Supporting Information

AIE-based bioprobe for differentiating early and late stages of apoptosis mediated by H_2O_2

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Table of Contents

Experimental Section

Materials and Method

THF (Labscan) was purified by simple distillation from sodium benzophenone ketyl under nitrogen immediately before use. 1-Bromo-3,5-dimethylbenzene, *N*-bromosuccinimide (NBS), benzoyl peroxide (BPO), N,N-Diisopropylethylamine (DIPEA), potassium carbonate, tetrakis (triphenylphosphine) palladium [$Pd(PPh_3)_4$], Zinc Nitrate, methanol, hydrogen peroxide solution, propidium iodide (PI) and other reagents were all purchased from Aldrich and used as received. Di-(2-picoyl) amine (DPA) was purchased from J&K and used as received. Carbon tetrachloride was purchased from Nacalai Tesque and used as received. Chloroform and dichloromethane (DCM) were purchased from Scharlau and used as received. The compounds **2**, **3** and **4** were prepared following the reported procedures[1].

^1H and ^{13}C NMR spectra were measured on a Bruker AV 400 spectrometer in DMSO-d_6. High-resolution mass spectra (HRMS) were recorded on a GCT premier CAB048 mass spectrometer operating in MALDI-TOF mode. The PL spectra were recorded on a PerkinElmer LS 55 spectrophotometer. UV spectra were measured on a Milton Ray Spectronic 3000 Array spectrophotometer

Minimum essential medium (MEM), Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), penicillin and streptomycinwere purchased from Life Technologies. Annexin V-FITC and its 5X binding solution were purchased from Promokine.

Synthesis

Prepararion of TPE-BDPA (5). A suspension of TPE-$B(OH)_2$ (**4**, 0.188 g, 0.5 mmol), bromo-BDPA (**3**, 0.17 g, 0.3 mmol), $Pd(PPh_3)_4$ (0.015 g, 0.025 mmol), K_2CO_3 (0.15 g, 2.08 mmol) in dry THF (25 mL) and distilled water (6 mL) were refluxed for 24 h. Afterward, the reaction mixture was cooled to room temperature and filtered. The filtrate was extracted with DCM and washed with brine for 3 times and the crude product was purified on an alumina column using DCM as eluent. Compound **4** was isolated as yellow solid in 80% yield. δ_H (400 MHz, DMSO-d_6): δ (TMS, ppm) 3.73 (s, 4H), 3.84 (s, 8H), 7.04-7.15 (m, 20H), 7.27 (s, 1H), 7.33-7.35 (d, 2H), 7.46 (s, 3H), 7.59-7.61 (m, 8H), 8.51-8.52 (dd, 4H). δ_C (400 MHz, $CDCl_3$): δ (TMS, ppm) 149.00, 143.79, 143.76, 141.14, 140.53, 139.53, 138.73, 136.44, 131.79, 131.46, 131.37, 128.07, 127.80, 127.72, 127.67, 126.51, 126.45, 126.24, 126.21, 122.78, 121.98, 60.14, 58.68. HRMS (MALDI-TOF): *m*/*z* 830.4097 [M+, calcd: 830.4051].

Prepararion of TPE-Zn_2BDPA (6). A methanolic solution of compound **5** (0.207 g, 0.25 mmol) and an aqueous solution of zinc nitrate (0.1521 g, 0.512 mmol) were mixed and stirred for 0.5 h. The solvent was removed and the residue lyophilized to

afford the complex **6** in quantitative yield. δ_H (400 MHz, DMSO-d_6) : δ (TMS, ppm) 3.75-3.79 (d, 4H), 3.96 (s, 4H), 4.38-4.42 (d, 4H), 6.99-7.22 (m, 17H), 7.32 (s, 1H), 7.55-7.68 (m, 12H), 8.06-8.10 (t, 4H), 8.65-8.66 (d, 4H). δ_C (400 MHz, DMSO-d_6) : δ (TMS, ppm) 149.27, 143.67, 143.58, 142.75, 141.23, 140.57, 139.81, 136.96, 131.90, 131.77, 131.21, 131.12, 129.28, 129.16, 128.38, 128.33, 128.26, 127.07, 126.35, 126.02, 122.86, 122.61, 59.70, 57.91. HRMS (MALDI-TOF): *m*/*z* 1144.2347 [M-NO_3+, calcd: 1144.2327]

Cell Culture and Imaging

Cell Culture HeLa cells and HepG2 cells were cultured in the MEM or DMEM containing 10% FBS and antibiotics (100 units/mL penicillin and 100 μg/mL streptomycin) in a 5% CO_2 humidity incubator at 37°C respectively.

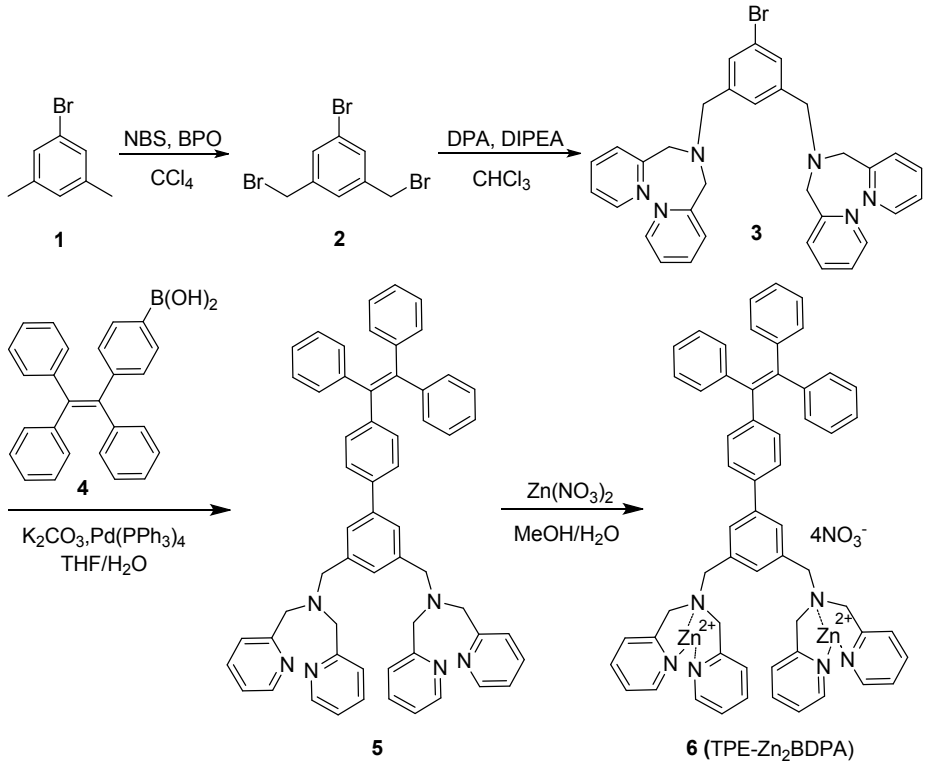
Cell Imaging HeLa cells were grown overnight on a 35 mm petri dish with a cover slip. The cells were stained with 5 μM of TPE-Zn_2BDPA for 15 min (by adding 1 μL of a 10 mM stock solution of TPE-Zn_2BDPA in DMSO to 2 mL culture medium) or 0.5 μM of PI for 30 min (by adding 0.5 μL of a 1 mM stock solution of PI in H_2O to 2 mL culture medium) or Annexin V- FITC solution for 15 mins (by adding 2 μL of Annexin V – FITC to 100 μL of 1X binding buffer). The cells were imaged under an FL microscope (BX41 Microscope) using different combination of excitation and emission filters for each dye: for TPE-Zn_2BDPA, excitation filter = 330–385 nm, dichroic mirror = 400 nm, and emission filter = 420 nm long pass. The cells were also imaged under confocal microscope (Zeiss Laser Scanning Confocal Microscope; LSM7 DUO) and different sets of filters. TPE-Zn_2BDPA was excited at 405 nm (100 % laser power) and the fluorescence was collected at 415–490 nm while Annexin V-FITC and PI used FITC and Texas Red filter respectively.

For photo-stability test, the cells were imaged by confocal microscope (Zeiss Laser Scanning Confocal Microscope; LSM7 DUO). TPE-Zn_2BDPA was excited at 405 nm (100 % laser power) and the fluorescence was collected at 415–490 nm. Annexin V using FITC filter was excited at 470 nm (100 % laser power) and fluorescence was collected at 500–550 nm.

Cell Viability Evaluated by MTT Assay. Viability of the cells was assayed using cell proliferation Kit I with the absorbance of 595 nm being detected using a Perkin-Elmer Victor plate reader. Five thousand cells were seeded per well in a 96-well plate. After overnight culture, various concentrations of TPE-Zn_2BDPA were added into the 96-well plate. After 24 h treatment, 10 μL of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/mL in phosphate buffer solution) was added into the each well. After 4 h incubation at 37 °C, 100 μL of solubilization solution containing 10%

SDS and 0.01 M HCl was added to dissolve the purple crystals. After 4 h incubation, the optical density readings at 595 nm were taken using a plate reader. Each of the experiments was performed at least 3 times.

Flow Cytometric Assay HeLa cells were cultured in a 60 mm petri dish. After overnight culture, HeLa cells were treated with 300 μM or 2000 μM H_2O_2 for 9h in the incubator. The treated cells were trypsinised and collected by centrifugation. The supernatant was removed and the cells were then stained with 5 μM of TPE-Zn_2BDPA for 15 min, 0.5 μM of PI for 30 min or 2 μL Annexin V-FITC solution for 15 mins. The staining solution was then removed. The cells were washed three times with PBS. The cells were re-suspended with PBS (pH 7.4). The fluorescence signals of the red, blue and green emission were collected in a flow cytometry (Becton Dickinson FACS Aria IIIu). Signals were collected from blue channel (λ_{ex}: 375 nm, λ_{em}: 450±20 nm), FITC green channel (λ_{ex}: 488 nm, λ_{em}: 530 ±15 nm) and PI red channel (λ_{ex} : 561 nm, λ_{em} : 610 ± 10 nm).



Scheme S1. Synthetic route to TPE-Zn_2BDPA.

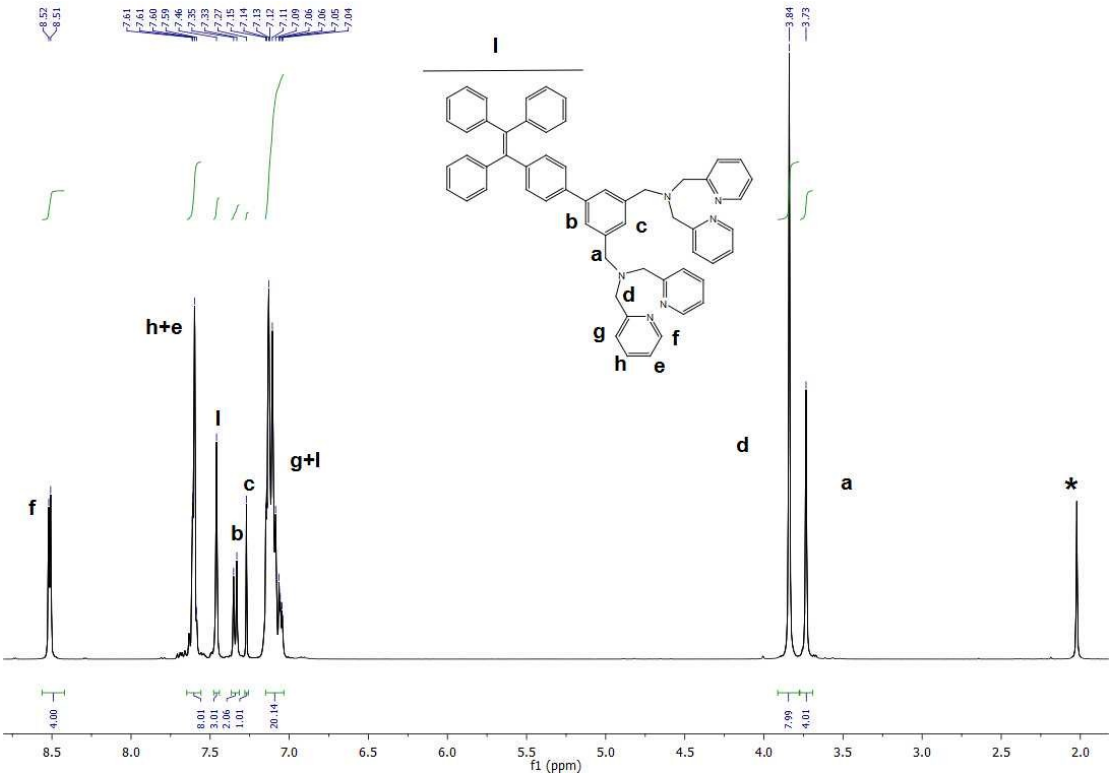


Fig. S1 ^{1}H NMR spectrum of **5** in DMSO-d_6.

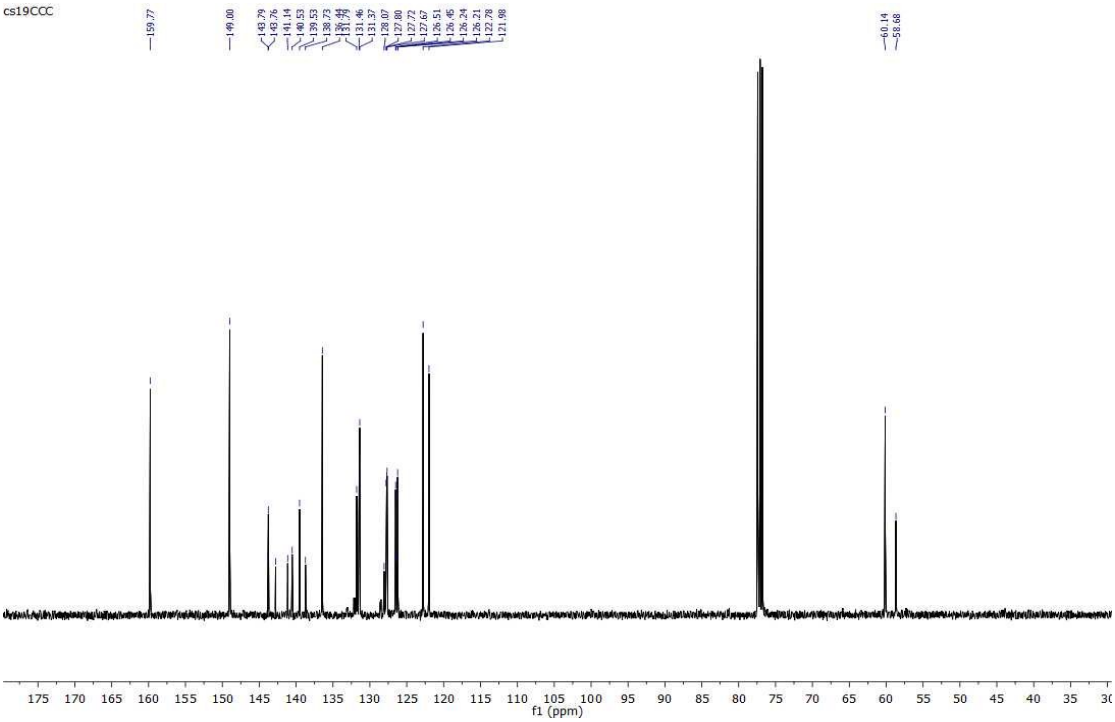


Fig. S2 ^{13}C NMR spectrum of **5** in DMSO-d_6.

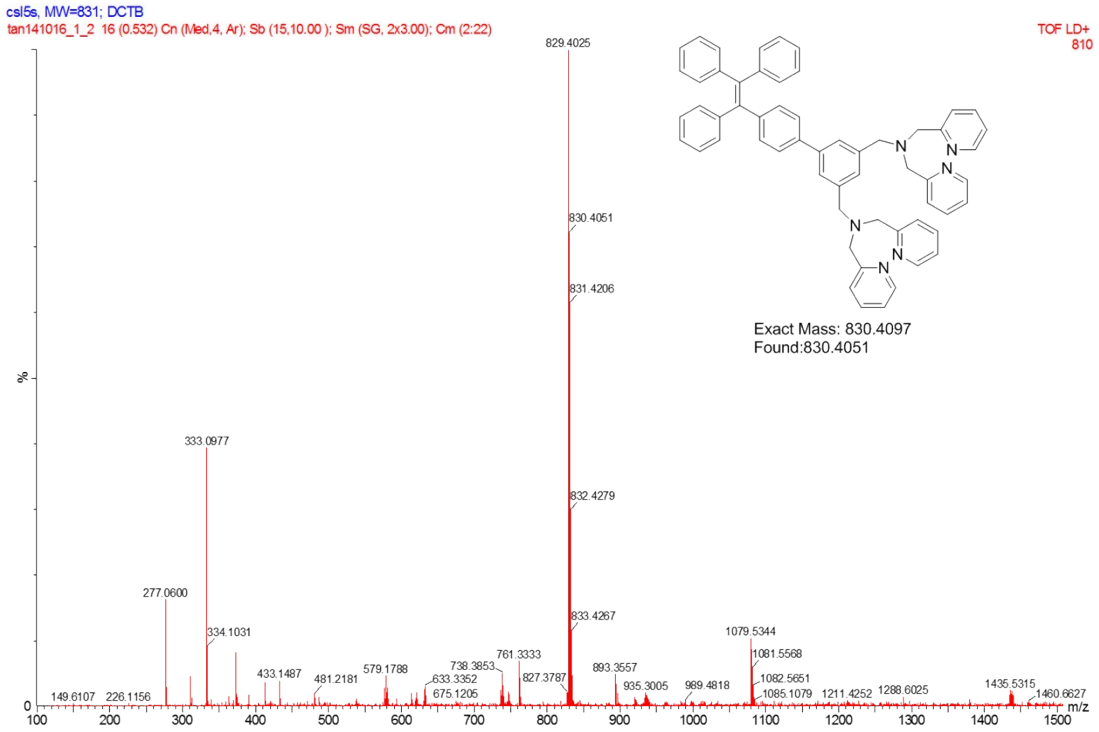


Fig. S3 High resolution mass spectrum of **5**.

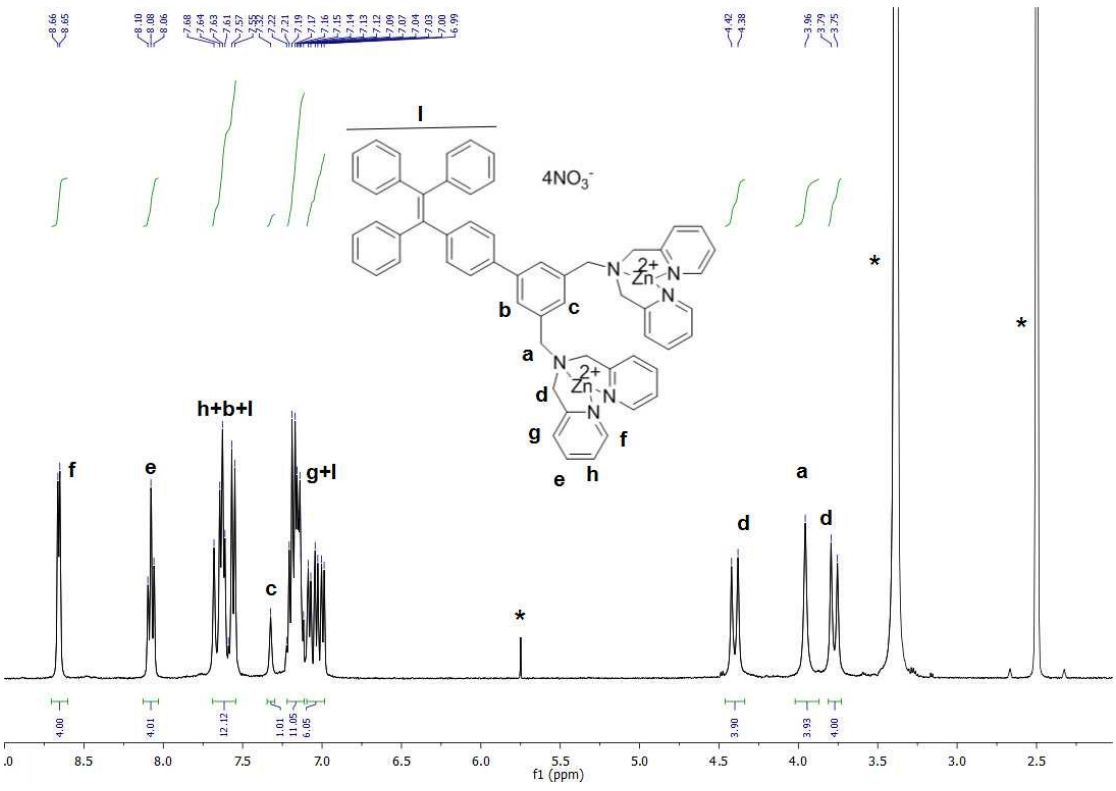


Fig. S4 ^{1}H NMR spectrum of TPE-Zn_2BDPA in DMSO-d_6.

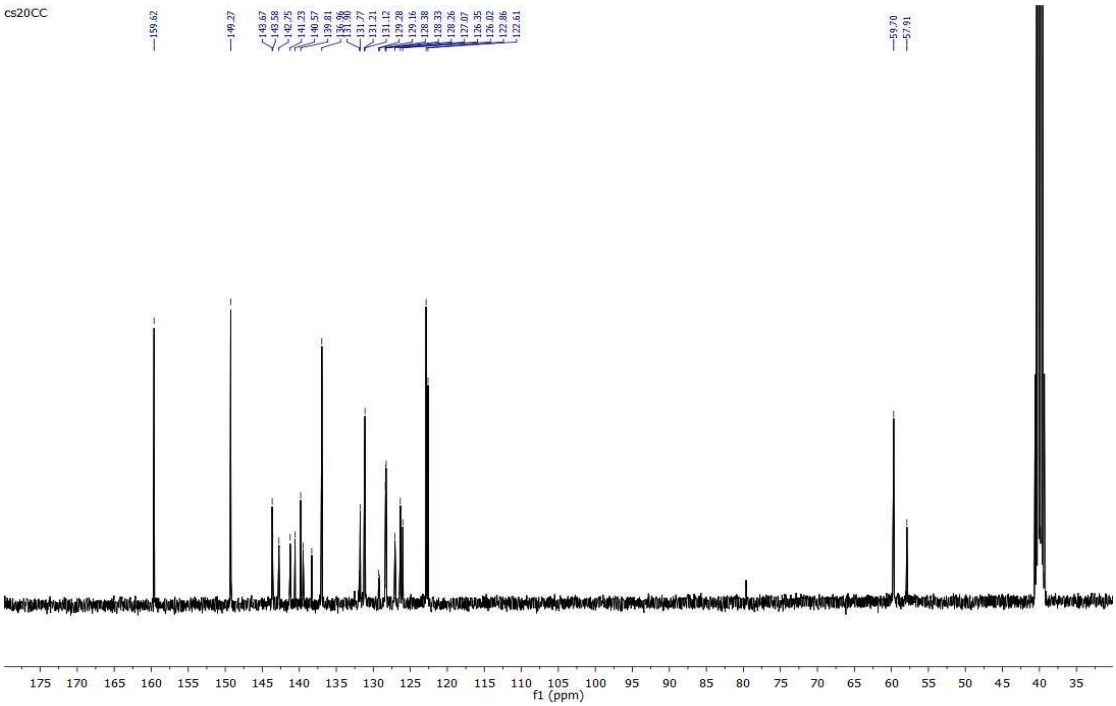


Fig. S5 ^{13}C NMR spectrum of TPE-Zn_2BDPA in DMSO-d_6.

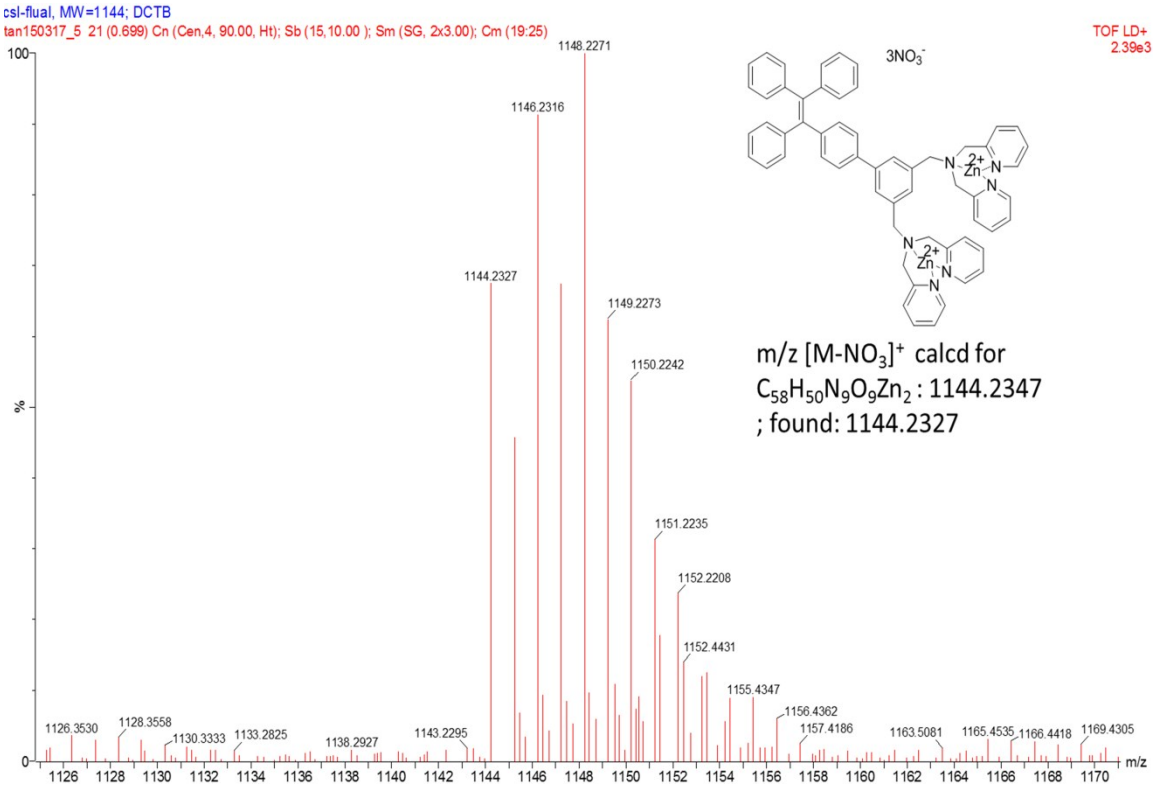


Fig. S6 HRMS of TPE-Zn_2BDPA.

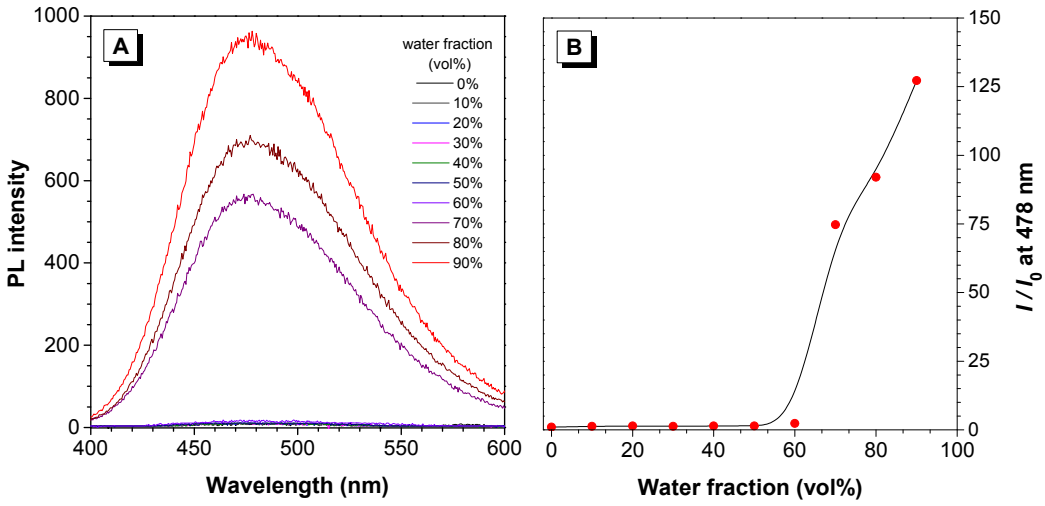


Fig. S7. (A) Emission spectra of TPE-Zn_2BDPA in DMSO/water mixtures with different water fractions. (B) Plot of relative PL intensity (I/I_0) at 478 nm versus the composition of the DMSO/water mixtures of TPE-Zn2BDPA, where I_0 is the PL intensity of TPE-Zn_2BDPA in pure DMSO. Concentration: 10 μM; λ_{ex}: 325 nm.

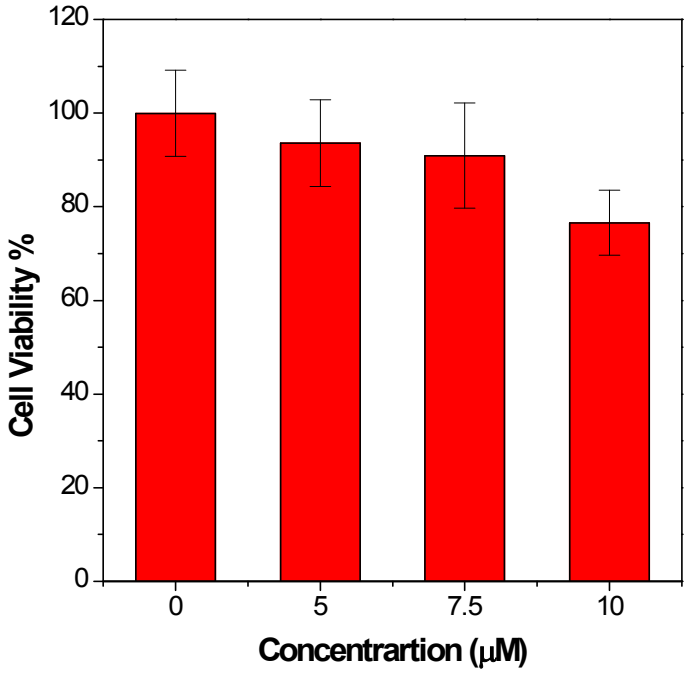


Fig. S8 Cytotoxicity of TPE-Zn_2BDPA evaluated by MTT assay

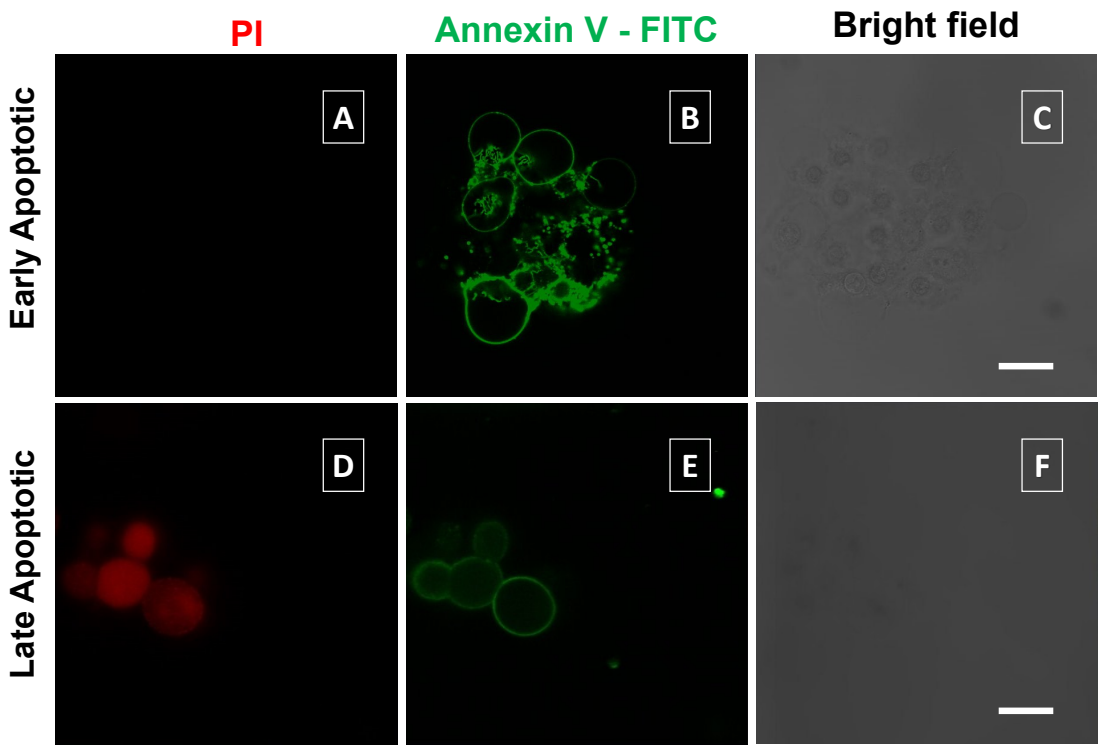


Fig. S9 (A–B and D–E) CLSM and (C and F) bright-field images of early apoptotic HeLa cells (A–C) induced by H_2O_2 (300 μM) and late apoptotic HeLa cells (D–F) induced by H_2O_2 (2000 μM), followed by co-staining with Annexin V-FITC (B and E) and PI (A and D). Concentration: 0.5 μM (PI) and 2 μL (Annexin V-FITC); Texas Red filter: λ_{ex}: 572±12.5 nm; band pass filter: 598–660 nm (A and D), FITC filter: λ_{ex}: 470±20 nm; band pass filter: 500–550 nm (B and E); scale bar = 20 μm.

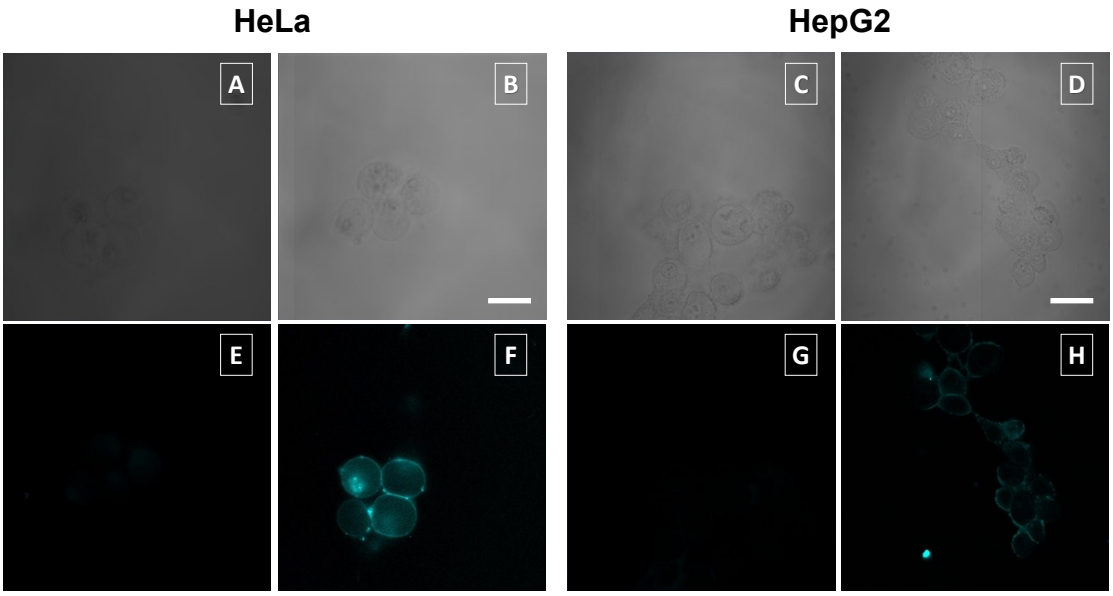


Fig. S10 (A–D) Bright-field (E–F) and confocal images of untreated (A and E) and H_2O_2-treated HeLa cells (B and F), and untreated (C and G) and H_2O_2-treated HepG2 cells (D and H) stained with TPE-Zn_2BDPA for 15 mins. Concentration: 5 μM (TPE-Zn_2BDPA), 2000 μM (H_2O_2; HeLa) and 3000 μM (H_2O_2; HepG2); λ_{ex}: 330–385 nm; scale bar: 30 μm.

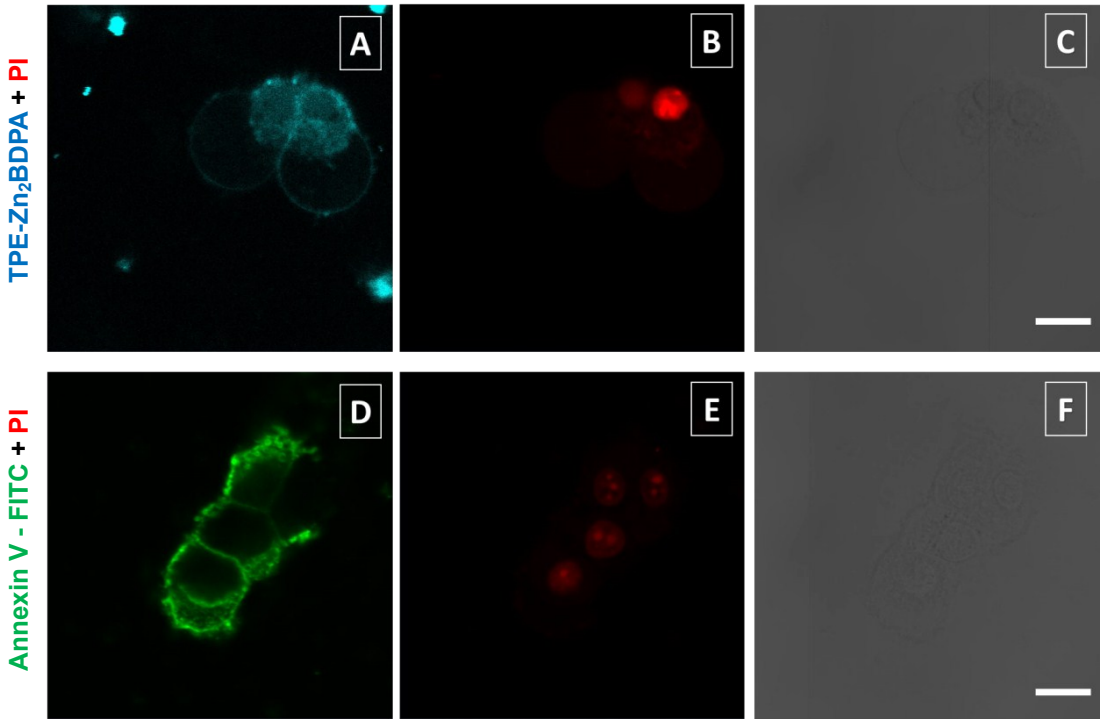


Fig. S11 (A, B, D and E) Confocal and (C and F) bright-field images of HepG2 cells pretreated with H_2O_2 (3000 μM) co-stained with PI and TPE-Zn_2BDPA (A–C), and co-stained with PI and Annexin V-FITC (D–F). Concentration: 0.5 μM (PI), 5 μM (TPE-Zn_2BDPA) and 2 μL (Annexin V-FITC); λ_{ex}: 405 nm; band pass filter: 415–490 nm (A), Texas Red filter: λ_{ex}: 572±12.5 nm; band pass filter: 598–660nm (B and E), FITC filter: λ_{ex}: 470±20 nm; band pass filter: 500–550 nm (D); scale bar = 20 μm.

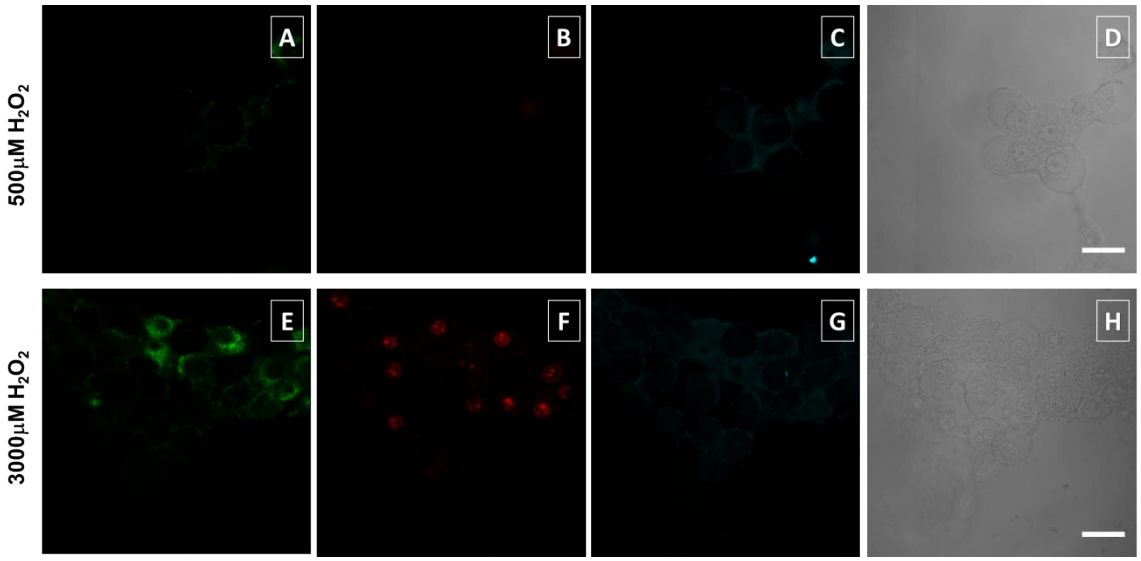


Fig. S12 (A–C and E–G) Confocal and (D and H) bright field images of HepG2 cells pretreated with H_2O_2 at concentration of 500 μM (A–D) and 3000 μM (E–H), and co-stained with TPE-Zn_2BDPA, PI and Annexin V-FITC. Concentration: 5 μM (TPE-Zn_2BDPA), 0.5 μM (PI) and 2 μL (Annexin V-FITC); λ_{ex}: 405 nm; band pass filter: 415–490 nm (C and G), Texas Red filter: λ_{ex}: 572±12.5 nm (B and F) ; FITC filter: λ_{ex}: 470±20 nm; band pass filter: 500–550 nm (A and E); scale bar: 20 μm.

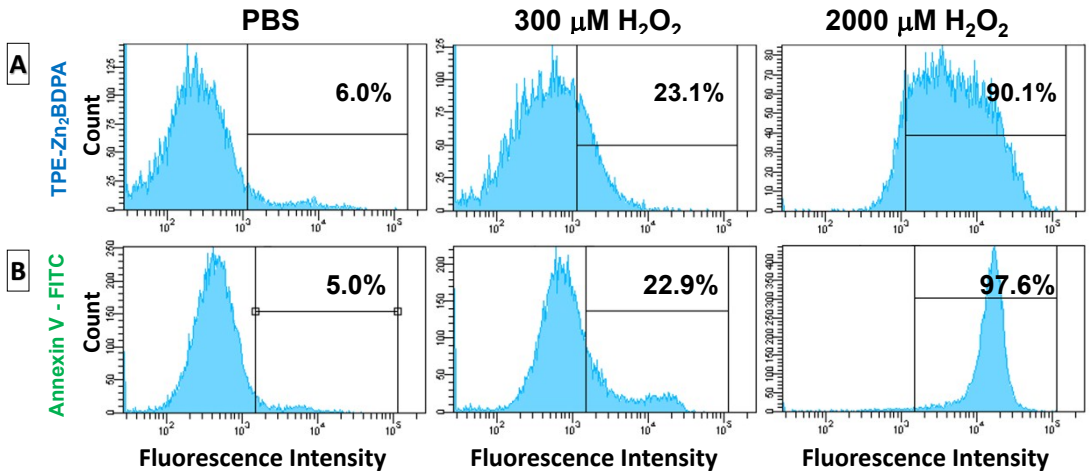


Fig. S13 Flow cytometry analysis of apoptotic HeLa cells induced by H_2O_2 at different concentrations for 9 h, stained with (A) TPE-Zn_2BDPA and (B) Annexin V-FITC.

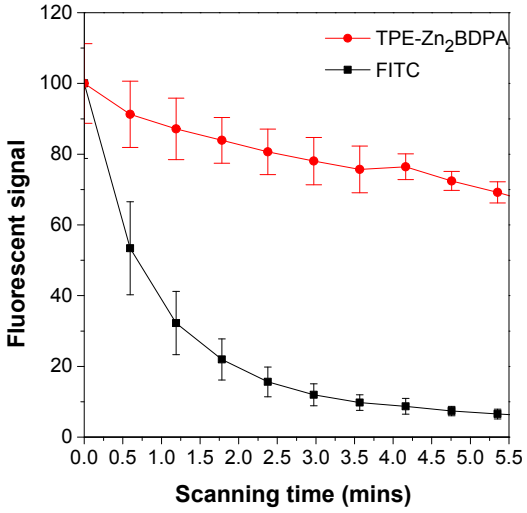


Fig. S14 Photostability of HeLa cells pretreated with 2000 μM H_2O_2 and stained with TPE-Zn_2BDPA and Annexin V-FITC with increasing scanning time. Concentration: 5 μM (TPE-Zn_2BDPA) and 2 μL (Annexin V-FITC); λ_{ex}: 405 nm; band pass filter 415–490 nm (TPE-Zn_2BDPA) and FITC filter: λ_{ex}: 470±20 nm; band pass filter: 500–550 nm (FITC).

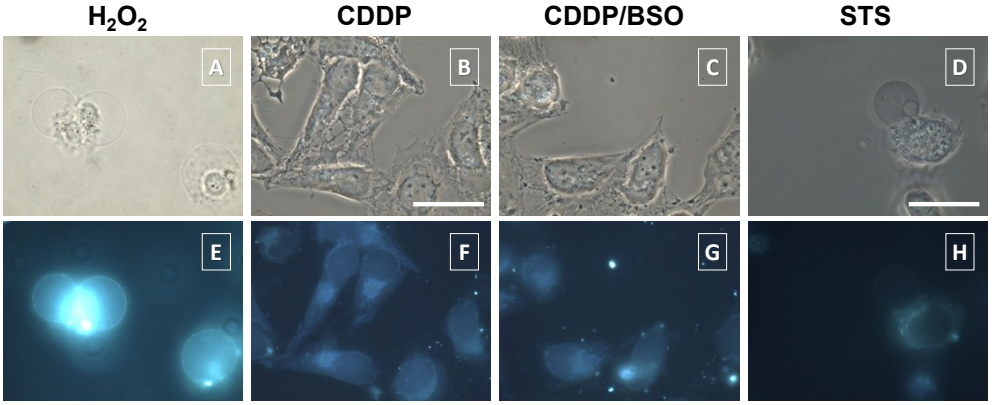


Fig. S15 (A–D) Bright-field and (E–H) fluorescent images of HeLa cells treated with H_2O_2 for 9 h and followed by staining of TPE-Zn_2BDPA for 15 min (A and E), treated with CDDP for 6 h and followed by staining with TPE-Zn_2BDPA for 30 mins (B and F), incubated in BSO for 24 h and reacted with CDDP for 6 h and followed by staining with TPE-Zn_2BDPA for 30 mins (C and G), treated with STS for 2 h before staining with TPE-Zn_2BDPA for 30 min (D and H). Concentration: TPE-Zn_2BDPA = 5 μM, H_2O_2 = 2 mM, BSO = 1 mM, CDDP = 0.5 mM; λ_{ex}: 330–385 nm; scale bar: 30 μm.

Reference

1. H. W. Rhee, H.Y. Choi, K. Han and J. I. Hong, *J. Am. Chem. Soc.*, 2007, **129**, 4524-4525.