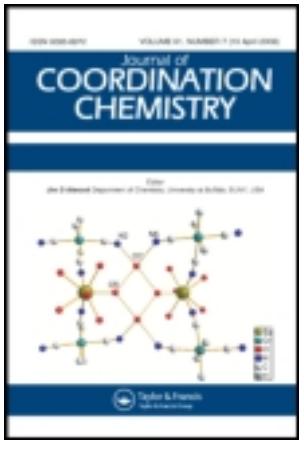

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# Synthesis, characterization, and cytotoxicity of mixed-ligand complexes of platinum(II) with 2,2′-bipyridine and 4-toluenesulfonyl-L-amino acid dianion

Jin Chao Zhang [a], Luwei Li [a], Lili Ma [a], Fangfang Zhang [a] & Shuxiang Wang [a]

[a] College of Chemistry and Environmental Science, Chemical Biology Key Laboratory of Hebei Province, Hebei University, Baoding 071002, PR China

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# Synthesis, characterization, and cytotoxicity of mixed-ligand complexes of platinum(II) with 2,2′-bipyridine and 4-toluenesulfonyl-L-amino acid dianion

JIN CHAO ZHANG*, LUWEI LI, LILI MA, FANGFANG ZHANG and SHUXIANG WANG*

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

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Five new platinum(II) complexes (**1**–**5**) with 4-toluenesulfonyl-L-amino acid dianion and 2,2′-bipyridine (bipy) have been synthesized and characterized by elemental analysis, IR, UV, $^{1}$H-NMR, $^{13}$C-NMR, and mass spectra. The crystal structure of **1** has been determined by X-ray diffraction analysis. Cytotoxicity was tested by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and sulforhodamine B (SRB) assays. The results indicate that **1**–**5** exert cytotoxic effects with selectivity against tested carcinoma cell lines; **5** displays better cytotoxicity against BGC-823, Bel-7402, and KB cell lines, while **1** has better cytotoxicity against KB cell line. The 4-toluenesulfonyl- L-amino acid dianions have important effects on cytotoxicity; when 4-toluenesulfonyl-L-amino acid dianions are 4-toluenesulfonyl-L-glycine and 4-toluenesulfonyl-L-phenylalanine, the complexes show better cytotoxicity.

*Keywords*: Platinum(II) complexes; Synthesis; Characterization; Cytotoxicity



## 1. Introduction

The landmark discovery of cisplatin by Rosenberg in 1965 heralded a new era of anticancer drug research based on metallopharmaceuticals [1]. Today, cisplatin is still one of the world's best-selling anticancer drugs. In addition, carboplatin and oxaliplatin have also received worldwide approval. Nedaplatin, lobaplatin, and heptaplatin have gained regionally limited approval and a few platinum drugs continue to be evaluated in clinical studies. Regardless of the achievements of current platinum drugs, there are some major drawbacks including a limited range of cancers, acquired or intrinsic resistance, and severe side-effects [2–4]. These problems have prompted chemists to develop new platinum anticancer drugs.

Bipy, quinoline, 1,10-phenanthroline (phen), and their derivatives are DNA intercalators. Amino acids are fundamental to life and the basis of metabolism. Introducing amino acid into antitumor drugs can improve their selectivities to tumor cells, enhance their liposolubilities, and remit their toxicities to normal cells. So, they

*Corresponding authors. Email: jczhang6970@yahoo.com.cn; wsx@hbu.edu.cn

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have been widely used in platinum anticancer drugs as ligands. Jin *et al*. synthesized and characterized nine complexes of platinum(II) with phen and amino acids (where amino acids are L-glycine (Gly), L-histidine (His), L-cysteine (Cys), L-isoleucine (Ile), L-alanine (Ala), L-proline (Pro), L-serine (Ser), L-aspartic acid (Asp), and L-glutamic acid (Glu).

The results indicated that most complexes were less cytotoxic than cisplatin, but the $IC_{50}$ of [Pt(phen)(Pro)]Cl · $2H_2O$ is similar to cisplatin [5]. Mital *et al*. reported the cytotoxicities of some platinum(II) complexes with phen and amino acids (where amino acids are Gly, Ala, L-leucine (Leu), and L-tyrosine (Tyr)). 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 [6]. In addition, Puthraya *et al*. [7] tested several platinum(II) complexes of bipy with amino acids against several tumor cells, where some complexes showed good cytotoxicity. In order to develop new platinum anticancer drugs, in this study, we present the synthesis, characterization, and cytotoxicity of five new platinum(II) complexes with bipy and 4-toluenesulfonyl-L-amino acid dianion.



## 2. Experimental

### 2.1. *Materials*

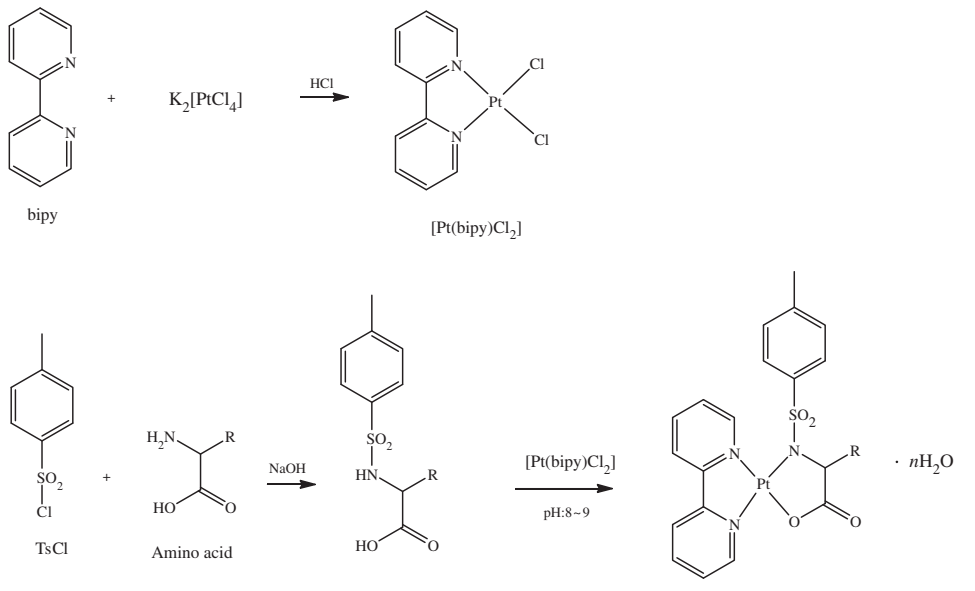
In this study, 4-Toluenesulfonyl chloride and $K_2[PtCl_4]$ were of chemical grade; bipy was of analytical grade. Commercially pure Gly, L-valine (Val), Leu, Ser, and L-phenylalanine (Phe) were purchased from Sigma. RPMI-1640 medium, trypsin and fetal bovine serum were purchased from Gibco. MTT, SRB, benzylpenicillin, and streptomycin were obtained from Sigma. Four different human carcinoma cell lines, HL-60 (immature granulocyte leukemia), Bel-7402(liver carcinoma), BGC-823 (gastro-carcinoma), and KB (nasopharyngeal carcinoma), were obtained from American Type Culture Collection.

### 2.2. *Instrumentation and measurement*

Elemental analyses were determined on an Elementar Vario EL III elemental analyzer. The electronic spectra in DMF were measured on a UV-3400 Toshniwal spectrophotometer. IR spectra were recorded using KBr pellets and a Perkin-Elmer Model-683 spectrophotometer. The $^1$H- and $^{13}$C-NMR spectra were recorded on a Bruker AVIII 600 NMR spectrometer. 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).

### 2.3. *Synthesis of compounds*

The platinum(II) complexes [Pt(bipy)(TsglyNO)] · $2H_2O$ (**1**), [Pt(bipy)(TsserNO)] · $H_2O$ (**2**), [Pt(bipy)(TsleuNO)] (**3**), [Pt(bipy)(TsvalNO)] (**4**), and [Pt(bipy)(TspheNO)] (**5**) were prepared by the reaction of [Pt(bipy)$Cl_2$] with 4-toluenesulfonyl-L-amino




**1**: R = –H; $n = 2$ **2**: R = –$CH_2OH$; $n = 1$ **3**: R = –$CH_2CH(CH_3)2$; $n = 0$

**4**: R = –$CH(CH_3)_2$; $n = 0$ **5**: R = –$CH_2(C_6H_5)$; $n = 0$

Figure 1. The synthetic routines of **1**–**5**.

acids: 4-toluenesulfonyl-L-glycine ($TsglyH_2$), 4-toluenesulfonyl-L-serine ($TsserH_2$), 4-toluenesulfonyl-L-leucine ($TsleuH_2$), 4-toluenesulfonyl-L-valine ($TsvalH_2$), or 4-toluenesulfonyl-L-phenylalanine ($TspheH_2$) in a mixture of $CH_3OH/H_2O$ (figure 1) [8].

**2.3.1. 4-Toluenesulfonyl-L-amino acids.** To a rapidly stirred solution of Gly (150 mg, 2.0 mmol) in 5.0 mL $H_2O$, 2.0 mL NaOH (1 mol $L^{-1}$) was added. Also, 4-Toluenesulfonyl chloride (380 mg, 2.0 mmol) was added to the solution, after 2.0 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 $TsglyH_2$. $^1$H-NMR (600 MHz, DMSO-$d_6$) $\delta_{(ppm)}$ 7.97 (t, $J = 6.1$ Hz, 1H, NH), 7.68 (d, $J = 8.2$ Hz, 2H, ArH), 7.38 (d, $J = 8.2$ Hz, 2H, ArH), 3.55 (s, 2H, $CH_2$), and 2.38 (s, 3H, $CH_3$).

$TsvalH_2$, $TsleuH_2$, $TsserH_2$, and $TspheH_2$ were carried out in an identical manner. $TsvalH_2$: $^1$H-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, H, NH), 3.78 (dd, $J = 9.8$, 4.7 Hz, 1H, CH), 2.40 (s, 3H, $CH_3$), 2.16–2.03 (m, 1H, CH), 0.94 (d, $J = 6.8$ Hz, 3H, $CH_3$), and 0.87 (d, $J = 8.2$ Hz, 3H, $CH_3$). $TsleuH_2$: $^1$H-NMR (600 MHz, $CDCl_3$) $\delta_{(ppm)}$ 7.73 (d, $J = 8.3$ Hz, 2H, ArH), 7.28 (d, $J = 8.0$ Hz, 2H, ArH), 5.33 (d, $J = 9.7$ Hz, 1H, NH),

3.84–4.00 (m, 1H), 2.41 (s, 3H, $CH_3$), 1.68–1.83 (m, 1H, CH), 1.59–1.42 (m, 2H, $CH_2$), 0.89 (d, $J = 6.7$ Hz, 3H, $CH_3$), and 0.81 (d, $J = 6.6$ Hz, 3H, $CH_3$). $TsserH_2$: $^1$H-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),and 3.53–3.45 (m, 1H), 2.37 (s, 3H). $TspheH_2$: $^1$H-NMR (600 MHz, DMSO-$d_6$) $\delta_{(ppm)}$ 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), 3.82–3.92 (m, 1H, CH), 3.00–2.87 (m, 1H, $CH_2$), 2.83–2.66 (m, 1H, $CH_2$), and 2.34 (s, 3H, $CH_3$).

**2.3.2. [Pt(bipy)$Cl_2$].** [Pt(bipy)$Cl_2$] was synthesized according to a published procedure [9]. Yield: 87.7%. Yellow solid. Anal. Calcd for $C_{10}H_8Cl_2N_2Pt$ (%): C, 28.45; H, 1.91; and N, 6.64. Found (%): C, 28.56; H, 1.98; and N, 6.80.

**2.3.3. [Pt(bipy)(TsglyNO)] · 2$H_2O$ (1).** [Pt(bipy)$Cl_2$] (21 mg, 0.05 mmol) was added to a 3 mL $CH_3OH/H_2O$ (volume 1 : 1) solution of $TsglyH_2$ (24 mg, 0.10 mmol). The solution was heated to 50°C, and the mixture was 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 by reduced pressure distillation. By evaporating the concentrated solution at room temperature, yellow crystals suitable for X-ray diffraction were obtained after a few weeks. Complex **1** separated from the solution. Yield: 65.7%. Yellow solid. IR (KBr, $cm^{-1}$): 1648, 1384, 420, and 537. $^1$H-NMR (600 MHz, DMSO-$d_6$) $\delta_{(ppm)}$ 9.33 (d, $J = 5.8$ Hz, 1H, ArH), 8.65 (d, $J = 4.6$ Hz, 1H, ArH), 8.61 (d, $J = 8.1$ Hz, 1H, ArH), 8.54 (d, $J = 7.8$ Hz, 1H, ArH), 8.45–8.37 (m, 2H, ArH), 7.90 (d, $J = 8.2$ Hz, 2H, ArH), 7.85–7.77 (m, 2H, ArH), 7.27 (d, $J = 8.1$ Hz, 2H, ArH), 3.75 (s, 2H, $CH_2$), and 2.34 (s, 3H, $CH_3$). $^{13}$C-NMR (150 MHz, DMSO-$d_6$) $\delta_{(ppm)}$ 182.52(C=O), 156.63, 156.21, 153.43, 148.71, 141.49, 141.27, 140.66, 139.85, 129.60, 128.31, 128.08, 127.62, 124.46, 124.10(Aryl-C), 54.47($CH_2$), 21.38($CH_3$). ESI–MS: 601.1 $[M + Na]^+$. Anal. Calcd for $C_{19}H_{21}N_3O_6PtS$ (%): C, 37.13; H, 3.44; N, 6.84. Found: C, 37.32; H, 3.35; N, 6.64.

**2.3.4. [Pt(bipy)(TsserNO)] · $H_2O$ (2).** The synthesis of **2** was carried out in an identical manner to **1** starting from [Pt(bipy)$Cl_2$] (19 mg, 0.045 mmol) and $TsserH_2$ (23 mg, 0.090 mmol). Yield: 58.0%. Yellow solid. IR (KBr, $cm^{-1}$): 1642, 1384, 420, 530. $^1$H-NMR (600 MHz, DMSO-$d_6$) $\delta_{(ppm)}$ 9.45 (d, $J = 5.8$ Hz, 1H, ArH), 8.69 (d, $J = 5.6$ Hz, 1H, ArH), 8.61 (d, $J = 7.9$ Hz, 1H, ArH), 8.54 (d, $J = 8.2$ Hz, 1H, ArH), 8.45–8.41 (m, 1H, ArH), 8.41–8.37 (m, 1H, ArH), 7.93–7.89 (m, 2H, ArH), 7.84–7.78 (m, 2H, ArH), 7.26 (d, $J = 7.9$ Hz, 2H, ArH), 4.74 (t, $J = 6.3$ Hz, 1H, OH), 3.83–3.80 (m, 1H, CH), 3.79–3.73 (m, 1H, $CH_2$), 3.65–3.59 (m, 1H, $CH_2$), 2.34 (s, 3H, $CH_3$). $^{13}$C-NMR (150 MHz, DMSO-$d_6$) $\delta_{(ppm)}$ 183.32(C=O), 156.49, 156.24, 154.12, 148.70, 141.33, 141.09, 140.44, 140.33, 129.46, 128.26, 128.03, 127.34, 124.45, 123.91(Aryl-C), 67.42($CH_2$), 64.86(CH), and 21.36($CH_3$). ESI–MS: 631.0 $[M + Na]^+$. Anal. Calcd for $C_{20}H_{21}N_3O_6PtS$ (%): C, 38.34; H, 3.38; and N, 6.71. Found: C, 38.63; H, 2.99; and N, 6.79.

**2.3.5. [Pt(bipy)(TsleuNO)] (3).** The synthesis of **3** was carried out in an identical manner to **1** starting from [Pt(bipy)$Cl_2$] (19 mg, 0.045 mmol) and $TsleuH_2$ (25 mg, 0.090 mmol). Yield: 75.5%. Yellow solid. IR (KBr, $cm^{-1}$): 1660, 1382, 422, and 533.

$^1$H-NMR (600 MHz, DMSO-$d_6$) $\delta_{(ppm)}$ 9.34 (d, $J = 5.1$ Hz, 1H, ArH), 8.67 (d, $J = 5.1$ Hz, 1H, ArH), 8.61 (d, $J = 8.1$ Hz, 1H, ArH), 8.55 (d, $J = 7.9$ Hz, 1H, ArH), 8.48–8.35 (m, 2H, ArH), 7.88 (d, $J = 8.2$ Hz, 2H, ArH), 7.86–7.83 (m, 1H, ArH), 7.80 (d, $J = 7.1$ Hz, 1H, ArH), 7.25 (d, $J = 8.0$ Hz, 2H, ArH), 3.77 (dd, $J = 9.9$, 4.3 Hz, 1H, CH), 2.33 (s, 3H, $CH_3$), 1.99–1.84 (m, 2H, $CH_2$), 1.52–1.37 (m, 1H, CH), 0.88 (d, $J = 6.6$ Hz, 3H, $CH_3$), and 0.82 (d, $J = 6.4$ Hz, 3H, $CH_3$). $^{13}$C-NMR (150 MHz, DMSO-$d_6$) $\delta_{(ppm)}$ 185.38(C=O), 156.70, 156.20, 153.05, 148.71, 141.30, 141.24 140.59, 140.33, 129.45, 128.36, 128.07, 127.72, 124.52, 124.18(Aryl-C), 63.23(CH), 45.23($CH_2$), 24.43(CH), 23.84($CH_3$), 21.91($CH_3$), and 21.37($CH_3$). ESI–MS: 656.7 $[M + Na]^+$. Anal. Calcd for $C_{23}H_{25}N_3O_4PtS$ (%): C, 43.53; H, 3.97; and N, 6.62. Found: C, 43.21; H, 3.89; and N, 6.79.

**2.3.6. [Pt(bipy)(TsvalNO)] (4).** The synthesis of **4** was carried out in an identical manner to **1** starting from [Pt(bipy)$Cl_2$] (19 mg, 0.045 mmol) and Tsval$H_2$ (24 mg, 0.090 mmol). Yield: 55.8%. Yellow solid. IR (KBr, $cm^{-1}$): 1665, 1381, 424, and 530. $^1$H-NMR (600 MHz, DMSO-$d_6$) $\delta_{(ppm)}$ 9.41 (d, $J = 5.5$ Hz, 1H, ArH), 8.69 (d, $J = 5.3$ Hz, 1H, ArH), 8.62 (d, $J = 8.1$ Hz, 1H, ArH), 8.55 (d, $J = 8.0$ Hz, 1H, ArH), 8.42 (dd, $J = 18.7$, 7.9 Hz, 2H, ArH), 7.89 (d, $J = 8.1$ Hz, 2H, ArH), 7.87 (d, $J = 7.0$ Hz, 1H, ArH), 7.84–7.77 (m, 1H, ArH), 7.24 (d, $J = 7.9$ Hz, 2H, ArH), 3.55 (d, $J = 6.2$ Hz, 1H, CH), 2.33 (s, 3H, $CH_3$), 2.09 (m, 1H, CH), 1.11 (d, $J = 6.7$ Hz, 3H, $CH_3$), and 1.00 (d, $J = 6.7$ Hz, 3H, $CH_3$). $^{13}$C-NMR (150 MHz, DMSO-$d_6$) $\delta_{(ppm)}$ 184.10(C=O), 156.65, 156.22, 153.14, 148.76, 141.18, 141.13, 140.52, 129.39, 128.33, 128.08, 127.70, 124.53, 124.14(Aryl-C), 70.00(CH), 32.4(CH), 21.36($CH_3$), and 20.19($CH_3$). ESI–MS: 643.2 $[M + Na]^+$. Anal. Calcd for $C_{22}H_{23}N_3O_4PtS$ (%): C, 42.58; H, 3.74; and N, 6.77. Found: C, 42.65; H, 3.64; and N, 6.82.

**2.3.7. [Pt(bipy)(TspheNO)] (5).** The synthesis of **5** was carried out in an identical manner to **1** starting from [Pt(bipy)$Cl_2$] (19 mg, 0.045 mmol) and Tsphe$H_2$ (29 mg, 0.090 mmol). Yield: 83.8%. Yellow solid. IR (KBr, $cm^{-1}$): 1657, 1385, 419, and 552. $^1$H-NMR (600 MHz, DMSO-$d_6$) $\delta_{(ppm)}$ 9.29 (d, $J = 5.8$ Hz, 1H, ArH), 8.61–8.53 (m, 2H, ArH), 8.50 (d, $J = 8.2$ Hz, 1H, ArH), 8.44–8.36 (m, 2H, ArH), 7.89–7.83 (m, 1H, ArH), 7.81–7.74 (m, 3H, ArH), 7.20 (d, $J = 7.9$ Hz, 2H, ArH), 7.12 (d, $J = 6.8$ Hz, 2H, ArH), 6.99–6.87 (m, 3H, ArH), 4.08 (dd, $J = 6.7$, 5.1 Hz, 1H, CH), 3.14 (dd, $J = 13.1$, 6.7 Hz, 1H, $CH_2$), 2.89 (dd, $J = 13.1$, 5.1 Hz, 1H, $CH_2$), and 2.33 (s, 3H, $CH_3$). $^{13}$C-NMR (150 MHz, DMSO-$d_6$) $\delta_{(ppm)}$ 184.30(C=O), 155.96, 153.20, 148.63, 141.23, 141.07, 140.44, 140.01, 138.47, 130.55, 129.42, 128.22, 127.83, 127.48, 126.25, 124.29, 123.90(Aryl-C), 66.35(CH), 41.07($CH_2$), and 21.37($CH_3$). ESI–MS: 691.2 $[M + Na]^+$. Anal. Calcd for $C_{26}H_{23}N_3O_4PtS$ (%): C, 46.70; H, 3.47; and N, 6.28. Found: C, 46.52; H, 3.56; and N, 6.13.

### 2.4. *X-ray structure determination of [Pt(bipy)(TsglyNO)]·2H$_2$O (1)*

The data collection of **1** was performed on a Bruker SMART APEX II CCD diffractometer equipped with graphite monochromated Mo-K$\alpha$ radiation ($\lambda = 0.71073$ Å) at 296(2) K. Multi-scan absorption corrections were applied using SADABS. The structure was solved by the direct method using SHELXS-97.

Table 1. Crystallographic data for **1**.

| | |
|---|---|
| Empirical formula | $C_{19}H_{21}N_3O_6PtS$ |
| Formula weight | 614.53 |
| Temperature (K) | 296(2) |
| Crystal system | Monoclinic |
| Space group | $C2/c$ |
| Unit cell dimensions (Å) | |
| $a$ | 15.981(3) |
| $b$ | 14.205(3) |
| $c$ | 18.680(4) |
| Volume (Å$^3$), $Z$ | 4.1308(15), 8 |
| Calculated density (Mg m$^{-3}$) | 1.973 |
| $F(000)$ | 2376 |
| Crystal size (mm$^3$) | $0.33 \times 0.23 \times 0.16$ |
| $\theta$ range for data collection (°) | 1.94–25.00 |
| Limiting indices | $-13 < h < 18$; $-16 < k < 16$; $-22 < l < 21$ |
| Data/parameters | 3640/272 |
| Goodness-of-fit on $F^2$ | 1.129 |
| Final $R$ indices [$I > 2\sigma(I)$] | $R_1 = 0.0809$, $wR_2 = 0.1988$ |

Refinements on $F^2$ were performed using SHELXL-97 by full-matrix least-squares with anisotropic thermal parameters for all non-hydrogen atoms. Table 1 lists the crystallographic details.

### 2.5. *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$^{-1}$ of penicillin, and 100 μg mL$^{-1}$ of streptomycin. Cells were maintained at 37°C in a humidified atmosphere of 5% $CO_2$ in air.

### 2.6. *Solutions*

The complexes were dissolved in DMSO at 5 mmol L$^{-1}$ as stock solution and diluted in culture medium at concentrations 1.0, 10, 100, and 500 μmol L$^{-1}$ as working solutions. To avoid DMSO toxicity, the concentration of DMSO was less than 0.1% (v/v) in all experiments.

### 2.7. *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 the 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 [10]. Upon completion of the incubation for 44 h, stock MTT dye solution (20 mL, 5 mg mL$^{-1}$) was added to each well. After 4 h incubation, 2-propanol

(100 mL) was added to solubilize the MTT formazan. The OD of each well was 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 [11]. 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 mmol $L^{-1}$ unbuffered Tris base (100 mL) and OD was measured at 540 nm as above. The $IC_{50}$ value was determined from plot of % viability against the dose of compounds added.



## 3. Results and discussion

### 3.1. *Characterization of the complexes*

Elemental analysis data of **1**–**5** are in good agreement with the calculated values. Mass spectra of **1**–**5** have molecular peaks, moreover, which provide support for the suggested composition and structures of the complexes.

Bipy has a maximal absorption peak at 283 nm, assigned to internal $\pi$–$\pi^*$ transition. After formation of the complexes, the absorption peak red shifts by *ca* 32 nm for **1**–**5** compared with bipy, caused by charge transfer transition (metal ~ ligand) from platinum d-orbital to a $\pi^*$ orbital of bipy.

The sulfonamides of $TsglyH_2$, $TsvalH_2$, $TsleuH_2$, $TsserH_2$, and $TspheH_2$ have a strong and sharp $\upsilon_{NH}$ at 3260–3290 $cm^{-1}$, which disappear for **1**–**5**, showing that the sulfonamide has been deprotonated. This is further confirmed by the sulfonamide (I) shifting from ~1630 to ~1550 $cm^{-1}$ and the disappearance of the sulfonamide (II) from the original region. New bands at 470 and 530 $cm^{-1}$ are assigned to $\upsilon_{Pt-N}$. The carboxylates of **1**–**5** show two bands, an intense antisymmetric carboxylate stretch $\upsilon_{(as,\,coo-)}$ and a symmetric carboxylate stretching $\upsilon_{(s,\ coo-)}$ at 1650 and 1380 $cm^{-1}$, respectively. The values of $_{\Delta}\upsilon_{(coo-)}(\upsilon_{(as,\ coo-)}-\upsilon_{(s,\ coo-)})$ are in the range of 245–284 $cm^{-1}$, greater than $_{\Delta}\upsilon_{(coo-)}$ of the corresponding sodium carboxylates, so the carboxylate may be monodentate [12]. This is further confirmed by the appearance of $\upsilon_{Pt-O}$ bands. These results are in agreement with the results revealed by X-ray crystal analysis.

The overall pattern of the $^1$H-NMR spectra of **1**–**5** resembles the free ligands closely, but the signals have shifted upon coordination. $TsvalH_2$ shows a doublet at $\delta = 5.35$, associated with the proton of the sulfonamide, but these peaks disappear for **4**, showing that the sulfonamide has been deprotonated. The methylene $^1$H resonances (amino acid) shift downfield as a result of deprotonated amide nitrogen coordinating to Pt(II). The $\alpha$-hydrogen of $TsvalH_2$ appears as a *dd*, but this proton is a doublet in **4**, which also shows deprotonation of amide (Supplementary material and figure 2). $^1$H-NMR spectra of **1**, **2**, **3**, and **5** are similar to that of **4**, further confirming that sulfonamide coordinates to platinum through deprotonated amide. The $^{13}$C-NMR spectra provide further support for the structures of the complexes (Supplementary material).

### 3.2. *Structural studies*

The molecular structure of $[Pt(bipy)(TsglyNO)]\cdot 2H_2O$ (**1**) is shown in figure 3 and selected bond lengths and angles are given in table 2. The platinum is square planar

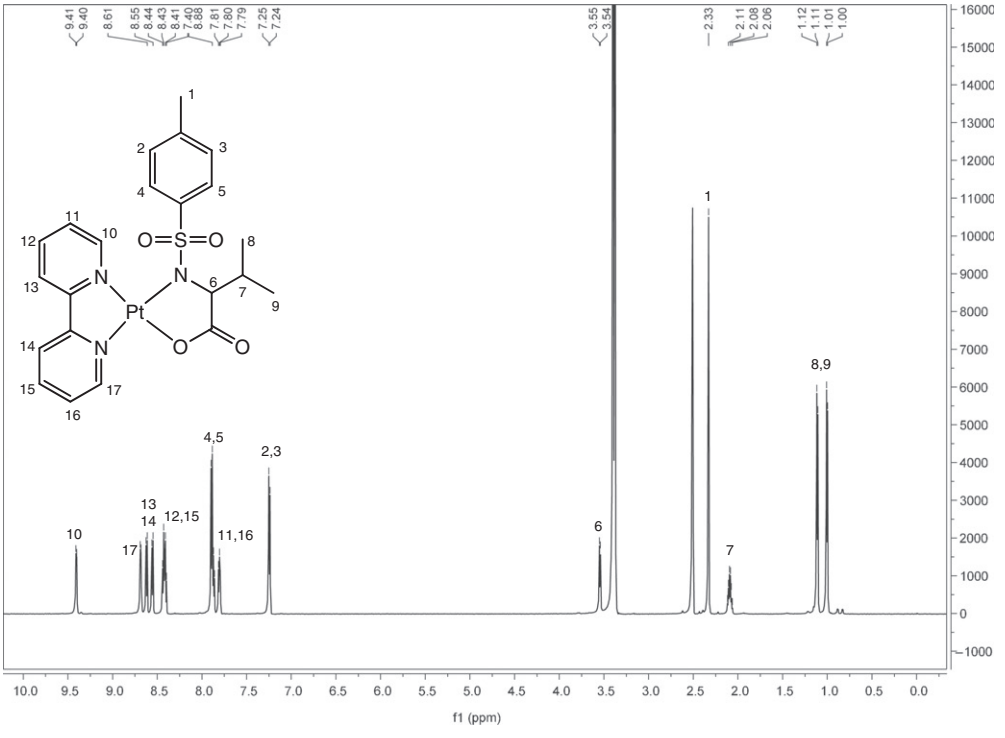



Figure 2. $^{1}$H-NMR spectra of **4** in DMSO-$d_6$.

with two nitrogens of bipy, one deprotonated sulfonamide and one carboxylic. The angle between planar N(2)–Pt(1)–N(3) and planar O(1)–Pt(1)–N(1) is 2.528(522)° 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 (2.050(13) Å) is similar to the Pt–N (bipy) bond lengths (2.013(15) and 2.008(14) Å), while it is longer than Pt–O (carboxylic oxygen) bond length (1.998(13) Å). Sigel *et al.* [13] reported that the coordinating qualities of deprotonated amide nitrogen were "O-like" as the deprotonated amide group is isoelectronic with carboxylate, and this has been confirmed by the stability constants of some complexes. Gong *et al.* [14] also reported that the deprotonated amide nitrogen was different from the ordinary amino nitrogen and its coordinating property might be "O-like". So, we deduce that the coordination of the deprotonated sulfonamide nitrogen may also be "O-like".

There is a weak $\pi$–$\pi$ interaction between bipy ligands. Bipy aromatic rings are arranged in an offset face-to-face stacking mode. The intermolecular centroid–centroid distances are approximately 4.01 Å, conforming to the approximate $\pi$–$\pi$ interaction. A similar $\pi$–$\pi$ stacking is observed between phenyl groups of TsglyNO with intermolecular centroid–centroid distances of 4.20 Å. The water in the crystal cell does not form hydrogen bonds; hence, we omit it for clarity (figure 4).

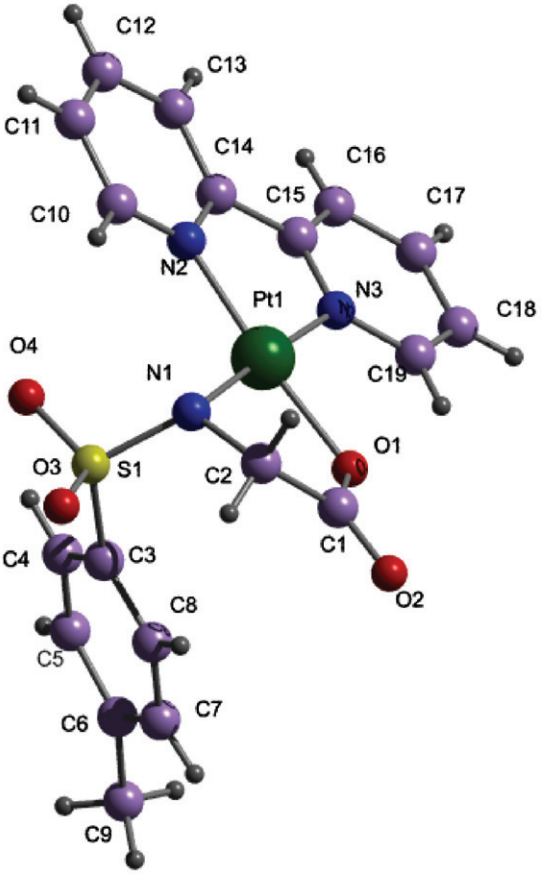



Figure 3. Molecular structure and atom-labeling scheme for **1** ($H_2O$ is omitted for clarity).

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

| | |
|---|---|
| Pt(1)–N(1) | 2.050(13) |
| Pt(1)–N(2) | 2.013(15) |
| Pt(1)–N(3) | 2.008(14) |
| Pt(1)–O(1) | 1.998(13) |
| N(1)–Pt(1)–N(2) | 103.3(6) |
| N(2)–Pt(1)–N(3) | 79.0(6) |
| N(3)–Pt(1)–O(1) | 95.9(6) |
| O(1)–Pt(1)–N(1) | 81.7(5) |
| O(1)–Pt(1)–N(2) | 174.4(5) |
| N(1)–Pt(1)–N(3) | 177.4(6) |

### 3.3. *Cytotoxic studies*

As listed in table 3, **1**–**5** exert cytotoxic effects with selectivity against tested carcinoma cell lines; **5** displays better cytotoxicity against BGC-823, Bel-7402, and KB cell lines, while **1** has better cytotoxicity against KB cell line; none of the complexes show higher cytotoxicity than cisplatin.

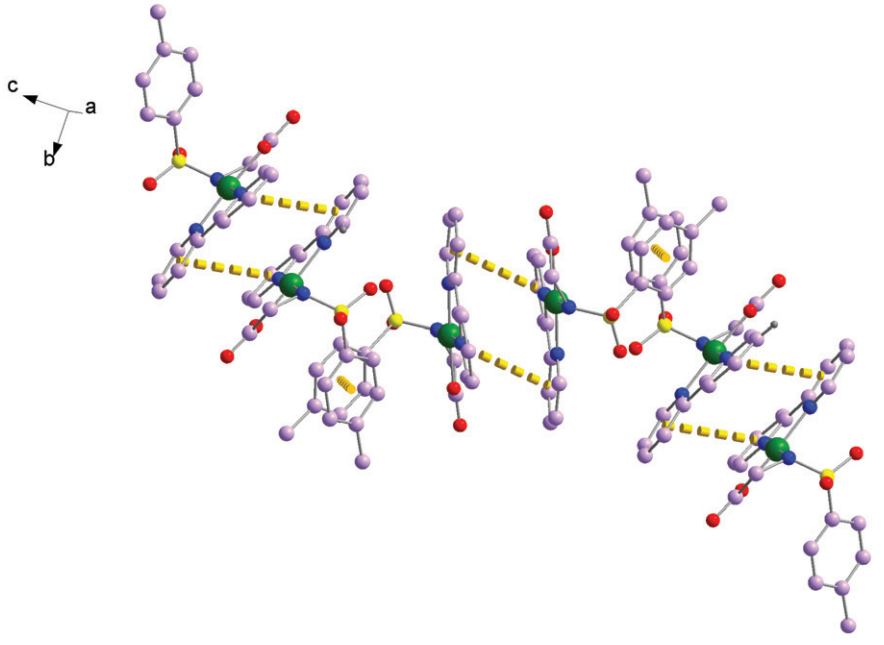



Figure 4. View showing the weak pairing of **1**.

Table 3. The cytotoxicities of complexes *in vitro* ($n = 5$).

| | $IC_{50}$ | | | |
|---|---|---|---|---|
| Complexes | HL-60 | BGC-823 | Bel-7402 | KB |
| **1** | $18.86 \pm 1.34$ | $39.35 \pm 3.56$ | $60.70 \pm 5.68$ | $4.34 \pm 1.09$ |
| **2** | $25.69 \pm 2.32$ | $26.73 \pm 2.05$ | $31.44 \pm 1.78$ | $22.40 \pm 2.76$ |
| **3** | $26.41 \pm 2.45$ | $22.02 \pm 2.32$ | $25.98 \pm 2.39$ | $13.60 \pm 1.23$ |
| **4** | $18.42 \pm 2.08$ | $26.53 \pm 2.12$ | $24.01 \pm 2.09$ | $15.61 \pm 1.34$ |
| **5** | $27.73 \pm 2.23$ | $10.03 \pm 1.76$ | $14.30 \pm 1.29$ | $6.98 \pm 1.01$ |
| Cisplatin | $2.89 \pm 0.34$ | $6.48 \pm 0.81$ | $8.12 \pm 0.97$ | $2.65 \pm 0.33$ |

Puthraya *et al*. reported that the side chain of the amino acids may influence the inhibitory activity for $[Pt(bipy)AA]^{n+}$ complexes (where AA is an anion of Cys, Asp, Glu, L-methionine (Met), His, L-arginine (Arg), Phen, Tyr, or L-tryptophan (Try), and $n = 0$ or 1). This inhibitory activity was found to decrease as follows: nonpolar hydrophobic > polar uncharged > charged side groups [7]. Da *et al*. reported that *cis*-$[Pt(Mor)(PhCH_2NH_2)Cl_2]$, *cis*-$[Pt(Mor)(PhCH_2CH_2NH_2)Cl_2]$ and *cis*-$[Pt(Mor)(OC_9H_6N)Cl]$ (Mor = morpholine) exhibited anticancer activities *in vitro* against human Hep-G and RD cell lines with $IC_{50} < 5\,mg\,mL^{-1}$ [15]. Corbi *et al*. reported that a platinum(II) complex with the amino acid L-alliin has moderate cytotoxic activity, inducing about 40% cell death at $400\,\mu mol\,L^{-1}$ [16]. Kavlakova *et al*. reported that three Pt(II) complexes with 4-amino-4H-1,2,4-triazole showed poorer cytotoxicity

than cisplatin against human tumor cell lines HL-60, BV-173, SKW-3, and HL-60 DOX [17]. In this study, platinum(II) complexes with 4-toluenesulfonyl-L-amino acid dianions and bipy, the 4-toluenesulfonyl-L-amino acid dianions have an important effect on cytotoxicity. With 4-toluenesulfonyl-L-glycine and 4-toluenesulfonyl-L-phenylalanine dianions, the complexes show better cytotoxicity. This will be valuable for the design of new metal-based antitumor agents.

## 4. Conclusions

We have synthesized and characterized five new platinum(II) complexes with 4-toluenesulfonyl-L-amino acid dianions and bipy. The cytotoxic experiment indicated that platinum(II) complexes with 4-toluenesulfonyl-L-amino acid dianions and bipy might be a promising source of metal-based antitumor agents. Current studies are ongoing in our laboratory to gain insight in the mechanism of action of these complexes, which may be helpful for the design of new metal-based antitumor agents.



## Supplementary material

Crystallographic data for the structural analysis of **1** have been deposited with the Cambridge Crystallographic Data Centre, CCDC – 794218. 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; www: http://www.ccdc.cam.ac.uk).

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