Research Article

Highly Selective Fluorescent Probe for the Detection of Copper (II) and Its Application in Live Cell Imaging

Zhihao Guo, Xiuji Wang, Pei Wei, Yihua Gao, and Qin Li

Analysis Center, Guangdong Medical University, Dongguan 523808, China

Correspondence should be addressed to Yihua Gao; gaoyh@gdmu.edu.cn

Received 5 February 2019; Revised 2 April 2019; Accepted 9 April 2019; Published 19 May 2019

Academic Editor: Guido Crisponi

Copyright © 2019 Zhihao Guo et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The development of fluorescent methods for the detection of metal ions is of great importance due to their diverse environmental and biological roles. Herein, a rhodamine 6G-based off-on fluorescent probe (L1) with a t-butyl pyrrole moiety as the recognition site was designed and synthesized. Photophysical studies show that L1 exhibits excellent sensitivity and selectivity towards Cu²⁺ to other metal ions in neutral acetonitrile aqueous media. Mechanism studies suggest that the recognition process may associate with a Cu²⁺ promoted hydrolysis reaction of L1. Furthermore, L1 has been successfully applied in fluorescence imaging of Cu²⁺ ion in living cells.

1. Introduction

The exploration of detection methods for environmentally and biologically important metal ions is of great interest to researchers currently [1, 2]. Among these metal ions, Cu²⁺ receives great attention because copper is the third most abundant essential metal (after iron and zinc) in the human body and plays an important role in a variety of physiological processes. For example, copper is integrated into various proteins and metalloenzymes that perform basic metabolic functions [3]. Copper deficiency could produce osteoporosis, hyperthyroidism, and coronary heart disease [4]. However, excessive accumulation of copper can cause central nervous system damage and increase the risk of neurodegenerative diseases such as Alzheimer’s, Parkinson’s, Menken’s, and Wilson’s diseases [5–8]. Hence, development of sensitive and selective analysis methods for copper ion, especially those that could be utilized in bio-imaging, is of great importance in the aspects of understanding the complex physiological functions of copper in the human body.

Traditionally, inductively coupled plasma atomic emission spectrometry (ICP-AES), atomic absorption spectroscopy (AAS), and inductively coupled plasma mass spectrometry (ICP-MS) are used to analyze copper ions [9–11]. However, these methods require sophisticated instruments and complicated and time-consuming processes of sample preparation. Thus, a simple and rapid detection method for quantifying copper ions is necessary. Fluorescence methods, with the advantages of sensitivity, simple operation, and real-time monitoring with fast response time, have been widely used in the detection of metal ions [12–21]. Due to the paramagnetic nature, Cu²⁺ was usually detected through fluorescence quenching of chemical sensors [22, 23], which may result in false-positive results and less-sensitive detection. Among the developed fluorophores, rhodamine derivatives, due to their excellent photophysical properties, such as large absorption coefficients, high fluorescence quantum yields, and long absorption and emission wavelengths, have attracted great attention from researchers [24–29]. In addition, it is well known that the fluorescence emission behaviors of rhodamine derivatives could be adjusted through a spirolactam ring-opening reaction. Spirocycle derivatives of rhodamine are colorless and nonfluorescent due to their nonconjugated structure. However, opening of the spirolactam ring, usually caused by metal ions, will produce intense fluorescence emission and a pink color change. Based on this spirolactam/ring-opened
amine equilibrium of rhodamine, through introducing proper recognition ligands, researchers have developed many turn-on fluorescent sensors for metal ions [30–40].

Taking into account the criteria mentioned above, we herein incorporated a tert-butyl pyrrole moiety into the rhodamine fluorophore to form a turn-on fluorescent probe L1 for the detection of Cu^{2+}. The molecular structure of L1 was verified by 1H NMR, 13C NMR, and MS spectra. With the addition of Cu^{2+} to L1 in a neutral CH3CN/H2O solution, metal-triggered ring-opening reaction of the spirolactam in L1 took place, resulting in sensitive colorimetric response and fluorescence emission. Mechanism studies suggest that the detection process may associate with a Cu^{2+} promoted hydrolysis reaction of L1. Furthermore, fluorescence microscopy experiments demonstrated that L1 could be used to image Cu^{2+} in living cells.

2. Materials and Methods

2.1. General Materials and Apparatus. All chemicals are purchased commercially and used directly without further purification. Deionized water was used throughout, and the pH was adjusted using diluted sodium hydroxide solution or hydrochloric acid. The pH value was measured with a Rex pH5-3E pH meter. Silica gel (200–300 mesh) was used for column chromatography. NMR spectra were obtained with a 600 MHz Bruker spectrometer, and tetramethylsilane (TMS) was used as the internal standard. High-resolution mass spectra were measured on an Agilent LCMS 6500 spectrometer. Absorbance spectra were measured on a Shimadzu UV-3101PC spectrometer. Measurements of fluorescence spectra were performed on a Shimadzu RF-5301PC spectrometer. Both excitation and emission slit widths were set at 3 nm. All experiments were operated at about 298 K.

2.2. Procedures of Sensing Experiments. A stock solution of probe L1 (2 × 10^{-4} M) was prepared in CH3CN. Stock solutions of metal ions (2 × 10^{-3} M) were prepared in deionized water from their chlorides salts or nitrate salt (Ag+). Working solution of L1 (10 μM, CH3CN/H2O, 1:1, v/v) was freshly prepared prior to spectroscopic experiments by diluting the high-concentration stock solution. In the sensing experiments, each time, a 3 mL working solution of L1 (10 μM, CH3CN/H2O, 1:1, v/v) was put in a quartz optical cell of 1 cm optical path length, and appropriate amounts of stock solutions of metal ions were added by a pipette. Spectral data were collected 2 min after the addition of the ions.

2.3. Synthesis of L1. To a 100 mL flask with three necks, rhodamine 6G hydrazine (2.8 mmol), which was synthesized according to reported methods [41], and 5-tert-butylpyrrole-2-carbaldehyde (3.3 mmol) were dissolved in 30 mL methanol. After addition of 0.1 mL acetic acid, the mixture was stirred and heated to reflux for 16 h. A pale yellow solid obtained was filtered off and washed using cold methanol. The solid was dried in vacuum and further purified by column chromatography (CH2Cl2/CH3OH = 150/1, v/v). Yield: 88.6%. 1H NMR (600 MHz, DMSO-d6), δ (ppm):

10.93 (s, 1H, CNHC), 8.16 (s, 1H, N=CH), 7.84 (d, J= 12 Hz, 1H, ArH), 7.50 (m, 2H, ArH), 6.94 (d, J= 12 Hz, 1H, ArH), 6.30 (s, 2H, ArH), 6.18 (s, 2H, ArH), 6.05 (d, J= 6 Hz, 1H, CCH(CH3)), 5.77 (d, J= 6 Hz, 1H, CCH(CH3)), 5.04 (t, J= 6 Hz, 2H, CH2CH2NH), 3.12 (q, J= 12 Hz, 4H, CH2CH2), 1.85 (s, 6H, ArCH3), 1.20 (m, 15H, CH3CH2, CCH3). 13C NMR (150 MHz, DMSO-d6), δ (ppm): 163.12, 151.96, 150.64, 147.66, 146.16, 143.13, 133.12, 128.41, 128.31, 126.64, 126.50, 123.24, 122.60, 118.11, 112.32, 105.03, 104.64, 95.91, 65.00, 37.44, 31.38, 29.98, 16.96, 14.13. HRMS: m/z calculated for M+1, C35H39N5O2, 561.3104. Found: 562.3192 (M+ 1).

2.4. Cell Culture and Fluorescence Imaging. The human breast adenocarcinoma cells MCF7 were cultured in DMEM supplemented with 10% fetal bovine serum at 37°C under an atmosphere containing 5% CO2. Before the experiments, the MCF7 cells were washed with PBS and then incubated with 10 μM L1 at 37°C for 30 min. After removal of excess probe and washed with PBS, the MCF7 cells were incubated with 20 μM CuCl2 for another 30 min. The MCF7 cells were rinsed with PBS again and live-cell imaging was conducted using an EVOS FL Auto microscope.

3. Results and Discussion

Compound L1 was easily synthesized from rhodamine 6G through a two-step reaction (Scheme 1). After purification by column chromatography (CH2Cl2/CH3OH = 150/1, v/v), L1 was obtained in an 88.6% yield. The molecular structure was verified by 1H NMR, 13C NMR, and MS spectra (Figures S1–S3). The m/z value of the molecular ion peak in the HRMS spectra was in good accordance with the accurate molecular weight with small derivation. Similar with other spirocycle derivatives of rhodamine, the solution of L1 in neutral CH3CN/H2O media was colorless and weakly fluorescent, indicating that it existed mainly in the form of spirolactam. In addition, a characteristic spirocycle carbon chemical shift at 65.0 ppm in the 13C NMR spectra was observed, which further supported this estimation [42]. The absorption spectrum of L1 (10 μM) in neutral CH3CN/H2O (1:1, v/v) solution is shown in Figure 1. No absorption peaks in the visible wavelength range was exhibited, suggesting that L1 existed with the structure of spirolactam. Once the solution of Cu^{2+} was added, a new peak was detected at 525 nm. With the increase of Cu^{2+} concentration, the intensity of the peak was gradually enhanced, which could be interpreted as the transform from a spirolactam structure to a ring-opened form of L1. Correspondingly, the color of the solution changed from colorless to purple, which help to achieve the naked-eye recognition of Cu^{2+} ion. As shown in Figure 1, with the increasing concentration of Cu^{2+}, the absorption value of L1 (10 μM) was found to increase linearly in the range of 2 μM–14 μM at 525 nm. The selective sensory studies of L1 (10 μM) in neutral CH3CN/H2O solution were then extended to other metal ions (Cr^{3+}, Al^{3+}, Fe^{3+}, Ca^{2+}, Ba^{2+}, Co^{2+}, Fe^{2+}, Hg^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Pd^{2+}, Zn^{2+}, Ag^{+}, Li^{+}, K^{+}, Na^{+}, and Sn^{2+}). When 1 equiv metal ions were added into the relevant
solution, only Cu^{2+} could induce a purple color and an obvious increase of absorbance at 525 nm (Figure 2). Al^{3+} exhibited weak absorbance response, and the other metal ions showed almost no absorbance increase in the same condition. The results indicated that L1 showed high selectivity towards Cu^{2+} in the detection of metal ions.

The fluorescence sensing behavior of L1 for Cu^{2+} in neutral CH₃CN/H₂O solution (10 μM, 1:1, v/v) was also investigated. When no Cu^{2+} was added, the free L1 solution had little fluorescence at the excitation wavelength of 500 nm due to the spirocyclic form of its molecular structure. However, similar to the results of the absorption experiments, once the solution of Cu^{2+} was added, an obvious fluorescence intensity increase was detected when the same amount of Cu^{2+} ion was added (Figure 4). In order to further evaluate the selectivity of L1 towards Cu^{2+} among other metal ions, the interference experiments were investigated. As shown in Figure 5, no obvious changes were observed in the Cu^{2+}-induced fluorescence emission of L1 when comparing the spectra data obtained in the presence and absence of other metal ions. These results indicated that L1 could be
Figure 4: Fluorescence spectra of L1 (10 μM) with addition of 10 μM various metal ions in CH3CN/H2O solution (1:1, v/v). Excitation was performed at 500 nm.

Figure 5: Fluorescence intensity at 545 nm of L1 (10 μM) with addition of 10 μM Cu2+ in the absence (blank) and presence of various metal ions (50 μM) in CH3CN/H2O solution (1:1, v/v). (1) blank, (2) Ag+, (3) Al3+, (4) Ba2+, (5) Ca2+, (6) Co2+, (7) Cr3+, (8) Fe2+, (9) Fe3+, (10) Hg2+, (11) K+, (12) Li+, (13) Mg2+, (14) Mn2+, (15) Na+, (16) Ni2+, (17) Pd2+, (18) Sn4+, and (19) Zn2+. Excitation was performed at 500 nm.

Scheme 2: The proposed reaction mechanism of L1 with Cu2+. 
used as a selective Cu²⁺ fluorescent sensor without interference from other metal ions.

The influence of solution pH on the fluorescence response towards Cu²⁺ of L₁ was studied. As shown in Figure S4, no obvious fluorescence could be found for free L₁ between pH 4.0 and 8.0, indicating that the spirolactam structure still dominated in this pH range. However, with the addition of Cu²⁺, fluorescence change of L₁ was observed with different fluorescence enhancement efficiency under different pH values (Figure S5). A marked fluorescence response towards Cu²⁺ was achieved in a pH range from 7 to 8. These results indicated that L₁ could be used as a fluorescent probe for the detection of Cu²⁺ in physiological pH conditions.

The effects of reaction media for the detection of Cu²⁺ by L₁ were also studied. As shown in Figure S6, in the presence of 1 equiv. Cu²⁺, the fluorescence signal value of L₁ reached the maximum when acetonitrile content was at 50%–60%. As a result, 50% aqueous acetonitrile was employed in all optical experiments. The response time of the detection system on fluorescence emission was studied to evaluate the sensitivity of L₁ towards Cu²⁺. After addition of 1 equiv. Cu²⁺, the fluorescence intensity of L₁ increased rapidly and reached maximum after two minutes and did not increase with the prolongation of reaction time (Figure S7). These results indicated that L₁ was sensitive for the detection of Cu²⁺ in the aqueous acetonitrile solution, and 2 min was selected as the detection time in this research.

In order to illustrate the interaction mechanism between Cu²⁺ and L₁, excess (10 equiv) Na₂EDTA was added to the solution of L₁ in neutral CH₃CN/H₂O solution (10 μM, 1:1, v/v) containing Cu²⁺ ion (10 μM). No obvious decrease in the fluorescent intensity or color change was observed after the addition, indicating that the detection of Cu²⁺ ion by L₁ was an irreversible process (Figure S8). HRMS experiments were carried out to analyze the reaction products of Cu²⁺ and L₁. The peak at m/z = 415.2025 was ascribed to rhodamine 6G (M⁺; m/z calculated for M⁺, C₂₆H₂₆N₂O₃, 414.1943), indicating rhodamine 6G as a final product (Figure S9). Moreover, the addition of Cu²⁺ into the solution of L₁ in pure CH₃CN resulted in no fluorescent emission or color change. Based on these experimental results, a mechanism involving Cu²⁺-promoted redox hydrolysis of L₁ was proposed (Scheme 2), which was similar to that of the sensing towards Cu²⁺ by rhodamine B hydrazide [44].

For further studying the practical application of L₁ in the detection of Cu²⁺, fluorescence imaging experiments were carried out in MCF7 cells. The MCF7 cells were incubated with L₁ for 30 min and washed with PBS. As shown in Figure 6(a), no intracellular fluorescence was observed in the image. However, after subsequent treatment with CuCl₂ at the same conditions for another 30 min, strong fluorescence in the MCF7 cells was observed (Figure 6(b)). These results suggest that L₁ could pass through the cell membrane and be used for the detection of Cu²⁺ in living cells. The cytotoxicity tests were studied by MTT assay with the concentrations of L₁
from 0 to 30 μM (Figure S10). Experimental results showed that more than 95% of the MCF7 cells were viable after incubation with L1 for 24 h at 37°C, indicating that L1 has low cytotoxicity to cells in this dosage range.

4. Conclusion

In summary, a new colorimetric and fluorescent probe L1 for Cu$^{2+}$ was developed through the combination of rhodamine 6G and a pyrrole moiety. As a turn-on fluorescent probe, L1 exhibited excellent sensitivity and selectivity for Cu$^{2+}$ detection in acetonitrile aqueous media with a low detection limit. Moreover, fluorescence bioimaging experiments confirmed that L1 could be used to detect intracellular Cu$^{2+}$ in living cells.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors report no conflicts of interest.

Authors’ Contributions

Zhihao Guo and Xiuji Wang contributed equally to this work.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (21502024 and 81503104) and the Doctoral Scientific Research Foundation of Guangdong Province (2014A030310471 and 2018A030310112).

Supplementary Materials

Figure S1: $^1$H NMR of compound L1 (600 MHz, DMSO-d$_6$). Figure S2: $^{13}$C NMR of compound L1 (150 MHz, DMSO-d$_6$). Figure S3: high-resolution mass spectra of L1. Figure S4: effect of pH on fluorescence intensity at 545 nm of L1 (10 μM) in CH$_3$CN/H$_2$O solution (1:1, v/v). Excitation was performed at 500 nm. Figure S5: effect of pH on fluorescence intensity at 545 nm of L1 (10 μM) in the presence of 10 μM Cu$^{2+}$ in CH$_3$CN/H$_2$O solution (1:1, v/v). Excitation was performed at 500 nm. Figure S6: effect of acetonitrile content on fluorescence intensity of L1 (10 μM) in the presence of 10 μM Cu$^{2+}$ in CH$_3$CN/H$_2$O solution. Excitation was performed at 500 nm. Figure S7: time course of the response of L1 (10 μM) to 1 equiv. of Cu$^{2+}$ in CH$_3$CN/H$_2$O solution. Figure S8: fluorescence spectra of L1 (10 μM) with addition of 10 μM Cu$^{2+}$ in the absence and presence of 10 equiv. Na$_2$EDTA in CH$_3$CN/H$_2$O solution (1:1, v/v). Figure S9: mass spectrum for the reaction products of Cu$^{2+}$ and L1 in CH$_3$CN/H$_2$O solution (1:1, v/v). Figure S10: the viability of MCF7 cells incubated with L1 of different concentrations. (Supplementary Materials)

References

[1] B. Champagne, A. Plaquet, J.-L. Pozzo, V. Rodriguez, and F. Castet, “Nonlinear optical molecular switches as selective cation sensors,” Journal of the American Chemical Society, vol. 134, no. 19, pp. 8101–8103, 2012.
[2] J. F. Zhang, Y. Zhou, J. Yoon, and J. S. Kim, “Recent progress in fluorescent and colorimetric chemosensors for detection of precious metal ions (silver, gold and platinum ions),” Chemical Society Reviews, vol. 48, no. 7, pp. 3416–3429, 2019.
[3] E. L. Que, D. W. Domaille, and C. J. Chang, “Metals in neurobiology: probing their chemistry and biology with molecular imaging,” Chemical Reviews, vol. 108, no. 5, pp. 1517–1549, 2008.
[4] M. Klatka, A. Blázewicz, M. Partyka, W. Kollątaj, E. Zienkiewicz, and R. Kocjan, “Concentration of selected metals in whole blood, plasma, and urine in short stature and healthy children,” Biological Trace Element Research, vol. 166, no. 2, pp. 142–148, 2015.
[5] M. Suneetha, P. Suman, S. M. Robinson, D. Shinya, W. A. Banks, and E. Nuran, “Copper complexing decreases the ability of amyloid beta peptide to cross the BBB and enter brain parenchyma,” Peptides, vol. 28, no. 7, pp. 1424–1432, 2007.
[6] E. Gaggelli, H. Kozłowski, D. Valensin, and G. Valensin, “Copper homeostasis and neurodegenerative disorders (Alzheimer’s, prion, and Parkinson’s diseases and atypical lateral sclerosis),” Chemical Reviews, vol. 106, no. 6, pp. 1995–2044, 2006.
[7] C. Deraeve, C. Boldron, A. Maraval et al., “Preparation and study of new poly-8-hydroxyquinoline chelators for an anti-Alzheimer strategy,” Chemistry—A European Journal, vol. 14, no. 2, pp. 682–696, 2008.
[8] I. A. Koval, P. Gamez, C. Belle, K. Selmeczi, and J. Reedijk, “Synthetic models of the active site of catechol oxidase: mechanistic studies,” Chemical Society Reviews, vol. 35, no. 9, pp. 814–840, 2006.
[9] M. Faraji, Y. Yamini, and S. Shariati, “Application of cotton as a solid phase extraction sorbent for on-line preconcentration of copper in water samples prior to inductively coupled plasma optical emission spectrometry determination,” Journal of Hazardous Materials, vol. 166, no. 2-3, pp. 1383–1388, 2009.
[10] N. Fournere and R. Hoveizavi, “Simultaneous preconcentration of Cu, Fe and Pb as methylthymol blue complexes on naphthalene adsorbent and flame atomic absorption determination,” Analytica Chimica Acta, vol. 549, no. 1-2, pp. 124–128, 2005.
[11] N. Pourmand, M. M. Sanagi, A. A. Naim, W. A. Wan Ibrahim, and U. Baig, “Dispersive micro-solid phase extraction method using newly prepared poly(methyl methacrylate) grafted agarose combined with ICP-MS for the simultaneous determination of Cd, Ni, Cu and Zn in vegetable and natural water samples,” Analytical Methods, vol. 7, no. 7, pp. 3215–3223, 2015.
[12] A. B. More, S. Mula, S. Thakare et al., “An acac-BODIPY dye as a reversible “ON-OFF-ON” fluorescent sensor for Cu$^{2+}$ and $S^{2-}$ ions based on displacement approach,” Journal of Luminescence, vol. 190, pp. 476–484, 2017.
[13] J. Park and Y. Kim, “A colorimetric probe for the selective naked-eye detection of Pb(II) ions in aqueous media,” Analyst, vol. 137, no. 14, pp. 3246–3248, 2012.
[14] Y. Wei, D. Cheng, T. Ren, Y. Li, Z. Zeng, and L. Yuan, “Design of NIR chromenyl-cyanine fluorophore library for
"switch-on" and ratiometric detection of bio-active species in vivo," *Analytical Chemistry*, vol. 88, no. 3, pp. 1842–1849, 2016.

[15] I. Roy, J.-Y. Shin, D. Shetty, J. K. Khedkar, J. H. Park, and K. Kim, "E-Bodipy fluorescent chemosensor for Zn 2+ ion," *Journal of Photochemistry and Photobiology A: Chemistry*, vol. 331, pp. 233–239, 2016.

[16] H. Lu, L. Xiong, H. Liu et al., "A highly selective and sensitive fluorescent turn-on sensor for Hg 2+ and its application in live cell imaging," *Organic & Biomolecular Chemistry*, vol. 7, no. 12, pp. 2554–2558, 2009.

[17] C. Baslak and A. N. Kursunlu, "Naked-eye fluorescent sensor for copper (II) ion based on naphtalene conjugate BODIPY dye," *Photochemical & Photobiological Sciences*, vol. 17, no. 8, pp. 1091–1097, 2018.

[18] W. Gao, H. Li, and S. Pu, "A highly selective fluorescent probe for Cu 2+ based on a diarylethene with a benzol[1,2,5] oxadiazol-4-ylamine Schiff base unit," *Journal of Photochemistry and Photobiology A: Chemistry*, vol. 364, pp. 208–218, 2018.

[19] S. Guo, G. Liu, C. Fan, and S. Pu, "A new diarylethene-derived probe for colorimetric sensing of Cu(II) and fluorometric sensing of Cu(II) and Zn(II): photochromism and high selectivity," *Sensors and Actuators B: Chemical*, vol. 266, pp. 603–613, 2018.

[20] Z. Shi, Y. Tu, R. Wang, G. Liu, and S. Pu, "Highly sensitive and selective turn-on fluorescent sensor for dual recognition of Cu 2+ and CN – based on a methylquinoline derivative," *Dyes and Pigments*, vol. 149, pp. 764–773, 2018.

[21] H. Kang, C. Fan, H. Xu, G. Liu, and S. Pu, "A highly selective fluorescence switch for Cu 2+ and Fe 3+ based on a new diarylethene with a triazole-linked rhodamine 6G unit," *Tetrahedron*, vol. 74, no. 33, pp. 4390–4399, 2018.

[22] K. A. Mccall and C. A. Fierke, "Colorimetric and fluorimetric assays to quantitate micromolar concentrations of transition metals," *Analytical Biochemistry*, vol. 284, no. 2, pp. 307–315, 2000.

[23] P. Chavez-Croker, N. Garrido, and G. A. Ahearn, "Copper transport by lobster hepatopancreatic epithelial cells separated by centrifugal elutriation: measurements with the fluorescent dye Phen Green," *Journal of Experimental Biology*, vol. 204, no. 8, pp. 1433–1444, 2001.

[24] F. Amat-Guerri, A. Costela, J. M. Figuera, F. Florido, and R. Sastre, "Laser action from rhodamine 6G-doped poly (2-hydroxyethyl methacrylate) matrices with different cross-linking degrees," *Chemical Physics Letters*, vol. 209, no. 4, pp. 352–356, 1993.

[25] X. Li, X. Gao, W. Shi, and H. Ma, "Design strategies for water-soluble small molecular chromogenic and fluorogenic probes," *Chemical Reviews*, vol. 114, no. 1, pp. 590–659, 2014.

[26] X. Chen, T. Pradhan, F. Wang, J. S. Kim, and J. Yoon, "Fluorescent chemosensors based on spiroirizing-opening of xanthenes and related derivatives," *Chemical Reviews*, vol. 112, no. 3, pp. 1910–1956, 2012.

[27] L. Yuan, W. Lin, K. Zheng, and S. Zhu, "FRET-based small-molecule fluorescent probes: rational design and bioimaging applications," *Accounts of Chemical Research*, vol. 46, no. 7, pp. 1462–1473, 2013.

[28] Y. Yang, Q. Zhao, W. Feng, and F. Li, "Luminescent chemodosimeters for bioimaging," *Chemical Reviews*, vol. 113, no. 1, pp. 192–270, 2013.

[29] D.-G. Cho and J. L. Sessler, "Modern reaction-based indicator systems," *Chemical Society Reviews*, vol. 38, no. 6, pp. 1647–1662, 2009.

[30] A. K. Bhanja, S. Mishra, K. Das Saha, and C. Sinha, "A fluorescence "turn-on" chemodosimeter for the specific detection of Fe 3+ by a rhodamine appended Schiff base and its application in live cell imaging," *Dalton Transactions*, vol. 46, no. 28, pp. 9245–9252, 2017.

[31] X. Bao, X. Cao, X. Nie et al., "A new selective fluorescent chemical sensor for Fe 3+ based on rhodamine B and a 1,4,7,10-tetraoxa-13-azacyclopentadecane conjugate and its imaging in living cells," *Sensors and Actuators B: Chemical*, vol. 208, pp. 54–66, 2015.

[32] J. Liu and Y. Qian, "A novel naphthalimide-rhodamine dye: intramolecular fluorescence resonance energy transfer and ratiometric chemodosimeter for Hg 2+ and Fe 2+," *Dyes and Pigments*, vol. 136, pp. 782–790, 2017.

[33] S. Guan, G. Wei, Z. Yan et al., "A novel turn-on fluorescent probe for multi-channel detection of Zn 2+ and Bi 3+ with different action mechanisms," *Analyt.,* vol. 143, no. 2, pp. 449–457, 2017.

[34] Y. Zheng, M. She, S. Ma et al., "Rhodamine based guanidino-benzimidazole functionalized fluorescent probe for tetra-valent tin and its application in living cells imaging," *Sensors and Actuators B: Chemical*, vol. 242, pp. 872–879, 2016.

[35] M. V. Tutov, A. A. Sergeev, P. A. Zadorozhny, S. Y. Bratskaya, and A. Y. Mironenko, "Dendrimeric rhodamine based fluorescent probe for selective detection of Au," *Sensors and Actuators B: Chemical*, vol. 273, pp. 916–920, 2018.

[36] Z. Xu, L. Zhang, R. Guo et al., "A highly sensitive and selective colorimetric and off-on fluorescent chemosensor for Cu 2+ based on rhodamine B derivative," *Sensors and Actuators B: Chemical*, vol. 156, no. 2, pp. 546–552, 2011.

[37] H. Xu, H. Ding, C. Fan, G. Liu, and S. Pu, "A multi-responsive diarylethene-rhodamine 6G derivative for sequential detection of Cr 3+ and CO 3 2−," *Tetrahedron*, vol. 74, no. 27, pp. 3489–3497, 2018.

[38] Y. Jiao, L. Zhou, H. He et al., "A novel rhodamine B-based "off-on" fluorescent sensor for selective recognition of copper (II) ions," *Talanta*, vol. 184, pp. 143–148, 2018.

[39] L. Xu, S. Wei, Q. Diao et al., "Sensitive and selective rhodamine-derived probes for fluorescent sensing of pH and colorimetric sensing of Cu 2+," *Sensors and Actuators B: Chemical*, vol. 246, pp. 395–401, 2017.

[40] X. Wang, J. Tao, X. Chen, and H. Yang, "An ultrasensitive and selective "off-on" rhodamine-based colorimetric and fluorescent chemodosimeter for the detection of Cu 2+," *Sensors and Actuators B: Chemical*, vol. 244, pp. 709–716, 2017.

[41] D. Wu, W. Huang, C. Duan, Z. Lin, and Q. Meng, "Highly sensitive fluorescent probe for selective detection of Hg 2+ in DMF aqueous media," *Inorganic Chemistry*, vol. 46, no. 5, pp. 1538–1540, 2007.

[42] Y. Xiang, A. Tong, P. Jin, and Y. Ju, "New fluorescent rhodamine hydrazone chemosensor for Cu(II) with high selectivity and sensitivity," *Organic Letters*, vol. 8, no. 13, pp. 2863–2866, 2006.

[43] M. Fischer and J. Georges, "Fluorescence quantum yield of rhodamine 6G in ethanol as a function of concentration using thermal lens spectrometry," *Chemical Physics Letters*, vol. 260, no. 1-2, pp. 115–118, 1996.

[44] V. Dujols, F. Ford, and A. W. Czarnik, "A long-wavelength fluorescent chemodosimeter selective for Cu(II) ion in water," *Journal of the American Chemical Society*, vol. 119, no. 31, pp. 7386–7387, 1997.