

## A Simple Carbazole-based Schiff Base as Fluorescence “off-on” Probe for Highly Selective Recognition of Cu<sup>2+</sup> in Aqueous Solution

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A carbazole-based Schiff base **CB2** was synthesized and applied as a highly selective and sensitive fluorescent probe for Cu<sup>2+</sup> in H<sub>2</sub>O-DMSO (8/2, v/v, pH = 7.4) solution. **CB2** exhibits an excellent selectivity to Cu<sup>2+</sup> over other examined metal ions with a prominent fluorescence “turn-on” at 475 nm. **CB2** and Cu<sup>2+</sup> forms a 1:2 binding ratio complex with detection limit of 9.5 μM. In addition, the Cu<sup>2+</sup> recognition process is hardly interfered by other examined metal ions.

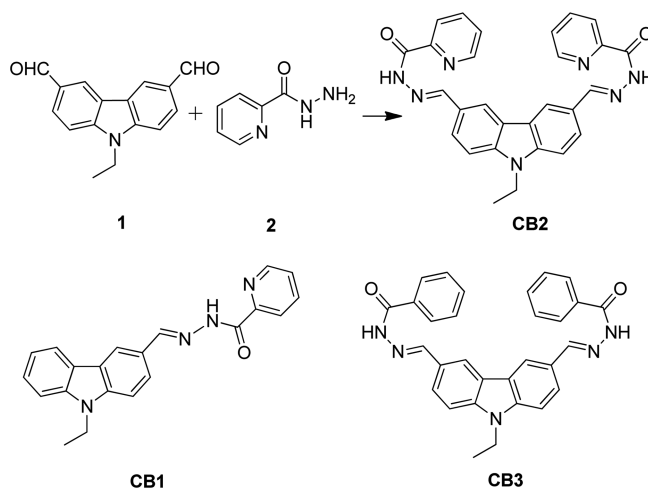
**Key Words** : Fluorescent probe, Carbazole, Copper(II) recognition, Schiff base

### Introduction

Selective detection of Cu<sup>2+</sup> has received considerable attention because it is not only an environmental pollutant at high concentrations<sup>1</sup> but also an essential trace element for many biological processes and systems.<sup>2</sup> Cu<sup>2+</sup> is the third most abundant metal after iron and zinc in human body,<sup>3</sup> it played pivotal biological roles as cofactor of many proteins, or as catalyst in oxido-reduction reactions.<sup>4</sup> However, excess Cu<sup>2+</sup> in human body has been reported to cause serious disease such as prion disease,<sup>5</sup> several neurodegenerative diseases including Menkes and Wilson diseases,<sup>6</sup> Alzheimer's and Parkinson's diseases.<sup>7</sup> In our daily lives, Cu<sup>2+</sup> is generated and accumulated in the environment and food chain with the development of various industries such as electroplating, wood, painting and paper industries. The limit of copper in drinking water is 1.3 ppm (~20 μM) set by US Environmental Protection Agency (EPA). Therefore, the development of fluorescence emission “off-on” probes for Cu<sup>2+</sup> sensing in aqueous media with high selectivity and sensitivity is still imperative.

To date, many excellent works associated with colorimetric or fluorescent probes for Cu<sup>2+</sup> sensing have been documented.<sup>8</sup> However, most of them displayed fluorescence “on-off” response to Cu<sup>2+</sup> owing to the paramagnetic nature of Cu<sup>2+</sup>. Recently, a number of fluorescence “off-on” Cu<sup>2+</sup> probes based on fluorophores such as rhodamine,<sup>9</sup> coumarin,<sup>10</sup> and 1,8-naphthalimide<sup>11</sup> have been reported. It is noteworthy that effective fluorescent probes derived from carbazole fluorophore are still rare.

Carbazole is a conjugated unit with interesting optical and electronic properties. A number of carbazole derivatives have been synthesized and employed as fluorescent probes for the recognition of Cd<sup>2+</sup>,<sup>12</sup> Hg<sup>2+</sup>,<sup>13</sup> and Pb<sup>2+</sup><sup>14</sup> ions. Very recently, we have reported a carbazole-based fluorescent probe **CB1** for Cu<sup>2+</sup> sensing in CH<sub>3</sub>CN-H<sub>2</sub>O (9:1, v/v) solution.<sup>15</sup> However, the Cu<sup>2+</sup> recognition by **CB1** suffers from the influence of Co<sup>2+</sup>. Inspired by the previous work, in this



**Scheme 1.** Synthesis of probe **CB2** and the structures of **CB1** and **CB3**.

work, we designed and synthesized a new carbazole-based fluorescent probe **CB2**, which contains two picolinohydrazide imines as Cu<sup>2+</sup> chelators. Probe **CB2** exhibits fluorescence “off-on” response to Cu<sup>2+</sup> in H<sub>2</sub>O-DMSO (8:2, v/v, pH = 7.4) solution with high selectivity and sensitivity.

### Experimental

**Reagents and Instruments.** Unless otherwise stated, solvents and reagents were analytical grade and used without further purification. Doubly distilled water was used for spectral detection. 9-Ethyl-9H-carbazole-3,6-dicarboxaldehyde (**1**)<sup>16</sup> was prepared by the literature method. <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were recorded on Agilent 400-MR spectrometer, chemical shifts (δ) were expressed in ppm and coupling constants (*J*) in Hertz. High-resolution mass spectroscopy (HRMS) was measured on a Bruker micrOTOF-Q mass spectrometer (Bruker Daltonik, Bremen, Germany). Low-resolution mass spectroscopy (LRMS) was measured on an Agilent 1100 series LC/MSD mass spectrometer.

Fluorescence measurements were performed on a Sanco 970-CRT spectrofluorometer (Shanghai, China). The pH values were measured with a Model PHS-25B meter (Shanghai Dapu instruments Co., Ltd., China).

The salts used in stock solutions of metal ions are  $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{Hg}(\text{NO}_3)_2$ ,  $\text{Ba}(\text{NO}_3)_2$ ,  $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{AgNO}_3$ ,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ,  $\text{KNO}_3$ ,  $\text{NaCl}$ ,  $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ ,  $\text{Mn}(\text{NO}_3)_2$ ,  $\text{Pb}(\text{NO}_3)_2$ ,  $\text{Sr}(\text{NO}_3)_2$ ,  $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ ,  $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{Cd}(\text{NO}_3)_2$ ,  $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ , respectively.

#### Synthetic Procedure.

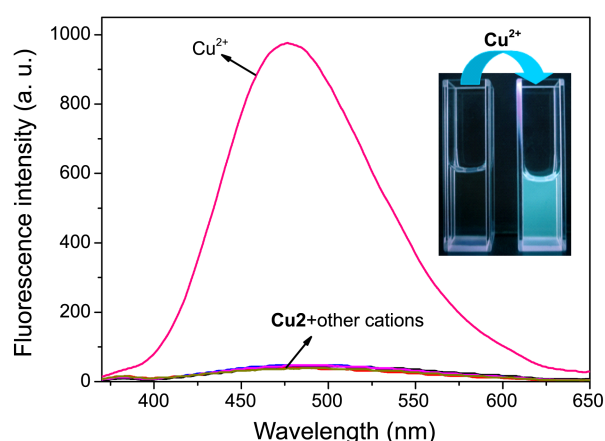
**Synthesis of CB2:** A mixture of compound **1** (251 mg, 1 mmol) and picolinohydrazide (**2**, 240 mg, 2.5 mmol) in 50 mL ethanol was stirred and heated at reflux for 4 h. After cooling to room temperature, the precipitates formed were collected and purified by silica gel column chromatography to give **CB2** as yellow solids. Yield: 75%. mp > 250 °C.  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  12.11 (s, 2H), 8.82 (s, 2H), 8.74 (d,  $J = 4.4$  Hz, 2H), 8.55 (s, 2H), 8.16 (d,  $J = 7.6$  Hz, 2H), 8.07 (t,  $J = 6.8$  Hz, 2H), 7.98 (d,  $J = 8.0$  Hz, 2H), 7.76 (d,  $J = 8.8$  Hz, 2H), 7.68 (dd,  $J_1 = 6.8$  Hz,  $J_2 = 4.8$  Hz, 2H), 4.52 (dd,  $J = 7.2$  Hz, 2H), 1.38 (t,  $J = 7.2$  Hz, 3H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ )  $\delta$  160.63, 150.59, 150.21, 148.97, 141.55, 138.45, 129.80, 127.33, 126.44, 125.17, 123.05, 122.73, 121.48, 110.56, 37.89, 14.32. HRMS (ESI<sup>+</sup>), calcd for  $\text{C}_{28}\text{H}_{24}\text{N}_7\text{O}_2$   $[\text{M}+\text{H}]^+$  490.1991, found 490.1981.

**Synthesis of CB3:** Control compound **CB3** was prepared following the similar procedures as depicted for **CB2** with the exception of benzoylhydrazine was used. Yield: 80%. mp 159–160 °C.  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  11.90 (s, 2H), 8.62 (s, 2H), 8.53 (s, 2H), 7.94 (m, 6H), 7.70 (d,  $J = 8.8$  Hz, 2H), 7.54 (m, 6H), 4.46 (dd, 7.2 Hz, 2H), 1.32 (t,  $J = 7.2$  Hz, 3H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ )  $\delta$  163.48, 149.39, 141.55, 134.12, 132.06, 128.89, 128.14, 126.51, 125.20, 122.84, 121.34, 110.54, 14.33. LRMS (ESI<sup>+</sup>), calcd for  $\text{C}_{28}\text{H}_{24}\text{N}_7\text{O}_2$   $[\text{M}+\text{H}]^+$  488, found 488.

**General Spectroscopic Methods.** Probe **CB2** was dissolved in  $\text{H}_2\text{O}$ -DMSO (8/2, v/v, pH = 7.4) to afford the test solution (10  $\mu\text{M}$ ). Titration experiments were carried out in 10-mm quartz cuvettes at 25 °C. Stock solutions of metal ions (as chloride or nitrate salts, 10 mM) were added to the host solution and used for the titration experiment. The excitation wavelength is 313 nm.

## Results and Discussion

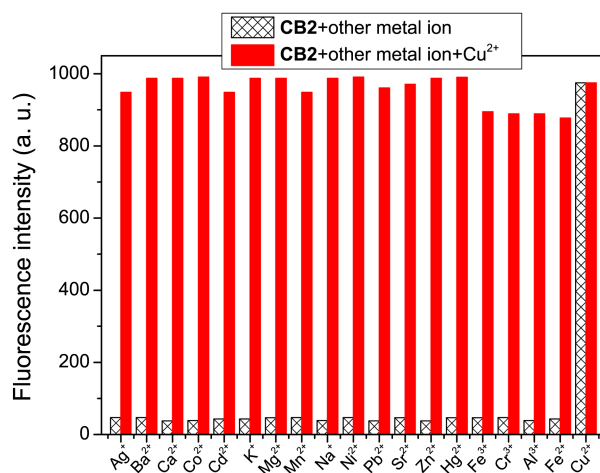
Probe **CB2** was prepared by the condensation of **1** and **2** in absolute ethanol and was structurally characterized by  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and HRMS spectroscopy. Then, the metal ion selectivity of **CB2** was investigated in  $\text{H}_2\text{O}$ -DMSO (8:2, v/v, pH = 7.4) solution (Fig. 1). Free **CB2** solution (10  $\mu\text{M}$ ) exhibited a very weak fluorescence emission at 475 nm, this may attributed to the C=N isomerization in the excited state. On addition of 2.0 equiv. of  $\text{Cu}^{2+}$ , a remarkable fluorescence enhancement at 475 nm was observed. This fluorescence enhancement can be mainly attributed to the inhibition of C=N isomerization after binding with  $\text{Cu}^{2+}$ . The chelation of



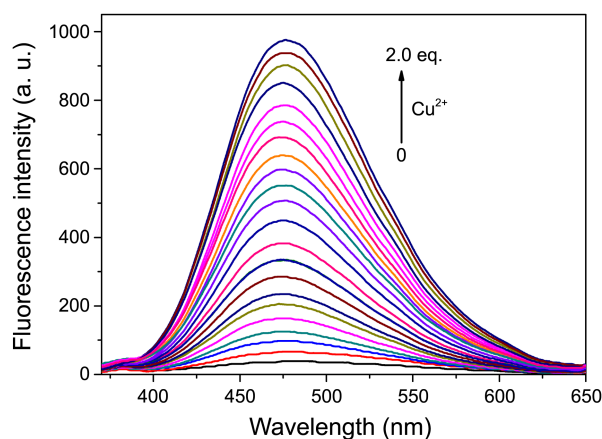
**Figure 1.** Fluorescence spectra of **CB2** (10  $\mu\text{M}$ ) in  $\text{H}_2\text{O}$ -DMSO (8/2, v/v, pH = 7.4) on addition of various metal ions. Inset: Fluorescence color changes of **CB2** before and after addition of  $\text{Cu}^{2+}$ .

**CB2** with  $\text{Cu}^{2+}$  also can induce increasing in rigidity of the receptor and leads to chelation-enhanced fluorescence (CHEF) of **CB2**. Whereas, addition of other transition and heavy metal ions including  $\text{Ag}^+$ ,  $\text{Ba}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Na}^+$ ,  $\text{Ni}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Sr}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Al}^{3+}$ , and  $\text{Fe}^{2+}$  promoted no significant fluorescence enhancement, which led to greatly improved selectivity over the previous probe **CB1**. Additionally, the fluorescence color changes of **CB2** before and after addition of  $\text{Cu}^{2+}$  was naked eye detectable (Fig. 1, inset). These results demonstrate that **CB2** has an excellent selectivity toward  $\text{Cu}^{2+}$  over other interested metal ions.

Besides the high selectivity of probe to the target metal ion, its anti-interference ability to other potential competitive metal ions is also important. Thus, the competition experiments were subsequently carried out. As shown in Figure 2, except  $\text{Cu}^{2+}$ , other tested metal ions (each cation was used as 2.0 equiv. to **CB2**) did not induce significant fluorescence



**Figure 2.** Fluorescence intensity of **CB2** (10  $\mu\text{M}$ ) in  $\text{H}_2\text{O}$ -DMSO (8/2, v/v, pH = 7.4) at 475 nm. The black bars represent the emission intensity of **CB2** in the presence of 2.0 equiv. of competing metal ion; the red bars represent the emission intensity of the above solution upon addition of 2.0 equiv. of  $\text{Cu}^{2+}$ .

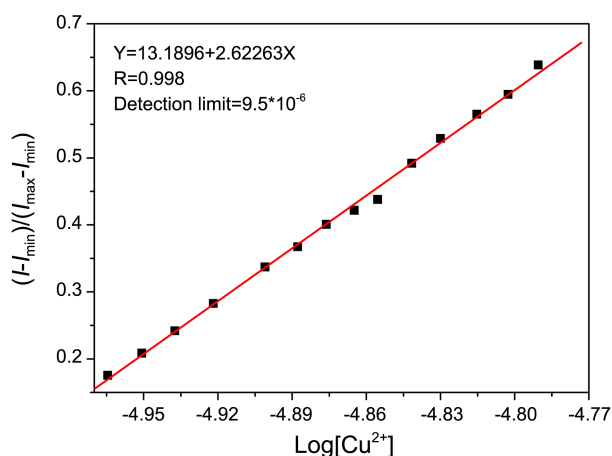


**Figure 3.** Fluorescence spectra changes of **CB2** (10  $\mu\text{M}$ ) in  $\text{H}_2\text{O}$ -DMSO (8/2, v/v, pH = 7.4) upon addition of  $\text{Cu}^{2+}$  (0 to 2.0 equiv.).

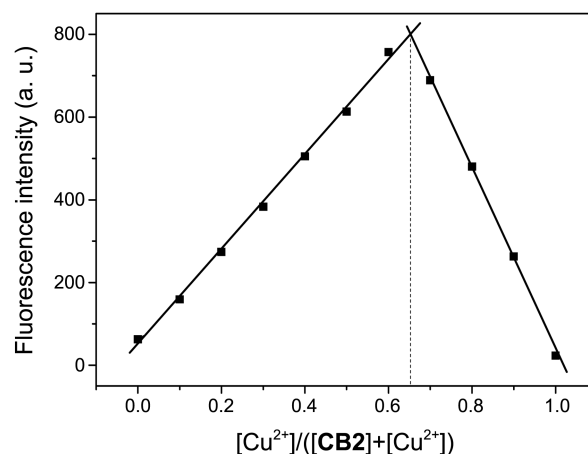
changes. Nevertheless, a dramatic fluorescence emission enhancement was observed on further addition 2.0 equiv. of  $\text{Cu}^{2+}$  to the above competitive metal ion containing solutions. These results show that the  $\text{Cu}^{2+}$  recognition event by **CB2** has a good anti-influence ability to other potential competitive metal ions.

To further investigate the interaction of probe **CB2** and  $\text{Cu}^{2+}$ , fluorescence titration experiment was conducted (Fig. 3). Upon addition of increasing amounts of  $\text{Cu}^{2+}$  ions, the fluorescence emission band centered at 475 nm gradually increased. The maximum emission intensity was obtained when 2.0 equiv. of  $\text{Cu}^{2+}$  ions was employed. Based on the titration data, the detection limit of **CB2** to  $\text{Cu}^{2+}$  was estimated to be 9.5  $\mu\text{M}$  (Fig. 4),<sup>17</sup> indicating that **CB2** is sufficiently sensitive to detect  $\text{Cu}^{2+}$  concentration in some aqueous system.

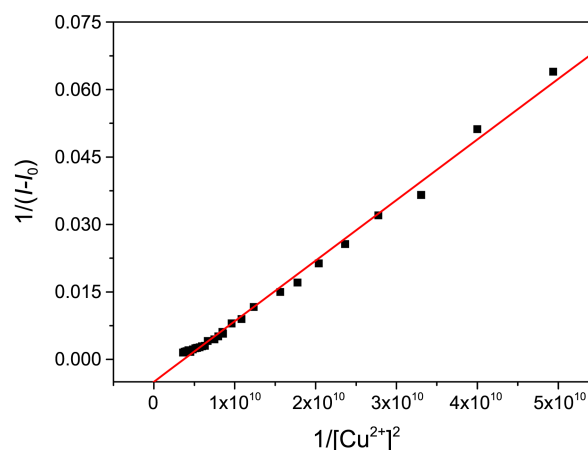
To get insight into the binding ratio of probe **CB2** with  $\text{Cu}^{2+}$ , the Job's plot was examined with a total concentration of **CB2** and  $\text{Cu}^{2+}$  as 20  $\mu\text{M}$ . An emission turning point was observed when the molar fraction of  $\text{Cu}^{2+}$  is about 0.67, suggesting the formation of a 2:1 binding stoichiometry between  $\text{Cu}^{2+}$  and probe **CB2** (Fig. 5). Linear fitting of the titration profiles using Benesi-Hildebrand plot based on a



**Figure 4.** Normalized fluorescence intensity of **CB2** (10  $\mu\text{M}$ ) as a function of  $\text{Log}[\text{Cu}^{2+}]$ .  $\lambda_{\text{em}} = 475 \text{ nm}$ .



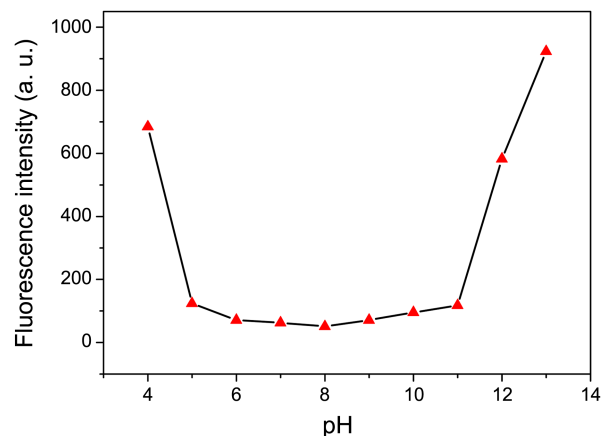
**Figure 5.** Job's plot showing the 2:1 binding of  $\text{Cu}^{2+}$  to **CB2**.



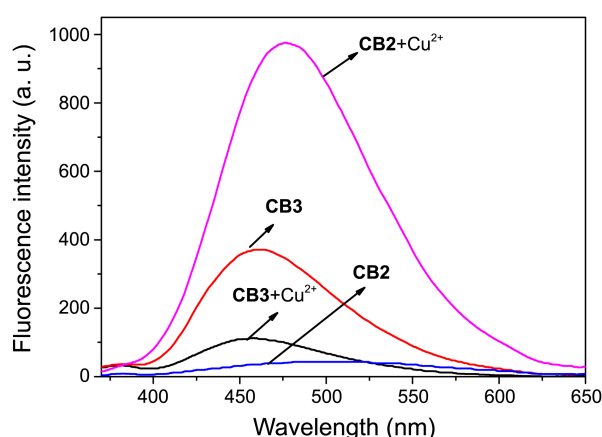
**Figure 6.** Benesi-Hildebrand plot **CB2** assuming 1:2 stoichiometry with  $\text{Cu}^{2+}$ .  $\lambda_{\text{em}} = 475 \text{ nm}$ .

2:1 binding mode results in a good linearity (correlation coefficient is over 0.99) (Fig. 6), which also strongly support the 2:1 binding stoichiometry of  $\text{Cu}^{2+}$  and **CB2**, and the apparent association constant ( $K_a$ ) is evaluated to be  $3.7 \times 10^9 \text{ M}^{-2}$ .

For practical application, the fluorescence intensity (at 475



**Figure 7.** Influences of pH on the fluorescence intensity (at 475 nm) of **CB2** (10  $\mu\text{M}$ ) in  $\text{H}_2\text{O}$ -DMSO (8/2, v/v) solution.



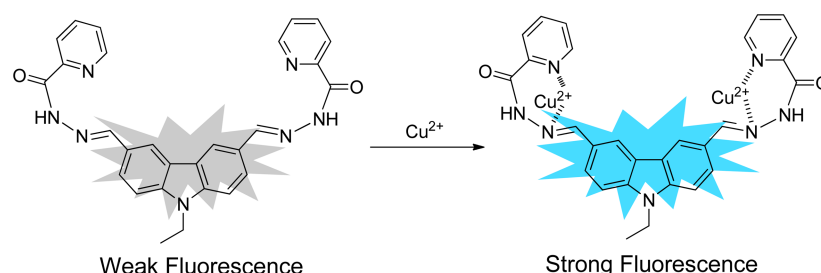
**Figure 8.** Different fluorescence responses of probe **CB2** and control compound **CB3** to  $\text{Cu}^{2+}$ .

nm) of free probe **CB2** at different pH values were explored (Fig. 7). The results showed that **CB2** displayed weak fluorescence emission from pH 5.0 to 11.0. Under strong acidic (pH < 5.0) and strong basic (pH > 11) conditions, the solutions displayed greatly enhanced fluorescence emissions. These results demonstrate that **CB2** can be used for  $\text{Cu}^{2+}$  recognition in a wide pH range.

To validate the binding function of pyridine nitrogen atom in **CB2**, a carbazole based Schiff-base **CB3** was prepared as a control compound, and its fluorescence responses to  $\text{Cu}^{2+}$  was also examined. Compound **CB3** (10  $\mu\text{M}$ ) in  $\text{H}_2\text{O}$ -DMSO (8/2, v/v, pH = 7.4) solution emitted a relative weak fluorescence. In the presence of  $\text{Cu}^{2+}$ , the fluorescence emission was greatly quenched rather than increased (Fig. 8). This response of **CB3** to  $\text{Cu}^{2+}$  is quite different from that of **CB2**, suggesting the pyridine moieties in **CB2** are requisite for its fluorescence “off-on”  $\text{Cu}^{2+}$  selectivity. The proposed binding mode of **CB2** and  $\text{Cu}^{2+}$  is illustrated in Scheme 2.

### Conclusions

In summary, we have developed a simple carbazole-based Schiff base **CB2** as a fluorescence “turn-on” probe for  $\text{Cu}^{2+}$  recognition in aqueous solution. Probe **CB2** displayed an extremely high selectivity toward  $\text{Cu}^{2+}$  over other interested metal ions with a 1:2 binding ratio. The low detection limit of **CB2** to  $\text{Cu}^{2+}$  makes it have a potential applicability in real water sample  $\text{Cu}^{2+}$  detection.



**Scheme 2.** Proposed bind mode of **CB2** with  $\text{Cu}^{2+}$ .

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