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## A DEOXYPODOPHYLLOTOXIN-BASED SENSOR FOR HIGHLY SELECTIVE RECOGNITION OF Hg<sup>2+</sup> ION

Juanjuan Wang, Yuanyuan Yan, and Hui Xu\*

Laboratory of Pharmaceutical Design & Synthesis, College of Sciences,  
Northwest A&F University, Yangling 712100, P. R. China

E-mail: orgxuhui@nwsuaf.edu.cn

Dedicated to Professor Dr. Victor Snieckus on the occasion of his 77<sup>th</sup> birthday

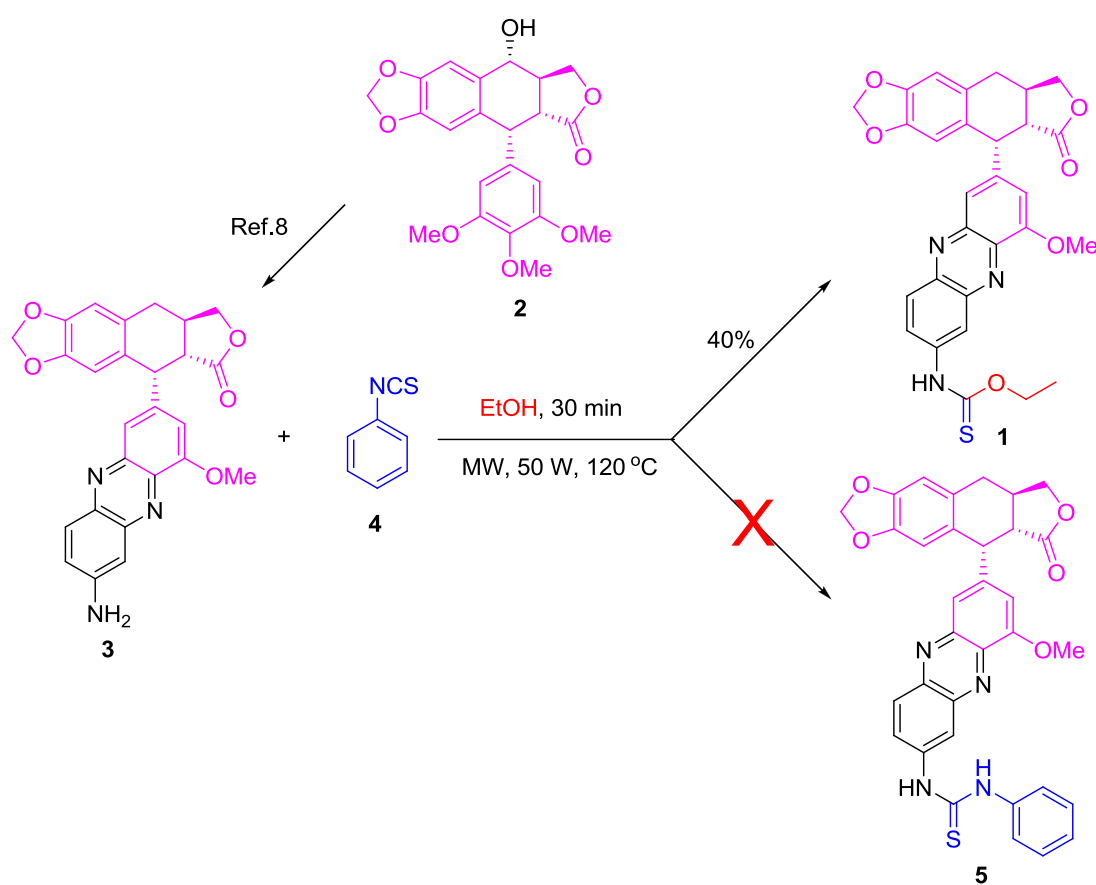
**Abstract** – A new deoxypodophyllotoxin-based probe **1** was prepared. Based on a selective and irreversible Hg<sup>2+</sup>-promoted desulfurization reaction, compound **1** exhibited high selectivity towards Hg<sup>2+</sup> ion over other metal ions, accompanied by transformation of an arylthiocarbamate moiety (green-yellow) to an arylcarbamate one (colorless). The present study may open up a new opportunity for development of natural-product-based probes for selective detection of some specific metal ions.

### INTRODUCTION

Podophyllotoxin (**2**, Scheme 1), a naturally occurring cyclolignan, is the main secondary metabolite isolated from the roots and rhizomes of *Podophyllum* species such as *P. hexandrum* and *P. peltatum*. Recently, extensive chemical structural modifications focused on compound **2** as anticancer,<sup>1</sup> insecticidal,<sup>2</sup> and antifungal<sup>3</sup> agents have been conducted. In continuation of our program aimed at the discovery and development of novel podophyllotoxin-based insecticidal agents,<sup>4</sup> we fortunately found that a new phenazine derivative of 4-deoxypodophyllotoxin (**3**)<sup>5</sup> exhibited strong fluorescence. On the other hand, mercury ions can cause great damage to the nervous system of human beings at very low concentrations, and could be bioaccumulated through the food chain, so mercury ions are considered to a highly toxic and dangerous pollutants for human beings.<sup>6</sup> Nowadays, it is still very desirable to

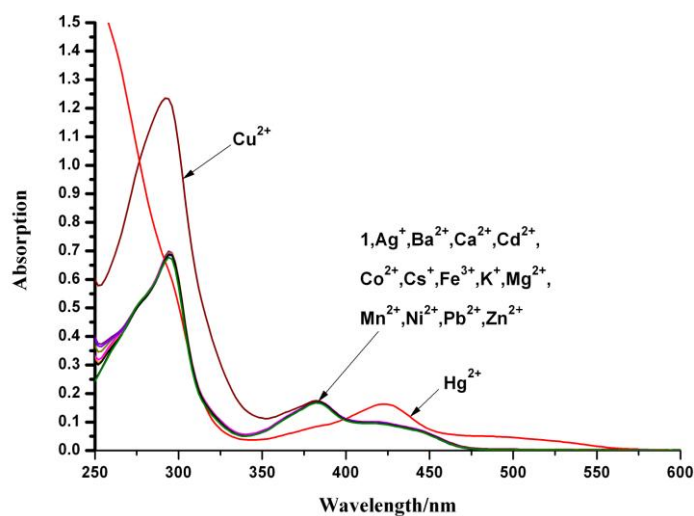
explore selective and sensitive chemosensors for detection of  $\text{Hg}^{2+}$  ion over other heavy and transition metal (HTM) ions. Recently, different kinds of excellent sensors for recognition of  $\text{Hg}^{2+}$  ion over other HTM ions have been developed with good performance.<sup>7</sup> Moreover, many attractive probes for detection of  $\text{Hg}^{2+}$  ion over other HTM ions based upon the specific mercury-promoted desulfurization reaction have also been described.<sup>8</sup>

Therefore, as part of our ongoing research program in developing selective chemosensors for detection of  $\text{Hg}^{2+}$  ion by a specific mercury-promoted desulfurization reaction,<sup>9</sup> in this paper, as shown in Scheme 1, we designed and tried to prepare a new thiourea derivative (**5**) by reaction of **3** with phenylisothiocyanate (**4**) under microwave irradiation. Interestingly, instead of **5**, an unexpected arylthiocarbamate derivative (**1**) was obtained in a 40% yield. The chemical structure of **1** was well characterized by  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy, and mass spectrometry (MS).<sup>10</sup> To the best of our knowledge, this is the first time to report the natural-product-based probe for recognition of  $\text{Hg}^{2+}$  ion based on the specific mercury-promoted desulfurization reaction.



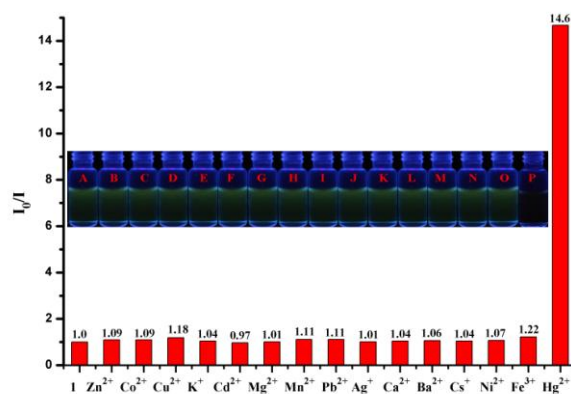
**Scheme 1.** Synthetic approach for probe **1**

## RESULTS AND DISCUSSION

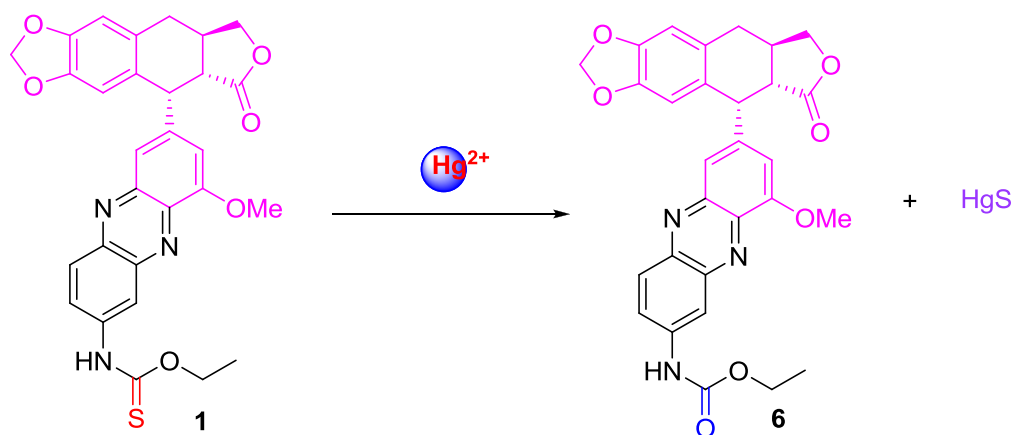


**Figure 1.** Variation of absorbance intensity of **1** ( $1.0 \times 10^{-5}$  M) in MeCN at 293 K in the presence of 10.0 equiv of different metal ions

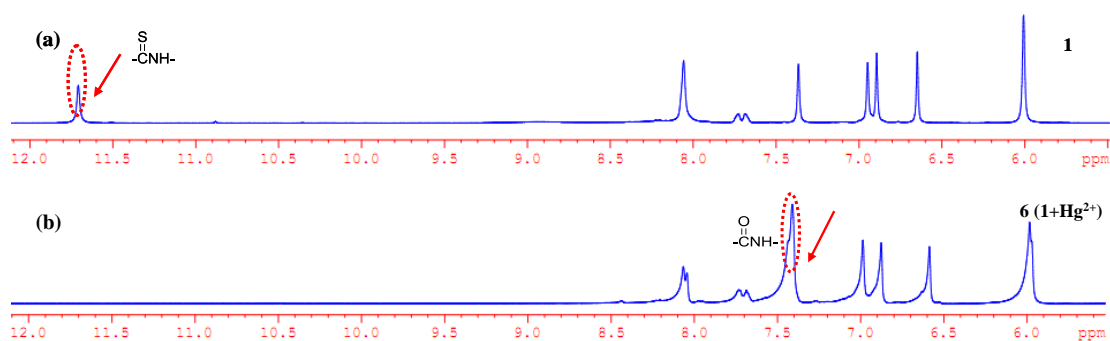
As described in Figure 1, the optical properties of **1** were examined in the absence or presence of various heavy and transition metal species. Compound **1** exhibited an absorption band at 384 nm. The metal species was then added to the solution of **1** respectively. As expected, the other tested metal ions, such as  $\text{Ca}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ ,  $\text{Cs}^+$  and  $\text{Ag}^+$ , showed almost no change. Only when  $\text{Hg}^{2+}$  ion was added to the solution of **1**, a 38 nm red-shift in the wavelength region from 384 to 422 nm was observed.



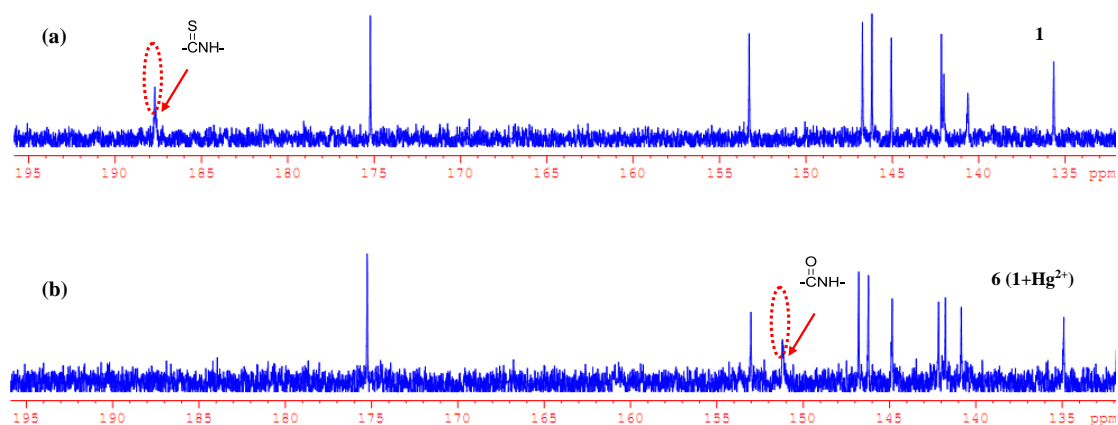
**Figure 2.** Fluorescent intensity ratio ( $I/I_0$ ) of **1** ( $1 \times 10^{-5}$  M) in MeCN at 293 K in the presence of 10.0 equiv of the respective metal ions. Inset: Photograph of **1** in the presence of various metal ions. A: **1** ( $5 \times 10^{-5}$  M); B-P: **1**+ $\text{Zn}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{K}^+$ ,  $\text{Cd}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Ag}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Cs}^+$ ,  $\text{Ni}^{2+}$ ,  $\text{Fe}^{3+}$ , and  $\text{Hg}^{2+}$  ( $5 \times 10^{-4}$  M)



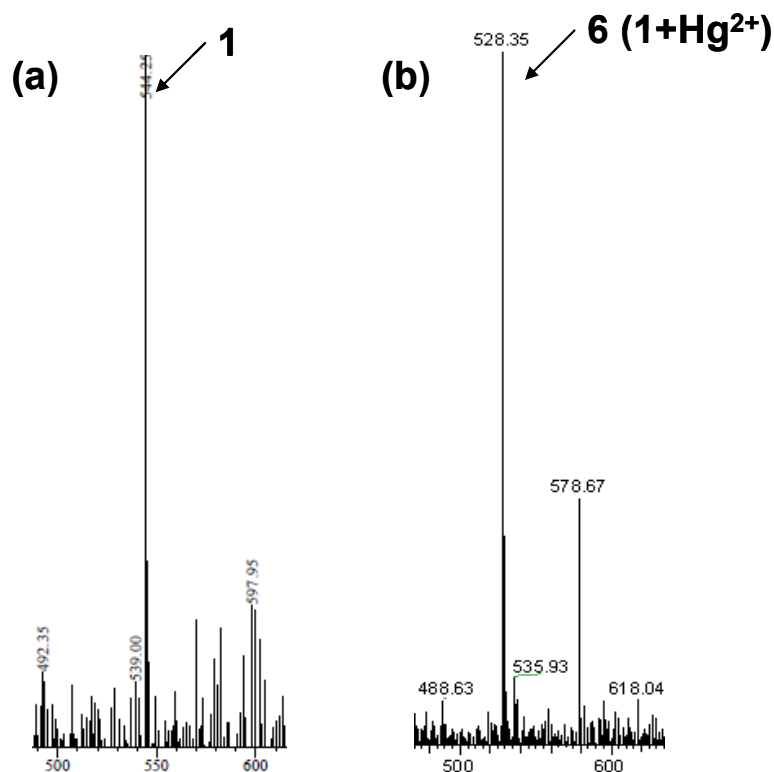
**Scheme 2.** Selective desulfurization of probe **1** in the presence of  $\text{Hg}^{2+}$  ion



**Figure 3.** Partial  $^1\text{H}$ NMR spectra of **1** (a), and **1**+1.2 equiv of  $\text{Hg}^{2+}$  (**6**, b) in  $\text{DMSO-}d_6$

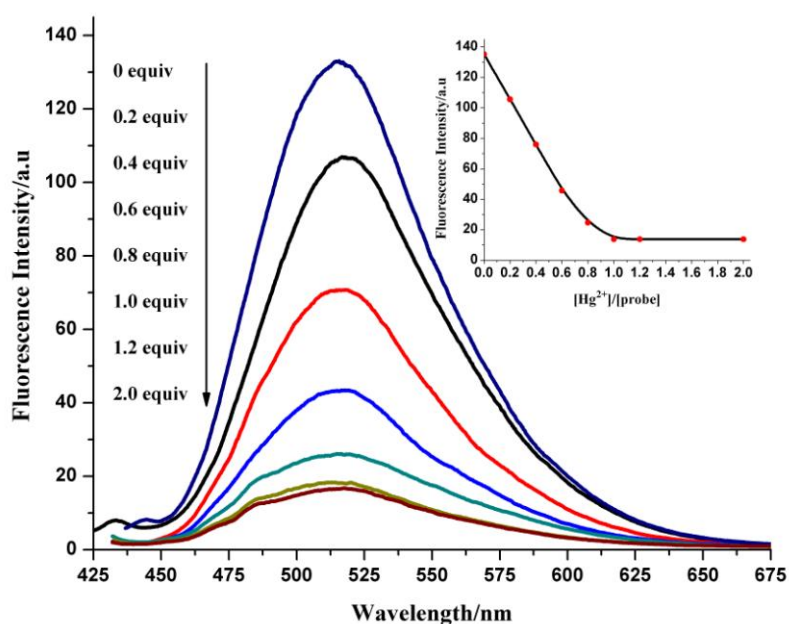


**Figure 4.** Partial  $^{13}\text{C}$  NMR spectra of **1** (a), and **1**+1.2 equiv of  $\text{Hg}^{2+}$  (**6**, b) in  $\text{DMSO-}d_6$



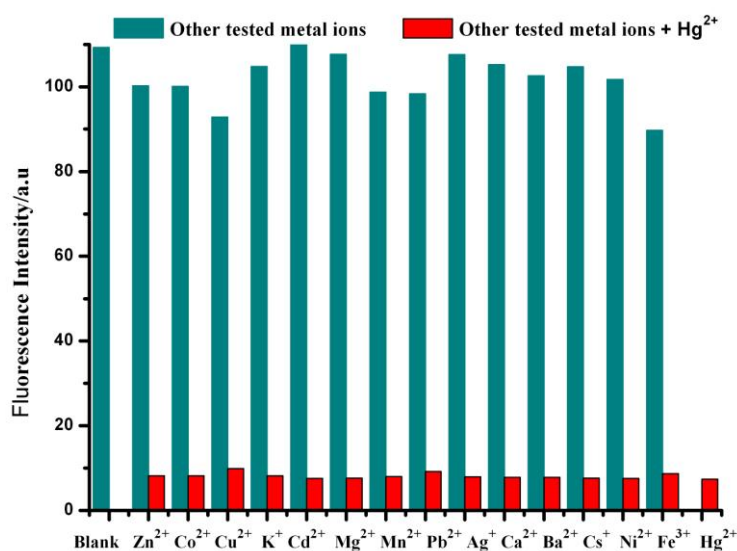
**Figure 5.** Partial ESI-MS spectra of **1** (a), and **1**+1.2 equiv of  $\text{Hg}^{2+}$  (**6**, b)

Figure 2 showed the fluorescent intensity ratio ( $I_0/I$ ) of **1** in the presence of  $\text{Ca}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Cs}^+$ ,  $\text{Ba}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Ag}^+$ ,  $\text{K}^+$ ,  $\text{Co}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Hg}^{2+}$  ions, respectively. It is noteworthy that only when  $\text{Hg}^{2+}$  ion was added to the solution of **1**, a 14.6-fold decrease of the fluorescent intensity was observed at 517 nm. It was because an arylthiocarbamate moiety of **1** was transformed to an arylcarbamate one in the presence of  $\text{Hg}^{2+}$  ion (Scheme 2), accompanied by transformation of a strong fluorescence (green-yellow, inset of Figure 2) to a weak fluorescence (colorless). Whereas the addition of other metal ions such as  $\text{Ca}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Cs}^+$ ,  $\text{Ba}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Ag}^+$ ,  $\text{K}^+$ ,  $\text{Co}^{2+}$ , and  $\text{Zn}^{2+}$ , did not lead to a significant change of the fluorescent intensity. The evidence for the proposed reaction was demonstrated by  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra. As depicted in the partial  $^1\text{H}$  NMR spectra of Figure 3, when 1.2 equiv of  $\text{Hg}^{2+}$  was added to the solution of **1**, the proton of NH was sharply shifted from 11.7 (**1**, (a)) to 7.41 ppm (**6**, (b), protons of benzene ring and NH were overlapped). Similarly, as shown in Figure 4, when 1.2 equiv of  $\text{Hg}^{2+}$  was added to the solution of **1**, the carbon chemical shift of C=S (arylthiocarbamate,  $\delta$ : 187.69 (**1**, (a))) was clearly decreased to 151.19 ppm (C=O, **6**, (b)). Solid evidence of the binding mode came from comparing the ESI-MS spectra of **1** and **6**. As described in Figure 5, the peak at  $m/z$  528.35 (b) was corresponding to  $[\mathbf{6}+\text{H}]^+$ , whereas probe **1** exhibited only a peak at  $m/z$  544.25 (a) corresponding to  $[\mathbf{1}+\text{H}]^+$ .



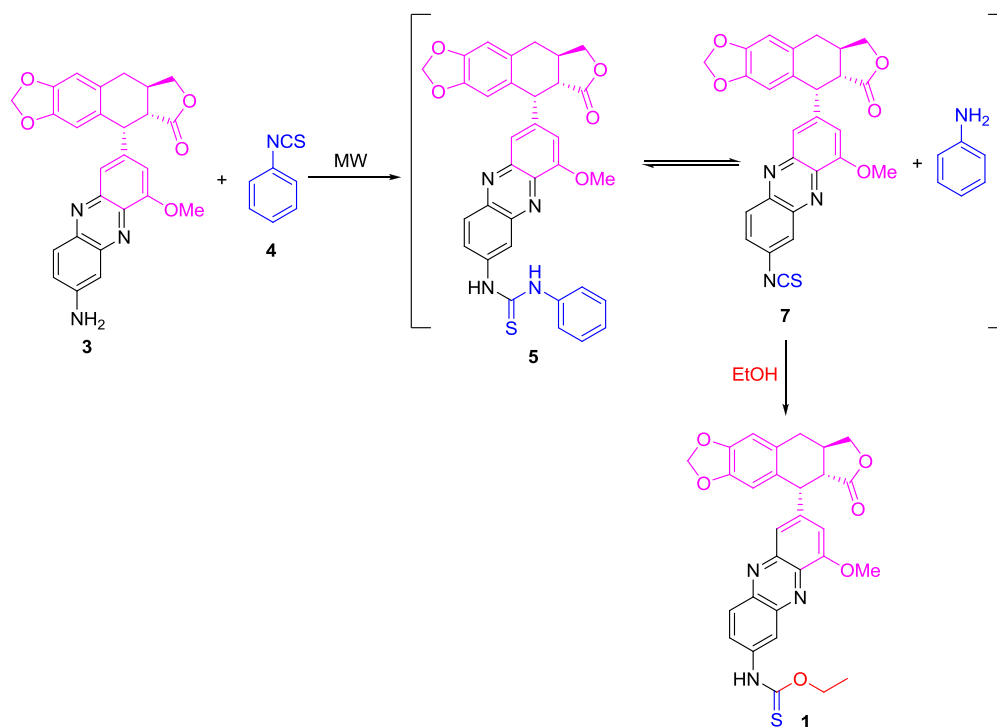
**Figure 6.** Fluorescence titration of **1** ( $1.0 \times 10^{-5}$  M) with  $\text{Hg}^{2+}$  in MeCN at 293 K with the concentration of  $\text{Hg}^{2+}$  ranging from 0 to  $2.0 \times 10^{-5}$  M;  $\lambda_{\text{ex}} = 422$  nm and the emission intensity was measured at 517 nm

The titration reaction curve of **1** toward the  $\text{Hg}^{2+}$  ion was investigated as shown in Figure 6. The fluorescent intensity of **1** gradually decreased in response to the increases in the concentration of the added  $\text{Hg}^{2+}$  ion, and the titration reaction curve showed a steady decrease until a plateau was reached when 1.0 equiv of  $\text{Hg}^{2+}$  ion was added.



**Figure 7.** Results of the competition experiments of probe **1** ( $1 \times 10^{-5}$  M) between  $\text{Hg}^{2+}$  ( $2 \times 10^{-5}$  M) and selected metal ions ( $2 \times 10^{-4}$  M) in MeCN at 293 K;  $\lambda_{\text{ex}} = 422$  nm and the emission intensity was measured at 517 nm

To investigate the utility of **1** as an ion-selective fluorescent sensor for  $\text{Hg}^{2+}$  ion, the cross-contamination experiments were conducted in the presence of  $\text{Hg}^{2+}$  ion at  $2 \times 10^{-5}$  M mixed with other metal ions at  $2 \times 10^{-4}$  M, respectively. It demonstrated that the selectivity of **1** towards  $\text{Hg}^{2+}$  ion was almost not affected by other competitive ions such as  $\text{Ca}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Cs}^+$ ,  $\text{Ba}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Ag}^+$ ,  $\text{K}^+$ ,  $\text{Co}^{2+}$ , and  $\text{Zn}^{2+}$  (Figure 7).



**Scheme 3.** The possible mechanism for synthesis of **1**

Additionally, the possible mechanism for synthesis of **1** was also proposed (Scheme 3). The thiourea **5** was firstly synthesized by reaction of **3** with **4** under microwave irradiation. Compound **5** was then transformed to the deoxypodophyllotoxin-derived isothiocyanate (**7**) by the reversible reaction, which further reacted with ethanol to offer the deoxypodophyllotoxin-derived thiocarbamate (**1**).

In conclusion, a new and highly  $\text{Hg}^{2+}$ -selective deoxypodophyllotoxin-like natural-product-based sensor **1** was prepared. Based on the  $\text{Hg}^{2+}$ -promoted selective desulfurization reaction, probe **1** exhibited high selectivity towards  $\text{Hg}^{2+}$  ion over other competitive metal ions, accompanied by transformation of an arylthiocarbamate moiety (green-yellow) to an arylcarbamate one (colorless). It was demonstrated by the  $^1\text{H}$ ,  $^{13}\text{C}$  NMR and MS spectroscopy. To the best of our knowledge, it is the first probe based on a natural product moiety (deoxypodophyllotoxin) for recognition of  $\text{Hg}^{2+}$  ion. Importantly, it is noteworthy that, due to a broad spectrum of chemical and functional diversity of natural products, this study may open up a new opportunity for development of natural-product-based probes for selective detection of some specific metal ions.

## EXPERIMENTAL

All solvents and reagents were used as obtained from commercial sources without further purification.  $\text{Zn}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{Fe}(\text{ClO}_4)_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{Ca}(\text{ClO}_4)_2 \cdot 4\text{H}_2\text{O}$ ,  $\text{Mg}(\text{ClO}_4)_2$ ,  $\text{Ba}(\text{ClO}_4)_2$ ,  $\text{Pb}(\text{ClO}_4)_2$ ,  $\text{Cd}(\text{ClO}_4)_2$ ,  $\text{Cu}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{Ni}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{Mn}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{KClO}_4$ ,  $\text{CsClO}_4$ ,  $\text{Co}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{Hg}(\text{ClO}_4)_2$  and  $\text{AgPF}_4$  were investigated as sources for metal ions. Analytical thin-layer chromatography (TLC) and preparative thin-layer chromatography (PTLC) were performed with silica gel plates using silica gel 60 GF<sub>254</sub> (Qingdao Haiyang Chemical Co., Ltd.). Microwave irradiation was performed in a CEM Discover Synthesis Unit (CEM Corp., Matthews, NC, made in USA). Nuclear magnetic resonance spectra (NMR) were recorded on a Bruker Avance DMX 400 MHz instrument in  $\text{DMSO}-d_6$  ( $^1\text{H}$  at 400 MHz and  $^{13}\text{C}$  at 100 MHz) using TMS (tetramethylsilane) as the internal standard. Electron impact ionization mass spectra (EI-MS) were carried out with HP 5988 instrument. Fluorescent spectra were determined on a Hitachi F-4500 spectrophotometer. UV-visible spectra were determined on a Hitachi U-3310 spectrophotometer. Compound **3** was prepared according to our previous paper.<sup>5</sup>

**Synthesis of compound 1.** A mixture of **3** (0.2 mmol) and phenylisothiocyanate (**4**, 0.3 mmol) in absolute EtOH (2 mL) was placed in a 10-mL glass tube, which was sealed with a septum and placed into the microwave cavity, and reacted at microwave irradiation at 120 °C with an output power of 50 W for 30 min. Then the vessel was allowed to cool to a temperature below 50 °C before removal from the microwave cavity and opening to the atmosphere. The mixture was concentrated and purified by PTLC to give 43.6 mg (40% yield) of **1** as a yellow solid. Mp 167-169 °C;  $[\alpha]_D^{20} = +54$  (*c* 2.5 mg/mL, acetone);  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$ : 11.70 (s, 1H, NH), 7.68-8.05 (m, 3H), 7.36 (s, 1H), 6.94 (s, 1H), 6.89 (s, 1H), 6.64 (s, 1H), 6.00 (s, 2H, OCH<sub>2</sub>O), 4.87 (d, *J* = 4.4 Hz, 1H, H-1), 4.63 (s, 2H, CH<sub>2</sub>CH<sub>3</sub>), 4.39-4.43 (m, 1H, H-11), 3.99-4.04 (m, 4H, 3'-OCH<sub>3</sub>, H-11), 3.22-3.27 (m, 1H, H-4), 3.08-3.11 (m, 1H, H-2), 2.69-2.84 (m, 2H, H-3, 4), 1.40 (t, *J* = 6.8 Hz, 3H, CH<sub>2</sub>CH<sub>3</sub>);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ )  $\delta$ : 187.7, 175.2, 153.3, 146.7, 146.2, 145.1, 142.2, 142.0, 140.6, 135.7, 131.6, 130.1, 129.4, 128.8, 121.9, 117.0, 111.5, 110.1, 108.7, 101.1, 71.8, 65.1, 55.9, 46.0, 43.7, 32.7, 32.0, 30.1, 14.2; MS (EI), *m/z* (%) 544.25 ( $[\text{M}+\text{H}]^+$ , 100).

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