Supporting information

Rational construction of two-photon NIR ratiometric fluorescent probe for the detection of bisulfite in live cells, tissues and foods

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1. General information

1.1. Reagents and instrumentation

Raw material for synthesis were get from commercial suppliers without further purification. NMR spectra, Mass spectra, UV-Vis absorption spectra and Fluorescence spectra were determined on the Bruker Avance III HD 500 instruments (TMS as an internal standard), Bruker Waters Acquity UPLC / Premier QTOF, Perkin Elmer Lambda 20 spectrophotometer and HORIBA Scientific FluoroMax spectrophotometer, respectively. Cytotoxicity asssays was evaluated by Biotek

Synergy TM H4, Fluorescence imaging with one-photon/two-photon in living cell and rat liver tissue was measured on Leica TCS SP8 STED 3X. Fluorescence imaging in vivo was measured on small animal optics & NIR imaging system (PerkinElmer, IVIS spectrum). The absolute quantum yield was measured by FLSL1000 in mixture solution of PBS and DMSO (3:7, pH=7.4 v/v) under 410 nm excitation.

1.2. Spectrophotometric experiments

Various analytes tested (10 mM) were prepared in distilled water and the probe (1 mM) was dissolved in 2.0 mL mixture solution of PBS and DMSO (3:7, pH=7.4 v/v). When measured, the analytes will be further diluted to the required concentration, the probe: (10 μM), The measurements of optical properties were conducted in 2.0 mL mixture solution of PBS and DMSO (3:7, pH=7.4 v/v). Without specifically mentioned, all fluorescence tests were measured in PBS and DMSO (3:7, pH=7.4 v/v) and the slit widths was 5 nm/5 nm, Excitation: 410 nm, time interval per scan: 3 min.

1.3. Cell Culture and cytotoxicity assay

Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 U/mL) at 37°C in a 95% humidity atmosphere under 5% CO_2 environment.

the cytotoxicity of the probe was evaluated using CCK8 assays. MCF-7 cells were seeded in the 96-well plate at the density of 5000 per well and incubated for overnight. After adherent, the old medium was replaced with the fresh medium containing different concentrations of the probe (0 μM, 10 μM, 25 μM, 50 μM, n=6). At different time points after incubation (0.5 h, 1 h, 2 h, 4 h), the medium was

removed and cells were washed with PBS (pH=7.4). CCK8 was then added and incubated with cells for another 2 h. After incubation, the absorbance at 450 nm of each well was read in the microplate reader. The cell viability was calculated as the ratio of absorbance of the experimental well and the control well.

1.4. Fluorescence imaging for the probe for HSO_3^-

Cellular Imaging：MCF-7 cells were seeded in the 12-well plate (5×10^4 per well) and incubated for 12 h. After adherence, the cells were divided into two groups and treated accordingly, In the first group, the old medium was replaced with the medium containing probe (50 μM), MCF-7 cells were incubated for 0.5 h and washed two times with PBS solution; In the second group, the old medium was replaced with the medium containing probe (50 μM), $NaHSO_3$ (500.0 μM) in succession (time interval:30 min), after each replacement, the treatment process is the same as the first group. Finally, Cell was observed under 618 nm excitation, one-photon (410 nm) excitation, two-photon (820 nm) excitation. Red channel: 630-700 nm; Green channel: 450-500 nm, Scan bar: 25μm;

Animal Imaging: Animal experiments meet the requirements of the Institutional Animal Care and Use Committee. The stock solution of the probe (1 mM) were in mixture solution of PBS and DMSO (3:7, pH=7.4 v/v), before injection, the stock solution of the probe (1 mM) was diluted into 20 μM by PBS buffer solution. Firstly, The Balb/c mice (5~6 weeks) was peritoneal injected with 20 μM of the probe (100 μL), and then 20 μL of $NaHSO_3$ (0.2 mM) were injected in the same region of interest

after 5 min, fluorescence variations was measured at different time points (0, 5, 10, 20 min) small animal optics & NIR imaging system (PerkinElmer, IVIS spectrum).

1.5. Liver tissue imaging

The liver tissue slices with a thickness of 200 μm were prepared from Balb/c mice by freezing microtome. the slices were incubated with the probe (50 μM) in at 37 ℃for 0.5 h and then washed with PBS three times, after completed, the slices were incubated with $NaHSO_3$ (50 μM) in at 37 ℃ for another 0.5 h at 37 ℃. Finally, the slices were washed with DPBS three times, and then two-photon fluorescence microscopy images, one-photon fluorescence and NIR fluorescence microscopy images were collected, The changes of fluorescence signal with different scan depths were determined by one/ two-photon fluorescence and NIR fluorescence in the Z -scan mode and the excitation wavelength of the probe was set at 618 nm with the emission wavelengths in the range of 630-700 nm. After adding $NaHSO_3$, one/two-photon excitation wavelength was set at 410/820 nm with the emission wavelengths in the range of 450-600 nm, respectively.

2. Fig S1-14

Fig. S1. ^{1}H NMR of 1-Hydroxy-2-(benzothiazol-2-yl)naphthalene (HBTN)

Fig. S2. ^{1}H NMR, and MS spectra of HBTN-CHO

Fig. S3. ^{1}H NMR, ^{13}C NMR and MS spectra of the probe

Fig. S4. Compared with others probes for HSO_3^- reported

Fig. S5. The changes of ^{1}H NMR spectrum the probe in the absence or the presence of $NaHSO_3$

Fig. S6. MS spectra of the probe and bisulfite

Fig. S7. The detection limit for HSO_3^- based on 3σ/κ, (y = 18139x + 385074, R_2 = 0.9751, σ=1283)

Fig. S8. (a) Fluorescence intensity of the probe (10 μM) and the mixture of the probe (10 μM) andHSO_3^- in the presence of various analytes (20 equiv) at 475 nm, Black bars: the emission intensity of probe in the presence of various analytes, Red bars : the emission intensity that occurs upon the subsequent addition of HSO_3^- to the above solution. From 1 to 19： Blank, Ac^-, Br^- , Cl^- , F^-, I^-, CO_3^{2-}, $H_2PO_4^{2-}$, HCO_3^{2-}, SO_4^{2-}, SO_3^{2-}, NO_2^-, NO_3^-, N_3^-, Cys, H_2O_2, ClO^- GSH, HS^- . (b) The fluorescent responses of the probe (10 μM) solution at 475 nm in the absence and presence of bisulfite (200 μM) at different pH values.

Fig. S9. Cytotoxicity assay

Fig. S10. MCF-7 cells images: (A) and (B) MCF-7 cells incubated with the probe (50.0 μM, 0.5 h) under 410 excitation and 618 nm excitation, (C) and (D) MCF-7 cells incubated with the probe (50.0 μM, 0.5 h) and $NaHSO_3$ (500.0 μM, 0.5h) under 618 nm excitation and 410 excitation, respectively. Scan bar:25μm.

Fig. S11. Fluorescence imaging of mice after peritoneal injection with 20 μM of the probe (100.0 μL) and $NaHSO_3$ (500.0 μM, 0.5 h) at different times (0, 5, 15, 20 min), Inset: Black circles are regions of interest. (Excitation/Emission filter: 618 nm/655 nm).

Fig.S12. The standard curves that the probe detect $NaHSO_3$ content in real samples (absorption bands at 618 nm) (y = -0.0163x + 0.6391, R_2 = 0.981).

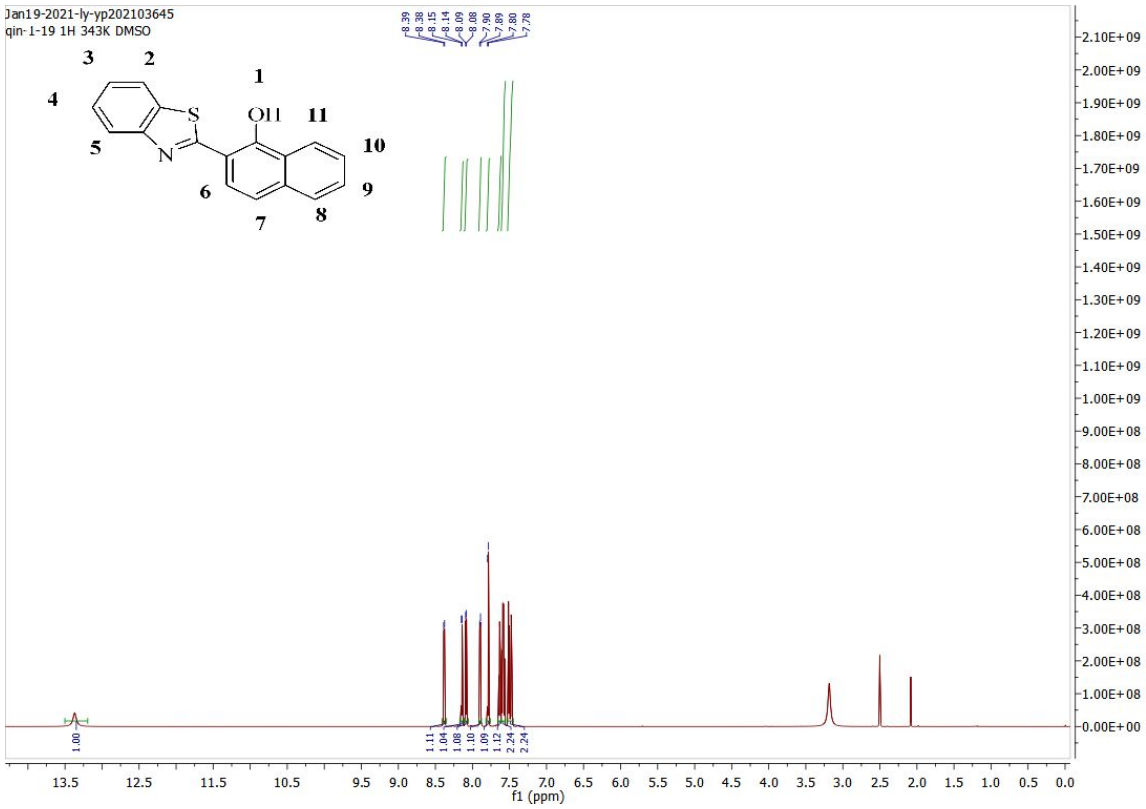


Fig. S1. ^{1}H NMR of 1-Hydroxy-2-(benzothiazol-2-yl)naphthalene (HBTN)

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm) =13.36 (s, 1H$_1$), 8.30 (d, J=8.1Hz, 1H$_{10}$), 8.15 (d, J=7.9 Hz, 1H$_8$), 8.09 (d, J=8.1Hz, 1H$_2$), 7.90 (d, J=8.0 Hz, 1H$_5$), 7.79 (d, J=8.6 Hz, 1H$_6$), 7.63 (m, 1H$_7$), 7.58 (m, 1H$_9$, 1H$_{10}$), 7.50 (m, 1H$_3$, 1H$_4$).

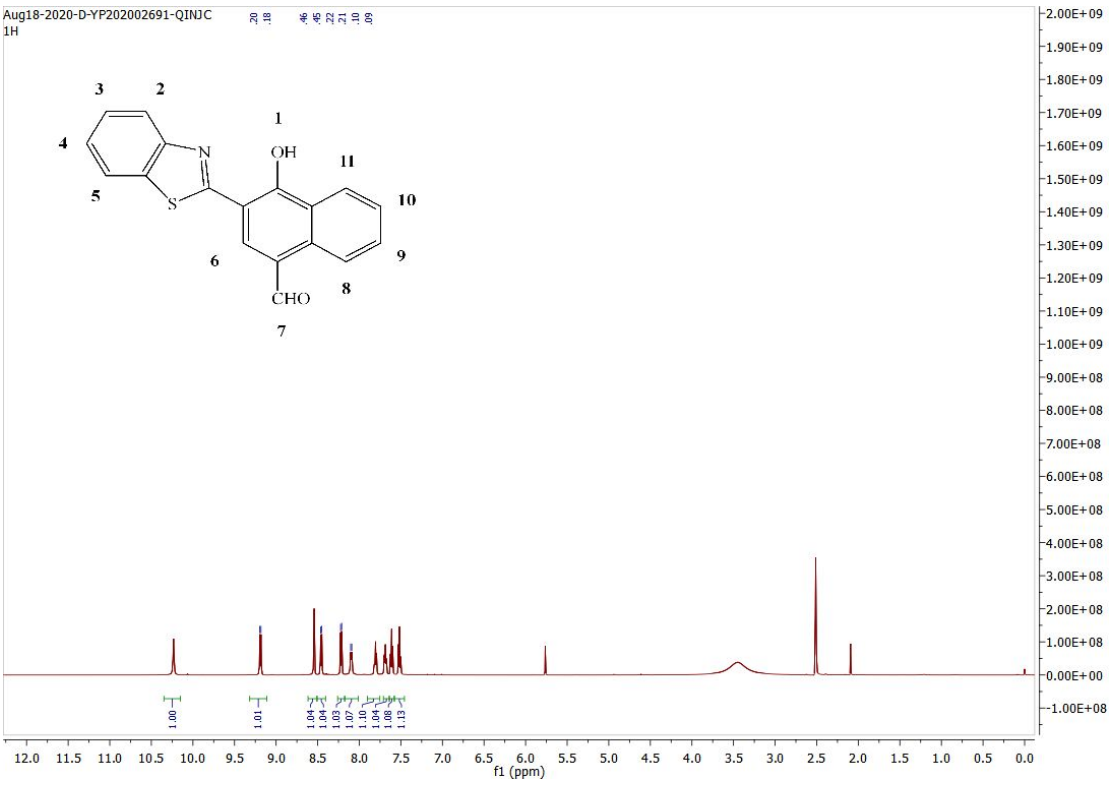


Fig. S2a. ^{1}H NMR of HBTN-CHO

^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) =10.25 (s, 1H$_7$), 9.21 (d, J=8.5Hz, 1H$_8$), 8.54 (s, 1H$_6$), 8.46 (d, J=8.2Hz 1H$_{11}$), 8.27 (d, J=8.2Hz, 1H$_2$), 8.12 (d, J=8.5Hz 1H$_8$), 7.79 (m, 1H$_9$), 7.70 (m, 1H$_{10}$), 7.60 (m, 1H$_3$), 7.49 (m, 1H$_4$).

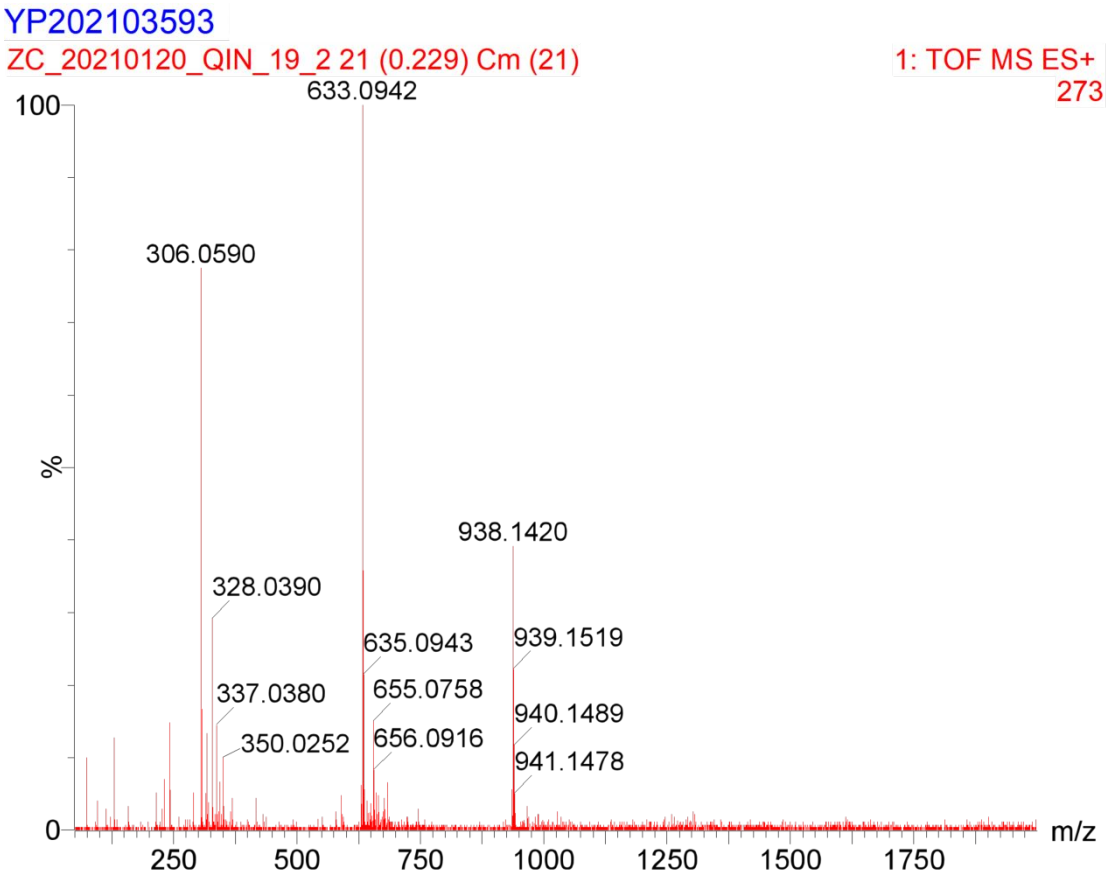


Fig. S2b. MS spectra of HBTN-CHO

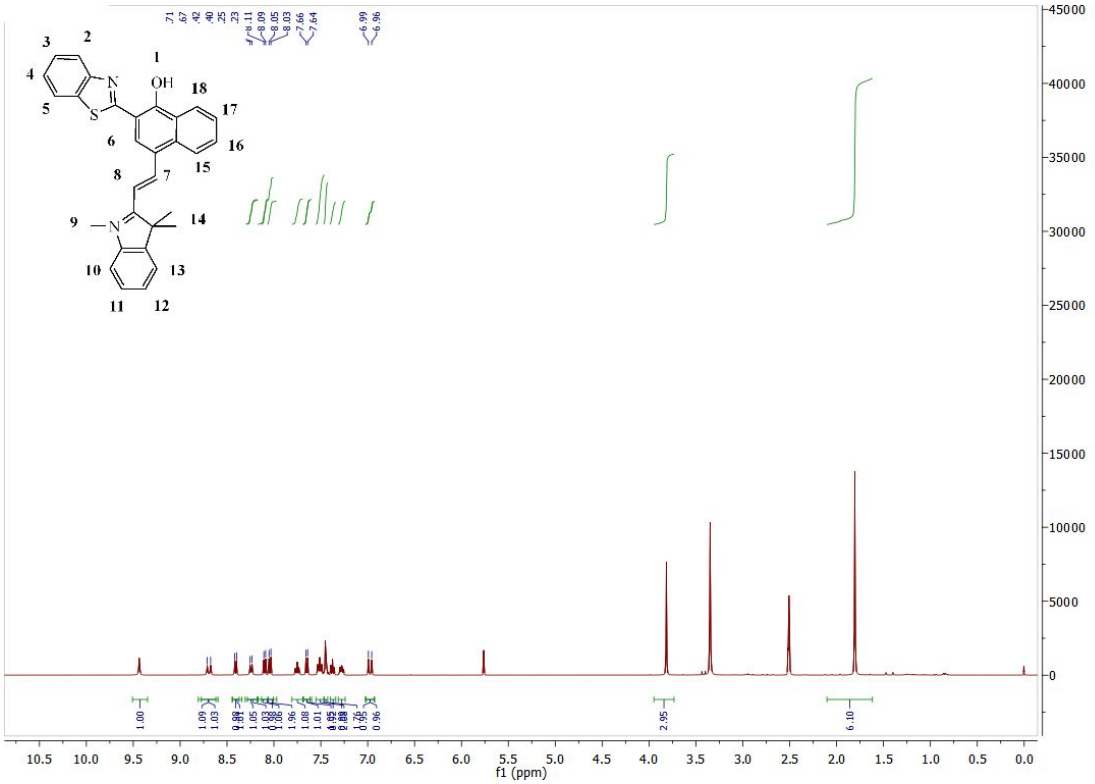


Fig. S3a. ^{1}H NMR of the probe

^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) = 9.44 (s, 1H$_6$), 8.69 (d, J=14.5Hz, 1H$_8$), 8.41 (d, J=8.0 Hz,1H$_{18}$), 8.24 (d, J=8.3Hz 1H$_2$), 8.10 (d, J=7.5Hz 1H$_5$), 8.04 (d, J=8.0 Hz 1H$_{15}$), 7.75 (m, 1H$_{16}$), 7.65 (m, 1H$_{17}$). 7.54–7.22 (m, 2H$_{3\text{-}4}$, 4H$_{10\text{-}13}$), 6.99 (d, J=14.5Hz, 1H$_7$), 3.81 (s, 3H$_9$), 1.80 (s, 6H$_{14}$).

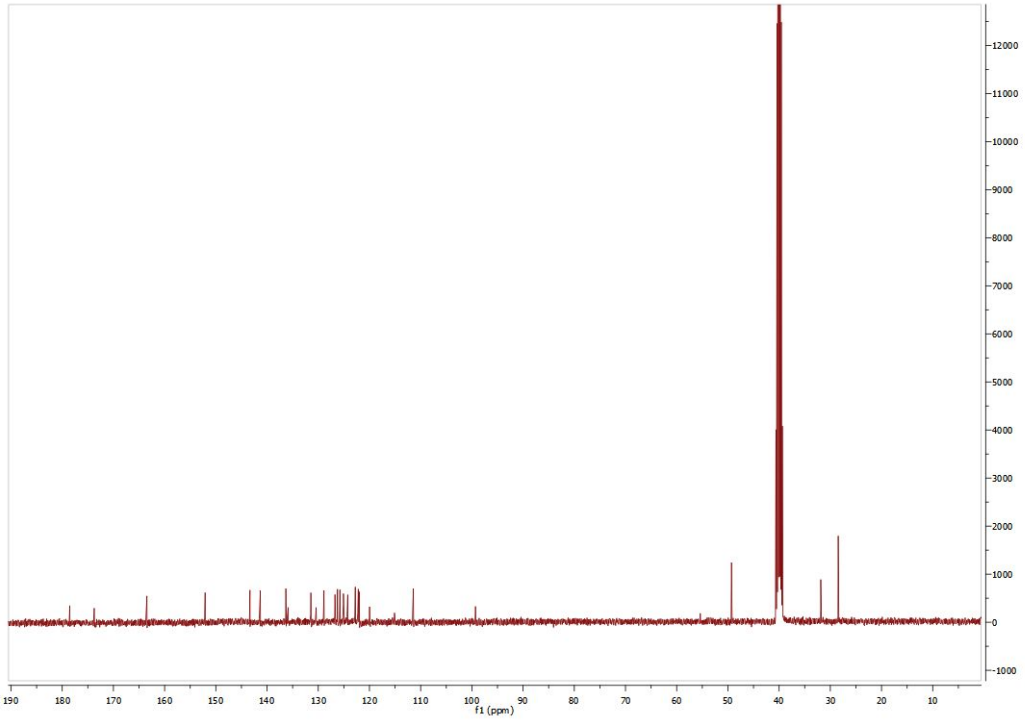


Fig. S3b. ^{13}C NMR of the probe

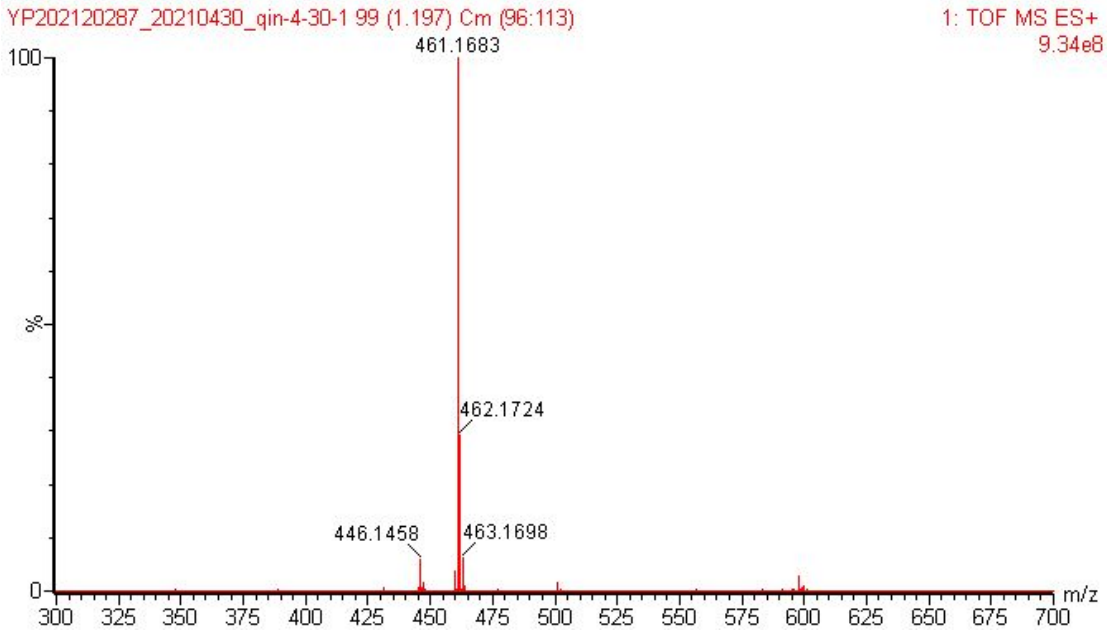


Fig. S3c. MS spectra of the probe

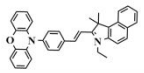
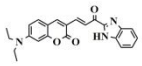
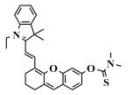
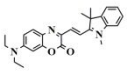
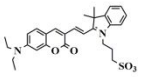
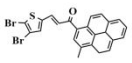
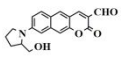
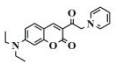
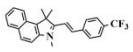
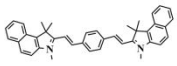
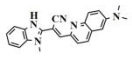
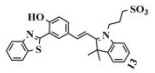
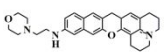
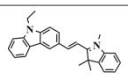
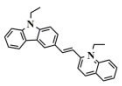
Structure	Imaging mode	(Emission wavelength (nm)	Application	Reference
	Turn-on One-photon	320/470	Cell imaging Zebrafish imaging	[16]
	Ratiometric Two-photon	415/(458/605) 800/(460/622)	Cell imaging Food sample	[27]
	Turn-on One-photon	375/450	Cell imaging	[18]
	Ratiometric One-photon	500/(560/717)	Cell imaging Food sample	[23]
	Turn-off One-photon	472/690	Zebrafish imaging Mouse imaging Food sample	[34]
	Ratiometric One-photon	450/(485/667)	Cell imaging Serum Food sample	[22]
	Ratiometric One-photon	366/(420/530)	Cell imaging Zebrafish imaging	[21]
	Ratiometric Two-photon	460/(562/625)	Cell imaging Serum	[26]
	Turn-on Two-photon	390/475	Cell imaging Tissue imaging	[20]
	Ratiometric One-photon	400/(465/571)	Food sample	[1]
	Ratiometric Two-photon	380/(480/600)	Cell imaging	[29]
	Ratiometric One-photon	380/475 460/625	Zebrafish imaging Mouse imaging Food sample	[35]
	Ratiometric One-photon	390/(450/590)	Cell imaging	[36]
		426/512 613/704	Cell imaging	[28]
	Ratiometric One-photon	375/(475/600)	Zebrafish imaging Onion tissues Food sample	[38]
	Turn-off One-photon	460/598	Zebrafish imaging Mouse imaging Food sample	[41]
This work	Ratiometric Two-photon	410/(475/655)	Cell imaging Tissue imaging Mouse imaging Food sample	

Fig. S4. Compared with others probes for HSO_3^- reported

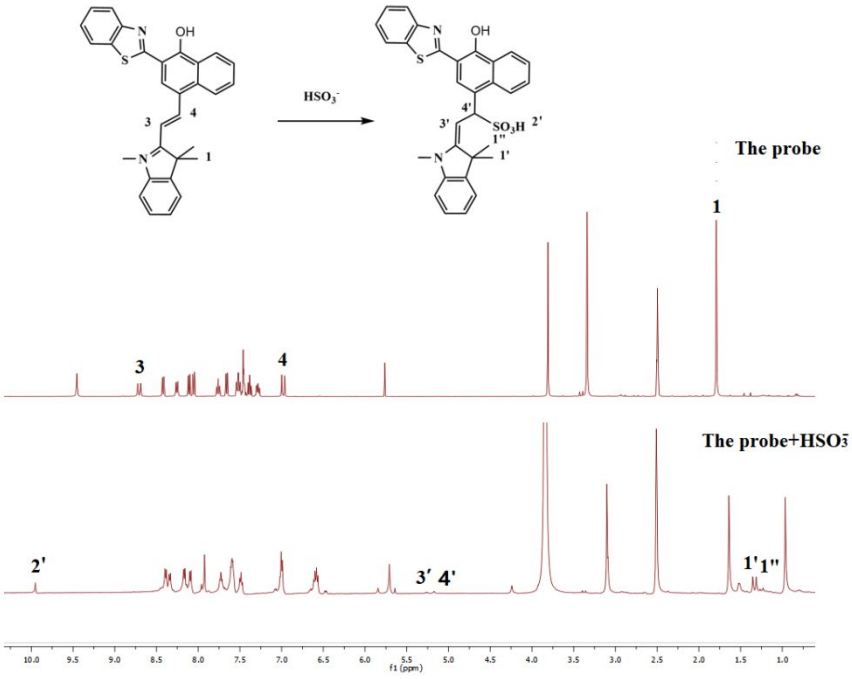


Fig. S5. The changes of ^{1}H NMR spectrum the probe in the absence or the presence of $NaHSO_3$

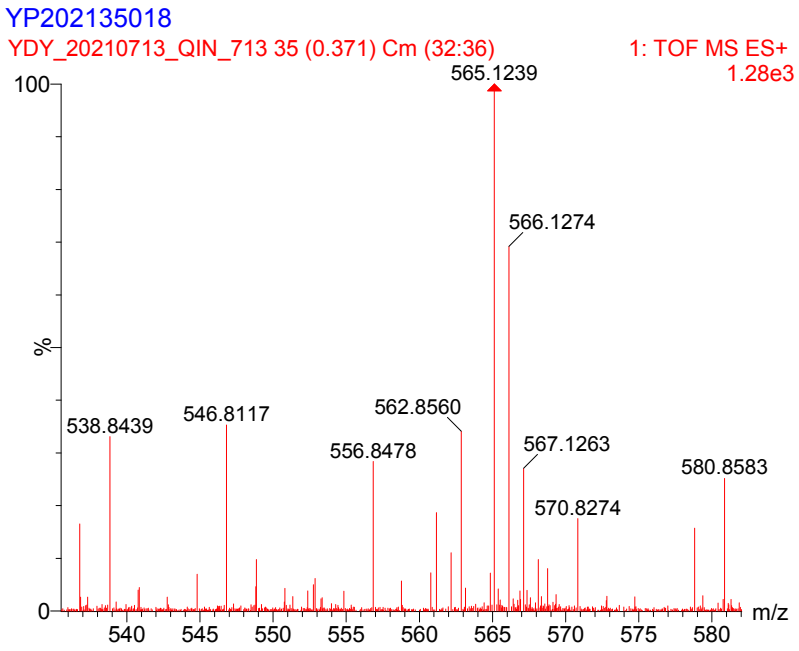


Fig. S6. MS spectra of the probe and $NaHSO_3$

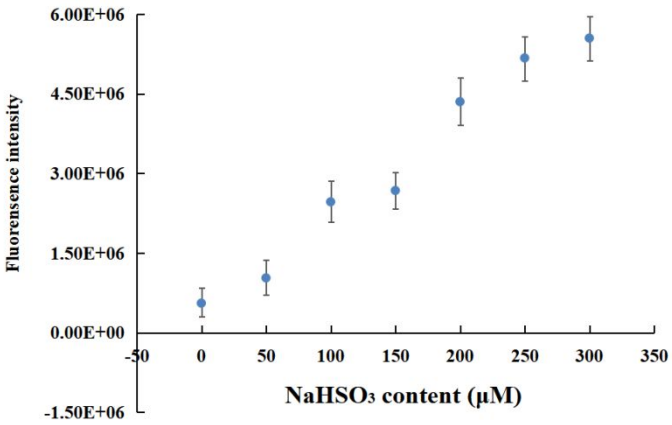


Fig. S7. The detection limit for HSO_3^- based on 3σ/κ, (y = 18139x + 385074, R_2 = 0.9751, σ=1283)

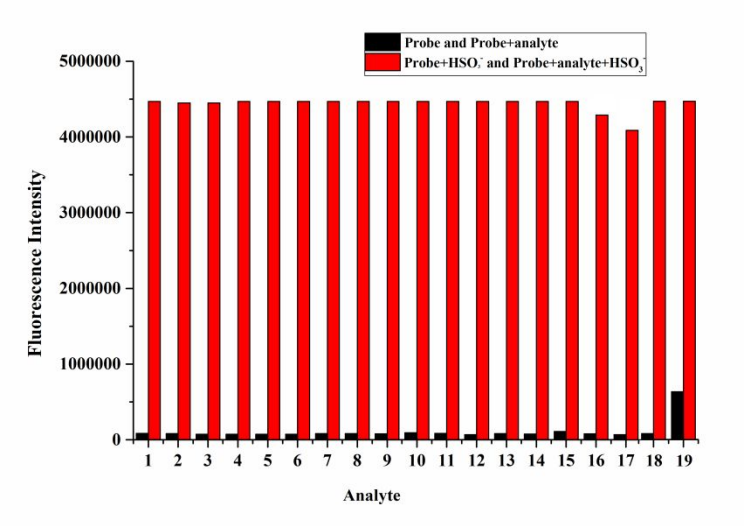
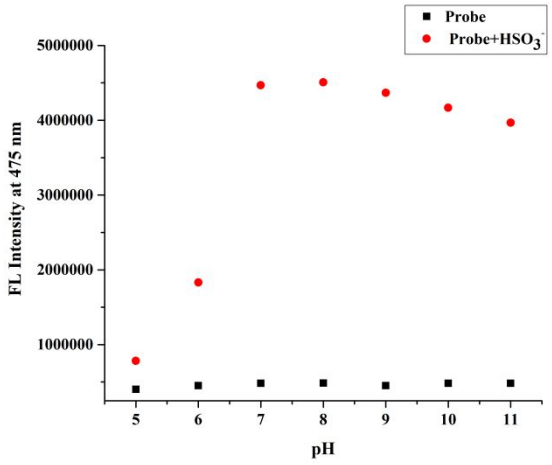


Fig. S8. (a) Fluorescence intensity of the probe (10 μM) and the mixture of the probe (10 μM) andHSO_3^- in the presence of various analytes (20 equiv) at 475 nm, Black bars: the emission intensity of probe in the presence of various analytes, Red bars : the emission intensity that occurs upon the subsequent addition of HSO_3^- to the above solution. From 1 to 19： Blank, Ac^-, Br^- , Cl^- , F^-, I^-, CO_3^{2-}, $H_2PO_4^{2-}$, HCO_3^{2-}, SO_4^{2-}, SO_3^{2-}, NO_2^-, NO_3^-, N_3^-, Cys, H_2O_2, ClO^- , GSH, HS^-.



(b) The fluorescent responses of the probe (10 μM) solution at 475 nm in the absence and presence of bisulfite (100 μM) at different pH values.

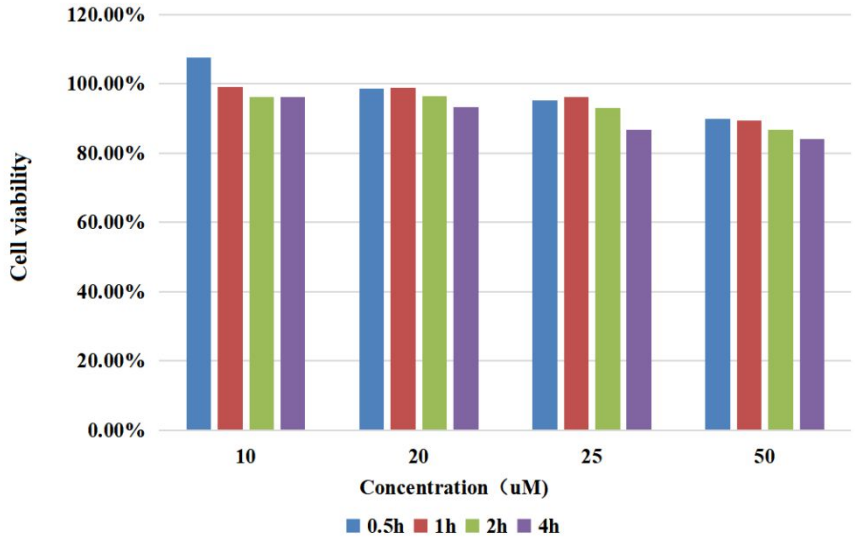


Fig. S9. Cytotoxicity assay

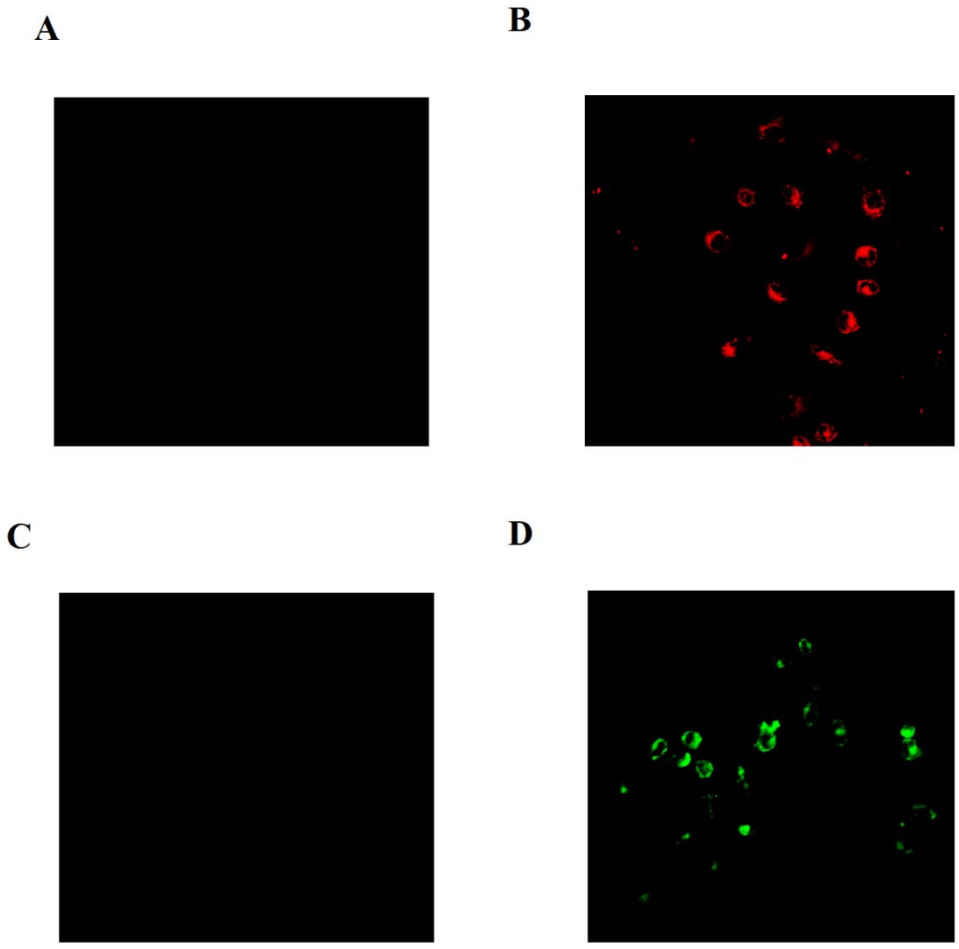
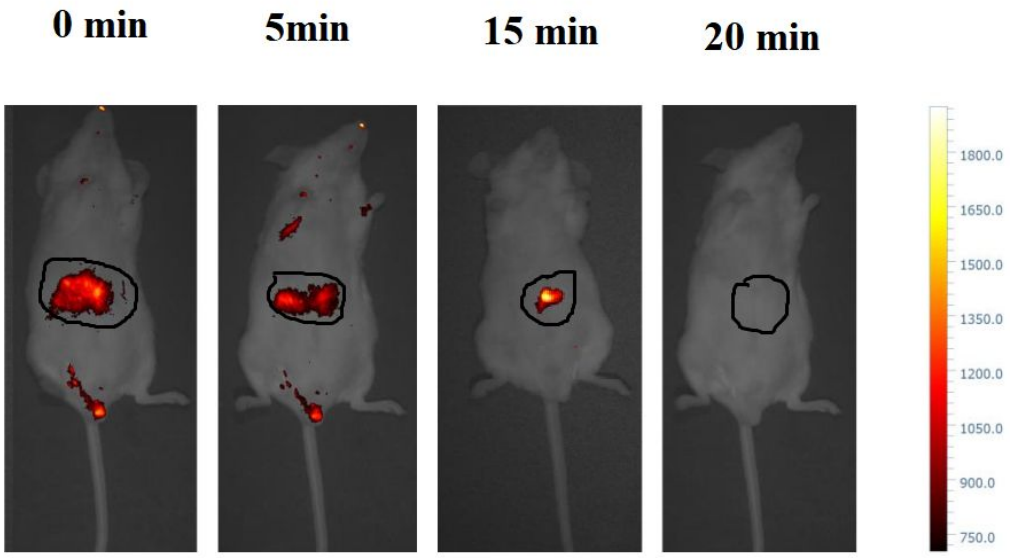


Fig. S10. MCF-7 cells images: (A) and (B) MCF-7 cells incubated with the probe (50.0 μM, 0.5 h) under 410 excitation and 618 nm excitation, (C) and (D) MCF-7 cells incubated with the probe (50.0 μM, 0.5 h) and $NaHSO_3$ (500.0 μM, 0.5h) under 618 nm excitation and 410 excitation, respectively. Scan bar:25μm.



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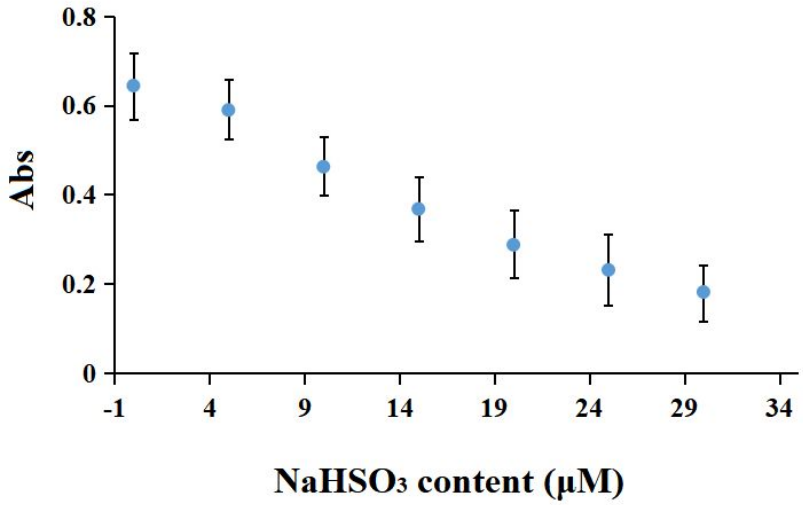

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