



A new fluorescent chemosensor for Zn^{2+} with facile synthesis: “Turn-on” response in water at neutral pH and its application for live cell imaging

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ABSTRACT

A series of salicylaldehyde-2-pyridinehydrazone derivatives were found to exhibit interesting “turn-on” fluorescence response toward Zn^{2+} in water at neutral pH, which could be prepared readily from commercially available materials through a one-step reaction in ethanol at room temperature. Among these compounds, **1** showed a 22-fold fluorescence enhancement in 99% water/DMSO (v/v) at pH 7.0 with a linear range of 0.10–1.00 $\mu\text{mol/L}$ and detection limit of 30 nmol/L Zn^{2+} . Especially, **1** was not affected by Cd^{2+} which usually induced a comparable fluorescence response to that of Zn^{2+} in other reported works. Moreover, live cell imaging showed that **1** could be useful for monitoring Zn^{2+} within biological samples.

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1. Introduction

The development of chemosensors for metal ions with high selectivity and sensitivity is an active field in analytical chemistry. Fluorescent chemosensors for metal ions have attracted particular attention due to their advantages such as low cost, simplicity, good sensitivity, capability of real-time detection and non-destructive emission signals [1–4]. Zinc is the second most abundant transition metal ion after iron in the human body, and is a very important ion species in many biological activities such as neural signal transmission, gene expression, cellular metabolism and DNA binding or recognition [5–8]. To date, numerous fluorescent chemosensors for Zn^{2+} have been developed using coumarin, rhodamine, fluorescein, BODIPY, etc. as fluorophores and using di-2-picolyamine, quino-line, bipyridine, etc. as receptors [9–12], which exhibited excellent selectivity and sensitivity toward Zn^{2+} and some of them could be used for biological imaging in a physiological environment. Recently, our group have also reported fluorescent chemosensors for Zn^{2+} based on salicylaldehyde Schiff-base [13,14]. Nevertheless, most of these reported chemosensors have some limitations. On one hand, the preparations of complicated fluorophores and receptors lead to multiple-step synthesis with high cost [15–23]; on the other hand, because of the poor water-solubility for most fluorescent sensors, high content of organic cosolvents are necessary to

exhibit satisfying analytical performances [13,14,17,24–31], which is not compatible for the detecting of Zn^{2+} in biologic system and practical samples. Moreover, many reported fluorescent chemosensors for Zn^{2+} encountered difficulty in distinguishing Zn^{2+} from Cd^{2+} , because Cd^{2+} is in the same group of the periodic table with Zn^{2+} and usually induces a comparable fluorescent response to that of Zn^{2+} [17,18,21,22,32–34]. Therefore, it is challenging to develop a facily synthesized, sensitive and selective fluorescent chemosensor for Zn^{2+} that could be useful for monitoring Zn^{2+} in aqueous solutions and biological samples.

In this paper, salicylaldehyde-2-pyridinehydrazone-based “turn-on” fluorescent chemosensor **1** (3,5-dichloro-2-hydroxybenzaldehyde-2-pyridinehydrazone) and its control compounds for detecting Zn^{2+} in water at neutral pH were reported. Upon the addition of Zn^{2+} to an aqueous solution of **1** at neutral pH, enhancement of blue fluorescence emission was observed, enabling fluorescence “turn-on” detection of Zn^{2+} by naked-eye under illumination with a UV lamp (Fig. 1b). Especially, **1** is a Zn^{2+} -selective fluorescent chemosensor that is less affected by physiological relevant metal ions including Cd^{2+} , thus, live cell imaging of Zn^{2+} was successfully performed with **1**.

2. Experimental

2.1. Materials and methods

Unless otherwise noted, all the materials of analytical grade were used in the experiments without further purification.

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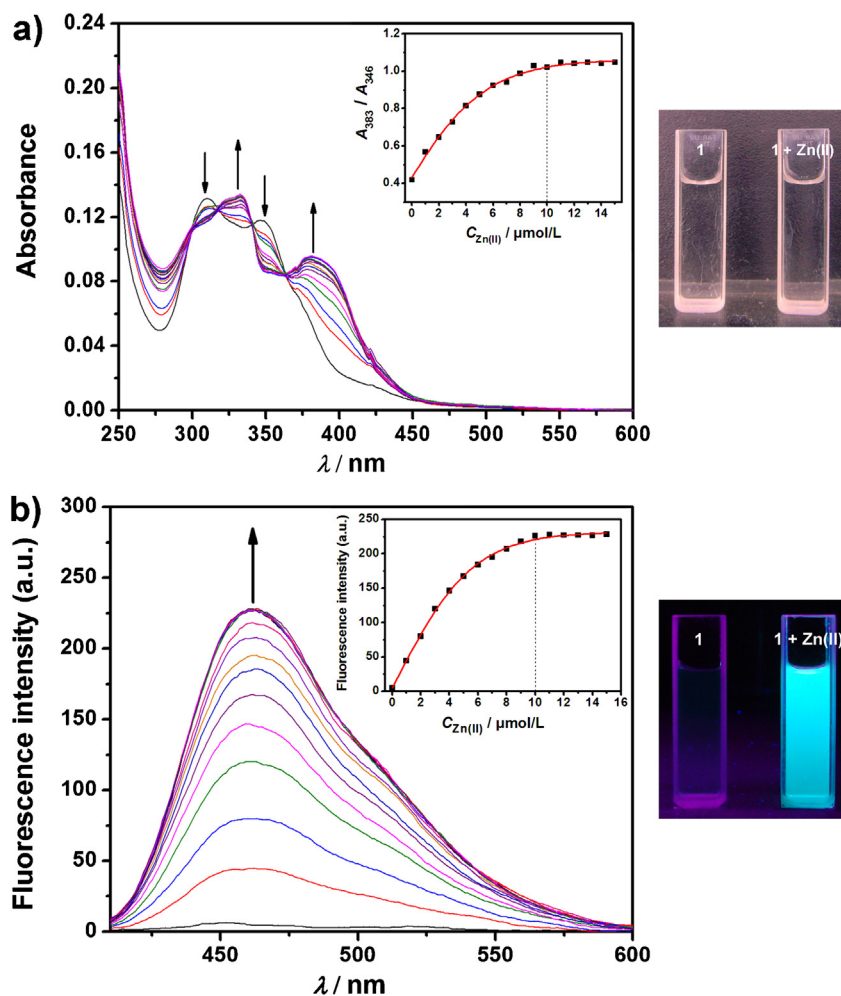


Fig. 1. (a) Absorption spectra of 10 $\mu\text{mol/L}$ **1** upon the addition of 0–15 $\mu\text{mol/L}$ Zn^{2+} . Inset: The absorbance ratio (A_{383}/A_{346}) as a function of Zn^{2+} concentration. (b) Fluorescence spectra of 10 $\mu\text{mol/L}$ **1** upon the addition of 0–15 $\mu\text{mol/L}$ Zn^{2+} . Inset: The fluorescence intensity at 460 nm as a function of Zn^{2+} concentration, excitation was at 364 nm. The photos on the right are **1** in the absence and presence of 1 equiv. Zn^{2+} in a glass cuvette excited by sun-light and UV light (365 nm). Conditions: [**1**] = 10 $\mu\text{mol/L}$, [Zn^{2+}] = 10 $\mu\text{mol/L}$, 99% water/DMSO (v/v) at pH 7.0 buffered by 10 mmol/L Tris–HCl.

3,5-Dichloro-2-hydroxybenzaldehyde, 3,5-dichlorobenzaldehyde, phenylhydrazine, pyriothione, 4-fluoro-2-hydroxybenzaldehyde, 5-chloro-2-hydroxybenzaldehyde, 5-bromo-2-hydroxybenzaldehyde, 2-hydroxybenzaldehyde, 4-methoxy-2-hydroxybenzaldehyde, 4-diethylamino-2-hydroxybenzaldehyde, tris(hydroxymethyl)aminomethane were purchased from Alfa Aesar Co., Tianjing, China. 2-Hydrazinopyridine was purchased from J&K Chemical Co., Beijing, China. Unless otherwise noted, all the other materials were purchased from Sinopharm Chemical Reagent Beijing Co., Beijing, China. Doubly distilled deionized water was used throughout the experiments. The solutions of all metal ions were prepared from their nitrate salts. Tris–HCl buffer solutions with different pH were prepared using 10 mmol/L Tris and proper amount of HCl or NaOH under adjustment by a pH meter. Absorption spectra were carried on a JASCO V-550 UV–vis spectrophotometer equipped with an ETC-505 peltier thermostatted single cell holder, 5 cm quartz cells. Fluorescence spectra were recorded on a JASCO FP-6500 spectrofluorimeter equipped with an ETC-273T peltier thermostatted single cell holder, 1 cm quartz cells. All pH measurements were made with a pH-3C pH meter (Shanghai Precision & Scientific instrument Co., Ltd, Shanghai, China). ^1H and ^{13}C NMR spectra were recorded using a JOEL JNM-ECA300 spectrometer operated at 300 MHz. ESI-MS spectra were carried on a HP 1100 LC–MS spectrometer without using the LC part. Elemental analyses were carried out on an Elementar vario

micro cube (Germany). Fluorescence imaging was observed under an Olympus FV-1000 confocal microscope.

2.2. Absorption and fluorescence measurements

Absorption and fluorescence titrations were recorded at 25 $^{\circ}\text{C}$, performed by addition of small aliquots of **1** and different metal ion working solutions to buffer stock solution (10 mmol/L Tris–HCl of desired pH) in a quartz cell. After being well mixed, the solutions were allowed to stand at 25 $^{\circ}\text{C}$ for 2 min, and then absorption or fluorescence spectra were recorded. For the analysis of Zn^{2+} containing water samples, 0.030 mL of 1 mmol/L stock solution of **1** and 0.600 mL of buffer stock solution (50 mmol/L Tris–HCl of pH 7.0) were added to 2.370 mL sample solutions, and then well mixed for 2 min before test.

2.3. Preparation of cells

HeLa Cells were obtained from NIH, and reagents for culture were purchased from HyClone. HeLa cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum, 1% penicillin and 1% streptomycin at 37 $^{\circ}\text{C}$ in a 5% CO_2 incubator. The cells were transferred to a glass-bottomed dish and incubated for 24 h before dye-loading.

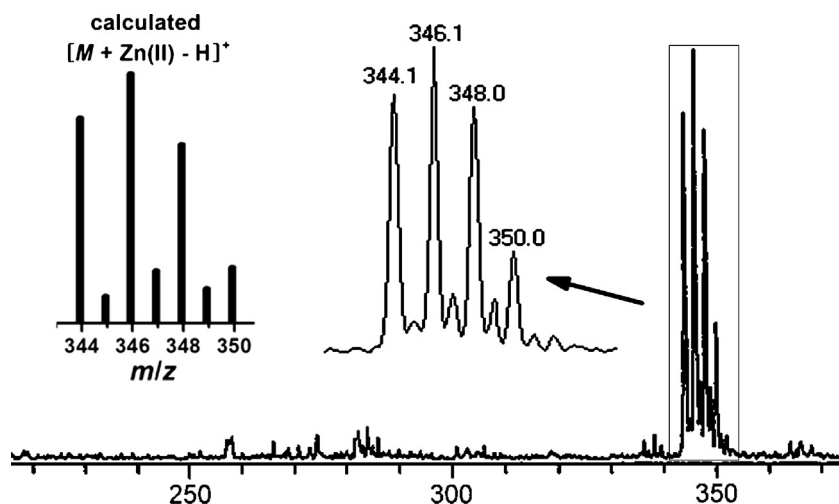
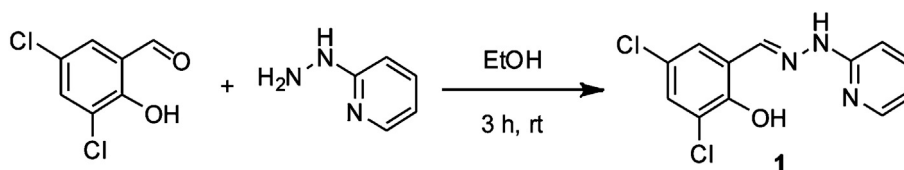


Fig. 2. ESI-MS spectrum of **1** in the presence of 2 equiv. Zn^{2+} . Inset: calculated isotopic patterns.



Scheme 1. Synthesis of **1**.

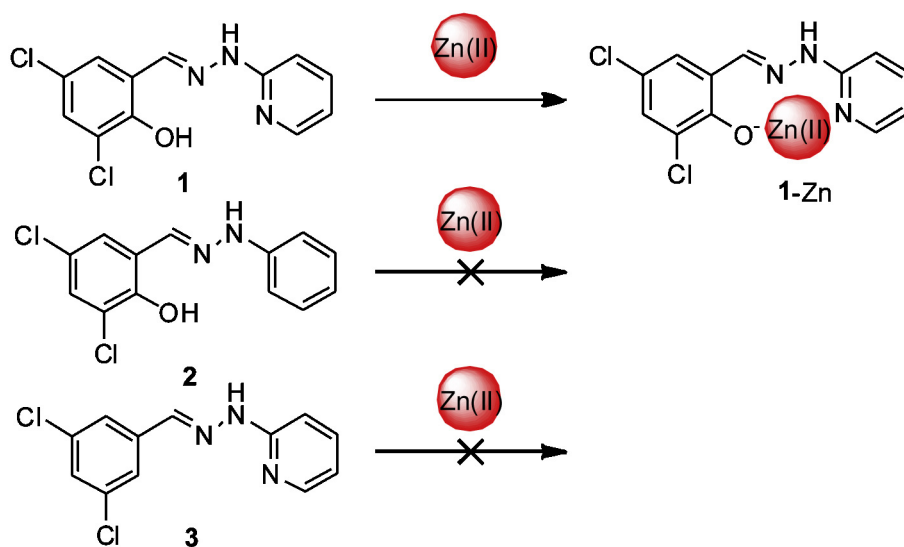
2.4. Synthesis of sensing molecules

General synthetic procedures of compounds **1–3** and **1a–f** are as follows. 1 mmol corresponding aldehyde (for **3**, 3,5-dichlorobenzaldehyde was used) and 1 mmol 2-hydrazinopyridine (for **2**, phenylhydrazine was used) were dissolved in 20 mL absolute ethanol. The mixture was stirred at room temperature for 3 h to form precipitate. The resulting precipitate was filtrated and washed with 20 mL of absolute ethanol three times. After being dried under reduced pressure, products were obtained in high yields (77–86%). The products were characterized by ESI-MS spectra, elemental analyses, ^1H and ^{13}C NMR spectra (see Supporting Information).

3. Results and discussion

3.1. Binding of **1** with Zn^{2+}

Absorption and fluorescence spectra were utilized for studying the binding mechanism of **1** with Zn^{2+} . Upon the addition of Zn^{2+} into 10 $\mu\text{mol/L}$ **1** in 99% water/DMSO (v/v) at pH 7.0 buffered by 10 mmol/L Tris-HCl, the absorption bands at 310 and 346 nm decreased gradually, new absorption bands at 333 and 383 nm emerged simultaneously. Four isobestic points could be found (Fig. 1a). In its fluorescence spectra under the same condition, more than 22-fold fluorescence enhancement at 460 nm occurred upon



Scheme 2. Proposed mechanism for the fluorescence change of **1** upon the addition of Zn^{2+} .

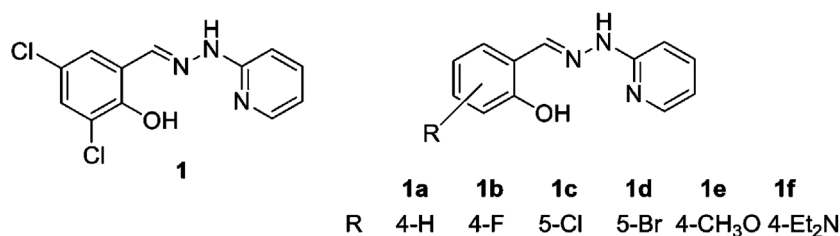
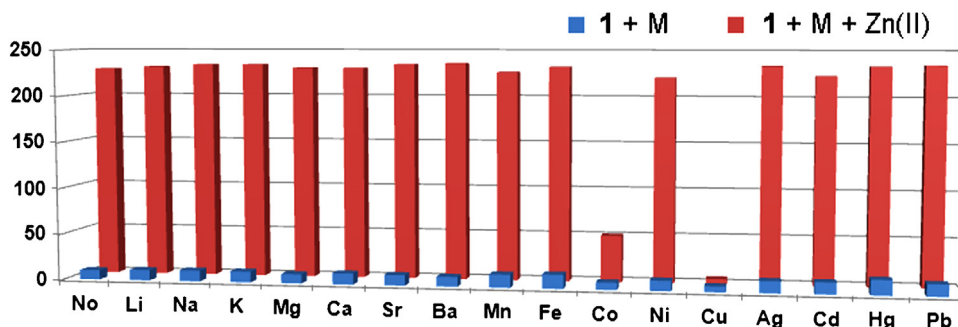
Scheme 3. Chemical structures of compounds **1** and **1a–f**.

Fig. 3. Fluorescence intensity of 10 $\mu\text{mol/L}$ **1** with 10 $\mu\text{mol/L}$ different metal ions in the absence and presence of 10 $\mu\text{mol/L}$ Zn^{2+} . Ions: blank, Li^+ , Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Sr^{2+} , Ba^{2+} , Mn^{2+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Ag^+ , Cd^{2+} , Hg^{2+} , Pb^{2+} . Conditions: 99% water/DMSO (v/v) at pH 7.0 buffered by 10 mmol/L Tris–HCl. Excitation and emission was at 364 and 460 nm, respectively.

the addition of Zn^{2+} (Fig. 1b). The nonlinear fitting of the titration curve revealed the binding of **1** and Zn^{2+} was most probably a 1:1 stoichiometry with an association constant K_a value about $5 \times 10^4 \text{ L/mol}$. To study the metal-to-ligand ratio, Job's plot was established from the fluorescence spectra of **1** and Zn^{2+} with a total concentration of 10 $\mu\text{mol/L}$. As shown in Fig. S1, the result suggests the formation of a 1:1 complex. Moreover, the ESI-MS spectrum of **1**– Zn^{2+} supplied a more direct evidence for the 1:1 metal-to-ligand ratio: in the presence of 2 equiv. Zn^{2+} , a clear peak at $m/z = 346.1$ ($[\text{M}+\text{Zn}(\text{II})-\text{H}]^+$, calcd 345.9) could be observed, and the isotopic patterns corresponded very well with the calculated ones (Fig. 2). Fluorescence emission enhancement and emission red shift from 452 to 460 nm of **1** upon binding with Zn^{2+} is considered to be due to the formation of **1**– Zn^{2+} complex: when complex formed the $\text{C}=\text{N}$ isomerization [36,37] and excited-state proton transfer (ESPT) [34,38] are inhibited and chelation-enhanced fluorescence (CHEF) [24,35] was observed (Scheme 1).

Control compounds **2** and **3** were synthesized which lack pyridine and hydroxyl group compared with **1**, respectively (Scheme 2). Both of them showed no response to Zn^{2+} in absorption under the same condition as **1** (Figs. S2 and S3). Thus, the pyridine and hydroxyl group of **1** were both indispensable for its Zn^{2+} binding property.

1a–f were also synthesized as control compounds to understand the effect of substituent on 2-hydroxybenzaldehyde (Scheme 3). All of these compounds showed fluorescence “turn-on” response toward Zn^{2+} in water at neutral pH except for **1f**, which contains an electron-donating group of diethylamino. Among these compounds, **1** shows the longest wavelength emission and the largest fluorescence enhancement, and **1**– Zn^{2+} complex has the best fluorescence quantum yield (Table S1). Furthermore, both of 3,5-dichloro-2-hydroxybenzaldehyde and 2-hydrazinopyridine are commercially available reagents of low cost.

3.2. Selectivity of **1** to Zn^{2+} over other metal ions

The influences of other metal ions on the fluorescence spectra of **1** were investigated to study the selectivity of **1** to Zn^{2+} . As shown in

Fig. 3, the response of **1** against those metal ions revealed a remarkable selectivity to Zn^{2+} . Even in the presence of Cd^{2+} , fluorescence enhancement was not detected. The high selectivity may be due to its specific binding affinity of **1** toward Zn^{2+} over Cd^{2+} . Other competitive metal ions showed no interference except for Co^{2+} and Cu^{2+} , which are paramagnetic metal ions leading to fluorescence quenching [4,39]. Although their quenching efficiency reached to 80% and 100%, respectively for Co^{2+} and Cu^{2+} , their presence in biological samples is much less than Zn^{2+} and these interferences could be negligible when **1** is applied for such sample analysis.

3.3. Optimizing the pH for fluorescence determination of Zn^{2+}

To investigate the pH effect, the fluorescence spectra of **1** in the presence of 1 equiv. Zn^{2+} were examined. As shown in Fig. S4, the fluorescence of **1**– Zn^{2+} complex reached to the maximum at pH

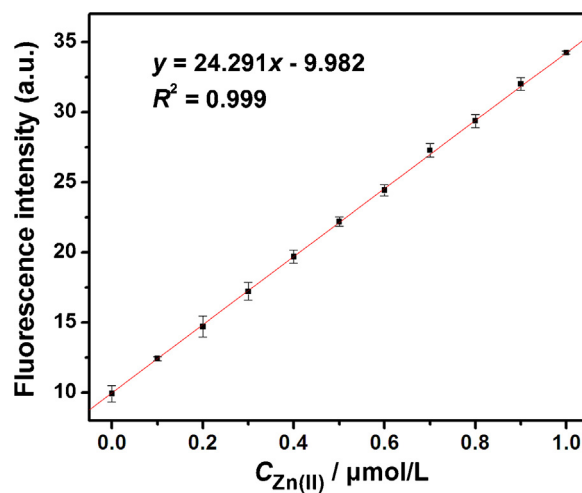


Fig. 4. Fluorescence intensity at 460 nm of **1** (10 $\mu\text{mol/L}$) as a function of Zn^{2+} concentration (0–1.00 $\mu\text{mol/L}$). Conditions: 99% water/DMSO (v/v) at pH 7.0 buffered by 10 mmol/L Tris–HCl. Excitation and emission was at 364 and 460 nm, respectively.

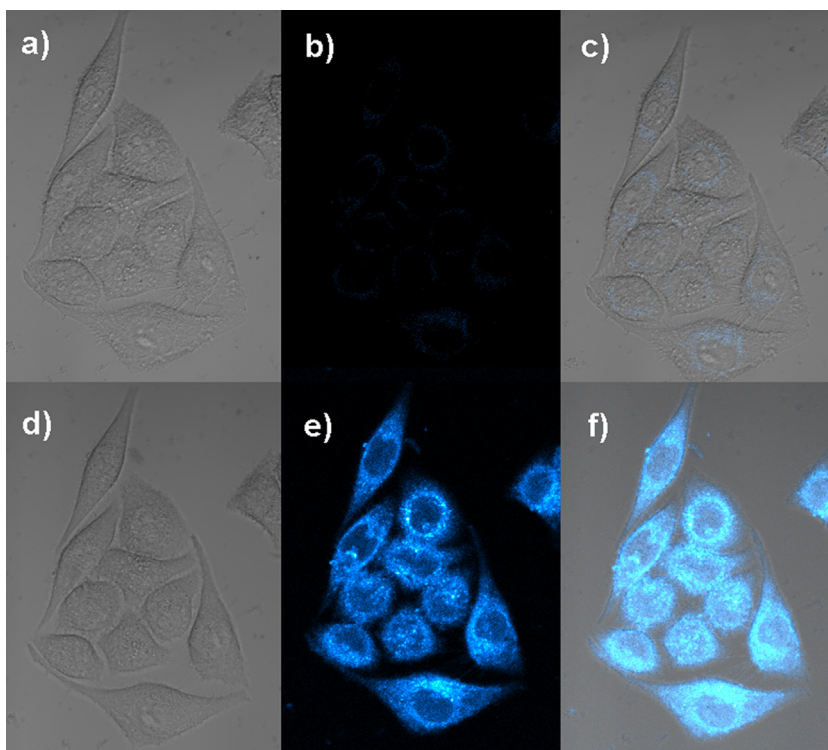


Fig. 5. Fluorescence images of live HeLa cells. From left to right are bright field images, fluorescence images and overlay images. Top (a–c): cells were incubated with **1** (10 $\mu\text{mol/L}$) for 20 min and washed with TBS twice. Bottom (d–f): cells were incubated with **1**, and then with Zn^{2+} (10 $\mu\text{mol/L}$) and pyruithione (10 $\mu\text{mol/L}$) for 20 min and washed with TBS twice. Emission was collected at 430–490 nm upon excitation at 405 nm.

7.0, while in acidic solutions the pyridine group may be protonated so that the Zn^{2+} binding should be weakened. The fluorescence of **1**– Zn^{2+} was decreased at $\text{pH} > 7$ due to the competition between OH^- and **1** in binding to Zn^{2+} . In order to obtain a higher signal-to-noise ratio and for compatibility with biological samples, pH 7.0 was employed for Zn^{2+} detection.

3.4. Analytical performance of **1** toward Zn^{2+}

According to the optimized conditions in 99% water/DMSO (v/v) at pH 7.0 buffered by 10 mmol/L Tris–HCl, the calibration curves for the determination of Zn^{2+} by **1** were constructed (Fig. 4). The linear range was at least 0.10–1.00 $\mu\text{mol/L}$ with a correlation coefficient of $R^2 = 0.999$ ($n = 3$). The detection limit, based on the definition by IUPAC ($C_{\text{DL}} = 3S_b/m$) from 10 blank solutions, was found to be 30 nmol/L. The relative standard deviation (R.S.D) for three repeated measurements of 0.50 $\mu\text{mol/L}$ Zn^{2+} was 1.6%. The content of Zn^{2+} in tap water sample and a synthesized water sample were analyzed by the proposed method under optimized condition. The results were summarized in Table S2, which showed satisfactory recovery and R.S.D. values for all of the samples.

3.5. Fluorescence imaging of intercellular Zn^{2+}

The application of **1** for fluorescence imaging of Zn^{2+} in live HeLa cells was investigated. As observed by a laser scanning confocal microscopy, HeLa cells incubated with 10 $\mu\text{mol/L}$ **1** for 20 min at 37 °C gave no intracellular fluorescence. Then, the cells were supplemented with 10 $\mu\text{mol/L}$ Zn^{2+} and 10 $\mu\text{mol/L}$ pyruithione, which is a zinc selective ionophore [10,32], for 20 min at 37 °C and washed with TBS twice, a significant fluorescence increase from the intracellular area was observed (Fig. 5). These results suggested that **1** may be used as an effective chemosensor for the imaging of Zn^{2+} in living cells.

4. Conclusions

In summary, we have developed a series of salicylaldehyde-2-pyridinehydrazone derivatives as fluorescent chemosensor for Zn^{2+} with facile synthesis. With a good selectivity to Zn^{2+} over other physiological relevant metal ions, **1** showed a “turn-on” fluorescence response toward Zn^{2+} in water at neutral pH. Moreover, confocal fluorescence microscopy experiments suggested that **1** may be used as a chemosensor for monitoring Zn^{2+} within biological samples.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.snb.2013.04.083>.

References

- [1] A.P. de Silva, H.Q.N. Gunaratne, T. Gunnlaugsson, A.J.M. Huxley, C.P. McCoy, J.T. Rademacher, T.E. Rice, Signaling recognition events with fluorescent sensors and switches, *Chemical Reviews* 97 (1997) 1515–1566.
- [2] V. Amendola, L. Fabbrizzi, F. Foti, M. Licchelli, C. Mangano, P. Pallavicini, A. Poggi, D. Sacchi, A. Taglietti, Light-emitting molecular devices based on transition metals, *Coordination Chemistry Reviews* 250 (2006) 273–299.
- [3] J.S. Kim, D.T. Quang, Calixarene-derived fluorescent probes, *Chemical Reviews* 107 (2007) 3780–3799.
- [4] Z. Xu, K.-H. Baek, H.N. Kim, J. Cui, X. Qian, D.R. Spring, I. Shin, J. Yoon, Zn^{2+} -triggered amide tautomerization produces a highly Zn^{2+} -selective, cell-permeable, and ratiometric fluorescent sensor, *Journal of the American Chemical Society* 132 (2010) 601–610.
- [5] T.V. Ohalloran, Transition-metals in control of gene-expression, *Science* 261 (1993) 715–725.

- [6] J.M. Berg, Y.G. Shi, The galvanization of biology: a growing appreciation for the roles of zinc, *Science* 271 (1996) 1081–1085.
- [7] M.P. Cuajungco, G.J. Lees, Zinc metabolism in the brain: relevance to human neurodegenerative disorders, *Neurobiology of Disease* 4 (1997) 137–169.
- [8] K.H. Falchuk, The molecular basis for the role of zinc in developmental biology, *Molecular and Cellular Biochemistry* 188 (1998) 41–48.
- [9] P. Carol, S. Sreejith, A. Ajayaghosh, Ratiometric and near-infrared molecular probes for the detection and imaging of zinc ions, *Chemistry – An Asian Journal* 2 (2007) 338–348.
- [10] E.M. Nolan, S.J. Lippard, Small-molecule fluorescent sensors for investigating zinc metalloneurochemistry, *Accounts of Chemical Research* 42 (2008) 193–203.
- [11] Z. Xu, J. Yoon, D.R. Spring, Fluorescent chemosensors for Zn^{2+} , *Chemical Society Reviews* 39 (2010) 1996–2006.
- [12] J. Wu, W. Liu, J. Ge, H. Zhang, P. Wang, New sensing mechanisms for design of fluorescent chemosensors emerging in recent years, *Chemical Society Reviews* 40 (2011) 3483–3495.
- [13] Z.F. Li, Y. Xiang, A.J. Tong, Ratiometric chemosensor for fluorescent determination of Zn^{2+} in aqueous ethanol, *Analytica Chimica Acta* 619 (2008) 75–80.
- [14] N. Li, Y. Xiang, X.T. Chen, A.J. Tong, Salicylaldehyde hydrazones as fluorescent probes for zinc ion in aqueous solution of physiological pH, *Talanta* 79 (2009) 327–332.
- [15] C. Lu, Z. Xu, J. Cui, R. Zhang, X. Qian, Ratiometric and highly selective fluorescent sensor for cadmium under physiological pH range: a new strategy to discriminate cadmium from zinc, *Journal of Organic Chemistry* 72 (2007) 3554–3557.
- [16] Z.-X. Han, X.-B. Zhang, L. Zhuo, Y.-J. Gong, X.-Y. Wu, J. Zhen, C.-M. He, L.-X. Jian, Z. Jing, G.-L. Shen, R.-Q. Yu, Efficient fluorescence resonance energy transfer-based ratiometric fluorescent cellular imaging probe for Zn^{2+} using a rhodamine spirolactam as a trigger, *Analytical Chemistry* 82 (2010) 3108–3113.
- [17] X. Lu, W. Zhu, Y. Xie, X. Li, Y. Gao, F. Li, H. Tian, Near-IR core-substituted naphthalenediimide fluorescent chemosensors for zinc ions: ligand effects on PET and ICT channels, *Chemistry – A European Journal* 16 (2010) 8355–8364.
- [18] Y.N. Hong, S.J. Chen, C.W.T. Leung, J.W.Y. Lam, J.Z. Liu, N.W. Tseng, R.T.K. Kwok, Y. Yu, Z.K. Wang, B.Z. Tang, Fluorogenic $Zn(II)$, chromogenic $Fe(II)$ sensors based on terpyridine-substituted tetraphenylethenes with aggregation-induced emission characteristics, *ACS Applied Materials & Interfaces* 3 (2011) 3411–3418.
- [19] F. Sun, G. Zhang, D. Zhang, L. Due, H. Jiang, Aqueous fluorescence turn-on sensor for Zn^{2+} with a tetraphenylethylene compound, *Organic Letters* 13 (2011) 6378–6381.
- [20] Z. Guo, G.-H. Kim, I. Shin, J. Yoon, A cyanine-based fluorescent sensor for detecting endogenous zinc ions in live cells and organisms, *Biomaterials* 33 (2012) 7818–7827.
- [21] M. Li, H.-Y. Lu, R.-L. Liu, J.-D. Chen, C.-F. Chen, Turn-on fluorescent sensor for selective detection of Zn^{2+} , Cd^{2+} , and Hg^{2+} in water, *Journal of Organic Chemistry* 77 (2012) 3670–3673.
- [22] Q.-Q. Zhang, B.-X. Yang, R. Sun, J.-F. Ge, Y.-J. Xu, N.-J. Li, J.-M. Lu, A near-infrared phenoxazinium-based fluorescent probe for zinc ions and its imaging in living cells, *Sensors and Actuators B: Chemical* 171 (2012) 1001–1006.
- [23] L. Sumin, L. Jae Hong, T. Pradhan, L. Chang Su, C. Bong Rae, S. Bhuniya, K. Sungtae, K. Jong Seung, Fluorescent turn-on Zn^{2+} sensing in aqueous and cellular media, *Sensors and Actuators B: Chemical* 160 (2011) 1489–1493.
- [24] L. Due, Q. Liu, H. Jiang, Ratiometric Zn^{2+} fluorescent sensor and new approach for sensing Cd^{2+} by ratiometric displacement, *Organic Letters* 11 (2009) 3454–3457.
- [25] P. Ashokkumar, V.T. Ramakrishnan, P. Ramamurthy, Photoinduced electron transfer (PET) based Zn^{2+} fluorescent probe: transformation of turn-on sensors into ratiometric ones with dual emission in acetonitrile, *Journal of Physical Chemistry A* 115 (2011) 14292–14299.
- [26] Y. Li, L. Shi, L.-X. Qin, L.-L. Qu, C. Jing, M. Lan, T.D. James, Y.-T. Long, An OFF–ON fluorescent probe for Zn^{2+} based on a GFP-inspired imidazolone derivative attached to a 1,10-phenanthroline moiety, *Chemical Communications* 47 (2011) 4361–4363.
- [27] E. Manandhar, J.H. Broome, J. Myrick, W. Lagrone, P.J. Cragg, K.J. Wallace, A pyrene-based fluorescent sensor for Zn^{2+} ions: a molecular 'butterfly', *Chemical Communications* 47 (2011) 8796–8798.
- [28] X.-L. Ni, X. Zeng, C. Redshaw, T. Yamato, Ratiometric fluorescent receptors for both Zn^{2+} and $H_2PO_4^-$ ions based on a pyrenyl-linked triazole-modified homooxacalix 3 arene: a potential molecular traffic signal with an R–S latch logic circuit, *Journal of Organic Chemistry* 76 (2011) 5696–5702.
- [29] S.A. Ingale, F. Seela, A ratiometric fluorescent On–Off Zn^{2+} chemosensor based on a tripropargylamine pyrene azide click adduct, *Journal of Organic Chemistry* 77 (2012) 9352–9356.
- [30] J. Wang, W. Lin, W. Li, Single fluorescent probe displays a distinct response to Zn^{2+} and Cd^{2+} , *Chemistry – A European Journal* 18 (2012) 13629–13632.
- [31] Z.-L. Gong, F. Ge, B.-X. Zhao, Novel pyrazoline-based selective fluorescent sensor for Zn^{2+} in aqueous media, *Sensors and Actuators B: Chemical* 159 (2011) 148–153.
- [32] K. Komatsu, Y. Urano, H. Kojima, T. Nagano, Development of an iminocoumarin-based zinc sensor suitable for ratiometric fluorescence imaging of neuronal zinc, *Journal of the American Chemical Society* 129 (2007) 13447–13454.
- [33] X.B. Yang, B.X. Yang, J.F. Ge, Y.J. Xu, Q.F. Xu, J. Liang, J.M. Lu, Benzo a phenoxazinium-based red-emitting chemosensor for zinc ions in biological media, *Organic Letters* 13 (2011) 2710–2713.
- [34] Y. Zhou, Z.X. Li, S.Q. Zang, Y.Y. Zhu, H.Y. Zhang, H.W. Hou, T.C.W. Mak, A novel sensitive turn-on fluorescent Zn^{2+} chemosensor based on an easy to prepare C-3-symmetric Schiff-base derivative in 100% aqueous solution, *Organic Letters* 14 (2012) 1214–1217.
- [35] T.H. Ma, M. Dong, Y.M. Dong, Y.W. Wang, Y. Peng, A unique water-tuning dual-channel fluorescence-enhanced sensor for aluminum ions based on a hybrid ligand from a 1,1'-binaphthyl scaffold and an amino acid, *Chemistry – A European Journal* 16 (2010) 10313.
- [36] J. Wu, W. Liu, J. Ge, H. Zhang, P. Wang, New sensing mechanisms for design of fluorescent chemosensors emerging in recent years, *Chemical Society Reviews* 40 (2011) 3483.
- [37] J.S. Wu, W.-M. Liu, X.Q. Zhuang, F. Wang, P.F. Wang, S.L. Tao, X.H. Zhang, S.K. Wu, S.T. Lee, Fluorescence turn on of coumarin derivatives by metal cations: A new signaling mechanism based on C=N isomerization, *Organic Letters* 9 (2007) 33.
- [38] X. Zhang, L. Guo, F.Y. Wu, Y.B. Jiang, Development of fluorescent sensing of anions under excited-state intermolecular proton transfer signaling mechanism, *Organic Letters* 5 (2003) 2667.
- [39] A.W. Varnes, E.L. Wehry, R.B. Dodson, Interactions of transition-metal ions with photoexcited states of flavins-fluorescence quenching studies, *Journal of the American Chemical Society* 94 (1972) 946–950.

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