Electronic Supporting Information

A Self-Assessed Photosensitizer: Inducing and Dual-Modal Phosphorescence Imaging of Mitochondria Oxidative stress

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Materials and measurements

Iridium chloride hydrate (Alfa Aesar), ppy (Sigma Aldrich), cisplatin (Sigma Aldrich), rapamycin (Sigma Aldrich, USA), NH_4PF_6 (Alfa Aesar), PI (propidium iodide, Sigma Aldrich), DMSO (dimethyl sulfoxide, Sigma Aldrich), 4-Amino-2,2,6,6-tetramethylpiperidine-1-Oxyl free radica (J&K Scientific Ltd.) , 1,10-Phenanthroline-5-carboxylic acid (Sigma Aldrich) , $FeSO_4.7H_2O$ (J&K Scientific Ltd.), H_2O_2 (J&K Scientific Ltd.), HOBT(1-Hydroxy benzotriazole, J&K scientific Ltd.), N,N'-Dicyclohexyl carbodie (DCC, J&K Scientific Ltd.), N,N-Diisopropylethy lamine (DIEA, J&K Scientific Ltd.) DPBF (1,3-diphenylisobenzofuran, Sigma Aldrich), MB (methylene, blue, Sigma Aldrich), DMSO(Sigma Aldrich), JC-1(Sigma Aldrich), H_2DCFDA (2',7'-dichloro dihydrofluorescein diacetate, Sigma Aldrich), Hoechst 33342(Sigma Aldrich), MTT (3-(4,5-dimeth ylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, Sigma Aldrich, USA), NAC (Sigma Aldrich), PMA (Propylene glycol monomethyl ether acetate, Sigma Aldrich), H_2O_2 (J&K Scientific Ltd.), The caspase 3/7 activity kit and ATP Assay were purchased from Promega (USA), Annexin-V/PI assay kit was purchased from Sigma Aldrich (USA).

All of the compounds tested were dissolved in DMSO immediately prior to the experiments, and the final concentration of DMSO was kept at 1% (v/v). NMR spectra were recorded on a Bruker Avance 400 spectrometer. Shifts were referenced relative to the internal solvent signals. Microanalysis (C, H, and N) was carried out using an Elemental Vario EL CHNS analyser (Germany). UV–vis spectra were recorded on a Varian Cary 300 spectrophotometer (USA). Emission spectra were recorded on a Shimadzu RF-5301 PC spectrofluorophotometer. ESI-MS spectra were recorded on a Thermo Finnigan LCQ DECA XP spectrometer (USA). The quoted m/z values represent the major peaks in the isotopic distribution. Conforcal images were captured by Conforcal Telescope (Germany, Carl Zeiss LSM 710), Lifetime was recorded by a DPC-23016 Channel Photon correlator (Becker &Hickl GmbH, UK).

Synthesis and Characterization of Ir(III) complexes

The dimeric iridium(III) precursors ($Ir_2(ppy)_4Cl_2$ and $Ir_2(dfppy)_4Cl_2$ were prepared according to the literature method.[1] Ligand **L** (2 eqv) was obtained from our previous work.[2] A mixture of $[Ir(ppy)_2Cl]_2$ or $[Ir(dbpz)_2Cl]_2$ (1 equiv) and **L** (2 equiv) in CH_2Cl_2/ CH_3OH (2:1, v/v) solution was heated and reflux overnight under N_2 protection in the dark. The solution was cooled to room temperature, 10-fold excess of NH_4PF_6 was added and stirred for 2 h, then the mixture was filtered and filtrate was evaporated to dryness with reduced pressure. The obtained solids were dissolved in CH_2Cl_2 and purified by column chromatography on silica gel eluted with CH_2Cl_2 and

CH_3OH (10:1 v/v), followed by recrystallization from CH_2Cl_2/diethyl ether.

Complex **1**: Light-yellow powder, yield: 0.640 g (70%). High resolution ESI-MS (CH_3OH): m/z 878.85 $[M-PF_6]^+$. Elemental analysis: calcd (%) for $C_{44}H_{41}N_6O_2PF_6Ir$: C, 51.66; H, 4.04; N, 8.21; found: C, 51.26; H, 4.20; N, 8.22; ^1H NMR (400 MHz, $CDCl_3$) δ 9.38 (s, 4H), 8.23 (s, 2H), 7.84 (d, J = 77.0 Hz, 8H), 7.37 (d, J = 44.1 Hz, 2H), 7.05 (d, J = 45.6 Hz, 5H), 6.45 (s, 3H), 1.89 (s, 17H); ^{13}C NMR (126 MHz, d_6-DMSO) δ 167.35, 167.29, 151.95, 151.39, 150.45, 150.14, 149.85, 146.87, 146.80, 144.50, 139.44, 137.65, 131.77, 130.83, 129.45, 128.92, 128.37, 128.30, 127.99, 125.67, 125.08, 124.92, 122.99, 120.65, 80.21, 79.72, 56.60, 19.31.

Complex **2**: Light-red powder, yield: 0.320 g (40%). High resolution ESI-MS (CH_3OH): m/z 1128.57 $[M-PF_6]^+$. Elemental analysis: calcd (%) for $C_{62}H_{46}N_8O_2PF_6Ir2H_2O$: C, 56.79; H, 3.85; N, 8.56; found: 56.57, H, 3.87; N, 8.53; ^1H NMR (400 MHz, $CDCl_3$) δ 9.45 (s, 6H), 8.59 (d, J = 7.4 Hz, 3H), 8.20 (t, J = 35.2 Hz, 6H), 7.88 (d, J = 31.4 Hz, 8H), 7.16 (s, 3H), 6.57 (s, 2H), 1.87 (s, 6H), 1.25 (s, 6H), 0.83 (s, 5H).^{13}C NMR (126 MHz, d_6-DMSO) δ 156.13, 151.56, 151.24, 150.70, 146.67, 144.78, 142.54, 141.14, 141.11, 140.83, 140.74, 139.91, 139.88, 138.15, 135.10, 133.00, 132.75, 132.54, 132.44, 131.47, 130.80, 130.07, 129.50, 129.27, 128.86, 128.62, 128.34, 126.30, 126.14, 124.58, 124.36, 122.38, 117.71, 110.02, 29.47, 29.20, 22.47.

Complex **1a**: Light-yellow powder, yield: 0.660 g (68%). High resolution ESI-MS (CH_3OH): m/z 681.55 $[M-PF_6]^+$. Elemental analysis: calcd (%) for $C_{34}H_{26}N_4IrPF_6$ $2H_2O$: C, 47.27; H, 3.5; N, 6.49; found: 47.35, H, 3.56; N, 6.44; ^1H NMR (400 MHz, d_6-DMSO) δ 8.90 (d, *J* = 8.2 Hz, 2H), 8.39 (s, 2H), 8.26 (d, *J* = 8.1 Hz, 2H), 8.21 (d, *J* = 5.0 Hz, 2H), 8.05 (d, *J* = 13.3 Hz, 2H), 7.95 (d, *J* = 7.6 Hz, 2H), 7.87 (t, *J* = 7.3 Hz, 2H), 7.46 (d, *J* = 5.4 Hz, 2H), 7.07 (t, *J* = 7.4 Hz, 2H), 7.03-6.90 (m, 4H), 6.30 (d, *J* = 7.3 Hz, 2H). ^{13}C NMR (126 MHz, d_6-DMSO) δ 167.32, 151.12, 150.32, 149.59, 146.57, 144.52, 139.30, 139.17, 131.73, 131.63, 130.68, 128.83, 127.61, 125.53, 124.33, 122.85, 120.44.

Complex **2a**: Red powder, yield: 0.430 g (51%). ESI-MS (CH_3OH): m/z 931.76 $[M-PF_6]^+$. Elemental analysis: calcd (%) for $C_{52}H_{32}N_6IrPF6$ $4H_2O$: C, 55.17; H, 3.38; N, 7.42; found: C, 55.15; H, 3.34; N, 7.40. ^1H NMR (400 MHz, d_6-DMSO) δ9.34 (s, 2H), 8.82 (s, 4H), 8.41 (d, J = 7.4 Hz, 2H), 8.31 (d, J = 8.1 Hz, 2H), 8.22 (s, 3H), 8.01 (s, 2H), 7.91 (s, 4H), 7.72 (s, 3H), 7.24 (s, 3H), 7.14 (s, 3H), 7.04 (s, 3H), 6.66 (d, J = 6.8 Hz, 2H); ^{13}C NMR (126 MHz, d_6-DMSO) δ 156.16, 151.50, 150.38, 146.45, 144.76, 142.51, 141.11, 139.98, 139.95, 135.06, 133.02, 132.96, 132.74, 132.72, 132.38, 132.32, 131.34, 131.14, 130.68, 129.51, 129.22, 128.73, 128.07, 126.28, 124.57, 122.38, 117.63, 117.54.

HPLC

A total of 0.1 μL of the solution of complex **1** and **2** was injected into an HPLC (Thermo, USA) . A Hypersil Gold Dim (100 × 2.1 mm, Thermo, USA) reverse phase column was used with a flow rate of 2 mL/min. The runs were performed with a Mobile phase: CH_3OH and H_2O (82:18, v/v) for **1**， CH_3OH and H_2O (97:3, v/v) for **2**.

$^1O_2$ production quantum yields in PBS

The $^1O_2$ quantum yields (Φ_Δ) of Ir(III) complexes in DMSO were detected according to the literature procedure with slight modifications.[3] The DMSO solutions containing **1**, **1a**, **2**, **2a** and DPBF (50 μM) were aerated for 10 min, then irradiated at 425 nm (2% *40 mW cm^{-2}). Every 2 s, the absorbance of DPBF at 418 nm was recorded. MB was used as the reference (Φ_Δ = 0.52).[4]

The $^1O_2$ quantum yields (Φ_Δ) of Ir(III) complexes in aerated PBS were evaluated using a steady-state method with ABDA as the $^1O_2$ indicator and $[Ru(bpy)_3]Cl_2$ as the standard (Φ_Δ = 0.18 in H_2O).[5] Briefly, air-equilibrated buffer solutions containing the tested complexes and ABDA (100 μM) were prepared in the dark and irradiated with 425 nm. The absorption maxima of ABDA were recorded every 10 s.

The absorbance at 425 nm of Ir (III) complexes, MB and $[Ru(bpy)_3]Cl_2$ was kept at 0.15. The $^1O_2$ quantum yields of Ir complexes were calculated according the following equation:

$$\Phi_{\Delta(x)} = \Phi_{\Delta(std)} \times \left(\frac{S_x}{S_{std}}\right) \times \left(\frac{F_{std}}{F_x}\right)$$

where S is the slope of a linear fit of the absorbance change of DPBF at 418 nm or ABDA absorption maxima 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).[6]

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'smedium, 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 DMSO (1%, v/v) only were used as the reference group .

Dark cytotoxicity[7]

Cells cultured in 96-well plates were grown to confluence. The compounds were dissolved in DMSO (1%, v/v), and diluted with fresh media immediately. The cells were incubated with a series of concentrations of the tested compounds for 44 h at 37 °C. 20 μL MTT solution then added to each well, and the plates were incubated for an additional 4 h. The media was removed, and DMSO was added (150 μL per well) and incubated for 10 min with shaking. The absorbance at 595 nm was measured using a microplate reader (Infinite M200 Pro, Tecan, Männedorf, Switzerland).

Phototoxicity

Cells were incubated with the tested compounds for 24 h. The media containing the Ir complexes was removed and fresh media without the complexes was added. Irradiated with a 425 nm LED light array (40 mW cm^{-2}) for 10 min (24 J cm^{-2}), incubated for another 20 h, MTT was added and incubated for an additional 4 h.

In the experiment investigating the elimination effect of NAC (5 and 10 mM) on phototoxicity, A549 cells were initially incubated with complex **1** for 24 h, media was removed and fresh media without the complex was added. NAC was added 1 h before light irradiation (425 nm, 24 J cm^{-2}), and then cell was incubated as described above for MTT assay.

Selective killing cancer cells during PDT treatment

A549 was labeled by Hoechst 33342 (5 μg/mL in PBS) in advance**.** A549 and LO2 cells were cultured in 60 mm dishes (Corning) to welt and treated with complexes **1** for 0.5 h. Photo-irradiation (425 nm, 40 mW·cm^{-2}, 10 min) was performed, and cell was then incubated under dark for 23h. The cells were washed twice with PBS, then 5 uL Annxin-V and 10 uL PI was added before cells were immediately viewed by a confocal microscopy.

Detection of apoptosis

Hoechst staining: A549 cells were seeded into 35 mm dishes (Corning) to welt and then treated with complex **1-2** for 2 h. Photo-irradiation (425 nm, 40 mW·cm^{-2}, 10 min) was performed and the cells were then incubated under dark for 22 h, and then washed twice with cold PBS and fixed with 4% paraformaldehyde at room temperature for 10 min. After washed with cold PBS, cells were labeled with Hoechst 33342 (5 μg/ml in PBS) for 5 min. The cells were analyzed immediately with a confocal laser-scanning microscope.

Annexin V/PI Assay: In flow cytometry, A549 cells were cultured in 6-well

tissue culture plates for 24 h and then treated with complex **1-2** at the indicated concentrations for 3 h. Photo-irradiation (425 nm, 40 $mW \cdot cm^{-2}$, 10 min) was performed and the cells were furthuer incubated under dark for another 3 h. The cells were harvested and stained using an Annexin -V /PI apoptosis detection kit (Sigma Aldrich) according to the manufacturer's instructions. Data were collected by flow cytometer and analyzed with FlowJo 7.6 software.

Cellular uptake and distribution

A549 cells were co-incubated with complexes **1** or **2** (10 μM) and MTDR (200 nM) or LTDR (150 nM) at 37 °C for 30 min. Cells were washed three times with PBS and visualized by confocal microscopy immediately (OPM: λex=405 nm and TPM λex=720 nm, λem=605 ± 20 nm for **1** and λem=650 ± 20 nm for **2**; MTDR and LTDR: λex=633 nm, λem= 665 ± 20 nm).

In a A549/LO2 co-cultured cell model, LO2 cells were labeled by Hoechst 33342 (5 μg/mL in PBS) in advance**.** A549 and LO2 cells were co-cultured in 60 mm dishes (Corning) to welt and treated with complexes **1** (10 μM) for 1 h, the cells were washed twice with PBS before viewed by confocal microscopy to compare the cellular uptake efficacy of cancer cells and normal cells.

ICP-MS

A549 cells were seeded in 10 cm tissue culture dishes to welt, the medium was removed and replaced with fresh medium containing the tested complexes (20 μM). For dark ICP-MS measurements, the cells were treated for 2 h under dark; For ICP-MS in the presence of light, cells were treated with Ir(III) complexes for 1 h under dark, irradiation (425 nm, 5 min) was operated followed by another 1 incubation. Then the cells were washed with PBS, trypsinized and collected after 24 h incubation. Cells were counted, and digested with HNO_3 (65%, 2 mL) at 60 °C for 1 h. The solution was then diluted to a final volume of 10 mL with Milli-Q water. The concentration of Iridium was measured using the XSERIES 2 ICP-MS.

One and Two photon confocal imaging of 1-treated zebrafish

Zebrafishes of 5-days-old were fed with **1** (50 μM) in PBS buffer at 28 ℃ for 6 h. All the fishes were terminally anaesthetized using methylcellulose(3%) for one-photon (λex = 405 nm) and two-photon (λex = 720 nm) bio-imaging.

The cytotoxicity for zebrafish was prepared with 5-day-old zebrafishes. Three hundred zebrafishes were divided into five groups, one group was used as control, each two groups was fed with different concentrations of **1** and **1a** (20, 40 μM) respectively. The total cultured time for **1** and **1a**-treated zebrafishes reached up to 72

hours, and the survival of zebrafishes was counted per 12 hours. The **1**-treated zebrafish viability was found over 98% in 72 hours at both concentrations; however, **1a**-treated zebrafish almost died in 12 hours even at 20 μM.

Colocalization of complexes and oxidative stress probe

A549 cells were cultured in 60 mm dishes for 24 h to welt. H_2O_2 or PMA was added to stimulate intracellular oxidative stress. Then cells were incubated with complex **1** or **1a** for 30 min, and then stained with oxidative stress probe Cell Rox Deep red (λex=640 nm, λem=665 nm) 5 μM and incubated for 30 min, the cell were washed twice with PBS before viewed by confocal microscopy.

PLIM imaging of induced oxidative stress

A549 cells seeded into 35 mm dishes (Corning) were pretreated with PMA or H_2O_2 at the indicated concentrations for 1.5 h and 30 min, respectively, to induce intracellular oxidative stress. Then the cells were treated with complex **1** for 30 min under dark and washed twice by PBS before confocal and PLIM imaging.

During PDT, A549 cells were treated with complex **1** for 30 min under dark and irradiation (425 nm, 40 $mW \cdot cm^{-2}$) with different light doses was performed to induce oxidative stress. In parallel experiments, A549 cells were pretreated with NAC at the indicated concentrations for 1 h before exposure to complex **1** and irradiation. Then all the cells were incubated under dark for another 30 min and washed twice with PBS before PLIM imaging.

Lifetime data were recorded by a DPC-23016 Channel Photon correlator and data was analyzed by a SPCM software.

Measurement of Intracellular ROS levels

A549 cells were cultured in 6-well tissue culture plates for 24 h and then treated with complex **1-2** at the indicated concentration for 3 h. PDT treatment was carried out as described before. Then cells were incubated under dark for another 3h and washed twice with serum-free DMEM, the fluorescence intensity of cells was measured immediately by flow cytometry with excitation at 488 nm and emission at 530 nm. Green mean fluorescence intensities were analyzed using FlowJo 7.6 software.

MMP Assay

Confocal microscopy:

A549 cells were treated with **1** and **2** (10 μM) for 3h in the absence or presence of irradiation (425 nm, 5min, 40 $mW \cdot cm^{-2}$). Then the cells were incubated under dark for another 3 h and stained with JC-1 (10 ug/mL) in the dark at 37 °C for 30 min. Cells were washed three times with PBS and then visualized by confocal microscopy (LSM 710, Carl Zeiss, Göttingen, Germany).Ex (JC-1 monomer) = 488 nm, Em (JC-1 monomer) = 529 nm ; Ex (JC-1 aggregate) = 543 nm, Em (JC-1 aggregate) = 590 nm.

Flow cytometry:

A549 cells were treated with **1** and **2** in the absence or presence of irradiation as described above. The cells were then collected and resuspended at 1×10^6/ml in pre-warmed PBS containing 5 μg/ml JC-1, and incubated for 30 min at 37 °C. Subsequently, the cells were washed twice with PBS and immediately analyzed in a flow cytometry. Fluorescence was monitored by measuring both the monomer and the aggregate forms of JC-1 following excitation at 488 nm. Red and green MFI were analyzed using Flow Jo 7.6 software (TreeStar, USA). 10, 000 events were acquired for each sample.

ATP Assay

ATP concentration of A549 was conducted by the CellTiter-Glo luminescent Cell Viability Assay(Promega, USA) according to the manufacturer's instructions. Cell were cultured in 96 round black well plate for 24h to welt and treated with complexes **1** or **2** for 3 h. Photo-irradiation (425 nm, 40 $mW \cdot cm^{-2}$, 10 min) was performed, and cell was then incubated under dark for another 3 h. Cell was washed by PBS once and balanced in PBS for 30 min and 100 μl CellTiter-Glo luminescent Cell Viability reagent was added into each well. The mixture was lysed for 2 min by a shaken machine, then incubated at room temperature for 10 min. The luminescence was measured using a TECAN Infinite M200 station. On the same condition, standard curve was obtained by the known concentration of standard ATP sample, Ribonucleotide Triphosphates (rRTPs) (10mM), we can obtain the ATP concentration of cells.

Real-time tracking of changes in mitochondrial morphology

A549 cells were cultured in 60 mm dishes (Corning) for 24 h and treated with complexes **1** (10 μM) for 1 h under dark. Irradiation (425 nm, 40 $mW \cdot cm^{-2}$, 10 min) was performed and confocal images were immediately collected every 5 min (λex=720 nm , λem=605 ± 20 nm).

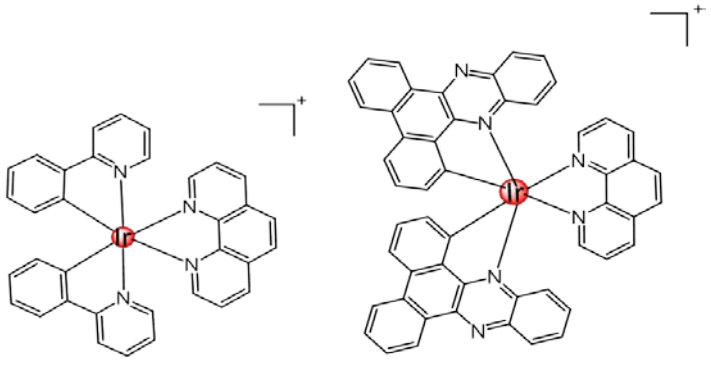
Transmission electron microscopy (TEM)

A549 cells were cultured in 60 mm dishes (Corning) and incubated with Complex **1** at indicated concentrations for 12 h,then Photo-irradiation (425 nm, 40 $mW \cdot cm^{-2}$, 10 min) was performed as mentioned above and the cells were incubated under dark for 12 h. The cells were washed by PBS and fixed overnight at 4℃ in phosphate buffer (pH 7.4) containing 2.5% glutaraldehyde. After treated with osmium tetroxide as post fixative, the cells were stained with uranyl acetate and lead citrate, then observed by a transmission electron microscope (JEM 100 CX, JEOL, Tokyo, Japan). Images were photographed using the Eversmart Jazz program (Scitex).

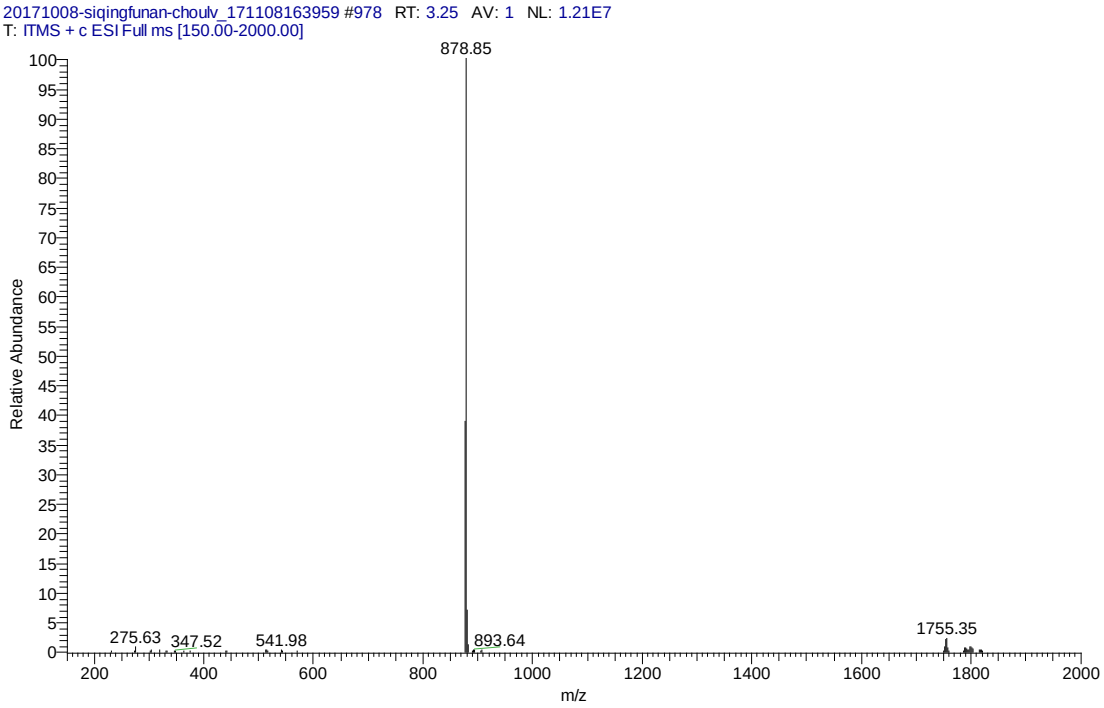
Statistical analysis

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



Scheme S1 Structures of nitroxide-free Ir(III) analogues, counter ion is PF_6.



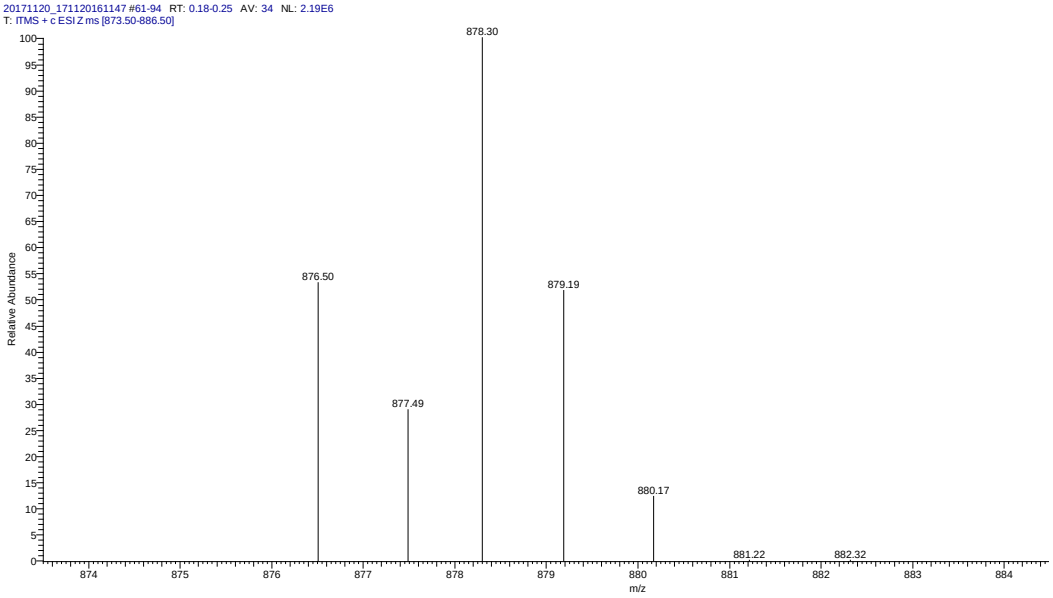


Figure S1 (A) High-resolution **ESI-MS spectrum of complex 1**, 878.85 $[M\text{-}PF_6]^+$

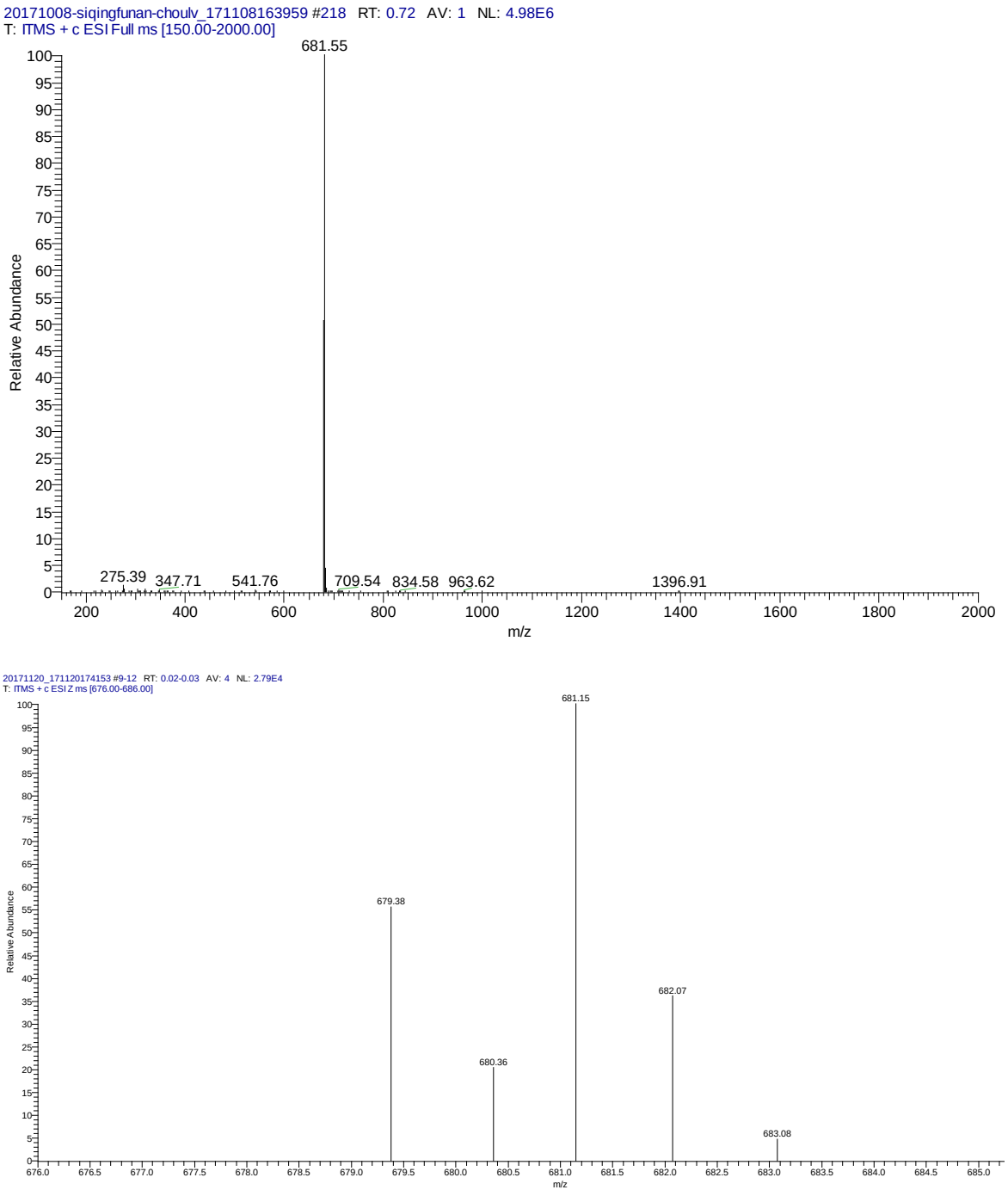


Figure S1 (B) High-resolution ESI-MS spectrum of complex **1a**, 681.55, $[M\text{-}PF_6]^+$

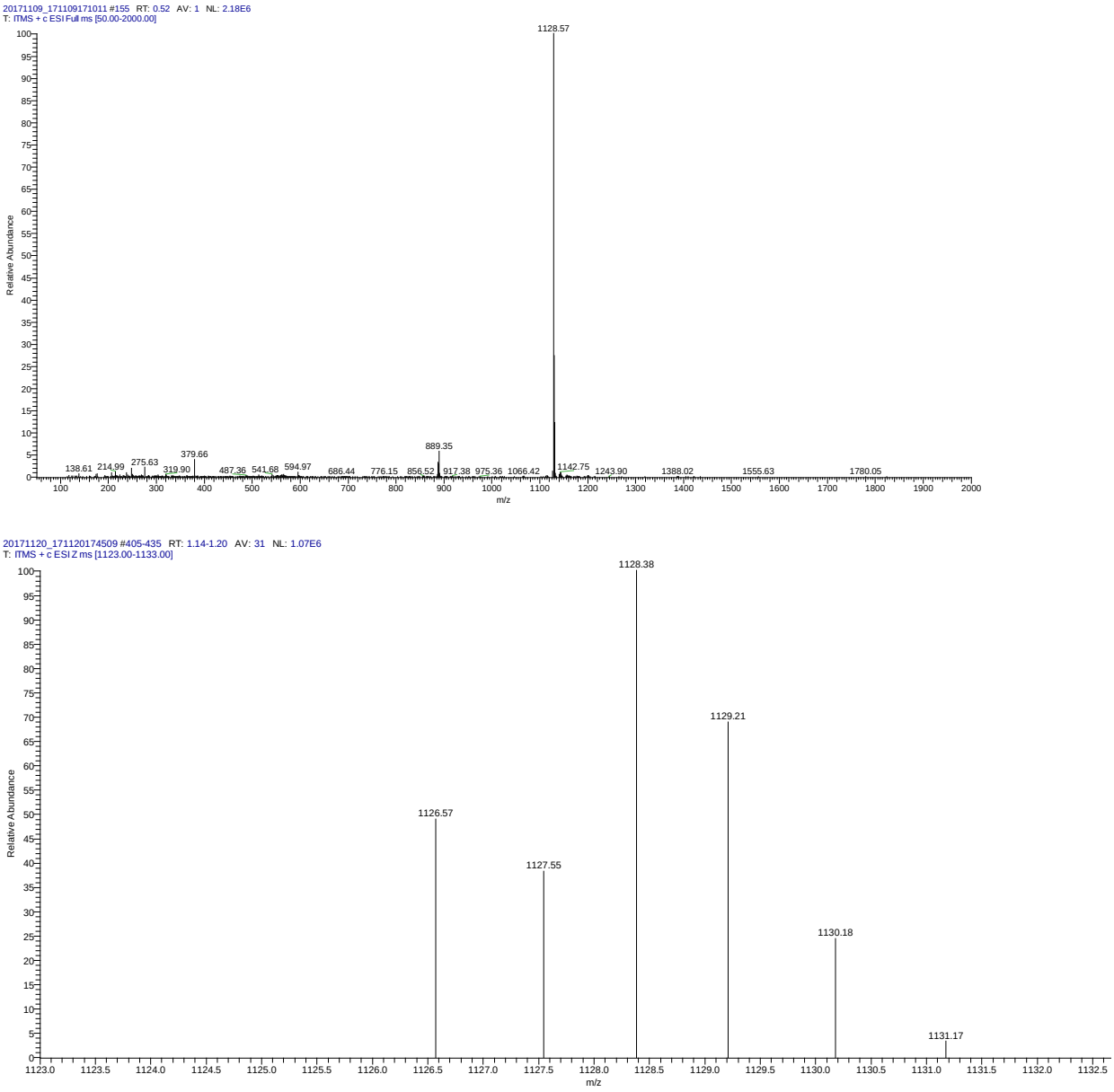


Figure S1 (C) High-resolution ESI-MS spectrum of complex **2**, 1128.57, $[M\text{-}PF_6]^+$

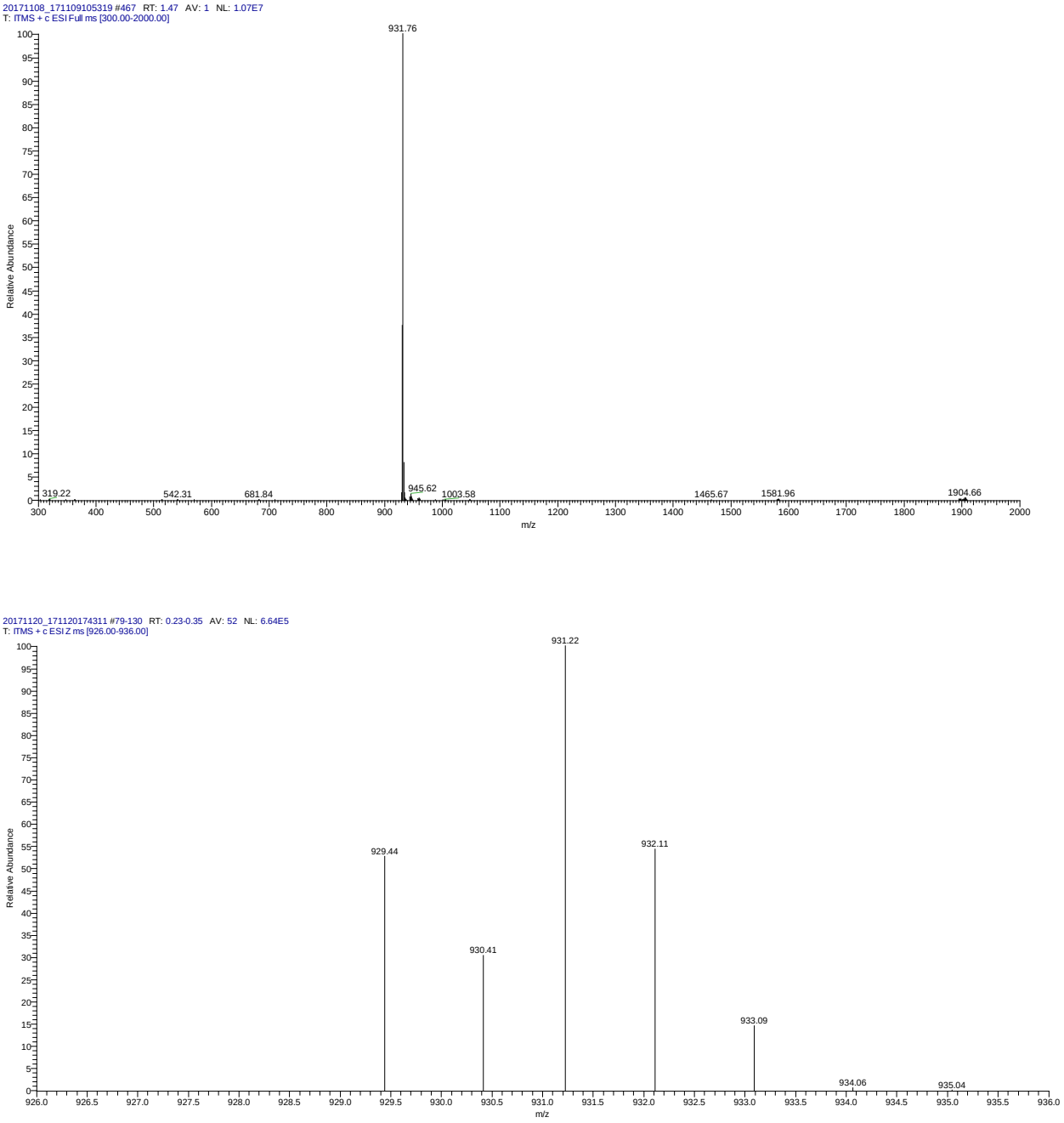


Figure S1 (D) High-resolution ESI-MS spectrum of complex **2a**, 931.76, $[M\text{-}PF_6]^+$

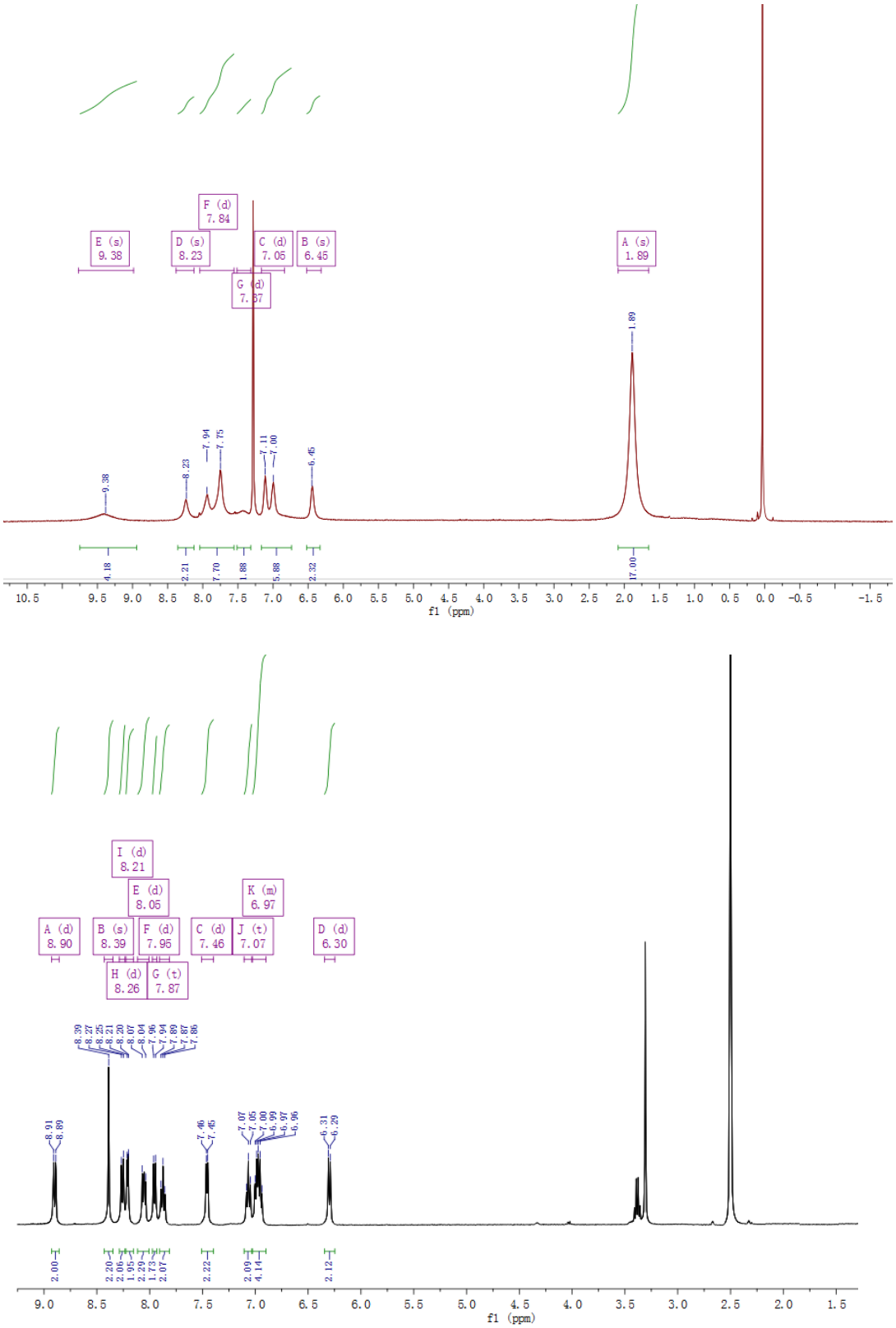


Figure S2 (A) ^{1}H NMR spectrum of complex **1** (in $CDCl_3$, top) and **1a** (d_6-DMSO, bottom)

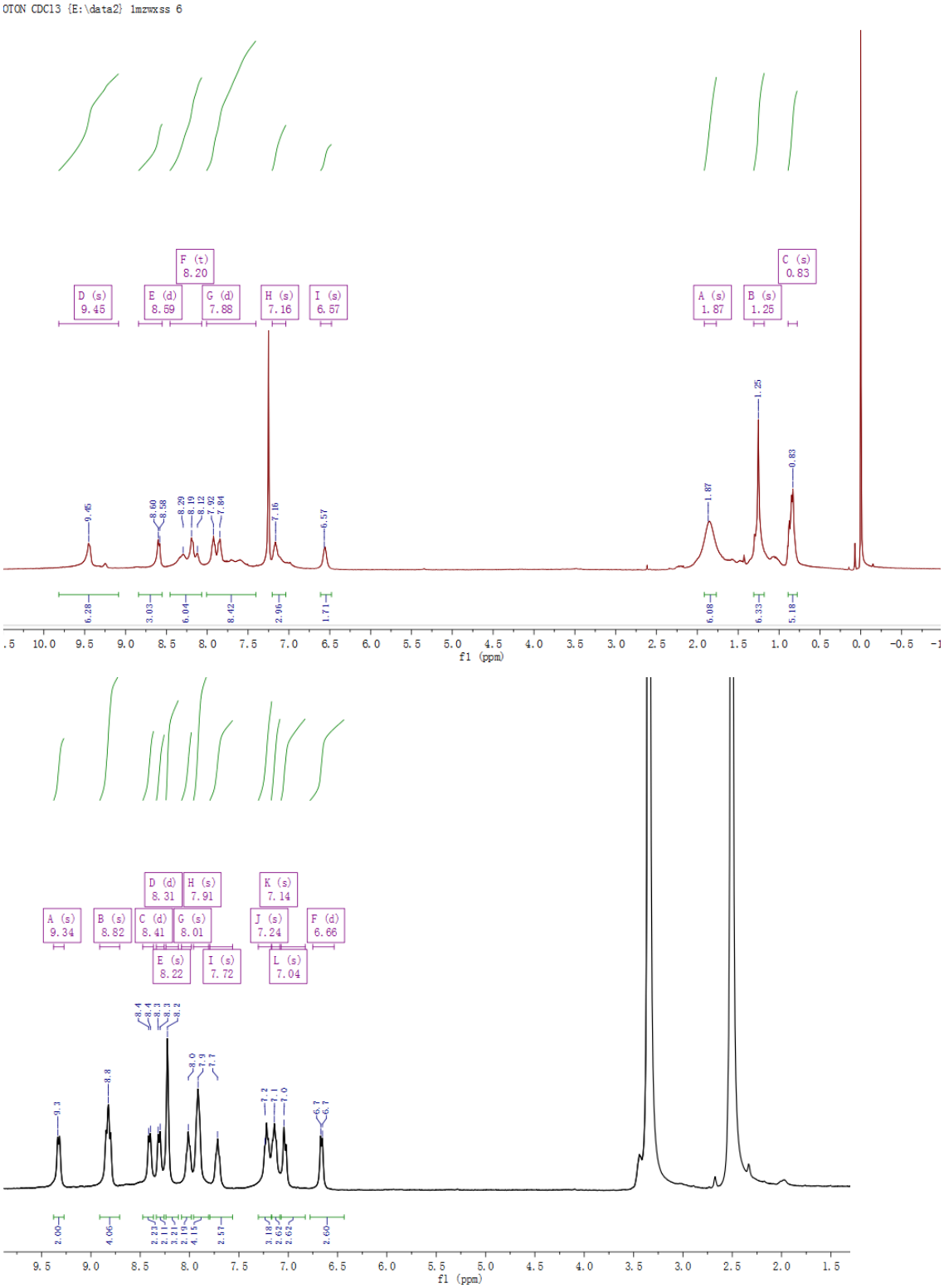


Figure S2 (B) ^{1}H NMR spectrum of complex **2** (in $CDCl_3$, top) and **2a** (d_6-DMSO, bottom)

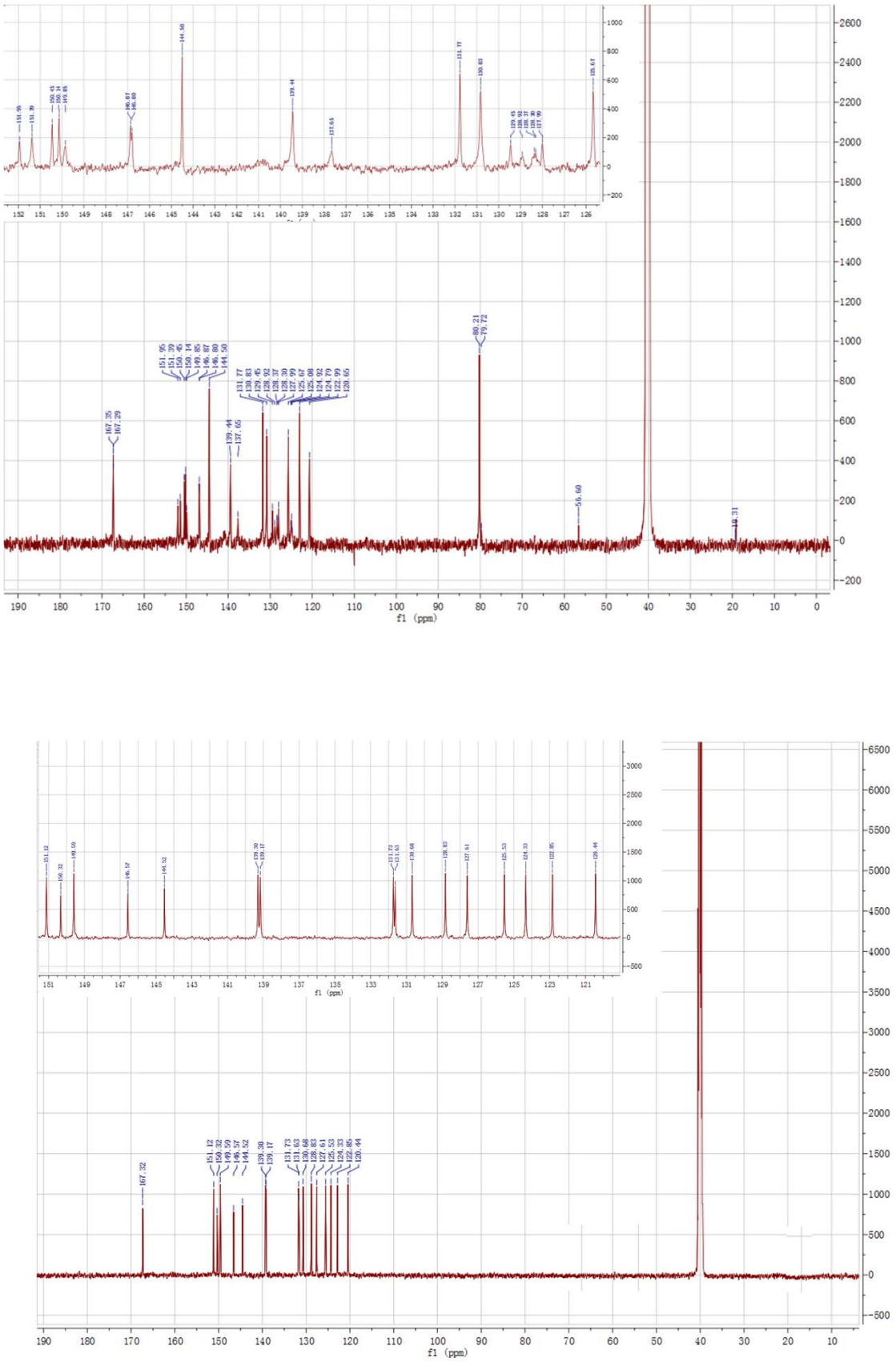


Figure S2 (C) ^{13}C NMR spectrum of complex **1** (top) and **1a** (bottom) in d_6-DMSO

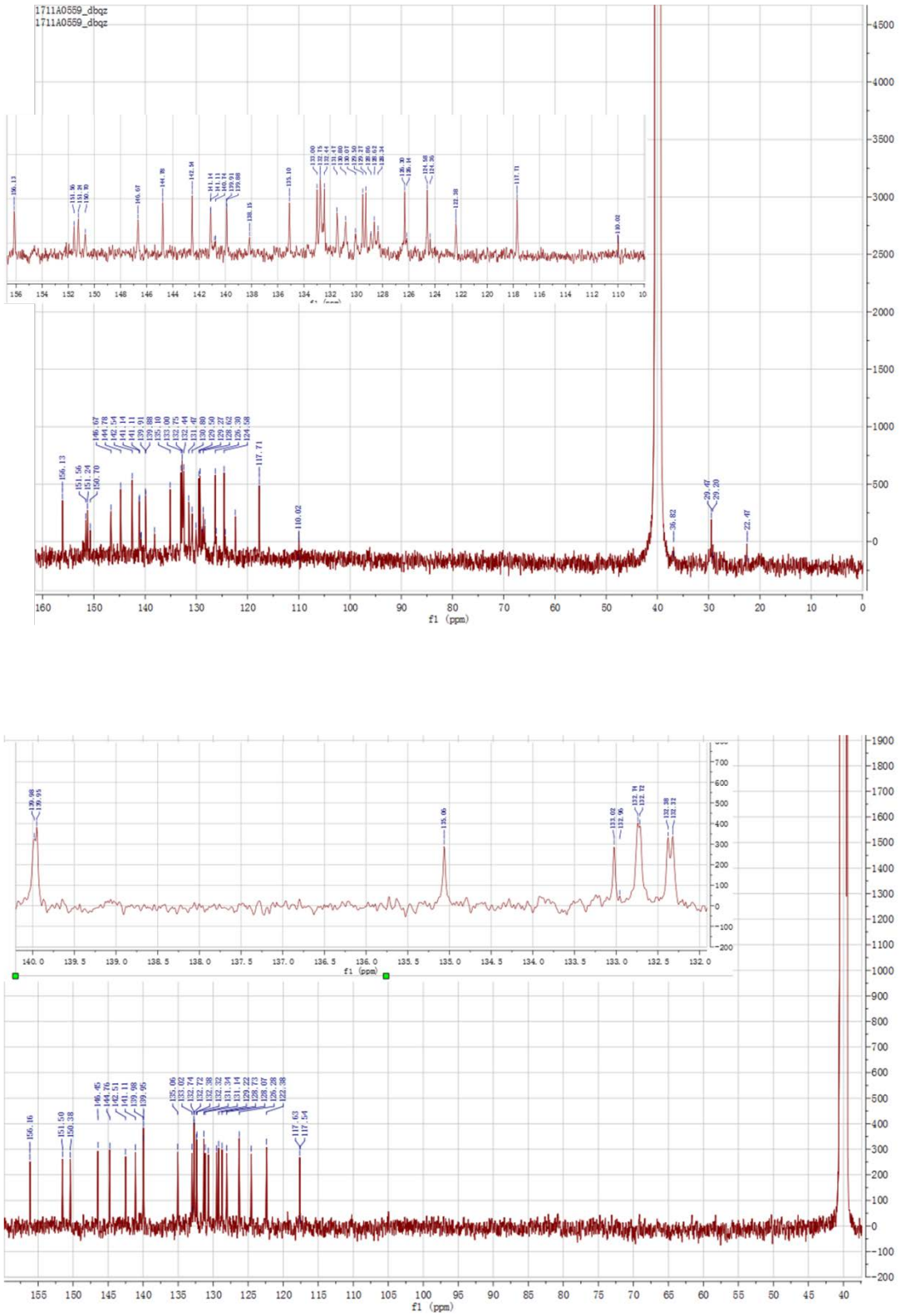
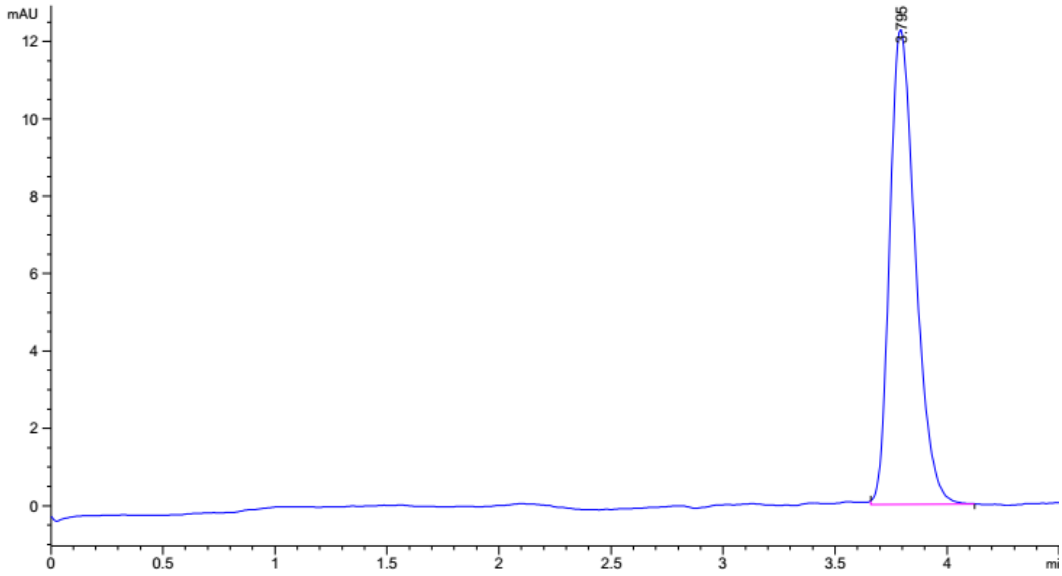
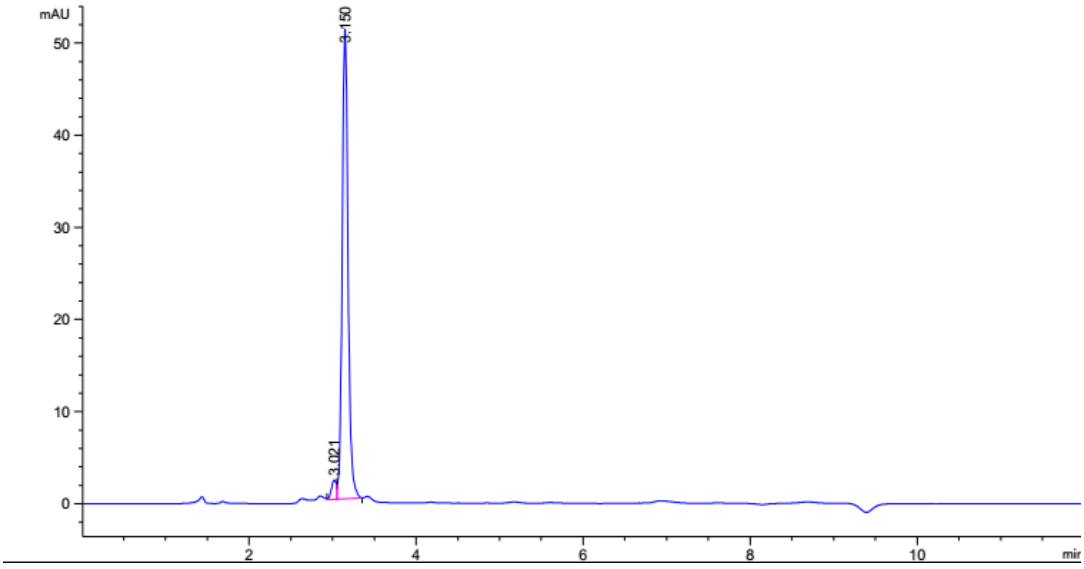


Figure S2 (D) ^{13}C NMR spectrum of complex **2** (top) and **2a** (bottom) in d_6-DMSO



#	[min]		[min]	[mAU*s]	[mAU]	%
1	3.795	BB	0.1248	97.68095	12.28405	100.0000

Figure S3 (A) HPLC chromatogram of complex **1** with UV detection at 280 nm. Mobile phase: CH_3OH and H_2O (82:18, v/v) pumped at a flow rate of 2.00 mL/min.



#	[min]		[min]	[mAU*s]	[mAU]	%
1	3.021	VV	0.0612	8.47728	2.09402	3.3003
2	3.150	VB	0.0726	248.38457	51.18304	96.6997

Figure S3 (B) HPLC chromatogram of complex **2** with UV detection at 300 nm. Mobile phase: CH_3OH and H_2O (97:3, v/v) pumped at a flow rate of 2.00 mL/min.

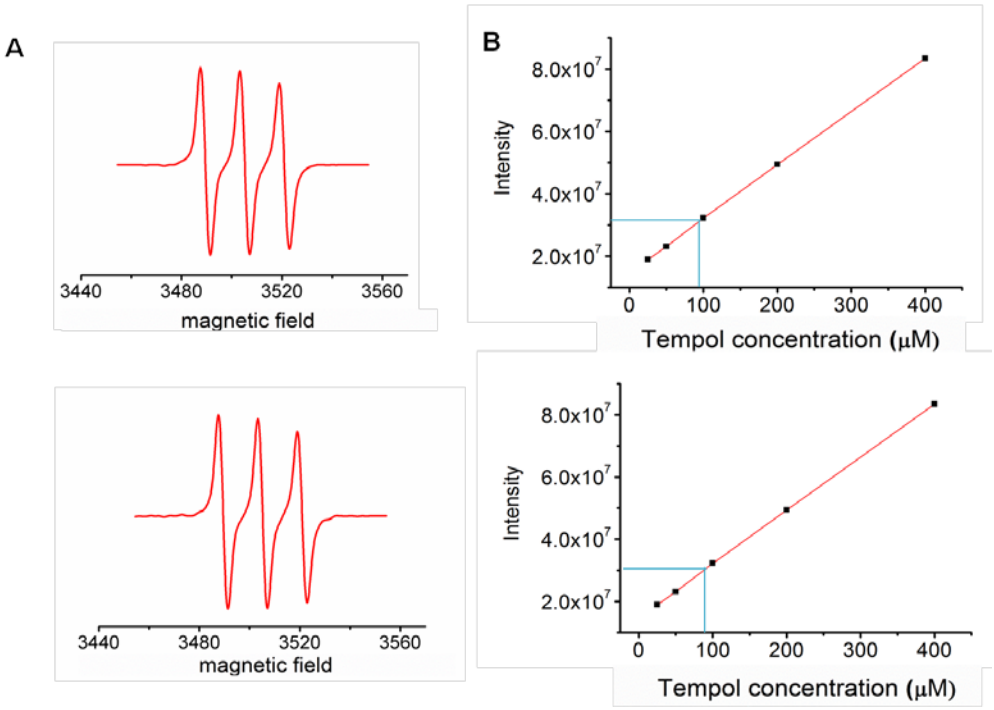


Figure S4 (A) EPR spectra of 1×10^{-4} M of complex **1** (upper panel) and **2** (bottom panel) in recorded in CH_3CN; (B) EPR standard curves show the intensity of the EPR signals as a function of the TEMPO concentration, accordingly determining the effective spin concentrations of complex **1-2** to be 97.2 % and 96.2%, respectively.

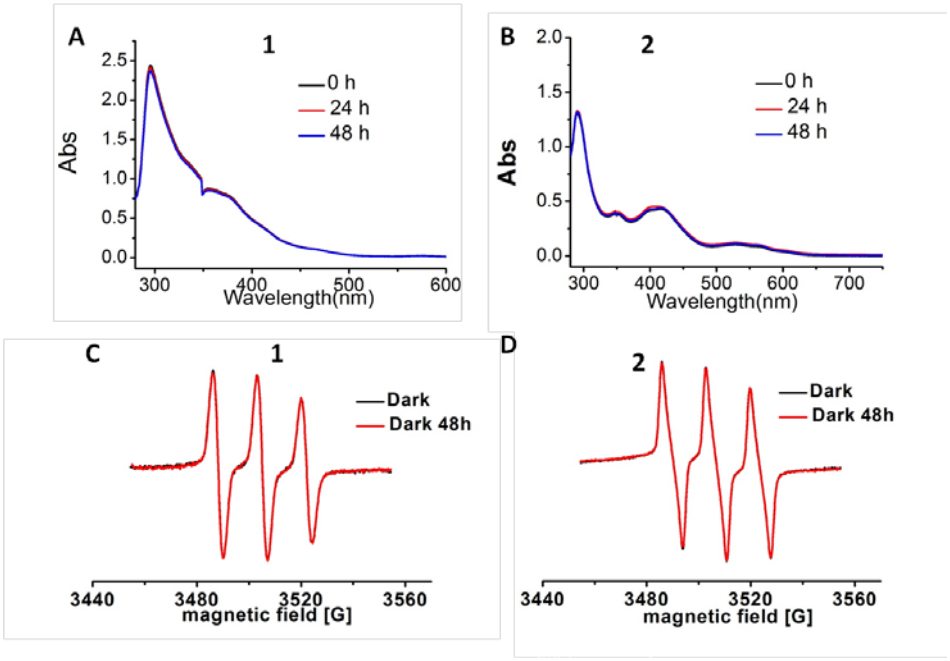


Figure S5 (A-B) UV-visible absorption and (C-D) EPR spectra of 20 μM complex **1** and **2** monitored in cell culture medium, indicating the good stability of nitroxide-containing complexes in at least 48 h.

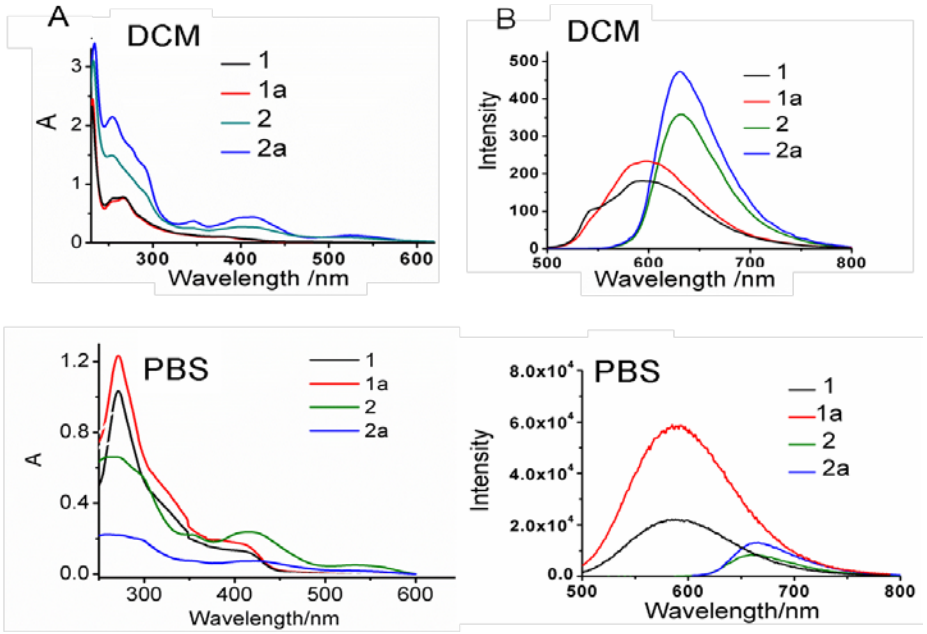


Figure S6 (A) UV/Vis and (B) Emission spectra (excitation at 405 nm) of complexes **1, 1a, 2, 2a (**10 μM) measured in CH_2Cl_2 and PBS at 298 K.

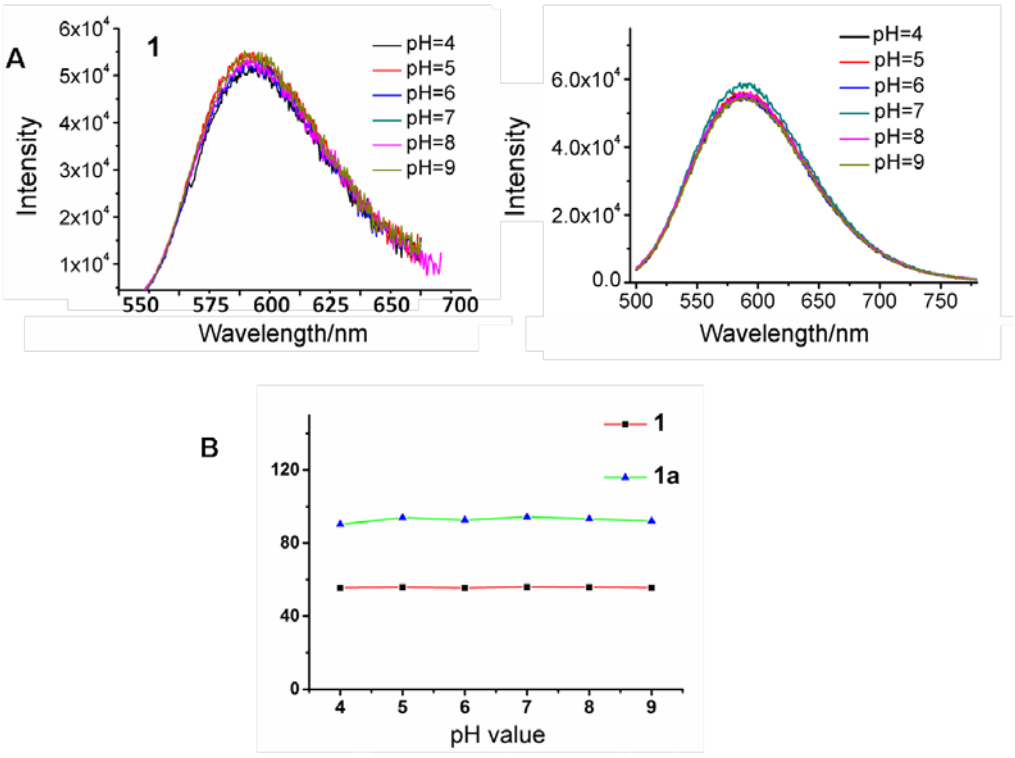


Figure S7 The effect of pH values on the (A) phosphorescence intensity and (B) lifetimes of **1** and **1a** (10 μM) in PBS at 298 K (λex = 405 nm).

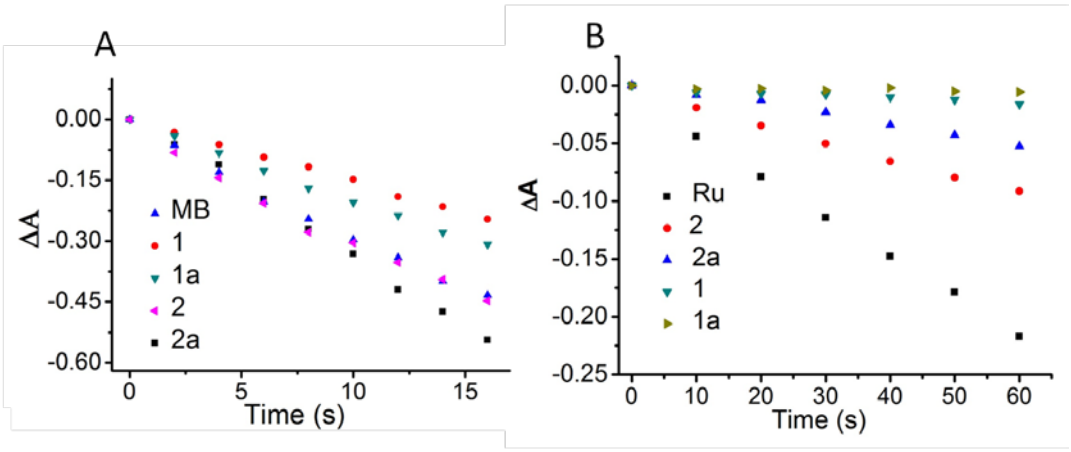


Figure S8 (A) The absorption changes of DPBF at 418 nm recorded in aerated DMSO in the presence of **1**, **2**, **1a**, **2a** upon irradiation at 425 nm. MB was used as standard; (B) Decay rate of ABDA sensitized by and Ir complexes in aerated PBS. $[Ru(bpy)_3]Cl_2$ was used as standard.

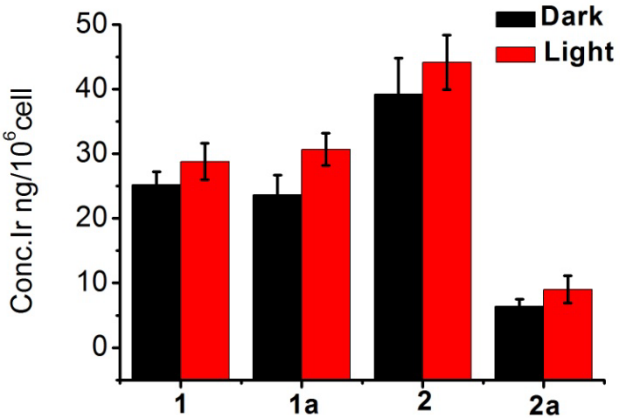


Figure S9 Cellular uptake of Ir(III) complexes (20 μM, 2 h) in A549 cells measured by ICP-MS in the absence and presence of light irradiation (425 nm, 5 min).

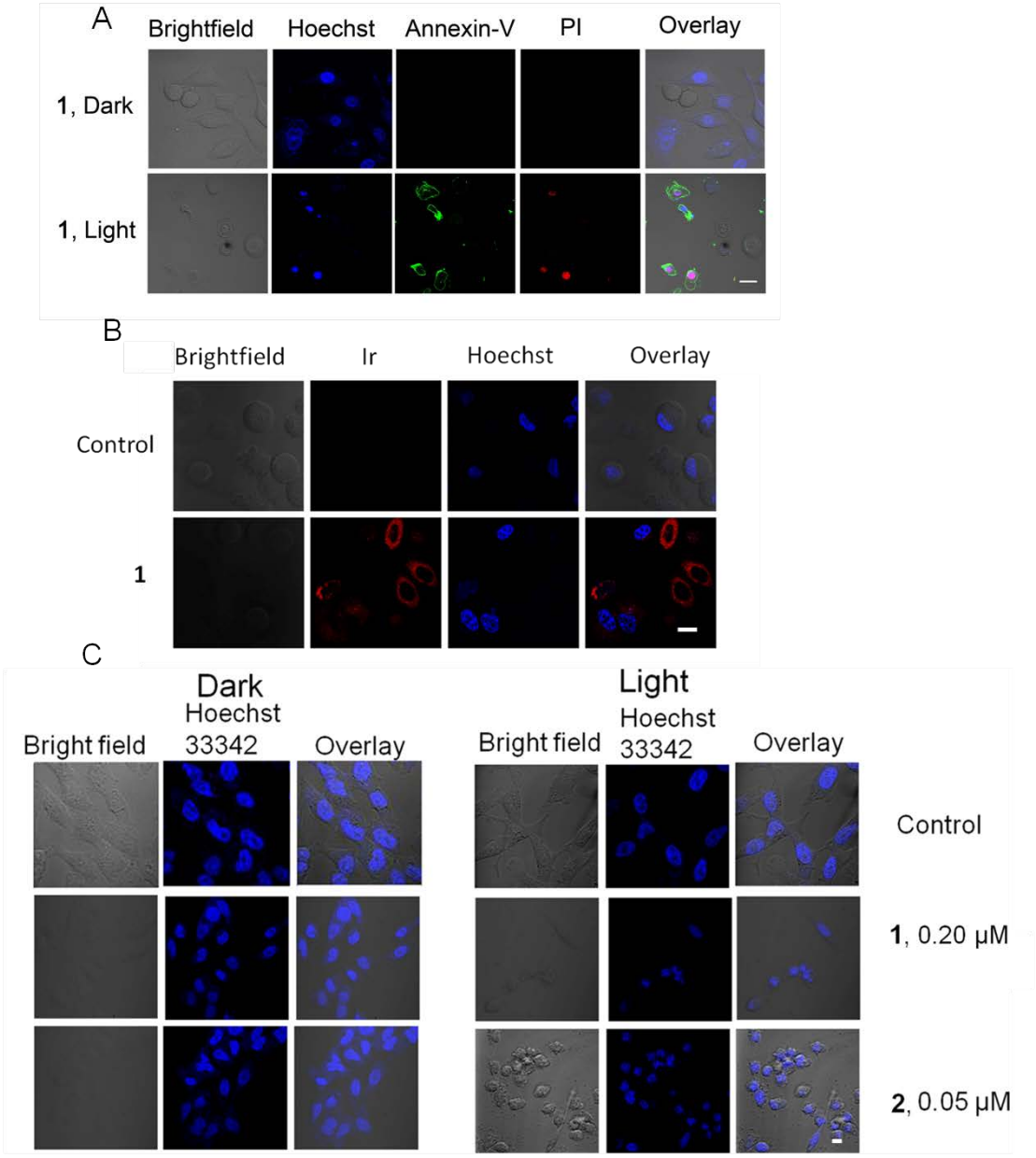


Figure S10 (A) The capability of complex **1** (0.1 μM, 24 h) to selectively induce cancer cell apoptosis in a A549/LO2 co-cultured cell model measured by annexin V/PI double staining. A549 cells were pre-labelled by Hoechst (blue); (B) Selective uptake of complex 1 (10 μM, 1 h) in a A549/LO2 co-cultured cell model. LO2 cells were pre-labeled by Hoechst (blue) (λex= 405 nm, λem= 605 ± 20 nm for **1**; Hoechst: λex= 405 nm, λem= 460 ± 20 nm); (C) Confocal microscopy of Ir(III)-induced cell apoptosis using Hoechst 33342 staining. A549 cells were incubated with complex **1-2** for 24 h in the absence and presence of light irradiation (425 nm, 40 $mW \cdot cm^{-2}$, 10 min). Scale bar: 10 μm.

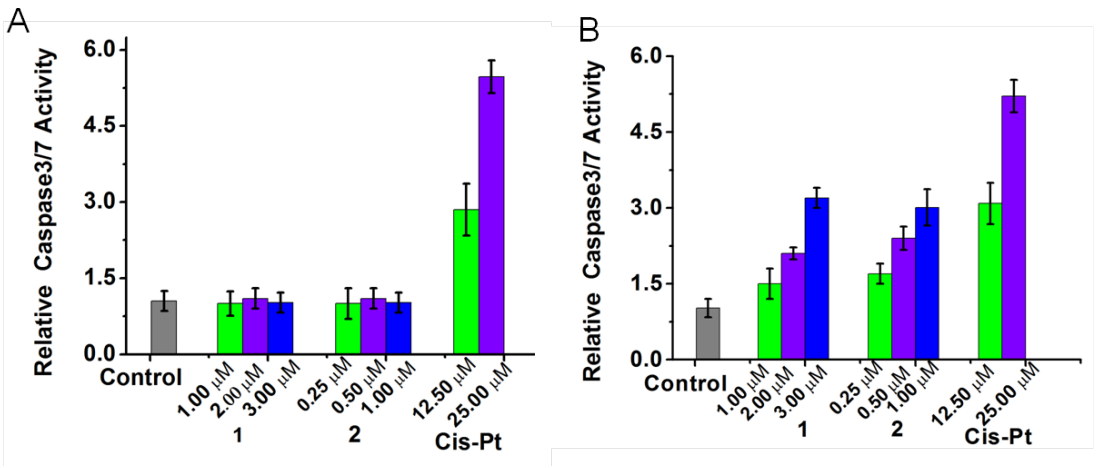


Figure S11 Caspase 3/7 activity of A549 cells treated with complexes **1-2** at the indicated concentrations in the absence and presence of light irradiation (425 nm, 40 $mW \cdot cm^{-2}$, 10 min).

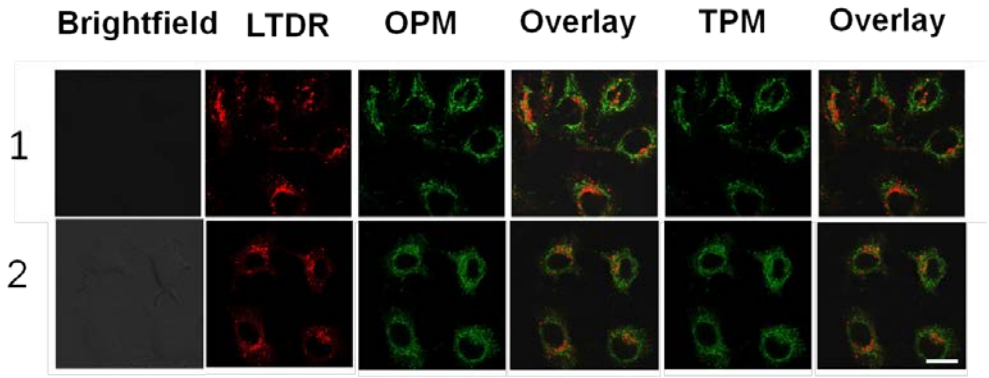


Figure S12 Colocalization of **1-2** (10 μM) with lysosome specific stain LTDR (150 nM) in A549 cells (OPM: λex= 405 nm; TPM: λex= 720 nm; λem = 605± 20 nm for **1** and 650± 20 nm for **2**； LTDR: λex= 633 nm, λem = 655 ± 20 nm). Scale bar: 10 μm.

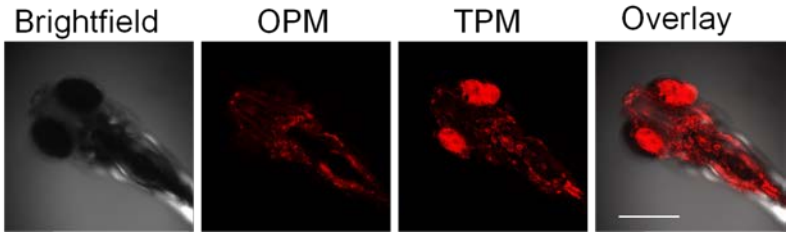


Figure S13 One-photon and two-photon fluorescence images of a 5-day-old zebrafish incubated with complex **1** (40 μM), after 6 h of incubation, and washed with PBS buffer. Compound **1** (40 μM) did not appear to induce toxicity over 72 h under dark and no zebrafish was found dead while over half amounts of zebrafish were found dead when incubated with **1a** (20 μM) in 12 h. Scale bar: 400 μm.

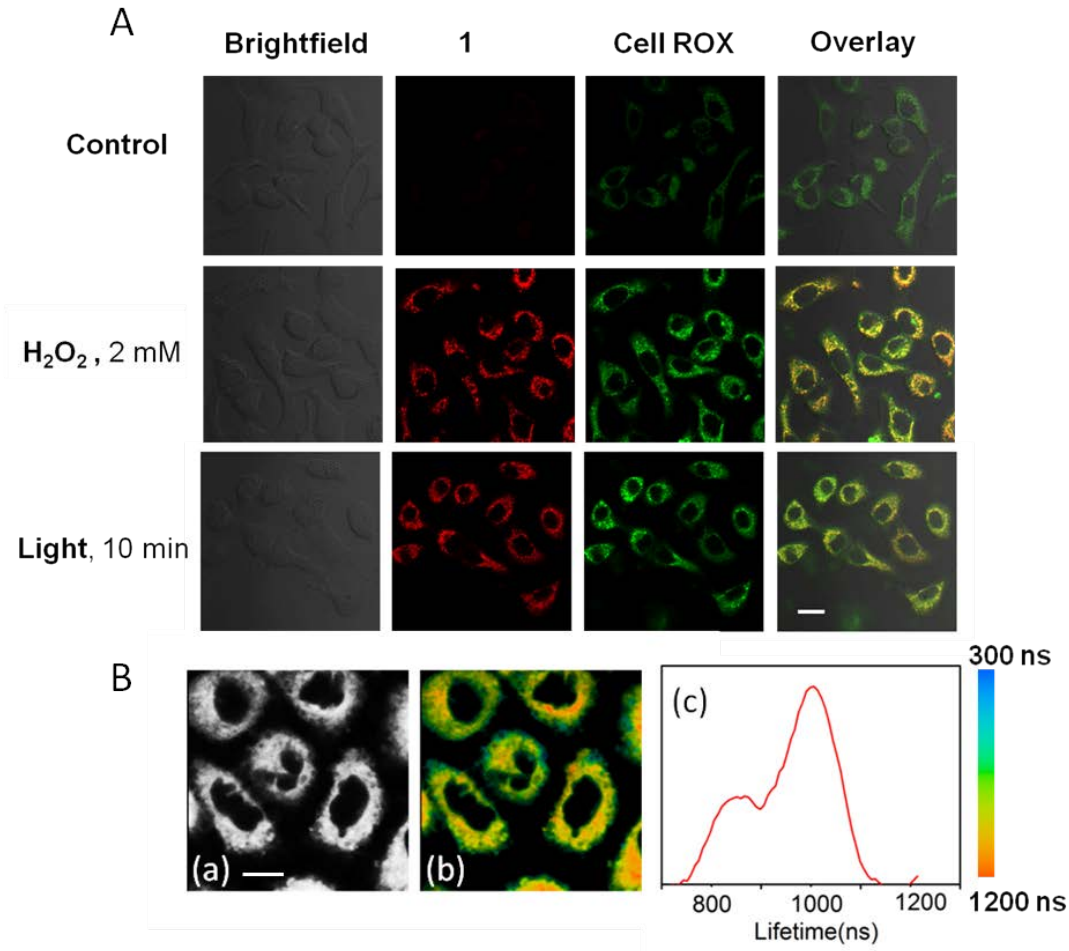


Figure S14 (A) Colocalization of **1** (5 μM) and oxidative stress probe Cell ROX in non-simulated (control), H_2O_2-simulated (2 mM, 30 min), and irradiation-simulated (425 nm, 40 mW·cm^{-2}) A549 cells (**1**: λex = 405 nm λem = 605± 20 nm; Cell ROX: λex = 644 nm, λem = 665 ± 20 nm); (B) Two-photon (λex = 720 nm) confocal, PLIM and luminescence lifetime distributions; of complex **1** (10 μM) in H_2O_2-simulated A549 cells. Scale bar: 10 μm

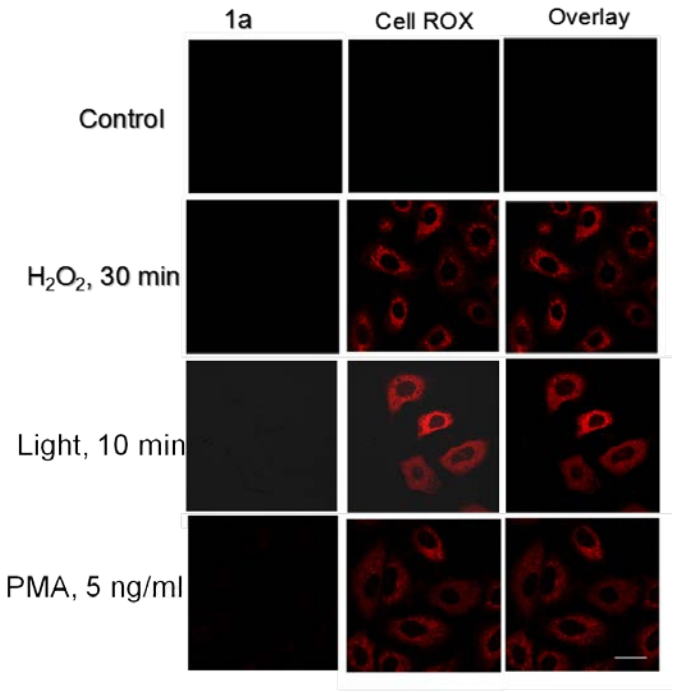


Figure S15 Confocal microscopy of **1a** (5 μM) and oxidative stress probe Cell ROX in non-simulated (control), H_2O_2-simulated (2 mM, 30 min), PMA-simulated (5 ng/ml, 2 h), and irradiation-simulated (425 nm, 40 mW·cm^{-2}) A549 cells (**1a**: λex = 405 nm λem = 605± 20 nm; Cell ROX: λex = 644 nm, λem = 665 ± 20 nm). Scale bar: 10 μm.

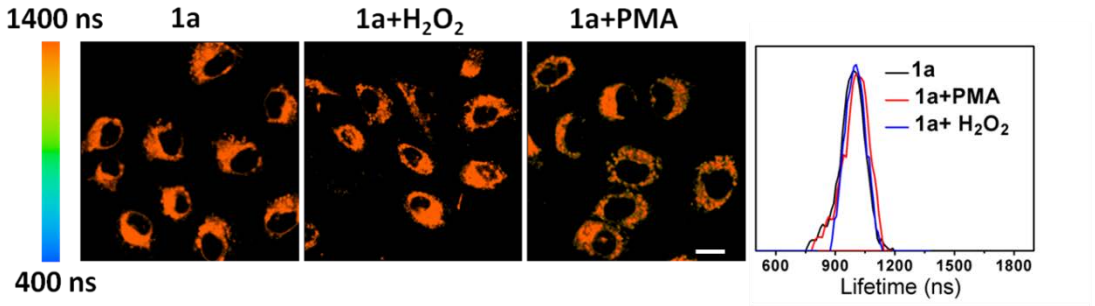


Figure S16 Two-photon PLIM images and luminescence lifetime distributions of complex **1a** in A549 cells (λex = 720 nm, λem = 605 ± 20 nm). For oxidative simulation, A549 cells were pre-treated with H_2O_2 (2 mM, 30 min) or PMA (5 ng/mL, 2h), respectively. Scale bar: 10 μm.

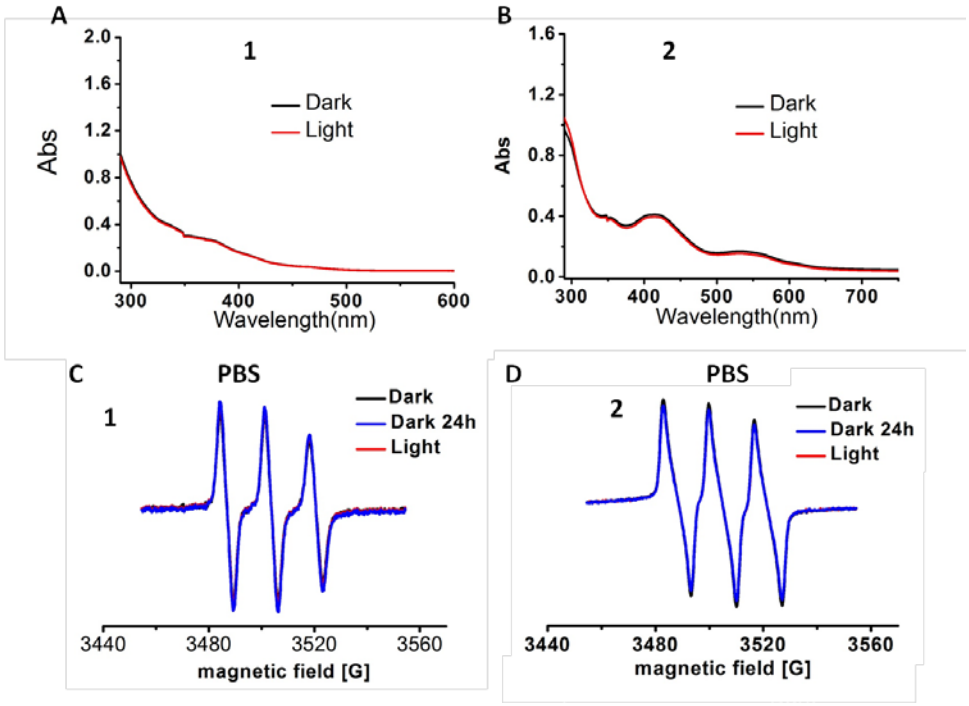


Figure S17 (A-B) UV-visible absorption and (C-D) EPR spectra of 20 μM complex **1** and **2** monitored in PBS in the absence and presence of light irradiation (425 nm, 5 min).

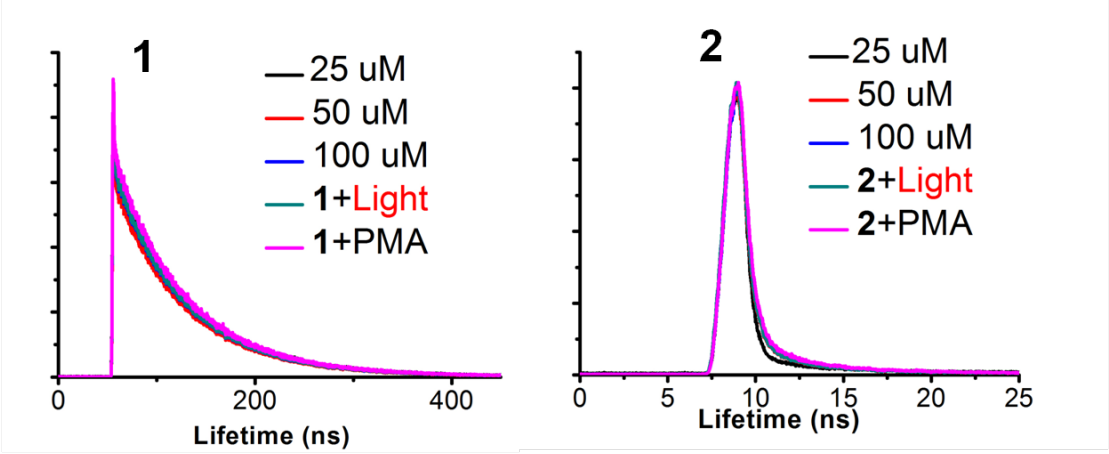


Figure S18 The influence of concentrations, light irradiation (425 nm, 5 min) and PMA (10 ng/ml) on the phosphorescence lifetimes of **1** and **2** in PBS at 25 °C (excitation at 375 nm).

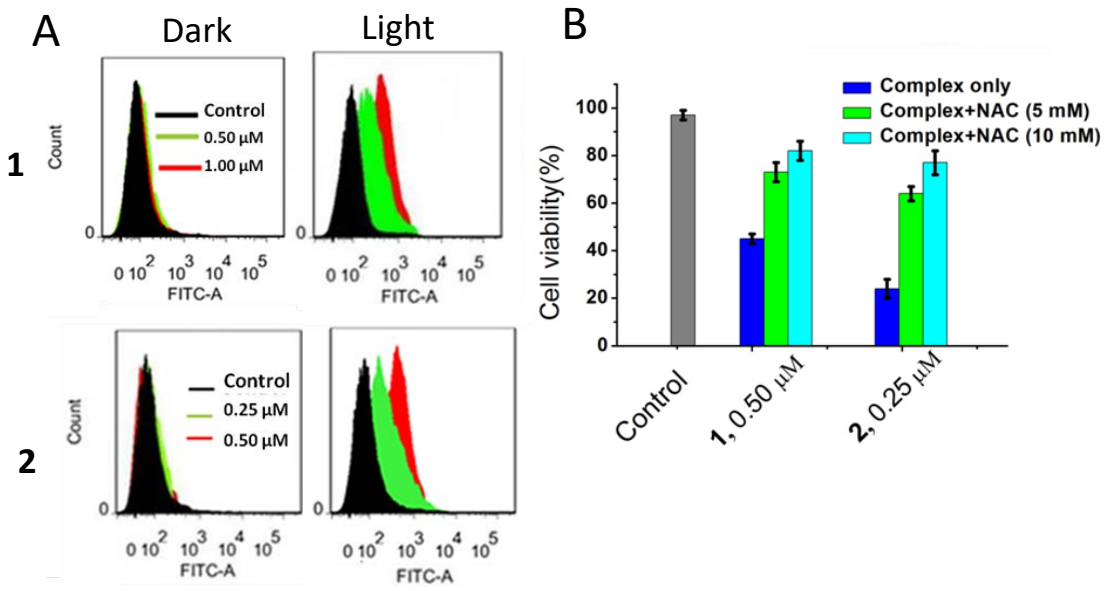


Figure S19 (A) Intracellular ROS levels in Ir(III)-treated A549 cells measured by DCF assay with flow cytometry in the absence and presence of light (425 nm, 5 min); (B) Dose-dependent inhibition of PDT-induced cell death upon incubation of A549 cells with ROS inhibitor NAC.

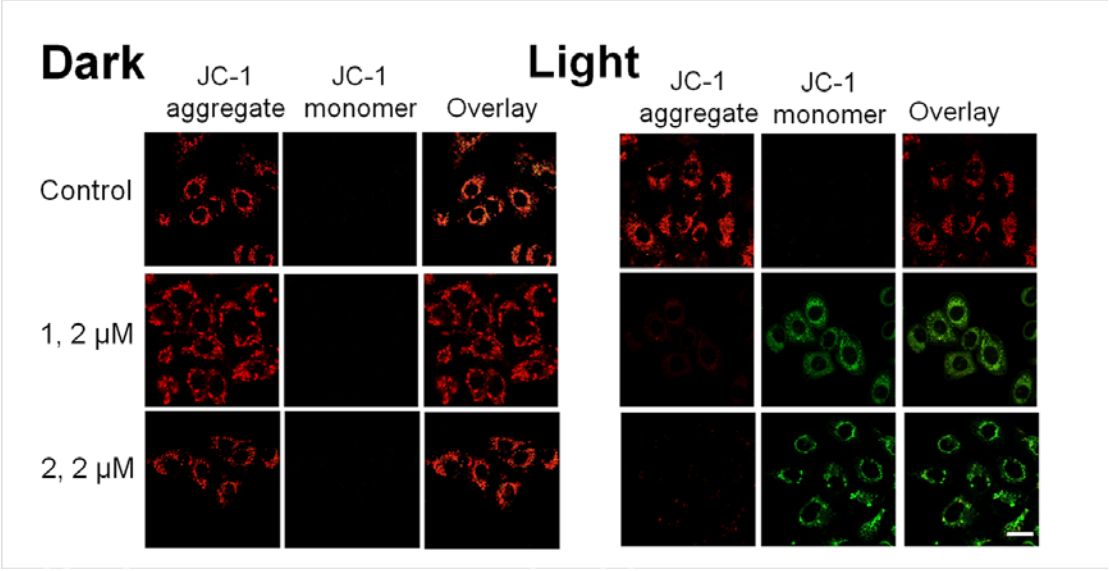


Figure S20 Confocal images of JC-1 labeled A549 cells incubated with complexes **1**-**2** at the indicated concentrations in the absence and presence of light irradiation (425 nm, 5 min), reflecting the mitochondrial membrane potential changes. Scale bar: 10 μm.

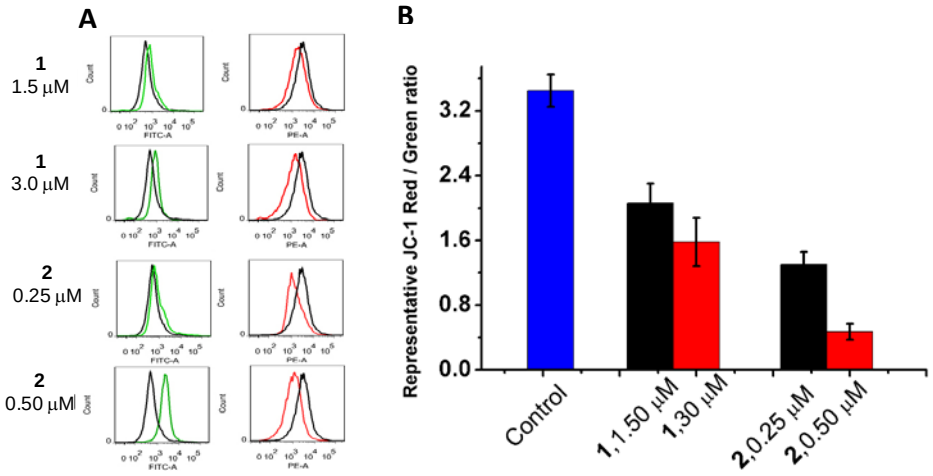


Figure S21 (A) Effect of **1-2** on the MMP in the presence of light irradiation; (B) The ratio of average intensity of Red/Green fluorescence of JC-1 in A549 cells treated with complexes **1-2** in the presence of light irradiation (425 nm, 5 min).

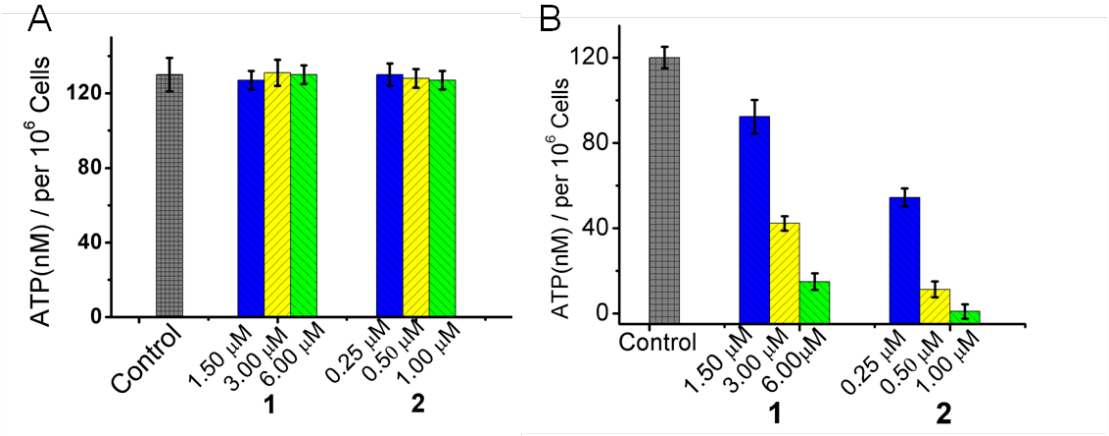


Figure S22 Intracellular ATP levels in A549 cells treated complexes **1-2** at the indicated concentrations for 6 h in the (A) absence and (B) presence of light irradiation (425 nm, 5 min).

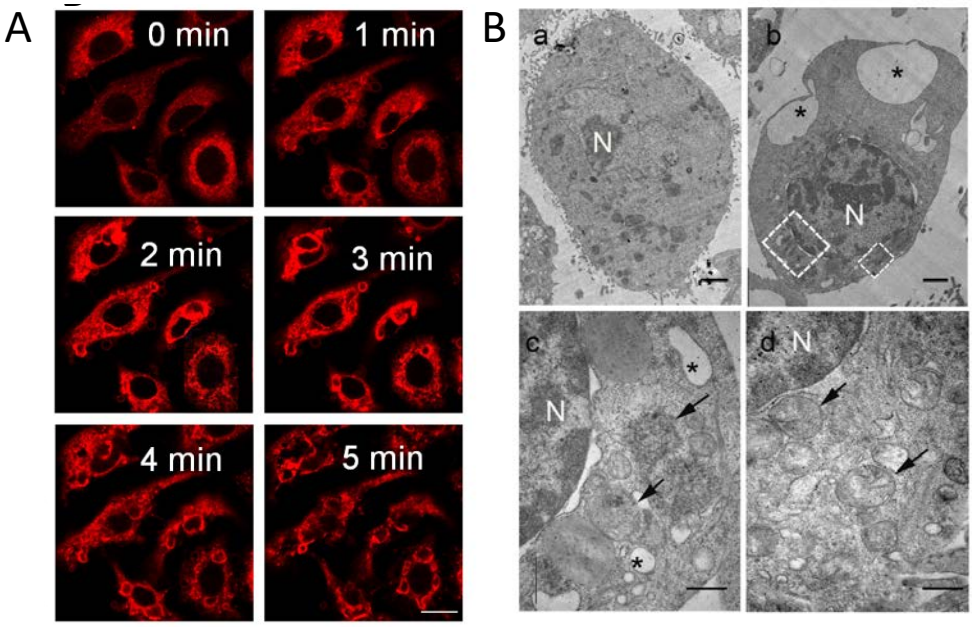


Figure S23 (A) Real-time tracking of the morphological changes of mitochondria in **1**-treated A549 cells after light irradiation (425 nm, 10 min); (B) Representative TEM images showing the morphological features of (a) untreated and (b) **1**-treated (0.1 μM, 24 h) A549 cells in the presence of irradiation (425 nm, 40 mW·cm^{-2}, 10 min). Figure c) and d) are the enlarged view of figure b) in the framed area respectively. Black arrows indicate swollen mitochondria; * indicates empty vacuoles in apoptotic cells. Scale bars: 2 μm.

Table S1 Photophysical data of complexes **1-2**, **1a-2a**.

Compounds	Medium	λabs, max (nm)	λem, max (nm)	τav/ns
1	PBS	405	604	56.83
	CH_2Cl_2	410	605	121.09
2	PBS	402	654	34.79
	CH_2Cl_2	415	655	542.08
1a	PBS	406	602	91.91
	CH_2Cl_2	410	606	119.68
2a	PBS	403	654	52.44
	CH_2Cl_2	411	656	536.01

Table S2 The $^1O_2$ quantum yields (Φ_Δ) of complexes **1-2**, **1a-2a** in aerated DMSO and PBS upon 425 nm irradiation.

Compounds	Φ_Δ[a]	
	DMSO	PBS
1	0.80	0.011
1a	0.71	0.004
2	0.58	0.047
2a	0.47	0.077

[a] MB (Φ_Δ = 0.52 in DMSO) and $Ru(bpy)_3Cl_2$ (Φ_Δ = 0.18 in H_2O) were used as the reference.

Table S3 IC_{50} (μM) values of tested compounds towards different cell lines.

	IC_{50} (μM)[a]							
	A549		**A549R**		**HeLa**	**LO2**		
	Dark	PI[c]	Dark	PI	Dark	PI	Dark	PI
Compound	(Light)[b]		(Light)		(Light)		(Light)	
1	41.3 ± 1.5	376	38.3 ± 1.5	348	40.7 ± 2.1	339	42.2 ± 1.3	9.9
	(0.11 ± 0.03)		(0.11 ± 0.04)		(0.12 ± 0.03)		(4.25 ± 0.42)	
1a	3.12 ± 0.11	78	2.70 ± 0.60	30	4.20 ± 0.60	42	3.25 ± 0.31	1.9
	(0.04 ± 0.01)		(0.09 ± 0.03)		(0.10 ± 0.04)		(1.75 ± 0.12)	
2	22.2 ± 0.9	1110	23.4 ± 2.3	1170	25.1 ± 2.4	697	20.1 ± 1.2	168
	(0.020 ± 0.008)		(0.020 ± 0.004)		(0.036 ± 0.010)		(0.12 ± 0.05)	
2a	1.56 ± 0.04	173	1.30 ± 0.80	130	1.40 ± 0.50	140	3.11 ± 0.14	82
	(0.010 ± 0.005)		(0.010 ± 0.004)		(0.010 ± 0.006)		0.038 ± 0.009	
L	> 100	1.0	> 100	1.0	> 100	1.0	> 100	1.0
	(> 100)		(> 100)		(> 100)		(> 100)	
cisplatin	28.1 ± 0.3	1.2	92.1 ± 3.4	1.1	27.2 ± 0.4	1.2	35.6 ± 1.1	1.2
	(23.2 ± 2.1)		(82.7 ± 2.4)		(24.6 ± 1.7)		(29.4 ± 1.0)	

[a] Cells were incubated with the indicated compounds for 48 h in total. [b] Irradiation with light at 425 nm for 10 min (24 J cm^{-2}) was applied after 24 h incubation and media refreshment. [c] PI (phototoxicity index) is the ratio of the IC_{50} values in dark to that obtained upon light irradiation.

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