Supporting Information

Highly selective and sensitive endoplasmic reticulum-targeted probe reveals HOCl- and cisplatin-induced H_2S biogenesis in live cells

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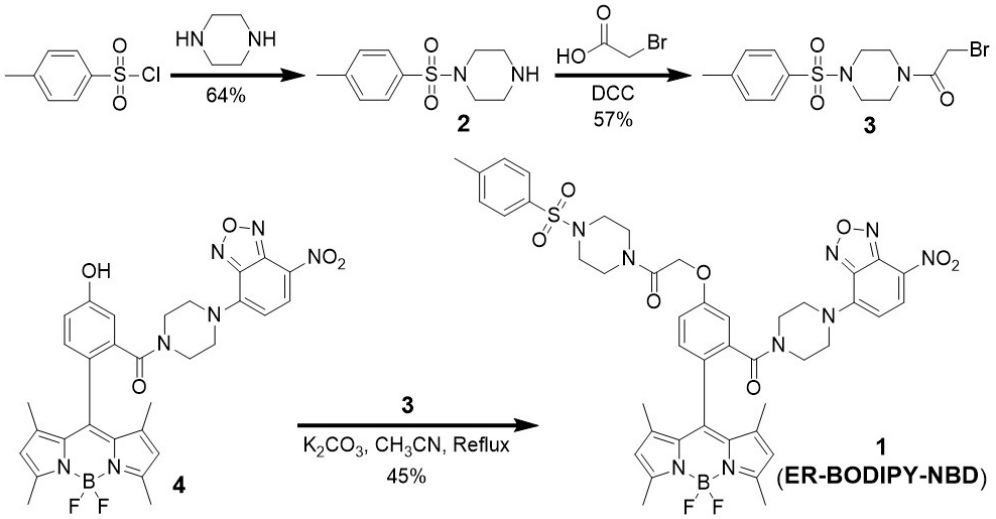
Table of contents

1. Reagents and Instruments

All chemicals and solvents used for synthesis were purchased from commercial suppliers and applied directly in the experiment without further purification. The progress of the reaction was monitored by TLC on pre-coated silica plates (Merck 60F-254, 250 μm in thickness), and spots were visualized by UV light. Merck silica gel 60 (70-200 mesh) was used for general column chromatography purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 400 spectrometer. Chemical shifts are reported in parts per million relative to internal standard tetramethylsilane ($Si(CH_3)_4$ = 0.00 ppm) or residual solvent peaks ($CDCl_3$ = 7.26 ppm, DMSO-d_6 = 2.50 ppm, CD_3CN = 1.96 ppm). ^{1}H NMR coupling constants (J) are reported in Hertz (Hz), and multiplicity is indicated as the following: s (singlet), d (doublet), t (triplet), m (multiplet). High-resolution mass spectrum (HRMS) was obtained on an Agilent 6540 UHD Accurate-Mass Q-TOFLC/MS. The UV-visible spectra were recorded on a UV-6000 UV-VIS-NIR-spectrophotometer (METASH, China). Fluorescence studies were carried out using F-280 spectrophotometer (Tianjin Gangdong Sci & Tech., Development. Co., Ltd). Cellular bioimaging was carried out on a confocal microscope (Olympus FV1000).

2. Synthesis of the Probe



Compound **2** was synthesized according to a published procedure.[1] To a solution of piperazine (516.8 mg, 6.0 mmol) in anhydrous CH_2Cl_2 (50 mL) was added slowly 4-

methylbenzene-1-sulfonyl chloride (381.3 mg, 2.0 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min and then concentrated under reduced pressure. The residue was purified by flash chromatography with CH_2Cl_2/CH_3OH (100/3) to afford compound **2** (308.8 mg, yield 64%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, J = 8.2 Hz, 2H), 7.30 (d, J = 8.0 Hz, 2H), 2.97–2.92 (m, 4H), 2.92–2.87 (m, 4H), 2.41 (s, 3H), 1.68 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 143.7, 132.5, 129.7, 127.9, 46.9, 45.4, 21.6. HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{11}H_{17}N_2O_2S^+$: 241.1005; found: 241.1025.

Compound **2** (480 mg, 2 mmol) was added to a solution of bromoacetic acid (417 mg, 3 mmol) and dicyclohexylcarbodiimide (DCC, 619 mg, 3 mmol) in anhydrous CH_2Cl_2 (15 mL). The reaction mixture was stirred at room temperature for 4 h and then concentrated under reduced pressure. The residue was purified by flash chromatography with CH_2Cl_2/CH_3OH (100/0.5) to afford compound **3** (412.8 mg, yield 57%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (d, J = 8.3 Hz, 2H), 7.37 (d, J = 8.0 Hz, 2H), 3.81 (s, 2H), 3.76–3.59 (m, 4H), 3.15–3.00 (m, 4H), 2.47 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.3, 144.4, 132.4, 130.1, 127.9, 46.3, 45.9, 45.7, 41.5, 25.5, 21.7. HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{13}H_{18}BrN_2O_3S^+$: 361.0216; found: 361.0217.

Compound **4** was synthesized based on our previous work.[2] A mixture of compound **4** (40 mg, 0.066 mmol), compound **3** (23.8 mg, 0.066 mmol), K_2CO_3 (13.7 mg, 0.099 mmol), and KI (0.6 mg) in 10 mL dry acetonitrile was stirred and refluxed for 12 h under nitrogen protection. After cooling, the solvent was removed under reduced pressure, and the resulted residue was purified by silica gel column chromatography with CH_2Cl_2/CH_3OH (100/1.2) to give an orange solid **ER-BODIPY-NBD** (**1**) (26.5 mg, yield 45%). ^{1}H NMR (400 MHz, CD_3CN) δ 8.46 (d, J = 9.0 Hz, 1H), 7.62 (d, J = 8.3 Hz, 2H), 7.39 (d, J = 8.0 Hz, 2H), 7.30 (d, J = 8.5 Hz, 1H), 7.12–7.05 (m, 2H), 6.38 (d, J = 9.0 Hz, 1H), 6.05 (s, 2H), 4.83 (s, 2H), 4.05–3.74 (m, 4H), 3.70–3.50 (m, 8H), 3.09–2.99 (m, 2H), 2.94–2.86 (m, 2H), 2.44 (s, 6H), 2.38 (s, 3H), 1.47 (s, 6H). ^{13}C NMR (101 MHz, CD_3CN) δ 167.7, 166.6, 159.4, 146.2, 146.0, 145.3, 136.6, 136.1, 133.4, 132.8, 130.8, 128.7, 126.1, 124.2, 122.2, 118.3, 115.1, 104.3, 67.5, 55.3,

49.6, 47.0, 46.8, 41.8, 30.3, 21.5, 15.0, 14.7. HRMS (ESI): m/z $[M-F]^+$ calcd. for $C_{43}H_{44}BFN_9O_8S^+$: 876.3105; found: 876.3102.

3. Spectroscopic Studies

All measurements were performed in degassed, sealed phosphate buffers (PBS, 50 mM, pH = 7.4) in a 3 mL corvette with 2 mL solution. Probe **1** was dissolved into DMSO to prepare the stock solutions with concentrations of 1-10 mM.

For the stability test, 1 μM probe **1** was incubated in PBS (50 mM, pH = 7.4, containing 30% CH_3CN) at 25 °C, and the time-dependent fluorescence spectra of the solutions were checked by excitation at 469 nm.

For the kinetic studies, **1** (10 μM) was incubated with H_2S (1.0 mM) in PBS (pH 7.4) at 25 °C, and the absorbance profiles were recorded at different time points. Alternatively, **1** (1 μM) was incubated with H_2S (100-400 μM) in PBS (pH 7.4) at 25 °C, and the time-dependent fluorescence spectra were recorded with excitation at 469 nm.

The selectivity was measured by fluorescence responses (λ_{em} = 520 nm) of probe **1** (1 μM) with various species in the absence or presence of H_2S for 1 h of incubation at 25 °C. H_2O_2 was diluted from 30% solution, and the concentration was determined from absorption at λ = 240 nm (ε = 43.6 $M^{-1}\cdot cm^{-1}$); HOCl was obtained from 14.5% NaOCl solution, and the concentration was determined from absorption at λ = 292 nm (ε = 350 $M^{-1}\cdot cm^{-1}$); other analytes (Fe^{2+}, Zn^{2+}, NO_2^-, $S_2O_3^{2-}$ and SO_3^{2-}) were obtained from related chloridate or sodium salt. All analytes were prepared as stock solutions in degassed water.

The detection limit was calculated by the $3\sigma/k$ method[3,4] where σ is the standard deviation of the fluorescence intensity of probe **1** in PBS; k is the slop between the fluorescence intensities at 520 nm of probe **1** versus H_2S concentrations. Upon addition of various concentrations of sub-micromolar H_2S, the probe solution was incubated at 37 ºC for 1 h before the fluorescence test. The fluorescent titrations were performed in triplicates, and the k value was determined as 43.06 (Fig. 3B). Nine

samples of 1 μM probe **1** in PBS (pH = 7.4) were incubated at 37 ºC for 1 h, and then the fluorescence intensity at 520 nm of each sample was recorded (17.47, 18.08, 17.73, 18.08, 18.36, 18.10, 17.74, 17.39, 18.60, 18.48, 18.15) for determination of the σ as 0.394. The detection limit was calculated as 27.4 nM.

For obtaining relative quantum yield, the reaction solutions of **1** with or without H_2S were measured in PBS (50 mM, pH = 7.4, containing 2% CH_3CN) with fluorescein in 0.1 M NaOH solution as the standard (Φ = 0.925).[5] The excitation wavelength was at 470 nm, and the emission range was 480-650 nm. The quantum yield was calculated using the following equation:

$$\Phi = \Phi_S \times (F/F_S) \times (A_S/A) \times (n^2/n_S^2)$$

where Φ 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. Subscript S refers to the standard of fluorescein.

4. HPLC Analysis

For HPLC studies, a mixture of probe **1** (50 μM) and H_2S (1 mM) in PBS (50 mM, pH = 7.4, containing 30% CH_3CN) was analyzed by HPLC at different reaction time. Conditions: ANGELA TECHNOLOGIES HPLC LC-10F; C_{18} column with 4.6 mm x 250 mm; detection wavelength: 500 nm. Buffer A: 0.1% (v/v) trifluoroacetic acid in water; buffer B: methanol; flow: 1 mL/min; elution conditions: 0-3 min, buffer B: 5-85%; 3-18 min, buffer B: 85%; 18-20 min, buffer B: 85-5%.

5. Cell Culture and MTT Assay

HeLa cells (human cervical cancer cells) were cultured at 37 ºC, 5% CO_2 in DMEM/HIGH GLUCOSE (GIBICO) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, 100 μg/mL streptomycin, and 4 mM L-glutamine. The HeLa cells were passaged every 2 days under standard cell culture conditions and used between passages 3 and 8.

Cells were seeded in a 96-well plate at the density about 1×10^4/well for 24 h of cultivation. The culture medium of HeLa cells was replaced with a fresh one containing different concentrations of probe **1** (0, 10, 20, 30, 40 and 50 μM) and incubated for 24 h. Then, 5 mg/mL MTT in PBS (20 μL) was added to each well and incubated for another 4 h. Finally, the culture medium was replaced with 150 μL DMSO to dissolve the purple formazan crystals. The absorbance intensity in each well was detected at 490 nm by a microplate spectrophotometer (SpectraMax M2E (Molecular Device, Inc.)).

6. Imaging of Live Cells

For the visualization of exogenous H_2S, HeLa cells were pre-incubated with 200 μM Na_2S for 30 min, and then washed and incubated with 10 μM probe **1** for 2 h. After that, the cells were incubated with ER-tracker Red (1 μM) for 15 min. Then, the cells were quickly washed with PBS, and then fixed with 4% paraformaldehyde solution for 10 min. Finally, the cells were washed using PBS and imaged using a confocal microscope (Olympus FV1000) with a 40 × objective lens. Emission was collected at the BODIPY channel (500-550 nm) with 488 nm excitation and at the red channel (570-670 nm) with 561 nm excitation.

The application of probe **1** to detect endogenous H_2S biogenesis in HeLa cells under ER stress conditions was evaluated by fluorescence imaging. Cisplatin was dissolved in DMF to provide a 20 mM stock solution. HeLa cells were treated simultaneously with probe **1** (10 μM) and different stimuli (HOCl (20-100 μM) or cisplatin (5-20 μM)) for 2 h. In control experiments, cells were pretreated with 200 μM ebselen or 200 μM DL-PAG for 1 h before incubation with **1** and HOCl or cisplatin, respectively. After incubation, the cells were quickly washed with PBS, and then fixed with 4% paraformaldehyde solution for 10 min. After that, the cells were washed with PBS and then treated with DAPI (2 μg/mL) for 10 min. Finally, the cells were washed using PBS and imaged using a confocal

microscope with a 40 × objective lens. Emission was collected at the blue channel (450-500 nm) with 405 nm excitation and at the BODIPY channel (500-550 nm) with 488 nm excitation.

In an additional bioimaging for the cisplatin-induced endogenous HOCl biogenesis, the HeLa cells were treated simultaneously with 2,7-dichlorodihydrofluorescein acetoacetic acid[6] (H2DCFDA, 10 μM) and cisplatin (20 μM) for 2 h before imaging. The control cells were pretreated with 200 μM ebselen or 200 μM DL-PAG for 1 h before incubation with probe H2DCFDA and cisplatin.

7. Supplementary Figures

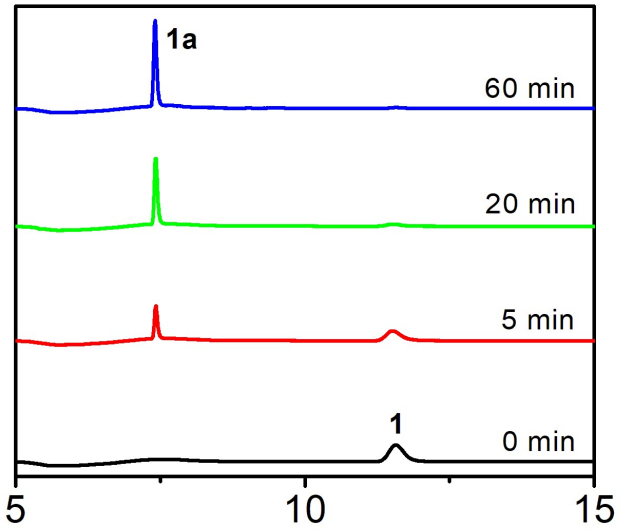


Fig. S1 HPLC traces of probe **1** (50 μM) in the absence or presence of H_2S (1.0 mM) in PBS (pH = 7.4, containing 30% CH_3CN) at 25 °C. The incubation time was indicated inset for each trace. Detection wavelength: 500 nm.

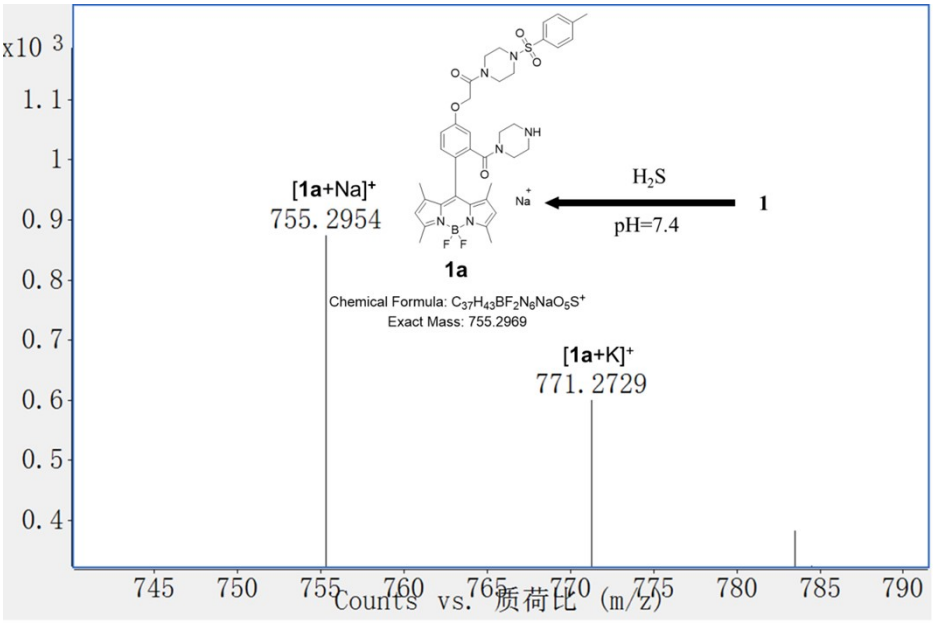


Fig. S2 HRMS spectrum of the reaction solution of **1** (0.3 mM) with H_2S (1.0 mM) in PBS buffer (pH = 7.4, containing 30% CH_3CN) after overnight incubation.

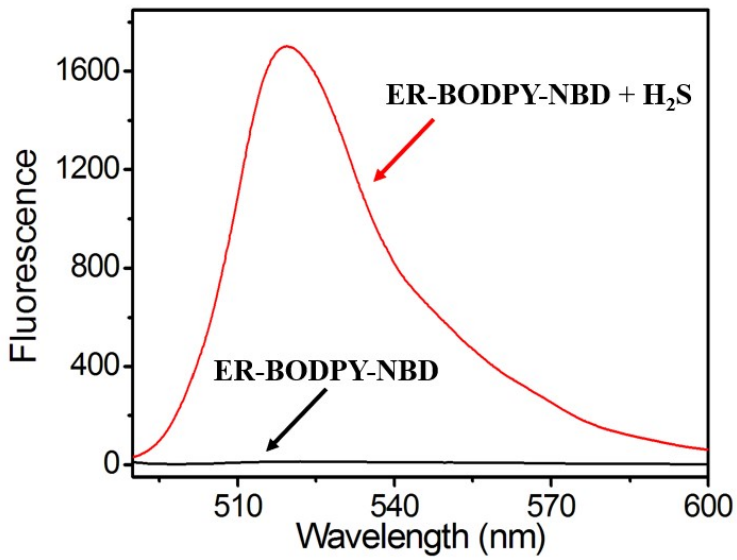


Fig. S3 Fluorescence spectra of **ER-BODIPY-NBD** (1 μM) in the presence and absence of H_2S (100 μM) for Fig. 2B in PBS (pH = 7.4) at 25 ℃. Excitation, 469 nm.

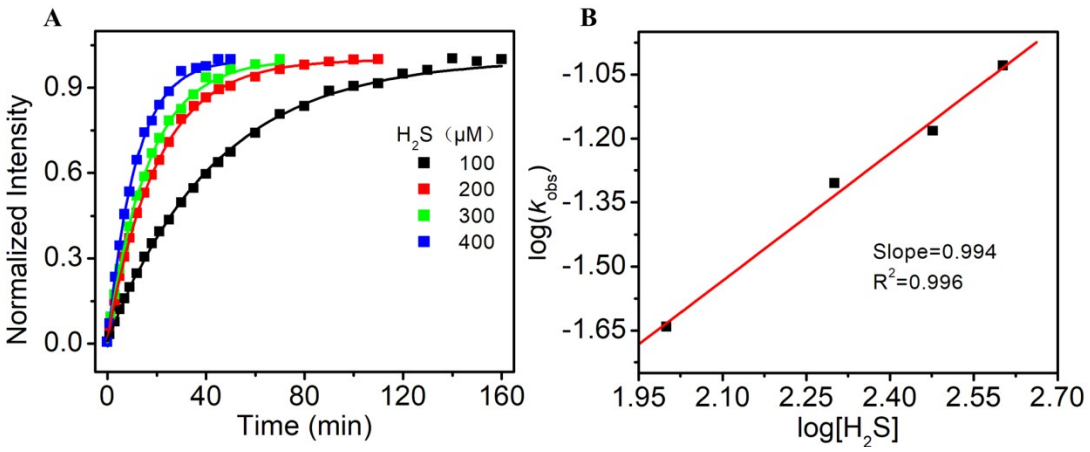


Fig. S4 (A) Time-dependent normalized fluorescence signals at 520 nm of probe **1** (1 μM) towards different concentrations of H_2S in PBS buffer (pH = 7.4, containing 30% CH_3CN) at 25 °C. The solid lines represent the best fitting with a single exponential function to give k_{obs}. (B) The linear-relationship plots of log(k_{obs}) versus log([H_2S]) from (A). The slope of 0.994 supports the first-order dependence in H_2S for the thiolysis of NBD amine.

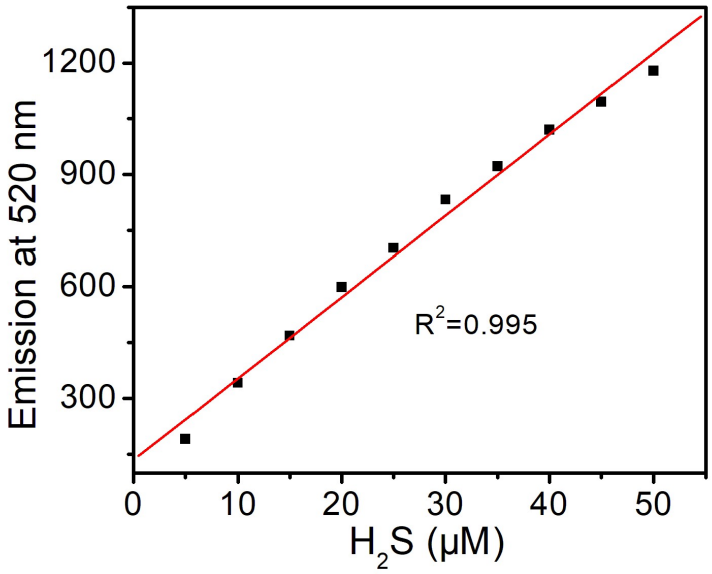


Fig. S5 Linear relationship of fluorescence intensities at 520 nm and the H_2S concentrations (5-50 μM) for the fluorescent titration in Fig. 3A.

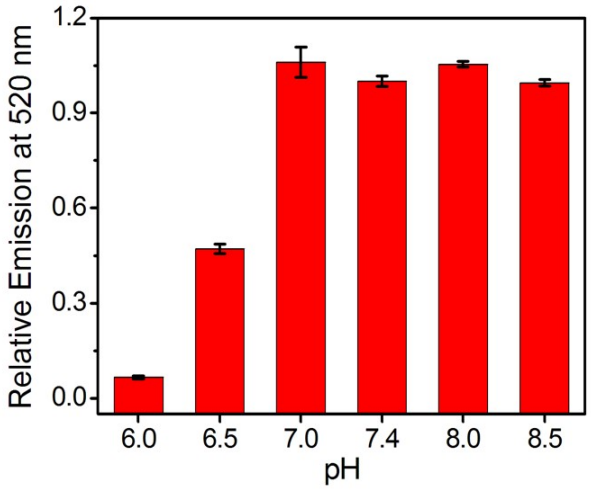


Fig. S6 Relative emission at 520 nm of **1** (1 μM) with H_2S (200 μM) for 1 h of incubation at different pH values in PBS at 25 ℃. Excitation, 469 nm.

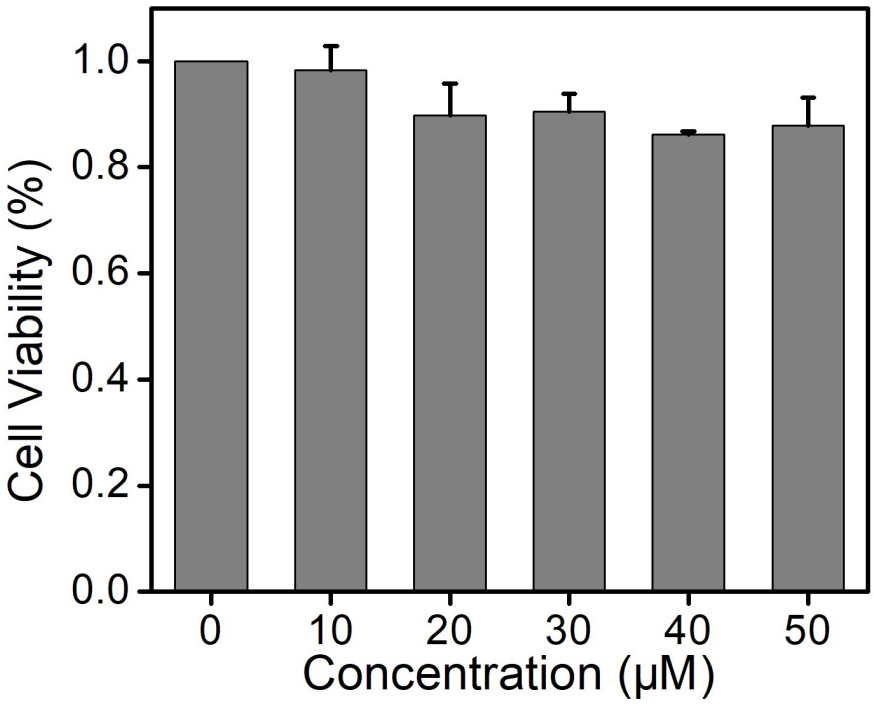


Fig. S7 Relative cell viability of HeLa cells with probe **1** (0-50 μM) for 24 h of incubation by using the MTT assay. The results are expressed as mean ± SD (n = 3).

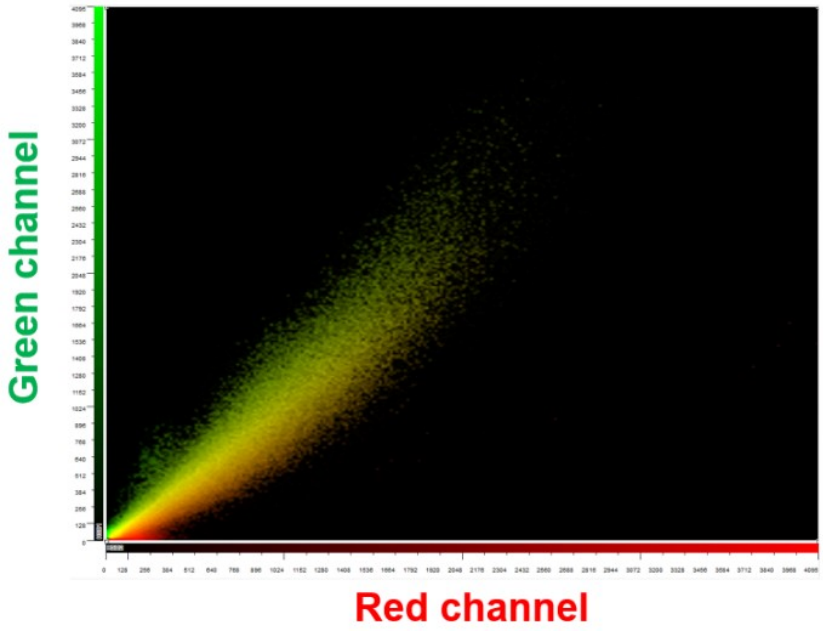


Fig. S8 Colocalization correlation scatter plots for Fig. 4.

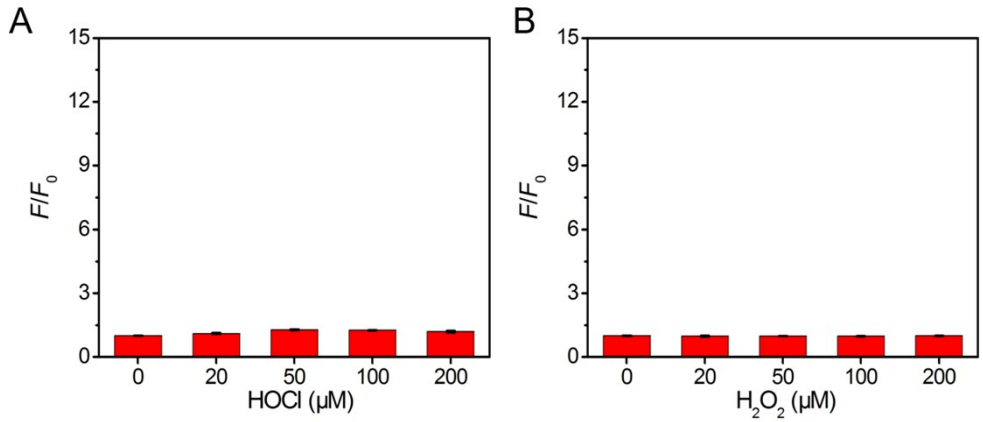


Fig. S9 Relative emission (F/F_0) at 520 nm of **1** (1 μM) toward 0-200 μM HOCl (A) or H_2O_2 (B) for 2 h of incubation at 25 °C. Excitation, 469 nm. The results imply that **ER-BODIPY-NBD** probe dose not react with the reactive oxygen species (ROS).

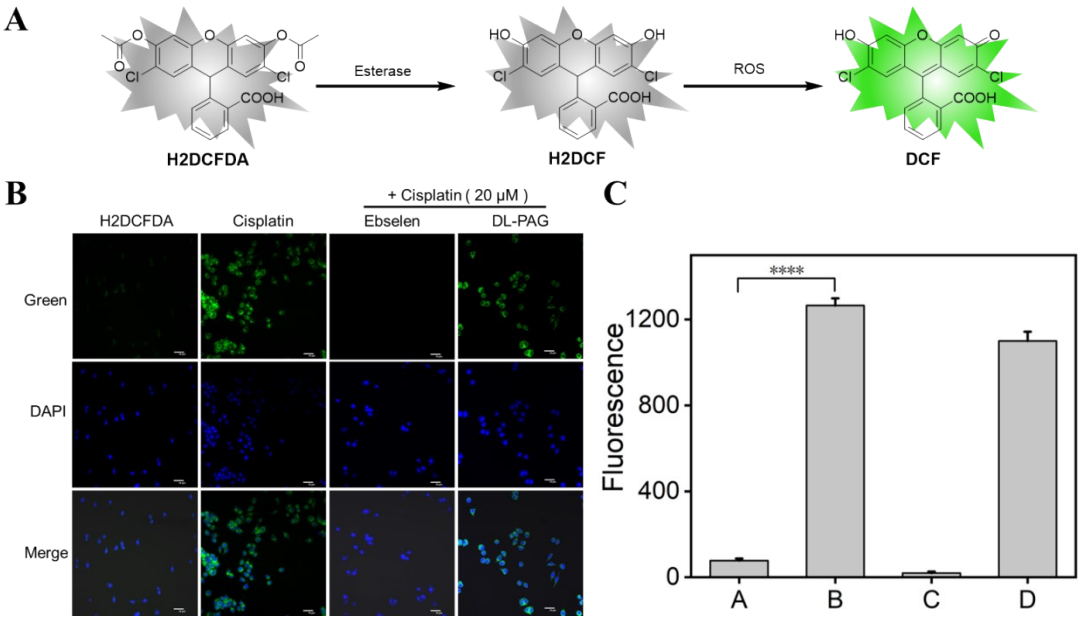
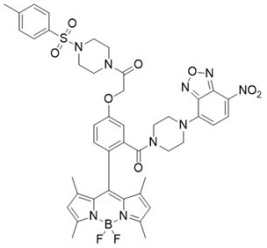
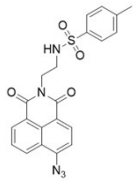
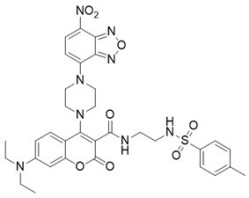
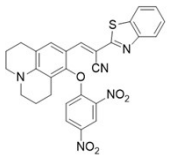
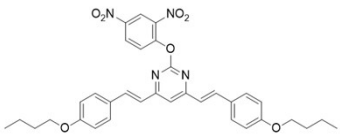
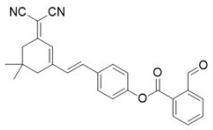
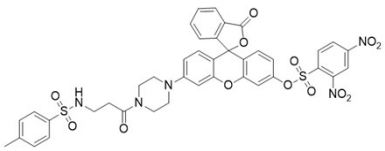
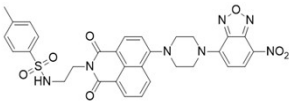


Fig. S10 Confocal fluorescence imaging of cisplatin-induced HOCl biogenesis in live cells. (A) Detection mechanism of the commercially available probe H2DCFDA for sensing ROS in live cells. (B) HeLa cells were co-incubated with H2DCFDA (10 μM) and cisplatin (20 μM) for 2 h, or pre-incubated with 200 μM ebselen or DL-PAG for 1 h and then co-incubated with H2DCFDA (10 μM) and cisplatin (20 μM) for 2 h. Fluorescent channels of green (500-550 nm) and DAPI (450-500 nm) were excited with 488 nm and 405 nm, respectively; merged images of the both fluorescent channels are also shown. Scale bar = 50 μm. (C) Relative green fluorescence intensities in (B). $N = 3$ fields of cells, error bars are means ± S.D. ****$P < 0.0001$.

Table S1. Summary of properties of ER-targeted H_2S fluorescence probes.

Probe	$\lambda_{ex}/\lambda_{em}$ (nm)	Fluorescence enhancement	Φ	LOD (μM)	k_2 $M^{-1}s^{-1}$	Ref
	469/520	~152	0.36	0.027[a]	3.8	This work
	440/545	~45	ND[b]	7.77	11.7[c]	[7]
	388/490	~6.5	ND	4.9	0.73[c]	[8]
	480/537	~32	0.45	0.15	12.6[c]	[9]
	494/506	~13	0.27	3.81[d]	ND	[10]
	480/650	~16.2[e]	ND	0.039	ND	[11]
	500/556	~100	ND	0.081	ND	[12]
	400/530	~205	ND	5.2	ND	[13]

[a] data measured at 37 °C; [b] ND: not detected. [c] data calculated from k_{obs}; [d] data measured in ethanol; [e] 16.2-fold from the change of the intensity ratio of the emission bands, F_{650}/F_{560}.

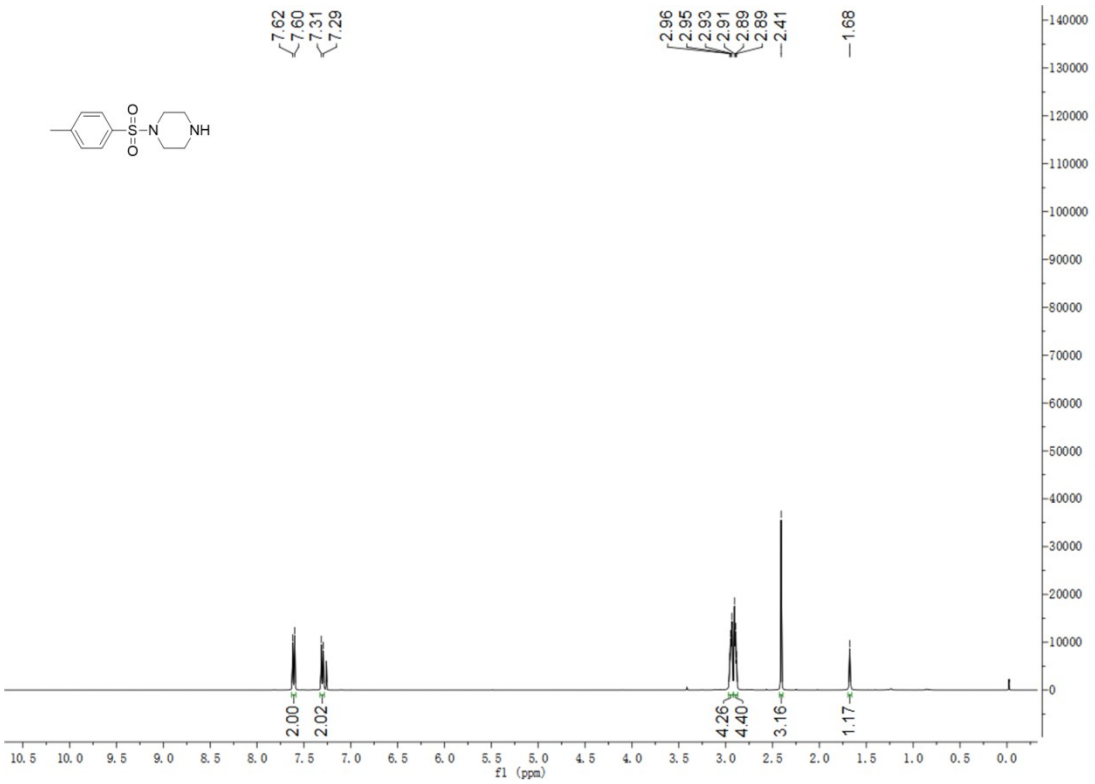


Fig. S11 ^{1}H NMR spectrum of **2**.

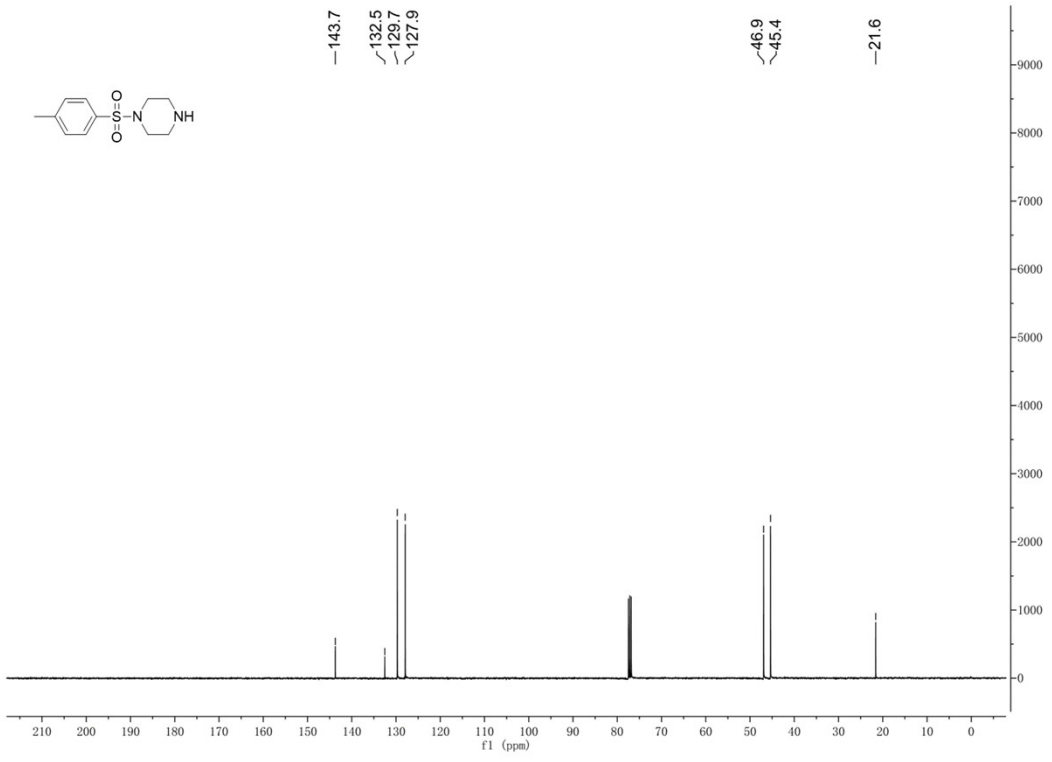


Fig. S12 ^{13}C NMR spectrum of **2**.

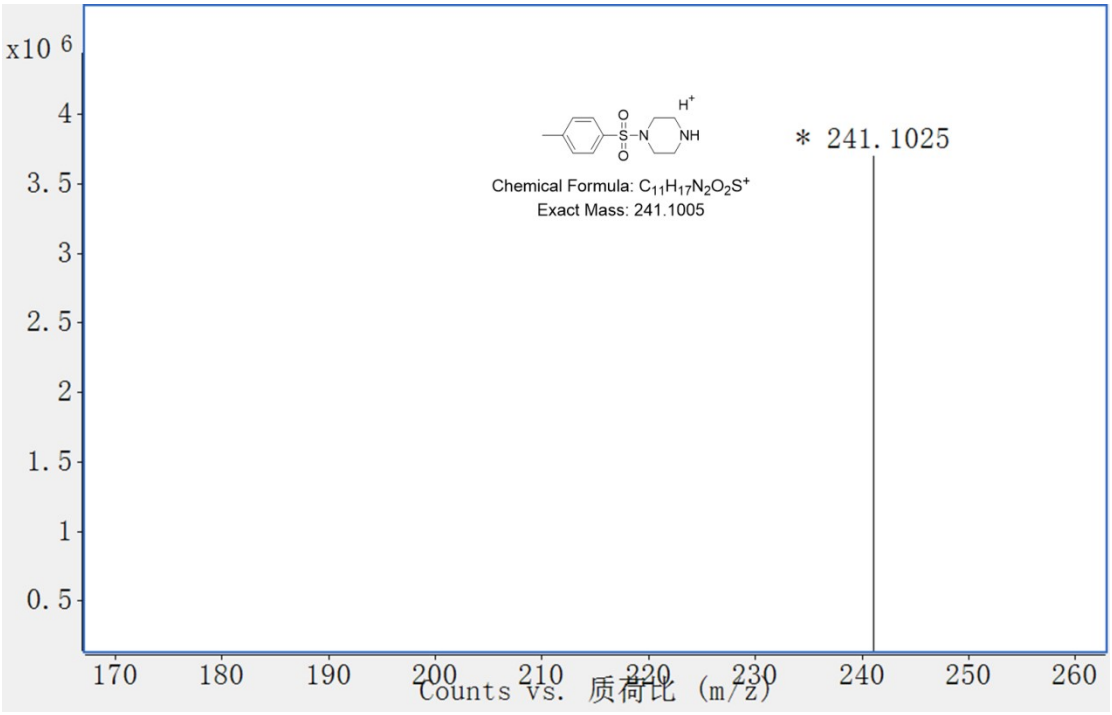


Fig. S13 HRMS spectrum of **2**.

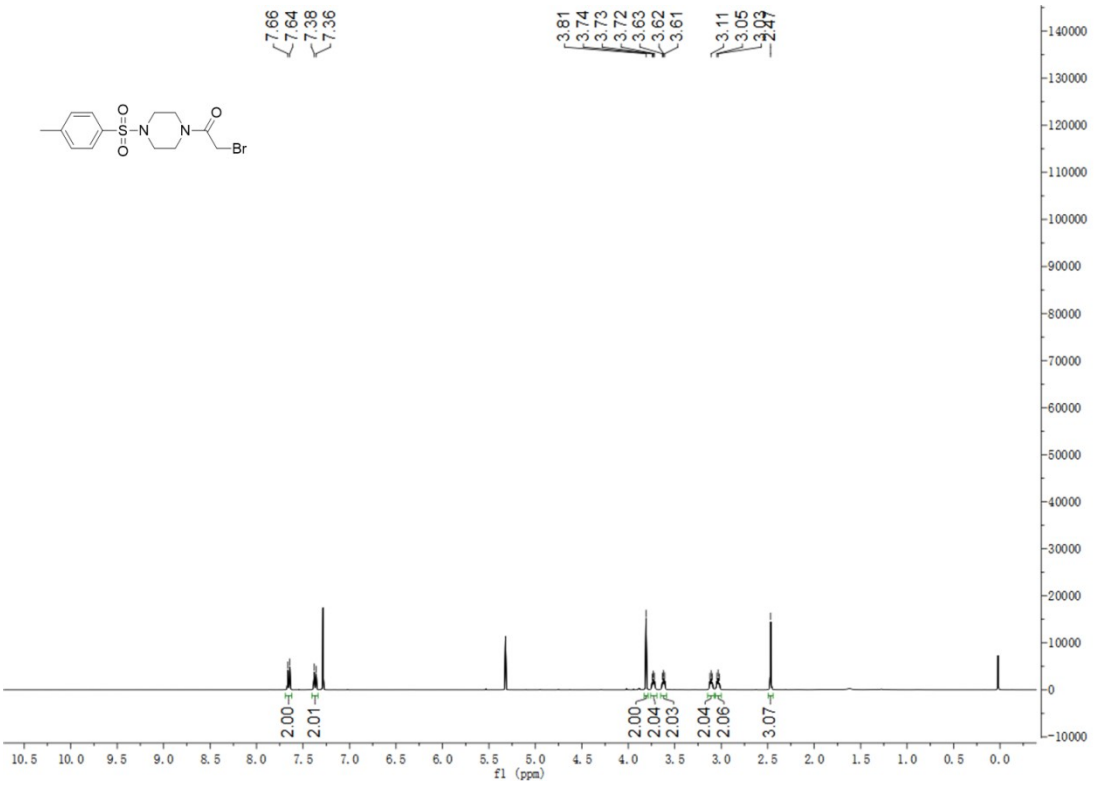


Fig. S14 ^{1}H NMR spectrum of **3**.

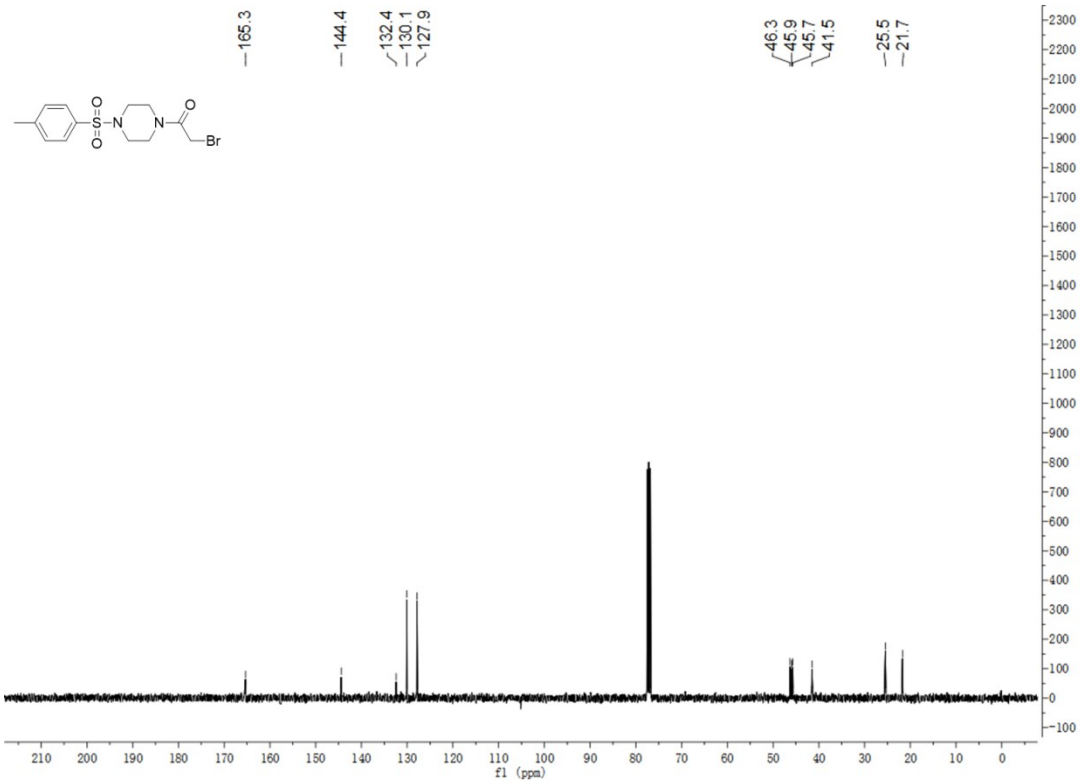


Fig. S15 ^{13}C NMR spectrum of **3**.

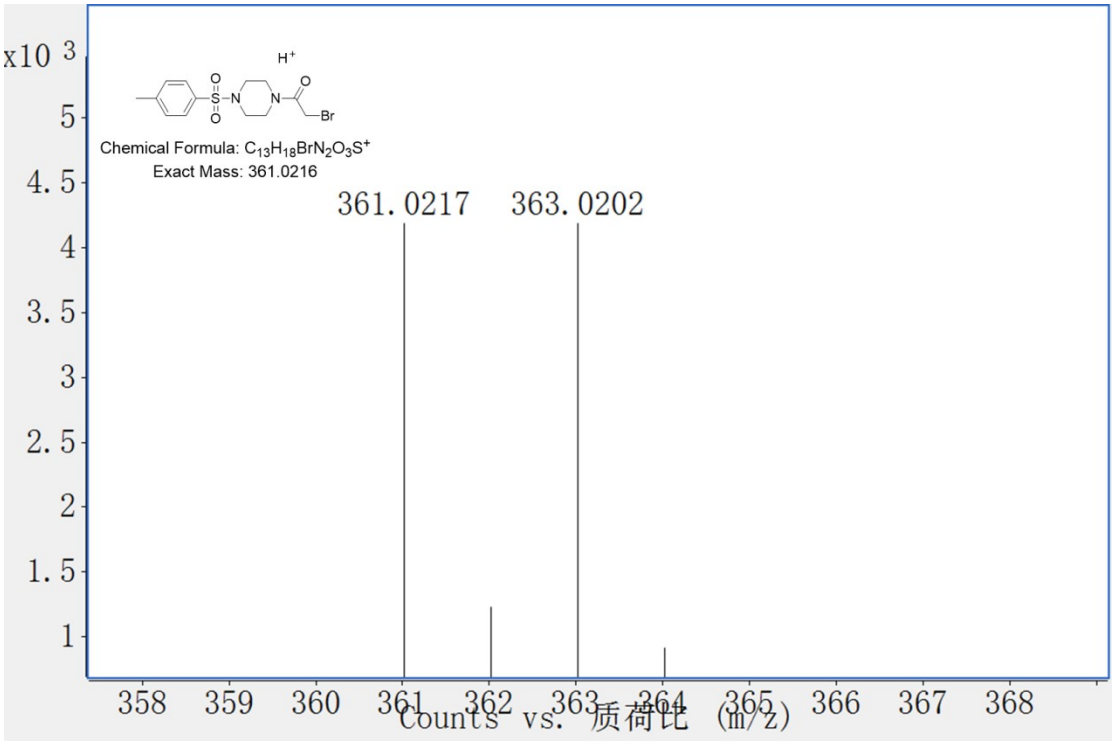


Fig. S16 HRMS spectrum of **3**.

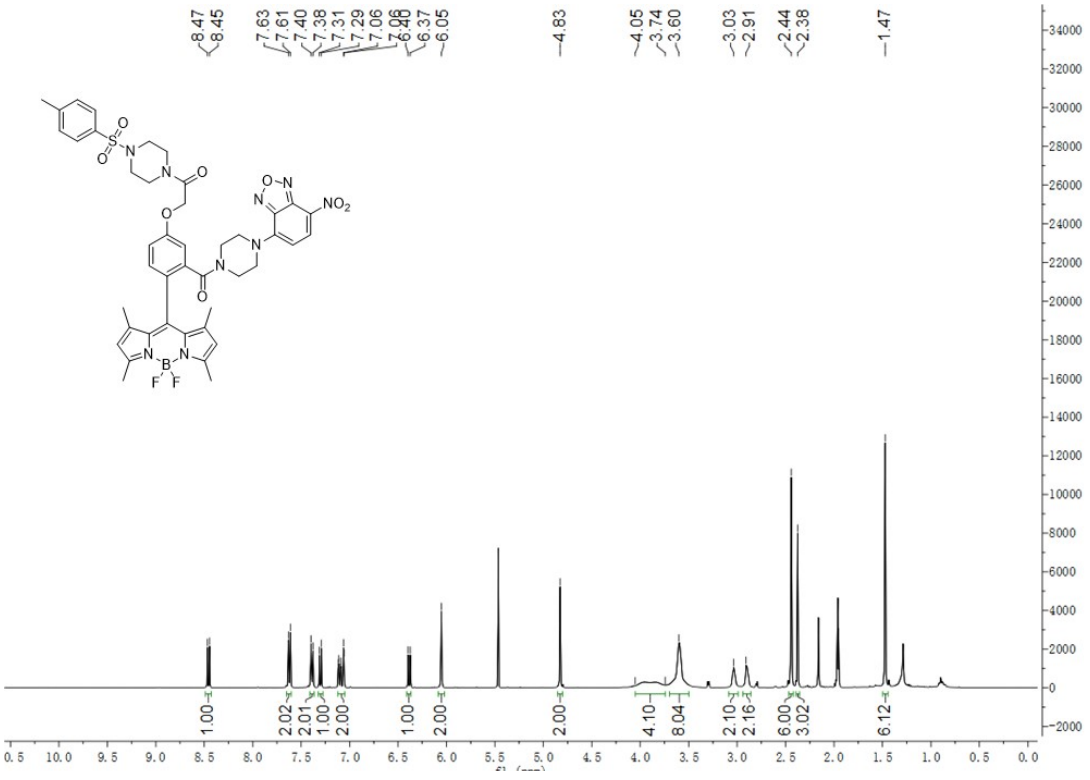


Fig. S17 ^{1}H NMR spectrum of **1**.

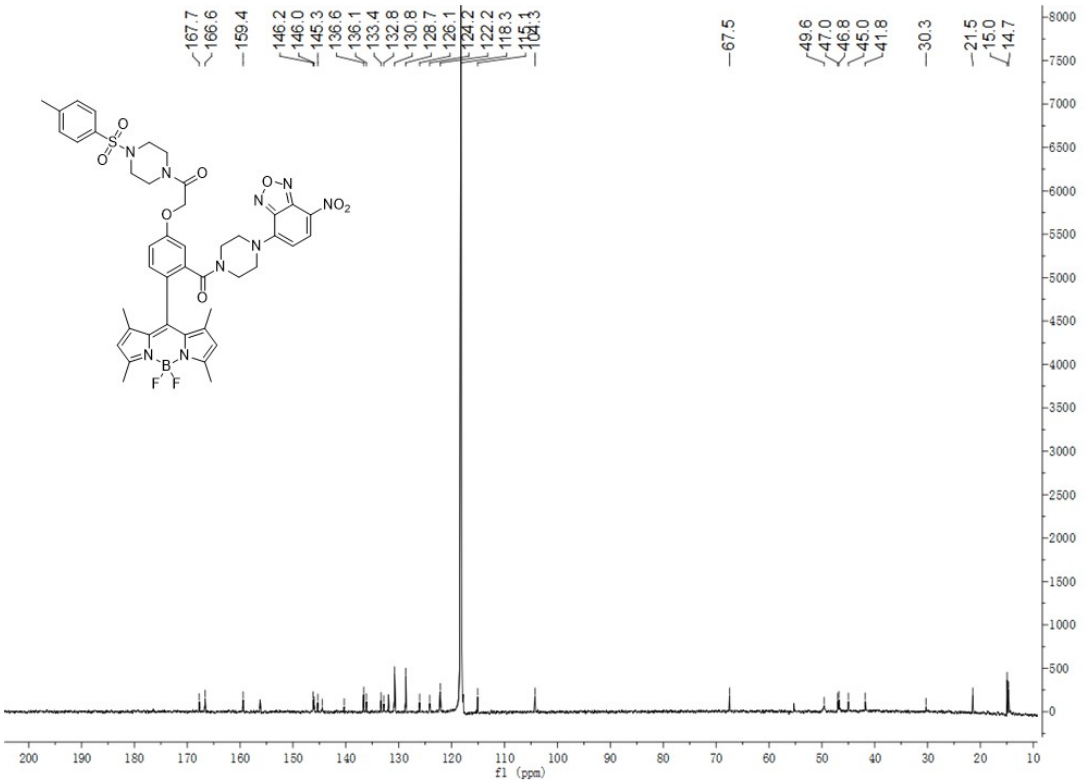


Fig. S18 ^{13}C NMR spectrum of **1**.

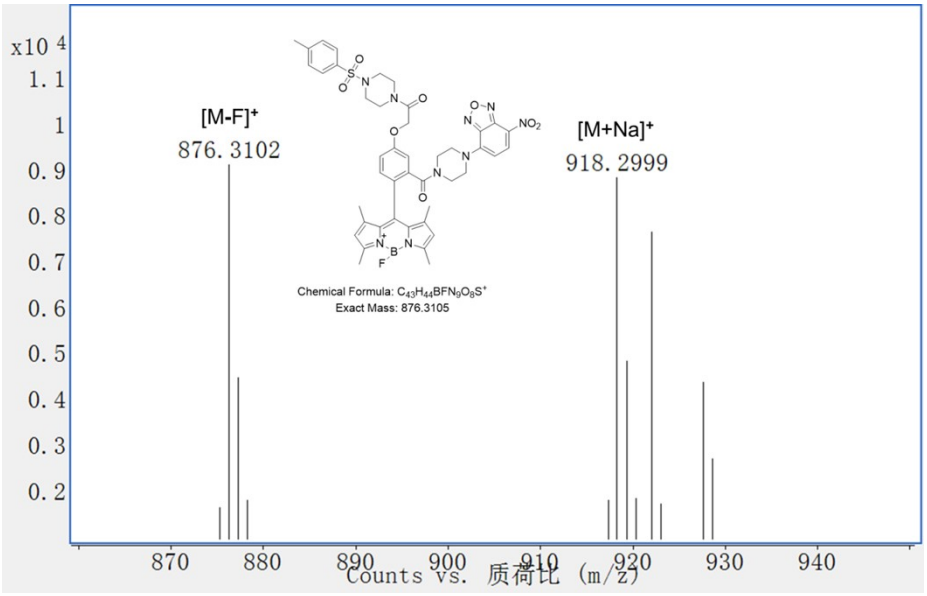


Fig. S19 HRMS spectrum of **1**.

8. References

[1] R. J. Man, D. J. Tang, X. Y. Lu, Y. T. Duan, X. .X Tao, M. R. Yang, L. L. Wang, B. Z. Wang, C. Xu and H. L. Zhu. *MedChemComm*, 2016, **7**, 1759-1767.

[2] H. Ye, L. Sun, Z. Pang, X. Ji, Y. Jiao, X. Tu, H. Huang, X. Tang, Z. Xi and L. Yi, *Anal. Chem.*, 2022, **94**, 1733-1741.

[3] S. K. Bae, C. H. Heo, D. J. Choi, D. Sen, E. H. Joe, B. .R Cho and H. M. Kim, *J. Am. Chem. Soc.*, 2013, **135**, 9915-9923.

[4] G. L. Long, J. D. Winefordner, *Anal. Chem.*, 1983, **55**, 712A-724A.

[5] L. Qiao, H. Nie, Y. Wu, F. Xin, C. Gao, J. Jing and X. Zhang, *J. Mater. Chem. B*, 2017, **5**, 525-530.

[6] O. G. Lyublinskaya, J. S. Ivanova, N. A. Pugovkina, I. V. Kozhukharova, Z. V. Kovaleva, A. N. Shatrova, N. D. Aksenov, V. V. Zenin, Yu. A. Kaulin, I. A. Gamaley and N. N. Nikolsky, *Redox Biol.*, 2017, **12**, 758-769.

[7] Y. Tang, A. Xu, Y. Ma, G. Xu, S. Gao and W. Lin, *Sci. Rep.*, 2017, **7**, 12944.

[8] H. Zhang, J. Chen, H. Xiong, Y. Zhang, W. Chen, J. Sheng and X. Song, *Org. Biomol. Chem.*, 2019, **17**, 1436-1441.

[9] Y. Zhang, B. Zhang, Z. Li, L. Wang, X. Ren and Y. Ye, *Org. Biomol. Chem.*, 2019, **17**, 8778-8783.

[10] H. Wang, L. Hu, B. Xu, H. Chen, F. Cai, N. Yang, Q. Wu, K. Uvdal, Z. Hu and L. Li, *Dyes Pigment*, 2020, **179**, 108390.

[11] W. Shu, S. Zang, C. Wang, M. Gao, J. Jing and X. Zhang, *Anal. Chem.*, 2020, **92**, 9982-9988.

[12] L. Zhou, Z. Q. Cheng, N. Li, Y. X. Ge, H. X. Xie, K. Zhu, A. Zhou, J. Zhang, K. M. Wang and C. S. Jiang, *Spectrochim. Acta, Part A*, 2020, **240**, 118578.

[13] Y. S. Kafuti, S. Zeng, X. Liu, J. Han, M. Qian, Q. Chen, J. Wang, X. Peng, J. Yoon and H. Li, *Chem. Commun.*, 2023, **59**, 2493-2496.