European Journal of Medicinal Chemistry 45 (2010) 5337–5344

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis, characterization and cytotoxicity of mixed-ligand complexes of palladium(II) with aromatic diimine and 4-toluenesulfonyl-L-amino acid dianion

Jinchao Zhang*, Luwei Li, Liwei Wang, Fangfang Zhang, Xiaoliu Li

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

ARTICLE INFO

Article history:
Received 22 May 2010
Received in revised form
25 August 2010
Accepted 26 August 2010

Keywords:
Palladium (II) complexes
Synthesis
Characterization
Cytotoxicity

ABSTRACT

Eight new palladium(II) complexes (**1a**–**2d**) with 4-toluenesulfonyl-L-amino acid dianion and diimine (bipy and phen) have been synthesized and characterized by elemental analysis, IR, UV, ^{1}H NMR, ^{13}C NMR and mass spectra techniques. Crystal structure of the complex (**2d**) has been determined by X-ray diffraction analysis. The cytotoxicity was tested by MTT and SRB assays. The results indicate that the complexes (**1a**–**2d**) have selectivity against tested carcinoma cell lines. The complex (**2c**) has the best cytotoxicity among the eight complexes, moreover its cytotoxicity is better than that of cisplatin against BGC-823, Bel-7402 and KB cell lines. It suggests that both aromatic diimine and 4-toluenesulfonyl-L-amino acid have important effect on cytotoxicity.

1. Introduction

The landmark discovery of cisplatin by Rosenberg in 1965 heralded a new era of anticancer drug research based on metallopharmaceuticals [1]. To date, cisplatin and its analogs are some of the most effective chemotherapeutic agents in clinical use as the first line of treatment in testicular and ovarian cancers. Unfortunately, they have several major drawbacks. Common problems include cumulative toxicities of nephrotoxicity and ototoxicity [2–5]. 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 particular, transition metals offer potential advantages over the more common organic-based drugs. For example, a wide range of coordination numbers and geometries, accessible redox states, 'tune-ability' of the thermodynamics and kinetics of ligand substitution. 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 [6–11]. Ligands like pyridine, quinoline, phenanthroline and their derivatives have been widely used because they have the ability to participate as DNA intercalators [12]. Numerous palladium complexes with aromatic N-containing ligands were shown to be effective against tumors *in vivo* [12]. Three planar palladium complexes *trans*-[$PdCl_2L_2$] (TH5, TH6 and TH7, where L = 3-hydroxypyridine, 2-hydroxypyridine and 4-hydroxypyridine) have been investigated for antitumor activity against ovarian cancer cell lines: A2780, A2780^{cisR} and A2780^{ZD0473R}, it can be seen that TH5, TH6 and TH7 are less active than cisplatin. However, the activity of TH6 against A2780^{cisR} cell line is found to be more active than that of cisplatin and the activity of TH6 against A2780^{ZD0473R} cell line is found to be similar to that of cisplatin. Among the three *trans*-palladium complexes, TH6 is found to be most active while TH7 is found to be least active. The resistance factors of TH5, TH6 and TH7 are found to be significantly less than those of cisplatin, indicating that they may be able to overcome resistance of cisplatin [13]. 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 [14]. Mital's group reported the synthesis and cytotoxicity of nine palladium(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). The palladium(II) complexes are found to

Abbreviations: bipy, 2,2′-bipyridine; phen, 1,10-phenanthroline; Ala, L-alanine; Val, L-valine; Leu, L-leucine; Phe, L-phenylalanine; $TsalaH_2$, 4-toluenesulfonyl-L-alanine; $TsvalH_2$, 4-toluenesulfonyl-L-valine; $TsleuH_2$, 4-toluenesulfonyl-L-leucine; $TspheH_2$, 4-toluenesulfonyl-L-phenylalanine; DMF, dimethyl formamide; DMSO, dimethyl sulphoxide; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; SRB, sulforhodamine B; OD, optical density; SD, standard deviation.

* Corresponding author. Tel./fax: +86 0312 5079480.
E-mail address: jczhang6970@yahoo.com.cn (J. Zhang).

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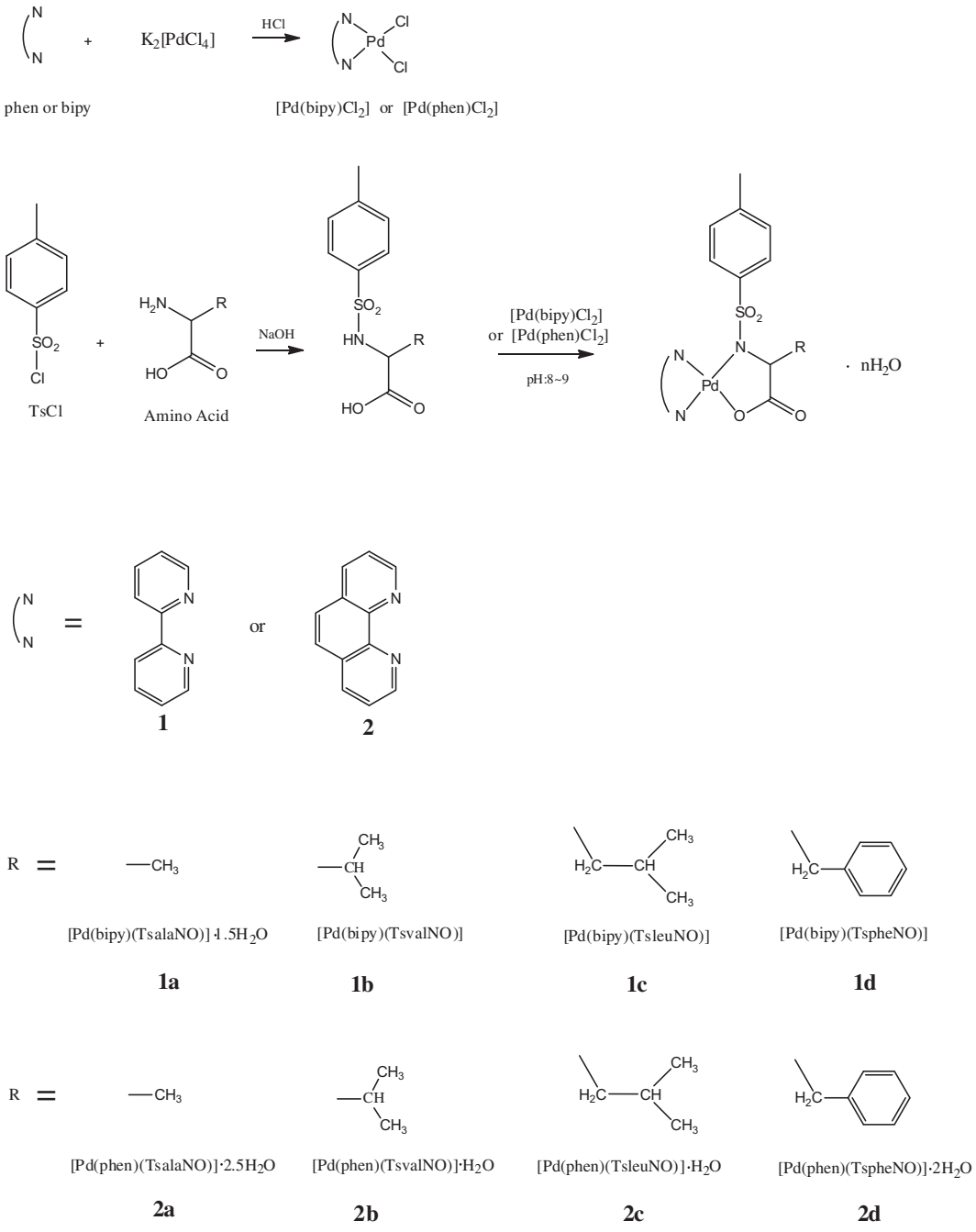
doi:10.1016/j.ejmech.2010.08.058

exhibit growth inhibition of P388 lymphocytic leukemic cells. The IC_{50} values for the palladium(II) complexes with glycine and L-valine are comparable to cisplatin, whereas the other palladium(II) complexes show higher IC_{50} values [15]. Until now, the cytotoxicity of mixed-ligand palladium(II) complexes with diimine and sulfonyl-L-amino acid dianion has not been reported, although some palladium(II) complexes with diimine and sulfonyl-L-amino acid dianion have been reported in order to characterize the type and stability constant of the complexes [16]. In the present work, we present the synthesis, characterization and cytotoxicity of eight new mixed-ligand palladium(II) complexes with 4-toluenesulfonyl-L-amino acid dianion and diimine (bipy and phen) for the first time.

2. Results and discussion

2.1. Synthesis and characterization

The palladium(II) complexes [Pd(bipy)(TsalaNO)]·1.5H_2O (**1a**), [Pd(bipy)(TsvalNO)] (**1b**), [Pd(bipy)(TsleuNO)] (**1c**), [Pd(bipy)(TspheNO)] (**1d**), [Pd(phen)(TsalaNO)]·2.5H_2O (**2a**), [Pd(phen)(TsvalNO)]·H_2O (**2b**), [Pd(phen)(TsleuNO)]·H_2O (**2c**) and [Pd(phen)(TspheNO)]·2H_2O (**2d**) have been prepared by the reaction of [Pd(bipy)Cl_2] or [Pd(phen)Cl_2] with 4-toluenesulfonyl-L-amino acids: TsalaH_2, TsvalH_2, TsleuH_2 or TspheH_2 in a mixture of CH_3OH/H_2O (See Scheme 1) [16].



Scheme. 1. The synthetic routines of the complexes (**1a**–**2d**).

The elemental analysis data of the complexes (**1a**–**2d**) are in good agreement with the calculated values. The mass spectra of the complexes (**1a**–**2d**) have molecular peaks, moreover, the peaks also provide support for the suggested composition and structures of the complexes.

Bipy and phen have a maximal absorption peak at 283 and 267 nm, which are assigned to internal $\pi-\pi^*$ type transition of bipy and phen, respectively. After formation of the complexes, the absorption peak red shifts by ca. 32 nm for the complexes (**1a**–**d**) compared with bipy, the absorption peak red shifts by ca. 10 nm for the complexes (**2a**–**d**) compared with phen, which may be caused by charge transfer transition (metal–ligand) from palladium d-orbital to a π^* orbital of bipy and phen.

The sulfonamide group of $TsalaH_2$, $TsvalH_2$, $TsleuH_2$ and $TspheH_2$ have a strong and sharp υ_{NH} in 3260–3290 cm^{-1} region. These peaks disappear for the complexes (**1a**–**2d**), showing that the sulfonamide group has been deprotonated. This is further confirmed by the sulfonamide (I) shifting from ~1630 cm^{-1} to ~1550 cm^{-1} and the disappearance of the sulfonamide (II) from original region. New bands appear at about 470 and 550 cm^{-1} and are assigned to υ_{Pd-N} and υ_{Pd-NAr}, respectively. The carboxylate group of the complexes (**1a**–**2d**) shows two bands, an intense antisymmetric carboxylate stretching $\upsilon_{(as,\ coo^-)}$ and a symmetric carboxylate stretching $\upsilon_{(s,\ coo^-)}$, at about 1650 and 1380 cm^{-1}, respectively. The values of $_{\Delta}\upsilon(coo^-)(\upsilon_{(as,\ coo^-)} - \upsilon_{(s,\ coo^-)})$ of the complexes (**1a**–**2d**) are in the range 248–281 cm^{-1}, which is greater than $_{\Delta}\upsilon(coo^-)$ of the corresponding sodium carboxylates, so the carboxylate group may be monodentate coordinated through oxygen atoms [17]. This is further confirmed by the appearance of the peaks of υ_{Pd-O}. These results are in good agreement with the results revealed by X-ray crystal analysis.

Although the overall pattern of the ^1H NMR spectra of the complexes (**1a**–**2d**) resembles very closely to that of the free ligand, the signals have been shifted upon coordination. $TsvalH_2$ show a doublet at $\delta = 5.35$, which is associated with the proton of the sulfonamide group, but these peaks disappear for the complexes, which shows that the sulfonamide group has been deprotonated (Fig. 1). The methylene ^1H resonances (amino acid) shifted to the down field as a result of deprotonated amide nitrogen coordinating to Pd(II). The β-hydrogen of $TsvalH_2$ appeared as a dd quartet, but in the complexes this proton appeared as a doublet, which also shows the deprotonation of amide group. These facts further confirmed that the sulfonamide group coordinates to palladium through deprotonated amide nitrogen atom (Fig. 2). The ^{13}C NMR spectra further provide support for the structures of the complexes (Fig. 3).

2.2. Structural studies

A view of the molecular structure of $[Pd(phen)(TspheNO)]\cdot 2H_2O$ (**2d**) is shown in Fig. 4. The selected bond lengths and angles of the complex are given in Table 1. The palladium atom shows

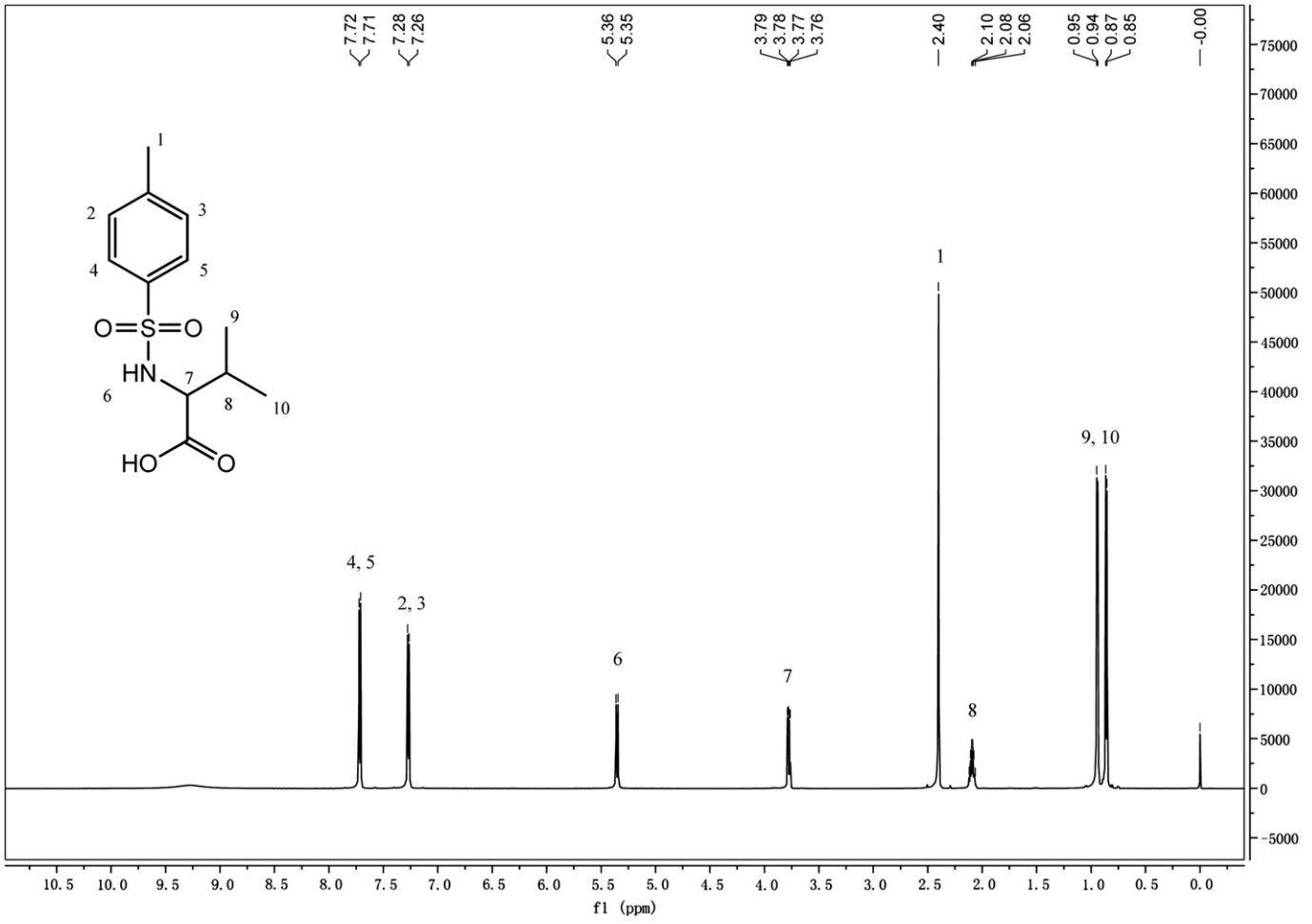


Fig. 1. ^1H NMR spectra of $TsvalH_2$ in $CDCl_3$.

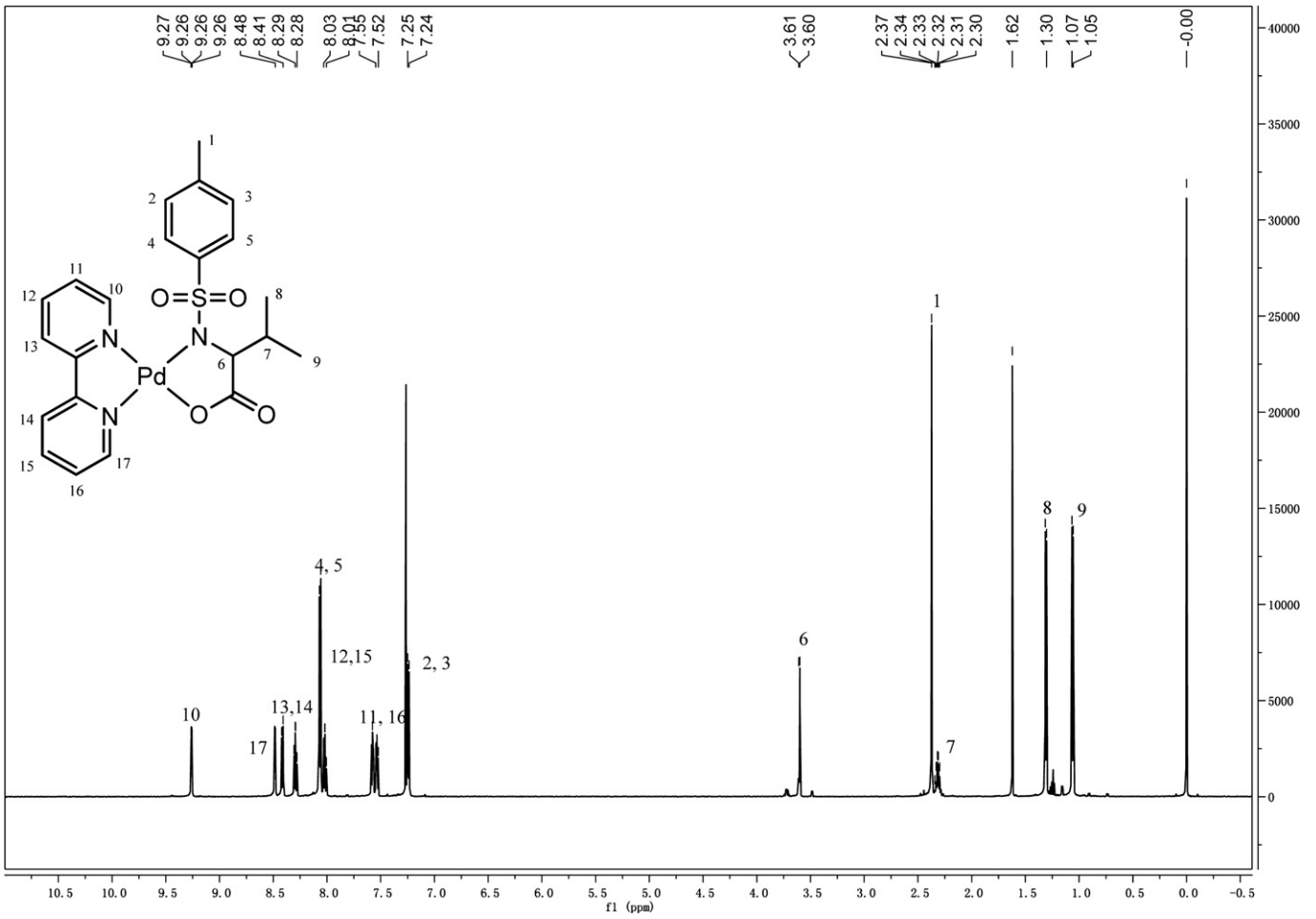


Fig. 2. ^{1}H NMR spectra of the complex (**1b**) in $CDCl_3$.

square-planar coordination given by two nitrogen atoms of phen, one deprotonated sulfonamide nitrogen atom and one carboxylic oxygen atom in each molecule. The angle between planar N(2)–Pd(1)–N(3) and planar O(1)–Pd(1)–N(1) is 2.592(102)° which indicates that the Pd(1)–O(1)–N(1)–N(2)–N(3) plane is slightly distorted. The Pd–N (deprotonated sulfonamide) bond length (2.0185(19) Å) is similar to the Pd–N (phen) bond lengths (2.003(2) and 2.029(2) Å), while it is longer than Pd–O (carboxylic oxygen) bond length (1.986(2) Å). Sigel et al. 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 confirmed by stability constants of some complexes [18]. Chen et al. also reported that the deprotonated amide nitrogen atom was exactly different from the ordinary amino nitrogen atom and its coordinating property may be "O-like" [19]. In the present work, a similar "O-like" coordinating property of the deprotonated sulfonamide nitrogen atom is also observed.

The π–π interaction link phen ligands into a stack chain along the a-axis. The aromatic rings are arranged in an offset face-to-face stacking mode. The intermolecular centroid–centroid distances are approximately 3.85 Å, and the interplanar dihedral angle is 3.64°, conforming to the approximate π–π interaction. A similar π–π stacking is also observed between the phenyl groups of the TspheNO ligand by the intermolecular and intramolecular, centroid–centroid distances which are 3.76 and 3.84 Å respectively. Hydrogen bonds are observed between H_2O molecular and the carboxylic oxygens, being the O…O distance approximately 3.13 Å. The other water molecular in the crystal cell cage does not form any hydrogen bond, hence we omitted it for clarity (Fig. 5).

2.3. Cytotoxic studies

As listed in Table 2, the complexes (**1a**–**2d**) exerted cytotoxic effects against tested carcinoma cell lines with a lower IC_{50} value (<50 μM), moreover, they have selectivity against tested carcinoma cell lines. Complex (**2c**) displayed the best cytotoxicity among the eight complexes against tested carcinoma cell lines. It can be seen that complex (**2c**) was more active than cisplatin against BGC-823, Bel-7402 and KB cell lines, but less active than cisplatin against HL-60 cell line. The BGC-823, HL-60 and KB cell lines demonstrated higher sensitivity to the complexes (**1c**, **2b** and **1d**) respectively. The complexes (**2a**–**d**) were more active than corresponding complexes (**1a**–**d**) against HL-60 cell line, the complexes (**2b**–**d**) demonstrated higher cytotoxicity than corresponding complexes (**1b**–**d**) against BGC-823 cell line, but the complexes (**1a**, **1c** and **1d**) were less active than the corresponding complexes (**2a**, **2c** and **2d**) against Bel-7402 cell line.

A series of palladium complexes with *N*,*N*′-dialkyl-1, 10-phenanthroline-dimathanamine shows significant cytotoxic activities against mouse leukemia L1210 and mouse liver carcinoma Bel-7402

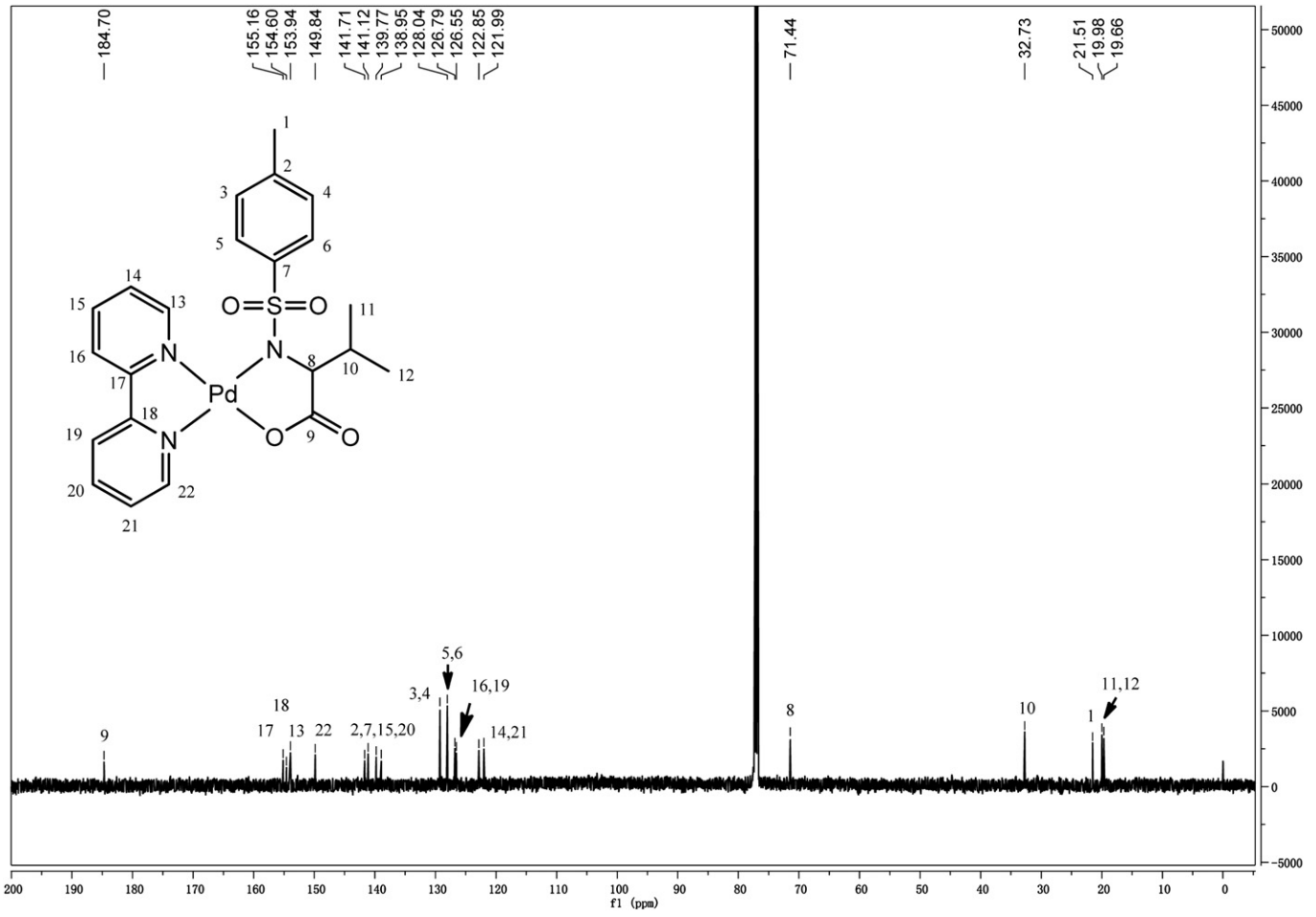


Fig. 3. ^{13}C NMR spectra of the complex (**1b**) in $CDCl_3$.

cell lines. The complexes show gradually increased cytotoxicity against the above mouse cell lines with an increase of lipophilicity of *R* groups from methyl, ethyl, propyl, tertbutyl, to cyclohexyl. The complexes containing propyl, tertbutyl, and cyclohexyl exhibit greater cytotoxic properties than cisplatin [20]. Puthraya et al. reported the synthesis and cytotoxicity of nine new palladium(II) complexes of the formula $[Pd(bipy)(AA)]^{n+}$ (where bipy is 2,2′-bipyridine, AA is an anion of L-cysteine, L-aspartic acid, L-glutamic acid, L-methionine, L-histidine, L-arginine, L-phenylalanine, L-tyrosine, or L-tryptophan, and $n = 0$ or 1). Among the effective $[Pt(bipy)AA]^{n+}$ complexes, side chain of the amino acids may influence the inhibitory activity. This inhibitory activity was found to be in decreasing order as follows: nonpolar hydrophobic > polar uncharged > charged side groups [21]. Mital et al. reported the synthesis and cytotoxicity of nine palladium(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). The IC_{50} values do not show definite correlation with variation of the amino acid side chains [15]. In our present work, for palladium(II) complexes with 4-toluenesulfonyl-L-amino acid dianion and bipy or phen, aromatic N-containing

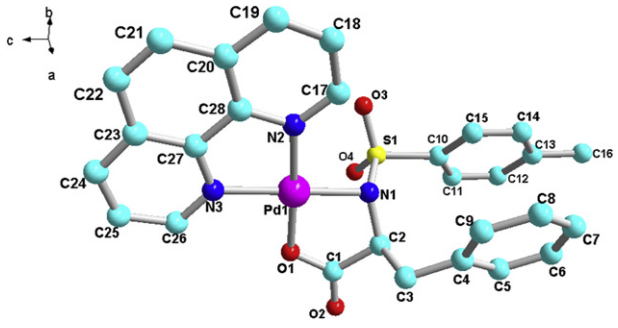


Fig. 4. Molecular structure and atom-labelling scheme for the complex (**2d**) (H_2O is omitted for clarity).

Table 1
Selected bond lengths (Å) and angles (°) for the complex (**2d**).

	2d
Pd(1)–N(1)	2.0185(19)
Pd(1)–N(2)	2.003(2)
Pd(1)–N(3)	2.029(2)
Pd(1)–O(1)	1.986(2)
N(1)–Pd(1)–N(2)	99.40(9)
N(2)–Pd(1)–N(3)	81.47(9)
N(3)–Pd(1)–O(1)	95.38(9)
O(1)–Pd(1)–N(1)	83.77(8)
O(1)–Pd(1)–N(2)	176.02(9)
N(1)–Pd(1)–N(3)	178.94(11)

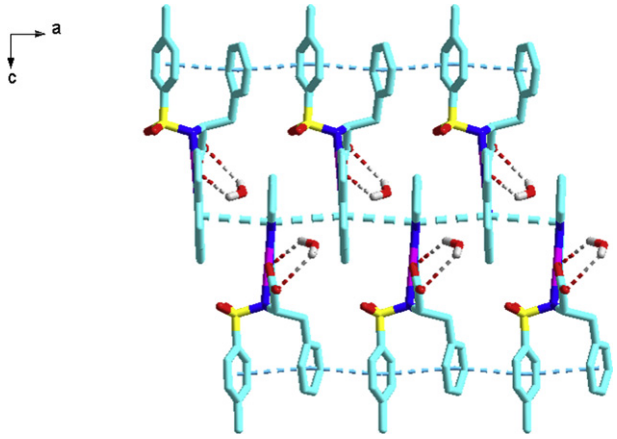


Fig. 5. View showing the weak pairing and hydrogen bonds of the complex (**2d**) molecule.

Table 2
The cytotoxicity of complexes *in vitro* ($n = 5$).

Complex	$IC_{50} \pm SD(\mu M)$			
	HL-60	BGC-823	Bel-7402	KB
1a	19.04 ± 2.11	21.31 ± 1.34	36.04 ± 2.87	36.33 ± 3.21
1b	12.70 ± 1.28	36.65 ± 2.59	39.79 ± 3.32	30.71 ± 2.64
1c	29.62 ± 2.20	7.54 ± 0.93	22.60 ± 1.67	28.27 ± 3.04
1d	18.44 ± 1.58	22.46 ± 1.33	38.41 ± 2.87	5.36 ± 0.78
2a	14.16 ± 1.25	23.00 ± 1.78	21.82 ± 1.14	36.55 ± 3.28
2b	7.95 ± 0.59	34.72 ± 3.04	44.14 ± 3.91	27.21 ± 3.85
2c	5.47 ± 0.32	4.48 ± 0.30	2.98 ± 0.19	2.08 ± 0.39
2d	7.24 ± 0.62	16.32 ± 2.13	15.40 ± 1.87	25.96 ± 1.91
Cisplatin	2.89 ± 0.34	6.48 ± 0.81	8.12 ± 0.97	2.65 ± 0.33

ligands have important effect on cytotoxicity. In general, the palladium(II) complexes with phen have better cytotoxicity than the corresponding palladium(II) complexes with bipy. In addition, although 4-toluenesulfonyl-L-amino acid has important effect on cytotoxicity, the IC_{50} values do not show definite correlation with variation of the amino acid.

Table 3
Crystallographic data for the complex (**2d**).

	2d
Formula	$C_{28}H_{27}N_3O_6PdS$
Fw	639.99
T(K)	296(2)
Cryst syst	Orthorhombic
Space group	P2(1)2(1)2(1)
a (Å)	7.5700(3)
b (Å)	11.2196(4)
c (Å)	32.4123(11)
V (nm^3)	2.75285(17)
Z	4
D_c (Mg m^{-3})	1.544
F(000)	1304
Cryst dimens (mm)	0.48 × 0.32 × 0.29
θ Range (deg)	1.92–26.32
hkl ranges	$-7 < h < 9$ $-13 < k < 14$ $-37 < l < 40$
Data/parameters	5592/370
Goodness-of-fit on F^2	1.025
Final *R* indices[$I > 2\sigma(I)$]	$R_1 = 0.0256$ $wR_2 = 0.0591$

3. Conclusions

In conclusion, the results indicated that palladium(II) complexes with 4-toluenesulfonyl-L-amino acid dianion and aromatic diimine might be a promising source of metal-based antitumor agents. Current studies are ongoing in our laboratory in order to gain a better insight in the mechanism of action of these palladium(II) complexes, which may be helpful for the design of new metal-based antitumor agents.

4. Experimental section

4.1. Materials

4-Toluenesulfonyl chloride and $K_2[PdCl_4]$ were of chemical grade, phen and bipy were of analytical grade. Commercially pure Ala, Val, Leu and Phe were purchased from Sigma. RPMI-1640 medium, trypsin and fetal bovine serum were purchased from Gibco. MTT and SRB, 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.

4.2. Instrumentation and measurement

Elemental analysis were 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 and ^{13}C NMR spectra were 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 optical density (OD) was measured on a microplate spectrophotometer (Bio-Rad Model 680, USA).

4.3. Synthesis of compounds

4-Toluenesulfonyl-L-amino acids were synthesized according to a published procedure. To a rapidly stirred solution of Ala (200 mg, 2.2 mmol) in 5.0 ml H_2O was added 2.2 ml NaOH (1 mol L^{-1}). 4-toluenesulfonyl chloride (427 mg, 2.2 mmol) was added to the solution, after 2.2 ml NaOH (1 mol L^{-1}) was added dropwise over 0.5 h. After further 6 h, the solution was cooled by ice and acidified to pH = 3 with HCl. The resulting white precipitate was filtered. The collected solid was washed with cold H_2O (50 ml) and dried to give $TsalaH_2$. $TsalaH_2$: ^1H NMR (600 MHz, DMSO, 25 °C) δ 1.14 (d, $J = 7.2$ Hz, 3H, CH_3), 2.37 (s, 1H, CH_3), 3.85–3.67 (m, 1H, CH), 7.37 (d, $J = 8.1$ Hz, 2H, ArH), 7.67 (d, $J = 7.9$ Hz, 2H, ArH), 8.04 (d, $J = 8.3$ Hz, 1H, NH).

$TsvalH_2$, $TsleuH_2$ and $TspheH_2$ were carried out in an identical manner to $TsalaH_2$. $TsvalH_2$: ^1H NMR (600 MHz, $CDCl_3$, 25 °C) δ 0.87 (d, $J = 8.2$ Hz, 3H, CH_3), 0.94 (d, $J = 6.8$ Hz, 3H, CH_3), 2.16–2.03 (m, 1H, CH), 2.40 (s, 3H, CH_3), 3.78 (dd, $J = 9.8$, 4.7 Hz, 1H, CH), 5.35 (d, $J = 9.8$ Hz, H, NH), 7.27 (d, $J = 8.0$ Hz, 2H, ArH), 7.72 (d, $J = 8.3$ Hz, 2H, ArH). $TsleuH_2$: ^1H NMR (600 MHz, $CDCl_3$, 25 °C) δ 0.81 (d, $J = 6.6$ Hz, 3H, CH_3), 0.89 (d, $J = 6.7$ Hz, 3H, CH_3), 1.59–1.42 (m, 2H, CH_2), 1.68–1.83 (m, 1H, CH), 2.41 (s, 3H, CH_3), 3.84–4.00 (m, 1H), 5.33 (d, $J = 9.7$ Hz, 1H, NH), 7.28 (d, $J = 8.0$ Hz, 2H, ArH), 7.73 (d, $J = 8.3$ Hz, 2H, ArH). $TspheH_2$: ^1H NMR (600 MHz, DMSO, 25 °C) δ 2.34 (s, 3H, CH_3), 2.83–2.66 (m, 1H, CH_2), 3.00–2.87 (m, 1H, CH_2), 3.82–3.92 (m, 1H, CH), 7.12 (s, 2H), 7.36–7.16 (m, 5H, ArH), 7.15–7.05 (m, 2H, ArH), 8.18 (d, $J = 5.0$ Hz, 1H, NH).

Precursor complexes [Pd(bipy)Cl_2] (**i**) and [Pd(phen)Cl_2] (**ii**) were synthesized according to a published procedure [22]. Yield: **i**: 72.6%, **ii**: 82.3%. **i**: Yellow solid. Anal. Calc. for $C_{10}H_8N_2Cl_2Pd$: C, 36.01; H, 2.42? N, 8.40. Found: C, 36.28; H, 2.63; N, 8.50. **ii**: Yellow solid. Anal. Calc. for $C_{12}H_8N_2Cl_2Pd$: C, 40.31; H, 2.26; N, 7.84. Found: C, 40.51; H, 2.35; N, 7.76.

4.3.1. Synthesis of [Pd(bipy)(TsalaNO)]·1.5H_2O (**1a**)

[Pd(bipy)Cl_2] (15 mg, 0.045 mmol) was added to a 3 ml CH_3OH/H_2O (volume 1:1) solution of TsalaH_2 (21 mg, 0.087 mmol) when the solution temperature was heated to 50 °C, the mixture was adjusted to pH = 8–9 by NaOH solution, then stirred for 2 h. The solution was heated in vacuo and concentrated to about 80% of the original volume. The complex (**1a**) was separated from the solution after a few days. Yield: 72.6%. Yellow solid. IR (KBr, cm^{-1}): 1641, 1375, 572, 468, 414. ^{1}H NMR (600 MHz, $CDCl_3$, 25 °C): δ1.60 (d, J = 7.2, 3H, CH_3), 2.40 (s, 3H, $ArCH_3$), 3.79 (q, J = 7.2, 1H, CH), 7.27 (s, 1H, ArH), 7.28 (s, 1H, ArH), 7.52–7.47 (m, 1H, ArH), 7.55 (m, 1H, ArH), 7.94 (m, 1H, ArH), 8.03 (s, 1H, ArH), 8.04 (s, 1H, ArH), 8.08 (d, J = 7.7, 1H, ArH), 8.38 (td, J = 7.9, 1.5, 1H, ArH), 8.41 (d, J = 5.5, 1H, ArH), 8.61 (d, J = 7.9, 1H, ArH), 9.13 (d, J = 5.6, 1H, ArH). ^{13}C NMR (150 MHz, $CDCl_3$) δ 184.97(C═O), 155.09, 154.77, 154.60, 150.10, 140.82, 140.17, 139.89, 138.69, 129.43, 127.95, 126.84, 126.75, 122.26, 121.75(Aryl-C), 55.91(CH_2), 21.51(CH_3), 18.02(CH_3). ESI-MS: 526.1 $[M + Na]^+$. Anal. Calc. for $C_{20}H_{22}N_3O_{5.5}PdS$ (530.89): C, 45.33; H, 3.99; N, 7.93. Found: C, 45.31; H, 4.20; N, 7.91.

4.3.2. Synthesis of [Pd(bipy)(TsvalNO)](**1b**)

The synthesis of **1b** was carried out in an identical manner to **1a** starting from [Pd(bipy)Cl_2] (15 mg, 0.045 mmol) and TsvalH_2 (24 mg, 0.090 mmol). Yellow solid. IR (KBr, cm^{-1}): 1658, 1384, 581, 478, 416. ^{1}H NMR (600 MHz, $CDCl_3$, 25 °C): δ 1.06 (d, J = 6.8, 3H, CH_3), 1.30 (t, J = 10.8, 3H, CH_3), 2.31 (m, 1H, CH), 2.37 (s, 3H, $ArCH_3$), 3.60 (d, J = 5.4, 1H, CH), 7.24 (s, 1H, ArH), 7.25 (s, 1H, ArH), 7.53 (m, 1H, ArH), 7.58 (m, 1H, ArH), 8.02 (m, 1H, ArH), 8.06 (d, J = 8.1, 3H, ArH), 8.29 (m, 1H, ArH), 8.42 (d, J = 8.0, 1H, ArH), 8.48 (dd, J = 5.4, 1.0, 1H, ArH), 9.26 (dd, J = 5.6, 0.9, 1H, ArH). ^{13}C NMR (150 MHz, $CDCl_3$) δ 184.70(C═O), 155.16, 154.60, 153.94, 149.84, 141.71, 141.12, 139.77, 138.95, 129.25, 128.04, 126.79, 126.55, 122.85, 121.99(Aryl-C), 71.44(CH), 32.73(CH), 21.51(CH_3), 19.98(CH_3), 19.66(CH_3). ESI-MS: 554.0 $[M + Na]^+$. Anal. Calc. for $C_{22}H_{23}N_3O_4PdS$ (531.04): C, 49.68; H, 4,36; N, 7.90. Found: C, 49.67; H, 4.48; N, 7.87.

4.3.3. Synthesis of [Pd(bipy)(TsleuNO)] (**1c**)

The synthesis of **1c** was carried out in an identical manner to **1a** starting from [Pd(bipy)Cl_2] (15 mg, 0.045 mmol) and TsleuH$_2$ (26 mg, 0.091 mmol). Yellow solid. IR (KBr, cm^{-1}): 1658, 1384, 572, 463, 408. ^{1}H NMR (600 MHz, DMSO, 25 °C): δ 0.73 (d, J = 6.5, 3H, CH_3), 0.88 (d, J = 6.7, 3H, CH_3), 1.53 (m, 1H, CH), 1.92–1.84 (m, 1H, CH_2), 2.13–2.05 (m, 1H, CH_2), 2.36 (s, 3H, $ArCH_3$), 3.48 (dd, J = 10.4, 4.3, 1H, CH), 7.29 (s, 1H, ArH), 7.30 (s, 1H, ArH), 7.80 (m, 1H, ArH), 7.91–7.86 (m, 1H, ArH), 7.94 (s, 1H, ArH), 7.95 (s, 1H, ArH), 8.31 (dd, J = 5.5, 0.9, 1H, ArH), 8.38 (m, 2H, ArH), 8.58 (d, J = 7.6, 1H, ArH), 8.62 (d, J = 8.0, 1H, ArH), 8.97 (dd, J = 5.7, 1.0, 1H, ArH). ^{13}C NMR (150 MHz, DMSO) δ 184.19(C═O), 155.37, 154.82, 153.88, 149.70, 141.48, 140.97, 139.90, 138.92, 129.01, 127.51, 127.12, 126.99, 123.32, 121.66(Aryl-C), 63.95(CH), 45.14(CH_2), 23.97(CH), 23.38(CH_3), 21.26(CH_3), 20.90 (CH_3). ESI-MS:568.1 $[M + Na]^+$. Anal. Calc. for $C_{23}H_{25}N_3O_4PdS$ (545.06): C, 50.60; H, 4.62; N, 7.70. Found: C, 50.69; H, 4.86; N, 7.46.

4.3.4. Synthesis of [Pd(bipy)(TspheNO)] (**1d**)

The synthesis of **1d** was carried out in an identical manner to **1a** starting from [Pd(bipy)Cl_2] (15 mg, 0.045 mmol) and TspheH$_2$ (29 mg, 0.091 mmol). Yellow solid. IR (KBr, cm^{-1}): 1640, 1384, 590, 463, 406. ^{1}H NMR (600 MHz, $CDCl_3$, 25 °C): δ 2.53–2.26 (m, 3H, $ArCH_3$), 3.07–2.98 (m, 1H, CH_2), 3.34 (m, 1H, CH_2), 4.11–4.01 (m, 1H, CH), 6.75 (d, J = 6.1, 1H, ArH), 6.78 (t, J = 7.2, 2H, ArH), 7.18 (d, J = 7.9, 2H, ArH), 7.22 (d, J = 5.0, 2H, ArH), 7.53–7.43 (m, 2H, ArH), 7.87 (t, J = 7.9, 2H, ArH), 7.91 (dd, J = 15.1, 7.5, 1H, ArH), 7.97 (d, J = 8.4, 1H, ArH), 8.18 (d, J = 4.9, 1H, ArH), 8.36–8.27 (m, 1H, ArH), 8.52–8.41 (m, 1H, ArH), 9.04 (d, J = 6.9, 1H, ArH). ^{13}C NMR (150 MHz, $CDCl_3$) δ 184.54(C═O), 154.96, 153.93, 153.61, 149.22, 141.74, 141.47, 139.45, 138.64, 138.14, 130.92, 129.29, 127.77, 127.35, 126.51, 125.95, 125.71, 123.26, 121.94(Aryl-C), 67.51(CH), 40.98(CH_2), 21.50(CH_3). ESI-MS:602.1 $[M + Na]^+$. Anal. Calc. for $C_{26}H_{23}N_3O_4PdS$ (579.04): C, 53.84; H, 4.00; N, 7.25. Found: C, 53.94; H, 4.02; N, 7.22.

4.3.5. Synthesis of [Pd(phen)(TsalaNO)]·2.5H_2O (**2a**)

The synthesis of **2a** was carried out in an identical manner to **1a** starting from [Pd(phen)Cl_2] (15 mg, 0.042 mmol) and TsalaH_2 (20 mg, 0.083 mmol). Yellow solid. IR (KBr, cm^{-1}): 1639, 1384, 573, 462, 407. ^{1}H NMR (600 MHz, $CDCl_3$, 25 °C): δ 1.75 (d, J = 7.2, 3H, CH_3), 2.38 (d, J = 21.3, 3H, ArH_3), 4.04 (q, J = 7.2, 1H, ArH), 7.23 (s, 1H, ArH), 7.24 (s, 1H, ArH), 7.87 (dd, J = 8.2, 5.1, 1H, ArH), 7.95 (dd, J = 8.2, 5.3, 1H, ArH), 8.08–8.01 (m, 2H, ArH), 8.15 (s, 1H, ArH), 8.17 (s, 1H, ArH), 8.59 (dd, J = 8.2, 1.2, 1H, ArH), 8.63 (dd, J = 8.2, 1.3, 1H, ArH), 8.94 (d, J = 5.1, 1H, ArH), 9.70 (d, J = 5.3, 1H, ArH). ^{13}C NMR (150 MHz, DMSO) δ 181.42(C═O), 154.05, 149.89, 146.59, 145.76, 141.55, 140.49, 140.27, 140.23, 130.35, 130.23, 129.70, 128.09, 127.97, 127.66, 126.54, 126.18(Aryl-C), 56.02(CH_2), 21.40(CH_3), 18.23(CH_3). ESI-MS: 550.0 $[M + Na]^+$. Anal. Calc. for $C_{22}H_{24}N_3O_{6.5}PdS$ (572.93): C, 46.12; H, 4.22; N, 7.33. Found: C, 46.19; H, 4.12; N, 7.31.

4.3.6. Synthesis of [Pd(phen)(TsvalNO)]·H_2O(**2b**)

The synthesis of **2b** was carried out in an identical manner to **1a** starting from [Pd(phen)Cl_2] (15 mg, 0.042 mmol) and TsvalH_2 (23 mg, 0.086 mmol). Yellow solid. IR (KBr, cm^{-1}): 1632, 1384, 583, 479, 403. ^{1}H NMR (600 MHz, $CDCl_3$, 25 °C): δ 1.23 (t, J = 11.5, 3H, CH_3), 1.41 (d, J = 6.7, 3H, CH_3), 2.35 (s, 3H, $ArCH_3$), 2.39 (m, 1H, CH), 3.79 (d, J = 5.3, 1H, CH), 7.21 (s, 1H, ArH), 7.22 (s, 1H, ArH), 7.86 (dd, J = 8.2, 5.1, 1H, ArH), 7.97 (dd, J = 8.2, 5.3, 1H, ArH), 8.08–8.01 (m, 2H, ArH), 8.15 (s, 1H, ArH), 8.16 (s, 1H, ArH), 8.60 (dd, J = 8.2, 1.3, 1H, ArH), 8.62 (dd, J = 8.2, 1.3, 1H, ArH), 8.91 (dd, J = 5.1, 1.3, 1H, ArH), 9.71 (dd, J = 5.2, 1.3, 1H, ArH). ^{13}C NMR (150 MHz, $CDCl_3$) δ 184.74 (C═O), 154.58, 150.12, 146.72, 145.98, 141.35, 140.00, 138.70, 138.34, 129.81, 129.74, 129.26, 127.84, 127.49, 126.76, 125.69, 125.31 (Aryl-C), 71.87 (CH), 32.93 (CH), 21.44 (CH_3), 20.03 (CH_3), 19.65 (CH_3). ESI-MS: 578.1 $[M + Na]^+$. Anal. Calc. for $C_{24}H_{25}N_3O_5PdS$ (573.96): C, 50.22; H, 4.39; N, 7.32. Found: C, 50.28; H, 4.28; N, 7.27.

4.3.7. Synthesis of [Pd(phen)(TsleuNO)]·H_2O(**2c**)

The synthesis of **2c** was carried out in an identical manner to **1a** starting from [Pd(phen)Cl_2] (15 mg, 0.042 mmol) and TsleuH$_2$ (24 mg, 0.084 mmol). Yellow solid. IR (KBr, cm^{-1}): 1665, 1384, 575, 474, 404. ^{1}H NMR (600 MHz, $CDCl_3$, 25 °C): δ 0.98 (s, 3H, CH_3), 0.99 (s, 3H, CH_3), 1.84 (m, 1H, CH), 2.16–2.09 (m, 1H, CH_2), 2.26 (m, 1H, CH_2), 2.35 (s, 3H, $ArCH_3$), 3.95 (dd, J = 10.3, 4.2, 1H, CH), 7.22 (s, 1H, ArH), 7.23 (s, 1H, ArH), 7.86 (dd, J = 8.2, 5.1, 1H, ArH), 7.95 (dd, J = 8.2, 5.2, 1H, ArH), 8.08–8.01 (m, 2H, ArH), 8.14 (s, 1H, ArH), 8.16 (s, 1H, ArH), 8.59 (m, 1H, ArH), 8.64–8.61 (m, 1H, ArH), 8.89 (dd, J = 5.0, 1.1, 1H, ArH), 9.68–9.63 (m, 1H, ArH). ^{13}C NMR (150 MHz, $CDCl_3$) δ 186.10(C═O), 154.50, 150.01, 146.71, 145.92, 141.47, 139.80, 138.82, 138.41, 129.80, 129.31, 127.83, 127.51, 126.81, 125.70, 125.36 (Aryl-C), 65.33(CH), 45.94(CH_2), 24.67(CH), 23.64(CH_3), 21.65(CH_3), 21.50(CH_3). ESI-MS: 592.0 $[M + Na]^+$. Anal. Calc. for $C_{25}H_{27}N_3O_5PdS$ (587.98): C, 51.07; H, 4.63; N, 7.15. Found: C, 51.07; H, 4.61; N, 7.09.

4.3.8. Synthesis of [Pd(phen)(TspheNO)]·2H_2O (**2d**)

The synthesis of **2d** was carried out in an identical manner to **1a** starting from [Pd(phen)Cl_2] (15 mg, 0.042 mmol) and TspheH$_2$

(27 mg, 0.084 mmol). Yellow solid. IR (KBr, cm^{-1}): 1635, 1385, 583, 474, 402. ^1H NMR (600 MHz, $CDCl_3$, 25 °C): δ 2.35 (s, 3H, $ArCH_3$), 3.16 (dd, $J = 13.2, 5.6$, 1H, CH_2), 3.42 (dd, $J = 13.2, 4.5$, 1H, CH_2), 4.34 (dd, $J = 5.4, 4.7$, 1H, CH), 6.32 (t, $J = 7.4$, 1H, ArH), 6.57 (t, $J = 7.6$, 2H, ArH), 7.19 (s, 1H, ArH), 7.21 (s, 1H, ArH), 7.34 (s, 1H, ArH), 7.35 (s, 1H, ArH), 7.79 (dd, $J = 8.2, 5.1$, 1H, ArH), 7.93 (dd, $J = 8.2, 5.2$, 1H, ArH), 8.03–7.97 (m, 2H, ArH), 8.06 (s, 1H, ArH), 8.07 (s, 1H, ArH), 8.55 (dd, $J = 4.3, 1.3$, 1H, ArH), 8.57 (dd, $J = 4.3, 1.2$, 1H, ArH), 8.66 (dd, $J = 5.0, 1.2$, 1H, ArH), 9.57 (dd, $J = 5.2, 1.1$, 1H, ArH). ^{13}C NMR (150 MHz, $CDCl_3$) δ 184.58(C═O), 154.29, 149.67, 146.38, 145.53, 141.47, 139.43, 138.74, 138.61, 138.18, 131.15, 129.50, 129.40, 129.29, 127.65, 127.34, 127.06, 126.75, 125.40, 125.36, 125.11(Aryl-C), 68.08(CH), 41.32 (CH_2), 21.48(CH_3). ESI-MS: 626.1 $[M + Na]^+$. Anal. Calc. for $C_{28}H_{27}N_3O_6PdS$ (640.02): C, 52.55; H, 4.25; N, 6.57. Found: C, 52.32; H, 4.32; N, 6.50.

4.4. Data collection and structural refinement of the complex (**2d**)

The data collection of the complex (**2d**) was performed on a Bruker SMART APEX II CCD diffractometer equipped with a graphite monochromatized Mo Kα radiation ($\lambda = 0.71073$ Å) at 296(2) K. 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. Table 3 lists crystallographic details. Crystallographic data for the structural analysis of **2d** have been deposited with the Cambridge Crystallographic Data Centre, CCDC-756219 (**2d**). 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.ukor http://www.ccdc.cam.ac.uk).

4.5. Cell culture

Four different human carcinoma cell lines: HL-60, Bel-7402, BGC-823 and KB were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 units/ml of penicillin and 100 μg/ml of streptomycin. Cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2 in air.

4.6. Solutions

The complexes were dissolved in DMSO at a concentration of 5 mM as stock solution, and diluted in culture medium at concentrations of 1.0, 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.

4.7. Cytotoxicity analysis

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 [23]. 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 [24]. 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.

Acknowledgements

This work was supported by the Special Foundation for State Major New Drug Research Program of China (Grant No. 2009ZX09103-139), the National Basic Research 973 Program (Grant No. 2010CB534913), the Research Project Foundation of Shijiazhuang Bureau of Science Technology (Grant No. 07120053A), the Research Project Foundation of Baoding Bureau of Science Technology (Grant No. 07F05), the Key Basic Research Special Foundation of Science Technology Ministry of Hebei Province (Grant No. 08966415D), the Research Project Foundation of Department of Education of Hebei Province (Grant No.2008311).

References

[1] B. Rosenberg, L. Van Camp, T. Krigas, Nature 205 (1965) 698–699.
[2] Y.P. Ho, S.C.F. Au-Yeung, K.K.W. To, Med. Res. Rev. 23 (2003) 633–655.
[3] I. Kostova, Recent Pat. Anticancer Drug Discov. 1 (2006) 1–22.
[4] M.A. Jakupec, M. Galanski, B.K. Keppler, Rev. Physiol. Biochem. Pharmacol. 146 (2003) 1–53.
[5] E. Wong, C.M. Giandomenico, Chem. Rev. 99 (1999) 2451–2466.
[6] L. Giovagnini, L. Ronconi, D. Aldinucci, D. Lorenzon, S. Sitran, D. Fregona, J. Med. Chem. 48 (2005) 1588–1595.
[7] A.G. Quiroga, C. Navarro Ranninger, Coord. Chem. Rev. 248 (2004) 119–133.
[8] S. Ray, R. Mohan, J.K. Singh, M.K. Samantaray, M.M. Shaikh, D. Panda, P. Ghosh, J. Am. Chem. Soc. 129 (2007) 15042–15053.
[9] F.V. Rocha, C.V. Barra, A.V.G. Netto, A.E. Mauro, I.Z. Carlos, R.C.G. Frema, S.R. Ananias, M.B. Quilles, A. Stevanato, M.C. da Rocha, Eur. J. Med. Chem. 45 (2010) 1698–1702.
[10] J. Ruiz, J. Lorenzo, C. Vicente, G. López, J.M. Ló pez-de-Luzuriaga, M. Monge, F.X. Avilés, D. Bautista, V. Moreno, A. Laguna, Inorg. Chem. 47 (2008) 6990–7001.
[11] E.J. Gao, M.C. Zhu, Y. Huang, L. Liu, H.Y. Liu, F.C. Liu, S. Ma, C.Y. Shi, Eur. J. Med. Chem. 45 (2010) 1034–1041.
[12] J.K. Barton, Science 233 (1986) 727–734.
[13] F. Huq, H. Tayyem, P. Beale, J. Yu, J. Inorg. Biochem. 101 (2007) 30–35.
[14] A. Matilla, J.M. Tercero, N.-H. Dung, B. Voissat, J.M. Pérez, C. Alonso, D. Martn-Ramos, J. Niclos-Gutierrez, J. Inorg. Biochem. 55 (1994) 235–247.
[15] R. Mital, T.S. Srivastava, H.K. Parekh, M.P. Chitnis, J. Inorg. Biochem. 41 (1991) 93–103.
[16] G.B. Gavioli, M. Borsari, L. Menabue, M. Saladini, G.C. Pellacani, M. Sola, J. Chem. Soc. Dalton Trans. 5 (1990) 1585–1587.
[17] G.B. Deacon, R.J. Phillips, Coord. Chem. Rev. 33 (1980) 227–250.
[18] H. Sigel, B.E. Fischer, B. Prijs, J. Am. Chem. Soc. 99 (1977) 4489.
[19] Y.Q. Gong, Y.F. Chen, J.M. Gu, X.R. Hu, Polyhedron 16 (1997) 3743–3746.
[20] G. Zhao, H. Sun, H. Lin, S. Zhu, X. Su, Y. Chen, J. Inorg. Biochem. 72 (1998) 173–177.
[21] K.H. Puthraya, T.S. Srivastava, A.J. Amonkar, M.K. Adwankar, M.P. Chitnis, J. Inorg. Biochem. 26 (1986) 45–54.
[22] F.A. Palocsay, J.V. Rund, Inorg. Chem. 8 (1969) 524–528.
[23] T. Mosmann, J. Immunol. Methods 65 (1983) 55–63.
[24] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney, M.R. Boyd, J. Natl. Cancer Inst. 82 (1990) 1107–1112.