氨·二甲胺·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* 赵学军 申 勇
(河北大学化学与环境科学学院,保定 071002)

摘要:本工作设计合成了 6 种新型混胺羧酸根合铂(Ⅱ)类配合物[Pt($\begin{matrix}CH_3\\CH_3\end{matrix}$>NH)($NH_3$)$X_2$](**a~f**){其中,X=$CH_3COO^-$(乙酸根),$CH_2ClCOO^-$(氯乙酸根),$CHCl_2COO^-$(二氯乙酸根),$C_6H_5COO^-$(苯甲酸根),*p*-$CH_3$-$C_6H_4$-$COO^-$(对甲基苯甲酸根),*p*-$CH_3O$-$C_6H_4$-$COO^-$(对甲氧基苯甲酸根)}。通过元素分析、摩尔电导、差热分析、红外光谱、紫外光谱和 ^1H 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,通过等离子体质谱研究了配合物与细胞 DNA 的键合量;体外抗肿瘤活性测试表明,配合物(**a~f**)对所测试的肿瘤细胞 MCF-7、HCT-8 和 BGC-823 没有表现出活性,但对 EJ 和 HL-60 两种肿瘤细胞表现出好的活性,而且配合物(**d~f**)对 HL-60 细胞的活性与顺铂相当。配合物(**a~f**)与 HL-60 细胞的 DNA 键合量与其作用浓度表现出一定的依赖性,从小到大的顺序为:cisplatin < **c** < **b** < **a** < **f** < **e** < **d**。

关键词:混胺铂(Ⅱ)配合物;细胞毒性;经典构效关系;Pt-DNA 键合
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Synthesis, Cytotoxicity and DNA-binding Levels of Ammine/Dimethylamine Platinum(Ⅱ) Complexes with Carboxylates

ZHANG Jin-Chao* ZHAO Xue-Jun SHEN Yong
(*College of Chemistry & Environmental Science, Hebei University, Baoding, Hebei* 071002)

Abstract: Six new ammine/dimethylamine platinum(Ⅱ) complexes with carboxylates (**a~f**) have been synthesized and characterized by elemental analysis, conductivity, thermal analysis, IR, UV, and ^1H NMR techniques. The cytotoxicity of the complexes was tested by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay. The levels of total platinum bound to DNA were measured by ICP-MS. The results show that the complexes (**a~f**) have selectivity against tested carcinomas, have no cytotoxicity against HCT-8, BGC-823 and MCF-7, but have better cytotoxicity against EJ and HL-60, moreover, cytotoxicity of complexes (**d~f**) against HL-60 is similar to that of cisplatin. The levels of total platinum bound to DNA in HL-60 are increased with increasing concentrations, and the levels of total platinum bound to DNA increase in the following sequence: cisplatin < **c** < **b** < **a** < **f** < **e** < **d**.

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

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*通讯联系人。E-mail:jczhang6970@yahoo.com.cn
第一作者:张金超,男,37 岁,博士后,教授;研究方向:无机药物化学、细胞生物无机化学、天然产物的提取分离与生物活性研究。

Cisplatin has been one of the most successful antineoplastic drugs. However, due to its drawbacks such as cumulative toxicities and inherent or treatment-induced resistant tumor to cell sub-populations, great efforts have been devoted to develop new derivatives with improved pharmacological properties. Among the over 30 platinum agents entered clinical trails after the onset of clinical studies with cisplatin, only carboplatin has received worldwide approval so far, oxaliplatin, nedaplatin, lobaplatin and SKI2053R have gained regionally limited approval, and a few drugs continue to be evaluated in clinical studies. Therefore, further work is still worthwhile[1~6].

The mixed ammine/amine platinum complexes with chloro ions as leaving groups have been reported and demonstrated better activity against cisplatin-resistant cells in vitro and much less toxicity than the parent cisplatin. For example, JM-216 has recently entered phase III studies[7~10]. 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 of carboplatin reduces the nephrotoxic and neurotoxic side-effects of cisplatin, and increases the efficacy of the drug and helps to circumvent resistance mechanism[11]. So carboxylate platinum complexes may be quite promising than the corresponding chloro analogs. So far, few mixed ammine/amine platinum complexes with carboxylates as leaving groups have been reported. Previously we reported the synthesis, characterization and cytotoxicity of platinum(Ⅱ) complexes of mixed ammine/amine with carboxylates[12~14]. In the present work, the synthesis, cytotoxicity and DNA-binding levels of new mixed ammine/dimethylamine platinum(Ⅱ) complexes with carboxylates are reportd and discussed.

1 Experimental

1.1 Reagents

All reagents and solvents were analytical reagent grade.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and Genomic DNA extraction kit were purchased from Sigma (USA). RPMI 1640 culture medium and fetal bovine serum (FBS) were from Gibco Industries Inc. (USA). Cisplatin was purchased from Qilu Pharmaceutical Factory, China.

1.2 Instrumentation and measurement

Elemental analyses were performed on a EA-1110 elemental analyzer. Molar conductances at room temperature were measured in 1 mmol·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. The ^1H NMR spectra in D_2O were recorded on an Brucker AV 400 NMR spectrometer. The thermal analysis was carried out between room temperature and 800 ℃ using RIGAKU 8150 meter. The OD(optical density) at 570 nm was measured on a microplate spectrophotometer (Bio-rad Model 680, USA). The levels of total platinum bound to DNA were measured by inductively coupled plasma mass spectrometry (ICP-MS, PE Elan-5000).

1.3 Preparation of complexes

Precursor complexes *cis*-{Pt $[(CH_3)_2NH]_2I_2$} (**i**), $[Pt(\begin{smallmatrix}CH_3\\CH_3\end{smallmatrix}>NH)I_2]_2$ (**ii**) and *cis*-$[Pt(\begin{smallmatrix}CH_3\\CH_3\end{smallmatrix}>NH)(NH_3)I_2]$ (**iii**) were synthesized according to the literatures[7,14].

$[Pt(\begin{smallmatrix}CH_3\\CH_3\end{smallmatrix}>NH)(NH_3)(OOCCH_3)_2]$ (**a**): *cis*-$[Pt(\begin{smallmatrix}CH_3\\CH_3\end{smallmatrix}>NH)(NH_3)I_2]$ (0.511 g, 1 mmol) was mixed with $AgNO_3$ (0.336 g, 1.98 mmol) in 15 mL of water. The mixture was allowed to stir overnight in the dark. The AgI precipitate was removed. When all the silver ions have been removed, a slight excess of the sodium salt of acetic acid was added to the filtrate. After 7 h, the mixture was evaporated to dryness under reduced pressure and washed a few times with a minimum quantity of very cold water. The final product $[Pt(\begin{smallmatrix}CH_3\\CH_3\end{smallmatrix}>NH)(NH_3)(OOCCH_3)_2]$ was dried over P_2O_5 under vacuum. Yield: 60%.

The synthetic procedure for $[Pt(\begin{smallmatrix}CH_3\\CH_3\end{smallmatrix}>NH)(NH_3)(OOCCH_2Cl)_2]$ (**b**), $[Pt(\begin{smallmatrix}CH_3\\CH_3\end{smallmatrix}>NH)(NH_3)(OOCCHCl_2)_2]$ (**c**),

[Pt($\begin{matrix}CH_3\\CH_3\end{matrix}$>NH)($NH_3$)(OOC-$C_6H_5$)$_2$] (**d**), [Pt($\begin{matrix}CH_3\\CH_3\end{matrix}$>NH)($NH_3$)(*p*-$CH_3$-$C_6H_4$-COO)$_2$] (**e**), [Pt($\begin{matrix}CH_3\\CH_3\end{matrix}$>NH)($NH_3$)(*p*-$CH_3O$-$C_6H_4$-COO)$_2$] (**f**) followed in general the same procedure as for (**a**).

1.4 Cell culture

Five different human carcinomas: human bladder carcinoma (EJ), human colon carcinoma (HCT-8), human gastricarcinoma (BGC-823), human immature granulocyte leukemia (HL-60), human galactophore carcinoma (MCF-7) were cultured in RPMI-1640 medium supplemented with 10% FBS, 100 units·mL^{-1} of penicillin and 100 μg·mL^{-1} of streptomycin. Cells were maintained at 37 ℃ in a humidified atmosphere of 5% CO_2 in air.

1.5 Cytotoxicity analysis

The complexes were dissolved in PBS(phosphate buffered saline) and diluted to the required concentration with culture medium before use. The cells harvested from exponential phase were plated equivalently into a 96-well plate, complexes were then added to the wells to achieve final concentrations ranging from 10^{-7} to 10^{-4} mol·L^{-1}. Control wells were prepared by addition of the culture medium. Wells containing culture medium without cells were used as blanks. The plates were incubated at 37 ℃ in a 5% CO_2 incubator for 48 h. The MTT assay was performed as described by Mosmmann[15]. Upon completion of the incubation, stock MTT dye solution (20 μL, 5 mg·mL^{-1}) was added to each well. After 4 h incubation, 2-propanol (100 μL) was added to solubilize the MTT formazan. The OD (Optieal 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 the dose of compounds added.

1.6 DNA binding

The levels of total platinum bound to DNA in HL-60 cells were performed as described by Mellish et al.[16]. Briefly, approximately 5 × 10^7 HL-60 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 the DNA extraction kit procedure. The purity and concentration of DNA were 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 be statistical different.

2 Results and discussion

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 found values. Low molar

Table 1　Elemental analysis data of the precursor and new complexes

Complex	Elemental analysis found (calcd.) / %				Λ_m / (S·cm^2·mol^{-1})
	C	N	H	Pt	
(**i**)	8.93(8.91)	5.19(5.20)	2.62(2.62)		
(**ii**)	4.85(4.86)	2.84(2.84)	1.43(1.43)		
(**iii**)	4.71(4.70)	5.47(5.48)	1.97(1.97)		
a	19.22(19.20)	7.45(7.47)	4.31(4.30)	51.89(51.98)	8.78
b	16.19(16.22)	6.30(6.31)	3.20(3.18)	43.95(43.92)	8.02
c	14.19(14.04)	5.48(5.46)	2.41(2.36)	38.18(38.03)	9.35
d	38.51(38.48)	5.59(5.61)	4.08(4.04)	39.16(39.06)	9.46
e	41.01(40.98)	5.35(5.31)	4.49(4.59)	36.87(36.99)	10.01
f	38.71(38.64)	5.12(5.01)	4.41(4.32)	34.68(34.87)	10.32

conductances for the complexes correspond to non-electrolytes[17].

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 dimethylamine. New band appears at about 470 cm^{-1} and is assigned to Pt-N stretching. 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 $\nu_{(s,coo^-)}$ and a symmetric stretching $\nu_{(s,coo^-)}$, at about 1 650 and 1 380 cm^{-1}, respevtively. The values of $\Delta\nu_{(coo^-)}(\nu_{(as,coo^-)} - \nu_{(s,coo^-)})$ of the complexes (**a~f**) are in the range of 223~273 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[18]. This is further confirmed by the appearance of the peaks of ν_{Pt-O}.

Table 2 Main IR spectral data (cm^{-1}) of the precursor and new complexes

Complex	ν_{NH}	δ_{NH}	$\nu_{(as,coo^-)}$	$\nu_{(s,coo^-)}$	$\Delta\nu_{(coo^-)}$	ν_{Pt-O}	ν_{Pt-N}
(**i**)	3 260 3 189	1 582					470
(**ii**)	3 210 3 142	1 578					480
(**iii**)	3 245 3 190	1 580					470
a	3 219 3 125	1 548	1 626	1 403	223	574	486
b	3 261 3 143	1 550	1 648	1 405	243	618	480
c	3 232 3 155	1 502	1 654	1 390	264	589	470
d	3 219 3 145	1 509	1 627	1 360	267	608	465
e	3 219 3 109	1 524	1 655	1 385	270	590	480
f	3 228 3 111	1 585	1 676	1 403	273	603	480

2.3 Electronic spectra

As listed in Table 3, after formation of the complexes, no absorption peak appears for complex (**a**), one new absorption peak appears for complex (**b**) at 197.0 nm, the absorption peak is red shifted by ca. 5.0 nm for complex (**c**), E_2 band is blue shifted by ca. 6.0, 14.0, 27.0 nm, B band blue shifted by *ca.* 26.0, 28.0, 26.0 nm for the complex (**d**), (**e**) and (**f**) as compared with the free ligands, respectively.

Table 3 UV spectral data of the complexes

Complex	λ / nm		
	$n \rightarrow \pi^*$	E_2 band	B band
a	—	—	—
b	197.0		
c	223		
d		189.0	224.0
e		196.0	237.0
f		200.0	245.0

2.4 ^{1}H NMR

As listed in Table 4, 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 dimethylamine are coordinated with platinum through oxygen and nitrogen atoms.

Table 4 ^{1}H NMR data of the complexes

Compound	Chemical shift δ / ppm
a	2.79(s, 6H, $-CH_3$), 2.33(s, 6H, $-CH_3$)
b	2.75(s, 6H, $-CH_3$), 4.15(s, 4H, $-CH_2Cl$)
c	2.78(s, 6H, $-CH_3$), 6.37(s, 2H, $-CHCl_2$)
d	2.74(s, 6H, $-CH_3$), 7.74~8.35(m, 10H, $-C_6H_5$)
e	2.80 (s, 6H, $-CH_3$), 7.48~8.03 (m, 8H, $-C_6H_4-$), 2.40(s, 6H, $-CH_3$)
f	2.78 (s, 6H, $-CH_3$), 7.17~8.10 (m, 8H, $-C_6H_4-$), 3.98(s, 6H, $-OCH_3$)

2.5 Thermal stability of the complexes

As listed in Table 5, the thermal behaviour of the complexes (**a~f**) was similar, there is a big endothermic peak on the DTA curve at about 300 ℃, corresponding to 47.96%~66.47% weight loss, this suggests that the residue may be platinum.

Based on the above studies and relative literature[8], we propose a tentative coordination structure for

the complexes(Fig.1).

Table 5　Thermal analytical data of the complexes

Complex	Dec. temp. / ℃		Total wt. loss / %	Residue
	T_1	T_2		
a	150	730	47.96	Pt
b	160	728	55.10	Pt
c	155	726	60.23	Pt
d	152	749	58.36	Pt
e	160	745	62.10	Pt
f	165	750	66.47	Pt

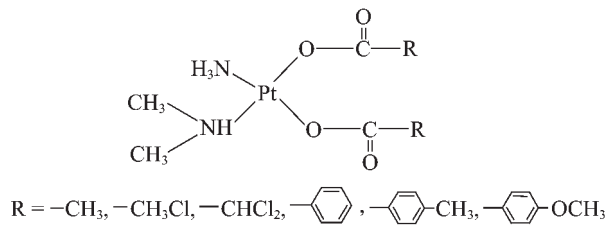
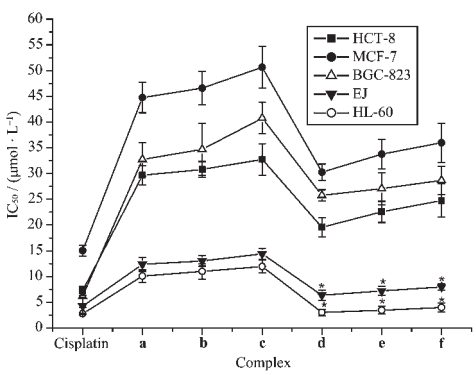


Fig.1　Possible structure of the complexes (**a~f**)

2.6　Cytotoxicity effect

As shown in Fig.2, the complexes (**a~f**) have selectivity against tested carcinomas, they have no cytotoxicity against HCT-8, BGC-823 and MCF-7, but have better cytotoxicity against EJ and HL-60, moreover, cytotoxicity of complexes (**d~f**) against HL-60 is similar to that of cisplatin.



*P > 0.05 compared with cisplatin, n=3

Fig.2　Cytotoxicity of the complexes against various cells

The mode of action of platinum anticancer drugs 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 proliferation and tumor growth. So the reactivity of leaving groups is an important factor to affect anticancer activity. When leaving groups are aromatic carboxylates, the complexes have better cytotoxicity, moreover, the substitution radical in benzene ring also influences cytotoxicity.

2.7　DNA binding

As shown in Fig.3, for the complexes (**a~f**), the levels of total platinum bound to DNA in HL-60 are increased with increasing concentrations, and the levels of total platinum bound to DNA increase in the following sequence: **c** < **b** < **a** < **f** < **e** < **d**. Moreover, their total DNA platination levels are higher than that of cisplatin under the same experimental conditions.

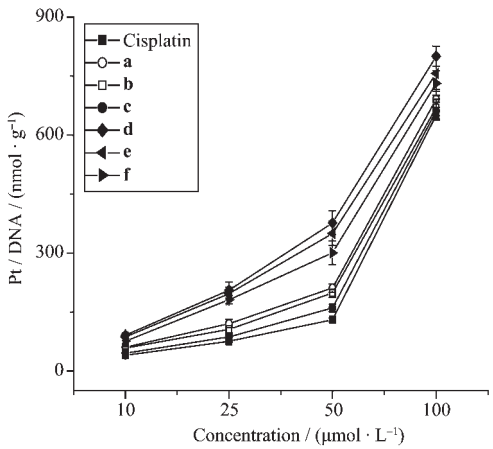


Fig.3　Levels of total platinum bound to DNA in HL-60 cells

It is accepted that DNA is a major target of platinum-based anticancer drugs. So far, it is controversial if the degree of cytotoxicity of platinum complexes correlates with the amount of DNA-platination. For cisplatin, in general the degree of cytotoxicity correlates with the amount of DNA-platination[19]. However, no association between cisplatin DNA-platination and drug sensitivity was found in a testicular cancer germ cell line and a breast cancer cell line[20,21]. With respect to a correlation between the cytotoxicity of oxaliplatin and DNA-platination, no correlation could be found between DNA-platination and cytotoxicity. Mellish et al.[16] reported that no significant correlation was also found between total DNA platination levels and cytotoxicity of the seven platinum based drugs in SKOV-3 or in CH1 cell lines. In the present work, we

found that there was significant correlation between total DNA platination levels and cytotoxicity for ammine/dimethylamine platinum (Ⅱ) complexes with carboxylates. Although the cytotoxicity of complexes (**a~f**) is weaker than that of cisplatin, their total DNA platination levels are higher than that of cisplatin under the same experimental conditions. So the results suggest that there may have no correlation between the total DNA platination levels and their cytotoxicity for different kinds of platinum complexes, but have significant correlation between the total DNA platination levels and their cytotoxicity for the same kind of platinum complexes. Because: (1) The total DNA platination levels contains some kinds of Pt-DNA adducts formed by platinum complex. This suggests that it is probably the level of specific DNA adducts that is important in determining the cytotoxicity of platinum based drugs[16]. (2) Although the mainstream of investigation has focused on DNA, platinum based drugs might have other important targets apart from nuclear DNA. It has been demonstrated that cisplatin have other non-DNA targets, it may react with phospholipids, inhibit amino acid transport, protein synthesis, ATPases, uncouple oxidative phosphorylation [22]. In summary, although the importance of these other targets in relation to cytotoxicity is unknown, these other targets may have important effect on cytotoxicity.

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