RSC Advances

PAPER

View Article Online
View Journal | View Issue

Published on 16 December 2013. Downloaded by Carleton University on 19/06/2014 16:36:08.

Cite this: *RSC Adv.*, 2014, **4**, 9784

Imine-linked chemosensors for the detection of Zn^{2+} in biological samples†

Preeti Saluja,[a] Vimal K. Bhardwaj,[a] Thangarasu Pandiyan,[c] Simanpreet Kaur,[b] Navneet Kaur*[b] and Narinder Singh*[a]

Received 16th November 2013
Accepted 13th December 2013

DOI: 10.1039/c3ra46759g

www.rsc.org/advances

A chemosensor **1** with a long hydrocarbon chain and polar end group is synthesized by the simple condensation reaction of a long chain amine with salicylaldehyde. A long chain hydrocarbon with a polar end group is used because of its solubility in an aqueous surfactant solution, which ensures that it can be used in a neutral water medium. The rationale for choosing an aryl aldehyde with –OH functionality is based upon the fact that a chelate ring consisting of an –OH group and an sp^2 nitrogen donor is always better for the selective recognition of Zn^{2+}. The sensor shows selective binding to Zn^{2+} in 1% Triton-X-100 solution. Binding of Zn^{2+} by sensor **3** leads to an approximately 300% enhancement in the fluorescence intensity of the sensor, due to the combined effects of excited state intramolecular proton transfer (ESIPT) and the inhibition of the photo-induced electron transfer (PET) process by the –OH group. The fluorescence emission profiles of sensor **1** show some changes in the low and high pH ranges, however the sensor remains stable in the pH range 4–9, which makes it appropriate for use in biological fluids.

Introduction

The design and synthesis of metal chemosensors with high selectivity and sensitivity is an active field of supramolecular chemistry.[1] The extraordinary spectroscopic properties of fluorescent chemosensors make them a promising alternative to other techniques for a variety of applications such as analytical chemistry, medical diagnostics and materials science.[2] Zinc is the second most abundant transition metal ion in the human body, and is an integral part of major biological processes such as gene transcription, immune function and brain function, *etc.*[3] Zinc is essential for the proper functioning of cellular metabolism, and it plays an important role in fertility in men and women. It stimulates the catalytic activity of about 100 enzymes in vital chemical reactions in living systems.[4] However, an excess of zinc can create disorder in metabolism and can further interfere in the absorption of iron, magnesium and copper. Doses of zinc supplements of above 40 mg per day can cause gastrointestinal upset, a metallic taste in the mouth, blood in urine, and lethargy.[5] Recently, many research groups have developed a variety of fluorescent sensors for Zn^{2+} with some success in imaging Zn^{2+} in living cells.[6] The Zn^{2+} sensor designs make use of various combinations of sp^2 hybridised nitrogen atoms in quinoline, bipyridyl, benzimidazole and benzothiazole as donor sites.[7] These types of sensors have an advantage, as these moieties can simultaneously act as metal binding sites as well as fluorophores.[8] This duality may help to reduce the size of the sensor and eventually reduce the burden of long synthetic strategies. Some of these sensors operate through a chelation enhanced fluorescence (CHEF) mechanism and exhibit enhanced fluorescence intensities.[9]

The formation of a chelate ring with the participation of donor sites from the fluorophore may change the HOMO–LUMO energy gap; in other words, metal binding close to the fluorophore generally affects internal charge transfer (ICT).[10] These modulations result in with a change in the wavelength, which is the basis of recently reported ratiometric sensors.[11] Although detailed mechanistic studies are available in the literature for Zn^{2+} sensors, some of these sensors have shortcomings such as poor water solubility, interference from other metal ions, the fact that they have not been evaluated for biocompatibility and problems monitoring the fluorescence changes at a conventional single wavelength.[12] In addition to these points, a low detection limit and wide detection range are basic requirements for sensor development.[13] A change of substituents and their positions with respect to the sp^2 nitrogen moiety may affect the sensitivity and selectivity of the sensor. This is the governing feature of many imine-linked chemosensors and their metal complexes.[14] With this intention, we synthesized sensor **1** by a simple condensation reaction. The long hydrocarbon chain with polar end groups may help to

[a]*Department of Chemistry, Indian Institute of Technology Ropar (IIT Ropar), Rupnagar, Punjab, 140001, India. E-mail: nsingh@iitrpr.ac.in*

[b]*Facultad de Química, Universidad Nacional Autónoma de México (UNAM), Ciudad Universitaria, Coyoacán, 04510 México D. F., Mexico*

[c]*Centre for Nanoscience and Nanotechnology (UIEAST), Panjab University, Chandigarh, 160014, India. E-mail: navneetkaur@pu.ac.in*

† Electronic supplementary information (ESI) available: NMR, IR spectra, UV-Vis absorption, fluorescence profiles and graphs for the fabrication and operation of ISE based upon **2a–c**. See DOI: 10.1039/c3ra46759g

This journal is © The Royal Society of Chemistry 2014

Published on 16 December 2013. Downloaded by Carleton University on 19/06/2014 16:36:08.

solubilise the sensor in an aqueous surfactant solution and thus will ensure neutral water solubility.

Results and discussion

The objective of the current investigation is to develop water soluble, biocompatible, selective and sensitive Zn^{2+} sensors, and compound **1** was chosen as a model compound for this study. The rationale for this choice is based upon the fact that a chelate ring consisting of an –OH group and an sp^2 nitrogen donor is effective for selective recognition of Zn^{2+}. The moieties present in **1** may show both chromogenic and fluorescent recognition to some extent as detailed in the literature.[15] The target compound **1** was synthesized by the condensation of oleylamine with the respective salicylaldehyde and was characterized by various spectroscopic techniques (Fig. S1–S4†). Due to the poor solubility of **1** in pure aqueous media, we used an "aqueous friendly" version of the surfactant medium. A number of surfactants were tried in order to dissolve **1** in an aqueous medium, however the best solubility of this compound was achieved with a 1% Triton-X-100 aqueous solution. Alteration of the pH can affect the UV-Vis absorption and fluorescence profiles of sensor **1**, as it has an sp^2 N and an –OH group that are prone to protonation and ionization respectively with variation in pH. The effect of the pH on sensor **1** was evaluated in 1% Triton-X-100 in water. However, the UV-Vis absorption and fluorescence profiles of sensor **1** remained almost unaltered in the pH range 4–9 (Fig. S5†), so the sensor is suitable for use in living systems. The effect of ionic strength on the UV-Vis absorption and fluorescence emission profiles of sensor **1** was excluded by recording its fluorescence and UV-Vis spectra in the presence of increasing concentrations of NaCl (Fig. S6†).

The literature reports highlighting the introduction of a salicylic group in fluorescent chemosensors encouraged us to evaluate the properties of **1** as a fluorescent metal chemosensor. Sensor **1** showed an emission band centred at 440 nm when a 5 μM concentration of **1** (in aqueous 1% Triton-X-100 solution) was excited at λ_{ex} = 320 nm. Fig. 1 shows the fluorescence spectra of **1** before and after addition of 50 μM concentration of various metal ions (Na^+, K^+, Ba^{2+}, Sr^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Cr^{3+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, Ag^+, Hg^{2+}, and Cd^{2+}). A comparison between the fluorescence signature of **1** with the fluorescence profiles of **1** upon addition of metal ions revealed that Zn^{2+} significantly increases the fluorescence intensity of sensor **1** (Fig. S7†). The remarkable influence of Zn^{2+} on the fluorescence emission of sensor **1** indicates a strong interaction between **1** and Zn^{2+}. The probe **1** exhibits dual channel emission with weak emission at λ_{max} = 355 nm and moderate emission at λ_{max} = 440 nm in the fluorescence signature of **1** recorded in aqueous 1% Triton-X-100 solution. This is due to the existence of **1** in both the keto and enol tautomeric forms produced through the excited state intramolecular proton transfer (ESIPT) mechanism, involving the –OH group and sp^2 nitrogen of the imine linkage.[16] The emission at lower wavelength is assigned to the enol form, while the keto form emits at the higher wavelength.

To gain more insight into the modulation of the fluorescence signatures of **1** due to structural features, DFT calculations were performed using Becke's three parameterized Lee–Yang–Parr (B3LYP) exchange functional with 6-31G* basis sets, using Gaussian-09 programs (Fig. 2).[17] The enol form possesses the correct symmetry to show H-bonding between the –OH group and the sp^2 hybridised nitrogen. The fully optimised structures of **1** indicate the energy equivalence for the enol and keto states (Table S1†). Therefore, both tautomeric forms should exist in equilibrium and **1** may exhibit the ESIPT phenomenon.[18] DFT calculations predict the keto form of **1** to be energetically more stable than the enol form. The observed phenomenon of fluorescence enhancement of **1** upon binding of Zn^{2+} is due to conversion of the enol form to the keto form, and the enhancement is due to inhibition of the PET process.[19] This change was even clearly visible when sensor **1** was viewed in the absence or presence of Zn^{2+} under 365 nm UV light. A solution of sensor **1** in aqueous 1% Triton-X-100 solution became fluorescent when Zn^{2+} was added to it as shown in the inset of Fig. 3B.

Silica strips coated with the sensor and polymer coated sensor strips dipped in Zn^{2+} were observed under 365 nm UV

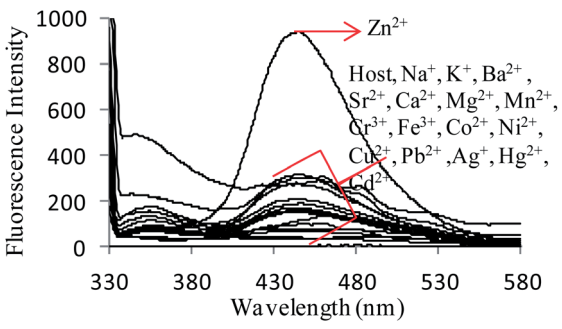


Fig. 1 Fluorescence emission spectra of **1** (5 μM) in the presence of different metal ions (50 μM) in aqueous 1% Triton-X-100 solution (excitation wavelength was 320 nm).

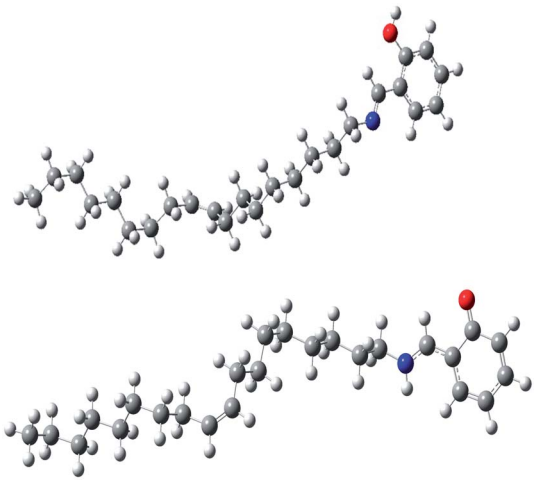


Fig. 2 Fully optimised structures of the enol and keto forms of sensor **1**; DFT calculations were performed at the B3LYP/6-31G* level (red, blue and grey spheres refer to O, N and C atoms respectively).

Published on 16 December 2013. Downloaded by Carleton University on 19/06/2014 16:36:08.

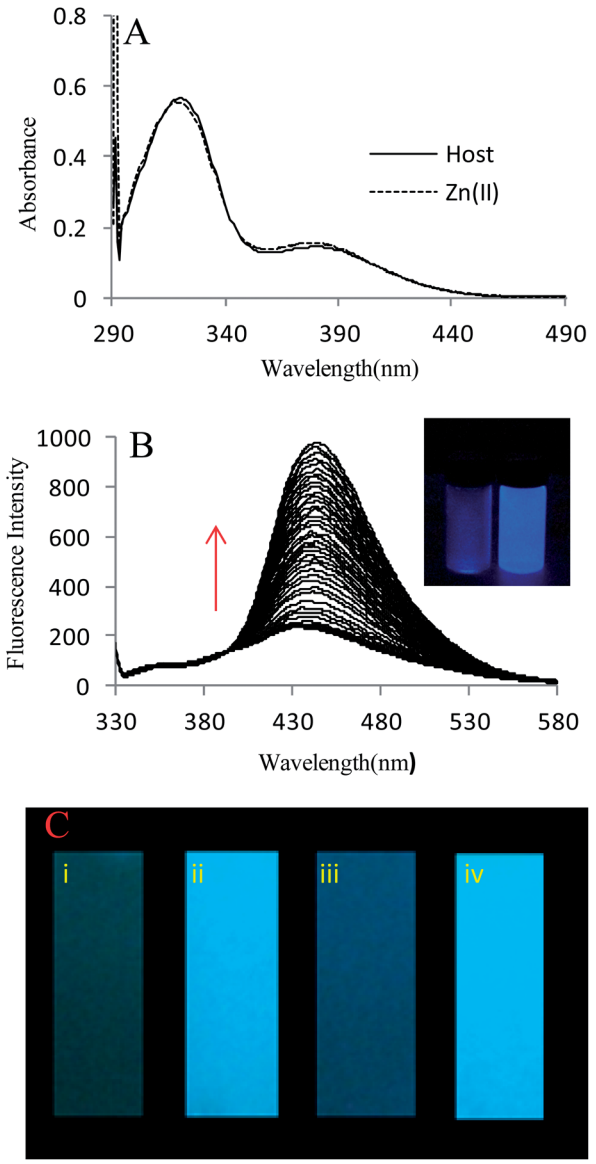


Fig. 3 (A) UV-Vis absorption spectra of sensor **1** in the presence and absence of Zn^{2+}; (B) fluorescence spectra of sensor **1** upon successive additions of Zn^{2+} (0–50 μM), inset of the figure shows the emission of a solution of sensor **1** in the presence and absence of Zn^{2+} under 365 nm UV light; (C) silica strips (i and ii) and polymer coated silica strips (iii and iv) dipped in sensor **1** (i–iv) and then in Zn^{2+} (ii and iv only) viewed under 365 nm UV light.

light. Fig. 3C shows that the strips became fluorescent in the presence of Zn^{2+}. The UV-Vis absorption spectrum of **1** in the presence of Zn^{2+} is almost the same as that of sensor **1**, as shown in Fig. 3A.

As there is no shift in the band maxima positions, it can be concluded that the change in fluorescence due to the binding of Zn^{2+} to sensor **1** is a consequence of excited state phenomena. Sensor **1** was titrated against Zn^{2+} (0–50 μM) in aqueous 1% Triton-X-100 solution with successive addition of aliquots of Zn^{2+}. The fluorescence intensity of the emission band of **1** at 440 nm increased steadily with subsequent additions of Zn^{2+}, as shown in Fig. 3B. The rise in fluorescence intensity is 322% of the fluorescence intensity in the absence of Zn^{2+}, demonstrating the wide range of Zn^{2+} concentrations that can be detected by sensor **1**. The mass spectrum of **1** shows a peak at *m*/*z* value 372.4, which corresponds to $[M + 1]$ where M is the molecular weight of **1** (Fig. S4†). The mass spectrum of complex **1**·Zn^{2+} shows a peak at *m*/*z* value 269.4, which corresponds to $[M + 1]^{2+}$, where M is $[\mathbf{1} + Zn^{2+} + NO_3^- + CH_3CN]$, suggesting that a 1 : 1 complex is formed between sensor **1** and Zn^{2+}. The observed stoichiometry of the complex was further confirmed with a Job's plot[20] (Fig. S8†). The binding constant for the formation of the **1**·Zn^{2+} supramolecular complex was calculated using a Benesi–Hildebrand plot of the fluorescence titration data, and it was calculated to be 1.05 (±0.3) × 10^5 (Fig. S9†), revealing strong binding between sensor **1** and Zn^{2+}.[21] Sensor **1** was found to be proficient in detecting Zn^{2+} concentration to a level of 8 nM (Fig. S10†). The detection limit was calculated from the fluorescence titration data using a known literature method, and the details are shown in the ESI.†[22]

To determine the binding sites of **1** responsible for the coordination of Zn^{2+}, an NMR titration of **1** with successive addition of Zn^{2+} was performed in a DMSO-d_6–D_2O (95 : 5, v/v) solvent system (Fig. 4). It was found that the addition of 3.0 equiv. of Zn^{2+} led to a shift of the signals ($\Delta\delta = 0.22$) corresponding to –CH=N. On the other hand, shifts were also observed for the aromatic signals (up to $\Delta\delta = 0.1$). These concurrent shifts led us to conclude the importance of the sp^2 nitrogen of the sensor. As the titration was conducted in DMSO-d_6–D_2O (95 : 5, v/v), our ability to determine the fate of the –OH signal during the course of the titration was limited.

The application of a probe as a sensor in biological systems requires the probe to be biocompatible. To address this, the cytotoxicity of **1** was determined using an MTT assay with HeLa cells cultured in a nutrient mixture. As per the standard protocol,[23] the cells were incubated at 37 °C in a 5% CO_2

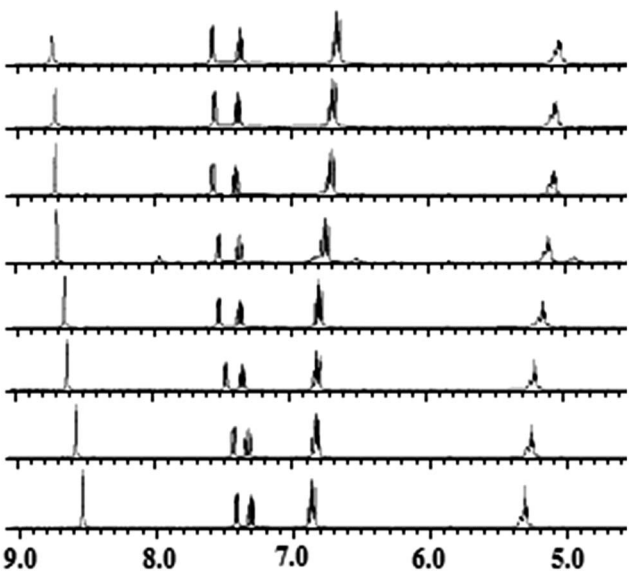


Fig. 4 Family of partial ^1H NMR spectra showing the change in the signals of **1** upon complexation of **1** (10 mM) with Zn^{2+} (5 equiv.) in a DMSO-d_6–D_2O (95 : 5, v/v) solvent system.

This journal is © The Royal Society of Chemistry 2014

Published on 16 December 2013. Downloaded by Carleton University on 19/06/2014 16:36:08.

environment for 24 h. Upon addition of **1**, only marginal cell death (~97% cell survival) was observed when the experiments were performed at the probe concentration used for recognition studies (5 μM). To investigate the potential cytotoxic effects of **1** at higher concentrations, dose dependent studies were performed. This experiment is mandatory because sometimes higher concentrations of the probe may be needed for certain biological applications. The results indicate that **1** is not very toxic to HeLa cells if the probe concentration remains under 22 μM, with ~85% cell survival over 24 h for a 22 μM concentration of **1**. At much higher concentrations of **1**, the probe became more toxic. For example, the IC_{50} value was found to be 56 μM over 24 h. To explore the application of **1** for Zn^{2+} recognition in real biological systems, we detected Zn^{2+} in blood serum, and the results were validated with atomic absorption spectra. A high Zn^{2+} concentration of 0.1 μM is naturally present in blood,[24] and deviation from the normal value is usually taken as an early diagnosis of certain illnesses.[25] Measurement of Zn^{2+} in red blood cells (RBCs) has been reported to be able to discriminate between hyperthyroid Grave's disease and transient thyrotoxicosis[26], and it may be a predictive indicator of sepsis syndrome in infancy.[27] The literature methods devised for the detection of Zn^{2+} ions in blood make use of atomic absorption spectroscopy (AAS),[28,29] such as graphite furnace AAS,[30] microwave assisted mineralization and flow injection AAS[31] and derivative microsampling flame AAS[32], or the inductively coupled plasma mass spectrometry (ICP-MS) method.[33,34] However, these methods are time consuming and fluorescence spectroscopy offers the advantage of being a simple to use method.

To investigate the practical application of **1** in Zn^{2+} recognition in biological systems, we cultured *Saccharomyces cerevisiae* in normal broth and in experimental media containing Zn^{2+}. The cells cultured in the media containing Zn^{2+} were treated with sensor **1** dissolved in aqueous 1% Triton-X-100 solution. Before performing microscopy observations, the microbe cells were washed with aqueous 1% Triton-X-100 solution. Microscopy images were taken of: (A) microbe cells cultured in normal medium, (B) microbe cells cultured in medium enriched with Zn^{2+}, (C) microbe cells cultured in normal medium and then treated with sensor **1**, and (D) microbe cells cultured in medium enriched with Zn^{2+} and treated with sensor **1** (Fig. 5). The microscopic investigations revealed that sensor **1** is capable of binding Zn^{2+} in a real cellular medium.

The roles of the sp^2 N and –OH binding sites were established by synthesizing compounds **2** and **3**, in which the hydroxyl group is missing in **2** and the imine linkage is missing in **3** (Fig. S11 and 12†). Neither showed any affinity for Zn^{2+} (Fig. S13 and 14†), confirming that the presence of both the sp^2 N and –OH binding sites is vital for the formation of a complex with Zn^{2+}. To check for any potential interference by any of the tested metal ions with the detection of Zn^{2+} by **1**, the ability of **1** to operate in solutions containing equimolar amounts of Zn^{2+} and other cations (10 equivalents) was tested. This experiment (as shown in Fig. 6) eliminated the possibility of any interference by the other tested cations with the recognition of Zn^{2+}.

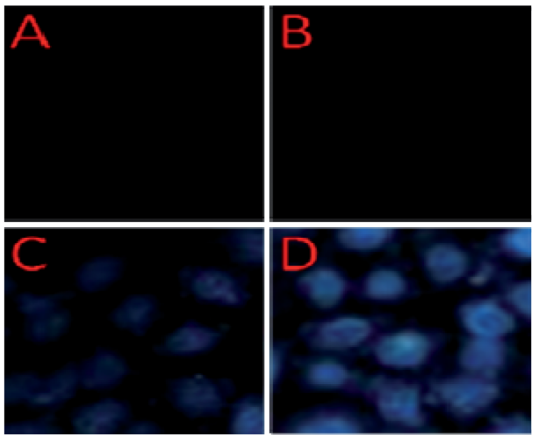


Fig. 5 Microscopic images of (A) microbe cells cultured in normal medium, (B) microbe cells cultured in medium enriched with Zn^{2+}, (C) microbe cells cultured in normal medium and then treated with sensor **1**, and (D) microbe cells cultured in medium enriched with Zn^{2+} and treated with sensor **1**. Before performing microscopy, the microbe cells were washed with aqueous 1% Triton-X-100 solution.

This implies that Zn^{2+} has a strong affinity for sensor **1** and it occupies the binding sites available in the sensor preferentially over other cations.

The effect of the pH on the binding affinity of sensor **1** for Zn^{2+} was also examined (Fig. S15†). A decrease in the pH inhibits the binding between sensor **1** and Zn^{2+}, however an increase in the pH up to 8.5 has no effect on the activity of sensor **1** for Zn^{2+} detection. However, at pH = 10, we observed the formation of a white ZnO precipitate.

Experimental section

Materials and methods

All the chemicals were purchased from commercial suppliers and were used without further purification. ^1H NMR and ^{13}C NMR spectra were recorded on an Avance-II (Bruker) instrument, which operated at 400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR. Fourier transform infrared (FT-IR) spectra of the dried

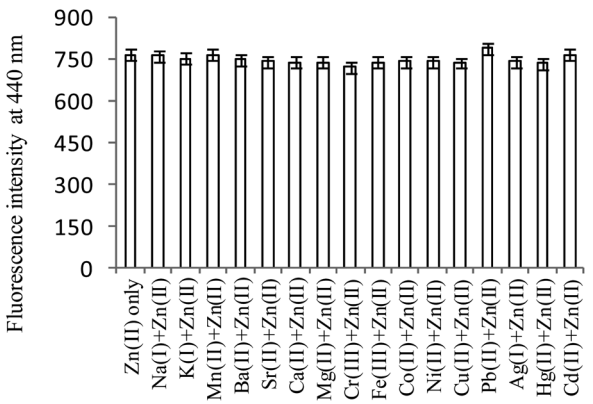


Fig. 6 Influence of the other tested metal ions on the sensor activity of **1** for Zn^{2+} binding.

Published on 16 December 2013. Downloaded by Carleton University on 19/06/2014 16:36:08.

compounds were measured on a Bruker Tensor 27 spectrophotometer, using a KBr pellet technique. For cation recognition studies, the UV-Vis absorption spectra were recorded using dilute solutions in quartz cells (1 cm path length) on a Specord 250 Plus Analytikjena spectrometer, and solid state spectra were recorded using fine coatings of the materials between the quartz plates. The fluorescence profiles of the sensor solutions were recorded on a Perkin Elmer L55 Fluorescence spectrophotometer using 1 cm path length quartz cells. The slit width for the excitation and emission was set at 10 nm and the scan speed was maintained at 200 scans per second throughout the experiments. The solid-state photoluminescence (PL) study was conducted using a setup that involved a solid sample holder. Scanning electron microscopic studies of aqueous solutions of the materials were carried out at a concentration of 200 μM. SEM images were recorded with a JEOL JSM-6610LV scanning electron microscope, which operated at 15 keV.

Synthesis of compound 1. A solution of oleylamine (267 mg, 1 mmol) and salicylaldehyde (183 mg, 1.5 mmol) in 50 ml dry methanol was stirred and refluxed for 4 h. Afterwards, the solvent was evaporated to 20 ml and the solution was kept at 0 °C. A yellow coloured solid separated out at low temperature. This solid was filtered and washed with cold methanol (three times), and the product (**1**) was obtained in 88% yield (327 mg). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 13.71 (br s, 1H, OH), 8.35 (s, 1H, CH=N), 7.29 (m, 2H, ArH), 6.98 (d, 1H, ArH), 6.88 (t, 1H, ArH), 5.38 (m, 2H, CH=CH), 3.60 (t, 2H, CH_2–N), 2.04 (m, 4H, C=C–CH_2), 1.71 (m, 2H, CH_2), 1.33–1.28 (m, 22H, CH_2), 0.89 (t, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 164.4, 161.4, 132.0, 131.1, 130.1, 129.8, 118.9, 118.4, 117.1, 59.6, 32.7, 31.9, 30.9, 29.8, 29.7, 29.6, 29.5, 29.5, 29.4, 29.3, 29.2, 29.1, 27.2, 22.8, 14.2; FTIR ν_{max} (KBr pellet): 1633 cm^{-1}. Anal. calcd for $C_{25}H_{41}NO$: C, 80.80; H, 11.12; N, 3.77. Found: C, 80.54; H, 11.31; N, 3.36; ESI-MS m/z = 372.4 (M + 1) (Scheme 1).

Synthesis of compound 2. A solution of oleylamine (267 mg, 1 mmol) and benzaldehyde (159 mg, 1.5 mmol) in 50 ml dry methanol was stirred and refluxed for 4 h. Afterwards, the solvent was evaporated to 20 ml and the solution was kept at 0 °C. An off-white coloured solid separated out at low temperature. This solid was filtered and washed with cold methanol (three times), and the product (**2**) was obtained in 82% yield (292 mg). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.29 (s, 1H, CH=N), 7.75 (m, 2H, ArH), 7.44 (m, 3H, ArH), 5.39 (m, 2H, CH=CH), 3.63 (t, 2H, CH_2–N), 2.03 (m, 4H, C=C–CH_2), 1.71 (m, 2H, CH_2), 1.35–1.28 (m, 22H, CH_2), 0.92 (t, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 160.6, 136.4, 130.4, 129.9, 129.8, 128.5, 128.1, 61.9, 32.7, 32.0, 31.8, 31.0, 29.9, 29.7, 29.6, 29.5, 29.47, 29.4, 29.3, 29.2, 29.1, 27.4, 27.3, 22.8, 14.2; FTIR ν_{max} (KBr pellet): 1647 cm^{-1}. Anal. calcd for $C_{25}H_{41}N$: C, 84.44; H, 11.62; N, 3.94. Found: C, 84.62; H, 11.51; N, 3.87 (Scheme 2).

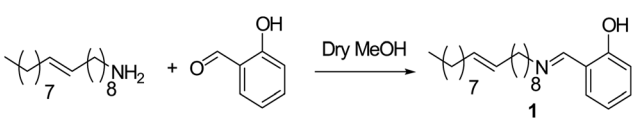
Synthesis of compound 3. Compound **1** (186 mg, 0.5 mmol) was reduced with $NaBH_4$ (76 mg, 2 mmol) in methanol (10 ml) at 50 °C for 8 h. After the completion of the reaction, the methanol was evaporated and the residue was dissolved in chloroform and washed with distilled water. The organic extract was separated, dried and evaporated to afford a white coloured solid compound (74% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.19 (t, 1H, ArH), 7.01 (d, 1H, ArH), 6.87 (d, 1H, ArH), 6.80 (t, 1H, ArH), 6.62 (br s, 1H, NH) 5.41 (m, 2H, CH=CH), 4.01 (s, 2H, N–CH_2), 2.70 (t, 2H, CH_2–N), 2.05 (m, 4H, C=C–CH_2), 1.56 (m, 2H, CH_2), 1.32 (m, 22H, CH_2), 0.93 (t, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 158.2, 129.8, 129.7, 128.6, 128.2, 122.2, 118.8, 116.3, 52.4, 48.5, 32.5, 31.8, 29.7, 29.6, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 27.1, 27.09, 27.0, 22.6, 14.0; FTIR ν_{max} (KBr pellet): 3702 cm^{-1}. Anal. calcd for $C_{25}H_{43}NO$: C, 80.37; H, 11.60; N, 3.75. Found: C, 80.52; H, 11.41; N, 3.82.

Metal recognition studies of 1–3

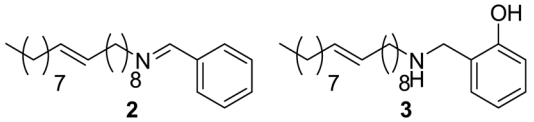
All the recognition studies were performed at 25 ± 1 °C, and before recording any spectra the samples were shaken for a sufficient time to ensure uniformity of the solutions. The cation binding abilities of **1–3** in 1% Triton-X-100 in water were determined by preparing standard solutions of **1–3** along with fixed amounts of the particular metal nitrate salts in 1% Triton-X-100 in water. The cation recognition behaviours of **1–3** were evaluated from the changes in photophysical properties of the sensors upon addition of that metal salt. The fluorescence spectra of **1–3** were recorded with the excitation wavelengths shown in the respective figures. For the titrations, volumetric flasks containing a standard solution of sensor **1** along with varying amounts of a particular metal nitrate salt in 1% Triton-X-100 in water were used. To evaluate any possible interference by other metal ions with the detection of Zn^{2+}, solutions were prepared containing sensor **1** (5 μM) along with a fixed concentration of Zn^{2+} (5 μM) both with and without other background cations (50 μM) in 1% Triton-X-100 in water. The fluorescence intensity of each solution was recorded. The effect of the pH on the UV-Vis absorption and fluorescence spectra of sensor **1** was investigated by recording spectra of sensor **1** in 1% Triton-X-100 in water with variable pH.

Stoichiometry determination

In order to determine the stoichiometry of the complex formed from receptor **1** and Zn^{2+}, solutions of **1** and Zn^{2+} were prepared with ratios of **1** : Zn^{2+} of 1 : 9, 2 : 8, 3 : 7, 4 : 6, 5 : 5, 6 : 4, 7 : 3, 8 : 2 and 9 : 1. These solutions were kept for 1 h, and were shaken occasionally. Fluorescence spectra were recorded for the Zn^{2+} complex. A plot of [HG] *vs.* [H]/[H] + [G] (H = host,



Scheme 1 Synthesis of sensor **1**.



Scheme 2 Chemical structures of **2** and **3**.

 This journal is © The Royal Society of Chemistry 2014

Published on 16 December 2013. Downloaded by Carleton University on 19/06/2014 16:36:08.

G = guest, HG = host-guest complex) was used to determine the stoichiometry of the complex formed. The fluorescence intensity at 440 nm was used for the calculations. The concentration of HG was calculated using the equation $[HG] = \Delta I/I_o \times [H]$.

Cytotoxicity of 1

The cytotoxicity of **1** was determined through an MTT assay, using HeLa cells seeded in a 96-well flat-bottomed microplate in growth medium (100 μl) and incubated at 37 °C under a 5% CO_2 atmosphere for 24 h. An analysis using **1** as the test compound and a blank analysis were performed, and 10 μl of MTT in PBS was added to each well. The microplate was incubated at 37 °C under a 5% CO_2 atmosphere for another 3 h. The medium was then removed, and DMSO (100 μl) was added to each well. The absorbance spectrum of each solution was measured at 570 nm. The dose dependent cytotoxicity of **1** was determined using different concentrations of **1** using the same conditions as mentioned above.

Fabrication of sensor strips

Two types of strip were used: one was simply a silica strip coated with sensor **1**, and the second consisted of a silica strip first coated with polymer polyethylene grafted maleic anhydride and then coated with sensor **1**. Both of these become fluorescent upon dipping into a solution of Zn^{2+}.

Conclusion

A highly selective sensor, **1**, with a chelate ring consisting of –OH and sp^2 N moieties for the detection of Zn^{2+} in an aqueous surfactant solution was synthesized. Sensor **1** displays an approximately 300% increase in fluorescence intensity upon binding to Zn^{2+}, through the collective effects of ESIPT and inhibition of PET.

Acknowledgements

This work was supported with a research grant (project no.: 01(2417)/10/EMR-II) from CSIR (New Delhi, India). V. K. B. is thankful to the DST for the INSPIRE Faculty grant.

Notes and references

1 (*a*) A. Coskun and E. U. Akkaya, *J. Am. Chem. Soc.*, 2005, **127**, 10464; (*b*) G. Kang, H. Son, J. M. Lim, H. S. Kweon, I. S. Lee, D. Kang and J. H. Jung, *Chem.–Eur. J.*, 2012, **18**, 5843; (*c*) S. Das, A. Sahana, A. Banerjee, S. Lohar, D. A. Safin, M. G. Babashkina, M. Bolte, Y. Garcia, I. Hauli, S. K. Mukhopadhyay and D. Das, *Dalton Trans.*, 2013, **42**, 4757; (*d*) C. Kaewtong, J. Noiseephum, Y. Uppa, N. Morakot, N. Morakot, B. Wanno, T. Tuntulanic and B. Pulpoka, *New J. Chem.*, 2010, **34**, 1104.

2 (*a*) J. Wu, W. Liu, J. Ge, H. Zhang and P. Wang, *Chem. Soc. Rev.*, 2011, **40**, 3483; (*b*) L. B. Desmonts, D. N. Reinhoudt and M. C. Calama, *Chem. Soc. Rev.*, 2007, **36**, 993; (*c*) G. P. C. Drummen, *Molecules*, 2012, **17**, 14067.

3 (*a*) Z. Xu, J. Yoon and D. R. Spring, *Chem. Soc. Rev.*, 2010, **39**, 1996; (*b*) Z. Xu, X. Liu, J. Pan and D. R. Spring, *Chem. Commun.*, 2012, **48**, 4764.

4 D. Bhowmik, B. Chiranjib and K. P. S. Kumar, *International Journal of Pharmacy and Biomedical Sciences*, 2010, **1**, 5.

5 (*a*) R. K. Chandra, *JAMA, J. Am. Med. Assoc.*, 1984, **252**, 1443; (*b*) M. Shannon, *Clin. Toxicol.*, 1999, **37**, 709; (*c*) C. A. D. Lewis, R. L. Lukman and R. K. Banks, *Comp. Med.*, 2011, **61**, 361.

6 (*a*) U. C. Saha, B. Chattopadhyay, K. Dhara, S. K. Mandal, S. Sarkar, A. R. Khuda-Bukhsh, M. Mukherjee, M. Helliwell and P. Chattopadhyay, *Inorg. Chem.*, 2011, **50**, 1213; (*b*) K. Hanaoka, K. Kikuchi, H. Kojima, Y. Urano and T. Nagano, *J. Am. Chem. Soc.*, 2004, **126**, 12470; (*c*) D. A. Pearce, N. Jotterand, I. S. Carrico and B. Imperiali, *J. Am. Chem. Soc.*, 2001, **123**, 5160; (*d*) C. He, W. Zhu, Y. Xu, Y. Zhong, J. Zhou and X. Qian, *J. Mater. Chem.*, 2010, **20**, 10755; (*e*) L. Xu, Y. Xu, W. Zhu, B. Zeng, C. Yang, B. Wu and X. Qian, *Org. Biomol. Chem.*, 2011, **9**, 8284; (*f*) N. C. Lim, L. Yao, H. C. Freake and C. Bruckner, *Bioorg. Med. Chem. Lett.*, 2003, **13**, 2251.

7 (*a*) L. E. McQuade and S. J. Lippard, *Inorg. Chem.*, 2010, **49**, 9535; (*b*) N. Y. Baek, C. H. Heo, C. S. Lim, G. Masanta, B. R. Cho and H. M. Kim, *Chem. Commun.*, 2012, **48**, 4546; (*c*) Z. Liu, C. Zhang, Y. Li, Z. Wu, F. Qian, X. Yang, W. He, X. Gao and Z. Guo, *Org. Lett.*, 2009, **11**, 795; (*d*) E. J. Choi, C. H. Kang, H. N. Choi and W. Y. Lee, *Bull. Korean Chem. Soc.*, 2009, **30**, 2387; (*e*) A. E. Dennisa and R. C. Smith, *Chem. Commun.*, 2007, 4641.

8 S. Comby, S. A. Tuck, L. K. Truman, O. Kotova and T. Gunnlaugsson, *Inorg. Chem.*, 2012, **51**, 10158.

9 Q. H. You, P. S. Chan, W. H. Chan, S. C. K. Hau, A. W. M. Lee, N. K. Mak, T. C. W. Mak and R. N. S. Wong, *RSC Adv.*, 2012, **2**, 11078.

10 Y. Zhang, X. Guo, L. Jia, S. Xu, Z. Xu, L. Zhenga and X. Qian, *Dalton Trans.*, 2012, **41**, 11776.

11 K. Hanaoka, Y. Muramatsu, Y. Urano, T. Terai and T. Nagano, *Chem.–Eur. J.*, 2010, **16**, 568.

12 (*a*) Z. Li, Y. Xiang and A. Tong, *Anal. Chim. Acta*, 2008, **619**, 75; (*b*) Y. Mei and P. A. Bentley, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 3131; (*c*) Z. Liu, B. Wang, Z. Yang, T. Li and Y. Li, *Inorg. Chem. Commun.*, 2010, **13**, 606; (*d*) L. Wang, W. Qin and W. Liu, *Inorg. Chem. Commun.*, 2010, **13**, 1122.

13 (*a*) E. Tomat and S. J. Lippard, *Inorg. Chem.*, 2010, **49**, 9113; (*b*) V. K. Bhardwaj, S. Sharma, N. Singh, M. S. Hundal and G. Hundal, *Supramol. Chem.*, 2011, **23**, 790.

14 (*a*) P. Saluja, H. Sharma, N. Kaur, N. Singh and D. O. Jang, *Tetrahedron*, 2012, **68**, 2289; (*b*) P. Saluja, N. Kaur, N. Singh and D. O. Jang, *Tetrahedron*, 2012, **68**, 8551; (*c*) V. K. Bhardwaj, P. Saluja, M. S. Hundal, G. Hundal, N. Singh and D. O. Jang, *Tetrahedron*, 2013, **69**, 1606.

15 (*a*) D. Maity and T. Govindaraju, *Chem.–Eur. J.*, 2011, **17**, 1410; (*b*) H. M. Abu-Shawish, *J. Hazard. Mater.*, 2009, **167**, 602; (*c*) A. Kumar, V. Kumar, U. Diwan and K. K. Upadhyay, *Sens. Actuators, B*, 2013, **176**, 420; (*d*) S. Anbu, S. Shanmugaraju, R. Ravishankaran, A. A. Karande and P. S. Mukherjee, *Inorg. Chem. Commun.*,

Published on 16 December 2013. Downloaded by Carleton University on 19/06/2014 16:36:08.

2012, **25**, 26; (*e*) Q. Li, Y. Guo, J. Xua and S. Shao, *Sens. Actuators, B*, 2011, **158**, 427; (*f*) D. K. Das, P. Goswami and S. Sarma, *J. Fluoresc.*, 2013, **23**, 503; (*g*) N. Li, W. Tang, Y. Xiang, A. Tong, P. Jin and Y. Ju, *Luminescence*, 2010, **25**, 445.

16 (*a*) F. S. Rodembusch, F. P. Leusin, L. B. Bordignon, M. R. Gallas and V. Stefani, *J. Photochem. Photobiol., A*, 2005, **173**, 81; (*b*) D. P. Murale, H. Kim, W. S. Choi and D. G. Churchill, *Org. Lett.*, 2013, **15**, 3946; (*c*) D. Maity, V. Kumar and T. Govindaraju, *Org. Lett.*, 2012, **14**, 6008; (*d*) A. J. A. Aquino, F. Plasser, M. Barbatti and H. Lischka, *Croat. Chem. Acta*, 2009, **82**, 105; (*e*) D. A. Yushchenko, V. V. Shvadchak, A. S. Klymchenko, G. Duportail, Y. Mely and V. G. Pivovarenko, *New J. Chem.*, 2006, **30**, 774; (*f*) D. LeGourriérec, V. A. Kharlanov, R. G. Brown and W. Rettig, *J. Photochem. Photobiol., A*, 2000, **130**, 101; (*g*) V. Luxami and S. Kumar, *RSC Adv.*, 2012, **2**, 8734.

17 (*a*) J. Jayabharathi, V. Thanikachalam and K. Jayamoorthy, *Spectrochim. Acta, Part A*, 2012, **89**, 168; (*b*) J. Jayabharathi, V. Thanikachalam, M. Vennila and K. Jayamoorthy, *Spectrochim. Acta, Part A*, 2012, **95**, 446.

18 M. J. Kim, K. Kaur, N. Singh and D. O. Jang, *Tetrahedron*, 2012, **68**, 5429.

19 (*a*) H. Sharma, H. J. Guadalupe, J. Narayanan, H. Hofeld, T. Pandiyan and N. Singh, *Anal. Methods*, 2013, **5**, 3880; (*b*) S. H. Mashraqui, R. Betkar, M. Chandiramani, C. Estarellas and A. Frontera, *New J. Chem.*, 2011, **35**, 57; (*c*) X. Chen, X. Tian, I. Shin and J. Yoon, *Chem. Soc. Rev.*, 2011, **40**, 4783; (*d*) S. K. Sahoo, D. Sharma, R. K. Bera, G. Crisponic and J. F. Callan, *Chem. Soc. Rev.*, 2012, **41**, 7195; (*e*) P. Saluja, N. Kaur, N. Singh and D. O. Jang, *Tetrahedron Lett.*, 2012, **53**, 3292.

20 P. Job, *Ann. Chim.*, 1928, **9**, 113.

21 H. Benesi and H. Hildebrand, *J. Am. Chem. Soc.*, 1949, **71**, 2703.

22 M. Shortreed, R. Kopelman, M. Kuhn and B. Hoyland, *Anal. Chem.*, 1996, **68**, 1414.

23 A. A. Van de Loosdrecht, R. H. J. Beelen, G. J. Ossenkoppele, M. G. Broekhoven and M. M. A. C. Langenhuijsen, *J. Immunol. Methods*, 1994, **174**, 311.

24 B. Xu, S. E. Chia and C. N. Ong, *Biol. Trace Elem. Res.*, 1994, **40**, 49.

25 J. Szpunar, J. Bettmer, M. Robert, H. Chassaigne, K. Cammann, R. Lobinski and O. F. X. Donard, *Talanta*, 1997, **44**, 1389.

26 N. Sayama, K. Oshida, K. Mori, H. Fukazawa, H. Hori, N. Nakazato, J. I. Tani, Y. Nakagawa and S. Ito, *Endocr. J.*, 1998, **45**, 767.

27 G. Saner, S. U. Baysal, E. Unuvar and T. Ozden, *J. Trace Elem. Exp. Med.*, 2000, **13**, 265.

28 A. Brockhaus, *VDI-Ber.*, 1973, **203**, 20.

29 T. Maeda, M. Nakagawa, M. Kawakatsu and Y. Tanimoto, *Shimadzu Hyoron*, 1980, **37**, 75.

30 M. Tuzen, *Trace Elem. Electrolytes*, 2002, **19**, 202.

31 J. L. Burguera, M. Burguera and M. R. Brunetto, *At. Spectrosc.*, 1993, **14**, 90.

32 X. Ji and J. Ren, *Analyst*, 2002, **127**, 416.

33 H. Vanhoe, C. Vandecasteele, J. Versieck and R. Dams, *Anal. Chem.*, 1989, **61**, 1851.

34 P. Schramel, J. Begerow and H. Emons, *Anal. Hazard. Subst. Biol. Mater.*, 1999, **6**, 1.

 This journal is © The Royal Society of Chemistry 2014