The hydrolysis products of *cis*-diamminedichloroplatinum(II) 6. A kinetic comparison of the *cis*- and *trans*-isomers and other *cis*-di(amine)di(chloro)platinum(II) compounds

Sian E. Miller, Kathryn J. Gerard and Donald A. House*
Department of Chemistry, University of Canterbury, Christchurch (New Zealand)

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Abstract

Di(amine)di(chloro)platinum(II) complexes react in aqueous acid solution to form an equilibrium mixture $L_2PtCl_2 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} L_2PtCl(OH_2)^+ + Cl^-$. Values of k_1, k_{-1} and the equilibrium constant K_1 ($=k_1/k_{-1}$) have been measured for the systems $L_2 = cis(NH_3)_2$, $trans(NH_3)_2$, $cis(py)_2$, en, *RR*-chxn, tn and Me_2tn, with varying temperature and ionic strength ($cis(NH_3)_2$ only). At 25 °C and $I = 0.1$ M, data for *cis*-$PtCl_2(NH_3)_2$ are $k_1 = 5.18 \times 10^{-5}$ s^{-1} ($\Delta H^{\#} = 86.7$ kJ mol^{-1}, $\Delta S^{\#} = -36$ J K mol^{-1}), $k_{-1} = 7.68 \times 10^{-3}$ M^{-1} s^{-1} ($\Delta H^{\#} = 72.7$, $\Delta S^{\#} = -41$) and $K_1 = 6.74 \times 10^{-3}$. Corresponding data for *trans*-$PtCl_2(NH_3)_2$ are $k_1 = 1.90 \times 10^{-5}$ s^{-1} ($\Delta H^{\#} = 92.2$, $\Delta S^{\#} = -26$), $k_{-1} = 3.05 \times 10^{-2}$ M^{-1} s^{-1} ($\Delta H^{\#} = 85.7$, $\Delta S^{\#} = +14$) and $K_1 = 6.22 \times 10^{-4}$. These data provide a kinetic explanation for the inactivity of the *trans*-isomer as an anti-cancer drug.

Introduction

The discovery [1, 2] that *cis*-$PtCl_2(NH_3)_2$ (*cis*-DDP) can be used for a positive response in the treatment of certain human cancers [3] has created a renaissance in platinium(II) chemistry. Even ten years ago [4] some 1.2×10^3 platinum-containing compounds had been specifically synthesised and screened for anti-cancer activity by the US National Cancer Institute. This screening process has produced a number of 'second-generation' anti-cancer platinum drugs [5] that are generally less toxic than *cis*-DDP, but hardly any are more potent.

Also as a result of this screening, a number of important structural requirements have been listed as necessary [4, 6, 7] for a Pt(II) complex to have anti-cancer activity.

There is, however, very little information available on the relative lability or reactivity of these Pt(II) complexes. Despite the frequent occurrence of schemes showing intact *cis*-DDP reacting directly with DNA [8], there is considerable evidence that the active species in solution is *cis*-$PtCl(NH_3)_2(OH_2)^+$ [9–12]. This hydrolysis product is formed from the parent dichloro according to eqn. (1). In acid solution,

$$\begin{array}{ccc} cis\text{-}PtCl_2(NH_3)_2 & \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} & cis\text{-}PtCl(NH_3)_2(OH_2)^+ + Cl^- \\ & & \uparrow\downarrow \; pK_a^3 = 6.85 \text{ [13]} \\ & & cis\text{-}PtCl(OH)(NH_3)_2 \end{array} \quad (1)$$

only the (aqua)(chloro) is formed, but as the pH is increased, conversion to the (chloro)(hydroxo) occurs. At pH = 7.4, $T = 37$ °C, $[Cl^-] \sim 0$ (inner cell physiological conditions), the (aqua)(chloro) and (chloro)(hydroxo) species are produced in an approximately 1:4 ratio [14].

As the (aqua)(chloro) ion is believed to be the active Pt(II) species, we will focus in this paper on the factors which control the amount and lability of an (aqua)(chloro)platinum(II) ion that can be delivered to the site of rapidly replicating DNA.

To this end we have measured k_1, k_{-1} and K_1 ($=k_1/k_{-1}$, the equilibrium constant for eqn. (1)) for *cis*-$PtCl_2(NH_3)_2$, *trans*-$PtCl_2(NH_3)_2$, *cis*-$PtCl_2(py)_2$, $PtCl_2$(en), $PtCl_2$(*R*,*R*-chxn), $PtCl_2$(tn) and $PtCl_2(Me_2tn)$**.

*Author to whom correspondence should be addressed.

**Abbreviations used: py = pyridine, en = $NH_2(CH_2)_2NH_2$, tn = $NH_2(CH_2)_3NH_2$, Me_2tn = $NH_2CH_2C(CH_3)_2CH_2NH_2$, *RR*-chxn = (−)-*trans*-(*R*,*R*)-1,2-diaminocyclohexane, DMSO = dimethylsulfoxide, CD = circular dichroism.

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Experimental

cis-$PtCl_2(NH_3)_2$, *trans*-$PtCl_2(NH_3)_2$, *cis*-$PtCl_2(py)_2$ and $PtCl_2$(*RR*-chxn) were purchased from Strem Chemical Co. and used as supplied. $PtCl_2$(en), $PtCl_2$(tn) and $PtCl_2(Me_2tn)$ were prepared from the diamines and *cis*-$PtCl_2(DMSO)_2$ according to literature [15–17] procedures.

Stock solutions of $L_2Pt(OH)_2$ ($(3–12)\times10^{-4}$ M) were prepared by suspending weighed amounts (30–90 mg) of the dichloro complexes in 0.01 M NaOH solution (250 ml) and allowing these to hydrolyse at 50 °C for 6 h and then overnight at room temperature. Such solutions appear to be stable for at least 3 months at room temperature, in the absence of light and CO_2.

Aliquots of these solutions (5.0 ml) were heated to the desired temperature in a water bath and 5.0 ml of 0.2 M $HClO_4$, containing known amounts of dissolved NaCl (6–50 mM), at the same temperature, were mixed. For *cis*$(py)_2$ the stock solution was diluted to 1/5 (6.3×10^{-5} M) before acidification.

The mixed solutions were transferred to a 4.00 cm, quartz spectrophotometer cell (10 ml capacity) in an electrically heated, temperature controlled (±0.1 °C) cell holder and absorbance versus time data were collected at appropriate wavelengths (Table 1) and time intervals, using a Perkin-Elmer λ-2 recording spectrophotometer.

Reactions were followed for 6–8 half-lives and pseudo-first-order rate constants (k_{obs}) could be calculated from expression (2)

TABLE 1. Isosbestic points for the reaction corresponding to k_{-2} (eqn. (4)) and k_{-1} (eqn. (1)).

L_2	λ (nm)		
$L_2Pt(OH_2)_2^{2+} + Cl^- \xrightarrow{k_{-2}} L_2PtCl(OH_2)^+$			
cis$(NH_3)_2$	230	270	
trans$(NH_3)_2$	246	275	
en	233	270	
RR-chxn	234	266	315
tn	233	258	303
Me_2tn	234	257	301
cis$(py)_2$	236	255	
$L_2PtCl(OH_2)^+ + Cl^- \xrightarrow{k_{-1}} L_2PtCl_2$			
cis$(NH_3)_2$	240	280	
trans$(NH_3)_2$	246	266	
en	241	286	
RR-chxn	242	286	
tn	240	273	326
Me_2tn	240	272	324
cis$(py)_2$	242	286	

TABLE 2. Observed rate constants ($10^4\times k_{obs}$, s^{-1}) for the chloride anation of $L_2PtCl(OH_2)^+$ ions in 0.1 M $HClO_4$ ($I=0.1$ M)[a]

L_2 = tn				$L_2 = Me_2$tn			
[Cl^-] (mM)	34.4 (°C)	45.0 (°C)	54.9 (°C)	[Cl^-] (mM)	34.1 (°C)	45.0 (°C)	54.9 (°C)
15.3	14.8	39.7		15.3	11.9	35.2	
10.3	10.2	28.5	66.5	10.3	8.56	26.1	60.6
7.3	7.83	22.5	53.7	7.3	7.32	20.2	48.8
5.3	6.41	18.8	45.7	5.3	5.56	16.8	41.4
3.3		14.7	36.0	3.3		12.7	33.3
L_2 = en				L_2 = *cis*$(py)_2$			
[Cl^-] (mM)	35.5 (°C)	45.0 (°C)	54.8 (°C)	[Cl^-] (mM)	35.5 (°C)	45.0 (°C)	54.7 (°C)
15.3	9.89	25.3	56.5	15.0	4.60	10.7	19.8
10.3	6.85	18.8	42.2	10.0	3.50	8.46	15.8
7.3	5.18	14.4	32.8	7.0	2.77	6.75	11.6
5.3	4.39	12.1	27.6	5.0		5.77	9.70
3.3		8.53	20.9	3.0		4.22	8.02

L_2 = *trans*$(NH_3)_2$

[Cl^-] (mM)	35.5 (°C)	40.1 (°C)	45.0 (°C)	49.7 (°C)	54.7 (°C)
15.3	16.7	27.9	46.2	69.4	
10.3	11.4	18.8	31.8	48.7	
7.3	8.21	13.2	23.8	34.5	65.0
5.3	6.22	10.8	17.7	26.3	46.8
3.3	4.78	6.82	11.5	19.4	32.3

L_2 = *cis*$(NH_3)_2$

[Cl^-] (mM)	35.5 (°C)	40.8 (°C)	45.0 (°C)	49.7 (°C)	54.7 (°C)
15.6	4.90	8.72	12.5	20.2	28.3
15.0		8.55			
10.6	4.00	7.04	10.2	16.3	24.4
7.6	3.29	5.90	8.69	14.1	21.3

L_2 = *RR*-(chxn)

[Cl^-] (mM)	15.4 (°C)	20.3 (°C)	25.2 (°C)	30.6 (°C)	35.3 (°C)	40.4 (°C)	45.0 (°C)	54.8 (°C)
200[b]	10.5	17.3	27.2					
150[b]	7.35	13.2	20.5					
100[b]		9.12	15.1	22.6				
80[b]		7.26	10.7	19.3				
50[b]			7.71	12.9	19.7			
30[b]			4.31	7.98	13.1	21.6		
20[b]				6.13	9.63	14.9		
15.3					9.60		25.0	55.7
10.3					6.39		17.6	41.3
10.0[b]					5.34	9.06		
7.3					5.11		14.3	33.6
5.3							11.6	27.0
3.3					3.20		8.64	21.9

[a]Mean of two to six determinations with a maximum deviation of 3%. [b]$I=0.3$ M, determined using changes in the CD spectra.

$$k_{obs}t = \ln[(A_\infty - A_0)(A_\infty - A_t)^{-1}] \quad (2)$$

as $[Cl^-]_i$ was always $> 10 \times (Pt(II))_i$ (Table 2).

Plots of k_{obs} versus $[Cl^-]$ were linear with slope $= k_{-1}$ and intercept $= k_1$ (Table 3) and the variation of k_1 and k_{-1} with temperature allowed the calculation of the activation parameters (Table 4). Similar procedures were used to determine the ionic strength dependence of k_{-1} for the *cis*-$PtCl(NH_3)_2(OH_2)^+$ ion except that the $HClO_4$ concentration was varied from 0.04–2.0 M, with dissolved NaCl at known concentrations (Table 5).

The forward reaction for *cis*-$PtCl_2$(*RR*-chxn) was measured directly by dissolving small samples in the appropriate aqueous medium and monitoring the extent of hydrolysis using changes in the CD spectrum with a JASCO-J20 recording spectropolarimeter. Acid hydrolysis reactions were measured at 282 nm and base hydrolysis reactions at 270 nm (Table 4). Both k_{-2} and k_{-1} (eqn. (3)) were obtained from the CD spectral changes of acidified solutions of $Pt(OH)_2$(*RR*-chxn) containing known (excess) concentrations of chloride ion using 315 nm for k_{-2} ($I = 0.1$ M) and 330 nm for k_{-1} ($I = 0.3$ M).

In both cases, plots of k_{obs} versus $[Cl^-]$ were linear with those for k_{-2} (Table 6) passing through the origin. The CD data for the k_{obs} associated with k_{-1} are combined with the spectrophotometrically determined data in Table 3.

Results

Reaction rates

The forward and reverse rate constants associated with reaction (1) (k_1 and k_{-1}) were determined from the reaction cycle (3).

$$\begin{array}{ccc} L_2PtCl_2 & \xrightarrow{OH^-} & 2Cl^- + L_2Pt(OH)_2 \\ k_{-1} \uparrow \downarrow k_1 & & \downarrow H^+ \\ L_2PtCl(OH_2)^+ & \underset{k_2}{\overset{k_{-2}}{\rightleftharpoons}} & L_2Pt(OH_2)_2^{2+} \end{array} \quad (3)$$

Treatment of the sparingly soluble dichloro compound with excess aqueous NaOH results in hydrolysis to give the soluble dihydroxo and two free chloride ions. This process proceeds to completion [20], and we have no evidence for any polymeric μ-hydroxo Pt(II) species being formed at pH = 12.

On acidification, the dihydroxo is immediately converted to the diaqua, which in turn follows two sequential chloride anation steps (4) and (5).

TABLE 3. Observed and calculated[a] values for k_1[b], k_{-1}[c] and K_1 (eqn. (1)) at various temperatures and $I = 0.1$ M

T °C [K]	$10^4 \times k_1$ (s^{-1})	$10^2 \times k_{-1}$ (M^{-1} s^{-1})	$10^3 \times K_1$
L_2 = tn			
54.9 [328.1]	22.2 (22.7)	43.1 (43.7)	5.19
45.0 [318.2]	7.26 (7.32)	20.7 (20.0)	3.66
34.0 [307.2]	1.91 (2.00)	8.24 (8.34)	2.29
25.0 [298.2]	(0.588)	(3.62)	1.62
L_2 = Me_2tn			
54.9 [328.1]	20.6 (19.2)	38.8 (40.6)	4.73
45.0 [318.2]	6.57 (7.26)	18.9 (17.3)	4.20
34.1 [307.3]	2.48 (2.32)	6.13 (6.37)	3.64
25.0 [298.2]	(0.840)	(2.61)	3.22
L_2 = en			
54.8 [328.0]	11.9 (12.2)	29.2 (29.9)	4.08
45.0 [318.2]	4.39 (4.00)	13.7 (13.2)	3.03
35.5 [308.7]	1.22 (1.25)	5.57 (5.69)	2.19
25.0 [298.2]	(0.320)	(2.11)	1.52
L_2 = *RR*-chxn			
54.8 [328.0]	12.4 (9.01)	28.8 (28.0)	3.22
53.3 [326.5][d]	8.78 (8.13)		
49.7 [322.9][d]	6.56 (6.33)		
47.6 [320.8][d]	4.88 (5.45)		
45.6 [318.8][d]	4.42 (4.72)		
45.0 [318.2]	4.24 (4.52)	13.4 (10.8)	4.22
42.0 [315.2][d]	3.49 (3.63)		
40.2 [313.4]	2.65 (3.06)	6.27 (6.70)	4.57
36.6 [309.8][d]	2.11 (2.26)		
35.2 [308.4]	2.66 (2.01)	3.46 (3.98)	5.05
30.6 [303.8]	1.88 (1.35)	2.14 (2.43)	5.55
25.2 [298.4]	0.715 (0.841)	1.33 (1.38)	6.09
25.0 [298.2]	(0.825)	(1.31)	6.30
20.3 [293.5]	0.747	0.827 (0.761)	9.82
15.4 [288.6]	0.490	0.457 (0.425)	11.5
L_2 = *cis*-$(NH_3)_2$			
55.1 [328.3][e]	14.3 (14.5)	14.1 (12.4)	11.7
54.7 [327.9]	15.0 (14.0)	8.59 (12.0)	11.7
50.2 [323.4][e]	9.05 (8.85)	9.85 (8.17)	10.8
49.7 [322.9]	8.28 (8.40)	7.62 (7.80)	10.8
45.8 [319.0][e]	4.98 (5.58)	6.47 (5.55)	10.1
45.0 [318.2]	5.13 (5.12)	4.54 (5.17)	9.90
41.0 [314.2][e]	3.21 (3.32)	3.90 (3.60)	9.22
40.1 [313.4]	3.22 (3.01)	3.30 (3.31)	9.09
35.5 [308.7]	1.84 (1.80)	1.97 (2.16)	8.33
25.0 [298.2]	(0.524)	(0.768)	6.82
L_2 = *cis*-$(py)_2$			
54.7 [327.9]	4.77 (5.00)	10.2 (10.5)	4.76
45.0 [318.2]	2.94 (2.60)	5.30 (5.02)	5.17
35.5 [308.7]	1.91 (1.31)	2.28 (2.33)	5.62
25.0 [298.2]	(0.587)	(0.942)	6.23
L_2 = *trans*-$(NH_3)_2$			
54.7 [327.9]	5.27 (6.04)	81.1 (76.5)	0.789
49.7 [322.9]	4.50 (3.53)	42.4 (46.4)	0.761
45.0 [318.2]	2.37 (2.09)	28.7 (28.5)	0.733
40.1 [313.3]	0.988 (1.20)	17.5 (17.0)	0.706
35.5 [308.7]	0.905 (0.696)	10.2 (10.2)	0.682
25.0 [298.2]	(0.190)	(3.05)	0.623

[a]Values in parentheses are calculated from the activation parameters cited in Table 4. [b]Estimated from the intercepts of the k_{obs} vs. $[Cl^-]$ plots. [c]Estimated from the slopes of the k_{obs} vs. $[Cl^-]$ plots. [d]Measured directly using the change in the CD spectra. [e]Data from Table 5.

TABLE 4. Kinetic parameters and equilibrium constants for the hydrolysis reactions of some square planar platinum(II) complexes at 25 °C

Complex	Medium	I (M)	$10^5 \times k_1$ (s^{-1})	$\Delta H_1^{\#}$ (kJ mol^{-1})	$\Delta S_1^{\#}$ (J K^{-1} mol^{-1})	$10^3 \times K_1$	Reference
cis-$PtCl_2(NH_3)_2$	H_2O	0.3	2.5	82.3	−59	3.3	18
	$HClO_4$	0.05	4.55[f]	91.5±5	−21±10	5.71	[a]
	$HClO_4$	0.1	5.18[f]	86.7±4	−36±8	6.74	[a]
	$HClO_4$	0.1	7.56[h]	73.7	−76		19
	$HClO_4$	0.1	5.24[d]	87.5±3	−34±6	6.82	[a]
	$HClO_4$	1.0	4.15[f]	93.6±8	−15±16	6.42	[a]
	$HClO_4$	1.0	6.32[h]	82.2	−49	10.1	19
	NaOH[b]	0.1	1.90	84.4	−52		20
cis-$PtBr_2(NH_3)_2$	$HClO_4$	1.0	~50	~75	~−60	~23	21
cis-$PtCl(OH)(NH_3)_2$	pH = 7.4	1.0	2.39	92.4	−75	25.5	22
trans-$PtCl_2(NH_3)_2$	H_2O	0.3	9.8				23
	HNO_3	0.01	6.62	75.2	−84	0.142	24
	$HClO_4$	0.1	1.90[f]	92.2±8	−26±16	0.622	[a]
$PtCl_2$(en)	HNO_3	0.01	5.13	92±6	−18±12	2.2	24
	$HClO_4$	0.1	3.20[f]	97.1±3	−5±6	1.5	[a]
	H_2O	0.318	3.4	85	−42	2.2	25
$PtCl_2$(*RR*-chxn)	$HClO_4$	1.0	10.3[c]	55.2±4	−136±8		[a]
	$HClO_4$	1.0[e]	5.69[c]	66.7±5	−102±10		[a]
	$HClO_4$	0.1	7.25[c]	67.8±2	−97±4		[a]
	$HClO_4$	0.1	3.68[f]	96.9±2	−5±4	1.81	[a]
	$HClO_4$	0.1–1.0	8.25[d]	64.4±4	−107±8		[a]
	NaOH[b]	0.1	3.81[c]	76.2±4	−74±8		[a]
$PtCl_2$(tn)	$HClO_4$	0.1	5.88[f]	97.0±3	−1±6	1.62	[a]
$PtCl_2(Me_2tn)$	$HClO_4$	0.1	8.40[f]	82.6±3	−46±6	3.22	[a]
cis-$PtCl_2(py)_2$	$HClO_4$	0.1	5.87[f]	56.2±4	−137±8	6.2	[a]
cis-$PtCl(NH_3)_2(OH_2)^+$	$HClO_4$	1.0	2.5			0.27	19
$PtCl(en)(OH_2)^+$	H_2O		4.4			0.27	25
$PtCl_3(NH_3)^-$	H_2O		5.6				23
$PtCl_4^{2-}$	H_2O		3.9				23

Anation

Complex	Nucleophile	I (M)	pH	$10^4 \times k_{Nu}$ (M^{-1} s^{-1})	$\Delta H_{-1}^{\#}$ (kJ mol^{-1})	$\Delta S_{-1}^{\#}$ (J K^{-1} mol^{-1})	Reference
cis-$PtCl(NH_3)_2(OH_2)^+$	Cl^-	1.0	<1	62.6	75.0	−36	19, 26
	Cl^-	1.0	<1	64.6	76.9±6	−29±12	[a]
	Cl^-	0.1	<1	76.8	72.7±8	−41±16	[a]
	Cl^-	0.05	<1	79.7	79.7±4	−18±8	[a]
	Hmal	1.0	4.3	9.90	74.8	−52	22
	gly	1.0	7.4	2.75	62.7	−103	22
trans-$PtCl(NH_3)_2(OH_2)^+$	Cl^-	0.01	2	4630	70.2	−25	24
	Cl^-	0.1	1	305	85.7±4	+14±8	[a]
cis-$PtBr(NH_3)_2(OH_2)^+$	Br^-	1.0	<1	205	74.9	−26	21
cis-$Pt(OH)(NH_3)_2(OH_2)^+$	Cl^-	1.0	7.4	9.37	61.7	−96	22
			7.8	12.7	39.5	−168	22
cis-$PtCl(en)(OH_2)^+$	Cl^-	0.32	<1	154	73.1	−33	25
		0.1	1	211	69.7±3	−43±6	[a]
cis-$PtCl$(*RR*-chxn)$(OH_2)^+$	Cl^-	0.03	<1	130[g]	74.8	−30	[a]
		0.1	1	203	69.5±5	−44±10	[a]
cis-$PtCl(tn)(OH_2)^+$	Cl^-	0.1	1	361	65.3±2	−53±4	[a]
cis-$PtCl(Me_2tn)(OH_2)^+$	Cl^-	0.1	1	261	72.1±6	−33±12	[a]
cis-$PtCl(py)_2(OH_2)^+$	Cl^-	0.1	1	94.2	63.5±6	−70±8	[a]

[a]This research. [b]Both chloro ligands lost to give $L_2Pt(OH)_2$ [20]. [c]Measured directly using changes in the CD spectra. [d]All data. [e]0.1 M $HClO_4$ plus 0.9 M $NaClO_4$. [f]Calculated from the temperature dependent intercepts of the k_{obs} vs. [Cl^-] plots (Table 3). [g]Measured using changes in the CD spectra. [h]Measured directly.

TABLE 5. Ionic strength variation of $10^4 \times k_{obs}$ for the reaction between *cis*-$PtCl(NH_3)_2(OH_2)^+$ and Cl^- [a,b]

Temperature (°C)	[Cl^-] (mM)					$10^2 \times k_{-1}$ (M^{-1} s^{-1})[d]
	20	25	30	35	40	
$I = 0.05$ M[c]						
41.0	11.4		15.2	17.8	20.3	4.18 (4.32)[e]
45.8	18.2		25.9	30.1	34.8	7.09 (6.93)
50.2	31.4		41.9	47.3	52.4	11.1 (10.6)
55.1	46.4		61.4	70.5	78.0	16.0 (16.7)
$I = 0.10$ M[c]						
41.0	11.2	12.6	14.6	17.1	18.8	3.90 (4.02)[e]
45.8	17.8	21.8	23.9	28.1	30.8	6.47 (6.31)
50.2	28.7	33.4	39.1	43.3	48.4	9.85 (9.45)
55.1	42.6	49.7		64.2	73.6	14.1 (14.6)
$I = 1.0$ M[c]						
41.0		11.4	12.8	14.2	15.9	3.20 (3.30)[e]
45.8	14.6	17.9	21.0	23.9	25.3	5.23 (5.21)
50.2	25.3	30.6	34.3	39.5	42.3	8.53 (7.84)
55.1	39.0	44.0	49.0	53.9	60.9	11.5 (12.2)

[a]$[Pt]_i = 0.61$ mM. [b]The reproducibility of k_{obs} is ±2%. [c]Adjusted with $HClO_4 + NaCl$. [d]Calculated from the slope of the k_{obs} vs. [Cl^-] plots. [e]The values in parentheses are calculated from the activation parameters cited in Table 4.

$$L_2Pt(OH_2)_2^{2+} + Cl^- \underset{k_2}{\overset{k_{-2}}{\rightleftharpoons}} L_2PtCl(OH_2)^+ \qquad (4)$$

$$L_2PtCl(OH_2)^+ + Cl^- \underset{k_1}{\overset{k_{-1}}{\rightleftharpoons}} L_2PtCl_2 \qquad (5)$$

The fact that $k_{-2} \geq 10 \times k_{-1}$ [19, 20] means that these two reactions can be followed separately at isosbestic points characteristic of the reaction not under investigation. Preliminary low temperature measurements established the isosbestic points for reaction (4) and these (Table 1) were used to monitor the anation of the (aqua)(chloro).

At high chloride ion concentration, the final absorption spectrum corresponded to that of the dichloro and, if the initial Pt(II) concentration is too high, the dichloro precipitates from solution. In this way, we were able to estimate the aqueous solubility of $PtCl_2$(*RR*-chxn) as 18 ± 2 mg/100 ml [27, 28] at 25 °C.

For a general reaction of the type (6), the equilibrium constant, K_n equals the forward and

$$L_2MXCl^{(n-1)+} \underset{k_{-n}}{\overset{k_n}{\rightleftharpoons}} L_2MX(OH_2)^{n+} + Cl^- \qquad (6)$$

reverse rate ratio ($K_n = k_n/k_{-n}$). In principle, both k_n and k_{-n} can be determined in the same set of experiments. Plots of k_{obs} versus [Cl^-] for the anation of $L_2MX(OH_2)^{n+}$ by chloride ion under pseudo-first-order conditions should give straight lines with slopes corresponding to k_{-n} and the intercepts, at [Cl^-] = 0, corresponding to k_n (Figs. 1–3). Thus, the intercept/slope ratio at any particular temperature will be a measure of K_n. In practice, there is frequently some difficulty with this procedure, as measuring intercepts close to zero is not particularly accurate (Fig. 1) and it may be better to measure k_n independently, assuming first-order kinetics.

Even this procedure is not without complications if K_n is small, and the kinetics are more accurately represented by eqns. (7) and (8) for reversible first- and second-order reactions [24, 29–31].

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B + C \qquad (7)$$

$$-d[A]/[dt] = k_1[A] - k_{-1}[B][C] \qquad (8)$$

TABLE 6. Spectropolarimetrically determined rate constants for the first step in the chloride anation of Pt(*RR*-chxn)$(OH_2)_2^{2+}$ ($I = 0.1$ M)[a]

[Cl^-] (mM)	25.3 °C		20.1 °C		14.9 °C		10.3 °C	
	$10^3 \times k_{obs}$ (s^{-1})	k_{-2} (M^{-1} s^{-1})	$10^3 \times k_{obs}$ (s^{-1})	k_{-2} (M^{-1} s^{-1})	$10^3 \times k_{obs}$ (s^{-1})	k_{-2} (M^{-1} s^{-1})	$10^3 \times k_{obs}$ (s^{-1})	k_{-2} (M^{-1} s^{-1})
4.28	1.44	0.337	0.906	0.212	0.545	0.127	0.342	0.0800
8.56	2.27	0.318	1.75	0.204	0.991	0.116	0.634	0.0740
17.1			3.30	0.193	2.10	0.123	1.24	0.0722
25.7					3.00	0.116	1.82	0.0708
Mean		0.328		0.203		0.121		0.0742
Calc.[b]		0.315		0.190		0.177		0.0699
Calc.[c]		0.331		0.201		0.120		0.0746

[a]$k_{-2} = k_{obs}[Cl^-]^{-1}$. These values were used in the calculation of the activation parameters. [b]Calculated from the slopes of the k_{obs} vs. [Cl^-] plots. [c]Calculated from the activation parameters: k_{-2} (25 °C, $I = 0.1$ M) = 32.2×10^{-2} M^{-1} s^{-1}, $\Delta H^{\#} = 67.4 \pm 1.2$ kJ mol^{-1}, $\Delta S^{\#} = -28.2 \pm 3$ J K^{-1} mol^{-1}. Corresponding values in the *cis*-$Pt(NH_3)_2(OH_2)_2^{2+} + Cl^-$ system are k_{-2} (25 °C, $I = 1.0$ M) = 9.09×10^{-2} M^{-1} s^{-1}, $\Delta H^{\#} = 72.7$ kJ mol^{-1} and $\Delta S^{\#} = -29.2$ J K^{-1} mol^{-1} [20]. The ionic strength dependence of k_{-2} is currently under investigation.

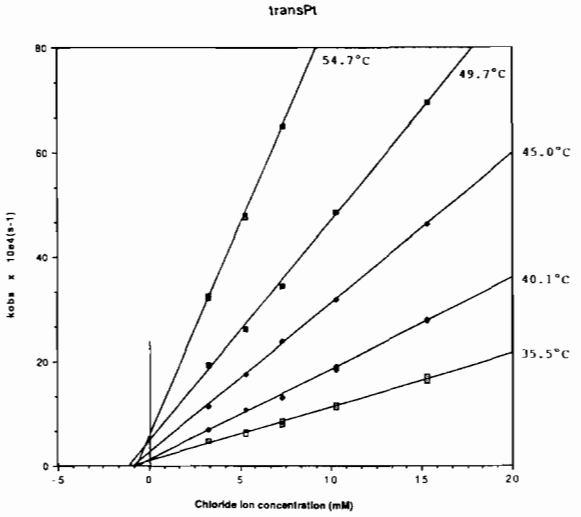


Fig. 1. Plots of k_{obs} vs. $[Cl^-]$ for the chloride ion anation of *trans*-$PtCl(NH_3)_2(OH_2)^+$ ($I=0.1$ M).

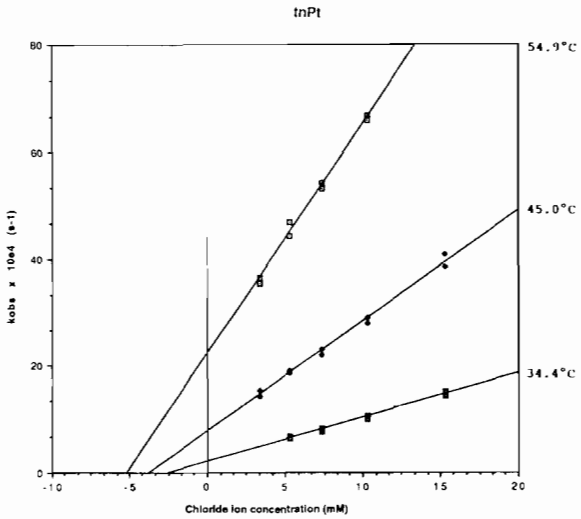


Fig. 2. Plots of k_{obs} vs. $[Cl^-]$ for the chloride ion anation of $PtCl(tn)_2(OH_2)^+$ ($I=0.1$ M).

For two of the systems described here (*cis*-DDP and $PtCl_2$(*RR*-chxn)), we have measured k_1 using first-order procedures as well as from the intercepts (Table 3) with reasonable agreement between the two methods.

Temperature variation

Plots such as Figs. 1–3 give estimates of K_n (= intercept/slope) at the temperature of the measurements. Consequently, activation parameters ($\Delta H^{\#}_{\pm n}$ and $\Delta S^{\#}_{\pm n}$) for k_n and k_{-n} can also be estimated from the temperature variation of the

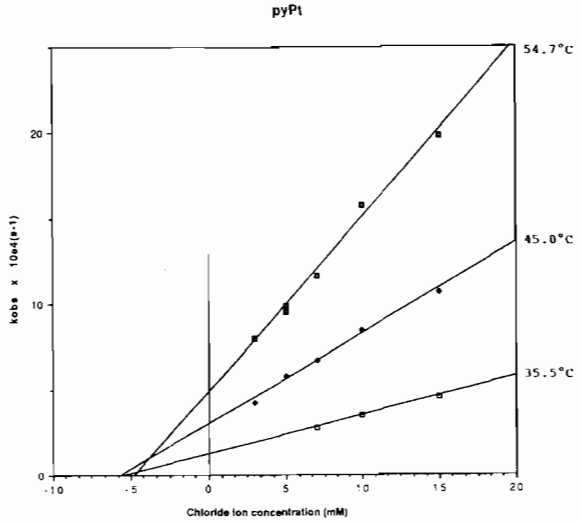


Fig. 3. Plots of k_{obs} vs. $[Cl^{-1}]$ for the chloride ion anation of *cis*-$PtCl(py)_2(OH_2)^+$ ($I=0.1$ M).

TABLE 7. Quasi-thermodynamic parameters (298.2 K, $I=0.1$ M) associated with eqn. (1)

Complex	$10^3 \times K_1$	ΔG_1° or $-\Delta G_{-1}^\circ$ (kJ mol^{-1})	ΔH_1° or $-\Delta H_{-1}^\circ$ (kJ mol^{-1})	ΔS_1° or $-\Delta S_{-1}^\circ$ (J K^{-1} mol^{-1})
cis-NH_3 [a]	10.1	11.4	+8±6	−13±18
cis-NH_3	6.82	12.4	+15±6	+8±18
cis-py	6.23	12.6	−7±8	−66±24
Me_2tn	3.22	14.2	+11±12	−12±36
tn	1.62	15.9	+32±5	+54±15
chxn	1.81	15.6	+27±8	+39±24
en	1.52	16.1	+27±6	+38±18
trans-NH_3	0.622	18.3	+7±12	−39±36

[a] $I=1.0$ M, ref. 19.

slope, respectively, using the Arrhenius expression [32] (Table 4).

If K_n were independent of temperature, the k_{obs} versus $[Cl^-]$ plots would generate a set of lines with a common focal point at some negative intercept on the abcissia, and ΔH° for the reaction would be zero. The data for *trans*-$PtCl_2(NH_3)_2$ (Fig. 1) approximates this situation.

However, as the temperature variation of K_n determines the enthalpy (ΔH°) of the reaction, two further situations are possible depending on the sign of ΔH°. These are shown in Fig. 2 ($PtCl_2$(tn) data) where ΔH° is positive (endothermic), and possibly in Fig. 3 (*cis*-$PtCl_2(py)_2$ data) where ΔH° is negative (exothermic) but with an experimental uncertainty that would also allow the $\Delta H^\circ = 0$ situation. For

endothermic reactions, the activation enthalpy ($\Delta H^{\#}$) associated with k_n is greater than that associated with k_{-n}. Other quasi-thermodynamic parameters (ΔG°_{298} and ΔS°_{298}) follow from the values of K_n (298) and ΔH°_{298} (Table 7).

Ionic strength variation

The reactions associated with k_{-1} (and k_{-2}) are reactions between ions of opposite charge and thus should exhibit a negative salt effect [33]. (A rate decrease with increasing ionic strength.) Our measurements here show this is indeed the case (Table 5), and confirm the negative salt effect reported for k_{-1} associated with *trans*-$PtCl_2(NH_3)_2$ [24]. All studies associated with k_1 indicate that the reaction rate is independent of ionic strength [19], as is to be expected for a reaction between two neutral molecules (L_2PtCl_2 and H_2O).

Discussion

Structural relationships

During the course of screening a large number of L_2PtCl_2 complexes for anti-cancer activity, a series of empirical *structural* requirements have emerged [4, 6, 7, 34]. These, in turn, have led to speculation as to why such structural features are important.

(a) Platinum should be in the +II oxidation state and have a four-coordinate, square-planar configuration. Some six-coordinate, octahedral, platinum(IV) complexes do appear to have anti-cancer activity [6] but there is speculation that reduction to Pt(II) is a precursor reaction.

(b) A comparison of *cis*- and *trans*-isomeric square-planar platinum(II) complexes indicates that the *cis*-isomers are much more effective anti-cancer agents [6].

(c) The most active *cis*-isomers are uncharged. In practise, this means that the complex must have two neutral and two uninegative donor atoms [15].

(d) The lability of the negative donor atoms is of considerable importance and Cl^- or RCO_2^- appear to be the most suitable.

(e) The activity of the complex is at its maximum when the neutral ligands are NH_3, primary or secondary amines. An NH proton appears to be a prerequisite for a good anti-cancer response.

Such structure reactivity correlations need not have any real validity [34]. The basis for this statement is that we still do not have any proper understanding of the pathways by which these platinum-containing drugs inhibit replicating DNA. In fact, all of the generalisations (a)–(e) have some exceptions, or can be questioned as to their relevance.

Equilibrium relationships

If a platinum-containing compound is to be an effective anti-cancer agent, then a reasonable concentration of substitution-labile platinum-containing material must be available at the site of the growing tumour. Although neither *cis*-$PtCl(OH)(NH_3)_2$ nor *cis*-$PtCl_2(NH_3)_2$ are considered to be able to react directly with replicating DNA, both can provide a source of $PtCl(NH_3)_2(OH_2)^+$. The amount of 'active platinum' available from any particular diaminedichloroplatinum(II) complex thus depends on the extent to which the equilibria represented by eqn. (1) occur, i.e. k_1, k_{-1}, K_1 and pK_a^3.

With these features in mind, it is of interest to see if variations of pK_a^3 or K_1 have been reported for the types of dichloroplatinum(II) that are both active and inactive in human cancer therapy.

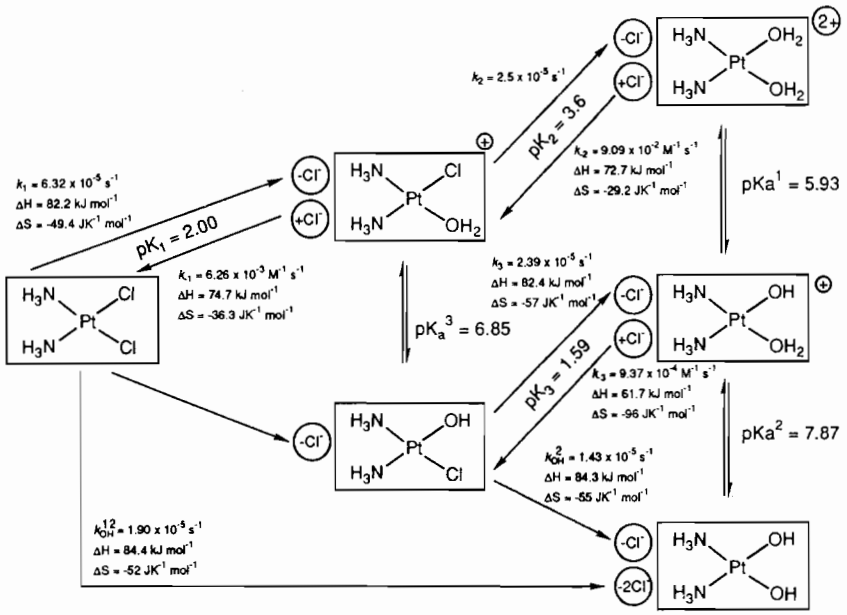
The acid–base equilibrium constant (K_a^3) in (1) is quite difficult to measure because of competing anation of the (aqua)(chloro) by chloride ion during any potentiometric titration procedure. It is only recently that values of $pK_a^3 = 6.85$ (25 °C) for *cis*-DDP and $pK_a^3 = 5.63$ (25 °C) for *trans*-DDP have been determined [13, 35]. pK_a^3 can also be estimated as being equal to the pH at which exactly 1.0 mole of NaOH is consumed/mole of Pt(II) after complete hydrolysis of *cis*-DDP. This procedure gives a value of $pK_a^3 = 7.25$ at 45 °C ($I = 0.2$ M) [14].

For other complexes, data for pK_a^3 are not available and some workers [36, 37] have used the arithmetic mean of K_a^1 and K_a^2 (Scheme 1) as an estimate. We can thus extend K_a^3 comparisons to $PtCl_2(en)$ where $pK_a^3(\text{est.}) = 6.7$ [12] a value not sensibly different from *cis*-DDP.

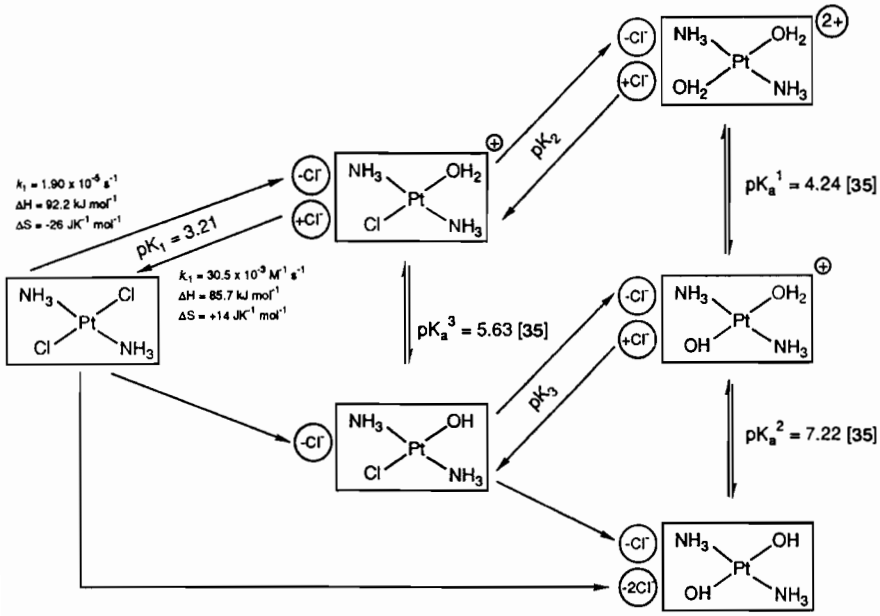
In the absence of more precise measurements, we are forced to use the speculative assumption that values for pK_a^3 will be similar (6.7 ± 0.2) for all the systems used in this investigation, except for *trans*-$PtCl_2(NH_3)_2$. A pK_a^3 value of 6.7 means that at physiological pH (=7.4) a '(chloro)(aqua)' solution will contain about 78% (chloro)(hydroxo), but with $pK_a^3 = 5.63$ (the *trans*-DDP value) the (chloro)-(hydroxo) proportion increases to about 98%.

Nevertheless the substitution inert (chloro)-(hydroxo) remains as an important source of active platinum, for, as the (chloro)(aqua) is removed from the system by complex formation, it will be constantly replaced from the (chloro)(hydroxo) pool in the proportion controlled by pK_a^3.

We now turn our attention to K_1, the chloride ion dependent hydrolysis equilibrium constant. K_1 data are relatively easy to determine as K_1 equals the forward and reverse rate ratio ($K_1 = k_1/k_{-1}$) and is a measure of the extent to which the production of $L_2PtCl(OH_2)^+$ will proceed from the initial



Scheme 1. Rate and equilibrium constants for the hydrolysis of *cis*-DDP ($I = 1.0$ M, $T = 25$ °C).



Scheme 2. Rate and equilibrium constants for the hydrolysis of *trans*-DDP ($I = 0.1$ M, $T = 25$ °C).

dichloro. If K_1 is small, then only a small amount of 'active platinum' will be available for reaction.

Our data for rates of the hydrolysis of *trans*-$PtCl_2(NH_3)_2$ and the chloride anation of *trans*-$PtCl(OH_2)(NH_3)_2^+$ are presented in Table 4 with $k_1 = 1.9 \times 10^{-5}$ s^{-1} and $k_{-1} = 305 \times 10^{-4}$ M^{-1} s^{-1} (0.1 M $HClO_4$, 25 °C), giving $K_1 = 0.622 \times 10^{-3}$. A previous estimate [24], gave values of $k_1 = 6.62 \times 10^{-5}$ s^{-1}, $k_{-1} = 4630 \times 10^{-4}$ M^{-1} s^{-1} and $K_1 = 0.142 \times 10^{-3}$ (0.01 M HNO_3, 25 °C). This latter study measured the extent of reaction to equilibrium (about 40%) by chloride release titration from *trans*-$PtCl_2(NH_3)_2$ at 0.4 mM, and analysing the data in terms of a system where the forward reaction is first order and the back reaction is second order. Other estimates of k_1 are 9.8×10^{-5} s^{-1} (H_2O, 25 °C) [38], 4×10^{-5} s^{-1} (pH = 11, 25 °C) [39] and 48.4×10^{-5} (DMSO, 26 °C) [40].

It is obvious that k_1 needs to be established with more certainty as others [41] have assumed k_1

($trans$) > k_1(cis), but the problem is made difficult by the low aqueous solubility of *trans*-$PtCl_2(NH_3)_2$ [42] and the unfavourable equilibrium.

A consideration of the values of K_1 (298.2 K, $I = 0.1$ M) for *cis*- and *trans*-$PtCl_2(NH_3)_2$ (viz. 6.31×10^{-3} and 6.22×10^{-4}, respectively (Table 7)) implies that there will be less $PtCl(NH_3)_2(OH_2)^+$ produced from the *trans*-isomer than from the *cis*. In the absence of any additional chloride ion, $I = 0.1$, $T = 25$ °C and with an initial $[PtCl_2(NH_3)_2]$ of 1 mM, the calculated equilibrium amounts of $PtCl(NH_3)_2(OH_2)^+$ produced are 88% and 54% for the *cis*- and *trans*-isomers, respectively. The relative amount of (chloro)(aqua) produced will increase as the $[Pt]_i$ is decreased and decrease with the addition of background chloride ion.

Thus we believe it is the unfavourable equilibrium concentration of *trans*-$PtCl(NH_3)_2(OH_2)^+$ relative to the *cis*-isomer, and the much lower pK_a[3] value, that prevents this species from functioning as an effective anti-tumor agent under physiological conditions.

There is just not sufficient *trans*-$PtCl(NH_3)_2(OH_2)^+$ available for effective attack on replicating DNA. Nevertheless, if reasonable amounts of *trans*-$PtCl(NH_3)_2(OH_2)^+$ can be supplied, then this isomer, too, can inhibit DNA synthesis [41, 43].

Scheme 2 summarises the available data for *trans*-$PtCl_2(NH_3)_2$. The other complexes listed in Table 7 all have K_1 values in the range $(1\text{–}6) \times 10^{-3}$ and on this basis should be slightly less effective anti-tumour agents than *cis*-$PtCl_2(NH_3)_2$.

Ionic strength dependence

K_1 is the ratio of the forward (k_1) and reverse (k_{-1}) rate constants associated with eqn. (1). The forward rate constant, k_1 is a reaction between two neutral species and has been found to proceed at a rate independent of ionic strength [19]. The reverse reaction is, however, a reaction between two singly charged species of opposite sign, and thus k_{-1} should decrease as the ionic strength increases [33]. This has been established previously for *trans*-$PtCl_2(NH_3)_2$ [24] and Table 5 reports our data for the *cis*-isomer, where again a negative salt effect is observed for k_{-1}.

Consequently K_1 will also be ionic strength dependent with a value increasing with increasing I (Table 4). We are now faced with the question as to the most appropriate value of K_1 for physiological conditions. Fortunately, the ionic strength variation of K_1 is not large (Table 4) and extracellular blood plasma has $I \sim 0.1$ M [44], thus values of K_1 obtained at 37 °C, $I = 0.1$ M are probably satisfactory for the biological situation.

The lability of the coordinated water molecule

The magnitude of k_{-1} gives a relative order of lability of the coordinated water molecule to nucleophilic substitution by chloride ion. Similar activation parameters (Table 4) associated with k_{-1}, for the systems under investigation, allow a direct comparison of the rate constants determined at any particular temperature. Thus, the second most labile system is *trans*-$PtCl(NH_3)_2(OH_2)^+$ and the least labile is the corresponding *cis*-isomer. The relatively substitution inert water molecule on *cis*-$PtCl(NH_3)_2(OH_2)^+$ gives this species a greater lifetime in the intercellular media, which is rich in spurious nucleophiles, and allows a greater concentration to be available for the target DNA. The small amount of labile *trans*-$PtCl(NH_3)_2(OH_2)^+$ available is probably more susceptible to wastage, although nucleophile discrimination for the *trans*-isomer has not yet been established [22].

Conclusions

Most of the discussion so far has concentrated on the *cis*- and *trans*-$PtCl_2(NH_3)_2$ systems. One other complex of particular interest is *cis*-$PtCl_2(py)_2$. This species lacks an N–H proton but there are confusing and conflicting reports as to the *in vitro* versus *in vivo* activity of the *cis*- and *trans*-$(py)_2$ isomers [45]. Our results indicate that the kinetic and equilibrium properties of *cis*-$PtCl_2(py)_2$ are quite similar to those of *cis*-$PtCl_2(NH_3)_2$ (Table 4) and we suspect that it is the rather low aqueous solubility of this complex that limits the anti-tumor activity.

All the other L_2PtCl_2 (L_2 = bidentate diamine) complexes studied here have reasonable anti-tumor activity and our kinetic and equilibrium measurements (Table 4) are in accord with these observations.

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