Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information for

A targetable fluorescent probe for imaging of mitochondria viscosity in living cells

Lixuan Dai, Mingguang Ren, Zihong Li, Li Wang, Weiying Lin*

Institute of Fluorescent Probes for Biological Imaging, School of Chemistry and Chemical Engineering, School of Materials Science and Engineering, University of Jinan, Jinan, Shandong 250022, P.R. China.

E-mail: weiyinglin2013@163.com

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Cell Culture and preparation of HeLa cells

HeLa cells were cultured in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO_2 and 95% air at 37 °C. Before the experiments, seed the HeLa cells in 35-mm glass-bottomed dishes at a density of 2×10^5 cells per dish in 2 mL of culture medium and incubate them inside an incubator containing 5% CO_2 and 95% air at 37 °C. Incubate the cells for 24 h. Cells will attach to the glass surface during this time.

Cytotoxicity assay

In vitro cytotoxicity was measured using the colorimetric methyl thiazolyl tetrazolium (MTT) assay on HeLa cells. Cells were seeded into the 96-well tissue culture plate in the presence of 100 μL Dulbecco's modifed eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 °C and 5% CO_2 atmosphere for overnight and then incubated for 24 h in the presence of **RM-V** at different concentrations (0, 1, 5, 10, 20, 30 μM). Then cells were washed with PBS buffer and 100 μL supplemented DMEM medium was added. Subsequently, 10 μL MTT (5 mg/mL) was added to each well and incubated for 4 h. Violet formazan was dissolved in 100 μL sodium dodecyl sulfate solution in the water-DMF mixture. Absorbance of the solution was measured at 570 nm using a microplate reader. The cell viability was determined by assuming 100% cell viability for cells without **RM-V.**

Synthesis

Systhesis of compound 1

Compound **1** was prepared according to the reported reference. A mixture of 2,3,3-triethylindole (3.25 g, 20 mmol, 1.0 eq) and iodine (3.34 g, 30 mmol, 1.5 eq) in 2 ml of acetonitrile was heated at 85 °C for 15 h. The crude product was filtered, washed with ether and dried under vacuum to give compound **1** (2.98 g, yield 56%) as a purple powder, which was used for the next step reaction without further purification.

Systhesis of compound 2

Compound **2** was prepared according to the reported reference. Benzil (1.05 g, 5 mmol, 1.0 eq), 1,4-phthalaldehyde (670 mg, 5 mmol, 1.0 eq) and ammonium acetate (3.1 g，40 mmol，8.0 eq) were dissolved in acetic acid, and reacted at 110 °C for 6 h with an inert atmosphere of nitrogen. After completly reacted, the reaction solution was cooled to room temperature, poured into ice water, suction filtered, washed with water and dried in vacuo. The resulting residue was purified by column chromatography on silica gel (petroleum ether to ethyl acetate/petroleum ether =1/10, v/v) to afford the compound **2** as a yellow powder (1.38 g, yield: 85%).

Synthesis of compound RM-V

Compound **1** (200 mg,0.75 mmol,1eq) and compound **2** (245 mg,0.75 mmol,1eq) were dissolved in 5ml N,N-dimethylformide (DMF), The reaction mixture was at 90°C for 6 h with an inert atmosphere of nitrogen, The reaction mixture was cooled to room temperature, then the reaction was poured into water, extracted with methylene chloride, and washed with water and then dried over anhydrous sodium sulfate. and then the solvent was removed under reduced pressure. The resulting residue was purified by column chromatography on silica gel "CH_2Cl_2 /methanol" = 20: 1, v/v to afford the compound **RM-V** as a magenta powder (210 mg, yield:56.6%). ^{1}HNMR (400 MHz, DMSO-d6) δ 13.34 (s, 1H), 8.53 (d, J = 16.2 Hz, 1H), 8.42(d, J = 8.9 Hz, 2H), 8.39 (d, J = 8.8 Hz, 2H), 7.98 (dd, J = 5.2, 3.7 Hz,1H), 7.94–7.90 (m, 1H), 7.82 (d, J =16.3 Hz, 1H), 7.67–7.62 (m, 2H),7.60–7.58 (m, 2H), 7.58–7.55 (m, 2H), 7.46 (t, J = 7.3 Hz, 2H), 7.41(d, J = 7.2 Hz, 1H), 7.34 (t, J = 7.4 Hz, 2H), 7.26 (t, J = 7.3 Hz, 1H),4.81 (q, J = 6.9 Hz, 2H), 1.84 (s, 6H), 1.48 (t, J = 7.2 Hz, 3H)· ^{13}C NMR (101 MHz, DMSO-d6) δ 181.74, 153.81, 144.94, 144.51, 140.91, 138.91, 135.37, 134.83, 134.42, 131.90, 131.06, 130.29, 129.90, 129.62, 129.09 (d, J = 6.3 Hz),128.73, 128.52, 127.75, 127.32, 126.02, 123.61,115.65, 112.64, 52.75, 42.72, 26.09, 14.35. HRMS (ESI): m/z calculated for $C_{35}H_{32}N_3^+$ 494.2591 $[M]^+$, found: 494.2592

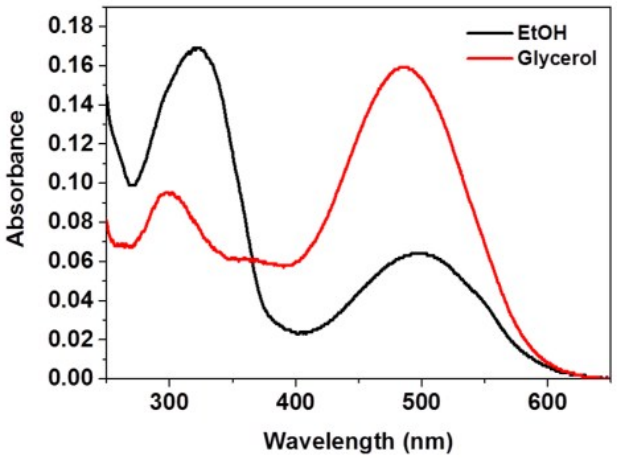

Fig.S1 Absorption spectra of **RM-V** in ethanol and glycerol.

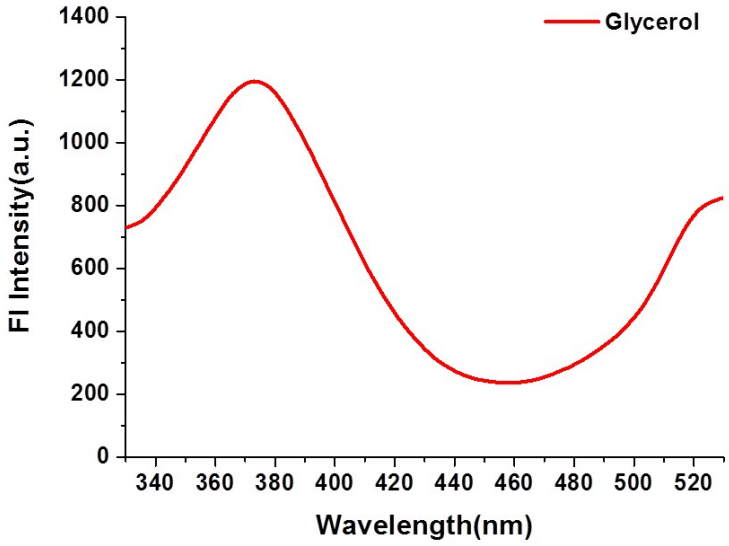

Fig. S2 The excitation spectrum of the probe **RM-V** (10 μM) in glycerol. (λ_{em}= 575 nm).

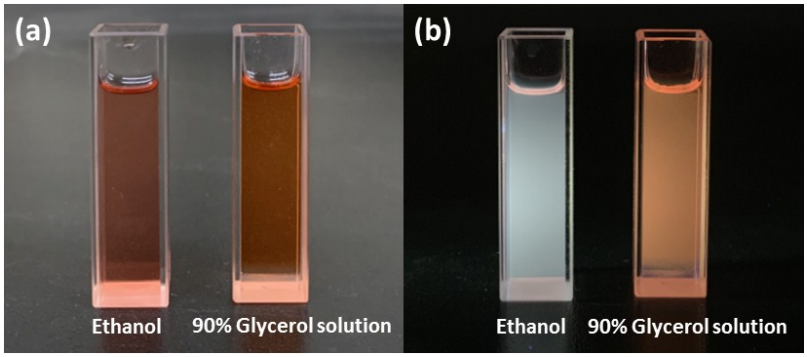

Fig. S3 (a) The color changes of the probe **RM-V** (10 μM) in ethanol and 90% glycerol solution.(b) The fluorescence changes of the probe **RM-V** (10 μM) in ethanol

and 90% glycerol solution with 365 nm ultraviolet light.

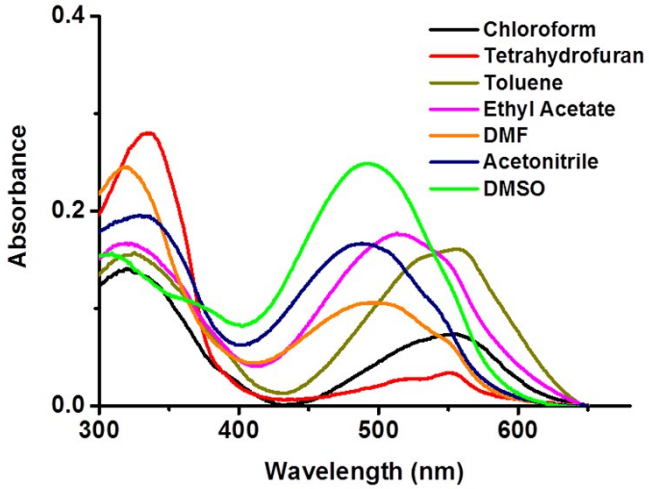

Fig. S4 Absorption spectrum of **RM-V** in different polarity of solvents DMF, DMSO, toluene, THF, chloroform, ethyl acetate, acetonitrile (λ_{ex}= 530 nm).

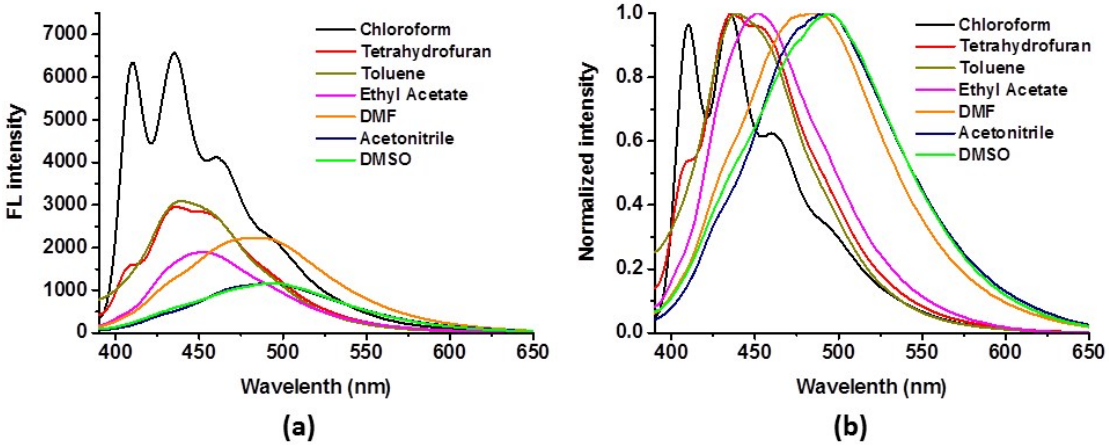

Fig. S5 (a) Fluorescence spectrum of Intensity of **RM-V** in different polarity of solvents DMF, DMSO, toluene, THF, chloroform, ethyl acetate, acetonitrile.(b) Fluorescence spectrum of normalized intensity of **RM-V** in different polarity of solvents DMF,DMSO, toluene, THF, chloroform, ethyl acetate, acetonitrile (λ_{ex}= 530 nm).

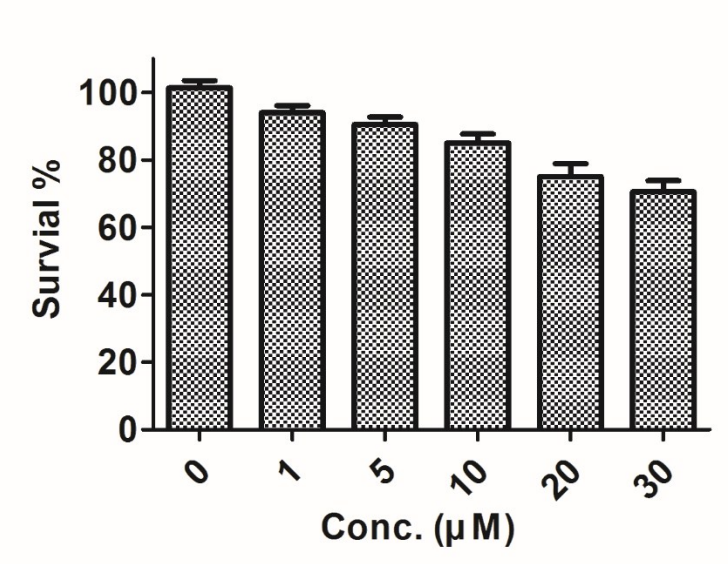

Fig. S6 Cytotoxicity assays of **RM-V** at different concentrations for HeLa cells in 24 h.

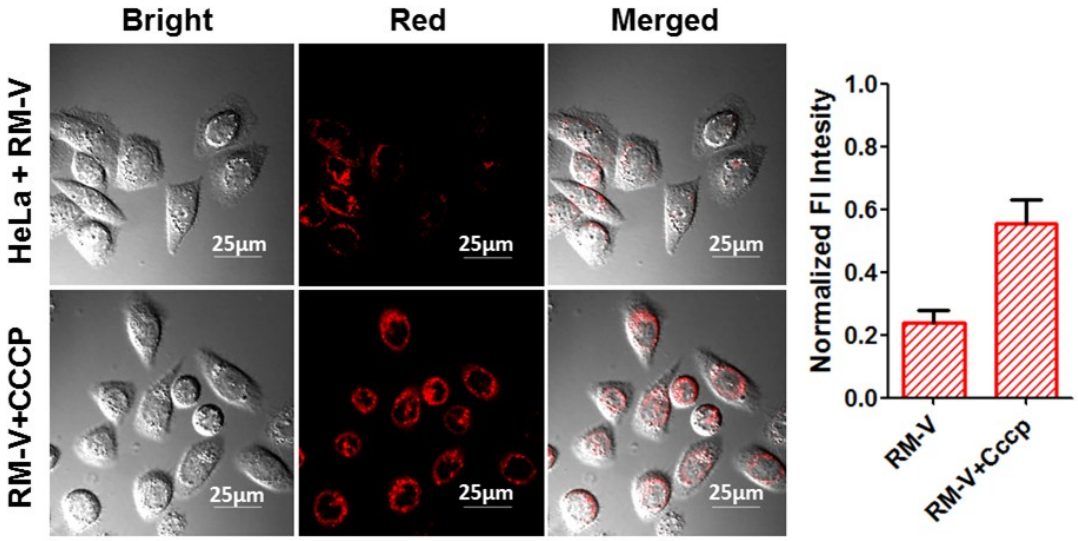

Fig. S7 (A) Fluorescence imaging of HeLa cells. (a1–a3) Images of HeLa cells stained with 5 μM **RM-V** for 30 min (b1–b3) HeLa cells incubated with **RM-V** (5 μM) for 30 min,and then treated with Cccp (100 μM) for 2 h. (a1–b1) Bright-field images of HeLa cells.(a2–b2) Fluorescence images of HeLa cells in the red channel. (a3–b3) The overlay of the bright-field and red channel.(B) Normalized fluorescence intensity of Hela cells in OP mode. Conditions:λex = 561 nm, λem =570–620 nm. Scale bar: 25 μm.

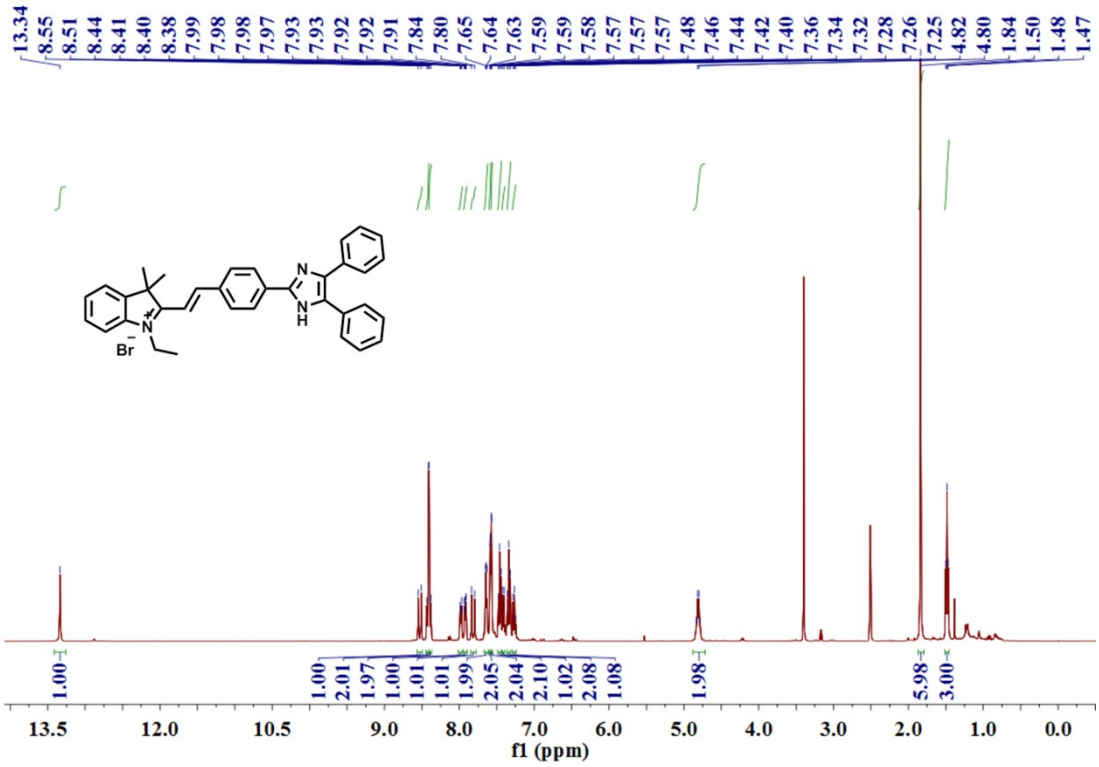

Fig. S8 ^{1}H-NMR (DMSO-d_6) spectrum of **RM-V.**

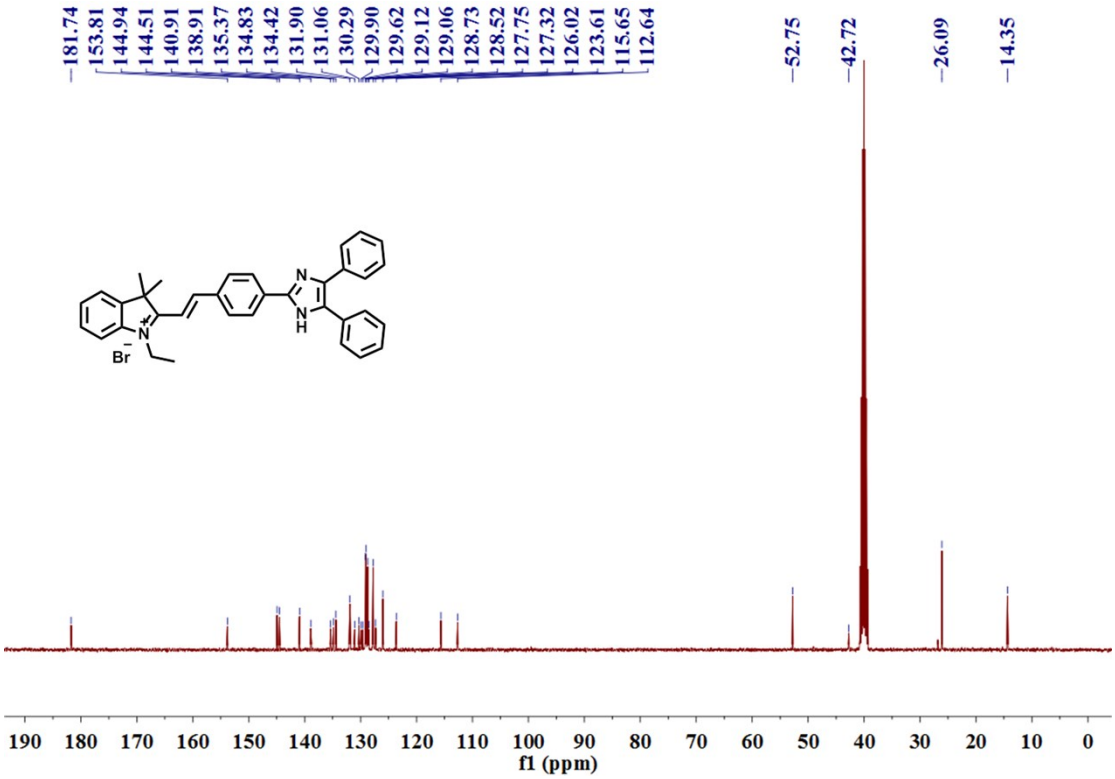

Fig. S9 ^{13}C-NMR (DMSO-d_6) spectrum of **RM-V.**

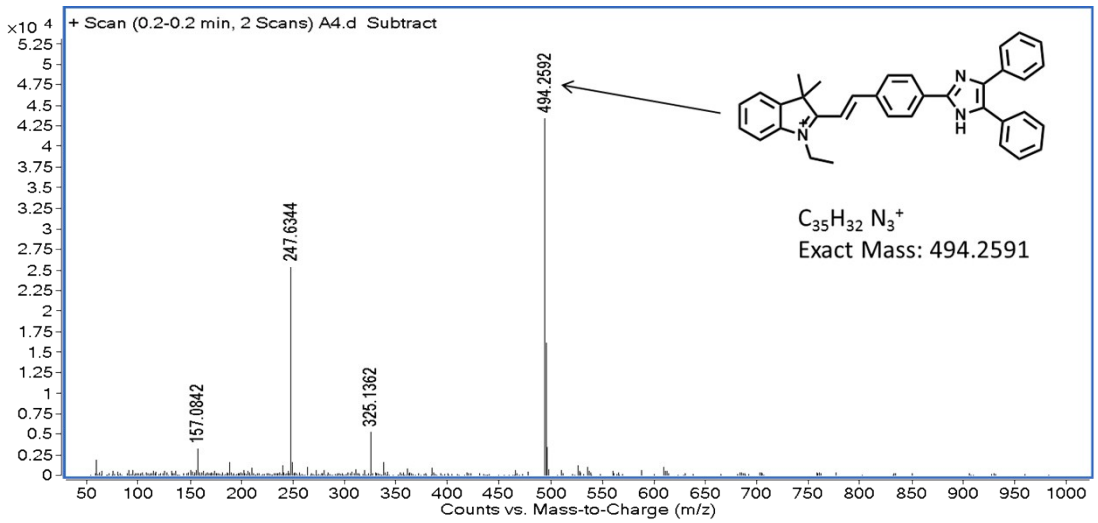

Fig. S10 HRMS (ESI) spectrum of **RM-V.**