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PII: S1386-1425(16)30529-7
DOI: doi:10.1016/j.saa.2016.09.011
Reference: SAA 14657

To appear in:

Received date: 20 April 2016
Revised date: 7 September 2016
Accepted date: 11 September 2016

Please cite this article as: Peng-Cheng Huang, Hao Fang, Jing-Jing Xiong, Fang-Ying Wu, Ultrasensitive turn-on fluorescence detection of Cu^{2+} based on p-dimethylaminobenzamide derivative and the application to cell imaging, (2016), doi:10.1016/j.saa.2016.09.011

Ultrasensitive turn-on fluorescence detection of Cu^{2+} based on p-dimethylaminobenzamide derivative and the application to cell imaging

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Abstract A new p-dimethylaminobenzamide derivative based compound BDIH has been synthesized. Cu^{2+} turned on the fluorescence of compound BDIH with a 1: 2 binding stoichiometry. The fluorescent color of compound BDIH shows an evident change from colorless to bright blue upon the addition of Cu^{2+}, which could be visibly detected by the naked eye under UV light at 365 nm. More importantly, the detection limit was found to be 0.64 nM which is far lower than the maximal allowed concentration of the WHO limit (31.5 μM) for drinking water. This selective "turn-on" fluorescence sensor was used to identify Cu^{2+} in living cells using confocal fluorescence microscopy, indicating that compound BDIH has a potential application for selective detection of Cu^{2+} in organism.

Keywords p-dimethylaminobenzamide derivative, fluorescence, Cu^{2+}, living cells

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1. Introduction

Acting as one of the most abundant transition metal ions in the biological environment and essential trace elements in the human body, Cu^{2+} has a significant influence on Cu^{2+} binding enzyme activities, such as oxidase, salivary amylase, tyrosinase, which related enzymes can play a critical role as an isomerous component and as a catalytic co-factor [1-3]. Deficiency intake of Cu^{2+} leads to Parkinson's disease [4], Alzheimer's disease [5] and Wilson's disease [6]. However, under overloading conditions, Cu^{2+} exhibits toxicity to the human body and leads to neurodegenerative diseases and oxidative stress probably by its involvement in the procedure of reactive oxygen species [7]. Furthermore, Cu^{2+} is a crucial environmental contaminant owing to its widespread use in agriculture and industries. It can be incorporated in the environment, water and biologic chain [8]. The World Health Organization (WHO) has set the maximum allowable level of copper in drinking water at 2 ppm (31.5 μM) [9]. Hence detection and recognition of Cu^{2+} has garnered much attention owing to its importance in biological and environmental systems in recent years.

Compared with some analytical methods including atomic absorption spectrometry [10], atomic emission spectrometry [11], and inductively coupled plasma-mass spectrometry [12], fluorescent techniques are powerful tools to monitor ions due to simplicity, convenience, low cost, high sensitivity, specificity, low background, fast response time, and rapid monitoring of target ions in practical biological and environmental applications [13-15, 16]. Metal-selective fluorescent sensors act as useful tools for exploitation of metal ions and thus have been extensively studied to explore biologically and environmentally relevant metal ions [17-22]. On surveying the literature, most of reported and investigated Cu^{2+} selective fluorescence probes have some drawbacks, for example, long response times [23], tedious synthetic procedures [24], large molecular structure [25], interference from other transition metal ions and high background with weak enhancement [26]. Particularly, there are still few examples of "turn-on" type sensors practicable [1, 27-30]. These would badly restrict their further applications in biological or environmental systems. Thus, to explore simple, easy to-make and highly sensitive and selective fluorescent probes for Cu^{2+} in biological and environmental system is still in an intense demand.

To fulfill the above-mentioned requirements, we herein report a compound of p-dimethylaminobenzamide derivative, benzoic acid-4-(dimethylamino)-

2-(1H-indol-3-ylmethylene)hydrazide (BDIH) (Scheme 1), to construct a fluorescent assay for Cu^{2+} with a very low detection limit, one-step synthetic process, and high chemical stability during at least 3 months. Compound BDIH displayed a selective response toward Cu^{2+} via significant turn-on fluorescence in aqueous media. Moreover, compound BDIH can also be used to quantify Cu^{2+} on the paper test strips and as a practical, visible fluorescent detection kit for Cu^{2+}. Finally, compound BDIH was further applied to cell imaging of Cu^{2+} using hepatoma carcinoma cells.

Scheme 1

2. Experimental Section

2.1 Reagents

Indole-3-carboxaldehyde is bought from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). $Cu(NO_3)_2$, $FeCl_3$, NaCl, $Ca(NO_3)_2$, $MgCl_2$, $NiCl_2$, $CdCl_2$, $AlCl_3$, $CrCl_3$, $HgCl_2$, $AgNO_3$, $Mn(NO_3)_2$, $CoCl_2$, $Pb(NO_3)_2$ and $ZnCl_2$ were purchased from Shanghai Qingxi Technology Co. Ltd. (Shanghai, China). All other reagents were of analytical grade and were used as supplied without further purification. All aqueous solutions were prepared using ultrapure water from a Milli-Q system.

2.2 Apparatus

All fluorescence measurements were carried out on an F-4500 spectrofluorimeter (Hitachi, Japan) equipped with a xenon lamp source and a 1.0 cm quartz cell, the scan speed was 2400 nm min^{-1} and the band pass of excitation and emission was set as 10 nm, respectively. Voltage was 400 V. Excitation wavelength was set at 352 nm. Absorption spectra were performed on a Shimadzu-2550 UV-Vis spectrophotometer (Shimadzu, Japan) using a 1.0 cm quartz cell. ESI-MS data were recorded on a Waters ZQ4000/2695 mass spectrometer (Waters, America). NMR spectra were recorded on an Agilent Technologies Plus-400 MR spectrometer (Bruker BioSpin, America) with DMSO-d_6 as the solvent and tetramethylsilane (TMS) as internal standard. Elemental analyses (C, H and N) were performed on an Elementary Vario EL analyzer (Elementar, Germany). Infrared spectra were taken in KBr disks on a Nicolet 5700 FTIR spectrometer (Nicolet,

America). Fluorescence imaging was performed using a Zeiss LSM 710 fluorescence microscope (Carl Zeiss, Germany).

2.3 Methods

General UV–vis and fluorescence spectra measurements: All measurements were carried out at room temperature. Stock solution of compound BDIH was prepared by dissolving 0.1 mM compound BDIH in DMSO. Further dilution was made to prepare 1.0 μM solution for the fluorescence experiments with MeCN-H_2O (v/v=4:1, pH=5) HAc-NaAc buffer solution. The UV–vis spectra were conducted with 10 μM of BDIH in MeCN-H_2O (v/v=4:1, pH=5) HAc-NaAc buffer solution.

General procedure for ^1H NMR experiments: For ^{1}H NMR titration, both compound BDIH and Cu^{2+} were prepared in DMSO-d_6. First of all, only compound BDIH in DMSO-d_6 was added into the NMR tube, and then Cu^{2+} was added. All solutions were mixed directly in the NMR tube.

2.4 Cell culture

The hepatoma carcinoma cells were seeded in the petri dish. The cells were incubated with BDIH overnight at 37 ℃ to determine the cell permeability of BDIH. After being washed with phosphate buffer solution (PBS) three times to remove the remaining BDIH, Cu^{2+} was added into the cells to incubate for 30 min at 37 ℃ as the case of BDIH (without PBS). Confocal fluorescence imaging was performed with a Zeiss LSM 710 microscope.

2.5 Synthesis of BDIH

p-dimethylaminobenzamide BDIH (CAS number: 540789-01-3) was prepared following a literature method [31,32]. The structure and synthesis procedure of compound BDIH is shown in Scheme 1. Indole-3-carboxaldehyde (0.1596 g, 1.1 mmol) and p-dimethylaminobenzamide (0.1791 g, 1.0 mmol) were dissolved in 25 mL of dry ethanol solution under reflux conditions at 77 ℃ for 3 h. A white precipitate was found. The reaction mixture was allowed to attain room temperature, and then the precipitate was collected through filtration. The residue was washed thoroughly with ethanol to isolate BDIH in pure form with 86% yield (the yield was calculated

based on the starting reagents). Compound BDIH was characterized by IR, NMR, ESI-mass spectrometry and element analysis data, which were consistent with the proposed formula. IR (KBr, cm^{-1}): ν=1608 cm^{-1} (C=O), 1577 cm^{-1} (C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 11.50 (s, 1H, N-H), 11.19 (s, 1H, NH), 8.57 (s, 1H, Ar-H), 8.28 (d, *J* = 6.6 Hz, 1H, Ar-H), 7.78 (dd, *J* = 22.5, 3H, Ar-H), 7.41 (d, *J* = 7.9 Hz, 1H, Ar-H), 7.27-7.09 (m, 2H,Ar-H), 6.74 (d, *J* = 8.7 Hz, 2H, Ar-H), 3.03-2.92 (s, 6H, N-CH_3); ^{13}C NMR (400 MHz, DMSO-d_6) δ(ppm): 162.75, 152.64, 143.93 137.43, 130.07, 129.26, 124.81, 122.94, 122.49, 120.64, 112.42, 112.17, 111.30. ESI-MS: calcd for $[C_{18}H_{18}N_4O + H]^+$ 307.2, found 307.2. Anal. calcd for $C_{18}H_{18}N_4O$: C 70.53, H 5.87, N 18.28; Found: C 70.75, H 5.79, N 18.14.

3. Results and discussion

3.1 Fluorescence spectral change of compound BDIH in the presence of various ions

To investigate binding properties of compound BDIH (1.0 μM) with the cations and anions, various ions including Na^+, Ni^{2+}, Pb^{2+}, Cr^{3+}, Zn^{2+}, Cu^{2+}, Ag^+, Al^{3+}, Mg^{2+}, Ca^{2+}, Cd^{2+}, Mn^{2+}, Co^{2+}, Fe^{3+}, Hg^{2+}, F^-, Cl^-, Br^-, I^-, HCO_3^-, $H_2PO_4^-$, HPO_4^{2-}, HSO_4^-, SO_4^{2-}, SO_3^{2-}, NO_3^-, SCN^-, Ac^-, CN^- were examined in the MeCN-H_2O (pH=5, v/v=4:1) HAc-NaAc buffer solution (Fig. 1). Fluorescence spectra were recorded after 8 min upon the addition of each of these ions. Compound BDIH itself exhibited no fluorescence. It was found that while the addition of 11 μM other ions did not show no fluorescence enhancement, Cu^{2+} could cause an evident fluorescence enhancement at 419 nm (excitation wavelength is set at 352 nm, Fig. S1). Meanwhile, upon the addition of Cu^{2+}, compound BDIH solution changed from colorless to blue under a UV lamp at 365 nm, and such a color change can be visibly observed by the naked eyes (Fig. 1 inset). The above observations support that compound BDIH is highly efficient in detecting Cu^{2+} over other foreign cations and anions existing in physiological and environmental samples. Therefore, compound BDIH can be potentially used to design a Cu^{2+}-selective turn-on fluorescent probe worked in aqueous solution.

Fig. 1

3.2 Optimization of the assay for Cu^{2+}

To estimate the high selectivity in the organic-aqueous mixture, we added various amounts of H_2O to the MeCN solution, achieving the percentage of 0 to 90 as indicated. As shown in Fig. S2, we explored the influence of Cu^{2+} on the fluorescence of BDIH with varying of H_2O: MeCN ratios (0:100-90:10). It can be seen that the most sensitive fluorescence response was observed at a MeCN: H_2O ratio of 4:1.

To study the practical applicability of the Cu^{2+}-probe in environmental or biological samples, the effect of various pH values on the fluorescence spectral change of compound BDIH towards Cu^{2+} was investigated. As revealed in Fig. S3, free BDIH showed no fluorescence at the pH range 3.5-8.1. The increase in the fluorescence intensity at low pH values (3.5-5) is owing to the formation of BDIH-Cu^{2+} complex. Under high pH conditions (pH> 5), the emission intensity at 419 nm gradually decreased, presumably due to the dissociation of complex BDIH-Cu^{2+} by the formation of the precipitation of $Cu(OH)_2$, reducing the concentration of Cu^{2+} in the sample solution. It should be noted that the probe could also give a similar response to Cu^{2+} in MeCN-H_2O (v/v=4:1) solution even if the buffer solution is not utilized. However, in order to maintain the solution stability, the pH of 5 was chosen to evaluate the response of compound BDIH to Cu^{2+}. We also studied the time course of the response of compound BDIH in the presence of 11 μM Cu^{2+}, and found that the fluorescence intensity remarkably increased to a maximum within 8 min (Fig. S4).

3.3 Fluorescence titration of compound BDIH with Cu^{2+}

Fluorescence spectra changes of BDIH upon titration with Cu^{2+} is shown in Fig. 2. Upon excitation at 352 nm, compound BDIH alone showed very weak fluorescence. The fluorescence titration experiment of BDIH for Cu^{2+} led to a remarkable enhancement at 419 nm with the solution emission color change from colorless to blue, implying a Cu^{2+}-selective turn-on fluorescence signal behavior. A good linear correlation was obtained for the fluorescence intensity of compound BDIH in the concentration range of Cu^{2+} between 0 and 15 μM with the detection limit as low as 0.64 nM, indicating this probe could be employed for quantitative detection of Cu^{2+} with ultrahigh sensitivity.

Fig. 2

3.4 Effect of coexisting components

To further study the selectivity of BDIH to Cu^{2+} in complicated systems, competitive experiments were performed with other ions in the case of 11 μM Cu^{2+}. As shown in Fig. 3, no obvious fluorescence change happened in the presence of 1000 equiv. different competitive cations (Na^+, Mg^{2+}, Al^{3+}, Cd^{2+}, Co^{2+}, Ca^{2+}, Zn^{2+}, Ni^{2+}, Mn^{2+}) and anions (F^-, Cl^-, Br^-, I^-, HCO_3^-, NO_3^-, $H_2PO_4^-$, HPO_4^{2-}, HSO_4^-, SO_4^{2-}, Ac^-, SO_3^{2-}, SCN^-, CN^-), and the data also displayed that Cr^{3+} (100 equiv.) Ag^+, Hg^{2+}, Pb^{2+} (10 equiv.), and Fe^{3+} (4 equiv.) caused negligible response to the fluorescence of BDIH-Cu^{2+}. The competitive experiments indicated that compound BDIH was capable of detecting Cu^{2+} in environmental and biological applications without the interference from most cations and anions.

Fig. 3

3.5 Absorption spectra of compound BDIH to Cu^{2+}

The absorption spectrum of BDIH showed the maximum peak at 336 nm in MeCN-H_2O (v/v=4:1, pH=5) HAc-NaAc buffer solution. Upon gradual addition of Cu^{2+} ions to the solution of BDIH, the absorption peak gradually decreased and reached a minimum at 24 equiv. of Cu^{2+} at 336 nm. At the same time a new absorption band at 265 nm slowly increased. Moreover, a clear isosbestic point at 293 nm was observed, implying the conversion of the free receptor BDIH to a copper complex (Fig. S5). To further study the sensing selectivity of compound BDIH toward Cu^{2+}, we carried out the selectivity experiments to various ions (Fig. S6). The absorption spectrum varied little by the presence of other cations and anions, except that Cu^{2+} induced an obvious decrease of the absorption peak at 336 nm.

3.6. "Naked-eye" and test strips measurement

As depicted in Fig. 4, color change was observed by separate addition of various metal ions (11 μM) into compound BDIH (1.0 μM) in MeCN-H_2O (v/v=4:1, pH=5) HAc-NaAc buffer solution. Under a UV lamp at 365 nm, compound BDIH showed an obvious color change from colorless to blue only upon binding with Cu^{2+}, whilst other metal ions did not induce any

remarkable color change under identical conditions. So the selective visual color change can be used for the "naked eye" detection of Cu^{2+} in aqueous media.

Fig. 4

To study the practical application of proposed compound BDIH, paper test strips were prepared for on-site detection of Cu^{2+}. In our experiment, Cu^{2+} solutions of five different concentrations, 0, 10^{-6}, 5×10^{-5}, 10^{-5}, 10^{-4} M, were prepared. For this purpose, filter papers were dipped in a 1.0 μM BDIH in MeCN-H_2O (v/v=4:1, pH=5) HAc-NaAc buffer solution for about 10 min and then dried in air. Under UV light at 365 nm, an obvious emission color change was observed clearly by naked eyes when the test strips were applied for sensing different Cu^{2+} concentrations. As revealed in Fig. 5, the color of paper test strips was converted from colorless to intense blue gradually as the concentration of Cu^{2+} increased, which was the same as that for the corresponding test vial. This enabled the sensor quite useful for immediate on-site detection of Cu^{2+}.

Fig. 5

3.7 The proposed reaction mechanism

The binding stoichiometry of BDIH with Cu^{2+} was investigated by a Job's plot (Fig. S7) in which the total concentration of BDIH and Cu^{2+} kept at 10 μM. The fluorescence $[1/(F - F_0)]$ at 419 nm varied as a function of $1/[Cu^{2+}]^2$ in a linear relationship (R = 0.992), and the analytical result confirmed a 2 : 1 stoichiometry of complex BDIH-Cu^{2+} with a binding constant of 1.33×10^{10} M^{-2} (Fig. S8). Moreover, the ligand BDIH was inherently poorly fluorescent owing to rapid isomerization of the O=C-N (¯O-C=N) bond in the excited state. In the presence of Cu^{2+}, compound BDIH could coordinate to Cu^{2+} with a strong fluorescence enhancement phenomenon. As shown in Scheme 2, these properties indicated that nitrogen atoms of the benzazole and Schiff base moiety and oxygen atoms of the carbanyl group bearing the lone pair of electrons in BDIH can efficiently chelate to Cu^{2+}. The binding of BDIH with Cu^{2+} obviously increased the fluorescence at 419 nm in the excited state due to the combined effect of suppression of ¯O-C=N isomerization. The fluorescence quantum yield of complex BDIH-Cu^{2+} was as high as 0.89, while

that of compound BDIH was only 0.15, both obtained with quinine sulfate solution in 0.05 M of H_2SO_4 (φ=0.55 at 363 nm excitation) as the reference [33], which also supporting significant fluorescence enhancement.

Scheme 2

To demonstrate the proposed binding of compound BDIH with Cu^{2+}, more direct evidence was provided by IR spectroscopy, ^{1}H NMR titrations and ESI-MS titration experiments. The IR spectrum of compound BDIH-Cu^{2+} showed a change in the bending vibration band of the secondary N-H corresponding to indole fragment relative to compound BDIH, that is, the peaks at 1577 and 766 cm^{-1} disappeared completely. Meanwhile the stretching vibration band of C=O corresponding to amide fragment shifted to a higher wavenumber from 1608 cm^{-1} to 1615 cm^{-1} (Fig. 6). The stretching vibration band of C=N group at 1528 cm^{-1} shifted to 1509 cm^{-1}. These changes suggested that Cu^{2+} coordinated to oxygen atoms of carbanyl group, the secondary amine of indole fragment and nitrogen atoms of Schiff base fragment, respectively.

Fig. 6

^{1}H NMR titration analysis was carried out to further illustrate the characteristic structural changes occurring upon interaction between BDIH and Cu^{2+} in DMSO-d_6. As shown in Fig. 7, for BDIH the imine proton in 11.21 ppm and methylene proton in 8.57 ppm consistent to the amide and Schiff base moiety gradually decreased and disappeared completely upon the addition of Cu^{2+} while the imine proton in 11.50 ppm corresponding to the indole moiety shifted to upfield at 11.49 ppm along with a slight decline of the proton signal. This result indicates that the binding with Cu^{2+} takes place with the carbanyl oxygen atom and nitrogen atom of Schiff base and amide fragment. Moreover, the ESI mass spectrum of BDIH (Fig. S9) displayed a peak at m/z = 307.2 (calcd=307.26) corresponding to $[BDIH+H]^+$. The complex BDIH-Cu^{2+} with the 2 : 1 complex formation between BDIH and Cu^{2+} was supported by showing a peak at m/z 675.2 (calcd.=675.02) corresponding to $[2BDIH+Cu-H]^+$ (Fig. S10).

Fig. 7

3.8 Fluorescence imaging of Cu^{2+} in living cells

The properties of BDIH for high selectivity and sensitivity towards Cu^{2+} have further prompted us to extend our research to detect Cu^{2+} in biological systems by live cell imaging experiments. The fluorescence imaging was carried out in hepatoma carcinoma living cells as showed in Fig. 8. When incubated with 10 μM of BDIH for 12 h at 37 °C, the cells showed no intracellular fluorescence. The cells were washed with PBS three times to remove free BDIH. Upon treating above cells with Cu^{2+} (20 μM) for 30 min at 37 °C (here PBS is discarded as its presence would influence fluorescence intensity), the intracellular blue fluorescence was significantly observed in hepatoma carcinoma cells. This result indicated that compound BDIH can be used as a fluorescent probe for the recognition of intracellular Cu^{2+} in living cells.

Fig. 8

4. Conclusion

In summary, we have successfully synthesized compound BDIH with a high yield and easy to-make which exhibited an obvious fluorescence enhancement response to Cu^{2+} at 419 nm enabling highly selective and sensitive detection of Cu^{2+} at a very low detection limit. Its application in quantitative detection of Cu^{2+} using simple paper-made test kits was performed. Accordingly, the probe was successfully used for fluorescence imaging of Cu^{2+} in living cells. It can be envisaged that BDIH could be one of the most promising alternatives for the detection of Cu^{2+} in biological and environmental systems.

Acknowledgement

This work is financially supported by Natural Science Foundation of China (No. 21365014 and 21505067). We greatly thank Dr. Xiaohong Zhao, College of Chemistry, Nanchang University, China, for helpful discussion.

References

[1] K.M.K. Swamy, S.-K. Ko, S.K. Kwon, H.N. Lee, C. Mao, J.-M. Kim, I. Shin, J. Yoon, Chem. Commun. 44 (2008) 5915-5917.

[2] E. Gaggelli, H. Kozlowski, D. Valensin, G. Valensin, Chem. Rev. 106 (2006) 1995-2044.

[3] B.P. Zietz, J. Dassel de Vergara, H. Dunkelberg, Germany, Environ. Res. 92 (2003) 129-138.

[4] J.C. Lee, H.B. Gray, J.R. Winkler, J. Am. Chem. Soc. 130 (2008) 6898-6899.

[5] Y.H. Hung, A.I. Bush, R.A. Cherny, Inorg. Chem. 15 (2010) 61-76.

[6] E. Madsen, J.D. Gitlin, Annu. Rev. Neurosci. 30 (2007) 317-337.

[7] K.J. Barnham, C.L. Masters, A.I. Bush, Nat. Rev. Drug Discovery 3 (2004) 205-214.

[8] C. Zong, K. Ai, G. Zhang, H. Li, L. Lu, Anal. Chem. 83 (2011) 3126-3132.

[9] G.R. You, G.J. Park, J.J. Lee, C. Kim, Dalton Trans. 44 (2015) 9120-9129.

[10] Y. Yamini, N. Alizadeh, M. Shamsipur, Anal. Chim. Acta 355 (1997) 69-73.

[11] S.L.C. Ferreira, A.S. Queiroz, M.S. Fernandes, H.C. dos Santos, Spectrochim. Acta, Part B, 57B (2002) 1939-50.

[12] C.F. Harrington, S.A. Merson, T.M. D' Silva, Anal. Chim. Acta 505 (2004) 247-254.

[13] W. Zhu, X. Huang, Z. Guo, X. Wu, H. Yu, H. Tian, Chem. Commun. 48 (2012) 1784-1786.

[14] Y. Kubo, T. Ishida, A. Kobayashi, T.D. James, J. Mater. Chem. 15 (2005) 2889-2895.

[15] Y.H. Lee, N. Park, Y.B. Park, Y.J. Hwang, C. Kang, J.S. Kim, Chem. Commun. 50 (2014) 3197-3200.

[16] H.N. Kim, W.X. Ren, J.S. Kim, J. Yoon, Chem. Soc. Rev. 41 (2012) 3210-3244.

[17] A. Coskun, E.U. Akkaya, J. Am. Chem. Soc. 128 (2006) 14474-14475.

[18] X. Peng, J. Du, J. Fan, J. Wang, Y. Wu, J. Zhao, S. Sun, T. Xu, J. Am. Chem. Soc. 129 (2007) 1500-1501.

[19] S.-K. Ko, Y.-K. Yang, J. Tae, I. Shin, J. Am. Chem. Soc. 128 (2006) 14150-14155.

[20] Y. Xiang, A. Tong, Org. Lett. 8 (2006) 1549-1552.

[21] J.-S. Wu, I.-C. Hwang, K.S. Kim, J.S. Kim, Org. Lett. 9 (2007) 907-910.

[22] X. Chen, S.-W. Nam, M.J. Jou, Y. Kim, S.-J. Kim, S. Park, J. Yoon, Org. Lett. 10 (2008) 5235-5238.

[23] J.-H. Ye, J. Xu, H. Chen, Y. Bai, W. Zhang, W. He, Tetrahedron Lett. 55 (2014) 6269-6273.

[24] J. Huang, M. Liu, X. Ma, Q. Dong, B. Ye, W. Wang, W. Zeng, RSC Adv. 4 (2014) 22964-22970.

[25] L. Liu, A. Wang, G. Wang, J. Li, Y. Zhou, Sens. Actuators, B 215 (2015) 388-395.

[26] Q. Liu, Q. Fei, Y. Fei, Q. Fan, H. Shan, G. Feng, Y. Huan, Spectrochim. Acta, Part A, 151 (2015) 785-789.

[27] Z. Xu, Y. Xiao, X. Qian, J. Cui, D. Cui, Org. Lett. 7 (2005) 889-892.

[28] S.H. Kim, J.S. Kim, S.M. Park, S.-K. Chang, Org. Lett. 8 (2006) 371-374.

[29] L. Zeng, E.W. Miller, A. Pralle, E.Y. Isacoff, C.J. Chang, J. Am. Chem. Soc. 128 (2006) 10-11.

[30] M. Royzen, Z. Dai, J.W. Canary, J. Am. Chem. Soc. 127 (2005) 1612-1613.

[31] F. Palace-Berl, K.F.M. Pasqualoto, S.D. Jorge, B. Zingales, R.R. Zorzi, M.N. Silva, A. K. Ferreira, R. A. d. Azevedo, S. F. Teixeira and L. C. Tavares, Eur. J. Med. Chem. 96 (2015) 330-339.

[32] A.R.B. Rao, S. Pal, J. Organomet. Chem. 696 (2011) 2660-2664.

[33] P. Huang, Q. Jiang, P. Yu, L. Yang, L. Mao, ACS Appl. Mater. Interfaces 5 (2013) 5239-5246.

Captions

Scheme 1 The structure and synthesis process of compound BDIH.

Fig. 1 Fluorescence spectra of compound BDIH (1.0 μM) upon the addition of 11 μM of Cu^{2+} and other cations and anions in the MeCN-H_2O (v/v=4:1, pH=5) HAc-NaAc buffer solution. The inset shows the photographs of the solution of BDIH before and after the addition of Cu^{2+} (11 μM) under a UV lamp at 365 nm.

Fig. 2 Fluorescence titration spectra of compound BDIH (1.0 μM) upon the addition of Cu^{2+} (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 equiv.) in MeCN-H_2O (v/v=4:1, pH=5) HAc-NaAc buffer solution. Inset: Linear plot of fluorescent intensity for BDIH upon the addition of Cu^{2+} concentration (0-15 equiv.) at 419 nm.

Fig. 3 Fluorescent selectivity of BDIH toward Cu^{2+} over various competitive cations and anions in MeCN-H_2O (v/v=4:1, pH=5) HAc-NaAc buffer solution.

Fig. 4 Photographs of the color of BDIH (1.0 μM) in MeCN-H_2O (v/v=4:1, pH=5) HAc-NaAc buffer solution in the presence of different metal ions under a UV lamp at 365 nm.

Fig. 5 Fluorescent photographs of the test strips according to the solution color of BDIH (1.0 μM) for the detection of various concentrations of Cu^{2+} from left to right: 0, 10^{-6}, 5×10^{-5}, 10^{-5}, 10^{-4} M.

Scheme 2 The proposed binding mode of compound BDIH with Cu^{2+}.

Fig. 6 IR spectra of BDIH and BDIH-Cu^{2+}.

Fig. 7 ^{1}H NMR spectra of BDIH and BDIH + Cu^{2+} measured in DMSO-d_6.

Fig. 8 Fluorescence microscopic images of hepatoma carcinoma cells incubated with 10 μM BDIH, adding 20 μM of Cu^{2+} to the BDIH-treated cells.

Scheme 1

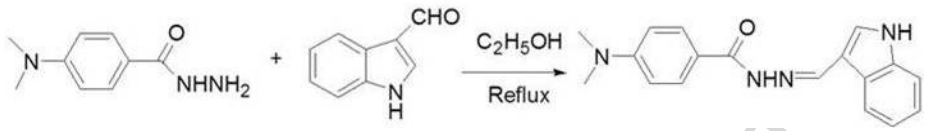


Fig. 1

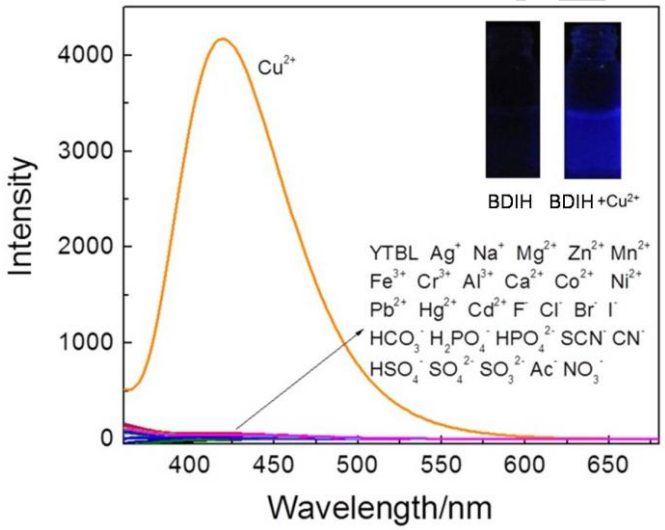


Fig. 2

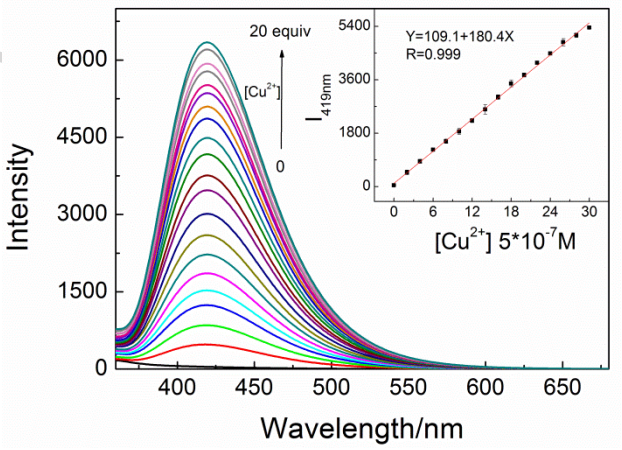


Fig. 3

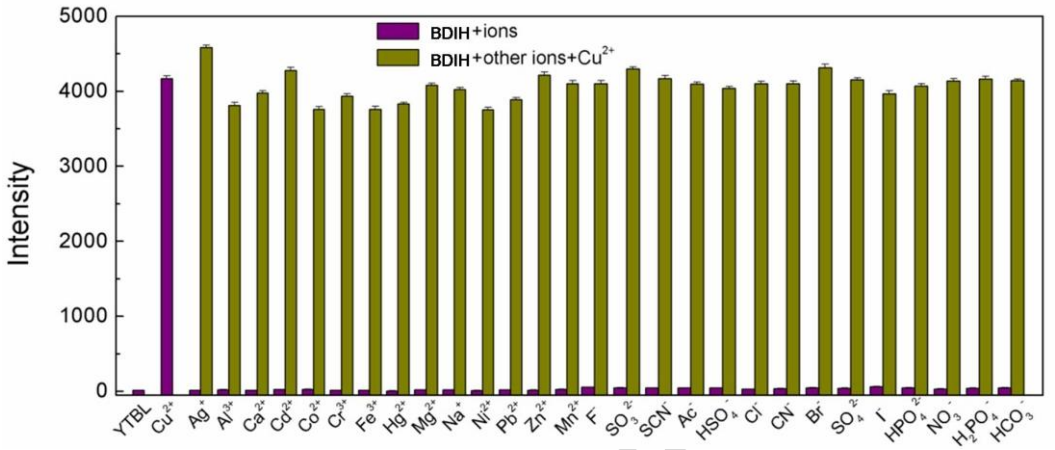


Fig. 4

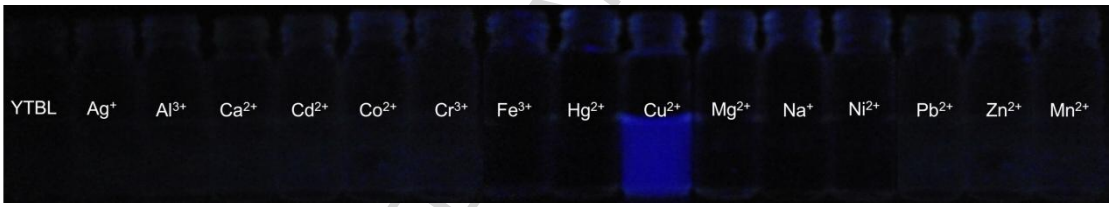
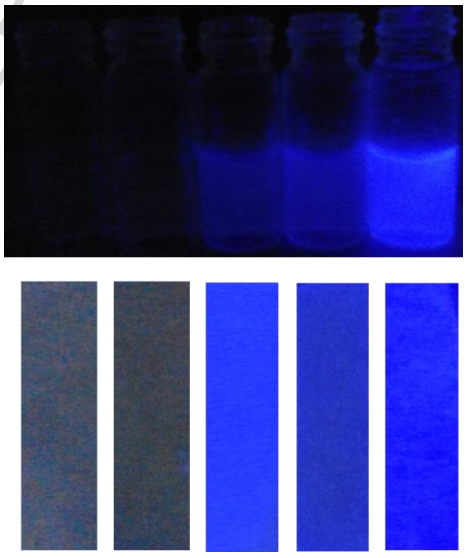


Fig. 5



Scheme 2

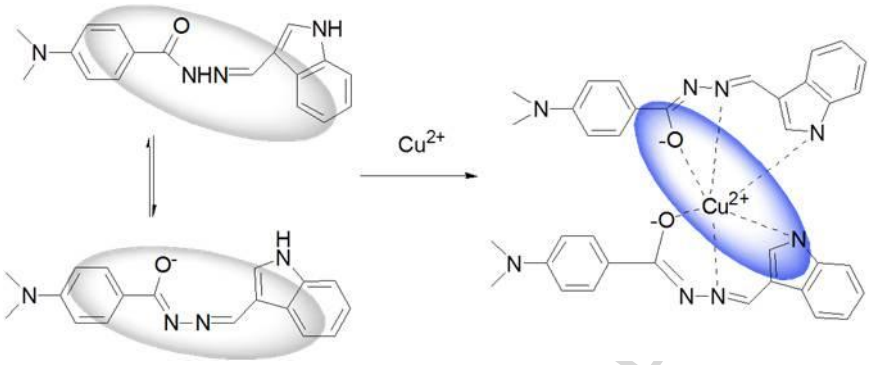


Fig. 6

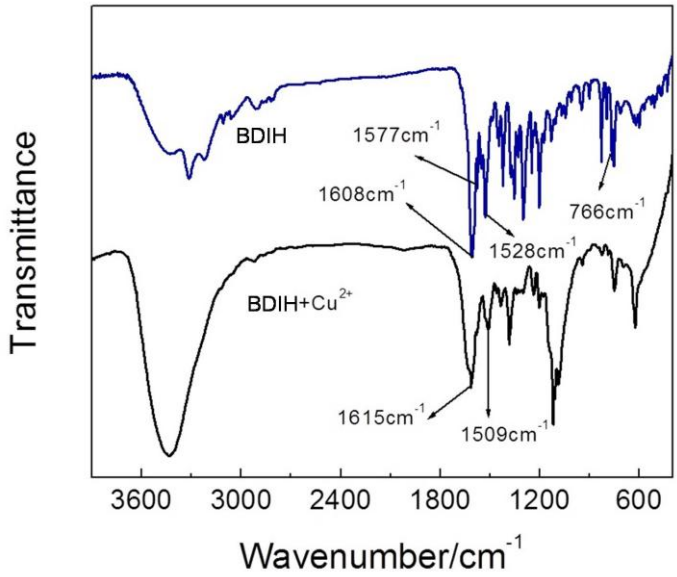


Fig. 7

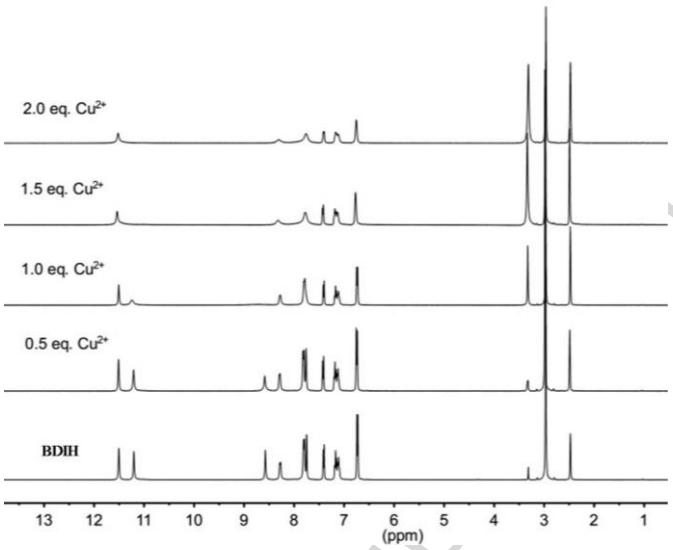
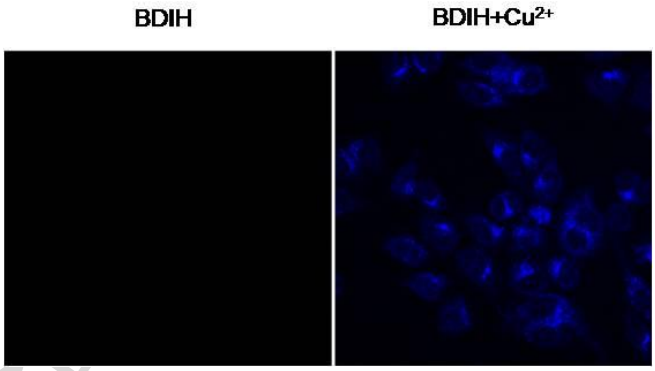
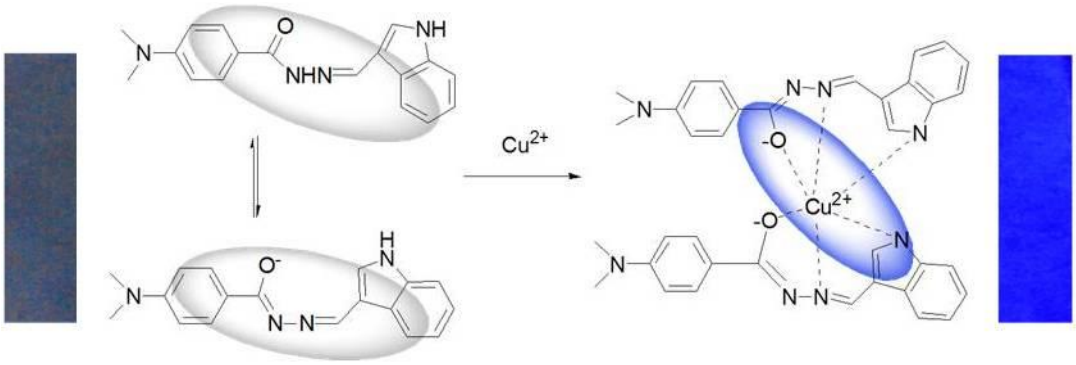
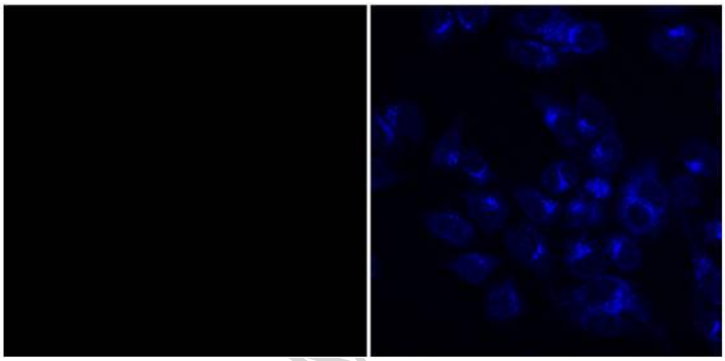


Fig. 8



Graphical abstract





Highlights

1. An ultrasensitive fluorescence "turn-on" assay for Cu^{2+} based on p-dimethylaminobenzamide-based derivative was presented.
2. The probe exhibits excellent selectivity and a very high tolerance to other common ions by the naked eye and fluorescence spectra.
3. The assay was applied to detect Cu^{2+} on the test kits and in living cells.
4. Compound BDIH showed a very low background.