Supporting Information for

pH-Activated NIR Fluorescent Probe for Sensitive Mitochondrial Viscosity Detection

Manlin Fu, Qingwen Li, Xue Chen, Zhenda Xie*, Jin-Feng Hu*

Institute of Natural Medicine and Health Products, School of Pharmaceutical Sciences, Zhejiang Provincial Key Laboratory of Plant Evolutionary Ecology and Conservation, Taizhou University, Zhejiang 318000, China

Corresponding Author:

Zhenda Xie: xiezd2019@tzc.edu.cn

Jin-Feng Hu: jfhu@tzc.edu.cn

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), 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:

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

Computational methodology

Density functional theory (DFT) calculations were employed using the B3LYP functional and 6-31+G(d, p)* basis set for all calculations. In the calculation, except for the specific instructions, other atom free optimization, the dihedral angle of bond length and bond angle are not fixed, and all optimization is carried out in vacuum environment by default.

Cytotoxicity of probe SSN

The cytotoxicity of probe **SSN** was tested by MTT method. HepG2 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 **SSN** (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 540 nm.

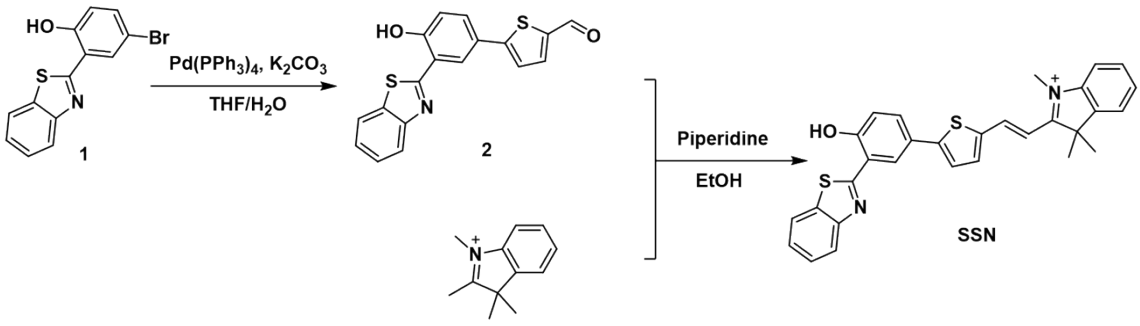
Cell culture and fluorescence imaging

DMEM containing 10% fetal bovine serum and 1% penicillin was used for HepG2 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

HepG2 cells, they were treated with 5 μM **SSN** for 30 min and then washed three times with PBS buffer.

Imaging in zebrafish

The 3-day-old zebrafish was incubated with **SSN** (10 μM) for 2 h, and then washed with PBS buffer and imaged as control group. The 3-day-old zebrafish firstly incubated with nystatin (50 and 100 mM, respectively) for 8 h, followed by **SSN** incubation for 2 h. Thereafter, the treated zebrafish was washed with PBS buffer three times and imaged using a confocal microscope ($\lambda_{ex}/\lambda_{em}$ = 561/700–760 nm).



Scheme S1. Synthetic routine for **SSN**.

Synthesis of Compound 1:

5-Bromo-2-hydroxybenzaldehyde (402 mg, 2 mmol), 2-aminothiophenol (300 mg, 2.4 mmol), and $Na_2S_2O_5$ (760 mg, 4 mmol) were added to a 200 mL round-bottom flask. The mixture was dissolved in 20 mL of DMF. Subsequently, the reaction was refluxed at 140°C for 2 hours. After cooling the mixture to room temperature, water was added, and the resulting solution was filtered to obtain a yellow solid compound 5 (367 mg, 60% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.71 (s, 1H), 8.39 (d, J = 2.4 Hz, 1H), 8.12 (dd, J = 27.5, 8.0 Hz, 2H), 7.56 (dd, J = 6.2, 2.5 Hz, 2H), 7.47 (t, J = 7.5 Hz, 1H), 7.07 (d, J = 8.8 Hz, 1H).

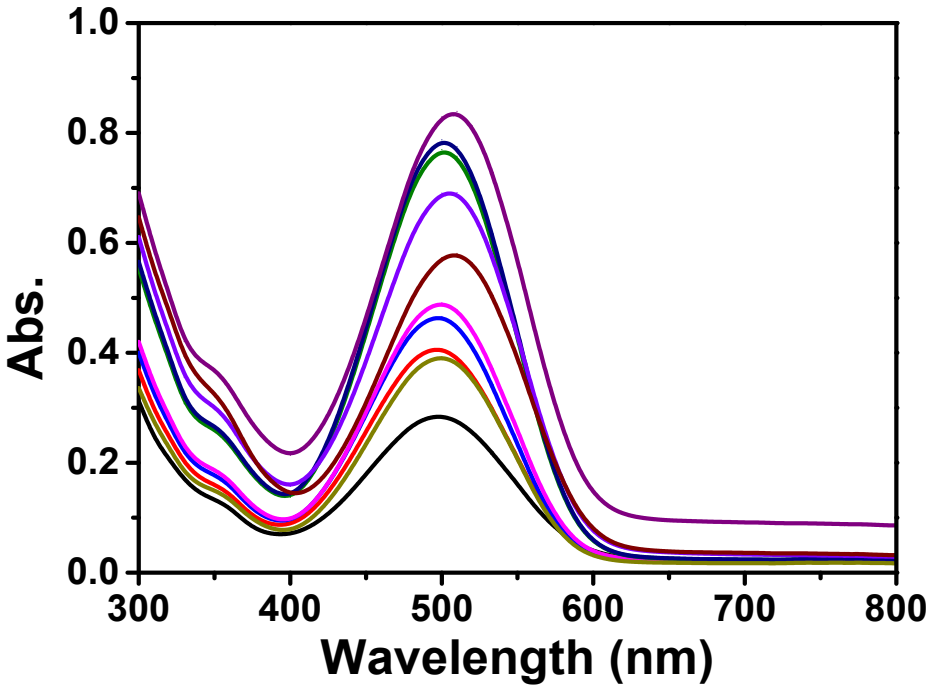


Fig.S1 Absorption spectra of **SSN** (5 μM) in different ratios of PBS/glycerol mixtures.

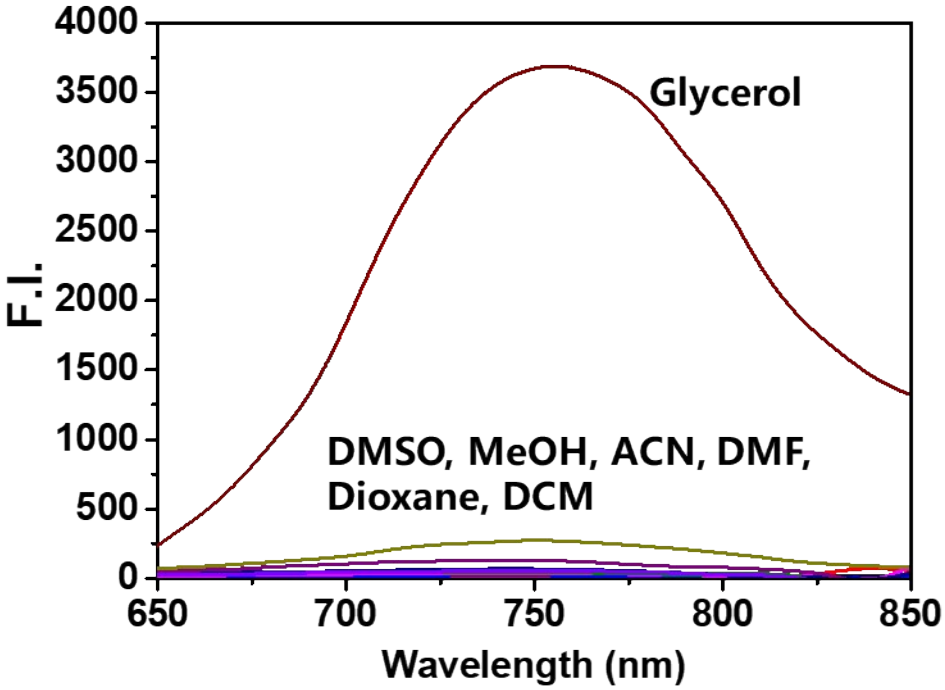


Fig. S2 The fluorescence spectra of probe **SSN** (5 μM) in different solvents.

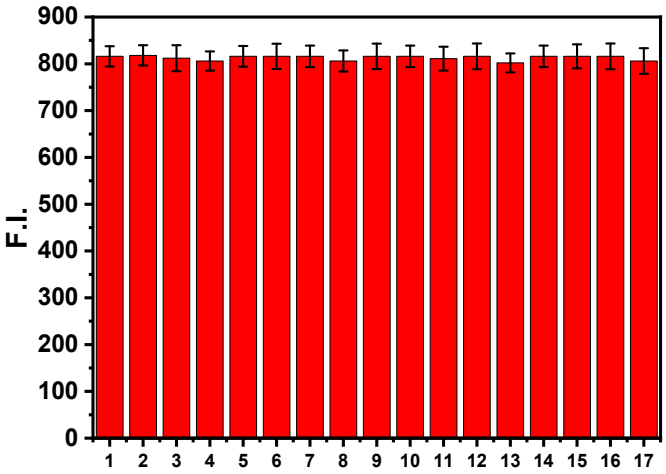


Fig. S3 Fluorescence intensity at 760 nm of 5 μM **SSN** toward various species (100 μM) in PBS/glycerol (v:v = 1:1) mixture. (1) Blank; (2)Ser; (3)Phe; (4)Arg; (5)Cys; (6)GSH; (7)Hcy; (8)BSA; (9)SO_3^{2-}; (10)SO_4^{2-}; (11)ClO^-; (12)ClO_4^-; (13)H_2O_2; (14)$ONOO^-$; (15)CO_3^{2-}; (16)OAc^-; (17) 50% Glyceral. The excitation wavelength was 520 nm.

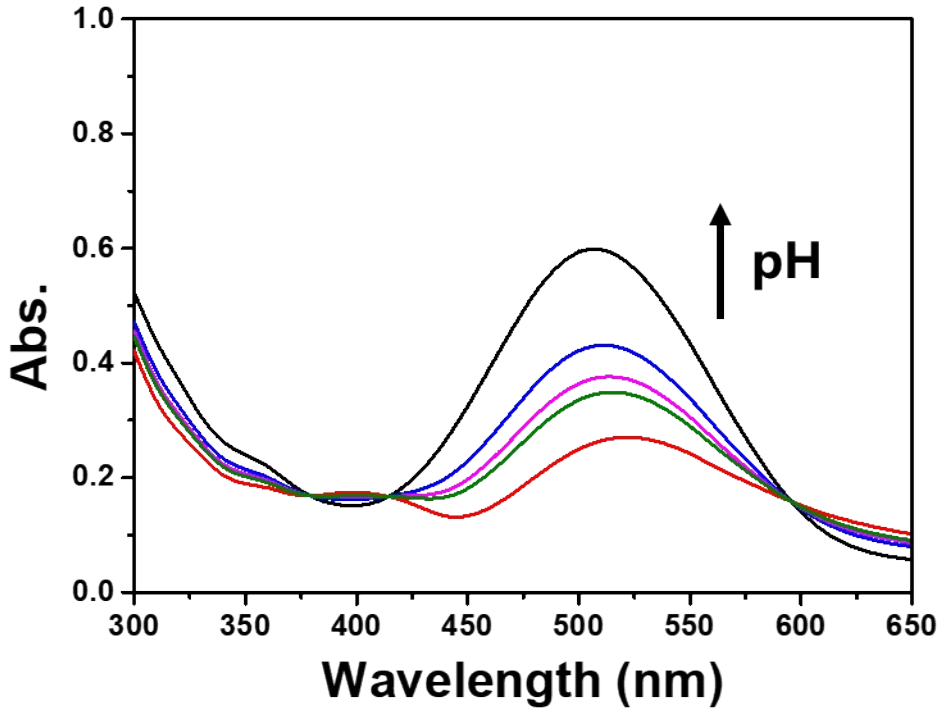


Fig.S4 Absorption spectra of **SSN** (5 μM) in different pH buffers.

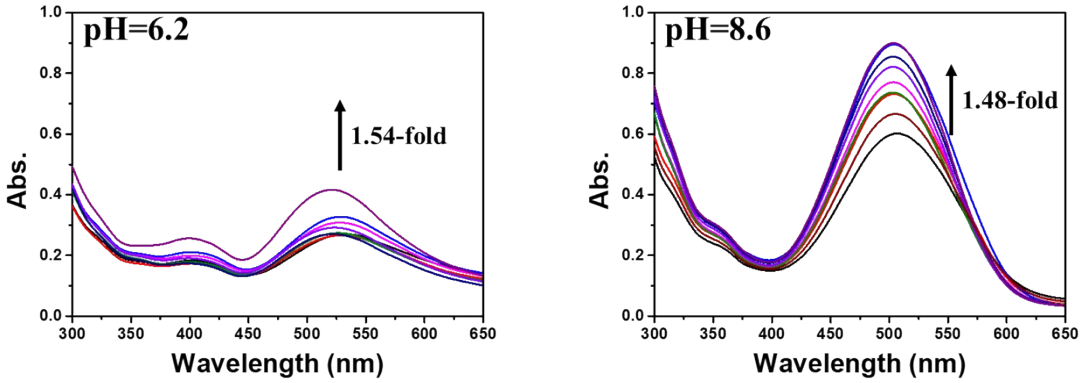


Fig.S5 Absorption spectra of **SSN** (5 μM) in different ratios of PBS/glycerol mixtures in pH =6.2 and pH =8.6, respectively.

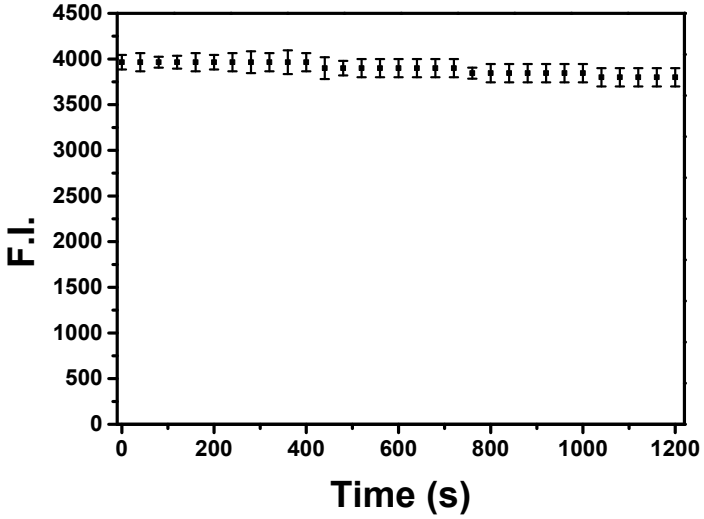


Fig. S6 Photobleaching of **SSN** (5 μM) in 95% glycerol solution under irradiation with 660 nm laser (300 mW/cm^2).

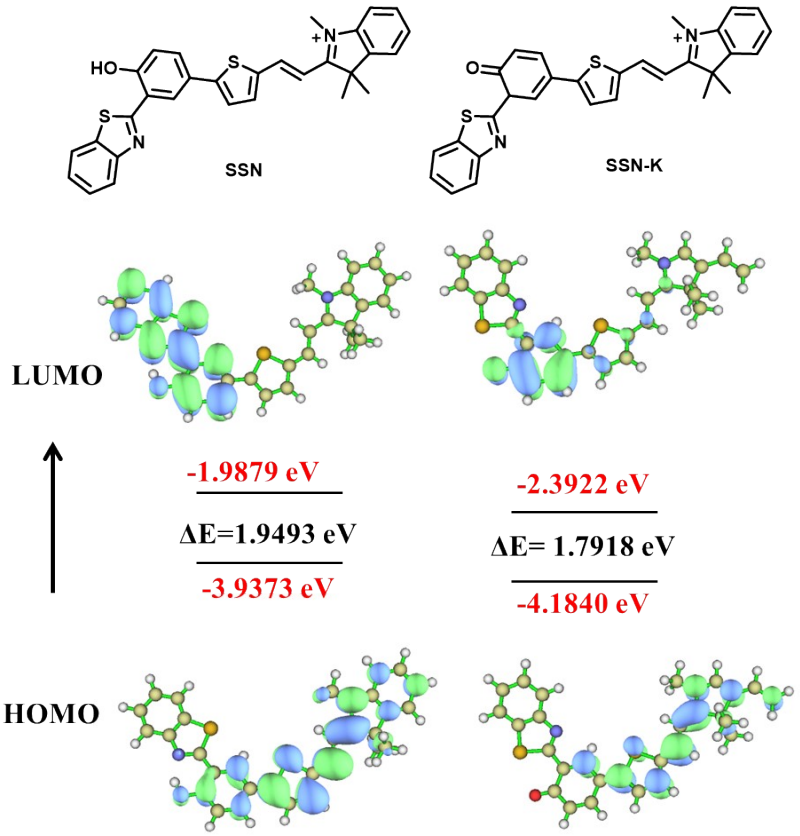


Fig. S7 Calculated electron-hole distributions during photoexcitation of **SSN**. The geometry of **SSN** and **SSN-K** is based on excited-state optimized structures.

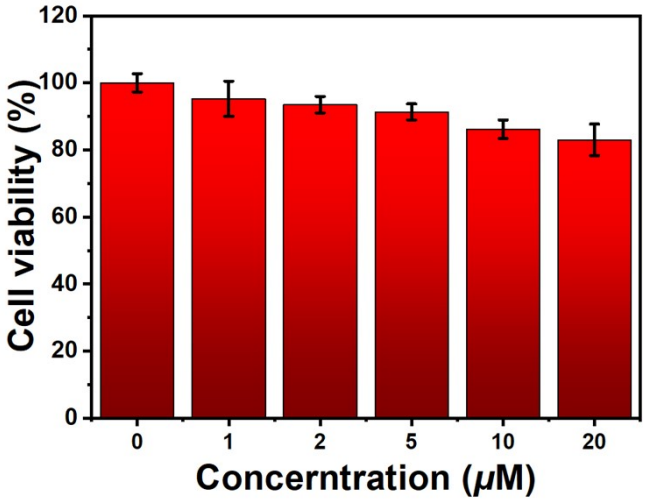


Fig. S8 MTT results of HepG2 cells viabilities after incubation with **SSN** for 24 h. Data are expressed as mean ± SD (*$p < 0.05$, experiment times n = 3).

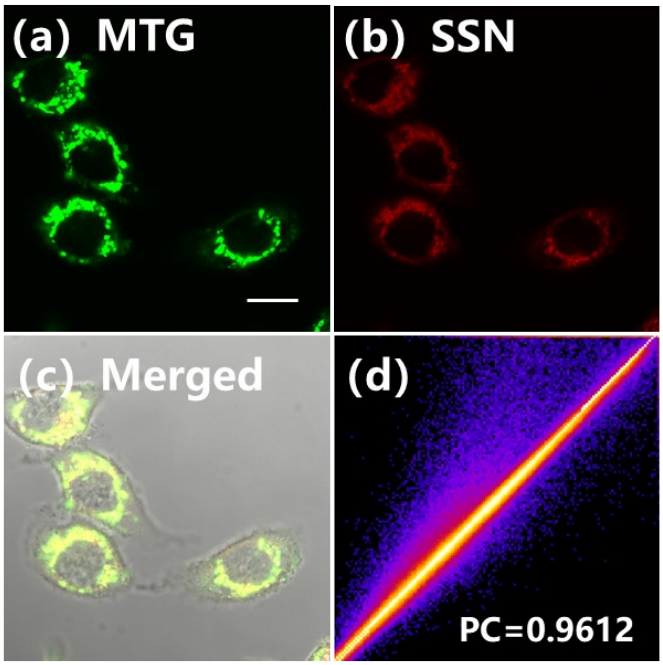


Fig. S9 Confocal HepG2 cell images co-stained by **SSN** (5 μM) and MTG (0.2 μM). (a) Image of MTG (λ_{em}: 500–550 nm); (b) Image of **SSN** (λ_{em}: 720–800 nm). (c) merged images with bright-field image. (d) Intensity scatter plot with Pearson's coefficient indicated (PC = 0.9612). Scale bar = 10 μm. Data are expressed as mean ± SD (*** $P<0.001$, experiment times n = 3).

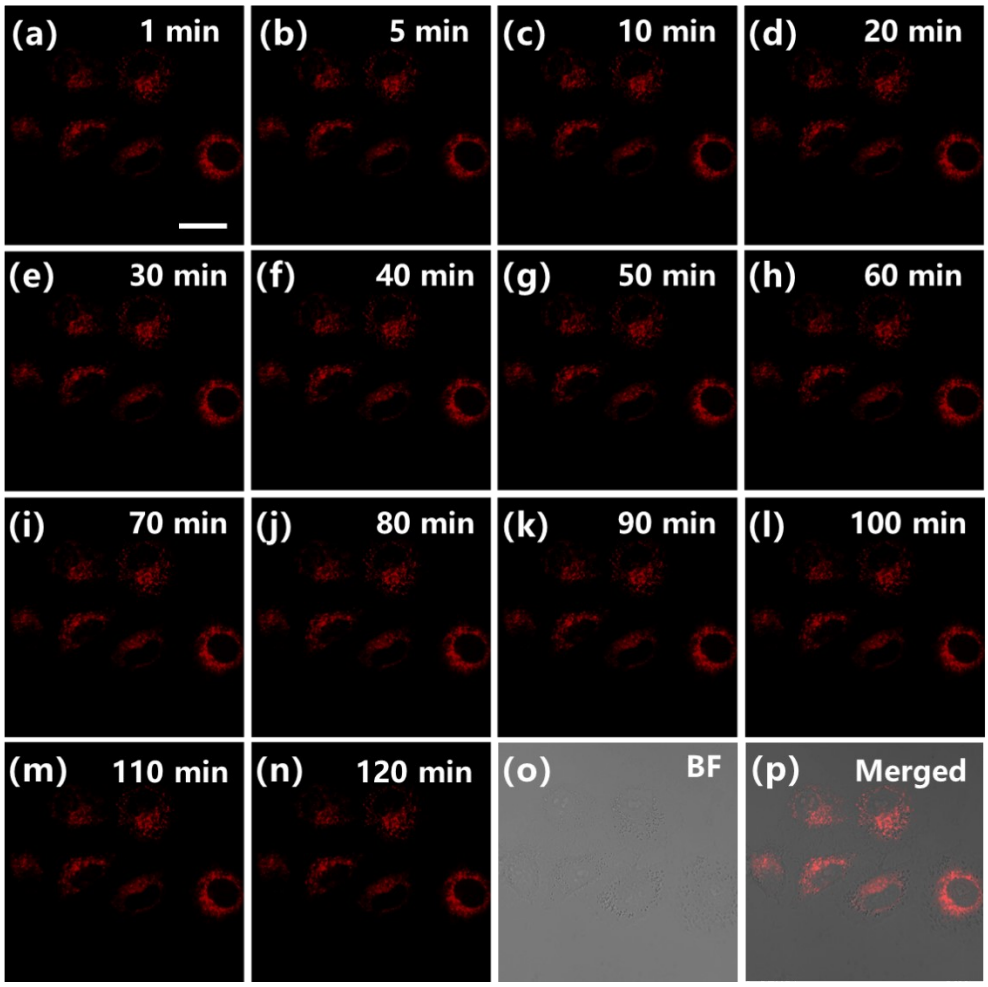


Fig. S10 Laser Scanning Confocal Microscopy (LSCM) images of HepG2 cells incubated with **SSN** (5 μM), captured at various time intervals. (a-n) Red channel; (o) BF; (p) Merged images. The scale bar = 20 μm.

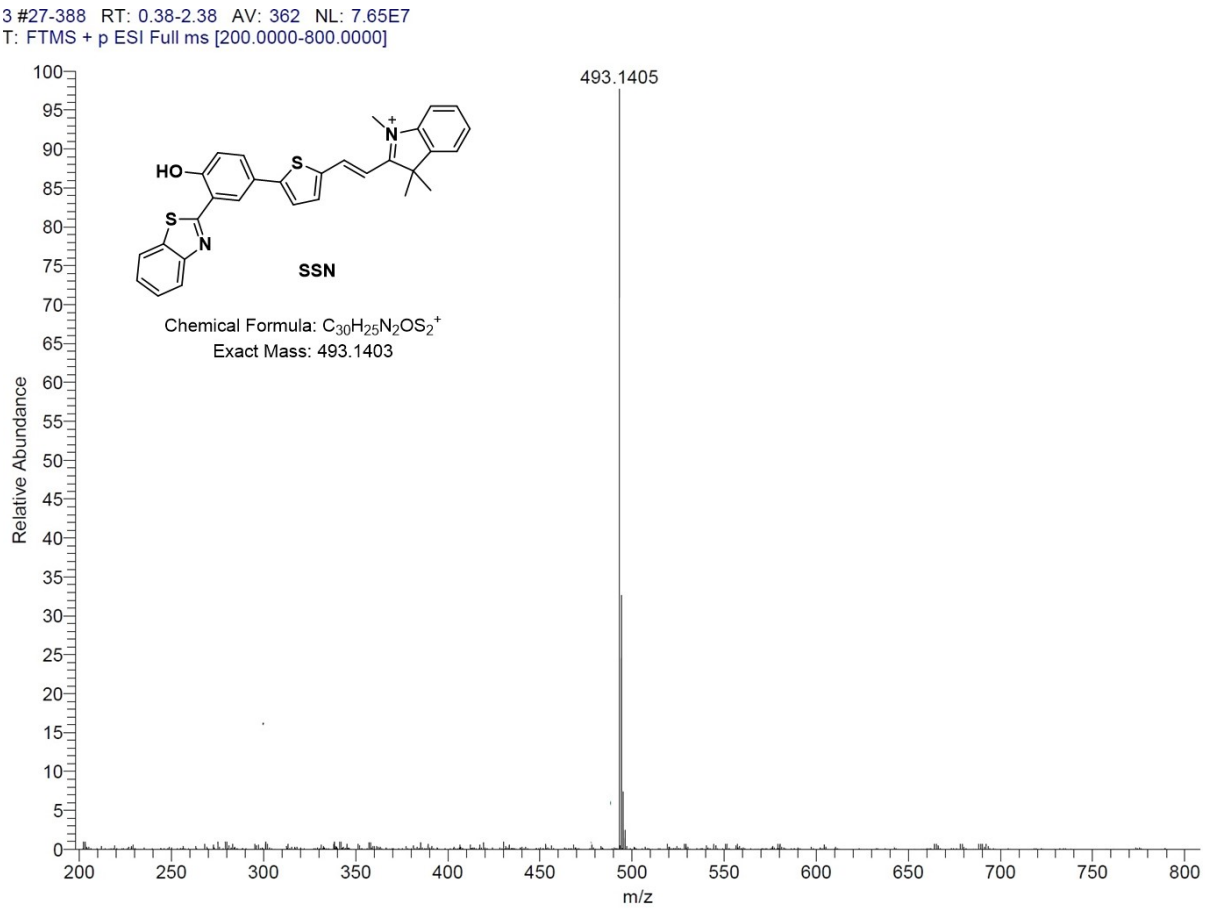


Fig. S11 Mass spectra of **SSN**.

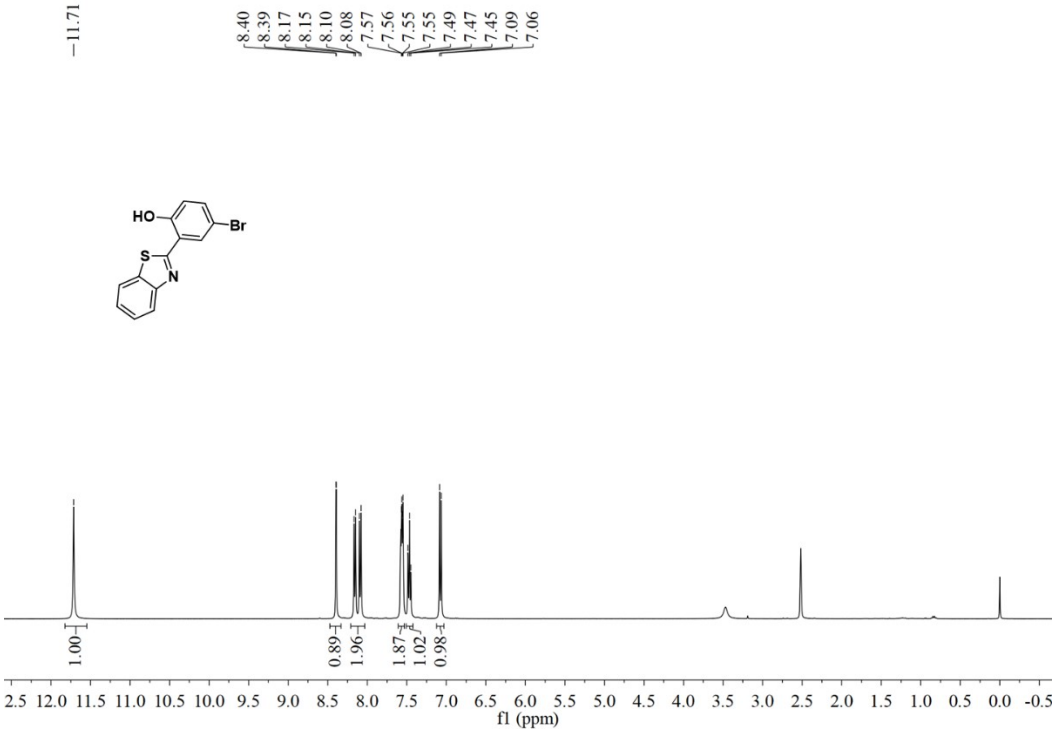


Fig. S12 ^{1}H NMR spectrum of **compound 1** in DMSO-d_6.

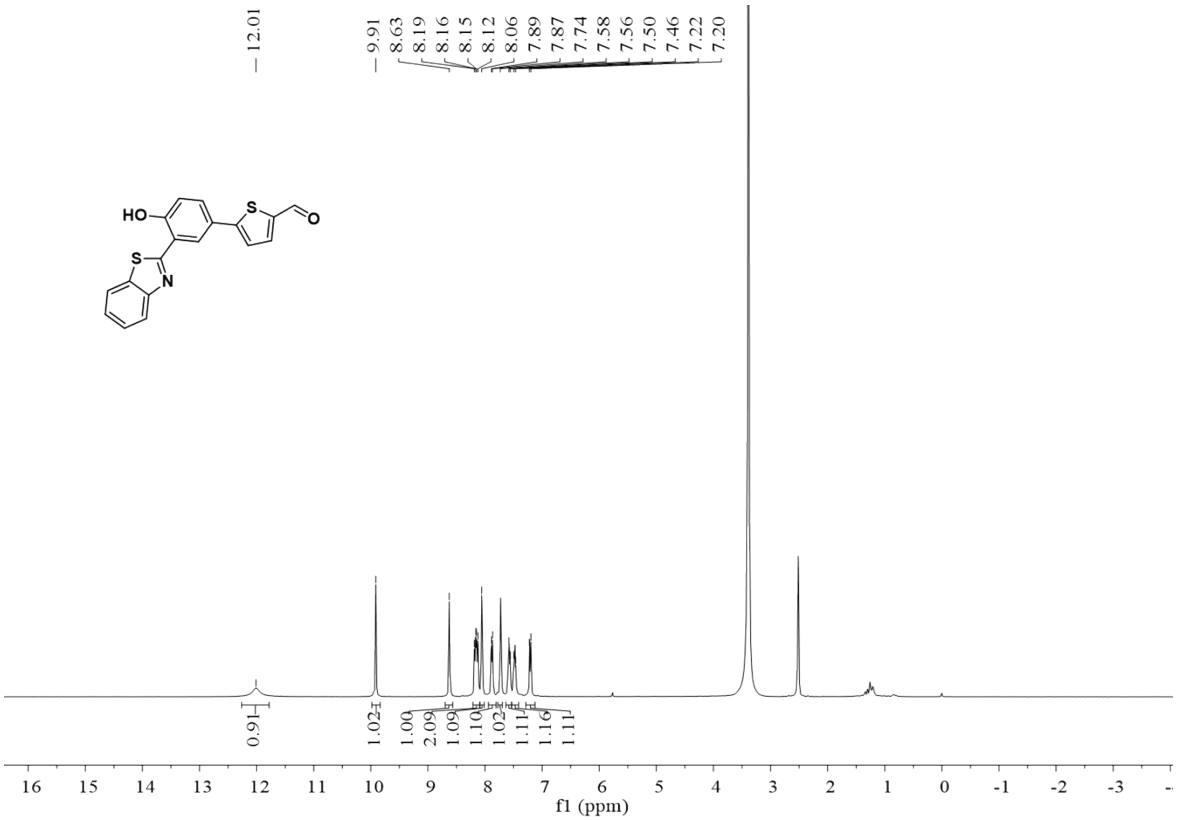


Fig. S13 [1]H NMR spectrum of **compound 2** in DMSO-d_6.

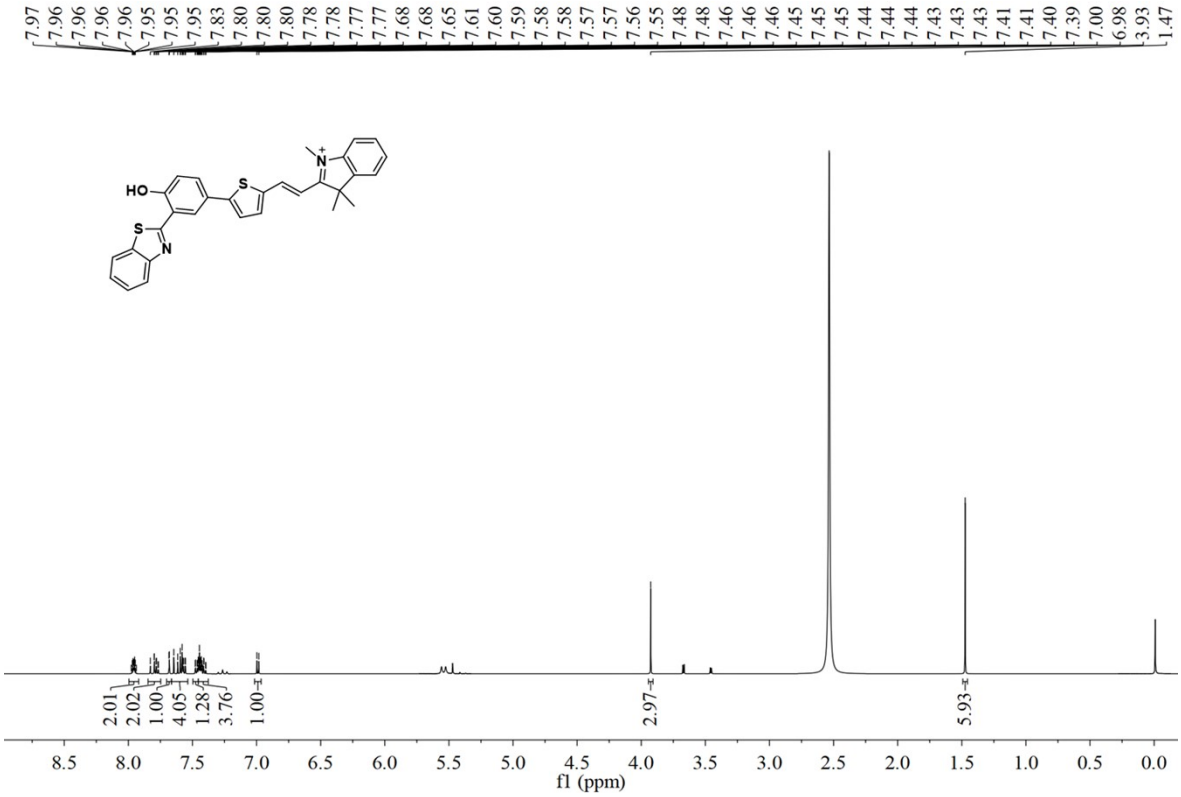


Fig. S14 [1]H NMR spectrum of **SSN** in DMSO-d_6.

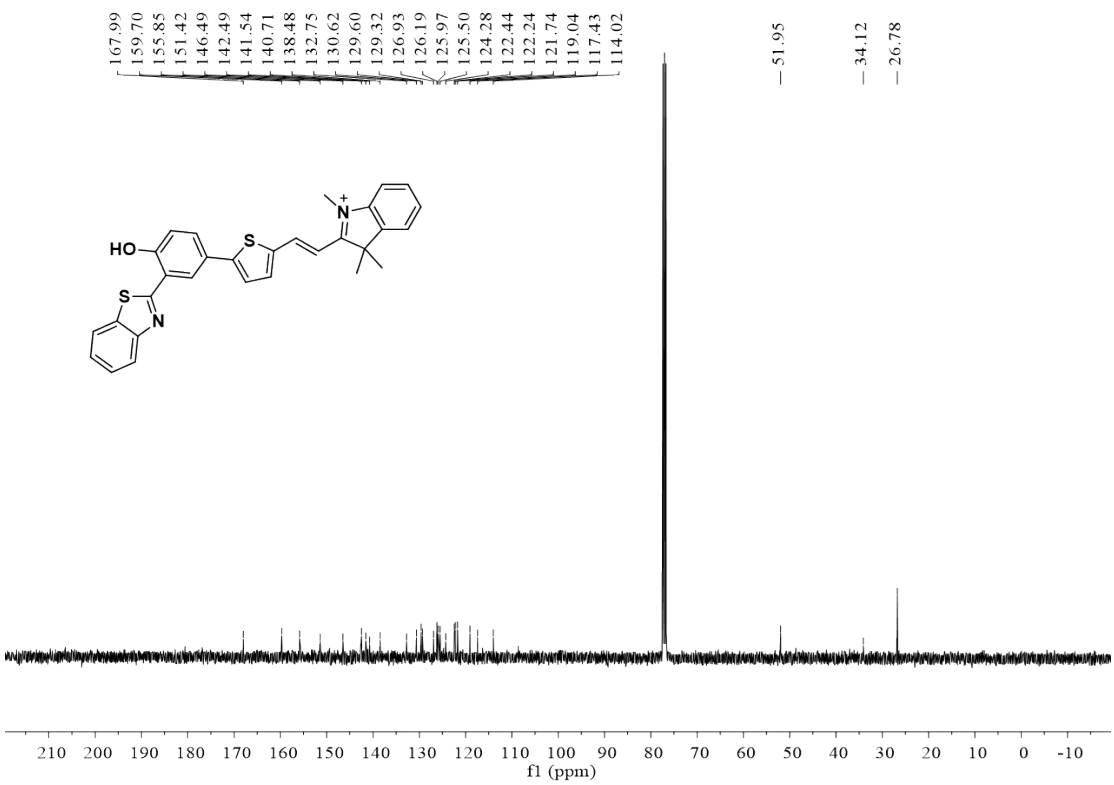


Fig. S15 ^{1}C NMR spectrum of **SSN** in DMSO-d_6.