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SCN^-桥联的铜配合物:抗肿瘤活性及对肿瘤细胞耗氧的影响

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摘要:合成和表征了一种新的 *N*-苯基二吡啶甲基胺 (phdpa)铜配合物[(phdpa)Cu(SCN)](ClO_4)。X-衍射晶体结构数据显示配合物中铜原子与配体 phdpa 中 3 个 N 原子、SCN^-中 S 原子和邻近分子中 SCN^- 中 N 原子配位,形成扭曲八面体结构。生物活性数据显示该铜配合物抑制 HepG-2 细胞的生长,半数抑制率为 10.4 μmol·L^{-1}。进一步机理研究数据显示这种 SCN^-桥联的铜配合物能诱导 HepG-2 细胞核的分裂,降低其耗氧量和 ROS 的含量,这表明该铜配合物是一种能影响 HepG-2 细胞氧代谢的多功能配合物。

关键词:铜配合物;抗肿瘤;ROS;耗氧量;晶体结构
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SCN-bridged Cu(Ⅱ) Complexes: Anticancer Activity and Effect on Attenuation of O_2 Consumption in HepG-2 Cells

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Abstract: A new copper(Ⅱ) complex of *N*-benzyl di(2-pyridylmethyl)amine (phdpa) was synthesized and characterized. X-ray crystal structure shows that the copper atom in [(phdpa)Cu(SCN)](ClO_4) is coordinated with a phdpa and a SCN^- forming a coordinated polymer. In vitro essay results show that this copper(Ⅱ) complex exhibits good inhibition on the proliferation of HepG-2 cells with IC_{50} of 10.4 μmol·L^{-1}. In addition, [(phdpa)Cu(SCN)](ClO_4) can induce the obvious nucleus fragmentation of HepG-2 cells, decrease the O_2 consumption and reactive oxygen species (ROS) level in HepG-2 cells, indicating that this SCN-bridged copper(Ⅱ) complex is a multi-functional copper(Ⅱ) complex. CCDC: 840495.

Key words: copper(Ⅱ) complex; antitumor; ROS; O_2 consumption; crystal structure

0 Introduction

Copper plays a key role in biological processes, and its complexes are used as metallopharmacons or preferred molecules for cancer inhibition because copper could accumulate in tumors due to the selective permeability of cancer cell membranes to copper compounds[1]. A number of copper complexes, which are artificial nucleases, have been screened for anticancer activity, and some of them were found to be active both *in vitro* and *in vivo*[2-3].

Except as artificial nucleases, copper (II) complexes are also reported as mimics of non-heme dioxygenases, which could induce apoptosis in cultured mammalian cells[4]. Copper(Ⅱ) complexes of N-substituted di(picolyl) amines, mimics of non-heme dioxygenase, are active against the proliferation of cancer cells[5]. The apoptosis mechanism of cancer

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cells is influenced by the structure and conformation of these copper(Ⅱ) complexes [6-7]. Oxygen scarceness limits the growth of tumors, because cancer cells-like most other cells in the organism-utilize oxygen to generate energy (at least in part) and as a substrate for a number of fundamental biochemical reactions including synthesis of macromolecules [7]. Oxidative therapy is a relatively new anticancer strategy [8]. The microenvironment of solid tumors is characterized by low p_{O_2} that is well below physiological levels. Intratumoral hypoxia is a major factor contributing to cancer progression and is exacerbated as a result of oxygen consumption by rapidly proliferating tumor cells near blood vessels, poor lymphatic drainage resulting in high interstitial pressure, and irregular blood supply through immature tumor. In solid tumors, energy production and synthesis of macromolecules are maximized with minimal reactive oxygen species (ROS) accumulation. Furthermore, a series of antioxidant enzymes are induced to mitigate the damaging effects of ROS[9]. The antitumor activities of the complex [(phdpa)Cu(H_2O)(Ac)](Ac) (phdpa=N-benzyl di(pyridylmethyl) amine) are related to their interference on the oxygen metabolite of cancer cells [10]. So, attenuation on oxygen related metabolic changes would be a synergistic approach to combination anti-cancer therapy and inhibition of cellular defenses against oxidative stress. We report here the synthesis, characterization of a new copper(Ⅱ) complex [(phdpa)Cu(SCN)](ClO_4). *In vitro* essay results show that the complex can inhibit the proliferation of cancer cells through interfering their O_2 consumption and ROS production.

1 Experimental

1.1 Chemical reagents, analysis and physical measurements

All chemicals used in the syntheses were of reagent grade and were used without further purification. *N*-benzyl di(pyridylmethyl)amine (phdpa) was synthesized according to the reported procedures [11]. Tris(hydroxymethyl)aminomethane (tris) was obtained from Sigma USA. Water was purified with a Millipore MIlli-Q system. The C, H and N microanalyses were performed on Vario EL elemental analyzer. The electronic absorption spectrum was recorded in the 900~190 nm region using the VARIAN CARY 50-BIO UV-Vis spectrophotometer. Infrared spectrum was recorded on a Nicolet-470 spectrophotometer in the wavenumber range of 4 000~400 cm^{-1} with KBr pellets. The fluorescence spectra were measured with Fp-750W Fluorometer.

1.2 Synthesis of [(phdpa)Cu(SCN)(ClO_4)] (1)

To a stirred ethanol solution (10 mL) of phdpa (289 mg, 1 mmol), a green solution of $Cu(ClO_4)_2\cdot 6H_2O$ (370.5 mg, 1 mmol) in ethanol (10 mL) was added dropwise. The mixture was stirred at 80 ℃ for 2 h and then cooled to room temperature. NH_4SCN (76.12 mg, 1 mmol) in ethanol (10 mL) was added to the above solution. The mixture was refluxed at 75 ℃ for 2 h then cooled to room temperature. After the solution was diffused with ethyl ether, blue crystals were obtained. Yield, 80% . Anal. Calcd. (%) for $C_{20}H_{19}ClCuN_4O_4S$: C, 47.06; H, 4.75; N, 10.98; Found (%): C, 47.13; H, 4.68; N, 11.04. IR (KBr) ν/cm^{-1}: ν(=CH) 3 109 m, ν(-CH_2-) 2 935 m, ν(-SCN) 2 106, ν(C=N) 1 608 s, ν(C=C) 1 574 s, ν(C=C) 1 442 s, ν(C=C) 1 452 m, ν(C=C) 1 359 m, ν(ClO_4^-) 1 165 s, 1 083 s, 1 000 s, δ(=CH, Ar) 765 s, 705 s, ν(ClO_4^-) 622 s.

1.3 X-ray crystal structure determinations

Crystallographic data for [phdpaCu(SCN)](ClO_4) are listed in Table 1. The blue prism crystals of the complex were selected for lattice parameter determination and collection of intensity at 298 K on Rigaku Mercury2 CCD Area Detector with monochromatized Mo $K\alpha$ radiation (λ=0.071 074 7 nm). The data was corrected for Lorenz and polarization effects during data reduction. A semi-empirical absorption correction from equivalents based on multi-scans was applied. The structure was solved by direct methods and refined on F^2 by full-matrix least-squares methods using SHELTL version 5.10[12]. All non-hydrogen atoms were refined anisotropically. All calculations were performed using the SHELX-97 programs.

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Table 1　Crystal data and structural refinements for [(phdpa)Cu(SCN)](ClO_4)

Empirical formula	$C_{20}H_{19}ClCuN_4O_4S$	Z	4
Color / shape	Blue / prism	D_c / (g·cm^3)	1.559
Formula weight	510.45	Absorption coeffcient / mm^{-1}	1.258
Temperature / K	293(2)	F(000)	1044
Wavelength / nm	0.0710747	Crystal size / mm	0.2×0.2×0.2
Crystal system / space group	Monoclinic / P21	θ range for data collection / (°)	3.08 to 26.02
a / nm	1.394 18(16)	Index ranges	$-11<h<16$, $-12<k<12$, $-19<l<18$
b / nm	1.040 71(11)	Reflections collected	4 427
c / nm	1.577 69(18)	Observed re?ns [$I>2\sigma(I)$]	3 579
α / (°)	90.000 0(0)	Goodness-of-?t on F^2	0.989
β / (°)	108.1399(26)	Final R indices [$I>2\sigma(I)$]	R_1=0.0421, wR_2=0.151 6
γ / (°)	90.000 0(0)	R indices (all data)	R_1=0.0497, wR_2=0.169 6
Volume / nm^3	2.175 4(8)		

1.4　Cytotoxicity testing

The cytotoxicity assay used two kinds of cell lines (A549 and HepG-2 cells). Cells were cultured in RPMI-1640 medium containing 4.8 g·L^{-1} of HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), 2.2 g·L^{-1} $NaHCO_3$ and supplemented with penicillin/streptomycin (1000 units·mL^{-1}) and 10% calf serum. A549 cells were cultured in Dulbecco′s Modified Eagle′s Medium (DMEM) containing 10% fetal bovine serum. All cells were grown at 37 ℃ in a humidified atmosphere in the presence of 5% CO_2. Test complexes were dissolved in DMSO and diluted with culture media. After 24 h, complexes were added and the same samples kept for 48 h. Cell viability was determined by the 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenpyltetrazolium bromide (MTT) assay by measuring the absorbance at 590 nm with an ELISA reader. DMSO was used as blank control and 5-Fluorouracil was used as reference control. IC_{50} was calculated using software provided by Nanjing University. Each test was performed in triplicate. Comparisons were made by one-way analysis of variance. Differences were considered to be significant when p <0.05. All experiments were repeated at least three times.

1.5　Nuclei observation on H2B-GFP-labled HepG-2 cells

H2B-labled HepG-2 cells were plated in 24-well plate at the density of 2.4×10^4. After incubating for 24 h with complexes, cells were stained with 4′, 6-diamidino-2-phenylindole (DAPI) and nuclei changes were visualized under fluorescence microscope (Nikon TE2000 inverted microscope)

1.6　Oxygen consumption and measurement of ROS in HepG-2 cells

HepG-2 cells were seeded at a density of 1×10^5 cells·mL^{-1} into sterile 96 well plates and grown in 5% CO_2 at 37 ℃ for 24 h. Test compounds were dissolved in H_2O or DMSO and diluted with culture media. After 24 h, complexes were added. Samples were transferred into Clark cell. After an equilibration time of 2 min (120 s), oxygen concentration was monitored by Clark oxygrtherm. Oxygen consumption rate was determined based on counting the cell numbers and density (nmol·(mL·min·10^5 cells)$^{-1}$). Each test was performed in triplicate. ROS levels were measured using the ROS Assay Kit from Beyotime (Beyotime, China), per the manufacturers protocol [13]. ROS% was calculated as the geometrical mean of the total green fluorescence of the oxidation product, 2,7-dichlorofluorescein (DCF) for per 10^4 cells.

2　Results and discussion

2.1　General characterization

The copper (Ⅱ) complex was characterized by elemental analysis, IR and UV spectra. The results suggest that the composition of the complex is [(phdpa)Cu (SCN)] (ClO_4). The IR spectra of the free ligand (phdpa) show two pyridyl ring bands at

approximately 1 589 cm^{-1} and 1 569 cm^{-1} and the δ(CH) vibration of the pyridyl ring at 762 cm^{-1}. The characteristic imine νC=N band at 1608 cm^{-1} for the complex shifts to lower frequencies due to metal coordination. The pyridyl bands δ(CH) are found at 765 cm^{-1}. These shifts indicate that the nitrogen atom of the pyridyl rings donates a pair of electrons to the central metal and then forms a coordinate bond. The sharp peak at 2 106 cm^{-1} indicates the presence of the SCN^-. The peaks at 1 083 cm^{-1} indicate the existence of ClO_4^-. The electronic spectra in the ultraviolet and visible region for the complex were recorded in ethanol solution. The strong bands at 217 nm and 255 nm are attributed to the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transition of the ligands, respectively. The broad band in the range of 600~700 nm, is characteristic of a copper (II) d-d transition in a tetragonal ligand field, in which the copper(Ⅱ) has a distorted octahedral coordination environment.

2.2 Crystal structure of complex

The molecular structure of [(phdpa)Cu(SCN)](ClO_4) with the atomic labeling scheme is shown in Fig.1, and the selected bond lengths and angles are listed in Table 2. The copper atom is five coordinated by three N atoms of phdpa (N1, N2, N3), one sulfur atom of SCN^- and one nitrogen atom of SCN^- from neighboring molecules with symmetry of (0.5+x, -0.5-y, z) as a coordinated polymer. The central copper atom in the complex has a distorted octahedron geometry, which is different from the trigonal bipyramidal complex [Cu(apme)(Cl)](BPh_4) (apme=tris (2-pyridylmethyl) amine)[14]. The N(1), N(2), N(3) and N (0A) form the equatorial plane with deviation 0.240 4, while the S2 occupies the apical positions. The coper (II) atom is located outside of the plane (shifted by 0.024 04 nm out of the equatorial plane towards N(3)). The bond distances of Cu(1)-N(0A) and Cu (1)-S (1) are 0.1949 (4) nm and 0.265 9 (9) nm, indicating that the S(1) is weakly coordinated with Cu (1). N(2)-Cu(1)-N(0A) and N(1)-Cu(1)-N(3) angles are 156.40 (10)°, 163.35(10)° , respectively.

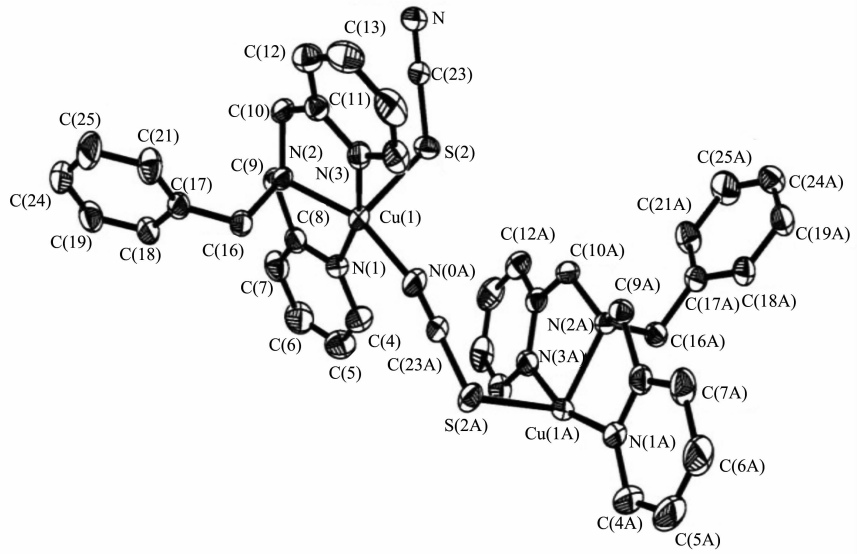


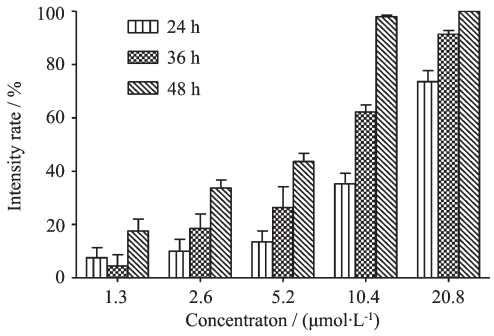
Fig.1 Crystal structure of $[(phdpa)Cu(SCN)]^+$, Thermal ellipsoids are drawn at 30% probability, Hydrogen atoms are omitted

Table 2 Selected bond lengths (nm) and bond angles (°) for [(phdpa)Cu(SCN)](ClO_4)

Bond distance					
Cu(1)-N(1)	0.199 1(2)	Cu(1)-N(3)	0.200 7(3)	Cu(1)-N(0A)	0.194 9(4)
Cu(1)-N(2)	0.202 5(2)	Cu(1)-S(1)	0.265 9(9)		
Bond angle					
N(2)-Cu(1)-N(1)	82.10(9)	N(2)-Cu(I)-N(0A)	156.40(10)	N(3)-Cu(1)-S(1)	91.01(8)
N(3)-Cu(1)-N(2)	81.27(9)	N(1)-Cu(1)-S(1)	94.29(4)		
N(1)-Cu(1)-N(3)	163.35(10)	N(2)-Cu(1)-S(1)	105.46(6)		

2.4 Inhibition on the proliferation of cancer cells

Copper(Ⅱ) complexes were reported to inhibit the proliferation of cancer cells A549 and HepG-2[5,14,16]. The complex [(phdpa)Cu(SCN)](ClO_4) was studied for their antitumor activity *in vitro* by determining the inhibitory percentage against growth of cancer cells A549 and HepG-2 using the method of MTT reduction. The IC_{50} data for 5-fluorouracil (a reference compound) is 32.0 μmolL^{-1}. The IC_{50} data for [(phdpa)Cu(SCN)] (ClO_4) on A549 and HepG-2 are 21.9 μmol·L^{-1} and 10.4 μmol·L^{-1} respectively. The IC_{50} values (10.4 μmol·L^{-1}) of [(phdpa)Cu(SCN)](ClO_4) on HepG-2 was smaller than the reported [(phdpa)Cu(H_2O)Ac)](Ac) (IC_{50}, 13.4 μmol·L^{-1} to HepG-2)[5]. However, the IC_{50} data (21.9 μmol·L^{-1}) for this complex on A549 is larger than that of phen-based complexes [$Cu(CH_3COO)_2$(phen)] (IC_{50}, 1.8 μmol·L^{-1} to A549)[14]. These show that [(phdpa)Cu(SCN)](ClO_4) (**1**) can inhibit selectively on the proliferation of HepG-2 cells. The dose and time-dependent anti-tumor effect for the complex (**1**) on HepG-2 cells is shown in Fig.2. The morphology of drug-treated cells was used to determine the extent of cytological effects. After incubating for 24 h with the complexes, HepG-2 cells was stained with DAPI, which is specific for visualisation of nuclear morphology and detection of DNA condensation. The untreated cells display a homogeneous morphology with nuclei evenly stained by DAPI. In contrast, after treatment with the complex



HepG-2 cells were exposed to complex (**1**) (0~20.8 μmol·L^{-1}). Cell proliferation was determined by MTT assay. Each column represents the mean of the data from three independent experiments. Standard deviation<0.06.

Fig.2 Dose and time-dependent anti-tumor effect of [(phdpa)Cu(SCN)] (ClO_4) (**1**)

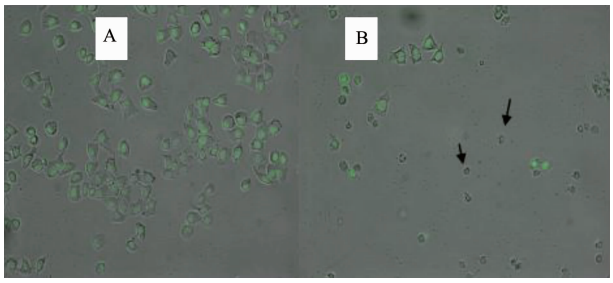


Fig.3 Change in nucleus morphology of HepG-2 cells. H2B-labled HeLa cells were exposed to [(phdpa)Cu(SCN)](ClO_4)(**1**) for 24 h, nuclei change was observed. A, control; B, the complex(**1**), 11 μmol·L^{-1}

for 24 h, most of the cell display fragment nuclei (Fig. 3). These indicate that the anticancer properties of [(phdpa)Cu(SCN)](ClO_4) is nucleus relevant.

Copper (Ⅱ) complexes of di (picolyl) amine derivatives are widely used as models of non-heme dioxygenase [15]. Previously, we found that complex [(phdpa)Cu(H_2O)(Ac)](Ac) interfere with the metabolite of cancer cells [10]. Here, the effect on the O_2 consumption for [(phdpa)Cu(SCN)](ClO_4) on the HepG-2 cells is shown in Fig.4. Experimental data show that this complex (11 μmol·L^{-1}) can decrease the O_2 consumption of HepG-2 cells in 6 h and the O_2 consumption does change with the time. These indicate that this complex could act on HepG-2 cell leading to the change of cell breath or oxygen involved metabolite. Oxygen scarceness limits the growth of tumors, because cancer cells-like most other cells in the organism-utilize oxygen to generate energy (at least in part) and as a substrate for a number of

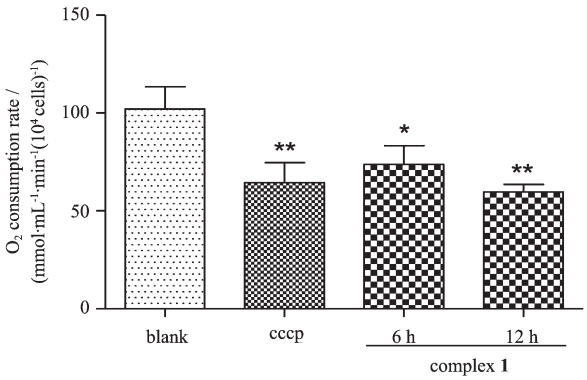


Fig.4 Effect on the O_2 consumption for [(phdpa)Cu(SCN)] (ClO_4) (**1**) on the HepG-2 cells. Complex (11 μmol·L^{-1}), cccp: 20 μmol·L^{-1} 6 h

fundamental biochemical reactions including synthesis of macromolecules [9]. So we deduce that strong interference on oxygenation of HepG-2 cells is a factor for the antitumor activity of [(phdpa)Cu(SCN)](ClO_4). Cancer cells use O_2 to generate excessive levels of the reactive oxygen species (ROS) and H_2O_2. This alteration in the metabolism of O_2 is a common feature of cancer cells and plays an important role in carcinogenesis. The effect of this complex on the level of ROS is shown in Fig.5. Extensive experimental results show that [(phdpa)Cu(SCN)](ClO_4) could decrease ROS level in HepG-2 cells in 6 h, indicating that the complex may initiate ROS-mediated death of cancer cells. The increase of ROS level in 12 h for the complex may be the result of the death of cancer cells signaling the production of ROS. So we deduce that this complex could induce cancer cells death through attenuation ROS signal.

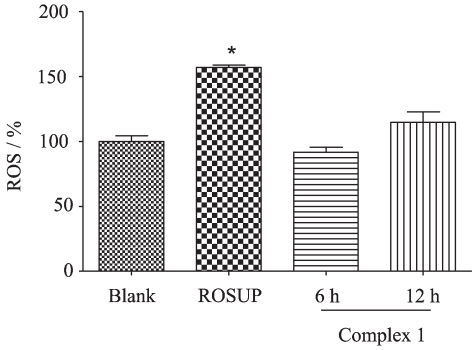


Fig.5 Effect on the ROS content in HepG-2 for [(phdpa)Cu(SCN)](ClO_4) (**1**, 15 μmol·L^{-1}); ROSUP (20 μmol·L^{-1}) was used as control experimental. ROS% was calculated based on the DCF fluorescence per 10^4 cell

3 Conclusions

A SCN-bridged copper(Ⅱ) complex of *N*-benzyl di(2-pyridylmethyl)amine, a non-planar aromatic heterocyclic rings ligand, could inhibit the proliferation of HepG-2 and cause the obvious nucleus fragmentation of HeLa cells. In addition, the complex could decrease the O_2 consumption and ROS level of HeLa cells in 6 h. The alteration in the metabolism of O_2 by copper(Ⅱ) complexes plays an important role in carcinogenesis. Experimental results show that attenuation on the metabolite of O_2 is an important factor for its anticancer activities. So, [(phdpa)Cu(SCN)](ClO_4) is a new complex to attenuate the nucleus and interfere the metabolite of O_2 signaling apoptosis of cancer cells.

References:

[1] Garbutcheon-singh K B, Grant M P, Harper B P, et al. *Curr. Top. Med. Chem.*, **2011**,**11**:521-542

[2] Dong X D, Wang X Y, Lin M X, et al. *Inorg. Chem.*, **2010**,**49**:2541-2549

[3] Ramakrishnan S, Shakthipriya D, Suresh E, et al. *Inorg. Chem.*, **2011**,**50**:6458-6471

[4] HUANG Juan(黄娟), CHEN Qiu-Yun(陈秋云), WANG Lin-Yun(王玲昀), et al. *Chinese J. Inorg. Chem.* (*Wuji Huaxue Xuebao*), **2009**,**25**(6):1077-1089

[5] CHEN Qiu-Yun(陈秋云) , HUANG Juan(黄娟), LI Jun-Fen(李军峰), et al. *Chinese Inorg. Chem.*(*Wuji Huaxue Xuebao*), **2008**,**24**(11):1789-1793

[6] Drummond G R, Selemidis S, Griendling K K, et al. *Nature Rev. Drug. Discov.*, **2011**,**10**:453-471

[7] Selvakumar B, Rajendiran V, Uma M P, et al. *J. Inorg. Biochem.*, **2006**,**100**:316-330

[8] Deegan C, McCann M, Devereux M, et al. *Cancer Lett.*, **2007**,**247**:224-233

[9] Nagasawa H. *J. Pharm. Sci.*, **2011**,**115**:446-452

[10]Chen Q Y, Fu H J, Zhu W H, et al. *Dalton Trans.*, **2011**,**40**:4414-4420

[11]Chen Q Y, Fu H J, Huang J, et al. *Spectrochim. Acta A.*, **2010**,**75**:355-360

[12]Sheldrick G M, *SHELXTL*-97, *Program for Crystal Structure Solution and Refinement*, University of Gottingen, Germany, **1997**.

[13]Liu H L, Xu J J, Dai X M, et al. *J. Appl. Toxicol.*, **2009**,**29**:489-495

[14]Utz D, Kisslinger S, Hampel F, et al. *J. Inorg. Biochem.*, **2008**,**102**:1236-1245

[15]Kruppa M, Konig B. *Chem. Rev.*, **2006**,**106**:3520-3560

[16]Li X W, Zheng Y J, Li Y T, et al. *Eur. J. Med. Chem.*, **2011**,**46**:3851-3857