Electronic Supporting Information

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

1. Materials and methods S1
2. Experimental procedure and characterisation S2
3. Absorption and emission spectra of $1a$ (3.4 μM) in water:MeOH (99:1, v:v) upon addition of Hg$^{2+}$ (0-3.39 μM) S13
4. Emission spectrum of $1b$ (3.4 μM) in water:MeOH (99:1, v:v) upon addition of Hg$^{2+}$ (0-3.39 μM) S14
5. Emission spectrum of $1c$ (3.4 μM) in water:MeOH (99:1, v:v) upon addition of Hg$^{2+}$ (0-3.39 μM) S14
6. Emission spectrum of $2$ (3.4 μM) in water:MeOH (99:1, v:v) upon addition of Hg$^{2+}$ (0-3.46 μM) S15
7. Emission spectrum of $3$ (3.4 μM) in water:MeOH (99:1, v:v) upon addition of Hg$^{2+}$ (0-3.39 μM) S15
8. Photophysical parameters of $1a$, $1b$, $1c$, $2$, $3$, $4a$, $4b$ & $4c$ in water:MeOH (99:1, v:v) S16
9. Power dependence of the two-photon excited upconversion emission intensity of $3$ in water:MeOH (99:1, v:v) on the input laser power S16
9. Two-photon excitation spectrum of $3$ and $3$+ Hg$^{2+}$ in water:MeOH (99:1, v:v) S17
10. Two-photon excited fluorescence spectrum of $3$ (12 μM) upon addition of Hg$^{2+}$ in water:MeOH (99:1, v:v) S17
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 isothiocyanate (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 (JX-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](image)

**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 CHCl₃: MeOH (6:4), to isolate the ADD-acid 6 (1.62 g, 85%) as a bright yellow crystalline solid. M.p.: 242-244°C; FTIR (KBr): ν = 3273 (br, NH), 3167 (br, OH), 1722 (s, acid CO), 1624 (vs, conj. CO), 1372 (s, -C=C-) cm⁻¹; ¹H NMR (500 MHz, DMSO-d₆, ppm): δ 0.85 and 1.01 (2s, 12H, gem-dimethyl), 1.98 and 2.18 (2d, 4H, J=16 Hz, C₂ and C₇-CH₂), 2.34 and 2.46 (2d, 4H, J= 16 and 17 Hz, C₄ and C₅-CH₂), 4.86 (s, 1H, C₉-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); ¹³C NMR (125 MHz, DMSO-d₆, 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 C₂₄H₂₇NO₄: 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 conc. H₂SO₄ was stirred at room temperature for 30 min. The reaction mixture was poured into water, the obtained solid was purified by recrystallisation from CHCl₃: MeOH (98:2, v/v) to isolate ADD-Ester 7 (1.86 g, 90%) as a pale yellow crystal. M.p.: 228-230°C; FTIR (KBr): ν = 3278 (br, NH), 1702 (s, ester CO), 1619 (vs, conj. CO), 1368 (s, -C=C-) cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆, ppm): δ 0.84 and 1.00, (2s, 12H, gem-dimethyl), 1.97 and 2.18 (2d, 4H, J= 16.2 Hz C₂ & C₇-CH₂), 2.33 and 2.47 (2d, 4H, J= 17.1 and 17.4 Hz, C₄ and C₅-CH₂), 3.78 (s, 3H, -OCH₃), 4.86 (s, 1H, C₉-H), 7.29 (d, 2H, J= 8.1 Hz, ArH), 7.77 (d, 2H, J= 8.1 Hz, ArH), 9.40 (s, 1H, -NH); ¹³C NMR (75 MHz, DMSO-d₆, 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 C₂₅H₂₉NO₄: 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 CHCl₃: MeOH (95:5, v/v) to isolate the pure ADD-hydrazide 8 (1.22 g, 81.3%), as a brown powder. M.p.: 243-245° C; FTIR (KBr): ν = 3279 (br, NH), 1623 (vs, conj. CO), 1573 (s, amide CO), 1367 (s, -C=C-) cm⁻¹; ¹H NMR (300 MHz; DMSO-d₆, ppm): δ 0.86 and 1.09 (2s, 12H, gem-dimethyl), 1.97 and 2.18 (2d, 4H, J= 16.2 Hz, C₂ and C₇ -CH₂), 2.34 and 2.47 (2d, 4H, J= 17.1 Hz, C₄ and C₅ -CH₂), 4.83 (s, 1H, C₉-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, D₂O exchangeable); ¹³C NMR (75 MHz, DMSO-d₆, ppm): δ 26.46, 29.08, 32.17, 33.27, 40.31, 50.22, 111.05, 126.45, 127.56, 128.32, 149.72, 150.12, 165.32, 194.52; MS (ESI): m/z = 407.64; elemental analysis (%) calcd for C₂₄H₂₉N₃O₃ (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 CHCl₃: MeOH (97:3, v/v) to separate pure compound 1a (0.77 g, 57.9 %) and bisamidothiourea derivative (0.22 g, 16.5%).

Hg²⁺ probe 1a

Yellow powder; M.p.: 196-198° C; FTIR (KBr): ν = 3346 (br, NH), 1618 (vs, conj. CO), 1365 (s, -C=C-) cm⁻¹; ¹H NMR (500 MHz; DMSO-d₆, ppm): δ 0.86 and 1.02 (2s, 12H, gem-dimethyl), 1.96 and 2.33 (2d, 4H, J= 16 and 17 Hz, C₂ and C₇ -CH₂), 2.19 and 2.48 (2d, 4H, J= 16 and 17 Hz, C₄ and C₅ -CH₂), 4.85 (s, 1H, C₉-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, D₂O exchangeable), 9.77 (s, 1H, -NH, D₂O exchangeable), 10.38 (s, 1H, -NH, D₂O exchangeable); ¹³C NMR (75 MHz; DMSO-d₆, 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 C₃₁H₃₄N₄O₃S₁ (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): ν = 3348 (br, NH), 1623 (vs, conj. CO), 1368 (s, -C=C-) cm⁻¹; ¹H NMR (400 MHz; DMSO-d₆, ppm): δ 0.87 and 1.02 (2s, 12H, gem-dimethyl), 1.97 and 2.19 (2d, 4H, J= 16.4 Hz, C₂ and C₇ -CH₂), 2.34 and 2.48 (2d, 4H, J= 17.2 Hz, C₄ and C₅ -CH₂), 3.74 (s, 3H, -OCH₃), 4.86 (s, 1H, C₉-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, D₂O exchangeable), 9.64 (s, 1H, -NH, D₂O exchangeable), 10.34 (s, 1H, -NH, D₂O exchangeable); ¹³C NMR (125 MHz; DMSO-d₆, 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 C₃₂H₃₆N₄O₄S₁: 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): ν = 3334 (br, NH), 1623 (vs, conj. CO), 1481 and 1332 (s, NO₂), 1368 (s, -C=C-) cm⁻¹; ¹H NMR (500 MHz; DMSO-d₆, ppm): δ 0.86 and 1.03 (2s, 12H, gem-dimethyl), 1.98 and 2.16 (2d, 4H, J= 16.4 Hz, C₂ and C₇ -CH₂), 2.31 and 2.47 (2d, 4H, J= 17.2 Hz, C₄ and C₅ -CH₂), 4.74 (s, 1H, C₉-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, D₂O exchangeable), 9.89 (s, 1H, -NH, D₂O exchangeable), 10.37 (s, 1H, -NH, D₂O exchangeable); ¹³C NMR (125 MHz; DMSO-d₆, 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 C₃₁H₃₃N₅O₅S₁: 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.\textsuperscript{15b}

**Hg\textsuperscript{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\textsubscript{3}: MeOH (97:3, v/v) to separate pure compound 3 (0.34 g, 53.2 %) and amidobisthiourea derivative (0.10 g, 15.6%).

Yield: 0.33 g, 51.7%; Yellow powder; M.p.: 205-207º C; FTIR (KBr): \(\nu = 3448\text{ (br, NH)}, 1631\text{ (vs, conj. CO)}, 1369\text{ (s, -C=C-)} \text{ cm}^{-1}; \text{\textsuperscript{1}H NMR (500 MHz; DMSO-d}_6, ppm): \delta 0.84\text{ and 1.05 (2s, 12H, gem-dimethyl)}, 1.99\text{ and 2.19 (2d, 4H, } J = 16.4\text{ Hz, C}_2\text{ and C}_7\text{ -CH}_2\text{), 2.40\text{ and 2.48 (2d, 4H, } J = 17.0\text{ Hz, C}_4\text{ and C}_5\text{ -CH}_2\text{), 2.82 (s, 3H, } \text{CH}_3\text{), 5.16 (s, 1H, C}_9\text{-H), 7.09 (t, 1H, ArH), 7.18 (m, 2H, ArH), 7.27 (d, 2H, } J = 7.0\text{ Hz, ArH), 7.40 (m, 4H, ArH), 7.58 (d, 2H, } J = 7.4\text{ Hz, ArH), 7.72 (d, 2H, } J = 7.0\text{ Hz, ArH), 9.64 (s, 1H, -NH, D}_2\text{O exchangeable), 9.72 (s, 1H, -NH, D}_2\text{O exchangeable), 10.36 (s, 1H, -NH, D}_2\text{O exchangeable); } \text{\textsuperscript{13}C NMR (125 MHz; DMSO-d}_6, ppm): \delta 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\textsubscript{38}H\textsubscript{40}N\textsubscript{4}O\textsubscript{3}S\textsubscript{1}Hg\textsubscript{2+}: C 72.12, H 6.37, N 8.85; found: C 72.21, H 6.39, N 8.83.

**Hg\textsuperscript{2+} dosimetric product (4a)**

A mixture of compound 1a (50 mg, 0.092 mmol) and Hg(ClO\textsubscript{4})\textsubscript{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\textsubscript{3}: MeOH (94:6, v/v) to isolate pure product 4 (45.4 mg, 96.9%) as a yellow powder; M.p.: 235-237º C; FTIR (KBr): \(\nu = 3452\text{ (br, NH)}, 1632\text{ (vs, conj. CO)}, 1370\text{ (s, -C=C-) cm}^{-1}; \text{\textsuperscript{1}H NMR (500 MHz; DMSO-d}_6, ppm): \delta 0.87\text{ and 1.02 (2s, 12H, gem-dimethyl)}, 1.99 and
2.19 (2d, 4H, J= 16 Hz, C2 and C7 -CH2), 2.35 and 2.48 (2d, 4H, J= 17 Hz, C4 and C5 -CH2), 4.87 (s, 1H, C9-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, D2O exchangeable); 13C NMR (100 MHz; DMSO-d6, 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 C31H32N4O3: C 73.21, H 6.34, N 11.02; found: C 73.24, H 6.35, N 11.01.

**Hg²⁺ dosimetric product (4b)**

Yield: 46.2 mg, 98.2%; M.p.: 221-223º C; FTIR (KBr): ν = 3467 (br, NH), 1626 (vs, conj. CO), 1367 (s, -C=C-) cm⁻¹; ¹H NMR (400 MHz; DMSO-d6, ppm): δ 0.85 and 1.00 (2s, 12H, gem-dimethyl), 1.98 and 2.18 (2d, 4H, J= 16 Hz, C2 and C7 -CH2), 2.34 and 2.47 (2d, 4H, J= 17 Hz, C4 and C5 -CH2), 3.72 (s, 3H, OCH3), 4.85 (s, 1H, C9-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, D2O exchangeable); 13C NMR (100 MHz; DMSO-d6, 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 C31H32N4O3: C 71.35, H 6.36, N 10.40; found: C 71.33, H 6.35, N 10.41.

**Hg²⁺ dosimetric product (4c)**

Yield: 28.9 mg, 61.3%; M.p.: 242-244º C; FTIR (KBr): ν = 3448 (br, NH), 1632 (vs, conj. CO), 1479 and 1330 (s, NO2), 1369 (s, -C=C-) cm⁻¹; ¹H NMR (500 MHz; DMSO-d6, ppm): δ 0.87 and 1.00 (2s, 12H, gem-dimethyl), 2.00 and 2.19 (2d, 4H, J= 16.0 Hz, C2 and C7 -CH2), 2.35 and 2.48 (2d, 4H, J= 17.0 Hz, C4 and C5 -CH2), 4.77 (s, 1H, C9-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, D2O exchangeable); 13C NMR (125 MHz; DMSO-d6, 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\textsubscript{31}H\textsubscript{32}N\textsubscript{4}O\textsubscript{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); $\lambda_{ex}$ = 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); $\lambda_{ex}$ = 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); $\lambda_{ex} = 393$ 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); $\lambda_{ex} = 391$ nm.
Fluorescence quantum yields were determined by exciting the sample at 366 nm using quinine sulfate as the standard ($\Phi_f = 0.546$ in 0.1N H$_2$SO$_4$; ± 3%; b) ± 6%)

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

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<thead>
<tr>
<th>Compound</th>
<th>Abs. max (nm)</th>
<th>Emi. max (nm)</th>
<th>Quantum yield ($\Phi_f$)$^a$</th>
<th>Lifetime ($\tau_f$) (ns)</th>
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<td>446</td>
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<td>9.34</td>
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<td>1b</td>
<td>386</td>
<td>446</td>
<td>0.81</td>
<td>9.30</td>
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<tr>
<td>1c</td>
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<td>446</td>
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[a] Fluorescence quantum yields were determined by exciting the sample at 366 nm using quinine sulfate as the standard ($\Phi_f = 0.546$ in 0.1N H$_2$SO$_4$); ± 3%; b) ± 6%

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; $\lambda_{ex} = 760$ nm.
**Fig. S7** Two-photon excitation spectra of $3$ and $3 + \text{Hg}^{2+}$ 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); $\lambda_{ex} = 760$ nm.