Electronic Supplementary Material (ESI) for Analytical Methods

Supporting Information

Mercuric–triggered hydrogen peroxide "turn–on" fluorescence detection in neuronal cells with novel fluorescein–based probe obtained in one pot

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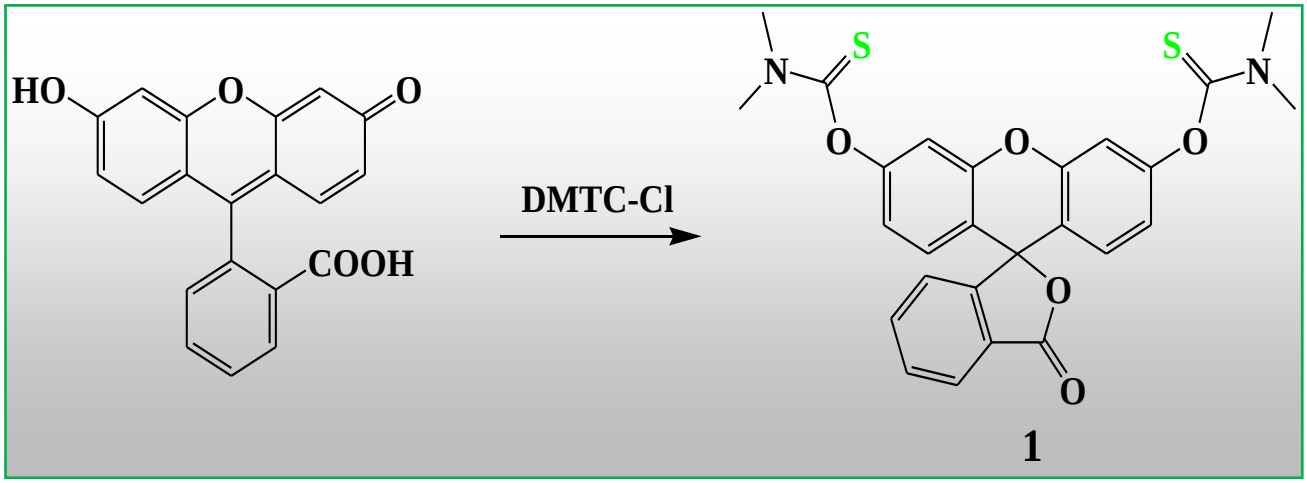
Experimental Section

General Remarks. All reagents used herein were used as received from the suppliers (Aldrich, Acros, and Junsei companies). ^{1}H and ^{13}C NMR spectra were acquired using a Bruker Avance 400 MHz spectrometer. TMS was used as an internal standard. ^{1}H and ^{13}C NMR spectral signals were calibrated internally by the respective protio impurity or carbon resonance of the NMR spectroscopic solvent *e.g.*, $CDCl_3$ (^{1}H: δ 7.24; ^{13}C: δ 77.0). ESI-mass spectrometry was performed on a VG AUTOSPEC ULTIMA by the research support staff at KAIST. This instrument possesses a trisector double focusing magnetic sector analyzer and was operated at a resolution of 80,000. Absorption spectra were measured using a JASCO V–530 UV/Vis spectrophotometer. Fluorescence measurements were carried out with a Shimadzu RF–5301pc spectrofluorophotometer.

Synthesis of probe 1:

In a 100 mL round bottom flask fluorescein (1 gm, 3.012 mmol) and 1,4-diazabicyclo[2.2.2]octane (1.34 gms. 6.024 mmol) were taken in 30 mL of dry DMF under an inert atmosphere of argon. After 15 mins. dimethylthiocarbamoyl chloride (1.5 gms. 6.024 mmol) was added as solid and reaction was continued at room temperature for 18 h. reaction was quenched by 150 mL of water and kept in refrigerator for 4h. After filtration reaction mixture was washed repeatedly by water and dried to get a yellow powder. (1.02 gms. 68 % yield).



^{1}H NMR spectroscopy: (400 MHz, $CDCl_3$, δ = 7.24): 7.97 (1H, d, J = 7.48 Hz), 7.64-7.56 (2H, m), 7.21 (1H, d, J = 7.64 Hz), 7.00 (2H, S), 6.78-6.73 (2H, m), 3.38 (6H, S), 3.27 (6H, S).

^{13}C NMR spectroscopy: (100 MHz, $CDCl_3$, δ = 77.0): 186.42, 168.92, 155.02, 152.37, 151.32, 135.15, 129.86, 128.28, 126.07, 124.90, 124.04, 118.85, 116.21, 111.42, 81.80, 43.08, and 38.69.

ES-MS: **Fig. S5** Compound **1** $[M+Na]^+$ = 529.0868 (cal.), 529.0866 (exp.)

ES-MS: **Fig. S6** Compound **1** + $\mathbf{Hg^{2+}}$ $[M+Na]^+$ = 497.1325 (cal.), 497.1310 (exp.)

ES-MS: **Fig. S7** Compound **1** + $\mathbf{Hg^{2+}}$ + $\mathbf{H_2O_2}$ $[M+H]^+$ = 333.0763 (cal.), 333.0756 (exp.)

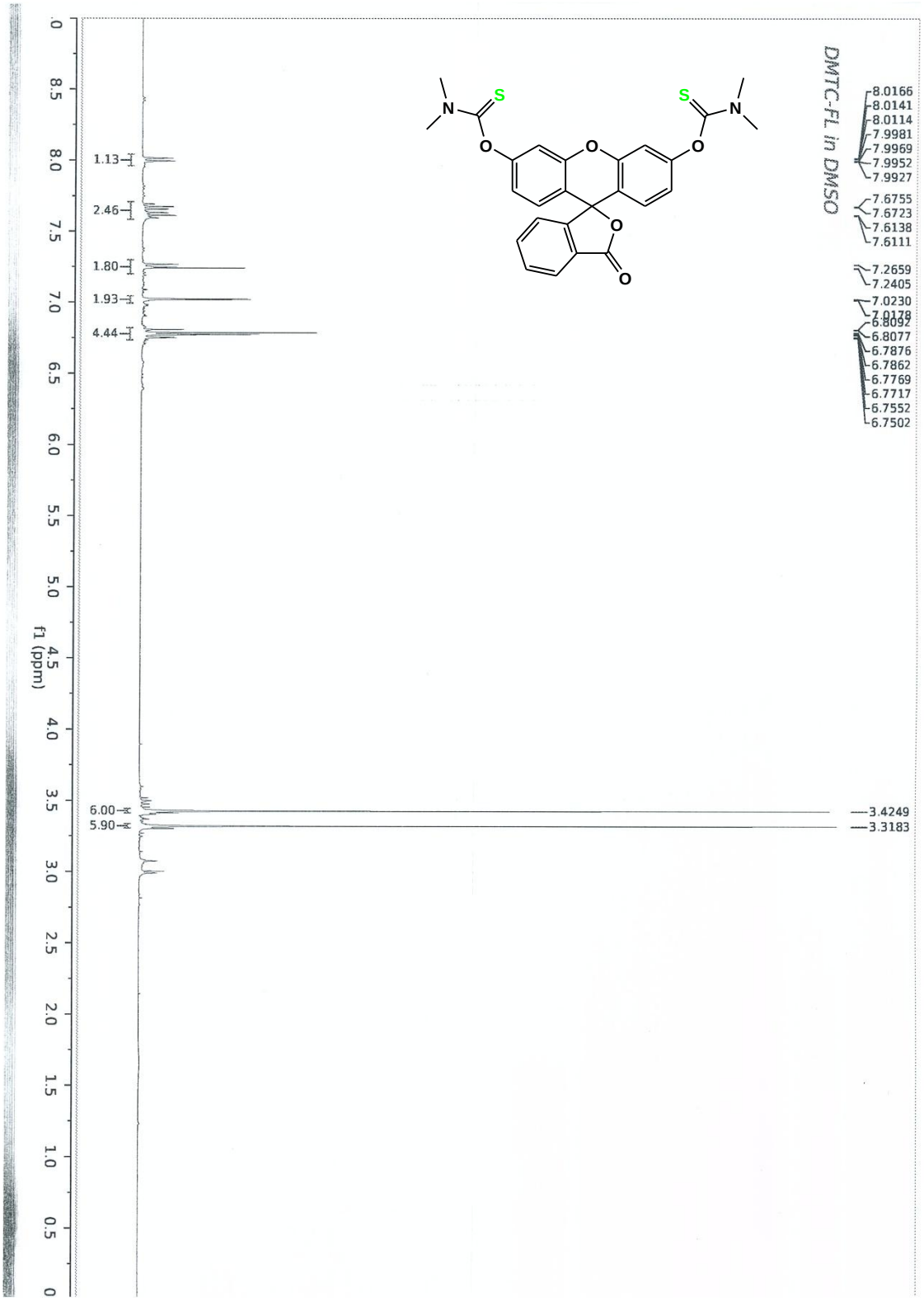


Fig. S1: ^{1}H NMR spectrum of compound **1.**

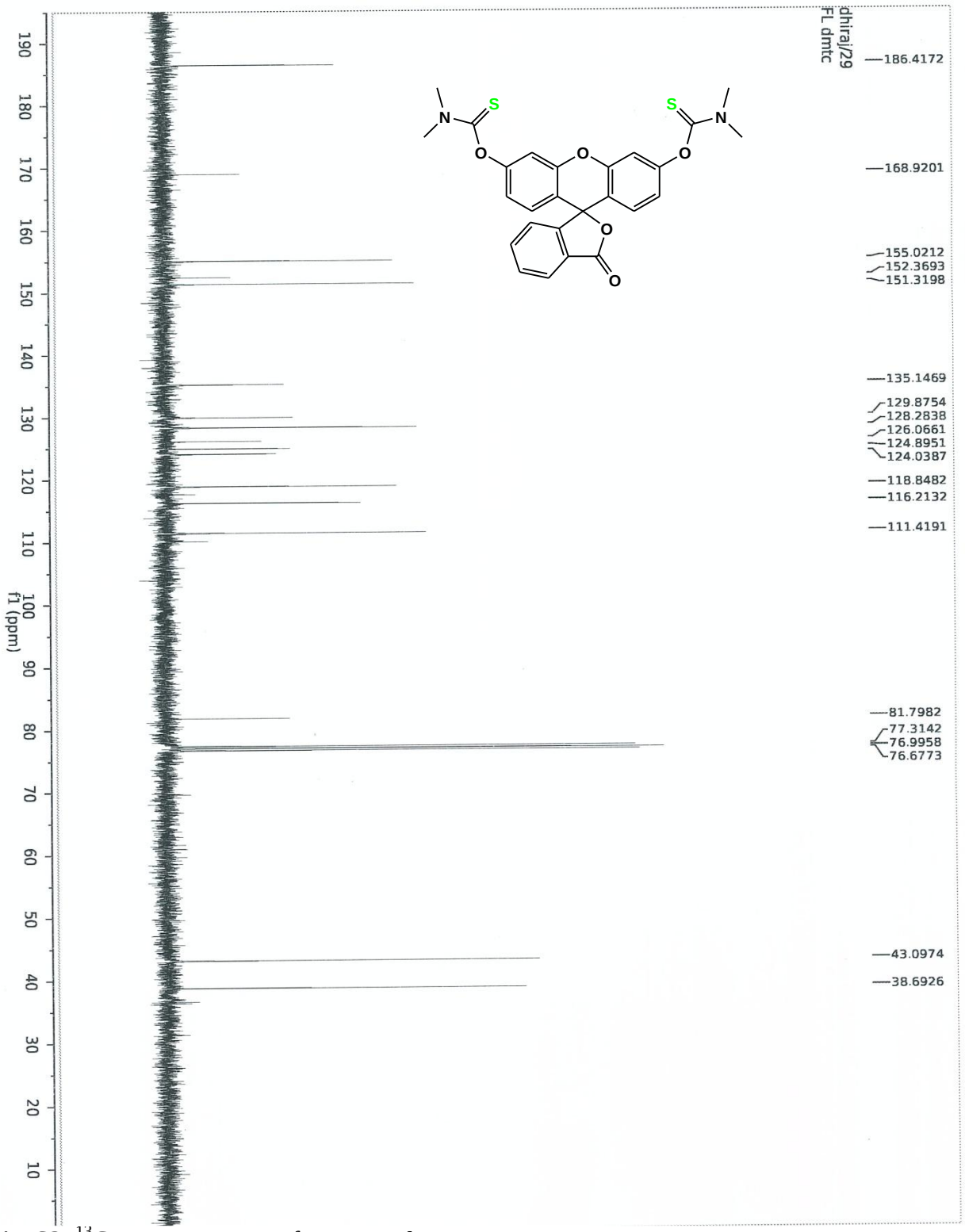


Fig. S2: ^{13}C NMR spectrum of compound **1.**

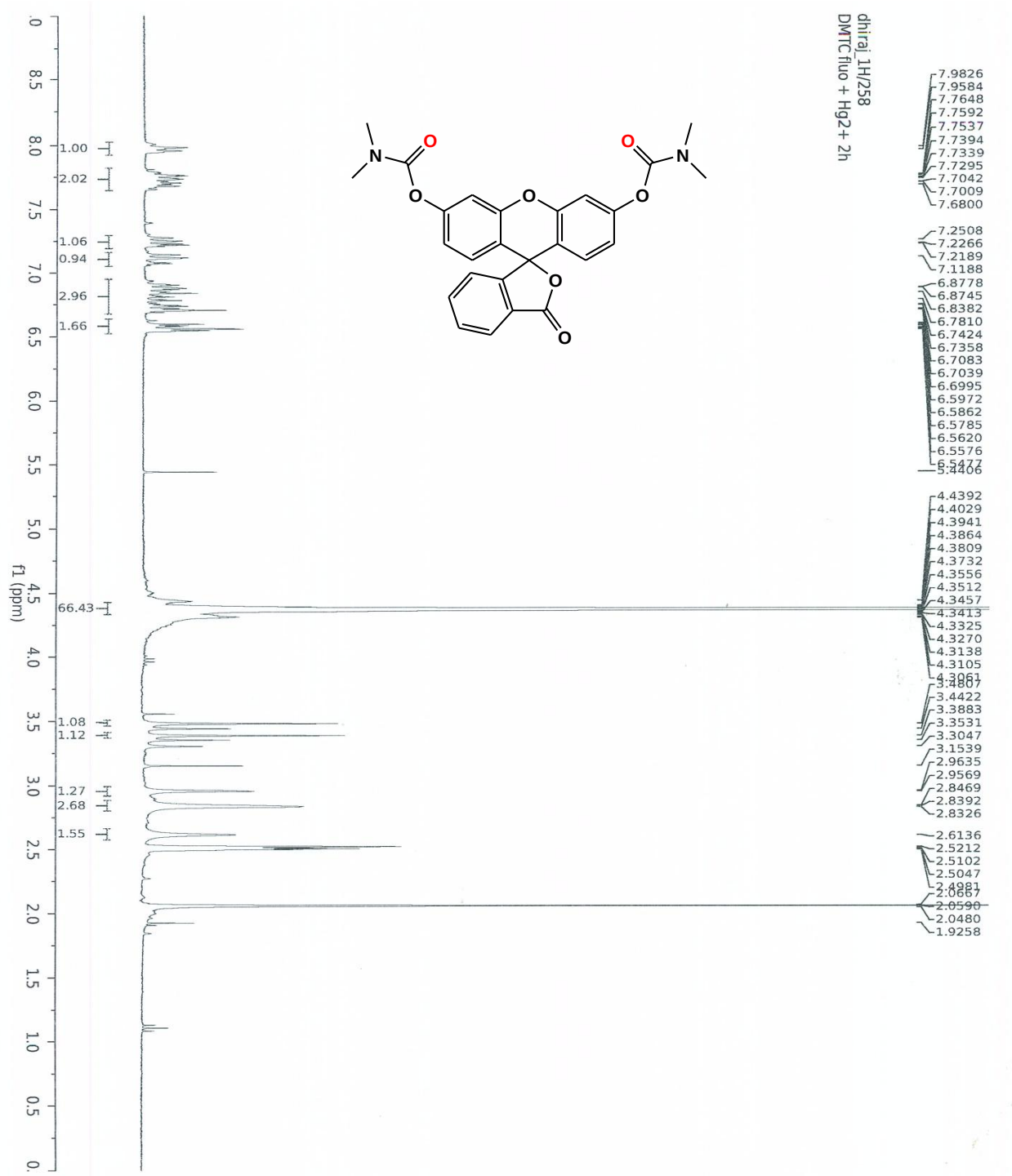


Fig. S3: ^{1}H NMR spectrum of compound **1** + Hg^{2+}.

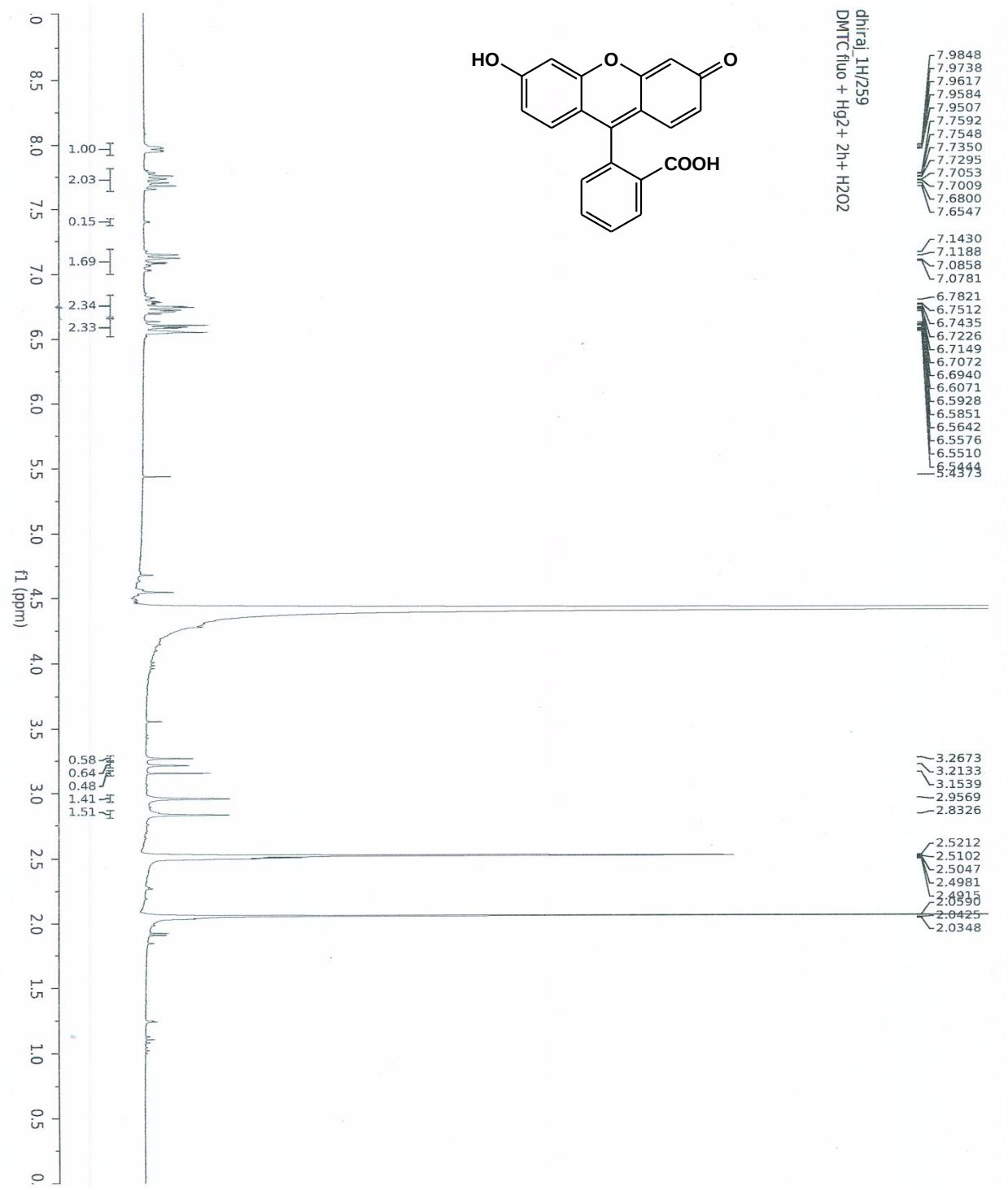


Fig. S4: ^{1}H NMR spectrum of compound **1 + Hg^{2+} + H_2O_2.**

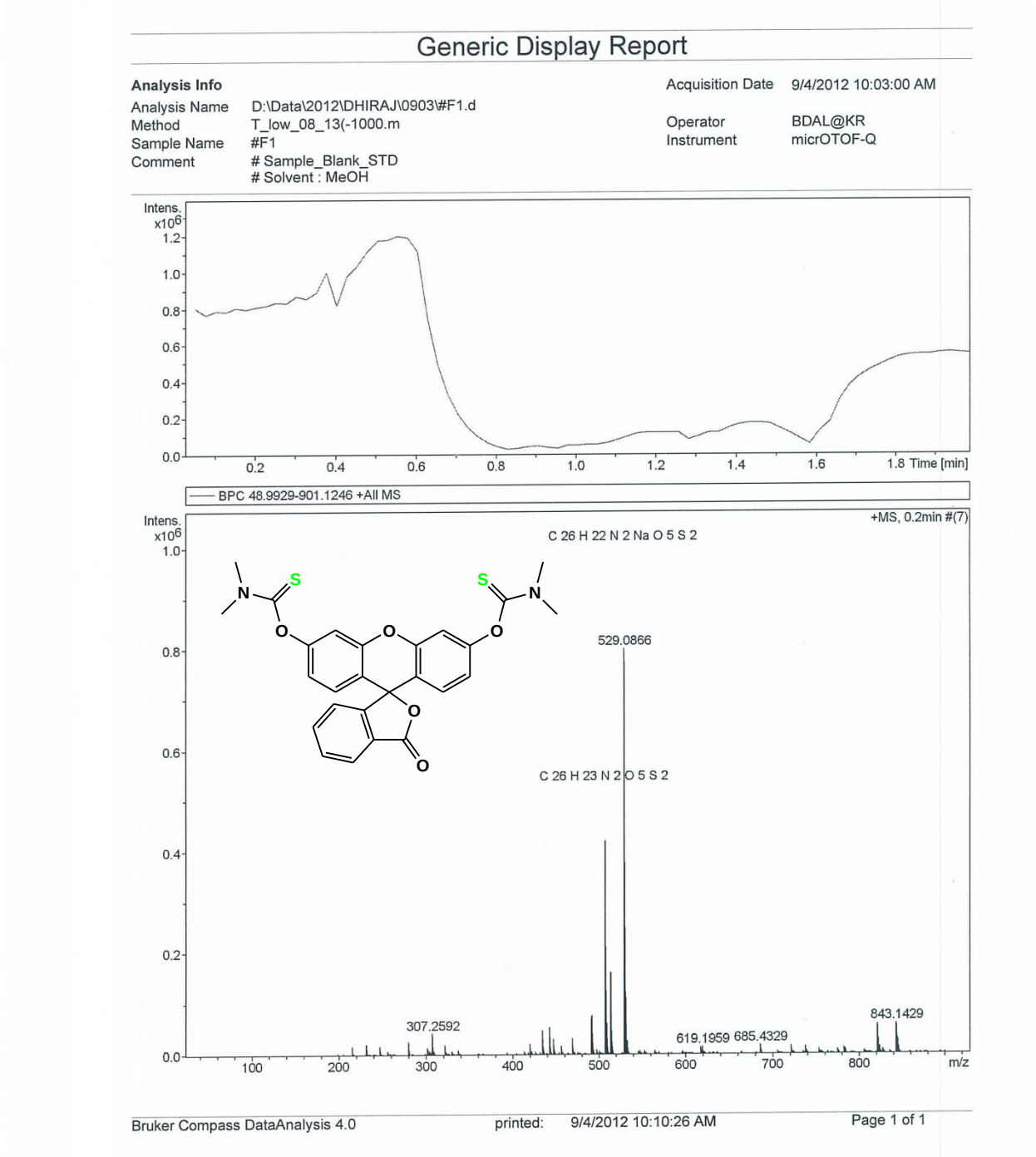


Fig. S5: ESI – mass spectrum of compound **1 .**

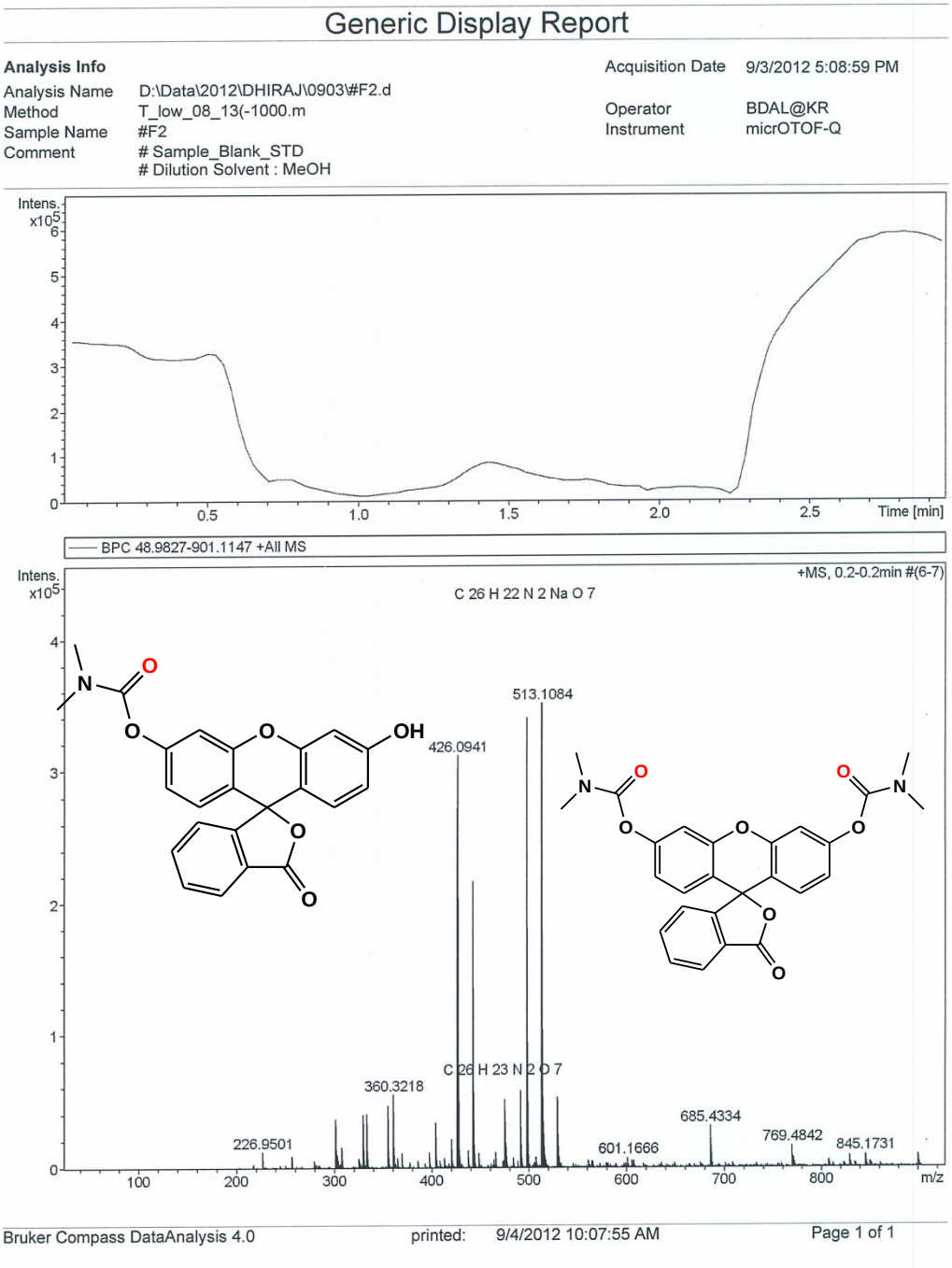


Fig. S6: ESI – mass spectrum of compound **1** + Hg^{2+}.

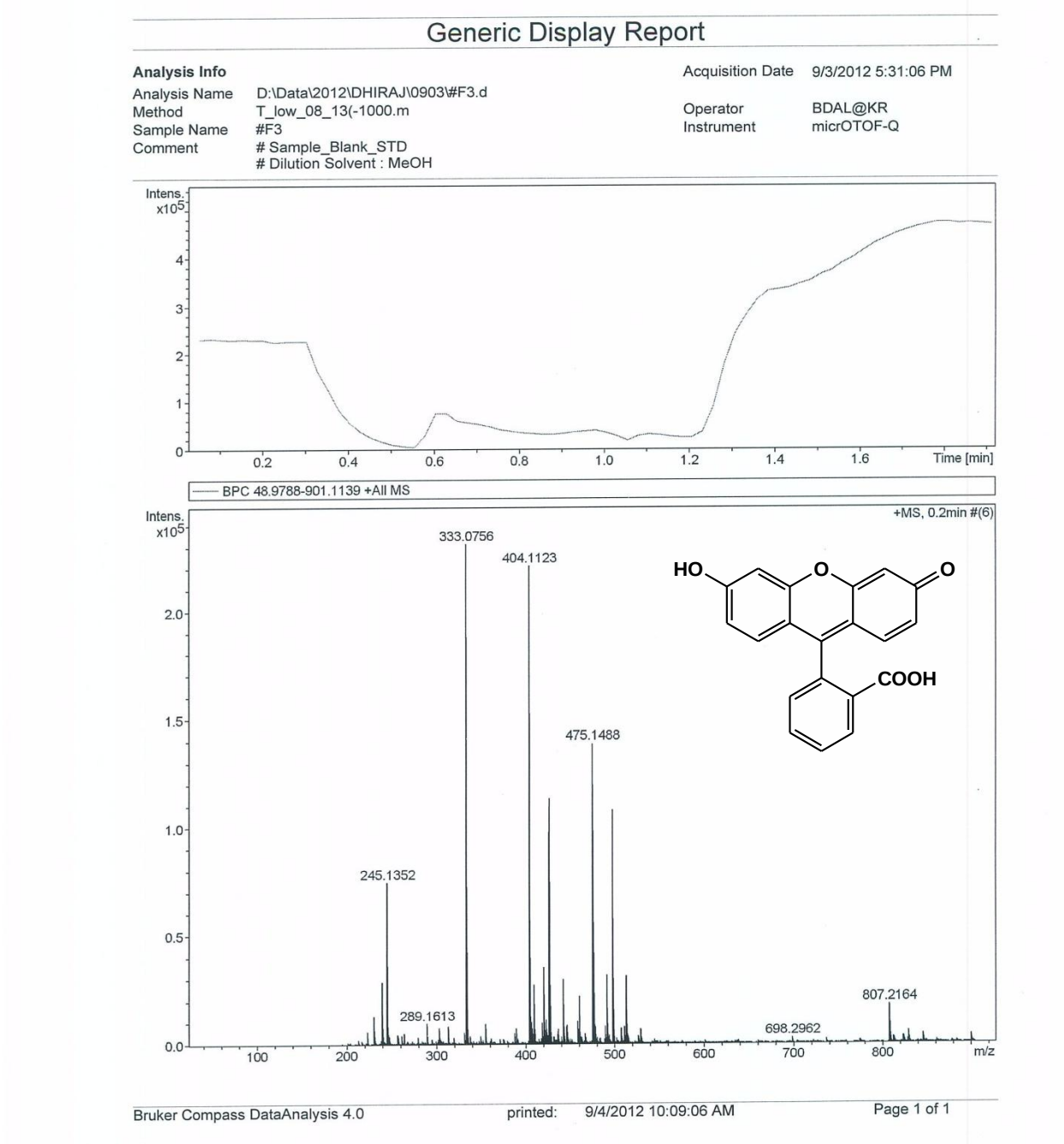


Fig. S7: ESI – mass spectrum of compound **1** + Hg^{2+} + H_2O_2.

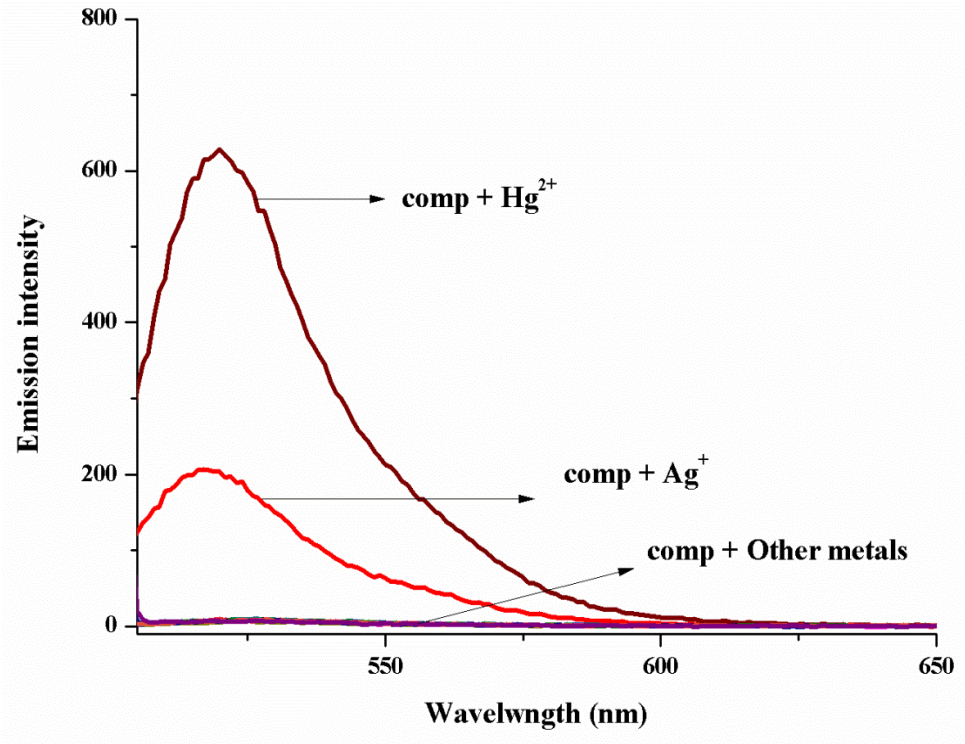


Fig. S8: Emission spectra of compound **1** (5×10^{-6} M, buffered H_2O: DMSO 80:20; pH 7.4; $buffer_{aq}$: 20 mM HEPES) with Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Mg^{2+}, Mn^{2+}, Pb^{2+}, Zn^{2+}, Ni^{2+}, Ag^{+} and Hg^{2+} (3.33×10^{-3} M in water) incubated for 30 min at RT. λ_{exci} = 490 nm.

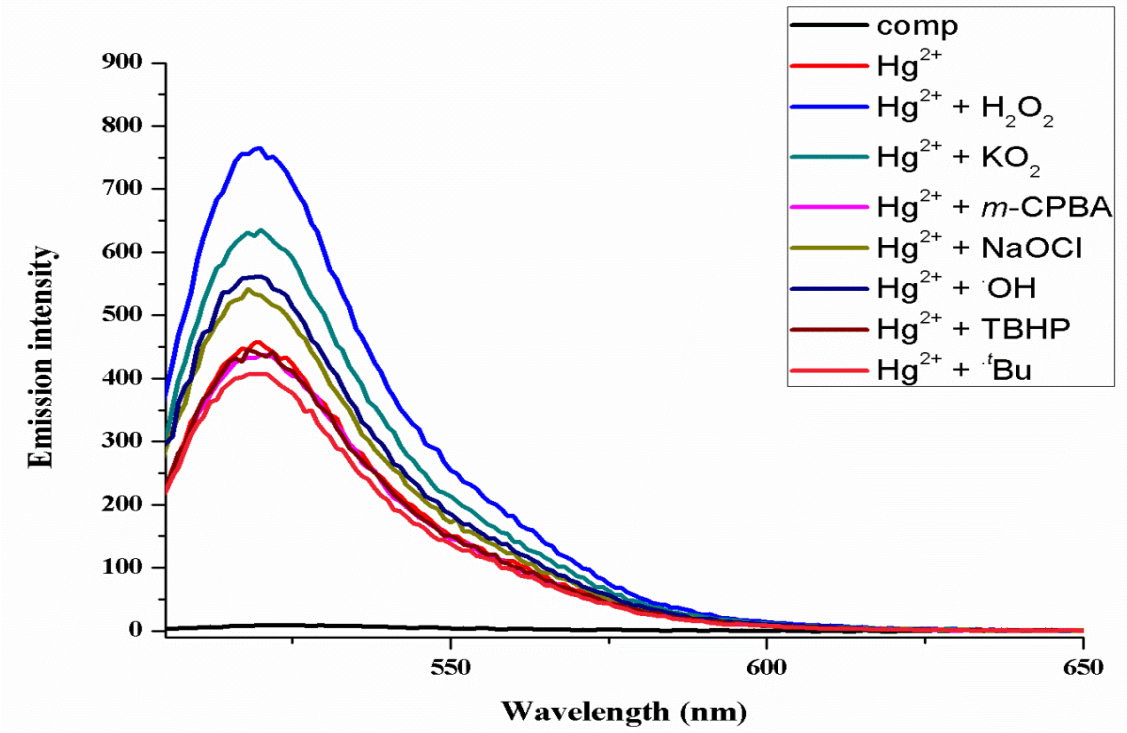


Fig. S9: Emission spectra of compound **1** (5×10^{-6} M, buffered H_2O: DMSO 80:20; pH 7.4; $buffer_{aq}$: 20 mM HEPES) with Hg^{2+} (3.33×10^{-3} M in water) incubated for 30 min at RT and different ROS (3.33×10^{-3} M in water) 30 min. at RT . λ_{exci} = 490 nm.

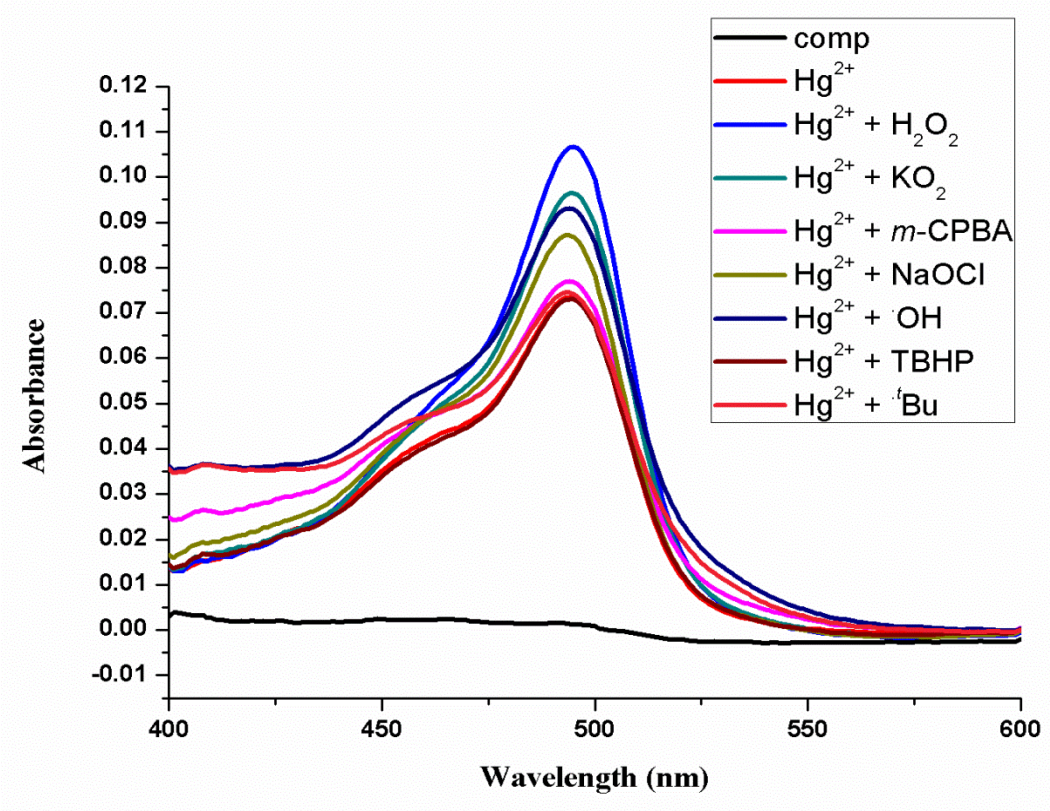


Fig. S10: Absorption spectra of compound **1** (5×10^{-6} M, buffered H_2O: DMSO 80:20; pH 7.4; $buffer_{aq}$: 20 mM HEPES) with Hg^{2+} (10 µL 0.1 M in water) incubated for 30 min at RT and different ROS (3.33×10^{-3} M in water) 30 min. at RT . λ_{exci} = 490 nm.

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