European Journal of Medicinal Chemistry 58 (2012) 281–286

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

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

Short communication

Synthesis, characterization and cytotoxicity of platinum(II)/palladium(II) complexes with 1,3-diaminopropane and 4-toluensulfonyl-L-amino acid dianion

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ARTICLE INFO

Article history:
Received 28 July 2012
Received in revised form
22 September 2012
Accepted 24 September 2012
Available online 23 October 2012

keywords:
Platinum(II)/palladium(II)
Synthesis
4-Toluenesulfonyl-L-amino acids
1,3-Diaminopropane
Cytotoxicity

ABSTRACT

Eight novel platinum(II)/palladium(II) complexes with 1,3-dap and 4-toluensulfonyl-L-amino acid dianion, [Pt(1,3-dap)(TsalaNO)]·0.5H_2O (**1a**), [Pt(1,3-dap)(TsvalNO)] (**1b**), [Pt(1,3-dap)(TspheNO)] (**1c**), [Pt(1,3-dap)(TsserNO)] (**1d**), [Pd(1,3-dap)(TsalaNO)]·1.5H_2O (**2a**), [Pd(1,3-dap)(TsvalNO)] (**2b**), [Pd(1,3-dap)(TspheNO)] (**2c**) and [Pd(1,3-dap)(TsileNO)] (**2d**) have been synthesized and characterized by elemental analysis, IR, UV, ^{1}H NMR and mass spectrometry techniques. Crystal structure of the complex **1b** has been determined by X-ray diffraction. The cytotoxicity was tested by MTT and SRB assays. The complexes (**1a**–**1d** and **2a**–**2d**) exert cytotoxicity against Bel-7402, HL-60, KB and BGC-823, but none of them is more active than cisplatin. The results suggest that metal ions, amino acids and aliphatic N-containing ligands have effect on cytotoxicity, while the IC_{50} values do not show definite correlation with variation of them.

1. Introduction

Nowadays, cisplatin is still one of the most successful antineoplastic drugs, which is used both alone and in combination with other drugs for the clinical treatment of numerous types of cancers including bladder, ovarian, head and neck, testicular and lung cancers [1]. Cisplatin administration is often limited by severe toxic side-effects, as well as the intrinsic and acquired resistance possessed by various cancers [2–4]. To overcome the shortages of cisplatin, numerous platinum-based compounds have been developed. Nevertheless, only carboplatin and oxaliplatin have received worldwide approval, nedaplatin, lobaplatin and heptaplatin have gained regionally limited approval and a few platinum drugs continue to be evaluated in clinical trials [5,6]. These drawbacks have spurred the chemists to find alternative chemotherapeutic strategies [7–9].

Based on the structural and thermodynamic similarity between platinum(II) and palladium(II) complexes, there is an increased interest in the study of palladium(II) derivatives as potential anticancer drugs [10,11]. Aromatic N-containing ligands, including pyridine, quinoline, phenanthroline and their derivatives, were introduced into metal-based anticancer drugs because of their ability to participate as DNA intercalators [12–14]. 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 [15]. A series of platinum(II)/palladium(II) complexes with aromatic N-containing ligands and amino acids have shown significant cytotoxic activities. Jin et al. synthesized and characterized nine complexes of platinum(II) with phen and amino acids (where amino acids are Gly, His, Cys, Ile, Ala, Pro, Ser, Asp and Glu). The results indicated that the IC_{50} value of [Pt(phen)(Pro)]$Cl_2\cdot H_2O$ was similar to cisplatin, though most complexes were less cytotoxic than cisplatin [16]. In addition, the cytotoxicity of some platinum(II) complexes with phen and amino acids (where amino acids are Gly, Ala, Leu, and Tyr) were reported by Mital et al. These complexes exhibit growth inhibition of P388 lymphocytic leukemic cells, but the IC_{50} values for the platinum(II) complexes are higher than those of cisplatin [17]. Puthraya et al. 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). Among the effective $[Pd(bipy)(AA)]^{n+}$ complexes, side chain of the amino acids may influence the inhibitory activity. This inhibitory activity was found to be in decreasing order as follows:

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http://dx.doi.org/10.1016/j.ejmech.2012.09.052

nonpolar hydrophobic > polar uncharged > charged side groups [12]. Mital et al. 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), but the IC_{50} values do not show definite correlation with variation of the amino acid side chains [18]. The cytotoxic study of $[Pd(AMBI)(AA)]^{n+}$ (where AA is an anion of Gly, Ala, Cys, Met, or Ser) was reported by A.A. El-Sherif. The result indicated that $[Pd(AMBI)(Met)]Cl\cdot H_2O$ showed significant activity against HCT116 cells with IC_{50} value of 0.74 μg mL^{-1}, while [Pd(AMBI)(Cys)] showed cytotoxicity against HEP2 with IC_{50} value of 0.60 μg mL^{-1}. Their cytotoxicity against HCT116 and HEP2 decreased in the sequences: Met > Ser > Ala > Gly > Cys, and Cys > Ser > Ala > Met > Gly, respectively [19]. We previously reported the synthesis and cytotoxicity of $[Pd(bipy)(TsAANO)]\cdot nH_2O$ and $[Pd(phen)(TsAANO)]\cdot nH_2O$ (where AA is Ala, Val, Leu, or Phe and $n = 0, 1, 1.5, 2$, or 2.5). The results indicated that the complexes had cytotoxicity against BGC-823, Bel-7402, KB and HL-60, moreover, the complex $[Pd(phen)(TsleuNO)]\cdot H_2O$ showed higher cytotoxicity than cisplatin against BGC-823, Bel-7402 and KB [20].

Considering that all the clinically used platinum-based anticancer drugs have aliphatic N-containing ligands as carrier groups, the structure–activity relationships of the five-, six- and seven-membered ring structure (which are abbreviated as 5-R, 6-R and 7-R, respectively) alky-1,4-butanediamine Pt(II) complexes with two different leaving groups (either dichloride or cyclobutane-1,1-dicarboxylate) toward L1210 cells were summarized. Among these complexes, 7-R had the highest cytotoxic activity [21]. We previously reported the synthesis and cytotoxicity of five palladium(II) complexes $[Pd(en)(TsAANO)]\cdot nH_2O$ (where AA is Ser, Gly, Ala, Leu, or Phe and $n = 0, 1, 1.5$, or 2). The results indicated that the complexes demonstrated the best cytotoxicity against HL-60, BGC-823, Bel-7402, and KB when amino acid was Phe [22]. In order to further explore the structure–activity relationships of platinum(II)/palladium(II) complexes with 4-toluenesulfonyl-L-amino acid dianion and N-containing ligands, and find new metal-based anticancer drugs, the synthesis, characterization and cytotoxicity of eight new platinum(II)/palladium(II) complexes (**1a**–**1d** and **2a**–**2d**) with 4-toluenesulfonyl-L-amino acid dianion and 1,3-dap are reported in this paper for the first time.

2. Result and discussion

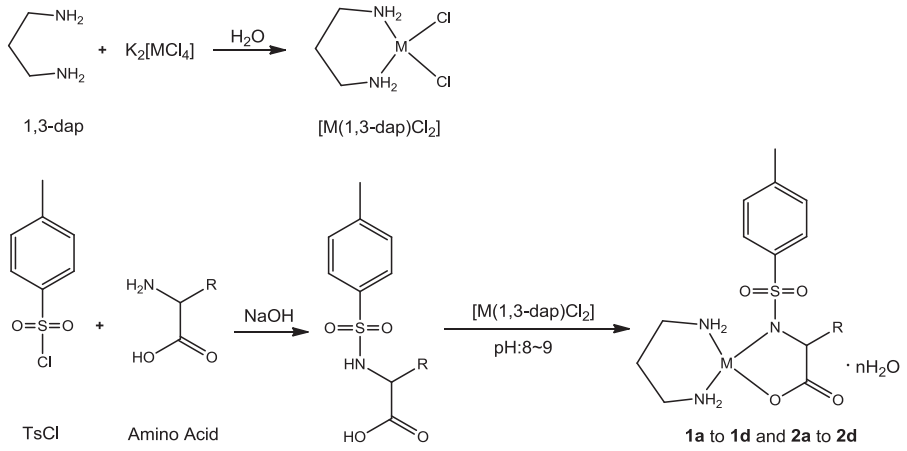
2.1. Synthesis and characterization

The platinum(II)/palladium(II) complexes $[Pt(1,3\text{-dap})(TsalaNO)]\cdot 0.5H_2O$ (**1a**), $[Pt(1,3\text{-dap})(TsvalNO)]$ (**1b**), $[Pt(1,3\text{-dap})(TspheNO)]$ (**1c**), $[Pt(1,3\text{-dap})(TsserNO)]$ (**1d**), $[Pd(1,3\text{-dap})(TsalaNO)]\cdot 1.5H_2O$ (**2a**), $[Pd(1,3\text{-dap})(TsvalNO)]$ (**2b**), $[Pd(1,3\text{-dap})(TspheNO)]$ (**2c**) and $[Pd(1,3\text{-dap})(TsileNO)]$ (**2d**) have been prepared by the reaction of $[Pt(1,3\text{-dap})Cl_2]$ or $[Pd(1,3\text{-dap})Cl_2]$ with 4-toluenesulfonyl-L-amino acids: $TsalaH_2$, $TsvalH_2$, $TspheH_2$, $TsserH_2$ or $TsileH_2$ in a mixture of CH_3OH/H_2O (see Scheme 1).

The complex **2c** was observed in the ESI-MS as singly charged $[M + Na]^+$ ion of m/z 520.0. The experimental isotope pattern for this ion matches theoretical prediction (see Fig. S1). ESI-MS of the other seven complexes is similar to that of the complex **2c**. These results provide evidence for the formation of the complexes (**1a**–**1d** and **2a**–**2d**). What's more, there is good agreement between calculated and measured values for elemental analysis of the complexes (**1a**–**1d** and **2a**–**2d**).

4-Toluenesulfonyl-L-amino acids have shoulders at 267 and 273 nm in UV–vis spectroscopy. After formation of the complexes, the shoulders turn into a single peak at 266 nm for the complexes (**1a**–**1d**) and at 268 nm for the complexes (**2a**–**2d**), respectively. In addition, there is also a broad MLCT peak at 360–365 nm for the complexes (**1a**–**1d**) and at 360–370 nm for the complexes (**2a**–**2d**), respectively, which further confirms the coordination of the platinum(II)/palladium(II) and 1,3-dap.

The IR spectra of the complexes (**1a**–**1d** and **2a**–**2d**) are similar. The sulfonamide groups of $TsalaH_2$, $TsvalH_2$, $TspheH_2$, $TsserH_2$ and $TsileH_2$ have a strong and sharp υ_{NH} in 3260–3290 cm^{-1} region. These bands disappear for the complexes (**1a**–**1d** and **2a**–**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 at about 550 cm^{-1} are assigned to $\upsilon_{Pd-N}/\upsilon_{Pt-N}$. The carboxylate group of the complexes (**1a**–**1d** and **2a**–**2d**) shows two bands, an intense antisymmetric carboxylate stretching $\upsilon_{(as,\ coo^-)}$ and a symmetric carboxylate



For **1a** to **1d**, M= Pt,

1a: R= -CH_3, n= 0.5; **1b**: R= -$CH(CH_3)_2$, n= 0; **1c:** R= -$CH_2(C_6H_5)$, n= 0; **1d**: R= -CH_2OH; n= 0;

For **2a** to **2d**, M= Pd,

2a: R= -CH_3, n= 1.5; **2b**: R= -$CH(CH_3)_2$, n= 0; **2c**: R= -$CH_2(C_6H_5)$, n= 0; **2d**: R= -$CH(CH_3)C_2H_5$, n= 0.

Scheme 1. Synthetic routines of the complexes (**1a**–**1d** and **2a**–**2d**).

stretching $\upsilon_{(s,\ coo^-)}$, at about 1620 and 1360 cm^{-1}, respectively. The values of $\Delta\upsilon_{(coo^-)}(\upsilon_{(as,\ coo^-)} - \upsilon_{(s,\ coo^-)})$ of the complexes (**1a**–**1d** and **2a**–**2d**) are in the range 217–328 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 [23]. This is further confirmed by the appearance of the bands of υ_{Pd-O} and υ_{Pt-O}. These results are in good agreement with the results revealed by X-ray crystal analysis.

The overall pattern of the ^{1}H NMR spectra of the complexes (**1a**–**1d** and **2a**–**2d**) resembles very closely to that of the free ligands. TsvalH$_2$ shows a doublet at $\delta = 5.35$, which is associated with the proton of the sulfonamide group, but these peaks disappear for the complex **1b**, which showing that the sulfonamide group has been deprotonated. The α-hydrogen of TsvalH$_2$ appears as a *dd* quartet, but this proton appears as a doublet in the complex **1b**, which also shows the deprotonation of sulfonamide group (see Fig. S2 and S3). ^{1}H NMR spectra of the complexes (**1a**, **1c**, **1d** and **2a**–**2d**) are similar to that of the complex **1b**. These facts further confirm that the sulfonamide group coordinates to platinum(II)/palladium(II) through deprotonated sulfonamide nitrogen atom.

2.2. Structural studies

A view of the molecular structure of [Pt(1,3-dap)(TsvalNO)] (**1b**) is exhibited in Fig. 1. The selected bond lengths and angles of the complex are given in Table 1. The platinum atom shows square-planar coordination given by two nitrogen atoms of 1,3-dap, one deprotonated sulfonamide nitrogen atom and one carboxylic oxygen atom in each molecule. The angle between planar N(2)–Pt(1)–N(3) and planar O(1)–Pt(1)–N(1) is 2.064(165)° which indicates that the Pt(1)–O(1)–N(1)–N(2)–N(3) plane is slightly distorted. The Pt–N (deprotonated sulfonamide) bond length (2.014(4) Å) is similar to the Pt–N (1,3-dap) bond lengths (2.026(4) and 2.048(5) Å), while it is shorter than Pt–O (carboxylic oxygen) bond length (2.023(4) 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 [24]. Gong et al. also reported that the deprotonated amide nitrogen atom was exactly different from the ordinary amino nitrogen atom and its coordinating property might be "O-like" [25]. So we deduce that the coordinating property of the deprotonated sulfonamide nitrogen atom may be also "O-like" (see Fig. 1).

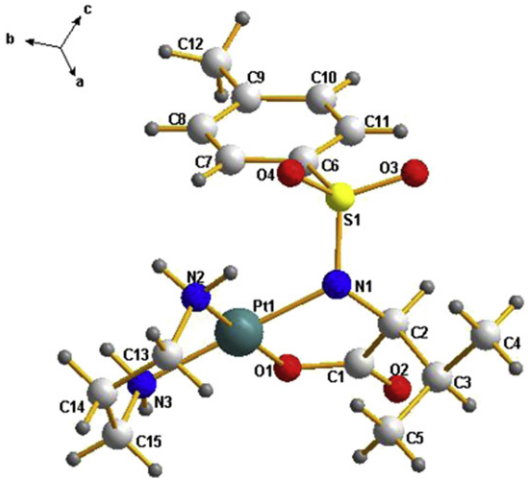


Fig. 1. Molecular structure and atom-labeling scheme for the complex **1b**.

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

	1b
Pt(1)–N(1)	2.014(4)
Pt(1)–N(2)	2.026(4)
Pt(1)–N(3)	2.048(5)
Pt(1)–O(1)	2.023(4)
N(1)–Pt(1)–N(2)	95.25(19)
N(2)–Pt(1)–N(3)	87.39(19)
N(3)–Pt(1)–O(1)	95.10(17)
O(1)–Pt(1)–N(1)	82.24(16)
O(1)–Pt(1)–N(2)	176.80(19)
N(1)–Pt(1)–N(3)	177.26(19)

2.3. Cytotoxic studies

In our present work, as listed in Table 2, most complexes exert cytotoxic effects against tested carcinoma cell lines; in addition, they have selectivity against tested carcinoma cell lines. But none of them shows higher cytotoxicity than cisplatin. The structure–activity relationships are summarized as follows:

(1) The metal ions have important effect on cytotoxicity. The palladium(II) complexes **2a** and **2c** show better activities than corresponding platinum(II) complexes **1a** and **1c** against HL-60, Bel-7402, BGC-823 and KB. In addition, the palladium(II) complex **2b** is more active than corresponding platinum(II) complex **1b** against Bel-7402, while it is less active than **1b** against HL-60.
(2) The amino acids also exert effects on the cytotoxicity. For the platinum(II) complexes **1a**–**1d** with the same 1,3-dap and different amino acids, the cytotoxicity against HL-60 and Bel-7402 decreases in the same sequences: Ser > Val > Phe > Ala. The sequences of their cytotoxicity toward BGC-823 and KB are: Phe > Val > Ser > Ala, and Val > Phe > Ser > Ala, respectively. For the palladium(II) complexes **2a**–**2d** with the same 1,3-dap and different amino acids, the cytotoxicity against HL-60 and Bel-7402 decreases in the same sequences: Ile > Ala > Phe > Val.
(3) The N-containing ligands have important effect on cytotoxicity. For platinum(II) complexes with same amino acid and different N-containing ligands, [Pt(1,3-dap)(TspheNO)] (**1c**) is less active than [Pt(bipy)(TspheNO)] against HL-60, BGC-823, Bel-7402, and KB. [Pt(1,3-dap)(TsvalNO)] (**1b**) has better cytotoxicity than corresponding complex [Pt(bipy)(TsvalNO)] against HL-60, BGC-823, and Bel-7402. [Pt(1,3-dap)(TsserNO)] (**1d**) has lower IC_{50} values than [Pt(bipy)(TsserNO)] against HL-60 and Bel-7402, but [Pt(bipy)(TsserNO)] shows higher cytotoxicity than [Pt(1,3-dap)(TsserNO)] (**1d**) against BGC-823 and KB [26]. For the palladium(II) complexes with 4-toluenesulfonyl-L-

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

Complexes	IC_{50} (μM)			
	HL-60	BGC-823	Bel-7402	KB
1a	119.78 ± 9.27	57.88 ± 4.41	85.70 ± 2.79	466.98 ± 9.37
1b	13.86 ± 1.02	26.19 ± 1.67	21.38 ± 1.42	24.22 ± 1.76
1c	31.85 ± 2.58	23.38 ± 2.04	24.14 ± 1.03	28.49 ± 2.37
1d	10.64 ± 0.78	27.93 ± 2.37	17.59 ± 0.93	31.07 ± 3.01
2a	16.47 ± 1.38	20.42 ± 1.58	15.40 ± 0.69	19.71 ± 0.93
2b	28.14 ± 1.48	–	18.46 ± 1.58	–
2c	25.19 ± 1.79	–	16.81 ± 1.45	–
2d	15.46 ± 1.35	16.36 ± 1.64	12.55 ± 1.15	11.32 ± 0.78
Cisplatin	2.89 ± 0.34	6.48 ± 0.81	8.12 ± 0.97	2.65 ± 0.33

amino acid dianion and N-containing ligands, when amino acids are Ala, Val, Phe, and Ile, aliphatic N-containing ligand is 1,3-dap, they exert higher cytotoxicity than corresponding complexes with 1,4-dab against Bel-7402. But [Pd(1,3-dap)(TsalaNO)]·1.5H_2O and [Pd(1,3-dap)(TsvalNO)] are less active than corresponding complexes [Pd(1,4-dab)(TsalaNO)] and [Pd(1,4-dab)(TsvalNO)] against HL-60. While [Pd(1,3-dap)(TspheNO)] and [Pd(1,3-dap)(TsileNO)] have higher cytotoxicity than corresponding complexes [Pd(1,4-dab)(TspheNO)]·0.5H_2O and [Pd(1,4-dab)(TsileNO)] against HL-60 [27,28].

In summary, for platinum(II)/palladium(II) complexes with 4-toluenesulfonyl-L-amino acid dianion and aliphatic N-containing ligands, all amino acids, aliphatic N-containing ligands and centre metal ions have significant effects on cytotoxicity. For the platinum(II) complexes (**1a**–**1d**), when amino acid is Ala, the complex has the least cytotoxicity. For the palladium(II) complexes (**2a**–**2d**), when amino acid is Ile, the complex has the best cytotoxicity. But the IC_{50} values do not show definite correlation with variation of them. In addition, the effect on cytotoxicity is also related to tumor cell type.

3. Conclusions

We have synthesized and characterized eight new palladium(II)/platinum(II) complexes with 4-toluenesulfonyl-L-amino acid dianions and 1,3-dap. Most complexes exert cytotoxic effects against tested carcinoma cell lines, but none of them shows higher cytotoxicity than cisplatin. The cytotoxic experiment indicates that metal ions, amino acids and aliphatic N-containing ligands have effect on cytotoxicity, but the cytotoxicity do not show definite correlation with variation of them. These results will be helpful for the design of new metal-based antitumor agents.

4. Experimental section

4.1. Materials

4-Toluenesulfonyl chloride, $K_2[PdCl_4]$ and $K_2[PtCl_4]$ were of chemical grade, 1,3-dap were of analytical grade. Commercially pure Ala, Val, Phe, Ile and Ser were purchased from Sigma. RPMI-1640 medium, trypsin and fetal bovine serum were purchased from Gibco. MTT, 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 was determined on an Elementar Vario EL III elemental analyzer. The IR spectra were recorded on a Perkin–Elmer Model-683 spectrophotometer using KBr pellets. The ^1H 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 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 the following procedure. To a rapidly stirred solution of Ala (232 mg, 2.6 mmol) in 5.0 mL H_2O was added 2.6 mL NaOH (1 mol L^{-1}). 4-Toluenesulfonyl chloride (500 mg, 2.6 mmol) was added to the solution, after 2.6 mL NaOH (1 mol L^{-1}) was added dropwise over 0.5 h. After further 8 h, the solution was cooled by ice and acidified to pH = 3–4 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-d_6) $\delta_{(ppm)}$ 8.04 (d, J = 8.3 Hz, 1H, NH), 7.67 (d, J = 7.9 Hz, 2H, ArH), 7.37 (d, J = 8.1 Hz, 2H, ArH), 3.67–3.85 (m, 1H, CH), 2.37 (s, 1H, CH_3), 1.14 (d, J = 7.2 Hz, 3H, CH_3). Melting point: 133.2–135.4 °C.

$TsvalH_2$, $TspheH_2$, $TsileH_2$ and $TsserH_2$ were carried out in an identical manner. $TsvalH_2$: ^1H NMR (600 MHz, $CDCl_3$) $\delta_{(ppm)}$ 7.72 (d, J = 8.3 Hz, 2H, ArH), 7.27 (d, J = 8.0 Hz, 2H, ArH), 5.35 (d, J = 9.8 Hz, 1H, NH), 3.78 (dd, J = 9.8, 4.7 Hz, 1H, CH), 2.16–2.03 (m, 1H, CH), 2.40 (s, 3H, CH_3), 0.94 (d, J = 6.8 Hz, 3H, CH_3), 0.87 (d, J = 8.2 Hz, 3H, CH_3). Melting point: 146.4–147.7 °C. $TspheH_2$: ^1H NMR (600 MHz, DMSO-d_6) 8.18 (d, J = 5.0 Hz, 1H, NH), 7.15–7.05 (m, 2H, ArH), 7.36–7.16 (m, 5H, ArH), 7.12 (s, 2H, ArH), 3.82–3.92 (m, 1H, CH), 3.00–2.87 (m, 1H, CH_2), 2.83–2.66 (m, 1H, CH_2), 2.34 (s, 3H, CH_3). Melting point: 150.4–152.9 °C. $TsileH_2$: ^1H NMR (600 MHz, $CDCl_3$) $\delta_{(ppm)}$ 7.72 (d, J = 8.1 Hz, 2H, ArH), 7.27 (d, J = 8.3 Hz, 2H, ArH), 5.32 (d, J = 9.7 Hz, 1H, NH), 3.82 (dd, J = 9.7, 4.9 Hz, 1H, CH), 1.86–1.77 (m, 1H, CH), 1.44–1.34 (m, 1H, CH_2), 1.20–1.10 (m, 1H, CH_2), 0.90 (d, J = 6.8 Hz, 3H, CH_3), 0.86 (t, J = 7.4 Hz, 3H, CH_3). Melting point: 134.1–134.5 °C. $TsserH_2$: ^1H NMR (600 MHz, DMSO-d_6) $\delta_{(ppm)}$ 7.89 (d, J = 8.6 Hz, 1H, NH), 7.68 (d, J = 7.9 Hz, 2H, ArH), 7.35 (d, J = 7.9 Hz, 2H, ArH), 3.80–3.69 (m, 1H, CH), 3.53–3.45 (m, 2H, CH_2), 2.37 (s, 3H, CH_3). Melting point: 223.9–224.9 °C.

Precursor complexes [Pt(1,3-dap)Cl_2] (**i**), [Pd(1,3-dap)Cl_2] (**ii**) were synthesized according to a published procedure [29]. Yield: **i**: Kelly solid, Yield: 45.4%, Anal. Calcd. for $C_3H_{10}Cl_2N_2Pt$ (%): C, 10.59; H, 2.96; N, 8.24. Found (%): C, 10.93; H, 2.70; N, 7.84; **ii**: Kelly solid, Yield: 65.4%, Anal. Calcd. for $C_3H_{10}Cl_2N_2Pd$ (%): C, 14.33; H, 4.01; N, 11.14. Found (%): C, 14.60; H, 3.68; N, 11.10.

4.3.1. [Pt(1,3-dap)(TsvalNO)] (*1b*)

[Pt(1,3-dap)Cl_2] (20.0 mg, 0.059 mmol) was added to a 6 mL CH_3OH/H_2O (volume 2:1) solution of $TsvalH_2$ (23.9 mg, 0.088 mmol) when the solution temperature was heated to 50 °C, the mixture was adjusted to pH = 9 by NaOH solution, then stirred for 3–4 h. The solution was concentrated to about 80% of the original volume by reduced pressure distillation. By evaporating the concentrated solution at room temperature, the colorless crystals suitable for X-ray diffraction were obtained after a few weeks and separated from the solution. IR (KBr, cm^{-1}): 1605, 1379, 548, 418. ^1H NMR (600 MHz, DMSO-d_6) $\delta_{(ppm)}$ 8.02 (d, J = 8.2 Hz, 2H, ArH), 7.29 (d, J = 8.1 Hz, 2H, ArH), 5.44–5.30 (m, 1H, NH_2), 5.30–5.15 (m, 1H, NH_2), 5.13–4.99 (m, 1H, NH_2), 4.92–4.85 (m, 1H, NH_2), 3.37 (d, J = 4.6 Hz, 1H, CH), 2.53–2.51 (m, 4H, CH_2), 2.36 (s, 3H, CH_3), 2.00–1.90 (m, 1H, CH), 1.73–1.65 (m, 1H, CH_2), 1.56–1.47 (m, 1H, CH_2), 1.08 (d, J = 6.9 Hz, 3H, CH_3), 1.05 (d, J = 6.9 Hz, 3H, CH_3). ESI-MS: 561.5 $[M + Na]^+$. Anal. Calcd. for $C_{15}H_{25}N_3O_4PtS$ (%): C, 33.45; H, 4.68; N, 7.80. Found: C, 33.64; H, 4.55; N, 7.91.

4.3.2. [Pt(1,3-dap)(TsalaNO)]·0.5H_2O (*1a*)

The synthesis of **1a** was carried out in an identical manner to **1b** starting from [Pt(1,3-dap)Cl_2] (20.0 mg, 0.059 mmol) and $TsalaH_2$ (21.5 mg, 0.088 mmol). White solid. IR (KBr, cm^{-1}): 1656, 1328, 557, 450. ^1H NMR (600 MHz, DMSO-d_6) $\delta_{(ppm)}$ 8.01 (d, J = 8.2 Hz, 2H, ArH), 7.32 (d, J = 8.0 Hz, 2H, ArH), 5.48–5.40 (m, 1H, NH_2), 5.40–5.25 (m, 1H, NH_2), 5.20–5.50 (m, 1H, NH_2), 5.05–4.90 (m, 1H, NH_2), 3.54 (q, J = 7.0 Hz, 1H, CH), 2.70–2.58 (m, 2H, CH_2), 2.58–2.52 (m, 2H, CH_2), 2.37 (s, 3H, CH_3), 1.75–1.67 (m, 1H, CH_2), 1.58–1.50 (m, 1H, CH_2), 1.28 (d, J = 7.0 Hz, 3H, CH_3). ESI-MS: 549.9 $[M-0.5H_2O + K]^+$. Anal. Calc. for $C_{13}H_{22}N_3O_{4.5}PtS$: C, 30.06; H, 4.27; N, 8.09. Found: C, 29.97; H, 4.13; N, 8.49.

*4.3.3. [Pt(1,3-dap)(TspheNO)] (**1c**)*

The synthesis of **1c** was carried out in an identical manner to **1b** starting from [Pt(1,3-dap)Cl_2] (20.0 mg, 0.059 mmol) and TspheH$_2$ (28.1 mg, 0.088 mmol). White solid. IR (KBr, cm^{-1}): 1644, 1349, 555, 429. ^{1}H NMR (600 MHz, DMSO-d_6) $\delta_{(ppm)}$ 7.98 (d, J = 8.1 Hz, 2H, ArH), 7.31–7.25 (m, = 15.3, 6H, ArH), 7.25–7.20 (m, 1H, ArH), 5.10–5.02 (m, 2H, NH_2), 5.01–4.98 (m, 1H, NH_2), 4.80–4.70 (m, 1H, NH_2), 3.83 (t, J = 5.2 Hz, 1H, CH), 3.02 (dd, J = 12.9, 4.7 Hz, 1H, CH_2), 2.91 (dd, J = 12.9, 5.7 Hz, 1H, CH_2), 2.63–2.55 (m, 1H, CH_2), 2.45–2.37 (m, 2H, CH_2), 2.37 (s, 3H, CH_3), 2.27–2.21 (m, 1H, CH_2), 1.57–1.50 (m, 2H, CH_2). ^{13}C NMR (150 MHz, DMSO-d_6) $\delta_{(ppm)}$ 185.09 (1C, COO), 141.84 (1C, Ar), 140.83 (1C, Ar), 138.09 (1C, Ar), 130.78 (2C, Ar), 129.45 (2C, Ar), 127.99 (2C, Ar), 127.12 (2C, Ar), 126.23 (1C, Ar), 64.26 (1C, CH), 43.44 (1C, CH_2), 43.40 (1C, CH_2), 42.14 (1C, CH_2), 28.00 (1C, CH_2), 21.36 (1C, CH_3). ESI-MS: 625.5 [M + K]$^+$. Anal. Calc. for $C_{19}H_{25}N_3O_4PtS$: C, 38.90; H, 4.30; N, 7.16. Found: C, 38.94; H, 4.36; N, 7.37.

*4.3.4. [Pt(1,3-dap)(TsserNO)] (**1d**)*

The synthesis of **1d** was carried out in an identical manner to **1b** starting from [Pt(1,3-dap)Cl_2] (20.0 mg, 0.059 mmol) and TsileH$_2$ (22.8 mg, 0.088 mmol). White solid. IR (KBr, cm^{-1}): 1611, 1391, 551, 423. ^{1}H NMR (600 MHz, DMSO-d_6) $\delta_{(ppm)}$ 8.04 (d, J = 8.2 Hz, 2H, ArH), 7.68 (d, J = 8.3 Hz, 2H, ArH), 5.50–5.40 (m, 1H, NH_2), 5.30–5.25 (m, 1H, NH_2), 5.10–5.00 (m, 1H, NH_2), 5.00–4.90 (m, 1H, NH_2), 4.30–4.25 (dd, J = 6.9, 5.1 Hz, 1H, CH), 3.64–3.60 (m, 1H, CH_2), 3.59–3.54 (m, 1H, CH_2), 3.48–3.44 (m, 1H, OH), 2.68–2.63 (m, 1H, CH_2), 2.62–2.61 (m, 1H, CH_2), 2.61–2.57 (m, 1H, CH_2), 2.57–2.54 (m, 1H, CH_2), 2.37 (s, 3H, CH_3), 1.73–1.67 (m, 1H, CH_2), 1.59–1.51 (m, 1H, CH_2). ESI-MS: 565.95 [M + K]$^+$. Anal. Calc. for $C_{13}H_{21}N_3O_5PtS$: C, 29.66; H, 4.02; N, 7.98. Found: C, 31.09; H, 4.17; N, 7.84.

*4.3.5. [Pd(1,3-dap)(TsalaNO)]·1.5H_2O (**2a**)*

The synthesis of **2a** was carried out in an identical manner to **1b** starting from [Pd(1,3-dap)Cl_2] (20.0 mg, 0.080 mmol) and TsalaH$_2$ (29.0 mg, 0.119 mmol). Yellow solid. IR (KBr, cm^{-1}): 1596, 1379, 549, 400. ^{1}H NMR (600 MHz, DMSO-d_6) $\delta_{(ppm)}$ 7.97 (d, J = 8.1 Hz, 2H, ArH), 7.35 (d, J = 7.9 Hz, 2H, ArH), 4.63–4.57 (m, 1H, NH_2), 4.45–4.39 (m, 1H, NH_2), 4.35–4.28 (m, 1H, NH_2), 4.20–4.12 (m, 1H, NH_2), 3.40 (q, J = 7.0 Hz, 1H, CH), 2.54–2.52 (m, 2H, CH_2), 2.48–2.41 (m, 2H, CH_2), 2.38 (s, 3H, CH_3), 1.72–1.64 (m, 1H, CH_2), 1.52–1.44 (m, 1H, CH_2), 1.29 (d, J = 7.0 Hz, 3H, CH_3). ESI-MS: 444.4 [M–H_2O + Na]$^+$. Anal. Calc. for $C_{13}H_{23}N_3O_5PdS$: C, 35.50; H, 5.27; N, 9.55. Found: C, 34.96; H, 4.98; N, 10.33.

*4.3.6. [Pd(1,3-dap)(TsvalNO)] (**2b**)*

The synthesis of **2b** was carried out in an identical manner to **1b** starting from [Pd(1,3-dap)Cl_2] (20.0 mg, 0.080 mmol) and TsvalH$_2$ (32.3 mg, 0.119 mmol). Yellow solid. IR (KBr, cm^{-1}): 1605, 1325, 558, 433. ^{1}H NMR (600 MHz, DMSO-d_6) $\delta_{(ppm)}$ 8.00 (d, J = 8.1 Hz, 2H, ArH), 7.33 (d, J = 8.0 Hz, 2H, ArH), 4.48–4.42 (m, 1H, NH_2), 4.33–4.21 (m, 2H, NH_2), 4.11–4.04 (m, 1H, NH_2), 3.27 (d, J = 4.3 Hz, 1H, CH), 2.54–2.52 (m, 1H, CH_2), 2.47–2.40 (m, 2H, CH_2), 2.38 (s, 3H, CH_3), 2.37–2.34 (m, 1H, CH_2), 1.98–1.90 (m, 1H, CH), 1.70–1.63 (m, 1H, CH_2), 1.50–1.41 (m, 1H, CH_2), 1.17 (d, J = 6.8 Hz, 3H, CH_3), 1.06 (d, J = 6.8 Hz, 3H, CH_3). ESI-MS: 487.6 [M + K]$^+$. Anal. Calc. for $C_{15}H_{25}N_3O_4PdS$: C, 40.05; H, 5.60; N, 9.34. Found: C, 39.67; H, 5.26; N, 9.23.

*4.3.7. [Pd(1,3-dap)(TspheNO)] (**2c**)*

The synthesis of **2c** was carried out in an identical manner to **1b** starting from [Pd(1,3-dap)Cl_2] (20.0 mg, 0.080 mmol) and TspheH$_2$ (38.0 mg, 0.119 mmol). Yellow solid. IR (KBr, cm^{-1}): 1628, 1346, 551, 420. ^{1}H NMR (600 MHz, DMSO-d_6) $\delta_{(ppm)}$ 8.01–7.83 (m, 2H, ArH), 7.32–7.10 (m, 7H, ArH), 5.11–4.96 (m, 3H, NH_2), 4.80–4.70 (m, 1H, NH_2), 3.85–3.73 (m, 1H, CH), 3.36–3.31 (m, 2H, CH_2), 3.04–2.94 (m, 1H, CH_2), 2.93–2.84 (m, 1H, CH_2), 2.34 (d, J = 21.1 Hz, 5H, CH_3, CH_2), 1.60–1.40 (m, 2H, CH_2). ESI-MS: 520.0 [M + Na]$^+$. Anal. Calc. for $C_{19}H_{25}N_3O_4PdS$: C, 45.83; H, 5.06; N, 8.44. Found: C, 45.98; H, 4.59; N, 8.34.

*4.3.8. [Pd(1,3-dap)(TsileNO)] (**2d**)*

The synthesis of **2d** was carried out in an identical manner to **1b** starting from [Pd(1,3-dap)Cl_2] (20.0 mg, 0.080 mmol) and TsserH$_2$ (30.9 mg, 0.119 mmol). Yellow solid. IR (KBr, cm^{-1}): 1643, 1331, 567, 442. ^{1}H NMR (600 MHz, DMSO-d_6) $\delta_{(ppm)}$ 7.99 (d, J = 8.1 Hz, 2H, ArH), 7.34 (d, J = 8.0 Hz, 2H, ArH), 4.52–4.45 (m, 1H, NH_2), 4.35–4.26 (m, 1H, NH_2), 4.25–4.20 (m, 1H, NH_2), 4.10–4.02 (m, 1H, NH_2), 2.55–2.52 (m, 1H, CH), 2.48–2.40 (m, 2H, CH_2), 2.38 (s, 3H, CH_3), 2.37–2.32 (m, 1H, CH), 1.77–1.69 (m, 2H, CH_2), 1.70–1.63 (m, 2H, CH_2), 1.52–1.41 (m, 2H, CH_2), 1.10 (d, J = 6.8 Hz, 3H, CH_3), 0.88 (t, J = 7.3 Hz, 3H, CH_3). ^{13}C NMR (150 MHz, DMSO-d_6) $\delta_{(ppm)}$ 182.84 (1C, COO), 142.77 (1C, Ar), 140.70 (1C, Ar), 129.59 (2C, Ar), 126.98 (2C, Ar), 68.47 (1C, CH), 41.72 (1C, CH_2), 41.39 (1C, CH_2), 40.76 (1C, CH_2), 28.56 (1C, CH), 25.74 (1C, CH_2), 21.37 (1C, CH_3), 16.41 (1C, CH_3), 12.45 (1C, CH_3). ESI-MS: 501.1 [M + K]$^+$. Anal. Calc. for $C_{16}H_{27}N_3O_4PdS$: C, 41.43; H, 5.87; N, 9.06. Found: C, 41.14; H, 5.88; N, 8.96.

*4.4. Data collection and structural refinement of the complex (**1b**)*

The data collection of the complex **1b** 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 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 the complex **1b** have been deposited with the Cambridge Crystallographic Data Centre, CCDC – 831020. 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).

Table 3
Crystallographic data for the complex **1b**.

	1b
Formula	$C_{15}H_{25}N_3O_4PtS$
Fw	538.53
T (K)	296(2)
Cryst syst	Tetragonal
Space group	$P4_32_12$
a (Å)	9.9122(4)
b (Å)	9.9122(4)
c (Å)	39.4933(15)
V (nm^3)	3880.3(3)
Z	8
Dc (Mg m^{-3})	1.844
F(000)	2096
Cryst dimens (mm)	0.41 × 0.21 × 0.02
θ range (°)	2.06–28.32
hkl ranges	$-13 < h < 13$ $-13 < k < 13$ $-52 < l < 34$
Data/parameters	4839/221
Goodness-of-fit on F^2	1.327
Final *R* indices [$I > 2s(I)$]	R_1 = 0.0303 wR_2 = 0.0619

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 [30]. 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 [31]. 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.

Acknowledgments

This work was supported by the Nature Science Fund for Distinguished Young Scholars of Hebei Province (Grant No. B2011201164), the Nature Science Fund of Hebei Province (Grant No. B2011201135), the Key Basic Research Special Foundation of Science Technology Ministry of Hebei Province (Grant No. 11966412D, 12966418D), the Pharmaceutical Joint Research Fundation of the Natural Science Foundation of Hebei Province and China Shiyao Pharmaceutical Group Co., LTD (B2011201174).

Abbreviations

1,3-dap	1,3-diaminopropane
AMBI	2-aminomethyl benzimidazole
Ala	L-alanine
Val	L-valine
Ile	L-isoleucine
Ser	L-serine
Phe	L-phenylalanine
Leu	L-leucine
Tyr	L-tyrosine
Pro	L-proline
Gly	L-glycine
Met	L-methionine
Cys	L-cysteine,
$TsalaH_2$	4-toluenesulfonyl-L-alanine
$TsvalH_2$	4-toluenesulfonyl-L-valine
$TsileH_2$	4-toluenesulfonyl-L-isoleucine
$TsserH_2$	4-toluenesulfonyl-L-serine
$TspheH_2$	4-toluenesulfonyl-L-phenylalanine
MLCT	metal-to-ligand charge transfer
DMF	dimethyl formamide
DMSO	dimethyl sulfoxide
SRB	sulforhodamine B
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
OD	optical density
SD	standard deviation

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2012.09.052.

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