Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry 11 (2003) 1669–1676

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis, Characterization and Antitumor Activity of Novel Octahedral Pt(IV) Complexes

Young-Ee Kwon,[a,*] Kyu-Ja Whang,[b] Young-Ja Park[c] and Kuk Hwan Kim[d]

[a]*Drug Discovery Institute of STC Life Science Center, 6-13, Nonhyun-Dong, Kangnam-Gu, Seoul, 135-010, South Korea*
[b]*College of Pharmacy, Sookmyung Women's University, Chungpa-dong, Yongsan-ku, Seoul, 140-742, South Korea*
[c]*Department of Chemistry, Sookmyung Women's University, Chungpa-dong, Yongsan-ku, Seoul, 140-742, South Korea*
[d]*College of Pharmacy, Dongduk Women's University, Worlkok-dong, Sungbuk-Gu, Seoul, 136-714, South Korea*

Received 18 September 2002; accepted 6 January 2003

Abstract—Novel platinum(IV) complexes were synthesized having octahedral structure for new antitumor agents. The series of (1,4-butanediamine)Pt(IV) complexes of the type *trans,cis*-[PtA_2Cl_2(1,4-butanediamine)] (A = hydroxo **9**, acetato **12**, trifluoroacetato **13** as axial ligands) and *trans*-[PtA_2(malonate)(1,4-butanediamine)] (A = hydroxo **16**, acetato **17**, trifluoroacetato **18**) were synthesized and characterized by IR, NMR and elemental analysis. The molecular structures of **12**, **13** and **18** have been determined by X-ray diffraction methods. The crystals are monoclinic, P2 1/c with $a = 21.165$ (5), $b = 9.050$ (3), $c = 15.293$ (3) Å, $\beta = 103.89$ (2)° and $Z = 8$ for **12,** $a = 10.178$ (5), $b = 12.894$ (9), $c = 12.182$ (8) Å, $\beta = 91.01$ (5)° and $Z = 4$ for **13** and $a = 10.460$ (5), $b = 11.199$ (8), $c = 15.641$ (7) Å, $\beta = 98.41$ (5)°, $Z = 4$ for **18**. Three crystallographically independent molecules of **12**, **13** and **18** have octahedral coordination around Pt(IV) cation. The *trans,cis*-[PtA_2Cl_2(1,4-butanediamine)] were prepared by acetylation or trifluoroacetylation of *trans,cis*-[$Pt(OH)_2Cl_2$(1,4-butanediamine)]. The *trans*-[PtA_2malonate(1,4-butanediamine)] **17** and **18** was prepared by a similar method. The in vitro cytotoxicity of theses Pt(IV) complexes have been evaluated against 12 cancer cell lines assayed by MTS method. The IC_{50} values of the compounds **12** and **13** were shown to be lower than those of cisplatin. The in vivo antitumor activity of the Pt(IV) complexes was evaluated using mice bearing L1210 leukemia, B16 melanoma and L1210/cis-DDP cancer animal models. The compound **18** was found to highest activity against cisplatin-resistant cancer cells, L1210/cis-DDP, in vivo.

Introduction

cis-Diamminedichloroplatinum(II) (cisplatin) **1**[1] is widely applied in the treatment of a various types of cancer such as testicular, ovarian and bladder carcinomas.[2–4] Cisplatin is also widely used in combination with other anticancer agents such as doxorubicin, etoposide, bleomycin and 5-fluorouracil, in treating head and neck cancer, lung carcinoma, stomach carcinoma, and so on.[5,6] However, the clinical usefulness of cisplatin has been frequently limited by its severe side effects such as nephrotoxicity, nausea, ototoxicity, neurotoxicity and myelotoxicity,[7,8] development of acquired resistance,[9] low activity against breast and colon cancer. Therefore, it is desirable to develop a new platinum-based anticancer drugs with broader spectrum of activity, improved clinical efficacy and reduced toxicity, better than cisplatin. One of the strategy is to replace the labile chloro ligand in cisplatin with other leaving groups and to extend the stable amine ligand to a series of either cyclic or acyclic alkylamines on the basis of the structure–activity relationship. Efforts over the past two decades have resulted in clinical trials of second-generation platinum complexes. Among them, *cis*-diamine (1,1-cyclobutanedicarboxylato) platinum(II) (carboplatin) **2** has proven to be second-generation platinum complex.[10] But carboplatin has a narrow spectrum of antitumor activity[11] and is not effective in the treatment of cancer cells resistant to cisplatin.[12] In addition to square-planar platinum(II) complexes, an attempt has also been made more recently to octahedral platinum(IV) complexes. Recently, the clinically studied platinum(IV) complexes are iproplatin **3**[13] and JM216 **4**. (Chart 1) [14] Another approach is to converting platinum (II) drugs to their platinum(IV) analogues.[15,16] The mechanism of cytotoxicity of cisplatin-resistant cell line

*Corresponding author. Tel.: +82-2-3438-0550; fax: +82-2-511-6705; e-mail: yekwon@stc365.com

0968-0896/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0968-0896(03)00044-0

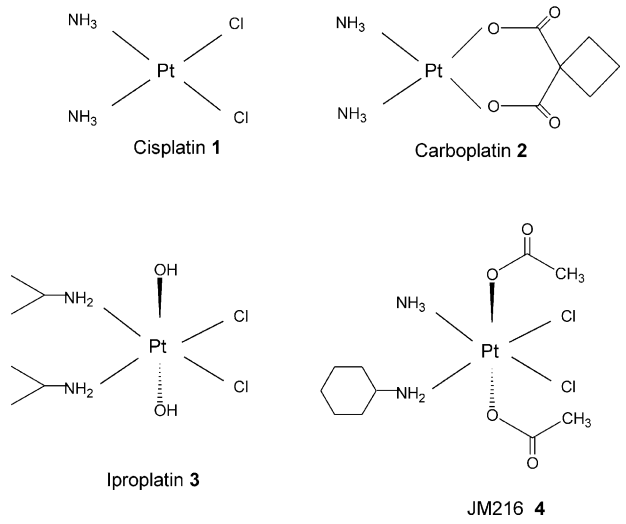


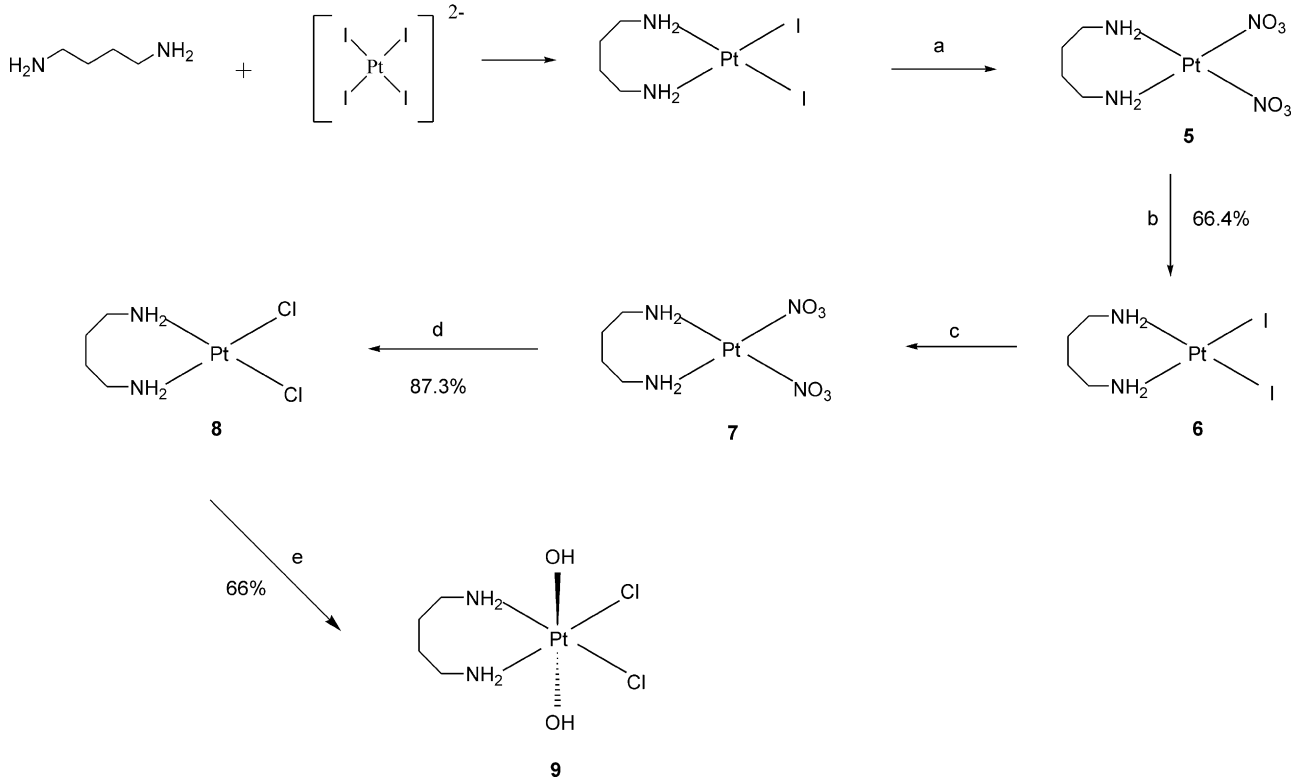
Chart 1.

was reported that Pt(IV) complex regulates p53 concerning cell cycle.[17] A broad spectrum of preclinical antitumor activity of Pt(IV) complexes have been reported having a different mechanism from Pt(II) complex and remain active against general cancer cell lines as well as other cell lines resistant to cisplatin.[18] The new Pt(IV) complexes having short step synthesized and gave high yield. The present study deals with synthesis, characterization including X-ray crystal structure and tested antitumor activities in vivo of the series of (1,4-butanediamine)Pt(IV) complexes containing *trans*-acetato or trifluoroacetato as axial ligands and *cis*-chloro or malonic acid as leaving groups.[19]

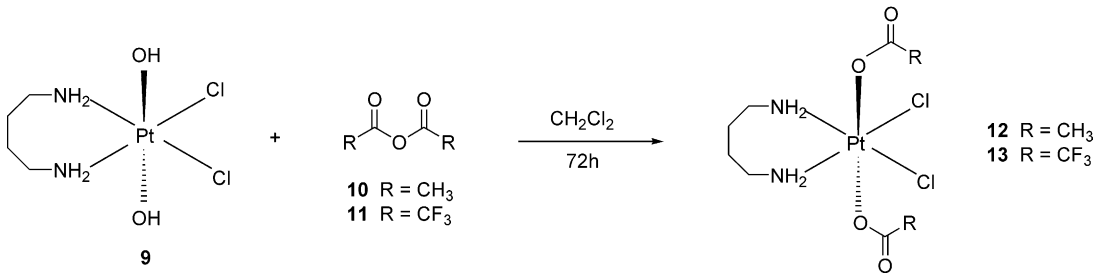
Results and Discussion

Chemistry

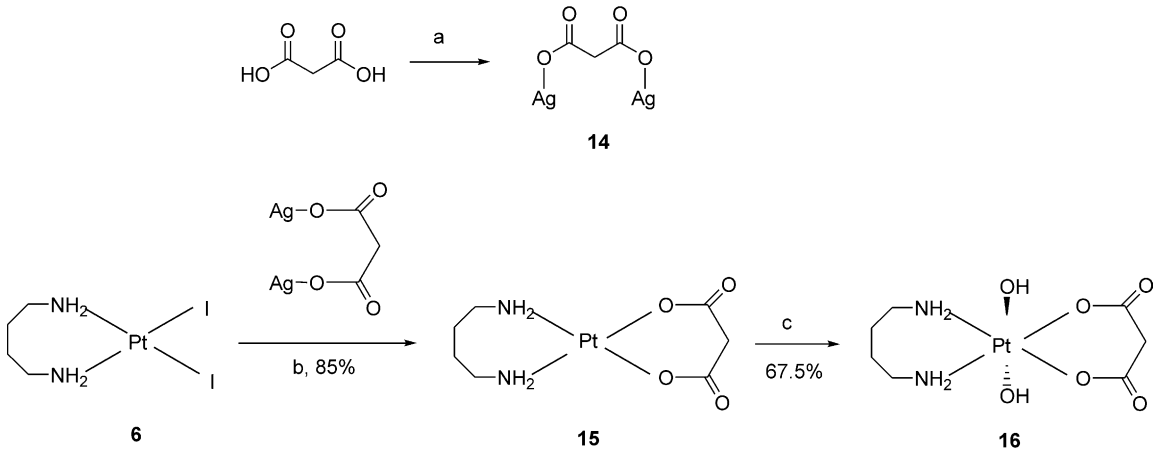
The syntheses of *trans,cis*-[PtA_2Cl_2(1,4-butanediamine)] (A = hydroxo **9**, acetato **12**, trifluoroacetato **13** as axial lignads) and *trans*-[PtA_2(malonate)(1,4-butanediamine)] (A = hydroxo **16**, acetato **17** trifluoro-acteato **18** as axial lignads) complexes were shown in Schemes 1, 2, 3 and 4. Pt(IV) complex **9** having axial hydroxyl group was prepared by the oxidation of *cis*-dichloro-1,4-butanediamine Pt(II) complex with 30% H_2O_2 at 80 °C. The complex **16** was synthesized by similar method as the complex **9** except the temperature. According to X-ray diffraction study, Pt(IV) complex having malonate as a leaving ligand contains intramolecular hydorgen bond, and its seven-membered ring conformation is not the most stable twist chair form but an unstable chair form. Therefore, if the reaction temperature becomes high, the hydrogen bond may be broken. Compounds **9** and **16** were reacted in an excess acetic anhydride, trifluoroacetic anhydride in CH_2Cl_2 in order to form Pt(IV)complexes (**12, 13, 17, 18**). Then, these complexes were recrystallized with methanol or methanol–water. The structures of each compounds were confirmed by IR, NMR, elemental analysis and X-ray diffraction method. The IR spectra of complexes **12** and **13** showed that the O–H peak around 3500 cm^{-1} disappeared, and a strong band due to (C=O) was observed at 1600 or 1700 cm^{-1}. This indicated the presence of an acetate or trifluoroacetate moieties. The differences in IR frequencies between **12, 17** and **13, 18** complexes indicated that the carbonyl attached to the trifluoro group was electron-deficient. ^1H NMR using DMSO-d_6 showed a peak for N–H bond was observed between 7.4 and 7.9



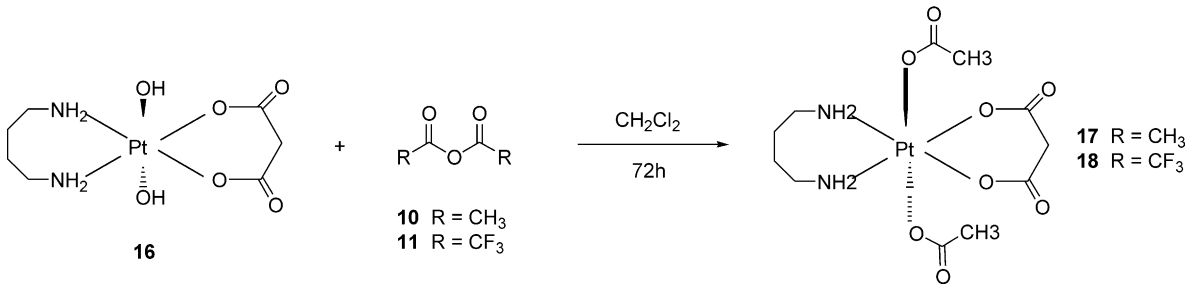
Scheme 1. Reagents and conditions: (a) $AgNO_3$, 60 °C; (b) KI; (c) $AgNO_3$; (d) NaCl; (e) 30% H_2O_2, 80 °C, 5 h.



Scheme 2.



Scheme 3. Reagents: (a) 0.6 N NaOH, $AgNO_3$; (b) H_2O; (c) 30% H_2O_2, 24 h.



Scheme 4.

and a peak for alkyl group between 1.6 and 3.9. The peak of alkyl group was not clear. Therefore, the sepectra was regarded in using CD_3OD to confirm the peak. Analysis of ^{13}C NMR spectra revealed that the carbonyl group were between 165.4 and 188. When proton and carbon peaks of **12** and **17** complexes were compared with those of **13** and **18** complexes, most of the peaks of compounds **13** and **18** were shifted to high magnetic field under the influence of an electrophilic effect of fluorine atom. The elemental analyses of Pt(IV) complexes were identical to the calculated values.

X-ray crystal structure analysis

The molecular structures of **12A, 13** and **18** with atomic numbering are shown in Figure 1. There are two molecules of **12** in the asymmetric unit and their molecular structures are similar. All four molecules consist of slightly distorted octahedral Pt(IV) cation. The seven-membered rings of **12A, 12B** and **13** are twist chair form. Molecule **18** shows that the seven-membered ring of Pt-amine ligand aquires a chair form and Pt-malonate six-membered ring has boat conformation. These three compounds have N–H- - -O type intramolecular hydrogen bonds. The X-ray crystal data of each compounds is described in Tables 1 and 2.

In vitro cytotoxicity against cancer cell lines

Anticancer activities of the platinum(IV) complexes were evaluated *in vitro* using various cancer cell lines such as human leukemia, HL-60; human colon cancer, HCT 116 and HCT 15; human lung cancer, A 549 and NCI-H23; human breast cancer, SK-BR3, MCF 7 and MDA-MB 231; human ovarian cancer, SKOV-3; human melanoma, SKMEL-2; human CNS cancer, XF-498; mouse leukemia, L1210. Anticancer activities of these compounds against each cancer cell lines in terms of IC_{50} of cisplatin, carboplatin, **9, 12, 13, 16, 17** and **18** were evaluated and shown in Table 3. *In vitro*, compounds **12** and **13** have excellent cytotoxicity which is about 2–15 times greater as compared to cisplatin.

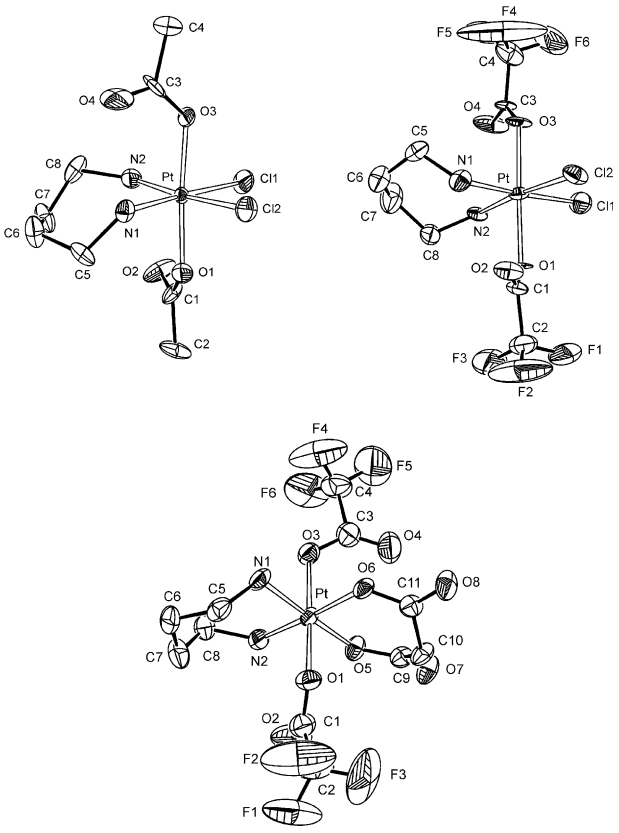


Figure 1. The molecular structures of **12A** (top, left), **13** (top right) and **18** (bottom) with atomic numbering (50% displacement ellipsoids).

Especially, compounds **12** and **13** with axial ligands were more potent to kill human breast and colon cancer cell lines than cisplatin. At present, mechanism of Pt(IV) complexes with axial ligands is not known. But it maybe because of enhanced intracellular accumulation due to an increase in the lipophilicity of the molecule.[20]

In vivo antitumor activity

The Pt(IV) complexes were evaluated for *in vivo* antitumor activity (Table 4). In B16 melanoma tumor model, T/C (%) values of cisplatin (4 mg/kg) and compound **12** (5 mg/kg) were found 171.9, 168.9, respectively. At similar drug concentration, antitumor activity of cisplatin and compound **12** was found similar but 10% mean decreased in body weight due to administraion of cisplatin whereas compound **12** have shown 2% mean increase. So, we could predict that toxicity of compound **12** could be less than that of cisplatin in effective drug concentrations. In cisplatin-resistant cancer cells L1210/*cis*-DDP, T/C (%) value of compound **18** (150 mg/kg) was 177.6. Compound **18** showed antitumor activity about all tested cancer cells in vivo. In further study, we will an try oral administration test of antitumor activity.

Experimental

General methods

These complexes were characterized by elemental analysis (C, H, N, O), IR, ^{1}H and ^{13}C NMR. Precoated Merck silica gel RP-18F_{254S} plates were used for thin-layer chromatography (TLC) and the spots were detected under UV light (254 nm). IR spectra was measured from KBr pellets on a Jasco FT/IR-430 spectrophotometer. ^{1}H– and ^{13}C NMR spectra were recorded in D_2O, CD_3OD or DMSO-d_6 solvents on a Varian Germini 300 and Bruker AMX500 spectrophotometers. Chemical shifts of proton are reported in ppm (δ), the coupling constants J in herz (Hz) and TMS as the internal referance. Elemental analyses were performed with a Carlo Erba EA-1108 elemental analyzer and the results lie within 0.4% deviation of theoretical values.

Table 1. Summary of X-ray crystal data of Pt(IV) complexes

	12	**13**	18
Formula	$PtC_8H_{18}Cl_2N_2O_4$	$PtC_8H_{12}F_6Cl_2N_2O_4$	$PtC_{11}H_{14}F_6N_2O_8$
Molecular weight	472.2	580.2	611.3
Crystal system	Monoclinic	Monoclinic	Monoclinic
Space group	$P2_1/C$	$P2_1/C$	$P2_1/C$
a, (Å)	21.165 (5)	10.178 (5)	10.460 (5)
b, (Å)	9.050 (3)	12.894 (9)	11.199 (8)
c, (Å)	15.293 (3)	12.182 (8)	15.641 (7)
β, °	103.89 (2)	91.01 (5)	98.41 (5)
V (Å^3)	2843.8 (12)	1598.5 (17)	1812.5 (18)
Z	8	4	4
μ (mm^{-1})	10.25	9.19	7.84
Density (g cm^{-3})	2.21 (calcd)	2.41 (calcd)	2.24 (calcd)
Goodness of fit	1.05	1.075	1.063
No. of indep. reflections	4971	1483	3189
No. of obs. reflections	3842	1369	2385
R(F)[a] for obs. reflections	0.071	0.035	0.03

trans,cis-Diacetato, dichloro-1,4-butanediamine Pt(IV) complex **12**. *trans,cis*-Ditrifluoroacetato,dichloro-1,4-butanediamine Pt(IV) complex **13**. *trans*-Ditrifluoroacetato, malonato-1,4-butanediamine Pt(IV) complex **18**.

[a] $R = \dfrac{\Sigma||F_o|-|F_c||}{\Sigma|F_o|}$.

Table 2. Conformation angles(°) of seven-membered ring of **12**, **13** and **18**

	12		13	18
	A	B		
N(2)-Pt-N(1)-C(5)	58	37	−49	67
Pt-N(1)-C(5)-C(6)	−86	−84	86	−86
N(1)-C(5)-C(6)-C(7)	71	82	−69	65
C(5)-C(6)-C(7)-C(8)	−75	−70	63	−75
C(6)-C(7)-C(8)-N(2)	96	82	−86	99
C(7)-C(8)-N(2)-Pt	−76	−83	82	−65
C(8)-N(2)-Pt-N(1)	12	33	−26	0

***cis*-Diiodo-1,4-butanediamine Pt(II) complex (6).** Twenty-four grams (145 mmol) of potassium iodide (50 mmol) in H_2O (100 mL) was added to 24 g (24 mmol) of potassium tetrachloroplatinate(II) dissolved in H_2O (250 mL) with stirring for 30 min at room temperature and cooled to 0 °C in the dark under the nitrogen atmosphere [solution A]. Separately, 2.12 g (24 mmol) of 1,4-butanediamine was dissolved in 300 mL of H_2O [solution B]. 800 mL of H_2O was placed in a brown flask and stirred at 60 °C under nitrogen, and then solution A and solution B were simultaneously added dropwise over a period 3 h at a constant rate. After 1 h, the yellowish-brown precipitate was filtered, washed sequentially with water, ethanol and ether, and dried in vacuo. The precipitate 11.56 g of crude *cis*-diiododiamine complex was obtained. For purification, 11.3 g (21 mmol) of this diiodoplatinum complex (II) was suspended in 10 mL of acetone and 200 mL of H_2O. $AgNO_3$ (39.91 mmol, 0.95 equiv) in H_2O (200 mL) was added into the suspension. The mixture was heated at 60 C for 3 h in the dark and filtered through 0.2 μm membrane filter. 20 g of KI was added the filtrate and stirred for 1 h at room temperature. The resulting yellow crystals were filtered off, washed with water and ethanol, and then dried to give 7.5 g of *cis*-diiodo-1,4-

Table 3. In vitro cytotoxicity of synthesized Pt(IV) complexes in 12 cancer cell lines

Compd	IC_{50} (μmol)											
	HL-60	L1210	HCT 116	HCT15	A549	NCI-H23	SK-BR-3	MCF7	MDA-MB 231	SK-OV-3	SK-MEL-2	XF498
9	13.62	13.78	19.94	16.48	25.05	24.04	28.43	42.48	21.25	34.78	31.5	13.26
12	1.15	1.23	1.95	1.75	2.38	2.71	1.83	1.03	2.31	4.65	2.98	1.15
13	0.76	0.87	0.85	0.76	1.39	1.44	1.06	0.8	1.19	3.16	2.68	1.26
16	71.7	63.24	92.13	98.59	93.52	83.36	96.28	77.98	>100	>100	>100	96.5
17	33.73	32.43	61.72	59.62	52.56	71.36	52.79	52.79	54.46	>100	95.47	66.82
18	18.96	17.87	15.83	15.93	24.03	24.29	22.05	23.58	25.83	66.72	15.83	37.2
Cisplatin	2.13	2.53	7.98	13.1	4.63	5.15	10.07	13.32	13.98	6.62	7.96	2.13
Carboplatin	23.24	44.05	69.44	69.6	40.77	51.14	45.43	62.19	68.26	22.16	41.86	28.97

This experimental result presented as micro-mole concentration of 50% cell growth inhibition (IC_{50}) of each drugs. Each IC_{50} values were calculated using quantal dose–response: Probits computer program edited by Ronald J. Tallarida et al. Compound **9** is *trans*, *cis*-dihydroxo, dichloro-1,4-butanediamine Pt(IV) complex. Compound **12** is *trans*, *cis*-diacetato, dichloro-1,4-butanediamine Pt(IV) complex. Compound **13** is *trans*, *cis*-ditrifluoroacetato, dichloro-1,4-butanediamine Pt(IV) complex. Compound **16** is *trans*-dihydroxo, malonato-1,4-butanediamine Pt(IV) complex. Compound **17** is *trans*-diacetato, malonato-1,4-butanediamine Pt(IV) complex. Compound **18** is *trans*-dtrifluoroacetato, malonato-1,4-butanediamine Pt(IV) complex.

Table 4. Antitumor activity against BDF1 mice bearing each of three cancer cells

Compd	Dose (mg/kg)	T/C (%)			Body weight changes (g)		
		L1210/cis-DDP	L1210	B16	L1210/cis-DDP	L1210	B16
12	20	82.9	105.4	Toxic	−6.4	−6.7	−7.6
	10	121.3	125.7	162.5	−2.1	−1.9	−4.8
	5	108.9	112.2	168.9	+4.0	+4.8	+0.4
13	10	108.9	141.9	Toxic	−3.4	−2.3	Toxic
	5	111.4	113.5	106.3	+1.0	+1.1	−3.5
	2.5	101.5	106.8	128.1	+3.3	+3.2	+0.4
17	150	102.5			−1.4		
	100	105.0	147.4	124.2	+0.4	+2.5	0
	60		111.4	115.2		+3.0	+0.6
	50	110.0			+3.0		
	30		105.7	109.1		+3.0	+1.1
18	150	177.6					
	100	146.1	180.0	163.6	−4.0	+1.0	−0.6
	60		161.4	154.5	−0.1	+2.2	+0.6
	50	109.2			+1.8		
	30		155.7	109.1		+3.1	+1.4
Cisplatin	4	123.8	167.6	171.9	−0.7	−0.5	−2.0
Carboplatin	60	113.2	147.4	166.7	+0.3	+2.1	−1.0
	30	106.6		124.2	+1.6	+2.5	+0.7

Animal is BDF1 mice. L1210/*cis*-DDP is cisplatin resistance mouse leukemia cell line. L1210 is mouse leukemia cell line. B16 is mouse melanoma cell line. Inoculum size of L1210 and L1210/*cis*-DDP is 1×10^5 cells per mouse. Inoculum size of B16 melanoma is 10:1 brei. Inoculum or treatment site is ip. Treatment time time is day 1, 5, 9 and parameter is mean survival time (test group survival/control group survival×100 = T/C%). Each groups were consist of 8 mice. Body weight changes (g) = mean body weight at day 1 − mean body weight at days 5 or 9.

butanediamine Pt(II) complex **6.** Yield: 66.4%, IR (KBr, cm^{-1}): 3205, 2928, 1591, 1183, ^1H NMR (300 MHz, DMSO-d_6): 4.9 (4H, m), 2.25 (4H, m), 1.4 (4H, m), Anal. calcd for $C_4H_{12}N_2I_2Pt$–C; 8.94%, H; 2.24%, N; 5.22%, found. C; 8.90%, H; 2.2%, N; 5.24%.

***cis*-Dichloro-1,4-butanediamine Pt(II) complex (8).** Six grams (11.18 mmol) of compound **6** were suspended in 1 mL of acetone and 30 mL of H_2O. 0.131 g of $AgNO_3$ (0.77 mmol, 0.98 equiv) in 10 mL H_2O was added into the suspension. The mixture was heated at 60 °C for 2 h in the dark and filtered. To this filtrate 0.2 g of NaCl was added and stirred for 2 h at room temperature. The resulting light-yellow precipitation was filtered off, washed with water, ethanol, and then dried to give 3.4 g of *cis*-dichloro-1,4-butanediamine Pt(II) complex **8**. Yield: 87.3%, IR (KBr, cm^{-1}): 3225, 1579, 1156, 508, ^1H NMR (300 MHz, DMSO-d_6): 4.9 (4H, m), 2.5–2.7 (4H, m), 1.8–2.0 (4H, m), Anal. calcd for $C_4H_{12}N_2Cl_2Pt$–C; 13.56%, H; 3.39%, N; 7.91%, found. C; 13.51%, H; 3.34%, N; 7.85%.

***cis*-Malonato-1,4-butandiamine Pt(II) complex (15).** Eight grams (14.91 mmol) of **6** in 5 mL of acetone and 300 mL of H_2O were added to 4.73 g (14.91 mmol) of malonato silver salt **14** in 50 mL of H_2O and stirred at room temperature in the dark overnight. The filtrate was concentrated under a reduced pressure to small volume and freeze-dried to give 5.12 g of malonato-1,4-butandiamine Pt(II) complex **15** as a white solid. Yield: 84.6%, IR (KBr, cm^{-1}): 3438, 3259, 3068, 1609, 1421, 1303, 745, ^1H NMR (300 MHz, D_2O): 4.9 (2H, m), 4.7 (4H, m), 2.8 (4H, m), 1.9–2.1 (4H, m), Anal. calcd for $C_7H_{14}N_2O_4Pt$–C; 21.82%, H; 3.64%, N; 7.27%, found. C; 21.8%, H; 3.61%, N; 7.24%.

***trans*, *cis*-Dihydroxo, dichloro-1,4-butanediamine Pt(IV) complex (9).** Three grams (8.48 mmol) of **8** in 100 mL of H_2O were stirred with 30 mL of 30% H_2O_2 at 80 °C for 5 h. The reaction mixture was concentrated under a reduced pressure to small volume and added 300 mL of methanol, then placed at 4 °C for 2 h. The resulting light-yellow precipitation was filtered off, washed with methanol, and then dried to give 2.17 g of *trans*, *cis*-dihydroxo, dichloro-1,4-butanediamine Pt(IV) complex **16**. Yield: 65.95%, IR (KBr, cm^{-1}): 3502, 3200, 1572, 1027, 545, ^1H NMR (300 MHz, D_2O): 2.8 (4H, m), 1.7 (4H, m), Anal. calcd for $C_4H_{14}N_2Cl_2O_2Pt$–C; 12.37%, H; 3.61%, N; 7.22%, O; 8.25%, found. C; 12.35%, H; 3.63%, N; 7.25%, O; 8.25%.

***trans*,*cis*-Diacetato, dichloro-1,4-butanediamine Pt(IV) complex (12).** One gram (2.577 mmol) of **9** in 100 mL methylenechloride was refluxed with 20 mL of acetic anhydride for 72 h. The reaction mixture was concentrated under a reduced pressure, dissolved in 20 mL of methanol and filtered. The filtrate was recrystallized to purify. The light-yellow crystals formed cubic shape were filtered off, washed with cold methanol, and then dried to give 0.96 g of *trans*,*cis*-diacetato,dichloro-1,4-butanediamine Pt(IV) complex **12**. Yield: 78.9%, IR (KBr, cm^{-1}): 3159, 3039, 1598, 1363, 1295, 986, 704, 503, ^1H NMR (500 MHz, CD_3OD): 2.92 (4H, t, $J=24.0$), 2.09 (6H, s), 1.85 (4H, m), ^{13}C NMR (125 MHz, CD_3OD): 183.1, 49.5, 49.3, 49.1, 49, 48.8, 48.6, 48.5, 48.2, 27.6, 23.6 Anal. calcd for $C_8H_{18}N_2Cl_2O_4Pt$–C; 20.34%, H; 3.81%, N; 5.93%, found. C; 20.33%, H; 3.80%, N; 5.93%.

***trans*,*cis* - Ditrifluoroacetato,dichloro - 1,4 - butanediamine Pt(IV) complex (13).** One gram (2.577 mmol) of **9** in methylenechloride (100 mL) was refluxed with 15 mL trifluoroacetic anhydride for 72 h and prepared by the method as applied for compound **12.** The resulting light-yellow cubic crystals were filtered off, washed with cold methanol, and then dried to give 1.22 g of *trans*,*cis*-ditrifluoroacetato,dichloro-1,4-butanediamine Pt(IV) complex **13**. Yield: 81.62%, IR (KBr, cm^{-1}): 3254, 3209, 3177, 1724, 1565, 1376, 1224, 1166, 739, ^1H NMR (500 MHz, CD_3OD): 2.95 (4H, dd, $J=22.21$, 20.3), 1.85 (4H, m), ^{13}C NMR (125 MHz, CD_3OD): 165.4, 165.1, 115, 112.7, 49.5, 49.3, 49.2, 49, 48.8, 48.5, 47.8, 26.9, Anal. calcd for $C_8H_{12}N_2Cl_2O_4F_6Pt$– C; 16.55%, H; 2.07%, N; 4.83%, found. C; 16.54%, H; 2.08%, N; 4.80%.

***trans*-Dihydroxo,malonato-1,4-butanediamine Pt(IV) complex (16).** Five grams (12.99 mmol) of **15** in 300 mL of H_2O were stirred with 30 mL of 30% H_2O_2 at room temperature for 72 h. The reaction solution was concentrated under a reduced pressure to small volume and added 300 mL methanol, and placed at 4 C for 2 h. The resulting white precipitation was filtered off, washed with methanol, and then dried to give 2.17 g of *trans*-dihydroxo,malonato-1,4-butanediamine Pt(IV) complex **16**. Yield: 67.43%, IR (KBr, cm^{-1}): 3525, 3205, 1660, 1370, 975, 753, 569, ^1H NMR (300 MHz, D_2O): 3.0 (2H, m), 2.8 (2H, m), 2.9 (4H, m), 1.8–2.0 (4H, m), Anal. calcd for $C_7H_{16}N_2O_6Pt$–C; 20.05%, H; 3.82%, N; 6.68%, Found. C; 19.98%, H; 3.79%, N; 6.62%.

***trans*-Diacetato,malonato-1,4-butanediamine Pt(IV) complex (17).** One and a half grams (3.58 mmol) of **16** were placed in 100 mL of methylene chloride and the next procedure was similar to that of compound **12**. White crystals of *trans*-diacetato,malonato-1,4-butanediamine Pt(IV) complex **17** were obtained by recrystalization. Yield: 54.42%, IR (KBr, cm^{-1}): 3444, 3172, 3049, 1660, 1362, 709, 532, ^1H NMR (500 MHz, D_2O): 2.95–2.75 (8H, m), 1.95–1.8 (8H, m), ^{13}C NMR (125 MHz, D_2O): 188, 183.9, 179.8, 179.2, 49.2, 48.7, 28.4, 28.1, 24.9, Anal. calcd for $C_7H_{16}N_2O_6Pt$–C; 26.24%, H; 3.98%, N; 5.57%, found. C; 26.19%, H; 3.95%, N; 5.53%.

***trans* - Ditrifluoroacetato, malonato - 1,4 - butanediamine Pt(IV) complex (18).** One and a half grams (3.58 mmol) of **16** were placed in 100 mL of methylene chloride and next procedure was similar to that of compound **13**. White crystals of *trans*-ditrifluoroacetato,malonato-1,4-butanediamine Pt(IV) complex **18** were obtained by recrystalization. Yield: 66.29%, IR (KBr, cm^{-1}): 3440, 3200, 3089, 1716, 1651, 1368, 1161, 739, 523, ^1H NMR (500 MHz, CD_3OD): 3.6 (2H, s), 2.9 (4H, dd, $J=20.4$, 18.26), 1.98–1.97 (4H, m), ^{13}C NMR (125 MHz,

CD_3OD): 176.4, 49.5, 49.3, 49.2, 49, 48.8, 48.6, 48.5, 47.4, 47.3, 26.5, Anal. calcd for $C_{11}H_{14}N_2O_8F_6Pt$–C; 21.6%, H; 2.29%, N; 4.58%, found. C; 21.58%, H; 2.30%, N; 4.57%.

X-ray structure determination of 12, 13 and 18

Crystals of **12, 13** and **18** were grown by slow evaporation from a dilute methylene chloride solution. Diffraction data were collected on an Enraf-Nonius CAD4 diffractometer with graphite-monochromated MoK_α radiation at 20 °C. All data were collected with the $\omega/2\theta$ scan mode. The structures of these compounds were solved by direct methods (SHELXS-97). Details of crystal structure and refinement data are given in Supplementary Data. Non-hydrogen atoms were refined by full-matrix least-squares methods (SHELXL-97).[21] The final R values were 0.071, 0.035 and 0.030 for observed reflections in the cases of **12**, **13** and **18**, respectively.

In vitro cytotoxicity against cancer cell lines

Twelve cancer cell lines namely HL-60, HCT 116, HT 15, A 549, NCI-H23, SK-BR3, MCF 7, MDA-MB 231, SKOV-3, SKMEL-2, XF-498 and L1210 were used. All the cancer cell lines were obtained from Korean Cell Line Bank (KCLB). These cell lines were grown in RPMI-1640 (GIBCO) or DMEM medium supplemented with 10% heat-inactivated fetal calf serum, penicillin (100 units/mL), and streptomycin (100 μg/mL) (RPMI-FCS) in a highly humidified atmosphere having 5% CO_2 at 37 °C to maintain the cell number between 2 and 5×10^5/mL. Cells of each cell line plated in 96-well microtiter plates, and the standard and growth curves of which were obtained by MTS assay. Each compound was dissolved in PBS (phosphate buffer saline) or ethanol or dimethylsulfoxide, adding PBS to be 2 mmol concentration, filtered with pore size 0.2 μm syringe filter for sterilization followed by a serial dilution in culture medium to predetermined concentrations. The final concentration of ethanol in cell culture medium was controlled to be below 0.1%. Each cell line of concentration 1×10^4/well were grown into 96 well plate and then Pt(IV) complexes of the dilute 10^{-4}–10^{-8} mol were added to each well. After cultivating cancer cell lines at 37 °C and 5% CO_2 for 72 h except L1210 cell line for 48 h. MTS reagent [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS, promega, USA] was added 20 μL/well, and an additional cultivation was performed at 37 °C and 5% CO_2 for 1 h. Then, absorption test using ELISA reader (Bio-Tek Instruments. Inc., Vermont, USA) was performed at 490 nm. For calibration, a blank test was performed on the same 96-well plate under the same conditions. The experiment was performed on more than three wells under the same conditions with different concentrations, and the same experiment was repeated over three times.

In vivo antitumor activity

L1210 leukemia cells, L1210/*cis*-DDP cisplatin-resistant leukemia cells and B16 melanoma cells were obtained from NCI (National Cancer Institute, USA) and maintained by serial passage in intraperitoneal cavities of DBA/2 or C58BL/6 mice. For in vivo activity against mouse leukemia L1210 and L1210/*cis*-DDP, 6 weeks aged BDF_1 male mice were inoculated ip with 1×10^5 cells/0.1 mL/mouse on day 0. For against mouse B16 melanoma, about 1 g of tumor was homogenized with a cold balanced salt solution (10:1 brei) and then BDF_1 male mice were inoculated ip with brei 0.5 mL/mouse. Test compounds were administrated ip on day 1, 5, 9 after the tumor implantation. Each drug-treated group for each dose level consisted of eight mice. The *in vivo* antitumor activity was evaluated by comparing the mean survival time of treated groups (T) with that of control groups (C) and was expressed by percentage value of T/C (% T/C). This antitumor activity test was evaluated in pharmaceutical screening division of Korea Research Institute of Chemical Technology.

Conclusion

The octahedral Pt(IV) complexes with axial ligands were synthesized for antitumor agents. The series of (1,4-butanediamine)Pt(IV) complexes of the type *trans*, *cis*-[PtA_2Cl_2(1,4-butanediamine)] and *trans*-[PtA_2(malonate)(1,4-butanediamine)] (A = acetato or trifluoroacetato) were synthesized and the molecular structures characterized. The compound **12** and **13** showed the exellent activity against breast and colon cancer cell lines, in vitro and compound **18** was found to highest activity against cisplatin-resistant cancer cells, L1210/*cis*-DDP, in vivo. Future studies will be continued following an advanced program.

Supplementary Data Available

Tables 1 and 2 giving details of the X-ray structure determination, including tables of crystal data, bond distances and angles, and positional and thermal parameters for compounds **12**, **13** and **18.** Compounds **12** (CCDC 199758), **13** (CCDC 199759) and **18** (CCDC 199760) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambrige Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk.

Acknowledgements

One of the authors (Y. J. Park) wishes to thank the Ministry of Science and Technology (Grant No. KISTEP;M1-0022-01-0002) and Sookmyung Women's University.

References and Notes

1. Rosenberg, B.; VanCamp, L.; Trosko, J. E.; Mansour, V. H. *Nature* **1969**, *222*, 385.

2. Einhorn, L. H.; Donohue, J. *Ann. Intern. Med.* **1977**, *87*, 293.
3. Ozols, R. F.; Young, R. C. *Semin. Oncol.* **1984**, *11*, 251.
4. Soloway, M. S. *J. Urol.* **1978**, *120*, 716.
5. Fiorentino, M. V.; Ghiotto, C. In *Platinum and Other Metal Coordination Compounds in Cancer Chemotherapy*; Nicolini, M., Ed.; Martinus Nijhoff: Boston, 1988; p 415.
6. Wagener, D. J.; Yap, S. H.; Wobbes, T.; Burghouts, J. T.; van Dam, F. E.; Hillen, H. F.; Hoogendoorn, G. J.; Scheerder, H.; van der Vegt, S. G. *Cancer Chemother. Pharmacol.* **1985**, *15*, 86.
7. Von Hoff, D. D.; Schilsky, R.; Reichert, C. M.; Reddick, R. L.; Rozencweig, M.; Young, R. C.; Muggia, F. M. *Cancer Treat. Rep.* **1979**, *63*, 1527.
8. Krakoff, I. H. *Cancer Treat. Rep.* **1979**, *63*, 1523.
9. Inoue, K.; Mukaiyama, T.; Mitsui, I.; Ogawa, M. *Cancer Chemother. Pharmacol.* **1985**, *15*, 208.
10. Foster, B. J.; Clagett-Carr, K.; Leyland-Jones, B.; Hoth, D. *Cancer Treat. Rev.* **1985**, *12* (Suppl. A), 43.
11. Canneta, R.; Rozencweig, M.; Carter, S. K. *Cancer Treat. Rev.* **1985**, *12*, 125.
12. Kikuchi, Y.; Iwano, I.; Miyauchi, M.; Kita, T.; Sugita, M.; Tenjin, Y.; Nagata, I. *Jpn. J. Cancer Res.* **1990**, *81*, 701.
13. Kadhim, R.; al-Hussany, A.; Ali, P. A.; Hancock, D. A.; el-Sharkawi, A. M. *Ann. N. Y. Acad. Sci.* **2000**, *904*, 263.
14. George, C. M.; Haraf, D. J.; Mauer, A. M.; Krauss, S. A.; Hoffman, P. C.; Rudin, C. M.; Szeto, L.; Vokes, E. E. *Invest. New Drugs* **2001**, *19*, 303.
15. Lee, Y. A.; Lee, S. S.; Kim, K. M.; Lee, C. O.; Sohn, Y. S. *J. Med. Chem.* **2000**, *43*, 1409.
16. Ali Khan, S. R.; Huang, S.; Shamsuddin, S.; Inutsuka, S.; Whitmire, K. H.; Siddik, Z. H.; Khokhar, A. R. *Bioorg. Med. Chem.* **2000**, *8*, 515.
17. Hagopian, G. S.; Mills, G. B.; Khokhar, A. R.; Bast, R. C., Jr.; Siddik, Z. H. *Clin. Cancer Res.* **1999**, *5*, 655.
18. Pendyala, L.; Kidani, Y.; Perez, R.; Wilkes, J.; Bernacki, R. J.; Creaven, P. J. *Cancer Lett.* **1995**, *97*, 177.
19. Kwon, Y. E.; Whang, K. J.; Kim, W. K. US Patent 6,340,770, 2002.
20. Kizu, R.; Nakanishi, T.; Yamamoto, S.; Hayakawa, K.; Matsuzawa, A.; Eriguchi, M.; Takeda, Y.; Akiyama, N.; Kidani, Y. *Anticancer Drugs* **1998**, *9*, 167.
21. Sheldrick, G. M. *SHELXS 97 and SHELXL 97*; University of Goettingen: Germany, 1997.