Tetrahedron Letters 51 (2010) 618–621

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Fluorescent chemosensor based on Schiff base for selective detection of zinc(II) in aqueous solution

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ARTICLE INFO

Article history:
Received 31 August 2009
Revised 9 November 2009
Accepted 17 November 2009
Available online 20 November 2009

ABSTRACT

A chemosensor **1**, based on the Schiff base, is easily prepared by reacting tryptophan and 2-hydroxy-1-naphthaldehyde in methanol. The optical properties of **1** are investigated in buffered aqueous solution, which displays specific recognition to Zn^{2+}, and especially avoids the interference of Cd^{2+} when **1** is tested against a range of physiological and environmentally relevant metal ions. Such a novel fluorescent probe can also be used to detect Zn^{2+} in live cells.

Zinc ion, the second most abundant transition-metal ion in the human body,[1] plays crucial roles in many important biological processes in acting as the structural and catalytic cofactors, neural signal transmitters or modulators, and regulators of gene expression, and apoptosis.[2] The negative effect of zinc ion appeared when its abnormal concentration existed in the natural and biological system. For example, in the environment an excessive concentration of zinc may reduce the soil microbial activity resulting in phytotoxic effect.[3] Moreover, it occurs commonly as an agricultural and food waste product in the environment. In the biological system, it is also known that disorders of zinc metabolism are closely associated with many severe neurological diseases such as Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), Guam ALS-Parkinsonism-dementia Parkinson's disease, hypoxia ischemia, and epilepsy.[4] Therefore, controlling the detection of Zn^{2+} is becoming essentially important in both the environment and biological systems.

Among the possible detection methods for Zn^{2+}, fluorescent chemosensors are becoming increasingly popular due to their ease of use in solution as well as their high selectivity and sensitivity to trace analytes. Recently, several typical chemosensors for Zn^{2+} have been developed.[5] However, in comparison with other transition-metal ions, the development of Zn^{2+} chemosensors has been limited due to the natural properties of zinc.[1a,6] The electron configuration of Zn^{2+} ($3d^{10}4s^0$) causes lack of intrinsic spectroscopic or magnetic signal. Specifically, although some zinc sensors have been developed, most of them confront various problems such as low water-solubility, bad selectivity, and/or few with fluorescent enhancements. Therefore, the design of effective and sensitive Zn^{2+} probe, especially that with both high water-solubility and fluorescent enhancements, has become crucially important.

It has been reported that the C=N isomerization was responsible for the predominant decay of the excited states of compounds containing an unbridged C=N structure, which usually resulted in weak fluorescence emission of the attached chromophore.[7] If this isomerization was prevented from by binding to metal ions, then enhancement of fluorescence emission could be achieved. In addition, it was also reported that a molecule possessing C=N structure generally has high affinity in coordinating with metal ions such as zinc and cadmium ions.[8] Despite being previously documented,[9] as a signal mechanism C=N isomerization has not been widely used in detecting metal ions in comparison with photoinduced electron/energy transfer (PET),[10] intramolecular charge transfer (ICT),[11] fluorescence resonance energy transfer (FRET),[12] and excimer mechanism.[13] Therefore, it was within our interests to develop a highly selective Zn^{2+} sensor by rationally moderating the structure of the corresponding groups of C=N. Therefore, we designed and synthesized a new Zn^{2+} chemosensor, 2-((2-hydroxynaphtha-

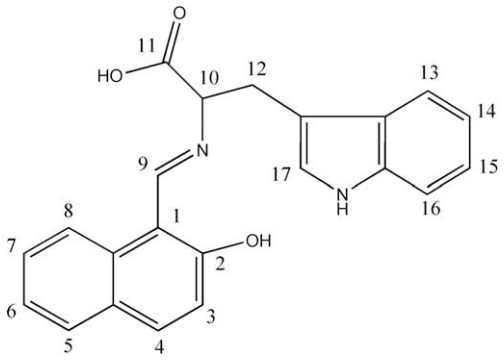

Figure 1. Molecular structure of 2-((2-hydroxynaphthalen-1-yl) methyleneamino)-3-(1*H*-indol-3-yl) propanoic acid (**1**).

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0040-4039/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2009.11.070

len-1-yl) methyleneamino)-3-(1*H*-indol-3-yl) propanoic acid (**1**) (Fig. 1), which possesses a complete solubility in aqueous environment, high selectivity to zinc ions, and easy synthesis. We choose the naphthalene group as the fluorophore due to its characteristic photophysical properties and the competitive stability in the environment. In addition, we combine 2-hydroxy-1-naphthaldehyde with tryptophan, which is inexpensive, water-soluble, and is expected to provide the binding site for the target metal ion. The structure of **1** was confirmed by its spectroscopic data (ESI (1)).

Under physiological conditions (10 mM Tris–HCl, pH 7.5), compound **1** alone had very weak fluorescence emission at 458 nm when it was excited at 408 nm. The addition of Zn^{2+} to the solution of **1** resulted in a great enhancement of fluorescence emission. As shown in Figure 2, upon small concentration increments of Zn^{2+} a significant enhancement of the emission band at 434 nm occurred, which resulted in a 28 nm blue-shift and a large increase in the intensity (saturated at 100 μM Zn^{2+} with a ~200-fold enhancement) of the fluorescence emission.

We also investigated the response of compound **1** to Zn^{2+} at various pHs. Over the pH range tested, compound **1** alone was not sensitive to pH as illustrated by fluorescence intensity at 434 nm (Fig. S1). However, in the presence of Zn^{2+} the complex of **1**/Zn^{2+} had a strong pH-dependence, which showed a sensitive fluorescence response in the pH range of 7.5–8.7. As pH increased the fluorescence intensity of **1**/Zn^{2+} approached close to maximum at pH 7.5 due to the fact that **1** has a pK_{a2} = 7.38 (Figs. S2, S3) and the deprotonation of the hydroxyl may promote the binding between **1** and Zn^{2+}.

The Zn^{2+}-specific response of **1** was confirmed by screening the other metal ions. It is clearly observed from Figure 3 that there is a slight increase in the emission intensity ratio of I/I_0 when Pb^{2+} is added to the solution of **1.** In contrast, the ratio of I/I_0 decreased when **1** was mixed to the solution containing Co^{3+} or Ni^{2+}, which is due to the fact that transition-metal ions with the unoccupied molecular d-orbital often quench the fluorescence of fluorophores.[14] Essentially, phenomena similar to those of Co^{3+} and Ni^{2+} were observed when **1** was mixed to the solution containing Cd^{2+}. Meanwhile, when the remaining metal ions were added to the solution of **1** it exhibited no obvious change of I/I_0. Additionally, similar properties of Cd^{2+} and Zn^{2+} generally cause a strong interference when they are placed in solution together, which makes it arduous to distinguish one from the other[15] Consequently, the interference experiment of Cd^{2+} on the detection of Zn^{2+} by **1** was performed specially, and the results showed a little interference. Therefore, the sensor **1** has displayed a considerable ability

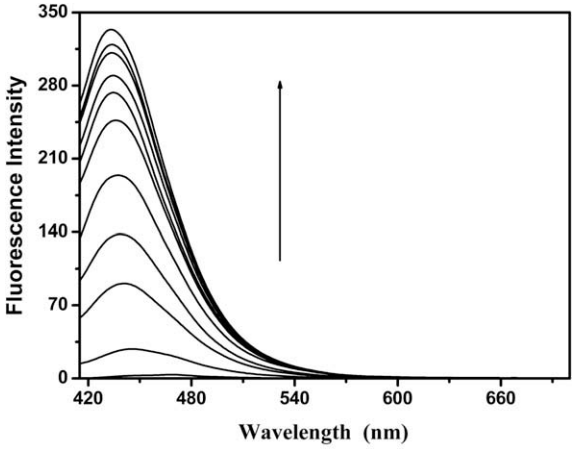

Figure 2. Fluorescence spectra of **1** (30 μM) upon addition of Zn^{2+} (0–100 μM) in buffer solution (10 mM, Tris–HCl, pH 7.5) with an excitation of 408 nm.

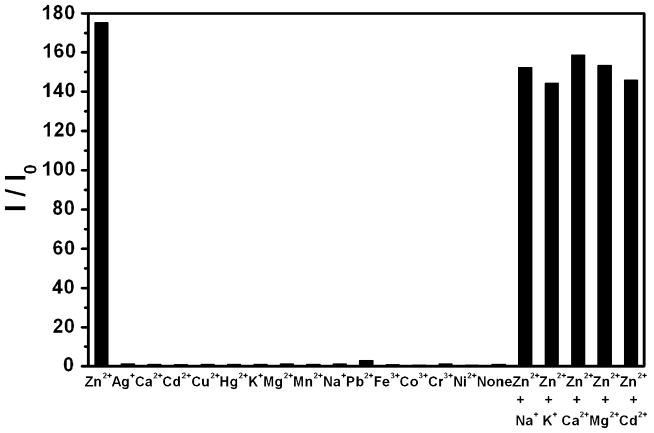

Figure 3. I/I_0 of **1** (30 μM) induced by the corresponding metal ions (2 equiv) in buffer solution (10 mM, Tris–HCl, pH 7.5). The concentration of **1** and that of metal ions used in interference measurement are the same.

to distinguish Zn^{2+} from Cd^{2+} in a common solution (as shown in Fig. 3). Furthermore, in exposing to high concentration of Na^+, K^+, Ca^{2+}, and Mg^{2+} (60 μM) in solution **1,** Zn^{2+} could be detected well, excluding the interferences of these ions as they are generally abundant in water and live cells. Therefore, **1** is a specific Zn^{2+} sensor in aqueous solution and may be useful in biological applications.

A study on the Zn^{2+}-sensing behaviors of **1** in B16-F10 cells was carried out by fluorescence microscopy. After incubation with **1** for 20 min at 37 °C, the cells displayed nonfluorescence (Fig. 4). However, after Zn^{2+} was introduced into the cells they exhibited visible fluorescence emission although naphthalene group has a relatively lower quantum yield. These results indicated that **1** can be used as a sensor to detect Zn^{2+} in live cells.

In order to explore the structural contribution of carboxylic acid in **1** to Zn^{2+} recognition, the involved proton was substituted by a methyl group, producing 2-((2-hydroxynaphthalen-1-yl) methyleneamino)-3-(1*H*-indol-3-yl) propanoate (**2**). After the addition of Zn^{2+} to **2**, only detectable quenching was induced rather than the enhancement or blue-shift of fluorescence emission (Fig. S4). Moreover, once the tryptophan of **1** was replaced by serine, histidine, or aspartic acid, slightly different but similar phenomena as **1** to metal ions were achieved. These results demonstrate that the carboxylate group of **1** plays a crucial role in the specific recognition of Zn^{2+}, but the indole ring residue may not be exclusively required for the recognition.

In exploring the binding model between **1** and Zn^{2+} Job's plot monitored by fluorescence emission shown in Figure 5 indicates the formation of a 1:1 complex. In addition, the H-B equation based on fluorescent titration spectra of the $I_0/(I - I_0)$ against $[Zn^{2+}]^{-1}$ (Fig. S5) resulted in the coordination of **1** with Zn^{2+} in a ratio of 1:1 with a binding constant (K_b) of 3.0×10^4 M^{-1} (R^2 = 0.9927).

Figure 4. Confocal fluorescence imaging of B16-F10 cells: (left) bright-field transmission image of cells labeled with **1** (100 μM) for 20 min incubation; (middle) fluorescence image of the same cells; (right) fluorescence image of the same cells after a further 20 min incubation with Zn^{2+} (1 equiv).

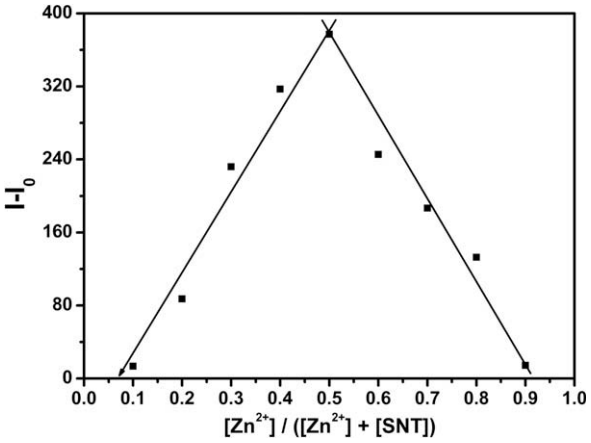

Figure 5. Job's plots according to the method for continuous variations. The total concentration of **1** and Zn^{2+} is 100 μM.

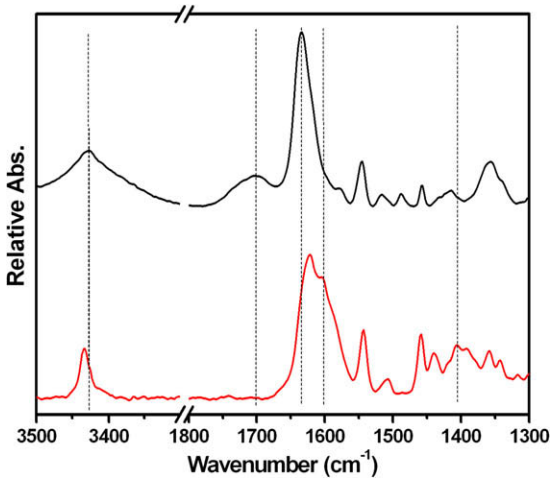

Figure 7. FT-IR spectra of **1** (top) and **1**/Zn^{2+} (bottom) in two representative regions.

To further understand the configuration of **1**/Zn^{2+}, density functional theory (DFT) calculations were done using B3LYP/6-31G level (GAUSSIAN03). The optimized configuration showed that one Zn^{2+} was occupied at the coordination center of **1** suitably and the complex formed a twisted tetrahedron (Fig. 6). The average bond length of Zn–O is reported as 1.98 Å (both for Zn–O(2) and Zn–O(11)), and those of Zn-$N_{-C=N}$ and Zn–Cl are reported as 2.06 Å and 2.24 Å, respectively. Based on these data, we proposed that the coordination of Zn^{2+} and $N_{-C=N}$ disrupted the decay of the excited state for compounds with an unbridged C=N structure, which led to the obvious fluorescence enhancement. The results of the theoretical calculation on the complex of **1**/Zn^{2+} appropriately answered the fluorescence emission phenomena of **1** in complexing with Zn^{2+} (Fig. 6).

In order to further validate the conjugation of **1**/Zn^{2+}, both FT-IR and ^{1}H NMR experiments (Table S1) were conducted on **1** and on the complex of **1**/Zn^{2+}. For the FT-IR spectra shown in Figure 7, after the coordination of **1** with Zn^{2+} the disappearance of the absorbance band of carboxylic acid ($\nu_{C=O}$ = 1702 cm^{-1}) and the appearance of the absorbance bands of carboxylate ($\nu^{as}_{COO^-}$ = 1604 cm^{-1}; $\nu^{s}_{COO^-}$ = 1406 cm^{-1}) are indicative of the participation of binding of the carboxylate group to Zn^{2+}. Furthermore, based on the previous reports[16] the large difference between $\nu^{as}_{COO^-}$ and $\nu^{s}_{COO^-}$ ($\Delta\nu$ = 198 cm^{-1}) accounted for a direct binding of the carboxylate to Zn^{2+} in the form of a monodentate. In addition, the vibrational mode of C–N (ν^{s}_{C-N} close to –C=N–) in **1** shifted from 1634 to 1621 cm^{-1}, reflecting the reduced conjugation effect between –C=N– and naphthyl after the direct binding between nitrogen atom and Zn^{2+}. For the hydroxyl group connected to naphthyl ring in **1**, a broad peak overlapping a sharp peak of ν_{NH} at the region of 3425 cm^{-1} was observed. After mixing with Zn^{2+}, the disappearance of the broad peak provided evidence for the direct interaction of O(2) and Zn^{2+} in the complex **1**/Zn^{2+}. In summary, the large differences of the IR spectra of **1** in the absence and presence of Zn^{2+} indicated that Zn^{2+} indeed directly interacted with O(2), O(11), and $N_{-C=N}$. Table S1 showed chemical shifts of several closely related protons of **1** in the presence and absence of Zn^{2+}. The relatively large changes of chemical shifts of H(9) (= 0.019 ppm) and H(10) (= 0.020 ppm) induced by Zn^{2+} (1 equiv) suggested the direct incorporation of nitrogen atom in –C=N and O(11) in the complex **1**/Zn^{2+}. Therefore, the data gained from the experiments were consistent with the theoretical calculation, indicating that the model established in Figure 6 was reasonable and appropriate for the complex **1**/Zn^{2+}.

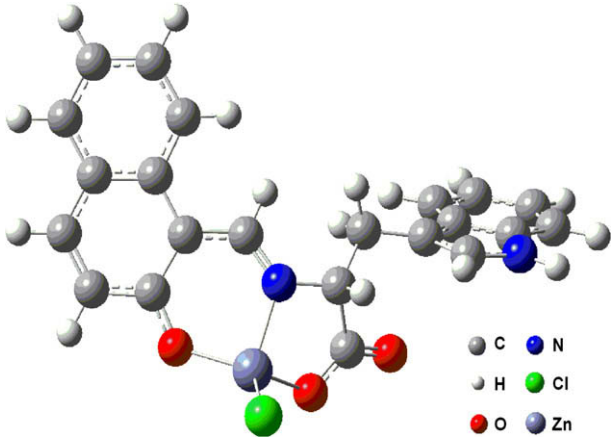

Figure 6. Conformation of **1**/Zn^{2+} optimized by density functional theory calculations.

In summary, we have designed and characterized a chemosensor **1** based on Schiff base, which showed high selectivity to Zn^{2+} over other metal ions of interest, especially Cd^{2+}. Moreover, although the cations of Na^+, K^+, Ca^{2+}, and Mg^{2+} are abundant in water as well as in live cells only a slight interference of them could be observed for Zn^{2+} detection. Further Zn^{2+}-sensing study in B16-F10 cells indicated that **1** could be used as a sensor to detect Zn^{2+} in live cells. All the results are agreeable with our original objectives and this strategy will facilitate the designing of more efficient chemosensors for other interesting metal ions in the future research.

Acknowledgements

The authors are grateful to the projects of the Natural Science Foundation of China (Nos. 20934002 and 20973073), the National Basic Research Program (2007CB808006), the Programs for New Century Excellent Talents in University (NCET), and the 111 Project (B06009). The authors also thank Mr. Greg R McKelvey, an exchanged undergraduate student from the University of West-Virginia, for correcting English.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.11.070.

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