Supporting Information for

A deep-red fluorescent probe for detection of viscosity in living cells and mice

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Synthesis of the probe HY

Compound **1** (1 mmol) and 2-benzothiazole acetonitrile (1 mmol) were dissolved in anhydrous ethanol (10 mL) and refluxed in the presence of piperidine for 6 hours. The reaction was stopped and cooled to room temperature. The filter cake was washed with ethanol for 2-3 times. The residue was dried in vacuum. The residue was separated by column chromatography. The eluent was dichloromethane/methanol (10:1), and the compound **HY** was obtained (yield 52%).^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.85 (s, 1H), 8.42 (s, 1H), 8.09 (d, *J* = 8.3 Hz, 1H), 7.89 (d, *J* = 8.0 Hz, 1H), 7.55 (m, 2H), 7.07 (s, 1H), 3.40 (s, 4H), 2.86 (d, *J* = 49.6 Hz, 4H), 2.02 (s, 4H).^{13}C NMR (101 MHz, Chloroform-*d*) δ161.57, 153.78, 152.45, 148.87, 142.66, 140.84, 134.68, 127.69, 126.63, 125.65, 123.53, 121.44, 119.90, 117.18, 110.75, 108.79, 106.31, 102.14, 50.49, 50.06, 29.70, 27.35, 21.12, 20.14, 19.97. HR-MS calculated for $C_{25}H_{19}N_3O_2S$ $[M+H]^+$ m/z 426.1285, found $[M+H]^+$ m/z 426.1282.

Preparation of solutions of probe HY

The stock solution of the probe **HY** was prepared by dissolving the requisite amount of **HY** in DMSO, the concentration of the stock solution was 1.0 mM.

Viscosity determination and fluorescence spectral measurement detection

The solvents with different viscosity were obtained by mixing methanol and glycerol in different proportions. Viscosity value was recorded by NDJ-8 rotational viscometer. The solutions of **HY** of different viscosity were prepared by adding the stock solution (1.0 mM) 30 μL to 3 mL of solvent mixture to obtain the final concentration of the probe **HY** (10.0 μM). These solutions were sonicated for 5 min to eliminate air bubbles. The spectra of the probe **HY** with different viscosity were measured in a UV spectrophotometer and a fluorescence spectrophotometer.

Reagents and instrumentations

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified by standard methods prior to use. Twice-distilled water was used throughout all experiments. High resolution mass spectrometric (HRMS) analyses were measured by using a Finnigan MAT 95 XP spectrometer. NMR spectra were recorded on a Bruker AVANCE III 400 MHz Digital NMR Spectrometer and using $CDCl_3$ as solvent and tetramethylsilane (TMS) as internal reference respectively. Absorption spectra were obtained on a Shimadzu UV-2600 UV-vis spectrophotometer. Fluorescent spectra were recorded at room temperature with a HITACHI F4600 fluorescence spectrophotometer. TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200–300), both of which were obtained from the Qingdao Ocean Chemicals. The fluorescence imaging of cells was performed using a Nikon confocal microscope.

MTT assays

HeLa cells line were cultured in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% FBS and 100 U/mL of penicillin and 100 μg/mL streptomycin in an atmosphere of 5% CO_2 and 95% air at 37 °C. The cells were then seeded into 96-well plates, and then 0-50.0 μM of **HY** (99.9% DMEM and 0.1% DMSO) was added respectively. Subsequently, the cells were incubated at 37 °C in an atmosphere of 5% CO_2 and 95% air for 24 h. Finally, the absorbance of the solution was acquired by using the microplate reader at 570 nm. The toxicity of **HY** was calculated by the following formula.

$$\text{The cell viability (\%)} = (OD_s\text{-}OD_b)/(OD_c\text{-}OD_b) \times 100\%.$$

OD_s denotes the cells incubated with various concentrations of the probe, OD_c denotes the cells without the probe, OD_b denotes the wells containing only the culture medium.

Cell culture and imaging

The cells including the Hela, HepG2 and MCF-7 were seeded up to appropriate density into a 35 mm glass-bottom culture dishes (Nest). Then the cells further incubated with the probe (the final concentration is 10 μM) for another 30 min at 37 ˚C. Then the cells were washed with PBS buffer (pH=7.4) three times, and the cells incubated with monensin, nystatin and LPS for more 30 min at 37 ˚C. Finally, the cells were washed three times with PBS buffer. The imaging experiments were recorded through a Nikon A1MP confocal microscopy inverted fluorescence microscopy equipped with a cooled CCD camera. The fluorescence emission was collected at 570-620 nm upon excitation at 561 nm.

Fluorescence in living mice

The 4-week old female balb/c mice were purchased from School of Pharmaceutical Sciences, Shandong University. The procedures for care and use of animals were approved by the Ethics Committee of the Institutional Animal Care and Use Committee (IACUC) and all applicable institutional and governmental regulations concerning the ethical use of animals were followed. All of the animal experiments were carried out according to the Animal Management Rules of the Ministry of Health in People's Republic of China (Document NO. 55, 2001) and the guidelines for the Care and Use of Animal Ethical Experimentation Committee of Shandong University.

Four-week-old female Balb/c mice were used for in vivo imaging of viscosity changes. Unshaved female Balb/c mice were intraperitoneally injected with 100 μL LPS (1 mg/L). After 24 h, the mice exhibited inflammation symptoms such as diarrhea, and then shaved the abdominal hair of mice. After that the mice were anesthetized by intraperitoneal injection of 20 μL 10% chloral hydrate solution. When the mice were comatose, 50 μL PBS solution of probe **HY** (0.2 μM) was injected by abdominal

injection. Then living imaging was taken out by using PerkinElmer IVIS Lumina III system with an excitation filter of 540 nm and an emission filter of 640 nm.

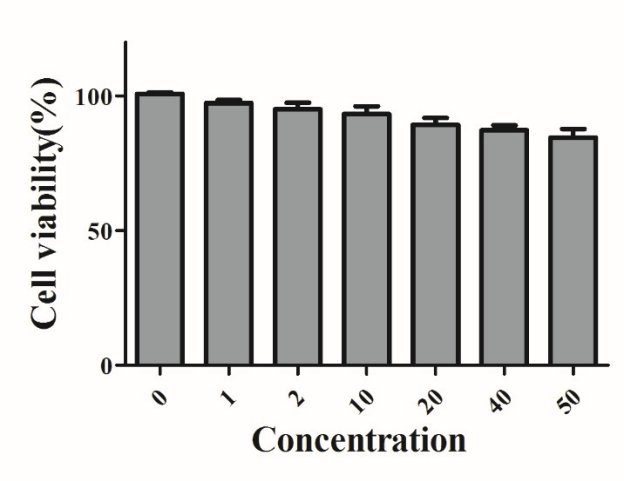

Figure S1. Cytotoxicity assays of **HY** at different concentrations (0 μM; 1μM; 2 μM; 10 μM; 20 μM; 40 μM; 50 μM) for HeLa cells

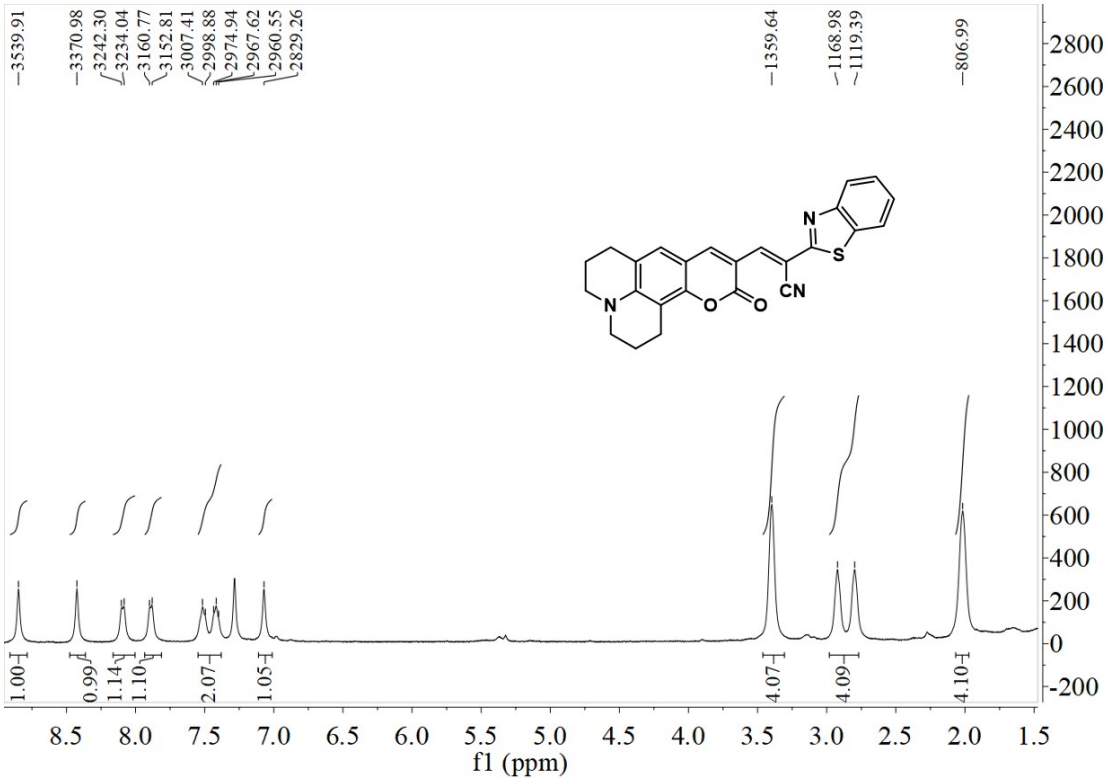

Figure S2. ^{1}H NMR ($CDCl_3$) spectrum of **HY.**

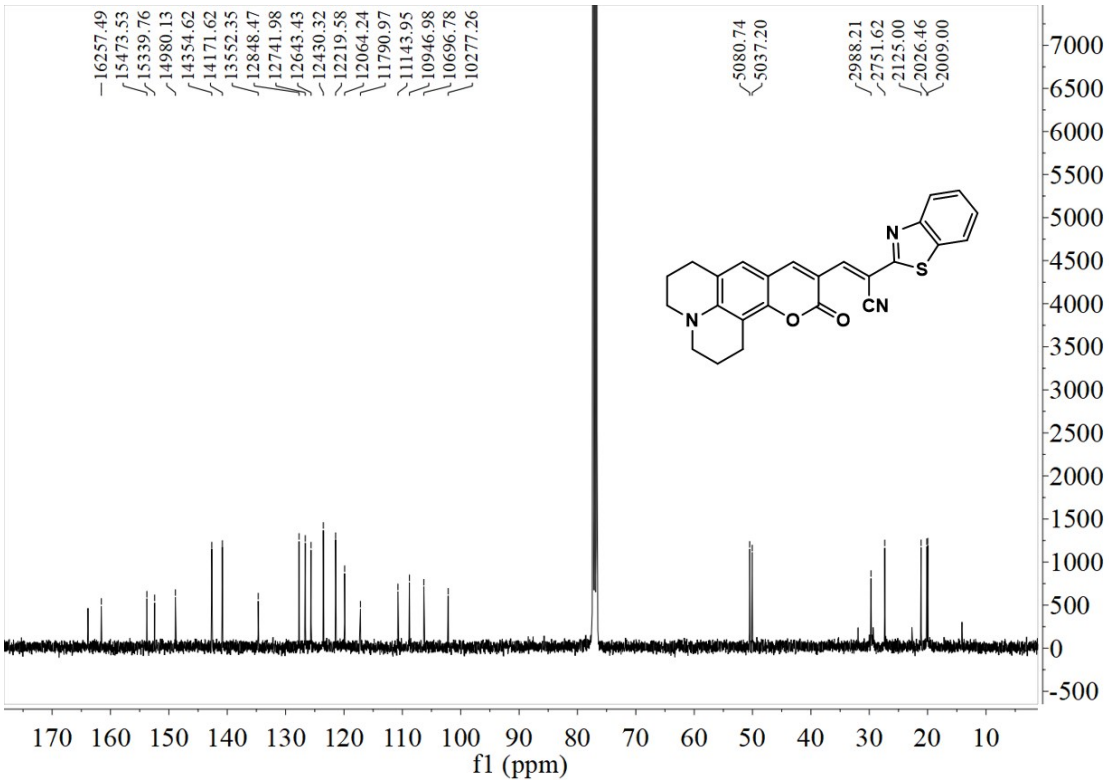

Figure S3. ^{13}C-NMR ($CDCl_3$) spectrum of **HY**.

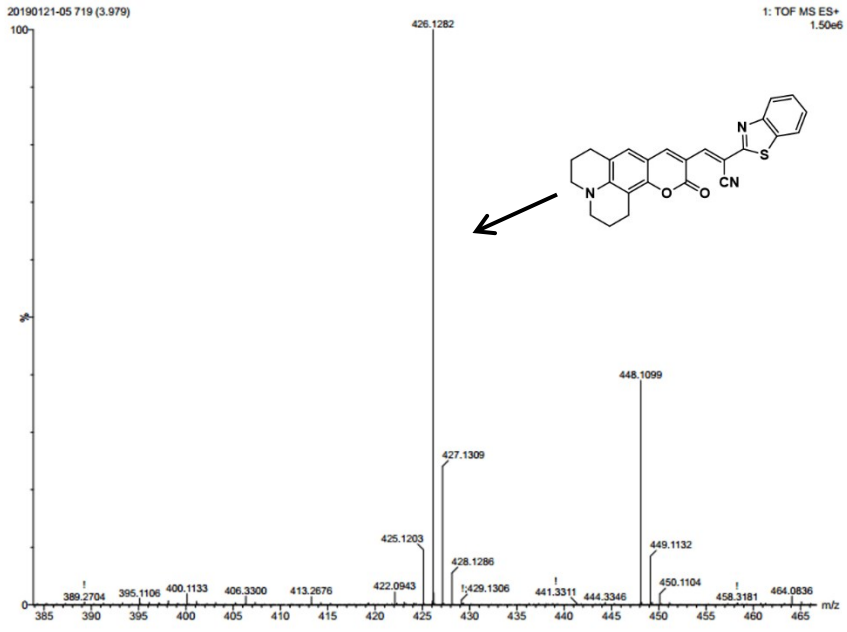

Figure S4. HRMS spectrum of the probe **HY**.

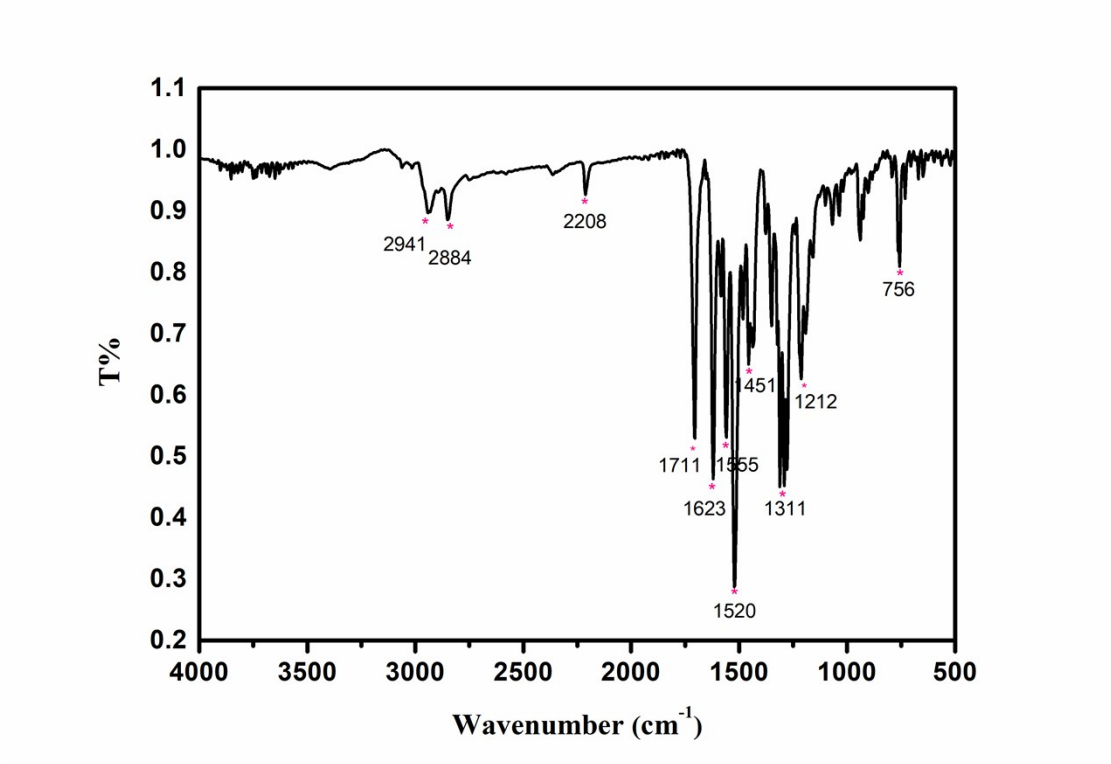

Figure S5. IR spectrum of the probe **HY**.

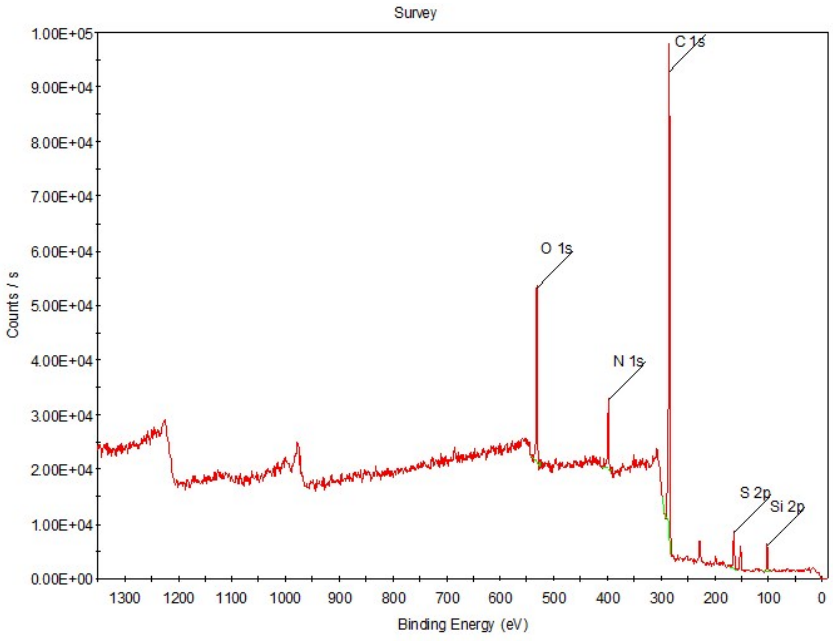

Figure S6. Elemental analysis of the probe **HY**.