Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

A Fluorescent Probe for Bisulfite Ion: Its Applications to Two-Photon Tissue Imaging

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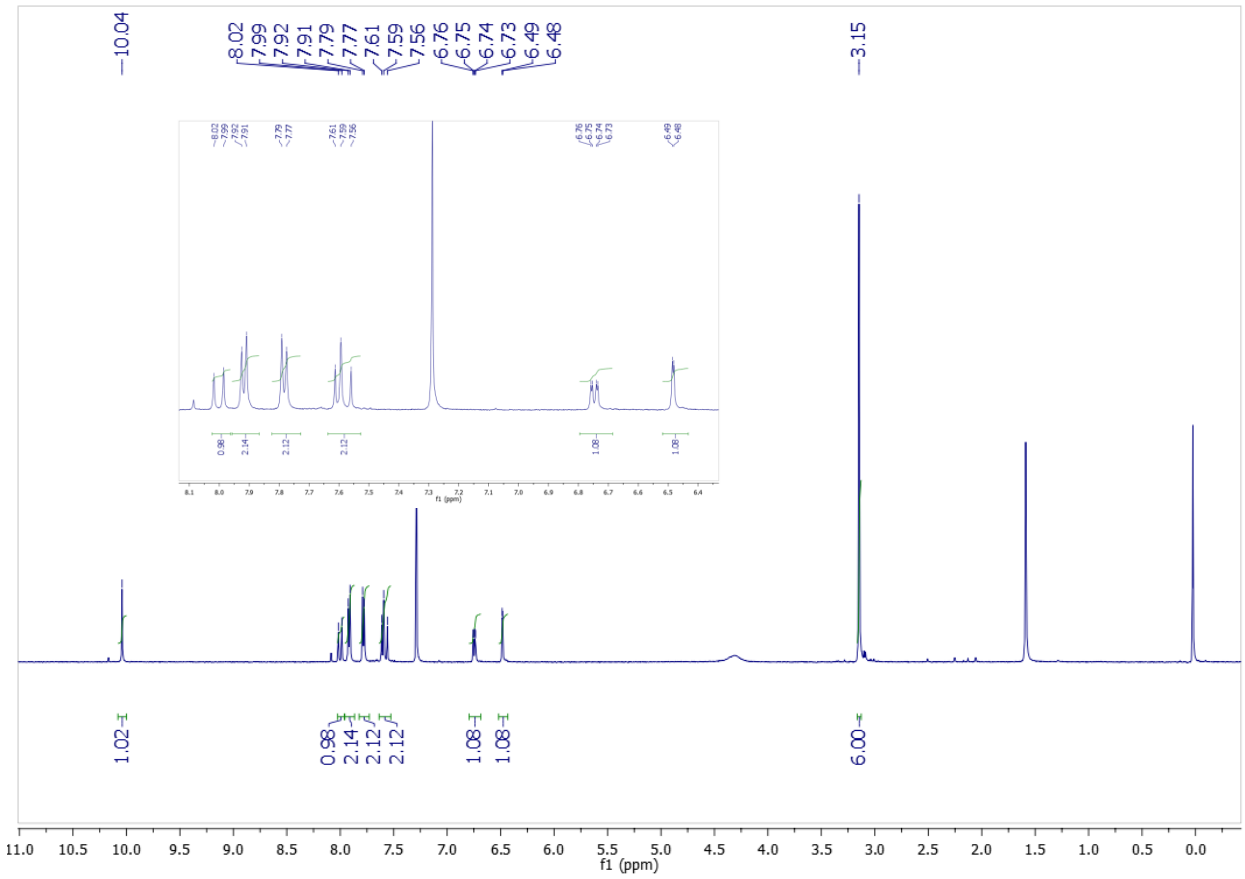
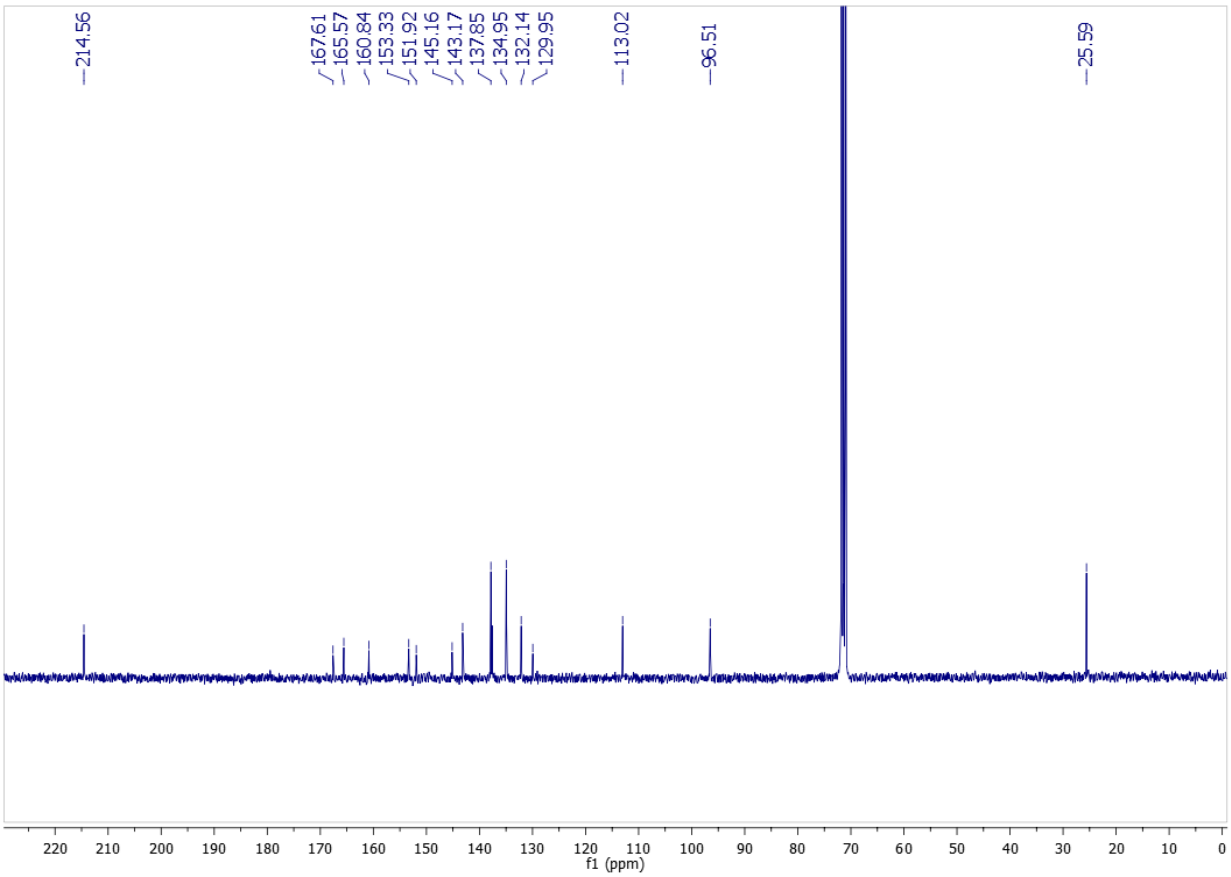
^{1}H NMR of **1:**



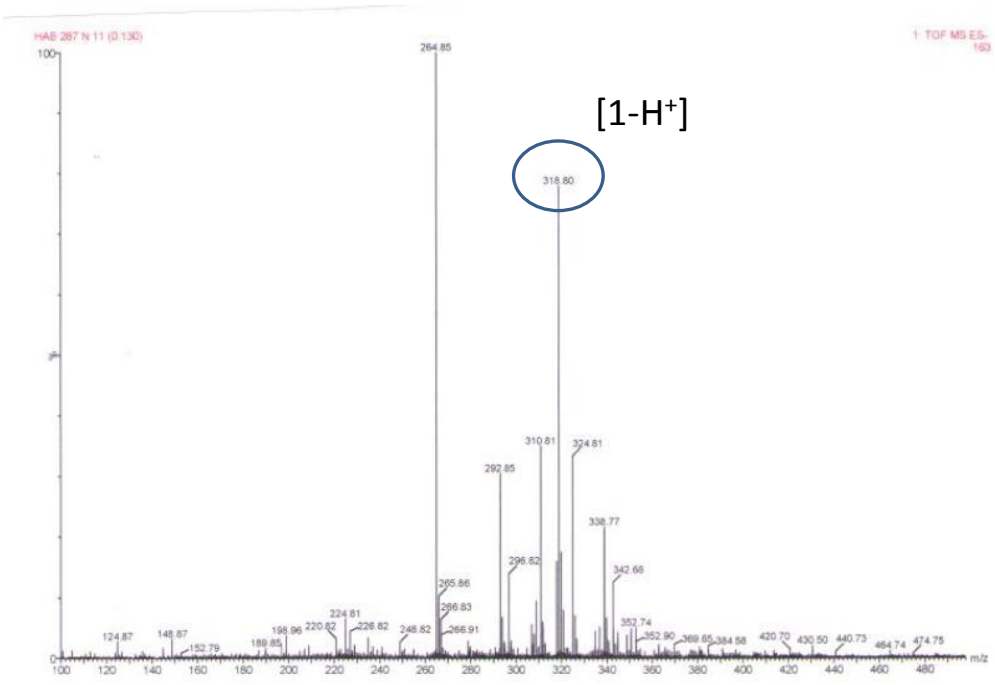
ESI figure S1. ^{1}H NMR spectra of **1** in $CDCl_3$.

^{13}C NMR spectra of **1**



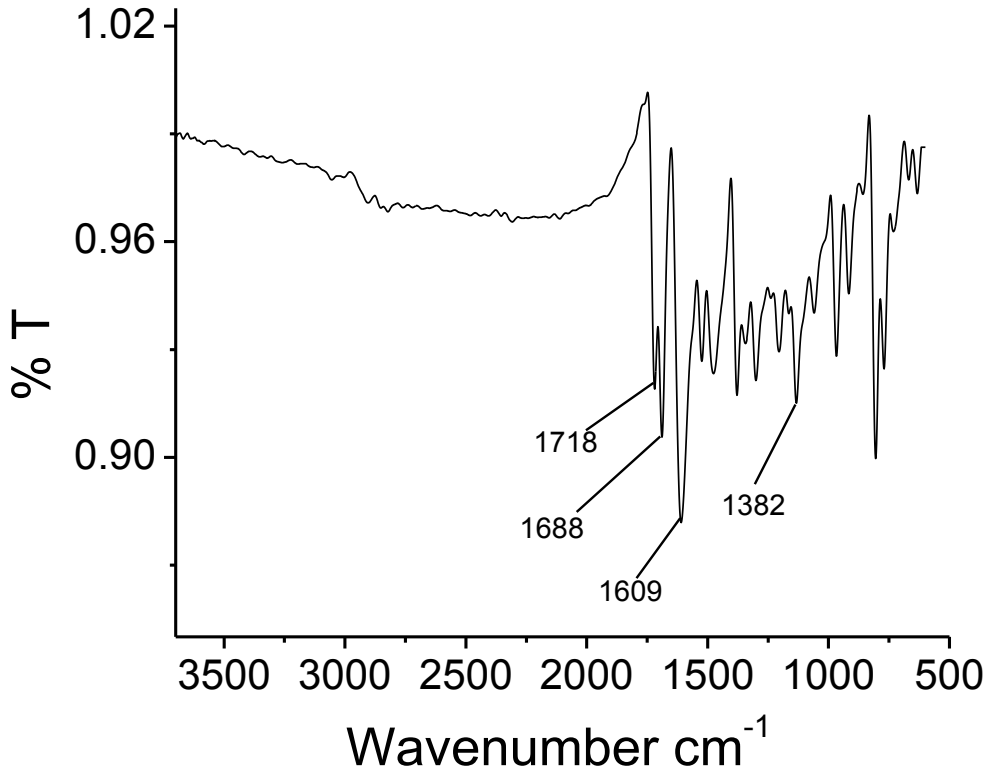
ESI figure S2. ^{13}C NMR spectra of **1** in $CDCl_3$.

Mass spectra of **1**



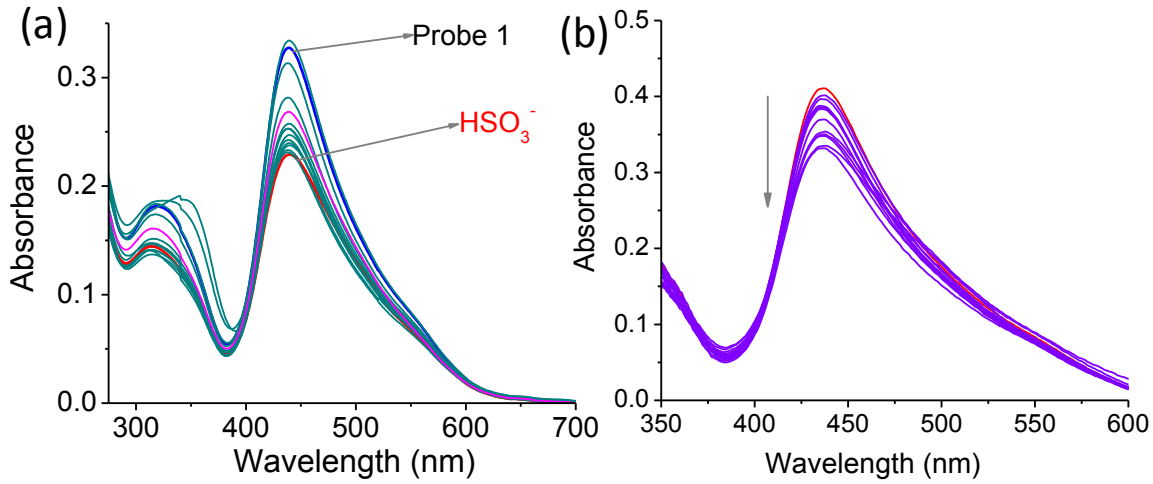
ESI Figure S3. Mass spectra of **1**

FTIR spectra of **1**



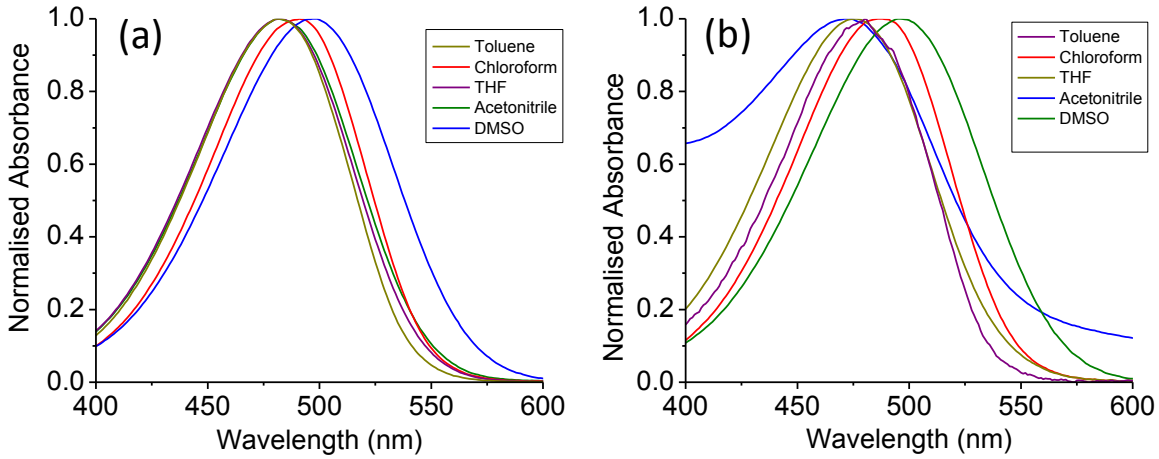
ESI Figure S4. FTIR spectra of **1**.

Uv-Vis Spectra of **1**



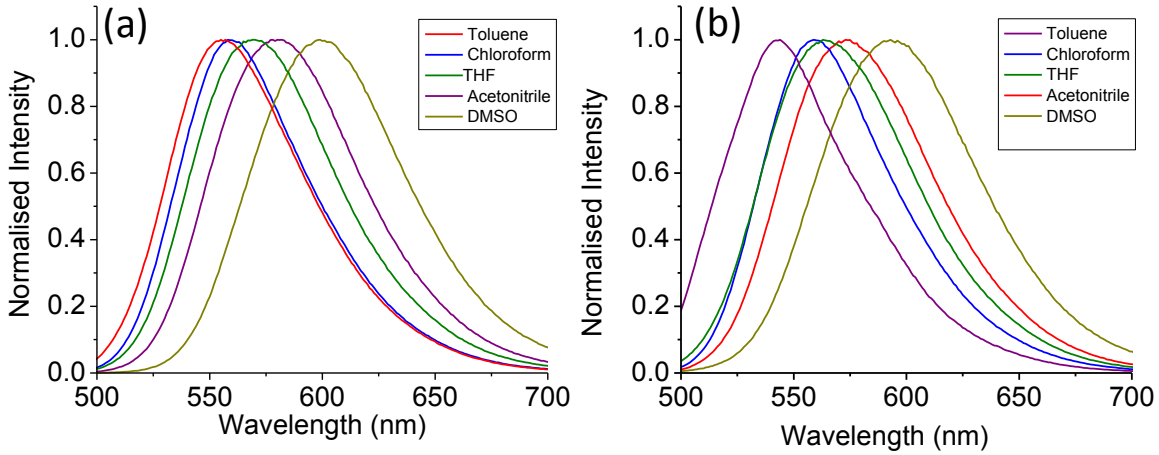
ESI Figure S5. (a) Uv-Vis spectra of **1** (20 μM) in presence of different anions and biothiols, (b) in presence of different concentration of HSO_3^-.

Solvent dependent UV-Vis spectra of **1**



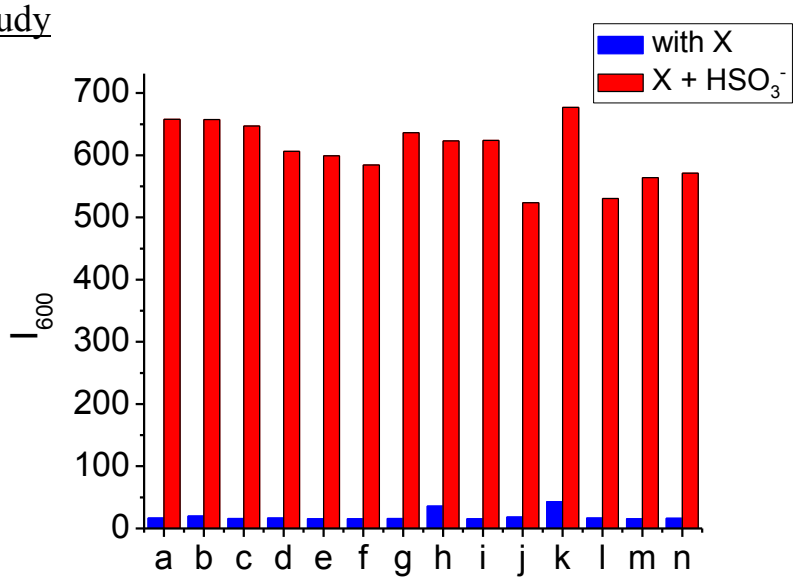
ESI Figure S6. Normalized absorption spectra of **1** (20 μM) in (a) absence (b) presence of 50 equivalent of HSO_3^- in solvents of different polarity.

Solvent dependent Emission spectra of **1**



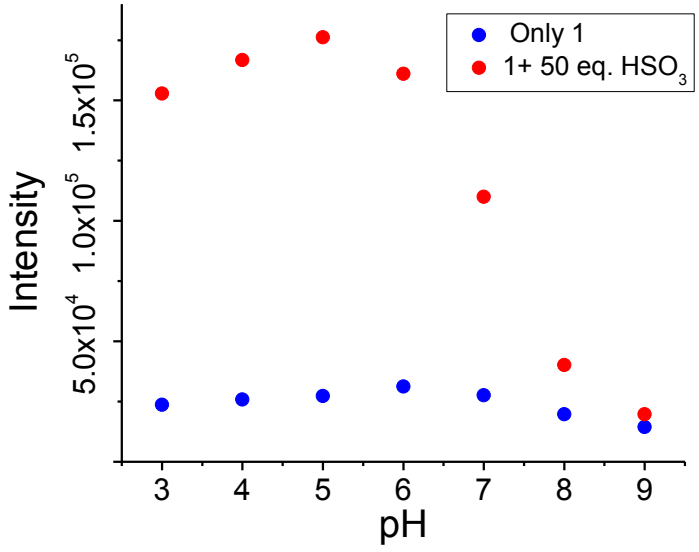
ESI Figure S7. Normalized emission spectra of **1** (20 μM) in (a) absence (b) presence of 50 equivalent of HSO_3^- in solvents of different polarity. All experiments were performed using excitation at 490 nm.

Interference Study



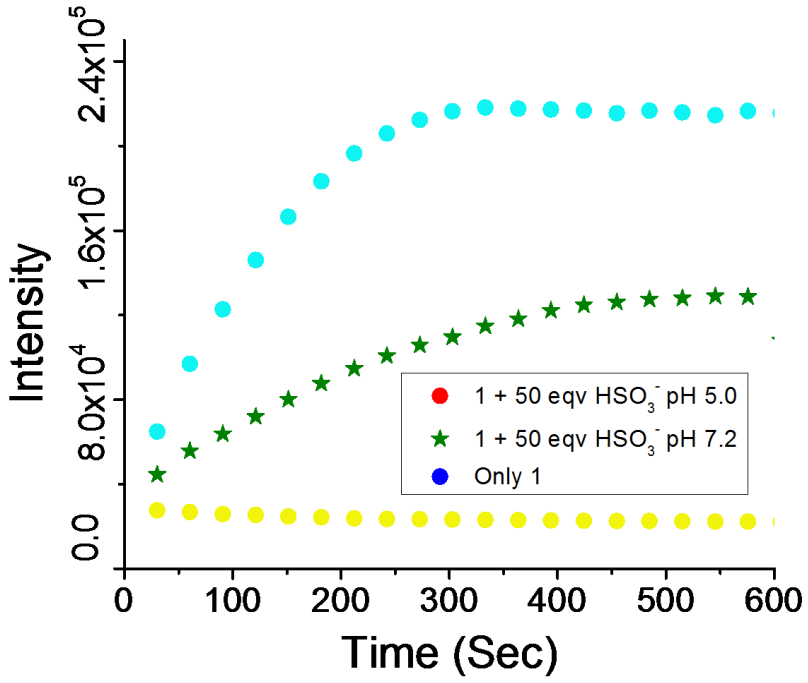
ESI Figure S8. Competitive graph in presence of 100 mole equivalent HSO_3^- and 200 mole equivalent of others [a=Cl^-, b= Br^-, c= F^-, d= I^-, e= OAc^-, f=NO_3^-, g= SO_4^-, h= $S_2O_3^{2-}$, i= $H_2PO_4^-$, j= N_3^-, k= Cys, l= Hcy, m=GSH, n= CN^-] in 200 mM Na_2HPO_4:citric acid aq. buffer solution pH 5.0 $\lambda_{Ext}/\lambda_{Em}$: 490/600nm.

pH dependent spectra



ESI Figure S9. pH dependent emission profile of **1** (20 μM) in absence and presence of 50 equivalents of HSO_3^- in 200 mM Na_2HPO_4:citric acid aq. buffer solution having pH 5.0, $\lambda_{Ext}/\lambda_{Em}$: 490/600nm.

Time-dependent spectra



ESI Figure S10. Time dependent emission profile of **1** (20 μM) in absence and presence of 50 equivalents of HSO_3^- in 200 mM Na_2HPO_4:citric acid aq. buffer solution pH 5.0 and pH 7.2 $\lambda_{Ext}/\lambda_{Em}$: 490/600nm.

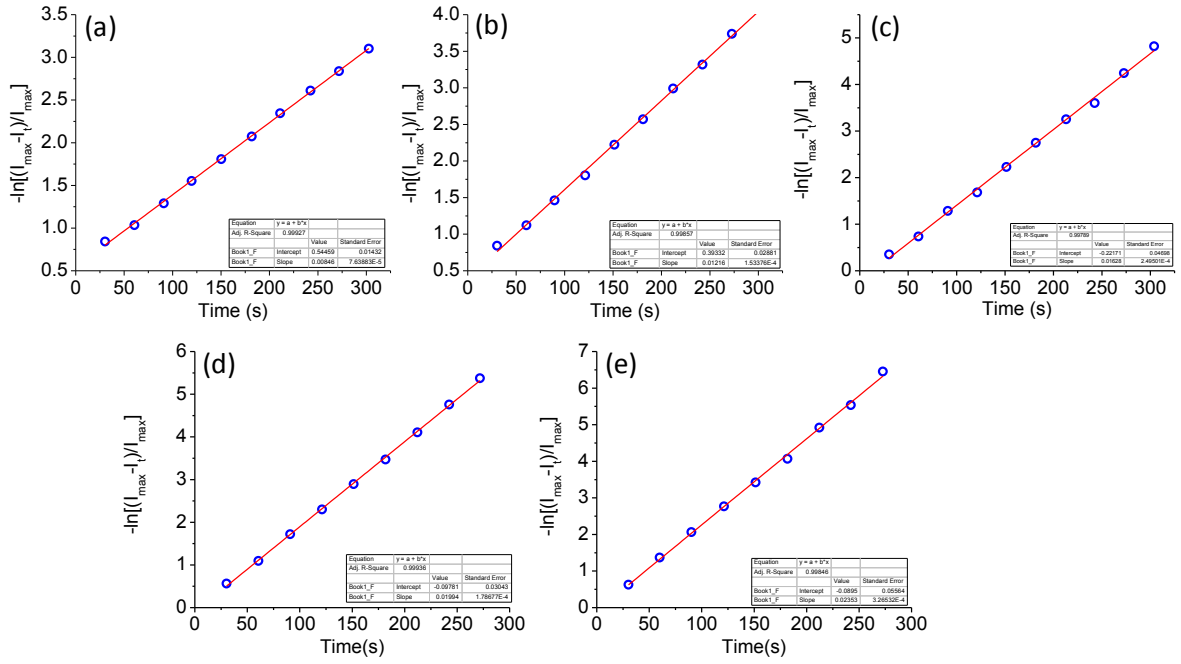
Kinetic Studies:

Time dependent studies of (20 μM) of **1** with different concentrations of HSO_3^-were carried out by mixing, and monitored by fluorescence measurements in 200 mM Na_2HPO_4: citric acid aq. buffer solution pH 5.0 $\lambda_{Ext}/\lambda_{Em}$: 490/600nm. Data were collected under pseudo-first-order conditions. The pseudo-first order rate constant for the reaction was determined by fitting the fluorescence intensity changes of the samples to the pseudo first-order equation:

$$\ln[(I_{max}-I_t)/I_{max}] = -k_{obs}\, t$$

Where, I_t and I_{max} represent the fluorescence intensities at times t and the maximum value obtained after the reaction was complete. k_{obs} is the observed rate constant of the reaction.

From the slope we get k_{obs} value for each reaction.



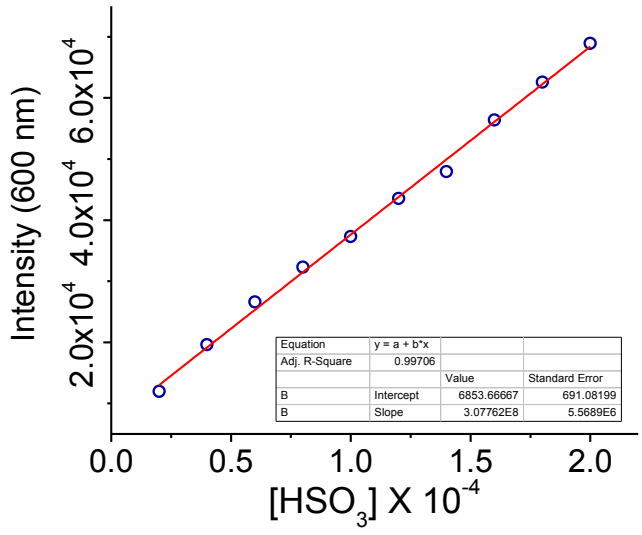
ESI Figure S11. Plot of–ln[$(I_{max}-I_t)/I_{max}$)] vs time with 20 μM of **1** in presence of different concentration of HSO_3^- (a) 2×10^{-4} M, (b) 4×10^{-4} M, (c) 6×10^{-4} M, (d) 8×10^{-4} M, (e) 10×10^{-4} M in 200 mM Na_2HPO_4:citric acid aq. buffer solution pH 5.0.

Calculation of Lowest Detection Limit:

The detection limit of HSO_3^- was calculated by following equation 2

DL = K. σ /s …..Equation 1

Where K= 2, σ is the standard deviation of blank measurement, s is the slope of intensity *vs.* $[HSO_3^-]$ plot.

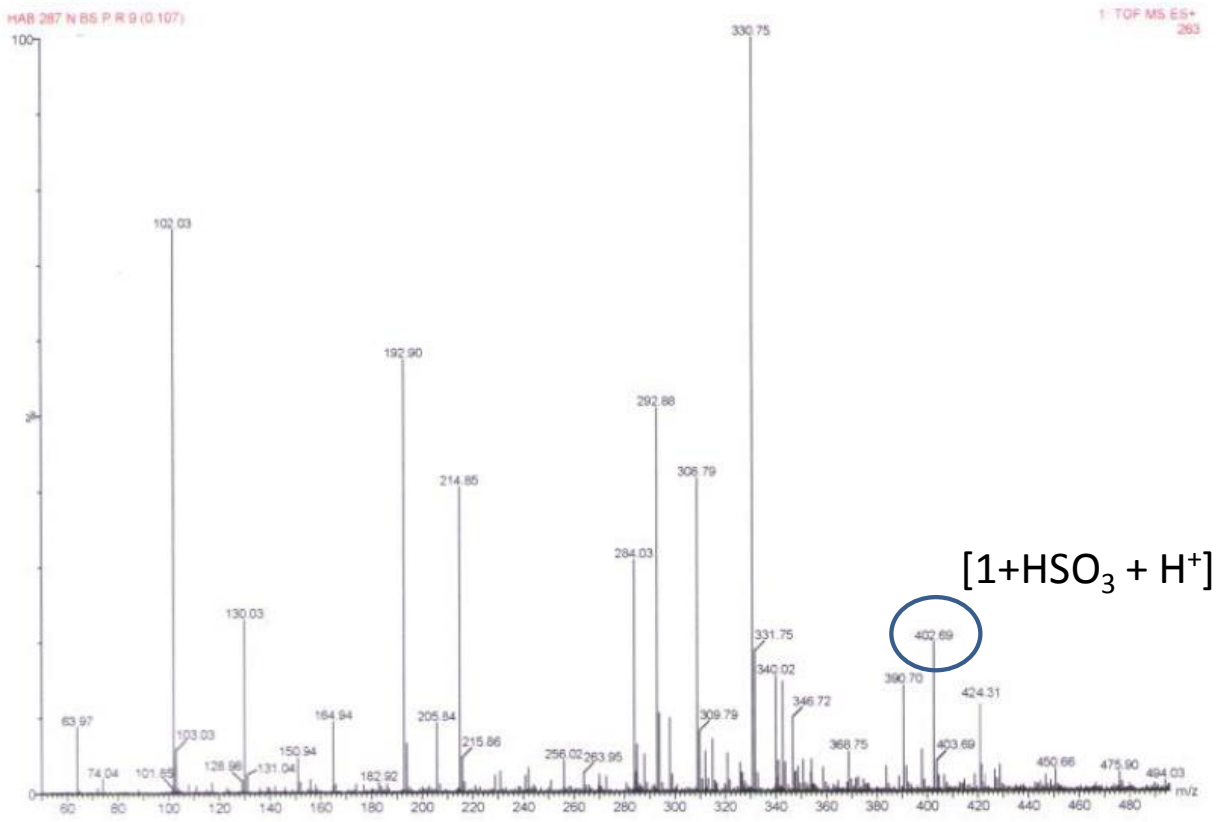


ESI Figure S12: Calibration curve for determining lowest detection limit. Measurements were performed in 200 mM Na_2HPO_4: citric acid aq. buffer solution pH 5.0 using $\lambda_{Ext}/\lambda_{Em}$: 490/600nm.

Here σ is found to be 286.11 and slope form the graph is 3.07×10^8

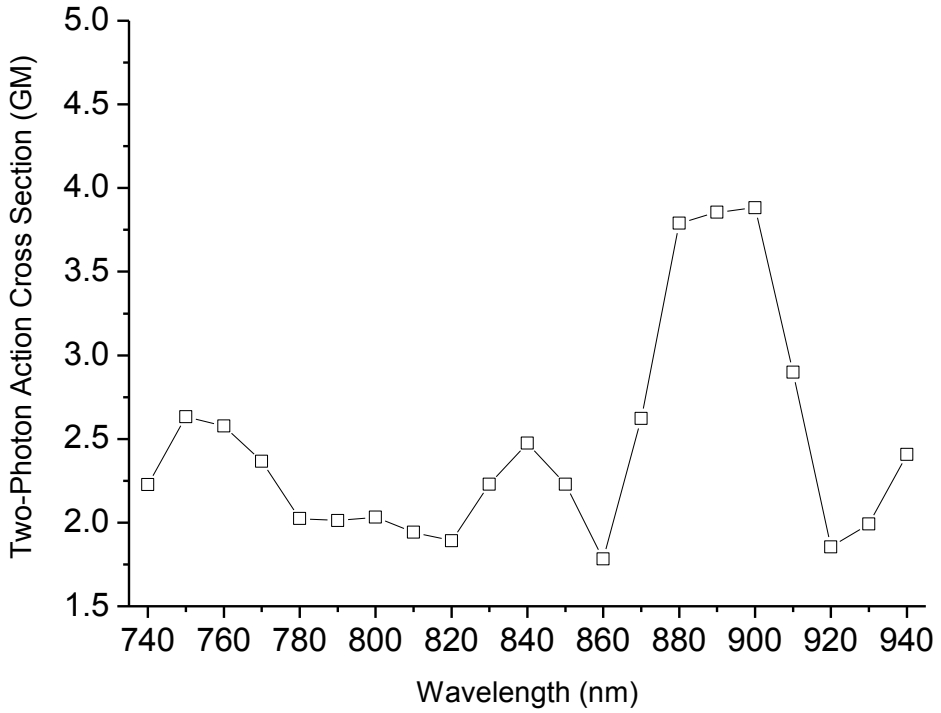
DL calculated is 1.86×10^{-6} M.

Mass Spectra of 1 with HSO_3^-



ESI Figure S13: Mass spectra of **1** in presence of HSO_3^-

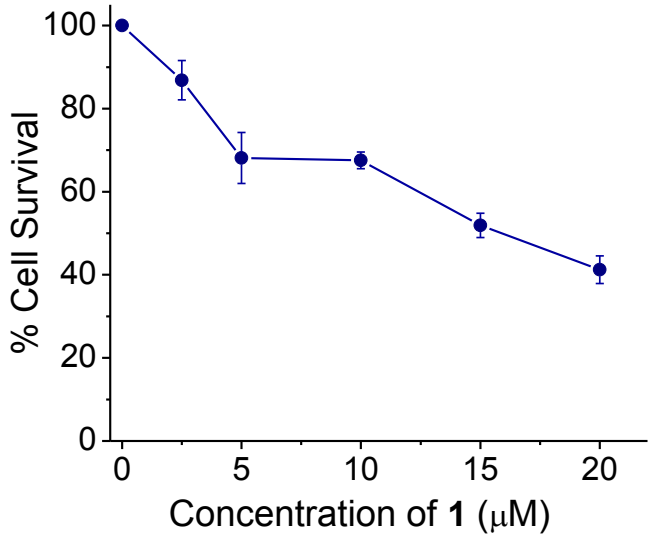
Two Photon Action Cross Section (TPACS):



ESI Figure S14: TPACS value under various two-photon excitation of the probe **1** (10 μM in pH 5.6 HEPES buffer in the presence of 200 equivalent of sodium bisulfite). The values are determined by using Rhodamine B (100 μM in methanol) as a reference dye.

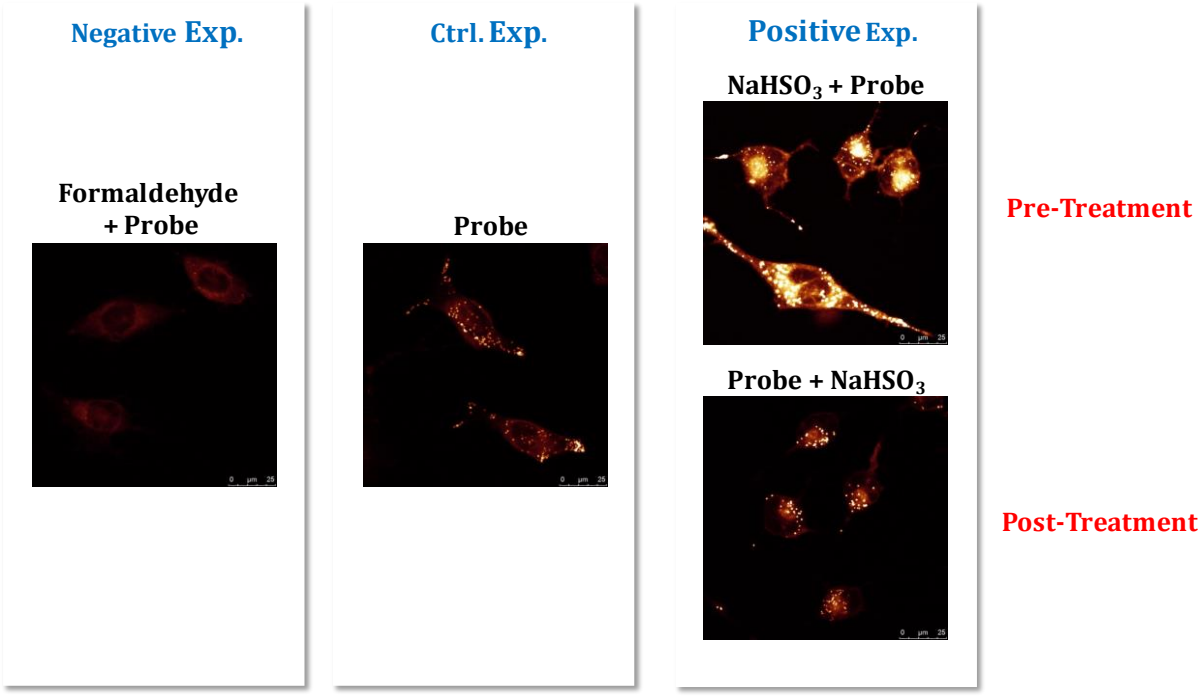
MTT assay of **1**

To determine cell cytotoxicity of **1** on Hct116 cell, MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, a yellow tetrazole) assay was performed. In a 96 well plate cells (5000) were seeded and cultured in a 37 °C incubator supplied with 5% CO_2. Cells were maintained in DMEM medium, supplemented with 10% FBS and antibiotics. Thereafter cells were treated with different concentration of **1** for 24 h, and then further treated with 0.5µg/µl of MTT reagent. Cells were incubated for 4 h at 37 °C and then later 100 µl of Isopropyl Alcohol was added to each well. The optical density was measured at 570 nm using Multiskan Go (Thermo Scientific) to find the concentration of the cell inhibition.



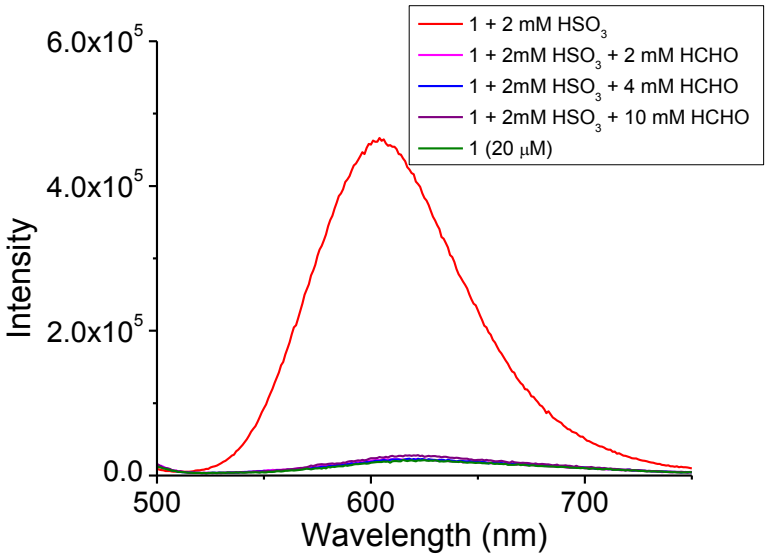
ESI Figure S15: Cell survival with different concentration of **1.**

TPM images of HeLa cells:



ESI Figure S16:TPM images of HeLa cells incubated with probe **1**(10 μM) for30 min before (post-treatment) and after (pre-treatment) treatment of bisulfite (1 mM) for 15 min. TPM imaging was conducted by exciting at 880 nm and by collecting fluorescence emission through two overlaid channels (500–550 nm; 565–605 nm). Scale bar is 25 μm.

Fluorescence spectra in presence of formaldehyde:



ESI Figure S17: Fluorescence spectra of **1 s** (20 μM) with 2 mM of $NaHSO_3$ in presence and absence of different concentration of formaldehyde.