Inorganic Chemistry Communications 25 (2012) 26–29

Contents lists available at SciVerse ScienceDirect

Inorganic Chemistry Communications

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

A phenanthrene based highly selective fluorogenic and visual sensor for Cu^{2+} ion with nanomolar detection limit and its application in live cell imaging

Sellamuthu Anbu [a], Sankarasekaran Shanmugaraju [a], Rajendran Ravishankaran [b], Anjali A. Karande [b], Partha Sarathi Mukherjee [a,*]

[a] Department of Inorganic and Physical Chemistry, Indian Institute of Science, Bangalore, 560 012, India
[b] Department of Biochemistry, Indian Institute of Science, Bangalore-560 012, India

ARTICLE INFO

Article history:
Received 11 July 2012
Accepted 14 August 2012
Available online 21 August 2012

Keywords:
Fluorogenic Cu^{2+} sensors
Visual sensors
Live cell imaging
Intracellular Cu^{2+} detection

ABSTRACT

A new phenanthrene based chemosensor has been synthesized and investigated to act as highly selective fluorescence and visual sensor for Cu^{2+} ion with very low detection limit of 1.58 nM; this has also been used to image Cu^{2+} in human cervical HeLa cancer cells.

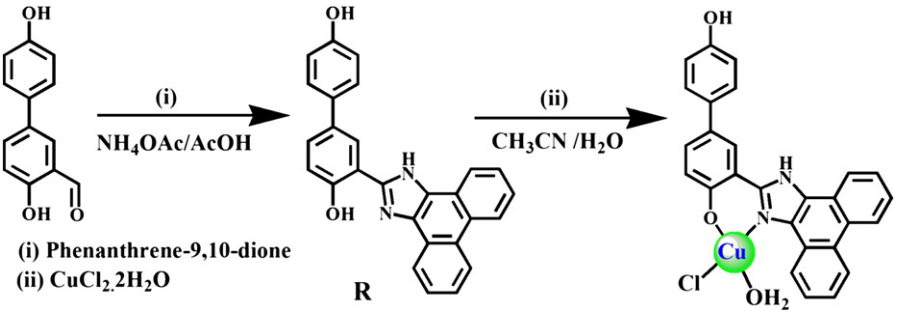
Research on highly selective and sensitive fluorescent probes for 3d-series metal ions has attracted great interest due to their vital roles in many biological processes [1,2]. In this regard, substantial efforts have been given and a large number of chemosensors have been developed for selective sensing of particular metal cations both *in vitro* and *in vivo* [3,4]. Fluorescent chemosensors have several advantages over the other optical sensors due to their intrinsic high sensitivity, easy handling and real-time monitoring with fast response time [5,6]. In recent time, the development of selective and sensitive imaging tools capable of rapid monitoring Cu^{2+} ions has attracted great attention due to the environmental and bio-relevant nature of Cu^{2+} [7–9]. Cu^{2+} is a vital (both useful and cytotoxic) trace ion in various enzymatic processes. Copper deficiency may lead to several neurological problems and excess copper in the body causes Alzheimer's, Wilson's, and Menke's *etc.* diseases [10,11]. Hence, search for selective fluorescent chemosensors for Cu^{2+} ions has become a great promising area of research. Although, several fluorescent sensors for Cu^{2+} are known, due to the slow response, low (mM or μM) sensitivity [12–15] lack of high selectivity and cytotoxicities of these sensors limit them to use for practical application [7–9]. In light of these issues, very recently an ethynyl based fluorescent Cu^{2+} sensor has been reported with the detection limit of ppb (0.1 μM) level [16]. Herein, we report highly sensitive (nanomolar), selective phenanthrene-based fluorescent and visual sensor (**R**) for Cu^{2+} ion with very low detection limit of ppt level (picomolar) and its potential application in human cancer cell bio-imaging. The fluorogenic receptor **R** (Scheme 1) was synthesized in a single step by reacting 5-(4-hydroxyphenyl)salicylaldehyde with 9,10-phenanthrenequinone in 58% yield (Scheme 1). **R** was characterized by FTIR, multinuclear (^1H, ^{13}C) NMR, HRMS and elemental analyses (Figs. S1–S4).

The binding behavior of the receptor **R** towards different metal cations as their chloride salts was monitored using UV–vis absorption and fluorescence spectroscopy. All the titration studies were carried out in H_2O/CH_3CN (8:2, v/v) solvent mixture. The electronic absorption spectrum of **R** (5 μM) in H_2O/CH_3CN (8:2, v/v) exhibited three sharp bands at 259, 351 and 366 nm (Fig. 1). Upon gradual addition of the aqueous solution of Cu^{2+} ion in increasing concentration (0–100 μM), the bands at 351 and 366 nm show slight enhancement in the initial absorption intensity and a new absorption band centered at 396 nm started to appear, which is attributed to the charge-transfer complex **R**–Cu^{2+} (Fig. 1).

The appearance of a well defined isobestic point centered at $\lambda = 282$ nm is consistent to an equilibrium between **R** and copper complex **R**–Cu^{2+} in solution. Furthermore, a perfect linear relationship was obtained from the absorption titration profile for the plot ($R = 0.9983$) of measured $[1/(A - A_0)]$ at 396 nm as a function of $1/[Cu^{2+}]$ using the well known linear Benesi–Hildebrand expression, which indicates a ~1:1 stoichiometry complex formation between **R** and Cu^{2+} ion in solution (Fig. 1). Calculated association constant is $K_a = 2.48 \times 10^3\ M^{-1}$. Notably, the addition of other metal cations (Na^+, K^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+} and Hg^{2+}) did not alter the initial absorption spectrum of the receptor **R** significantly. From these UV–vis studies, it is clear that receptor **R** shows very high selective binding affinity in the ground state only for Cu^{2+} ion even in the presence of different other metal ions (Fig. 1).

* Corresponding author. Tel.: +91 80 2293 2827; fax: +91 80 2360 1552.
E-mail address: psm@ipc.iisc.ernet.in (P.S. Mukherjee).

1387-7003/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.inoche.2012.08.012



Scheme 1. Synthesis of phenanthrene-based receptor **R** and its Cu^{2+} complex.

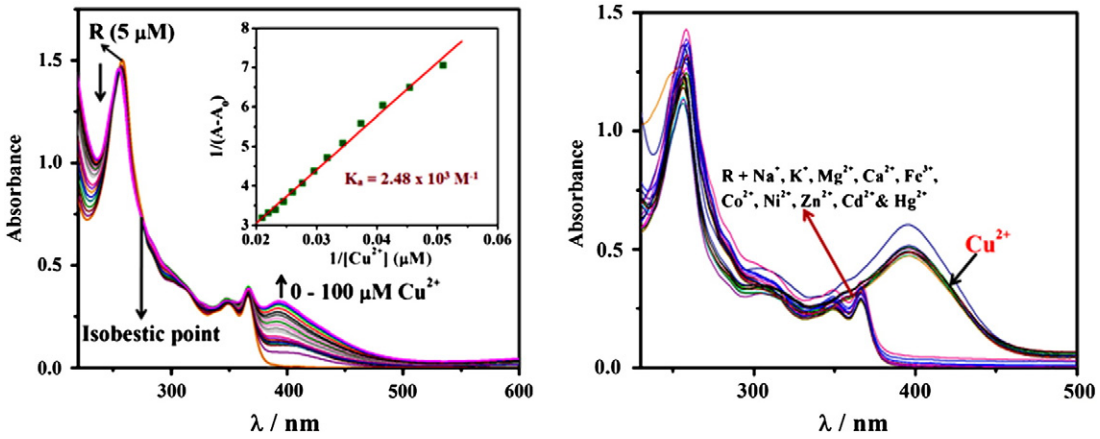


Fig. 1. Change in the absorption spectrum (left) of **R** (5 μM) in the presence of increasing concentration (0–100 μM) of Cu^{2+} ions (inset: the Benesi–Hilder Brand plot) and change in the absorption spectrum (right) of **R** upon mixing with different other metal cations.

The fluorescence spectrum of the receptor **R** (5 μM) exhibits a strong emission at 435 nm in H_2O/CH_3CN (8:2, v/v) medium. Upon gradual addition of increasing amounts of aqueous Cu^{2+} solution (0–10 μM) to the solution of **R**, the fluorescence emission at 435 nm is almost completely quenched after the addition of ~1.0 equivalent of Cu^{2+} (Fig. 2). This dramatic quenching of initial fluorescence intensity of **R** induced by Cu^{2+} ion is attributed to the reverse photo-induced electron transfer from phenanthrene moiety to the phenolic-OH and imidazole-N atoms due to the decrease in electron density upon the metal ion complexation [17]. Furthermore, the time resolved fluorescence study showed no changes in life time (2.13 ns) of **R** upon the gradual titration with Cu^{2+}, which support that the observed fluorescence quenching follows static quenching mechanism *via* the ground state complex (**R**–Cu^{2+}) formations (Fig. S5). The stoichiometry plot (Fig. 2) analysis of the fluorescence titration profile of **R** (5 μM) revealed a 1:1 stoichiometry between **R** and Cu^{2+} species and the calculated Stern–Volmer binding constant is 166.5×10^3 M^{-1}. The formation of a 1:1 complex was also indicated by ESI-MS, where obtained spectra show a peak at *m/z* of 518.53 corresponding to the expected [**R** + $CuCl(H_2O)$] complex (Fig. S6).

In order to prove the selectivity of receptor **R** towards Cu^{2+}, we carried the fluorescence titration experiment of **R** with other alkali

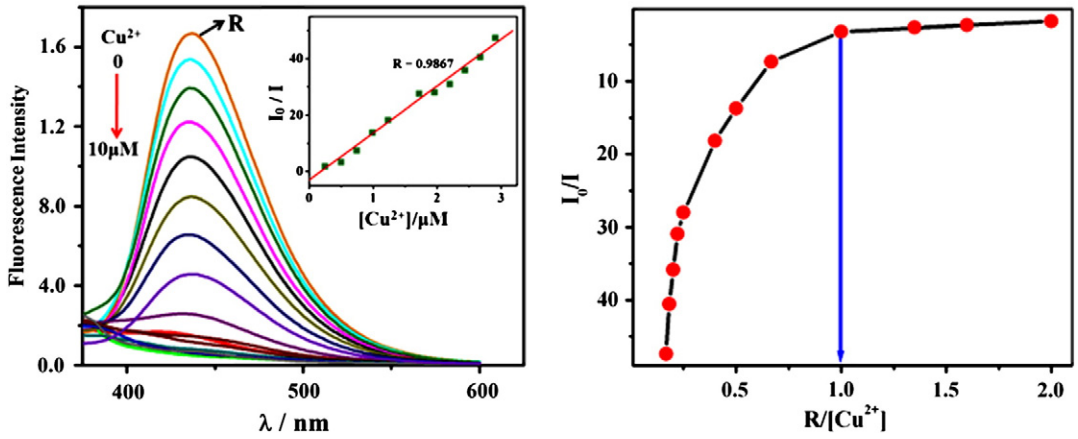


Fig. 2. Reduction in the initial fluorescence intensity of **R** (5 μM) upon gradual addition of Cu^{2+} solution (0–10 μM) (inset: Stern–Volmer plot) (left) and its stoichiometry plot (right).

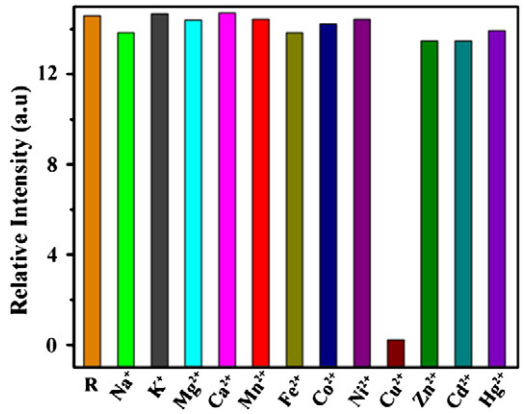


Fig. 3. Change in the initial fluorescence intensity of receptor **R** (5 μM) in the presence of 1.0 equiv of different metal cations in H_2O/CH_3CN (8:2, v/v) medium.

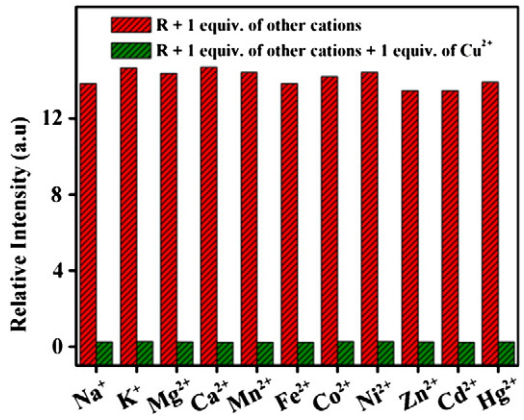


Fig. 4. Competitive selective binding affinity of **R** (5 μM) towards Cu^{2+} ions in the presence of 1.0 equiv of different metal cations in H_2O/CH_3CN (8:2, v/v) medium.

(Na^+, K^+), alkaline-earth (Mg^{2+}, Ca^{2+}) and 3d-series (Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+} and Hg^{2+}) metal ions. As shown in Fig. 3, only Cu^{2+} elicited a dramatic fluorescence quenching response, while the other tested metal ions such as Na^+, K^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+} and Hg^{2+} exhibited almost no fluorescence quenching response (Fig. S7) under the identical spectroscopic conditions as used for Cu^{2+}. The possible reason for the high selectivity of **R** might be due to the paramagnetic and unfilled d-shell of Cu^{2+} ion. That eventually makes the Cu^{2+} ion to exhibit discernable quenching of the fluorescence intensity *via* electron and/or energy transfer process [18,19]. Thus, receptor **R** could be used as a highly selective fluorescence sensor for Cu^{2+} ion over other metal species in aqueous medium.

To corroborate the practical applicability of receptor **R** as a selective fluorescence probe for Cu^{2+} ion, we carried out a competitive fluorescence titration study with other competing metal ions. As shown in Fig. 4, the initial fluorescence intensity of **R** did not changed significantly (red bar) upon mixing **R** with one equivalent of different other metal cations (Na^+, K^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+} and Hg^{2+}). But the subsequent addition of one equivalent of Cu^{2+} solution elicited a prominent fluorescence quenching (green bar), which further confirmed the excellent selectivity of the sensor **R** for Cu^{2+} ion in aqueous medium even in the presence of other aforesaid interfering metal cations.

Moreover, search for visual sensors for the trace detection of desired analytes has been a popular target in modern chemistry owing to their ease of interpretation and more suitable tool to practice in field. The ability of receptor **R** as a colorimetric probe for Cu^{2+} ion was imaged using a hand-held camera in the presence of other competing metal cations. As depicted in Fig. S8, **R** exhibited a distinct visual color change from colorless to green (under room light) and blue emission to almost dark (under UV light) after the addition of Cu^{2+} solution. However, there were no observable color changes noticed upon the mixing of **R** with other interfering metal cations (Na^+, K^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+} and Hg^{2+}) solutions. Thus, receptor **R** can be used as selective colorimetric sensor for Cu^{2+} ion over other competing metal ions in various environmental and biological systems.

As far as real-time application is concerned, the sensing process of a probe molecule must be a highly reversible one. To examine whether sensing process of **R** is reversible, one equivalent of ethylenediamine (en) solution was added into the solution of **R** which is pre-incubated with one equivalent of Cu^{2+} solution. After the addition of en solution, the initial emission intensity of **R** was almost recovered immediately from non-fluorescent **R**–Cu^{2+} complex including a sharp visual color change (Fig. 5). This result suggests the high reversibility of **R** towards Cu^{2+} sensing and potential in application of real-time monitoring. In addition, the fluorescence titration profile also demonstrates that **R** has a detection limit of 1.58 nM (100.33 ppt) for Cu^{2+} (Fig. 5), which is higher than that of other reported Cu^{2+} chemosensors [12–15] and this level of detection limit is sufficient enough to sense Cu^{2+} ion

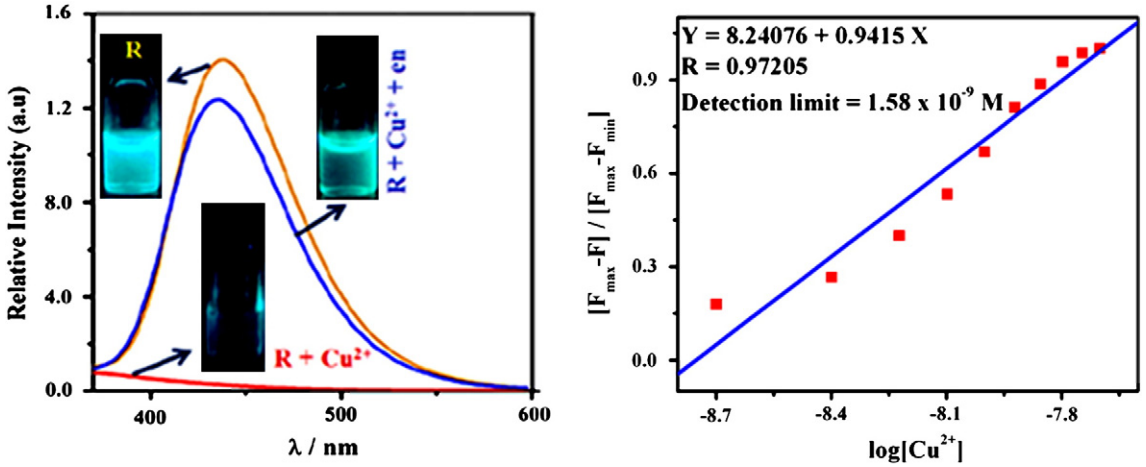


Fig. 5. Changes in fluorescence intensity (left) of **R** (5 μM, orange line) in the presence of Cu^{2+} (red line) and Cu^{2+} + en (blue line) in H_2O/CH_3CN 8:2 (v/v) and detection limit plot (right).

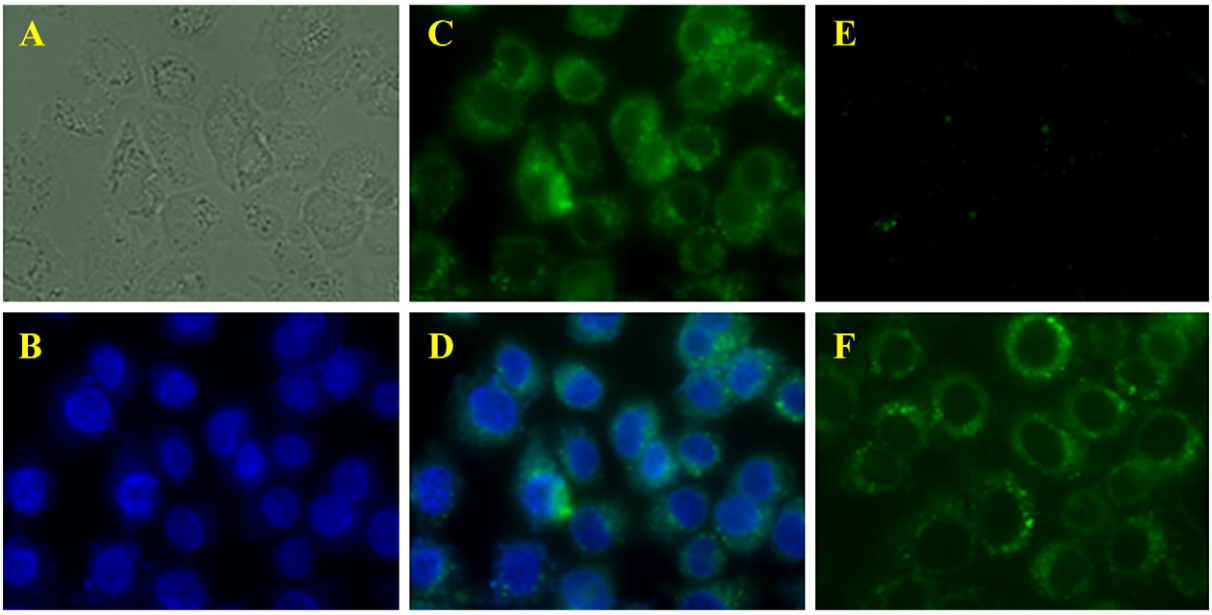


Fig. 6. Confocal fluorescence images of Cu^{2+} in HeLa cells (ApoTome [ZEISS] Fluorescence microscope). (A) Brightfield transmission images of HeLa cells. (B) Hoechst 33342 stained fluorescence images of HeLa cells incubated with **R** (5 μM). (C) Fluorescence image of HeLa cells incubated with **R** (5 μM). (D) Merged images of (B and C). (E) Cells supplemented with **R** (5 μM) in the growth media for 0.5 h at 37 °C and then incubated with $CuCl_2$ (10 μM) for 1 h at 37 °C. (F) Reversibility of fluorescence was achieved by addition of EDTA (20 μM). $\lambda_{ex} = 435$ nm; fluorescence images are recorded at single (465 ± 20 nm) channel.

even in the biological systems. Because the average concentration of Cu^{2+} in blood is 100–150 μg/L (15.7–23.6 μM) [20].

Having studied the interesting photophysical properties of **R** such as high sensitivity, selectivity and fast-response towards Cu^{2+} ion, we further extended our study to evaluate its potential use in imaging Cu^{2+} in living cells. The human cervical HeLa cancer cell lines incubated for 0.5 h at 37 °C with different concentrations of **R** (1.0 and 5.0 μM) showed bright fluorescence due to the accumulation of **R** within the cells (Figs. 6 and S9). But in contrast, the staining of pre-incubated cell with Cu^{2+} (5.0 and 10.0 μM) for 1 h at 37 °C exhibited almost no fluorescence and subsequent addition of EDTA (5.0 and 10.0 μM) regenerated the initial emission intensity of **R**. This result implies that receptor **R** is reversible and highly cell membrane permeable and thus **R** can be used as bio-sensor to probe the intracellular Cu^{2+} concentration and investigate its bioactivity in living cells.

In conclusion, we have synthesized a new phenanthrene-based visible and fluorescent sensor **R**, which shows highly selective, sensitive and reversible fluorescence quenching response towards Cu^{2+} in aqueous medium. In addition, we further demonstrated that receptor **R** can be utilized in live cell imaging of Cu^{2+} ion. To the best of our knowledge this represents a fluorescence and visual sensor with lowest detection limit of nanomolar in solution. The excellent detection limit [100.33 ppt] of this sensor would be useful in detection of trace quantity of Cu^{2+} in biological and environmental samples.

Acknowledgment

S. A. is grateful to UGC for D. S. Kothari fellowship, and S. S is thankful to CSIR, India for research fellowship. Financial support from the DST, New Delhi, India, is gratefully acknowledged.

Appendix A. Supplementary material

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.inoche.2012.08.012.

References

[1] H.N. Kim, M.H. Lee, H.J. Kim, J.S. Kim, J. Yoon, J. Chem. Soc. Rev. 37 (2008) 1465–1472.
[2] D.Y. Sasaki, D.R. Shnek, D.W. Pack, F.H. Arnold, Angew. Chem. Int. Ed Engl. 34 (1995) 905–907.
[3] A.P. de Silva, H.Q.N. Gunaratne, T. Gunnlaugsson, A.J.M. Huxley, C.P. McCoy, J.T. Rademacher, T.E. Rice, Chem. Rev. 97 (1997) 1515–1566.
[4] K. Rurack, U. Resch-Genger, Chem. Soc. Rev. 31 (2002) 116–127.
[5] R. Martinez-Manez, F. Sancenon, Chem. Rev. 103 (2003) 4419–4476.
[6] J.S. Kim, D.T. Quang, Chem. Rev. 107 (2007) 3780–3799.
[7] J. Wang, W. Lin, L. Yuan, J. Songa, W. Gaoa, Chem. Commun. 47 (2011) 12506–12508.
[8] L. Huang, J. Cheng, K. Xie, P. Xi, F. Hou, Z. Li, G. Xie, Y. Shi, H. Liu, D. Bai, Z. Zeng, Dalton Trans. 40 (2011) 10815–10817.
[9] Z. Xu, Y. Xiao, X. Qian, J. Cui, D. Cui, Org. Lett. 7 (2005) 889–892.
[10] B. Sarkar, Chem. Rev. 99 (1999) 2535–2544.
[11] K.J. Barnham, C.L. Masters, A.I. Bush, Nat. Rev. Drug Discov. 3 (2004) 205–214.
[12] H. Qin, J. Ren, J. Wang, E. Wang, Chem. Commun. 46 (2010) 7385–7387.
[13] X.Y. Zhao, Y. Liu, K.S. Schanze, Chem. Commun. (2007) 2914–2916.
[14] M. Beltramello, M. Gatos, F. Mancin, P. Tecillab, U. Tonellatoa, Tetrahedron Lett. 42 (2001) 9143–9146.
[15] L. Huang, F. Chen, P. Xi, G. Xie, Z. Li, Y. Shi, M. Xu, H. Liu, Z. Maa, D. Bai, Z. Zeng, Dyes Pigments 90 (2011) 265–268.
[16] S. Sirilaksanapong, M. Sukwattanasinitt, P. Rashatasakhon, Chem. Commun. 48 (2012) 293–295.
[17] M. Kumar, R. Kumar, V. Bhalla, P.R. Sharma, T. Kaur, Y. Qurishi, Dalton Trans. 41 (2012) 408–412.
[18] 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, J.S. Kim, J. Am. Chem. Soc. 131 (2009) 2008–2012.
[19] Z. Li, L. Zhang, L. Wang, Y. Guo, L. Cai, M. Yu, L. Wei, Chem. Commun. 47 (2011) 5798–5800.
[20] W.T. Tak, S.C. Yoon, Korean J. Nephrol. 20 (2001) 863–871.