Supplementary Information (SI) for Analyst.

Supporting information for

A Ratiometric Green to Blue Emitting Hg^{2+} Ions Responsive Ionic Liquids-based Micro-optode

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Experimental section

General methods and instrumentation

Chemical reagents were procured from Sigma-Aldrich, India, and applied without any further purification. Anhydrous solvents and HPLC-grade solvents for spectroscopy were obtained from Merck, India. We have purchased all of the cationic and anionic salts from Sigma-Aldrich in India, including trihexyltetradecylphosphonium chloride ([TTP]Cl), 8-hydroxy pyranine-1, and 3-trisulfonic acid trisodium salt [HPTS]. Also, Deuterated chloroform ($CDCl_3$) was purchased from Sigma Aldrich, India, and employed for NMR spectroscopic analysis. Fourier transform infrared (FT-IR) spectra were recorded using a Bruker Optik GMBH (Germany) FT-IR spectrometer under ambient conditions. Mass spectrometric analysis was performed on a Quattro Micro API (MICROMASS, UK) coupled with an LC-WATERS 2695 system equipped with a PDA2998 detector ESI_Negative with capillary voltage 3 kV, Cone -30 V and extractor-3 V employing source and dissolving temperature -90℃ and -250℃ respectively using dissolving gas-450 Litre/hour with flow rate-10 μL/min. We used a HITACHI U-2910 spectrophotometer for monitoring UV-visible absorption spectra, while fluorescence measurements were performed on a HITACHI F-7100 fluorimeter, employing a 5 nm slit width for both excitation and emission. During steady-state emission studies, the excitation wavelength was set at 330 nm, and emission was monitored over the range of 330–640 nm, respectively. The size and shape of the **mHPTIL** are estimated by field emission scanning electron microscopy (SEM) (ZEISS) employing an operating voltage of 50 kV by drop casting the required amounts of **mHPTIL** (20 μg/mL) on the carbon-coated copper grids. The average particle size is determined by considering the size of more than 100 particles. The hydrodynamic radius of **mHPTIL** is measured employing the dynamic light scattering (DLS) technique (Anton Paar Litesizer 500). The zeta potential (ζ) of nanoparticles is also obtained by this instrument using a capillary ζ-cell.

Before the analysis, the stock solution of **HPTIL** was prepared instantly (1×10^{-3} M in DMSO). The solution of numerous inorganic metal salts like $AlCl_3$, $BaCl_2$, $CaCl_2$, $CdCl_2$, $CoCl_2$, $CuCl_2$, $ZnCl_2$, $FeCl_3$, $HgCl_2$, $MgCl_2$, $MnCl_2$, $NiCl_2$, $PbCl_2$, and $FeCl_2$ including all the inorganic and organic anion salt like $AlPO_4$, K_2CO_3, KBr, KCl, NaF, $NaBPh_4$, Na_2HPO4, NaH_2PO_4, Na_3PO_4, KPF_6, S^{2-} and Na_2SO_4 were prepared instantly in (1×10^{-3} M) concentration in water.

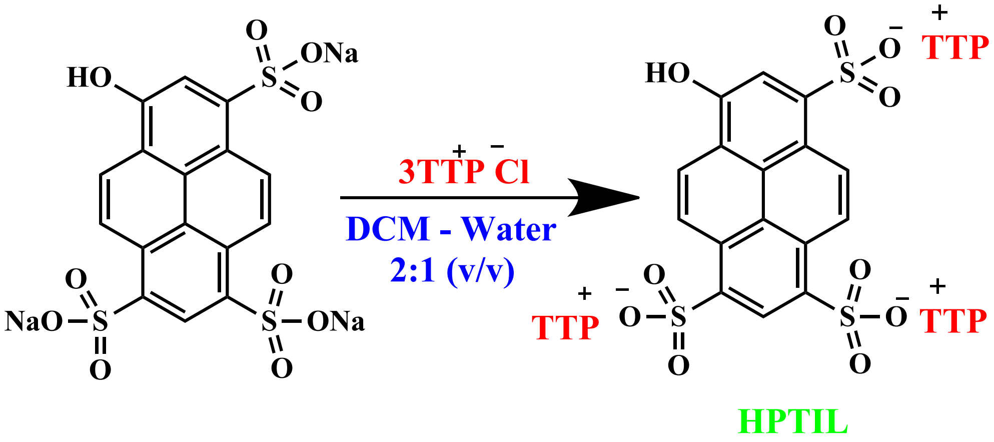
For the fabrication of a paper-based test kit, Whatman-41 filter paper is cut into strips, which are fully immersed in **mHPTIL** aqueous solution (29 µM) for a while. Finally, the test strips are removed and dried in a hot air oven at 60 °C. Some drops of all the metal ions solution (10^{-3} M) are added to these filter papers.

Synthesis and characterization of HPTIL

We have used an optimized ion-exchange methodology to prepare **HPTIL** as a room-temperature ionic liquid (RTIL) in quantitative yield, developed by Bakers and colleagues[1] with some modifications. (**Scheme S1**). In a 1:3 ratio, 8-hydroxy pyranine-1, 3, 6-trisulfonic acid trisodium salt [HPTS], and trihexyltetradecylphosphonium chloride ([TTP]Cl) are mixed in a mixture of dichloromethane (DCM)-Water of 2/1 (v/v) ratio with overnight stirring. A bluish fluorescence is seen under the 365 nm UV lamp with a distinct color change from colorless to yellowish in the necked eye. Successful ion exchange is indicated when the water layer (upper layer) becomes colorless. The DCM layer is washed with water several times to remove the byproduct, NaCl, thoroughly. The aqueous layer is further tested with $AgNO_3$ solution to ensure the complete removal of NaCl and continues until no white precipitate due to the formation of AgCl has appeared. The upper water layer is removed, and to ensure the complete removal of water from DCM, anhydrous Na_2SO_4 is added to absorb the trace amount of water. By evaporating the DCM layer and the remaining water, **HPTILs** are obtained under a vacuum (**Scheme S1**). The as-prepared ILs, **HPTIL,** are fully characterized by NMR, FTIR, and LC-MS analysis.

Synthesis and characterization of mHPTIL

The nano-GUMBOS **mHPTIL** has been fabricated using the reprecipitation method, and with the help of various analytical techniques, it has been characterized. A stock solution of **HPTIL** (1 mg/mL) was prepared in dimethyl sulfoxide (DMSO) by dissolving the appropriate amount of **HPTIL** in the solvent. Aqueous suspended nanoparticles were formed by quickly injecting 60 µL of the HPTIL solution into water, followed by sonication for approximately 30 minutes. The formation of nanoparticles, **mHPTIL,** is confirmed by SEM, DLS, UV−visible absorption, and emission spectral analysis.



Scheme S1. Synthetic procedure of **HPTIL.**

Characterization: (^{1}H NMR, $CDCl_3$, 400 MHz, in ppm units): 10.02 (s, 1H), 9.30 (s, 1H), 9.29 (s, 1H), 9.26 (s, 1H), 9.19 (d, 1H), 8.83 (s, 1H), 8.50 (d, 1H), 1.9 - 0.8 (various m, 204H) (**Fig. S1**).

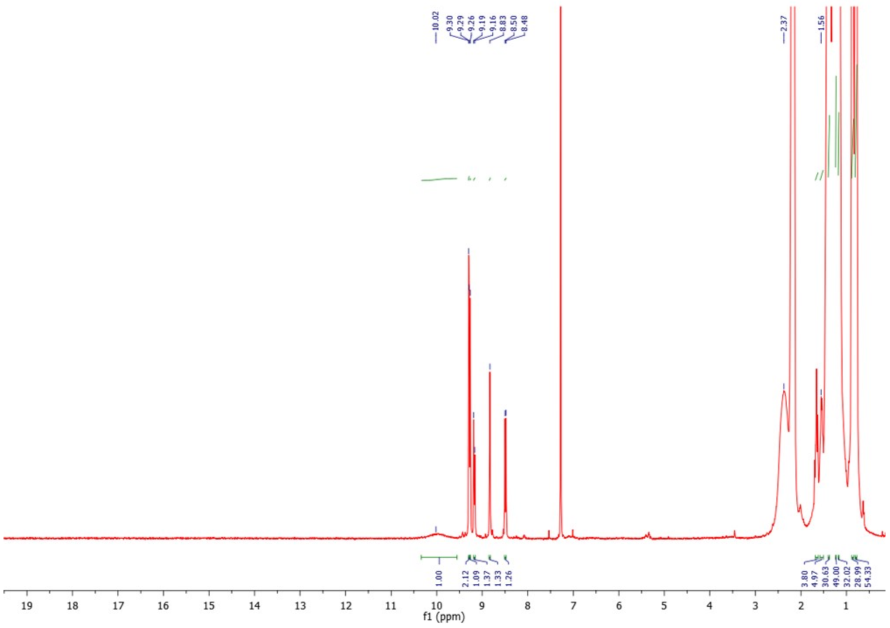
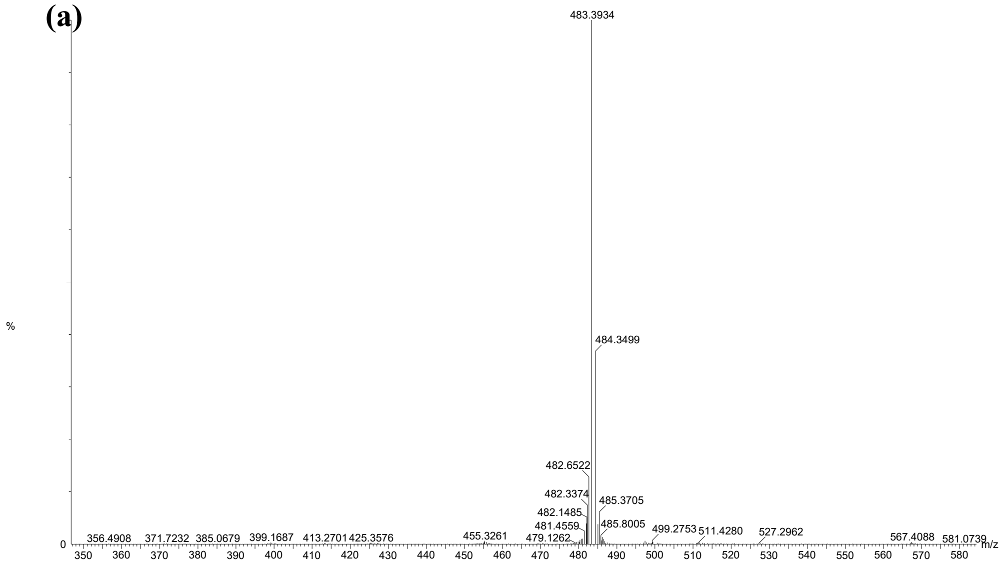


Fig. S1 ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of **HPTIL.**



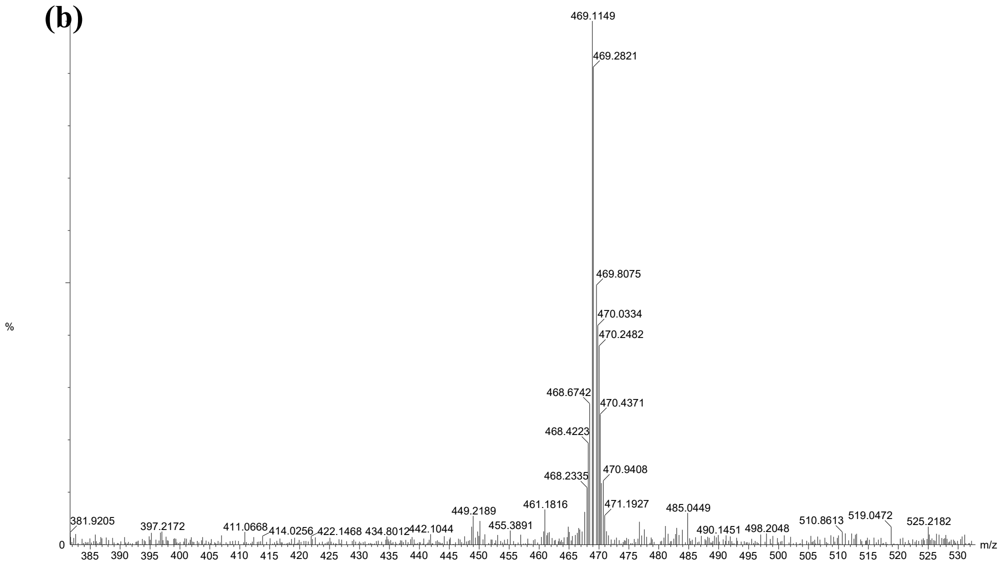


Fig. S2 LC-MS spectra of **HPTIL (a)** positive ion mode and **(b)** negative ion mode.

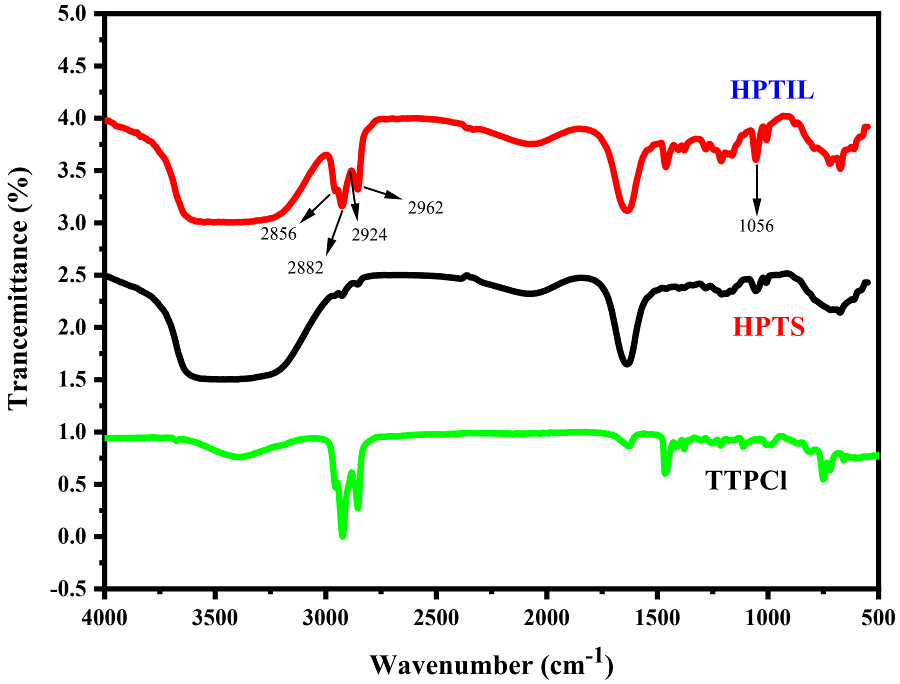


Fig. S3 FTIR spectra of TTPCl - IL, HPTS, and **HPTIL.**

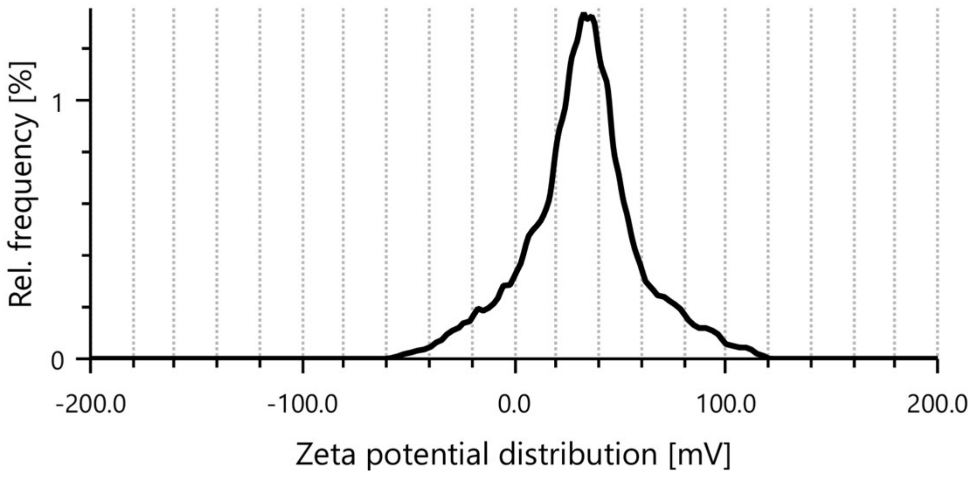


Fig. S4 The relative frequency vs. zeta potential distribution curve for the determination of the zeta potential of **mHPTIL**.

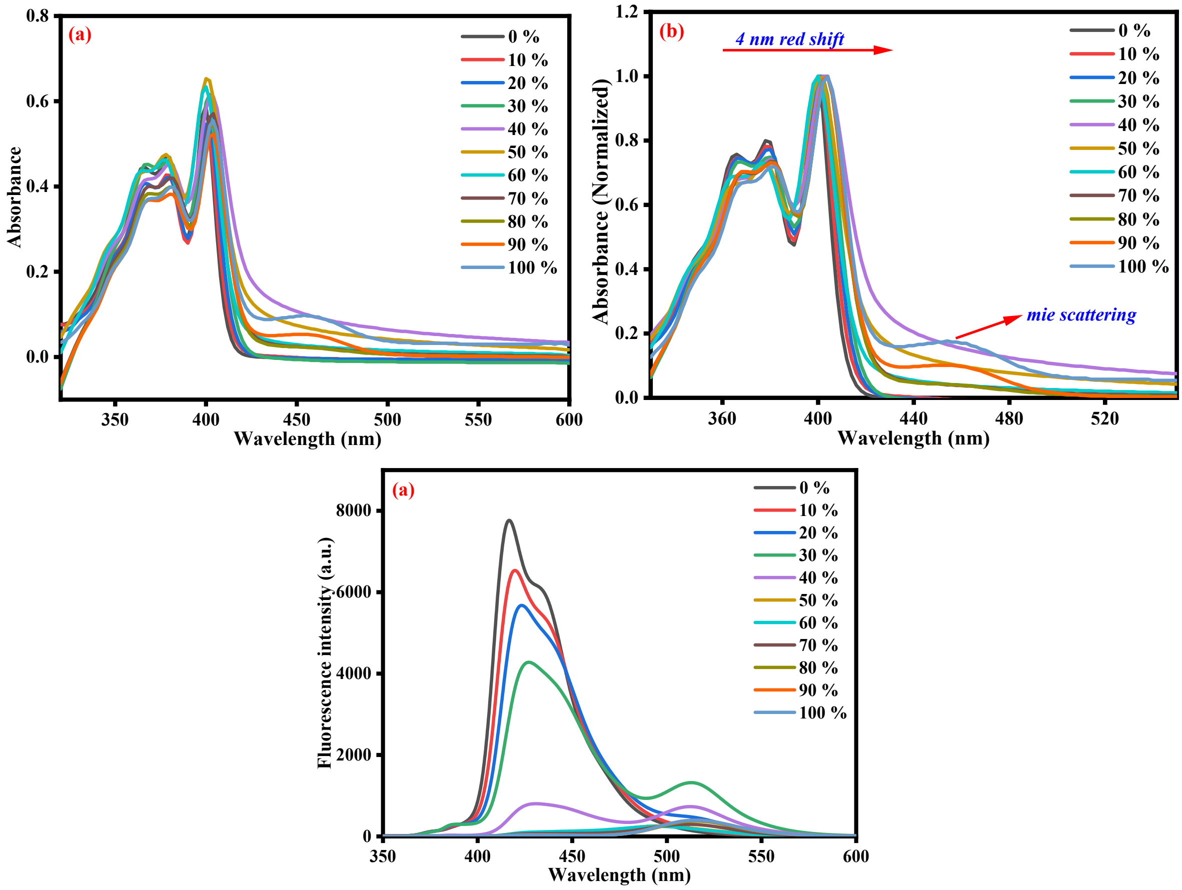


Fig. S5 (a) UV-visible absorption spectra of optode **HPTIL** in different percentages of water. **(b)** UV-vis absorption spectra with normalized absorbance of optode **HPTIL** in different percentages of water demonstrating the distinct 4 nm red shift with an increase in Mie scattering. **(c)** Fluorescence spectra of **HPTIL** in water-DMSO mixed solvents demonstrating ACQ.

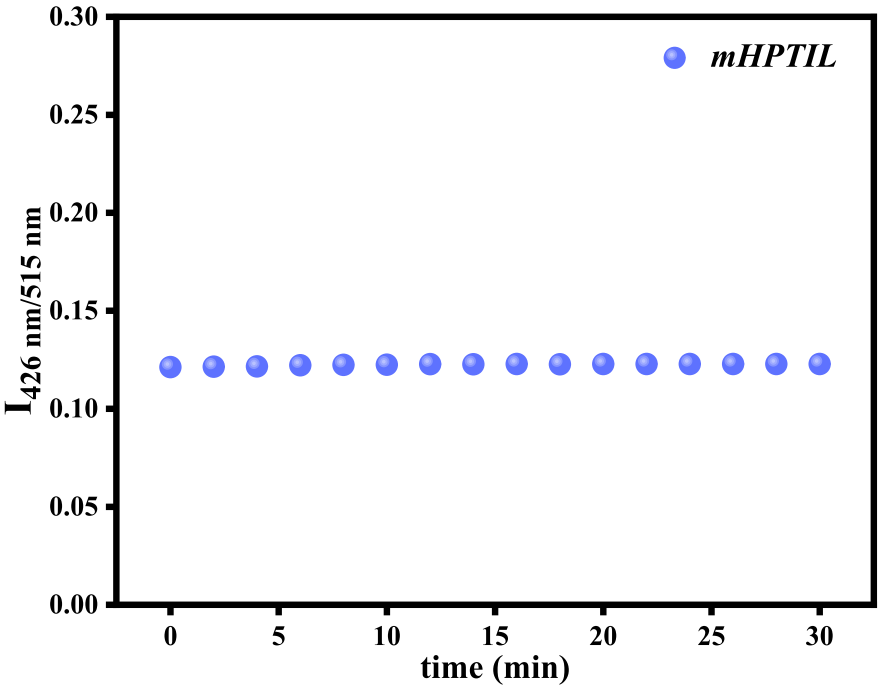


Fig. S6 Time-dependent stability of **mHPTIL** under continuous UV exposure.

Table. S1 Calculated ΔG values in various temperatures.

Temperature(℃)	**ΔG (**J mol^{-1}**)**	**ΔH (**J mol^{-1}**)**	**ΔS (**J mol^{-1} K^{-1}**)**
15	-24728.52686	-23440.58	85.86
25	-25587.15626		
35	-26445.78567		
45	-27304.41507		
55	-28163.04448		
65	-29021.67388		

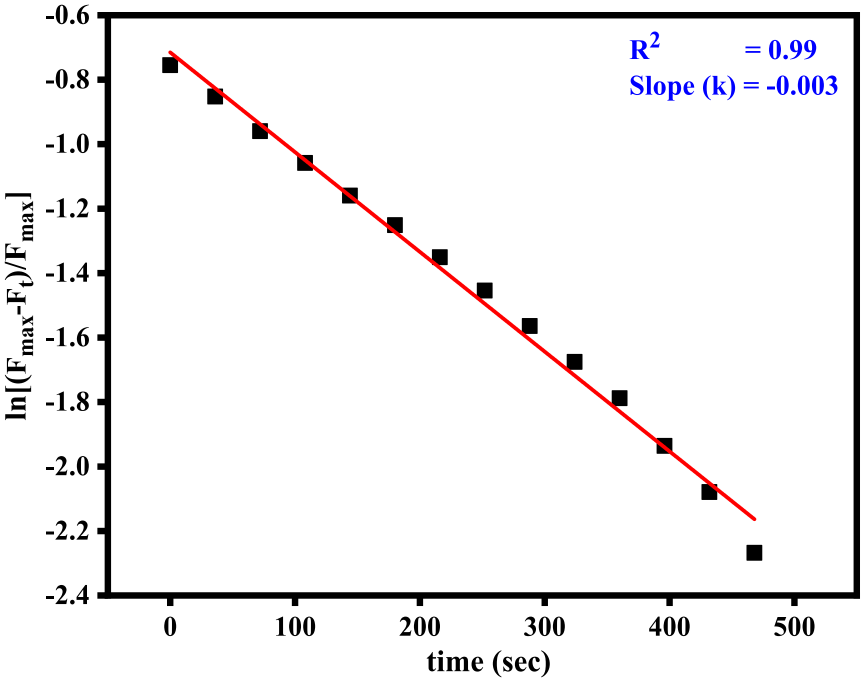


Fig. S7 Kinetics plot of **mHPTIL** towards Hg^{2+} showing pseudo-first-order kinetics

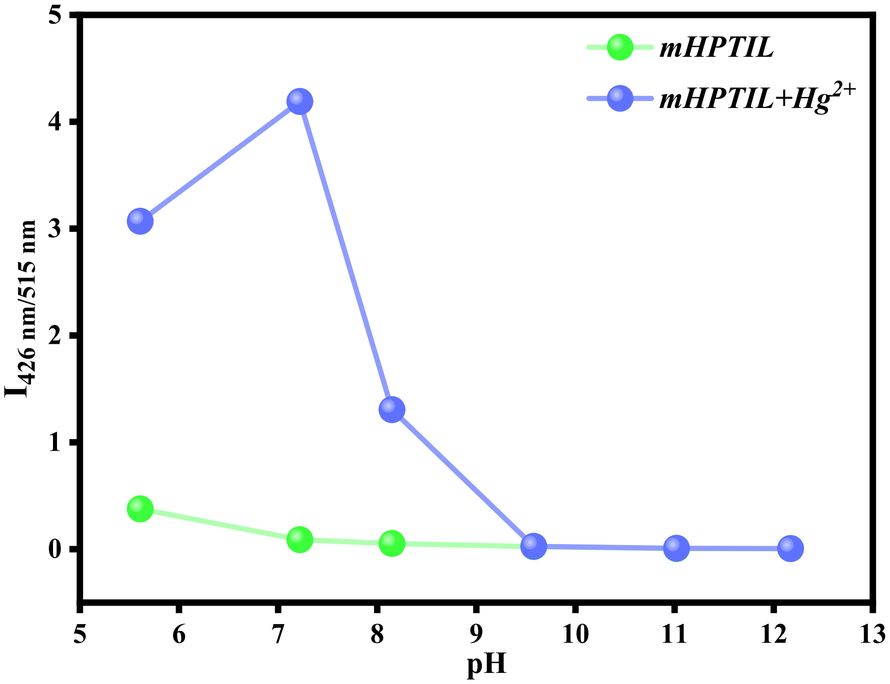


Fig. S8 The impact of pH on photoluminosity of **mHPTIL** with and without Hg^{2+} ions at 25 °C.

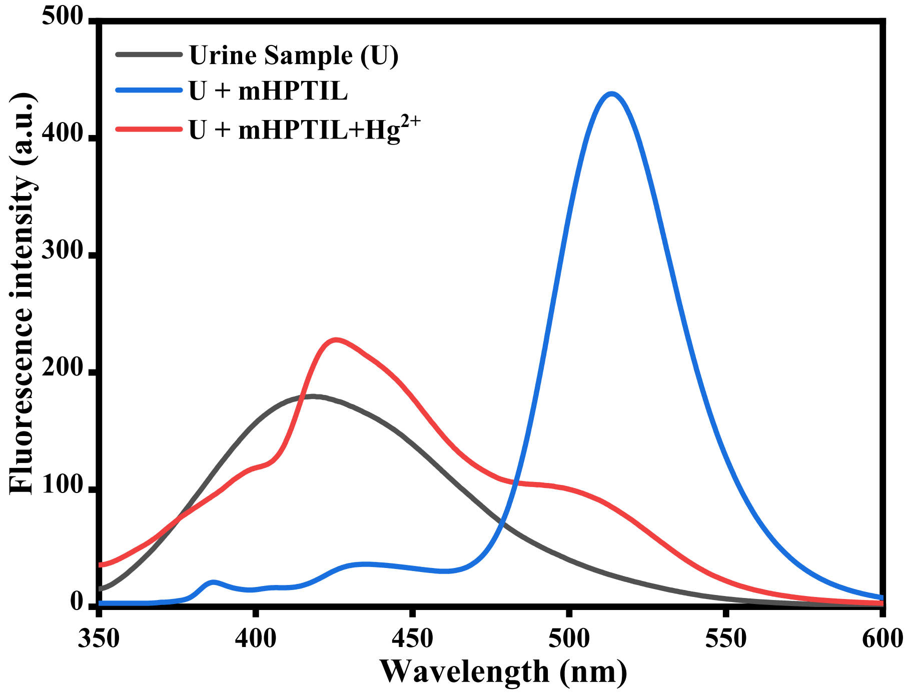
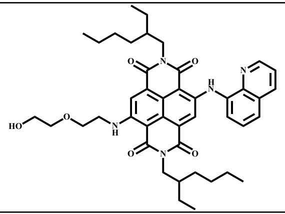
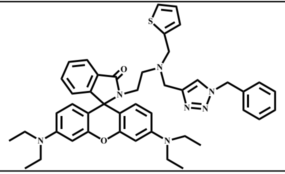
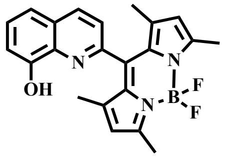
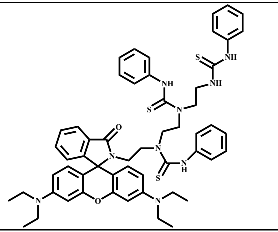
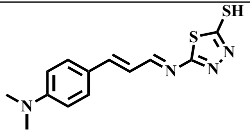
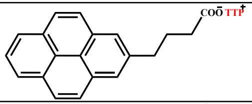
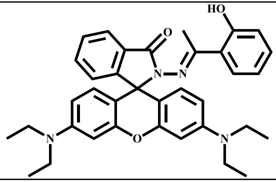
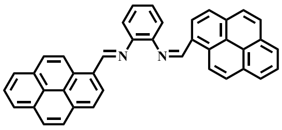
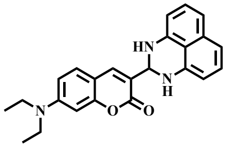
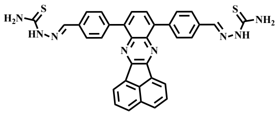
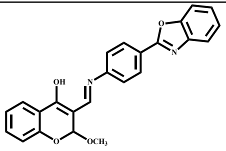
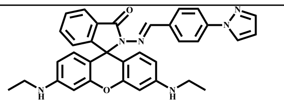
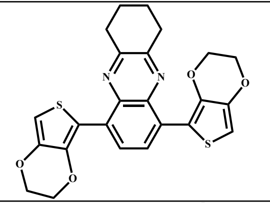
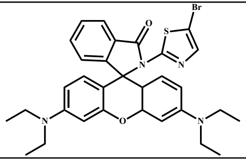
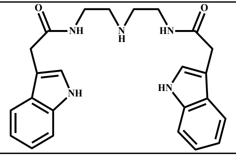
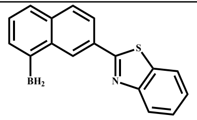
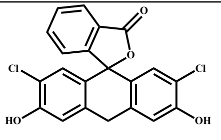
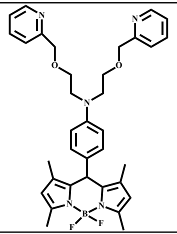
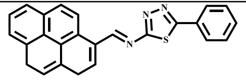
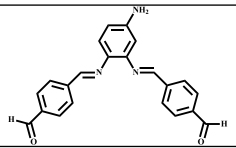
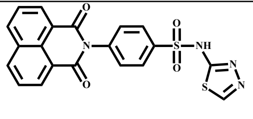


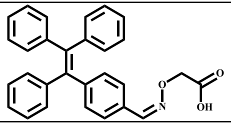
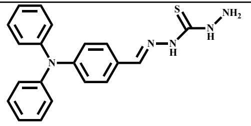
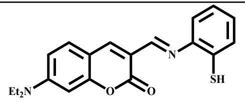
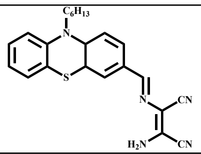
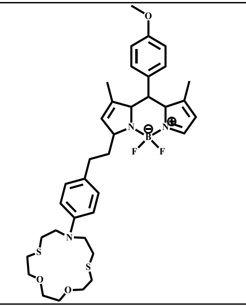
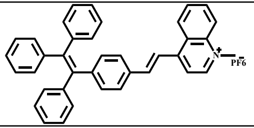
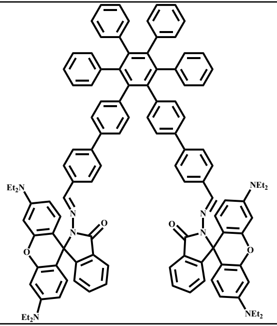
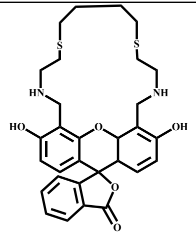
Fig. S9 Distinctions in photoluminescence intensity detected in urine, urine mixed with **mHPTIL,** and urine mixed with **mHPTIL** and Hg^{2+} ions.

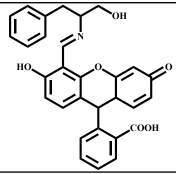
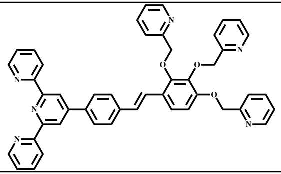
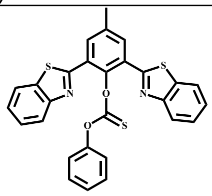
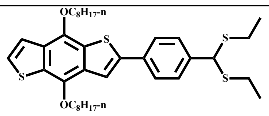
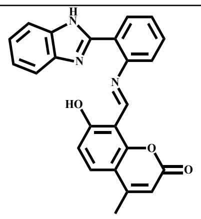
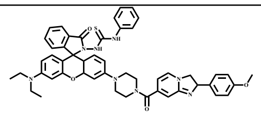
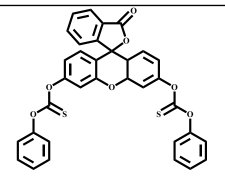
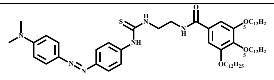
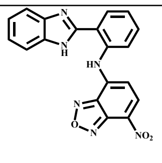
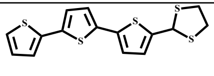
Table. S2 Comparison with the detection limits of sensing Hg^{2+} ions by various sensors

Sl. No	**Sensor**	**Solvent**	**Sensor type**	**LOD**	**LOQ**	**Portability &cost-effective**	**Application**	**Ref.**
1		Acetone medium	Turn-on	3 µM	10 µM	YES	NA	[2]
2		ACN	Turn-on	16 nM	53.3 nM	NA	Metal ion sensor and real sample analysis	[3]
3		dioxane-water (1:3, v/v) solvent	Turn-off	5 µM	16.6 µM	NA	Metal ion sensor	[4]

4		MeCN–HEPES (9 : 1, v/v) medium	Turn-on	30 µM	100 µM	NA	Water sample analysis	5
5		1:1 (v/v) acetonitrile/HEPES Buffer (10mM, pH 7.4)	Turn-on	2 µM	6.66 µM	NA	Water sample	6
6		THF	Turn-off	17 nM	56.66 nM	YES	Real sample analysis	7
7		EtOH–water (2 : 1, v/v)	Turn-on	150 nM	500 nM	NA	NA	8
8		aqueous MeCN	Turn-off	90 nM	300 nM	YES	Paper-test strip application	9
9		CH_3CN/H_2O (v/v = 3/7) solutions	Turn-on	1.08 µM	3.6 µM	NA	Bioimaging in living cells and zebrafish	10
10		DMSO/H_2O (v/v=9/1) buffered by 50mM Tris-HCl at pH=7.0.	Turn-off	0.90 µM	3 µM	YES	Water sample	11
11		50% (1:1, v/v) H_2O/DMSO	Turn-on	0.4 µM	1.33 µM	YES	Real sample analysis	12

12		DMSO –water (1 : 1, v/v)	Turn-on	20.7 nM	69 nM	YES	Water sample analysis	13
13		Mixture of THF and water	Turn-off	8.0 ppm	26.6 ppm	YES	Paper-based sensor	14
14		MeCN/ H_2O (8:2 v/v)	Turn-on	6.9 µM	23 µM	NA	Real-time monitoring	15
15		H_2O: EtOH= 7:1, v/v	Turn-off	22.5 µM	75 µM	NA	NA	16
16		Dioxane- H_2O (9:1, v/v)	Turn-off	30 µM	100 µM	NA	NA	17
17		Acetate buffer aqueous 10% DMSO	Turn-off	15 µM	50 µM	NA	NA	18
18		CH_3CN -H_2O (1:1)	Turn-on	18.1µM	60.33 µM	YES	NA	19
19		Buffer-CH_3CN (3:7, v/v, 10 mM, pH=7.4)	Turn-on	36 nM	120 nM	YES	NA	20
20		H_2O (Ph 7.4)	Turn-off	0.061 µM	0.2 µM	YES	NA	21
21		DMSO-Water (1:99, v/v, HEPES buffer	Ratio-metric	14.7 nM	49 nM	NA	NA	22

		pH=7.2						
22		Ethanol-water (3:7)	Ratiometric	45.4 nM	151.33 nM	NA	Real sample analysis	23
23		DMSO/Tris-HCl (8:2, v/v, pH=7.0)	Turn-off	31 nM	103.33 nM	YES	Real sample analysis	24
24		HEPES buffer (20 mM, 3:7 CH_3CN/H_2O)	Turn-off	50 nM	166.67 nM	YES	Solid sensor	25
25		Ethanol-water (6:4, v/v)	Turn-off	17.8 nM	59.33 nM	NA	NA	26
26		CH_3CN-H_2O (5:95, v/v)	Turn-on	99 ppm	330 ppm	NA	NA	27
27		Aqueous (1% DMSO)	Turn-on	591.9 nM	1.9 µM	NA	NA	28
28		MeCN–water (1 : 1, v/v)	Turn-on	100 nM	0.33 µM	NA	NA	29
29		MeOH-Tris-HCl (95:5, v/v, pH=7.2)	Turn-off	7.38 nM	24.6 nM	NA	NA	30

30		Aqueous medium	Turn-off	0.34 µM	1.13 µM	YES	Real-time sensing	31
31		Aqueous medium	Ratiometric	0.138 ppm	0.46 ppm	YES	Real sample analysis	32
32		EtOH–water (5/5, v/v, HEPES pH = 7.4)	Turn-on	55 nM	183.3 nM	NA	NA	33
33		THF–water (1 : 1, v/v)	Ratiometric	0.31 µM	1.03 µM	NA	NA	34
34		HEPES buffer/ DMSO (v/v = 9 : 1, pH = 7.2)	Turn-on	70 nM	0.23 µM	NA	biological systems	35
35		PBS/EtOH (9 : 1, v/v) medium	Turn-on	9.1 nM	30.33 nM	NA	Real sample analysis	36
36		HEPES buffer solution (20 mM, pH 7.4, 1% EtOH)	Turn-on	40 nM	133.3 nM	NA	NA	37
37		ACN	Colorimetric	9.22 nM	30.73 nM	NA	Multiple metal sensor	38
38		methanol-water (1 : 1) mixture	Colorimetric	47 µM	156.66 µM	YES	Real sample analysis	39
39		EtOH–water (1 : 1,	Turn-on	10.3 nM	34.33 µM	YES	Real sample analysis	40

		v/v)						
40	**mHPTIL**	**100% water**	**Ratiometric**	**1.67 nM**	**5.56 nM**	**YES**	**Real sample analysis**	**This work**

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