A "switching on" fluorescent chemodosimeter of selectivity to Zn^{2+} and its application to MCF-7 cells†

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A highly sensitive and selective 1,8-naphthyridine-based fluorescent chemodosimeter for Zn^{2+} has been designed, synthesized, and applied to the detection of intracellular Zn^{2+}.

The design and synthesis of fluorescent probes with high selectivity and sensitivity is a vibrant field of supramolecular chemistry for their fundamental role in medical, environmental and biological applications.[1] The zinc ion (Zn^{2+}), the second most abundant transition metal in the human body, plays vital roles in numerous biological processes, such as gene transcription, regulation of metalloenzymes, and neural signal transmission.[2] Hence, the development of Zn^{2+}-probes is a promising field. Rapid progress has been made in the development of fluorescent Zn^{2+}-probes in solution,[4,5] due to their simplicity, high sensitivity and instantaneous response,[3] and based on ion-induced fluorescence changes. However, reports of their intracellular application are rare.[5] It is desired to develop new fluorescent indicators with improved properties, especially with high efficiency in the spectral visible region and study of Zn^{2+}-probes in cell.

The aim of this communication is to design and construct a fluorometric assay to highly effectively detect Zn^{2+} both in solution and cell. Because of their various photoluminescent properties and short metal···metal distance formed in their metal building blocks, 1,8-naphthyridine and its derivatives have been widely used as guanine recognition regents and bidentate ligands.[6] However, there are very few reports about the derivatives of 1,8-naphthyridine as fluorescent probes for transition metals. In our previous work, a 1,8-naphthyridine-based Zn^{2+}-chemodosimeter was designed and studied.[7] Considering the above, 1,8-naphthyridine group was used herein as the reaction unit with Zn^{2+}. On the other hand, in general, compounds containing acyclic C=N bonds are non-fluorescent, and cyclic C=N bonds significantly fluorescent.[8] (*E*)-2-(4-Hydroxyphenylimino)-4-methyl-7-acetamidyl-1,8-naphthyridine (**1**; Chart 1) was designed, in which the naphthyridine group and the C=N bond can react with Zn^{2+} to make the acyclic C=N bond become to cyclic C=N bond, resulting in a fluorescent product.

Herein, we report the Zn^{2+}-selective recognizing properties of compound **1**. Compound **1** possesses an efficient Zn^{2+}-selective OFF–ON fluorescent behavior both in solution and cell.

Compound **1** was synthesized according to the reported method.[9] The synthetic route (Scheme S1), detailed characterization and the crystal structure of **1**[10] (Fig. S1) are shown in the ESI.†

The absorption spectra change of **1** in ethanol upon addition of Zn^{2+} is shown in Fig. S2.† Upon gradual addition of Zn^{2+} into the ethanol solution of **1**, a new absorption peak at about 428 nm appeared and its intensity increased while the original absorption peak at 378 nm weakened ($\varepsilon = 1.99 \times 10^4$ M^{-1} cm^{-1}). The red-shift of the absorption peak can be ascribed to reformed electronic orbits and new energy gaps in the product of compound **1** and Zn^{2+}.

Compound **1** hardly exhibits fluorescence emission in ethanol upon excitation at 370 nm (Fig. 1). Upon titration of Zn^{2+}, a new fluorescence emission peak at about 505 nm appeared and the intensity was dramatically enhanced, indicating a Zn^{2+}-selective fluorescent signaling behavior. Because of the great enhancement of emission at 505 nm ($F/F_0 = 15$) upon addition of zinc ions, compound **1** indicates an efficient Zn^{2+}-selective OFF–ON fluorescent behavior. The increased emission intensity is probably due to the complex formed between $ZnCl_2$ and **1**, in which the acetamide group was hydrolyzed to amino group and the rotation of acyclic C=N is inhibited.[4c,7] The possible reaction mechanism of Zn^{2+} and **1** is depicted in Scheme 1, which was further confirmed by ESI data measured after addition of Zn^{2+} into the ethanol solution of **1**.[11] The insets in Fig. 1 demonstrate the relationship between the concentration of Zn^{2+} and the emission intensity at a special wavelength.

To validate the proposed reaction mechanism of **1** and Zn^{2+}, ^1H NMR spectra of **1** in DMSO-d_6, **1** in the mixture of CD_3OD and DMSO-d_6, and **1** with Zn^{2+} in the mixture of CD_3OD and DMSO-d_6 were measured, as shown in Fig. 2. The difference between Fig. 2a and b is the decrease of signal

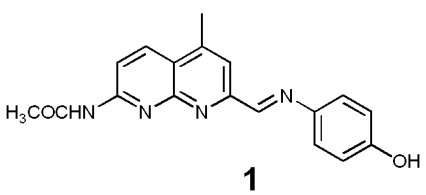

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Chart 1

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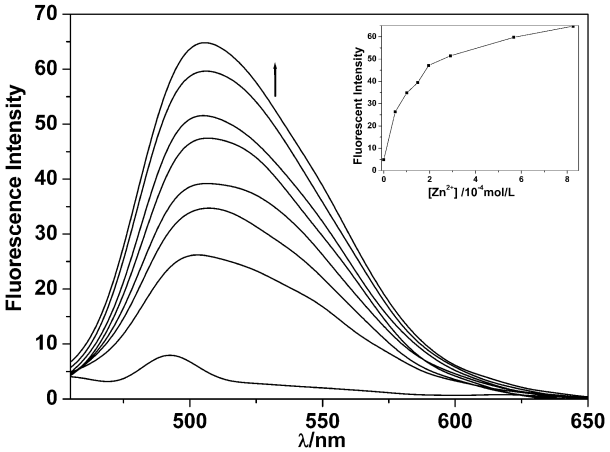
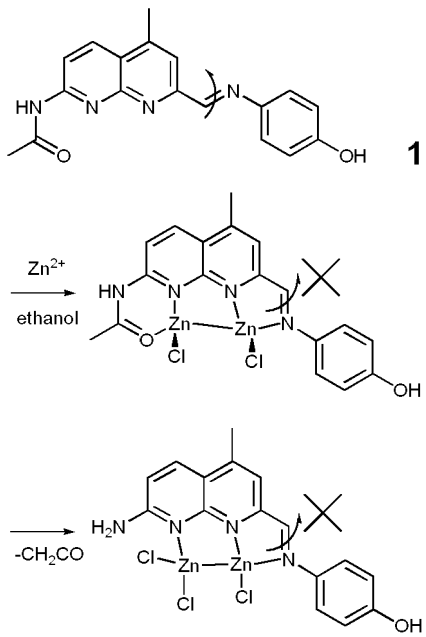


Fig. 1 Changes in fluorescence emission spectra of **1** (5.0×10^{-5}M) in ethanol upon addition of Zn^{2+}. The final ratio of zinc ion to **1** is 12 equiv. Inset: Emission intensities of **1** at 505 nm (black dot) as a function of addition of Zn^{2+}. The concentration of Zn^{2+} changed from 0 to 6.0×10^{-4} M and excitation was at 370 nm.



Scheme 1 Proposed reaction mechanism of Zn^{2+} with **1**.

intensity at around 9.73, which was assigned to the proton exchange between H_b and CD_3OD. After addition of Zn^{2+} into DMSO-d_6 and CD_3OD solution of **1**, the decrease of the signal intensity at around 9.73 and the shift to downfield of the signal at around 2.17 (Fig. 2b and c) are the most significant. Such changes are ascribed to the reaction between acetylamide group and Zn^{2+}. Combined with the ESI data, the reaction mechanism of Zn^{2+} and **1** was further confirmed. Moreover, the ^{1}H NMR spectra of **1** and Zn^{2+} (Fig. 2) and Job's plot (Fig. S7, Table S1†) reveal a 1 : 2 binding model between **1** and Zn^{2+}. The binding constants of **1** with Zn^{2+}, Cu^{2+}, Co^{2+} and Ni^{2+} were calculated (Fig. S7–S10, Table S1†).

The experiments of the counterion effect on the selective properties of Zn^{2+} demonstrate that nitrate and acetate anions have little influence on the selective effect of **1** on

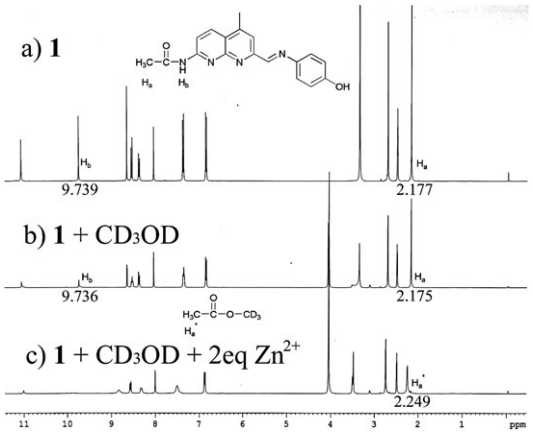


Fig. 2 ^{1}H NMR spectra (400 MHz, DMSO-d_6, 298 K) of (a) **1**; (b) **1** + CD_3OD-d_4; (c) **1** + CD_3OD-d_4 + 2 eq Zn^{2+}.

Zn^{2+} (Fig. S4–S6†). As for the sulfate anion, the addition of $ZnSO_4$ enhanced the fluorescence of **1** very little, which may be ascribed to the uncoordinated ability of sulfate anion.

The selectivities of **1** to the specific metal ions were examined in ethanol. As illustrated in Fig. S3† and in the left part of Fig. 3, upon addition of the same amount of the various metal ions, only Zn^{2+} enhanced the emission of **1**. Compound **1** did not respond distinctly to alkali and alkaline earth metal ions such as Ca^{2+}, Mg^{2+}, Na^+, and K^+. As for the first-row transition-metal ions, Fe^{2+}, Cu^{2+}, Co^{2+} and Ni^{2+} decreased the emission intensity while Mn^{2+} and Cr^{2+} had little influence on the fluorescence intensity of **1**. The selectivity of **1** toward Zn^{2+} suggests that **1** has potential application in sensing Zn^{2+}.

Because Ca^{2+}, Mg^{2+}, Na^+ and K^+ exist at high concentration under physiological conditions, it is necessary to

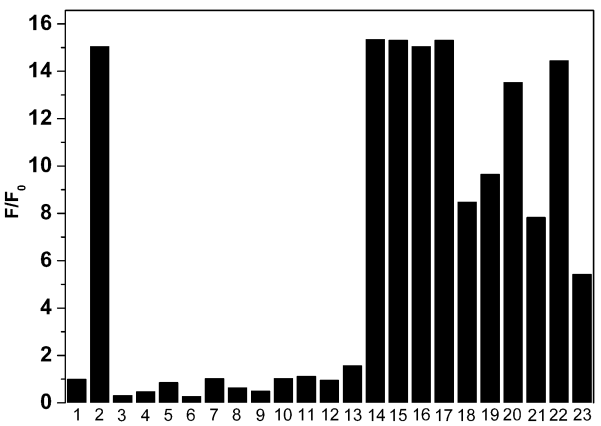


Fig. 3 Fluorescence responses of **1** to various metal ions (left part from 1 to 13 represents compound **1** only, Zn^{2+}, Cu^{2+}, Co^{2+}, Cr^{3+}, Fe^{2+}, Hg^{2+}, Mn^{2+}, Ni^{2+}, Na^+, Mg^{2+}, K^+ and Ca^{2+}, respectively) and fluorescence change of the mixture of **1** and Zn^{2+} after addition of an excess of the appropriate metal ions (right part from 14 to 23 represents Na^+, Mg^{2+}, K^+, Ca^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+}, Co^{2+}, Cr^{3+} and Ni^{2+}, respectively. The equiv. ratio of Zn^{2+} to each of Ca^{2+} Mg^{2+}, Na^+, K^+ is 1 : 10 and the equiv. ratio of Zn^{2+} to each of the other competing metal ions is 5) (right part from Zn^{2+} represented in Fig. 3). Bars represent the ratio of the difference between F to F_0. F and F_0 represent the emission intensity at 505 nm. The overall emission spectra were measured at excitation of 370 nm.

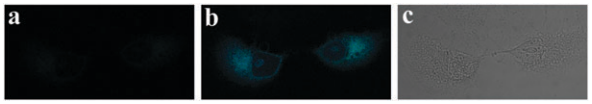


Fig. 4 The intracellular Zn^{2+} was imaged in living cells at 37 °C with use of confocal microscopy. (a) MCF-7 cells incubated with 10 μM of compound **1** solution (the volume ratio of ethanol and water is 4 to 6) for 30 min. (b) MCF-7 cells in part a 10 min after being treated with 2×10^{-2} μmol of Zn^{2+} aqueous solution. (c) Bright field image of living MCF-7 cells in parts a and b.

explore the disturbance of such metal ions on the detection of Zn^{2+}. The titration of Zn^{2+} and **1** in the presence of various metal ions was conducted, and the experimental results (the right part in Fig. 3) indicate that the fluorescence of the complex resulting from the reaction of Zn^{2+} and **1** was not influenced by the alkali and alkaline earth metal ions, even at a high concentration of 6 mM. Furthermore, the other physiological necessary metal ions such as Fe^{2+}, Cu^{2+}, Mn^{2+}, Cr^{2+}, Co^{2+}, Ni^{2+} were also applied to explore their disturbance on the detection of Zn^{2+}. When the ratio of Zn^{2+} to such metal ions is no less than 5, no influence was observed on the detection of Zn^{2+}.

The sensitivity of **1** to Zn^{2+} was examined in living cells by using confocal microscopy. Fluorescence images were recorded with excitation at 408 nm by an diode laser, Spinhole aperture, 100% gain of detector, and an oil objective with 60× magnification and 1.40 NA. The qualitatively *in vitro* results are exhibited in Fig. 4. After MCF-7 cells were incubated with 10^{-3} μmol **1** and 1 mL PBS for 30 min at 37 °C, no obvious fluorescence can be imaged (Fig. 4a). At the same experimental conditions, 10 min after 2×10^{-2} μmol of Zn^{2+} was introduced into the same MCF-7 cells, the strong fluorescence was imaged (Fig. 4b), which resulted from the reaction of **1** and Zn^{2+}. The bright field transmission images of these MCF-7 cells in Fig. 4c is exactly the same as the fluorescence image in Fig. 4b, confirming an intracellular fluorescence imaged.

In summary, a novel 1,8-naphthyridine-based fluorescent Zn^{2+}-chemodosimeter **1** has been designed and synthesized, and it displays high selectivity and sensitivity for Zn^{2+} in the presence of competing metal ions. In the presence of Zn^{2+}, significant fluorescence enhancement is achieved. The reason is probably that the acetamide group of **1** was hydrolyzed to amino group and the rotation of acyclic C=N is frozen. The sensitivity of **1** to Zn^{2+} was demonstrated in living cells, indicating its potential application for the intracellular imaging of Zn^{2+}.

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10 Crystal data for **1**: $C_{36}H_{32}N_8O_5$, M = 656.70, orthorhombic, space group $P\bar{1}$, a = 7.5496(15) Å, b = 13.657(3) Å, c = 30.427(6) Å, α = 90°, β = 90°, γ = 90°, V = 3137.1(11) Å^3, Z = 4, D_c = 1.390 Mg m^{-3}, F000 = 1376, MoKa radiation, λ = 0.71073 Å, T = 273(2) K, $2\theta_{max}$ = 55.64. 32 427 reflections measured, 13 839 unique (R_{int} = 0.0456). The structures were solved by direct methods and refined by a full-matrix least-squares technique on F2 using the SHELXH97 program. Final GooF = 1.195, R_1 = 0.0937, wR_2 = 0.1995, R indices based on 8981 reflections and 912 refined parameters, with $I > 2\sigma(I)$. CCDC 783331.

11 ESI data of the final compound from reaction of **1** and $ZnCl_2$: m/z) 514.5 [M], 279 [$M^+ + 1 - ZnCl_2 - ZnCl - CH_2CO$].