ORGANIC LETTERS

2012
Vol. 14, No. 19
5070–5073

Controlling Ion-Sensing Specificity of *N*-Amidothioureas: From Anion-Selective Sensors to Highly Zn^{2+}-Selective Sensors by Tuning Electronic Effects

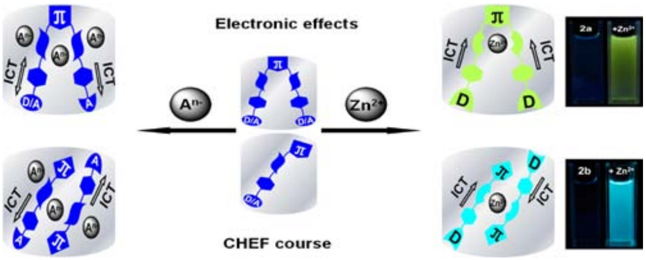
Hua-Long Chen, Zhi-Fo Guo, and Zhong-lin Lu*

College of Chemistry, Beijing Normal University, Xinjiekouwai Street 19, Beijing, 100875, China

luzl@bnu.edu.cn

Received August 19, 2012

ABSTRACT



***N*-Amidothioureas bearing nitro groups showed different sensitivities to various anions, while those with methoxy moieties exhibited the sensitive and selective fluorescence turn-on property for Zn^{2+} in aqueous solution and in cells. *N*-Amidothioureas can therefore be adjusted from anion-selective sensors to Zn^{2+}-selective sensors by varying the electronic effects of their substituents.**

Due to their ability to form multiple inter- and intramolecular hydrogen bonds, especially pairs of N−H...S hydrogen bonds,[1] amidoureas and amidothioureas have been successfully developed as colorimetric and fluorescent anion sensors in recent years. Various mono- and disubstituted *N*-amidothiourea receptors have been designed and synthesized for their selective and efficient binding of different anions in both organic solvents and aqueous media.[2] The mechnisms for anion sensing by these compounds are suggested to be either through hydrogen bonding and/or via deprotonation. Based on the reported examples, cleft-like bis-*N*-amidothiourea receptors show enhanced affinities and colorimetric sensing for anions as compared to monosubstituted receptors.[3,4] It is also known that electron-withdrawing substituents such as nitro and trifluoromethyl groups attached to the *N*-phenyl rings reinforce the anion binding and afford high sensitivity.[4] However, to the best our knowledge, there hae been no reports of these compounds acting as sensors for the detection of cations based on coordination mode.[5] Different from anion recognition, which relies on both the acidity of the thioureido NH protons and the electron-deficient

---

(1) (a) Moragues, M. E.; Martínez-Máñez, R.; Sancenón, F. *Chem. Soc. Rev.* **2011**, *40*, 2593. (b) Jiang, Q. Q.; Darhkijav, B.; Liu, H.; Wang, F.; Li, Z.; Jiang, Y. B. *Chem.—Asian J.* **2010**, *5*, 543. (c) Li, A. F.; Wang, J. H.; Wang, F.; Jiang, Y. B. *Chem. Soc. Rev.* **2010**, *39*, 3729. (d) Evans, L. S.; Gale, P. A.; Light, M. E.; Quesada, R. *Chem. Commun.* **2006**, *42*, 965. (e) Zeng, Z. Y.; He, Y. B.; Wu, J. L.; Wei, L. H.; Liu, X.; Meng, L. Z.; Yang, X. *Eur. J. Org. Chem.* **2004**, *13*, 2888.

(2) (a) Nie, L.; Li, Z.; Han, J.; Zhang, X.; Yang, R.; Liu, W. X.; Wu, F. Y.; Xie, J. W.; Zhao, Y. F.; Jiang, Y. B. *J. Org. Chem.* **2004**, *69*, 6449. (b) Liu, W. X.; Yang, R.; Li, A. F.; Li, Z.; Gao, Y. F.; Luo, X. X.; Ruan, Y. B.; Jiang, Y. B. *Org. Biomol. Chem.* **2009**, *7*, 4021.

(3) (a) Duke, R. M.; O'Brien, J. E.; McCabe, T.; Gunnlaugsson, T. *Org. Biomol. Chem.* **2008**, *6*, 4089. (b) Duke, R. M.; Gunnlaugsson, T. *Tetrahedron Lett.* **2010**, *51*, 5402. (c) Duke, R. M.; McCabe, T.; Schmitt, W.; Gunnlaugsson, T. *J. Org. Chem.* **2012**, *77*, 3115. (d) Wei, W.; Zhang, Y. M.; Wei, T. B. *Chin. J. Chem.* **2008**, *26*, 1935.

(4) (a) Evans, L. S.; Gale, P. A.; Light, M. E.; Quesada, R. *New J. Chem.* **2006**, *30*, 1019. (b) Wu, J. L.; He, Y. B.; Wei, L. H.; Liu, S. Y.; Meng, L. Z.; Hu, L. *Supramol. Chem.* **2004**, *16*, 353. (c) Wei, L. H.; He, Y. B.; Wu, J. L.; Wu, X. J.; Meng, L. Z.; Yang, X. *Supramol. Chem.* **2004**, *16*, 561.

(5) Some Hg^{2+} sensors are due to the desulfurization reactions; see: (a) Yang, Y. K.; Yook, K. J.; Tae, J. *J. Am. Chem. Soc.* **2005**, *127*, 16760. (b) Mahapatra, A. K.; Roy, J.; Sahoo, P.; Mukhopadhyay, S. K.; Chattopadhyay, A. *Org. Biomol. Chem.* **2012**, *10*, 2231. (c) Liu, J.; Yu, M.; Wang, X. C.; Zhang, Z. *Spectrochim. Acta. A* **2012**, *93*, 245. (d) Koteeswari, R.; Ashokkumar, P.; Malar, E. J. P.; Ramakrishnan, V. T.; Ramamurthy, P. *Chem. Commun.* **2011**, *47*, 7695.

10.1021/ol302313x

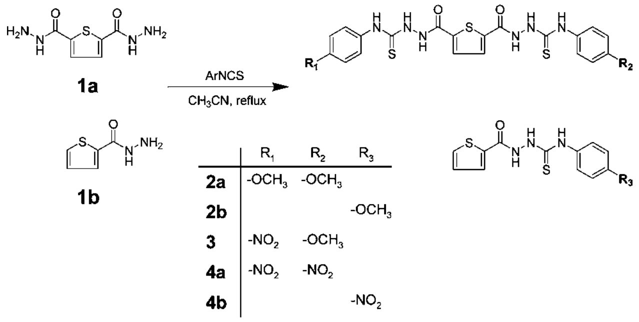
thiourea center for high sensitivity, an elelctron-rich thiourea center in the *N*-amidothiourea compounds may lead to an increase in sensitivity for cations. Given the thioaffinity of zinc(II) ions, *N*-amidothioureas with elelctron-donating substituentes should serve as good sensors for Zn^{2+}. On the other hand, Zn^{2+}, as the second most abundant d-block metal ion in the human body, plays critical roles in regulating enzyme activities as well as in structure and function, neural signal transmission, gene expression, and apoptosis. Although various Zn^{2+} sensors have been developed,[6] highly Zn^{2+}-selective, water-soluble, cell permeable, and easily accessible fluorescent sensors are still in high demand.

Herein, we report the synthesis of a series of *N*-amidothiourea compounds with different substituents and the switchable anions/cations sensing behavior of these compounds. While *N*-amidothioureas containing elelctron-withdrawing nitro substituents are able to recognize many biologically important anions and show almost no response to metal ions, those with electron-donating methoxyl moieties exhibited a high selectivity and sensitivity for Zn^{2+} ions in aqueous solution as well as in cells. Ours are the first series of Zn^{2+} sensors based on *N*-amidothioureas.

The syntheses of the new *N*-amidothioureas were straightforward (Scheme 1). All new compounds were fully characterized with ^{1}H, ^{13}C NMR, mass spectroscopy and IR spectra (see Supporting Information). A single crystal of **2b** was analyzed by X-ray diffraction. (Figure S1a–S1b). The 3D supramolecular frameworks was established by H-bondings and strong $\pi-\pi$ interaction between adjacent thiophene planes (3.725 Å) located in the two dimers.

The sensing abilities of the five *N*-amidothiourea receptors toward anions such as $H_2PO_4^-$, HPO_4^{2-}, $P_2O_7^{4-}$, AMP, ADP, ATP, OAc$^-$, and F$^-$ were determined in 4:1 DMSO/H_2O following the reported procedure.[3a] It was found that their absorption spectra of these conpounds showed clear but different changes upon the addition of the above-mentioned anions (Figures 1 and S3a).

Scheme 1. Synthesis of the *N*-Amidothiourea Compounds



	R$_1$	R$_2$	R$_3$
2a	-OCH$_3$	-OCH$_3$	
2b			-OCH$_3$
3	-NO$_2$	-OCH$_3$	
4a	-NO$_2$	-NO$_2$	
4b			-NO$_2$

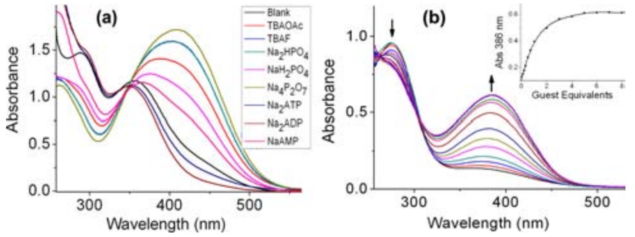


Figure 1. (a) Changes in the UV–vis of **4a** (25 μM) in 4:1 DMSO/H_2O upon addition of various anions. (b) Changes in the UV–vis of **2a** upon addition of HPO_4^{2-} (0→8.0 equiv) in DMSO.

Several points are noteworthy: (1) The sensitivity of the five receptors to the anions follows essentially the same direction, i.e., it varies in the order $P_2O_7^{4-} > HPO_4^{2-} \geq F^- > CH_3CO_2^- > HPO_4^{2-} > AMP \gg ADP \sim ATP$. Their recognition of pyrophosphate is very interesting. An insignificant change in their absorption spectra upon the addition of ADP and ATP was observed. (2) The absorption spectra of the nitro-containing amidothioureas **3**, **4a**, and **4b** displayed a new strong band at *ca.* 420 nm, which is visible to naked eyes since the color of the solution changed from colorless to yellow. For the methoxyl-containing **2a** and **2b**, their sensitivity toward anions clearly decreased as shown by the changes of the absorption spectra and the apperance of new bands at short wavelengths. Among the anions tested, only $P_2O_7^{4-}$, HPO_4^{2-}, OAc$^-$, and F$^-$ were obviously differentiated with new bands appearing at λ_{max} = 390 and 350 nm, respectively (Figure S3a).

Results from the anion titrations (Figure S3b) clearly indicated that *N*-amidothioureas with electron-withdrawing substituents showed strong affinity toward these ions. ^{1}H NMR titrations (Figure S7a–7c) demonstrated that both hydrongen-bonding and/or deprotonation interactions are responsible for anion sensing. These results are in good agreement with those in the literatures.[3] The distinct selectivity for anions can be attributed to the presence of the multihydrogen bonding interactions. The presence of electron-withdawing substituents in the *N*-phenyl ring favors the recognition of anions due to the complementary electrostatic interactions, and the enhanced internal charge transfer (ICT) which shifted the spectra to a longer wavelength.[3]

In the sensing of cations using the above *N*-amidothioureas, the perchlorate salts of Li$^+$, Ag$^+$, Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Hg^{2+}, K$^+$, Mg^{2+}, Na$^+$, Ni^{2+}, Pb^{2+}, and Zn^{2+} ions were examined in aqueous buffer (DMSO/H_2O, 1:99, v/v, tris-HCl, 10 mM, 0.1 M KNO_3, pH 7.4). The three nitro-containing ligands showed no sigiunificant fluorescence changes upon the addition of the 14 cations (Figure S4). However, for **2a** (Φ_{free} = 0.043) and **2b** (Φ_{free} = 0.030),

---

(6) For recent reviews for Zn^{2+} sensors, see: (a) Nolan, E. M.; Lippard, S. J. *Acc. Chem. Res.* **2009**, *42*, 193. (b) Zhang, J. F.; Zhou, Y.; Yoon, J. Y.; Kim, Y. M.; Kim, S. J.; Kim, J. S. *Org. Lett.* **2010**, *12*, 3852. (c) Zhang, J. F.; Kim, S. Y.; Han, J. H.; Lee, S. J.; Pradhan, T.; Cao, Q. Y.; Kang, C. H.; Kim, J. S. *Org. Lett.* **2011**, *13*, 5294. (d) Yamaguchi, S.; Miuraa, C.; Kikuchi, K.; Celino, F. T.; Agusa, T.; Tanabe, S.; Miura, T. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 10859. (f) Que, E. L.; Domaille, D. W.; Chang, C. J. *Chem. Rev.* **2008**, *108*, 1517. (g) Wu, J. S.; Liu, W. M.; Zhuang, X. Q.; Wang, P. F.; Wu, S. K.; Lee, S. T. *Org. Lett.* **2007**, *9*, 33. (h) Xu, Z.; Yoon, J.; Spring, D. R. *Chem. Soc. Rev.* **2010**, *39*, 1996.

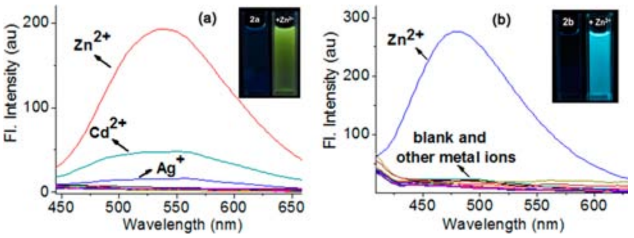


Figure 2. Fluorescence spectra of **2a** (a) and **2b** (b) at 10 μM in the absence and presence of 1.0 equiv of different metal ions (Li^+, Ag^+, Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Hg^{2+}, K^+, Mg^{2+}, Na^+, Ni^{2+}, Pb^{2+}, Zn^{2+}). Inset: Fluorescent emission change irradiated at 365 nm by fluorescent lamp.

upon the addition of Zn^{2+}, the probes immediately gave a yellow-green (λ_{max} = 541 nm, *ca.* 14-fold) and a bright-blue emission enhancement (λ_{max} = 479 nm, *ca.* 19-fold) (Figure 2). For **2a**, the addition of Cd^{2+} and Ag^+ showed a slight enhancement in emission, while only Zn^{2+} triggered a sharp fluorescence enhancement for **2b** ($\Phi_{\mathbf{2a}\text{-Zn(II)}}$ = 0.270, and $\Phi_{\mathbf{2b}\text{-Zn(II)}}$ = 0.352).

Competing experiments among metal ions (Figure S6) proved that Li^+, Na^+, K^+, Ca^{2+}, Mg^{2+}, Ag^+, and Fe^{3+} showed no interference in the recognition of Zn^{2+}. For Pb^{2+}, Hg^{2+}, and Cd^{2+} ions, the fluorescence were not completely restored by the addition of Zn^{2+}, but strong enhancements were still observed. In the case of Cu^{2+}, Ni^{2+}, and Co^{2+}, the quenched fluorescence was not recovered after the addition of Zn^{2+}, which is common for most Zn^{2+} sensors. It needs to be pointed out that the influence of these ions could be neglected due to their low concentration in vivo.

A fluorescence titration experiment (Figure 3) revealed that a linear enhancement with increasing [Zn^{2+}] up to 10 μM (1:1) was observed. Higher Zn^{2+} concentrations only caused insignificant emission enhancement for **2a**. The emission enhancement of **2b** plateaued with an excess of 5 μM of Zn^{2+} (2:1). The 1:1 and 2:1 stoichiometric ratios between **2a** and **2b** to Zn^{2+} were also derived from Job's plot (Figure S5). The binding constant K_a of **2a** for Zn^{2+} was determined to be 7.87×10^4 M^{-1}, which is higher than that of **2b** (2.16×10^2 M^{-2}, Figure S9) because of the cleft effect. The detection limits were found to be 1.05 and 2.30 μM for **2a** and **2b**. ESI-MS results also confirmed the formation of 1:1 (**2a**:Zn) and 2:1 (**2b**:Zn) complexes (Figure S8).

IR spectra indicated that the amide-I stretching vibrations of free **2a** and **2b** were blue-shifted by 39 and 14 cm^{-1} in those of the two complexes, illustrating the coordination of the amido-carbonyl oxygen atom to Zn^{2+}.[7a,b] The C=S stretching at 1462, 740 cm^{-1} in **2a** and 1415, 732 cm^{-1} in **2b** were split and shifted to 1447, 1382, 720 cm^{-1} and 1413, 730, and 693 cm^{-1}, respectively, indicating that the coordination involved the sulfur atom in the Zn^{2+} complexes.[7c,d]

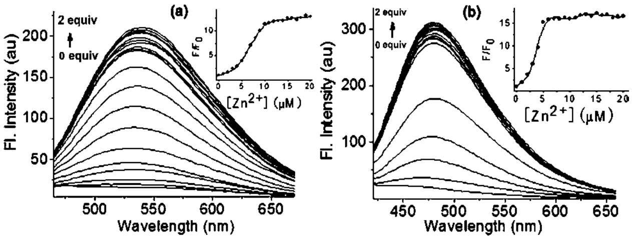


Figure 3. Fluorescence titration spectra of **2a** (a) and **2b** (b) at 10 μM upon addition of Zn^{2+} at 0–20 μM (0–2.0 equiv) in DMSO/H_2O (1:99, v/v). Inset: ratiometric fluorescence intensity [F/F_0] as a function of [Zn^{2+}].

^1H NMR titration experiments of **2a** and Zn^{2+} in DMSO-d_6 found that the three signals at 10.69, 9.78, and 9.65 ppm, derived from the amide and the two thiourea protons on each arm of the ligand, had no significant change upon the addition of Zn^{2+} (0–10 equiv),[3a,4a] indicating that there was no significant amide deprotonation observed; i.e., no enolation occurred in the metal coordination sites (Figure S7d–S7e).

The emission intensities of **2a** and **2b** in the presence of Zn^{2+} ion were stable in the pH range 6.0 –10.0, which would be beneficial for biological applicability (Figure S2). For the absorption spectra of **2a** and **2b**, the addition of Zn^{2+} ions resulted in the appearance of new bands at 448 and 336 nm. Complexes with stoichiometries of 1:1 and 1:2 were clearly formed (Figure S3c).

Table 1 showed the results from molecular orbital calculations for these ligands. In HOMOs, the charge was mainly located on the center of all compounds. However, in LUMOs, the extent of π-electron delocalization of the nitro-containing ligands was drastically increased, which clearly weakens the coordination capability to metal ions.

However, such delcocalization should enhance interactions with anions. In contrast, the π-electrons of **2a** and **2b** were mainly found on the electron-deficient moieties of these ligands (—(C=O)—NH—NH—(C=S)—) due to the electron-donating effect of the methoxyl groups, which greatly improved the ability of chelating metal ions by these compounds (Tables S5–S6).[8]

A likely mechanism for the sensing of anions and Zn^{2+} is shown in Figure 4. The anion–recepotor interaction can be both a hydrongen-bonding and/or deprotonation course as reported in the literatures.[3,9] Zn^{2+} chelation

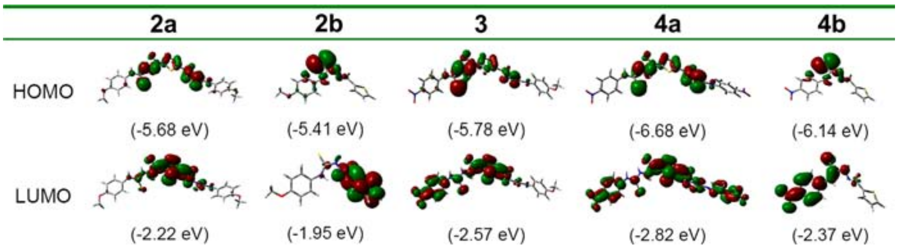
---

(7) (a) Desseyn, H. O.; Jacob, W. A.; Herman, M. A. *Spectrochim. Acta* **1969**, *25A*, 1685. (b) Nishat, N.; Ahamad, T.; Zulfequar, M.; Hasnain, S. *J. Appl. Polym. Sci.* **2008**, *110*, 3305. (c) Swaminathan, K.; Irving, H. M. N. H. *J. Inorg. Nucl. Chem.* **1964**, *26*, 1291. (d) Pedrido, R.; Romero, M. J.; Bermejo, M. R.; González-Noya, A. M.; García-Lema, I.; Zaragoza, G. *Chem.—Eur. J.* **2008**, *14*, 500.

(8) (a) Yousef, T. A.; El-Gammal, O. A.; Ghazy, S. E.; Abu El-Reash, G. M. *J. Mol. Struct.* **2011**, *1004*, 271. (b) Hudson, G. A.; Cheng, L.; Yu, J.; Yan, Y.; Dyer, D. J.; McCarroll, M. E.; Wang, L. *J. Phys. Chem. B* **2010**, *114*, 870.

(9) For the hydrogen bonding and deprotonation mechanism of the *N*-Amidothioureas compounds with anions, see: (a) Perez-Casas, C.; Yatsimirsky, A. K. *J. Org. Chem.* **2008**, *73*, 2275. (b) Gunnlaugsson, T.; Kruger, P. E.; Lee, T. C.; Parkesh, R.; Pfeffer, F. M.; Hussey, G. M. *Tetrahedron Lett.* **2003**, *44*, 6575. (c) Camiolo, S.; Gale, P. A.; Hursthouse, M. B.; Light, M. E. *Org. Biomol. Chem.* **2003**, *1*, 741.

Table 1. Calculated HOMOs and LUMOs of the Derivatives Using DFT Method (6-31G* Basis Sets)



	2a	2b	3	4a	4b
HOMO	(-5.68 eV)	(-5.41 eV)	(-5.78 eV)	(-6.68 eV)	(-6.14 eV)
LUMO	(-2.22 eV)	(-1.95 eV)	(-2.57 eV)	(-2.82 eV)	(-2.37 eV)

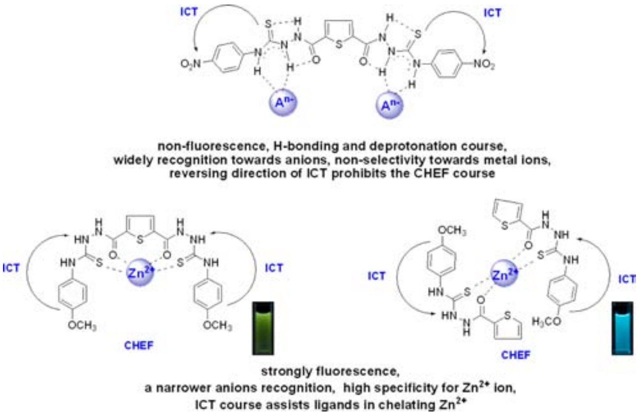


Figure 4. Proposed binding mode and sensing process of the amidothioureas with anions and Zn^{2+}.

resulted in better planarity and rigidity of the amidothioureas and hence inhibited the distortion of the molecular structures. This promoted strong ICT from $-OCH_3$ moieties to the thiourea center and fulfilled the CHEF course.

Furthermore, intracellular Zn^{2+} sensing and imaging were investigated using **2b** in live HepG2 cells as shown in Figure 5. When the cells were stained with only **2b** (20 μM), a negligible intracellular fluorescence was detected. In contrast, with the addition of Zn^{2+} (10 μM), a significant enhancement of the intracellular blue fluorescence were observed. In view of the good match between Figure 5b and 5c, it can be concluded that the probe has excellent membrane permeability. The binding of **2b** with Zn^{2+} happened within HepG2 cells.

In summary, this work clearly demonstrated that tuning the electronic properties of the substituents in *N*-amidothioureas results in the conversion of sensors for anions to those for cations. *N*-Amidothioureas containing electron-withdrawing nitro groups are responsive to a host of biologically important anions, whereas those containing electron-donating methoxy moieties can recoginize Zn^{2+} with high selectivity and sensitivity. To the best of our knowledge, ours is also the first example that “turns on” fluorescent sensors for Zn^{2+} based on amidothioureas, a class of compounds that are easily available, highly selective, water-soluble, and cell-permeable.

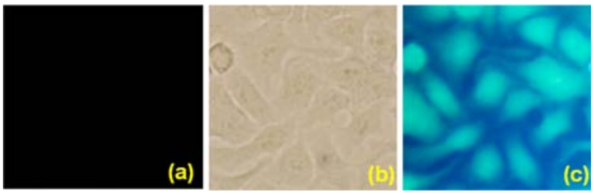


Figure 5. Intracellular Zn^{2+} was imaged in living cells at 37 °C. (a) HepG2 cells incubated with **2b** (20 μM) buffer solution for 30 min. (b) Bright field transmission image of living HepG2 cells in parts a and c. (c) HepG2 cells further incubated with Zn^{2+} (10 μM) for 30 min, then washed three times with PBS.

Acknowledgment. We are thankful for funding from the NSFC (20972019), NCET-08-0054, and 2009SC-1. The authors thank Prof. B. Gong at NYSU, Buffalo, for his helpful discussions.

Supporting Information Available. Experimental details, compound characterizations, and the X-ray single crystal diffraction data for compound **2b**. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.