Supporting Information

Human peripheral blood mononuclear cells targeted multidimensional switch for selective detection of bisulphite anion

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CONTENTS

1. Determination of detection limit:

The detection limit was calculated based on the fluorescence titration. To determine the S/N ratio, the emission intensity of **CM** without HSO_3^- was measured by 10 times and the standard deviation of blank measurements was determined. The detection limit (DL) of **CM** for HSO_3^- was determined from the following equation: DL = K × Sb_1/S, where K = 2 or 3 (we take 3 in this case); Sb_1 is the standard deviation of the blank solution; S is the slope of the calibration curve. For HSO_3^- :

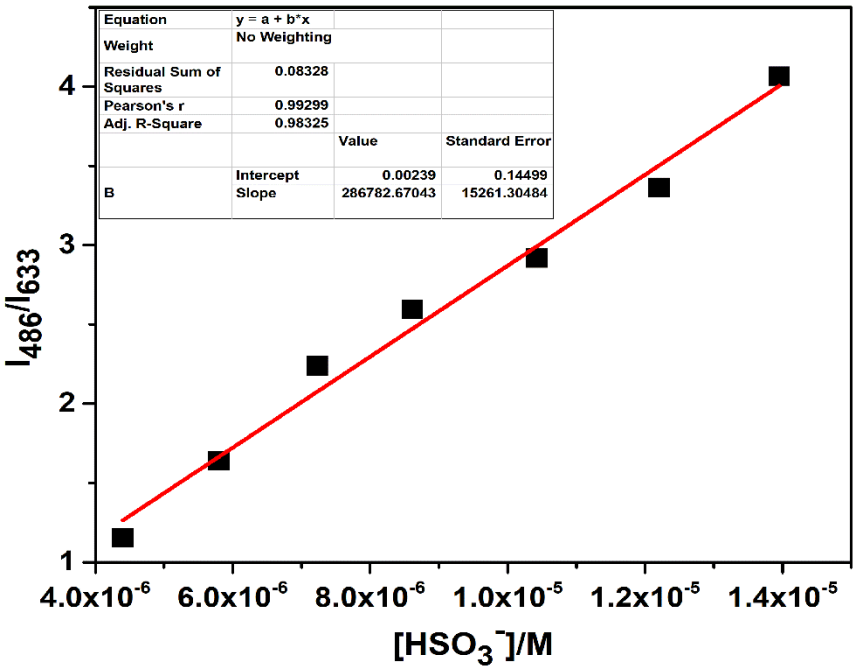

Figure S1: Emission intensity ratio I_{486}/I_{633} of **CM** depending on the concentration of HSO_3^-

From the graph we get slope = 286782.670, and Sb_1 value is 0.00116

Thus using the formula we get the Detection Limit = 1.21 × 10^{-8} M i.e. CM can detect HSO_3^- in this minimum concentration through fluorescence method.

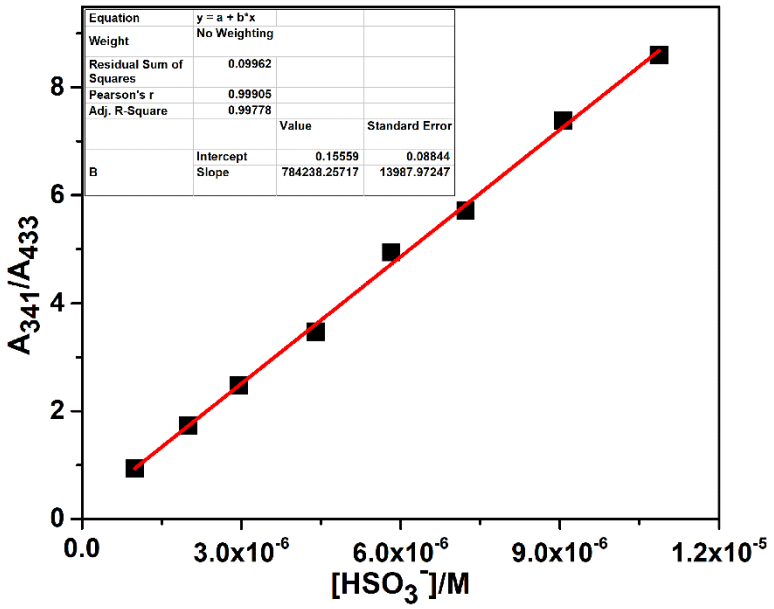

Figure S2: Absorbance intensity ratio A_{341}/A_{433} of **CM** depending on the concentration of HSO_3^-

From the graph we get slope = 784238.30, and Sb_1 value is 0.0215

Thus using the formula we get the Detection Limit = 8.224×10^{-8} M i.e. **CM** can detect HSO_3^- in this minimum concentration through UV-vis method.

2. Linear responsive curve of CM depending on HSO_3^- concentration:

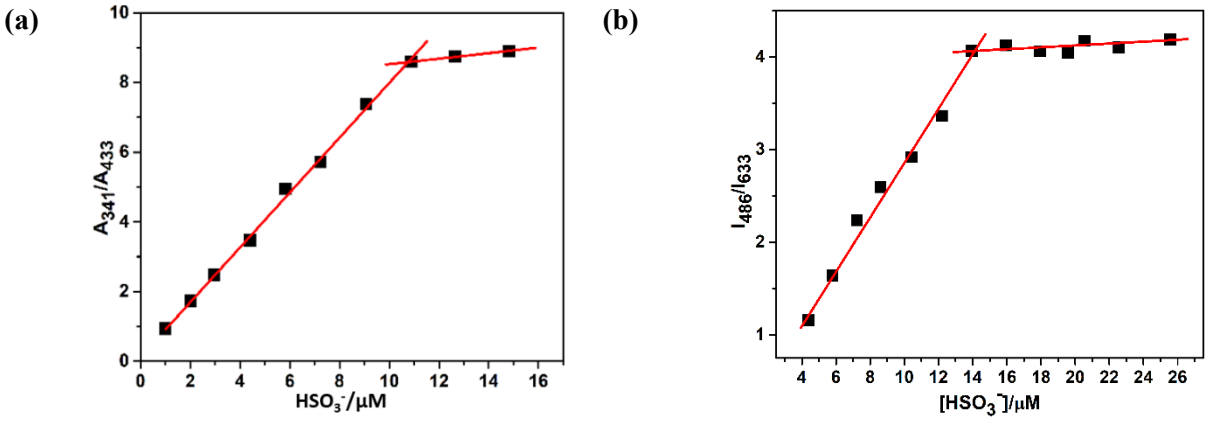

Figure S3: The response curve of (a) absorbance intensity ratio (A_{341}/A_{433}) and (b) intensity ratio (I_{486}/I_{633}) of **CM** depending on the HSO_3^- concentration.

3. Determination of fluorescence Quantum Yields (Φ) of CM and its complex with HSO_3^- :

For measurement of the quantum yields of CM (and CA) and its complex with HSO_3^-, we recorded the absorbance of the compounds in methanol solution. The emission spectra were recorded using the maximal excitation wavelengths, and the integrated areas of the fluorescence-corrected spectra were measured. The quantum yields were then calculated by comparison comparison with fluorescein (Φs = 0.97 in basic ethanol) as reference using the following equation:

$$\Phi x = \Phi s \times \left(\frac{Ix}{Is}\right) \times \left(\frac{As}{Ax}\right) \times \left(\frac{nx}{ns}\right)^2$$

Where, x & s indicate the unknown and standard solution respectively, Φ is the quantum yield, *I* is the integrated area under the fluorescence spectra, *A* is the absorbance and *n* is the refractive index of the solvent.

We calculated the quantum yield of CM, CM-HSO_3^- using the above equation and the value is 0.19 and 0.44 respectively and for CA it was found 0.14.

4. Solvatochromic change and fluorescence quantum yields in different solvents for **CM** and **CA**

Table S1: Absorbance, Emission peaks and fluorescence quantum yields in different solvents for **CM**.

Solvents	n-hexane	Toluene	DEE	THF	DCM	CH_3CN	MeOH
Absorbance Peak (nm)	439	451	455	449	462	445	446
Emission Peak (nm)[a]	524	548	574	611.5	614.5	676	676.5
Fluorescence Quantum Yield (Φ)[b]	0.19	0.22	0.25	0.27	0.29	0.30	0.33
	[a] excitation wavelength (nm) is 450 nm; [b] Φ was obtained by compared with anthracene (Φ = 0.19 in ethanol)						

Table S2: Absorbance, Emission peaks and fluorescence quantum yields in different solvents for **CA**.

Solvents	n-hexane	Toluene	THF	DCM	CH_3CN	DMSO	CH_3OH
Absorbance Peak (nm)	372	379	378	386	379	387	383
Emission Peak (nm)[a]	437	454.5	482.5	508.5	535.5	540	593.5
Fluorescence Quantum Yield (Φ)[b]	0.14	0.15	0.16	0.18	0.20	0.21	0.22
[a] excitation wavelength (nm) is 380 nm; [b] Φ was obtained by compared with anthracene (Φ = 0.14 in ethanol)							

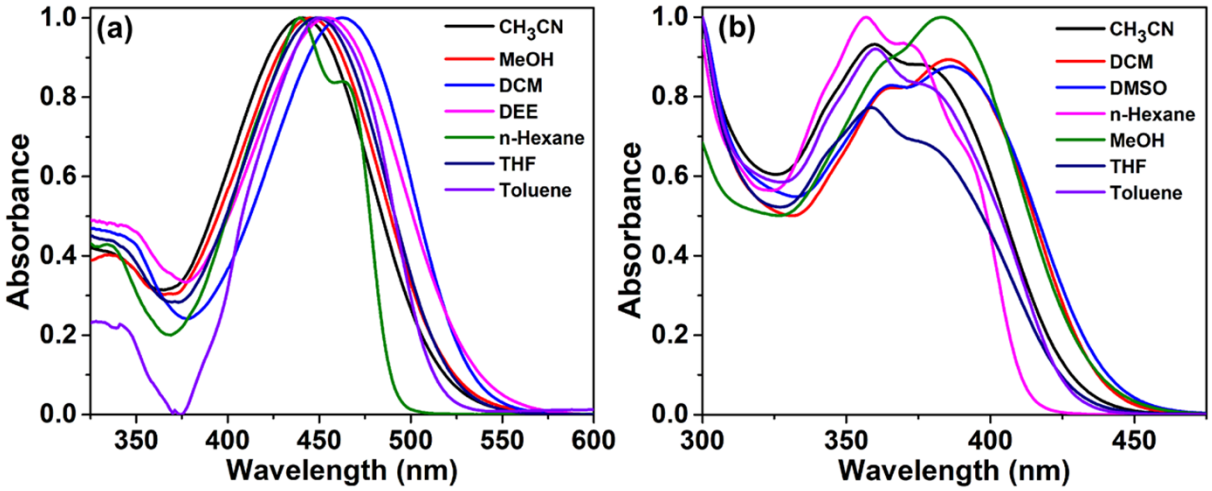

Figure S4: Solvent-dependent emission spectra of (a) CM and (b) CA (5 μM)

5. pH dependent study:

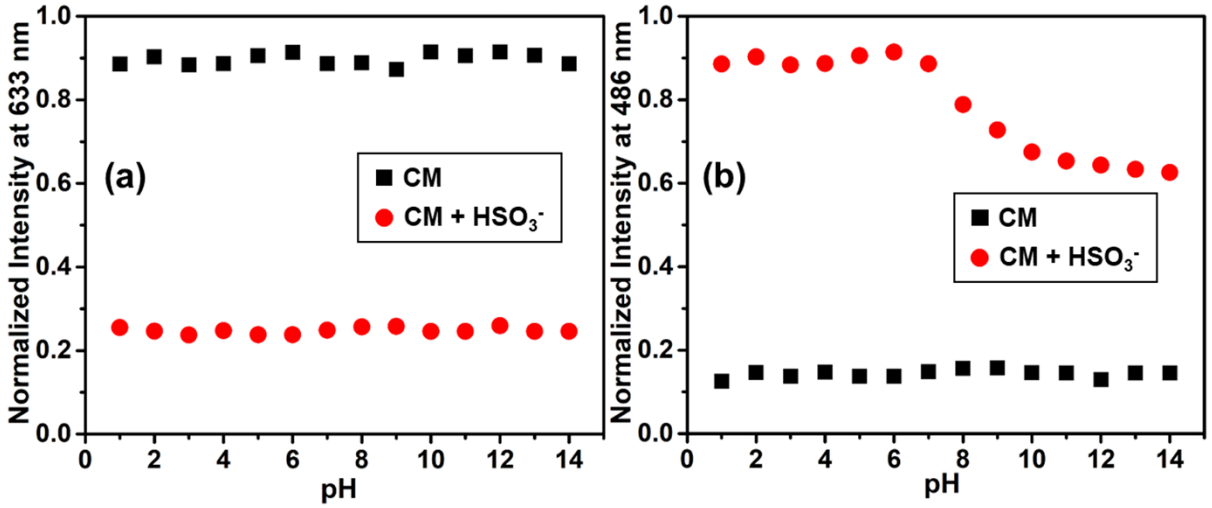

Figure S5: Fluorescence response of only CM and CM + HSO_3^- at (a) 633 nm and (b) 486 nm as a function of pH in MeOH/ H_2O (1/ 1, *v/v*), pH is adjusted by using aqueous solutions of 1 M HCl or 1 M NaOH. [HQCN] = 10 μM, [HSO_3^-] = 50 μM. λ_{ex} = 400 nm.

6. *CM and CA as viscosity sensor*

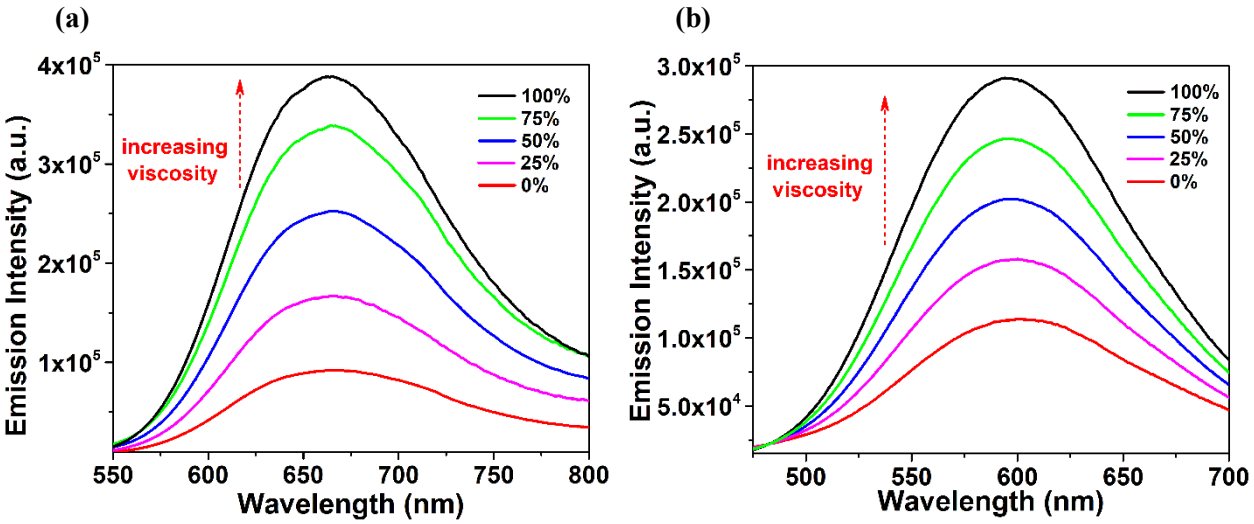

Figure S6: Fluorescence spectra of (a) CM and (b) CA in Mehanol-PBS-glycerin mixture with different volume fractions of glycerol (10 μM; λex = 530 nm and 480 nm respectively for CM and CA)

7. Time dependent fluorescence spectra of CM with added HSO_3^-

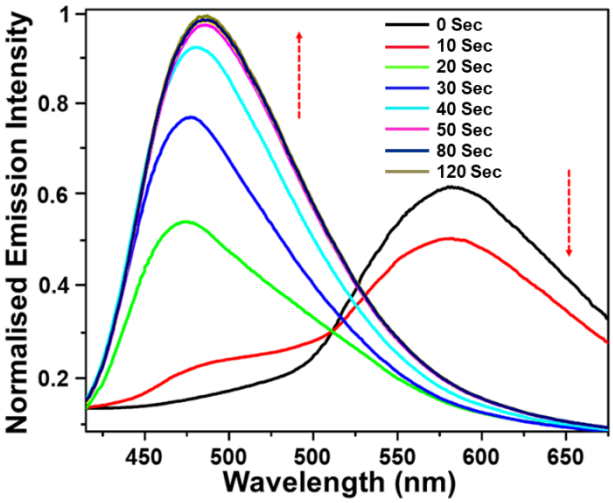

Figure S7: Change of emission spectra of CM (10 μM) upon addition of HSO_3^- (2 equivalents)

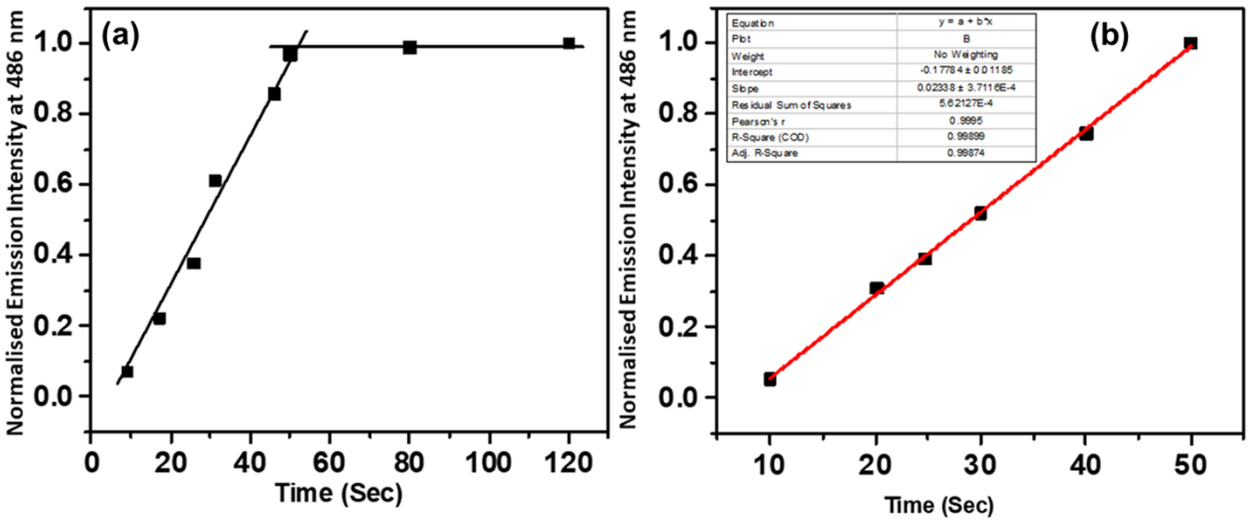

Figure S8: Time dependent fluorescence spectra of CM after interaction HSO_3^- with time.

8. ^{1}H NMR spectrum of CM

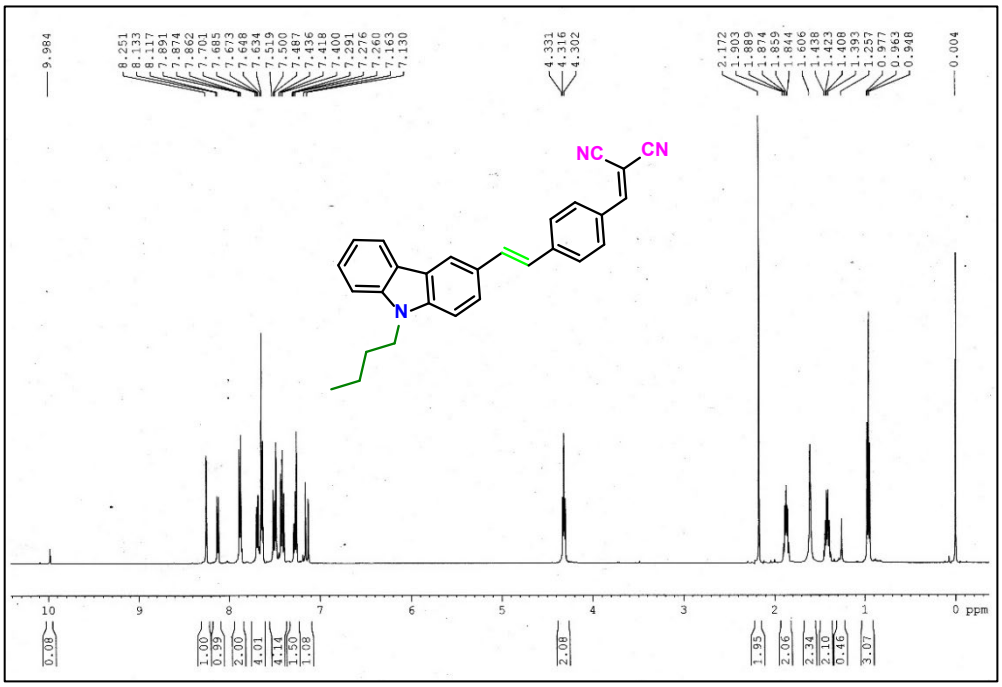

Figure S9: ^{1}H NMR (500 MHz) spectrum of CM in $CDCl_3$

8. ^{13}C NMR spectrum of CM

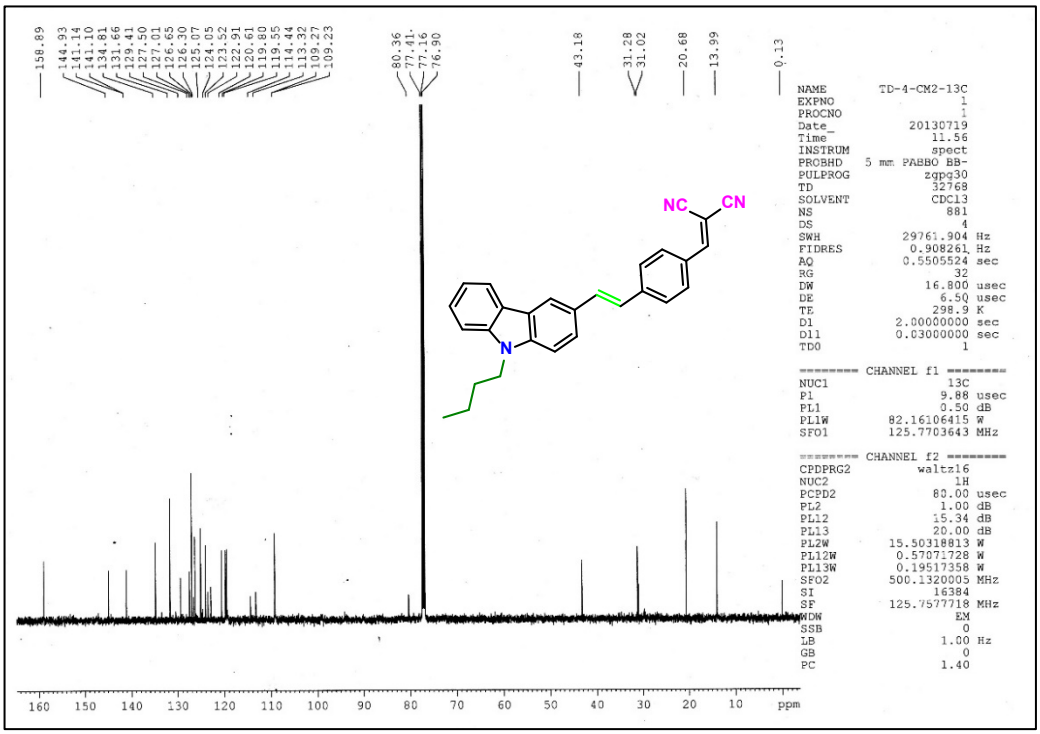

Figure S10: ^{13}C NMR (125 MHz) spectrum of CM in $CDCl_3$

9. Mass spectrum (HRMS) of CM

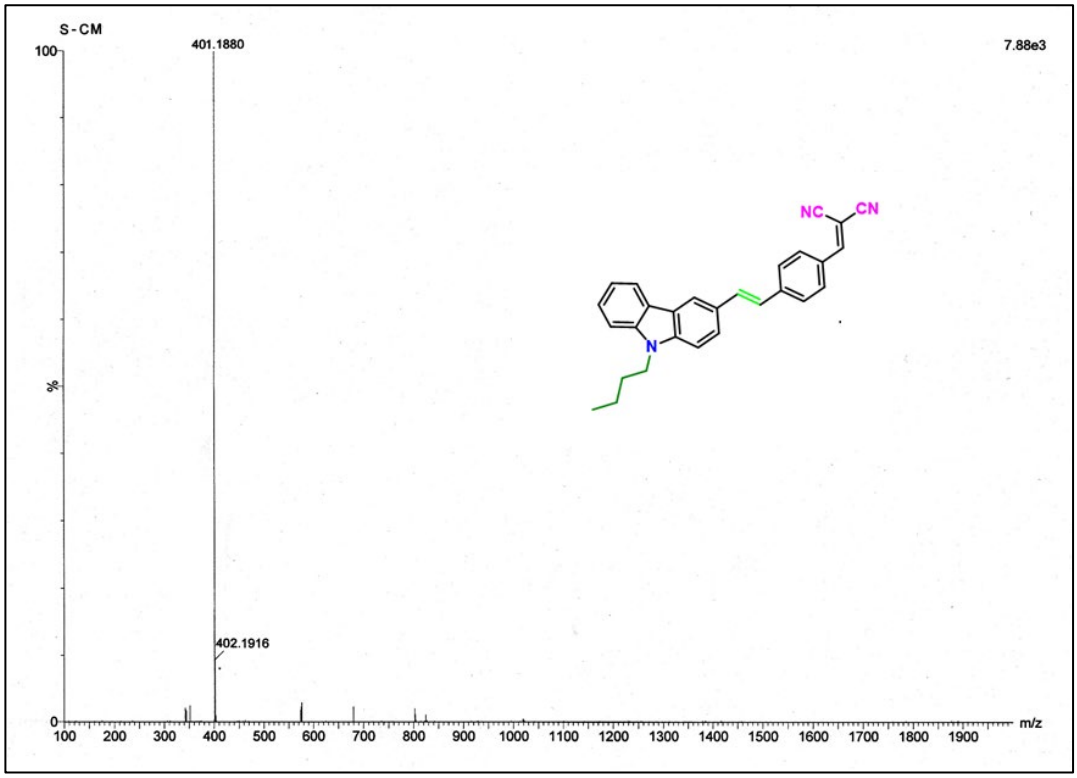

Figure S11: HRMS of CM.

10. MS spectrum of the product (CM with HSO_3^-)

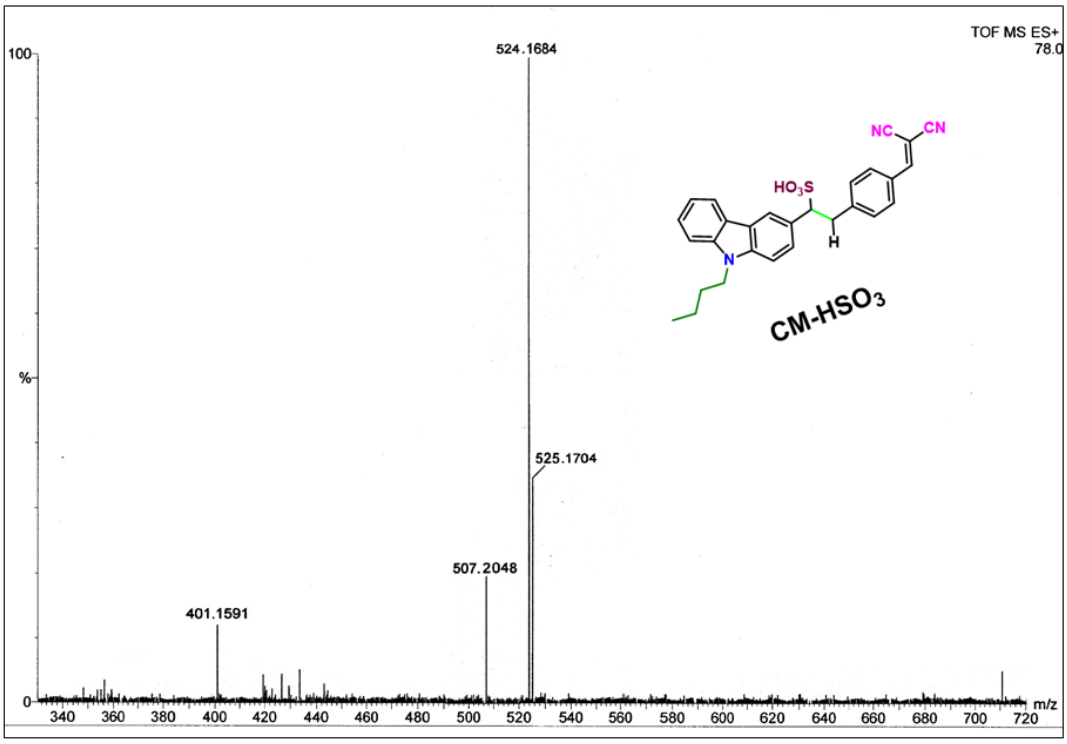

Figure S12: HRMS of CM-HSO_3 Complex.

11. ^{1}H NMR spectra of (CM with HSO_3^-)

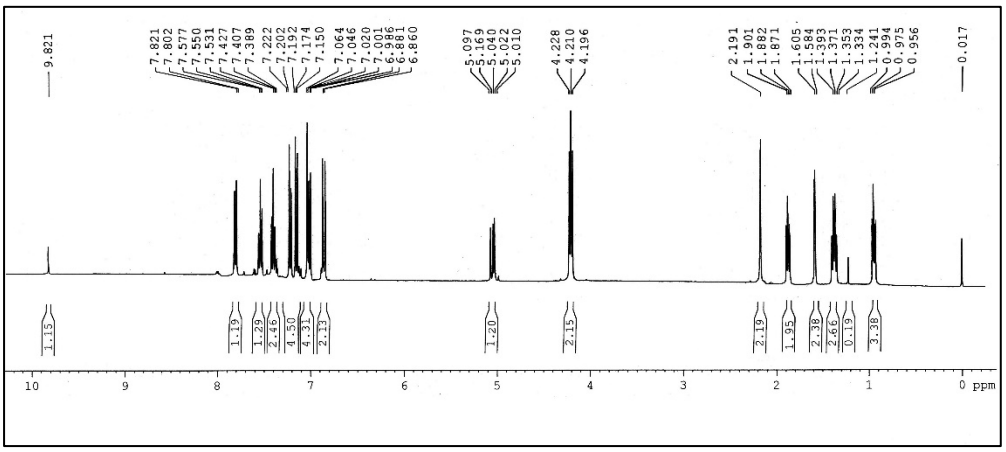

Figure S13: ^{1}H NMR (400 MHz) spectra of [**CM** + **HSO_3^-**] in $CDCl_3$.

12. **Table S3** Fluorescence life time data of CM

Entry	Φ	τ (ns)	k_r ($10^8 \times s^{-1}$)	k_{nr} ($10^8 \times s^{-1}$)
CM	0.19	1.7	1.11	4.76
CM-HSO_3^-	0.44	9.32	0.47	0.6

13. **Table S4** Detection of HSO_3^- in Food Samples:

Granulated sugar	Bisulfite content (μmol/L)	Added (μmol/L)	Found (μmol/L)	Recovery (%)
Sample 1	6.45	5 6	11.35 12.10	99.12 97.18
Sample 2	4.50	3 4	7.32 8.31	97.6 97.76

14. Materials and methods

Details of bio-imaging

Venous blood (3ml) was obtained by venepuncture from a healthy male volunteer donor (age - 30 years) with informed consent. The research program was approved by Calcutta University Biosafety and Ethics Committee. Peripheral blood mononuclear cells were isolated with histopaque-1077 gradient [SIGMA] through density gradient centrifugation. PBMCs were washed in ice cold PBS for two times and resuspended in the same with a cell density of 3 X 10^6. PBMCs were treated with or without $NaHSO_3$ (25 μM) and CM (10 μM) and incubated for 30 minutes at 37 ^{0}C in dark. CM samples were prepared in DMSO and PBS (1:1). The endogenous fluorescence intensity was measured in fluorescence microscope (Carl Zeiss HBO 100) under 40X magnification with fluorescence emissions at 633 nm (Red channel, Filter set 42) nm and 486 nm (Green channel, Filter Set 9) respectively. The relative fluorescence intensities were quantitated using ImageJ software.

15. MTT assay:

To observe the cell viability against CM, PBMCs were treated with varied concentrations of CM solution, concentration ranging from 5-50 μM, with or without the presence of HSO_3^- (25 μM). The cells were incubated for 1 hour at 37^{0}C against control cell suspension without CM. Cell density were 0.05 x 10^6 cells per well in a 96- well plate. 100 μl of MTT solution (5 mg/ml) were added in both control and treated wells, and incubated for 4 hours at 37^{0}C. The purple colored formazan crystals were

dissolved with 100 µl of DMSO and the absorbance were measured at 570 nm. Cell viability was calculated using the following calculation:

$$\% \text{ of Cell Viability} = \frac{(\text{Absorbance of treatment group - blank})}{(\text{Absorbance of control group - blank})} \text{ X } 100$$

16. Comparison Table S5

Sr. No	Fluorophore Used	Solid state fluorescence	Ratiometric Fluorescence Change with Detection Limit	Bioimaging Studies	Food samples Analysis	solvatofluorochromic	Reference
1.	Carbazole – quinolinium	Yes	Not ratiometric (turn-off) 18.1 nM	Yes	Yes	No	J. Agric. Food Chem., 2019, 67, 4375−4383
2.	1,2,4,5-tetrazinebased	No	Turn off colorimetric change 3.8 µM.	No	Yes	No	RSC Adv., 2018, 8, 33459 –33463
3.	Carbazole based Polymer micelle	No	Ratiometric Fluorescence Change 1.1 µM	Yes	No	No	ACS Appl. Bio Mater., 2019, 2, 1, 236–242
4.	Diformyl phenol and diformyl bisphenol	No	Not given	Yes	Yes	No	Journal of Photochemistry and Photobiology A: Chemistry, 2020, 389, 112214
5.	biscyclometalated Ir(III) complex	No	Ratiometric Fluorescence Change LOD 0.9 µM	Yes	Yes	No	Analyst, 2018,143, 3670-3676
6.	Ethylcarbazole-3vinyl)-benzothiazolium iodide	No	Ratiometric Fluorescence Change LOD 0.53 µM	Yes	No	No	J. Mater. Chem. B, 2016, 4, 3703-3712
7.	Quinolone-benzimidazole	No	Ratiometric Fluorescence Change	Yes	Yes	No	ACS Omega 2020, 5, 10, 5452–5459

			LOD 0.29 μM				
8.	semi-cyaninecoumarin hybrid dye	No	Ratiometric Fluorescence Change LOD 27.6 nM	No	No	No	Dyes and Pigments, 2016, 134, 190-197
9.	Benzimidazole and Hemicyanine	No	Ratiometric Fluorescence Change LOD 40 nM	Yes	No	No	Analytica Chimica Acta 2019, 1055, 133-139
10.	coumarin–thiazole compound	No	1.22 μM,	Yes	No	No	Journal of Photochemistry & Photobiology A: Chemistry, 2019, 372, 212–217
11.	Biotin and Coumarin	No	Ratiometric Fluorescence Change LOD 72 nM	Yes	No	No	ACS Sens. 2016, 1, 166−172
12.	1,8-naphthalic anhydride and morpholinoethanamine	No	Fluorescence quenching and ratiometric change in the absorption spectra LOD 3.2 nM	Yes	No	No	J. Fluorescence, 2020, 30, 977-983
13.	Carbazol-thiazol-3-ium iodide	No	Turn on 3.3 nM	No	Yes	No	Journal of Photochemistry & Photobiology, A: Chemistry 411 (2021) 113201
14.	Maleonitrile conjugated carbazole dye with an intervening p-styryl spacer	Yes	Ratiometric Fluorescence Change LOD 1.21×10^{-8} M	Yes	Yes	Yes	Present Work