# Synthesis of Platinum Complexes of 2-Aminomethylpyrrolidine Derivatives for Use as Carrier Ligands and Their Antitumor Activities

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**In order to study a new antitumor platinum complex, various platinum complexes were prepared from 2-aminomethylpyrrolidine derivatives synthesized to serve as carrier ligands and tested for their antitumor activity against Colon 26 carcinoma (s.c.–i.p. system) and P388 leukemia (i.p.–i.p. system) in mice.**

**2-Aminomethylpyrrolidine proved to be the most effective carrier ligand in its amine derivatives. The structure–activity relationships of the carrier ligands in the platinum complexes with dichloro, oxalato, 1,1-cyclobutanedicarboxylato and dichlorodihydroxo as leaving group were clearly shown on the Colon 26 carcinoma screen and were as follows: the antitumor activity of the platinum complexes with any leaving groups was considerably decreased by the substitution of hydrogen by alkyl group (Me, Et) on nitrogen of aminomethyl and the effects of 1,1-cyclobutanedicarboxylato Pt(II) complexes completely disappeared with the same substitution on nitrogen of pyrrolidine. In all the tested platinum complexes 2-aminomethylpyrrolidine(1,1-cyclobutanedicarboxylato)platinum(II) (15) exhibited the most potent antitumor activity. 15 was superior to 1,1-cyclobutanedicarboxylatodiammineplatinum(II) (CBDCA) and similar to *cis*-diamminedichloroplatinum(II) (CDDP) on the Colon 26 carcinoma screen but it was inferior to CBDCA and CDDP on the P388 leukemia screen.**

**Furthermore, 15 showed more potent antitumor activity than CBDCA against Colon 38 carcinoma (s.c.–i.p. system).**

**Keywords** platinum complex; 2-aminomethylpyrrolidine; antitumor activity; Colon 26 carcinoma; P388 leukemia; structure–activity relationship

In the course of our studies on the development of antitumor agents, various platinum complexes of unsymmetrical bidentate diamines, such as 2-aminomethyl-*N*-heterocycles, were prepared as carrier ligands and screened for antitumor activity against P388 leukemia and Colon 26 carcinoma in mice.[1] Among them, 2-aminomethylpyrrolidine(1,1-cyclobutanedicarboxylato)platinum(II) (**15**) proved to be the most promising candidate for development as an antitumor agent.

The relationships between structure and antitumor activities are of great interest, because the substituents on 2-aminomethylpyrrolidine are thought to play an important role in antitumor activities.

In this paper, we describe the synthesis of platinum complexes of 2-aminomethylpyrrolidine derivatives as carrier ligands and evaluate their antitumor activities.

**Chemistry** The diamines used as carrier ligands were prepared by standard synthesis techniques (Chart 1). 2-Alkylaminomethylpyrrolidines (**2a—c**) were prepared by catalytic hydrogenation of 2-alkylaminomethylpyrrole (**1a—c**).[2] The diamine (**5**) was also prepared by catalytic hydrogenation of 2-acetylpyrroloxime (**4**), obtained by treating 2-acetylpyrrol (**3**) with hydroxylamine, by Putokhin's method.[3] 2-Aminomethyl-1-methylpyrrolidine

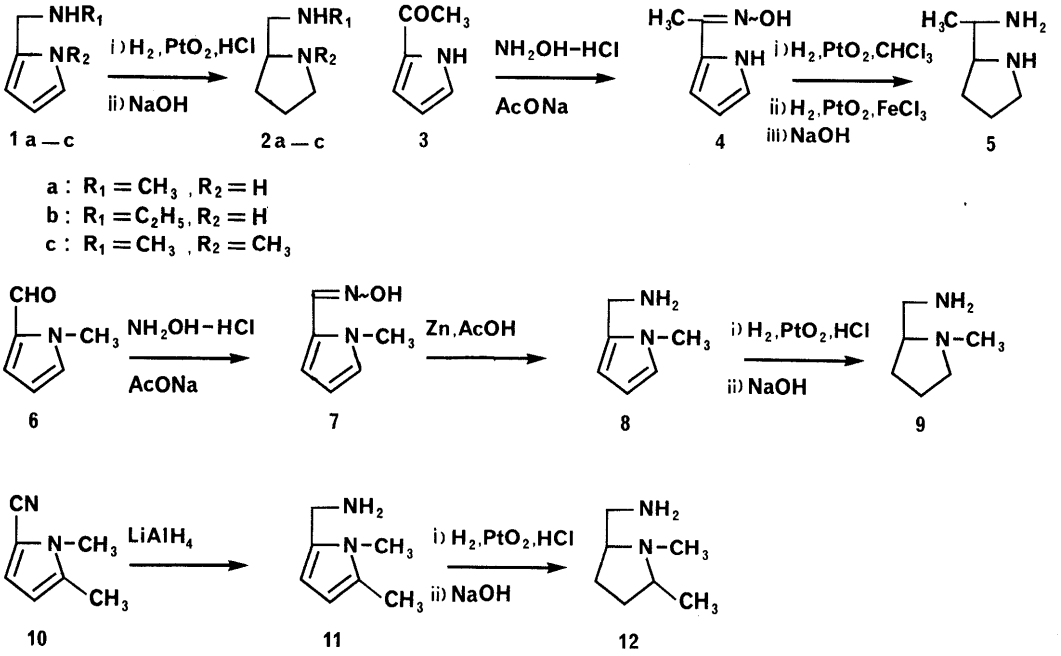
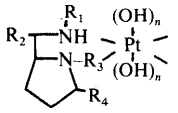



Chart 1

TABLE I. Synthesized Platinum Complexes of 2-Aminomethylpyrrolidine Derivatives




| Compd. No. | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $n$ | Leaving group | Yield (%) | mp (°C, dec.) | IR $\nu_{max}^{KBr}$ cm$^{-1}$ | Formula | Analysis (%) Calcd (Found) C | H | N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **13** | H | H | H | H | 0 | Cl, Cl | | | Ref. 1*c* | | | | |
| **14** | H | H | H | H | 1 | Cl, Cl | 38[b] | 225—230 | 3550, 3500, 540 | $C_5H_{14}Cl_2N_2O_2Pt$ | 15.00 (15.19) | 3.53 3.37 | 7.00 6.70) |
| **15** | H | H | H | H | 0 | OCO, OCO | | | Ref. 1*c* | | | | |
| **16** | H | H | H | H | 0 | OCO, OCO | | | | | | | |
| **17** | H | H | H | H | 0 | Br, Br | 78[b] | 285—289 | 3220, 3170 | $C_5H_{12}Br_2N_2Pt$ | 13.19 (13.45) | 2.66 2.81 | 6.15 5.89) |
| **18** | H | H | H | H | 1 | Br, Br | 22[c] | 217—222 | 3550, 3480, 3090, 535 | $C_5H_{14}Br_2N_2O_2Pt$ | 12.27 (12.55) | 2.88 2.94 | 5.73 5.46) |
| **19** | $CH_3$ | H | H | H | 0 | Cl, Cl | 24[a] | 243—247 | 3150 | $C_6H_{14}Cl_2N_2Pt$ | 18.95 (18.91) | 3.71 3.58 | 7.37 7.30) |
| **20** | $CH_3$ | H | H | H | 1 | Cl, Cl | 62[b] | 200—205 | 3560, 3450, 540 | $C_6H_{16}Cl_2N_2O_2Pt$ | 17.39 (17.26) | 3.89 3.87 | 6.76 6.47) |
| **21** | $CH_3$ | H | H | H | 0 | OCO, OCO | 23[b] | 272—277 | 3140, 1660, 1610 | $C_{12}H_{20}N_2O_4Pt$ | 31.92 (31.64) | 4.46 4.65 | 6.20 6.08) |
| **22** | $CH_3$ | H | H | H | 0 | OCO, OCO | 59[b] | 277—281 | 3120, 1691, 1662, 1638 | $C_8H_{14}N_2O_4Pt$ | 24.17 (24.01) | 3.55 3.61 | 7.05 6.93) |
| **23** | $C_2H_5$ | H | H | H | 0 | Cl, Cl | 48[a] | 285—290 | 3130 | $C_7H_{16}Cl_2N_2Pt$ | 21.32 (21.16) | 4.09 4.01 | 7.10 7.23) |
| **24** | $C_2H_5$ | H | H | H | 0 | OCO, OCO | 20[b] | 272—277 | 3140, 1660, 1610 | $C_{13}H_{22}N_2O_4Pt$ | 33.54 (33.34) | 4.76 4.81 | 6.02 5.91) |
| **25** | $C_2H_5$ | H | H | H | 0 | OCO, OCO | 55[b] | 265—270 | 3120, 1690, 1660 | $C_9H_{16}N_2O_4Pt$ | 26.27 (26.03) | 3.92 4.11 | 6.81 6.74) |
| **26** | $CH_3$ | H | $CH_3$ | H | 0 | Cl, Cl | 15[a] | 245—249 | 3160 | $C_7H_{16}Cl_2N_2Pt$ | 21.32 (21.15) | 4.09 4.03 | 7.10 7.25) |
| **27** | $CH_3$ | H | $CH_3$ | H | 0 | OCO, OCO | 38[b] | 260—263 | 3110, 1655, 1625 | $C_{13}H_{22}N_2O_4Pt$ | 33.54 (33.38) | 4.76 4.89 | 6.02 5.92) |
| **28** | $CH_3$ | H | $CH_3$ | H | 0 | OCO, OCO | 64[b] | 263—268 | 3140, 1690, 1670, 1645 | $C_9H_{16}N_2O_4Pt$ | 26.27 (25.99) | 3.92 4.10 | 6.81 6.70) |
| **29** | H | $CH_3$ | H | H | 0 | Cl, Cl | 73[a] | 247—252 | 3430, 3210, 3150 | $C_6H_{14}Cl_2N_2Pt$ | 18.95 (18.88) | 3.71 3.69 | 7.37 7.21) |
| **30** | H | $CH_3$ | H | H | 1 | Cl, Cl | 31[b] | 300 | 3520, 3460, 545 | $C_6H_{16}Cl_2N_2O_2Pt$ | 17.39 (17.27) | 3.89 3.78 | 6.76 6.68) |
| **31** | H | $CH_3$ | H | H | 0 | OCO, OCO | 58[b] | 253—257 | 3140, 3036, 1630, 1600 | $C_{12}H_{20}N_2O_4Pt$ | 31.92 (31.65) | 4.46 4.68 | 6.20 6.19) |
| **32** | H | $CH_3$ | H | H | 0 | OCO, OCO | 48[b] | 265—270 | 3120, 1690, 1660, 1630, 1605 | $C_8H_{14}N_2O_4Pt$ | 24.17 (23.89) | 3.55 3.78 | 7.05 7.04) |
| **33** | H | H | $CH_3$ | H | 0 | Cl, Cl | 85[a] | 212—216 | 3180, 3110 | $C_6H_{14}Cl_2N_2Pt$ | 18.95 (18.92) | 3.71 3.64 | 7.37 7.35) |
| **34** | H | H | $CH_3$ | H | 1 | Cl, Cl | 67[b] | 195—197 | 3540, 3080, 540 | $C_6H_{16}Cl_2N_2O_2Pt$ | 17.39 (17.10) | 3.89 3.81 | 6.76 6.59) |
| **35** | H | H | $CH_3$ | H | 0 | OCO, OCO | 58[b] | 234—239 | 3150, 3070, 1630, 1595 | $C_{12}H_{20}N_2O_4Pt$ 1/2 $H_2O$ | 31.31 (31.07) | 4.60 4.55 | 6.08 5.89) |
| **36** | H | H | $C_2H_5$ | H | 0 | Cl, Cl | 88[a] | 250—255 | 3300, 3280, 3120 | $C_7H_{16}Cl_2N_2Pt$ | 21.32 (21.26) | 4.09 4.04 | 7.10 6.68) |
| **37** | H | H | $C_2H_5$ | H | 1 | Cl, Cl | 75[b] | 183—185 | 3590, 3490, 530 | $C_7H_{18}Cl_2N_2O_2Pt$ | 19.63 (19.70) | 4.24 4.16 | 6.54 6.51) |
| **38** | H | H | $C_2H_5$ | H | 0 | OCO, OCO | 28[b] | 269—274 | 3150, 3080, 1620 | $C_{13}H_{22}N_2O_4Pt$ | 33.54 (33.38) | 4.76 4.62 | 6.02 5.94) |
| **39** | H | H | $C_2H_5$ | H | 0 | OCO, OCO | 22[b] | 264—269 | 3155, 3080, 1695, 1670, 1650 | $C_9H_{16}N_2O_4Pt$ | 26.27 (25.99) | 3.92 3.62 | 6.81) 6.53) |
| **40** | H | H | $CH_3$ | $CH_3$ | 0 | Cl, Cl | 78[a] | 245—249 | 3240, 3190, 3110 | $C_7H_{16}Cl_2N_2Pt$ | 21.32 (21.06) | 4.09 4.00 | 7.10 6.85) |

TABLE I. (continued)

| Compd. No. | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $n$ | Leaving group | Yield (%) | mp (°C, dec.) | IR $\nu_{max}^{KBr}$ $cm^{-1}$ | Formula | Analysis (%) Calcd (Found) C | H | N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **41** | H | H | $CH_3$ | $CH_3$ | 1 | Cl, Cl | 11[b] | 199—201 | 3550, 3510, 535 | $C_7H_{18}Cl_2N_2O_2Pt$ | 19.63 (19.60) | 4.24 4.17 | 6.54 6.25) |
| **42** | H | H | $CH_3$ | $CH_3$ | 0 | OCO, OCO | 66[b] | 280—285 | 3250, 3220, 3140, 1700, 1680, 1660, 1615 | $C_9H_{16}N_2O_4Pt$ | 26.27 (26.16) | 3.92 3.90 | 6.81 6.78) |

*a*) Calculated with regard to the corresponding diamine. *b*) Calculated with regard to the corresponding dichloroplatinum(II) complex. *c*) Calculated with regard to **17**.

(**9**) was prepared by the following method. The aldoxime (**7**) obtained by the reaction of the aldehyde (**6**) and hydroxylamine was reduced with Zn–AcOH, and then catalytic hydrogenation to give **9**. 2-Aminomethyl-1,5-dimethylpyrrolidine (**12**) was prepared by reducing the nitrile (**10**) with $LiAlH_4$, followed by catalytic hydrogenation. All the carrier ligand diamines were racemic and the diamines (**5** and **12**) were used as carrier ligands without the separation of the stereoisomers.

The platinum complexes listed in Table I were prepared from the corresponding diamines (**2a—c**, **5**, **9** and **12**) by standard methods.[4]

As leaving groups, dichloro, oxalato, 1,1-cyclobutanedicarboxylato, and dichlorodihydroxo were used because in previous papers[1,5] they were shown to be effective. In the platinum complex of 2-aminomethylpyrrolidine, the dibromo derivatives were also synthesized to see whether bromo was effective in the platinum complex of this species of carrier ligand.

We could not synthesize the platinum complexes of dichlorodihydroxo of **2b** and **2c**, or oxalato of **9**, or 1,1-cyclobutanedicarboxylato of **12** although we attempted to do so.

## Results and Discussion

The compounds listed in Table I were tested against Colon 26 carcinoma (s.c.–i.p. system) in mice and the effect of their carrier ligands was examined. Among the dichloro Pt complexes, **13** exhibited the most potent antitumor activity. The next most potent were **29**, **33**, **36** and **40**. Compounds **19** and **26** had less antitumor activity, and **23** was not evaluated because all of the mice given it died. The substitution of hydrogen by the alkyl groups (Me, Et) in 2-aminomethylpyrrolidine decreased the antitumor activity of the mother compound **13**, and particularly the substituents on $R_1$ (see Table I) remarkably decreased the antitumor activity.

It is said that the antitumor platinum complexes exert their cytotoxic effects by direct interaction with cellular deoxyribonucleic acid (DNA), and that the DNA binding involves ligand (leaving group) displacement.[6]

Apparently the Pt complexes of these substituted carrier ligands probably increase the steric hindrance and make interaction with DNA difficult. In particular, it seems that the substitution of hydrogen by the alkyl groups (Me, Et) on $R_1$ (see Table I), which is near the Pt, greatly increases the steric hindrance and $R_1$ substituents play an important part in the interaction with DNA. But we do not understand why the Pt complexes (**33** and **36**) of the carrier ligands substituted for the alkyl groups (Me, Et) on $R_3$ (Table I), which like $R_1$ were near the Pt, exhibited potent antitumor activity. In the dichlorodihydroxo Pt(IV) complexes, the substitution of the carrier ligand in 2-aminomethylpyrrolidine influenced antitumor activity almost as much as the dichloro Pt(II) complexes did. Namely, compound **14** of the carrier ligands ($R_1=R_2=R_3=H$ in Table I) exhibited the most potent antitumor activity, and **30**, **34** and **37** of the carrier ligands ($R_1=R_3=H$, $R_2=Me$: **30**, $R_1=R_2=H$, $R_3=Me$: **34** and $R_1=R_2=H$, $R_3=Et$: **37** in Table I) exhibited the next most potent antitumor activity.

TABLE II. Antitumor Activity of Platinum Complexes against Colon 26 Carcinoma (s.c.–i.p. System)

| Compd. | Dose (mg/kg) | GIR (%) | Compd. | Dose (mg/kg) | GIR (%) |
|---|---|---|---|---|---|
| **13** | 10 | 52 | **29** | 10 | 45 |
| | 30 | 89 | | 20 | 64 (1/5)[a] |
| | 60 | Died[b] | | 30 | Died[b] |
| **14** | 40 | 75 | **30** | 20 | 7 |
| | 80 | 76 | | 40 | 48 |
| | 160 | 91 | | 80 | 74 |
| **15** | 40 | 97 | **31** | 40 | 54 |
| | 60 | 98 | | 80 | 72 |
| | 80 | 98 (1/5)[a] | **32** | 15 | 63 (1/5)[a] |
| **16** | 15 | 57 | | 30 | 80 (3/5)[a] |
| | 30 | 74 (1/5)[a] | **33** | 40 | 76 |
| | 60 | Died[b] | | 80 | Died[b] |
| **17** | 40 | 10 | **34** | 40 | 44 |
| | 80 | 46 (4/5)[a] | | 80 | 67 |
| **18** | 20 | 24 | **35** | 20 | −5 |
| | 40 | 33 | | 40 | 11 |
| | 80 | 70 | | 80 | 1 |
| **19** | 40 | −9 | **36** | 40 | 71 |
| | 80 | 49 | | 80 | 78 (3/5)[a] |
| **20** | 20 | 10 | | 160 | Died[b] |
| | 40 | 37 | **37** | 30 | −6 |
| | 80 | 59 | | 60 | 76 |
| **21** | 20 | −3 | | 80 | 88 (2/5)[a] |
| | 40 | −6 | **38** | 80 | −7 |
| | 80 | 8 | | 160 | −1 |
| **23** | 40 | Died[b] | **39** | 80 | 28 |
| | 80 | Died[b] | | 160 | 60 |
| **24** | 20 | 5 | **40** | 20 | 19 |
| | 40 | −16 | | 40 | 25 |
| | 80 | −10 | | 80 | 74 |
| **26** | 40 | −7 | **41** | 20 | 6 |
| | 80 | 27 | | 40 | 39 |
| **27** | 20 | −14 | | 80 | 67 (5/6)[a] |
| | 40 | 1 | CDDP | 12 | 97 |
| | 80 | 0 | CBDCA | 60 | 72 |

*a*) Number of mice having a toxic death/number of mice used. *b*) All mice administered the drug died.

Compound **20** of the carrier ligand ($R_1$=Me, $R_2$=$R_3$=H in Table I) had less antitumor activity than the above dichlorodihydroxo Pt(IV) complexes. These results do not contradict the reports[7] that the dichlorodihydroxo Pt(IV) complexes can serve as prodrugs for Pt(II) complexes and that they were reduced to the dichloro Pt(II) complexes by ubiquitous biological reducing agents like ascorbic acid or cysteine and then interacted with DNA.

In the 1,1-cyclobutanedicarboxylato Pt(II) complexes, **15** of the carrier ligands ($R_1$=$R_2$=$R_3$=H in Table I) showed the most potent antitumor activity. Compound **31** of the carrier ligand ($R_1$=$R_3$=H, $R_2$=Me in Table I) had the next most potent antitumor activity, and **21**, **24** and **27** of the carrier ligand substituted by the alkyl groups (Me, Et) on $R_1$ (Table I) did not exhibit antitumor activity just like the results of the dichloro Pt complexes. However **35** and **38**, which have the effective carrier ligands ($R_1$=$R_2$=H, $R_3$=Me: **35** and $R_1$=$R_2$=H, $R_3$=Et: **38** in Table I) in the dichloro Pt complexes, contrary to our expectation did not have antitumor activity. It seems impossible that the quantity of the 1,1-cyclobutanedicarboxylato Pt(II) complexes taken into the cell was much smaller than the corresponding dichloro Pt complexes, since **15** had more potent antitumor activity than **13**. Thus it was inferred that the mechanism of the antitumor activity of the 1,1-cyclobutanedicarboxylato Pt(II) complexes is different from that of the dichloro Pt(II) complexes. Dichloro Pt(II) complexes, such as *cis*-$Pt(NH_3)_2Cl_2$,[5a] are said to bind to DNA after changing into the aqua complexes, such as *cis*-$Pt(NH_3)_2(H_2O)^+(OH)$, in the intracellular fluid.[8] If the 1,1-cyclobutanedicarboxylato Pt(II) complexes also react with DNA through the aqua path as the dichloro Pt(II) complexes do, **35** and **38** should have potent antitumor activity like **33** and **36**. These Pt complexes with 1,1-cyclobutanedicarboxylato probably can not change into the aqua Pt complex because it strongly binds to the Pt. Actually, the optically active substance (*R* form: **15***R*) of **15** is really stable in water and forms the adduct of two molar guanosine by displacement of the leaving group directly when **15***R* is mixed with two molar guanosine at room temperature in an aqueous solution.[9] It is thought that **15**, in exerting its potent antitumor activity, reacts with DNA by the *SN*2 alkylation mechanism, while the Pt complexes with the alkyl groups (Me, Et) on $R_1$ or $R_3$ (Table I) may produce the steric hindrance in the *SN*2 alkylation of DNA.

The oxalato Pt complexes, **16**, **32** and **39** were screened for antitumor activity. Compounds **16** and **32** of these carrier ligands ($R_1$=$R_2$=$R_3$=H: **16**, $R_1$=$R_3$=H, $R_2$=Me: **32** in Table I) had potent antitumor activity although some mice died at low doses. Compound **39** of the carrier ligand ($R_1$=$R_2$=H, $R_3$=Et in Table I) showed antitumor activity although it was weaker than **16** or **32**. The other oxalato Pt complexes were not screened because any mice given them were expected to die at low doses as those in previous experiments[1c] with the Pt complexes of 2-aminoalkyl heterocycles did. Compounds **17** and **18**, in which the leaving group was dibromo, showed less antitumor activity than the corresponding dichloro Pt complexes (**13** and **14**). These results showed that among the platinum complexes of 2-aminomethylpyrrolidine derivatives, compound **15** had the most potent antitumor activity against Colon 26 carcinoma. The GIR (growth inhibitory ratio) of **15** was 98%, and it was superior to 1,1-cyclobutanedicarboxylatodiammineplatinum (II) (CBDCA)[5c] and *cis*-diamminedichloroplatinum (II) (CDDP).[5a,10] In the screening against P388 leukemia (i.p.–i.p system), the compounds examined did not have much antitumor activity on the whole (Table III) and, the structure–activity relationships in the carrier ligands were not definite. However, it is significant that, just as in the screening against Colon 26 carcinoma (s.c.–i.p. system), the 1,1-cyclobutanedicarboxylato Pt(II) complexes (**35** and **38**) had no antitumor activity although the dichloro Pt(II) complexes (**33** and **36**), which have the same carrier ligand as **35** and **38**, had antitumor activity. In this screening against P388 leukemia, **15** showed the most antitumor activity and the ILS (increase in life span) was 53%.

The three compounds **14**, **15** and **37**, which showed potent antitumor activity against Colon 26 carcinoma (s.c.–i.p. system) were tested for antitumor activity against Colon

TABLE III. Antitumor Activity of Platinum Complexes against P388 Leukemia (i.p.–i.p. System)

| Compd. | Dose (mg/kg) | ILS (%) | Compd. | Dose (mg/kg) | ILS (%) |
|---|---|---|---|---|---|
| **13** | 10 | 39 | **33** | 10 | 28 |
| | 20 | 43 | | 20 | 42 |
| | 40 | N.E. (6/6)[a] | | 30 | 52 |
| **14** | 40 | 23 | **34** | 20 | 23 |
| | 80 | 23 | | 40 | 25 |
| | 160 | N.E. (4/7)[a] | | 80 | 34 |
| **15** | 20 | 36 | **35** | 40 | 8 |
| | 40 | 53 | | 80 | 10 |
| | 80 | N.E. (5/6)[a] | **36** | 20 | 36 |
| **16** | 20 | 45 | | 40 | 51 |
| | 40 | N.E. (5/6)[a] | | 60 | 50 |
| | 60 | N.E. (6/6)[a] | **37** | 20 | 25 |
| **20** | 40 | 16 | | 40 | 39 (1/6)[b] |
| | 80 | 33 | | 80 | N.E. (4/6)[a] |
| **21** | 40 | 14 | **38** | 40 | 2 |
| | 80 | 30 | | 80 | 3 |
| **24** | 40 | 14 | | 160 | 10 |
| | 80 | 20 | **39** | 40 | 27 |
| **27** | 40 | 5 | | 80 | 46 |
| | 80 | 9 | | 160 | 44 |
| **29** | 10 | 45 | **41** | 40 | 38 |
| | 20 | N.E. (6/6)[a] | | 80 | 7 (3/6)[a] |
| | 40 | N.E. (6/6)[a] | CDDP | 4 | 54 |
| **30** | 40 | 31 | | 8 | 72 |
| | 80 | 36 | | 12 | 110 (1/5)[b] |
| **31** | 20 | 18 | CBDCA | 20 | 63 |
| | 40 | 30 | | 40 | 85 |
| | 80 | 34 | | 80 | 72 (2/6)[a] |
| **32** | 15 | 46 | | | |
| | 30 | 45 (1/5)[a] | | | |
| | 40 | N.E. (4/5)[a] | | | |

*a*) Number of mice having a toxic death/number of mice used. *b*) Number of mice that survived 30 d after receiving agent/number of mice used. N.E.: Not effective.

TABLE IV. Antitumor Activity of Platinum Complexes against Colon 38 Carcinoma (s.c.–i.p. System)

| Compd. | Dose (mg/kg/d) | GIR (%) |
|---|---|---|
| **14** | 40 | 35 |
| **15** | 40 | 85 |
| **37** | 30 | 23 |
| CBDCA | 40 | 55 |

38 carcinoma (s.c.–i.p. system). Compound **15** showed potent antitumor activity and it was much stronger than CBDCA. Compounds **14** and **37** showed less antitumor activity (Table IV).

In the above screenings, 2-aminomethylpyrrolidine proved to be a better carrier ligand than its derivatives, and **15** was the best platinum complex for antitumor activity. Furthermore, we examined the antitumor spectrum of **15** in mice and the nephrotoxicity-inducing activity in normal $BDF_1$ mice, as indicated by changes in blood urea nitrogen (BUN) at almost the MTD (100 mg/kg).[11] On the whole it exhibited potent antitumor activities against M 5076 ovarian tumor, B16 melanoma and various other tumors in a systemic screening system, and significantly, it had no effect on BUN levels.

## Experimental

**Chemicals** All melting points were taken on a Yanagimoto micro melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Hitachi 260—30 spectrophotometer. Proton nuclear magnetic resonance ($^1$H-NMR) spectra were recorded on a Hitachi 24B (60 MHz) spectrometer with tetramethylsilane as an internal standard. Thin layer chromatography (TLC) was done on silica gel as Merck $F_{254}$ (0.25 mm).

**2-Methylaminomethylpyrrolidine (2a)** 2-Methylaminomethylpyrrole (**1a**) (8.0 g, 73 mmol) dissolved in 10% HCl–ethanol (100 ml) and $H_2O$ (20 ml) was hydrogenated using $PtO_2$ (1.0 g, 4.4 mmol) at room temperature for 5 h. After filtration of the reaction mixture, the filtrate was basified with 5 N NaOH. The generated oil was extracted with chloroform, and the extract was dried over $MgSO_4$. Evaporation of the solvent and distillation of the residue gave **2a** (5.08 g, 61%) as a colorless oil. bp 46 °C (4 mmHg). $^1$H-NMR ($CDCl_3$) $\delta$: 3.47—2.40 (5H, m), 2.42 (3H, s, $CH_3$), 2.03—1.14 (4H, m), 1.47 (2H, s, NH × 2). *Anal.* Calcd for $C_6H_{14}N_2$: C, 63.11; H, 12.36; N, 24.53. Found: C, 62.01; H, 12.33; N, 24.50. This method was used to prepare compounds **2b** (7.26 g, 70%) and **2c** (6.51 g, 63%) from **1b** (10 g, 81 mmol) and **1c** (10.0 g, 81 mmol) respectively.

**2-Ethylaminomethylpyrrolidine (2b)** Colorless oil. bp 51 °C (4 mmHg). $^1$H-NMR ($CDCl_3$) $\delta$: 2.45—2.37 (7H, m), 2.08—1.32 (4H, m), 1.58 (2H, s, NH × 2), 1.09 (3H, t, $J$=6.5 Hz, $CH_3$). *Anal.* Calcd for $C_7H_{16}N_2$: C, 65.57; H, 12.58; N, 21.85. Found: C, 65.50; H, 12.64; N, 21.60.

**1-Methyl-2-methylaminomethylpyrrolidine (2c)** Colorless oil. bp 32—34 °C (4 mmHg). $^1$H-NMR ($CDCl_3$) $\delta$: 3.20—2.75 (1H, m, CH), 2.68—1.90 (4H, m), 2.45 (3H, s, $CH_3$), 2.33 (3H, s, $CH_3$), 1.90—1.50 (4H, m) 1.12 (1H, br s, NH). *Anal.* Calcd for $C_7H_{16}N_2$: C, 65.57; H, 12.58; N, 21.85. Found: C, 65.51; H, 12.66; N, 21.59.

**2-(1-Aminoethyl)pyrrolidine (5)** To a solution of hydroxylaminehydrochloride (29.3 g, 422 mmol), sodium acetate (29.3 g, 375 mmol) in $H_2O$ (180 ml) and ethanol (90 ml) was added a solution of 2-acetylpyrrole (**3**) (25.0 g, 229 mmol) in ethanol (300 ml) with stirring. After stirring for 4 d at room temperature, the reaction mixture was evaporated under reduced pressure, and the residue was extracted with ether and dried over $Na_2SO_4$. Evaporation of the solvent gave 2-acetylpyrroloxime (**4**) (22.5 g) as a crude product. The crude **4** (12.0 g) dissolved in ethanol (5 l) and chloroform (48 ml) was hydrogenated using $PtO_2$ (2.0 g, 8.8 mmol) at room temperature for 2 d. In this time the spot of starting material (**4**) disappeared and two new spots ($Rf$=0.1, 0.2) appeared on TLC ($CHCl_3$ : MeOH, 4 : 1). After filtration of the reaction mixture the filtrate was evaporated under reduced pressure. To the above residue dissolved in ethanol (100 ml) was added a solution of ferric chloride (31.5 g, 194 mmol) in acetic acid (100 ml) and ethanol (100 ml), and it was hydrogenated using $PtO_2$ (1.8 g, 7.9 mmol) at room temperature for 1 d. After filtration of the reaction mixture, the filtrate was evaporated and distilled to give **5** (5.7 g, 52%) as a colorless oil. bp 42—45 °C (12—13 mmHg). $^1$H-NMR ($CDCl_3$) $\delta$: 3.05—2.50 (4H, m), 2.05—0.80 (7H, m), 1.45 (3H, s, NH and $NH_2$). *Anal.* Calcd for $C_6H_{14}N_2$: C, 63.11; H, 12.36; N, 24.53. Found: C, 63.00; H, 12.42; N, 24.46.

**2-Aminomethyl-1-methylpyrrolidine (9)**[12] 1-Methylpyrrole-2-carboxyaldehyde (**6**) (25.0 g, 229 mmol) dissolved in ethanol (11 ml) and $H_2O$ (44 ml) was added to a solution of hydroxylaminehydrochloride (15.8 g, 0.227 mol) and sodium acetate (18.5 g, 0.225 mol) in $H_2O$ (66 ml) with stirring. After stirring for 1 h at room temperature, the resulting white precipitate was collected by filtration, and washed with water to give 1-methylpyrrol-2-carboxaldoxime (**7**) (28.1 g) as a colorless powder. To a solution of compound **7** in acetic acid (390 ml) and $H_2O$ (110 ml) zinc powder (46.8 g, 0.716 mol) was added gradually with stirring under ice cooling. The resulting mixture was stirred for 1 h at 0 °C, then 5 N NaOH was added to basify. After the mixture was further stirred for 30 min, the aqueous layer was extracted with chloroform, and the extract was dried over $K_2CO_3$. Evaporation of the solvent gave 2-aminomethyl-1-methylpyrrole (**8**) (8.3 g) as a colorless oil, which was converted into **9** (3.6 g, 42%) as colorless oil in the same manner as described for **2a**. bp 52—55 °C (30 mmHg) lit.[12] 56 °C (18 mmHg). $^1$H-NMR ($CDCl_3$) $\delta$: 3.24—2.86 (1H, m), 2.71 (2H, d, $J$=4.8 Hz, $CH_2NH_2$), 2.30 (3H, s, $CH_3$, 2.50—1.05 (6H, m), 1.42 (2H, s, $NH_2$). *Anal.* Calcd for $C_6H_{16}N_2$: C, 63.11; H, 12.36; N, 24.53. Found: C, 62.88; H, 12.19; N, 24.48.

**2-Aminomethyl-1,5-dimethylpyrrolidine (12)** To a stirred suspension of lithium aluminum hydride (14.2 g, 374 mmol) in dry tetrahydrofuran (500 ml) was added dropwise a solution of 1,5-dimethyl-2-pyrrolecarbonitrile (**10**) (45.0 g, 375 mmol) in dry tetrahydrofuran (200 ml) under ice cooling. After completion of the addition, the mixture was refluxed for 1 h, permitted to cool in an ice bath, and then added dropwise to 28% aqueous $NH_3$ (40 ml). The resulting mixture was filtered and the filtrate was extracted with chloroform. The extract was dried over $K_2CO_3$ to a concentrated dryness. The obtained residue was distilled under reduced pressure to give 2-aminomethyl-1,5-dimethylpyrrole (**11**) (36.1 g, 78%) as a colorless oil. bp 84—86 °C (7 mmHg). $^1$H-NMR ($CDCl_3$) 5.75 (1H, d, $J$=4 Hz, aromatic H), 5.64 (1H, d, $J$=4 Hz, aromatic H), 3.66 (2H, s, $CH_2$), 3.39 (3H, s, $NCH_3$), 2.15 (3H, s, $CH_3$), 1.25 (2H, s, $NH_2$). *Anal.* Calcd for $C_7H_{12}N_2$: C, 67.70; H, 9.74; N, 22.56. Found: C, 67.60; H, 9.68; N, 22.39. Compound **11** (20.0 g, 161 mmol) was converted into **12** (10.6 g, 51%) as a colorless oil in the same manner as described for **2a**. bp 39—40 °C (7—8 mmHg). $^1$H-NMR ($CDCl_3$) $\delta$: 2.65 (2H, d, $J$=4.8 Hz, $CH_2NH_2$), 2.50—1.25 (6H, m), 2.20 (3H, s, $NCH_3$), 1.11 (2H, s, $NH_2$), 1.05 (3H, d, $J$=6 Hz, $CHCH_3$). *Anal.* Calcd for $C_7H_{16}N_2$: C, 65.57; H, 12.58; N, 21.85. Found: C, 65.41; H, 12.62; N, 21.79.

**[2-(1-Aminoethyl)pyrrolidine]dichloroplatinum(II) (29)** **5** (4.6 g, 0.04 mol) dissolved in $H_2O$ (600 ml) was added to a solution of $K_2PtCl_4$ (16.9 g, 0.04 mol) in $H_2O$ (30 ml), and the reaction mixture was stirred at room temperature for 16 h. The resulting precipitate was collected by filtration, washed with water, and then dried under vacuum to give **29** (11.2 g, 73%) as a pale yellow powder. This method was used to prepare compounds **19**, **23**, **26**, **33**, **36** and **40** from their corresponding amines.

**[2-(1-Aminoethyl)pyrrolidine]oxalatoplatinum(II) (32)** Silver nitrate (3.5 g, 20.6 mmol) was added to a suspension of **29** (4.0 g, 10.5 mmol) in $H_2O$ (1.5 l) and the reaction mixture was stirred at room temperature under light shielding for 3 d. The resulting white precipitate of silver chloride was removed by filtration using a millipore filter (0.22 $\mu$m). The filtrate, which contained the unreacted silver nitrate, was treated with 10% aqueous NaCl. The resulting silver chloride was removed by filtration, and the filtrate was evaporated below 40 °C under reduced pressure into 21 ml. To 4 ml of the above solution was added disodium oxalate (0.22 g, 1.64 mmol), and the reaction mixture was stirred at room temperature for 8 d. The resulting precipitate was collected by filtration and recrystallized from $H_2O$ to give **32** (0.38 g, 48%) as a colorless crystal. This method was used to prepare compound **22**, **25**, **28**, **39** and **42** from **19**, **23**, **26**, **36** and **40** respectively, and similarly **21**, **24**, **27**, **31**, **35** and **38** were prepared from their corresponding dichloroplatinum(II) derivatives (**19**, **23**, **26**, **29**, **33** and **36**) and disodium 1,1-cyclobutanedicarboxylate.

**(2-Aminomethylpyrrolidine)-*cis*-dichloro-*trans*-dihydroxoplatinum(IV) (14)** To the suspension of **13** (4.0 g, 2.73 mmol) in $H_2O$ (3 ml) was added 31% aqueous $H_2O_2$ (45 ml) with stirring under ice cooling. The reaction mixture was stirred at room temperature for 20 min and then at 80 °C for 1.5 h. After cooling in an ice bath, the resulting precipitate was collected by filtration, washed with cold $H_2O$ and dried under reduced pressure to give **14** (2.2 g, 50%) as a light brown powder. This method was used to prepare compounds **18**, **20**, **30**, **34**, **37** and **41** from **17**, **19**, **29**, **33**, **36** and **40**.

**(2-Aminomethylpyrrolidine)dibromoplatinum(II) (17)** Silver nitrate (1.8 g, 10.6 mmol) was added to a suspension of **13** (2.0 g, 5.5 mmol) in $H_2O$ (740 ml) and the reaction mixture was stirred at room temperature under light shielding for 4 d. The resulting white precipitate of silver chloride was removed by filtration using a millipore filter (0.22 $\mu$m). The filtrate, which contained the unreacted silver nitrate, was treated with 10% aqueous NaCl. The resulting silver chloride was removed by filtration and the filtrate was evaporated below 40 °C under reduced pressure to 39 ml. To 10 ml of the above solution was added potassium bromide (1.1 g, 9.2 mmol) and the reaction mixture was stirred at room temperature for 6 h. The resulting precipitate was collected by filtration, washed with $H_2O$, and dried under vacuum to give **17** (0.5 g, 78%) as a yellow powder. mp

286—289 °C (dec.). IR $cm^{-1}$: 3220, 3170 (NH). *Anal.* Calcd for $C_5H_{12}Br_2N_2Pt$: C, 13.19; H, 2.66; N, 6.15.; Found C, 13.48; H, 2.81; N, 5.89.

**Antitumor Activity** Colon 26 Carcinoma (s.c.–i.p. System): Colon 26 carcinoma was maintained by continuous passage in syngenic mice. This tumor was transplanted subcutaneously in 2 $mm^3$ fragments by trocar into male $CDF_1$ mice (6—7 weeks of age) on day 0. Four days after the implantation, mice with almost the same size tumor 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 of only 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 GIR by the following.

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

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

P388 Leukemia (i.p.–i.p. System): P388 leukemia was maintained by continuous passage in syngeneic mice. This tumor was inoculated ($10^6$cell/mouse) intraperitoneally in male $CDF_1$ mice (6—7 weeks of age) on day 0. On the next day after 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 ILS by the following equation.

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

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

Colon 38 Carcinoma (s.c.–i.p. System): Colon 38 carcinoma was maintained by continuous passage in syngenic mice. This tumor was transplanted subcutaneously in 2 $mm^3$ fragments by trocar into male $CDF_1$ mice on day 0. Four days after the implantation, mice with almost the same size tumor were divided into groups of 7 members each, and the drugs were intraperitoneally administered on days 4,6 and 8. Drugs were prepared in a vehicle of 0.25 ml of 0.2% Tween 80 in saline. Tumor-bearing mice administered 0.25 ml of only 0.2% Tween 80 in saline were used as a control group. The tumors were removed and weighed on day 14. Drug efficacy was expressed as GIR (%).

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