Synthesis and application of a highly selective copper ions fluorescent probe based on the coumarin group

Guangjie He, Xiangli Liu, Jinhe Xu, Liguo Ji, Linlin Yang, Aiying Fan, Songjun Wang, Qingzhi Wang

PII: S1386-1425(17)30747-3
DOI: doi: 10.1016/j.saa.2017.09.028
Reference: SAA 15462

To appear in: *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*

Received date: 12 July 2017
Revised date: 18 August 2017
Accepted date: 12 September 2017

Please cite this article as: Guangjie He, Xiangli Liu, Jinhe Xu, Liguo Ji, Linlin Yang, Aiying Fan, Songjun Wang, Qingzhi Wang , Synthesis and application of a highly selective copper ions fluorescent probe based on the coumarin group, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* (2017), doi: 10.1016/j.saa.2017.09.028

Synthesis and application of a highly selective copper ions fluorescent probe based on the coumarin group

Guangjie He[a*], Xiangli Liu[a], Jinhe Xu[a], Liguo Ji[a*], Linlin Yang[a], Aiying Fan[a], Songjun Wang[b], Qingzhi Wang[a]

[a] *Department of Forensic Medicine, Xinxiang Medical University, Jinsui Road No. 601, Xinxiang, 453003, Henan Province, P. R. China*

[b] *Hebei Key Laboratory of Forensic Medicine, Hebei Medical University, East Zhongshan Road No. 361, Shijiazhuang, 050017, Hebei Province, P. R. China*

* Corresponding author: Tel./Fax: +86-373-3831183

E-mail: guangjiehe@163.com; liguoji_academy@126.com

Absract

A highly selective copper ions fluorescent probe based on the coumarin-type Schiff base derivative **1** (probe) was produced by condensation reaction between coumarin carbohydrazide and 1H-indazole-3-carbaldehyde. The UV-vis spectroscopy showed that the maximum absorption peak of compound **1** appeared at 439 nm. In the presence of Cu^{2+} ions, the maximum peak decreased remarkably compared with other physiological important metal ions and a new absorption peak at 500 nm appeared. The job's plot experiments showed that complexes of 1:2 binding mode were formed in CH3CN:HEPES (3:2, v/v) solution. Compound 1 exhibited a strong blue fluorescence. Upon addition of copper ions, the fluorescence gradually decreased and reached a plateau with the fluorescence quenching rate up to 98.73%. The detection limit for Cu^{2+} ions was estimated to 0.384 ppm. Fluorescent

microscopy experiments demonstrated that probe **1** had potential to be used to investigate biological processes involving Cu^{2+} ions within living cells.

Keywords: fluorescent probe, Cu^{2+} ions probe, coumarin, cell imaging

1. Introduction

Copper ions as an important existing form of copper element are prevalent in lives; its content in the body is only less than iron and zinc[1, 2]. It plays significant roles in pathophysiology metabolism and metabolic activities of various enzymes in the organisms. For instance, copper ions as a catalytic cofactor are involved in mitochondrial respiration, iron absorption and redox process of a large number of enzymes such as SOD, dopamine β-hydroxylase, lysyloxidase, tyrosinase, cytochrome oxidase, etc[3-5]. The normal concentration of the copper ions in human body is about 15.7 ~ 23.6 μM[6]. Thus abnormalities of the copper ions may lead to some severe neurodegenerative diseases and disorders of growth and metabolism[7]. Especially in recent years the environment pollution exposed people for a long time in the circumstances of high concentration of Cu^{2+} and induce to an emergence of series of neurodegenerative diseases caused by copper metabolism disorders[8-10], such as Alzheimer's diseases (AD), Menkes syndrome, Parkinson's diseases (PD), Huntington diseases (HD), familial amyotrophic lateral sclerosis (ALS) and many others[11-13]. Therefore, design of a rapid, convenient, highly selective and

sensitive detection method for copper ion is significant and always attracts a great deal of attentions[14-20].

The fluorescent analysis method relative to the electrochemical analysis method, inductively coupled plasma mass spectrometry[21], flame atomic absorption spectrometry[22], flow injection analysis[23], spectrophotometry, etc., serving for determination of copper ions has the advantages of high sensitivity, high selectivity, low cost, easy operation, fast analysis, less sample size and real time in situ detection. In addition, the detection of copper ions in the body could be realized by fluorescence imaging technology[24, 25]. Thus a further research work is also needed although some relate works have been reported.

Coumarin molecules possessed high fluorescence intensity, strong light stability, good solubility, easy preparation, high molar absorption coefficient and high fluorescence quantum yield, thus they were usually served as chromophoric group to synthesis fluorescent probes[26-31]. Based on this, a new fluorescent probe of coumarin-type schiff derivative (compound **1**) was synthesized by the condensation reaction between coumarin carbohydrazide and 1H-indazole-3-carbaldehyde. This probe was characterized by NMR and ESI-MS spectrums, and manifested obviously selective to Cu^{2+} ions in the following experiments results. Meanwhile, the interaction of this fluorescent compound with other metal ions was also discussed by UV-vis absorption and fluorescent

spectroscopy.

2. Experiment section

2.1 Instruments

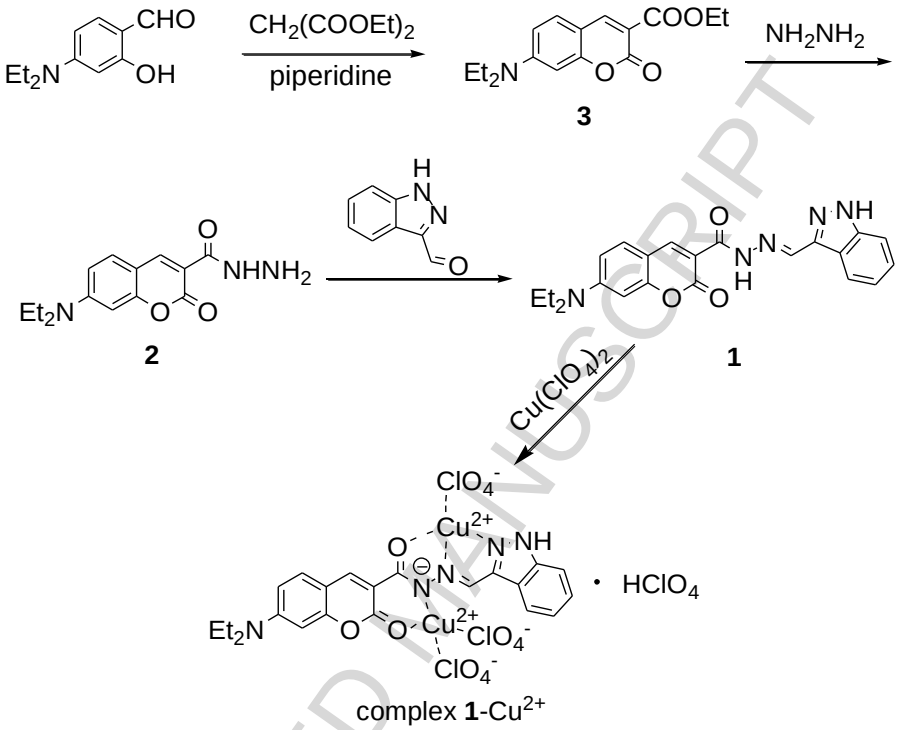
The NMR spectra data were recorded with chemical shifts as ppm with TMS as internal standard measured on a Bruker Ascend™ 400 spectrometer. Mass spectrometric data were measured on a Bruker Microtof-QIII spectrometry. UV-vis absorption spectra data were obtained by Shimadzu UV2600 spectrophotometer. Fluorescence spectra were recorded with Edinburgh Instruments FS-5 fluorescence spectrophotometer. Cell imaging was recorded with Leica DMI8 inverted fluorescence microscope.

2.2 Reagents

All compounds were analytical grade and directly used as received. 4-(diethylamino)-2-hydroxybenzaldehyde, diethyl malonate, piperidine, hydrazine hydrate (80%) and the other chemical reagents were purchased from Energy Chemical Co. Ltd. Stock solutions (2.0×10^{-2} M) of the perchlorate Mn^{2+}, Fe^{3+}, Fe^{2+}, K^{+}, Mg^{2+}, Pb^{2+}, Al^{3+}, Zn^{2+}, Ag^{+}, Ni^{2+}, Cd^{2+}, Ca^{2+}, Na^{+}, Cr^{3+}, Co^{2+}, Hg^{2+} and Cu^{2+} ions were prepared in aqueous solutions. In the spectral measurements, the stock solutions of compound **1** were prepared in CH_3CN:HEPES (3:2, v/v) solutions. A 3 mL solution of compound **1** was filled in a quartz cell of 1 cm optical path length each

time, by using a micro-syringe different stock solutions of metal ions were added into the quartz cell gradually.

2.3 Synthetic procedures



Scheme.1 synthetic route of compound **1** and the presumable structure of complex **1**-Cu^{2+}

The synthetic procedures were shown in Scheme 1. The coumarin hydrazine (compound **2**) and ethyl-7-(diethylamino)-2-oxo-2H-chromene -3-carboxylate (compound **3**) were synthesized according to the previous work[32, 33]. Compound **1** (probe) was synthesized conveniently from the reaction of compound 2 with 1H-indazole-3-carbaldehyde and characterized by ^{1}H NMR, ^{13}C NMR, and ESI-MS (Fig.S1, S2, S3), and the complex **1**-Cu^{2+} was prepared by compound **1** and $Cu(ClO_4)_2 \cdot 6H_2O$ under reflux condition for 3 h. The experimental details could be found in Supplementary Information.

3. Results and Discussions

3.1 The experiment of water solubility

The application of the probe in vivo was obviously influenced by its water solubility, so a fluorescent stability test of the probe was proceeded in varied solutions in which the ratio of CH_3CN and HEPES buffer were changed (Fig.S4). The results indicated that the fluorescent intensity was stable until the proportion of the HEPES in the solution was up to 40%. A drastically decreasing was observed when more HEPES solution was added. So the buffer solution (CH_3CN:HEPES = 3:2, v/v) was used to proceed the following tests.

3.2 The pH dependence of the probe

For reducing the system error caused by the pH, the pH dependent experiment of the compound **1 a**nd compound **1**-Cu^{2+} were proceeded in the solutions (CH_3CN:HEPES = 3:2, v/v) (Fig.S5). The results demonstrated that the fluorescent intensity of both compounds was no obviously changed in the pH 5.5-7.5. Thus the pH range of the solutions in the following tests was controlled in 7.2-7.4 and which also indicated that the probe could be applied in physiological condition.

3.3 The UV-vis spectra responses of the probe

Metal ions selectivity of the probe was investigated by UV-vis spectrometry. Various equivalent metal ions such as Mn^{2+}, Fe^{3+}, Fe^{2+}, K^{+},

Mg^{2+}, Pb^{2+}, Al^{3+}, Zn^{2+}, Ag^{+}, Ni^{2+}, Cd^{2+}, Ca^{2+}, Na^{+}, Cr^{3+}, Co^{2+}, Hg^{2+}, Cu^{2+} (as ClO_4^- salts) were added into solutions (10 μmol/L, CH_3CN:HEPES = 3:2, v/v) of compound **1.** The intensity of maximum absorption peak (439 nm) weaken and slightly red shift was induced by addition of Cu^{2+} and Hg^{2+} ions while other metal ions were not observed obviously (Fig.S6). These results indicated that the probe could selectively recognized Cu^{2+} ions. Then the UV-vis spectra titration experiment of the probe 1 with Cu^{2+} ions was also proceeded (Fig.1). With the increasing concentration of the Cu^{2+} ions, obviously, the sharp decreasing and red shift of the maximum absorption peak at 439 nm were observed in the titration experiments, approximately 6 equivalents Cu^{2+} ions could induce equilibrium. Meanwhile, a new absorption peak at 500 nm appeared which indicated that a new species was formed in the solution.

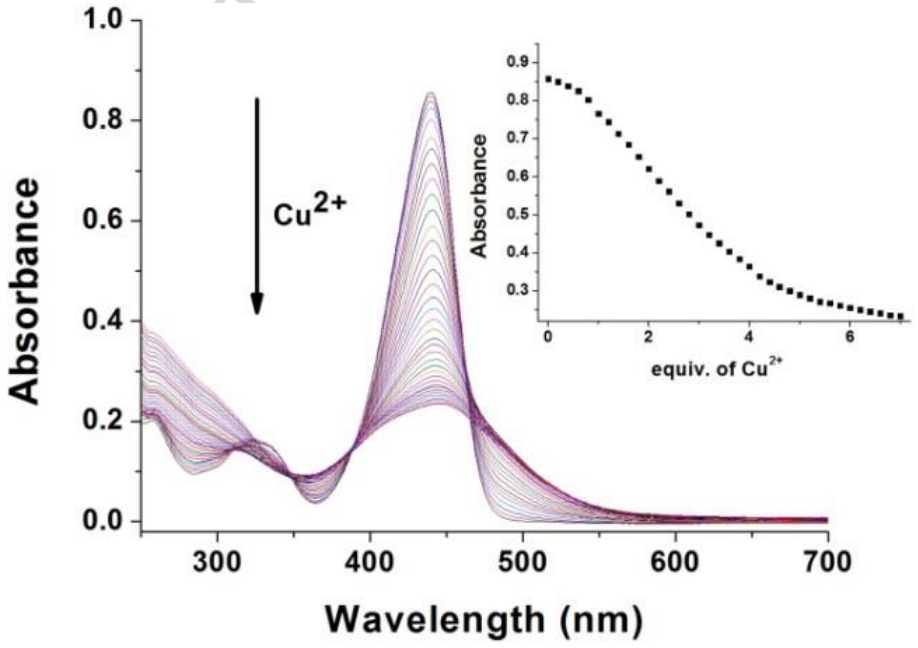


Fig.1 The UV-vis spectra titration experiments of the probe 1 (10 μmol/L) with Cu^{2+} ions in

solution (CH_3CN:HEPES = 3:2, v/v). Insert: UV-vis titration profile of compound 1 upon addition of Cu^{2+}, the absorption spectra were recorded at 439 nm.

For further understanding the binding mode of the compound **1** with the copper ions, a job's plot experiment was carried on (Fig.S7) in a solution (CH_3CN:HEPES = 3:2, v/v). The results indicated that the binding mode of the compound **1** relative to Cu^{2+} was 1:2. The binding constant was also calculated from the UV-vis spectra titration dates by employing the Benesi–Hildebrand (B-H) relation[34-36]. The constant value was calculated as $K = 1.18\times 10^9\ M^{-2}$ (R=0.994) (Fig.S8).

3.4 The fluorescent spectra responses of the probe

The fluorescence experiments of the probe were also investigated. The results demonstrated that the maximum emission peak of the compound 1 appeared at 483 nm (excited at 439 nm). Only the copper ions could lead the fluorescence of the compound **1** (10 μmol/L) to quench dramatically, which was similar to the results that obtained in the UV-vis spectra experiments. The fluorescent quenching rate in the solution (CH_3CN:HEPES = 3:2, v/v) was evaluated up to 98.73%. Other metal ions such as Mn^{2+}, Fe^{3+}, Fe^{2+}, K^+, Mg^{2+}, Pb^{2+}, Al^{3+}, Zn^{2+}, Ag^+, Ni^{2+}, Cd^{2+}, Ca^{2+}, Na^+, Cr^{3+}, Co^{2+}, Hg^{2+}, Cu^{2+} (as ClO_4^- salts) induced the fluorescence almost no responses (Fig.2). As to the mechanism of fluorescence of probe **1** was quenched by Cu^{2+} ions, it might be attributed to the photoinduced electron transfer (PET) mechanism or d-d electron

paramagnetic quenching mechanism of Cu^{2+} ions[37-40].

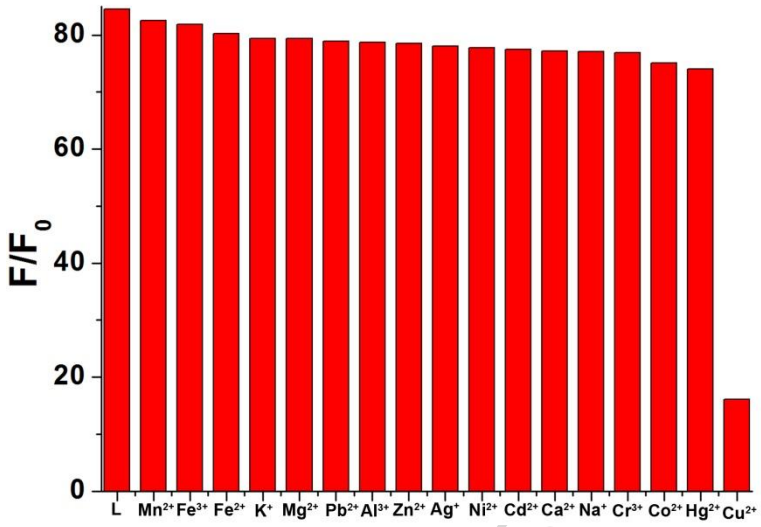


Fig.2 The fluorescence intensity variation (at 483 nm) of the compound **1 (**10 μmol/L) in solutions (CH_3CN:HEPES = 3:2, v/v) after addition of various metal ions (as ClO_4^- salts)

For further proving the selectivity of probe to copper ions, the competitive fluorescent experiments of compound **1** with copper ions under the existences of other different metal ions were carried on (Fig.S9). The fluorescence intensity (at 483 nm) of the probe (10 μmol/L, CH_3CN:HEPES = 3:2, v/v) was not changed distinctly under equivalent various metal ions such as Mn^{2+}, Fe^{3+}, Fe^{2+}, K^+, Mg^{2+}, Pb^{2+}, Al^{3+}, Zn^{2+}, Ag^+, Ni^{2+}, Cd^{2+}, Ca^{2+}, Na^+, Cr^{3+}, Co^{2+} and Hg^{2+} (as ClO_4^- salts). An obviously decreasing of the fluorescent intensity (at 483 nm) relative to compound **1** was observed when the copper ions were added subsequently. This was coincident with the results observed in UV-vis experiments that the probe had a good selectivity to copper ions relative to other metal ions.

The fluorescent titration experiments were further investigated in

solution (CH_3CN:HEPES = 3:2,v/v) (Fig.3). It was indicative that an obviously decreasing of the fluorescent intensity was observed along with the copper ions were added. Approximately 4 equivalents copper ions added lead the fluorescence quenching completely, instead of 2 equivalents that observed in the job's plot experiments, which might be due to the moderate strength binding ability between compound **1** and copper ions. For further interpreting this point, the binding constant (K) for the complex formed between compound 1 and Cu^{2+} ions was determined by employing Benesi–Hildebrand (B-H) relation[34-36], it was calculated as K = 1.91×10^9 M^{-2} (R=0.994) (Fig.S10), which was similar to the result obtained from the UV-vis spectra titration dates.

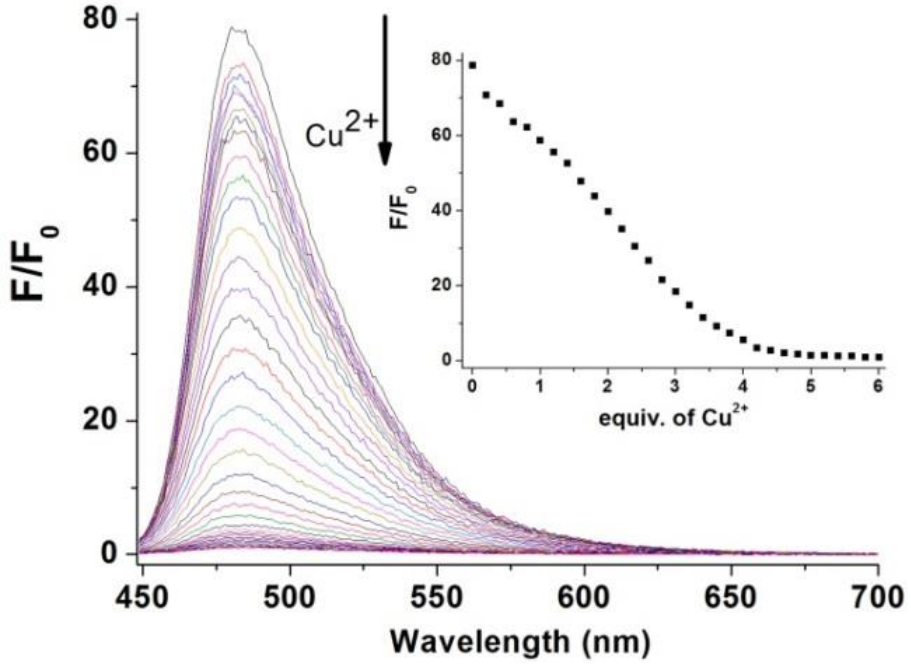


Fig.3 The fluorescence spectra titration experiments of the compound **1** (10 μmol/L) with Cu^{2+} ions (as ClO_4^- salts) in the solution (CH_3CN:HEPES = 3:2, v/v). Insert: the fluorescece titration profile of compounds 1 upon addition of Cu^{2+} (excited at 439 nm, recorded at 483 nm).

In order to investigate the sensitivity of the probe to copper ions, the minimum limit test was performed in the solution (CH_3CN:HEPES = 3:2, v/v). The minimum limit was calculated to 6 μM (0.384 ppm, decreased up to 11.67%) when the concentration of probe was 1 μM. A good fitting curve demonstrated that there was a good linear relationship between the fluorescent intensity of probe and the concentration of copper ions (Fig.4).

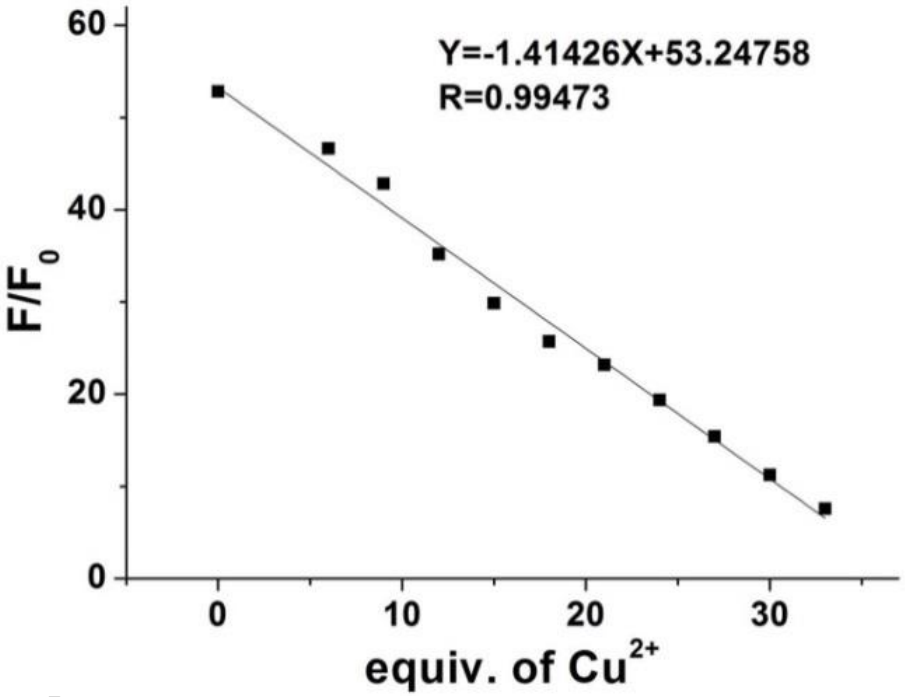


Fig.4 The fitting curve of the fluorescent intensity (at 483 nm) of the probe (1 μM) relative to copper ions in the solution (CH_3CN:HEPES = 3:2, v/v).

3.5 The Fluorescent images of probe in living cells

The fluorescent methods to monitor the biological species are significant. Fluorescence images of HepG-2 cells were observed under a Leica DMI8 inverted fluorescence microscopy. Firstly, HepG-2 cells were

incubated for 24 hours in RPMI-1640 culture medium containing 10% fetal bovine serum. Then compound **1** (2 μM) was added and continually incubated for 3 hours. It showed a clear green-blue intracellular fluorescence (Fig 5a), which demonstrated that compound **1** was cell permeable. After stained with the probe for 3 hours and rinsed with PBS buffer three times, HepG-2 cells were supplemented with 5 equivalents of Cu^{2+} for another 5 minutes; a remarkable decreasing of the fluorescence intensity was observed (Fig 5b). The cells were still alive and the probe showed no obviously toxic and side effects during the imaging experiments (approximately 2 hours). These results were indicative that compound **1** could be used to detect intracellular Cu^{2+} ions. Therefore, this probe had potential application value to investigate biological processes involving Cu^{2+} ions within living cells.

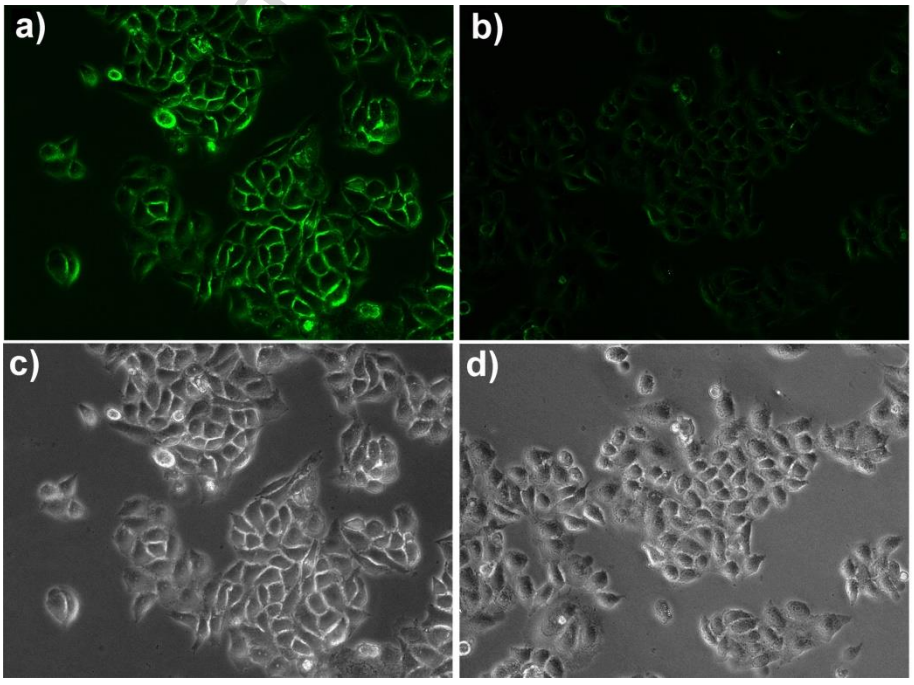


Fig.5 Fluorescence images of HepG-2 cells incubated with the probe (2 μM) (a), further incubated with addition of $Cu(ClO_4)_2$ (5 equivalents) (b) , and their corresponding bright field images (c, d).

4. Conclusions

In summary, based on coumarin carbohydrazide and 1H-indazole-3-carbaldehyde, a new fluorescent probe **1** was synthesized for detection of Cu^{2+} ion. The UV-vis and fluorescent spectral experiments indicated that the probe has excellent selectivity and sensitivity toward Cu^{2+} ions. The 1:2 binding ratio of the compound **1** with Cu^{2+} ions was confirmed by the jobs' plot experiments. The results of competitive experiments further confirmed its outstanding selectivity in the presence of other metal ions. The detection limit of the probe to copper ion was 0.384 ppm. The Fluorescent microscopy experiments demonstrated that probe **1** could be used to investigate biological processes involving Cu^{2+} ions within living cells. Its other applications in life science are still underway.

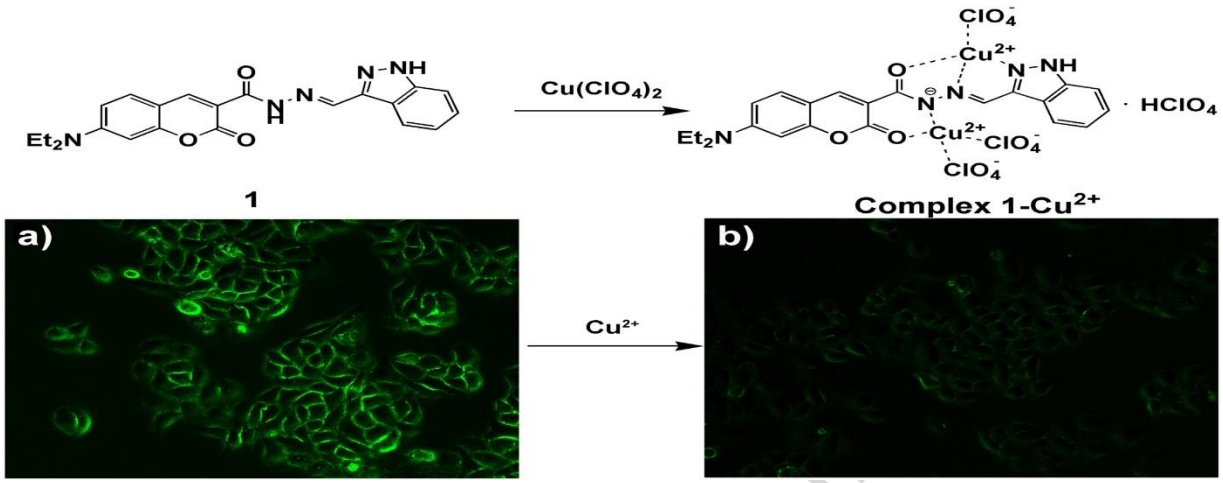
Acknowledgments:

This work was financially supported by the National Natural Science Foundation of China (No.21371148), the Doctoral Scientific Research initiation Foundation of Xinxiang Medical University (No.XYBSKYZZ201649), the Hebei Key Laboratory of Forensic Medicine (No.KF201601), and the Disciplinary group of Psychology and Neuroscience, Xinxiang Medical University.

Referrences

1. Y. Li, X. Zhang, B. Zhu, J. Xue, Z. Zhu and W. Tan, *Analyst*, 2011, **136**, 1124-1128.
2. M.-Z. Tian, M.-M. Hu, J.-L. Fan, X.-J. Peng, J.-Y. Wang, S.-G. Sun and R. Zhang, *Bioorganic & Medicinal Chemistry Letters*, 2013, **23**, 2916-2919.
3. E. Wang, Y. Zhou, Q. Huang, L. Pang, H. Qiao, F. Yu, B. Gao, J. Zhang, Y. Min and T. Ma, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2016, **152**, 327-335.
4. J. H. Kim, J. Y. Noh, I. H. Hwang, J. Kang, J. Kim and C. Kim, *Tetrahedron Letters*, 2013, **54**, 2415-2418.
5. W. Wang, A. Fu, J. You, G. Gao, J. Lan and L. Chen, *Tetrahedron*, 2010, **66**, 3695-3701.
6. H. S. Jung, P. S. Kwon, J. W. Lee, J. I. Kim, C. S. Hong, J. W. Kim, S. Yan, J. Y. Lee, J. H. Lee, T. Joo and J. S. Kim, *Journal of the American Chemical Society*, 2009, **131**, 2008-2012.
7. C. Liu, X. Jiao, S. He, L. Zhao and X. Zeng, *Organic & Biomolecular Chemistry*, 2017, **15**, 3947-3954.
8. S. Lutsenko, A. Gupta, J. L. Burkhead and V. Zuzel, *Archives of Biochemistry and Biophysics*, 2008, **476**, 22-32.
9. S. G. Kaler, *Nat Rev Neurol*, 2011, **7**, 15-29.
10. D. G. Barceloux, *Journal of Toxicology: Clinical Toxicology*, 1999, **37**, 217-230.
11. X. Guan, W. Lin and W. Huang, *Organic & Biomolecular Chemistry*, 2014, **12**, 3944-3949.
12. C.-Y. Chou, S.-R. Liu and S.-P. Wu, *Analyst*, 2013, **138**, 3264-3270.
13. S. Yin, V. Leen, S. V. Snick, N. Boens and W. Dehaen, *Chemical Communications*, 2010, **46**, 6329-6331.
14. P. Ding, S. Li, L. Yang, J. Cheng, Z. Wang, Y. Zhao and Y. Ye, *Journal of Fluorescence*, 2015, **25**, 15-24.
15. P. Das, S. Ganguly, M. Bose, S. Mondal, A. K. Das, S. Banerjee and N. C. Das, *Materials Science and Engineering: C*, 2017, **75**, 1456-1464.
16. D. Liu, S. Ji, H. Li, L. Hong, D. Kong, X. Qi and D. Ding, *Faraday Discussions*, 2017, **196**, 377-393.
17. S. Gharami, D. Sarkar, S. Acharyya and T. K. Mondal, *Journal of Fluorescence*, 2016, **26**, 2113-2118.
18. Q. Li, C. Xie, R. Zeng, X. Li and D. Tang, *Inorganic Chemistry Communications*, 2017, **78**, 9-12.
19. Y. Singh, S. Arun, B. K. Singh, P. K. Dutta and T. Ghosh, *RSC Advances*, 2016, **6**, 80268-80274.
20. B. K. Momidi, V. Tekuri and D. R. Trivedi, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2017, **180**, 175-182.
21. J. S. Becker, A. Matusch, C. Depboylu, J. Dobrowolska and M. V. Zoriy, *Analytical Chemistry*, 2007, **79**, 6074-6080.
22. Z. Fang, T. Guo and B. Welz, *Talanta*, 1991, **38**, 613-619.
23. Q. U. Ying-Juan, Y. H. Zhai, Y. N. Wang and S. O. Chemistryamp, *Chinese Journal of Spectroscopy Laboratory*, 2013, **30**, 2283-2286.

24. D. T. Quang and J. S. Kim, *Chemical Reviews*, 2010, **110**, 6280-6301.
25. M.-J. Peng, Y. Guo, X.-F. Yang, L.-Y. Wang and J. An, *Dyes and Pigments*, 2013, **98**, 327-332.
26. J. Li, C.-F. Zhang, S.-H. Yang, W.-C. Yang and G.-F. Yang, *Analytical Chemistry*, 2014, **86**, 3037-3042.
27. G. He, J. Li, Z. Wang, C. Liu, X. Liu, L. Ji, C. Xie and Q. Wang, *Tetrahedron*, 2017, **73**, 272-277.
28. G. He, J. Li, L. Yang, C. Hou, T. Ni, Z. Yang, X. Qian and C. Li, *PLOS ONE*, 2016, **11**, e0148026.
29. P. Wu, M. Jiang, X. Hu, J. Wang, G. He, Y. Shi, Y. Li, W. Liu and J. Wang, *RSC Advances*, 2016, **6**, 27944-27951.
30. O. García-Beltrán, B. Cassels, C. Pérez, N. Mena, M. Núñez, N. Martínez, P. Pavez and M. Aliaga, *Sensors*, 2014, **14**, 1358.
31. M. H. Kim, H. H. Jang, S. Yi, S.-K. Chang and M. S. Han, *Chemical Communications*, 2009, DOI: 10.1039/B908638B, 4838-4840.
32. Y. Ma, W. Luo, P. J. Quinn, Z. Liu and R. C. Hider, *Journal of Medicinal Chemistry*, 2004, **47**, 6349-6362.
33. H. Takechi, Y. Oda, N. Nishizono, K. Oda and M. Machida, *CHEMICAL & PHARMACEUTICAL BULLETIN*, 2000, **48**, 1702-1710.
34. Y. Xiang, A. Tong, P. Jin and Y. Ju, *Organic Letters*, 2006, **8**, 2863-2866.
35. S. Dalapati, M. A. Alam, S. Jana and N. Guchhait, *Journal of Fluorine Chemistry*, 2011, **132**, 536-540.
36. C. Zhang, B. Gao, Q. Zhang, G. Zhang, S. Shuang and C. Dong, *Talanta*, 2016, **154**, 278-283.
37. R. Krämer, *Angewandte Chemie International Edition*, 1998, **37**, 772-773.
38. L. Fabbrizzi, M. Licchelli, P. Pallavicini, A. Perotti and D. Sacchi, *Angewandte Chemie International Edition in English*, 1994, **33**, 1975-1977.
39. L. Fabbrizzi, M. Licchelli, P. Pallavicini, A. Perotti, A. Taglietti and D. Sacchi, *Chemistry – A European Journal*, 1996, **2**, 75-82.
40. A. W. Varnes, R. B. Dodson and E. L. Wehry, *Journal of the American Chemical Society*, 1972, **94**, 946-950.



Graphical abstract

Highlights:

a) Based on the coumarin-type Schiff base derivative a novel fluorescent probe for copper ions detection was produced.
b) The probe displayed high selective and low detection limit for Cu^{2+} ions.
c) The probe could be used to investigate biological processes involving Cu^{2+} ions within HepG-2 cells.