Electronic Supporting Information

Highly selective, sensitive and quantitative detection of Hg^{2+} in aqueous medium under broad pH range

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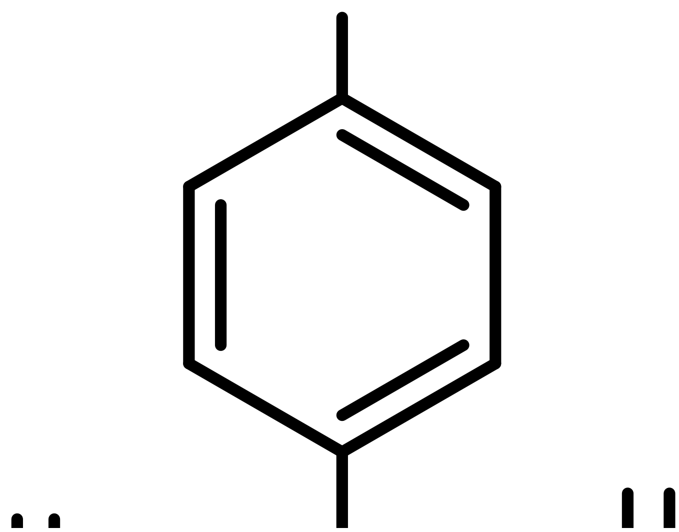
Contents

Materials and methods

Dimedone (98%) and hydrazine monohydrate (99+%) were purchased from Alfa Aesar (India), Ltd. 4-Carboxybenzaldehyde (97%), 4-methoxyphenyl isothiocyanate (98%), 4-nitrophenyl isothiocyanatae (98%) and *p*-toluidine (99%) were purchased from Sigma Aldrich Chemicals Pvt. Ltd. Phenyl isothiocyanate (99%) and ammonium acetate (GR) were purchased from Sisco Research Laboratory (SRL) and Merck Chemicals Pvt. Ltd., respectively. Metal ions as their chlorides or perchlorates were purchased from SRL and Sigma Aldrich Chemicals Pvt. Ltd. Methanol (HPLC grade) was purchased from SRL and deionized water is used throughout the experiment. NMR spectra were recorded on Bruker Avance III 500 MHz and Bruker 300 MHz instruments in deuterated solvents as indicated; TMS or the residual solvent peaks were used as internal standards. Chemical shifts are reported in ppm and coupling constants ($J_{X\text{-}X'}$) are reported in Hz. ESI-MS were performed on an ECA LCQ Thermo system with ion-trap detection in positive and negative mode. Elemental analyses (C, H and N) were taken on a Euro EA Elemental analyzer. Absorption spectra were recorded on an Agilent 8453 diode array spectrophotometer. Emission spectra were recorded on a HORIBA JOBIN YVON Fluoromax 4P spectrophotometer. The two photon excitation was carried out using a mode locked femtosecond Ti: sapphire laser from Spectra Physics (Tsunami), with a repetition rate of 82 MHz and a pulse width of about 100 femtoseconds. The tuning range of the Ti-Sapphire laser is 690 nm to 1080 nm. The output laser beam, with an average power of 0.6– 0.8 W, is vertically polarized. In the present study the standard optics with the output from 740 nm to 850 nm was used to excite the sample. The mode locked femtosecond laser is reflected into a 10x objective, which focuses the laser beam into the sample and causes fluorescence. The multi-photon excited fluorescence was collected perpendicularly to the incident beam direction and measured using a single channel charge coupled detector spectrometer (Ocean Optics, model – USB4000-VIS-NIR) with the grating tuning range 350 to 1000 nm. The two photon excited emission was recorded using the Ocean Optics spectroscopy software (Spectrasuite). The two photon nature of the observed processes was verified by measuring the multi-photon excited emission dependence on the excitation laser irradiance (power variation). Fluorescence images of the cells were taken by LEICA - TCS SP2 SE Laser scanning confocal microscope using Ti-sapphire laser with the excitation at 760 nm.

Experimental Procedures



Scheme 1 Synthesis of ADD linked amidothiourea derivatives.
(In addition to the expected amidothiourea (**1a-c**), amidobisthiourea was also formed, which did not show any interaction with Hg^{2+}; hence, we have not included the bisthiourea derivatives in the present paper. The bisthiourea derivatives show a novel spectral response with anions, which will be published later).

A mixture of tetraketone **5** (2.0 g, 4.85 mmol) and ammonium acetate (1.5 g, 19.5 mmol, excess) was kept under reflux in acetic acid (20 ml) for 6 hours. After the completion of the reaction as indicated by TLC, the reaction mixture was cooled and poured into crushed ice. The solid obtained was purified by recrystallisation from $CHCl_3$: MeOH (6:4), to isolate the ADD-acid **6** (1.62 g, 85%) as a bright yellow crystalline solid. M.p.: 242-244º C; FTIR (KBr): $\bar{\nu}$ = 3273 (br, NH), 3167 (br, OH), 1722 (s, acid CO), 1624 (vs, conj. CO), 1372 (s, -C=C-) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6, ppm): δ 0.85 and 1.01 (2s, 12H, *gem*-dimethyl), 1.98 and 2.18 (2d, 4H, J=16 Hz, C_2 and C_7 -CH_2), 2.34 and 2.46 (2d, 4H, J= 16 and 17 Hz, C_4 and C_5 -CH_2), 4.86 (s, 1H, C_9-H), 7.27 (d, 2H, J= 8.0 Hz, ArH), 7.76 (d, 2H, J= 8.5 Hz, ArH), 9.38 (s, 1H, -NH) 12.73 (s, 1H, -COOH); ^{13}C NMR (125 MHz, DMSO-d_6, ppm): δ 26.34, 29.04, 32.09, 33.36, 40.25, 50.11, 110.87, 127.79, 128.10, 128.80, 149.64, 151.95, 167.27, 194.35; MS (ESI): m/z = 393.51; elemental analysis (%) calcd for $C_{24}H_{27}NO_4$ (393.48): C 73.26, H 6.92, N 3.56; found: C 73.31, H 6.90, N 3.54.

A solution of ADD-acid **6** (2.0 g, 5.09 mmol) in methanol (20 ml) with a few drops of con. H_2SO_4 was stirred at room temperature for 30 min. The reaction mixture was poured into water, the obtained solid was purified by recrystallisation from $CHCl_3$: MeOH (98:2, v/v) to isolate ADD-Ester **7** (1.86 g, 90%) as a pale yellow crystal. M.p.: 228-230º C; FTIR (KBr): $\bar{\nu}$ = 3278 (br, NH), 1702 (s, ester CO), 1619 (vs, conj. CO), 1368 (s, -C=C-) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 0.84 and 1.00, (2s, 12H, *gem*-dimethyl), 1.97 and 2.18 (2d, 4H, J= 16.2 Hz C_2 & C_7 -CH_2), 2.33 and 2.47 (2d, 4H, J= 17.1 and 17.4 Hz, C_4 and C_5 -CH_2), 3.78 (s, 3H, -OCH_3), 4.86 (s, 1H, C_9-H), 7.29 (d, 2H, J= 8.1 Hz, ArH), 7.77 (d, 2H, J= 8.1 Hz, ArH), 9.40 (s, 1H, -NH); ^{13}C NMR (75 MHz, DMSO-d_6, ppm): δ 26.31, 29.04, 32.09, 33.44, 40.41, 50.10, 51.84, 110.79, 126.93, 127.98, 128.65, 149.67, 152.41, 166.15, 194.32; MS (ESI): m/z = 407.60; elemental analysis (%) calcd for $C_{25}H_{29}NO_4$ (407.50): C 73.68, H 7.17, N 3.44; found: C 73.64, H 7.18, N 3.43.

ADD-ester **7** (1.5g, 3.68 mmol) and hydrazine monohydrate (99%) (0.22 g, 4.39 mmol) in MeOH (20 ml) was kept under reflux for 14 hours. After the completion of the reaction, as indicated by TLC, the reaction mixture was cooled and poured into crushed ice. The solid obtained was purified by column chromatography over silica gel and eluting with $CHCl_3$: MeOH (95:5, v/v) to isolate the pure ADD-hydrazide **8** (1.22 g, 81.3%), as a brown powder. M.p.: 243-245º C; FTIR (KBr): $\bar{\nu}$ = 3279 (br, NH), 1623 (vs, conj. CO), 1573 (s, amide CO), 1367 (s, -C=C-) cm^{-1}; ^{1}H NMR (300 MHz; DMSO-d_6, ppm): δ 0.86 and 1.09 (2s, 12H, *gem*-dimethyl), 1.97 and 2.18 (2d, 4H, J= 16.2 Hz, C_2 and C_7 -CH_2), 2.34 and 2.47 (2d, 4H, J= 17.1 Hz, C_4 and C_5 -CH_2), 4.83 (s, 1H, C_9-H), 7.20 (d, 2H, J= 7.8 Hz, ArH), 7.60 (d, 2H, J= 7.8 Hz, ArH), 9.43 (s, 1H, -NH), 9.64 (s, 1H, -NH, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6, ppm): δ 26.46, 29.08, 32.17, 33.27, 40.31, 50.22, 111.05, 126.45, 127.56, 128.32, 149.72, 151.20, 165.32, 194.52; MS (ESI): m/z = 407.64; elemental analysis (%) calcd for $C_{24}H_{29}N_3O_3$ (407.51): C 70.74, H 7.17, N 10.31; found: C 70.83, H 7.14, N 10.28.

A solution of ADD-hydrazide **8** (1.0 g, 2.46 mmol) and phenyl isothiocyanate (0.33 g, 2.44 mmol) in ethanol (25 ml) was stirred at 5-10˚C for 45 min. After evaporating the solvent, the crude product was chromatographed over a silica gel column and eluted with $CHCl_3$: MeOH (97:3, v/v) to separate pure compound **1a** (0.77 g, 57.9 %) and bisamidothiourea derivative (0.22 g, 16.5%).

Hg^{2+} probe 1a

Yellow powder; M.p.: 196-198º C; FTIR (KBr): $\bar{\nu}$ = 3346 (br, NH), 1618 (vs, conj. CO), 1365 (s, -C=C-) cm^{-1}; ^{1}H NMR (500 MHz; DMSO-d_6, ppm): δ 0.86 and 1.02 (2s, 12H, *gem*-dimethyl), 1.96 and 2.33 (2d, 4H, J= 16 and 17 Hz, C_2 and C_7 -CH_2), 2.19 and 2.48 (2d, 4H, J= 16 and 17 Hz, C_4 and C_5 -CH_2), 4.85 (s, 1H, C_9-H), 7.14 (t, 1H, ArH), 7.26 (d, 2H, J= 8 Hz, ArH), 7.31 (m, 2H, ArH), 7.41, (br, 2H, ArH), 7.76 (d, 2H, J= 8 Hz, ArH), 9.36 (s, 1H, -NH), 9.67 (s, 1H, -NH, D_2O exchangeable), 9.77 (s, 1H, -NH, D_2O exchangeable), 10.38 (s, 1H, -NH, D_2O exchangeable); ^{13}C NMR (75 MHz; DMSO-d_6, ppm): δ 26.36, 29.11, 32.10, 33.41, 40.32, 50.15, 111.00, 123.39, 124.80, 127.35, 127.50, 127.86, 129.79, 139.24, 149.50, 151.02, 165.85, 174.78, 194.27; MS (ESI): m/z = 542.73; elemental analysis (%) calcd for $C_{31}H_{34}N_4O_3S_1$ (542.69): C 68.61, H 6.31, N 10.32; found: C 68.55, H 6.33, N 10.30.

Hg^{2+} probe 1b

Yield: 0.83 g, 59.0%; Yellow powder; M.p.: 180-182º C; FTIR (KBr): $\bar{\nu}$ = 3348 (br, NH), 1623 (vs, conj. CO), 1368 (s, -C=C-) cm^{-1}; ^{1}H NMR (400 MHz; DMSO-d_6, ppm): δ 0.87 and 1.02 (2s, 12H, *gem*-dimethyl), 1.97 and 2.19 (2d, 4H, J= 16.4 Hz, C_2 and C_7 -CH_2), 2.34 and 2.48 (2d, 4H, J= 17.2 Hz, C_4 and C_5 -CH_2), 3.74 (s, 3H, -OCH_3), 4.86 (s, 1H, C_9-H), 6.88 (d, 2H, J= 8.8 Hz, ArH), 7.26 (s (br), 4H, ArH), 7.76 (d, 2H, J= 8 Hz, ArH), 9.34 (s, 1H, -NH), 9.54 (s, 1H, -NH, D_2O exchangeable), 9.64 (s, 1H, -NH, D_2O exchangeable), 10.34 (s, 1H, -NH, D_2O exchangeable); ^{13}C NMR (125 MHz; DMSO-d_6, ppm): δ 26.48, 29.23, 32.10, 33.40, 40.14, 50.17, 55.16, 111.02, 113.12, 123.44, 127.35, 127.47, 129.83, 132.10, 149.49, 150.99, 156.65, 165.84, 175.04, 194.25; MS (ESI): m/z = 572.81; elemental analysis (%) calcd for $C_{32}H_{36}N_4O_4S_1$ (572.72): C 67.11, H 6.34, N 9.78; found: C 67.17, H 6.35, N 9.76.

Hg^{2+} probe 1c

Yield: 0.44 g, 30.5%; Yellow powder; M.p.: 208-210º C; FTIR (KBr): $\bar{\nu}$ = 3334 (br, NH), 1623 (vs, conj. CO), 1481 and 1332 (s, NO_2), 1368 (s, -C=C-) cm^{-1}; ^{1}H NMR (500 MHz; DMSO-d_6, ppm): δ 0.86 and 1.03 (2s, 12H, *gem*-dimethyl), 1.98 and 2.16 (2d, 4H, J= 16.4 Hz, C_2 and C_7 -CH_2), 2.31 and 2.47 (2d, 4H, J= 17.2 Hz, C_4 and C_5 -CH_2), 4.74 (s, 1H, C_9-H), 7.25 (d, 2H, J= 8.0 Hz, ArH), 7.54 (d, 2H, J= 8.4 Hz, ArH), 7.75 (d, 2H, J= 8 Hz, ArH), 8.14 (d, 2H, J= 8.4 Hz, ArH), 9.36 (s, 1H, -NH), 9.78 (s, 1H, -NH, D_2O exchangeable), 9.89 (s, 1H, -NH, D_2O exchangeable), 10.37 (s, 1H, -NH, D_2O exchangeable); ^{13}C NMR (125 MHz; DMSO-d_6, ppm): δ 26.35, 29.57, 32.14, 33.44, 40.34, 50.13, 111.06, 123.43, 124.83, 127.48, 128.31, 129.80, 144.63, 146.76, 149.53, 151.04, 165.84, 175.01, 194.89; MS (ESI): m/z = 588.77 (M+1); elemental analysis (%) calcd for $C_{31}H_{33}N_5O_5S_1$ (587.70): C 63.36, H 5.66, N 11.92; found: C 63.21, H 5.69, N 11.89.

Synthesis and characterization of compound **2** has been already reported.[15b]

Hg^{2+} probe 3

By using the above similar procedure, reaction of N-tolyl substituted ADD-hydrazide (0.50 g, 1.01 mmol) and phenyl isothiocyanate (0.14 g, 1.03 mmol) in ethanol (15 ml) yielded the desired product **3**, which was purified by column chromatography over silica gel and eluted with $CHCl_3$: MeOH (97:3, v/v) to separate pure compound **3** (0.34 g, 53.2 %) and amidobisthiourea derivative (0.10 g, 15.6%).

S5

Yield: 0.33 g, 51.7%; Yellow powder; M.p.: 205-207° C; FTIR (KBr): $\bar{\nu}$ = 3448 (br, NH), 1631 (vs, conj. CO), 1369 (s, -C=C-) cm^{-1}; ^{1}H NMR (500 MHz; DMSO-d_6, ppm): δ 0.84 and 1.05 (2s, 12H, *gem*-dimethyl), 1.99 and 2.19 (2d, 4H, J= 16.4 Hz, C_2 and C_7 -CH_2), 2.40 and 2.48 (2d, 4H, J= 17.0 Hz, C_4 and C_5 -CH_2), 2.82 (s, 3H, CH_3), 5.16 (s, 1H, C_9-H), 7.09 (t, 1H, ArH), 7.18 (m, 2H, ArH), 7.27 (d, 2H, J= 7.0 Hz, ArH), 7.40, (m, 4H, ArH), 7.58 (d, 2H, J= 7.4 Hz, ArH), 7.72 (d, 2H, J= 7.0 Hz, ArH), 9.64 (s, 1H, -NH, D_2O exchangeable), 9.72 (s, 1H, -NH, D_2O exchangeable), 10.36 (s, 1H, -NH, D_2O exchangeable); ^{13}C NMR (125 MHz; DMSO-d_6, ppm): δ 21.1, 26.6, 29.9, 30.4, 32.4, 40.5, 49.6, 108.7, 109.8, 124.1, 125.6, 126.2, 127.7, 128.1, 129.1, 138.9, 139.8, 142.3, 146.4, 151.6, 154.6, 165.5, 175.4, 194.5; MS (ESI): m/z = 632.77; elemental analysis (%) calcd for $C_{38}H_{40}N_4O_3S_1$ (632.81): C 72.12, H 6.37, N 8.85; found: C 72.21, H 6.39, N 8.83.

Hg^{2+} dosimetric product (4a)

A mixture of compound **1a** (50 mg, 0.092 mmol) and $Hg(ClO_4)_2$ (0.022 g, 0.055 mmol) was stirred in acetonitrile for 15 min at room temperature. The solvent was evaporated under reduced pressure and the crude product was purified by column chromatography on silica gel using $CHCl_3$: MeOH (94:6, v/v) to isolate pure product **4** (45.4 mg, 96.9%) as a yellow powder; M.p.: 235-237° C; FTIR (KBr): $\bar{\nu}$ = 3452 (br, NH), 1632 (vs, conj. CO), 1370 (s, -C=C-) cm^{-1}; ^{1}H NMR (500 MHz; DMSO-d_6, ppm): δ 0.87 and 1.02 (2s, 12H, *gem*-dimethyl), 1.99 and

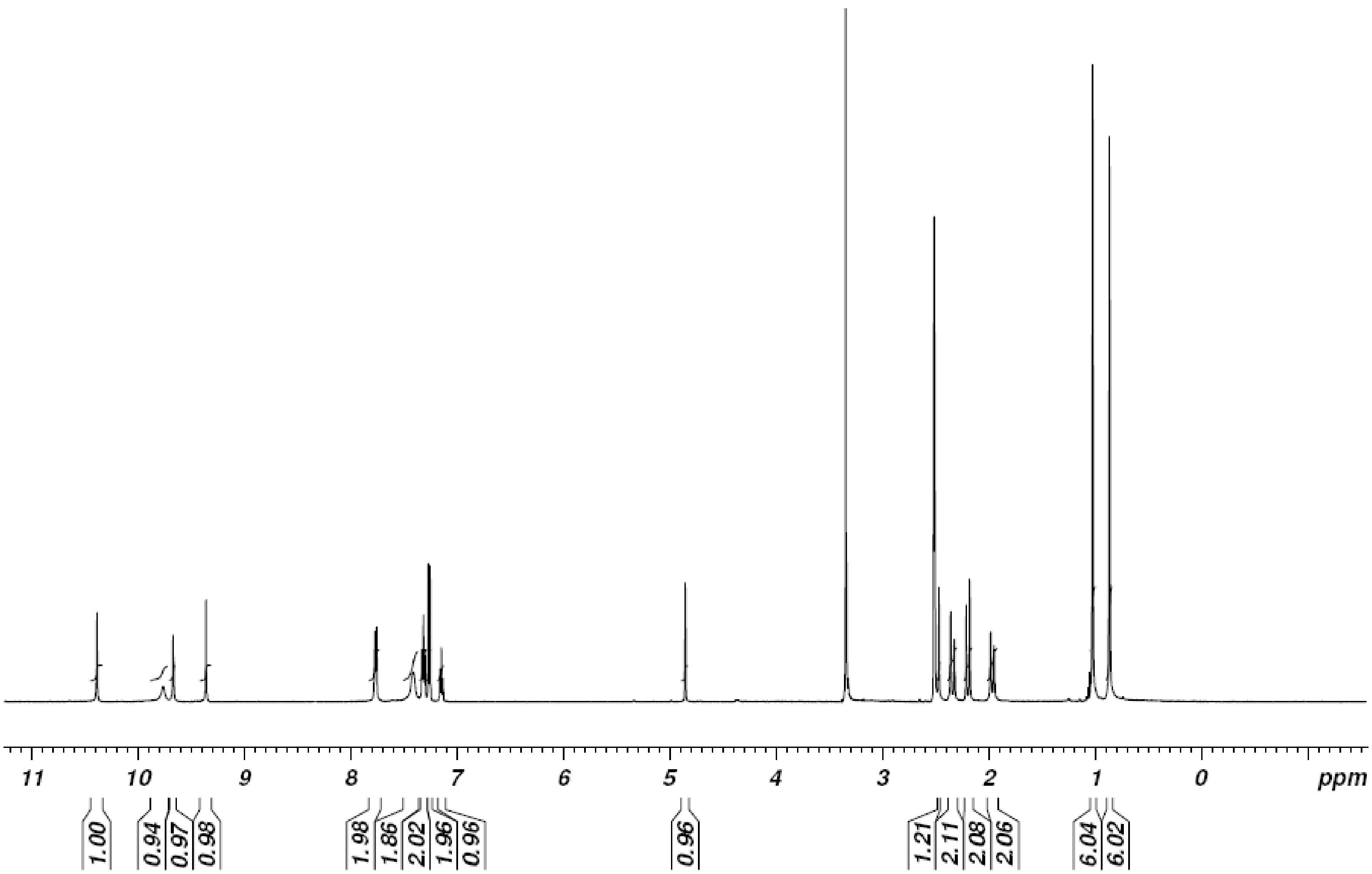
2.19 (2d, 4H, J= 16 Hz, C_2 and C_7 -CH_2), 2.35 and 2.48 (2d, 4H, J= 17 Hz, C_4 and C_5 -CH_2), 4.87 (s, 1H, C_9-H), 7.01 (t, 1H, ArH), 7.34 (m, 4H, ArH), 7.60 (d, 2H, J= 8.5 Hz, ArH), 7.72 (d, 2H, J= 8.5 Hz, ArH), 9.39 (s, 1H, -NH), 10.64 (s, 1H, -NH, D_2O exchangeable); ^{13}C NMR (100 MHz; DMSO-d_6, ppm): δ 26.4, 29.00, 32.13, 33.39, 39.99, 50.11, 110.87, 117.02, 121.08, 121.85, 125.07, 127.90, 128.60, 129.06, 138.55, 149.70, 157.80, 159.58, 194.45; MS (ESI): m/z = 508.67; elemental analysis (%) calcd for $C_{31}H_{32}N_4O_3$ (508.62): C 73.21, H 6.34, N 11.02; found: C 73.24, H 6.35, N 11.01.

Hg^{2+} dosimetric product (4b)

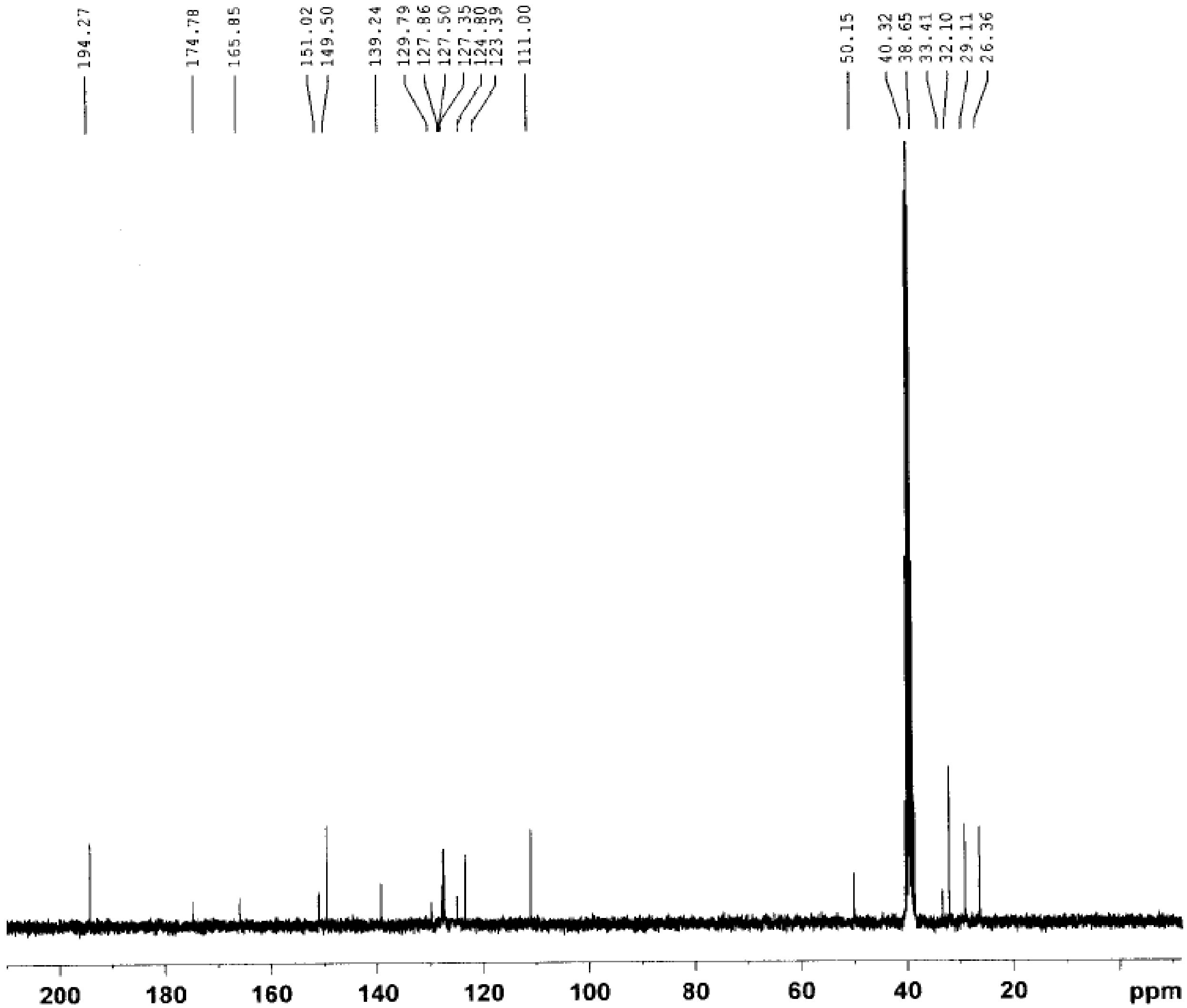
Yield: 46.2 mg, 98.2%; M.p.: 221-223º C; FTIR (KBr): $\bar{\nu}$ = 3467 (br, NH), 1626 (vs, conj. CO), 1367 (s, -C=C-) cm^{-1}; ^{1}H NMR (400 MHz; DMSO-d_6, ppm): δ 0.85 and 1.00 (2s, 12H, *gem*-dimethyl), 1.98 and 2.18 (2d, 4H, J= 16 Hz, C_2 and C_7 -CH_2), 2.34 and 2.47 (2d, 4H, J= 17 Hz, C_4 and C_5 -CH_2), 3.72 (s, 3H, -OCH_3), 4.85 (s, 1H, C_9-H), 6.94 (d, 2H, J= 9.2 Hz, ArH), 7.34 (d, 2H, J= 9.2 Hz, ArH), 7.49 (d, 2H, J= 9.2 Hz, ArH), 7.69 (d, 2H, J= 8.4 Hz, ArH), 9.42 (s, 1H, -NH), 10.55 (s, 1H, -NH, D_2O exchangeable); ^{13}C NMR (100 MHz; DMSO-d_6, ppm): δ 26.36, 28.98, 32.11, 33.37, 39.88, 50.08, 55.19, 110.86, 113.73, 118.90, 121.00, 125.04, 128.58, 131.50, 149.80, 150.13, 154.62, 157.57, 159.78, 194.60; MS (ESI): m/z = 538.68; elemental analysis (%) calcd for $C_{31}H_{32}N_4O_3$ (538.64): C 71.35, H 6.36, N 10.40; found: C 71.33, H 6.35, N 10.41.

Hg^{2+} dosimetric product (4c)

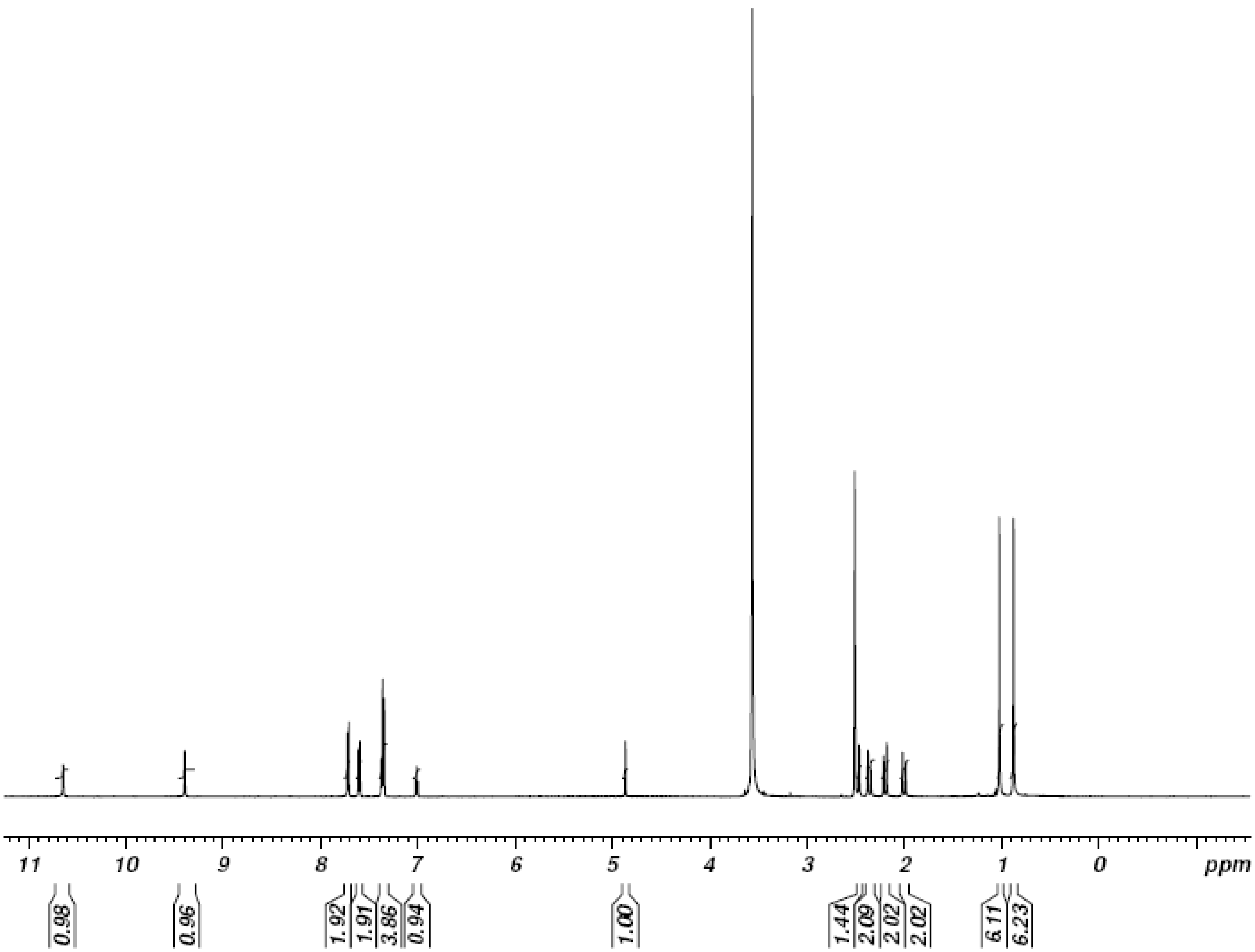
Yield: 28.9 mg, 61.3%; M.p.: 242-244º C; FTIR (KBr): $\bar{\nu}$ = 3448 (br, NH), 1632 (vs, conj. CO), 1479 and 1330 (s, NO_2), 1369 (s, -C=C-) cm^{-1}; ^{1}H NMR (500 MHz; DMSO-d_6, ppm): δ 0.87 and 1.00 (2s, 12H, *gem*-dimethyl), 2.00 and 2.19 (2d, 4H, J= 16.0 Hz, C_2 and C_7 -CH_2), 2.35 and 2.48 (2d, 4H, J= 17.0 Hz, C_4 and C_5 -CH_2), 4.77 (s, 1H, C_9-H), 7.25 (d, 2H, J= 7.5 Hz, ArH), 7.61 (d, 2H, J= 8.5 Hz, ArH), 7.76 (d, 2H, J= 7.5 Hz, ArH), 7.34 (d, 2H, J= 9.2 Hz, ArH), 9.41 (s, 1H, -NH), 10.70 (s, 1H, -NH, D_2O exchangeable); ^{13}C NMR (125 MHz; DMSO-d_6, ppm): δ 26.26, 28.98, 32.12, 33.38, 39.98, 50.11, 110.86, 120.23, 122.29, 125.02, 128.61, 129.07, 130.02, 137.90, 144.65, 149.71, 157.87, 159.68, 194.46; MS (ESI): m/z = 553.74; elemental analysis (%) calcd for $C_{31}H_{32}N_4O_3$ (553.61): C 67.26, H 5.64, N 12.65; found: C 67.39, H 5.61, N 12.62.



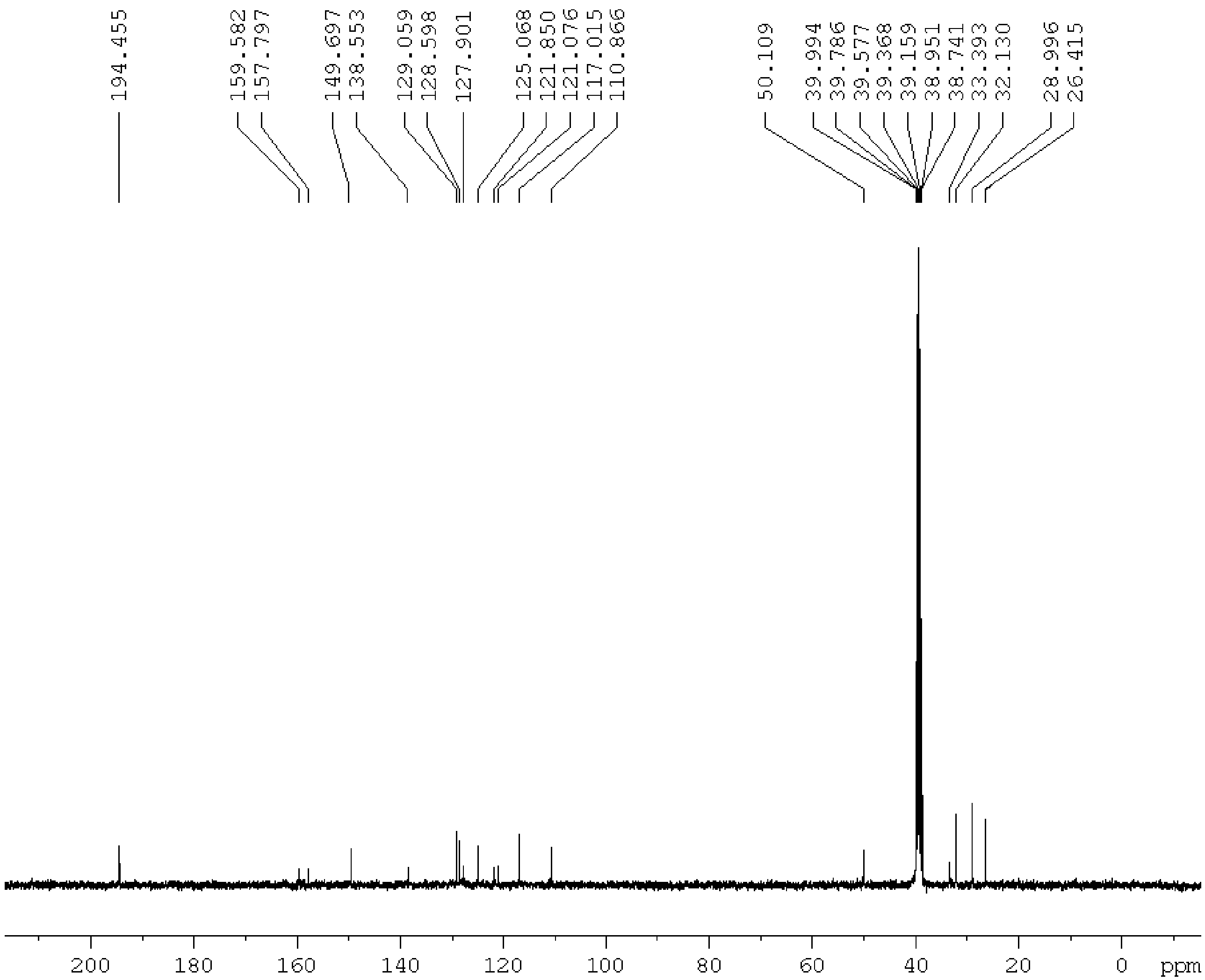
[1]H-NMR spectrum of **1a** in DMSO-d_6.



^{13}C-NMR spectrum of **1a** in DMSO-d_6.



[1]H-NMR spectrum of **4a** in DMSO-d_6.



^{13}C-NMR spectrum of **4a** in DMSO-d_6.

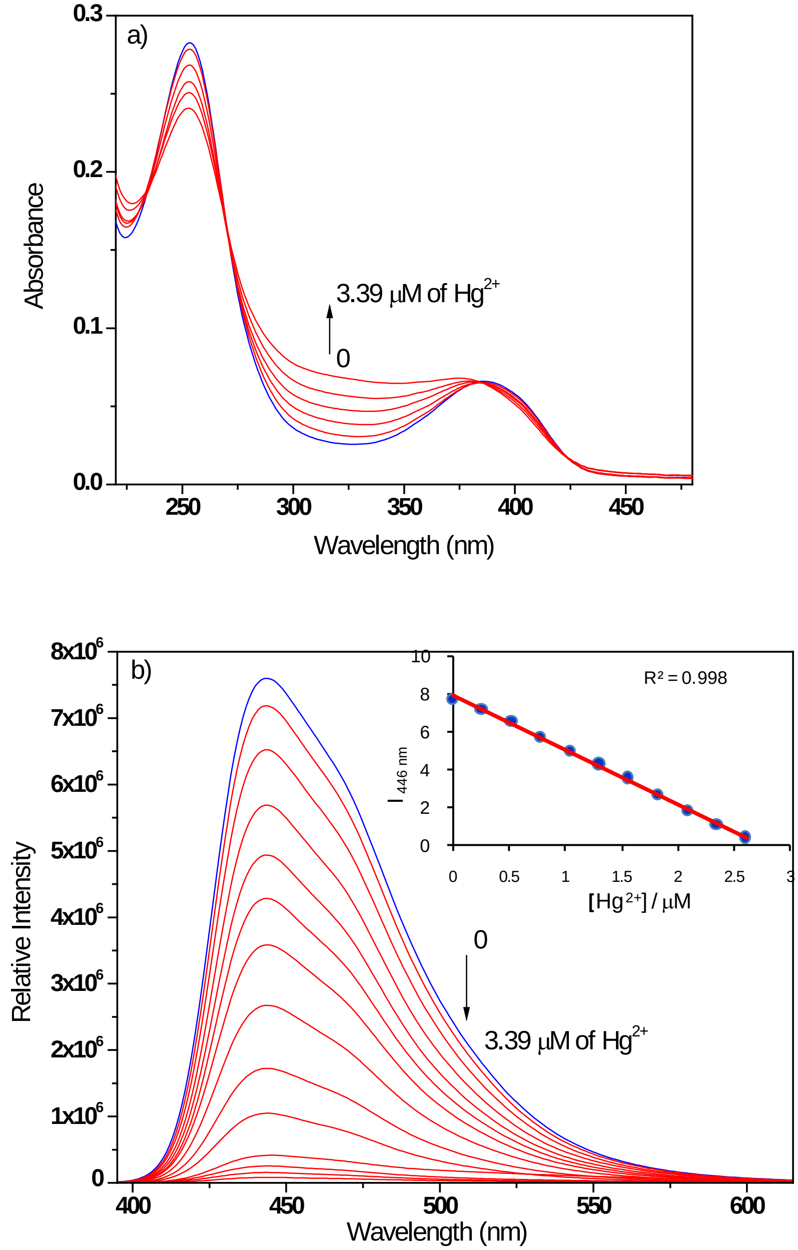


Fig.S1 a) Absorption, b) Emission spectra of **1a** (3.4 μM) in water:MeOH (99:1, v:v) upon addition of Hg^{2+} (0- 3.39 μM); λ_{ex}= 385 nm. Inset shows the changes in the fluorescence intensity at 446 nm upon addition of Hg^{2+} (0- 2.61 μM).

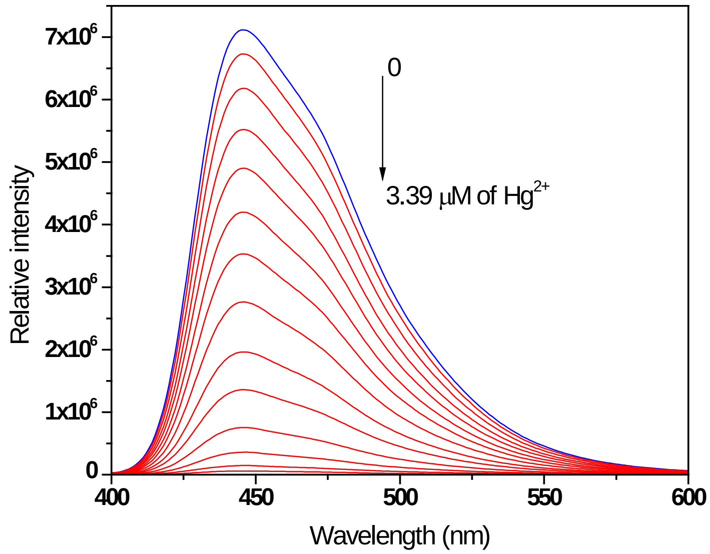


Fig.S2 Emission spectra of **1b** (3.4 μM) in water:MeOH (99:1, v:v) upon addition of Hg^{2+} (0- 3.39 μM) ; λ_{ex}= 385 nm.

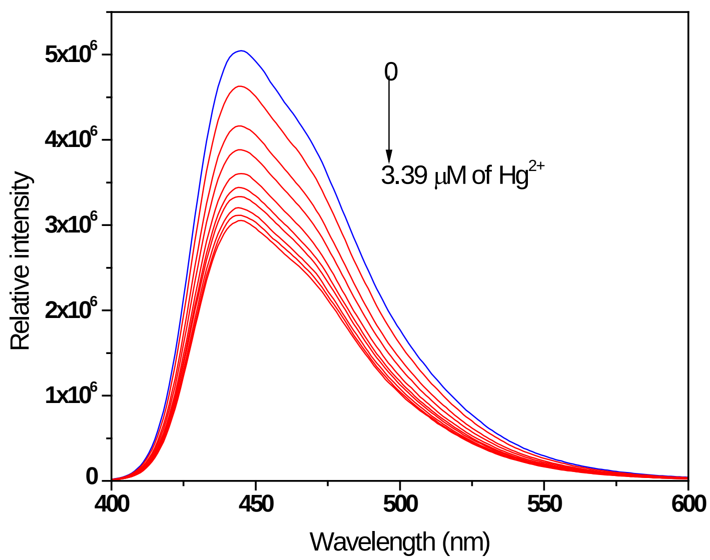


Fig.S3 Emission spectra of **1c** (3.4 μM) in water:MeOH (99:1, v:v) upon addition of Hg^{2+} (0- 3.39 μM) ; λ_{ex}= 385 nm.

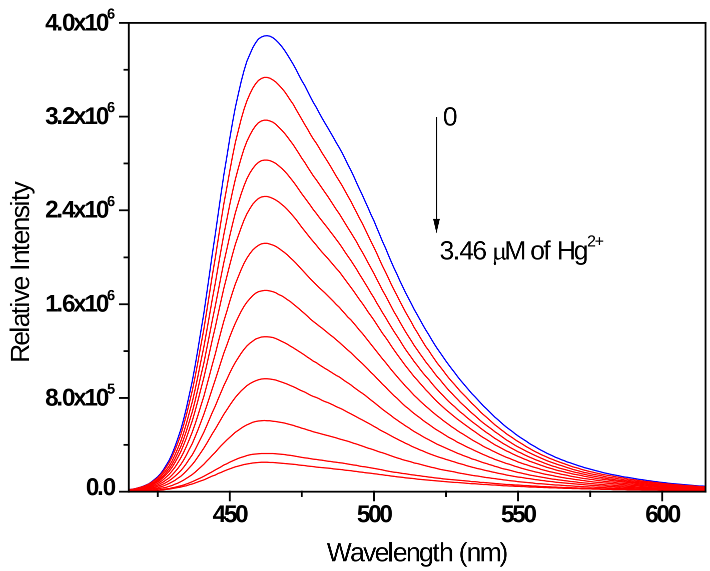


Fig.S4 Emission spectra of **2** (3.4 μM) in water:MeOH (99:1, v:v) upon addition of Hg^{2+} (0- 3.46 μM) ; λ_{ex}= 393 nm.

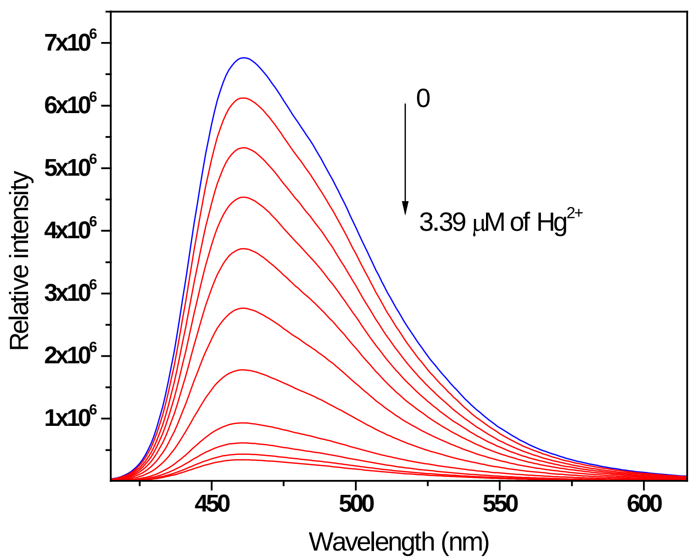


Fig.S5 Emission spectra of **3** (3.4 μM) in water:MeOH (99:1, v:v) upon addition of Hg^{2+} (0- 3.39 μM) ; λ_{ex}= 391 nm.

Compound	Abs. max (nm)	Emi. max (nm)	Quantum yield (ϕ_f)[a]	Lifetime (τ_f) (ns)
1a	386	446	0.83	9.34
1b	386	446	0.81	9.30
1c	386	446	0.80	9.27
2	392	462	0.86	9.78
3	390	458	0.84	9.54
4a	384	446	0.011[b]	0.23
4b	385	446	0.009[b]	0.18
4c	384	446	0.62	4.44

[a] Fluorescence quantum yields were determined by exciting the sample at 366 nm using quinine sulfate as the standard (Φ_f = 0.546 in 0.1N H_2SO_4); ± 3%; b) ± 6%

Table S1 Photophysical parameters of **1a, 1b**, **1c**, **2, 3, 4a, 4b** & **4c** in water:MeOH (99:1, v:v).

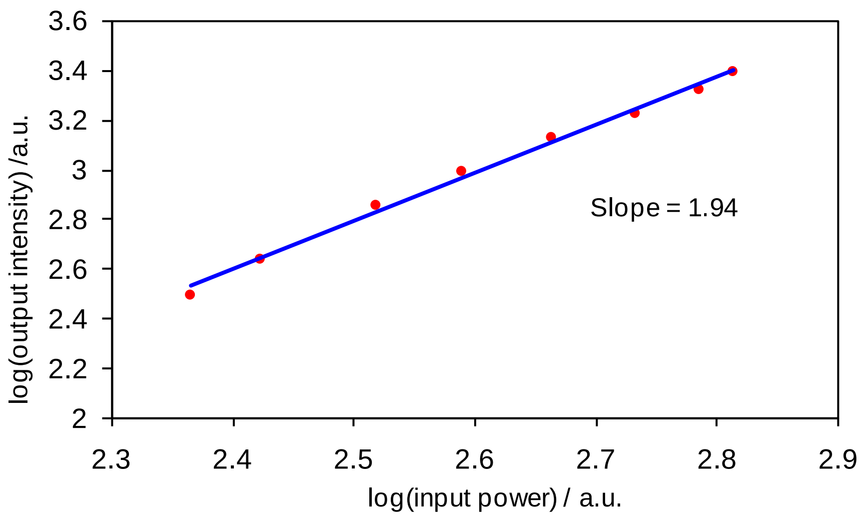


Fig.S6 Power dependence of the two-photon excited upconversion emission intensity of **3** in water:MeOH (99:1, v:v) on the input laser power; λ_{ex}= 760 nm.

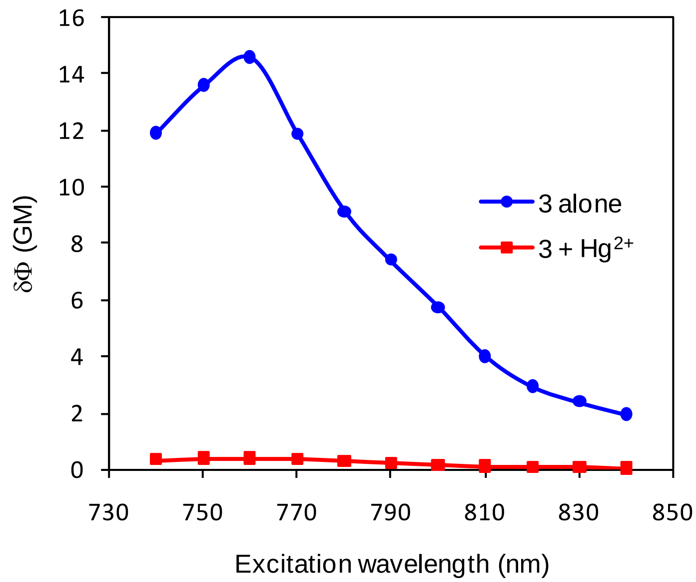


Fig.S7 Two-photon excitation spectra of **3** and **3**+ Hg^{2+} in water:MeOH (99:1, v:v).

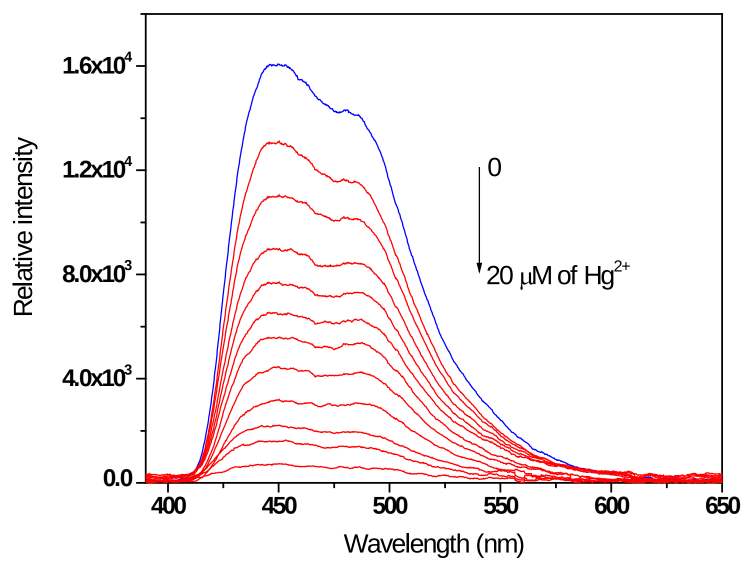


Fig.S8 Two-photon excited fluorescence spectrum of **3** (12 μM) upon addition of Hg^{2+} in water:MeOH (99:1, v:v); λ_{ex}= 760 nm.