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Carbazole–Thioether Conjugates for Highly Selective and Sensitive Fluorescent Sensing of Hg^{2+} Ions

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1. **Characterisation data**

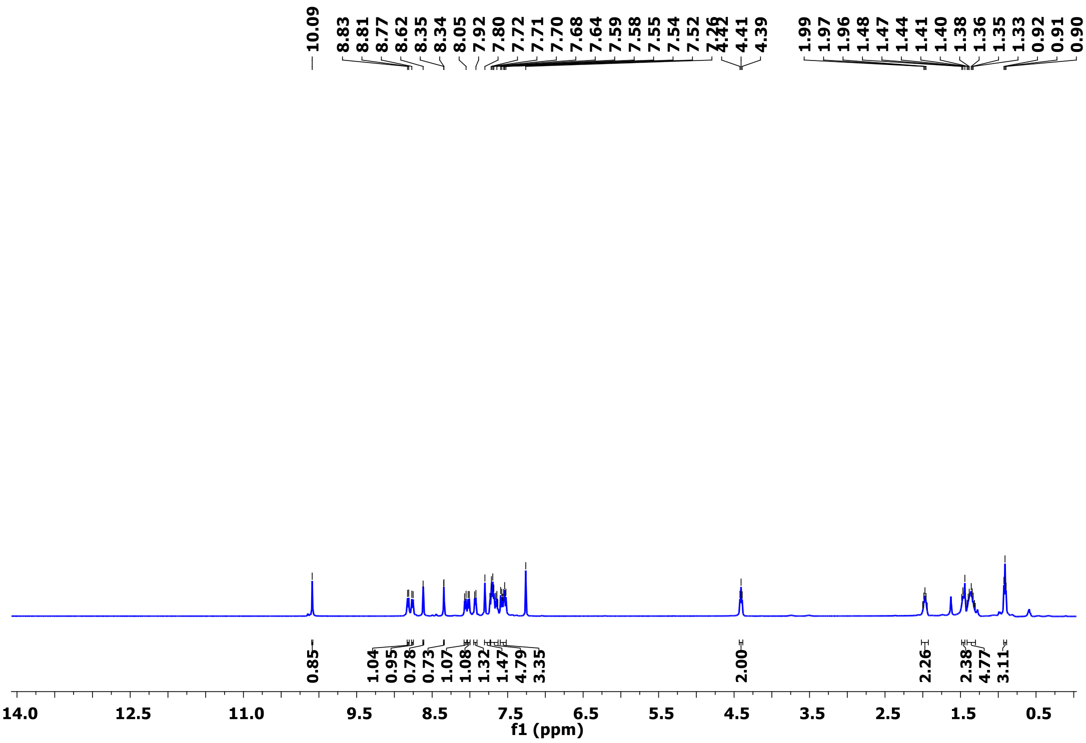

Figure S1: ^{1}H NMR of **3**

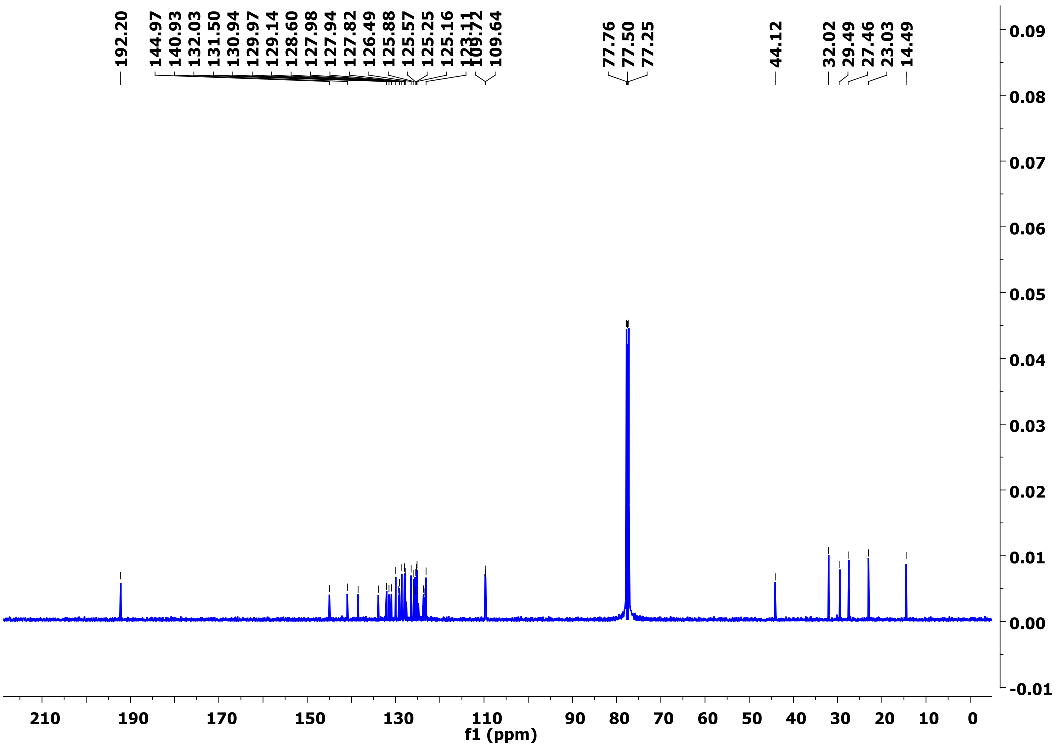

Figure S2: ^{13}C NMR of **3**

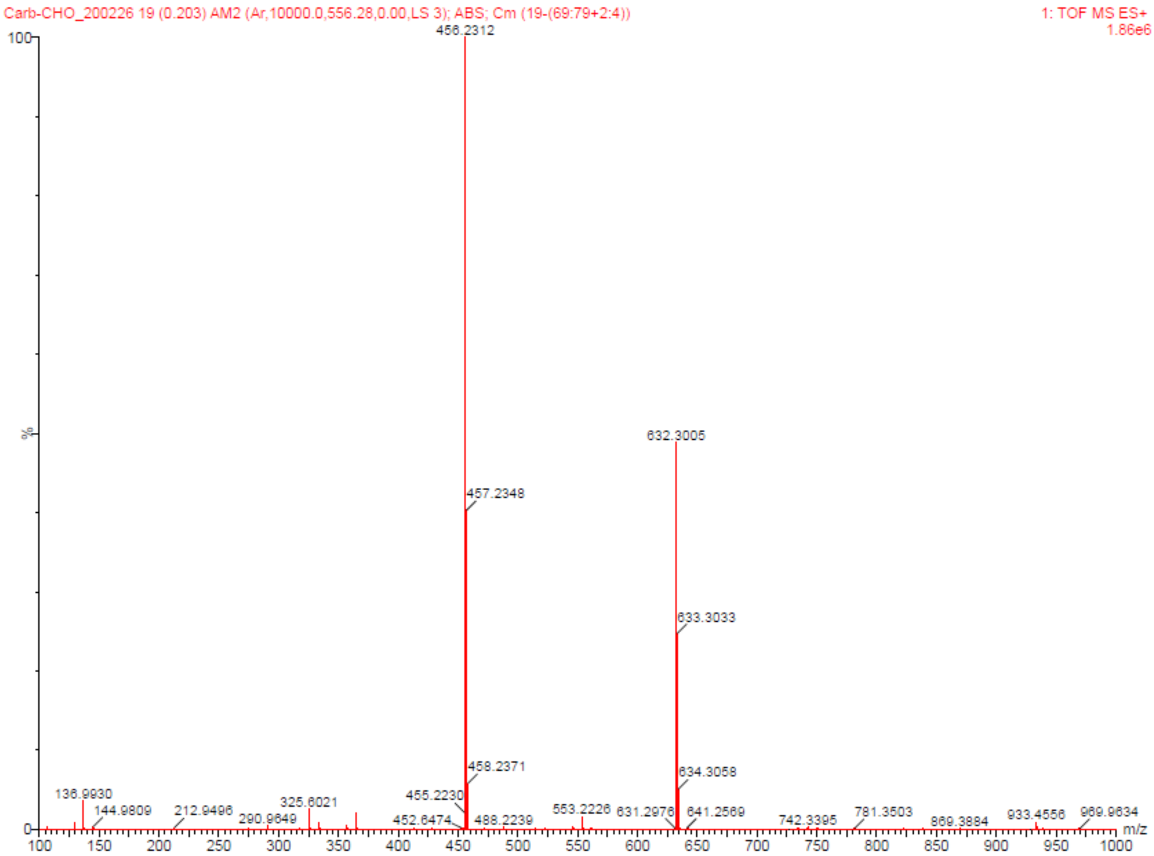

Figure S3: HRMS spectrum of **3**

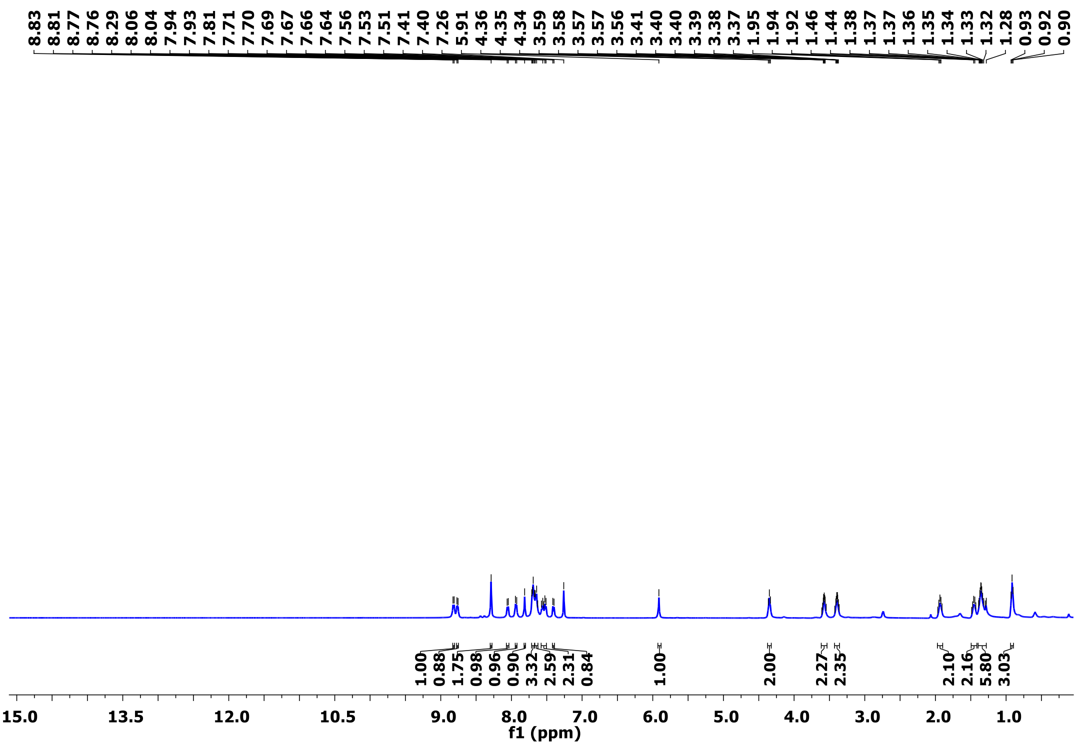

Figure S4: ^{1}H NMR of **Thiol-1**

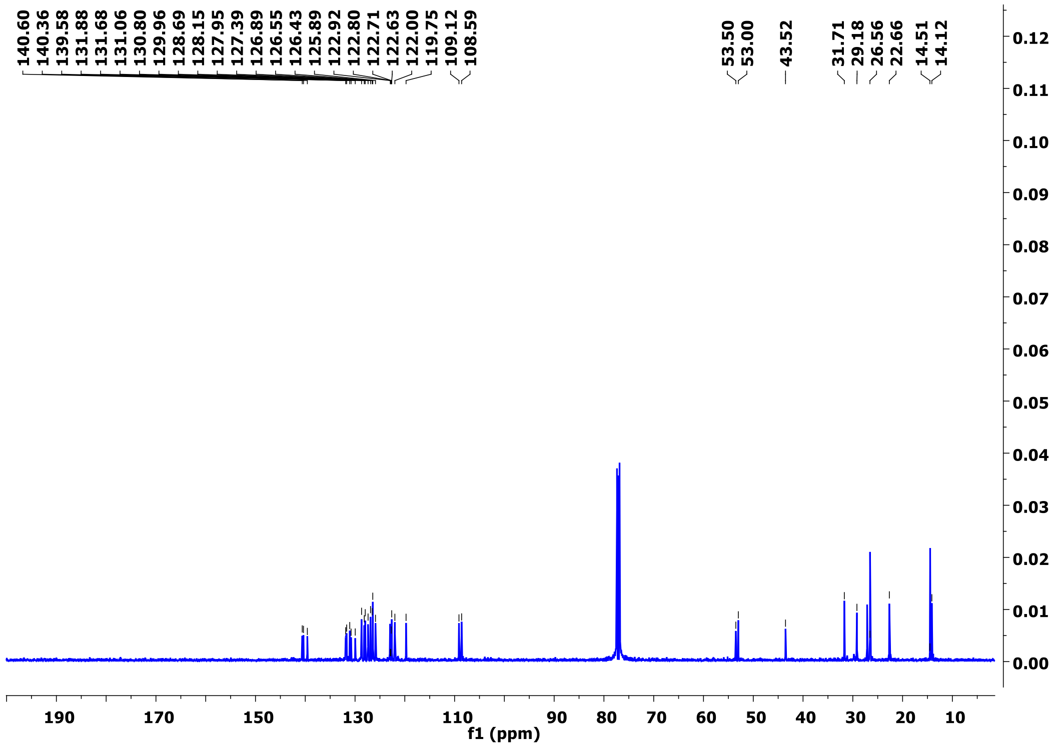

Figure S5: ^{13}C NMR of **Thiol-1**

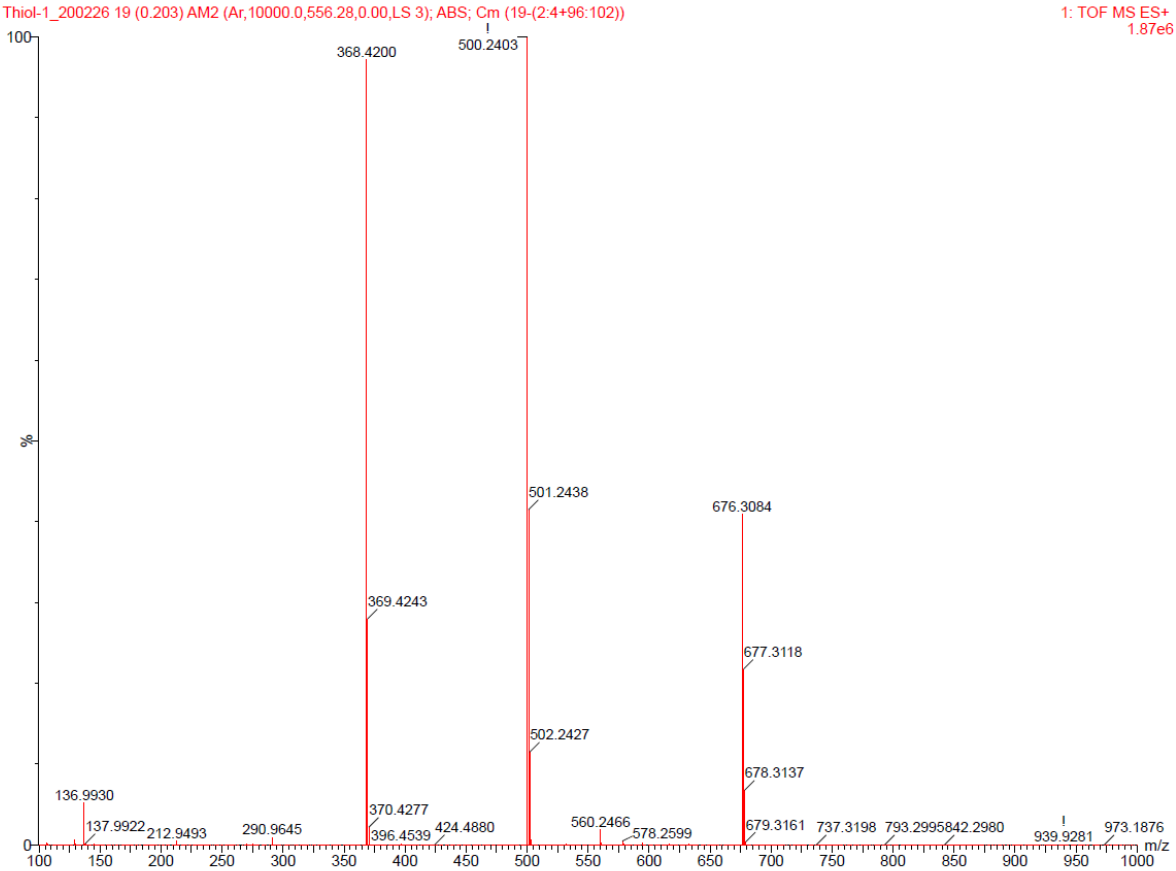

Figure S6: HRMS spectrum of **Thiol-1**

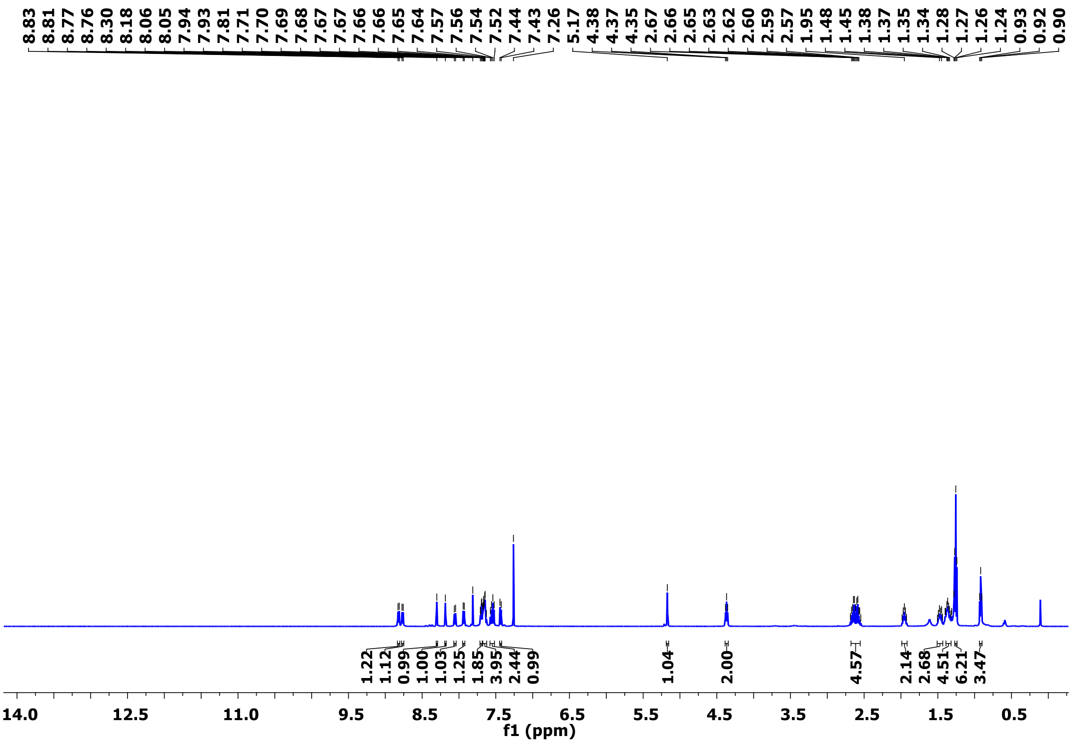

Figure S7: ^{1}H NMR of **Thiol-2**

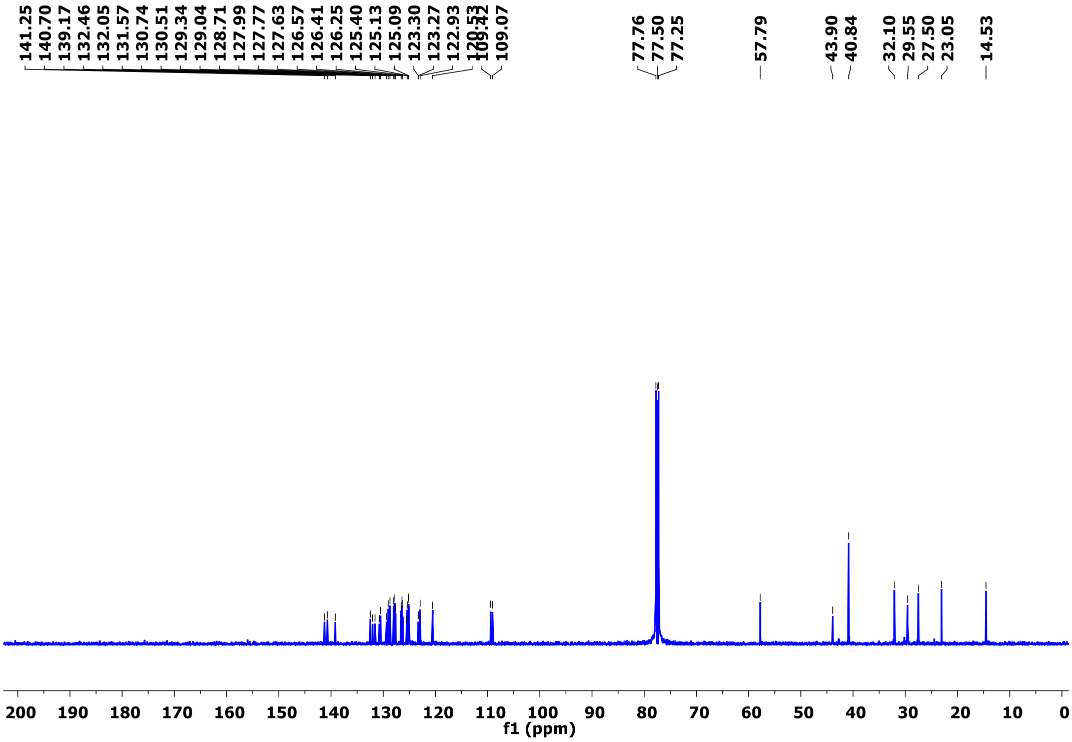

Figure S8: ^{13}C NMR of **Thiol-2**

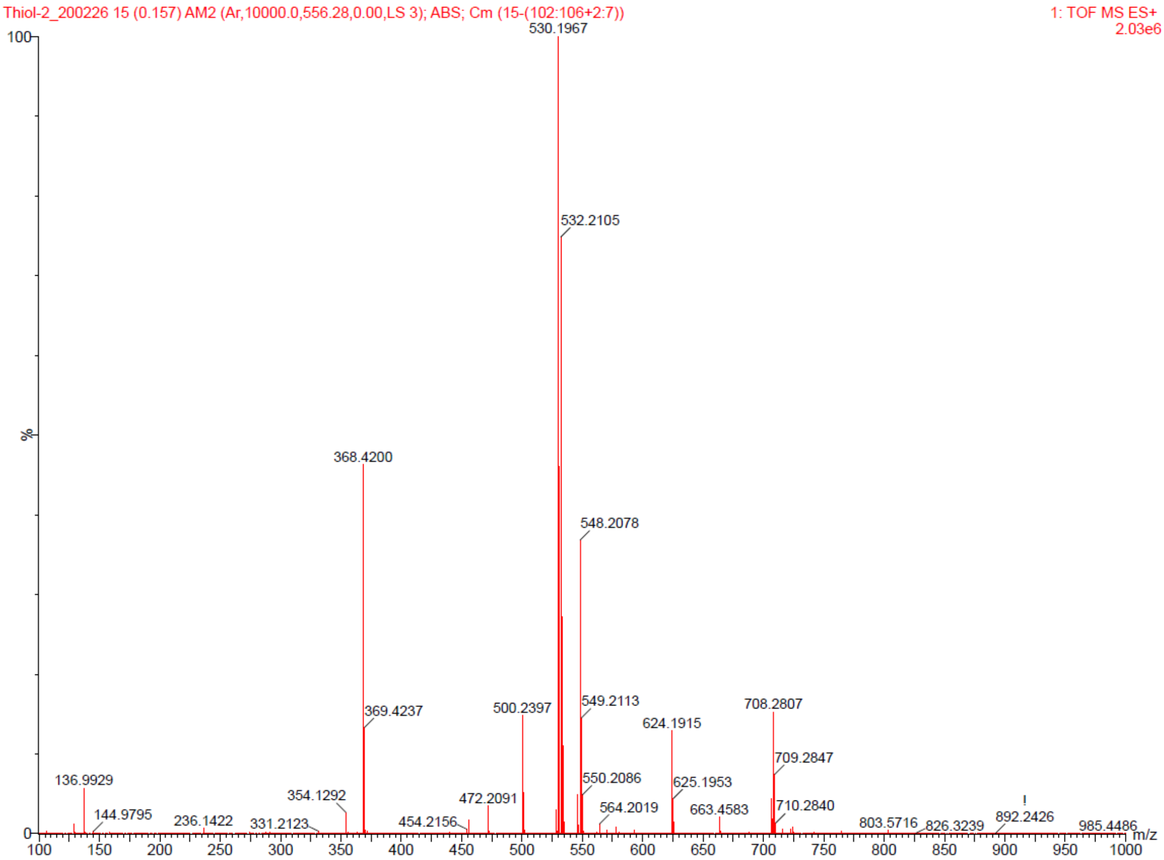

Figure S9: HRMS spectrum of **Thiol-2**

2. Photophysical and Sensing studies

2.1 UV–Visible Titration studies

UV–Visible titration of **Thiol-1** and **Thiol-2** (1 mL, 1×10^{-5} M) were carried out by placing the ligands in a cuvette and was titrated with metal ion (5×10^{-4} M) solution in DMF and PBS buffer (1:1, *v*/v, pH 7.4). Stock solutions of various cations (Na^+, K^+, Al^{3+}, Fe^{3+}, Cd^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Ag^+, Cu^{2+}, $Zn^{2+}$and Hg^{2+}) were prepared in deionized water.

2.2 Fluorescence titration studies

The fluorescence titration experiments were carried in DMF and PBS buffer (1:1, *v*/v, pH 7.4). The stock solution of **Thiol-1** and **Thiol-2** is 1×10^{-5} M placing 1 mL quartz cuvette and followed metal ions 1×10^{-4} M were added gradually increasing amount. For each addition, at least three fluorescence spectrums were recorded repeatedly at 298 K to obtain a concordant value. **Thiol-1** and **Thiol-2** were excited at 310 nm and the emission was monitored at 475 nm respectively, whereas slit width was kept 10 nm for excitation and 5 nm for emission.

2.3 Details about the association constant[1,2]

For compound Thiol-1:

DMF and PBS buffer (1:1, *v*/v, pH 7.4) of **Thiol-1** (1 mL, 1 × 10^{-5} M) were placed in a cuvette and was titrated with incremental amount of metal ions (1×10^{-4} M). The changes associated with the absorption band at 465 nm were monitored. The calculation for **Thiol-1** is shown below.

Thiol-1+ [Metal] ⟷ **Thiol-1**•[Metal]

I = k_s[**Thiol-1**] + k_p[**Thiol-1**•[Metal]]

I_0 = k_s[**Thiol-1**]$_0$

[**Thiol-1**]$_0$ = [**Thiol-1**] + [**Thiol-1**•[Metal]]

K = [**Thiol-1**•[Metal]^2]/ [**Thiol-1**] [Metal] (K is association constant)

I/I_0 = (k_s[**Thiol-1**] + k_p[[Metal ion]• **Thiol-1**]) / k_s[**Thiol-1**]$_0$

= (k_s[**Thiol-1**] + k_p[[Metal ion]• **Thiol-1**]) / k_s ([**Thiol-1**] + [Metal ion• **Thiol-1**])

I/I_0 = (1 + (k_p/k_s) K[Metal])/(1 + K[Metal]^2) (1 – I/I_0)/ [Metal]

(1-I/I_0) = K[Metal] (I/I_0)- αK[Metal]

(1-I/I_0)/ [Metal] = K(I/I_0)- αK

Here, I_0 is initial absorbance of respective band, I is absorbance of respective band I upon addition of eqve of metal ions.

The association constant is obtained from the slope of 1/I_0-I vs 1/[Metal].

A= εCl, here ε= molar extension constant, C= concentration of sample, l = path length

= εl [C]

=Ks [C] for substrate

=Kp [C] for product

2.4 The detection limit of Thiol-1

The Limit of Detection of the ligands **Thiol-1** toward Hg^{2+} ions were evaluated through absorbance titration experiments. The detection limits were calculated based on the changes in fluorescence at 475 nm as a function of increasing concentrations of Hg^{2+} ions. A calibration curve was constructed by plotting the change in fluorescence at 475 nm against the corresponding metal ion concentration. The slope of this linear plot was determined via least-squares fitting and used in the subsequent calculations. The limit of detection (LOD) was calculated using the standard equations:

LOD=3×SD/Slope

where **SD** represents the standard deviation of the blank measurements (i.e., the baseline fluorescence in the absence of metal ions), and the **Slope** corresponds to the sensitivity derived from the linear portion of the calibration curve. These parameters provide quantitative insights into the lowest concentration of metal ions that can be reliably detected (LOD) using the respective ligand systems. The observed LOD values underscore the effectiveness of the thiol based chemosensor in recognizing trace levels of Hg^{2+} ions, highlighting their potential utility in analytical sensing applications.

Table S1. Calculation of standard deviation

	Thiol-1
1	12359
2	13247
3	12945
4	11982
5	13024
6	12541
	σ = 473.4

Table S2. Photophysical properties of **Thiol-1** with Hg^{2+} ions

S. No	Compounds	Association constants[b] ($\times10^6$ M^{-1})	Detection limit (nM)[c]	Quantum yileds[a]
1	**Thiol-1**	--	--	0.035
2	**Thiol-2**	--	--	0.064
2	**Thiol-1+Hg^{2+}**	4.7	31	0.24

[a]Quantum yields are calculated using quinine sulphate (0.1 M in H_2SO_4, Φ_F = 0.577) solution as reference and using the following formula $\Phi = \Phi_F \times I/I_R \times A_R/A \times \eta^2 / \eta_R^2$ where Φ = quantum yield, I = intensity of emission, A = absorbance at λ_{ex}, η = refractive index of solvent. c) The quantum yields of compounds are determined in a DMSO. [a]the detection limits calculated via fluorescence titration data. [b] The association constant calculated via UV-Visible titration data.

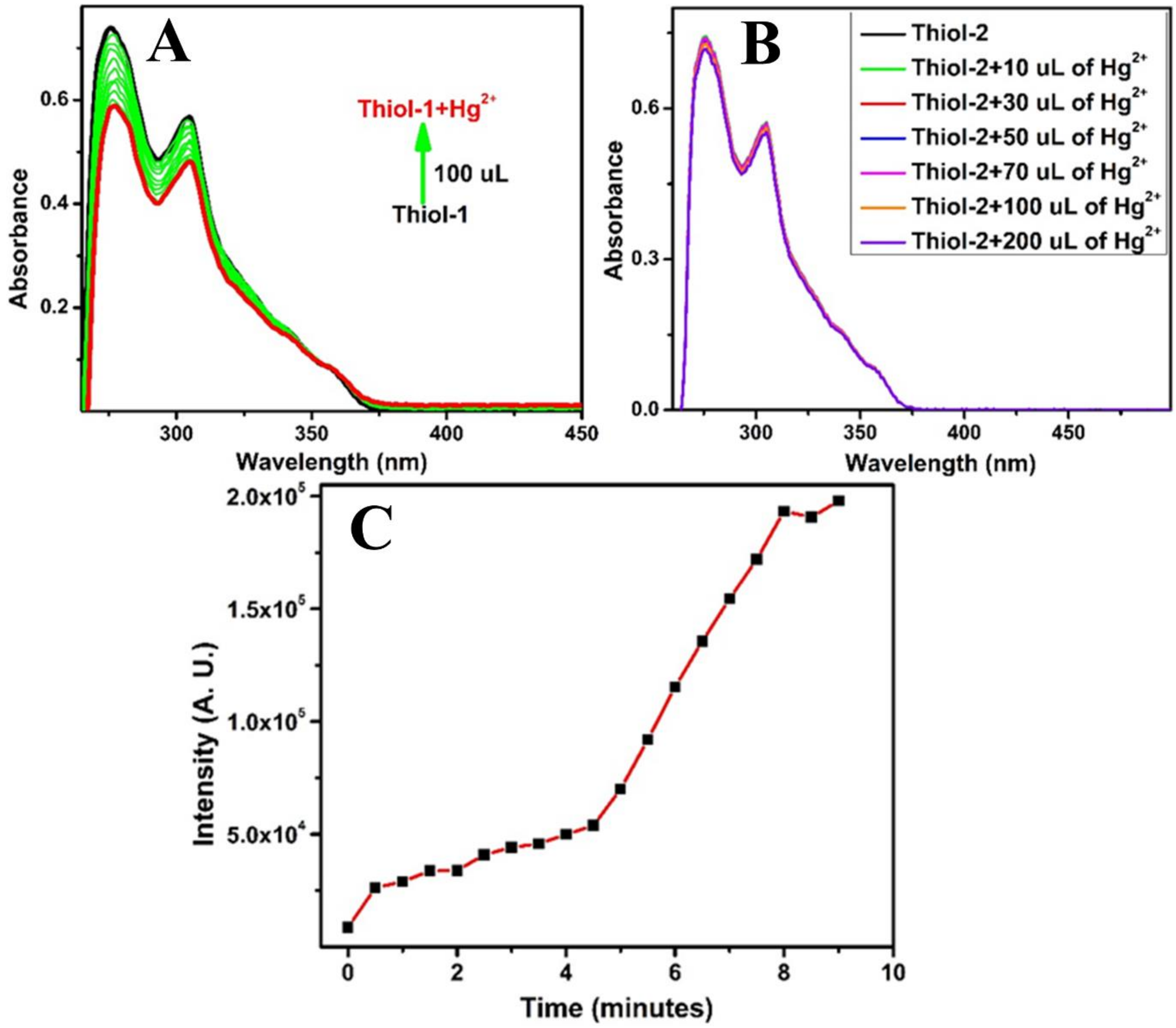

Figure S10. UV-Visible titration of **Thiol-1** (A), **Thiol-2** (B) and response time (C) of **Thiol-1** with Hg^{2+} ions.

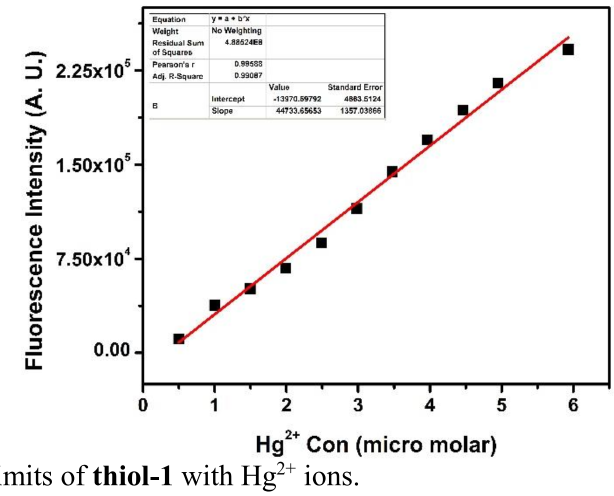

Figure S11. Detection limits of **thiol-1** with Hg^{2+} ions.

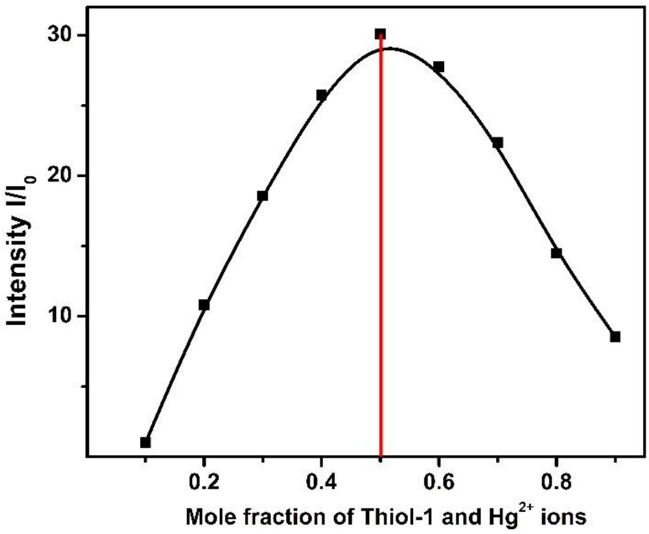

Figure S12. Job plot according to the method of continuous variations, indicating the 1:1 (ligand and metal) stoichiometry of **Thiol-1** with Hg^{2+} ions.

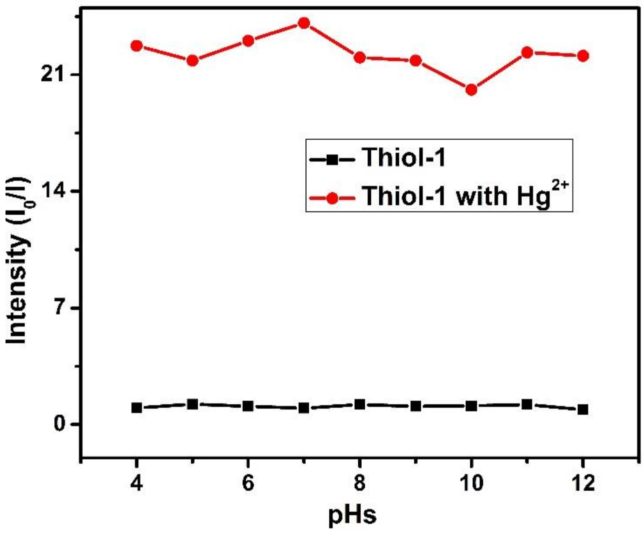

Fig. S13. Different pHs of **Thiol-1** and **Thiol-1+Hg^{2+}**

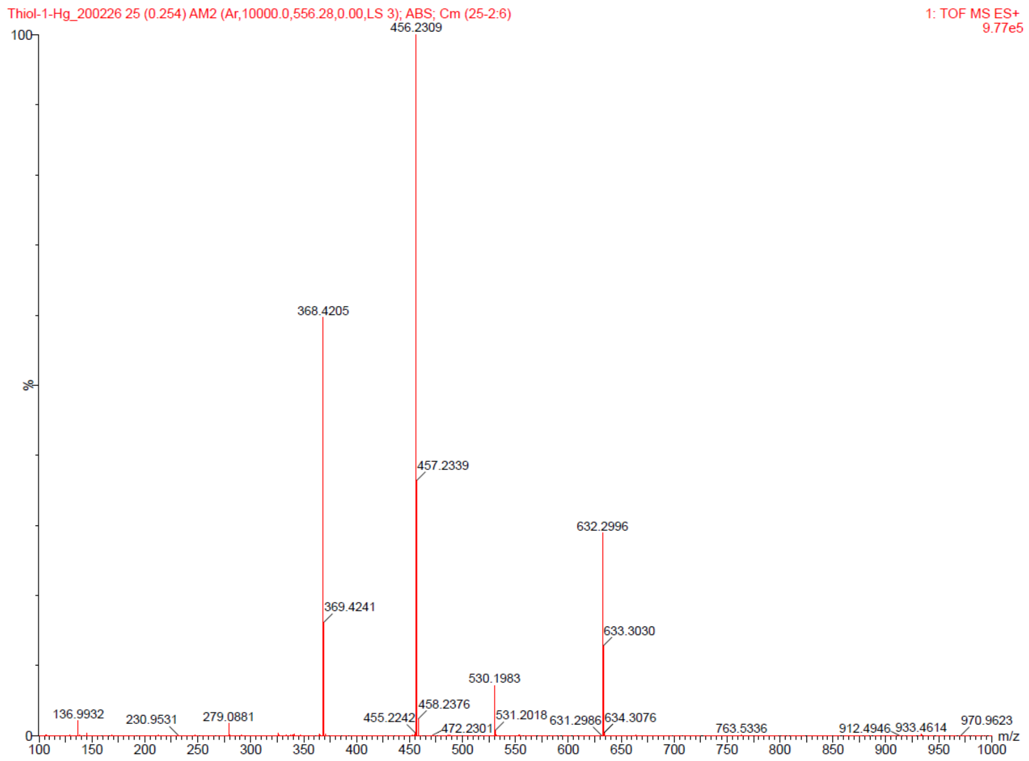

Figure S14. ESI-MS of **Thiol-1** after addition of Hg^{2+} ions.

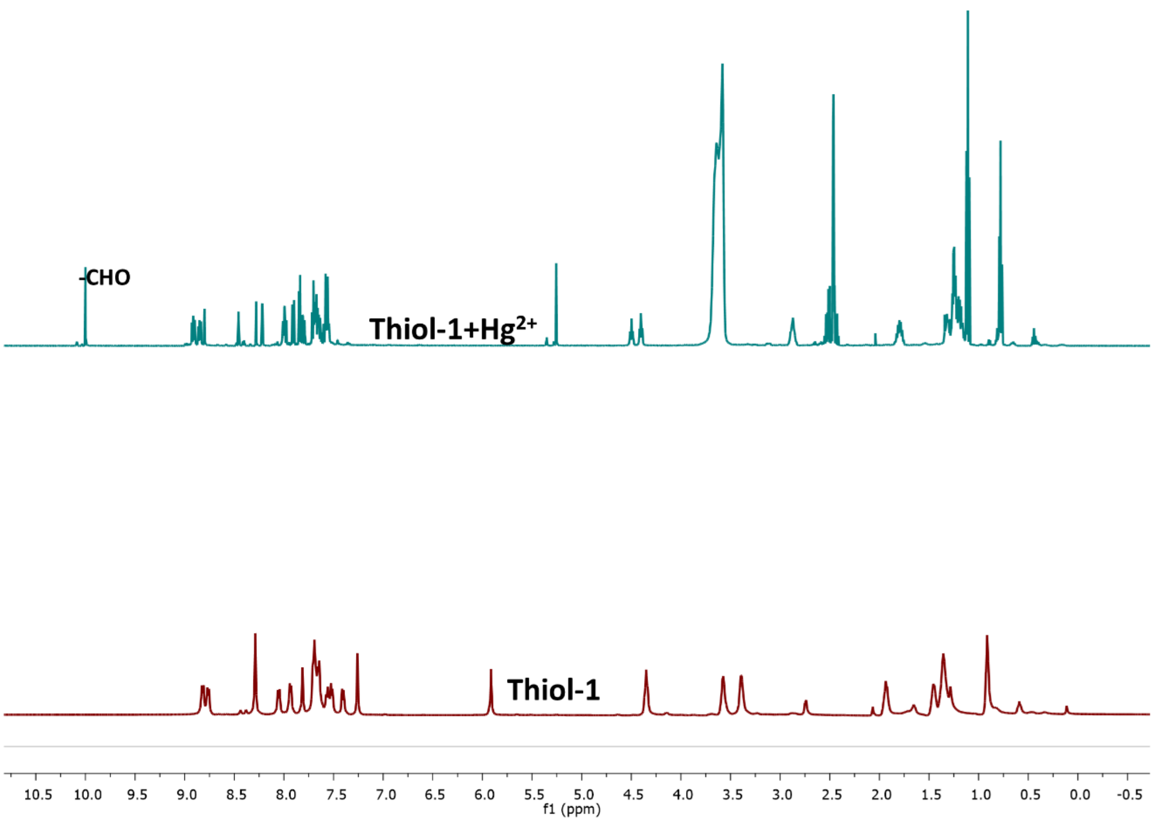

Figure S15. The compared 1H NMR of **Thiol-1** and after addition of **Thiol-1+Hg^{2+}** ions

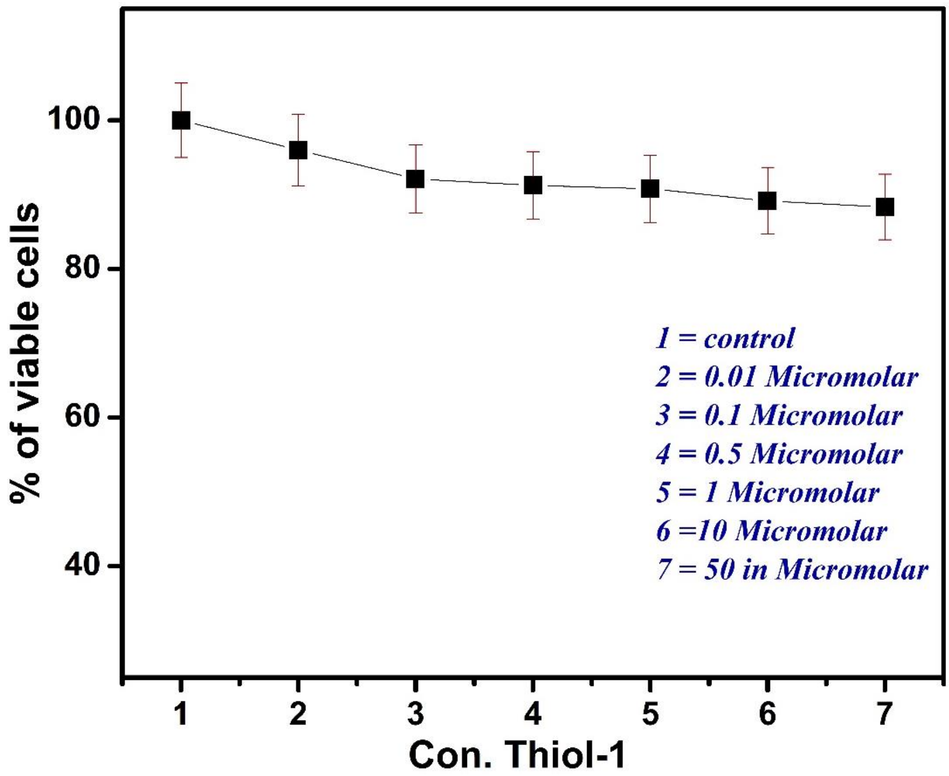

Figure S16. Cell viability of **thiol-1**

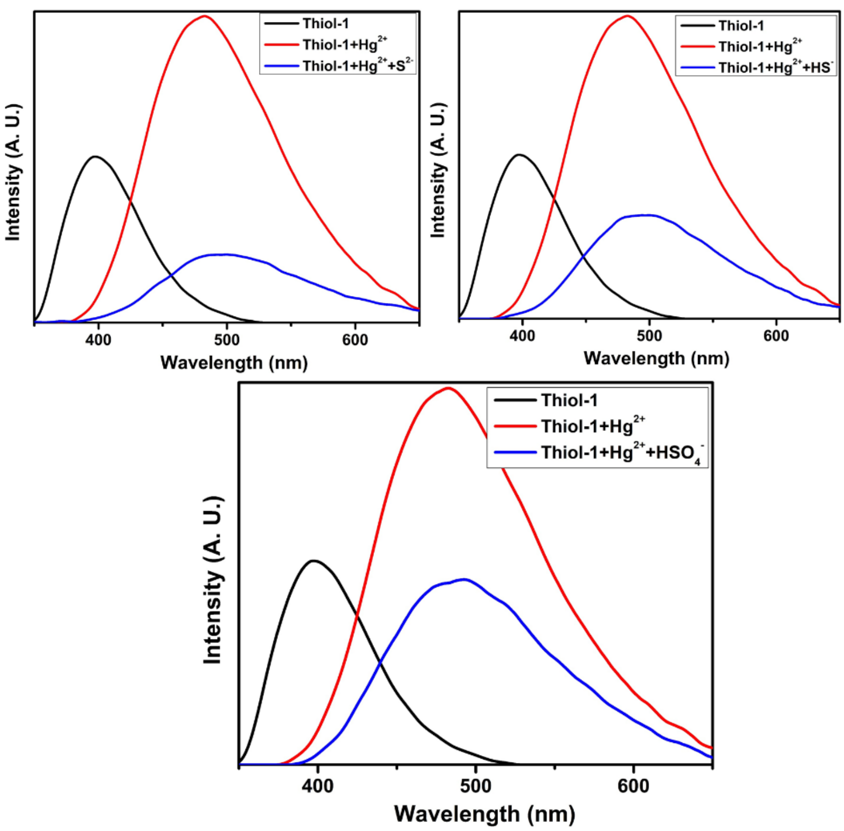

Figure S17. Sensing studies of Thiol-1, Hg2+ and followed by interfering anions such as SH^-, S^{2-}, and HSO_4^-.

3. DFT Calculations

Table S3. Coordination of **Thiol-1**

6	-2.441601000	2.349640000	0.700079000
6	-1.339188000	3.175350000	0.897753000
6	-0.065410000	2.621657000	0.734996000
6	0.099901000	1.254759000	0.389138000
6	-1.025363000	0.446857000	0.201638000
1	-3.437785000	2.759968000	0.838107000
1	-1.477834000	4.214536000	1.180964000
1	-0.912863000	-0.598956000	-0.071440000
6	2.157325000	2.255927000	0.606245000
6	3.551687000	2.372979000	0.613423000
6	4.304784000	1.245921000	0.304958000
6	3.700644000	0.006619000	-0.000554000
6	2.308277000	-0.097790000	0.001877000
6	1.526175000	1.021200000	0.304529000
1	4.043830000	3.311554000	0.849912000
1	5.388002000	1.316961000	0.288125000
1	1.834290000	-1.050038000	-0.225785000
7	1.184811000	3.219046000	0.850115000
6	1.435096000	4.594524000	1.251813000
1	0.622474000	5.212052000	0.854442000
1	2.350278000	4.927908000	0.751091000
6	1.556149000	4.775676000	2.768954000
1	1.742487000	5.827908000	3.012305000
1	0.636461000	4.463242000	3.274164000
1	2.382223000	4.178583000	3.168496000
6	4.540414000	-1.213725000	-0.304841000
1	3.883234000	-2.036976000	-0.604826000
6	5.992602000	-3.428009000	0.753175000
1	6.631835000	-3.353512000	-0.130710000
1	5.114868000	-4.029980000	0.490821000
6	4.625542000	-0.766525000	-3.099977000
1	4.030081000	-1.680836000	-3.206481000

1	3.941655000	0.061607000	-2.888130000
6	6.747313000	-4.060129000	1.922514000
1	7.081844000	-5.067417000	1.649381000
1	7.631578000	-3.471615000	2.189138000
1	6.112613000	-4.143678000	2.811218000
6	5.423695000	-0.496483000	-4.375494000
1	6.109837000	-1.320307000	-4.599399000
1	4.742695000	-0.382334000	-5.226701000
1	6.012612000	0.422483000	-4.287227000
16	5.426980000	-1.740170000	1.225308000
16	5.771408000	-0.961760000	-1.672219000
6	-2.308421000	0.984826000	0.351869000
6	-3.506783000	0.107709000	0.211468000
6	-3.613153000	-0.999542000	1.007589000
6	-4.560332000	0.391256000	-0.748841000
6	-4.737512000	-1.883549000	0.962440000
1	-2.829726000	-1.209787000	1.731721000
6	-5.710082000	-0.457691000	-0.815710000
6	-4.461152000	1.476329000	-1.653864000
6	-4.803675000	-3.008588000	1.818197000
6	-5.808364000	-1.616220000	0.062698000
6	-6.716100000	-0.146851000	-1.760694000
1	-3.572771000	2.097349000	-1.629919000
6	-5.459920000	1.748072000	-2.568840000
6	-5.892800000	-3.856503000	1.795685000
1	-3.975946000	-3.193398000	2.499010000
6	-6.911804000	-2.501645000	0.065869000
6	-6.603053000	0.932629000	-2.616330000
1	-7.600203000	-0.771121000	-1.828929000
1	-5.356683000	2.585897000	-3.253179000
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1	-5.931852000	-4.717731000	2.457097000
1	-7.748421000	-2.330435000	-0.602769000
1	-7.394436000	1.141353000	-3.331261000

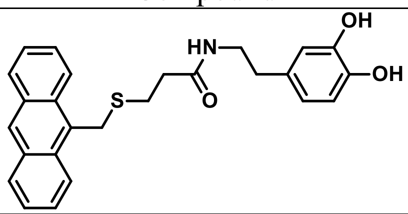
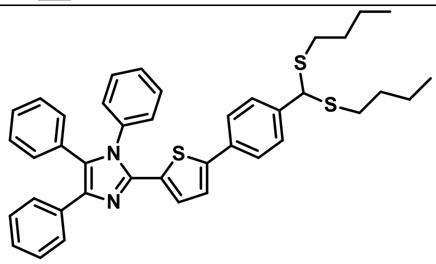
1	-7.817416000	-4.258319000	0.888583000

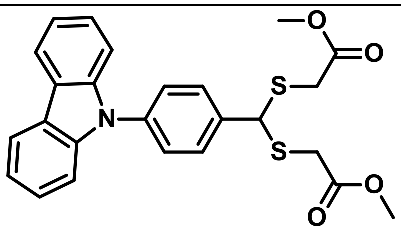
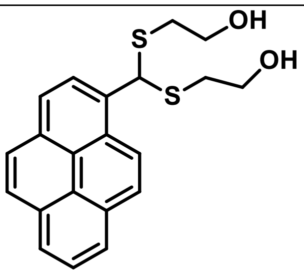
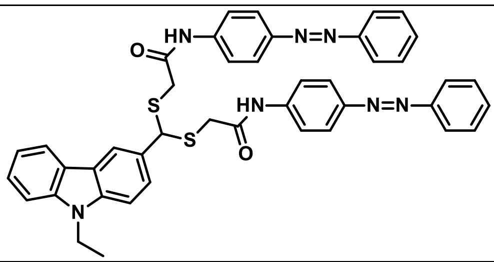
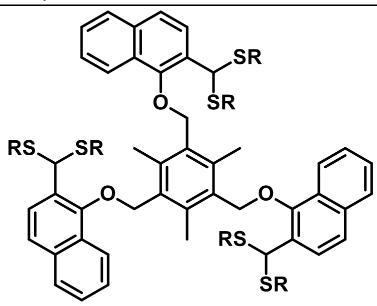
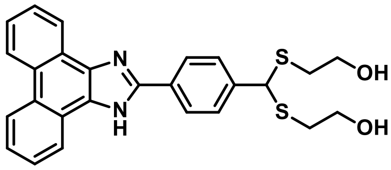
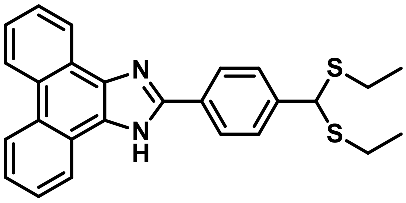
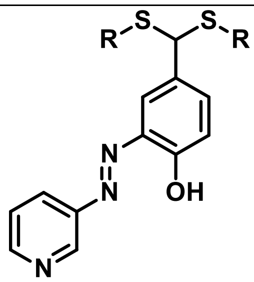
Table S4. Coordination of **Thiol-1**

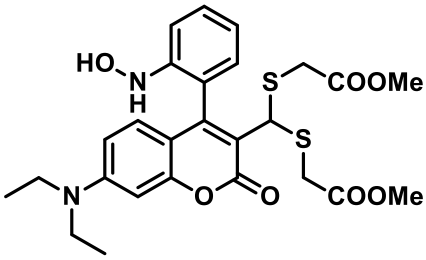
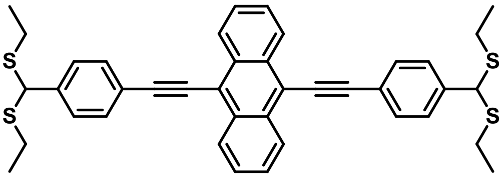
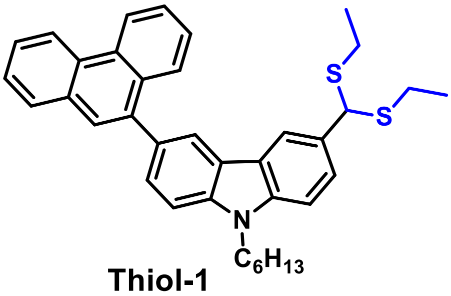
6	0.681685000	0.329714000	-0.065362000
1	2.949821000	2.818573000	-0.552058000
1	0.906167000	4.183786000	-0.742473000
1	0.632594000	-0.739256000	0.123833000
6	-2.602834000	1.983635000	-0.275091000
6	-4.002625000	2.021058000	-0.263990000
6	-4.686412000	0.831459000	-0.049316000
6	-4.009224000	-0.390777000	0.154417000
6	-2.613868000	-0.415547000	0.134790000
6	-1.899295000	0.766614000	-0.083065000
1	-4.548895000	2.945828000	-0.423602000
1	-5.771829000	0.833442000	-0.042331000
1	-2.085207000	-1.353538000	0.288447000
7	-1.690250000	3.016159000	-0.453081000
6	-2.022410000	4.401820000	-0.746248000
1	-1.237087000	5.031688000	-0.315050000
1	-2.944904000	4.645843000	-0.208802000
6	-2.180331000	4.688157000	-2.243592000
1	-2.976951000	4.074191000	-2.676114000
1	-2.432181000	5.742616000	-2.404200000
1	-1.252831000	4.469806000	-2.782759000
6	-4.764240000	-1.675077000	0.394458000
1	-4.046785000	-2.489260000	0.516498000
6	-6.929632000	-2.932921000	1.384326000
6	-7.359846000	-2.631144000	-0.050781000
1	-6.425142000	-3.902315000	1.449082000
1	-7.787037000	-2.939100000	2.065603000
1	-7.811560000	-3.511588000	-0.519745000
1	-8.082463000	-1.811015000	-0.071042000
16	-5.875082000	-2.165862000	-1.037876000
16	-5.801907000	-1.596769000	1.920820000
6	3.177725000	0.133778000	-0.149102000

6	3.323764000	-0.900262000	-1.032677000
6	4.240986000	0.403644000	0.804357000
6	4.498484000	-1.715486000	-1.086916000
1	2.532798000	-1.099817000	-1.751631000
6	5.439775000	-0.376758000	0.773900000
6	4.105728000	1.406225000	1.795821000
6	4.604562000	-2.763703000	-2.031662000
6	5.579270000	-1.454264000	-0.197285000
6	6.453655000	-0.081209000	1.715376000
1	3.182518000	1.972609000	1.844358000
6	5.113610000	1.664562000	2.704627000
6	5.742684000	-3.541285000	-2.106840000
1	3.768279000	-2.945470000	-2.702789000
6	6.733177000	-2.266144000	-0.301573000
6	6.303247000	0.918575000	2.657743000
1	7.374105000	-0.654563000	1.711183000
1	4.981956000	2.437626000	3.456940000
6	6.815853000	-3.286193000	-1.232411000
1	5.812077000	-4.343669000	-2.836278000
1	7.578692000	-2.096002000	0.356030000
1	7.101806000	1.117493000	3.367479000
1	7.715961000	-3.892575000	-1.287484000

Table S5: Comparative table for the thioacetal based chemodosimeter for the Hg2+ ions

S. No	Compound	Detection limit	Ref
1		1.1×10^{-6} M	1
2		6.7×10^{-8} M	2

3		5.1×10^{-8} M	3
4		1.80 nM	4
5		1.8×10^{-8} M	5
6		3.5×10^{-7} M	6
7		1.16 nM	7
8		5.22×10^{-7} M	8
9		$1.1 \text{ x } 10^{-6}$ M	9

10		4.04×10^{-9} M	10
11		59 nM	11
12	 Thiol-1	31 nM	Present work

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