# Synthesis and spectral studies of a novel benzoxazole-derived fluorescent probe for $Hg^{2+}$

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A novel turn-off fluorescent probe bearing a benzoxazole fluorophore has been synthesised and characterised. Its recognition properties towards different metal ions have been researched by spectrometry. The probe was highly selective and sensitive to $Hg^{2+}$ and showed a complexation ratio towards $Hg^{2+}$ of 1:1. Its fluorescence intensity varied almost linearly with the concentration of $Hg^{2+}$ (1.6～8.6 μmol $L^{-1}$), and the detection limit of $Hg^{2+}$ was estimated to be 0.68 μmol $L^{-1}$.

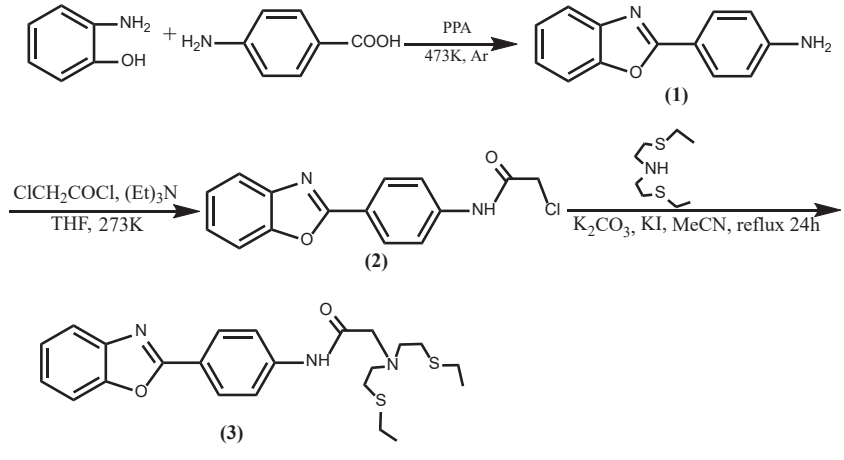
**Keywords:** benzoxazole, $Hg^{2+}$, fluorescent probe, selectivity, sensitivity

Heavy metals have attracted considerable attention because of their wide use and subsequent impact on the environment and human health. One of the very toxic and frequently used heavy metals is mercury. Mercurial species may be released into the environment through coal-fired power plants and into water streams from chemical, gold mining and chlor-alkali industrial plants.[1] It's reported that exposure to $Hg^{2+}$ can cause immune, sensory, neurological, motor and behavioural dysfunctions through the food chain.[2,3] Therefore, it is important to develop new methods for the detection and quantification of $Hg^{2+}$. Recently, $Hg^{2+}$ probes have been highly valuable because of their high sensitivity and selectivity, quick response time and easy signal detection.[4–7] Although many types of $Hg^{2+}$ ion-sensing ionophores have been introduced to detect mercuric ions in chemical and biological systems, there is still need for more probes for the analysis of this ion in varying types of samples in terms of the sample matrix and concentration ranges.[8,9] Therefore, the development of $Hg^{2+}$-selective probes is still a challenge.[10–12] Benzoxazole compounds are widely used as optical probes, fluorescent brighteners and laser dyes due to their good photophysical and photochemical properties.[13–15] Herein, we report a sensitive and selective fluorescent probe bearing a benzoxazole fluorophore for the detection of $Hg^{2+}$.

## Results and discussion

The benzoxazole-derived fluorescent probe **3** was synthesised by a nucleophilic substitution reaction of a primary halogenoalkane and secondary amine, as depicted in Scheme 1. Probe **3** was fully characterised by $^{1}H$ NMR, $^{13}C$ NMR, IR, MS and elemental analysis, and its recognition of $Hg^{2+}$ was studied by spectrometry. Metal-binding properties showed that **3** is highly selective and sensitive to $Hg^{2+}$ in aqueous EtOH solution.

The maximum absorption wavelength of **3** was 320 nm, and its maximum excitation ($\lambda_{ex}$) and emission ($\lambda_{em}$) wavelengths were 325 and 412 nm, respectively. Different common metal ions were used to evaluate the metal ion binding properties and selectivity of **3** (10 μmol $L^{-1}$) by fluorescence spectra. As shown in Fig. 1, upon the addition of $K^+$, $Ca^{2+}$, $Na^+$, $Mg^{2+}$, $Mn^{2+}$, $Fe^{2+}$, $Fe^{3+}$, $Co^{2+}$, $Ni^{2+}$, $Zn^{2+}$, $Cd^{2+}$ or $Ag^+$ (5 equiv.), no obvious effect on the fluorescence emissions were observed, whereas $Cu^{2+}$ and $Pb^{2+}$ caused a weak decrease in the fluorescent intensity. In contrast, the addition of $Hg^{2+}$ resulted in a significant quenching in the fluorescent emission of **3**, indicating that **3** displayed a high $Hg^{2+}$ selectivity. The selectivity of **3** for $Hg^{2+}$ was further proved by metal ion competition experiments, which were examined by use of binary mixtures of $Hg^{2+}$ and various metal ions (Fig. 2). The fluorescence intensity of **3** (10 μmol $L^{-1}$) in the presence of 1 equiv. of $Hg^{2+}$ was hardly affected by the addition of 10 equiv. of competing metal ions. It can be seen that **3** displayed a high selectivity for recognition of $Hg^{2+}$. That was attributed to the far higher binding affinity and stability of **3** to $Hg^{2+}$ compared to other metal ions. It was also found that the UV-Vis spectra of **3** showed a strong absorption band at 320 nm, but its absorption intensity decreased upon addition of the same amount of $Hg^{2+}$, and the maximum absorption wavelength of the Hg-complex remained almost unchanged (Fig. 3).




**Scheme 1** Synthesis of fluorescent probe **3**.

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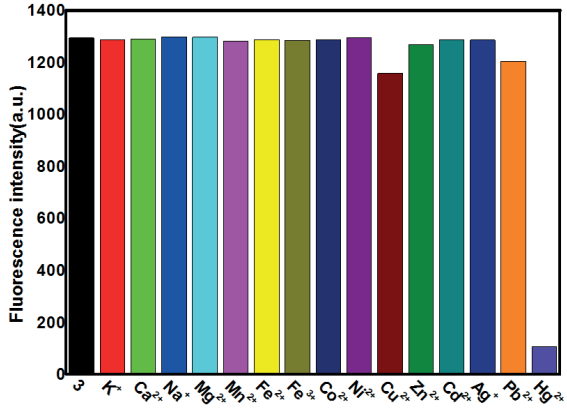



**Fig. 1** The effect of metal ions (50 μM) on the fluorescent properties of probe **3** (10 μM) in 100 mM Tris-HCl buffer (pH 7.4, $H_2O$:EtOH = 4:1) with an excitation at 325 nm. The samples were incubated for 10 min at room temperature prior to measurements.

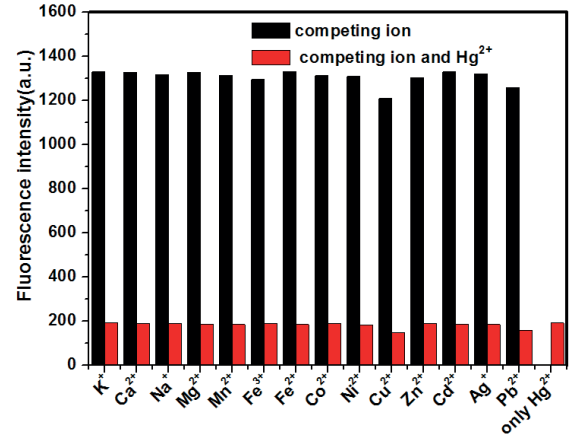



**Fig. 2** The effect of $Hg^{2+}$ on **3** (10 μmol $L^{-1}$) in the presence of different metal ions (100 μmol $L^{-1}$).

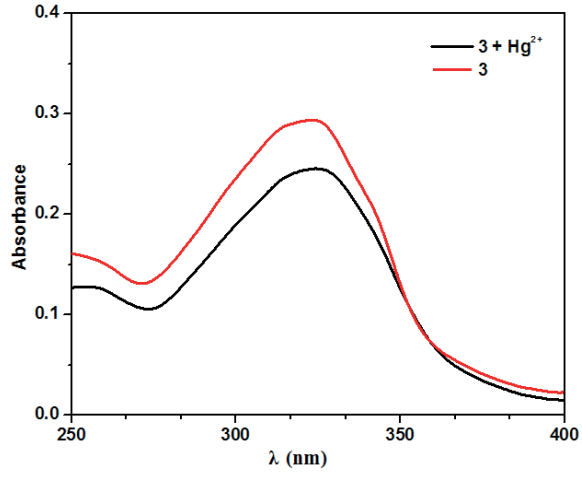



**Fig. 3** Change in UV-Vis spectra of **3** upon interaction with $Hg^{2+}$.

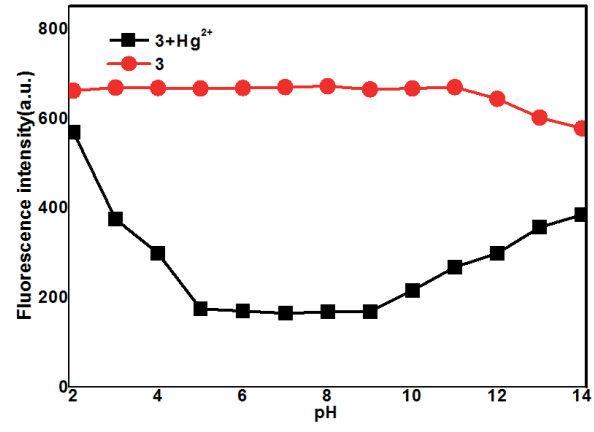



**Fig. 4** The fluorescence intensity of **3** (10 μmol $L^{-1}$) and **3** + $Hg^{2+}$ over a pH range from 2 to 14 at room temperature.

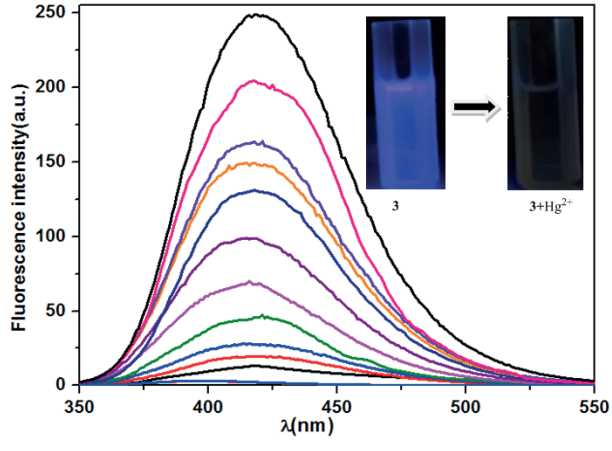



**Fig. 5** The emission intensity of **3** (10 μmol $L^{-1}$) changes with increasing concentrations of $Hg^{2+}$ (0 ~ 30 μmol $L^{-1}$); the inset shows photograph of **3** (10 μmol $L^{-1}$) in the absence and presence of 3 equiv. $Hg^{2+}$ under UV light (365 nm).

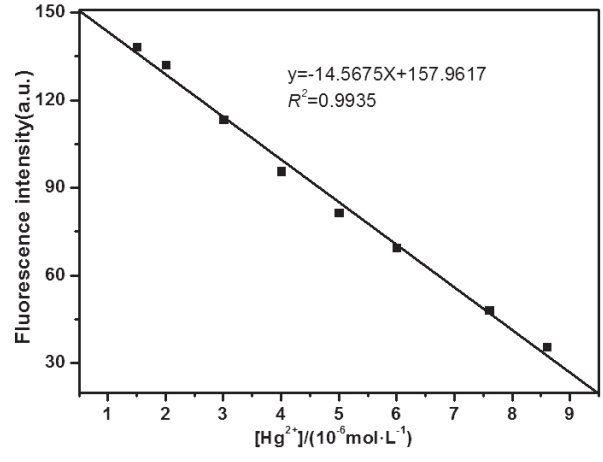



**Fig. 6** Fluorescence intensity of **3** as a function of concentration of $Hg^{2+}$ (1.6 ~ 8.6 μmol $L^{-1}$).

The fluorescence spectrum of **3** was highly stable over a pH range of 2–11 (Fig. 4). Upon addition of $Hg^{2+}$, **3** remained unaffected with increasing pH from 5 to 9, indicated its potential applications in biological systems. Therefore, **3** can be used for detection of $Hg^{2+}$ without the adverse influence of other metal ions and pH variations close to physiological conditions.

The sensitivity of the fluorescence response of **3** towards $Hg^{2+}$ was investigated by fluorescent titration. Fig. 5 shows the gradual change of the fluorescence spectra of **3** upon addition of $Hg^{2+}$; clear "on–off" fluorescence changes of **3** to $Hg^{2+}$ were observed. When $Hg^{2+}$ was added to the solution, a significant decrease of the fluorescence intensity at 412 nm was observed. It was found that the fluorescence of **3** was quenched almost completely after the addition of 3 equiv. of $Hg^{2+}$. As illustrated in the inset photograph shown in Fig. 5, an obvious fluorescence change from bright blue to colourless can be observed under irradiation at 365 nm after the

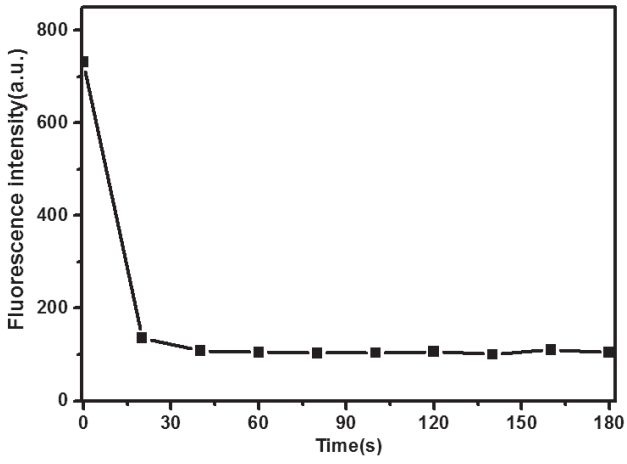



**Fig. 7** Changes of fluorescence intensity of **3** (10 μmol $L^{-1}$) in the presence of 1.0 equiv. of $Hg^{2+}$ as a function of time.

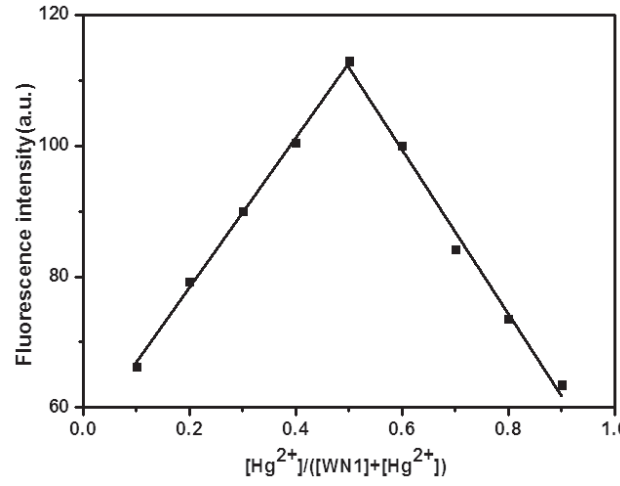
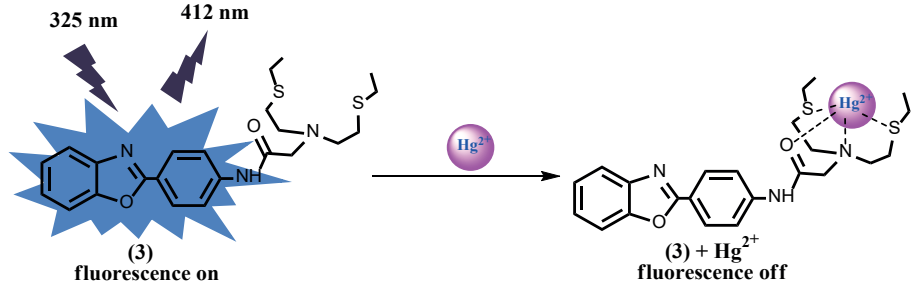



**Fig. 8** Job's plot of **3** and $Hg^{2+}$ ([**3**] + [$Hg^{2+}$] = 10 mmol $L^{-1}$).




**Scheme 2** The proposed sensing mechanisms of probe **3** for $Hg^{2+}$.

addition of 3 equiv. of $Hg^{2+}$ to the solution of **3**. Interestingly, the decremental fluorescence emission intensity was found to be proportional to the concentration of $Hg^{2+}$ ion in the range from 1.6 to 8.6 μmol $L^{-1}$ (Fig. 6), so a linear regression equation y = –14.5675x + 157.9617 (x is the concentration of $Hg^{2+}$, and y is the fluorescence intensity, linear correlation coefficient $R^2$ = 0.9935) could be applied. From the changes in $Hg^{2+}$-dependent fluorescence intensity, the detection limit of **3** to $Hg^{2+}$ was determined to be 0.68 μmol $L^{-1}$ according to the calculation method reported in the literature, thus **3** exhibited a high sensitivity toward $Hg^{2+}$.[16] The response time of **3** to $Hg^{2+}$ was also demonstrated to be short (Fig. 7). The intensity of the fluorescent signal at 412 nm quickly reached the minimum level, and was unchanged as time went by, therefore, **3** could be used for real-time tracking of $Hg^{2+}$. Furthermore, the stoichiometry of the complex formation of **3** with $Hg^{2+}$ was determined by a Job's plot at room temperature. The emission changes at 412 nm were plotted against molar fractions of **3** under the conditions of an invariant total concentration (10 μmol $L^{-1}$). As a result, when the molar fraction of [**3**] / ([**3**] + [$Hg^{2+}$]) was about 0.5, the changes of fluorescence emission approached a maximum, indicating formation of a 1:1 complex between **3** and $Hg^{2+}$ (Fig. 8). On the basis of a 1:1 stoichiometry, the association constant of **3** for $Hg^{2+}$ was determined to be $1.08 \times 10^6$ $L \cdot mol^{-1}$ according to the Benesi–Hildebrand equation.[17]

Based on above experiments and some recent reports, we propose a possible complexation mechanism between **3** and $Hg^{2+}$, as shown in Scheme 2.[18–20] In the chelation by **3**, two sulfur atoms, one amino–nitrogen atom and one oxygen atom of amide might bind with $Hg^{2+}$, the sulfur, oxygen and nitrogen atoms donating their lone pair of electrons to the empty orbitals of $Hg^{2+}$. The capture of $Hg^{2+}$ caused electron or energy transfer between the chelating unit and the benzoxazole fluorophore, thus resulting in the fluorescence quenching of the benzoxazole fluorophore.

In conclusion, we have synthesised a benzoxazole-based fluorescent probe **3**, which is selective and sensitive to $Hg^{2+}$. The detection limit of **3** to $Hg^{2+}$ was determined to be 0.68 μmol $L^{-1}$, and **3** forms a 1:1 complex with $Hg^{2+}$.

## Experimental

*General*

Unless otherwise noted, all reagents were purchased from commercial companies and directly used without further purification. The melting point was determined with an XT4A micromelting point apparatus and was uncorrected. The $^1$H NMR and $^{13}$C NMR spectra were recorded on a Mercury Plus-400 spectrometer in $CDCl_3$. Electrospray ionisation mass spectra (ESI-MS) were acquired on an Applied Biosystems API 2000 LC/MS/MS system. IR spectra were performed on a Perkin-Elmer Spectrum BX FT-IR instrument in tablets with potassium bromide. Elemental analyses were carried out on a Perkin-Elmer 2400 instrument. Fluorescence spectra were performed on a FluoroMax-P spectrofluorimeter.

*Synthesis*

The intermediates 4-(benzo[*d*]oxazol-2-yl)aniline (**1**) and bis[2-(ethylthio)ethyl]amine were prepared according to the reported procedure.[21,22]

N-*[4-(benzo[*d*]oxazol-2-yl)phenyl]-2-chloroacetamide* (**2**): Chloroacetyl chloride (1.12 g, 10 mmol) was added dropwise to a solution of the intermediate **1** (1.05 g, 5 mmol) and triethylamine (1.01 g, 10 mmol) in anhydrous tetrahydrofuran (40 mL), cooled in an ice bath. The resulting mixture was stirred rapidly for 4 h, then the solvent was evaporated to give the crude product, which was further purified *via* column chromatography

(silica, petroleum ether/EtOAc, 5:1) to afford a pale yellow solid (1.29 g, 90%); m.p. 189–190 °C; IR (KBr): 3150, 2935, 1685, 1525, 1440, 1248 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$, ppm): δ 4.38 (s, 2H), 7.18 (s, 1H), 7.38 (d, *J* = 7.6 Hz, 2H), 7.72 –7.82 (m, 4H), 7.92 (d, *J* = 8.7 Hz, 2H); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 168.4, 160.6, 150.2, 142.4, 139.6, 128.2, 127.5, 125.6, 124.8, 120.6, 119.8, 112.3, 44.1; ESI-MS: *m/z* 286.2 [$M^+$]. Anal. calcd for $C_{23}H_{29}N_3O_2S_2$: C, 62.84; H, 3.87; N, 9.77; found: C, 62.55; H, 3.54; N, 9.56%.

N-*[4-(benzo[*d*]oxazol-2-yl)phenyl]-2-{bis[2-(ethylthio)ethyl] amino}acetamide* (**3**): The intermediate **2** (0.58 g, 2 mmol), $K_2CO_3$ (0.28 g, 2 mmol) and a catalytic amount of KI (0.02 g) were added to a solution of bis[2-(ethylthio)ethyl]amine (0.38 g, 2 mmol) in acetonitrile (100 mL). The mixture was refluxed for 24 h under stirring. The progress of the reaction was monitored by TLC. After concentration under reduced pressure, the yellow crude product was purified *via* column chromatography (silica, petroleum ether/EtOAc, 1:1) to give a yellow solid (0.58 g, 65%); m.p. 59–60 °C; IR (KBr): 3136, 2968, 1682, 1545, 1445, 1386, 1292, 1114 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$, ppm): δ 1.26 (t, 6H, *J* = 7.6 Hz), 2.52–2.94 (m, 12H), 3.32 (s, 2H), 7.29 (s, 1H), 7.37 (d, 2H, *J* = 7.2 Hz), 7.59–7.93 (m, 4H), 8.25 (d, 2H, *J* = 7.8 Hz); $^{13}C$ NMR (100 MHz, $CDCl_3$, ppm): δ 169.9, 162.9, 150.7, 142.1, 140.1, 128.6, 127.8, 124.5, 122.4, 119.8, 119.4, 110.5, 59.4, 54.3, 30.1, 25.8, 14.7; ESI-MS: *m/z* 443.2 [$M^+$]; Anal. calcd for $C_{23}H_{29}N_3O_2S_2$: C, 62.27; H, 6.59; N, 9.47; found: C, 62.42; H, 6.88; N, 9.22%.

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