Electronic Supplementary Information

Reductive cleavage of C=C bond as a new strategy to turn on dual fluorescence for effective sensing H_2S

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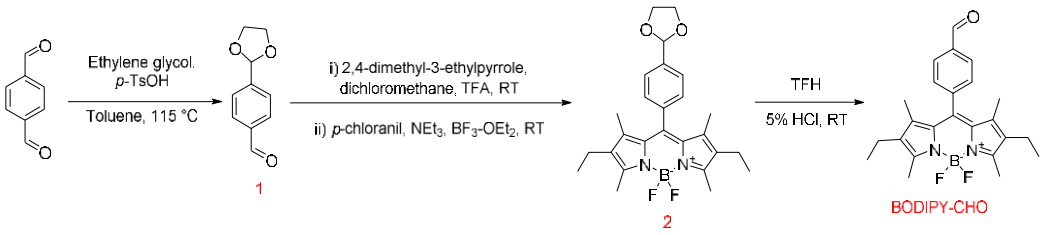
1. Materials and Methods.

Chemicals, such as 4-pyridinecarboxaldehyde, 2,2'-bipyridine-4-carbaldehyde, 1-Isoindolinone, 2,4-dimethyl-3-ethylpyrrole, 4-formylphenylboronic acid and analytes, including Na_2SO_4, K_3PO_4, Phenylalanine, Arginine, Glutamic, Cysteine and Glutathione were provided by Dieckman (Hong Kong) chemical industry company Ltd (Hong Kong, China). Fetal bovine serum (FBS) was purchased from Gibco/BRL (Grand Island, USA). 1640 cell culture medium and other chemicals were obtained from Sigma-Aldrich (St. Louis, USA). Besides, all other reagents were AR grade and used without further purification. Distilled water was purified with a Milli-Q water purification system provided by Millipore (Bedford, USA).

NMR spectra detection were recorded by a Bruker AV-400 instrument (Bruker, Germany) and reported in ppm downfield. High resolution mass spectra (HRMS) were obtained by a Xevo G2-XS QTof spectrometer (Waters, UK). All tested compounds were determined through a reverse phase C18 column by peak area integration (Agilent HPLC 1260, USA). Mass spectra were also collected by MALDI-TOF system (Microflex LT, Bruker, Germany). UV/Vis absorption and fluorescence spectroscopy were determined by UV spectrophotometer (UV-1800, Shimadzu, Japan) and fluorescence spectrophotometer (FluoroMax-4, Horiba, Japan). Cell and C. elegans imaging experiments were evaluated with fluorescence microscope (Carl Zeiss, Germany).

2. Synthesis



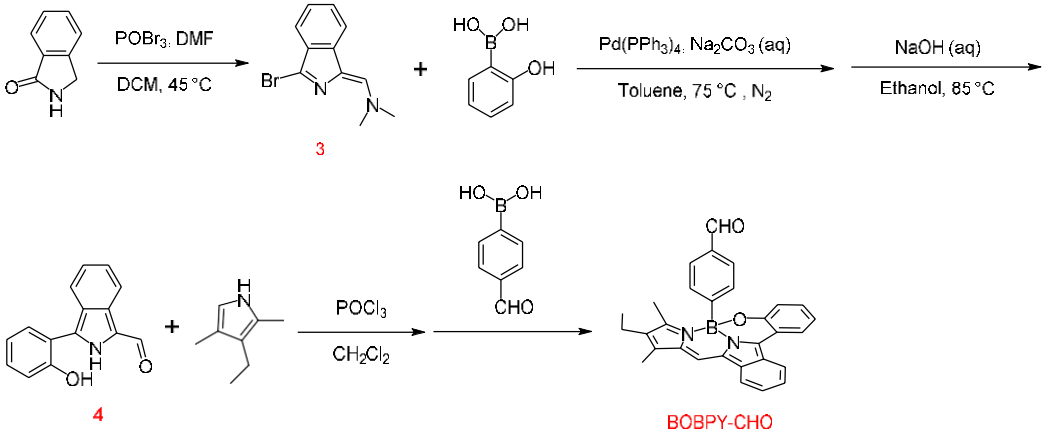
Scheme S1 Synthetic route of BODIPY-CHO.

Synthesis of compound 1. As described in Scheme S1, protection of aldehyde group was consulted with previous study.[1] A mixture of benzene-1,4-dicarbaldehyde (1.34 g, 10 mmol), ethylene glycol (0.75 g, 12 mmol) dissolved in toluene (40 mL), catalytic quantity of p-toluene sulfonic acid was added and the solution was refluxed for 10 h. After that, the solvent was removed under reduce pressure to get the crude product, and purified by silica gel column (dichloromethane/n-hexane=10:1) to obtain compound 1 in 74.36% as yield. ^{1}H NMR (400 MHz, DMSO) δ=10.03 (s,

1H), 8.01-7.90 (m, 2H), 7.73-7.60 (m, 2H), 5.84 (s, 1H), 4.09-4.04 (m, 2H), 4.01-3.96 (m, 2H).

Synthesis of compound 2. Compound 2 was synthesized via one step reaction as previously reported.[2] A solution of 2,4-dimethyl-3-ethylpyrrole (6.6 mmol, 0.81 g) and compound 1 (3 mmol, 0.54 g) dissolved in anhydrous dichloromethane (40 mL) under nitrogen atmosphere. The solution was stirred at room temperature for nearly 4 h, after one drop of trifluoroacetic acid was added. Subsequently, p-chloranil (3 mmol, 68 g) was added and stirred overnight. Finally, 2 ml of triethylamine and 2 ml of boron trifluoride diethyl etherate was added for stirring about 3 h. To the reaction solution, NaOH solution (1 M) was added and HCl solution (1M) was also used to bring pH around 7. The mixture was extracted with dichloromethane, the organic layer was separated and concentrated. Crude product was purified by silica gel column using dichloromethane/n-hexane=5:1 to get compound 2 (yield 56.75%). ^{1}H NMR (400 MHz, Acetone) δ=7.71-7.67 (m, 2H), 7.44-7.39 (m, 2H), 5.86 (s, 1H), 4.17-4.10 (m, 2H), 4.09-4.02 (m, 2H), 2.49 (s, 6H), 2.35 (q, J=7.6, 4H), 1.32 (s, 6H), 0.98 (t, J=7.6, 6H).

Synthesis of 8-(4-Formylphenyl)-1,3,5,7-tetramethyl-2,6-diethyl-4,4-difluoro-4-bora- 3a,4a-diaza-s-indacene (BODIPY-CHO). Compound 2 (1 mmol, 0.45 g) was dissolved in tetrahydrofuran (50 ml) containing 5% HCl. Then the mixture was stirred at room temperature for almost 8 h. After the reaction completed, NaOH 1 N solution was added until pH was around 7. The mixture was extracted with dichloromethane and solvent was removed. The residue was purified by silica gel column (dichloromethane/n-hexane=3:1) to get BODIPY-CHO (yield 90%). ^{1}H NMR (400 MHz, Acetone) δ=10.19 (s, 1H), 8.16 (d, J=8.4, 2H), 7.67 (d, J=8.0, 2H), 2.51 (s, 6H), 2.35 (q, J=7.6, 4H), 1.32 (s, 6H), 0.98 (t, J=7.6, 6H).



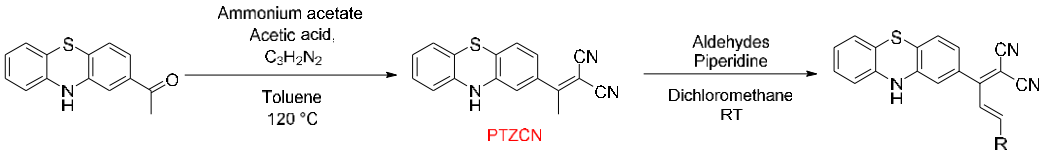
Scheme S2 Synthetic route of BOBPY-CHO.

Synthesis of compound 3. Compound 3 was synthesized according to previous literatures by modification[3] shown in Scheme S2. A mixture of $POBr_3$ (20 mmol, 5.73 g) in anhydrous dichloromethane (5 mL) was added dropwise to DMF (20 mmol, 1.46 g) with anhydrous dichloromethane (15 mL) at 0 ˚C. The mixture was stirred at room temperature for 30 min. Then a mixture of 1-Isoindolinone (10 mmol, 1.33 g) in anhydrous dichloromethane (50 mL) was added to the mixture at 0 ˚C. Subsequently, the reaction solution was refluxed for 6 h. After cooling, the solvent was removed by reduced pressure. Ice water was added, and pH of the mixture was adjusted by adding aqueous NaOH (5 M) to around 8. Black solid precipitated and the mixture was stirred overnight, then compound 3 (yield 84.21%) was collected by filtration. ^{1}H NMR (400 MHz, $CDCl_3$) δ=7.58 (t, J=8.1, 2H), 7.34-7.28 (m, 1H), 7.25-7.19 (m, 2H), 4.03-3.08 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ=140.67, 138.69, 133.52, 132.34, 126.09, 124.30, 123.03, 120.37, 115.74.

Synthesis of compound 4 and BOBPY-CHO. Compound 4 and BOBPY-CHO were synthesized by referring previous study.[4] To compound 3 (8 mmol, 2 g), 2-hydroxyphenyboronic acid (17.5 mmol, 2.41 g) and $Pd(PPh_3)_4$ (0.25 mmol, 0.29 g) in a Schlenk flask under nitrogen was added aqueous Na_2CO_3 (1 M) and toluene (50 mL). This reaction mixture was then degassed via three freeze-pump-thaw cycles before filled with nitrogen. The reaction solution was heated to 75 ˚C for 24 h. After cooling to room temperature, the reaction mixture was washed with water. Organic layers were combined, dried with anhydrous Na_2SO_4, and evaporated under vacuum. The crude product was refluxed for 3 h in ethanol containing aqueous NaOH (4 M). Solvent was removed in vacuum. The resultant solid was dissolved in ethyl acetate, neutralized with HCl (3 M). Organic layers were combined, dried under anhydrous Na_2SO_4, filtrated and concentrated under vacuum, the residual product was purified by silica gel column chromatography (dichloromethane/ethyl acetate=1:1) to afford compound 4 (yield 75.83%).

$POCl_3$ (5.2 mmol, 0.8 g) was added to a dichloromethane solution (15 mL) of 2,4-dimethyl-3-ethylpyrrole (10.4 mmol, 1.28 g) at 0 ˚C. Then a solution of compound 4 (5.2 mmol, 1.23 g) in dichloromethane (25 mL) was added dropwise to the reaction mixture at 0 ˚C. The reaction mixture was stirred at room temperature for 4 h. To this solution, 4-formylphenylboronic acid (52 mmol, 7.80 g) was dissolved in THF and added. Then the reaction mixture was stirred for another 4 h. After evaporation of the solvent, the residual product was purified by silica gel column chromatography (dichloromethane/n-hexane=2:1) to give BOBPY-CHO (yield 71.28%). ^{1}H NMR (400 MHz,

$CDCl_3$) δ=9.81 (s, 1H), 8.12 (d, J=8.3, 1H), 7.97 (dd, J=7.9, 1.5, 1H), 7.92 (d, J=8.1, 1H), 7.52 (t, J=7.4, 3H), 7.45 (d, J=4.1, 2H), 7.40-7.29 (m, 4H), 6.99 (s, 1H), 2.45 (s, 3H), 2.38 (dd, J=7.6, 2.3, 2H), 2.29 (s, 3H), 1.04 (t, J=7.6, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ=157.11, 154.20, 150.96, 143.18, 135.34, 133.23, 133.00, 132.90, 132.10, 130.36, 128.77, 128.43, 126.35, 125.65, 125.38, 123.43, 120.13, 119.65, 119.57, 119.08, 115.92, 114.10, 67.99, 25.62, 17.50, 13.07, 9.59.



Scheme S3 General synthetic route of phenothiazine ethylidene malononitrile derivatives.

Synthesis of PTZCN. Firstly, synthesis of PTZCN was according to previous studies with some modifications.[5,6] A mixture of malononitrile (20 mmol, 1.32 g), ammonium acetate (4 mmol, 0.3 g), 2-acetyl phenothiazine (20 mmol, 4.84 g), acetic acid (22 mmol, 1.25 mL), was dissolved in toluene (60 mL) and refluxed overnight. After that, the reaction was cooled to room temperature and washed with saturation $NaHCO_3$ solution. The organic layer was dried with $MgSO_4$ and concentrated in vacuo to get PTZCN (yield 88.35%). ^{1}H NMR (400 MHz, DMSO) δ=8.87 (s, 1H), 7.11-6.97 (m, 3H), 6.92 (dd, J=7.7, 1.3, 1H), 6.84 (d, J=1.8, 1H), 6.78 (dd, J=7.5, 1.2, 1H), 6.68 (dd, J=7.9, 1.1, 1H), 2.54 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ=176.57, 142.49, 141.46, 135.57, 128.50, 126.92, 126.78, 122.81, 121.60, 115.69, 115.16, 113.89, 112.97, 82.70, 24.42.

General Procedure for phenothiazine ethylidene malononitrile derivatives. Consulting previous literature,[7] a solution of PTZCN (1.1 mmol) and aldehydes (1 mmol) in dichloromethane was added of piperidine as catalyst (100 μL). The mixture was stirred at room temperature overnight. Evaporation of dichloromethane gave the crude product and purified by silica gel column to get products.

PTZ-P1. Yield 65.13%. ^{1}H NMR (400 MHz, DMSO) δ=8.86 (s, 1H), 8.65 (dd, J=4.5, 1.5, 2H), 7.73-7.63 (m, 3H), 7.17-7.09 (m, 2H), 7.01 (td, J=7.7, 1.4, 1H), 6.93 (ddd, J=12.5, 7.8, 1.6, 2H), 6.79 (td, J=7.5, 1.2, 1H), 6.74-6.65 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ=169.74, 151.06, 145.60, 142.66, 141.72, 141.62, 132.30, 128.70, 128.47, 127.17, 126.83, 123.17, 122.85, 122.67, 121.85, 115.84, 115.18, 114.79, 114.13, 113.33, 83.61. HRMS (ESI): calcd for $C_{23}H_{14}N_4S$ $[M+H]^+$, 379.0939; found, 379.1132.

PTZ-P2. Yield 62.31%. ^{1}H NMR (400 MHz, DMSO) δ=8.87 (s, 1H), 8.76 (d, J=5.1, 1H), 8.71 (dd, J=3.2, 2.4, 1H), 8.62 (s, 1H), 8.40 (d, J=7.9, 1H), 7.97 (td, J=7.8, 1.8, 1H), 7.78 (dd, J=5.1, 1.6, 1H), 7.72 (d, J=15.7, 1H), 7.49 (ddd, J=7.5, 4.8, 1.1, 1H), 7.29 (d, J=15.7, 1H), 7.14 (d, J=7.9, 1H), 7.01 (td, J=7.7, 1.4, 1H), 6.95 (d, J=1.8, 2H), 6.79 (s, 1H), 6.75 (d, J=1.8, 1H), 6.69 (d, J=1.0, 1H). ^{13}C NMR (101 MHz, DMSO) δ=169.81, 156.71, 155.07, 150.83, 149.83, 145.78, 142.99, 142.64, 141.64, 137.96, 132.26, 128.81, 128.46, 127.14, 126.82, 125.08, 123.21, 122.93, 122.82, 121.82, 121.15, 119.21, 115.86, 115.17, 114.86, 114.16, 113.45, 83.64. HRMS (ESI): calcd for $C_{28}H_{17}N_5S$ $[M+H]^+$, 456.1204, found, 456.1383.

PTZ-P3. Yield 60.78%. ^{1}H NMR (400 MHz, DMSO) δ=8.87 (s, 1H), 7.95 (s, 2H), 7.61 (d, J=15.7, 1H), 7.46 (d, J=8.3, 2H), 7.24 (d, J=15.6, 1H), 7.14 (d, J=7.9, 1H), 7.04-6.99 (m, 1H), 6.94 (s, 2H), 6.80 (td, J=7.5, 1.3, 1H), 6.75 (d, J=1.8, 1H), 6.68 (dd, J=7.9, 1.1, 1H), 2.44 (s, 6H), 2.29 (d, J=7.5, 4H), 1.28 (s, 6H), 0.94 (t, J=7.5, 6H). ^{13}C NMR (101 MHz, DMSO) δ=207.16, 176.52, 153.99, 147.72, 142.53, 141.48, 135.56, 133.21, 133.07, 132.51, 130.10, 129.63, 128.46, 126.88, 126.73, 122.79, 121.55, 115.71, 115.15, 113.82, 112.96, 82.71, 55.31, 24.36, 16.84, 14.91, 12.65, 11.96, 11.70. HRMS (ESI): calcd for $C_{41}H_{36}BF_2N_5S$ $[M+H]^+$, 680.2752; found 680.2795. Calcd for $C_{41}H_{36}BF_2N_5S$ $[M-F]^+$, 660.2846; found 660.2631.

PTZ-P4. Yield 50.23%. ^{1}H NMR (400 MHz, DMSO) δ=8.74 (s, 1H), 8.34 (d, J=8.4, 1H), 8.24 (d, J=8.1, 1H), 8.14 (s, 2H), 7.62 (t, J=7.5, 1H), 7.53-7.41 (m, 2H), 7.37(d, J=8.2, 2H), 7.27 (s, 2H), 7.14 (d, J=8.1, 2H), 7.05 (d, J=7.8, 3H), 6.92 (s, 2H), 6.78 (ddd, J=13.9, 6.3, 4.7, 2H), 6.61 (d, J=1.8, 2H), 2.42 (s, 3H), 2.35 (dd, J=14.9, 7.2, 2H), 2.28 (s, 3H), 0.99 (t, J=7.5, 3H). ^{13}C NMR (101 MHz, DMSO) δ=170.72, 156.47, 150.70, 149.63, 142.70, 142.53, 141.59, 135.34, 134.78, 134.08, 133.05, 132.67, 132.25, 130.48, 129.69, 128.48, 128.09, 127.20, 127.03, 126.81, 126.61, 125.90, 124.03, 123.42, 123.07, 122.81, 121.42, 121.05, 120.89, 120.34, 118.77, 118.61, 115.86, 115.14, 114.79, 80.05, 31.43, 22.53, 17.29, 15.20, 14.44, 13.27, 9.76. MALDI-TOF: calcd for $C_{47}H_{34}BN_5OS$ $[M-H]^-$, 726.257; found, 726.218.

3. Proposed mechanism of PTZ-P1 by reacting with H_2S.

PTZ-P1 (0.5 mmol, 0.19 g) was dissolved in 10 mL DMSO and added dropwise with NaHS (0.5 mmol, 0.028 g) dissolved in 10 mL distilled water. Then the mixture was stirred for 4 h at room temperature. After reaction completed, distilled water was added and extracted with ethyl acetate.

The organic layer was separated and dried with Na_2SO_4. After concentrating in vacuo, the crude product was purified by silica gel column (dichloromethane/ethyl acetate=10:1) to get PTZCNSF (yield 18.79%). ^{1}H NMR (400 MHz, DMSO) δ=8.73 (s, 1H), 7.25 (s, 2H), 6.95 (s, 2H), 6.91 (d, J=1.8, 2H), 6.83 (d, J=1.7, 1H), 6.77 (t, J=7.5, 1H), 6.71 (d, J=7.3, 1H), 6.45 (s, 1H); ^{13}C NMR (101 MHz, DMSO) δ=166.88, 142.67, 142.22, 138.39, 134.34, 128.11, 126.83, 126.71, 122.38, 120.91, 116.92, 116.66, 116.54, 115.01, 113.10, 105.23, 83.40. HRMS (ESI): calcd for $C_{17}H_{11}N_3S_2$ $[M+H]^+$, 322.0394; found, 322.0497.

4. Preparation of the test solution.

Sodium hydrosulfide (NaHS) was dissolved in distilled water to 10 mM. A stock solution (1 mM) of PTZ-P1, PTZ-P2, PTZ-P3 and PTZ-P4 were both prepared in DMSO. Take PTZ-P1 for example, test solutions of PTZ-P1 were diluted to 2 mL with a mixture of distilled water and DMSO (1:1, V/V), while small aliquots of NaHS solution was added for detecting UV-vis absorption and fluorescence property.

The solutions of various analytes were prepared from H_2O_2, HClO, NaCl, KCl, $CaCl_2$, $NaHSO_3$, $Na_2S_2O_3$, Na_2SO_3, Na_2SO_4, K_3PO_4, Phenylalanine, Arginine, Glutamic, Cysteine and Glutathione respectively. Small aliquots of each testing species solution were added. The resulting solution was shaken well and incubated for 10 min at room temperature before recording the fluorescent spectra.

5. Analysis of Cell Viability by MTT.

HeLa cells were cultured in complete 1640 medium, containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37 ˚C in atmosphere containing 5% CO_2. After 90% confluence, the cells were cultured into 96-well plates (5000 cells/well, 100 μL). Cells were treated with different concentrations of PTZ-P4 (80, 70, 60, 50, 40, 30, 20, 10 μM) for 24 h. Then, MTT stock solution (5.0 mg/mL, 100 μL) was added to each well for incubating 4 h to form purple crystal formazan. At the end of incubation, DMSO (100 μL) was added for 10 min microvibration after removing medium. The absorbance was measured at 570 nm on a microplate reader (Thermo, USA).

6. Cell treatment and imaging exogenous H_2S.

After 90% confluence, cells were seeded in confocal culture dishes for 24 h. Aqueous NaHS was used as exogenous H_2S source. Cells were preincubated with 20 μM PTZ-P4 for 5 h. Subsequently, cells were washed with PBS (pH=7.4) for three times and incubated with H_2S (100 μM) for 3 h.

Residual medium was removed by washing with PBS (pH=7.4) for three times and fixed. Images were collected by fluorescence microscope.

7. C. elegans treatment and imaging endogenous H_2S.

C. elegans strains used in this study included Bristol N2 (wild type), glp-1 (e2144) (higher level of endogenous H_2S), and cth-1 (ok3319) (lower level of endogenous H_2S), and all nematodes were maintained at 20 ˚C on nematode growth media (NGM) plates seeded with Escherichia coli OP50 bacteria. Synchronized L1 worms were achieved by hypochlorite treatment of gravid worms using a bleaching solution (20 % alkaline hypochlorite, 0.2 M NaOH in H_2O), and were cultured at 25 ˚C on NGM plates containing 200 μM PTZ-P4 for 48 h. PTZ-P4 was mixed in OP50 bacteria and added to NGM plates at the indicated concentrations 1 d before worms were added. Finally, worms were exposed to anesthesia and images were collected by fluorescence and confocal microscopes.

8. Limit of detection

It is reported that variable estimates of physiological relevant H_2S ranging from nano- to millimolar levels, but the exact H_2S concentrations remain controversial.[8-12] According to previous study with Equation (1),[13] we calculated the limit of detection of PTZ-P4 to H_2S, which was 4 μM satisfying well with this range.

Equation (1): $C_L = kS_{bi}/m$

Where, m is the slope of the linear regression equation shown in Figure S1. S_{bi} is the standard deviation of the blank measures. Generally, k=3, $P < 0.01$, so we obtained $C_L = 4$ μM.

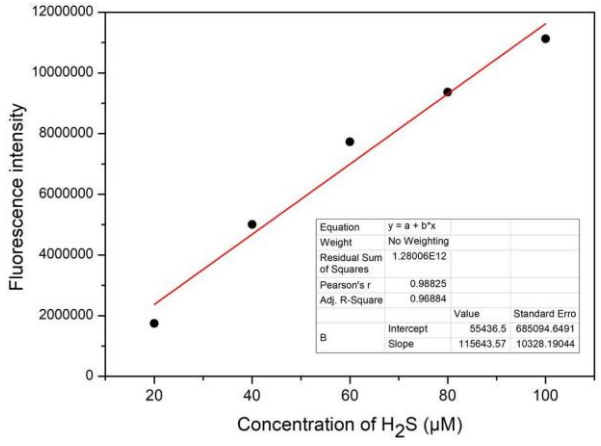


Figure S1 The corresponding linear relationship between the fluorescent intensity of PTZ-P4 (10 μM) and different concentrations of H_2S in PBS buffer (pH=7.4)/DMSO (1/2, 2% v/v PEG 400)

solution.

9. Data availability.

The authors declare that all relevant data supporting the findings of this study are available within the article and in the Electronic Supplementary Information document, or from the corresponding author on request. The X-ray crystallographic coordinates for PTZ-P1 and PTZCNSF can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif under deposition numbers CCDC 1846921 and 1846922, respectively.

Table S1 Crystal data and structure refinement.

Compounds	PTZ-P1	PTZCNSF
CCDC No.	1846921	1846922
Empirical formula	$C_{23}H_{14}N_4S$	$C_{17}H_{11}N_3S_2$
Formula weight	378.4530	321.4160
Temperature	293 K	293 K
Wavelength	1.54178	1.54178
Crystal system	P 21 21 21	P 21
space group	orthorhombic	monoclinic
a/Å	7.8612	8.7916
b/Å	9.8518	5.6255
c/Å	27.1605	14.7562
α/(°)	90	90
β/(°)	90	95.407
γ/(°)	90	90
V/Å3	2103.50	726.55
Z	4	2
Dc/g m^{-3}	1.252	1.465
μ/mm^{-1}	1.530	3.302
F (000)	824.0	330.0
Final R indices[I>2σ(I)]	R_1=0.0548 wR_2= 0.1638	R_1 = 0.0311 wR_2 = 0.0834
Goodness-of-fit on F^2	1.092	1.037

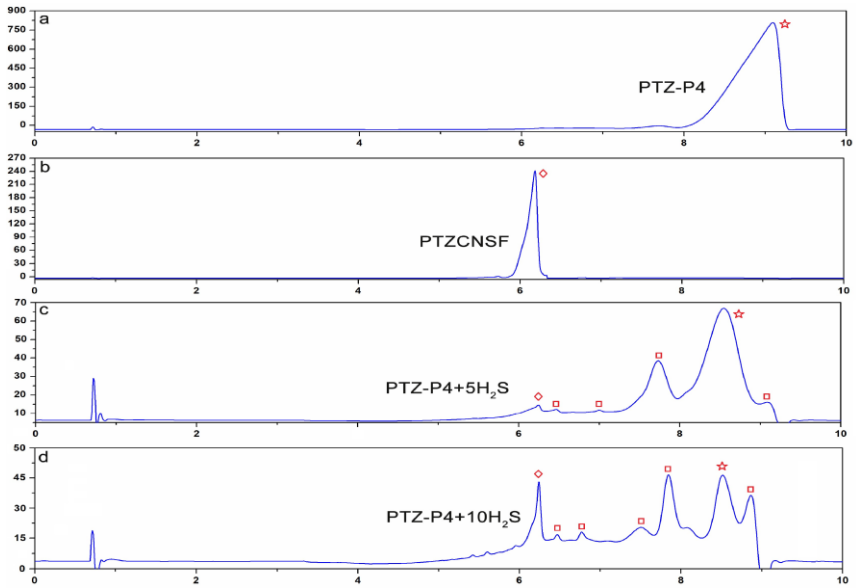


Figure S2 HPLC chromatogram. (a) Chromatogram of PTZ-P4. (b) Chromatogram of PTZCNSF. (c) Chromatogram of PTZ-P4 added with 5 equivalent H_2S. (d) Chromatogram of PTZ-P4 added with 10 equivalent H_2S.

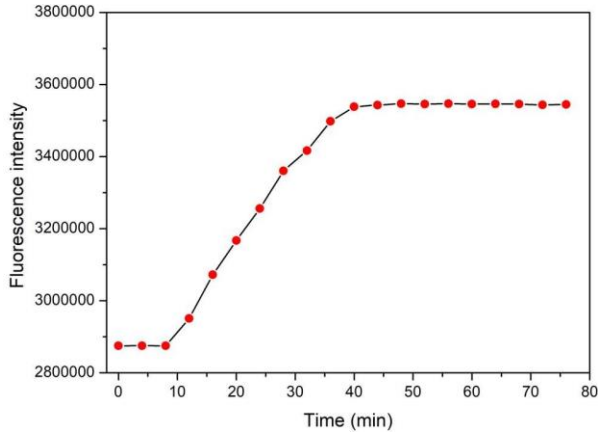


Figure S3 Time-dependent fluorescence spectra of PTZ-P4 (10 μM) towards 20 μM H_2S with the fluorescence emission changes at 638 nm (λ_{ex}=580 nm).

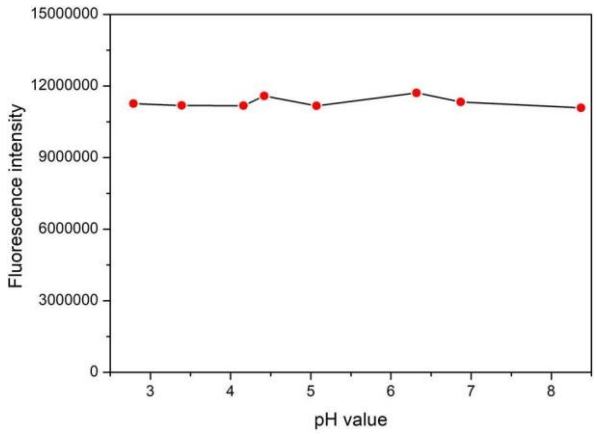


Figure S4 Effect of PTZ-P4 in PBS buffer solution with different pH values. The fluorescence emission changes at 638 nm with the pH titration curve of 10 μM PTZ-P4 (λ_{ex}=580 nm), pH value: 2.79, 3.39, 4.16, 4.42, 5.07, 6.32, 6.87, 8.37 (from left to right).

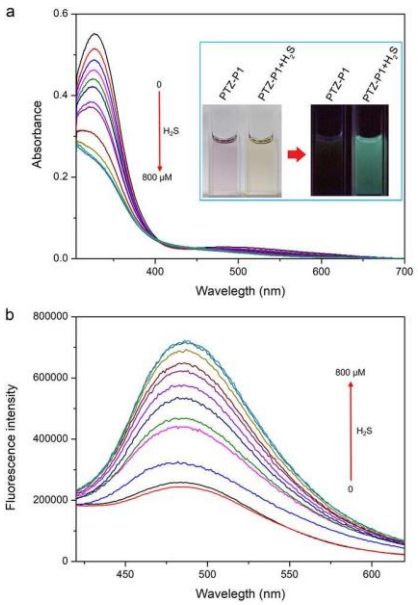


Figure S5 (a) Absorption spectra of PTZ-P1 (20 μM) in PBS buffer (pH=7.4)/DMSO (1/2, 2% v/v PEG 400) adding with H_2S (0-800 μM), inset: photoimages of probe with and without H_2S in daylight (left) and under an ultraviolet lamp (365 nm; right). (b) Fluorescent spectra of PTZ-P1 (20 μM) in PBS buffer (pH=7.4)/DMSO (1/2, 2% v/v PEG 400) adding with H_2S (0-800 μM), λ_{ex}=330 nm.

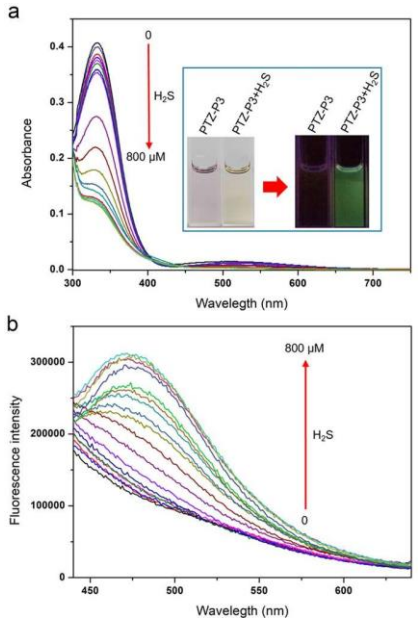


Figure S6 (a) Absorption spectra of PTZ-P2 (10 μM) in PBS buffer (pH=7.4)/DMSO (1/2, 2% v/v PEG 400) in the presence of H_2S (0-800 μM), inset: photoimages of probe with and without H_2S in

daylight (left) and under an ultraviolet lamp (365 nm; right). (b) Fluorescent spectra of PTZ-P2 (10 μM) in PBS buffer (pH=7.4)/DMSO (1/2, 2% v/v PEG 400) in the presence of H_2S (0-800 μM), λ_{ex}=330 nm.

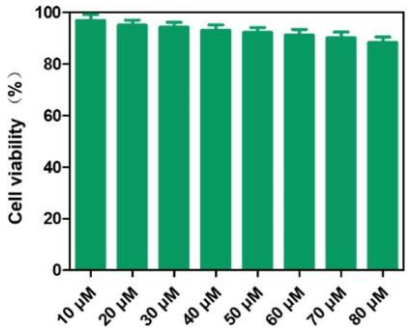


Figure S7 Viabilities of HeLa cells after incubation with different concentrations of PTZ-P4 for 24 h.

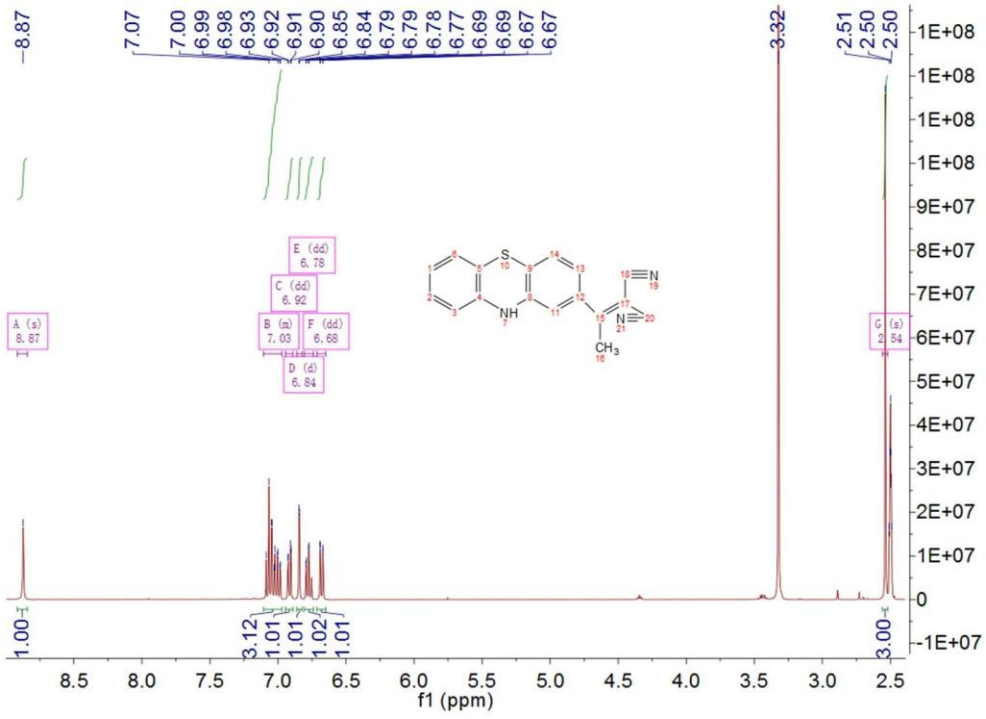


Figure S8 ^{1}H NMR spectrum of PTZCN in DMSO.

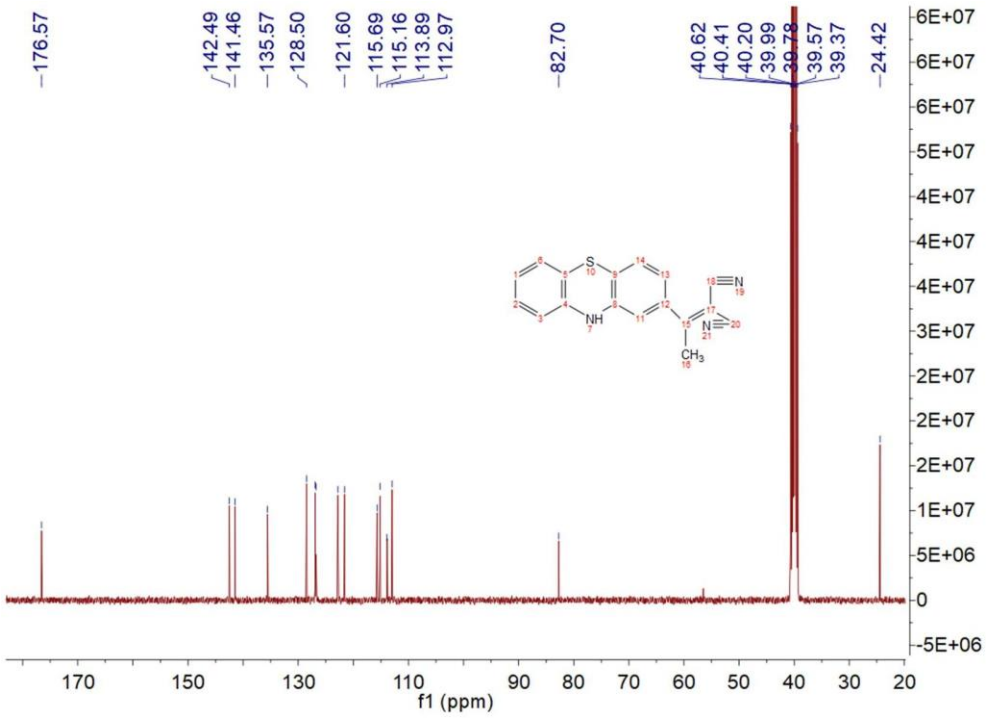


Figure S9 ^{13}C NMR spectrum of PTZCN in DMSO.

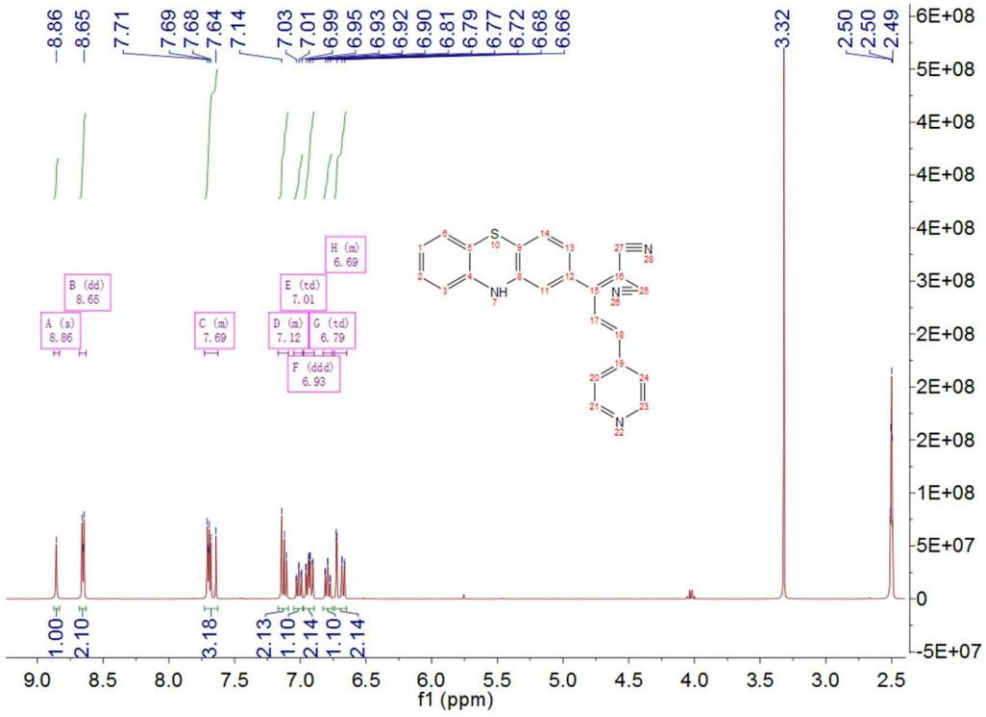


Figure S10 ^{1}H NMR spectrum of PTZ-P1 in DMSO.

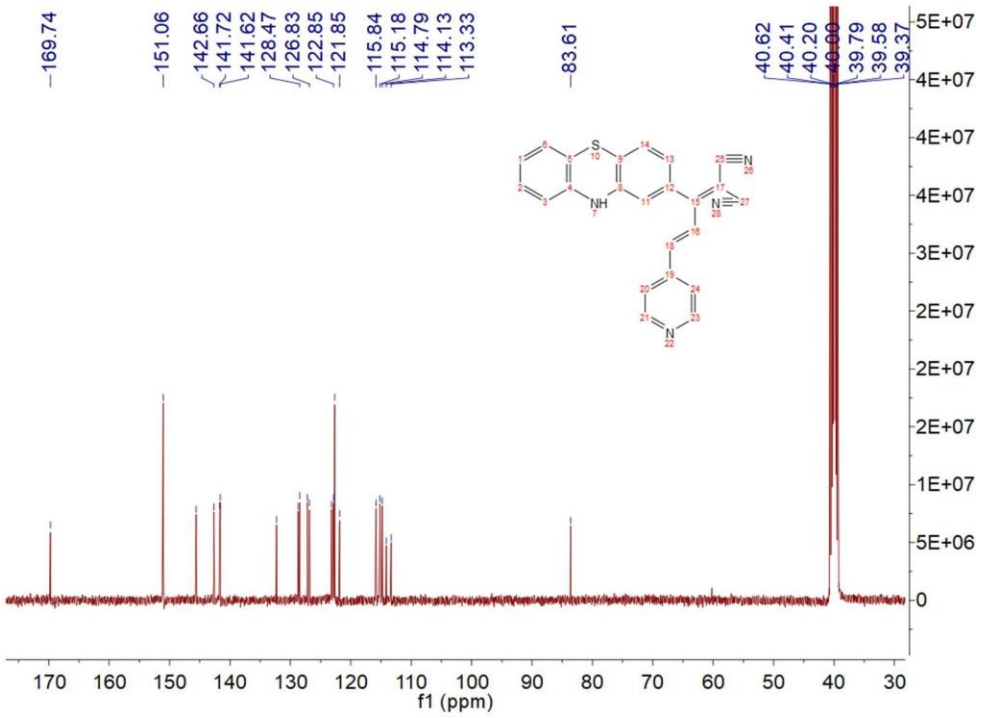


Figure S11 ^{13}C NMR spectrum of PTZ-P1 in DMSO.

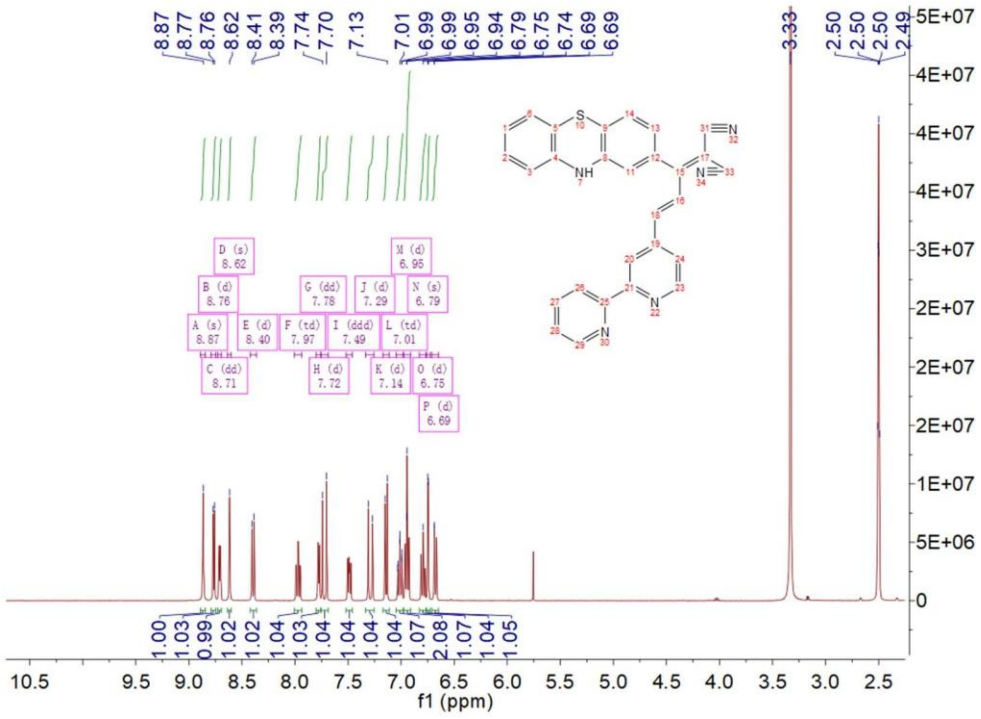


Figure S12 ^{1}H NMR spectrum of PTZ-P2 in DMSO.

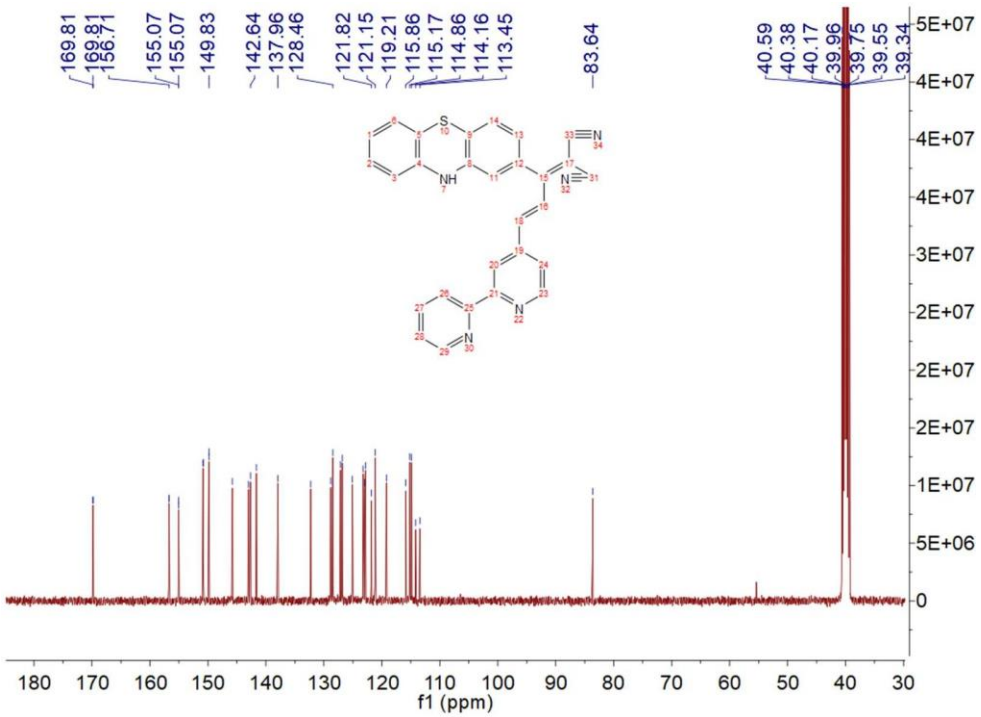


Figure S13 ^{13}C NMR spectrum of PTZ-P2 in DMSO.

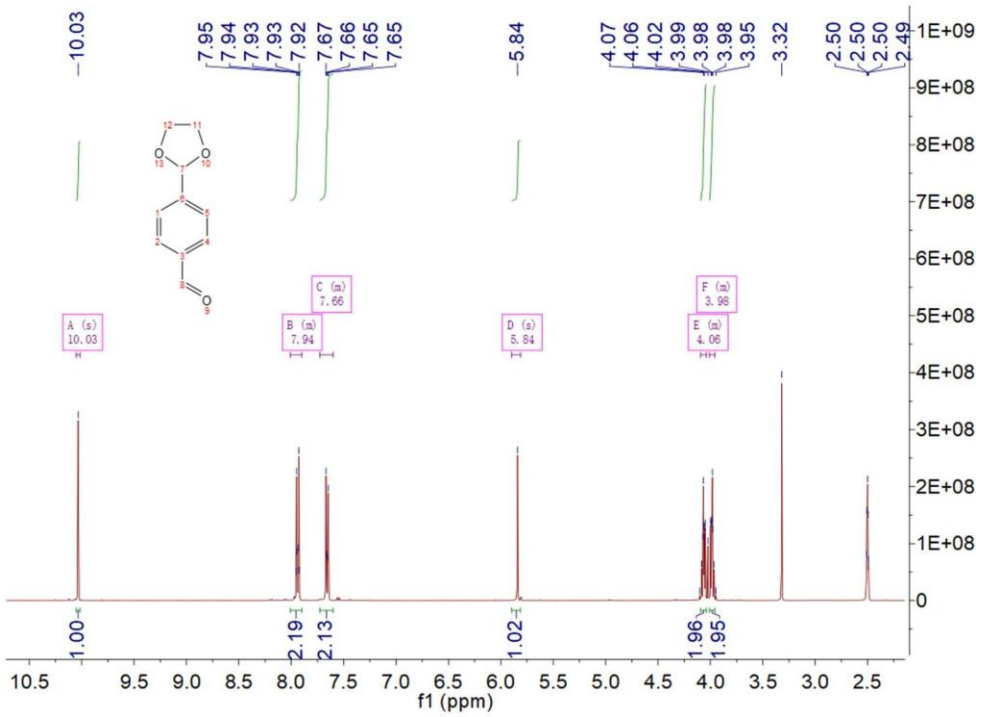


Figure S14 ^{1}H NMR spectrum of compound 1 in DMSO.

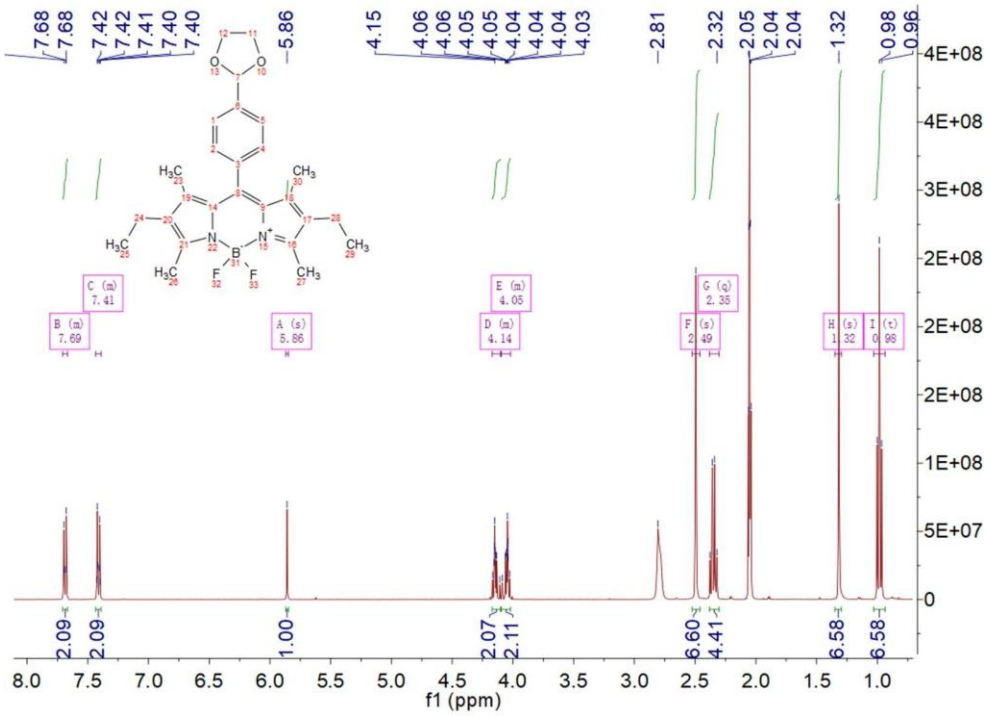


Figure S15 ^{1}H NMR spectrum of compound 2 in Acetone.

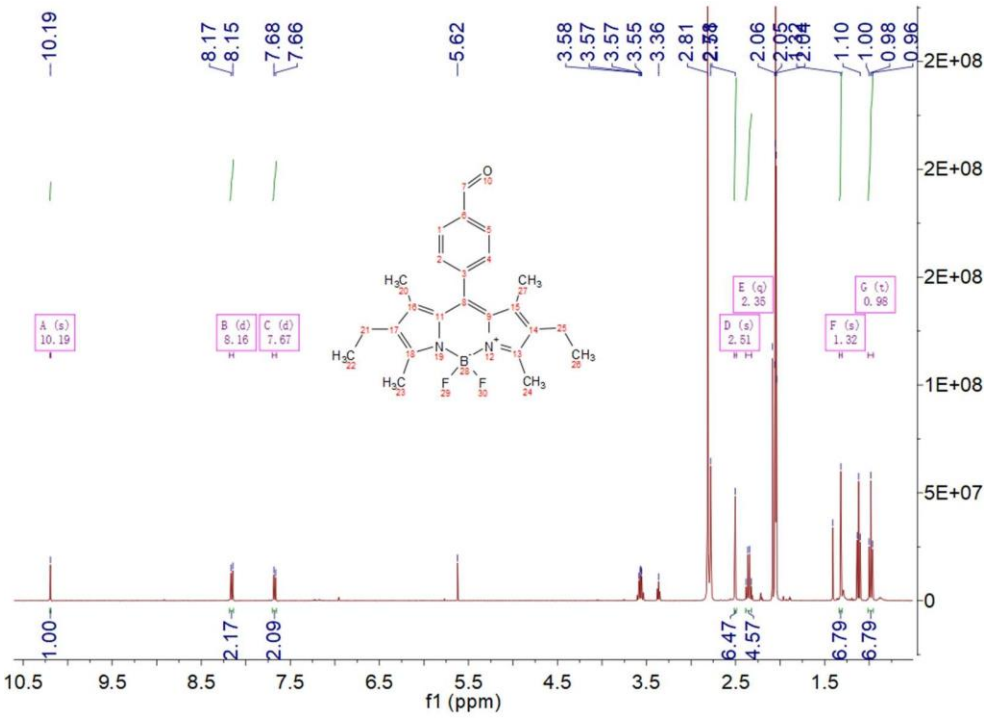


Figure S16 ^{1}H NMR spectrum of BODIPY-CHO in Acetone.

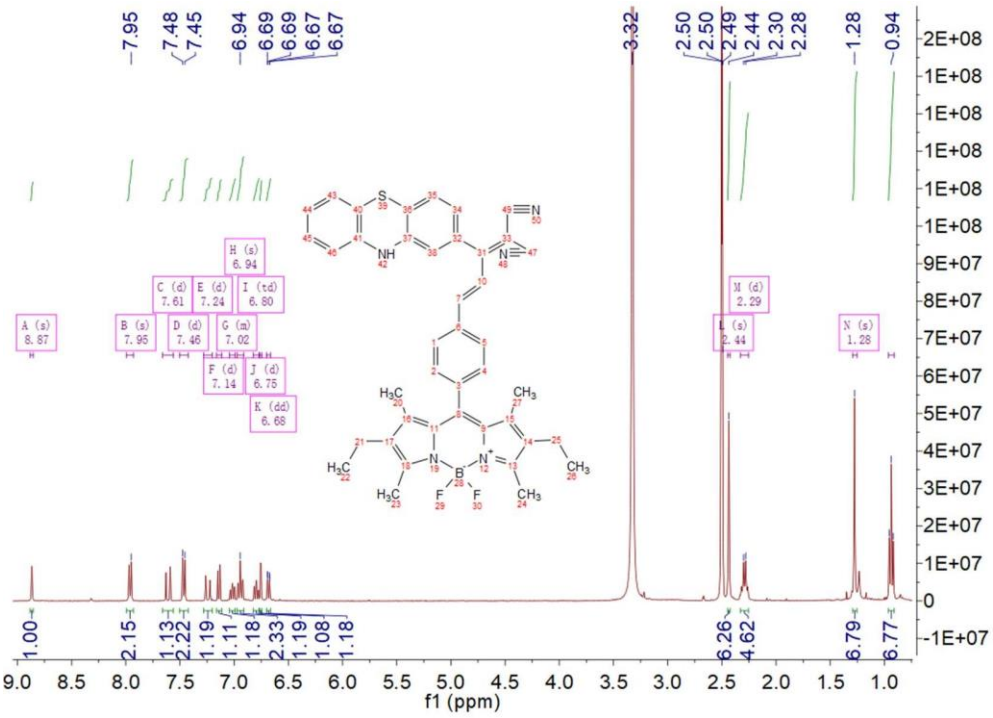


Figure S17 ^{1}H NMR spectrum of PTZ-P3 in DMSO.

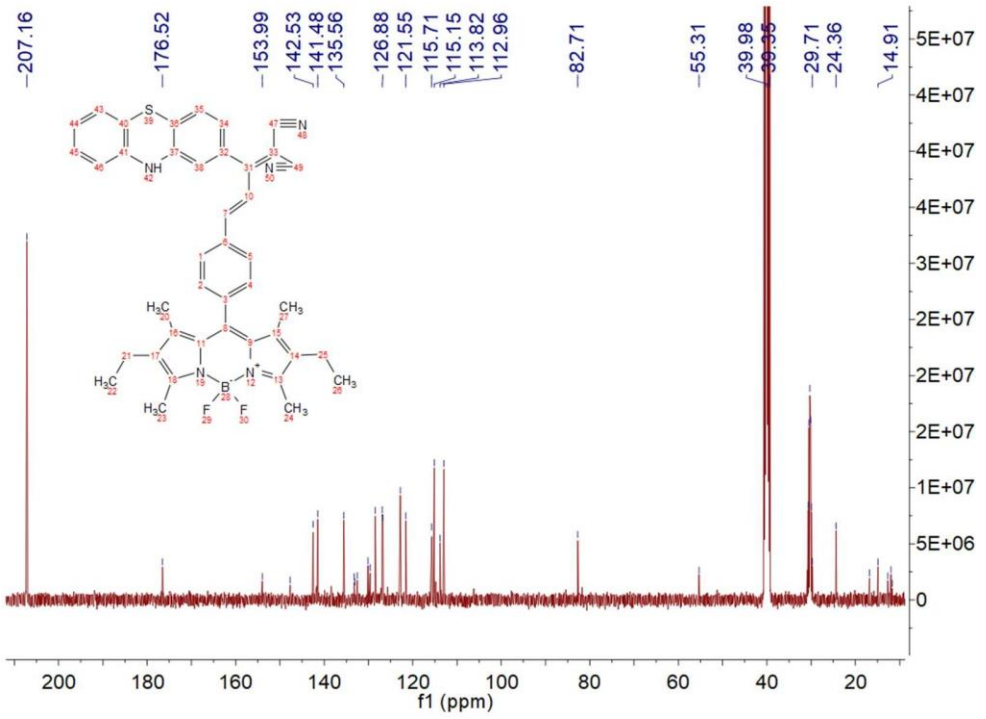


Figure S18 ^{13}C NMR spectrum of PTZ-P3 in DMSO.

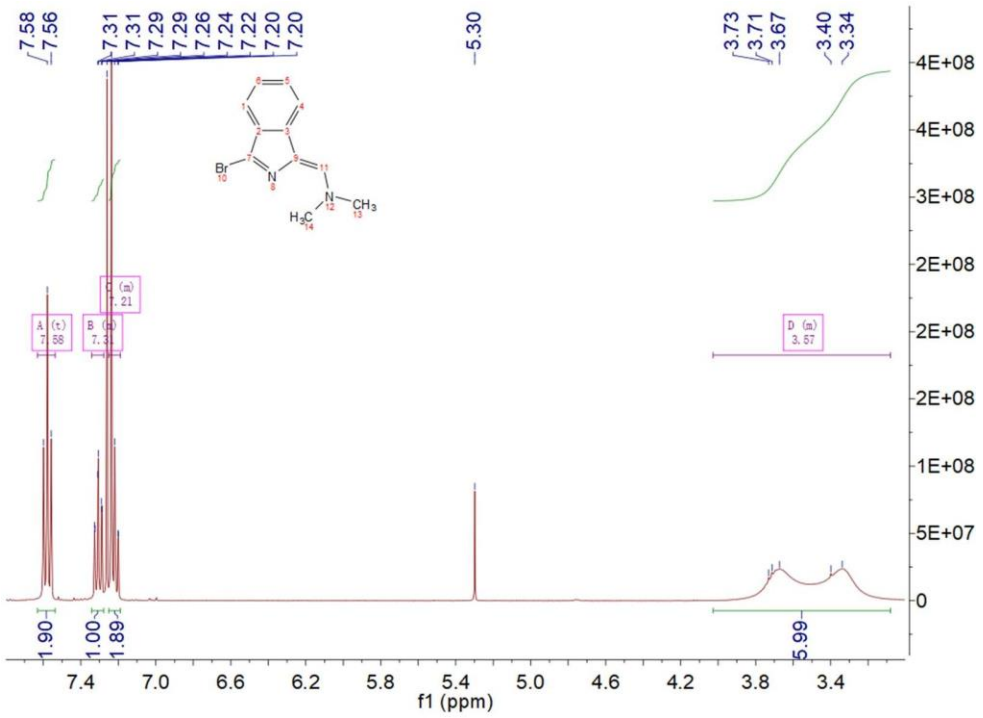


Figure S19 ^{1}H NMR spectrum of compound 3 in $CDCl_3$.

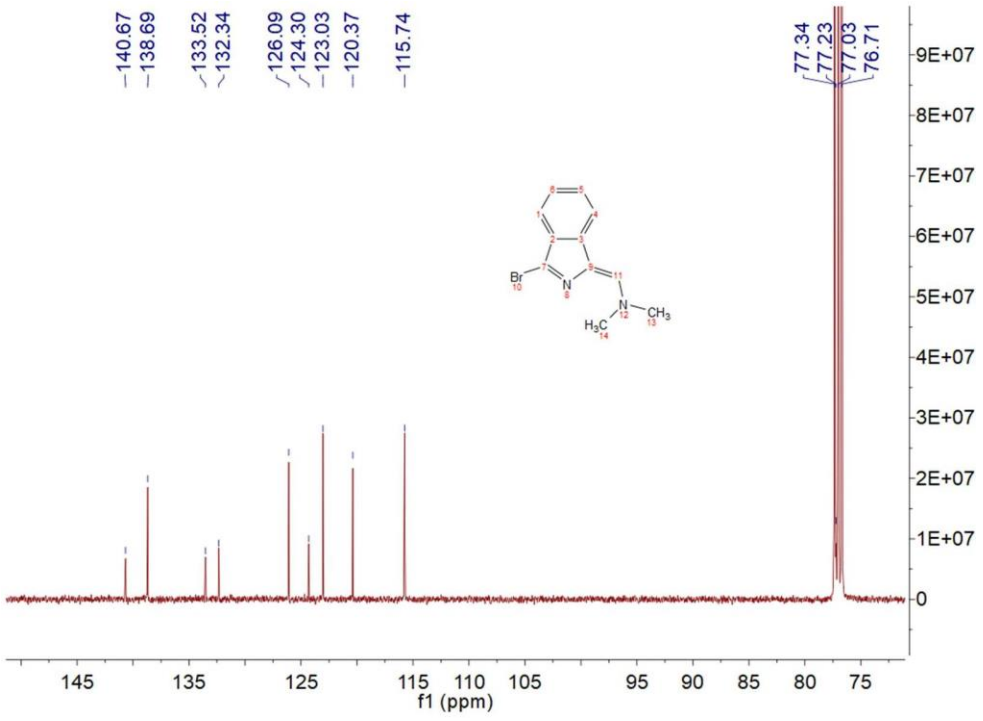


Figure S20 ^{13}C NMR spectrum of compound 3 in $CDCl_3$.

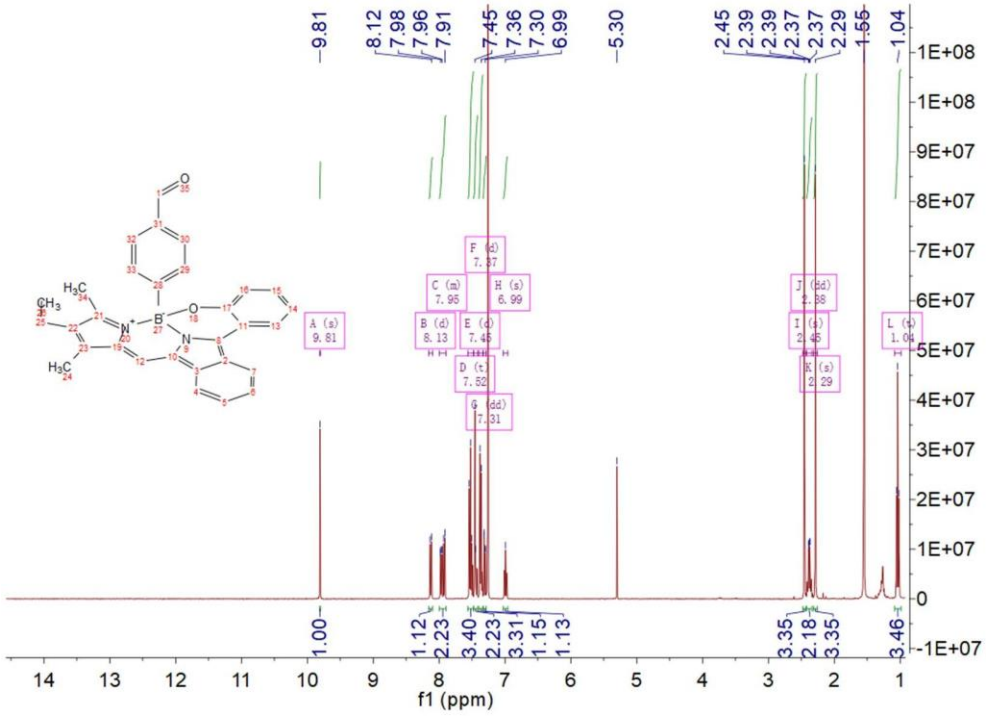


Figure S21 ^{1}H NMR spectrum of BOBPY-CHO in DMSO.

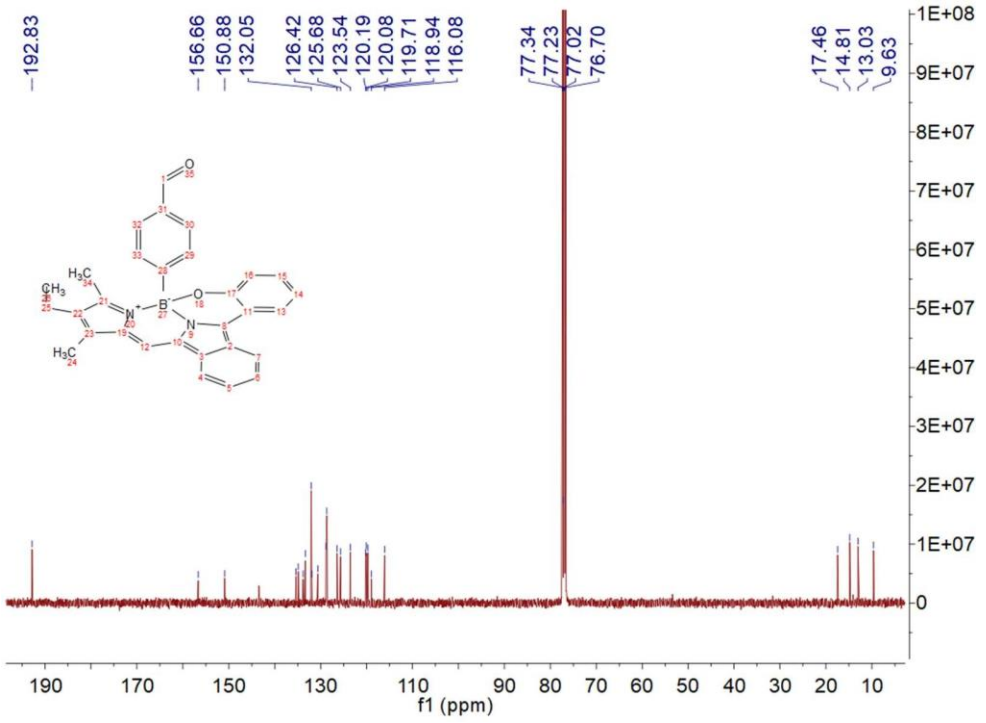


Figure S22 ^{13}C NMR spectrum of BOBPY-CHO in DMSO.

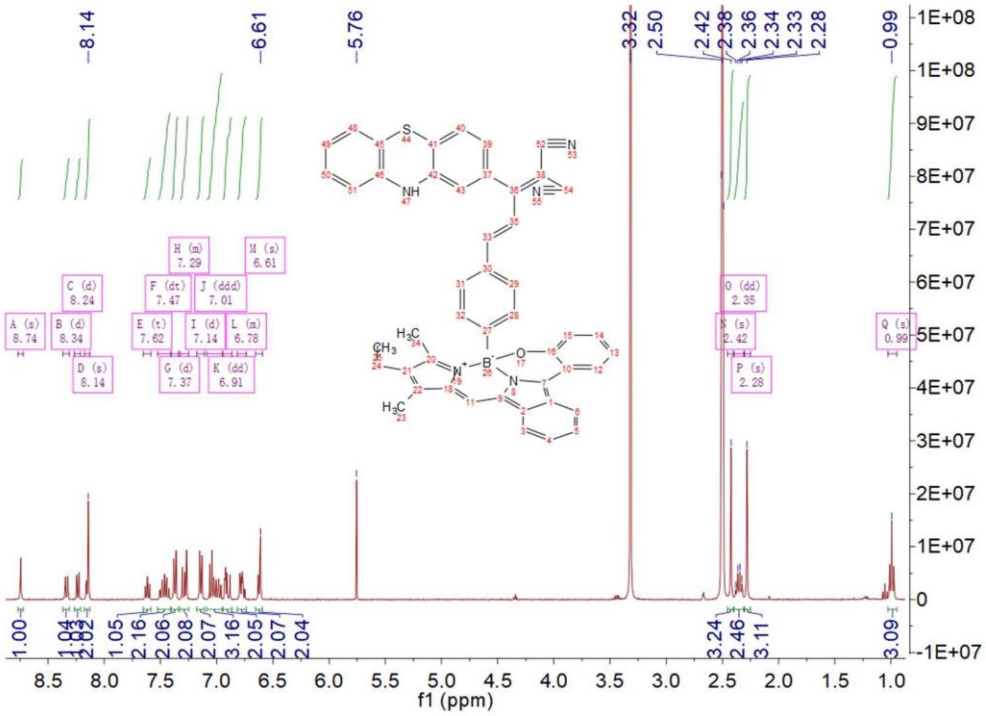


Figure S23 ^{1}H NMR spectrum of PTZ-P4 in DMSO.

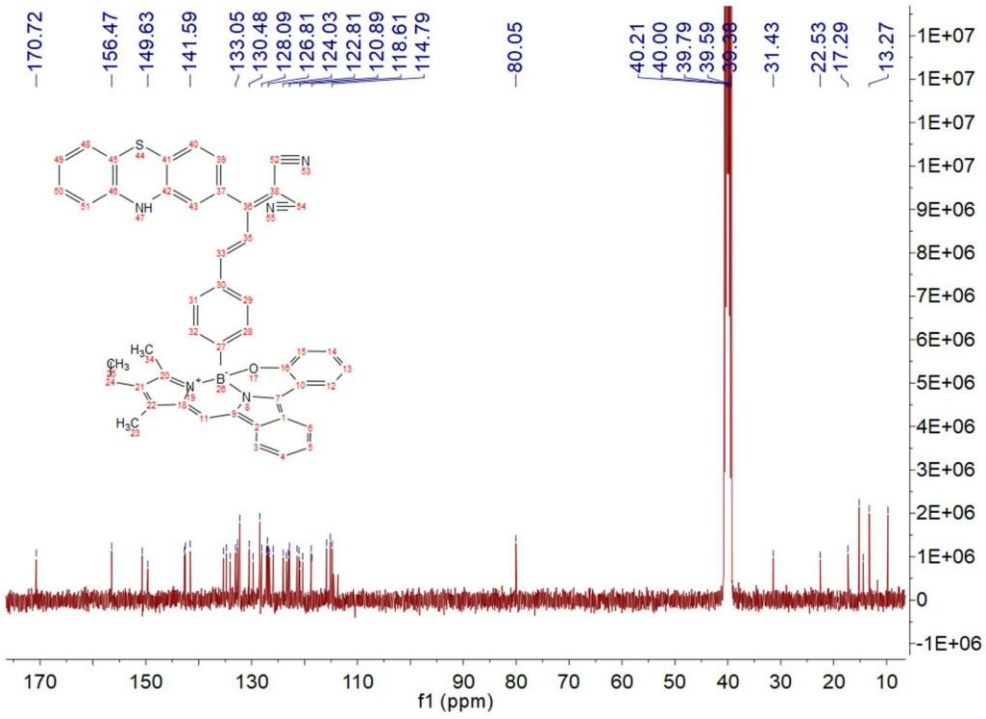


Figure S24 ^{13}C NMR spectrum of PTZ-P4 in DMSO.

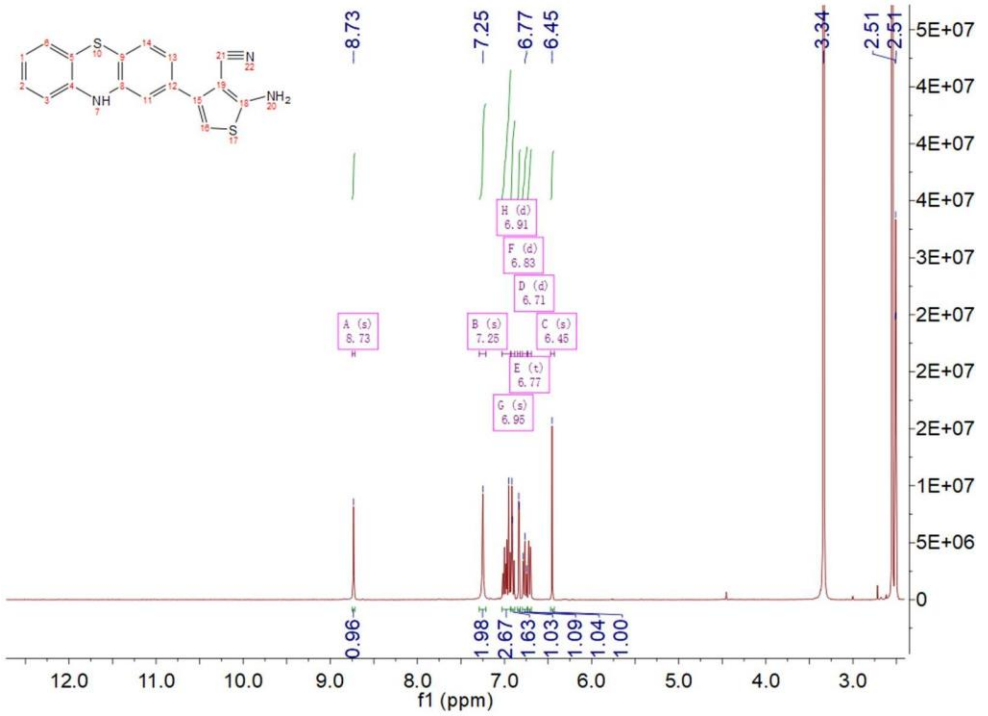


Figure S25 ^{1}H NMR spectrum of PTZCNSF in DMSO.

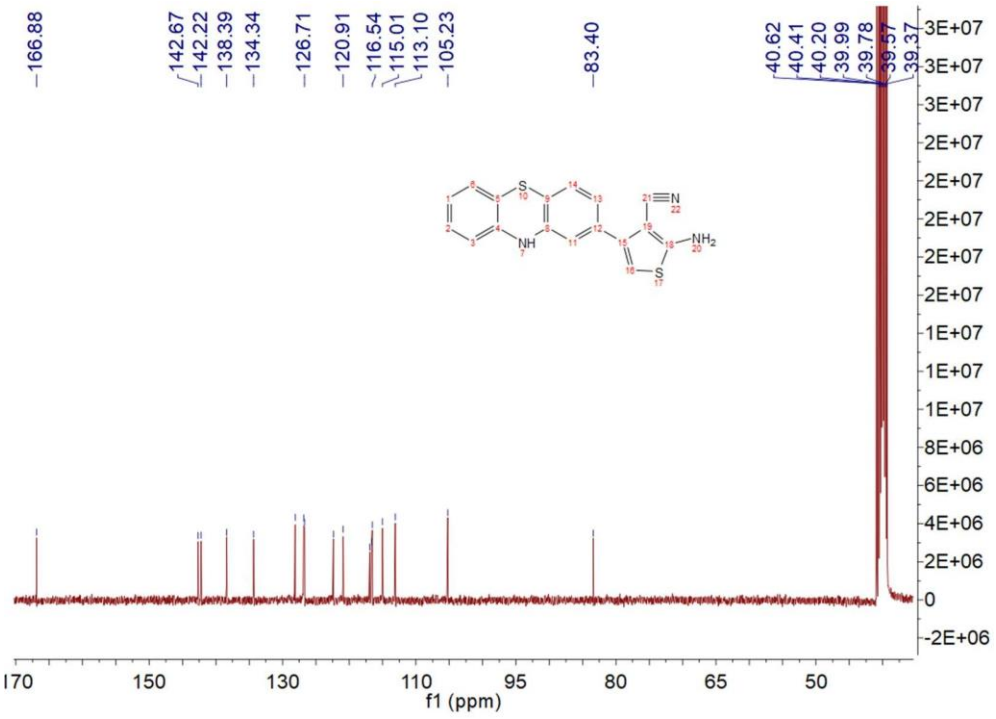


Figure S26 ^{13}C NMR spectrum of PTZCNSF in DMSO.

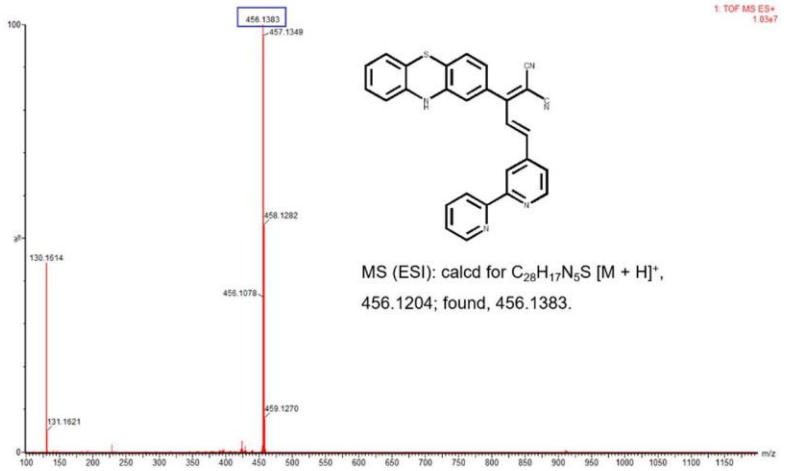


Figure S27 HRMS spectrum of PTZ-P2.

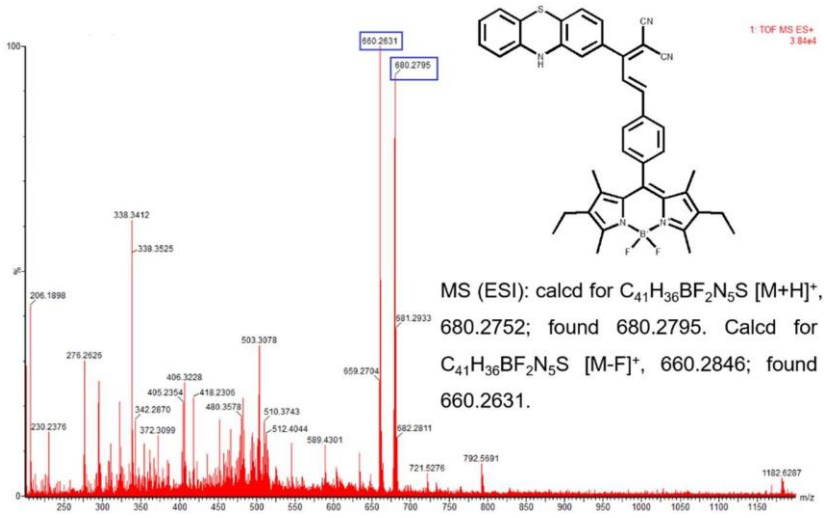


Figure S28 HRMS spectrum of PTZ-P3.

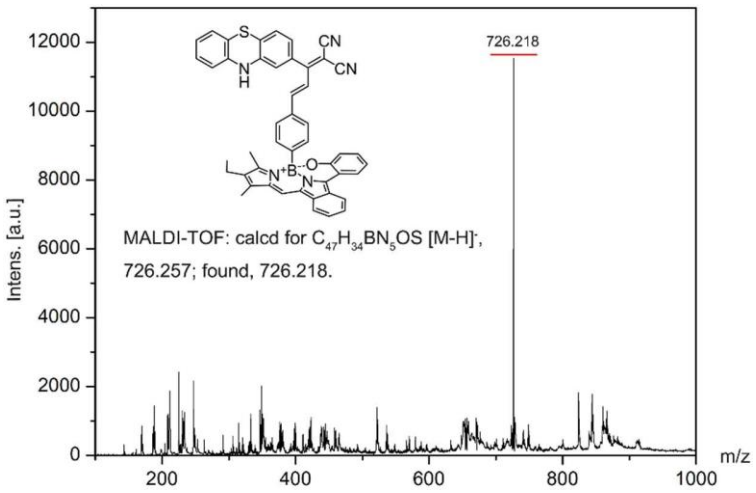


Figure S29 MALDI-TOF spectrum of PTZ-P4.

19. References

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