Synthesis and Antitumor Activities of Platinum Complexes of Unsymmetrical Alicyclic Diamines as Carrier Ligands

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Received June 30, 1989, from the *Exploratory Research Laboratories, Chugai Pharmaceutical Company, Ltd., 1-135 Komakado, Gotemba-shi, 412, Japan.* Accepted for publication November 1, 1989.

Abstract □ The synthesis and biological activities of the platinum complexes of 2-aminomethylaziridine, 2-aminomethylazetidine, 2-aminomethylpyrrolidine, and 2-aminomethylpiperidine as carrier ligands are described. The platinum complexes of 2-aminomethylazetidine and 2-aminomethylpyrrolidine are significantly effective against murine tumors. In particular, 2-aminomethylazetidine(1,1-cyclobutanedicarboxylato)platinum II (**13**) and 2-aminomethylpyrrolidine(1,1-cyclobutanedicarboxylato)platinum II (**16**) exhibited potent antitumor activity and were soluble in water, and their antitumor activities against Colon 26 carcinoma (sc-ip system) were superior to CBDCA and comparable to CDDP.

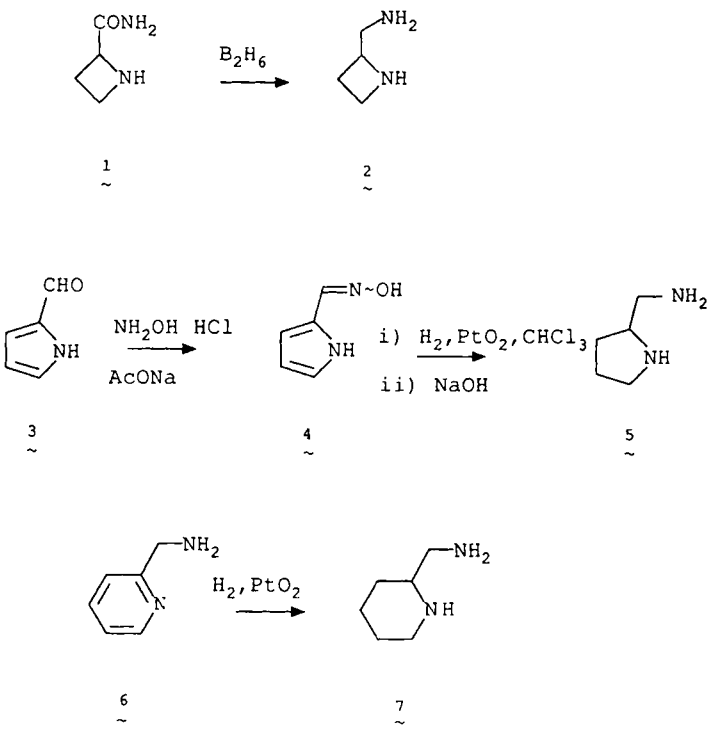
Since the discovery of the antitumor activity of *cis*-diamminedichloroplatinum II (CDDP),[1] many platinum complexes have been synthesized and tested for similar activity. The structure–activity relationships (SAR) between the amines as carrier ligands and the antitumor activity have been studied. It was reported that the general order of the antitumor activity of carrier ligands is $NH_3 \simeq RNH_2 > R_2NH > R_3N$, and the platinum complexes of aromatic heterocyclic amines like pyridine exhibit weak antitumor activity.[2] However, these SARs were discussed for the platinum complexes of symmetrical diamines as carrier ligands. Although some platinum complexes of unsymmetrical diamines as carrier ligands have been synthesized and examined for their anticancer activity,[2,3] the SAR of unsymmetrical diamine carrier ligands has not been previously investigated. Therefore, to determine how unsymmetrical diamines as carrier ligands affect antitumor activity, we synthesized various platinum complexes of 2-aminoalkylpyridine derivatives, which are unsymmetrical bidentate carrier ligands, and examined their SAR by tests with P388 leukemia (ip-ip system) in mice. Some of the platinum complexes of 2-aminomethylpyridine derivatives had potent antitumor activity.[4] This indicated that the above hypothesis about the SAR of the carrier ligand may not necessarily apply to the platinum complexes of the unsymmetrical bidentate diamines, leading us to examine these complexes further.

The effective platinum complexes of 2-aminomethylpyridine derivatives described above were tested for antitumor activity against Colon 26 carcinoma in an ip-ip system and a sc-ip system. They exhibited antitumor activity against Colon 26 carcinoma in an ip-ip system, but had less antitumor effect against it in a sc-ip system.[5] It was thought that one of the reasons why they had less antitumor effect in a systemic system was the low stability of the weak bond strength between platinum and pyridine. Kidani et al. reported that the platinum complexes containing amines with higher pK_a values, therefore having stronger bonding between the platinum and the amine, were more potent against tumors.[6]

Based on these results we synthesized the platinum complexes of unsymmetrical carrier ligands containing aliphatic heterocyclic amines (aziridine, pK_a: 7.88; azetidine, pK_a: 11.29; pyrrolidine, pK_a: 11.11; piperidine, pK_a: 11.20). These amines have higher pK_a values than pyridine (pK_a: 5.23), so they might be expected to improve the antitumor effectiveness of the 2-aminomethylpyridine platinum complexes.

Here we report the synthesis and antitumor activity of the platinum complexes which contain aliphatic heterocyclic amines substituted by aminomethyl at the 2-position. The dichloro-, oxalato-, and 1,1-cyclobutanedicarboxylate leaving groups were used because they were effective in our previous studies.[4] The synthesized platinum complexes were evaluated using two kinds of screening systems: P388 leukemia (ip-ip system) and Colon 26 carcinoma (sc-ip system).

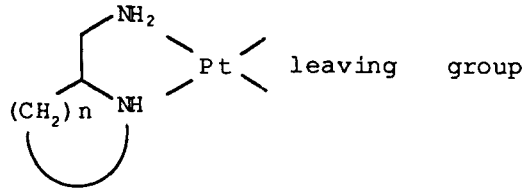
The diamines used as carrier ligands were synthesized by the following methods (Scheme I). 2-Aminomethylaziridine was prepared according to the method of Spivack and Valley.[7] 2-Aminomethylazetidine (**2**) was prepared by reduction of 2-azetidinecarboxamide (**1**)[8] with B_2H_6. 2-Aminomethylpyrrolidine (**5**) and 2-aminomethylpiperidine (**7**) were respectively prepared by catalytic hydrogenation of 2-pyrrolcarboxaldoxime (**4**)[9] obtained from 2-pyrrolcarboxaldehyde (**3**) and 2-aminomethylpyridine (**6**). The platinum complexes (Table I)



Scheme I

0022-3549/90/0800-0750$01.00/0

Table I—Synthesized Platinum Complexes



Compound	n	Leaving Group	Yield, %	mp, °C[a]	IR max (KBr), cm^{-1}	Formula[b]	Solubility in Water, mg/mL
8	1	Cl Cl	83[c]	270–273	3460, 3180, 3100	$C_3H_8Cl_2N_2Pt$	1.4
9	1	OCO OCO	31[d]	265–268	3140, 1690, 1670	$C_5H_8N_2O_4Pt$	2.8
10	1	OCO OCO	30[d]	272–276	3160, 1600	$C_9H_{14}N_2O_4Pt$	12.3
11	2	Cl Cl	69[c]	270–273	3450, 3235, 3170, 3110	$C_4H_{10}Cl_2N_2Pt$	1.0
12	2	OCO OCO	66[d]	265–270	3460, 3160, 3120, 1690, 1660, 1610	$C_6H_{10}N_2O_4Pt$	3.0
13	2	OCO OCO	35[d]	230–235	3170, 3100, 1640, 1600	$C_{10}H_{16}N_2O_4Pt$	25.0
14	3	Cl Cl	88[c]	252–255	3420, 3230, 3170	$C_5H_{12}Cl_2N_2Pt$	2.0
15	3	OCO OCO	44[d]	271–275	3120, 1690, 1665, 1635, 1600	$C_7H_{12}N_2O_4Pt$	5.0
16	3	OCO OCO	80[d]	245–250	3480, 3420, 3190, 3100, 1635, 1600, 1590	$C_{11}H_{18}N_2O_4Pt \cdot H_2O$	17.0
17[e]	4	Cl Cl	67[c]	300	3420, 3260, 3190, 3100,	$C_6H_{14}Cl_2N_2Pt$	0.25
18[e]	4	OCO OCO	61[d]	290–295	3480, 3440, 3240, 3060, 1705, 1655, 1625	$C_8H_{14}N_2O_4Pt \cdot H_2O$	1.7
19	4	OCO OCO	76[d]	285–290	3200, 3100, 1635, 1610,	$C_{12}H_2ON_2O_4Pt$	3.2

[a] With decomposition. [b] All compounds were analyzed for C, H, and N and were within ±0.4% of the calculated values. [c] Calculated with regard to the corresponding diamine. [d] Calculated with regard to the corresponding dichloroplatinum. [e] Reference 3a.

were synthesized according to standard methods.[10]

Result and Discussion

The compounds listed in Table I were tested against P388 leukemia (ip-ip system) and Colon 26 carcinoma (sc-ip system) in mice. The 2-aminomethylaziridine carrier ligand was shown to be less effective than the other ligands in the P388 leukemia screen (ip-ip system). In the study of the effect of modification of the leaving group on antitumor activity, the 1,1-cyclobutanedicarboxylato platinum complexes had the most potent antitumor effect of all except for the 2-aminomethylaziridine platinum complexes. In this screen, the most effective compounds were **13** and **19**; they achieved an average survival time of all the mice and maximal ILS (increase in life span) values of 108% (Table II).

In the Colon 26 carcinoma screen (sc-ip system), 2-aminomethylazetidine and 2-aminomethylpyrrolidine platinum complexes were the most effective, 2-aminomethylpiperidine was the next most effective, and 2-aminomethylaziridine had no antitumor effect (Table III). This order of the efficacy of the leaving groups was the same as the order found in the P388 leukemia screen (ip-ip system). In this screen, **13** and **16** exhibited potent antitumor activity and their GIR (growth inhibitory ratios) were 95%. Although **19** exhibited potent antitumor activity against P388 leukemia (ip-ip system), it failed to show significant antitumor activity in the Colon 26 carcinoma system even at a high dose (160 mg/kg).

In both the Colon 26 carcinoma and P388 leukemia screens, the MTD (maximum tolerated dose) of the complexes having an oxalato leaving group was lowest and that of the platinum complexes having a 1,1-cyclobutanedicarboxylato leaving group was highest among the three kinds of the leaving groups.

Lastly, the solubility of the platinum complexes in water was examined (Table I). Among the carrier ligands, 2-aminomethylpiperidine platinum complexes were the least soluble. The other complexes were about equally soluble. Among the leaving groups, the order of the solubility was

Table II—Antitumor Activity of Platinum Complexes on P388 Leukemia as Determined in an ip-ip System

Compound	Dose, mg/kg	ILS, %
8	20	28
	40	36
	80	40
9	5	12
	10	20
	20	N.E.[a] (3/5)[b]
10	20	9
	40	15
	80	18
11	10	64
	20	62 (1/5)[b]
	40	43 (3/5)[b]
		(1/5)[c]
12	5	76
	10	41 (3/5)[b]
		(1/5)[c]
	20	N.E.[a] (5/5)[b]
13	20	84
	40	108
	80	N.E.[a] (5/5)[b]
14	10	39
	20	43
	40	N.E.[a] (6/6)[b]
15	20	45
	40	N.E.[a] (5/6)[b]
	60	N.E.[a] (6/6)[b]
16	20	36
	40	53
	80	N.E.[a] (5/6)[b]
18	40	51
	80	N.E.[a] (6/6)[b]
	160	N.E.[a] (6/6)[b]
19	40	56
	80	75
	160	108
CDDP	4	54
	8	72
	12	110 (1/5)[c]
CBDCA	20	63
	40	85
	80	72 (2/6)[b]

[a] Not effective. [b] Number of mice of toxic death/number of mice used. [c] Number of mice that survived 30 days after receiving agent/number of mice used.

Table III—Antitumor Activity of Platinum Complexes on Colon 26 Carcinoma as Determined in a sc-ip System

Compound	Dose, mg/kg	GIR, %
8	20	13
	40	46
	80	73
9	5	12
	10	20
	20	died
10	20	14
	40	26
	80	36
11	10	68
	20	72
	40	died
12	5	47
	10	died
13	20	52
	40	96
	80	died
14	10	52
	30	89
	60	died
15	15	57
	30	74 (1/5)[a]
	60	died
16	20	36
	40	97
	80	98 (1/5)[a]
17	20	5
	40	39
	60	68 (1/5)[a]
18	40	49
	80	died
19	80	14
	160	68
CDDP	12	97
CBDCA	60	72

[a] Number exhibiting mice of toxic death/number of mice used.

1,1-cyclobutanedicarboxylato > oxalato > dichloro.

In the above tests, the platinum complexes of the aliphatic heterocyclic amine substituted with aminomethyl at the 2-position exhibited antitumor activity against Colon 26 carcinoma in an sc-ip system, while 2-aminomethylpyridine platinum complexes exhibited less antitumor activity.[5] We cannot conclude the correlation between the carrier ligand amine pK_a value and the antitumor activity in the systemic system because the pK_a values of the three amines among the four amines used in this report were similar. However, it was significant that the platinum complexes of 2-aminomethylaziridine, with the lowest pK_a value of the four kinds of carrier ligands tested, exhibited less antitumor activity than the platinum complexes of the other carrier ligands.

Consequently, **13**, **16**, and **19** were found to exhibit potent antitumor activity in both screening systems. In particular, **13** and **16** showed the most potent antitumor activity in a sc-ip system (against Colon 26 carcinoma), which was a systemic screening system. Their antitumor activities were comparable to CBDCA,[11] a second-generation platinum complex that did not exhibit the dose-limiting nephrotoxicity of CDDP.

We also examined the antitumor spectrum of **16** in mice and its nephrotoxicity in normal BDF_1 mice.[12] It exhibited potent antitumor activities against Colon 38 carcinoma, M5076 ovarian tumor, B16 melanoma, and Colon 26 carcinoma in a systemic screening system, and it had no effect on BUN (blood urine nitrogen) levels at a concentration close to the MTD (100 mg/kg).

Experimental Section

Melting points were taken on a Yanagimoto micro melting point apparatus and are uncorrected. Infrared spectra were recorded with a Hitachi 260-30 spectrophotometer. Proton NMR spectra were recorded on a Hitachi R-24B(60MHz) spectrometer, and $Me_4Si(CDCl_3)$ was used as internal standard. Chemical shifts are reported on the δ scale with peak multiplicities as follows: S, singlet; d, doublet; t, triplet; m, multiplet. Elemental analyses were performed by Analytical Research Laboratory of Chugai Pharmaceutical Company, Ltd. All analyses (C,H,N) were within ± 0.4% of the theoretical values. The TLC was done on silica gel 60 Merck F_{254} (0.25 mm).

2-Aminomethylazetidine (2)—A solution of 100 mL of 1 M borane in anhydrous tetrahydrofuran was added in a dropwise manner with stirring in a nitrogen atmosphere to a suspension of 2-azetidinecarboxamine (**1**; 2.7 g, 0.027 mol) in anhydrous tetrahydrofuran (50 mL). After completion of the addition, the mixture was refluxed for 77 h. The reaction mixture was permitted to cool in an ice bath and methanol (17 mL) was added to the mixture. After evaporation of the solvent under reduced pressure, diethyl ether (75 mL) and 2 M HCl (25 mL) was added to the residue, and the mixture was stirred for 1 h. The aqueous layer was basified with 40% aqueous NaOH under ice cooling and extracted with chloroform (100 mL × 4). The combined extract was dried over K_2CO_3. Evaporation of the solvent and distillation of the residue gave **2** (0.70 g, 30.1%) as a colorless oil: bp 53–55 °C (33–35 mmHg); ^1H NMR($CDCl_3$): δ 1.55(S, 3H, NH and

NH_2), 1.95–2.30 (m, 2H, $CHCH_2CH_2$), 2.7 (d, J = 6 Hz, 2H, CH_2NH_2), and 3.06–4.05 ppm (m, 3H, $CHNHCH_2$).

Anal.—Calc for $C_4H_{10}N_2$: C,H,N.

2-Aminomethylpyrrolidine (5)—To a solution of hydroxylamine hydrochloride (36.2 g, 0.521 mol) and sodium acetate (51.8 g, 0.631 mol) in H_2O (150 mL) was added a solution of 2-pyrrolcarboxaldehyde (50.0 g, 0.526 mol) dissolved in ethanol (25 mL) and H_2O (100 mL). After stirring at room temperature for 1 h, the resulting white precipitate was collected by filtration and washed with water to give 2-pyrrolcarboxaldoxime[8] (4; 53.0 g) as crude product. The above crude 4 (43.0 g) dissolved in ethanol (6.4 L) and chloroform (194 mL) was hydrogenated using PtO_2 (10.0 g) at room temperature for 57 h. After filtration of the reaction mixture, the filtrate was evaporated under reduced pressure. The residue was dissolved in water (25 mL) and basified with NaOH (35.0 g, 0.875 mol). The aqueous layer was extracted with chloroform, and the extract was dried over $MgSO_4$. Evaporation of the solvent and distillation of the residue gave 5 (19.5 g, 46.6%) as a colorless oil: bp 74–80 °C (27–30 mmHg), lit.[13] 65 °C (11 mmHg), 50 °C (7 mmHg); ^1H NMR($CDCl_3$): δ 1.0–2.1 (m, 7H, $NHCHCH_2CH_2CH_2$ and NH_2), and 2.3–3.3 ppm (m, 5H, $NH_2CH_2CHNHCH_2$).

2-Aminomethylpiperidine (7)—A solution of 2-aminomethylpyridine (2.16 g, 0.020 mol) in ethanol (25 mL) containing 10% HCl and H_2O (7 mL) was hydrogenated using PtO_2 (0.15 g) at room temperature for 2.5 h. After filtration of the reaction mixture, the filtrate was basified with 5 M NaOH. The solution was extracted with chloroform, and the extract was dried over $MgSO_4$. Evaporation of the solvent and distillation of the residue gave 7 (0.64 g, 28.1%) as a colorless oil: bp 33–37 °C (7 mmHg), lit.[14] 80–81 °C (18 mmHg); ^1H NMR($CDCl_3$): δ 1.30 (S, 3H, NH and NH_2), 0.80–1.90 (m, 6H, $NHCHCH_2CH_2CH_2$), and 2.25–3.25 ppm (m, 5H, $NH_2CH_2CHNHCH_2$).

(2-Aminomethylpyrrolidine)dichloroplatinum II (14)—To a solution of K_2PtCl_4 (28.1 g, 0.068 mol) in water (600 mL) was added a solution of 5 (6.8 g, 0.068 mol) in water (120 mL), and the reaction mixture was stirred at room temperature for 24 h. The resulting precipitate was collected by filtration, washed with water, and dried under reduced pressure to give 14 (21.9 g, 88.2%) as a pale yellow crystal.

This method was used to prepare 8, 11, and 17 (Table I) from their corresponding amines.

2-Aminomethylpyrrolidine(1,1-cyclobutanedicarboxylato) platinum II (16)—Silver nitrate (3.6 g, 0.0212 mol) was added to a suspension of 14 (4.0 g, 11.0 mmol) in water (1.5 L), and the reaction mixture was stirred at room temperature under light shielding for 3 days. The resulting white precipitate of silver chloride was removed by filtration using a millipore filter (0.22 m). The filtrate, which contained the unreacted silver nitrate, was treated with an aqueous solution of sodium chloride. The resulting silver chloride was removed by filtration, and the filtrate was evaporated below 40 °C under reduced pressure to 80 mL. To the above solution was added disodium 1,1-cyclobutanedicarboxylate (1.8 g, 8.5 mmol), and the reaction mixture was stirred at room temperature for 24 h and evaporated below 40 °C to 4 mL. The above solution was stirred at room temperature for 2 h. The resulting precipitate was collected by filtration and recrystallized from H_2O to give 16 (3.80 g, 79.5%) as a colorless crystal.

This method was used to prepare 10, 13, and 19 from 8, 11, and 17, respectively. Similarly, 9, 12, 15, and 18 were prepared from their corresponding dichloroplatinum II compounds (8, 11, 14, and 17) and sodium oxalate.

Biological Evaluation—*P388 Leukemia (ip-ip System)*—The P388 leukemia was maintained by continuous passage in syngeneic mice. This tumor was inoculated (10^6 cells/mouse) intraperitoneally in male CDF_1 (6–7 weeks of age) mice on day 0. On the next day of the inoculation, mice were randomly divided into groups of 5–6 members each, and the drugs were intraperitoneally administered once on day 1. Drugs were prepared in a vehicle of 0.25 mL of 0.2% Tween 80 in saline. Tumor-bearing mice, administered only 0.25 mL of 0.2% Tween 80 in saline, were used as a control group. Drug efficacy was expressed as increase in life span (ILS) by the following equation:

$$\text{ILS}(\%) = \left(\frac{\text{mean survival time of treated group}}{\text{mean survival time of control group}} - 1\right) \times 100 \quad (1)$$

The criteria for significant therapeutic response, according to the NCI, is >25% ILS.

Colon 26 Carcinoma (sc-ip System)—Colon 26 carcinoma was maintained by continuous passage in syngeneic mice. This tumor was transplanted subcutaneously in 2-mm^3 fragments by trocar into male CDF_1 (6–7 weeks of age) mice on day 0. Four days after the implantation, mice with comparable size tumors were divided into groups of 5–6 members each, and the drugs were intraperitoneally administered once on day 4. Drugs were prepared in a vehicle of 0.25 mL of 0.2% Tween 80 in saline. Tumor-bearing mice, administered 0.25 mL only of 0.2% Tween 80 in saline, were used as a control group. The tumors were removed and weighed on day 14 after the tumor inoculation. Drug efficacy was expressed as growth inhibitory ratios (GIR) by the following:

$$\text{GIR}(\%) = \left(1 - \frac{\text{mean tumor weight of treated group}}{\text{mean tumor weight of control group}}\right) \times 100 \quad (2)$$

The criteria for significant therapeutic response, according to the NCI, is >50% GIR.

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Acknowledgments

We thank Mrs. Mika Komori for preparation of the manuscript.