Supporting information

A BODIPY-based fluorescent probe for the differential recognition of Hg(II) and Au(III) ions

*Ceren Cantürk, Muhammed Üçüncü and Mustafa Emrullahoğlu**

Department of Chemistry, Faculty of Science, İzmir Institute of Technology, Urla, 35430, Izmir, Turkey

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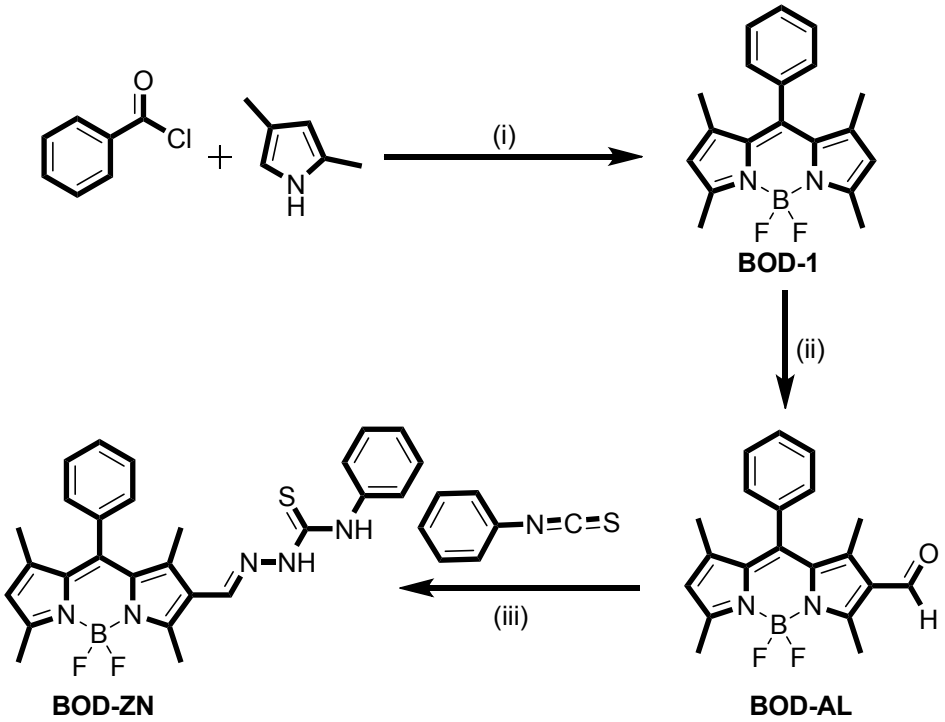
1. General methods

All reagents were purchased from commercial suppliers (Aldrich and Merck) and they were used without further purification. ^{1}H NMR and ^{13}C NMR were measured on a Varian VNMRJ 400 Nuclear Magnetic Resonance Spectrometer. UV absorption spectra were obtained on Shimadzu UV-2550 Spectrophotometer. Fluorescence measurements were performed by using Varian Cary Eclipse Fluorescence spectrophotometer. Samples were contained in 10.0 mm path length quartz cuvettes (2.0 mL volume). Upon excitation at 460 nm, the emission spectra were integrated over the range 480 nm to 750 nm. The slit width was 5 nm for both excitation and emission. Melting points were determined by using an Electrothermal Melting Point Apparatus 9200. The pH was recorded by HI-8014 instrument (HANNA). All measurements were conducted at least in triplicate.

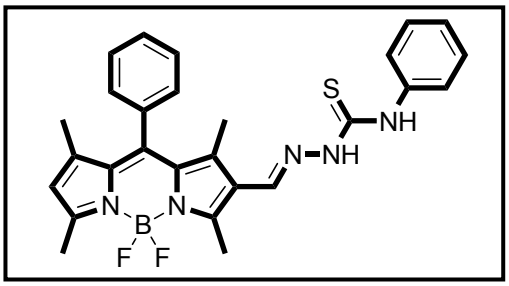
2. Synthesis section

The synthesis pathway for **BOD-ZN** was shown in Scheme S1. **BOD-1** and **BOD-AL** were synthesized according to the literature procedure[1]. **BOD-1** was converted to its aldehyde form by using the well known Vilsmeier Haack's formylation reaction. By using hydrazine hydrate, phenyl isothiocyanate and catalytic amount of acetic acid in methanol, **BOD-ZN** was synthesized.



Scheme S1: Synthesis pathway of **BOD-ZN. (i)** DCM, RT, overnight, then Et_3N and BF_3OEt_2 **(ii)** $POCl_3$, DMF, 0°C, then DCE, 60°C, overnight, **(iii)** Hydrazine hydrate, phenyl isothiocyanate, methanol, reflux.

Synthesis of BOD-ZN



To a mixture of **BOD-AL** (52.1 mg, 0.15 mmol) and hydrazine hydrate (12 µL, 0.18 mmol) in 5 ml of methanol, phenyl isothiocyanate (23 µL, 0.18 mmol) was injected. Then, a drop of glacial acetic acid was added and resulting solution was refluxed for an hour. After cooling room temperature, the solvent was removed under reduced pressure. The resultant residue was purified by column chromatography (10:1 (Hexane:Ethyl acetate)) to afford **BOD-ZN** as purple solid (29.4 mg, 40 % yield)[2]. Mp: 228-231 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.92 (s, 1H), 8.95 (s, 1H), 7.97 (s, 1H), 7.60 (d, J=8.0 Hz, 2H), 7.52-7.51 (m, 3H), 7.36 (t, J=8.0 Hz, 2H), 7.28-7.26 (m, 2H), 7.21 (t, J=7.2 Hz 1H), 6.09 (s, 1H), 2.75 (s, 3H), 2.59 (s, 3H), 1.50 (s, 3H), 1.40 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.7, 159.4, 153.9, 145.9, 142.5, 139.6, 138.1, 137.9, 134.5, 133.0, 130.5, 129.4, 128.7, 127.9, 125.9, 123.9, 123.1, 122.1, 14.9, 14.7, 14.1, 12.5. HRMS (EI) calc. for $C_{27}H_{26}BF_2N_5S$ $[M-H]^+$ 500.18918, found 500.18066.

3. Determination of quantum yields

Fluorescence quantum yields of **BOD-ZN** were determined by using optically matching solutions of Rhodamine 6G (Φ_F=0.95 in water) as a standard[3]. The quantum yield was calculated according to the equation;

$$\Phi_{F(X)} = \Phi_{F(S)} (A_S F_X / A_X F_S) (n_X / n_S)^2$$

Where Φ_F is the fluorescence quantum yield, A is the absorbance at the excitation wavelength, F is the area under the corrected emission curve, and n is the refractive index of the solvents used. Subscripts S and X refer to the standard and to the unknown, respectively.

4. Cell imaging

A549 Human Lung Adenocarcinoma cell lines were grown in DMEM supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5 % CO_2 at 37 °C. The cells were plated on 12 mm cover glasses in 6-well plate and allowed to grow for 24h. Before the experiments, the cells were washed with PBS buffer, and then the cells were incubated **BOD-ZN** (10 μM) for 20 min at 37 °C then washed with PBS three times. After incubating with Au^{3+} and Hg^{2+} (10 μM) for 20 min at 37 °C, cells were rinsed with PBS three times, and DAPI for 10 min at 37°C then washed with PBS three times. Then, the fluorescence images were acquired through an Olympus IX71 fluorescence microscope.

5. Determination of the association constant and stoichiometry

The association constant of [Hg^{2+}] was determined by using fluorescence titration data with the help of following equation;[4]

$$\ln[(F - F_0) / (F_{max} - F_0)] = n \ln[Hg^{2+}] + n \ln(K_{asscn}) \quad \text{.....(1)}$$

where n is the number of mercury ions associating with each molecule of **BOD-ZN**, K_{assoc} is the association constant, F_0 is the fluorescence of the free probe, F_{max} is the fluorescence intensity at saturation point, and F is the fluorescence of probe obtained with Hg^{2+} addition,

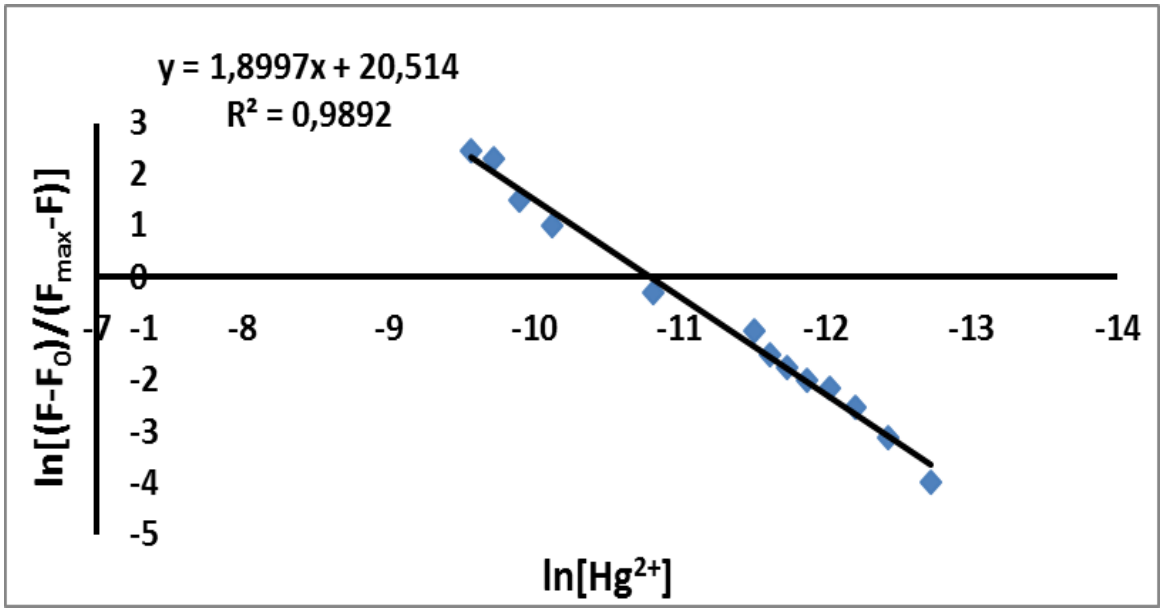


Figure S1: Plot of $\ln[(F-F_0)/(F_{max}-F)]$ against $\ln[Hg^{2+}]$; the stoichimetry of **BOD-ZN** Hg^{2+} association, obtained directly from the slope, is 1.9 ≅ 2. Following equation 1, the intercept gave an association constant of **BOD-ZN** as 4.2 x 10^4 M^{-2} (in 0.1 M potassium phosphate buffer, pH 7.0/EtOH (v/v, 1:4) (λ_{ex}: 460 nm, λ_{em} = 480 nm at 25 °C).

6. Determination of detection limits

a) For Au^{3+};

The detection limit was calculated based on the fluorescence titration[1]. To determine the S/N ratio, the emission intensity of **BOD-ZN** (10 μM) without Au^{3+} was measured by 10 times and the standard deviation of blank measurements was determined. Under the present conditions, a good linear relationship between the fluorescence intensity and Au^{3+} concentration could be obtained in the 0.1-1.0 μM (R = 0.9915). The detection limit is then calculated with the equation: detection limit = 3σbi/m, where σbi is the standard deviation of blank measurements; m is the slope between intensity versus sample concentration. The detection limit was determined to be 128.0 nm at S/N = 3.

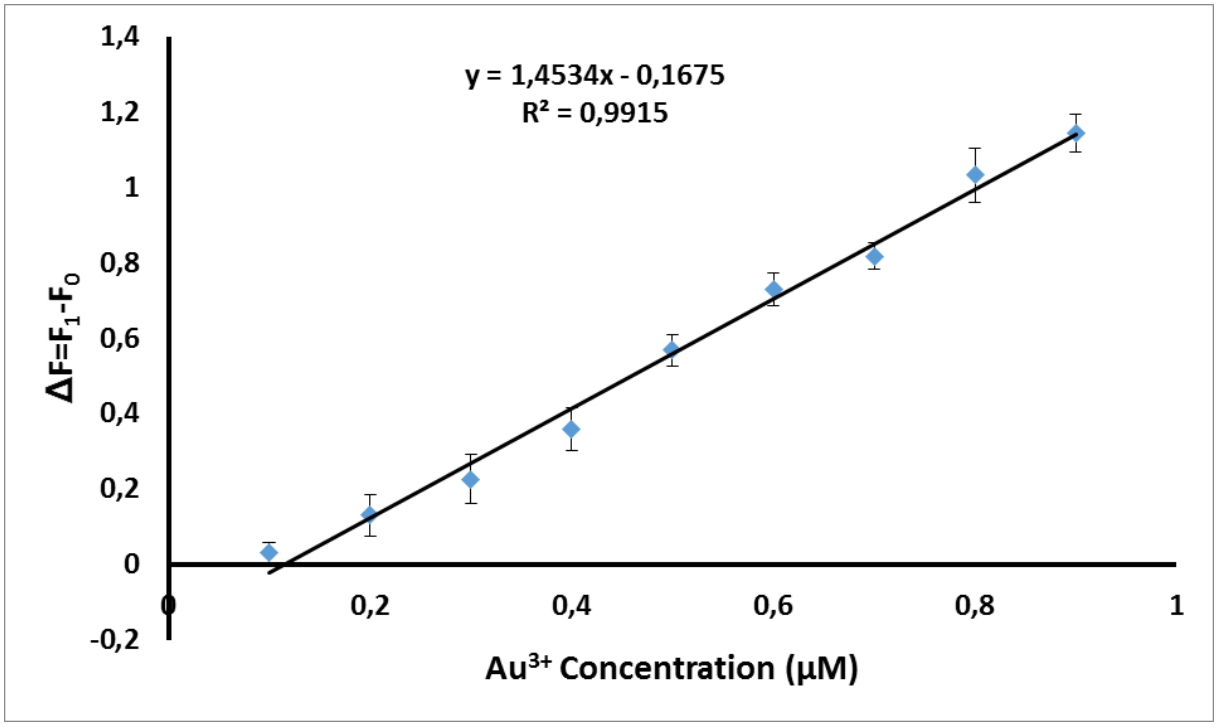


Figure S2: Fluorescence changes of **BOD-ZN** (10 μM) in 0.1 M phosphate buffer/EtOH (pH = 7.0, v/v, 1:4) upon the addition of Au^{3+} (0.01 to 0.1 equiv.) (0.1-1 μM)

b) For Hg^{2+};

Under the present conditions, a good linear relationship between the fluorescence intensity and Hg^{2+} concentration could be obtained in the 0.1-1.0 μM (R = 0.9931). The detection limit was measured to be 160.0 nM at S/N = 3.

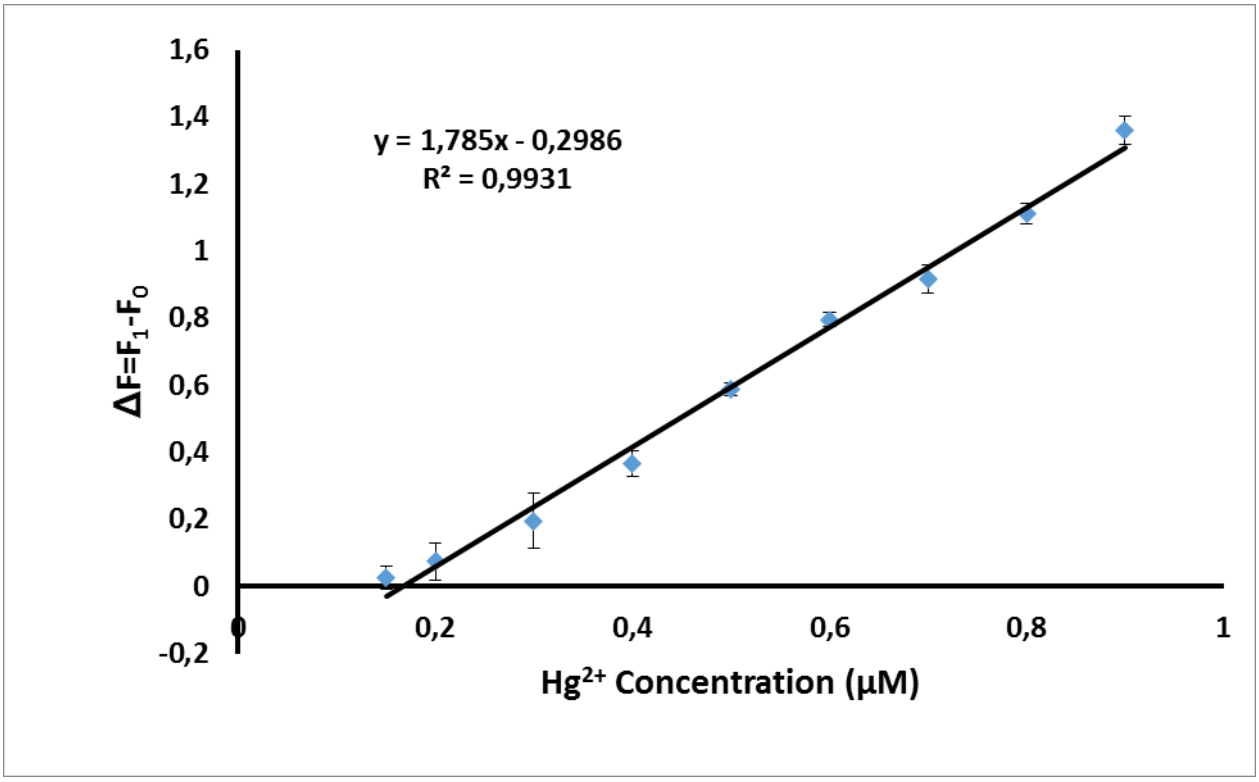


Figure S3: Fluorescence changes of **BOD-ZN** (10 μM) in 0.1 M phosphate buffer/EtOH (pH = 7.0, v/v, 1:4) upon addition of Hg^{2+} (0.01 to 0.1 equiv.) (0.1-1 μM)

6. References

1. M. Emrullahoğlu, M. Ucuncu and E. Karakus, *Chem. Commun.*, 2013, **49**, 7836.
2. S. Cunha and T.L. Da Silva, *Tetrahedron*, 2009, **50**, 2090.
3. A. M. Brouwer, *Pure Appl. Chem.*, 2011, **83**, 2213.
4. a) H. A. Benesi and J. H. Hildebrand, *J. Am. Chem. Soc.* **1949**, 71, 2703; b) J. Hatai, S. Pal, G. P. Jose, T. Sengupta and S. Bandyopadhyay, *Rsc Adv.* **2012**, 2, 7033.

7. Absorption and fluorescence spectra of BOD-ZN with Au^{3+}

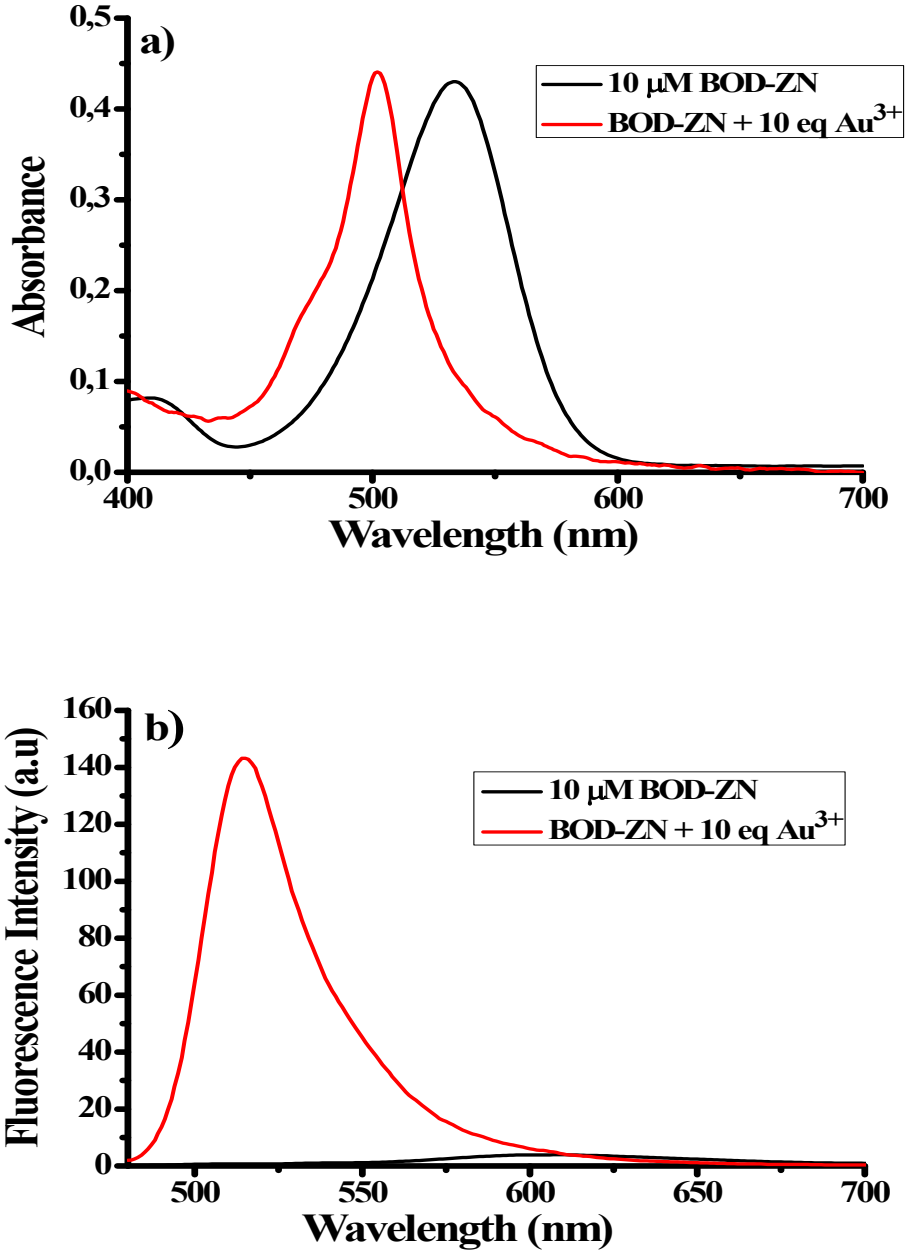


Figure S4: a) Absorbance and **b)** fluorescence spectra of **BOD-ZN** (10 μM) in the presence of 10.0 equiv. (100 μM) of $AuCl_3$ measured in 0.1 M phosphate buffer/EtOH (pH = 7.0, v/v, 1:4) (λ_{ex} = 460 nm, λ_{em} = 480 nm)

8. Time-dependent fluorescence change of BOD-ZN with Au^{3+}

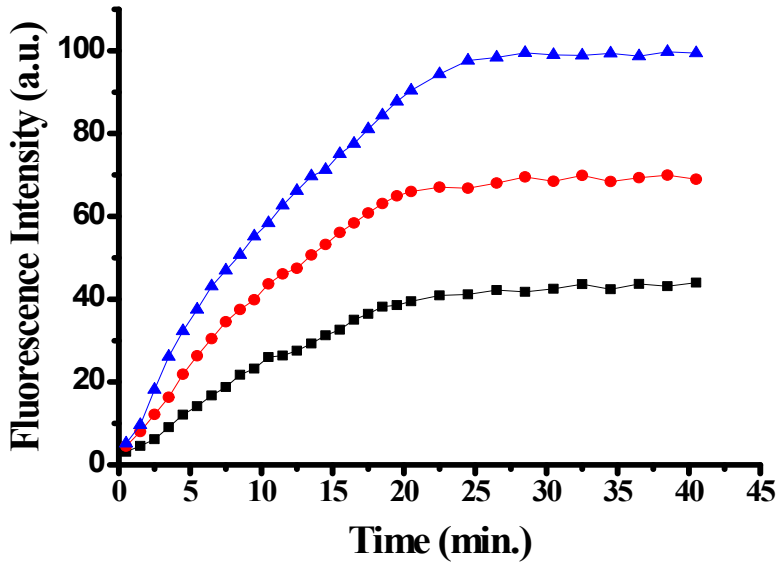


Figure S5: Time-dependent fluorescence change of **BOD-ZN** (10 μM) in the presence of 1.0 (■), 3.0 (●) and 5.0 (▲) equiv. (10, 30 and 50 μM) of $AuCl_3$ measured in 0.1 M phosphate buffer/EtOH (pH = 7.0, v/v, 1:4) (λ_{ex} = 460 nm, λ_{em} = 480 nm)

9. Fluorescence titration of BOD-ZN with Au^{3+}

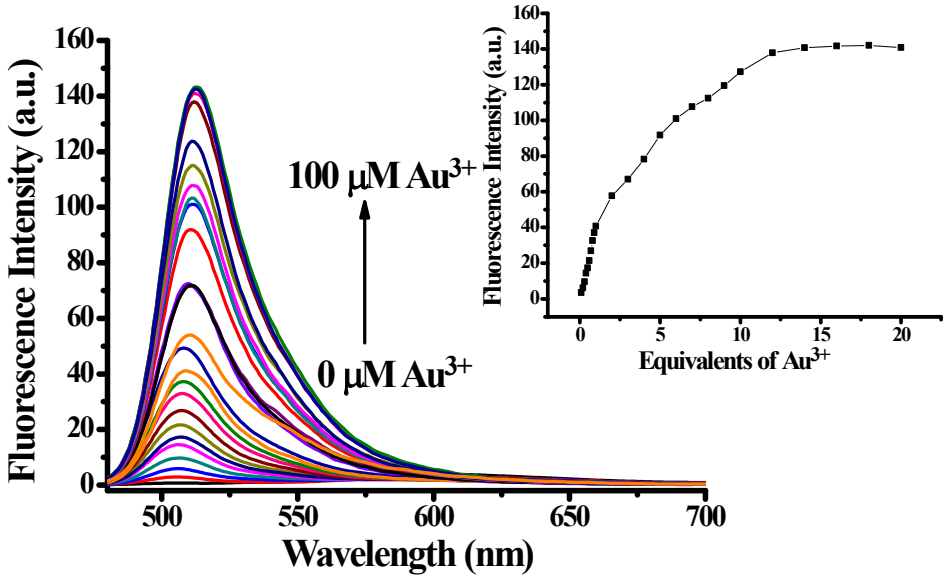


Figure S6: Fluorescence spectra of **BOD-ZN** (10 μM) in 0.1 M phosphate buffer/EtOH (pH = 7.0, v/v, 1:4) in the presence of Au^{3+} (mole equivalents = 0.01-10.0) (0.1-100 μM) (λ_{ex} = 460 nm, λ_{em} = 480 nm) Inset: Fluorescence intensity changes of **BOD-ZN** vs. equivalents of Au^{3+} .

10. The fluorescence responses of BOD-ZN with Hg^{2+}, Au^{3+}, and other metal ions

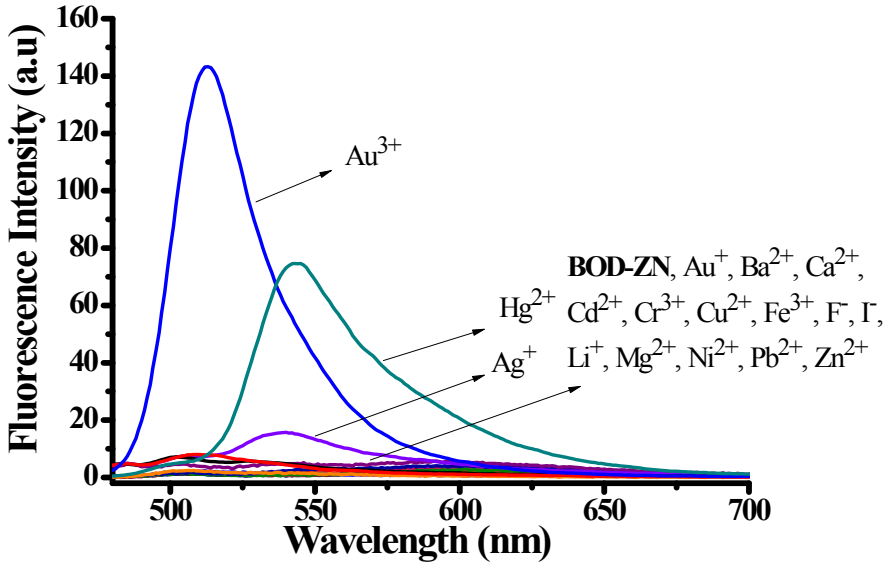


Figure S7: Fluorescence intensities of **BOD-ZN** (10 μM) in 0.1 M phosphate buffer/EtOH (pH = 7.0, v/v, 1:4) in the presence of 20.0 equiv. (200 μM) of the cations interests: **BOD-ZN** only , Au^{+} , Ba^{2+}, Ca^{2+}, Cd^{2+}, Cr^{3+}, Cu^{2+}, Fe^{3+}, F^{-}, I^{-}, Li^{+}, Mg^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+} (λ_{ex} = 460 nm, λ_{em} = 480 nm)

11. The fluorescence responses of BOD-ZN in the presence of Au^{3+} and other metal ions

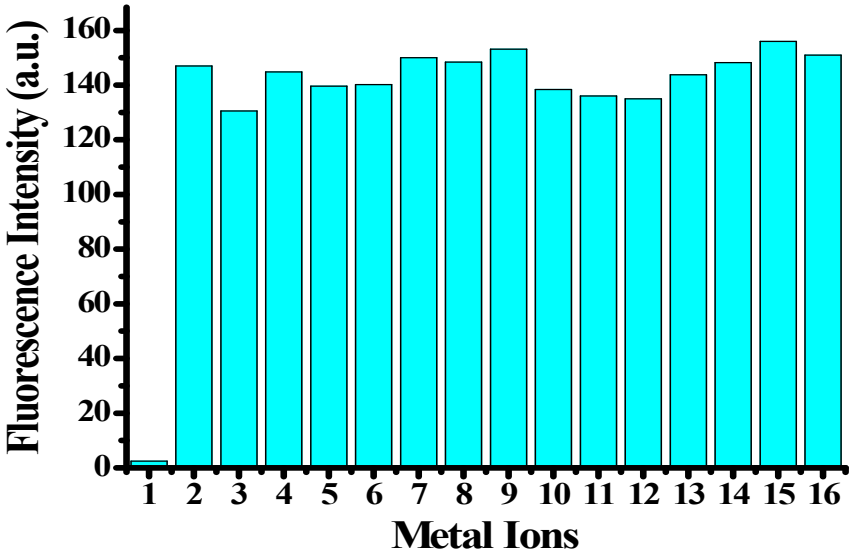


Figure S8: Fluorescence intensities of **BOD-ZN** (10 μM) in 0.1 M phosphate buffer/EtOH (pH = 7.0, v/v, 1:4) in the presence of 20.0 equiv. (200 μM) of the cations interest : 1, **BOD-ZN** only; 2, Au^{3+}; 3, Au^{+} ; 4, Ba^{2+}; 5, Ca^{2+}; 6, Cd^{2+}; 7, Cr^{3+}; 8, Cu^{2+}; 9, Fe^{3+}; 10, K^{+}; 11, Li^{+}; 12, Mg^{2+}; 13, Ni^{2+}; 14, Zn^{2+}; 15, Ag^{+}; 16, F^{-} (λ_{ex} = 460 nm, λ_{em} = 480 nm)

12. Effect of pH

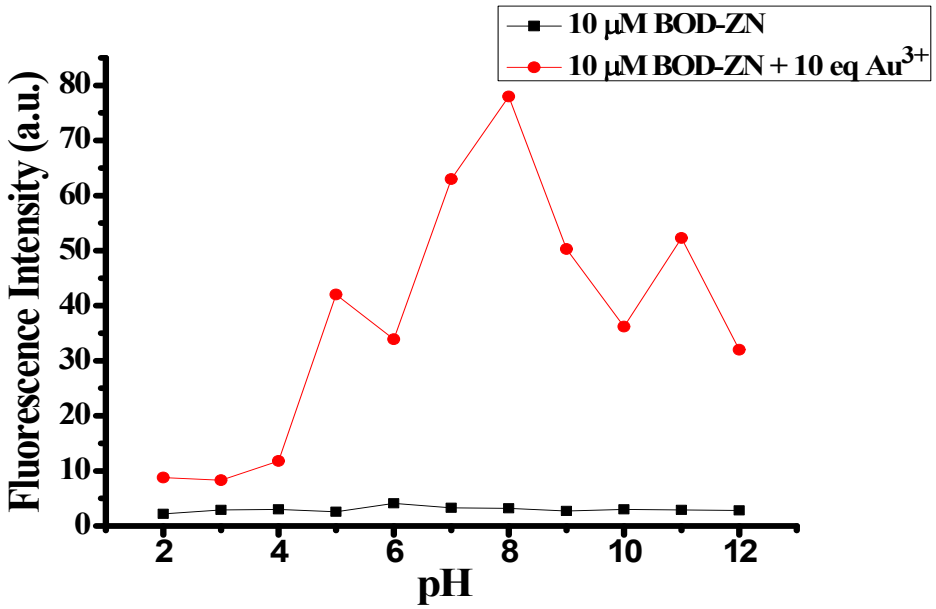


Figure S9: Effect of pH on the fluorescence intensity of **BOD-ZN** (10 μM) in 0.1 M phosphate buffer/EtOH (v/v, 1:4) in the absence and presence of Au^{3+} (10.0 equiv., 100 μM) (λ_{ex} = 460 nm, λ_{em} = 480 nm)

13. Effect of fraction of water

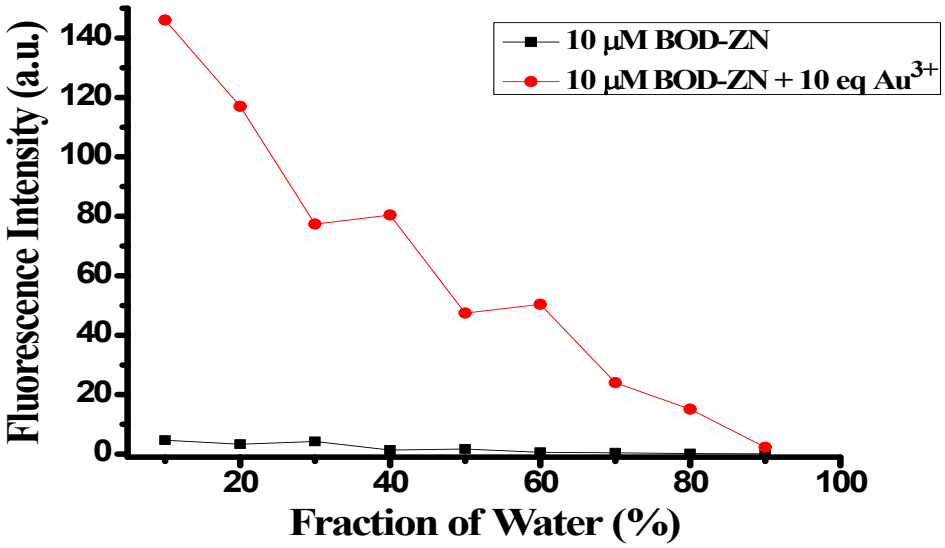


Figure S10: Effect of fraction of water on the fluorescence intensity of **BOD-ZN** (10 μM) in 0.1 M phosphate buffer/EtOH (pH = 7.0, v/v, 1:4) in the absence and presence of Au^{3+} (10.0 equiv., 100 μM) (λ_{ex} = 460 nm, λ_{em} = 480 nm)

14. Absorption and fluorescence spectra of BOD-ZN with Hg^{2+}

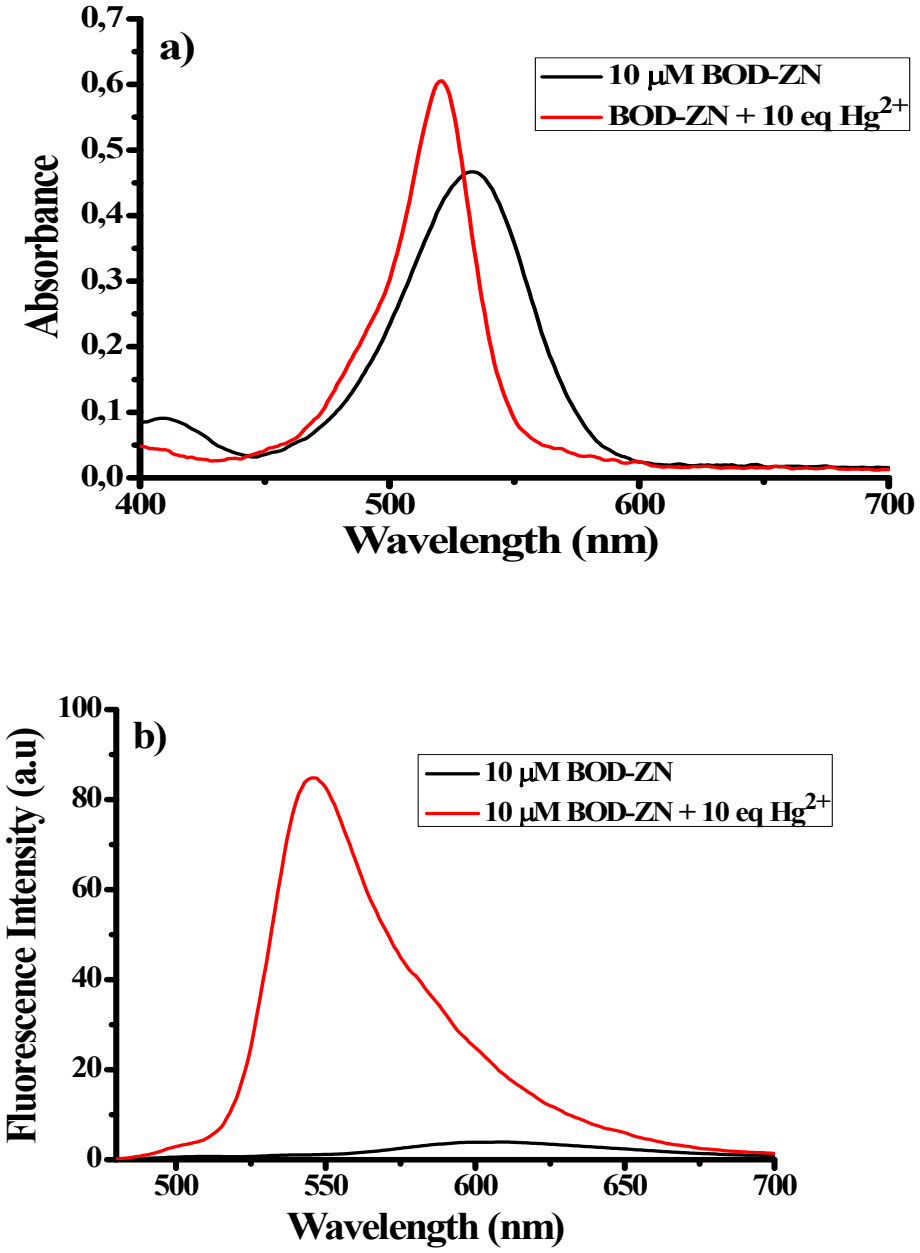


Figure S11: a) Absorbance and **b)** fluorescence spectra of **BOD-ZN** (10 μM) in the presence of 10.0 equiv. (100 μM) of $HgCl_2$ measured in 0.1 M phosphate buffer/EtOH (pH = 7.0, v/v, 1:4)

15. Time-dependent fluorescence change of BOD-ZN with Hg^{2+}

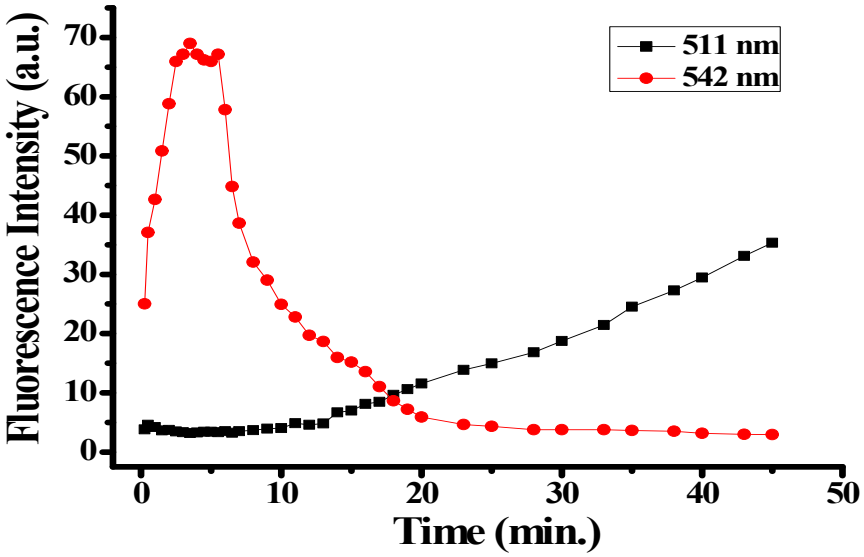


Figure S12: Time-dependent fluorescence change of **BOD-ZN** (10 μM) measured in 0.1 M Phosphate Buffer/EtOH (pH = 7.0, v/v, 1:4) in the presence of 10 equiv. (100 μM) of $HgCl_2$ (λ_{ex} = 460 nm, λ_{em} = 480 nm)

16. Fluorescence titration of BOD-ZN with Hg^{2+}

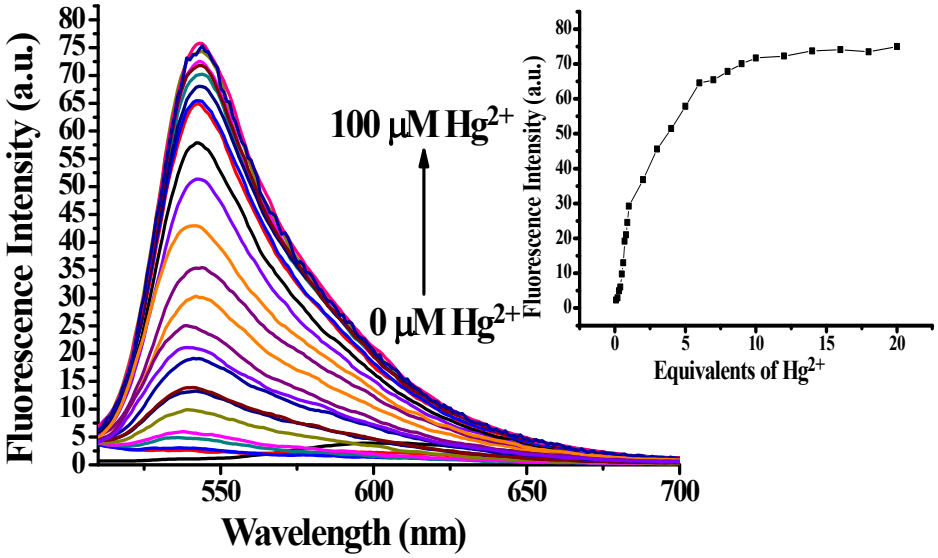


Figure S13: Fluorescence spectra of **BOD-ZN** (10 μM) in 0.1 M phosphate buffer/EtOH (pH = 7.0, v/v, 1:4) in the presence of Hg^{2+} (mole equivalents = 0.01-10.0) (0.1-100 μM) (λ_{ex} = 460 nm, λ_{em} = 480 nm) Inset: Fluorescence intensity changes of **BOD-ZN** vs. equivalents of Hg^{2+}

17. The fluorescence responses of BOD-ZN in the presence of Hg^{2+} and other metal ions

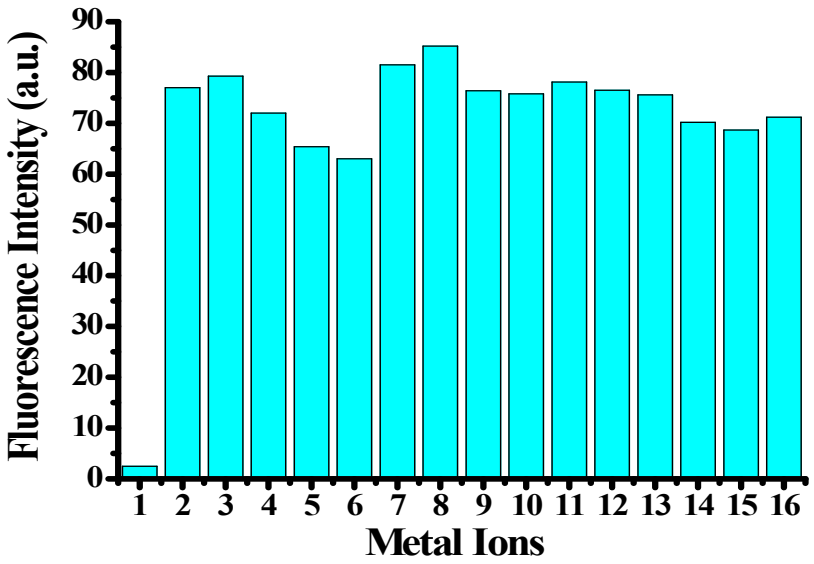


Figure S14: Fluorescence intensities of **BOD-ZN** (10 μM) in 0.1 M phosphate buffer/EtOH (pH = 7.0, v/v, 1:4) in the presence of 20.0 equiv. (200 μM) of the cations interest : 1, **BOD-ZN** only; 2, Hg^{2+}; 3, Au^{+}; 4, Ba^{2+}; 5, Ca^{2+}; 6, Cd^{3+}; 7, Cr^{2+}; 8, Cu^{2+}; 9, Fe^{2+}; 10, I^{-}; 11, Mg^{2+}; 12, Ni^{2+}; 13, Pb^{2+}; 14, Zn^{2+}; 15, Ag^{+}; 16, F^{-} (λ_{ex} = 460 nm, λ_{em} = 480 nm)

18. The fluorescence intensity changes of BOD-ZN in the presence of Hg^{2+} ions and Na_2S

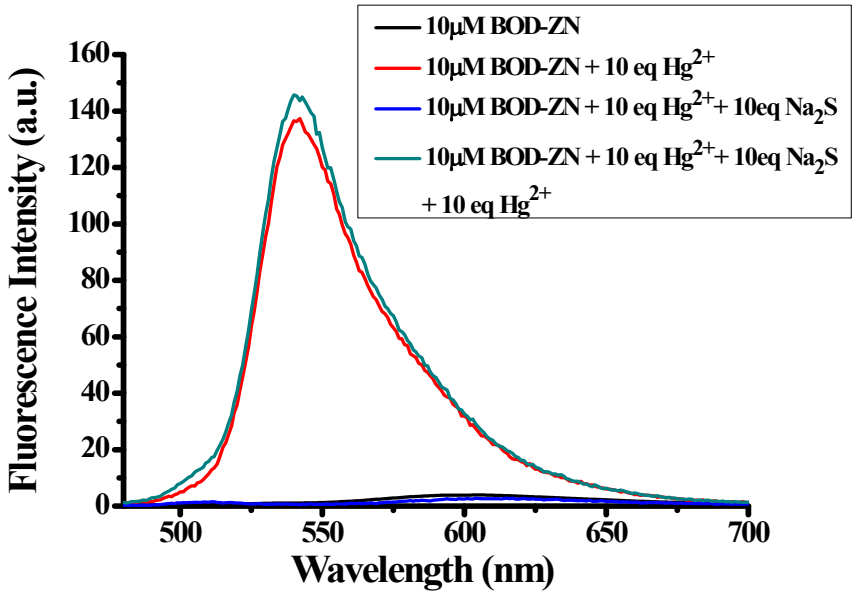


Figure S15: Fluorescence intensity changes of **BOD-ZN** (10 μM) in EtOH at pH = 7.0 at λ_{max} =542 nm after addition of 10 equiv. Hg^{2+}, 10 equiv. Hg^{2+} + 10 equiv. Na_2S, 10 equiv. Hg^{2+} + 10 equiv. Na_2S + 10 equiv. Hg^{2+}, respectively. (λ_{ex} = 460 nm, λ_{em} = 480 nm)

19. Effect of pH

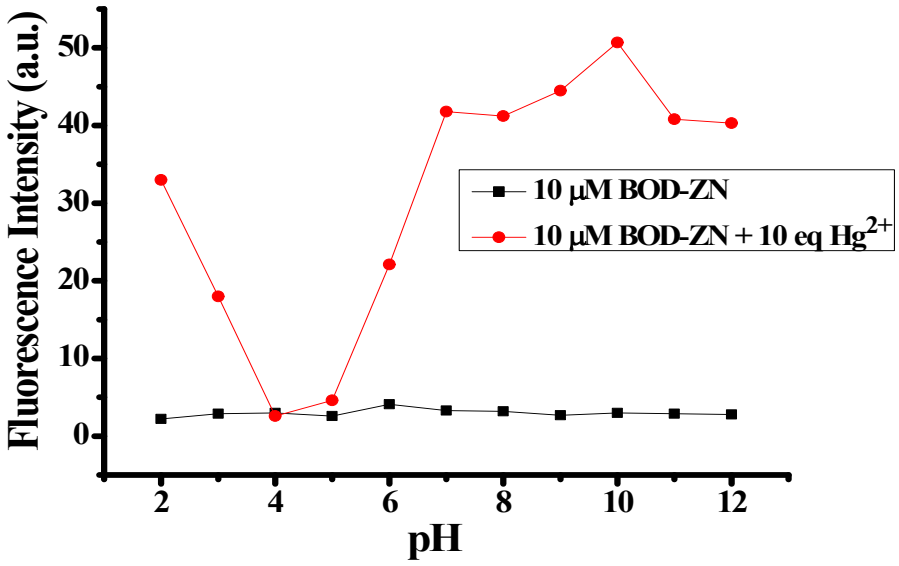


Figure S16: Effect of pH on the fluorescence intensity of **BOD-ZN** (10 μM) in 0.1 M phosphate buffer/EtOH (v/v, 1:4) in the absence and presence of Hg^{2+} (10.0 equiv., 100 μM) (λ_{ex} = 460 nm, λ_{em} = 480 nm)

20. Effect of fraction of water

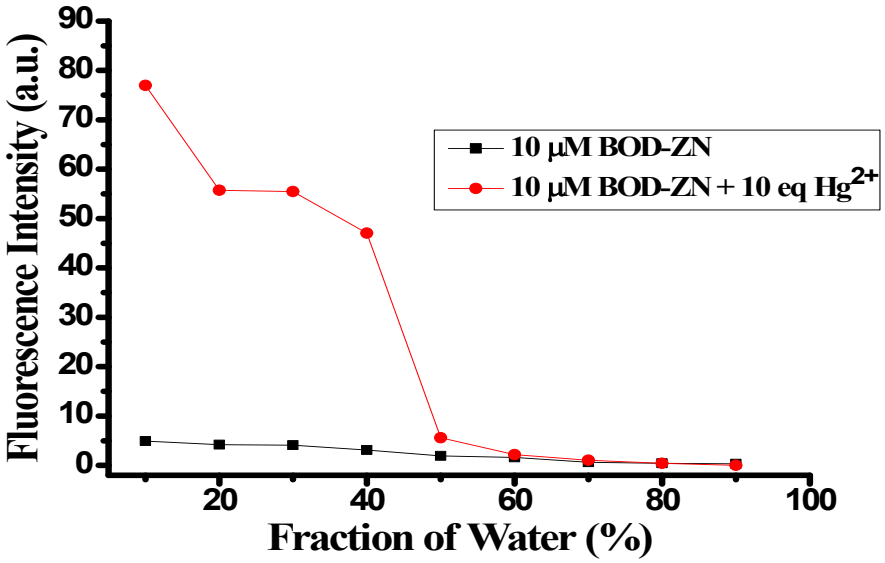


Figure S17: Effect of fraction of water on the fluorescence intensity of **BOD-ZN** (10 μM) in 0.1 M phosphate buffer/EtOH (pH = 7.0, v/v, 1:4) in the absence and presence of Hg^{2+} (10.0 equiv., 100 μM) (λ_{ex} = 460 nm, λ_{em} = 480 nm)

21. Selectivity of BOD-ZN in the presence of both Hg^{2+} and Au^{3+} ions

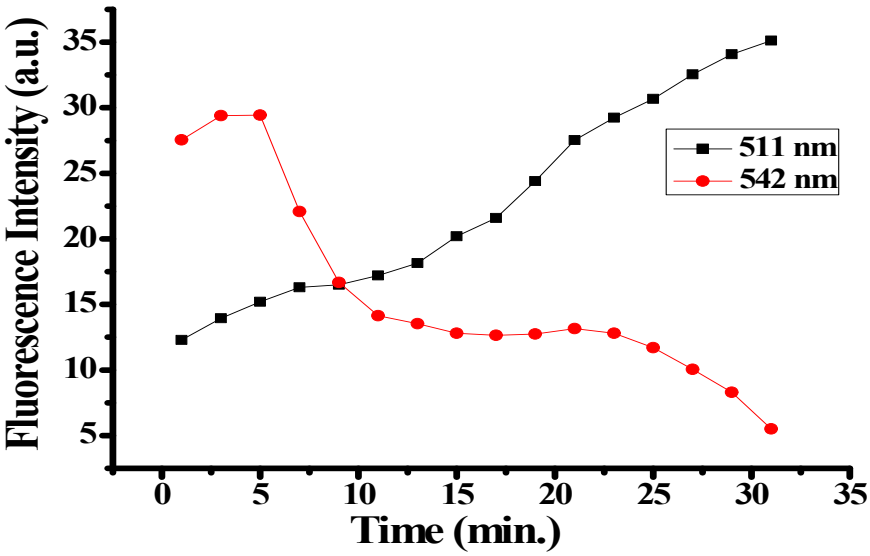


Figure S18: Selectivity of **BOD-ZN** (10 μM) in the presence of both Hg^{2+} and Au^{3+} (10 equiv., 100 μM) in 0.1 M phosphate buffer/EtOH (pH 7.0, v/v, 1:4) (λ_{ex} = 460 nm, λ_{em} = 480 nm)

22. Photograph image of the hydrolysis reaction of BOD-ZN mediated by Au(III)

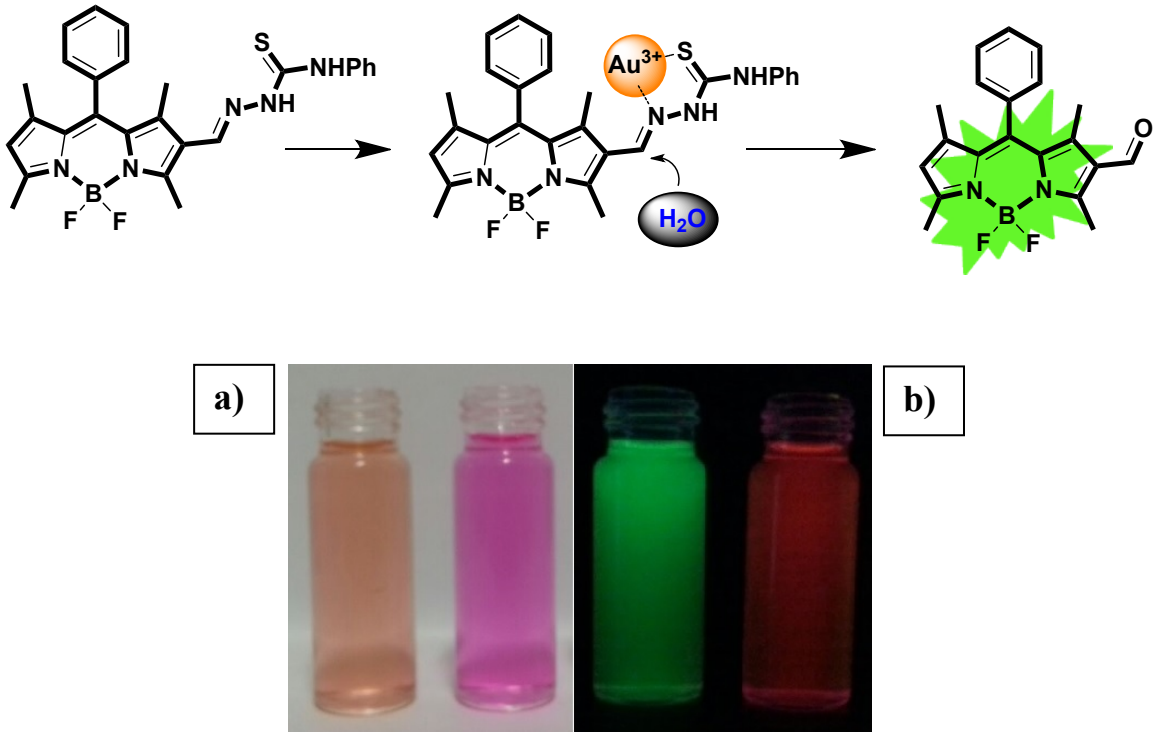


Figure S19: a) Day light photograph image of **BOD-ZN** + Au(III) (left), **BOD-ZN** (right), **(b)** fluoresccence image of **BOD-ZN** + Au(III) (left), **BOD-ZN** (right)

23. Photograph image of the coordination of BOD-ZN mediated by Hg(II) ions

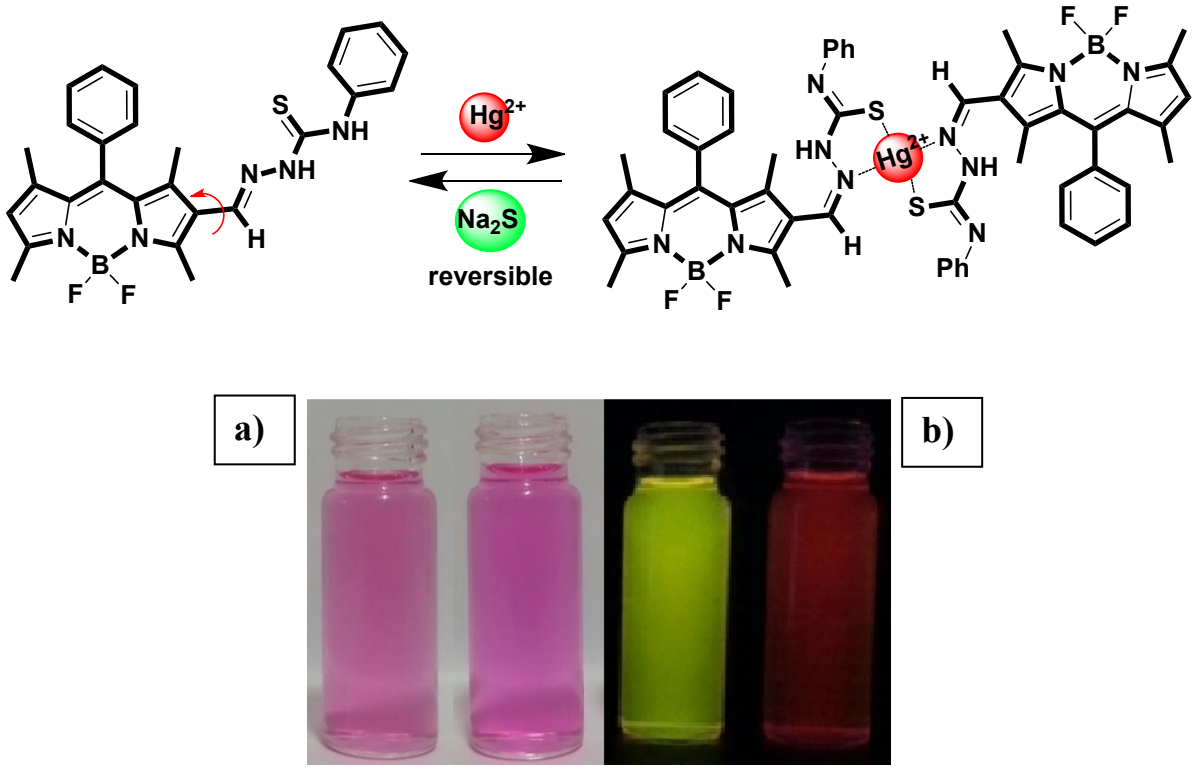
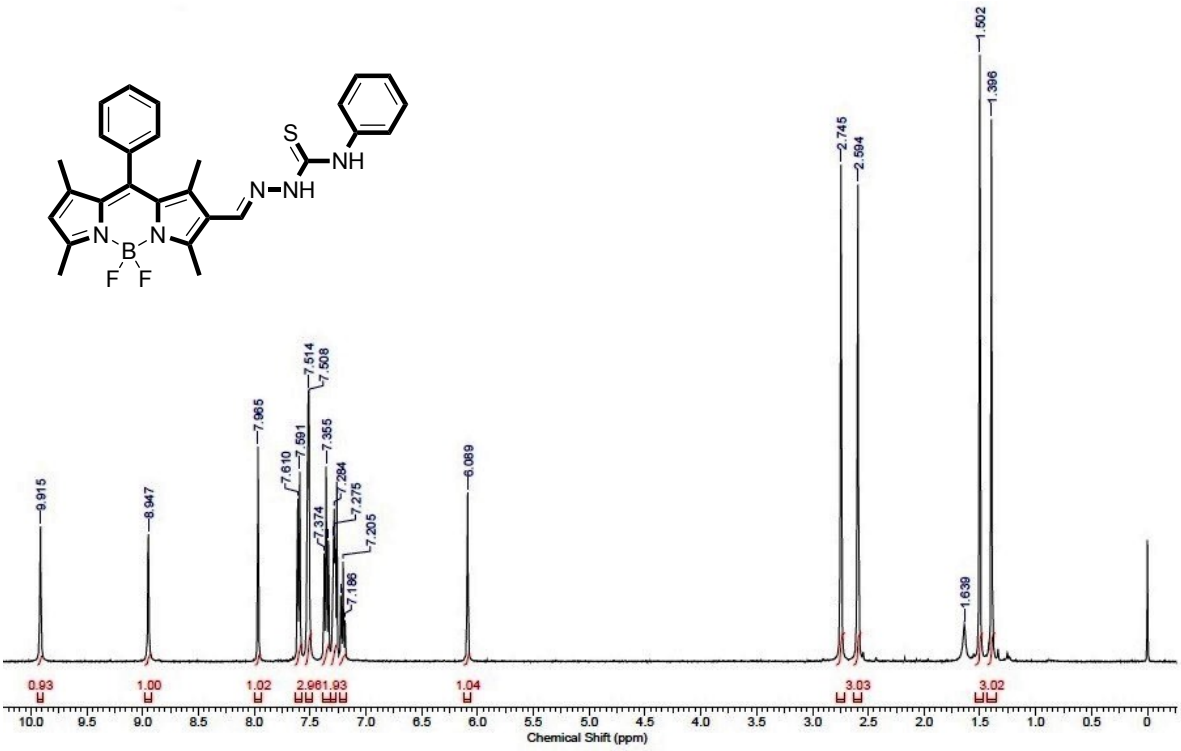
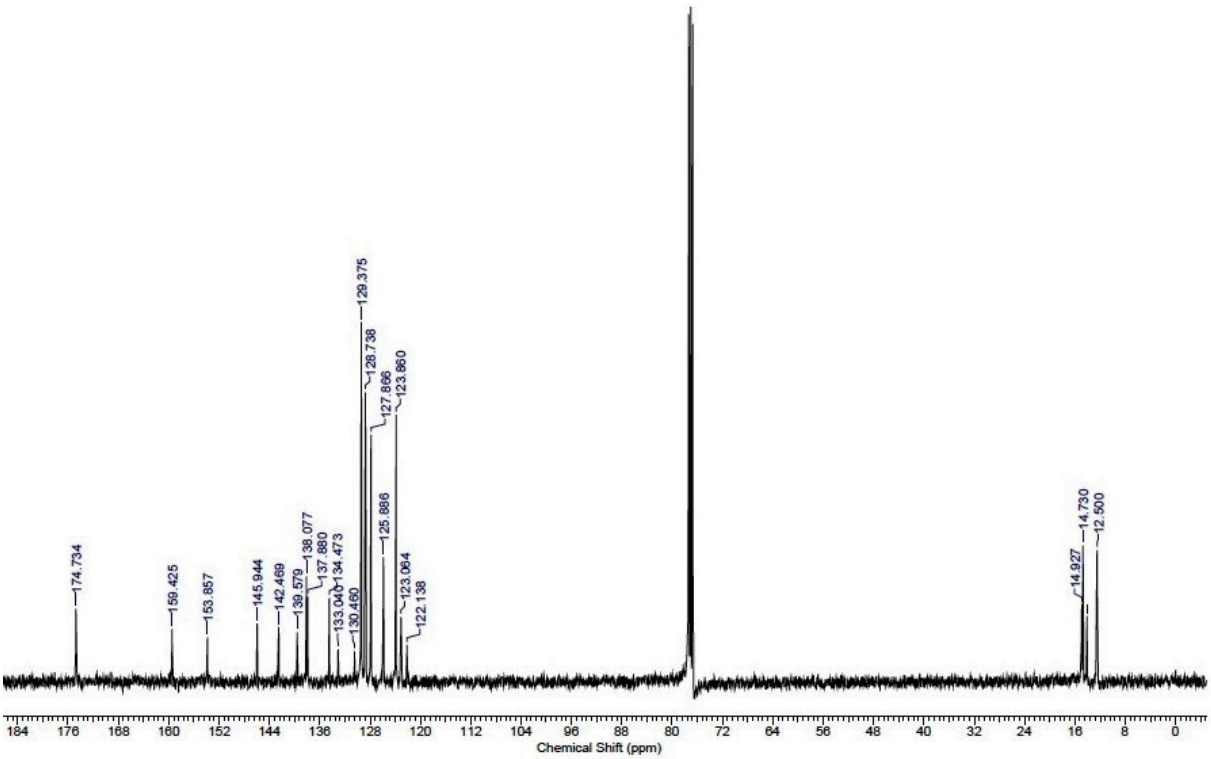


Figure S20: a) Day light photograph image of **BOD-ZN** + Hg(II) (left), **BOD-ZN** (right), **(b)** fluoresccence image of **BOD-ZN** + Hg(II) (left), **BOD-ZN** (right)

24. ^{1}H and ^{13}C NMR spectra





25. HRMS spectra of BOD-ZN in the absence and presence of Hg^{2+} ion