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SYNTHESIS AND *IN VITRO* CYTOTOXICITY OF *CIS*-DICHLORO[(2*S*,3*R*,4*S*)-2-AMINOMETHYL-3,4-(*O*-ISOPROPYLIDENE)DIHYDROXY- or -3,4-DIHYDROXYPYRROLIDINE]PLATINUM(II)

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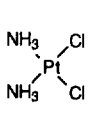
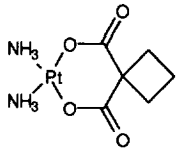
Abstract : *cis*-Dichloro[(2*S*,3*R*,4*S*)-2-aminomethyl-3,4-(*O*-isopropylidene)dihydroxypyrrolidine]-platinum(II) (**1**) and *cis*-dichloro[(2*S*,3*R*,4*S*)-2-aminomethyl-3,4-dihydroxypyrrolidine]platinum(II) (**2**) have been synthesized and evaluated for their *in vitro* cytotoxicity against cisplatin-sensitive and -resistant L1210 murine leukemia cell lines and human tumor cell lines. The complex **1** showed high cytotoxicity against human cancer cell lines, A 549, SK-OV-3, and XF 498 and much lower cross-resistance against cisplatin-resistant L1210 cells than cisplatin and carboplatin.

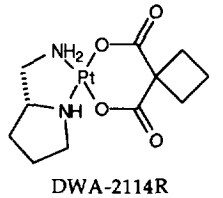
cis-Dichlorodiammineplatinum(II) (cisplatin)[1] is one of the most widely used chemotherapeutic agents, either alone or, more often, in combination with other agents, in the treatment of various human cancers.[2] However, its clinical usefulness has frequently been limited by severe side effects, such as nephrotoxicity, gastrointestinal toxicity, ototoxicity, and neurotoxicity,[3] and by the emergence of cancer cells resistant to cisplatin after an initial response.[4] *cis*-Diammine(1,1-cyclobutanedicarboxylato)platinum(II) (carboplatin) shows the same level of activity as cisplatin in treating some kinds of cancers, such as ovarian cancer and small-cell lung cancer, and is much less nephrotoxic and emetic than cisplatin.[5] Carboplatin, however, exhibits rather the narrow spectrum of antitumor activity than cisplatin and is not effective in the treatment of cancer cells resistant to cisplatin due to its cross-resistance with cisplatin.[6] To overcome these unfavorable drawbacks of cisplatin and carboplatin, extensive efforts have been made to develop new cisplatin analogues with equivalent or greater antitumor activity and lower toxicity.[7] A new platinum complex, (–)-(*R*)-2-aminomethylpyrrolidine(1,1-cyclobutanedicarboxylato)platinum(II) monohydrate (DWA-2114R)[7c] showed activity superior to cisplatin and carboplatin against



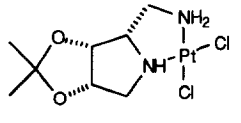
Cisplatin



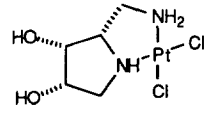
Carboplatin



DWA-2114R

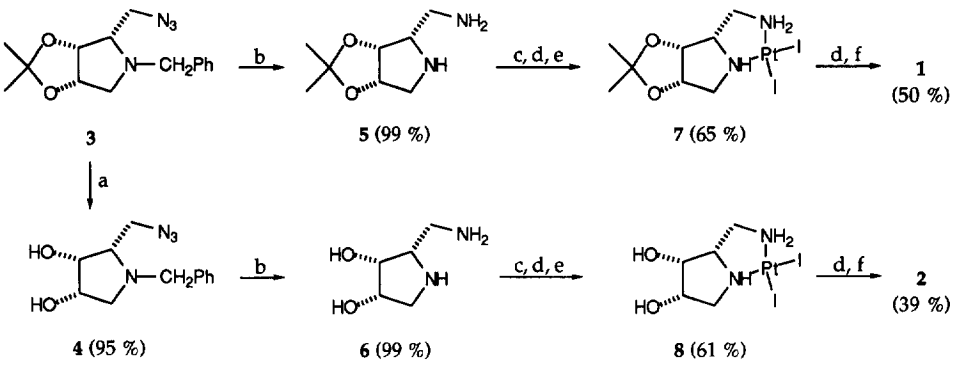


1



2

Scheme 1[a]



[a](a) TFA–H_2O (4:1), rt, 2 h; (b) 10 % pd/C, H_2 (50 psi), EtOH, 50 °C, 3 h; (c) K_2PtCl_4 (1 equiv.), KI (6 equiv.), H_2O, 60 °C, 1 h, N_2 atmosphere; (d) $AgNO_3$ (2 equiv.), H_2O, 60 °C, 2 h; (e) KI (10 equiv.), 0 °C, 1 h; (f) NaCl (10 equiv.), 40 °C.

ovarian and prostate cancers in phase II clinical trials and relatively low cross-resistance to cisplatin against cisplatin-resistant murine leukemia P388 and L1210 cells and human leukemia K562 cells.[8] DWA-2114R, however, is less potent than cisplatin and carboplatin in terms of the clinical trial dose administered; the recommended doses of cisplatin, carboplatin, and DWA-2114R for human are 60–120 mg/m^2, 350–450 mg/m^2, and 800–1000 mg/m^2, respectively.[9] In an attempt to develop more potent analogues of DWA-2114R, we have now prepared *cis*-dichloro[(2*S*,3*R*,4*S*)-2-aminomethyl-3,4-(*O*-isopropylidene)dihydroxypyrrolidine]platinum(II) (**1**) and *cis*-dichloro[(2*S*,3*R*,4*S*)-2-aminomethyl-3,4-dihydroxypyrrolidine]platinum(II) (**2**). Replacement of a bidentate leaving ligand, 1,1-cyclobutanedicarboxylate with chloride in DWA-2114R would increase its original potency, and introduction of a 1,3-dioxolane moiety or two hydroxy groups in the 2-aminomethylpyrrolidine carrier ligand may render the organoplatinum species more water-soluble than the simple 2-aminomethylpyrrolidine complex, thus being less toxic owing possibly to a more facile excretion *via* the kidney, as previously shown by us[7a].

The synthesis of the dichloro platinum(II) complexes **1** and **2** is outlined in Scheme 1. Hydrolysis of (2*S*,3*R*,4*S*)-2-azidomethyl-1-benzyl-3,4-(*O*-isopropylidene)dihydroxypyrrolidine (**3**), prepared from D-ribose according to the published procedure[10], with 80 % trifluoroacetic acid at room temperature for 2 h produced **4** in 95 % yield. Reductive hydrogenation (50 psi) of **3** and **4** in the presence of 10 % pd/C in EtOH at 50 °C for 3 h afforded 2-aminomethylpyrrolidines **5** and **6** in almost quantitative yields. The compounds **5** and **6** were reacted with an equimolar amount of *in situ* generated potassium tetraiodoplatinate(II) to produce the crude diiodo platinum(II) complexes **7** and **8**, which were subsequently treated with an aqueous silver nitrate solution, followed by potassium iodide to give the pure diiodo platinum(II) complexes in 61–65 % yields. Reaction of **7** and **8** with an aqueous silver nitrate solution followed by treatment of the resulting an aqueous solution of diaquo complexes with sodium chloride afforded the corresponding *cis*-dichloro complexes **1** and **2**[11] in 50 and 39 % yields, respectively. Compound **1** showed 2.3 times higher solubility in H_2O compared to cisplatin (2.3 *vs.* 1.0 mg/mL at 25 °C) and compound **2** was highly water-soluble (33.3 mg/mL).

The cytotoxicity of **1** and **2** along with cisplatin and carboplatin against cisplatin-sensitive and -resistant L1210 leukemia cell lines *in vitro* was tested by trypan blue dye-exclusion method[12] (Table 1).

Table 1. Cytotoxicity of Platinum(II) Complexes against Cisplatin-sensitive and -resistant L1210 Leukemia Cell Lines *in vitro*[a]

	IC_{50} (μM)[b]		
compound	L1210/parent	L1210/CPR	relative resistance[c]
1	0.3	1.6	5.3
2	1.4	10.9	7.8
cisplatin	0.1	3.4	34.0
carboplatin	2.0	45.3	22.7

[a]Tested by trypan blue dye-exclusion method. [b]Mean value of 3 experiments. [c]IC_{50} resistant subline/IC_{50} parent cell line.

The relative resistance for these complexes in comparison with those for cisplatin and carboplatin is defined by the ratio of IC_{50} of the resistant subline to that of the sensitive one. L1210/CPR cells were found to be 34.0- and 22.7-fold cross-resistant to cisplatin and carboplatin, respectively, in comparison with L1210 cells, whereas L1210/CPR cells were only 5.3- and 7.8-fold cross-resistant to the complexes **1** and **2**, respectively.

Table 2. Cytotoxicity of Platinum(II) Complexes against Human Cancer Cell Lines *in vitro*[a]

	IC_{50} (μM)[b]		
compound	A 549[c]	SK-OV-3[d]	XF 498[e]
1	10.9	7.8	6.2
2	32.5	15.4	14.8
cisplatin	3.2	4.4	2.9
carboplatin	37.7	13.9	13.6

[a]Tested by SRB protein assay. [b]Mean value of 3 experiments. [c]Non-small cell lung cancer cell line. [d]Ovarian carcinoma cell line. [e]CNS cancer cell line.

The cytotoxicity of these complexes **1** and **2** was further tested toward three human cancer cell lines, A 549 (non-small cell lung cancer), SK-OV-3 (ovarian carcinoma), and XF 498 (CNS cancer), by the sulforhodamine B (SRB) protein assay[13] (Table 2). Although the complex **1** was 1.8–3.4-fold less potent than cisplatin in terms of IC_{50}, it was 1.8–3.5-fold more potent than carboplatin. The complex **2** was almost equally cytotoxic to carboplatin against all three cancer cell lines tested.

In conclusion, it has been shown that the complex **1** has high cytotoxicity against human cancer cell lines, A 549, SK-OV-3, and XF 498 and much lower cross-resistance against L1210/CPR than cisplatin and carboplatin. Since we have previously shown that the 2-substituents in the 4,5-bis(aminomethyl)-1,3-dioxolane carrier ligands considerably influenced their cytotoxicity,[7a] modification at the C–2 in the 1,3-dioxolane moiety of **1** is currently undergoing in our laboratory.

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11. **1**: yellow needles (EtOH–H_2O); ^{1}H NMR (DMF-d_7/TMS) δ 1.32 (s, 3 H), 1.98 (s, 3 H), 2.90 (m, 2 H) 3.04 (m, 1 H), 3.16 (m, 1 H), 3.74 (m, 1 H), 4.55 (br s, 1 H), 4.92 (m, 2 H), 5.43 (m, 1 H), 7.03 (m, 1 H); ^{13}C NMR (DMF-d_7/TMS) δ 23.97, 25.84, 49.17, 54.64, 69.33, 80.38, 81.06, 113.15; FAB-MS *m/z* 438 (M^+); *Anal.* Calcd for $C_8H_{16}Cl_2N_2O_2Pt$: C, 21.93; H, 3.68; N, 6.39. Found: C, 21.85; H, 3.72; N, 6.20. **2**: yellow needles (EtOH–H_2O); ^{1}H NMR (DMF-d_7/TMS) δ 2.75 (m, 1 H), 3.08–3.30 (m, 2 H), 3.38–3.57 (m, 2 H), 4.16–4.32 (m, 2 H), 5.24 (d, J = 5.4 Hz, 3 H), 5.32 (d, J = 4.8 Hz, 1 H), 6.60 (br s, 1 H); ^{13}C NMR (DMF-d_7/TMS) δ 50.15, 56.60, 68.89, 71.14, 71.46; FAB-MS *m/z* 399 (M^+ + H); *Anal.* Calcd for $C_5H_{12}Cl_2N_2O_2Pt$: C, 15.08; H, 3.04; N, 7.04. Found: C, 14.82; H, 3.10; N, 6.91.
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