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烷氧基酸为离去基团的铂(Ⅱ)配合物的体外活性研究

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摘要:合成了 5 个以直链和带支链烷氧基乙酸为载体配基的顺铂类配合物,通过红外光谱、核磁共振氢谱和质谱对配合物进行了表征,并测试了化合物对肺腺癌 SPC-A1 和胃腺癌 BGC823 的体外抗肿瘤活性。生物活性测试结果表明,配合物的活性与离去基团有很大关系。配合物 **4**(顺-二(异丙氧基乙酸根)·[(1*R*,2*R*)-1, 2-反式环己二胺]合铂(Ⅱ))在 2 个细胞系中均显示最高的体外抗肿瘤活性,甚至超过顺铂。

关键词:铂(Ⅱ)配合物;抗肿瘤活性;烷氧基酸根
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In vitro Cytotoxicity Study on Platinum(Ⅱ) Complexes with Alkoxycarboxylates as Leaving Groups

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Abstract: Five cisplatin-typed platinum complexes, characteristic of linear and branched alkoxycarboxylates as leaving groups, were synthesized and characterized by infrared, electro-spray ionization MS and ^{1}H NMR spectroscopy. In vitro cytotoxicities of these complexes against SPC-A1 human lung adenocarcinoma and BGC823 human gastric adenocarcinoma cell lines were evaluated. Biological tests reveal that the cytotoxicities of these compounds are highly related to the nature of leaving groups. Complex **4**, *cis*-(*trans*-1*R*, 2*R*-diaminocyclohexane) bis (2-isopropoxyacetate) platinum (Ⅱ), shows the highest cytotoxicity against both cell lines, and its cytotoxicity is higher than that of cisplatin.

Key words: Pt(Ⅱ) complexes; anti-tumor activity; alkoxycarboxylates

Cisplatin is the representative of the classical Pt complexes, widely applied as antitumor drugs at present[1~7], however, the usefulness is also limited by its serious toxicity[8~10], narrow spectrum of activity, both inherent and acquired resistance, and low aqueous solubility[11~19]. Much effort has been made to overcome these limitations on the grounds of its structure. Generally, there are two categories. One is to replace its carrier ligand with enantiomeric amines so as to produce specific diastereoisomeric intera-ctions between the platinum-AA′ moiety and chiral DNA[20~24]. The work of Yang et al. shows that platinum complexes with diamines of *R* or *RR* absolute configurations are usually more active than those with

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the corresponding diamines of *S*, *SS* or *RS* configurations[25]. A successful example is clinically approved oxaliplatin which shows no cross-resistance in some cisplatin-resistant cell lines. The lack of cross-resistance is attributed to the *trans*-1*R*, 2*R*-diaminocyclohexane(DACH) carrier ligand[4]. The other way is to substitute the chloride anions of cisplatin by appropriate leaving groups with well-balanced solubility in both water and liposome, since the substitution is greatly helpful to transport drugs into target cells and reduce drug-related toxicities[26~31]. In our previous work, chloride anions were substituted into diverse carboxylic acids as carboxylato ligands to promote the aqueous solubility of the related platinum complexes. The results indicated that most of them showed good *in vitro* cytotoxicities against the selected cell lines in addition to appropriate solubility[32~34].

More often, both strategies are involved simultaneously. In this study, the carrier ligand is selected and fixed as *trans*-1*R*, 2*R*-diaminocyclohexane since it has been demonstrated to be an excellent carrier ligand. The leaving groups were evolved into alkoxyacetates with branched alkoxy groups to distinguish from those with linear alkoxy groups. Platinum complexes with linear alkoxyacetates as leaving groups exhibit not only reasonable aqueous solubility and liposolubility but also high *in vitro* cytotoxicities against selected cell lines[33]. Upon further modulating the activity and toxicity via the structural variety of alkoxyacetates, we have designed and synthesized alkoxyacetates with branched aliphatic chains with expectation of higher antitumor activities and lower toxicity (**4** and **5** in Fig.1). As a developing effort, the new designed cisplatin analogues together with previous linear ones(**1~3**) were evaluated for their in vitro cytotoxicity toward SPC-A1 human lung adenocarcinoma and BGC823 human gastric adenocarcinoma cell lines.

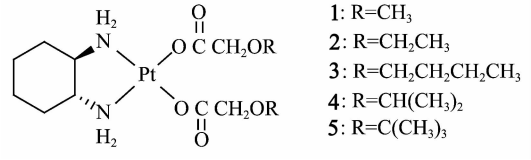

Fig.1　Structures of platinum(Ⅱ) complexes **1~5**

1 Experimental

1.1 Instruments

All complexes **1~5** were characterized by IR(Bruker Vector 22 spectrophotometer), ^{1}H NMR (Bruker DRX500 spectroscopy) and ESI-MS(Finnigan MAT SSQ 710) spectromery[35]. All reagents and solvents were analytical reagent grade. Cisplatin and *trans*-1*R*, 2*R*-diaminocyclohexane both were purchased from Alfa Aesar.

1.2 Preparation of complexes

Platinum complexes **1~5** containing alkoxy carboxylic anions(CH_3OCH_2COOH(Ⅰ), $CH_3CH_2OCH_2COOH$(Ⅱ), $CH_3CH_2CH_2CH_2OCH_2COOH$(Ⅲ), $CH_3)_2CHOCH_2COOH$(Ⅳ),$(CH_3)_3COCH_2COONa$(**Ⅴ**)) were prepared by the pre-viously reported methods[33].

1.3 *In vitro* cytotoxicity study

The *in vitro* cytotoxicities of compounds **1~5** toward SPC-A1 human lung adenocarcinoma and BGC823 human gastric adenocarcinoma cell lines were determined by Propidium Iodide-flow cytometry (PI-FCM) assay[36,37], performed by the School of Medicine, Nanjing University. The cytotoxicities of these compounds were compared with those of cisplatin. In this study, the tumor cells were continuously exposed to the tested compounds **1~5** and cisplatin for 24 h at four different concentrations: 50, 25, 10 and 1 g·mL^{-1}, respectively. Consequently, the cell apoptosis was investigated by using flow cytometry (FCM) with PI (abbreviation of Propidium Iodide) staining. The percentages of tumor cell death were analyzed by Winmdi software afterwards. The duplicate tests were performed.

2 Results and discussion

2.1 IR

The main IR data of the complexes(**1~5**) are listed in Table 1. The bands of ν_{NH_2} and δ_{NH_2} shift to lower frequencies comparing with the free amino group, demonstrating that they are coordinated with platinum through nitrogen atoms. This is further confirmed by the appearance of the bands of ν_{Pt-N}. The C=O absorption shifts from a free carboxylic acids near 1 730 cm^{-1}

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

Complex	ν_{OH}	ν_{NH_2}	δ_{NH_2}	ν_{CH_2}	$\nu_{C=O}$	ν_{Pt-O}	ν_{Pt-N}
Ⅰ					1 735		
Ⅱ					1 735		
Ⅲ					1 734		
Ⅳ					1 733		
Ⅴ					1 601		
1	3 449	3 218	1 633	2 937	1 633	615	441
2	3 442	3 199	1 620	2 936	1 620	609	440
3	3 449	3 199	1 614	2 957	1 614	625	440
4	3 443	3 239	1 631	2 934	1 631	610	440
5	3 434	3 244	1 629	2 934	1 629	615	441

to a band near 1 662~1 614 cm^{-1}. The peak of ν_{Pt-O} appears at about 440 cm^{-1}. Thus it proves that the carboxylate anion is combined with the metal atom in each case.

2.2 ^{1}H NMR

As shown in Table 2, the molecular structures of all compounds are also confirmed by their related ^{1}H NMR spectral data.

Table 2 ^{1}H NMR data of the complexes(ppm)

Complex	Carrier ligand		Leaving group			
	$4CH_2$ of DACH	2CH of DACH	$COCH_2O$	CH	CH_2	CH_3
Ⅰ			4.01(s,2H)			3.30(s,3H)
Ⅱ			4.05(s,2H)		3.48~3.53(m,2H)	1.07~1.10(m,3H)
Ⅲ			4.05(s,2H)		3.46~3.49(m, 2H)	0.77~.80(m, 3H)
					1.44~1.49(m, 2H)	
					1.20~1.27(m, 2H)	
Ⅳ			4.10(s, 2H)	3.67~3.71(m, 1H)		1.18~1.20(d, 6H)
Ⅴ			3.72(s, 2H)			1.08(s, 9H)
1	1.08(m, 2H)	3.42(m,2H)	3.66~3.78(m, 4H)			3.12(m, 6H)
	1.08(m, 2H)					
	1.82(m, 2H)					
	2.16(m, 2H)					
2	1.25(m, 2H)	3.40~3.90(m,2H)	3.40~3.90(m, 4H)		3.40~3.9(m, 4H)	1.06(m, 6H)
	1.60(m, 2H)					
	1.96(m, 2H)					
	2.35(m, 2H)					
3	1.11(m, 2H)	2.33(m, 2H)	3.84~3.97(m, 4H)		3.45(m, 8H)	0.84(m, 6H)
	1.29(m, 6H)					
	1.49(m, 6H)					
	1.99(m, 2H)					
4	1.04(m, 2H)	2.34(m, 2H)	3.51(s, 4H)	3.59(m, 2H)		1.04(m, 12H)
	1.33(m, 2H)					
	1.50(m, 2H)					
	1.92(m, 2H)					
5	1.04(m, 2H)	2.35(m, 2H)	3.47(s, 2H)			1.10(d, 18H)
	1.32(m, 2H)		3.79(s, 2H)			
	1.50(m, 2H)					
	1.92(m, 2H)					

2.3 ESI-MS

As listed in Table 3, complex **1** has a peak of $[M+Na]^+$, complexes **2**, **3** give $[M-X+H_2O]^+$ peaks. Both complexes **4** and **5** are found with three peaks, i.e.$[M+H]^+$, $[M+Na]^+$, $[M-X+CH_3OH]^+$.

Table 3　ESI-MS data of the complexes (Da with relative abumdance, %)

Complex	$[M+H]^+$	$[M+Na]^+$	$[M-X+CH_3OH]^+$	$[M-X+H_2O]^+$
1	—	510(100%)	—	—
2	—	—	—	430(100%)
3	—	—	—	458(100%)
4	544(36%)	566(100%)	458(34%)	—
5	572(60%)	594(88%)	472(100%)	—

2.4 Anti-tumor cytotoxicity

The results are summarized in Fig.2 and 3, respectively. As depicted in Fig.2 and 3, the order of the cytotoxicities in SPC-A1 is **4**>**5**, cisplatin>**3**>**2**>**1**, and in BGC823 is cisplatin>**3**, **4**, **5**>**2**>**1**.

The above biological results suggest that SPC-A1 cell is more active to those platinum analogues treatment. The majority of compounds **1** to **5** show cytotoxicity against both SPC-A1 and BGC823. The cytotoxicity of compounds **3**~**5** against both cell lines is comparable to that of cisplatin. In particular, compound **4** displays the highest cytotoxicity against SPC-A1 cell line, much better than cisplatin at a higher concentration.

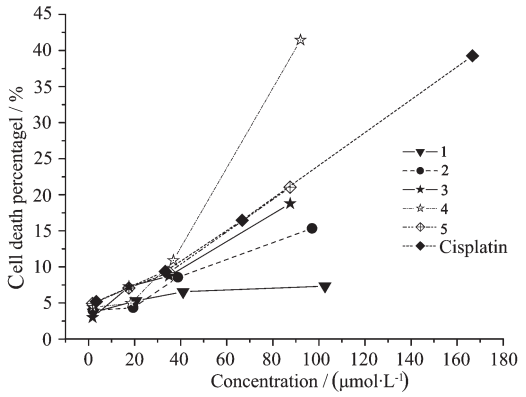

Fig.2　Prcentages of cell death of SPC-A1 human lung adenocarcinoma cell after being treated with those complexes of four different concentrations

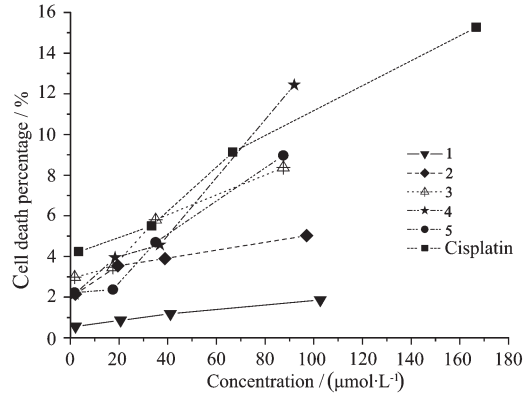

Fig.3　Prcentages of cell death of BGC823 human gastric adenocarcinoma cell after being treated with those complexes of four different concentrations

Since the tested platinum complexes possess the same carrier ligand but exhibit totally different percentages of cell apoptosis, the structure of the leaving group is much more significant to cytotoxic activity. As for linear aliphatic chains, the order of cytotoxicities in both SPC-A1 and BGC823 is **3**>**2**>**1**. This means that platinum complexes with long aliphatic chains in the alkoxy carboxylic moiety are more active against both cell lines than those with short ones. However, the order is the opposite when towards Ramos lymphoma, 3AO ovarian carcinoma and A549 lung cancer carcinoma cell lines, that is **2** >**3** [33]. As the aliphatic chain of alkoxy moieties changing from liner to brangched, the cytotoxicity of platinum complex with 2-isopropoxyacetate is enhanced significantly, but the improvement for the complex with 2-*tert*-butoxyacetate is not much. As seen from Fig.2, the cytotoxicity of **4** is better than that of **5**, and the latter is equal to cytotoxicity of cisplatin. Accordingly, the branched aliphatic chain may be helpful to reach higher cytotoxicity due to better liposolubility, but this is not always true. The cytotoxicity will go down if the aliphatic chain is extremely branched and consequently reduces its aqueous solubility(**4**>**5**).

In conclusion, most synthetic compounds exhibit

good cytotoxic activity against both SPC-A1 and BGC823 cell lines. Complex **4**, *cis*-(*trans*-1*R*, 2*R*-diaminocyclohexane)bis (2-isopropoxyacetate)platinum (Ⅱ) , displays the greatest potency toward both cell lines. With not only high *in vitro* cytotoxicity but also well-balanced solubility in both water and liposome, it is much promising to be further studied.

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References:

[1] Carter S K. In: Hacker M P, Douple E B, Krakoff IH (eds). *Platinum Coordination Complexes in Cancer Chemotherapy*. Boston: Martinus Nijhoff, **1984**.359~376

[2] Durant J R. In: Prestayko A W, Crooke ST, Karter SK(eds). *Cisplatin, Current Status and New Developments*. New York: Academic Press, **1980**.317

[3] Trimmer E E, Essigmann J M. *Essays. Biochem*., **1999**,**34**: 191~211

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

[5] Guo Z J, Sadler P J. *Angew. Chem. Int. Ed*., **1999**,**38**:1512 ~1531

[6] Lebwohl D, Canetta R. *Eur. J. Cancer*., **1998**,**34**:1522~1534

[7] Jamieson E R, Lippard S J. *Chem. Rev*., **1999**,**99**:2467~2498

[8] Krakoff IH. *Cancer. Treat. Rep*., **1979**,**63**:1523~1525

[9] Von Hoff D D, Schilsky R, Reichert C M, et al. *Cancer. Treat. Rep*., **1979**,**63**:1527~1531

[10]Loehrer P J Sr, Williams S D, Einhorn L H. *J. Natl. Cancer. Inst*., **1988**,**80**:1373~1382

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

[12]Pasini A, Zunino F. *Angew. Chem*., **1987**,**99**:632~641

[13]Kelland L R, Sharp S Y, O′Neill C F et al. *J. Inorg. Biochem*., **1999**,**77**:111~115

[14]Bitha P, Carvajal S G, Citarella R V. et al. *J. Med. Chem*., **1989**,**32**:2063~2067

[15]Ho Y P, To K K W, Au-Yeung S C F, et al. *J. Med. Chem*., **2001**,**44**:2065~2068

[16]Lee Y A, Chung Y K, Sohn Y S. *Inorg. Chem*., **1999**,**38**:531 ~537

[17]Lee Y A, Chung Y K, Sohn Y S. *J. Inorg. Biochem*., **1997**,**68**: 289~294

[18]Kim Y S, Song R, Kim D H, et al. *Bioorg. Med. Chem*., **2003**, **11**:1753~1760

[19]Song R, Kim Y S, Lee C O, et al. *Tetrahedron. Lett*., **2003**,**44**: 1537~1540

[20]WANG Lian-Hong(王联红), LIU Yun(刘 芸), GOU Shao-Hua(苟少华), et al. *Chemistry*(*Huaxue Tongbao*), **2003**,**66**(12): 828~836

[21]WANG Lian-Hong(王联红), LIU Yun(刘 芸), GOU Shao-Hua(苟少华), et al. *Chinese J. Inorg. Chem.*(*Wuji Huaxue Xuebao*), **2004**,**20**(7):775~780

[22]Nowatari H, Kuroda Y, Hayami H, et al. *Chem. Pharm. Bull*., **1989**,**37**:2406~2409

[23]Morikawa K, Honda M, Endoh K, et al. *J. Pharm. Sci*., **1991**, **80**:837~842

[24]Noji M, Okamoto K, Tashiro T, et al. *J. Med. Chem*., **1981**,**24**: 508~515

[25]Yang M, Hu Q, Zhang L, et al. *Afr. J. Chem*., **1997**,**50**:227~231

[26]Calvert A H, Harland S J, Newell D R, et al. *Cancer Chemother. Pharmacol*., **1982**,**9**:140~147

[27]Foster B J, Clagett-Carr K, Leyland-Jones B, et al. *Cancer. Treat. Rev*., **1985**,**12**:43~49

[28]Carter S K, Canetta R, Rozencweig M. *Cancer Treat. Rev*., **1985**,**12**:145~152

[29]Canetta R, Rozencweig M, Carter S K. *Cancer Treat. Rev*., **1985**,**12**:125~136

[30]MacLean D S, Khokhar A R, Perez-Soler R. *Neutron. Cancer Biother. Radiopharm*., **2000**,**15**:253~259

[31]Kishimoto S, Miyazawa K, Terakawa Y, et al. *Jpn. J. Cancer Res*., **2000**,**91**:1326~1332

[32]Wang L H, Gou S H, Chen Y J, et al. *Bioorg. Med. Chem. Lett*., **2005**,**15**:3417~3422

[33]Cui K, Wang L H, Gou S H, et al. *Bioorg. Med. Chem. Lett*., **2006**,**16**:2937~2942

[34]Liu X, Shen H, Gou S H, et al. *Bioorg. Med. Chem. Lett*., **2007**,**17**:3831~3834

[35]XIN Zhao-Ping(辛召平), XU Cai-Fu(许才绂), HUANG Yu-Xin(黄裕新), et al. *World Chin. J. Digestol.*(*Huaren Xiaohua Zazhi*), **1998**,**6**(10):844~846

[36]HE Yue-Ming(何跃明), LÜ Xin-Sheng(吕新生), AI Zhong-Li (艾中立), et al. *Chin. J. Gen. Surg.*(*Zhongguo Putong Waike Zazhi*), **2004**,**13**(3):179~184