Supporting Information for

A Cabazole Based "Turn on" Fluorescent Sensor for Selective Detection of Hg^{2+} in an Aqueous Medium

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Contents:

1. Experimental Section

1.1. Reagents

Starting materials and reagents such as carbazole, 1-bromooctane, N-methylformanilide, and barbituric acid were purchased from Sigma-Aldrich and used as received. The solvents used in the synthesis procedures were obtained from Spectrochempvt.Ltd. and distilled before use.Spectroscopic grade solvents from Spectrochempvt. Ltd. were used for photophysical studies.

1.2. Experimental General

The ^{1}H NMR and ^{13}C NMR spectra were recorded on 400 MHz on Bruker FT-NMR spectrometer with tetramethylsilane (TMS) as internal standard. Chemical shifts were reported in parts per million (ppm) downfield to tetramethylsilane. High resolution mass spectra of new compounds were obtained using a WATERSSYNAPTG2S spectrometer.

1.3.Synthesis of CBA

CBA was synthesized according to a reported procedure[1] by Knoevenagel condensation between 9-octyl-9H-carbazole-3-carbaldehyde and barbituric acid in ethanol yielded 85%. ^{1}H NMR (dmso d_6) d (ppm): 0.80 (t, 3H), 1.17–1.28 (m, 10H), 1.75–1.81 (m, 2H), 4.45 (t, 2H), 7.31 (t, 1H), 7.52 (t, 1H, J ¼ 7.3 Hz), 7.65 (dd, 2H), 8.18 (d, 1H, J ¼ 7.7 Hz), 8.53 (s, 1H), 8.60 (dd, 1H), 11.15 (s, 1H), 11.27 (s, 1H); ^{13}C NMR (DMSO d6) d (ppm): 13.84, 21.94, 26.34, 28.45, 28.51, 28.62, 31.08, 42.61, 109.24, 110.22, 114.06, 120.42, 120.47, 122.18, 122.38, 123.43, 126.69,129.46, 133.27, 140.75, 143.12, 150.23, 156.86, 162.37, 164.18; HRMS (ESI MS) m/z: theoretical: 418.2125, found: 418.2115 ([M + H]+ detected.

1.4. General photophysical studies

A stock solution of CBA with the concentration of 4.3×10^{-3}M was prepared in tetrahydrofuran (THF) and stored in a cold and dark place. This stock solution was used for all spectrofluoremetric titrations after appropriate dilution. Absorption spectra were recorded using

Evolution 201 UV-visible spectrophotometer. Fluorescence emission spectrum of a sample was measured using a Perkin Elmer luminescence spectrophotometer (model LS 45).

1.4.1. Determination of Fluorescence Quantum Yield

Quantum yield of fluorescence of CBA and CBA-Hg-CBA complex in 1:1 MeOH/H_2O was determined by the method of relative actinometry reported elsewhere.[2] The reference used was N,N'-Bis(2-ethylhexyl)-3,4,9,10-perylenetetracarboxylic Diimide in chloroform (Φ_f=0.99).[2] The quantum yield of CBA and CBA-Hg-CBA was calculated as 0.002 and 0.195 respectively. Appropriate corrections for the refractive index and absorbance at the excitation wavelength (408 nm) were incorporated in the calculation.

1.5. Determination of association constant

$$CBA + Hg^{2+} \overset{K_{11}}{\rightleftharpoons} CBA\text{-}Hg^{2+}$$

$$CBA\text{-}Hg^{2+} + CBA \overset{K_{21}}{\rightleftharpoons} CBA\text{-}Hg\text{-}CBA$$

The association constant was calculated based on the fluorescence titration curve of CBA with metal ions. Association constant was determined by a nonlinear least squares fit of the data with the following equation (See Figure S8) as described elsewhere.[3]

$$y = \frac{x}{2ab(1-x)2} + \frac{xb}{2}$$

Where ***x*** is $\mathbf{I\text{-}I_o/I_{max}\text{-}I_o}$,**y** is the concentration of Hg^{2+} ions, **a** is the association constant K_{21}, and **b** is the concentration of CBA. Here I is the emission intensity at particular Hg^{2+} concentration, I_0 is the initial emission intensity and I_{max} is the final emission intensity from thefluorescence titration of CBA with Hg^{2+}

1.6. Job Plot by UV-Vis method[4]

A series of solutions containing CBA and $Hg(OAc)_2$ were prepared keeping sum of concentration of [Hg^{2+}] ion and [CBA] a constant, whereby, the mole fraction (X) of Hg^{2+} was varied from 0.1 to 1.0. The Job's plot is obtained by plotting the absorbance (abs460 x $X_{Hg^{2+}}$) at 460 nm against the mole fraction of the Hg^{2+} (see Figure S5). The value of mole fraction

corresponding to the maximum on the Job's plot thus obtained was 0.4 indicative of a 2:1 binding stoichiometry.[5]

1.7. Detection limit calculations experimental procedure[5]

The detection limit was calculated based on the fluorescence titration. To calculate the S/N ratio, the emission intensity of CBA in the absence of Hg (II) was measured 10 times and the standard deviation (**σ**) of blank measurements was determined. Three independent duplication measurements of emission intensity at 593 nm in the presence of Hg(II) and the average value of the intensities was plotted against concentration of Hg(II) for determining the slope (**m**). The detection limit is then calculated with the following equation.

$$\textbf{Detection limit} = 3\sigma/m$$

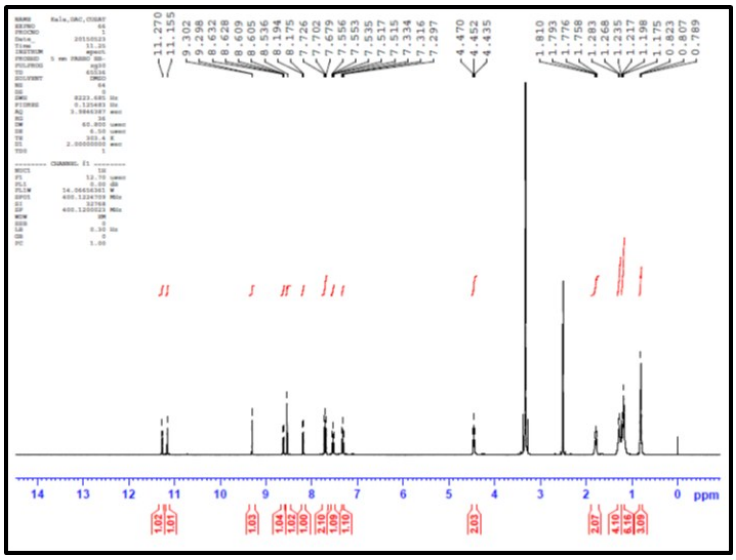


Figure S1. ^{1}H NMR of CBA

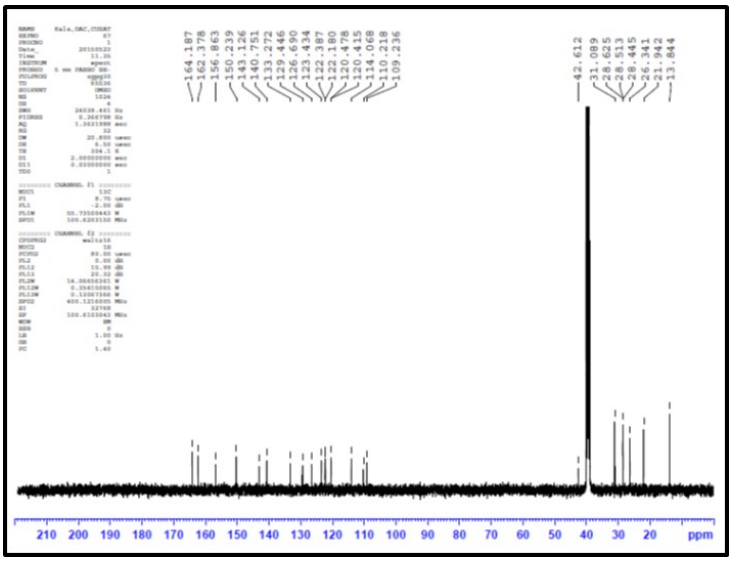


Figure S2. ^{13}C NMR of CBA

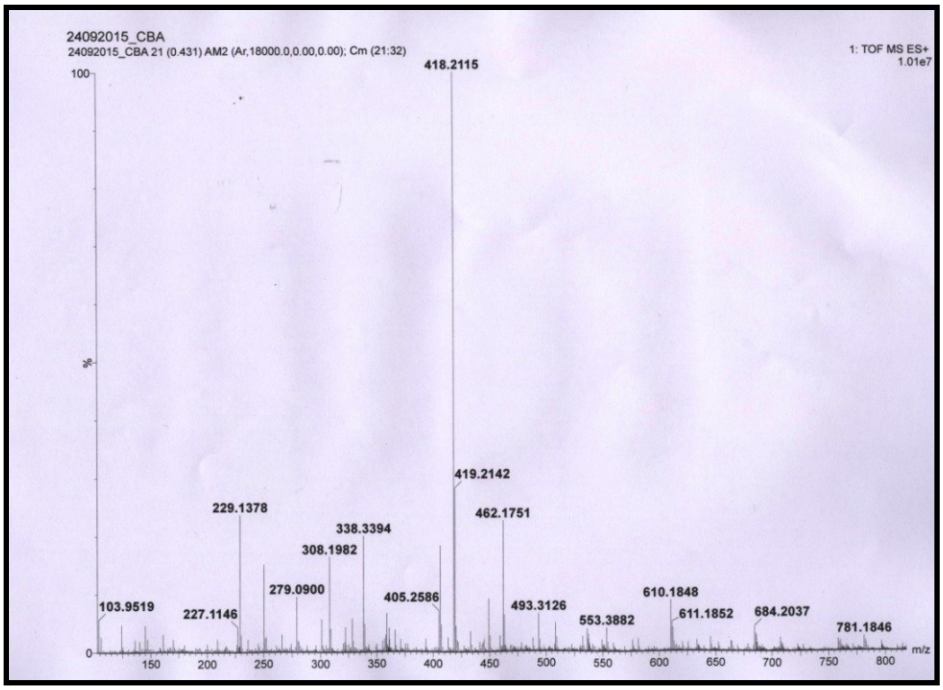


Figure S3. ESI mass spectrum of CBA

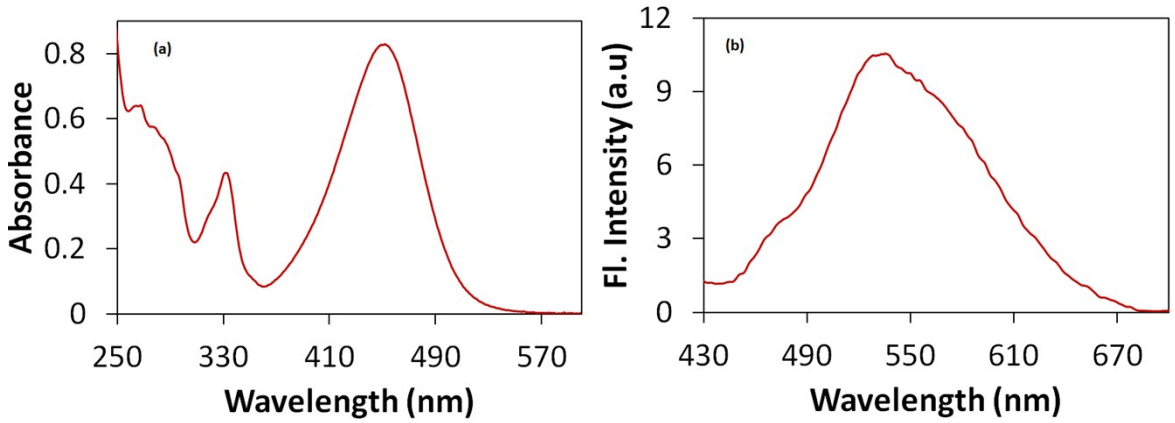


Figure S4 Absorption (a) and emission spectrum (b) of CBA in 1:1 MeOH H_2O. λ_{ex} 408 nm

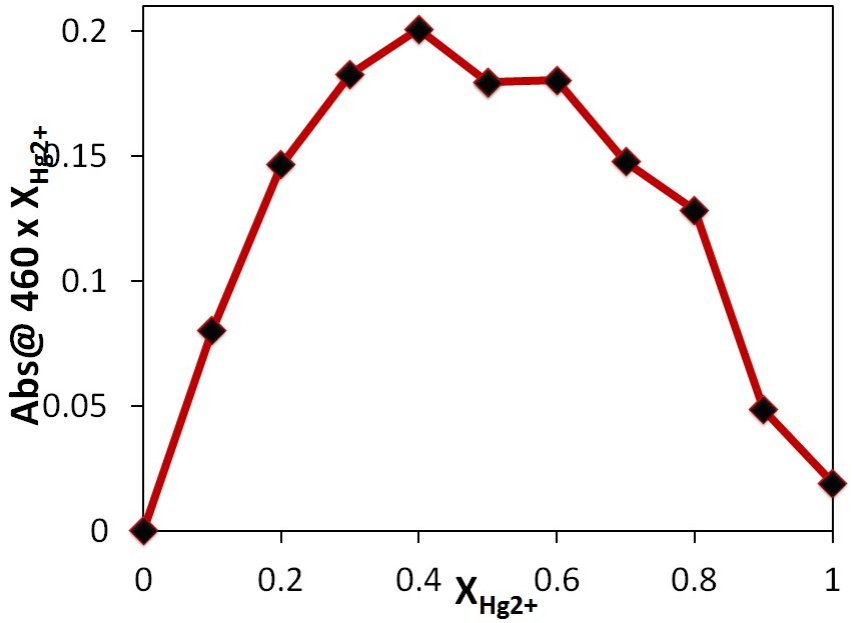


Figure S5: Job's plot analysis of CBA with Hg^{2+} showing a 2:1 binding geometry.

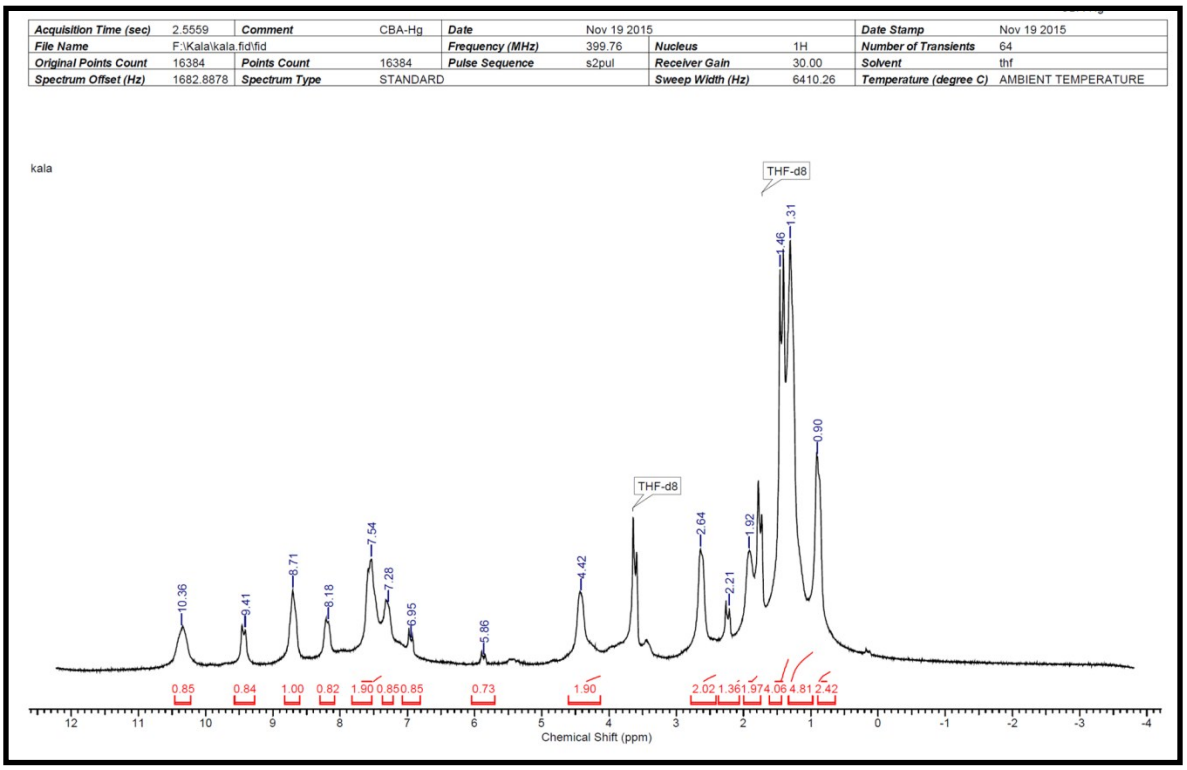
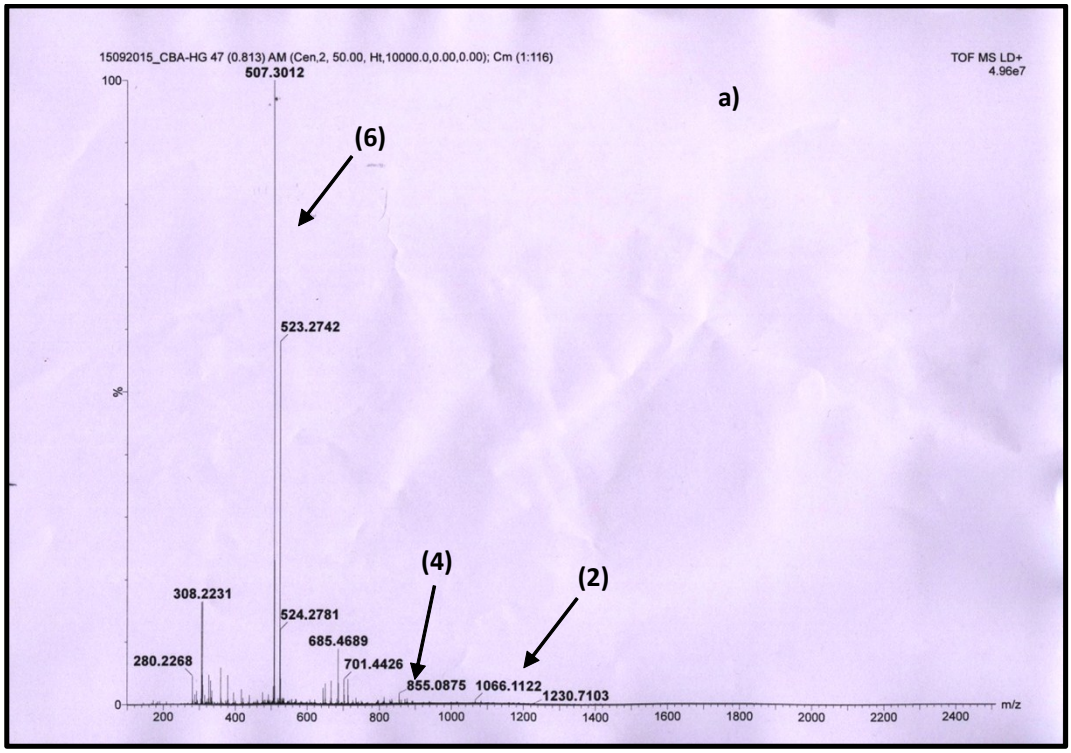


Figure S6. ^{1}H NMR of CBA-Hg-CBA in THF-d8



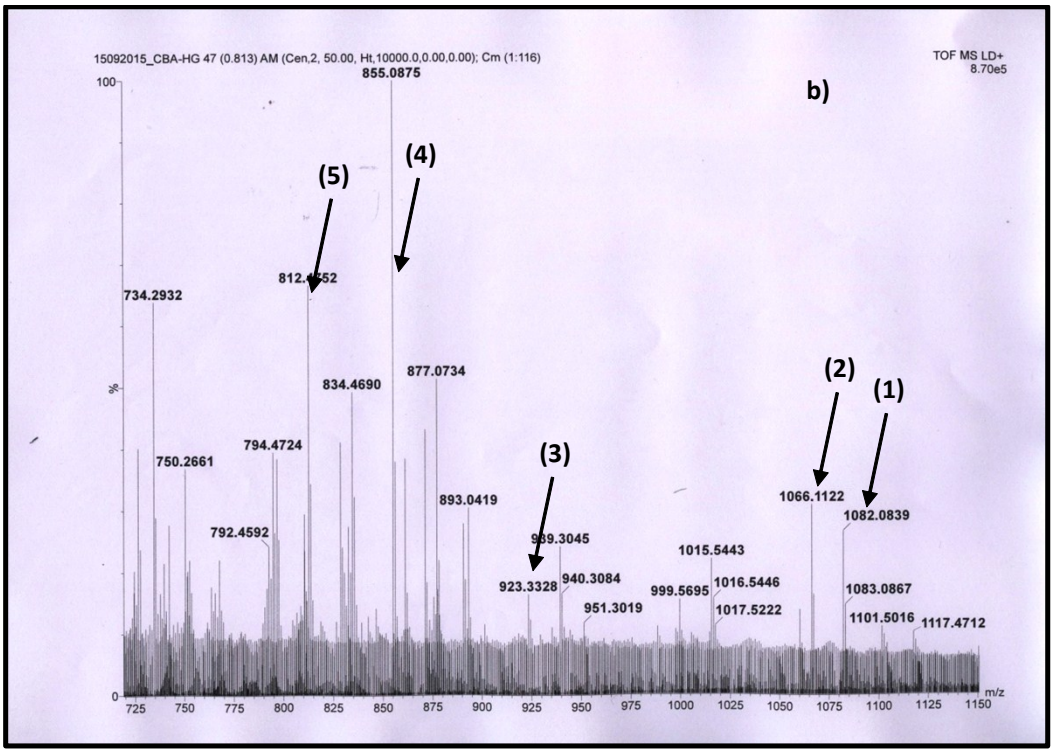
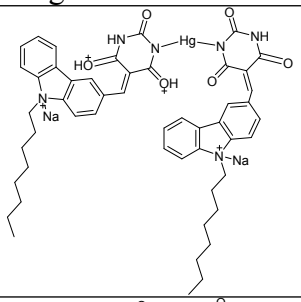
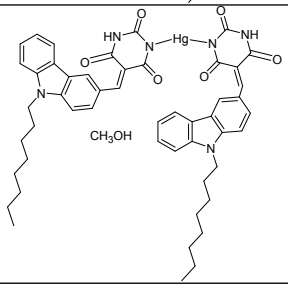
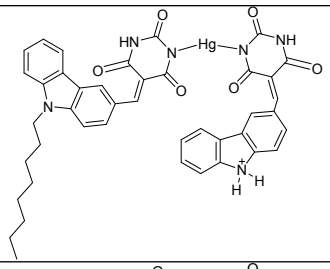
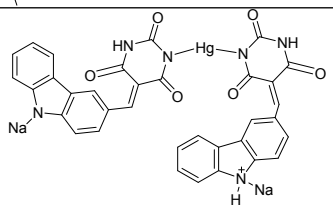
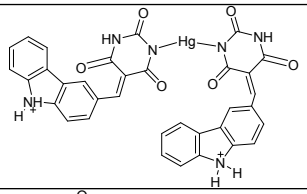
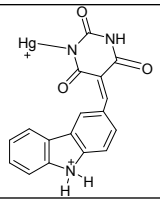


Figure S7: MALDI- TOF mass spectrum of CBA –Hg complex

Table S1 Fragment ions observed in MALDI-TOF (Refer Figure S6 for Peak No.)[6-7]

Peak No.	Fragment	Expected Mass	Observed Mass
1		1082.3584	1082.0839
2		1066.3917	1066.1122

3		923.2475	923.3328
4		855.0862	855.0875
5		812.1296	812.4752
6		507.0496	507.3012

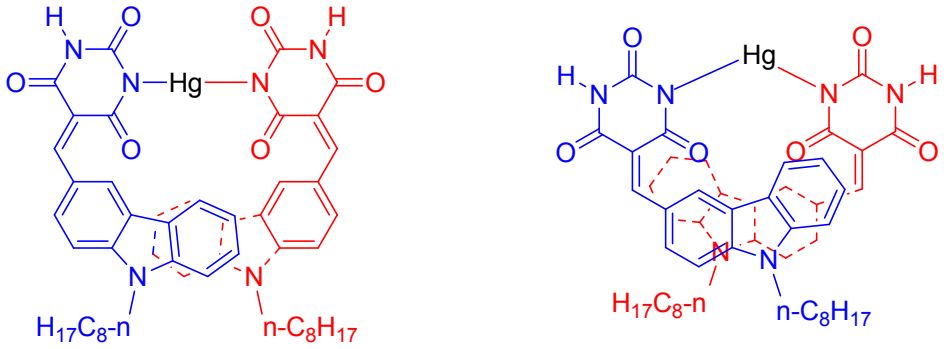


Figure S8. Proposed geometry for the CBA-Hg-CBA complex

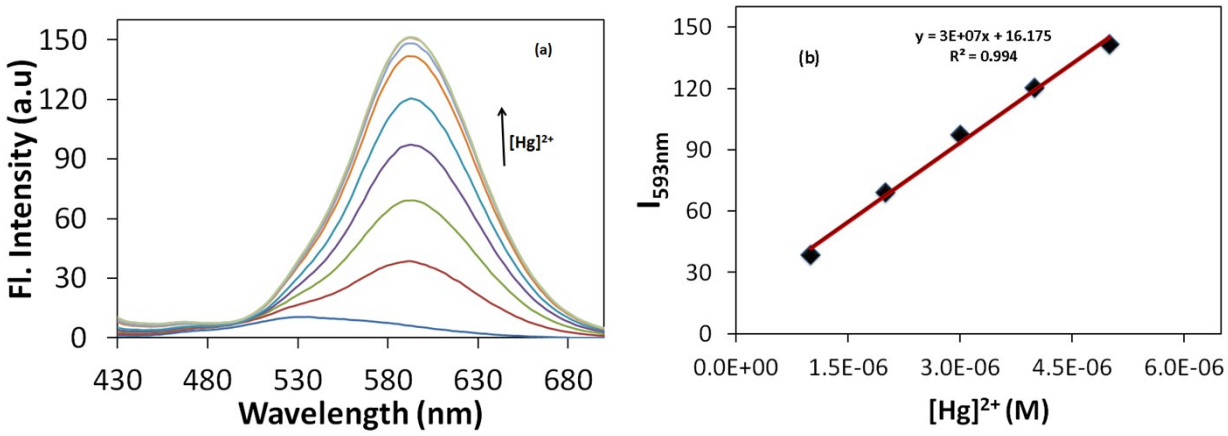


Figure S9. (a) Fluorescence spectra of CBA (10μM) in the presence of different concentration of $Hg(OAc)_2$ ($1x10^{-6}$, $2x10^{-6}$, $3x10^{-6}$, $4x10^{-6}$, $5x10^{-6}$, $6x10^{-6}$, $7x10^{-6}$, $8x10^{-6}$) and (b)fluorescence intensity at 593nm as the concentrations of Hg(II) in MeOH/H_2O (1:1, v/v).

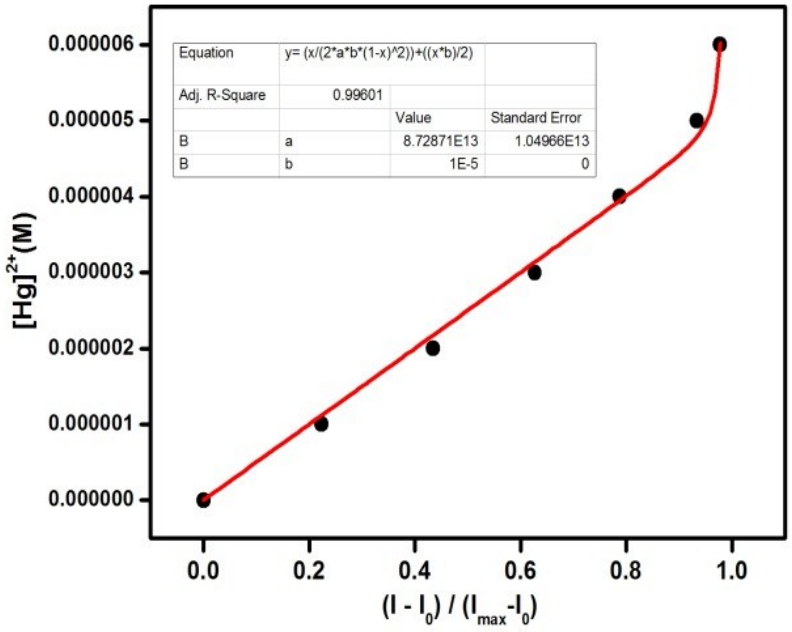


Figure S10: A plot of fluorescence intensity ratio (I-I_0/I_{max}-I_0) vs. concentrations of Hg^{2+} in (1:1) MeOH/H_2O.

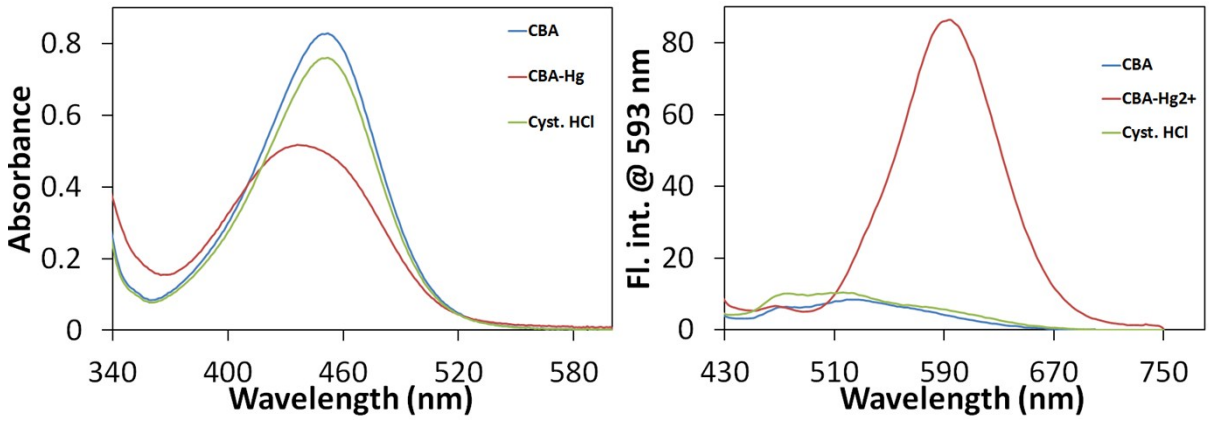


Figure S11: Absorption and emission spectrum of CBA, CBA-Hg-CBA complex and CBA-Hg-CBA complex in the presence of cysteamine hydrochloride. λ_{ex} 408 nm

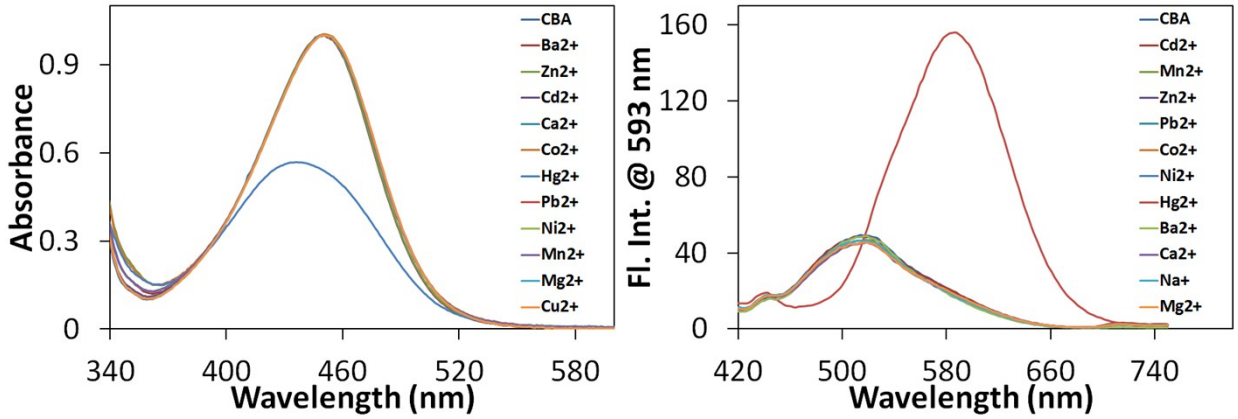


Figure S12: Absorption and emission spectra of CBA recorded in the presence of various metal ions (Metal ion concentration = 35μM, [CBA] = 26μM) in MeOH/H_2O (1:1). λ_{ex} 408 nm

References

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