A ruthenium(II) complex as turn-on Cu(II) luminescent sensor based on oxidative cyclization mechanism and its application in vivo

Yunfei Zhang1*, Zonglun Liu1*, Kui Yang1, Yi Zhang2, Yongqian Xu1, Hongjuan Li1, Chaoxia Wang3, Aiping Lu4 & Shiguo Sun1

1College of Science, Northwest A&F University, Yangling, Shaanxi, 712100, China, 2College of Plant Protection, Northwest A&F University, Yangling, Shaanxi, China, 3Key Laboratory of Eco-Textile, Ministry of Education, School of Textile and Clothing, Jiangnan University, 1800 Lihu Avenue Wuxi, 214122, China, 4School of Chinese Medicine, Hong Kong Baptist University, Kowloon Tong, Hong Kong, China.

Copper ions play a vital role in a variety of fundamental physiological processes not only in human beings and plants, but also for extensive insects and microorganisms. In this paper, a novel water-soluble ruthenium(II) complex as a turn-on copper(II) ions luminescent sensor based on o-(phenylazo)aniline was designed and synthesized. The azo group would undergo a specific oxidative cyclization reaction with copper(II) ions and turn into high luminescent benzotriazole, triggering significant luminescent increases which were linear to the concentrations of copper(II) ions. The sensor distinguished by its high sensitivity (over 80-fold luminescent switch-on response), good selectivity (the changes of the emission intensity in the presence of other metal ions or amino acids were negligible) and low detection limit (4.42 nM) in water. Moreover, the copper(II) luminescent sensor exhibited good photostability under light irradiation. Furthermore, the applicability of the proposed sensor in biological samples assay was also studied and imaged copper(II) ions in living pea aphids successfully.

As an essential transition metal ion not only for human beings and plants but also for extensive insects and microorganisms, Cu(II) plays a vital role in a variety of fundamental physiological processes including neurotransmission, energy generation, iron transportation, pigmentation and scavenging of free radicals1–3. Moreover, the internal concentrations of Cu2+ in normal organisms are tightly regulated and disruption of the copper homeostasis often cause disease states or pathophysiological conditions1–4. For humans, alterations in the copper homeostasis can be connected to some serious neurodegenerative diseases5–8 and may cause gastrointestinal disturbance or damages to liver and kidney9. While, for insects such as aphids, excess or deficiency in Cu(II) not only hinders their normal growth and development but also affects their plant responses10–13, which is closely related to the damage extent with their host plants. In addition, due to their widespread use in industry and agriculture, cupric ions are also considered to be a significant environmental pollutant14. Consequently, developing robust and versatile methods to investigate the biological and environmental roles of copper(II) ions have been attracted extensive attentions.

Among the reported methods for copper ions detection, luminescent probes are extensively employed owing to their distinct advantages in sensitivity and biological imaging15. However, due to the intrinsic fluorescence quenching property of Cu2+ stemming from its paramagnetic nature, most hitherto reported Cu2+ sensors have shown a “turn-off” response via an electron/energy transfer process16–18. Although some luminescence “off-on” Cu2+ sensors with high selectivity19,20, nanomolar sensitivity19,20, good water solubility20,23, excellent photostability24 and long emission wavelength20,24 have been reported, sensors combining all these features are rare up to now25. Furthermore, the probes for Cu2+ detection in biological systems, such as different cancer cells24,27, rat hippocampal slices25,26, zebrafish25,28, human tissues27,28, herb leaves26 have been investigated, however, copper(II) imaging in insects is rarely reported. Definitely, developing new Cu2+-selective turn-on luminescent probes with excellent performance for diverse biological systems is still of importance and necessity.
Ruthenium(II) complexes are one type of potential candidates for environmental and biological Cu$^{2+}$ probing, due to their good water solubility, high chemical and photocatalytic ability, intense polarized luminescence, red emission, large Stokes shifts, and long lifetimes$^{25,26}$. To date, some ruthenium(II) complex based luminescent probes for Cu$^{2+}$ have been developed$^{27–34}$. Unfortunately, as far as we know, there is only one example of luminescence enhancement Cu$^{2+}$ sensor based on a ruthenium(II) complex (Ru(bpy)3Pt) reported by Gopidas’s group, which could detect micromolar amounts of Cu$^{2+}$ in acetonitrile solution$^{35}$. Herein, in this paper, we focus on the development of a turn-on ruthenium(II) complex based luminescent sensor with superior performance for Cu$^{2+}$ detection and imaging.

Lee et al. reported a fluorescence turn-on chemodosimeter for Cu$^{2+}$ based on oxidative cyclization of a non-emissive azoaniline into a highly fluorescent benzotriazole product, which can detect μM-level concentrations of Cu$^{2+}$ in water at room temperature with the green emission$^{36}$. Given the relatively high detection limit of the reported sensor, here, we designed and synthesized a novel ruthenium(II) complex RuMAZO (Fig. 1) with o-(phenylazo)aniline group as a turn-on luminescent sensor for Cu$^{2+}$. The non-emissive RuMAZO in presence of copper(II) ions undergoes oxidative cyclization to form a highly luminescent product RuTAZO (Fig. 1).

**Results**

The non-emissive RuMAZO in presence of copper(II) ions undergoes oxidative cyclization to form a highly luminescent product RuTAZO (Fig. 1). This cyclization reaction can be triggered by nM-level (4.42 nM) concentrations of Cu$^{2+}$ in a HEPES (HEPES = 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer, to exhibit >80-fold enhancement in a red emission at $\lambda_{\text{max,em}}$ = 599 nm. The chloride salt of RuTAZO exhibits $\lambda_{\text{max,em}}$ value at 599 nm with a quantum yield of 4.7%, using [Ru(bpy)$_3$]$^{2+}$ (bpy = 2,2-bipyridine) as a standard (see supplementary information part)$.^{37}$ Moreover, this probe has been proved to be an appropriate luminescent Cu$^{2+}$ imaging reagent in live pea aphids. To the best of our knowledge, this is the first report on developing a ruthenium(II) complex-based luminescent sensor for luminescence enhancement detecting Cu$^{2+}$ in aqueous solution with high selectivity and sensitivity and imaging Cu$^{2+}$ in insects.

Luminescence enhancement was clearly evident up to 30 min in a HEPES buffer solution (20 mM, pH 7.4, 37°C), then no further significant changes occurred, indicating that the optimal reaction time for Cu$^{2+}$ detection via oxidative cyclization for this sensor is around 30 min (Fig. S1). In addition, the luminescence properties of RuMAZO were checked under the same conditions. As shown in Fig. S2, after treatment with different concentrations (0–3 equiv.) of Cu$^{2+}$ at a physiological temperature 37°C, the ligand absorption of RuMAZO (10 μM) at around 263 nm apparently increased and the metal-to-ligand charge transfer (MLCT) absorption at around 452 nm decreased, whereas a new ligand absorption peak at about 294 nm appeared. Correspondingly, within 30 min of reaction under the same conditions, the emission intensity at 599 nm increased to over 80 fold upon excitation at 465 nm with only 1 equiv. of Cu$^{2+}$ (Fig. 2a). The Stokes shift of RuTAZO is 134 nm. These results indicate that the o-(phenylazo)aniline group of RuMAZO can be efficiently converted into luminescent benzotriazole. Furthermore, the dose-dependent luminescence enhancement followed a good linear relationship with very low Cu$^{2+}$ concentrations in the range of 0.1–2.0 μM (Fig. 2b) and the limit of detection (LOD) for Cu$^{2+}$ with RuMAZO (10 μM) was determined to be 4.42 × 10$^{-9}$ M (see supplementary information part), lower or comparable to those of most previously reported highly sensitive sensors$^{38}$. Thus, the broad linear range and low detection limit make RuMAZO suitable for environmental or biological copper(II) detection and imaging.

For further biological applications, the cytotoxicity of RuMAZO and Cu$^{2+}$ to the HeLa cell lines was investigated with an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay after a 24 h treatment (Fig. S10). RuMAZO did not exhibit obvious cytotoxicity towards the HeLa cell lines at the concentrations employed. Confirming RuMAZO can be a suitable luminescence chemosensing probe for Cu$^{2+}$ detection in vivo.

To investigate the practical applicability of RuMAZO as a Cu$^{2+}$ sensor in the luminescence imaging of living cells, HeLa cells were incubated with RuMAZO (10 μM) for 2 h at 37°C in a PBS (phosphate buffer solution, pH = 7.4). After washed with PBS to remove the remaining RuMAZO, no obvious luminescence could be observed from the confocal laser scanning microscopy (Fig. S11a). However, the intracellular luminescence showed a clear red luminescence after incubated with Cu$^{2+}$ (20 μM) and PDTC (pyrrolidine dithiocarbamate, 100 μM) for 2 h at 37°C (Fig. S11b). PDTC$^{39}$ was used to increase the intracellular level of Cu$^{2+}$. The results revealed that RuMAZO could be used as an off-on luminescent probe for imaging Cu$^{2+}$ in living cells.

To examine the applicability of the sensor for visualizing Cu$^{2+}$ in living organisms, four-day-old pea aphids were selected and divided into three groups. The first two groups were given skin-pop injections at the bottom of the middle legs with Cu$^{2+}$ (300 nL, 5 mM in a HEPES buffer solution (20 mM, pH 7.4)) or RuMAZO (300 nL, 25 μM in a HEPES buffer solution (20 mM, pH 7.4)) respectively as the control. The third group was given a hypodermic injection of 25 μM RuMAZO and then 50 μM Cu$^{2+}$ (300 nL, 20 mM HEPES)

---

**Figure 1** | Synthesis of RuMAZO and the proposed mechanism of response of RuMAZO to Cu$^{2+}$ ions.
immediately. All samples were imaged using a Confocal Laser Scanning Microscope with a 488 nm excitation laser after incubation for 6 h. As shown in Fig. 3, pea aphids in the experimental group exhibited distinct luminescence signal over the entire bodies. While no apparent emission was observed in the control groups, illustrating that RuMAZO could detect Cu(II) in vivo without the interference of background signals. Taken together, RuMAZO is proved to be a desired turn on imaging agent for visualizing the distribution of Cu(II) in insects, based on this, a reliable method could be established for investigating the functions of Cu(II) on the plant response of aphids, the work is ongoing now.

Discussion

To investigate the sensing mechanism of RuMAZO to Cu(II), the reaction product of RuMAZO with Cu(II) in ethanol/H2O mixture was isolated and characterized by 1H NMR, 13C NMR and HR-MS (Fig. S19–S21). Furthermore, the isolated product exhibited nearly identical UV-vis and luminescence spectra with those of the testing mixture of RuMAZO and Cu(II) incubated at 37°C for 30 min (Fig. S3). The result of the EDTA (EDTA = ethylene diamine tetraacetic acid) competitive experiment provided further evidence on the non-binding interaction between RuMAZO and Cu(II) (Fig. S4). All these demonstrated the above mentioned proposed mechanism.

To verify the selectivity of RuMAZO towards Cu(II), the influence of other metal ions on the sensing of Cu(II) was determined. As shown in Fig. 4 and Fig. S5, the changes of the emission intensity of RuMAZO in the presence of 10.0 equivalents of other metal ions were negligible. Upon the addition of only 1.0 equivalent of Cu(II) to the 1 : 10 mixture of RuMAZO and other metal ions, a significant luminescence enhancement was observed, indicating that the existence of those metal ions in testing samples did not interfere copper(II) detection and imaging. Different copper salts (CuSO4, CuCl2, Cu(NO3)2 and Cu(OAc)2) were also tested, not much affection can be observed on the response of RuMAZO to Cu(II) ions with the presence of different counter anions (Fig. S6).

RuMAZO was observed to exhibit good photostability under the irradiation of 500 W iodine-tungsten lamp for 2 h (Fig. S7), this is beneficial for long-time luminescence tracking. In addition, the influence of pH on the luminescence of RuMAZO and RuTAZO

Figure 2 | (a) Luminescence intensity of RuMAZO (10 μM) with various concentrations of Cu(II) (0–30 μM) in a HEPES buffer solution (20 mM, pH 7.4); Insert: the changes of luminescence intensity at 599 nm with various concentrations of Cu(II); (b) A linear correlation between emission intensity of RuMAZO at 599 nm and concentrations of Cu(II) (0.1–2.0 μM).

Figure 3 | Confocal luminescence images of pea aphids given a subcutaneous injection of Cu(II) (a, 300 nL, 5 mM in a HEPES buffer solution (20 mM, pH 7.4)); 25 μM RuMAZO (b, 300 nL, 20 mM HEPES), 25 μM RuMAZO and 50 μM Cu(II) (c, 300 nL, 20 mM HEPES). Images were taken after incubation for 6 h. Left: Bright field images. Middle: Dark field images. Right: Merged images. λex = 488 nm.

Figure 4 | Luminescence changes of RuMAZO (10 μM) upon the addition of various metal ions (100 μM) and 10 μM Cu(II). Left-hand bars represent the luminescence response towards metal ions (blank, Li+, Na+, K+, Ca2+, Mg2+, Mn2+, Fe2+, Fe3+, Co2+, Ni2+, Cd2+, Ba2+, Pb2+, Pd2+, Cd2+, Ag+); right-hand bars represent the subsequent addition of 10 μM Cu(II) to the aforementioned solutions.
was examined by luminescence titration under different pH value. As shown in Fig. S8, no obvious signal changes were observed over the pH range of 2–13, confirming that the luminescence of RuMAZO and RuTAZO was independent of pH and expected to work well under physiological conditions.

Amino acids were also examined as potential interfering factors for bioimaging applications of the probe. The result demonstrated that the presence of amino acids had no interference with the sensitive detection of Cu²⁺ by RuMAZO (Fig. S9). All these proved that RuMAZO is appropriate for biological Cu²⁺ sensing and imaging.

In summary, a fully water-soluble ruthenium(II) complex (RuMAZO) with o-(phenylazo)aniline group as reactive site has been developed as a turn on copper(II) luminescence sensor. Under a physiological environment (20 mM HEPEs buffer solution, pH 7.4; 37°C), non- emissive RuMAZO can be efficiently transformed into high luminescent RuTAZO by an oxidative cyclization reaction with Cu²⁺ within 30 min, which can be triggered by nM level (4.42 nM) concentration of Cu²⁺ with excellent selectivity. Moreover, the probe has been employed to image Cu²⁺ in live pea aphids with a turn-on luminescence signal.

Methods

All solvents and chemical reagents employed for synthesis were analytical grade and purchased from commercial suppliers. The solutions of EDTA and metal ions were prepared in double-distilled water. HEPES buffered aqueous solution (20 mM, pH 7.4) was prepared on an Olympus FV1000 confocal microscope (Japan). Emission spectra were measured with a Shimadzu RF-5301 fluorescence spectrophotometer (Japan). Mass spectra were recorded on a Bruker 500 AVANCE III spectrometer with chemical shifts reported in ppm at room temperature. Mass spectra were obtained with Thermo Fisher LCQ Fleet mass spectrometer (USA) and a LC/Q-Tof MS spectrometry (USA). The pH of the testing systems was determined by a PHS-3C pH Meter (China).

Compound 1 was prepared according to the literature. The MAZO ligand was prepared through the coupling reaction of compound 1 with phenyl diazonium salt. The ruthenium(II) complex was obtained in a satisfactory yield (89%) through direct cyclization of Azoaromatics as Fluorescence Turn-On Signaling Mechanism.

RuMAZO was then converted to the chloride salt by dissolving in a minimum amount of acetone, and then dropped to a saturated solution of tetrabutylammonium chloride in acetone, stirred for 15 minutes. The chloride salt was filtered, washed with acetone, and dried under vacuum. Yield: 0.340 g, 92%. ESI-MS: 798.00 [M–Cl]+; 380.97 [M–2Cl]+.

RuTAZO. RuMAZO (53 mg, 0.05 mmol) was dissolved in 10 mL ethyl alcohol and water (2/3, V/V), then CuSO₄·5H₂O (25 mg, 0.1 mmol) was added to the mixture. It was refluxed for 1 h. Then the mixture was concentrated and purified by chromatography to get red orange solid 50 mg, 94.7%. H NMR (500 MHz, Acetone-d₆, δ ppm) 9.17 (s, 1H), 8.52 (dd, J = 3.3, 5.3 Hz, 2H), 8.25 (dd, J = 3.9, 7.7 Hz, 2H), 7.74 (d, J = 8.0 Hz, 2H). 7.66 (t, J = 7.4 Hz, 1H). 4H-NMR (125 MHz, Acetone-d₆) δ 153.46, 153.20, 153.08, 150.10, 148.01, 147.92, 146.61, 139.83, 137.16, 137.15, 138.10, 131.70, 129.80, 128.6, 127.34, 126.30, 126.27, 124.55, 124.52, 120.08. HR-MS: 904.1257; [M–PF₆]⁺: 379.5697; [M–2PF₆]⁻: 379.5697.

1. Peña, M. M. O., Lee, R. & Thiele, D. J. A Delicate Balance: Homeostatic Control of Copper Uptake and Distribution. J. Nutr. 129, 1251–1260 (1999).
2. Festa, R. A. & Thiele, D. J. Copper: An essential metal in biology. Curr. Biol. 21, R877–R883.
3. Kim, H., Wu, X. & Lee, J. SLC31 (CTR) family of copper transporters in health and disease. Molecular Aspects of Medicine 34, 561–570 (2013).
4. Arguello, J. M., Raimunda, D. & Padilla-Benavides, T. Mechanisms of Copper Homeostasis in Bacteria. Front. Cell. Infect. Microbiol. 3, 1–14 (2013).
5. Bush, A. I. Metals and neuroscience. Curr. Opin. Chem. Biol. 4, 184–191 (2000).
6. Barnham, K. J., Masters, C. L. & Bush, A. I. Neurodegenerative diseases and oxidative stress. Nat. Rev. Drug Discov. 3, 205–214 (2004).
7. Brünnl, E. J., Millar, T. M. & Cleveland, D. W. Unraveling the mechanisms involved in motor neuron degeneration in ALS. Annu. Rev. Neurosci. 27, 723–749 (2004).
8. Siggs, O. M. et al. Disruption of copper homeostasis due to a mutation of AtPta delays the onset of prion disease. PNAS 109, 13733–13738 (2012).
9. Georgopoulos, P. G. et al. Environmental copper: Its Dynamics and Human Exposure Issues. J. Toxicol. Environ. Health B Crit. Rev. 4, 341–394 (2001).
10. Auclair, J. L. & Srivastava, P. N. Some mineral requirements of the pea aphid, acyrthosiphon pisum (homoptera: aphididae). Can. Entomol. 104, 927–936 (1972).
11. Crawford, L. A., Hodkinson, I. D. & Lepp, N. W. The Effects of Elevated Host-Plant Cadmium and Copper on the Performance of the Aphid Aphis fabae (Homoptera: Aphididae). Appl. Ecol. 32, 528–533 (1995).
12. Giordanengo, P. et al. Compatible plant-aphid interactions: How Aphids manipulate plant responses. C. R. Biol. 333, 516–523 (2010).
13. Giordanengo, P. et al. Compatible plant-aphid interactions: How Aphids manipulate plant responses. C. R. Biol. 333, 516–523 (2010).
14. Ghrefat, H. & Yusuf, N. Assessing Mn, Fe, Cu, Zn, and Cd pollution in bottom sediments of Wadi Al-Arab Dam, Jordan. Chemosphere 65, 2114–2121 (2006).
15. Pal, A. & Jo, J. Cyclization of Azoaromatics as Fluorescence Turn-On Sensing and Imaging.
16. Bergonzì, R., Fabbrizzi, L., Licchelli, M. & Mangano, C. Molecular switches of Cu²⁺ sensing and imaging. Curr. Opin. Chem. Biol. 6, 734–748 (2006).
17. Giordanengo, P. et al. Compatible plant-aphid interactions: How Aphids manipulate plant responses. C. R. Biol. 333, 516–523 (2010).
18. Ghrefat, H. & Yusuf, N. Assessing Mn, Fe, Cu, Zn, and Cd pollution in bottom sediments of Wadi Al-Arab Dam, Jordan. Chemosphere 65, 2114–2121 (2006).
19. Pal, A. & Jo, J. Cyclization of Azoaromatics as Fluorescence Turn-On Sensing and Imaging.
20. Giordanengo, P. et al. Compatible plant-aphid interactions: How Aphids manipulate plant responses. C. R. Biol. 333, 516–523 (2010).
21. Crawford, L. A., Hodkinson, I. D. & Lepp, N. W. The Effects of Elevated Host-Plant Cadmium and Copper on the Performance of the Aphid Aphis fabae (Homoptera: Aphididae). Appl. Ecol. 32, 528–533 (1995).
22. Giordanengo, P. et al. Compatible plant-aphid interactions: How Aphids manipulate plant responses. C. R. Biol. 333, 516–523 (2010).
23. Giordanengo, P. et al. Compatible plant-aphid interactions: How Aphids manipulate plant responses. C. R. Biol. 333, 516–523 (2010).
24. Ajayakumar, G., Sreenath, K. & Gopidas, K. R. Phenothiazine attached Ru(bpy)₃²⁺ fluorescent probe for Cu²⁺ ions in aqueous solution with high sensitivity and selectivity. J. Am. Chem. Soc. 129, 9838–9839 (2007).
25. Xu, Z., Xiao, Y., Qian, X., Cui, J. & Cui, D. Ratiometric and Selective Fluorescent Sensor for Cull Based on Internal Charge Transfer (ICT). Org. Lett. 7, 889–892 (2005).
26. Ren, Z., Yang, R., He, H. & Jiang, Y. A highly selective charge transfer fluoroionophore for Cu²⁺. Chem. Commun. 1, 106–108 (2005).
27. Jo, J. et al. Reactivity-Based Detection of Copper(II) Ion in Water: Oxidative Cyclization of Azoraomacins as Fluorescence Turn-On Signaling Mechanism. J. Am. Chem. Soc. 134, 16000–16007 (2012).
28. Ajayakumar, G., Sreenath, K. & Gopidas, K. R. Phenothiazine attached Ru(bpy)₃²⁺ derivative as highly selective "turn-on" luminescence chemosensor for Cu²⁺. Dalton Trans. 7, 1180–1186 (2009).
29. Li, P. et al. A near-infrared fluorescent probe for detecting copper(ii) with high sensitivity and selectivity and its biological imaging applications. Chem. Commun. 47, 7755–7757 (2011).
30. Ballesteros, E. et al. A New Selective Chromogenic and Turn-On Fluorescent Probe for Copper(II) in Water—Acrinotetra 1:1 Solution. Org. Lett. 11, 1269–1272 (2009).
27. Kang, D. E. et al. Two-Photon Probe for Cu\textsuperscript{2+} with an Internal Reference: Quantitative Estimation of Cu\textsuperscript{2+} in Human Tissues by Two-Photon Microscopy. * Anal. Chem.* **86**, 5353–5359 (2014).

28. Swamy, K. M. K. et al. Boronic acid-linked fluorescent and colorimetric probes for copper ions. * Chem. Commun.* **45**, 5915–5917 (2009).

29. Zhou, L. et al. Molecular Engineering of a TEBT-Based Two-Photon Fluorescent Probe for Ratiometric Imaging of Living Cells and Tissues. *J. Am. Chem. Soc.* **136**, 9838–9841 (2014).

30. Yao, J. et al. Efficient Ratiometric Fluorescent Probe Based on Dual-Emission Quantum Dots Hybrid for On-Site Determination of Copper Ions. * Anal. Chem.* **85**, 6461–6468 (2013).

31. Balzani, V., Bergamini, G., Marchioni, F. & Ceroni, P. Ru(II)-bipyridine complexes in supramolecular systems, devices and machines. *Coord. Chem. Rev.* **230**, 1254–1266 (2006).

32. Bolletta, F. et al. A [Ru\textsuperscript{II}(bipy)\textsubscript{3}](1,9-diamino-3,7-diazanonane-4,6-dione) two-component system, as an efficient ON-OFF luminescent chemosensor for Ni\textsuperscript{2+} and Cu\textsuperscript{2+} in water, based on an ET (energy transfer) mechanism. *Dalton Trans.* **9**, 1381–1386 (1999).

33. Comba, P., Kramer, R., Mokhir, A., Naing, K. & Schatz, E. Synthesis of New Phenanthroline-Based Heteroditopic Ligands—Highly Efficient and Selective Fluorescence Sensors for Copper(II) Ions. *Eur. J. Inorg. Chem.* **2006**, 4442–4448 (2006).

34. Gel German Sz Ligature Er, B. & Alsfasser, R. Probing the aqueous copper(ii) coordination chemistry of bifunctional chelating amino acid ligands with a luminescent ruthenium chromophore. *Dalton Trans.* **4**, 612–618 (2003).

35. Li, X. et al. A New Luminescent Ruthenium(II) Polyphenylpyridine-derived Dipicolylamidine Complex as a Sensor for Cu\textsuperscript{2+} Ions. *Chinese J. Chem.* **29**, 1947–1950 (2011).

36. Lin, Q., Pei, L., Xu, W., Chao, H. & Ji, L. [Ru(bpy)\textsubscript{2}(pipdpda)]\textsuperscript{3+} as a highly sensitive and selective luminescent chemosensor for Cu\textsuperscript{2+} in aqueous solution. *Inorg. Chem. Commun.* **16**, 104–106 (2012).

37. Muegge, B. D. & Richter, M. M. Electrochemiluminescent Detection of Metal Cations Using a Ruthenium(II) Bipyridyl Complex Containing a Crown Ether Moiety. *Anal. Chem.* **74**, 547–550 (2001).

38. Patra, S. Boricha, V. P. Sreenidhi, K. R. Suresh, E. & Paul, P. Luminescent metallo receptors with pendant macrocyclic ionophore: Synthesis, characterization, electrochemistry and ion-binding study. *Inorg. Chem. Acta.* **363**, 1639–1648 (2010).

39. Rawle, S. C., Moore, P. & Alcock, N. W. Synthesis and coordination chemistry of 1-[2-(prime or minute)2[prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute]prime or minute}