Asian Journal of Chemistry; Vol. 27, No. 1 (2015), 47-50

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2015.16681

Synthesis, Characterization and Antitumor Studies of Two Copper(II) Schiff Base Complexes Derived from Glutathione and *o*-Vanillin

YAOYAO ZHU, YUHUA FAN, CAIFENG BI*, XIA ZHANG, PENGFEI ZHANG and XINGCHEN YAN

Key Laboratory of Marine Chemistry Theory and Technology, Ministry of Education, College of Chemistry and Chemical Engineering, Ocean University of China, Qingdao 266100, Shandong Province, P.R. China

*Corresponding author: E-mail: bicaifeng301@163.com

Received: 12 November 2013; *Accepted*: 4 April 2014; *Published online*: 26 December 2014; AJC-16503

Two new copper Schiff base complexes ($Cu_2C_{19}H_{31}N_3O_{11}S$ (**1**), $Cu_2(C_{18}H_{23}N_3O_8S)(C_{12}H_8N_2)_2$) (**2**) [$C_{18}H_{23}N_3O_8S$ = HL (Schiff base derived from glutathione and *o*-vanillin), $C_{12}H_8N_2$ = 1,10-phenanthroline] were synthesized and characterizated by elemental analysis, IR spectroscopy, ^{1}H NMR and thermogravimetric analysis. The test of cell proliferation observed that complex **2** binding with 1,10-phenanthroline are more potent apoptosis inducers in MDA MB 231 cells than complex **1**. Furthermore, our study suggests that complex **2** is capable of inhibiting tumor cellular proteasome activity and induce cancer cell-specific apoptotic death.

Keywords: Glutathione, Copper, Schiff base, Ternary complexes, Antitumor.

INTRODUCTION

Glutathione is a low molecular weight tripeptide, composed of glutamine, cysteine and glycine[1]. It is an important intracellular antioxidant responsible for several vital roles within a cell including maintenance of the redox state, drug detoxification and cellular protection from damage by free radicals, peroxides and toxins[2]. Glutathione is the most abundant thiol species at a concentration range of 1-10 mM in the cytoplasm that has been used as a releasing reagent *in situ* in living cells due to its major reducing capability in biochemical processes[3]. However, glutathione may be important in the aspect of cancer drugs.

Copper is an important cofactor in many proteins and enzymes and it has been known for angiogenesis[4,5]. Angiogenesis plays a key role in growth, development and wound healing processes. Besides, angiogenesis also assists in tumor cell proliferation and metastasis[6-9]. However, reducing the copper content *in vivo* to inhibit the angiogenesis without destruction of normal cellular function has been a hot topic for the treatment of cancer and the mechanism of angiogenic sensitivity to copper is still not fully understand. The complex of copper and the glutathione can fully reconstitute the enzyme in a very efficient process apparently involving a Cu·GSH·protein intermediate and glutathione may be able to donate Cu to the copper free enzyme *in vivo*[10].

Some Schiff base copper complexes such as L-glutamine Schiff base copper, taurine Schiff base copper complexes have been reported, could inhibit proteasome activity and cell proliferation, as well as induce apoptosis in breast cancer and prostate cancer cells[11-14].

The ubiquitin-proteasome pathway plays an important role in proteining degradation of eukaryotic cells[15-17]. Chymotrypsin-like activity has been known to be one of the proteolytic activities and it's only inhibition which is tightly associated with induction of tumor cell death programs[18-21].

In the current study, the copper complex with the Schiff base derived from glutathione and *o*-vanillin as ligands was synthesized and characterized. Its ternary complex using 1,10-phenanthroline as the second ligand was also synthesized and characterized. Based on the characterization, their structures were inferred. Then we investigated the profile of cancer cell growth-inhibitory activity and their structure-activity relationships of two copper complexes (Fig. 1). We have found that the complex **2**, which contain 1,10-phenanthroline as the second ligand, could inhibit proliferation and induce apoptosis in MDA MB 231 cells through inhibition of the ubiquitin-proteasome pathway.

EXPERIMENTAL

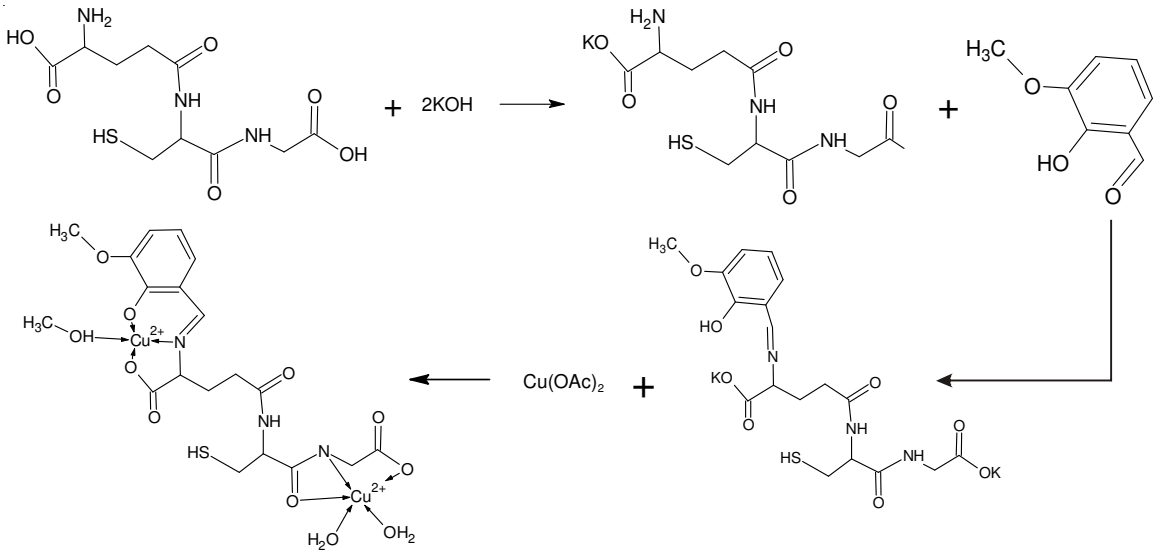
Meterials and physical measurement: The chemical agents, reduced glutathione (glutathione), *o*-vanillin and 1,10-phenanthroline were purchased from Aladdin (Los Angeles, CA). The chemical agents, dimethyl sulfoxide and 3-[4,5-dimethyltiazol-2-yl]-2, 5-diphenyl-tetrazolium bromide (MTT)

were purchased from Sigma-Aldrich (St. Louis, MO, USA) and all used without further purification. All compounds were dissolved as 50 mM stocks in DMSO and stored at 4 °C. Elemental analysis (C, H and N) was performed with a PerkinElmer model 2400 analyzer. IR spectra were recorded as KBr pellets with a Nicolet 170SX spectrophotometer in the 4,000-400 cm^{-1} region. ^1H NMR spectra were recorded with a AVANCE-III (600 MHz) spectrometer. TG analysis was carried out with a Netzsch STA409PC instrument at a heating rate of 10 K/min from 20 to 800 °C under nitrogen (flow rate = 60 mL/min).

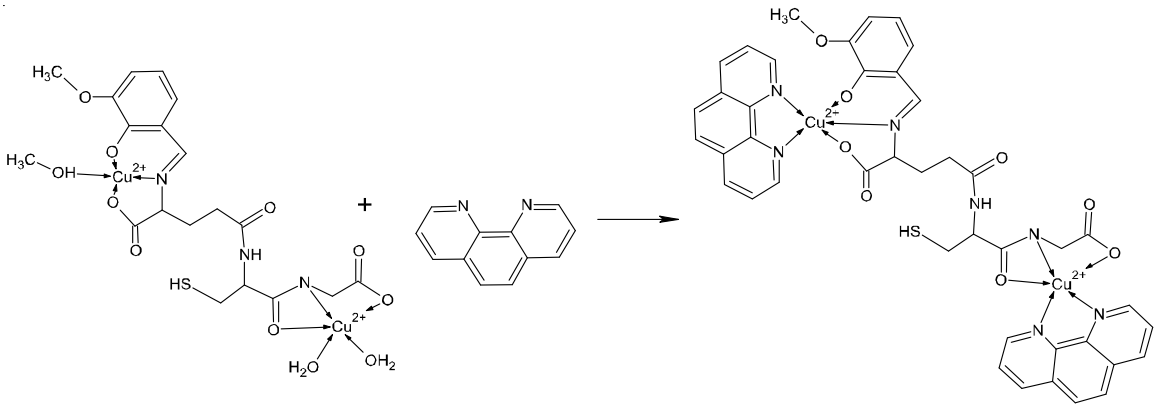
Synthesis of the complex 1: Glutathione (1 mmol) was dissolved in 20 mL of methanol and KOH (1 mmol) was added to give a solution. *o*-Vanillin (1 mmol) dissolved in 5 mL of methanol was added drop wise to the above solution with stirring and refluxed for 4 h to give a yellow solution, followed by addition of Cu $(CH_3COOH)_2{\cdot}H_2O$ (1 mmol) dissolved in 10 mL of methanol and reacted for 3 h to give a gray-green precipitate and then filtered off to give the complex (**Scheme-I**). Anal. calcd. for $Cu_2C_{19}H_{31}N_3O_{11}S$ (%); C, 35.54; H, 4.83; N, 6.54; S, 4.98. Found (%): C, 35.50; H, 4.80; N, 6.48; S, 5.01. IR data (KBr, ν_{max}, cm^{-1}): 1636.03 s, ν(N=C); 1603.65 m, ν_{as}(COO-); 1388.21 m, ν_s(COO-); 532.11 m, ν(Cu-N); 742.69 m, ν(Cu-O). ^1H NMR (DMSO, 600 MHz): δ (ppm) 10.325 (1H, s, NH); 9.378 (1H, s, OH); 8.609 [1H, s,= C(1)H]; 7.721 [1H, s = C(2)H]; 6.917 [1H, s = C(3)H]; 4.308 [8H, d, C(4)H2]; 3.770 [2H, d, C(5)H]; 3.304 [1H, m, C(6)H]; 3.281 [3H, m, $OC(7)H_3$]; 2.967 [3H, s, $C(8)H_3$]; 2.944 [4H, s, OH_2].

Synthesis of complex 2: Glutathione (1 mmol) was dissolved in 20 mL of methanol and KOH (1 mmol) was added to give a solution. *o*-Vanillin (1 mmol) dissolved in 5 mL of methanol was added drop wise to the above solution with stirring and refluxed for 4 h to give a yellow solution, followed by addition of Cu $(CH_3COOH)_2{\cdot}H_2O$ (1 mmol) dissolved in 10 mL of methanol and reacted for 3 h to give a gray-green precipitate, then added in 1,10-phenanthroline (1 mmol) dissolved in 5 mL of methanol and reacted for 4 h to give a green precipitate and then filtered off to give the complex (**Scheme-II**). Anal. calcd. for $Cu_2(C_{18}H_{23}N_3O_8S)(C_{12}H_8N_2)_2$ (%); C, 38.42; H, 4.16; N, 10.55; S, 3.42. Found (%): C, 39.01; H, 4.20; N, 9.86; S, 3.53. IR data (KBr, ν_{max}, cm^{-1}): 1637 s, ν(N=C); 1606.58 m, ν_{as}(COO-); 1401.2 m, ν_s(COO-); 543.11 m, ν(Cu-N); 720.56 m, ν(Cu-O) NMR (DMSO, 600 MHz): δ (ppm) 13.572 [2H, s, -SH]; 10.687 [3H, s, C(1)H] 8.324 [16H, s, C(2)H]; 6.648 [6H, s, $C(3)H_2$]; 4.808[6H, s, C(4)H]; 3.517[3H, s, OCH_3].

Cell culture and whole cell extract preparation: MDA MB 231 human breast cancer cells were purchased from the American Type Culture Collection (ATCC) and cultured in RPMI-1640 or 1:1 Dulbecco's modified Eagle's medium/F-12 medium, respectively, supplemented with 10 % fetal bovine



Scheme-I



Scheme-II

serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin (Life Technologies, Carlsbad, CA USA). Cells were cultured in an atmosphere containing 5 % CO_2 at 37 °C. Whole-cell extracts were prepared as described previously[22]. Briefly, cells were harvested, washed with PBS (phosphate-buffered saline), homogenized in a lysis buffer [50 mM *tris*-HCl, pH 8, 150 mM NaCl, 0.5 % NP_40], vortexed for 0.5 min at 4 °C and centrifuged at 12,000 g for 12 min. The supernatants were collected as whole-cell extracts and used or measurement of chymotrypsin-like activity.

Cell proliferation assay: The effect of each compound on cell proliferation was determined by 1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan, thiazolyl (MTT) assay. MDA MB 231 cells was seeded in triplicate in a 96-well plate and incubated at 37 °C until 70-80 % confluent, then treated with the indicated concentration of each compound for 24 h. Media was then removed and MTT solution (1 mg/mL) was added followed by a 2 h incubation period. MTT was then removed and 100 μL DMSO added to dissolve the metabolized MTT product, followed by measuring the absorbance values on a Victor 3 multi-label plate reader (PerkinElmer Wellesley, MA). Cellular morphological analysis Cellular changes were observed using an Axiovert 25 microscope (Carl Zeiss Microscopy, Thornwood, FL USA). Rounded and detached cells were considered apoptotic cells.

Analysis of proteasomal chymotrypsin-like activity in cell extracts: Whole cells extracts (10 μg) were incubated for 2 h at 37 °C in 100 μL of assay buffer (50 mM *tris*-HCl, pH = 7.5), with 20 μmol/L fluorogenic peptide substrate Suc-LLVY-AMC for assessment of proteasomal chymotrypsin-like activity. After incubation, production of hydrolyzed AMC groups was measured with a Wallac Victor 3 Multilabel counter with an excitation filter of 365 nm and emission filter of 460 nm. Changes in fluorescence were calculated against the DMSO treated control.

RESULTS AND DISCUSSION

The IR spectrum of complex **1** displays a strong absorption at 1636.03 cm^{-1}, which is assigned to ν(C=N). In addition to, the complex **1** displays two peaks at 1603.65 and 1388.21 cm^{-1}, which could be attributed to $\nu_{as}(COO^-)$ and $\nu_s(COO^-)$. The magnitude of $\nu_{as}(COO^-)$ and $\nu_s(COO^-)$ was more than 200 cm^{-1} in the complex, indicating that COO^- group was coordinated to the metal ion in a monodentate fashion[23]. The absorption bands at 532.11 cm^{-1} in complex **1** and 543.11 cm^{-1} in complex **2** are attributed to ν(Cu-N). The absorption at 742.69 cm^{-1} in complex **1** is attributed to ν(Cu-O). Comparing complex **2** with 1,10-phenanthroline, the absorption bands at 1515.3, 847.2 and 726 cm^{-1} all have a moving towards long wavenumber, suggesting that 1,10-phenanthroline participated in the coordination.

^{1}H NMR spectrum: In the complexes, we found that the hydrogen atom of -COOH was displaced by the metal ion and a new complex was formed by coordination of copper ion, which is also supported by the IR spectra.

TG-analysis: TG-analysis of these complexes was recorded in the range of 20-800 °C. These complexes were both decomposed primarily in two steps.

Complex 1: In the first step, 11.11-12.50 % (calculated as 11.22 %) of the complex **1** were lost, which was attributed to the loss of two molecules, one H_2O molecule and one CH_3OH molecule. The residual mass of the complex was 23.69 % (calculated as 24.94 %).

Complex 2: In the first step, 18.80-19.31 % (calculated as 19.21 %) of the complex **2** were lost, which was contributed to the loss of 1,10-phenanthroline. The residual mass of the complex was 18.20 % (calculated as 17.45 %).

Copper complexes inhibit cell proliferation and proteasome activity in human breast cancer cells: To investigate and compare the effects of complex **1** and complex **2** on cancer cell growth. MDA MB 231 cells were treated with 15, 30, 60 μM for 24 h, followed by MTT assay (Fig. 1). Cells treated with the solvent DMSO were used as control. We found that the two complexes both had growth-inhibitory activity, complex **1** inhibited cell proliferation by 11, 22 and 20 % at 15, 30 and 60 μM and complex **2** could inhibit cell proliferation by 92 % at 15 and at 30 and 60 μM and it inhibited almost 99 % of breast cancer cell growth. As a comparison, the complex **2** had sharp growth-inhibitory potency to MDA MB 231 cells and with the increasing concentration, they are more potent apoptosis inducers in MDA MB 231 cells. To assess the ability of complex **2** to induce apoptosis, we studied morphological changes in the same experiment based on the MTT results. (Fig. 2) At 24 h, we found that apoptosis-associated cellular morphology changes (rounding and shrinkage) were also observed in cells treated with complex **2**, at concentrations as low as 10 μM. We found that complex **2** has an obvious induction of apoptosis of cancer cells and with the increase of the concentration, the effect was more in-depth.

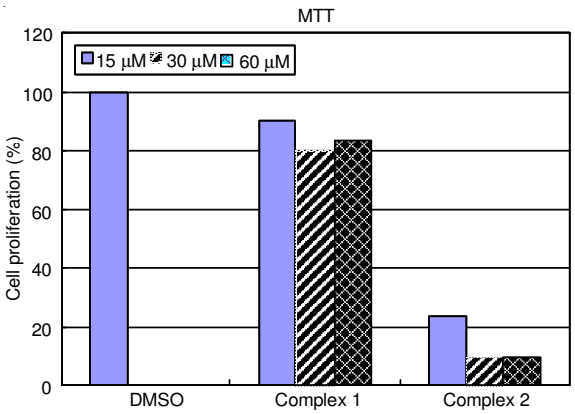


Fig. 1. Bromide (MTT) assay of MDA MB 231 cells treated with complex **1** and complex **2**. MDA MB 231 cells were treated with the complexes for 24 h at various concentrations as indicated. After 24 h, the medium was removed and the cells were treated with MTT solution, as described in "Experiment". Dimethyl sulfoxide (DMSO) was used as a control

MDA MB 231 cells were treated using 5, 10, 20, 30, 40 μM of complex **2** for 24 h and the effect on proteasomal CT-like activity. The CT-like activity assay results that with the concentration increasing, complex **2** as potent inhibitor, with a increasing drop in CT-like in the breast cancer cells (Fig. 3). In addition to, we treated MDA MB 231 cells with complex **2** at 30 μm for different time. We found that with the time going on, there is a increasing drop in CT-like in the breast cancer

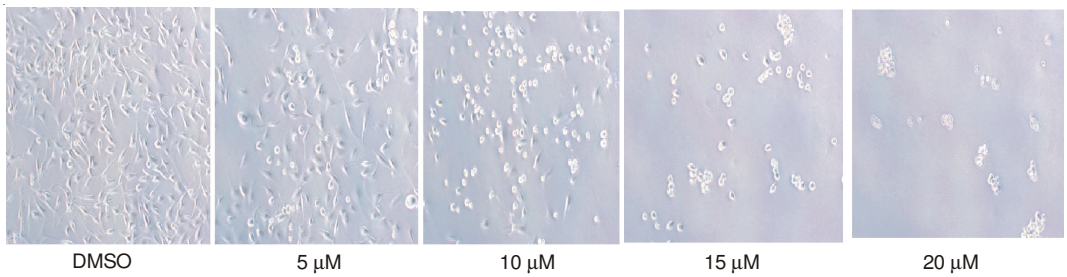


Fig. 2. Cellular morphologic changes

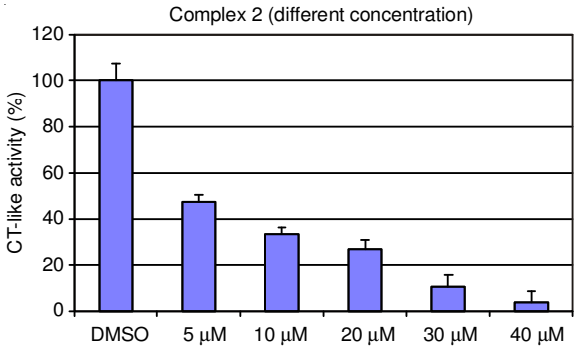


Fig. 3. Concentration effects of complex **2** on breast negative cancer MDA MB 231 cells. MDS MB 231 cells were treated with DMSO (as control) or different concentrations of complex **2** for 24h, followed by the chymotrysin-like activity assay

cells. Fig. 4 not all copper complexes are able to induce apoptosis and only those complexes that can carry copper ions into tumor cells and can prevent copper from interacting with many nonspecific proteins could induce apoptosis[24]. The results suggest that complex **2** couldn't only inhibit the growth of cancer cells, but also could get into the cells to induce the proteasome inhibition and apoptosis.

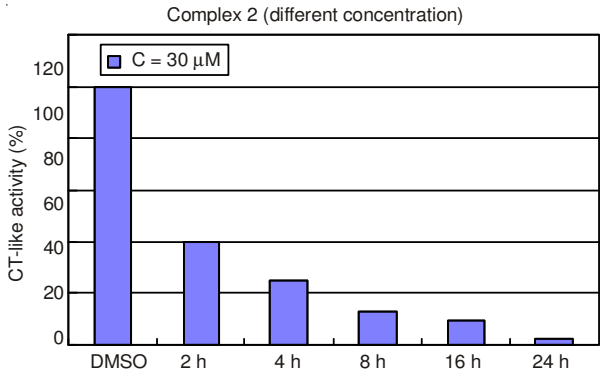


Fig. 4. Time effects of complex **2** on breast negative cancer MDA MB 231 cells. MDS MB 231 cells were treated with DMSO (as control) at different times of complex 2 at 30 μm, followed by the chymotrysin-like activity assay

Conclusion

Two new complexes were synthesized and characterized by elemental analysis, IR spectroscopy, ^{1}H NMR and thermogravimetric analysis, from which their structures were inferred. Then we investigate their effects to MDA MB 231 cells and found that both of the two complexes could inhibit cell proliferation. Compared to complex **1**, the effect of complex **2** to the cancer cells was deeper. According to the research of cell proliferation analysis, CT-like activity analysis and cellular morphological analysis, we found that complex **2** could kill almost all of the cancer cells. This result suggests that the 1,10-phenanthroline may play a key role in the effect of the apoptosis and complex **2** may be a potential anti-cancer medicine.

ACKNOWLEDGEMENTS

This research was supported by the National Natural Science Foundation of China to C.F. Bi (No.21371161 and No. 21071134) and to Y.H. Fan (No. 20971115), the Specialized Research Fund for the Doctoral Program of Higher Education of China to Y.H. Fan (No. 20120132110015), the Natural Science Foundation of Shandong Province to X. Zhang (No. ZR2012BQ026), the Special Foundation for Young Teachers of Ocean University of China to X. Zhang (No. 201113025).

REFERENCES

1. H. Sies, *Free Radic. Biol. Med.*, **27**, 916 (1999).
2. S. Singh, A.R. Khan and A.K. Gupta, *J. Exp. Therap. Oncol.*, **9**, 303 (2012).
3. (a) J.H. Ryu, R.T. Chacko, S. Jiwpanich, S. Bickerton, R.P. Babu and S. Thayumanavan, *Am. J. Chem. Soc.*, **132**, 17227 (2010); (b) A.M. Sauer, A. Schlossbauer, N. Ruthardt, V. Cauda, T. Bein and C. Bräuchle, *Nano Lett.*, **10**, 3684 (2010).
4. M. Linder, Biochemistry of Copper, Plenum Press, New York (1991).
5. L. Finney, S. Vogt, T. Fukai and D. Glesne, *Clin. Exp. Pharmacol. Physiol.*, **36**, 88 (2009).
6. M.M. Eatock, A. Schatzlein and S.B. Kaye, *Cancer Treat. Rev.*, **26**, 191 (2000).
7. J.P. Dutcher, *Curr. Oncol. Rep.*, **3**, 353 (2001).
8. S.B. Fox, G. Gasparini and A.L. Harris, *Lancet Oncol.*, **2**, 278 (2001).
9. M. Gourley and J.S. Williamson, *Curr. Pharm. Des.*, **6**, 417 (2000).
10. M.R. Ciriolo, A. Desideri, M. Paci and G. Rotilio, *J. Biol. Chem.*, **265**, 11030 (1990).
11. D. Chen, Q.C. Cui, H.J. Yang and Q.P. Dou, *Cancer Res.*, **66**, 10425 (2006).
12. X. Zhang, C. Bi, Y. Fan, Q. Cui, D. Chen, Y. Xiao and Q.P. Dou, *Int. J. Mol. Med.*, **22**, 677 (2008).
13. K.G. Daniel, P. Gupta, R.H. Harbach, W.C. Guida and Q.P. Dou, *Biochem. Pharmacol.*, **67**, 1139 (2004).
14. Y. Xiao, C. Bi, Y. Fan, C. Cui, X. Zhang and Q.P. Dou, *Intl. Oncol.*, **33**, 1073 (2008).
15. M. Hochstrasser, *Curr. Opin. Cell Biol.*, **7**, 215 (1995).
16. R.Z. Orlowski and E.C. Dees, *Breast Cancer Res.*, **5**, 1 (2002).
17. M. Hochstrasser, *Curr. Opin. Cell Biol.*, **7**, 215 (1995).
18. J. Adams, V.J. Palombella, E.A. Sausville, J. Johnson, A. Destree, D.D. Lazarus, J. Maas, C.S. Pien, S. Prakash and P.J. Elliott, *Cancer Res.*, **59**, 2615 (1999).
19. S. Imajoh-Ohmi, T. Kawaguchi, S. Sugiyama, K. Tanaka, S. Omura and H. Kikuchi, *Biochem. Biophys. Res. Commun.*, **217**, 1070 (1995).
20. K. Shinohara, M. Tomioka, H. Nakano, S. Tone, H. Ito and S. Kawashima, *Biochem. J.*, **317**, 385 (1996).
21. H.C. Drexler, *Proc. Natl. Acad. Sci. USA*, **94**, 855 (1997).
22. C.L. Fattman, B. An and Q.P. Dou, *J. Cell. Biochem.*, **67**, 399 (1997).
23. K. Nakamoto, D.R. Huang and Q.R. Wang, Infrared and Raman Spectra of Inorganic and Coordination Compounds, Chemical Industry Press, Beijing (1988).
24. Z. Zhang, C. Bi, S.M. Schmitt, Y. Fan, L. Dong, J. Zuo and Q.P. Dou, *J. Biol. Inorg. Chem.*, **17**, 1257 (2012).