

Electronic Supplementary Information

for

A triphenylamine–based fluorescent probe with phenylboronic acid for highly selective detection of Hg²⁺ and CH₃Hg⁺ in ground water

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1. Materials, instruments, and methods

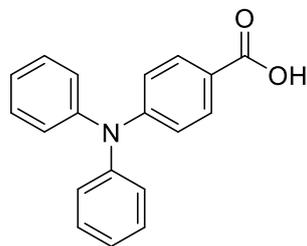
All raw materials and solvents were purchased through commercial channels, unless otherwise mentioned. ^1H NMR and ^{13}C NMR spectra were recorded by a nuclear magnetic resonance spectrometer (AV-400, Bruker, Germany). The UV-Vis spectra and fluorescence spectra were monitored via a UV-Vis spectrophotometer (UV-3900, Hitachi, Japan) and fluorescence spectrometer (FL-7000, Hitachi, Japan).

The limit of detection (LOD) was calculated based on fluorescence titrations. A plot of the measured fluorescence intensity at the emission band 440 nm versus concentration of $\text{Hg}^{2+}/\text{CH}_3\text{Hg}^+$ added allowed calculation of the limit of detection from equation $\text{LOD} = 3\sigma / k$, where σ is the standard deviation of the emission of a blank solution, which was measured 20 times, and k is the slope of the calibration curve. In the fluorescence detection system, the concentration of **TPA-PBA** is 1 μM . First, 1 mM probe solution is prepared by dissolving **TPA-PBA** in DMSO. Next, HEPES buffer solution (pH 7.4, 10% DMSO, 10 mM) was prepared as the concentration diluent. The fluorescence emission spectra of all detection systems were recorded in the wavelength range of 400–600 nm under 344 nm excitation.

Detailed protocol for the detection of mercury in the wastewater using the **TPA-PBA** prob

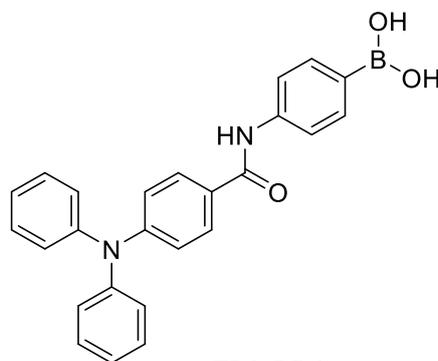
Firstly, 1 mL of ultrapure water and 1 mL of 2 μM **TPA-PBA** solution (20% DMSO, pH=7.40, 20 mM HEPES) were mixed well, and $\text{Hg}^{2+}/\text{CH}_3\text{Hg}^+$ was added accordingly while the resulting fluorescence spectra were recorded. Then, the fluorescence intensity at 440 nm was linearly fitted to the added $\text{Hg}^{2+}/\text{CH}_3\text{Hg}^+$ concentration to obtain the corresponding standard curve. Next, after the insoluble impurities in the actual water sample were removed by filter membrane, 1 mL of the actual water sample and 1 mL of 2 μM **TPA-PBA** solution (20% DMSO, pH=7.40, 20 mM HEPES) were mixed well, and different concentrations of $\text{Hg}^{2+}/\text{CH}_3\text{Hg}^+$ were added respectively. The stabilized fluorescence intensities were substituted into the resulting standard curves, and the resulting standard concentrations were compared with the actual addition to calculate the recoveries, and each group of experiments was tested for three times.

2. Synthesis



TPA-COOH

TPA - COOH: 4-(diphenylamino)benzaldehyde (2 g, 7.34 mmol) was dissolved in 100 mL acetone-H₂O (v/v=4:1) and 0.96 g KMnO₄ was added in batches. the reaction mixture was stirred at 80 °C for 4 h. The reaction process was detected by TLC, the mixture was filtered and the filtrate was washed with 1 M HCl aqueous solution to give a white precipitate. The precipitate was filtered, washed with water and dried under vacuum to give a white solid 4-diphenylaminobenzoic acid, TPA-COOH, in 58% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ = 12.52 (s, 1H), 7.78 (d, *J* = 8.8 Hz, 2H), 7.37 (t, *J* = 7.9 Hz, 4H), 7.17 (d, *J* = 7.2 Hz, 2H), 7.12 (d, *J* = 8.7 Hz, 4H), 6.87 (d, *J* = 8.8 Hz, 2H).



TPA-PBA

TPA - BPA: TPA-COOH (0.5 g, 1.73 mmol) and p-aminophenylboronic acid hydrochloride (0.3 g, 1.73 mmol) were dissolved in 20 mL of ethylene glycol ether at room temperature and reacted for 10 min. followed by the addition of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM) (0.51 g, 1.73 mmol). The reaction was monitored by TLC for 3 h at room temperature. The reaction mixture was poured into water and extracted with dichloromethane. The organic phases were combined and washed with saturated sodium carbonate, 1 M HCl aqueous solution and saturated salt water in turn, and dried with anhydrous Na₂SO₄. The

crude product was purified by column chromatography to give 472 mg of pale yellow solid TPA-B (67%). ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ = 10.07(s, 1H), 7.91 (s, 2H), 7.85 (d, J = 8.8 Hz, 2H), 7.78 – 7.69 (q, J = 8.1 Hz, 4H), 7.38 (t, J = 7.1 Hz, 4H), 7.16 (d, J = 7.5 Hz, 2H), 7.11 (d, J = 7.4 Hz, 4H), 6.96 (d, J = 8.8 Hz, 2H). ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ = 165.02, 150.34, 146.43, 141.10, 134.73, 129.86, 128.69, 127.28, 125.30, 124.38, 120.09, 118.97. HRMS (ESI) m/z 407.1567 $[\text{M-H}]^-$ calcd for $\text{C}_{25}\text{H}_{20}\text{BN}_2\text{O}_3$, found 407.1570.

3. Characterization

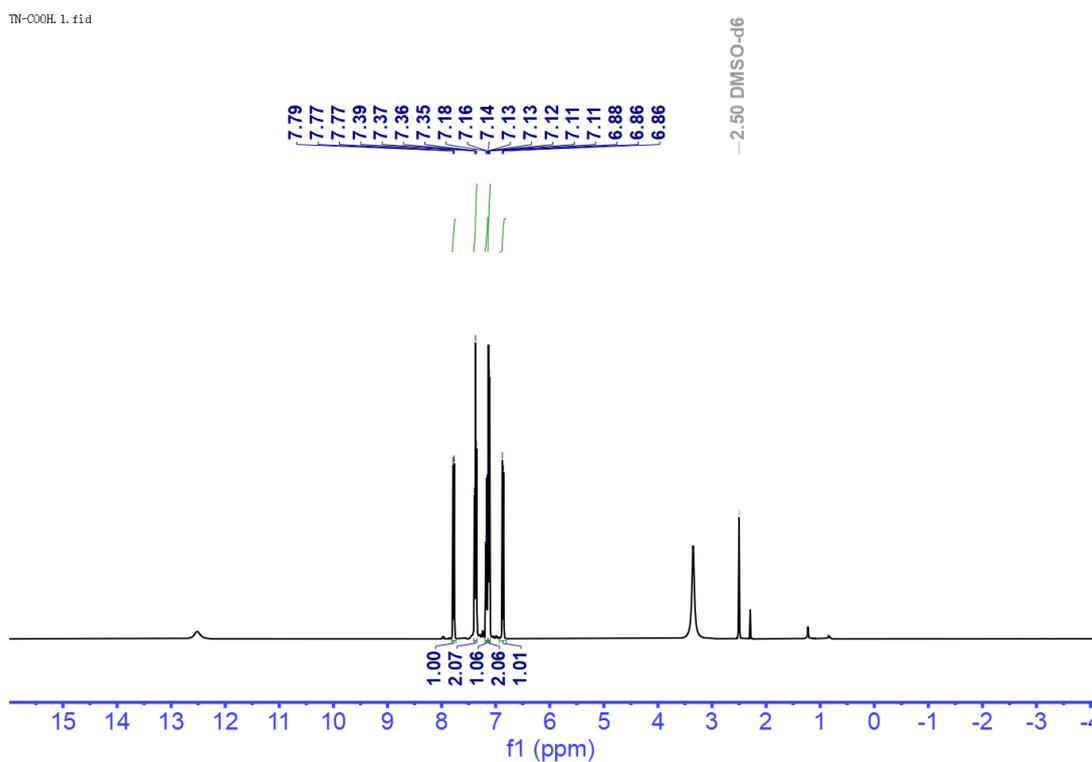


Fig. S1. ^1H NMR spectrum of TPA - COOH

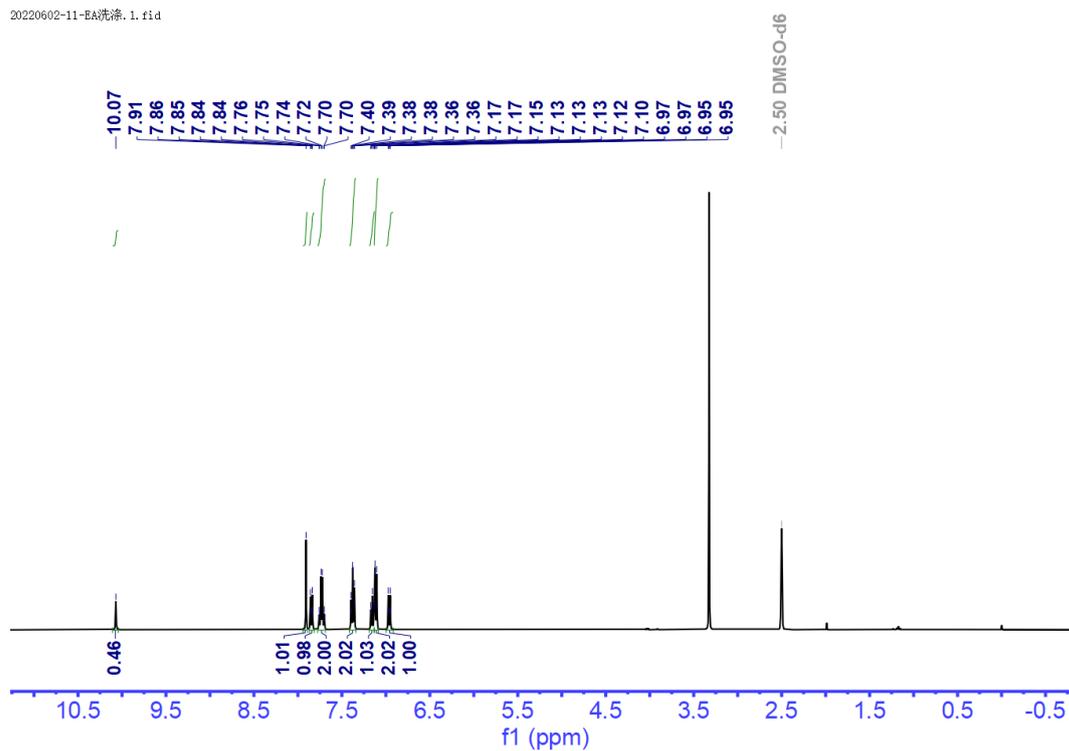


Fig. S2. ^1H NMR spectrum of TPA - PBA

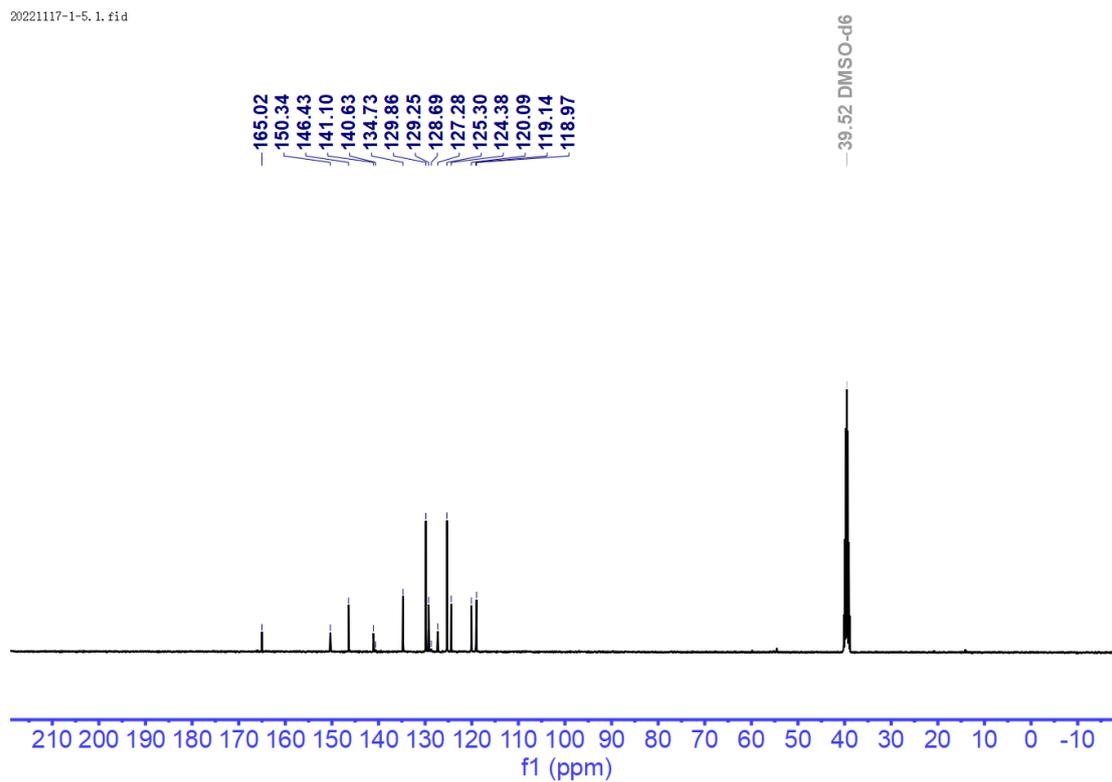


Fig. S3. ^{13}C NMR spectrum of TPA - PBA

TPN #20 RT: 0.27 AV: 1 NL: 5.08E4
T: FTMS (1,2) - p ESI Full ms [150.00-2000.00]

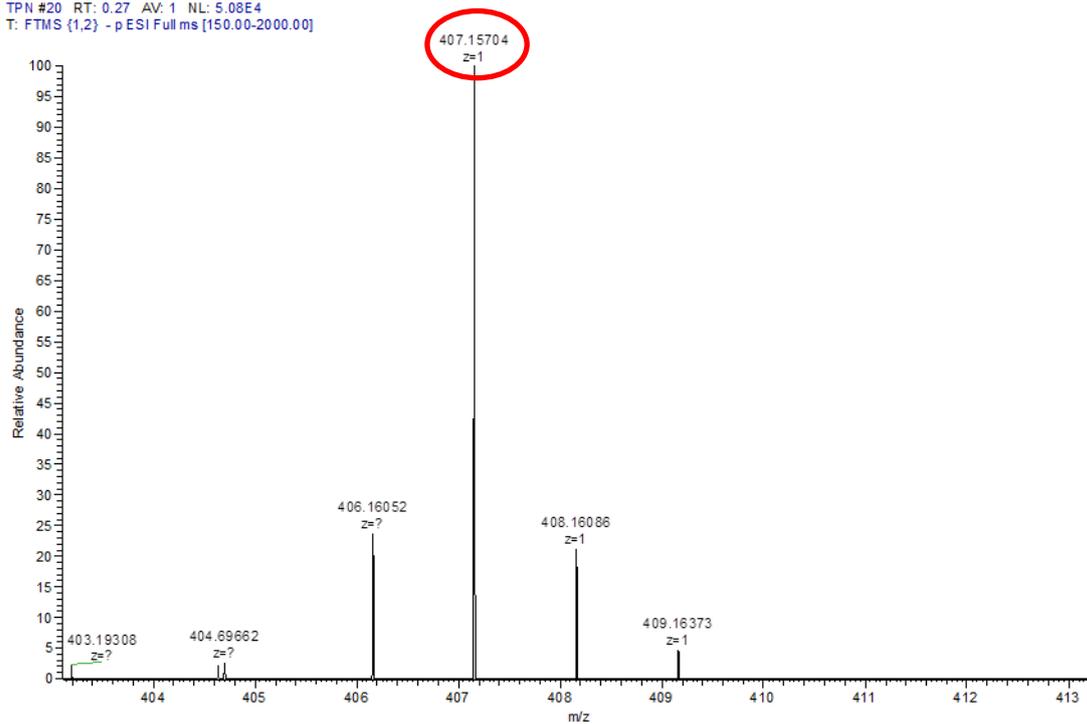


Fig. S4. ESI-MS spectrum of TPA - PBA

4. Spectroscopic analysis

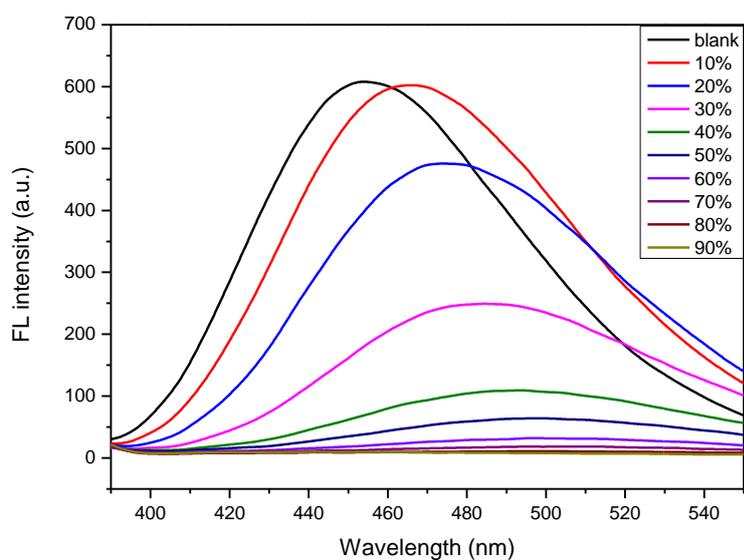


Fig. S5. Fluorescence spectra of TPA-PBA (1 μM) in DMSO system with different water content.

$\lambda_{\text{ex}}=344$ nm. Slit: 2.5 nm/2.5 nm.

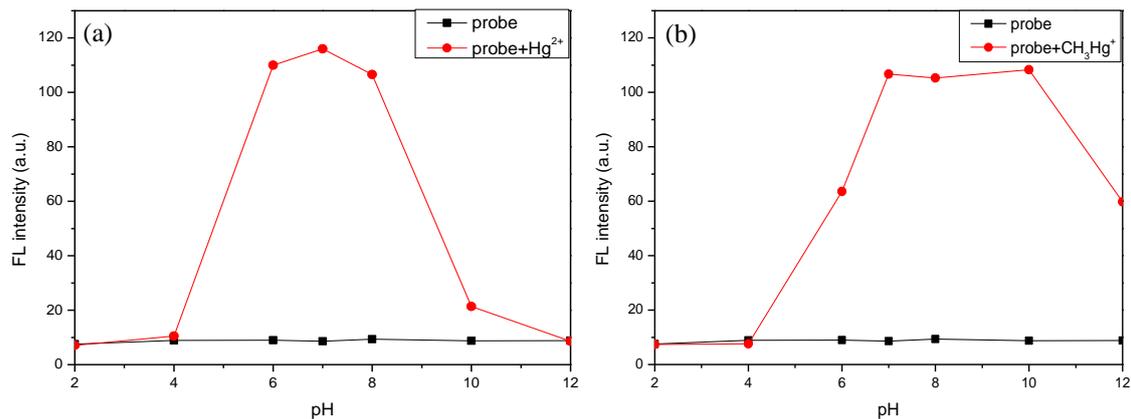


Fig. S6. pH effect on the fluorescence emission of TPA-PBA and (a) TPA-PBA/ Hg^{2+} and (b) TPA-PBA/ CH_3Hg^+ in DMSO/ H_2O ($f_w = 90\%$, pH 7.4, 10 mM HEPES) $\lambda_{\text{ex}} = 344$ nm, Slit: 2.5 nm/2.5 nm.

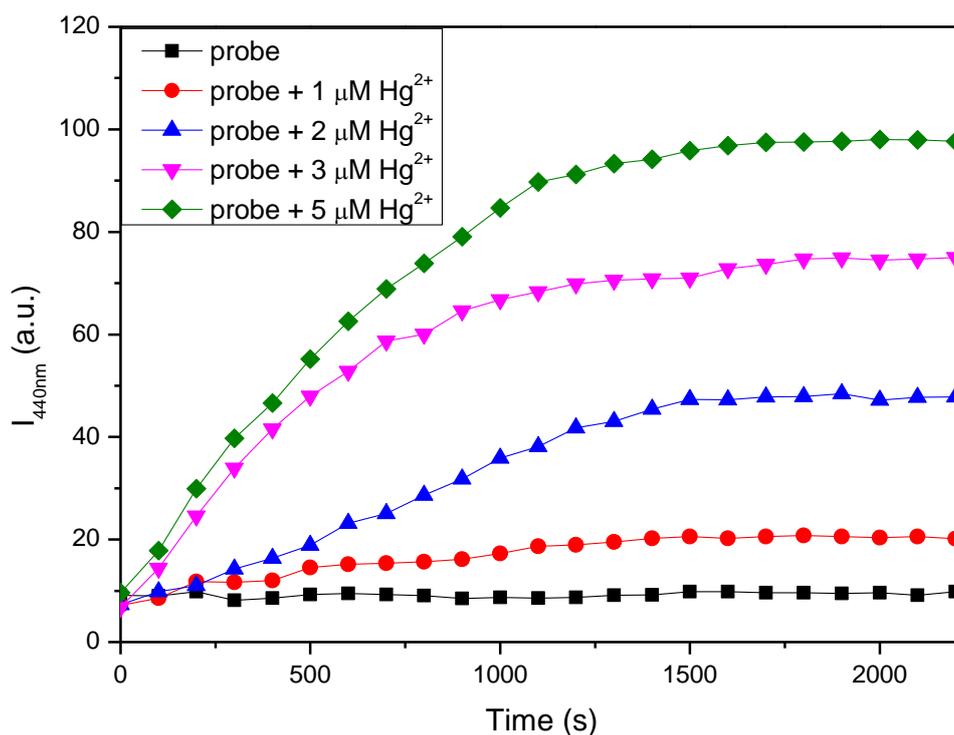


Fig. S7 Time-dependent emission intensity at 440 nm of TPA-PBA (1 μM) with Hg^{2+} (0–5 equiv.) in aqueous buffered solution (10 mM, HEPES, pH 7.4) containing 10% DMSO ($\lambda_{\text{ex}} = 344$ nm).

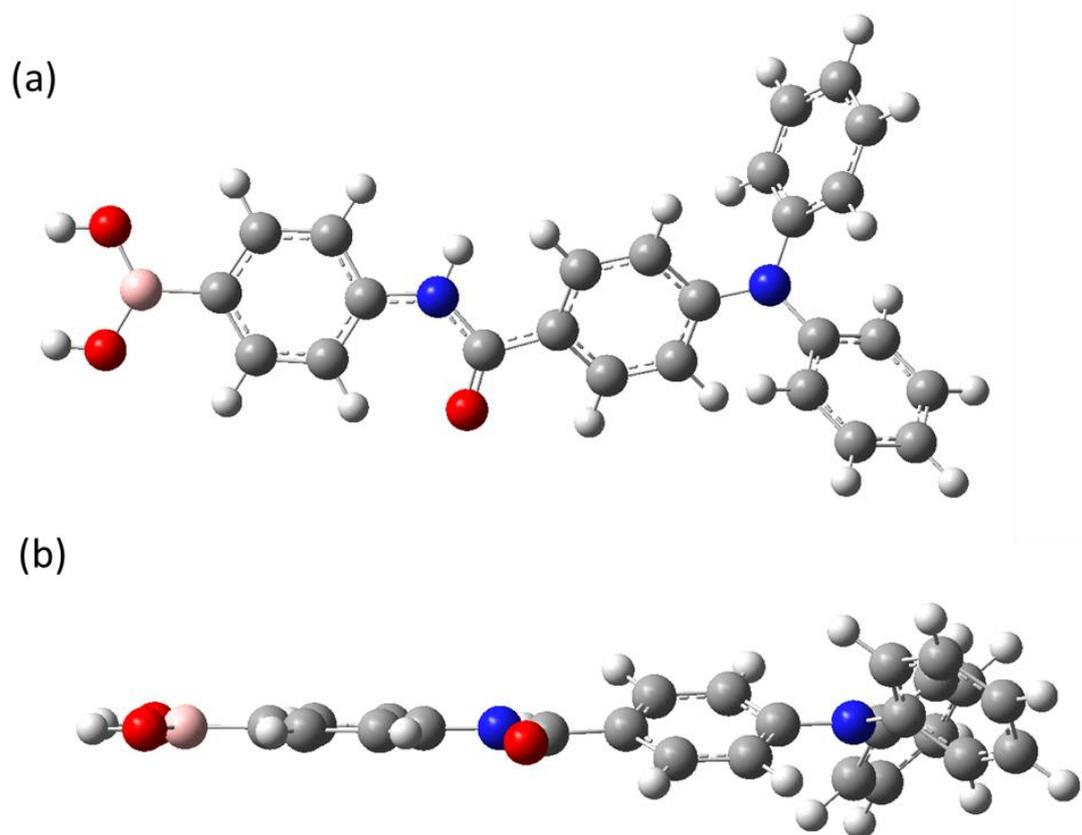


Fig. S8 DFT-optimized structure of probe TPA-PBA: (a) top view and (b) front view.

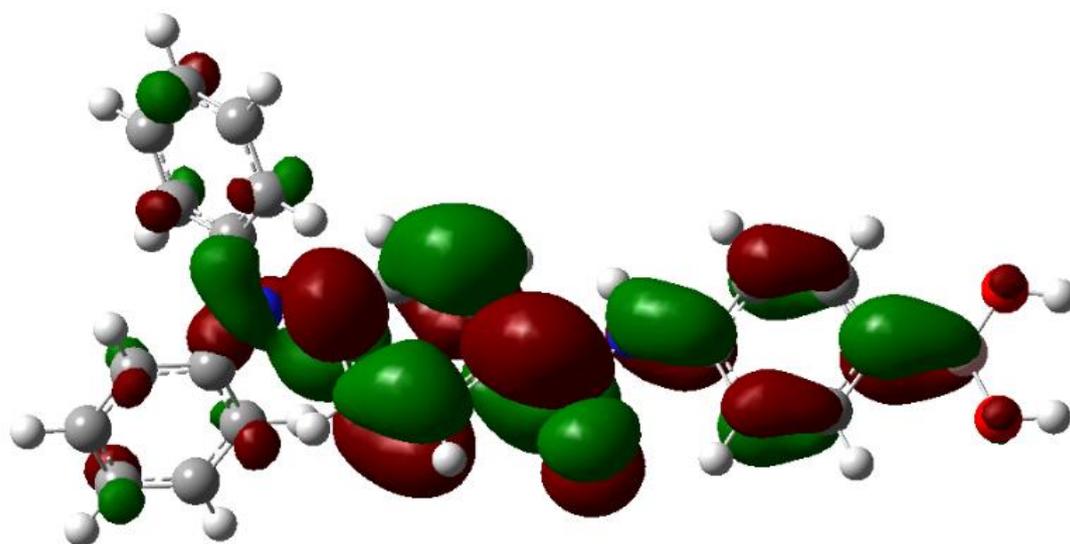


Fig. S9 The LUMO of TPA-PBA probe.

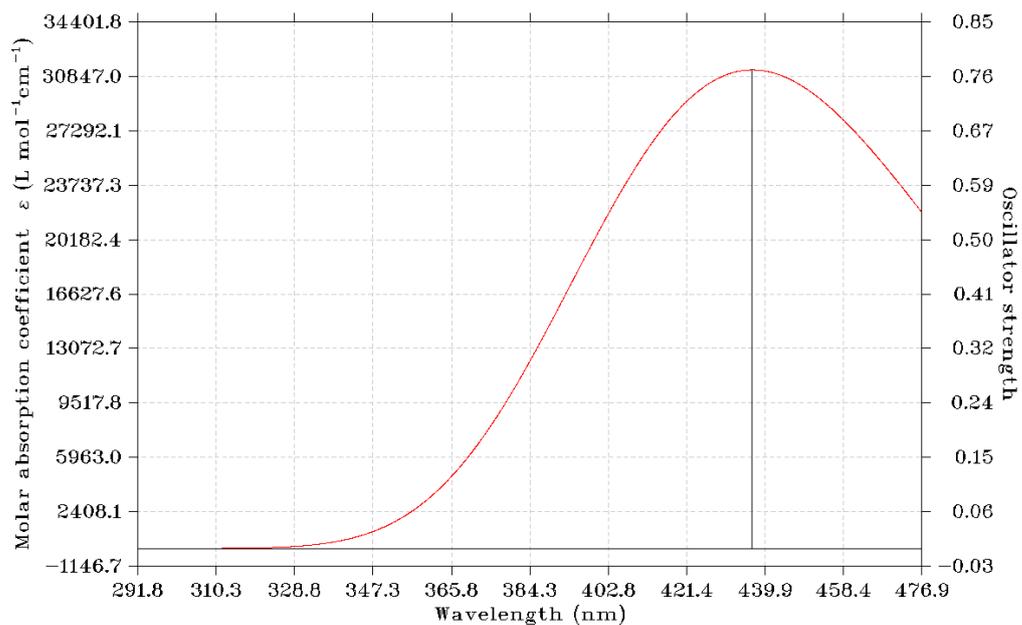


Fig. S10 Theoretical fluorescent spectrum of probe **TPA-PBA** in water, showing the emission maxima at about 440 nm as well.

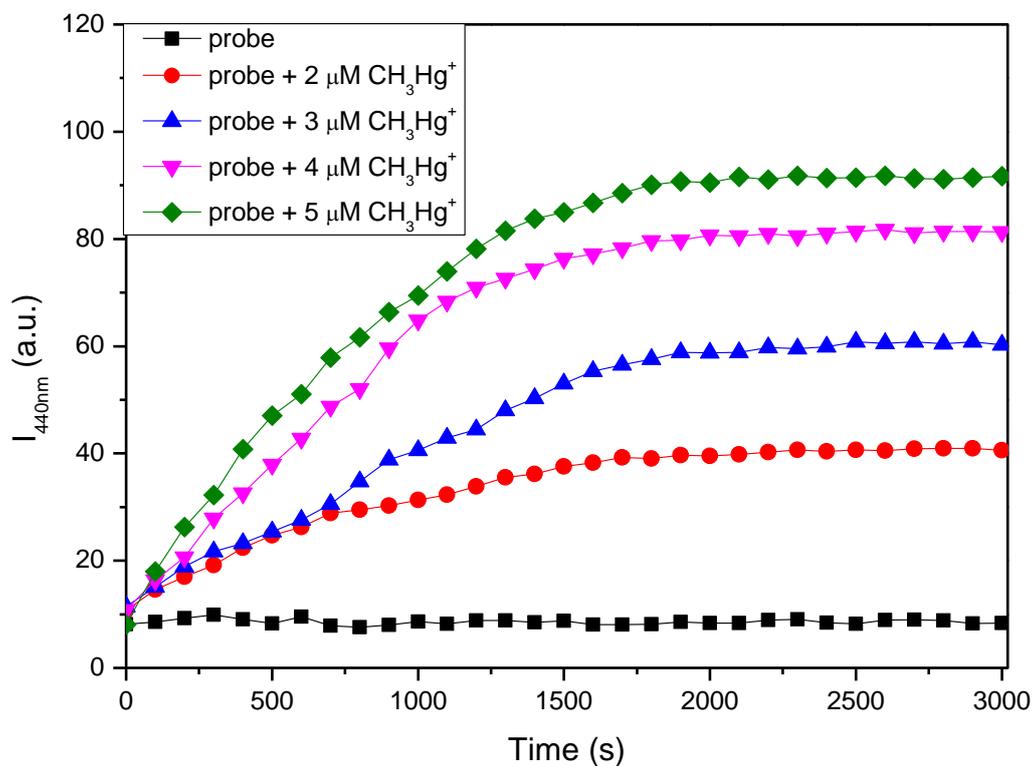


Fig. S11 Time-dependent emission intensity at 435 nm of **TPA-PBA** (1 μM) with MeHg^+ (0–5 equiv.) in aqueous buffered solution (10 mM, HEPES, pH 7.4) containing 10% DMSO ($\lambda_{\text{ex}} = 344$ nm).

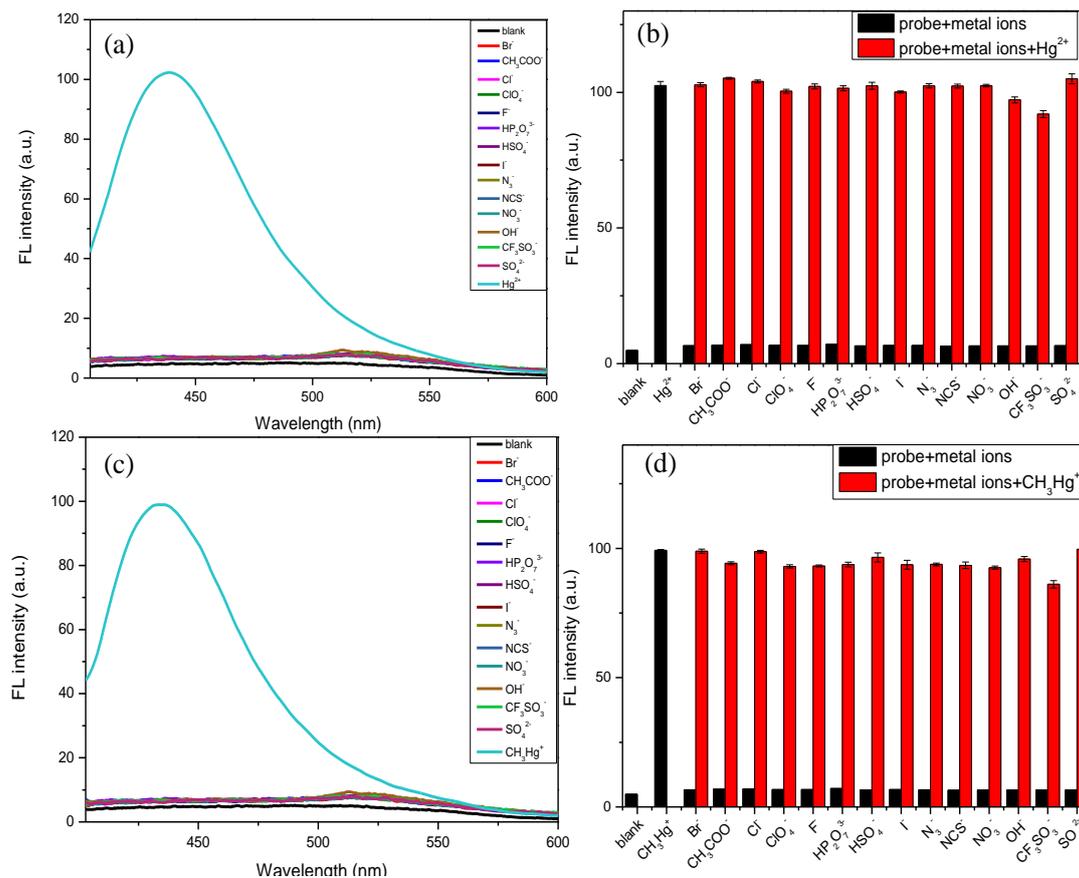


Fig. S12. Selective experiments of TPA-PBA (1.0 μM) in DMSO/H₂O ($f_w = 90\%$, pH 7.4, 10 mM HEPES) medium after the addition of (a) Hg²⁺ (10 μM) and (c) CH₃Hg⁺ (10 μM) and other interfering ions (1000 μM), respectively; The interference experiments of TPA-PBA (1 μM) in DMSO/H₂O ($f_w = 90\%$, pH 7.4, 10 mM HEPES) medium with (b) Hg²⁺ (10 μM) and (d) CH₃Hg⁺ (10 μM) and other interfering ions (1000 μM), respectively. $\lambda_{\text{ex}}=344$ nm. Slit: 2.5 nm/2.5 nm.

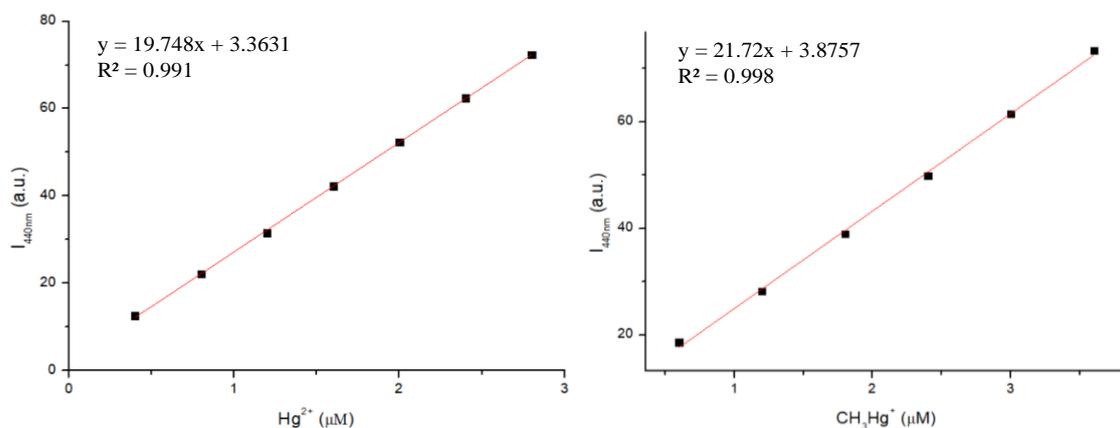


Fig. S13. The fluorescence intensity of TPA - PBA at 440 nm as a function of (a) Hg²⁺ (0–3 μM) and (b) CH₃Hg⁺ (0–3.6 μM) in pure water. $\lambda_{\text{ex}}=344$ nm. Slit: 2.5 nm/2.5 nm.

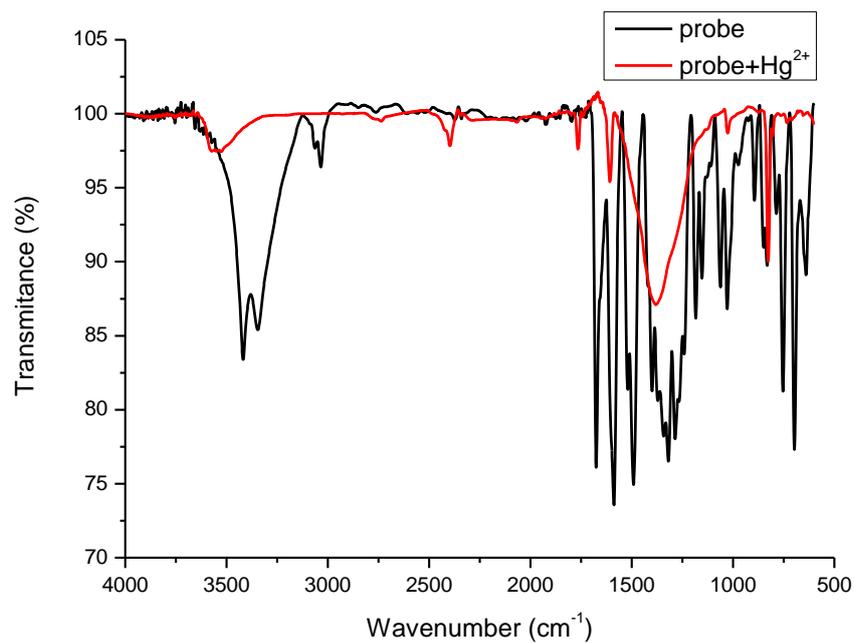


Fig. S14. FTIR spectrum of the product of TPA - PBA with Hg^{2+} (5eq)

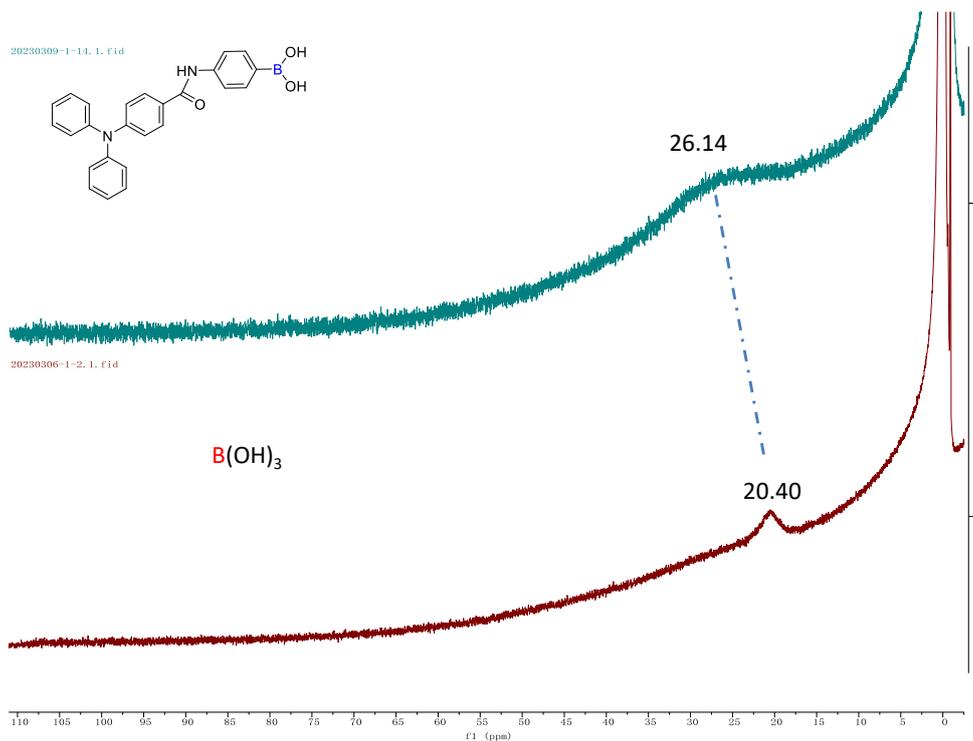


Fig. S15. Partial ^{11}B NMR spectra of probe TPA-PBA and a mixture of TPA-PBA and Hg^{2+} in $\text{DMSO}-d_6$.

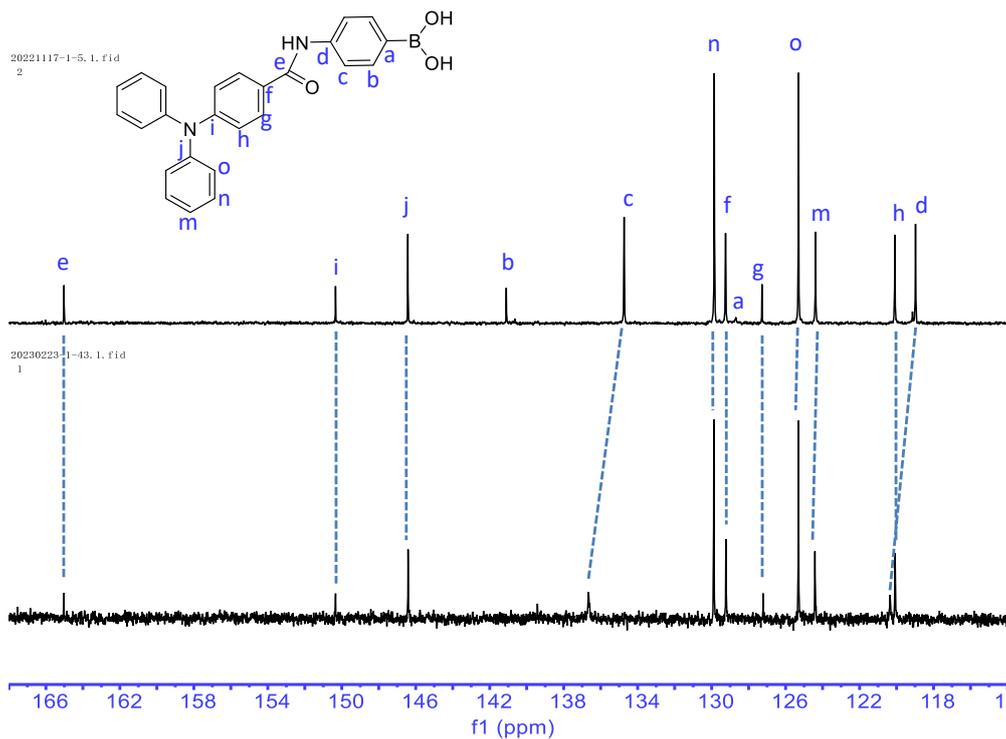


Fig. S16. Partial ^{13}C NMR spectra of probe **TPA-PBA** and a mixture of **TPA-PBA** and Hg^{2+} in $\text{DMSO-}d_6$.

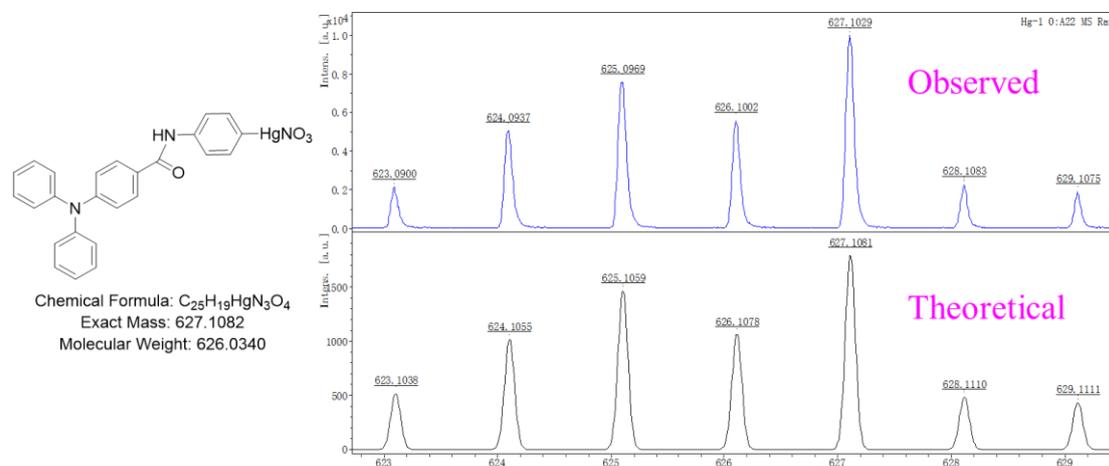


Fig. S17. MS (EI) of the reaction product of **TPA-PBA** with 5 equiv Hg^{2+}