# Reduction and Anticancer Activity of Platinum(IV) Complexes

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A series of $Pt^{IV}$ anticancer complexes with chloro leaving groups have been investigated for the effects of axial and carrier ligands on the reduction and cytotoxicity. The reduction rates of the $Pt^{IV}$ complexes such as Pt(*d,l*)-(1,2-$(NH_2)_2C_6H_{10}$)$Cl_4$ (tetraplatin, Pt(dach)$Cl_4$; dach = diaminocyclohexane), *cis,trans,cis*-[Pt($(CH_3)_2CHNH_2)_2(OH)_2$-$Cl_2$] (iproplatin, Pt(ipa)$(OH)_2Cl_2$; ipa = isopropylamine), *cis,trans,cis*-[Pt$(NH_3)(C_6H_{11}NH_2)(OCOCH_3)_2Cl_2$] (JM-216, Pt(a,cha)$(OCOCH_3)_2Cl_2$; a = ammine, cha = cyclohexylamine), *cis,trans,cis*-[Pt$(NH_3)(C_6H_{11}NH_2)(OCOC_3H_7)_2Cl_2$] (JM-221, Pt(a,cha)$(OCOC_3H_7)_2Cl_2$), *cis,trans,cis*-[Pt(en)$(OH)_2Cl_2$], Pt(en)$Cl_4$ (en = ethylenediamine), *cis,trans,cis*-[Pt(en)$(OCOCH_3)_2Cl_2$], and *cis,trans,cis*-[Pt(en)$(OCOCF_3)_2Cl_2$] by ascorbate and cathodic reduction potentials strongly depend on the electron-withdrawing power and the steric hindrance of the axial and carrier ligands. Beginning with $Pt^{IV}$ complexes bearing en carrier ligands, reduction rates and reduction potentials increase in the following order of axial ligand substitutions: OH < $OCOCH_3$ < Cl < $OCOCF_3$, coinciding with increasing electron-withdrawing power of the axial ligand. $Pt^{IV}$ complexes with en carrier ligands tend to show slower reduction rates than the corresponding complexes with ipa or cha carrier ligands. Ascorbic acid does not reduce Pt(en)$(OH)_2Cl_2$, but reduces Pt(ipa)$(OH)_2Cl_2$. The reduction rate of Pt(a,cha)$(OCOCH_3)_2Cl_2$ is about 12 times higher than that of Pt(en)$(OCOCH_3)_2Cl_2$. Overall, there is no strong correlation between reduction rate and cytotoxicity toward cisplatin-sensitive L1210/0 cells among the eight complexes studied. However, when the four compounds with en carrier ligands were compared with one another, the one with the fastest reduction rate exhibited the highest cytotoxicity. The cytotoxicity increases with axial ligand substitution in the order OH < $OCOCH_3$ < Cl < $OCOCF_3$, following the same trend as reduction rate. Comparing complexes having different carrier ligands but the same axial ligands reveals that the compound with the faster reduction rate exhibits the higher cytotoxicity. Reduction rate and cytotoxicity increase in the order Pt(en)$(OH)_2Cl_2$ < Pt(ipa)$(OH)_2Cl_2$, Pt(en)$(OCOCH_3)_2Cl_2$ < Pt(a,cha)$(OCOCH_3)_2Cl_2$, Pt(en)$Cl_4$ < Pt(dach)$Cl_4$.

## Introduction

There is a growing interest in six-coordinate $Pt^{IV}$ complexes because of their anticancer activity, especially since these complexes are toxic to tumors which are resistant to cisplatin.[1−3] Depending on the axial and equatorial ligands, there is a wide range of anticancer activity.[3] The anticancer activity mechanism of these $Pt^{IV}$ complexes has not been studied in detail. However, it is generally believed that since $Pt^{IV}$ compounds are inert in ligand substitution reactions relative to their $Pt^{II}$ analogues,[4a] they must be reduced to $Pt^{II}$ species before binding to DNA. Numerous experimental results support that $Pt^{IV}$ drugs are reduced by both extracellular and intracellular reducing agents.[5] Moreover, $Pt^{IV}$ complexes do not bind to closed-circular DNA[5b,c] or 5′-GMP (5′-guanosine monophosphate).[5d] The reactivity of $Pt^{IV}$ complexes toward DNA is enhanced in the presence of reducing agent such as ascorbic acid,[5d] intracellular glutathione,[3e,6a] and a protein sulfhydryl.[5e] These results suggest that $Pt^{IV}$ metal

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centers are reduced by cellular components to form the $Pt^{II}$ analogues that bind to DNA.

On the other hand, there are other experimental results which cannot be entirely explained by simple $Pt^{IV}$ reduction to the active $Pt^{II}$ analogue prior to DNA binding. Some $Pt^{II}$ analogues do not show any of the activity or selectivity of their $Pt^{IV}$ counterparts.[3a] For a series of different $Pt^{IV}$ complexes that upon reduction putatively yield the same $Pt^{II}$ analogue, there is an 800-fold range in activity.[3d] Moreover, there are a few papers reporting that $Pt^{IV}$ can bind to DNA and RNA fragments without being reduced.[2]

These experimental results point to the importance of reduction mechanism in determining anticancer activity of $Pt^{IV}$ complexes. Depending on the nature of the ligands and reduction conditions, some $Pt^{IV}$ complexes may undergo different DNA binding modes. The possible importance of the reduction rate in determining the cytotoxicity of $Pt^{IV}$ complexes with hydroxo and chloro axial ligands has been recognized by Siddik's group.[3b−e] However, without quantitative reduction rates for the $Pt^{IV}$ complexes having different axial and equatorial ligands, it was not possible to make any correlation. So far only two quantitative reduction rates have been reported for $Pt^{IV}$ anticancer drugs, one for tetraplatin ($Pt(dach)Cl_4$)[6a] and the other for iproplatin ($Pt(ipa)(OH)_2Cl_2$)[11] (dach = diaminocyclohexane; ipa = isopropylamine). Therefore in this study we have obtained the rate of reduction and the reduction potential of a series of $Pt^{IV}$ complexes with varying axial and carrier ligands. We also have obtained the cytotoxicity toward cisplatin-sensitive L1210/0 cells and have examined if there is any correlation between the reduction and cytotoxicity.

## Experimental Section

**$Pt^{IV}$ Complexes.** The series of $Pt^{IV}$ complexes with variable axial and equatorial carrier ligands, but with the same chloride leaving groups, are shown in Figure 1. The $Pt(en)(OH)_2Cl_2$, $Pt(en)(OCOCH_3)_2Cl_2$, $Pt(en)Cl_4$, and $Pt(en)(OCOCF_3)_2Cl_2$ complexes were synthesized following literature procedures[3d] (en = ethylenediamine). Each complex was characterized by elemental analysis (Atlantic Microlab, Norcross, GA), IR (Mattson Cygnus 100), and $^{13}C$ NMR in $D_2O$ solution (GE GN−Omega 300 MHz). IR spectra were obtained in the diffuse reflectance mode using KBr as a diluent. The $Pt(ipa)(OH)_2Cl_2$ (iproplatin) and $Pt(dach)Cl_4$ (tetraplatin) complexes were obtained from the National Cancer Institute, Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment. The $Pt(a,cha)(OCOCH_3)_2Cl_2$ (JM-216) and $Pt(a,cha)(OCOC_3H_7)_2Cl_2$ (JM-221) complexes were kindly provided by Dr. Chris Giandomenico of Johnson-Matthey Inc. (a = ammine, cha = cyclohexylamine).

**Kinetic Studies.** Buffers were not used to avoid complications arising from buffer coordination to platinum.[7] The pH of the solution was adjusted to 7.1 (±0.1) with 0.1 M NaOH or 0.1 M HCl. The $pK_{a1}$ of ascorbic acid is 4.2, so, at pH 7.1 and concentrations between 1 and 15 mM, it exists predominantly as the monoanionic ascorbate. Stock ascorbate solutions were treated with Chelex 100 resin (Biorad) to remove trace iron.[8] Ascorbate concentrations were determined by absorbance at 265 nm ($\epsilon = 14\,500\ M^{-1}\ cm^{-1}$). Stock platinum complex solutions were prepared by dissolving platinum complexes in deionized water at room temperature. Stock ascorbate and platinum complex solutions were freshly prepared for each experiment.

For slow reactions, the stock ascorbate and platinum complex solutions were mixed to give initial concentrations of 7.5 mM ascorbate and 0.75 mM platinum. The solution in the 1 cm path length cell was placed in a jacketed cuvette holder, and the reaction was monitored using an OLIS 17 spectrophotometer. Sample temperature was maintained by circulating water through the jacketed cuvette holders from a RM 6 Lauda Brinkmann bath.

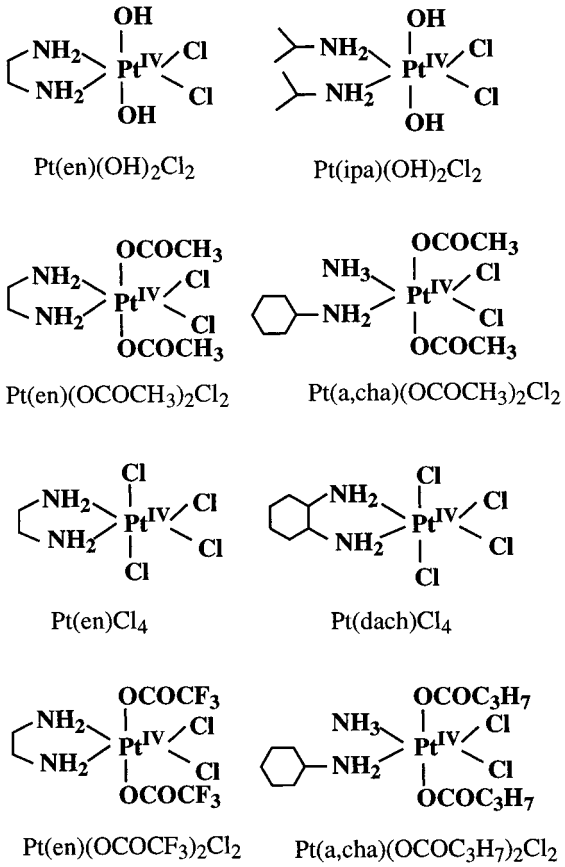


**Figure 1.** Molecular structures of $Pt^{IV}$ complexes studied in this research.

Study of the rapidly reduced $Pt(dach)Cl_4$ and $Pt(en)(OCOCF_3)_2Cl_2$ complexes required the use of a stopped-flow spectrophotometer (RX 1000 rapid kinetics spectrometer accessory from Applied Photophysics, Leatherhead, U.K., attached to an OLIS118 spectrophotometer). A solution of 15 mM ascorbic acid was placed in one drive syringe of the stopped-flow apparatus, and a solution of 1.5 mM platinum complex was placed in the other drive syringe. The initial concentrations immediately after mixing were 7.5 and 0.75 mM ascorbate and platinum, respectively.

Both slow and rapid reactions were monitored by observing the decrease in absorbance ($A_t$) at 330 nm except for the $Pt(en)Cl_4$ complex, which was monitored at 350 nm. Plots of $\ln(A_t - A_\infty)$ versus time, where $A_t$ and $A_\infty$ are the absorbances at time $t$ and at infinity, respectively, were linear. Pseudo-first-order rate constants were obtained from the slopes. Second-order rate constants were calculated by dividing the observed first-order constant by the ascorbic acid concentration. The experimental error which might have occurred due to oxidation of ascorbic acid by dissolved $O_2$ (~0.3 mM) was minimized by removing trace amounts of transition metals with Chelex 100 and by using a relatively high concentration of ascorbic acid (7.5 mM). The activation parameters ($\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$) were calculated in the usual way.

**Electrochemistry.** Each platinum complex was dissolved to a final concentration of 1.0 mM in 0.1 M KCl, and the pH was adjusted to be 7.0. Cyclic voltammetric (CV) measurements were performed using a Bioanalytical System (West Lafayette, IN) BAS 100 electrochemical analyzer with a scan rate of 100 mV/s. Nitrogen was bubbled through the solution to remove oxygen. The working electrode was a glassy carbon electrode, the reference electrode was Ag/AgCl, and the auxiliary electrode was a platinum wire.

**Cytotoxicity Assay.** The cisplatin-sensitive murine leukemia L1210/0 cell line was kindly provided by Dr. Miles Hacker (Department of

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Pharmacology, University of Vermont Medical School, Burlington, VT) and Dr. Alan Eastman (Department of Pharmacology, Dartmouth Medical School, Hanover, NH). Stock cells were stored in liquid nitrogen, and fresh cells were obtained when necessary. The cells were maintained in RPMI 1640 medium supplemented with 15% fetal bovine serum (FBS) and 1% penicillin/streptomycin. The medium was buffered with 24 mM $NaHCO_3$ and 25 mM HEPES (pH = 7.4). The cells were grown in an incubator with 5% $CO_2$ and 95% relative humidity at 37 °C. Stock cells were subcultured every 3 days. All reagents were obtained from GibcoBRL (Gaithersbery, MD) with the exception of the FBS (Sigma, St. Louis, MO).

The sensitivities of the L1210/0 cells to the eight $Pt^{IV}$ complexes were determined by measuring the amount of growth inhibition caused by exposure for a 3 day incubation period. A fresh 1 mM solution of platinum complex was prepared in 0.1 M NaCl. The solution was sterilized by filtration through a sterile filter (0.8/0.2 $\mu$m, Acrodisc PF, Gelman Sciences), and 140 $\mu$L of this solution was added to 10 mL of the cell suspension ($\sim 1 \times 10^5$ cells/mL) for a final drug concentration of 7 $\mu$M. A control group was treated with 140 $\mu$L of 0.1 M NaCl solution that did not contain any platinum complex. After the drug was introduced into the media, the cell concentrations were determined at 0, 24, 48, and 72 h. Two hundred microliters of cell suspension was removed from the media and added to an equivalent volume of trypan blue dye solution. After staining, live cells appeared translucent, whereas dead cells were colored blue. The solution was allowed to stand for 5−15 min, and then the concentration of live cells was determined using a hemocytometer.[9] The four corner boxes and the center box were counted for each sample removed. The calculated volume for each box was $1 \times 10^{-4}$ mL. For each vial, five samples were collected and counted, and an average cell count was determined. A minimum of two trials were performed for each platinum complex and for the control groups. The percentage of cell growth inhibition was determined using the following equation: % inhibition = $100[1 - (N_{Dt}/N_{D0})/(N_{Ct}/N_{C0})]$, where $N_{Dt}$, $N_{D0}$, $N_{Ct}$, and $N_{C0}$ are the number of cells treated with a platinum drug and saline solution (control) at time $t$ and 0, respectively.

## Results and Discussion

**Kinetics.** The absorbance at 330 nm of the Pt(a,cha)-$(OCOCH_3)_2Cl_2$ complex in the presence of a 10-fold excess of ascorbic acid versus time is shown in Figure 2a. The plot of $\ln(A_t - A_\infty)$ versus time is linear ($R^2 = 0.998$) (Figure 2b), indicating that the reduction of $Pt^{IV}$ by ascorbic acid in the presence of a 10-fold excess of ascorbic acid is pseudo-first-order. While the general mechanism for $Pt^{IV}$ reduction has not been established, there is ample evidence that $Pt^{IV}$ reductions may be catalyzed by $Pt^{II}$,[4b,c] which would produce a complicated autocatalytic kinetic curve. None of the eight $Pt^{IV}$ compounds in this study have shown autocatalytic kinetic curves within the first 10 min of the reaction. We have, however, seen a deviation from pseudo-first-order behavior in the reaction between the $Pt(ipa)(OH)_2Cl_2$ complex and ascorbic acid at 40 °C after 40 min which may be due to autocatalysis. We did not do any further analysis of the curve because we were interested only in the relative reduction rates among the eight $Pt^{IV}$ compounds. The kinetics of reduction by Cr(II)[10] of ammine and ethylenediamine $Pt^{IV}$ complexes and by ascorbic acid of $Pt(dach)Cl_4$[6a] and $Pt(ipa)(OH)_2Cl_2$[11] have been analyzed under the assumption of a second-order reaction (first-order with respect to $Pt^{IV}$ and first-order with respect to the reducing agent). Therefore, we obtained rate constants by monitoring the reaction in the early stages (5 or 10 min) and assuming that ascorbic acid and the $Pt^{IV}$ complex reaction is second-order. The rate-limiting step of $Pt^{IV}$ + ascorbic acid → $Pt^{II}$ + dehydroascorbic acid has a rate law of $-d[Pt^{IV}]/dt = k[\text{ascorbic acid}][Pt^{IV}]$. The second-order rate constants, calculated from averaging three experiments, and the activation parameters, $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$, are summarized in Table 1.

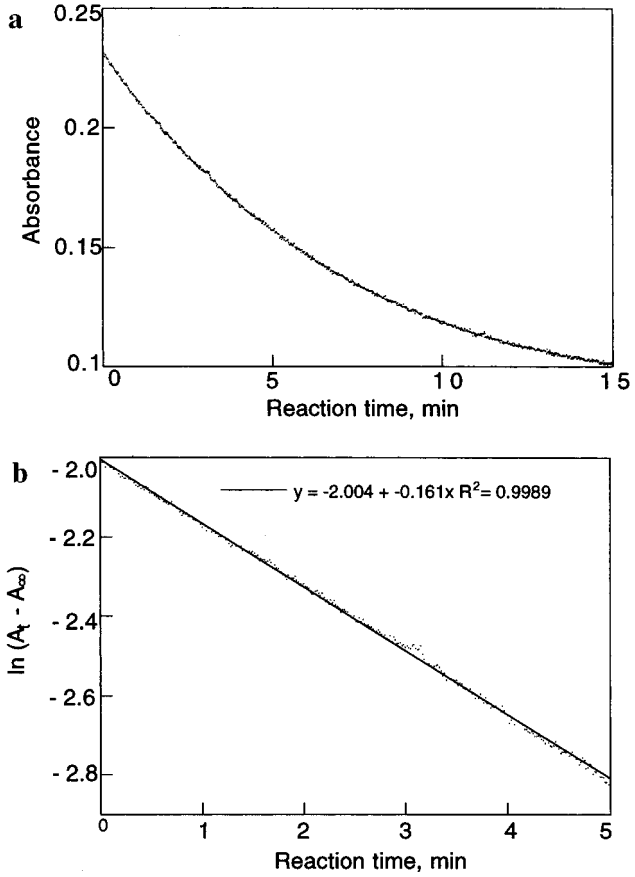


**Figure 2.** (a) Plot of absorbance at 330 nm versus reaction time for $Pt(a,cha)(OCOCH_3)_2Cl_2$ (0.75 mM) and ascorbic acid (7.5 mM) at pH = 7.1. (b) Plot of $\ln(A_t - A_\infty)$ versus time for $Pt(a,cha)(OCOCH_3)_2Cl_2$ (0.75 mM) and ascorbic acid (7.5 mM) at pH = 7.1. $A_t$ = Absorbance at 330 nm at time $t$. $A_\infty$ = absorbance at 330 nm after 30 min.

Our rate constant of 225 $M^{-1}$ $s^{-1}$ for the reduction of $Pt(dach)Cl_4$ by ascorbic acid is five times higher than the previous value of 43 $M^{-1}$ $s^{-1}$ reported by Gibbons et al.[6a] In their experiment, they used very low concentrations (10 $\mu$M) of $Pt(dach)Cl_4$ and ascorbic acid. At these concentrations, the oxidation of ascorbic acid by dissolved $O_2$ from air may have interfered. Our rate constant of 0.33 $M^{-1}$ $s^{-1}$ for the reduction of $Pt(ipa)(OH)_2Cl_2$ by ascorbic acid agrees reasonably well with the 0.103 $M^{-1}$ $s^{-1}$ value obtained by Evans et al.[11] Our value may be more trustworthy. While Evans used ascorbic acid concentrations (3−12 mM) near ours, they did not filter their solution through Chelex 100 to remove iron which autocatalyzes the ascorbic acid oxidation by $O_2$.[8]

The reduction rates vary widely among the $Pt^{IV}$ complexes tested. The platinum complexes with hydroxo axial ligands are reduced very slowly, those with acetate ligands moderately slowly, and those with chloro ligands show fast reduction. $Pt(en)(OH)_2Cl_2$ was not reduced at all by ascorbic acid, while $Pt(dach)Cl_4$ showed a very high reduction rate with $k$ = 225 $M^{-1}$ $s^{-1}$. The very slow reduction rates of $Pt^{IV}$ complexes containing hydroxo axial ligands such as $Pt(en)(OH)_2Cl_2$ ($k$ = 0 $M^{-1}$ $s^{-1}$) and $Pt(ipa)(OH)_2Cl_2$ ($k$ = 0.33 $M^{-1}$ $s^{-1}$) compared to the ones containing chloro axial ligands such as $Pt(en)Cl_4$ ($k$ = 164 $M^{-1}$ $s^{-1}$) and $Pt(dach)Cl_4$ (225 $M^{-1}$ $s^{-1}$) are consistent with the previous findings: the reduction rate of *trans*-$[Pt(NH_3)_4ClOH]^{2+}$ by tris(bipyridine)Cr(II) is less than 1/1000 as rapid as that of *trans*-$[Pt(NH_3)_4Cl_2]^{2+}$.[10a] The complexes with trifluoroacetate ligands have a slightly faster reduction rate than those with chloro ligands. In summary, for a series of $Pt^{IV}$

**Table 1.** Reduction and Cytotoxicity Data of $Pt^{IV}$ Complexes

| compound | $k$(40 °C) ($M^{-1}$ $s^{-1}$) (mean ± SD) ($N$)[a] | $\Delta S^{\ddagger}$ (J $K^{-1}$ $mol^{-1}$)[b] | $\Delta H^{\ddagger}$ (kJ $mol^{-1}$)[b] | cathodic potential (mV) (mean ± SD) ($N$)[a] | % inhibn on L1210 (mean ± SD) ($N$)[a] |
|---|---|---|---|---|---|
| $Pt(en)(OH)_2Cl_2$ | 0 | −720 ± 144 | 45 ± 6 | −884[c] | 45 ± 6(2) |
| $Pt(ipa)(OH)_2Cl_2$ | 0.33 ± 0.05(3) | −206 ± 46 | 15.1 ± 2.2 | −730 ± 103(3) | 87 ± 3(2) |
| $Pt(en)(OCOCH_3)_2Cl_2$ | 0.54 ± 0.04(3) | −192 ± 27 | 18.3 ± 1.6 | −546[c] | 49 ± 6(3) |
| $Pt(a,cha)(OCOCH_3)_2Cl_2$ | 6.4 ± 0.8(3) | −8.8 ± 0.9 | 69.0 ± 20.2 | −250 ± 60(3) | 94 ± 1(2) |
| $Pt(a,cha)(OCOC_3H_7)_2Cl_2$ | 14.7 ± 0.3(3) | −26.2 ± 3.4 | 61.3 ± 20.0 | −150 ± 80(3) | 90 ± 1(2) |
| $Pt(en)Cl_4$ | 164 ± 32.8(3) | −59.4 ± 14.9 | 46.1 ± 14 | −160 ± 53(3) | 71 ± 14(5) |
| $Pt(en)(OCOCF_3)_2Cl_2$ | 209 ± 20.1(3) | 203 ± 26.4 | 127 ± 24.1 | 0 ± 25(3) | 74 ± 14(2) |
| $Pt(dach)Cl_4$ | 225 ± 15.8(3) | 114 ± 32 | 99.4 ± 13.5 | −90 ± 19(3) | 95 ± 6(3) |

[a] SD: standard deviation. $N$: number of experiments. [b] The error was obtained from the standard deviation of the slope. [c] Data from Ellis et al.[2a]

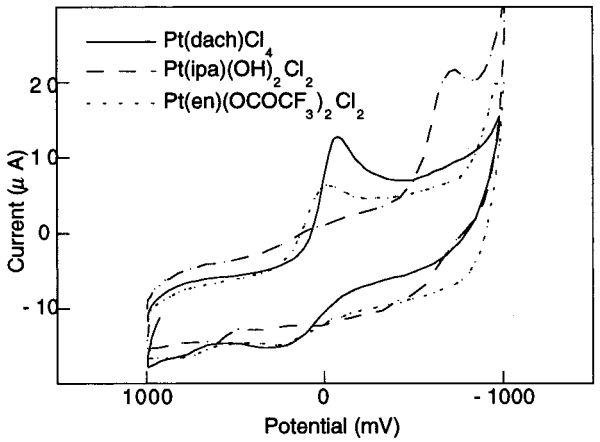


**Figure 3.** Cyclic voltammograms of $Pt(dach)Cl_4$, $Pt(en)(OCOCF_3)_2Cl_2$, and $Pt(ipa)(OH)_2Cl_2$. Conditions: [Pt] = 1 mM, 0.1 M KCl supporting electrolyte, pH 7.0; glassy carbon working electrode; Ag/AgCl reference electrode; scan rate = 100 mV/s.

complexes with the same equatorial ligands, the rate increases in the following order of axial ligands: OH < $OCOCH_3$ < Cl < $OCOCF_3$, which coincides with their electron-withdrawing power (electronegativity). The more electronegative ligands promote destabilization of the $Pt^{IV}$ state, which results in a faster reduction of the higher oxidation state.

The reduction rate also depends on the bulkiness of the axial and equatorial ligands. The $Pt(a,cha)(OCOC_3H_7)_2Cl_2$ complex is reduced twice as fast as the $Pt(a,cha)(OCOCH_3)_2Cl_2$ complex due to the bulkier ligand, $OCOC_3H_7$. $Pt^{IV}$ complexes with an en carrier ligand have a slower reduction rate than those with ipa or a,cha carrier ligands. Ascorbic acid could not reduce the $Pt(en)(OH)_2Cl_2$ complex but could reduce the $Pt(ipa)(OH)_2Cl_2$ complex, although very slowly. The reduction of the $Pt(a,cha)(OCOCH_3)_2Cl_2$ complex is about 12 times as rapid as that of the $Pt(en)(OCOCH_3)_2Cl_2$ complex. Ethylenediamine may cause less steric hindrance than either ipa or a,cha, stabilizing the six-coordinated state. Bulky ligands destabilize the six-coordinated $Pt^{IV}$ state, which results in a faster rate of reduction to the four-coordinate $Pt^{II}$ state.

The entropy of activation varies widely among the $Pt^{IV}$ complexes tested. The $Pt(en)(OCOCF_3)_2Cl_2$ and $Pt(dach)Cl_4$ complexes exhibit a large positive $\Delta S^{\ddagger}$ indicating a dissociative-type mechanism, while the other complexes show a negative $\Delta S^{\ddagger}$ indicating an associative-type mechanism.

**Electrochemistry.** The CVs of some of the $Pt^{IV}$ complexes studied are displayed in Figure 3. Their features are generally the same as reported earlier.[2a] Since reduction involves loss of the axial ligands, the reduction is irreversible in all cases. The cathodic potentials of all the complexes studied are listed in Table 1. The variation in the cathodic potential follows the trend found in reduction rate. The cathodic reduction potential

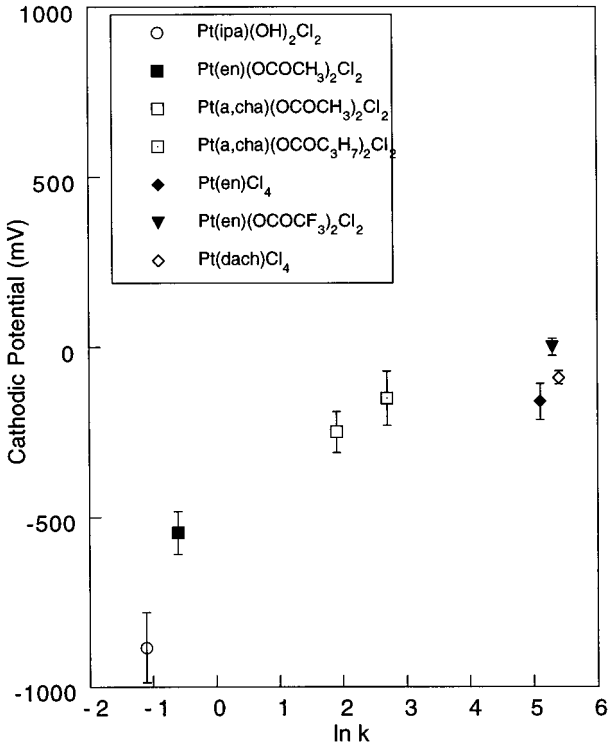


**Figure 4.** ln $k$ versus cathodic reduction potential of $Pt^{IV}$ complexes studied.

depends on the electron-withdrawing power of the axial ligands and the bulkiness of the axial or carrier ligands.[2a] The $Pt(en)(OCOCF_3)_2Cl_2$ complex has a higher reduction potential than the $Pt(en)Cl_4$ complex due to the stronger electron-withdrawing power of trifluoroacetato ligands than chloro ligands. The $Pt(a,cha)(OCOC_3H_7)_2Cl_2$ complex has (CVs not shown) the highest reduction potential among the $Pt^{IV}$ complexes with dicarboxylato axial ligands due to the bulky $OCOC_3H_7$ axial and bulky a, cha carrier ligands. This trend has been also observed among $Pt(en)(OCOR)_2Cl_2$ series[2a] where the order of reduction potential is R = $C_3H_7$ > $C_2H_5$ > $CH_3$. The $Pt(ipa)(OH)_2Cl_2$ complex, with the more sterically hindered carrier ligands than the $Pt(en)(OH)_2Cl_2$ complex, has the higher reduction potential.

The anodic potential of ascorbic acid at pH 7.0 was measured to be +560 mV. This may explain why ascorbic acid could not reduce the $Pt(en)(OH)_2Cl_2$ complex with a reduction potential of −884 mV. The rate of reduction by ascorbic acid correlates roughly with cathodic reduction potential (Figure 4). A similar correlation between electrochemical and chemical reduction has been noted for other $Pt^{IV}$ complexes such as *trans*-$[Pt(en)_2Cl_2]^{2+}$, $[Pt(en)_3]^{4+}$, *trans*-$[Pt(en)_2ClOH]^{2+}$, and *trans*-$[Pt(en)_2(OH)_2]^{2+}$.[10a] Such a correlation is predicted by the

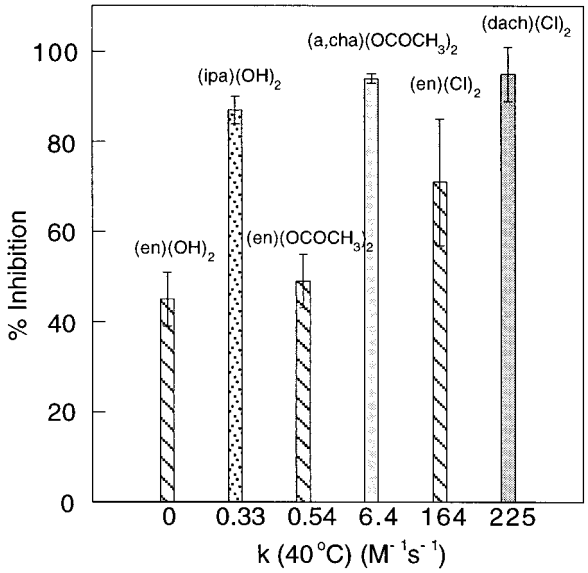


**Figure 5.** Percent growth inhibition of L1210/0 cells by $Pt^{IV}$ complexes versus reduction rate constant ($k$, 40 °C) of $Pt^{IV}$ complexes by ascorbic acid; % inhibition = $100[1 - (N_{Dt}/N_{D0})/(N_{Ct}/N_{C0})]$, where $N_{Dt}$, $N_{D0}$, $N_{Ct}$, and $N_{C0}$ are the number of cells ($\sim 10^5-10^6$ cells/mL) treated with a $Pt^{IV}$ compound (7 $\mu$M) and saline solution (0.1 M NaCl) at time $t$ (3 days) and 0, respectively. Each value represents the mean ± the standard error of at least two independent experiments.

Marcus theory. The rate-determining step in the reduction of the $Pt^{IV}$ complexes is an outer-sphere, one-electron transfer to generate a $Pt^{III}$ intermediate.[10a] This prediction cannot be ascertained from the present results.

**Cytotoxicity and Reduction Rate.** Cytotoxicities, expressed as percent growth inhibitions in L1210/0 cell lines, are listed in Table 1. The percent inhibition versus reduction rate by ascorbic acid is plotted in Figure 5. Overall, there is no strong correlation between the cytotoxicity and the reduction rate. For example, the $Pt(ipa)(OH)_2Cl_2$ complex, with a very low reduction rate, shows much higher percent inhibition compared to the $Pt(en)Cl_4$ complex, which has a very high reduction rate. However, when the four compounds with en carrier ligands were compared with one another, the one with the fastest reduction rate exhibited the highest cytotoxicity. The cytotoxicity increases with axial ligand substitution in the order OH < $OCOCH_3$ < Cl < $OCOCF_3$, following the same trend as reduction rate. Also, when the en complex is compared with the ipa, a,cha, or dach complex, the en complex shows both the lower rate and lower cytotoxicity. Reduction rate and cytotoxicity increase in the order $Pt(en)(OH)_2Cl_2$ < $Pt(ipa)(OH)_2Cl_2$, $Pt(en)(OCOCH_3)_2Cl_2$ < $Pt(a,cha)(OCOCH_3)_2Cl_2$, $Pt(en)Cl)_4$ < $Pt(dach)Cl_4$. The low cytotoxicity shown by the en compound may not be entirely due to its low reduction rate, but it is interesting to note the trend between the reduction rate and the percent inhibition. The dependency of cytotoxicity, especially toward cisplatin-sensitive L1210/0 cells, on the reduction rate is also seen in the literature. In a $Pt^{IV}$ a,cha,[3a,b] dach[3c] and en[3d] homologous series, the compounds showing higher antitumor activity possess either more powerful electron-withdrawing or bulkier axial ligands, hence they are predicted to have higher reduction rates.

On the other hand, there is another experimental result which is opposite to this trend, particularly for cisplatin resistant cells, L1210/cisplatin.[3b] Although $Pt(a,cha)(OH)_2Cl_2$ shows lower anticancer acitivity on L1210/0 than $Pt(a,cha)Cl_4$, it shows higher anticancer acitivity on L1210/cisplatin than $Pt(a,cha)Cl_4$.[3b] Explanation of why the order of potency toward L1210/cisplatin cells is reversed compared to that toward L1210/0 cells requires additional studies.

## Conclusion

We have shown that reduction rate and reduction potential of a series of $Pt^{IV}$ complexes depend on the electron-withdrawing power of axial ligands and the steric hindrance of axial and carrier ligands. The complex with a bulkier and more electron withdrawing ligand shows a higher reduction rate and reduction potential. There is no strong correlation between reduction rate and anticancer activity toward L1210/0 cells among the eight compounds tested. However, in a homologous series of compounds differing in only one ligand position, the correlation exists: the one undergoing the fastest reduction exhibits the highest cytotoxicity.

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