

Rhodamine based Dual Probes for Selective Detection of Mercury and Fluoride Ions in Water using Two Mutually Independent Sensing Pathways

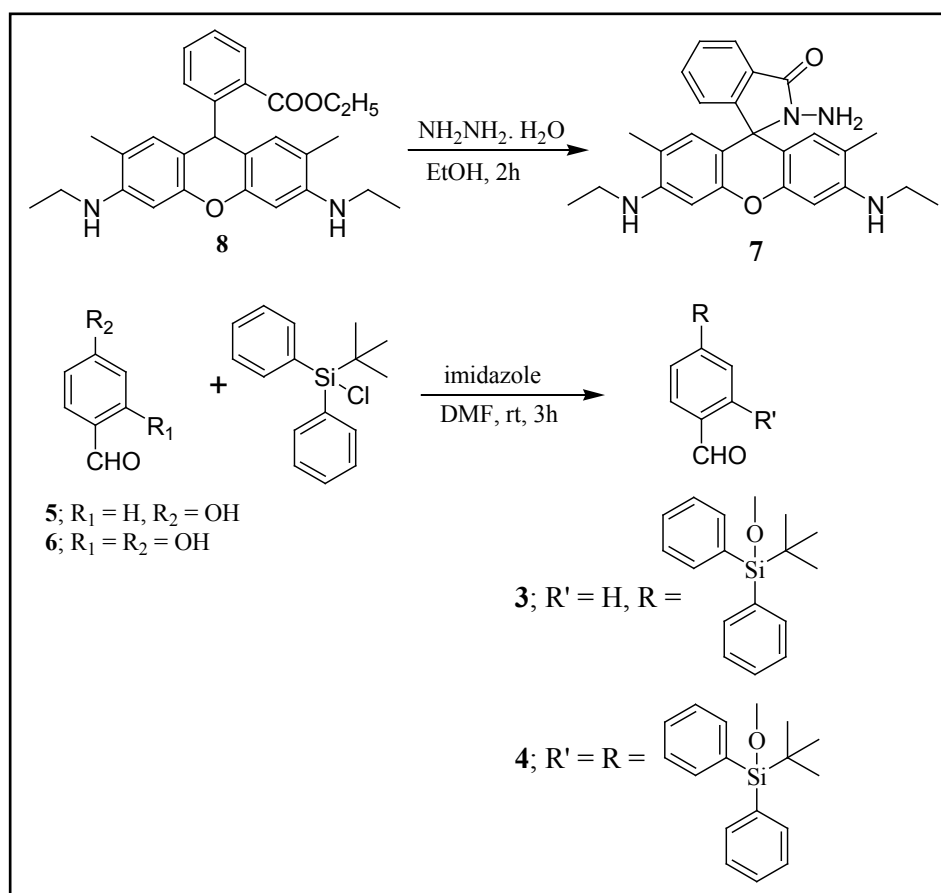
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Synthesis of starting material:



Scheme S1. Synthetic procedure of starting materials.

Synthesis of rhodamine hydrozone (7). Rhodamine-6G hydrozone (7) was prepared according to a procedure described in literature.¹ In a 50 mL round bottomed flask, Rhodamine-6G (0.48 g, 1 mmol) was dissolved in 15 mL ethanol. To that 1.5 mL (excess) hydrazine monohydrate (85%) was added dropwise with vigorous stirring at room temperature. After the addition, the stirred mixture was refluxed for 2h, and then cooled overnight. The resulting precipitate was filtered and washed 3 times with 10 mL EtOH/water. After drying under vacuum, the reaction afforded Rhodamine-6G hydrozone. Yield 0.37 g, 80 %; IR (neat, cm^{-1}) 3370.6, 2921.5, 1621.4, 1516.6, 1270.3, 1203.6, 1017.8, 742.4; ^1H NMR (400 MHz, CDCl_3) δ (ppm): 1.32 (t, $J = 7.1$ Hz, 6H), 1.92 (s, 6H), 3.22 (q, $J = 6.9$ Hz, 4H), 3.56 (br, 4H), 6.26 (s, 2H), 6.39 (s, 2H), 7.06 (t, $J = 3.3$ Hz, 1H), 7.45 (t, $J = 4.6$ Hz, 2H), 7.96 (t, $J = 3.2$ Hz, 1H); HRMS m/z calcd. for $\text{C}_{26}\text{H}_{28}\text{N}_4\text{O}_2$ ($\text{M}+\text{Na}$)⁺ 451.2110, found 451.2106.

Synthesis of 4-(tert-Butyldiphenylsilyloxy)benzaldehyde (3).² 4-hydroxybenzaldehyde (0.5 g, 4.1 mmol), imidazole (0.42 g, 6.1 mmol) and *tert*-butyldiphenylsilyl chloride (1.28 mL, 4.6 mmol) were taken in DMF and stirred at rt for 3 h. After that the reaction mixture was diluted with brine and the aqueous layer was extracted with ether. The combined organic extracts were washed with water, dried (using anhydrous Na₂SO₄) and concentrated to get an oily residue, which was further purified by silica gel column chromatography (5% EtOAc/hexane) to get the silyl ether as white crystals. Yield 1.41 g, 96%; IR (neat, cm⁻¹) 3433.6, 2859.4, 2859.4, 1701.9, 1599, 1508.2, 1274.1, 1115.1, 911.2, 701.2; ¹H NMR (400 MHz, CDCl₃) δ (ppm): 1.11 (s, 9H), 6.86 (d, *J* = 8.4 Hz, 2H), 7.39 (d, *J* = 7.2 Hz, 4H), 7.44 (d, *J* = 7.2 Hz, 4H), 7.64 (d, *J* = 8.4 Hz, 2H), 7.70 (d, *J* = 7.2 Hz, 2H), 9.80 (s, 1H); HRMS *m/z* calcd for C₂₃H₂₄O₂Si (M+Na)⁺ 383.1443, found 383.1443.

Synthesis of 2, 4-(tert-Butyldiphenylsilyloxy)benzaldehyde (4). 2, 4-hydroxybenzaldehyde (0.25 g, 1.8 mmol), imidazole (0.42 g, 6.1 mmol) and *tert*-butyldiphenylsilyl chloride (1.3 mL, 4.7 mmol) were taken in DMF and stirred at rt for 3 h. After that the reaction mixture was diluted with brine and the aqueous layer was extracted with ether. The combined organic extracts were washed with water, dried (using anhydrous Na₂SO₄) and concentrated to get an oily residue, which was further purified by silica gel column chromatography (5% EtOAc/hexane) to get the silyl ether as light yellow oily material. Yield 1.13 g, 90%; IR (neat, cm⁻¹) 3450.2, 2959.3, 2859.4, 1686.0, 1594.6, 1428.2, 1112.9, 998.0, 822.5, 700.8; ¹H NMR (400 MHz, CDCl₃) δ (ppm): 1.04 (s, 18H), 7.20 (s, 2H), 7.37 (t, *J* = 4.8 Hz, 3H), 7.42-7.39 (m, 12H), 7.50 (d, *J* = 7.6 Hz, 2H), 7.68 (t, *J* = 6.4 Hz, 4H), 10.5 (s, 1H); HRMS *m/z* calcd. for C₃₉H₄₂O₃Si₂ (M+Na)⁺ 637.2570, found 637.2571.

1. (a) Y.-K. Yang, K.-J. Yook and J. Tae, *J. Am. Chem. Soc.*, 2005, **127**, 16760. (b) X.-F. Yang, X.-Q. Guo and Y.-B. Zhao, *Talanta*, 2002, **57**, 883.
2. K. C. Lee, B. S. Moon, J. H. Lee, K.-H. Chung, J. A. Katzenellenbogen, D. Y. Chi, *Bioorg. Med. Chem.*, 2003, **11**, 3649.

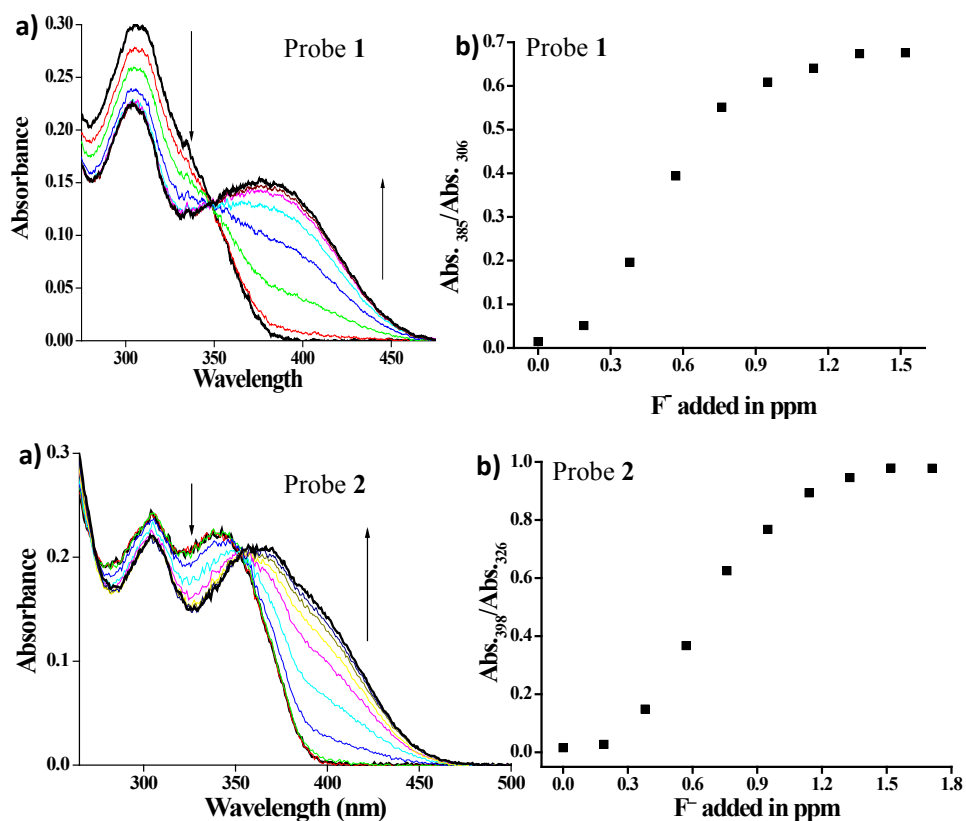


Fig. S1 (a) UV-vis titration of probe (10 μ M) with fluoride ion (TBAF) in acetonitrile. (b) Plot of absorbance ratio with the added fluoride ion.

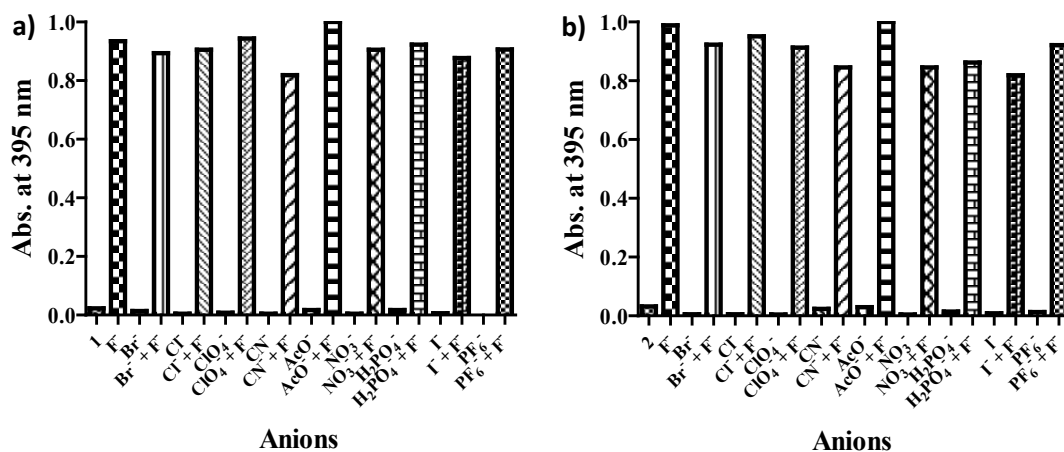


Fig. S2 Normalized plot of (a) Absorbance at 395 nm for **1** (10 μ M) in CH_3CN upon addition of fluoride ion (TBAF) (20 equiv.) in presence of excess of other anions (50 equiv.). (b) Absorbance at 395 nm for **2** (10 μ M) in acetonitrile upon addition of fluoride ion (TBAF) (20 equiv.) in presence of excess of other anions (50 equiv.).

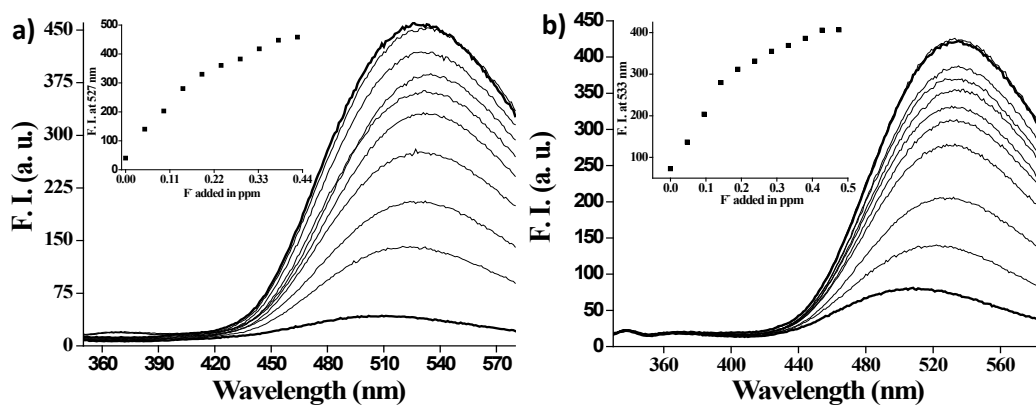


Fig. S3 Fluorescence titrations of (a) **1** (5 μM) and (b) **2** (5 μM) with the F⁻ ion in CH₃CN. ($\lambda_{\text{excit.}} = 305 \text{ nm}$) [Inset: plot of F. I. of the probe at (a) 527 nm and (b) 533 nm with the added F⁻ ion.]

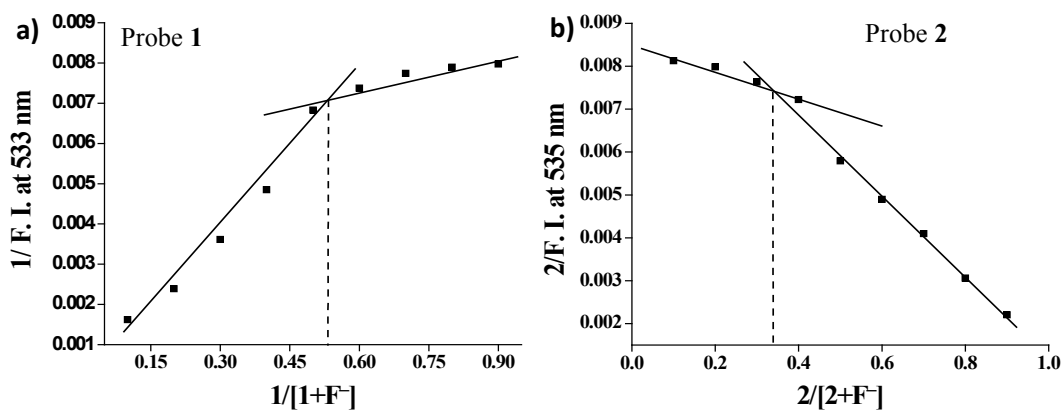


Fig. S4 Stoichiometry determination of (a) **1** and (b) **2** with F⁻ ion in CH₃CN. [The total concentration [Probe] + [F⁻] = $1.0 \times 10^{-4} \text{ M}$.]

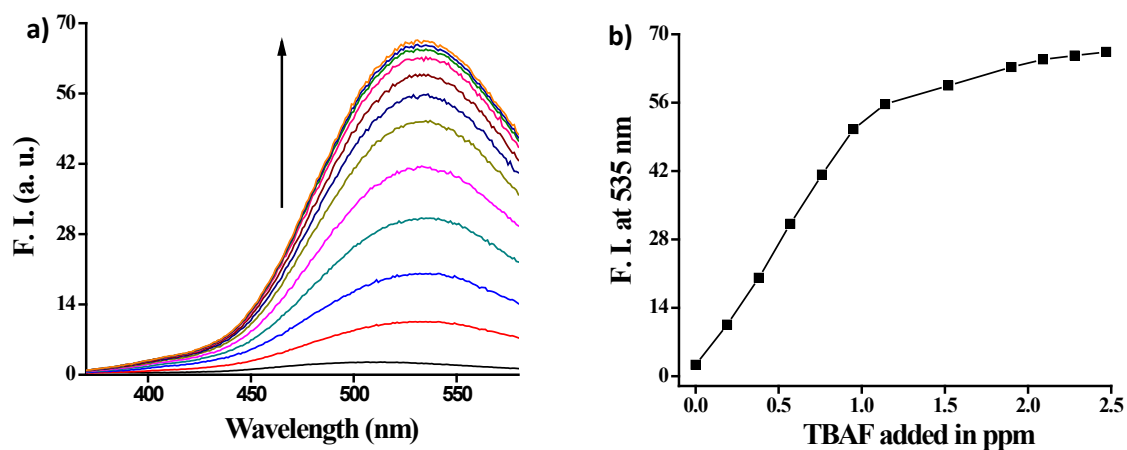


Fig. S5 (a) Fluorescence titration of **1** (5 μM) with TBAF in 9:1 (DMF-water) ($\lambda_{\text{ex.}} = 305$ nm); b) Plot of fluorescence emission intensity at 535 nm with the added F^- ion.

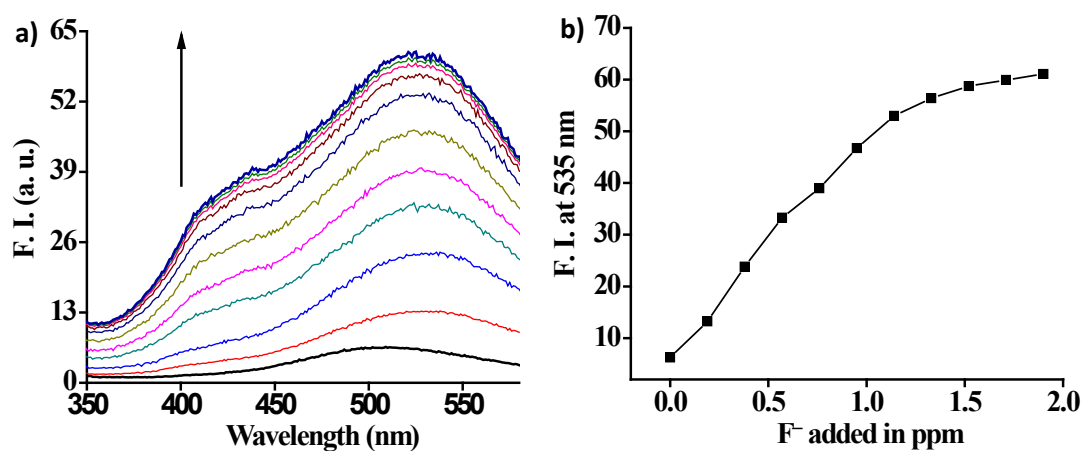


Fig. S6 (a) Fluorescence titration of **2** (5 μM) in 8:2 (DMF-Water) at $\lambda_{\text{ex.}} = 305$ nm. (b) Plot of fluorescence emission intensity at 535 nm with the added fluoride ion.

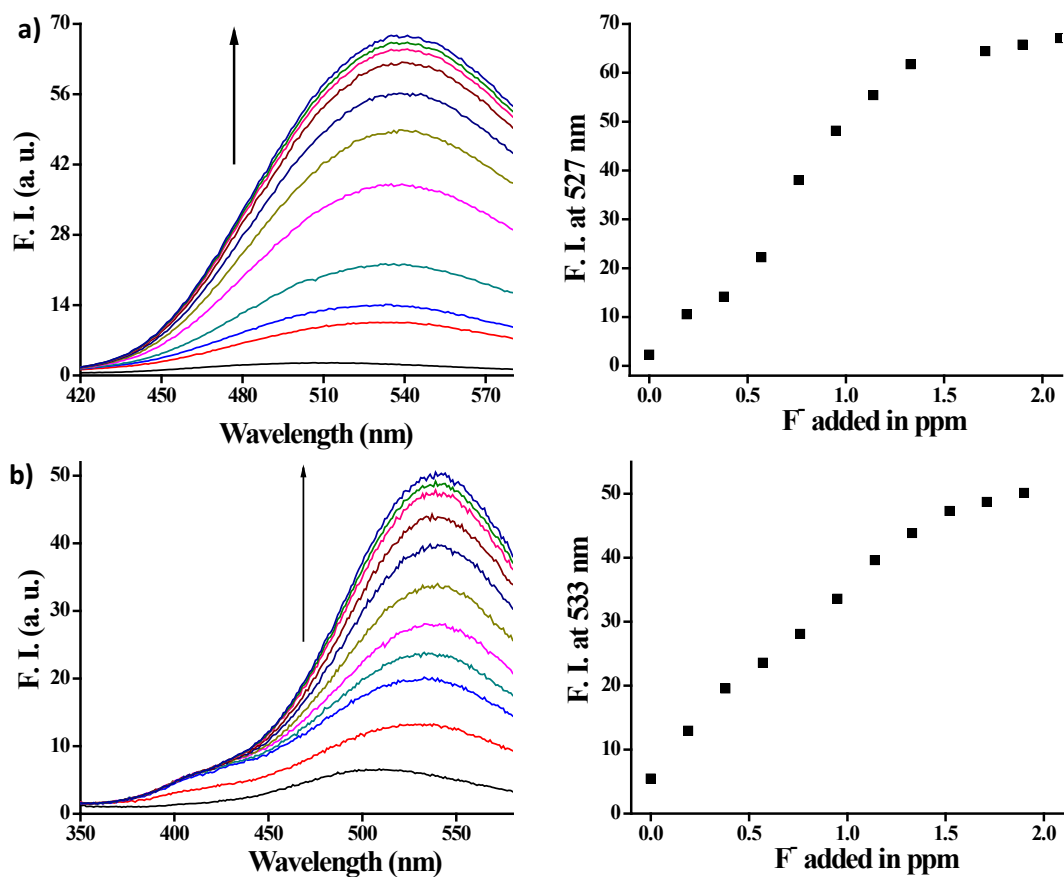


Fig. S7 (a) Fluorescence titration of **1** (5 μ M) ($\lambda_{\text{ex.}}$ = 305 nm) with KF (0-2 ppm) in 9:1 (DMF-Water). (b) Fluorescence titration of **2** (5 μ M) ($\lambda_{\text{ex.}}$ = 305 nm) with KF (0-2 ppm) in 8:2 (DMF-water).

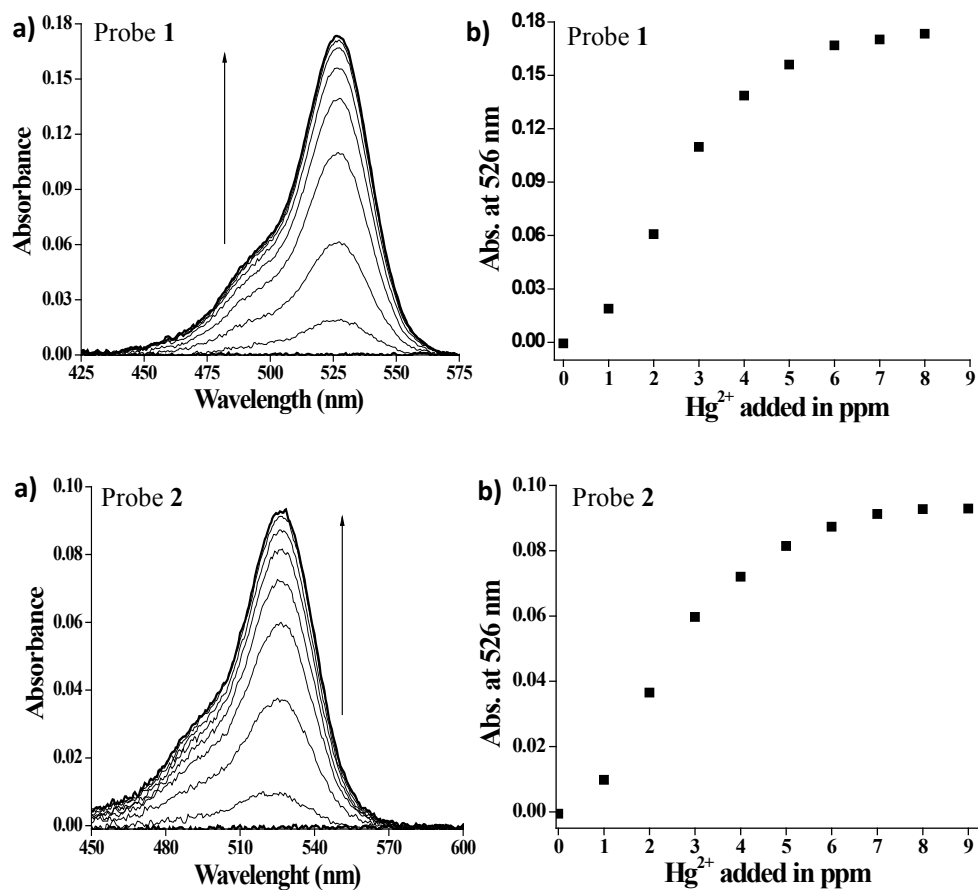


Fig. S8 (a) UV-vis titration of probe (10 μM) with Hg^{2+} ion in CH_3CN . (b) Plot of the absorbance at 526 nm of probe with the added Hg^{2+} ion.

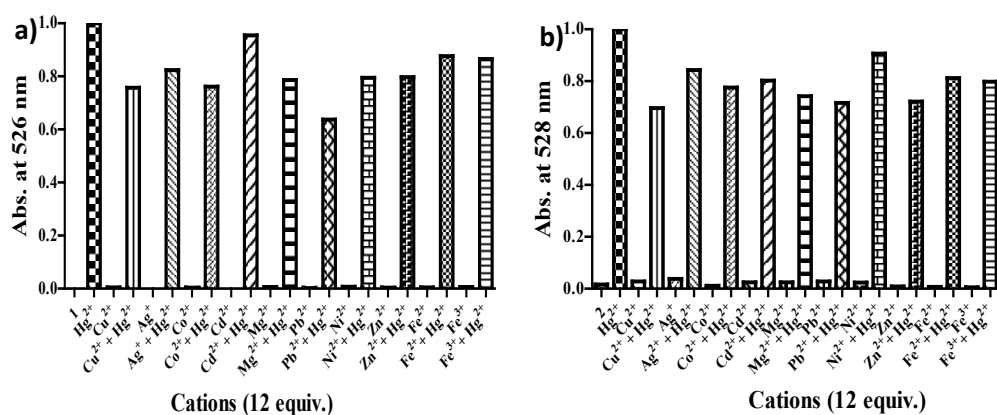


Fig. S9 Normalized plot of the (a) absorbance at 526 nm for **1** (10 μM) in CH_3CN with Hg^{2+} (4 equiv.) and in presence of excess of other cations (12 equiv.). (b) Absorbance at 528 nm for **2** (10 μM) in CH_3CN with Hg^{2+} (4 equiv.) and in presence of an excess of other cations (12 equiv.).

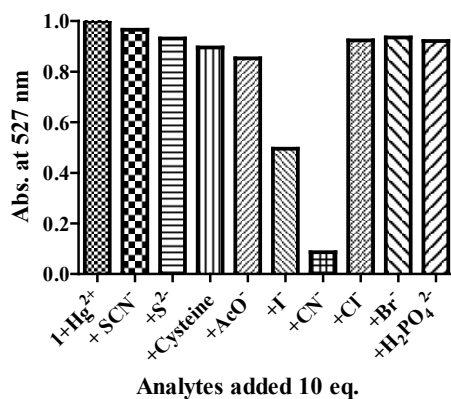


Fig. S10 Normalized absorbance of **1** (10 μ M) at 527 nm with 5 equiv. of added Hg²⁺ ions and to that 10 equiv of added ions/molecules.

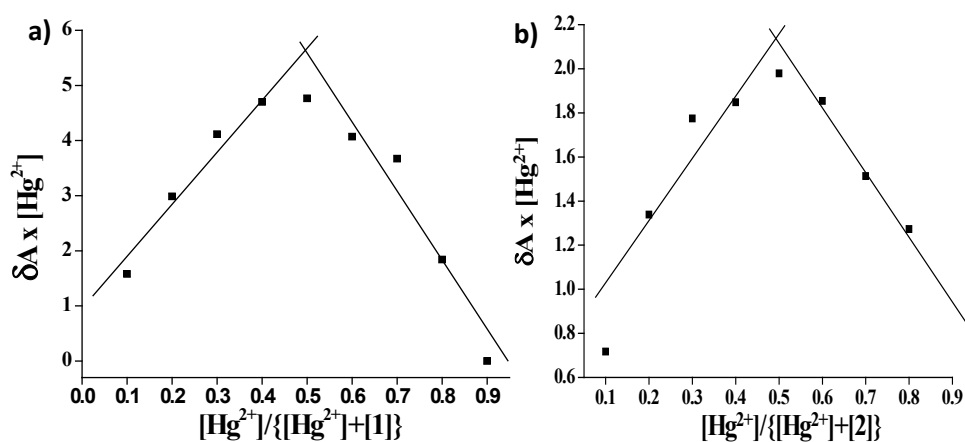


Fig. S11 Job plot analyses of (a) **1** and (b) **2** with Hg²⁺ ion in CH₃CN. [(δA = change in absorbance); the total concentration [probe] + [Hg²⁺] = 1.0×10^{-4} M.]

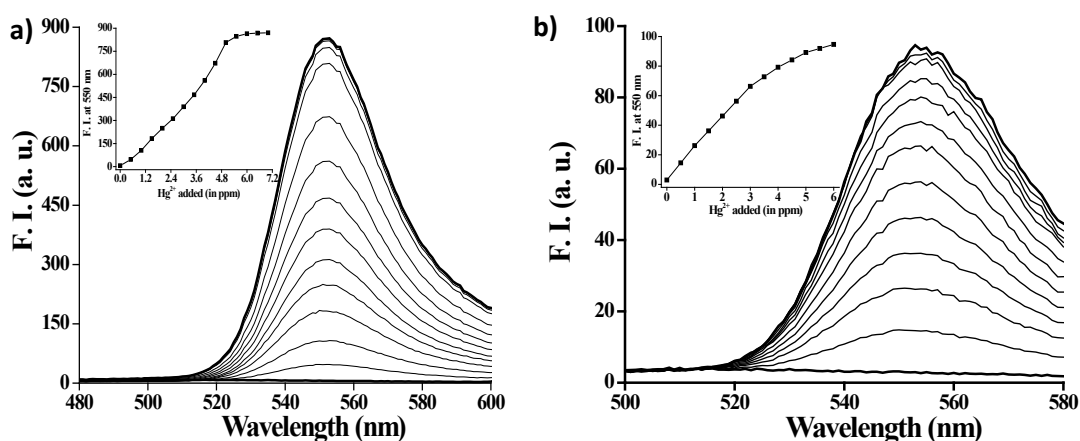


Fig. S12 Fluorescence titration of (a) **1** (5 μ M) and (b) **2** (5 μ M) with Hg²⁺ ion (0-2 equiv.) in CH₃CN (λ_{ex} = 305 nm).

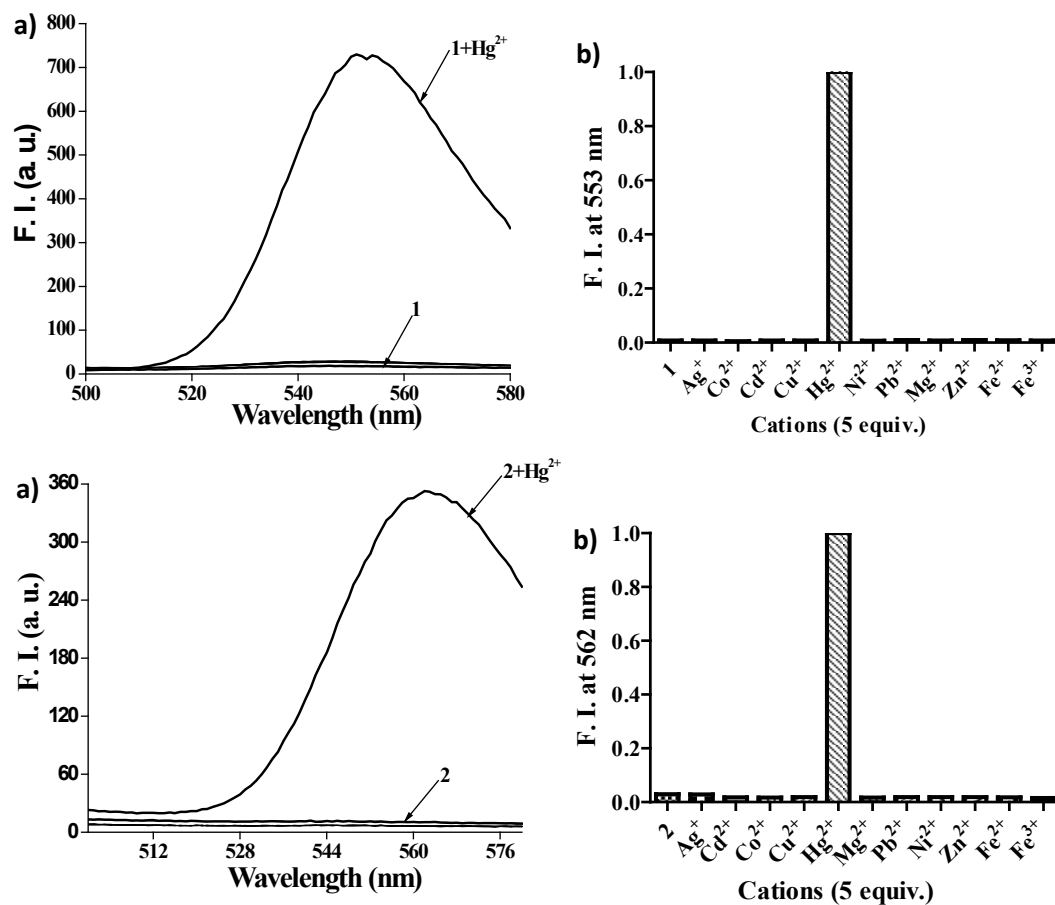


Fig. S13 (a) Fluorescence spectra of probe (5 μM) in 3:2 HEPES (pH 7.4)-CH₃CN mixture at $\lambda_{\text{ex.}} = 305$ nm, upon addition of various cations (5 equiv.). (b) Normalized plot of the fluorescence intensity with different cations added.

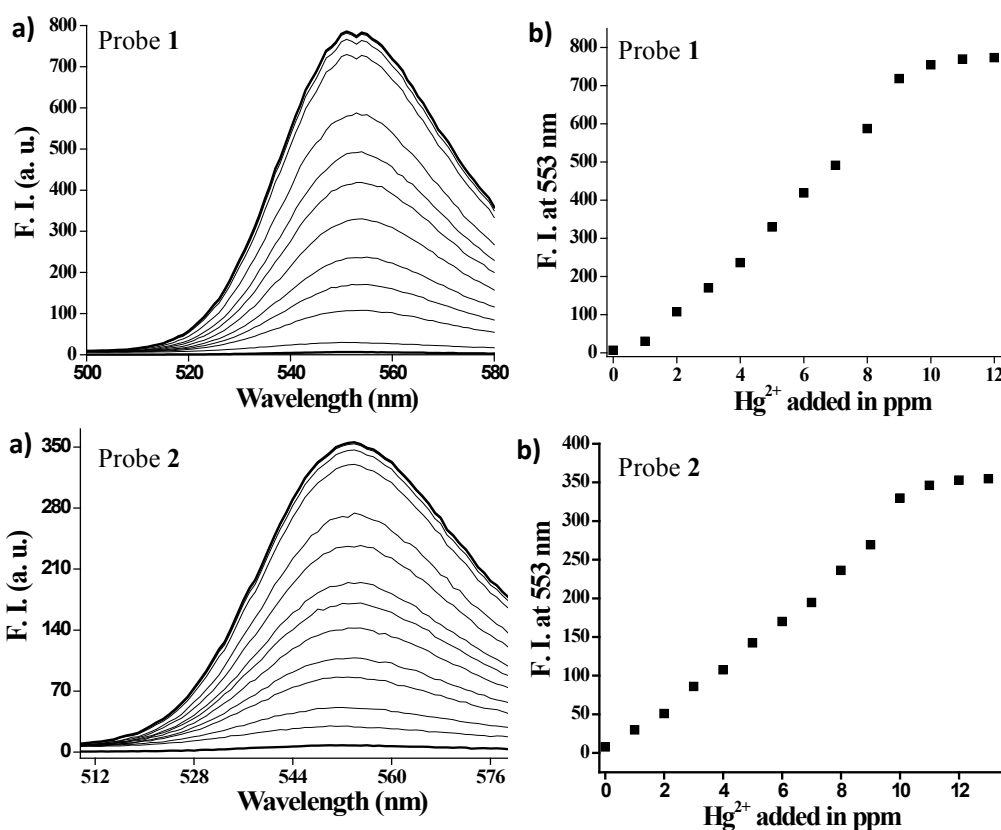


Fig. S14 (a) Fluorescence titration of probe 1/2 (5 μM) with Hg^{2+} in 3:2 [buffer (pH 7.4)-acetonitrile mixture] ($\lambda_{\text{ex.}} = 305 \text{ nm}$) (b) plot of F. I. at 553 nm with the added Hg^{2+} ions.

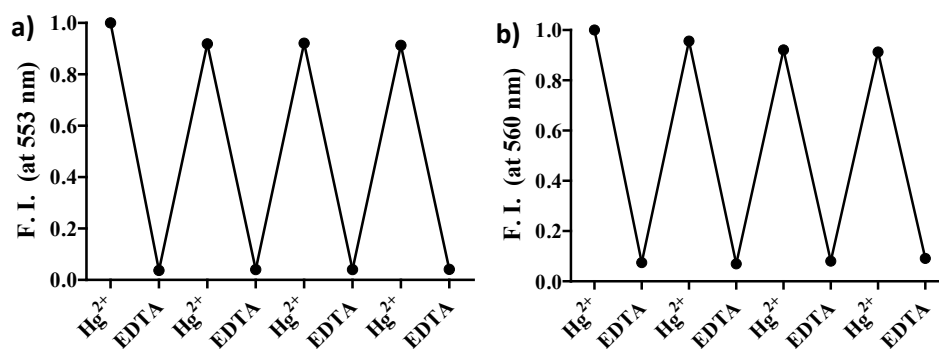


Fig. S15 Recovery of molecular fluorescence of (a) 1 and (b) 2 after adding EDTA (10 equiv.) after each addition of 5 equiv. of Hg^{2+} to the probes (10 μM) in 6:4 (buffer-acetonitrile mixture).

Table S1. Binding constant of **1** with Hg^{2+} and F^- ion in different media according to Benesi-Hildebrand equation based on a 1:1 stoichiometry.

Analyte	Medium	$\log K$
Hg^{2+}	Acetonitrile	4.45 ± 0.01
Hg^{2+}	(2:3) CH_3CN -HEPES buffer	4.17 ± 0.01
TBAF	Acetonitrile	4.89 ± 0.02
TBAF	(9:1) DMF-HEPES buffer	2.54 ± 0.02
KF	(9:1) DMF-HEPES buffer	2.71 ± 0.01

Table S2. Binding constant of **2** with Hg^{2+} for 1:1 stoichiometry and F^- ion for 1:2 stoichiometry in different medium according to Benesi-Hildebrand equation.

Analyte	Medium	$\log K$
Hg^{2+}	Acetonitrile	4.87 ± 0.01
Hg^{2+}	(2:3) CH_3CN -HEPES buffer	4.69 ± 0.01
TBAF	Acetonitrile	10.25 ± 0.02
TBAF	(8:2) DMF-HEPES buffer	9.32 ± 0.04
KF	(8:2) DMF-HEPES buffer	8.60 ± 0.05

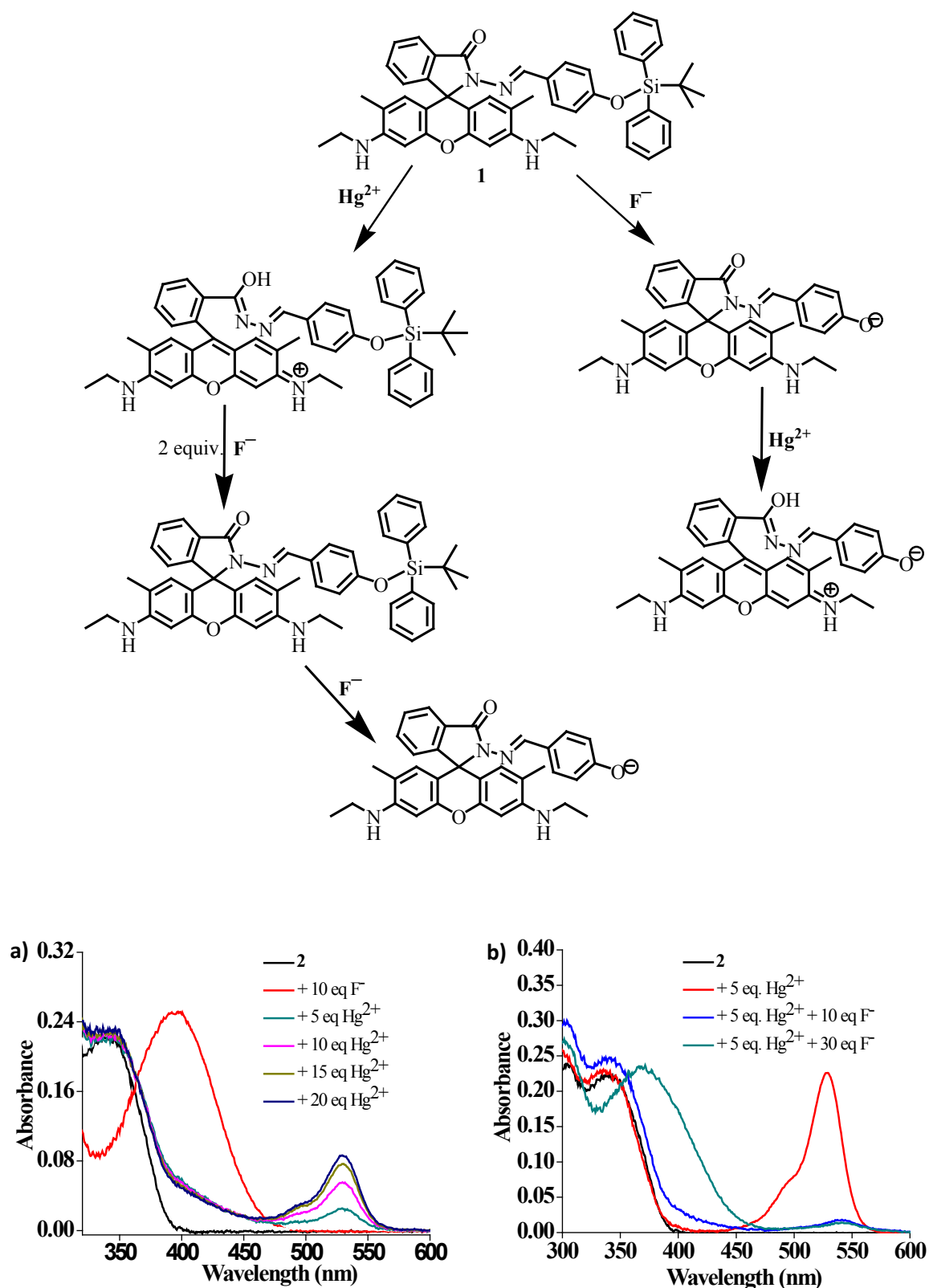


Fig. S16 UV-vis spectra of (a) **2** (10 μM) in presence of F^- ions and to which titration was performed with progressive addition of Hg^{2+} ions. (b) **2** (10 μM) in presence of Hg^{2+} ions and to which titration was performed with progressive addition of F^- ions.

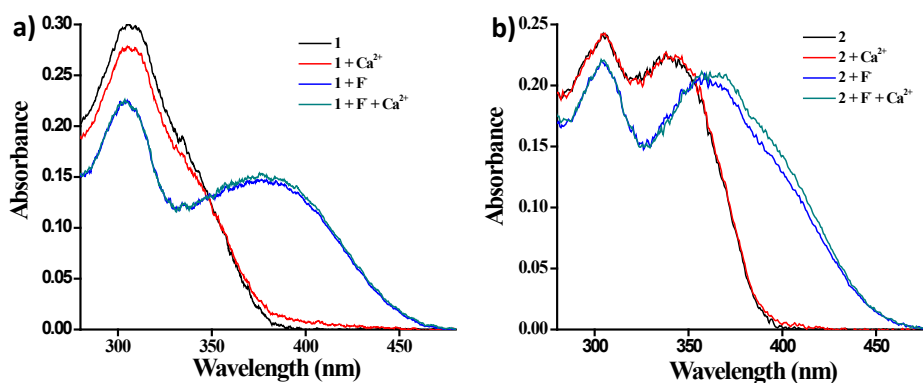


Fig. S17 UV-vis spectra of (a) **1** (10 μM) and (b) **2** (10 μM) in acetonitrile upon addition of 5 equiv. of Ca²⁺ ion after adding F⁻ (10 equiv.).

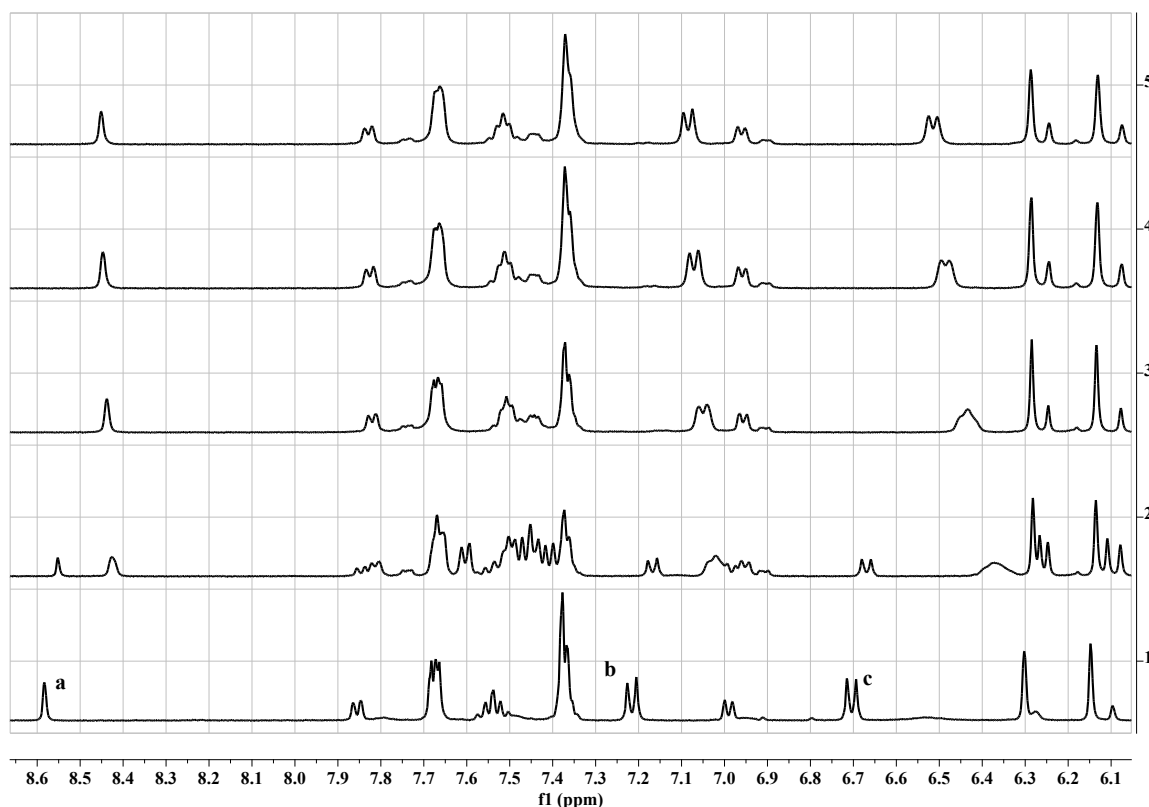
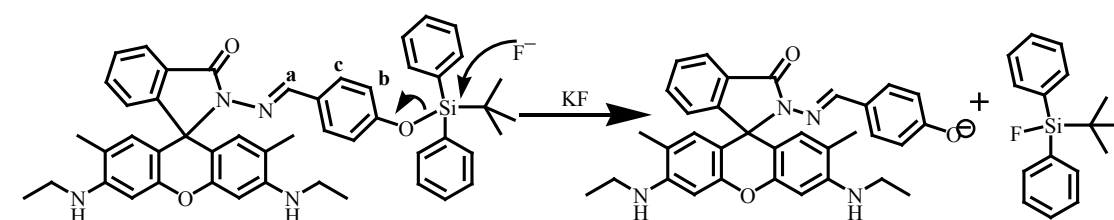


Fig. S18 Partial ¹H NMR (400 MHz) spectra of **1** in DMSO-*d*₆ in the presence of [0, 0.25, 0.5, 0.75 and 1 equiv. (1-4)] of F⁻ (KF in D₂O).

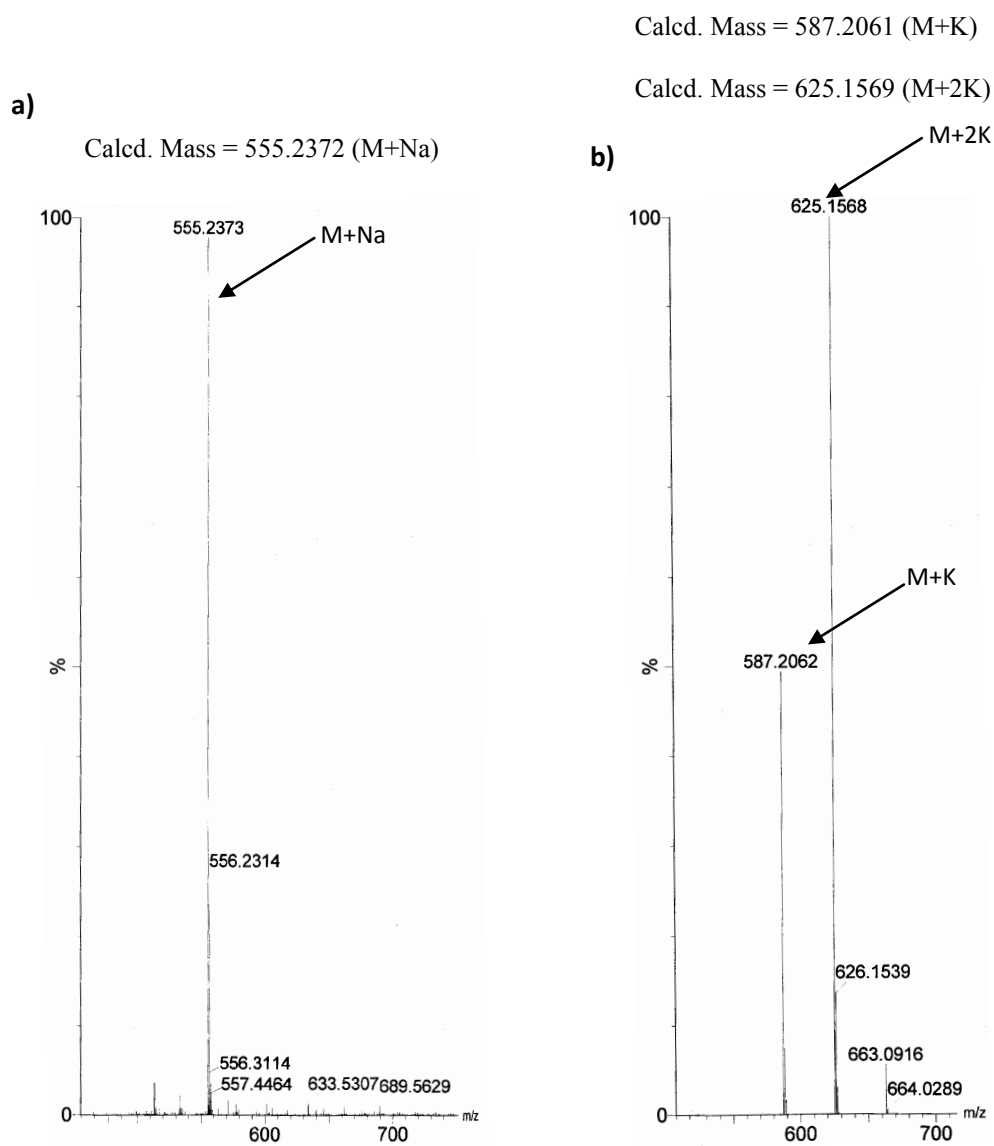


Fig. S19 Mass spectra of (a) **1** and (b) **2** after addition of F^- ion.

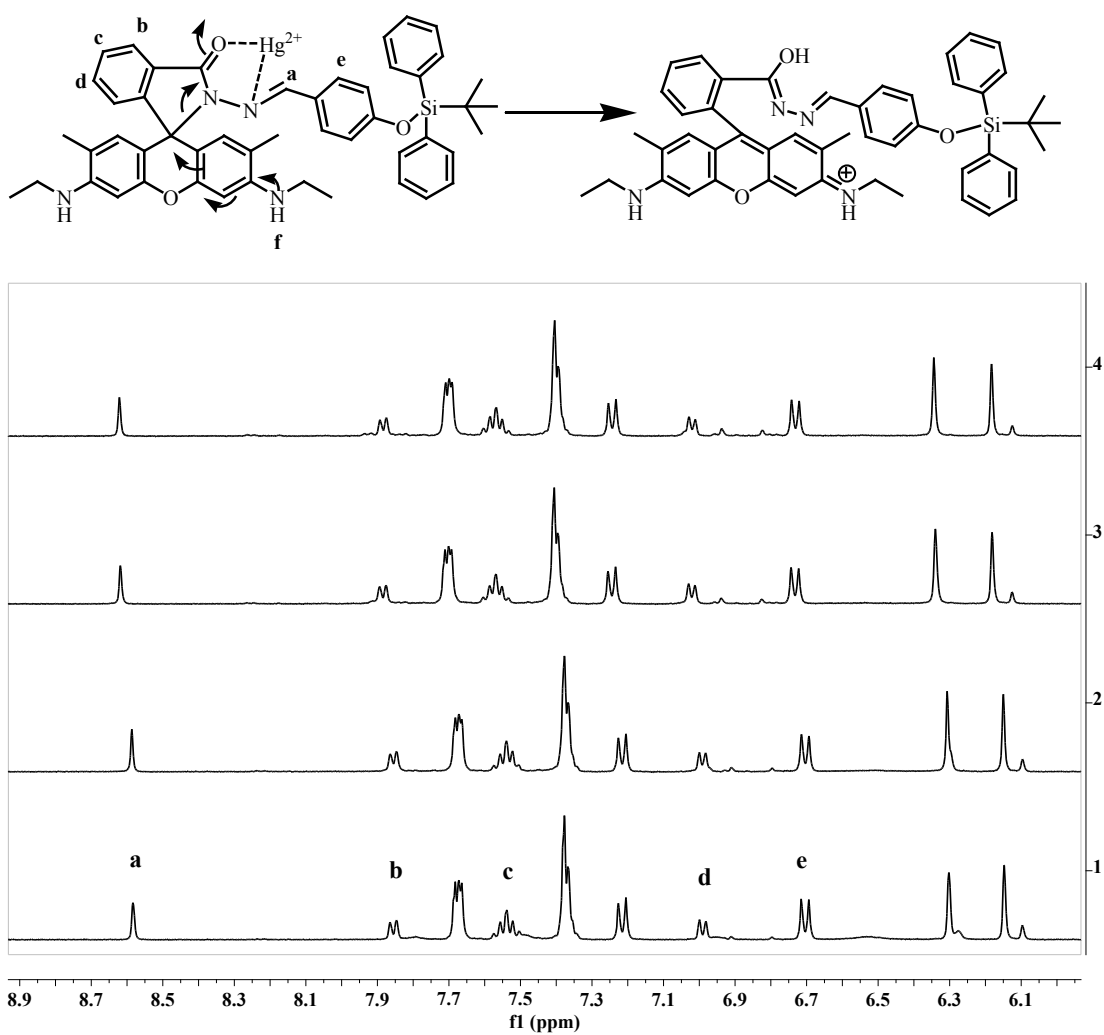


Fig. 20 ¹H NMR titration of **1** (8 mM) with [0, 0.25 eq., 0.5 eq. 0.75 eq. and 1 eq. (1-5)] Hg²⁺ (Hg(ClO₄)₂) in DMSO-*d*₆.

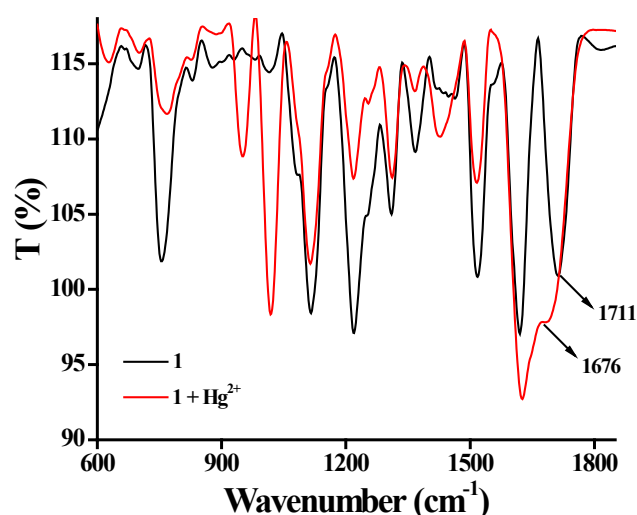


Fig. S21 IR spectra of 1 and 1-Hg²⁺.

Protocol for the detection of Fluoride ion in toothpaste. The toothpaste was weighed different amounts (4 samples; 3, 4, 5 and 6 mg) in small vials. The samples were dried overnight in oven (Temperature = 75 °C). Then to each sample 3 mL of water was added. After that the solutions were sonicated at 50 °C for 15 minute and equilibrated for 2 hours at room temperature. Then they were centrifuged and filtered to get clear solution. This solution was treated with the probe solution and the corresponding emission spectra were recorded in 8:2 CH₃CN-Water mixture. In each case enhancement of emission of probe 2 at 535 nm was observed upon addition of toothpaste solution.

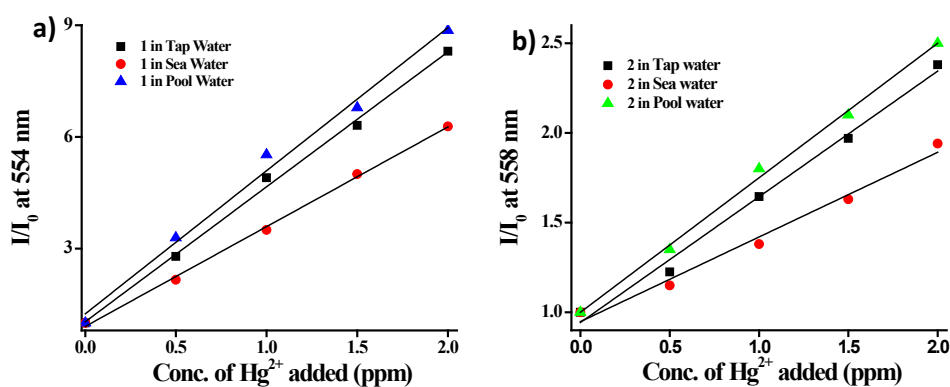


Fig. S22 Changes in the emission intensity ratio at 554 nm of (a) 1 (5 μM) ($\lambda_{\text{ex.}} = 305 \text{ nm}$) and (b) 2 (5 μM) ($\lambda_{\text{ex.}} = 305 \text{ nm}$) with the added Hg²⁺ in tap water, sea water, and swimming pool water.

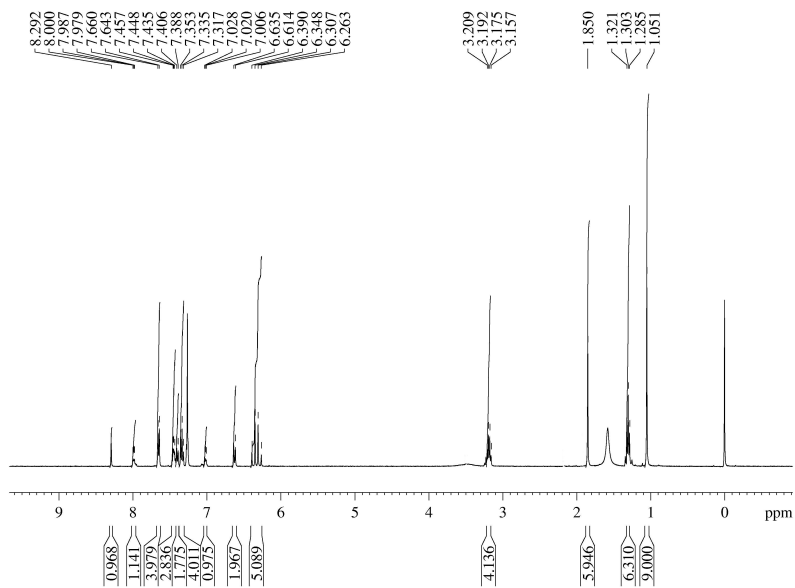


Fig. S23 ^1H NMR of compound 1.

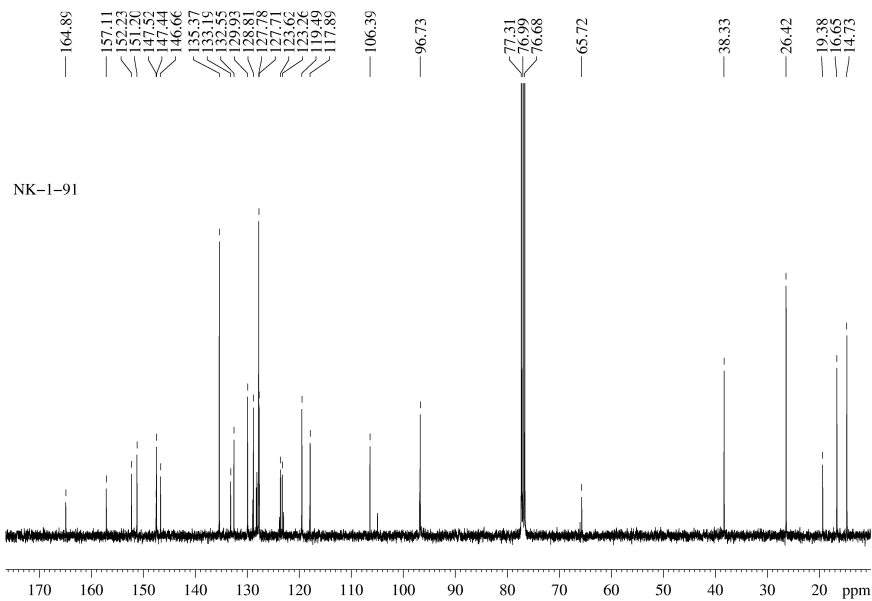


Fig. S24 ^{13}C NMR of compound 1.

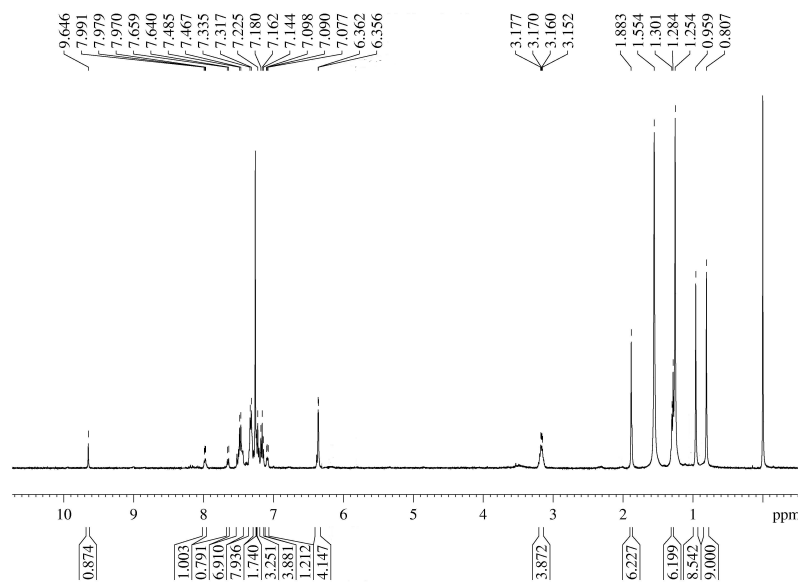


Fig. S25 ^1H NMR of compound **2**.

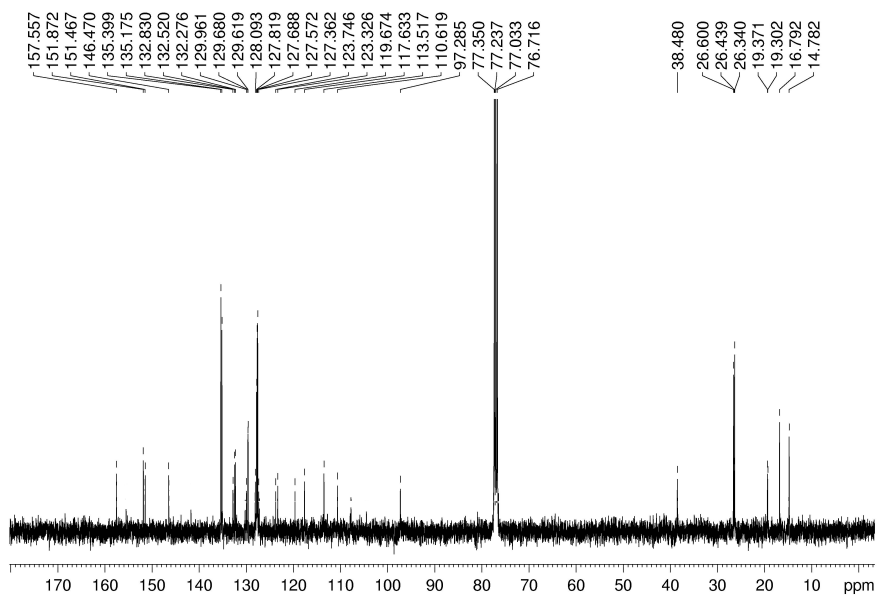


Fig. S26 ^{13}C NMR of compound **2**.