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Supporting information

Kumujian-C based highly selective fluorescence turn-on probe enables detection of sulfite in real samples and living cells

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Experimental section

1. Synthesis

Synthetic procedure of 1-(dimethoxymethyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-*b*]indole-3-carboxylic acid (A): A mixture of L-tryptophan (1 g, 4.9 mmol) and 2,2-dimethoxy acetaldehyde (60% aq. solution in water, 1 mL) was dissolved in (5 ml) dry DCM and stirred for 5-10 min at room temperature. After stirring, a solution of TFA (1 ml) in DCM (10 ml) was added dropwise through pressure equalizing funnel and the reaction mixture was stirred at room temperature for 2 h. The completion of the reaction was confirmed by TLC and excess DCM was evaporated under reduced pressure. The residue was poured into ice cold water and quenched by slow addition of saturated $NaHCO_3$ solution under stirring. The pH was carefully adjusted to slight alkaline and the precipitation of product was observed which was filtered through sintered funnel and washed with cold water and dried in vacuum. Recrystallised with ethanol to yield the pure product **A** as white crystals.[1] Yield: 1.2 g (85 %); ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 10.22 (s, 1H), 7.40 (t, J = 9.0 Hz, 2H), 7.03 (t, J = 7.0 Hz, 1H), 6.40 (t, J = 7.5 Hz, 1H), 4.47 (d, J = 6.5 Hz, 1H), 4.30 (d, J = 6.5 Hz, 1H), 3.60 – 3.58 (m, 1H), 3.44 (s, 6H), 2.99 (d, J = 15.0 Hz, 1H), 2.64 (t, J = 13.5 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ (ppm) 173.4, 136.8, 130.9, 126.7, 121.5, 118.9, 118.0, 112.2, 108.4, 105.6, 56.3, 55.7, 55.3, 54.4, 24.9. HRMS calculated for $\mathbf{C_{15}H_{18}N_2O_4}$ $(M+H)^+$: 291.1339, found 291.1317.

Synthetic procedure of 1-(dimethoxymethyl)-9H-pyrido[3,4-*b*]indole (B): To a mixture of compound **A** (1 g, 3.4 mmol) and triethyl amine (1.3 ml, 8.5 mmol) in anhydrous DMF, a solution of N-chlorosuccinimide (1 g, 6.8 mmol) in DMF was added drop wise through dropping funnel over 15 min and the reaction mixture was stirred for 30 min in room temperature. After completion of the reaction confirmed by TLC, the reaction mixture was quenched with ice cold water. The content was extracted with ethyl acetate and washed with saturated aqueous $NaHCO_3$ solution. The combined organic layer was washed with water, brine solution, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The resulting crude product was successfully purified by silica gel (230-400 mesh) column chromatography using petroleum ether/ethyl acetate (3:2) as the eluent to afford the pure product **B** as yellowish liquid. [1] Yield: 658 mg (80 %); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 9.30 (s, 1H), 8.43 (d, J = 5.0 Hz, 1H), 8.09 (d, J = 5.0 Hz, 1H), 7.92 (d, J = 5.0 Hz, 1H), 7.54 – 7.49 (m, 2H), 7.25 (t, J = 9.0 Hz,

1H), 5.76 (s, 1H), 3.49 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 140.3, 139.9, 137.8, 133.4, 130.1, 128.5, 121.5, 120.9, 119.8, 114.9, 111.5, 106.2, 54.1. HRMS calculated for $\mathbf{C_{14}H_{14}N_2O_2}$ $(M+H)^+$: 243.1128, found 243.1138.

Synthetic procedure of Kumujian C (Kum-C): A solution of **B** (650 mg, 2.7 mmol) and iodine (68.5 mg, 0.27 mmol) in anhydrous acetone was stirred for 2 h in a teflon capped pressure tube. After completion of the reaction (monitored by TLC), acetone was removed under reduced pressure and the resulting mixture was successively worked-up using aqueous solution of sodium thiosulfate pentahydrate and ethyl acetate. The organic phase was then dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting crude product was purified by silica gel (230-400 mesh) column chromatography using petroleum ether and ethyl acetate as the eluent to afford the pure Kumujian C as yellowish solid.[2] Yield: 480 mg (91%); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 10.37 (s, 1H), 10.09 (s, 1H), 8.65 (d, J = 4.5 Hz, 1H), 8.19–8.17 (m, 2H), 7.65–7.29 (m, 2H), 7.37 (t, J = 7.5 Hz, 1H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 196.1, 141.6, 140.0, 136.3, 135.6, 132.0, 130.0, 122.3, 121.5, 120.8, 119.7, 112.4. HRMS calculated for $\mathbf{C_{12}H_8N_2O}$ $(M+H)^+$: 197.0709, found 197.0561.

2. Spectroscopic studies

UV-Vis spectroscopic studies were carried out with a spectrophotometer (Model: UV 2700, Shimadzu, Japan) and steady-state fluorescence measurements were executed in a spectrofluorometer (Model: Fluoromax-4, Horiba, Japan). In both the cases the sample were kept in a 10 mm x 10 mm quartz cuvette. In all the SS fluorescence studies the dye samples were excited at 295 nm, to avoid any changes in optical density of the samples upon formation sulfite adduct. Time Correlated Single Photon Counting (TCSPC) spectrometer purchased from Edinburgh Instruments, U.K was utilized to monitored the time-resolved fluorescence decays traces for the samples where a 405 nm pulsed diode laser (EPL-405, pulse width ~60 ps with repetition rate of 10 MHz) was used for sample excitation and the fluorescence decays were recorded at 458 nm maintaining a magic angle (54.7 ^{o}C) configuration to circumvent any effect associated with rotational diffusion or anisotropy. Light scattered from aqueous silica suspension (ludox) provided the instrument response function (IRF) having the full-width at half-maximum (FWHM) ~290 ps.

Analysis of the decay traces were performed with a multi-exponential function, having the following form,[3]

$$I(t) = I(0)\sum \alpha_i \exp(-t/\tau_i) \quad \text{(S1)}$$

The calculations of the average excited-state lifetime were carried out using the equation,[3]

$$<\tau> = \sum A_i \tau_i \text{ where, } \quad A_i = \alpha_i \tau_i / \sum \alpha_i \tau_i \quad \text{(S2)}$$

NMR spectroscopic studies were carried out in 500 MHz Varian FT-NMR instruments (500 MHz). Multiplicity of 1H NMR signals have been abbreviated as, s = singlet, d = doublet, dd = doublet of doublet, m = multiplet. J values, which denoted the proton coupling constants, are expressed in Hertz (Hz).

3. Procedure of sulfite detection in real samples

Water samples collected from different sources (tap water of three different places at different times). The collected water samples were diluted to 50% by adding equal volume of 10 mM phosphate buffer, pH ~7.4. Then known concentrated stock of Kum-C was added into it to make dye concentration ~15 μM. Afterwards, known concentrations of sulfite were added into the different water samples and the concentrations were estimated by standard addition method.

For the sugar sample 727 mg sugar, obtained from local super market was dissolving in 6.6 ml phosphate buffer (10 mM, pH ~7.4) to make ~110 mg/ml sugar solution. Then 48 μl Kum-C (1.6 mM in MeOH) added to 5 ml of the sugar solution to make ultimate dye concentration ~15 μM. A known concentration of sulfite was added into it which was afterwards determined using standard addition method. Same protocol was followed for the salt solution too. In this case concentration of salt was ~100 mg/ml.

4. Cytotoxicity assay with MTT and Fluorescence imaging in live cell

MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide] assay was carried out to evaluate the cytotoxicity of Kum-C dye on U2OS cells. The cells were seeded in 96-well plate (6000 cells/well) followed by Kum-C dye treatment with increasing concentrations, ranging between 0 μM to 100 μM, and the cells were incubated for 24 hours in complete media. Then,

the dye containing media was removed and 100 µl of MTT solution (0.5 mg/ml) was added with 2 hours incubation. The formazan crystals formed, were solubilised with DMSO and the OD values were taken with Omega Polar star Multiplate reader.

For fluorescence imaging of the live cells, U2OS cells were pre-incubated with 200 µM Na_2SO_3 for 30 minutes in complete media, followed by PBS (phosphate buffer saline) wash. Then, the cells were treated with 100 µM Kum-C dye for 15 minutes followed by PBS wash. The cells were then take for image acquisition using Zeiss confocal microscope and the intracellular SO_3^{2-} detecting capability of the Kum-C was assessed. A 355 nm laser was used for excitation, whereas 488 nm filter was used for detection, the detection spectral limit was set as 444 nm to 593 nm.

Equation of binding constant or binding affinity of Kum-C towards SO_3^{2-}

$$\Delta I_f = \Delta I_f^{\infty}\left(1-\frac{\{K_b[Dye]_0 - K_b[H]_0 - 1\} + \sqrt{(K_b[Dye]_0 + K_b[H]_0 + 1)^2 - 4(K_b)^2[Dye]_0[H]_0}}{2[Dye]_0 K_b}\right) \quad \text{(S3)}$$

Where, $\Delta I^{\infty}_{Dye.H}$ is the ultimate change in fluorescence intensity on formation of the sulfite adduct, $[Dye]_0$ is the total dye concentration used and $[H]_0$ is the total sulfite (Na_2SO_3) concentration used in the solution at any given stage.

Determination of quantum yield

The quantum yield (φ) of Kum-C was estimated by comparing the integrated emission intensities of Kum-C with that of quinine sulfate in 0.5 M H_2SO_4 using the following equation,

$$\varphi = \varphi_{QN} \times \frac{FA_{Kum-c}}{FA_{QN}} \times \frac{A_{QN}}{A_{Kum-c}} \times \left(\frac{n_{Kum-C}}{n_{QN}}\right)^2 \quad \text{(S4)}$$

where, φ_{QN} is Fluorescence quantum yield of quinine sulfate in 0.5 (M) H_2SO_4 = 0.54;[4] A denotes the absorbance of the solution, FA is the integrated area under the fluorescence spectrum at the excitation wavelength (λ_{ex} = 295 nm) and n is the refractive index of the solvent.

^{1}H and ^{13}C NMR

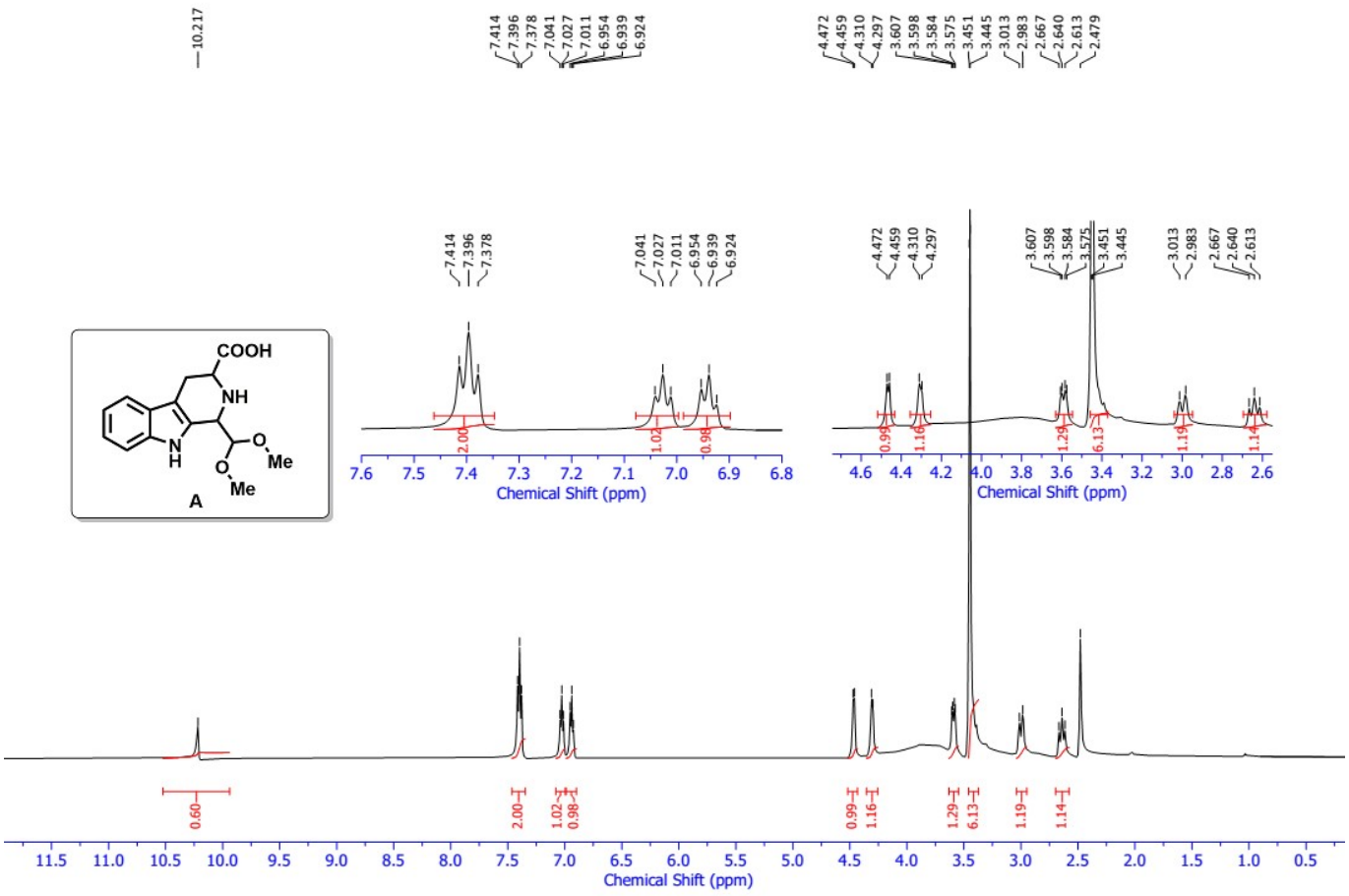


Figure S1. ^{1}H NMR of compound A in DMSO-d_6.

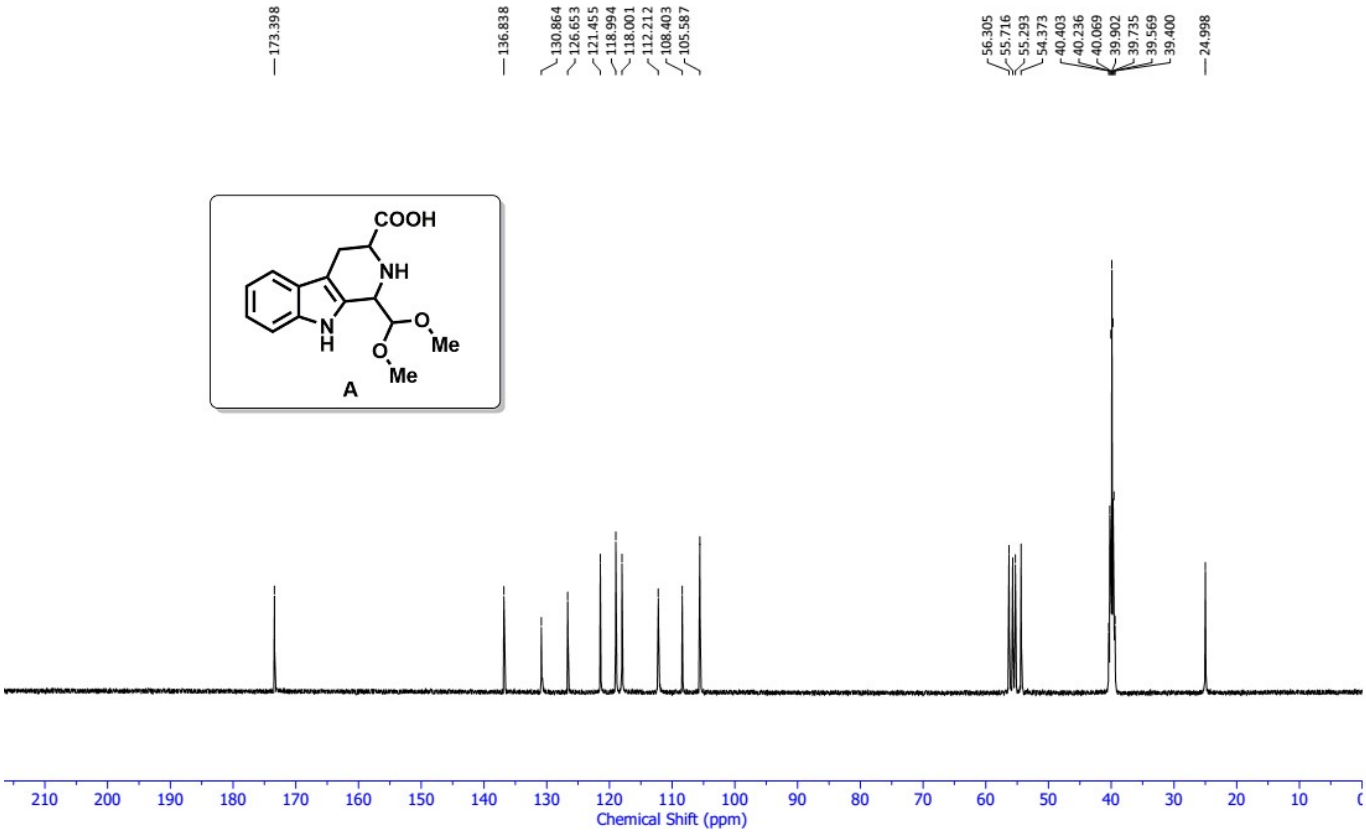


Figure S2. ^{13}C NMR of compound A in DMSO-d_6.

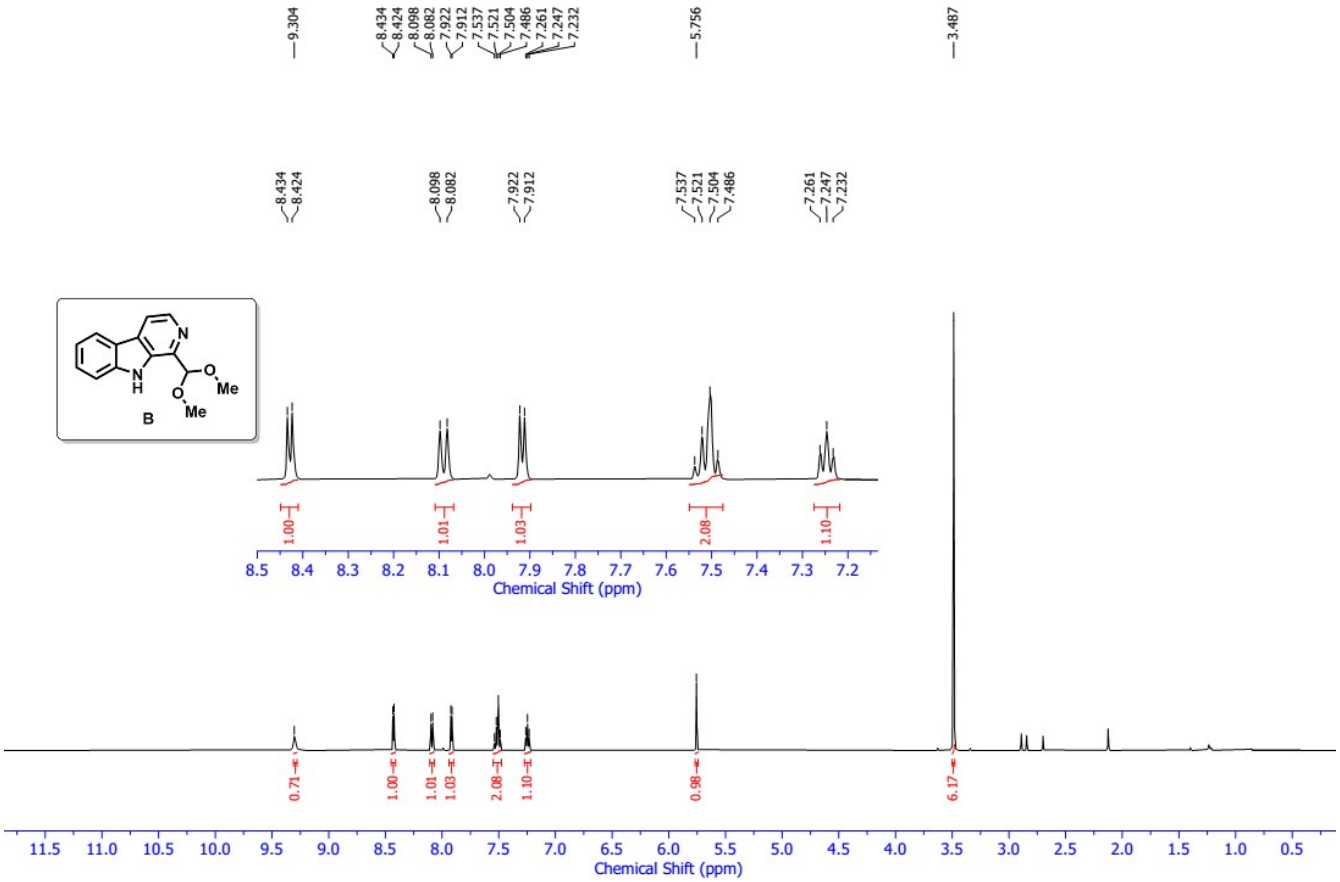


Figure S3. ^{1}H NMR of compound B in $CDCl_3$.

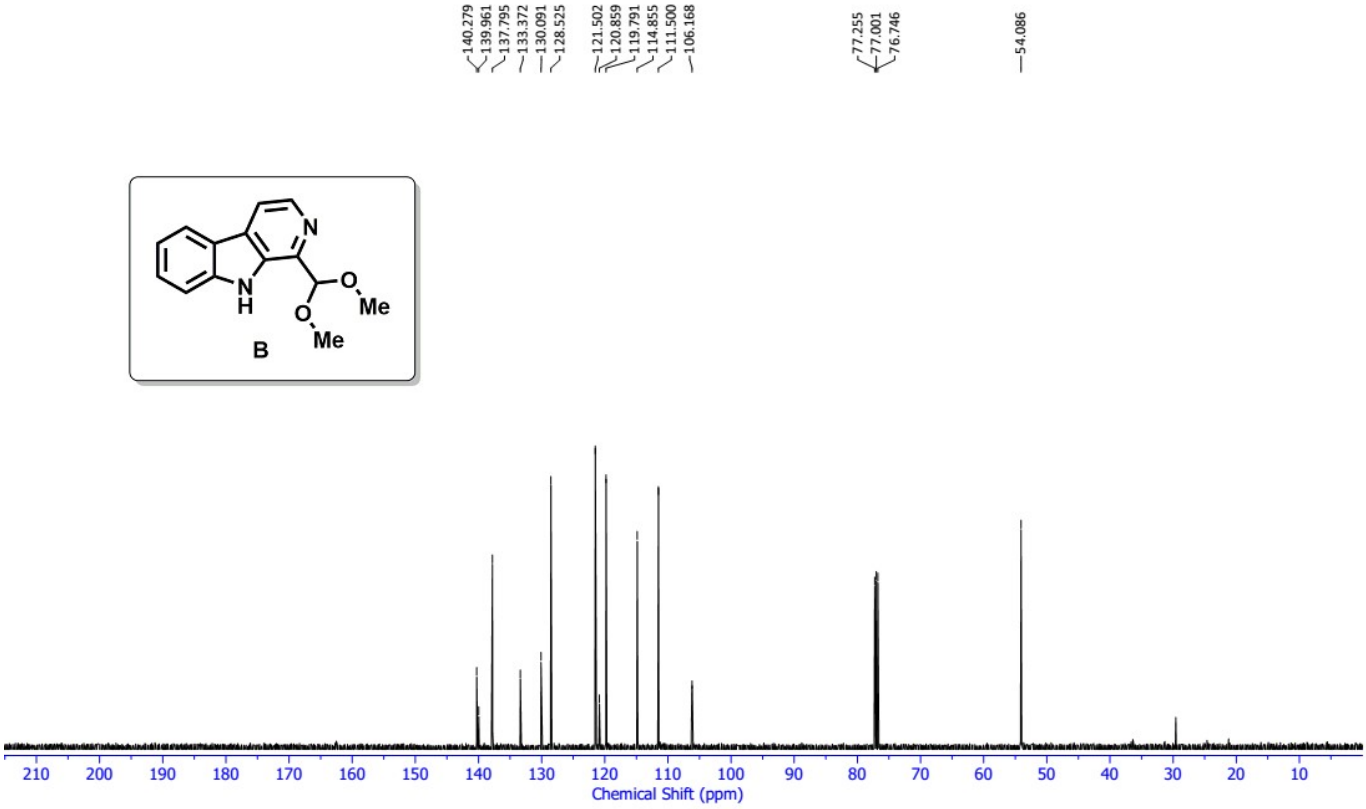


Figure S4. ^{13}C NMR of compound B $CDCl_3$.

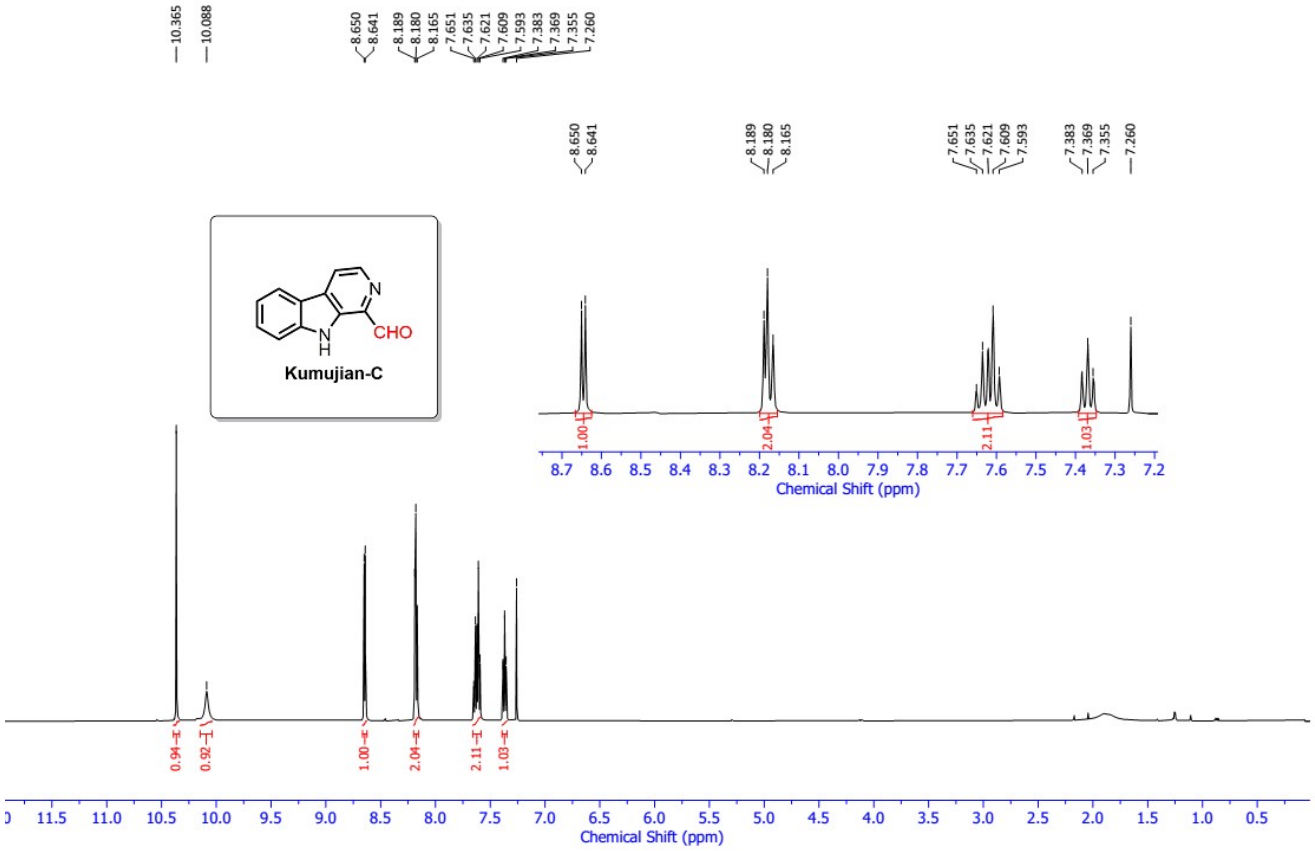


Figure S5. ^{13}C NMR of Kum-C in $CDCl_3$.

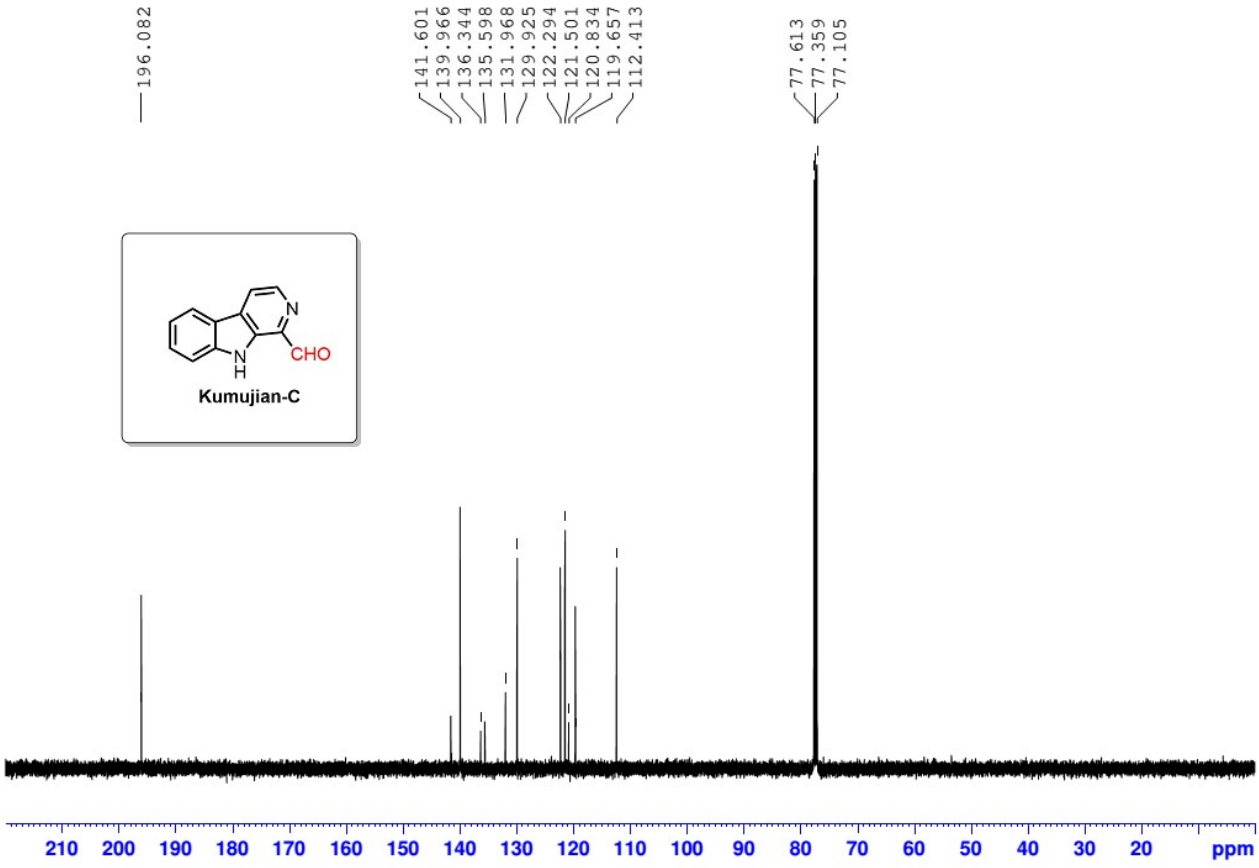


Figure S6. ^{13}C NMR of Kum-C in $CDCl_3$.

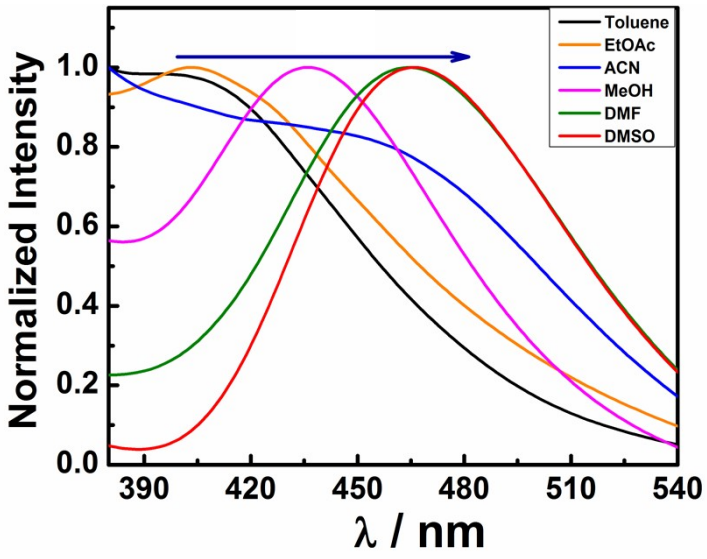
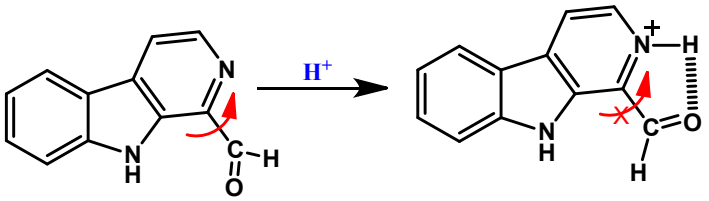


Figure S7. Solvent dependent normalized emission plot of Kum-C. λ_{ex} = 295 nm.



Restriction of -CHO group about C-C bond due to the formation of intramolecular H-bond

Figure S8. Intramolecular H-bond formation in the protonated form of Kum-C.

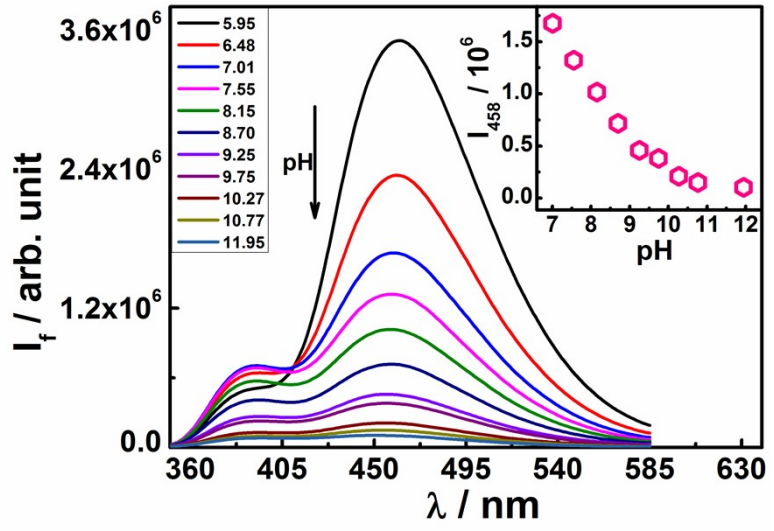


Figure S9. pH dependent emission spectra of Kum-C-sulfite adduct (15 μM Kum-C+1.5 mM Na_2SO_3) in water. Inset: Change in emission intensity at 458 nm for Kum-C-sulfite adduct with pH of the solution. λ_{ex} = 295 nm.

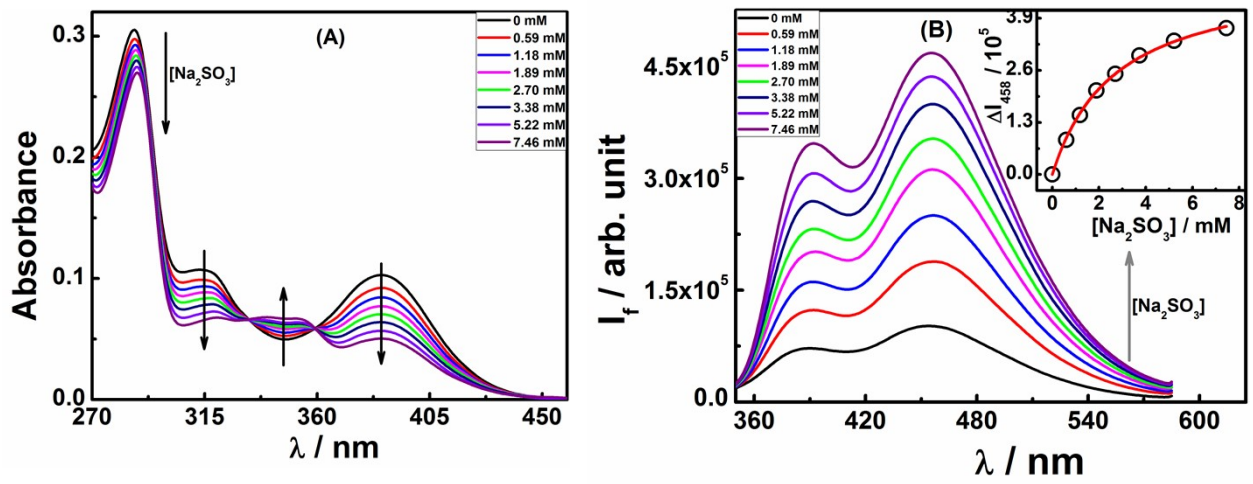


Figure S10. Modulations in **(A)** absorption and **(B)** emission spectrum of Kum-C (15 μM) in response to the changing concentration of sodium sulfite (from 0 to 7.64 mM) in 10 mM bicarbonate-carbonate buffer (pH ~9.2), λ_{ex} = 295 nm. Inset: Non-linear fitting of the binding isotherm of Kum-C with sulfite. Binding constant (K_b) = (3.69.3±0.15) x 10^2 M^{-1}.

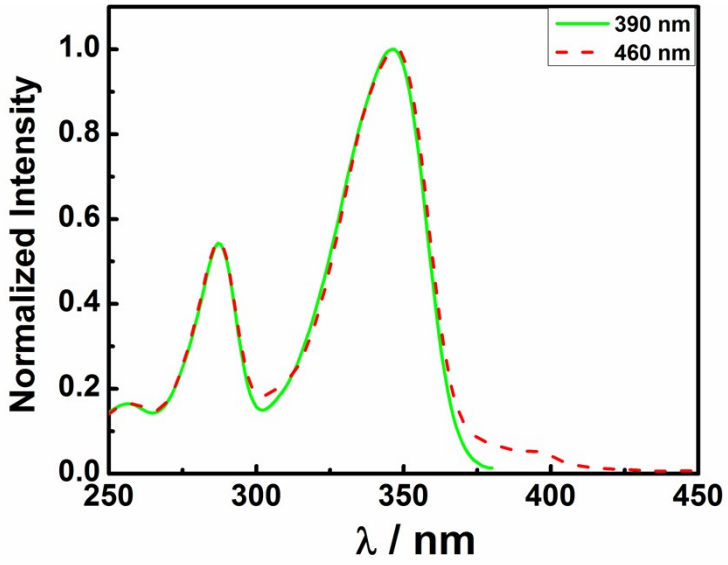


Figure S11. Excitation spectra of Kum-C in 10 mM phosphate buffer (pH ~7.4) keeping emission wavelengths at 390 nm and 460 nm, respectively.

Discussion: Whether only one individual compound absorbs light in solvents and in water, in absence of HSO_3^-; we recorded the excitation spectra for the dye fixing its emission at 390 nm and 460 nm, respectively. The excitation spectra at both the emission wavelengths are found to

be the same, which explicitly indicate that only one individual compound absorbs light in solvents and in water, in absence of HSO_3^-.

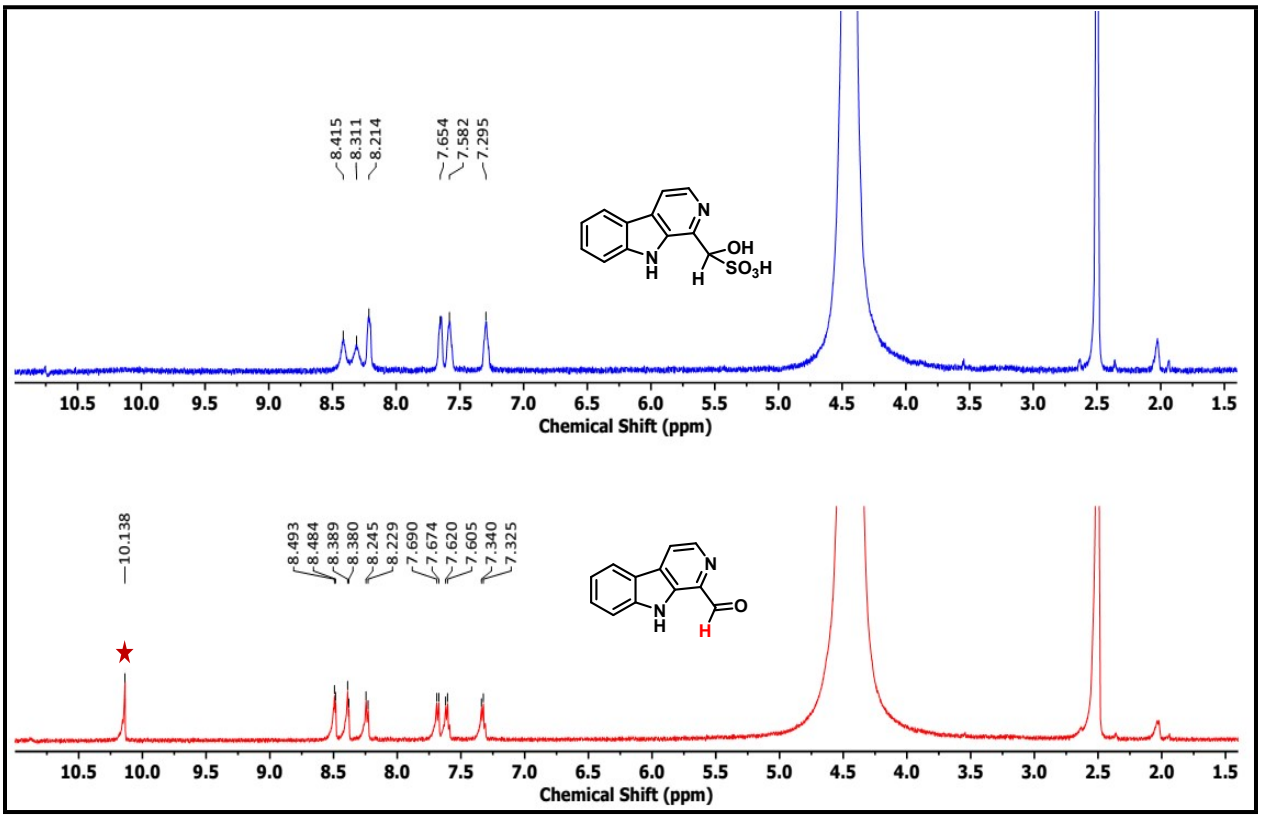


Figure S12. 1H NMR spectra of Kum-C (below) and its adduct (above) in a mixture of DMSO-d_6 and D_2O (at 45% DMSO-d_6 and 55% D_2O). [Kum-C] = 1.5 mM and [Na_2SO_3] = 2.5 mM. Peak 2.65 ppm and ~4.69 ppm indicate solvent peaks of DMSO-d_6 and D_2O respectively. Star marked peak is the signal for the proton of the aldehyde group which is absent in the adduct.

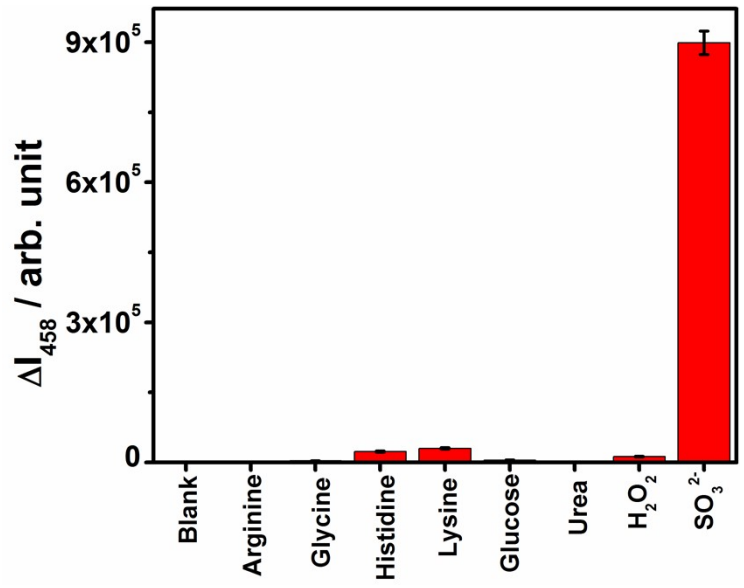


Figure S13. Relative fluorescence responses of Kum-C (15 μM) towards various analytes (1.5 mM) in buffer. Error bar indicates standard deviation, which is within 2% of the reported readings.

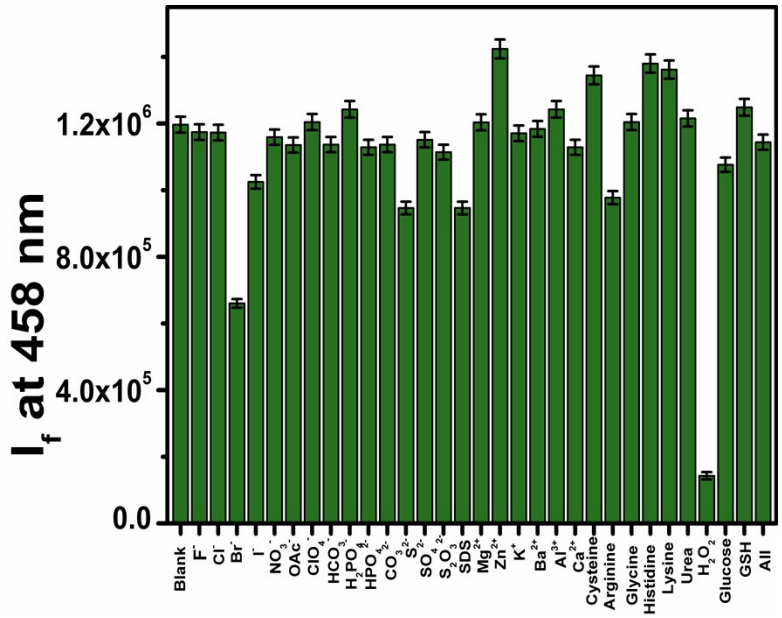


Figure S14. Relative fluorescence responses of Kum-C-sulfite adduct (15 μM Kum-C + 1.5 mM Na_2SO_3) towards various analytes (1.5 mM) in buffer.

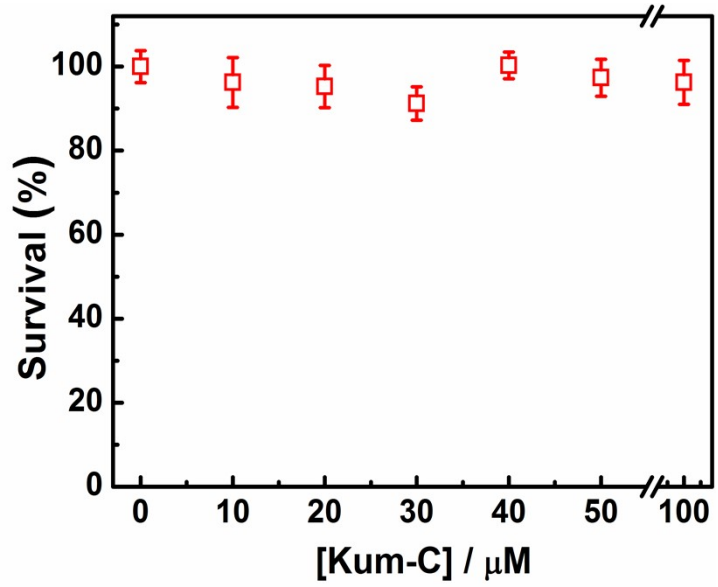


Figure S15. Plot of cell survival against concentration of Kum-C obtained from MMT assay after incubation for 24 hours.

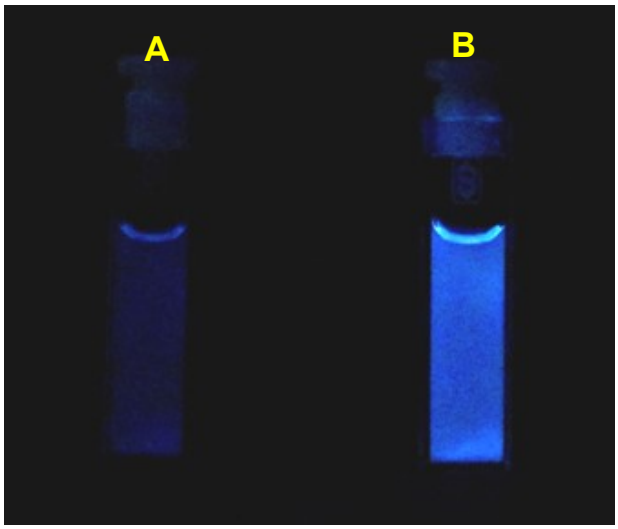


Figure S16. Photographic image of **(A)** Kum-C and **(B)** Kum-C-sulfite adduct under UV illumination (Concentration of Kum-C = 15 μM and Na_2SO_3 = 1.5 mM, λ_{irr} = 254 nm).

References

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