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Synthesis, Characterization, and Cytotoxicity of Mixed-Ligand Complexes of Palladium(II) with 1,10-Phenanthroline and *N*-Carbonyl-*L*-Isoleucine Dianion[1]

J. C. Zhang*, L. W. Wang, S. Y. Liu, F. F. Zhang, J. L. Du, L. W. Li, S. X. Wang, S. H. Li, and G. Q. Zhou**

College of Chemistry & Environmental Science, Chemical Biology Key Laboratory of Hebei Province, Hebei University, Baoding, 071002 P.R. China

Key Laboratory of Medicinal Chemistry and Molecular Diagnosis of the Ministry of Education, Hebei University, Baoding, 071002 P.R. China

*e-mail: * jczhang6970@yahoo.com.cn; ** wsx@hbu.edu.cn*

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Abstract—Three novel palladium(II) complexes [Pd(Phen)(Bzile)] (**I**), [Pd(Phen)(*p*-mBzile)] (**II**), and [Pd(Phen)(*p*-NBzile)] · H_2O (**III**), where Bzile = *N*-benzoyl-*L*-isolecine), *p*-MBzile = *N*-(*p*-methylbenzoyl)-*L*-isolecine), *p*-NBzile = *N*-(*p*-nitrobenzoyl)-*L*-isolecine), have been synthesized and characterized by elemental analysis, ES-MS, and IR. The crystal and molecular structure of the complex **II** has been determined by single crystal X-ray diffraction. The cytotoxicity was tested against carcinoma cell lines: KB, BGC-823, Bel-7402 and HL-60 by MTT assay. The results indicated that these complexes exerted cytotoxicity, but none of them showed higher cytotoxicity than cisplatin. The cytotoxicity against HL-60, BGC-823, Bel-7402 and KB cell lines decreases in the sequence: *p*-MBzile > Bzile > *p*-NBzile. It suggests that the acylated groups have important impact on the cytotoxicity of complexes.

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INTRODUCTION

Nowadays cisplatin and its derivatives are the most widely used clinical anticancer drugs. Unfortunately, they have several major drawbacks. Common problems include cumulative toxicities of nephrotoxicity and ototoxicity [1, 2]. In addition to the serious side effects, the therapeutic efficacy is also limited by inherent or treatment-induced resistant tumor cells. 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. On the basis of the structural and thermodynamic analogy between platinum(II) and palladium(II) complexes, there is also much interest in the study of palladium(II) derivatives as potential anticancer drugs [3–6]. It was reported that 1,10-phenanthroline (Phen) derivatives had the ability to participate as DNA intercalators. Amino acids are the fundamental matters of life and material base of metabolism. Introducing amino acids into the antitumor drug molecules can improve their selectivity to tumor cells, enhance their liposolubility, and remit their toxicities to normal cells. In addition, owing to higher lability of palladium versus platinum analogs, amino acid ligands, which do not dissociate easily in aqueous solution, have been used to synthesize palladium anticancer complexes. These palladium complexes with amino acid ligands are expected to be useful for the treatment of tumors of the gastrointestinal region because of their little interaction with chloride ions compared with cisplatin [7]. Besides, less kidney toxic than cisplatin is expected because of the difficulty of replacing the tightly bound bidentate amino acids in these complexes by protein bound sulphydryl groups of kidney tubule cells [8]. So Phen and amino acid have been widely used in palladium anticancer drugs as ligands. Mital's group reported the synthesis and cytotoxicity of nine palladium(II) complexes of type $[Pd(Phen)(AA)]^+$ (where AA is an anion of *L*-glycine (Gly), *L*-alanine (Ala), *L*-leucine (Leu), *L*-phenylalanine (Phe), *L*-tyrosine (Tyr), *L*-tryptophan (Try), *L*-valine (Val), *L*-proline (Pro) or *L*-serine (Ser)). The palladium(II) complexes are found to exhibit growth inhibition of P388 lymphocytic leukemic cells. The IC_{50} values for the palladium(II) complexes with Gly and Val are comparable to cisplatin, whereas the other palladium(II) complexes show higher IC_{50} values [9].

Because the coordination behavior of *N*-carbonyl-amino acids show some similarities to that of the O-terminal end of peptides and proteins, great attention has been paid to the coordination properties of these ligands. The deprotonated Pt(II)/Pd(II) complexes, in which *N*-acetylglycine acts as an N,O-

[1] The article is published in the original.

chelating ligand, are obtained in [10]. Several palladium(II) complexes with deprotonated sulfonamides nitrogen have been synthesized in [11]. Four new mixed-ligand complexes of palladium(II) with *N*-benzoyl-α-amino acid dianion and ethyldiamine, 2,2'-bipyridine (Bipy) or Phen were synthesized [12]. Until now, the cytotoxicity of mixed-ligand complexes of palladium(II) with *N*-carbonyl amino acid dianion and diamine has not been reported. In the present work, we present the synthesis, characterization and cytotoxicity of three mixed-ligand palladium(II) complexes with *N*-carbonyl-*L*-isoleucine dianion and Phen for the first time.

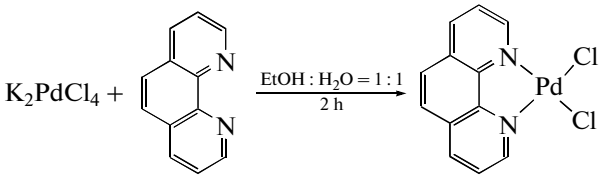
EXPERIMENTAL

Materials and methods. Benzoyl chloride, *p*-methylbenzoyl chloride, *p*-nitrobenzoyl chloride and K_2PdCl_4 were of chemical grade, Phen and *L*-isolecine (Ile) 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), benzyl penicillin and streptomycin were from Sigma. Four different human carcinoma cell lines: HL-60 (immature granulocyte leukemia), Bel-7402 (liver carcinoma), BGC-823 (gastro carcinoma) and KB (nasopharyngeal carcinoma) were obtained from American Type Culture Collection.

Elemental analysis was determined on an Elementar Vario EL III elemental analyzer. The IR spectra were recorded using KBr pellets and a PerkinElmer Model-683 spectrophotometer. The mass spectra were measured by LC-MS apparatus Agilent 1200-6310. X-ray single crystal structure was performed on a Bruker SMART APEX II CCD diffractometer. The OD was measured on a microplate spectrophotometer (Bio-Rad Model 680, USA).

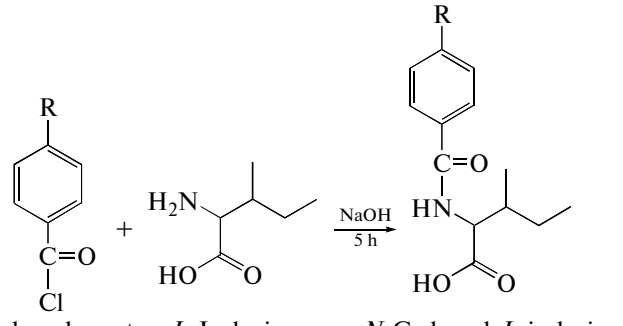
Synthesis of complexes I–III. *N*-carbonyl-*L*-isolecine and $[Pd(Phen)Cl_2]$ were prepared by the reported methods [13, 14]. Reaction of $[Pd(Phen)Cl_2]$ with two equivalents of *N*-carbonyl-*L*-isolecine produced the title complexes (**I**–**III**) [11]. Complex **II** (15 mg, 0.028 mmol) was dissolved in 3 mL mixture CH_3OH–$CHCl_3$ (v/v = 2 : 1). After several days, yellow crystals suitable for X-ray studies were obtained by the slow evaporation. The synthetic routines of complexes **I**–**III** are given below:

Step 1:



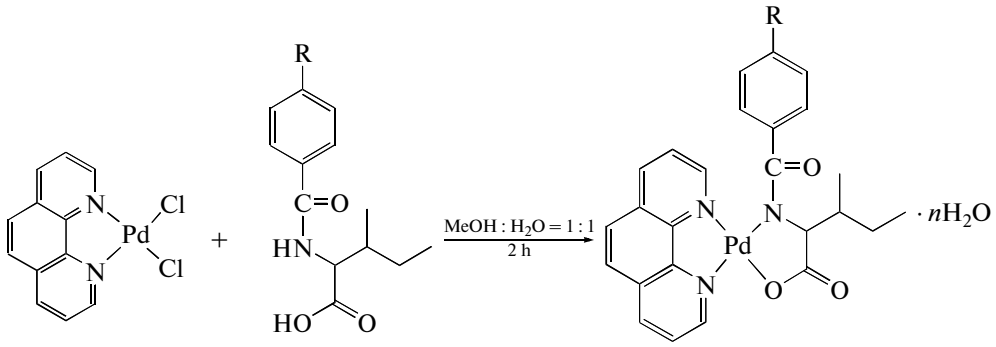
1,10-Phenanthroline Pd(Phen)Cl_2

Step 2:



Carbonyl agent *L*-Isolecine *N*-Carbonyl-*L*-isolecine

Step 3:



Pd(Phen)Cl_2 *N*-Carbonyl-*L*-isolecine

I. $n = 0$, R = H;
II. $n = 0$, R = CH_3;
III. $n = 1$, R = NO_2.

IR for $BzileH_2$, *p*-$MBzileH_2$, *p*-$NBzileH_2$ (KBr; ν, cm^{-1}): 3352, 3356, 3348 ν(NH-amide), 1639, 1630, 1642 ν(amide I), 1536, 1543, 1545 ν(amide II), 1726, 1724, 1711 $\nu(OCO)_{as}$, 1208, 1206, 1346 $\nu(OCO)_s$, respectively.

For $C_{25}H_{23}N_3O_3Pd$ (**I**, $M = 519.89$)

anal. calcd., %:	C, 57.76;	N, 8.08;	H, 4.46.
Found, %:	C, 57.76;	N, 8.16;	H, 4.36.

ES-MS (*m/z*): 542.89 $[M + Na]^+$.

IR (KBr; ν, cm^{-1}): 1535 ν(amide I), 1642 $\nu(OCO)_{as}$, 1394 $\nu(OCO)_s$, 547 ν(Pd–N), 469 ν(Pd–O).

^1H NMR (600 MHz; $CDCl_3$; δ, ppm): 0.84–0.78 (m., 3H, CH_3), 1.20 (t., $J = 5.26$ Hz, 3H, CH_3), 1.48 (d., $J = 6.81$ Hz, 2H, CH_2), 2.17 (s., 1H, CH), 4.00–3.97 (m., 1H, CH), 7.33–8.60 (13H, Ar–H).

For $C_{26}H_{25}N_3O_3Pd$ (**II**, $M = 533.91$)

anal. calcd., %:	C, 58.49;	N, 7.87;	H, 4.72.
Found, %:	C, 58.35;	N, 7.83;	H, 4.70.

ES-MS (*m/z*): 556.89 $[M + Na]^+$.

IR (KBr; ν, cm^{-1}): 1545 ν(Amide I), 1636 $\nu(OCO)_{as}$, 1384 $\nu(OCO)_s$, 552 ν(Pd–N), 479 ν(Pd–O).

^1H NMR (600 MHz; $CDCl_3$; δ, ppm): 0.82 (q., $J =$ 7.38, 6.98 Hz, 3H, CH_3), 1.25 (d., $J = 7.06$ Hz, 3H, CH_3), 1.46 (d., $J = 6.75$ Hz, 2H, CH_2), 2.35 (s., 3H, Ar–CH_3), 3.74–3.69 (m., 1H, CH), 4.01–3.98 (m., 1H, CH), 7.30–8.63 (12H, Ar–H).

For $C_{25}H_{22}N_4O_5Pd$ (**III**, $M = 564.89$)

anal. calcd., %:	C, 53.16;	N, 9.92;	H, 3.93.
Found,%:	C, 51.51;	N, 9.67;	H, 4.04.

ES-MS (*m/z*): 565.89 $[M + H]^+$.

IR (KBr; ν, cm^{-1}): 3420 ν(water), 1554 ν(amide I), 1638 $\nu(OCO)_{as}$, 1382 $\nu(OCO)_s$, 562 ν(Pd–N), 474 ν(Pd–O).

X-ray crystallography. The data collection of the complex **II** was performed on a Bruker SMART APEX II CCD diffractometer equipped with a graphite monochromatized MoK_α radiation ($\lambda = 0.71073$ Å) at 296(2) K. The program SAINT was used for integration of the diffraction profiles [15]. Multiscan absorption corrections were applied using the SADABS program. The structures were 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 [16]. The hydrogen atoms were located from different Fourier maps. Supplementary material for structure **II** has deposited with the Cambridge Crystallographic Data Centre (no. 771187; deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk).

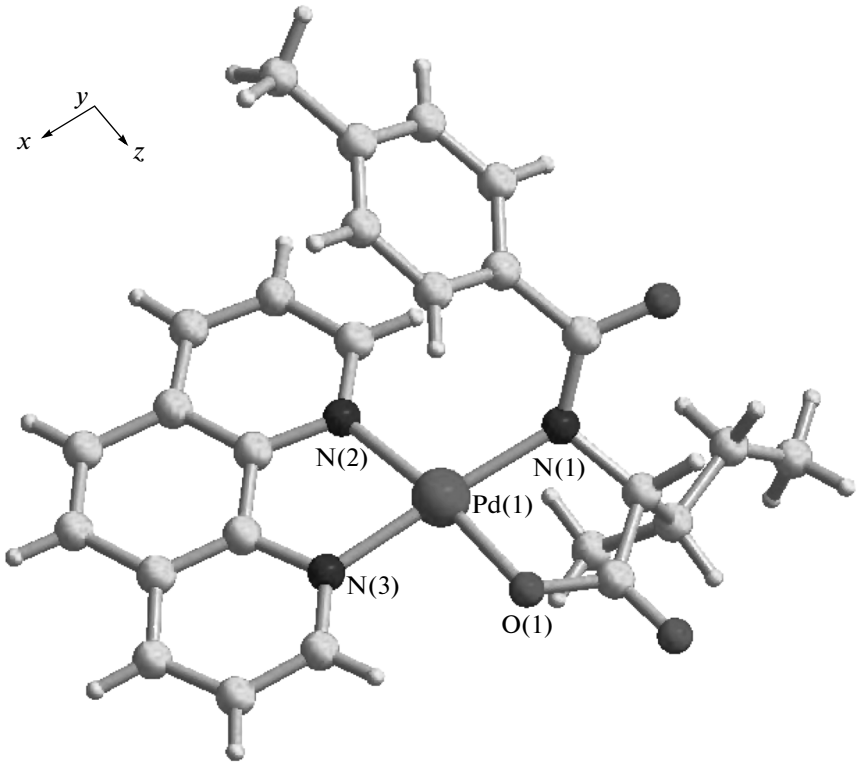
In vitro cytotoxicity study. The complexes were dissolved in DMSO at a concentration of 5 mmol/L as stock solutions and diluted in culture medium at concentrations of 1.0, 10, 100, and 500 μmol/L as working-solution. To avoid DMSO toxicity, the concentration of DMSO was less than 0.1% (v/v) in all experiments.

The cells harvested from exponential phase were seeded equivalently into a 96-well plate, and then the complexes were 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. All experiments were performed in quintuplicate. The MTT assay was performed as described by Mosmann for HL-60 [17]. Upon completion of the incubation for 44 h, stock MTT dye solution (20 mL, 5 mg/mL) 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 measured on a microplate spectrophotometer at a wavelength of 570 nm. The SRB assay was performed as previously described for Bel-7402, BGC-823, and KB [18]. Upon completion of the incubation for 44 h, the cells were fixed in 10% trichloroacetic acid (100 mL) for 30 min at 4°C, washed five times 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 mM 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.

RESULTS AND DISCUSSION

The elemental analysis and mass showed that all the complexes, isolated as crystalline solids, were of high purity. There is good agreement between calculated and found values for complexes **I**–**III**.

The amide groups of $BzileH_2$, *p*-$MBzileH_2$ and *p*-$NBzileH_2$ have a strong and sharp ν(NH) at about 3350 cm^{-1}. This peak disappears in complexes **I**–**III**, showing that the amide group has been deprotonated. This is also confirmed by the amide **I** shifting from ~1640 to 1535–1554 cm^{-1} and the disappearance of the amide **II** from ~540 cm^{-1} region. New bands appeared at 547–562 cm^{-1} are assigned to ν(Pd–N). The carboxylate group of the complexes **I**–**III** shows two bands, an intense antisymmetric carboxylate stretching $\nu(COO–)_{as}$ and a symmetric carboxylate stretching $\nu(COO–)_s$ at about 1640 and 1380 cm^{-1}, respectively. The values of $_\Delta\nu(COO–)$ ($\nu(COO–)_{as} - \nu(COO–)_s$) of complexes **I**–**III** are in the range 248–256 cm^{-1}, which is greater than $_\Delta\nu(COO–)$ of the corresponding sodium carboxylates, so the carboxylate group may be



The molecular structure of complex **II**.

Table 1. Crystallographic data and refinement details for complex **II**

Parameter	Value
Formula weight	533.89
Crystal system, space group	Monoclinic, $P2_1$
Unitcell dimensions:	
a, Å	11.8184(12)
b, Å	11.1624(11)
c, Å	18.2371(19)
β, deg	102.489(2)
V, Å^3	2348.9(4)
ρ_{calcd}, mg/cm^3	1510
Z	4
Crystal size, mm	0.19 × 0.16 × 0.07
μ, mm^{-1}	0.823
F(000)	1088
θ Range for data collection, deg	1.14–28.28
Limiting indices	$-13 \le h \le 15$, $-13 \le k \le 14$, $-23 \le l \le 24$
Reflections collected/unique	14497/9090 ($R_{int} = 0.0312$)
Completeness, %	97.2
Parameters	602
Goodness-of-fit on F^2	1.462
Final R indices, $I > 2\sigma(I)$	$R_1 = 0.0438$, $wR_2 = 0.0609$
R indices, all data	$R_1 = 0.0787$, $wR_2 = 0.0677$
Largest diff. peak and hole, e/Å^3	1.105 and −0.807

monodentate coordinated through oxygen atoms [19, 20]. This is further confirmed by the appearance of the peaks of ν(Pd–O). These results are in good agreement with the results revealed by X-ray crystal analysis.

The crystal structures and numbering schemes of the complex **II** are shown in figure. The crystal data, data collection, and structural solution refinement parameters for complex **II** are summarized in Table 1. Selected bond distances and angles are listed in Table 2. The crystal data reveal that the geometry around Pd(II) of two complexes is approximately square planar, composed of two nitrogen atoms from Phen and one carbonyl amide nitrogen atom and one

Table 2. Selected bond lengths (Å) and angles (deg) for complex **II**

Bond	d, Å	Bond	d, Å
Pd(1)–O(1)	1.989(5)	Pd(1)–N(2)	2.039(6)
Pd(1)–N(1)	1.985(6)	Pd(1)–N(3)	2.032(6)
Angle	**ω, deg**	**Angle**	**ω, deg**
O(1)Pd(1)N(1)	82.1(2)	O(1)Pd(1)N(2)	170.5(2)
O(1)Pd(1)N(3)	92.6(2)	N(1)Pd(1)N(2)	104.2(3)
N(1)Pd(1)N(3)	173.1(4)	N(3)Pd(1)N(2)	81.5(3)

Table 3. Cytotoxity against four cancer cell lines: KB, BGC-823, Bel-7402 and HL-60

Compound	IC_{50}, mmol/L			
	HL-60	BGC-823	Bel-7402	KB
[Pd(Phen)(BzileNO)] (**I**)	16.99	37.90	38.32	23.58
[Pd(Phen)(*p*-MBzileNO)] (**II**)	9.54	34.68	27.35	19.52
[Pd(Phen)(*p*-NBzileNO)] · H_2O (**III**)	23.22	46.32	42.78	31.46
cis-DDP	2.89	6.48	8.12	2.65

carboxylic oxygen of the amino acid molecule. Thus Pd has planar square coordination geometry.

The angle between planar N(2)–Pd(1)–N(3) and planar O(1)–Pd(1)–N(1) is 8.624(246)° 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 (1.985(6) Å) is shorter than the Pd–N(Phen) bond lengths (2.032(6) and 2.0392(6) Å), while it is similar to Pd–O (carboxylic oxygen) bond length (1.989(5) Å). In [21], it was reported that the coordinating qualities of the deprotonated amide nitrogen atoms were "O-like" as the deprotonated amide group is isoelectronic with the carboxylate group, and this has been conrmed by stability constants of some complexes. In [20], it was also reported that the deprotonated amide nitrogen atom was exactly different from the ordinary amino nitrogen atom and its coordinating property maybe "O-like".

As listed in Table 3, the complexes **I**–**III** exerted cytotoxic effects against tested carcinoma cell lines with a lower IC_{50} value (<50 μmol/L), the complex **II** has best activity against the four cancer cell lines, but none of them show higher cytotoxicity than cisplatin. The acylated groups have important impact on the cytotoxicity of complexes. The cytotoxicity against HL-60, BGC-823, Bel-7402 and KB cell lines decrease in the sequence: *p*-MBzile > Bzile > *p*-NBzile. In summary, this study can enrich inorganic medicine area, these results will be very helpful for designing new anticancer drugs.

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