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A NEW CARBAZOLE-BASED FLUORESCENCE SENSOR FOR HIGH SELECTIVE DETECTION OF COPPER(II) IN AQUEOUS SOLUTIONS

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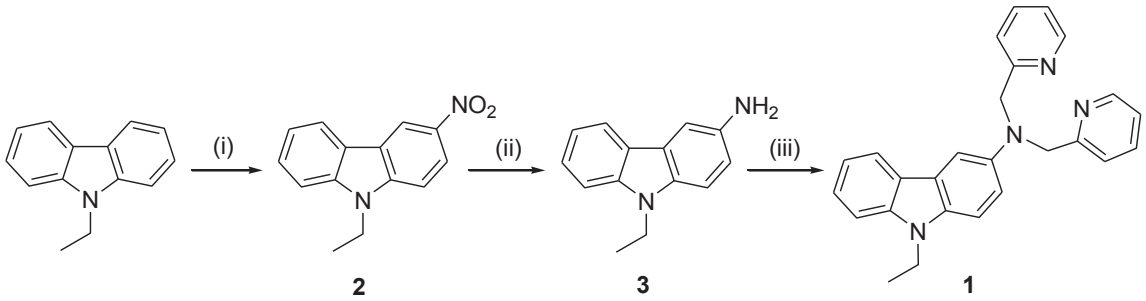
Abstract – A new fluorescent sensor based on 9-ethylcarbazol and di-2-picolylamine was designed and synthesized. Its structure was confirmed by IR, NMR, MS spectra and elemental analyses. Its binding properties investigated by fluorescence spectroscope showed that it can selectively bind Cu^{2+} in anquous solution with fluorescence quenching.

The design and synthesis of chemosensors for highly noxious, heavy, and transition metal ions are currently a task of prime importance for medical, environmental, and biological applications.[1] Copper is an essential trace element in biological systems,[2] many proteins use copper ions as a cofactor for electron transport, or as a catalyst in oxido-reduction reactions. However, an excess of copper ions in living cells can result in several serious diseases, including Alzheimer's disease,[3] Wilson's disease,[4] Parkinson's disease,[5] and prion disease.[6] Moreover, copper is also a common metal pollutant due to its extensive applications in our daily lives. Thus, much effort has been devoted to the design of various chemosensors specific for Cu^{2+} detection.[7-12]

Carbazoles are a distinguished class of aromatic heterocyclic nuclei. The molecular and optical properties of carbazoles can be engineered by structural modifications on the C-2, -3, -6, -7, and -9 positions.[13] Carbazole-based heterocyclic compounds are known for their good chemical stability and high fluorescence quantum yield, therefore they have been widely used as a scaffold in chemosensors. Recently, a number of carbazole-based ligands have been found to be effective as anion receptors[14] and metal ions receptors.[15-17] However, studies on carbazole-based chemosensors for fluorescent recognition of Cu^{2+} is still rare.[18] Herein, we designed and synthesized a new fluorescent sensor 9-ethyl-N,N-bis(pyridin-2-ylmethyl)-9*H*-carbazol-3-amine (**1**, **Scheme 1**). The sensor is constructed via two functional moieties: 9-ethyl-9*H*-carbazole acts as a fluorophore for its excellent photophysical property, and

bis(pyridin-2-ylmethyl)amine (dpa) linked to 9-ethyl-9*H*-carbazole provides the recognition and binding site for metal ions. Sensor **1** displays high selectivity for Cu^{2+} ion among the metal ions examined and exhibits fluorescence quenching upon binding of Cu^{2+} ion with an "on–off" type fluoroionophoric switching property.

The synthetic route of compound **1** was shown in **Scheme 1**. The structure of the compound **1** was determined by IR, NMR, EI-MS, and Elemental analyses.



Schem 1. Synthetic route to **1**. Reagents and conditions: i) $CO(NH_2)_2 \cdot HNO_3$, AcOH; ii) $SnCl_2 \cdot H_2O$, HCl, EtOH, reflux 2 h; iii) 2-(chloromethyl)pyridine hydrochloride, KI, K_2CO_3, MeCN, reflux, 5 h.

The binding properties of molecule **1** were investigated by the UV-vis absorption spectra and fluorescence measurement. The UV–vis absorption spectra of **1** and its titration with Cu^{2+} were measured in DMSO/water (95:5, v/v) containing HEPES buffer (10 mM, pH=7.0), as shown in **Figure 1**. With the addition of increasing amounts of Cu^{2+} to a solution of receptor **1**, two absorbances at 309 nm and 383 nm decreased gradually, and concomitantly, a rising new absorbance that peaked at 331 nm appeared. Three isosbestic points were clearly observed at 303, 319, and 314 nm, indicating the formation of a new complex between **1** and Cu^{2+}.

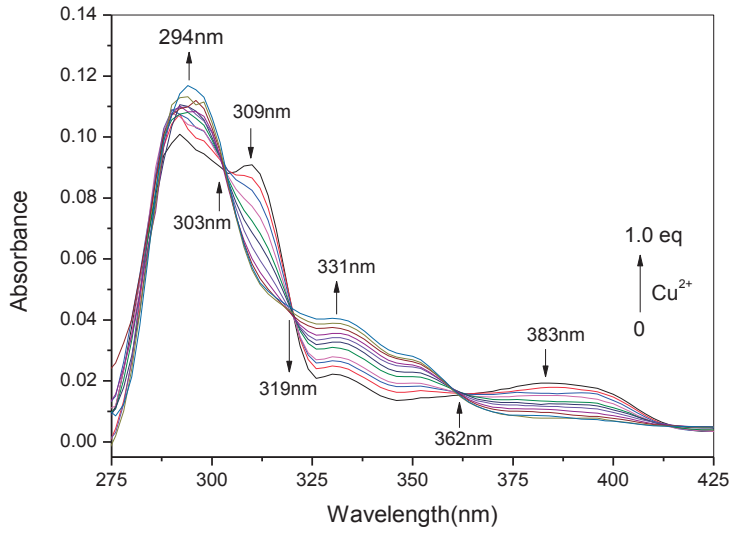


Figure 1. UV–vis spectral changes of compound **1** (10 μM) in DMSO/water (95:5, v/v) containing HEPES buffer (10 mM, pH=7.0) with increasing amount of Cu^{2+} (as its chloride salt).

Fluorescence spectra were measured and recorded simultaneously as shown in **Figure 2**. Emission spectrum of **1** in DMSO/water (95:5, v/v) containing HEPES buffer (10 mM, pH=7.0) displayed a typical broad ICT band with a maximum at 435 nm upon excitation at 376 nm. With the addition of increasing amounts of Cu^{2+} to a solution of compound **1**, the fluorescence intensity of compound **1** gradually decreased with no noticeable change in the shape of the spectra. When the amount of Cu^{2+} added was about **1** eqiv of compound **1**, the fluorescence intensity of **1** almost reached minimum.

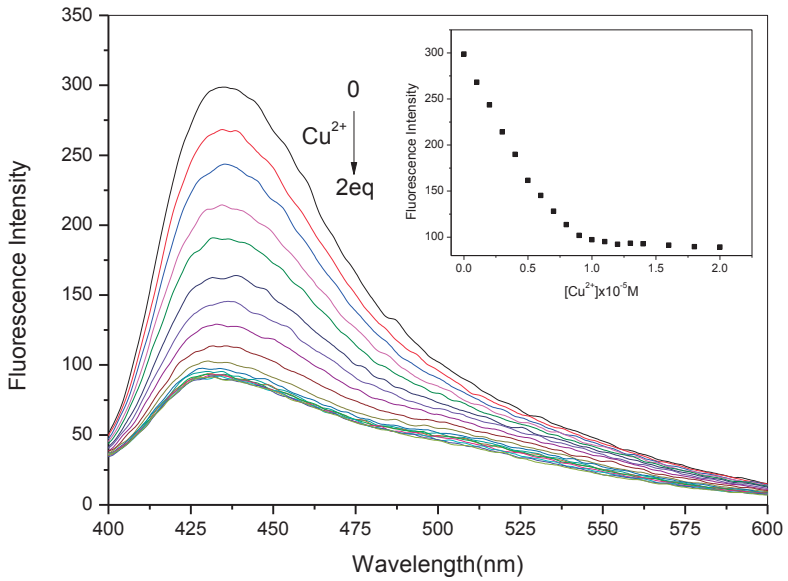


Figure 2. Emision spectra of **1** (10 μM) in DMSO/water (95:5, v/v) containing HEPES buffer (10 mM, pH=7.0) upon the addition of Cu^{2+} (0–2 eq) at 25 °C. Inset shows the spectral fitting of the titration curve (λ_{ex}=376 nm).

To determine the binding stoichiometry of the **1**–Cu^{2+} complex, the Job's plot[19] for the system was also performed in aqueous solution by keeping the total concentration of **1** and Cu^{2+} at 10 μM and changing the molar ration of Cu^{2+} ([Cu^{2+}]/[Cu^{2+}+ 1]) from 0 to 1. As shown in **Figure 3**, the result shows that a maximum at a molar fraction of 0.5, indicating the formation of 1:1 complex of **1** and Cu^{2+}. The ESI mass spectrum of a mixture of **1** and $CuCl_2$ also revealed the formation of a 1:1 metal–ligand complex through the metal coordination interaction where there is a major signal at *m/z* 490.10 (calculated value, 490.1) assigned to the species[Cu(**1**)Cl]$^+$ with the loss of one Cl^- anion (ESI, **Figure S4**).

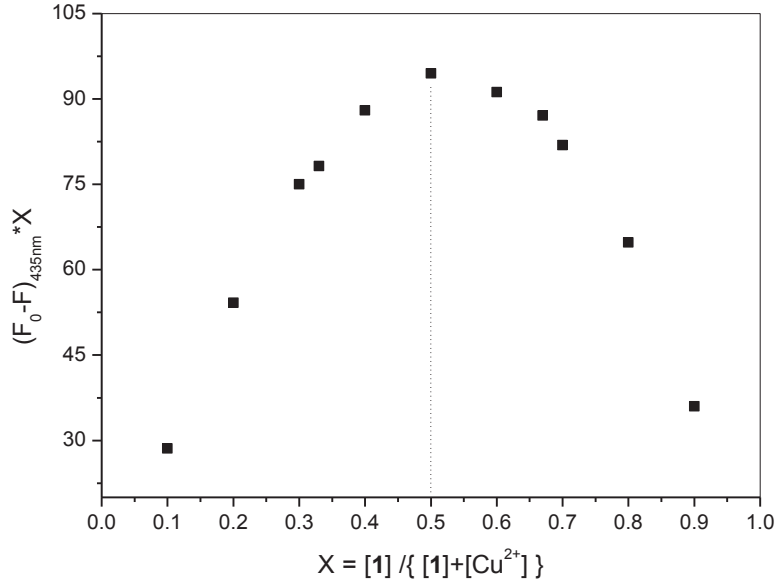


Figure 3. Job' plot of **1** and Cu^{2+}. The total concentration of **1** and Cu^{2+} was kept at a fixed 10 μM. The data are consistent with 1:1 Cu^{2+}–**1** complex.

The binding constant between **1** and the Cu^{2+} ion was calculated from the fluorescence titration result following Benesi–Hildebrand equation[20] and was found to be 7.3×10^4 M^{-1} (**Figure 4**). The detection limit, based on the definition by IUPAC (CDL=3*Sb*/m),[21] was found to be 2.2×10^{-7} M from 10 blank solutions, lower than the limit of copper in drinking water (~20 μM). These results suggest that compound **1** has a high selectivity to Cu^{2+}, and could be exploited as a fluorescence sensor for Cu^{2+}.

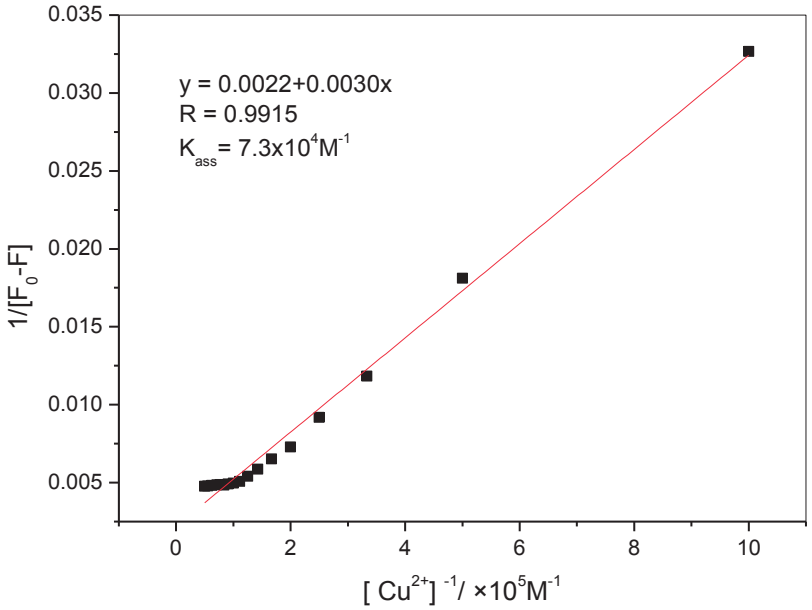


Figure 4. Benesi–Hildebrand plot of sensor **1** with Cu^{2+}

The selectivity and tolerance of compound **1** for Cu^{2+} ion over other metal cations such as Ba^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Hg^{2+}, K^{+}, Mg^{2+}, Mn^{2+}, Na^{+}, Ni^{2+}, Pb^{2+}, Zn^{2+}, Al^{3+}, and Fe^{3+} ions were investigated by adding metal cations (10 μM) to the solution of compound **1** (10 μM). As depicted in **Figure 5**, the emission spectrum of **1** displays a sharp band at 435 nm upon excition at 376 nm. No significant changes of the spectra were observed for compound **1** upon addition of most of above metal ions except for Ni^{2+} and Cu^{2+}. Upon addition of 1 equiv of Ni^{2+}, the fluorescence intensity of **1** was reduced to 75.1% of the initial one. However, the fluorescence intensity of **1** could be reduced to 30.7% of the initial one upon addition of 1 equiv of Cu^{2+}. Such a significant difference in fluorescence intensity between Cu^{2+} and other metal ions indicates that the function group DPA of **1** is more suitable to bind Cu^{2+} than other metal ions observed.

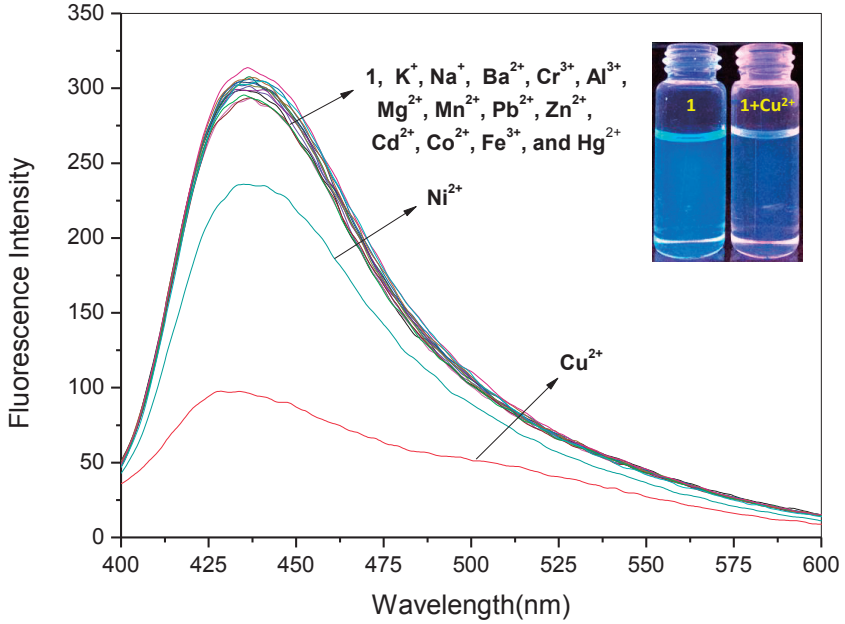


Figure 5. Emission spectral changes of compound **1** (10 μM) in DMSO/water (95:5, v/v) containing HEPES buffer (10 mM, pH=7.0) upon additions of various metal ions (10 μM). λ_{ex}=376 nm. Inset: Photos of **1** in DMSO/water (95:5, v/v) containing HEPES buffer (10 mM, pH=7.0) without and with addition of Cu^{2+} under their radiation of UV light at 365 nm.

To further gauge selectivity for Cu^{2+} ion over other metal ions, competition experiments of Cu^{2+} ion mixed with other metal ions were carried out from fluorescence spectra and the results are shown in **Figure 6**. The fluorescence intensity of **1** (10 μM) in the presence of 1 equiv of the Cu^{2+} ion was almost unaffected by the addition of 1 equiv of competing metal ions (Ba^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Hg^{2+}, K^{+}, Mg^{2+}, Mn^{2+}, Na^{+}, Ni^{2+}, Pb^{2+}, Zn^{2+}, Al^{3+}, and Fe^{3+}). These results suggested that compound **1** could be used as Cu^{2+} selective fluorescent chemosensor.

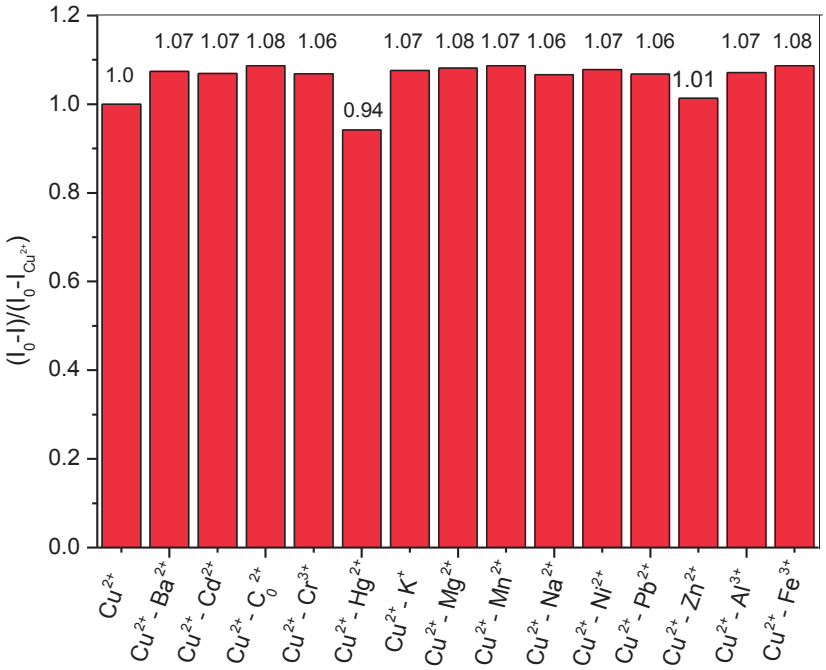
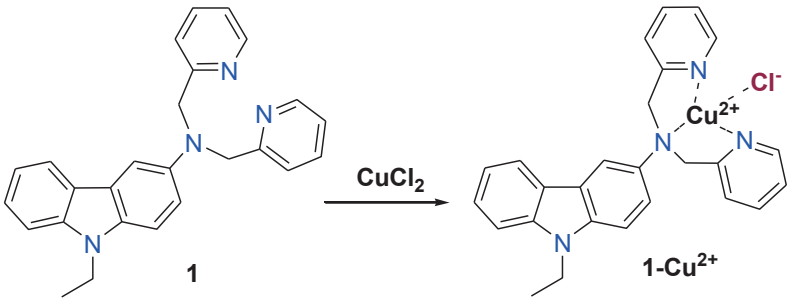


Figure 6. Competitive experiments in the **1**+Cu^{2+} system with interfering metal ions. [**1**]=10 μM, [Cu^{2+}]= 10 μM, and [M^{n+}]=10 μM. λ_{ex}=376 nm.

Based on the above experiments, a proposed binding mechanism of Cu^{2+} with **1** was shown in **Scheme 2**. It should be noted that in the square-planar Cu^{2+} complex with the sensor **1**, four coordination centers probably come from three N atoms of DPA moiety and a Cl^-. Because of the paramagnetic nature of Cu^{2+} ion ($3d^9$),[22] the capture of Cu^{2+} resulted in the electron or energy transfer from **1** to Cu^{2+}; thus, compound **1** showed quenching of the fluorescence for Cu^{2+}. As to the other tested metal ions, Ni^{2+} maybe also can form complexes with **1**, but it do not undergo effective fluorescence quenching of the excited state of **1**.



Scheme 2. Proposed 1:1 binding model of **1** with Cu^{2+}

In summary, a new selective fluorescent sensor based on 9-ethyl-9*H*-carbazole and di-2-picolylamine was designed and synthesized which was capable to detect Cu^{2+} ion with high selectivity in anquous DMSO. This sensor formed a 1:1 complex with Cu^{2+} and showed a fluorescent quenching.

EXPERIMENTAL

The NMR was recorded on a Bruker 300 spectrometer with TMS as internal reference and $CDCl_3$ as solvent. IR were recorded on a Perkin–Elmer PE–983 infrared spectrometer as KBr pellets with absorption in cm^{-1}. Mass spectra were measured on a LCQ Advantage MAX (ESI). The elemental analyses were performed with a Vario EL-III instrument (Germany). UV spectra were measured on a SP-1900 spectrophotometer. Fluorescence spectra were determined on a Hitachi F-4500.

Starting materials. 9-Ethyl-3-nitro-9*H*-carbazole (**2**)[23] and 9-ethyl-9*H*-carbazol-3-amine (**3**)[24] were synthesized according to the previously reported procedure. All other chemicals used in this study were commercially available.

Synthesis of compounds 1: A mixture of 9-ethyl-9*H*-carbazol-3-amine (**3**) (0.21 g, 1.0 mmol), 2-(chloromethyl)pyridine hydrochloride (0.37 g, 2.3 mmol), KI (0.50 g, 3.0 mmol), and K_2CO_3 (0.97 g, 7.0 mmol) was refluxed in MeCN (10 mL) for 5 h. After concentrating the mixture under reduced pressure, the resulting residue was dissolved in $CHCl_3$ and washed with water. The organic layer was dried ($MgSO_4$) and concentrated to give a crude product, which was purified by column chromatography (SiO_2) eluting with a mixture of petroleum/EtOAc (20:1, V/V) to afford compound **1** (0.28 g, 72.3%) as a yellow soil solid (**Scheme 1**). IR (ν_{max}, KBr, cm^{-1}): 3440, 2359, 1634, 1588, 1484, 1334, 1230, 1087, 749, 471; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.48-8.47 (m, 1H), 7.77-7.80 (m, 1H), 7.39-7.45 (m, 2H), 7.32-7.39 (m, 1H), 7.21-7.25 (m, 2H), 7.10-7.16 (m, 1H), 7.04-7.07 (m, 1H), 6.94-7.04 (m, 4H), 6.85-6.89 (m, 1H), 4.79 (s, 4H), 4.08 (q, 2H, *J*=7.2 Hz), 1.19 (t, 3H, *J*=7.2 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.3, 149.3, 142.0, 140.2, 136.6, 133.6, 125.2, 123.4, 122.4, 121.7, 121.1, 120.2, 117.8, 113.7, 108.8, 108.1, 104.5, 58.4, 37.2, 13.6. ESI-MS: *m/z* 393.04 ($[M+H]^+$), 807.04 ($[2M+Na]^+$). Anal. Calcd for $C_{26}H_{24}N_4$: C, 79.56; H, 6.16; N, 14.27. Found: C, 79.52; H, 6.13; N, 14.24.

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