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SYNTHESIS AND *IN VITRO* CYTOTOXICITY OF 1,3-DIOXOLANE-2-(2-ETHANAMINE)-2-METHANAMINE PLATINUM(II) COMPLEXES

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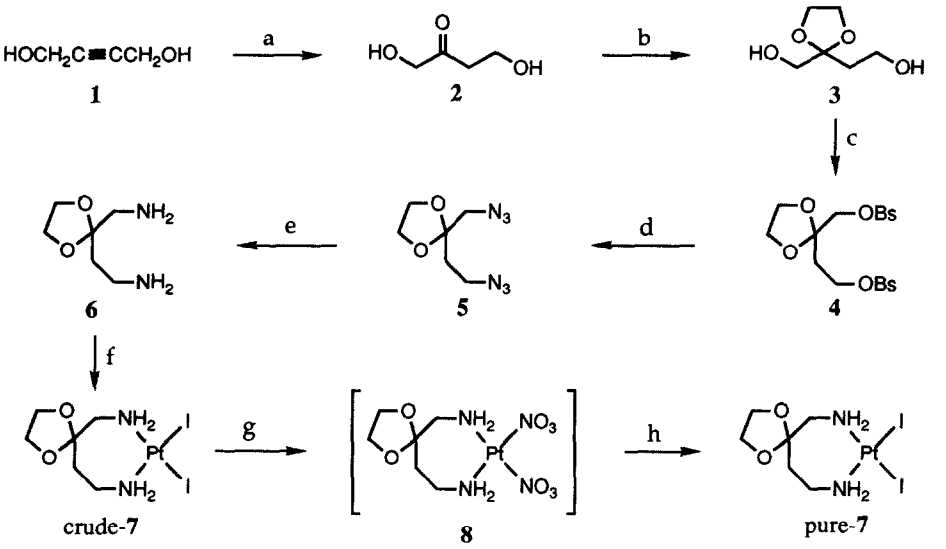
Abstract : The synthesis and *in vitro* cytotoxicity of novel 1,3-dioxolane-2-(2-ethanamine)-2-methanamine platinum(II) complexes having a seven-membered ring structure are described. It has been demonstrated that cisplatin-resistant murine L1210 leukemia cells have lower cross-resistance to this class of compounds than to cisplatin and carboplatin, and the human stomach cancer cell lines, SNU-1, SNU-5, and SNU-16, are highly sensitive to the members of this class.

Since the discovery of the antitumor properties of platinum compounds by Rosenberg *et al.*[1], cisplatin has demonstrated a remarkable chemotherapeutic potential in a large variety of human solid cancers, such as testicular, ovarian, bladder, lung, and stomach carcinomas.[2,3] However, the adverse effects that are observed in patients receiving cisplatin, such as nephrotoxicity, gastrointestinal toxicity, ototoxicity, and neurotoxicity[4] as well as the low activity for certain kinds of cancers, such as breast and colon cancers[2] strongly limit its clinical use. Furthermore, the development of acquired resistance to cisplatin is frequently observed during chemotherapy.[5] In order to overcome these drawbacks of cisplatin, numerous analogues have been synthesized and evaluated to develop alternative active agent with equivalent or greater antitumor activity and lower toxicity than cisplatin. Among them, carboplatin has proven to be the only second generation platinum complex commercially available at present. Carboplatin has modified the problems of renal and gastrointestinal toxicities of cisplatin.[6] Carboplatin, however, has not achieved the enhanced therapeutic efficacy[7] and has not possessed the property to overcome cross-resistance to cisplatin[8]. Recently, it has been reported that two different classes of compounds, bis(platinum) complexes[9] and platinum(IV) ammine/amine dicarboxylates[10], showed significant activity against a number of cisplatin-resistant murine and human tumor cell lines, however, these complexes did not overcome resistance completely. Therefore, the search for the new potent platinum complexes that possess a broader spectrum of the antitumor activity, lower toxicity, and lack of cross-resistance is continuing.

Most of the platinum complexes reported to date have five-membered ring or six-membered ring structures between a bidentate carrier ligand and a platinum atom.[11] The reason why reports are scarce on the synthesis of seven-membered ring complexes seems to be that the

chelate effect in a seven-membered ring structure is considered to be weaker than that in a five- or six-membered ring structure.[12] The target compounds **9-12** have been designed to have a seven-membered ring structure based on the recent findings of Nowatari *et al.*[12], in which 1,4-butanediamine platinum(II) complexes (seven-membered ring) have exhibited the higher antitumor activity against L1210 cells *in vivo* and *in vitro* than ethylenediamine platinum(II) complexes (five-membered ring) and 1,3-propanediamine platinum(II) complexes (six-membered ring). A 1,3-dioxolane ring moiety has been introduced in the carrier ligand to render the organoplatinum species more water-soluble, thereby facilitating intravenous administration and being possibly less toxic due to a more facile excretion *via* the kidney. The reactivity of platinum(II) complexes is known to be reduced by steric hindrance around the reactive center, platinum,[13] therefore, a 1,3-dioxolane ring has been incorporated into the β-position of 1,4-diaminobutane rather than the α-position.

Scheme 1[a]

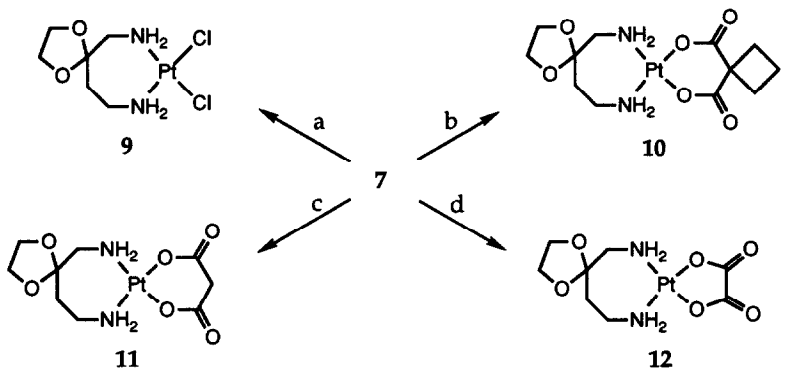


[a](a) $Hg(OAc)_2$, H_2O, reflux, 5 h; (b) (i) $HO(CH_2)_2OH$, $BF_3{\cdot}Et_2O$, rt, 1.5 h, (ii) K_2CO_3, rt, 20 min, (iii) continuous extraction with $CHCl_3$; (c) $C_6H_5SO_2Cl$, pyridine, rt, 3 h; (d) NaN_3, DMF, 100 °C, 16 h; (e) 10% Pd-C, H_2 (50 psi), EtOH, 40 °C, 2 h; (f) K_2PtCl_4, KI, H_2O, 60 °C, 1 h; (g) $AgNO_3$, H_2O, 60 °C, 2 h; (h) KI, 0 °C, 1 h

The synthesis of key intermediate, diiodo platinum(II) complex **7**, is outlined in Scheme 1. 1,4-Dihydroxy-2-butanone **2**[14], which was easily prepared from 2-butyne-1,4-diol by hydration in the presence of mercuric acetate, was reacted with ethylene glycol and boron trifluoride etherate to afford 2-(2-hydroxyethyl)-2-hydroxymethyl-1.3-dioxolane **3**[15] in 59% yield after a combination of continuous extraction with $CHCl_3$ from the aqueous phase and flash column chromatography. Treatment of dihydroxy compound **3** with benzenesulfonyl chloride in

pyridine yielded bis(benzenesulfonate) **4**[16] in 91% yield. Compound **4** was reacted with sodium azide in DMF to give 2-(2-azidoethyl)-2-azidomethyl-1,3-dioxolane **5**[17] in 75% yield, which was then reduced with hydrogen in the presence of 10% palladium on activated carbon in an alcoholic medium to afford 2-(2-aminoethyl)-2-aminomethyl-1,3-dioxolane **6**[18] in quantitative yield. The diamino compound **6** was reacted with an equimolar amount of *in situ* generated potassium tetraiodoplatinate(II) to produce a crude diiodo platinum(II) complex **7**, which was subsequently treated with an aqueous silver nitrate solution, followed by KI to yield a pure product **7**[19] in 56% yield. Conversion of complex **7** into *cis*-dichloro[1,3-dioxolane-2-(2-ethanamine)-2-methanamine]platinum(II) **9** was accomplished in 72% yield by the similar procedure described for the purification of **7**.

Scheme 2[a]



[a](a) (i) $AgNO_3$, H_2O, 60 °C, 2 h, (ii) NaCl, 0 °C ,1 h; (b) 1,1-cyclobutanedicarboxylic acid disilver salt, H_2O, 60 °C, 16 h; (c) malonic acid disilver salt, H_2O, 60 °C, 16 h; (d) oxalic acid disiver salt, H_2O, 60 °C, 16 h

Treatment of **7** with the disilver salt of 1,1-cyclobutanedicarboxylic acid, malonic acid, and oxalic acid afforded the corresponding 1,1-cyclobutanedicarboxylato platinum(II) complex **10**, malonato platinum(II) complex **11**, and oxalato platinum(II) complex **12**, in 72%, 86%, and 55% yields, respectively. The synthesized platinum complexes **9-12** were characterized by spectral data and elemental analysis.[20] The FAB mass spectra of these complexes showed typical three protonated molecular ion peaks because of the isotopes ^{194}Pt (33%), ^{195}Pt (35%), and ^{196}Pt (25%). The purity of the target compounds was further determined by analytical reverse-phase HPLC, and all complexes were found to be sufficiently pure (>98%) for biological evaluation.[21] The dichloro complex **9** showed 6.1 times higher solubility in H_2O compared to cisplatin (6.1 *vs.* 1.0 mg/mL at 25 °C). Compound **11** was highly water-soluble (28.2 mg/mL), while compounds **10** and **12** were marginally water-soluble (1.3 and 1.0 mg/mL, respectively).

In order to evaluate the antitumor property of these complexes to overcome acquired cisplatin-resistance, we have established a cisplatin-resistant subline of L1210 (L1210/CPR) by

continuous exposing L1210 cells to the increasing concentrations of cisplatin.[22] The cytotoxicity of the complexes **9-12** along with cisplatin and carboplatin against cisplatin-sensitive and -resistant L1210 leukemia cell lines *in vitro* were tested by trypan blue dye-exclusion method[23], and the results are shown in Table I.

Table I. Cytotoxicity of Platinum(II) Complexes against Cisplatin-sensitive and -resistant L1210 Murine Leukemia Cell Lines *in vitro*

	IC_{50} (μM)[a]		
compound	L1210/parent	L1210/CPR	relative resistance[b]
9	0.29	0.82	2.8
10	2.42	8.98	3.7
11	0.72	4.35	6.0
12	0.98	5.40	5.5
cisplatin	0.10	3.27	32.7
carboplatin	1.83	44.20	24.1

[a]Mean value of 3 experiments. [b]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 32.7- and 24.1-fold cross-resistant to cisplatin and carboplatin, respectively, in comparison with L1210 cells, while L1210/CPR cells were only 2.8- and 3.7- fold cross-resistant to the complexes **9** and **10**, respectively.

Table II. Cytotoxicity of Platinum(II) Complexes against Human Stomach and Lung Cancer Cell Lines *in vitro*

	IC_{50} (μM)[a]				
compound	SNU-1[b]	SNU-5[b]	SNU-16[b]	PC-9[c]	PC-14[c]
9	2.51	0.32	2.59	14.56	2.91
10	7.86	3.65	16.07	105.71	50.06
11	4.89	0.83	5.34	29.55	10.83
12	6.06	0.54	5.05	39.60	8.15
cisplatin	2.67	1.00	5.00	1.00	1.33
carboplatin	20.11	9.16	13.65	21.82	15.62

[a]Mean value of 3 experiments. [b]Stomach adenocarcinoma. [c]Lung adenocarcinoma.

The cytotoxicity of the complexes **9-12** were further tested toward three human stomach cancer cell lines, SNU-1, SNU-5 and SNU-16[24], and two human non-small cell lung cancer cell lines, PC-9 and PC-14, by MTT assay[25] (Table II).[26] It was found that the human stomach cancer

cell lines were much more sensitive to these complexes than the human non-small cell lung cancer cell lines. In particular, the dichloro complex **9** was even more potent against all three stomach cancer cell lines tested than cisplatin.

In conclusion, it has been shown that cisplatin-resistant murine L1210 leukemia cells have lower cross-resistance to these 1,3-dioxolane-2-(2-ethanamine)-2-methanamine platinum(II) complexes than to cisplatin and carboplatin, and the human stomach cancer cell lines, SNU-1, SNU-5, and SNU-16, are highly sensitive to this class of compounds. Based on these results, the dichloro complex **9** has been selected for further evaluation to demonstrate its *in vivo* antitumor activity and target organ toxicity profiles.

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15. **3** : a colorless oil; IR (neat) 3384 cm^{-1} (OH); ^1H NMR ($CDCl_3$/TMS) δ 2.00 (t, *J* = 5.4 Hz, 2 H, CH_2CH_2OH), 2.56 (br s, 1 H, OH), 2.84 (br s, 1 H, OH), 3.55 (s, 2 H, CH_2OH), 3.78 (t, *J* = 5.4 Hz, 2 H, CH_2CH_2OH), 4.03 (s, 4 H, OCH_2CH_2O); ^{13}C NMR ($CDCl_3$) δ 37.01, 58.35, 65.09, 65.29, 110.30.
16. **4** : a colorless oil; IR (neat) 1362, 1188 cm^{-1} (O-SO_2); ^1H NMR ($CDCl_3$/TMS) δ 2.05 (t, *J* = 6.6 Hz, 2 H, CH_2CH_2OBs), 3.85 (s, 4 H, OCH_2CH_2O), 3.87 (s, 2 H, CH_2OBs), 4.14 (t, *J* = 6.6 Hz, 2 H, CH_2CH_2OBs), 7.50-7.70 (m, 6 H, Ar), 7.85-7.95 (m, 4 H, Ar); ^{13}C NMR ($CDCl_3$) δ 34.06, 65.33, 65.55, 70.06, 106.27, 127.78, 127.82, 129.22, 133.75, 133.88, 135.80, 135.98.

17. **5** : a colorless oil; IR (neat) 2105 cm^{-1} (N_3); ^1H NMR ($CDCl_3$/TMS) δ 2.02 (t, *J* = 7.2 Hz, 2 H, $CH_2CH_2N_3$), 3.24 (s, 2 H, CH_2N_3), 3.39 (t, *J* = 7.2 Hz, 2 H, $CH_2CH_2N_3$), 3.95-4.15 (m, 4 H, OCH_2CH_2O); ^{13}C NMR ($CDCl_3$) δ 34.63, 46.03, 55.28, 65.74, 108.83.
18. **6** : a colorless oil; IR (neat) 3368 cm^{-1} (NH_2); ^1H NMR ($CDCl_3$/TMS) δ 1.30 (br s, 4 H, $2NH_2$), 1.83 (t, *J* = 7.2 Hz, 2 H, $CH_2CH_2NH_2$), 2.74 (s, 2 H, CH_2NH_2), 2.81 (t, *J* = 7.2 Hz, 2 H, $CH_2CH_2NH_2$), 3.99 (s, 4 H, OCH_2CH_2O); ^{13}C NMR ($CDCl_3$) δ 37.29, 38.92, 47.37, 65.31, 110.84.
19. **7** : yellow crystals; IR (KBr) 3508, 3226, 1591 cm^{-1}; FAB-MS *m/z* 595 $(MH)^+$; Anal. Calcd for $C_6H_{14}N_2O_2I_2Pt$: C, 12.11; H, 2.37; N, 4.71. Found: C, 12.02; H, 2.39; N, 4.58.
20. **9** : pale yellow crystals; IR (KBr) 3447, 3212, 1587 cm^{-1}; FAB-MS *m/z* 413 $(MH)^+$; Anal. Calcd for $C_6H_{14}N_2O_2Cl_2Pt$: C, 17.48; H, 3.42; N, 6.80. Found: C, 17.35; H, 3.48; N, 6.64. **10** : white crystals; IR (KBr) 3387, 1672, 1636, 1611 cm^{-1}; ^1H NMR (DMSO-d_6/TMS) δ 1.65 (quintet, *J* = 7.8 Hz, 2 H, $CH_2CH_2CH_2$), 2.13 (m, 2 H, $CH_2CH_2NH_2$), 2.55-2.77 (m, 8 H, CH_2NH_2, $CH_2CH_2NH_2$ and $CH_2CH_2CH_2$), 3.87 (s, 4 H, OCH_2CH_2O), 5.13 (br s, 2 H, NH_2), 5.25 (br s, 2 H, NH_2); ^{13}C NMR (DMSO-d_6) δ 14.84, 30.27, 36.51, 40.56, 52.01, 55.51, 64.14, 106.87, 177.28, 177.30; FAB-MS *m/z* 484 $(MH)^+$; Anal. Calcd for $C_{12}H_{20}N_2O_6Pt$: C, 29.82; H, 4.17; N, 5.80. Found: C, 29.88; H, 4.13; N, 5.56. **11** : white crystals; IR (KBr) 3441, 3193, 3127, 1680, 1641 cm^{-1}; ^1H NMR (DMSO-d_6/TMS) δ 2.12 (m, 2 H, $CH_2CH_2NH_2$), 2.55-2.80 (m, 4 H, CH_2NH_2 and $CH_2CH_2NH_2$), 3.22 (s, 2 H, CH_2), 3.87 (s, 4 H, OCH_2CH_2O), 5.15 (br s, 2 H, NH_2), 5.28 (br s, 2 H, NH_2); ^{13}C NMR (DMSO-d_6) δ 36.43, 40.63, 50.23, 51.95, 64.16, 106.86, 173.99; FAB-MS *m/z* 444 $(MH)^+$; Anal. Calcd for $C_9H_{16}N_2O_6Pt$: C, 24.38; H, 3.64; N, 6.32. Found: C, 24.21; H, 3.58; N, 6.25. **12** : white crystals; IR (KBr) 3473, 3234, 3158, 1699, 1674, 1616 cm^{-1}; ^1H NMR (DMSO-d_6/TMS) δ 2.13 (m, 2 H, $CH_2CH_2NH_2$), 2.55-2.80 (m, 4 H, CH_2NH_2 and $CH_2CH_2NH_2$), 3.88 (s, 4 H, OCH_2CH_2O), 5.40 (br s, 2 H, NH_2), 5.49 (br s, 2 H, NH_2); ^{13}C NMR (DMSO-d_6) δ 36.45, 40.72, 51.81, 64.16, 106.84, 166.03, 166.07; FAB-MS *m/z* 430 $(MH)^+$; Anal. Calcd for $C_8H_{14}N_2O_6Pt$: C, 22.38; H, 3.29; N, 6.53. Found: C, 22.15; H, 3.25; N, 6.45.
21. The purity for tested platinum(II) complexes was assessed by analytical reverse-phase HPLC on a Waters Associates system (consisting of a 600E pump, a 712WISP automated injector, and a Model 990 photodiode array detector), using a μBondapak C_{18}, 10μm particle size, 125Å pore size column, 3.9x300 mm. The mobile phase utilized was MeOH-H_2O system and the flow rate was 1.5 mL/min, with monitoring the peak at 220 nm.
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26. SNU-1, SNU-5, and SNU-16 were obtained from Professor J.-G. Park, Cancer Research Institute, Seoul National University College of Medicine, Korea, and PC-9 and PC-14 were kindly provided by Dr. W.-S. Hong, National Cancer Center Hospital and Research Institute, Korea.

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