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Synthesis and spectral studies of 2-[(*N*-ethyl carbazole)-3-sulfonyl ethylenediamine]-1-*N*,*N*-2-(2-methypyridy) as a fluorescence probe for Zn^{2+}

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ABSTRACT

A novel Zn^{2+} fluorescence probe, 2-[(*N*-ethyl carbazole)-3-sulfonyl ethylenediamine]-1-*N*,*N*-bis(2-methypyrbidy), was designed and synthesized via simple steps, and its structure was confirmed by IR and ^1H NMR. The probe gives significant fluorescence enhancement immediately following Zn^{2+} addition at neutral pH and exhibits improved selectivity for Zn^{2+} compared to the other metal ions in aqueous solution. The spectra and fluorescence quantum yield of the synthesized compound were carefully investigated by UV–vis absorption and fluorescence spectra in various solvents.

Because the availability of better Zn^{2+}-specific probes would provide additional insight into the cell biology of Zn^{2+}, interest in the field remains high, and many fluorescent sensors for Zn^{2+} have been described in the past decade.[1–3] Many factors determine the performance of fluorescence probe in vivo, including their photophysical properties, sensitivity, and selectivity for the analyte of interest, affinity for the analyte and other species in the biological milieu.[4] In the current study, there is still scope for improvement in the design of such sensors as they often suffer from disadvantages such as sensitivity to H^+, Ca^{2+} and Mg^{2+}, short excitation and emission wavelengths, small stokes shifts and cumbersome synthesis.[5]

Some fluorophore or its modificated derivatives exhibit a weak emission in the free state but they fluoresce intensely accompanied by a spectral shift when bound to Zn^{2+} or other metal ions.[6,7] This property is desirable for fluorescence ratiometric determination of the metal ions. Carbazole derivatives are fluorescent material possessing excellent fluorescent characteristics, which have been widely used in construction of light-emitting device and fluorescent detection.[8–10] With perfect planarity and conjugation in molecular structure, carbazole may be desirable for fluorescence ratiometric determination of Zn^{2+}, although there have been very few studies related to carbazole derivatives as fluorescent probes for metal ions. It is also known that one of the most outstanding properties of Zn^{2+} ion is a strong affinity for aromatic sulfonamides or *N*,*N*,*N*′,*N*′-tetrakis (2-pyridylmethyl)-ethylenediamine (TPEN). Therefore, if aromatic sulfonamides and TPEN were assembled together with carbazole, the target compound should be a good fluorescence probe for Zn^{2+}.

In previous papers, we reported synthesis and spectrum characteristic of new organic fluorescent dyes of pyrazoline compounds and determination of human serum albumin using an intramolecular charge transfer fluorescence probe: 4′-Dimethylamino- 2,5-dihydroxychalcone.[11,12] The direct interaction of alkali metal or alkaline earth metal ions not only with Rhodamine B base or a fluoran-based color former but also with 1-(2-pyridylazo)-2-naphthol and its derivative has been demonstrated.[13,14]

The present work aims to describe the synthesis, chemical characterization and spectral properties of a new compound containing TPEN derivative, *N*,*N*-bis(2-pyridylmethyl)ethylenediamine linked to aromatic sulfonamides units which are connected carbazole (fluorescent signaling probe) units (see compound **e**). As we know, nitrogen-rich tri- and tetra-dentate ligands coordinate with Zn^{2+} with high affinity. We have synthesized the new derivative of carbazole (compound **e**), exploiting methodology for coupling TPEN with carbazole. This approach provides a straightforward and convenient means to access a compound that otherwise would require a difficult, multistep route using conventional organic techniques. The structures of the target compound have been fully characterized by IR, ^1H NMR and fluorescence techniques. The synthesized compound was used as a fluorescent probe for Zn^{2+} in aqueous media under physiological buffer conditions and showed its outstanding characteristics including nice sensitivity and selectivity for zinc over other metal ions.

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The synthesis route of compound 2-[(*N*-ethyl carbazol)-3-sulfonyl ethylenediamine]-1-*N*,*N*-2-(2-methypyridy) is shown in Scheme 1.

Figure 1 shows the drastic changes in the fluorescence spectrum from the parent compound, carbazole, to the product (**e**). From carbazole to *N*-ethyl carbazole, a shift towards longer wavelengths in the fluorescence-emission spectra and an increase in fluorescence intensity are observed. It is known that the molecular structure of the carbazole possesses a good conjugated system and rigid plane. According to the intramolecular charge transfer (ICT) theory, a conjugate connection between the fluorophore and electron donor is formed by the introduction of electron-donating ethyl group, results in a ICT from donor to acceptor, and then reduces the energy levels of electronic transitions in the molecule, which causes the changes in fluorescence spectra.

From *N*-ethyl carbazole to *N*-ethyl carbazole-3-sulfonyl chloride, significantly reduced fluorescence intensity and red shift in fluorescence spectra are detected. These phenomenon can be explained by the electron withdrawing effect caused by the directly connection of strong electron-accepter chlorosulfonyl group with carbazole at the benzene ring.

From *N*-ethyl carbazole-3-sulfonyl chloride to 2-[(*N*-ethyl carbazole)-3-sulfonyl ethylenediamine, the introduction of two electron-donating amidocyanogen weakens the electron withdrawing effect of chlorosulfonyl group, which are responsible for the slight recovery of fluorescence intensity and the red shift in fluorescence spectra.

As for the spectrum change from 2-[(*N*-ethyl carbazole)-3-sulfonyl ethylenediamine to 2-[(*N*-ethy carbazole)-3-sulfonyl ethylenediamine]-1-*N*,*N*-2-(2-methypyridy), a large conjugated structure is resulted in when two methypyridy groups are joined. The strong ICT occurs in the first excited singlet state can induce large molecular polarizability, making a red shift of the fluorescence emission wavelength and a concomitant increase of half-band-width value. As seen in Figure 1 for compound (**e**), the fluorescence intensity is moderately weaker comparing with other compounds which suggests that the formed compound system is so large that the rigid planar structure of molecular may have been distorted to some extent.

The spectra of product were studied in four solvents of different polarity: ethanol, tetrahydrofuran, chloroform and carbon tetrachloride. As shown in Figure 2, the absorption spectra of target compound (**e**) exhibits a redshift in chloroform but a blueshift in ethanol and tetrahydrofuran respectively compared to carbon tetrachloride (nonpolar solvent). The blueshift value is 62 nm in ethanol and 44 nm in tetrahydrofuran. The redshift value is 24 nm in chloroform. The spectral behavior suggests that the compound (**e**) is relatively sensitive to variations in the solvent polarity. Such sensitivity to microenvironment may be an advantage toward achieving a sensor with good photophysical properties and affinity or Zn^{2+} selectivity.

The fluorescence spectrum of the probe compound (**e**) in various solvents is shown in Figure 3. By a detailed analysis of the absorption and fluorescence spectra, it is clear that the fluorescence spectra follow the same trend as the absorption spectra. Judging from the blueshift in both fluorescence and absorption spectra, it can be concluded that a hydrogen-bonding complex is formed between the solvent and the fluorophore in ground state and the slight blue shift in the fluorescence spectra can be rationalized by the hydrogen bonding interactions.

The hydrogen bond donor-acceptor ability and the polarity of a solvent will generally influence the actual fluorescence spectral shift of fluorophores.[15] In addition, the hydrogen-bonding interaction between the solvents and the compound molecules partly inhibits the excited-state charge transfer between the lone-pair electrons on the electron donating substituents and aromatic ring in the compounds

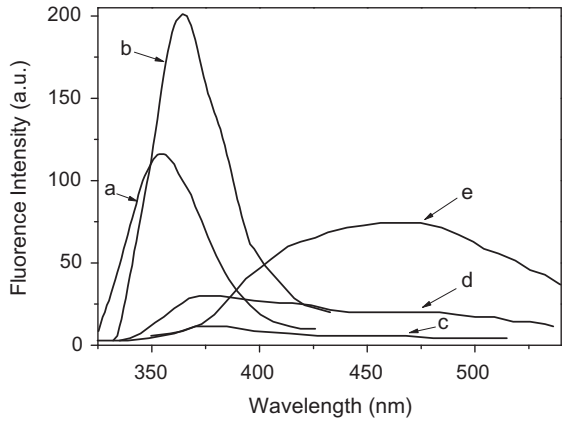
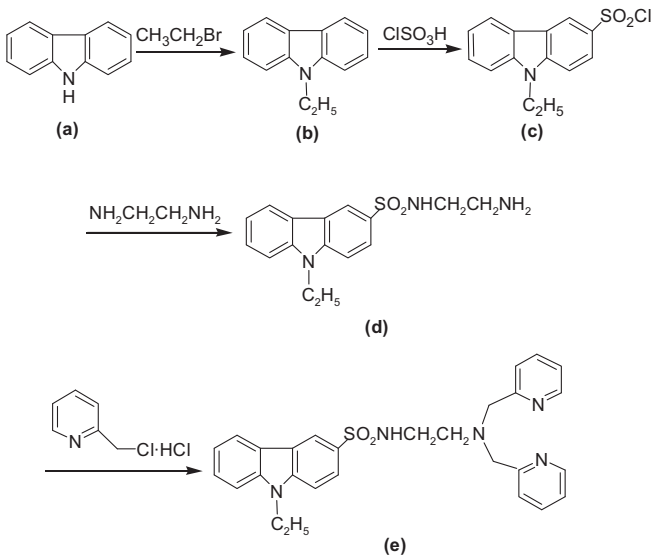


Figure 1. The fluorescent emission spectra of (a) carbazole (1.2×10^{-7} M), (b) *N*-ethyl carbazole (3.2×10^{-7} M), (c) *N*-ethyl carbazole-3-sulfonyl chloride (4.1×10^{-6} M), (d) 2-[(*N*-ethy carbazole)-3-sulfonyl ethylenediamine (2.4×10^{-7} M), (e) 2-[(*N*-ethy carbazole)-3-sulfonyl ethylenediamine]-1-*N*,*N*-2-(2-methypyridy) (5.6×10^{-7} M) in CH_2Cl_2.



Scheme 1. Synthesis of 2-[(*N*-ethyl carbazole)-3-sulfonylethylenediamine]-1-*N*,*N*-2-(2-methypyridy).

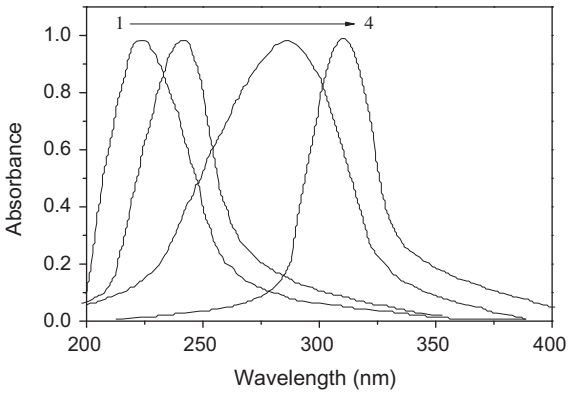


Figure 2. Normalized absorption spectrum of the probe compound (**e**) in various solvents: (1) ethanol, (2) tetrahydrofuran, (3) carbon tetrachloride, (4) chloroform.

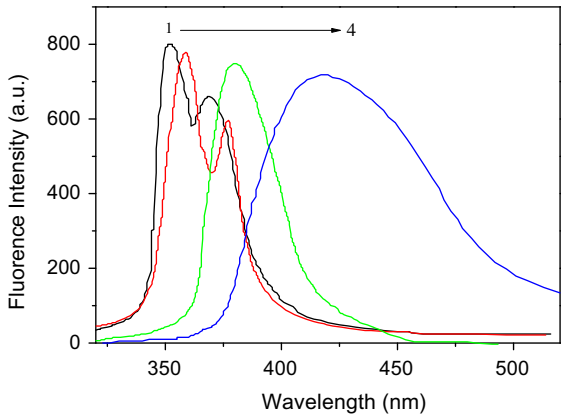


Figure 3. Fluorescence emission spectra of the probe compound (**e**) (1×10^{-5} M) in various solvents. (1) ethanol (λ_{ex} = 224 nm, λ_{em} = 352/368 nm), (2) tetrahydrofuran (λ_{ex} = 242 nm, λ_{em} = 360/375 nm), (3) carbon tetrachloride(λ_{ex} = 286 nm, λ_{em} = 380 nm), (4) chloroform(λ_{ex} = 310 nm, λ_{em} = 417 nm).

molecules, causing thus the blue shift of fluorescence spectra in the solvents. On the other hand, the presence of hydrogen bonds may increase the molecular coplanarity in excited state, which promote ICT and thus exhibit a red shift in the fluorescence spectra. Therefore, the actual measured shift of fluorescence spectral is the net result of two operations, that is, the combination of solvent effect and hydrogen bonding interactions on solute molecules. As shown in Figure 3, the high fluorescence intensities are exhibited in strong polar solvents accompanied by the appearance of vibrational fine structure (the typical two sub-peaks) in fluorescence spectrum, while the low fluorescence intensities are displayed in weakly polar solvents with the disappearance of vibrational fine structure.

The ability of the molecules to emit the absorbed light energy is characterized quantitatively by the fluorescence quantum yield. The photoluminescent quantum yields of different compounds were measured by relative method using Quinine sulfate as the standard. The quantum yield was calculated from the following Eq. 1:

In the above expression, Y_u and Y_s are the fluorescent quantum yield of test and reference materials, F_u and F_s are the integration of the emission intensities of test and reference materials, A_u and A_s are the absorbance of the test and reference material solutions

$$Y_u = Y_s \cdot \frac{F_u}{F_s} \cdot \frac{A_s}{A_u} \quad (1)$$

at the exciting wavelength, respectively.

Under the same conditions, the fluorescence quantum yields of the goal product and the other compounds were measured with the standard value of 0.55 at the excitation wavelength of 313 nm for quinine sulfate.[16] Table 1 details the quantum yields of the different compounds in various solvents.

When the planar configuration is distorted by the increased size of the molecules, the fluorescence of products are gradually weakened, due to an increase of the non-radioactive decay rate induced by hampered ICT. Relatively poor fluorescence quantum yields for dichloromethane compared to ethanol is understandable in view of a much weaker hydrogen bonding or protonation effects. This behavior of compound (**e**) is also in accord with many known fluoroionophores carrying nitrogen heterocyclic, which also display highly quenched fluorescence.[17,18]

Table 1
The fluorescence quantum yields of the compounds in various solvents

Compound	Solvent	Fluorescence quantum yield
Carbazole (a)	Ethanol	0.57
Ethyl carbazole (b)	Ethanol	0.64
Compound (**e**)	Ethanol	0.19
Compound (**e**)	Dichloromethane	0.11

Figure 4 shows the effect of Zn^{2+} ion on the fluorescence emission spectra of the synthesized compound (**e**) in solution of physiological pH. The excitation, emission wavelength of compound (**e**) is 303, 422 nm, respectively. The fluorescence quantum yield of compound (**e**) is found to be 0.037. When $ZnSO_4$ solution was added, a significant linear increase in fluorescence intensity was observed, and the quantum yield increased to 0.10 with only minor changes in the maximum absorption and emission, indicating that a complex may be formed between compound (**e**) and Zn^{2+}.

When concentration ratio of Zn^{2+}/compound (**e**) reaches 1, the fluorescence intensity of the solution does not change basically with increasing concentration of Zn^{2+}. The dissociation constant K_d of the Zn^{2+} complex of compound (**e**) were determined as 1.2 nM by fitting the fluorescence data with a 1:1 association equation. That is to say, the probe can be used to quantitatively determine the concentration of Zn^{2+} around the sub-nanomolar range, which affords sufficient sensitivity for application in mammalian cells. The fluorescent response to Zn^{2+} by compound (**e**) can be attributed to a inhibition of photoinduced electron transfer process. It is possible that incorporation of Zn^{2+} ion enhances the rigidity of molecular thus inhibiting some of non-radiative transitions between electronic states or shuts down the photoinduced electron-transfer pathway of the excited free ligand.

The interference from other metal ions with the detection of the Zn^{2+} ion was evaluated, which shows that other ions do not hinder the Zn^{2+} detection. As can be seen from Table 2, the results clearly demonstrate the selectivity for Zn^{2+} over the other metal ions. The fluorescence of compound (**e**) is not influenced by cations such as Na^+, K^+, Ca^{2+}, Mg^{2+}, which exist at high concentration under physiological conditions, even at 9 mM. Thus, this molecule can be used even under biological conditions involving an increase of Ca^{2+} concentration. The alkaline metals or alkaline earth metals ions have no effect on the detection of Zn^{2+} ion, which can be attributed to the poor complexation of these metal ions with the synthesized fluorescent substance. What's more, the fluorescence of this molecule can be quenched to some extent by Fe^{3+}, Cu^{2+}, Co^{2+}, probably because there is electron or energy transfer between metal cation

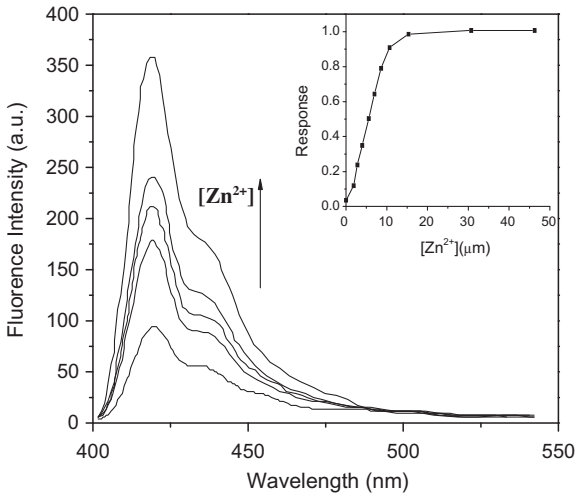


Figure 4. Fluorescence spectra of the compound (**e**) (1×10^{-5} M) in H_2O (HEPES: 100 mM, pH7.4, I = 0.1 (KNO_3), 10 mM NTA) with incremental addition of Zn^{2+} (0–5×10^{-5} M). Inset: Determination of K_d by the increase in I_f as a function of Zn^{2+} concentration.

Table 2
Relative fluorescent intensities of the compound (**e**) with various biologically important metal cations

Entry	Metal cations	Relative intensity (I_f/I_0)[a]
1	None	1.0
2	9×10^{-3} M Na^+	1.0
3	9×10^{-3} M K^+	1.0
4	9×10^{-3} M Ca^{2+}	1.0
5	9×10^{-3} M Mg^{2+}	1.0
6	1×10^{-5} M Zn^{2+}	3.6
7	1×10^{-5} M Fe^{3+}	0.5
8	1×10^{-5} M Cu^{2+}	0.3
9	1×10^{-5} M Ni^{2+}	1.0
10	1×10^{-5} M Co^{2+}	0.2
11	1×10^{-5} M Mn^{2+}	1.0
12	1×10^{-5} M Cd^{2+}	1.2
13	1×10^{-5} M Zn^{2+} + 9×10^{-3} M Na^+	3.6
14	1×10^{-5} M Zn^{2+} + 9×10^{-3} M K^+	3.6
15	1×10^{-5} M Zn^{2+} + 9×10^{-3} M Ca^{2+}	3.6
16	1×10^{-5} M Zn^{2+} + 9×10^{-3} M Mg^{2+}	3.6

[a] I_0 and I_f indicate the average fluorescence intensities of fluorescence probe compound (**e**) (1×10^{-5} M) in the absence and presence of various metal cations, respectively.

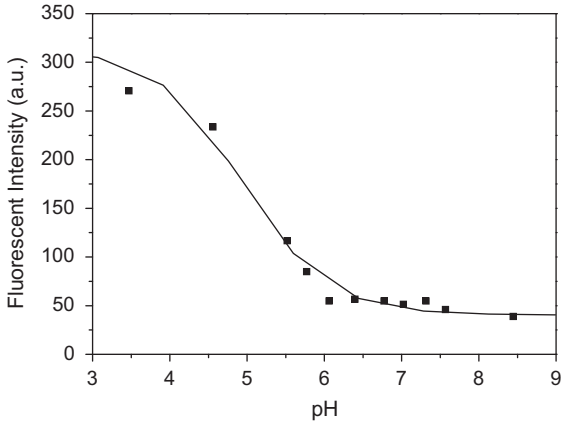


Figure 5. Effect of pH on the fluorescence intensity of compound (**e**) (1.0×10^{-5} M) in the presence of Zn^{2+} (the concentration of Zn^{2+} was fixed at 1.0×10^{-5} M). Measured at 25 °C in DMSO–H_2O (1:9,v/v).

and fluorophore upon excitation, which is known as the fluorescence quenching mechanism.[19]

Fluorescence turn-on by protonation has been a common problem for fluorescent Zn^{2+} sensors, because protonation diminishes the sensitivity to zinc by increasing the background signal intensity.[20] Compared with some conventional Zn^{2+} probe, the fluorescence response of compound (**e**) is proved much less pH-sensitive within the biologically relevant window. Figure 5 shows a slight fluorescence decrease occurs when the solution pH increases from 6.0 to 8.0.

To determine the cell permeability of probe, cultured macrophages (RAW 264.7) will be incubated with phosphate-buffered saline (PBS) containing the probe. The change of the fluorescences could be tested using confocal laser scanning microscope by the addition of Zn^{2+} and pyrithione, which is a zinc-selective ionophore, and followed by the TPEN, a high affinity zinc chelator. If the cells were not stained, indicating that probe could not permeate through the cell membrane. And then, a ethyl ester derivative of probe can be prepare to improve the lipophility so that it could be permeated into the cell where it will be transformed into probe by esterase in the cytosol.[21]

In conclusion, a novel fluorescence probe 2-[(*N*-ethyl carbazole)-3-sulfonyl ethylenediamine]-1-*N*,*N*-2-(2-methypyridy) has been synthesized. It should be emphasized that the probe can be easily synthesized in four steps from readily available starting materials. The reactivity of the fluorescence probe with Zn^{2+} has been examined in solution of physiological pH by fluorescence spectroscopy. The UV/vis spectra of the compound in various solvents were measured and the interactions between the probe and solvents were also discussed. Finally, it should be noted that the target fluorescent probe, due to its unique fluorescence properties and high selectivity, could be very promising for further research on the Zn^{2+} analysis under physiological conditions. We are currently in the process of modifying carbazole compound (**e**) to shift its excitation and emission spectra in the visible to protect biological sample from photodamage upon UV irradiation region as well as to circumvent the spectral window with pronounced cellular autofluorescence and we will report the findings subsequently.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.11.004.

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