Supporting Information

8-Hydroxyquinoline-modified ruthenium(II) polypyridyl complexes for JMJD inhibition and photodynamic antitumor

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Supporting Methods

$^1O_2$ detection

The DMSO solutions containing **Ru1** or **Ru2** and DPBF (50 μM) were blown several times with the gun tip and then irradiated with a light source at 450 nm (30 J cm^{-2}). The change in the absorption spectra of DPBF at 418 nm was recorded at 2 s intervals. MB was used as the reference compound (Φ_Δ = 0.52). The absorbance of **Ru1**, **Ru2** and MB at 450 nm was adjusted to be around 0.15 at the beginning of the experiments. The Φ_Δ of the **Ru1** or **Ru2** were calculated by the following equation:

$$\Phi_\Delta^{Ru(II)} = \Phi_\Delta^{MB} \times (s^{Ru(II)} \times F^{MB})/(s^{MB} \times F^{Ru(II)})$$

The equation s is the slope of the absorbance curve of DPBF at 418 nm with time, and F is the absorption correction factor ($F = 1\text{-}10^{-OD}$, OD is the optical density at the irradiation wavelength).

Stability test

The stability of **Ru1** and **Ru2** in PBS and 0.1% DMSO solution was evaluated by analyzing their UV–Vis spectra at 0 h, 24 h, and 48 h.

ICP-MS determination

A549 cells were incubated overnight in 6-well plates, then the medium was removed and replaced with fresh medium containing **Ru1** (20 μM) or **Ru2** (20 μM). After 6 hours of incubation, the cells were collected, counted, and subsequently digested with HNO_3. Finally, the samples were detected by ICP-MS.

Binding of Ru1 or Ru2 with Fe^{2+}

The binding of **Ru1** or **Ru2** with Fe^{2+} was recorded by UV–Vis absorption spectra and fluorescence emission spectra. Firstly, 1 mM stock solution of **Ru1** or **Ru2** was prepared by dissolving in DMSO, then which was diluted to 10 μM with PBS. Secondly, the indicated concentrations of $FeSO_4$ solutions were prepared in distilled water. The different concentrations of Fe^{2+} solutions were added to **Ru1** or **Ru2** solutions, followed by incubation at room temperature for 3 min. After that, the changes of UV–Vis absorption spectra and fluorescence emission spectra of **Ru1** and **Ru2** were monitored. The excitation wavelength of the Ru(II) compounds was 450 nm.

The fluorescence quenching data of **Ru1** or **Ru2** upon addition of Fe^{2+} were analysed using the Stern–Volmer equation[1, 2]:

$$F_0/F = 1 + K_{SV}[Q]$$

Where F_0 and F are the fluorescence intensities of **Ru1** or **Ru2** in the absence and presence of Fe^{2+}, respectively, K_{SV} is the Stern-Volmer quenching constant, and [Q] is the concentration of quencher *i.e.*, Fe^{2+} (0–200 μM).

Cell lines and culture conditions

HeLa, HepG2, A549, A549R, MCF-7 and LO2 cell lines were purchased from Nanjing KeyGen Biotechnology Co., Ltd., and PC3 cell line was purchased from Kunming Cell Bank, Chinese Academy of Sciences. Cell lines were cultured in DMEM or RPMI 1640 complete medium at 37 ℃ with 5% CO_2 atmosphere.

Phototoxicity test in vitro

The phototoxicities of **Ru1** and **Ru2** were analyzed in HeLa, HepG2, A549, A549R, PC3, MCF-7 and LO2 cell lines and determined by MTT assay. A series of preliminary experiments were used to determine the optimal light dose, based on the criteria that an obvious photocytotoxicity in cells could be achieved for **Ru1** and **Ru2** and no statistical difference in viability was observed between the dark and the irradiated samples. The final light treatment condition was 450 nm, 30 J cm^{-2}, 3 min. Cells were placed in 96-well plates and cultured for 24 h at 37 ℃ in a 5% CO_2 incubator. After replacing the culture medium with fresh medium containing **Ru1** or **Ru2** at the indicated concentrations, cells were cultured for 12 h. And then the media was replaced with fresh media, cells were irradiated at 450 nm for 3 min. After another 36 h of incubation, 20 μL MTT was added to each well 4 h before the end of the incubation. Subsequently, the liquid in the cell wells was poured out and 150 μL/well DMSO was added to dissolve the MTT-formazan crystals. The absorbance of living cells at 570 nm was detected.

To investigate the impact of different ROS scavengers on antiproliferative activities of **Ru1** and **Ru2**, A549 cells were seeded in 96-well plates for 24 h ant then incubated with the ROS scavengers (D-mannitol: 50 mM; Trion: 5 mM; Sodium pyruvate: 10 mM) for 1 h. Then the cells were incubated with **Ru1** and **Ru2** (5 μM) for 44 h. 20 μL MTT (5 mg/mL) was added to each well and incubated for another 4 h. The cell viability was measured as described above.

Light treatment methods for cells in different biological experiments

For Western blot analysis, Annexin V staining or Hoechst 33342 staining assay: A549 cells seeded in confocal dishes or 6-well plates were cultured for 24 h and then washed with PBS. The indicated concentrations of **Ru1** or **Ru2** in fresh medium were added to each well and incubated for

12 h. Then, medium was removed and fresh RMPI 1640 containing 10% FBS was added. The plates were then irradiated at 450 nm (30 J cm^{-2}) for 3 min. The cells were incubated for another 12 h.

For Measurement of intracellular ROS or MMP: A549 cells were seeded in 6-well plates for 24 h and then washed with PBS. The indicated concentrations of **Ru1** or **Ru2** in fresh medium were added to each well and incubated for 3 h. Then, medium was removed and fresh RMPI 1640 containing 10% FBS was added. The plates were then irradiated at 450 nm (30 J cm^{-2}) for 3 min. The cells were incubated for another 3 h.

Western blot analysis

A549 cells were treated with the indicated concentrations of **Ru1** or **Ru2** in absence or presence of light. After 24 h, cells were harvested in lysis buffer and then centrifuged at 12,000 g (4 °C) for 10 min. The protein concentration was determined using the BCA assay kit. Equal amounts of protein were ran on SDS-PAGE and then transferred to the PVDF membrane. After 2 h of closure with milk powder, the membrane was incubated with primary antibodies against H3K9me3 (Cell Signaling Technology, USA, #13969, 1:1000), caspase-3 (Cell Signaling Technology, USA, #14220, 1:1000) and PARP (Cell Signaling Technology, USA, #9532, 1:1000) overnight, followed by 1 h with secondary antibodies (Beyotime Biotechnology, China, A0208, 1:1000). Finally, the images were displayed by CLINX ChemiScope S6 imager.

Annexin V-FITC Staining

After treatment with the indicated concentrations of **Ru1** or **Ru2** in absence or presence of light, cells were harvested from the plates using 0.25% trypsin-EDTA and resuspended in 500 μL binding

buffer containing 5 μL annexin V. Subsequently they were incubated for 10 min at 25 °C in the dark. Cells were analyzed by flow cytometry immediately (λ_{ex} = 488 nm, λ_{em} = 530 nm ± 20 nm).

Hoechst 33342 staining assay

A549 cells were treated with the indicated concentrations of **Ru1** or **Ru2** in absence or presence of light. After 24 h, cells were fixed with 4% paraformaldehyde and then labelled with Hoechst 33342 (5 μg/mL) at 37 °C for 10 min. After washing twice with PBS, cells were detected by confocal microscopy (λ_{ex} = 405 nm, λ_{em} = 460 ± 20 nm).

Measurement of intracellular ROS

A549 cells were treated with the indicated concentrations of **Ru1** or **Ru2** in absence or presence of light. After 6 h incubation, the cells were stained with 10 μM of H_2DCFDA at 37 °C for 10 min, then washed three times with PBS, and detected by confocal microscopy (λ_{ex} = 488 nm, λ_{em} = 530 ± 20 nm).

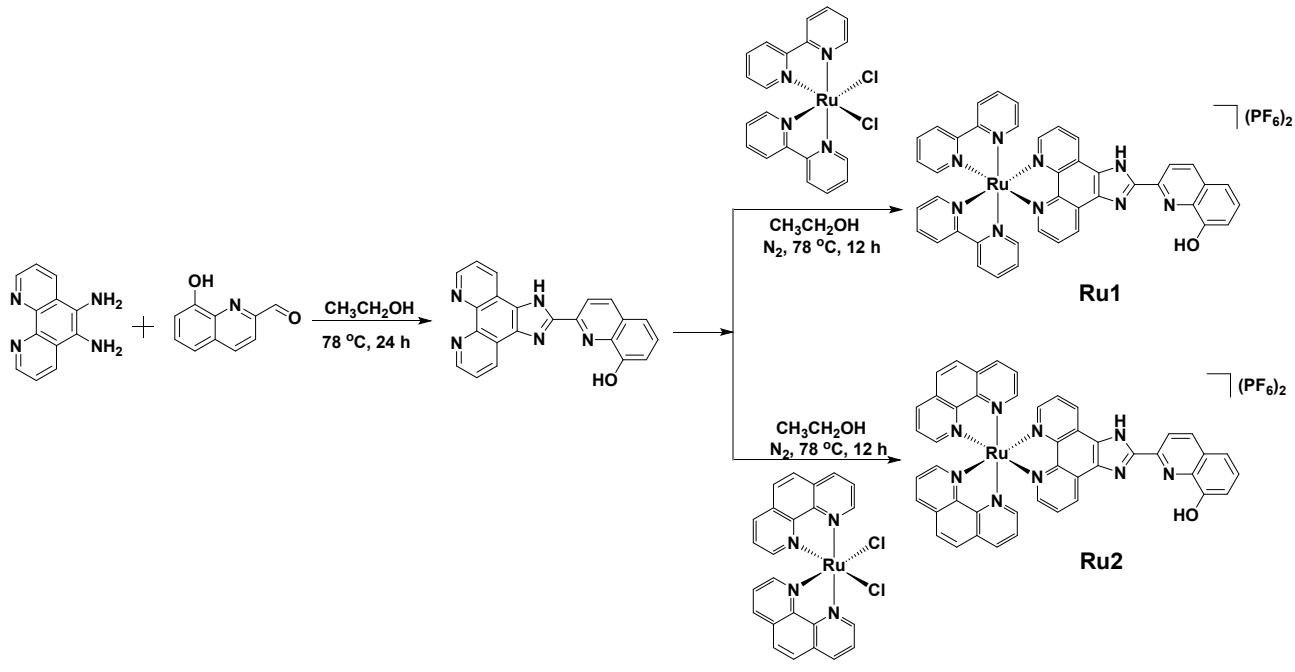
Measurement of MMP

A549 cells were treated with the indicated concentrations of **Ru1** or **Ru2** in absence or presence of light. After 6 h incubation, the cells were incubated with Rh123 (1 μg/mL) at 37 °C for 30 min, then washed with PBS twice. Cells were detected by confocal microscopy (λ_{ex} = 488 nm, λ_{em} = 530 ± 20 nm).

Statistical analysis

Biological experiments were repeated at least 3 times and the results were presented as means ± SD.

Supporting Figures



Scheme S1. Synthetic routes of complexes **Ru1** and **Ru2**.

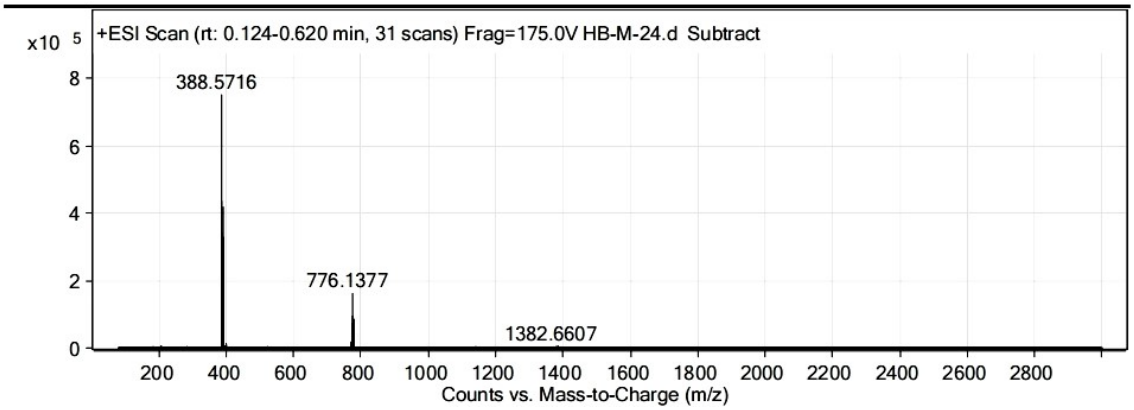


Fig. S1. ESI-MS characterization of **Ru1**. ESI-MS (CH_3CN): m/z 388.5716 $[M-2PF_6]^{2+}$, 776.1377 $[M-PF_6]^{+}$.

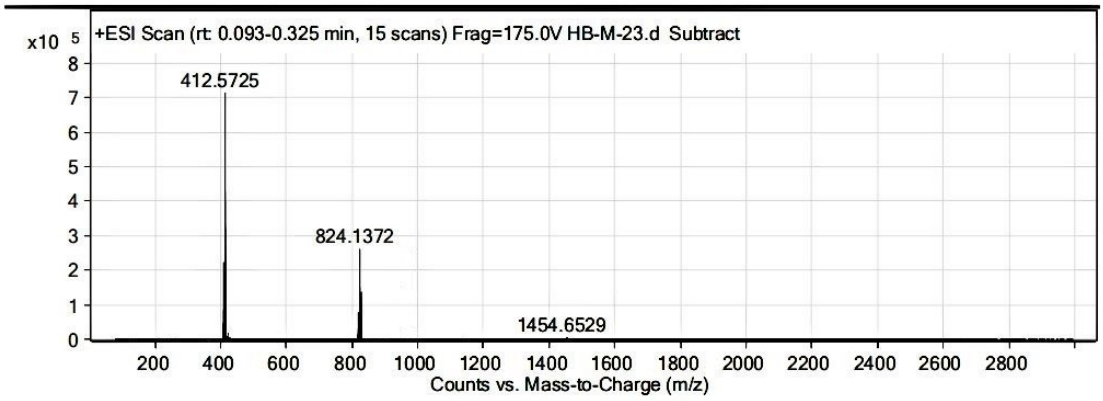


Fig. S2. ESI-MS characterization of **Ru2**. ESI-MS (CH_3CN): m/z 412.5725 $[M-2PF_6]^{2+}$, 824.1372 $[M-PF_6]^{+}$.

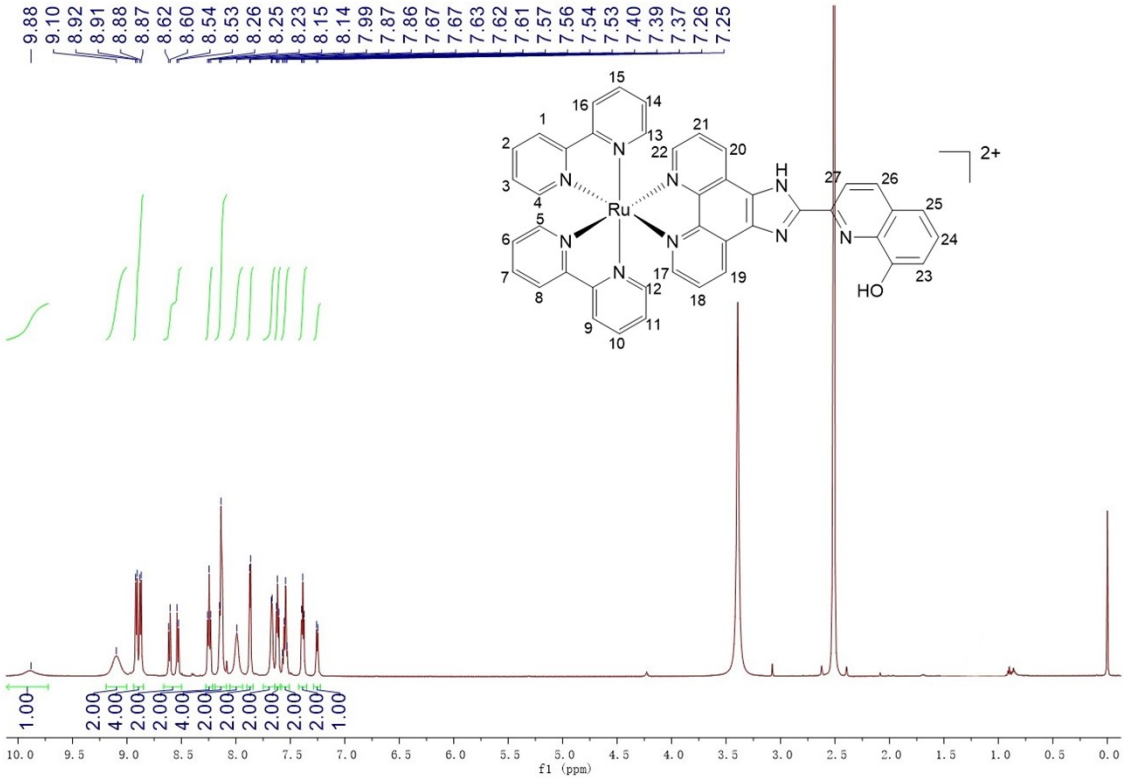


Fig. S3. ^{1}H NMR spectrum of **Ru1** in $(CD_3)_2SO$.

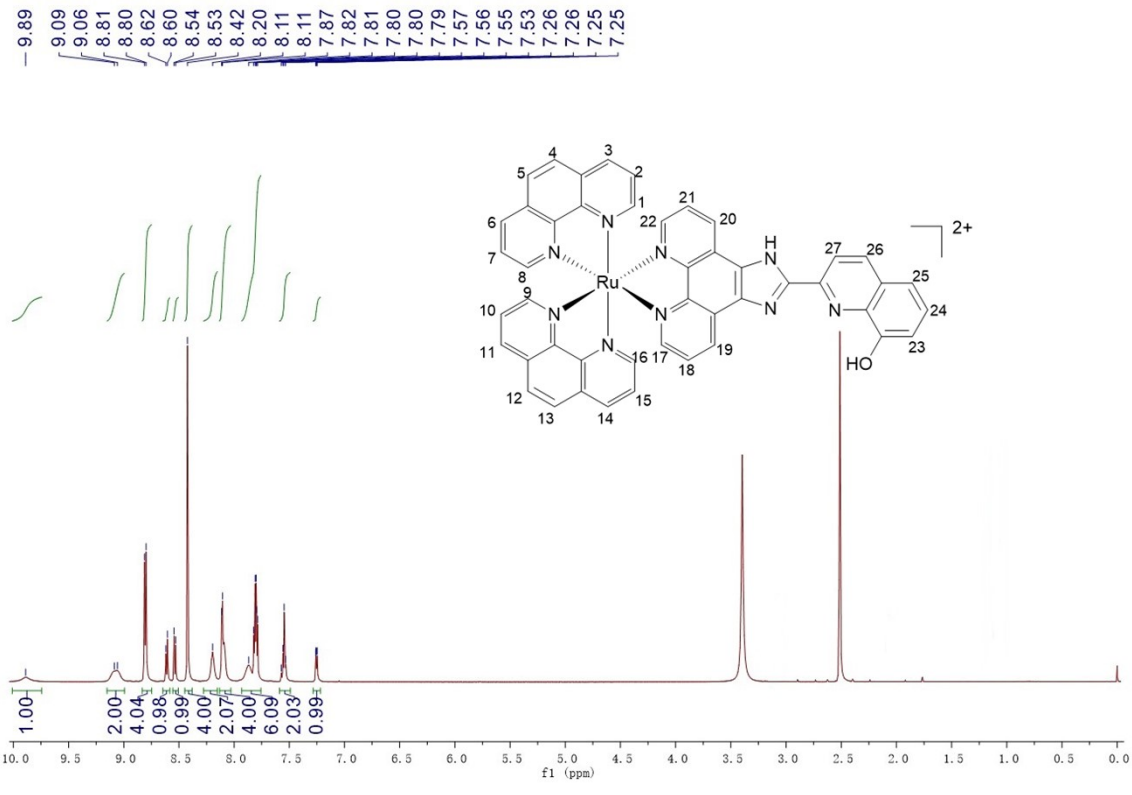


Fig. S4. ^{1}H NMR spectrum of **Ru2** in $(CD_3)_2SO$.

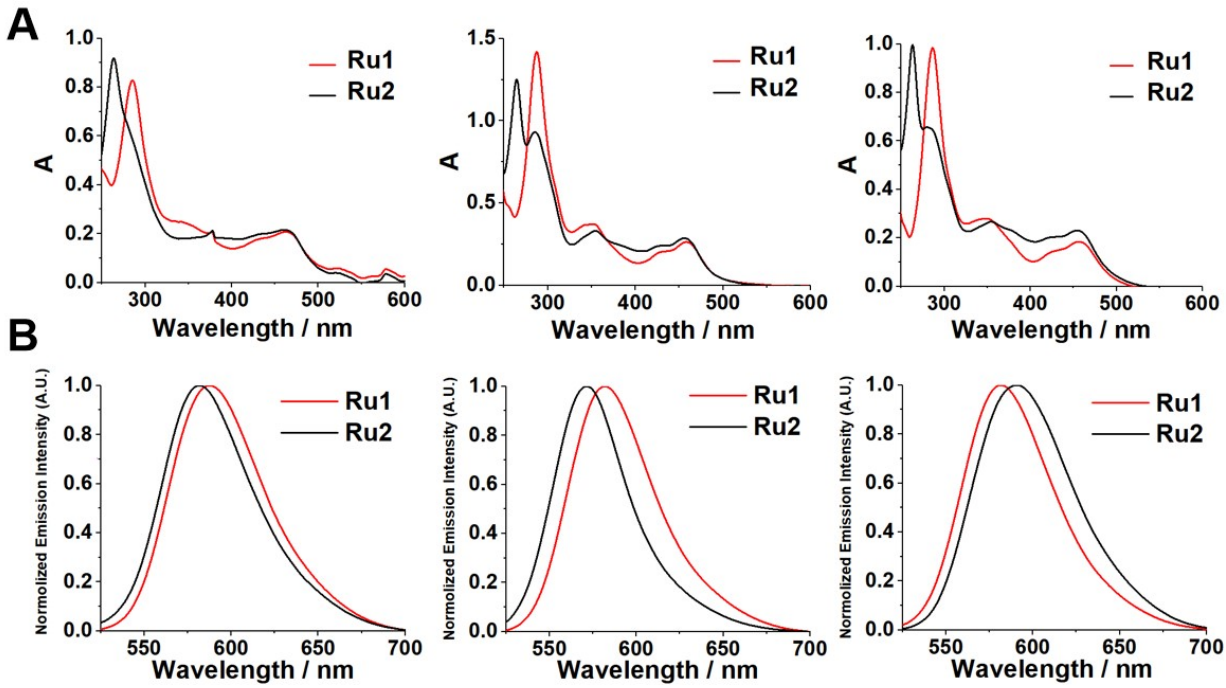


Fig. S5. UV/Vis (A) and emission spectra (B) of **Ru1** (10 μM) and **Ru2** (10 μM) measured in PBS, CH_2Cl_2 and CH_3CN at 298 K.

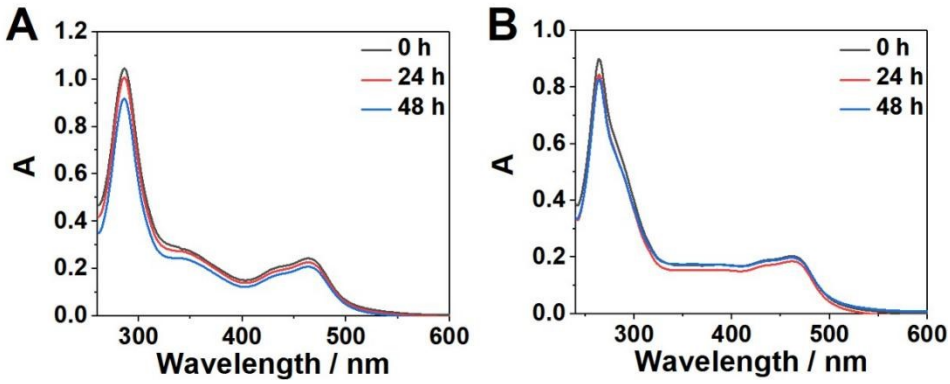


Fig. S6. UV-Vis absorption spectra of **Ru1** (A) and **Ru2** (B) in PBS solution collected at 0, 24, and 48 h, respectively.

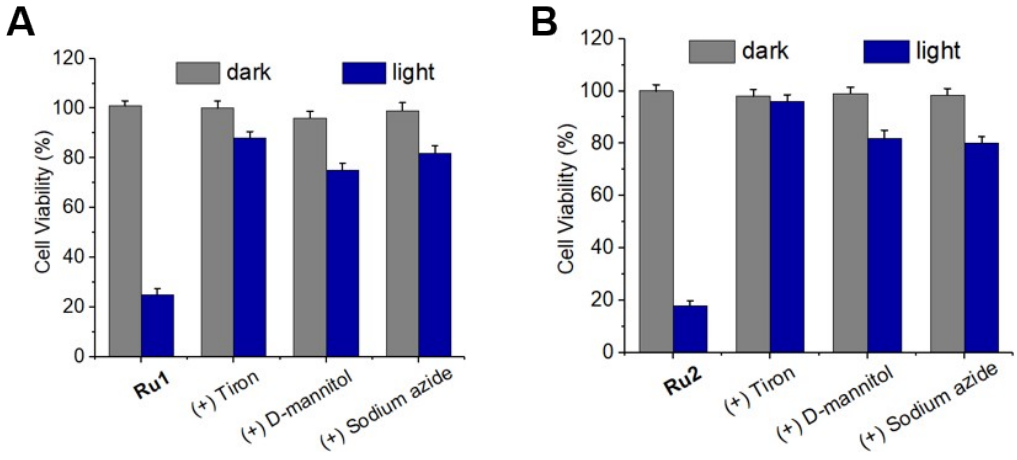


Fig. S7. The viability of A549 cells with off/on irradiating conditions (450 nm, 30 J cm^{-2}, 3 min) after **Ru1** or **Ru2** (5 μM, 48 h) treatment with or without different ROS scavengers.

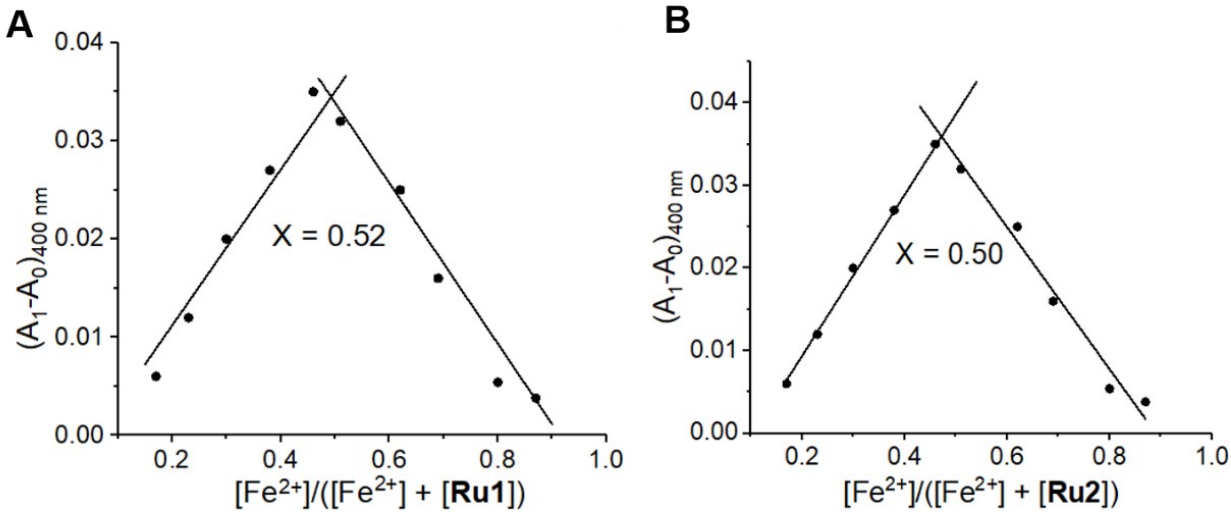


Fig. S8. Job's plot for **Ru1** (A) or **Ru2** (B) and Fe^{2+}, indicating the formation of 1:1 complex.

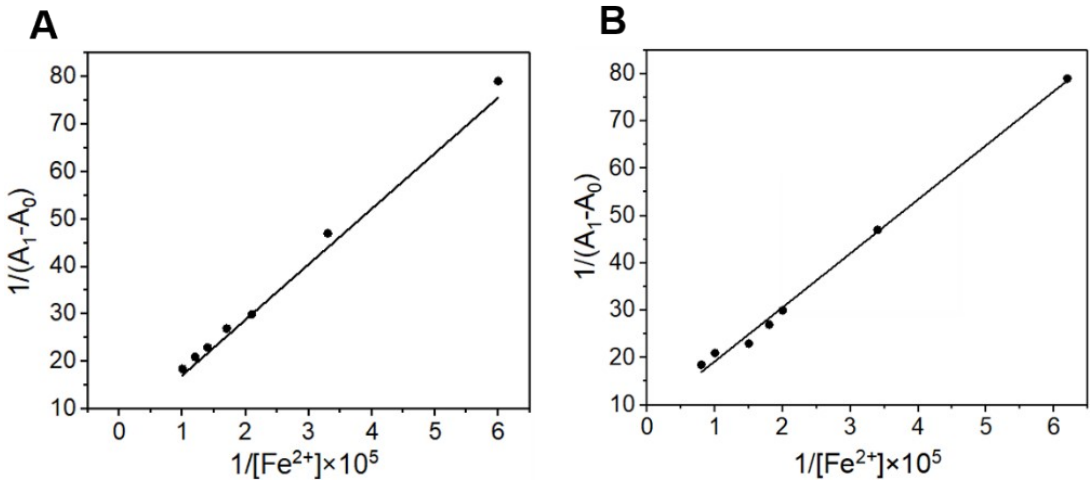


Fig. S9. The binding constant values of Fe^{2+} with **Ru1** (A) or **Ru2** (B) have been determined from the UV-Vis titration data following the modified Benesi-Hildebrand equation.

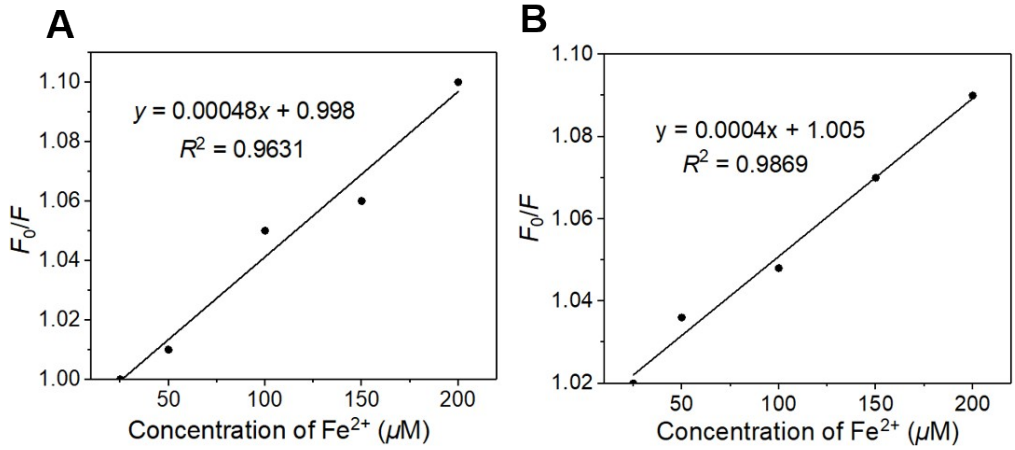


Fig. S10. The Stern–Volmer plot for **Ru1**-Fe^{2+} system (A) and **Ru2**-Fe^{2+} system (B).

Table S1 Photophysical data of the complexes in different solutions.

Compounds	Medium	$\lambda_{abs,\ max}$ (nm)	$\lambda_{em,\ max}$ (nm)
Ru1	PBS	463	588
	CH_2Cl_2	458	582
	CH_3CN	456	582
Ru2	PBS	460	582
	CH_2Cl_2	456	573
	CH_3CN	454	591

Table S2 IC_{50} values of tested compounds in different cell lines [a]

Compound	IC_{50} (μM)							
	HepG2		PC3		MCF-7		HeLa	
	dark (light)	PI [b]	dark (light)	PI	dark (light)	PI	dark (light)	PI
Ru1	> 100 (7.0 ± 0.8)	> 14.2	21.3 ± 1.8 (8.7 ± 0.5)	2.4	> 100 (4.7 ± 0.5)	> 21.2	50.1 ± 3.8 (5.0 ± 0.6)	10.0
Ru2	> 100 (3.9 ± 2.8)	> 25.6	21.7 ± 0.6 (2.8 ± 0.2)	7.7	50.0 ± 1.8 (2.1 ± 0.4)	23.8	44.6 ± 0.6 (3.7 ± 0.5)	12.0
Cisplatin	23.5 ± 2.0 (21.3 ± 0.8)	1.1	21.5 ± 3.2 (20.3 ± 0.8)	1.0	35.8 ± 2.8 (34.2 ± 0.7)	1.0	18.3 ± 0.5 (15.2 ± 0.6)	1.2

[a] IC_{50} values are drug concentrations necessary for 50% inhibition of cell viability. The data are presented as mean ± SD and cytotoxicity is assessed after 48 h of incubation.

[b] PI refers to the phototoxicity index, which is the ratio between the IC_{50} values in the dark upon light irradiation.

Supporting References

1. T. Senthilkumar, N. Parekh, S. B. Nikam and S. K. Asha, *J. Mater. Chem. B.*, 2016, **4**, 299-308.
2. S. Konar, D. Samanta, S. Mandal, S. Das, M. K. Mahto, M. Shaw, M. Mandal and A. Pathak, *RSC Adv.*, 2018, **8**, 42361-42373.