



Supporting Information

for

The fluorescence of a mercury probe based on osthol

Guangyan Luo, Zhishu Zeng, Lin Zhang, Zhu Tao and Qianjun Zhang

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General information and descriptions of the methods

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Experimental

Reagents

Osthol (analytical purity $\geq 98\%$) was purchased from Shanghai Yuanye Biochemical Technology Co., Ltd. The water is the secondary re-steaming water. Methanol was analytical pure. Hydrochloric acid (analytical purity $\geq 99.5\%$) was purchased from Tianjin Kaixin Chemical Industry Co., Ltd. Deuterated heavy water (analytical purity $\geq 99.9\%$) was purchased from SAAN Chemical Technology (Shanghai) Co., Ltd.

Mercury nitrate was purchased from Aladdin Ltd. Other metal perchlorates of

Na⁺, K⁺, Al³⁺, Ca²⁺, Fe²⁺, Ba²⁺, Cu²⁺, Ag⁺, Fe³⁺, Cr³⁺, Mn²⁺, Cd²⁺, Ni²⁺, Zn²⁺, Mg²⁺, CO²⁺, and Pb²⁺ were purchased from Aladdin Co., Ltd.

Instruments

A UV-2700 dual-beam UV–vis spectrophotometer (Shimadzu Instruments Co., Ltd.), VARIANCARYE-CLIPSE fluorescence spectrophotometer (Varian, USA), Agilent 6545 Q-TOF LC/MS, PHS-25 digital pH meter (Shanghai INESA Scientific Instrument Co., Ltd.), AKHL-III-08 Eco laboratory ultrapure water machine (Chengdu Eco Water Treatment Equipment Co., Ltd.), FA2204N electronic balance (Shanghai Jinghai Instrument Co., LTD.), Nano ITC isothermal calorimeter (TA company, USA), JNM-ECZ400s MHz NMR system (JEOL), SHA-IIIS constant temperature oscillator (Zhengzhou Great Wall Technology Industry and Trade Co., Ltd.), and 101-1AB electric heating blast drying oven (Tianjin Test Instrument Co., Ltd.) were used.

Methods

OST fluorescence probe selectivity to metal ions

A VARIANCARYE-CLIPSE fluorescence spectrophotometer was used to measure the selectivity to the metal ions. Secondary water was used as the solvent. All the metal ions were prepared as a 0.2 mol · L⁻¹ stock solution. Methanol was used as the solvent to prepare the OST probe reserve solution with a concentration of 1.0 × 10⁻³ mol·L⁻¹. At pH 7.0, a H₂O/CH₃OH 97:3, v/v solution of the OST (3.0 × 10⁻⁵ mol·L⁻¹) was prepared for characterization. To obtain fluorescence spectra, known quantities of ion solutions were added to

the OST. In aqueous solution, the fluorescence spectra were obtained by excitation at 325 nm, with 10 nm emission and excitation bandwidths, and the emission intensity was monitored at 300–540 nm at room temperature [1].

Interference and competitive experiment of metal ions

3 ml of a $3.0 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$ H₂O/CH₃OH 97:3, v/v solution of the OST probe at pH 7 and an equimolar concentration of Hg²⁺ were added into a quartz fluorescence cuvette. Then, added $1.2 \times 10^{-4} \text{ mol}\cdot\text{L}^{-1}$ of various metal ions to the OST–Hg²⁺ system. The excitation wavelength was 325 nm and the slit was 10:10. Fluorescence measurement was carried out under the conditions [2].

OST probe fluorescence titration of mercury

3 mL of a $3.0 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$ solution of the OST probe were added into a quartz fluorescence cuvette with a width of 1 cm, and then $n(\text{Hg}^{2+})/n(\text{OST}) = 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0$, respectively, were added, mixed well, and the fluorescence intensity was detected. The excitation wavelength was 325 nm and the slit was 10:10. Fluorescence measurements were carried out under the conditions found in Reference [3].

¹H NMR and MS spectroscopy

A JEOL JNM-ECZ400s spectrometer was used to record the ¹H NMR spectra at 25 °C. ¹H NMR titration experiments of OST with Hg²⁺ were performed in D₂O/CD₃OD 97:3, v/v. That is, the concentration of OST was set as $5.0 \times 10^{-4} \text{ mol}\cdot\text{L}^{-1}$, and Hg²⁺ was gradually added dropwise to the solution by used of the molar ratio method.

ESITOF mass spectra of the OST–Hg²⁺ complex were recorded on an Agilent 6545 Q-TOF spectrometer at room temperature. For that, a mixed alcohol/water solution of OST–Hg²⁺ was prepared at a concentration of $1.0 \times 10^{-4} \text{ mol}\cdot\text{L}^{-1}$. Then, the solution was filtered and tested by MS.

Impact of the pH value of the solution

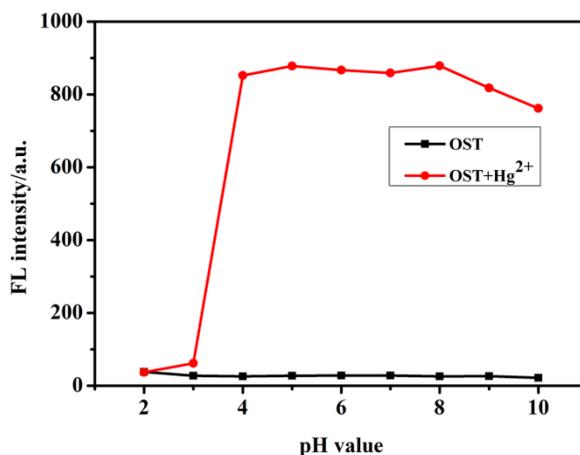


Figure S1: Effects of different pH values on OST and OST–Hg²⁺.

Effect of the solvent

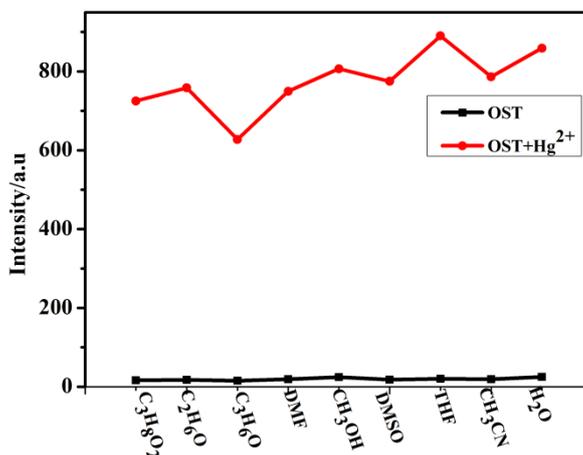


Figure S2: Fluorescence spectra of OST–Hg²⁺ ($3.0 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$) in different solvents.

UV-vis measurements

UV-vis spectra of samples in 1 cm quartz cells were recorded on a UV-2700 double beam UV-vis spectrophotometer. The equimolar continuous change method (Job method) was used to determine the binding ratio. A series of solutions of $n(\text{OST})/n(\text{OST} + \text{Hg}^{2+}) = 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9,$ and 1.0, respectively, was prepared, and the corresponding solutions were used without Hg^{2+} as blank controls. The absorption was measured at room temperature.

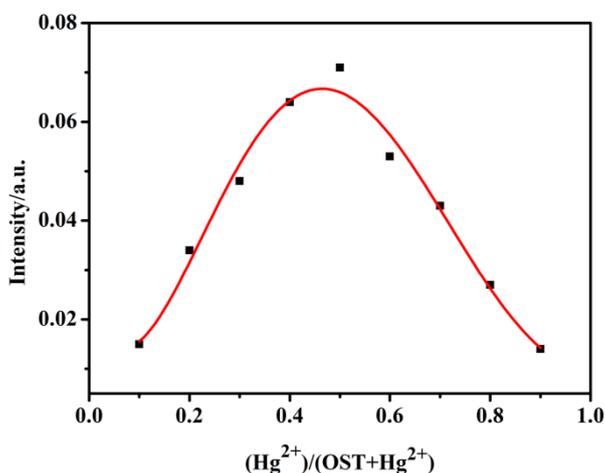


Figure S3: The Job plot data of the probe OST with Hg^{2+} present.

Isothermal titration calorimetry (ITC) measurements [4]

Thermodynamic parameters were determined by the isothermal titration calorimeter Nano ITC (TA, USA). An aqueous solution of Hg^{2+} with a concentration of $2.0 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$ and a solution of OST (pH = 7, containing 1.0% methanol) with a concentration of $1.0 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$ were prepared and mixed well before used. The OST probe solution was titrated with the Hg^{2+}

solution to determine the binding constant. The measurement conditions used on the Nano ITC device were: 25 °C, 6 $\mu\text{L}\cdot\text{d}^{-1}$, the interval time was 250 s, and the stirring speed was 250 $\text{r}\cdot\text{min}^{-1}$.

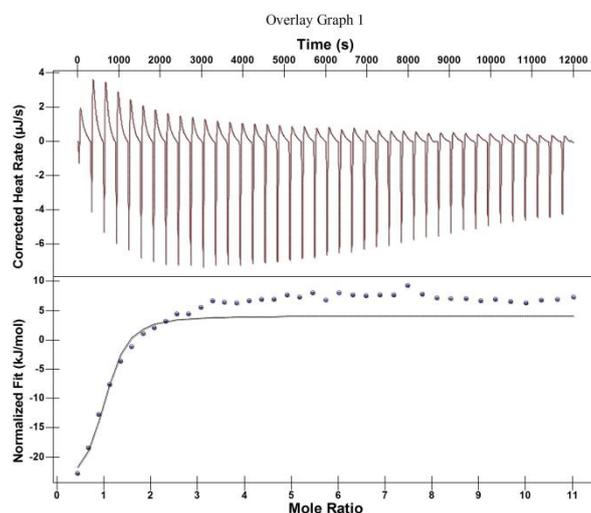


Figure S4: The interaction between the OST probe and Hg^{2+} .

Table S1: Thermodynamic binding data for OST– Hg^{2+} .

Hg^{2+}	K ($\text{L}\cdot\text{mol}^{-1}$)	ΔH ($\text{kJ}\cdot\text{mol}^{-1}$)	n	$T\cdot\Delta S$ ($\text{kJ}\cdot\text{mol}^{-1}$)	K_D ($\text{L}\cdot\text{mol}^{-1}$)
OST	1.552×10^5	-29.70	0.972	0.065	6.441×10^{-6}

Measurement of the fluorescence quantum yield

The fluorescence quantum yield (Y) is the ratio of the number of photons emitted by the fluorescent material after absorbing light to the number of photons of the excited light absorbed. The fluorescence quantum yield was measured by the reference method. By measuring the integral fluorescence intensity and the absorbance of the diluted solution of the tested substance and the reference substance at the same excitation wavelength, the fluorescence

quantum yield of the tested substance was calculated according to a formula from Reference [5]: $Y_u = Y_s \cdot F_u/F_s \cdot A_s/A_u$. Among them, Y_u and Y_s represent the fluorescence quantum yield of the test substance and the reference standard substance, respectively. F_u and F_s represent the comprehensive fluorescence intensity of the test product and the reference product, respectively. A_u and A_s represent the absorbance of the incident light at the excitation wavelength of the test substance and the reference substance, respectively ($A = \epsilon \cdot b \cdot c$).

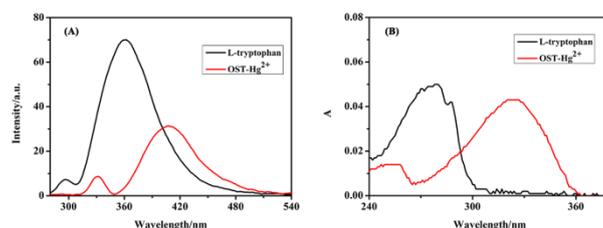


Figure S5: Fluorescence spectra of (A) L-tryptophan and OST-Hg²⁺ and (B) UV-vis absorption spectra.

References

1. Wang, X.; Ma, X.; Wen, J. *Talanta*, **2019**, *207*, 120-311.
2. Lin, Q.; Fu, Y.P.; Chen, P. *Dyes & Pigments*, **2013**, *96*, 1-6.
3. Wen, J.; Geng, Z.; Yin, Y. *Dalton Trans*, **2011**, *40*, 1984-1989.
4. Michael A, A.; O'Neill, Gaisford, S. *Int J Pharm*, **2011**, *417*, 83-93.
5. Kumari, A. ; Kumar, A.; Sahu, S. K. *Sensors & Actuators B Chemical*, **2017**, *254*, 197-205.