Supporting Information

For

Substituent effect on fluorescence signalling of the HSO_4^- 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

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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 λ_{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.

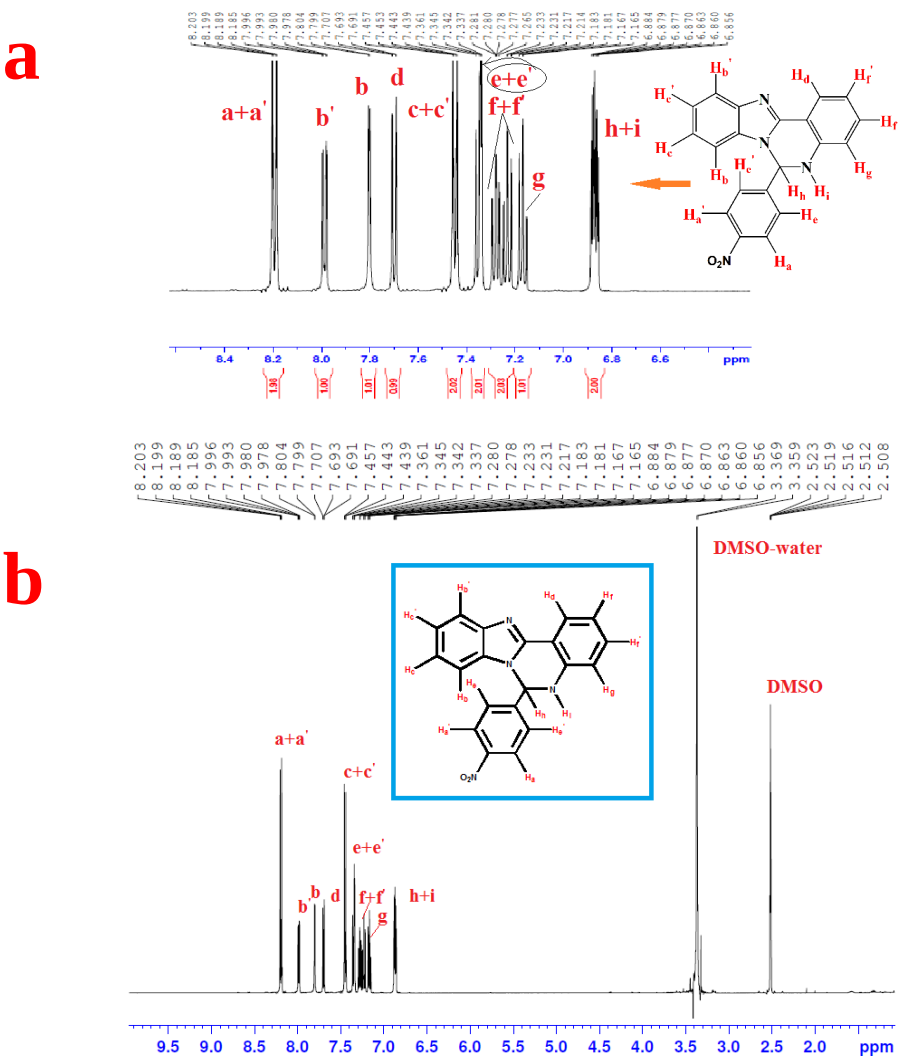


Fig. S1 a) Expansion of aromatic region **b)** ^{1}HNMR of **L_1H**

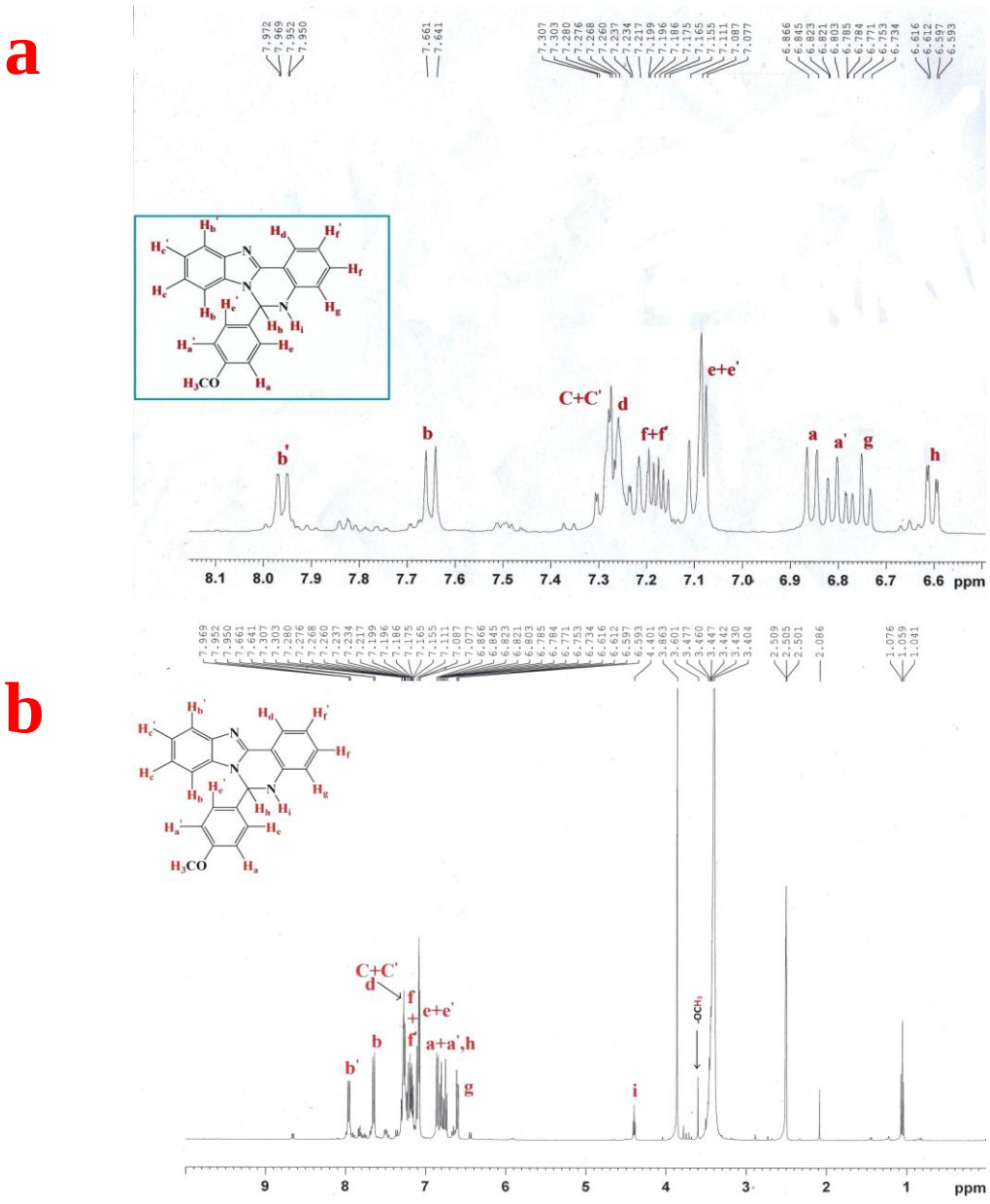


Fig. S2 a) Expansion of aromatic region **b)** ^{1}HNMR of **L_2H**

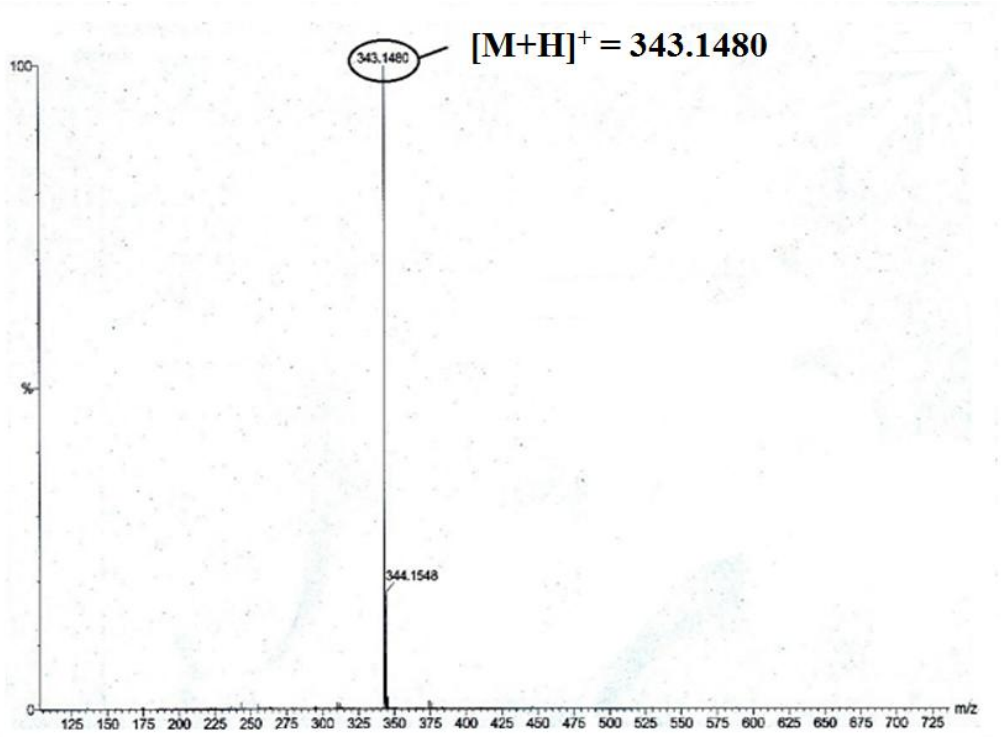


Fig. S3. Mass spectrum of $\mathbf{L_1H}$

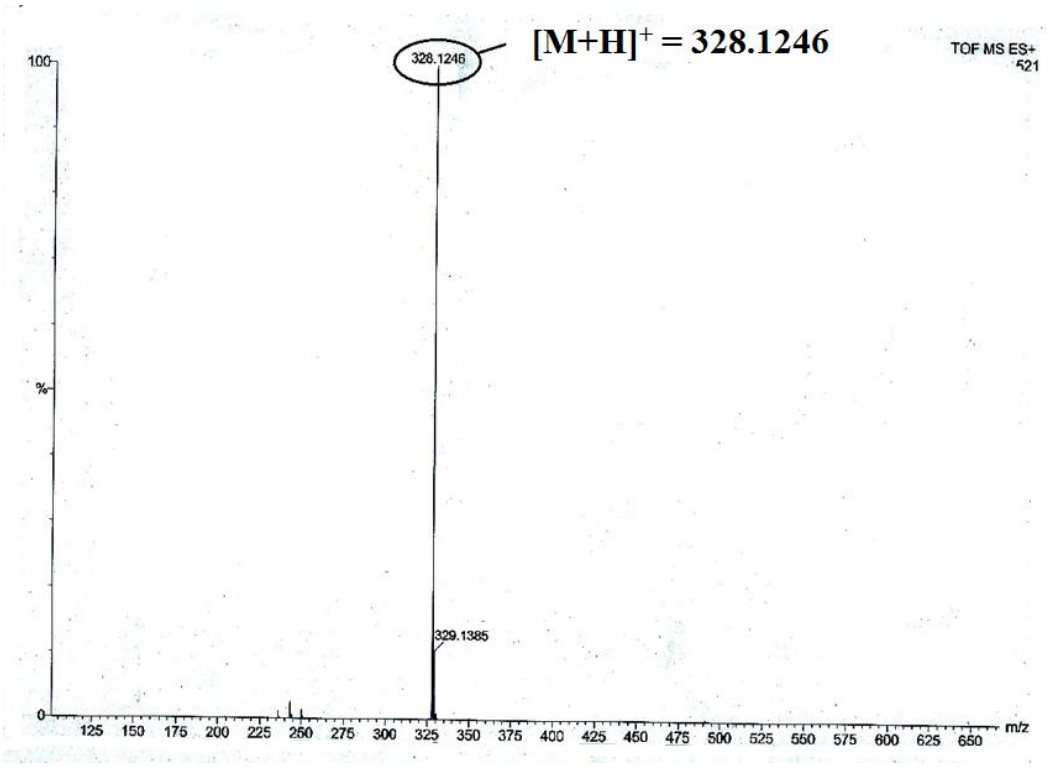


Fig. S4 Mass spectrum of $\mathbf{L_2H}$

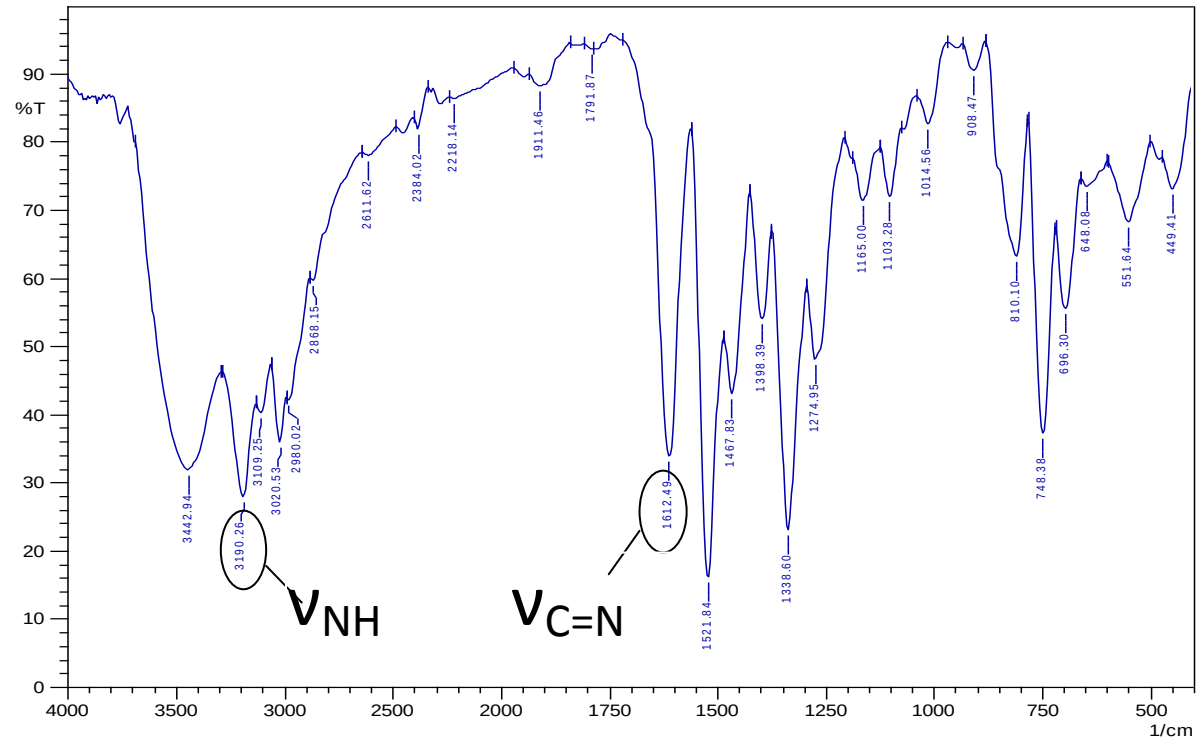


Fig. S5 IR spectrum of **L_1H**

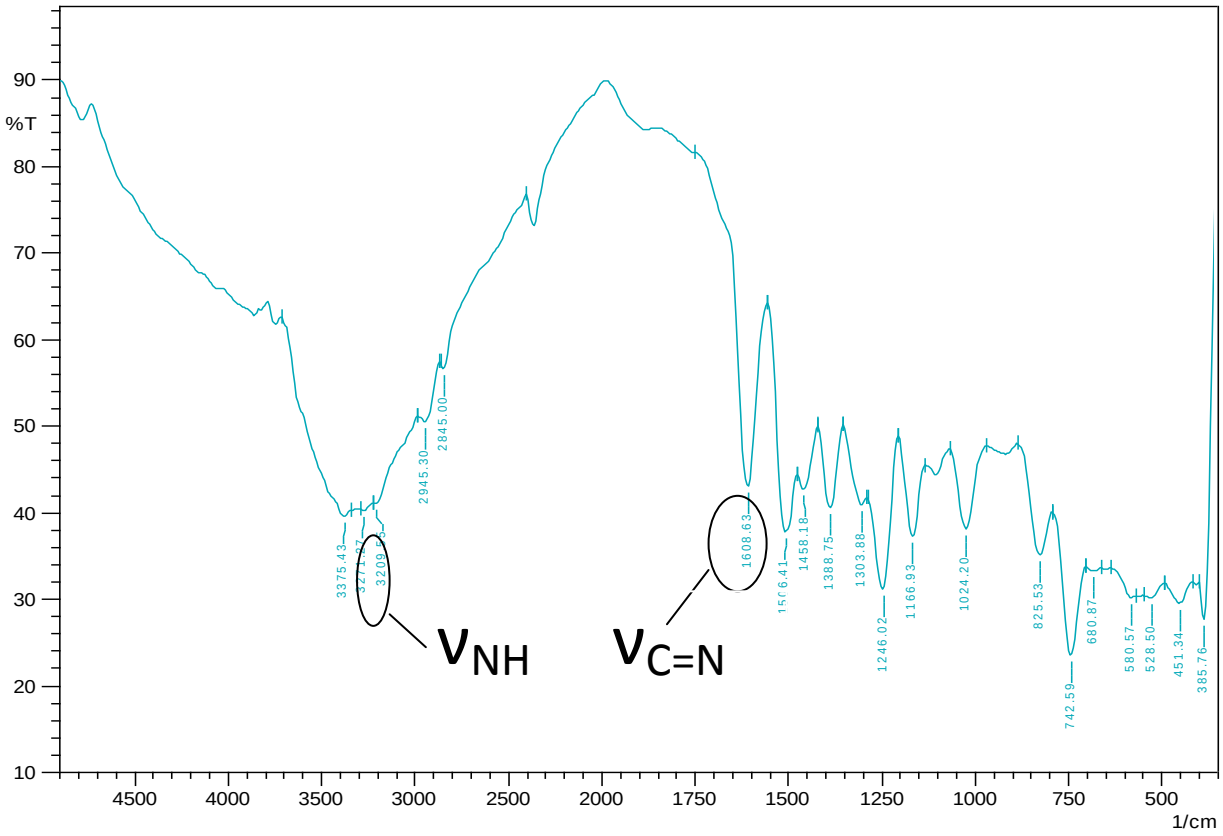


Fig. S6 IR spectrum of **L_2H**

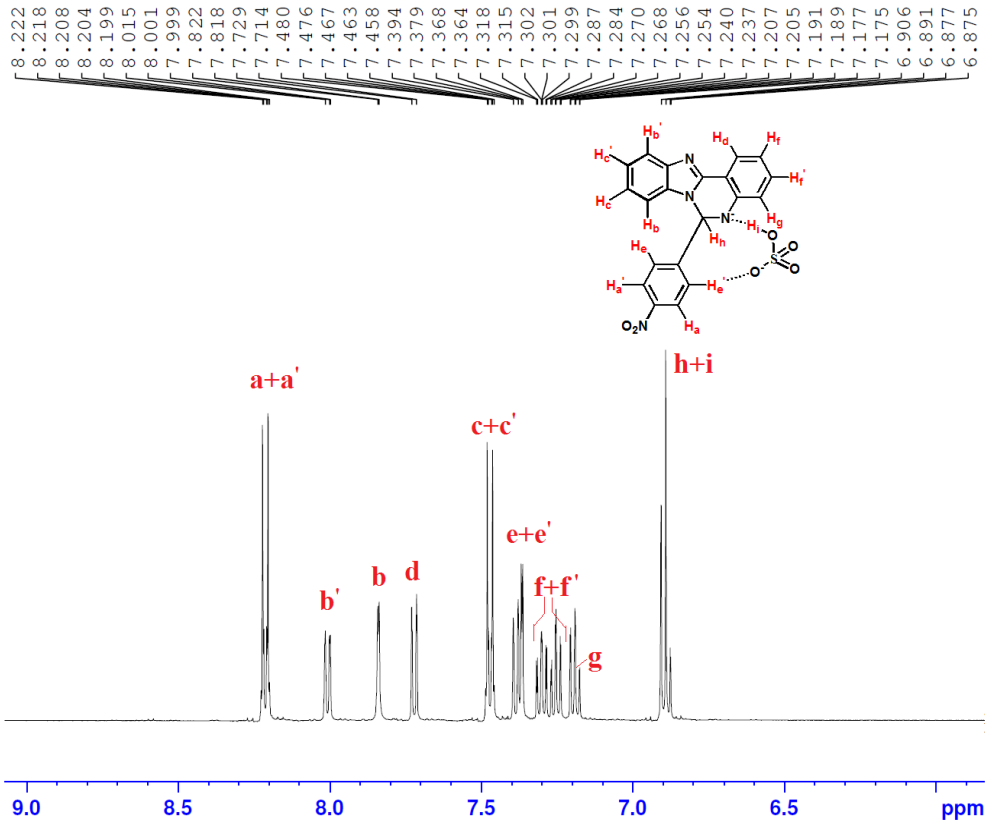


Fig. S7 ^{1}HNMR expansion of **K[L_1H-HSO_4]**

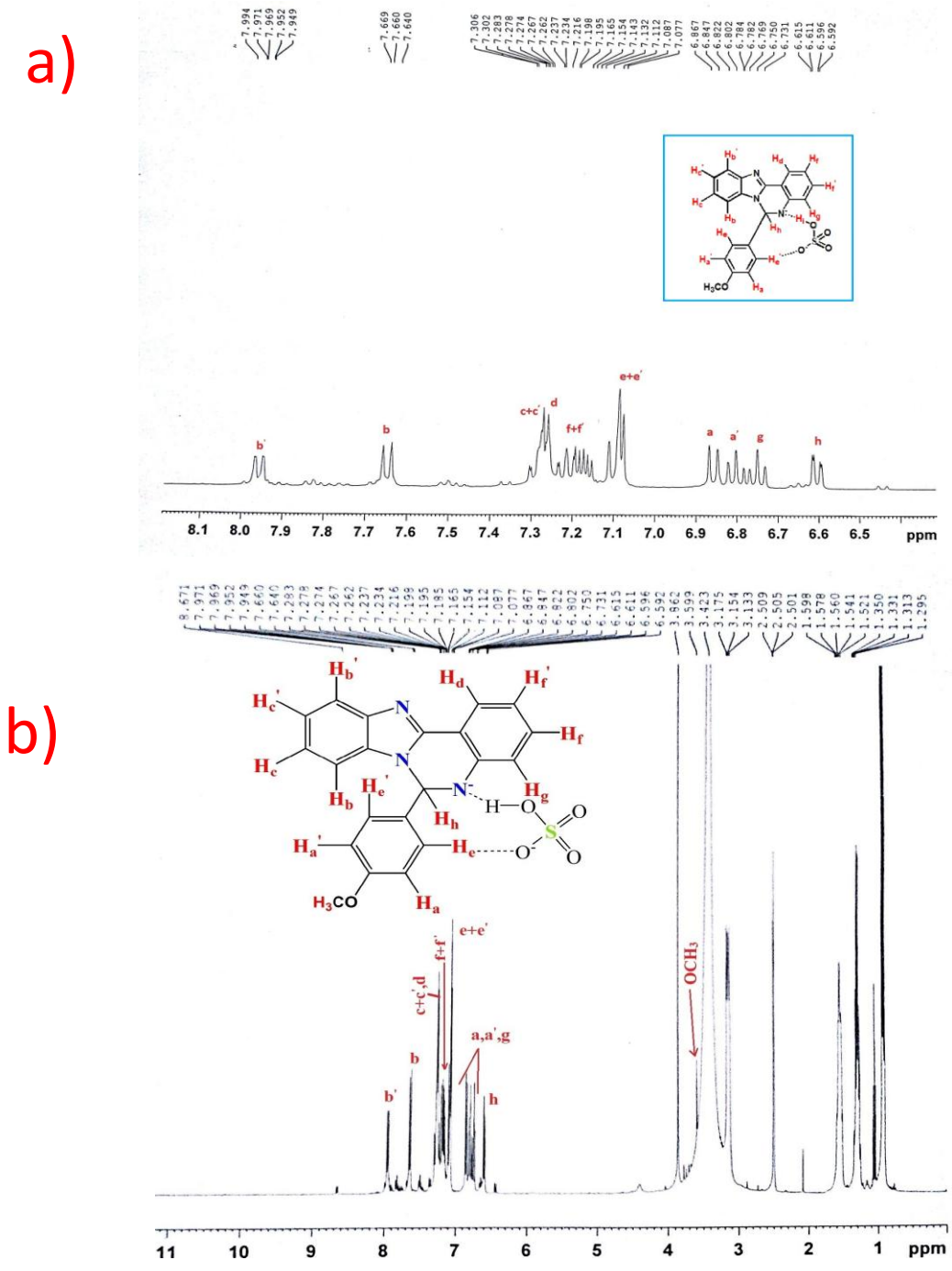


Fig. S8 a) Expansion of aromatic region **b)** ^{I}HNMR of **K[L_2H-HSO_4]**

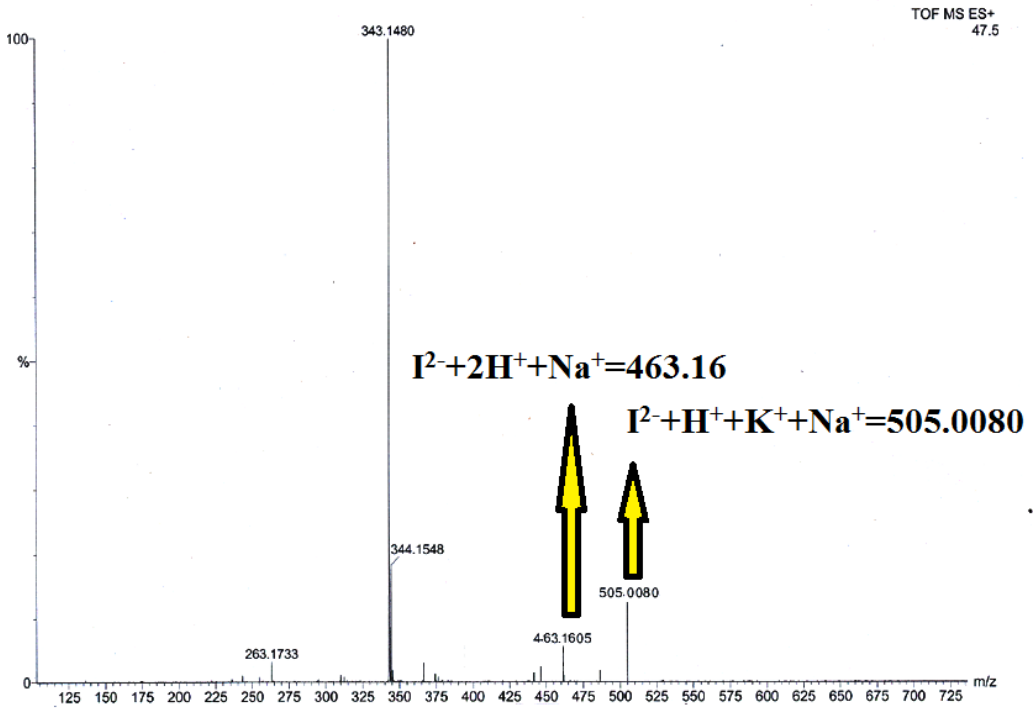


Fig. S9 Mass spectrum of **K[L_1H-HSO_4]**

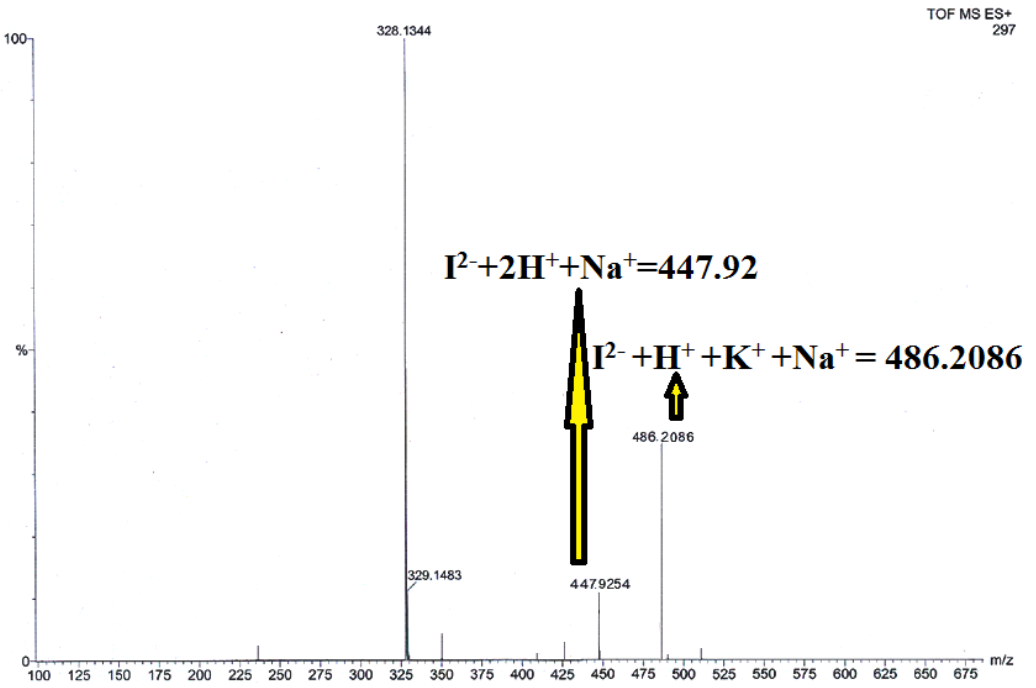


Fig. S10 Mass spectrum of **K[L_2H-HSO_4]**

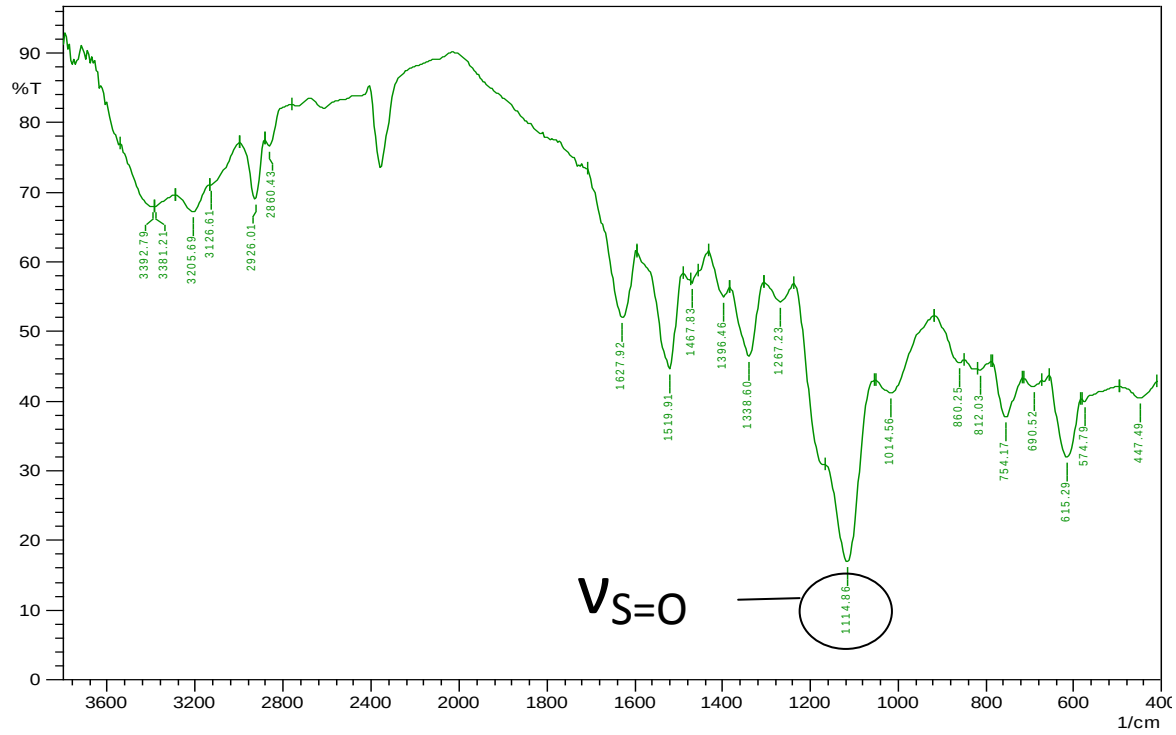


Fig. S11 IR spectrum of **K[L_1H-HSO_4]**

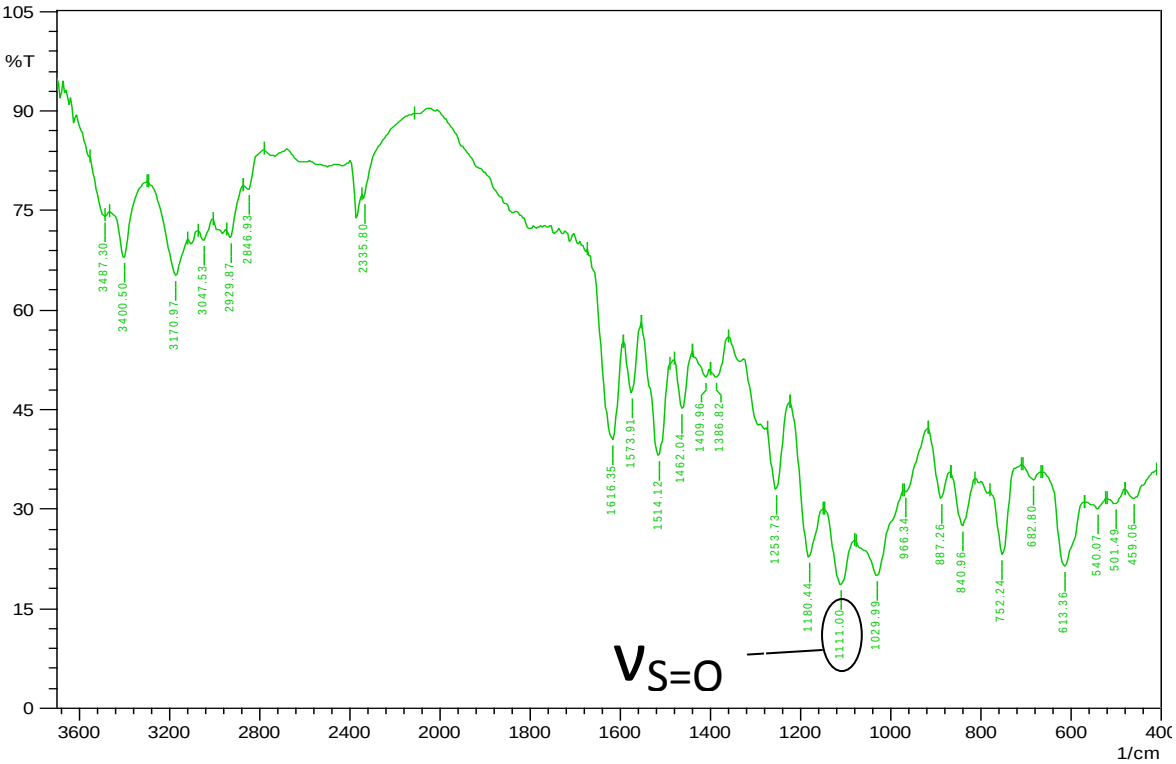


Fig. S12 IR spectrum of **K[L_2H-HSO_4]**

Table S1. [1]Hnmr titration data of **L_1H**

	H_a , $H_{a'}$ (2H,d-d)	$H_{b'}$ (1H,d-d)	H_b (1H,d)	H_d (1H,d-d)	H_c , $H_{c'}$ (2H,m)	H_e , $H_{e'}$ (2H,m)	H_f ,$H_{f'}$,H_g (3H,m)	H_h , H_i (2H,m)
L_1H	8.194	7.986	7.801	7.704	7.457-7.439	***7.361,7.345*** *7.342,7.337*	7.281-7.165	6.884,6.879, 6.877,6.870, ***6.863,6.860, 6.856***
L_1-HSO_4	8.213	8.007	7.82	7.720	7.480-7.458	***7.394,7.39*** *7.368,7.364*	7.318-7.170	6.906,6.891, 6.877,6.875

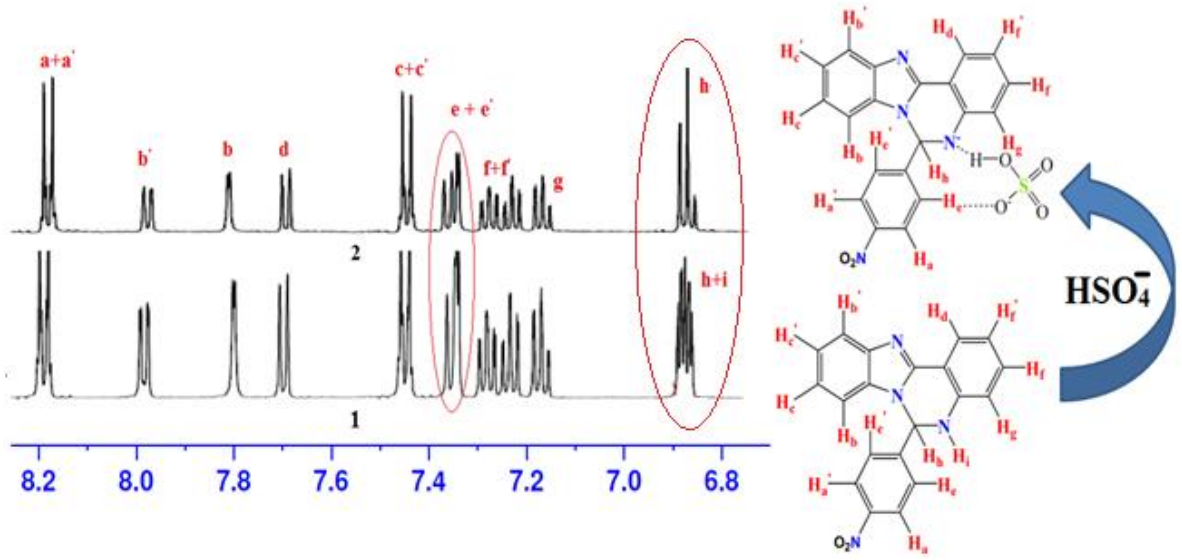


Fig. S13 [1]HNMR titration of **L_1H**

Table S2. [I]HNMR titration data of **L_2H**

	$H_{b'}$ (1H,d-d)	H_b (1H,d)	H_d ,H_c, $H_{c'}$, H_e , $H_{e'}$, H_f , $H_{f'}$(7H,m)	H_a , $H_{a'}$, H_g (3H,m)	H_h (1H,d-d)	H_i (1H,s)	-OCH_3 (3H,s)
L_2H	7.96	7.651	7.307-7.077	6.866-6.734	6.604	***4.401***	3.601
L_2- HSO_4	7.9605	7.645	7.306-7.077	6.867-6.731	6.594	-	3.601

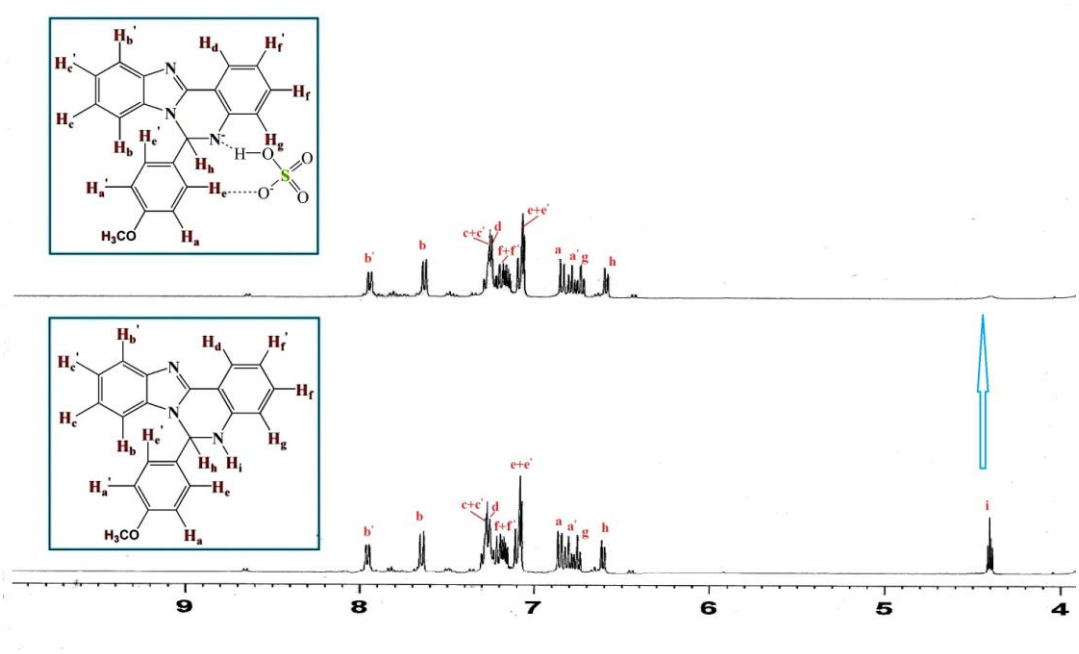


Fig. S14 ^{1}HNMR titration of **L_2H**

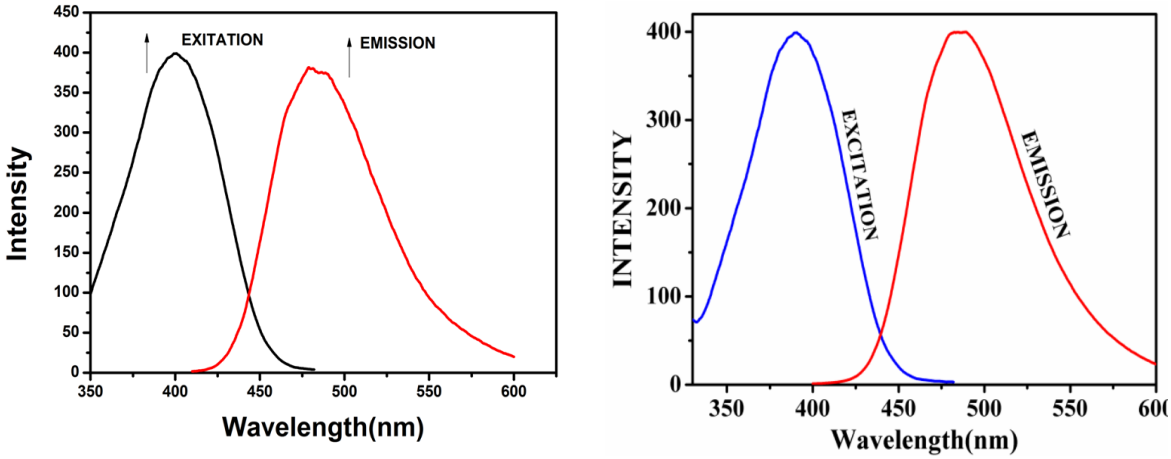
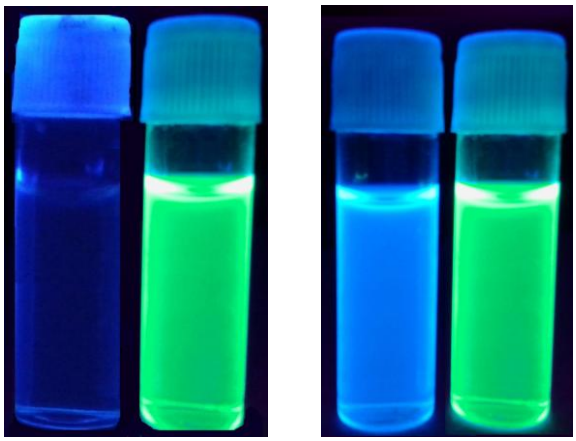


Fig. S15 Absorption and emission spectra of 25 μM of the **L_1H** (left) and **L_2H** (right)in 100 mM HEPES buffer (ethanol/water 1:5, v/v) at 25°C



(a) **(b)**

Fig. S16 (a).Fluorescence colour of the **L_1H** in absence (left) and presence(right) of HSO_4^- ion. (b). Fluorescence colour of the **L_2H** in absence (left) and presence(right) of HSO_4^- ion.

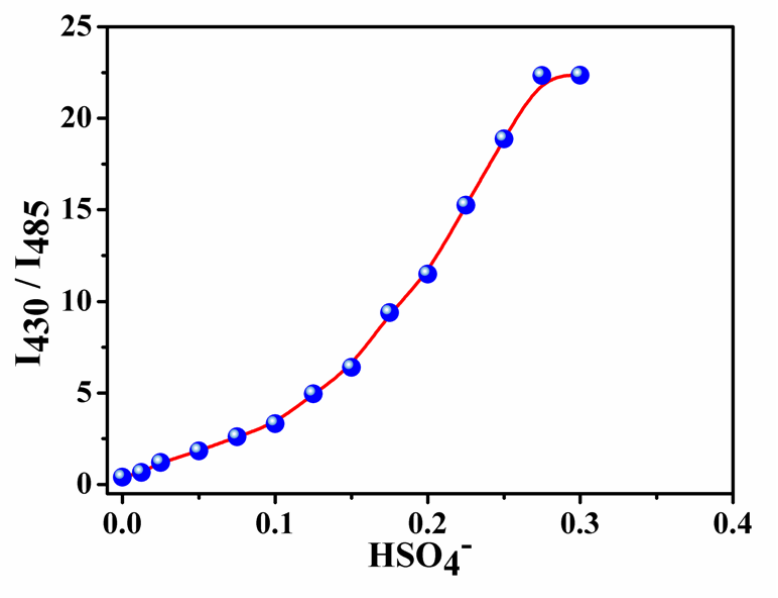


Fig. S17 Ratiometric signalling output of **L_2H**

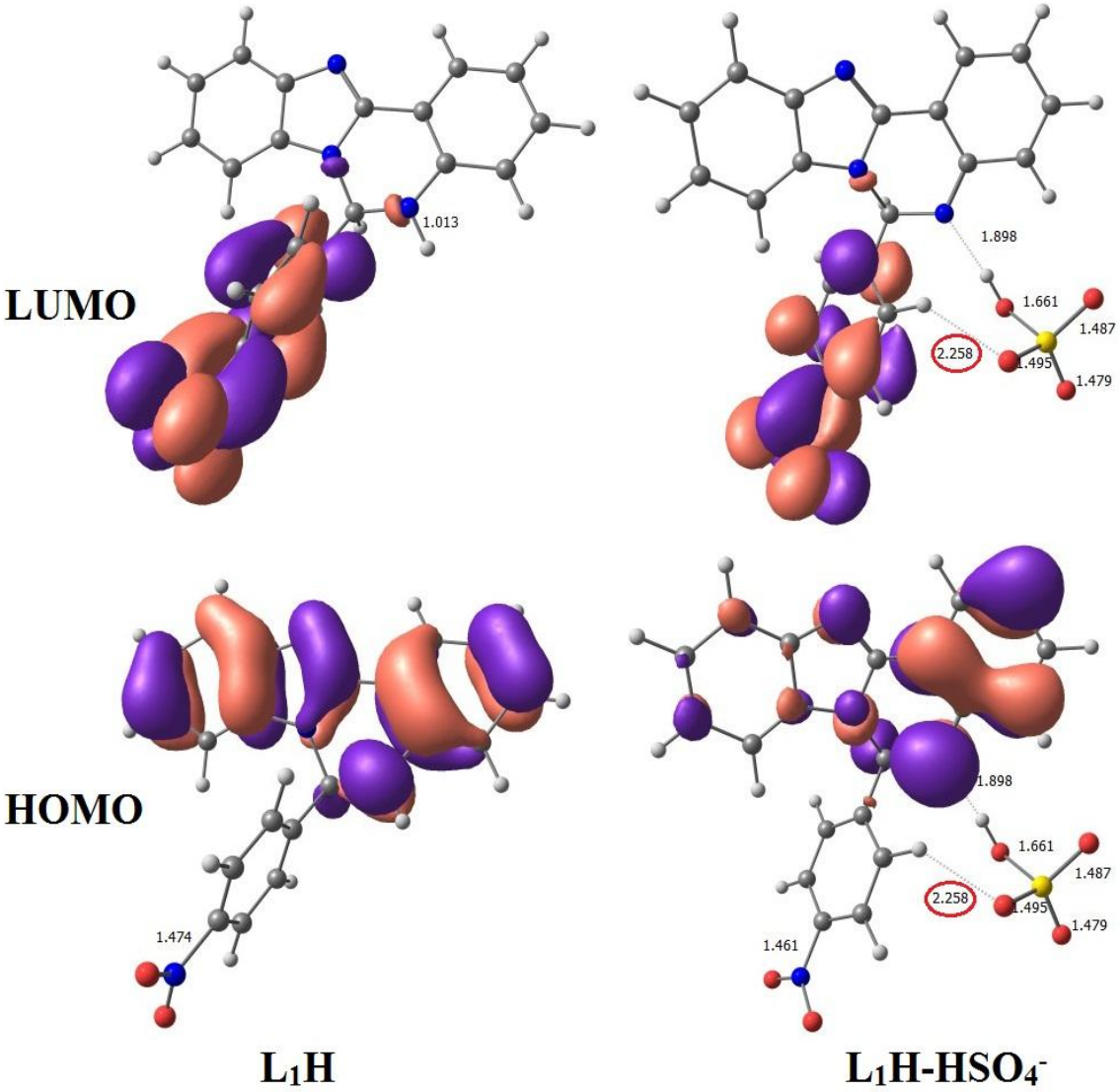


Fig. S18 Computational study of probe **L_1H** and it's adduct with **HSO_4^-**

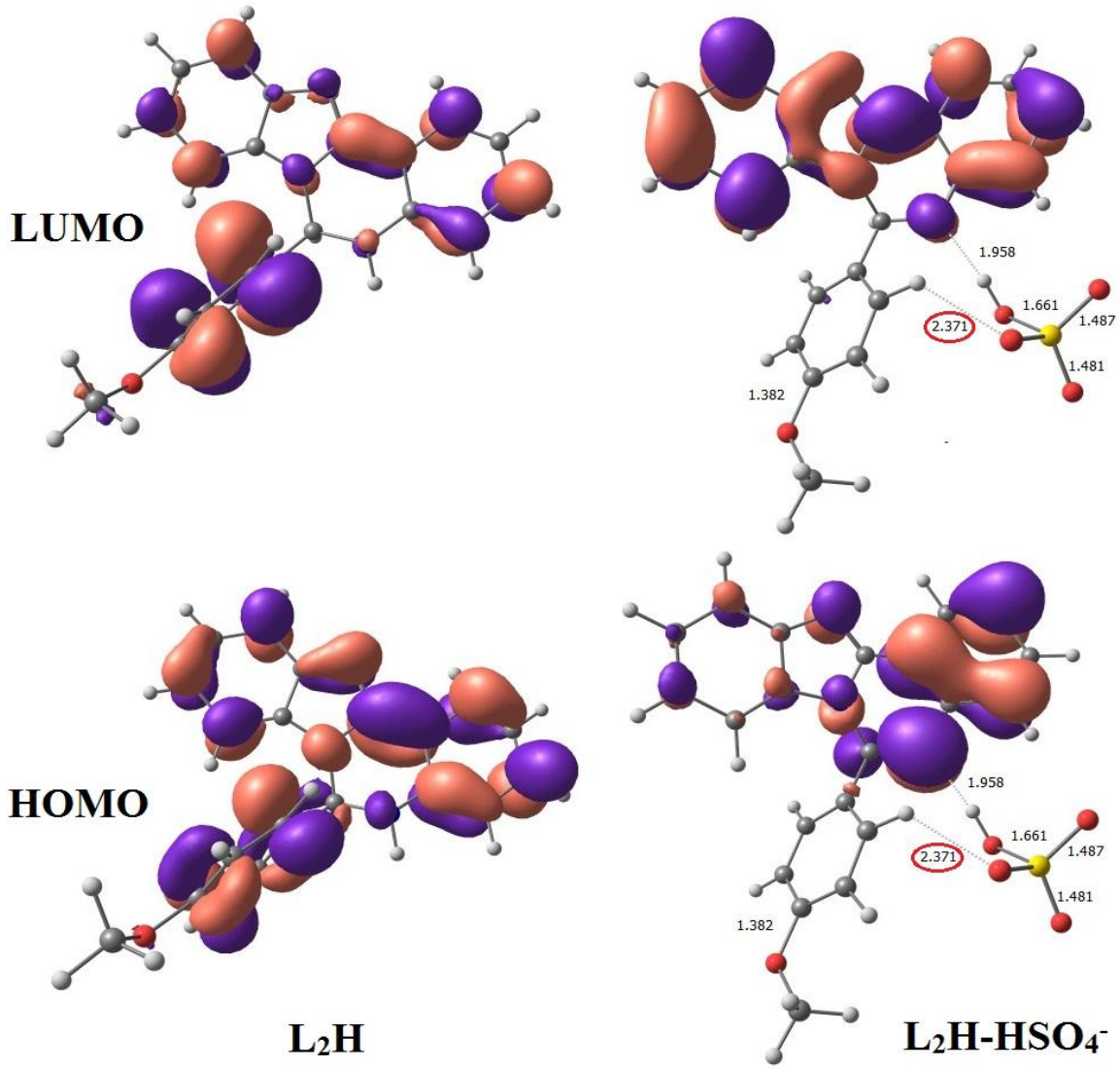


Fig. S19 Computational study of probe **L_2H** and it's adduct with **HSO_4^-**

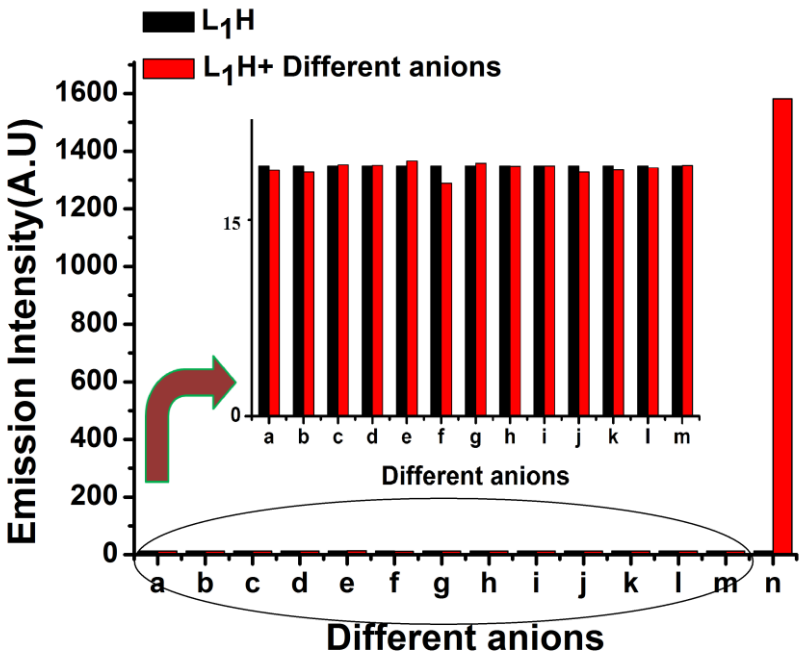


Fig. S20.Fluorescence intensity of **L_1H** in presence of different anions in HEPES buffer (100 mM, pH 7.4; ethanol/water: 1/5, v/v) at 25 °C, (a) OAc^-, (b) F^-, (c) I^-, (d) $H_2PO_4^-$, (e) ClO_4^-, (f) N_3^-, (g) Br^-, (h) $H_2AsO_4^-$, (i) Cl^-, (j) SO_4^{2-}, (k) S^{2-}, (l) CN^-, (m) NO_3^-, (n) HSO_4^-

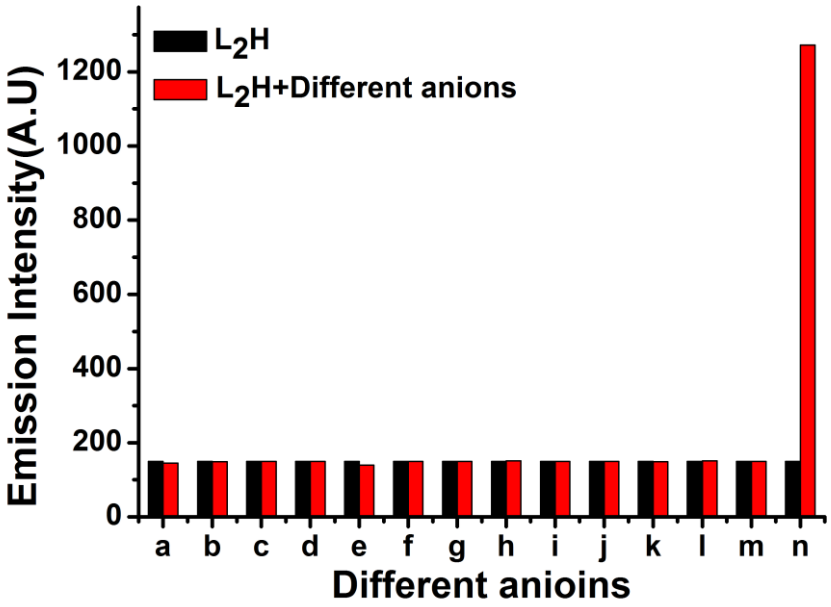


Fig. S21.Fluorescence intensity of **L_2H** in presence of different anions in HEPES buffer (100 mM, pH 7.4; ethanol/water: 1/5, v/v) at 25 °C, (a) Cl^-, (b) Br^-, (c) I^-, (d) F^-, (e) OAc^-, (f) $H_2PO_4^-$, (g) N_3^-, (h) ClO_4^-, (i) $H_2AsO_4^-$, (j) SO_4^{2-}, (k) S^{2-}, (l) CN^-, (m) NO_3^-, (n) HSO_4^-

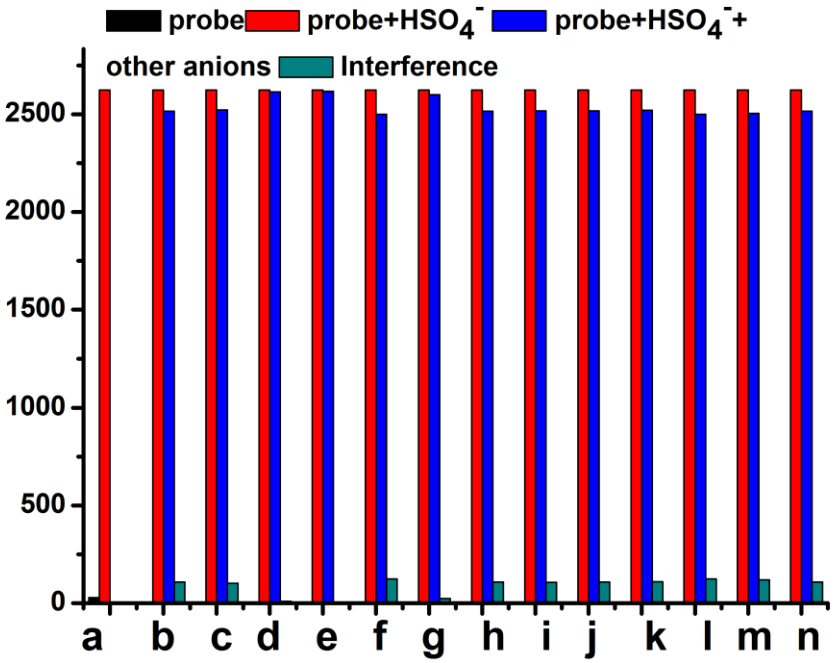


Fig. S22 Change of relative fluorescence intensity profile of $\mathbf{L_1H}$ in presence of different anions in ethanol: water (1: 5, v/v) at room temperature (λ_{ex}= 400 nm) where (a) HSO_4^-, (b) OAc^-, (c) F^-, (d) I^-, (e) $H_2PO_4^-$, (f) ClO_4^-, (g) N_3^-, (h) Br^-, (i) $H_2AsO_4^-$, (j) NO_3^-, (k) SO_4^{2-}, (l) S^{2-}, (m) CN^-

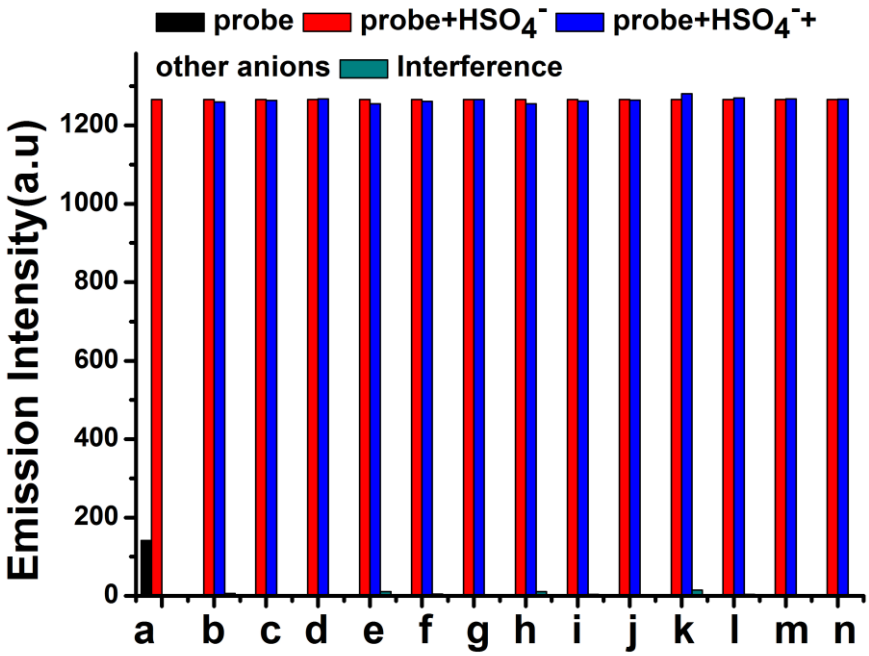


Fig. S23 Change of relative fluorescence intensity profile of $\mathbf{L_2H}$ in presence of different anions in ethanol: water (1: 5, v/v) at room temperature (λ_{ex}= 390 nm). (a) HSO_4^-, (b) Cl^-, (c) Br^-, (d) I^-, (e) F^-, (f) OAc^-, (g) $H_2PO_4^-$, (h) $H_2AsO_4^-$, (i) ClO_4^-, (j) N_3^-, (k) SO_4^{2-}, (l) S^{2-}, (m) CN^-, (n) NO_3^-

Table S3. Life time detail of **L_1H**

	B_1	B_2	T_1(ns)	T_2(ns)	T_{av}(ns)	X^2	ϕ	K_r	K_{nr}
(L_1H)	5.07	94.93	1.74	8.67	8.32	1.082	0.0055	0.00066	0.1195
L_1+HSO_4^- (1:0.5)	29.28	70.72	1.93	12.53	9.42	1.028			
L_1+HSO_4^- (1:1)	18.98	81.02	1.86	12.84	10.75	1.064	0.355	0.033	0.06

Table S4. Life time detail of **L_2H**

	B_1	B_2	T_1(ns)	T_2(ns)	T_{av}(ns)	X^2	φ	K_r	K_{nr}
(L_2H)	33.61	66.39	7.30	10.96	8.26	1.068	0.065	0.00785	0.113
L_2+HSO_4^- (1:0.5)	14.67	85.33	6.76	8.89	8.58	1.031	-	-	-
L_2+HSO_4^- (1:1)	6.55	93.45	3.65	12.36	11.79	1.078	0.48	0.04	0.04411

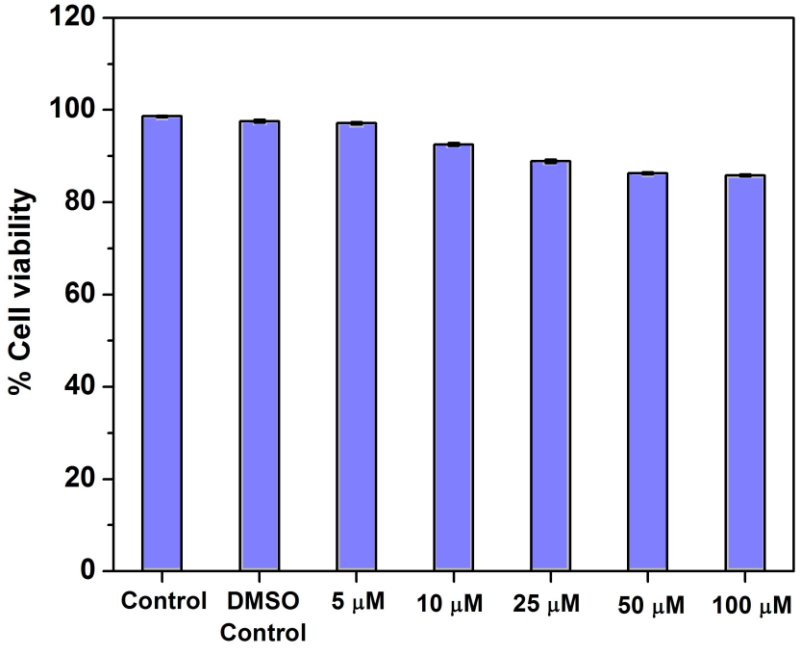


Fig. S24 Cytotoxic effect of $\mathbf{L_1H}$ (5, 10, 25, 50 and 100 μM) in HeLa cells incubated for 8 h

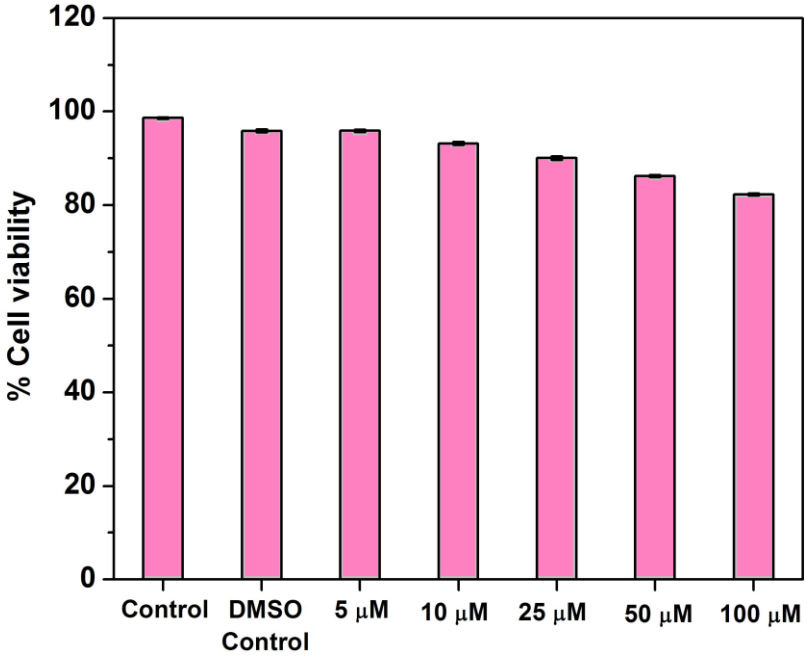


Fig. S25 Cytotoxic effect of $\mathbf{L_2H}$ (5, 10, 25, 50 and 100 μM) in HeLa cells incubated for 8 h