Supporting Information for

Development of a Novel Near-Infrared Molecule Rotator for Early Diagnosis and Visualization of Viscosity Changes in Acute Liver Injury Models

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Experimental section

Quantum yield calculation

The fluorescence quantum yield Φ_s was estimated from the absorption and fluorescence spectra of probe according to equation, where the subscript s and r stand for the sample and reference (fluorescein as standard $\Phi_F = 0.85$), respectively. Φ is the quantum yields, A represents the absorbance at the excitation wavelength, S refers to the integrated emission band areas and n_D is the solvent refractive index. The fluorescence quantum yields (Φ_F) were estimated with equation as follows:

$$\varphi_s = \varphi_r \frac{S_s\ A_r\ n_{DS}^2}{S_R\ A_S\ n_{Dr}^2}$$

Cytotoxicity of probe DJM

The cytotoxicity of probe **DJM** was tested by MTT method. Miha cells were seeded at a density at 1×10^4 cells per well into 96-well plate, and incubated in 37 °C cell incubator (containing 5% CO_2) for 12 hours. The culture medium was high glucose DMEM with fetal bovine serum and appropriate antibodies (penicillin and streptomycin). Then the probe **DJM** (0, 1, 2, 5, 10, 20 μM) was added and incubated for 24 h. 50 μL MTT was added to each pore and the cells were incubated at 37 °C and 5% CO_2 for 4 h. Then, removed the medium and replaced it with DMSO (150μL), and detected the absorption values at 490 nm.

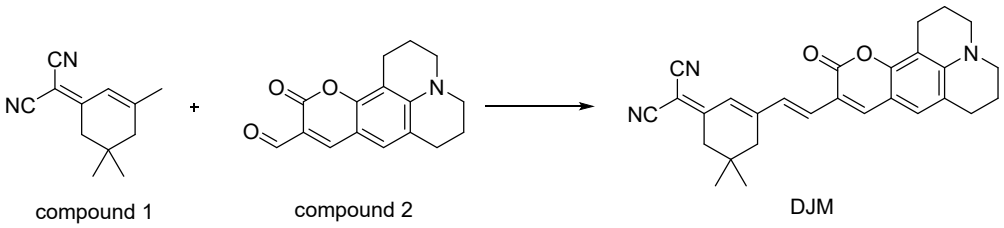
Cell culture and fluorescence imaging

DMEM containing 10% fetal bovine serum and 1% penicillin was used for Miha cell culture in an incubator supplemented with 95% air and 5% CO_2 at 37 °C. Then, nutrient solution was removed and cells were washed three times with PBS buffer (pH=7.0, 10 mM) before imaging. To compare the difference in viscosity level of Miha cells, they were treated with 10 μM **DJM** for 15 min and then washed three times with PBS buffer.

Imaging in zebrafish

The 3-day-old zebrafish was incubated with **DJM** (10 μM) for 30 min, and then washed with PBS buffer and imaged as control group. The 3-day-old zebrafish firstly

incubated with APAP (200 μM) for 4 h, then incubated with **DJM** (10 μM) for 30 min, washed with PBS buffer and imaged as APAP-treated group. The third group is firstly treated with APAP (200 μM) for 4 h, and then treated with NAC (100 μM) for 1h. After that, these zebrafish was then incubated with **DJM** (10 μM) for 30 min, washed with PBS buffer and imaged as APAP+NAC group. Thereafter, the treated zebrafish was washed with PBS buffer three times and imaged using a confocal microscope. Fluorescence images were acquired with Nikon A1R confocal microscope.



Scheme S1. Synthesis route of **DJM.**

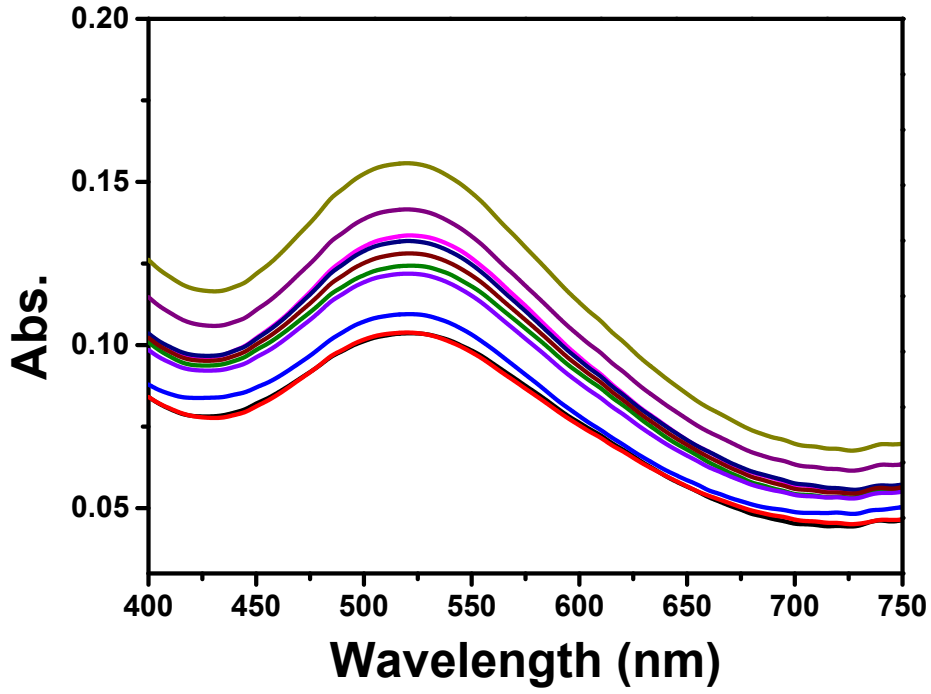


Fig.S1 Absorption spectra of **DJM** (10 μM) in different ratios of PBS/glycerol mixtures.

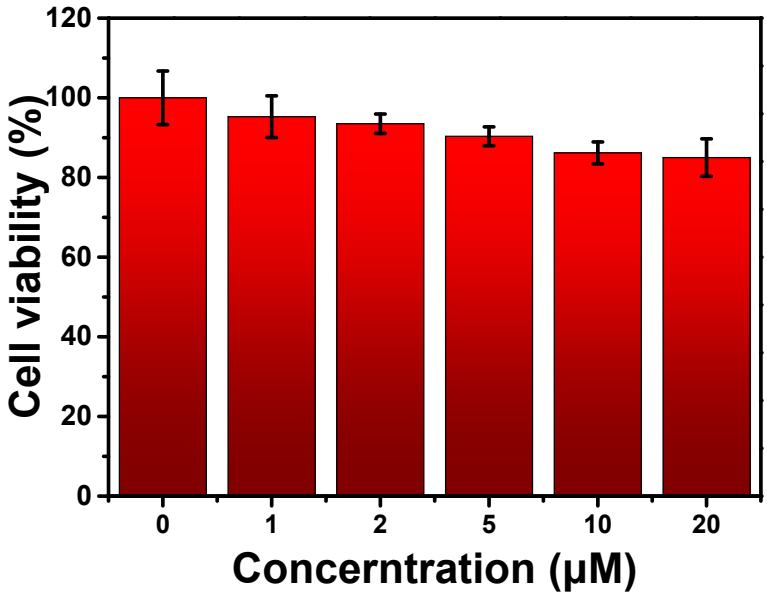


Fig. S2 MTT results of Miha cells viabilities after incubation with **DJM** for 24 h. Data are expressed as mean ± SD (*$p < 0.05$, experiment times n = 3).

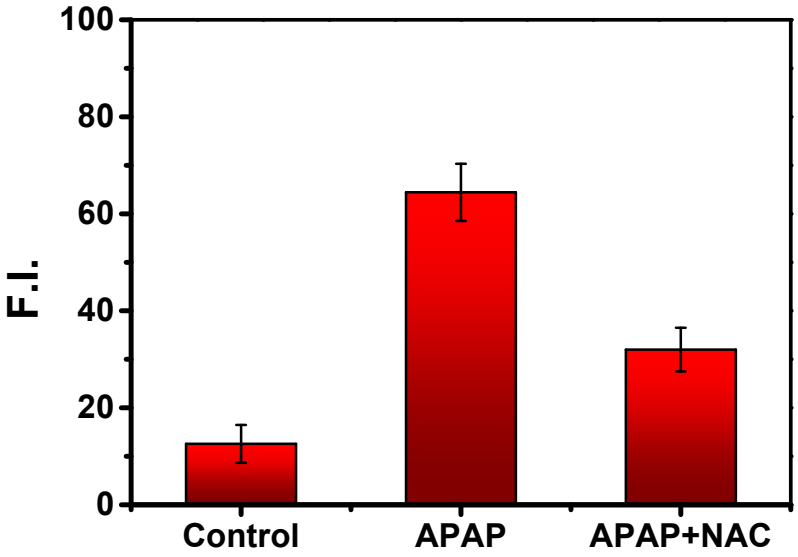


Fig. S3 Quantitative analysis of Miha cell labeled with **DJM** (10 μM) for 2 h (a) control; (b) after treatment with APAP (200 μM) for 4 h; (c) after treatment with APAP (200 μM) for 4 h, and then treated with NAC (100 μM) for 1h.

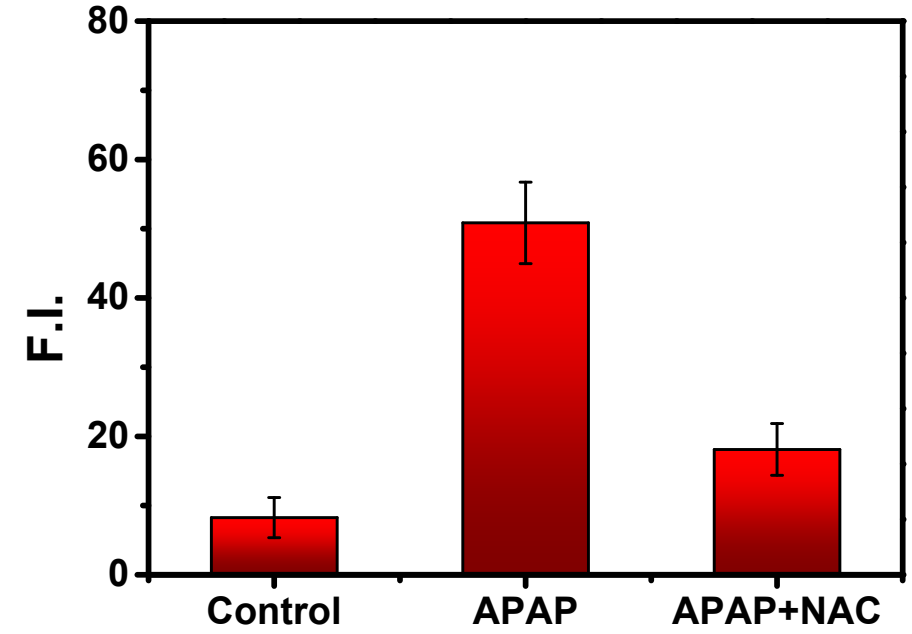


Fig. S4 Quantitative analysis of living 3-day-old zebrafish. (a) only labeled with **DJM** (10 μM) for 2 h. (b) after treatment with APAP (200 μM) for 4 h. (c) after treatment with APAP (200 μM) for 1 h, and then treated with NAC (100 μM) for 1h.

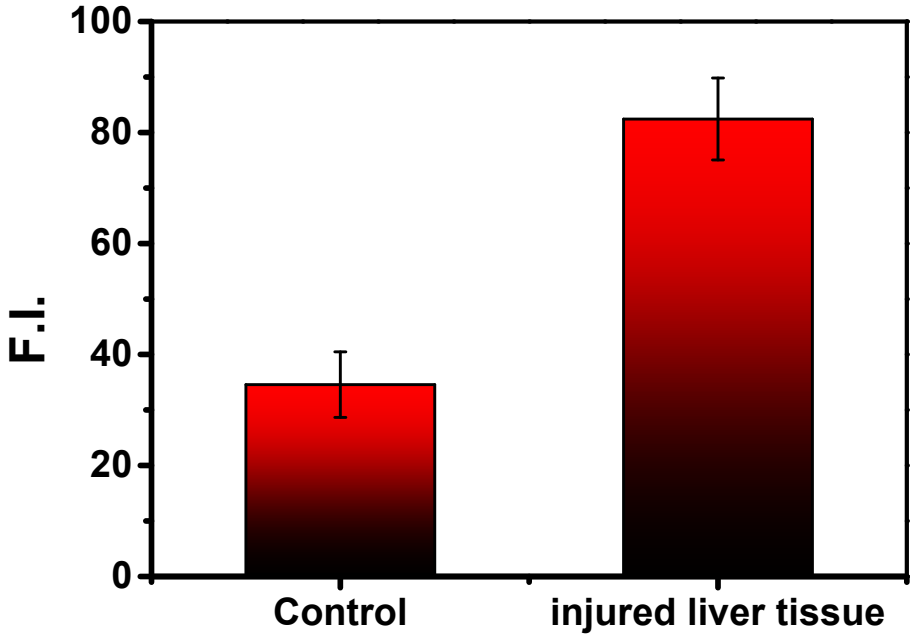


Fig. S5 Quantitative analysis of normal and APAP-induced liver tissue in mice.

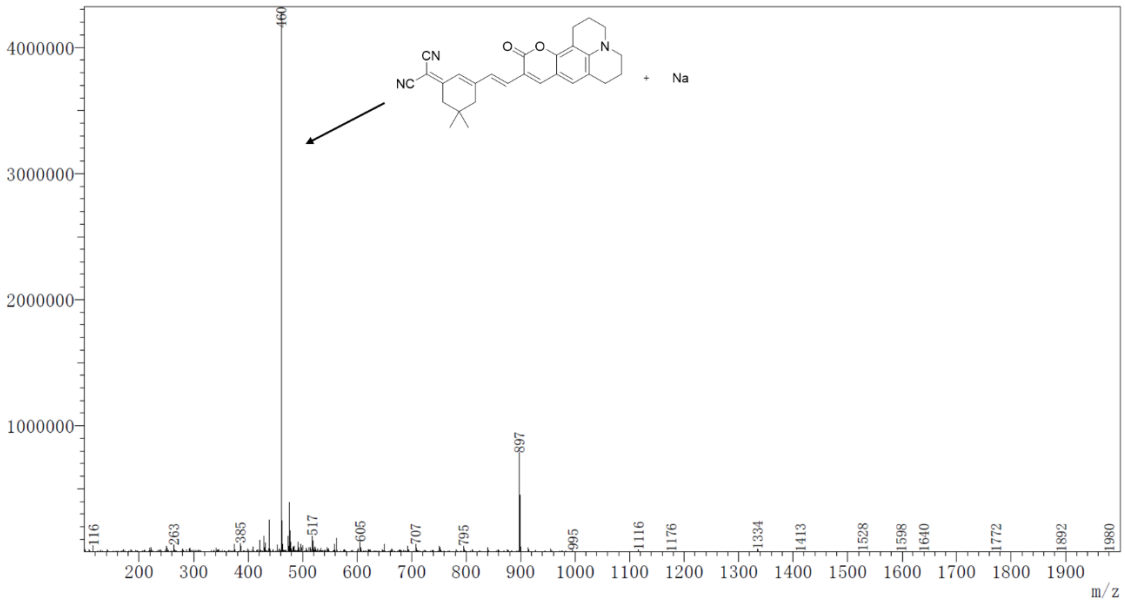


Fig. S6 Mass spectra of **DJM**.

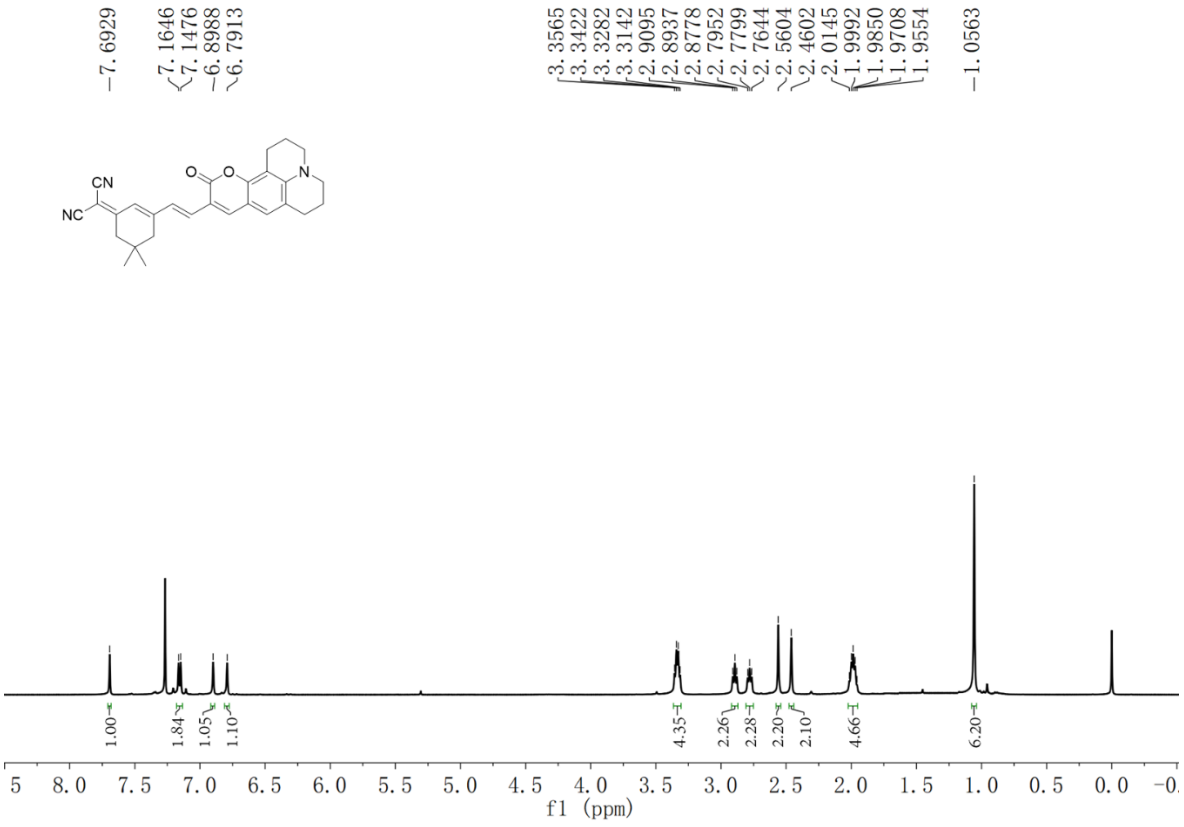


Figure S7 ^{1}H NMR spectrum of **DJM** in $CHCl_3$-d_6.

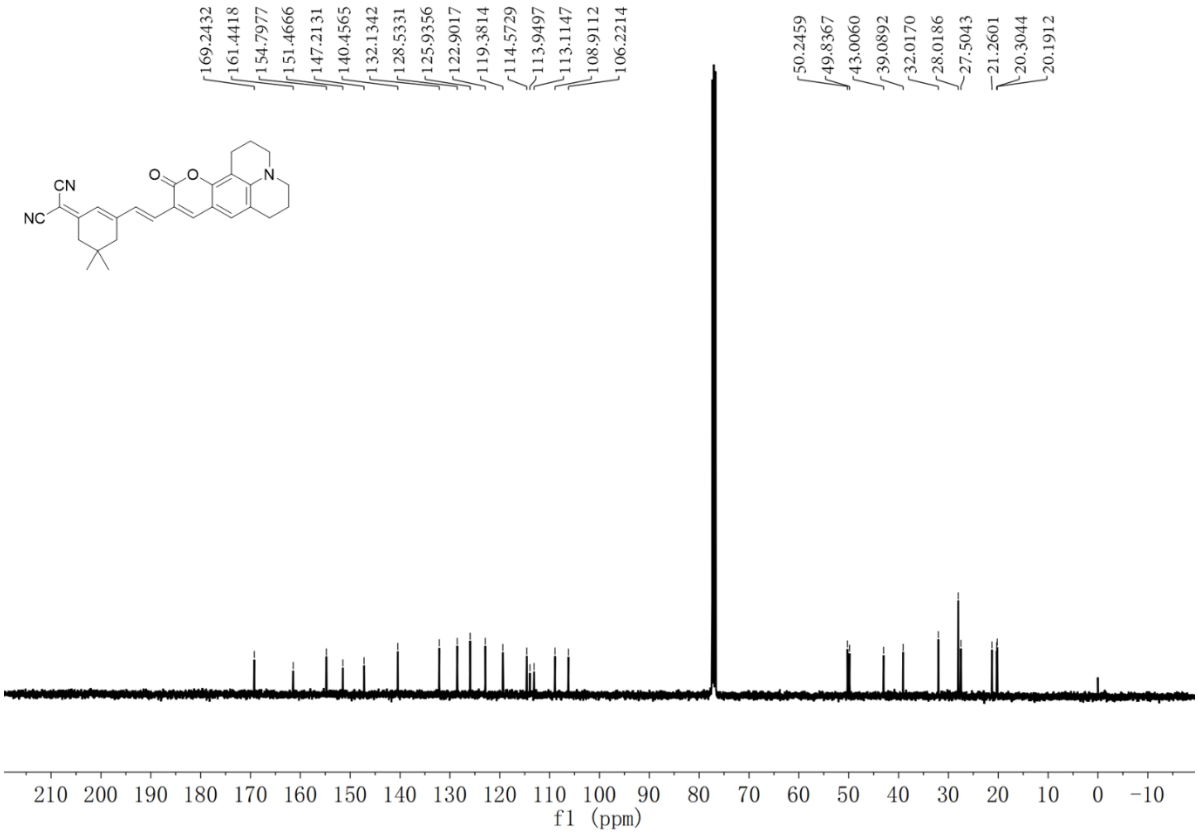


Figure S8 ^{1}C NMR spectrum of **DJM** in $CHCl_3$-d_6.