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芳香亚胺与 *N*-(4-甲基苯甲酰)-*L*-缬氨酸双阴离子合钯(Ⅱ)配合物的合成、晶体结构及体外抗肿瘤活性

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摘要: 本文首次报道了 2 个钯(Ⅱ)的配合物[Pd(bipy)(4-CH_3Bzval-N,O)] (**1**)和[Pd(phen)(4-CH_3Bzval-N,O)] (**2**)(bipy=2,2′-联吡啶,phen=1,10-菲咯啉,4-CH_3Bzval-N,O=*N*-(4-甲基苯甲酰)-*L*-缬氨酸双阴离子)的合成及晶体结构,利用 MTT 法和 SRB 法研究了配合物的体外抗肿瘤活性。配合物 **2** 属单斜晶系 $P2_1/n$ 空间群,其中 a=1.162 92(8) nm,b=1.074 03(7) nm,c=1.821 14(12) nm,V=2.232 8(3) nm^3,Z=4。结果显示:2 个配合物对 HL-60, BGC-823,Bel-7402 和 KB 4 种人的肿瘤细胞表现出一定的活性和选择性,但其活性均小于顺铂。

关键词: *N*-酰化-*L*-缬氨酸双阴离子; 钯(Ⅱ)配合物; 单晶结构; 抗肿瘤活性

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Synthesis, Crystal Structure and Cytotoxicity of Palladium(Ⅱ) Complexes with *N*-(4-methylbenzoyl)-*L*-valine Dianion and Aromatic Diimine

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Abstract: Two novel palladium(Ⅱ) complexes, [Pd(bipy)(4-CH_3Bzval-N,O)] (**1**) and [Pd(phen)(4-CH_3Bzval-N,O)] (**2**) (bipy =2,2′ -bipyridine, phen =1,10-phenanthroline, 4-CH_3Bzval-N,O =*N*-(4-methylbenzoyl)-*L*-valine dianion)have been prepared and structurally characterized, the cytotoxicity in vitro has also been investigated by MTT and SRB assays. The complex **2** crystallizes in the hexagonal system, space group $P2_1/n$ with cell parameters a=1.162 92(8) nm, b=1.074 03(7) nm, c=1.821 14(12)nm, V=2.232 8(3) nm^3 and Z=4. The complexes (**1** and **2**) presented cytotoxic effects and selectivity, but were less active than cisplatin against HL-60, BGC-823, Bel-7402 and KB cell lines. CCDC: 761046.

Key words: *N*-acylated-*L*-valine dianion; Pd(Ⅱ) complexe; crystal structure; cytotoxicity

0 Introduction

Now cisplatin and its analogues are some of the most effective chemotherapeutic agents in clinical use as the first line of treatment in testicular and ovarian cancers[1]. Unfortunately, they have several major drawbacks. Common problems include cumulative toxicities, the serious side effects and inherent or

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treatment-induced resistant tumor cells[2]. These drawbacks have provided the motivation for alternative chemotherapeutic strategies.

Metals, in particularly, transition metals offer potential advantages over the more common organic-based drugs. The notable analogy between the coordination chemistry of platinum(Ⅱ) and palladium(Ⅱ) complexes has advocated studies of Pd(Ⅱ) complexes as antitumor drugs. The hydrolysis of the leaving ligands in palladium complexes is too rapid. They dissociate readily in solution leading to very reactive species that are unable to reach their pharmacological targets. This implies that if an antitumor palladium drug is to be developed, it must somehow be stabilized by a strongly coordinated nitrogen ligand and a suitable leaving group. Amino acid, bipyridine and phen or their derivatives have been widely used to synthesize palladium anticancer complexes because amino acid ligands do not dissociate easily in aqueous solution and bipyridine or phen has the ability to participate as DNA intercalators[3-4]. Some mixed-ligand palladium(Ⅱ) complexes of 2,2′-bipyridine and amino acids have been synthesized[5]. These complexes have also shown growth inhibition against L1210 lymphoid leukemic, P388 lymphocytic leukemic, Sarcoma 180, and Ehrlich ascitic tumor cells. Mital et al. reported the synthesis and cytotoxicity of nine paIladium(Ⅱ) complexes of type $[Pd(phen)(AA)]^+$ (where AA is an anion of glycine, *L*-alanine, *L*-leucine, *L*-phenylalanine, *L*-tyrosine, *L*-tryptophan, *L*-valine, *L*-proline, or *L*-serine). They are found to exhibit growth inhibition of P388 lymphocytic leukemic cells[6]. We previously reported the synthesis and cytotoxicity of a novel palladium (Ⅱ) complex $[Pd(Phen)(TsserNO)]\cdot H_2O$ (Phen =1,10-phenanthroline; TsserNO = 4-toluenesulfonyl-*L*-serinate dianion), its cytotoxicity is equal to that of cisplatin against BGC-823 and Bel-7402 cells lines, however it is less potent than cisplatin against HL-60 and KB cell lines[7]. Until now, the cytotoxicity of mixed-ligand palladium (Ⅱ) complexes with *N*-acylated-*L*-amino acid dianion and aromatic diimine has not been reported. In the present work, we present the synthesis, characterization and cytotoxicity of two novel mixed-ligand palladium (Ⅱ) complexes with *N*-(4-methylbenzoyl)-*L*-valine dianion and aromatic diimine for the first time.

1 Experimental

1.1 Materials and instruments

4-Methylbenzoyl chloride, $K_2[PdCl_4]$ and all reagents were of chemical grade, 1,10-phenanthroline (phen) and *L*-valine were of analytical grade. RPMI-1640 medium, trypsin and fetal bovine serum were purchased from Gibco. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), SRB (sulforhodamine B), benzylpenicillin and streptomycin were from Sigma. Four different human carcinoma cell lines: HL-60 (immature granulocyte leukemia), Bel-7402 (liver carcinoma), BGC-823 (gastrocarcinoma) and KB (nasopharyngeal carcinoma) were obtained from American Type Culture Collection.

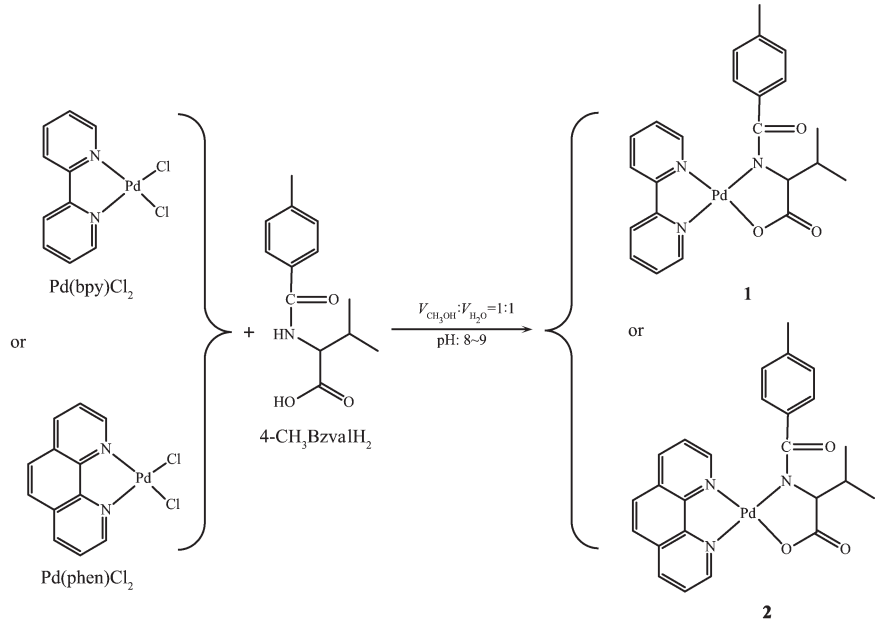
Elemental analysis were determined on a Elementar Vario EL Ⅲ elemental analyzer. IR spectra were recorded in the solid state (KBr pellets) in the range 4 000~400 cm^{-1} using a Perkin-Elmer Model-683 spectrophotometer. The ^1H NMR spectra were measured on a Bruker AV Ⅲ 600 NMR spectrometer in dimethyl sulfoxide-d_6 with solvent peaks as references. X-ray single crystal structure was performed on a Bruker SMART APEX Ⅱ CCD diffractometer. The optical density (OD) at 570 nm was measured on a microplate spectrophotometer (Bio-Rad Model 680, USA).

1.2 Preparation of complexes

N-(4-methylbenzoyl)-*L*-valine (4-CH_3BzvalH$_2$), [Pd(bipy)Cl_2] and [Pd(phen)Cl_2] were synthesized by the reported procedures[8-9].

The complex **1** was prepared as follows: [Pd(bipy)Cl_2] (15 mg, 0.045 mmol) was added to a 3 mL CH_3OH/H_2O (volume 1∶1) solution of 4-CH_3BzvalH$_2$ (21 mg, 0.090 mmol) when the solution temperature was heated to 48 ℃, the mixture was adjusted to pH=8~9 by NaOH solution, then stirred for 2 h. The solution was heated in vacuo and concentrated it to about 80% of the original volume.

The complex **1** was separated from the solution after a few days, but the crystal suitable for X-ray diffraction was not obtained (Scheme 1).



Scheme 1　Synthetic routines of the complexe **1** and **2**

Elemental analysis calc. for $C_{23}H_{23}N_3O_3Pd$ (%): C, 55.71; H, 4.68; N, 8.47. Found (%): C, 55.66; H, 4.57; N, 8.51. IR (KBr, cm^{-1}): 1 547; 1 635; 1 388; 547; 466.

^1H NMR (600 MHz, DMSO-d_6, ppm) δ: 8.58~8.49 (m, 2H), 8.42~8.32 (m, 2H), 8.18 (d, J=7.9 Hz, 2H), 8.11~8.03 (m, 1H), 7.88~7.80 (m, 1H), 7.55 (d, J=5.4 Hz, 1H), 7.22~7.15 (m, 1H), 6.86 (d, J=7.9 Hz, 2H), 4.57 (d, J=6.4 Hz, 1H), 2.43~2.36 (m, 1H), 2.05 (s, 3H), 1.25 (d, J=6.7 Hz, 2H), 1.18 (d, J=6.7 Hz, 2H).

The synthesis of the complex **2** was carried out in an identical manner to the complex **1** starting from [Pd(phen)Cl_2] (20.00 mg, 0.06 mmol) and 4-CH_3BzvalH$_2$ (34.00 mg, 0.12 mmol) (Scheme 1). By evaporating the filtered solutions at room temperature, the yellow crystal suitable for X-ray diffraction was obtained after a few days.

Elemental analysis calc. for $C_{25}H_{23}N_3O_3Pd$(%): C, 57.76; H, 4.46; N, 8.08. Found(%): C, 57.56; H, 4.37; N, 8.12. IR (KBr, cm^{-1}): 1 542; 1 634; 1 392; 556; 460.

^1H NMR (600 MHz, DMSO-d_6, ppm) δ: 8.97 (d, J= 8.1 Hz, 1H), 8.87 (d, J=5.7 Hz, 1H), 8.69 (d, J=8.3 Hz, 1H), 8.27~8.22 (m, 2H), 8.18 (d, J=8.8 Hz, 1H), 8.16~8.12 (m, 1H), 7.79 (d, J=5.0 Hz, 1H), 7.55~7.50 (m, 1H), 7.26 (d, J=7.8 Hz, 1H), 6.80 (d, J=7.4 Hz, 2H), 4.62 (d, J=6.0 Hz, 1H), 2.40~2.36 (m, 1H), 2.00 (s, 3H), 1.28 (d, J=6.7 Hz, 2H), 1.21 (d, J=6.6 Hz, 2H).

1.3　Crystal structure determination

The single crystal of the complex with approximate dimensions of 0.45 mm ×0.33 mm ×0.33 mm was selected for X-ray diffraction analysis. Data collection was performed on a Bruker SMART APEX Ⅱ CCD diffractometer equipped with a graphite monochromatized Mo $K\alpha$ radiation (λ=0.071 073 nm) at 296(2) K. A total of 11 067 reflections were collected in the range of $1.92° \leqslant \theta \leqslant 28.22°$ for the complex, of which 3 939 (R_{int}=0.013 3) reflections were unique, and reflections were considered as observed ($I>2\sigma(I)$). The maximum and minimum transmission factors are 0.763 8 and 0.695 3, respectively. Multi-scan absorption corrections were applied using the SADABS program. The structure was solved by the direct method using the SHELXS-97 program. Refinements on F^2 were performed using SHELXL-97 by the full-matrix least-squares method with anisotropic thermal parameters for all non-hydrogen atoms. The hydrogen atoms of the ligand were generated geometrically, while the H atoms of the coordination water molecules were located from difference Fourier synthesis and refined with restraint parameters. A summary of crystallographic data and refinement parameters is given in Table 1.

CCDC: 761046.

Table 1 Crystallographic data for complex 2

Empirical formula	$C_{25}H_{23}N_3O_3Pd$	V / nm^3	2.232 8(3)
Formula weight	519.86	Z	4
Temperature / K	296(2)	Crystal size / mm	0.45×0.33×0.33
Color	Yellow	F(000)	1 056
Crystal system	Monoclinic	D_c / (g·cm^{-3})	1.547
Space group	$P2_1/n$	Absorption coefficient / mm^{-1}	0.863
θ range for data collection / (°)	1.92~28.22	Reflections collected	11 067
a / nm	1.16292(8)	Independent reflections (R_{int})	3 939 (0.013 3)
b / nm	1.074 03(7)	Goodness-of-fit on F^2	1.059
c / nm	1.821 14(12)	Final R indices ($I>2\sigma(I)$)	R_1=0.019 3, wR_2=0.051 3
β / (°)	101.007 0(10)	R indices (all data)	R_1=0.021 6, wR_2=0.052 3

1.4 Cell culture

Four human carcinoma cell lines were used for cytotoxicity determination: HL-60, Bel-7402, BGC-823 and KB. They were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 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 cells harvested from exponential phase were seeded equivalently into a 96-well plate, complexes were then added to the wells to achieve final concentrations. Control wells were prepared by addition of 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 Mosmann[10]. Upon completion of the incubation, stock MTT dye solution (20 mL, 5 mg·mL^{-1}) was added to each well. After 4 h incubation, 2-propanol (100 mL) was added to solubilize the MTT formazan. The OD of each well was then measured on a microplate spectrophotometer at a wavelength of 570 nm. The SRB assay was performed as previously described[11]. Upon completion of the incubation, the cells were fixed in 10% trichloroacetic acid (100 mL) for 30 min at 4 ℃, washed five times in tap water and stained with 0.1% SRB in 1% acetic acid (100 mL) for 15 min. The cells were washed four times in 1% acetic acid and air-dried. The stain was solubilized in 10 mmol·L^{-1} unbuffered Tris base (100 mL) and OD was measured at 540 nm as above. The IC_{50} value was determined from plot of % viability against dose of compounds added.

2 Results and discussion

2.1 Chemical characterization

The elemental analysis data of the complexes **1** and **2** are in good agreement with the calculated values. This provides support for the suggested composition of the complexes.

In IR spectra, the amide group of 4-CH_3BzvalH_2 has a sharp and strong ν_{NH} in 3 313 cm^{-1} region. This peak disappears for both complexes, indicating that the amide group has been deprotonated. The amide group deprotonated and coordinating to metal ion is also indicated by the amide (Ⅰ) shifting from ~1 611 to ~1 543 cm^{-1} and the disappearance of the amide(Ⅱ) from ~1 546 cm^{-1} region. The carboxylate group of the complexes **1** and **2** shows two bands, an intense antisymmetric carboxylate stretching ν_{as,COO^-} and a symmetric carboxylate stretching ν_{s,COO^-}, at about 1 630 and 1 385 cm^{-1}, respectively. The values of $\Delta\nu_{COO^-}$($\nu_{as,COO^-} - \nu_{as,COO^-}$) of the complexes are in the range 240~250 cm^{-1}, which is greater than $\Delta\nu_{COO^-}$ of the corresponding sodium carboxylates, so the carboxylate group may be monodentate coordinated through oxygen atoms. This is further confirmed by the appearance of the peaks of $\nu_{Pd\text{-}O}$.

4-CH_3BzvalH_2 show a doublet at δ=6.63, which is associated with the proton of the amide group, but these peaks disappear for the complexes, which showing that the amide group has been deprotonated. The methylene

^{1}H resonances (*L*-valine) shifted to the down field as a result of deprotonated amide nitrogen coordinating to Pd(Ⅱ). The β-hydrogen of 4-CH_3BzvalH$_2$ appeared as a dd quartet, but in the complexes this proton appeared as a doublet, which also confirmed the deprotonation of amide group.

2.2 Crystal structure

Complex **2** crystallizes in the monoclinic system and space group $P2_1/n$. A diagram of the crystal structure of complex **2** is presented in Fig.1. the Pd^{2+} ion is coordinated by two nitrogen atoms of phen, one deprotonated amide nitrogen and one carboxylic oxygen. The deprotonated ligand 4-CH_3BzvalH$_2$ acts as a bidentate ligands combining with Pd^{2+} ion through one carboxyl oxygen atom and one deprotonated amide nitrogen atom, which leads to a five member chelating cycle. The angle between planar N(2)-Pd(1)-N(3) and planar O(1)-Pd(1)-N(1) is 8.459(51)° which indicates that the Pd(1)-O(1)-N(1)-N(2)-N(3) plane is slightly distorted. The Pd-N (deprotonated amide) bond length (0.199 35(15) nm) is similar to the Pd-N (phen) bond lengths (0.200 48(16) and 0.202 01(15) nm), while it is longer than Pd-O (carboxylic oxygen) bond length (0.198 14(13) nm) (Table 2).

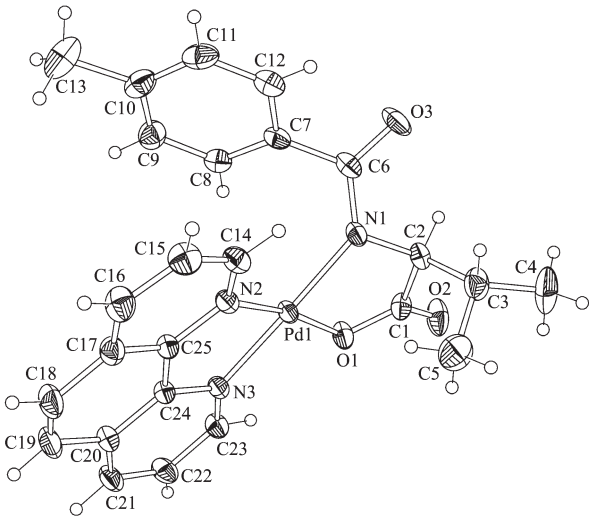


Fig.1 Molecular structure of complex **2**, showing displacement ellipsoids at 30% probability level and the atom numbering scheme

Table 2 Selected bond lengths (nm) and angles (°) for complex 2

Pd(1)-O(1)	0.198 14(13)	Pd(1)-N(1)	0.199 35(15)	Pd(1)-N(2)	0.200 48(16)
Pd(1)-N(3)	0.202 01(15)				
O(1)-Pd(1)-N(1)	81.80(6)	O(1)-Pd(1)-N(3)	93.80(6)	O(1)-Pd(1)-N(2)	171.46(5)
N(2)-Pd(1)-N(3)	81.02(6)	N(2)-Pd(1)-N(1)	103.87(6)	N(1)-Pd(1)-N(3)	173.69(6)

2.3 Cytotoxic studies

As listed in Table 3, The complexes **1** and **2** exerted cytotoxic effects against HL-60, BGC-823, Bel-7402 and KB cell lines with a lower IC_{50} value (<50 μmol·L^{-1}), but they were less active than cisplatin. Complex **1** displayed the better cytotoxicity than complex **2** against the tested carcinoma cell lines. It suggests that aromatic diimine has important effect on cytotoxicity, the palladium(Ⅱ) complexes with bipy have better cytotoxicity than the corresponding palladium(Ⅱ) complexes with phen.

Table 3 Cytotoxicity of complexes *in vitro* (*n*=5)

Complex	(IC_{50} ± SD) / (μmol·L^{-1})			
	HL-60	BGC-823	Bel-7402	KB
1	19.99±2.03	16.81±1.56	22.27±2.54	25.29±2.13
2	20.26±2.42	20.31±2.01	38.30±3.45	31.38±3.25
Cisplatin	2.89±0.34	6.48±0.81	8.12±0.97	2.65±0.33

3 Conclusions

In summary, two novel palladium(Ⅱ) complexes, [Pd(bipy)(4-CH_3Bzval-N,O)] (**1**) and [Pd(phen)(4-CH_3Bzval-N,O)] (**2**) have been synthesized and structurally characterized. Crystal structure of the complex **2** has been determined by X-ray diffraction analysis. The Pd^{2+} ion is coordinated by two nitrogen

atoms of phen, one deprotonated amide nitrogen atom and one carboxylic oxygen atom. Cytotoxic data indicate that two complexes display cytotoxic effects against HL-60, BGC-823, Bel-7402 and KB cell lines, moreover, the palladium (Ⅱ) complexes with bipy have better cytotoxicity than the corresponding palladium (Ⅱ) complexes with phen. This suggests that it may be a new class metal-based anticancer drugs.

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