# A dual-ratiometric fluorescent probe for individual and continuous detection of $H_2S$ and HClO in living cells

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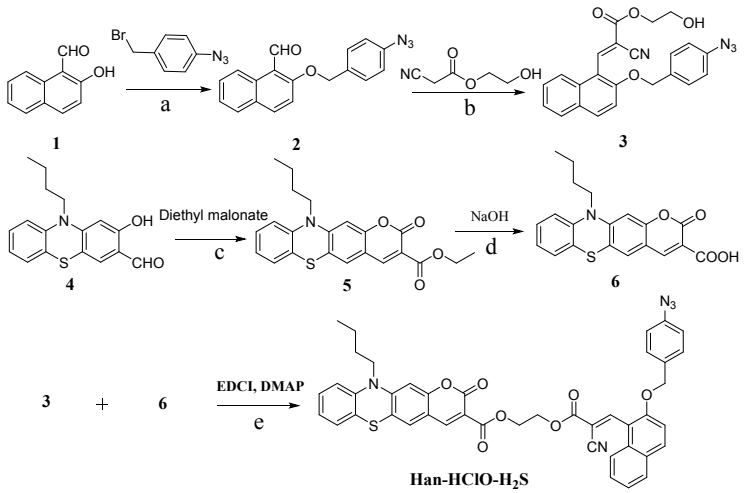
## 1. Experimental

### *1.1. Materials and instruments*

Chemicals and reagents were purchased from the supplier and used without further purification. Solvents were analytical pure. Twice-distilled water was used in the experiments. NaClO and $Na_2S$ were used as the sources of HClO and $H_2S$, respectively. $^1H$ NMR and $^{13}C$ NMR spectra were obtained using a Bruker 400 spectrometer with chemical shifts reported in ppm (TMS as an internal standard). Mass spectra were obtained on a Bruker Daltonics micr-OTOF-Q II mass spectrometer. Emission spectra were recorded on a Hitachi F-7000 fluorometer, and UV-vis absorption spectra were recorded on an Agilent UV-2450 spectrophotometer. A Leici PHS-3C meter was used for the pH measurements. Fluorescence imaging experiments were conducted on an operetta CLS from the company of PerkinElmer. MCF-7 cells were provided by the State Key Laboratory of Chemo/Biosensing and Chemometrics, Hunan University, China.

### *1.2. Synthesis*




**Scheme S1.** The synthetic route of probe **Han-HClO-$H_2S$**. (a) $K_2CO_3$, $CH_3CN$, reflux for 4 h, 78% yield; (b) piperidine, $CH_3CH_2OH$, 25 °C, 4 h, 64% yield; (c) piperidine, $CH_3CH_2OH$, 25 °C, 2 h, 63% yield; (d) NaOH, $H_2O$, reflux for 0.5 h, 91% yield; (e) EDCI, DMAP, $CH_2Cl_2$, 25 °C, 5 h, 69% yield.

**Synthesis of compounds 4 and 5**.

Compounds **4** and **5** were synthesized according to the reported methods [1-2].

**Synthesis of compound 2**

Compound **1** (172 mg, 1 mmol), 1-azido-4-(bromomethyl) benzene (211 mg, 1 mmol), and $K_2CO_3$ (414 mg, 3 mmol) were dissolved in anhydrous $CH_3CN$ (10 mL) at room temperature under argon atmosphere. The reaction mixture was stirred at 80 ºC for 4 h. After cooling to room temperature, the mixture was extracted with 30 mL $CH_2Cl_2$ three times. The organic layer was dried over $Na_2SO_4$, and the solvent was evaporated under a reduced pressure. The crude product was chromatographed on silica gel to give as a yellow solid (238 mg, 78% yield). $^1H$ NMR (400 MHz, $CDCl_3$) δ 10.93 (s, 1H), 9.27 (d, $J$ = 8.7 Hz, 1H), 8.03 (d, $J$ = 9.1 Hz, 1H), 7.77 (d, $J$ = 8.1 Hz, 1H), 7.62 (t, $J$ = 7.3 Hz, 1H), 7.43 (t, $J$ = 6.9 Hz, 3H), 7.30 (d, $J$ = 9.1 Hz, 1H), 7.06 (d, $J$ = 8.4 Hz, 2H), 5.28 (s, 2H). $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 191.96, 162.97, 140.30, 137.55, 132.56, 131.55, 129.99, 129.10, 128.78, 128.27, 125.05, 125.02, 119.42, 117.35, 113.91, 71.01. HRMS (ESI) m/z calcd for $C_{18}H_{13}N_3O_3$ $[M+Na]^+$: 326.0900; found 326.0905.

**Synthesis of compound 3**

To a stirred solution of 2-hydroxyethyl-2-cyanoacetate (65 mg, 0.5 mmol) and compound **2** (128 mg, 0.42 mmol) in ethanol (4 mL) was added piperidine (11 μL, 0.110 mmol) at room temperature under argon. The reaction mixture was allowed to stir at room temperature for 4 h. Following the removal of the solvent under a reduced pressure, the crude product was chromatographed on silica gel to give as a pale yellow solid (112 mg, 64% yield); $^1H$ NMR (400 MHz, $CDCl_3$) δ 8.81 (s, 1H), 7.88 (t, $J$ = 9.1 Hz, 1H), 7.78 (t, $J$ = 7.5 Hz, 1H),

7.73 (d, $J$ = 8.5 Hz, 1H), 7.57 – 7.48 (m, 1H), 7.45 – 7.36 (m, 3H), 7.24 (t, $J$ = 11.4 Hz, 1H), 7.01 (t, $J$ = 10.3 Hz, 2H), 5.29 (s, 2H), 4.47 – 4.39 (m, 2H), 3.97 – 3.87 (m, 2H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 162.46, 155.31, 152.10, 139.98, 133.96, 132.99, 131.74, 129.03, 128.98, 128.80, 128.68, 128.21, 124.82, 123.51, 119.34, 115.22, 115.14, 114.07, 109.71, 70.71, 68.08, 60.68. HRMS (ESI) m/z calcd for $C_{23}H_{18}N_4O_4$ $[M+Na]^+$: 437.1220; found : 437.1233.

**Synthesis of compound 6**

To a solution of compound **5** (790 mg, 2 mmol) in MeOH was added NaOH (240 mg, 72.6 mmol), and the reaction mixture was refluxed for 0.5 h. Then the solvent was removed under a reduced pressure and the residue was dissolved in $CH_2Cl_2$ (50 mL) and was acidified to pH 3-4 with 10% HCl. After washing with brine and water, the organic layer was dried over anhydrous sodium sulfate and was concentrated in vacuum to give compound **6** as an oxblood red solid (672 mg, 91% yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 7.29 (s, 1H), 7.21 (t, J = 7.7 Hz, 1H), 7.11 (d, J = 7.5 Hz, 1H), 7.03 (t, J = 7.5 Hz, 1H), 6.94 (d, J = 8.2 Hz, 1H), 6.77 (s, 1H), 3.91 (t, J = 7.3 Hz, 2H), 1.88 – 1.76 (m, 2H), 1.51 (dt, J = 14.8, 7.4 Hz, 2H), 0.99 (t, J = 7.3 Hz, 3H).

### *1.3. Spectral measurements*

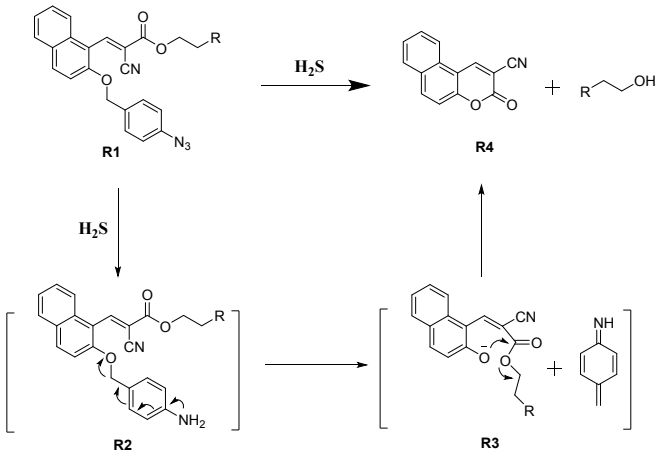
The stock solution of probe **Han-HClO-$H_2S$** (1.0 mM) was prepared in $CH_3CN$. The stock solutions of various testing species (10.0 mM) were prepared in distilled water. The media used in all the spectral measurements except pH study was PBS buffer (pH 7.4, 10 mM, containing 50% $CH_3CN$). For the titration experiments, different amounts of $H_2S$ or/and HClO were added into the solution of the probe (5.0 μM) in a 2.0 mL PBS buffer (pH 7.4, 10 mM, containing 50% $CH_3CN$). The resulting solutions were shaken well and then was incubated for 100 min at 25 °C before the spectra were measured.

### *1.4. Cell culture*

MCF-7 cells were seeded in culture dishes in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin and cultured in a humidified incubator containing 5% $CO_2$ and 95% air at 37 ˚C for 24 h. Before imaging, cells were cultured in an 18 mm glass dish, during which dead cells and cell metabolites were washed away with physiological PBS buffer.

MTT assay was performed using MCF-7 cells, which were inoculated into 96-well plate and was cultured in a cell culture tank. After cell attachment was completed, different concentrations (0.0, 5.0, 10.0, 15.0, 20.0 μM) of probe **Han-HClO-$H_2S$** were added into the 96-well plate and incubated in 5% $CO_2$ humidified incubator for 24 h. The MTT solution (1.0 mg/mL in PBS) was then added to each well and cells were incubated in a cell culture tank for another 4 h. Finally, MTT solution was dumped and DMSO (100.0 μL) was added to each well. The absorbance was determined at 490 nm and the cell viability was calculated using the following formula: cell viability = (mean absorbance of test wells - mean absorbance of medium control wells) / (mean absorbance of untreated wells - mean absorbance of medium control wells) × 100%.

### *1.5. Sensing mechanism*




**Scheme S2.** The proposed sensing mechanism of probe **Han-HClO-$H_2S$** for $H_2S$.

## 2. Spectroscopic Property

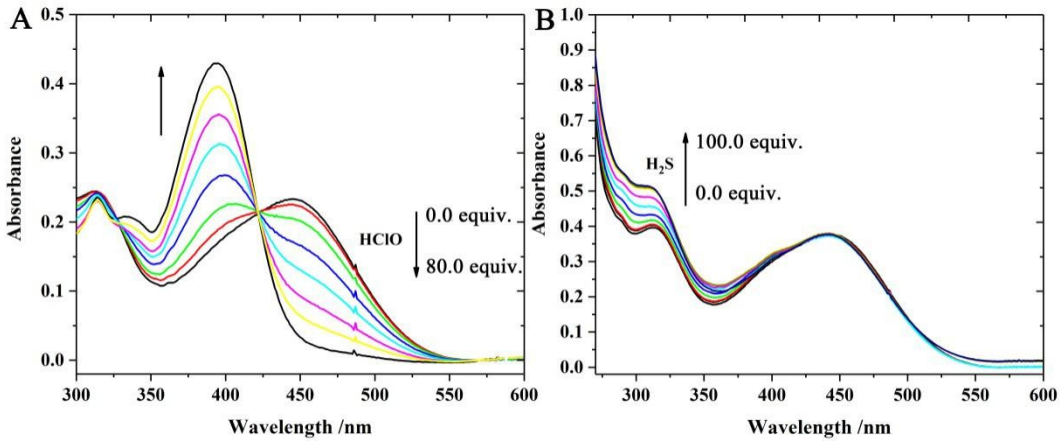



**Fig. S1** Absorption spectra of probe **Han-HClO-$H_2S$** (5.0 μM) in the absence and presence of HClO for 10 min (A) and $H_2S$ for 120 min (B).

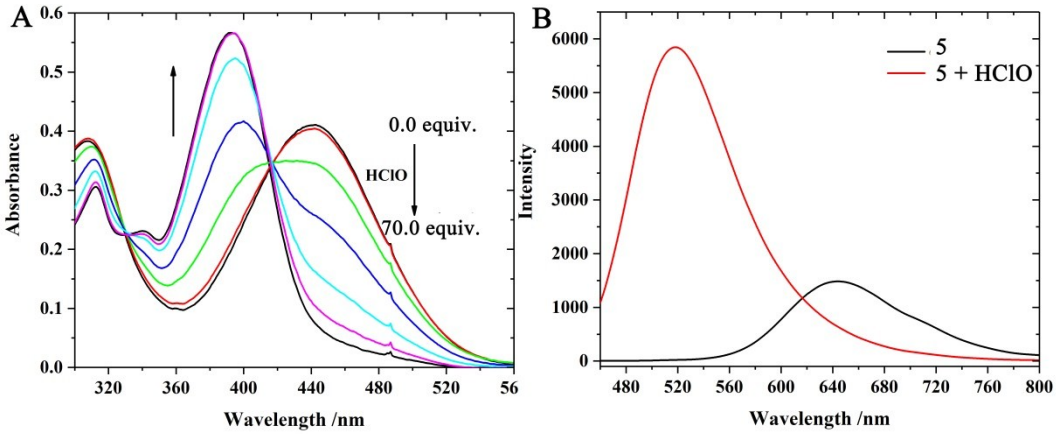



**Fig. S2** Absorption (A) and emission (B) spectra of compound **5** (5.0 μM) in the absence and presence of HClO for 10 min.

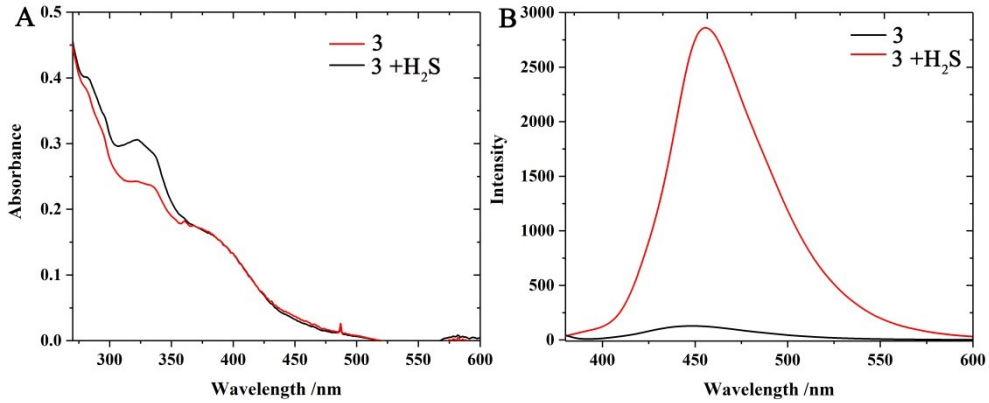



**Fig. S3** Absorption (A) and emission (B) spectra of compound **3** (5.0 μM) in the absence and presence of $H_2S$ for 120 min.

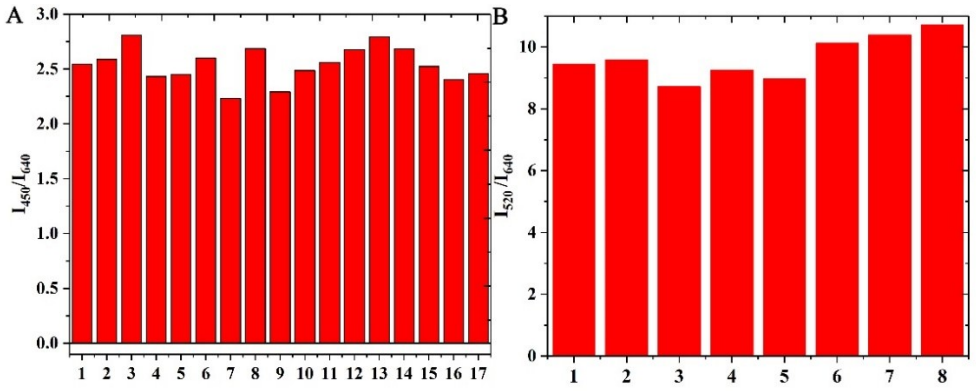



**Fig. S4** (A) The fluorescence intensity ratio ($I_{450}/I_{640}$) of probe **Han-HClO-$H_2S$** (5.0 μM) in the presence of $H_2S$ (500.0 μM) and biologically relevant species (500.0 μM): Cys, Hcy, GSH, $Na_2S_2O_3$, $Na_2S_2O_5$, NaCl, $NaNO_2$, $SCN^-$, $Na_2SO_3$, KI, $Na_2CO_3$, NaF, $ZnCl_2$, $Na_2SO_4$, $NaHCO_3$ and $H_2S_2$ with 120 min incubation. (B) The fluorescence intensity ratio ($I_{520}/I_{640}$) of probe **Han-HClO-$H_2S$** (5.0 μM) in the presence of HClO (400.0 μM) and biologically relevant species (400.0 μM): NO, $ONOO^-$, ·OH, $^1O_2$, $O^{2-}$, ROO· and t-BuO· with 10 min incubation.

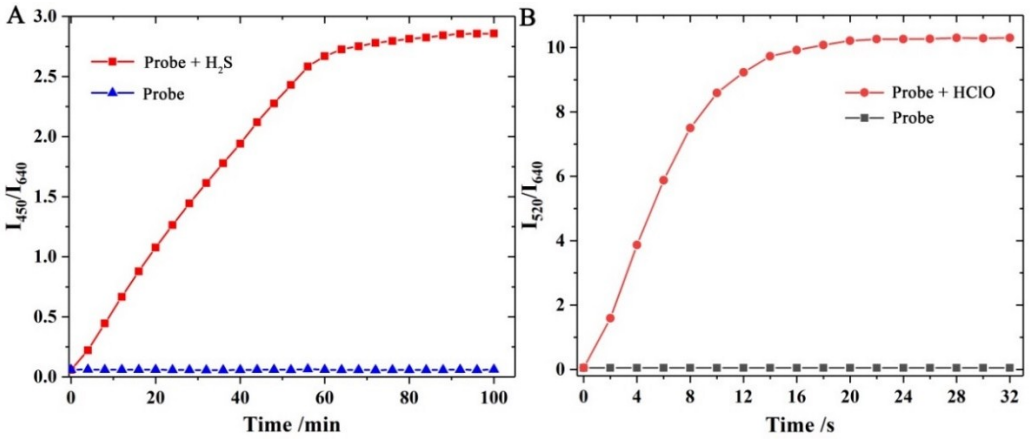



**Fig. S5** (A) Time-dependent fluorescence intensity ratio ($I_{450}/I_{640}$) of probe **Han-HClO-$H_2S$** (5.0 μM) in the presence and absence of $H_2S$ (100.0 equiv.); (B) time-dependent fluorescence intensity ratio ($I_{520}/I_{640}$) of probe **Han-HClO-$H_2S$** (5.0 μM) in the presence and absence of HClO (80.0 equiv.).

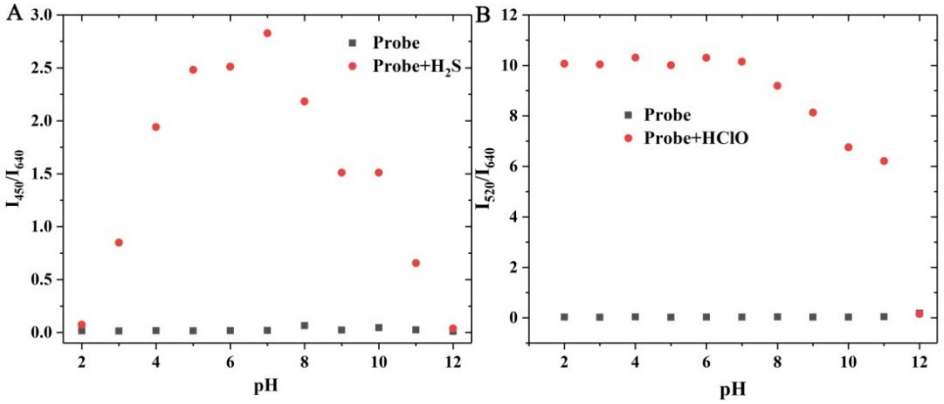



**Fig. S6** pH effects on the fluorescence intensity ratio ($I_{450}/I_{640}$) (A) and ($I_{520}/I_{640}$) (B) of probe **Han-HClO-$H_2S$** (5.0 μM) in the absence and presence of $H_2S$ (100.0 equiv.) for 120 min and HClO (80.0 equiv.) for 10 min, respectively.

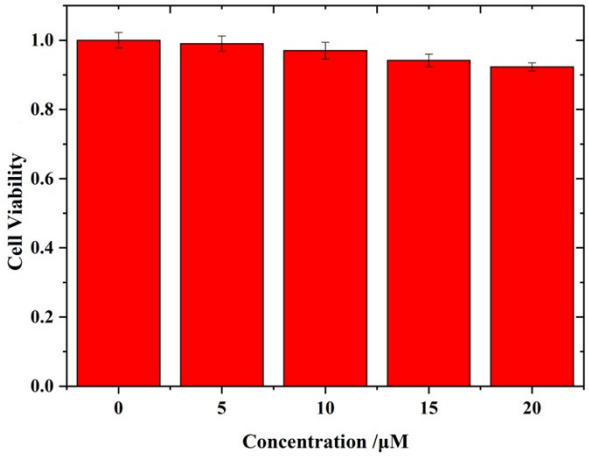



**Fig. S7** The cytotoxicity assay of MCF-7 cells with different concentrations of **Han-HClO-$H_2S$**.

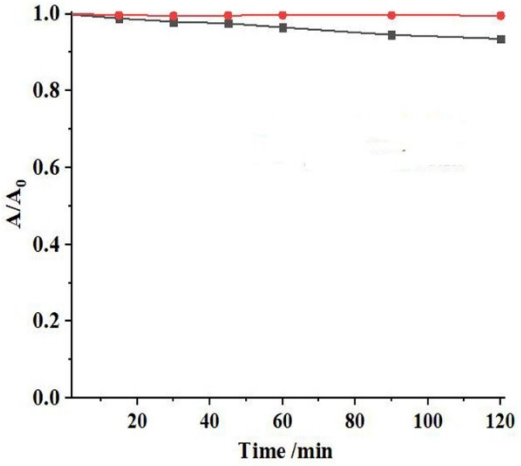



**Fig. S8** Time-dependent absorption changes of probe **Han-HClO-$H_2S$** (5.0 μM) under dark (red line) and a 500 W Xe light irradiation (black line) in PBS buffer (10.0 mM, pH=7.4, containing 50% acetonitrile). Distance between the light source and the sample: 20 cm.

## 3. $^{1}$H NMR, $^{13}$C NMR and HRMS spectra

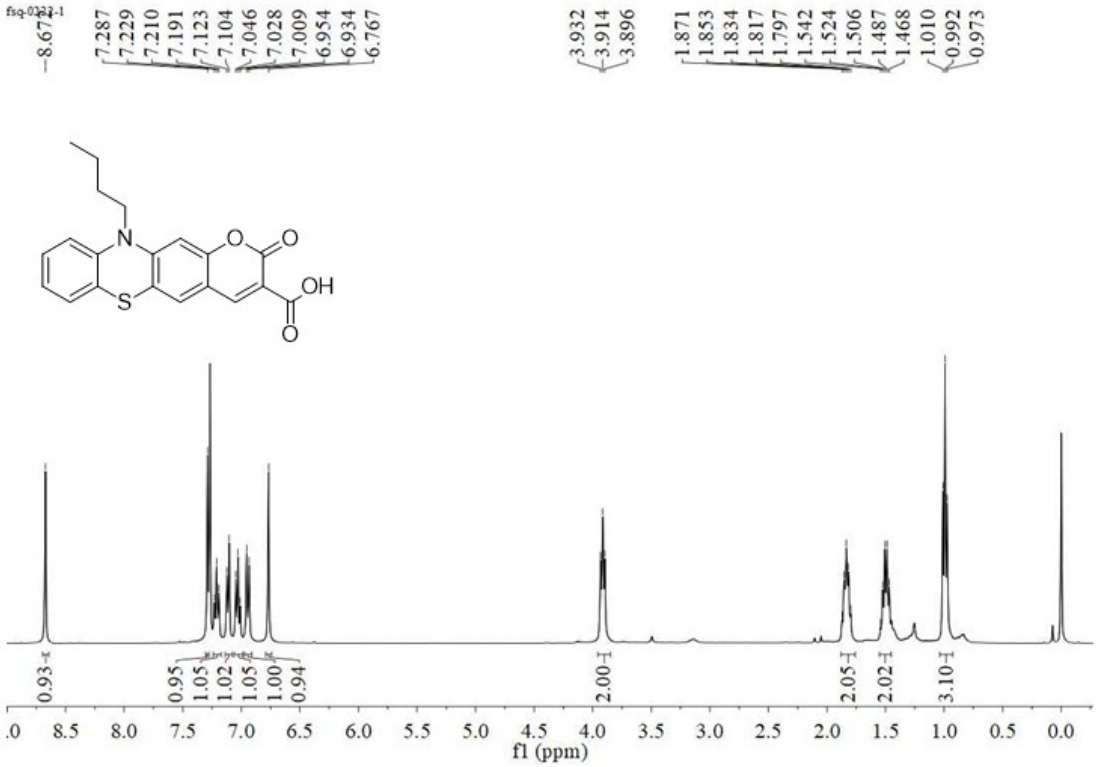



**Fig. S9** $^{1}$H NMR spectrum of compound **6** in $CDCl_3$.

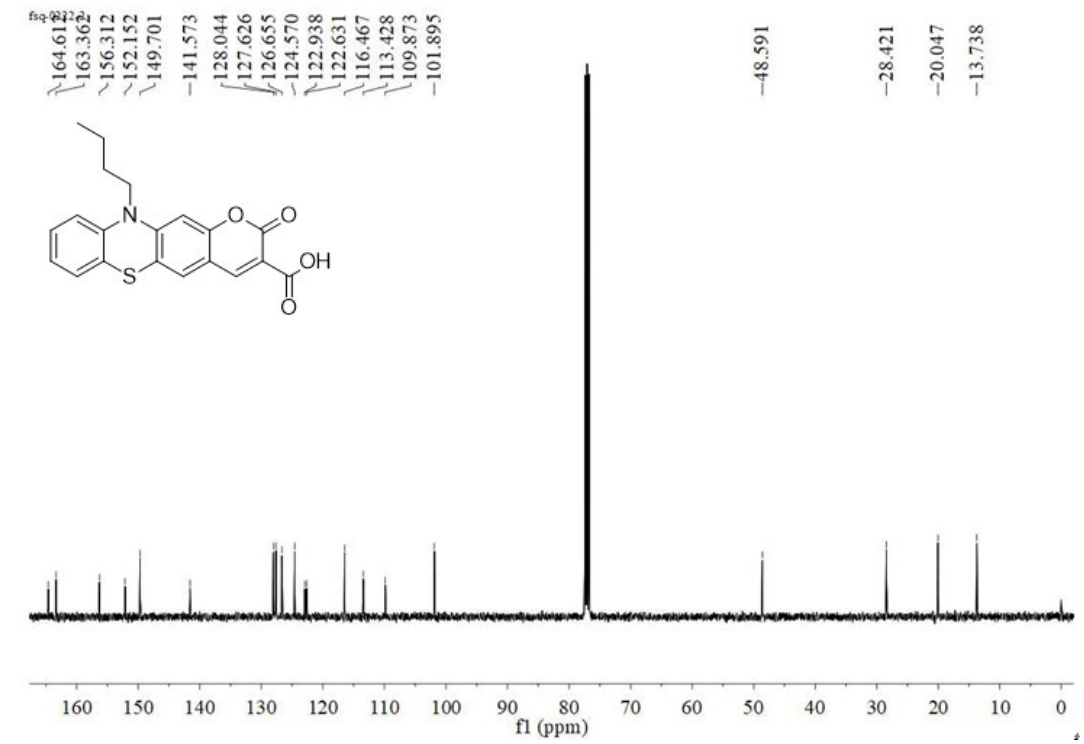



**Fig S10** $^{13}$C NMR spectrum of compound **6** in $CDCl_3$.

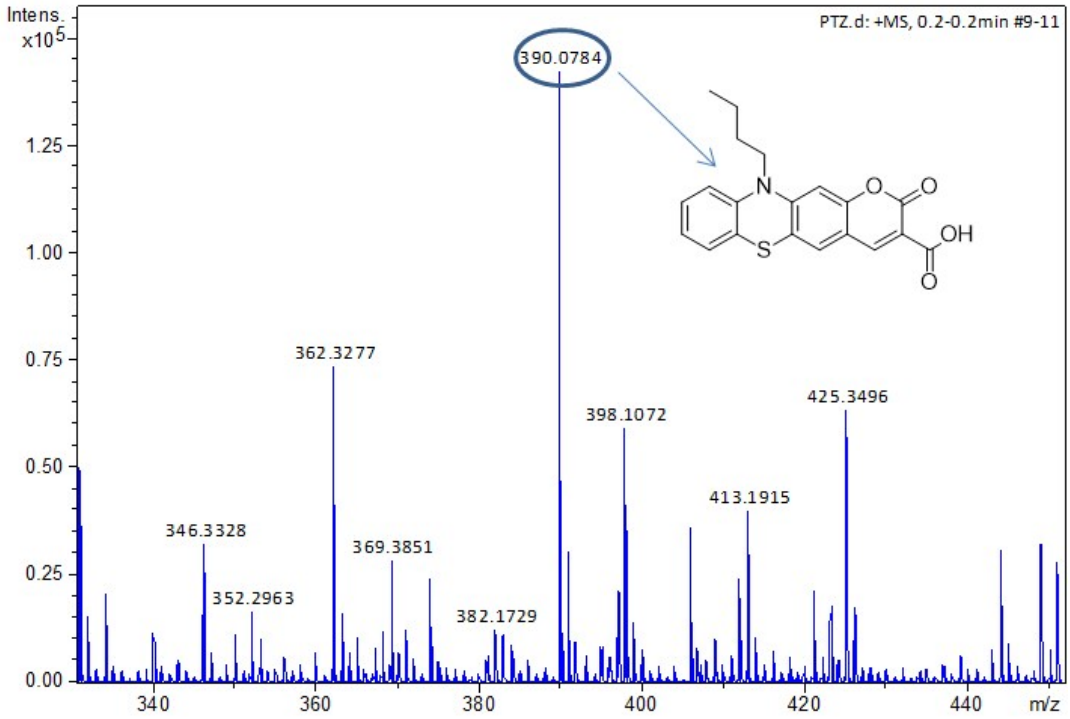



**Fig. S11** HR-MS spectrum of compound **6**.

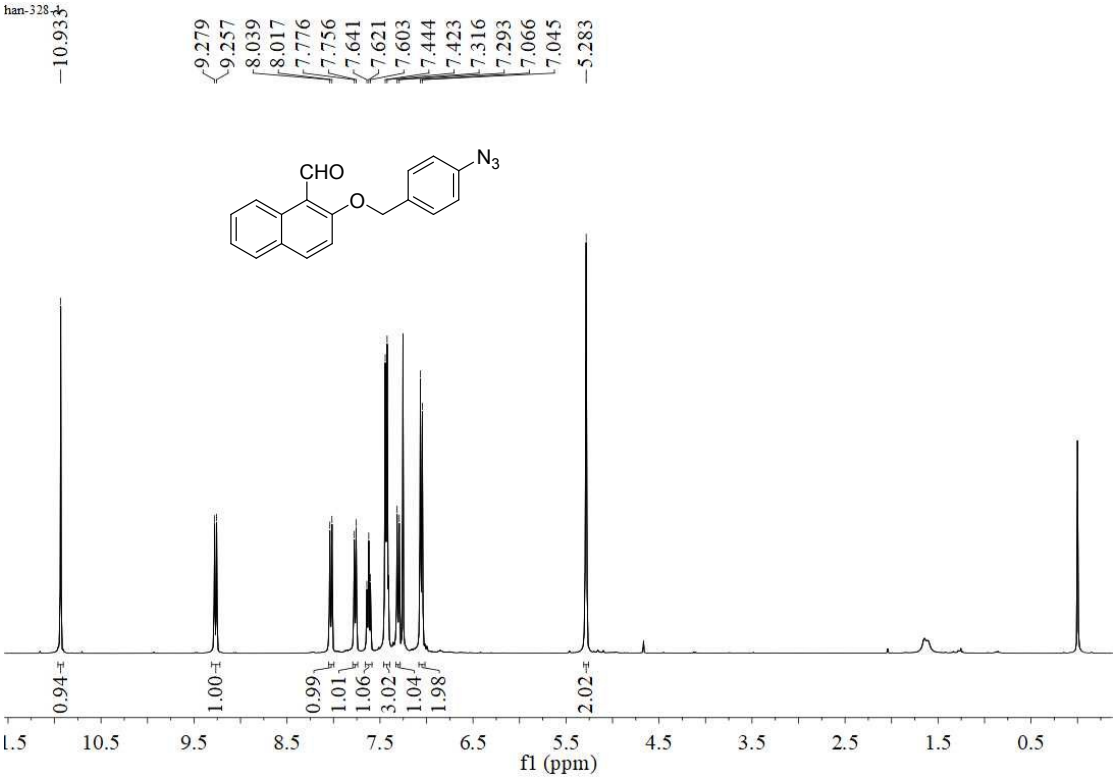



**Fig. S12** $^{1}$H NMR spectrum of compound **2** in $CDCl_3$.

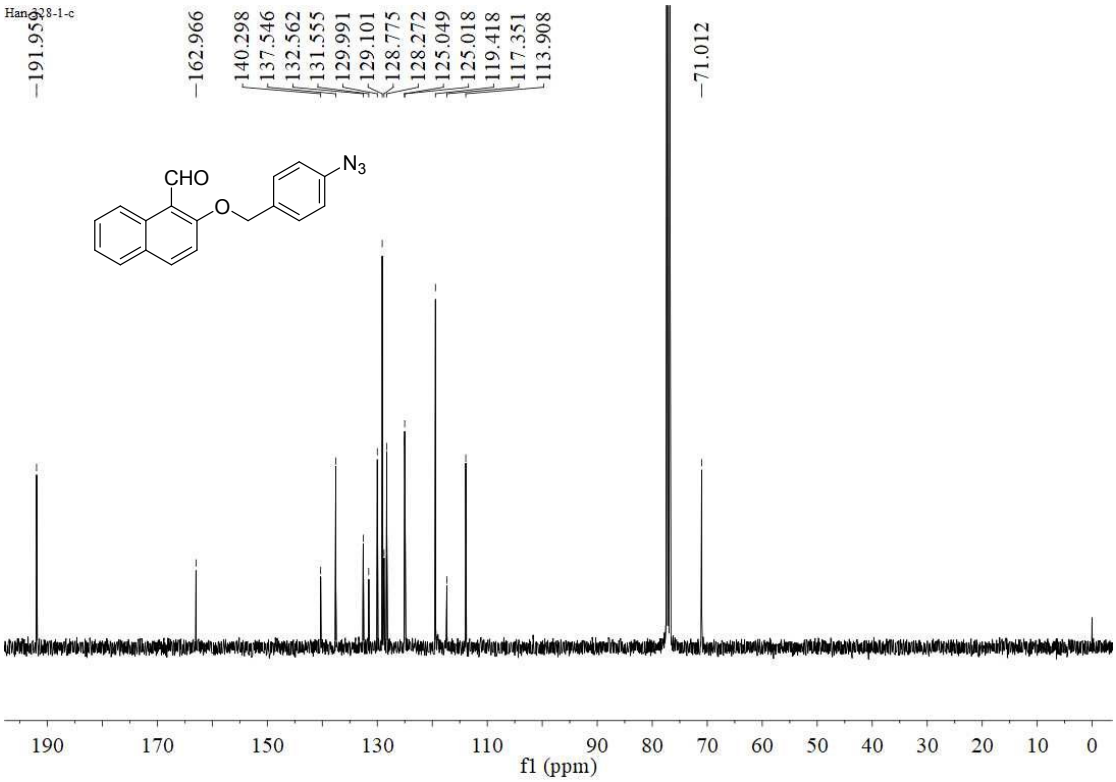



**Fig. S13** $^{13}C$ NMR spectrum of compound **2** in $CDCl_3$.

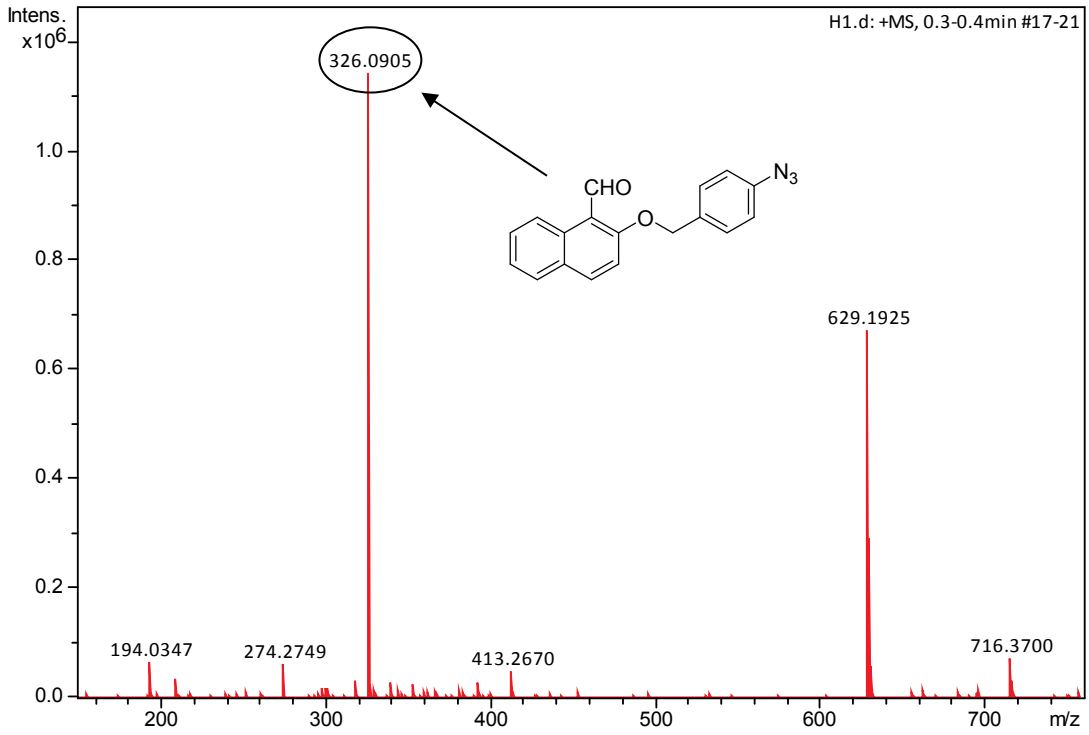



**Fig. S14** HR-MS spectrum of compound **2**.

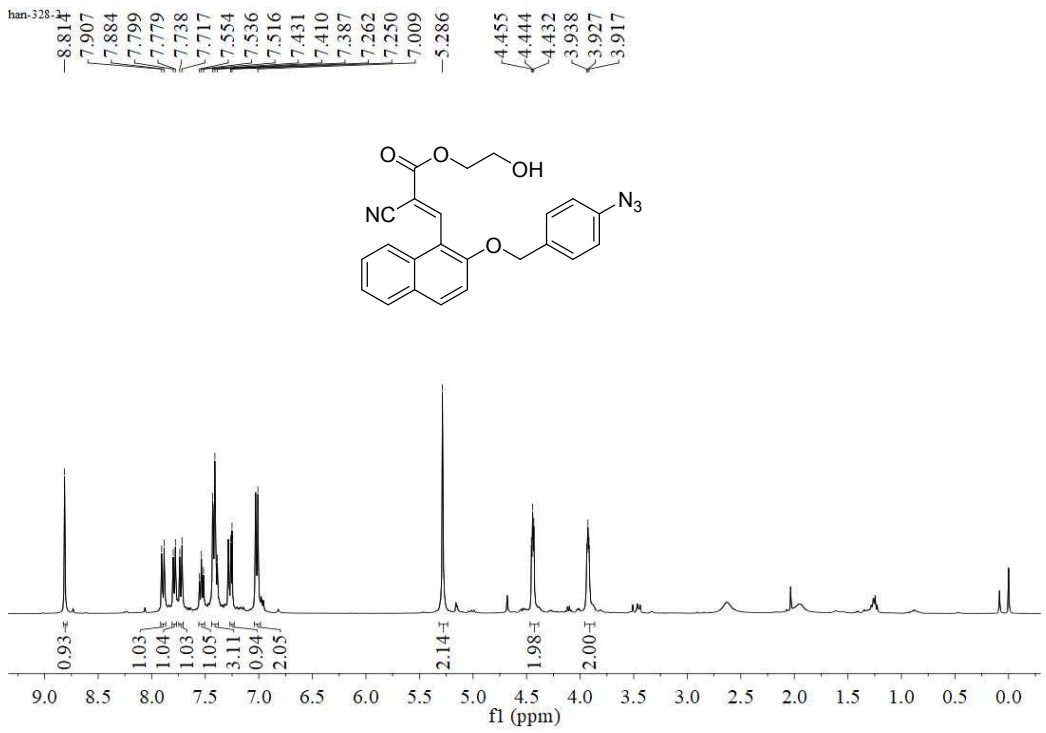



**Fig. S15** $^{1}$H NMR spectrum of compound **3** in $CDCl_3$.

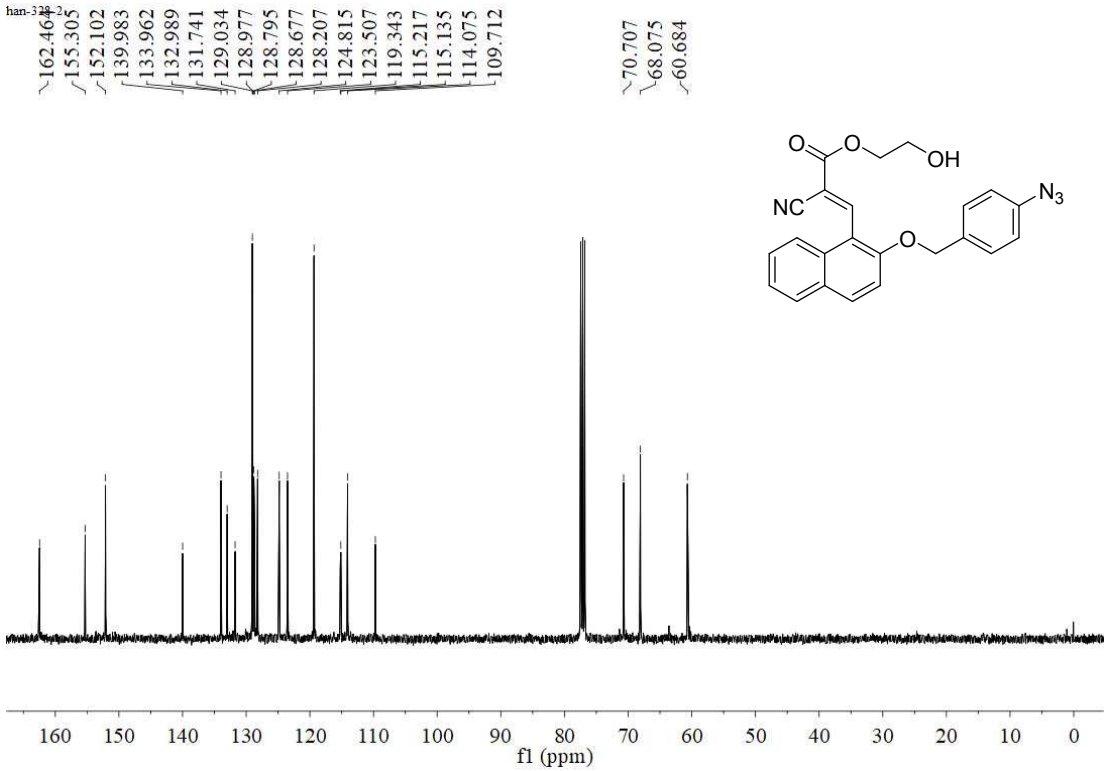



**Fig. S16** $^{13}$C NMR spectrum of compound **3** in $CDCl_3$.

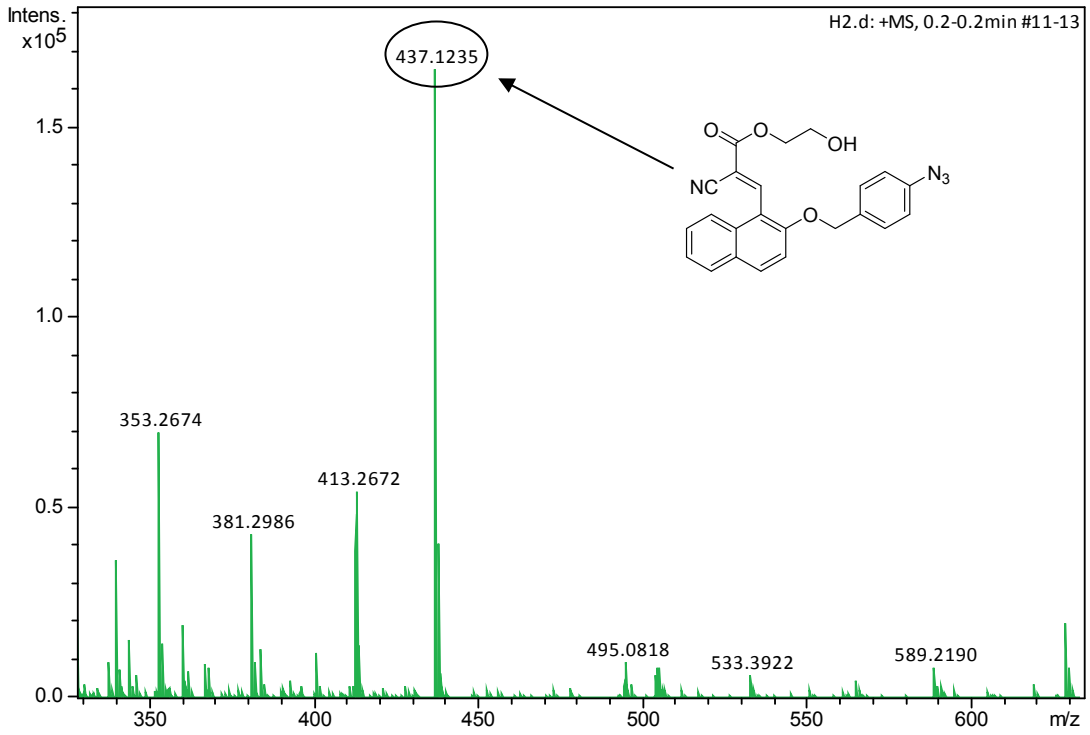



**Fig. S17** HR-MS spectrum of compound **3**.

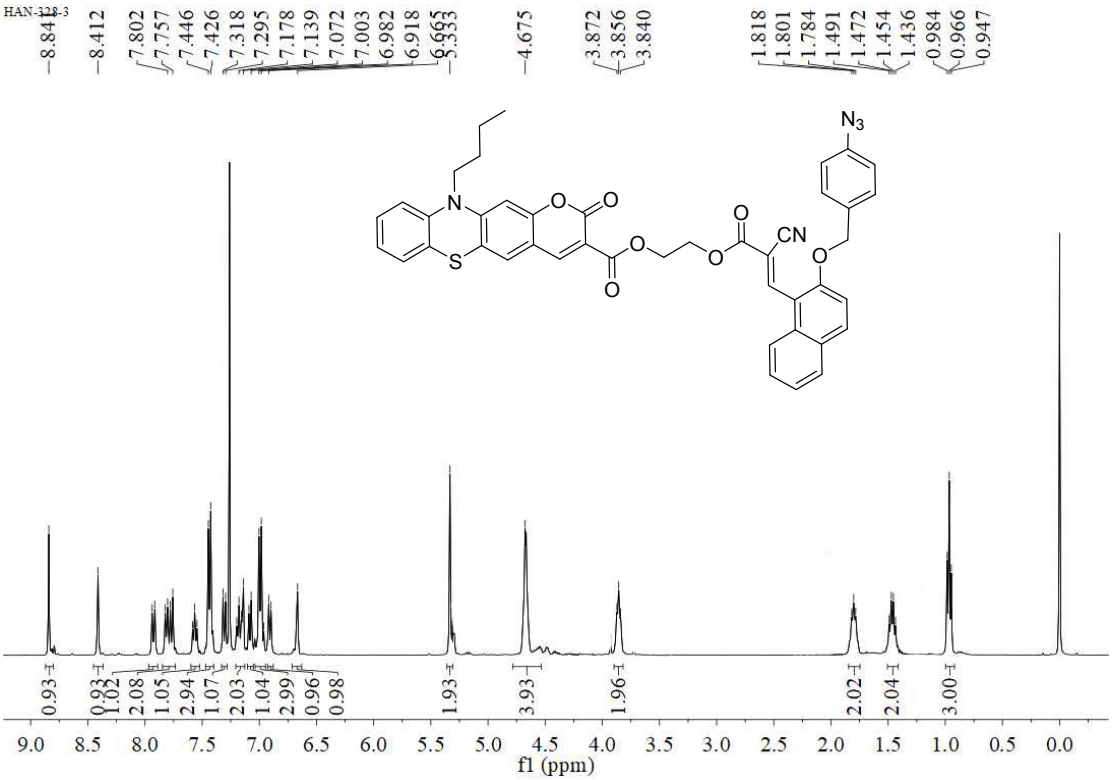



**Fig. S18** $^1$H NMR spectrum of probe **Han-HClO-$H_2S$** in $CDCl_3$.

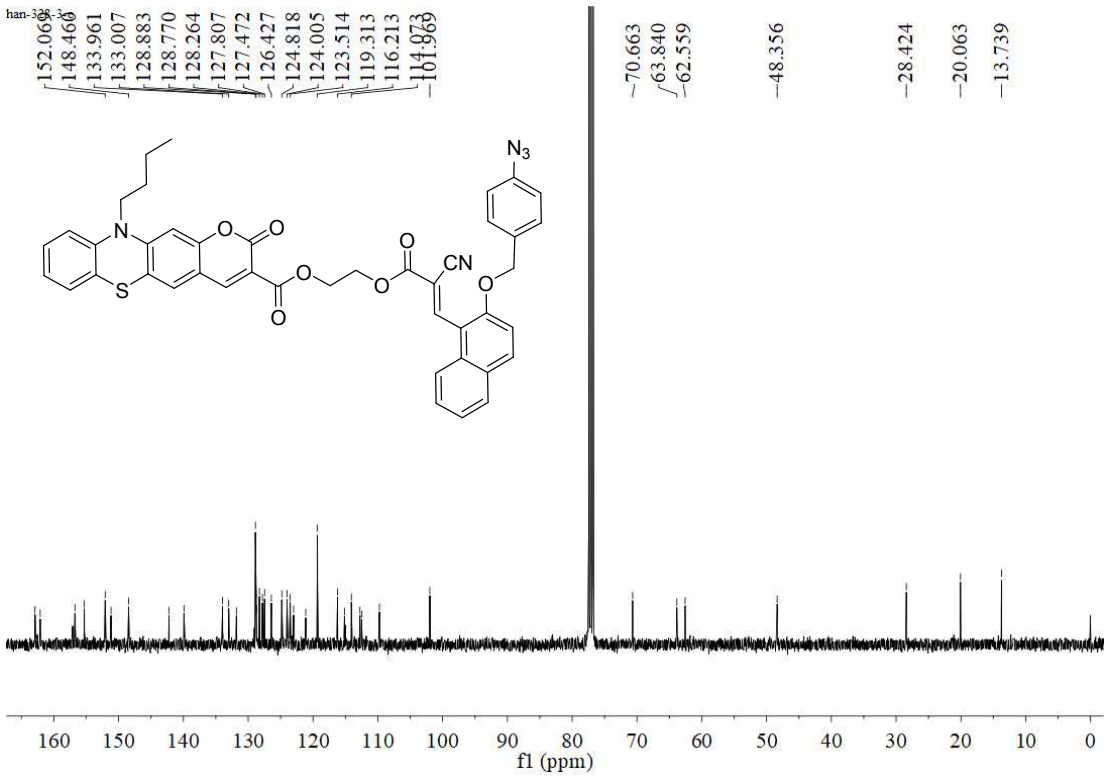



**Fig. S19** $^{13}C$ NMR spectrum of probe **Han-HClO-$H_2S$** in $CDCl_3$.

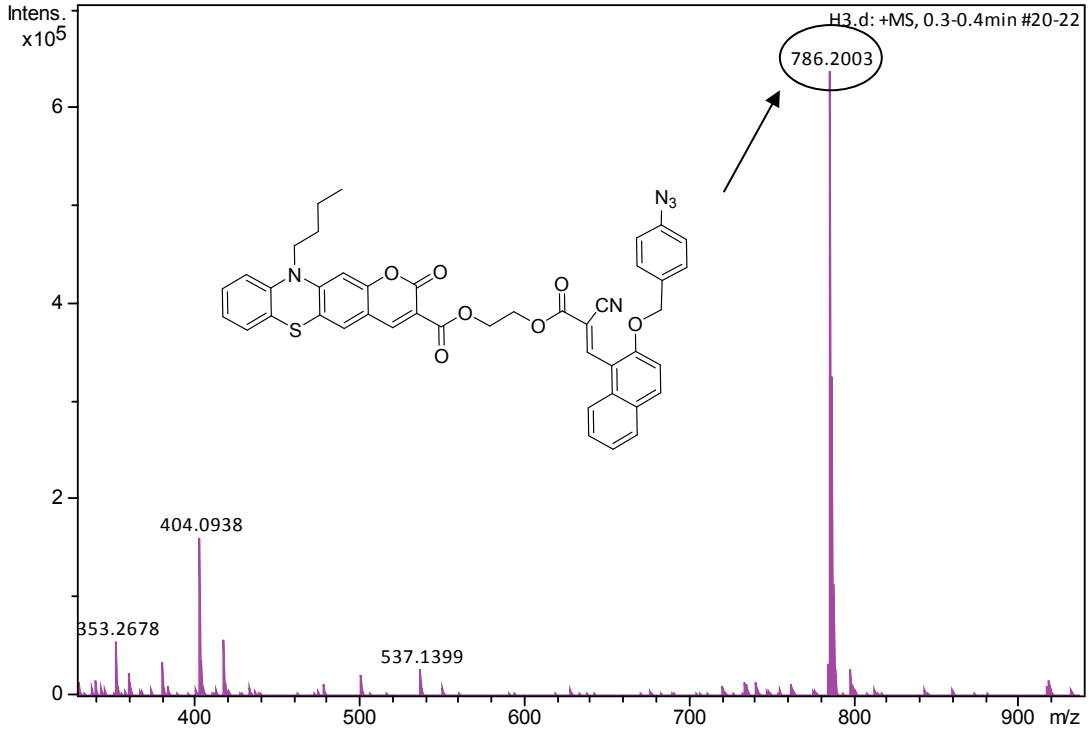



**Fig. S20** HR-MS spectrum of probe **Han-HClO-$H_2S$**.

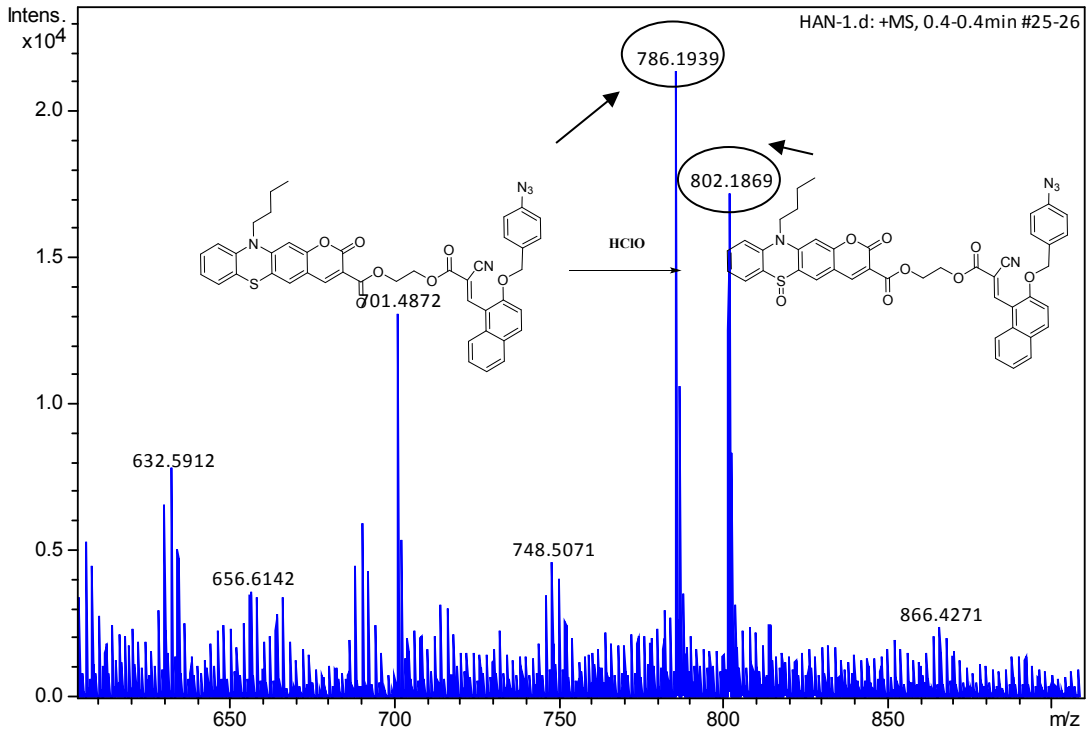



**Fig. S21** HR-MS spectrum of the reaction product of probe **Han-HClO-$H_2S$** with HClO.

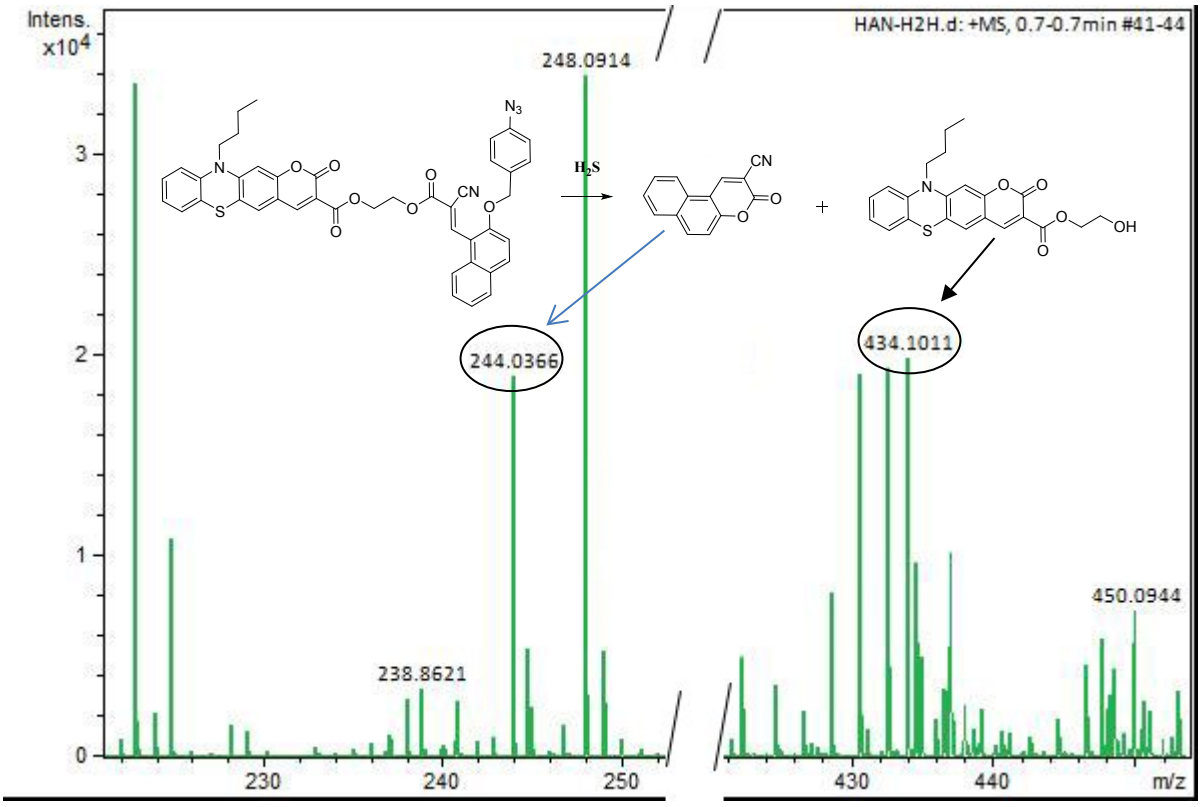



**Fig. S22** HR-MS spectrum of the reaction products of probe **Han-HClO-$H_2S$** with $H_2S$.

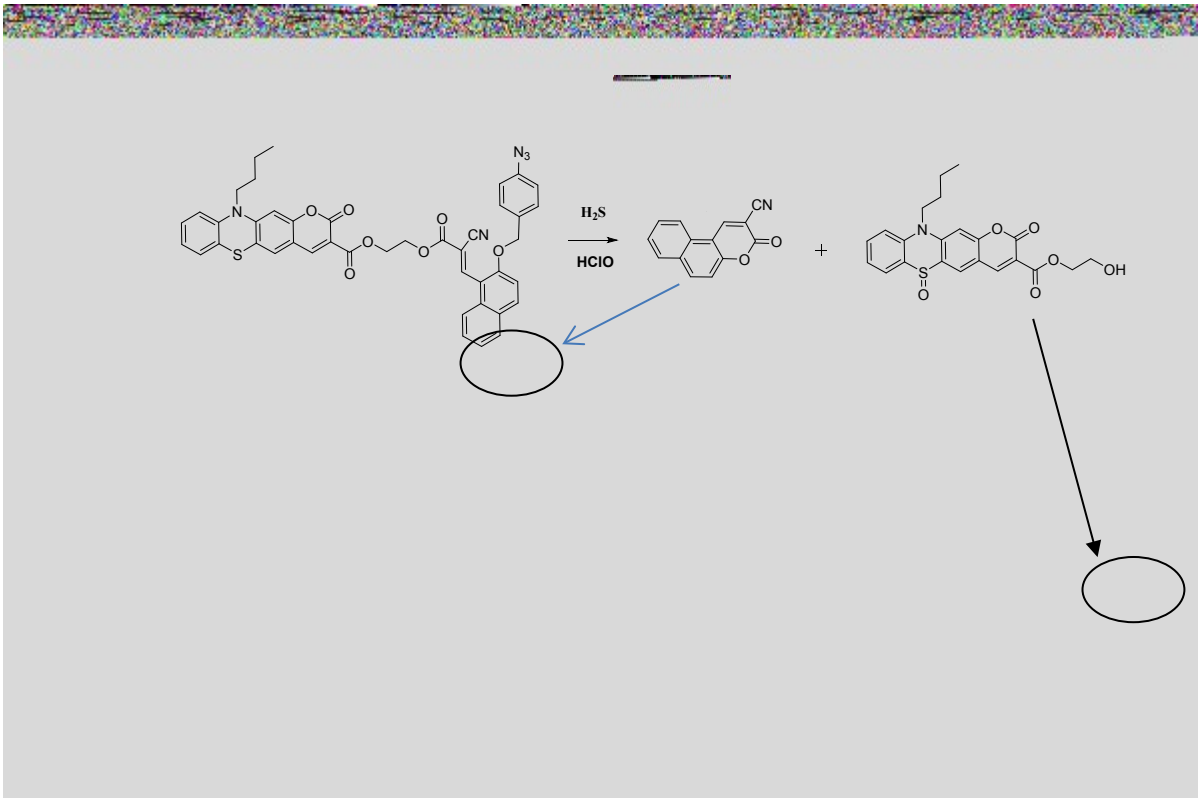



**Fig. S23** HR-MS spectrum of the reaction products of probe **Han-HClO-$H_2S$** with $H_2S$ and HClO.

1. W. Chen, L. Zhu, Y. Hao, X. Yue, J. Gai, Q. Xiao, S. Huang, J. Sheng and X. Song, *Tetrahedron*, 2017, **73**, 4529-4537.

2. L. Dinparast, S. Hemmati, G. Zengin, A. A. Alizadeh, M. B. Bahadori, H. S. Kafil and S. Dastmalchi, *ChemistrySelect*, 2019, **4**, 9211-9215.