Synthesis, crystal structure and fluorescent properties of a novel benzothiazole-derived fluorescent probe for Zn²⁺

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A novel turn-on fluorescent probe containing a benzothiazole fluorophore has been synthesised and its structure confirmed by single crystal X-ray diffraction. The probe exhibited high selectivity to Zn^{2+} over other metal ions and demonstrated high sensitivity for Zn^{2+} with a 12-fold fluorescence enhancement. It also showed a fast response to Zn^{2+} and a complexation ratio towards Zn^{2+} of 1:1.

Keywords: benzothiazole, crystal structure, Zn²⁺, fluorescent probe, selectivity, sensitivity

Zinc is the second most abundant d-block metal after iron in the human body. It plays an important role in various biological processes including structural cofactors, gene expression, catalytic centres, regulators of enzymes and neural signal transmission.¹⁻³ In industry, zinc is thought to be an environmental contaminant, an excess of Zn²⁺ may reduce soil microbial activity and produce phytotoxic effects.^{4,5} Therefore, the use of Zn²⁺ chemosensors in environmental and biological systems is of great interest. Many recent studies have reported the successful application of Zn²⁺ fluorescent probes based on quinoline, fluorescein, rhodamines, coumarin, indole and naphthalimide.6-12 Although there are several commercially available Zn²⁺ probes to satisfy different needs, the search for readily accessible Zn²⁺ fluorescent probes with high selectivity and sensitivity is still a challenge.¹³ We now report a new fast-response, highly selective and sensitive fluorescent probe for Zn²⁺.

Results and discussion

Benzothiazole is often used as a fluorophore for designing fluorescence probes to detect metal ions due to its good photophysical properties.^{14,15} We have designed a benzothiazole-based fluorescent probe **3**, in which the benzothiazole group is linked to a chelating moiety 2-picolylamine (Scheme 1). Compound **3** was synthesised by a nucleophilic substitution reaction of a primary halogenoalkane and secondary amine, and it was fully characterised by IR, NMR, MS, elemental analysis and single crystal X-ray diffraction. Metal-binding properties toward cations showed that **3** is highly selective and sensitive to Zn^{2+} in a aqueous EtOH solution.

Yellow crystals of **3** were obtained by slow evaporation of an ethyl acetate solution at room temperature. In its crystal structure, the benzothiazole ring system and neighbouring benzene ring comprise the fluorescent moiety of **3**; they are nearly coplanar, and the dihedral angle between them is 13.85 (2)° (Fig. 1). In

the chelating moiety of **3**, the dihedral angle of the two pyridine rings is 37.17 (2)°, and their centroid-to-centroid distance is 5.826 nm. The angle C(16)–N(3)–C(22) is 114.19(2)°, which leads to the V-shape of the chelating moiety. The C–N bond distances range from 1.294(2) to 1.463(2) Å, and the C–N (benzothiazolyl) bonds are shorter than the C–N(amino) bonds. The bond length of C(13)-N(2) is longer than that of C(14)-N(2), due to the fact that C(14) is *sp*² hybridised, whereas C(13) is part of the aromatic ring. The C(7)–S(1) bond length [1.747 (2) Å] is also found to be longer than the C(1)–S(1)bond length [1.722(2) Å] for the same reason.

The maximum excitation (λ_{ex}) and emission (λ_{em}) wavelengths of **3** in EtOH/tris-HCl buffer (V/V = 1/4, pH 7.4) are 355 and 470 nm, respectively. The selectivity of **3** (10 μ mol L⁻¹) toward various cations (K⁺, Ca²⁺, Na⁺, Mg²⁺, Mn²⁺, Fe²⁺, Fe³⁺, Co²⁺, Ni²⁺, Cu²⁺, Zn²⁺, Ag⁺, Pb²⁺, Cd²⁺, Cr³⁺ and Hg²⁺) was investigated using fluorescent spectrometry. As illustrated in Fig. 2, significant fluorescent enhancement of 3 was observed on addition of Zn^{2+} (30 µmol L⁻¹). Under identical conditions, the other tested cations showed nearly negligible fluorescence changes, except that Cu²⁺ and Co²⁺ responded with different extent of decrease in the fluorescent intensity. Moreover, ion interference experiments further illustrated that 3 (10 μ mol L⁻¹) was selective for the recognition of Zn²⁺ by the addition of 10 equiv. of competing metal ions (Fig. 3). The fluorescence enhancement of 3 caused by most of the mixtures of Zn²⁺ with other cations was similar to that caused by Zn2+ alone. A fluorescence quenching effect was found when Zn^{2+} was mixed with Cu^{2+} or Co^{2+} , demonstrating that these paramagnetic metal ions might compete with Zn2+ for binding with 3. These facts suggested that probe 3 could recognise Zn^{2+} with high selectivity.

To get an insight into the sensitivity of **3** to Zn^{2+} , fluorescence titration experiments were performed (Fig. 4). Probe **3** displayed a weak fluorescence with a quantum yield (Φ) of



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Fig. 1 An ORTEP drawing of 3 with displacement ellipsoids shown at the 30% probability level.



Fig. 3 Fluorescence response of 3 (10 $\mu mol~L^{1})$ to $Zn^{2^{+}}$ in the presence of different metal ions (100 $\mu mol~L^{-1}).$



Fig. 2 Fluorescence intensities of probe 3 (10 μ mol L⁻¹) upon addition of various metal ions (30 μ mol L⁻¹) in CH3CH2OH/Tris-HCl buffer (1/4, V/V, pH 7.4).



Fig. 4 The fluorescence spectral changes of 3 (10 µmol L⁻¹) in the presence of increasing Zn^{2+} concentrations (0-30 µmol L⁻¹), the inset shows the linear relationship between the emission intensity of 3 and concentrations of Zn^{2+} (0.2-3.2 µmol L⁻¹).



Fig. 5 Effect of pH on the fluorescence intensity of 3 (10 $\mu mol~L^{-1})$ measured with and without $Zn^{2+}.$



Fig. 6 Response of fluorescence intensity of 3 (10 μ mol L⁻¹) in the presence of Zn²⁺ as a function of time, the inset shows the visual fluorescence colour of 3 before (left) and after (right) incubation with Zn²⁺ under UV light (365 nm).



Fig. 7 Job's plot of probe 3 and Zn^{2+} ([3]+ [Zn^{2+}] = 10 mmol L⁻¹).

0.05. On addition of Zn²⁺, the fluorescence intensity increased remarkably. When the concentration of Zn²⁺ increased to 3 equiv., the fluorescence response reached a maximum point, and an approximately 12-fold fluorescence enhancement could be observed ($\Phi = 0.58$). As depicted in the inset in Fig. 4, the fluorescent intensity of **3** was nearly proportional to the concentration of Zn²⁺ in the range of 0.2–3.2 µmol L⁻¹ (linear correlation coefficient $R^2 = 0.9913$). The detection limit of **3** to Zn²⁺ was determined to be 0.68 µmol L⁻¹ according to the calculation method reported in the literature.¹⁶

The influence of pH on the fluorescence of **3** was also examined (Fig. 5). The fluorescence intensity of **3** was nearly invariable over a wide pH value range of 2–12, however, upon addition of Zn^{2+} , it was enhanced between pH 6 and 10, indicating that it could be applied to the detection of Zn^{2+} in pH values close to physiological conditions. Furthermore, the response time of **3** (10 µmol L⁻¹) to Zn^{2+} was explored (Fig. 6). It was found that the fluorescence enhancement reached a plateau after 30 s, indicating the quick response of **3** to Zn^{2+} . This fluorescence change was also observable by the naked eye (Fig. 5 inset). Under irradiation at 365 nm, the fluorescence colour of **3** turned from pale blue to bright blue after the addition of Zn^{2+} .

In order to determine the binding stoichiometry between **3** and Zn²⁺, a Job's plot experiment was employed. In Fig. 7, the emission intensity at 470 nm is plotted as a function of the molar fraction of Zn²⁺, and the total molar concentration of **3** and Zn²⁺ was 10 μ mol·L⁻¹. Maximum fluorescence intensity was reached when the mole fraction was 0.5, indicating that Zn²⁺ formed a 1:1 complex with **3**.

Based on above results and some recent reports, we propose a possible sensing mechanisms of probe **3** for Zn^{2+} (Scheme 2).^{17,19} The weak fluorescence emission of probe **3** can be attributed to the emission of its normal excited state, and the photoinduced electron transfer (PET) from aliphatic amine nitrogen to the excited state of the phenylbenzothiazole moiety (fluorophore) decreased its emission intensity. Zn^{2+} binding to **3** could not only induce a conformation restriction but also block PET quenching of the singlet excited state of the fluorophore, thus a drastic fluorescence enhancement was observed.

In conclusion, we have prepared a benzothiazole-based fluorescent probe **3** for Zn^{2+} , which has been characterised by single crystal X-ray diffraction. Probe **3** was sensitive and selective to Zn^{2+} , even in presence of an excess of other metal ions. It also displayed rapid recognition of Zn^{2+} and formed a 1:1 complex with Zn^{2+} .

Scheme 2 The proposed sensing mechanisms of probe 3 for Zn²⁺.

Experimental

All reagents were purchased from commercial companies and directly used without further purification, unless otherwise noted. Melting points were determined with an XT4A micro melting point apparatus and were uncorrected. IR spectra were performed on a PerkinElmer Spectrum BX FT-IR instrument in tablets with potassium bromide. The ¹H NMR spectra were recorded on a Mercury Plus-400 spectrometer in CDCl₃. Electrospray ionisation mass spectra (ESI-MS) were acquired on an Applied Biosystems API 2000 LC/MS/MS system. Elemental analyses were carried out on a PerkinElmer 2400 instrument. Fluorescence spectra were measured on a FluoroMax-P spectrofluorimeter.

Synthesis

The intermediates 1 and 2 were synthesised according to reported procedures.^{17,20} Fluorescent probe **3**, 2-picolylamine (0.40 g, 2 mmol) and the intermediate 2 (0.60 g, 2 mmol) were dissolved in acetonitrile (100 mL), then K₂CO₃ (0.28 g, 2 mmol) and a catalytic amount of KI (0.05 g) were added to the resulting solution. After stirring and refluxing for 24 h until the reaction was complete as detected by TLC, the mixture was cooled to room temperature, and the solvent was removed under reduced pressure. The residue was purified using column chromatography (silica, AcOEt/CH₂Cl₂, 5/1, v/v) to give a yellow solid. Yield 78%; m.p. 116-117 °C. IR (KBr): 3430, 3150, 2935, 1675, 1568, 1525, 1440 cm⁻¹. ¹H NMR (400 MHz, CDCl₂, ppm): 8.66 (d, J = 7.8 Hz, 2H), 7.86–8.21 (m, 5H), 7.25–7.56 (m, 10H), 4.06(s, 4H), 3.44(s, 2H). ¹³C NMR (75 MHz, CDCl₃): 170.8, 166.6, 158.1, 154.6, 148.3, 139.5, 138.8, 134.7, 129.2, 128.8, 127.9, 127.6, 125.7, 125.6, 125.2, 124.9, 122.6, 121.8, 116.4, 63.2, 59.8. ESI-MS: m/z 465.2 (M⁺). Anal. calcd for C₂₇H₂₃N₅OS: C, 69.65; H, 4.98; N, 15.04; found: C, 69.28; H, 5.18; N, 15.32%.

X-ray diffraction study of 3

A yellow crystal of **3** having approximate dimensions of 0.12 mm × 0.10 mm ×0.10 mm was mounted on a glass fibre in a random orientation at 298(2) K. The determination of unit cell and the data collection were performed using MoK α radiation ($\lambda = 0.71073$ Å) on a Bruker APEX-II CCD diffactometer. A total of 22280 reflections were collected in the range of 1.81 < θ < 30.00° at room temperature. The structure was solved by direct methods and semi-empirical absorption corrections were applied. The non-hydrogen atoms were refined anisotropically and the hydrogen atoms were determined by theoretical calculation. The final cycle of full matrix least-squares refinement was based on 6689 independent reflections [I > 2 σ (I)] and 308 variable parameters with R¹ = 0.0477, wR² = 0.1333. All calculations were carried out on a PC using SHELXS–97 and SHELXL–97 programs.^{21,22} *Crystal data*: C₂₇H₂₂N₅OS, *M* = 465.56,

Monolinic, space group P 2(1)/C, a = 9.4764(17) Å, b = 22.451(4) Å, c = 10.919(2) Å, $\alpha = 90^{\circ}$, $\beta = 98.013(3)^{\circ}$, $\gamma = 90^{\circ}$, V = 2300.3(7) Å³, Z = 4, Dc = 1.344 Mg m⁻³, $\mu = 0.172$ mm⁻¹. The data have been deposited as CCDC 1416988 which contains the supplementary crystallographic information for this paper. These data can be obtained free of charge at <u>www.ccdc.cam.ac. uk/conts/ retrieving.html</u> or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; E-mail: deposit@ccdc.cam.ac.uk.

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