

## **Electronic Supplementary Information (ESI)**

### High selective fluorescence imaging of zinc distribution in HeLa cell and Arabidopsis using naphthalene-based fluorescent probe

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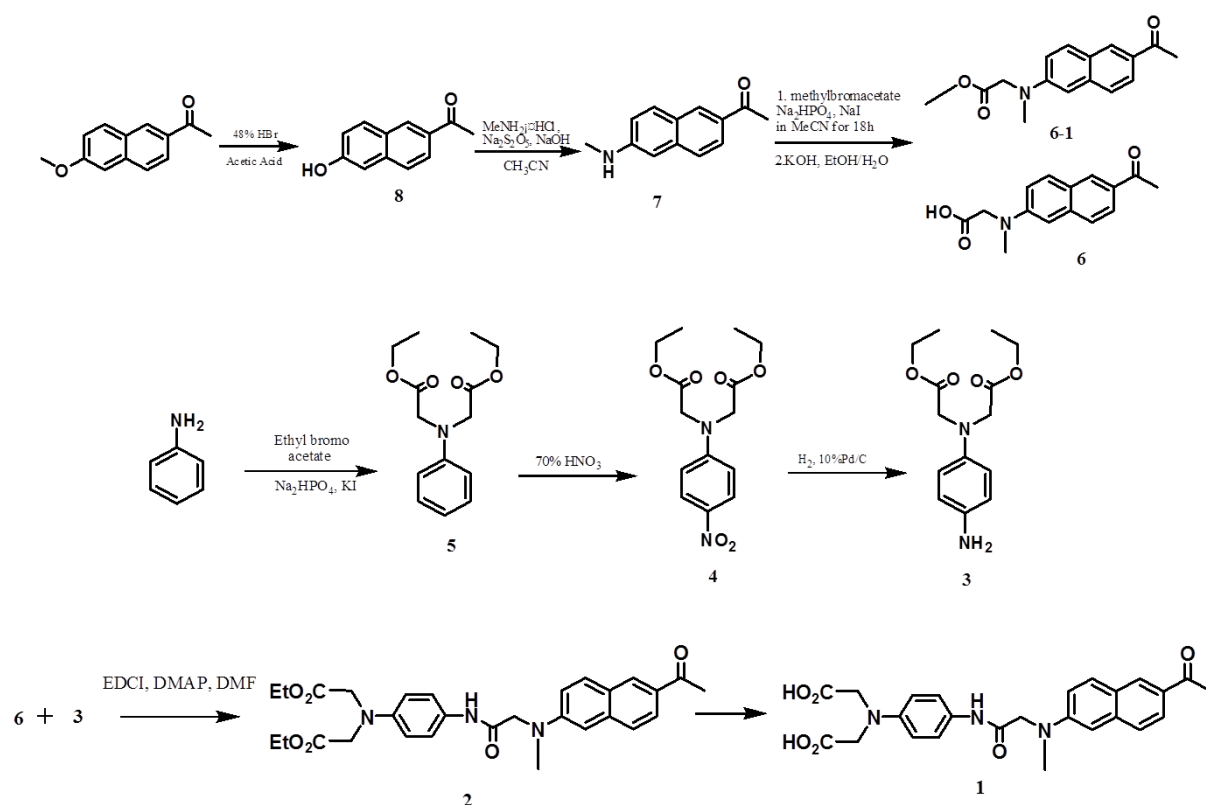
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## Experimental sections

**Characterization:**  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were measured with a Bruker ARX 300 MHz spectrometer. MS spectra were obtained with a JEOL JMS-700 mass spectrometer. IR spectra were obtained for KBr pellets, in the range  $400\text{--}4000\text{ cm}^{-1}$ , with a Shimadzu FTIR 8400S instrument. Transmission electron microscopy (TEM) images were taken with a JEOL JEM-2100F instrument operated at 200 kV. Images were recorded on 2k CCD (Gatan Inc. USC 1000). All fluorescence spectra were recorded in RF-5301PC spectrophotometer. UV-Vis absorption spectra were recorded with Sinco S-3100 spectrophotometers.



**Scheme S1.** Synthesis of compound **1**.

**Compound 5.** To the mixture of aniline (0.93 g, 10 mmol),  $\text{Na}_2\text{HPO}_4$  (2.84 g, 20 mmol), and KI (1.66 g, 10 mmol) in anhydrous acetonitrile (MeCN), bromoethylacetate (5.56 ml, 50 mmol) was added. The reaction mixture was refluxed overnight under nitrogen atmosphere. After completion of reaction (as monitored on TLC) solvent was evaporated to dryness under reduced pressure, and cold water was added to it and then extracted with chloroform. The organic layer was separated and dried over anhydrous  $\text{Na}_2\text{SO}_4$ . Filtered and chloroform was evaporated under reduced pressure to obtain a viscous liquid (1.98 g, 75%).  $R_f$  ( $\text{CHCl}_3$ ) 0.61  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 7.21 (t, 2H,  $J$ /7.8, 7.5 Hz), 6.80 (m, 1H), 6.62 (d, 2H,  $J$ /7.8 Hz), 4.27 (m, 4H), 3.89 (s, 4H), 1.32 (t, 6H,  $J$ /6.9, 6.9 Hz).

**Compound 4.** Compound **5** (6.00 g, 22.72 mmol) was taken into 50 ml of acetic acid and stirred at 0 °C. To this, 3 mL of HNO<sub>3</sub> was added. After 15 min, the reaction mixture was poured over ice, filtered and the resulting solid recrystallized from ethanol. This gave **4** as yellow brown needles in 76% yield. Mp = 160-162 °C MS (ES+) *m/z* = 310 (M<sup>+</sup>). Anal. calc. for C<sub>14</sub>H<sub>18</sub>N<sub>2</sub>O<sub>6</sub>: C, 54.19; H, 5.85; N, 9.03. Found: C, 54.12; H, 5.85; N, 8.98%. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): *d* 1.30 (t, 6H, *J* = 7.0 Hz), 4.21 (s, 4H), 4.27 (q, 4H, *J* = 7.0 Hz), 6.6 (d, 2H, *J* = 9.5 Hz), 8.13 (d, 2H, *J* = 9.5 Hz). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): *d* 13.74, 53.00, 61.29, 110.82, 125.55, 138.52, 152.23, 168.86. FT-IR (KBr, cm<sup>-1</sup>): 3474, 3121, 3098, 2976, 2908, 2696, 2614, 2426, 2231, 1918, 1893, 1751, 1591, 1516, 1420, 1272, 1117, 1026, 961, 918, 871, 828, 757, 736, 696, 632, 586, 535, 559.72.

**Compound 3.** A solution of **4** (1.0 g, 3.22 mmol) in methanol (50 mL) was hydrogenated at 1 atm for 15 min using Pd-C (10% w/w, 0.07 g). After completion the catalyst was filtered off and the solvent removed under reduced pressure to yield **3** (0.85 g, 95%) as a brown liquid. MS (ES+) *m/z* = 281 (MH)<sup>+</sup>. Anal. calc. for C<sub>14</sub>H<sub>20</sub>N<sub>2</sub>O<sub>4</sub>: C, 59.99; H, 7.19; N, 9.99. Found: C, 59.25; H, 7.03; N, 9.84%. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): *d* 1.28 (t, 6H, *J* = 6.8 Hz), 4.09 (s, 4H), 4.18-4.23 (q, 4H, *J* = 6.8 Hz), 6.57 (d, 2H, *J* = 8.9 Hz), 6.66 (d, 2H, *J* = 8.9 Hz). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): *d* 170.88, 140.94, 138.02, 116.26, 114.54, 60.49, 53.68, 13.79. IR (max, NaCl, cm<sup>-1</sup>): 3330, 2981, 2930, 2355, 1733, 1616, 1519, 1448, 1412, 1347, 1255, 1188, 1097, 1025, 974, 918, 817, 729, 521.

**Compound 8.** To a solution containing 6-acyl-2-methoxynaphthalene (10.4 g, 52 mmol) in glacial acetic acid (100 mL), 48 % HBr (43.0 g, 0.53 mol) was added. The mixture was stirred at 100°C for 12 hr. Excess acetic acid was removed in vacuo, and the residue was taken up in ethyl acetate and washed with dilute NaHCO<sub>3</sub> and brine. The organic layer was dried with MgSO<sub>4</sub> and the solvent was removed in vacuo. The product was purified by column chromatography using ethyl acetate/hexane (1:1) as the eluent. Yield 7.2 g (74 %); mp 173 °C; IR (KBr): 3362, 1664 cm<sup>-1</sup> <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 8.41 (d, 1H, *J* = 2 Hz), 7.99 (dd, 1H, *J* = 9, *J* = 2 Hz), 7.87 (d, 1H, *J* = 9 Hz), 7.70 (d, 1H, *J* = 9 Hz), 7.20 (d, 1H, *J* = 2 Hz), 7.18 (dd, 1H, *J* = 9, *J* = 2 Hz), 5.70 (br s, 1H), 2.71 (s, 3H). Anal. Calcd for C<sub>12</sub>H<sub>10</sub>O<sub>2</sub>: C, 77.40; H, 5.41. Found: C, 77.52; H, 5.46.

**Compound 7.** MeNH<sub>2</sub>·HCl (14.2 g, 0.17 mol) was added to a mixture of **8** (6.5 g, 35 mmol), Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub> (13.3 g, 70 mmol), NaOH (7.0 g, 0.17 mol), and H<sub>2</sub>O (200mL) in a steel-bomb reactor and the mixture was stirred at 140 °C for 48 h. The product was collected by filtration, washed with water, and purified by flash column using chloroform/ethyl acetate (50:1) as the eluent. It was further purified by recrystallization from MeOH. Yield 5.9 g (85 %); mp 181 °C; IR (KBr): 3347, 1663 cm<sup>-1</sup> <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 8.30 (d, 1H, *J* = 2 Hz), 7.93 (dd, 1H, *J* = 9, *J* = 2 Hz), 7.72 (d, 1H, *J* = 9 Hz), 7.63 (d, 1H, *J* = 9 Hz), 6.91 (dd, 1H, *J* = 9, *J* = 2 Hz), 6.77 (d, 1H, *J* = 2 Hz), 4.17 (br s, 1H), 2.97 (s, 3H), 2.67 (s, 3H). Anal. Calcd for C<sub>13</sub>H<sub>13</sub>NO: C, 78.36; H, 6.58 N, 7.03. Found: C, 78.32; H, 6.56; N, 7.08.

**Compound 6-1.** A mixture of **7** (4.5 g, 23 mmol), methyl bromoacetate (5.2 g, 34 mmol), Na<sub>2</sub>HPO<sub>4</sub> (4.8 g, 34 mmol), NaI (1.4 g, 9.2 mmol) in MeCN (150 mL) was refluxed under N<sub>2</sub> for 18 h. The product was extracted with ethyl acetate, washed with brine, and purified by flash column using chloroform/ethyl acetate (30:1) as the eluent. Yield 5.2 g (83 %); mp 92 °C; IR (KBr): 1754, 1671 cm<sup>-1</sup> <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 8.32 (d, 1H, *J* = 2 Hz), 7.92

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(dd, 1H,  $J = 9, J = 2$  Hz), 7.80 (d, 1H,  $J = 9$  Hz), 7.64 (d, 1H,  $J = 9$  Hz), 7.08 (dd, 1H,  $J = 9, J = 2$  Hz), 6.88 (d, 1H,  $J = 2$  Hz), 4.23 (s, 2H), 3.74 (s, 3H), 3.21 (s, 3H), 2.67 (s, 3H); Anal. Calcd for C<sub>16</sub>H<sub>17</sub>NO<sub>3</sub>: C, 70.83; H, 6.32; N, 5.16; Found: C, 70.82; H, 6.30; N, 5.17.

**Compound 6.** A mixture of **6-1** (2.0 g, 7.4 mmol) and KOH (0.8 g, 14 mmol) in EtOH/H<sub>2</sub>O (50/10 mL) was stirred for 5h. The resultant solution was diluted with ice-water (100 mL) and concentrated HCl(aq) was added slowly at < 5 °C until pH = 3. The resulting precipitate was collected, washed with distilled water and purified by crystallization from MeOH. Yield 1.6 g (84 %); mp 158 °C; IR (KBr): 2906, 1739, 1678 cm<sup>-1</sup> <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD): δ 8.39 (d, 1H,  $J = 2$  Hz), 7.86 (dd, 1H,  $J = 9, J = 2$  Hz), 7.84 (d, 1H,  $J = 9$  Hz), 7.64 (d, 1H,  $J = 9$  Hz), 7.18 (dd, 1H,  $J = 9, J = 2$  Hz), 6.93 (d, 1H,  $J = 2$  Hz), 4.27 (s, 2H), 3.19 (s, 3H), 2.65 (s, 3H); <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD): δ = 199.3, 173.2, 149.8, 138.2, 130.9, 130.9, 130.8, 126.4, 125.7, 124.1, 116.0, 105.5, 53.5, 38.7, 25.4 ppm; Anal. Calcd for C<sub>15</sub>H<sub>15</sub>NO<sub>3</sub>: C, 70.02; H, 5.88; N, 5.44. Found: C, 70.08; H, 5.79; N, 5.45.

**Compound 2.** A mixture of **6** (0.50 g, 1.9 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide HCl (0.44 g, 2.3 mmol) in DMF (20 mL) was stirred for 20 min. To this mixture, **3** (0.71 g, 2.1 mmol) and 4-dimethylaminopyridine (0.033 g, 0.29 mmol) were added and stirred for 12 h under N<sub>2</sub>. The product was extracted with ethyl acetate, dried over MgSO<sub>4</sub>, and the solvent was removed in vacuo. The product was purified by column chromatography using chloroform/ethyl acetate (1:1) as the eluent. It was further purified by recrystallization from MeOH to obtain a white solid. Yield 0.64 g (58 %); mp 120 °C; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): 8.39 (s, 1H); 8.22 (s, 1H); 8.02 (dd,  $J = 8.68, 1.73$  Hz, 2H), 7.92 (d,  $J = 9.01$  Hz, 1H), 7.75 (d,  $J = 8.69$  Hz, 1H), 7.35 (d,  $J = 9.02$  Hz, 2H), 7.24 (dd,  $J = 9.04, 2.54$  Hz, 1H), 7.18 (d,  $J = 2.14$  Hz, 1H), 6.58 (d,  $J = 9.06$  Hz, 2H), 4.21 (q,  $J = 7.13, 7.12, 7.12$  Hz, 4H), 4.14 (s, 2H), 4.11 (s, 4H), 3.26 (s, 3H), 2.71 (s, 3H), 1.28 (t,  $J = 7.13, 7.13$  Hz, 6H). Anal. Calcd for C<sub>30</sub>H<sub>33</sub>N<sub>3</sub>O<sub>9</sub>: C, 67.04; H, 6.40; N, 8.09. Found: C, 67.02; H, 6.50; N, 8.1.

**Compound 1.** This ester (0.5 g, 0.86 mmol) was hydrolyzed by the method described for **6**. The resulting precipitate was collected, washed with distilled water, and purified by crystallization from MeOH. Yield 0.32 g (69 %); mp 148 °C; IR (KBr): cm<sup>-1</sup> <sup>1</sup>H NMR (300 MHz, DMSO): δ 8.38 (d, 1H,  $J = 2$  Hz), 7.84 (d, 1H,  $J = 9$  Hz), 7.83 (d, 1H,  $J = 9$  Hz), 7.63 (d, 1H,  $J = 9$  Hz), 7.22 (s, 1H), 7.18 (dd, 1H,  $J = 9, J = 2$  Hz), 7.03 (dd, 1H,  $J = 9, J = 2$  Hz), 6.97 (d, 1H,  $J = 2$  Hz), 6.85 (d, 1H,  $J = 9.0$  Hz), 4.61 (s, 2H), 4.22 (s, 2H), 4.06 (s, 4H), 3.20 (s, 3H), 2.62 (s, 3H); <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD): δ = 199.2, 174.3, 171.1, 169.7, 150.2, 149.9, 138.0, 135.9, 133.3, 130.9, 130.8, 130.7, 126.3, 125.8, 124.0, 120.0, 116.2, 113.8, 107.1, 105.8, 65.3, 56.4, 54.3, 39.1, 25.2 ppm; Anal. Calcd for C<sub>27</sub>H<sub>27</sub>N<sub>3</sub>O<sub>9</sub>: C, 60.33; H, 5.06; N, 7.82. Found: C, 60.31; H, 5.12; N, 7.78.

**Photospectroscopy.** Fluorescence emission spectra were recorded with a Shimadzu RF-5301-PC instrument. For all measurements, excitation was at 370 nm, with excitation and emission slit widths of 1.5 nm. The pH value was adjusted by using 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (20 mM, pH 7). Fluorescence quantum yields were determined by reference to rhodamine 6G ( $\Phi = 0.76$ ).<sup>1,2</sup>

**Method of fluorescence titration.** Stock solutions of the **1** (1.0 x 10<sup>-5</sup> M) was prepared in aqueous solution at pH=7. The solution of the guest cation (Zn<sup>2+</sup>, 1.0 x 10<sup>-4</sup> M) were prepared in aqueous solution at pH=7. Fluorescence spectra were initially recorded of **1** solution and

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adding increasing amount of guest cation solution to it. Binding constant was calculated according to the Benesi-Hildebrand equation.  $K_a$  was calculated following the equation stated below.<sup>3</sup>

$$1/(A-A_0) = 1/\{K(F_{\max}-F_0) [Zn^{2+}]_n\} + 1/[F_{\max}-F_0]$$

Here  $F_0$  is the fluorescence of receptor in the absence of guest,  $F$  is the fluorescence recorded in the presence of added guest cation,  $F_{\max}$  is fluorescence in presence of added  $[Zn^{2+}]_{\max}$  and  $K$  is the association constant ( $M^{-1}$ ). The association constant ( $K$ ) could be determined from the slope of the straight line of the plot of  $1/(F-F_0)$  against  $1/[Zn^{2+}]_n$ . The association constant ( $K_a$ ) as determined by fluorescence titration method for **1** with  $Zn^{2+}$  is found to be  $7.51 \times 10^4 M^{-1}$ .

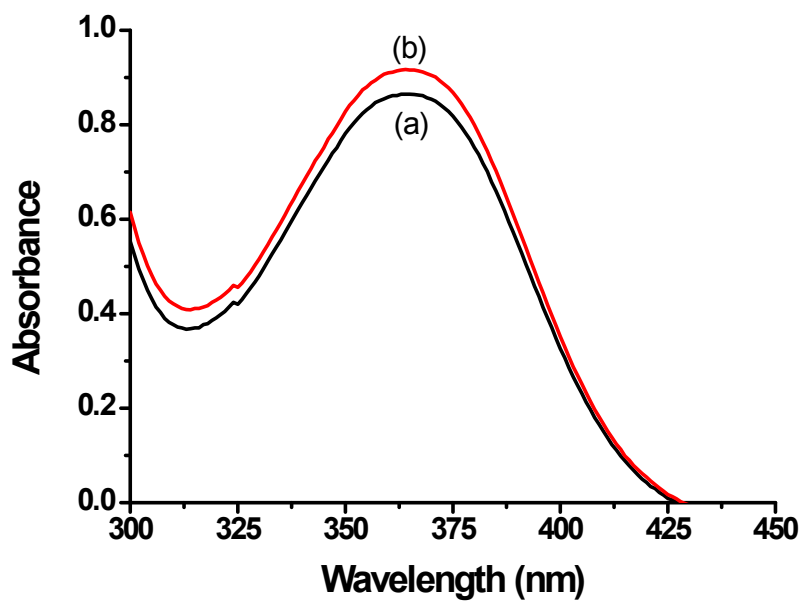
**Plant materials and growth conditions.** Surface-sterilized seeds of *Arabidopsis thaliana* (ecotypes, Col-0) were germinated and grown on the Murashige-Skoog (MS) medium without  $ZnSO_4$ . All plants were grown at  $24^\circ C$  under long-day conditions (light/dark regime of 16 h/8 h, white light,  $100 \mu mol photons m^{-2}s^{-1}$ ).

**Treatment and fluorescence imaging.** Five-day-old seedlings were transferred to MS medium containing different concentration of  $ZnSO_4$ . After treatment of  $ZnSO_4$  for 6 hours, seedlings were vacuum-infiltrated with  $100 \mu M$  **1** probe for 5 min, and then incubated in the dark condition for 10 min. The seedlings were washed with water, before fluorescence microscopy. Fluorescence was visualized using Olympus confocal laser scanning microscope (model FV1000, Tokyo, Japan) with following filter setup: excitation 405 nm and emission BF 442-514 nm.

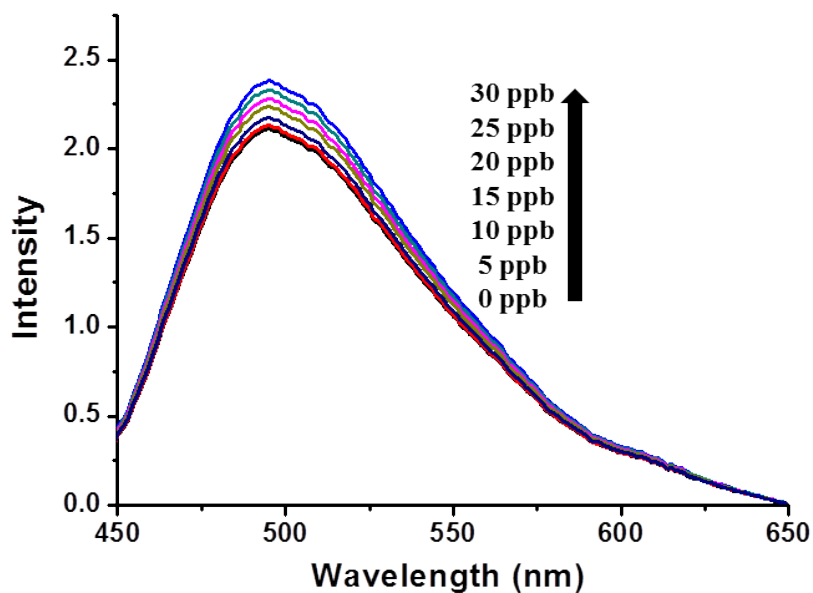
**Cell culture and imaging.** HeLa cells (KCLB, Seoul, Korea) were cultured in DEME (Invitrogen) supplemented with 10% FCS (Invitrogen). One day before imaging, the cells were passaged and seeded onto Delta T Dishes (Bioprotechs) and maintained under a humidified atmosphere of 5/95 (v/v) of  $CO_2$ /air at  $37^\circ C$ . The next day, the cells were incubated with compound **1** ( $5 \mu M$ ) for 30 min or pre-incubated with pyrithion-Zn 1:1 complex ( $20 \mu M$ ) for 40 min at  $37^\circ C$  under 5%  $CO_2$ . Fluorescence images of HeLa cells were obtained using a multiphoton confocal microscope (Leica TCS SP2 model) fitted with a  $100\times$  oil lens (numerical aperture = 1.30). Other information including excitation wavelength and emission filter are available in the figure captions.

## References

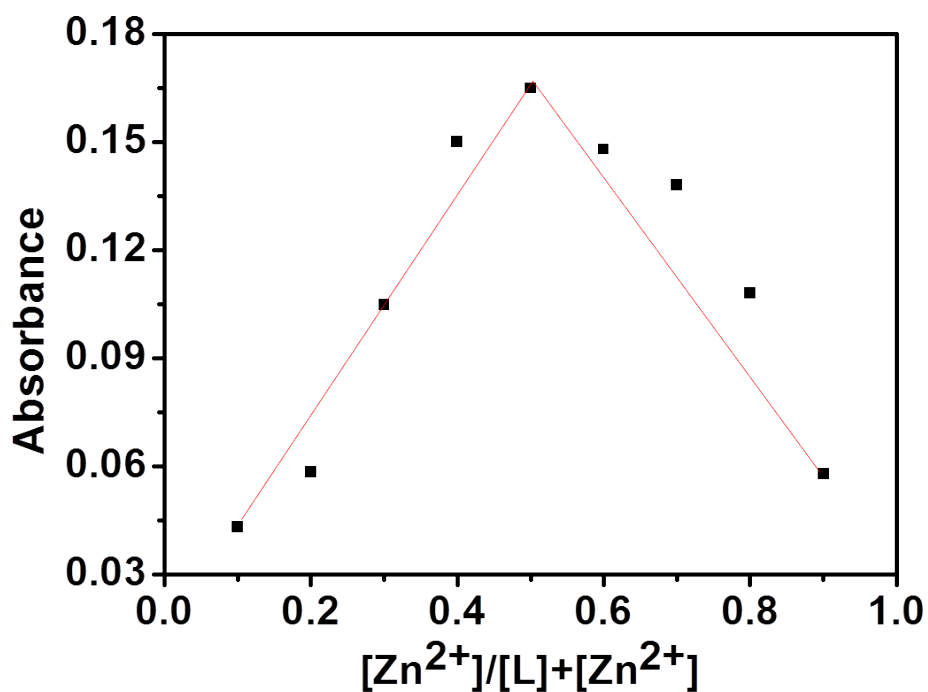
1. W. Qin, T. Rohand, M. Baruah, A. Stefan, M. V. der Auweraer, W. Dehaen and N. Boens, *Chem. Phys. Lett.*, **2006**, *420*, 562-568.
2. J. Olmsted III, *J. Phy. Chem.* **1979**, *83*, 2581-2584.
3. (a) H. A. Benesi and J. H. Hildebrand, *J. Am. Chem. Soc.*, 1949, **71**, 2703-2707; (b) K. A. Connors, *Binding Constants, The Measurement of Molecular Complex Stability*; Wiley: New York, **1987**.



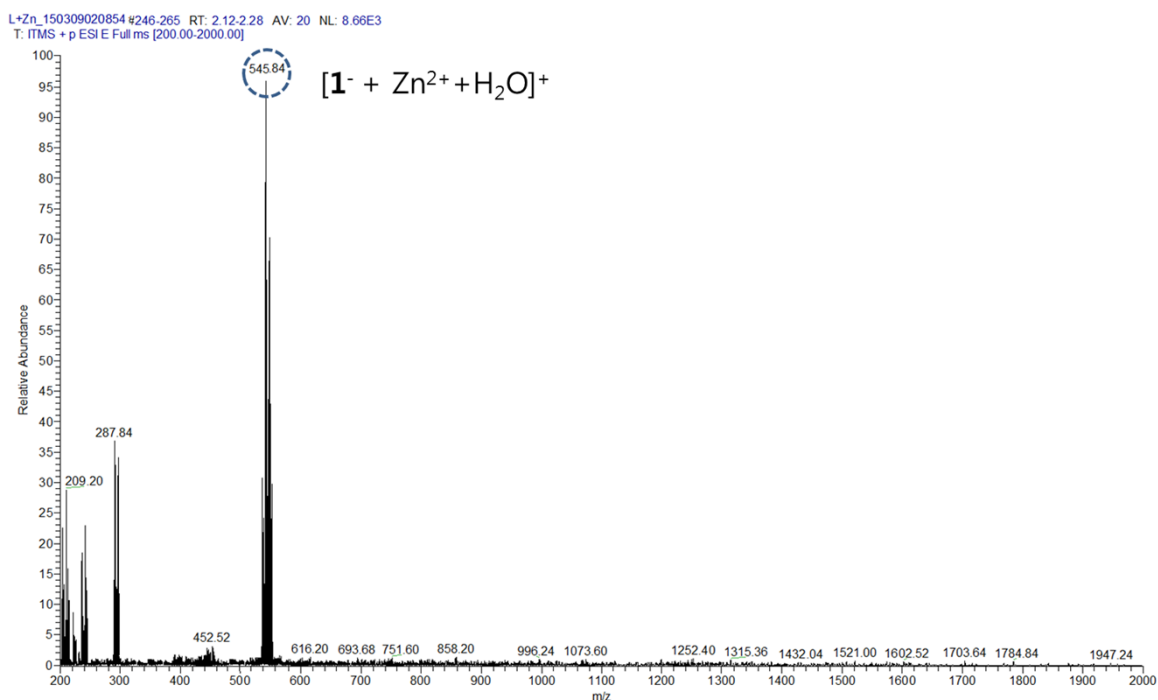
**Fig. S1** UV-vis spectrum of (a) **1** ( $1.0 \times 10^{-5}$  M) and (b) in the present of  $\text{Zn}(\text{NO}_3)_2$  ( $1.0 \times 10^{-4}$  M) in aqueous solution at pH=7.



**Fig. S2** Fluorescence spectra of **1** ( $1.0 \times 10^{-8}$  M) upon addition of increasing  $\text{Zn}(\text{NO}_3)_2$  concentrations in aqueous solution at pH 7.0.



**Fig. S3** Job's plot of **1** ( $1.0 \times 10^{-4}$  M) by using the  $Zn(NO_3)_2$  ( $1.0 \times 10^{-4}$  M) absorption changes in aqueous solution at pH=7.



**Fig. S4** ESI MS spectrum of compound **1** with  $Zn(NO_3)_2$ .

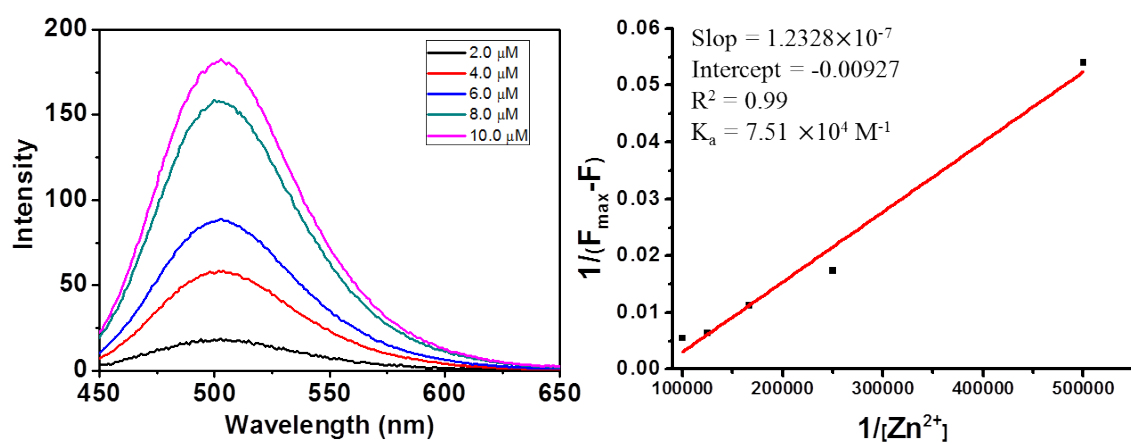


Fig. S5 The association constant ( $K_a$ ) of **1** with  $Zn^{2+}$ .

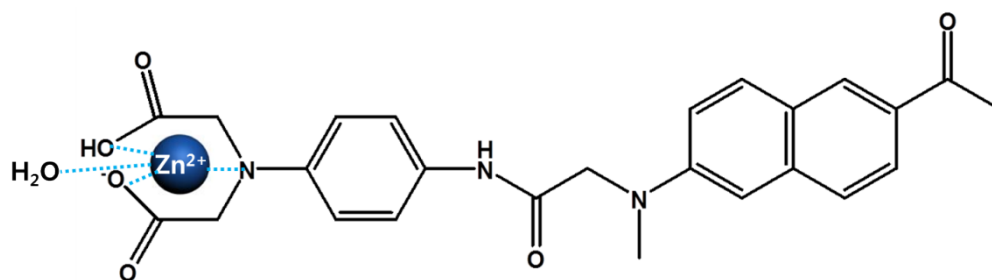
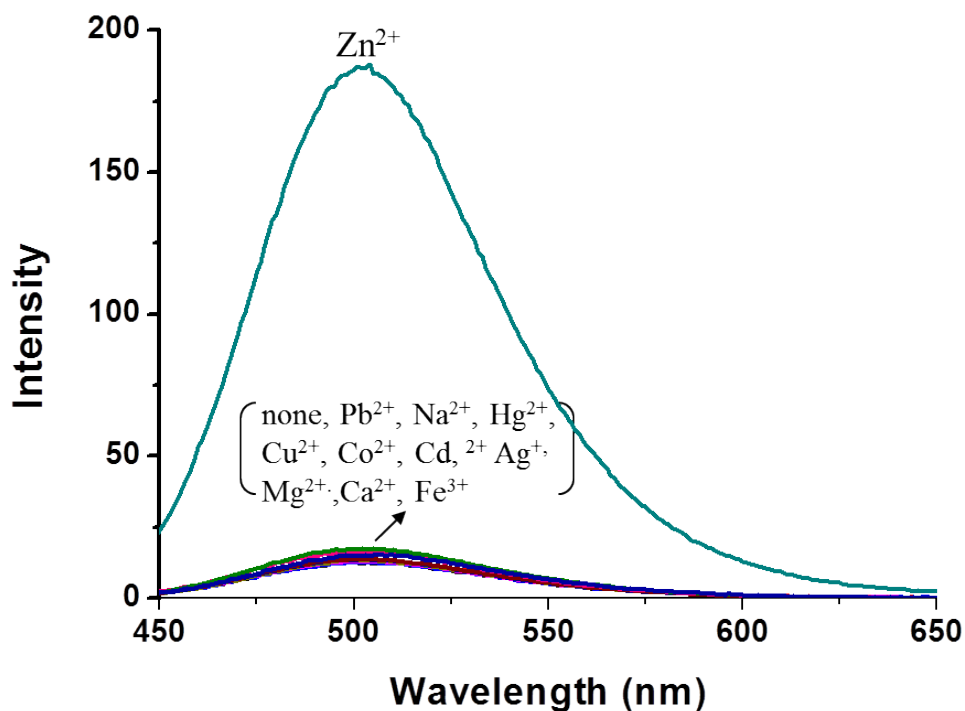
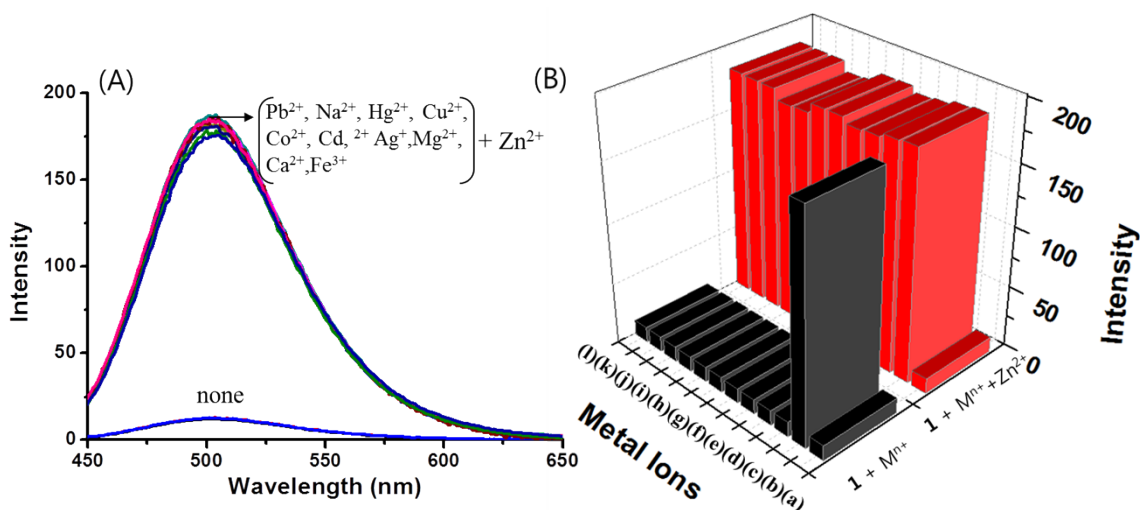


Fig. S6 Schematic of proposed bonding between **1**- $Zn^{2+}$ .

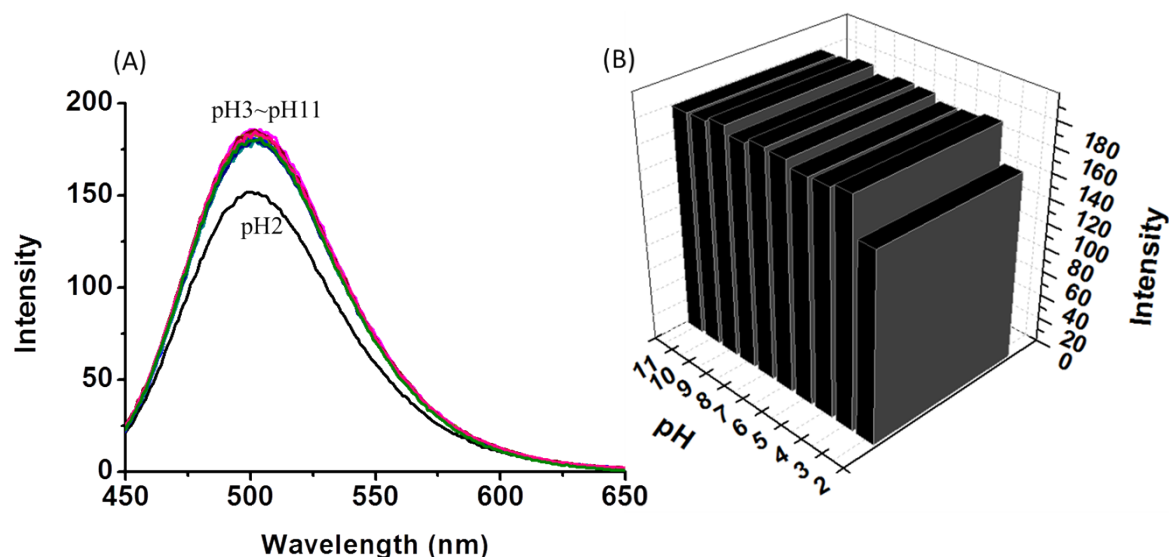




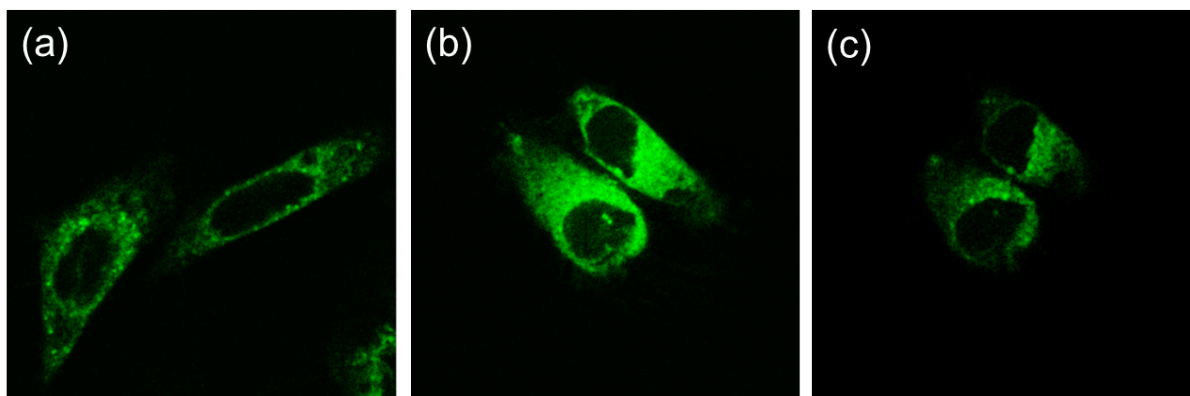
**Fig. S7** Fluorescence responses of **1** ( $1.0 \times 10^{-5}$  M) upon addition of  $\text{Zn}(\text{NO}_3)_2$  ( $1.0 \times 10^{-4}$  M; 10 equivalents) and addition of other metal ions (100 equivalents) in aqueous solution at pH=7



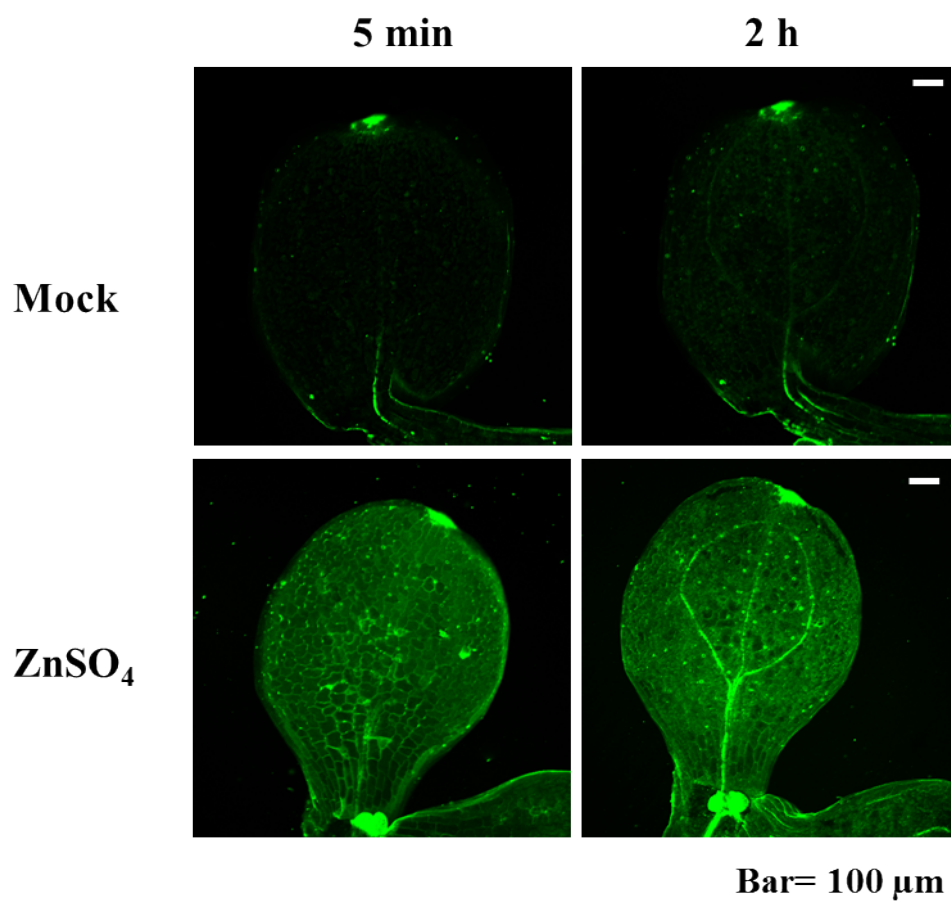
**Fig. S8** Fluorescence responses of **1** ( $1.0 \times 10^{-5}$  M) upon addition of (a) none, (b)  $\text{Zn}^{2+}$ , (c)  $\text{Co}^{2+}$ , (d)  $\text{Na}^+$ , (e)  $\text{Cu}^{2+}$ , (f)  $\text{Ag}^+$ , (g)  $\text{Hg}^{2+}$ , (h)  $\text{Mg}^{2+}$ , (i)  $\text{Pb}^{2+}$ , (j)  $\text{Cd}^{2+}$ , (k)  $\text{Ca}^{2+}$  and (l)  $\text{Fe}^{3+}$  (100 equivalents) and subsequent addition of  $\text{Zn}^{2+}$  (10 equivalents) in aqueous solution at pH=7.



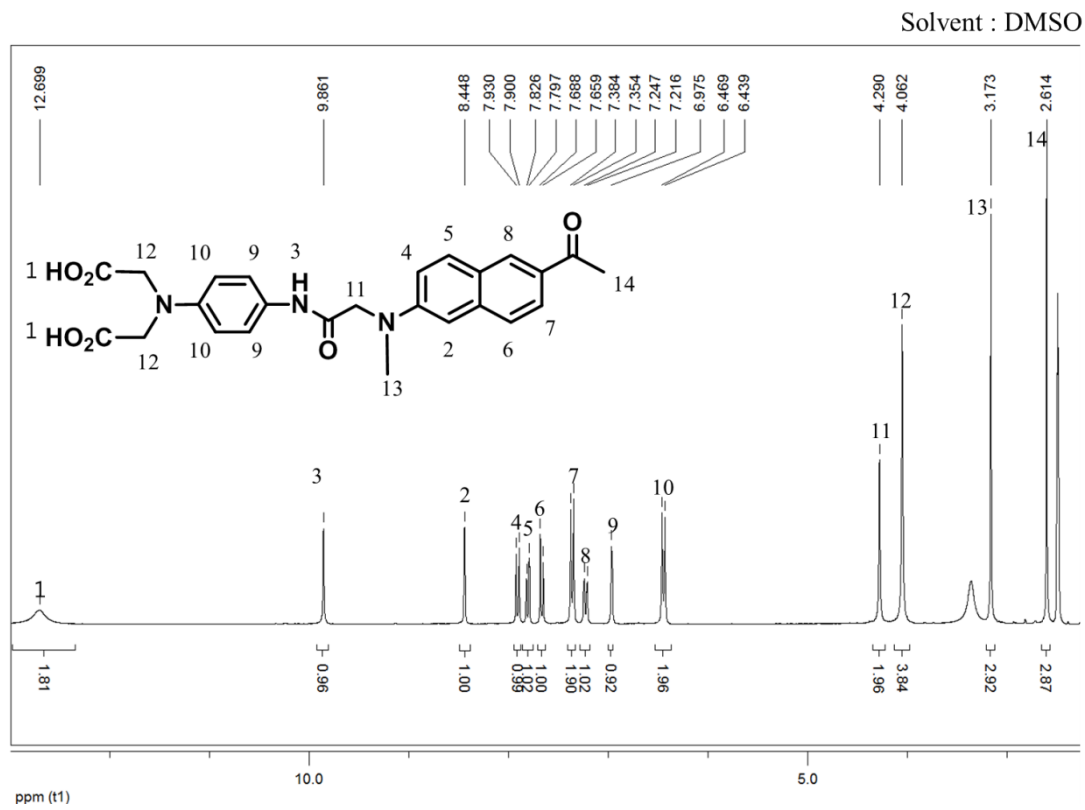
**Fig. S9** Fluorescence responses of **1** ( $1.0 \times 10^{-5}$  M) with  $\text{Zn}(\text{NO}_3)_2$  ( $1.0 \times 10^{-4}$  M) in aqueous solution at different pH.



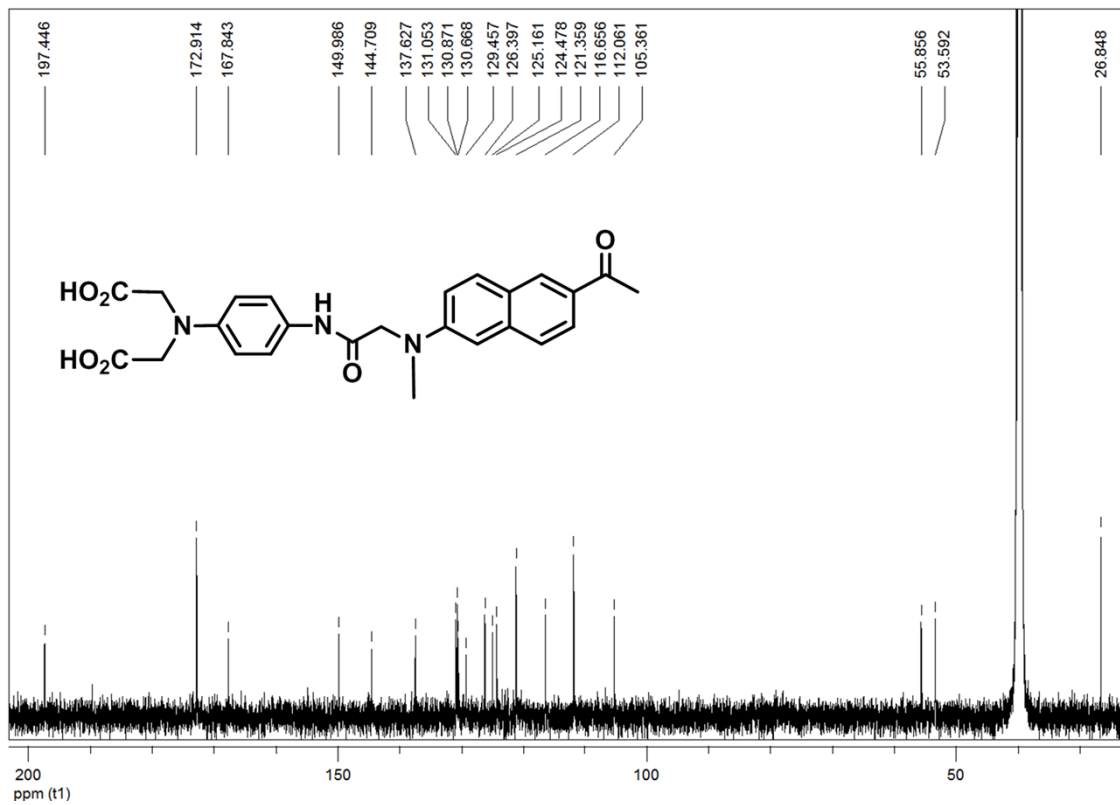
**Fig. S10** (a) Confocal fluorescent images of HeLa cell incubated with  $5 \times 10^{-6}$  M of **1** for 30 min,  $\lambda_{\text{ex}} = 740$  nm and (b) 40 min pretreatment with  $\text{Zn}^{2+}$ /pyrithione ( $2 \times 10^{-5}$  M, 1:1 ratio) and (c) After addition of  $1 \times 10^{-4}$  M TPEN.



**Fig. S11** Fluorescence images of plants treated with different time of ZnSO<sub>4</sub> ( $1.0 \times 10^{-3}$  M) in the presence of probe **1** (100  $\mu$ M).



**Fig. S12** <sup>1</sup>H NMR spectrum of compound **1**.



**Fig. S13** <sup>13</sup>C NMR spectrum of compound **1**.