# ***Supporting Information***

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

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## 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 $HSO_3^-$ :

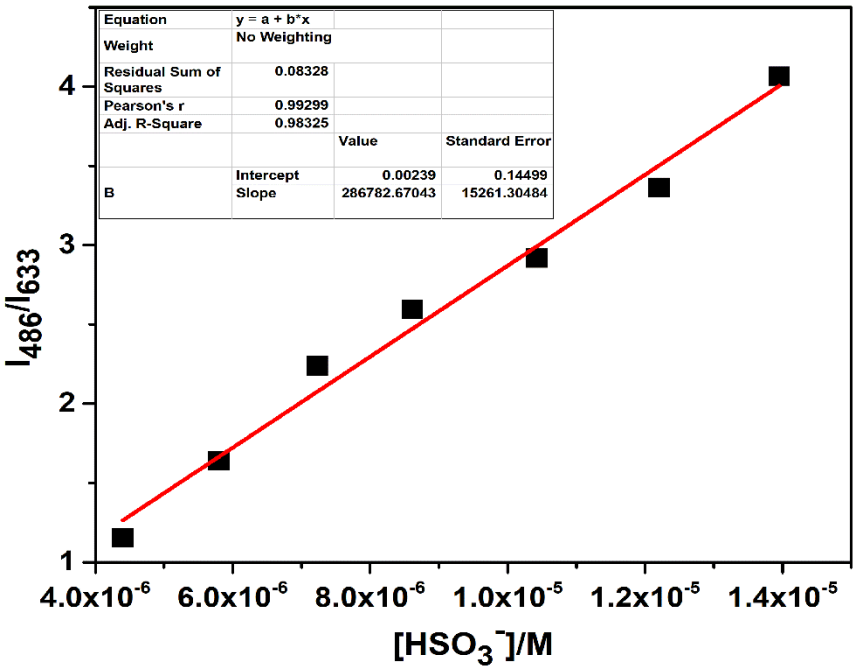


**Figure S1:** Emission intensity ratio $I_{486}/I_{633}$ of **CM** depending on the concentration of $HSO_3^-$

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.

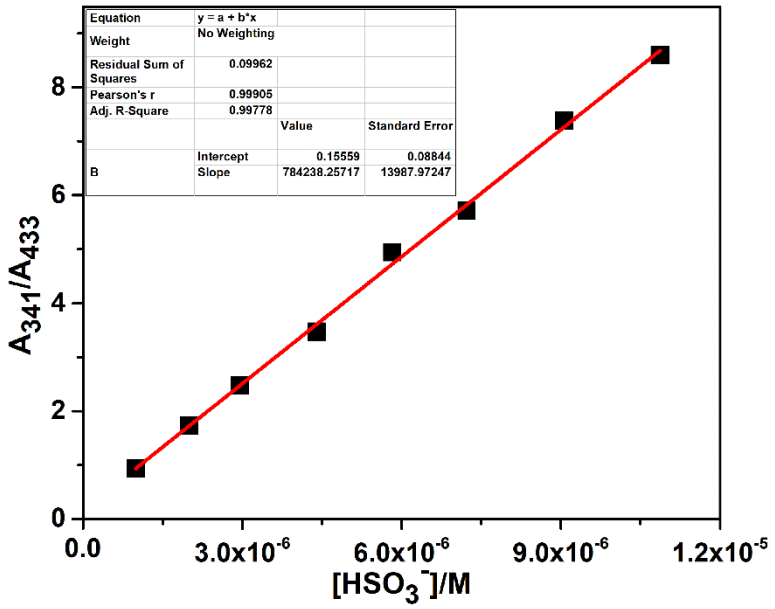


**Figure S2:** Absorbance intensity ratio $A_{341}/A_{433}$ of **CM** depending on the concentration of $HSO_3^-$

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

Thus using the formula we get the Detection Limit = $8.224 \times 10^{-8}$ 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:

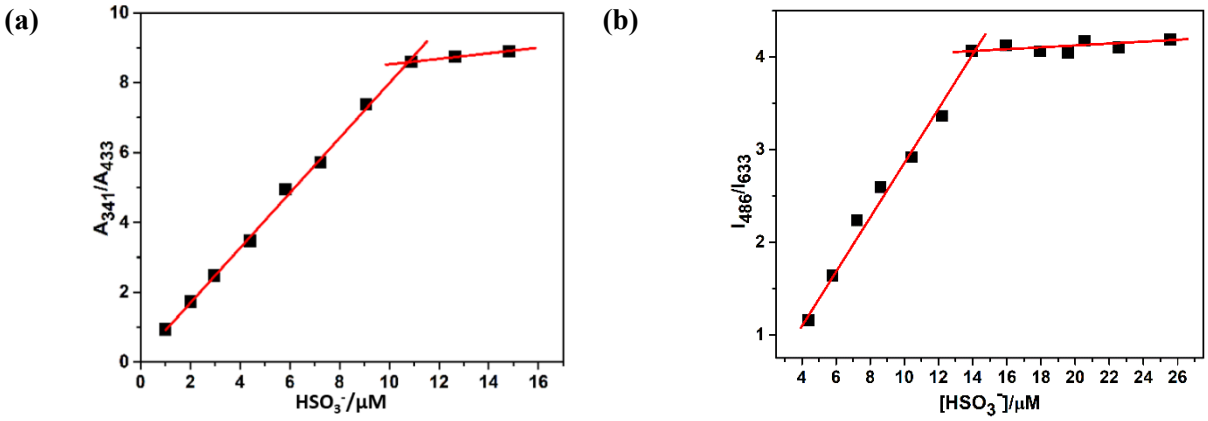


**Figure S3:** The response curve of (a) absorbance intensity ratio ($A_{341}/A_{433}$) and (b) intensity ratio ($I_{486}/I_{633}$) of **CM** depending on the $HSO_3^-$ concentration.

### 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 ($\Phi$s = 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) | | | | | | | |

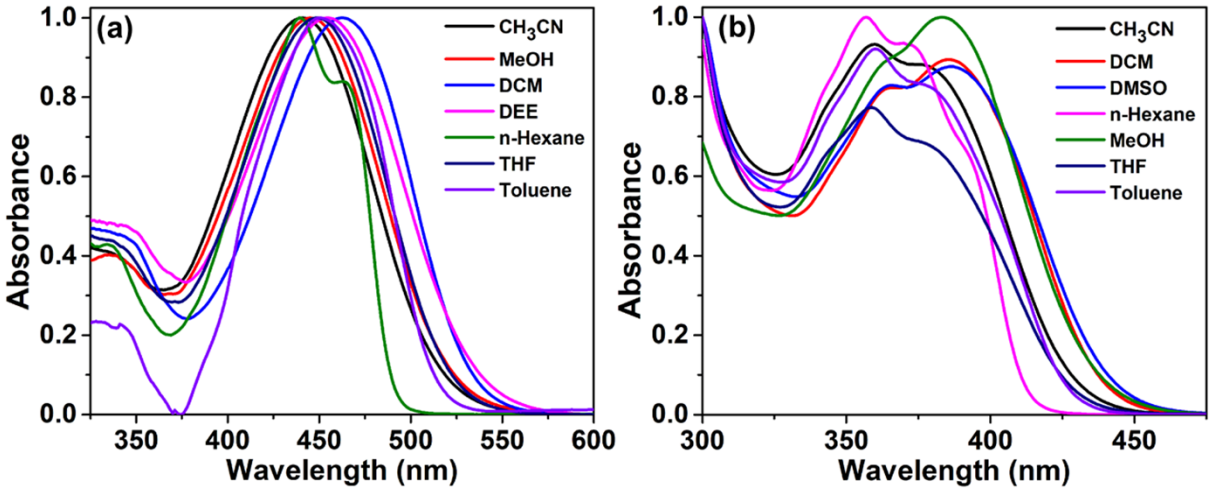


**Figure S4**: Solvent-dependent emission spectra of (a) CM and (b) CA (5 μM)

## 5. pH dependent study:

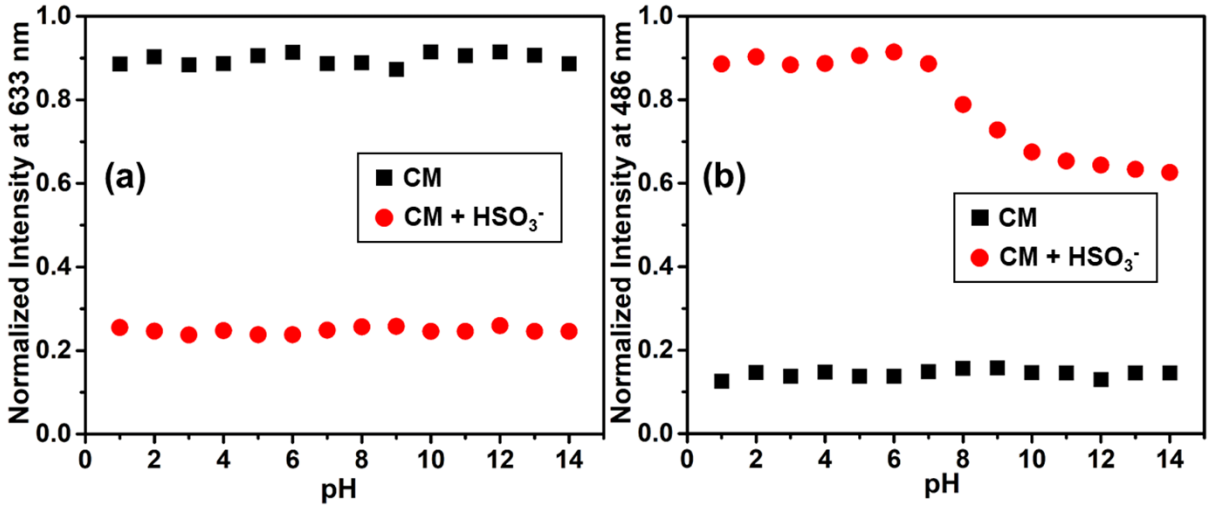


**Figure S5**: Fluorescence response of only CM and CM + $HSO_3^-$ at (a) 633 nm and (b) 486 nm as a function of pH in MeOH/ $H_2O$ (1/ 1, *v/v*), pH is adjusted by using aqueous solutions of 1 M HCl or 1 M NaOH. [HQCN] = 10 μM, [$HSO_3^-$] = 50 μM. $\lambda_{ex}$ = 400 nm.

## 6. *CM and CA as viscosity sensor*

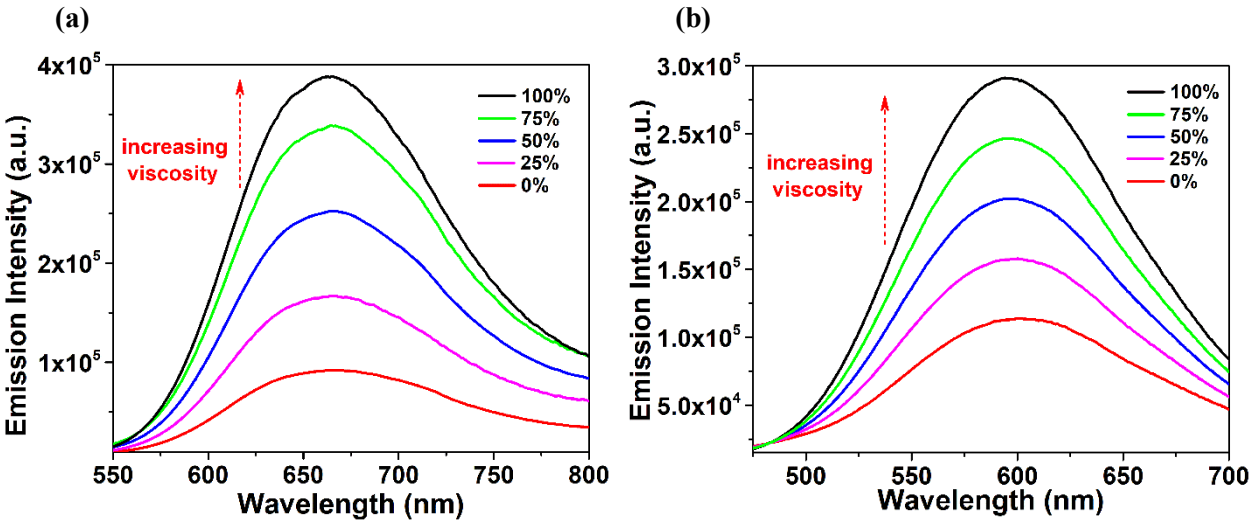


**Figure S6:** Fluorescence spectra of (a) CM and (b) CA in Mehanol-PBS-glycerin mixture with different volume fractions of glycerol (10 μM; λex = 530 nm and 480 nm respectively for CM and CA)

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

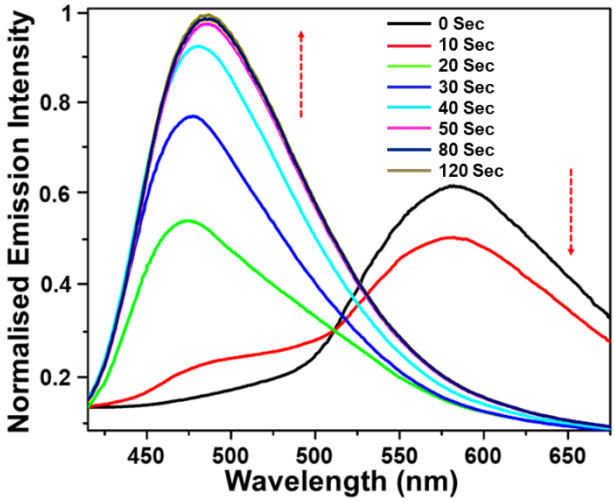


**Figure S7:** Change of emission spectra of CM (10 μM) upon addition of $HSO_3^-$ (2 equivalents)

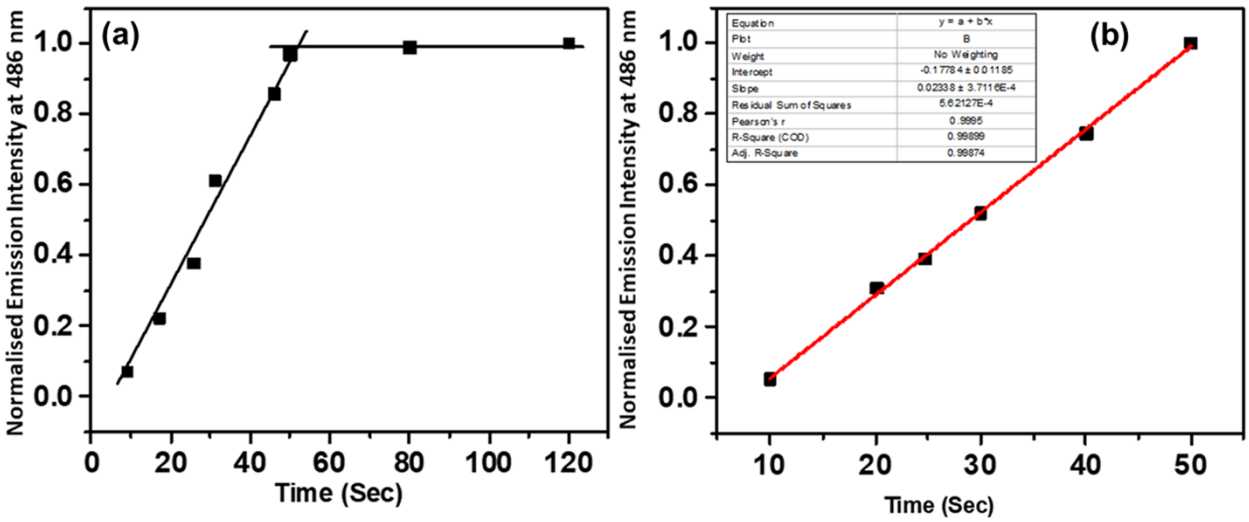


**Figure S8:** Time dependent fluorescence spectra of CM after interaction $HSO_3^-$ with time.

## 8. $^1$H NMR spectrum of CM

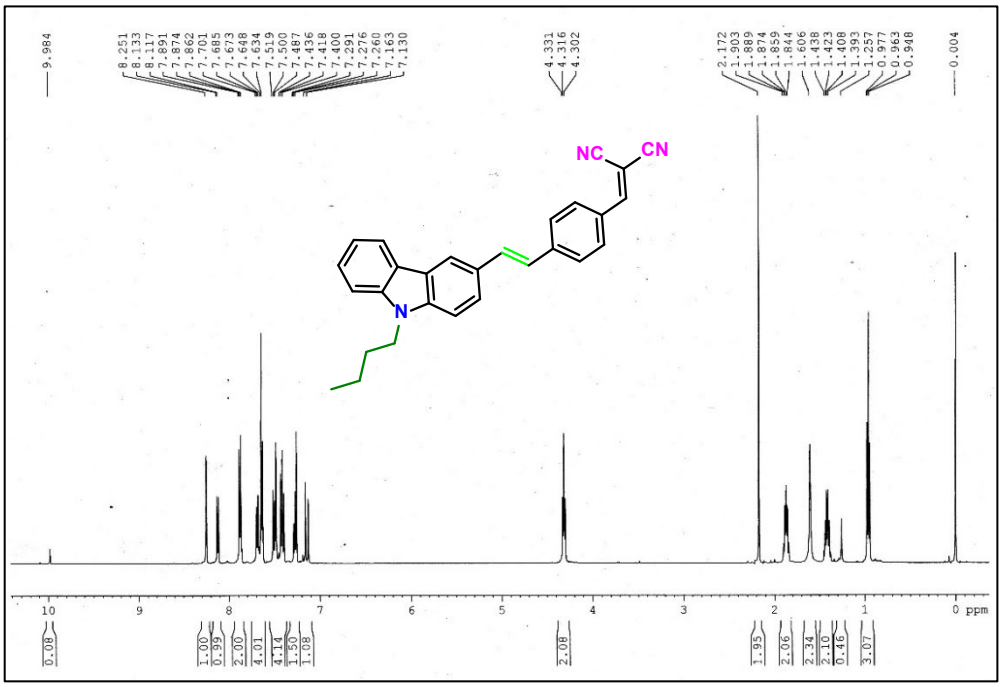


**Figure S9:** $^1$H NMR (500 MHz) spectrum of CM in $CDCl_3$

## 8. $^{13}$C NMR spectrum of CM

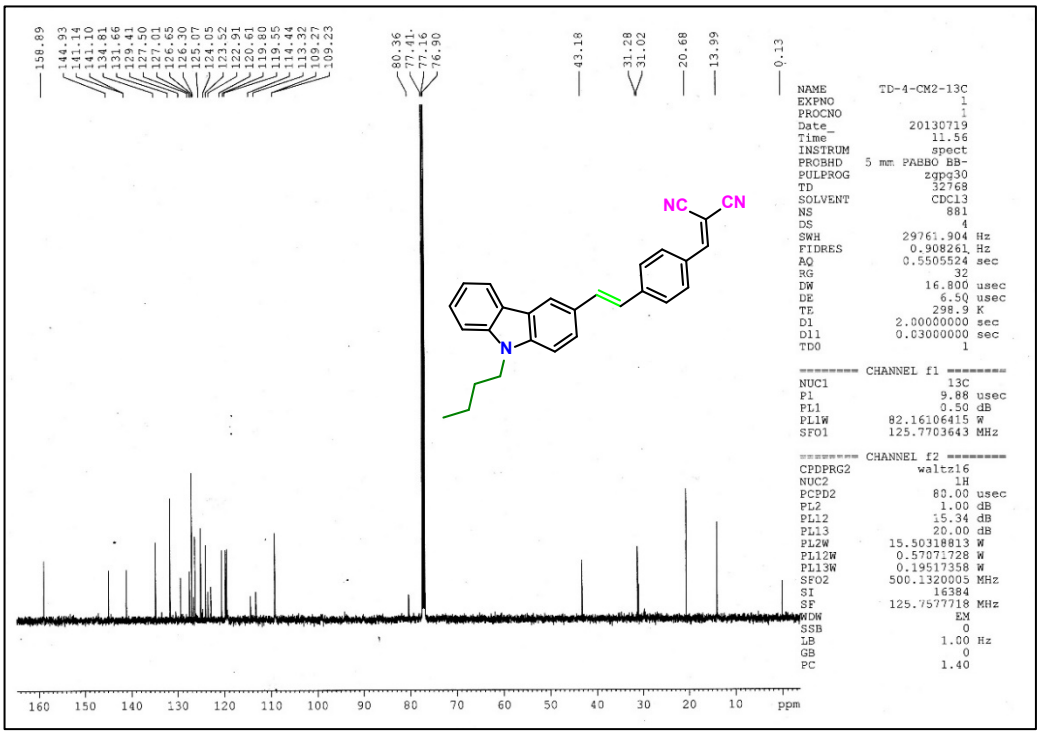


**Figure S10:** $^{13}$C NMR (125 MHz) spectrum of CM in $CDCl_3$

## 9. Mass spectrum (HRMS) of CM

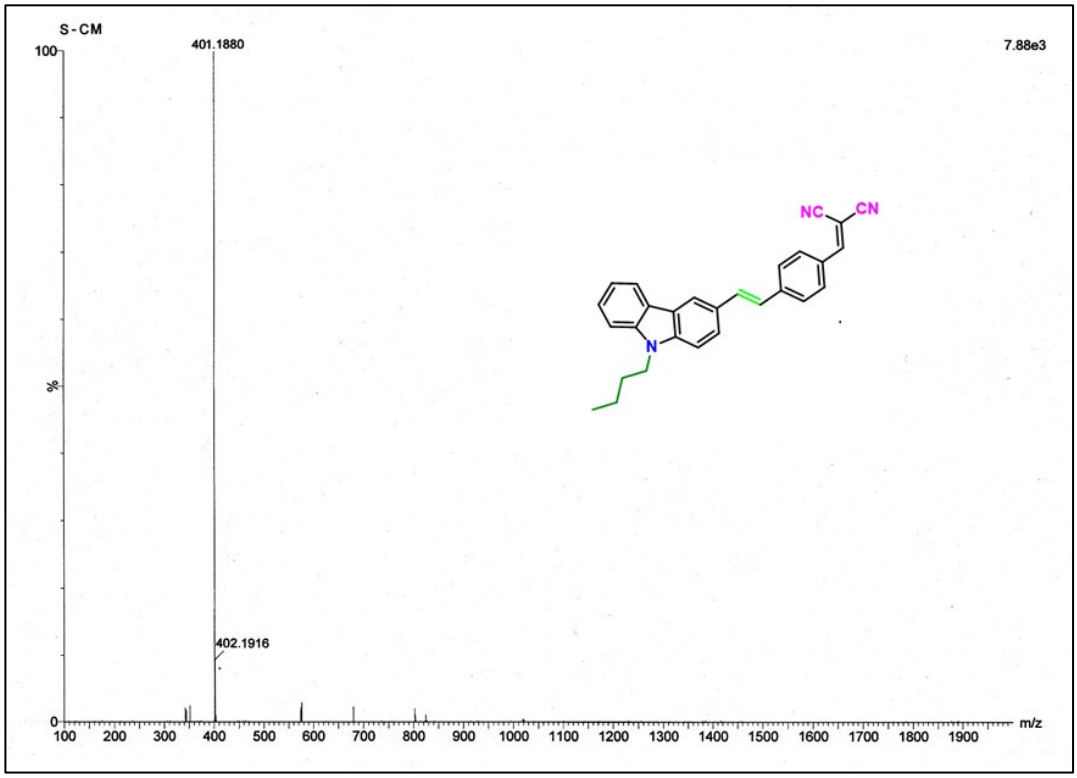


**Figure S11:** HRMS of CM**.**

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

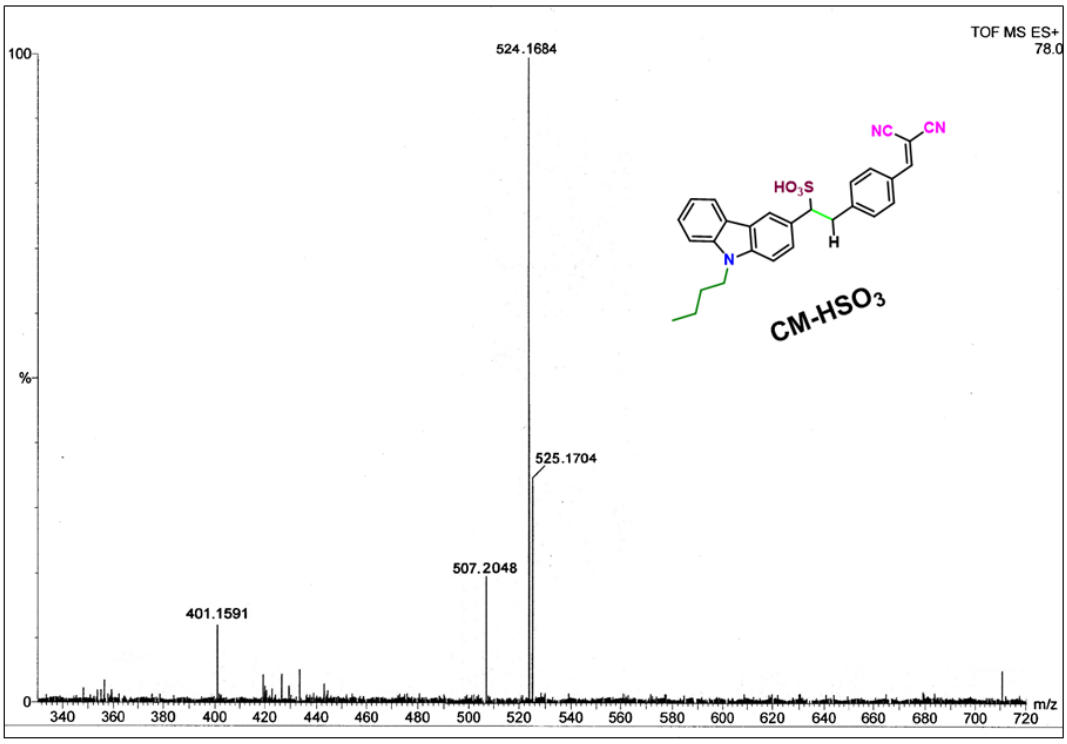


**Figure S12:** HRMS of CM-$HSO_3$ Complex.

## 11. $^1$H NMR spectra of (CM with $HSO_3^-$)

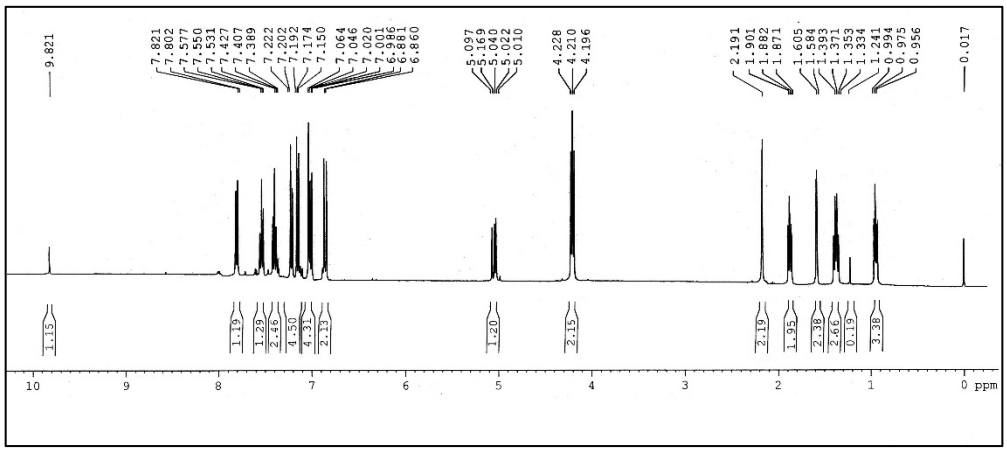


**Figure S13:** $^1$H NMR (400 MHz) spectra of [**CM** + **$HSO_3^-$**] in $CDCl_3$.

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

| Entry | $\Phi$ | 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<br>6 | 11.35<br>12.10 | 99.12<br>97.18 |
| Sample 2 | 4.50 | 3<br>4 | 7.32<br>8.31 | 97.6<br>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 $1.21 \times 10^{-8}$ M | Yes | Yes | Yes | Present Work |