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Supplementary Material

A Red-Emissive AIE Probe for Targeting Mitochondria in Living Cells

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S1 Materials and measurements

All reagents and solvents were available commercially and purified according to the standard method.

The ^{1}H NMR spectra were obtained on Bruker 400 Ultrashield spectrometer. Mass spectroscopy was recorded with a Micromass GCT-MS (ESI source). FT-IR spectra (KBr pressed pellets) were recorded on the Nicolet FT-IR-870SX spectrophotometer. UV-vis absorption and one-photon fluorescence spectra were obtained on UV-1700 and Hitachi F-4600 fluorescence spectrophotometer, respectively. SEM was obtained using a Hitachi S-4800 scanning electron microscope.

A Bruker SMART CCD area detector was used to demonstrate the X-ray diffraction measurements which use graphite monochromated Mo-Kα radiation (λ = 0.71069 Å) at 298 (2) K. Intensity data were collected in the variable ω-scan mode. The structures were solved by direct methods and difference Fourier syntheses. The non-hydrogen atoms were refined anisotropically and hydrogen atoms were introduced geometrically. Calculations were performed with the HELXTL-97

program package.

Cell Imaging

Hela cells were seeded in 24-well glass-bottom plates at a density of 1×10^5 cells per well and grown for 48 h. For live-cell imaging, cells were incubated with **M** at 10 μM in cell medium containing 10 % Fetal Bovine Serum (FBS) and maintained at 37°C in an atmosphere of 5% CO_2 and 95% air for 30 min. The cells were then washed with PBS three times. The cells were imaged using water immersion lenses on a confocal laser scanning microscopy. Co-staining was performed using 1 μM Mitotracker Red for 30 min.

Fluorescence microscopy

Luminescent Hela cells were imaged with Leica TCS SP8 confocal laser scanning microscope using magnification 63× objective lenses for monolayer cultures. Image data processing was performed using Image J.

MTT assay

The cytotoxicity of **M** was measured using the MTT assay. Hela cells were seeded in 96-well plates at a density of 5000 cells/well and incubated for 3 days at 37 ^oC under 5 % CO_2. **M** was then added at indicated concentrations to triplicate wells. Prior to the compounds' treatment, the cell culture medium was changed, and aliquots of the compounds' stock solutions were diluted to obtain the final concentrations of 5, 10, 15, 20 and 30 μM in the growth medium. After incubation for 24 h, the medium was replaced with fresh DMEM medium. Subsequently, cells were treated with 5 mg/mL MTT (10 μL/well) and incubated for an additional 4 h (37 ^oC, 5% CO_2). After MTT medium removal, the formazan crystals were dissolved in DMSO (100 μL/well) and the absorbance was measured at 490 nm using an INFINITE 200 PRO.

Table S1 Crystallographic data and structure refinement parameters of compound **M**.

Empirical formula	$C_{86}H_{76}N_{10}O_9S_2$

Formula weight	1457.70
Crystal system	Triclinic
Space group	$P\bar{1}$
a(Å)	9.573(4)
b(Å)	18.591(7)
c(Å)	23.577(9)
a[°]	101.211(5)
b[°]	92.527(6)
γ[°]	91.182(6)
V(Å^3)	4110(3)
Z	2
R_1, wR_2 [$I \geq 2\sigma(I)$]	0.1010, 0.1971
R_1, wR_2 [all data]	0.2527, 0.2323
S on F^2	0.998
CCDC	1989197

Table S2 Selected bond parameters of compound **M**.

Bond distance	**Å**	**Bond angle**	**°**
C1-S1	1.751	O2-S1-O1	112.2
N1-C3	1.496	O2-S1-O3	113.7
C6-C9	1.506	O1-S1-O3	113.2
C9-C10	1.257	C6-C9-C10	126.9
C10-C11	1.486	C9-C10-C11	125.6
N2-C14	1.414	C14-N2-C15	121.5
N2-C36	1.437	C14-N2-C36	119.8

N2-C15	1.424	C15-N2-C36	118.6
C18-C11	1.469	C23-N3-C24	117.3
C23-C31	1.489	C25-N4-C26	116.7
C24-C25	1.484	C31-N5-C32	117.5
C44-S2	1.774	O5-S2-O6	115.4
N6-C46	1.517	O4-S2-O6	111.7
C49-C52	1.464	O4-S2-O5	111.3
C52-C53	1.297	C49-C52-C53	126.5
C53-C54	1.462	C52-C53-C54	126.9
N7-C57	1.410	C57-N7-C79	118.1
N7-C79	1.439	C57-N7-C58	121.7
N7-C58	1.431	C58-N7-C79	120.0
C61-C64	1.477	C66-N8-C67	118.4
C67-C68	1.454	C69-N9 –C68	120.0
C66-C74	1.489	C74-N10-C75	116.7

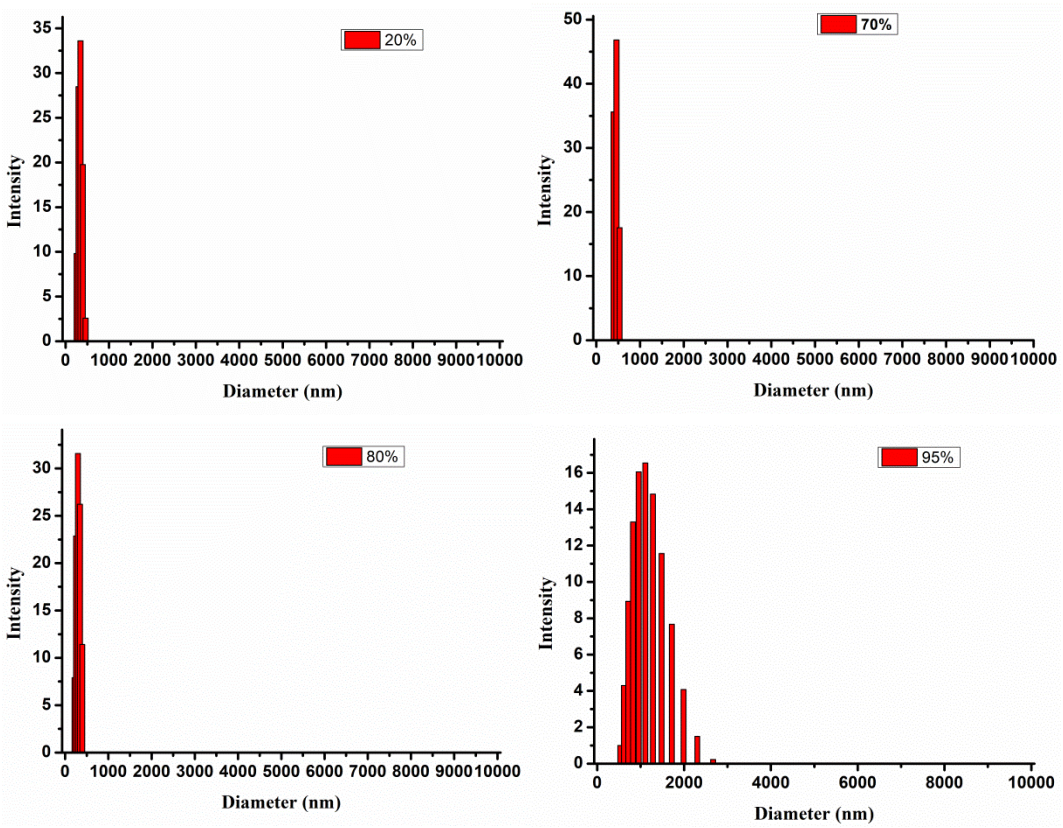

Figure S1 Particle size distributions of **M** in ethanol-water mixtures with 20%, 70%, 80% and 90% water fractions.

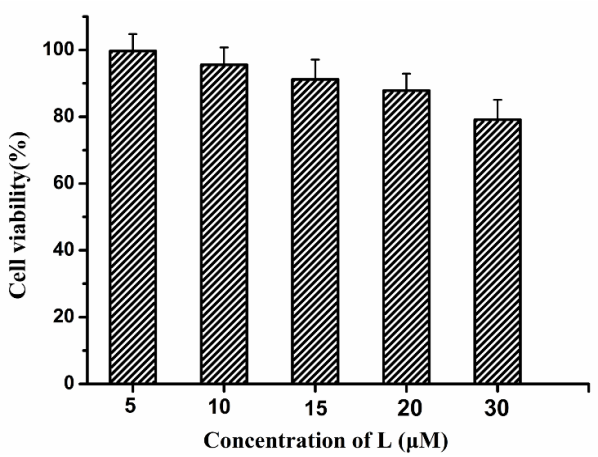

Figure S2 Cytotoxicity data results obtained from the MTT assay at different concentrations for 24 h.

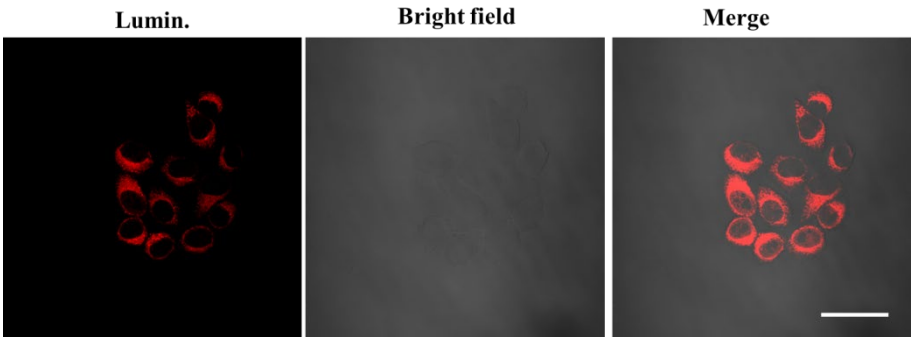

Figure S3 Fluorescence microscopy images of Hela cells treated with 10 μM of the compound **M** (λ_{ex}=460 nm, λ_{em}=580-620 nm). Bar=20 μm.

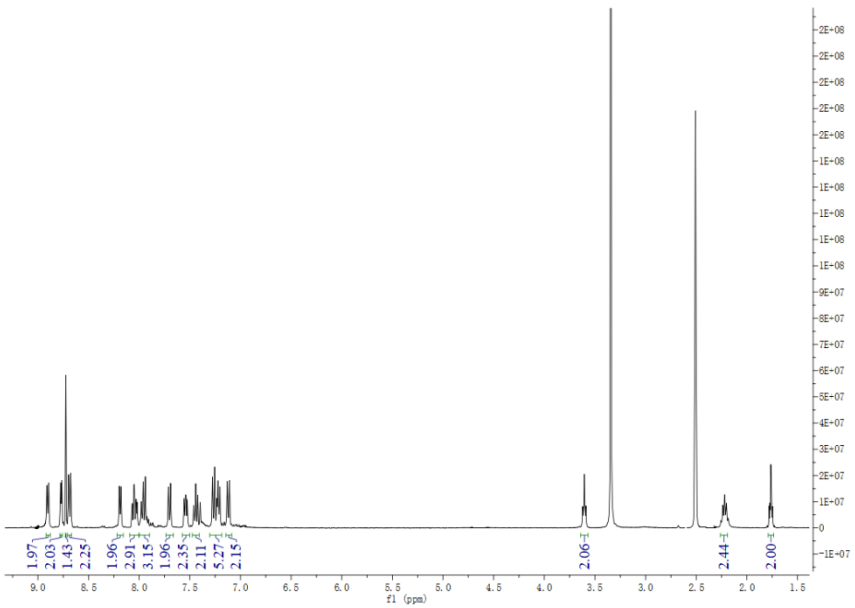

Figure S4 ^{1}H NMR of **M**.

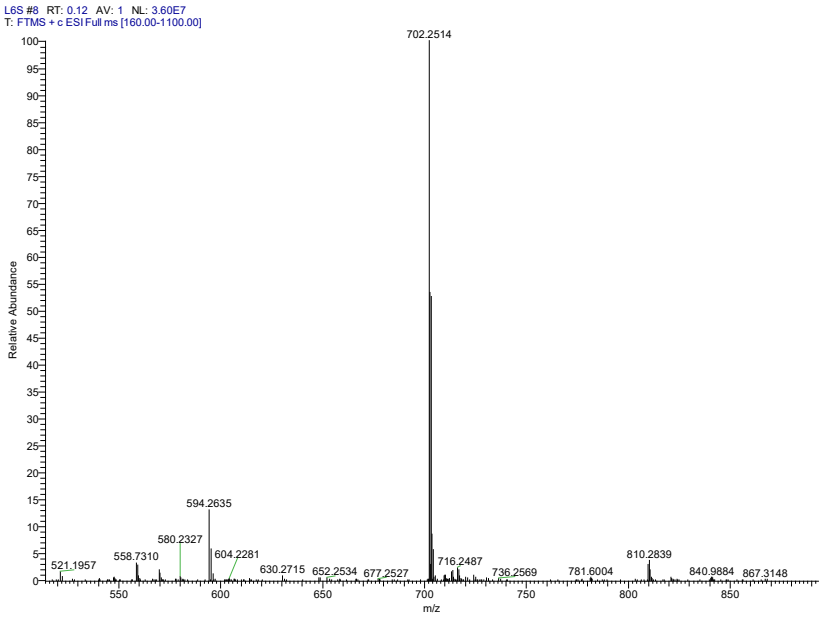

Figure S5 ESI-MS spectra of **M**.