

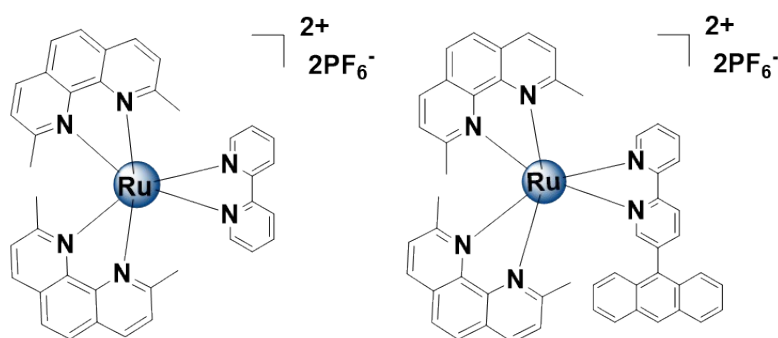
Supporting Information

Novel Ru(II) complexes with multiple anticancer photoreactivity: ligand exchange, photoredox catalysis, reactive oxygen generation and endoperoxide formation

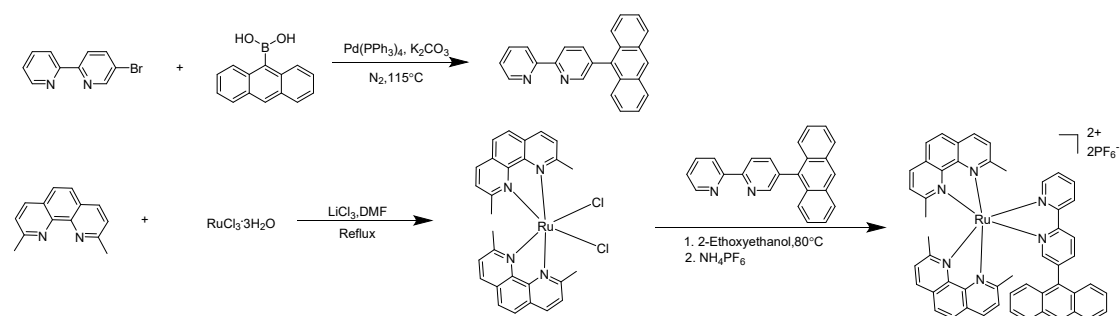
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1. Schemes

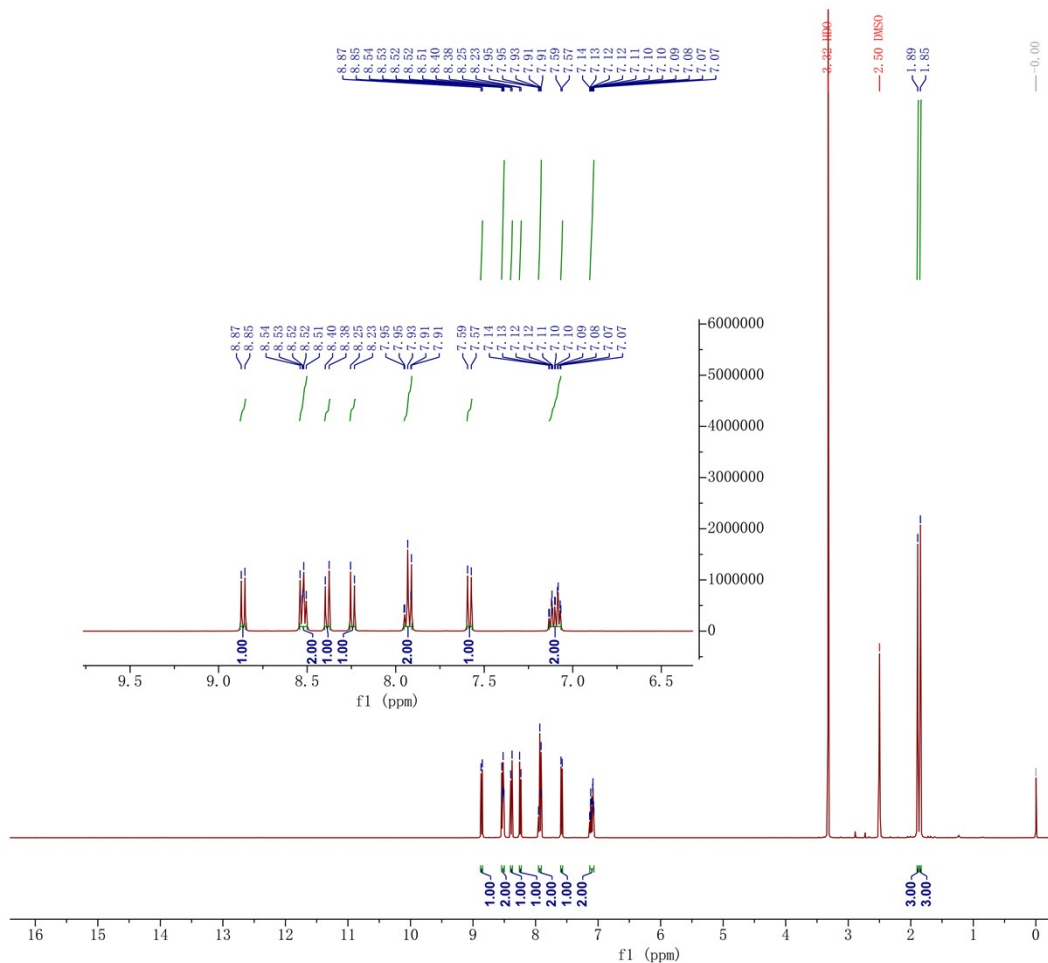


Scheme S1. Structure of Ru1 and Ru2.



Scheme S2. Synthetic scheme for complex Ru2.

2. Figures



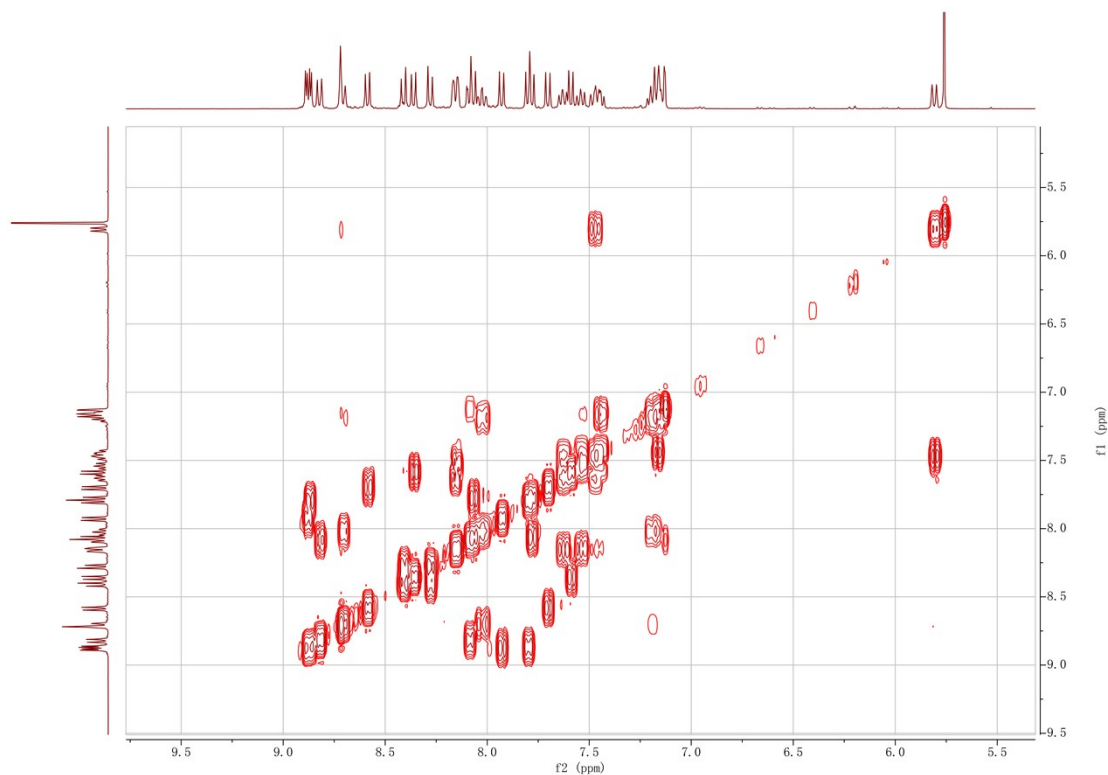


Fig. S2. ^1H - ^1H COSY spectra (500 MHz, $\text{DMSO-}d_6$) of **Ru1** and **Ru2**.

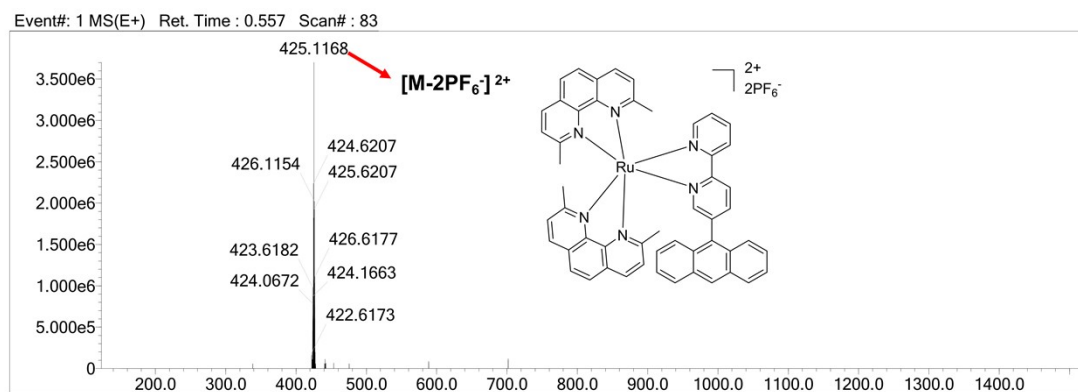


Fig. S3. HR-ESI-MS spectra of **Ru2**.

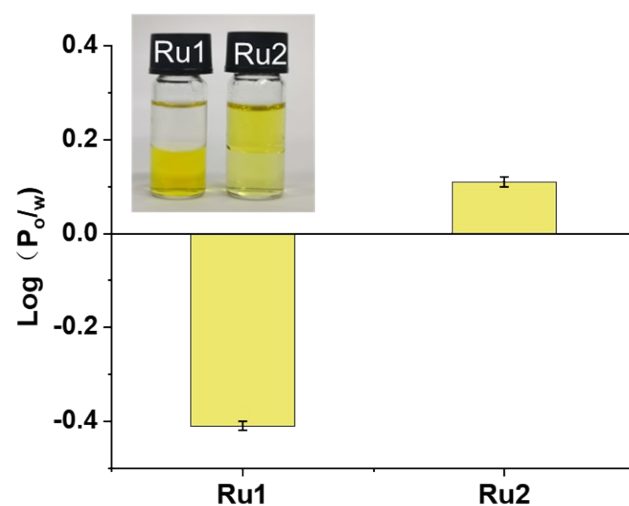


Fig. S4. Octanol/water partition coefficients of **Ru1** and **Ru2**.

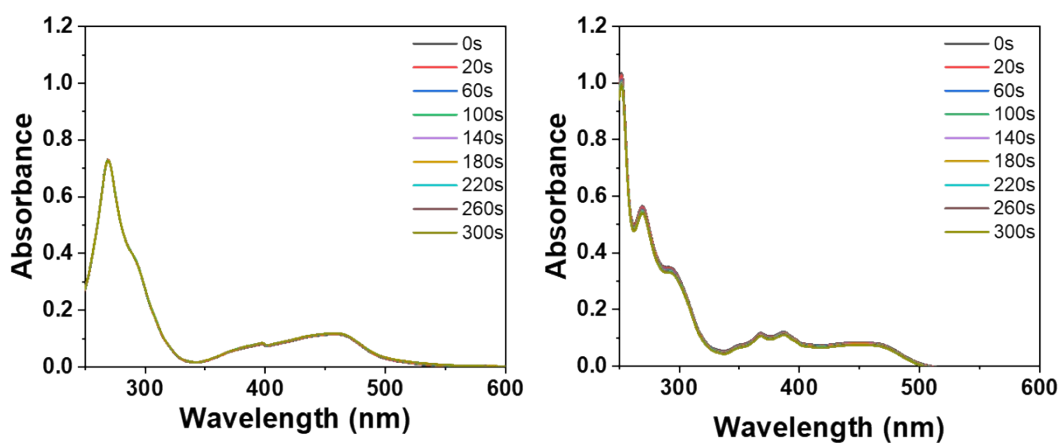


Fig. S5. Dark stability of **Ru1** (Left) and **Ru2** (Right) in PBS.

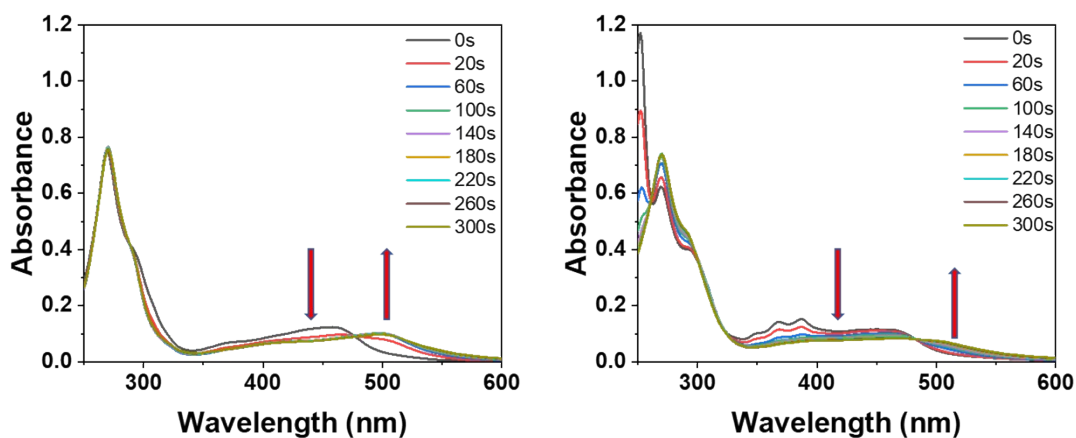


Fig. S6. Absorption spectra changes of **Ru1**(Left) and **Ru2**(Right) upon white light irradiation (11.6 J/cm^2).

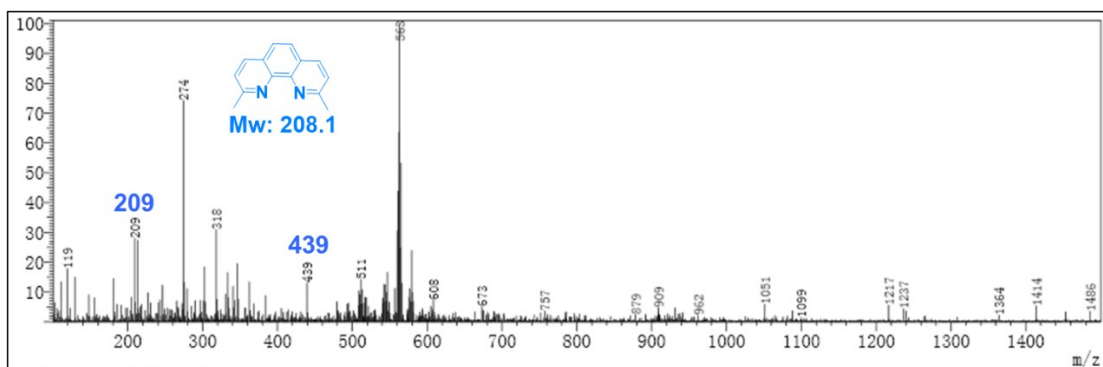


Fig. S7. Mass spectra showing ligand dissociation of **Ru1** in methanol after illumination (11.6 J/cm^2).

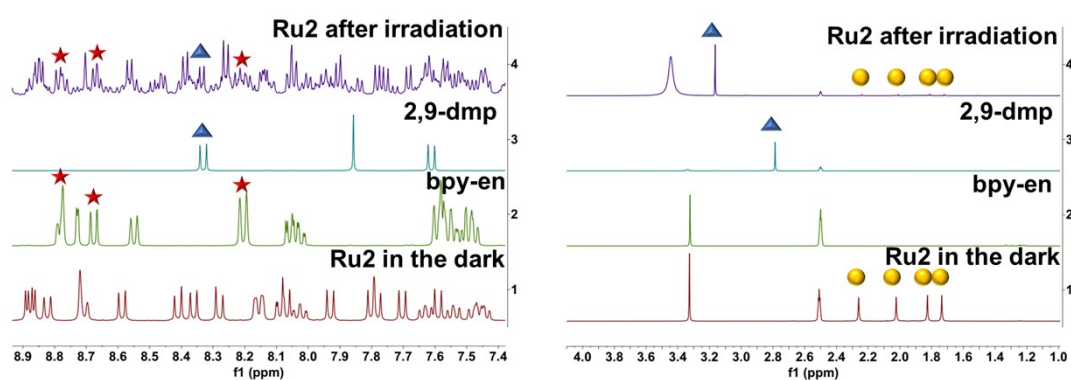


Fig. S8. ^1H NMR spectra showing ligand dissociation of **Ru2** after illumination (11.6 J/cm^2).

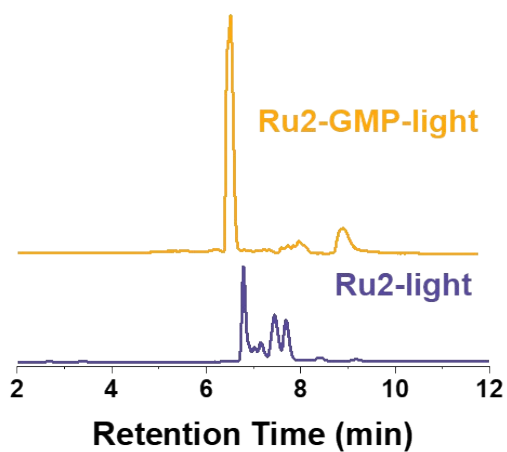


Fig. S9. HPLC of GMP and **Ru2** after irradiation. (11.6 J/cm^2).

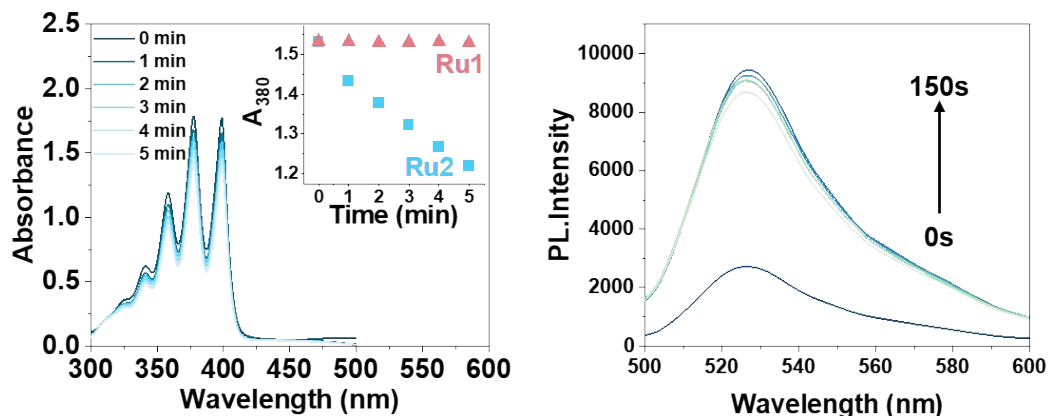


Fig. S10. $^1\text{O}_2$ generation by **Ru2** ($10\ \mu\text{M}$) upon white light irradiation ($38.6\ \text{mW}/\text{cm}^2$) with ABDA (left) and SOSG (right).

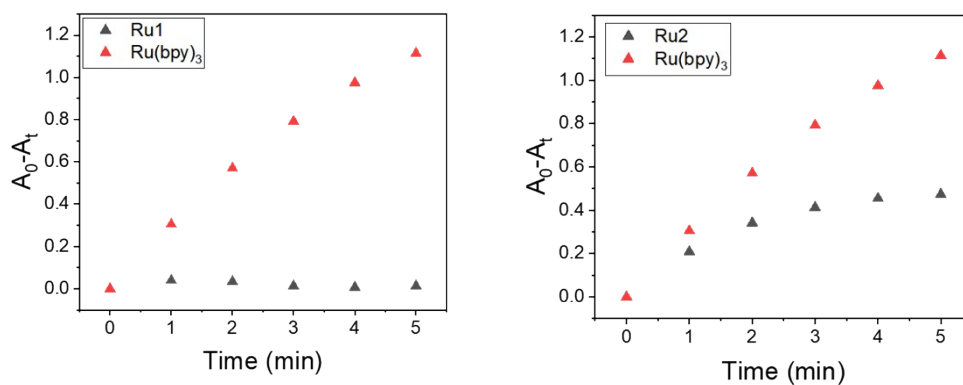


Fig. S11. Determination of the quantum yield for the $^1\text{O}_2$ generation by the complex **Ru1** and **Ru2** or $\text{Ru}(\text{bpy})_3\text{Cl}_2$ (as reference) in PBS under white light irradiation.

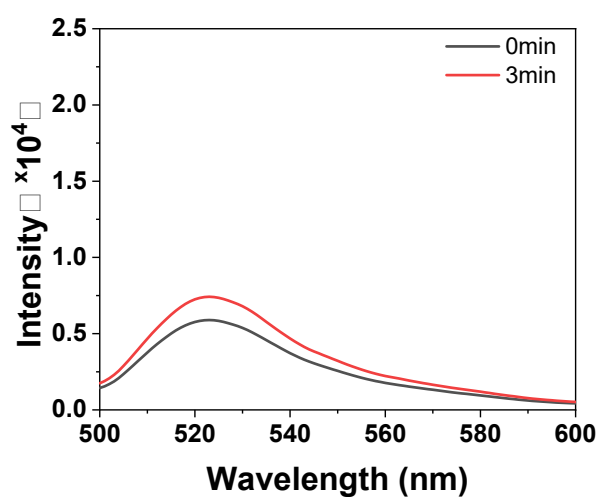


Fig. S12. Fluorescence intensity changes of DHR123 ($10\ \mu\text{M}$) in the dark.

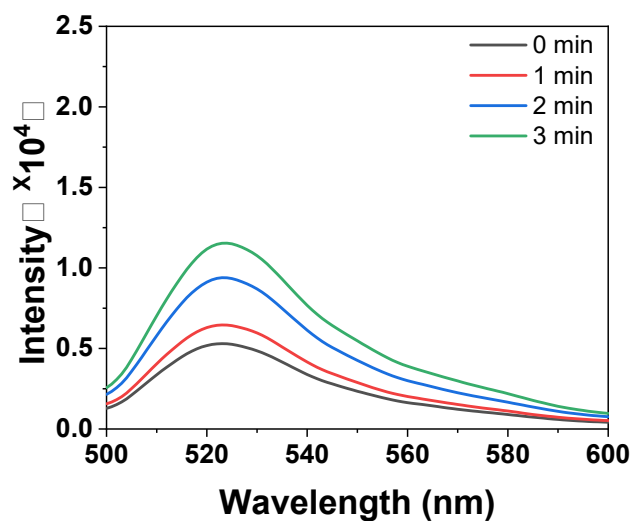


Fig. S13. $O_2^{\cdot -}$ detection using the DHR123 ($10 \mu M$) assay for Ru1 ($5 \mu M$) in aqueous solution after white light irradiation ($7 J/cm^2$).

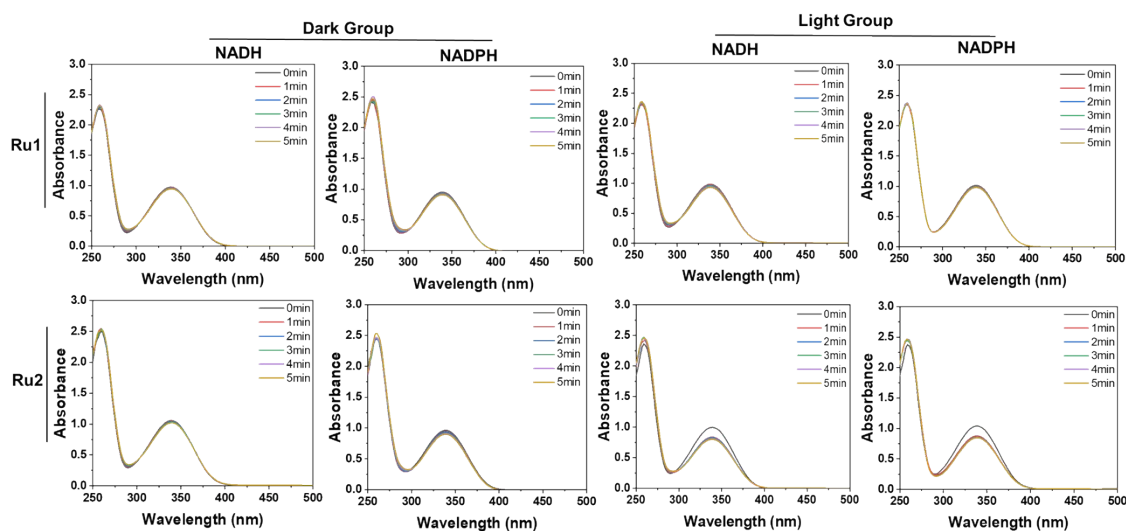


Fig. S14. UV-vis spectra showing the photocatalytic oxidation of NADH ($160 \mu M$) and NADPH ($160 \mu M$) by Ru1 and Ru2 ($10 \mu M$) in aqueous solution.

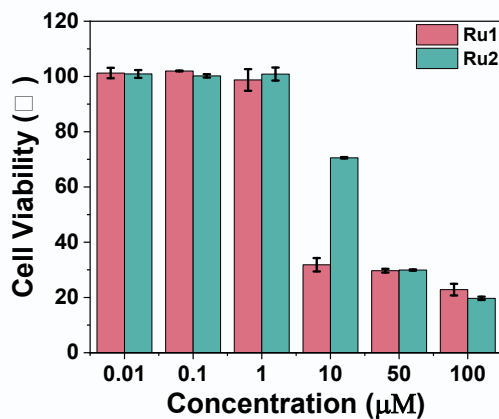


Fig. S15. Dark toxicity of Ru2 toward non-tumorigenic cell lines HEK-293T.

	A549(21% O ₂)			A549(5% O ₂)		
	Dark ^a	Light ^b	PI ^c	Dark ^a	Light ^b	PI ^c
Ru1	28.3±1.6	8.8±0.7	3.2	>100	40.7±2.1	2.5
Ru2	65.0±1.1	0.29±0.04	224.1	81.3±3.1	10.3±2.8	7.9
Cisplatin	2.4±0.2	1.8±0.1	1.3	3.7±1.4	4.1±1.5	0.9
5-FU	67.1±1.4	66.8±1.1	1.0	88.3±0.9	86.4±1.2	1.0
Ce6	22.4±0.7	0.21±0.08	106.7	17.4±2.3	8.4±1.7	2.1

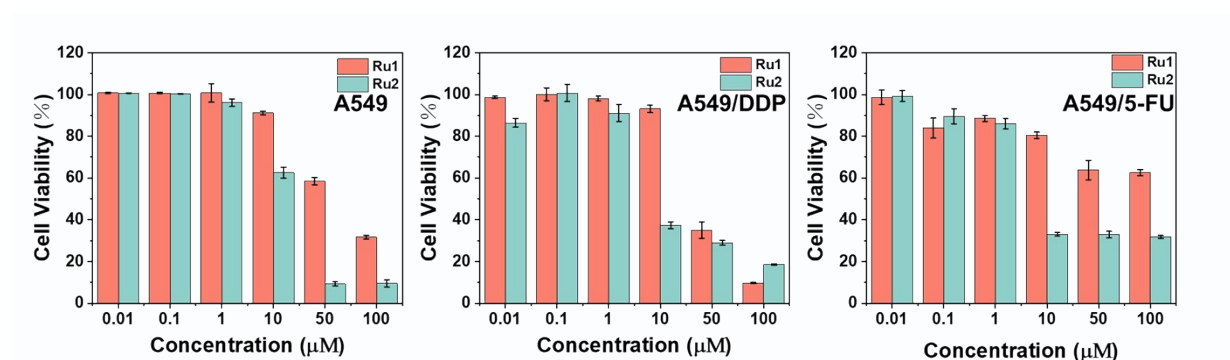


Fig. S16. Antiproliferative activity of **Ru1** and **Ru2** against different tumor cell lines under hypoxia (5% O₂) conditions.

3. Tables

Table S1. Dark and photo IC₅₀ values (μM) and photo-cytotoxicity index (PI = IC₅₀ dark/IC₅₀ light) in A549 cells under normoxic and hypoxic conditions

^a48 h drug exposure in the dark.

^b16h drug exposure in the dark, followed by white light irradiation (11.6 J/cm²) for 5 min and further 32 h incubation.

^cPI = IC₅₀(Dark^a) / IC₅₀(Light^b).

5-FU =5-Fluorouracil, n.a. = Not applicable.

Table S2. Dark and photo IC₅₀ values (μM) and photo-cytotoxicity index (PI = IC₅₀ dark/IC₅₀ light) in A549/5-FU cells under normoxic and hypoxic conditions

	A549/5-FU(21% O ₂)			A549/5-FU(5% O ₂)		
	Dark ^a	Light ^b	PI ^c	Dark ^a	Light ^b	PI ^c
Ru1	66.6±3.7	21.6±2.4	3.1	>100	53.7±2.4	1.9
Ru2	67.2±2.1	0.66±0.09	101.8	>100	4.2±1.9	23.8
5-FU	>200	>200	n.a.	>200	>200	n.a.
Ce6	32.4±1.6	0.25±0.02	129.6	17.5±1.6	1.4±1.1	12.5

^a48 h drug exposure in the dark.

^b16h drug exposure in the dark, followed by white light irradiation (11.6 J/cm²) for 5 min and further 32 h incubation.

^cPI = IC₅₀(Dark^a) / IC₅₀ (Light^b).

5-FU =5-Fluorouracil , n.a. = Not applicable.

	A549/DDP(21% O ₂)			A549(5% O ₂)		
	Dark ^a	Light ^b	PI ^c	Dark ^a	Light ^b	PI ^c
Ru1	51.4±3.2	11.8±1.9	4.4	>100	>100	n.a.
Ru2	>100	0.13±0.03	769.2	62.8±2.3	1.2±0.6	52.3
Cisplatin	15.5±2.2	11.8±0.4	1.3	77.6±2.9	74.1±3.1	1.0
Ce6	63.5±1.7	0.36±0.2	176.4	21.4±2.4	8.0±1.2	2.7

^a48 h drug exposure in the dark.

^b16h drug exposure in the dark, followed by white light irradiation (11.6 J/cm²) for 5 min and further 32 h incubation.

^cPI = IC₅₀(Dark^a) / IC₅₀(Light^b).

n.a. = Not applicable.

Table S3. Dark and photo IC₅₀ values (μM) and photo-cytotoxicity index (PI = IC₅₀ dark/IC₅₀ light) in A549/DDP cells under normoxic and hypoxic conditions

Table S4. Photo IC₅₀ values (μM) in A549 cells under different photo dose.

	5.8 J/cm ²	11.6 J/cm ²	23.2 J/cm ²
Light IC ₅₀ (μM)	0.93±0.08	0.29±0.04	0.26±0.009

Table S5. Photo IC₅₀ values (μM) and photo-cytotoxicity index (PI = IC₅₀ dark/IC₅₀ light) in A549 cells of various Ruthenium complexes.

	Light IC ₅₀ (μM)	PI
Ru (Reported by Glazer) ¹	1.1 ± 0.3	136
Ru (Reported by Glazer) ¹	1.2 ± 0.1	208
Ru (Reported by Bonnet) ²	6.5	9.1
Ru (Reported by Zhou) ³	11.2 ± 0.2	3
Ru2	0.29±0.04	224

4. References

- 1 B. S. Howerton, D. K. Heidary and E. C. Glazer, *J. Am. Chem. Soc.*, 2012, **134**, 8324-8327.
- 2 C. Chen, J. A. Cuello-Garibo, L. Bretin, L. Zhang, V. Ramu, Y. Aydar, Y. Batsiun, S. Bronkhorst, Y. Husiev, N. Beztsinna, L. Chen, X. Zhou, C. Schmidt, I. Ott, M. J. Jager, A. M. Brouwer, B. E. Snaar-Jagalska and S. Bonnet, *Chem.Sci.*, 2022, **13**, 6899-6919.
- 3 C. Zhang, X. Guo, X. Da, Z. Wang, X. Wang and Q. Zhou, *Dalton Trans.*, 2021, **50**, 10845-10852.

