A Benzothiazole-Based Fluorescence Turn-on Sensor for Copper(II)

Gyeongjin Kim \(^1\) · Donghwan Choi \(^1\) · Cheal Kim \(^1\)

Received: 30 March 2021 / Accepted: 20 May 2021 / Published online: 26 May 2021
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2021

Abstract

A new benzothiazole-based chemosensor BTN (1-((Z)-((E)-3-methylbenzo[d]thiazol-2(3H)-ylidene)hydrazono)methyl)naphthalen-2-ol) was synthesized for the detection of Cu\(^{2+}\). BTN could detect Cu\(^{2+}\) with “off-on” fluorescent response from colorless to yellow irrespective of presence of other cations. Limit of detection for Cu\(^{2+}\) was determined to be 3.3 \(\mu\)M. Binding ratio of BTN and Cu\(^{2+}\) turned out to be a 1:1 with the analysis of Job plot and ESI-MS. Sensing feature of Cu\(^{2+}\) by BTN was explained with theoretical calculations, which might be owing to internal charge transfer and chelation-enhanced fluorescence processes.

Keywords
Benzothiazole · Fluorescence chemosensor · Copper ion · Turn on

Introduction

Copper is one of the pivotal transition metals in human body [1–3]. It exists diverse enzymes like cytochrome oxidase and tyrosine, and plays an essential key role in vital metabolisms, such as redox system and physiological response [4]. However, excessive level of copper can cause neurodegenerative troubles like Parkinson’s, Wilson’s and Alzheimer’s disease [5–7]. Furthermore, copper can also be a major source of environmental pollutants because it is widely used in industrial and agricultural practices [8]. Accordingly, it is essential to develop probes that can selectively detect copper with high sensitivity, selectivity and quick response.

Till now, a number of fluorescence chemosensors detecting Cu\(^{2+}\) have been developed because of the features such as great selectivity, versatility, sensitivity and relatively simple handling [9–13]. However, many fluorescent sensors for detecting Cu\(^{2+}\) are based on “turn-off” system, owing to the paramagnetic character of Cu\(^{2+}\) [14]. Therefore, less examples were reported for fluorescence “turn-on” for detecting Cu\(^{2+}\) [15]. Fluorescence sensors which have “turn-on” system shows better sensing properties at their sensitivity, selectivity and easy observation than “turn-off” system [16–18]. Moreover, the fluorescence “turn-off” system induced by a fluorescence quenching may give false results from other quenchers in practical samples and then, is less suitable for analytical applications [19]. Thus, the development of “turn-on” chemosensors for Cu\(^{2+}\) has been receiving substantial attention.

Many chromophore/fluorophore systems such as quinoline [20, 21], rhodamine [22], bis-thiophene [23], phenazine [24], thiazole ring [25] and BODIPY [26] have been reported and developed for sensing Cu\(^{2+}\). Among the systems, benzothiazole and its derivatives are sometimes used for the design of fluorescent probes due to the features of large Stokes shift and good quantum yield [27, 28]. Meanwhile, naphthol group is a good fluorophore that can interact with various metal ions [29–31]. Thus, the sensor containing both benzothiazole and naphthol moieties might be a suitable platform that can detect Cu\(^{2+}\) with turn-on system [32–41].

Herein, we demonstrated the design and application of a “turn-on” fluorescence probe (BTN) for detecting Cu\(^{2+}\). In particular, BTN can sense selectively Cu\(^{2+}\) in samples with metal ions like Hg\(^{2+}\), Fe\(^{3+}\), Co\(^{2+}\) and Ni\(^{2+}\) having a fluorescence quenching property. The sensing mechanism and binding structure of BTN toward Cu\(^{2+}\) were explained by using Job plot, FT-IR, ESI-MS analysis and theoretical calculations.

Experiments

General Information

\(^1\)H and \(^{13}\)C NMR were recorded using a Varian spectrometer. Using the Perkin Elmer spectrometers, absorption and fluorescent spectral data were obtained. ESI-mass was provided by
an ACQUITY QDa. FT-IR spectra were recorded on Agilent Cary 670 spectrometer.

**Synthesis of BTN (1-(((Z)-((E)-3-Methylbenzo[d]
Thiazol-2(3H)-Ylidene))Hydrazono) Methyl)
Naphthalen-2-Ol)**

(E)-2-hydrazono-3-methyl-2,3-dihydrobenzo[d]thiazole
(0.11 g, 5 × 10⁻⁴ mol) was dissolved in methanol (5 mL) and
2-hydroxy-1-naphthaldehyde (0.09 g, 5.1 × 10⁻⁴ mol) was
added into the solution. The light yellow power was immedi-
ately formed and the reaction mixture was stirred for 3 h at
22 °C. The product was filtered and washed with dichloro-
methane and ether (yield: 78%). ¹H NMR (DMSO-d₆), δ (ppm):
12.31 (s, 1H), 9.43 (s, 1H), 8.50 (d, J = 8.8 Hz, 1H),
7.90 (m, 2H), 7.74 (d, J = 7.6 Hz, 1H), 7.55 (m, 1H), 7.39 (m,
3H), 7.25 (d, J = 8.8 Hz, 1H), 7.16 (s, 1H), 3.65 (s, 3H). ¹³C
NMR (DMSO-d₆), δ (ppm): 164.6 (1C), 158.0 (1C), 151.9
(1C), 140.8 (1C), 132.5 (1C), 131.6 (1C), 128.8 (1C), 127.8
(1C), 127.6 (1C), 126.9 (1C), 123.5 (1C), 122.6 (1C), 122.3
(1C), 122.1 (1C), 121.5 (1C), 117.6 (1C), 110.5 (1C), 109.2
(1C), 31.0 (1C). ESI-mass for [BTN + H⁺ + 2·MeCN]⁺, calcd,
416.15 (m/z); found, 415.77.

**Fluorescent and UV-Visible Tests**

Fluorescent and UV-visible tests were checked in acetonitrile.
A BTN stock (5 mM) was made in DMSO (1 mL) and 6 μL of
BTN (5 mM) was diluted with 2.997 mL of MeCN to make
10 μM. In titration experiments, 0.75–10.5 μL of a stock
Cu(NO₃)₂ (2 × 10⁻² M) in MeCN were added to BTN (2 × 10⁻⁵ M). After mixing the solutions for 3 s, UV-vis and fluo-
rescence spectra were obtained.

**Quantum Yield**

To compare the quantum yields of BTN and BTN-Cu²⁺,
fluoresceine (Φ = 0.54 in 100 mM H₂SO₄ solution) was used
as reference fluorophore [42]. With the following equation,
quantum yield was calculated [42].

\[
Φ_{FS} = Φ_{FR} \times \frac{A_R}{A_S} \times \frac{F_S}{F_R} \times \left(\frac{R_S}{R_R}\right)^2
\]

(Φₚ = fluorescence quantum yield, F = integrated fluores-
cence emission, A = absorbance, n = refractive index of the
solution, R = reference material and S = test sample)

**Job Plot**

BTN (1.7 mg, 5 × 10⁻³ mmol) was diluted in DMSO (1 mL). Copper(II) nitrate (47.4 mg, 4 × 10⁻⁴ mol) was dissolved in
10.0 mL of CH₃CN. To make 20 mL of Cu²⁺ solution
(20 μM) and 20 mL of BTN solution (20 μM), 80 μL of the
stock BTN (5 × 10⁻³ M) was diluted in 19.92 mL of CH₃CN.
Likewise, 20 μL of the stock Cu²⁺ (20 mM) was diluted in

![Fig. 1 Fluorescent response of BTN to diverse cations. Inset: Fluorescent image of BTN and BTN-Cu²⁺](image1.png)

![Fig. 2 Fluorescent variations of BTN (1 × 10⁻⁵ M) with different concentrations of Cu²⁺](image2.png)
19.98 mL of CH₃CN. Then, 0.3–2.7 mL of BTN were added to each quartz. 0.3–2.7 mL of Cu²⁺ was added into the quartz to give a total 3 mL volume. After stirring them for 3 s, fluorescence measurements were executed.

**Competition Test**

A BTN stock (5 mM) was made in DMSO (1 mL). In cells with 3 mL of MeCN, 12 μL of other metal ions stocks (In³⁺, Fe⁷⁺, K⁺, Fe⁵⁺, Al³⁺, Mn²⁺, Ga³⁺, Zn²⁺, Ni²⁺, Cd²⁺, Mg²⁺, Na⁺, Cr³⁺, Hg⁺, Ag₂⁺, Co²⁺, Ca²⁺ and Pb²⁺; 20 mM) was diluted into each cell to make 8 equiv. 12 μL of Cu²⁺ (2 × 10⁻² M) was added into each cell. Then, 6 μL of BTN (5 × 10⁻³ M) was added into the cell, which was stirred for a few seconds. The cations were selected, because they may be frequently exposed to our environment system.

**Theoretical Studies**

Calculations were executed with Gaussian 16 program [43]. DFT method was applied for geometry optimizations [44, 45]. B3LYP was used for the hybrid functional, and the 6-31G (d,p) basis set was applied to all atoms except for Cu²⁺ [46, 47]. For BTN-Cu²⁺ complex, the LANL2DZ basis set was employed for applying effective core potential to Cu²⁺ [48–50]. Imaginary frequencies were not found for both BTN and BTN-Cu²⁺, representing the local minima of the structures. The solvent effect of acetonitrile was regarded with IEFPCM [51]. With energy-optimized forms of BTN and BTN-Cu²⁺, the possible UV-vis transition states were calculated by using TD-DFT method with twenty lowest singlet states.

**Results and Discussion**

Compound BTN was produced by the coupling reaction between 2-hydroxy-1 naphthaldehyde and (E)-2-hydrazono-3-methyl-2,3-dihydrobenzo[d]thiazole (Scheme 1). BTN was confirmed by ¹H and ¹³C NMR and ESI-MS (Figs. S1-S3).

**Fluorescent Sensing of BTN to Cu²⁺**

The fluorescence spectral measurements to varied metal ions were executed to confirm the fluorescence sensing capability of BTN (Fig. 1). BTN showed little fluorescence emission. After the addition of Cu²⁺ to BTN, the fluorescence emission at 539.5 nm remarkably increased (λₑₓ = 397 nm) and its solution color varied to yellow under UV light. Quantum yields of BTN and BTN-Cu²⁺ were given to be 0.0778 and 0.7919, respectively. By contrast, the presence of other cations with BTN did not show any variation. On the other hand, the photo-stability of BTN was examined (Fig. S4). Sensor BTN was stable enough for 24 h.

To find out the sensing process of BTN towards Cu²⁺, fluorescent titration was carried out (Fig. 2). With addition of Cu²⁺, the fluorescence at 539.5 nm was consistently enhanced. UV-vis spectral tests were carried out to demonstrate the binding property of BTN and Cu²⁺ (Fig. 3). The absorbance at 300 and 500 nm significantly increased and that at 380 nm gradually decreased. An obvious isosbestic point observed at 420 nm meant that the binding of BTN with Cu²⁺ produced a single product.

The Job plot was executed to investigate the complexation ratio of BTN and Cu²⁺ (Fig. 4). It displayed a 1:1 complexation ratio of BTN and Cu²⁺, which was verified by ESI-MS (Fig. S5). The peak of 435.87 (m/z) was expressive to [BTN-H⁺ + MeCN + Cu²⁺]⁺ (calcd: 436.04).
Additionally, to illustrate the binding structure of BTN with Cu$^{2+}$, the FT-IR investigation was performed (Fig. S6). The broad O-H peak (2800–3200 cm$^{-1}$) disappeared after the complexation of BTN-Cu$^{2+}$, demonstrating that the hydroxy group in BTN was deprotonated. The C=N peak at 1614 cm$^{-1}$ shifted to 1574 cm$^{-1}$ after BTN was bound to Cu$^{2+}$. These outcomes illustrated that the sulfur, the nitrogen and the deprotonated oxygen of BTN might coordinate to Cu$^{2+}$ (Scheme 2). An association constant of BTN with Cu$^{2+}$ was analyzed to be $2.0 \times 10^3$ M$^{-1}$ ($R^2 = 0.9906$) with the equation of Benesi-Hildebrand [52] (Fig. S7). With the fluorescence titration, detection of limit for Cu$^{2+}$ turned out to be 3.3 μM using $3\sigma/K$ (Fig. 5) [53]. This is only the fourth benzothiazole-based fluorescent "turn-on" probe for detecting Cu$^{2+}$, while the number of benzothiazole-based fluorescent "turn-off" sensors have been reported (Table S1).

Competitive test was executed to confirm a sensing capability of BTN (Fig. 6). BTN was not interfered by other cations and exhibited the constant fluorescence emission at 539.5 nm. This indicated that BTN can detect Cu$^{2+}$ without being disturbed by other metal ions, resulting in high selectivity. Therefore, BTN could be a very effective fluorescence sensor for sensing Cu$^{2+}$ in samples containing other metal ions.

### Calculations

Optimized forms of BTN and BTN-Cu$^{2+}$ were calculated, based on the Job plot, ESI-mass and IR data (Fig. 7). BTN has a distorted structure with bending of the naphthol group, while BTN-Cu$^{2+}$ exhibits a nearly planar structure. With the structural change during the complex formation, the dihedral angle (1S, 2 N, 3 N, 4O) changed from 154.190° to 18.894°.

With energy-optimized forms of BTN-Cu$^{2+}$ and BTN, TD-DFT calculations were executed to investigate molecular orbitals and transition energies. For BTN, the major absorption band stemmed from the HOMO $\rightarrow$ LUMO transition (380.48 nm, Figs. S8 and S9), which meant intramolecular charge transfer (ICT) from the benzothiazole group to the naphthol one. With BTN-Cu$^{2+}$, the main transition at 427.12 nm stemmed from the HOMO $\rightarrow$ LUMO ($\alpha$) and HOMO $\rightarrow$ LUMO+1 ($\beta$), which showed ICT characteristics (Fig. S10 and S11). Given the experiment and calculation results, the fluorescent turn-on process of BTN to Cu$^{2+}$ may be CHEF effect [54, 55]. Free vibration and rotation of BTN, non-radiative transitions, were restricted due to the complex formation with Cu$^{2+}$. The red shift shown in the experimental UV-vis spectra was consistent with the decreased energy gap. Based on calculations and experimental results, proper structure of BTN-Cu$^{2+}$ was proposed (Scheme 2).
Conclusion

We presented a great efficient fluorescent turn-on chemosensor BTN synthesized from the combination of 3-methyl-2-benzothiazolinone hydrazone and 2-hydroxy-1-naphthaldehyde. BTN can work as one of a few benzothiazole-based fluorescent “turn-on” probes for detecting Cu$^{2+}$, while the number of benzothiazole-based fluorescent “turn-off” sensors has been addressed. Limit of detection for Cu$^{2+}$ was 3.3 μM. In particular, in samples with metal ions like Hg$^{2+}$, Fe$^{3+}$, Co$^{2+}$ and Ni$^{2+}$ having a fluorescent quenching property, BTN can selectively sense Cu$^{2+}$. Detection process of Cu$^{2+}$ by BTN was demonstrated to be CHEF and ICT processes through theoretical calculations.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10895-021-02752-x.

Acknowledgments The National Research Foundation of Korea (NRF) (2018R1A2B6001686 and NRF-2020R1A6A1A03042742) is gratefully acknowledged.

Availability of Data and Material (Data Transparency) Not applicable.

Code Availability (Software Application or Custom Code) Not applicable.

Authors’ Contributions Gyeongjin Kim (60% contributions), Donghwan Choi (10% contributions), Cheal Kim (30% contributions).

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Zhao Y, Zhang XB, Han ZX, Qiao L, Li CY, Jian LX, Shen GL, Yu RQ (2009) Highly sensitive and selective colorimetric and off-on fluorescent chemosensor for Cu$^{2+}$ in aqueous solution and living cells. Anal Chem 81:7022–7030
2. Tapiero H, Townsend DM, Tew KD (2003) Trace elements in human physiology and pathology. Copper. Biomed Pharmacother 57:386–398
3. Lee DY, Singh N, Jang DO (2010) A benzimidazole-based single molecular multianalyte fluorescent probe for the simultaneous analysis of Cu$^{2+}$ and Fe$^{3+}$. Tetrahedron Lett 51:1103–1106
4. Que EL, Domaile DW, Chang CJ (2008) Metals in neurobiology: probing their chemistry and biology with molecular imaging. Chem Rev 108:1517–1549
5. Smith DP, Ciccotosto GD, Tew DJ, Fodero-Tavoletti MT, Johansen T, Masters CL, Barnham KJ, Cappai R (2007) Concentration dependent Cu$^{2+}$ induced aggregation and dityrosine formation of the Alzheimer’s disease amyloid-β-peptide. Biochemistry 46:2881–2891
6. You GR, Lee JJ, Choi YW, Lee SY, Kim C (2016) Experimental and theoretical studies for sequential detection of copper(II) and cysteine by a colorimetric chemosensor. Tetrahedron 72:875–881
7. Gaetke LM, Chow CK (2003) Copper toxicity, oxidative stress, and antioxidant nutrients. Toxicology 189:147–163
8. Seo H, An M, Kim BY, Choi JH, Helal A, Kim HS (2017) Highly selective fluorescent probe for sequential recognition of copper(II) and iodide ions. Tetrahedron 73:4684–4691
46. Harihan PC, Pople JA (1973) The influence of polarization functions on molecular orbital hydrogenation energies. Theor Chim Acta 28:213–222
47. Francl MM, Pietro WJ, Hehre WJ et al (1982) Self-consistent molecular orbital methods. XXIII A polarization-type basis set for second-row elements. J Chem Phys 77:3654–3665
48. Hay PJ, Wadt WR (1985) \textit{Ab} initio effective core potentials for molecular calculations. Potentials for the transition metal atoms Sc to Hg. J Chem Phys 82:270–283
49. Wadt WR, Hay PJ (1985) \textit{Ab} initio effective core potentials for molecular calculations. Potentials for main group elements Na to Bi. J Chem Phys 82:284–298
50. Hay PJ, Wadt WR (1985) \textit{Ab} initio effective core potentials for molecular calculations. Potentials for K to Au including the outermost core orbitals. J Chem Phys 82:299–310
51. Klamt A, Moya C, Palomar J (2015) A comprehensive comparison of the IEFPCM and SS(V) PE continuum solvation methods with the COSMO approach. J Chem Theory Comput 11:4220–4225
52. Mukhopadhyay M, Banerjee D, Koll A, Mandal A, Filarowski A, Fitzmaurice D, Das R, Mukherjee S (2005) Excited state intermolecular proton transfer and caging of salicylidine-3,4,7-methyl amine in cyclodextrins. J Photochem Photobiol A Chem 175:94–99
53. Goswami S, Aich K, Das S, Das Mukhopadhyay C, Sarkar D, Mondal TK (2015) A new visible-light-excitable ICT-CHEF-mediated fluorescence “turn-on” probe for the selective detection of Cd\textsuperscript{2+} in a mixed aqueous system with live-cell imaging. Dalton Trans 44:5763–5770
54. Slassi S, Aarjane M, El-Ghayoury A, Amine A (2019) A highly turn-on fluorescent CHEF-type chemosensor for selective detection of Cu\textsuperscript{2+} in aqueous media. Spectrochim Acta - Part A Mol Biomol Spectrosc 215:348–353
55. Chang Y, Li B, Mei H, Yang L, Xu K, Pang X (2020) Indole-based colori/fluorimetric probe for selective detection of Cu\textsuperscript{2+} and application in living cell imaging. Spectrochim Acta - Part A Mol Biomol Spectrosc 226:117631

\textbf{Publisher's Note} Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.