氨·环己胺·羧酸根合铂(Ⅱ)类配合物的合成、抗肿瘤活性和与 DNA 的键合水平

张金超*,[1]　申　勇[1]　杨梦苏[2]
([1]河北大学化学与环境科学学院化学系,保定　071002)
([2]香港城市大学生物及化学系,香港)

摘要： 本工作设计合成了 6 种新型氨·环己胺·羧酸根合铂(Ⅱ)类配合物[Pt(NH_3)(◇-NH_2)X_2](**a~f**){其中,X=CH_3COO^-(乙酸根),CH_2ClCOO^-(氯乙酸根),C_6H_5-COO^-(苯甲酸根),*p*-CH_3O-C_6H_4-COO^-(对甲氧基苯甲酸根),*p*-CH_3-C_6H_4-COO^-(对甲基苯甲酸根),*p*-NO_2-C_6H_4-COO^-(对硝基苯甲酸根)}。通过元素分析、摩尔电导、红外光谱、紫外光谱和 ^{1}H 核磁共振谱对配合物进行了表征。通过 MTT 法研究了配合物的体外抗肿瘤活性,通过流式细胞仪以及等离子体质谱研究了配合物对细胞周期的影响以及与细胞 DNA 的键合量;体外抗肿瘤活性测试表明,配合物(**c~f**)对 EJ 和 HL-60 2 种肿瘤细胞表现出好的活性,而且配合物(**c**),(**d**)和(**e**)对 EJ 和 HL-60 2 种肿瘤细胞的活性高于临床用药顺铂; 配合物(**a~f**)对 MCF-7、HCT-8 和 BGC-823 3 种肿瘤细胞的活性低于顺铂;它们能阻止 HL-60 和 EJ 细胞 G_2 + M → G_1 期的进行;配合物(**a~f**)与 HL-60 和 EJ 细胞的 DNA 键合量从大到小的顺序为:**c** > **d** > **e** > cisplatin > **f** > **a** > **b**。

关键词： 混胺铂(Ⅱ)配合物; 抗肿瘤活性; 经典构效关系; 细胞周期; Pt-DNA 键合
中图分类号： O614.82　　**文献标识码：** A　　**文章编号：** 1001-4861(2006)05-0823-09

Synthesis, Cytotoxicity and DNA-Binding Levels of Amminecyclohexy/Aminecarboxy/Platinum(Ⅱ) Complexes

ZHANG Jin-Chao*,[1]　SHEN Yong[1]　YANG Meng-Su[2]
([1]*Department of Chemistry, College of Chemistry & Environmental Science, Hebei University, Baoding, Hebei* 071002)
([2]*Department of Biology and Chemistry, City University of Hong Kong, Hong Kong*)

Abstract: Six new amminecyclohexy/aminecarboxy/platinum(Ⅱ) complexes with carboxylates (**a~f**) have been synthesized and characterized by elemental analysis, conductivity, IR, UV, and ^{1}H NMR spectra techniques. The cytotoxicity of the complexes was tested by MTT assay. The cell cycle analysis and the levels of total platinum bound to DNA were measured by flow cytometry and ICP-MS. The results show that complexes (**c**), (**d**), (**e**) and (**f**) have excellent cytotoxicity against EJ and HL-60 and complexes (**c**), (**d**) and (**e**) demonstrate cytotoxicity superior to that of the clinically established cisplatin. Complexes (**a~f**) have poor cytotoxicity against HCT-8, MCF-7 and BGC-823 than that of cisplatin. The complexes (**a~f**) induce a concentration-dependent accumulation of HL-60 and EJ cells in the G_2/M phase of the cell cycle as cisplatin. The levels of total platinum bound to DNA in HL-60 and EJ cells decrease in the sequence: **c** > **d** > **e** > cisplatin > **f** > **a** > **b** under the same experimental conditions.

Key words: mixed amine platinum(Ⅱ) complexes; antitumor activity; classical structure-activity relationships; cell cycle; Pt-DNA binding

收稿日期:2005-10-31。收修改稿日期:2006-01-04。
*通讯联系人。E-mail:jczhang6970@yahoo.com.cn
第一作者:张金超,男,37 岁,博士,教授;研究方向:无机药物化学和细胞生物无机化学。

0 Introduction

Cisplatin is one of the most active chemotherapeutic agents available for the treatment of a variety of malignancies, especially testicular and ovarian. However, the drug′s clinical application is restricted by both toxicological and tumour resistance. Since the introduction of cisplatin, thousands of Pt compounds have been synthesized and evaluated as potential antitumor agents. Among the 33 platinum complexes which have entered clinical trials after the onset of clinical studies with cisplatin in the early 1970s, only carboplatin has received worldwide approval so far. In addition, four drugs (oxaliplatin, nedaplatin, lobaplatin and SKI2053R) have also gained regionally limited approval and another eight drugs continue to be evaluated in clinical studies. Two of these (JM-216 and ZD0473) have recently entered phase Ⅲ studies[1~5]. Many valuable works have been done in China[6~9]. Previously, we reported the synthesis and antitumor activity of mixed ammine/methylamine platinum(Ⅱ) complexes with bicarboxylates, binuclear platinum(Ⅱ) complexes, diaqua platinum(Ⅱ) complexes with carboxylates[10~12].

The structure-activity relationships summarized by Cleare and Hoeschele dominated Pt drug design for over 20 years and remained valid until recently[13]. This is reflected in the fact that all Pt compounds that have entered clinical trials so far adhere to this set of guidelines. However, it has become quite evident that mere analogues of cisplatin or carboplatin will not probably offer any substantial clinical advantages over the existing drugs. A number of researchers have taken a completely different approach to Pt drug design and have prepared compounds that violate the structure-activity relationships but yet show antitumor activities. Therefore, the search continues for an improved Pt antitumor agent, motivated by the desire to design a less toxic, orally active compound that is non-cross-resistant with cisplatin and carboplatin[2,14,15].

The mixed ammine/amine platinum complexes with chloro ion have been reported and demonstrated better activity against cisplatin-resistant cells in vitro and much less toxicity than the parent cisplatin[16,17]. For example, JM-216 has recently entered phase Ⅲ studies. The possible advantage of platinum anticancer drugs with decreased reactivity of leaving group is an established approach which commenced with the clinical success of carboplatin. It is reported that the decreased reactivity reduces the nephrotoxic and neurotoxic side-effects of cisplatin. Moreover, decrease in reactivity may also lead to reduced detoxification reactions by intracellular thiols. This may increase the efficacy of the drug and helps to circumvent resistance mechanism such as overexpression of glutathione. So carboxylate platinum complexes seem to be quite promising than the corresponding chloro analogs. So far, many mixed ammine/amine platinum complexes with chloro ion have been reported. But few mixed ammine/amine platinum complexes with carboxylates are reported[18,19]. We previously reported the synthesis and antitumor activity of platinum(Ⅱ) complexes of mixed ammine/methylamine with bidentate carboxylates[10]. Here, the synthesis, cytotoxicity and DNA-binding levels of new mixed ammine/cyclohexylamine platinum(Ⅱ) complexes with carboxylates are reportd and discussed.

1 Experimental

1.1 Reagents

All reagents and solvents were analytical reagent grade and purchased from Beijing Chemical Reagents Company.

MTT(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), Tris-HCl (tris-(hydroxymethyl)-aminomethane, hydrochloride), PI (propidium iodide), RNase and Genomic DNA extraction kit were purchased from Sigma. RPMI 1640 culture medium and FBS(fetal bovine serum)was from Gibco. Cisplatin was purchased from Qi Lu Pharmaceutical Factory in China.

1.2 Instrumentation and measurement

Elemental analyses were done on a EA-1110 elemental analyzer. Molar conductances at room temperature were measured in 10^{-3} mol·L^{-1} aqueous solutions using a DSS-11A type conductivity meter. The IR spectra were recorded in the 400~4 000 cm^{-1} range using KBr pellets and a Perkin-Elmer Model-683

Spectrophotometer. The electronic spectra in H_2O were measured on an UV-3400 Toshniwal Spectrophotometer(wavelengh range: 190~900 nm, spectra width: 0.1~5 nm, resolution: 0.1 nm). The 1H NMR spectra in D_2O was recorded on an Brucker AV 400 NMR Spectrometer (DSS as internal standard). Cell cycle analysis was performed on a BECScan Flow Cytometry (Becton Dickinso). The levels of total platinum bound to DNA were measured by ICP-MS (Inductively Coupled Plasma Mass Spectrometry, PE Elan-5000) (cooling gas flow: 12 $L\cdot min^{-1}$, plasma gas flow: 1.0 $L\cdot min^{-1}$, nebulizer pressure: 2.8×10^5 Pa, sample uptake flow: 0.75 $mL\cdot min^{-1}$).

1.3 Preparation of complexes

Precursor complexes *cis*-[Pt(cyclohexylamine)$_2$I$_2$] (**i**), [Pt(cyclohexylamine)I$_2$]$_2$ (**ii**) and *cis*-[Pt(cyclohexylamine)(NH$_3$)I$_2$] (**iii**) were synthesized according to the literatures[20,21].

Pt(NH$_3$)(cyclohexylamine)(COOC-CH$_3$)$_2$ (**a**): *cis*-[Pt(cyclohexylamine)(NH$_3$)I$_2$] (**iii**) (0.647 g, 1 mmol) was mixed with $AgNO_3$ (0.331 g, 1.95 mmol) in 15 mL of water. The mixture was allowed to stir overnight in the dark. The AgI precipitate was removed by filtration and the filtrate was checked by NaCl solution until free from immediate precipitation of AgCl. When all the silver ions have been removed, a slight excess of the sodium salt of the acetic acid was added to the filtrate. After 6 h, the mixture was evaporated to dryness and washed with diethyl ether. The final product Pt(NH$_3$)(cyclohexylamine)(OOC-CH$_3$)$_2$ was dried over P_2O_5 under vacuum. Yield: 65%.

The synthetic procedure for Pt(NH$_3$)(cyclohexylamine)(OOC-CH$_2$Cl)$_2$ (**b**), Pt(NH$_3$)(cyclohexylamine)(OOC-C$_6$H$_5$)$_2$ (**c**), Pt(NH$_3$)(cyclohexylamine)(*p*-CH$_3$O-C$_6$H$_4$-COO)$_2$ (**d**,) Pt(NH$_3$)(cyclohexylamine)(*p*-CH$_3$-C$_6$H$_4$-COO)$_2$ (**e**), Pt(NH$_3$)(cyclohexylamine)(*p*-NO$_2$-C$_4$H$_6$-COO)$_2$ (**f**) were similar. The synthetic routines of the amminecyclohexy/aminecarboxy/platinum(Ⅱ) complexes are given below (Fig.1).

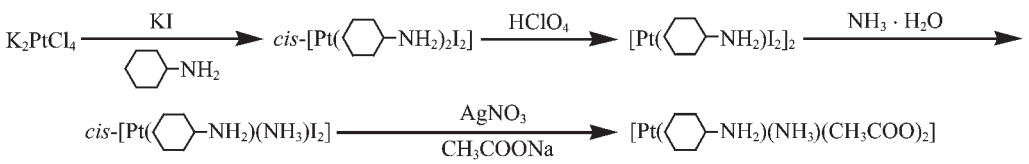

Fig.1　Synthetic routines of the series of Pt(NH$_3$)(cyclohexylamine)(carboxylate)$_2$

1.4 Cytotoxicity analysis

The complexes were dissolved in phosphate buffered saline (PBS) and diluted to the required concentration with culture medium when used. The cytotoxicity was evaluated by MTT assay[22]. Briefly, cells were plated in 96-well microassay culture plates (10^4 cells per well) and grown overnight at 37 ℃ in a 5% CO_2 incubator. Test compounds were then added to the wells to achieve final concentrations ranging from 10^{-7} to 10^{-4} $mol\cdot L^{-1}$. Control wells were prepared by addition of culture medium. Well containing culture medium without cells were used as blanks. The plates were incubated at 37 ℃ in a 5% CO_2 incubator for 48 h. Upon completion of the incubation, stock MTT dye solution(20 μL, 5 $mg\cdot mL^{-1}$) was added to each well. After 4 h incubation, 2-propanol (100 μL) was added to solubilize the MTT formazan. The optical density of each well was then measured on a microplate spectrophotometer at a wavelength of 570 nm. The IC_{50} value was determined from plots of % viability against dose of compound added. Five different human carcinomas were the subjects in this study: EJ (bladder carcinoma), HCT-8 (colon carcinoma), BGC-823 (gastricarcinoma), HL-60 (immature granulocyte leukemia) and MCF-7 (galactophore carcinoma).

1.5 Flow cytometry analysis

The cell cycle analysis was performed as described by Ferlini et al.[23]. Cells were treated with platinum complexes for the indicated times and harvested by centrifugation at 1 200 $r\cdot min^{-1}$ for 5 min at room temperature. Cell pellets were rinsed with PBS, suspended in a 1∶1 (*V*/*V*) solution of PBS and 0.2 $mol\cdot L^{-1}$ Na_2HPO_4-0.1 $mol\cdot L^{-1}$ citric acid (pH=7.5), and fixed with cold ethanol at 4 ℃ for 1 h. Fixed cells were washed with PBS and resuspended in a staining solution containing PI (10 $\mu g\cdot mL^{-1}$) and

DNase-free Rnase (100 μg·mL^{-1}). The cell suspensions were incubated at 37 ℃ for 1 h in the dark and analyzed on a Flow cytometry. Data were collected by ModFit LT 2.0 for power software.

1.6 DNA binding

The levels of total platinum bound to DNA in HL-60 and EJ cells were performed as described by Mellish et al.[24]. Briefly, approximately 5 × 10^7 HL-60 and EJ cells were seeded in tissue-culture flasks, then the complexes were added in a concentration gradient, each concentration in triplicate, and the final concentrations were maintained at 10, 25, 50 and 100 μmol·L^{-1}, respectively. They were incubated at 37 ℃ in 5% CO_2 for 4 h. Cells were then harvested, and DNA was extracted according to DNA extraction kit procedure. The DNA was dissolved in 300 μL water. DNA purity was confirmed and concentration was measured by UV spectroscopy. An aliquot of the remaining sample was sonicated and subjected to platinum analysis by ICP-MS.

1.7 Statistical analysis

Data were collected from at least three separate experiments. The results are expressed as mean ± SD. The statistical differences were analyzed using SPSS′ *t*-test. p values less than 0.05 were considered to have significant statistical differences.

2 Results

2.1 Physical properties of the complexes

The physical properties of the complexes are presented in Table 1. There is good agreement between the caculated and the found values Low molar conductances for the complexes correspond to non-electrolytes[25].

Table 1 Physical properties of the complexes

Complex	Colour	Found(Calcd.) / %				Mol. wt.	A_M / ($\Omega^{-1}\cdot cm^2\cdot mol^{-1}$)
		C	N	H	Pt		
(**i**)	Yellow	22.20(22.27)	4.30(4.33)	4.01(4.05)	—	—	—
(**ii**)	Red brown	13.09(13.15)	2.50(2.56)	2.34(2.39)	—	—	—
(**iii**)	Yellow	12.70(12.75)	4.80(4.96)	2.70(2.85)	—	—	—
a	Pale yellow	27.85(27.97)	6.39(6.53)	5.02(5.16)	45.21(45.44)	429.40	4.25
b	Pale yellow	24.25(24.10)	5.89(5.73)	4.12(4.04)	39.12(39.15)	498.28	5.02
c	Pale yellow	43.58(43.39)	5.23(5.06)	4.88(4.73)	35.36(35.25)	553.53	4.98
d	Pale yellow	43.25(43.06)	4.49(4.57)	4.86(4.93)	32.00(31.80)	613.58	4.65
e	Pale yellow	45.59(45.43)	5.01(4.82)	5.32(5.20)	33.80(33.55)	581.58	5.36
f	Pale yellow	37.21(37.33)	8.86(8.71)	3.95(3.76)	30.36(30.32)	643.53	5.32

2.2 IR spectra

The IR spectra of the complexes (**a~f**) are similar, the main bands with tentative assignments are listed in Table 2. The bands of ν_{NH} and δ_{NH} in the precursor complexes (**i~iii**) and new complexes (**a~f**) shift to lower frequencies than those of free ammine and cyclohexylamine. New band appears at 444~490 cm^{-1} and is assigned to Pt-N strectching. Thus it indicates that they are coordinated with platinum through nitrogen atoms. The carboxylate group of the complexes (**a~f**) shows two bands, an intense antisymmetric carboxylate stretching ν_{as,COO^-} and a symmetric stretching ν_{s,COO^-}, at about 1 650 and 1 350 cm^{-1}, respevtively. The values of $\Delta\nu_{COO^-}$ ($\nu_{as,COO^-}-\nu_{s,COO^-}$) of the complexes (**a~f**) are in the range 241~285 cm^{-1}, which is greater than $\Delta\nu_{COO^-}$ of the corresponding sodium carboxylates, so we may suggest that the carboxylate group is monodentate coordinated through oxygen atoms[26]. This is further confirmed by the appearance of the peaks of $\nu_{Pt\text{-}O}$.

2.3 Electronic spectra

The results are listed in Table 3. After formation of the complexes, one new peak appears at 198.0 and 200.0 nm in the spectra of the complex (**a**) and (**b**), respectively. E_2 band blue shifts by *ca.* 9.0, 27.0, 15.0 and 20.0 nm, B band blue shifts by *ca.* 30.0, 26.0, 30.0 and 6.0 nm in the spectra of the complex (**c**), (**d**), (**e**) and (**f**), respectively.

Table 2　IR data of the complexes (cm^{-1})

Complex	ν_{NH}	δ_{NH}	ν_{as,COO^-}	ν_{s,COO^-}	$\Delta\nu_{COO^-}$	ν_{Pt-O}	ν_{Pt-N}
(**i**)	3 195	1 566					444
	3 109						
(**ii**)	3 236	1 570					470
	3 200						
(**iii**)	3 270	1 510					480
	3 200						
a	3 260	1 550	1 648	1 405	243	586	490
	3 165						
b	3 258	1 549	1 630	1 389	241	569	485
	3 156						
c	3 209	1 535	1 610	1 325	285	578	470
	3 125						
d	3 265	1 489	1 620	1 355	265	565	472
	3 156						
e	3 245	1 532	1 635	1 352	283	569	468
	3 168						
f	3 268	1 523	1 625	1 356	269	574	483
	3 158						

Table 3　UV data of the ligands and complexes

Compound	λ / nm		
	$n \rightarrow \pi^*$	E_2 band	B band
Acetic acid			
Chloroacetic acid			
Benzoic acid		195.0	250.0
p-methoxylbenzoic acid		227.0	271.0
p-methylbenzoic acid		210.0	265.0
p-nitrobenzoic acid		212.0	271.0
a	198.0		
b	200.0		
c		186.0	220.0
d		200.0	245.0
e		195.0	235.0
f		192.0	265.0

2.4　^{1}H NMR

The results of the ^{1}H NMR spectra are listed in Table 4. After formation of the complexes, the hydrogen protons of the complexes shift to low field compared with those of free ligands. This is also further confirmed that the carboxylate and cyclohexylamine are coordinated with platinum through oxygen and nitrogen atoms.

Based on the above studies, we propose a tentative coordination structure for the complexes (Fig.2).

2.5　Cytotoxicity effect

As listed in Fig.3, Cytotoxicity decreases in the sequence: **c** > **d** > **e** > cisplatin > **f** > **a** > **b**. Complexes (**c**), (**d**), (**e**) and (**f**) have excellent cytotoxicity and complexes (**c**), (**d**) and (**e**) demonstrate better cytotoxicity than that of the clinically established cisplatin against EJ and HL-60 cell lines. Complexes (**a**~**f**) have poor cytotoxicity against HCT-8, MCF-7 and

Table 4 ^{1}H NMR spectra of the ligands and complexes

Compound	Chemical shift (δ) / ppm
Cyclohexylamine	2.52 (br, 1H, CH (methine)), 0.9~2.0 (m, 10H, CH(alkyl))
Acetic acid	2.10 (s, 3H, -CH_3,)
Chloroacetic acid	4.00(s, 2H, -CH_2-)
Benzoic acid	7.45~8.12(m, 5H, -C_6H_5)
p-methoxybenzoic acid	7.07~7.91(m, 4H, -C_6H_4-), 3.84(s, 3H, -OCH_3)
p-methylbenzoic acid	7.30~7.87(m, 4H, -C_6H_4-), 2.34(s, 3H, -CH_3)
p-nitrobenzoic acid	8.24~8.38(m, 4H, -C_6H_4)
a	2.91 (br, 1H, CH (methine)), 1.0~2.2 (m, 10H, CH(alkyl)), 2.35 (s, 6H, -CH_3)
b	2.92 (br, 1H, CH (methine)),1.1~2.4 (m, 10H, CH(alkyl)), 4.12(s, 4H, -CH_2-)
c	2.89 (br, 1H, CH (methine)), 1.0~2.3 (m, 10H, CH(alkyl)), 7.75~8.29(m, 10H, -C_6H_5)
d	2.84 (br, 1H, CH (methine)), 1.0~2.2 (m, 10H, CH(alkyl)), 7.15~8.12(m, 8H, -C_6H_4-), 3.98(s, 6H, -OCH_3)
e	2.88 (br, 1H, CH (methine)), 1.0~2.3 (m, 10H, CH(alkyl)), 7.45~8.01(m, 8H, -C_6H_4-), 2.49(s, 6H, -CH_3)
f	2.93 (br, 1H, CH (methine)), 1.0~2.5 (m, 10H, CH(alkyl)), 8.36~8.50(m, 8H, -C_6H_4)

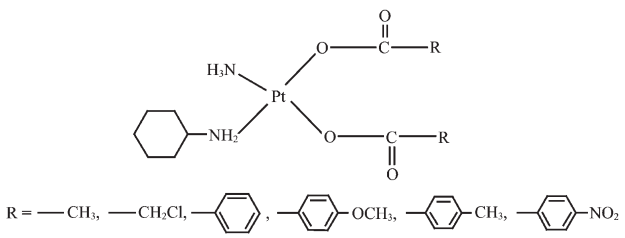

Fig.2 Chemical structure of the complexes (**a~f**)

BGC-823 cell lines than that of cisplatin.

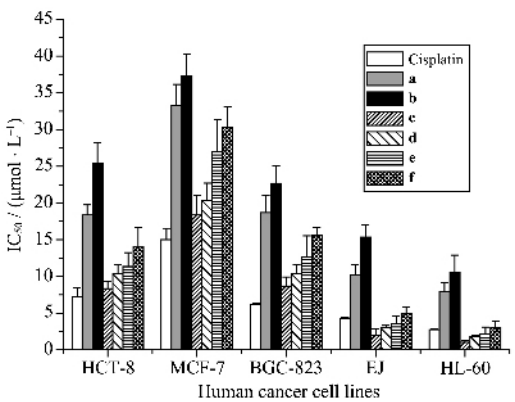

Fig.3 Cytotoxicity of complexes against various human carcinomas

2.6 DNA binding

As listed in Fig.4 and 5, the levels of total platinum bound to DNA in HL-60 and EJ cells decrease in the sequence: **c** > **d** > **e** > cisplatin > **f** > **a** > **b** under the same experimental conditions.

2.7 Cell cycle analysis

The effects of the complexes on cell cycle are given in Table 5 and 6. The complexes (**a~f**) induced

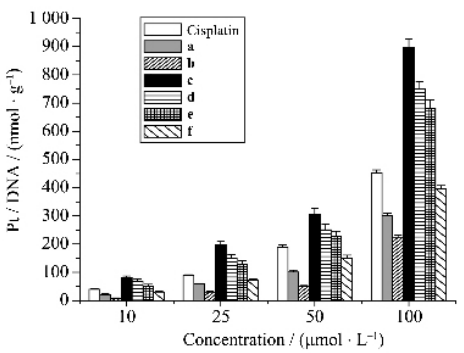

Fig.4 Levels of total platinum bound to DNA in HL-60 cell after 4 h exposure to platinum complexes

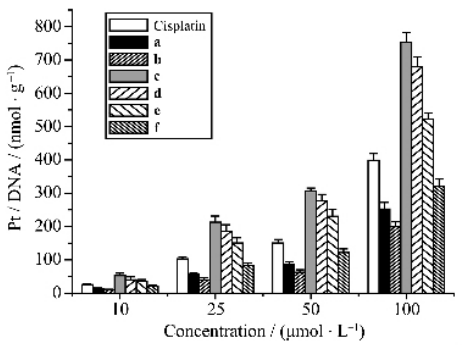

Fig.5 Levels of total platinum bound to DNA in EJ cell after 4 h exposure to platinum complexes

Table 5　Effect of the platinum complexes on cell cycle in HL-60 cells (n=4)

Complex	Concentration / (μmol·L^{-1})	Cell cycle / % ($\bar{x}$ ± SD)		
		G_1	S	G_2/M
Control	0.0	31.34 ± 1.06	60.85 ± 1.41	7.80 ± 1.18
Cisplatin	1.5	25.36 ± 1.25**	50.36 ± 1.35**	24.28 ± 2.35**
	3.0	18.98 ± 1.02**	45.68 ± 2.87**	35.34 ± 2.87**
a	1.5	30.24 ± 3.56*	60.23 ± 1.06*	9.53 ± 1.23*
	3.0	28.35 ± 2.13*	58.65 ± 4.56*	13.00 ± 1.02**
b	1.5	31.02 ± 2.13*	60.21 ± 5.32*	8.77 ± 1.02*
	3.0	29.32 ± 2.53*	58.23 ± 4.25*	12.45 ± 0.89**
c	1.5	12.32 ± 0.25**	25.69 ± 3.56**	61.99 ± 0.98**
	3.0	6.21 ± 0.69**	10.35 ± 3.69**	83.44 ± 1.01**
d	1.5	15.69 ± 1.02**	30.35 ± 3.56**	53.96 ± 2.65**
	3.0	9.65 ± 1.03**	15.68 ± 3.89**	74.67 ± 3.89**
e	1.5	20.68 ± 1.02**	45.68 ± 2.36**	33.64 ± 2.56**
	3.0	15.98 ± 1.23**	35.08 ± 4.23**	48.94 ± 3.02**
f	1.5	28.65 ± 3.21**	53.69 ± 2.69**	17.66 ± 0.89**
	3.0	22.63 ± 1.24**	48.98 ± 3.25**	28.39 ± 1.89**

*P > 0.05, **P < 0.05 compared with the control group.

Table 6　Effect of the platinum complexes on cell cycle in EJ cells (n=4)

Complex	Concentration / (μmol·L^{-1})	Cell cycle / % ($\bar{x}$ ± SD)		
		G_1	S	G_2/M
Control	0.00	25.68 ± 1.36	52.69 ± 2.69	21.63 ± 2.68
Cisplatin	2.5	19.86 ± 1.96**	42.36 ± 5.36**	37.78 ± 4.69**
	5.0	12.35 ± 1.03**	22.68 ± 1.96**	64.97 ± 6.89**
a	2.5	24.98 ± 2.98*	52.09 ± 5.68*	22.93 ± 1.65*
	5.0	22.69 ± 3.09*	49.69 ± 5.03*	27.62 ± 2.69**
b	2.5	25.03 ± 2.08*	52.31 ± 5.82*	22.66 ± 1.36*
	5.0	24.36 ± 2.09*	50.36 ± 4.86*	25.28 ± 2.68**
c	2.5	10.69 ± 1.68**	20.58 ± 2.58**	68.73 ± 8.59**
	5.0	5.36 ± 1.02**	11.35 ± 2.07**	83.29 ± 7.56**
d	2.5	12.35 ± 1.03**	25.69 ± 3.56**	61.96 ± 5.36**
	5.0	7.69 ± 1.23**	15.36 ± 2.35**	76.95 ± 7.69**
e	2.5	17.89 ± 1.32**	32.86 ± 3.56**	49.25 ± 4.02**
	5.0	10.35 ± 1.06**	19.65 ± 2.03**	70.00 ± 6.58**
f	2.5	20.65 ± 2.65**	46.38 ± 3.69**	32.97 ± 2.35**
	5.0	15.79 ± 1.03**	28.69 ± 2.89**	55.52 ± 5.68**

*P > 0.05, **P < 0.05 compared with the control group.

a concentration-dependent accumulation of HL-60 and EJ cells in the G_2 / M phase of the cell cycle as cisplatin.

3　Discussion

The mode of action of platinum anticancer drugs is still not completely understood but it is thought to depend on hydrolysis reactions where the leaving group is replaced by a water molecule adding a positive charge on the molecule. The hydrolysis product is believed to be the active species reacting mainly with glutathione in the cytoplasm and the DNA in the nucleus thus inhibiting replication, transcription and other nuclear functions and arresting cancer cell pro-

liferation and tumor growth. So the reactivity of leaving groups is an important factor to affect anticancer activity. For ammine/cyclohexylamine platinum (Ⅱ) complexes with carboxylates, when leaving group is aromatic carboxylates, the complexes have better cytotoxicity, moreover, the substitution radical species in benzene also have effects on cytotoxicity[5].

It is generally been accepted that platinum-based drugs exert their cytotoxic effects through the formation of platinum-DNA adducts. This occurs predoiminantly at the N7 position of guanosine. The vast majority of cisplatin-DNA adducts are of the intrastrand type between adjacent guanines[27]. In general, the degree of cytotoxicity of cisplatin correlates with the amount of DNA-platination[28]. However, a study in testicular cancer germ cell lines found no association between cisplatin DNA-platination and drug sensitivity and a similar observation was made in a breast cancer cell line[29,30]. With respect to a correlation between the cytotoxicity of oxaliplatin and DNA-platination, the literature is limited. No correlation could be found between DNA-platination and cytotoxicity. Mellish et al reported that no significant correlation was found between total DNA platination levels and cytotoxicity of the seven platinum based drugs in SKOV-3 or in CH1 cell lines[24]. Roberts et al reported that that significant correlation was found between total DNA platination levels and cytotoxicity of polynuclear platinum complexes in L1210/0 cell line[31]. In our work, we found that there was also significant correlation between total DNA platination levels and cytotoxicity of six ammine/cyclohexylamine platinum complexes with carboxylates in HL-60 and EJ cell lines. The total DNA platination levels contains some kinds of Pt-DNA adducts formed by platinum complex such as 1, 2-intrastrand adducts between adjacent guanines or adjacent adenine.This suggests that it is probably the level of specific DNA adducts that is important in determining the cytotoxicity of platinum based drugs[24]. In addition, platinum based drugs might have other important targets apart from nuclear DNA. It has been demonstrated that cisplatin and oxaliplatin can induce apoptosis independent of the cell nucleus. It is interesting to note that mitochondrial DNA has been shown to have a two to fifty times greater propensity to be platinated than nuclear DNA. Cisplatin reacts with phospholopids, inhibits amino acid transport, protein synthesis, ATPases, uncouples oxidative phosphorylation, causes calcium efflux from the mitochondria and selectively alters the intracellular concentrations of calcium and potassium[32~34]. Although the importance of these other targets in relation to cytotoxicity is completely unknown, these other targets may have important effect on cytotoxicity. It remains to be further studied.

References:

[1] Sadler P J, Guo Z J. *Pure & Appl. Chem.*, **1998**,**70**(4):863~871

[2] Wong E, Giandomenico C M. *Chem. Rev.*, **1999**,**99**:2451~2466

[3] Ho Y P, Au-Yeung S C F, Kenneth K W T. *Med. Res. Rev.*, **2003**,**23**(5):633~655

[4] Jakupec M A, Galanski M, Keppler B K. *Rev. Physiol. Biochem. Pharmacol.*, **2003**,**146**:1~53

[5] Boulikas T, Vougiouka M. *Oncology Rep.*, **2003**,**10**:1663~1682

[6] Tang W X, Qu J, Dai A B, et al. *Nanjing Daxue Xuebao, Ziran Kexue Ban*(*Journal of Nanjing University*, *Natural Science Edition*), **1984**,**3**:471~478

[7] Wang L H, Liu Y, Yuan F P, et al. *Wuji Huaxue Xuebao* (*Chinese J. Inorg. Chem.*), **2004**,**20**(7):775~780

[8] Yang P, Guo M L. *Coord. Chem. Rev.*, **1999**,**185~186**:189~211

[9] Wang K. *Pure & Appl. Chem.*, **1988**,**60**:1279~1285

[10]Zhang J C, Gong Y Q, Zheng X M. *Synth. React. Inorg. Met.-Org. Chem.*, **2002**,**32**(1):49~57

[11]Zhang J C, Lin W X, Gong Y Q, et al. *Zhejiang Daxue Xuebao, Lixue Ban*(*Journal of Zhejiang University*, *Science Edition*), **2003**,**30**(2):192~195

[12]Zhang J C, Gong Y Q, Zheng X M, et al. *Zhejiang Daxue Xuebao, Lixue Ban*(*Journal of Zhejiang University*, *Science Edition*), **2005**,**32**(2):189~193

[13]Cleare M J, Hoeschle J D. *Bioinorg. Chem.*, **1973**,**2**:187~210

[14]Farrell N. *Cancer Invest.*, **1993**,**11**:578~589

[15]Hambley T W. *Coord. Chem. Rev.*, **1997**,**166**:181~223

[16]Yoshida M, Khokhar A R, Siddik Z H. *Cancer Res.*, **1994**,

54:4691~4697

[17]Orr R M, O′Neill C F, Nicolson M C, et al. *Br. J. Cancer*, **1994**,**70**:415~420

[18]Giandomenico C M, Abrams M J, Murrer B A, et al. *In Sixth International Symposium on Platinum and Other Metal Coordination Compounds in Cancer Chemotherapy*, San Diego, CA, January 23~26, **1991**.58

[19]Harrap K R, Morgan S E, Murrer B A, et al. *In Seventh NCI-EORTC Symposium on New Drugs in Cancer Therapy*, Amsterdam, March 17~20, **1992**.116

[20]Khokhar A R, Deng Y, Al-baker S, et al. *J. Inorg. Biochem.*, **1999**,**51**:677~687

[21]Rochon F D, Kong P C. *Can. J. Chem.*, **1986**,**64**:1894~1896

[22]Mosmmann L M. *Immunol. Methods*, **1983**,**65**(1~2):55~63

[23]Ferlini C, Cesare D S, Rainaldi G, et al. *Cytometry.*, **1996**, 24:106~115

[24]Mellish K J, Barnard C F, Murrer B A, et al. *Int. J. Cancer*, **1995**,**62**:717~723

[25]Greary W J. *Coord. Chem. Rev.*, **1971**,**7**:81~122

[26]Deacon G B, Phillips R J. *Coord. Chem. Rev.*, **1980**,**33**:227~250

[27]Reedijk J. *Proc. Natl. Acad. Sci. USA.*, **2003**,**100**(7):3611~3616

[28]Yang Z, Faustino P J, Andrews P A. *Cancer Chemother. Pharmacol.*, **2000**,**46**:255~262

[29]Luo X, Budihardjo I, Zou H. *Cell*, **1998**,**94**:481~490

[30]Mueller T, Voigt W, Simon H. *Cancer Res.*, **2003**,**63**:513~521

[31]Roberts J D, Peroutka J, Farrell N. *J. Inorg. Biochem.*, **1999**, **77**:51~57

[32]Gourdier I, Grabbe L, Andreau K. *Oncogene*, **2004**,**23**(45): 7449~7457

[33]Mandic A, Hansson J, Linder S. *J. Biol. Chem.*, **2003**,**278** (11):9100~9106

[34]Speelmans G, Sips W H H M, Grisel R J H. *Biochim. Biophys. Acta*, **1996**,**1283**:60~66