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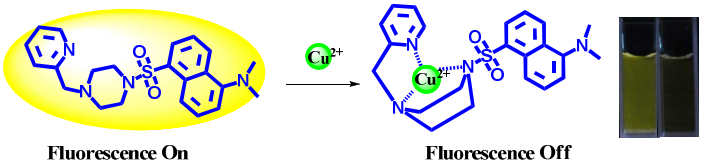
Graphical Abstract



Fluorescence On Fluorescence Off

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ARTICLE

A dansyl-based fluorescent probe for selectively detecting Cu^{2+} and imaging in living cells

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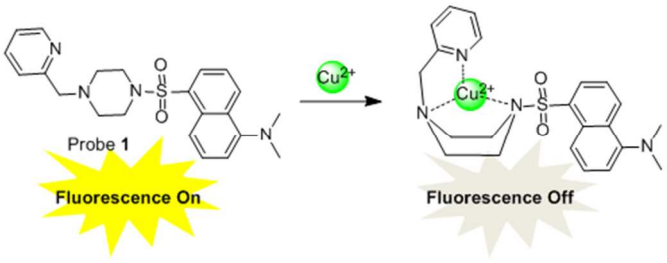
Copper is a crucial transition metal ion that plays an essential role in environmental, biological, and chemical systems. Therefore an efficient method for detection of copper ions is significant. In this work, a sulfonamide-based probe containing a dansyl fluorophore was synthesized in good yield. This probe exhibited good selectivity and sensitivity for copper ions in aqueous solution without any interference from other metal ions. And it displayed a very low detection limit (2.91×10^{-8} M) for Cu^{2+}, which supported its further application in bioimaging. Its good water-solubility, bio-compatibility and cell permeability leads it to be employed as an efficient biomarker to monitor the level of Cu^{2+} in living cells. The binding model between fluorescent probe and Cu^{2+} was evaluated by density functional theory calculations.

Introduction

Copper ions, the third most abundant metal ions in the human body play a vital role in numerous fundamental biological systems, such as enzyme function.[1] An appropriate high level of Cu^{2+} in food supplements significantly increases the flexibility of body tissues and prevents hair bleaching and albinism. However, excess Cu^{2+} in the body can cause a series of diseases which influence normal physiological metabolism, leading to neurodegenerative diseases including Parkinson's disease, Wilson's disease and Alzheimer's disease, while its deficiency in the body may lead to hematological manifestations and a wide variety of neurological problems.[2] Thus, simple and rapid sensing of copper ions in biological and environmental systems is very significant.

Currently, some methods have been used to detect Cu^{2+}.[3] Among of them, the method based on fluorescent response is considered to be one of the most effective strategies due to its high sensitivity, simplicity and operability. Over the past deceades, the fluorescence-dependent techniques and methods have been widely applied in many different fields such as luminescence material, fluorescent markers, fluorescent sensors, drug release and so on.[4] Cu^{2+} as a paramagnetic ion, can strongly quench the fluorescence of fluorophores by electron or energy transfer. In recent years, a number of fluorescent probes have been used in the detection of Cu^{2+}. Howver, many fluorescent probes usually suffered from some disadvantageous such as complicated synthesis of probes, poor water-solubility, low sensitivity, high detection limits, strong interference and so on. Therefore, the development of a simple and efficient fluorescent probe has potential value in practical applications.

The structure of dansyl-based fluorophore presents donor-acceptor system owing to it containing electron-donating and electron-withdrawing moieties. As a consequence, its derivatives show strong fluorescence with high emission quantum yields.[5] More, sulfonyl chloride as a commercial available fluorophore has very high reactivity and can treat with a variety of amines to afford corresponding dansylsulfonamide derivatives, and numerous researches have shown that they can be employed as fluorescence probes to detect metal cations.[6] Moreover, the affording metal complexes have further application ascribing to the fact that the metal complexes can be used as a tool to monitor some other biological species such as anions, amino acids and so on.[7, 8] Therefore, developing the dansyl-based fluorescent probes for metal ions contracts more attention. Herein, in the present work, we reported a dansyl-based fluorescence probe containing a piperazine-linking pyridine, as shown in Scheme 1, which was capable of detecting copper (II) and exhibited high selectivity and low interference due to co-existent metal ions in Scheme 1. Importantly, compared with other probes, our probe has the advantages of simple synthesis and low detection limits. These excellent properties support the use of this probe in living cells to monitor the level of copper ions.



Scheme 1 Schematic illustration of probe **1** for Cu^{2+} detection.

Experimental

Instruments and reagents

All reactions were taken place by using standard Schlenk techniques under an argon atmosphere, unless elaborated. All reagents and materials were purchased commercially and without further purification. Dansyl chloride (97%) and 1-pyridin-2-yl-methyl-piperazine (95%) are obtained by commercial approach from J&K Scientific Ltd. CH_2Cl_2 was dried with CaH_2, and then distilled under nitrogen atmosphere. Column chromatography was performed over silica gel (200-300 mesh). NMR spectra were obtained on American Varian Mercury Plus 600 spectrometer (600 MHz) or 400 MHz and their chemical shifts are relative to TMS. Electrospray (EI) mass spectra were carried on Firmigan Trace. UV-Vis spectra were obtained on U-3310 UV Spectrophotometer. Fluorescence spectra were taken on a Fluoromax-P luminescence spectrometer (HORIBA JOBIN YVON INC.). All the density functional theory calculations were carried out at the B3LYP/6-31G* level using the Gaussian 09 suite of programs.

Characterizations

A stock solution of probe **1** (1 mM) was prepared in DMSO, and a stock solution of metal ions (10 mM) were dissolved in distilled water. We selected a wide range of metal ions, such as Ca^{2+}, Ni^{2+}, Li^{+}, Al^{3+}, Hg^{2+}, Cd^{2+}, K^{+}, Ag^{+}, Zn^{2+}, Pb^{2+}, Mn^{2+}, Cu^{2+}, Fe^{3+}, Na^{+}. For selectivity, the work solution of **1** (10 μM) was prepared by mixing 1 mM of **1** and 0.1 mM of metal ions (10 mM) in 0.02 M HEPES buffer/DMSO (8/2, v/v). For titration, the work solution of **1** (10 μM) was obtained by mixing 1 mM of **1** in 0.02 M HEPES buffer/DMSO (8/2, v/v) with varying concentration of Cu^{2+} from 0 to 700 μM.. For all fluorescence spectra were collected from 380 to 700 nm excitation with 360 nm, slit width at 5 nm.

Calculation of dissociation constant[1]

The dissociation constant (Kd), was calculated by nonlinear fitting of Eq. (1)

$$K_d = \frac{(F_{max} - F)[Cu^{2+}]}{(F - F_{min})} \quad (1)$$

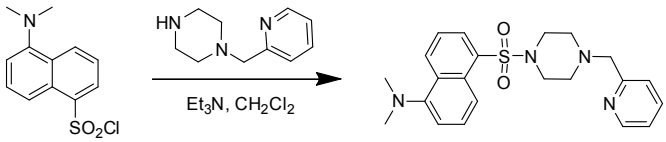
F is the fluorescence intensity of $[Cu^{2+}]$. F_{min} and F_{max} is the fluorescence intensity at minimal and maximal concentration of $[Cu^{2+}]$

Cell culture and imaging

DMEM (Dulbecco's modified Eagle's medium) was used to dilute the concentration of probe **1**. A stock solution of probe **1** (10 mM) was prepared in DMSO, and then using the DMEM to dilute the probe **1** from 10 mM to 100 μM. And then added it to Hela cells incubated at 37 ℃ in humidified air and 5% CO_2. After that DMEM was removed, washed with PBS (phosphate-buffered saline), and added PBS again. Experiments for Cu^{2+} ion were incubated for 30 min in the same medium.

Synthesis of probe 1

5-(dimethylamino) naphthalene-1-sulfonyl chloride was added dropwise at 0 ℃ to a solution of 1-benzylpiperazine in CH_2Cl_2 and Et_3N (2 mL). The mixture was stirred for 12 h at room temperature. And then the solvent was removed in vacuum. The residue was purified by silica gel column chromatography to obtain the target compound as a yellow solid in 60% yield. ^1H NMR (600 MHz, $CDCl_3$): δ (ppm): 8.56 (d, 1H, Ph-H), 8.52 (d, 1H, Ph-H), 8.46 (d, *J = 6 Hz*, 1H, Ph-H), 8.19 (d, *J = 6 Hz*, 1H, Ph-H), 7.61 (t, *J = 6 Hz,* 1H, Ph-H), 7.54-7.50 (m, *J = 6 Hz*, 2H, Ph-H), 7.27 (s, 1H, Ph-H), 7.18 (d, *J = 6 Hz,* 1H, Ph-H), 7.15-7.13 (m, *J = 6 Hz*, 1H, Ph-H), 3.61 (s, 2H, CH_2), 3.23 (t, 4H, CH_2), 2.89 (s, 6H, CH_3), 2.55 (t, 4H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 157.46, 151.48, 149.24, 136.30, 132.28, 130.42, 129.87, 127.78, 123.12, 122.98, 122.07, 119.64, 115.04, 63.91, 52.34, 45.49, 45.27. EI MS m/z= 410.18 [M]; calculated exact mass= 410.46.



Scheme 2 Synthesis of probe **1**

Results and discussion

UV-Vis spectrum

To investigate the sensitivity of **1** to different metal ions including Ca^{2+}, Ni^{2+}, Li^{+}, Al^{3+}, Hg^{2+}, Cd^{2+}, K^{+}, Ag^{+}, Zn^{2+}, Pb^{2+}, Mn^{2+}, Cu^{2+}, Fe^{3+}, Na^{+}, UV-Vis absorption was employed in DMSO/HEPES (2/8, v/v). From the instrument data, the absorption peak at 336 nm vanished dramatically upon addition the Cu^{2+}, while no significant changes happened in other metal ions (Figure 1S). This result indicated that the probe has high selectivity towards Cu^{2+}.

Fluorescence spectrum

Subsequently, we further explore the fluorescence changes in the presence of metal ions. No drastically fluorescence changes were observed upon the addition of other metal ions, but there was almost 10 fold decrease in fluorescence intensity when the Cu^{2+} was added as shown in Figure 1(b). It indicated **1** had a high selectivity towards Cu^{2+}. And this phenomenon can be confirmed by the naked eye at the UV light resulting that the fluorescence of **1** was quenched by Cu^{2+} in Figure 1(a).

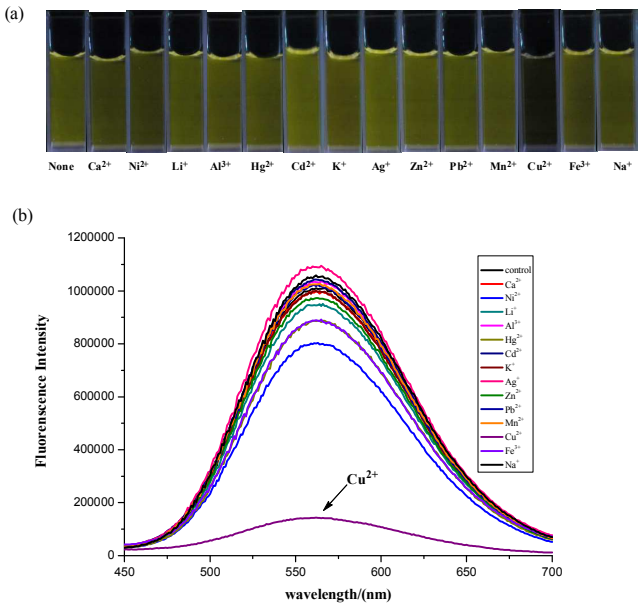


Fig. 1 (a) Photograph of **1** with various metal ions under UV light; (b) Fluorescence spectra of **1** (10 μM) with various metal ions (100 μM) in HEPES buffer (0.02 M, pH=7.0)/DMSO (8/2, v/v) excited at 360 nm.

Additionally, an interference experiment of **1** toward Cu^{2+} in the presence of other metal ions was investigated. From Figure 2, there were negligible fluorescence changes in the presence of other cations, which showed that there was no inference in detection of **1** toward Cu^{2+}. The result demonstrated that this probe can discriminate Cu^{2+} from other metals ions.

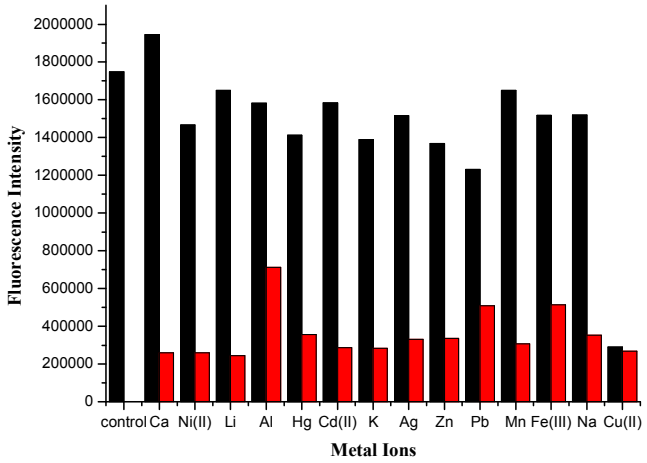


Fig. 2 The fluorescence change of **1** with various metal ions. Black bars is **1** with other metal ions; red bars is **1** with Cu^{2+} in the present of other metal ions.

In a titration experiment, there was a marked decrease from 0.2 to 5.0 eq. at 564 nm following the addition of Cu^{2+}, and no further decreases in fluorescence intensity were observed with further additions of Cu^{2+} (Figure 3). When the concentration of Cu^{2+} was 5.0 eq. total quenching occurred. When the concentration of 1 ranged from 2 μM to 20 μM, a good linear relationship between **1** and Cu^{2+} was observed. The linear regression equation was $F=1.41\times10^{6}-3.91\times10^{6}E$ (E is the equiv. of Cu^{2+}) (Figure 2S, inset). From the linear equation of the titration plot, the limit of detection for **1** was calculated to be as low as 2.91×10^{-8} M, according to the equation $DL=3\sigma/m$, where σ is the standard deviation of the blank solution and m is the slope of the calibration curve. The low detection limit made it possible to detect the level of Cu^{2+} in cells using this probe. The dissociation constant between **1** and Cu^{2+} was also calculated and was estimated to be 3.27×10^{-6} M.

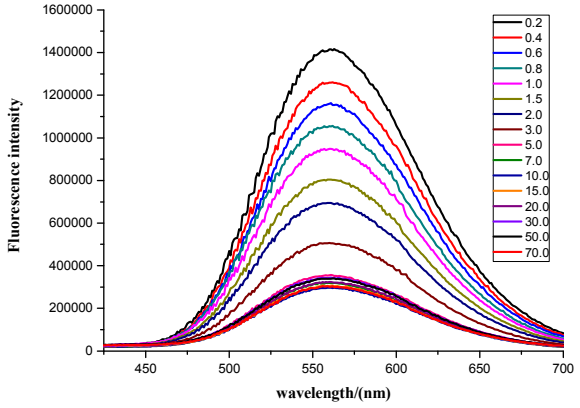


Fig. 3 The fluorescence titration spectra of **1** with various concentration of Cu^{2+} in HEPES buffer (0.02 M, pH=7.0)/DMSO (8/2, v/v) excited at 360 nm

Binding mode

To further investigate the binding mode of probe **1** and Cu^{2+}, we have gone the ratio experiment which turned out to be 1:1 using the Job's plot in Figure 3S. The fluorescence intensity of probe **1** reached the minimum value at 560 nm when the concentration of Cu^{2+} was near to 5 μM, indicating the formation of a 1:1 complex between probe **1** and Cu^{2+}. The result indicated that introducing 1-pyridin-2-ylmethyl-piperazine group to dansyl fluorophore is useful to bind metal ions. So we could infer Cu^{2+} binding at the pyridyl and piperazine nitrogen atoms, which formed a tridentate coordination site as shown in Scheme 1.[3a, 9] Subsequently, the binding mode was further proved by density functional theory (DFT) calculations. From the frontier molecular orbitals, we could observe the electron density, as shown in Figure 4. For probe **1**, the electron density chiefly located in dansyl group in both HOMO and LUMO orbitals possible due to the donor-acceptor system of dansyl fluorophore. However, for **1**-Cu^{2+} complex, the molecular orbitals contained both Alpha and Beta orbitals. The electron density distributed to the whole molecule, and Beta orbital which played a major role to the quenching of fluorescence had the lower energy gap compared to Alpha orbital. Furthermore, the binding also influenced the molecular donor-acceptor system, which resulted in the fluorescence quenching.

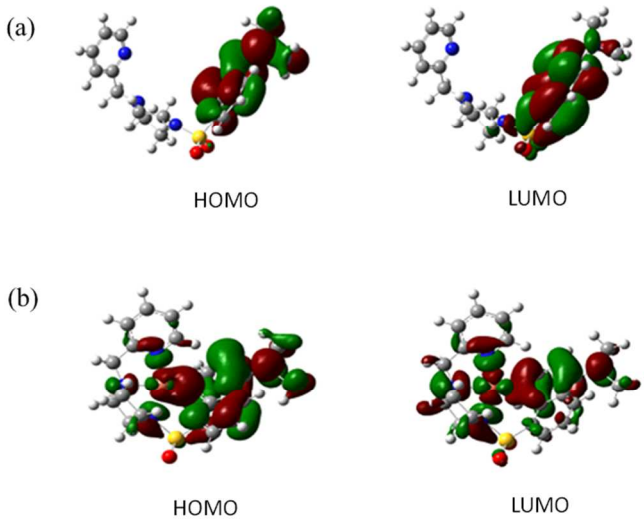


Fig. 4 (a) Calculated HOMOs and LUMOs of **1**; (b) Calculated HOMOs and LUMOs of **1** - Cu^{2+} complex.

Cell imaging

As we know, high concentration of Cu^{2+} is harmful to biological system, leading various diseases.[10] The excellent properties of probe such as good selectivity, high sensitivity and low detect limit made it is possible to apply to living cells. As Hela cell was easy to incubate, we select HeLa cell to do the experiment. The ability of **1** to detect the level of Cu^{2+} ion in cell was demonstrated by fluorescence microscope images. To observe the cell permeability of **1**, Hela cells were incubated with **1** (100 μM) at humidified atmosphere of 5% CO_2, 37 ℃ for 6 h. As displayed in Figure 5(b), probe **1** displayed good adaptability in this environment, suggesting the low cytotoxicity and good cell permeability of probe **1**. After removed the PBS, 1 mM $CuCl_2$ with DMEM was added to the Hela cells incubated in 5% CO_2, 37 ℃ for 30 min, Figure 5(c)

indicated that the fluorescence of **1** was quenching by Cu^{2+} ion. These results showed that **1** could be used as a Cu^{2+}-selective probe in living cells.

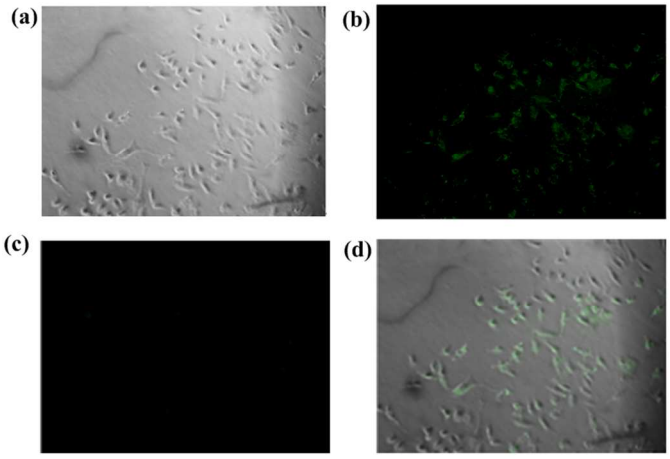


Fig. 5 Fluorescence microscope images of living HeLa cells. (a) Bright-field image of the live HeLa cells; (b) Hela cells incubated with 100 μM **1** for 6 h at 5% CO_2 , 37 ℃; (c) Hela cells incubated with 100 μM **1** for 6 h at 5% CO_2 , 37 ℃, then added 1 mM $CuCl_2$ incubated for 30 min; (d) Bright-field view of overlay image of A and B.

Conclusions

In summary, we developed a sulfonamide-based probe containing a dansyl fluorophore, which exhibited high selectivity and sensitivity without interference from other metal ions, and a low detection limit for Cu^{2+}. Significantly, its corresponding copper complex could be used as a fluorescence indicator to detect amino acids. According to DFT calculations, we explored the proposed quenching mechanism. Furthermore, the probe was successfully used in biological systems to visualize the level of copper ions. Further studies will focus on the development of novel efficient probes

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Notes and references

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