Supporting Information

Amphiphilic Cationic Triscyclometalated Iridium(III) Complex-Peptide Hybrids Induce Paraptosis-like Cell Death of Cancer Cells via an Intracellular Ca^{2+}-Dependent Pathway

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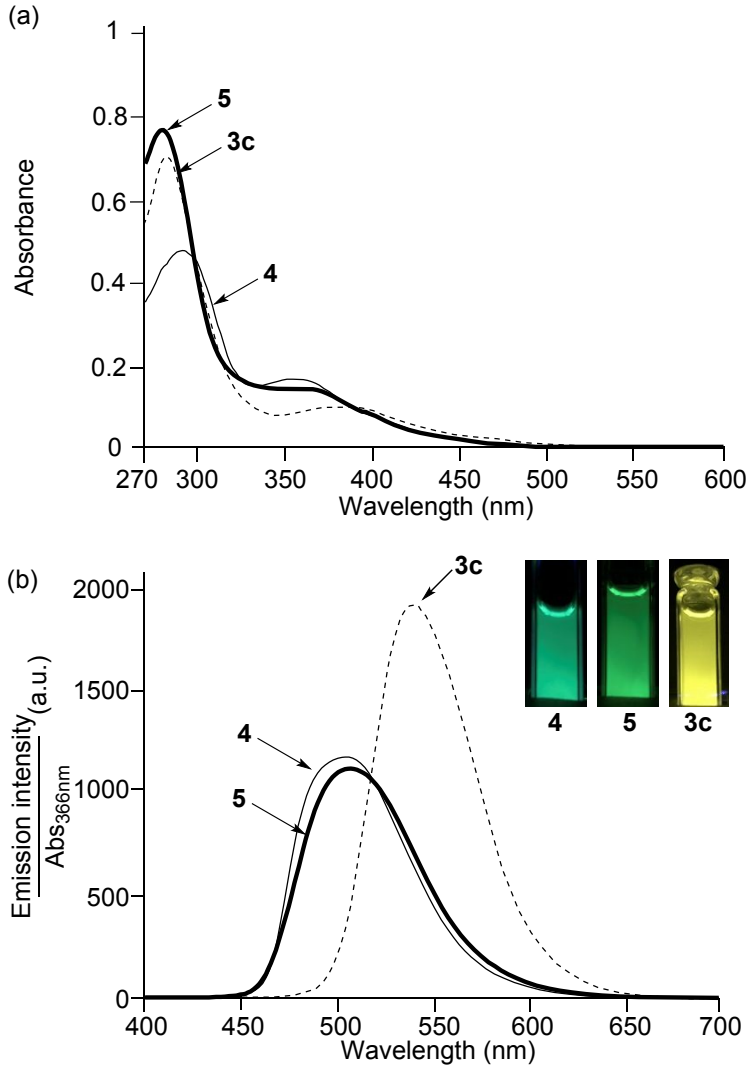


Figure S1. (a) UV/Vis absorption and (b) emission spectra of **3c** (dashed curve), **4** (plain curve) and **5** (bold curve) in degassed 100 mM HEPES (pH 7.4) at 25 ºC. [Ir complex] = 10 μM, excitation at λ = 366 nm. a.u. = arbitrary units. The photographs show the emission of **4**, **5**, and **3c** (excitation at λ = 366 nm).

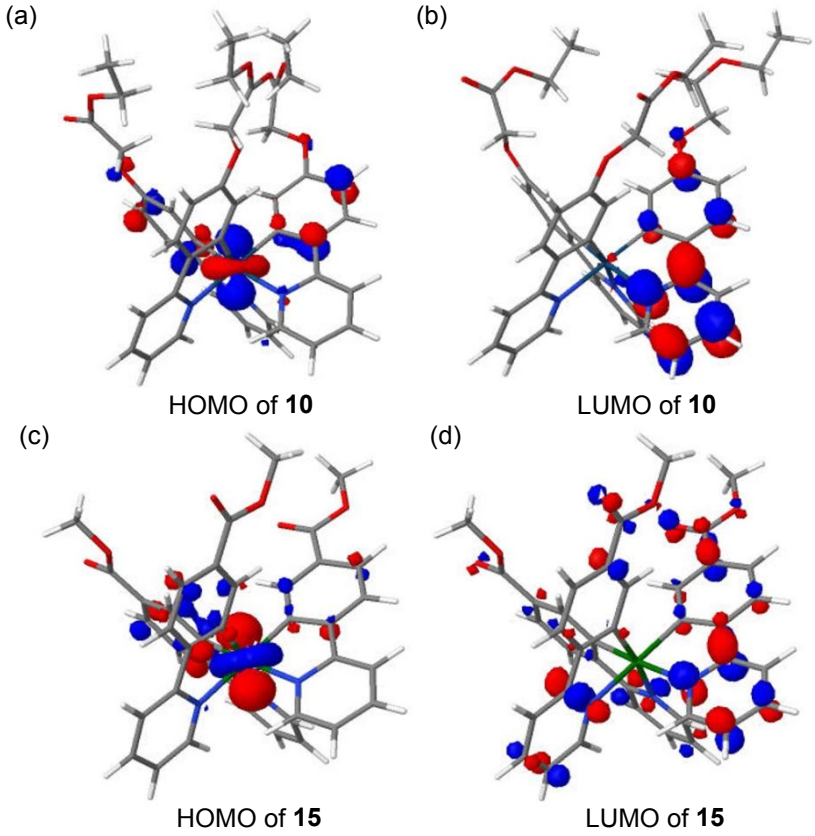


Figure S2. HOMO and LUMO surfaces of **10** (a and b) and **15** (c and d) calculated by the DFT method with the B3LYP hybrid functional together with the LanL2DZ basis set for the Ir atoms and the 6-31G basis set for the H, C, O, and N atoms (green: iridium, gray: carbon, red: oxygen, blue: nitrogen; white: hydrogen).

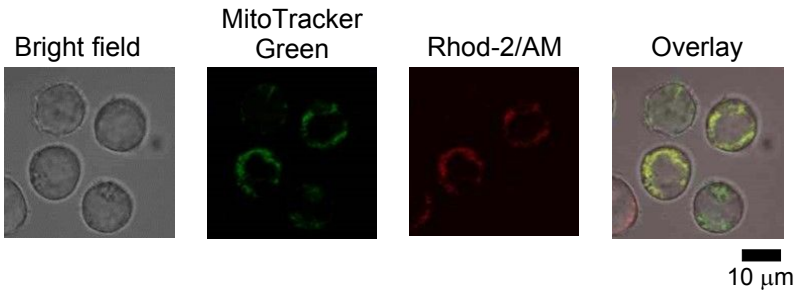


Figure S3. Typical luminescence confocal microscopy images of Jurkat cells treated with MitoTracker Green and Rhod-2/AM. Jurkat cells (2.0×10^5 cells) were treated with 5 μM Rhod-2/AM (Dojindo) in RPMI 1640 medium with 10% FBS at 37 ºC under 5% CO_2 for 30 min, followed by 500 nM MitoTracker Green (Invitorogen) for 30 min. After washing with PBS three times, the cells were observed by congocal fluorescent microscopy (Fluoview, FV-1000, Olympus). Excitation wavelength was 478 nm for MitoTracker green and 559 nm for Rhod-2.

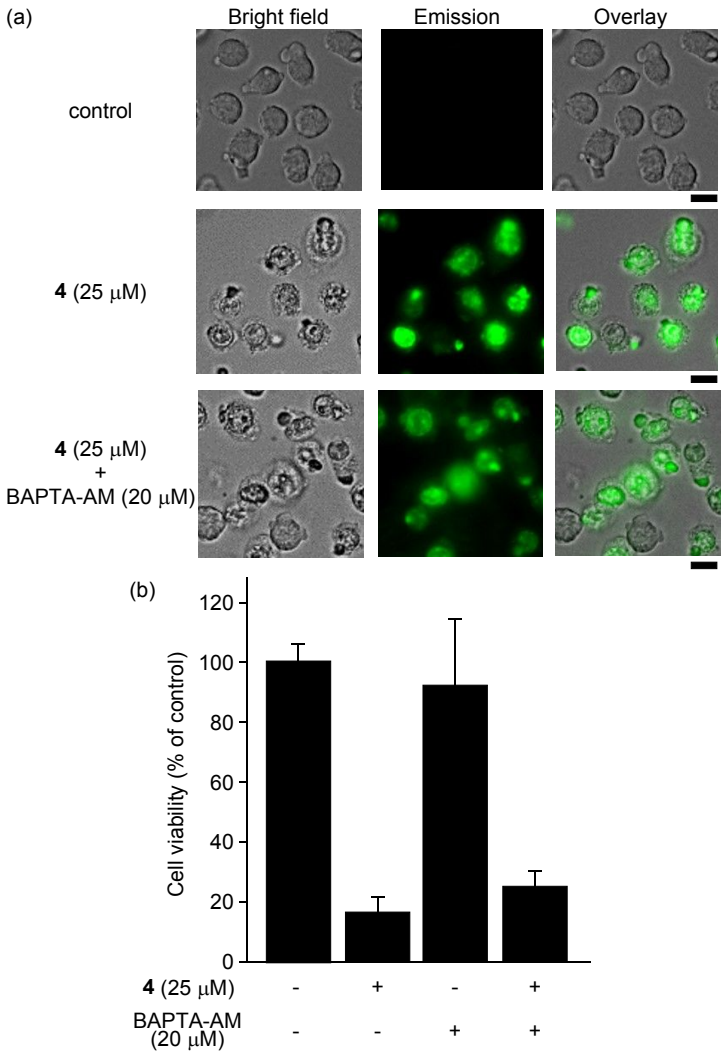


Figure S4. Effect of BAPTA-AM, a cytosolic Ca^{2+} chelator. (a) Typical luminescence microscopy images of Jurkat cells treated with BAPTA-AM (20 μM). Scale bar (black) = 10 μm. (b) MTT assays of Jurkat cells in the presence and/or absence of **4** (25 μM), and BAPTA/AM (20 μM)

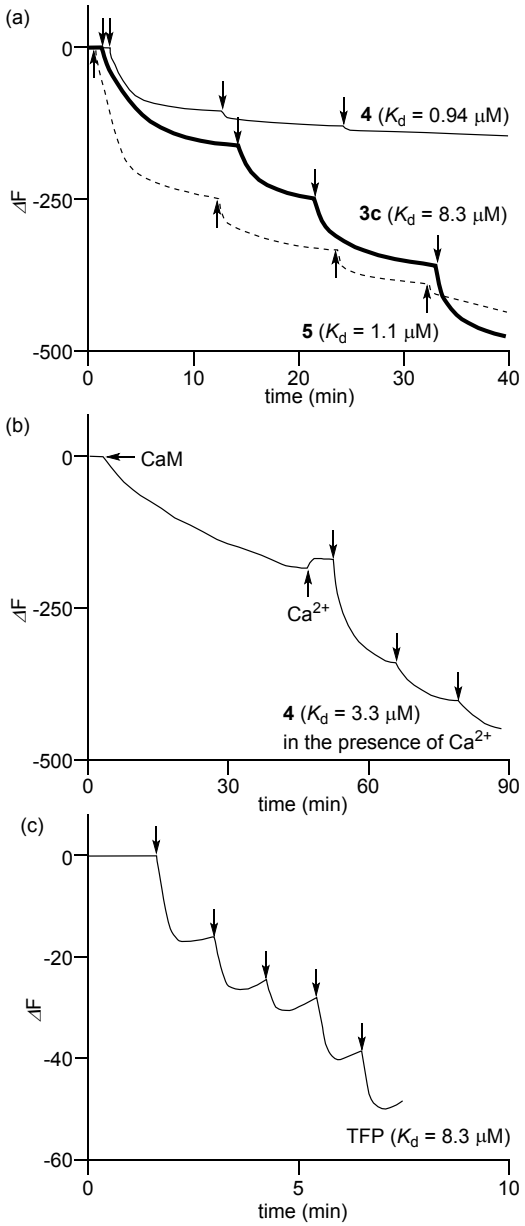


Figure S5. Time course for the frequency change (ΔF (Hz)) of an Ir complex–calmodulin (CaM) or TFP–CaM complexation on 27 MHz QCM in the presence (80 μM) or absence of Ca^{2+} at 25 °C in phosphate buffer saline (PBS). (a) An aliquot of a solution of **4** (390 μM), **5** (500 μM), and **3c** (410 μM) was added to CaM fixed on the sensor chip (a) in the absence or (b) absence of Ca^{2+}. (c) Trifluoperazine (2 mM) in H_2O was added to CaM fixed on the sensor chip. Addition times of analyses to CaM are indicated by the plain arrows.

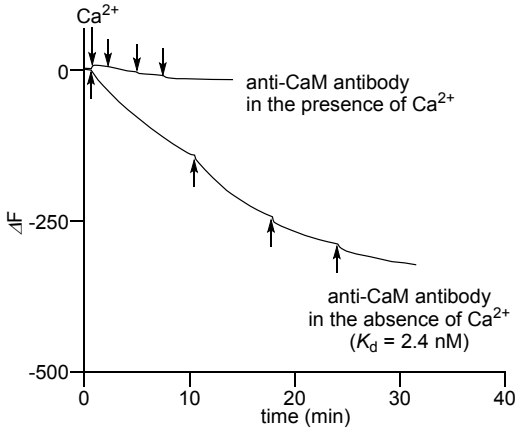


Figure S6. Time course for the frequency change (ΔF (Hz)) of an anti-CaM antibody-CaM complexation in the absence or presence of Ca^{2+} on 27 MHz QCM at 25 ºC in phosphate buffer saline (PBS). Addition times of analyses to CaM are indicated by the plain curve.

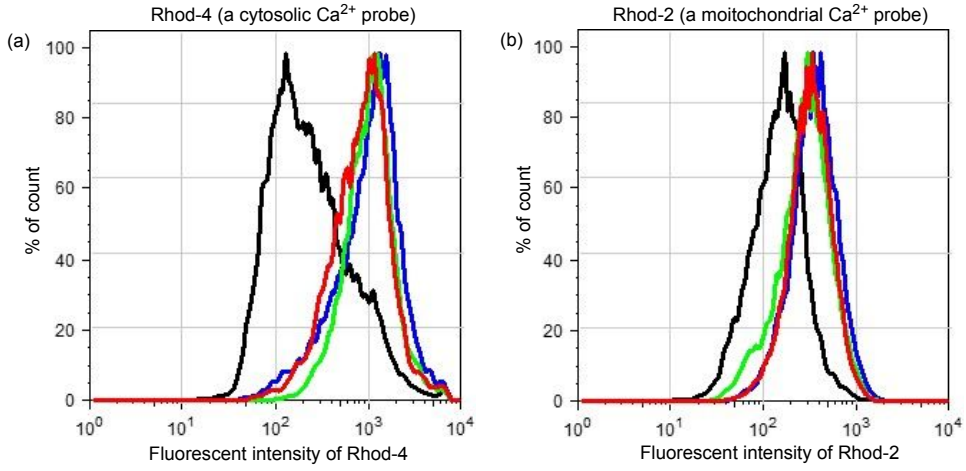


Figure S7. Flow cytometric analysis of Jurkat cells treated with celastrol (30 μM) in RPMI 1640 medium with 10% FBS at 37 ºC under 5% CO_2 for 1-5 h, and then stained with (a) Rhod-2 (5 μM) or (b) Rhod-4 (5 μM) in RPMI 1640 medium with 10% FBS at 37 ºC under 5% CO_2 for 30 min. Different colors responds to the incubation time of celastrol: control (black), 1 h (blue), 3 h (light green), and 5 h (red).

Table S1. Photophysical properties of **1a**, **1c**, **15**, **3c**, **10**, **4**, and **5**. [Ir complex] = 10 μM in degassed 100 mM HEPES (pH = 7.4), CH_2Cl_2 or DMSO at 25 ºC).

Compound	λ_{max} (absorption)	λ_{max} (emission)	Φ	τ
1a (in CH_2Cl_2)[a]	287 nm, 373 nm	512 nm[b]	0.50	2.0 μs[c]
1c[d]	289 nm, 360 nm	495 nm[b]	0.65	1.8 μs
15 (in DMSO)	287 nm, 391 nm	543 nm[b]	0.24	1.0 μs
3c	283 nm, 383 nm	541 nm[b]	0.11	1.0 μs
10 (in DMSO)	290 nm, 360 nm	499 nm[b]	0.37[e]	0.65 μs[f]
4	292 nm, 356 nm	505 nm[b]	0.43[e]	0.78 μs[f]
5	280 nm, 360 nm	507 nm[b]	0.41[e]	1.4 μs[f]

[a]Ref. 18. [b]Excitation at 366 nm. [c]Ref. 2. [d]Ref. 19. [e]Quinine sulfate in 0.1 M H_2SO_4 (Φ = 0.55) was used as a reference compound. [f]A 495 nm longwave pass filter was used.

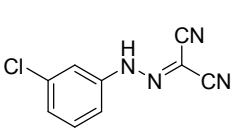
Table S2. Calculated triplet transition states of **10** and **15** using TD-DFT calculations at the B3LYP (LanL2DZ/6-31G) level.

Compound	λ_{em} (nm) exp.	*E* (eV) exp.	*E* (eV) TD-DFT	State
10	499	2.49	2.64	T_1
15	543	2.28	2.37	T_1

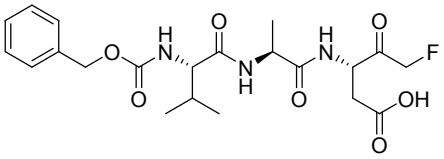
Table S3. Complexation constants of anti-CaM antibody with calmodulin (assuming 1:1 complexation)

Analytes	K_{app} (M^{-1})	K_d
anti-CaM antibody in the absence of Ca^{2+}	$(4.22 \pm 0.15) \times 10^8$	2.4 ± 0.1 nM
anti-CaM antibody in the presence of Ca^{2+} (80 μM)	$< 1.0 \times 10^3$	> 1 mM

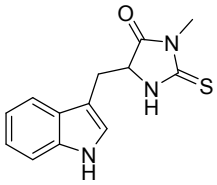
Chart S1.



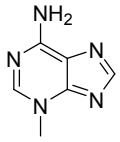
CCCP



Z-VAD-fmk

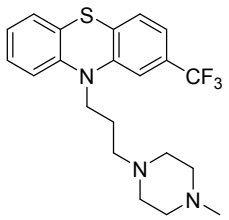


necrostatin-1



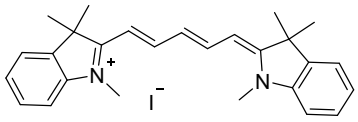
3-methyladenine

Chart S2.



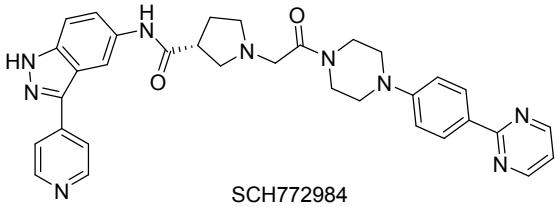
trifluoperazine
(TFP)

Chart S3.

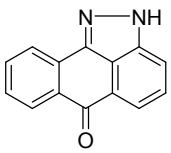


DilC1(5)

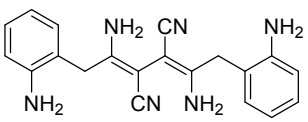
Chart S4.



SCH772984
(ERK inhibitor)



SP600125
(JNK inhibitor)



U0126
(MEK inhibitor)