This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this Accepted Manuscript with the edited and formatted Advance Article as soon as it is available.

You can find more information about Accepted Manuscripts in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal’s standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.
Metal Ion-assisted Ring-opening of Quinazoline-based Chemosensor: Detection of Copper(II) in Aqueous Media

Wei Cao, Xiang-Jun Zheng,* De-Cai Fang,* and Lin-Pei Jin

A quinazoline-based fluorescence chemosensor for highly selective recognition of Cu(II) in aqueous media was synthesized. The combination of experiments and theoretical calculations confirmed that Cu(II) ion assisted the ring-opening of the quinazoline derivative, forming a Cu(II) Schiff base during the detection.
Metal Ion-assisted Ring-opening of Quinazoline-based Chemosensor: Detection of Copper(II) in Aqueous Media

Wei Cao, Xiang-Jun Zheng,∗ De-Cai Fang,* and Lin-Pei Jin

Received Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

 DOI: 10.1039/b000000x

A quinazoline-based fluorescence chemosensor, 6-phenol-2-yl-(5,6-dihydrobenzimidazo[1,2-c])quinazoline (HL) for highly selective recognition of Cu(II) in aqueous media was synthesized. The detection limit was in the order of 10⁻⁶ M. The crystal structures of the Cu(II) and Cd(II) complexes showed that HL changed to a Schiff base when it reacts with metal salts, and that the metal ions coordinate with two nitrogen atoms and one hydroxyl oxygen atom from the Schiff base. The theoretical computations at B3LYP-SCRF/6-31G(d) confirmed that it is Cu(II) ion that assisted the ring-opening of the quinazoline derivative, forming a Cu(II) Schiff base complex during the detection. And LMCT leads to the disappearance of fluorescence. Cell imaging study indicated that HL could be used to detect the intracellular Cu²⁺ ion.

Introduction

Fluorescent chemosensor for biologically important metal ions has received considerable attention due to their high selectivity and sensitivity. Cu²⁺ plays an important role in many fundamental physiological processes, but excessive Cu²⁺ will lead to severe neurodegenerative disease. The U. S. Environmental Protection Agency (EPA) has set the limit of copper in drinking water to be 1.3 ppm (about 20 µM). Also, the average concentration of blood copper in the normal group is 100-150 µg/dL (15.7-23.6 µM). Therefore, the design and synthesis of chemosensors for detection of Cu²⁺ ion in aqueous media are very important. Quinazoline derivatives play an important role in the field of pesticides and pharmaceuticals due to their extensive biological and pharmaceutical activities. This suggests that quinazoline derivatives generally have a low hazard to environmental and organisms. The quinazoline derivatives synthesized by different aldehydes and 2-(2-aminoaryl)benzimidazole could be used as fluorescence chemosensors for metal ions, such as Pb²⁺, Hg²⁺, Cr³⁺, Zn²⁺, Fe²⁺, Fe³⁺, and Al³⁺, indicating that the quinazoline derivatives can act as excellent probes for detection of metal ions.

To increase the aqueous solubility and the coordination ability of the quinazoline derivative, we selected salicylaldehyde with a hydroxyl group to react with 2-(2-aminoaryl)benzimidazole to prepare the target compound, 6-phenol-2-yl-(5,6-dihydrobenzimidazo[1,2-c])quinazoline (HL).

Also, during the recognition process, it was reported that a solvent-assisted [1,5] sigmatropic-type shift of the secondary N-H proton from the quinazoline derivative probably occurred to result in a metal Schiff-base complex. Generally, the breakage of C-N bond needs the energy of 60-90 kcal/mol. It is really a challenge for solvent to break C-N bond of the quinazoline ring only with the assistance of solvent. The transformation mechanism from quinazoline derivatives to Schiff bases to form the metal complexes has not been solved yet. In this context, investigating the response mechanism of quinazoline derivatives as chemosensors has become particularly important. In this paper, the detection of Cu(II) by 6-phenol-2-yl-(5,6-dihydrobenzimidazo[1,2-c]) quinazoline (HL) was investigated. The response mechanism was discussed with a combination of experiments and theoretical calculations in detail. Also HL can be used to detect the Cu²⁺ ion in living cell by bioimaging.

Fig. 1 Fluorescence spectra of HL (10 µM) upon the addition of 1 equiv. metal ions in DMSO/H₂O (1:9, v/v, HEPES 20 mM, pH = 7.4) (λex = 352 nm).
From the fluorescence spectra (Fig.1), it can be seen that HL can emit blue light with an emission peak at 437 nm. There was no remarkable change of fluorescence intensity when Li$^+$, Na$^+$, K$^+$, Mg$^{2+}$, Ca$^{2+}$, Al$^{3+}$, Cr$^{3+}$, Mn$^{2+}$, Fe$^{3+}$, Ni$^{2+}$, Zn$^{2+}$, Cd$^{2+}$, Pb$^{2+}$ or Hg$^{2+}$ was added, respectively (Fig. 1 and Fig. S2a). But addition of Cu$^{2+}$ to HL quenched the fluorescence completely. Therefore HL was used to detect Cu$^{2+}$ in DMSO/H$_2$O (1:9, v/v, HEPES 20 mM, pH = 7.4) solution. The competition experiments indicated that other metal ions did not interfere with the selective recognition of HL toward Cu$^{2+}$ (Fig. S2b). HL could act as an efficient chemosensor for the detection of Cu$^{2+}$. From UV-vis absorption spectra (Fig. S2c) we can see that compared to the absorption of HL, there are no significant changes upon addition of metal ions except Cu$^{2+}$. HL shows two bands at ca. 300 nm and a broad band at 350 nm. Upon addition of Cu$^{2+}$ to HL, the band at 350 nm disappeared, two bands at ca. 300 nm became one broad band, and a new band emerged at 404 nm which could be attributed to the ligand-metal charge transfer (LMCT),$^8$ accompanied by the color change from colorless to yellow. With the increasing amount of Cu$^{2+}$, an isosbestic point could be observed at 388 nm, indicating a clear conversion of HL into the Cu(II) complex (Fig. S3).

Species formed in the solution

To identify the species formed in the response system, three methods were adopted to obtain the Cu$^{2+}$ complex. First, one-pot reaction of CuCl$_2$ with salicylaldehyde and 2-(2-aminophenyl)benzimidazole under solvothermal condition gave simple crystal of 1. Crystallographic data, and selected bond distances and angles are summarized in Tables 1 and 2, respectively. The result of single-crystal X-ray diffraction shows that it’s a Cu(II) Schiff base (L$^1$) complex. Cu$^{2+}$ ion is four- coordinated with hydroxyl oxygen atom, imine nitrogen and benzimidazole nitrogen atom from L$^1$ and one Cl$^-$ in a square geometry (Fig. 2a).

In addition, the mixed solution of CuCl$_2$ and HL gave red crystals either under solvothermal condition (2) or after slow evaporation at room temperature (3). Crystals of 2 and 3 were characterized by elemental analyses, IR, and powder X-ray diffraction. (Fig.2b). The results show that 2 and 3 are the same complex as 1, which implies that the C-N bond in HL was broken to become a benzimidazole-based Schiff base (HL$^-$) with more chelating sites to bind Cu$^{2+}$, resulting in the formation of complex 1.

Furthermore, it was observed that the absorption spectrum of the system HL + Cu$^{2+}$ was different from that of HL, but the same as that of the complex 1 (Fig. 2c), indicating the complex 1 was formed in the system. The Job’s plot indicated that HL binds Cu(II) to form a 1:1 Cu-L$^1$ complex (Fig.S4). To gain an insight into the stoichiometry of the Cu-L$^1$ complex, the electrospray ionization mass spectrum was recorded (Fig. S5). A peak appearing at m/z 375.02 was attributed to Cu-L$^1$ with a 1:1 binding mode (calculated [CuL$^1$]$^+$/m/z is 374.90), while the peak at m/z 453.03 can be ascribed to [CuL$^1$DMSO]$^+$ (calcd. 453.03). All the above results indicated that upon the addition of Cu$^{2+}$ to the solution of HL, HL changed to HL$^-$, and that the formation of 1 resulted in the fluorescence quenching of HL. Based on the fluorescence titrations, the detection limit for Cu$^{2+}$ was 7.1 µM and the association constant (log K$^a_2$) deduced for 1:1 stoichiometry was 4.7 (Fig. S6).$^7$ The detection limit was lower than the limit of copper in drinking water defined by U.S. EPA limit (~20 µM).$^7$

![Fig. 2](image1.png)

![Fig. 3](image2.png)

Considering copper has changeable valence in complexes, we selected a d$^{10}$ metal ion, Cd$^{2+}$, to investigate its corresponding complexes, the preparations of which are similar to those of complexes 1 and 2. Orange and yellow crystals were obtained and characterized by single-crystal X-ray diffraction to be [CdL$^1$(Ac)$_2$] (4) and [CdL$^1$(Ac)(CH$_3$OH)$_2$] (5), respectively. They...
have binuclear molecular structure (Fig. 3a, 3b). For Cd$^{2+}$ ion, similarly to Cu$^{2+}$, after one-pot reaction of tricomponent or direct reaction of Cd$^{2+}$ ion with HL, a Schiff base complex was formed. The Schiff base L$^1$ adopts a tetradentate coordination mode to link two Cd$^{2+}$ ions. Cd$^{2+}$ ions in 4 and 5 are both six-coordinated in a distorted octahedral geometry. The structures of 1 - 5 directly support the ring-opening of the quinazoline compound HL to become the Schiff-base in the Cu(II) and Cd(II) complexes. The results indicated that ring-opening of HL is assisted by binding of 10 HL with metal ions.

Ring-opening and fluorescence quenching of HL by Cu$^{2+}$

Fig. 4 The relative energy (kcal/mol) at B3LYP-SCRF/6-31G(d) for the stationary points along the reaction of HL+CuCl$_2$→[CuLCl]+HCl.

To explore the probable reaction of HL+CuCl$_2$→[CuLCl]+HCl, B3LYP-SCRF/6-31G(d) method has been employed to characterize the energies, structures and frequencies. The probable reaction pathway and relative energy is schematically described in Fig. 4, from which one can see that the formation of COM1 would release free energy of 29.7 kcal/mol. After that one of Cl atom in CuCl$_2$ would take hydrogen atom in OH group to form HCl molecule, and then the hydrogen atom in HCl molecule could be easily transferred into N atom in imidazole ring to form INT2 without any transition state, releasing the free energy of 9.3 kcal/mol. Breaking C-N bond is the rate-controlling step in this reaction, with free energy barrier being 21.8 kcal/mol, which is easily to overcome at experimental condition. As we know, the bond energy of C-N bond in HL has been calculated to be 69.3 kcal/mol, which is hardly to overcome without the assistance of CuCl$_2$. In INT2, TS2 and INT3, the Cl atom bonded to hydrogen atom holds the charge of ca. -0.85e, and thus it is in fact Cl, which is only loosely bonded to NH and probably could be free moved in solution. Therefore, the formation of INT3a is also a favorable process, releasing the free energy of 3.8 kcal/mol, and then the releasing of HCl molecule would further lower the free energy of 21.2 kcal/mol, indicating that this is an automatic process.

Fig. 5 The frontier orbits and their orbital energies for [CuLCl]$^+$.

Table 1. Crystal data and structure refinement parameters of complexes 1, 4 and 5.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Formula</th>
<th>Fw</th>
<th>Crystal system</th>
<th>Space group</th>
<th>a (Å)</th>
<th>b (Å)</th>
<th>c (Å)</th>
<th>α (°)</th>
<th>β (°)</th>
<th>γ (°)</th>
<th>F (Å$^{-1}$)</th>
<th>Z</th>
<th>calculated density (Mg/m$^3$)</th>
<th>F (000)</th>
<th>Reflections collected/unique</th>
<th>Goodness-of-fit on F$^2$</th>
<th>Final R indices [/2σ(I)]</th>
<th>R indices all data</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C$<em>{20}$H$</em>{15}$ClN$_4$O$_6$Cu</td>
<td>411.33</td>
<td>Triclinic</td>
<td>P-1</td>
<td>8.064(6)</td>
<td>10.188(8)</td>
<td>10.856(9)</td>
<td>90.52(1)</td>
<td>105.14(1)</td>
<td>94.42(1)</td>
<td>858.2(1)</td>
<td>2</td>
<td>1.592</td>
<td>418</td>
<td>5244 / 3812</td>
<td>1.123</td>
<td>R$_1$ = 0.0375, wR$_2$ = 0.0961</td>
<td>R$_1$ = 0.0508, wR$_2$ = 0.1182</td>
</tr>
<tr>
<td>4</td>
<td>C$<em>{20}$H$</em>{14}$N$_6$O$_6$Cd$_2$</td>
<td>967.59</td>
<td>Monoclinic</td>
<td>P2(1)c</td>
<td>10.445(7)</td>
<td>21.759(1)</td>
<td>8.695(6)</td>
<td>90</td>
<td>101.74(1)</td>
<td>90</td>
<td>1934.9(2)</td>
<td>2</td>
<td>1.661</td>
<td>968</td>
<td>11656 / 4449</td>
<td>1.036</td>
<td>R$_1$ = 0.0304, wR$_2$ = 0.0721</td>
<td>R$_1$ = 0.0433, wR$_2$ = 0.0961</td>
</tr>
<tr>
<td>5</td>
<td>C$<em>{20}$H$</em>{14}$N$_6$O$_6$Cd$_2$</td>
<td>1031.68</td>
<td>Monoclinic</td>
<td>P2(1)n</td>
<td>11.633(6)</td>
<td>14.607(7)</td>
<td>14.607(7)</td>
<td>90</td>
<td>90.26(1)</td>
<td>90</td>
<td>2097.9(2)</td>
<td>2</td>
<td>1.034</td>
<td>1040</td>
<td>13857 / 4798</td>
<td>0.1182</td>
<td>R$_1$ = 0.0520, wR$_2$ = 0.0558</td>
<td></td>
</tr>
</tbody>
</table>

$R_1 = \frac{\sum||F_o||-|F_c||}{\sum|F_o|}$; $wR_2 = \left(\frac{\sum(w|F_o|^2-|F_c|^2)^2/\sum(w|F_o|^2)}{n}\right)^{1/2}$.
In order to elucidate the fluorescence quenching of HL upon the addition of Cu$^{2+}$, the LMCT mechanism for the complex [CuL$_1$Cl] was calculated. The frontier orbitals and energy levels are depicted in Fig. 5, from which one can realize that HOMO is mainly on the phenyl ring of ligand (L$_1$) and LUMO is an empty d orbital, therefore, the overlap of HOMO and LUMO is almost zero, i.e., the excitation of HOMO to LUMO is not allowed. Although the overlap between HOMO and LUMO+1 is not very big, the excitation between HOMO and LUMO+1 seems possible. Once one electron is excited to LUMO+1, the electron could be transferred into LUMO orbital (d orbital of Cu) from LUMO+1(1 on L$^1$), and then the electron could not return to HOMO, leading to the disappearance of fluorescence.

### Cell Imaging Studies

![Cell Imaging Studies](image)

Fig. 6 Live-cell imaging of HeLa cells treated with HL before (A) and after (B) incubation with CuCl$_2$. (a) and (d) represent the bright-field images, (b) and (e) represent the fluorescence images, and (c) and (f) represent the overlay images ($\lambda_{ex} = 405$ nm).

Owing to the encouraging selectivity and sensitivity of HL toward Cu$^{2+}$ ion, bioimaging experiments were conducted to prove the ability of HL to detect Cu$^{2+}$ in living cells. HeLa cells were first incubated with 20 µM HL for 2 h, and then treated with 10 µM CuCl$_2$ for 15 min. As shown in Fig. 6, intensive fluorescence was observed when HeLa cells were exposed to HL, while the bright fluorescence can be quenched completely by further incubation of the cells with Cu$^{2+}$. These results demonstrated that HL is cell-permeable and can respond to copper(II) ions within living cells.

### Conclusions

In summary, a quinazoline derivative 6-phenol-2-y1-(5,6-dihydrobenzimidazo[1,2-c]quinazoline (HL) was synthesized and characterized. HL showed highly selective and sensitive fluorescence ‘on-off’ behaviour toward Cu$^{2+}$, and it can be used to detect the Cu$^{2+}$ ion in living cell by bioimaging. The fluorescence quenching of HL upon the addition of Cu$^{2+}$ ion was due to LMCT in I. The combination of the experimental observations and the theoretical calculations indicated that it is the binding of HL with metal ions that leads to the ring-opening of HL.

### Experimental Section

#### General Information and Materials

All solvents and reagents (analytical grade) were used as...
was replaced by Cd(Ac)$_2$.

Synthesis of [CdL($\text{Ac}$)]($\text{CH}_2\text{OH}$)$_2$ (5)

5 was synthesized as the complex 2 except that 0.1mmol CuCl$_2$·2H$_2$O was replaced by 0.05mmol Cd(Ac)$_2$·2H$_2$O. The yellow crystals suitable for X-ray crystallography were obtained (yield 40.2%). Anal. Calcld for C$_3$H$_7$_N$_2$O$_3$Cd (%): C, 53.55; H, 4.10; N, 8.15. Found: C, 53.67; H, 4.14; N, 8.08.

X-ray Crystallography

Single-crystal X-ray diffraction data of HL, 1, 4 and 5 were collected on a Bruker APEXI CCD diffractometer with graphite monochromated Mo-K$_\alpha$ radiation (\(\lambda = 0.71073\) Å) at 293 K. The structures were solved by the direct method and refined by full matrix least squares based on \(F^2\) using the SHELX 97 program.$^{[10]}$ All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were placed in calculated positions. CCDC nos. 1030199-1030201 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/data_request/cif.

Methods for Cell Imaging

HeLa cell line was cultured in DMEM (Dulbecco’s Modified Eagle Medium). Cells were incubated with 20 \(\mu\)M of HL at 37 \(^\circ\)C for 2 h. After washing with PBS three times to remove the remaining HL, the cells were then incubated with 7.5 \(\mu\)M CuCl$_2$·2H$_2$O for 15 min at room temperature. The incubated cells were washed with PBS and mounted onto a glass slide. The fluorescent images of the mounted cells were obtained using a confocal laser scanning microscope with 405 nm excitation.

Calculation methods

In this work, the quantum chemical calculations were carried out using the Gaussian 09 program package.$^{[11]}$ The possible ground state structures have been optimized with density functional theory (DFT) at B3LYP/6-31G(d) level, in which the effect of solvent has been considered using polarized continuum model (PCM)$^{[13]}$ with corresponding solvent, such as methanol, DMF and DMSO. On the basis of optimized configuration for the ground state, TD-DFT$^{[14]}$ calculations were performed using the B3LYP functional (TD-B3LYP-SCRF) within the adiabatic approximation to predict the excitation energies, which will provide the information of fluorescence properties of studied species.

Acknowledgment

This work is supported by the National Natural Science Foundation of China (20971015) and the Fundamental Research Funds for the Central Universities.

Notes and references
People’s Republic of China.

Job’s plot and ESIxMS of HL+Cu competition experiments of HL with Cu
stationary points located; ESIxMS, changes of absorption spectra of HL
ions; fluorescence titration. See

DOI: 10.1039/b000000x/


