Electronic Supplementary Information (ESI)

Mitochondria-nucleolus migration fluorescent probe for monitoring of mitochondrial membrane potential and identification of cell apoptosis

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Instruments and Materials

UV-vis absorption spectra were measured in 1 cm quartz cells with a TU-1901 (PERSEE) UV-vis spectrophotometer. A F7000 fluorescence spectrophotometer (HITACHI) at 298 K was used for fluorescence measurements with a 600 V PMT voltage.^{1}H NMR and ^{13}C NMR were measured on an Agilent AM400 NMR spectrometer using TMS as internal standard. High resolution electrospray ionization mass spectra (HR-ESI-MS) were recorded on a BrukerDaltonics Bio TOF mass spectrometer. Fluorescence imaging was conducted on a ZEISS LSM 780 confocal laser scanning microscope.

Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. All of solvents used were analytical-reagent grade in synthesis and HPLC in the optical spectroscopic studies.

DNase and RNase Treatment

HeLa cells were fixed at −20 °C for 10 min in a cold MeOH solution and then washed with PBS three times. DNase (20 μg/mL) or RNase (25 μg/mL) solutions were added into the samples and incubated for 3 h in the cell culture incubator. Then cell samples were incubated with 5 μM **Cz-Bz-1** for another 30 min. The cells were washed with PBS three times and imaged.

Synthesis

Synthesis of **Cz-Bz-1**: Compound **3**[1] (2.0 mmol, 0.47 g) and **2**[2] (5 mmol, 1.76 g) were dissolved in 50 mL EtOH. 1 drop of piperidine was added and the mixture was refluxed for 12 hours. After cooling to room temperature, the solvent was removed under reduced pressure; the crude product was purified by silica gel column (DCM:MeOH 100:1) to give the product as a red solid (0.54 g) in 30% yield. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 9.39 (s, 2H), 8.73 (d, *J* = 16.0 Hz, 2H), 8.49 (t, *J* = 9.4 Hz, 4H), 8.31 (d, *J* = 8.8 Hz, 2H), 8.23 (d, *J* = 8.0 Hz, 2H), 8.14 (d, *J* = 8.8 Hz, 2H), 7.95 (d, *J* = 8.4 Hz, 2H), 7.91-7.80 (m, 4H), 7.73 (t, *J* = 7.0 Hz, 2H), 4.37 (s, 6H), 4.09 (s, 3H), 2.10 (s, 12H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 182.0 (s), 153.3 (s), 144.5 (s), 139.6 (s), 137.6 (s), 133.0 (s), 130.8 (s), 130.1 (s), 128.4 (s), 127.2 (s), 127.0 (s),126.7 (s), 124.6 (s), 123.2 (s), 113.3 (s), 111.3 (s), 110.1 (s), 53.5 (s), 35.3 (s), 30.1 (s), 25.5 (s). HRMS (m/z): $[M-2I]^{2+}$ calcd. for $C_{47}H_{43}N_3^{2+}$, 324.6723, found 324.6707.

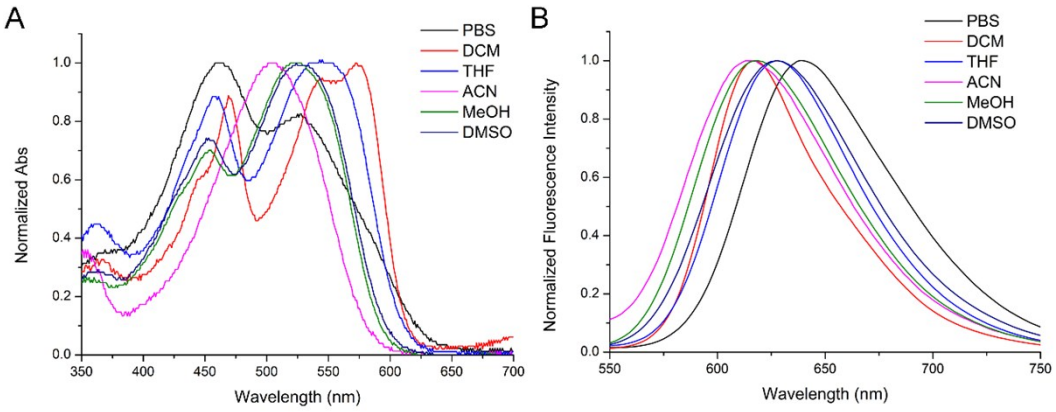

Fig. S1 Normalized absorption (A) and fluorescence spectra (B) of **Cz-Bz-1** (2 μM) in different solvents. λ_{ex} = 460 nm.

Table S1: Photophysical properties of **Cz-Bz-1** in different solvents.

Solvents	**Maxima λ_{ab}**	**Maxima λ_{em}**	**ε ($M^{-1} \cdot cm^{-1}$)**	**Φ (%)**
PBS	460	640	43000	1.5
DCM	572	617	49000	4.8
THF	534	628	43500	2.1
ACN	501	614	37500	--
MeOH	519	619	58500	4.1
DMSO	524	628	56500	5.8

ε (M^{-1} cm^{-1}): molar absorption coefficients at maximum absorption wavelengths, Φ:absolute fluorescence quantum yields in different solvents.

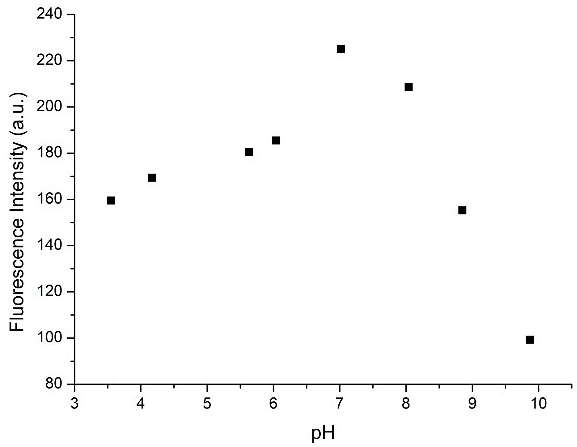

Fig. S2 Fluorescence intensity (λ_{em}= 640 nm) of **Cz-Bz-1** (2 μM) at different pH in PBS. λ_{ex} = 460 nm.

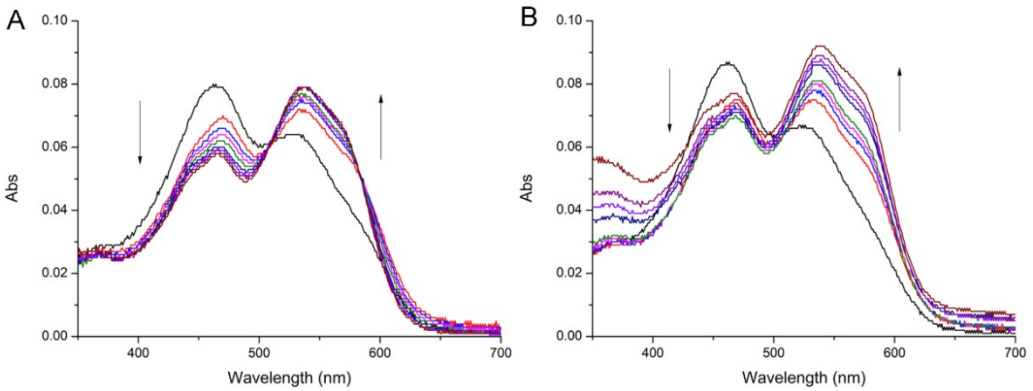

Fig. S3 Absorption titrations of **Cz-Bz-1** (2 μM) in PBS with increasing concentrations of DNA (A) and RNA (B). DNA and RNA concentrations: 0-300 μg/mL.

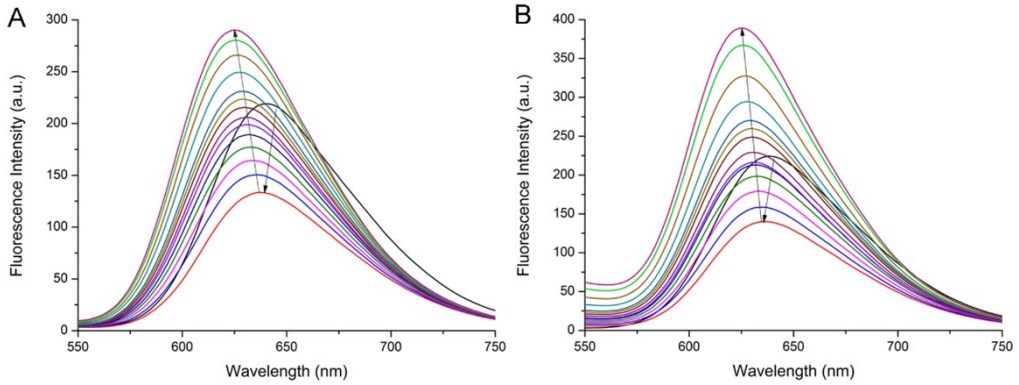

Fig. S4 Fluorescence titrations of **Cz-Bz-1** (2 μM) in PBS with increasing concentrations of DNA (A) and RNA (B). DNA and RNA concentrations: 0-300 μg/mL, λ_{ex} = 460 nm.

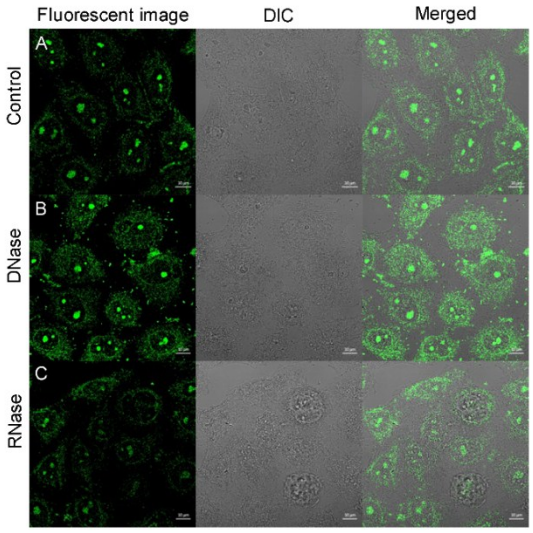

Fig. S5 Fluorescent images of DNase and RNase digest experiments for **Cz-Bz-1** (5 μM). λ_{ex} = 488 nm, λ_{em} = 600−650 nm; scale bar = 10 μm.

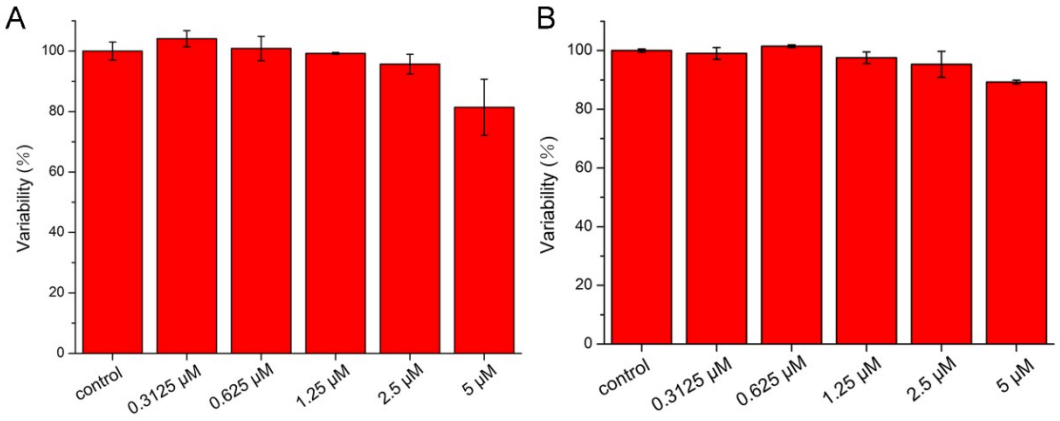

Fig. S6 MTS (A) and ATP detection (B) results of HeLa cell viabilities after incubation with **Cz-Bz-1** for 24 h at different incubation concentrations. The viability of cells without **Cz-Bz-1** is defined as 100%. The results are expressed as the mean ± SD of three separate measurements.

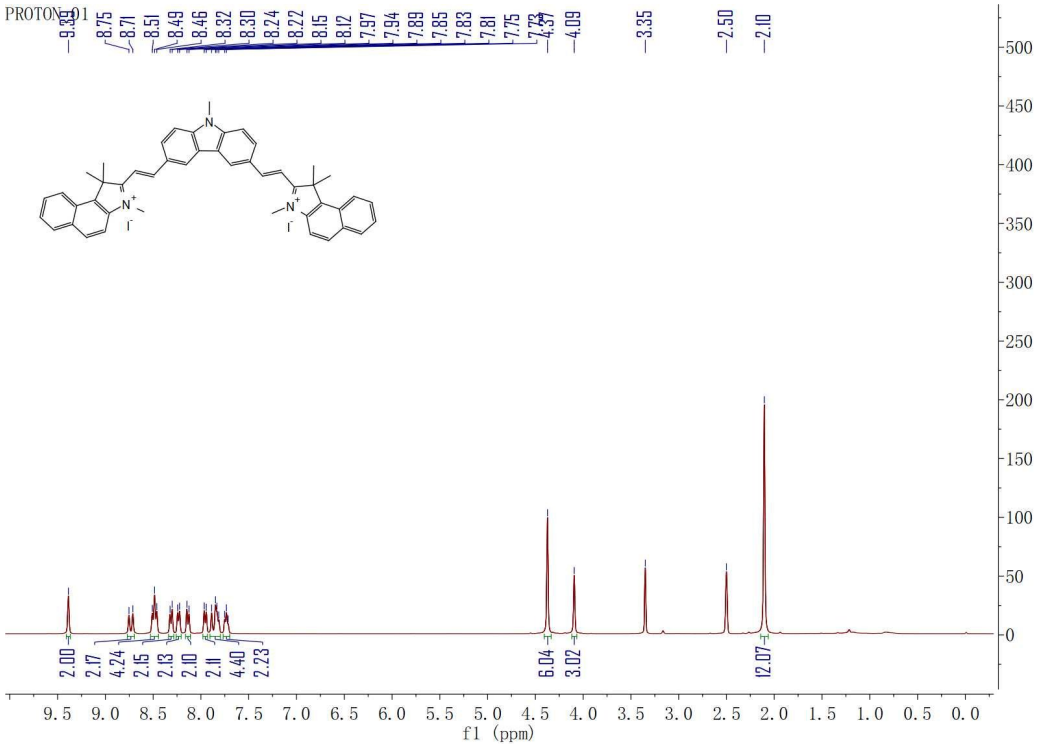

Fig. S7 ^{1}H NMR spectrum of **Cz-Bz-1**.

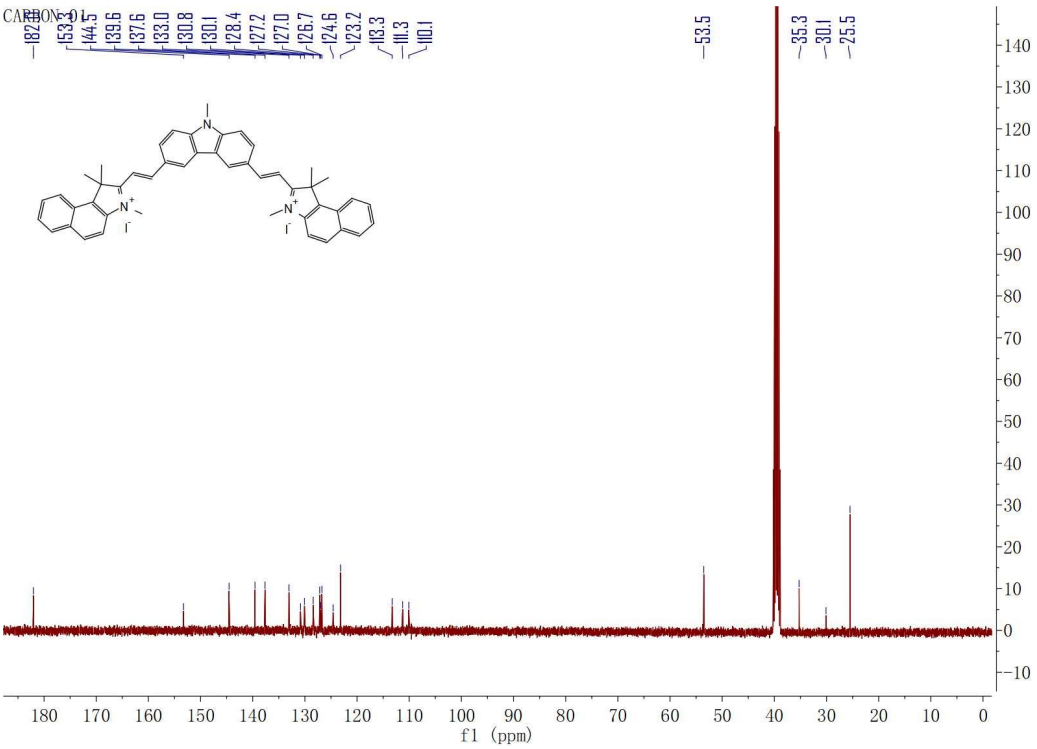

Fig. S8 ^{13}C NMR spectrum of **Cz-Bz-1**.

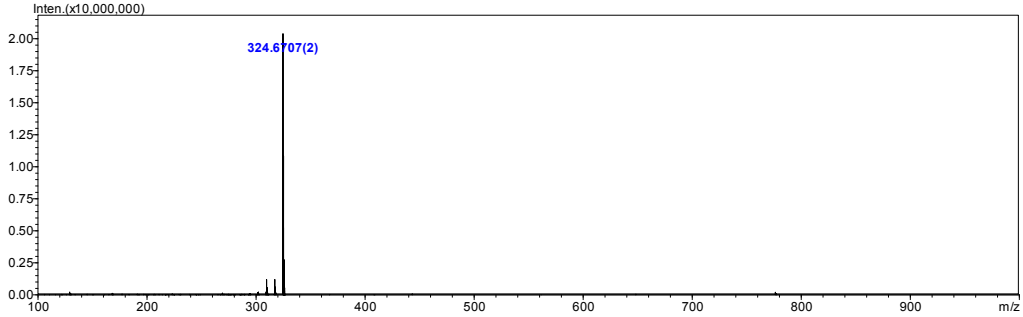

Fig. S9 HRMS spectrum of **Cz-Bz-1**.

Reference

1. M.-Y. Li, P.-C. Cui, K. Li, J.-H. Feng, M.-M. Zou, X.-Q. Yu, *Chin. Chem. Lett.*, 2016, **27**, 330–334.

2. N. Narayanan, G. Patonay, *J. Org. Chem.*, 1995, **60**, 2391-2395.