Supplementary Information

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

Weiping Liu,[†] Xizhu Chen, [†] Qingsong Ye, [†] Yongping Xu, [‡] Chengying Xie, [‡] Mingjin Xie,[§]

Qiaowen Chang [†] Liguang Lou,*[,‡]

Experimental Section

General

All commercially available reagents were of high purity and were used without any further purification. 3-Hydroxy-1,1-cyclo-butanedicarboxylic acid and (4*R*,5*R*)-4,5-bis(amino-methyl)-2-isopropyl-1,3-dioxolane (A_2) were prepared using previously reported methods[3,10] with some modifications. Chemical analyses for C, H, and N were performed using a Carlo-Ebra Instrument, whereas platinum was determined according to the method described in United States Pharmacopeia 24. Mass spectrometry studies were carried out on a VG-Autospec Spectrometer in the FAB^+ (fast atom bombardment positive-ion source) mode using glycerol as a matrix. FT-IR spectra were recorded in the 4000–400 cm^{-1} region on a Perkin Elmer 880 spectrometer with KBr pellets. ^{13}C-NMR spectra were obtained in dimethyl sulfoxide (DMSO) on a Brucker AV400 (100.62 MHz) using tetramethylsilane as an external standard.

Syntheses

LLC-0601. To a suspension of cis-{Pt[(4*R*,5*R*)-4,5-bis(amino-methyl)-2-isopropyl-1,3-dioxolane]I_2} (10 mmol) in 120 mL water was added 10 mmol of the disilver salt of 3-hydroxy-1,1-cyclobutanedicarboxylic acid, and the reaction mixture was stirred at 40°C for 36 hours. After AgI formed, it was filtrated off and the filtrate was condensed at 45°C under reduced pressure to 20 mL. The white crystalline product that precipitated was then filtrated off, washed with water and ethanol, and dried in a vacuum oven at 60°C. The yield was 4.1 g (76%). Analysis found the following (% calculated for

$C_{14}H_{24}N_2O_7Pt$): C, 31.7 (31.9); N, 5.34 (5.31); H, 4.61 (4.55); Pt, 37.2 (37.0). FAB^+-MS (m/e, RI): 528 (M^+, 100%) , 368 (M^+-$C_6H_6O_5$, 20%), 368 (A_2^+, 60%). IR (cm^{-1}, KBr): 3225(s, νNH_2), 2968-2896 (w, νCH), 1623 (ws, $\nu_{as}(COO)$), 1392 (s, $\nu_s(COO)$). ^{13}C-NNR (DMSO, ppm): 16.5, 16.6 (CH_3, isopropyl), 31.4 (CH, isopropyl), 41.8, 41.9 ($2CH_2$, cyclobutane), 48.0, 47.8 (*C*-1, cyclobutane), 60.1 (*C*-3 cyclobutane), 78.0, 77.9 ($2CH_2NH_2$), 79.5, 79.6 (*C*-4, *C*-5, 1,3-dioxolane), 106.9 (*C*-2, 1,3-dioxolane), 177.3, 177.4 ($2COO^-$). The specific rotatory power ($[\alpha]_D^{25℃}$) was -41º (C=20.45mg/ml, Water)

Single crystal X-ray data collection and structure determination

Single crystals of LLC-0601 were obtained by slow evaporation from aqueous solutions at room temperature. Intensity data for a single crystal of the complex were collected on a BRUKER SMART APEX II CCD detector with graphite-monochromatized Mo Kα radiation (λ = 0.071073 nm). The structures were solved by direct methods using the program SHELXS-97 and subsequent Fourier difference techniques, and refined anisotropically by full matrix least-squares on F^2 using SHELXL-97 [G. M. Sheldrick, Acta Crystallogr. A64 (2008) 112-122.]. Crystal data and structural refinements of LLC-0601 are shown in **Table S2**.

MTT assay

The cytotoxicity of compounds was determined by MTT assay. Cells were plated in duplicate in 96-well microtiter plates and then treated with different concentrations of

compounds for 72 hours. At the end of treatment, formazan crystals formed were solubilized in DMSO and read at 490 nm using a SPECTRA MAX 190 spectrophotometer.

In vivo efficacy study

Female nude mice (Balb/cA-nude, 5–6 weeks old) and female Kunming mice were purchased from Shanghai Laboratory Animal Center, Chinese Academy of Sciences, Shanghai, China. Human tumor xenografts of A549 and SGC-7901, and the mouse Sarcoma 180 tumor were established by subcutaneously inoculating cells into female mice. When tumor volumes reached 100–300 mm^3, the mice were randomly assigned to control and treatment groups and treated accordingly. Tumor volume was calculated as (length × $width^2$)/2. For Sarcoma 180 experiments, agents and vehicle controls were administered on the day following inoculation. Animal experiments were conducted in accordance with the Institutional Animal Care and Use Committee guidelines of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

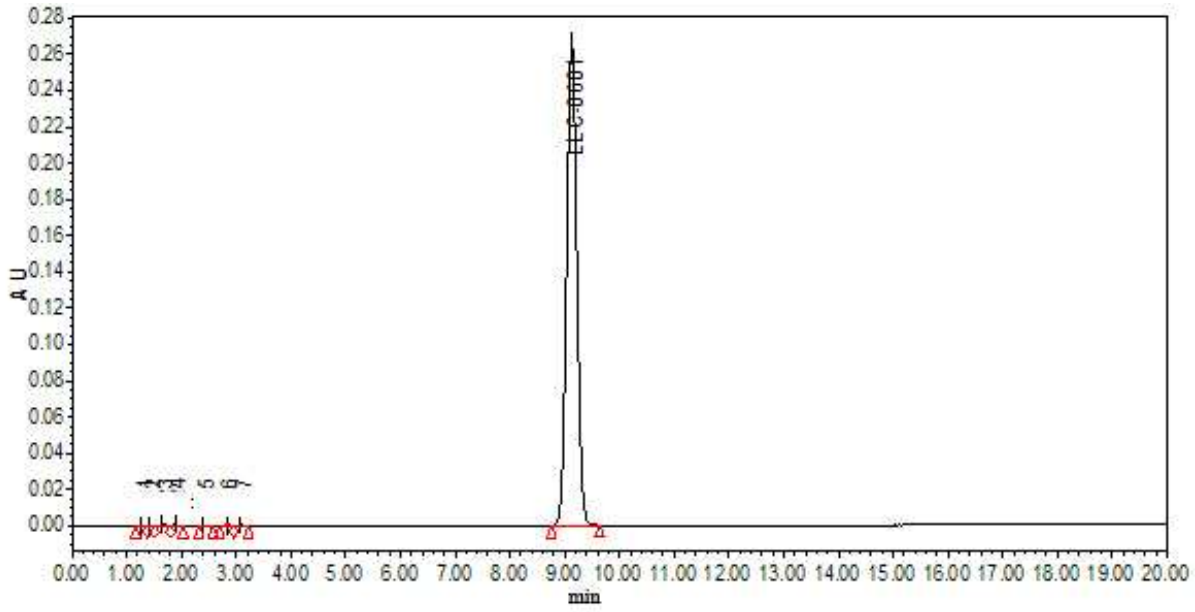

Figure S1. HPLC analysis of LLC-0601.

The purity for LLC-0601 was assessed by analytical reverse-phase column chromatography (RP-HPLC) on a Waters Associates system (consisting of a 1525 pump, a 717 automated injector, and a Model 2998 photodiode array detector), using Kromasil-C_{18}, 5-μm particle size, , 4.6×250 mm column. The mobile phase was a MeOH-H_2O (30:70) system, and the flow rate was 1.0 ml/min, with monitoring of the peak at 230 nm.

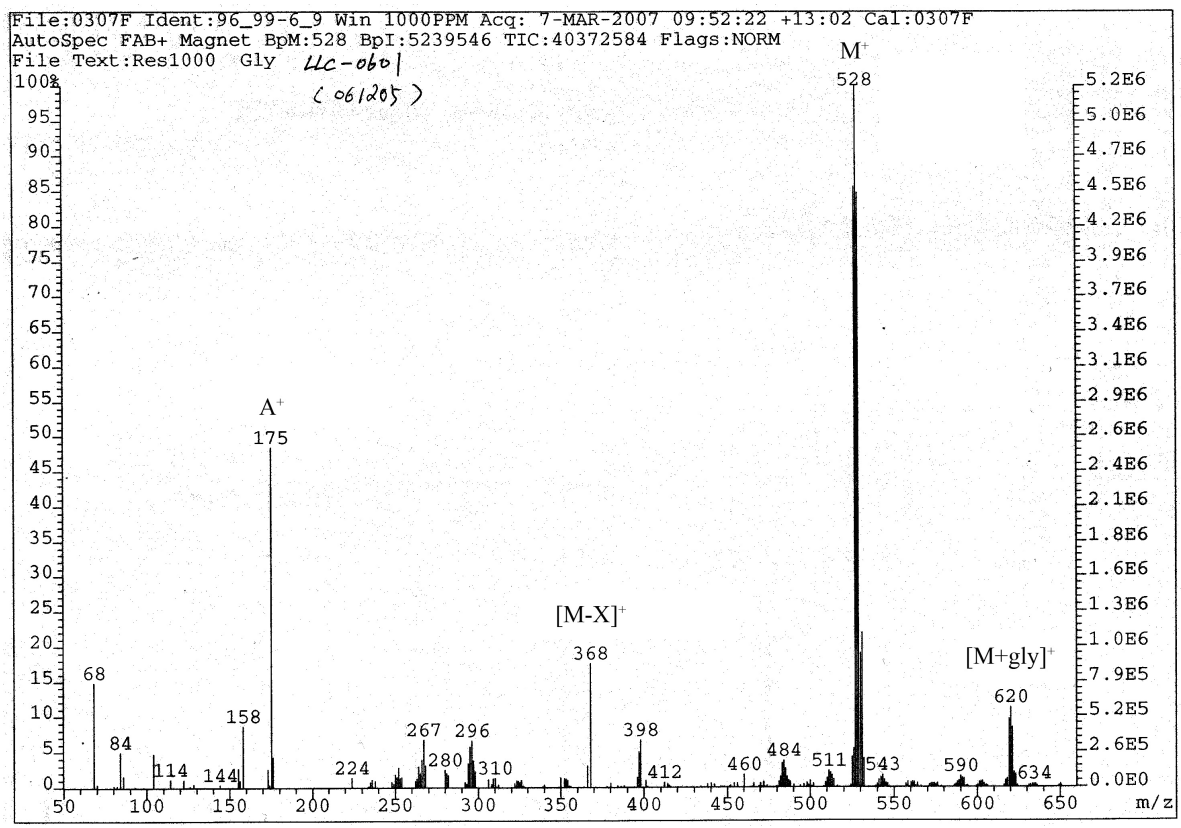

Figure S2. FAB$^+$-MS of LLC-0601

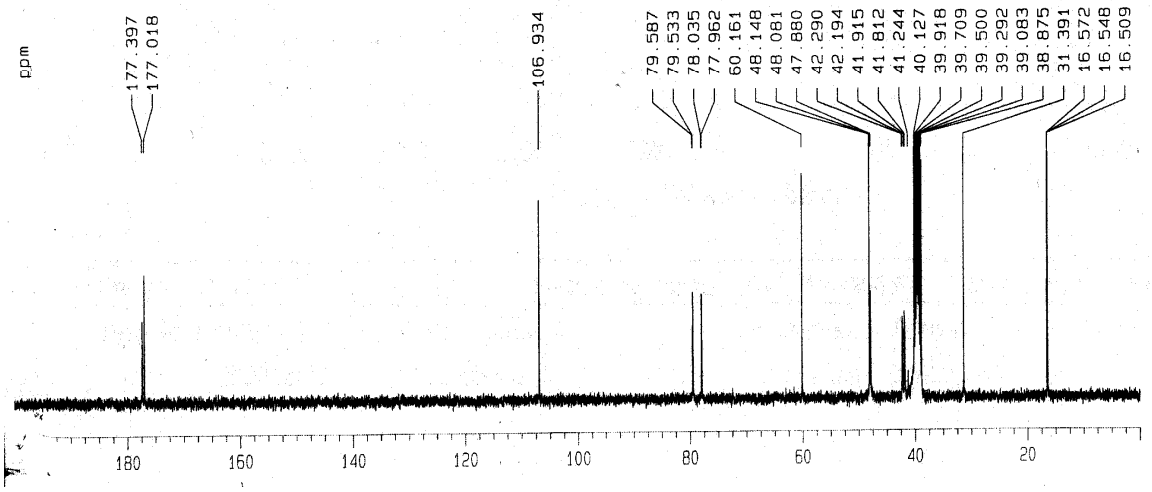

Figure S3. ^{13}C-NMR of LLC-0601

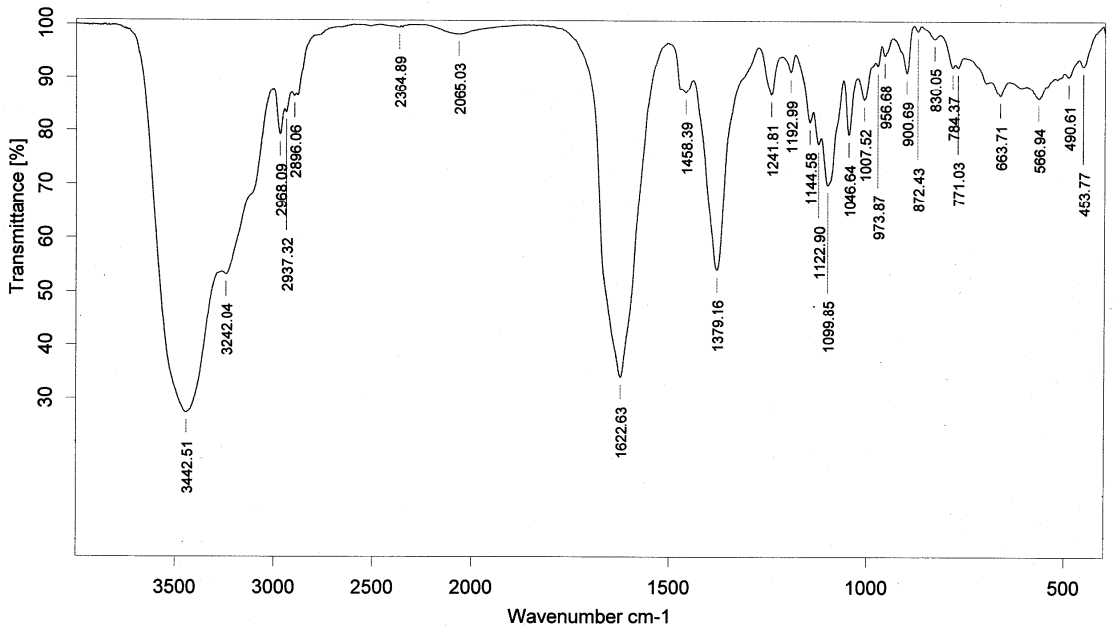

Figure S4. IR of LLC-0601

Table S1. Selected bond lengths [Å] and angles [°] for LLC-0601

Pt(1)-N(1)	2.021(9)	Pt(1)-N(2)	2.017(10)
Pt(1)-O(1)	2.025(8)	Pt(1)-O(2)	1.983(8)
O(2)-Pt(1)-N(2)	176.0(4)	N(1)-Pt(1)-O(1)	177.5(4)
O(2)-Pt(1)-N(1)	87.5(4)	O(2)-Pt(1)-N(1)	87.5(4)
N(2)-Pt(1)-N(1)	91.6(4)	O(2)-Pt(1)-O(1)	90.7(3)

Table S2. Hydrogen bonds for 081017b [Å] and angles [°]

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
N(1)-H(1A)...O(10)#1	0.90	2.05	2.924(14)	162.2
N(1)-H(1A)...O(8)#1	0.90	2.61	3.258(13)	130.0
N(1)-H(1B)...O(15)#2	0.90	2.09	2.934(17)	155.2
N(2)-H(2B)...O(10)#1	0.90	2.36	3.171(14)	149.2
N(2)-H(2B)...O(2)#3	0.90	2.61	3.154(13)	119.6
N(3)-H(3A)...O(15)#4	0.90	2.09	2.972(17)	165.0
N(3)-H(3B)...O(3)	0.90	2.03	2.891(13)	158.5
N(3)-H(3B)...O(1)	0.90	2.58	3.240(14)	131.0
N(4)-H(4A)...O(3)	0.90	2.17	3.031(13)	160.2
N(4)-H(4B)...O(16)#5	0.90	2.38	3.139(15)	141.4
O(5)-H(5)...O(15)	0.82	2.12	2.939(17)	174.6
O(12)-H(12)...O(6)#6	0.82	1.98	2.68(2)	142.7

Symmetry transformations used to generate equivalent atoms:

#1 x,y+1,z #2 -x+1,-y+2,-z #3 -x+2,-y+2,-z

#4 x+1,y,z #5 -x+1,-y+1,-z+1 #6 -x+2,-y+1,-z+1

Table S3. Crystal data and structural refinements for LLC-0601

Empirical formula	$C_{14}H_{26}N_2O_8Pt$
M_r	$[Pt(C_6H_6O_5)(C_8H_{18}O_2N_2)\cdot H_2O]$
Temperature	545.45
Wavelength	293(2) K
Crystal system	0.71073 A
space group	Triclinic
a (Å)	P-1
b (Å)	11.1235(12)
c (Å)	13.6608(15)
α (°)	13.8965(15)
β (°)	73.0970(10)
γ (°)	75.1660(10)
V (Å^3)	86.5720(10)
Z	1952.8(4)
D_{calc} (g·cm^{-3})	4
μ (mm^{-1})	1.843
F(000)	7.225
	1050

Crystal size (mm^3)	$0.16 \times 0.14 \times 0.10$
θ range (º)	1.56 to 28.36
Limiting indices	$-14 \leq h \leq 14$, $-17 \leq k \leq 18$, $-18 \leq l \leq 18$
Reflections collected / unique	16773
Independent reflection, R_{int}	8810, 0.0581
Max. and min. transmission	1.0000 and 0.5374
Data / restraints / parameters	8810 / 0 / 473
Goodness-of-fit on F^2	0.955
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0611$, $wR_2 = 0.1544$
R indices (all data)	$R_1 = 0.1066$, $wR_2 = 0.1792$
$\Delta\rho_{max/min}$ ($e \cdot Å^{-3}$)	2.270 / -2.000

Table S4 Acute toxicity of platinum(II) complexes in ICR mice

	Lethal dose [mg/kg]	
	LD_{10}	LD_{50}
LLC-0601	216.5	372.7
Heptaplatin	154.5	196.2

Table S5 Antitumor activity of platinum(II) complexes in mice with Sarcoma180

Group	Dose mg/kg	Schedule	No.of animals d0	No.of animals d8	Mean body weight(g) d0	Mean body weight(g) d8	Tumor weight X±SD(g)	T/C %
Control	-	ip, d1, d4	14	14	21.8	28.6	1.80±0.32	
Heptaplatin	40(≈ ¼LD_{10})		7	7	21.9	25.4	1.73±0.30	96.1
	80(≈ ½LD_{10}) [a]		7	7	21.7	23.2	1.37±0.52	76.1
LLC-0601	60(≈ ¼LD_{10})		7	7	21.8	25.9	1.36±0.44	75.6
	120(≈½LD_{10})		7	7	21.7	23.5	0.87±0.23	48.3**

T/C =treated/control. [a] 80 mg/kg is the maximum dose of heptapatin which can be ip administrated to mice, due to the low water solubility. **$p<0.01$ vs. control.

Table S6 Antitumor activity of platinum(II) complexes in BALB/cA-nude mice with A549 xenografts

Group	Dose (mg/kg)	Schedule	No.of animals d0/d21	TV(X±SD, mm^3) d0	d21	RTV X±SD	T/C (%)
Control	-	ip, Q4D × 4	10/10	253±60	939±275	3.86±1.22	
Heptaplatin	80		6 /6	224±52	522±233	2.29±0.69	59.3*
LLC-0601	60		6 /6	223±69	489±146	2.31±0.92	59.8*
	120		6 /6	210±28	347±119	1.67±0.65	43.3**

TV=tumor volume, RTV=relative tumor volume, *p<0.05, **p<0.01vs. control.

Table S7 Antitumor activity of platinum(II) complexes in BALB/cA-nude mice with SGC-7901 xenografts

Group	Dose (mg/kg)	Schedule	No.of animals d0/d21	TV(X±SD, mm^3) d0	d21	RTV X±SD	T/C (%)
Control	-	ip, Q4D × 4	12/12	220±40	917±268	4.20±1.28	
Heptaplatin	80		6 /6	226±15	718±171	3.21±0.84	76.4
LLC-0601	120		6 /6	209±24	519±219	2.57±1.24	61.2*

TV=tumor volume, RTV=relative tumor volume, *$p<0.05$ vs. control.

Table S8 Toxicological data of LLC-0601 following a 8-repeated dose injection to healthy SD rats

Group	Dose (mg/kg)	Schedule	No.of Rats d0/d28	Body Weight (g) d0	Body Weight (g) d28	Blood Cell Count (d28) WBC(10^9/L)	Blood Cell Count (d28) RBC(10^{12}/L)	Blood Cell Count (d28) PLT(10^9/L)	Serum Biochemistry (d28) Cr(μM)	Serum Biochemistry (d28) Urea(mM)	Serum Biochemistry (d28) ALT(U/L)	Serum Biochemistry (d28) AST(U/L)
Control	-	iv, d1, d2,	10/10	224±11	290±15	7.53±1.54	6.09±0.99	730±124	23.8±2.03	6.98±0.75	54.1±11.9	128±24
Heptaplatin	40	d8,d9, d15,	10 /8	222±15	244±27*	0.66±0.37**	2.65±1.55**	26±20.8**	37.8±2.06*	17.9±3.2**	78.6±10.8*	134±42
LLC-0601	40	d16, d22,d23	10 /10	223±12	270±22	3.90±1.21**	4.67±0.40*	826±190	26.4±2.31	8.24±0.91	55.4±8.9	137±16
	70		10 /10	223±13	259±12	3.93±1.44**	4.82±1.33*	482±233*	24.3±3.28	6.93±1.87	56.9±12.0	130±32.

WBC=white blood cells, RBC=red blood cells, PLT=platelet, Cr=creatinine, ALT=alanine transaminase, AST=aspartate transaminase. *p<0.05, **p<0.01vs control.