Supporting Information for

A Highly sensitive Red-emitting Probe for the detection of viscosity changes in living cells, zebra fish and Diabetic Blood Samples

Key Laboratory of Bioorganic Synthesis of Zhejiang Province, College of Biotechnology and Bioengineering,

Zhejiang University of Technology, Hangzhou 310014, P. R. China

Email: zhuq@zjut.edu.cn.

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1. Further experimental details

Test method for fluorescence quantum yield

For the measured fluorescence quantum yield (Φ_u), fluorescein was used as a standard in aqueous NaOH (pH 13, λ ex = 490 nm, λ ex = 510 nm, Φ S = 0.49). The value is calculated according to the following formula: $\Phi_u = \Phi_s (n_u^2 E_u A_s)/(n_s^2 E_s A_u)$, where the subscripts u and s refer to the sample and the reference compound, respectively. A is the absorbance at the excitation wavelength, E is the integrated area under the emission spectrum, and n is the refractive index of the solvent.

Cytotoxicity Assay

The cytotoxicity was evaluated by MTT assay. Hela cells and MCF-7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) in 96-well microplates at 37 °C under 5% CO_2 for 12 h. The medium was next replaced by fresh medium containing various concentrations of **DJH** (0-20 μM). Each concentration was tested in three replicates. Cells were rinsed twice with phosphate buffer saline 24 h later and incubated with 0.5 mg/mL MTT reagent for 4h at 37 °C. 150 μL DMSO was then added to dissolve formazan. The absorbance at 510m was measured in a microplate reader. Cell viability (%) was calculated according to following equation: Viability = (mean Abs. of treated wells/mean Abs. of control wells) ×100%.

Imaging in zebrafishes

The 3-day-old zebrafish exposed to 1000 μg/L and 100 μg/L 50-μm polystyrene microplastics was incubated with **DJH** (10 μM) for 30 min, and then washed with PBS buffer and imaged as control groups for the experiment. The 3-day-old normal zebrafish followed by addition of **DJH**(10 μM) for 30 min incubation. Thereafter, the incubated zebrafish was washed three times with PBS buffer and imaged using a confocal microscope. Fluorescence images were acquired with Nikon A1R confocal microscope.

Blood viscosity detection in hyperglycemia mice

C57bl/6j mice (male) were purchased from the Slacacas Laboratory Animal Center (Shanghai, China). This study was conducted in accordance with the Animal Care and Institutional Ethical Guidance in China. Diabetic mice were obtained according to the literature by treating normal mice with streptozotocin (STZ). When the blood sugar is more than 16.7 mM, diabetes was confirmed. Finally, fresh blood was obtained from the normal mice and the diabetic mice. 60μL mice plasma samples were diluted 10-fold with phosphate buffer (0.1 M, pH 7.5) for detection. The fluorescence spectra were measured within the wavelength range of 580–750 nm under 510 nm excitation. The band-slits of excitation and emission were all set as 5.0 nm. The fluorescence intensity of **DJH** at 675 nm was used for the quantitative analysis of blood viscosity detection.

Blood viscosity detection in human clinical samples

We collected 65 male and female adult patients volunteers who have been hospitalized or treated in from Jinhua Central Hospital and have been clinically diagnosed with diabetes and hypertension. Adults with a history of vascular diseases (coronary artery disease, stroke, peripheral vessel occlusive disease, autoimmune disease, thrombosis), anemia (male <12 g/dL, female <11 g/dL) or a history of transfusions within a month were excluded. Then, the baseline characteristics of three groups (Thirty patients who were clinically diagnosed with hypertension were assigned as group A, 35 normotensive diabetic patients were assigned as group B, and the other 20 healthy patients were assigned as group C) are described for further study. Finally, fresh blood was obtained from these patients.

60μL human blood samples were diluted 10-fold with phosphate buffer (0.1 M, pH 7.5) before detection. The

concentration of DJH was kept at 5 μM in phosphate buffer (0.1 M, pH 7.5) with gentle shaking. The fluorescence spectra were measured within the wavelength range of 580–750 nm under 510 nm excitation. The band-slits of excitation and emission were all set as 5.0 nm. The fluorescence intensity of DJH at 675 nm was used for the quantitative analysis of human blood

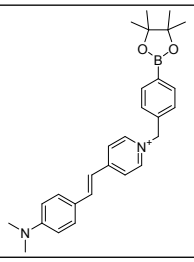
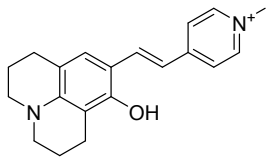
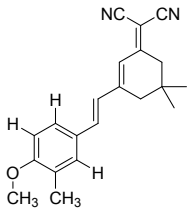
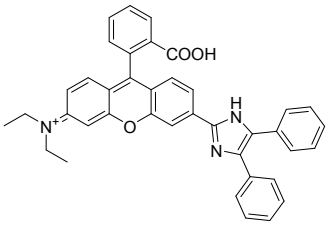
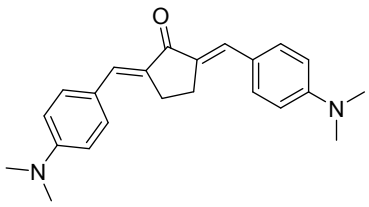
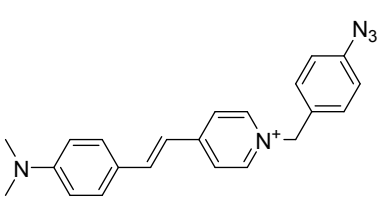
Statistical analysis using SPSS 19.0 software. Those who were tested for normal distribution by Kolmogorov-Smirnov were expressed as mean ± standard deviation, and the mean comparison between groups was analyzed by one-way analysis of variance.

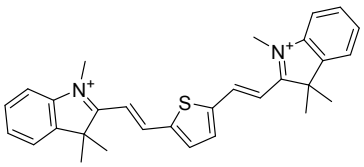
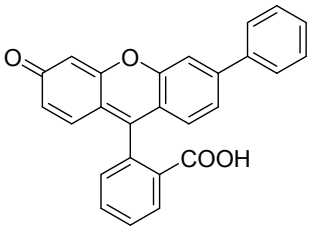
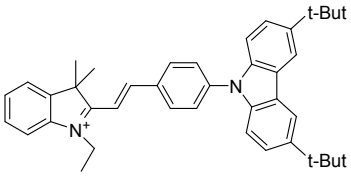
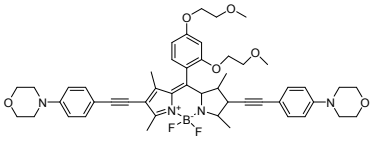
Whole blood viscosity detection in human clinical samples

Clinical data were collected from medical records. Blood samples were collected once for each patient, regardless of disease stage or therapy. The samples were used for determination of WBV using FOR on a ReoRox Jr. (MediRox AB, Nyköping, Sweden). WBV were measured at three kinds of shear rate at high (150/s), middle (50/s), low (10/s) and oscillation frequency 11 Hz, at a temperature of 38 °C. we then collected these blood viscosity values for each group of shear rates data.

2. Supporting figures and tables

Table S1 previous viscosity probes and this work

Probes	λ_{ex}/nm	λ_{em}/nm	Stokes shift/nm	sensitivity	Bioimaging application	ref
	500	607	107	16-fold	Cells	[1]
	530	620	90	Around 20-fold, not mention in the article	Cells	[2]
	405	600	195	approximately 20-fold	Cells	[3]
	570	655	85	48.5-fold	cells zebra fish mice	[4]
	450	508	58	less than 10-fold	Cells	[5]
	500	610	110	7-fold	Cells	[6]

	525	595	70	Not mention	Cells	[7]
	480	540	60	5-fold	Cells	[8]
	545	628	83	approximately 16-fold	Cells	[9]
	550	586	36	Nearly 20-fold	Cells	[10]
This work	**510**	**675**	**165**	**Nearly 400-fold**	**Cells, zebra fish, clinical blood samples**	

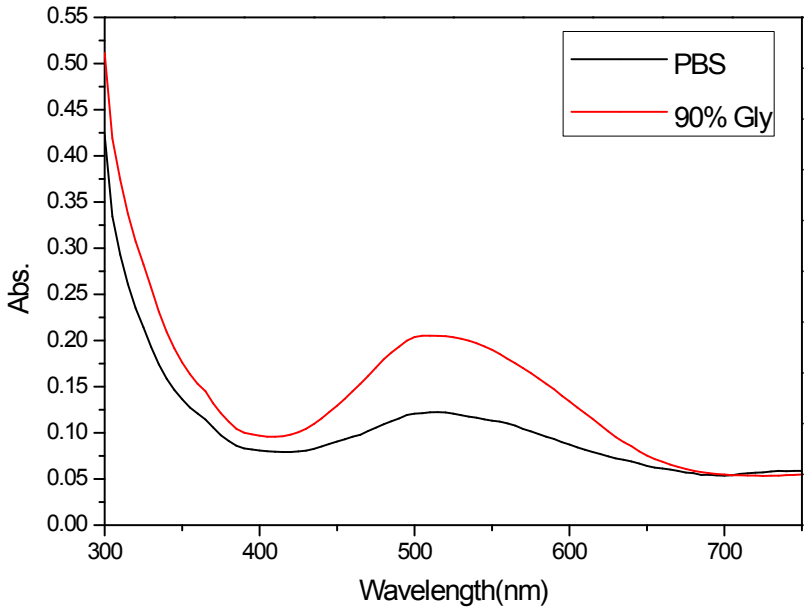


Figure S1 Absorbance of the probe **DJH** (10 μM) in PBS buffer and 90% glycerol.

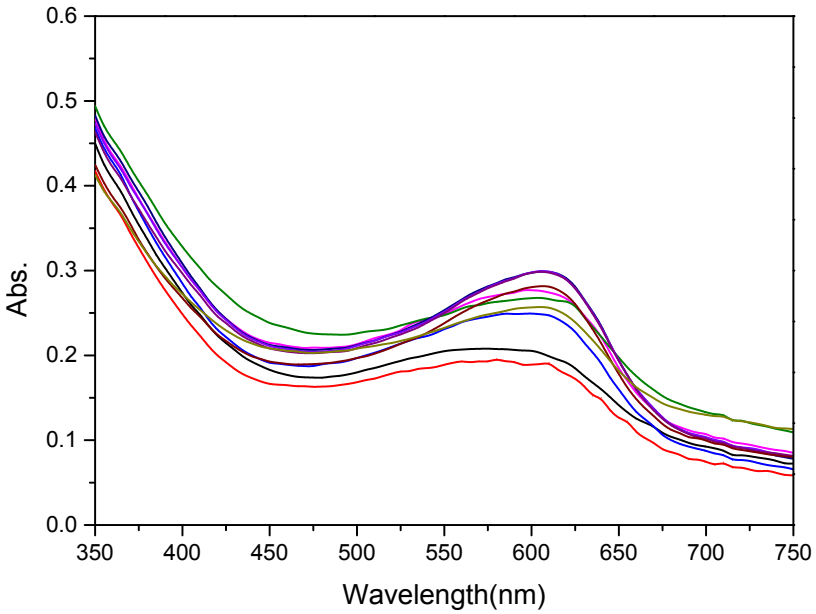


Figure S2 Absorbance of the probe **DJN** (10 μM) in PBS buffer to 90% glycerol.

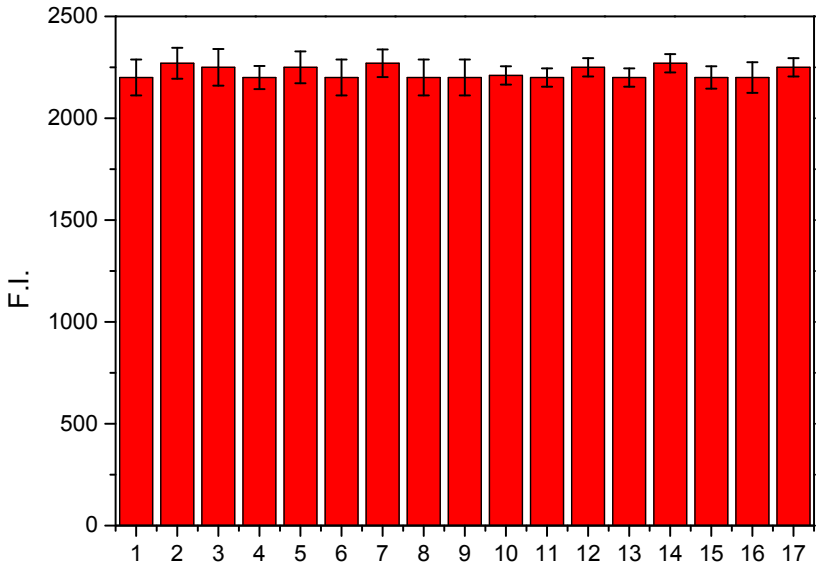


Figure S3 Fluorescence intensity of probe DJH (10 μM) in 50% Gly solution with the presence of various analytes, 50μM (1) Blank; (2) Cys, (3) GSH, (4) Hcy, (5) H_2S; (6) HSO^{3-}; (7) CO_3^{2-}; (8) OAc^-; (9) NO_3^- (10) $ONOO^-$, (11) H_2O_2, (12) TBHP, (13) HClO, (14) Fe^{2+}, (15) Ca^{2+}, (16) Zn^{2+}, (17) Mg^{2+}

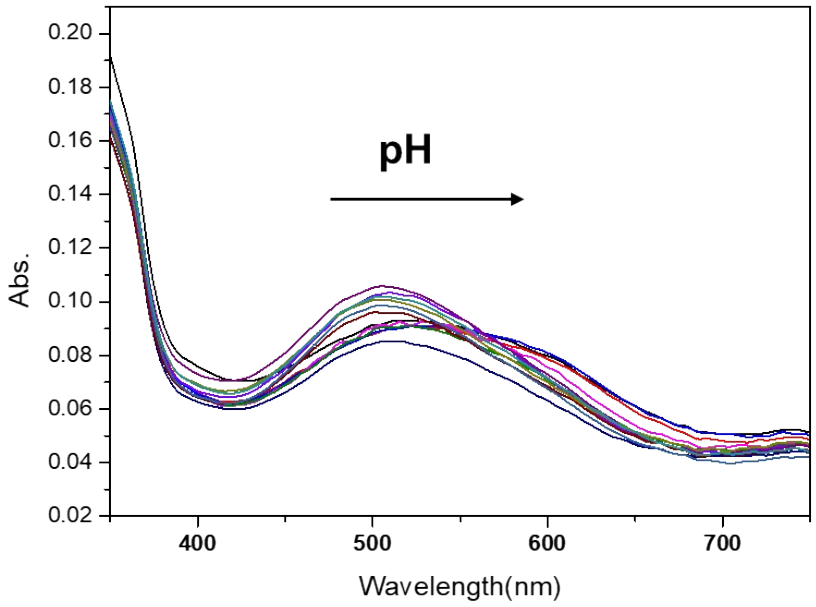


Figure S4 Absorbance of the probe **DJH** (10 μM) in different pH solution from 2.1-10.8.

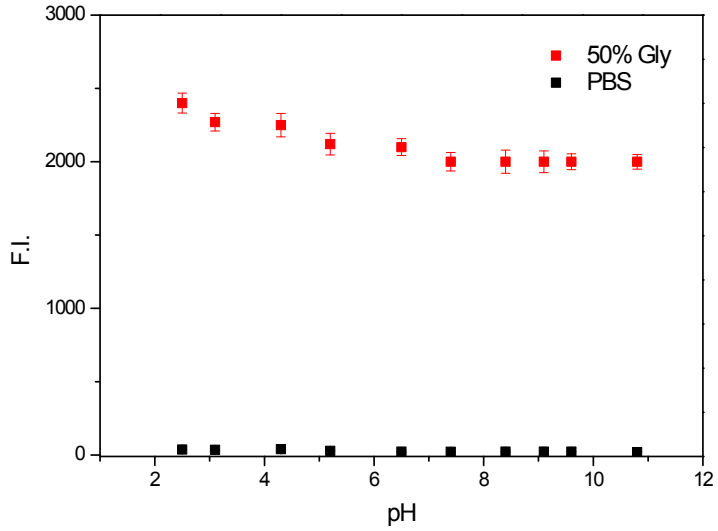


Figure S5 pH effects on the probe DJH. (10 μM)

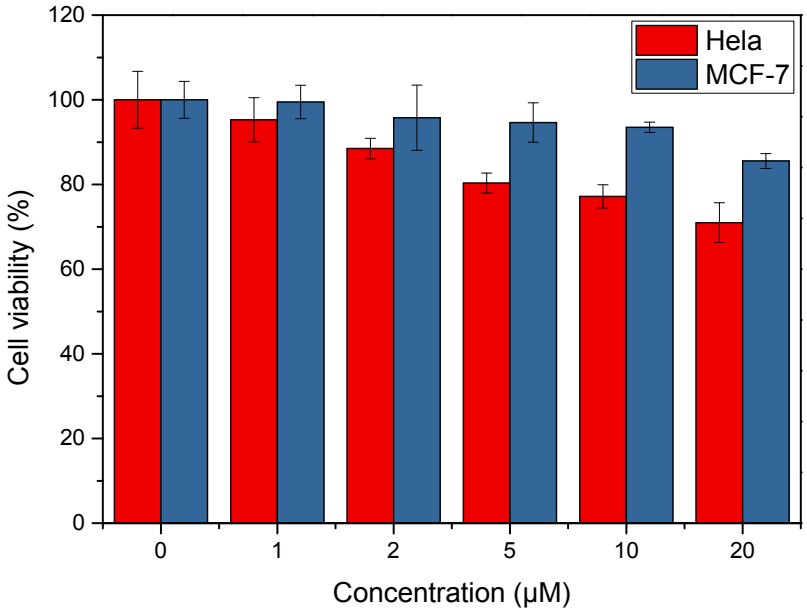


Figure S6. MTT results of Hela and MCF-7 cells viabilities after incubation with **DJH** for 24 h. Data are expressed as mean ± SD (*$p < 0.05$, experiment times n = 3).

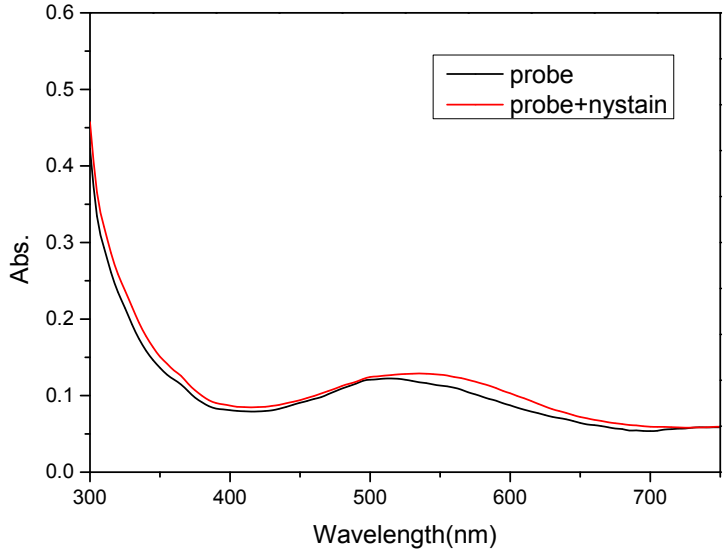


Figure S7 Absorbance of the probe **DJH** (10 μM) in PBS buffer with and without 10 μm nystatin.

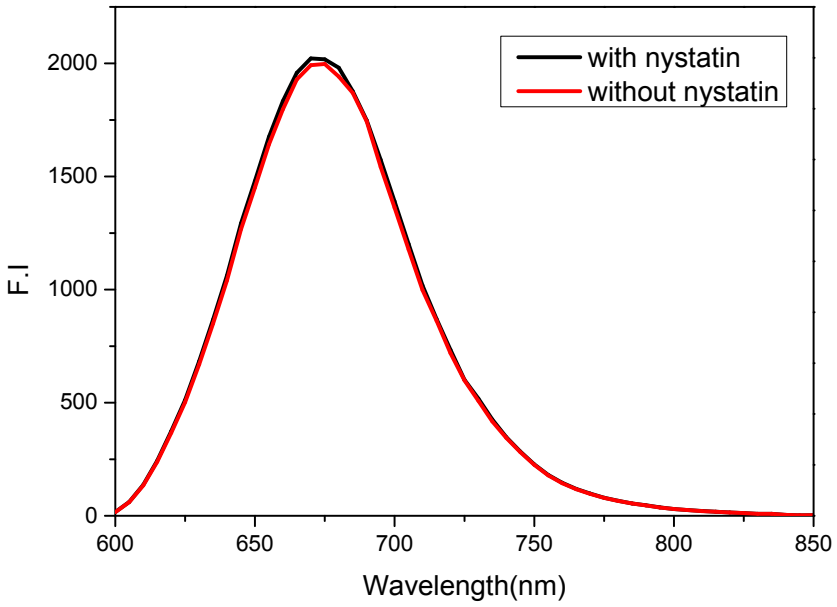


Figure S8 The fluorescence spectral of the probe DJH (10 μM) in Gly: PBS=1:1 solution with and without 10 μm nystatin

Variables	Group A= 30 Group B= 35 Control= 20	P□
Age(years)	43.6±9.7	>0.05
Height(cm)	170.5±5.5	>0.05
Body weight (kg)	70.3±10.4	>0.05
Body mass index (kg/m^2)	24.2±3.0	>0.05

Table S2. Baseline characteristics of subgroups of clinical samples selected according to the exclusion criteria of the present study. (Results were expressed as mean ± SD. P□ values by independent t-test.). Group A: Hypertensive patient. Group B: Normotensive dietetic patients. Group A: Healthy people.

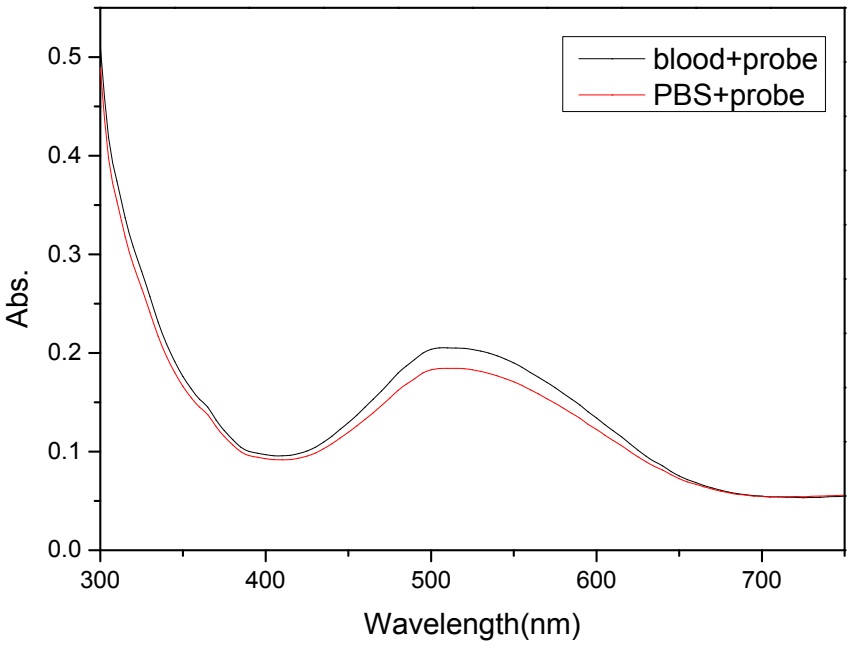


Figure S9 Absorbance of the probe **DJH** (10 μM) in PBS buffer and fresh blood of healthy samples.

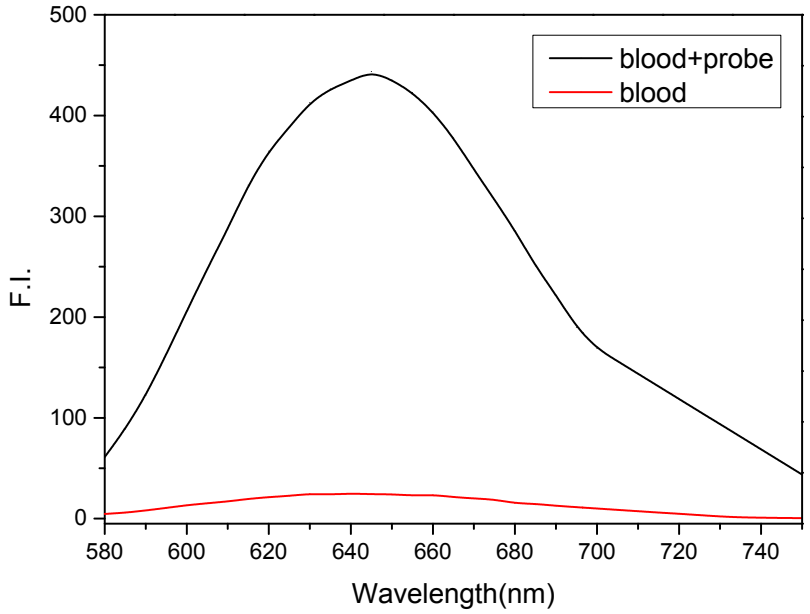


Figure S10 The fluorescence spectral changes of the clinic samples of hypertension patients with and without probe **DJH** (10 μM).

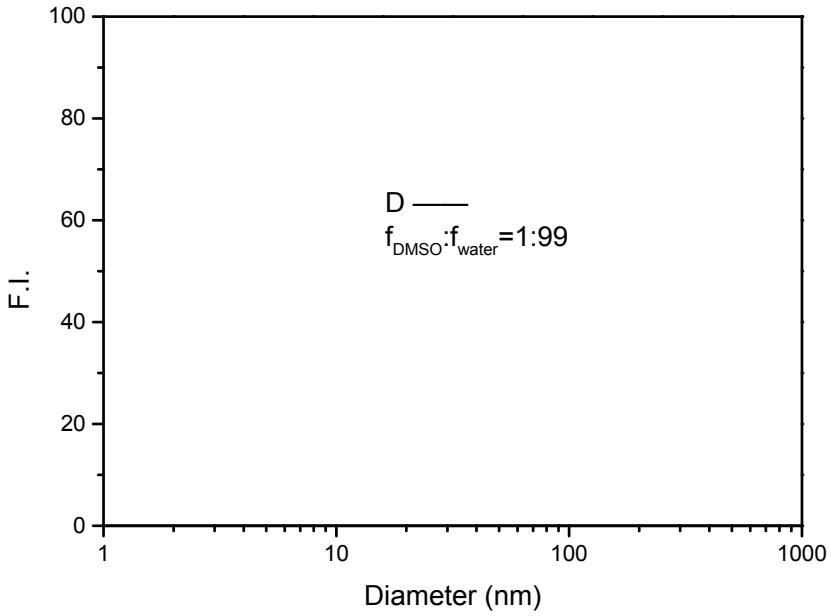


Figure S11 Particle size distribution (DLS) of DJH in DMSO: PBS=1:99 solution

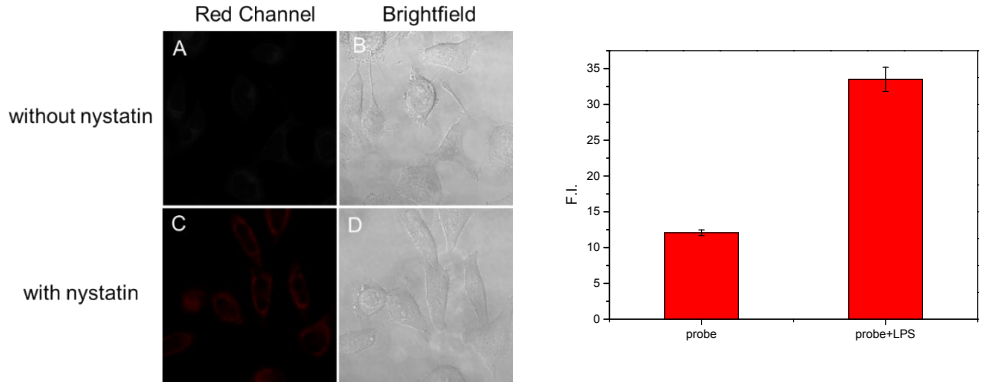


Figure S12 Laser Scanning Confocal Microscopy (LSCM) images of HeLa cells with or without LPS treatment upon one-photon excitation at 510 nm.

Formula (M)	Ion Formula	m/z	Calc m/z	Diff (ppm)
C25 H27 N3 O	C25 H26 N3 O	384.2093	384.2081	-3.02

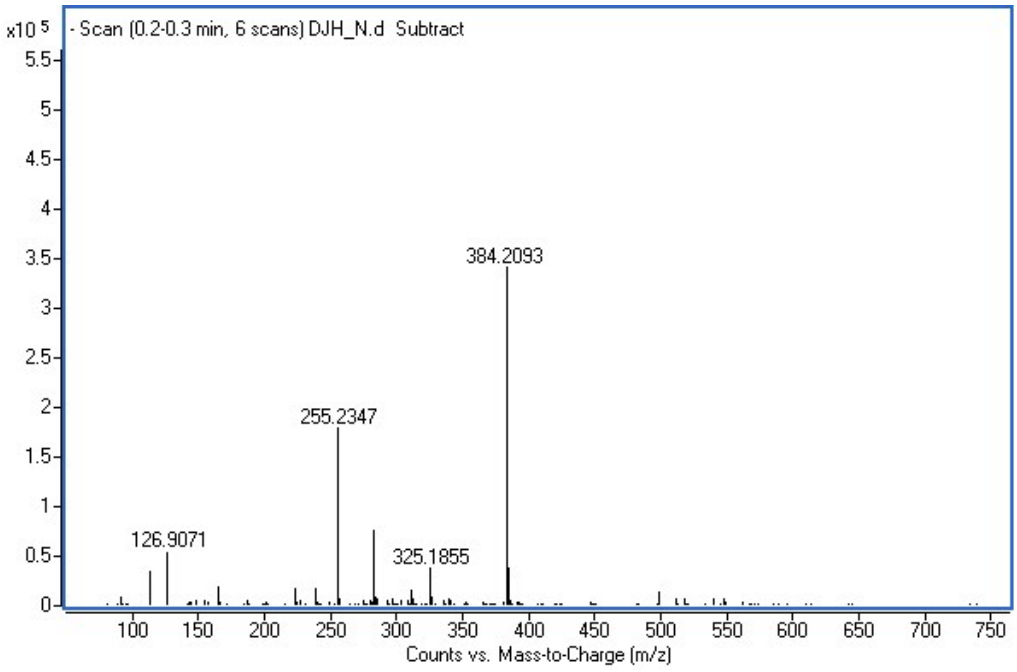


Fig. S13. HRMS spectrum of DJH

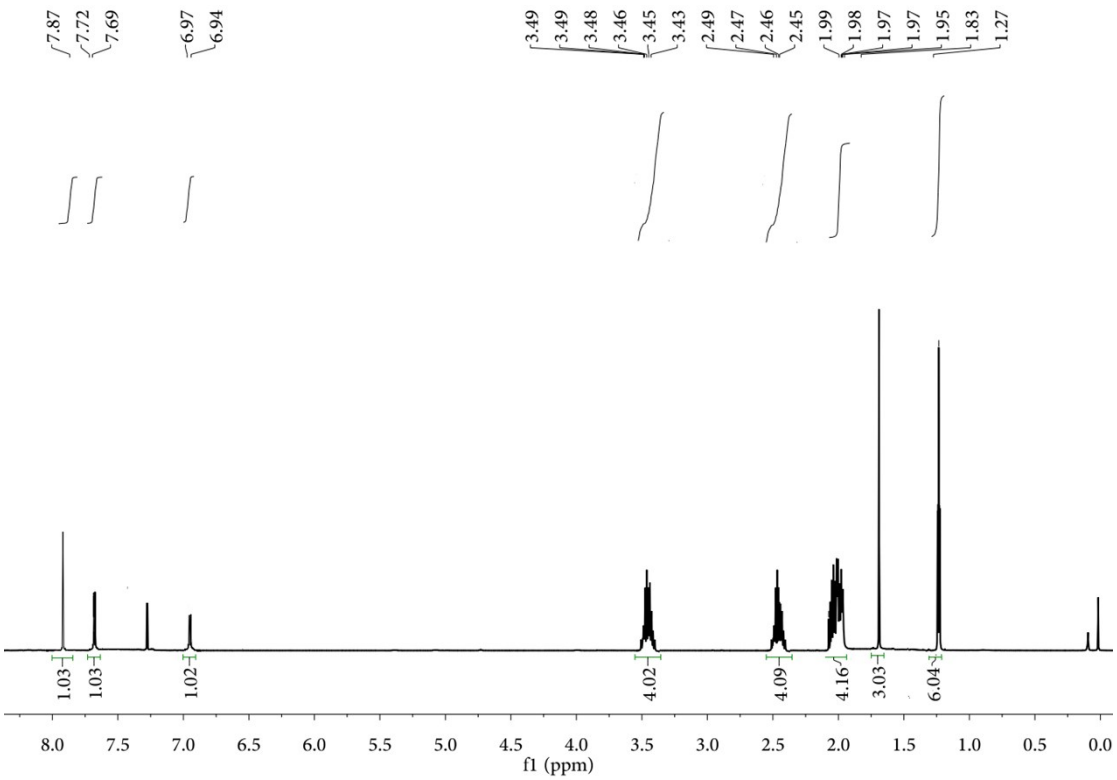


Fig. S14. ^{1}H NMR spectrum of DJH in $CDCl_3$

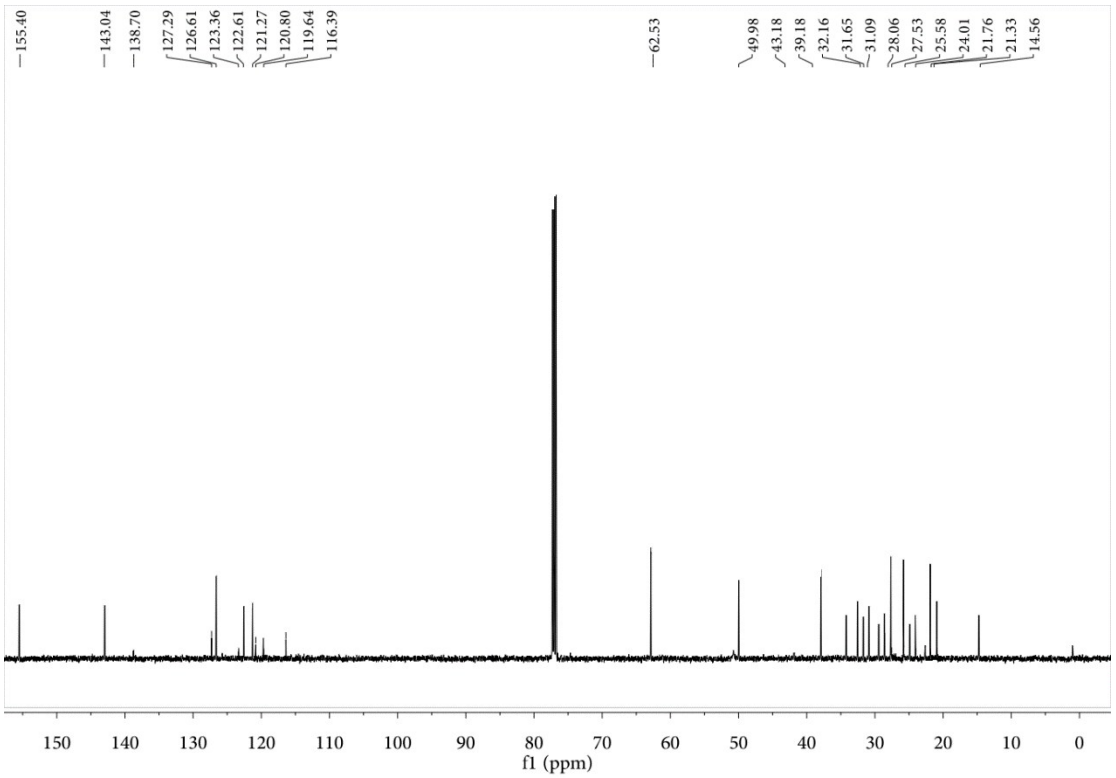


Fig. S15. ^{13}C NMR spectrum of DJH in $CDCl_3$

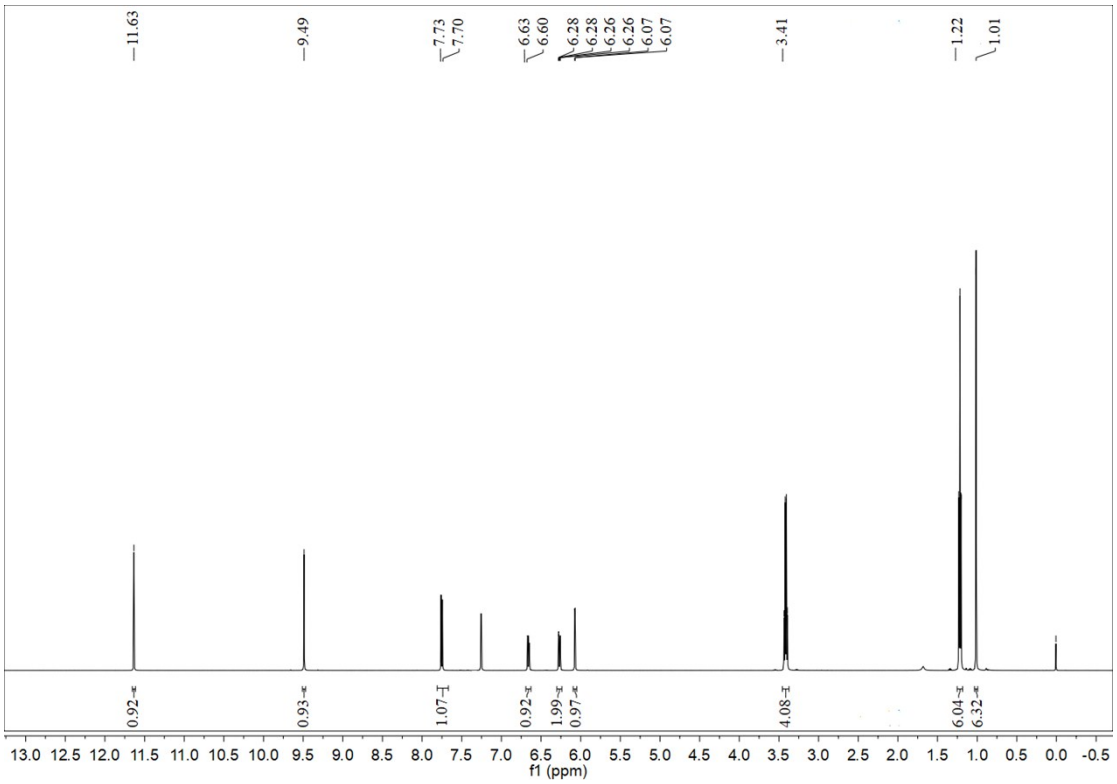


Fig. S16. ^{1}H NMR spectrum of DJN in $CDCl_3$

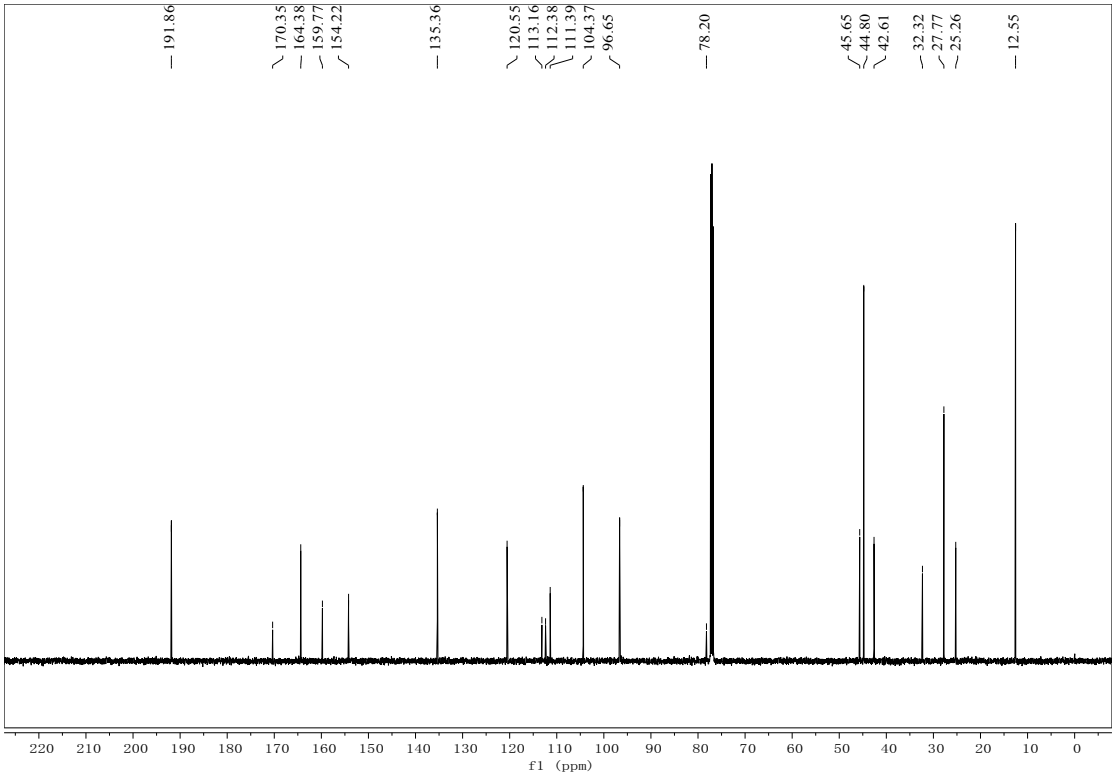


Fig. S17. ^{13}C NMR spectrum of DJN in $CDCl_3$

[1] M. Ren, B. Deng, K. Zhou, X. Kong, J. Y. Wang, W. Lin, *Anal Chem* **2017**, *89*, 552-555.

[2] G. Zhang, Y. Sun, X. He, W. Zhang, M. Tian, R. Feng, R. Zhang, X. Li, L. Guo, X. Yu, S. Zhang, *Anal Chem* **2015**, *87*, 12088-12095.

[3] S. C. Lee, C. L. Lee, J. Heo, C. U. Jeong, G. H. Lee, S. Kim, W. Yoon, H. Yun, S. O. Park, S. K. Kwak, S. H. Park, O. P. Kwon, *Chemistry* **2018**, *24*, 2888-2897.

[4] R. Guo, J. Yin, Y. Ma, Q. Wang, W. Lin, *Journal of Materials Chemistry B* **2018**, *6*, 2894-2900.

[5] X. Dai, B. Dong, M. Ren, W. Lin, *Journal of Materials Chemistry B* **2018**, *6*, 381-385.

[6] Q. Zhao, C. Yin, J. Kang, Y. Wen, F. Huo, *Dyes and Pigments* **2018**, *159*, 166-172.

[7] Y. Baek, S. J. Park, X. Zhou, G. Kim, H. M. Kim, J. Yoon, *Biosens Bioelectron* **2016**, *86*, 885-891.

[8] R. M. Yusop, A. Unciti-Broceta, M. Bradley, *Bioorg Med Chem Lett* **2012**, *22*, 5780-5783.

[9] K. Zhou, M. Ren, B. Deng, W. Lin, *New Journal of Chemistry* **2017**, *41*, 11507-11511.

[10] L.-L. Li, K. Li, M.-Y. Li, L. Shi, Y.-H. Liu, H. Zhang, S.-L. Pan, N. Wang, Q. Zhou, X.-Q. Yu, *Analytical Chemistry* **2018**, *90*, 5873-5878.