Supporting Information
For
Substituent effect on fluorescence signalling of the HSO₄⁻ receptors through single point to ratiometric response in green solvent†

Manjira Mukherjee, a Siddhartha Pal, a Buddhadeb Sen, a Somenath Lohar, a Samya Banerjee, b Snehasis Banerjee c and Pabitra Chattopadhyay a*

Department of Chemistry, Burdwan University, Golapbag, Burdwan-713104, West Bengal, India
E-mail: pabitracc@yahoo.com

CONTENTS

1. Fig. S1 ¹H NMR spectrum of L₁H in DMSO-d6
2. Fig. S2 ¹H NMR spectrum of L₂H in DMSO-d6
3. Fig. S3 Mass spectrum of L₁H
4. Fig. S4 Mass spectrum of L₂H
5. Fig. S5 IR spectrum of L₁H
6. Fig. S6 IR spectrum of L₂H
7. Fig. S7 ¹H NMR spectrum of K[L₁H-HSO₄]
8. Fig. S8 ¹H NMR spectrum of K[L₂H-HSO₄]
9. Fig. S9 Mass spectrum of K[L₁H-HSO₄]
10. Fig. S10 Mass spectrum of K[L₂H-HSO₄]
11. Fig. S11 IR spectrum of K[L₁H-HSO₄]
12. Fig. S12 IR spectrum of K[L₂H-HSO₄]
13. Fig. S13 ¹Hnmr titration of L₁H and Table S1
14. Fig. S14 ¹Hnmr titration of L₂H and Table S2
15. Fig. S15 Absorption and emission spectra of 25 µM of the L₁H and L₂H in 100 mM HEPES buffer (ethanol/water 1:5, v/v) at 25°C
16. Fig. S16 Fluorescence colour of the L₁H and L₂H in absence and presence of HSO₄⁻ ion
17. Fig. S17 Ratiometric signaling of fluorescence output
18. **Fig. S18** Computational study of probe $L_1H$ and its adduct with $\text{HSO}_4^-$

19. **Fig. S19** Computational study of probe $L_2H$ and its adduct with $\text{HSO}_4^-$

20. **Fig. S20** Fluorescence intensity of $L_1H$ in presence of different anions

21. **Fig. S21** Fluorescence intensity of $L_2H$ in presence of different anions

22. **Fig. S22** Change of relative fluorescence intensity profile of $L_1H$ in presence of different anions

23. **Fig. S23** Change of relative fluorescence intensity profile of $L_2H$ in presence of different anions

24. **Table S3** Life time details of $L_1H$

25. **Table S4** Life time details of $L_2H$

**Materials and Instruments**

All of the solvents were of analytical grade. The elemental analyses (C, H and N) were performed on a Perkin Elmer 2400 CHN elemental analyzer. A Shimadzu (model UV-1800) spectrophotometer was used for recording electronic spectra. IR spectra were recorded using Prestige-21 SHIMADZU FTIR spectrometer preparing KBr disk. $^1$HNMR spectrum of organic moiety was obtained on a Bruker Avance DPX 300 spectrometer using DMSO−d$_6$ solution. Electrospray ionization (ESI) mass spectra were recorded on a Qtof Micro YA263 mass spectrometer. A Systronics digital pH meter (model 335) was used to measure the pH of the solution and the adjustment of pH was done using either 50 mM HCl or KOH solution. Steady-state fluorescence emission and excitation spectra were recorded with a Hitachi-4500 spectrofluorimeter. Time-resolved fluorescence lifetime measurements were performed using a HORIBA JOBIN Yvon picosecond pulsed diode laser-based time-correlated single-photon counting (TCSPC) spectrometer from IBH (UK) at $\lambda_{ex}= 370$ nm and MCP-PMT as a detector. Emission from the sample was collected at a right angle to the direction of the excitation beam maintaining magic angle polarization (54.71). The full width at half-maximum (FWHM) of the instrument response function was 250 ps, and the resolution was 28.6 ps per channel. Data were fitted to multi exponential functions after de convolution of the instrument response function by an iterative re convolution technique using IBH DAS 6.2 data analysis software in which reduced w2 and weighted residuals serve as parameters for goodness of fit.
Fig. S1 a) Expansion of aromatic region  b) $^1$HNMR of L$_1$H
Fig. S2  a) Expansion of aromatic region  b) $^1$HNMR of L$_2$H
Fig. S3. Mass spectrum of $L_1H$

$[M+H]^+ = 343.1480$

Fig. S4. Mass spectrum of $L_2H$

$[M+H]^+ = 328.1246$
Fig. S5 IR spectrum of L₁H

Fig. S6 IR spectrum of L₂H
Fig. S7 $^1$H NMR expansion of K[L$_1$H-HSO$_4$]
Fig. S8 a) Expansion of aromatic region  b) $^1$HNMR of K$_2$H$_2$HSO$_4$
**Fig. S9** Mass spectrum of K\([L_1H\text{-HSO}_4]\)

\[
I^2+2H^++Na^+=463.16
\]
\[
I^2+H^++K^++Na^+=505.0080
\]

**Fig. S10** Mass spectrum of K\([L_2H\text{-HSO}_4]\)

\[
I^2+2H^++Na^+=447.92
\]
\[
I^2+H^++K^++Na^+=486.2086
\]
Fig. S11 IR spectrum of $\text{K}[\text{LiH-HSO}_4]$

Fig. S12 IR spectrum of $\text{K}[\text{Li}_2\text{H-HSO}_4]$
Table S1. $^1$Hnmr titration data of $L_1H$

<table>
<thead>
<tr>
<th></th>
<th>$H_a$, $H_{a'}$</th>
<th>$H_b$</th>
<th>$H_c$, $H_{c'}$</th>
<th>$H_d$, $H_{d'}$, $H_e$, $H_{e'}$, $H_f$</th>
<th>$H_{g}$</th>
<th>$H_{h}$</th>
<th>$H_{i}$, $H_{i'}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L_1H$</td>
<td>8.194</td>
<td>7.986</td>
<td>7.801</td>
<td>7.704</td>
<td>7.457-7.439</td>
<td>7.281-7.165</td>
<td>6.884, 6.879,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.877, 6.870,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.863, 6.860,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.856</td>
</tr>
<tr>
<td>$L_1^-$</td>
<td>8.213</td>
<td>8.007</td>
<td>7.82</td>
<td>7.720</td>
<td>7.480-7.458</td>
<td>7.318-7.170</td>
<td>6.906, 6.891,</td>
</tr>
<tr>
<td>HSO$_4$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.877, 6.875</td>
</tr>
</tbody>
</table>

Fig. S13 $^1$HNMR titration of $L_1H$

Table S2. $^1$HNMR titration data of $L_2H$

<table>
<thead>
<tr>
<th></th>
<th>$H_{b'}$</th>
<th>$H_b$</th>
<th>$H_d$, $H_c$, $H_e$, $H_{g}$</th>
<th>$H_a$, $H_{a'}$, $H_f$</th>
<th>$H_h$</th>
<th>$H_i$</th>
<th>$-OCH_3$</th>
</tr>
</thead>
</table>
Fig. S14 $^1$HNMR titration of $L_2H$

Fig. S15 Absorption and emission spectra of 25 μM of the $L_1H$ (left) and $L_2H$ (right) in 100 mM HEPES buffer (ethanol/water 1:5, v/v) at 25°C
Fig. S16 (a). Fluorescence colour of the \( L_1H \) in absence (left) and presence (right) of \( \text{HSO}_4^- \) ion. (b). Fluorescence colour of the \( L_2H \) in absence (left) and presence (right) of \( \text{HSO}_4^- \) ion.

Fig. S17 Ratiometric signalling output of \( L_3H \)
**Fig. S18** Computational study of probe $L_1H$ and its adduct with $HSO_4^-$
**Fig. S19** Computational study of probe L$_2$H and its adduct with HSO$_4$\textsuperscript{-}
Fig. S20. Fluorescence intensity of L₁H in presence of different anions in HEPES buffer (100 mM, pH 7.4; ethanol/water: 1/5, v/v) at 25 °C, (a) OAc⁻, (b) F⁻, (c) I⁻, (d) H₂PO₄⁻, (e) ClO₄⁻, (f) N₃⁻, (g) Br⁻, (h) H₂AsO₄⁻, (i) Cl⁻, (j) SO₄²⁻, (k) S²⁻, (l) CN⁻, (m) NO₃⁻, (n) HSO₄⁻.

Fig. S21. Fluorescence intensity of L₂H in presence of different anions in HEPES buffer (100 mM, pH 7.4; ethanol/water: 1/5, v/v) at 25 °C, (a) Cl⁻, (b) Br⁻, (c) I⁻, (d) F⁻, (e) OAc⁻, (f) H₂PO₄⁻, (g) N₃⁻, (h) ClO₄⁻, (i) H₂AsO₄⁻, (j) SO₄²⁻, (k) S²⁻, (l) CN⁻, (m) NO₃⁻, (n) HSO₄⁻.
**Fig. S22** Change of relative fluorescence intensity profile of **L₁H** in presence of different anions in ethanol: water (1: 5, v/v) at room temperature (λ_{ex}= 400 nm) where (a) HSO₄⁻, (b) OAc⁻, (c) F⁻, (d) I⁻, (e) H₂PO₄⁻, (f) ClO₄⁻, (g) N₃⁻, (h) Br⁻, (i) H₂AsO₄⁻, (j) NO₃⁻, (k) SO₄²⁻, (l) S²⁻, (m) CN⁻.

**Fig. S23** Change of relative fluorescence intensity profile of **L₂H** in presence of different anions in ethanol: water (1: 5, v/v) at room temperature (λ_{ex}= 390 nm), (a) HSO₄⁻, (b) Cl⁻, (c) Br⁻, (d) I⁻, (e) F⁻, (f) OAc⁻, (g) H₂PO₄⁻, (h) H₂AsO₄⁻, (i) ClO₄⁻, (j) N₃⁻, (k) SO₄²⁻, (l) S²⁻, (m) CN⁻, (n) NO₃⁻.
### Table S3. Life time detail of $L_1H$

<table>
<thead>
<tr>
<th></th>
<th>$B_1$</th>
<th>$B_2$</th>
<th>$T_1$(ns)</th>
<th>$T_2$(ns)</th>
<th>$T_{av}$(ns)</th>
<th>$\chi^2$</th>
<th>$\phi$</th>
<th>$K_r$</th>
<th>$K_{nr}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$(L_1H)$</td>
<td>5.07</td>
<td>94.93</td>
<td>1.74</td>
<td>8.67</td>
<td>8.32</td>
<td>1.082</td>
<td>0.0055</td>
<td>0.0006</td>
<td>0.119</td>
</tr>
<tr>
<td>$L_1+HSO_4^-$ (1:0.5)</td>
<td>29.28</td>
<td>70.72</td>
<td>1.93</td>
<td>12.53</td>
<td>9.42</td>
<td>1.028</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$L_1+HSO_4^-$ (1:1)</td>
<td>18.98</td>
<td>81.02</td>
<td>1.86</td>
<td>12.84</td>
<td>10.75</td>
<td>1.064</td>
<td>0.355</td>
<td>0.033</td>
<td>0.06</td>
</tr>
</tbody>
</table>

### Table S4. Life time detail of $L_2H$

<table>
<thead>
<tr>
<th></th>
<th>$B_1$</th>
<th>$B_2$</th>
<th>$T_1$(ns)</th>
<th>$T_2$(ns)</th>
<th>$T_{av}$(ns)</th>
<th>$\chi^2$</th>
<th>$\phi$</th>
<th>$K_r$</th>
<th>$K_{nr}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$(L_2H)$</td>
<td>33.61</td>
<td>66.39</td>
<td>7.30</td>
<td>10.96</td>
<td>8.26</td>
<td>1.068</td>
<td>0.065</td>
<td>0.00785</td>
<td>0.113</td>
</tr>
<tr>
<td>$L_2+HSO_4^-$ (1:0.5)</td>
<td>14.67</td>
<td>85.33</td>
<td>6.76</td>
<td>8.89</td>
<td>8.58</td>
<td>1.031</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>$L_2+HSO_4^-$ (1:1)</td>
<td>6.55</td>
<td>93.45</td>
<td>3.65</td>
<td>12.36</td>
<td>11.79</td>
<td>1.078</td>
<td>0.48</td>
<td>0.04</td>
<td>0.044</td>
</tr>
</tbody>
</table>

18
**Fig. S24** Cytotoxic effect of L₁H (5, 10, 25, 50 and 100 μM) in HeLa cells incubated for 8 h

**Fig. S25** Cytotoxic effect of L₂H (5, 10, 25, 50 and 100 μM) in HeLa cells incubated for 8 h