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A new fluorescent probe based on imidazole[2,1-*b*]benzothiazole for sensitive and selective detection of Cu^{2+}

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Abstract: A simple fluorescent chemosensor **WS1** (*N'*-[(*Z*)-1*H*-indazol-3-ylmethylidene]imidazo[2,1-*b*][1,3]benzothiazole-2 -carbohydrazide) based on imidazole[2,1-*b*]benzothiazole was designed and synthesized. The probe showed specific recognition towards Cu²⁺ through colorimetric and "turn-off" fluorescence response in MeOH/H₂O (9:1 V/V) buffer solution (10 mM Tris, pH = 7.4) with detection limit of 4.32×10^{-8} M. In addition, WS1 was a reversible fluorescence probe for the detection of Cu²⁺, the fluorescence intensity recovered to the similar state of free WS1 as PPi was added. And it showed a favorable potency for actual water monitoring. The proposed binding mode of **WS1** with Cu^{2+} was verified by DFT calculation using Gaussian 09.

1. Instruction

Recently, the research of fluorescent probes have been made remarkable progress in fields of physiological and environmental monitoring because of their specific recognition for target analytes [1-4]. Especially, fluorescence probes for transition metal ions have attracted considerable attention due to their crucial applications in chemical, biological and environmental processes [5-9]. Among the essential heavy metals, copper is third most abundance element in human bodies and plays a crucial role in various fundamental physiologic processes [10, 11]. It was reported that suitable concentration of copper in human body can keep the normal physiological movement operating, whereas, disorders of copper metabolism could lead to an increasing risk of various diseases [12, 13]. Copper deficiency is likely to result in anemia-like symptoms as well as hypopigmentation, irregularity in cholesterol metabolisms and skeletal deformity, and excessive amounts of copper in the body could lead to increase morbidity rate of various neurodegenerative diseases, such as Alzheimer's and Wilson's disease [14-18]. Also, copper have wide applications in lots of industrial fields such as electronics manufacturing, petroleum industry, printing, medical equipment [19-22].

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Numerous fluorescence probes for the detection of copper ion were reported based on quinolone, coumarin, BODIPY, naphthoamide and Rhodamine in last decade [23-27], but there was no report using imidazo[2,1-*b*][1,3]benzothiazole derivative copper sensitive as chemsensor. Imidazo[2,1-*b*]benzothiazole often used was as pharmaceutical intermediate and play a significant role in drug discovery [28-30]. In fact, the framework of imidazole[2,1-b]benzothiazole has structural potential to be a fluorescent group because of its molecular delocalization planarity and electrons [31,32]. Hence, imidazo[2,1-b][1,3]benzothiazole is actual a potential platform for the development of optical sensor for metal ion recognition. So, it is of great meaning in scientific and applicable aspect if it is possible to develop new fluorescence probes for detection of copper with satisfactory sensitivity and selectively with imidazo[2,1-*b*][1,3]benzothiazole as fluorophore.

Accordingly, in this report, a new Schiff base **WS1** was designed and synthesized based on imidazo[2,1-b][1,3]benzothiazole-2-carbohydrazide and 1H-indazole-3-carbaldehyde. The molecule of **WS1** possessed a large electron delocalization might lead to a sufficient fluorescence, meanwhile, the "N", "O" atoms of the molecule could act as binding sites for recognizing metal ions [33, 34]. Just as predicted, **WS1** showed ideal colorimetry and fluorescent response towards copper ion in MeOH/H₂O

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(9:1 V/V) buffer solution (10 mM Tris, pH = 7.4).

2. Experimental

2.1 Materials and reagents

All reagents and organic solvents were purchased from commercial suppliers and used without further purification. Metal ions used were stored in the salt solutions of NaCl, Li₂SO₄, KCl, MgCl₂·6H₂O, CaCl₂, ZnCl₂, CdCl₂·2.5H₂O, HgCl₂, AgNO₃, CuCl₂·2H₂O, CoCl₂·6H₂O, NiCl₂·6H₂O, MnCl₂·4H₂O, CrCl₃·6H₂O, AlCl₃·6H₂O, FeCl₃, FeSO₄. The stock solutions of aforementioned were prepared in distilled water.

2.2 Experimental apparatus

¹H MNR spectra were recorded on a Bruker AV III 400MHz NMR spectrometer and ¹³C MNR spectra data were obtained by a Bruker AV III 100MHz NMR spectrometer with TMS as an internal standard and DMSO- d_6 as solvent. Fluorescence measurements were carried out using a HITACHI Instruments F-4600 Fluorescence spectrophotometer (EX WL: 385nm, EX slit: 10, EM slit: 10). UV-vis absorption spectra data were taken on a SHimmadzu 3100 spectrometer. Infrared spectra were determined on a Bruker Vertex 70 FT-IR spectrometer with KBr pellets. Mass spectrum was performed under ESI mode on a Thermo Fisher Scientific Exactive Plus mass spectrometer. All pH measurements were made with a pH-10C digital pH meter. All reactions were monitored with

thin layer chromatography (TLC).

2.3 Experimental details for spectral studies

All tests described in this work were performed at room temperature $(25^{\circ}C)$. **WS1** was dissolved in MeOH/H₂O (9:1 V/V) buffer solution (10 mM Tris, pH = 7.4) to afford the test solution $(1 \times 10^{-5} \text{ M})$. All the cations solutions were prepared from NaCl, Li₂SO₄, KCl, MgCl₂, CaCl₂, ZnCl₂, CdCl₂, HgCl₂, AgNO₃, CuCl₂, CoCl₂, NiCl₂, MnCl₂, CrCl₃, AlCl₃, FeCl₃ and FeSO₄ in distilled water with a concentration of 3×10^{-2} M, respectively. The sensing for tap water sample was showed in MeOH/tap water (9:1 V/V) solution. The Cu²⁺ solution were prepared from dissolved CuCl₂·H₂O in tap water with a concentration of 3×10^{-2} M.

2.4 Theoretical calculation

DFT (density functional theory) structural was optimized with the Gaussian 09 program. In all instances, the structures were optimized using the B3LYP functional and the mixed basis set 6-31G/(d). Each structure was subsequently subjected to TDDFT (time dependent density functional theory) calculation using the B3LYP functional. 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 Gauss View 5.0 program.

2.5. Calculation of quantum yield

The quantum yield was determined according to the following equation

[26b]:

$$\Phi_u = \Phi_s \frac{F_u A_s n_u^2}{F_s A_u n_s^2}$$

 Φ , *F*, *A*, *n* represents the quantum yield, the integrated area under the corrected emission spectra, the absorbance intensity at the excitation wavelength and the refractive index of solvent, respectively. In addition, *s* refers to Rhodamine B as standard one, and *u* refers to the target one. The quantum yield (Φ) of Rhodamine B dissolved in anhydrous ethanol is 0.97.

2.6. Calculation of the association constant

The association constant of complex **WS1** with Cu^{2+} was calculated by the Benesi–Hildebrand equation [35]:

$$\frac{1}{F - F_0} = \frac{1}{K_a (F_{min} - F_0) [Cu^{2+}]} + \frac{1}{F_{min} - F_0}$$

F is the fluorescence intensity of the solution of **WS1** + Cu^{2+} complex in accordance with the concentration of Cu^{2+} at 465 nm. *F*₀ is the fluorescence intensity of free **WS1**. *F_{min}* is the fluorescence intensity of **WS1** + Cu^{2+} complex in the presence of the maximum concentration of Cu^{2+} .

ethyl-imidazo[2,1-b][1,3]benzothiazole-2-carboxylate (1): 1 was

synthesized following previously reported method [36].

Synthesis of imidazo[2,1-*b*][1,3]benzothiazole-2-carbohydrazide(2): 3 ml 80% hydrazine hydrate was added dropwise to 15 ml ethanol solution of 142 mg (0.58 mmol) compound **1** and the mixture was stirred for 6 hours at room temperature. Then the precipitate was filtered via vacuum filtration and the filter cake was washed with ethanol (3 × 5 ml) to obtain 109 mg (0.47 mmol) compound **2** as a white solid. Yield: 82%. ESI-MS (Theoretical molecular weight: 232.04): m/z = 233.0456 [M + H⁺]⁺. FTIR (KBr, cm⁻¹): v = 1369 (C–N), 1654 (C=O), 3112 (N–H), 3423 (N–H). ¹H NMR (400 MHz, DMSO-*d*₆, ppm): δ = 9.42 (s, 1H, –CH–), 8.85 (s, 1H, –NH–), 8.14 (d, *J* = 7.9 Hz, 1H, –CH–), 8.05 (d, *J* = 7.9 Hz, 1H, –CH–), 7.57 (t, *J* = 7.4 Hz, 1H, –CH–), 7.47 (t, *J* = 7.7 Hz, 1H, –CH–), 4.45 (s, 2H, –NH, –NH). ¹³C NMR (100 MHz, DMSO-*d*₆, ppm): δ = 161.34, 146.84, 141.33, 132.00, 129.90, 127.25, 126.27, 125.53, 115.68, 114.55.

Synthesis

of

N'-[(Z)-1H-indazol-3-ylmethylidene]imidazo[2,1-b][1,3]benzothiazole -2-carbohydrazide (WS1): 95 mg (0.65 mmol) 1H-indazole-3-carbaldehyde was added to 15 ml ethanol suspension of 109mg (0.47 mmol) compound 2 and the mixture was stirred for 12 hours at room temperature. After the reaction was completed which was monitored with TLC, the mixture was filtered via vacuum filtration and the filter cake was washed with ethanol (3 × 5 ml) to obtain 101 mg (0.28 mmol) compound **WS1** as a yellow solid. Yield: 60%. ESI-MS (Theoretical molecular weight: 360.07): m/z = 361.0785 [M + H⁺]⁺, 383.0596 [M + Na⁺]⁺. FTIR (KBr, cm⁻¹): v= 1617 (C=N), 1690 (C=O), 3115 (C–N), 3435 (N–H). ¹H NMR (400 MHz, DMSO- d_6 , ppm): δ = 13.43 (s, 1H, –NH–), 12.02 (s, 1H, –NH–), 9.07 (s, 1H, –CH–), 8.88 (s, 1H, –CH–), 8.37 (d, *J* = 8.0 Hz, 1H, –CH–), 8.23 (d, *J* = 8.2 Hz, 1H, –CH–), 8.09 (d, *J* = 8.0 Hz, 1H, –CH–), 7.63 – 7.59 (m, 1H, –CH–), 7.58 (d, *J* = 4.2 Hz, 1H, –CH–), 7.50 (t, *J* = 7.7 Hz, 1H, –CH–), 7.47 – 7.42 (m, 1H, –CH–), 7.28 (t, *J* = 7.4 Hz, 1H, –CH–). ¹³C NMR (100 MHz, DMSO- d_6 , ppm): δ = 158.28, 147.08, 143.88, 141.64, 141.50, 141.10, 131.96, 130.04, 127.35, 127.33, 126.50, 125.58, 122.94, 122.14, 120.58, 117.31, 114.84, 110.87.

3. Results and discussion

3.1 Synthesis and structural characteristics of WS1

The synthesized route of **WS1** was shown in scheme 1. **WS1** was synthesized by the dehydration condensation of compound **2** with *1H*-indazole-3-carbaldehyde in ethanol at room temperature. The chemical structure of compound **2** and probe **WS1** was confirmed by ¹H NMR, ¹³C NMR, FTIR and ESI-MS spectrum. All the data in the spectra were well accordance with the structures.



Scheme 1. The synthetic route of sensor WS1.

3.2 The absorbance response of WS1 toward metal ions

The colorimetric detectability of **WS1** was measured with the addition of various metal ions in MeOH/H₂O (9:1 V/V) buffer solution (10 mM Tris, pH = 7.4) of **WS1** (1 × 10⁻⁵ M). As shown in Fig. 1a, the solution of free **WS1** (1 × 10⁻⁵ M) showed a higher energy absorption band centered at 325nm which was attributed to π - π * transition. Whereas, in the presence of 5 equiv. Cu²⁺, due to the complexion between **WS1** and Cu²⁺, the absorption intensity at 325nm decreased and a new absorption band appeared which centered at 390 nm. However, the addition of equal concentrations other metal ions (Ni²⁺, Co²⁺, Li⁺, Na⁺, K⁺, Mg²⁺, Ca²⁺, Zn²⁺, Cd²⁺, Hg²⁺, Ag⁺, Mn²⁺, Fe³⁺, Fe²⁺, Cr³⁺ and Al³⁺) caused inconspicuous change of absorption. And the color of test samples were shown in Fig. 1c, it could be found that only the solution with 5 equiv. Cu²⁺ changed from colorless to yellow which could be observed by naked



eyes. This consequence suggested that **WS1** revealed colorimetric specific recognition for Cu^{2+} .

Fig. 1 (a) UV-vis absorption spectra of **WS1** (1×10^{-5} M) in MeOH/H₂O (9:1 V/V) buffer solution (10 mM Tris, pH = 7.4) with various metal ions (5×10^{-5} M). (b) The absorption titration spectra of **WS1** (1×10^{-5} M) in the presence of different concentrations of Cu²⁺ (0 - 5 equiv.) in MeOH/H₂O (9:1 V/V) buffer solution (10 mM Tris, pH = 7.4). (C) The image of visual colorimetric response with test metal ions.

In continuation of the above findings, UV-vis titration was carried out in MeOH/H₂O (9:1 V/V) buffer solution (10 mM Tris, pH = 7.4) of **WS1** (1×10^{-5} M) with gradual addition of Cu²⁺. As shown in Fig. 1b, with the increasing of the concentrations of Cu²⁺, a new absorption band at 390 nm appeared and the absorbance gradually increased, meanwhile, the absorption at 325 nm exhibited slight decreased. Concomitantly there were two isosbestic points at 292nm and 351nm, respectively. As shown in Fig. S10, a spinodal appeared when the concentration of Cu²⁺ reached to 1×10^{-5} M, it was found that the complex ratio between WS1 and Cu²⁺ is 1:1. And based on the data of Fig. S11, the detection limit was calculated to be 4.63×10^{-7} M according to the equation LOD = $3\sigma/s$ [37], and the value was lower than the drinking water standard of WHO [38]. This consequence suggested that WS1 could be applied in colorimetric detection of Cu²⁺ selectively and sensitively.

3.3 The fluorescence response of **WS1** toward metal ions

The fluorescence detectability of WS1 was investigated by examination the fluorescence intensity of WS1 with excitation at 385nm in the presence of various metal ions. As shown in Fig. 2a, the MeOH/H₂O (9:1 V/V) buffer solution (10 mM Tris, pH = 7.4) of free **WS1** (1×10^{-5} M) exhibited a strong fluorescence centered at 465nm with a quantum yield (Φ) of 0.097, and a blue fluorescence color was observed (in Fig. 2c). After the addition of 5 equiv. of Cu²⁺, WS1 showed a significant fluorescence quenching. Whereas, equal concentrations of other metal ions caused negligible changes in fluorescence intensity. As shown in Fig. 2b, upon the addition of 5 equiv. Cu^{2+} , the fluorescence intensity at 465nm had declined around 80%. However, the addition of other metal ions resulted unconspicuous change of fluorescence intensity. Therefore, **WS1** showed a highly selectivity towards Cu^{2+} over other metal ions.



Fig. 2 (a) The emission spectra of **WS1** (1×10^{-5} M) in MeOH/H₂O (9:1 V/V) buffer solution (10 mM Tris, pH = 7.4) with various metal ions (5×10^{-5} M). (b) The emission intensity of **WS1** (1×10^{-5} M) at 465nm in MeOH/H₂O (9:1 V/V) buffer solution (10 mM Tris, pH = 7.4) with various metal ions (5×10^{-5} M). (c) The image of fluorescence response with the test metal ions. EX = 385nm.

fluorescence titration the Subsequently, conducted was in MeOH/H₂O (9:1 V/V) buffer solution (10 mM Tris, pH = 7.4) of WS1 (1 \times 10⁻⁵ M) to systematically measure the spectral activity for Cu²⁺. As shown in Fig. 3, the fluorescence intensity at 465nm gradually decreased and displayed a remarkable fluorescence quenching with the increasing of the concentration of Cu^{2+} (0 - 5equiv.). Fig. S12 showed the changes of fluorescence intensity as the concentration of Cu^{2+} increased from 0 to 5 \times 10⁻⁵ M, and an excellent linear relationship was observed. As shown in Fig. S13, the limit of detection (LOD) was calculated to be 4.32×10^{-8} M according to the equation $LOD = 3\sigma/s$. In addition, Fig. S14 showed the satisfactory linear relationship verifying the 1:1 binding ratio hypothesis and the association constant as an estimated standard for the binding

ability was calculated to be 5.75×10^5 M⁻¹ according to the formula (2). These consequences suggested that **WS1** could act as a fluorescence probe for detection of Cu²⁺ with high selectivity and sensitivity by fluorescence "trun off" response.



Fig. 3 The emission titration spectrum of **WS1** (1×10^{-5} M) in the presence of different concentrations of Cu²⁺ (0 - 5equiv) in MeOH/H₂O (9:1 V/V) buffer solution (10 mM Tris, pH = 7.4). EX = 385nm.

3.4. The competitive experiments

The competitive experiment was executed in MeOH/H₂O (9:1 V/V) buffer solution (10 mM Tris, pH = 7.4) for investigating the anti-interference ability of **WS1** toward Cu²⁺. As shown in Fig. 4, no obvious changes of fluorescence intensity were observed when 5 equiv. competitive metal ions (Li⁺, Na⁺, K⁺, Mg²⁺, Ca²⁺, Ni²⁺, Zn²⁺, Cd²⁺, Hg²⁺, Ag⁺, Mn²⁺, Fe³⁺, Fe²⁺, Co²⁺, Cr³⁺ and Al³⁺) were added into the **WS1** solutions, respectively. Subsequently, when 5 equiv. Cu²⁺ were added to the above solutions, the fluorescence intensity at 465 nm decreased

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obviously. Fig. 4 showed that **WS1** exhibited high selectivity for Cu^{2+} and the recognition toward Cu^{2+} was not interfered by other concomitant metal ions in the test system. This result indicated that **WS1** had a possibility for specific recognition for Cu^{2+} in actual detection.



Fig. 4 Metal ions competition test of **WS1** (1×10^{-5} M) in MeOH/H₂O (9:1 V/V) buffer solution (10 mM Tris, pH = 7.4). The red bars represent emission of **WS1** and 5 equiv. of other metal ions. The black bars represent fluorescence changes that occur upon addition of 5 equiv. of other metal ions to solution containing **WS1** and 5 equiv. Cu²⁺. EX = 385 nm.

3.5. The pH effect of probe WS1

Fig. 5 showed the effect of pH on the fluorescence intensity of **WS1** and **WS1** + Cu^{2+} complex in MeOH/H₂O (9:1 V/V) solution. **WS1** exhibited a stationary fluorescence in the pH range from 2 to 11, and the fluorescent quenching caused by Cu^{2+} was most conspicuous at pH = 7.4. In addition, the sensor had an accepted fluorescence response toward Cu^{2+} in the pH range of 5 - 8. When the pH less than 5 or more than 8, the fluorescence quenching was gradually unconspicuous. The reasons for these phenomenon were the protonation process of **WS1** in acidic condition, and the higher concentration of OH^- in alkaline condition could disturb the complex between **WS1** and Cu^{2+} . This consequence suggested that **WS1** had a potential for the detection of Cu^{2+} in actual samples.



Fig. 5 Effect of pH on fluorescence intensity at 465 nm for free **WS1** (1 \times 10⁻⁵ M) and **WS1** + Cu²⁺ in MeOH/H₂O (9:1 V/V) buffer solution (10 mM Tris, pH = 7.4). EX = 385 nm.

3.6 The reversibility of **WS1** for recognizing toward Cu^{2+}

In addition, the reversibility of **WS1** was testified with the fluorescence response of [**WS1** + Cu²⁺] for various anions. As shown in Fig. S15, the fluorescence intensity at 465nm decreased dramatically after the addition of 5 equiv. Cu²⁺. Subsequently, the fluorescence intensity recovered to the similar state of free **WS1** as PPi was added. But with the addition of other anions (BF₄⁻, F⁻, Cl⁻, B^{r-}, I⁻, NO₃⁻, NO₂⁻, S²⁻, HSO₃⁻, S₂O₃²⁻, SO₄²⁻, PO₄³⁻, CrO₄²⁻, HCO₃⁻, H₂PO₄⁻) to the solution of the **WS1** + Cu²⁺, the fluorescence was not recovered yet as that happened to PPi. Moreover, Fig. 6 showed **WS1** still displayed an acceptable fluorescence

response to Cu^{2+} after 4 cycles when Cu^{2+} and PPi was added in turn. This consequence demonstrated that **WS1** was a reversible fluorescence probe for the detection of Cu^{2+} .



Fig. 6. Fluorometric reversibility of **WS1** upon sequential addition of Cu^{2+} and PPi in MeOH/H₂O (9:1 V/V) buffer solution (10 mM Tris, pH = 7.4). EX = 385nm.

3.7 The probable binding mechanism and the DFT calculation of WS1 and $[WS1+Cu^{2+}]$

In order to discuss the possible binding mechanism, the Job's plot was obtained on the basis of fluorescence emission spectrum. As shown in Fig. 7, the fluorescence emission intensity at 465nm reached the maximum when the molar fraction of Cu^{2+} was 0.5. This result demonstrated that the stoichiometry between **WS1** and Cu^{2+} was 1:1. And the ESI-MS spectrum date in Fig. S9 also verified the stoichiometry is 1:1 because of the peak at m/z = 423.0087 [**WS1** + Cu^{2+}]²⁺. Based on the above data of emission and ESI-MS spectrum, a probable binding mechanism between **WS1** and Cu^{2+} was proposed as shown in scheme 2.

Upon the addition of Cu^{2+} , the coordination between nitrogen atoms of the imine (C=N), imidazole (C=N) and indazole (C=N) with paramagnetic Cu^{2+} lead to the fluorescence quenching [39-42].



Fig. 7 Job's plots of the complexation between **WS1** with Cu^{2+} .

the density functional In addition, theory (DFT) and time-dependent density functional theory (TDDFT) calculations of WS1 and complex $[WS1 + Cu^{2+}]$ have been performed to acquire the optimized structure of ground state and energy optimize structure of excited state using the Gaussian 09 program at the B3LYP/6-31G basic sets. Fig. S16 showed the coplanarity of $[WS1 + Cu^{2+}]$ optimized structure decreased compared with that of WS1. Then as shown in Fig. 8, the HOMO and LUMO orbital of **WS1** were spread over the indazole moiety, therefore, electrons which excited to LUMO can transfer back to the HOMO orbital which caused that WS1 exhibited a strong fluorescence. After the addition of Cu^{2+} , the frontier orbital of complex [WS1 + Cu^{2+}] became

relatively different form free **WS1** due to the paramagnetism of Cu^{2+} . The HOMO orbital was centered on the imidazole[2,1-*b*]benzothiazole moiety, while the LUMO orbital was located at the indazole moiety. The HOMO and LUMO orbital was distributed by the different moieties, this corresponded to electron cloud distribution of PET mechanism process. The occurrence of PET process lead to the conspicuous fluorescence quenching. The energy gap between HOMO and LUMO orbitals of free **WS1** and complex [**WS1** + Cu²⁺] were 4.182 eV and 3.341 eV, respectively. The binding of **WS1** with Cu²⁺ stabilized the system which was certified from the lower energy gap of complex [**WS1** + Cu²⁺]. These calculation results support the probable binding mechanism in scheme 2.



Scheme. 2 The probable binding mode of **WS1** for Cu²⁺.



Fig. 8 Frontier molecular orbitals (MOs) and energy diagrams of free WS1 and complex $[WS1 + Cu^{2+}]$.

3.8 The sensing test in actual water samples

To explore the application of **WS1** in practical environmental monitoring, the sensing test for Cu^{2+} in tap water was performed. As shown in Table 1, **WS1** exhibited satisfactory performance for Cu^{2+} in tap water at low concentrations. Compared with the standard curve of Cu^{2+} , an acceptable recovery (96.75% – 107%) and RSD (1.86% – 4.24%) of samples in tap water were obtained. Thus, this consequence demonstrated that **WS1** showed a favorable potency for the quantitative detection of Cu^{2+} in actual water.

Sample	Cu ²⁺ added (mol/L)	Cu ²⁺ recovered (mol/L)	Recovery (%)	RSD (%)
1	3×10^{-6}	$3.23 imes 10^{-6}$	107	4.24
2	$5 imes 10^{-6}$	$5.13 imes 10^{-6}$	102.6	1.08
3	$8 imes 10^{-6}$	$7.74 imes10^{-6}$	96.75	2.74
4	1×10^{-5}	1.034×10^{-5}	103.4	1.86

Table 1 the sensing test for Cu^{2+} in tap water samples.

4. Conclusion

In summary, a fluorescence probe WS1 was designed and synthesized based imidazo[2,1-*b*][1,3]benzothiazole-2-carbohydrazide on and 1H-indazole-3-carbaldehyde. The chemical structure of WS1 was confirmed by ¹H NMR, ¹³C NMR, FT-IR and ESI-MS. WS1 exhibited high selectivity and sensitivity toward Cu²⁺ over other metal ions in MeOH/H₂O (9:1 V/V) buffer solution (10 mM Tris, pH = 7.4). Upon the addition of Cu²⁺, a new absorption band appeared at 390nm with the solution changed from colorless to yellow, and the colorimetric limit of detection was 4.63×10^{-7} M. The fluorescence quenching occured because WS1 complexed with Cu^{2+} , the association constant of WS1 to Cu^{2+} was calculated to be $5.57 \times 10^5 M^{-1}$ and the limit of detection was 4.32×10^{-8} M which was sufficiently low to enable the detection of Cu²⁺ in practical applications. WS1 was a reversible fluorescence probe for the detection of Cu²⁺ and the Job's plot analysis supported the stoichiometry between WS1 and Cu^{2+} as 1:1. In addition, due to its satisfactory performance for Cu^{2+} , WS1 was of the potential possibility to be applied in the domains of biochemistry and biomedicine in the future.

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- 1. The probe showed specific recognition towards Cu²⁺ through two pathways (colorimetric and fluorescence response).
- 2. The limit of detection for Cu^{2+} can be calculated to be 4.63×10^{-7} M and 4.32×10^{-8} M by colorimetric and fluorescence response, respectively.
- 3. The proposed binding mode of WS1 with Cu²⁺ was verified by DFT calculation using Gaussian 09.
- 4. WS1 can detect Cu^{2+} in tap water samples with acceptable recovery and RSD.

Journal Prevention

Dear Editor:

This work are no conflicts to declare.

Yours sincerely,

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