Supporting information

Design and Synthesis of Luminescent Cyclometalated Iridium(III) Complex Having *N*,*N*-Diethylamino Group that Stains Acidic Intracellular Organelles and Induces Cell Death by Photoirradiation

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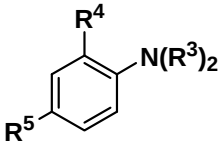
Table S1. pK_a values of model compounds



Model compound

		6a, **7a**, **8a** (R^3 = H)	**6b**, **7b**, **8b** (R^3 = Me)	**6c**, **7c**, **8c** (R^3 = Et)	**6d**, **7d**, **8d** (R^3 = *n*-Pr)	**6e**, **7e**, **8e** (R^3 = *n*-Bu)
6a-e (R^4 = R^5 = H)	pK_a	4.5[a] (4.6)[b] 4.6[c]	4.9[a] (5.1)[b] 5.1[c]	6.5[a] (6.7)[b] 6.6[c]	*n.d.*[e] (5.7)[b] 5.6[c]	*n.d.*[e] (6.3)[b] ~5.7[c]
	Δ pK_a[d]	0	+ 0.4	+ 2.0	-	-
7a-e (R^4 = Me, R^5 = H)	pK_a	4.3[a] (4.5)[b] 4.4[c]	- (5.7)[b] 5.9[c]	7.2[a] (7.2)[b] 7.2[c]	*n.d.*[e] (6.2)[b] -	*n.d.*[e] (6.8)[b] -
	Δ pK_a[d]	0	-	+ 2.9	-	-
8a-e (R^4 = Me, R^5 = OMe)	pK_a	5.4[a] (5.1)[b]	(6.4)[b]	7.6[a] (7.9)[b]	*n.d.*[e] -	*n.d.*[e] -
	Δ pK_a[d]	0		+ 2.2	-	-

[a] The pK_a values determined by potentiometric pH titration in MeCN/H_2O(5/95) with I = 0.1 ($NaNO_3$) at 25 °C. [b] pK_a values in parentheses were cited from the SciFinder database. [c] Brown, H. C.; McDaniel, D. H.; Häflinger, O. In *Determination of Organic Structures by Physical Methods*; Braude, E. A., Nachod, F. C., Eds.; Academic Press: New York, 1955. [d] Δ pK_a = (pK_a value of *N*,*N*-alkylaniline derivative)–(pK_a value of the corresponding anilines) based on the pK_a values determined by potentiometric pH titrations. [e] Not determined due to the low solubility of the compounds in aqueous solution.

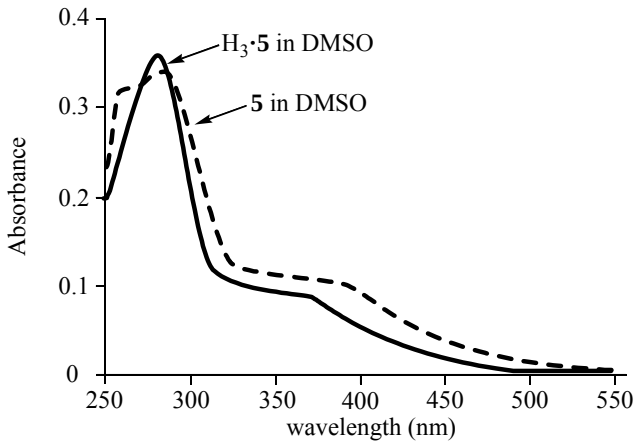


Figure S1. UV/vis spectra of **5** (10 μM) in DMSO and H_3·**5** (10 μM) in DMSO.

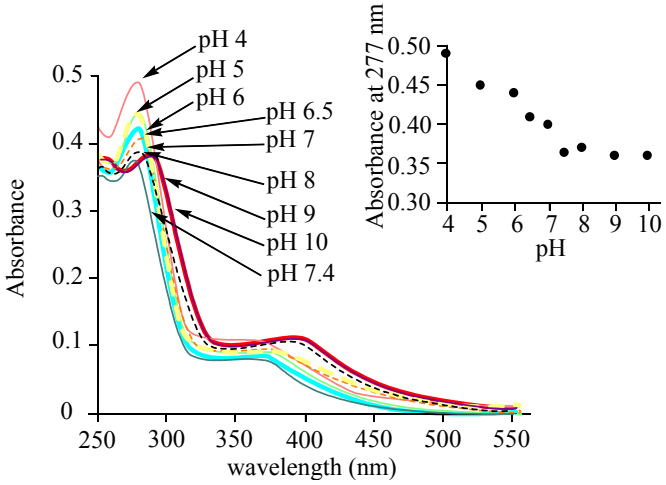


Figure S2. pH-Dependent change in UV/vis spectra of **5** (10 μM) in DMSO/10 mM buffer (from pH 4 to 10) (1/1) at 25 °C. pH 4 (pink plain curve), pH 5 (green thin curve), pH 6 (yellow bold dashed curve), pH 6.5 (blue bold curve), pH 7 (orange dashed curve), pH 7.4 (gray thin curve), pH 8 (black plain dashed curve), pH 9 (purple plain curve) and pH 10 (red bold curve). (Inset) pH-dependent change (from pH 4 to pH 10) in the absorbance of **5** at 277 nm.

Table S2. Photochemical properties of **5** in DMSO at 298K.

Complex	λ_{max} (absorption) [a]	λ_{max} (emission) [b]	ϕ [a,c]	$t^{1/2}$
5	260 nm, 283 nm, 385 nm	554 nm	9.8×10^{-3}	2.9 μsec
H_3·**5**	282 nm, 375 nm	497 nm	0.29	1.5 μsec

[a] [**5**] = [H_3·**5**] = 10 μM, [b] [**5**] = [H_3·**5**] = 1 μM, [c] quantum yield of emission.

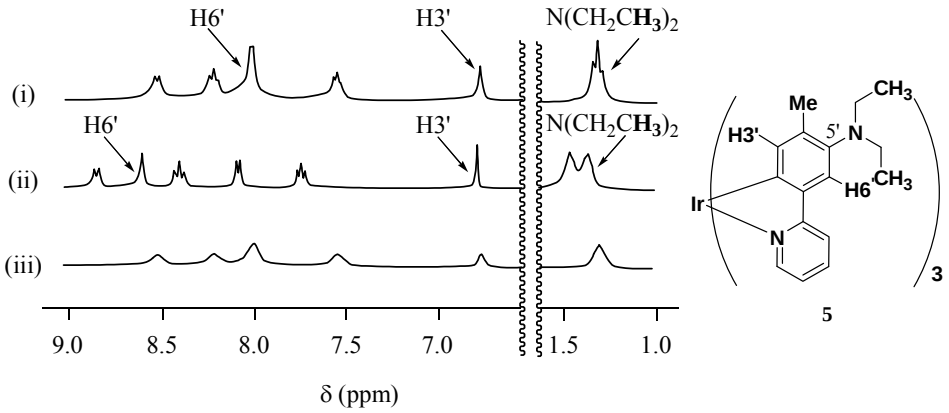


Figure S3. Change in ^{1}H NMR of **5** (4 mM) in DMSO-d_6/D_2O (9/1) (300 MHz/TSP) upon the addition of acid (1N DCl in DMSO-d_6/D_2O(9/1)) and base (1N NaOD in DMSO-d_6/D_2O(9/1). (i) acid-free **5**, (ii) (i) + DCl, (iii) (ii) + NaOD. TSP: sodium salt of 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid.

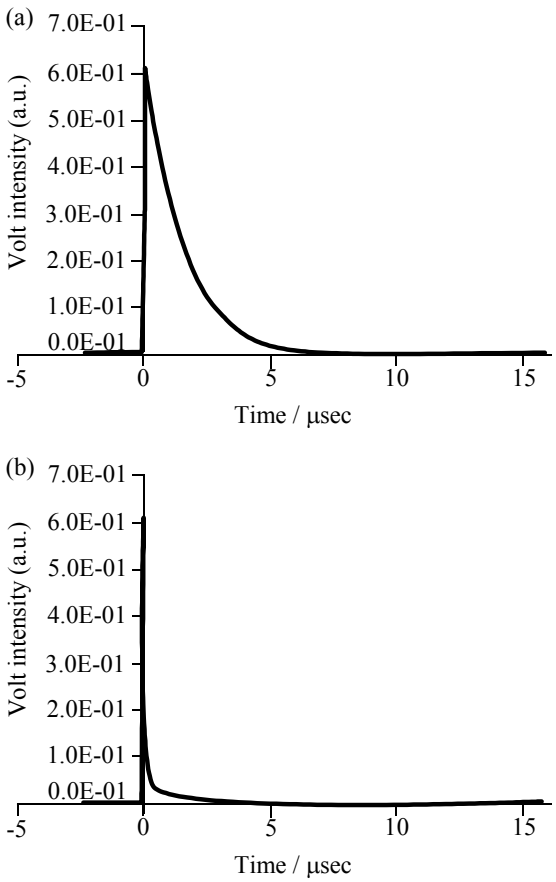


Figure S4. Luminescence decay of (a) **5** (5 μM) (in degassed DMSO/100 mM MES (pH 6.0) (1/4)) and (b) **5** (5 μM) (in degassed DMSO/100 mM HEPES (pH 7.4) (1/4)) at 25 °C (excitation at 355 nm). A.u. is arbitrary unit.

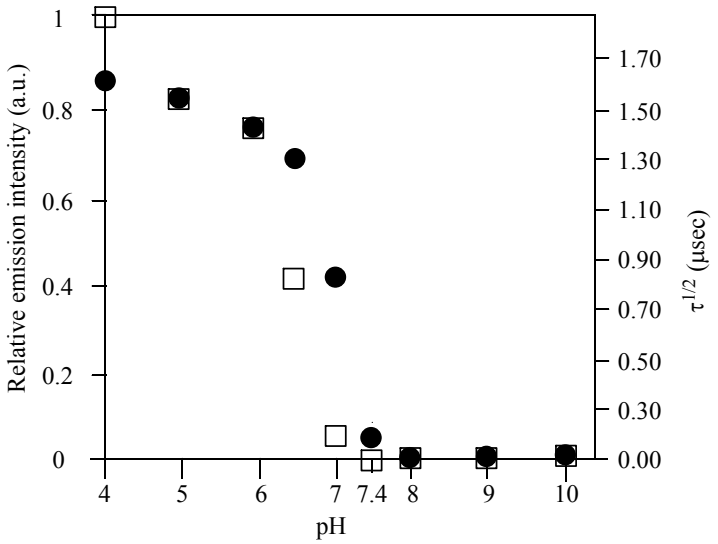


Figure S5. Comparison of pH-dependent change in emission intensity of **5** (1 μM) (open squares) (excitation at 366 nm and emission at 494 nm) and pH-dependent change in lifetime of **5** (5 μM) (closed circles). Luminescence decay of **5** (pH 6) and **5** (pH 7.4) are shown in Figure S4.

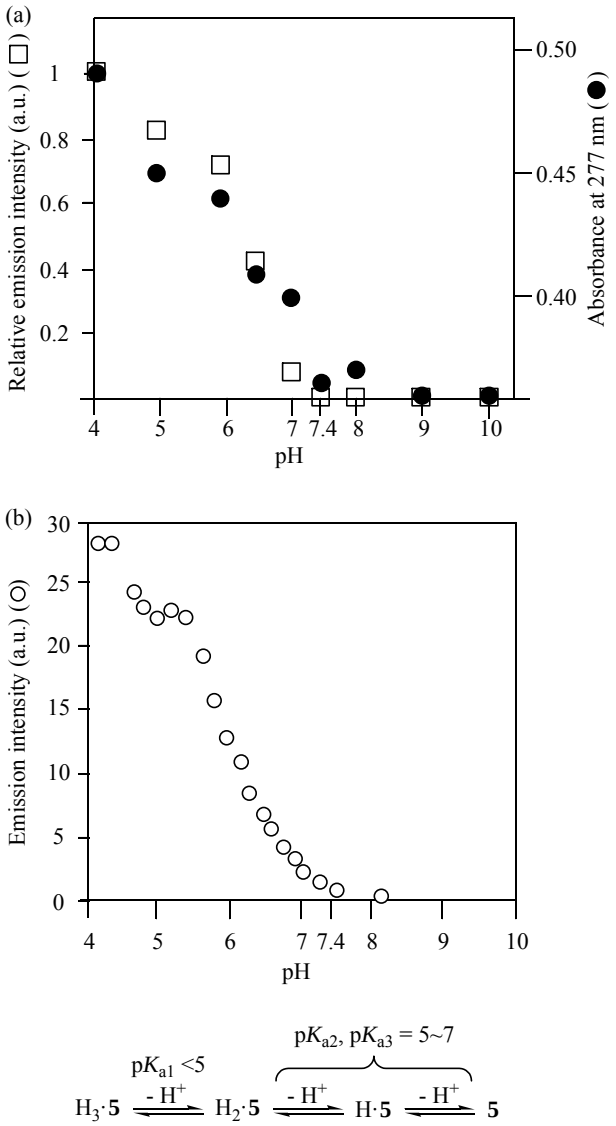


Figure S6. (a) Comparison of pH-dependent change in absorbance of **5** (10 μM) at 277 nm (closed circles) and that in emission intensity of **5** (1 μM) (open squares) (excitation at 366 nm and emission at 494 nm). (b) pH-dependent change in emission intensity of **5** (1 μM) (open circles) in DMSO/100 μM MES buffer (1/4) at 25 °C. The pH value of a mixture was adjusted to pH 8 at the start point and changed from pH 8 to 4 by addition of 10 mM HCl aq. (10 μL each, which is 0.125 eq. against MES molecule in a solution). The relative emission intensity of **5** in Figure S6a was calculated based on 1 at pH 4.

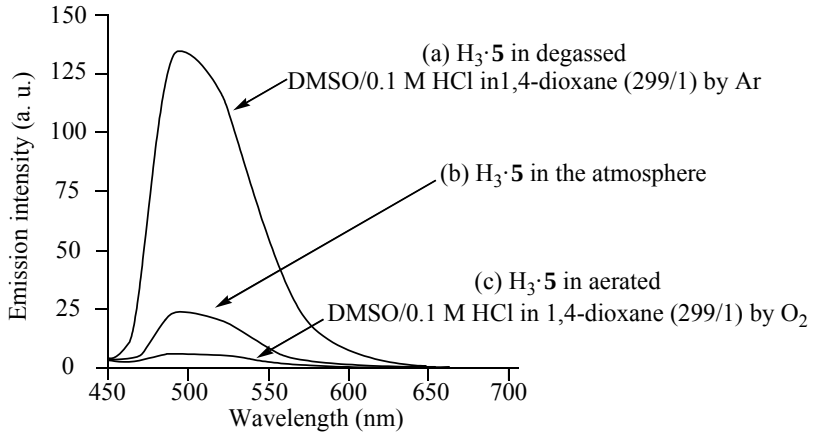


Figure S7. Emission spectra of H_3·**5** (1 μM) in DMSO/0.1 M HCl in 1,4-dioxane (299/1, v/v) (a) in deggased DMSO/0.1 M HCl in 1,4-dioxane (299/1, v/v) by Ar, (b) in the atmosphere, (c) in the aerated DMSO/0.1 M HCl in 1,4-dioxane (299/1, v/v) by $^1O_2$ at 25 °C. Excitation at 366 nm. A.u. is in arbitrary units.

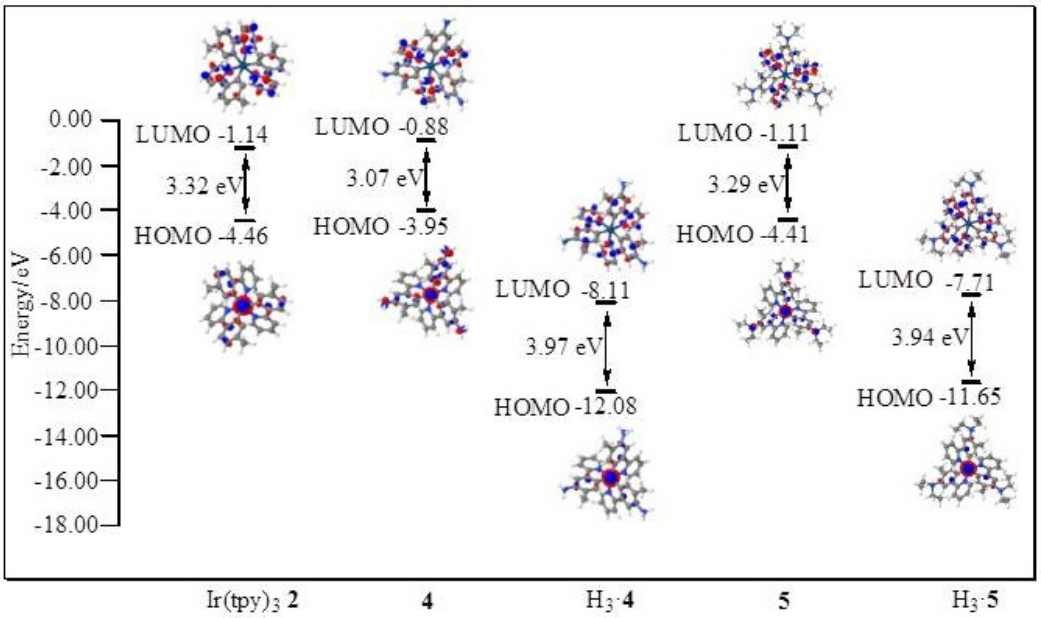


Figure S8. The HOMO−LUMO energy gap of **2**, **4**, H_3·**4**, **5**, and H_3·**5** calculated by the Gaussian03 program using the B3LYP hybrid functional together with the LanL2DZ basis set for Ir atom and the 6-31G basis set for H, C, N atoms.

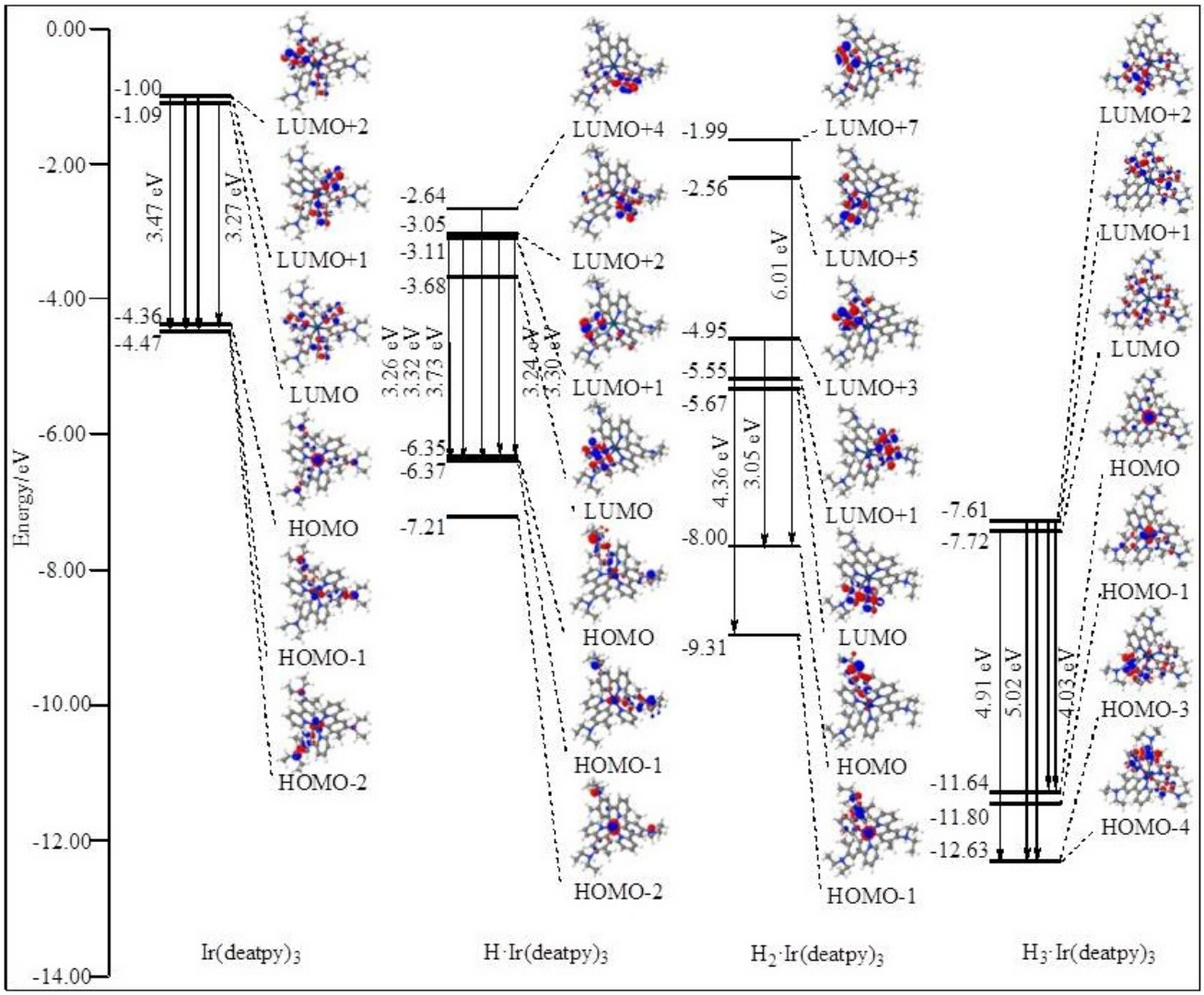


Figure S9. TD-DFT calculation of **5**, H·**5**, H_2·**5**, and H_3·**5** calculated by the Gaussian03 program using the B3LYP hybrid functional together with the LanL2DZ basis set for Ir atom and the 6-31G basis set for H, C, N atoms.

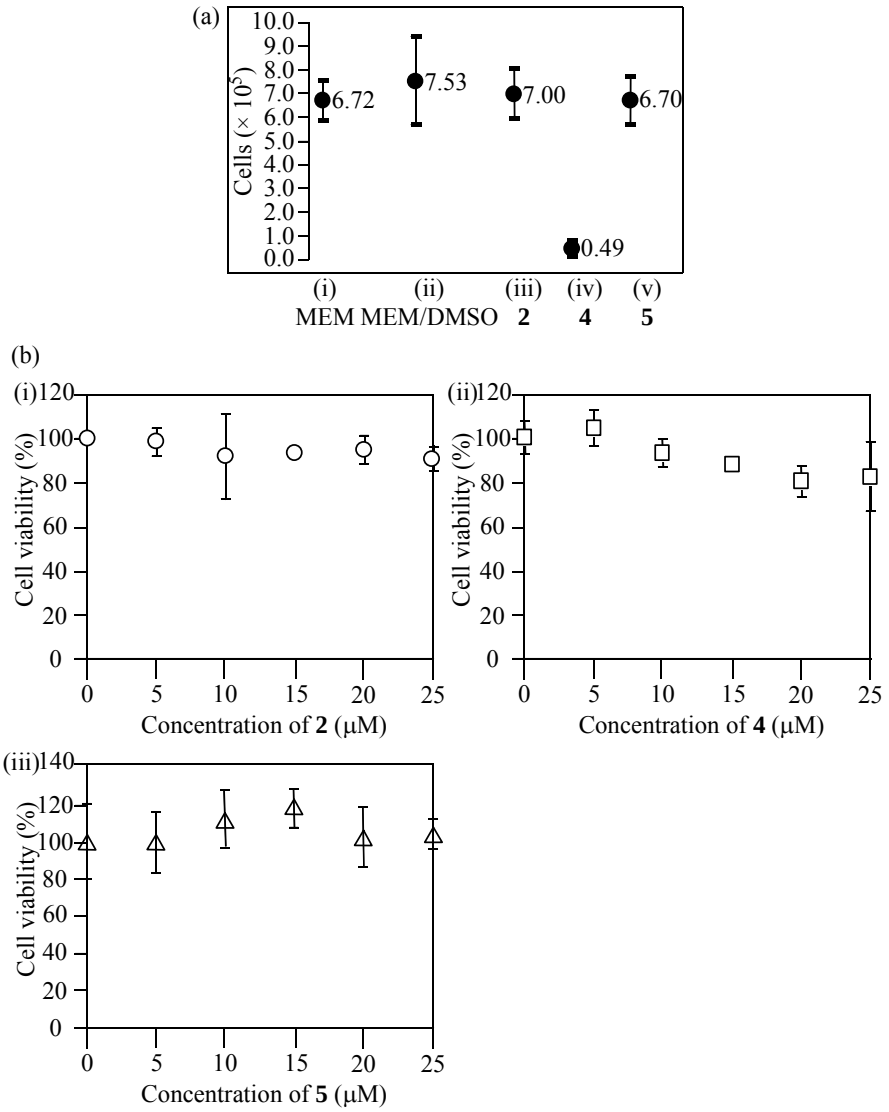


Figure S10. (a) HeLa-S3 cells growth in the absence and presence of Ir complexes (counted by trypan blue staining) at 37 °C for 4 days, (i) in MEM, (ii) in MEM/DMSO (99/1, v/v), (iii) with **2** in MEM/DMSO (99/1, v/v), (iv) with **4** in MEM/DMSO (99/1, v/v), and (v) **5** in MEM/DMSO (99/1, v/v) ([Ir complex] = 10 μM). (b) Results of MTT assay of HeLa-S3 cells in the presence of Ir complexes at 37 °C for 16 hours, with **2** (i), **4** (ii), and **5** (iii) in MEM/DMSO (99/1, v/v).

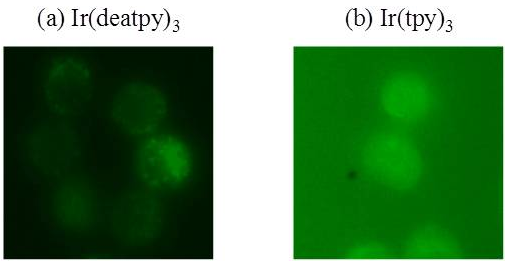


Figure S11. Fluorescence microscope images of HeLa-S3 cells (×40) after incubation with (a) **5** and (b) **2** in MEM/DMSO (99/1, v/v) at 37 °C for 1 min without successive PBS wash ([Ir complex] = 10 μM in medium, excitation at 377 nm).

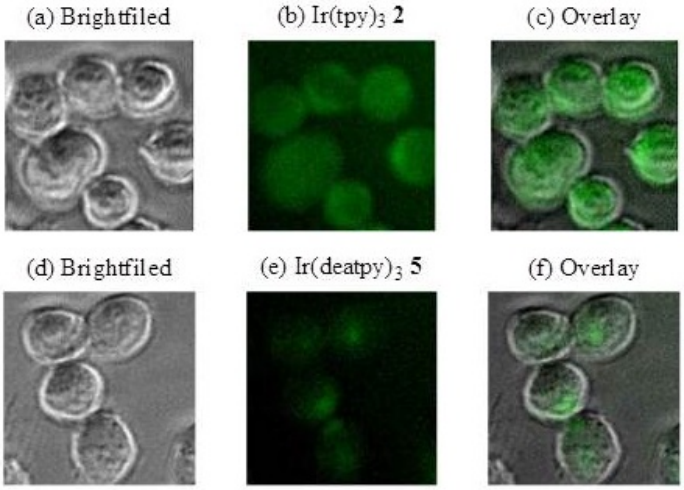


Figure S12. Luminescence microscopic images (BIOREVO BZ-9000, Keyence) of HeLa-S3 cells (×40) incubated with **2** (10 μM) and **5** (10 μM) in MEM/DMSO (99/1, v/v) *at 4 °C* for 30 min respectively, excitation at 377 nm. (a) brightfield image of HeLa-S3 with **2**, (b) emission image of **2**, (c) overlay image of (a) + (b), (d) brightfield image of **5**, (e) emission image of **5**, (f) overlay image of (d) + (e).

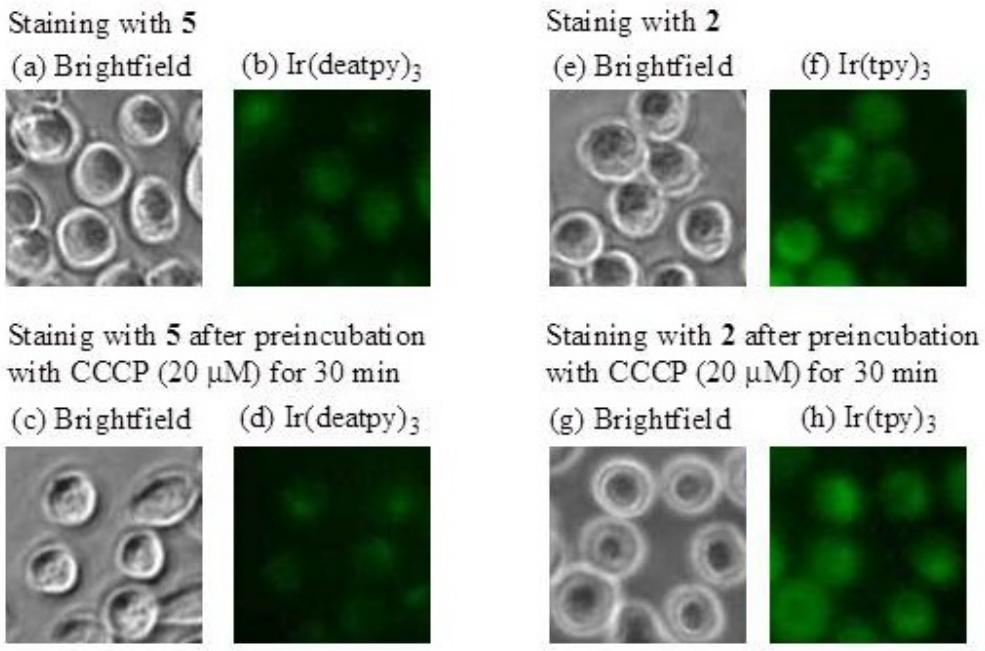


Figure S13. Fluorescence microscope images of HeLa-S3 cells (×40) incubated with CCCP (20 μM) at 37 °C for 30 min, and then stained with **5** (10 μM) and **2** (10 μM) in MEM/DMSO (99/1, v/v) at 37 °C for 30 min, (excitation at 377 nm). (a), (c), (e), and (g) brightfield image of HeLa-S3, (b) emission image of **5**, (d) emission image of **5** after preincubation with CCCP, (f) emission image of **2**, (h) emission image of **2** after preincubation with CCCP.

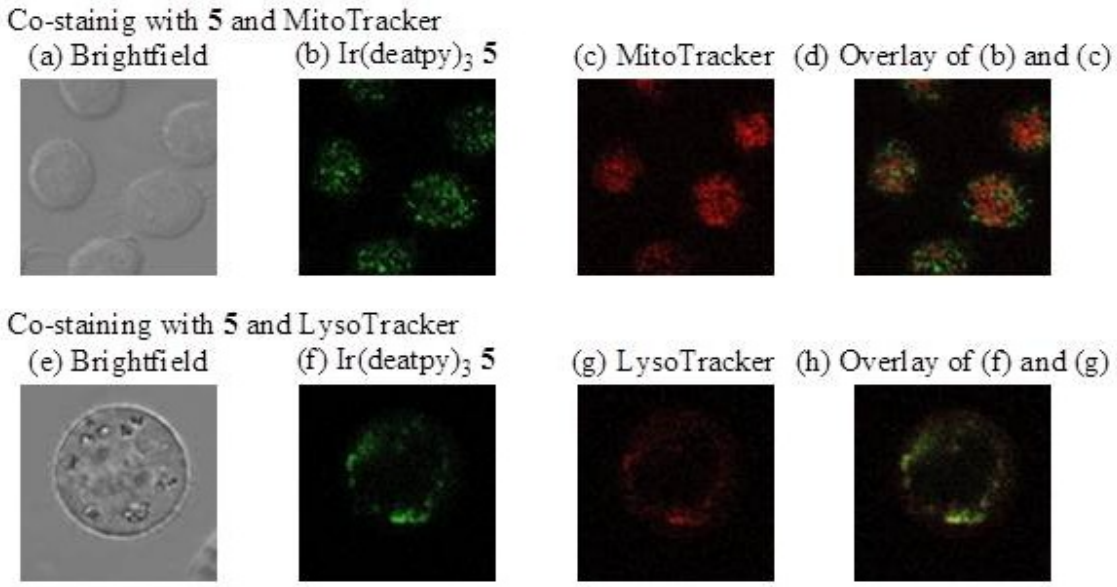


Figure S14. Confocal microscope images (Leica TCS SP2, Leica) of HeLa-S3 cells (×63) co-stained with **5** (10 μM) and MitoTracker (10 nM) or LysoTracker (100 nM) in MEM/DMSO (99/1, v/v) at 37 °C for 30 min. (a) and (e) brightfield images of HeLa-S3, (b) and (f) emission images of **5**, (c) emission images of MitoTracker, (g) emission images of LysoTracker, (d) overlay of (b) and (c), and (h) overlay of (f) and (g). Excitation at 350 nm and 360 nm for **5** and excitation at 568 nm for MitoTracker and LysoTracker.

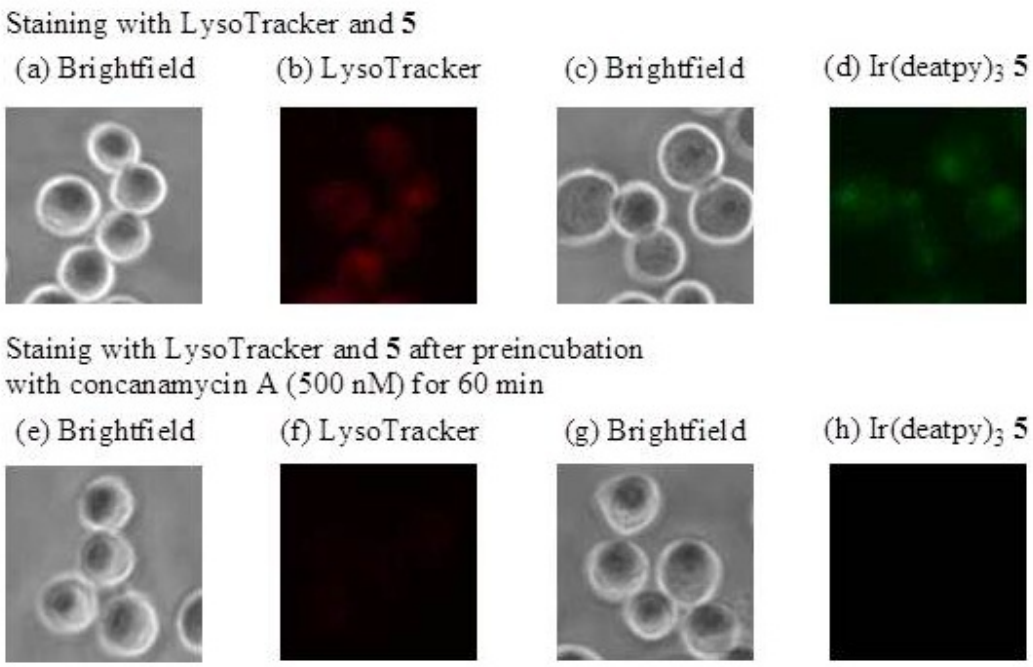


Figure S15. Fluorescence microscope images of HeLa-S3 cells (×40) incubated with LysoTracker (100 nM) and **5** (10 μM) in MEM/DMSO (99/1, v/v) at 37 °C for 30 min after preincubation with concanamycin A (500 nM) at 37 °C for 60 min, excitation at 377 nm for **5** and 540 nm for LysoTracker. (a), (c), (e), and (g) brightfield image of HeLa-S3, (b) emission image of LysoTracker, (d) emission image of **5**, (f) emission image of LysoTracker after preincubation with concanamycin A, (h) emission image of **5** after preincubation with concanamycin A.

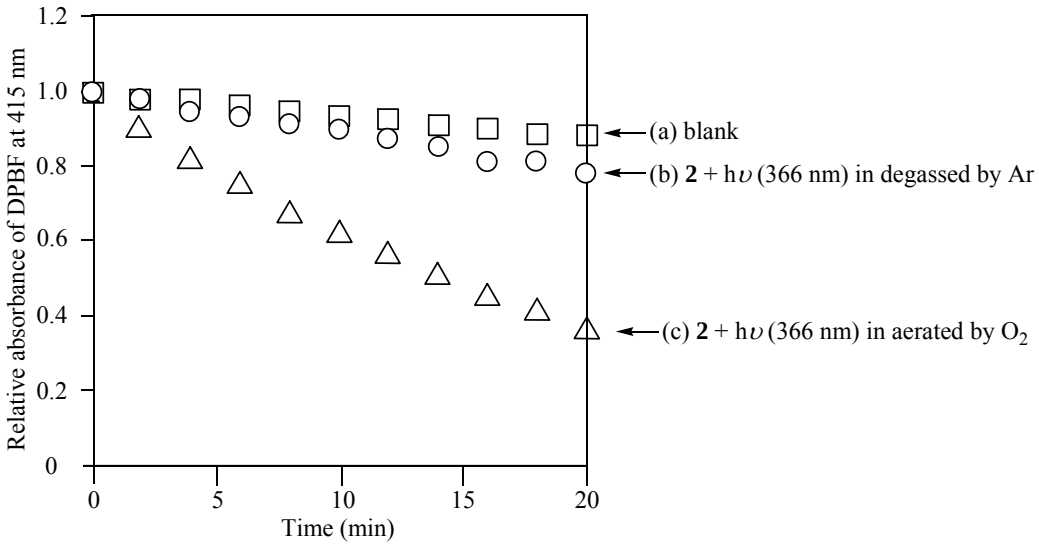


Figure S16. Comparative photooxidation of 1,3-diphenylisobenzofuran (DPBF) (50 μM) in DMSO/H_2O (3/2), (a) blank in DMSO/H_2O (3/2) (open squares), (b) **2** (10 μM) in DMSO/H_2O (3/2) in degassed by Ar (open circles), (c) **2** (10 μM) in DMSO/H_2O (3/2) in degassed by O_2 (open triangles). Excitation at 366 nm for **2**.

Table S3. Induction of cell membrane swelling upon photoirradiation in the presence of Ir complexes

Entry	Ir complex (μM)	Solvent	Conditions[a]	microscopy (filter)[b]	result[c]
1	blank	MEM/DMSO (99/1)	30 min, 37°C	BIOREVO (FF01)	-
2	**2** (10 μM)	MEM/DMSO (99/1)	30 min, 37°C	BIOREVO (FF01)	+
3	**4** (10 μM)	MEM/DMSO (99/1)	60 min, 37°C	BIOREVO (FF01)	-
4	**5** (10 μM)	MEM/DMSO (99/1)	30 min, 37°C	BIOREVO (FF01)	+
5	blank	MEM/DMSO (99/1)	30 min, 37°C	BIOREVO (GFP-BP)	-
6	**2** (10 μM)	MEM/DMSO (99/1)	30 min, 37°C	BIOREVO (GFP-BP)	+
7	**5** (10 μM)	MEM/DMSO (99/1)	30 min, 37°C	BIOREVO (GFP-BP)	+
8	methylene blue (10 μM)	MEM/DMSO (99/1)	30 min, 37°C	BIOREVO (Cy5)	+

(*a*) Conditions show excitation time and incubation temperature. (*b*) FF01 filter : Ex = 377/50 nm, Em = 520/35 nm. GFP-BP filter : Ex = 470/40 nm, Em = 535/50 nm. Cy5 filter : Ex = 620/60 nm, Em = 700/75 nm. (*c*) + : cell membrane swelling was observed. – : cell membrane swelling was not observed.