Inorganic Chemistry Communications 46 (2014) 310–314

Contents lists available at ScienceDirect

Inorganic Chemistry Communications

journal homepage: www.elsevier.com/locate/inoche

A novel selective fluorescent and colorimetric chemosensor for the visual detection of Pd^{2+} and application of imaging in living cells

Meipan Yang, Yinjuan Bai, Wenfei Meng, Zhao Cheng, Na Su, Bingqin Yang *

Key Laboratory of Synthetic and Natural Functional Molecule Chemistry, Ministry of Education, College of Chemistry and Materials Science, Northwest University, Xi'an 710069, PR China

ARTICLE INFO

Article history:
Received 24 April 2014
Received in revised form 7 June 2014
Accepted 25 June 2014
Available online 26 June 2014

Keywords:
Pd^{2+}
Chemosensor
Naked-eye
Test strips
Bioimaging

ABSTRACT

A rhodamine-based fluorescent chemosensor was synthesized and characterized, which demonstrated excellent specificity toward Pd^{2+}. Upon addition of Pd^{2+}, the ring-opening process of rhodamine spirolactam resulted in remarkably enhanced fluorescent accompanied by distinct color changes. Commonly coexistent metal ions displayed little interference to the sensor. Obviously, these unique results suggested that the chemosensor could serve as a potential naked-eye sensor for Pd^{2+}. The detection limit was 0.19 μM. In addition, we prepared the test strips of sensor for Pd^{2+} detection, which provided a simple and convenient method for determination of Pd^{2+} in palladium-containing water samples. Importantly, the sensor was successfully used for fluorescence imaging in HepG2 cells.

Among the rare transition metals, palladium plays a critical role in materials and chemistry. It is extensively being used to prepare dental crowns, catalytic converters, fuel cells, alloys and jewelry [1–3]. At the same time, Pd-catalyzed reactions are becoming increasingly popular in pharmacy because of the powerful transformations for the synthesis of complex molecules [4–7]. However, their large use leads to a high level of residual palladium. For example, the release of palladium from catalytic converters of automobiles results in a great quantity of palladium in complex environmental system, from soil to plants, rivers and oceans, which also influences our health in an adverse way [8–11]. Governmental restrictions on the levels of residual heavy metals in end products are very rigorous and strict. The proposed dietary intake of palladium is less than 1.5–15 μg/day per person and the threshold in drugs is 5–10 ppm [12]. Thus, a considerable effort has been devoted to the development of the efficient methods to detect and analyze palladium. Conventional detection methods include atomic absorption spectroscopy (AAS), atomic emission spectrometry (ICP-AES), X-ray fluorescence etc., which usually require sophisticated instrumentation and complicated sample-pretreatment procedures [12–15]. Fluorescence analysis offers significant advantages over other methods for metal ion detection due to its simplicity, high sensitivity, low cost and instantaneous response [16–21]. Whereas most of the reported Pd^{2+} fluorescent chemosensors show "on–off" signal upon the binding of Pd^{2+} due to the fact that it usually acts as typical fluorescence quenchers, which is not as sensitive as a fluorescence enhancement response [22–25]. Importantly, most reported "off–on" fluorescent chemosensors have not been applied to detecting Pd^{2+} in biological systems [26–30]. Therefore, it is still an outstanding challenge to create new "off–on" fluorescent Pd^{2+} chemosensors which demonstrated value of practical applications in biological systems.

Recently, a number of research groups have proposed various chemosensors based on rhodamine conjugates [31–42]. Rhodamine derivatives provide an ideal model for the design of metal ion sensing with light "off–on" switching by virtue of the equilibrium between spirolactam (nonfluorescence) and ring-opened amide (fluorescence) forms. For our role, here we presented the design and synthesis of sensor R1, which can be used for "naked-eye" detection with the switch-on fluorescence and significant color changes. The sensor showed highly sensitive spectroscopic response to Pd^{2+}. Soaked on paper, R1 exhibited an acute color response, which provided a simple and convenient method for determination of Pd^{2+} in palladium-containing water samples. What's more, the bioimaging capability of the sensor was further tested successfully in HepG2 cells.

The chemosensor R1 was prepared by using a two-step procedure (See Scheme 1.) High selectivity for the analyte and suitability of the sensor are crucial in designing modern chemosensors. In Fig. 1, the color-on reaction of R1 showed a high selectivity toward Pd^{2+} only, rather than other examined ions, such as Fe^{3+}, NH_4^+, Sn^{2+}, Ag^+, Ba^{2+}, Al^{3+}, Cd^{2+}, Cr^{3+}, Cu^{2+}, Hg^{2+}, K^+, Mg^{2+}, Mn^{2+}, Na^+, Ni^{2+}, Pb^{2+} and Zn^{2+}. These unique results suggested that R1 could serve as a potential naked-eye chemosensor for Pd^{2+}. The UV–vis and fluorescence properties of R1 were investigated toward different cations in methanol/PBS (1:1, v/v, pH 7.4) solution (Fig. S1). Interestingly, upon addition of Pd^{2+} to the solution of chemosensor, a significant absorbance appeared

---

* Corresponding author.
E-mail addresses: yangbq@nwu.edu.cn, ymp1111@163.com (B. Yang).

http://dx.doi.org/10.1016/j.inoche.2014.06.027
1387-7003/

Fig. 1. Pictures of R1 as a selective naked-eye chemosensor (top) and the visual fluorescence emissions by using a UV lamp (365 nm) (bottom) for Pd^{2+}. From the left to the right: Pd^{2+}, Mn^{2+}, Hg^{2+}, Zn^{2+}, Mg^{2+}, Ni^{2+}, Al^{3+}, Fe^{3+}, NH_4^+, Cd^{2+}, Cr^{3+}, K^+, Ba^{2+}, Cu^{2+}, Sn^{2+}, Ag^+, Na^+, Li^+, blank.

at around 555 nm. At the same time, a remarkable fluorescence enhancement at 578 nm was observed. In contrast, addition of other metal cations did not display the similar behavior. This demonstrated the opening of the spirolactam ring of rhodamine B moiety upon Pd^{2+} binding. The competition experiments were also carried out. As shown in Fig. S2, the Pd^{2+}-induced fluorescence response was unaffected in by 2 equiv. of other coexistent metals ions. Obviously, these results confirmed that our proposed chemosensor R1 had remarkably high selectivity toward Pd^{2+}.

In order to get more insight on the binding behavior of R1 toward Pd^{2+}, absorption and fluorescence spectrum titrations were performed (Fig. 2). Upon the addition of increasing concentrations of Pd^{2+}, the absorbance and fluorescence increased significantly. A reasonable linear relationship for Pd^{2+} detection was obtained. Based on the definition of detection limit [43], the limit of detection for Pd^{2+} was up to 0.19 μM (Fig. S3). Compared with other reported sensors [27–29], the limit of detection was relatively low. In fact, the range of residual Pd^{2+} during organic synthesis is 5–100 ppm, which means that the sensor could be used for residual Pd^{2+} detection in reactor. What's more, the sensor showed the potential application for Pd-contaminated water and soil sample analysis. The association constant K [44] of R1 with Pd^{2+} was estimated to be $2.40 \times 10^4\ M^{-1}$ (Fig. S4).

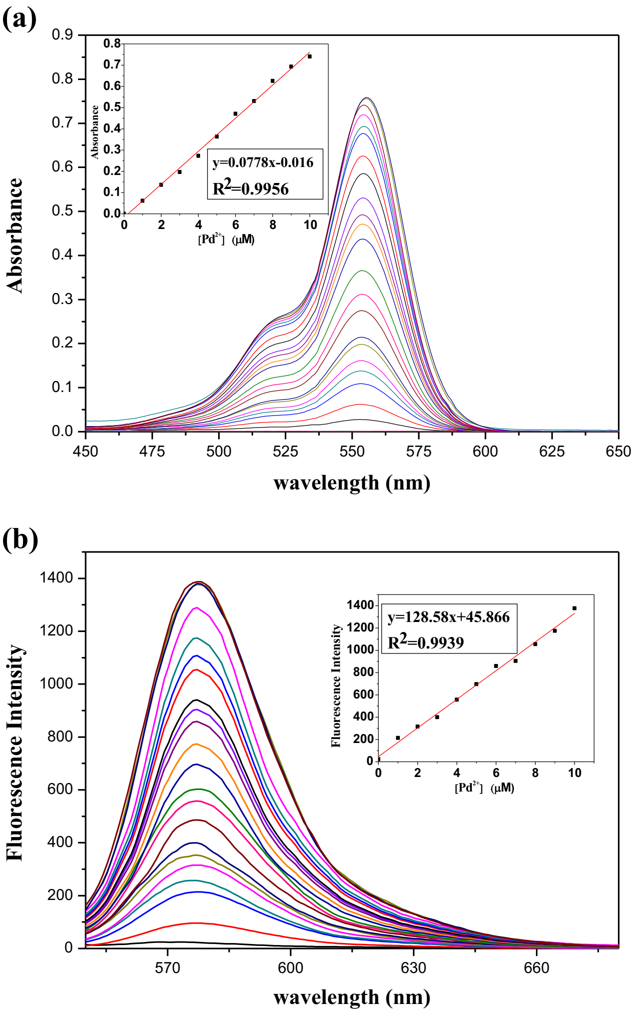


Fig. 2. Changes in UV–vis absorption spectra (a) and fluorescence spectra (b) of R1 (10 μM) with various amounts of Pd^{2+} in methanol/PBS (1:1, v/v, pH 7.4) solution. $\lambda_{ex} = 535$ nm. Inset: the linear relationships of R1 toward Pd^{2+}.

For practical application, the fluorescence spectrum response of R1 in the absence and presence of Pd^{2+} in different pH values was evaluated. Upon the addition of Pd^{2+} ions, there was an obvious fluorescence emission of R1 under pH range 6.0–8.0. When pH was lower than 4.0, the fluorescence intensity of R1 increased with decreasing pH values, which might be caused by the H^+-induced ring opening of rhodamine spirolactam (Fig. S5). The emission spectrum of R1 had no obvious change in the pH range 12.0–4.0. These data showed that the fluorescence of the sensor is pH independent at pH > 4, demonstrating that they can work in a wide physiological pH range, which gave R1 more advantage over some previous work [19,28,30]. As a result, R1 could work in real environmental and biological samples with very low background fluorescence. Therefore, the methanol/PBS (1:1, v/v, pH 7.4) solution was used as the ideal experimental medium.

The Job's plot (Fig. S6) revealed that the binding mode was 2:1 stoichiometry. The species formed between R1 and Pd^{2+} was more accurately determined by ESI-MS (Fig. 4). The peak at m/z 561.2504 that corresponds to $[2R1 + Pd^{2+}]^{2+}$ (Fig. 3) was clearly observed to support the 2:1 stoichiometry. In order to further confirm why the significant absorbance and fluorescence of R1 changed, the IR spectrum experiments were carried out [45]. IR spectra of R1 and R1-Pd^{2+} were taken in KBr disks, respectively. As demonstrated in Fig. S7, the carbonyl absorption of R1 at 1689 cm^{-1} shifted to a lower frequency at 1652 cm^{-1} upon the addition of Pd^{2+}. This supported that carbonyl oxygen was actually involved in the coordination with Pd^{2+}. According to these results, a possible coordination mode for R1 with Pd^{2+} was proposed (Scheme 2).

To investigate the practical application of chemosensor R1, the test strips were prepared by immersing filter papers ($3 \times 1\ cm^2$) in a methanol solution of R1 (5.0 mM). The stock solution of Pd^{2+} was diluted to different concentrations with deionized water. After dried in air, the test strips were directly utilized in the detection of Pd^{2+}. The obvious color changes were observed when the test strips coated with R1 which were soaked with solutions of Pd^{2+} of different concentrations, similar to the measurement of pH test strips (Fig. 4). Thus, such a way is more convenient than spectroscopic instrumental methods.

In order to further evaluate the potential of the sensor for imaging Pd^{2+} in living cells, cultured HepG2 cells were incubated with the sensor for 1 h at 37 °C. The cells incubated with only sensor (100 μM) exhibited almost no fluorescence (Fig. 5a). By contrast, the cells stained

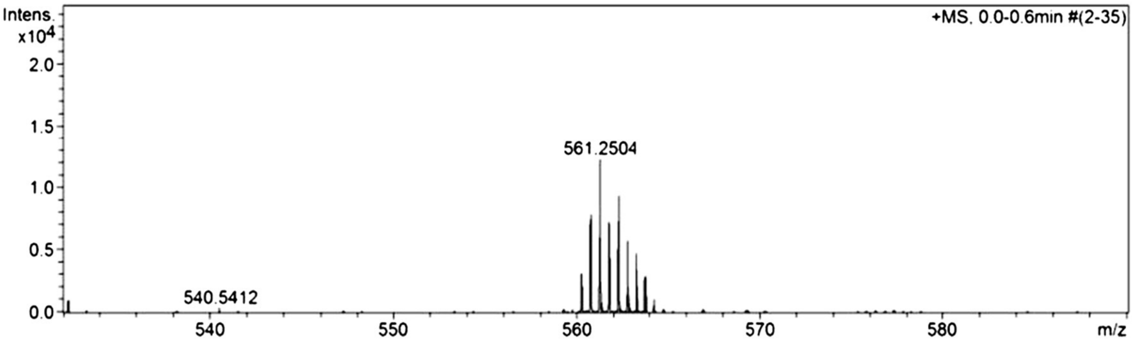
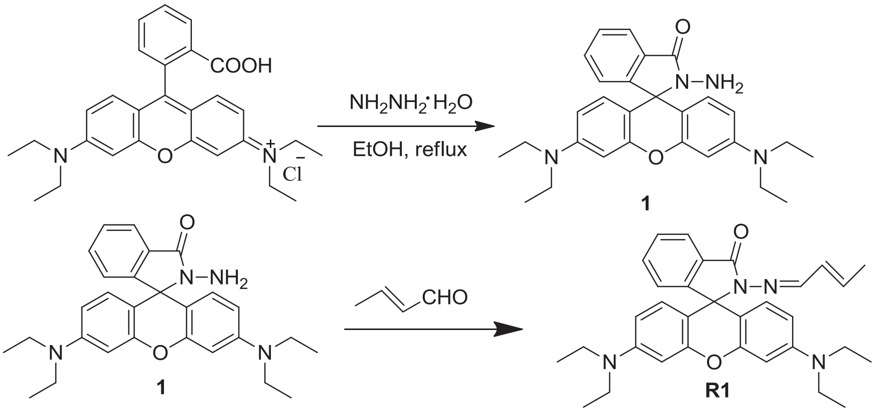


Fig. 3. Mass spectrum of R1 in the presence of Pd^{2+}.



Scheme 1. Synthesis of R1.

with both the sensor and further incubated with $PdCl_2$ (200 μM) displayed bright red fluorescence (Fig. 5b). Bright-field transmission images of cells treated with $PdCl_2$ and sensor revealed that the cells were viable throughout the imaging experiments (Fig. 5c). Taken together, these results demonstrate that sensor R1 was cell membrane-permeable and effective intracellular imaging agent for Pd^{2+} ions.

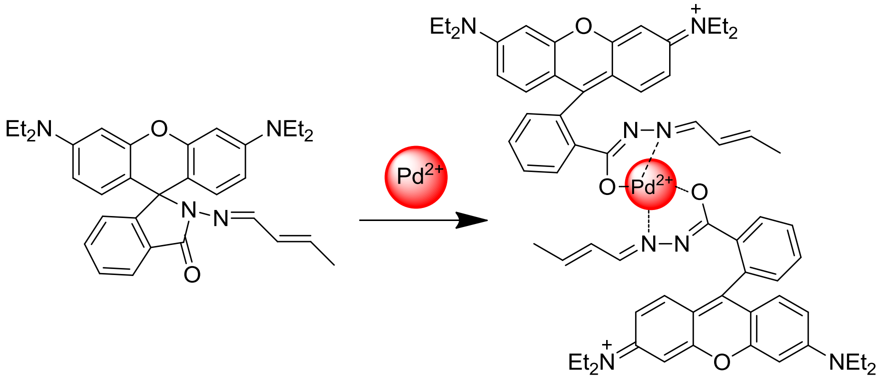
In summary, we synthesized a rhodamine based derivatives and investigated the properties for the detection of Pd^{2+}. The sensor exhibited high selectivity to Pd^{2+} over other metal cations. The detection limit was 0.19 μM. With addition of Pd^{2+}, the obvious color change could be easily observed by naked-eyes. For practical application, the paper-made test strips of R1 could be employed as portable sensing tools for Pd^{2+} detection. The results from confocal laser scanning microscopy experiments further showed that sensor could be used to detect Pd^{2+} in living cells.

Acknowledgment

This work was financially supported by the National Natural Science Foundation of China (Grant No. 21172178).

Appendix A. Supplementary material

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.inoche.2014.06.027. These data include MOL file



Scheme 2. Proposed mechanism for the fluorescent changes of R1 upon the addition of Pd^{2+}.

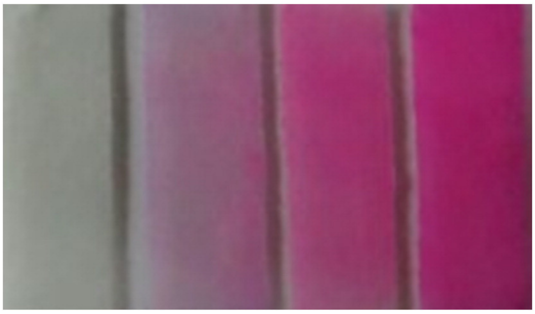


Fig. 4. Photographs of the test strips made from R1 for the detection of Pd^{2+} with different concentrations: left to right: 0, 30 μM, 50 μM, 100 μM.

and InChiKey of the most important compounds described in this article.

References

[1] J.L. Bars, U. Specht, J.S. Bradley, D.G. Blackmond, A catalytic probe of the surface of colloidal palladium particles using Heck coupling reactions, Langmuir 15 (1999) 7621–7625.

[2] T. Iwasawa, M. Tokunaga, Y. Obora, Y. Tsuji, Homogeneous palladium catalyst suppressing Pd black formation in air oxidation of alcohols, J. Am. Chem. Soc. 126 (2004) 6554–6555.

[3] M. Lafrance, K. Fagnou, Palladium-catalyzed benzene arylation: incorporation of catalytic pivalic acid as a proton shuttle and a key element in catalyst design, J. Am. Chem. Soc. 128 (2006) 16496–16497.

[4] G. Zeni, R.C. Larock, Synthesis of heterocycles via palladium π-olefin and π-alkyne chemistry, Chem. Rev. 104 (2004) 2285–2309.

[5] L.F. Tietze, H. Ila, H.P. Bell, Enantioselective palladium-catalyzed transformations, Chem. Rev. 104 (2004) 3453–3516.

[6] K.C. Nicolaou, P.G. Bulger, D. Sarlah, Palladium-catalyzed cross-coupling reactions in total synthesis, Angew. Chem. Int. Ed. 44 (2005) 4442–4489.

[7] X. Chen, K.M. Engle, D.H. Wang, J.Q. Yu, Palladium(II)-catalyzed C–H activation/C–C cross-coupling reactions: versatility and practicality, Angew. Chem. Int. Ed. 48 (2009) 5094–5115.

[8] S.H. Yu, H.W. Rhee, J.I. Hong, Fluorescent sensing system for palladium(II) based on the Heck reaction, Tetrahedron Lett. 52 (2011) 1512–1514.

[9] T.Z. Liu, S.D. Lee, R.S. Bhatnagar, Toxicity of palladium, Toxicol. Lett. 4 (1979) 469–473.

[10] B. Bocca, A. Alimonti, A. Cristaudo, E. Cristallini, F. Petrucci, S. Caroli, Monitoring of the exposure to platinum-group elements for two Italian population groups through urine analysis, Anal. Chim. Acta. 512 (2004) 19–25.

[11] M.A. Palacios, M. G´omez, M. Moldovan, B. G´omez, Assessment of environmental contamination risk by Pt, Rh and Pd from automobile catalyst, Microchem. J. 67 (2000) 105–113.

[12] J.S. Carey, D. Laffan, C. Thomson, M.T. Williams, Analysis of the reactions used for the preparation of drug candidate molecules, Org. Biomol. Chem. 4 (2006) 2337–2347.

[13] K.V. Meel, A. Smekens, M. Behets, P. Kazandjian, R.V. Grieken, Determination of platinum, palladium, and rhodium in automotive catalysts using high-energy secondary target X-ray fluorescence spectrometry, Anal. Chem. 79 (2007) 6383–6389.

[14] C. Locatelli, D. Melucci, G. Torsi, Determination of platinum-group metals and lead in vegetable environmental bio-monitors by voltammetric and spectroscopic techniques: critical comparison, Anal. Bioanal. Chem. 382 (2005) 1567–1573.

[15] B. Dimitrova, K. Benkhedda, E. Ivanova, F. Adams, Flow injection on-line preconcentration of palladium by ion-pair adsorption in a knotted reactor coupled with electrothermal atomic absorption spectrometry, J. Anal. At. Spectrom. 19 (2004) 1394–1396.

[16] D. Kalný, A.M. Albrecht-Gary, J. Havel, Highly sensitive method for palladium(II) determination as a porphyrinato complex by capillary zone electrophoresis, Anal. Chim. Acta. 439 (2001) 101–105.

[17] R.J.T. Houk, K.J. Wallace, H.S. Hewage, E.V. Anslyn, A colorimetric chemodosimeter for Pd(II): a method for detecting residual palladium in cross-coupling reactions, Tetrahedron 64 (2008) 8271–8278.

[18] J. Jiang, H. Jiang, W. Liu, X.L. Tang, X. Zhou, W.S. Liu, R.T. Liu, A colorimetric and ratiometric fluorescent probe for palladium, Org. Lett. 13 (2011) 4922–4925.

[19] S. Goswami, D. Sen, N.K. Das, H.K. Fun, C.K. Quah, A new rhodamine based colorimetric “off–on” fluorescence sensor selective for Pd^{2+} along with the first bound X-ray crystal structure, Chem. Commun. 47 (2011) 9101–9103.

[20] S. Mukherjee, S. Chowdhury, A.K. Paul, R. Banerjee, Selective extraction of palladium(II) using hydrazone ligand: a novel fluorescent sensor, J. Lumin. 131 (2011) 2342–2346.

[21] F.L. Song, A.L. Garner, K. Koide, A highly sensitive fluorescent sensor for Palladium based on the allylic oxidative insertion mechanism, J. Am. Chem. Soc. 129 (2007) 12354–12355.

[22] A. Tamayo, L. Escriche, J. Casabó, B. Covelo, C. Lodeiro, Synthesis, complexation and spectrofluorometric studies of a new NS$_3$ anthracene-containing macrocyclic ligand, Eur. J. Inorg. Chem. 2006 (2006) 2997–3004.

[23] J.R. Matthews, F. Goldoni, H. Kooijman, A.L. Spek, A.P.H.J. Schenning, E.W. Meijer, Metal coordination and aggregation properties of chiral polythiophenes and polythienylethynylenes, Macromol. Rapid Commun. 28 (2007) 1809–1815.

[24] L.P. Duan, Y.F. Xu, X.H. Qian, Highly sensitive and selective Pd^{2+} sensor of naphthalimide derivative based on complexation with alkynes and thio-heterocycle, Chem. Commun. 8 (2008) 6339–6341.

[25] B. Liu, Y.Y. Bao, F.F. Du, H. Wang, J. Tian, R. Bai, Synthesis and characterization of a fluorescent polymer containing 2,6-bis(2-thienyl)pyridine moieties as a highly efficient sensor for Pd^{2+} detection, Chem. Commun. 47 (2011) 1731–1733.

[26] M.E. Jun, K.H. Ahn, Fluorogenic and chromogenic detection of palladium species through a catalytic conversion of a rhodamine B derivative, Org. Lett. 12 (2012) 2790–2793.

[27] H. Li, J. Fan, J. Du, K. Guo, S. Sun, X. Liu, X. Peng, A fluorescent and colorimetric probe specific for palladium detection, Chem. Commun. 46 (2010) 1079–1081.

[28] H. Li, J. Fan, F. Song, H. Zhu, J. Du, S. Sun, X. Peng, Fluorescent probes for Pd^{2+} detection by allylidene-hydrazone ligands with excellent selectivity and large fluorescence enhancement, Chem. Eur. J. 16 (2010) 12349–12356.

[29] Y. Zhou, J. Zhang, H. Zhou, Q. Zhang, T. Ma, J. Niu, A highly sensitive and selective “off–on” chemosensor for the visual detection of Pd^{2+} in aqueous media, Sensors Actuators B Chem. 171–172 (2012) 508–514.

[30] H. Li, J. Cao, H. Zhu, J. Fan, X. Peng, Optical Pd^{2+} sensing by rhodamine hydrazone ligands: different stoichiometries in aqueous/nonaqueous environments, Tetrahedron Lett. 54 (2013) 4357–4361.

[31] J. Zhang, C.W. Yu, S.Y. Qian, G. Lu, J.W. Chen, A selective fluorescent chemosensor with 1,2,4-triazole as subunit for Cu(II) and its application in imaging Cu(II) in living cells, Dyes Pigments 92 (2012) 1370–1375.

[32] Q.J. Ma, X.B. Zhang, X.H. Zhao, Z. Jin, G.J. Mao, G.L. Shen, R.Q. Yu, A highly selective fluorescent probe for Hg^{2+} based on a rhodamine–coumarin conjugate, Anal. Chim. Acta. 663 (2010) 85–90.

[33] S.X. Wang, X.M. Meng, M.Z. Zhu, A naked-eye rhodamine-based fluorescent probe for Fe(III) and its application in living cells, Tetrahedron Lett. 52 (2011) 2840–2843.

[34] X.F. Yang, M.L. Zhao, G. Wang, A rhodamine-based fluorescent probe selective for bisulfite anion in aqueous ethanol media, Sensors Actuators B Chem. 151 (2011) 8–13.

[35] X. Zhang, Y. Shiraishi, T. Hirai, A new rhodamine-based fluorescent chemosensor for transition metal cations synthesized by one-step facile condensation, Tetrahedron Lett. 48 (2007) 5455–5459.

[36] H. Kim, S. Lee, J. Lee, J. Tae, Rhodamine triazole-based fluorescent probe for the detection of Pt^{2+}, Org. Lett. 12 (2010) 5342–5345.

[37] M. Kumar, N. Kumar, V. Bhalla, H. Singh, P.R. Sharma, T. Kaur, Naphthalimide appended rhodamine derivative: through bond energy transfer for sensing of Hg^{2+} ions, Org. Lett. 13 (2011) 1422–1425.

[38] W.Y. Lin, X.W. Cao, Y.D. Ding, L. Yuan, L.L. Long, A highly selective and sensitive fluorescent probe for Hg^{2+} imaging in live cells based on a rhodamine–thioamide–alkyne scaffold, Chem. Commun. 46 (2010) 3529–3531.

[39] J.F. Zhang, Y. Zhou, J. Yoon, Y. Kim, S.J. Kim, J.S. Kim, Naphthalimide modified rhodamine derivative: ratiometric and selective fluorescent sensor for Cu^{2+} based on two different approaches, Org. Lett. 12 (2010) 3852–3855.

[40] Q.A. Best, R.S. Xu, M.E. McCarroll, L.C. Wang, D.J. Dyer, Design and investigation of a series of rhodamine-based fluorescent probes for optical measurements of pH, Org. Lett. 12 (2010) 3219–3221.

[41] M.H. Lee, T.V. Giap, S.H. Kim, Y.H. Lee, C. Kang, J.S. Kim, A novel strategy to selectively detect Fe(III) in aqueous media driven by hydrolysis of a rhodamine 6G schiff base, Chem. Commun. 46 (2010) 1407–1409.

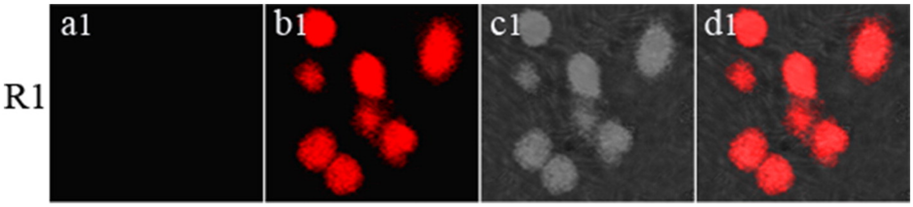


Fig. 5. Bioimaging application of chemosensor in HepG2 cells. Representative fluorescence images of HepG2 cells treated with chemosensor (100 μM) in either the absence (a) or the presence (b) of 200 μM Pd^{2+} for 1 h at 37 °C; (c) bright-field image of cells shown in panel; (d) overlay image of (b) and (c).

[42] X.Y. Hu, J. Wang, X. Zhu, D.P. Dong, X.L. Zhang, S. Wu, C.Y. Duan, A copper(II) rhodamine complex with a tripodal ligand as a highly selective fluorescence imaging agent for nitric oxide, Chem. Commun. 47 (2011) 11507–11509.

[43] Z. Xu, L. Zhang, R. Guo, T. Xiang, C. Wu, Z. Zhang, F. Yang, A highly sensitive and selective colorimetric and off–on fluorescent chemosensor for Cu^{2+} based on rhodamine B derivative, Sensors Actuators B Chem. 156 (2011) 546–552.

[44] Z.K. Song, B. Dong, G.J. Lei, M.J. Peng, Y. Guo, Novel selective fluorescent probes for sensing Zn^{2+} ions based on a coumarin schiff-base, Tetrahedron Lett. 54 (2013) 4945–4949.

[45] F.Y. Yan, M. Wang, D.L. Cao, N. Yang, Y. Fu, L. Chen, L.G. Chen, New fluorescent and colorimetric chemosensors based on the rhodamine detection of Hg^{2+} and Al^{3+} and application of imaging in living cells, Dyes Pigments 98 (2013) 42–50.