Supplementary Materials

Sensitive detection of bisulfite by a novel quinoxalinyl-based fluorescent probe and its applications in food detection and bioimaging

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Table of Contents

1. Materials and methods 1

1.1. Materials.....1

1.2. Methods 1-2

2. Calculation of detection limit.....2

3. Figures 3-15

4. Reference.....15

1. Materials and methods

1.1. Materials

All chemical reagents needed in the synthesis process were of analytical grade without further purification unless otherwise noted. Dimethyl sulfoxide for spect ral testing, camphorquinone, 4,5-DibroMo-1,2-phenylenediaMine, 4-(N,N-Dimethy lamino) Phenylboronic Acid, Pinacol Ester, 4-Formylphenylboronic acid, 1,2,3,3-Tetramethyl-3H-indolium iodide, tetrakis(triphenylphosphine) palladium were pur chased from Adamas Co., Ltd.. The sodium bisulfite was purchased from Shan ghai Aladdin Biochemical Technology Co., Ltd. and the purity was 99%. All t he deionized water was adopted for preparing the aqueous solution. NMR spect ra were recorded on a Bruker AV 500 spectrometer. High-resolution mass spec tra (HRMS) spectra were acquired by using an America Agilent 5975c mass s pectrometer. The ultraviolet-visible (UV-vis) absorption spectra were tested by using Shimadzu UV-2450 spectrophotometer. The fluorescence spectra were me asured by PerkinElmer LS55 spectrophotometer. Fluorescent images were taken using a Carl Zeiss LSM-710 confocal laser scanning fluorescence microscope.

1.2. Methods

Synthesis of compound **DTTP**: Camphorquinone (0.498 g, 3 mmol) was mixed with 4, 5-dibromo-1, 2-phenylenediamine (0.798 g, 3 mmol), and nitrogen was vented for 0.5 h. Then add 10 mL acetic acid and react at 90 °C overnight. After the reaction, the pH of the reaction liquid was adjusted to neutral with saturated sodium bicarbonate solution, and then extracted with dichloromethane and water. After the organic phase was dehydrated with anhydrous sodium sulfate, the crude product was obtained after filtration, spin steaming, and drying. Finally, compound **DTTP** was purified by column chromatography (PE:EA = 30:1, v/v). Yield: 80%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.39 (s, 1H), 8.35 (s, 1H), 3.06 (m, 1H), 2.28 (m, 1H), 2.09 – 2.05 (m, 1H), 1.31 (s, 3H), 1.08 (s, 3H), 0.91 (m, 1H), 0.78 (m, 1H), 0.52 (s, 3H). ^{13}C NMR (150 MHz, DMSO) δ 166.89, 165.17, 142.09, 140.58, 132.78, 132.69, 123.32, 53.90, 53.64, 52.38,

31.25, 24.07, 20.02, 18.12, 10.95, 9.97. HRMS (m/z): $[M + H]^+$ calcd for $C_{16}H_{16}Br_2N_2 + H^+$, 394.9753; found, 394.9762.

Synthesis of compound **BTTD**: Compound **DTTP** (0.396 g, 1 mmol), 4-formylphenylboronic acid (0.180 g, 1.2 mmol) and anhydrous potassium carbonate (0.276 g, 2 mmol) were added to a three-necked flask, and tetrakis (triphenylphosphine) palladium (0.116 g, 0.1 mmol) was added under nitrogen atmosphere. After passing nitrogen for 30 min to eliminate oxygen, 18 mL of solvent (1,4-dioxane:water = 5:1, v/v) was added, and heated at 90 °C for about 12 h. After the reaction completed, the reaction solution was distilled to obtain the crude **BTTD**. The pure compound **BTTD** was acquired by purification using silica gel column (PE:EA = 5:1, v/v), a white solid, yield: 60%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.11 (s, 1H), 8.02 (s, 1H), 7.83 (s, 1H), 7.74 (d, J = 2.2 Hz, 1H), 7.64 – 7.60 (m, 1H), 7.55 (d, J = 5.6Hz, 1H), 7.45 (d, J = 6.2 Hz, 1H), 3.10 – 3.04 (m, 1H), 2.30 (m, 1H), 2.11 – 2.03 (m, 1H), 1.34 (s, 3H), 1.22 (m, 1H), 1.17 (m, 1H), 1.09 (s, 3H), 0.55 (s, 3H). ^{13}C NMR (150 MHz, DMSO) δ 192.69, 166.58, 164.62, 140.78, 140.34, 140.14, 139.67, 138.97, 130.18, 130.03, 129.19, 129.17, 129.14, 128.63, 128.56, 53.31, 52.14, 31.10, 31.04, 23.86, 19.75, 17.88, 9.72. HRMS (m/z): $[M + H]^+$ calcd for $C_{16}H_{16}BrN_2O + H^+$, 421.0910; found, 421.0917.

2. Calculation of detection limit

The detection limit was calculated according to the fluorescence titration method. The fluorescence emission spectra of the probe **DPTI** were detected 10 times without adding HSO_3^-. To obtain the slope, the standard curve of the probe sensitivity was drawn with the fluorescence intensity ratio (I_{437}/I_{532}) as the vertical axis and the concentration of HSO_3^- as the horizontal axis. The formula for calculating the detection limit was:

Detection limit (LOD) =3 σ/k.

"σ" was the standard deviation of the blank sample measurement, and "k" was the slope of the fluorescence intensity ratio (I_{437}/I_{532}) and HSO_3^- concentration at 486nm.

3. Figures

Fig. S1. ^{1}H NMR spectra of compound **DTTP**.

Fig. S2. ^{13}C NMR spectra of compound **DTTP**.

Fig. S3. HRMS spectra of compound **DTTP**.

Fig. S4. ^{1}H NMR spectra of compound **BTTD**.

Fig. S5. ^{13}C NMR spectra of compound **BTTD**.

Fig. S6. HRMS spectra of compound **BTTD**.

Fig. S7. ^{1}H NMR spectra of compound **DPTB**.

Fig. S8. ^{13}C NMR spectra of compound **DPTB**.

Fig. S9. HRMS spectra of compound **DPTB**.

Fig. S10. ^{1}H NMR spectra of compound **DPTI.**

Fig. S11. ^{13}C NMR spectra of compound **DPTI**.

Fig. S12. HRMS spectra of compound **DPTI**.

Fig. S13. (a) The fluorescence spectra of **DPTI** (10 μM) in different ratios of DMF/PBS solutions. λ_{ex} = 380 nm. (b) Fluorescence emission spectra of **DPTI** (10 μM) with HSO_3^- (100 μM) in different ratios mixing solvents of DMF/PBS (λ_{ex} = 380 nm). (c) The fluorescence spectra of **DPTI** (10 μM) in different organic solvents. (d) The fluorescence spectra of **DPTI** (10 μM) with HSO_3^- (100 μM) in different organic solvents.

Fig. S14. Change of fluorescence intensity ratio (I_{437}/I_{532}) of probe **DPTI** in different organic solvents before and after adding HSO_3^- (100 μM).

Fig. S15. Fluorescence intensity ratio (I_{437}/I_{532}) of probe **DPTI** (10 μM) in the absence and presence of HSO_3^- (100 μM) under continuous irradiation of a 365 nm UV lamp. λ_{ex} = 380 nm.

Fig. S16. HRMS changes before and after adding HSO_3^- to the probe **DPTI**.

Fig. S17. Cell viability of HepG2 cells after incubation with different concentrations of **DPTI** (0–40 μM).

Table S1. Comparison of **DPTI** with the reported fluorescent probes for the detection of HSO_3^-.

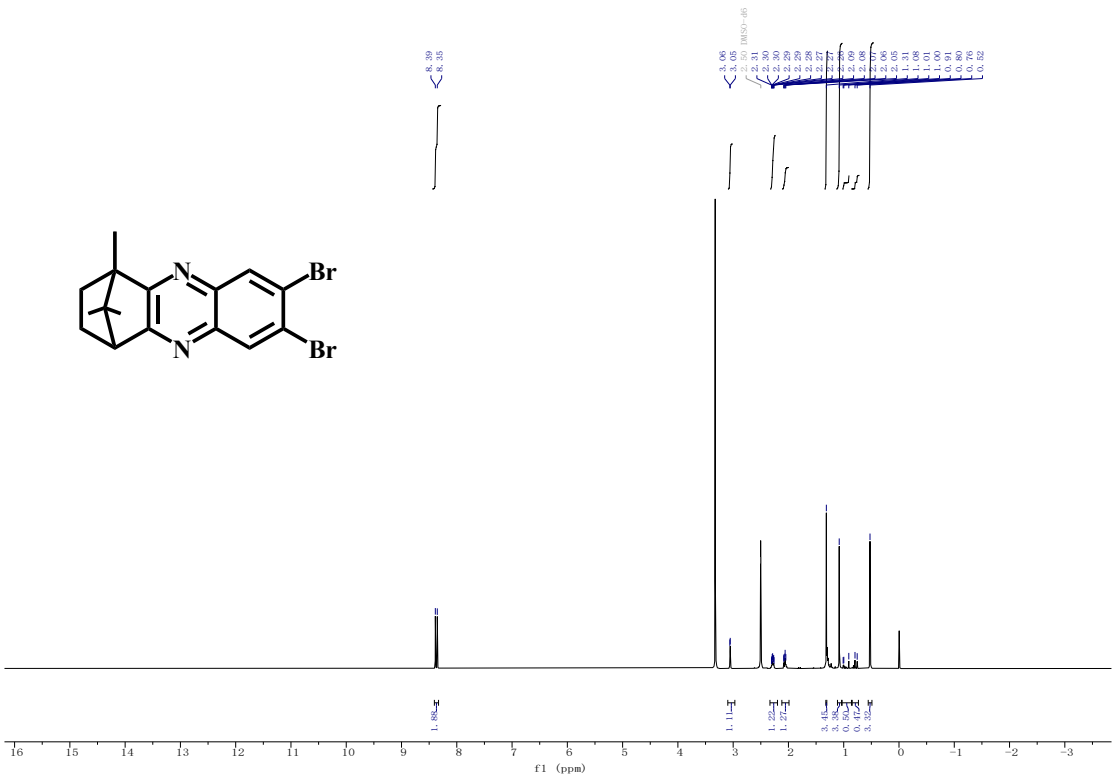


Fig. S1 ^{1}H NMR spectra of compound **DTTP** in DMSO-d_6.

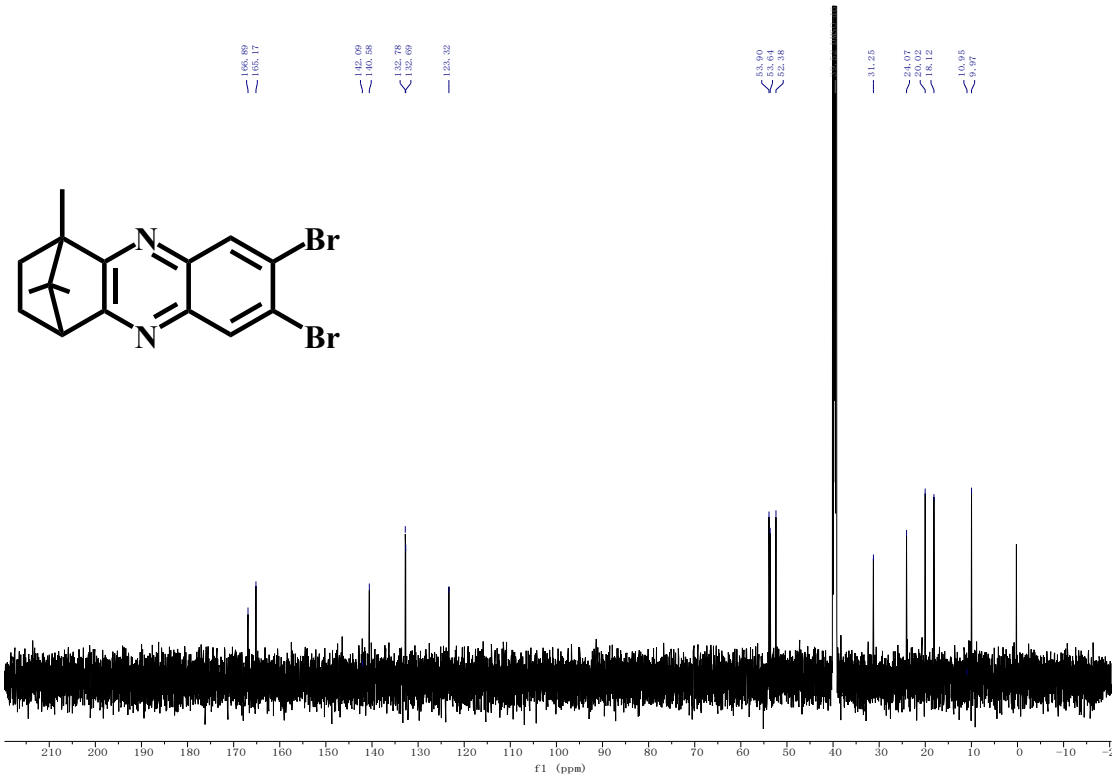


Fig. S2 ^{13}C NMR spectra of compound **DTTP** in DMSO-d_6.

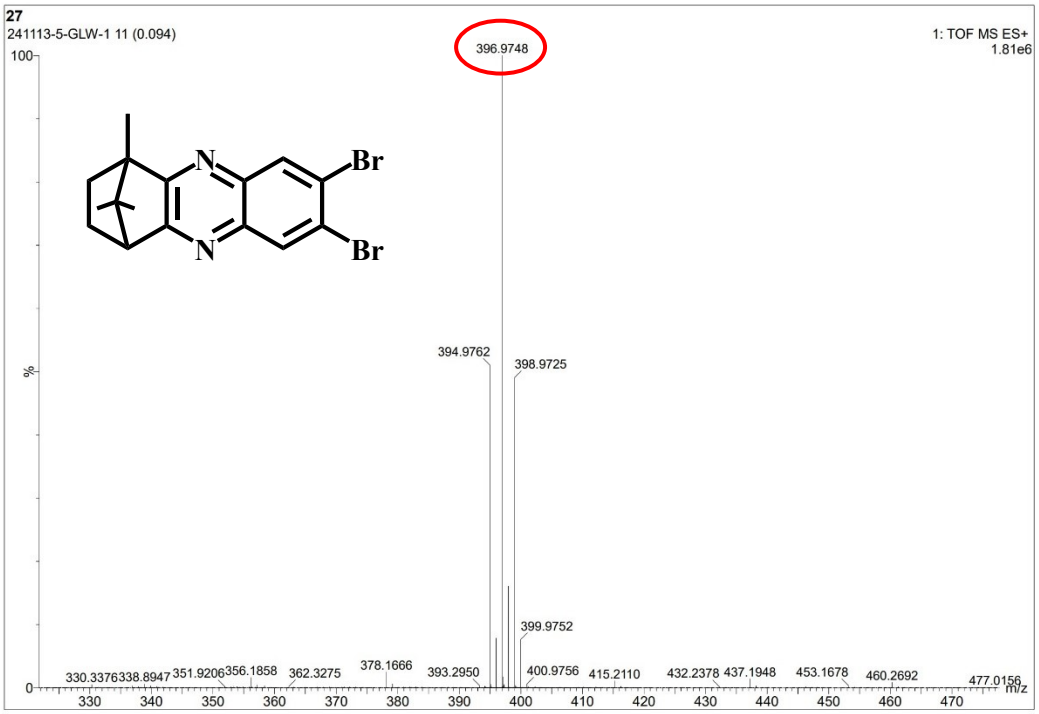


Fig. S3 HRMS spectra of compound **DTTP**.

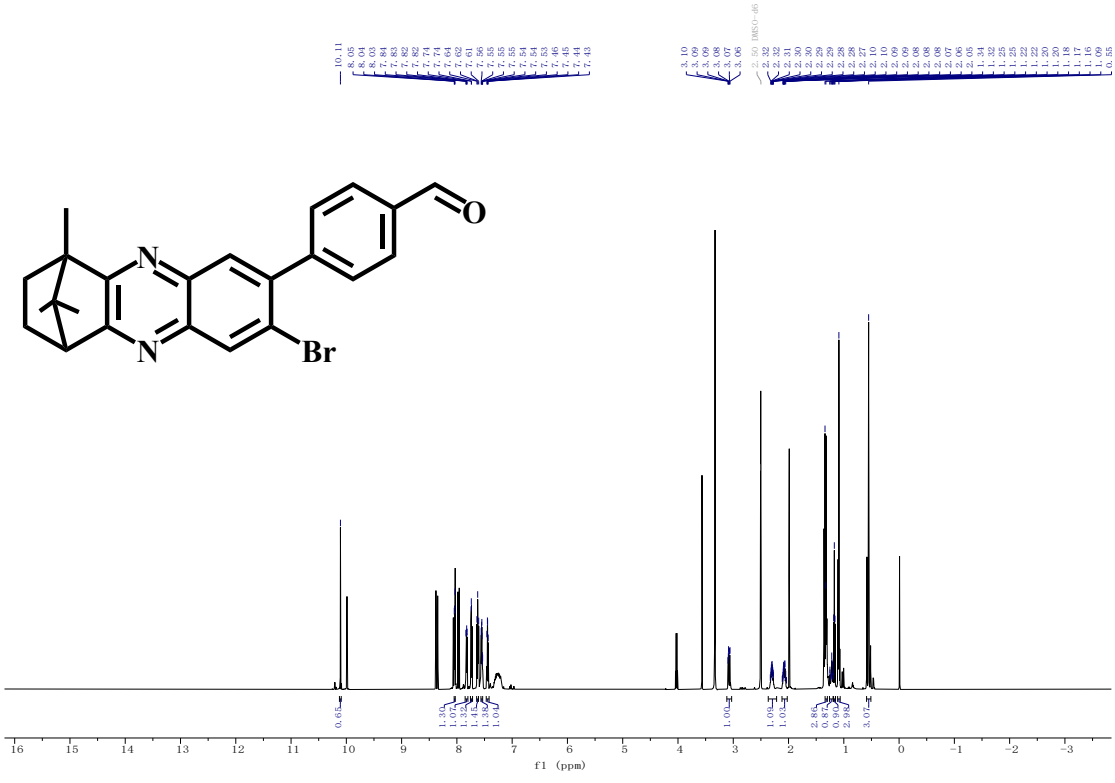


Fig. S4 ^{1}H NMR spectra of compound **BTTD** in DMSO-d_6.

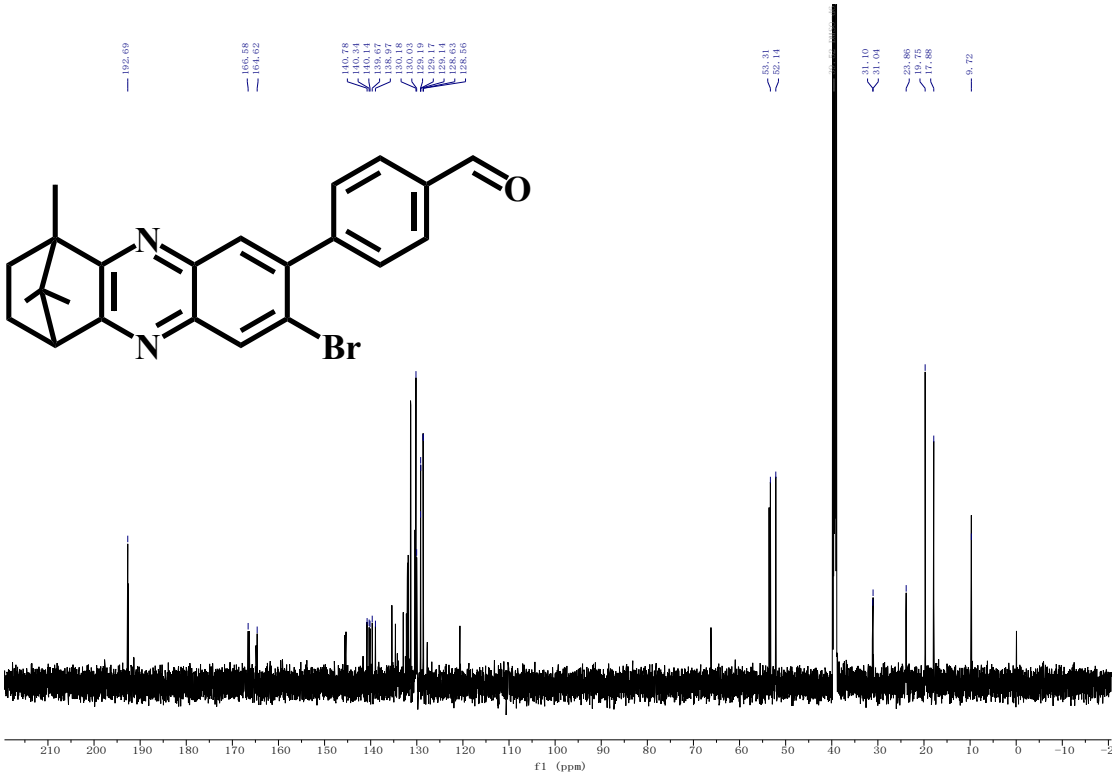


Fig. S5 ^{13}C NMR spectra of compound **BTTD** in DMSO-d_6.

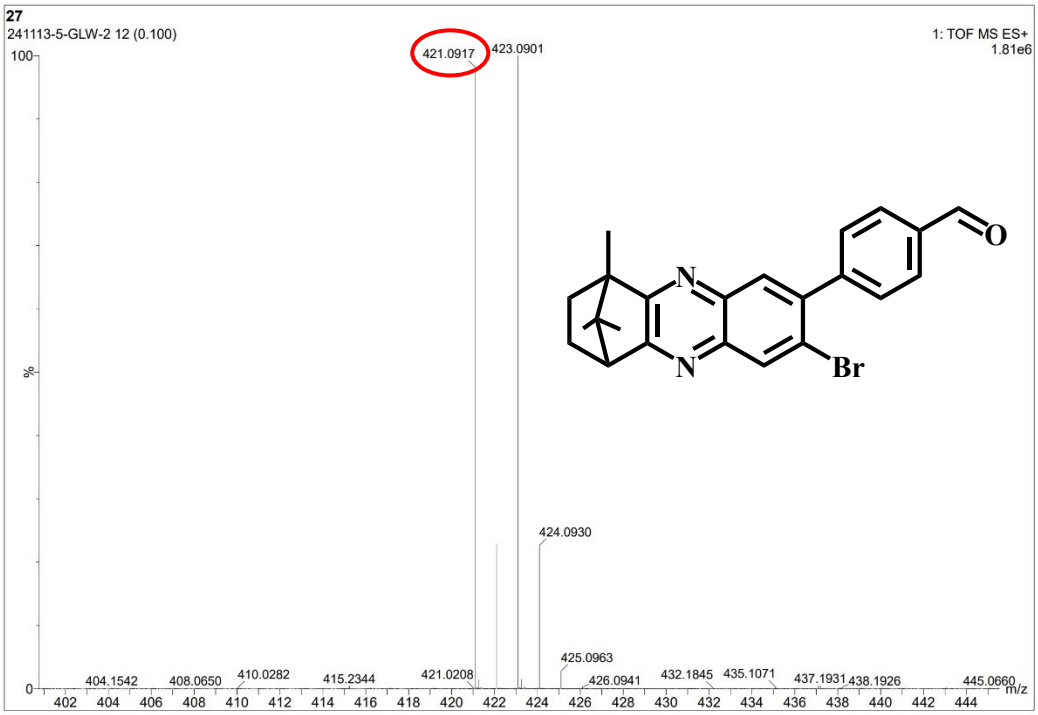


Fig. S6 HRMS spectra of compound **BTTD**.

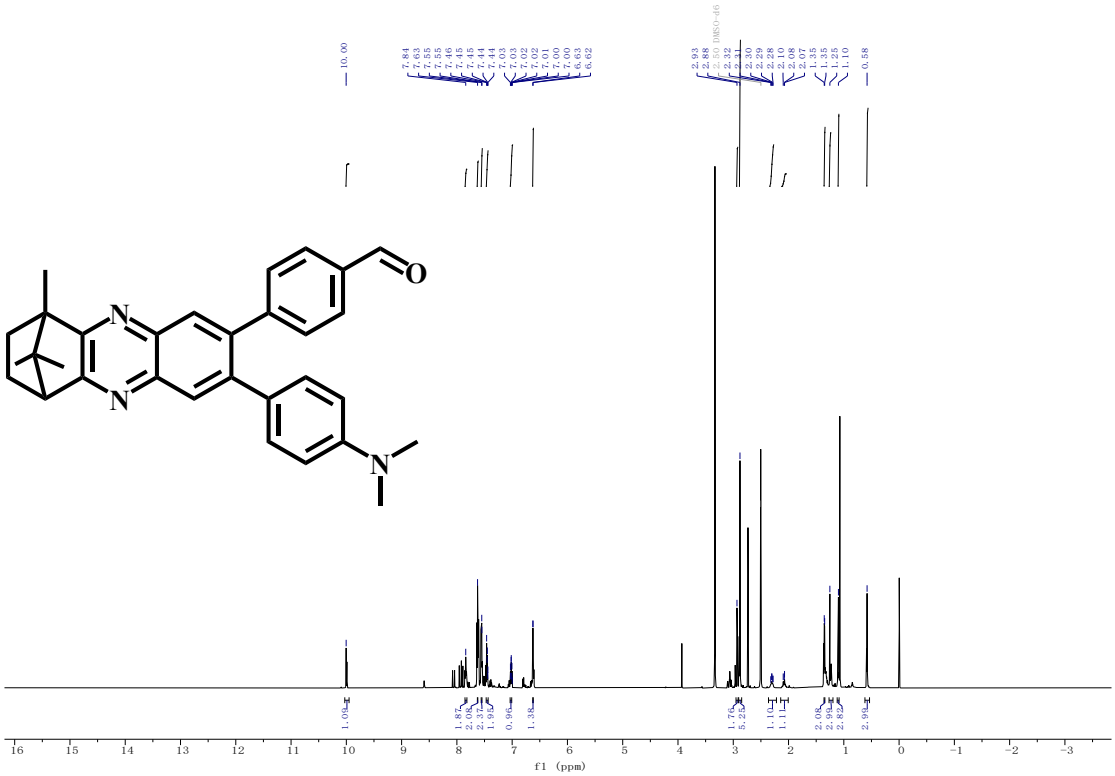


Fig. S7 ^{1}H NMR spectra of compound **DPTB** in DMSO-d_6.

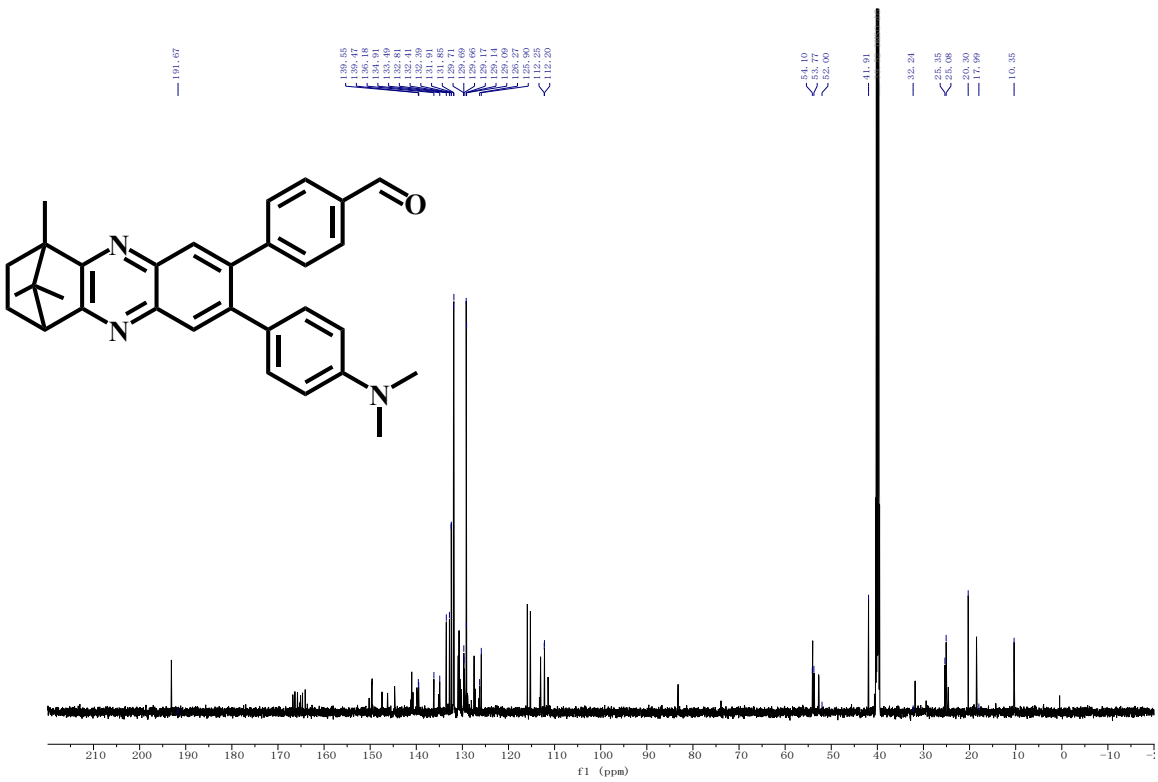


Fig. S8 ^{13}C NMR spectra of compound **DPTB** in DMSO-d_6.

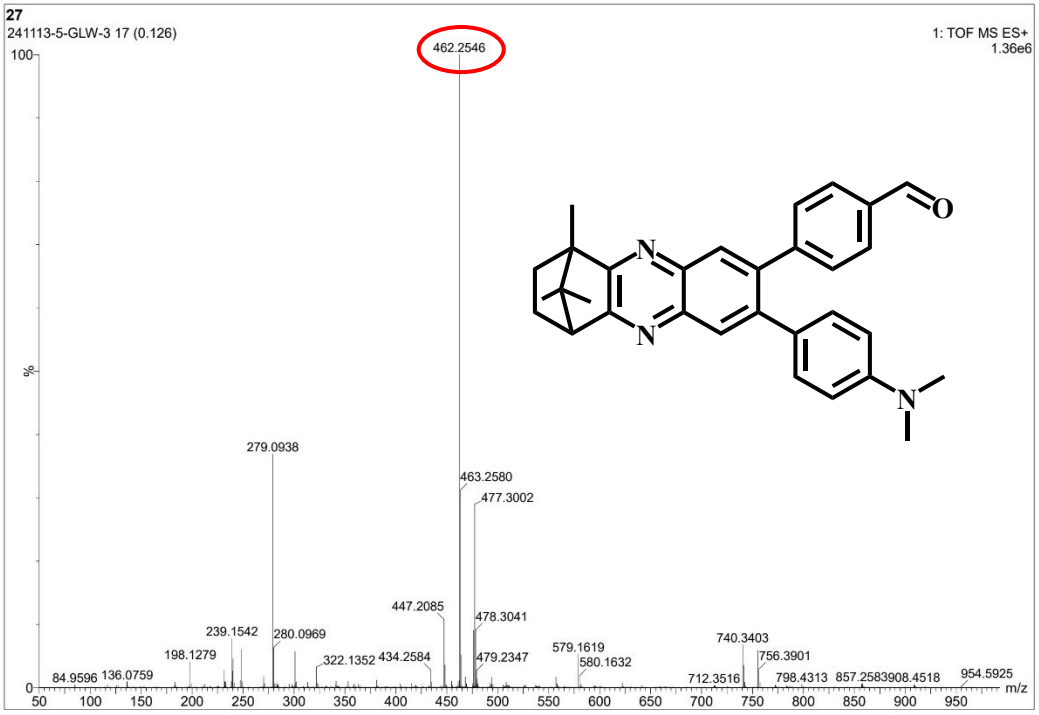


Fig. S9 HRMS spectra of compound **DPTB**.

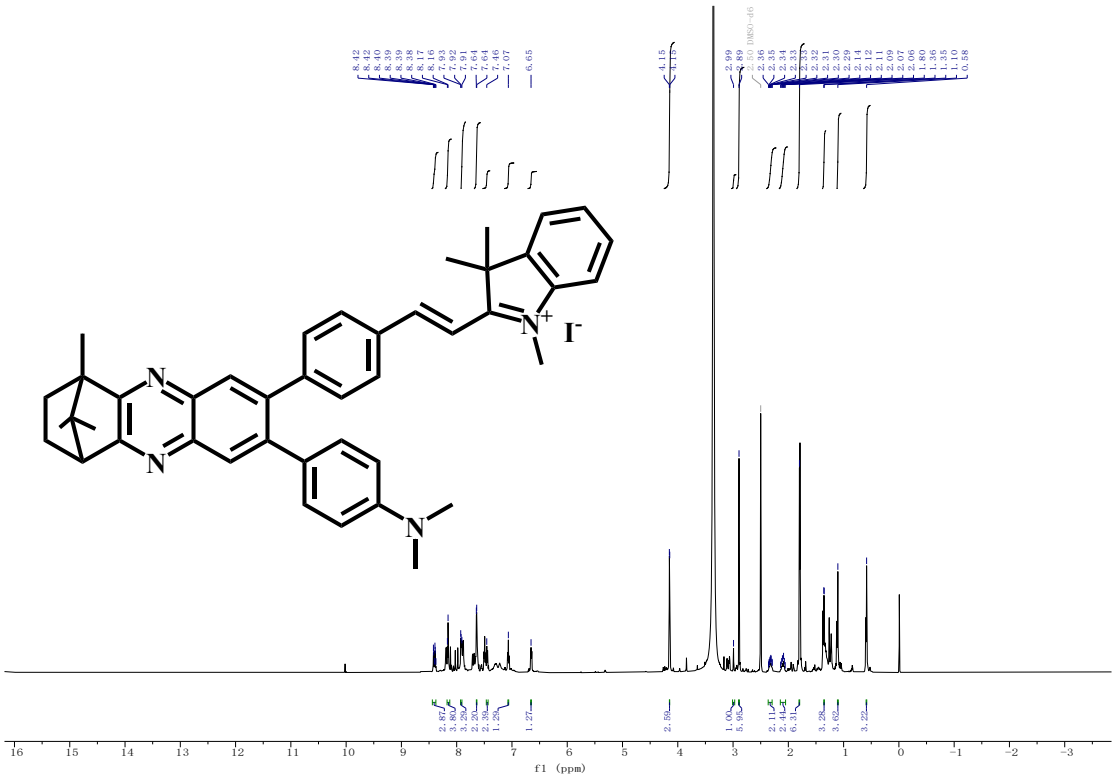


Fig. S10 ^{1}H NMR spectra of compound **DPTI** in DMSO-d_6.

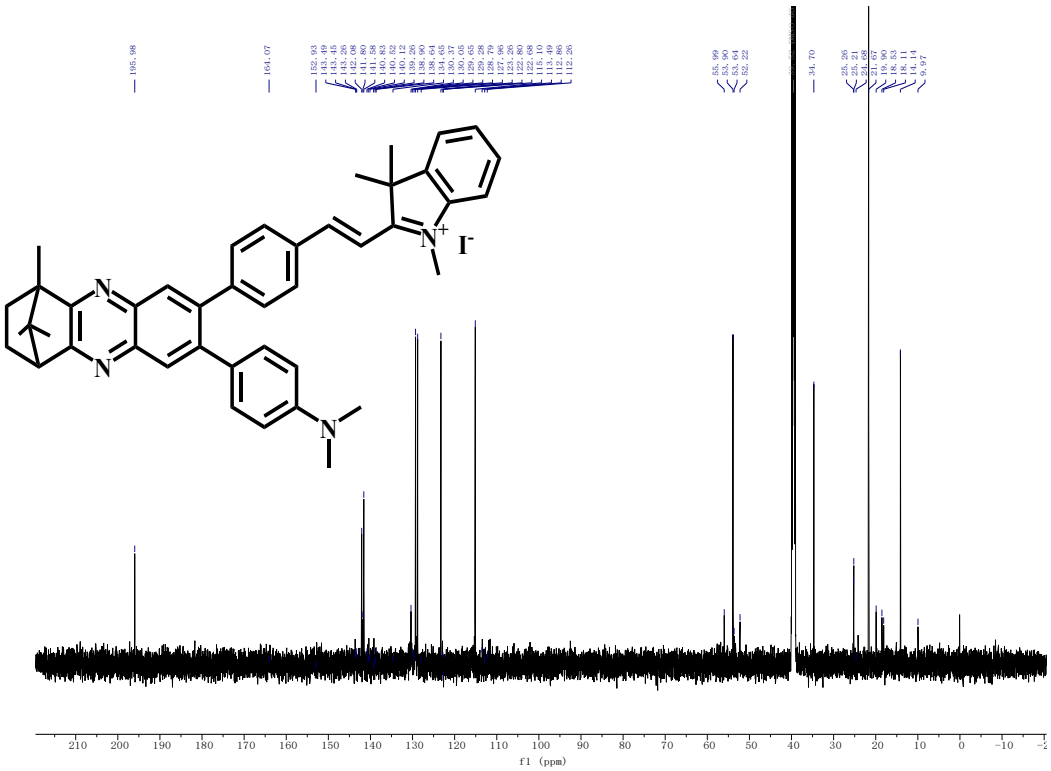


Fig. S11 ^{13}C NMR spectra of compound **DPTI** in DMSO-d_6.

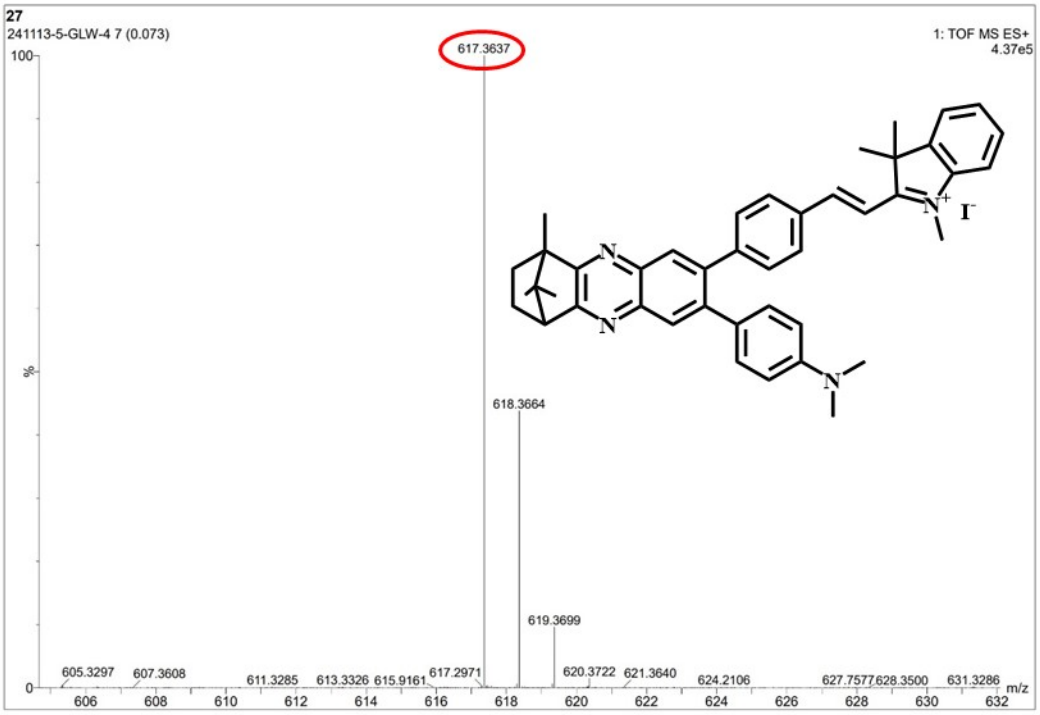


Fig. S12 HRMS spectra of compound **DPTI**.

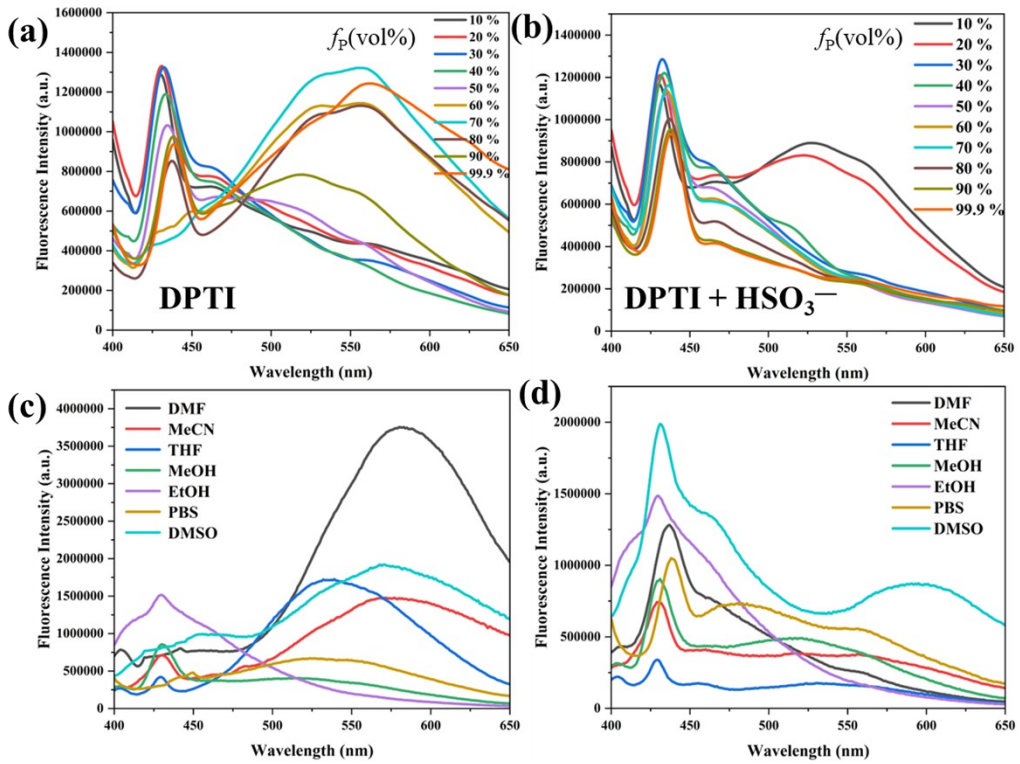


Fig. S13 (a) The fluorescence spectra of **DPTI** (10 μM) in different ratios of DMF/PBS solutions. λ_{ex} = 380 nm. (b) Fluorescence emission spectra of **DPTI** (10 μM) with HSO_3^- (100 μM) in different ratios mixing solvents of DMF/PBS (λ_{ex} = 380 nm). (c) The fluorescence spectra of **DPTI** (10 μM) in different organic solvents. (d) The fluorescence spectra of **DPTI** (10 μM) with HSO_3^- (100 μM) in different organic solvents.

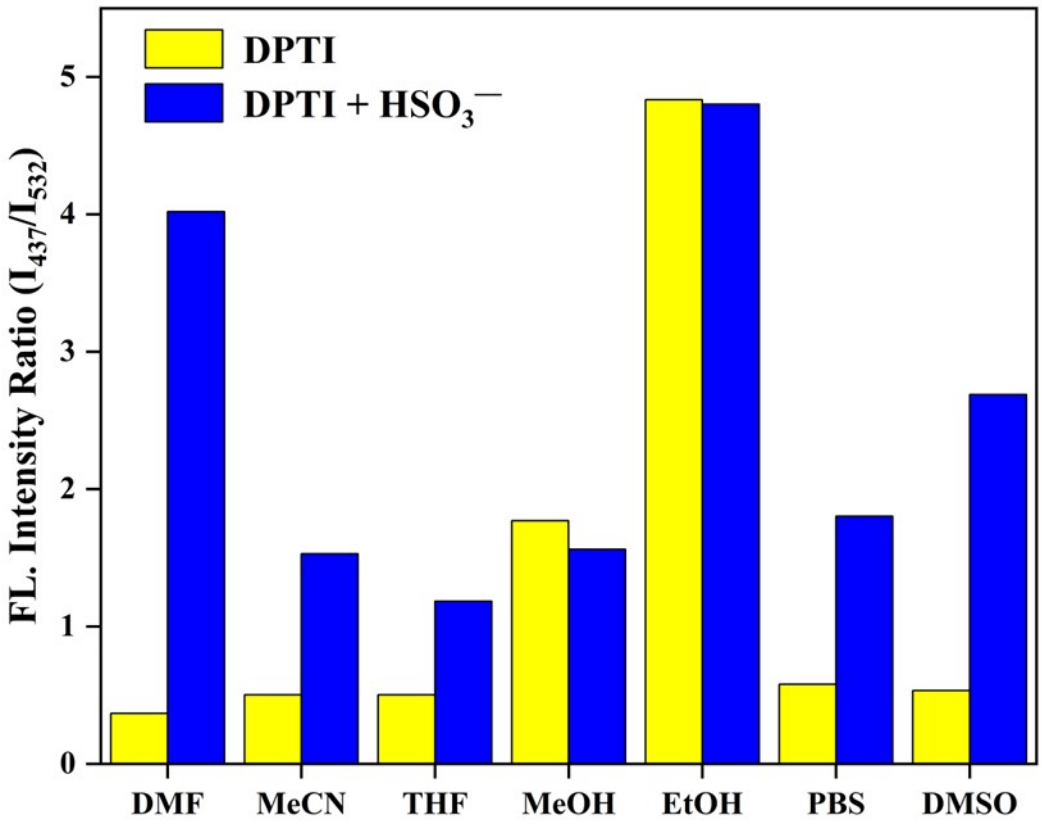


Fig. S14 Change of fluorescence intensity ratio (I_{437}/I_{532}) of probe **DPTI** in different organic solvents before and after adding HSO_3^- (100 μM).

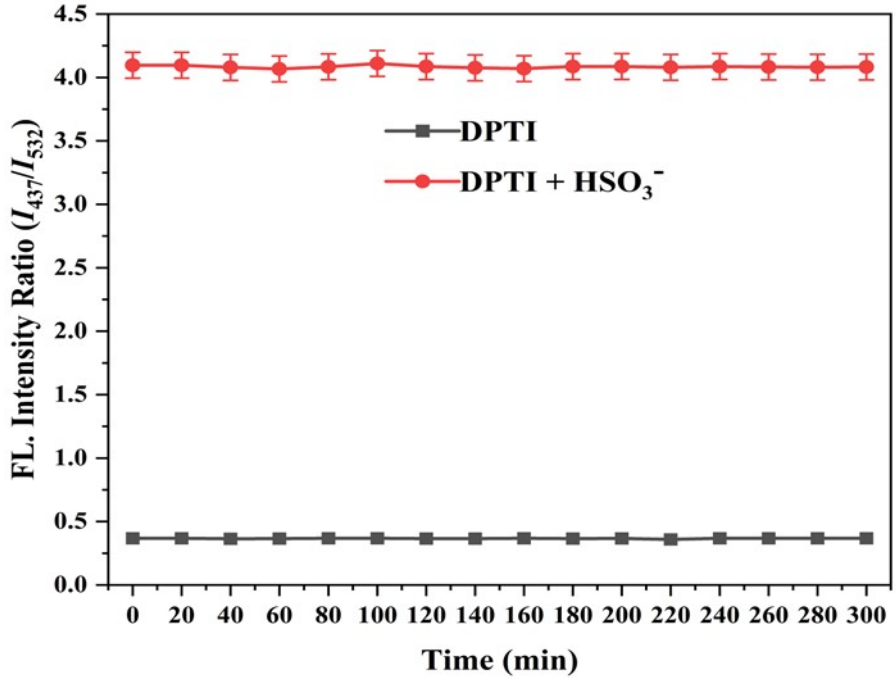


Fig. S15 Fluorescence intensity ratio (I_{437}/I_{532}) of probe **DPTI** (10 μM) in the absence and presence of HSO_3^- (100 μM) under continuous irradiation of a 365 nm UV lamp.

λ_{ex} = 380 nm.

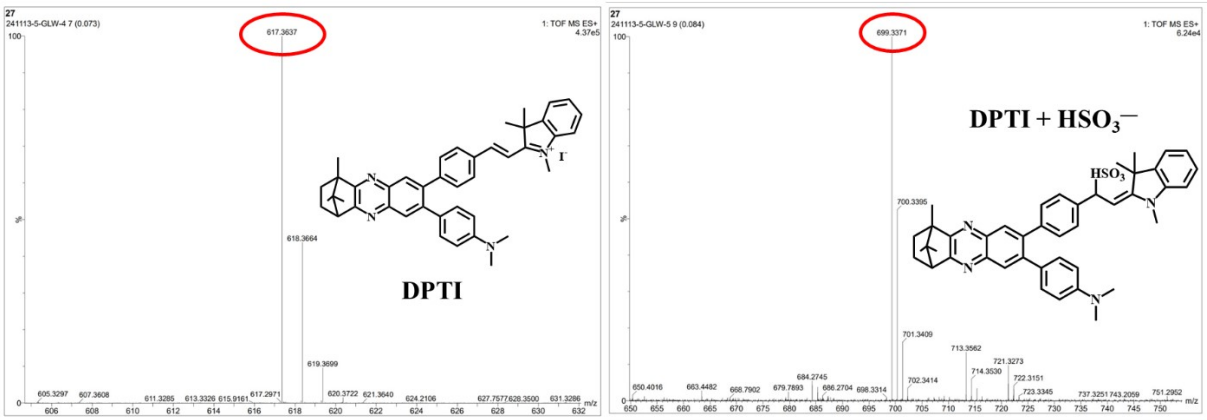


Fig. S16 HRMS changes before and after adding HSO_3^- to the probe **DPTI.**

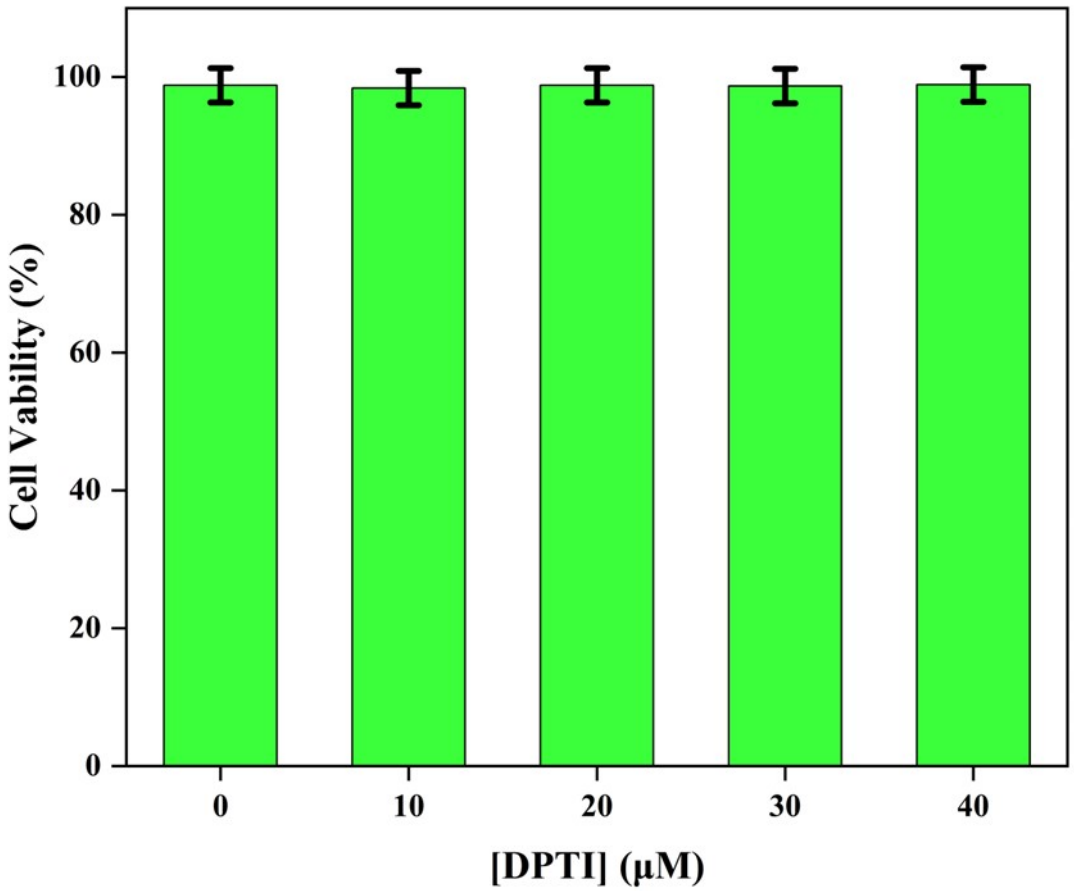
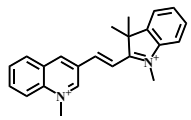
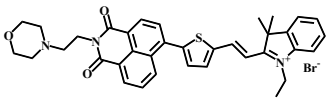
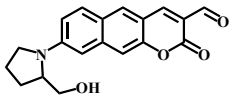
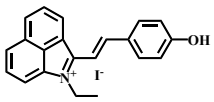
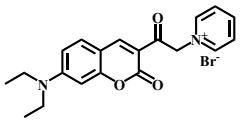
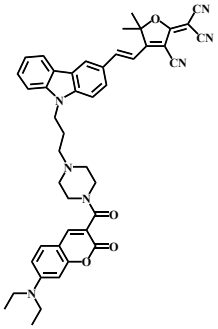
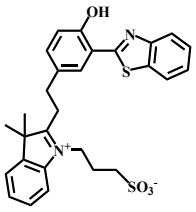
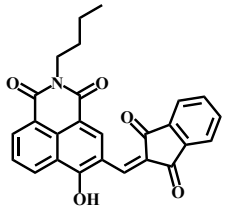


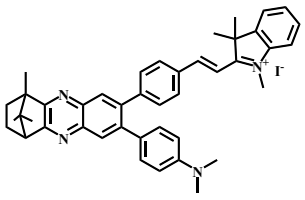
Fig. S17 Cell viability of HepG2 cells after incubation with different concentrations of **DPTI** (0–40 μM).

Table S1. Comparison of **DPTI** with the reported fluorescent probes for the detection of HSO_3^-.

Probe	Detection limit	Response time	Detection mode	Linear range	Applications	Reference
	0.288 μM	Within 5s	Turn-on	0.1-5 μM	Food detection; Bioimaging	1
	20.7 nM	3 min	Turn-on	0-100 μM	Bioimaging	2
	0.08 μM	Within 30s	Ratiometric	0-1 μM	Bioimaging	3
	8.4 nM	5 min	Turn-on	0-5 μM	Food detection; Bioimaging	4
	0.1 μM	15 min	Turn-on	0-70 μM	Bioimaging	5
	18 nM	Within 10 min	Ratiometric	0-5 μM	Bioimaging	6
	0.34 μM	15 min	Ratiometric	0-50 μM	Bioimaging	7
	2.05 μM	1.87 min	Turn-off	0-10 μM-	Bioimaging	8

续表 S1

Probe	Detection	Response	Detection	Linear	Applications	Reference

	limit	time	mode	range		
	0.07 μM	10 min	Ratiometric	0-0.7 μM	Food detection; Bioimaging	This work

4. Reference

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