Supporting Information for

Single fluorescent probe for reversible detecting copper ions and cysteine in pure water system

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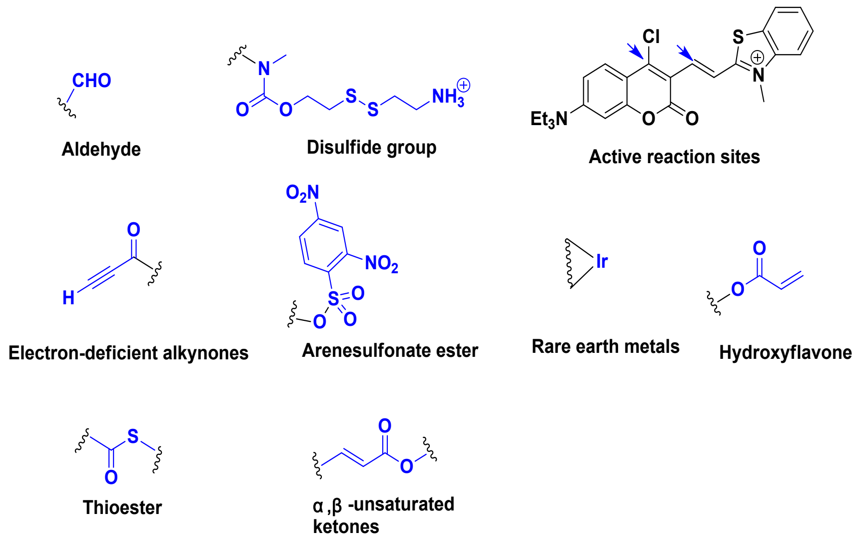
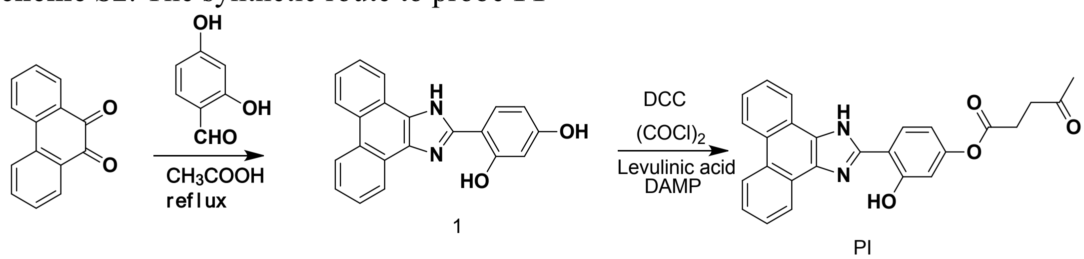
Several recognition cys sites



Scheme S1. Several recognition Cys sites of previous reported

Synthesis

Scheme S2. The synthetic route to probe **PI**



Synthesis 4-(1H-phenanthro[9,10-d] imidazol-2-yl)benzene-1,3-diol (1):

A mixture of 2,4-dihydroxybenzaldehyde(0.28g, 2 mmol), 9,10-phenanthroquinone (0.21 g, 1 mmol), and ammonium acetate (1.54g, 20 mmol) in glacial AcOH (10 mL) was heated to 100 ℃ for 30 min. The hot solution was cooled to room temperature, and the resulting yellow solid was collected by filtration and washed with acetate acid, dilute sodium hydrogen carbonate solution, and water. The white solid was further dried under reduced vacuum, and then purified by silica gel column chromatography using acetone as eluent to afford the pure product. ^{1}H NMR (400 MHz, d_6-DMSO), δ (ppm): 8.79 (d, J = 8.2 Hz, 2H), 8.54 (d, J = 7.9 Hz, 2H), 7.84 – 7.55 (m, 6H), 7.40 (d, J = 0.9 Hz, 1H), 6.96 (s, 1H). ^{13}C NMR (400 MHz, DMSO-*d6*), δ (ppm): 160.31, 162.70, 133.73, 132.18, 130.80, 129.19, 127.55, 123.98, 120.01, 118.81, 115.80.

Synthesis of 3-hydroxy-4-(1H-phenanthro[9,10-d]imidazol-2-yl)phenyl4-oxopentanoate (PI):

A mixture of **1 (**0.20g, 0.6 mmol), levulinic acid (0.17g, 1.5 mmol), DCC (0.24g, 1.2

mmol), and DAMP (0.007g, 0.06mmol) in dichloromethane (30mL) at 25 ℃ for 8h.

The organic phase was washed with water and saturated brine, dried over anhydrous magnesium sulfate overnight. After CH_2C1_2 was removed, the crude product was purified by column chromatography with dichloromethane / methanol (20:1) as eluent, finally the blue solid was obtained for **PI** with a yield of 78%.^{1}H NMR (400 MHz, *d_6-DMSO*), δ (ppm): 1H NMR (400 MHz, DMSO) δ 13.75 (s, 1H), 13.45 (s, 1H), 8.91 (s, 2H), 8.55 (d, *J* = 21.8 Hz, 2H), 8.28 (d, *J* = 8.5 Hz, 1H), 7.90 – 7.52 (m, 4H), 7.02 – 6.66 (m, 2H), 2.89 (t, *J* = 6.3 Hz, 2H), 2.76 (dd, *J* = 13.4, 7.3 Hz, 2H), 2.18 (s, 3H), 2.09 (s, 1H). ^{13}C NMR (400 MHz, DMSO-*d6*), δ (ppm): ^{13}C NMR (100 MHz, DMSO-*d6*) δ 160.26, 159.17, 158.27, 156.54, 152.33, 148.73, 133.75, 126.51, 112.74, 110.82, 110.30, 107.35, 103.07, 47.42, 37.45, 33.26, 29.44, 27.83, 25.23, 24.36. HRMS (m/z): $[M]^+$ calcd for $C_{26}H_{19}N_2O_4$, 423.1400; found: 423.1467.

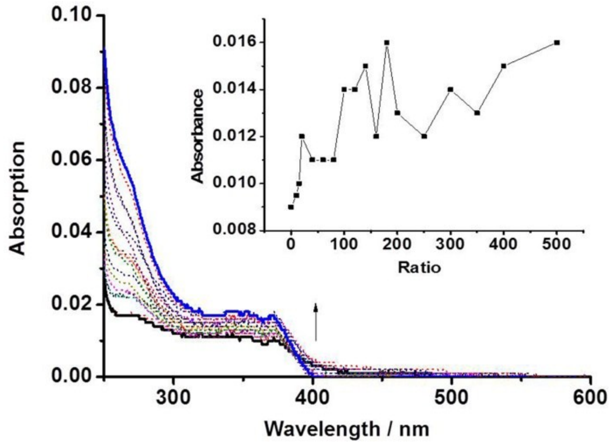


Fig. S1. Absorption spectra of compound **PI** (10 μM) with the increasing concentrations of Cu^{2+} ions (0-500 equiv) in pH 7.4 PBS at around 365 nm; inset, ofchange trend of absorption spectra at around 365 nm.

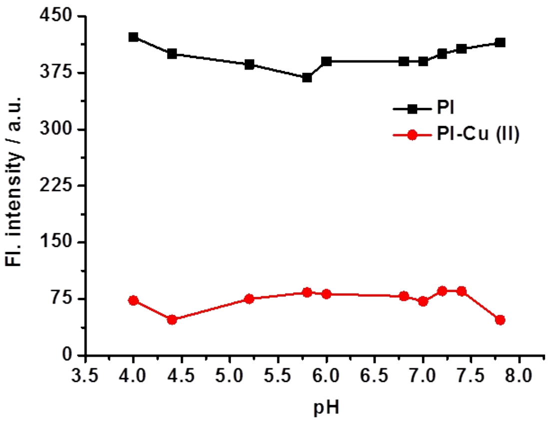


Fig. S2. Fluorescence changes of compound **PI** (10 μM) and **PI-Cu(II)** ensemble (**PI**/Cu(II)=1/250, v:v) at different pH values. Excitation wavelength: 365 nm

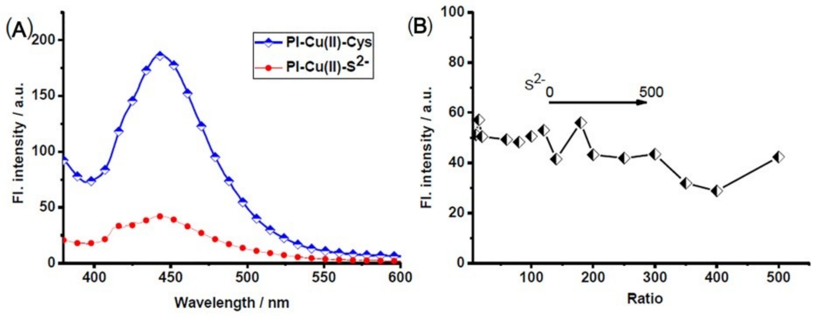


Fig. S3. (A) The **PI-Cu(II)** ensemble of fluorescent response for Cys and sulfur ions ; (B) Fluorescence changes of the **PI-Cu(II)** ensemble with the increasing concentrations of sulfur ions (0-500 equiv) in pH 7.4 PBS.

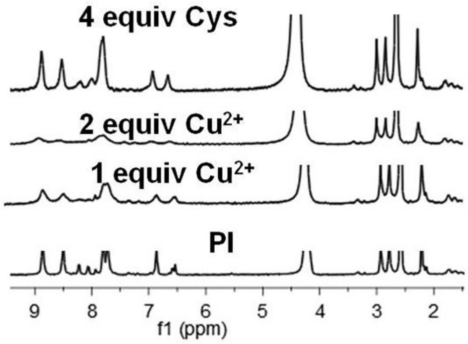


Fig. S4. The [1]H NMR spectrum of the addition of 1-2 equiv of Cu^{2+} ions to dye **PI** and addition of 4 equiv Cys to the ensemble in d_6- DMSO/D_2O (4/1).

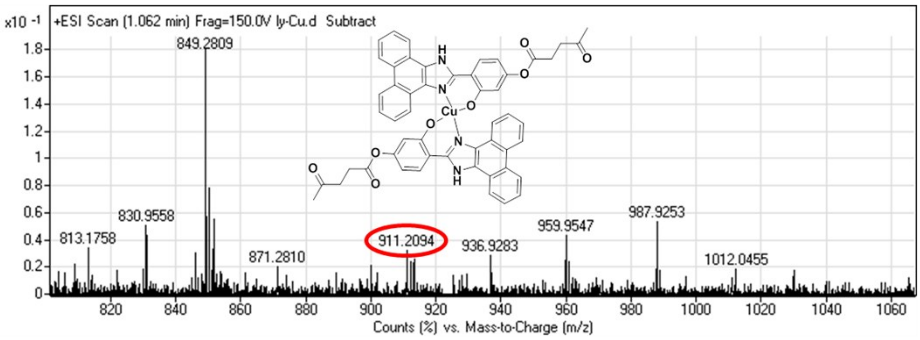


Fig. S5. Intense peak at m/z 911.2 corresponding to (2**PI- Cu(II)** + H)$^+$ is present in the HMRS spectrum.

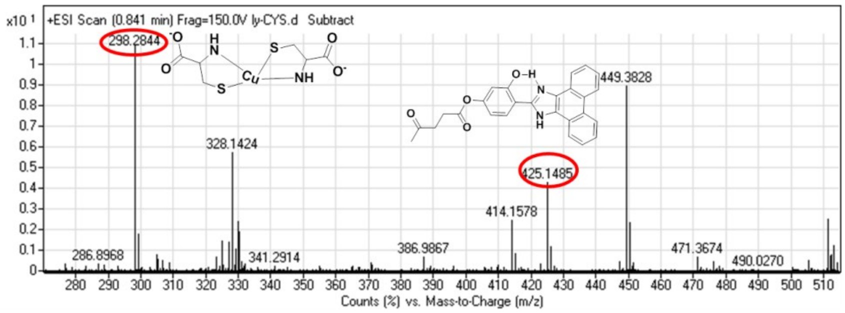


Fig. S6. Intense peak at m/z 298.2 and 425.1 corresponding to (Cys-Cu(II))$^+$ and (**PI**)$^+$ is present in the HMRS spectrum, respectively.

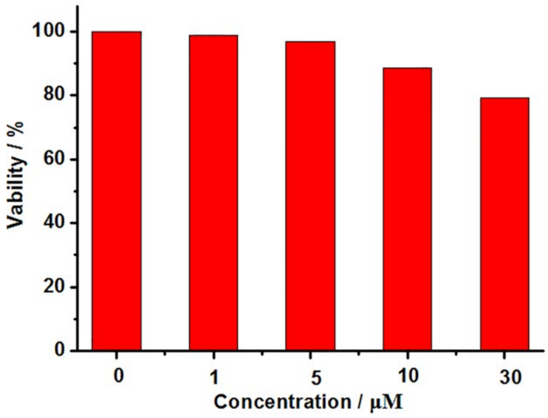


Fig. S7. MTT assay

Spectral Characterization

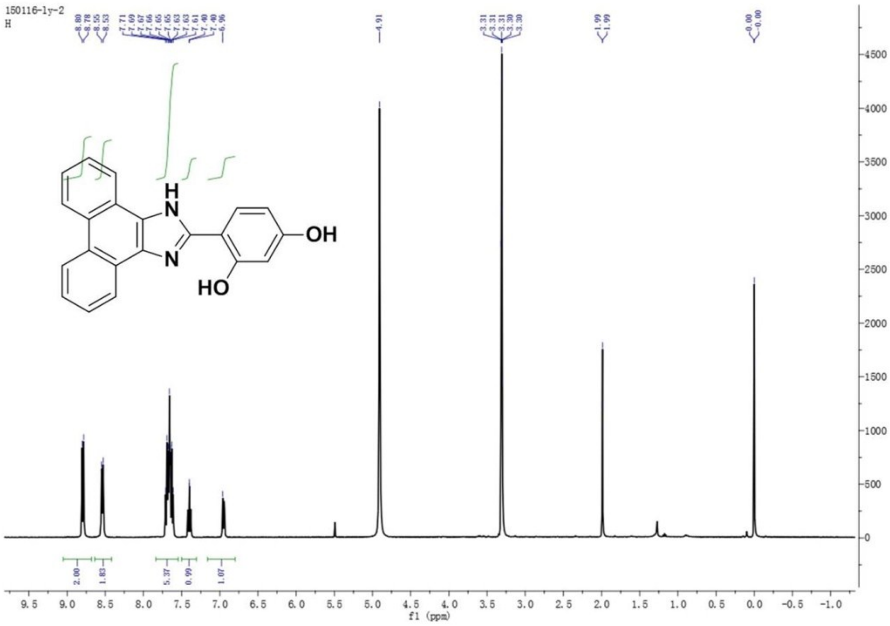


Fig. S8. [1]H NMR spectrum of compound **1** in CD_3Cl.

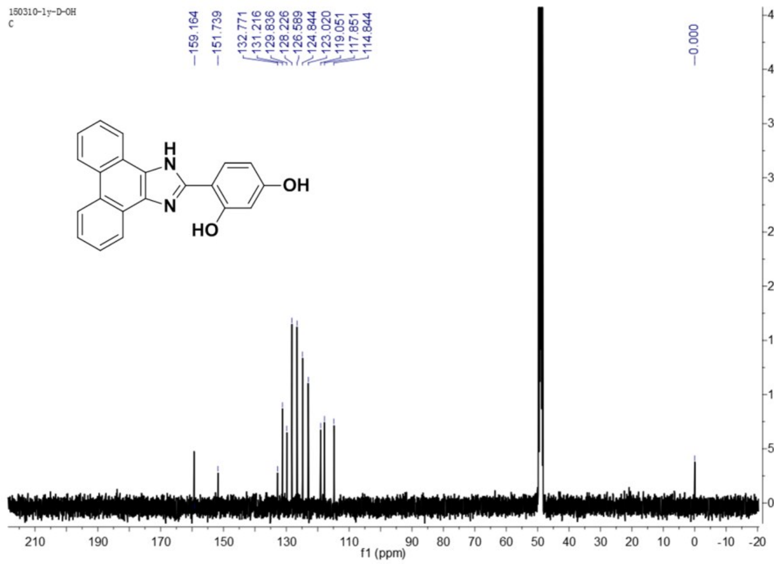


Fig. S9. [13]C NMR spectrum of compound **1** in CD_3Cl.

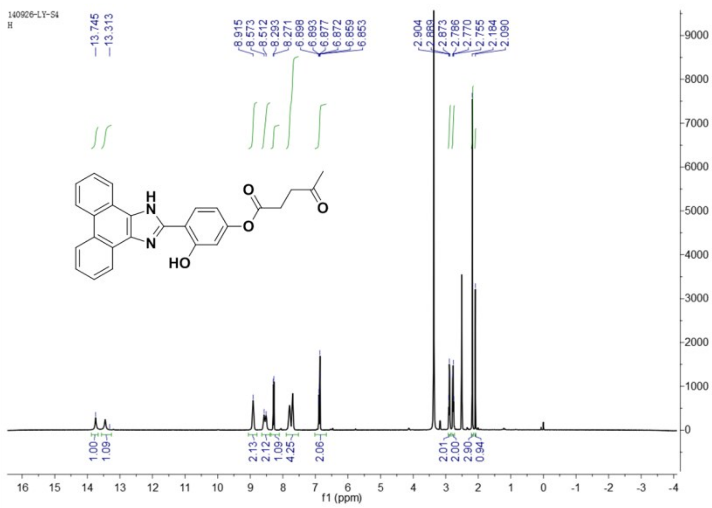


Fig. S10 ^{1}H NMR spectrum of compound **PI** in d_6-DMSO.

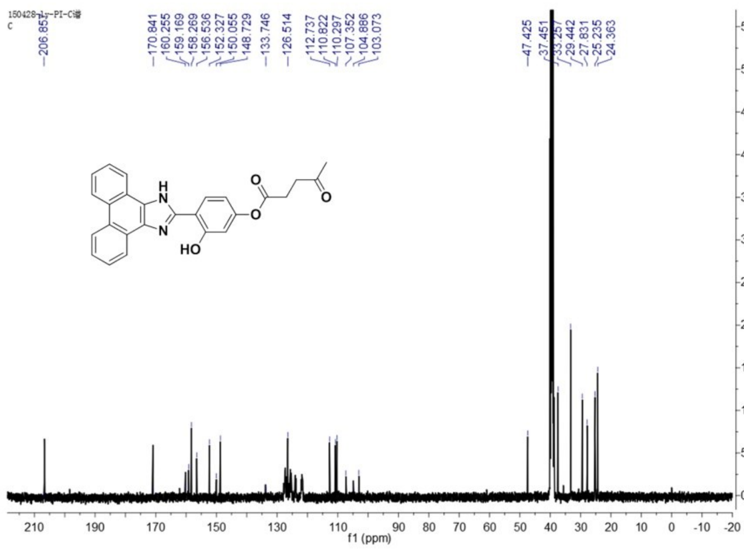


Fig. S11. ^{13}C NMR spectrum of compound **1** in **PI** in d_6-DMSO.

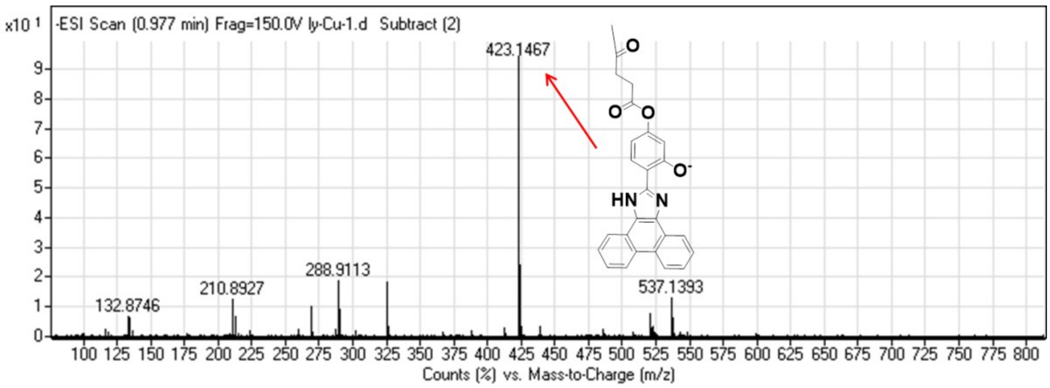


Fig. S12. ESI-MS spectrum of compound **PI**.