A highly selective, sensitive and reversible fluorescence chemosensor for Zn$^{2+}$ and its cell viability†

Anoop Kumar Saini,a Mansi Srivastava,b Vinay Sharma,b Veenu Mishraa and Shaikh M. Mobina,b,c

A new imine conjugate Schiff base ligand ($H_2L$) was prepared and evaluated for its sensing properties. $H_2L$ detects Zn$^{2+}$ selectively among the wide range of metal ions. The sensing behavior of $H_2L$ was identified by UV-vis, fluorescence and $^1$H NMR titration. $H_2L$ shows fluorescence switch ON with the Zn$^{2+}$ ion among 18 other metal/heavy metal ions with a detection limit of 1.47 μM. The binding of Zn$^{2+}$ was confirmed by single crystal X-ray studies, which reveal the formation of binuclear complex (1). The packing diagram of $H_2L$ reveals the presence of rare linear C–H···C interactions (bond distance 2.79 Å and bond angle 180°) which form 1D-polymeric chains. Furthermore, the cytotoxicity of $H_2L$ and 1 has been assessed against MCF-7 and A375 cell lines and both are found to have marginal toxicity.

Introduction

Zinc(II) is the second most abundant trace element found in the human body and is present as metalloproteins.1–4 Zn$^{2+}$ plays a major role in various human diseases, such as Parkinson's disease, infantile diarrhea, Alzheimer's disease, and ischemic stroke.5–9 Compounds containing Zn$^{2+}$ have potential applications as tumor photosensitizers, antibacterials, anti-diabetics, insulin mimetics, and radioprotective and anticancer agents.10 To monitor the movement and activity of Zn$^{2+}$ in the human body, the development of advanced detection methods involving fluorescence sensors and chemosensors are highly desirable. Due to the low cost, faster response time, and low detection limit with high selectivity and sensitivity, fluorescent chemosensors have recently gained considerable interest.11–13 However, most of the presently reported Zn$^{2+}$ sensors suffer from some limitations like low signal continuity, high light scattering, auto-fluorescence and low Zn$^{2+}$ binding affinity.

So far the fluorescence sensors synthesized for the selective detection of Zn$^{2+}$ contain some commonly used ligands such as fluorescein, coumarin, dipicolylamine, quinoline, and 4-nitrobenzoxadiazole as receptors in addition to their tedious reaction conditions.14–17 On the other hand, simple Schiff base originated imine (HC=C==N−) group ligands are known for their metal binding affinity.18–22 However, very few reports are available for the construction and design of a novel probe for Zn$^{2+}$ sensors.23

Herein, we report the synthesis and sensing behavior of ligand $H_2L$ by using UV-visible, fluorescence and NMR titration. The binding features of $H_2L$ towards Zn$^{2+}$ have also been discussed by using single crystal X-ray studies. The cytotoxicity associated with metal complexes is a key factor for their in vivo applicability; hence the cytotoxicity of $H_2L$ and 1 was also evaluated against MCF-7 and A375 cell lines.

Results and discussion

A new ligand 1,1’-(1E,1’E)-(2,4,6-trimethyl-1,3-phenylene)bis-(azan-1-yl-1-ylidene)bis(methan-1-yl-1-ylidene)dinaphthalen-2-ol ($H_2L$) was obtained by the reaction of 2,4,6-trimethylbenzene-1,3-diamine with 2-hydroxy-1-naphthaldehyde (1:2) in methanol under reflux conditions for 4 h (Scheme 1). $H_2L$ has been characterized by elemental analysis, NMR and ESI-MS spectroscopic techniques and further authenticated by single crystal X-ray studies.

$H_2L$ crystallizes in the monoclinic C2/c space group with a crystallographically imposed inversion center (Fig. 1 and Table 1). The central trimethyl benzene ring is observed to be slightly tilted from the plane by 2.87°, and the presence of two arms of 2-hydroxy-1-naphthyl moieties adjacent to each other...
are arranged in a \textit{trans}-fashion. A closer look on the packing features in \ce{H2L} reveals the presence of interesting C–H...C interactions, which leads to the formation of 1D-polymeric  
chains (Fig. 2). Further, these 1D polymeric chains are linked to each other through C(14)–H(14B)...O(1), 2.313 Å interactions  

**Table 1** Crystallographic parameters of \ce{H2L} and 1  

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**Scheme 1** Schematic representation showing the synthesis of ligand \ce{H2L}.

**Fig. 1** Molecular structure of \ce{H2L}.

along the c-axis forming a supramolecular 2D-network (Fig. S1 and Table S2†).

**Photophysical behavior of \ce{H2L}**

The absorption spectra of \ce{H2L} in an ACN–H₂O mixture (7 : 3 v/v) in 10 µM HEPES buffer at pH = 7.4) were dominated by two absorption peaks at 315 nm and 370 nm wavelength corresponding to the π-π* and π-π transitions, respectively. Further, different metal ions viz., \ce{Ag⁺}, \ce{Na⁺}, \ce{K⁺}, \ce{Li⁺}, \ce{Mn^{2+}}, \ce{Cd^{2+}}, \ce{Hg^{2+}}, \ce{Mg^{2+}}, \ce{Ca^{2+}}, \ce{Cr^{3+}}, \ce{Ni^{2+}}, \ce{Co^{2+}}, \ce{Cu^{2+}}, \ce{Zn^{2+}}, \ce{Pb^{2+}}, \ce{Fe^{3+}}, \ce{Al^{3+}} and As^{3+} at varying concentrations were introduced to the \ce{H2L} solution. The subsequent addition of \ce{Zn^{2+}} results in a gradual decrease of absorption maxima at 370 nm with the concomitant formation of two new absorption peaks at 394 and 412 nm (Fig. 3a). This bathochromic shift between two isosbestic points at 434 nm and 384 nm is the evidence for the strong binding nature of \ce{H2L} towards \ce{Zn^{2+}} ions. However, titration of \ce{H2L} with other metal ions shows no significant change in the absorbance (Fig. 3b).

In the case of emission studies \ce{H2L} shows weak fluorescence at 454 nm which is attributed to the isomerization of the imine bond (–HC=–N) and excited state intramolecular proton transfer (ESIPT) from the –OH group to the imine nitrogen at 370 nm excitation in the ACN–H₂O mixture (7 : 3 v/v) in 10 µM HEPES buffer at pH = 7.4. The titration of \ce{H2L} with \ce{Zn^{2+}} revealed that the gradual fluorescence enhancement at 454 nm (Fig. 4a) may be due to the formation of a rigid system on binding with \ce{Zn^{2+}} ions. Further, titration of \ce{H2L} against different metal ions viz., \ce{Ag⁺}, \ce{Na⁺}, \ce{K⁺}, \ce{Li⁺}, \ce{Mn^{2+}}, \ce{Cd^{2+}}, \ce{Hg^{2+}}, \ce{Mg^{2+}}, \ce{Ca^{2+}}, \ce{Cr^{3+}}, \ce{Ni^{2+}}, \ce{Co^{2+}}, \ce{Cu^{2+}}, \ce{Zn^{2+}}, \ce{Pb^{2+}}, \ce{Fe^{3+}}, \ce{Al^{3+}} and As^{3+} was performed and the resulting emission spectra show no enhancement of fluorescence intensity at 454 nm. However, on addition of \ce{Zn^{2+}} the sensor showed fluorescence turn-ON with about 9 fold enhancement in fluorescence intensity (Fig. 4b).

The probable reasons for the fluorescence turn-ON may be due to (i) the deprotonated form of \ce{H2L} by addition of \ce{Zn^{2+}} ions, (ii) a new band in the absorption spectrum and (iii) the photoinduced electron transfer (PET) suppressed by \ce{Zn^{2+}} coordination. Thus, the results obtained from absorption and emission studies validate the high sensing behavior of \ce{H2L} specific to \ce{Zn^{2+}} ions.

The binding affinity of \ce{H2L} with \ce{Zn^{2+}} has been calculated using the Benesi–Hildebrand equation and found to be 1.13 × 10⁷ M⁻¹ and 1.21 × 10⁷ M⁻¹ by absorption (Fig. S2†) and emission (Fig. S3†) methods, respectively. The limit of detection (LOD) is calculated using the following equation:\[ 26 \text{ LOD} = \frac{3.3}{σ} \text{m(S)}, \]where σ is the standard error and S is the slope of calibration.

The LOD was determined to be 1.47 µM. The effect of the influence of other active metal ions viz., \ce{Ag⁺}, \ce{Na⁺}, \ce{K⁺}, \ce{Li⁺}, \ce{Mn^{2+}}, \ce{Cd^{2+}}, \ce{Hg^{2+}}, \ce{Mg^{2+}}, \ce{Ca^{2+}}, \ce{Cr^{3+}}, \ce{Ni^{2+}}, \ce{Co^{2+}}, \ce{Cu^{2+}}, \ce{Pb^{2+}}, \ce{Fe^{3+}}, \ce{Al^{3+}} and As^{3+} with \ce{H2L} in the presence of \ce{Zn^{2+}} was studied with concentration four times the concentration of \ce{Zn^{2+}} (Fig. 5), which shows that only \ce{Zn^{2+}} ion exhibits fluorescence turn-ON. This indicates that none of the metal ions interfere in the fluorescence intensity of the \ce{H2L–Zn} complex.
Fig. 2  C–H⋯C interaction supported 1D-polymeric chains in H₂L.

Fig. 3  (a) UV-vis absorption titration spectra of H₂L (c = 1.0 x 10⁻⁵ M) in aq. ACN (ACN/H₂O = 7 : 3 v/v, 10 µM HEPES buffer, pH = 7.4) with Zn²⁺ metal ions (c = 1.0 x 10⁻⁴ M). (b) Competitive absorption spectra of H₂L in the presence of different metal ions (nitrates, chlorides) salts of Ag⁺, Na⁺, K⁺, Mn²⁺, Cd²⁺, Hg²⁺, Mg²⁺, Ca²⁺, Cr²⁺, Ni²⁺, Co²⁺, Cu²⁺, Zn²⁺, Pb²⁺, Fe²⁺, Al³⁺ and As³⁺ in aq. ACN (ACN/H₂O = 7 : 3 v/v, 10 µM HEPES buffer, pH = 7.4).

Fig. 4  (a) Fluorescence emission spectra obtained during the titration of H₂L (c = 1.0 x 10⁻⁵ M) in aq. ACN (ACN/H₂O = 7 : 3 v/v, 10 µM HEPES buffer, pH = 7.4) with Zn²⁺ ions (c = 1.0 x 10⁻⁴ M); the inset shows the relative fluorescence intensity (I/I₀) as a function of [Zn²⁺]/[H₂L] mole ratio. (b) Effect of fluorescence intensity at 454 nm with addition of Zn²⁺ and with other metal ions.
Further, to understand the mode of complexation of H$_2$L with Zn$^{2+}$ ions, $^1$H NMR titration was performed in acetone-D$_6$ and D$_2$O solvents. On subsequent addition of Zn$^{2+}$ to H$_2$L, the naphthyl –OH peak (15.34 ppm) decreases and finally at 0.8 eq. of Zn$^{2+}$, it was observed that the –OH peak completely disappeared (Fig. 6), which indicates the complex formation of H$_2$L with Zn$^{2+}$.

In order to authenticate the complex formation as indicated by $^1$H NMR an attempt was made to crystallize the Zn$^{2+}$ + H$_2$L compound. The reaction of H$_2$L with Zn(NO$_3$)$_2$·6H$_2$O in MeOH at RT yields single crystals of dimeric [Zn(C$_{31}$H$_{24}$N$_2$O$_2$)]$_2$ (1) in two weeks (Scheme 2).

1 crystallizes in the trigonal R3 space group with a crystallographically imposed inversion centre (Fig. 7 and Table 1). Each Zn$^{2+}$ ion is in a N$_2$O$_2$ tetracoordinated environment from each naphthyl group which leads to the distorted tetrahedral geometry around each Zn$^{2+}$ ion. The distance between two Zn$^{2+}$ centres is 6.958 Å with a cavity size of 6.958 × 3.882 Å. The crystal structure of 1 is in agreement with the Job plot which indicates 1 : 1 complexation (Fig. 8) stoichiometry between H$_2$L and Zn$^{2+}$.

The packing diagram of 1 reveals the presence of C–H⋯O and C–H⋯π interactions. The C(17)–H(17)⋯O(1), 2.718 Å, intermolecular hydrogen bonding interactions involve the donor oxygen atom of the naphthyl group and the hydrogen atom of the adjacent naphthyl ring leading to the formation of 1D-polymeric chains (Fig. S4†), which further extend via C(19)–H(19)⋯π interactions between two adjacent layers forming a 2D-network along the a-axis (Fig. 9).

The electronic spectra of 1 (Fig. S5†) show peaks at 414 (ε, 1.9 × 10$^3$ M$^{-1}$ cm$^{-1}$), 396 (ε, 1.9 × 10$^3$ M$^{-1}$ cm$^{-1}$) and 318 nm (ε, 2.0 × 10$^3$ M$^{-1}$ cm$^{-1}$) corresponding to the π–π$^*$ transition and n–π$^*$ transition, respectively. The quantum yield and average lifetime of 1 were found to be 0.121 and $<$$\tau$$>$ = 1.36 ns, respectively (Table 2, S3 and Fig. S6†).

**Reversibility of sensor**

The reversibility of chemosensor H$_2$L was studied by using a chelating agent EDTA. The enhanced fluorescence intensity in the titration of H$_2$L with Zn$^{2+}$ ions was observed to be quenched as expected on addition of EDTA, demonstrating the binding of H$_2$L with Zn$^{2+}$ as reversible (Scheme 3 and Fig. 10, S7†).

**Cytotoxicity studies**

The cytotoxicity of H$_2$L and 1 was investigated on two different cell lines MCF-7 and A375. The concentration was tested in the range of 0–300 μM of H$_2$L and 1. A two-step toxicity effect was found for both H$_2$L and 1, when the concentration was increased from 0 μM to 40 μM, a considerable decrease in cell viability was observed but on further increasing the concentration from 40 μM up to 300 μM, a substantial effect was found. In the case of both MCF-7 and A375, more than 60% cells were alive up to a concentration of 300 μM. Although the IC$_{50}$ could not be determined up to the tested concentration (300 μM) but considering the reduced viability at low concentrations up to 40 μM, marginal toxicity of H$_2$L and 1 can be concluded. The comparison with the recently reported cytotoxic agents, the toxicity of H$_2$L and 1 was found to be relatively less. 27,28 The results of the MTT assay are depicted in Fig. 11.

To visualize the extent of live and dead cells after treatment with H$_2$L and 1, a dual staining study was performed, where treated and control cell samples were stained with Hoechst 33342 and propidium iodide. Hoechst is a cell permeable dye and it effectively stains all cells while propidium iodide is a membrane impermeable dye and can only stain cells with a compromised cell membrane. Hence dual staining was used to distinguish between the live and dead cells and to examine their relative proportion. The fluorescence microscopy images revealed that a significantly large number of live cells were present as compared to dead cells in both H$_2$L and 1 treated wells. It is clearly visible from Fig. 12 that H$_2$L and 1 have almost similar and marginal toxicity to the A375 and MCF-7 cells and hence can be used in biological systems.
Conclusion

A new chemosensor $\text{H}_2\text{L}$ has been designed and synthesized. The $\text{H}_2\text{L}$ sensor was employed for metal sensing properties and found to be sensitive, selective and reversible in nature, which showed fluorescence turn-ON with the Zn$^{2+}$ ion among 18 other metal ions. The binding mode of the $\text{H}_2\text{L}$ sensor was confirmed by single crystal X-ray studies that reveal the formation of a binuclear Zn(II) complex (1). Furthermore, the cytotoxicity of $\text{H}_2\text{L}$ and 1 has been evaluated against MCF-7 and A375 cell lines and found to be compatible in biological systems. Thus, $\text{H}_2\text{L}$ and 1 can be potential candidates for...
some in vivo or in vitro biological studies in future. H2L shows the presence of unusual linear C–H⋯C contacts which lead to the formation of 1D-polymeric chains, which cleave on binding with Zn(II). Moreover, H2L as a ligand can be considered to be a favorable synthon for different metal ion complexations.

### Experimental details

#### Materials

Commercially available materials and reagent grade solvents were used as received. The common reagents and solvents were procured from Merck and S. D. Fine Chem. Ltd. The solvents were dried and distilled following the standard literature.
procedures prior to their use. 2-Hydroxy-1-naphthaldehyde and 2,4,6-trimethylbenzene-1,3-diamine were purchased from Sigma Aldrich Chemical Co., USA and used as received without further purifications.

**Physical measurements**

$^1$H NMR (400 MHz) and $^{13}$C NMR (100 MHz) spectra were recorded on a Bruker Avance (III) instrument by using CDCl$_3$. $^1$H NMR chemical shifts are reported in parts per million (ppm) relative to the solvent residual peak (CDCl$_3$, 7.26 ppm). $^{13}$C NMR chemical shifts are reported relative to the solvent residual peak (CDCl$_3$, 77.36 ppm). IR spectra [4000–400 cm$^{-1}$] were recorded with a Bio-Rad FTS 3000MX instrument on KBr pellets. Elemental analyses were carried out with a Thermo-Flash 2000 elemental analyser. Spectrophotometric measurements were performed on a Varian UV-vis spectrophotometer (model: Cary 100) using a quartz cuvette with a path length of 1 cm. The excitation and emission slits were 5/5 nm for the emission measurements. The mass spectra were recorded on a Bruker-Daltonics, micrOTOF-QII mass spectrometer. The life time measurement was recorded on a TCSPC system from Horiba Yovin (model: Fluorocube-01-NL). The samples were excited at 375 nm using a picosecond diode laser (model: Pico Brite-375L) with the repetition rate of 5 MHz. The signals were collected at magic angle (54.70) polarization using a photomultiplier tube (TBX-07C) as the detector, which has a dark count less than 20 cps. The instrument response function of our setup was 150 ps. The data analysis was performed using IBH DAS (version 6, HORIBA Scientific, Edison, NJ) decay analysis software.

**X-ray crystallography**

Data were collected at 293 K using graphite-monochromated Mo Kα ($\lambda$ = 0.71073 Å) and Cu Kα ($\lambda$ = 1.54814 Å). The strategy for the data collection was evaluated by using the CrysAlisPro CCD software. The data were collected by using the standard phi-omega scan techniques and were scaled and reduced using CrysAlisPro RED software. The structures were solved by direct methods using SHELXS-97 and refined by full matrix least squares with SHELXL-97, refining on $\mathbf{F}^2$. The positions of all the atoms were obtained by direct methods. All non-hydrogen atoms were refined anisotropically. The remaining hydrogen atoms were placed in geometrically constrained positions and refined with isotropic temperature factors, generally $1.2 \times U_{eq}$ of their parent atoms. All the H-bonding interactions, mean plane analyses, and molecular drawings were obtained using the program Mercury (ver 3.1) and Diamond (ver 3.1d). The crystal and refinement data are summarized in Table 1 and selected bond distances and bond angles are shown in
Table S1.† In 1 disordered CH₂Cl₂ was observed which was omitted by using the SQUEEZE option in the Platon program.

Synthesis of chemosensor (H₂L)

2,4,6-Trimethylbenzene-1,3-diamine (0.189 g, 1 mmol) dissolved in dry methanol (10 ml) was added to a solution of 2-hydroxy-1-naphthaldehyde (0.334 g, 2 mmol) in methanol (15 ml). The content of the flask was heated under reflux for 3 h, and a yellow precipitate was obtained. The solvent was evaporated under vacuum and washed with hexane. The needle shaped yellow crystals were grown by recrystallization in DCM/CHCl₃ (2 : 1) within two days. Elemental analysis for C₁₃H₁₈N₂O₂ (Mₑ, 248.54) (found: C, 81.20; H, 5.80; N, 6.12%).

Quantum yield calculation

The fluorescence quantum yield (Φ) was calculated using eqn (1) by the steady-state comparative method using quinine sulfate as the standard (Φₛₜ = 0.54)²⁰

Φₛₜ = Φₛₜ × Sₛₜ/Sₛₜ × Aₛₜ/Aₛₜ × n²ₛₜ/dₛₜ × n²ₛₜ (1)

where Φₛₜ is the emission quantum yield of the sample, Φₛₜ is the emission quantum yield of the standard, Aₛₜ and Aₛₜ represent the absorbance of the standard and the sample at the excitation wavelength, respectively, while Sₛₜ and Sₛₜ are the integrated emission band areas of the standard and the sample, and u and st refer to the unknown and the standard, respectively.

Average life time measurement

The amplitude weighted lifetime was calculated using the following equation:

<τ> = α₁τ₁ + α₂τ₂ (2)

where <τ> is the average fluorescence lifetime of 1. τ₁ and τ₂ are the average lifetimes of various fluorescent forms of compound 1 and α₁ and α₂ are the normalized pre-exponential factors. To obtain the best fitting in all the cases the χ² was maintained near to unity.

Acknowledgements

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References