A novel ratiometric fluorescent probe based on thienocoumarin and its application of selective detection of hypochlorite in real water samples and in vivo

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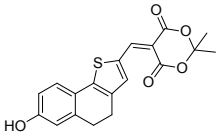
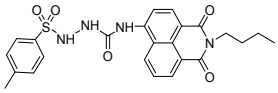
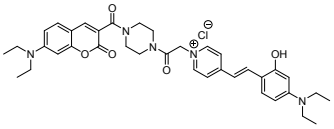
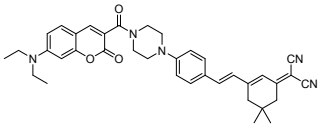
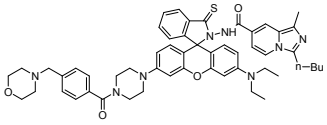
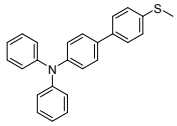
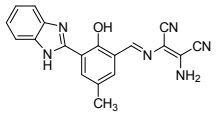
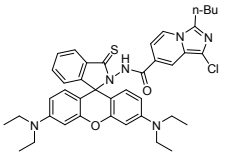
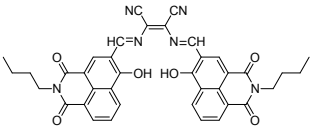
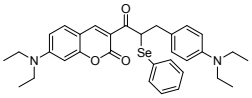
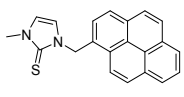
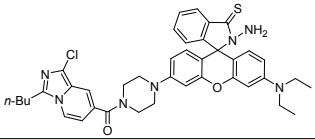
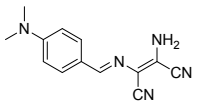
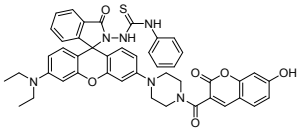
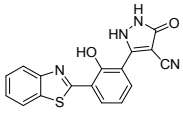
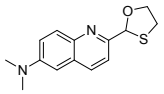
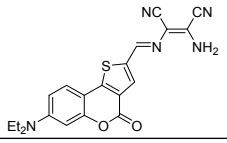
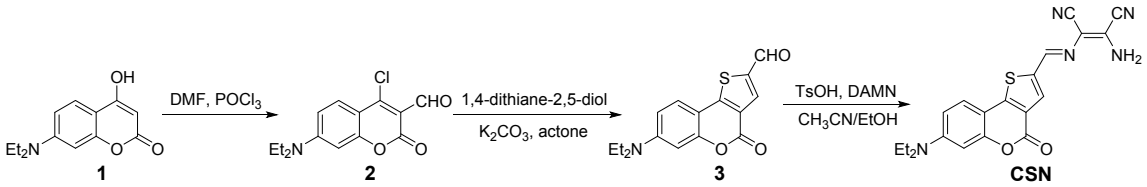
Table S1. The development of ratiometric fluorescent probes for the detection of HClO/ClO^- in recent two years.

The structures of probes	λ_{ex}, λ_{em} (nm)	Emision shifts	Detection limit	Response Time	Water detection	Reference
	440, 555/635	80 nm	78 nM	1 s	No	[1]
	412, 488/542	54 nm	61 nM	2 min	Yes	[2]
	420, 478/610	132 nm	25 nM	13 s	No	[3]
	410, 470/672	202 nm	73 nM	1 min	No	[4]
	370, 462/589	127 nm	10.2 nM	40 s	No	[5]
	334, 437/497	60 nm	16 nM	40 s	No	[6]
	340, 485/600	115 nm	14.6 nM	30 s	No	[7]
	400, 462/587	125 nm	2.08 μM	30 s	No	[8]
	425/585, 515/640	125 nm	26 nM	7 min	No	[9]
	475, 495/618	123 nm	4.6 nM	5 s	No	[10]
	355, 378/482	104 nm	200 nM	2 min	No	[11]
	370, 465/585	120 nm	24 nM	20 s	No	[12]

	485, 521/635	114 nm	0.5 nM	30 s	No	[13]
	420/575, 460/585	125 nm	42 nM	5 s	No	[14]
	400, 468/600	132 nm	7.8 nM	60 s	No	[15]
	360, 492/562	70 nm	89 nM	60 s	No	[16]
	413, 470/640	170 nm	94 nM	60 s	Yes	This work



Scheme S1. The synthetic route and structure of probe **CSN**

2. Synthesis of probe CS

Reagents and instruments.

All reagents used were commercially available chemicals. For ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra, Bruker AVANCE III spectrometer was involved. For high-resolution mass spectrometry (HRMS), the ESI-Q-TOF mass spectrometer (Thermo, TSQ Quantum Ultra; Waters, SYNAPT G2-Si) was employed. For the UV–Vis and fluorescent spectra, the Hitachi U-4100 spectrophotometer and Edinburgh F35 luminescence spectrophotometer were used.

Synthesis of Compound 2[17]

Compound **1** was synthesized according to the previous literature.[17]

Under the nitrogen atmosphere, $POCl_3$ (2.8 mL) was added dropwise to the fresh distilled DMF (2.8 mL) at room temperature and stirred for 30 minutes to yield a pink suspension. Then a solution of compound **1** (2.33 g, 10 mmol) in 13 mL DMF was added dropwise to the above suspension. The resulting mixture was stirred at 60 ºC overnight. Subsequently cooling to room temperature, the reaction mixture was poured into the ice water and adjusted to neutral with the aqueous solution of sodium hydroxide (5 mol/L). The yellow precipitation occurred was filtered, washed with water and dried. Then the crude product of compound **2** was used in the

next step without further purifications (1.20 g, 88.2% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.33 (s, 1H), 7.87 (d, J = 9.2 Hz, 1H), 6.72 (dd, J = 9.6 Hz & J = 2.0 Hz, 1H), 6.47 (d, J = 2.4 Hz, 1H), 3.51 (q, J = 7.2 Hz, 4H), 1.29 (t, J = 7.2 Hz, 6H).

Synthesis of Compound 3

K_2CO_3 (830 mg, 6.0 mmol) and 1,4-dithiane-2,5-diols (183 mg, 1.20 mmol) were added to the solution of compound **2** (560 mg, 2.0 mmol) in acetone (40 mL). Then the mixture was stirred at room temperature for 1 h and 45 ^{o}C for another 3 h. Subsequently cooling in the refrigerator (0 ^{o}C), a yellow precipitate was collected by filter and washed with cold ethanol (10 mL × 4 times). After that, the obtained solids was re-dissolved by CH_2Cl_2 and filtered to remove the insoluble substance. The filtrate was concentrated under reduced pressure to give the compound **3** as the yellow solids (494 mg, 82% yield).

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.96 (s, 1H), 8.42 (s, 1H), 7.72 (d, J = 8.8 Hz, 2H), 6.79 (dd, J = 8.80 Hz & J = 2.0 Hz, 1H), 6.64 (d, J = 2.0 Hz, 1H), 3.47 (q, J = 6.8 Hz, 4H), 1.15 (t, J = 6.8 Hz, 6H).^{13}C NMR (150 MHz, DMSO-d_6) δ 184.0, 156.51, 154.96, 154.28, 151.15, 139.16, 137.27, 125.94, 120.60, 109.70, 103.79, 97.00, 44.13, 12.25. MS-ESI ($C_{16}H_{15}NO_3S$): calcd. for $[M+H]^+$ 301.09; found: 302.1.

Synthesis of probe CSN

The diaminomaleonitrile (DAMN, 359 mg, 3.3 mmol) and *p*-toluene sulfonic acid (TsOH, 8 mg, 0.05 mmol) were dissolved in ethanol (20 mL). Then a solution of compound **3** (100 mg, 0.33 mmol) in acetonitrile (40 mL) was added dropwise and the resulting mixture was stirred at room temperature for 1 h. Subsequently, the reaction mixture was concentrated and recrystallized with absolute ethanol. The red solids were collected by filtration and then purified by column chromatography (CH_2Cl_2 : ethyl acetate = 20 : 1) to give probe **CSN** as the red solid (72 mg, 55% yield).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.49 (s, 1H), 8.09 (s, 1H), 7.88 (bs, 2H), 7.52 (d, J = 8.8 Hz, 1H), 6.80 (d, J = 9.2 Hz, 1H), 6.63 (s, 1H), 3.46 (q, J = 6.8 Hz, 4H), 1.15 (t, J = 6.8 Hz, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 156.90, 154.29, 153.05, 150.89, 148.52, 138.91, 132.31, 126.80, 125.43, 121.38, 114.82, 114.16, 110.13, 104.76, 103.04, 97.58, 44.61, 12.84. HRMS-ESI ($C_{20}H_{16}O_2N_5S$): calcd. for $[M-H]^-$ 390.10302; found: 390.1038.

Synthesis of Compound 4

The probe **CSN** (40 mg, 1.0 mmol) was dissolved in methanol (40 mL) and stirred at room temperature. Then, a solution of NaClO (13.4 mM, 0.6 mL; 10.7 mmol) was added dropwise until the red fluorescence of the resulting solution disappeared (about 10 min). Subsequently, the mixture was concentrated, and the crude product was purified by column chromatography (CH_2Cl_2 : EtOH = 20 : 1) to give compound **4** as the white solid (14 mg, 36% yield).

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.07 (bs, 1H), 7.60 (d, J = 8.8 Hz, 1H), 7.57 (s, 1H), 6.75 (d, J = 9.2 Hz, 1H), 6.62 (s, 1H), 3.44 (q, J = 6.8 Hz, 4H), 1.15 (t, J = 6.8 Hz, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 156.89, 153.23, 152.43, 149.49, 148.25, 136.53, 124, 57, 120.89, 119.64, 118.91, 115.88, 109.35, 104.77, 97.26, 44.03, 12.33. HRMS-ESI ($C_{20}H_{14}O_2N_5S$): calcd. for $[M-H]^-$ 388.08737; found: 388.08827.

3. General procedures for spectra measurements

A stock solution of probe **CSN** (1.0 mM) was prepared in DMF and stored at 0 °C in the dark. Stock solutions (200 μM) of various testing species, including NaF, ZnAcO, $PbNO_3$, $MnSO_4$, $CaCl_2$, $FeCl_3$ and $Al_2(SO_4)_3$, were prepared in twice-distilled water. Various reactive oxygen species (ROS) and reactive nitrogen species (RNS), including NO, ·HO, t-BuOOH, H_2O_2, $ONOO^-$ and NaClO, were prepared according to the previous reports.[18-20]

A typical test solution (10.0 mL) of probe **CSN** was prepared by dilution of its stock solution (100 μL) with PBS buffer solution (10 mM) containing CTAB (1.5 mM) to afford the desired concentration of 10 μM. Then, each analyte of corresponding concentrations was added and shaken well for 1 min. All the measurements of fluorescence and absorbance spectra were conducted at room temperature. For the fluorescence study, the samples were excited at 413 nm and the fluorescence emission ranged from 420 nm to 800 nm. The excitation and emission slit widths were set as 1.0 and 2.2 nm, respectively.

In addition, the absolute fluorescence quantum yield (Φ) values of probe **CSN** were determined by the integrating sphere according to the previous literature.[21]

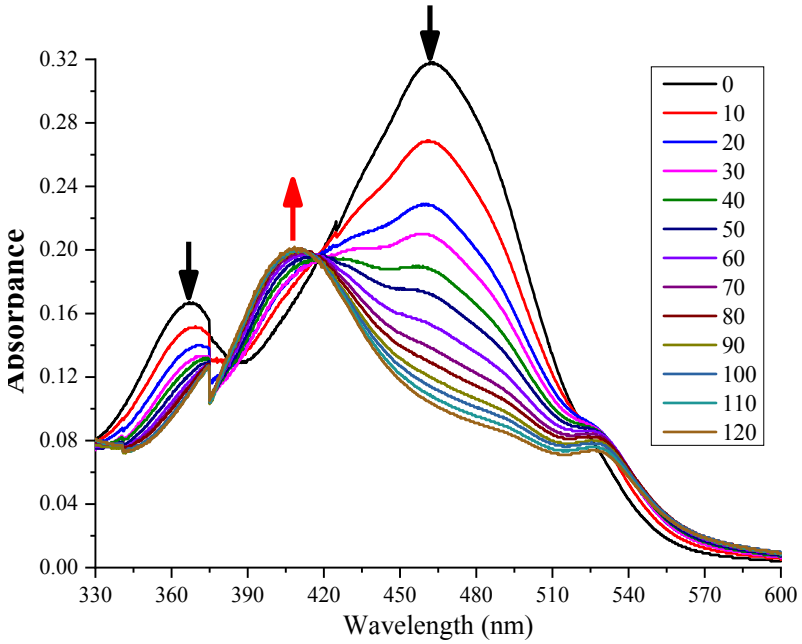


Fig. S1. The absorption spectra of **CSN** (20 μM) toward varying concentrations of NaClO (0-10 eq) in PBS buffer (20 mM, pH = 7.4) containing CTAB (1.5 mM). Slit widths: 2.0 nm / 5.0 nm.

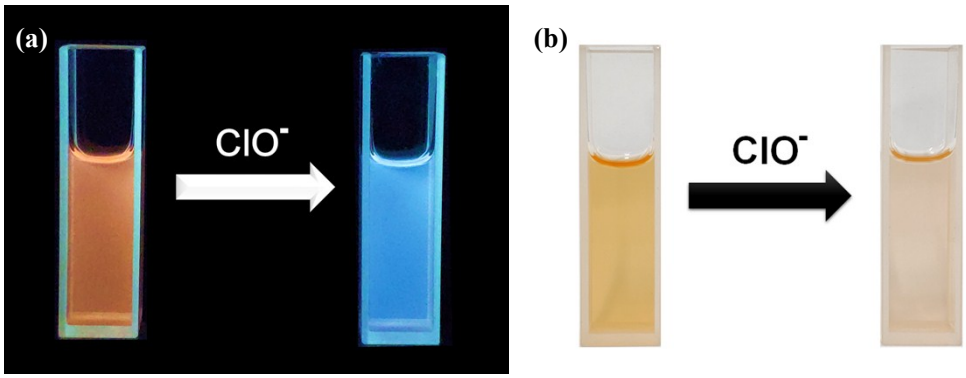


Fig. S2. Under the irradiation light of 365 nm (a) and sunlight (b), the photographs of probe **CSN** (20 μM) with or without addition of NaClO (10 eq) in PBS buffer (20 mM, pH = 7.4) containing CTAB (1.5 mM).

Fig. S3. Under the irradiation light of 365 nm (a) and sunlight (b), the photographs of probe **CSN** (10 μM) with the addition of NaClO (0~11 eq, left to right) in PBS buffer (20 mM, pH = 7.4, containing 1.5 mM CTAB).

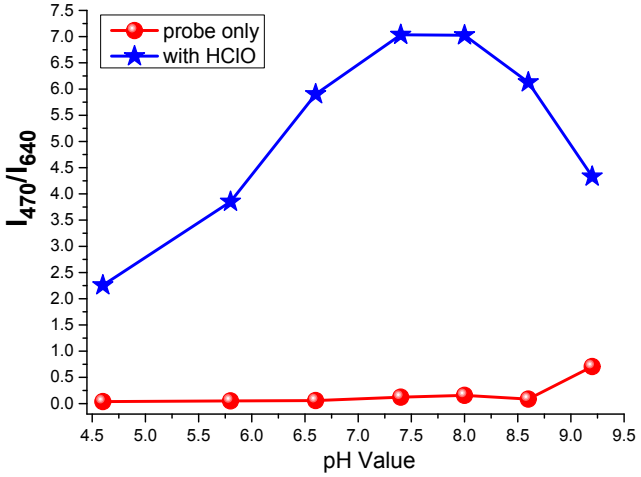


Fig. S4. The pH effects on the fluorescence intensity ratio (I_{470}/I_{640}) of probe **CSN** (10 μM) in the absence/presence of NaClO (100 μM).

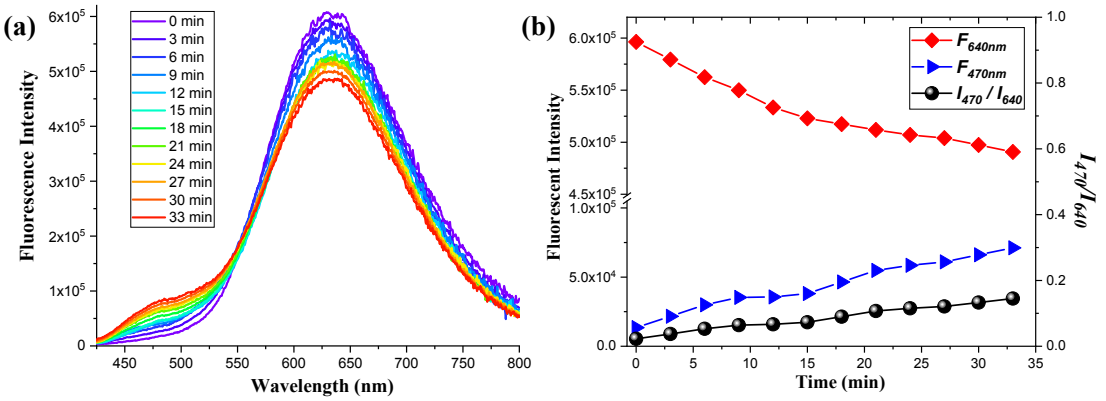


Fig. S5. (a) The fluorescence spectra of probe **CSN** (10 mM) in PBS buffer (10 mM, pH = 7.4, containing 1.5 mM CTAB) under the irradiation of 150 W Xe lamp; (b) the relationship of the fluorescence intensity and the fluorescence ratios (I_{470}/I_{640}) of **CSN** versus the irradiating time.

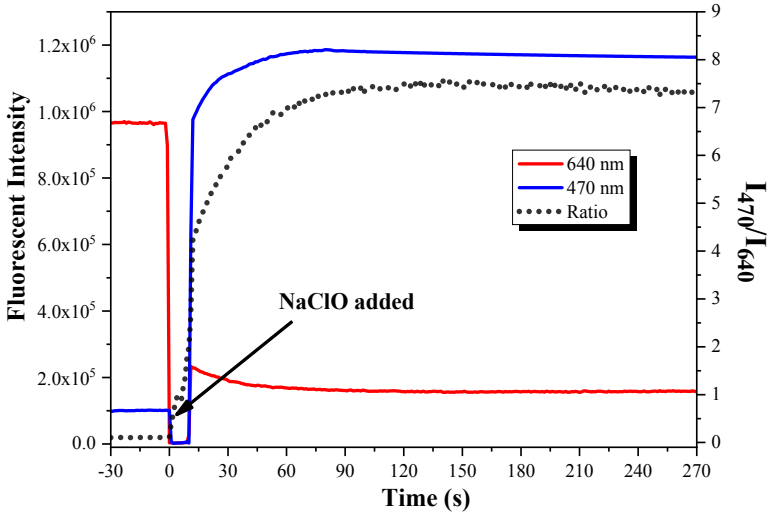
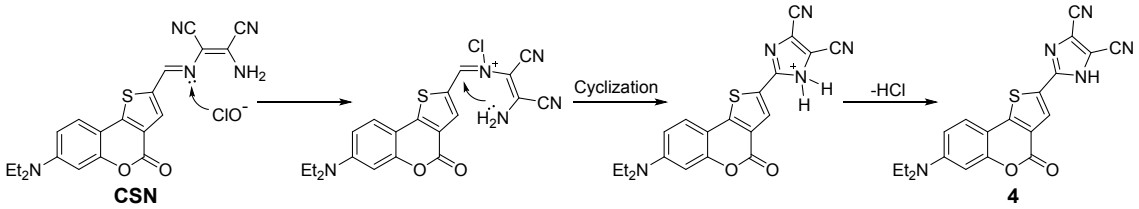


Fig. S6. The kinetic curve of probe **CNS** (10 μM) upon the addition of NaClO (100 μM) in PBS solution (20 mM, pH = 7.4) containing CTAB (1.5 mM). The kinetic studies were conducted at room temperature.



Scheme S2. The proposed sensing mechanism for probe **CSN** toward HClO/ClO^-.

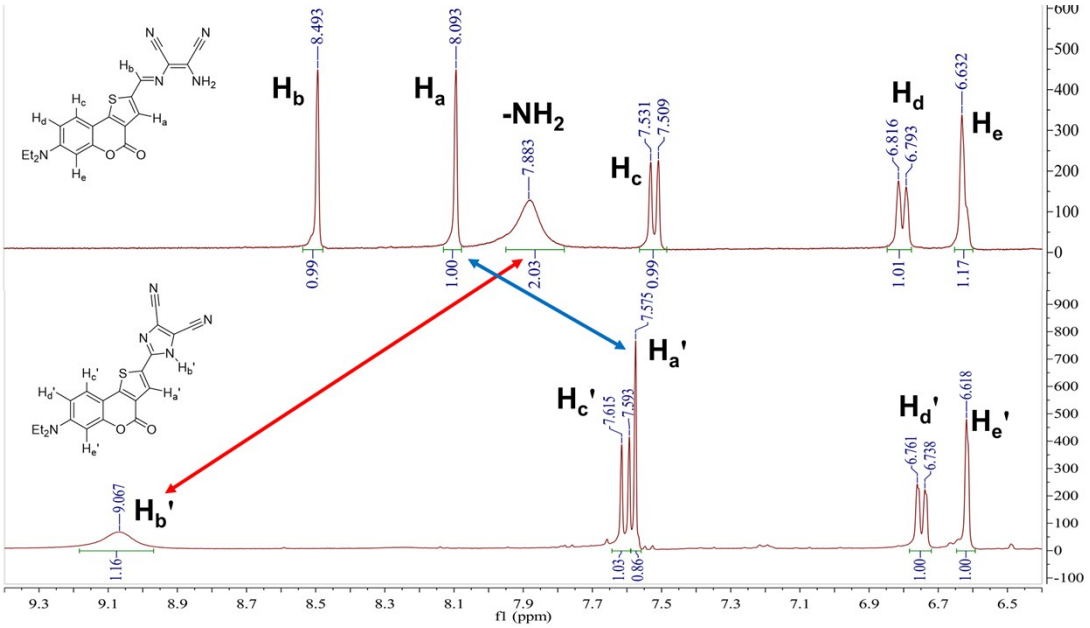


Fig. S7. The comparison of ^{1}H NMR spectra of compound **CSN** and **4** in in d_6-DMSO.

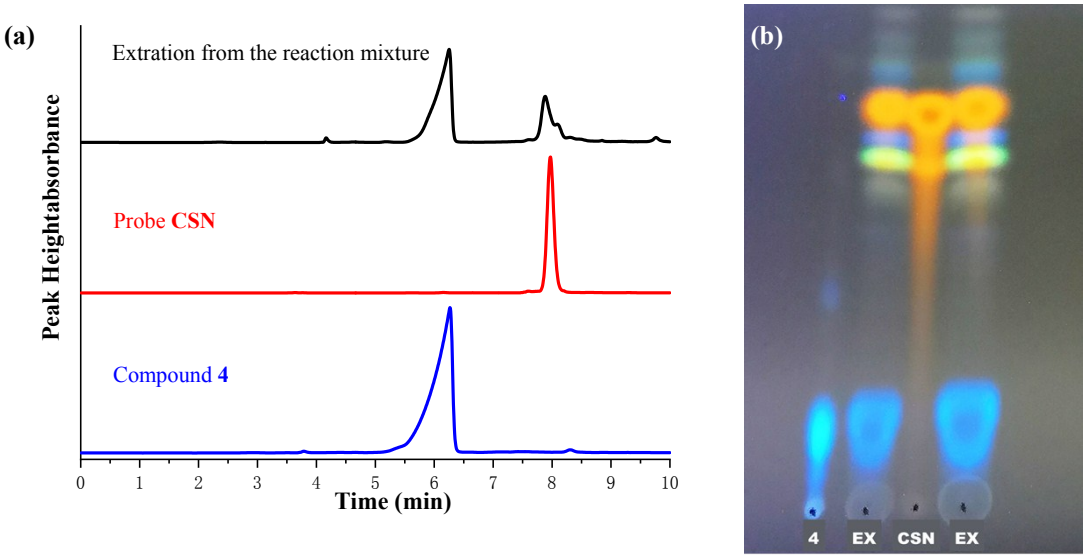


Fig. S8. (a) The HPLC spectrum of the extraction from the testing solution (Agilent 1290; mobile phase: methanol). Blackline: CH_2Cl_2 (10 mL) was used to extract the testing solution of probe **CSN** (10 μM) and NaClO (100 μM) in PBS solution (50 mL, 10 mM, pH = 7.4) containing CTAB (1.5 mM), and then the organic layer was blown away, redissolved with MeOH and analyzed by HPLC method. Redline: the solution of probe **CSN** in MeOH. Blueline: the solution of compound **4** in MeOH. (b) The photography of the thin layer silica gel plate under UV light of 365 nm. **EX**, referred to as the extraction solution from the testing solution of probe **CSN** and NaClO.

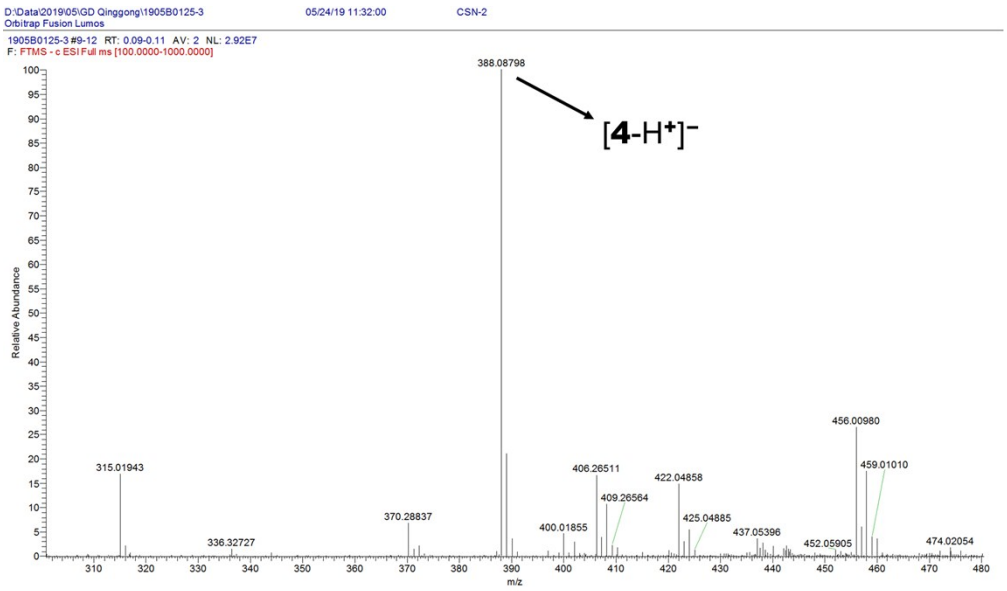


Fig. S9. The HRMS of the extraction from the testing solution. The CH_2Cl_2 (10 mL) was used to extract the testing solution of probe **CSN** (10 μM) and NaClO (100 μM) in PBS solution (50 mL, pH = 7.4) containing CTAB (1.5 mM), followed by evaporation and dissolution with MeOH, which was injected and analyzed by the mass spectrometer.

Table S2. Determination of $HClO/ClO^-$ in real water samples

Samples	Spiked (μM)	Detected (μM)	Recovery (%)
Tap water	0	1.17 ± 0.11	0
	5	6.10 ± 0.21	98.60%
	10	11.12 ± 0.34	99.50%
	20	21.58 ± 0.37	102.05%
	40	42.06 ± 0.41	102.23%
Mineral water	5	4.74 ± 0.19	94.80%
	10	9.87 ± 0.16	98.70%
	20	19.41 ± 0.41	97.05%
	40	39.42 ± 0.51	98.55%
River water	5	4.63 ± 0.22	92.60%
	10	9.23 ± 0.35	92.30%
	20	19.07 ± 0.41	95.35%
	40	38.42 ± 0.76	96.05%

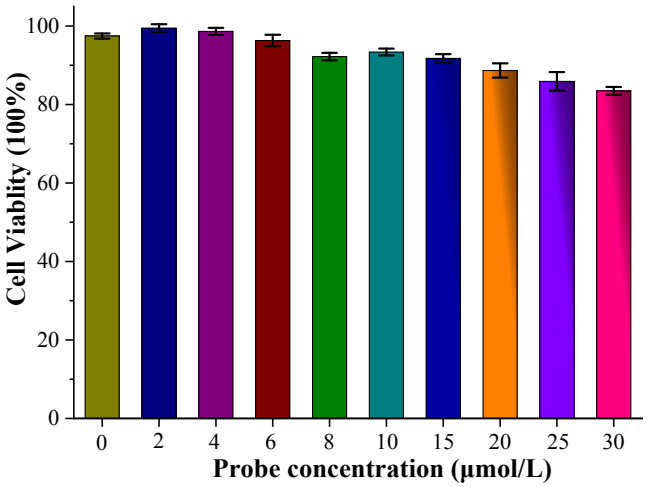


Fig. S10. The cell survival rate of probe **CSN** at the concentrations (0-40 μM). The result is the mean values ± standard deviation of five separate measurements.

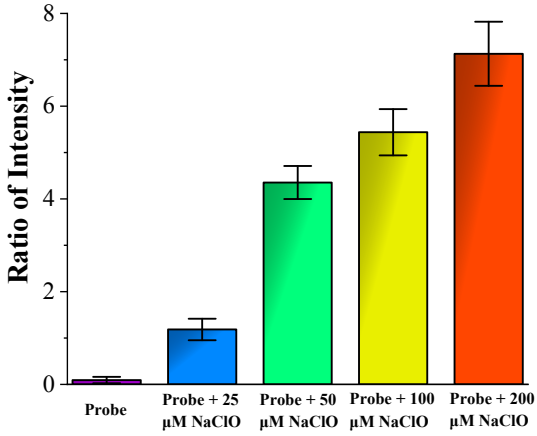


Fig. S11. The fluorescence ratio (I_{blue}/I_{red}) for the images shown in Fig. 5.

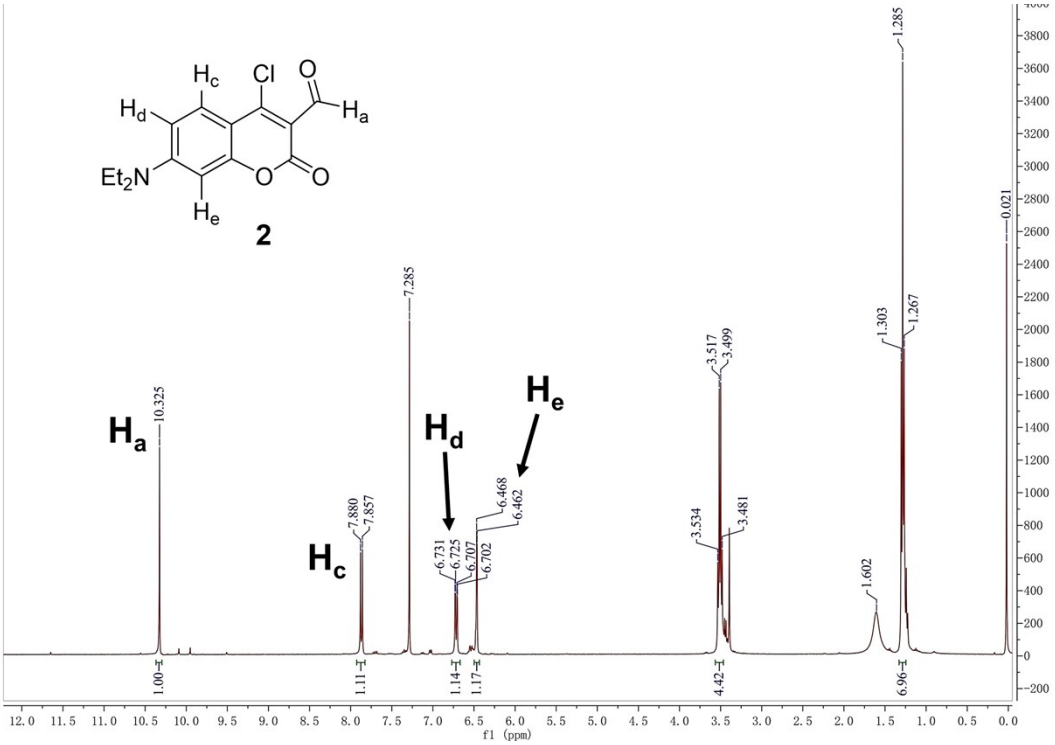


Fig. S12. ^{1}H NMR of compound **2** in $CDCl_3$.

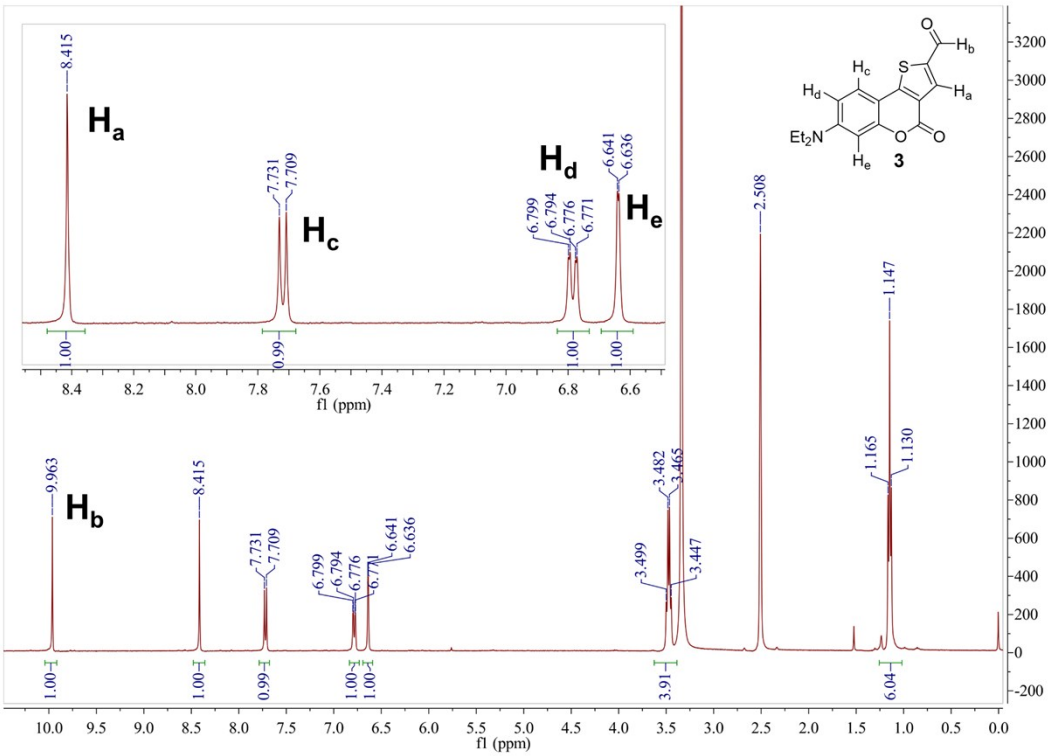


Fig. S13. ^{1}H NMR of compound **3** in d_6-DMSO.

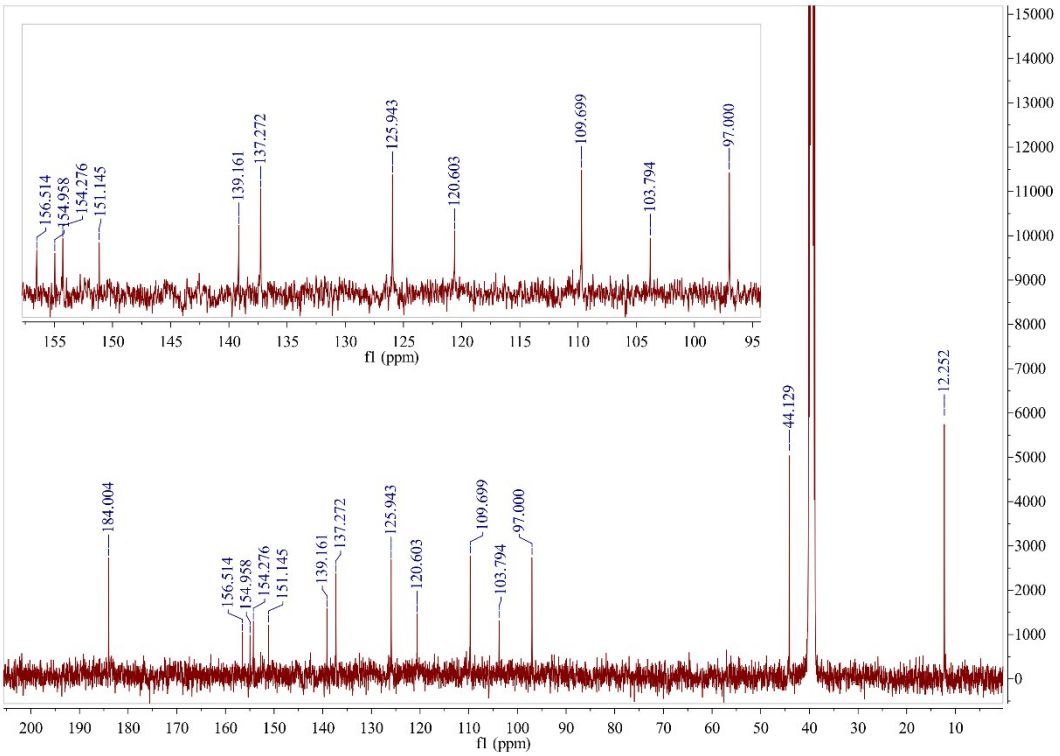


Fig. S14. ^{13}C NMR of compound **3** in d_6-DMSO.

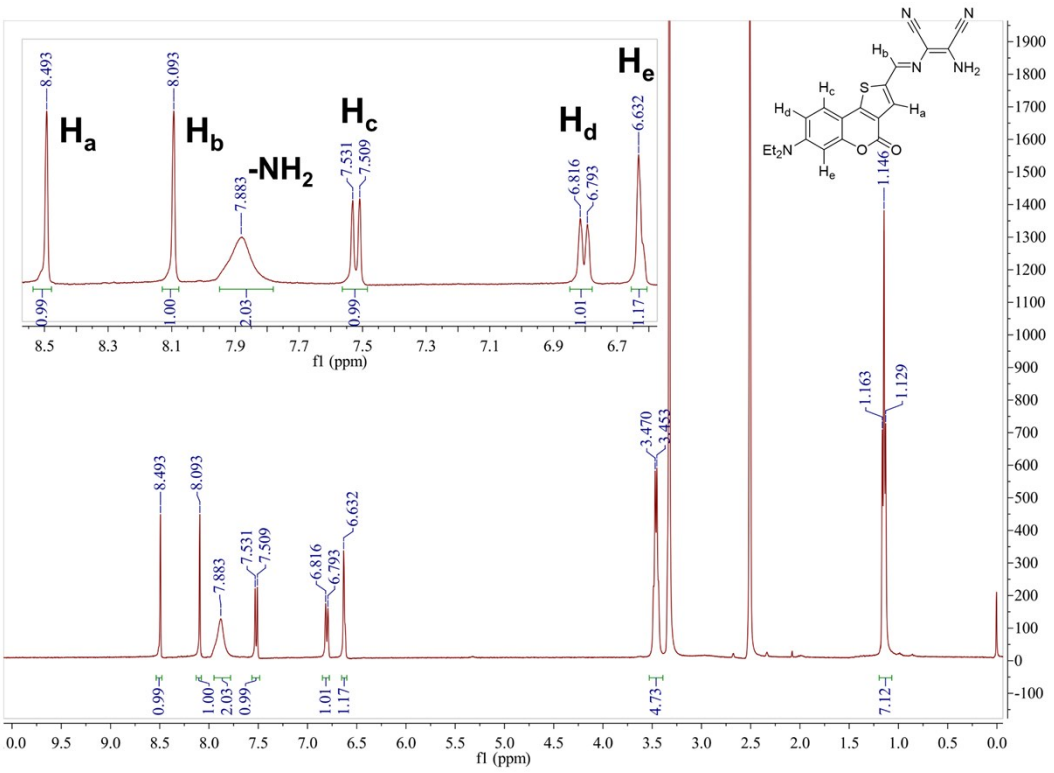


Fig. S15. ^{1}H NMR of probe **CSN** in d_6-DMSO.

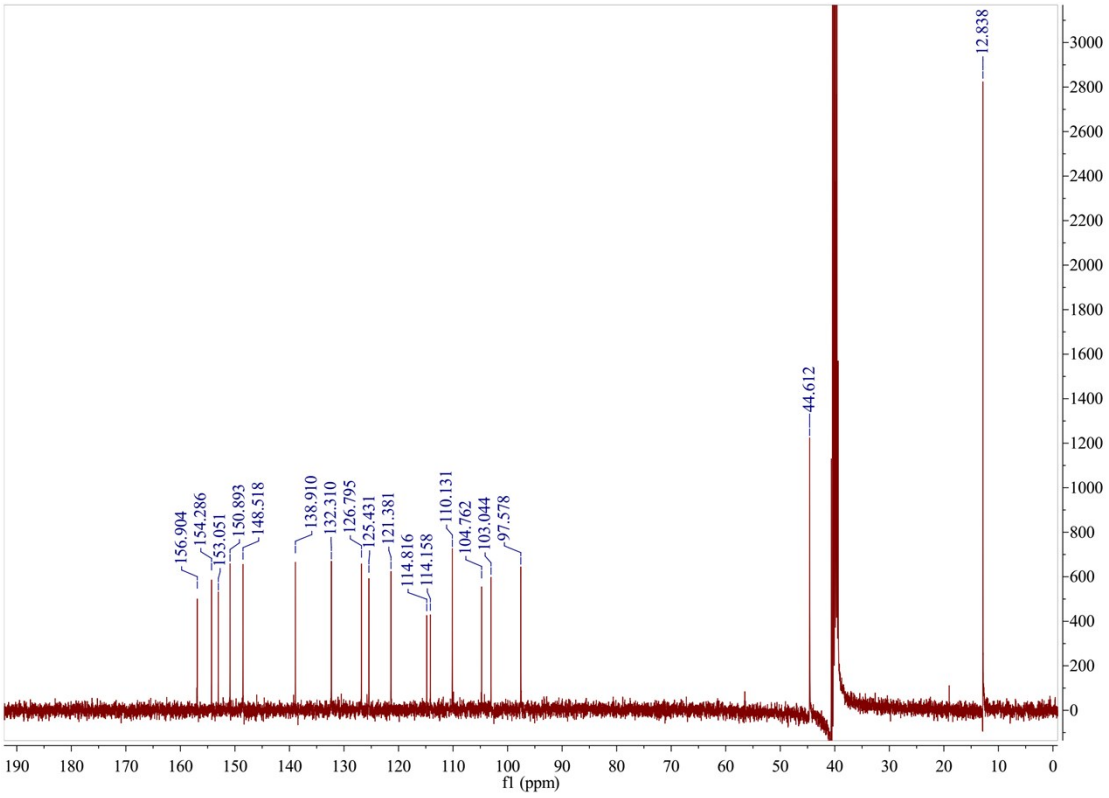


Fig. S16. ^{13}C NMR of probe **CSN** in d_6-DMSO.

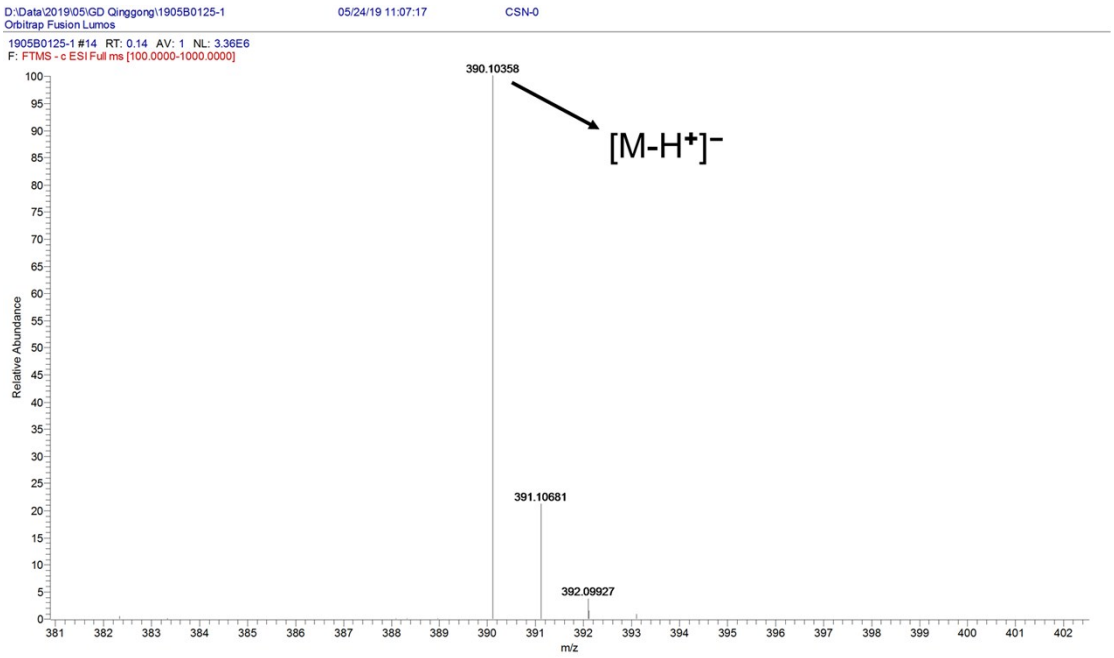


Fig. S17. HRMS(ESI) spectrum of probe **CSN**.

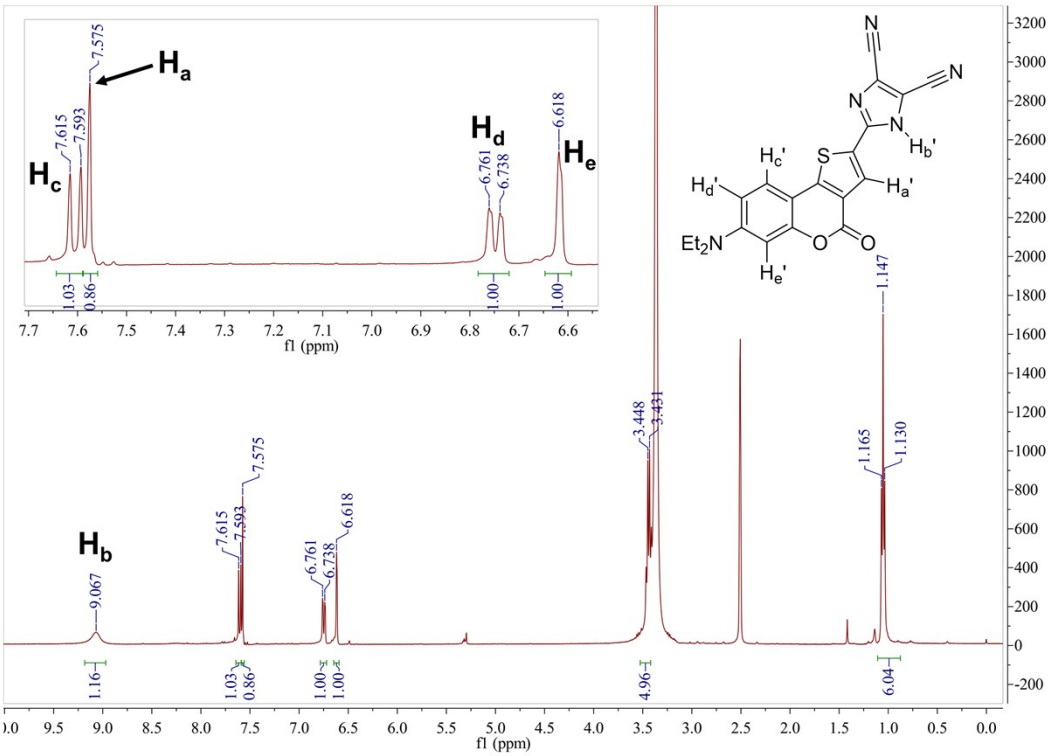


Fig. S18. ^{1}H NMR of compound **4** in d_6-DMSO.

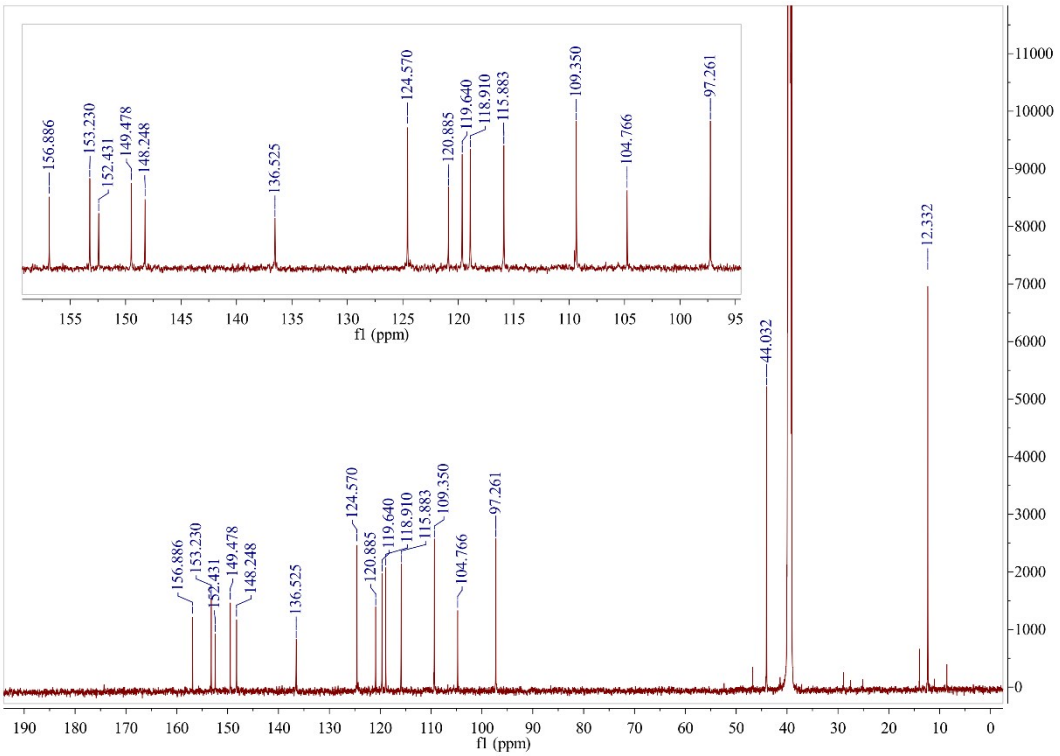


Fig. S19. ^{13}C NMR of compound **4** in d_6-DMSO.

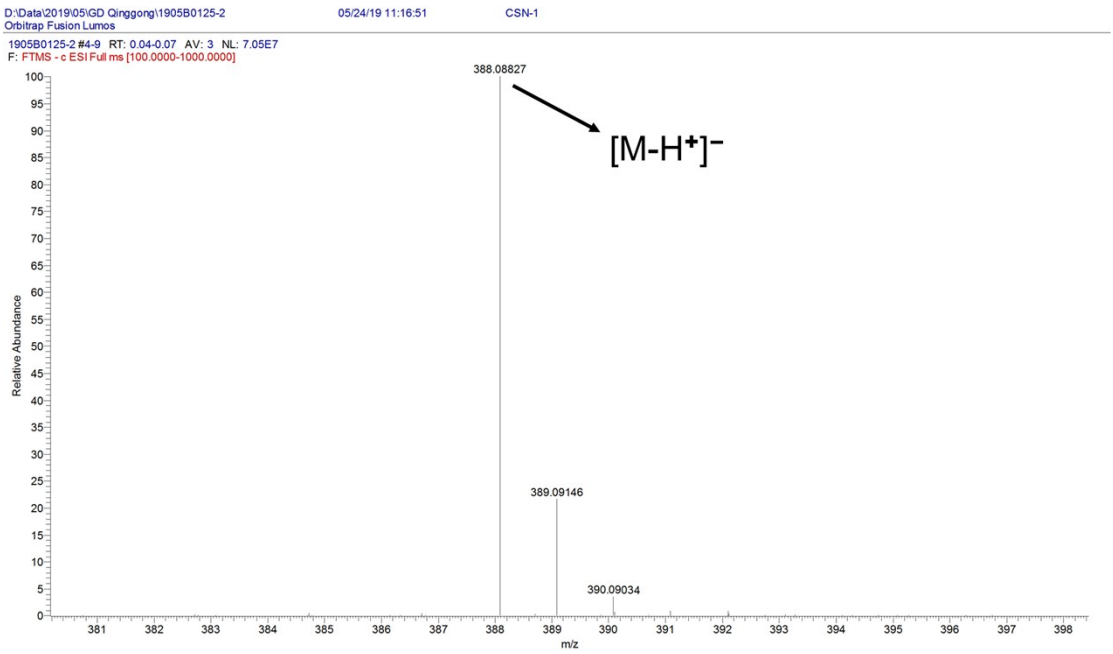


Fig. S20. HRMS(ESI) spectrum of compound **4**.

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