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A Novel Water-Soluble Heptaplatin Analogue with Improved Antitumor Activity and Reduced Toxicity

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Supporting Information

ABSTRACT: A novel water-soluble heptaplatin analogue, *cis*-[(4*R*,5*R*)-4,5-bis-(aminomethyl)-2-isopropyl-1,3-dioxolane](3-hydroxy-1,1-cyclobutanedicarboxylato)platinum-(II), has been synthesized and biologically evaluated. The complex shows more activity and less toxicity than its parent drug heptaplatin, exhibiting the great potential for further development.

Heptaplatin, *cis*-[(4*R*,5*R*)-4,5-bis(aminomethyl)-2-isopropyl-1,3-dioxolane]malonatoplatinum(II), is a third-generation platinum antitumor drug developed by SK Pharmaceuticals.[1,2] As a platinum complex, heptaplatin shows equivalent antitumor activity and less toxicity compared to cisplatin.[3] In addition, it is effective against cisplatin-resistant L1210 leukemia cells, probably owing to its unique amine carrier. Heptaplatin has been approved in the Republic of Korea for the treatment of advanced gastric cancer and lung cancer.[4−6] Unfortunately, treatment with heptaplatin still causes significant side-effects including nephrotoxicity, myelosuppression, and heptatoxicity, and nephrotoxicity is considered dose-limiting.[2,7] The water solubility of heptaplatin is only 4−5 mg/mL at room temperature, lower than that for carboplatin (17.5 mg/mL), a factor that we believe is an important contributor to the neprotoxicity associated with heptaplatin. Increasing the water solubility of platinum antitumor comlexes has been an important practical objective of many drug development programs,[8,9] reflecting the general view that greater water solubility may reduce the side-effects, particularly nephrotoxicity, of platinum antitumor complexes. In our effort to retain the excellent antitumor properties of heptaplatin while improving their aqueous solubility, we have recently prepared a series of more water-soluble heptaplatin analogues, as shown in Figure 1, and screened them for their anticancer activity in an S180 animal model. All of the analogues were found to be soluble and stable in water (>15 mg/mL), but only one, LLC-0601, had antitumor activity that was superior to that of heptaplatin. Therefore, this analogue was selected for further investigation.

Synthesis of LLC-0601 is outlined in Schemes 1−3. 3-Hydroxy-1,1-cyclobutanedicarboxylic acid (X) and (4*R*,5*R*)-4,5-bis-(aminomethyl)-2-isopropyl-1,3-dioxolane (A) were prepared by using the reported methods[3,10] with some modification and improvement. K_2PtCl_4 was converted in situ to K_2PtI_4 by treatment with KI. Upon the addition of A, *cis*-[Pt(II)AI_2] was formed. The quantitative reaction with Ag_2X yielded the final product, which was then recrystallized from a 1:1 mixture of water and ethanol to obtain the sample for structural characterization and biological tests. The purity was determined by RP-HPLC to be >99.0% (see Figure S1, Supporting Information). The structural characterization was performed by elemental analysis, FT-IR, ^{13}C NMR, FAB$^+$-MS spectroscopy (Figures S2−S4, Supporting Information) along with X-ray crystallography. The data are in good agreement with the corresponding structure of LLC-0601. The water solubility was determined to be 25.7 mg/mL at 25 °C, and $[\alpha]_D^{25°C}$ was −41° (*C* = 20.45 mg/mL, water)

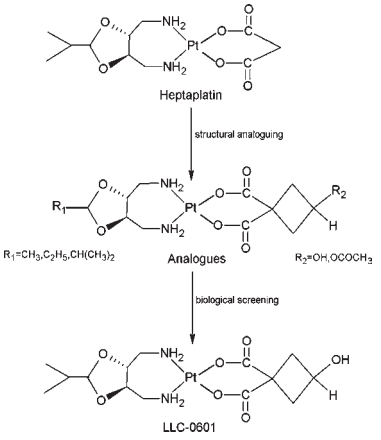
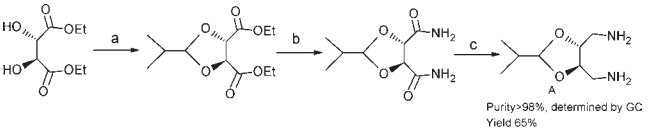


Figure 1. Chemical structures of heptaplatin and LLC-0601.

Scheme 1. Preparation of (4*R*,5*R*)-4,5-Bis(aminomethyl)-2-iso-propyl-1,3-dioxolane[a]



[a] (a) $(CH_3)_2CHCHO$, CH_3SO_3H, cyclohexane, 24 h; (b) NH_3, C_2H_5OH, 4 °C, 20 h; (c) $LiAlH_4$, THF, reflux, 2.5 h.

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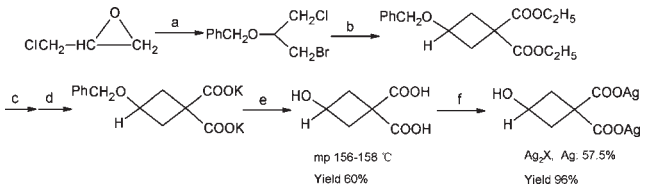
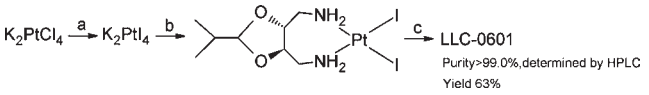
Scheme 2. Preparation of 3-Hydroxy-1,1-cyclobutanedicarboxylic Acid[a]



[a] (a) $PhCH_2Br$, $HgCl_2$, 160 °C, 9 h; (b) $CH_2(COOC_2H_5)_2$, NaH, dioxane, N_2, reflux, 60 h; (c) KOH, C_2H_5OH, reflux; (d) HCl; (e) H_2, 5% Pd/C, 30 °C, 5 h; (f) $NaHCO_3$, $AgNO_3$, 20−25 °C, 2 h.

Scheme 3. Synthesis of LLC-0601[a]



[a] (a) KI, 35−40 °C, 5 h; (b) A, 22−25 °C, 2 h; (c) Ag_2X, 50−55 °C, 24 h.

Single crystals of LLC-0601 were obtained by slow evaporation from aqueous solutions at room temperature. The structure of LLC-0601, as determined from single-crystal X-ray diffraction studies, is illustrated in Figure 2. The selected bond lengths and angles are listed in Table S1, Supporting Information. The Pt(II) atom is coordinated on a distorted square by two N atoms of the (4*R*,5*R*)-4,5-bis(aminomethyl)-2-isopropyl-1,3-dioxolane ligand and two O atoms of the 3-hydroxy-1,1-cyclobutane dicarboxylate anion with the metal at about 0.02 Å out of the mean plane defined by the four donor atoms. The average Pt−N distance is 2.019 Å, and the Pt−O distance is 2.004 Å, while the N−Pt−N and O−Pt−O angles are 91.6° and 90.7°, respectively. All of these agree well with the data of similar platinum complexes described in the literatures.[11−14] The six-membered chelate ring formed with the Pt(II) atom adopts the boat conformation, and the cyclobutane ring is nearly perpendicular to the Pt(II) coordination plane. The seven-membered chelate ring formed with the Pt(II) atom also adopts the boat conformation. The complex molecules are linked by molecular interactions involving the O atoms in the carboxylate groups and the hydrogen atoms in the water molecules and NH_2 groups, giving rise to a three-dimensional network motif. Extensive intermolecular hydrogen bonds were observed in this crystal, with ammine groups as hydrogen bond donors and the oxygen atoms of the carboxylate as acceptors. The hydrogen bonds formed with water molecules may contribute to a great solubility of the complex in water.

The cytotoxicities of LLC-0601 and its parent heptaplatin as a control were assessed with the standard MTT assay[13,15] using human tumor cell lines as well as human normal cell lines. The results are summarized in Table 1.

IC_{50} is the concentration of platinum complexes required to induce 50% inhibition of cell growth. The values of IC_{50} revealed that LLC-0601 had comparable activity to heptaplatin against A549/ATCC and SGC-7901 cancer cells. However, for selected human normal renal and liver cells, it was nearly 2 times less cytotoxic than heptaplatin on the basis of IC_{50} values.

Acute toxicity is an adverse nonspecific effect that occurs in a healthy animal within two weeks after being IP-injected with a single dose of the drug. The acute toxicity tests of LLC-0601 and heptaplatin were carried out in healthy ICR mice according to the standard procedure.[16] The toxicity was measured according to the values of LD_{10} (dose causes 10% death of animals) and LD_{50} (dose causes 50% death). As seen from Table S4 (Supporting Information), the LD_{10} and LD_{50} of LLC-0601 were found to be 216.5 and 372.5 mg/kg, respectively, which are much larger than the corresponding values of heptaplatin (LD_{10} = 154.5 mg/kg, LD_{50} = 196.2 mg/kg), indicating that LLC-0601 was less toxic than heptaplatin in animals following IP adminstration. The histological post-mortem examinations of mice revealed death caused by these platinum complexes mainly resulted from myelosuppression.

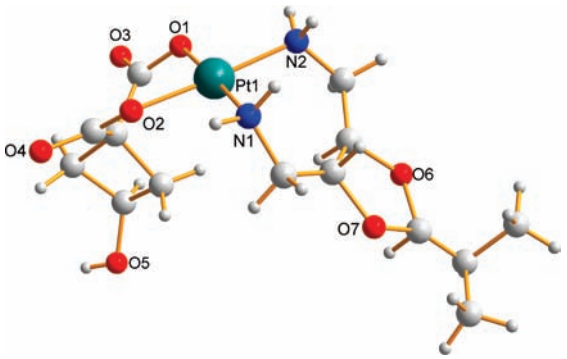


Figure 2. A view of the molecular structure of LLC-0601 with the atomic labeling scheme.

Table 1. *In Vitro* Cytotoxicities of Platinum(II) Complexes[a]

	IC_{50} [μM]			
	A549/ATCC	SGC-7901	HK-2	L-02
LLC-0601	10.7 ± 1.2	2.7 ± 0.32	168.2 ± 10.0	36.6 ± 7.6
Heptaplatin	9.80 ± 1.0	2.0 ± 0.28	90.0 ± 4.9	16.5 ± 3.3

[a] A549/ATCC = human lung cancer cell line, SGC-7901 = human gastric carcinoma cell line, HK-2 = human normal renal tubular epithelial cell line, L-02 = human normal fetal liver cell line. Data are presented as mean ± SD from three independent experiments.

The antitumor activity of LLC-0601 and heptaplatin was further compared in mouse S180 sarcoma as well as human tumor A549 and SGC-7901 xenografts in accordance with well-established methods,[13,16−18] and the results are presented in Tables 2 and S5−S7 (Supporting Information). Treatment with LLC-0601 following tumor transplant caused a dose-dependent reduction of tumor weight in mice with an S180 tumor and A549 xenograft. The dose required to produce a statistically significant antitumor effect ($p < 0.05$) was 120 mg/kg both in S180 and SGC-7901 tumors and 60 mg/kg in the A549 tumor. In contrast, heptaplatin only exhibited significant activity against the A549 xenograft at its maximum dose of 80 mg/kg, due to its low water solubility. In general, a 60 mg/kg dose of LLC-0601 had *in vivo* antitumor activity approximately equal to that of 80 mg/kg of heptaplatin. Given that the *in vitro* cytotoxicity of LLC-0601 was similar to that of heptaplatin against A549/ATCC and SGC-7901 cancer cells, LLC-0601 may have more favorable pharmacokinetic characteristics than heptaplatin.

A repeated-dose toxicity study[19] was conducted in order to further explore the potential advantage of LLC-0601 over heptaplatin. Sprague−Dawley rats were intravenously (IV) administrated

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Table 2. Antitumor Activity of LLC-0601 in Mouse Tumor Models

		tumor growth inhibition (%)		
compound	dose (mg/kg)	S180	A549	SGC-7901
LLC-0601	60 (~1/4LD$_{10}$)	24.4	40.2[a]	
	120 (~1/2LD$_{10}$)	51.7[a]	56.7[b]	38.9[a]
heptaplatin	40 (~1/4LD$_{10}$)	3.9		
	80 (~1/2LD$_{10}$)	23.9	40.7[a]	23.6

[a] $p < 0.05$. [b] $p < 0.01$ vs control.

eight doses of either LLC-0601 or heptaplatin over 4 weeks. The doses used, in terms of mg/m^2 unit, were equal to 1.1−2 times the optimal dose determined in the A549 xenograft. At the end of the study, all rats were anesthetized, and blood samples were collected for biochemical analysis. The results are summarized in Table S8 (Supporting Information). All animals survived except for the heptaplatin-treated group, in which two rats died in the last week due to serious myelosuppression, as evidenced by histopathological examination. Increases in body weight were observed across all groups, but three treated groups had a lower weight gain, with only the heptaplatin-treated group exhibiting a significant difference compared with the control group. Blood cell counts are the intuitive indictor of bone marrow cell proliferation, and myelosuppression, a major side-effect of cytotoxic anticancer drugs, leads to a decrease of blood cell counts, especially white blood cell and platelet numbers. On the basis of the values of blood cell counts given in Table S8, both LLC-0601 and heptaplatin had myelosuppresive effects, causing lower blood cell counts, but the effects induced by heptaplatin were much more pronounced, with thrombocytopaenia being very severe. Serum blood urea and creatinine levels are an important measure of nephrotoxicity. As shown from these data, LLC-0601 did not elevate serum urea or creatinine levels, whereas heptaplatin produced a significant increase in both serum urea (17.9 mM) and creatinine (37.8 μM), indicating that 40 and 70 mg/kg LLC-0601 were less nephrotoxic than 40 mg/kg heptaplatin. In addition, the study also revealed a 25% increase of serum alanine transaminase level in the heptaplatin-treated group compared to the control group, refecting damaged hepatic functions in these rats, whereas LLC-0601 had no effect on serum alanine transaminase levels, even at the highest dose.

In conclusion, a water-soluble heptaplatin analogue, LLC-0601, was developed in our lab. The analogue has shown greater anticancer activity and less toxicity than its parent compound heptaplatin and has been selected for preclinical development. The design and synthesis of new platinum anticancer drugs remains an important field in inorganic chemistry, and the past decade has witnessed a shift in focus toward nonclassical platinum compounds represented by picoplatin, polynuclear complexes, trans-platinum complexes, and Pt(IV) complexes.[20−22] However, the outcomes of clinical trials of these complexes remained below expectations, and none of these complexes has been approved for clinical application.[2] Our present study suggests that analoguing (direct modification) of the clinically established platinum drugs is still an effective way to find new platinum drugs that are more potent and less toxic than their parent complexes.[23]

■ ASSOCIATED CONTENT

Supporting Information. Experimental section, HPLC analysis, FAB$^+$-MS, ^{13}C NMR, IR, selected bond lengths, hydrogen bonds and angles, crystal data and structural refinements, acute toxicity of platinum(II) complexes, antitumor activity of platinum(II), and toxicological data. This material is available free of charge via the Internet at http://pubs.acs.org.

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