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Novel zinc fluorescent probe bearing dansyl and aminoquinoline groups†

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A novel fluorescent chemosensor (L) demonstrates a remarkable selectivity and sensitivity for zinc(II) ion as evidenced from the solution characterisations and *in vitro* experiments using Hela cell lines.

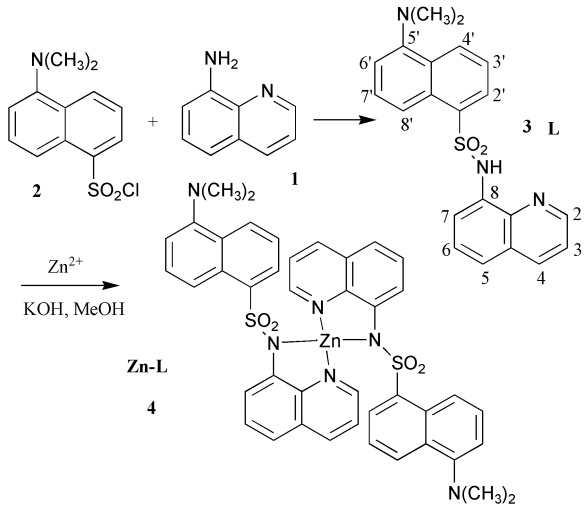
Zinc(II) ion is the second most abundant transition metal essential for the human body with a concentration ranging from sub-nM to ~0.3 mM.[1] Recent work showed that Zn(II) is closely associated with severe pathological disease, such as Alzheimer's disease and familial amyotrophic lateral sclerosis,[2] and its intracellular concentration is under tight control.[3] However, the detection of zinc has always been problematic due to its inherent d^{10} shell and the lack of spectroscopic characteristics. Although most of zinc ions are tightly bound to proteins such as zinc fingers, pools of chelatable zinc ions have been imaged in living cells.[4] It is believed that fluorescence method is likely to be the most effective way to detect zinc, and so far five types of probes have been reported.[4–14] 6-Methoxy-(8-*p*-toluenesulfonamido)quinoline (TSQ) and its derivatives (Zinquin series) have been tested in cell lines, but their ultraviolet band excitation wavelength and relative low membrane permeability may hinder their *in vivo* application. Our approach is to improve excitation wavelength and fluorescence property by incorporating dansyl and aminoquinoline groups into one ligand.

Fluorescent chemosensors for cations can be classified into two basic categories according to their fluorescence mechanisms: photoinduced electron-transfer (PET) cation fluoroionophore and photoinduced charge-transfer (PCT) cation fluoroionophore.[15,16] In general, the PET sensors result in the increase in intensity while the PCT ones lead to the shift of the excitation and emission bands. The combination of both properties in one compound could potentially improve the fluorescence property. Considering the extensive use of 8-aminoquinoline (**1**) in the design of zinc probes and the strong fluorescence of dansyl chloride (**2**), we have synthesized compound **L** by conjugating (**1**) and (**2**) (Scheme 1). The dansyl-based fluorophore belongs to the PET range, while quinoline-based fluorophore always exhibits a fluorescence enhancement when combined to zinc ion. Meanwhile, the sulfon ester group has strong lipophilicity that could facilitate the cross-membrane of **L**.

The novel ligand, [8-(5′-dimethylamino-1′-naphthalene)sulfonamidoquinoline] (**L**), was prepared according a reported procedure for similar ligands.[9] Compound **L** is fully characterised by FT-IR, elemental analysis, ESMS, NMR and X-ray crystallography. The crystallographic data (ESI†) show that the dansyl plane and quinoline plane in **L** are nearly perpendicular to each other, similar to the zinc complex of 2-MeTSQ.[9]

The 1:2 zinc(II) complex with Zn(II), $\mathbf{ZnL_2}$ (**4**) was prepared by refluxing **L** and $Zn(ClO_4)_2{\cdot}6H_2O$ in 1:2 molar ratio in methanol.[17] The complex $\mathbf{ZnL_2}$ was characterized by ESMS, IR and NMR spectroscopy. The ESMS spectrum of $\mathbf{ZnL_2}$ shows a peak at *m*/*z* 875.0 which matches perfectly with the calculated value for $[\mathbf{ZnL_2}]^-$. In the IR spectrum of $\mathbf{ZnL_2}$, the ν_{N-H} band of free **L** at 3255 cm^{-1} disappeared, which may be due to the coordination of the amide group to the zinc ion.[9] The 2D NMR experiments further support the formation of $\mathbf{ZnL_2}$. By comparing the 2D ^1H COSY, TOCSY and NOESY spectra of ligand **3** and complex **4** in DMSO-d_6, all the proton resonances have been fully assigned. It is noted that the H2 signal of quinoline was shifted to lower field by 0.23 ppm suggesting the coordination of quinoline N to zinc ion. Therefore, it can be concluded that the Zn(II) is situated in a N_4 coordination sphere similar to those observed in Zinquin series probes.[9] The reaction process of **L** with Zn(II) was initially studied by UV titration. The following metal salts were used: $ZnAc_2$, $Zn(ClO_4)_2$, $CuAc_2$, $CaCl_2$, KCl, NaCl, $MnCl_2$, $CoAc_2$, $NiAc_2$, $Fe_2(SO_4)_3$ and $(NH_4)_2Fe(SO_4)_2$. The data show that the coordination of the ligand **L** to Zn(II) is completed within seconds. It is found that sodium, potassium or calcium ions do not react with the ligand, while the transition metal ions do but with much lower reactivity. The spectral changes during the titration of zinc are shown in Fig. 1. Two isosbestic points at 271 and 336 nm were observed which corresponded to the binding of the ligand with zinc ion. The band at 321 nm for free **L** is shifted to 349 nm when **L** is bound to Zn(II) (at λ_{321}, ε_L = 688.98 $m^2\ mol^{-1}$, $\varepsilon_{\mathbf{ZnL_2}}$ = 1144.1 $m^2\ mol^{-1}$; at λ_{349}, ε_L = 522.66 $m^2\ mol^{-1}$, $\varepsilon_{\mathbf{ZnL_2}}$ = 1531.4 $m^2\ mol^{-1}$). The obvious red shift of the band can be explained as follows: the binding of **L** to zinc forms a five-membered chelate ring with the aminoquinoline moiety through two nitrogen atoms, which enlarges the conjugated ring; moreover, the binding of zinc to **L** increases the dipole moment, which results in the higher electron mobility in the π orbital. The larger conjugated system reduces the energy difference between n and π* orbitals. According to the titration data, the formation constant of $\mathbf{ZnL_2}$ was calculated to be 6.46×10^8.

The fluorescence properties of **L** and $\mathbf{ZnL_2}$ were also studied in a DMSO–H_2O mixture, but the concentration was 1 order magnitude lower (10^{-5} M) compared to the UV studies. The fluorescent experiment reveals that essential metal ions such as



Scheme 1

† Electronic supplementary information (ESI) available: NMR spectra and assignment, UV titration details, crystal structure and competitive fluorescent experiments of L. See http://www.rsc.org/suppdata/cc/b2/b202976f/

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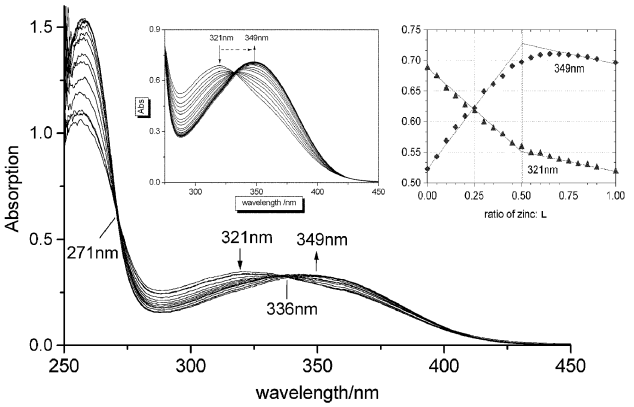

Fig. 1 UV titration spectra of **L** (1×10^{-4} M) with $ZnAc_2$ (1×10^{-3} M) in DMSO–H_2O (80 : 20) [Arrow indicates the direction of change upon Zn(II) addition], a red shift from 321 to 349 nm occurs and there is a plateau when Zn(II) : **L** approaches 1 : 2. Left inset: selected absorption titration spectra; right inset: fit (◆) of 349 nm absorption increment, (▲) of 321 nm absorption decrement.

copper(II), iron(II), iron(III), cobalt(II), manganese(II) and nickel(II) ions (10^{-5} M) quenched the fluorescence of the ligand while zinc ion enhanced the fluorescence. The fluorescence spectra of **L** and $\mathbf{ZnL_2}$ are shown in Fig. 2, with the excitation spectra on the left part of the figure and emission spectra on the right part. The maximum excitation band of **L** is at 357 nm with a shoulder at 381 nm. Whatever the excitation wavelength between 300–450 nm is chosen, the maximum of the emission is always at 545 nm. There is a significant band shift and increase in fluorescence intensity for $\mathbf{ZnL_2}$. The maximum excitation of the zinc complex is at 395 nm, and maximum emission is at 469 nm. As can be seen from Fig. 2, the fluorescence intensity at 469 nm is *ca.* 2.4 times of that of the ligand at 545 nm. Moreover, the fluorescence of $\mathbf{ZnL_2}$ at 469 nm is 24 times stronger than that of the free ligand at the same wavelength, which means there is no interference from the ligand at this wavelength. Therefore, the 395 nm excitation and 469 ± 10 nm emission may be suitable wavelengths for the detection of Zn(II) with **L**, because the nearly visible light excitation is less harmful to the living body. The competitive fluorescent experiment shows that other ions do not interfere with the detection of Zn(II) (see ESI†).

As discussed above, the new probe synthesized in this work shows a combination of both PCT and PET properties. Therefore ligand **L** possesses a unique fluorescence property with both fluorescence enhancement and emission band shift upon binding to the target metal ion.

Preliminary *in vitro* studies using **L** was carried out with Hela cells.‡ Fig. 3 shows the fluorescence micrograph of the Hela

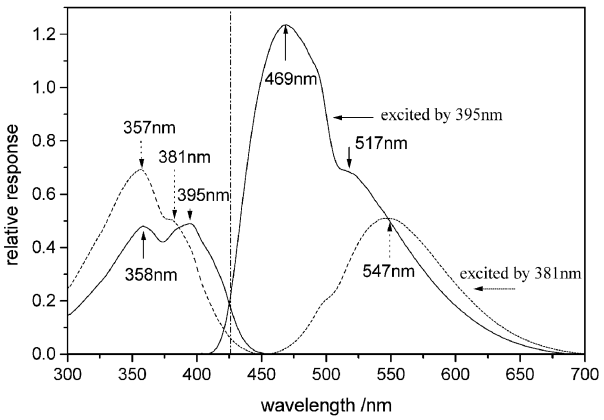

Fig. 2 Fluorescence spectra of **L** and $\mathbf{ZnL_2}$ at 293 K in DMSO–H_2O (80 : 20) (left halves are excitation spectra and right halves are emission spectra). The dashed line corresponds to the ligand and the solid line to the zinc complex, which shows that 395 nm excitation and 469 ± 10 nm monitoring may be a good choice to detect zinc ion with this novel probe.

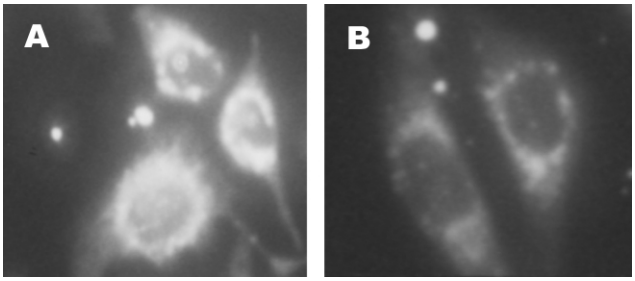

Fig. 3 Fluorescence photos for Hela cells with **L** (> 440 nm emission was permitted to pass). A: cells cultured in jpj2; B: cells cultured in **L** and TPEN at same time (TPEN crosses the cell membrane and chelates zinc ions). The background fluorescence in B arises from the probe itself, which can be eliminated by screening with a more suitable filter.

cells cultured with **L** in the absence (A) or the presence (B) of TPEN. The addition of **L** in the culture media gives a strong fluorescence in the inner part of the cell, and the addition of EDTA or EGTA together with the probe at the same time does not affect the fluorescence of the cell. However, the addition of TPEN does reduce the fluorescence of the cells. This may be due to the fact that EGTA and EDTA are highly charged (+4), while TPEN is neutral.[5,6,9] Further studies with specific wavelength filters are underway, and *in vivo* fluorescence experiments will be carried out to correlate the diseased case with Zn(II) distribution.

In summary, we have designed and synthesized a novel zinc ion probe which not only shows a remarkable fluorescent selectivity and sensitivity, but also demonstrated a combined property of both PET and PCT fluorophores. The preliminary *in vitro* studies with Hela cells exhibited the potential application of the probe.

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Notes and references

‡ Hela cells (5×10^4 cells ml^{-1}) were grown in MEM on poly-lysine precoated cover-glasses. These slides were then treated with 25 μM TPEN, EDTA or EGTA, respectively, before incubation with 25 μM **L** for 30 min at 37 °C. Cells were examined under a NIKON microscope with V-2A filter.

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