Electronic Supplementary Information (ESI)

A Solo Fluorogenic Probe for Real-time Sensing of SO$_3^{2-}$ and SO$_4^{2-}$/ HSO$_4^-$ in Aqueous Medium and Live Cells by Distinct Turn-On Emission Signals

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■ EXPERIMENTAL SECTION:

General Information and Materials: All the materials for synthesis were purchased from commercial suppliers and used without further purification. The absorption spectra were recorded on a Perkin-Elmer Lamda-25 UV−Vis spectrophotometer using 10 mm path length quartz cuvettes in the range of 300–800 nm wavelength, while fluorescence measurements were performed on a Horiba Fluoromax-4 spectrofluorometer using 10 mm path length quartz cuvettes with a slit width of 3 nm at 298 K. The mass spectrum of the ligand L was obtained using Waters Q-ToF Premier mass spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Advance 600 MHz instrument. The chemical shifts were recorded in parts per million (ppm) on the scale. The following abbreviations are used to describe spin multiplicities in $^1$H NMR spectra: s = singlet; d = doublet; t = triplet; q= quartet, m = multiplet.

Synthesis of the probe L: 10mmol (2.09g) of 1,1,2-Trimethylbenz[e]indole was dissolved in 50 mL of acetonitrile and 12 mmol of iodoethane (1.87g) was added to this solution. The mixture was refluxed for 10 h and the resultant solution was then kept for slow evaporation to get crystalline solid product of 3–ethyl–1,1,2–trimethyl–1H–benzo[e]indol–3–ium (first step of the Scheme S1); which was then filtered and washed with cold methanol.

Scheme S1: Design and synthesis of probe L

In the next step, 1.0 mmol of this 3–ethyl–1,1,2–trimethyl–1H–benzo[e]indol–3–ium was dissolved in dry EtOH and 1.1 mmol of 4-(Dimethyl-amino)cinnamaldehyde was added to it. Resultant mixture was then refluxed for 12 h to yield the dark blue colour product.
Calculated yield: 64%. \(^1\)H NMR [600 MHz, CD\(_3\)OD, J (Hz), \(\delta\) (ppm)]: 8.41-8.26 (3H, m), 8.17 (1H, d, J=7.8), 8.12(1H, t= 7.8), 7.85 (1H, d, J=9.0), 7.75 (1H, d, J=7.2), 7.66 (2H, d, J=8.4), 7.33 (1H, t, J=10.2), 7.00-6.93 (2H, m), 6.83 (1H, t, J=7.8), 4.89 (s, H\(_2\)O in CD\(_3\)OD), 4.58 (1H, d, J=6.6), 3.62 (2H, q, J=6.6), 3.31 (6H, s), 3.15 (m, CD\(_3\)OD), 2.03 (6H, s), 1.19 (3H, t, J=7.2).

\(^{13}\)C NMR [150 MHz, CDCl\(_3\), TMS, \(\delta\) (ppm)]: 179.34, 156.14, 153.33, 138.32, 137.09, 133.19, 132.82, 131.55,130.41, 128.48, 127.90, 126.75, 124.89, 123.99, 122.67, 112.40, 111.92, 111.62, 110.72, 52.82, 42.60, 40.37, 27.43, 14.11. ESI-MS (positive mode, m/z) Calculated for C\(_{28}\)H\(_{31}\)N\(_2^+\): 395.2487. Found: 395.2507.

**UV–Vis and fluorescence spectroscopic studies:** Stock solutions of various analytes (1 × 10\(^{-1}\) mol·L\(^{-1}\)) were prepared in methanol or Millipore water depending upon the preferred solubility of the analytes chosen. Na or (NH\(_4\))\(_4\) or tetrabutyl ammonium (TBA) salts of various anions were chosen for the selectivity experiments which encompassed TBAF, TBACL, TBABr, TBAI, NaNO\(_2\), TBANO\(_3\), TBA(OAc), TBAH\(_2\)PO\(_4\), NaPpi, TBAHCO\(_3\), (TBA)_\(_2\)SO\(_4\), TBAHSO\(_4\), TBAPF\(_6\), TBAClO\(_4\), NH\(_4\)SCN, Na\(_2\)S\(_2\)O\(_3\), Na\(_2\)S\(_2\)O\(_8\), NaC\(_6\)H\(_7\)O\(_6\) (Na-ascorbate), Na\(_2\)SO\(_3\) and NaSH. A stock solution of \(L\) (5 × 10\(^{-3}\) mol·L\(^{-1}\)) was prepared in DMSO. The solution of \(L\) was then diluted to 10 × 10\(^{-6}\) mol·L\(^{-1}\) with Millipore water for spectral studies by taking only 4 µL stock solution of \(L\) and making the final volume 2mL adding Millipore water. In fluorescence selectivity experiment, the test samples were prepared by placing appropriate amounts of the stock solutions of the respective anions/analytes into 2.0 mL of probe solution (10 × 10\(^{-6}\) mol·L\(^{-1}\)). For UV-Visible and fluorescence titration experiments another sets of anions (SO\(_3^{2-}\) and SO\(_4^{2-}\)) standard solutions having 5mM concentrations were prepared by diluting the earlier prepared stock solutions (1 × 10\(^{-1}\) mol·L\(^{-1}\)) in Millipore water. Quartz optical cells of 1 cm path length were filled with 1.0 mL and 2.0 mL solutions of \(L\) for UV-Visible and fluorescence titration experiments respectively, to which the 5mM anions (SO\(_3^{2-}\) and SO\(_4^{2-}\)) stock solutions were gradually added using a micropipette. For fluorescence measurements, excitation was provided at 380 nm, and emission was acquired from 400 nm to 650 nm for usual selectivity and titration experiments. However to ascertain the ratio-metric fluorescence change in case of titration experiment with SO\(_3^{2-}\), another excitation was provided at 580 nm, and emission was acquired from 630 nm to 800 nm. Spectral data were recorded within 1 minute after addition of the analytes.
**Detection Limit:** The detection limits were calculated on the basis of the fluorescence titration. The fluorescence emission spectrum of \( L \) was measured 10 times, and the standard deviation of blank measurement was achieved. To gain the slope, the fluorescence emission at 442 nm was plotted as a concentration of \( \text{SO}_3^{2-} \) and the fluorescence emission at 511 nm was plotted as a concentration of \( \text{SO}_4^{2-} \). The detection limits were calculated using the following equation:

\[
\text{Detection limit} = \frac{3\sigma}{k} \quad (1)
\]

where \( \sigma \) is the standard deviation of blank measurement, and \( k \) is the slope between the fluorescence emission intensity versus \([\text{SO}_3^{2-}]\) or, emission intensity versus \([\text{SO}_4^{2-}]\).

**Dynamic light scattering studies:** The particle size of \( L \), \( L-\text{SO}_4^{2-} \) aggregate were measured by dynamic light scattering (DLS) experiments on a Malvern Zetasizer Nano ZS instrument equipped with a 4.0 mW He–Ne laser operating at a wavelength of 633 nm. The samples and the background were measured at room temperature (25°C). DLS experiments were carried out with optically clear solutions of \( L \) (10 μM) in aqueous medium, both in the absence and presence of 20 equivalents of \( \text{SO}_4^{2-} \) to determine the change in particle size on interaction of \( L \) with \( \text{SO}_4^{2-} \). The solution was equilibrated for 30 minutes before taking the measurements.

**Atomic Force Microscope (AFM) Studies:** The overall morphology of the \( L-\text{SO}_4^{2-} \) aggregate was investigated from NT-MDT micro-40 AFM instrument using a semicontact mode at a scan rate of 1 Hz. To a solution of \( L \) (10 μM) in aqueous medium 20 equivalents of \( \text{SO}_4^{2-} \) was added and mixed well then it was drop-casted on a cover slip and left open to atmosphere for 12 h, followed by desiccation prior to acquiring AFM images.

**Cytotoxicity assay**

HeLa cells (human cervical carcinoma cells) were initially cultured in a 25 cm² tissue culture flask in DMEM medium supplemented with 10% (v/v) FBS, penicillin (100 μg/mL) and streptomycin (100 μg/mL) under a humidified atmosphere of 5% CO₂ until the cells were approximately 80% confluent. Prior to MTT assay, cells were trypsinized and seeded into 96 well tissue culture plates at \( 10^4 \) cells per well and incubated with varying concentrations (0.5 μM-50 μM) of the probe \( L \) made in DMEM and incubated for a period of 24 h under 5% CO₂. Solvent control samples (cells incubated in 0.1% methanol) were also included in separate sets. Following incubation, the growth media was carefully removed, and fresh DMEM containing
MTT solution was added to the wells. The plate was subsequently incubated for 4 h at 37°C. Following incubation, the supernatant was carefully collected and the insoluble colored formazan product was solubilized in DMSO and its absorbance was measured in a microtiter plate reader (Infinite M200, TECAN, Switzerland) at 550 nm. For each concentration of the test samples, MTT assay was performed in six independent sets. Data analysis and determination of standard deviation was performed with Microsoft Excel 2010 (Microsoft Corporation). In the MTT assay, the absorbance for the solvent control cells was considered as 100% cell viability and the absorbance for the treated cells was compared to ascertain % cell viability with respect to the solvent control. MTT assay was also conducted in separate set of experiments to determine the cytotoxic potential of \( \text{L-SO}_3^{2-} \) and \( \text{L-SO}_4^{2-} \) ensemble in cells. To this end, HeLa cells seeded into 96 well tissue culture plates at \( 10^4 \) cells per well and incubated with varying concentrations 0.5 μM -5.0 μM of the probe \( \text{L} \) made in DMEM for 30 min under 5% CO₂. Subsequently, sodium sulphite and sodium sulfate salts were added in separate sets to HeLa cells pre-incubated with \( \text{L} \) so as to achieve an \( \text{L} \)-sulfite and \( \text{L} \)-sulfate ensemble ratio of 1:5 and 1:10. The cells were then incubated for a period of 24 h under 5% CO₂ following which MTT assay was performed for the samples as mentioned before.

**Cell imaging studies**

HeLa cells were initially cultured in a 25 cm² tissue culture flask containing DMEM medium supplemented with 10% FBS, penicillin (100 μg/ mL) and streptomycin (100 μg/mL) in a CO₂ incubator at 37°C. Prior to performing cell imaging studies, HeLa cells were seeded into a 6 well plate and grown in DMEM medium at 37°C till 80% confluency in a CO₂ incubator. Subsequently, the cells were washed thrice with sterile phosphate buffered saline (PBS) and DAPI stain (6.0 μM in sterile PBS) was added to the cells and incubated for 5 min. The cells were again washed thrice with sterile PBS in order to eliminate excess DAPI stain. The cells were further incubated for 1 h in separate sets with 25 μM each of sodium sulfite and tetrabutylammonium sulfate (to achieve the
final ratio of 1:5 L-analyte ratio) prepared in sterile PBS. The images of the cells were again acquired with a fluorescence microscope. The colocalization of the images was performed using ImageJ software and the JACoP plugin.\textsuperscript{S1}

Reference:

\textbf{Figure S1.} \textsuperscript{1}H-NMR spectra of L in CD\textsubscript{3}OD
Figure S2. $^{13}$C-NMR spectra of L in CDCl$_3$

Figure S3. Mass spectrum of L

Calculated m/z = 395.2487

Obtained m/z = 395.2507
Figure S4. (A) UV-Visible spectra of L (10 µM) in aqueous medium in presence of excess of (20 equivalents) SO$_3^{2-}$ and SO$_4^{2-}$. (B) Visual changes in color of the solution of L in presence of different anions under day light; (C) UV-Visible spectra of L (10 µM) in presence of varying concentration of SO$_3^{2-}$; INSET: Changes in the absorbance at 581 nm with addition of equivalents of SO$_3^{2-}$.

Figure S5. Fluorescence spectra of L (10 µM) in presence of (SO$_3^{2-}$ + SO$_4^{2-}$) mixture with varying SO$_3^{2-}$ & SO$_4^{2-}$ concentrations; $\lambda_{ex} = 380$ nm.
**Figure S6.** Changes in the emission intensity of L at 442 nm with time upon interaction with SO$_3^{2-}$; $\lambda_{ex} = 380$ nm

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Figure S9: Fluorescence spectra of L (10 µM) in presence of varying concentration of SO$_3^{2-}$; INSET: Changes in the emission intensity at 695 nm with addition of equivalents of SO$_3^{2-}$; $\lambda_{ex} = 580$ nm.
Figure S10: Fluorescence intensity vs. concentration of $\text{SO}_3^{2-}$ plot for determination of detection limit.

$y = 2E+10x - 180437$

$R^2 = 0.9881$

Figure S11: Fluorescence intensity vs. concentration of $\text{SO}_4^{2-}$ plot for determination of detection limit.

$y = 4E+09x + 59890$

$R^2 = 0.999$
Figure S12: (A) UV-Visible spectra and (B) Fluorescence spectra ($\lambda_{ex} = 380$ nm) of L (10 $\mu$M) in different solvents.

Figure S13: Fluorescence spectra of L (10 $\mu$M) in presence of 20 equivalents of $SO_3^{2-}$ in different solvents; $\lambda_{ex} = 380$ nm.
Figure S14: Mass spectrum of L in presence of Na$_2$SO$_3$.

Calculated mass m/z = 498.1953
Obtained mass m/z = 498.2915

Figure S15: NMR spectra (400 MHz) of L and L+ SO$_3^{2-}$ in DMSO-d$_6$+D$_2$O (3:2; v/v) mixed solvent
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**Figure S18:** MTT assay to evaluate the cytotoxic effect of the probe L on HeLa cells.

**Figure S19:** MTT assay to evaluate the cytotoxic effect of the probe L-SO$_3$$^-$$^2$ ensemble on HeLa cells.
**Figure S20:** MTT assay to evaluate the cytotoxic effect of the probe $\text{L-SO}_4^{2-}$ ensemble on HeLa cells.

**Figure S21.** Co-localization coefficient (Pearson's coefficient) of (A) $\text{L-SO}_3^{2-}$ adduct (Pearson coefficient is 0.59) and (B) $\text{L-SO}_4^{2-}$ ensemble (Pearson coefficient is 0.82); based on green and blue channel emission.