Evaluation of HOCl-generating anticancer agents by an ultrasensitive dual-mode fluorescent probe

Donglei Shi,[‡a] Shuqiang Chen,[‡b] Biao Dong,[‡c] Yanhui Zhang,[a] Chunquan Sheng,[b] Tony D. James,[d] and Yuan Guo*[a]

[a]*Key Laboratory of Synthetic and Natural Functional Molecule Chemistry of the Ministry of Education, National Demonstration Center for Experimental Chemistry Education, College of Chemistry and Materials Science, Northwest University, Xi'an 710127, China*

[b]*School of Pharmacy, Second Military Medical University, 325 Guohe Road, Shanghai 200433, China*

[c]*State Key Laboratory on Integrated Optoelectronics, College of Electronic Science and Engineering, Jilin University, Changchun 130012, China*

[d]*Department of Chemistry, University of Bath, Bath BA2 7AY, United Kingdom*

*To whom correspondence should be addressed.

E-mail: guoyuan@nwu.edu.cn

‡These authors contributed equally to this work.

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Materials and General Methods

All highest commercial quality reagents were purchased from commercial suppliers and used without further purification. All solvents were spectral grade unless otherwise noted. All inorganic salts used were of analytical grade. Distilled water was used throughout the experiments. The fluorescence spectra were recorded by a Hitachi F-2700 fluorescence spectrometer (Tokyo, Japan) with a 10 mm quartz cuvette and absorption spectra were determined by a Shimadzu UV-2550 spectrometer. The ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Varian Unity INOVA-400 spectrometer with tetramethylsilane (TMS) as internal standard. High-resolution mass spectra were performed with a Bruker micrOTOF-Q II mass spectrometer (Bruker Daltonics Corp, Germany) in electrospray ionization (ESI) mode. The pH measurements were carried out on a FE28-Standard pH meter. The single crystal structure was determined by X-ray crystallography with a Bruker SMART APEXCCD system. The fluorescence imaging studies of cells and *C. elegans* were observed with an Olympus FV1000 confocal microscope and Nikon A1 confocal microscope. And the fluorescent imaging of mice were recorded by IVIS Spectrum small animal *in vivo* imaging system.

X-ray crystallography details

Single-crystal X-ray experiments were performed by a Bruker Smart Apex CCD diffractometer equipped with a graphite-monochromatized Mo Kα radiation (λ = 0.71073 Å) using ω and φ scan mode at room temperature. The data integration and reduction were processed with SAINT software. The structures were solved by the direct method using SHELXS and refined by a full-matrix least-squares method on F^2 with the SHELXL-2014 program.[1,2] Hydrogen atoms were placed in geometrically calculated positions. A summary of the crystallographic data and the structure refinement parameters of **A**, **C1**-**C7** and **C7c** are given in Tables S3 and S4. The crystallographic data for **A**, **C1**-**C7** and **C7c** can be found in the CCDC with the numbers 1586906, 1571172, 1586907, 1571173, 1863813, 1863814, 1863815, 1863816 and 1863817.

Cytotoxicity assay

The cytotoxic potential of HepG 2 cells were identified by the methyl thiazolyl tetrazolium (MTT) assays with **C1** and **C3**-**C7**. 90% Confluence HepG2 cells were seeded into a 96-well cell-culture plate with density of 5000 cells per well. After incubating for 24 h, different concentrations of **C1** or **C3**-**C7** (0, 5, 10, 15, 20, 25 μM) were added to the each well, respectively. The cells were treated and incubated at 37 °C under 5% CO_2 for another 24 h. 10 mL MTT solution was added to each well and incubated for 4 h in the same conditions. The MTT solution was removed and yellow precipitates (formazan) observed in the plates were dissolved in DMSO. The absorbance was recorded at 490 nm using the microplate spectrophotometer system (Varioskan Flash). The viability of the cells was calculated by the ratio of mean of the absorbance value of the treatment group and the control group.

Absorpation and emission spectra

Probes **C1**-**C7** were dissolved in DMSO to obtain 1 mM stock solutions. These stock solutions were separately diluted with PBS buffer and HEPES buffer to a final concentration of 10 μM. Absorption spectra were obtained with a UV-2550 UV/Vis spectrometer (Shimadzu) and fluorescence spectra were obtained with an F-2700 fluorescence spectrophotometer (Hitachi).

Determination of detection limit

The detection limit was determined from the fluorescence titration data based on a reported and widely used method.[3] According to the results of the titrating experiment, the fluorescent intensity data were plotted as a concentration of HOCl. A linear regression curve was then fitted to these data, and then the detection limit was calculated using the following equation:

$$\text{Detection limit} = 3\sigma/k$$

Where σ is the standard deviation of eleven blank measurements, and k is the slope of the linear equation.

Determination of the fluorescence quantum yield

Fluorescence quantum yields were determined using solutions of rhodamine 6G (Φ_F =0.31 in water)[2] and quinine sulphate (Φ_s = 0.53 in 0.05 M H_2SO_4) as a standard.[4] The quantum yield was calculated using the following equation:

$$\Phi_{F(X)} = \Phi_{F(S)}(A_S F(X)/ A_X F(S))(nx/ns)^2$$

Where Φ_F is the fluorescence quantum yield, A is the absorbance at the excitation wavelength, F is the area under the corrected emission curve, and n is the refractive index of the solvents used. Subscripts S and X refer to the standard and to the unknown, respectively.

Table S1. Fluorescence quantum yields of probes.

	Φ_S (probe)	Φ_S (probe/HOCl)
C1	0.01	0.07
C2	0.06	0.13
C3	0.02	0.62
C4	0.13	0.51
C5	0.01	0.15
C6	0.07	0.11
C7	0.04	0.10
C7[a]	0.03	0.15

[a]The fluorescence quantum yields of probe **C7** were determined using quinine sulphate (Φ_s = 0.53 in 0.05 M H_2SO_4) as a standard at micromolar level of HOCl.

Spectrophotometric measurements

The stock solutions of probes (1.0 mM) were prepared in DMSO. Stock solutions (10 mM) of various analytes were prepared by dissolving an appropriate amount of testing species in water. Other typical ROS, reactive nitrogen RNS, representative anions and amino acids were prepared according to the previous reports.[5] All UV/vis and fluorescence experiments were performed for at room temperature. And any changes in the fluorescence intensity were monitored using a fluorescence spectrometer.

Cell culture and fluorescence imaging

HepG2 and A549 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, penicillin (100 μg/mL), and streptomycin (100 μg/mL) at 37 °C in a humidified incubator, and culture media were replaced with fresh media every day. The cells were further incubated with diverse concentration of probes in culture media at 37 °C and then washed 3 times with PBS buffer before cell fluorescence imaging experiments with confocal laser scanning microscopy.

***C. elegans* strain and culture conditions**

Young gravid worms were placed on a NGM agar plate seeded with OP50 (*Escherichia coli)* at 20 °C and allowed to lay eggs for 4 h. Then these synchronized eggs were cultured under standard conditions until the worms reached the young adult, then picked immediately prior to the next experiment.

Spectroscopic Properties of C1-C7

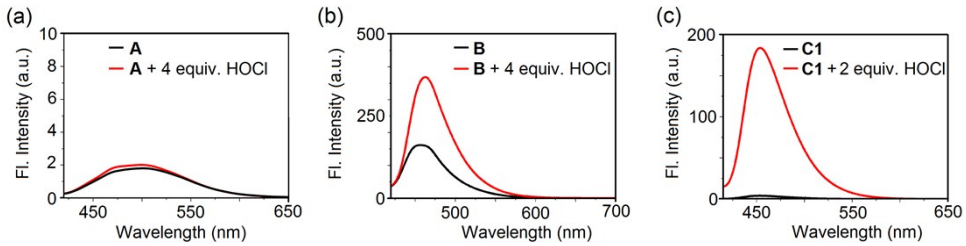


Fig. S1 (a) Changes in fluorescence emission spectra of **A** treated with 4 equiv. HOCl in PBS buffer (10 mM, pH = 7.4); (b) Changes in fluorescence emission spectra of **B** treated with 4 equiv. HOCl in PBS buffer (10 mM, pH = 7.4); (c) Changes in fluorescence emission spectra of **C** treated with 2 equiv. HOCl in PBS buffer (10 mM, pH = 7.4).

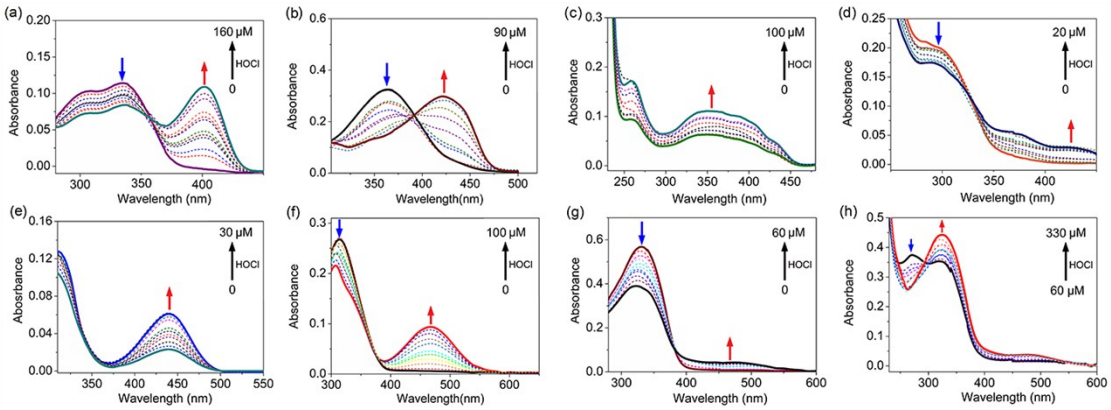


Fig. S2 (a) UV-vis absorption spectra of **C1** (10 μM) as the titration of HOCl from 0 to 160 μM in PBS buffer (10 mM, pH = 7.4); (b) UV-vis absorption spectra of **C2** (10 μM) as the titration of HOCl from 0 to 90 μM in PBS buffer (10 mM, pH = 7.4); (c) UV-vis absorption spectra of **C3** (10 μM) as the titration of HOCl from 0 to 100 μM in PBS buffer (10 mM, pH = 7.4); (d) UV-vis absorption spectra of **C4** (10 μM) as the titration of HOCl from 0 to 20 μM in PBS buffer (10 mM, pH = 7.4); (e) UV-vis absorption spectra of **C5** (10 μM) as the titration of HOCl from 0 to 30 μM in PBS buffer (10 mM, pH = 7.4); (f) UV-vis absorption spectra of **C6** (10 μM) as the titration of HOCl from 0 to 100 μM in PBS buffer (10 mM, pH = 7.4); (g) UV-vis absorption spectra of **C7** (10 μM) in HEPES buffer (10 mM, pH = 7.4) in the presence of low concentration range of HOCl (0-60 μM) and (h) in the presence of high concentration range of HOCl (60-330 μM).

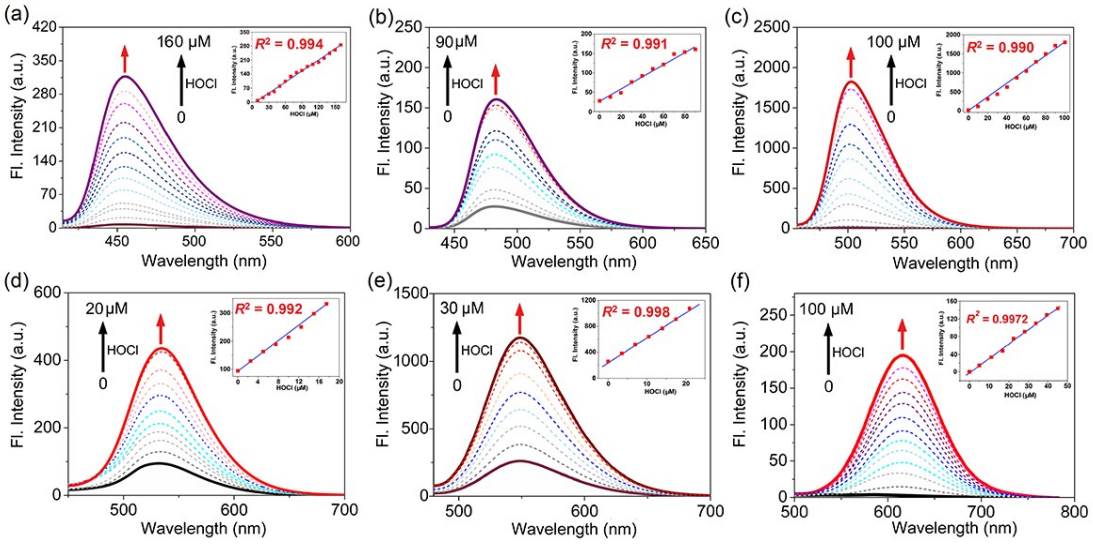


Fig. S3 Changes in fluorescence emission spectra of probes treated with HOCl. (a) Dose-dependent emission spectra of **C1** (10 μM) toward HOCl (0-160 μM). Inset: Plots of fluorescence intensity of **C1** *vs.* HOCl concentrations; (b) Dose-dependent emission spectra of **C2** (10 μM) toward HOCl (0-90 μM). Inset: Plots of fluorescence intensity of **C2** *vs.* HOCl concentrations; (c) Dose-dependent emission spectra of **C3** (10 μM) toward HOCl (0-100 μM). Inset: Plots of fluorescence intensity of **C3** *vs.* HOCl concentrations; (d) Dose-dependent emission spectra of **C4** (10 μM) toward HOCl (0-20 μM). Inset: Plots of fluorescence intensity of **C4** *vs.* HOCl concentrations; (e) Dose-dependent emission spectra of **C5** (10 μM) toward HOCl (0-30 μM). Inset: Plots of fluorescence intensity of **C5** *vs.* HOCl concentrations; (f) Dose-dependent emission spectra of **C6** (10 μM) toward HOCl (0-100 μM). Inset: Plots of fluorescence intensity of **C6** *vs.* HOCl concentrations.

Table S2. The fluorescence changes of probes before and after the addition of HOCl.

Probes	λ_{ex} (nm)	Fold increase
C1	420	41
C2	400	6
C3	420	223
C4	440	8
C5	435	5
C6	475	74
C7	480	20
C7[a]	400	48

[a]The fluorescence ratio of probe **C7** (I_{468}/I_{630}) exhibit a 48-fold change.

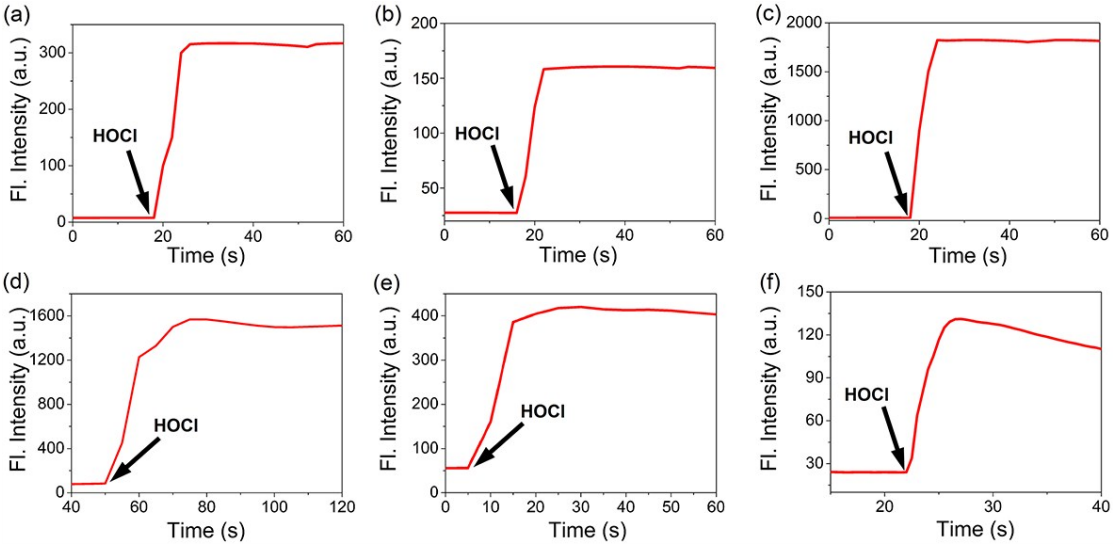


Fig. S4 (a) Time course of fluorescence intensity of **C1** after addition of HOCl; (b) Time course of fluorescence intensity of **C2** after addition of HOCl; (c) Time course of fluorescence intensity of **C3** after addition of HOCl; (d) Time course of fluorescence intensity of **C4** after addition of HOCl; (e) Time course of fluorescence intensity of **C5** after addition of HOCl; (f) Time course of fluorescence intensity of **C6** after addition of HOCl.

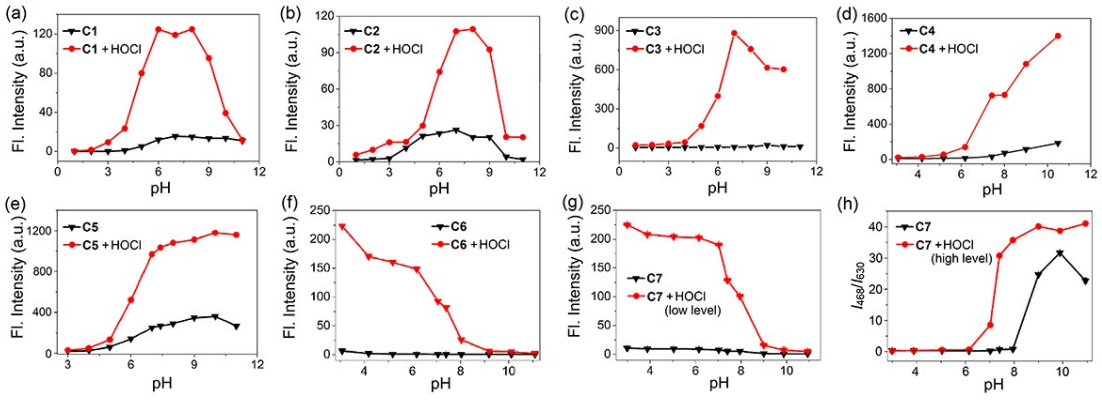


Fig. S5 (a) Influence of pH on the response of **C1** to HOCl; (b) Influence of pH on the response of **C2** to HOCl; (c) Influence of pH on the response of **C3** to HOCl; (d) Influence of pH on the response of **C4** to HOCl; (e) Influence of pH on the response of **C5** to HOCl; (f) Influence of pH on the response of **C6** to HOCl; (g) Plots of fluorescence intensity of **C7** and (h) plot of I_{468}/I_{630} of **C7** *vs.* pH values.

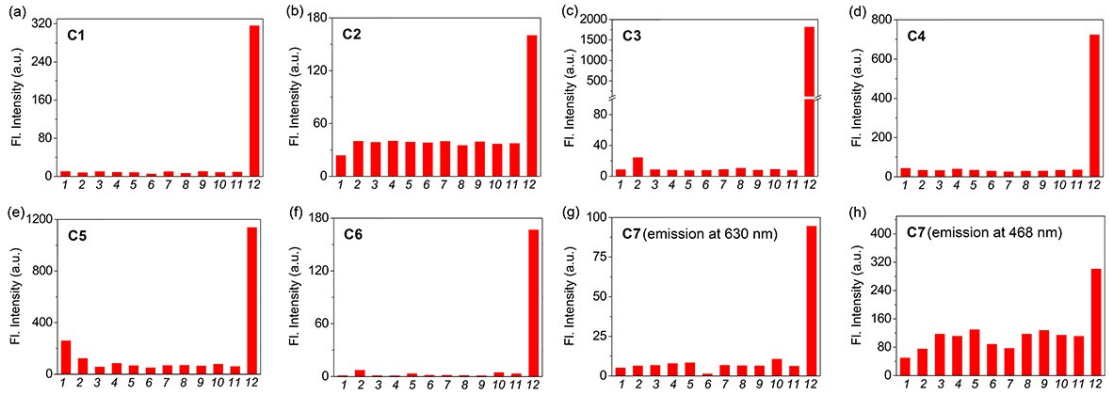


Fig. S6 The fluorescence response of probes **C1-C7** to various metal ions. (1) Blank, (2) Ag^{+}, (3) Ca^{2+}, (4) Co^{2+}, (5) Cr^{3+}, (6) Cu^{2+}, (7) Fe^{2+}, (8) Fe^{3+}, (9) Ni^{2+}, (10) Pb^{2+}, (11) Zn^{2+}, (12) HOCl.

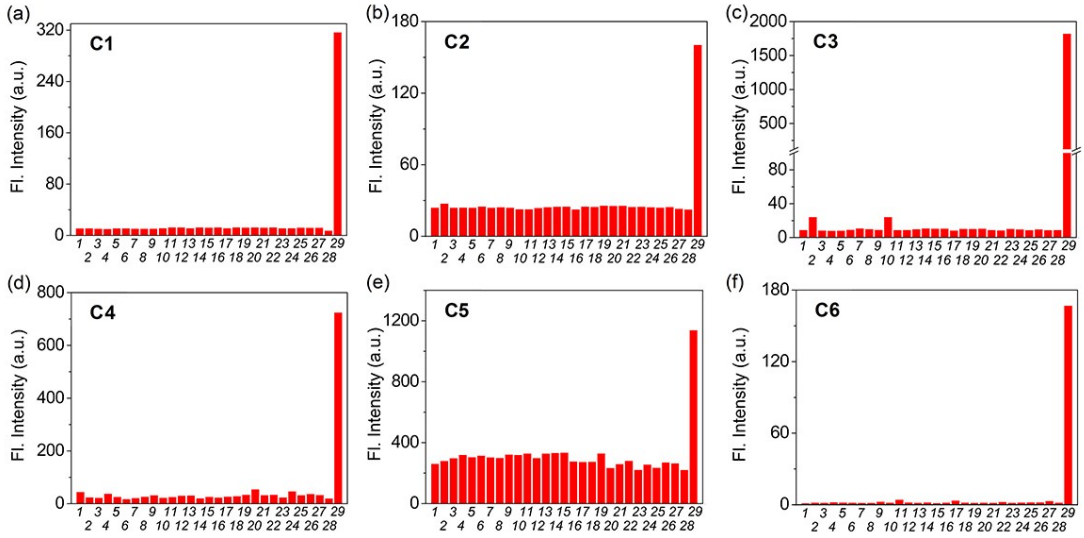
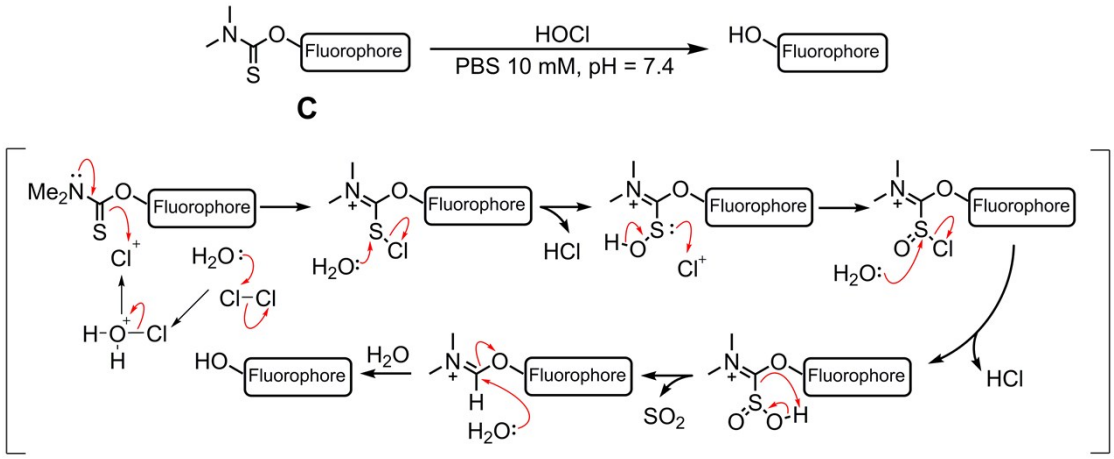


Fig. S7 Fluorescence responses of probes **C1-C6** to various anion species and biothiols. (1) Blank, (2) H_2O_2, (3) $^{\bullet}OH$, (4) $O_2^{\bullet-}$, (5) $^1O_2$, (6) $NO^{\bullet}$, (7) $TBO^{\bullet}$, (8) TBHP, (9) $ONOO^-$, (10) HSO_3^-, (11) F^-, (12) Cl^-, (13) SCN^-, (14) NO_2^-, (15) HSO_4^-, (16) $S_2O_3^{2-}$, (17) HPO_4^-, (18) I^-, (19) PO_4^{3-}, (20) Br^-, (21) HCO_3^-, (22) SO_4^{2-}, (23) $H_2PO_4^{2-}$, (24) CO_3^{2-}, (25) NO_3^-, (26) Hcy, (27) GSH, (28) Cys, (29) HOCl.

Scheme S1. Proposed entropy-driven reaction mechanism of **C** to HOCl.



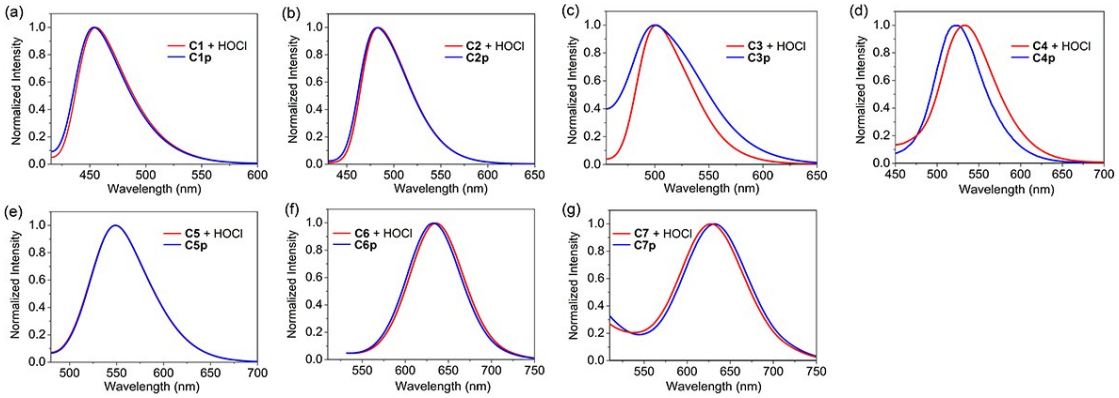


Fig. S8 (a) The normalized emission intensity of **C1p** and **C1** treated with HOCl; (b) the normalized emission intensity of **C2p** and **C2** treated with HOCl; (c) the normalized emission intensity of **C3p** and **C3** treated with HOCl; (d) the normalized emission intensity of **C4p** and **C4** treated with HOCl; (e) The normalized emission intensity of **C5p** and **C5** treated with HOCl; (f) The normalized emission intensity of **C6p** and **C6** treated with HOCl; (g) The normalized emission intensity of **C7p** and **C7** treated with low concentrations of HOCl.

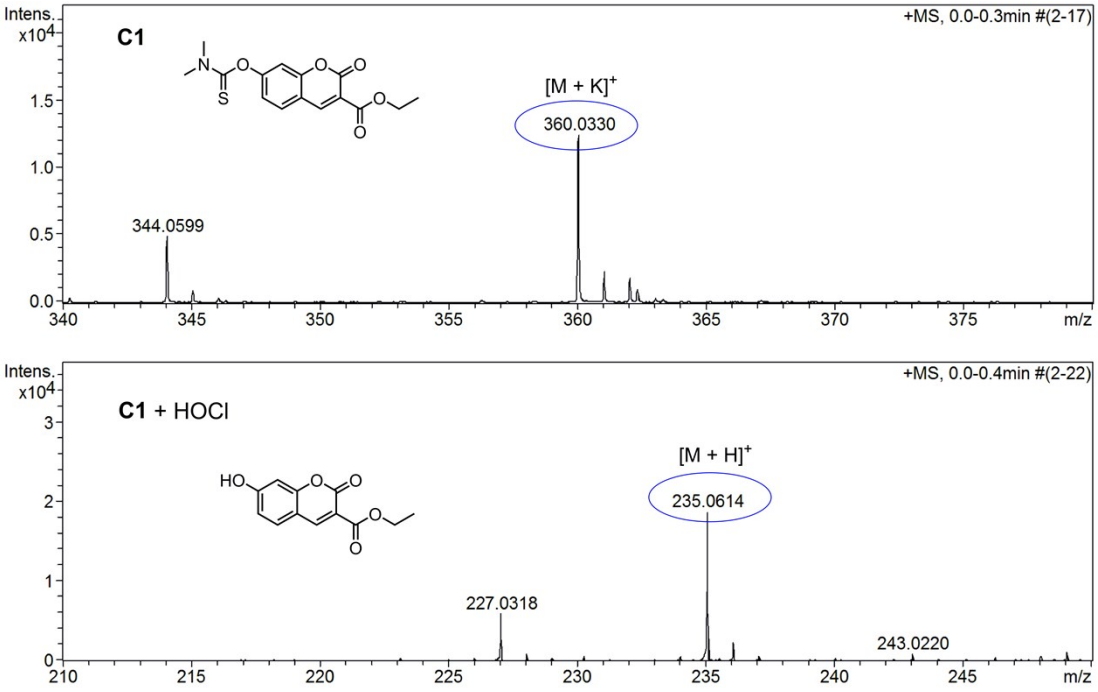


Fig. S9 The MS spectra of **C1** before and after adding HOCl.

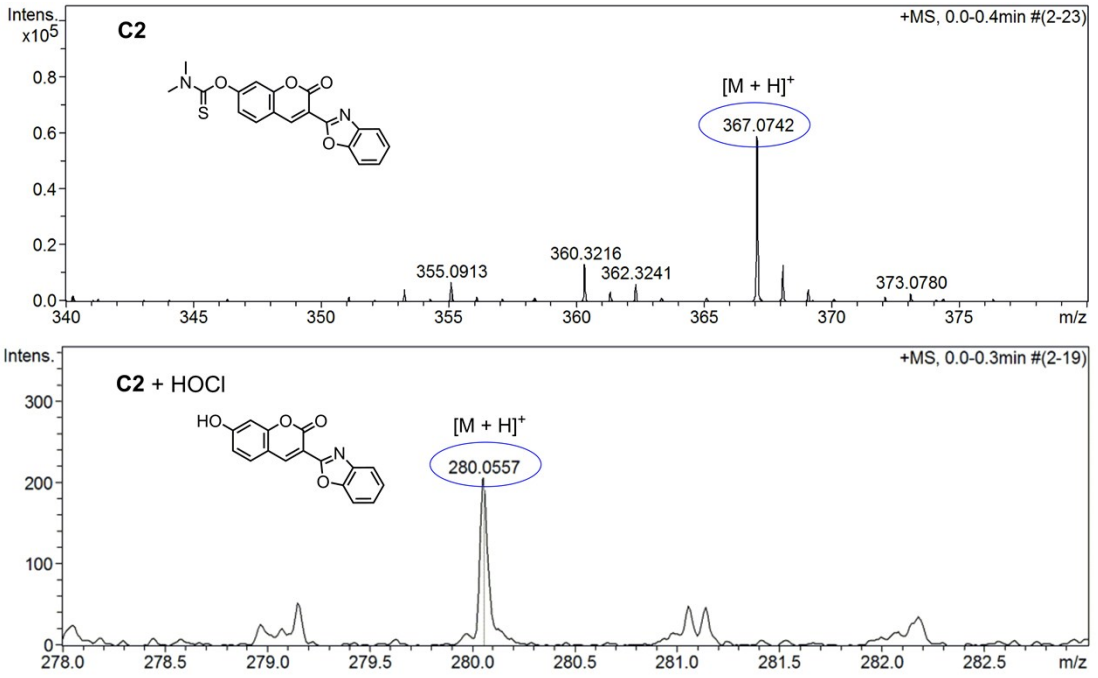


Fig. S10 The MS spectra of **C2** before and after adding HOCl.

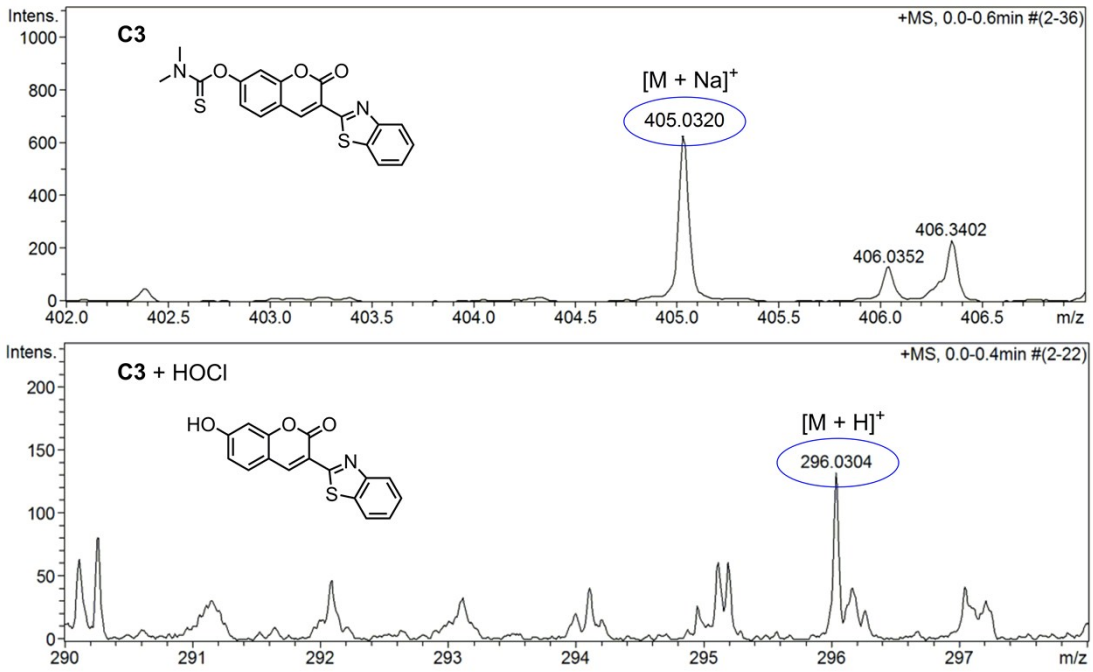


Fig. S11 The MS spectra of **C3** before and after adding HOCl.

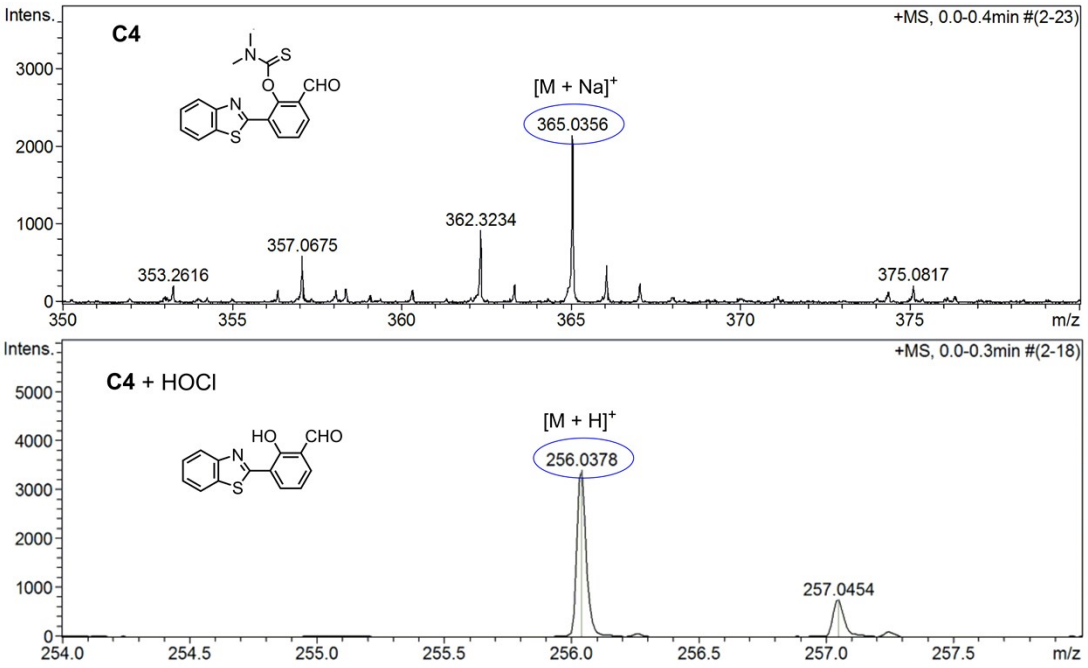


Fig. S12 The MS spectra of **C4** before and after adding HOCl.

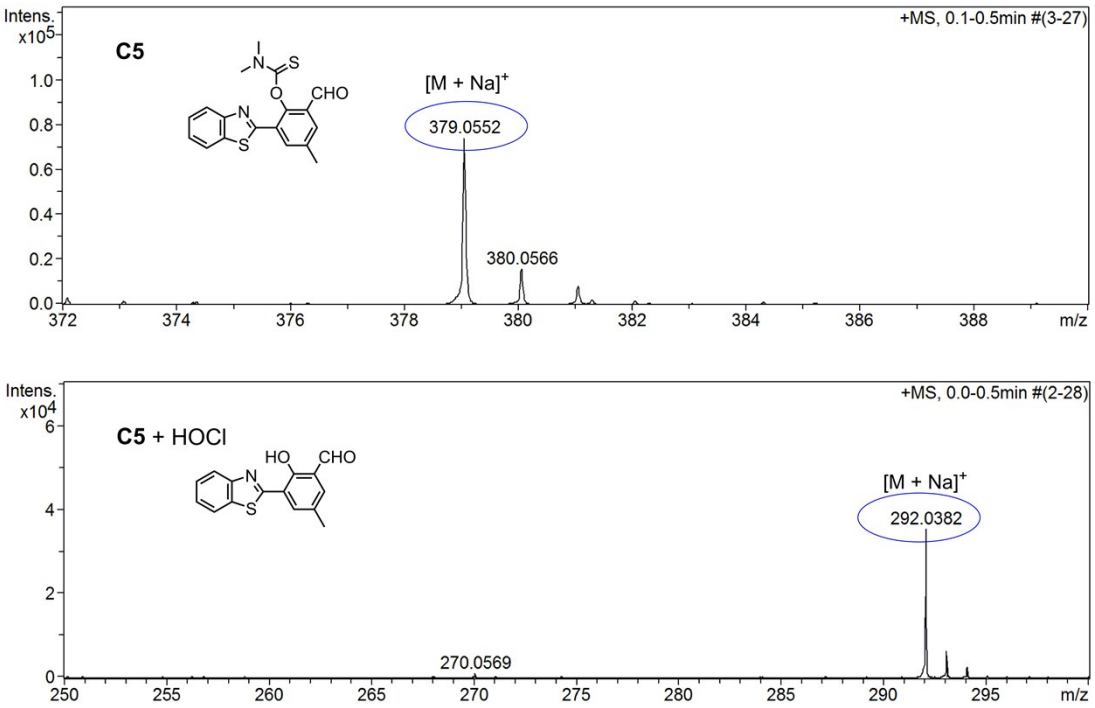


Fig. S13 The MS spectra of **C5** before and after adding HOCl.

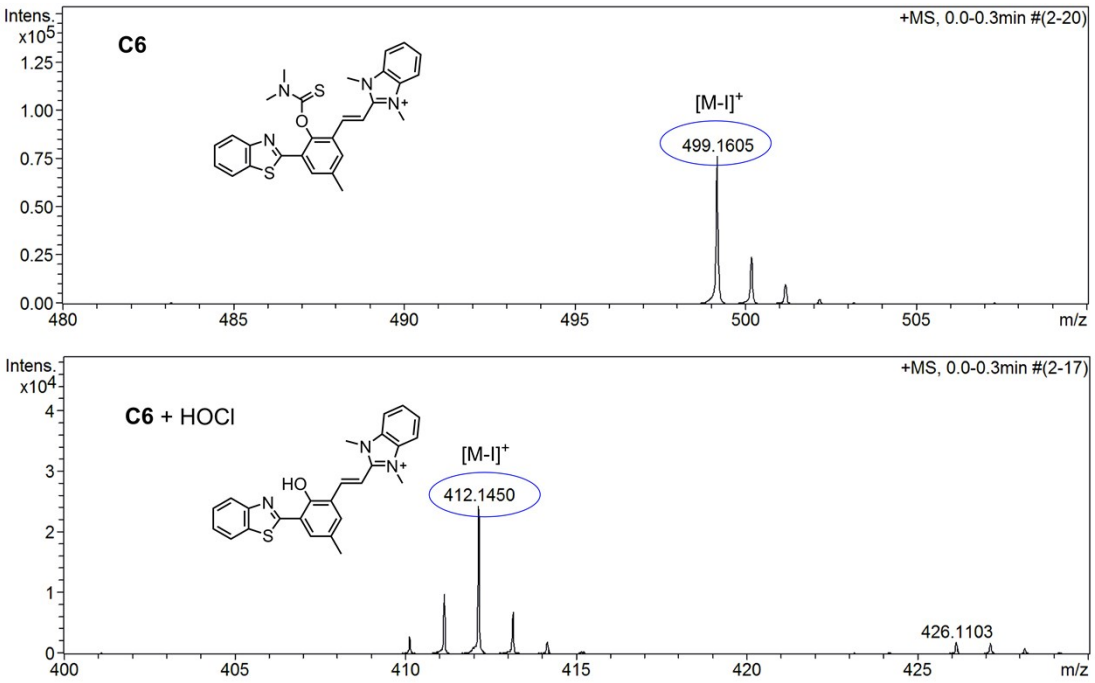


Fig. S14 The MS spectra of **C6** before and after adding HOCl.

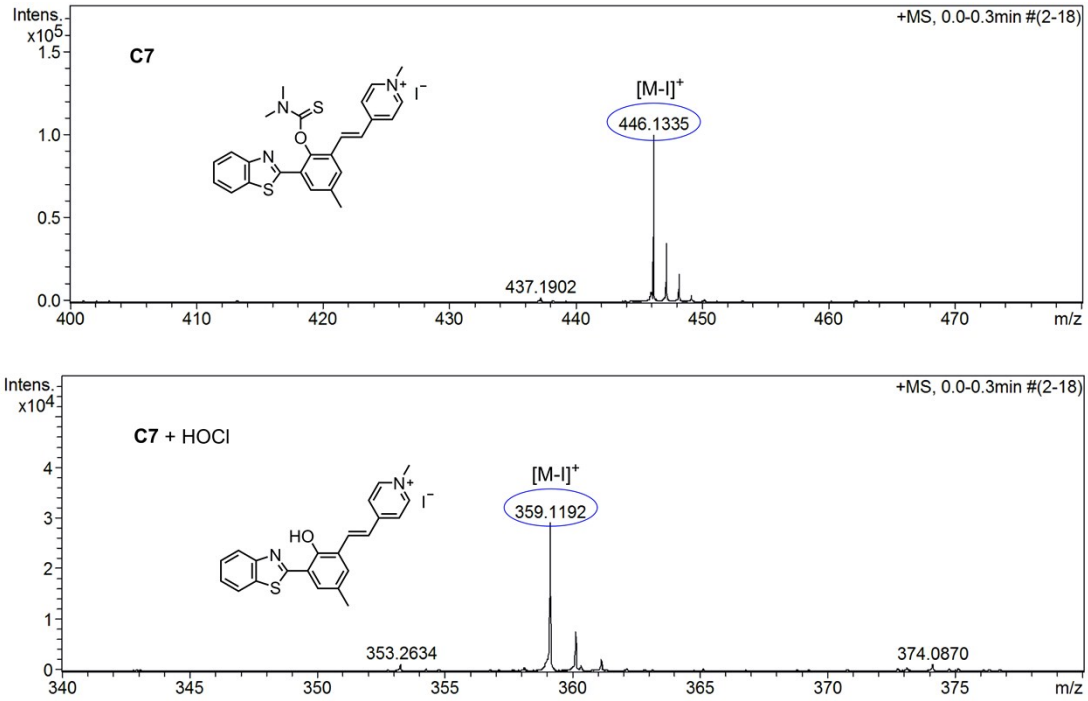


Fig. S15 The MS spectra of **C7** before and after adding HOCl.

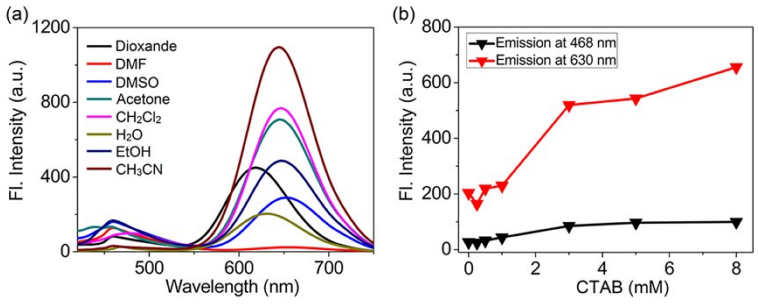


Fig. S16 (a) Emission spectra of **C7p** (10 μM) in different solvents; (b) Plot of fluorescence intensity at 468 nm and 630 nm of **C7p** (10 mM) *vs.* CTAB.

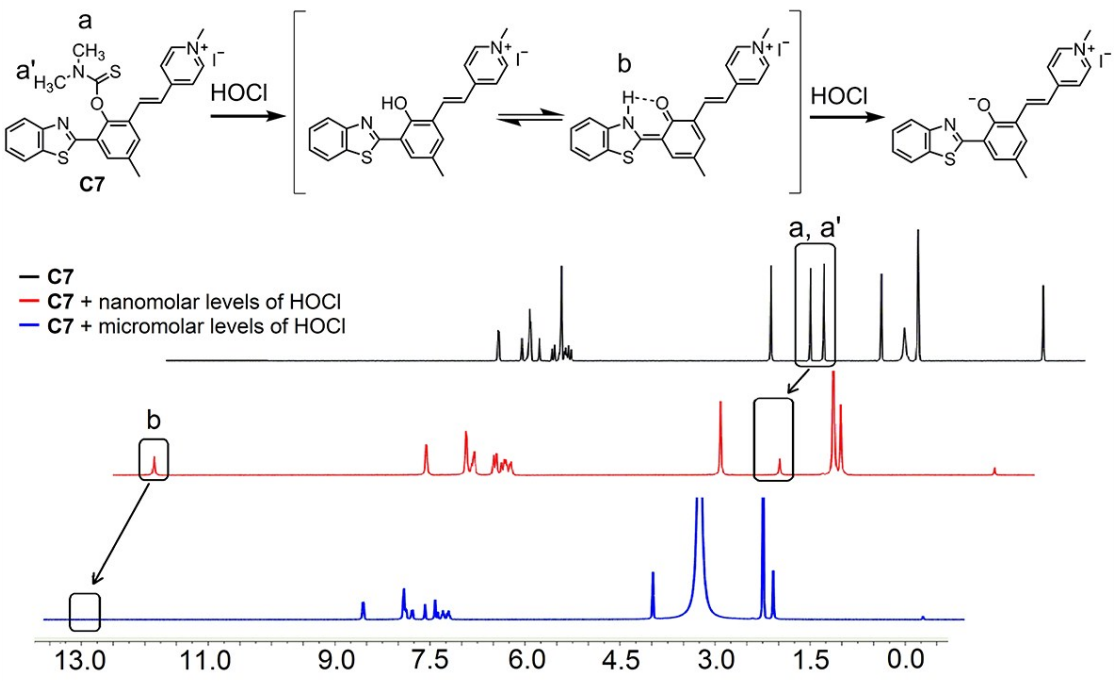


Fig. S17 ^{1}H NMR spectra of **C7**, **C7** treated with nanomolar concentrations of HOCl and **C7** treated with micromolar concentrations of HOCl in DMSO-d_6.

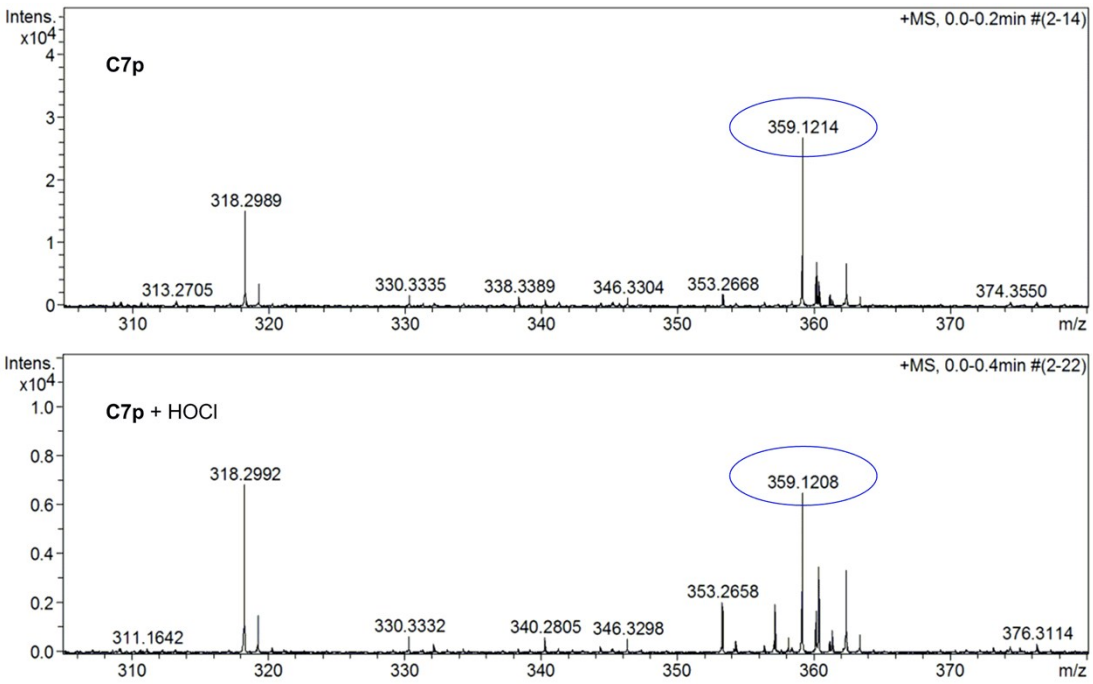


Fig. S18 The MS spectra of **C7p** before and after adding micromolar levels of HOCl.

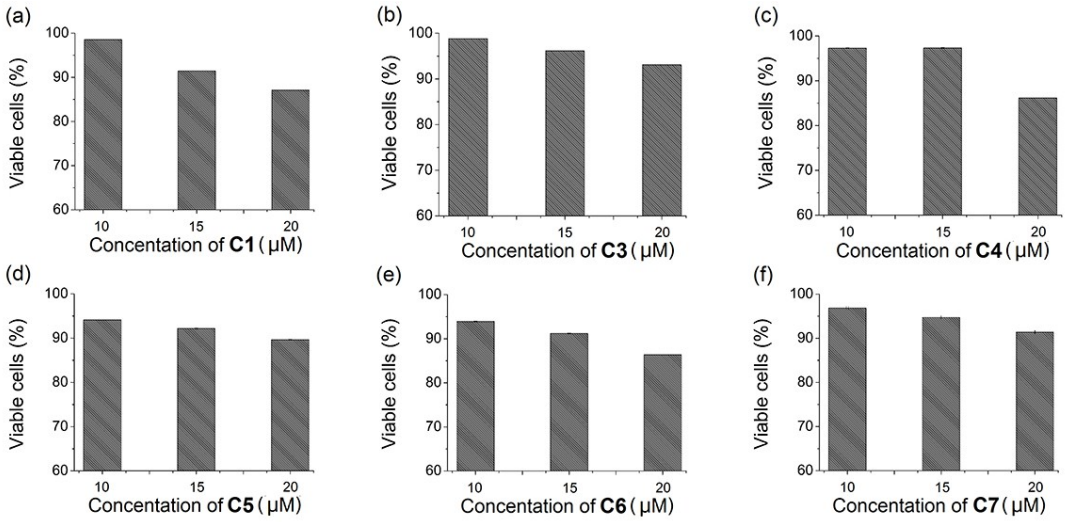


Fig. S19 Percentage of HepG2 cells viability remaining after cells treatment with **C1** and **C3-C7** (untreated cells were considered to have 100% survival).

Confocal Imaging

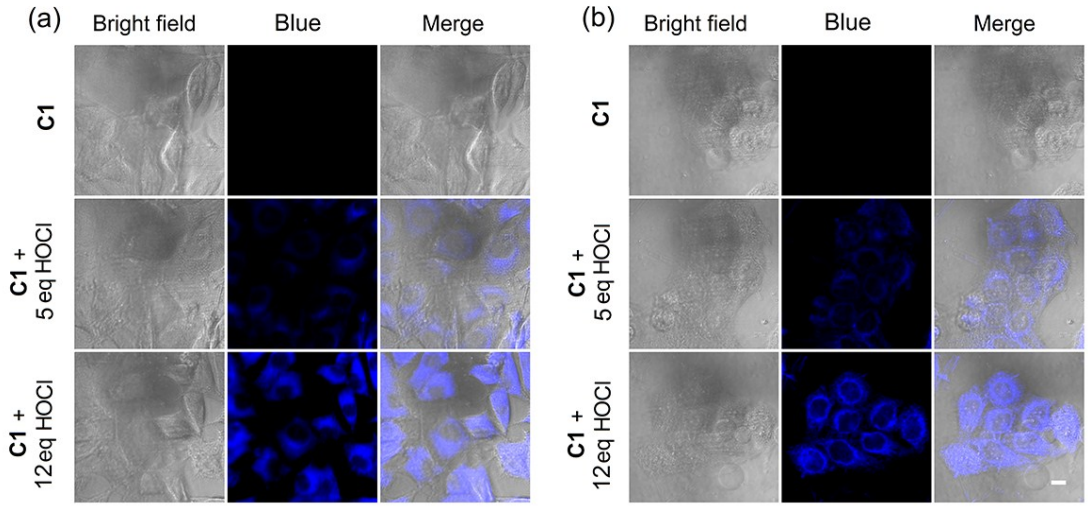


Fig. S20 Dose-dependent fluorescence images of (a) A549 cells and (b) HepG2 cells. The cells were incubated with 20 μM **C1**, and then treated with different amount of HOCl (50 μM and 120 μM). Blue channel: collected at 440-480 nm, λ_{ex} = 405 nm, scale bar = 10 μm.

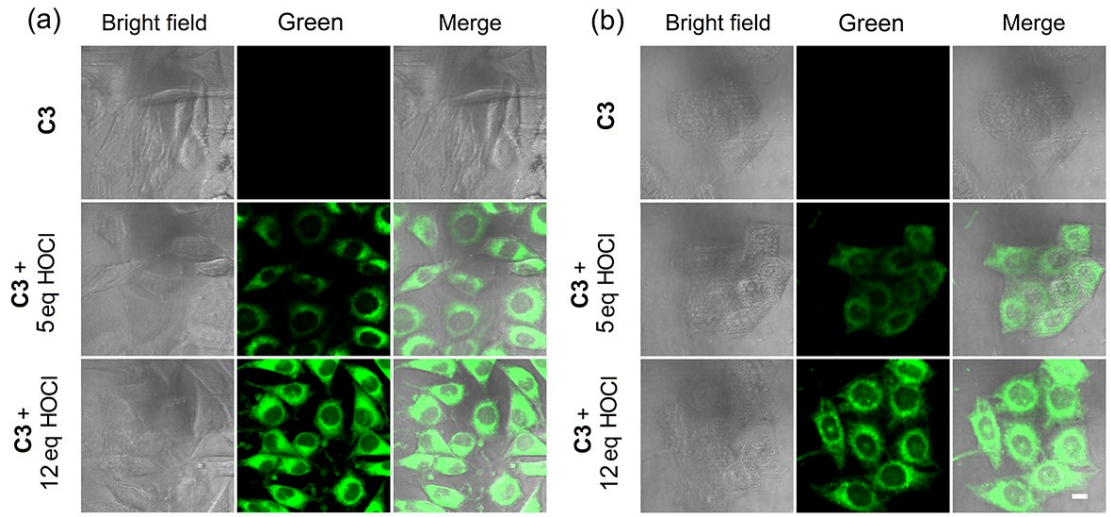


Fig. S21 Dose-dependent fluorescence images of (a) A549 cells and (b) HepG2 cells. The cells were incubated with 10 μM **C3**, and then treated with different amount of HOCl (50 μM and 120 μM). Green channel: collected at 480-520nm, λ_{ex} = 405 nm, scale bar = 10 μm.

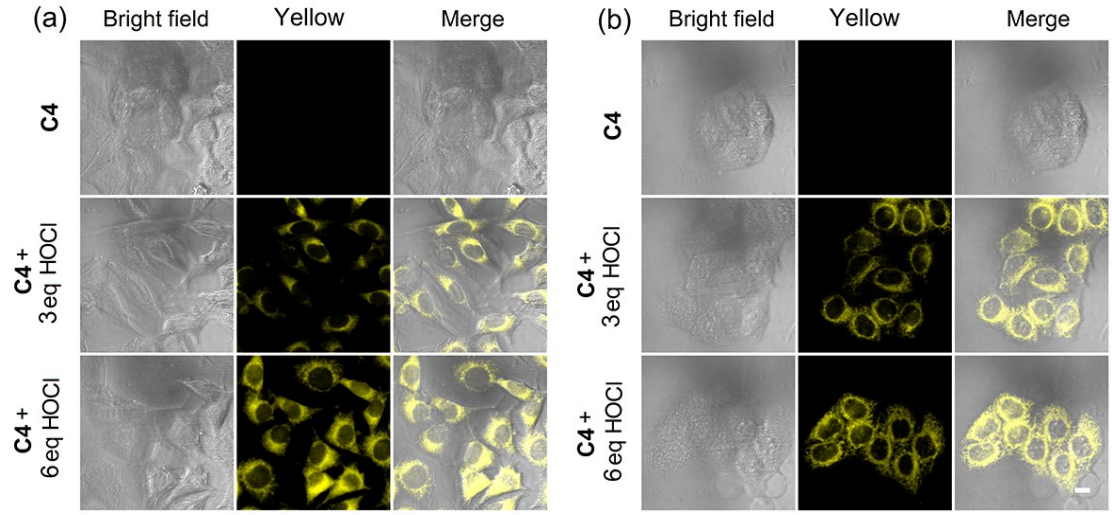


Fig. S22 Dose-dependent fluorescence images of (a) A549 cells and (b) HepG2 cells. The cells were incubated with 10 μM **C4**, and then treated with different amount of HOCl (30 μM and 60 μM). Yellow channel: collected at 530-580 nm, λ_{ex} = 488 nm, scale bar = 10 μm.

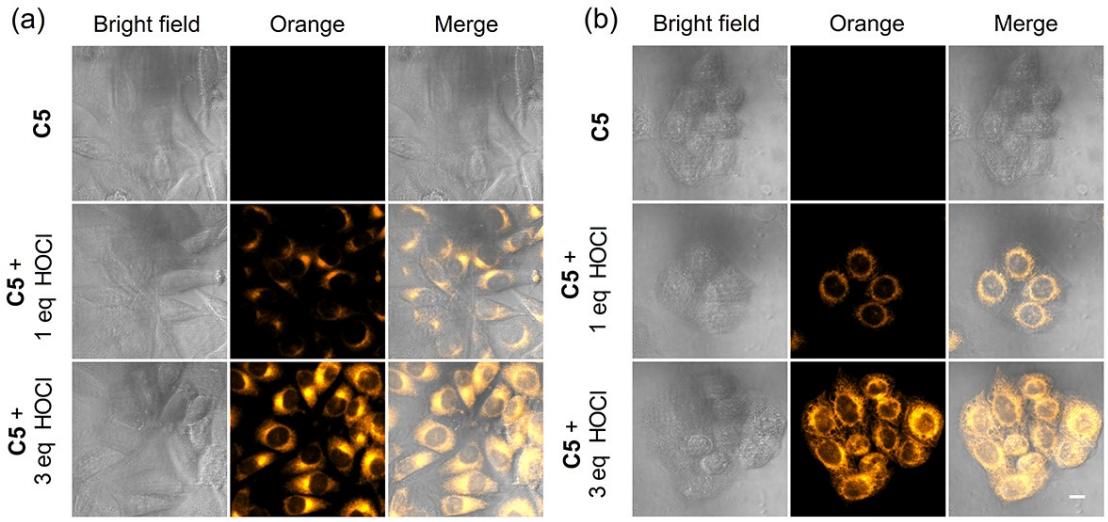


Fig. S23 Dose-dependent fluorescence images of (a) A549 cells and (b) HepG2 cells. The cells were incubated with 10 μM **C5**, and then treated with different amount of HOCl (10 μM and 30 μM). Orange channel: collected at 540-580 nm, λ_{ex} = 488 nm, scale bar = 10 μm.

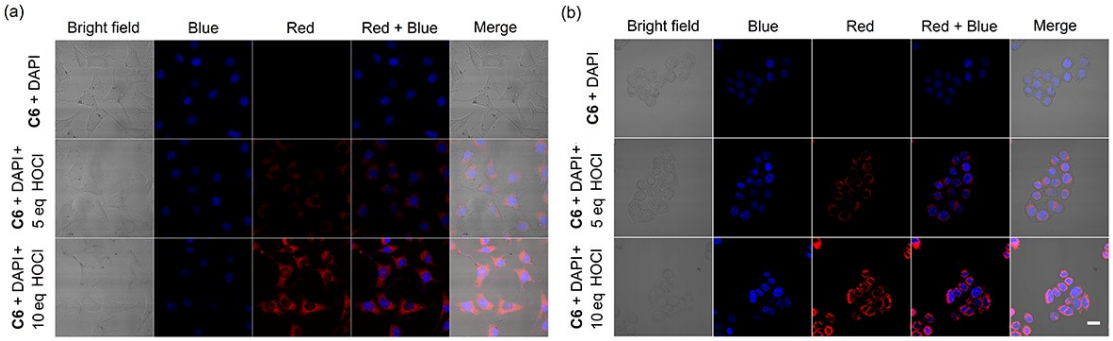


Fig. S24 Confocal fluorescence images of (a) A549 cells and (b) HepG2 cells preloaded 10 μM on **C6**, 2 μg DIPA and further incubated with various concentration of HOCl (50 μM, 100 μM). Blue channel: collected at 420-480 nm, λ_{ex} = 405 nm; Red channel: collected at 595-635 nm, λ_{ex} = 488 nm, scale bar = 20 μm.

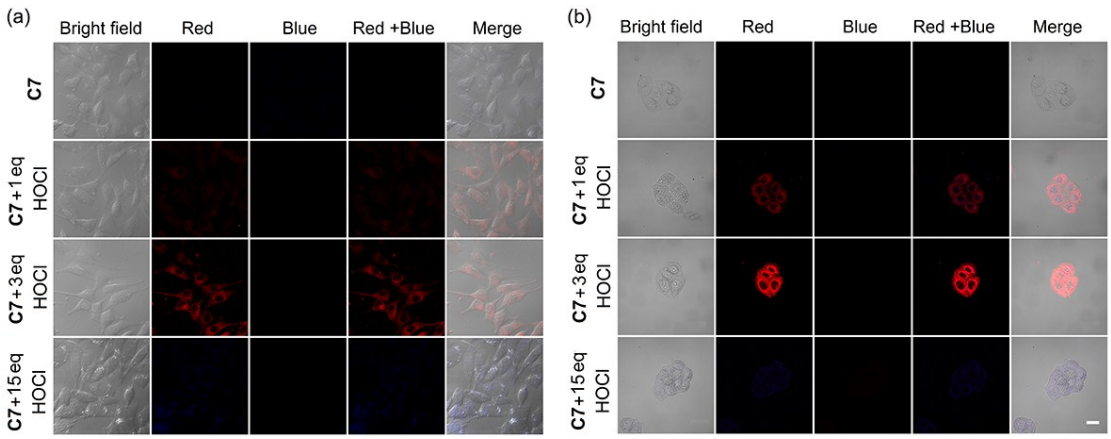


Fig. S25 Confocal fluorescence images of (a) A549 cells and (b) HepG2 cells preloaded on 10 μM **C7** and further incubated with various concentration of HOCl (10 μM, 30 μM, 150 μM). Blue channel: collected at 440-490 nm, λ_{ex} = 405 nm; Red channel: collected at 610-650 nm, λ_{ex} = 405 nm, scale bar = 20 μm.

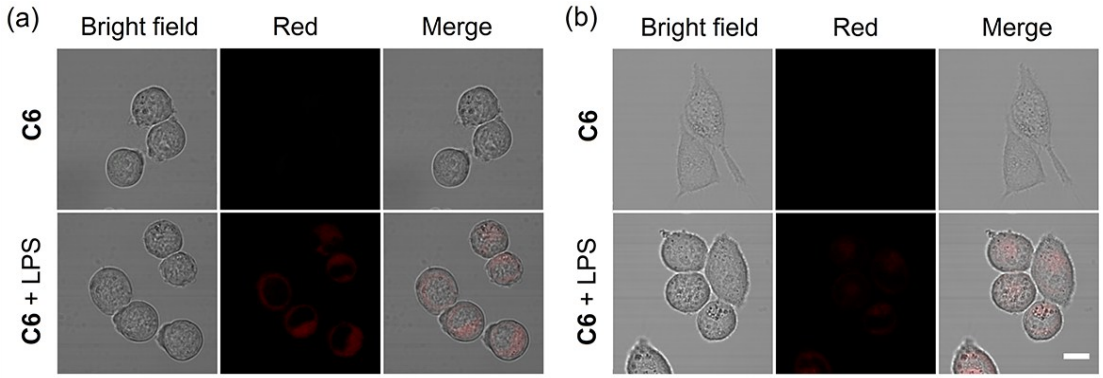


Fig. S26 Confocal fluorescence images of (a) HepG2 cells and (b) A549 cells preloaded on LPS (1 μg/ml, 3 h) and further incubated in buffers with **C6** (10 μM, 10 min). λ_{ex} = 488 nm, λ_{em} = 595-635 nm, Scale bar: 10 μm.

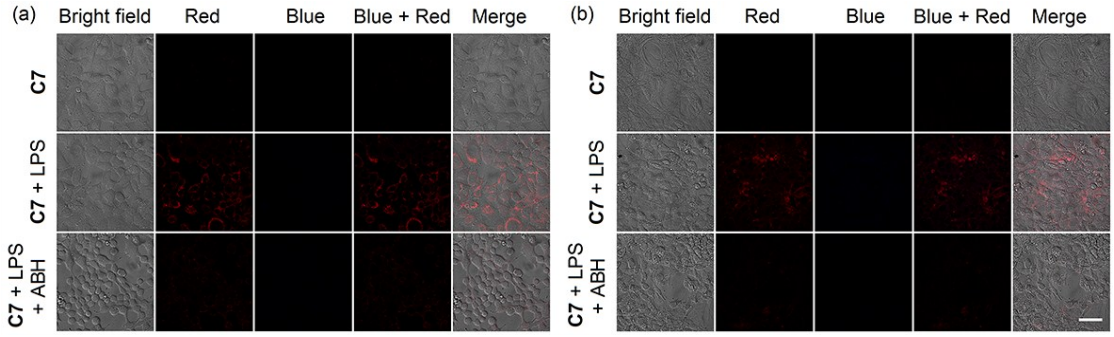


Fig. S27 Confocal fluorescence images of (a) HepG2 cells and (b) A549 cells. From top to bottom: Cells were incubated with **C7** (10 μM, 10 min); Cells were stimulated with LPS (1 μg/ml, 3 h) and further incubated with **C7** (10 μM, 10 min); Cells were stimulated with LPS (1 μg/ml) and ABH (250 μM) for 3 h, and then incubated with **C7** (10 μM, 10 min). λ_{ex} = 405 nm, λ_{em} = 610-650 nm. Scale bar: 50 μm.

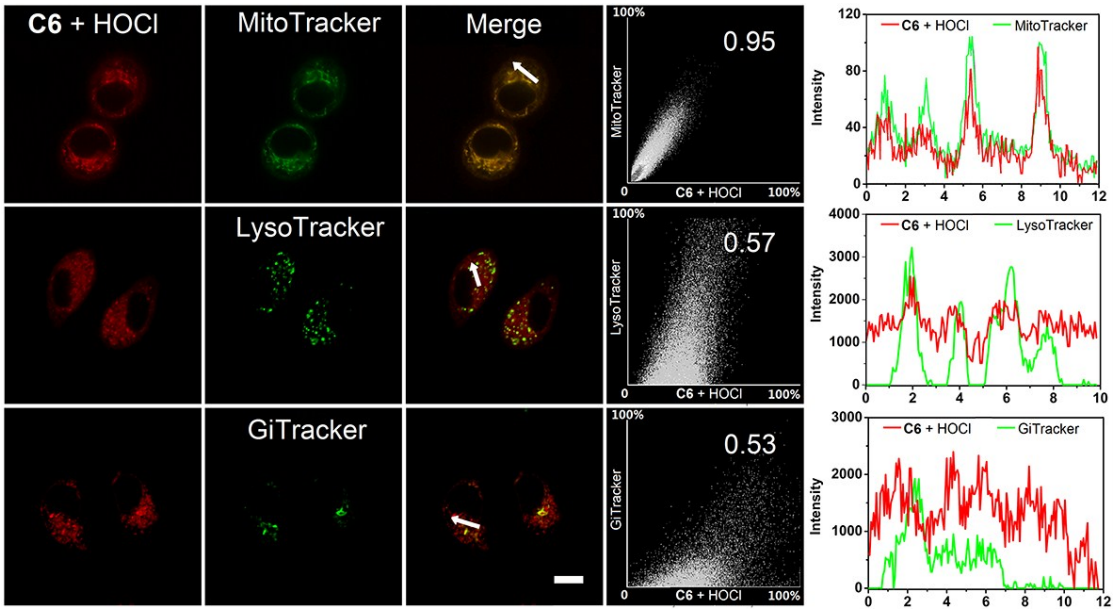
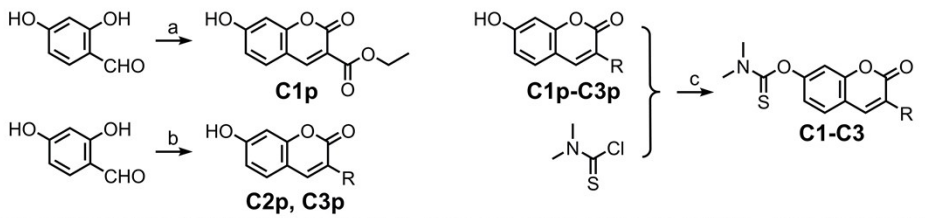


Fig. S28 Intracellular localization of **C6** in HepG2 cells. Fluorescence imaging of HepG2 cells incubated with 20 μM **C6**, and then stained with MitoTracker Green FM (200 nM), LysoTracker Green DND-26 (7.5 nM) and GolgiTracker Green (NBD C6-ceramide, 3 μM), respectively. Cells were incubated with dyes and then treated with HOCl before confocal imaging. Plots in the column 4 represent the intensity correlation plot of dyes and probes. Column 5 represents cross-sectional analysis along the white line in the insets. Red channel: λ_{ex} = 488 nm, λ_{em} = 595-635 nm; Green channel: λ_{ex} = 488 nm, λ_{em} = 490-550 nm. Scale bar: 10 μm.

Synthetic Procedures

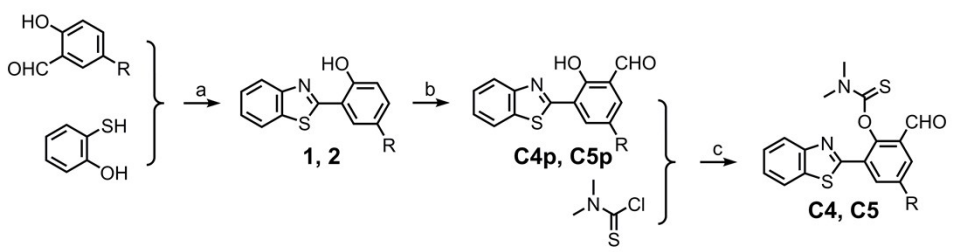
Scheme S2. Synthesis of **C1**-**C7**, **C1p**-**C7p** and control compounds (**A**, **B** and **C7c**).

(a)



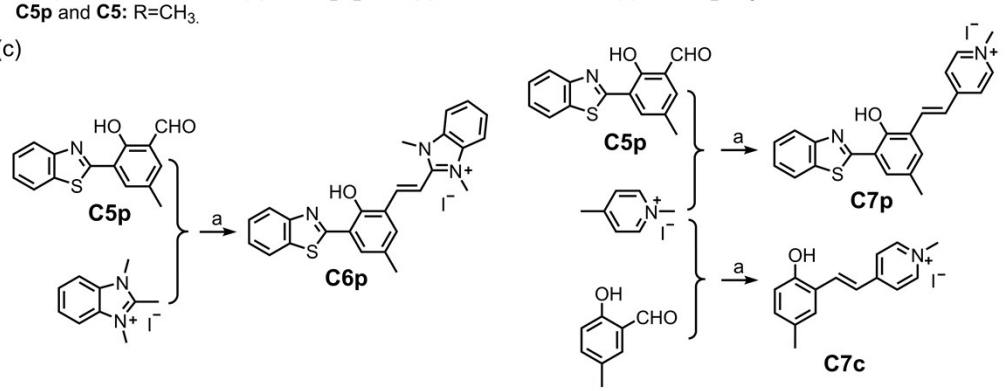
Reagents and conditions: (a) Diethyl malonate, Et_3N, acetic acid, EtOH, reflux; (b) *n*-BuOH, ethyl cyanoacetate, benzoic acid, 1,2-diaminobenzene (**C1p**) or 2-aminophenol (**C2p**) or 2-aminothiophenol (**C3p**); (c) DMF, K_2CO_3, r. t.; **C1p, C1**: R=$COOCH_2CH_3$; **C2p**, **C2**: R=Benzoxazole; **C3p**, **C3**:R=Benzothiazole.

(b)



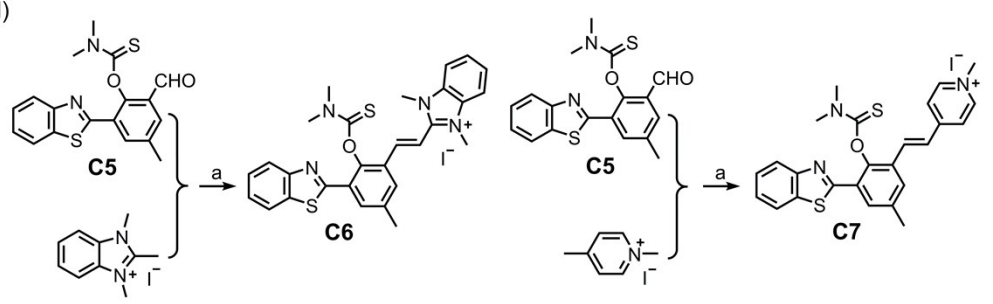
Reagents and conditions: (a) HCl, H_2O_2, r. t.; (b) HMTA, TFA, 80 ºC; (c) DMF, K_2CO_3, r. t.; **1, C4p** and **C4**: R=H; **2**, **C5p** and **C5:** R=CH_3.

(c)



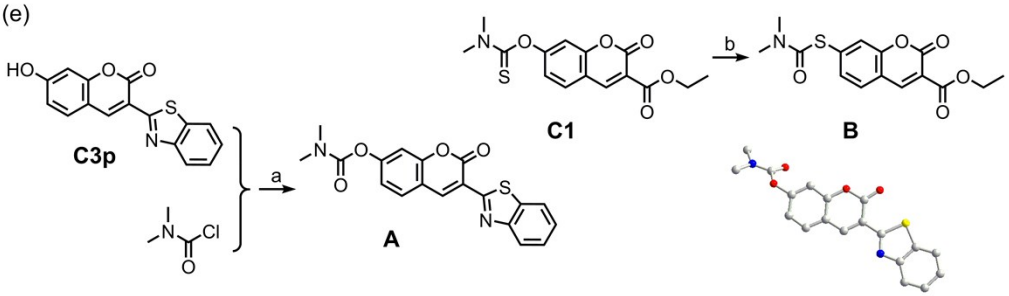
Reagents and conditions: (a) EtOH, piperidine, reflux.

(d)



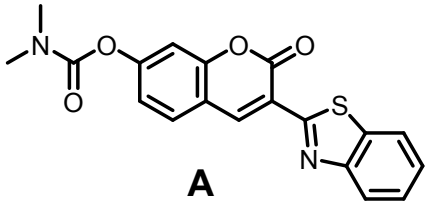
Reagents and conditions: (a) NaOAc, $(Ac)_2O$, reflux.

(e)



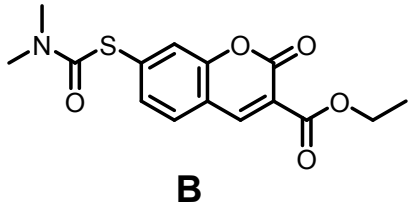
Reagents and conditions: (a) DMF, K_2CO_3, r. t.; (b) DMF, reflux.

The X-ray crystal structure of **A**



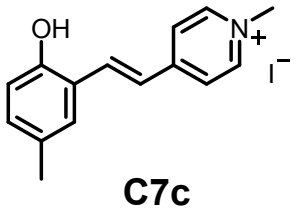
A

Synthesis of A. A mixture of 29.5 mg (0.1 mmol) compound **C3p**, 21.5 mg (0.2 mmol) dimethylcarbamyl chloride and 30.4 mg (0.22 mmol) potassium carbonate were taken in 5 mL of DMF and stirred at 70 °C monitoring with TLC. After the reaction was complete, the mixture solution was cooled to room temperature and then poured in ice-cold water (30 mL), the precipitate was filtered, washed with brine, and then dried under vacuum to give the crude product, which was further purified by chromatography on a silica gel column (CH_2Cl_2:CH_3OH = 400:1, v:v) to give a product **A** (24.8 mg) in 67.6% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 3.09 (s, 3H), 3.17 (s, 3H), 7.23 (d, J = 10.4 Hz, 1H), 7.29 (s, 1H), 7.46 (t, J = 7.7 Hz, 3H), 7.57 (t, J = 7.8 Hz, 3H), 7.74 (d, J = 8.5 Hz, 1H), 8.01 (d, J = 7.9 Hz, 1H), 8.12 (d, J = 8.2 Hz, 1H), 9.10 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 36.6, 36.9, 110.1, 116.1, 119.2, 119.4, 121.7, 122.8, 125.3, 126.5, 129.9, 136.8, 141.1, 152.4, 153.5, 154.5, 155.4, 159.7, 159.9, 162.5; HRMS (ESI): *m/z* found $[M+H]^+$ for $C_{19}H_{15}N_2O_4S^+$ 367.0739, calcd 367.0747.



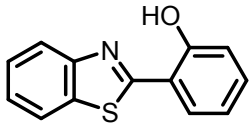
B

Synthesis of B. 12.7 mg (0.1 mmol) compound **C1** was dissolved in 10 ml DMF and then heated to 153°C for 4 h. The crude product was recrystallized from C_2H_5OH to give **B** (23.2 mg) as a yellow solid, yield 82.3%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.40 (t, J = 7.1 Hz, 3H), 3.04 (s, 3H), 3.10 (s, 3H), 4.40 (q, J = 7.1 Hz, 2H), 7.44 (d, J = 8.0 Hz, 1H), 7.50 (s, 1H), 7.57 (d, J = 8.1 Hz, 1H), 8.50 (s, 1H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ (ppm): 14.3, 36.9, 61.98, 117.9, 118.7, 122.6, 129.1, 131.1, 137.1, 147.9, 154.5, 156.2, 162.8, 164.5; HRMS (ESI): *m/z* found $[M+Na]^+$ for $C_{15}H_{15}NO_5SNa^+$ 344.0565, calcd 344.0563; FT-IR (KBr, cm^{-1}): 2995, 1795, 1712, 1660, 1600, 1522, 1539, 1290, 1253, 1112, 1018, 891, 794, 694.



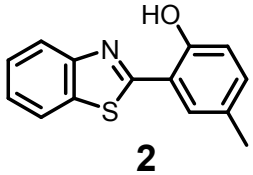
C7c

Synthesis of compound C7c. 2-hydroxy-5-methylbenzaldehyde (136 mg, 1 mmol) and 1,2,3-trimethyl-1H-benzoimidazol-3-ium iodide (576 mg, 2 mmol) were dissolved into 5 ml EtOH, then piperidine (0.1 ml, 1.08 mmol) were added sequentially. And the reaction mixture heated at reflux for 6 h. After the reaction was complete, the mixture solution was cooled to room temperature. The light yellow solid formed was filtered and washed with a small amount of methanol. 250 mg compound **C7c** was dried in vacuum and then obtained with a yield of 70.8%. ^{1}H NMR (400 MHz, CD_3CN) δ (ppm): 8.59 (d, J = 8.0 Hz, 1H), 8.54 (d, J = 4.0 Hz, 1H), 8.10 (d, J = 8.0 Hz, 1H), 7.81 (d, J = 4.0 Hz, 2H), 7.75 (d, J = 16.0 Hz 1H), 7.43 (d, J = 16.0 Hz, 1H), 7.30 (d, J = 8.0 Hz, 1H), 7.07 (d, J = 8.0 Hz, 1H), 4.24 (s, 3H), 2.38 (s, 3H); NMR (100 MHz, CD_3CN): 144.9, 144.2, 136.5, 134.0, 132.2, 128.4, 127.6, 124.7, 124.3, 2, 123.2, 47.4, 21.2; HRMS: *m/z* found $[M-I]^+$ for $C_{15}H_{16}NO^+$ 226.1220, calcd 226.1226.



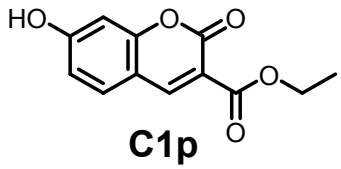
1

Synthesis of compound 1. To a mixture of *o*-aminobenzenethiol (126 mg, 1.1 mmol), salicylaldehyde (122 mg, 1.0 mmol), 30% hydrogen peroxide (680 μL, 0.6 mmol), and 37% hydrochloric acid (250 μL, 0.3 mmol) were added in anhydrous ethanol (5 mL). The reaction solution was stirred at room temperature for 6 h. Once the solid had been obtained and filtered, it was washed with anhydrous ethanol and dried under vacuum, giving a yellow-green solid (127 mg, 56.0% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.00 (d, J = 8.4 Hz, 1H), 7.91 (d, J = 8.1 Hz, 1H), 7.70 (d, J = 7.8 Hz, 1H), 7.51 (t, J = 7.2 Hz, 1H), 7.44-7.37 (m, 2H), 7.11 (d, J = 8.9 Hz, 1H), 6.96 (t, J = 6.6 Hz, 1H); HRMS: *m/z* found $[M+H]^+$ for $C_{13}H_{10}NOS^+$ 228.0475, calcd 228.0478.



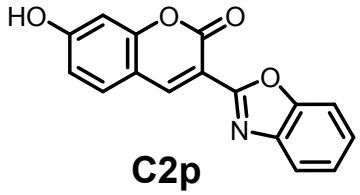
2

Synthesis of compound 2. To a mixture of *o*-aminobenzenethiol (126 mg, 1.1 mmol), 2-hydroxy-5-methylbenzaldehyde (136 mg, 1.0 mmol), 30% hydrogen peroxide (680 μL, 0.6 mmol), and 37% hydrochloric acid (250 μL, 0.3 mmol) were added in anhydrous ethanol (5 mL). The reaction solution was stirred at room temperature for 6 h. Once the solid had been obtained and filtered, it was washed with anhydrous ethanol and dried under vacuum, giving a yellow-green solid (145 mg, 60.2% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 12.31 (s, 1H), 7.98 (d, J = 8.1 Hz, 1H), 7.90 (d, J = 7.92 Hz, 1H), 7.52-7.48 (m, 2H), 7.40(t, J = 7.6 Hz, 1H), 7.19 (d, J = 8.4 Hz, 1H), 7.01 (d, J = 8.4 Hz 1H), 2.36 (s, 3H); HRMS (ESI): *m/z* found $[M+H]^+$ for $C_{14}H_{12}NOS^+$ 242.0632, calcd 242.0634.



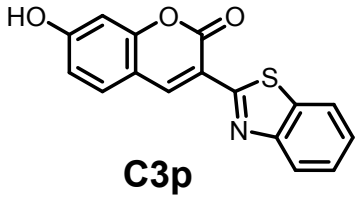
C1p

Synthesis of C1p. 2,4-dihydroxybenzaldehyde (919 mg, 6.6 mmol), diethyl malonate (0.6 mL, 4.0 mmol), 1 mL triethylamine and 1.00 mL acetic acid were dissolved in 5 mL absolute ethyl alcohol and reaction mixture heated at reflux for 12 h. After the reaction was complete, the mixture solution was cooled to room temperature. The light yellow solid formed was filtered and washed with a small amount of methanol several times. Compound **C1p** was dried in vacuum and then obtained with a yield of 54.56% (624 mg). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.31 (t, J = 7.6 Hz, 3H), 4.27 (q, J = 7.2 Hz, 2H), 6.74 (s, 1H), 6.85 (d, J = 8.4 Hz, 1H), 7.77 (d, J = 8.6 Hz, 1H), 8.69 (s, 1H), 11.08 (s, 1H); HRMS (ESI): *m/z* found $[M+H]^+$ for $C_{12}H_{11}O_5^+$ 235.0614, calcd 235.0606.



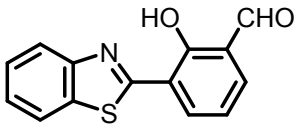
C2p

Synthesis of C2p. 2,4-dihydroxybenzaldehyde (1.82 g, 13.20 mmol), cyanoacetic acid ethyl ester (1.63 g, 14.4 mmol), 2-aminophenol (1.43 g, 13.20 mmol) and benzoic acid (0.54g, 4.40 mmol)dissolved in 65 mL *n*-butyl alcoholand reaction mixture heated at reflux for 12 h. Finally, the compound **C2p** was obtained (2.52g, 61.8%). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 6.77 (s, 1H), 6.86 (d, J = 8.1 Hz, 1H), 7.41 (m, 2H), 7.77 (d, J = 9.1 Hz, 1H), 7.79 (d, J = 7.2 Hz, 1H), 7.81 (d, J = 10.2 Hz, 1H), 8.92 (s, 1H) 11.14 (s, 1H); HRMS (ESI): *m/z* found $[M+H]^+$ for $C_{16}H_{10}NO_4^+$ 280.0557, calcd 280.0610.



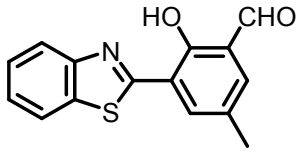
C3p

Synthesis of C3p. 1.82 g (13.20 mmol) 2,4-dihydroxybenzaldehyde, 1.63 g (14.4 mmol) cyanoacetic acid ethyl ester, 1.65 g (13.20 mmol) 2-aminothiophenol and 0.54g (4.40 mmol) benzoic acid dissolved in 65 mL *n*-butyl alcohol and reaction mixture heated at reflux for 12 h. After the reaction was complete, the mixture solution was cooled to room temperature and then poured in ice-cold water (100 mL) washed with brine, and then dried under vacuum to give the crude product, which was further purified by chromatography on a silica gel column (CH_2Cl_2:CH_3OH = 50:1, v:v) to give a product (2.83 g) in 72.45% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 6.82 (s, 1H), 6.89 (d, J = 7.4 Hz, 1H), 7.42 (t, J = 6.6 Hz, 1H), 7.53(t, J = 6.0 Hz, 1H), 7.89 (d, J = 8.0 Hz, 1H), 8.01 (d, J = 7.4 Hz, 1H), 8.12 (d, J = 7.4 Hz, 1H) 9.12 (s, 1H); HRMS (ESI): *m/z* found $[M+H]^+$ for $C_{16}H_{10}NO_3S^+$ 296.0304, calcd 296.0381.



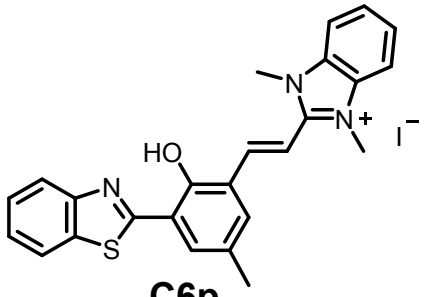
C4p

Synthesis of C4p. Compound **1** (908 mg, 4.0 mmol) and hexamethylenetetramine (560 mg, 4.0 mmol) were stirred in trifluoroacetic acid (10 mL) for 15 h at 80 ºC. After completion of the reaction, 1 mol/L NaOH was added slowly with stirring until the pH of the solution reached 7.0. The crude product was filtered, and further purified by silica gel flash chromatography (petroleum ether/ethyl acetate, 15:1, v/v) to afford compound **C4p** as a yellow solid (900 mg, 87.0% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 10.53 (s, 1H), 9.93 (s, 1H), 8.07-8.00 (m, 2H), 7.94-7.89 (m, 2H), 7.54 (t, J = 7.4 Hz, 1H), 7.45 (t, J = 7.1 Hz, 1H), 7.08 (t, J = 7.6 Hz, 1H); HRMS: *m/z* found $[M+Na]^+$ for $C_{14}H_9NO_2SNa^+$ 278.0251, calcd 278.0246.



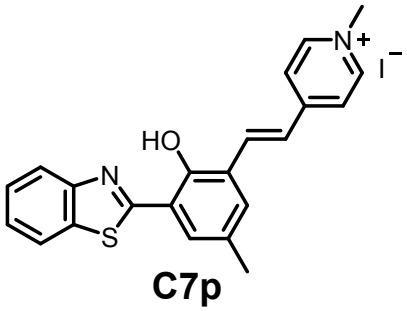
C5p

Synthesis of C5p. Compound **2** (964 mg, 4.0 mmol) and hexamethylenetetramine (560 mg, 4.0 mmol) were stirred in trifluoroacetic acid (10 mL) for 5 h at 80 ºC. After completion of the reaction, 1 mol/L NaOH was added slowly with stirring until the pH of the solution reached 7.0. The crude product was filtered, and further purified by silica gel flash chromatography (petroleum ether/ethyl acetate, 15:1, v/v) to afford **C5p** as a yellow solid (226 mg, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 10.48 (s, 1H), 8.02 (d, J = 8.12 Hz, 1H), 7.93 (d, J = 7.7 Hz, 1H), 7.88 (s, 1H), 7.70(s, 1H), 7.53 (t, J = 7.4 Hz, 1H), 7.44 (d, J = 7.6 Hz, 1H), 2.40 (s, 3H); HRMS (ESI): *m/z* found $[M+Na]^+$ for $C_{15}H_{11}NO_2SNa^+$ 292.0410, calcd 292.0403.



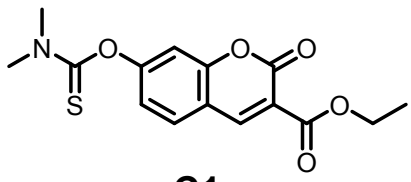
C6p

Synthesis of C6p. C5p (26.9 mg, 0.1 mmol) and 1,2,3-trimethyl-1H-benzoimidazol-3-ium iodide (57.6 mg, 0.2 mmol) were dissolved into 5 ml EtOH, then piperidine (0.05 ml, 0.54 mmol) were added sequentially. And the reaction mixture heated at reflux for 6 h. After the reaction was complete, the mixture solution was cooled to room temperature. The light yellow solid formed was filtered and washed with a small amount of methanol several times. Compound **C6p** was dried in vacuum and then obtained with a yield of 51.85% (27.9 mg). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.44 (s, 3H), 4.16 (s, 6H), 7.53 (t, J = 8.0 Hz, 1H), 7.61 (t, J = 8.0 Hz, 1H), 7.71 (s, 3H), 7.91 (s, 1H), 8.00 (t, J = 8.0 Hz, 2H), 8.13 (d, J = 8.0 Hz, 1H), 8.23 (d, J = 8.0 Hz, 1H), 13.25 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 168.6, 151.3, 149.0, 141.5, 133.1, 132.4, 132.0, 127.7, 126.9, 126.5, 123.3, 123.1, 123.0, 122.9, 122.4, 121.6, 117.9, 113.4, 109.2, 109.1, 109.1, 33.3, 20.4; HRMS (ESI): *m/z* found $[M\text{-}I]^+$ for $C_{25}H_{22}N_3OS^+$ 412.1448, calcd 412.1478.



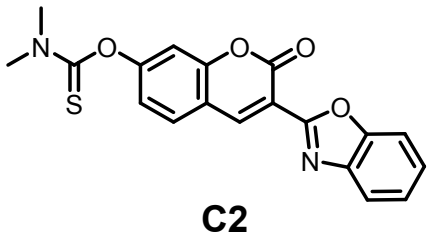
C7p

Synthesis of C7p. C5p (26.9 mg, 0.1 mmol) and 1,4-dimethylpyridin-1-ium iodide (46.8 mg, 0.2 mmol) were dissolved into 5 ml EtOH, then piperidine (0.05 ml, 0.54 mmol) were added sequentially. And the reaction mixture heated at reflux for 6 h. After the reaction was complete, the mixture solution was cooled to room temperature. The light yellow solid formed was filtered and washed with a small amount of methanol several times. Compound **C7p** was dried in vacuum and then obtained with a yield of 54.0% (25.3 mg). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.39 (s, 3H), 4.26 (s, 3H), 7.53 (t, J = 8.0 Hz, 1H), 7.61 (t, J = 8.0 Hz, 1H), 7.67 (d, J = 8.0 Hz, 1H), 7.80 (s, 2H), 8.09 (s, 2H), 8.23 (s, 3H), 8.85 (s, 2H), 13.09 (s, 1H); HRMS (ESI): *m/z* found $[M\text{-}I]^+$ for $C_{22}H_{19}N_2OS^+$ 359.1198, calcd 359.1213.



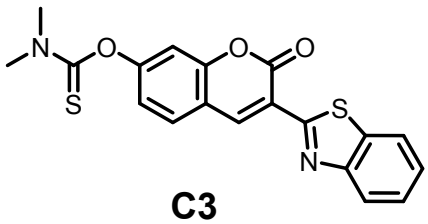
C1

Synthesis of C1. A mixture of 23.4 mg (0.1 mmol) compound **C1p**, 24.7 mg (0.20 mmol) N, N-dimethylaminothioformyl chloride and 30.4 mg (0.22 mmol) potassium carbonate were taken in 5 mL of DMF and stirred at 70 °C monitoring with TLC. After the reaction was complete, the mixture solution was cooled to room temperature and then poured in ice-cold water (50 mL), the precipitate was filtered, washed with brine, and then dried under vacuum to give the crude product, which was further purified by chromatography on a silica gel column (CH_2Cl_2:CH_3OH = 300:1 v:v) to give a product **C1** (7.3 mg) in 20.2% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.36 (t, J = 7.6 Hz, 3H), 3.33 (s, 3H), 3.41 (s, 3H), 4.36 (q, J = 7.2 Hz, 2H), 7.04 (s, 1H), 7.06 (d, J = 8.4 Hz, 1H), 7.59 (d, J = 8.6 Hz, 1H), 8.49 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 14.4, 39.3, 43.3, 61.8, 111.7, 115.5, 117.5, 120.4, 120.8, 130.2, 148.7, 155.8, 156.5, 158.3, 163.0, 186.1; HRMS (ESI): *m/z* found $[M+K]^+$ for $C_{15}H_{15}NO_5SK^+$ 360.0330, calcd 360.0303.



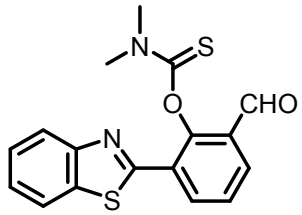
C2

Synthesis of C2. A mixture of compound **C2p** (27.9 mg, 0.1 mmol), N, N-dimethylaminothioformyl chloride (12.7 mg, 0.1 mmol) and potassium carbonate (60.7 mg, 0.44 mmol) were taken in 5 mL of CH_2Cl_2. Finally, the probe **C2** was obtained (20.5 mg, 56%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 3.39 (s, 3H), 3.47 (s, 3H), 7.13 (d, J = 7.4 Hz, 1H), 7.15 (s, 1H), 7.40 (m, 2H), 7.63 (d, J = 8.2 Hz, 1H), 7.68 (d, J = 6.6 Hz, 1H), 7.86 (d, J = 7.0 Hz, 1H), 8.77 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 39.1, 43.3, 110.9, 111.7, 114.4, 116.3, 120.8, 124.9, 126.3, 129.5, 134.4, 134.9, 141.8, 144.6, 150.6, 155.0, 156.3, 158.0, 186.3; HRMS (ESI): *m/z* found $[M+H]^+$ for $C_{19}H_{15}N_2O_4S^+$ 367.0742, calcd 367.0753.



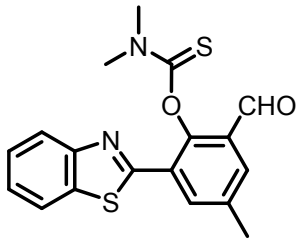
C3

Synthesis of C3. The probe **C3** was also prepared by referring to the preparation procedure of **C1**. In brief, a mixture of compound **C3p** (12.7 mg, 0.43 mmol), N, N-dimethylaminothioformyl chloride (27.9 mg, 0.1 mmol) and potassium carbonate (60.7 mg, 0.44 mmol) were taken in 5 mL of CH_2Cl_2. Finally, the probe **C3** was obtained (10.2 mg, 61.8%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 3.34 (s, 3H), 3.53 (s, 3H), 7.20 (d, J = 8.4 Hz, 1H), 7.24 (s, 1H), 7.49 (t, J = 7.8 Hz, 1H), 7.59 (m, 1H), 7.79 (m, 1H), 8.04 (m, 1H), 8.15 (d, J = 8.2 Hz, 1H), 9.15 (s, 1H); HRMS (ESI): *m/z* found $[M+Na]^+$ for $C_{19}H_{14}N_2O_3S_2Na^+$ 405.0320, calcd 405.0344.



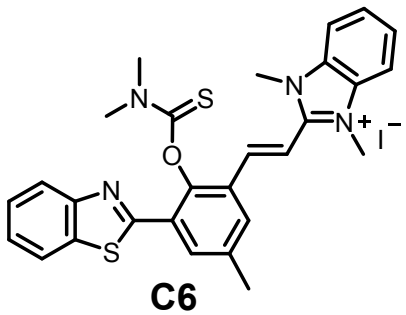
C4

Synthesis of C4. N, N-dimethylaminothioformyl chloride (369 mg, 3 mmol), **C4p** (269 mg, 1 mmol) and K_2CO_3 (276 mg, 2 mmol) were dissolved in anhydrous DMF. After stirring at room temperature for 2 h, distilled water was added to the mixed solution and stirred at room temperature for 5 min. The solid was collected by filtration, washed with ether for 3 times, and dried under vacuum to give **C4** (190 mg, 52.3%) as a canary yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.05 (s, 1H), 8.63 (d, J = 8.0 Hz, 1H), 8.19 (d, J = 8.0 Hz, 1H), 8.12 (d, J = 8.0 Hz, 1H), 8.08 (d, J = 8.0 Hz, 1H), 7.66 (t, J = 6.0 Hz, 1H), 7.58 (t, J = 6.0 Hz, 1H), 7.50 (t, J = 6.0 Hz, 1H), 3.60 (s, 3H), 3.42 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): 43.7, 43.8, 122.7, 123.5, 126.3, 127.2, 127.6, 128.2, 130.8, 132.6, 134.6, 135.4, 152.4, 161.2, 189.2, 192.4; HRMS (ESI): *m/z* found for $C_{18}H_{16}N_2O_2S_2Na^+$ $[M+Na]^+$ 365.0356, calcd 365.0394.



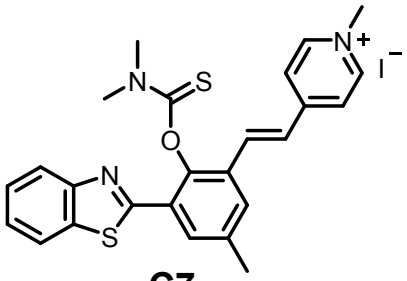
C5

Synthesis of C5. N, N-dimethylaminothioformyl chloride (369 mg, 3 mmol), **C5p** (269 mg, 1 mmol) and K_2CO_3 (276 mg, 2 mmol) were dissolved in anhydrous DMF. After stirring at room temperature for 2 h, distilled water was added to the mixed solution and stirred at room temperature for 5 min. The solid was collected by filtration, washed with ether for 3 times, and dried under vacuum to give **C5** as a canary yellow solid (180 mg, 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 10.05 (s, 1H); 8.41 (s, 1H); 8.08 (d, J = 8.0 Hz, 1H); 7.92 (d, J = 8.0 Hz, 1H); 7.82 (s, 1H); 7.51 (t, J = 8.0 Hz, 1H); 7.43 (t, J = 6.0 Hz, 1H); 3.58 (s, 3H); 3.50 (s, 3H); 2.51 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): 188.3, 186.4, 161.2, 150.4, 136.0, 126.5, 125.6, 123.4, 43.8, 39.6, 20.9; HRMS (ESI): *m/z* found $[M+Na]^+$ for $C_{18}H_{16}N_2NaO_2S_2^+$ 379.0552, calcd 379.0551.



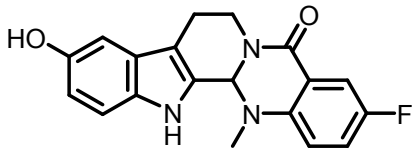
C6

Synthesis of C6. C5 (35.6 mg, 0.1 mmol) was dissolved into 2.5 ml acetic anhydride, then sodium acetate (27.2 mg, 0.2 mmol) and 1,2,3-trimethyl-1H-benzo imidazol-3-ium iodide (57.6 mg, 0.2 mmol) were added sequentially. After the mixture was stirred at 80 °C for 24 h, ether was added into the solution and the product was collected by filtration as a yellow solid (34.4 mg, 55%). ^{1}H NMR (400 MHz, $CDCl_3$ & CF_3COOD) δ (ppm): 8.45 (d, J = 8.0 Hz, 1H); 8.30 (s, 1H); 8.14 (d, J = 8.0 Hz, 1H); 8.09 (s, 1H); 7.88 (t, J = 8.0 Hz, 1H); 7.81 (t, J = 8.0 Hz, 1H); 7.72 (s, 4H); 7.52 (d, J = 20.0 Hz, 1H); 7.31 (d, J = 20.0 Hz, 1H); 4.11 (s, 6H); 3.53 (s, 3H); 3.43 (s, 3H); 2.57 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): 184.5, 168.1, 148.2, 147.0, 140.9, 139.4, 133.3, 132.0, 130.9, 129.5, 128.3, 127.5, 123.2, 121.4, 118.7, 118.4, 115.9, 113.0, 112.8, 112.2, 110.2, 44.2, 40.0, 33.6, 32.0, 20.5; HRMS (ESI): *m/z* found $[M-I]^+$ for $C_{28}H_{27}N_4OS_2^+$ 499.1605, calcd 499.1621.



C7

Synthesis of C7. C5 (35.6 mg, 0.1 mmol) was dissolved into 2.5 ml acetic anhydride, then sodium acetate (27.2 mg, 0.2 mmol) and 1,4-dimethylpyridin-1-ium iodide (46.8 mg, 0.2 mmol) were added sequentially. After the mixture was stirred at 80 °C for 24 h, ether was added into the solution and the product was collected by filtration as a yellow solid (34.3 mg, 60%).^{1}H NMR (400 MHz, $CDCl_3$ & CD_3CN) δ (ppm): 8.52 (d, J = 8.0 Hz, 2H); 8.14 (s, 1H); 8.02 (t, J =6.0 Hz, 4H); 7.87 (s, 1H); 7.67 (d, J = 12.0 Hz, 1H); 7.48 (t, J = 8.0 Hz, 1H); 7.41 (d, J =16.0 Hz, 1H); 4.24 (s, 3H); 3.63 (s, 3H); 3.42 (s, 3H); 2.52 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): 185.2, 173.6, 162.4, 152.7, 152.1, 148.0, 145.9, 136.8, 135.5, 133.2, 132.4, 131.9, 130.8, 130.5, 127.8, 127.2, 126.6, 126.2, 124.5, 123.4, 122.7, 47.6, 43.9, 23.1, 21.0; HRMS (ESI): *m/z* found $[M-I]^+$ for $C_{25}H_{24}N_3OS_2^+$ 446.1335, calcd 446.1355.



HF-EVO

Synthesis of HF-EVO. The compound **HF-EVO** was synthesized according to a reported procedure.[6] ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.83 (s, 1H), 8.72 (s, 1H), 7.51 (d, J = 8.0 Hz, 1H), 7.31-7.35 (m, 1H), 7.13-7.15 (m, 1H), 6.77 (s, 1H), 6.62 (d, J = 8.0 Hz, 1H), 6.04 (s, 1H), 4.56-4.61 (m, 1H), 3.14-3.2 (m, 1H), 2.78-2.82 (m, 1H), 2.73-2.74 (m, 1H), 2.68 (s, 3H). HRMS (ESI): *m/z* found $[M+H]^+$ for $C_{19}H_{17}FN_3O_2^+$ 338.1373, calcd 338.1305.

Characterization of Compounds

Table S3. Crystal data and structure refinement summary for **A**, **C1-C4**.

	A	**C1**	**C2**	**C3**	**C4**
Empirical formula	$C_{19}H_{14}N_2O_4$	$C_{15}H_{15}NO_5S$	$C_{19}H_{14}N_2O_4S$	$C_{19}H_{14}N_2O_3S_2$	$C_{17}H_{14}N_2O_2S_2$
Formula weight	366.38	321.34	366.38	382.44	342.42
Crystal system	Triclinic	Triclinic	Triclinic	Triclinic	trigonal
Space group	*P*-1	*P*-1	*P*-1	*P*-1	*R*-3
a (Å)	7.806(2)	6.2237(15)	8.087(2)	6.515(8)	28.700(4)
b (Å)	12.459(4)	10.551(3)	9.953(3)	11.778(16)	28.700(4)
c (Å)	17.312(5)	12.693(3)	12.356(5)	22.99(3)	11.8086(19)
A (°)	85.886(6)	98.868(4)	111.892(6)	90	90
B (°)	89.534(6)	99.615(4)	94.737(6)	99.01(4)	90
γ (°)	76.326(5)	106.235(4)	110.387(4)	90	120
V (Å^3)	1631.7(8)	771.0(3)	838.8(5)	1742(4)	8423(3)
Z	4	2	2	4	18
D_c (g m^{-3})	1.491	1.384	1.451	1.458	1.215
μ (mm^{-1})	0.228	0.232	0.221	0.328	0.293
F (000)	760	336	380	792	3204.0
Reflns collected	9138/6526	4227/3034	4597/3261	8215/3063	14319/3327
R_{int}	0.0338	0.0187	0.0273	0.1577	0.0487
GOF on F^2	0.961	1.032	1.011	0.823	1.021
R_1[a] [$I > 2\sigma(I)$]	0.0642	0.0526	0.0493	0.0629	0.0468
wR_2[b] (all data)	0.1592	0.1423	0.1359	0.1693	0.1240
CCDC	1586906	1571172	1586907	1571173	1863813

Table S4. Crystal data and structure refinement summary for **C5**-**C7** and **C7c**.

	C5	**C6**	**C7**	**C7c**
Empirical formula	$C_{18}H_{16}N_2O_2S_2$	$C_{28}H_{27}IN_4O_2S_2$	$C_{25}H_{22}IN_3OS_2$	$C_{15}H_{16}INO$
Formula weight	356.45	642.56	571.47	353.19
Crystal system	monoclinic	Monoclinic	orthorhombic	triclinic
Space group	$P2_1/c$	P2(1)/c	Pbca	*P*-1
a (Å)	11.9911(19)	11.4238(13)	13.4772(13)	7.2725(11)
b (Å)	16.171(3)	20.241(2)	20.032(2)	10.5716(17)
c (Å)	11.1317(18)	13.3064(14)	20.904(2)	10.5761(16)
A (°)	90	90	90	88.060(2)
B (°)	117.048(3)	115.300(2)	90	74.048(2)
γ (°)	90	90	90	70.689(2)
V (Å^3)	1922.5(5)	2781.7(5)	5643.6(10)	736.4(2)
Z	4	4	8	2
D_c (g m^{-3})	1.232	1.534	1.345	1.593
μ (mm^{-1})	0.288	1.334	1.302	2.163
F (000)	744.0	1296	2288.0	348.0
Reflns collected	10165/3784	13959/4944	29198/5662	4107/2982
R_{int}	0.0342	0.0487	0.0512	0.0156
GOF on F^2	1.021	1.033	1.040	1.034
R_1[a] [$I > 2\sigma(I)$]	0.0498	0.0553	0.0535	0.0319
wR_2[b] (all data)	0.1313	0.1795	0.1523	0.0798
CCDC	1863814	1863815	1863816	1863817

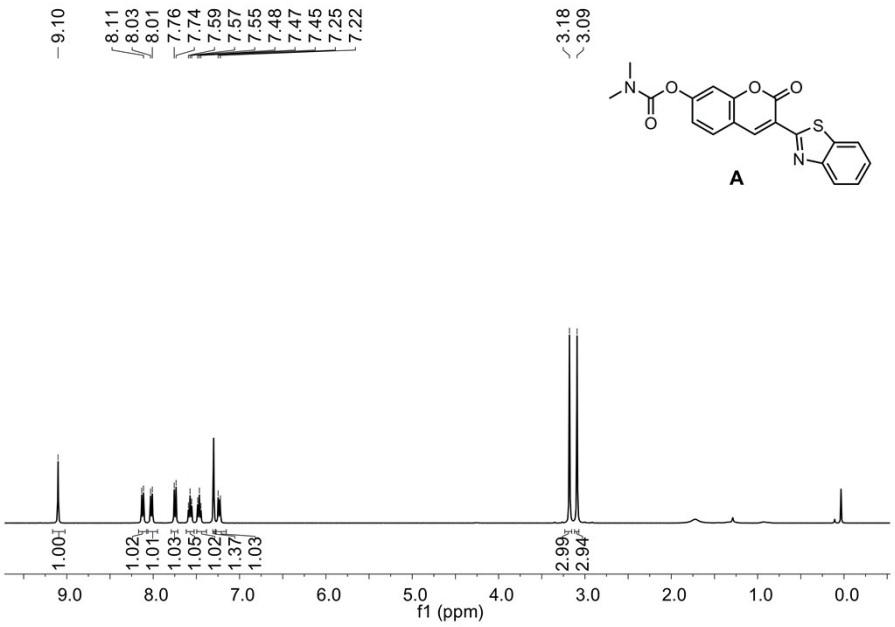


Fig. S29 ^{1}H NMR spectrum of **A** recorded in $CDCl_3$.

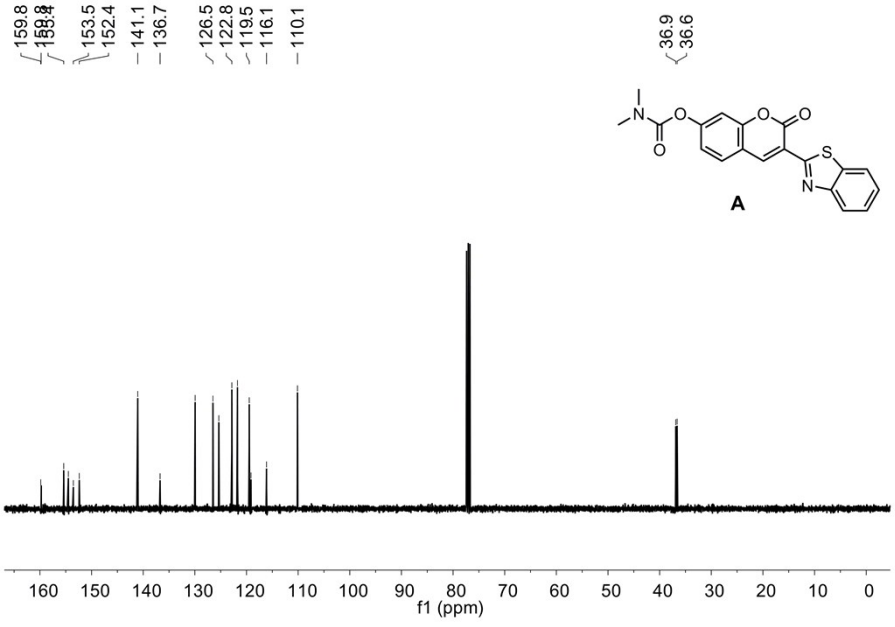


Fig. S30 ^{13}C NMR spectrum of **A** recorded in $CDCl_3$.

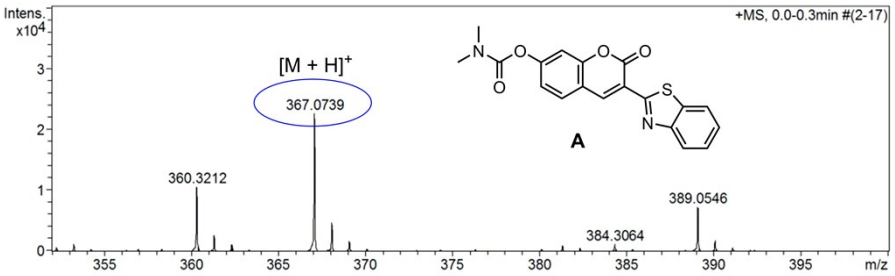


Fig. S31 ESI-MS spectrum of **A**.

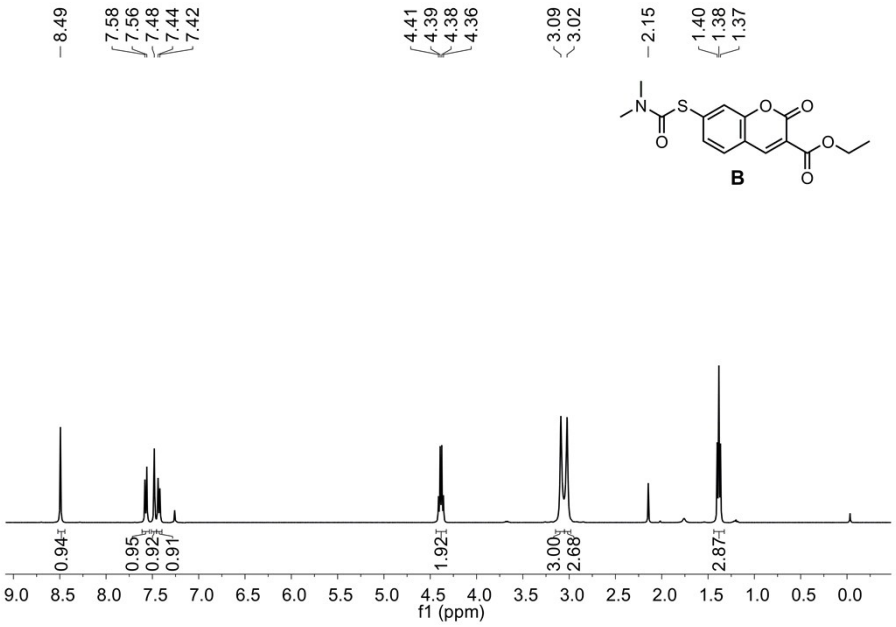


Fig. S32 ^{1}H NMR spectrum of **B** recorded in $CDCl_3$.

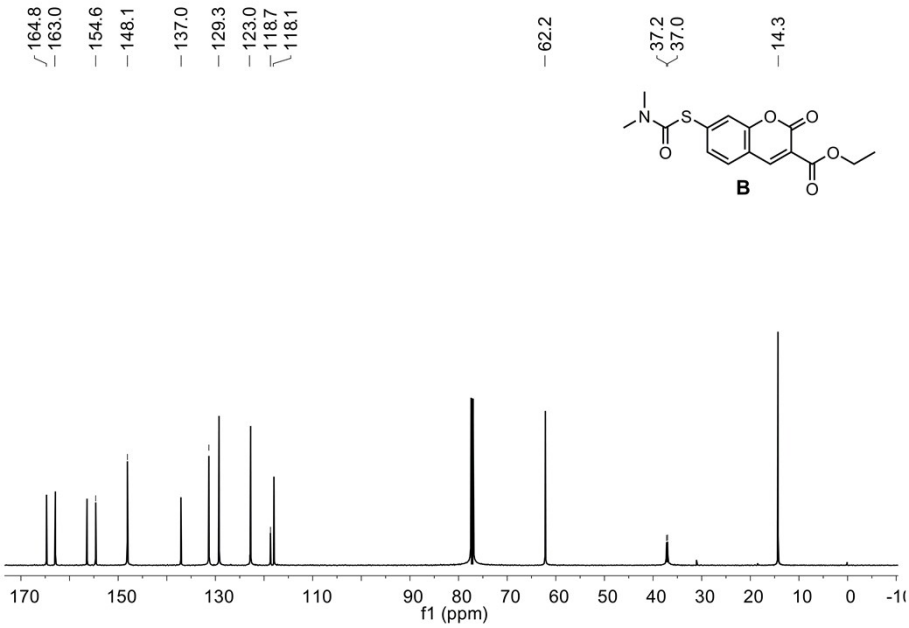


Fig. S33 ^{13}C NMR spectrum of **B** recorded in $CDCl_3$.

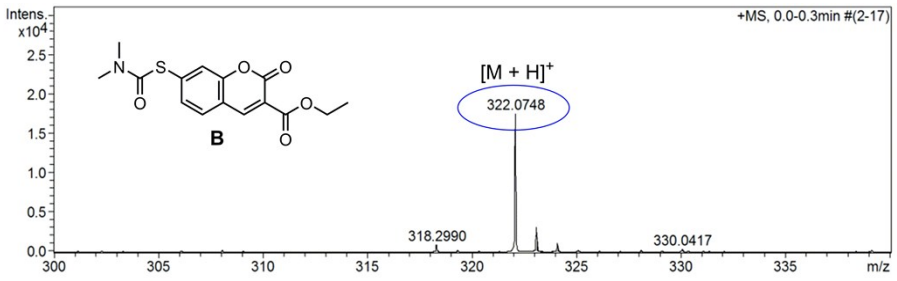


Fig. S34 ESI-MS spectrum of **B**.

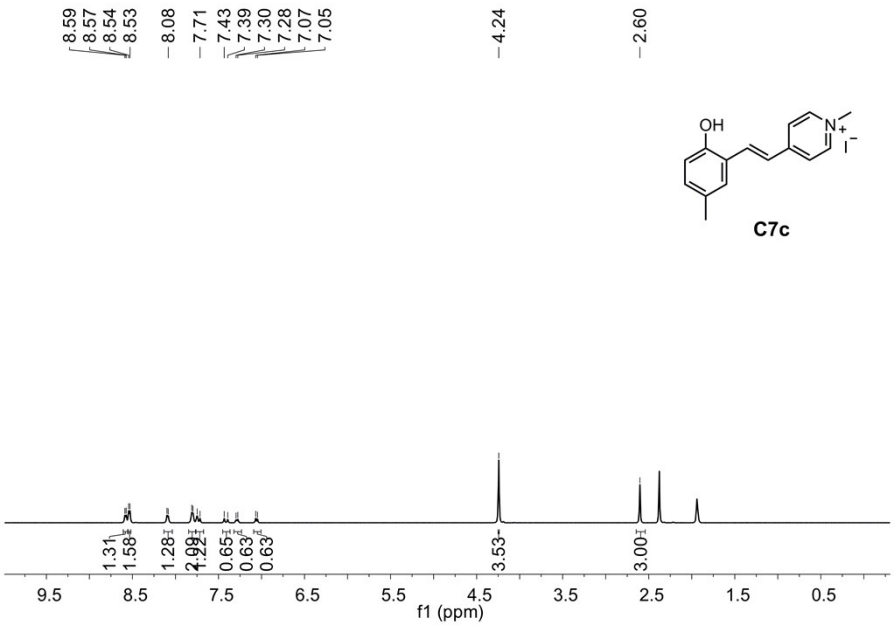


Fig. S35 ^{1}H NMR spectrum of **C7c** recorded in CD_3CN.

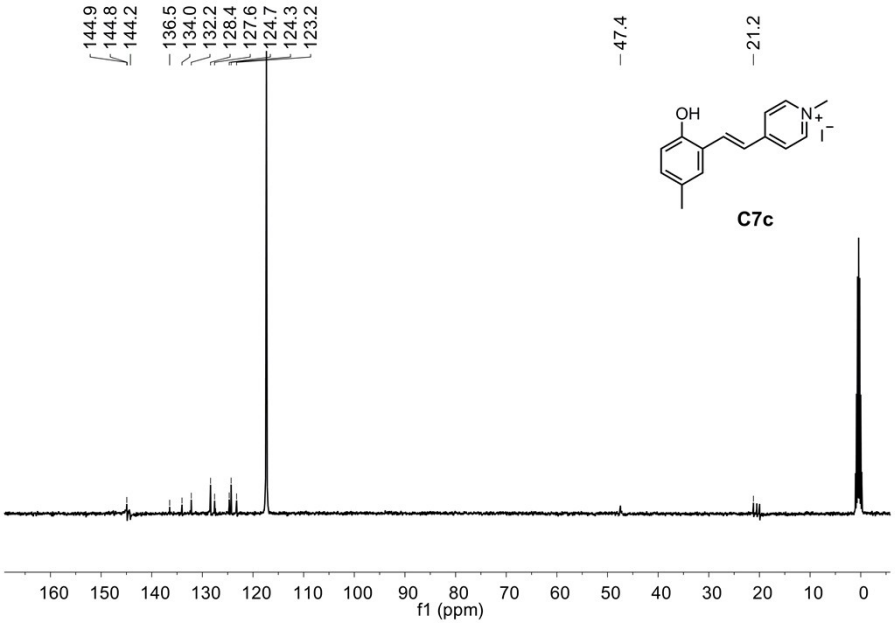


Fig. S36 ^{13}C NMR spectrum of **C7c** recorded in CD_3CN.

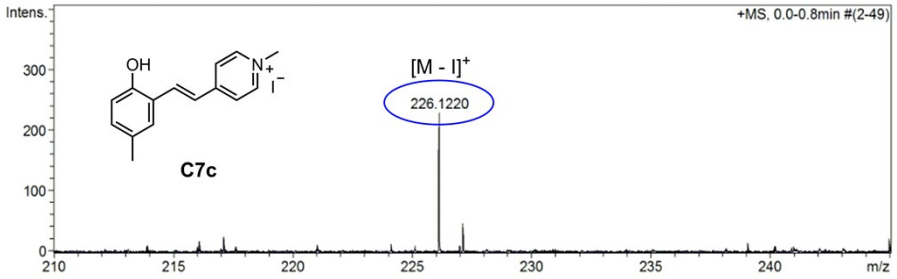


Fig. S37 ESI-MS spectrum of **C7c**.

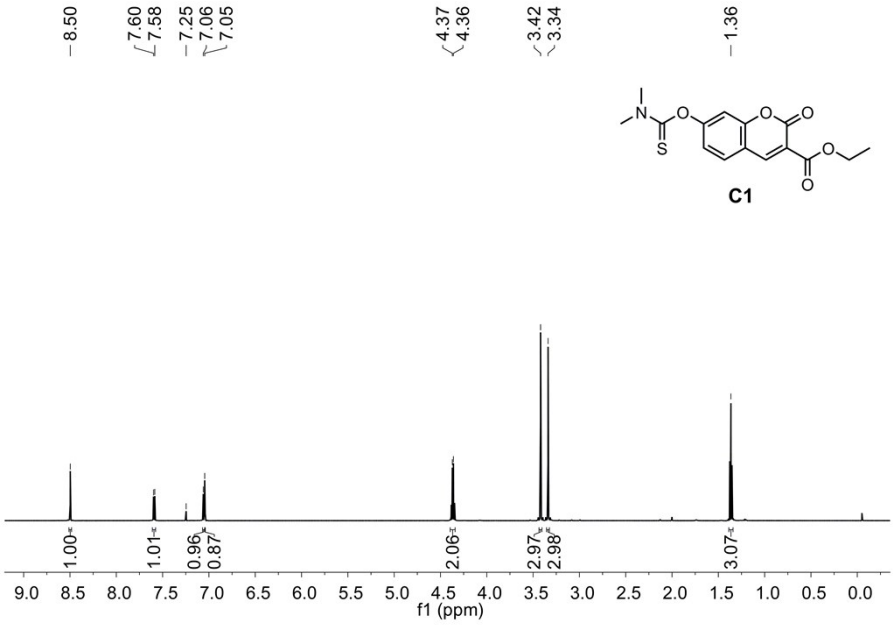


Fig. S38 ^{1}H NMR spectrum of probe **C1** recorded in $CDCl_3$.

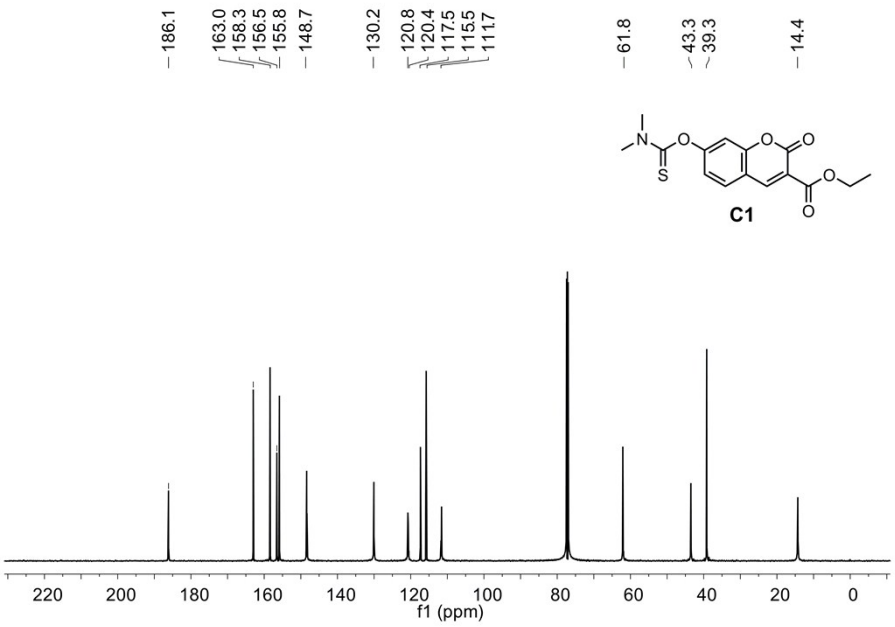


Fig. S39 ^{13}C NMR spectrum of probe **C1** recorded in $CDCl_3$.

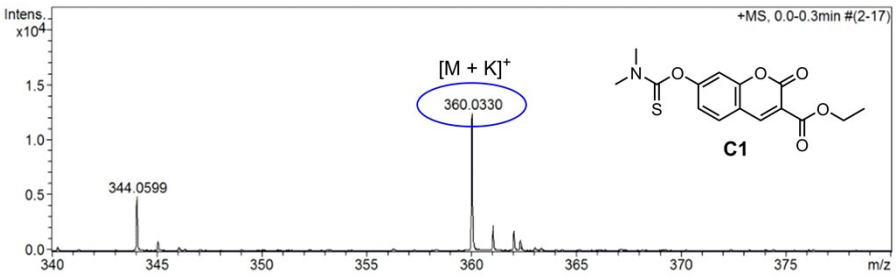


Fig. S40 ESI-MS spectrum of probe **C1**.

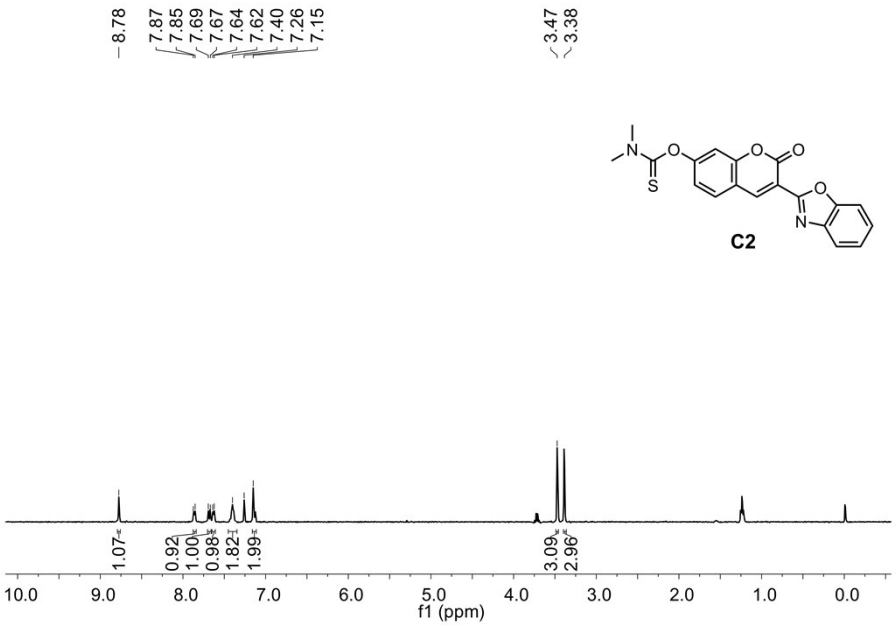


Fig. S41 ^{1}H NMR spectrum of probe **C2** recorded in $CDCl_3$.

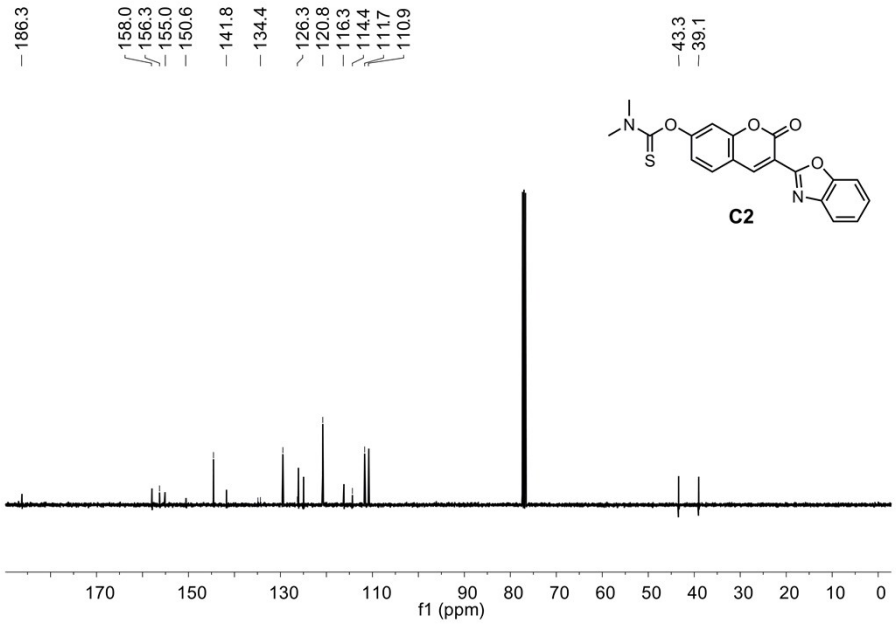


Fig. S42 ^{13}C NMR spectrum of probe **C2** recorded in $CDCl_3$.

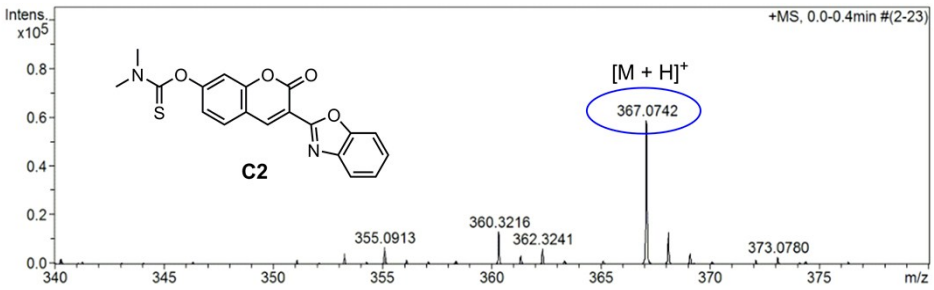


Fig. S43 ESI-MS spectrum of probe **C2**.

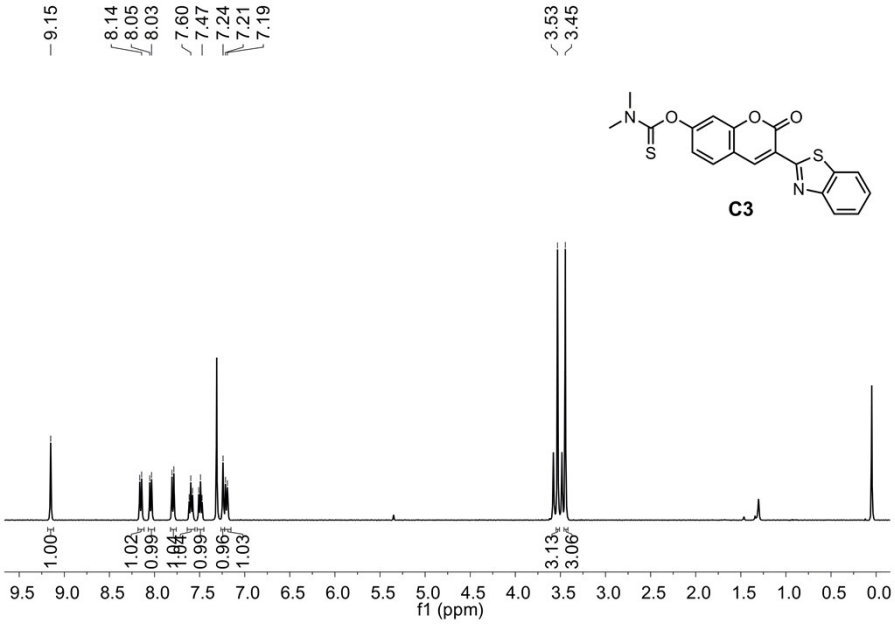


Fig. S44 ^{1}H NMR spectrum of probe **C3** recorded in $CDCl_3$.

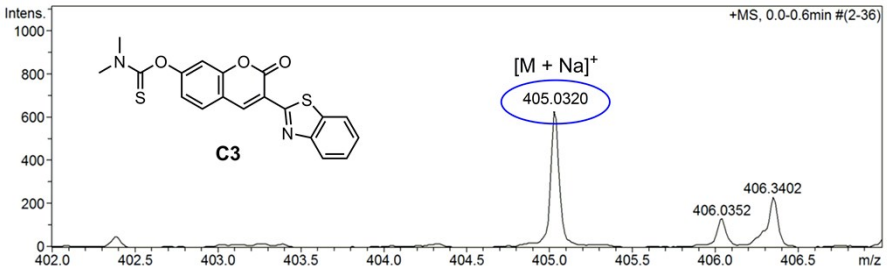


Fig. S45 ESI-MS spectrum of probe **C3**.

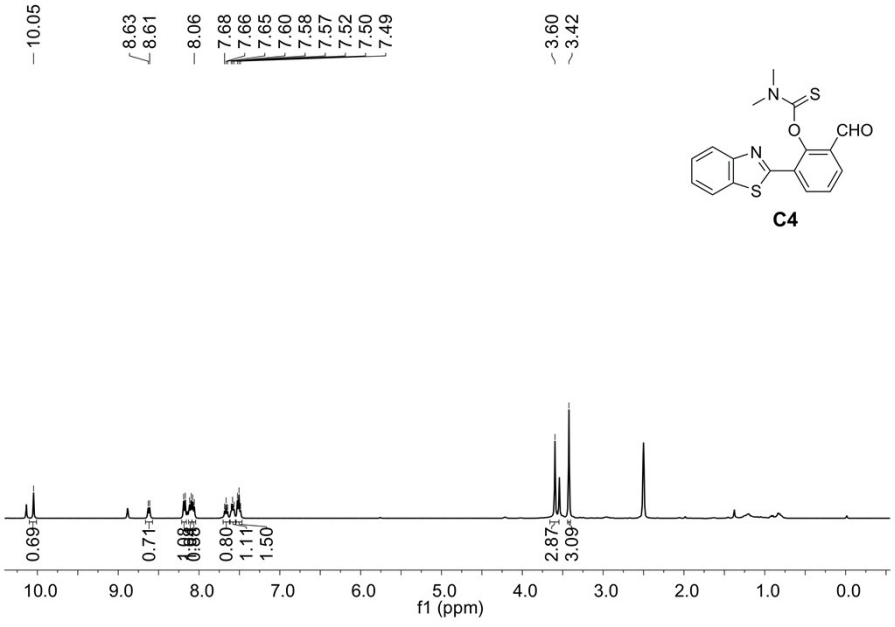


Fig. S46 ^{1}H NMR spectrum of probe **C4** recorded in DMSO-d_6.

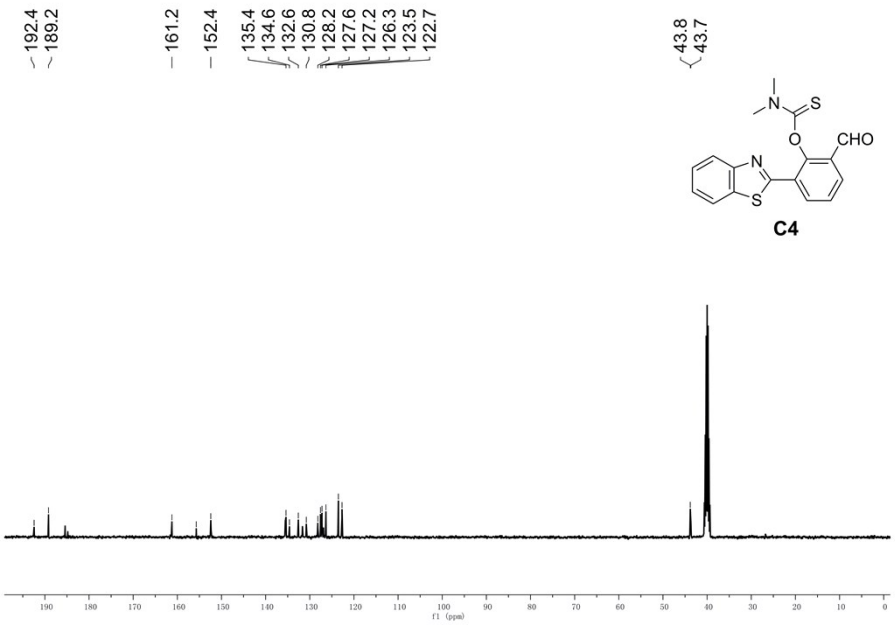


Fig. S47 ^{13}C NMR spectrum of probe **C4** recorded in DMSO-d_6.

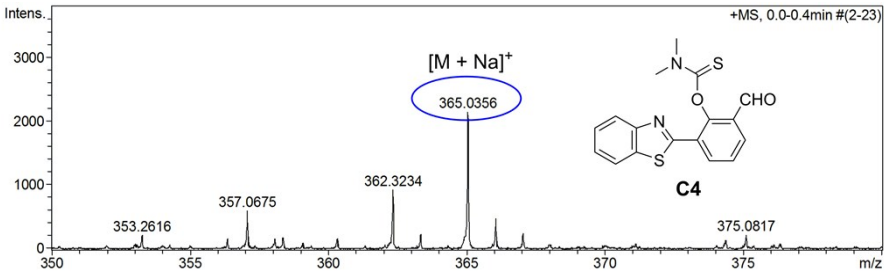


Fig. S48 ESI-MS spectrum of probe **C4**.

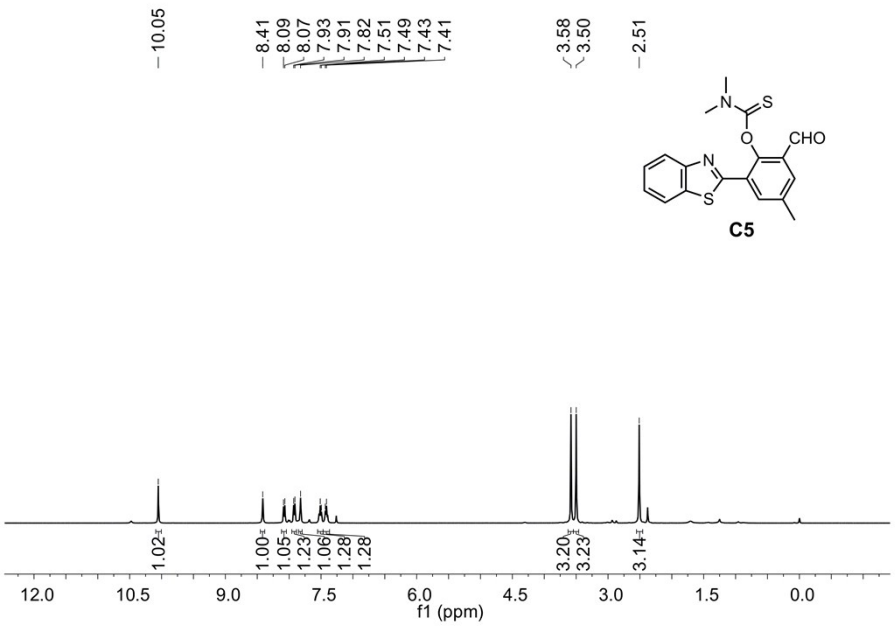


Fig. S49 ^{1}H NMR spectrum of probe **C5** recorded in $CDCl_3$.

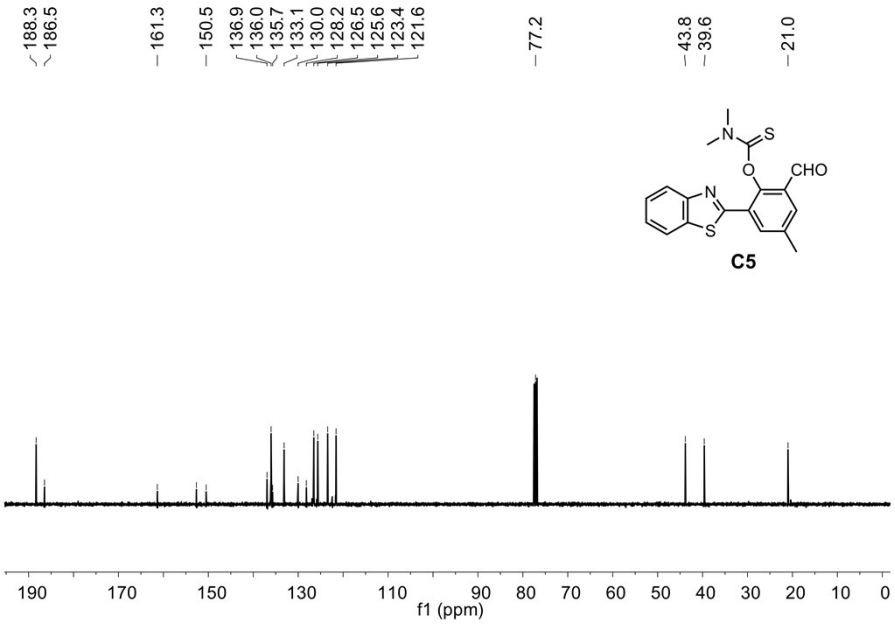


Fig. S50 ^{13}C NMR spectrum of probe **C5** recorded in $CDCl_3$.

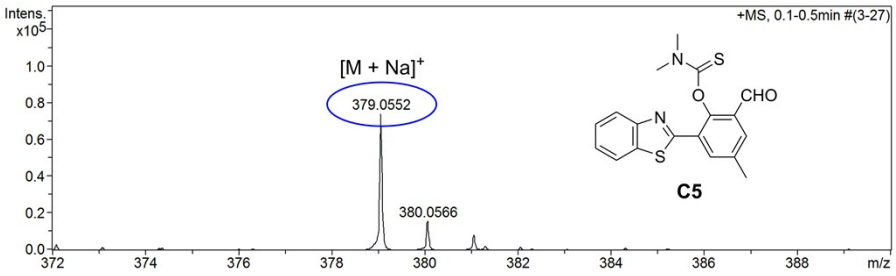


Fig. S51 ESI-MS spectrum of probe **C5**.

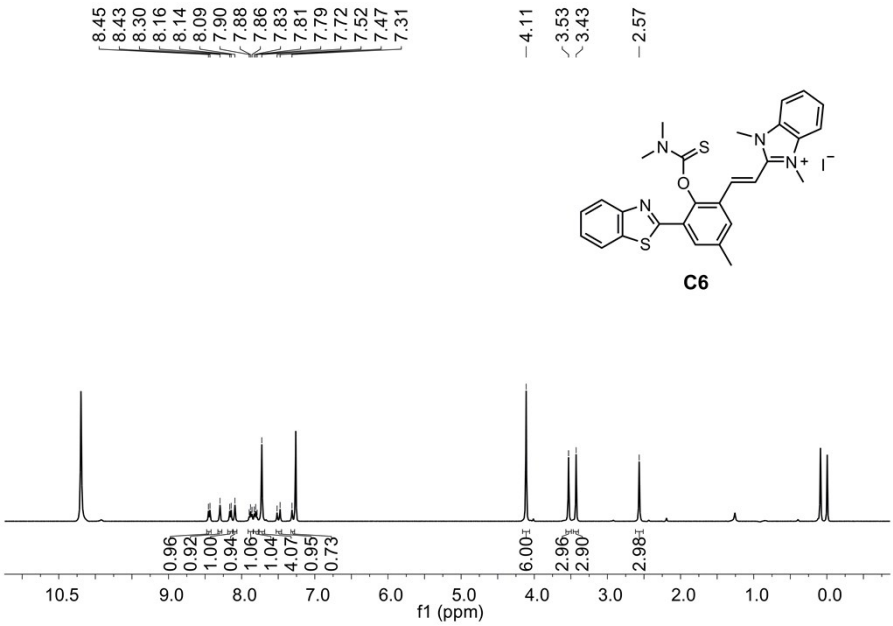


Fig. S52 [1] H NMR spectrum of probe **C6** recorded in $CDCl_3$ and CF_3COOD.

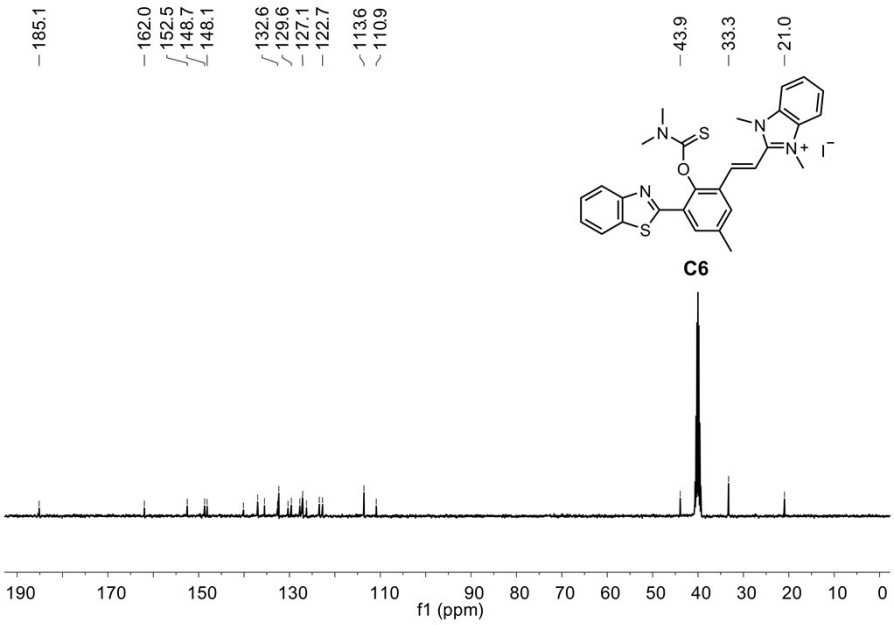


Fig. S53 ^{13}C NMR spectrum of probe **C6** recorded in DMSO-d_6.

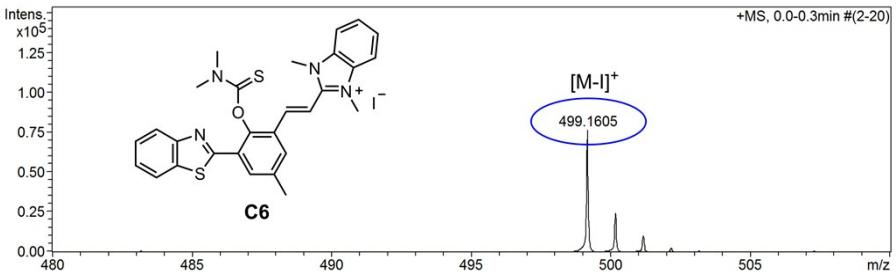


Fig. S54 ESI-MS spectrum of probe **C6**.

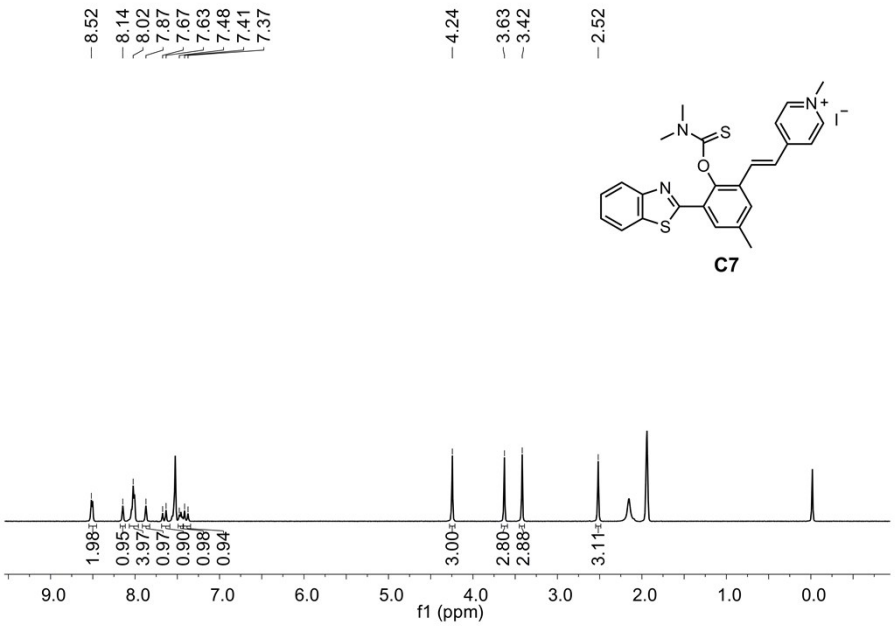


Fig. S55 ^{1}H NMR spectrum of probe **C7** recorded in $CDCl_3$ and CD_3CN.

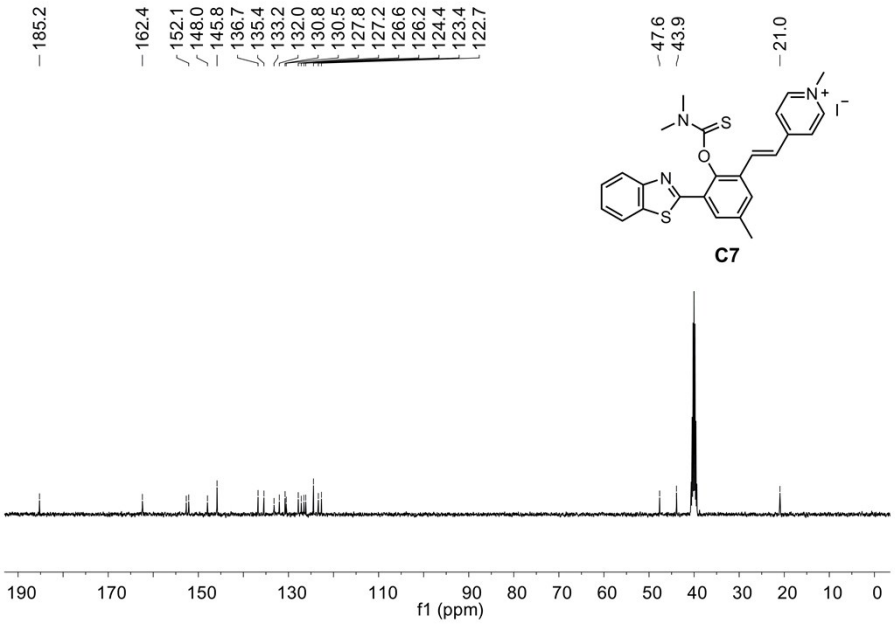


Fig. S56 ^{13}C NMR spectrum of probe **C7** recorded in DMSO-d_6.

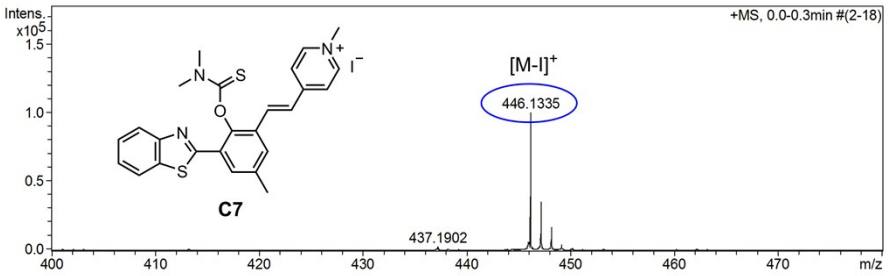


Fig. S57 ESI-MS spectrum of probe **C7**.

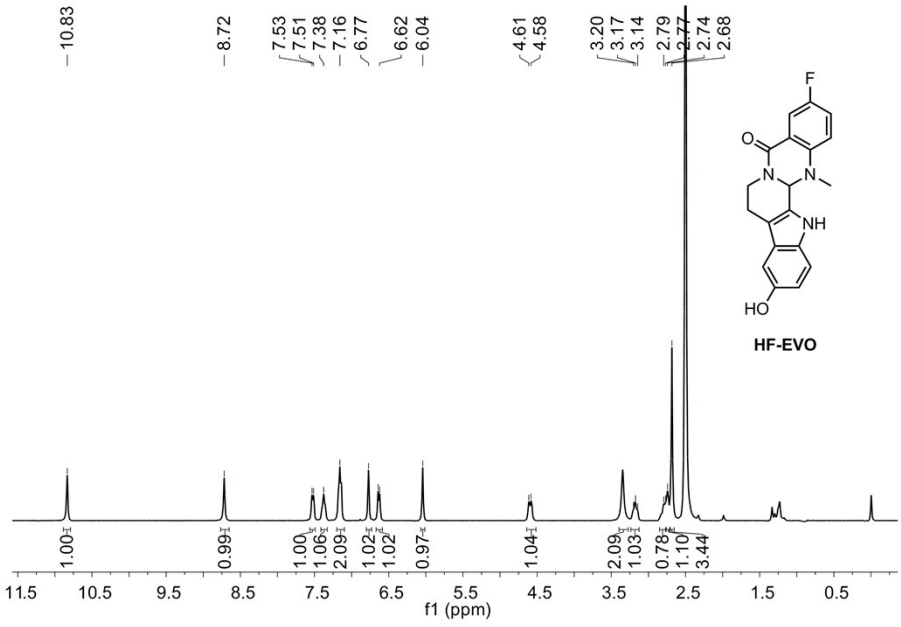


Fig. S58 ^{1}H NMR spectrum of **HF-EVO** recorded in DMSO-d_6.

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