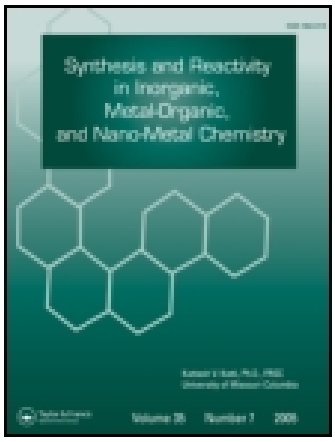

Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lsrt20

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Accepted author version posted online: 13 Jan 2015.

To cite this article: Li-Wei Wang, Si-Yuan Liu, Jin-Jie Wang, Wen Peng, Sheng-Hui Li, Guo-Qiang Zhou, Xin-Ying Qin, Shu-Xiang Wang & Jin-Chao Zhang (2015) Synthesis, Characterization, and Cytotoxicity of Palladium(II) Complexes With Diimine/Diamine and N-Carbonyl-L-Phenylalanine Dianion, Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry, 45:7, 1049-1056, DOI: 10.1080/15533174.2013.862652

To link to this article: http://dx.doi.org/10.1080/15533174.2013.862652

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ISSN: 1553-3174 print / 1553-3182 online
DOI: 10.1080/15533174.2013.862652

Synthesis, Characterization, and Cytotoxicity of Palladium(II) Complexes With Diimine/Diamine and *N*-Carbonyl-*L*-Phenylalanine Dianion

LI-WEI WANG[1], SI-YUAN LIU[1], JIN-JIE WANG[1], WEN PENG[1], SHENG-HUI LI[1,2], GUO-QIANG ZHOU[1,2], XIN-YING QIN[1,2], SHU-XIANG WANG[1,2] and JIN-CHAO ZHANG[1,2]

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Received 28 July 2013; accepted 2 November 2013

Six palladium(II) complexes, [Pd(bipy)(Bzphe-N,O)] (I-a), [Pd(bipy)(*p*-Mbzphe-N,O)]·2H_2O (I-b), [Pd(bipy)(*p*-Nbzphe-N,O)]·2H_2O (I-c), [Pd(phen)(Bmined by X-ray diffraction. The cytotoxicity test indicates that the complexes exert cytotoxic effects against KB, BGC-823, Bel-7402, and HL-60, but none of them shows higher cytotoxicity than cisplatin. The structure-activity relationship suggests that bomined by X-ray diffraction. The cytotoxicity test indicates that the complexes exert cytotoxic effects against KB, BGC-823, Bel-7402, and HL-60, but none of them shows higher cytotoxicity than cisplatin. The structure-activity relationship suggests that both *N*-containing ligands and *N*-carbonyl reagent have important effect on cytotoxicity, however, the IC_{50} values do not show definite correlation with their variation.

Keywords: Palladium (II) complex, *N*-carbonyl-*L*-phenylalanine dianion, diimine, cytotoxicity

Introduction

The landmark discovery of cisplatin heralded a new era of anticancer drug research based on metallopharmaceuticals.[1] Now cisplatin, carboplatin, and oxaliplatin are commonly used for the treatment of lung, colorectal, ovarian, breast, head and neck, bladder, and testicular cancers. In addition, nedaplatin, lobaplatin, and heptaplatin have gained regional approval, a few platinum drugs continue to be evaluated in clinical studies. The major drawbacks of current platinum drugs, including a limited range of cancers, acquired or intrinsic resistance, and severe side effects prompted chemists to develop new metal anticancer drugs.[2,3]

Due to the structural and thermodynamic similarities between palladium(II) and platinum(II) complexes, there is an increased interest in the study of palladium(II) derivatives as potential anticancer drugs.[4–6] It was found that the hydrolysis of the leaving ligands in palladium complexes was too rapid, thus the complexes dissociate readily in solution leading to very reactive species that are unable to reach their pharmacological targets. This implies that if anticancer palladium drugs will be developed, they must be stabilized by strongly coordinated nitrogen ligands and suitable leaving ligands. Amino acid, bipy, 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 bipy or phen has the ability to participate as DNA intercalator.[7,8] Numerous palladium complexes with aromatic *N*-containing and *L*-amino acid ligands were shown to be effective against tumors. For example, Puthraya et al.[9] reported the synthesis and cytotoxicity of palladium(II) complexes of the formula $[Pd(bipy)(AA)]^{n+}$ (where AA is an anion of Cys, Asp, Glu, Met, His, Arg, Phe, Tyr, or Try, and n = 0 or 1). The results indicated that these palladium(II) complexes showed growth inhibition against L1210 lymphoid leukemic, P388 lymphocytic leukemic, Sarcama 180, and Ehrlich ascites tumor cells. IC_{50} values of some complexes were comparable to or lower than that of cisplatin.[9] Mital et al.[10] reported the synthesis and cytotoxicity of palladium(II) complexes of type $[Pd(phen)(AA)]^{+}$ (where AA is an anion of Gly, Ala, Leu, Phe, Tyr, Try, Val, Pro, or Ser), only two complexes, $[Pd(phen)(Gly)]^{+}$ and $[Pd(phen)(Val)]^{+}$, showed comparable cytotoxicity as cisplatin.[10] The cytotoxic study of $[Pd(AMBI)(AA)]^{n+}$ (where AA is an anion of Gly, Ala, Cys, Met, or Ser) was reported

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Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/lsrt.

by El-Sherif.[11] The results indicated that [Pd(AMBI)(Met)]Cl·H_2O showed significant activity against HCT116 cells with IC_{50} value of 0.74 $\mu g \cdot mL^{-1}$, while [Pd(AMBI)(Cys)]Cl·H_2O showed cytotoxicity against HEP2 cells with IC_{50} value of 0.60 $\mu g \cdot mL^{-1}$.[11] We previously reported the synthesis and cytotoxicity of four platinum(II) complexes with bipy and *N*-benzoyl-*L*-amino acid dianion. The results indicated that these complexes exerted cytotoxicity against HL-60, Bel-7402, BGC-823, and KB cell lines.[12] In order to develop new metal anticancer drugs, in this study, we present the synthesis, characterization, and cytotoxicity of six palladium(II) complexes with diimine (phen and bipy) or diamine (en) and *N*-carbonyl-*L*-phenylalanine dianion for the first time.

Experimental

Materials

Benzoyl chloride, *p*-methyl benzoyl chloride, *p*-nitro benzoyl chloride, and $K_2[PdCl_4]$ were of chemical grade: bipy, en, and phen were of analytical grade. MTT, SRB, benzylpenicillin, streptomycin, and Phe were purchased from Sigma. RPMI-1640 medium, trypsin, and fetal bovine serum were purchased from Gibco. Four different human carcinoma cell lines: HL-60 (immature granulocyte leukemia), Bel-7402 (liver carcinoma), BGC-823 (gastric carcinoma), and KB (nasopharyngeal carcinoma) were obtained from American Type Culture Collection.

Instrumentation and Measurement

Elemental analysis was determined on an Elementar Vario EL III elemental analyzer. The electronic spectra in DMF were measured on an UV-3400 Toshniwal spectrophotometer. The IR spectra were recorded using KBr pellets and a Perkin Elmer Model-683 spectrophotometer. The ^1H NMR was recorded on a Bruker AVIII 600 NMR spectrometer. 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 Compounds

Six palladium(II) complexes [Pd(bipy)(Bzphe-N,O)] (**I-a**), [Pd(bipy)(*p*-Mbzphe-N,O)]·$2H_2O$ (**I-b**), [Pd(bipy)(*p*-Nbzphe-N,O)]·$2H_2O$ (**I-c**), [Pd(phen)(Bzphe-N,O)]·$2H_2O$ (**II-a**), [Pd(en)(Bzphe-N,O)]·H_2O (III-**a**), and [Pd(en)(*p*-Nbzphe-N,O)]·$2H_2O$ (III-**c**) were prepared by adding precursor complex [Pd(bipy)Cl_2], [Pd(phen)Cl_2] or [Pd(en)Cl_2] with *N*-carbonyl-*L*-pheylalanine in a mixed solution of CH_3OH/H_2O at 50°C and pH 8–9. The mixture was stirred till to a clear solution. Then the solution was filtered and concentrated to 3 mL at 50°C by a rotary evaporator. By evaporating the filtered solution (or the filtrate) at room temperature, yellow crystal or sediment was obtained in 1–2 weeks (Figure 1).

N-*Carbonyl*-L-*Phenylalanine Ligands*

N-carbonyl-*L*-pheylalanine was prepared by a modified method.[13] To a vigorously stirred solution of *L*-phenylalanine (200 mg, 1.2 mmol) and 6.0 mL of NaOH (0.2 mol•L^{-1}) water solution in an ice bath, benzoyl chloride (0.15 mL, 1.2 mmol) dissolved in 8 mL of $CHCl_3$ and 6 mL of NaOH (0.2 mol•L^{-1}) solution were added slowly at the same time. The reaction was held for 5-6 h. Then the solution was acidified to pH = 3∼4 with HCl (0.2 mol•L^{-1}) and the aqueous layer was extracted with $CHCl_3$ (3 × 8 mL). Organic layers were combined, dried with anhydrous sodium sulfate, and concentrated to 3 mL by a rotary evaporator at 40°C. By evaporating the solution at room temperature, white solid was generated. After filtration, the white solid was recrystallized twice from ethanol-water (V/V = 1:1) mixtureand dried to give $BzpheH_2$, m.p: 185∼186°C (found); 187°C (lit[13]); IR (KBr, cm^{-1}): $\nu_{(NH\text{-}amide)}$ 3327, $\nu_{(Amide\text{ I})}$ 1612, $\nu_{(Amide\text{ II})}$ 1538, $\nu_{(OCO)a}$ 1722, $\nu_{(OCO)s}$ 1228.

p-$MbzpheH_2$ was prepared following a procedure similar to $BzpheH_2$. IR (KBr, cm^{-1}): $\nu_{(NH\text{-}amide)}$ 3324, $\nu_{(Amide\text{ I})}$ 1618, $\nu_{(Amide\text{ II})}$ 1544, $\nu_{(OCO)a}$ 1718, $\nu_{(OCO)s}$ 1232; ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 2.39 (s, 3H, CH_3), 3.29 (dd, J = 14.1, 5.7 Hz, 1H, CH_2-H), 3.38 (dd, J = 14.1, 5.7 Hz, 1H, CH_2-H), 5.11 (dd, J = 13.2, 6.0 Hz, 1H, CH), 6.52 (d, J = 7.2 Hz, 1H, NH), 7.22 (t, J = 7.2 Hz, 4H, Ar-H), 7.33–7.30 (m, 1H, Ar-H), 7.60 (d, J = 8.4 Hz, 2H, Ar-H), 7.99 (d, J = 8.4 Hz, 2H, Ar-H).

p-$NbzpheH_2$ was prepared following a procedure similar to $BzpheH_2$. IR (KBr, cm^{-1}): $\nu_{(NH\text{-}amide)}$ 3351, $\nu_{(Amide\text{ I})}$ 1600, $\nu_{(Amide\text{ II})}$ 1530, $\nu_{(OCO)a}$ 1710, $\nu_{(OCO)s}$ 1299; ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 3.33–3.27 (m, 1H, CH_2-H), 3.40 (dd, J = 14.4, 5.4 Hz, 1H, CH_2-H), 5.13 (dd, J = 13.2, 5.4 Hz, 1H, CH), 6.64 (d, J = 7.8 Hz, 1H, NH), 7.20 (d, J = 6.6 Hz, 2H, Ar-H), 7.35–7.29 (m, 2H, Ar-H), 7.85 (d, J = 8.4 Hz, 2H, Ar-H), 8.29–8.26 (m, 2H, Ar-H), 8.33 (d, J = 9.0 Hz, 1H, Ar-H).

Precursor Complexes

Precursor complexes [Pd(bipy)Cl_2], [Pd(phen)Cl_2], and [Pd(en)Cl_2] were synthesized according to published procedures.[14,15] [Pd(bipy)Cl_2]**:** Yellow solid, yield 63%. Anal. Calcd. for $C_{10}H_8Cl_2N_2Pd$: C, 36.01; H, 2.42; N, 8.40. Found: C, 36.28; H, 2.63; N, 8.50. [Pd(phen)Cl_2]: Yellow solid, yield 95%. Anal. Calcd. for $C_{12}H_8Cl_2N_2Pd$: C, 40.31; H, 2.26; N, 7.88. Found: C, 40.51; H, 2.32; N, 7.79. [Pd(en)Cl_2]: Brown yellow solid, yield 84%. Anal. Calcd. for $C_{12}H_6Cl_2N_2Pd$: C, 10.12; H, 3.40; N, 11.80. Found: C, 10.16; H, 3.41; N, 11.65.

[Pd(bipy)(Bzphe-N, O)] (I-a)

To a vigorously stirred solution of $BzpheH_2$ (32.32 mg, 0.12 mmol) in 8 mL CH_3OH/H_2O (V:V = 1:1), [Pd(bipy)Cl_2] (20 mg, 0.06 mmol) was added. The mixture was heated to 50°C and adjusted to pH = 8–9 by NaOH solution, and then stirred for 2 h. The solution was concentrated to about 80% of the original volume. The complex **I-a** was separated from the solution after a few days. Yellow crystalline, yield:

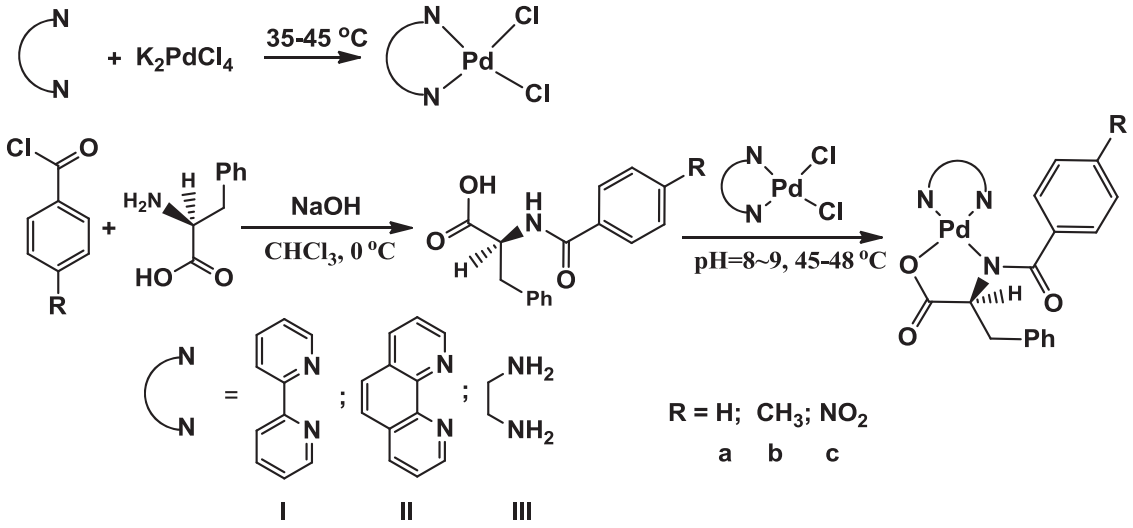

Fig. 1. The synthesis routes of the six Pd(II) complexes.

68%. IR (KBr, cm^{-1}): $\nu_{(Amide\square)}$1551, $\nu_{(OCO)a}$ 1632, $\nu_{(OCO)a}$ 1383, $\nu_{(Pd\text{-}N)}$ 566, $\nu_{(Pd\text{-}O)}$ 465. ^1H NMR (600 MHz, d_6-DMSO) δ (ppm): 3.08 (dd, J = 13.1, 4.5 Hz, 1H, CH_2-H), 3.29 (dd, J = 13.1, 4.5 Hz, 1H, CH_2-H), 5.08–5.05 (m, 1H, CH), 6.65 (t, J = 7.5 Hz, 1H, Ar-H), 6.73 (t, J = 7.5 Hz, 2H, Ar-H), 7.09 (d, J = 3.0 Hz, 3H, Ar-H), 7.13 (d, J = 5.4 Hz, 1H, Ar-H), 7.23 (d, J = 7.2 Hz, 2H, Ar-H), 7.28 (d, J = 5.4 Hz, 1H, Ar-H), 7.78–7.75 (m, 1H, Ar-H), 8.02 (t, J = 7.8 Hz, 1H, Ar-H), 8.26 (d, J = 7.8 Hz, 1H, Ar-H), 8.30 (t, J = 6.9 Hz, 4H, Ar-H), 8.42 (d, J = 7.8 Hz, 1H, Ar-H). ESI-MS: 568.03 $[M+K]^+$. Anal. Calcd. for [Pd(bipy)(Bzphe-N, O)] ($C_{26}H_{21}N_3O_3Pd$, 529.06): C, 58.93; H, 3.99; N, 7.93. Found: C, 58.84; H, 4.04; N, 7.84.

*[Pd(bipy)(*p*-Mbzphe-N, O)]·2H$_2$O (I-b)*

The synthesis of **I-b** was carried out in an identical manner to **I-a** starting from [Pd(bipy)Cl_2] (20 mg, 0.06 mmol) and *p*-MbzpheH$_2$ (34.00 mg, 0.12 mmol). Yellow solid, yield: 62%. IR (KBr, cm^{-1}): $\nu_{(OH)}$ 3430, $\nu_{(Amide\square)}$ 1530, $\nu_{(OCO)a}$ 1641, $\nu_{(OCO)a}$ 1385, $\nu_{(Pd\text{-}N)}$ 572, $\nu_{(Pd\text{-}O)}$ 455. ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 2.07 (s, 3H, CH_3), 3.07 (dd, J = 14.1, 5.7 Hz, 1 H, CH_2-H), 3.09 (dd, J = 14.1, 5.7 Hz, 1H, CH_2-H), 5.05–5.02 (m, 1H, CH), 6.65 (t, J = 7.2 Hz, 1H, Ar-H), 6.72 (t, J = 7.2 Hz, 2H, Ar-H), 6.89 (d, J = 7.8 Hz, 3H, Ar-H), 7.15–7.11 (m, 1H, Ar-H), 7.22 (d, J = 7.2 Hz, 3H, Ar-H), 7.27 (d, J = 4.8 Hz, 1H, Ar-H), 7.78–7.74 (m, 1H, Ar-H), 8.04 (t, J = 7.5 Hz, 1H, Ar-H), 8.21 (d, J = 7.8 Hz, 2H, Ar-H), 8.26 (d, J = 7.8 Hz, 1H, Ar-H), 8.41 (d, J = 8.4 Hz, 1H, Ar-H). ESI-MS: 580.11 $[M+H]^+$. Anal. Calcd. for [Pd(bpy)(*p*-Mbzphe-N,O)]·2H_2O ($C_{27}H_{27}N_3O_5Pd$, 579.10): C, 55.92; H, 4.69; N, 7.25. Found: C, 56.91; H, 4.68; N, 7.25.

*[Pd(bipy)(*p*-Nbzphe-N, O)]·H$_2$O (I-c)*

The synthesis of **I-c** was carried out in an identical manner to **I-a** starting from [Pd(bipy)Cl_2] (20 mg, 0.06 mmol) and *p*-NbzpheH$_2$ (37.71 mg, 0.12 mmol). Yield: 62%, yellow solid. IR (KBr, cm^{-1}): $\nu_{(OH)}$ 3428, $\nu_{(Amide\square)}$ 1552, $\nu_{(OCO)a}$ 1640, $\nu_{(OCO)a}$ 1397, $\nu_{(Pd\text{-}N)}$ 565, $\nu_{(Pd\text{-}O)}$ 479. ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 3.11 (dd, J = 12.9, 5.7 Hz, 1H, CH_2-H), 3.44 (dd, J = 12.9, 5.7 Hz, 1H, CH_2-H), 5.12–5.06 (m, 1H, CH-H), 7.19–7.13 (m, 2H, Ar-H), 7.27 (d, J = 7.2 Hz, 2H, Ar-H), 7.31 (d, J = 5.4 Hz, 1H, Ar-H), 7.81–7.74 (m, 2H, Ar-H), 7.95 (d, J = 9.0 Hz, 2H, Ar-H), 8.03 (t, J = 8.1 Hz, 1H, Ar-H), 8.35–8.26 (m, 4H, Ar-H), 8.43 (d, J = 8.4 Hz, 1H, Ar-H), 8.52 (d, J = 8.4 Hz, 2H, Ar-H). ESI-MS: 592.05 $[M]^+$. Anal. Calcd. for [Pd(bipy)(*p*-Nbzphe-N, O)]·H_2O ($C_{26}H_{22}N_4O_6Pd$, 592.06): C, 52.67; H, 3.74; N, 9.45. Found: C, 52.75; H, 3.89; N, 9.29.

*[Pd(phen)(Bzphe-N, O)]·2H$_2$O (*II*-a)*

The synthesis of **II-a** was carried out in an identical manner to **I-a** starting from [Pd(phen)Cl_2] (20 mg, 0.056 mmol) and BzpheH$_2$ (30.16 mg, 0.112 mmol). Yield: 66%, yellow crystalline. IR (KBr, cm^{-1}): $\nu_{(OH)}$ 3428, $\nu_{(Amide\square)}$ 1549, $\nu_{(OCO)a}$ 1637, $\nu_{(OCO)a}$ 1384, $\nu_{(Pd\text{-}N)}$ 562, $\nu_{(Pd\text{-}O)}$ 474. ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 3.14 (dd, J = 14.1, 5.7 Hz, 1H, CH_2-H), 3.31 (dd, J = 14.1, 5.7 Hz, 1H, CH_2-H), 5.13–5.08 (m, 1H, CH), 6.26 (d, J = 7.2 Hz, 1H, Ar-H), 6.52 (t, J = 7.4 Hz, 2H, Ar-H), 7.07 (d, J = 4.8 Hz, 3H, Ar-H), 7.23 (d, J = 7.2 Hz, 2H, Ar-H), 7.50 (s, 1H, Ar-H), 8.08 (dd, J = 7.8, 5.2 Hz, 1H, Ar-H), 8.13 (d, J = 8.8 Hz, 1H, Ar-H), 8.20 (d, J = 8.8 Hz, 1H, Ar-H), 8.39 (m, 2H, Ar-H), 8.65 (dd, J = 12.8, 5.2 Hz, 2H, Ar-H), 8.91 (d, J = 8.0 Hz, 1H, Ar-H). ESI-MS: $[M+H]^+$ 590.10. Anal. Calcd. for [Pd(phen)(Bzphe-N, O)]·2H_2O ($C_{28}H_{25}N_3O_5Pd$, 589.08): C, 57.01; H, 4.27; N, 7.12. Found: C, 57.11; H, 4.17; N,7.05.

*[Pd(en)(Bzphe-N, O)]·H$_2$O (*III*-a)*

The synthesis of **III-a** was carried out in an identical manner to **I-a** starting from [Pd(en)Cl_2] (20 mg, 0.084 mmol) and BzpheH$_2$ (45.24 mg, 0.168 mmol). Yield: 66%, yellow

powder. IR (KBr, cm^{-1}): $\nu_{(OH)}$ 3430, $\nu_{(Amide\square)}$ 1535, $\nu_{(OCO)a}$ 1640, $\nu_{(OCO)a}$ 1380, $\nu_{(Pd\text{-}N)}$ 552, $\nu_{(Pd\text{-}O)}$ 460. ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 1.97 (s, 2H), 2.33 (s, 2H), 2.83 (dd, $J = 12.80$, 5.8 Hz, 2H), 2.97 (dd, $J = 12.80$, 5.8 Hz, 2H), 3.12 (dd, $J = 12.8$, 6.4 Hz, 1H), 3.24 (dd, $J = 12.8$, 6.4 Hz, 1H), 4.48–4.42 (m, 1H), 6.63 (d, $J = 7.2$ Hz, 3H), 6.97–6.88 (m, 3H), 7.28 (d, $J = 5.6$ Hz, 2H), 7.37 (d, $J = 7.2$ Hz, 1H), 7.66 (d, $J = 7.2$ Hz, 1H). ESI-MS: $[M]^+$ 451.08. Anal. Calcd. for [Pd(en)(Bzphe-N, O)]·H_2O ($C_{18}H_{23}N_3O_4Pd$, 451.07): C, 47.85; H, 5.13; N, 9.30. Found: C, 47.75; H, 5.05; N, 9.27.

[Pd(en)(p-Nbzphe-N, O)]·2H$_2$O (Ш-c)

The synthesis of **Ш-c** was carried out in an identical manner to **I-a** starting from [Pd(en)Cl_2] (20 mg, 0.084 mmol) and *p*-NbzpheH_2 (52.80 mg, 0.168 mmol). Yield: 82%, yellow powder. IR (KBr, cm^{-1}): $\nu_{(OH)}$ 3427, $\nu_{(Amide\square)}$ 1528, $\nu_{(OCO)a}$ 1636, $\nu_{(OCO)a}$ 1398, $\nu_{(Pd\text{-}N)}$ 566, $\nu_{(Pd\text{-}O)}$ 465. ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 1.94 (s, 2H), 2.31 (s, 2H), 2.87 (dd, $J = 12.8$, 5.8 Hz, 2H), 2.94 (dd, $J = 12.8$, 5.8 Hz, 2H), 3.15 (dd, $J = 12.8$, 6.4 Hz, 1H), 3.21 (dd, $J = 12.8$, 6.4 Hz, 1H), 4.39–4.52 (m, 1H), 6.59 (d, $J = 7.2$ Hz, 3H), 6.93–6.78 (m, 3H), 7.31 (d, $J = 5.6$ Hz, 2H), 7.68 (d, $J = 7.2$ Hz, 1H). ESI-MS: $[M+Na]^+$ 537.06. Anal. Calcd. for [Pd(en)(*p*-Nbzphe-N, O)]·2H_2O ($C_{18}H_{24}N_4O_7Pd$, 514.07): C, 41.99; H, 4.70; N, 10.88. Found: C, 41.86; H, 4.72; N, 10.83.

Crystallography

The data collection of the complex **I-a** was performed on a Bruker SMART APEX II CCD diffractometer equipped with a graphite monochromatized Mo Kα radiation (λ = 0.71073 Å) at 296(2) K. Multi-scan 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. Table 1 lists crystallographic details.

Table 1. Crystallographic data for the complex **I-a**

Complex	**I-a**
Chemical formula	$C_{24}H_{24}N_3O_3Pd$
Formula weight	508.86
T/K	296(2)
Crystal dimensions/mm	0.46 × 0.37 × 0.33
Crystal system	Monoclinic
Space group	P2(1)/c
a/Å	10.4931(4)
b/Å	18.9224(7)
c/Å	11.6646(5)
α (°)	90
β (°)	103.8690(10)
γ (°)	90
V/Å^3	2248.54(15)
Z	4
Dc/g•cm^{-3}	1.503
F(000)	1036
θ range for collection (°)	2.00–28.30
Reflections collected	16315
Independent reflections	5585
Parameters/restraints	298/0
R (int)	0.0181
R,W*R*2 (all)	0.0357, 0.0958
R,W*R*2[$I>2\sigma(I)$]	0.0305, 0.0917
GOF on F^2	1.052

Cytotoxicity Analysis

The complexes were dissolved in DMSO at a concentration of 5 mM as stock solution, and diluted in culture medium at concentrations of 1, 10, 100, and 500 μM 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, 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.[16] Upon completion of the incubation for 44 h, stock MTT dye solution (20 μL, 5 mg/mL) was added to each well. After 4 h incubation, DMSO (100 μL) 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 for Bel-7402, BGC-823, and KB.[17] Upon completion of the incubation for 44 h, the cells were fixed in 10% trichloroacetic acid (100 μL) for 30 min at 4°C, washed five times and stained with 0.1% SRB in 1% acetic acid (100 μL) for 15 min. The cells were washed four times in 1% acetic acid and air-dried. The staining was solubilized in 10 mM unbuffered Tris base (100 μL) and OD was measured at 540 nm as mentioned previously. The IC_{50} value was determined from plot of% viability against dose of complexes.

Results and Discussion

Characterization of Complexes

The elemental analysis showed that there was good agreement between calculated and found values for six complexes, this indicated that all the complexes were of high purity.

Bipy and Phen have a strong absorption peak at 282 and 273 nm, respectively, which is assigned to internal π-π* transition. After formation of the complexes, the absorption peak red shifts by ca. 32 nm for complexes (**I-a** ~ **I-c** and **П-a**) compared with that of bipy or phen, which may be caused by charge transfer transition (metal ~ ligand) from palladium *d*-orbital to π* orbital of bipy or phen.

The amide groups of BzpheH_2, *p*-MbzpheH_2, and *p*-NbzpheH_2 have a strong sharp υ_{NH} near 3320 cm^{-1}, which disappears after formation of the complexes, showing that

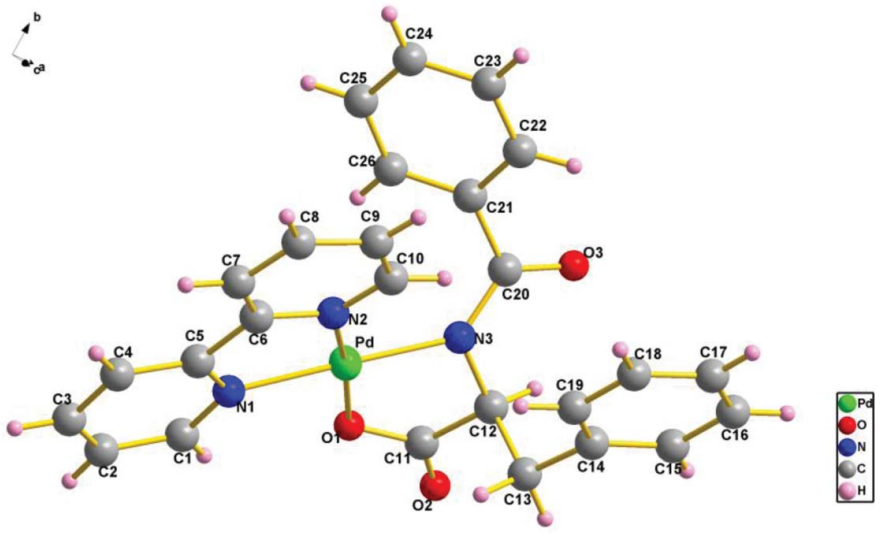

Fig. 2. Molecular structure and atom-labeling scheme for the complex **I-a**.

the amide groups have been deprotonated. This is also further confirmed by the amide (I) shifting from ~1610 cm^{-1} to 1530~1551 cm^{-1} and the disappearance of the amide (II) in the ~1540 cm^{-1} region. New bands appear at 564~572 cm^{-1} and are assigned to $\upsilon_{Pd\text{-}N}$. The carboxylate group of these complexes shows two bands, an intense antisymmetric carboxylate stretching $\upsilon_{(as,\ coo^-)}$ and a symmetric carboxylate stretching $\upsilon_{(s,\ coo^-)}$, at about 1640 and 1380 cm^{-1}, respectively. The values of $_{\Delta}\upsilon_{(coo^-)}(\upsilon_{(as,\ coo^-)} - \upsilon_{(s,\ coo^-)})$ of these complexes are in the range 241–256 cm^{-1}, which is greater than $_{\Delta}\upsilon_{(coo\text{-})}$ of the corresponding sodium carboxylates, so the carboxylate group may be monodentate coordinated through oxygen atoms.[18] This is further confirmed by the appearance of the peaks of $\upsilon_{Pd\text{-}O}$. These results are in good agreement with the results revealed by X-ray crystal analysis.

$BzpheH_2$, *p*-$MbzpheH_2$ and *p*-$NbzpheH_2$ show signal as doublet at $\delta = \sim 6.5$ in ^1H NMR, which are associated with the proton of the amide groups, but these peaks disappeared for these complexes, which further confirmed that the amide groups had been deprotonated. The methylene ^1H resonances (amino acid) of the complexes shifted to the lower field as a result of deprotonated amide nitrogen coordinating to Pd(II).

X-Ray Structure of the Complex I-a

The crystal structure and numbering scheme of complex **I-a** were shown in Figure 2. Selected bond distances and angles were listed in Table 2. Complex **I-a** crystallized in a monoclinic unit cell, space group P2(1)/c. The geometry around Pd(II) is approximately square planar, composed of two nitrogen atoms from the bipy, one carbonyl amide nitrogen atom, and one carboxylic oxygen atom of the amino acid molecule.

For complex **I-a**, there is weak interaction between bipy ring side and the other bipy ring side from the neighbor molecule, which happens in the distance of 3.548 and 3.664 Å. π-π stacking is also observed between the benzene rings of Phe and bipy ligands considering that the intermolecular centroid-centroid distance is 4.317 Å (Figure 3).

Cytotoxicity Studies

As listed in Table 3, the six complexes exerted cytotoxic effects against HL-60, BGC-823, Bel-7402, and KB. However, none of them showed higher cytotoxicity than cisplatin. Comparing to other complexes, the complexes **II-a** and **III-a** exerted higher cytotoxicity against HL-60 with IC_{50} values of 8.50 and 5.95 μmol/L. For BGC-823, complex **I-c** displayed higher cytotoxity. For Bel-7402 and KB, complex **II-a** displayed higher cytotoxity.

For $[Pd(bipy)(AA)]^{n+}$ complexes, Puthraya et al.[9] reported that side chain of the amino acids might affect the inhibitory activity. This inhibitory activity was found to be in decreasing order as follows: nonpolar hydrophobic > polar uncharged > charged side groups.[9] However, for $[Pd(phen)(AA)]^+$ complexes, Mital et al.[10] reported that the IC_{50} values did not show definite correlation with variation of the amino acid side chains.[10] El-Sherif[11] reported that the cytotoxicity of $[Pd(AMBI)(Met)]Cl{\cdot}H_2O$ against HCT116 and HEP2 decreased in the sequences: Met > Ser > Ala > Gly > Cys, and Cys > Ser > Ala > Met > Gly, respectively.[11] We

Table 2. Selected bond lengths (Å) and angles (°) for the complex **I-a**

Bond distances/Å		Angles/°	
Pd–O(1)	1.9953(17)	O(1)-Pd-N(1)	94.18(8)
Pd–N(1)	2.0095(19)	O(1)-Pd-N(3)	81.40(8)
Pd–N(2)	2.019(2)	N(2)-Pd-N(3)	104.47(8)
Pd–N(3)	2.0030(19)	O(1)-Pd-N(2)	172.20(8)
		N(1)-Pd-N(2)	80.08(8)
		N(3)-Pd-N(1)	175.30(8)

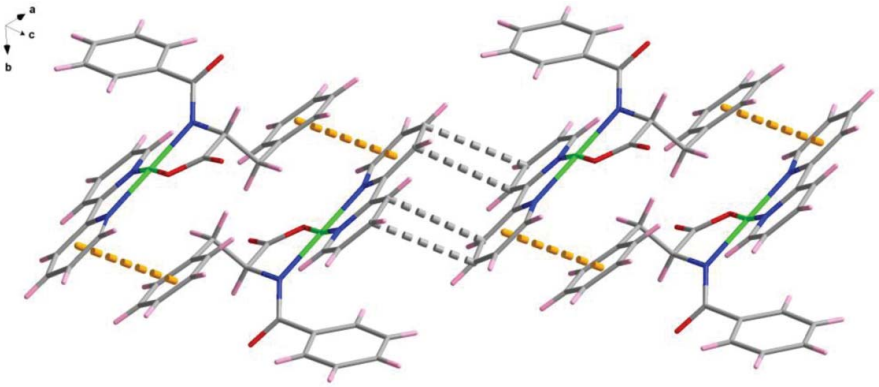

Fig. 3. View showing the weak pairing of the complex **I-a**.

previously reported the synthesis and cytotoxity of palladium (II) complexes with 4-toluenesulfonyl-L-amino acid dianion and diimine/diamine ligands. Our results indicated that these complexes exerted cytotoxic effects with selectivity against tested carcinoma cell lines, some complexes displayed better cytotoxity than cisplatin. Both amino acids and diimine/diamine ligands have important effect on cytotoxicity, but the IC_{50} values do not show definite correlation with variation of the amino acids and diimine/diamine ligands, the cytotoxicity of complexes is also related to tumor cell species.[19–24] For example, for palladium(II) complexes with 1,4-dab and different amino acids, the cytotoxicity against Bel-7402 and HL-60 decreases in the sequences: Gly > Ser > Ile > Val > Phe > Thr > Leu and Ser > Gly > Val > Thr > Phe > Leu > Ile, respectively. For palladium(II) complexes with bipy and different amino acids, the cytotoxicity against HL-60 decreases in the sequence: Val > Phe > Ala > Leu, but the cytotoxicity against Bel-7402 decreases in the sequence: Leu > Ala > Phe > Val. For palladium(II) complexes with phen and different amino acids, when the amino acid is Leu, the complex has the best cytotoxicity against HL-60 and Bel-7402. For palladium(II) complexes with en and different amino acids, the cytotoxicity against HL-60 and Bel-7402 decreases in the same sequence: Phe > Leu > Ala > Gly > Ser. For the palladium(II) complexes with Val and different diimine/diamine ligands, the cytotoxicity against HL-60 decreases in the sequence: phen > bqu > bipy > 1,4-dab, and the cytotoxicity against Bel-7402 decreases in the sequence: 1,4-dab > bqu > bipy > phen. For the palladium (II) complexes with ser and different diimine/diamine ligands, the cytotoxicity against HL-60 and Bel-7402 decreases in the sequences: 1,4-dab > bqu> 1,3-dap > en, and 1,4-dab > 1,3-dap > bqu > en. For the palladium(II) complexes with phe and different diimine/diamine ligands, the cytotoxicity against HL-60 decreases in the sequence: phen> bqu > en > bipy >1,4-dab, the cytotoxicity against Bel-7402 decreases in the sequence: phen > bqu >1,4-dab > en > bipy. In this study, for palladium(II) complexes with *N*-carbonyl-L-amino acid dianion and diimine/diamine ligands, the nature of diimine/diamine and *N*-carbonyl reagent have important effects on cytotoxicity. For palladium(II) complexes (**I-a**, **II-a**, and **III-a**) with Bzphe and different diimine/diamine, the cytotoxicity against BGC-823, Bel-7402 and KB decreases in the sequence: phen > bipy > en, the cytotoxicity against HL-60 decreases in the sequence: en > phen > bipy. For palladium(II) complexes (**I-c** and **III-c)** with *p*-Nbzphe and different diimine/diamine, the cytotoxicity against HL-60 and BGC-823 cell lines decreases in the sequence: bipy > en; the cytotoxicity against Bel-7402 and KB cell lines decreases in the sequence: en > bipy. For palladium(II) complexes (**I-a**, **I-b**, and **I-c**) with bipy and different *N*-carbonyl-L-phenylalanine dianion, the cytotoxicity against HL-60 and

Table 3. The cytotoxicities of the complexes *in vitro*

No.	Compound	IC_{50} (μmol/L)			
		HL-60	BGC-823	Bel-7402	KB
Cisplatin	*Cis*-$[Pt(NH_3)_2Cl_2]$	2.89	6.48	8.12	2.65
I-a	[Pd(bipy)(Bzphe-N,O)]	16.98	37.89	38.31	23.57
I-b	[Pd(bipy)(*p*-Mbzphe-N,O]·$2H_2O$	15.33	18.49	29.21	50.08
I-c	[Pd(bipy)(*p*-Nbzphe-N,O)]·H_2O	17.31	13.35	57.59	22.17
II-a	[Pd(phen)(Bzphe-N,O)]·$2H_2O$	8.50	24.85	19.74	10.19
III-a	[Pd(en)(Bzphe-N,O)]·H_2O	5.95	47.32	42.85	44.51
III-c	[Pd(en)(*p*-Nbzphe-N,O)]·$2H_2O$	27.45	23.33	35.45	17.41
V-4	[Pt(bipy)(Bzphe-N,O)]·$4.5H_2O$ [12]	26	39	33	12

Bel-7402 decreases in the sequence: *p*-Mbzphe > Bzphe > *p*-Nbzphe; the cytotoxicity against BGC-823 decreases in the sequence: *p*-Nbzphe > *p*-Mbzphe > Bzphe; the cytotoxicity against KB decreases in the sequence: *p*-Nbzphe > Bzphe > *p*-Mbzphe. For palladium(II) complexes (**III-a** and **III-c**) with en and different *N*-carbonyl-L-phenylalanine dianion, the cytotoxicity against HL-60 decreases in the sequence: Bzphe > *p*-Nbzphe; the cytotoxicity against BGC-823 decreases in the sequence: *p*-Nbzphe > Bzphe; the cytotoxicity against Bel-7402 and KB decreases in the sequence: *p*-Nbzphe > Bzphe. In addition, the metal ion has also important effect on cytotoxicity. For complexes (**I-a** and **V-4)** with bipy and Bzphe, the cytotoxicity against HL-60 and BGC-823 decreases in the sequence: Pd > Pt; the cytotoxicity against Bel-7402 and KB decreases in the sequence: Pt > Pd.[12]

In summary, the nature of *N*-containing ligands and *N*-carbonyl reagent has important effect on cytotoxicity. However, the IC_{50} values do not show definite correlation with variation of *N*-containing ligands and *N*-carbonyl reagent, the cytotoxicity of complexes is related to tumor cell species.

Conclusion

ne (phen and bipy) or diamine (en). The crystal structure of complex **I-a** was determined by single-crystal X-ray diffractine (phen and bipy) or diamine (en). The crystal structure of complex **I-a** was determined by single-crystal X-ray diffraction. Cytotoxic studies showed that six complexes exhibited cytotoxity against different cell lines, but none of them showed higher cytotoxicity than cisplatin. The results suggest that both *N*-containing ligands and *N*-carbonyl reagent have important effect on the cytotoxity.

Abbreviations

en = ethylenediamine
bipy = 2,2'-bipyridine
phen = 1,10-phenanthroline
AMBI = 2-aminomethyl benzimidazole
1,4-Dab = 1,4-diaminobutane
Bqu = 2, 2′-biquinoline
1,3-Dap = 1,3-diaminopropane
Ala = *L*-alanine
Arg = *L*-arginine
Asp = *L*-aspartic acid
Glu = *L*-glutamic acid
Gly = *L*-glycine
His = *L*-histidine
Leu = *L*-leucine
Phe = *L*-phenylalanine
Try = *L*-tryptophan
Tyr = *L*-tyrosine
Met = *L*-methionine
Cys = *L*-cysteine
Val = *L*-valine
Pro = *L*-proline
Ser = *L*-serine
Ile = *L*-isoleucine
$BzpheH_2$ = *N*- benzoyl-*L*-pheylalanine
p-$MbzpheH_2$ = *N*-*p*-methylbenzoyl-*L*-phenylalanine
p-$NbzpheH_2$ = *N*-*p*-nitrolbenzoyl-*L*-phenylalanine
DMF = dimethyl formamide
DMSO = dimethyl sulfoxide
MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide
SRB = sulforhodamine B
OD = optical density

Supplementary Material

Crystallographic data for the structural analysis of **I-a** have been deposited with the Cambridge Crystallographic Data Centre, CCDC-870815. Copies of this information may be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44 1223 336033; E-mail: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk)

Funding

The authors gratefully acknowledge financial support by the Nature Science Foundation for Distinguished Young Scholars of Hebei Province (No. B2011201164), Natural Science Foundation of Hebei Province (No. B2011201135), the Key Basic Research Special Foundation of Science Technology Ministry of Hebei Province (No. 11966412D, 12966418D), the Key Research Project Foundation of Department of Education of Hebei Province (No. ZH2012041), the Pharmaceutical Joint Research Foundation of the Natural Science Foundation of Hebei Province and China Shiyao Pharmaceutical Group Co., LTD (No. B2012201044, B2011201174).

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