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# Mercaptooxazole–phenazine based blue fluorescent sensor for the ultra-sensitive detection of mercury(II) ions in aqueous solution†

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Herein, a mercury(II) ion fluorescent sensor (Z-3) with high sensitivity and immediate response is designed and synthesized. The sensor uses the phenazine group as a luminophore and sulfhydryl as a recognition moiety. The sensor is easily synthesized and it exhibits a remarkable blue shift with  $\text{Hg}^{2+}$ . Correspondingly, its fluorescence color changes from yellow to blue. In addition, the low naked eye detection limit ( $10^{-5}$ ) of the sensor allows the identification of concentration limits. Moreover, the sensor could detect mercury(II) ions over a wide pH range (from 2 to 8), which indicates that the detection can be carried out in aqueous systems. In addition, test strips are fabricated, which could act as a convenient pathway for the recognition of  $\text{Hg}^{2+}$ .

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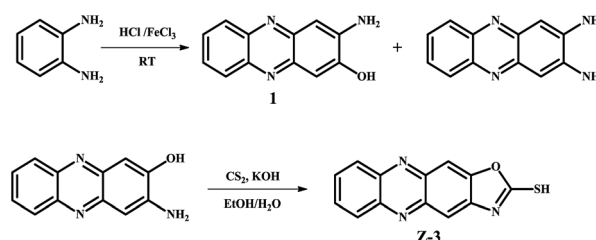
## Introduction

Mercury plays an important role in electrical and chemical applications.<sup>1–3</sup> Its electrical applications include thermometers, barometers, sphygmomanometers and other related instruments. In the chemical industry, mercury is used to electrolyze a salt for the preparation of high purity chlorine and caustic soda.<sup>4–11</sup> However, excessive consumption of mercury can lead to irreversible damage to the environment and human body.<sup>12–15</sup> As a toxic metal, mercury and its compound could cause vital organ dysfunction such as DNA damage,<sup>16</sup> mitosis impairment,<sup>17</sup> and nervous system defects.<sup>18</sup> The emission of mercury ions has been increasing due to the full speed development of the mercury industry.<sup>19,20</sup> Mercury ions enter the body through the food chain and accumulate gradually, which directly affects the safety of human life. To date, many sensors have been reported for the detection of  $\text{Hg}^{2+}$ . However, some sensors are made by complex synthesis processes and expensive raw materials or are difficult to synthesize,<sup>21–24</sup> and thus their practical applications are limited in certain aspects. Due to their rapid testing, strong practicality and easy operation, colorimetric and fluorescent sensors are the best choice for the detection of mercury(II) ions in physiological and environmental samples.<sup>25–29</sup>

Among the reported sensors, phenazine and its derivatives have been widely employed in the design of fluorescent sensors due to their stable optical properties, easy synthesis, cheap raw materials and large conjugate systems.<sup>30–32</sup> They act as an important skeleton in fluorescence sensors used to identify amino acids, citrate and other ions.<sup>33–35</sup>

According to these superior properties and our goals,<sup>36</sup> we designed and synthesized a phenazine derivative Z-3 (Scheme 1), which could rapidly select  $\text{Hg}^{2+}$  in aqueous solutions. Based on its deprotonation mechanism, the fluorescent sensor could detect  $\text{Hg}^{2+}$  with blue fluorescence in a wide pH range (from 2–8). It also exhibits high selectivity ( $2.14 \times 10^{-9}$  M) and other common metal ions have nearly no influence on its sensing behavior. Moreover, its naked eye minimum detection limits are  $10^{-5}$  and  $10^{-4}$  for solution and test strips, respectively, which greatly improve the practicality of this sensor. Moreover, the test strips also can act as a reliable means for the detection of  $\text{Hg}^{2+}$  in real-time.

Fluorescence spectra were measured with different metal ions in DMSO/ $\text{H}_2\text{O}$  (v/v, 3 : 2, buffered with HEPES, pH = 7.20). As shown in Fig. 1a, the addition of  $\text{Hg}^{2+}$  induced a distinct



Scheme 1 Synthesis of compound Z-3.

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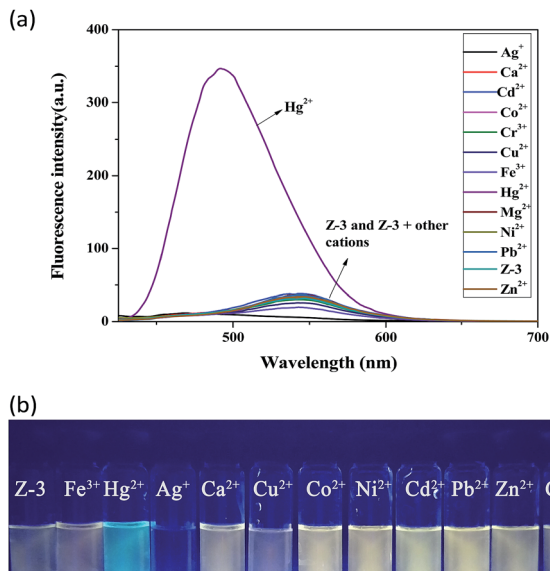


Fig. 1 (a) Fluorescence spectra ( $\lambda_{\text{ex}} = 400 \text{ nm}$ ) of Z-3 ( $2 \times 10^{-5} \text{ M}$ ) with different metal ions:  $\text{Fe}^{3+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Ag}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cr}^{3+}$  and  $\text{Mg}^{2+}$  (10 equiv., respectively) in DMSO/ $\text{H}_2\text{O}$  (v/v, 3 : 2; buffered with HEPES, pH = 7.20). (b) Visual fluorescence emission of the Z-3 sensor upon the addition of different metal ions.

fluorescence enhancement with an obvious blue-shift (from 544 to 496 nm). Moreover, the fluorescence color of the solutions changed from yellow to blue under illumination with a 365 nm UV lamp (Fig. 1b). We speculated that the sensor complexed with  $\text{Hg}^{2+}$ , which resulted in the fluorescence colour change. All these facts indicate that the sensor could be used for the selective identification of  $\text{Hg}^{2+}$  among other cations.

The most important aspect of selective recognition is that the target analyte is detected over other possible interfering metal ions, and thus a competition experiment was carried out. In Fig. 2a and b, in the presence of other metal ions, such as  $\text{Fe}^{3+}$ ,  $\text{Ag}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cr}^{3+}$  and  $\text{Mg}^{2+}$ , there was no evident fluorescence emission peak shift and color change. Therefore, it is clear that the interference of other ions is negligible during the detection of  $\text{Hg}^{2+}$ . These results further suggest that Z-3 has a strong ability to complex with  $\text{Hg}^{2+}$  over other metal ions and could be used as a sensor for  $\text{Hg}^{2+}$  over a wide range of cations.

We also performed a fluorescence titration to verify the recognition behavior sensitivity (Fig. 3). In the fluorescence spectrum, upon the addition of  $\text{Hg}^{2+}$  to Z-3, the emission peak gradually blue shifted from 544 nm to 496 nm with a fluorescence color change from yellow to blue. The detection of Z-3 for  $\text{Hg}^{2+}$  calculated on the basis of  $3\sigma/m$  for the fluorescence spectra (Fig. S5<sup>†</sup>) is  $2.14 \times 10^{-9} \text{ M}$ ,<sup>37</sup> which is lower than the EPA guideline of 10 nM.<sup>38</sup> This result also shows that Z-3 has higher sensitivity for  $\text{Hg}^{2+}$  compared with other reported  $\text{Hg}^{2+}$  sensors (Table 1).

In order to improve the actual operating performance of the detection behavior, we studied the pH scope of Z-3 +  $\text{Hg}^{2+}$  and fabricated comparison test strips. As shown in Fig. 4, Z-3 with  $\text{Hg}^{2+}$  exhibits strong fluorescence intensity in the pH range of 2–

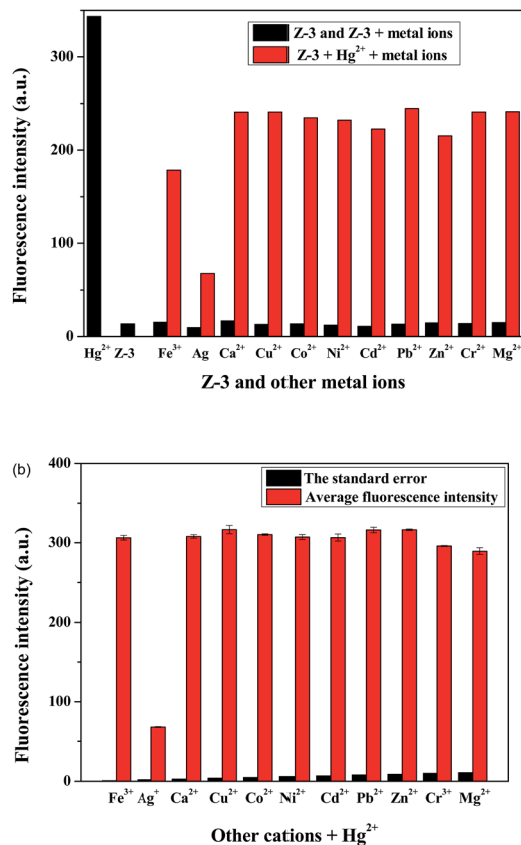


Fig. 2 (a) Fluorescence spectra of Z-3 and Z-3 +  $\text{Hg}^{2+}$  in the presence of 10 equiv. various metal ions in DMSO/ $\text{H}_2\text{O}$  (v/v, 3 : 2; buffered with HEPES, pH = 7.20) solutions; (b) error bar of Z-3 +  $\text{Hg}^{2+}$  in the presence of various cations.

8, while the fluorescence intensity increases obviously at pH > 8, which may result from the fact that too high pH will result in a reaction between  $\text{Hg}^{2+}$  and  $-\text{OH}$  to form the corresponding metal hydroxides. This fact indicates that the sensor could be used in a wide pH range from 2 to 8 for the detection of  $\text{Hg}^{2+}$ .

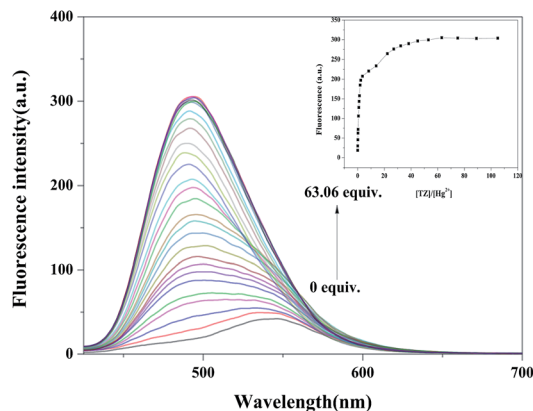


Fig. 3 Fluorescence spectra of Z-3 with different concentrations of  $\text{Hg}^{2+}$  in DMSO/ $\text{H}_2\text{O}$  (v/v, 3 : 2; buffered with HEPES, pH = 7.20) solutions. Insert: plot of fluorescence intensity vs. concentration of  $\text{Hg}^{2+}$  in the range of 0 to 63.06 equiv. ( $\lambda_{\text{ex}} = 400 \text{ nm}$ ).



Table 1 A comparison of the detection limits for Hg<sup>2+</sup>

Method	Analyte	Solvent	Detection limit
Ref. 39	Hg <sup>2+</sup>	1-Propanol-cyclohexane	1.7 × 10 <sup>-8</sup> M
Ref. 40	Hg <sup>2+</sup>	MeOH-H <sub>2</sub> O	2.1 × 10 <sup>-7</sup> M
Ref. 41	Hg <sup>2+</sup>	THF-HEPES	8 × 10 <sup>-8</sup> M
Ref. 42	Hg <sup>2+</sup>	CH <sub>3</sub> CN-H <sub>2</sub> O	9 × 10 <sup>-8</sup> M
Our work	Hg <sup>2+</sup>	DMSO-HEPES	2.14 × 10 <sup>-8</sup> M

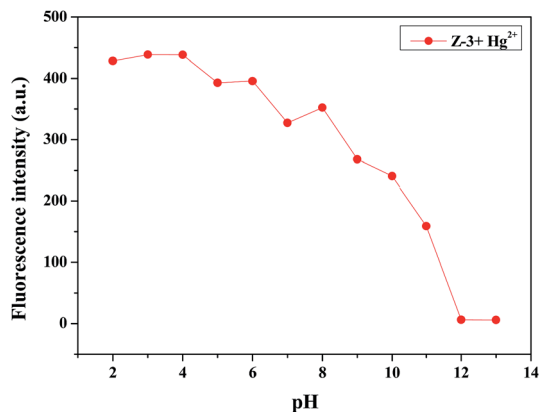


Fig. 4 Fluorescence intensity ( $\lambda_{\text{ex}} = 400$  nm and  $\lambda_{\text{em}} = 496$  nm) of the Z-3 + Hg<sup>2+</sup> complex at various pH values in DMSO/H<sub>2</sub>O (v/v, 3 : 2; buffered with HEPES, pH = 7.20) solution.

Test strips were prepared by immersing filter paper into a DMSO solution of Z-3 ( $2 \times 10^{-4}$  M) and then drying them in air. The test strips containing Z-3 were utilized to sense Hg<sup>2+</sup>. As shown in Fig. 5, with a decrease in the Hg<sup>2+</sup> concentration, the test paper fluorescence color gradually becomes yellow. The minimum identification limit of the test strips is  $10^{-4}$  M. Moreover, the test strips could act as a convenient method for the detection of Hg<sup>2+</sup> in an aqueous solution. We also conducted the experiments with the minimum detection limit with respect to naked eyes. As shown in Fig. 6, when the concentration of Hg<sup>2+</sup> is  $10^{-6}$  M, the fluorescence color of the solution is consistent with the sensor (Z-3). We thought that the lowest detection limit of Hg<sup>2+</sup> was  $10^{-5}$  M.

In order to study the combination of mercury ions(II) with Z-3, we first studied the isomerization of Z-3. As shown in Fig. S1,† the peak at 14.41 ppm is ascribed to the -NH proton in the <sup>1</sup>H NMR spectrum. Moreover, we also observed a peak at  $2675 \text{ cm}^{-1}$  in the IR spectrum (Fig. S2†), which is the characteristic peak of



Fig. 5 Photographs of test strips (the concentration of Hg<sup>2+</sup> is 0 M,  $10^{-1}$  M,  $10^{-2}$  M,  $10^{-3}$  M,  $10^{-4}$  M,  $10^{-5}$  M, both under an UV lamp at 365 nm).



Fig. 6 Naked-eyes detection limit under UV lamp at 365 nm. From left to right, the concentration of Hg<sup>2+</sup> is  $10^{-1}$  M,  $10^{-2}$  M,  $10^{-3}$  M,  $10^{-4}$  M,  $10^{-5}$  M,  $10^{-6}$  and 0 M.

-SH. Based on these facts, we believe that Z-3 has two tautomers of thioketone (=S) and thiols (-SH), and the structure of thione (=S) is the main form in solution. From the <sup>1</sup>H NMR titration (Fig. 7), the peak at 14.41 ppm disappeared with the addition of Hg<sup>2+</sup>, which suggests that the complexation occurred after the -NH proton was fully deprotonated. Amazingly, the solution precipitated solid on the addition of 0.2 equivalents of mercury ions. We speculate that it may be from the perchlorate, and its molecular formula may be [Z-3 + Hg]<sup>+</sup>ClO<sub>4</sub><sup>-</sup>. In addition, we also carried out an IR experiment on the solid of Z-3 and Z-3 + Hg<sup>2+</sup>. As shown in Fig. S3,† the peak at  $2675 \text{ cm}^{-1}$ , which was recognized as the characteristic peak of -SH, disappeared upon the addition of Hg<sup>2+</sup>. All these results indicate that the mercury ions coordinated with the sulfur atom and nitrogen atom on the oxazole ring after the protonation of the sensor.

Moreover, in order to clarify the stoichiometric relationship between Hg<sup>2+</sup> and Z-3, the Job plot was obtained. As shown in Fig. 8, the fluorescence intensity at 496 nm was plotted against molar fraction of Z-3 sensor. The maximum emission intensity was reached when the molar fraction was 0.4. This result manifests a 1 : 1 ratio for the Z-3 + Hg<sup>2+</sup> complex.

Based on all these facts, we propose a reasonable mechanism for the detection of Hg<sup>2+</sup> by the sensor in an aqueous solution (Fig. 9). We hypothesize that the thiocarbonyl and secondary amine nitrogen of the oxazole motif coordinate with Hg<sup>2+</sup>, and then the complex and perchloric acid form a salt and precipitate.

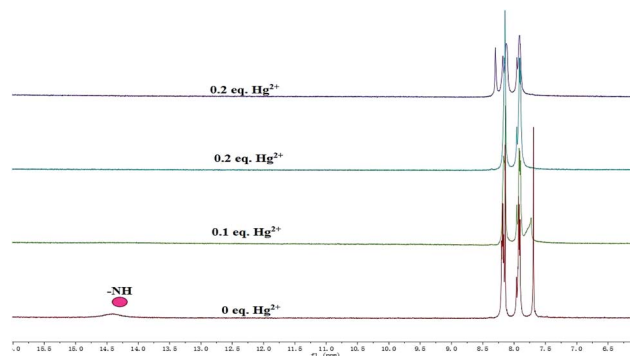


Fig. 7 Partial <sup>1</sup>H NMR spectra of Z-3 in DMSO-*d*<sub>6</sub> with different concentrations of Hg<sup>2+</sup>.



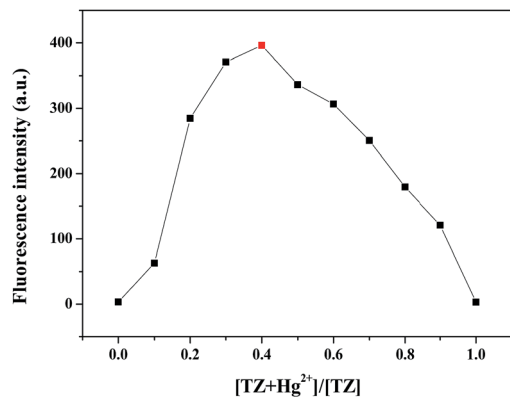


Fig. 8 Job plot examining the relationship between Z-3 and Hg<sup>2+</sup>.

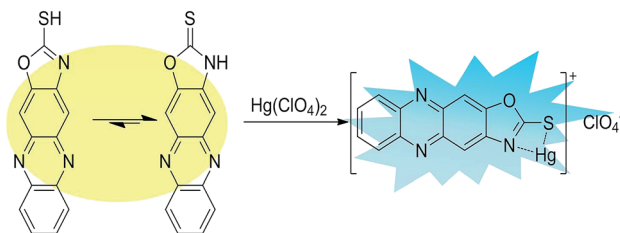


Fig. 9 Possible binding mode of Z-3 with Hg<sup>2+</sup>.

In conclusion, we obtained a fluorescent sensor Z-3 via a simple method for the detection of Hg<sup>2+</sup>, which displays high selectivity and sensitivity in the recognition process. The sensor exhibits an obvious fluorescence colour change from yellow to blue, and its detection behaviour operates in a wide pH range. Moreover, its test strips and naked-eyes minimum detection limit greatly improve the practicality of Z-3. Hence, this study also implies that phenazine and its derivatives are potential candidates for the detection of ions.

## Conflicts of interest

There are no conflicts to declare.

## Acknowledgements

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