Electronic Supplementary Information

A red-emissive mitochondria probe for imaging of the viscosity in living cells

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Experiment section

Measurements and apparatus

All reagents were obtained commercially and used as supplied. ^{1}H-NMR and ^{13}C-NMR spectra were obtained on a Bruker Avance 400 spectrometer (TMS as internal standard in NMR). Mass spectrum was measured on HRMS-LTQ Obritrap XL (ESI source). IR spectra were recorded on a Nicolet FT-IR-is5 spectrophotometer in the 4000-400 cm^{-1} range with samples prepared as KBr pellets. UV spectra were recorded on a UV-1700 spectrophotometer. The fluorescence spectra were measured by using a HITACHI F-4600 fluorescence spectrophotometer. The concentration of a sample solution was 1.0×10^{-5} mol/L. The fluorescence quantum yields (Φ) were determined by using rhodamine B as the reference according to the literature method.

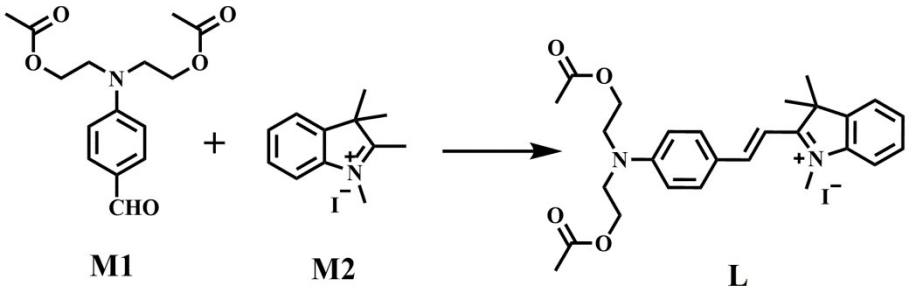
Cell Imaging

Hela cells were seeded in 24-well glass botton plates at a density of 1×10^{4} cells per well and grown for 96 hours. For live cell imaging, cells were incubated with L at 10 μM in cell medium containing 10% Fetal Bovine Serum (FBS) and maintained at

37°C in an atmosphere of 5% CO_2 and 95% air for 20 min. The cells were then washed with PBS three times. The cells were imaged using water immersion lenses on a confocal laser scanning microscopy. Co-staining was performed using 1 μM Mitotracker Red for 30 min.

Cytotoxicity assays in cells

The cytotoxicity of **L** was evaluated using the MTT assay. Hela cells were seeded in 96-well plates at a density of 5000 cells/well and incubated for 3 days at 37 ^{o}C under 5 % CO_2. L was then added at indicated concentrations to triplicate wells. Prior to the compounds' treatment, cell culture medium was changed, and aliquots of the compounds stock solutions were diluted to obtain the final concentrations of 5, 10, 15, 20 and 25 μM in growth medium. To ensure the same volume of compound solution to be added into each well with desired different concentrations, we started with different stock solutions. However, DMSO was controlled below 1% in the medium to minimize its effects on live cell imaging. For example, the higher trace of DMSO was present in 5 μM treatment group. To gain 5 μM working concentration, 1 mM stock solution (in DMSO) was first 1:10 (V:V) diluted in DMEM medium and further diluted 1:20 in DMEM medium. After incubation for 24 h, the medium was replaced with fresh DMEM medium. Subsequently, cells were treated with 5 mg/mL MTT (10 μL/well) and incubated for an additional 4 h (37 ^{o}C, 5% CO_2). After MTT medium removal, the formazan crystals were dissolved in DMSO (100 μL/well) and the absorbance was measured at 490 nm using an INFINITE 200 PRO.



M1 M2 L

Scheme S1. Synthetic route for compound **L**.

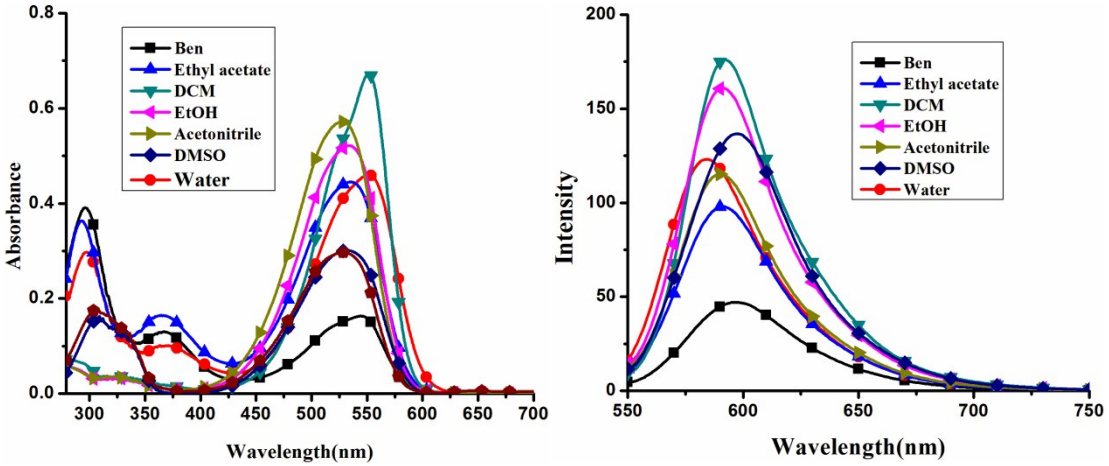


Figure S1. Absorption spectra (left) and fluorescence spectra (right) of compound **L** (10 μM) in different solvents.

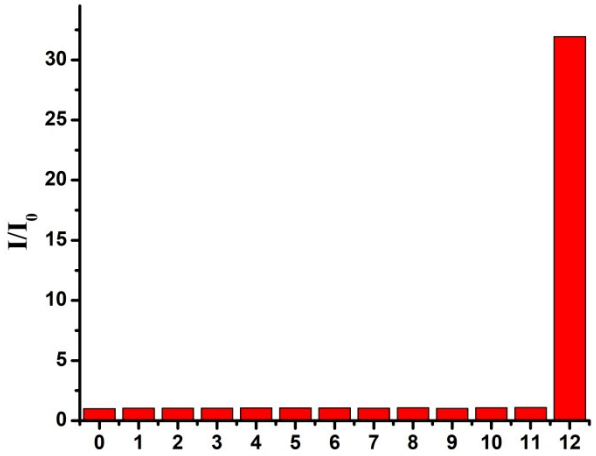


Figure S2. Fluorescence intensities of compound **L** (10 μM) treated with various species. (0) blank, (1) BSA, (2) Cys, (3) DNA, (4) GSH, (5) Histidine, (6) Proline, (7) RNA, (8) Tryptophan, (9) Serine, (10) Threonine, (11) Valine, (12) Glycerol.

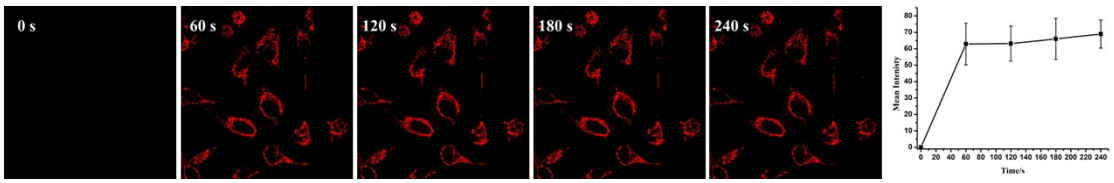


Figure S3. Time dynamics of the penetration and the intracellular fluorescence intensity correspondingly.

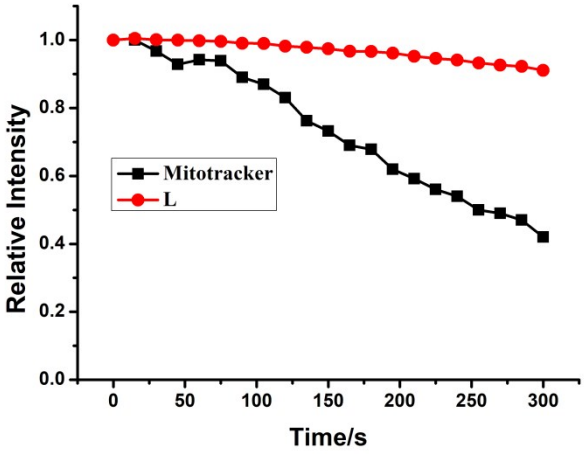


Figure S4. Photostability of compound **L** and Mitotracker Red in living cells.

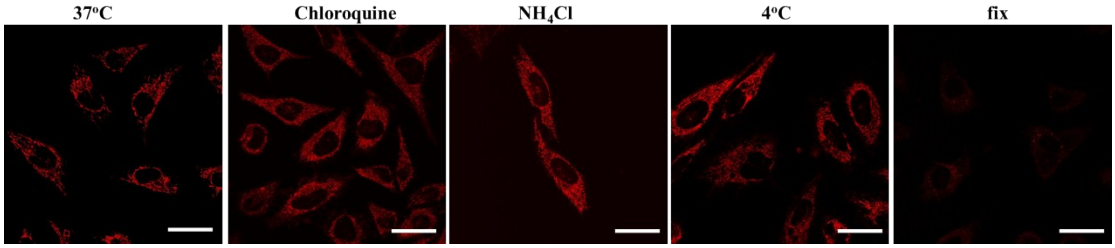


Figure S5. Effect of incubation temperature (37 °C and 4 °C), chloroquine (50 μM), NH_4Cl (50 μM) and fixed Hela cells on cellular uptake of **L** (10 μM, 20 min) measured by confocal microscopy. Scale bar = 20 μm.

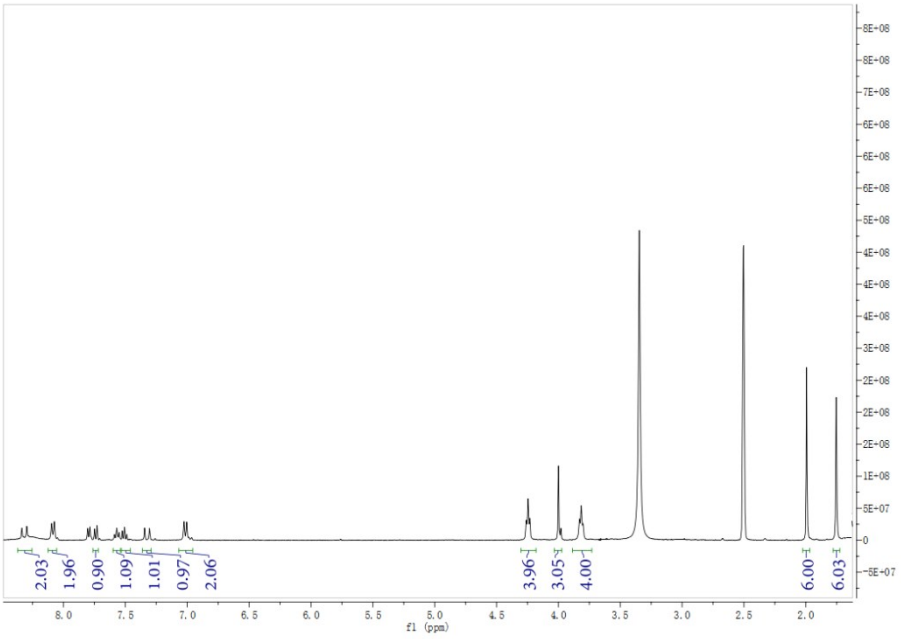


Figure S6. ^1H NMR spectra of **L**.

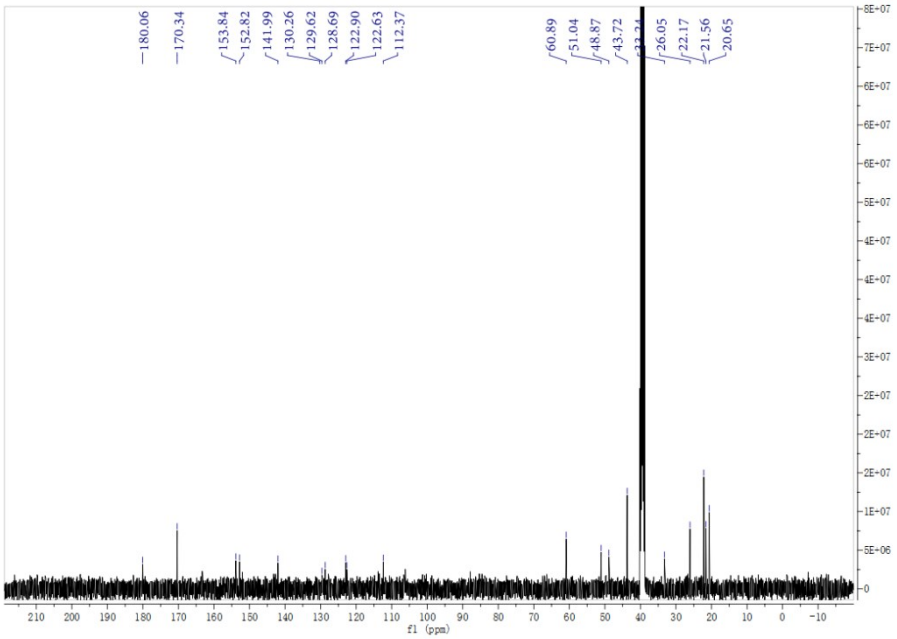


Figure S7. ^{13}C NMR spectra of **L**.

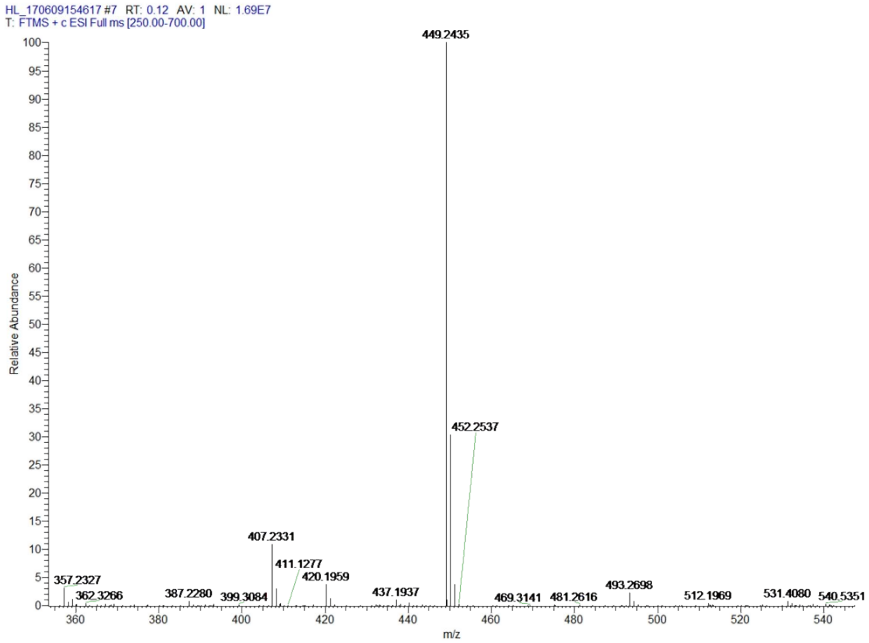


Figure S8. ESI-MS spectra of **L**.

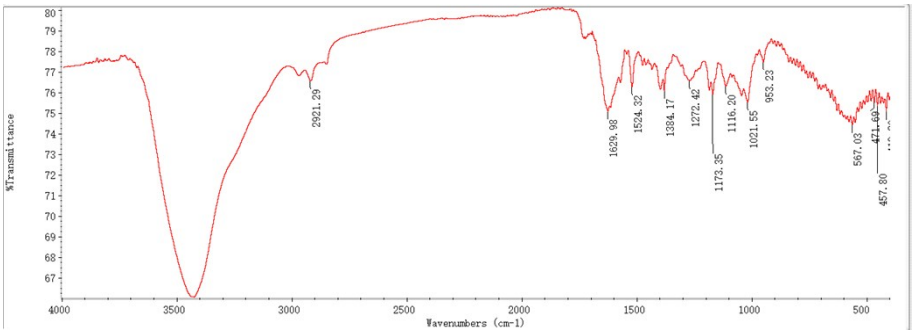


Figure S9. IR spectra of **L**.