± 0.3) | 1.0 | > 100 (> 100) | - |
| Cisplatin | 13.0 ± 1.0 (11.6 ± 1.0) | 1.1 | 83.5 ± 3.0 (90.0 ± 2.9) | 0.9 | 32.2 ± 2.1 (20.9 ± 1.8) | 1.5 |

[a] $IC_{50}$ values are drug concentrations necessary for 50% inhibition of cell viability. Data are presented as means ± standard deviations obtained in three independent experiments.

[b] Cells were incubated with the indicated compounds in the dark for 48 h.

[c] Cells were incubated with the indicated compounds for 12 h in the dark and then irradiated with light at 365 nm.

[d] PI = Phototoxicity Index, PI is the ratio of the $IC_{50}$ values in dark to those obtained upon light irradiation.

**Table S4** Photocytotoxicity ($IC_{50}$/μM) (λ = 425 nm) of the compounds towards A549, A549R and LO2 cell lines[a]

| Compounds | A549 | PI[b] | A549R | PI | LO2 | PI |
|---|---|---|---|---|---|---|
| **1** | 12.9 ± 1.0 | > 7.8 | 10.1 ± 1.0 | > 9.9 | 50.4 ± 3.7 | > 2.0 |
| **2** | 9.1 ± 0.8 | > 11.0 | 5.6 ± 0.2 | > 17.9 | 65.3 ± 4.2 | > 1.5 |
| **3** | 6.9 ± 0.5 | > 14.5 | 5.3 ± 0.3 | > 18.9 | 54.4 ± 1.7 | 1.6 |
| **4** | 4.4 ± 0.2 | 20.3 | 2.6 ± 0.2 | 18.8 | 25.7 ± 1.2 | 1.7 |
| **L** | > 100 | - | > 100 | - | > 100 | - |
| SAHA | 4.1 ± 0.3 | 1.0 | 2.9 ± 0.2 | 1.0 | > 100 | - |
| Cisplatin | 11.4 ± 1.0 | 1.1 | 82.1 ± 3.2 | 1.0 | 31.3 ± 1.3 | 1.0 |

[a] $IC_{50}$ values are drug concentrations necessary for 50% inhibition of cell viability. Data are presented as means ± standard deviations obtained in at least three independent experiments.

[b] PI = Phototoxicity Index, PI is the ratio of the $IC_{50}$ values in dark to those obtained upon light irradiation.

**Table S5** Effect of **1** in the absence and presence of light on the distribution of HeLa cells in cell cycle populations after 24 h of treatment[a]

| Compounds | sub-G1 | G0/G1 | S | G2/M |
|---|---|---|---|---|
| Control, dark | - | 52.8 ± 2.4 | 33.2 ± 1.5 | 14.0 ± 0.7 |
| **1**, 5 μM, dark | 6.1 ± 0.8 | 52.6 ± 2.6 | 29.4 ± 1.5 | 18.0 ± 0.9 |
| **1**, 10 μM, dark | 12.4 ± 1.0 | 59.7 ± 2.5 | 28.9 ± 1.7 | 11.4 ± 1.1 |
| **1**, 20 μM, dark | 15.9 ± 0.8 | 53.2 ± 2.6 | 32.9 ± 1.7 | 13.9 ± 1.0 |
| Control, light | - | 48.4 ± 2.4 | 36.2 ± 1.5 | 15.4 ± 0.7 |
| **1**, 5 μM, light | 10.3 ± 0.6 | 54.7 ± 2.8 | 29.0 ± 1.5 | 16.3 ± 1.1 |
| **1**, 10 μM, light | 21.4 ± 1.5 | 48.5 ± 1.6 | 34.3 ± 1.0 | 17.2 ± 0.9 |
| **1**, 20 μM, light | 41.5 ± 2.7 | 40.7 ± 1.1 | 41.0 ± 0.8 | 18.3 ± 0.9 |

[a] Data shown are mean values ± standard deviations of three independent experiments for each treatment.

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