# Supporting Information

## Light-activated "cycle reversible ICT" fluorescent probe: Monitoring of pHi trace-change induced by UV in programmed cell death

Tao Jiang,[a] Xin Wang,[a] Ge Wang,[b] Yafu Wang,[a] Kui Wang,[b] Xiaopeng Xuan,[a] Changpo Chen,[a*] Kai Jiang [a] and Hua Zhang[a,*]

[a] Henan Key Laboratory of Green Chemical Media and Reactions, Ministry of Education; Key Laboratory of Green Chemical Media and Reactions; Collaborative Innovation Center of Henan Province for Green Manufacturing of Fine Chemicals; School of Chemistry and Chemical Engineering Institution, Henan Normal University, Xinxiang 453007, China

[b] School of Basic Medical Sciences, Xinxiang Medical University.

Corresponding Author Email: zhanghua1106@163.com Tel/Fax: +86-373-3329030.

## Contents

## 1. Procedures Section

The reagent grade of solvents and reagents were used in the synthesis and testing experiments. Column chromatographic filled with 200-300 mesh of silica gel was used to purify the intermediate and target products. 400/600 MHz of NMR spectra (Bruker Avance II) was employed to obtain 1H NMR and 13C NMR of all compounds. In all spectral experiments, a PerkinElmer Lambd 950 spectrophotometer (USA) and a PerkinElmer LS-55 spectrophotometer (USA) were used to measure UV-absorption spectra and fluorescence spectra, respectively. Gaussian 09 and the High Performance Computing Center of Henan Normal University were used in the theoretical calculation. In cell imaging, Olympus FV1200 spectral confocal multiphoton microscope was employed for all living imaging. BD flow cytometer (FACSCanto II, USA) was employed to analyze and sort cells. In all experiments, the final data was the average values of the five the experiment results, separately. A.R. grade of solvents and reagents were used in this work. Column chromatographic was used to purify compounds and silica gel (200-300 mesh) was used as fillers. HPLC(Agilent 1290 Infinity)-HRMS (Bruker microToF II, Bruker Co., Switzerland) with an auto sampler operated in-line with a quantum triple quadrupole instrument was carried out in mass spectral studies on ESI positive or negative ion mode. NMR spectra were obtained from Avance 400 and 600 MHz spectrometer (Bruker Co., Switzerland).

**Light-activated ultrasensitive fluorescent probe synthesis**: The synthetic routes and steps of **UV-SP** were listed (see the Supporting Information, SI). **UV-SP** was characterized by NMR and mass spectrometry.

**To detect absorption and fluorescence specific spectral response:** The probe **UV-SP** was dissolved into DMSO to form 1.0 Mm of stock solution for all the specific spectral detections.

a. The absorption and fluorescence spectrum were detected in buffer.

b. The molar extinction coefficient (ε): according to the eq (1), the ε of **UV-SP** in different conditions was calculated. The eq (1) as follow:

$\varepsilon = a/bc$ (1)

Among, every symbols represent:

a = the maximum absorbance;

b = optical path length, 1.0 cm was used in this experiment;

c = the sample solution concentration.

c. Fluorescence quantum yield (Φ): according to the eq (2), the Φ of **UV-SP** in different conditions was calculated by reference method. The eq (2) as follow:

$$\Phi_x = \Phi_s(F_X/F_S)(A_s/A_x)(\lambda ex_s/\lambda ex_X)(n_X/n_S)^2 \quad (2)$$

Among, every symbols represent:

F = the integrated area in the emission spectrum;

A = the maximum absorbance;

λex = the excitation wavelength;

n = the refractive index of the solution;

subscripts x = the sample to be tested, s = the standard sample;

Rhodamine B (Φs = 0.97, methanol solution) as reference solution was used in the detection of fluorescence quantum yield. In all spectral test solution, the final solutions only contained <5‰ of DMSO.

**Quantum calculations**: All the quantum calculation data, the energy of ground-and excited-state and simulated spectrum, were obtained by the Gaussian 09 suite (Zhang et al. 2013a, Zhang et al. 2013b, Xu et al. 1996, Frisch et al. 2009.). The geometry of **UV-SP, UV-HCY, UV-HCY-H$^+$** and **UV-HCY-OH$^-$** were optimized by the B3-LYP functional and 6-31G basis set. There are no constraints on bonds/angles/dihedral angles. The TD-DFT theory (B3LYP/6-31G level) was used to calculate the

electronic transition energies and simulated spectrum (Gross et al. 1985; Stratmann et al. 1998.).

**Cell culture and cytotoxicity:**

a. Cell culture All cell lines i.e. HepG2 cell line and NIH 3T3 cell line were purchased from the Institute of Basic Medical Sciences of the Chinese Academy of Medical Sciences. Cell supernatants, DMEM and MEM, were added with penicillin/streptomycin and 10% fetal bovine serum. The all cell lines were cultured by the mentioned above the cell supernatants under a 5.0 wt %/vol $CO_2$ incubator at 37 °C. Before imaging 24 h, cell lines were cultured into a dish (the diameter of dish = 35 mm, the diameter of glass bottomed = 20 mm). After 24 h, the cells were stained with concentrated **UV-SP**.

b. cytotoxicity The different concentrations of **UV-SP** were used to incubate dyes for different time. And then, after 100 μL of MTT was added into each well the cells were incubated for 4 h. Subsequently, a microplate reader was used to detect the optical density (OD) of each well at 570 nm and 630 nm with. Cytotoxicity was determined by cell survival number. The cell viability (CV%) was calculated by eq (3) as follow:

$$CV\% = (OD_{\text{UV-SP}} - OD_{K\ \text{UV-SP}}) / (OD_{\text{control}} - OD_{K\ \text{control}}) \times 100 \qquad (3)$$

**Confocal microscopic imaging** To use An Olympus FV1200 spectral confocal microscope obtains the cell imaging. The imaging parameters as follow: internal PMTs: 16 bit with 1024 × 1024 pixels, excitation wavelength = 405 nm and 488 nm. The scan range: the yellow channel = 580-610 nm, the red channel = 650-680 nm and the green channel = 550-580 nm.

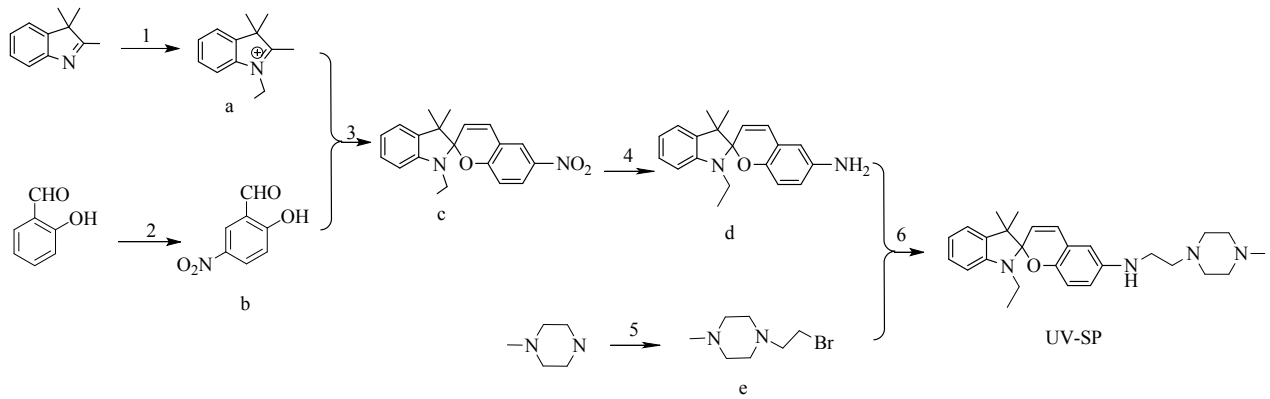
**Flow cytometry** The cells incubated by **UV-SP** were evenly dispersed in 2.0 mL of PBS buffer. And then, it was analyzed by flow cytometry with FACS Diva software. Samples were illuminated with a sapphire laser at 488 nm, the collected signal bands were 580-610 nm, 650-680 nm and 550-580 nm, respectively. Basic signal data were collected using 10,000 cells at rate of 150 events/second.

**Photostability in solution:** **UV-SP** was dissolved in DMSO-water (5:5 v/v). The solutions were irradiated by a 500W iodine-tungsten lamp situated 250 mm away for 6.0 h. An aqueous solution of

sodium nitrite (50 g/L) was placed between the samples and the lamp as a light filter (to cut off the light shorter than 400 nm) and as a heat filter. The photostabilities were expressed in terms of remaining absorption (%) calculated from the changes of absorbance at the absorption maximum before and after irradiation by iodine-tungsten lamp. The absorbance was determined on a Lambd 950 spectrophotometer (PerkinElmer, USA). The data were obtained from replicate experiments (n=5).

**Cells imaging in HepG2 cells by UV-SP (5.0 μM) at different PBS buffer:** HepG2 cells were cultured by DMEM with penicillin/streptomycin and 10% fetal bovine serum under a 5.0 wt %/vol $CO_2$ incubator at 37 °C. Before imaging 24 h, cell lines were cultured into a dish (the diameter of dish = 35 mm, the diameter of glass bottomed = 20 mm). After 24 h, the cells were washed by PBS. And then, the cells were cultured by different PBS buffer at 6.0, 7.4 and 8.0, respectively. After 4.0 h, the cells were stained with concentrated UV-SP for 30min. The yellow channel (emission wavelength = 580-610 nm). And green channel (emission wavelength = 550-580 nm) and red channel (emission wavelength = 650-680 nm) were obtained by excited at 488 nm after photoactivation by 375 nm. Furthermore, the pHi at 6.0, 7.4 and 8.0 were detected by microacidity meter after cell homogenate.

## 2. Synthetic route of UV-SP




**Supplementary Scheme S1**. The synthetic route of **UV-SP**. (1) 2,3,3-Trimethylindolenine, iodoethane, reflux, 60%; (2) $HNO_3$, $CH_3COOH$, room temperature,83%; (3) Compound a, 5-nitrosalicylaldehyde, reflux, 74%; (4) Compound c, Absolute ethanol, zinc powder, reflux ,40%;(5) Methylpiperazine, 1,2-Dibromoethane,anhydrous sodium carbonate,reflux, 56%;(6) Compound a, Compound e, anhydrous sodium carbonate,reflux, 30%.

## 3. Synthetic procedures of UV-SP

**The synthesis of a and b** Compound a and b were prepared by the literature methods [1, 2]. To 2,3,3-Trimethylindolenine (8 mmol) in hot Trichloromethane (20 ml), iodoethane (1.3 mmol) was added and the mixture was refluxed for 24 h and then allowed to cool. Remove the solvent to to yield the desired purple-red crystal compound a. Yield 60%.

Add 10 mmoL of salicylaldehyde to 10 mL of glacial acetic acid, and slowly add 9 mmoL of fuming nitric acid within 30 min at 15 °C. After the addition is complete, react at 45 °C for 1.5 h. The system was poured into 20 mL of water, and the pH was adjusted to neutral with NaOH solution, and a primrose yellow solid was precipitated and filtered off, yield the desired compound b. Yield 83%.

**The synthesis of c** To compound a (5 mmol) and compound b (5 mmol) in hot Absolute ethanol(10 ml), triethylamine (1.3 mmol) was added and the mixture was refluxed for 2 h and then allowed to cool. Remove the solvent and column chromatography; to yield the desired Bright orange crystal compound c. Yield 74%.

**The synthesis of d** To compound c (9 mmol) in hot Absolute ethanol(10 ml), zinc powder and anhydrous calcium chloridewas added and the mixture was refluxed for13 h and then allowed to cool.Filter, remove the solvent and column chromatography;to yield the desired Earth brown crystal compound d. Yield 40%.

**The synthesis of e** Compound e was prepared by the literature methods [3,4]. To methylpiperazine (10 mmol) in hot DMSO (10 ml), 1,2-Dibromoethane, anhydrous sodium carbonate, added and the mixture was refluxed for10 h and then allowed to cool. Filter, remove the solvent and column chromatography; to yield the desired wine red compound e. Yield 56%.

**The synthesis of UV-SP** To compound d(5 mmol) in hot DMSO (10 ml), compound e, anhydrous sodium carbonate, added and the mixture was refluxed for16 h and then allowed to

cool.Filter, remove the solvent and column chromatography; to yield the desired wine red compound e. Yield 30%.

## 4. Characterization of UV-SP

$^{1}$H NMR ($CD_3OD$, 600 MHz): δ 8.92 (*J* = 8.3 Hz, 1H), 8.65 (*J* = 7.9 Hz, 1H), 8.08 (*J* = 8.6 Hz, 1H), 7.96 (4H), 7.70 (*J* = 16.2, 7.9, 5.0 Hz, 2H), 7.10 (*J* = 9.3 Hz, 1H), 4.18 – 4.09 (2H), 3.67 (2H), 3.54 – 3.43 (3H), 1.61(2H), 1.35 (*J* = 6.9 Hz, 3H), 1.26 (*J* = 19.3 Hz, 6H), 0.88-0.84 (8H).

$^{13}$C NMR (MeDO, 151 MHz): 13.62, 18.30, 39.48, 46.45, 47.15, 52.57, 55.91, 57.94, 108.60, 109.28, 114.39, 114.64, 115.70, 117.82, 121.43, 126.45, 127.15, 127.82, 137.61, 141.62, 142.04, 149.42.

ESI-MS: m/z calcd for $C_{27}H_{36}N_4O$+H: 433.2967, found: 433.2967.

## 5. The selectivity of UV-SP for pH

Besides ultra-sensitive of spectral response for pH, the specific selectivity of **UV-SP** as another crucial parameter must be evaluated. To evaluate the selectivity of **UV-SP** for pH under light-activation, we investigated the fluorescence spectra of **UV-SP** (5.0 μM) in response to relevant interfering species including 11 kind ions and 18 kind bioactive small molecules. As shown in **Figure S1a** and **Figure S1b**, no interfering effects were observed in present of those interfering species. The probe appears the spectral change like Figure 1b and 1c must under the synergistic effect of protonation-deprotonation. However, all the interfering species did not offered the environment for the protonation-deprotonation. So, there are no any interference in **Figure S1a** and **Figure S1b**.

The photostability of **UV-SP** was one of the evaluation parameters which were evaluated by residual fluorescence intensity. **Figure S1c** shown the fluorescence intensity of **UV-SP** in the water solution had no obvious change after it was continuously irradiated by an iodine-tungsten lamp (500 w)

for 7.0 h. Even if it was continuously irradiated by a laser at 488 (the original optical power is 4.0 mW), its fluorescence intensity maintains excellent stability for 1000 s (**Figure S1d**). These results showed that **UV-SP** revealed specific selectivity and strong photostability.

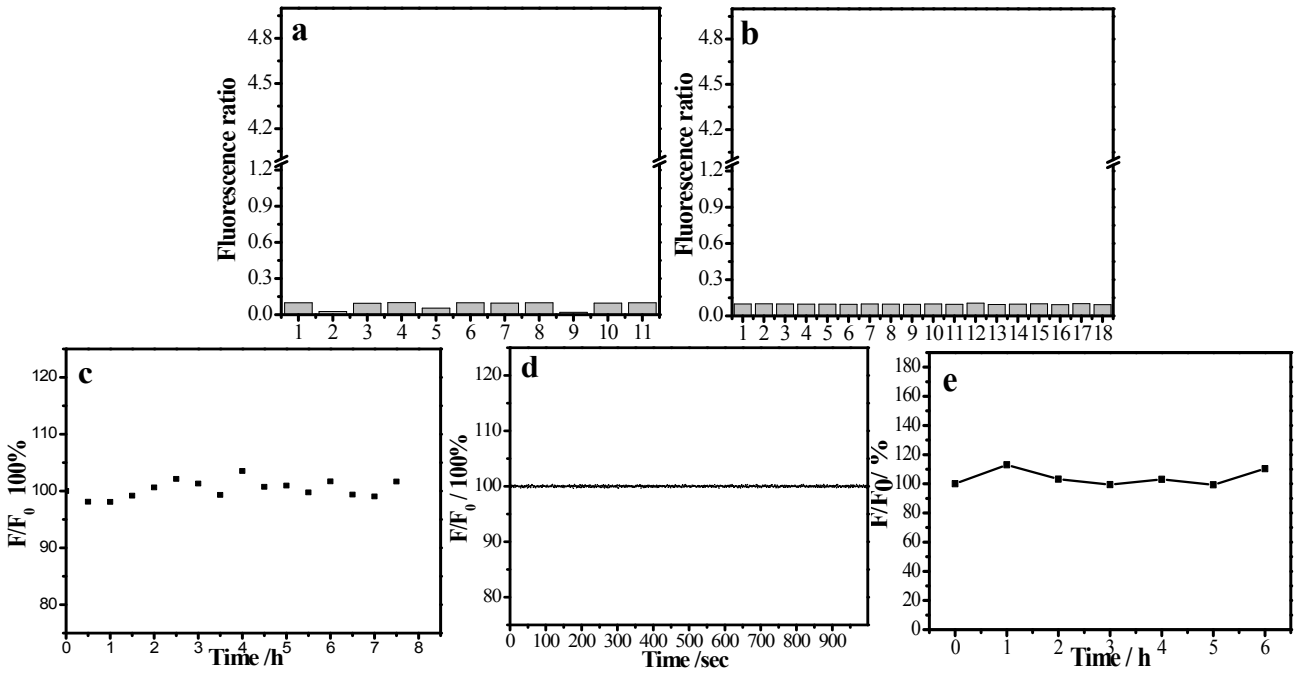



**Figure S1**. Specific selectivity and strong photostability of **UV-SP** (5.0 μM). a. Specific selectivity under the interference of 11 kind ions after photoactivation. **UV-SP** (5.0 μM) under irradiation by UV-light (375 nm) forms **UV-HCY**. The ion concentrations: 1,$Ca^{2+}$ (3.0 mM); 2, $Li^{+}$ (0.1 mM); 3, $Ni^{2+}$ (0.1 mM); 4, $Mg^{2+}$ (3.0 mM); 5, $Cu^{2+}$ (0.1 mM); 6, $Na^{+}$ (3.0 mM);7, $Na^{+}$ (3.0 mM); 8, $K^{+}$(3.0 mM); 9, $Ag^{+}$ (0.1 mM); 10, $Hg^{2+}$ (0.1 mM); 11, $Zn^{2+}$ (0.1 mM) in PBS buffer (pH = 7.4). Excitation wavelength = 487 nm, fluorescence emission wavelength = 595 nm. b. Specific selectivity under the interference of 18 kind bioactive small molecules after photoactivation. **UV-SP** (5.0 μM) under irradiation by UV-light (375 nm) forms **UV-HCY**. The concentration of all the bioactive small molecules is 200 μM in PBS buffer (pH = 7.4). 1, Glycidyl-DL-phenylalanine; 2, lysine; 3, DL-Threonine; 4, glutamine; 5, cysteine; 6, arginine; 7, Glycidyl-DL-tyrosine; 8, methionine; 9, (S)-2-Amino-3-mercaptopropionic acid; 10, glycine; 11, DL-Leucine; 12, serine; 13, homocysteine; 14,DTT; 15, glutamic acid; 16, hypoxanthine; 17, L-aspartate; 18, Valine. Excitation wavelength = 487 nm, fluorescence emission wavelength = 595 nm. c. The photostability of **UV-SP** (5.0 μM) under an iodine-tungsten lamp (500 w) for 7.0 h in DMSO-water (5:5 v/v). Excitation wavelength = 487 nm, fluorescence emission wavelength = 595 nm. d. The photostability of **UV-SP** (5.0 μM) under a laser at 488 (the original optical power is 4.0 mW) for 1000 s. Excitation wavelength = 487 nm, fluorescence emission wavelength = 595 nm.e. The photostability of **UV-SP** (5.0 μM) under an iodine-tungsten lamp (500 w) for 6.0 h in PBS buffer (pH = 7.4). Excitation wavelength = 487 nm, fluorescence emission wavelength = 595 nm.

## 6. The intracellular localization of UV-SP

Figure S2a shown that pka of **UV-SP** is 7.46 because it contains other groups, such as imide and phenol hydroxyl. Therefore, probe molecules cannot be specifically localized in lysosomes. Furthermore, the colocalization imaging (Figure S2b) further verified that the probe cannot localize in lysosomes. The Pearson coefficient is only 0.017.

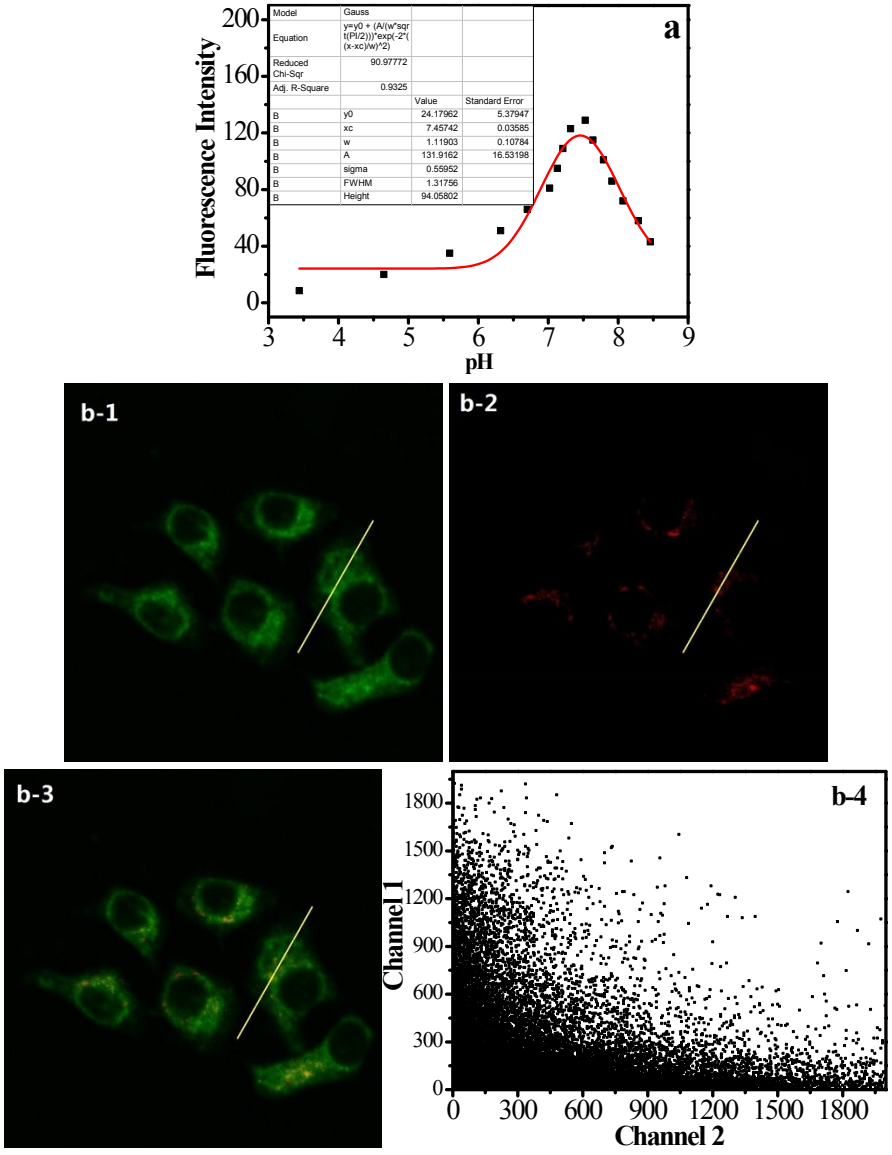
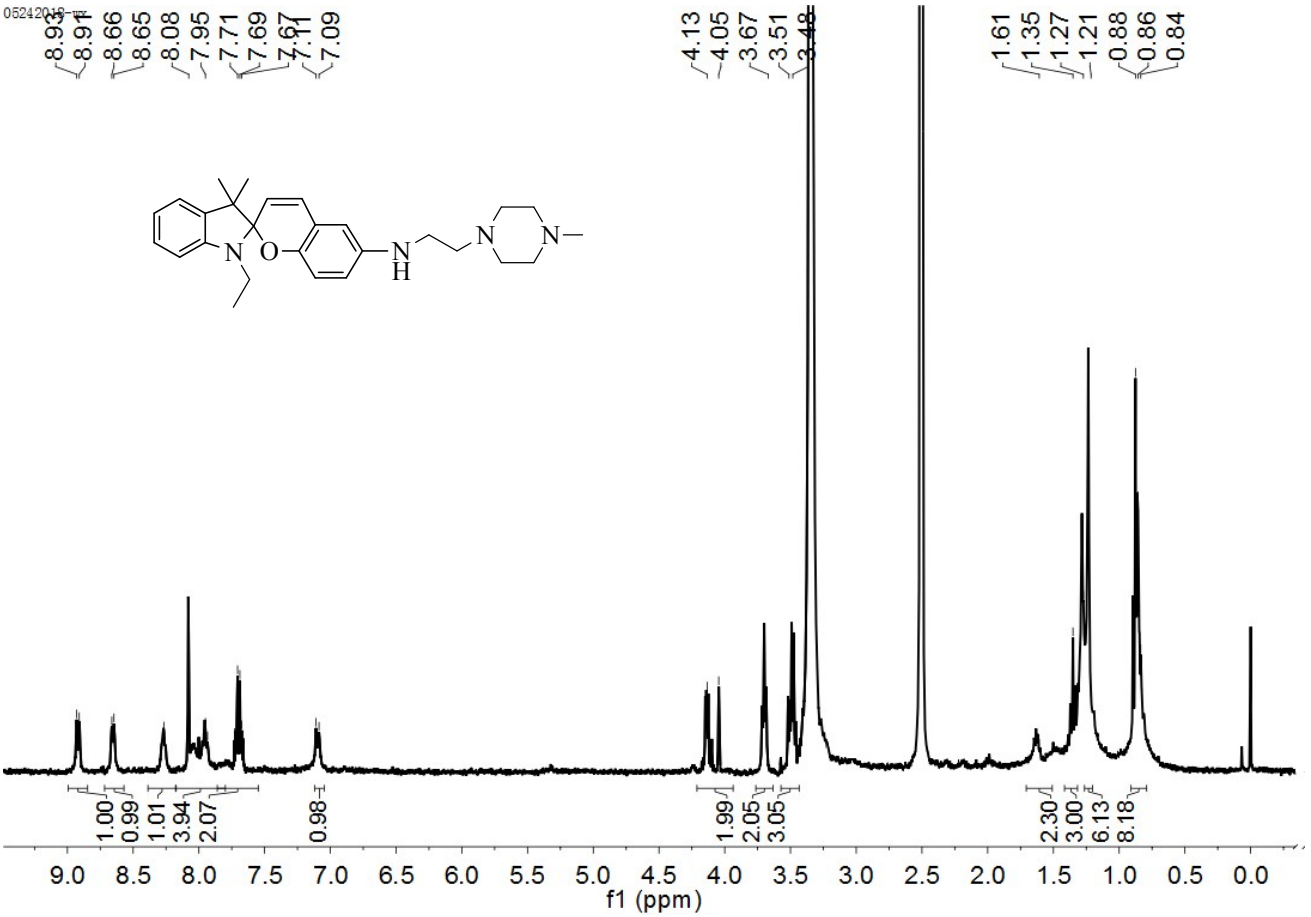



**Figure S2**. a. Fluorescence intensity of **UV-SP** at 595 nm changes with pH changes. b. The intracellular localization of **UV-SP** (5.0 μM) with Lyso-Tracker Red (1.0 μM). b-1. The green channel (emission wavelength = 550-570 nm) was obtained by excited at 488 nm after photoactivation by 375 nm. b-2. The red channel (emission wavelength = 570-620nm) were obtained by excited at 559 nm. b-3. The overlay channel. b-4. Colocation analysis.

## Spectrum of $^{1}H$ NMR



## Spectrum of $^{13}C$ NMR