Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Development of a mitochondria-targeted fluorescent probe for hydrazine monitoring in living cells

Contents

1. General remarks for experimental

^{1}H-NMR, ^{13}C-NMR spectra were measured on a Bruker AM400 NMR spectrometer. Proton Chemical shifts of NMR spectra were given in ppm relative to internals reference TMS (1H, 0.00 ppm). ESI-MS and HRMS spectral data were recorded on a Finnigan LCQ^{DECA} and a BrukerDaltonics Bio TOF mass spectrometer, respectively. All pH measurements were performed with a pH-3c digital pH-meter (Shanghai Lei Ci Device Works, Shanghai, China) with a combined glass-calomel electrode. Fluorescence emission spectra were obtained using FluoroMax-4 Spectrofluorophotometer (HORIBA JobinYvon) at 298 K. Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. All the solvents were dried according to the standard methods prior to use. All of the solvents were either HPLC or spectroscopic grade in the optical spectroscopic studies.

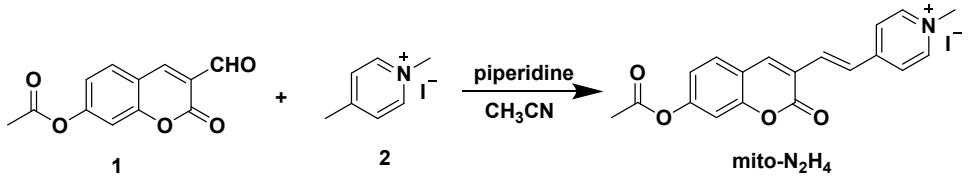
2. Fluorescence analysis

Probe **mito-N_2H_4** was prepared in DMSO at a concentration of 5mM. All UV/Vis and fluorescence titration experiments were performed using 5μM **mito-N_2H_4** PBS buffer solution (10 mM, pH 7.4) with varying concentrations of analytes at room temperature. The time dependences of the response of **mito-N_2H_4** (5μM) to hydrazine(50μM) were determined by mixing the two reactants in PBS buffer solution / DMSO (1:9, v/v).

3. Co-localization imaging of cells.

HepG2 cells were cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum and 1% Antibiotic-Antimycotic at 37^{o}C in a 5% CO_2/95% air incubator. For fluorescence imaging, cells (4×10^3/well) were passed on a 6-well plate and incubated for 24h. Immediately before the staining experiment, cells were washed twice with PBS, incubated with 5μM **mito-N_2H_4** for 30 min at 37^{o}C. Then, 50μM hydrazine or isopyknic PBS was added and incubated for another 30 min. After the cells were washed twice with PBS, Mito Tracker Deep Red(1μM) was added and incubated for 15 min and the confocal fluorescent images were captured.

4. Preparation and Characterization of mito-N_2H_4



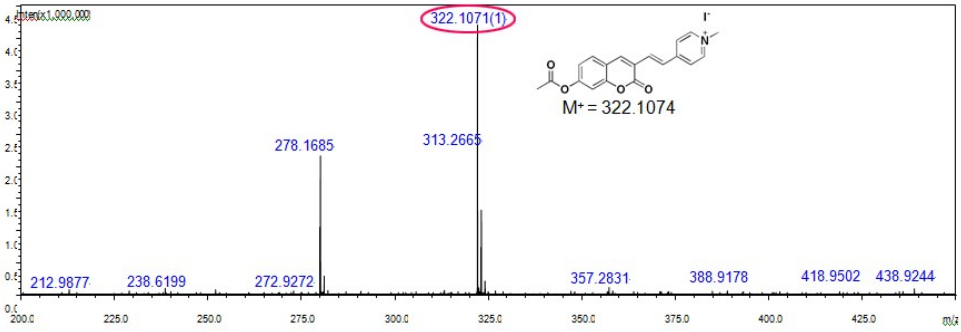
Compound **1** and **2** was synthesized according to the literature[1,2].

To a solution of compound **2**（1.247g，5.3mmol）and compound **1**(1.026g，4.4mmol) dissolved in 50mL acetonitrile, 3~5drops of piperidine was added and then the reaction solution was refluxed for 24h. After cooling to room temperature, the solvent was evaporated and the

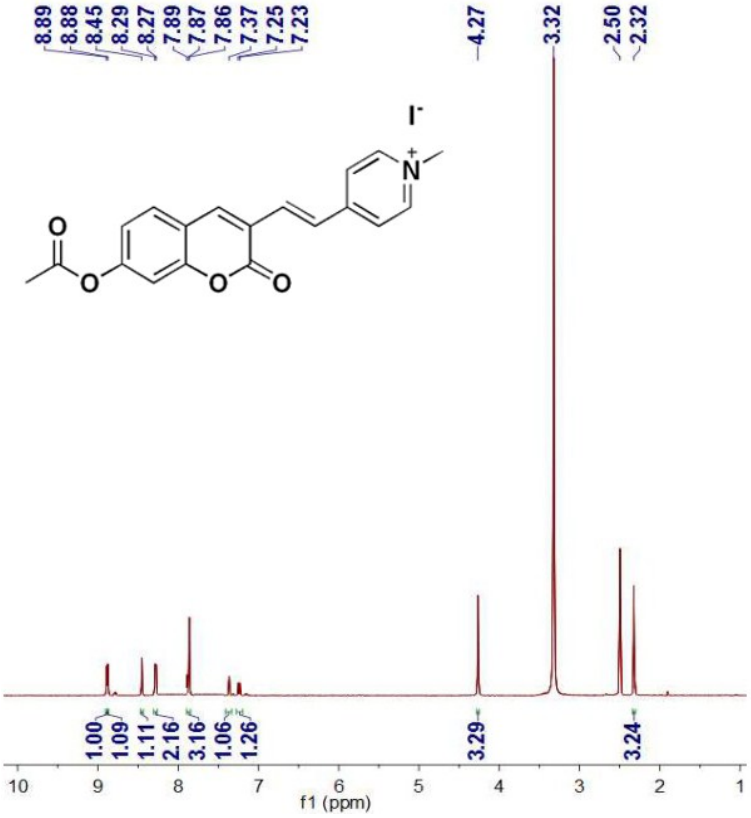
residue was then purified by column chromatography over silica gel eluting with petroleum ether/ethyl acetate to afford **mito-N_2H_4** as brown solid 397mg. Yield: 20%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ8.89(s, 1H), 8.88(s,1H), 8.45(s, 1H), 8.27-8.29(d, *J*=8Hz, 2H), 7.86-7.89(d, *J*=12Hz, 3H), 7.37(s, 1H), 7.23-7.25(d, *J*=8Hz, 1H), 4.27(s, 3H), 2.32(s, 3H). ^{13}C-NMR (100 MHz, DMSO-d_6) δ169.1, 159.1, 154.3, 154.1, 152.4, 145.7, 144.1, 135.1, 130.6, 127.4, 124.4, 121.9, 119.7, 117.5, 110.6, 47.5, 21.7. **Mito-N_2H_4**: m/z [M]$^+$ 322.1071 found, 322.1074 calcd.

5. Product Analysis

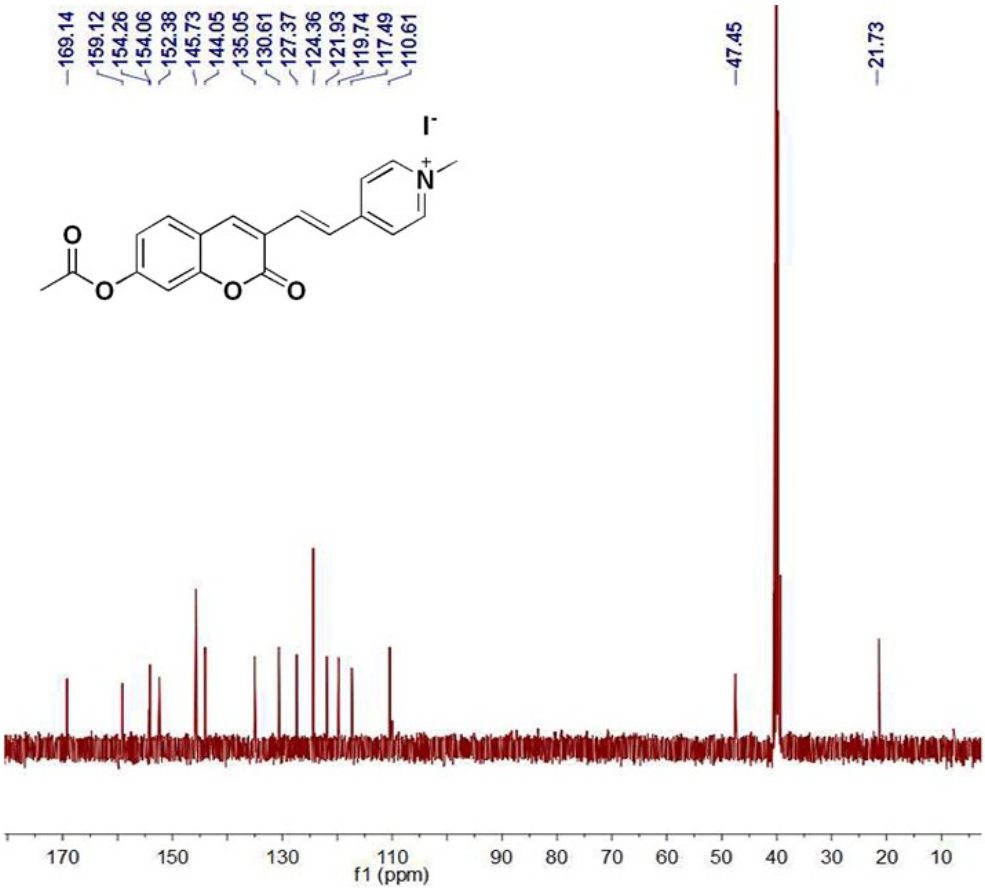
ESI-MS of **mito-N_2H_4**



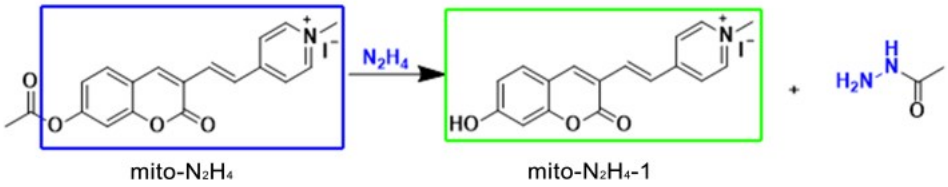
^{1}H-NMR spectra of **mito-N_2H_4** in DMSO-d_6



^{13}C-NMR spectra of **mito-N_2H_4** in DMSO-d_6



6. Mechanism study by ESI-MS



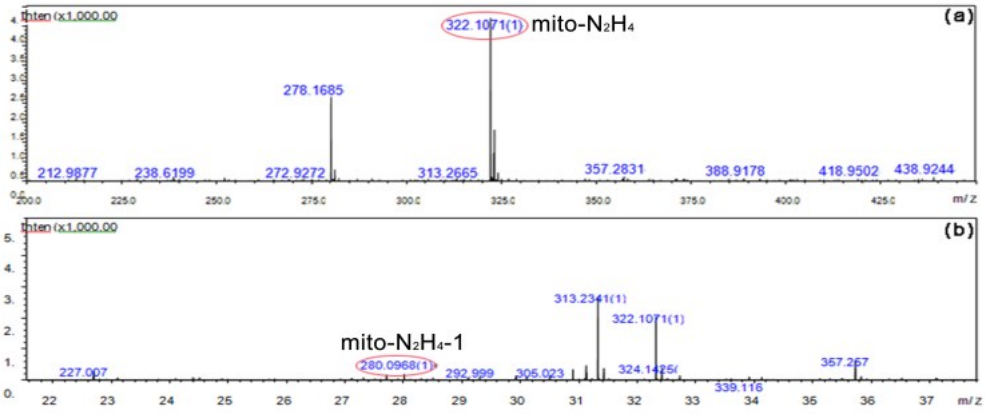


Fig. S1 ESI-MS spectrum of **mito-N_2H_4** in DMSO. (a) **mito-N_2H_4** only. (b) **mito-N_2H_4** + hydrazine (2 equiv.). [**mito-N_2H_4**] = 5μM.

7. Reference

1 J.-T. Hou, K. Li, B.-Y. Liu, Y.-X. Liao and X.-Q. Yu, *Tetrahedron*, 2013, **69**, 2118.

2 G. Zhang, Y.-M. Sun, X.-Q. He, W.-J. Zhang, M.-G. Tian, R.-Q. Feng, R.-Y. Zhang, X.-C. Li, L.-F. Guo, X.-Q. Yu and S.-L. Zhang, *Anal. Chem.*, 2015, **87**, 12088.