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A New Quinoline-Based Acylhydrazone for Highly Selective Fluorescence Recognition of Cu(II) and Sulfide in Aqueous Solution

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A new quinoline-based acylhydrazone (**1**) has been synthesized and applied as a fluorescent probe. Probe **1** exhibits high selectivity and sensitivity to Cu^{2+} with fluorescence "ON-OFF" behavior in HEPES buffered (1‰ DMSO, HEPES 20 mM, pH = 7.4) solution. The on-site generated **1**-Cu^{2+} complex displays excellent selectivity to sulfide ions with fluorescence "OFF-ON" performance through copper displacement approach.

Key Words : Copper detection, Sulfide recognition, Copper displacement, Acylhydrazone

Introduction

As the third-most abundant transition metal in human body, copper(II) ion plays critical roles in the fundamental physiological processes of organisms ranging from bacteria to mammals.[1] Whereas, overloading of copper in the neuronal cytoplasm can lead to Wilson's disease, Alzheimer's disease and Menke's disease.[2] In recent years, the development of artificial probes for Cu^{2+} has received immense attention.[3] A Cu^{2+} bound fluorophore usually exhibits fluorescence quenching due to the paramagnetic nature of Cu^{2+}, and this issue is always regarded as a drawback in fluorescent Cu^{2+} sensing processes. From another viewpoint, if the fluorescence inactive Cu^{2+}-fluorophore complex could act as a highly selecitve anion sensor through Cu^{2+} dispalcement approach, the resulted fluorescence revival of the released fluorophore can provide an indirect approach for fluorescence "turn-on" anion recognition.[4]

Sulfide is widely spread in the environment and has many applicable utilities including the areas of manufacture of sulfur and sulfuric acid, dyes and cosmetics.[5] However, exposure to high level of sulfide can lead to various physiological and biochemical problems, for instance, irritation in mucous membranes, unconsciousness, and respiratory paralysis.[5,6] In recent years, sulfide anion detection has received tremendous interest[7] and a variety of detection strategies have been developed for sulfide anions, such as spectroscopy, electrochemical methods, titration, ion chromatography, and chemiluminescence methods, *etc*.[8] Among the employed methods, sulfide sensing by fluorescence spectrometry is an increasingly popular method due to its high sensitivity and easy operability.

Although a great number of S^{2-} ion selective fluorescent probes have been developed,[9] fluorescent sensing of sulfide anions in water solution still remains a challenging task. The strong hydration nature of anions weakens the interaction of probes with the target anions.[10] An effective method to overcome this issue is the displacement (ensemble) approach,[11] and several sulfide selective probes based on Cu^{2+} displacement approach have been documented.[9c-h,12] However, most of them were applied in organic/water mix-solvent, sulfide selective probes that could be operated in water solution are still rare.[9c,9g,9h,12a] For potential applicability in environmental and biological areas, the development of simple and effective sulfide selective fluorescent probes that can be applied in near 100% water solution is still highly desirable.

Herein we report the preparation and ion recognition properties of a new quinoline-based acylhydrazone (**1**), which exhibits highly selective sequential recognition of Cu^{2+} and sulfide anion in HEPES buffer (1‰ DMSO, HEPES 20 mM, pH = 7.4) solution.

Experimental

Apparatus and Reagents. Unless otherwise stated, all the solvents and reagents were of analytical grade from commercial sources and used as received. Column chromatography was performed on silica gel (200-300 mesh). 8-Methoxyquinoline-2-carbaldehyde (**2**) was prepared according to literature method.[13] NMR spectra were recorded on Agilent 400-MR spectrometer. High resolution mass spectrum (HRMS) was measured on an Agilent 1200 time-of-flight mass spectrometer (Bruker, micrOTOF-Q). Single crystal X-ray diffraction data was collected on a Bruker Smart Apex-II CCD area detector. UV-vis absorption spectra were measured on a SP-1900 spectrophotometer (Shanghai, China). Fluorescence measurements were performed on a Sanco 970 CRT spectrofluorometer (Shanghai, China). pH measurements were made with a Model PHS-25B meter.

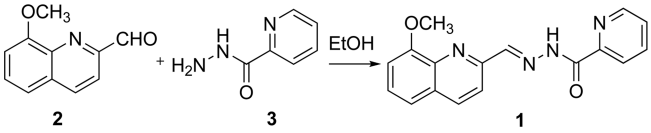
Synthesis of Sensor 1. Compound **2** (200 mg, 1.156 mmol) and picolinohydrazide (**3**) (190 mg, 1.387 mmol) were dissolved in 25 mL of absolute ethanol, the solution was heated to reflux and stirred for 1 h. After cooled to room temperature, the pale yellow precipitates formed was collected by filtration, which was washed with ethanol and dried under

vacuum to give **1** in 74% yield. mp 182.5-183 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.69 (s, 1H), 8.77 (s, 1H), 8.72 (d, 1H, J = 4.4 Hz), 8.33 (d, 1H, J = 4.8 Hz), 8.13 (t, 2H, J = 9.2 Hz), 8.05 (t, 1H, J = 7.2 Hz), 7.67 (dd, 1H, J_1 = 6.8 Hz, J_2 = 5.2 H), 7.54-7.48 (m, 2H), 7.20 (d, 1H, J = 6.8 Hz), 3.96 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.35, 155.58, 152.93, 149.78, 149.52, 149.02, 139.51, 138.55, 136.93, 129.40, 128.26, 127.67, 123.45, 119.82, 118.37, 109.36, 56.02. HRMS (ESI+) cacld for $C_{17}H_{14}N_4O_2Na$: 329.1014, found: *m/z* 329.1017 $[M+Na]^+$.

Results and Discussion

Probe **1** was synthesized in good yield by condensation of compounds **2** and **3** in refluxed ethanol (Scheme 1). The structure of **1** was characterized by ^{1}H NMR, ^{13}C NMR and HRMS data. Our preliminary experiments demonstrate that probe **1** is free soluble in HEPES buffered (1‰ DMSO, HEPES 20 mM, pH = 7.4) solution with a strong emission peak at 523 nm. The pH effect on fluorescence of **1** solution was examined and the results showed that **1** displays strong fluorescence from pH 5 to 8 (Fig. S1, Supporting Information). Thus, the 1‰ DMSO HEPES buffer at pH 7.4 was selected as the working moiety in the following study.

Optical Recognition of Cu^{2+}. The optical response of **1** to different metal ions was then evaluated. As shown in Figure 1, addition of metal ions such as Ni^{2+}, Hg^{2+}, Ba^{2+}, Mg^{2+}, Fe^{2+}, K^+, Al^{3+}, Mn^{2+}, Na^+, Sr^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+}, Cr^{2+}, Fe^{2+}, and Ag^+ (1 equiv. of each) could not signicantly alter the initial fluorescence spectrum of **1** solution (1 μM), addition of Pb^{2+} only induced a slight quenching effect. Whereas,



Scheme 1. Synthesis of probe **1**.

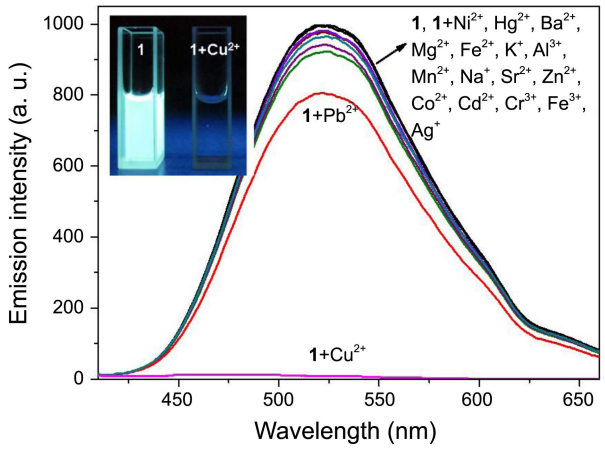


Figure 1. Fluorescent intensity of solution **1** (1 μM) in HEPES buffer (1‰ DMSO, HEPES 20 mM, pH = 7.4) in the presence of different metal ions (1 equiv. of each, λ_{ex} = 340 nm). Inset: visible emission change of **1** (10 μM) before and after Cu^{2+} addition under 365 nm with portable UV-lamp.

upon addition of 1 equiv. of Cu^{2+} ion, an almost complete fluorescence quenching (quenching eciency $(I_0\text{-}I)/I_0 \times 100\%$ = 99.6%) was observed, which indicates that probe **1** exhibits a specific response to Cu^{2+} ion. This fluorescence quench may attribute to the chelation-enhanced fluorescence quenching (CHEQ) effect.[14]

To obtain insight into the sensing property of **1** to Cu^{2+}, fluorescence titration experiments were subsequently carried out. Upon incremental addition of Cu^{2+} ions (0-1 μM) to **1** solution, the fluorescence intensity was gradually decreased and reached saturation when 1.0 equiv. of Cu^{2+} was employed (Fig. 2). In addition, the UV-vis absorption spectra studies also manifest the selective response of **1** to Cu^{2+}. Probe **1** solution (20 μM) exhibited absorption bands at 290 and 340 nm, respectively. Amongst the tested metal ions, only the addition of Cu^{2+} could induce dramatic absorption spectrum changes, which caused absorption intensity decrease at 290 nm and 340 nm, concomitantly, a new absorption band at 395 nm was developed (Fig. S2, SI). On incremental addition of Cu^{2+} ions, the absorption bands at 290 nm and 340 nm gradually decreased, and the newly formed absorption band at 395 nm gradually increased accordingly (Fig. S3, SI).

According to the Benesi-Hildebrand expression,[15] the measured $1/(I\text{-}I_0)$ at 523 nm varied as a function of $1/[Cu^{2+}]$ with a nice linear relationship (R = 0.9936) (Fig. S4, SI), indicative of the 1:1 stoichiometry between **1** and Cu^{2+} ion, which is in good agreement with the result of Job plot (Fig. S5, SI). The association constant for **1** with Cu^{2+} was evaluated to be 7.64×10^5 M^{-1}. Plotting of $(I_{min}\text{-}I)/(I_{min}\text{-}I_{max})$ versus $\log[Cu^{2+}]$ afforded a nice linear relationship (R = 0.9963), and the detection limit of **1** for Cu^{2+} ions was estimated to be 0.3 μM (Fig. S6, SI).[16] These results reveal that sensor **1** is sensitive enough to monitor the Cu^{2+} concentration in real water samples.

To get further insight into the high selectivity of **1** to Cu^{2+}, fluorescence competition experiments were also conducted (Fig. 3). The results indicate that coexistence of metal ions such as Ni^{2+}, Hg^{2+}, Ba^{2+}, Mg^{2+}, Fe^{2+}, K^+, Al^{3+}, Mn^{2+}, Na^+,

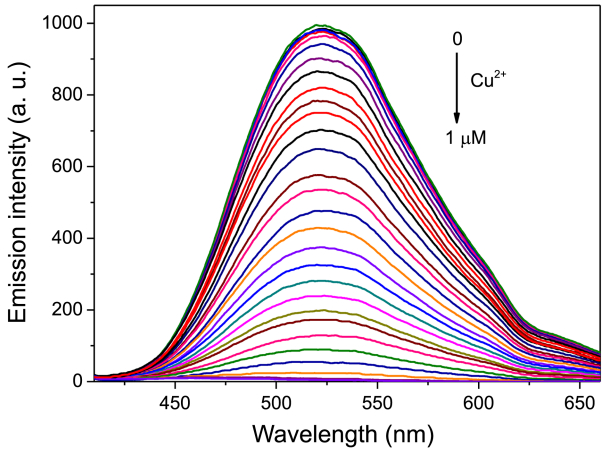


Figure 2. Fluorescence emission spectra of **1** solution (1 μM) in HEPES buffer (1‰ DMSO, HEPES 20 mM, pH = 7.4) upon addition of Cu^{2+} (0 to 1 equiv.).

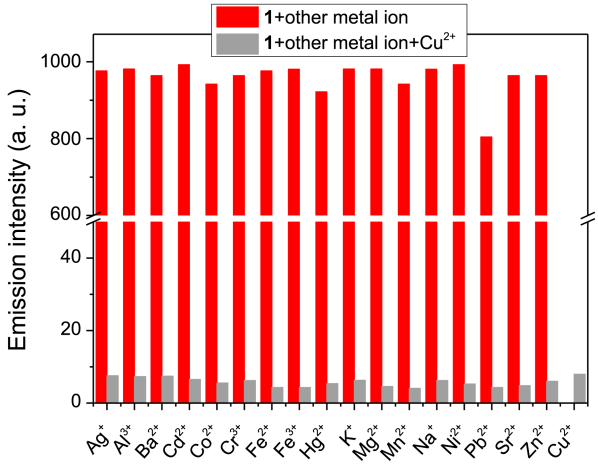


Figure 3. Changes in fluorescence intensity of **1** solution (1 μM) at 523 nm to various metal ions. The red bars represent the fluorescence intensity of **1** in the presence of 1 equiv. of miscellaneous metal ions; the gray bars represent the fluorescence intensity of the above solution upon further addition of 1 equiv. of Cu^{2+}.

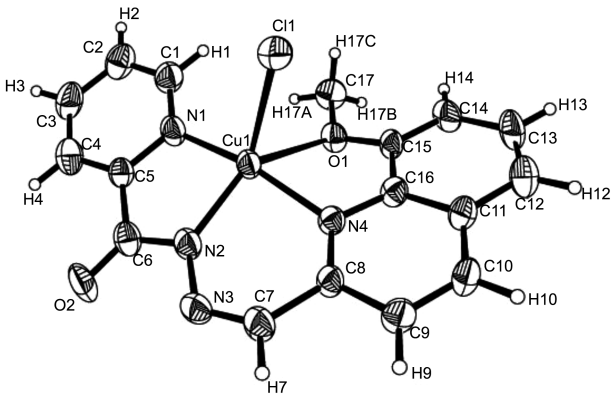


Figure 4. X-ray crystal structure of **1**-Cu^{2+}.

Sr^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+}, Cr^{2+}, Fe^{2+}, Ag^{+}, Pb^{2+}, did not behave significant interference on Cu^{2+} recognition.

The binding mode of **1** with Cu^{2+} was explicitly disclosed by X-ray diffraction analysis of **1**-Cu^{2+} single crystal structure (Fig. 4). The single crystal of **1**-Cu^{2+} suitable for crystallographic analysis was obtained by slow evaporation of DMSO-MeOH (1:10, v/v) solution of **1** in the presence of Cu^{2+}. As depicted in Figure 4, **1** and Cu^{2+} form a complex with 1:1 stoichiometry. The nitrogen atoms in pyridine, quinoline and amide groups as well as the methoxyl oxygen atom are all participated in coordination with Cu^{2+}. In addition, a chloride is also bond to Cu^{2+}. It is noteworthy that the hydrogen atom on amide N is removed by Cu^{2+} interaction.

Optical Recognition of S^{2-} by 1-Cu^{2+} Complex. In view of the high affinity of sulfide to Cu^{2+} ion,[17] the fluorescence quenched on-site formed **1**-Cu^{2+} complex was considered as a promising ensemble for S^{2-} detection. Thus, the selectivity of **1**-Cu^{2+} for a variety of anions was evaluated to assess the value of **1**-Cu^{2+} as a sensor. A remarkable fluorescence enhancement (Fig. 5) was observed when 500 equiv. of S^{2-} ions was added to **1**-Cu^{2+} solution (1 μM). However,

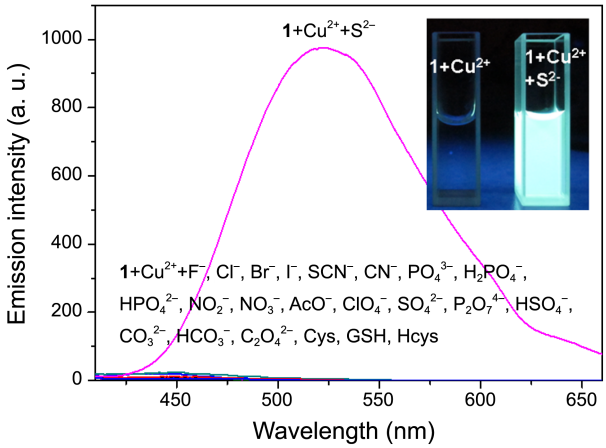


Figure 5. Changes in absorbance of receptor **1**-Cu^{2+} (1 μM) upon addition of various anions (500 μM of each) in HEPES buffer (1‰ DMSO, HEPES 20 mM, pH = 7.4). Inset: visible emission change of **1**-Cu^{2+} (10 μM) before and after S^{2-} addition under 365 nm with portable UV-lamp.

addition of other anions such as F^{-}, Cl^{-}, Br^{-}, I^{-}, SCN^{-}, CN^{-}, PO_4^{3-}, H_2PO^{4-}, HPO_4^{2-}, NO_2^{-}, NO_3^{-}, AcO^{-}, ClO_4^{-}, SO_4^{2-}, $P_2O_7^{4-}$, HSO_4^{-}, CO_3^{2-}, HCO_3^{-}, $C_2O_4^{2-}$, as well as some thiol containing amino acids including L-cysteine (Cys), reduced glutathione (GSH) and L-homocysteine (Hcys) did not induce any noticeable fluorescence enhancement. These results demonstrate that **1**-Cu^{2+} complex has an excellent selectivity to S^{2-}.

To further understand the sensing behavior of **1**-Cu^{2+} to S^{2-} anion, we conducted the S^{2-} titration experiment. Upon gradual addition of S^{2-}, the fluorescence intensity of **1**-Cu^{2+} solution increased gradually and almost reverted to the original emission state of free **1** when 500 equiv. of S^{2-} was added (Fig. 6). The Uv-vis absorption spectrum changes of **1**-Cu^{2+} solution (20 μM) on addition of different anions also support the high selectivity of **1**-Cu^{2+} to S^{2-} (Fig. S7, SI). Addition of increasing concentrations of S^{2-} ions to **1**-Cu^{2+} solution led to a gradual spectrum modification, which led to the reappearance of the initial absorption shape of free **1**

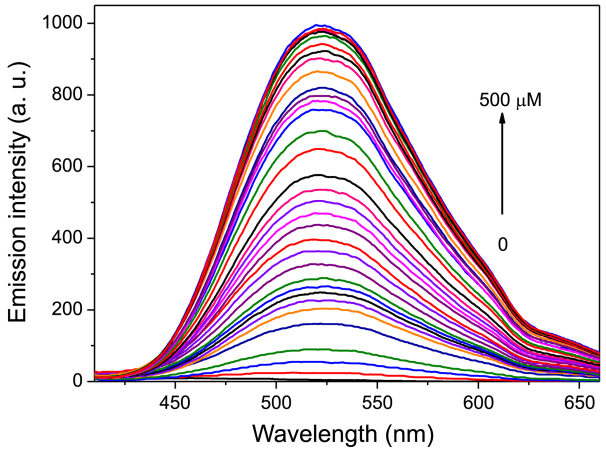


Figure 6. Fluorescence spectra changes of **1**-Cu^{2+} (1 μM) upon gradual increase in S^{2-} concentration (0-500 μM).

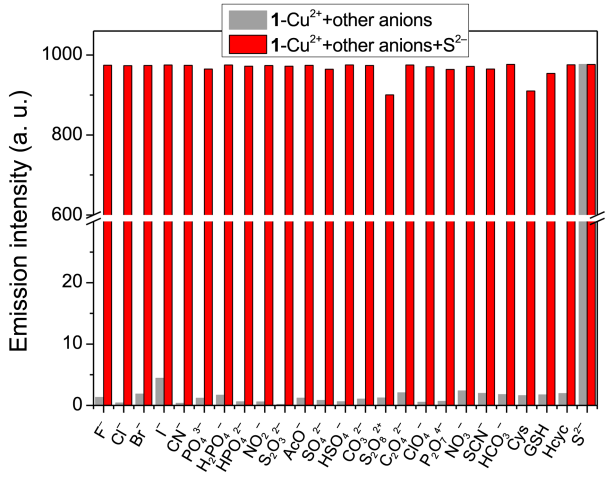


Figure 7. Changes in fluorescence intensity of **1**-Cu^{2+} solution (1 μM) at 523 nm to various metal ions. The gray bars represent the fluorescence intensity of **1**-Cu^{2+} in the presence of 500 equiv. of miscellaneous anions; the red bars represent the fluorescence intensity of the above solution upon further addition of 500 equiv. of S^{2-}.

(Fig. S8, SI). The revival of fluorescence and absorption spectra of **1**-Cu^{2+} solution to the original states of free **1** strongly support the S^{2-} induced Cu^{2+} displacement process.[18] Based on the fluorescence titration profile, the detection limit of **1**-Cu^{2+} to S^{2-} was calculated to be 2.82×10^{-5} M (Fig. S9, SI).

To evaluate the anti-interference ability of **1**-Cu^{2+} for S^{2-} recognition, the competition experiments were then performed. As shown in Figure 7, the initial fluorescence intensities of **1**-Cu^{2+} did not change significantly (gray bars) upon mixing with 500 equiv. of alternative anions (F^-, Cl^-, Br^-, I^-, SCN^-, CN^-, PO_4^{3-}, $H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}, $P_2O_7^{4-}$, NO_2^-, NO_3^-, AcO^-, ClO_4^-, SO_4^{2-}, HSO_4^-, CO_3^{2-}, HCO_3^-, $C_2O_4^{2-}$) and the thiol-containing amino acids of Cys, GSH, and Hcys. The subsequent addition of 500 equiv. of S^{2-} elicited a dramatic fluorescence enhancement (red bars), which further confirmed the excellent selectivity of sensors **1**-Cu^{2+} to S^{2-} ions.

Conclusions

In summary, a new quinoline-based acylhydrazone probe (**1**) was designed and synthesized. Probe **1** displays highly selective sequential recognition of Cu^{2+} and S^{2-} in HEPES buffered (1‰ DMSO, HEPES 20 mM, pH = 7.4) solution through fluorescence and Uv-vis dual mode responses. Probe **1** interacts with Cu^{2+} through 1:1 stoichiometry, and the in situ generated **1**-Cu^{2+} complex displays excellent selectivity toward sulfide ions with fluorescence "off-on" behavior via copper displacement approach. This highly selective Cu^{2+} and S^{2-} sequential recognition property make **1** a promising sensor for Cu^{2+} and S^{2-} detection in aqueous samples.

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Supporting Information. Figures S1 to S9.

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