第 24 卷第 11 期 无 机 化 学 学 报 Vol.24 No.11
2008 年 11 月 CHINESE JOURNAL OF INORGANIC CHEMISTRY 1789~1793

双核锰配合物$[Mn_2(Adpa)_2Cl_4]$的合成、与线粒体和癌细胞的作用

陈秋云*,[1] 黄 娟[1] 李军峰[1] 高 静[2]
([1]江苏大学化学化工学院,镇江 212013)
([2]江苏大学药学院,镇江 212013)

摘要:本文以 *N*-烯丙基二吡啶甲基胺为配体,合成了一个新的锰配合物。晶体结构显示其为双核锰(Ⅱ,Ⅱ)配合物。用 MTT 法研究了双核锰配合物体外与线粒体作用和对肿瘤细胞生长的抑制作用。实验结果表明双核锰(Ⅱ,Ⅱ)配合物对癌细胞 ECA-109 有较强的抑制作用,且化合物能抑制过量钙离子引起的线粒体肿胀。

关键词:双核锰配合物;癌细胞;线粒体肿胀
中图分类号:O614.7+11 **文献标识码**:A **文章编号**:1001-4861(2008)11-1789-05

Synthesis, Interaction with Mitochondrial and Cancer Cells of a Dinuclear Manganese(Ⅱ) Complex: $Mn_2(Adpa)_2Cl_4$

CHEN Qiu-Yun*,[1] HUANG Juan[1] LI Jun-Feng[1] GAO Jing[2]
([1]*School of chemistry and chemical engineer, Jiangsu University, Zhenjiang, Jiangsu* 212013)
([2]*Institute of medicine, Jiangsu University, Zhenjiang, Jiangsu* 212013)

Abstract: A dinuclear manganese(Ⅱ, Ⅱ) complex $Mn_2(Adpa)_2Cl_4$ (Adpa=*N*-allyl di(picolyl)amine) was synthesized and the crystal structure was determined using X-ray diffraction crystal structure analysis. The bioassay results show complex was active against the four kinds of cancer cells (HepG 2, Eca-109, LoVo, A549) with IC_{50} in the range 25~65 μmol·L^{-1}, and it was found that the complex activated the proliferation of cancer cell A549 in the range of 1~20 μmol·L^{-1}. The interaction between ct-DNA and complex is weak, but the complex can inhibit the induced swelling of Ca^{2+}-loaded mitochondria swelling in a dose-dependent manner *in vitro*. CCDC: 658318.

Key words: dinuclear manganese(Ⅱ) complex; cancer cells; mitochondria swelling

Mitochondria play a central role in cell life and death and are known to be important in a wide range of disease including the cancer, diabetes, cardiovascular disease, etc [1]. The unique structural and functional characteristics of mitochondria enable the selective targeting of drugs designed to modulate the function of this organelle for therapeutic gain[2]. Gold(Ⅰ) phophine complex and [Pt(*o*,*o*-acac)(gamma-acac)(dmso)] were found exerting fast cytotoxity in MCF breast cancer cells via the mito-chondrial apoptotic pathway [3,4]. Manganese (Ⅱ) is a requ-ired co-factor for many ubiquitous enzymes. Mitochond-rial can accumulate Mn through an ATP-dependent Ca transporter[5]. Manganese (Ⅱ) complexes of containing thiosemicarbazone or hydrazone groups were reported as antitumor agents[6,7]. But no report on the interaction of manganese(Ⅱ) metal com-plexes to interact with mitochondrial was reported. Di (picolyl)amine (dpa) was used as neutral, nondeproton-ated chelating ligands to capable of complexing Zn(Ⅱ), Cu(Ⅱ), Fe(Ⅱ, Ⅲ) ions to recognize

收稿日期:2008-04-21。收修改稿日期:2008-08-10。
国家自然基金(No.20777029B0702),南京大学配位化学国家重点实验室基金和江苏大学高级人才基金资助(No.06JDG050)。
*通讯联系人。E-mail:chenqy@ujs.edu.cn
第一作者:陈秋云,女,42 岁,教授;研究方向:无机药物化学。

proteins[8,9]. The utility of these ligands is enhanced by the ease with which substituents may be introduced on the imino nitrogen atom, thus allowing the controlled modification of solubility and molecular conformation through the non-bonding interactions [10]. *N*-allyl di (picolyl)amine (Adpa) was active against the proliferation of cancer cells[11]. In order to find new complex to target mito-chondrial, a new manganese (Ⅱ) complex $Mn_2(Adpa)_2Cl_4$ were synthesized and characterized. The bioactivities of complex $Mn_2(Adpa)_2Cl_4$ were studied and compared with the reported complex $Mn(bpa)_2Cl$.

1 Experimental section

1.1 Materials and instruments

All chemicals and solvents used in this work were of analytical reagent and were used without further purification. The complex $Mn(dpa)_2Cl_2$ and *N*-allyl di (picolyl)amine were synthesized as reported [11,12]. Di (picolyl)amine (dpa) was purchased form Sigma Aldrich company. The Calf thymus DNA and Tris-HCl (tris (hydroxymethyl)amino-methane) buffer (pH 7.0) stock standard solution was purchased from Sigma. Carbon, hydrogen and nitrogen were determined using an Elemental Vario EL elemental analyzer. The electronic absorption spectra were recorded in the 800~200 nm region using the UV-Vis spectrophotometer. Infrared spectrum was recorded on a Nicolet-470 spectrophotometer in the spectral range 4 000~400 cm^{-1} pellets. The fluorescence were measured with Fp-750w Fluorometer. DNA bind experiment was performed as reported[13].

1.2 Preparation of $Mn_2(Adpa)_2Cl_4$

A solution of $MnCl_2$ (0.025 g, 0.2 mmol) in 10 ml methanol was added dropwise to a stirred solution of N-allyl di (picolyl)amine (0.086 g, 0.2 mmol) in 10 mL methanol. The resulting solution was refluxed for 2 hours resulting yellow solution. After evaporated at room temperature for 7 days, white yellow crystals suitable for X-ray diffraction structure analysis formed from the solution. Yield: 80% . Anal. Calc. for $C_{30}H_{34}Cl_4Mn_2N_6$(%): C 49.34, H 4.69, N 11.51; Found (%): C 49.40, H 4.53, N 11.46. IR (cm^{-1}): 3 074s ν(=C-H); 2 922s ν(C-H); 1 608, 1 571, 1 479s ν(C=C, py). UV-Vis (λ_{max} / nm), CH_3OH): 262(9 620 $L\cdot mol^{-1}\cdot cm^{-1}$), 208 (26 300 $L\cdot mol^{-1}\cdot cm^{-1}$).

1.3 Crystal structure determination and refinement

The X-ray diffraction data (Table 1) was collected at 293(2) K on Smart Apex CCD X-ray single crysal diffractometer with Mo $K\alpha$ radiation (λ=0.071 073 nm). A total of 8 964 reflections were collected in range of $2.35°<\theta<26.00°$, of which 3 268 (R_{int}=0.048 5) were independent, and 2 854 reflections were observed with $I>2\sigma(I)$. The structure was solved using direct methods with SHELXS-97 program, and the non-hydrogen atoms were refined anisotropically with SHELXL-97 using full-matrix least-squares procedures based on F^2 values[14,15]. The hydrogen atom positions were fixed geometrically and allowed to ride on the parent atoms in the refinement.

CCDC: 658318.

Table 1 Crystal data and structural refinement parameters for $Mn_2(Adpa)_2Cl_4$

Empirical formula	$C_{30}H_{34}Cl_4Mn_2N_6$	D_c / ($g\cdot cm^{-3}$)	1.454
Color / shape	Yellow / plate	Absorption coefficient / mm^{-1}	1.107
Formula weight	730.31	F(000)	748
Temperature / K	293(2)	Crystal size / mm	0.26 × 0.22 × 0.10
Wavelength / nm	0.071073	θ range for data collection / (°)	2.355 to 26.00
Crystal system	Monocline	Index ranges	$-12 \leqslant h \leqslant 12, -20 \leqslant k \leqslant 20, -13 \leqslant l \leqslant 6$
Space group	$P2_1/n$	Reflections collected	8 964
a / nm	0.998 72(9)	Reflns. unique (R_{int})	3 268 (0.048 9)
b / nm	1.640 30(14)	Observed reflns [$I>2\sigma(I)$]	2 854
c / nm	1.092 46(9)	Goodness-of-fit on F^2	1.071
β / (°)	111.2120 (10)	R indices (all data)	R_1=0.029 3; wR_2=0.070 2
Volume / nm^3	1.668 4(2)	Final R indices [$I>2\sigma(I)$]	R_1=0.025; wR_2=0.068 2
Z	2		

1.4 Assay for cytotoxic activity

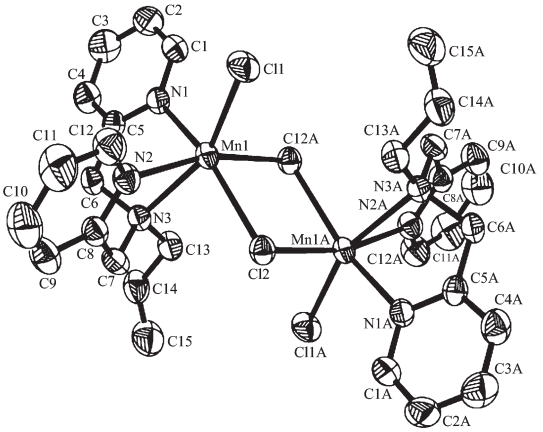
The cytotoxity assay was in four kinds of cells line (hepatocellular carcinoma HepG 2, human esophageal cancer cell Eca-109, human colon carcinoma cancer line LoVo and human lung adenocarcinoma A549 cells). Cells were cultured at 37 ℃ under a humidified atmosphere of 5% CO_2 in PRMI 1640 medium supplemented with 10% fetal serum and dispersed in replicate 96-well plates with 1×10^4 cells/well. Compounds were then added. After 24, 48, 72 h exposure to the toxins, cells viability were determined by the 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenpyltetrazolium bromide (MTT) assay by measuring the absorbance at 570 nm with ELISA reader. Each test was performed in triplicate.

1.5 Mitochondrial swelling

Liver mitochondria were isolated by conventional differential centrifugation from adult rats. The livers were homogenized in 250 mmol·L^{-1} sucrose, 1 mmol·L^{-1} EGTA, and 10 mmol·L^{-1} Hepes buffer (pH 7.4). The mitochondrial suspension was washed twice in the same medium containing 0.1 mmol·L^{-1} EGTA, and the final pellet was resuspended in 250 mmol·L^{-1} sucrose to a final protein concentration of 80~100 mg·mL^{-1}. Mitochondria (0.4 mg of protein) were incubated in 1.5 mL of a medium containing 125 mmol·L^{-1} sucrose, 65 mmol·L^{-1} KCl, 10 mmol·L^{-1} HEPES-KOH, pH 7.4, 5 mmol·L^{-1} potassium succinate (+2.5 μmol·L^{-1} rotenone), and 10 μmol·L^{-1} $CaCl_2$ at 25 ℃. Mitochondrial swelling was estimated from the decrease in absorbance at 540 nm measured by a Hitachi U-2000 Spectrophotometer.

2 Results and discussion

The complex $Mn_2(Adpa)_2Cl_4$ was characterized by elemental analysis, IR, UV and X-ray crystal structure analysis. Data shows the existence of the $Mn_2(Adpa)_2Cl_4$. The complex was soluble in polar organic solvents such as MeOH, MeCN, CH_2Cl_2 and had mild solubility in aqueous solvents. An ORTEP drawing of [$Mn_2(Adpa)_2Cl_4$] with the atomic numbering scheme is shown in Fig. 1. Selected bond distances and angles for complex [$Mn_2(Adpa)_2Cl_4$] are given in Table 2.



Symmetry code: A: 2–x; 1–y; 2–z

Fig.1 Structure of the title complex (Ellipsoids drawn at 50% probability; H atoms have been omitted for clarity)

Crystallographic analysis of the complex reveal that each Mn(Ⅱ) ion is coordinated by three N atoms of the *N*-allyl di(picolyl)amine and by three Cl atoms. Both Mn atoms are in a distorted octahedral environment and also the two Mn atoms are centrosymmetric. The Mn-N distances are in the range 0.226 39(13)~0.228 89(13) nm, which is in the range of 0.218~0.237 nm for Mn_2(Ⅱ, Ⅱ) complexes[9]. The distances between the Mn atoms and the bridging clorine atoms are 0.254 74 and 0.258 71(5) nm, respectively; and the corresponding

Table 2 Selected bond distances (nm) and angles (°) for [$Mn_2(Adpa)_2Cl_4$]

Mn(1)-N(1)	0.226 39(13)	Mn(1)-N(3)	0.238 36(13)	Mn(1)-Cl(2)	0.254 74(5)
Mn(1)-N(2)	0.228 89(13)	Mn(1)-Cl(1)	0.242 34(5)	Mn(1)-Cl(2a)	0.258 71(5)
Mn(1)-Cl(2)-Mn(1A)	96.684(15)	Cl(1)-Mn(1)-N(2)	94.21(4)	Cl(2)-Mn(1)-N(3)	90.75(3)
N(1)-Mn(1)-N(2)	99.27(15)	Cl(1)-Mn(1)-N(3)	157.65(3)	Cl(1)-Mn(1)-Cl(2)	105.03(3)
N(1)-Mn(1)-N(3)	72.98(5)	Cl(2)-Mn(1)-N(1)	160.3(4)	Cl(2)-Mn(1)-Cl(2A)	83.316(15)
N(2)-Mn(1)-N(3)	71.05(5)	Cl(1)-Mn(1)-Cl(2)	98.206(17)		
Cl(1)-Mn(1)-N(1)	93.74(4)	Cl(2)-Mn(1)-N(2)	85.24(4)		

Atoms labelled with the suffixes A is at the symmetry positions 2–x; 1–y; 2–z.

Mn(1)-Cl(2)-Mn(1A) angles (*ca.* 96.684) are larger than those observed (0.210 nm and 107 ℃, respectively) in the Mn (Ⅱ, Ⅱ)$_2$ complex of a heptadentate phenol[9]. Two crystallographically equivalent manganese atoms are bridged by two Cl anions lead to a dinuclear manganese unit with a Mn-Mn distance 0.383 6 nm.

Complexes were studied for their antitumor activity *in vitro* by determining the inhibitory percentage against growth of cancer cells HepG2, A549, LoVo, and ECA-109 by using the method of 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenpyltetrazolium bromide reduction (MTT method). The $Mn(dpa)_2Cl_2$ complex can inhibit the proliferation of LoVo cells in 100 μmol·L^{-1}, but it has no obvious inhibition on the proliferation of cells HepG2, A549 and Eca109 with IC_{50} large than 100 μmol·L^{-1}. The dinuclear $Mn_2(Adpa)_2Cl_4$ complex was active against the four cancer cells with IC_{50} in the range 25~65 μmol·L^{-1} (Table 3). The cytotoxicity assay for $Mn_2(Adpa)_2Cl_4$ complex on the cancer cells A549 and LoVo in the range 1~100 μmol·L^{-1} after 48 h was shown in Fig.2. The cytotoxicity of $[Mn_2(Adpa)_2Cl_4]$ complex was dependent on the concentration. It activates the proliferation of cancer cell A549 in the range of 1~20 μmol·L^{-1}, but has no activation on the proliferation of cancer cell LoVo in the same range.

Table 3 Inhibition on the growth of human cancer cells for Mn(Ⅱ) complexes

Tested complexes	$IC^*_{50\text{-HepG2}}$ / (μmol·L^{-1})	$IC^*_{50\text{-LoVo}}$ / (μmol·L^{-1})	$IC^*_{50\text{-A549}}$ / (μmol·L^{-1})	$IC^*_{50\text{-ECA109}}$ / (μmol·L^{-1})
$Mn(dpa)_2Cl_2$	>100	55	>100	>100
$Mn_2(Adpa)_2Cl_4$	65	45	25	30

*All IC_{50} values were expressed as mean ±S.D. values of the three experiments.

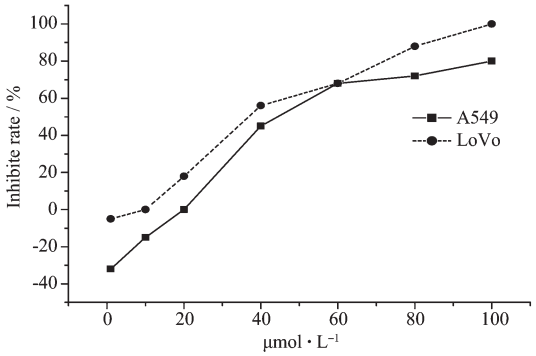
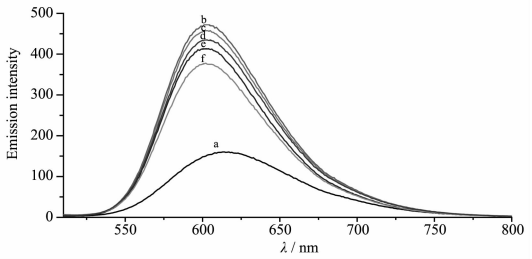


Fig.2 Inhibition for $Mn_2(Adpa)_2Cl_4$ complex on the cancer cells A549 (line) and LoVo (dash line) in the range 1~100 μmol·L^{-1} after 48 h

The relative binding of the complex to calf thymus DNA has been studied by the fluorescence method using the emission intensity of ethidium bromide (EB). Ethidium bromide alone or in the presence of metal complex shows weak emission in Tris buffer medium due to fluorescence quenching of the free EB by solvent molecules (Fig.3, line a). In the presence of DNA, EB shows enhanced emission intensity due to its intercalative binding to DNA (Fig.3, line b). Adding of manganese(Ⅱ) complex to the solution of calf thymus DNA -bound EB lead to a little decrease of the emission intensity of EB (Fig.3, line c, d, e, f) showing the bind of manganese(Ⅱ) complex to DNA is weak compared with that of copper or cisplatin complexes[16].



C_{DNA}=1×10^{-5} mol·L^{-1}, C_{EB}=5.1×10^{-6} mol·L^{-1}, $C_{[Mn_2(Adpa)_2Cl_4]}$ (×10^{-5} mol·L^{-1}, a → f)=0, 0, 5, 10, 20, 40

Fig.3 Emission spectra for EB (a), EB+DNA (b), EB+DNA+$[Mn_2(Adpa)_2Cl_4]$ (c → f), in Tris buffer

Mitochondrial swelling was a important method to detect mitochondria functions[17]. The interaction for the complexes $Mn(dpa)_2Cl_2$ and $Mn_2(Adpa)_2Cl_4$ with mitochondria was studied by measured the Ca^{2+}-loaded mitochondrial swelling. The $Mn(dpa)_2Cl_2$ shows short time (short than 0.5 min) inhibition in the 100 μmol·L^{-1}, but the complex $Mn_2(Adpa)_2Cl_4$ can inhibit the induced swelling of Ca^{2+}-loaded mitochondria swelling in a dose-dependent manner. Inhibition for the $Mn_2(Adpa)_2Cl_4$ complex on the swelling of Ca^{2+}-loaded mitochondria in the presence of 10 μmol·L^{-1} $CaCl_2$ was

shown in Fig.4. The Ca^{2+}-loaded mitochondria swelling was inhibited completely in the presence of 80~100 μmol·L^{-1} complex $Mn_2(Adpa)_2Cl_4$. Since Ca^{2+} is a key inducer of permeability transition pores (PTP) (in the inner mitochondrial membrane) opening, attenuation of Ca^{2+} overload may provide protection against PTP opening [5]. The experimental results indicate that the $Mn_2(Adpa)_2Cl_4$ complex may be used as inhibitor of the PTP opening.

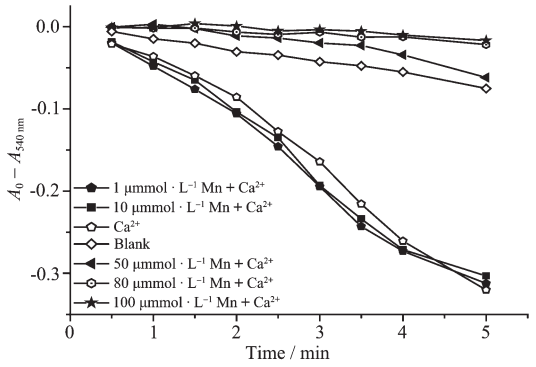


Fig.4　Inhibition for the $Mn_2(Adpa)_2Cl_4$ complex on the swelling of Ca^{2+}-loaded mitochondria in the presence of 10 μmol·L^{-1} $CaCl_2$

3　Conclusion

The dinuclear manganese(Ⅱ, Ⅱ) complex $Mn_2(Adpa)_2Cl_4$ was synthesized and characterized. Cytotoxicity assay shows the complex was more active than the reported complex $Mn(dpa)_2Cl_2$ of the unsubstituted ligand di(picolyl)amine (dpa). The complex $Mn_2(Adpa)_2Cl_4$ was active against the four kinds of cancer cells (HepG 2, LoVo, ECA-109, A549), but it activates the proliferation of cancer cell A549 in the range of 1~20 μmol·L^{-1}. The interaction between the complex and ct-DNA is weak, but the complex can inhibit the induced swelling of Ca^{2+}-loaded mitochondria swelling in a dose-dependent manner in vitro. The experimental results indicate that the $Mn_2(Adpa)_2Cl_4$ complex may be developed as antitumor complex to target mitochondrial.

References:

[1] Armstrong J S. British J. *Pharm.*, **2007**,**151**:1154~1165

[2] Hagand H, Nikolaisen J, Hodneland L I, et al. *Expert Opin. Ther. Target*, **2007**,**11**:1055~1069

[3] Rackham O, Nichols S J, Leedman P J, et al. *Biochem. Pharm.*, **2007**,**74**:992~1002

[4] Muscella A, Calabriso N, Fanizzi E P, et al. *British J. Pharm.*, **2008**,**153**:34~49

[5] Javadov S, Karmazyn M. *Cell. Physiol. Biochem.*, **2007**,**20**:01~22

[6] Yin G, Danby A M, Kitto D, et al. *Inorg. Chem.*, **2007**,**46**:2173~2180

[7] Higuchi C, Sakiyama H, Okwa H. *J. Chem. Soc, Dalton Trans.*, **1995**,**11**:4015~4020

[8] Wahnon D, Hynes R C, Chin J. *Chem. Commun.*, **1994**,**12**:1441~1142

[9] Goto M, Koga N, Ohse Y, et al. *Inorg. Chem.*, **2004**,**43**:5120~5127

[10]Kruppa M, Konig B. *Chem. Rev.*, **2006**,**106**:3520~3580

[11]Chen Q Y, Zhang R X, Liu Q S, et al. *Chem. Res. Chin. Uni.*, **2007**,**23**:284~289

[12]Bose D, Mostafa G, Fun H K, et al. *Polyhedron*, **2005**,**24**:747~758

[13]Qin D D, Yang Z Y, Wang B D. *Spectrochim. Acta Part A*, **2007**,**68**:912~917

[14]Sheldrick G M. *SHELXS*-97, *Program for the Solution of Crystal Structure*, University of Göttingen, Götingen, Germany, **1997**.

[15]Sheldrick G M. *SHELXL*-97, *Program for the Refinement of Crystal Structure*, University of Göttingen, Götingen, Germany, **1997**.

[16]Dhar S, Nethaji M, Chakravarty A R. *Inorg. Chem.*, **2006**,**45**:11403~11409

[17]Mohammadi B A, Ghazi K M, Mosmann T. *J. Immunol Methods*, **1983**,**65**:55~63