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Synthesis, Characterization and Antitumor Activity of Novel Cadmium Complex with Laminine Ligand

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A novel cadmium(Cd) complex $[Cd(C_9H_{20}N_2O_2)_2{\cdot}(CH_3COO)_2{\cdot}2H_2O]$ ($C_9H_{20}N_2O_2$: laminine) was synthesized and characterized by elemental analysis, IR spectroscopy, ^1H NMR and thermogravimetric analysis. The anticancer activity of the laminine-Cd complex on MDA-MB-231 breast cancer cells was also investigated. The results showed that the laminine-Cd complex could inhibit the chymotrypsin-like activity of cell-free proteasome *in vitro* and induce apoptosis in cultured breast cancer MDA-MB-231 cells.

Keywords: Laminine, Cadmium complex, Proteasome inhibitor, Anticancer activity.

INTRODUCTION

The induction of protein degradation is facilitated by two independent pathways *i.e.*, lysosome-mediated and ubiquitin-proteasome pathway[1]. The latter is significant in the degradation of most endogenous proteins related to gene transcription, cell cycle, apoptosis and other major cellular processes. Since cancer cells are much more dependent on the ubiquitin-proteasome pathway than the normal ones, it becomes more valuable to develop proteasome inhibitors as selective anticancer drugs[2-7]. Chymotrypsin-like activity has been known as one of the proteolytic activities and it's only inhibition which is tightly concerned with induction of tumor cell death programs[8,9].

Recently there have been a great amount of reports emphasizing the use of transition metal-based complexes as anticancer agents. Probably the best known of these is cisplatin [*cis*-diamminedichloroplatinum(II)]. It has been widely used to treat various types of cancers, especially testicular cancer, with a 70 to 90 % cure rate. When combined with other drugs, it has been used successfully in the treatment brain, ovarian, bladder and breast cancer[10]. It has also reported that mixtures of disulfiram and cadmium can selectively inhibit proteasome activity in human breast cancer cells, but not non-tumorigenic cells and finally result in apoptosis[11]. Our laboratory has developed a number of metal-based anticancer drugs, including organic copper-, zinc- and gold-based complexes, all of which are able to inhibit proteasome and proliferation of the tumor cells, thereby inducing cancer cell death[12-14]. Cadmium, more harmful than necessary to people, is a widespread environmental pollutant with increasing worldwide concern. It is associated with continual consumption of contaminated food and water. Cadmium accumulates in the human body with a half-life exceeding 25 years once absorbed. For non-occupationally exposing people, Cd is absorbed into the body from dietary sources and cigarette smoking[15]. Cadmium has been shown to play an important role in carcinogenesis by enhancing DNA mutation rates and stimulating mitogenic signaling pathways and expression of proteins which control cellular proliferation[16,17].

To adapt to special living situation, most of the marine organisms produce and accumulate a large number of substances with special chemical structure and biological activity in their body. Laminine (5-amino-5-carboxy-N,N,N-trimethyl-1-pentanaminium inner salt) is a small molecule with good bioactivity, obtained from marine organism. It is a kind of non-protein amino acid which has special physiological functions in seaweed, such as lowering cholesterol, reducing blood lipids, anti-platelet condensed and preventing atherosclerosis[18].

In the current study, we describe the preparation and characterizations of a novel cadmium complex with laminine and investigate the potential proteasomal inhibition activities and apop-tosis induction in human breast cancer cells. Our study suggests the potential use of laminine as an agent to transform the carcinogen Cd to an anticancer drug through proteasome inhibition.

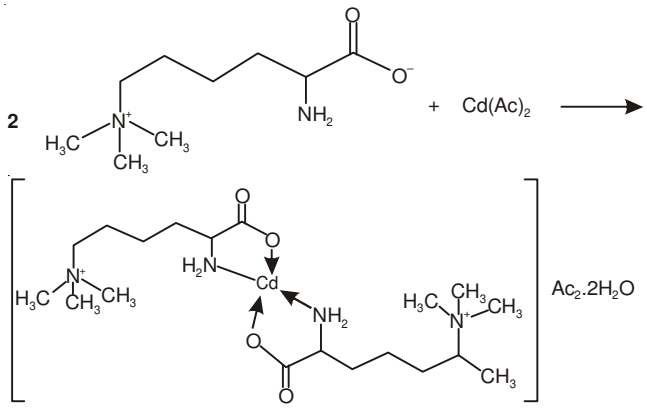
EXPERIMENTAL

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 prepared at a stock concentration of 50 mM in DMSO and stored at 4 °C. Elemental analysis (C, H and N) was performed with a Perkin Elmer 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 Bruker 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).

Preparation of the ligand: Laminine was synthesized in the Key Laboratory of Marine Chemistry Engineering and Technology (Ocean University of China, Qingdao, China).

Preparation of the cadmium complex: Laminine (0.189 g, 1 mmol) was dissolved in 20 mL of methanol with magnetic stirring and then 1 mmol (0.266 g) of cadmium acetate dissolved in 10 mL of anhydrous methanol were added dropwise to the solution. The mixture was stirred at 50 °C for 4 h to give a milky precipitate. The resulting solution was cooled at room temperature and then filtered to produce the final complex. Anal. calcd. (%) for $[Cd(C_9H_{20}N_2O_2)_2{\cdot}(CH_3COO)_2{\cdot}2H_2O]$ (mol. wt. 643.02): C, 41.06; H, 7.84; N, 8.71. Found (%): C, 41.22; H, 7.67; N, 8.93. IR data (KBr pellets, ν_{max}, cm^{-1}): 3321.11 (N-H); 1609.23, 1401.55 (COO^-); 668.90 (Cd-N); 565.71 (Cd-O). ^1H NMR (DMSO-d_6, 600 MHz) δ (ppm): 7.443 (s, 4H, $2NH_2$); 3.684 (s, 18H, 2^+ N $(CH_3)_3$); 3.821 (t, 2H, 2CH); 3.077 (t, 4H, $2CH_2$); 1.508-1.939 (m, 12H, $6CH_2$). TG analysis: lost 5.43 % (calcd. 5.60 %, $2H_2O$) in the first step at 70-150 °C; residue 19.21 % (calcd. 19.97 %, CdO).

The reaction equation is shown as follows:



Cell culture: MDA-MB-231 human breast cancer cells were obtained from American Type Culture Collection (Manassas, VA, USA) and cultured in RPMI 1640 or 1:1 Dulbecco's modified Eagle's medium/F-12 medium. All media was supplemented with 10 % fetal bovine serum (FBS), 100 μg/mL penicillin and 100 μg/mL streptomycin (Life Technologies, Carlsbad, CA USA). Cells were maintained at 37 °C in a humidified incubator with an atmosphere of 5 % CO_2.

Cell proliferation assays: The MTT assay was used to measure the effect of the compound on the cell proliferation of MDA-MB-231 human breast cancer cells. Cells were seeded in triplicate in 96-well plates and grown to 70 to 80 % confluent, then treated with the indicated concentration of the compound for 24 h. Media were then removed and MTT solution (1 mg/mL) was added. After 2 h incubation period at 37 °C, 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)[19].

***in vitro* Proteasomal activity assay:** Cell-free proteasome (10 μg) was incubated in 100 μL of assay buffer (20 mM *tris*-HCl, pH 7.5) and 20 μM of chymotrypsin-like substrate Suc-LLVY-AMC, using different concentrations of the laminine-Cd complex or DMSO as a vehicle control at 37 °C for 2 h. Following incubation, proteasome CT-like activity was measured using the Wallac Victor 3 Multi-label Counter with an excitation filter of 365 nm and emission filter of 460 nm.

Cellular morphology analysis: A Zeiss (Thornwood, NY) Axiovert 25 microscope was available for observing microscopic imaging with phase contrast for cellular morphology, as previously described[20].

RESULTS AND DISCUSSION

Some of the cadmium complexes could selectively inhibit proliferation and induce apoptosis in human breast cancer cells, but not in normal cells[3]. In this study, we synthesized a new cadmium complex first. The complex was characterized by elemental analysis, ^1H NMR spectroscopy, IR spectroscopy and thermogravimetric analysis.

Elemental analysis and ^1H NMR spectroscopy: The carbon, hydrogen and nitrogen contents in the synthetic complex were performed using elemental analyzer and compared with the calculated values which were described in the experimental section. From the ^1H NMR spectra, the $-NH_2$ protons appeared as singlet at δ 7.443 ppm, indicating that the coordination of nitrogen in $-NH_2$ to the metal ion.

IR spectra: The strong $-NH_2$ absorption peaks appeared at 3321.11 cm^{-1}, with obvious shift compared to the ligand (3235.92 cm^{-1}). This experimental result indicated that nitrogen and metal ions formed a coordination bond. The shift of $\nu_{as}(COO^-)$ and $\nu_s(COO^-)$ from 1655.23 and 1379.52 cm^{-1} in the ligand to 1621.11 and 1401.54 cm^{-1} in the complex, respectively, indicated the coordination of the oxygen in the carboxylate group to the metal ions. Furthermore, the magnitude of $\nu_{as}(COO^-)$ and $\nu_s(COO^-)$ was more than 200 cm^{-1}, which suggested that the oxygen in the $-COO^-$ group was coordinated to the metal ion in a monodentate fashion[21]. New bands at 668.90 and 565.71 cm^{-1} could be as a result of ν(Cd-N) and ν(Cd-O), respectively.

Thermal decomposition studies: TG-analysis of the complex was recorded in the range of 20-800 °C. On the basis of the TG and DTG curves, the complex was decomposed primarily in three steps. In the first step, decomposition temperature range was 70-150 °C, with weight lose of 5.43 % (calcd. 5.60 %), which corresponded to the two molecules of water. The fact that the water molecule was lost at a low temperature

suggests that the water was crystalline water[22]. The second weight loss stage had decomposition temperature range of 150-380 °C and 22.99 % (calcd. 22.52 %) of the Cd complex was lost, corresponding to the loss of two molecule acetate ($-CH_3COO^-$). Here after, the complex decomposed sequentially. The weight percentage of 19.21 % (calcd. 19.97 %) of the original sample remained, CdO is the final residue.

Cadmium compound inhibit cell proliferation and proteasome activity in MDA-MB-231 breast cancer cells: It has been shown that inhibition of tumor cellular proteasome activity is associated with apoptosis induction[6]. First of all, we started to measure their effect on cell proliferation respectively treating the MDA-MB-231 cells with 30 and 60 μM of ligand and Cd complex for 24 h, then a MTT cell proliferation assay was performed. DMSO was used as a control in this experiment. Results of the MTT cell proliferation assay using breast cancer cells showed that Cd complex caused more than 84 % growth decline at 60 μM (Fig.1), indicating that Cd complex is a responsibly potent inhibitor. In contrast, the ligand failed to inhibit the MDA-MB-231 cell proliferation under the same experimental condition.

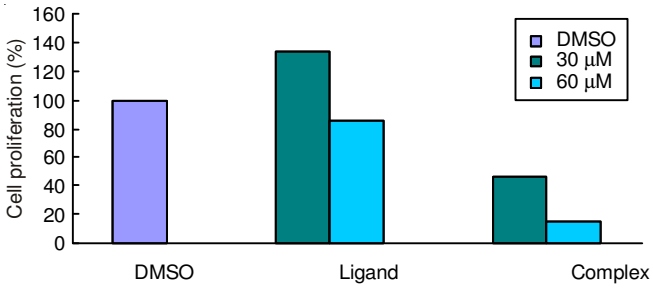


Fig. 1. Anti-proliferation activities of the ligand and complex on MDA-MB-231 cells.MDA-MB-231 cells were treated with each indicated compound at 30 and 60 μM for 24 h

Following MTT cell proliferation assay, we investigated the ability of the Cd complex to inhibit proteasomal activity and induce apoptotic cell death. MDA-MB-231 cells were treated with different concentrations (20, 40 and 60 μM) of compound for 24 h, followed by the effect on proteasomal CT-like activity. The CT-like activity assay indicated that Cd complex was a potent inhibitor, with 73.1 % inhibition at 20 μM, 83.2 % inhibition at 40 μM and 85.3 % inhibition at 25 μM. In comparison, cells treated with free ligand had no or little proteasome inhibitory effect (Fig. 2), which agreed with the MTT assay result.

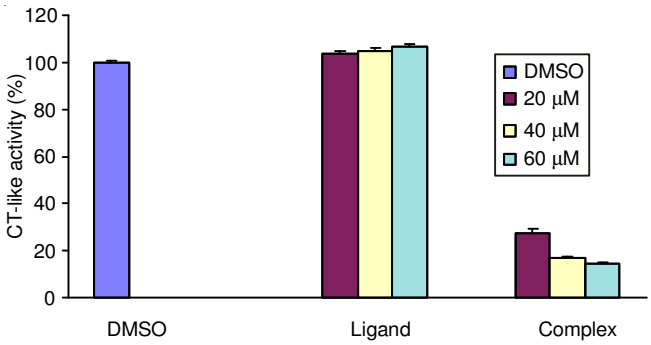


Fig. 2. Inhibition of proteasomal CT-like activity by $Cd(C_9H_{20}N_2O_2)_2{\cdot}(CH_3COO)_2{\cdot}2H_2O$

Concentration-dependent effect of cadmium complex on apoptosis induction in MDA-MB-231 cells: Though Cd complex was able to inhibit cell proliferation and proteasome activity in MDA-MB-231 breast cancer cell, we next tried to find out whether this complex could induce apoptosis on human breast cancer cells. MDA-MB-231 cells were treated with the Cd complex at concentrations of 5, 10, 20 and 50 μM for 24 h. Cells treated with DMSO were used as a vehicle control.

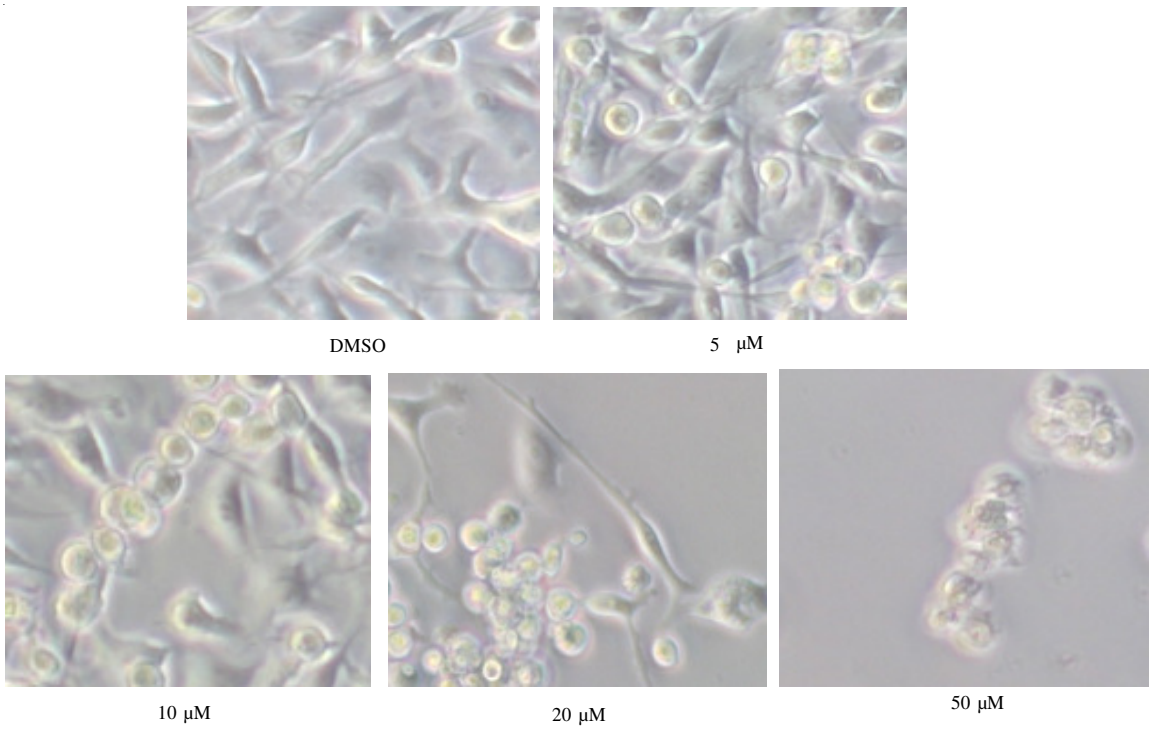


Fig. 3. Cellular morphological changes of MDA-MB-231 cells treated with $Cd(C_9H_{20}N_2O_2)_2{\cdot}(CH_3COO)_2{\cdot}2H_2O$ for 24 h

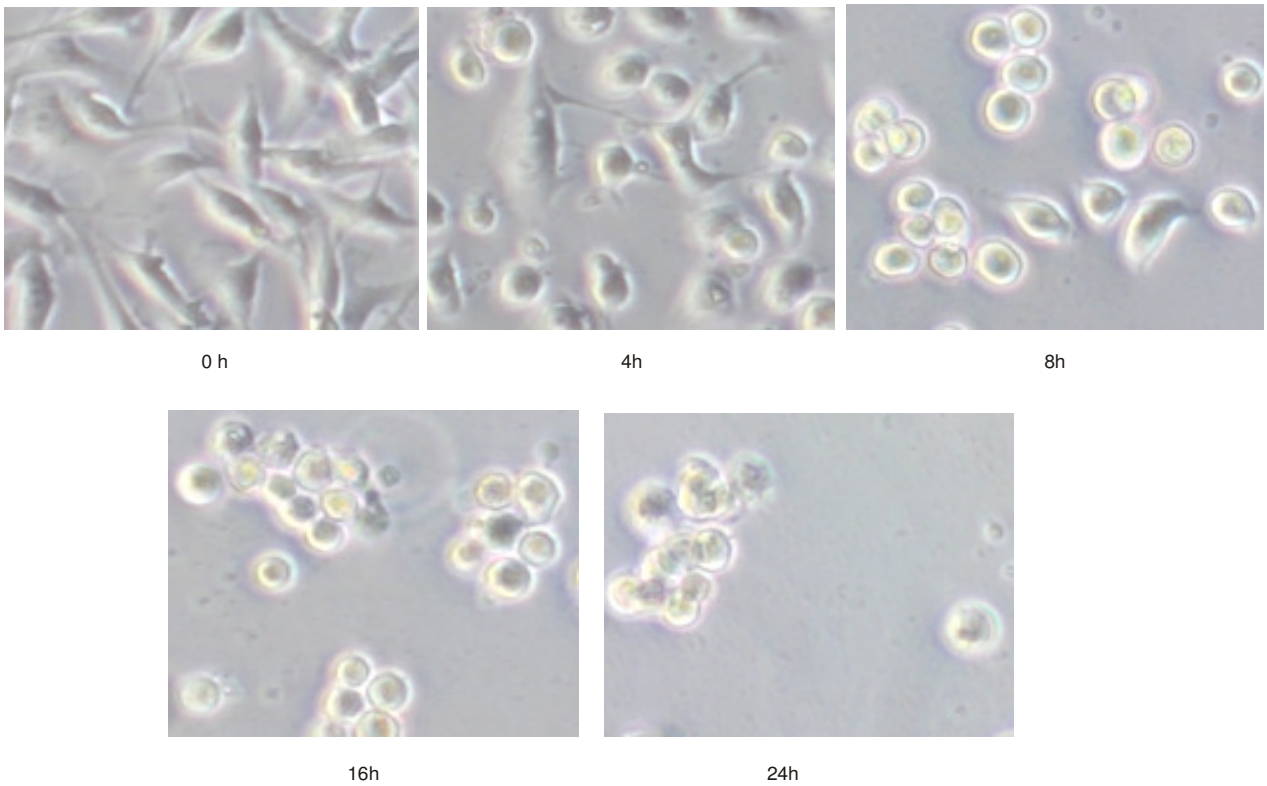


Fig. 4. Cellular morphological changes of MDA-MB-231 cells treated with $Cd(C_9H_{20}N_2O_2)_2{\cdot}(CH_3COO)_2{\cdot}2H_2O$ at 50 μM

Consistently, morphological changes (detached, shrunken and apoptotic blebbing) were observed in cells treated with 50 μM of Cd complex and a significant but less extent at 5 μM (Fig. 3). We could come to a conclusion that the Cd complex inhibited proliferation and induced apoptosis of MDA-MB-231 cells in a concentration-dependent manner.

Cadmium complex induces time-dependent apoptosis in MDA-MB-231 cell: To further confirm the kinetic effect of apoptosis induction, MDA-MB-231 cells were treated with 50 μM of Cd complex over different time points (4-24 h). We observed that apoptotic morphological changes appeared after 4 h treatment with the complex. Apoptosis induction at later time points was also typified with the appearance of aberrant morphological changes (detached, shrunken and apoptotic blebbing) (Fig. 4). Our results distinctly pointed out the idea that Cd complex induce apoptosis in breast tumor cells.

Conclusion

In the current study we synthesized a novel Cd complex with laminine and characterized by elemental analysis, IR spectroscopy, ^{1}H NMR and thermogravimetric analysis. Furthermore, we examined the proteasome inhibition activity and apoptosis induction of the Cd complex using MDA-MB-231 breast cancer cells. It is found that Cd complex could suppress the proliferation and inhibit proteasome activity in MDA-MB-231 breast cancer cells and they could be capable of inducing apoptosis in breast cancer cells in a concentration- and time-dependent fashion. The research of the antitumor activity of those small molecules with good bioactivity of marine organism and their complexes may be important to the finding of new antitumor drugs and the utilization of marine resources.

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REFERENCES

1. X. Yan, C.F. Bi, Y.H. Fan, C. Cui, X. Zhang and Q.P. Dou, *Int. J. Oncol*., **33**, 1073 (2008).
2. U.G. Lopes, P. Erhardt, R. Yao and G.M. Cooper, *J. Biol. Chem*., **272**, 12893 (1997).
3. S.S. Hindo, M. Frezza, D. Tomco, M.J. Heeg, L. Hryhorczuk, B.R. McGarvey, Q.P. Dou and C.N. Verani, *Eur. J. Med. Chem*., **44**, 4353 (2009).
4. X. Zhang, C.F. Bi, Y.H. Fan, D. Chen, X. Yan and Q.P. Dou, *Int. J. Mol. Med*., **22**, 677 (2008).
5. D. Chen, C. Cui, H.J. Yang, R.A. Barrea, F.H. Sarkar, S.J. Sheng, B. Yan, G.P.V. Reddy and Q.P. Dou, *Cancer Res.*, **67**, 4841 (2007).
6. Z. Zhang, C.F. Bi, S.M. Schmitt, Y.H. Fan, L.L. Dong, J. Zuo and Q.P. Dou, *J. Biol. Inorg. Chem*., **17**, 1257 (2012).
7. B. Cvek, V. Milacic, J. Taraba and Q.P. Dou, *J. Med. Chem*., **51**, 6256 (2008).
8. 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).
9. X. Zhang, M. Frezza, V. Milacic, L. Ronconi, Y.H. Fan, C.F. Bi, D. Fregona and Q.P. Dou, *J. Cell. Biochem*., **109**, 162 (2010).
10. C. Deegan, M. McCann, M. Devereux, B. Coyle and D.A. Egan, *Cancer Lett*., **247**, 224 (2007).

11. L. Li, H. Yang, D. Chen, C. Cui and Q. Ping Dou, *Toxicol. Appl. Pharmacol.*, **229**, 206 (2008).
12. V. Milacic and Q.P. Dou, *Coord. Chem. Rev.*, **253**, 1649 (2009).
13. M. Frezza, S.S. Hindo, D. Tomco, M.M. Allard, C. Cui, M.J. Heeg, D. Chen, Q.P. Dou and C.N. Verani, *Inorg. Chem.*, **48**, 5928 (2009).
14. G.H. Zhao and H.K. Lin, *Curr. Med. Chem.*, **5**, 137 (2005).
15. Z. Zhang, C.F. Bi, D. Buac, Y.H. Fan, X. Zhang, J. Zuo, P.F. Zhang, N. Zhang, L.L. Dong and Q.P. Dou, *J. Inorg. Biochem.*, **123**, 1 (2013).
16. A. Shrivastav, N.K. Singh and G. Srivastava, *Bioorg. Med. Chem.*, **10**, 2693 (2002).
17. N. Zhang, Y.H. Fan, Z. Zhang, J. Zuo, P.F. Zhang, Q. Wang, S.B. Liu and C.F. Bi, *Inorg. Chem. Commun.*, **22**, 68 (2012).
18. J.P. Girard, C. Marion, M. Liutkus, M. Boucard, E. Rechencq, J.P. Vidal and J.C. Rossi, *Planta Med.*, 193 (1988).
19. K.G. Daniel, D. Chen, S. Orlu, C. Cui, F.R. Miller and Q.P. Dou, *Breast Cancer Res.*, **7**, 897 (2005).
20. K.G. Daniel, P. Gupta, R.H. Harbach, W.C. Guida and Q.P. Dou, *Biochem. Pharmacol.*, **67**, 1139 (2004).
21. D.J. Liu, Z.R. Liu and C.F. Bi, *J. Rare Earths*, **22**, 54 (2004).
22. Y.H. Fan, C.F. Bi, S.Q. Liu, L.R. Yang, F. Liu and X.K. Ai, *J. Radioanal. Nucl. Chem.*, **267**, 89 (2005).