Electronic Supplementary Material (ESI) for Analytical Methods.

Fabrication of a Fluorescent Probe for Reversibly Monitoring Mitochondrial Membrane Potential in Living Cells

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Materials and apparatus

Unless otherwise stated, all the used reagents were acquired from commercial suppliers without further purification. UV−vis absorption spectra were measured on a Shimadzu UV-2700 spectrophotometer and fluorescence spectra were recorded with a HITACHI F4600 fluorescenc spectrophotometer. Fluorescence imaging experiments were performed with Nikon A1MP confocal microscopy. TLC analysis carried out on silica gel plates and column chromatography was conducted over silica gel (mesh200−300), both of them were purchased from the Qingdao Ocean Chemicals. ^{1}H and ^{13}C NMR spectra were performed on an AVANCE III 400 MHz digital NMR spectrometer. High resolution mass spectrometric (HRMS) analyses were carried out on an Agilent 1100 HPLC/MSD spectrometer.

General procedure for spectral measurements

The stock solution of **REP** (5 mM) was prepared in methanol. For optical measurements, suitable amount of **REP** stock solutions were added into methanol, ethanol, or buffer solutions to obtain working solutions of **REP** (10.0 μM). These solutions were sonicated for 5 min to remove air bubbles. Afterwards, the absorption and emission spectra were acquired with UV spectrophotometer and fluorescence spectrophotometer. The excitation wavelength was 488 nm to measure the emission spectra.

Cell culture

HepG 2 and 4T1 cells were purchased from Procell Life Science & Technology Co., Ltd., which were grown in H-DMEM (Dulbecco's Modified Eagle's Medium, High Glucose) supplemented with 10% FBS (Fetal Bovine Serum) in a 5% CO_2 incubator at 37 ˚C. For cell imaging experiments, live HepG 2 cells were suspended and diluted in the culture medium with cell concentration of 10000 cells/mL. 1 mL of the cell suspension solution was added into glass bottom dish and cultured for 24 h to allow adhesion.

Cell imaging experiments

The live cells adhered in the glass bottom dish was initially cultured in 1 mL of culture medium. Then the culture medium was removed and 1 mL of 2 μM **REP** dispersed in culture medium was added in. The cells were incubated with the probe for 20 min, which were directly placed under Nikon A1R confocal microscope without further washing procedure. The fluorescence signals were collected in red channel. λ_{ex} = 488 nm; λ_{em} = 570–620 nm.

Cytotoxicity test

The cytotoxicity of the **REP** has been evaluated with HepG 2 cells using the reagent MTT. A 96-well plate was used to perform the cell viability experiment. Suspension of HepG 2 cells with cell concentration of 10000 cells/mL was firstly prepared, and the suspension was added to the plate (200 μL per well). At the same time, culture medium without cells was also introduced into the wells (200 μL per well) as blank. The cells were incubated with 2 μM of **REP** for different time. Change the culture medium of all the wells, and after that 10 μL of MTT (5mg/mL) was added to each well. The well plate was incubated for 4 h, and then the culture medium in each well was removed. 200 μL of DMSO was added to dissolve the formazan, and finally the absorbance was measured with a microplate reader at 620 nm. The cell survival rates were finally calculated following the equation below:

$$Survival(\%) = \frac{(A_s - A_b)}{(A_c - A_b)} \times 100\% \quad (2)$$

Where A_s is the absorbance of the wells with cells treated with the probes, A_b is the absorbance of wells pretreated with only culture medium, and A_c is the absorbance of wells with cells treated with no reagents.

Synthesis of compound REP

4-(Diethylamino) salicylaldehyde (200 mg, 1 mmol) and 1-indanone (150 mg, 1.13 mmol) were dissolved in trifluoroacetic acid (TFA) (3 mL). The reaction mixture was refluxed for 6 hours. After cooled to room temperature and evaporation of the solvent, the residue was purified by column chromatography (dichloromethane: methanol =

30:1, v/v) to give red solid (yield 50%).^{1}HNMR (400 MHz, MeOD) δ 8.84 (s, 1H), 8.20 (d, J = 7.8 Hz, 1H), 8.03 (d, J = 9.4 Hz, 1H), 7.89 (d, J = 7.7 Hz, 1H), 7.81 (td, J = 7.5, 1.1 Hz, 1H), 7.72 (t, J = 7.6 Hz, 1H), 7.52 (dd, J = 9.4, 2.4 Hz, 1H), 7.38 (d, J = 2.0 Hz, 1H), 4.18 (s, 2H), 3.80 (q, J = 7.2 Hz, 4H), 1.39 (t, J = 7.2 Hz, 6H). ^{13}CNMR (101 MHz, MeOD) δ 159.22, 156.14, 148.17, 145.41, 133.80, 132.72, 132.55, 128.56, 126.91, 126.04, 122.21, 118.44, 117.52, 96.41, 45.74, 32.94, 11.28.HRMS (ESI): m/z calculated for $C20H20NO^{+}$ 290.1539 [M^{+}], found: 290.1548.

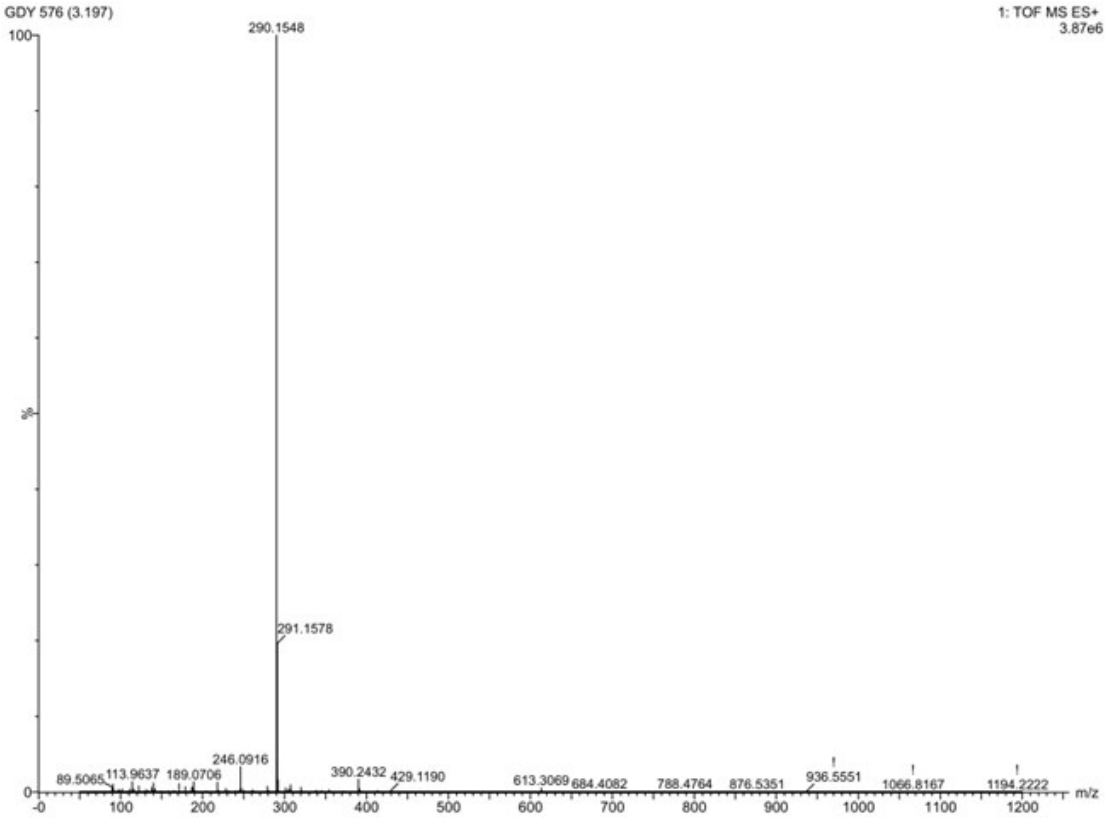

Figure S1. The HRMS spectrum of **REP**.

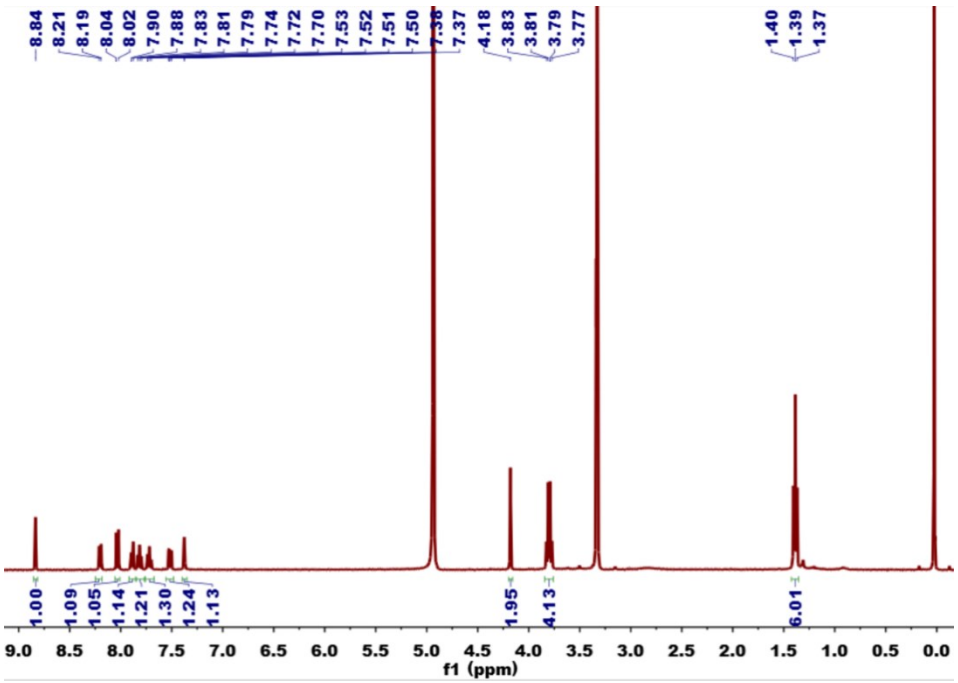

Figure S2. The ^{1}HNMR spectrum of **REP** in Methanol-d_4.

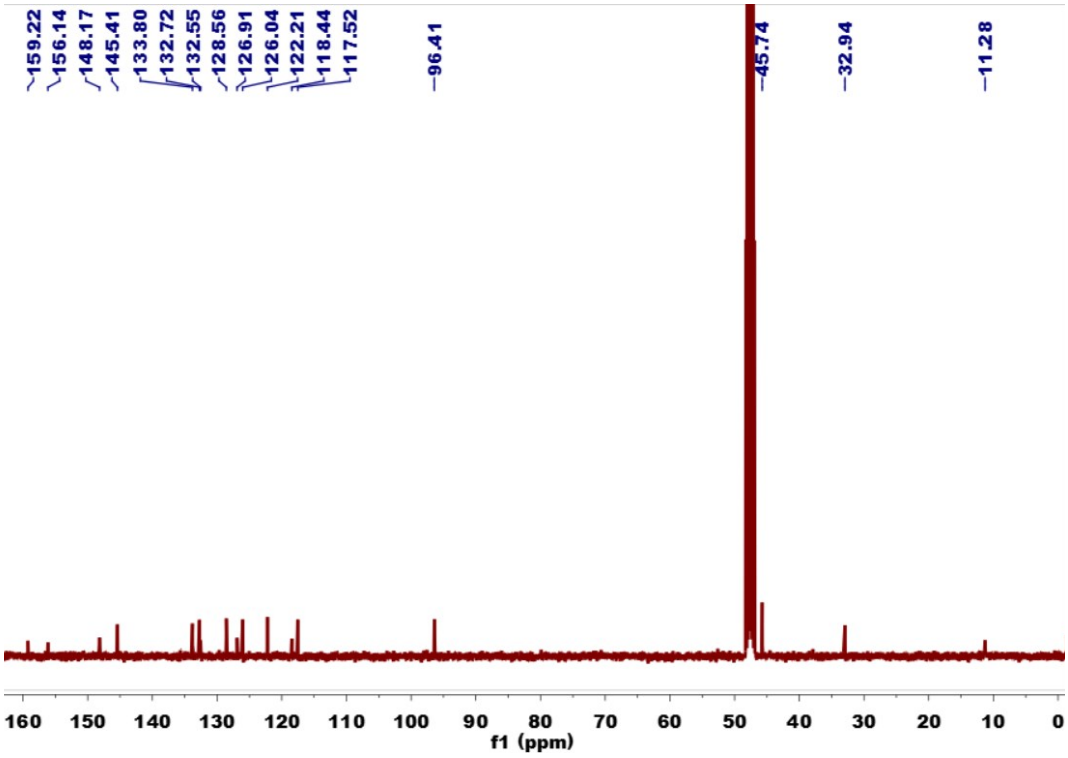

Figure S3. The ^{13}C NMR spectrum of **REP** in Methanol-d_4.

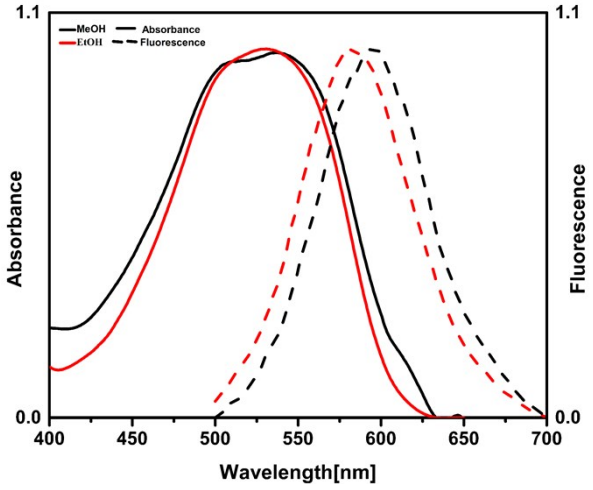

Figure S4. The normalized absorption and emission spectra of 10 μM **REP** in methanol and ethanol. Excitation wavelength: 488 nm.

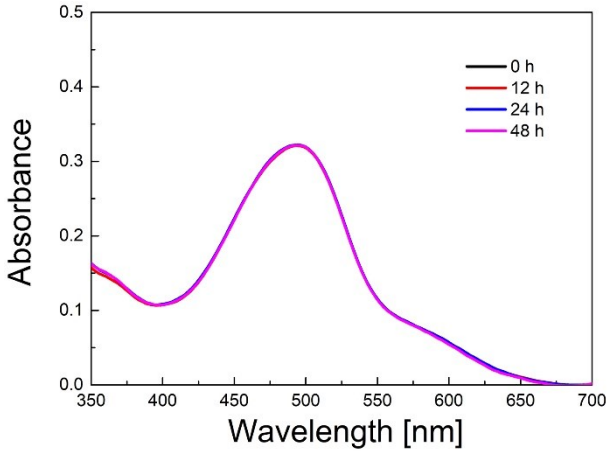

Figure S5. The absorbance of **REP** (10 μM) suspended in PBS buffer solutions for different time (0~48 h).

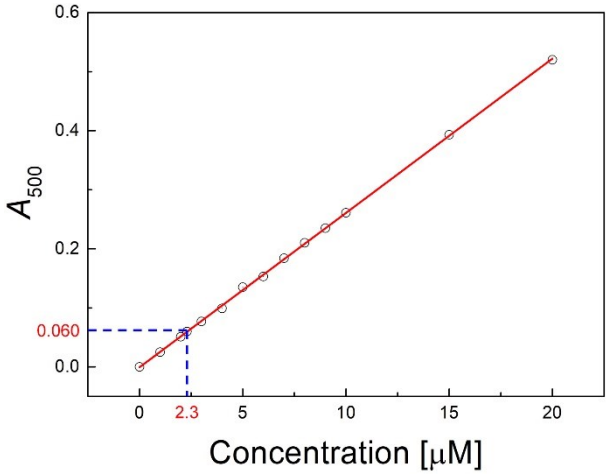

Figure S6. The plot of absorbance at 500 nm versus REP concentration in PBS buffer solution containing 1% DMSO, and the measured absorbance and concentration of REP saturated in PBS buffer solution.

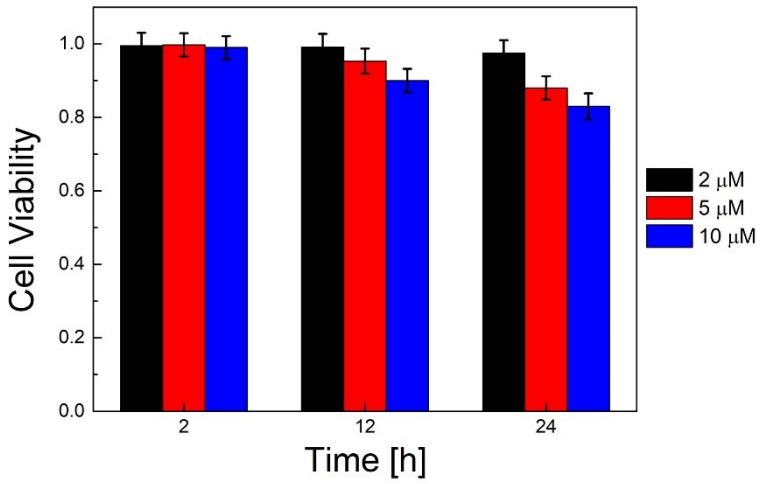

Figure S7. The cell viability of HepG 2 cells incubated with different concentration (2~10 μM) of **REP** for different time (2~24 h).