Synthesis, Characterization and Cytotoxicity of Dihalogeno-platinum(II) Complexes with L-Histidine Ligand

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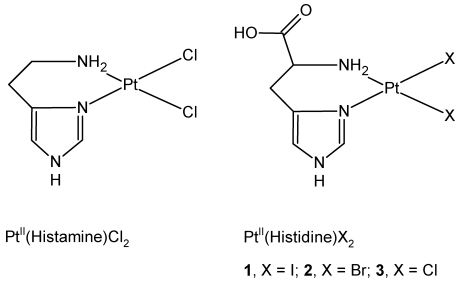
Diiodo-, dibromo- and dichloro-platinum(II) complexes containing L-histidine ligand were prepared. Their spectra and X-ray crystal structure of the dibromo-platinum(II) complex were described. Only the dichloro-platinum(II) complex showed comparable cytotoxic activity with carboplatin against A549/ATCC, HT-29, and LNcap cell lines. Nevertheless the complexes with COOH-substituted ligands histidine may be good starting materials to synthesize targeting platinum complexes since they could be easily linked to suitable carrier molecules *via* esterification.

Key words platinum(II) complex; L-histidine; histamine; cytotoxic activity; crystal structure

Since its discovery over four decades ago, cisplatin, *cis*-dichlorodiammineplatinum(II), has become one of the most widely used anticancer drugs in the treatment of a variety of human tumors. Driven by the impressive impact of cisplatin on cancer therapy, as well as to overcome its severe toxic side effects and drug resistances, numerous platinum complexes have been synthesized and tested for their anticancer activities, resulting in two platinum-based drugs, carboplatin and oxaliplatin, received worldwide approval and achieved routine clinical use.[1—7] In recent years, three other platinum-based drugs, nedaplatin, lobaplatin and SKI2053R, have received limited approval for use in Japan, China and South Korea, respectively.[5] As of yet, they have not demonstrated any clear advantages over cisplatin although some of them showed potential activity against cisplatin-resistant cells and tumors during preclinical evaluations.[1,5] Therefore, the search continues for the new potent platinum complexes with high antitumor activity, less toxic and lack of cross-resistance to cisplatin.

One noteworthy approach in the development of novel platinum anticancer drugs is to use physiologically active biomolecules as ligands.[8—10] Studies on these platinum complexes have yielded interesting results, for example, bile acid- and steroid-platinum complexes have been proved to improve antitumor activity and reduce toxicity by selective accumulation in tumor tissues.[11—14] Reedijk and Kralingen and Garnuszek *et al.* prepared a new platinum complex, Pt^{II}(histamine)Cl_2 (Fig. 1), containing histamine biomolecule as the carrier ligand, but did not report its anticancer activity.[15,16] In our previous study, we tested for its cytotoxicity against A549/ATCC, HT-29, and LNcap human carcinoma cell lines. The complex showed significant cytotoxic activity, however, it is nearly insoluble in water (0.3 mg/ml) at 25 °C.[17] In order to improve the solubility, we also synthesized the corresponding dicarboxylate histamine-platinum(II) complexes. Unfortunately, they were inactive against the tested cell lines.[17]



Pt^{II}(Histamine)Cl_2 Pt^{II}(Histidine)X_2

1, X = I; **2**, X = Br; **3**, X = Cl

Fig. 1. Chemical Structures of Pt^{II}(Histamine)Cl_2 and Pt^{II}(L-Histidine)X_2 Complexes

During recent years, much attention in the design of new platinum anticancer drugs has been focused on platinum(II) complexes with OH- and COOH-substituted ligands,[5,18,19] because the ligands are potential to act as donors or acceptors for hydrogen bonds, which could play an important role in the binding of platinum complexes to DNA, the major target of platinum-based chemotherapy. Moreover, these platinum complexes were also used as starting materials for further derivatization, with the aim of attaching suitable carrier molecules for drug targeting.[8—10] In this context, we have synthesized a series of platinum(II) complexes with OH-substituted ligands, 2-hydroxy-1,3-propanediamine, as their carrier ligands. These complexes show significant cytotoxic activity against the tested cell lines.[20] The excellent activity inspires us further investigation of platinum(II) complexes containing COOH-substituted ligands.

Based on the above findings, we designed and synthesized Pt^{II}(L-histidine)X_2 (**1**, X=I; **2**, X=Br; **3**, X=Cl) complexes (Fig. 1) with the attempt to increase the solubility and cytotoxicity of Pt^{II}(histamine)Cl_2. L-Histidine, an essential amino acid, could be converted to histamine *via* decarboxylation in the human body. In the last few years several authors have shown interest in the structure of Pt^{II}(L-histidine)X_2 complexes. Caubet *et al.*[21] have prepared dichloro-(L-histidine)-platinum(II) complex and Baidina *et al.*[22] have reported X-ray crystal structure of diiodo-(L-histidine)platinum(II) complex. However, less information is available about their spectra and cytotoxicity. In this paper we report synthesis, characterization and *in vitro* cytotoxic activity of complexes **1**—**3** (Fig. 1), along with X-ray crystal structure of complex **2**.

Experimental

Materials and Instruments K_2PtCl_4 and L-histidine were purchased

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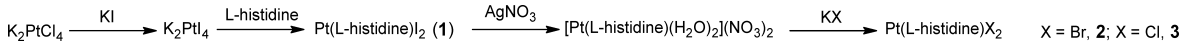

Chart 1

from Aldrich (Sigma-Aldrich China Inc. Shanghai, China). All reagents were of high purity and used without any further purification. Chemical analyses for C, H and N were performed with a Carlo-Ebra Instrument (Carlo Erba, Milan, Italy), whereas platinum was determined according to the method in USP24. Mass spectrometry studies were carried out on a VG-Auto300 Spectrometry in the FAB^+ mode using glycerine as matrix. ^{1}H-NMR was obtained on Brucker AV-400 (Bruker Bioscience, Billerica, MA, U.S.A.) relative to tetramethylsilane (TMS) as an external standard.

Synthesis The complexes **1**—**3** were prepared by using an extension of Dhara's method (Chart 1).[23,24]

cis-Diiodo(L-histidine)platinum(II) (**1**): K_2PtCl_4 (2.075 g, 5 mmol) was dissolved in distilled water (40 ml) and treated with KI (30 mmol). After stirring in the dark for 40 min at room temperature, a solution of L-histidine (0.776 g, 5 mmol) in 25 ml distilled water was added dropwise. The mixture was stirred for 4 h at 40 °C and the yellow precipitate was filtrated off, washed with distilled water and ethanol and dried under vacuum at 60 °C. Yield 70% (2.11 g). Found (Calcd for $C_6H_9I_2N_3O_2Pt$): Pt 31.56 (32.30), C 11.82 (11.93), H 1.45 (1.50), N 6.91 (6.96). MS-FAB^+ *m/z*: $[M]^+$ 604 (25%), $[L\text{-histidine}+H]^+$ 156 (100%). ^{1}H-NMR (DMSO, 400 MHz) δ (ppm): 2.96 (m, 2H, $\underline{CH}_2$), 3.16 (m, 1H, $\underline{CH}CH_2$), 5.82, 6.24 (two m, 2H, $\underline{NH}_2$), 7.30 (s, 1H, C_5-$\underline{H}$), 8.68 (s, 1H, C_4-$\underline{H}$), 13.20 (s, 1H, $\underline{NH}$).

cis-Dibromo(L-histidine)platinum(II) (**2**): To a suspension of complex **1** (0.604 g, 1 mmol) in 20 ml distilled water, 0.34 g $AgNO_3$ (2 mmol) dissolved in 5 ml distilled water was added, and the mixture was stirred for 24 h at room temperature. After AgI formed was filtrated off, 0.25 g (2.1 mmol) KBr was added to the filtrate giving an off-white precipitate. The off-white precipitate was removed by filtration. The yellow filtrate was concentrated to 4 ml under reduced pressure, a yellow crystal product precipitated and then it was filtrated off, washed with distilled water and ethanol and dried under vacuum at 60 °C. Yield 25% (0.13 g). Found (Calcd for $C_6H_9Br_2N_3O_2Pt$): Pt 38.01 (38.25), C 14.08 (14.13), H 1.76 (1.78), N 8.19 (8.24). MS-FAB^+ *m/z*: $[M+H]^+$ 511 (35%), $[L\text{-histidine}+H]^+$ 156 (100%). ^{1}H-NMR (DMSO, 400 MHz) δ (ppm): 2.95 (m, 2H, $\underline{CH}_2$), 3.11 (m, 1H, $\underline{CH}CH_2$), 5.45, 5.91 (two m, 2H, $\underline{NH}_2$), 7.20 (s, 1H, C_5-$\underline{H}$), 8.42 (s, 1H, C_4-$\underline{H}$), 13.02 (s, 1H, $\underline{NH}$).

cis-Dichloro(L-histidine)platinum(II) (**3**): The synthetic procedure was similar to that used for complex **2**. Yield 21% (0.09 g). Found (Calcd for $C_6H_9Cl_2N_3O_2Pt$): Pt 46.10 (46.32), C 17.06 (17.11), H 2.14 (2.15), N 9.95 (9.98). MS-FAB^+ *m/z*: $[M+H]^+$ 422 (32%), $[L\text{-histidine}+H]^+$ 156 (100%). ^{1}H-NMR (DMSO, 400 MHz) δ (ppm): 2.81 (m, 2H, $\underline{CH}_2$), 2.96 (m, 1H, $\underline{CH}CH_2$), 5.06, 5.63 (two m, 2H, $\underline{NH}_2$), 7.14 (s, 1H, C_5-$\underline{H}$), 8.26 (s, 1H, C_4-$\underline{H}$), 12.80 (s, 1H, $\underline{NH}$).

X-Ray Crystallographic Study Single crystals of complex **2** suitable for X-ray analysis were obtained by slow evaporation from aqueous solutions at room temperature. Intensity data were collected on a SMART APEX II CCD diffractometer at room temperature. For extracting intensities from CCD images the program Nonius was used. The structure were solved by direct methods and refined by full-matrix least-squares techniques. Non-hydrogen atoms were refined with anisotropic displacement parameters. H atoms were calculated and allowed to ride. Computer programs: structure solution, SHELXS-97,[25] refinement, SHELXS-97,[26] molecular diagrams, ORTEP.[27] The crystal structure data are shown in Tables 1—3.

***In Vitro* Cytotoxicity Assays** The cellular survival was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method.[24,28] Cells were plated onto 96-well sterile plates in 100 μl of medium at a density of 2×10^3 cells per well and incubated for 48 h at 37 °C in a 7% CO_2-containing incubator. The title complexes, Pt^{II}(histamine)Cl_2, cisplatin and carboplatin were added in final concentrations ranging from 0 to 100 μM (The compounds were dissolved in water.). After 72 h, 50 μl MTT in PBS (5 mg/ml) was added to each well and the plates were incubated for 2—3 h at 37 °C. The solution was carefully removed and the remaining crystals dissolved in 100 μl of DMSO. Cell survival was evaluated by measuring the absorbance at 590 nm. All cytotoxicity tests were performed three times in quadruplicate. The IC_{50} values were calculated from curves constructed by plotting cell survival (%) *versus* compound concentration (in μM).

Results and Discussion

Complex **1** was synthesized by direct reaction of K_2PtI_4 and histidine. Complexes **2** and **3** were prepared by using an extension of Dhara's method (Chart 1).[23,24] We also attempted to prepare complex **3** by direct reaction of K_2PtCl_4 and histidine as Caubet *et al.* reported,[21] but in this reaction we only obtained an off-white precipitate. The precipitate is insoluble in most common solvents, and no attempt has been made to identify it. By using Dhara's method, the procedures of preparation of complexes **2** and **3** also formed off-white precipitates, but we successfully prepared them as yellow crystals by removed the precipitates though the yields were low.

All three complexes were characterized by elemental analysis, FAB^+-MS and ^{1}H-NMR. The elemental analysis re-

Table 1. Crystal Data and Structure Refinement for Complex **2**

Chemical formula	$C_6H_9Br_2N_3O_2Pt$
M_r	510.07
Crystal system	Monoclinic
Space group	*P*2(1)
a [Å]	7.6310(9)
b [Å]	14.2527(16)
c [Å]	10.5921(12)
β [°]	96.0890(10)
V [Å^3]	1145.5(2)
Z	2
Density (calcd, g cm^{-3})	2.958
Diffraction radiation type	Mo$K\alpha$ (λ=0.71073 Å)
μ [mm^{-1}]	19.205
T [K]	273(2)
Crystal dimension [mm]	0.08×0.07×0.04
F(000)	920.0
R_{int}	0.0295
T_{max}/T_{min}	0.5138/0.3087
Reflections with $I>2\sigma(I)$	4229
$R[F^2>2\sigma(F^2)]$	0.0352
w$R(F^2)$	0.1036
Goodness-of-fit	0.691
$\Delta\rho_{max}$ [e Å^{-3}]	1.367
$\Delta\rho_{min}$ [e Å^{-3}]	−1.074

Table 2. Selected Bond Distances [Å] and Angles [°] for Complex **2**

Pt(1)–N(2)	2.019(11)	N(2)–Pt(1)–N(1)	89.9(5)
Pt(1)–N(1)	2.023(8)	N(2)–Pt(1)–Br(1)	174.0(3)
Pt(1)–Br(1)	2.4353(15)	N(1)–Pt(1)–Br(1)	85.1(4)
Pt(1)–Br(2)	2.4522(12)	N(2)–Pt(1)–Br(2)	92.9(3)
		N(1)–Pt(1)–Br(2)	177.2(4)
		Br(1)–Pt(1)–Br(2)	92.23(6)

Table 3. Hydrogen Bonds for Complex **2**

D–H···A	d(D–H)	d(H···A)	d(D···A)	∠(DHA)
N(6)–H(6)···Br(4)$^{a)}$	0.86	2.94	3.538(12)	128.6
N(6)–H(6)···Br(3)$^{a)}$	0.86	2.64	3.423(13)	151.3
N(4)–H(4A)···Br(2)$^{b)}$	0.90	2.63	3.514(10)	166.2
N(3)–H(3)···Br(1)$^{c)}$	0.86	2.95	3.546(12)	128.2
N(3)–H(3)···Br(2)$^{c)}$	0.86	2.58	3.368(11)	152.8
O(4)–H(4)···O(1)$^{d)}$	0.82	1.83	2.636(15)	167.9
O(2)–H(2)···O(3)$^{e)}$	0.82	1.88	2.636(14)	152.7

Symmetry transformations used to generate equivalent atoms: *a*) $-x+2$, $y+1/2$, $-z+2$; *b*) $x+1$, y, z; *c*) $-x+1$, $y-1/2$, $-z+1$; *d*) x, y, $z-1$; *e*) x, y, $z+1$.

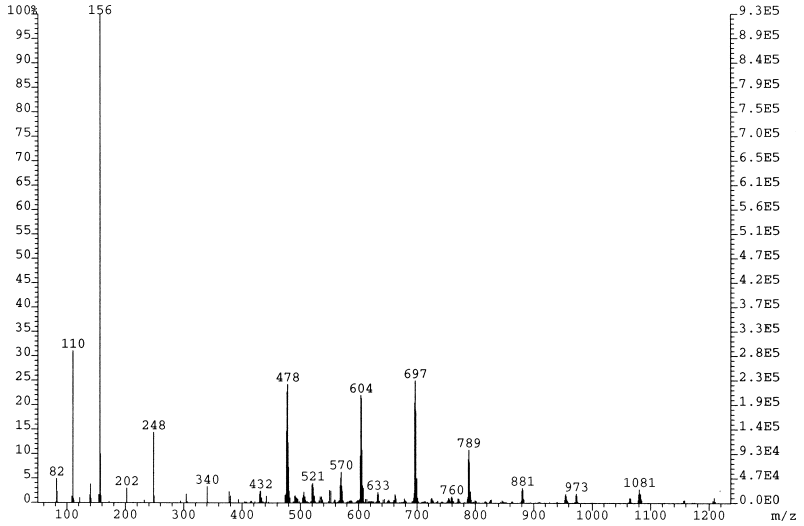

Fig. 2. The Mass Spectra of Complex **1**

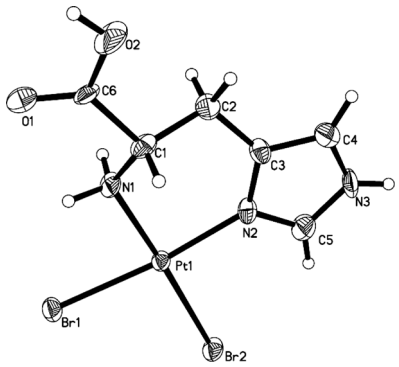

Fig. 3. ORTEP Diagram of Complex **2** Displaying Thermal Ellipsoids at 30% Probability

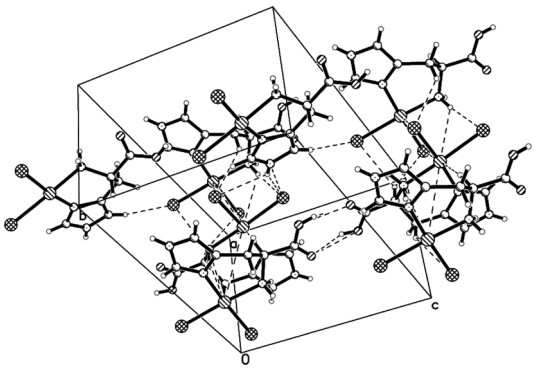

Fig. 4. View Showing the Weak Pairing of Complex **2** Molecules within the Unit Cell

sults are in good agreement with the calculated values. The complexes showed $[M]^+$ or $[M+H]^+$ peaks (25—35%) (Fig. 2), corresponding to their molecular weights. The ^{1}H-NMR spectra of the complexes were consistent with their corresponding protons both in the chemical shifts and the number of hydrogens. Compared with free histidine, the signal of the methine proton (H) of the complexes shifts to upfield, whereas of H of imidazole shifts to downfield as a consequence of the ligand coordination to the platinum atom.

The ORTEP drawing of complex **2** is shown in Fig. 3, and selected bond distances and angles are listed in Table 2. The Pt(II) atom is coordinated on a distorted square by two bromide atoms and the bidentate histidine ligand bound to the Pt atom *via* the two N atoms in the amino and imidazole group. As shown in Table 2, the average Pt–N distance is 2.02 Å and Pt–Br distance is 2.44 Å, while the N–Pt–N and Br–Pt–Br angles are 89.9° and 92.2°, respectively, all of these agreeing well with the data of similar platinum complexes described in the literatures.[29—31] The six-membered chelate ring formed with the Pt(II) atom adopts the boat conformation and the imidazole ring is nearly parallel to the Pt(II) coordination plane. The complex molecules are linked by molecular interactions involving the bromine atoms and the hydrogen atoms in the OH, NH_2 and NH groups (Fig. 4, Table 3), giving rise to a three-dimensional network motif.

Complexes **1**—**3**, as expected, have higher solubility in water (>5 mg/ml) than Pt^{II}(histamine)Cl_2. The *in vitro* cytotoxicities of the complexes were evaluated by MTT colorimetric assay[24,28] using A549/ATCC human lung carcinoma, HT-29 human colon carcinoma and LNcap human prostate adenocarcinoma cell lines. As can be seen from Table 4, complexes **1** and **2** did not show any cytotoxic activity against the tested cell lines, while complex **3**, unlike Pt^{II}(histamine)Cl_2, was less active than cisplatin and only showed comparable activity to carboplatin. However, the COOH-substituted ligands could easily link suitable carrier molecules *via* esterification, so complex **3** may be a good starting material for further derivatization for drug targeting.

Table 4. *In Vitro* Cytotoxicity against Selected Human Cancer Cell Lines of Complexes **1**—**3**

Complexes	IC_{50} (μM)		
	A549/ATCC	HT-29	LNcap
1	>100	>100	>100
2	>100	>100	>100
3	77.4	57.2	22.7
Pt^{II}(histamine)Cl_2	13.8	25.1	10.5
Carboplatin	73.2	>100	38.2
Cisplatin	4.5	17.3	2.4

In conclusion, we have synthesized and characterized

dihalogeno-(L-histidine)platinum(II) complexes. L-Histidine ligand significant improves the solubility of the platinum complexes in water compared to the corresponding histamine platinum complexes. However, the biological evaluation showed the COOH-substituted ligand histidine as carrier ligands could not improve the cytotoxic activity of platinum(II) complexes.

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