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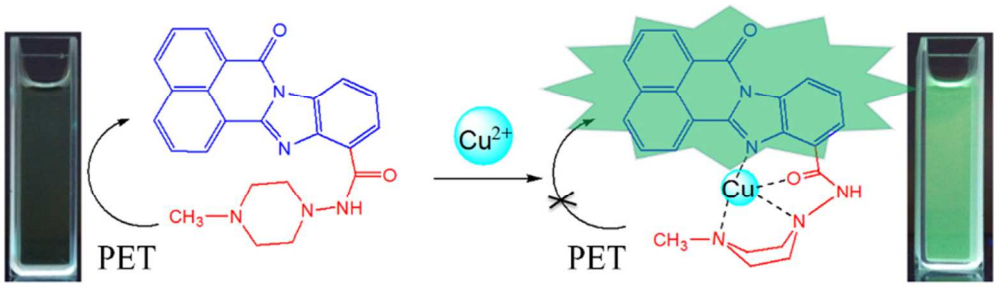

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ARTICLE TYPE

Novel fluorescent sensors based on benzimidazo[2,1a]benz[de] isoquinoline-7-one-12-carboxylic acid for Cu^{2+}

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Fluorescent sensors of N-(4-methylpiperazin-1-yl)benzimidazo[2,1-a]benz[de]isoquinoline-7-one-12-carboxylamide (**C1**) and its corresponding quaternary ammonium salt (**C2**) were prepared. **C1** showed 45-fold fluorescence turn-on response towards Cu^{2+} with a detection limit of 5.7×10^{-8} mol/L in acetonitrile-H_2O (9:1) buffer solution and **C2** showed 18-fold fluorescence enhancement towards Cu^{2+} with a detection limit of 3.4×10^{-7} mol/L in the same condition. The Cu^{2+} sensing of **C1** and **C2** were both based on the photoinduced electron transfer (PET) process. Such behaviors confirmed that the benzimidazo[2,1-a]benz[de]isoquinoline-7-one-12-carboxylic acid based **C1** and **C2** could be utilized as fluorescent sensors for Cu^{2+}. The mechanism of fluorescence enhancement of **C1** towards Cu^{2+} was verified by DFT/TDDFT calculation using Gaussian 03. In addition, obvious color change was observed when water solution of **C2** was treated with aqueous NaOH. Such behavior confirms that **C2** could be used as fluorescent OH^- sensor in water.

Introduction

Chemosensors are compounds that have significant changes in electrical, electronic, magnetic or optical signal when binding with specific guest counterparts.[1-3] Among various chemsensors, fluorescent sensors have attracted special interests because they enjoyed superb sensitivity, selectivity, rapidity and portability, etc.[4-6] The most commonly exploited approach for design of fluorescent sensor was photoinduced electron transfer (PET) process using "fluorophore-spacer-receptor" format. When the receptor was unbound, the assembled molecule lost its fluorescence due to PET process from receptor to the fluorophore.[7] However, upon binding with protons or suitable metal ions, a large chelation-enhanced fluorescence (CHEF) effect was observed because the protonation or chelation abrogated the PET process. In other words, the presence of the guest was signaled by fluorescence enhancement of the system.[8]

As we all known, Cu^{2+} played an important role in various biological processes.[9-12] Exposure to a high level of Cu^{2+} could cause a wide variety of symptoms (gastrointestinal disease, Wilson's disease, dyslexia, hypoglycemia, and infant liver damage), suggesting that Cu^{2+} affected multiple targets in various physiological processes.[13-16] Synthesis and application of fluorescent sensors for Cu^{2+} will give help to clarify how Cu^{2+} work in vivo and how to give rise to these severe diseases. Thus, a useful chemsensor with excellent sensitivity and selectivity for Cu^{2+} is requisite. Cu^{2+} complexation was well known to induce intrinsic fluorescence quenching, while chemosensors with fluorescence enhancement were more encouraging because of their simplicity in practical applications.[17-22] So fluorescent sensors which have 'turn-on' response in the presence of analytes are much more grateful than those of 'turn-off' sensors. Therefore, fluorescence 'turn-on' chemosensors with high selectivity and sensitivity towards Cu^{2+} are highly desirable.

To date, a plenty of effective fluorescent sensors have been successfully developed, and most of them consisted of familiar fluorophores (which included coumarin, rhodamine, naphthalimide, fluorescein, distyryl ketone...) and similar macrocyclic receptors.[23-26] For that reason, it is of significance to design and synthesize new sensors which exhibit fluorescence enhancement, ideal selectivity, as well as highly sensitivity towards target analytes. Consequently, a new selective chemosensor including a suitable fluorophore with visible light excitation for pH and Cu^{2+} become our purpose. Clearly, contributions to this finding are helpful to extend the realm of fluorescence probes.

Benzimidazo[2,1-a]benz[de]isoquinoline-7-one, which contained five conjugated rings in its molecule, was a developmental fluorophore based on 1,8-naphthalimides. The heterocyclic compound with both benzimidazo and naphthalimide group in its molecule rendered it a stronger extent of conjugation and a biological ability of the naphthalimide at the same time. These excellent properties also gave it a broad potential applied as a fluorophore in the field of chemsensors. To date, fewer sensors based on the fluorophore have been reported because of their complication in preparation and purification.[27-30] We believe benzimidazo[2,1-a]benz[de]isoquinoline-7-one-12 carboxylic acid, which was formed by introduction of carboxylic group on the C-12 position of the fluorophore benzimidazo[2,1-a]benz[de]isoquinoline-7-one formally, will be an efficient intermediate of chemosensors. Also the carboxylic group can be easily modified with different electron-donating receptors and leads to potential intensive fluorescence by forming a PET system. Encouraged by this idea, the intermediate of benzimidazo[2,1-a]benz[de]isoquinoline-7-one-12 carboxylic acid was prepared with simple procedure, and then **C1** and **C2** with aminopiperizine as receptors were synthesized. Research of their optical properties revealed that the sensors absorbed light and transfered their excitation electrons from receptors to the fluorophore efficiently,

which mean the "off" state of the compounds. Then they exhibited a strong fluorescence enhancement when binding with Cu^{2+} or protons.

Experimental section

Materials

All metal salts such as $CuCl_2 \cdot 2H_2O$, $NiCl_2 \cdot 6H_2O$, $BaCl_2 \cdot 3H_2O$, $AlCl_3 \cdot 6H_2O$, $AgNO_3$, $CdCl_2 \cdot 2.5H_2O$, $PbCl_2$, $CoCl_2 \cdot 6H_2O$, $SrCl_3 \cdot 6H_2O$, $CrCl_3 \cdot 6H_2O$, $ZnCl_2$, $HgCl_2$, $MnCl_2 \cdot 4H_2O$ and LiCl were analytical grade and used without further purification. All other organic reagents were purchased and used as received.

Measurements

UV-vis spectra were recorded on a Shimadzu 3100 spectrometer. Fluorescence measurements were carried out using an Edinburgh Instruments Ltd-FLS920 fluorescence spectrophotometer. ^1H NMR spectra were recorded on a Bruker AV III 400 MHz NMR spectrometer and ^{13}C NMR spectra were recorded on a Bruker AV III 100 MHz NMR spectrometer with tetramethysilane (TMS) as an internal standard. Infrared spectra were recorded using a Bruker Vertex 70 FT-IR spectrometer with KBr pellets.

Sample preparation

All tests were carried out at room temperature (25 °C) with distilled water. In the experiments of titration with various metal ions, the sensors were dissolved in HEPES acetonitrile-H_2O (9:1) buffer solution or water to afford the test solution (1×10^{-5} M). Stock solutions (1×10^{-5} M) of the mental salts of $HgCl_2$, $CuCl_2$, $PbCl_2$, $AlCl_3$, $CrCl_3$, $SrCl_3$, $NiCl_2$, $BaCl_2$, LiCl, $CoCl_2$, $ZnCl_2$, $CdCl_2$, $AgNO_3$ and $MnCl_2$ in water were prepared.

Computational details

The quantum yield of sensor **C1** was determined according to the following equation:

$$\phi_u = \phi_s \frac{F_u A_s n_u^2}{F_s A_u n_s^2}$$

where ϕ is fluorescence quantum yield; F is integrated area under the corrected emission spectra; A is the absorbance at the excitation wavelength; n is the refractive index of the solution; the subscripts u and s refer to the unknown and the standard, respectively. Rhodamine B in ethanol solution was used as the standard, which has a quantum yield of 0.97.

Density functional theory (DFT) structural optimizations were performed with the Gaussian 03 program. In all cases, the structures were optimized using the B3LYP functional and the mixed basis set 6-31+G(d). Each structure was subsequently subjected to TD-DFT calculation using the B3LYP functional.[31] For all optimized structures, frequency calculations were carried out to confirm the absence of imaginary frequencies. The molecular orbitals were visualized and plotted with the GaussView 5.0 program.

Synthesis

Benzimidazo[2,1-a]benz[de]isoquinoline-7-one-12-carboxylic acid (1). 2,3-diaminobenzoic acid (1.98 g, 10.00 mmol) and 1,8-Naphthalic anhydride (1.52 g, 10.00 mmol) were mixed in 50 ml acetic acid and refluxed for 20 h. The suspension was filtered and the cake was washed with small amount of acetic acid. Then dried in the air and dissolved in 100 ml NaOH solution (5%). The black unsoluble substance was removed by filtration, and concentrated HCl was added to the filtrate until pH was less than 5. The resulting suspension was filtered, and the filter cake was washed with boiling water (50 ml×5). **1** was obtained as yellow solid after drying in vacuum. Yield: 2.19 g, 62.6%. Ms (ESI): m/z = 315.08 $[M+H]^+$. FTIR (KBr, cm^{-1}): 1700 (C=O), 1230 (C-N). ^1H NMR (400 MHz, DMSO-d6) δ 13.06 (s, 1H), 8.85 (d, J = 6.9 Hz, 1H), 8.75 (d, J = 7.0 Hz, 1H), 8.69 (d, J = 7.9 Hz, 1H), 8.59 (d, J = 8.0 Hz, 1H), 8.45 (d, J = 8.4 Hz, 1H), 8.05 – 7.91 (m, 3H), 7.60 (t, J = 7.9 Hz, 1H). Element analysis for $C_{19}H_{10}N_2O_3$ (%): C 72.40, H 3.22, N 8.88, calculated C 72.61, H 3.18, N 8.91.

Benzimidazo[2,1-a]benz[de]isoquinoline-7-one-12-carbonyl chloride (2). Benzimidazo[2,1-a]benz[de]isoquinoline-7-one-12-carboxylic acid (2.00 g, 6.37 mmol) was suspended in 80 ml methylene dichloride and 4 drops of DMF was added, then 6 ml oxalyl chloride was added dropwise and the mixture was stirred overnight at room temperature. The suspension was filtered, and the filter cake was washed with small amount of methylene dichloride. The crude acyl chloride was obtained as yellow solid. Yield: 2.03 g, 95.9%. It was used for next reaction without further purification.

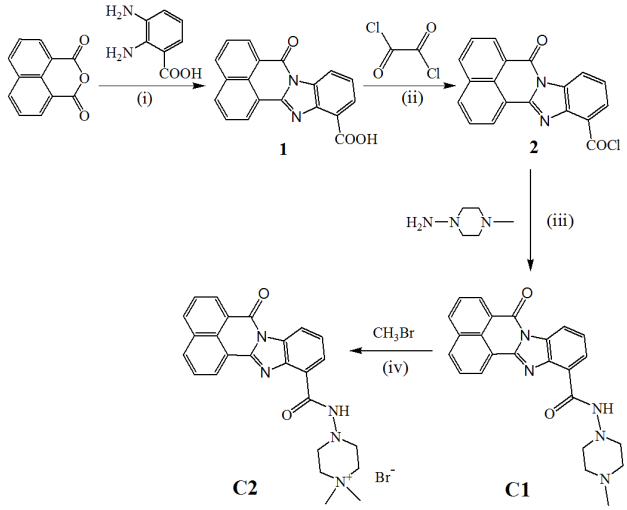
N-(4-methylpiperazin-1yl)benzimidazo[2,1a]benz[de] isoquinoline-7-one-12 carboxylamide (C1). **2** (1.00 g, 3.01 mmol) was suspended in 40 ml chloroform and then 4-methylpiperazin-1-amine (0.40 g, 3.48 mmol) was added dropwise. 5 ml triethylamine was added to the solution as acid scavenger. The mixture was stirred at room temperature for 30 min. Then the reaction mixture was washed with aqueous Na_2CO_3 (5%) (50 ml×3) and dried over anhydrous Na_2SO_4. Solvent was removed by evaporation, and the residue was washed with methanol thoroughly to give **C1**. Yield: 1.16 g, 93.5%. Ms (ESI): m/z = 412.23 $[M+H]^+$. FTIR (KBr, cm^{-1}): 1660 (C=O), 1230 (C-N). ^1H NMR (400 MHz, $CDCl_3$-d) δ 10.70 (s, 1H), 8.79 (d, J = 7.2 Hz, 1H), 8.74 (d, J = 7.2 Hz, 1H), 8.65 (d, J = 8.1 Hz, 1H), 8.35 (d, J = 7.7 Hz, 1H), 8.30 (d, J = 8.2 Hz, 1H), 8.18 (d, J = 8.1 Hz, 1H), 7.82 (d, J = 14.6, 7.8 Hz, 2H), 7.55 (t, J = 7.9 Hz, 1H), 3.26 (s, 4H), 2.82 (s, 4H), 2.46 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$-d): 162.508, 160.518, 149.261, 140.700, 135.799, 132.866, 132.336, 132.204, 131.785, 127.834, 127.418, 127.374, 127.223, 127.051, 125.457, 122.738, 119.561, 119.034, 55.810, 54.258, 45.757. Element analysis for $C_{19}H_{10}N_2O_3$ (%): C 70.01, H 5.13, N 17.05, calculated C 70.07, H 5.11, N 17.03.

4-[(benzimidazo[2,1-a]benz[de]isoquinoline-7-one-12-carbonyl)amino]-1,1-dimethylpiperzinium bromide (C2). **C1** (0.50 g, 1.22 mmol) was dissolved in 50 ml chloroform and then bromomethane (0.20 g, 2.13 mmol) was added. The reaction mixture was stirred at room temperature overnight. Yellow solid precipitated from the solvent. The suspension was filtrated and

the filter cake was washed with chloroform thoroughly, **C2** was obtained. Yield: 0.49 g, 80.1%. Ms (ESI): m/z = 426.2057 [M+H-Br$^-$]$^+$. FTIR (KBr, cm^{-1}): 1660 (C=O), 1230 (C-N). ^{1}H NMR (400 MHz, DMSO-d6) δ 10.70 (s, 1H), 8.99 (d, *J* = 7.2 Hz, 1H), 8.75 (d, *J* = 7.2 Hz, 1H), 8.67 – 8.56 (m, 2H), 8.48 (d, *J* = 8.0 Hz, 1H), 8.09 (d, *J* = 7.7 Hz, 1H), 8.00 (td, *J* = 7.8, 3.0 Hz, 2H), 7.62 (t, *J* = 7.9 Hz, 1H), 3.67 (d, *J* = 4.9 Hz, 3H), 3.50 (s, 4H), 3.29 (s, 6H). Element analysis for $C_{19}H_{10}N_2O_3$ (%): C 59.23, H 5.09, N 17.05, calculated C 59.29, H 5.11, N 17.03.

Results and discussion

Synthesis of C1 and C2



Scheme 1. Synthetic routes of **C1** and **C2**. Conditions: (i) CH_3COOH, at 118□ for 20 h; (ii) CH_2Cl_2, at room temperature overnight; (iii) $CHCl_3$, at room temperature for 30 min; (iv) $CHCl_3$, at room temperature overnight.

C1 and **C2** were synthesized in moderate yield according to the synthetic route shown in Scheme 1. 2,3-diaminobenzoic acid reacted with 1,8-Naphthalic anhydride in acetic acid to give the initial fluorophore **1** (benzimidazo[2,1-a]benz[de]isoquinoline-7-one-12-carboxylic acid). Reaction of fluorophore **1** and oxalyl chloride was carried out in methylene chloride to give compound **2** (benzimidazo[2,1-a]benz[de]isoquinoline-7-one-12-carbonyl chloride), which reacted with 4-methylpiperazin-1-amine to give **C1**. The quaternary ammonium salt **C2** was prepared by quaterisation of **C1** with bromomethane. The chemical structures of the synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR, FTIR and mass spectrum. Ideal ^{13}C NMR spectra of compound **1** and **C2** were failed to get because of their limited solubility and they were only characterized by ^{1}H NMR, FTIR, and mass spectrum. All of the data in the spectra were in good accordance with the structures.

The H$^+$ effect

The influences of H$^+$ upon the fluorescence intensity of **C1** and **C2** were performed in acetonitrile-H_2O (9: 1). For this purpose the sensors at the concentration of 10^{-5} M were titrated with different amount of HCl. The H$^+$ effect on the fluorescence of

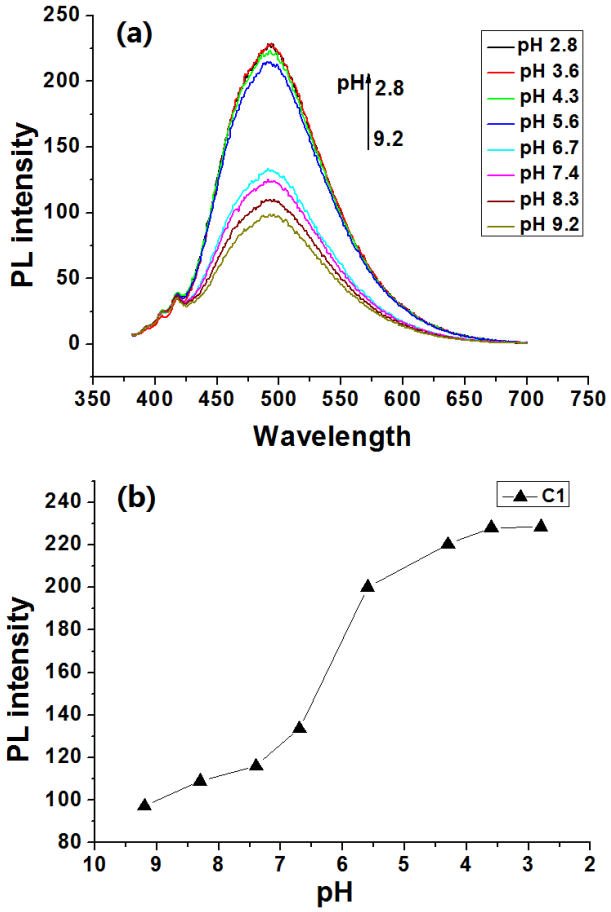


Figure 1. (a) Changes in the PL intensity of **C1** (1×10^{-5} M) in acetonitrile-H_2O (9:1) solution upon acidification. Excitation is at 370 nm. (b) Emission intensity of **C1** versus the different pH values. Emission wavelength is at 490 nm.

sensor **C1** (excited at 370 nm) was presented in Fig. 1. It was found that free sensor displayed very weak fluorescence (quantum yield: 0.008). The figure showed that, upon addition of aqueous HCl to **C1**, around 2.5-fold fluorescence enhancement centered at 490 nm was observed with the pH value changed from 9.2 to 2.8. In contrast to **C1**, the fluorescence of the quaternary ammonium salt **C2** showed no obvious change upon addition of aqueous HCl (Figure S1). It is interesting that the maximum fluorescence intensity of protonated **C1** at pH 2.8 is same as that of **C2** (Figure S2).

The different fluorescence enhancement of **C1** and **C2** toward H$^+$ was possibly due to the different substituent groups at C-12 position of the fluorophore. PET process in **C1** was directed from the receptor of 4-methylpiperazine group towards the fluorophore of benzimidazo[2,1-a]benz[de]isoquinoline-7-one, which led to a fluorescence quenching. Upon addition of aqueous HCl, the protonation took place in the terminal amino group in piperazine, so the PET process between fluorophore and receptor was partially inhibited which led to an enhancement of fluorescence of **C1**. The explanation was also supported by the fluorescent behavior of **C2**. **C2** was a quaternary ammonium salt with no lone pair electrons in the terminal amino group in piperazine ring. So the addition of HCl to **C2** showed no influence on its

fluorescent intensity, and the maximum fluorescent of **C2** was almost the same as that of protonated **C1** at pH 2.8.

The OH^- effect

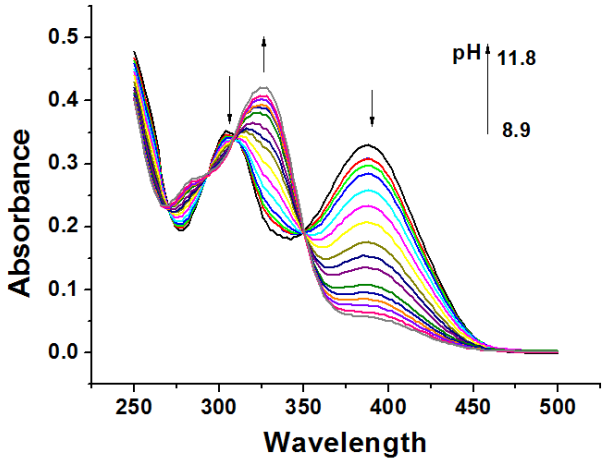


Figure 2. pH-dependence of the absorption spectra of sensor **C2** (1×10^{-5} M) in pure water. The arrow indicates the change of pH increases from 8.9 to 11.8 with the titration of NaOH.

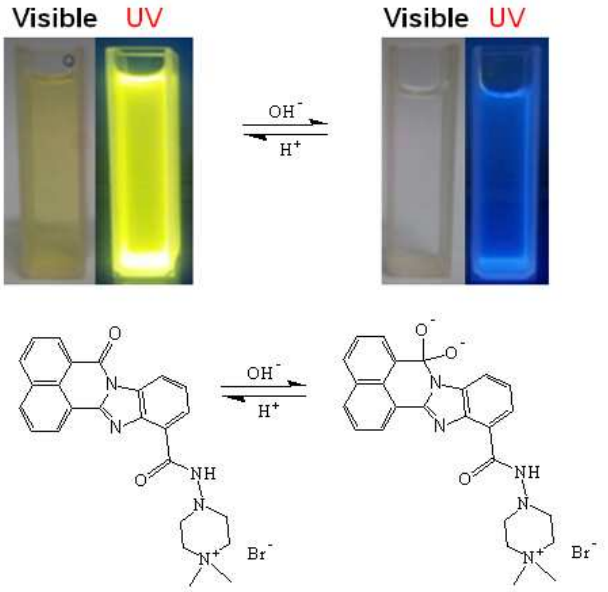


Figure 3. The mechanism of reversible transformation between sensor **C2** and mixture **C2**+OH^- and the color change under visible and UV light.

The influence of hydroxide on UV–vis absorption of **C2** was investigated in aqueous solution. The pH value was adjusted by addition of aqueous NaOH and the titration was carried out in the pH range 8.9-11.8. As shown in Fig. 2, **C2** showed two characteristic UV-vis absorbance bands centered at 304 and 388 nm. With the increase of pH value from 8.9 to 11.8, the intensity of absorption bands at 304 and 388 nm gradually decreased and a simultaneous new absorption band at 326 nm appeared, with concomitant formation of three well obvious isosbestic points (296 nm, 306 nm and 350 nm). Fluorescence emission spectra of **C2** at basic conditions were then measured with excitation wavelength of 370 nm (Fig. S3). Upon adding aqueous OH^- (pH from 8.9 to 11.8), the emission intensity of **C2** at 515 nm decreased clearly, while the emission density at 415 nm increased simultaneously. In agreement with above results, the solution color of **C2** changed gradually from yellow to colorless under visible light and changed from yellow to blue under UV light with addition of NaOH solution (Fig. 3). In contrast to the quaternary ammonium salt **C2,** sensor **C1** was unsoluble in water, and it showed no OH^- response in acetonitrile-water solution.

We assumed that a reversible chemical reaction between **C2** and hydroxide ions should account for such a phenomenon. Both the changes of absorption and fluorescence indicated that the original conjugated structure was transferred into a new chemical species. The color change might be originated from the hydration of the carbonyl group (C=O) of the fluorophore under basic conditions as show in Fig. 3, which was in consist with the similar research reported previously.[32]

The Cu^{2+} sensing

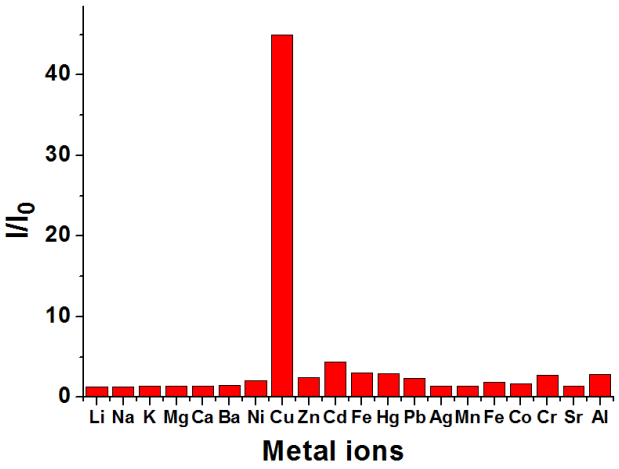


Figure 4. The relative PL intensity (I/I_0) of **C1** (1×10^{-5} M) in the presence of 20 equiv of Cu^{2+} (1×10^{-5} M) and 40 equiv of various metal ions (Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Fe^{2+}, Hg^{2+}, Pb^{2+}, Ag^+, Mn^{2+}, Fe^{3+}, Co^{2+}, Cr^{3+}, Sr^{3+} and Al^{3+}) in acetonitrile-H_2O (9:1) containing HEPES (5 mM, pH=7.4) at 25 °C, respectively. Excitation is at 370 nm, and emission is monitored at 494 nm.

The selectivity of sensors for Cu^{2+} was investigated firstly through fluorescence spectroscopy by adding various metal ions (Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Fe^{2+}, Hg^{2+}, Pb^{2+}, Ag^+, Mn^{2+}, Fe^{3+}, Co^{2+}, Cr^{3+}, Sr^{3+} and Al^{3+}) to **C1** and **C2**, respectively. After addition of 20 equiv of Cu^{2+} to **C1** and **C2** in acetonitrile-H_2O (9:1) buffer solution (pH=7.4) at 25 °C, significant fluorescence enhancement was induced. While after addition of various other metal ions to the solution of **C1** and **C2**, almost negligible enhancement of fluorescence intensity was induced (Fig. 4 and Fig. S4). To obtain an insight into the sensing properties of **C1** and **C2** toward Cu^{2+}, the fluorescent titration of Cu^{2+} in HEPES buffer solution was investigated. As shown in Fig. 5 and Fig. S5, upon the incremental addition of Cu^{2+} into **C1** and

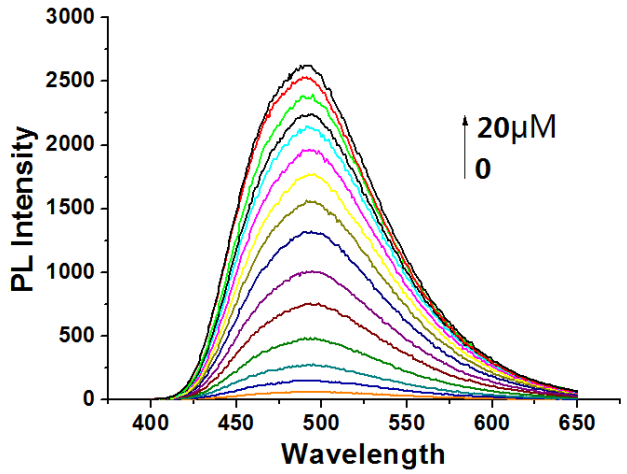
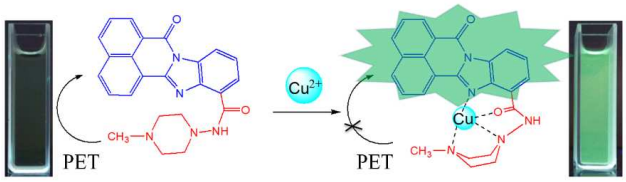


Figure 5. Changes in the PL intensity of **C1** (1×10^{-5}M) in acetonitrile-H_2O (9:1) containing HEPES (5mM, pH=7.4) upon titration with Cu^{2+} (1×10^{-5}M). Excitation is at 370 nm.



Scheme 2. The proposed binding mode of sensor **C1** with Cu^{2+}. Insert: the concomitant on-off color change under UV light. Excitation is at 370 nm.

C2, the fluorescence emission maximum at 490 nm gradually increased. The fluorescence enhancements of **C1** and **C2** toward Cu^{2+} were 45-fold and 18-fold respectively. Particularly, the fluorescence intensity of **C1** linearly increased as the concentration of Cu^{2+} changed from 3 μM to 10 μM and that of **C2** linearly increased as the concentration of Cu^{2+} changed from 2 μM to 8 μM (Fig. S6 and Fig. S7). By linearly fitting the changes of fluorescence as the function of concentration of Cu^{2+}, we obtained the slope as 3.7×10^5 and 8.8×10^4 for **C1** and **C2**, respectively. The detection limit (LOD) of **C1** for Cu^{2+} of 5.7×10^{-8} mol/L and that of **C2** of 3.4×10^{-7} were obtained based on LOD= 3σ/s, where σ is the standard deviation of blank measurements, and s is the slope between fluorescence intensity versus Cu^{2+} concentration.[33] Furthermore, a clear fluorescence enhancement by 55%-fold could be observed when the concentration of Cu^{2+} of solution **C1** reached 2.00×10^{-8} M. Correspondingly, with the titration of Cu^{2+}, the solution color of **C1** changed gradually from colorless to green under UV light as shown in Scheme 2. Consequently, sensor **C1** and **C2** could be applied as typical fluorescence sensors for Cu^{2+}.

In addition, the activity of **C1**, **C2** toward Cu^{2+} were also examined with absorption spectroscopy. The free sensors **C1** and **C2** displayed three similar absorption bands at 296, 306 and 390 nm in acetonitrile-H_2O (9:1) buffer solution (pH=7.4) at 25 °C (Fig. S8 and Fig. S9). With the addition of Cu^{2+} (from 0 to 20 μM) to **C1**, the absorption band at 306 nm decreased gradually, and a new absorption peak at 288 nm appeared with a pronounced isosbestic point at 300 nm. But for sensor **C2**, upon adding Cu^{2+} (from 0 to 20 μM), the absorption bands had no obvious change except a slightly increase of the absorption intensity centered at 296, 306 and 390 nm.

Compared with sensor **C2**, **C1** showed the obvious absorbance change and larger fluorescence enhancement with the titration of Cu^{2+}, this indicated that **C1** was more suitable as a Cu^{2+} fluorescence turn-on chemosensor in acetonitrile-H_2O (9:1) media.

For investigation of the fluorescent selectivity of **C1** towards Cu^{2+}, competition experiments were carried out in acetonitrile-H_2O (9:1) buffer solution. There was almost no obvious fluorescence change when **C1** was treated with 40 equiv. (40 μM) of other common metallic ions (Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Fe^{2+}, Hg^{2+}, Pb^{2+}, Ag^+, Mn^{2+}, Fe^{3+}, Co^{2+}, Cr^{3+}, Sr^{3+} and Al^{3+}) (Fig. S10). However, a strong fluorescence enhancement was observed with no shift of maximum emission when 5 equiv (50 μM) of Cu^{2+} was added to the above mixture. The results indicated that the selectivity and sensitivity of **C1** for Cu^{2+} was very remarkable.

Based on the results of fluorescence and absorbance titration, we proposed a plausible binding mode of sensor **C1** with Cu^{2+} as shown in scheme 2. Remarkable fluorescence enhancement (45-fold, 18-fold for **C1** and **C2,** respectively) induced only by Cu^{2+} verified that the nitrogen atoms in piperazine ring in sensor **C1** and **C2** played an indispensable role in Cu^{2+} binding.

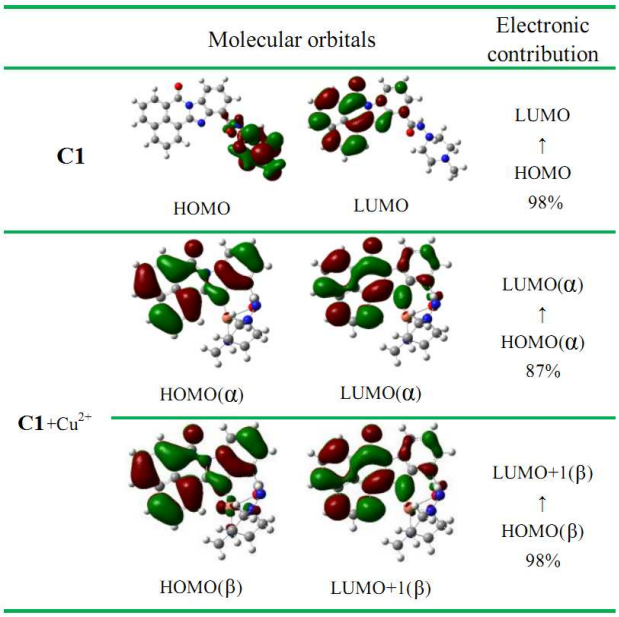


Figure 6. Molecular orbitals and electronic contributions of the relevant excitations for **C1** and **C1**+Cu^{2+}.

To verify the mechanism for the changes of fluorescence and the proposed interaction of Cu^{2+} with sensor **C1**, electronic properties of ground state and excited state of **C1** and **C1**+Cu^{2+} complex were studied with ab initiomolecular orbital calculation. The calculation was performed on TDDFT using a B3LYP/6-31G(d) basis set within the Gaussian 03 programs. From this calculation, it was noticed that the fluorescence enhancement by Cu^{2+} could be rationalized in terms of the occupancy of the frontier orbitals. The lowest singlet electronic transition for **C1**

was HOMO-LUMO transition and the lowest doublet electronic transitions for **C1**+Cu^{2+} complex were HOMO(α)-LUMO(α) and HOMO(β)-LUMO+1(β) (Table S1).

Fig. 6 showed the molecular orbital which were relevant to the excitations and the contributions of orbital transitions for **C1** and **C1**+Cu^{2+} complex. In **C1**, the electron densities of HOMO were only distributed over the receptor moiety, while those of LUMO were distributed over the fluorophore moiety. Upon excitation of the free probe, an electron would be transferred from the receptor to the fluorophore, resulting in the quenching of **C1**. Thus, a PET mechanism was demonstrated. For **C1**+Cu^{2+} complex, the orbital were localized on florophore for both HOMO(α) and LUMO(α), HOMO(β) and LUMO+1(β), so there was no electron transfer upon excitation and the fluorescence was enhanced comparing with that of free sensor **C1**, these were in full agreement with experimental observations.

Conclusions

In summary, two new fluorescent sensors **C1** and **C2** were designed and synthesized based on benzimidazo[2,1-a]benz[de]isoquinoline-7-one-12 carboxylic acid. The sensors **C1** and **C2** exhibited a PET mechanism caused by the Donor-Acceptor interaction between the fluorophore and receptors. The emission of **C1** and **C2** were very sensitive and selective toward Cu^{2+}. Dramatically, the solution colour of **C2** changed gradually from yellow to colorless under visible light and changed from yellow to blue under UV light with addition of NaOH solution. Such behaviors demonstrated excellent photophysical characteristics of the innovative sensor intermediate which could be easily modified depending on the substituent nature at carboxylic position. So, the intermediate might be competitive to many of the available large fluorescent markers in the field of sensors. Our future efforts will be focused on developing fluorescent chemosensors, which can function in aqueous systems and living cells with high affinities for Cu^{2+} and other metal cations. In addition, a lot of research work based on this subject is on the way in our lab, and will be reported soon.

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Notes and references

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‡ Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

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