Journal of Inorganic Biochemistry 140 (2014) 126–130

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

journal homepage: www.elsevier.com/locate/jinorgbio

Synthesis, anticancer activity and toxicity of a water-soluble *4S,5S*-derivative of heptaplatin, *cis*-{Pt(II)[(*4S,5S*)-4,5-bis(aminomethyl)-2-isopropyl-1,3-dioxolane]·(3-hydroxyl-cyclobutane-1,1-dicarboxylate)}

Weiping Liu [a], Jing Jiang [a], Chengying Xie [b], Shuqian Hou [a], Haitian Quan [b], Qingsong Ye [a], Liguang Lou [b,*]

[a] State Key Laboratory for Platinum Group Metals, Kunming Institute of Precious Metals, 650106 Yunnan, PR China
[b] Shanghai Institute of Materia Medica, Chinese Academy of Science, Shanghai 201203, PR China

ARTICLE INFO

Article history:
Received 27 May 2014
Received in revised form 14 July 2014
Accepted 14 July 2014
Available online 22 July 2014

Keywords:
Platinum complexes
Anticancer activity
Toxicity
Heptaplatin
Derivative

ABSTRACT

A water-soluble *4S,5S*-derivative of heptaplatin, *cis*-{Pt(II)[(*4S,5S*)-4,5-bis(aminomethyl)-2-isopropyl-1,3-dioxolane]·(3-hydroxyl-cyclobutane-1,1-dicarboxylate)} was synthesized. The anticancer activity and toxicity were evaluated by comparing its interaction with DNA, cytotoxicity against four human cancer cell lines, antitumor efficiency in human gastric carcinoma NCI-N87 xenografts in nude mice, and preliminary side-effects in rats to those of its *4R,5R*-optical isomer which is under preclinical development. Both isomers induce condensation of DNA to the same extent and have similar cytotoxicity, but show different antitumor activity and toxicity, probably owing to the difference in respective pharmacokinetic profiles. *4S,5S*-Isomer seems to exhibit superior antitumor activity and less toxicity than *4R,5R*-optical isomer as well as the parent heptaplatin. These results imply that *4S,5S*-configuration as a new drug candidate may be better than *4R,5R*-counterpart.

1. Introduction

Platinum-based drugs, represented by cisplatin (DDP), carboplatin and oxaliplatin, have been key players in systemic anticancer chemotherapy since cisplatin was approved in 1978 by the Food and Drug Administration for clinical application [1–3]. In addition, three regionally approved platinum complexes, nedaplatin and heptaplatin and lobaplatin, are also available for clinical options [4–6]. However, the unfavorable toxicity and drug resistance associated with these drugs severely hamper their clinical use. Reducing toxicity and overcoming resistance of platinum chemotherapy are still the most important objectives in the drug development [7–12].

Heptaplatin was developed in 1999 by Sunkyong Industries Co., Ltd., Republic of Korea, and is used particularly in the clinical treatment of advanced gastric cancer [13–15]. It shows an altered antitumor profile in comparison with the cisplatin and is active in the cisplatin-resistant L1210 model due to its unique carrier (*4R,5R*)-4,5-bis(aminomethyl)-2-isopropyl-1,3-dioxolane [16–18]. Hepatotoxicity and nephrotoxicity are two major dose-limiting side effects of heptaplatin [19,20], which is considered to be closely related to its low water solubility (4–5 mg/ml). In our previous studies, a new water-soluble analogue of heptaplatin, *cis*-{Pt(II)[(*4R,5R*)-4,5-bis(aminomethyl)-2-isopropyl-1,3-dioxolane]·(3-hydroxyl-cyclobutane-1,1-dicarboxylate)} was synthesized. It possesses greater antitumor activity and much lower nephrotoxicity than the parent heptaplatin, therefore has been selected for preclinical development [21,22].

Structurally, 4,5-bis(aminomethyl)-2-isopropyl-1,3-dioxolane (A_2) has two asymmetric carbon centers, consequently platinum complexes of A_2 as a carrier group can exist as *4R,5R*- and *4S,5S*-optical isomers, as illustrated in Fig. 1. The importance of isometric configuration to antitumor activity of chiral platinum complexes was highlighted by many researchers [16,23–26]. From this point of view, a comparative study of two optical isomers is of great interest and is also a must in drug development. In the present study, we have synthesized **1a**-(*S,S*) and compared its anticancer activity and preliminary toxicity with those of **1b**-(*R,R*).

2. Experimental section

2.1. Chemistry

2.1.1. Materials and instrument

Potassium tetrachloroplatinate(II) and L-tartaric acid diethyl ester, two starting materials, were purchased from Alfa Aesar. All other chemicals obtained from commercial suppliers were of analytical grade and used as received. Water was distilled prior to use. The synthetic procedures were carried out in light protected environment

* Corresponding author. Tel./fax: +86 21 50806056.
E-mail address: liguanglou1@126.com (L. Lou).

http://dx.doi.org/10.1016/j.jinorgbio.2014.07.013
0162-0134/

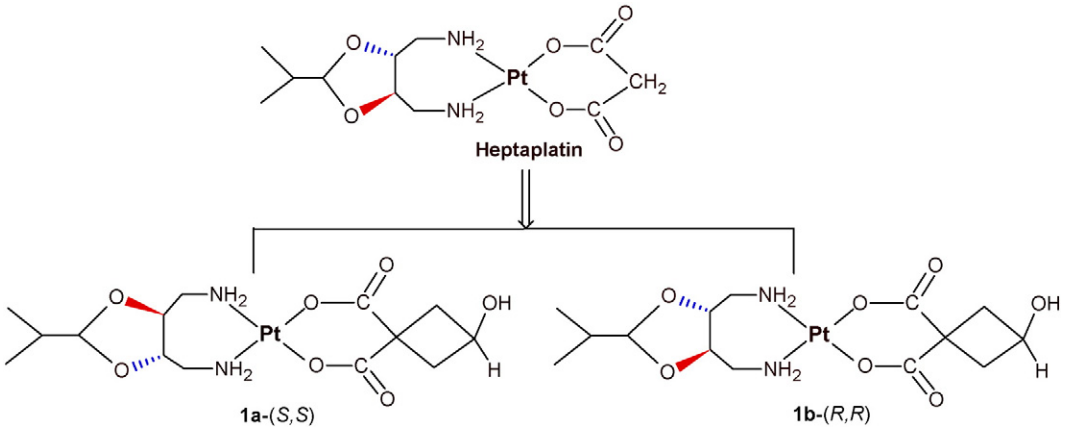

Fig. 1. Chemical structures of heptaplatin and two optical isomers.

when platinum complexes were involved. Composition analyses for C, H and N were performed with a Carlo-Erba instrument, whereas the content of platinum was analyzed according to the method in EP6.5. FT-IR spectra were measured in KBr pellets with a Perkin Elmer 880 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded in DMSO on Brucker AV-400 MHz relative to TMS (tetramethylsilane) as an external standard. Electrospray ionization mass spectra (ESI-MS) were recorded on Agilent G6230 TOF MS equipped with an electrospray ion source type.

The specific optical rotation was determined on an AP-300 Automatic Polarimeter. The purity was determined 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. The peak was monitoring at λ = 230 nm.

*2.1.2. Synthesis of **1a**-(S,S)*

1a-(*S,S*) was synthesized from L-tartaric acid diethyl ester and K_2PtCl_4(II) as the starting chemicals by following the same procedure as previously described for **1b**-(*R,R*) [21]. Briefly, K_2PtCl_4 (5 g, 12 mmol) was mixed at 45 °C with KI (12 g, 6 × 12 mmol) in 100 ml H_2O for 2 h, and then (*4S,5S*)-4,5-bis(aminomethyl)-2-isopropyl-1,3-dioxolane (2.09 g, 12 mol) was dropwise added with vigorous stirring. After standing for 4 h, the resulting yellow precipitate—diaminediiodoplatinum(II) was collected by filtration, washed with water and ethanol, and dried in vacuo at 65 °C. The yield was 93% (6.6 g). Freshly prepared disilver 3-hydroxyl-cyclobutane-1,1-dicarboxylate (3.60 g, 9.62 mmol) [27] was mixed with diaminediiodoplatinum(II) (6.01 g, 9.64 mmol) in 150 ml H_2O for 36 h with stirring at 37 °C. After AgI was filtrated off, the solution was condensed at 45 °C under reduced pressure to 20 ml to precipitate a white crystalline product — **1a**-(*S,S*). It was collected, washed successively with icy water and ethanol, and dried in vacuo at 45 °C. Yield: 73% (3.71 g). $[\alpha]_D^{25\,^{\circ}C} = +39.7°$ (*C* = 20.5 mg/ml in water). Found (% calculated for $C_{14}H_{24}N_2O_7Pt$): C 31.6(31.9), H 4.59(4.55), N 5.27(5.31), Pt 36.7(37.0); ESI^+-MS (m/z, RI): 550($[C_{14}H_{24}N_2O_7PtNa]^+$, 100%) 528($[M + 1]^+$,4%). EI-HRMS calcd. = 550.1129 for $C_{14}H_{24}N_2O_7PtNa$, Found = 550.1117; IR(KBr, cm^{-1}): 3416(s, ν_{O-H}), 3240, 3211(m, ν_{N-H}), 2966–2877(w, ν_{C-H}), 1595(vs, $\nu_{as(COO)}$), 1365(vs, $\nu_{a(COO)}$). ^{1}H NMR (dmso, δ): 0.84(6H, $2CH_3$), 1.72(1H, CH, isopropyl), 2.30, 2.57(4H, $2CH_2$, cyclobutane), 2.98, 3.07(4H, $2C\underline{H}_2NH_2$), 3.83(1H, CH, cyclobutane), 4.40, 4.47(2H, 2CH, 1,3-dioxolane), 4.78(1H, CH, 1,3-dioxolane), 4.97(1H, C–OH), 5.36, 5.46(4H, $2CH_2N\underline{H}_2$); ^{13}C NMR (dmso, δ): 16.5, 16.6($2CH_3$, isopropyl), 31.4(CH, isopropyl), 41.9, 42.2($2CH_2$, cyclobutane), 48.1(C-1, cyclobutane), 60.1(C-3, cyclobutane), 77.9, 78.0($2CH_2NH_2$), 79.5, 79.6(C-4, C-5, 1,3-dioxolane), 107.0(C-2, 1,3-dioxolane), 177.1, 177.5($2COO^-$).

2.2. Interaction with λ-DNA

2.2.1. Magnetic tweezer apparatus

The magnetic tweezer setup was purchased from Pico Twist Company (France). In brief, it was made up of an inverted microscope objective, a microfluidics flow cell and a pair of permanent magnets. Under the flow cell, the microscope objective (Olympus 1006, numerical aperture [NA] = 1.2, oil immersion) was used to observe the beads in real time. In the flow cell, the DNA was bound to the bottom of the cell at one end. The other end was tethered to a super-paramagnetic bead (MyOne, Dynabeads, Invitrogen). Above the flow cell, there was a set of magnets producing a strong field gradient to exert a force on beads. The force was varied by changing the position of the magnets relative to the beads. The beads can be rotated through rotating the magnets.

2.2.2. λ-DNA preparation for magnetic tweezers study

The bacteriophage λ-DNA (New England Biolabs), which has two 12-nt cohesive termini, was separately annealed with two 12-nt labeled oligomers (labeled by biotin and digoxigenin, respectively). These oligomers have complementary sequences to the overhangs. The 12-nt oligomers were obtained from Sangon Biotech (Shanghai).

2.2.3. Single molecule measurement by magnetic tweezers

1a-(*S,S*) or **1b**-(*R,R*) at a concentration of 400 mM was injected into the flow cell and incubated with DNA while keeping DNA stretched by a large constant force (approximately 6 pN). The stretching force prevented the formation of micro-loops and long range cross-links. A changing curve of force-extension length of DNA with the incubation time was recorded for at least two times for each complex.

2.3. In vitro cytotoxicity

2.3.1. Cell culture

Human cancer cell lines NCI-N87, SK-OV-3 were purchased from the American Type Culture Collection (Manassas, VA, USA), and, SGC-7901 were obtained from the Cell Bank of the Shanghai Institute for Biological Sciences, Chinese Academy of Science (Shanghai, China), whereas cisplatin-resistant SK-OV-3 cell line (SK-OV-3/DDP) was kindly provided by Chinese Academy of Medical Sciences (Beijing, China). Cells were grown in DMEM or RPMI-1640 medium containing 10% fetal bovine serum and supplemented with 100 units/ml of penicillin and 100 μg/ml of streptomycin. Cells were maintained at 37 °C in a humidified incubator with an atmosphere of 5% CO_2.