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Supplementary Information

A Reusable Ratiometric Two-Photon Chemodosimeter for Hg^{2+} based on ESIPT and Its Application in Bioimaging

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Contents

1. Preparation of chemodosimeter 2

2-(benzo[d]oxazol-2-yl)phenol (7)

This compound was synthesized according to the literature procedure [1].

2-(2-(allyloxy)phenyl)benzo[d]oxazole (8)

To a solution of 2-(benzo[d]oxazol-2-yl)phenol **7** (1.34 g, 10.0 mmol) in 30 mL acetone was added 3-bromoprop-1-ene (1.06 mL, 20.0 mmol) and K_2CO_3 (1.75 g, 20.0 mmol). The mixture was stirred for 6 h at reflux temperature and cooled down to room temperature, inorganic salt was filtrated off. Then the crude product was washed with water and CH_2Cl_2 and dried at 60 ˚C to afford **8**. Yield: 1.53 g, (96%). M.p.55.8-57.1 ˚C.

^{1}H NMR (400 MHz, $CDCl_3$). δ 8.14 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.83-7.76 (m, 1H), 7.61-7.54 (m, 1H), 7.47 (ddd, *J* = 8.9, 7.5, 1.7 Hz, 1H), 7.37-7.30 (m, 2H), 7.12-7.02 (m, 2H), 6.11 (ddt, *J* = 17.2, 10.6, 4.7 Hz, 1H), 5.68-5.28 (m, 2H), 4.74 (dt, *J* = 4.3, 1.7 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.31, 156.72, 149.20, 140.06, 136.49, 136.45, 133.84, 128.52, 125.39, 125.32, 125.04, 119.33, 119.25, 116.01, 110.73, 110.20, 34.19. ESI-MS *m/z* [$(M + H)^+$]: 251.7.

2-allyl-6-(benzo[d]oxazol-2-yl)phenol (2)

8 (1.50 g, 7.5 mmol) was dissolved in N-methylpyrrolidone (10 mL) and refluxed for 2.5 h at 220 ˚C under Ar. After evaporation of the solvent, the product was purified with silica gel chromatography, eluted with petroleum ether to afford **2**. Yield: 1.23 g, (82%). m.p.83.6-84.2 ˚C.

^{1}H NMR (400 MHz, $CDCl_3$). δ 11.75 (s, 1H), 7.91 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.71

(ddd, J = 6.1, 3.2, 0.6 Hz, 1H), 7.60 (ddd, J = 5.9, 3.2, 0.6 Hz, 1H), 7.37 (dd, J = 6.0, 3.2 Hz, 2H), 7.33-7.29 (m, 1H), 6.95 (t, J = 7.7 Hz, 1H), 6.13-6.01 (m, 1H), 5.16-5.07 (m, 2H), 3.52 (d, J = 6.6 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.31, 156.72, 149.20, 140.06, 136.49, 136.45, 133.84, 128.52, 125.39, 125.32, 125.04, 119.33, 119.25, 116.01, 110.73, 110.20, 34.19. ESI-MS *m/z* [$(M + H)^+$]: 251.8.

2. Fluorescence spectra

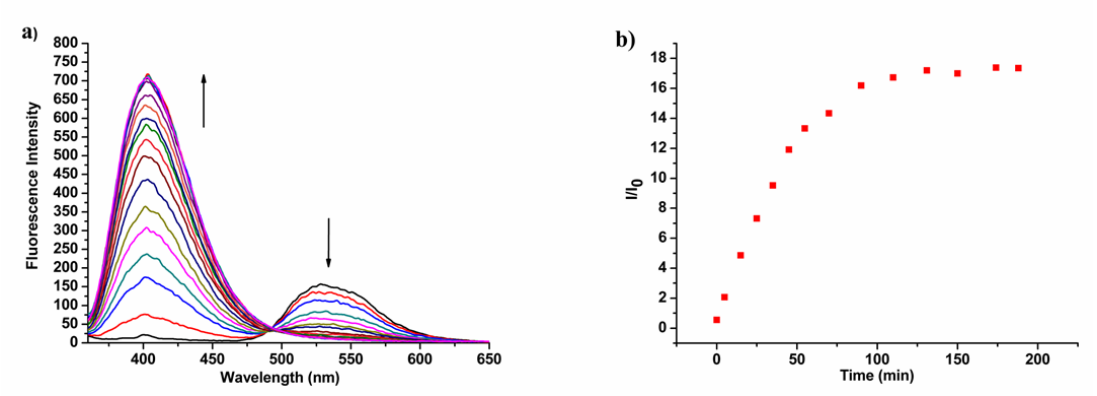

Fig. S1. a) The time-dependent fluorescence change acquired for a mixture of 10 uM **1** and 10 uM $HgCl_2$ in aqueous solution; λ_{ex} = 350 nm. Slit: 2.5 nm/2.5 nm. b) Plot of the fluorescence intensity ratio changes as a function of the reaction time.

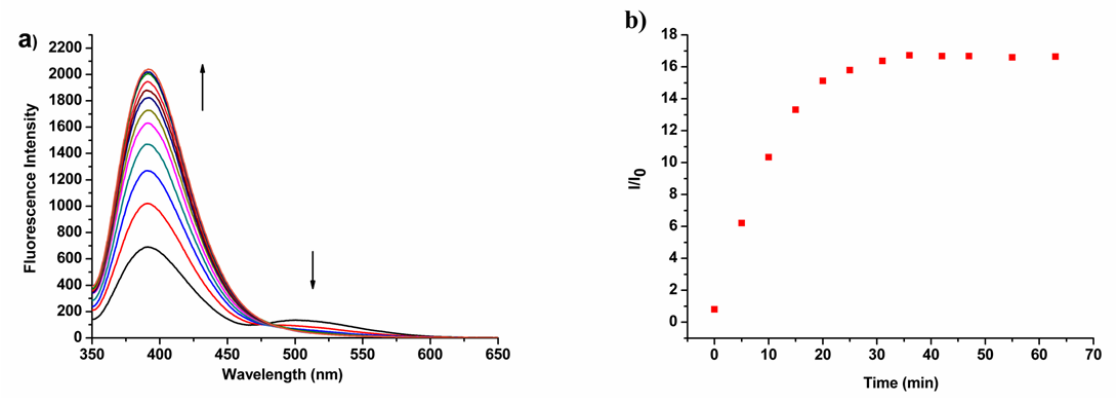

Fig. S2. a) The time-dependent fluorescence change acquired for a mixture of 10 uM **2** and 10 uM $HgCl_2$ in aqueous solution; λ_{ex} = 340 nm. Slit: 2.5 nm/2.5 nm. b) Plot of the fluorescence intensity ratio changes as a function of the reaction time.

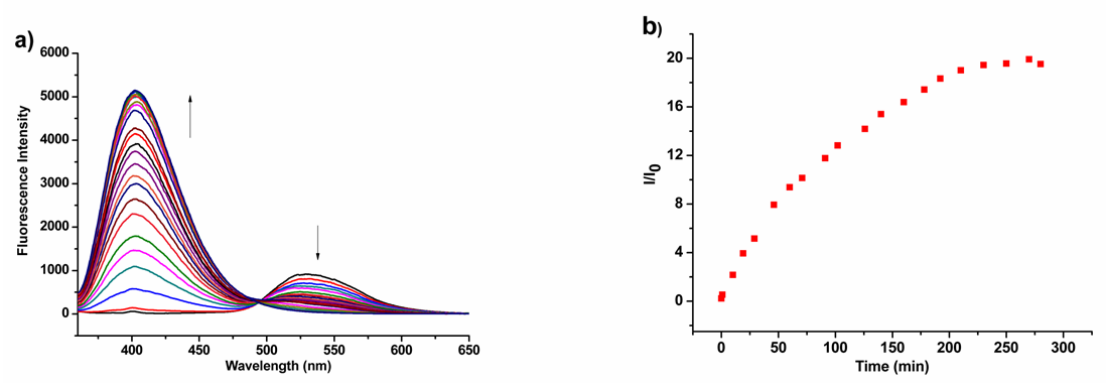

Fig. S3. a) The time-dependent fluorescence change acquired for a mixture of 10 uM **1** and 10 uM $HgCl_2$ in PBS buffer solution (pH = 7.4, containing 0.5% CH_3CN); λ_{ex} = 350 nm. Slit: 2.5 nm/5.0 nm. b) Plot of the fluorescence intensity ratio changes as a function of the reaction time.

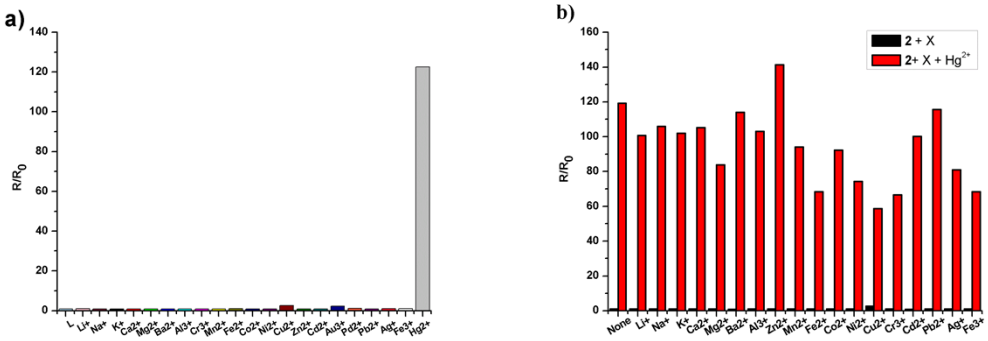

Fig. S4. a) Fluorescence spectra of **2** in the absence and presence of different metal ions Li^+, Na^+, K^+, Ca^{2+}, Mg^{2+}, Ba^{2+}, Al^{3+}, Zn^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Cr^{3+}, Cd^{2+}, Pb^{2+}, Ag^+, Fe^{3+} and Hg^{2+} in aqueous solution (containing 0.5% CH_3CN). b) Changes in fluorescence of **2** (10 uM) upon addition of Hg^{2+} (1 equiv.) with various mental ions (1 equiv.). Bars represent the fluorescence intensity ratio in the presence (R) and absence (R_0) of various mental ions. λ_{ex} = 340 nm. Slit: 2.5 nm/2.5 nm; R = I_{390nm}/I_{480nm}. Each spectrum was acquired 30 min after mental ions addition.

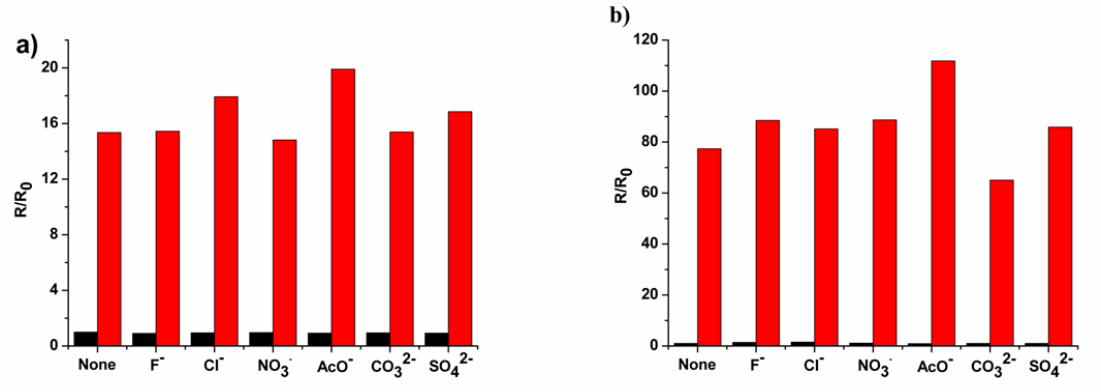

Fig. S5. Fluorescence responses of a) **1**, **1**-Hg^{2+} b) **2**, **2**-Hg^{2+} (10 μM) with various anions (10 μM) : F^-, Cl^-, NO_3^-, AcO^-, CO_3^{2-} and SO_4^{2-} in a) in PBS buffer solution (pH = 7.4, containing 0.5% CH_3CN) b) in aqueous solution (containing 0.5% CH_3CN). Bars represent the fluorescence intensity ratio in the presence (R) and absence (R_0) of various mental ions. a) $R = I_{401nm}/I_{494nm}$ b) $R = I_{390nm}/I_{480nm}$. Each spectrum was acquired 30 min after anions addition.

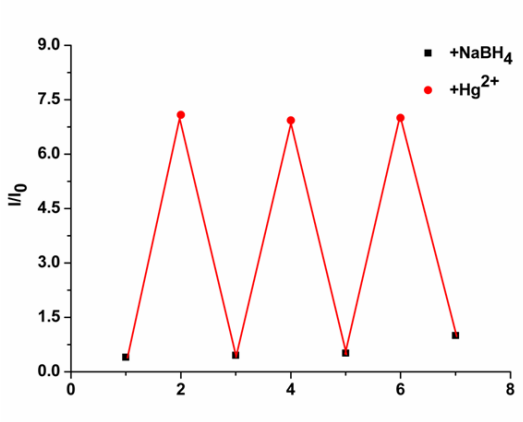

Fig. S6. The reversibility of **2** (10 μM) reacting with Hg^{2+} (10 μM) in aqueous solution (containing 0.5% CH_3CN). The spectrum was acquired 30 min after Hg^{2+} addition.

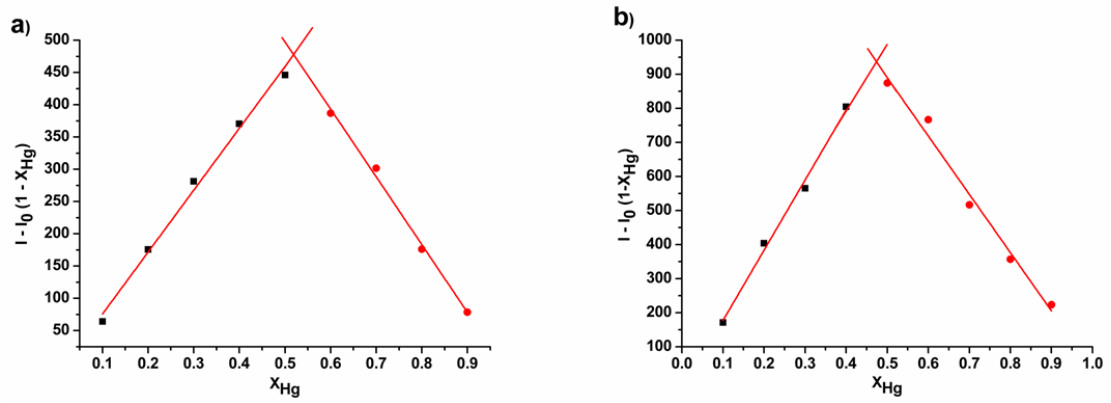

Fig. S7. a) Job's plot for determining the stoichiometry of **1** and Hg^{2+} in PBS buffer solution (pH = 7.4, containing 0.5% CH_3CN); the total concentration of **1** and Hg^{2+} was 10 μM; X_{Hg} = $[Hg^{2+}]/([Hg^{2+}]+$ [**1**]. b) Job's plot for determining the stoichiometry of **2** and Hg^{2+} in aqueous solutions; the total concentration of **2** and Hg^{2+} was 10 μM; X_{Hg} = $[Hg^{2+}]/([Hg^{2+}]+$ [**2**]. Each spectrum was acquired 30 min after Hg^{2+} addition.

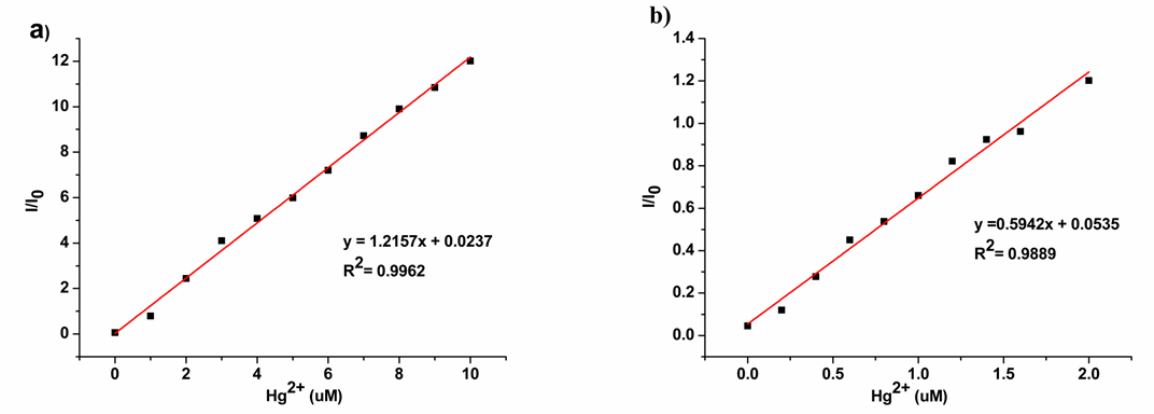

Fig. S8. a) The linear relationship of **2** between the concentration of Hg^{2+} within the range 0-10 μM and the fluorescence intensity ratios at 390 nm and 480 nm (I_{390nm}/I_{480nm}) in aqueous solution (containing 0.5% CH_3CN). b) The linear relationship of **2** between Hg^{2+} concentration within the range 0-2 μM and the flurescence intensity ratios (I_{390nm}/I_{480nm}) in drinking water.

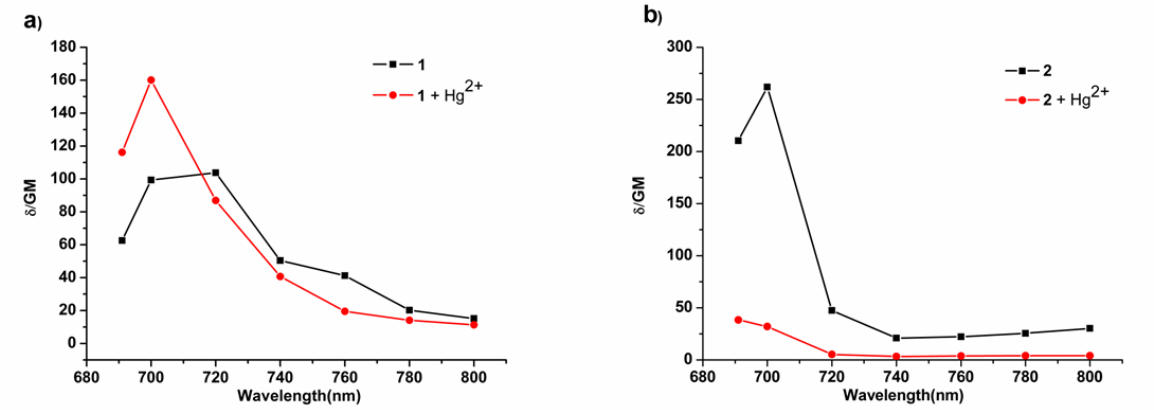

Fig. S9. a) Two-photon excitation spectra of **1** (10 μM) with and without Hg^{2+} (10 μM) in aqueous solutions. b) Two-photon excitation spectra of **2** (10 μM) with and without Hg^{2+} (10 μM) in aqueous solutions.

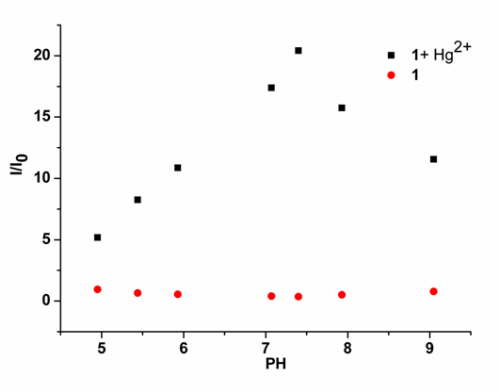

Fig. S10. The fluorescence intensity ratios (I_{401nm}/I_{494nm}) of 10 μM **1**, **1**-Hg^{2+} as a function of pH in PBS buffer solution (pH = 7.4, containing 0.5% CH_3CN). Each spectrum was acquired 30 min after **1**, **1**-Hg^{2+}addition.

3. TP Bioimaging

3.1. Cell cytotoxicity

The cytotoxic effect of **1** and **1**−Hg complex was determined by an MTT assay following the manufacturer instruction (Sigma-Aldrich, MO). HeLa cells were initially propagated in a 25 cm^2 tissue culture flask in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS), penicillin (100 μg/mL), and streptomycin (100 μg/mL) in a CO_2 incubator. For cytotoxicity assay, cells were seeded into 96-well plates (approximately 104 cells per well), and various concentrations of **1** and **1**−Hg complex (10, 20, 30, 50 and 100 μM) made in DMEM were added to the cells and incubated for 24 h. Solvent control samples (cells treated with CH_3CN alone) and cells treated with 10μM $HgCl_2$ alone were also included in parallel sets. Following incubation, the growth media was removed, and fresh DMEM containing MTT solution was added. The plate was incubated for 2−3 h at 37 ˚C. Subsequently, the supernatant was removed, the insoluble colored formazan product was solubilized in CH_3CN, and its absorbance was measured in a microtiter plate reader (Infinite M200, TECAN, Switzerland) at 550 nm. The assay was performed in six sets for each concentration of **1** and **1**−Hg complex. Data analysis and calculation of standard deviation was performed with Microsoft Excel 2010 (Microsoft Corporation). For statistical analysis, a one way analysis of variance (ANOVA) was performed using Sigma plot.

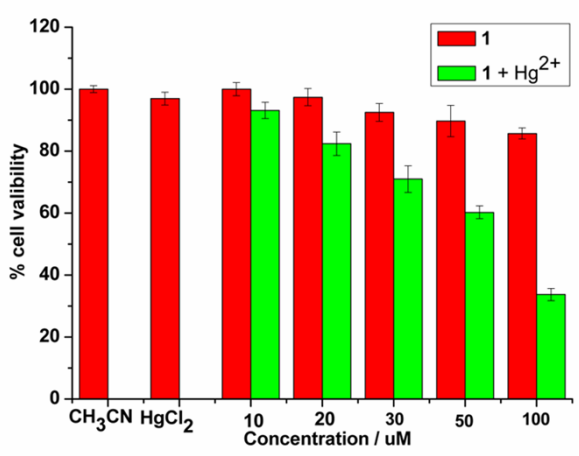

Fig. S11. MTT assay to determine the cytotoxic effect of **1** and **1**−Hg^{2+} complex on HeLa cells. Statistically significant values derived by ANOVA are indicated by bar marks.

3.2. Cell culture and concentration gradient experiment.

HeLa cells were procured from the biomedical engineering center of Lanzhou University (Lanzhou, China). The cells were propagated in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum, penicillin (100 μg/mL), and streptomycin (100 μg/mL). Cells were maintained under a humidified atmosphere of 5% CO_2 and at 37 ˚C incubator as mentioned before. For cell imaging studies, cells were seeded into a confocal dish and incubated at 37 °C in a CO_2 incubator for one day. Then the cells were washed three times with PBS buffer (pH = 7.4) and divided into several groups. For group one cells were washed three times with phosphate buffered saline (pH = 7.4) and incubated with 10 μM **1** in DMEM at 37 ˚C for 30 minutes in a CO_2 incubator and observed under Olympus FV1000 laser confocal microscope IX81. For group two cells incubated with 10 μM **1** were again washed thrice with PBS (pH = 7.4) to remove the free probe, and then incubated with 10 μM $HgCl_2$ for 60 minutes. Again, images were taken using Olympus FV1000 laser confocal microscope IX81. Then, fluorescence microscopic images were acquired. Also the concentration based imaging detected as the previous procedure.

3.3 Tissue culture and imaging.

Tissue slices were prepared from HeLa cancer cells. A total of 2×10^6 Hela cancer cells diluted in 100 μL of serum-free PBS medium were injected subcutaneously into the right flank of 6- to 8-week-old BALB/c-nude mice to inoculate tumors. After HeLa cancer cells inoculation was for 15 days, mice were sacrificed. Tumors were transferred and embedded with O.C.T (Sakura Finetek, USA, Torrance, CA) for frozen sections. The tissues were cut into 500 μm- thick slices using a vibrating-blade microtome. Slices were incubated with 50 μM of probe **1** for 12 h at 4 ˚C. After washing with PBS for three times, the slices were mounted with 10% glycerol and sealed with nail varnish on a glass substrate.

4. Determination of the detection limit

The detection limit was calculated based on the fluorescence titration. The fluorescence emission spectrum of **1** and **2** was measured by ten times and the standard deviation of blank measurement was achieved. To gain the slope, the fluorescence intensity ratios I_{401nm}/I_{494nm} and I_{390nm}/I_{480nm} were plotted as a concentration of Hg^{2+}. So the detection limits of **1** and **2** were calculated with the following equation:

Detection limit = $3\sigma/k$

Where σ is the standard deviation of blank measurement, k is the slope between the fluorescence intensity versus Hg^{2+}.

5. NMR and ESI-mass Data

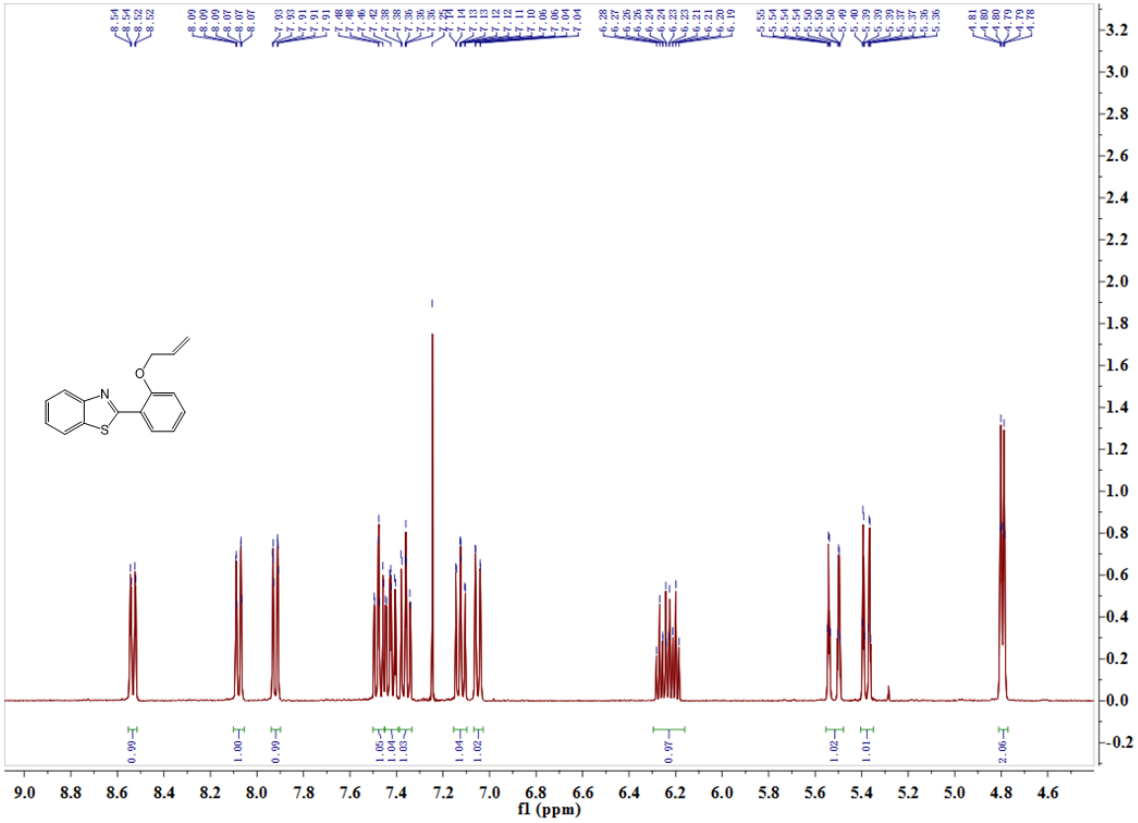

Fig. S12 ^{1}H NMR spectrum of 6 ($CDCl_3$)

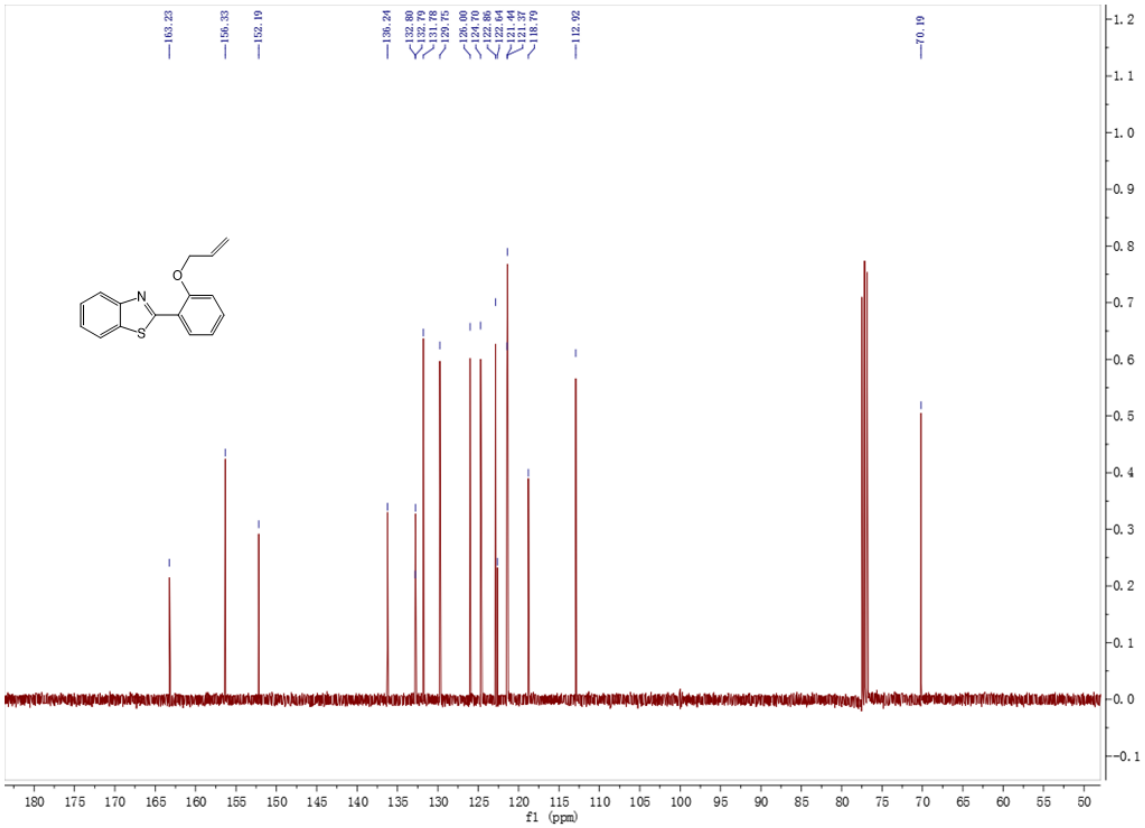

Fig. S13 ^{13}C NMR spectrum of 6 ($CDCl_3$)

Fig. S14 ESI-mass spectrum of 6

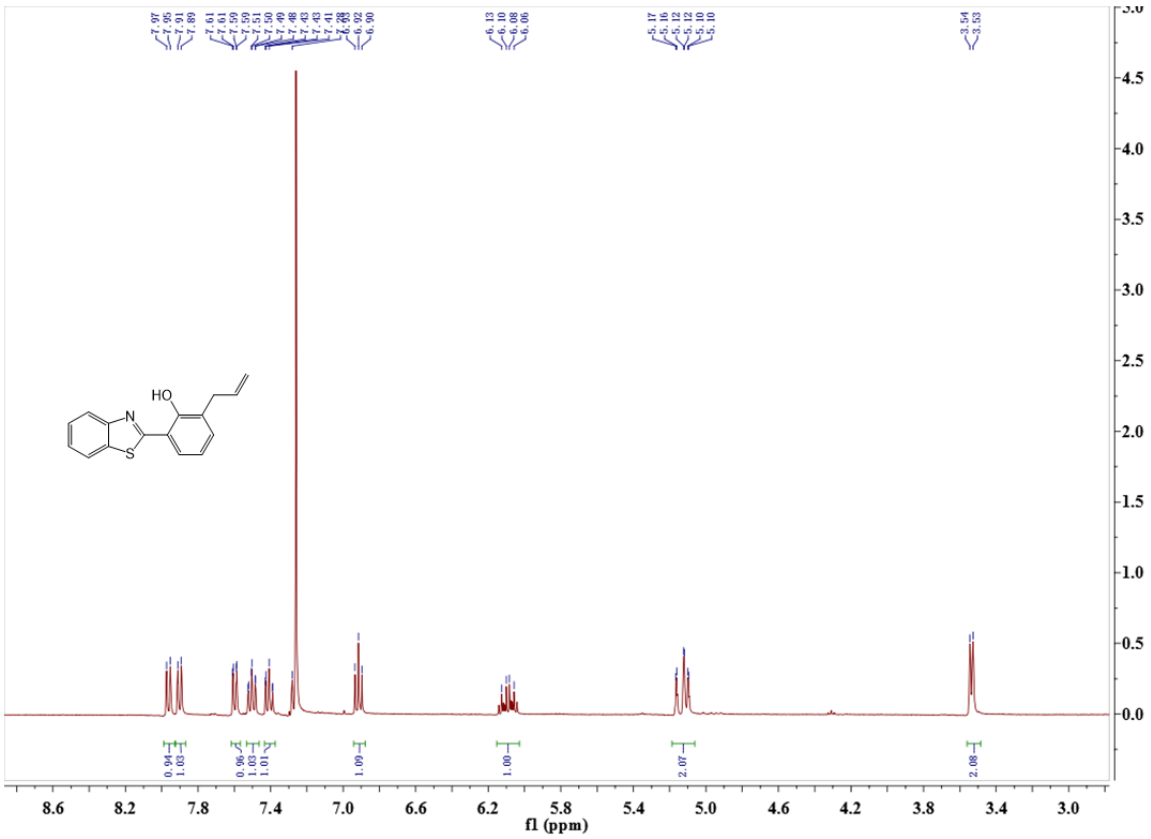

Fig. S15 ^{1}H NMR spectrum of **1** ($CDCl_3$)

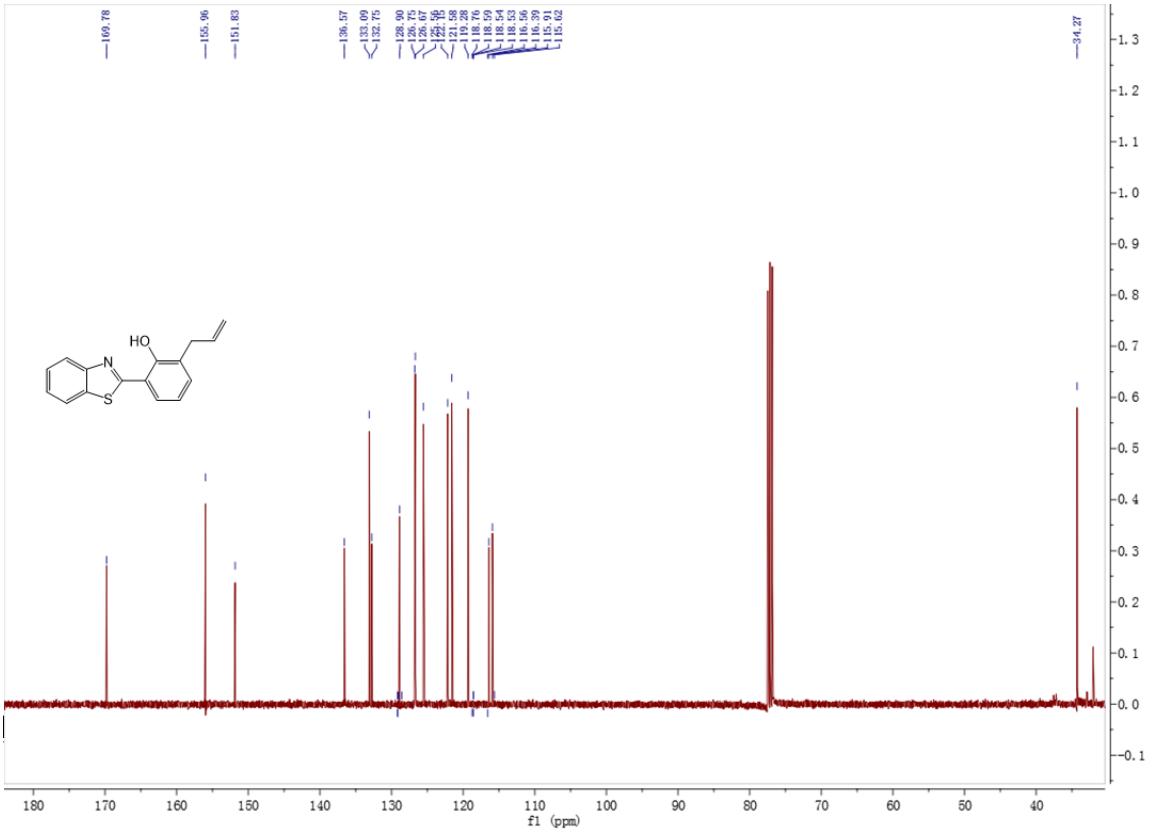

Fig. S16 ^{13}C NMR spectrum of **1** ($CDCl_3$)

Fig. S17 ESI-mass spectrum of **1**

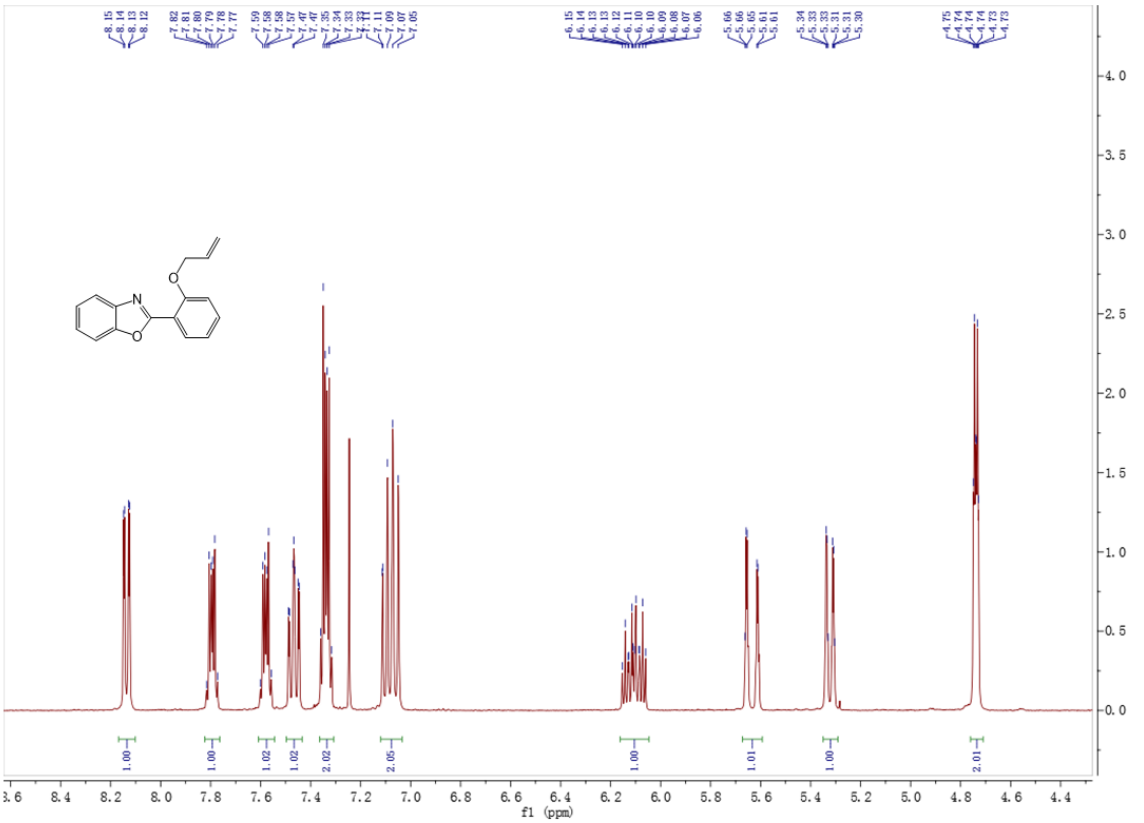

Fig. S18 ^{1}H NMR spectrum of 8 ($CDCl_3$)

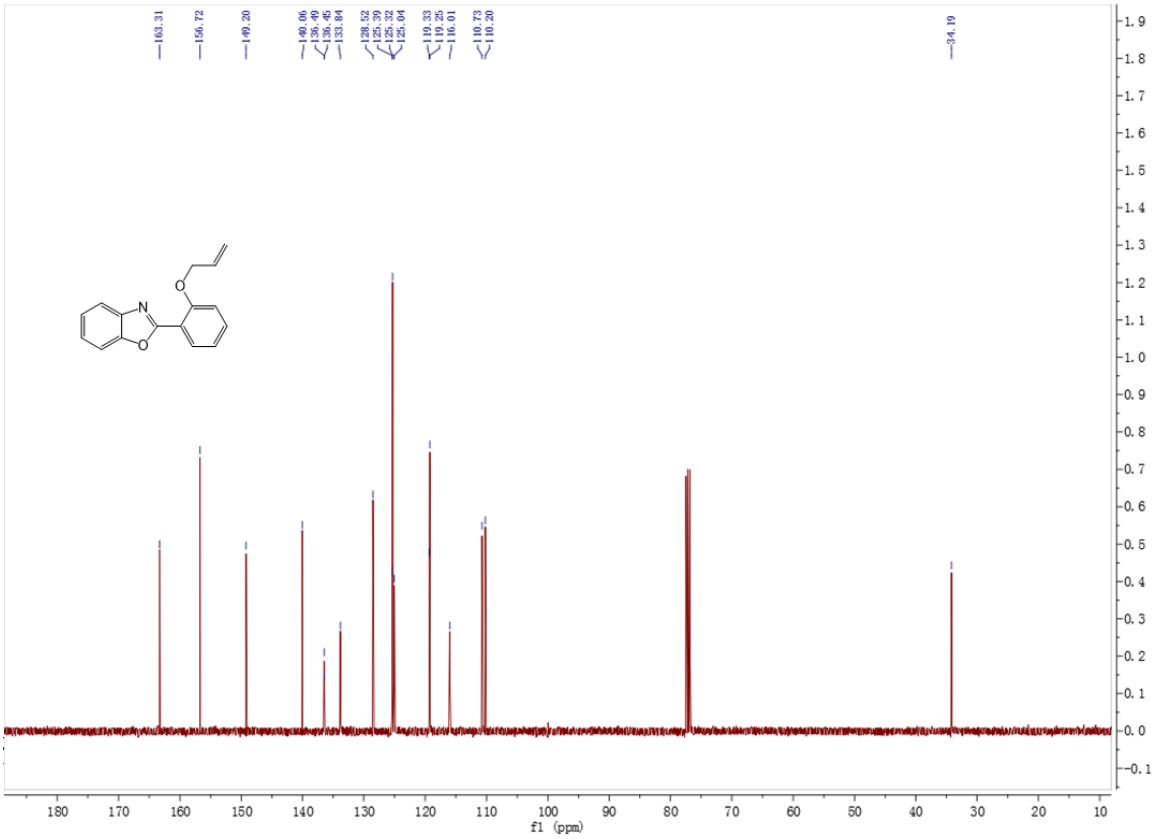

Fig. S19 ^{13}C NMR spectrum of 8 ($CDCl_3$)

Fig. S20 ESI-mass spectrum of 8

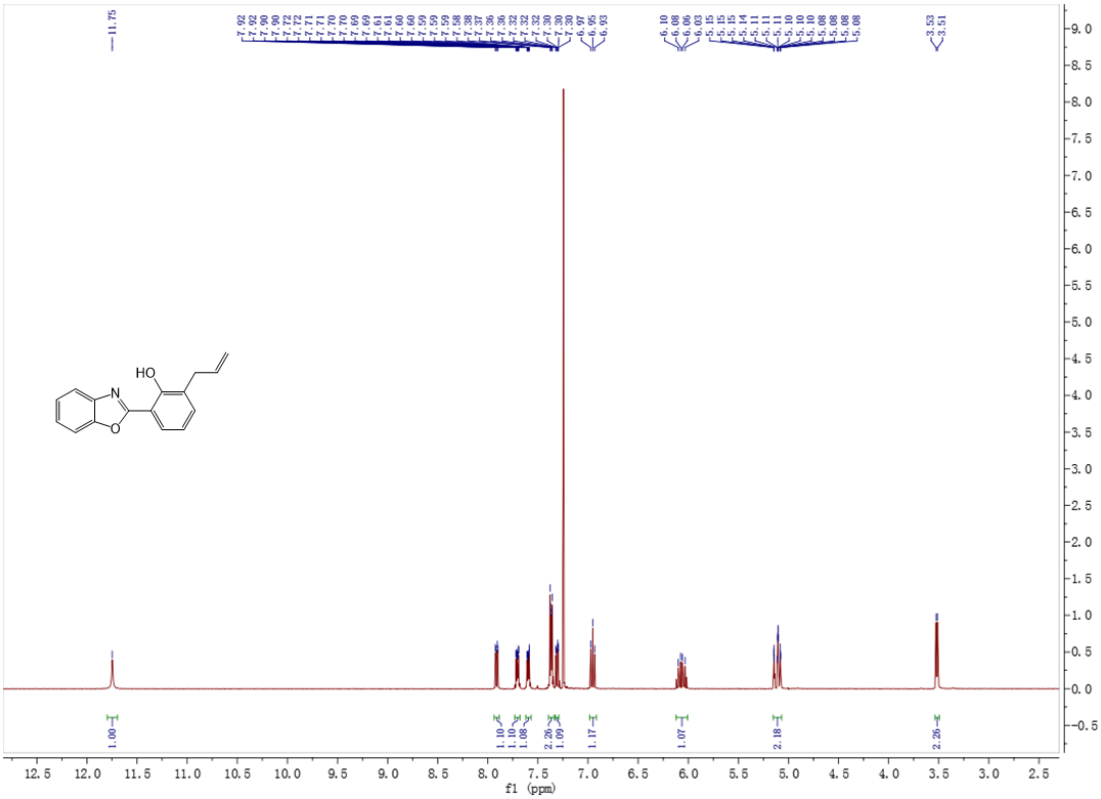

Fig. S21 ^{1}H NMR spectrum of **2** ($CDCl_3$)

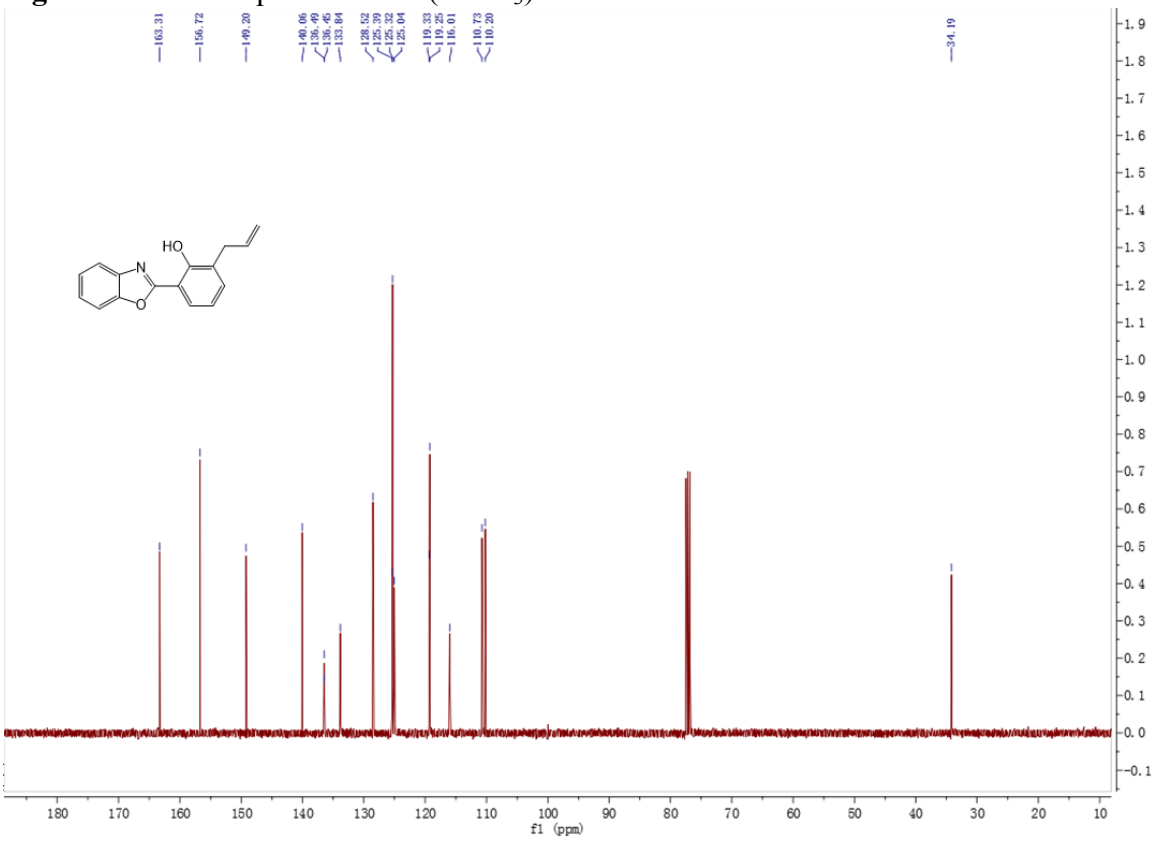

Fig. S22 ^{13}C NMR spectrum of **2** ($CDCl_3$)

Fig. S23 ESI-mass spectrum of **2**

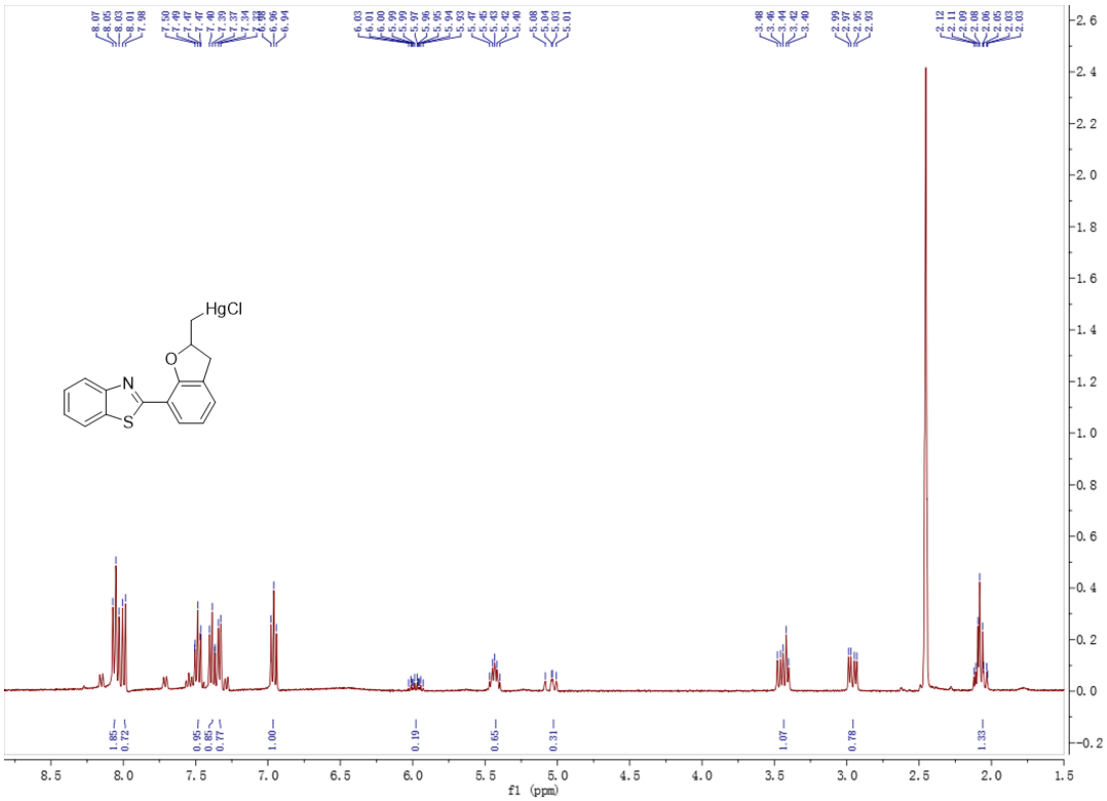

Fig. S24 ^{1}H NMR spectrum of **1-Hg^{2+}** (d_6-DMSO)

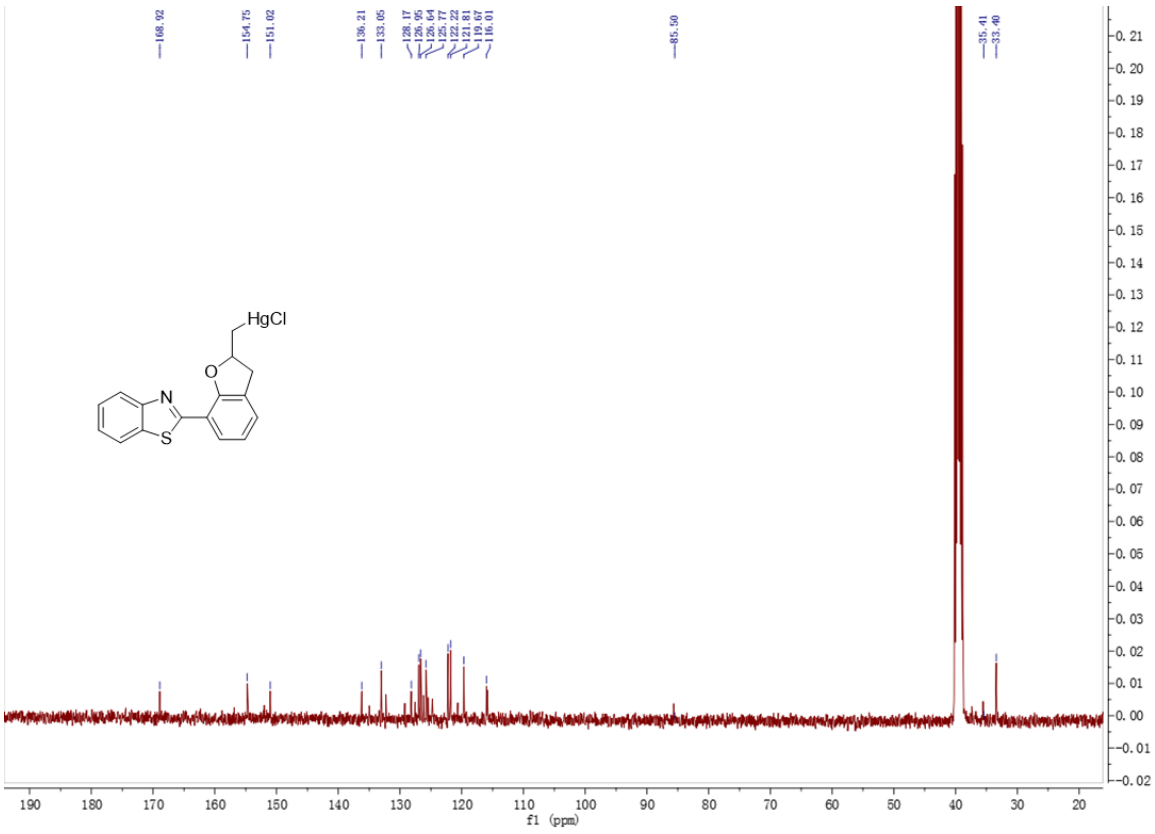

Fig. S25 ^{13}C NMR spectrum of **1-Hg^{2+}** (d_6-DMSO)

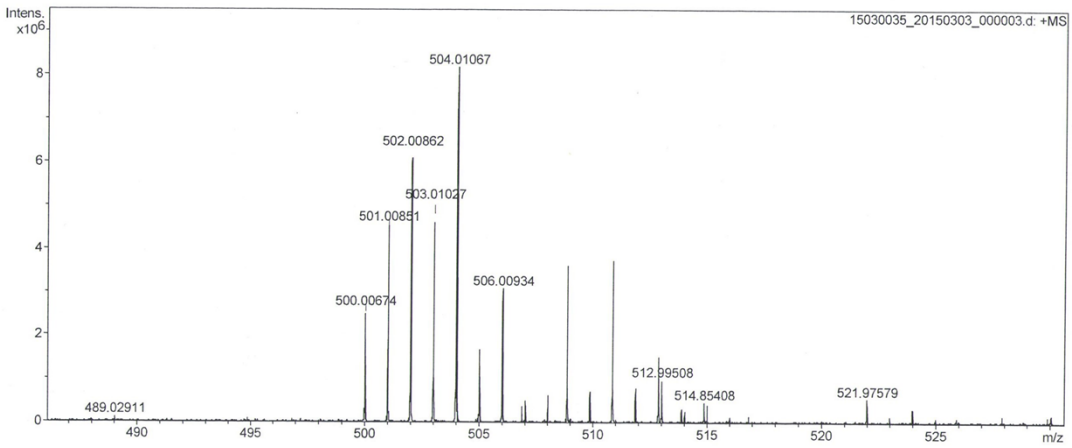

Fig. S26 HRMS spectrum of **1-Hg^{2+}**

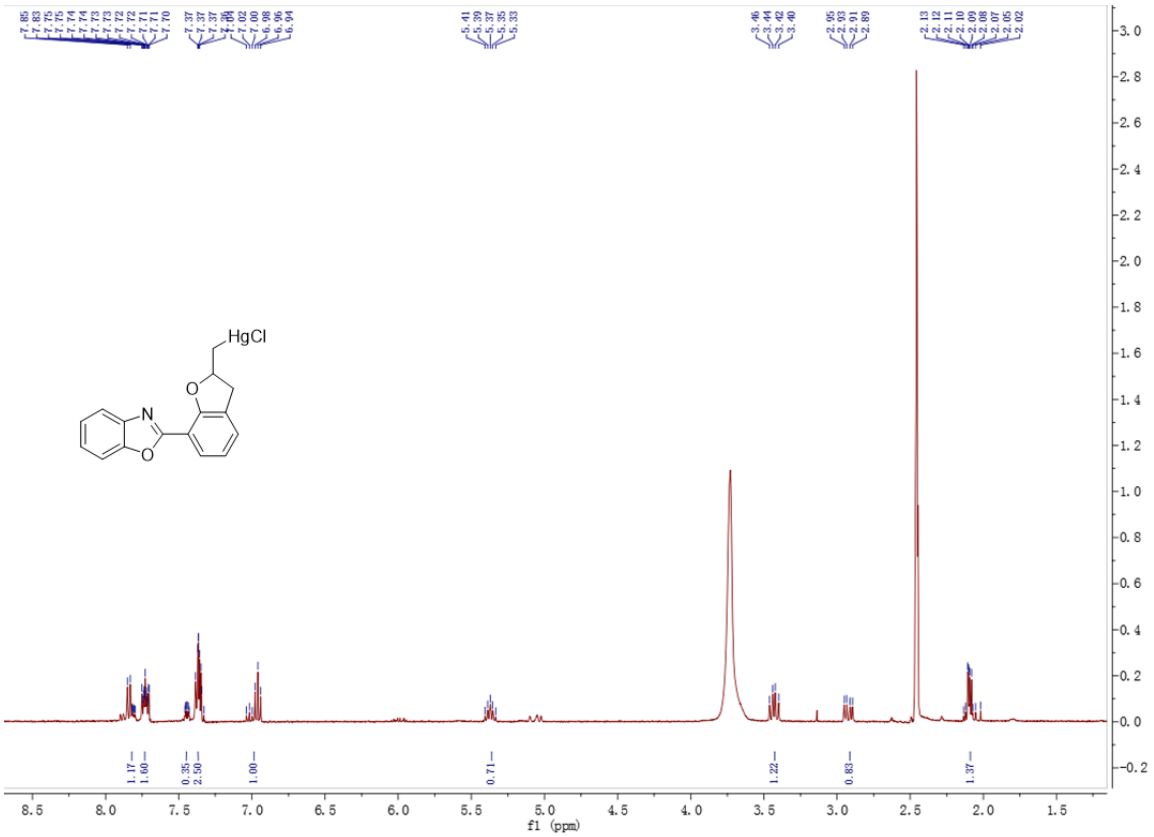

Fig. S27 ^{1}H NMR spectrum of **2-Hg^{2+}** (d_6-DMSO)

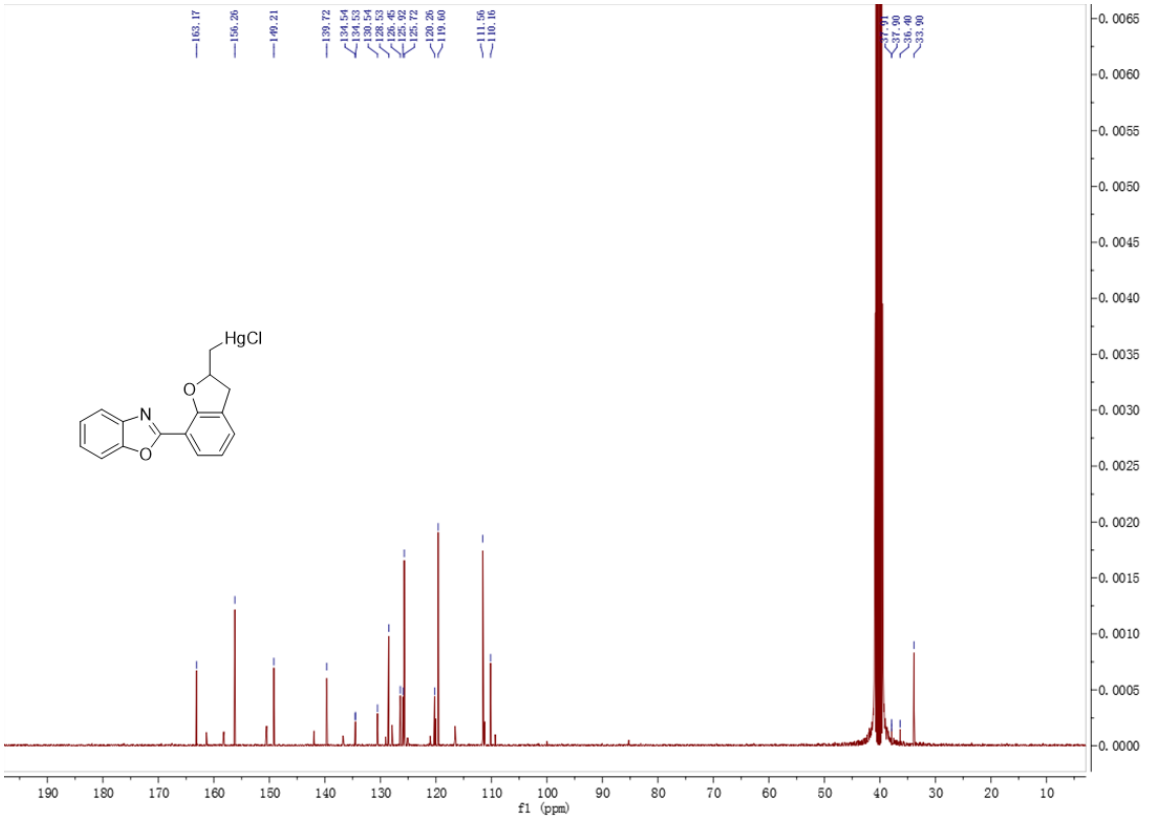

Fig. S28 ^{13}C NMR spectrum of **2-Hg^{2+}** (d_6-DMSO)

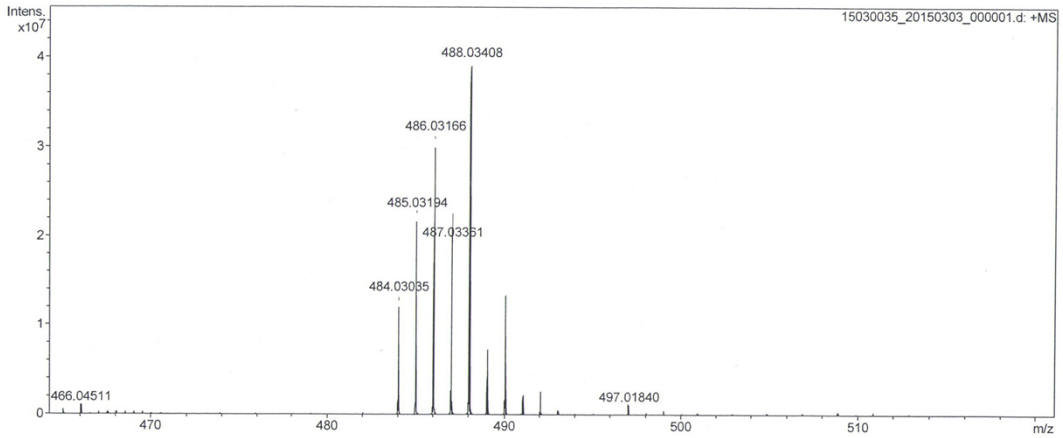

Fig. S29 HRMS spectrum of **2-Hg^{2+}**

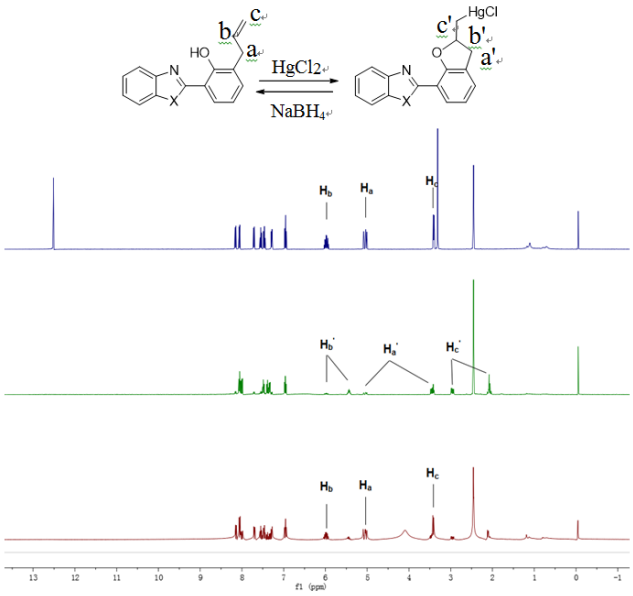

Fig. S30. ^{1}H NMR spectra of **1**, **1**-$HgCl_2$ (1.0 equiv.) and product of **1**-$HgCl_2$ with $NaBH_4$ in DMSO-d_6, X = S.

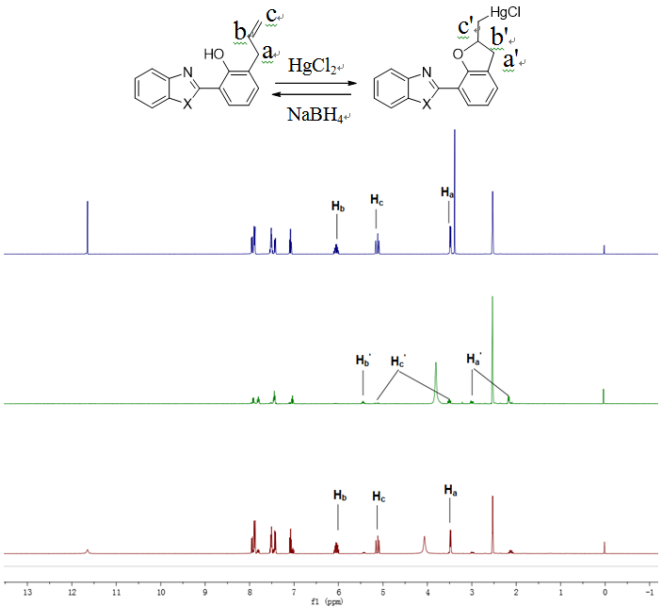

Fig. S31. ^{1}H NMR spectra of **2**, **2**-$HgCl_2$ (1.0 equiv.) and product of **2**-$HgCl_2$ with $NaBH_4$ in DMSO-d_6, X = O.

References:

[1] F. S. Rodembusch, L. F. Campo, F. P. Leusinn and V. *J.* Stefani, *Lumin.*, 2007, **126**, 728-734.