CYTOTOXICITY AND DNA BINDING ABILITY OF TWO NOVEL GOLD(III) COMPLEXES

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*The interaction of two gold(III) complexes [Au(phen)Cl_2](Cl) (**1**) and [Au(pdon)Cl_2](Cl) (**2**) with calf thymus-DNA (CT-DNA) has been investigated by absorption and fluorescence emission. Both complexes **1** and **2** show medium interaction ability with CT-DNA with the intrinsic binding constants K_b of 4.98×10^5 and 1.98×10^5 M^{-1} at room temperature, respectively, which is the same as earlier reports for typical classical intercalators. Moreover, complex **1** demonstrates a better antitumor effect on the tested cancer cells.*

Keywords: *potential anticancer agents, DNA binding, metal complexes, fluorescence spectroscopy.*

ЦИТОТОКСИЧНОСТЬ И СПОСОБНОСТЬ СВЯЗЫВАНИЯ С ДНК ДВУХ НОВЫХ КОМПЛЕКСОВ ЗОЛОТА(III)

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*Взаимодействие двух комплексов золота(III) [Au(phen)Cl_2](Cl) (**1**) и [Au(pdon)Cl_2](Cl) (**2**)) с ДНК тимуса теленка исследовано с помощью поглощения и флуоресцентного излучения. Комплексы **1** и **2** взаимодействуют с ТМ-ДНК при комнатной температуре с константами связывания $K_b = 4.98\times10^5$ и 1.98×10^5 M^{-1}. Комплекс **1** демонстрирует лучший противоопухолевый эффект на тестируемые раковые клетки.*

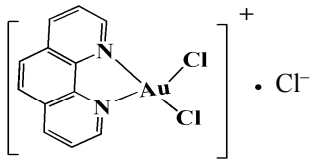
Ключевые слова: *потенциальные противораковые агенты, связывание ДНК, комплексы металлов, флуоресцентная спектроскопия.*

Introduction. Cancer is one of the most severe diseases, triggered by excessive cell proliferation in the body [1]. Large numbers of chemotherapy drugs have been developed to treat cancer [2–4], and among them, platinum-based complexes, being the most basic ones, are extensively used due to their considerable advantages [5, 6] such as low cost, broad spectrum, high efficiency, and so on. Although platinum compounds exert a certain influence on the treatment of lung, cervical, and head and neck cancer, their major defects are severe renal toxicity [7–10] and drug resistance [11–13]. The development of cisplatin has also led scientists to develop other metal complexes [14, 15]. Gold complexes have become a hot topic for researchers because of their ability to block cell growth and fight HIV [16–18].

Trivalent gold complexes are of great interest to researchers because their structure is similar to cisplatin [19, 20]. Lum reported a series of trivalent gold complexes highly active against cisplatin-resistant cell lines [21]. Marzano et al. [22] made a significant contribution to the animal experiments and clinical studies of carbamate-based Au(III) complexes with better anti-cancer effects. However, some gold complexes are unstable under physiological conditions and exhibit serious side effects in clinical trials [23, 24]. Additionally, al-

though many studies have illustrated that gold complexes have a significant anti-tumor effect, the exact mechanism of cytotoxicity is still uncertain [25–27]. So it is necessary to develop more Au(III) complexes [28, 29].

With the aim to overcome the strong side effects and cross-resistance defects of cisplatin and to explore more stable, less toxic gold complexes, in this paper, two novel Au(III) complexes $[Au(phen)Cl_2](Cl)$ (**1**) and $[Au(pdon)Cl_2](Cl)$ (**2**) (phen = 1,10-phenanthroline; pdon = 1,10-phenanthroline-5,6-dione) were prepared from the reaction of phen or pdon with $KAuCl_4 \cdot 2H_2O$, respectively, according to the previously reported procedure [30, 31].



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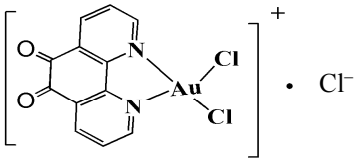

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The interaction of **1** and **2** with DNA was studied by fluorescence and UV-vis spectroscopy. By the results of cytotoxicity, complex **1** exhibited a strong inhibition on HepG-2 cells.

Experimental. All chemical solvents were dehydrated and distilled by standard methods before use. Ethidium bromide (EB) and calf thymus DNA (CT-DNA) were purchased from Sigma.

IR spectra were recorded on a Nicolet 380 spectrometer as KBr pellets in the range 4000–400 cm^{-1} with OMNIC software. Electrospray ionization mass spectrometry (ESI-MS) was analyzed using a Triple TOF^{TM} 5600$^+$ system with an ions spray source in the positive-ion mode. Uv-Vis spectra were measured by a Hewlett Packard HP-8453 spectrophotometer. Fluorescence spectral were obtained by RF-5301 fluorescence spectrophotometer at the approved temperature.

*Synthesis of [Au(phen)(Cl)$_2$]Cl (**1**).* The precursor was prepared according to the procedure [30, 31] with some modification. $KAuCl_4 \cdot 2H_2O$ (414.2 mg, 1.0 mmol) was dissolved in water (10 mL), 1,10-phenanthroline (375.3 mg, 2.0 mmol) in methanol (10 mL) was added, and the reaction mixture was stirred for about 6 h. The crude product of Au(III) metal complex **1** was obtained and thrice washed by distilled water and methanol. Elemental analysis for $[Au(phen)(Cl)_2]Cl$: calculated, %: C 29.81, H 1.67, N 5.79; found, %: C 29.78, H 1.81, N 5.78. Electrospray ionization mass spectrometry (ESI-MS): *m*/*z* 479.00 [**1**-Cl + $CH_3OH]^+$ 479.1649.

*Synthesis of [Au(pdon)Cl$_2$]Cl (**2**).* A similar method was used to prepare complex **2**. One mmol (210.1 mg) of 1,10-phenanthroline-5,6-dione was dissolved in CH_3OH (10 mL) to make pdon completely dissolve, and then $KAuCl_4 \cdot 2H_2O$ (414.3 mg, 1.0 mmol) in 10 mL water was added dropwise to the resulting solution and left stirring at room temperature for 8 h. The crude product of Au(III) metal complex **2** was obtained and washed with distilled water and methanol three times. Elemental analysis for $[Au(pdon)(Cl)_2]Cl$: calculated, %: C 30.15, H 1.27, N 5.86; found, %: C 30.41, H 2.13, N 5.84. Electrospray ionization mass spectrometry (ESI-MS): *m*/*z* 476.95 [**2**-Cl]$^+$ 476.8321.

DNA and BSA binding experiments. UV and fluorescence emission spectra relative to the binding of the complex to calf thymus (CT-DNA) were proceed in a 5 mM Tris-HCl–50 mM NaCl buffer (pH 7.20). Each nucleotide of DNA concentration was determined by absorption spectrometry using the molar absorption coefficient (ε = 6600 $M^{-1} \cdot cm^{-1}$ at 260 nm) [32]. The stock solutions were stored at 4°C and used over no more than 4 days. Absorption titrations, were proceed by fixing the complex concentration (10 μM) constant and changing the CT-DNA concentration (0–17.5 μM). The fluorescence emission spectra were obtained using a quartz cuvette with a 1 cm optical path length, and the excitation and emission slits were 5 and 5 nm, respectively. For this experiment, DNA was pretreated by EB in a [DNA]/[EB] ratio of 20 for 5 min at room temperature. The competitive binding experiments were carried out in the buffer by keeping [DNA]/[EB] and varying amounts of **1** or **2**. The fluorescence spectrum adopted 510 nm as the excitation wavelength and the emission range was set at 530~700 nm. After adding **1** or **2** to each measurement, let the mixture for 5 min.

Results and discussion. *DNA-binding mode and affinity.* Absorption spectral titration is a useful way to investigate the binding of intercalation between metal complexes and DNA [30]. From Fig. 1a, upon incremental additions of CT-DNA to complex **1** in a 5 mM Tris-HCl/50 mM NaCl buffer (pH 7.2), the peak at 276 nm increased gradually. Significant changes in the Uv-Vis spectrum indicate the major binding patterns between complex 1 and DNA, such as insertions, and suggest superimposing interactions between aromatic

ligands and DNA base pairs [33, 34]. The intrinsic binding constant K_b for the interaction of complex **1** and CT-DNA was calculated using the equations [35]:

$$\frac{\varepsilon_a - \varepsilon_f}{\varepsilon_b - \varepsilon_f} = \frac{b - \sqrt{b^2 - 2K_b{}^2 C_t [DNA]/s}}{2K_b C_t}, \quad (1)$$

$$b = 1 + K_b C_t + K_b[\mathrm{DNA}]/2s, \quad (2)$$

where ε_a: the absorption intensity after given DNA concentration, ε_f: the absorption intensity of free **1** or **2**, ε_b: the absorption intensity of **1** or **2** when fully bound to DNA (it was assumed that the absorption intensity would not changed by further addition of DNA), K_b: the binding constant, C_t: [DNA]total, s:the binding site. Both K_b and s are calculated from the best fit line.

Intrinsic binding constants K_b of complexes of **1** and **2** were obtained as 4.98×10^5 and 1.98×10^5 M^{-1} from Eq. (1) and (2), respectively (Fig. 1b), which indicated a medium binding strength of the two complexes with CT-DNA. The K_b values of complexes **1** and **2** with CT-DNA are similar to the early reports for typical classical intercalative, such as ethylene blue with CT-DNA (3.3×10^5 M^{-1}), proflavin with *Escherichia coli* DNA (4.1×10^5 M^{-1}), phenosafranine with CT-DNA (3.81×10^5 M^{-1}), and the complex $C_{20}H_{16}Cl_2N_2Pt\cdot H_2O$ with CT-DNA (8.2×10^5 M^{-1}) [30, 36–38].

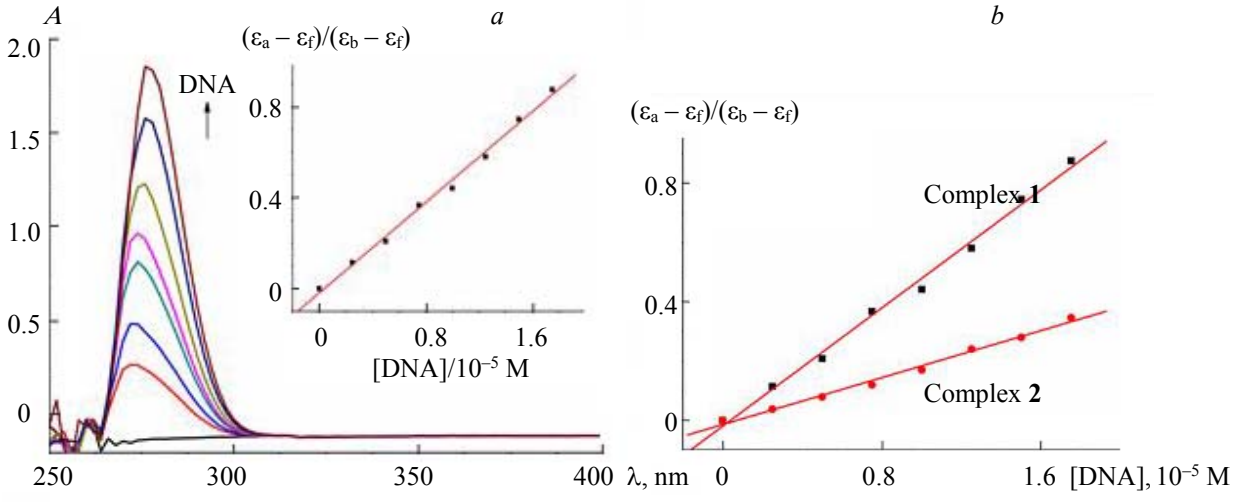

Fig. 1. (a) Absorption spectra of **1** (10 μM) in the absence and presence of increasing amounts of CT-DNA (2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5 μM) in 5 mM Tris-HCl/50 mM NaCl buffer (pH 7.2). The arrow shows the absorbance changes at increasing the DNA concentration. Inset: Plot of $(\varepsilon_a - \varepsilon_f)/(\varepsilon_b - \varepsilon_f)$ versus [DNA] for the titration of DNA to **1**. (b) Plot of $(\varepsilon_a - \varepsilon_f)/(\varepsilon_b - \varepsilon_f)$ versus [DNA] for the titration of DNA to **1** and **2**.

*Effect of the **1** and **2** on the fluorescence spectra of CT-DNA.* The fluorescence spectrum is another convenient method widely used to analyze and verify the formation of interaction between metal complexes and CT-DNA. No emission band was observed about complexes **1** and **2** either with or without CT-DNA at ambient temperature. Due to EB strong intercalation between the adjacent DNA base pairs, the fluorescence of DNA-EB could be quenched by the other molecule [39]. Therefore, the relative binding propensity of complexes **1** and **2** to CT-DNA was investigated with EB bound to the CT-DNA solution in a 5 mM Tris-HCl/50 mM NaCl buffer (pH 7.20), as shown in Fig. 2a. The results revealed that the addition of complexes **1** to the DNA bound with EB could release EB molecules, and consequently the emission intensity at 580 nm (510 nm excitation) decreased gradually [40]. Thus, the results further confirmed that the interaction between DNA and complex **1** was intercalation.

Let the classical Stern–Volmer formula $F_0/F = 1 + K_{SV}[Q]$ be used. Here F_0 and F are fluorescence intensity in the absence and presence of DNA, respectively; K_{SV} is the Stern–Volmer binding constant; $[Q]$ is the concentration of the **1** or **2**. As shown in Fig. 2b, the obtained apparent binding constant (K_{app}) are 7.82×10^5 and 3.35×10^5 M^{-1}, respectively, which is accord with the values of K_b values obtained by UV spec-

troscopy. The calculated K_b is less than 10^7 M^{-1} [41, 42], indicating that the binding effect between complex **1** or **2** and DNA is medium. The binding constant is the same as our early reported results [30].

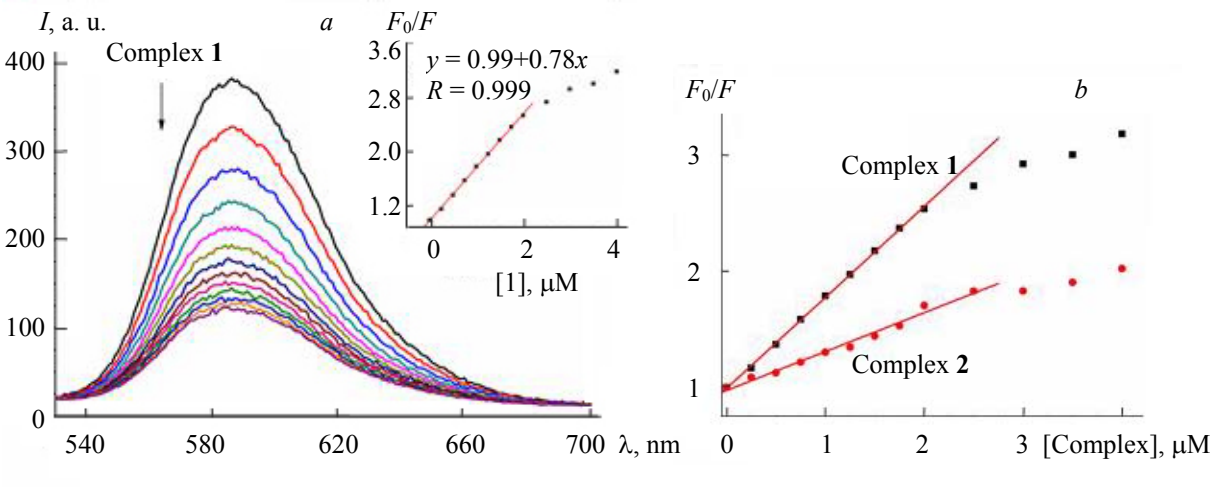

Fig. 2. (a) Emission spectra of EB-CT-DNA in the absence (black line) and in the presence (other lines) of **1** with increasing amounts (2.5, 5.0, 7.5, 10.0, 12.5, 15, 17.5, 20.0, 22.5, 25.0, 27.5, and 30.0 μM). Inset: plot of F_0/F versus [**1**] showing linearity. (b) plot of F_0/F versus [**1**] and [**2**].

MTT assay. The cytotoxic effects of complex **1** on the viability of a HepG2 cancer cell line were examined by MTT assay. HepG2 cells were treated with complex **1** and incubated for 48 h with the increasing concentration. As showed in Fig. 3, complex **1** demonstrated a better antitumor effect on the tested cancer cells.

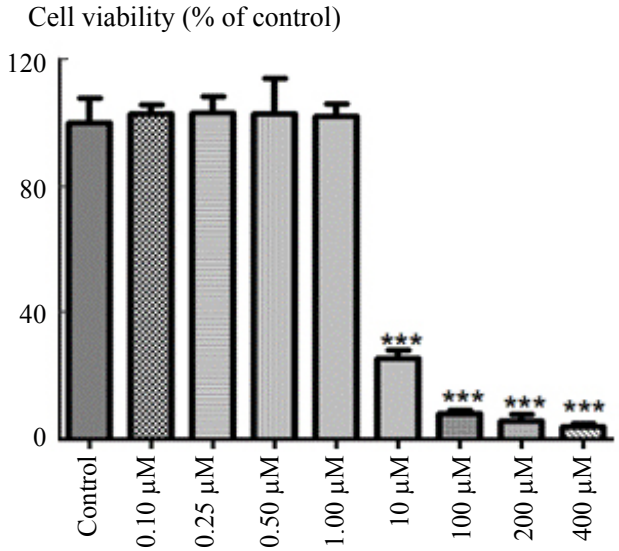

Fig. 3. Cell viability of HepG2 cells treated with complex **1** at 0, 0.1, 0.25, 0.5, 1.0, 10, 100, 200, and 400 μM for 48 h (*** P<0.0001).

Conclusion. Two novel coordination complexes ([Au(phen)Cl_2]Cl (**1**) and [Au(pdon)Cl_2]Cl (**2**)) were prepared and characterized by IR, EAs, and ESI-MS. Complexes 1 and 2 binding to DNA were studied by Uv-Vis absorption and fluorescence spectroscopy. The results revealed that complexes **1** and **2** could interact with CT-DNA through the intercalation mode and showed a medium binding strength, with $K_b = 4.98\times10^5$ and 1.98×10^5 M^{-1} for complexes **1** and **2**, respectively. Moreover, the in vitro cytotoxic activities showed that

1 is active against HepG2. These results indicate that interactions between the complexes and DNA actually take place.

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REFERENCES

1. L. Sleire, H. E. Forde-Tislevoll, I. A. Netland, L. Leiss, B. S. Skeie, P. O. Enger, *Pharmacol. Res.*, **124**, 74–91 (2017).
2. B. Jönsson, N. Wilking, *J. Cancer Policy*, **2**, 45–62 (2014).
3. Y. L. Chen, M. C. Chang, W. F. Cheng, *Cancer Lett.*, **400**, 282–292 (2017).
4. M. S. Kinch, *Drug. Discov. Today*, **21**, 1046–1050 (2016).
5. J. P. Delord, C. Puozzo, F. Lefresne, R. Bugat, *Anticancer Res.*, **29**, 553–560 (2009).
6. D. S. Hsu, B. S. Balakumaran, C. R. Acharya, V. Vlahovic, K. S. Walters, K. Garman, *J. Clin. Oncol.*, **25**, 4350–4357 (2007).
7. M. T. Sener, E. Sener, A. Tok, B. Polat, I. Cinar, H. Polat, A. Fatih, S. Halis, *Pharmacol. Rep.*, **64**, 594–602 (2012).
8. P. D. Sanchez-Gonzalez, F. J. Lopez-Hernandez, F. Perez Barriocanal, A. I. Morales, J. M. Lopez-Novoa, *Nephrol. Dial. Transpl.*, **26**, 3484–3495 (2011).
9. S. I. Sohn, H. K. Rim, Y. H. Kim, J. H. Choi, J. H. Park, J. W. Choi, S. D. Kim, S. Y. Jeong, K. T. Lee, *Biol. Pharm. Bull.*, **34**, 1508–1513 (2011).
10. A. A. Fouad, A. I. Al-Sultan, S. M. Refaie, M. T. Yacoubi, *Toxicology*, **274**, 49–56 (2010).
11. R. P. Miller, R. K. Tadagavadi, G. Ramesh, W. B. Reeves, *Toxins* (Basel), **2**, 2490–2518 (2010).
12. C. Zhao, X. Chen, D. Zang, X. Lan, S. Liao, C. Yang, P. Q. Zhang, J. J. Wu, X. F. Li, N. N. Liu, Y. N. Liao, H. B. Huang, X. P. Shi, L. L. Jiang, X. H. Liu, Z. M. He, X. J. Wang, J. B. Liu, *Biochem. Pharmacol.*, **116**, 22–38 (2016).
13. T. S. Reddy, S. H. Priver, N. Mirzadeh, S. K. Bhargava, *J. Inorg. Biochem.*, **175**, 1–8 (2017).
14. N. P. E. Barry, P. J. Sadler, *Chem. Commun.*, **49**, 5106–5131 (2013).
15. G. Gasser, N. Metzler-Nolte, *Curr. Opin. Chem. Biol.*, **16**, 84–91 (2012).
16.T. Zou, C. T. Lum, C. N. Lok, J. J. Zhang, C. M. Che, *Chem. Soc. Rev.*, **44**, 8786–8801 (2015).
17. F. Trudu, F. Amato, P. Vaňhara, T. Pivetta, E. M. Peña-Méndez, J. Havel, *J. Appl. Biomed.*, **13**, 79–103 (2015).
18. S. Medici, M. Peana, V. M. Nurchi, J. I. Lachowicz, G. Crisponi, M. A. Zoroddu, *Coordin. Chem. Rev.*, **284**, 329–350 (2015).
19. M. N. Patel, B. S. Bhatt, P. A. Dosi, *Inorg. Chem. Commun.*, **29**, 190–193 (2013).
20. T. Zou, C. T. Lum, C. N. Lok, J. J. Zhang, C. M. Che, *Chem. Soc. Rev.*, **44**, 8786–8801 (2015).
21. C. T. Lum, Z. F. Yang, H. Y. Li, R. Wai-Yin Sun, S. T. Fan, R. T. Poon, M. C. M. Lin, C. M. Che, H. F. Kuang, *Int. J. Cancer*, **118**, 1527–1538 (2006).
22. C. Marzano, L. Ronconi, F. Chiara, M. C. Giron, I. Faustinelli, P. Cristofori, A. Trevisan, D. Fregona, *Int. J. Cancer*, **129**, 487–496 (2011).
23. A. Casado-Sanchez, C. Martin-Santos, J. M. Padron, R. Mas-Balleste, C. Navarro-Ranninger, J. Alemánc, S. Cabrera, *J. Inorg. Biochem.*, **174**, 111–118 (2017).
24. M. F. Tomasello, C. Nardon, V. Lanza, G. Di Natale, N. Pettenuzzo, S. Salmaso, D. Milardi, P. Caliceti, G. Pappalardo, D. Fregona, *Eur. J. Med. Chem.*, **138**, 115–127 (2017).
25. M. C. Gimeno, H. Goitia, A. Laguna, M. E. Luque, M. D. Villacampa, C. Sepulveda, M. Meireles, *J. Inorg. Biochem.*, **105**, 1373–1382 (2011).
26. B. Alberto, R. M. Pia, S. Guido, G. Chiara, C. Angela, M. Luigi, *Coord. Chem. Rev.*, **253**, 1692–1707 (2009).
27. S. Urig, K. Becker, *Semin. Cancer Biol.*, **16**, 452–465 (2006).
28. Y. Wang, Q. Y. He, R. W. Sun, C. M. Che, J. F. Chiu, *Eur. J. Pharmacol.*, **554**, 113–122 (2007).
29. T. C. Fuchs, P. Hewitt, *AAPS J.*, **13**, 615–631 (2011).
30. Q. M. Wang, L. Yang, J. H. Wu, H. Wang, J. L. Song, X. H. Tang, *Biometals*, **30**, No. 1, 17–26 (2017).
31. Q. M. Wang, H. Mao, W. L. Wang, H. M. Zhu, L. H. Dai, Y. L. Chen, X. H. Tang, *Biometals*, **30**, No. 4, 575–587 (2017).
32. S. Satyanarayana, J. C. Dabroniak, J. B. Chaires, *Biochemistry*, **31**, No. 39, 9319–9324 (1992).

33. P. Baldini, M. Belicchi-Ferrari, F. Bisceglie, P. P. Dall'Aglio, G. Pelosi, S. Pinelli, P. Tarasconi, *Inorg. Chem.*, **43**, 7170–7179 (2004).
34. A. Silvestri, G. Barone, G. Ruisi, D. Anselmo, S. Riela, V. T. Liver, *J. Inorg. Biochem.*, **101**, 841–848 (2007).
35. L. F. Tan, H. Chao, Y. F. Zhou, L. N. Ji, *Polyhedron*, **26**, No. 13, 3029–3036 (2007).
36. H. Li, X. Y. Le, D. W. Pang, H. Deng, Z. H. Xu, Z. H. Lin, *J. Inorg. Biochem.*, **99**, No. 11, 2240–2247 (2005).
37. Mudasir, N. Yoshioka, H. Inoue, *J. Inorg. Biochem*., **77**, 239 (1999).
38. P. T. Tamil-Selvi, H. Stoeckli-Evans, M. J. Palaniandavar, *Inorg. Biochem.*, **99**, 2110–2118 (2005).
39. A. Gohel, M. B. McCarthy, G. Gronowicz, *Endocrinology*, **140**, 5339–5347 (1999).
40. J. K. Barton, J. M. Goldberg, C. V. Kumar, N. J. Turro, *J. Am. Chem. Soc.*, **108**, 2081–2088 (1986).
41. B. D. Wang, Z. Y. Yang, Q. Wang, T. K. Cai, P. Crewdson, *Bioorg. Med. Chem.*, **14**, 1880–1888 (2006).
42. H. Laitinen, V. P. Hytonen, V. R. Nordlund, M. S. Kuloma, *Cell Mol. Life Sci.*, **63**, 2992–3017 (2006).