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ORIGINAL ARTICLE

A New Fluorescent “Turn-Off” Coumarin-Based Chemosensor: Synthesis, Structure and Cu-Selective Fluorescent Sensing in Water Samples

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Abstract We report the synthesis and characterization two coumarin-based fluorescence probes, N′-{[7-(diethylamino)-2-oxo-2H-chromen-3-yl]carbonyl}pyridine-3-carbohydrazide (**3**) and N′-benzoyl-7-(diethylamino)-2-oxo-2H-chromene-3-carbohydrazide (**4**), proposed as a novel fluorescent chemosensor. The two probes designed showed an instant turn-off fluorescence response to Cu^{2+} over other metal ions in ethanol-water mixture based on intramolecular charge transfer (ICT). It was found that pyridine-analogue coumarin is highly selective and sensitive sensor for Cu^{2+}. The **3** sensor coordinates Cu^{2+} in 1:1 stoichiometry with a binding constant, $K_a = 5.22\ M^{-1}$ and the detection limit was calculated 1.97×10^{-9} M.

Keywords Fluorescence · Copper (II) sensor · Coumarin · ICT

Introduction

Although copper is an essential element for proteins and receptors, accumulation of copper over time in human body can cause serious illnesses such as Alzheimer’s and Parkinson’s disease [1, 2]. The average concentration of copper in human body is 100–150 mg/dL (15.7–23.6 mM) [3], and the recommended intake is 0.9 mg/day [4, 5]. The acceptable limit of copper, by U.S. Environmental Protection Agency (EPA), is 1.3 ppm in drinking water [3]. Thus, copper levels of drinking water have to be monitored efficiently. To this end, the design of new optical materials such as organic fluoroionophores [6–8] and functionalized quantum dots [9–11] or nanowires [12, 13] is currently a task of synthetic chemistry. Coumarin derivatives are one of the most investigated fluorophores because of strong emission in the visible region, large Stokes shifts, high photostability, and high fluorescence quantum yields [14–16].

Coumarin fluorophores could act as fluoroionophores as a result of non-radiative processes such as excited-state intramolecular proton transfer (ESIPT), intramolecular charge transfer (ICT), photo-induced electron transfer (PET), and isomerization mechanism. Because the carbon in the 3-position has a partial negative charge in coumarins, intramolecular charge transfer (ICT) mechanism could be modulated by the introduction of an electron-donating substituent at the 7-position [17] or an electron-withdrawing substituent at the 3-position [3]. Attachment of a hydrazide chain to coumarin moiety at 3-position could both enhance the ICT and set up donor set selective for copper via the carbonyl oxygen and amide nitrogen.

Herein, we report a new coumarin-based fluorescent Cu^{2+} probes, **3** and **4**, including electron-withdrawing hydrazide chain group at 3-position by using cross-validation data for 2-pyridinecarboxylic and benzoyl hydrazine obtained from literature. To the best of our knowledge, only a few coumarin-based fluorescent sensors have been reported for detection of Cu^{2+} in water [18]. We also studied selective cation binding properties of sensors in ethanol-water mixture in 1:100 ratio.

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Experimental

Material and Method

1×10^{-3} M stock solution of the Na^+, K^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{3+}, Ni^{2+}, Cu^{2+}, Zn^{2+} and Cd^{2+} (purchased from Merck), and Cr^{3+}, Co^{2+} (from Advanced Resarche Chemicals) ions were prepared from 1000 ppm ICP-OES standards stabilized with 0.1 HNO_3. The detection and quantification limits (LOD and LOQ) of the compounds for Cu^{2+} were determined from the following equation:

$$\mathrm{Limit} = \mathrm{K} \times \mathrm{Sd}/\mathrm{S}$$

Where K = 3 for LOD and 10 for LOQ, Sd is the standard deviation of the blank solution; and S is the slope of the calibration curve [19]. The melting points were measured on capillary tubes on a Stuart SMP30 melting point apparatus and uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on Agilent 400 MHz spectrometer in DMSO-d_6. Chemical shifts are reporded relative to TMS. The elemental compositions were evaluated on a Leco Truspec Micro analyzer; the experimental values were in agreement (±0.4%) with calculated ones. All reactions were monitored by TLC using precoated aluminum sheets (silica gel 60 F 2.54 0.2 mm thickness). 7-(diethylamino)-2-oxo-2H-chromene-3-carboxylic acid (**1**) and 3-(1H–benzotriazol-1-ylcarbonyl)-7-(diethylamino)-2H–chromen-2-one (**2**) were prepared according to literature method [20, 21]. Analytical parameters of Cu^{2+} selective sensors were obtained from the work conducted by Zhou and his team [22].

Synthesis of Sensors

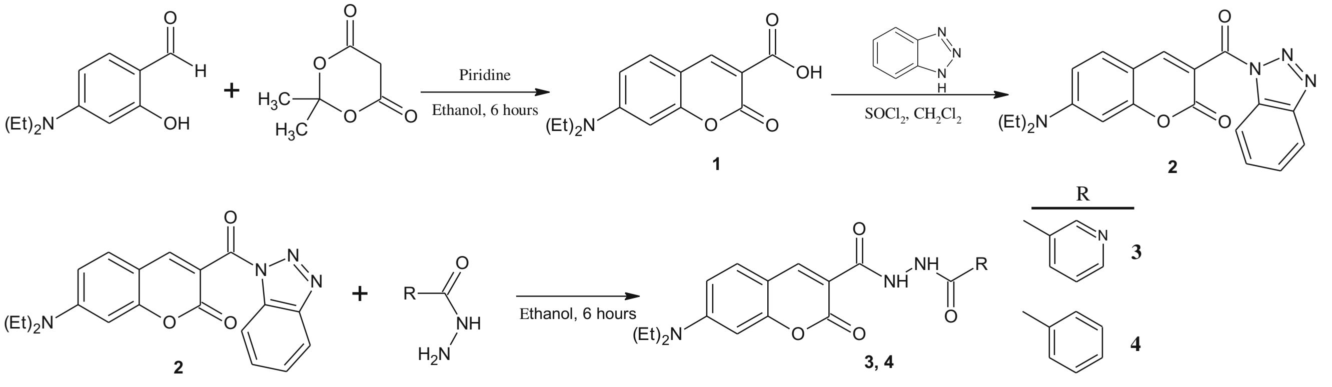
Synthesis Compounds 3 and 4

N′-Benzoyl-7-(diethylamino)-2-oxo-2H-chromene-3-carbohydrazide (**4**) was synthesized following the same procedure adopted for N′-{[7-(diethylamino)-2-oxo-2H-chromen-3-yl]carbonyl}pyridine-3-carbohydrazide (**3**).

A solution of 3-(1*H*–benzotriazol-1-ylcarbonyl)-7-(diethylamino)-2*H*–chromen-2-one (**2**) (0.01 mol) and nicotinic hydrazide or benzhydrazide (0.011 mol) in ethanol (10 mL) was placed in a round-bottom flask. The mixture was refluxed for 6 h (monitored by TLC), and the mixture was cooled to room temperature. The solid product was filtrated off and washed with hot ethanol to obtain a microcrystalline product.

N′-{[7-(Diethylamino)-2-oxo-2H-chromen-3-yl]carbonyl}pyridine-3-carbohydrazide (3): Yield: 2.54 g (67%), M.p.: 266–267 °C, ^{1}H NMR (400 MHZ, DMSO-d_6): 1.13 (t, J = 6.8 Hz, 6H, CH_3), 3.90 (q, J = 6.8 Hz, 4H, CH_2), 6.63 (s, 1H, Ar-H), 6.82 (d, J = 6.4 Hz, 1H, Ar-H), 7.55 (d, J = 6.8 Hz, 1H, Ar-H), 7.73 (d, J = 9.2 Hz, 1H, Ar-H), 8.24 (d, J = 7.6 Hz, 1H, Ar-H), 8.74 (d, J = 8.4 Hz, 2H, Ar-H), 9.03 (s, 1H, Ar-H), 10.47 (s, 1H, NH), 11.12 (s, 1H, NH). ^{13}C NMR (100 MHZ, DMSO-d_6): 12.77 (CH_3), 44.87 (CH_2), 96.38, 108.13, 108.29, 110.81, 124.06, 128.45, 132.33, 135.79, 149.01 (coumarin C-4), 153.31 (coumarin-C-3), 157.94 (C = O), 161.65 (C = O), 163.79 (C = O). Anal. Calcd. For $C_{20}H_{20}N_4O_4$: C, 63.15; H, 5.30; N, 14.73. Found: C, 63.08; H, 5.25; N, 14.68.

N′-Benzoyl-7-(diethylamino)-2-oxo-2H-chromene-3-carbohydrazide (4): Yield: 2.73 g (72%), M.p.: 263–264 °C, 1H NMR (400 MHZ, DMSO-d_6): 1.12 (t, J = 6.8 Hz, 6H, CH_3), 3.48 (q, J = 6.8 Hz, 4H, CH_2), 6.62 (s, 1H, Ar-H), 6.81 (d, J = 6.8 Hz, 1H, Ar-H), 7.49 (t, J = 7.2 Hz, 2H, Ar-H), 7.57 (d, J = 7.2 Hz, 1H, Ar-H), 7.72 (d, J = 6.8 Hz, 1H, Ar-H), 7.92 (d, J = 6.8 Hz, 2H, Ar-H), 8.70 (s, 1H, Ar-H), 10.40 (s, 1H, NH), 10.85 (s, 1H, NH). ^{13}C NMR (100 MHZ, DMSO-d_6): 12.75 (CH_3), 44.87 (CH_2), 96.36, 108.11, 108.41, 110.79, 128.01, 128.91, 132.31, 132.66, 148.89 (coumarin C-4), 153.28 (coumarin-C-3), 157.91 (C = O), 161.76 (C = O), 165.27 (C = O). Anal. Calcd. For $C_{21}H_{21}N_3O_4$: C, 66.48; H, 5.58; N, 11.08. Found: C, 66.39; H, 5.50; N, 11.01.



Scheme 1 Synthetic route of sensors

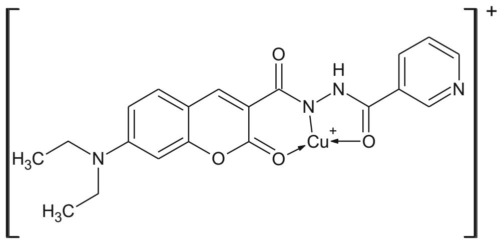


Fig. 1 Proposed coordination mode of Cu^{2+} ion to **3** sensor

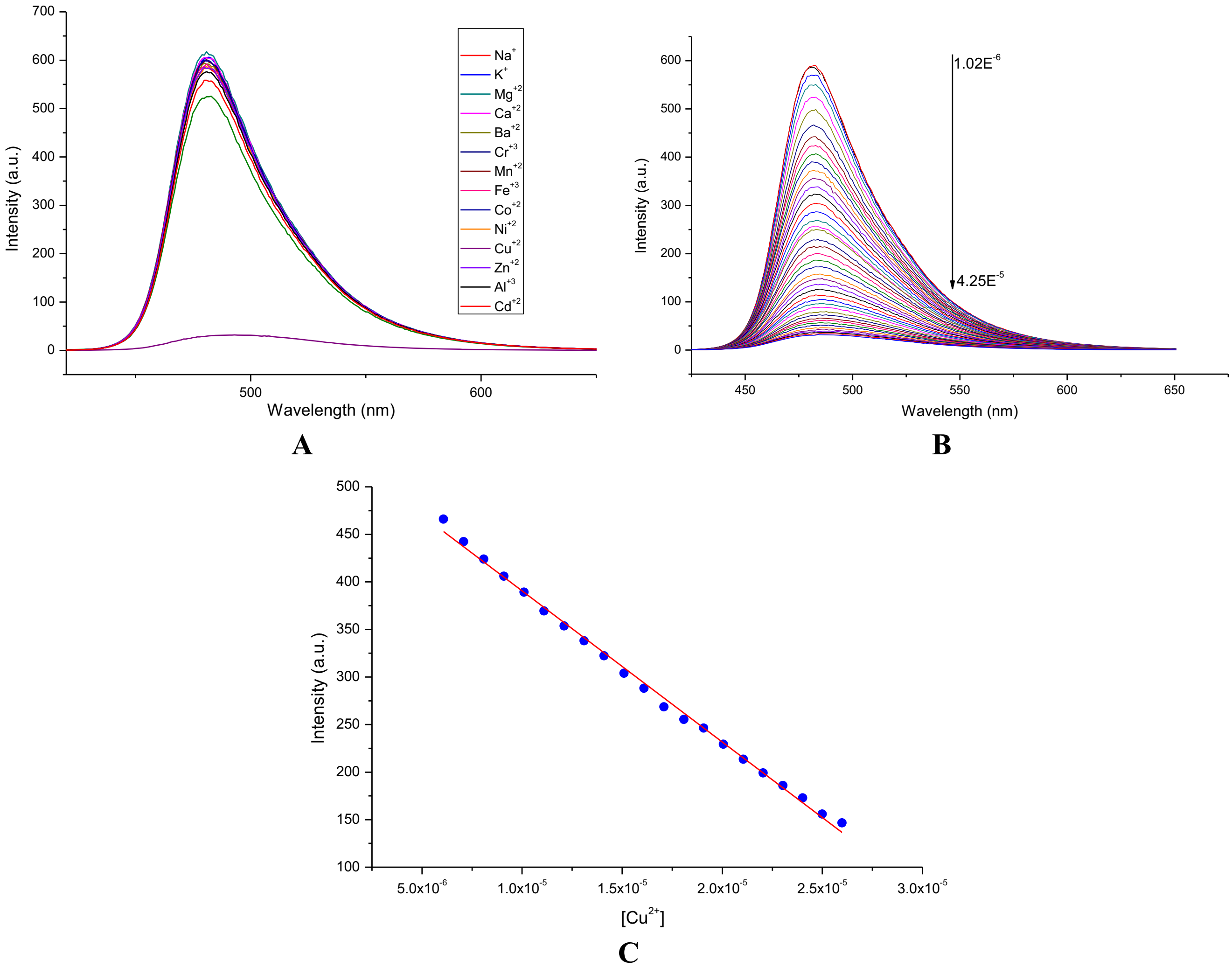
Fluorometric Assays

Flourometric measurements of the sensors were performed on Agilent Cary Eclipse in a 1-cm quartz fluorescence cuvette at ambient temperature. A typical flourometric measurement was recorded after an incubation time of 10 min at 680 V PMT voltage. Excitation and emission slits were 5 nm. The sensors were investigated for pH 4–8, and the optimum pH value was determined as pH 5. Because of the low solubility of the ligand in water, the fluorometric experiments were investigated in ethanol-acetate buffer solution (pH 5, 50 mM) in 1:100 ratio. 100 μL solution of the Cu^{2+} ion was gradually added in to the quartz cuvette initially containing 0.2 mL solution of **3** and **4**, and 2.7 mL buffer solution.

Results and Discussion

Chemistry

Firstly, 7-(diethylamino)-2-oxo-2H-chromene-3-carboxylic acid (**1**) was prepared by the reaction of 4-(diethylamino) salicylaldehyde and 2,2-dimethyl-1,3-dioxane-4,6-dione in ethanol (containing 0.5 mL of pyridine, as catalyst). Then 7-(diethylamino)-2-oxo-2*H*-chromene-3-carboxylic acid (**1**)



A

B

C

Fig. 2 **a** Change in fluorescence spectrum of **3** in the presence of some metal ions in ethanol/water mixture with excitation at 332 nm; **b** fluorescence titration of **3** with Cu^{2+}; **c** linear working curve of **3**/Cu^{2+} titration experiments

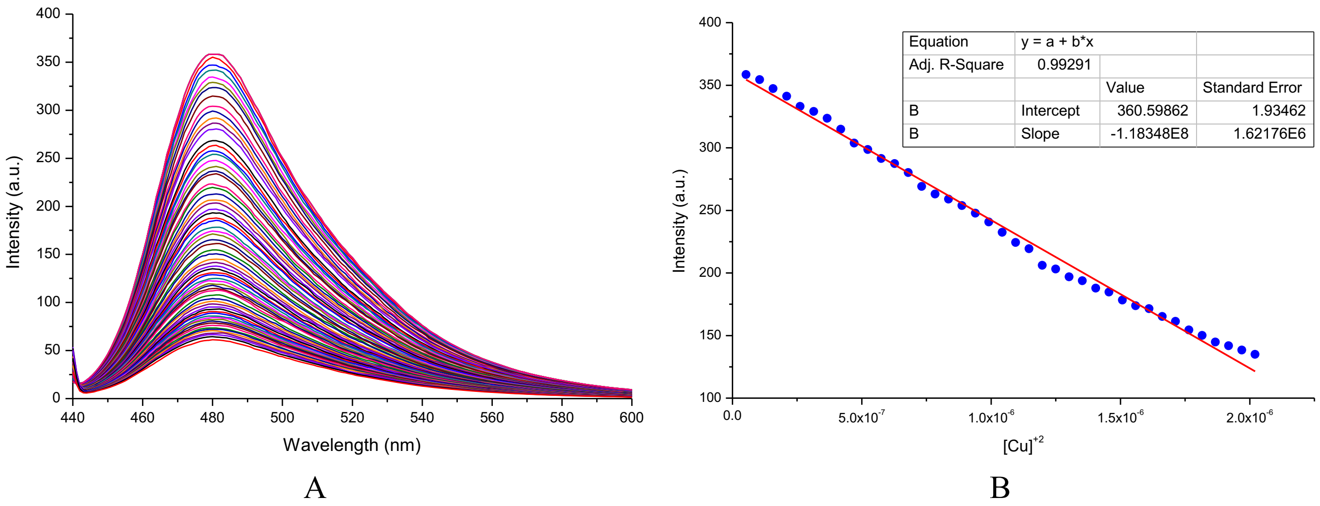


Fig. 3 **a** fluorescence titration of 4 with Cu^{2+} with excitation at 330 nm; **b** lineer working curve of 4/Cu^{2+} titration experiments

was reacted with 1H–benzotriazole in dichloromethane in the presence of thionyl chloride to obtain 3-(1*H*–benzotriazol-1-ylcarbonyl)-7-(diethylamino)-2*H*–chromen-2-one (**2**). The benzotriazole group is known as an easy leaving group. This group offers many advantages to researchers on synthetic applications [21]. The treatment of 3-(1*H*–benzotriazol-1-ylcarbonyl)-7-(diethylamino)-2*H*–chromen-2-one (**2**) with nicotinic hydrazide or benzhydrazide in ethanol resulted in the target compounds **3** and **4** (Scheme 1).

Fluorometric Assays

In order to verify the selectivity, photochemical properties of the sensors were investigated by the addition of metal ions in ethanol-water (1:100) solution. As seen in Fig. S5, the compounds exhibited high Stokes shifts (~150 nm), excitation and emission wavelengths of **3** is recorded as 332 and 480 nm (λ_{ex} = 330 and λ_{em} = 480 for **4**), respectively. There were not a significant change of the emission band in the presence of Na^+, K^+, Mg^{2+}, Ca^{2+}, Cr^{3+}, Mn^{2+}, Co^{2+}, Fe^{3+}, Ni^{2+}, Zn^{2+} and Cd^{2+}, and the fluorescence intensity of the sensors was quenched in emission intensity of the **3** totally quenched by coordination of Cu^{2+} (Fig. 1a). These results can be explained by fluorescence-quenching nature of paramagnetic Cu^{2+} ion via enhanced spin–orbital coupling, or an energy or electron transfer mechanism [23, 24]. A primary advantage of the sensor is that it enables higher sensitivity with very low amount of organic solvent. To obtain analytical parameters of the **3**, the fluorescence titration experiments were conducted in the same media (Fig. 2b and c). **3** showed no change in fluorescence peak position located at 480 nm during the titration experiments. The LOD and LOQ values of the **3** toward Cu^{2+} ion are calculated as 1.97 × 10^{-9} M and 6.58 × 10^{-9}, respectively. The obtained LOD value for **3** was found comparable to other reported values of the coumarin-based ICT sensors and optical nanomaterials (see Table S1) [25–28]. In order to determine the K_d value, the Benesi-Hildebrand method was used for sensors. The K_d value of the sensor toward Cu^{2+} ion calculated from the plot of log $(I-I_o)/I_{max}-I$ versus log $[Cu^{2+}]$. The slope value (1.21) indicates that 1:1 binding ratio of Cu^{2+} ions to **3** and log K_d value is calculated as 5.22 M^{-1}.

Table 1 The LOD and LOQ values of investigated sensors

Derivative	LOD	LOQ
3-pyridinecarboxylic (3)	1.97 × 10^{-9}	6,58 × 10^{-9}
2-pyridinecarboxylic[(a)]	0.1 × 10^{-9}	—[(b)]
Benzoyl hydrazine (4)	0.3 × 10^{-9}	0.9 × 10^{-9}
	0.6 × $10^{-9(1)}$	— [(b)]

[a] from UV-vis.-based literature data [22]

[b] not available in literature data

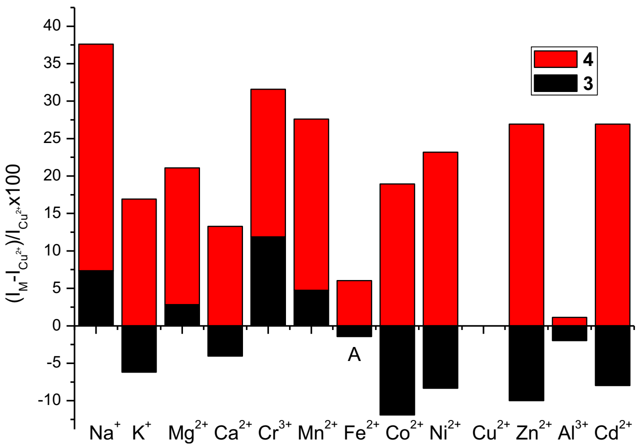


Fig. 4 The column diagram of the relative intensity of **3** (*black bars*) and **4** (*red bars*) at aqueous media

Fluorescence titration method was also used to determine the analytical values of 4 (Fig. 3). The LOD and LOQ values of 4 toward Cu^{2+} were calculated as 0.3×10^{-9} and LOQ 0.90×10^{-9}, respectively. The K_d value and metal:ligand ratio of the Cu^{2+} were calculated as 6.03 and 1:2 by Benesi-Hildebrand method, respectively. The LOD and LOQ values of investigated sensors were tabulated in Table 1 with the cross-validation data set.

The competitive binding experiments with different metal ions and Cu^{2+} ion were also studied. In Fig. 4, the black bars represent relative intensity of **3** in the presence of Cu^{2+} ions in aqueous solutions together with other metal ions in 1:1 M ration such as Na^+, K^+, Mg^{2+}, Ca^{2+}, Cr^{3+}, Mn^{2+}, Co^{2+}, Fe^{3+}, Ni^{2+}, Zn^{2+} and Cd^{2+}. While it is clearly seen from Fig. 4 that no significant variation was observed in the fluorescence quenching efficiency for **3**, the large number of the metal ions interfered with the quenching of Cu^{2+} by **4** (selectivity experiments of the 2-pyridinecarboxylic and benzoyl hydrazine derivatives in [22] were monitored by UV-vis spectroscopy for Ca^{2+}, Fe^{3+}, Mg^{2+}, Na^+, and Ni^{2+}).

Conclusion

In summary, we have successfully developed a novel coumarin-based fluorescent ratiometric chemosensor with ICT mechanism for recognition of Cu^{2+} in ethanol–water solutions (1:100, *v*/v, acetate, pH = 5.0). The fluorescence titration experiments have been conducted for validation of analytical data of benzoyl hydrazine (**4**), and the result was found acceptable in nM levels despite different analysis media. The effect of the position of N atom in pyridine to sensitivity was also investigated by means of the LOD value, and it was found that 2-pyridinecarboxylic derivative showed ~20 fold sensitivity compared to 3-pyridinecarboxylic derivative (LOD: 1.97×10^{-9} M). The competitive selectivity experiments of compounds have been examined for Na^+, K^+, Mg^{2+}, Ca^{2+}, Cr^{3+}, Mn^{2+}, Co^{2+}, Fe^{3+}, Ni^{2+}, Zn^{2+} and Cd^{2+} ions, and it was found that **3** has higher selectivity toward Cu^{2+} than validation compounds.

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References

1. Yokel RA (2002) Brain uptake, retention, and efflux of aluminum and manganese. Environ Health Perspect 110:699–704
2. Yasui M, Kihira T, Ota K (1992) Calcium, magnesium and aluminum concentrations in Parkinson's disease. Neurotoxicology 13: 593–600
3. Helal A, Or Rashid MH, Choi C-H, Kim H-S (2011) Chromogenic and fluorogenic sensing of Cu2+ based on coumarin. Tetrahedron 67:2794–2802. doi:10.1016/j.tet.2011.01.093
4. Georgopoulos PG, Roy A, Yonone-Lioy MJ, Opiekun RE, Lioy PJ (2001) Environmental copper: its dynamics and human exposure issues. J Toxicol Environ Heal Part B 4:341–394. doi:10.1080/109374001753146207
5. Rafter GW (1987) Rheumatoid arthritis: a disturbance in copper homeostasis. Med Hypotheses 22:245–249. doi:10.1016/0306-9877(87)90190-3
6. Yang J, Yuan Z, Yu G et al (2016) Single chemosensor for double analytes: spectrophotometric sensing of Cu2+ and fluorogenic sensing of Al3+ under aqueous conditions. J Fluoresc 26:43–51. doi:10.1007/s10895-015-1710-2
7. Kong F, Liu Q, Wu X et al (2011) 2-(4-Formylphenyl) phenanthroimidazole as a colorimetric and fluorometric probe for selective fluoride ion sensing. J Fluoresc 21:1331–1335. doi:10.1007/s10895-011-0858-7
8. Tang R, Lei K, Chen K et al (2011) A rhodamine-based off–on fluorescent chemosensor for selectively sensing Cu(II) in aqueous solution. J Fluoresc 21:141–148. doi:10.1007/s10895-010-0698-x
9. Yuan C, Liu B, Liu F et al (2014) Fluorescence "turn on" detection of mercuric ion based on Bis(dithiocarbamato)copper(II) complex functionalized carbon Nanodots. Anal Chem 86:1123–1130. doi:10.1021/ac402894z
10. Salinas-Castillo A, Ariza-Avidad M, Pritz C et al (2013) Carbon dots for copper detection with down and upconversion fluorescent properties as excitation sources. Chem Commun 49:1103. doi:10.1039/c2cc36450f
11. Wang F, Gu Z, Lei W et al (2014) Graphene quantum dots as a fluorescent sensing platform for highly efficient detection of copper(II) ions. Sensors Actuators B Chem 190:516–522. doi:10.1016/j.snb.2013.09.009
12. Tang B, Niu J, Yu C et al (2005) Highly luminescent water-soluble CdTe nanowires as fluorescent probe to detect copper(ii). Chem Commun 4184. doi:10.1039/b502978c
13. Mu S, Chang JC, Lee S-T (2008) Silicon nanowires-based fluorescence sensor for Cu(II). Nano Lett 8:104–109. doi:10.1021/nl072164k
14. Bekhradnia A, Domehri E, Khosravi M (2016) Novel coumarin-based fluorescent probe for selective detection of Cu(II). Spectrochim Acta Part A Mol Biomol Spectrosc 152:18–22. doi:10.1016/j.saa.2015.07.029
15. Song Z-K, Dong B, Lei G-J et al (2013) Novel selective fluorescent probes for sensing Zn2+ ions based on a coumarin Schiff-base. Tetrahedron Lett 54:4945–4949. doi:10.1016/j.tetlet.2013.07.015
16. Wang H, Guo LE, Li XM et al (2015) Coumarin-based turn-on fluorescence probes for highly selective detection of Pi in cell culture and *Caenorhabditis elegans*. Dyes Pigments 120:293–298. doi:10.1016/j.dyepig.2015.04.031
17. Lin W, Yuan L, Cao X et al (2008) A coumarin-based chromogenic sensor for transition-metal ions showing ion-dependent bathochromic shift. Eur J Org Chem 2008:4981–4987. doi:10.1002/ejoc.200800667
18. Khatua S, Choi SH, Lee J et al (2009) Highly selective fluorescence detection of Cu 2+ in water by chiral dimeric Zn 2+ complexes through direct displacement. Inorg Chem 48:1799–1801. doi:10.1021/ic802314u
19. Ma Y, Chen H, Wang F et al (2014) A highly sensitive and selective ratiometric fluorescent sensor for Zn2+ ion based on ICT and FRET. Dyes Pigments 102:301–307. doi:10.1016/j.dyepig.2013.11.011
20. Katritzky A, Ibrahim T, Tala S et al (2011) Synthesis of coumarin conjugates of biological thiols for fluorescent detection and estimation. Synthesis-Stuttgart 2011:1494–1500. doi:10.1055/s-0030-1259991

21. Kahveci B, Yilmaz F, Mentese E, Ülker S (2015) Microwave-assisted synthesis of some new coumarin derivatives including 1,2,4-triazol-3-one and investigation of their biological activities. Chem Heterocycl Compd 51:447–456. doi:10.1007/s10593-015-1714-5
22. Ma L, Xu Y, Wang K et al (2015) Synthesis and recognition properties for copper ions and cyanide anions of two coumarin hydrazide compounds. Inorg Chem Commun. doi:10.1016/j.inoche.2015.05.019
23. Lin W, Yuan L, Cao Z et al (2009) Fluorescence enhancement of coumarin–quinoline by transition metal ions: detection of paramagnetic Ni2+ and Co2+. Dyes Pigments 83:14–20. doi:10.1016/j.dyepig.2009.03.006
24. Allendorf MD, Bauer CA, Bhakta RK, Houk RJT (2009) Luminescent metal–organic frameworks. Chem Soc Rev 38:1330. doi:10.1039/b802352m
25. Mukherjee S, Talukder S (2016) A coumarin-based luminescent chemosensor for recognition of Cu2+ and its In-situ complex for CN− sensing via Cu2+ displacement approach. J Fluoresc. doi:10.1007/s10895-016-1974-1
26. Karuk Elmas ŞN, Ozen F, Koran K et al (2016) Coumarin based highly selective "off-on-off" type novel fluorescent sensor for Cu2+ and S2− in aqueous solution. J Fluoresc. doi:10.1007/s10895-016-1972-3
27. Yeh J-T, Chen W-C, Liu S-R, Wu S-P (2014) A coumarin-based sensitive and selective fluorescent sensor for copper(ii) ions. New J Chem 38:4434. doi:10.1039/C4NJ00695J
28. Razi SS, Srivastava P, Ali R et al (2015) A coumarin-derived useful scaffold exhibiting Cu2+ induced fluorescence quenching and fluoride sensing (on-off-on) via copper displacement approach. Sensors Actuators B Chem 209:162–171. doi:10.1016/j.snb.2014.11.082