Synthesis and *in Vitro* and *in Vivo* Antitumor Activity of a Series of *Trans* Platinum Antitumor Complexes

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The synthesis of a series of platinum complexes of *trans* coordination geometry [centered around the general formula, *trans*-ammine(amine)dichlorodihydroxoplatinum(IV) plus corresponding tetrachloroplatinum(IV) or Pt(II) counterparts] is described as part of a drug discovery program to identify more effective platinum-based anticancer drugs, particularly targeted toward the circumvention of resistance to cisplatin. Complexes have been evaluated for antitumor activity using *in vitro* and *in vivo* tumor models. *In vitro* against a panel of cisplatin-sensitive and -resistant human tumor cell lines (predominantly ovarian), many of the *trans* platinum complexes studied (e.g., **1**, R = cyclohexyl) exhibited comparable potency to cisplatin and also overcame acquired cisplatin resistance, where resistance was due mainly to either reduced drug uptake or enhanced platinum–DNA adduct removal. Moreover, 14 *trans* complexes showed significant *in vivo* antitumor activity against the subcutaneous murine ADJ/PC6 plasmacytoma model; all were platinum(IV) complexes, 13/14 possessing axial hydroxo ligands the other possessing axial ethylcarbamato ligands. Where tested, all of their respective platinum(II) or tetrachloroplatinum(IV) counterparts were inactive. Notably, three dihydroxoPt-(IV) complexes (**18**, **29**, **34**) (R = c-hexyl, c-heptyl, and 1-adamantyl) retained some efficacy against a cisplatin-resistant variant of the ADJ/PC6. Compounds **18** {*trans*-[$PtCl_2(OH)_2NH_3$-(RNH_2)]} R = c-C_6H_{11}, **22**, R = Me_3C, **27**, R = *n*-C_6H_{13}, **28**, R = $PhCH_2$, and **36** {*trans*-[$PtBr_2(OH)_2NH_3$(c-$C_6H_{11}NH_2$)]} also produced evidence of antitumor activity (>5 days growth delay) against subcutaneously grown advanced stage human ovarian carcinoma xenografts. These data demonstrate that a series of *trans*-ammine(amine)dichlorodihydroxoplatinum(IV) complexes are active *in vivo* against both murine and human subcutaneous tumor models and represent potential leads to a new generation of platinum-based anticancer drug.

Introduction

Since the successful introduction of cisplatin into oncology practice over 20 years ago, many analogs have been synthesized in attempts to either overcome the particularly toxic side effects of the parent drug (especially nephrotoxicity) or to broaden its clinical spectrum of antitumor activity. Our previous studies have led to the introduction of the less toxic analog, diammine-1,1-cyclobutanedicarboxylatoplatinum(II), carboplatin,[1] and, more recently, the first orally administrable platinum drug, bis(acetato)amminedichlorocyclohexylamineplatinum(IV), JM216,[2] into clinical practice. As tumor resistance to cisplatin and carboplatin commonly limit their clinical efficacy, our current efforts are focused on the urgent need to discover new platinum-based agents capable of overcoming resistance.

There is compelling evidence to suggest that both cisplatin and carboplatin (which both possess *cis*-ammine carrier ligands) exert their antitumor effects through binding to DNA to produce a similar spectrum of monofunctional and bifunctional (both intra- and interstrand) adducts.[3,4] In addition, since enhanced removal of cisplatin (and carboplatin)-induced DNA adducts has been shown to contribute to tumor cell resistance to these drugs,[5] a logical strategy aimed at the circumvention of resistance might be to rationally design novel platinum complexes which interact with DNA in a manner distinct from that of the parent drugs. One platinum agent that is known to possess DNA binding properties distinct from those of cisplatin is its *trans* isomer, transplatin.[6–8] However, early structure–activity relationship studies of platinum-based coordination complexes showed that transplatin (as well as other *trans* platinum isomers studied), is inactive as an antitumor agent.[9] Regardless of this finding, in recent years there has been renewed interest in attempts to obtain activity for the *trans* geometry of platinum complexes.[10–14]

Our recently reported studies[14] have identified JM335 [compound **18**; *trans*-amminedichlorocyclohexylamine-dihydroxoplatinum(IV)] as a promising lead to the further development of active *trans* platinum complexes. This study reports the synthesis and comparative *in vitro* and *in vivo* antitumor activity (against murine ADJ/PC6 and human ovarian tumor models, both sensitive and resistant to cisplatin) of a novel series of over 25 *trans* platinum complexes, including JM335 (**18**). Comparison has been made with cisplatin and transplatin and various *cis* congeners.

Chemistry

The synthesis of *trans* isomers of platinum(II) complexes (see Figure 1) followed a well-established route[15] which utilizes the difference in the *trans* effect of halide and amine ligands in platinum(II) complexes to achieve

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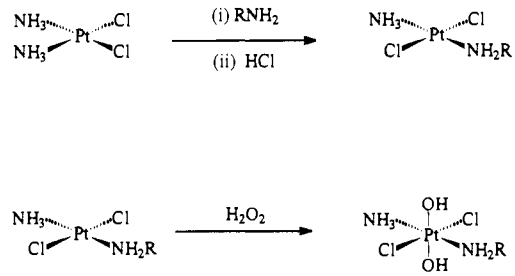

Figure 1. Synthesis of *Trans* Platinum Complexes.

selective substitution and thus control of stereochemistry. Variations in the experimental conditions established for *trans*-[$PtCl_2(NH_3)_2$] are required, however, due to the low water solubility of the higher amines and their platinum complexes. Thus, while purely aqueous methods work satisfactorily for C_1-C_6 amines, mixed aqueous/organic systems were more successful for C_7-C_{10} amines. For secondary amines and for bis-amine complexes, reaction in a polar organic solvent such as *N,N*-dimethylacetamide proved necessary.

The oxidation of platinum(II) complexes with hydrogen peroxide yields platinum(IV) complexes in which the stereochemistry of the platinum(II) complex is retained and *trans* hydroxo ligands are added.[16-18] The isomeric structure of the reaction product was confirmed by X-ray crystallography for [$PtCl_2(OH)_2(NH_3)_2$].[18] While the isomerization of *trans,trans,trans*-[$PtCl_2(OH)_2(NH_3)_2$] to *cis,cis,trans*-[$PtCl_2(OH)_2(NH_3)_2$] on heating in aqueous solution was reported in that study, there is no evidence for isomerization of the amine ligands in such complexes under mild conditions. Distinction between the two *trans* amine isomers is difficult in the absence of both compounds but, as the majority of platinum(IV) complexes prepared for this study were isolated directly from peroxide solution, it is believed that they have *all-trans* stereochemistry. This is supported by evidence from reaction of **18** with acetic anhydride. The assignment of the *cis,cis,trans* stereochemistry to **40** is supported by IR and Raman spectral data (IR 358, Raman 367, 359 cm^{-1} symmetric Pt–Cl; IR 301, Raman 301 cm^{-1} asymmetric Pt–Cl) which suggest *cis* chloride ligands. Chromatographic analysis of the reaction liquor during the course of the reaction indicates that the *all-trans* isomer of **40** is formed as the initial product and subsequently rearranges. As the reaction of **18** with acetic anhydride is not expected to lead to any change in stereochemistry[19] this supports the assignment of *all-trans* stereochemistry to **18** and the related compounds.

As for the preparation of the platinum(II) complexes, the reaction conditions for oxidation must be altered to take account of the hydrophilicity of the platinum compounds. *N,N*-Dimethylacetamide is generally a more suitable solvent than water for the hydrophobic complexes of the higher amines.

Results

***In Vitro* Growth Inhibitory Properties.** The effect of each *trans*-dihydroxoplatinum(IV) complex (plus some comparative data for corresponding *cis* isomers, *trans*-tetrachloro-Pt(IV) counterparts, cisplatin and transplatin) on the *in vitro* growth inhibition of a panel of human ovarian carcinoma cell lines is shown in Tables 1 and 2. This panel contains examples of tumor cells possessing either intrinsic (HX/62 and SKOV-3) or acquired (41McisR, CH1cisR, and A2780cisR) resistance to cisplatin and has been used previously in attempts to identify novel platinum complexes capable of circumventing resistance to cisplatin.[2,12,20,21]

Results (Table 1) show that while transplatin (mean IC_{50} of 127 μM) was an average of over 40-fold less potent than cisplatin (mean IC_{50} of 3.1 μM), the remaining four *trans* complexes (**1**, **18**, **21**, **22**) showed growth inhibitory potencies similar to cisplatin. Moreover, compounds **18**, **21**, and **22** were generally more potent than their corresponding *cis* isomers, especially against the two intrinsically cisplatin-resistant HX/62 and SKOV-3 cell lines [the Pt(II) complex **1** was less potent than its *cis* isomer]. For example, **22** (the mean IC_{50} of 1.9 μM) was 28- and 7-fold more potent than its *cis* isomer (mean IC_{50} of 13.8 μM) against the HX/62 and SKOV-3 cell lines, respectively. In addition, the four active *trans* complexes showed a similar pattern of response against the three cell lines possessing acquired cisplatin resistance; no cross-resistance was observed to 41McisR, partial cross-resistance was observed to CH1cisR, and relatively high levels of cross-resistance were observed to A2780cisR.

Table 2 contains examples of analogous Pt(II) and Pt(IV) (dihydroxo or tetrachloro) complexes. While, in general, the *in vitro* growth inhibitory potency of Pt(II) and Pt(IV) counterparts was similar (e.g., **10** *versus* **27**, R = hexyl; **11** *versus* **29**, R = cycloheptyl; **13** *versus* **32**, R = *N*-methylcyclohexyl), in two instances (**7** *versus* **24**, R = diethyl and **12** *versus* **31**, R = quinuclidine) the Pt(II) complex was inactive (IC_{50} > 50 μM). Interestingly, the tetrachloro-Pt(IV) counterpart within the quinuclidine series (**39**) showed a flat IC_{50} response across all

Table 1. *In Vitro* Growth Inhibition of Some *Cis*, *Trans* Pairs of Platinum Complexes[a]

	cell line (IC_{50}, μM)							
compound	HX/62	SKOV-3	41M	41McisR	CH1	CH1cisR	A2780	A2780cisR
cisplatin	12.6 ± 2.9	4.4 ± 3.1	0.26 ± 0.078	1.2 ± 0.4 (4.7)	0.11 ± 0.02	0.71 ± 0.2 (6.4)	0.33 ± 0.1	5.2 ± 1.3 (15.7)
transplatin	245 ± 45	255 ± 77	57 ± 16	70 ± 13 (1.2)	30 ± 3.8	68.5 ± 11 (2.3)	32 ± 8	260 ± 46 (8.1)
cis-[$PtCl_2(NH_3)$(c-$C_6H_{11}NH_2$)]	2.0	2.1	0.26	0.28 (1.1)	0.066	0.38 (5.7)	0.083	0.4 (4.8)
1	4.1	7.2	1.7	1.5 (0.9)	1.1	3.3 (3)	1.2	5.7 (4.7)
cis-[$PtCl_2(OH)_2(NH_3)$-(c-$C_6H_{11}NH_2$)]	30 ± 2.7	16.6 ± 0.8	3.5 ± 0.3	3.2 ± 0.2 (0.9)	0.57 ± 0.05	2 ± 0.3 (3.5)	0.8 ± 0.27	1.9 ± 0.1 (2.4)
18	4.4 ± 0.5	6 ± 0.9	1.3 ± 0.13	1.4 ± 0.12 (1.1)	1.1 ± 0.33	2.1 ± 0.2 (1.9)	0.42 ± 0.07	3.0 ± 0.26 (7.1)
cis-[$PtCl_2(OH)_2(NH_3)$-(Me_2CHNH_2)]	50	30.5	3.9	9.2 (2.4)	1.4	4.9 (3.5)	0.99	7.2 (7.3)
21	14	9.2	3.2	4.6 (1.4)	2.4	5.5 (2.3)	0.54	4.7 (8.7)
cis-[$PtCl_2(OH)_2(NH_3)$-(Me_3CNH_2)]	50	34	4.5	8 (1.8)	1.1	3.4 (3.1)	0.8	8.5 (10.7)
22	1.8	4.7	1.1	0.9 (0.8)	0.74	1.62 (2.2)	0.21	4.2 (20)

[a] Values are IC_{50} in μM (96 h drug exposure). Values are means from at least two experiments. Values in parentheses are resistance factors (RF: IC_{50} resistant line/IC_{50} parent line). Where indicated, errors = SD, n = 3.

Table 2. *In Vitro* Activity of Some *Trans* Platinum(II) and Platinum(IV) Complexes[a]

compound	cell line (IC_{50}, μM)							
	HX/62	SKOV-3	41M	41McisR	CH1	CH1cisR	A2780	A2780cisR
				(A) Pt(II) Complexes				
7	>50	>50	>50	>50	>50	>50	>50	>50
10	4.8	7.1	4.5	4.1 (0.9)	4	4.4 (1.1)	2.3	4.8 (2.1)
11	4.2	10	2.4	1.7 (0.7)	4.3	4.7 (1.1)	1.3	4.6 (3.4)
12	>50	>50	>50	>50	>50	>50	26	>50
13	2.2	3.9	0.78	0.48 (0.6)	0.38	1.1 (2.8)	0.46	3.6 (7.8)
15	0.8	1.5	0.49	1.2 (2.4)	0.21	0.43 (2)	0.4	1.4 (3.6)
17	10.9	11.5	4	3.2 (0.8)	4	5 (1.2)	1.9	5.4 (2.8)
				(B) Pt(IV) Complexes				
19	110	72	5.4	33 (6.1)	12.5	30.5 (2.4)	6.2	64 (10.3)
20	42	45	9.4	13.3 (1.4)	10.3	14 (1.4)	4.5	26.5 (5.9)
23	100	47	26	54 (2.1)	17.3	28.3 (1.6)	10.9	47 (4.3)
24	3.3	12.1	1.8	0.76 (0.4)	3.3	4.5 (1.4)	1.9	11.6 (6.2)
25	10.2	7	1.1	1.3 (1.2)	1.1	1.9 (1.7)	0.95	5.2 (5.5)
26	12.5	13.5	5.5	4.3 (0.8)	4.8	4.8 (1)	2.7	5.7 (2.1)
27	14.2	10.3	4.6	4.4 (1)	3.7	5.4 (1.4)	1.9	6.4 (3.3)
28	10.2	8.2	1.3	1.3 (1)	1.1	1.9 (1.7)	1.1	4.2 (4)
29	3.4	3.9	1.3	1.4 (1.1)	1.1	1.7 (1.5)	0.61	2.5 (4.2)
30	5.6	4.4	1.4	1.5 (1)	1.4	1.7 (1.2)	0.25	3 (12)
31	40	27	14.4	9.5 (0.7)	10.2	14.3 (1.4)	3.2	16 (5)
32	4.1	6.9	2.3	1.6 (0.7)	1.3	2.9 (2.2)	1.3	5 (3.7)
33	1.3	1.8	0.48	1 (2.1)	0.36	0.55 (1.5)	0.4	2.2 (5.5)
34	1.2	2.5	0.84	1.3 (1.5)	0.44	0.62 (1.4)	0.49	1.6 (3.3)
35	6.9	2.5	0.8	1.2 (1.5)	0.55	0.6 (1.1)	0.8	3.2 (4)
36	3.2	4.7	2.1	1.7 (0.8)	1.6	2.8 (1.7)	1.4	3.6 (2.5)
37	4.4	9.3	3.1	4.1 (1.3)	3.9	4.7 (1.2)	1.5	5.3 (3.4)
38	11	14.5	5.3	4.1 (0.8)	4.7	4.8 (1)	3.2	5.6 (1.7)
39	23	29	16.5	16 (1)	15.5	15.5 (1)	14	16 (1.1)
40	3.1	4.8	1.3	1.2 (0.9)	1.1	1.5 (1.4)	0.3	3 (10)
41	14	19	6.2	4.3 (0.7)	4.6	5.6 (1.2)	3.2	10.6 (3.3)

[a] Values are IC_{50} in μM (96 h drug exposure). Values in parentheses are resistance factors (RF: IC_{50} resistant line/IC_{50} parent line).

Table 3. *In Vivo* Antitumor Activity of Some *Cis* and *Trans* Pairs of Platinum Complexes Using a Single ip Administration against the Murine ADJ/PC6 sc Plasmacytoma

compound	LD_{50} (mg/kg)	ED_{90} (mg/kg)	therapeutic index
cisplatin	11.3	1.6	7.1
transplatin	32	–[a]	–
cis-[$PtCl_2(NH_3)$(c-$C_6H_{11}NH_2$)]	17.5	1.4	13
1	35	–	–
cis-[$PtCl_2(OH)_2(NH_3)$(c-$C_6H_{11}NH_2$)]	17.5	1.4	12.5
18	8.8	0.76	11.6
cis-[$PtCl_2(OH)_2(NH_3)(Me_2CHNH_2)$]	35	3	11.6
21	18	10.5	1.7
cis-[$PtCl_2(OH)_2(NH_3)(Me_3CNH_2)$]	35.5	4.9	7.2
22	35	4.7	7.4

[a] – = 90% tumor inhibition not obtained.

eight human ovarian carcinoma cell lines with no cross-resistance to all three acquired cisplatin resistant cell lines. For the cyclohexylamine series, **1** [dichloro-Pt(II)], **17** [dibromo-Pt(II)], **18** [dichlorodihydroxo-Pt(IV)], **36** [dibromodihydroxo-Pt(IV)], and **37** [tetrachloro-Pt(IV)] mean IC_{50} values were generally similar: 3.2, 5.7, 2.4, 2.6, and 4.5 μM, respectively. Typically, the *trans* complexes exhibited *in vitro* IC_{50} values in the low micromolar range and showed no cross-resistance for 41McisR, a low level of cross-resistance for CH1cisR (resistance factor of around 1.5), and a higher level of cross-resistance for A2780cisR.

***In Vivo* Antitumor Activity.** The *in vivo* antitumor activity of the pairs of isomers described in Table 1 is shown in Table 3 for a single intraperitoneal administration to animals bearing the murine ADJ/PC6 plasmacytoma subcutaneous tumor model. As shown previously, transplatin was toxic to the animals but did not produce any antitumor effect even at a toxic dose.[9] As expected, all of the *cis* complexes including cisplatin exhibited activity against this highly cisplatin-sensitive tumor model. However, the three *trans*-dihydroxo-Pt(IV) complexes, **18**, **21**, and **22**, all showed *in vivo* antitumor activity. Compound **18** (R = cyclohexyl) showed a similar toxicity to cisplatin and its *cis* isomer and an ED_{90} of less than 1 mg/kg; compound **22** (R = *tert*-butyl) was less toxic and less active (higher ED_{90}) than **18**; compound **21** (R = isopropyl) was less effective (therapeutic index, TI, LD_{50}/ED_{90} of 1.7). The corresponding Pt(II) complex to **18** (compound **1**) did not exhibit any efficacy.

Table 4 summarizes the *in vivo* ADJ/PC6 data for a further 22 *trans* platinum complexes. Eleven of these complexes showed *in vivo* antitumor activity. Notably, 10 of these active complexes were dihydroxo-Pt(IV) compounds, the exception being the ethylcarbamato-Pt(IV) complex **41** (R = cyclohexyl). As for the Pt(II) and Pt(IV) counterparts **1** and **18** described in Table 3, **13** was inactive while its Pt(IV) counterpart **32** (R = *N*-methylcyclohexyl) was active (TI of 3). In addition, the tetrachloro-Pt(IV) (**37**) and bis(acetato)-Pt(IV) (**40**) counterparts of **1** and **18** were inactive. However, not all of the dihydroxo-Pt(IV) complexes were active; generally, where R = a short aliphatic chain, there was a loss of *in vivo* activity (e.g., **19** R = methyl; **20** R = ethyl; **24** R = diethyl). In addition, activity was lost where R = morpholino (**23**), pyridine (**26**), and quinuclidine (**31**). From Tables 3 and 4, a total of 14 active *trans* complexes has been described; those possessing alicyclic R substituents (cyclopentyl, **25**; cyclohexyl, **18**, **36**; and cycloheptyl, **29**) were most active (ED_{90} of <3 mg/kg and a therapeutic index >5).

A number of *trans* complexes have also been evaluated *in vivo* against an acquired cisplatin resistant subline of the ADJ/PC6 plasmacytoma.[22] Notably, three

Table 4. *In Vivo* Antitumor Activity of Some *Trans* Platinum(II) and Platinum(IV) Complexes Using a Single ip Administration against the Murine ADJ/PC6 sc Plasmacytoma

compound	LD_{50} (mg/kg)	ED_{90} (mg/kg)	therapeutic index
	(A) Pt(II)		
13	71	–[a]	–
	(B) Dihydroxo-Pt(IV)		
19	8.8	–	–
20	50	–	–
23	82	94	0.9
24	35	–	–
25	15	2.5	6
26	42	–	–
27	>100	39	>2.6
28	71	22	3.2
29	42	2.9	14.5
30	17.5	4.9	3.6
31	7.4	–	–
32	29	9.4	3
33	29	9.6	3
34	115	22	5.2
35	>100	40	>2.5
36	17.5	2.7	6.5
	(C) Tetrachloro-Pt(IV)		
37	14.5	–	–
38	>100	–	–
39	17.5	–	–
	(D) Bis(acetato)-Pt(IV)		
40	35	–	–
	(E) Bis(ethylcarbamato)-Pt(IV)		
41	465	185	2.5

[a] – = 90% tumor inhibition not obtained.

Table 5. *Trans* Compounds Exhibiting Additional *in Vivo* Antitumor Activity

(A) Against the Acquired Cisplatin-Resistant ADJ/PC6 Plasmacytoma (ADJ/PC6cisR)

compound	LD_{50} (mg/kg)	ED_{90} (mg/kg)	therapeutic index
cisplatin	11	–	–
cis-$[PtCl_2(OH)_2(NH_3)(c\text{-}C_6H_{11}NH_2)]$	8.8	–	–
18	10.5	5.6	1.9
29	29	18	1.6
34	140	46	3

(B) Against Various Human Ovarian Carcinoma Xenografts

compound	dose (mg/kg)[a]	tumor	growth delay[b] (days)
18	2	HX/110	25.5
18	2	PXN/109T/C	17
18	2	PXN/109T/CC	5.4
18	2	OVCAR-3	12
18	4	OVCAR-3	16.8
18	2	PXN/100	23.9
18	4	PXN/100	63.7
18	4	SKOV-3	7
22	10 (×2)	PXN/109T/C	13.4
24	24 (×2)	PXN/109T/C	3.4
27	40	PXN/109T/C	8.8
27	50	PXN/109T/CC	4.5
27	60 (×1)	SKOV-3	10.4
28	20	PXN/109T/C	8.3
32	8	PXN/109T/CC	1.1
35	50	PXN/109T/CC	1.8
36	16 (×2)	PXN/109T/C	10.7
36	8	PXN/109T/CC	3.0
39	4	PXN/109T/C	0

[a] Doses administered ip weekly for 4 weeks unless otherwise indicated in parentheses. [b] Growth delay = difference in time required for treated versus control tumors to double in starting volume.

complexes (again all dihydroxo-Pt(IV) compounds, **18**, R = cyclohexyl; **29**, R = cycloheptyl; **34**, R = 1-adamantyl) retained activity against this cisplatin-resistant tumor (Table 5). In addition, several *trans* Pt(IV) complexes have been evaluated against examples of our previously described panel of cisplatin-sensitive or -resistant advanced stage human ovarian carcinoma xenografts.[23] As for the ADJ/PC6 data, the only compounds which exhibited any marked antitumor activity (e.g., growth delay of >5 days) were dihydroxo-Pt(IV) complexes; compound **39** (tetrachloro, R = quinuclidine) was inactive. In particular, compound **18** (R = cyclohexyl) exhibited *in vivo* antitumor activity against a broad spectrum of human ovarian carcinoma xenografts with growth delays ranging from 5.4 days for the acquired cisplatin resistant PXN/109T/CC (the *in vivo* counterpart of the CH1cisR cell line) to 64 days for the cisplatin-sensitive PXN/100. In addition, indications of xenograft activity were apparent for **22** (R = *tert*-butyl), **27** (R = hexyl), **28** (R = benzyl), and **36** (R = cyclohexyl, dibromo).

Discussion

The original structure–activity rules for platinum-based antitumor complexes indicated that, where *in vivo* antitumor activity occurred, this was only found in *cis* rather than *trans* isomers; e.g., cisplatin *versus* transplatin.[9] However, in recent years, we[14] and others[10–13] have shown that activity is achievable for the *trans* geometry of platinum coordination complexes, especially in terms of *in vitro* growth inhibition of tumor cells. Since these complexes should bind to DNA in a manner distinct from that of cisplatin (and carboplatin), they provide potentially attractive leads to the discovery of new platinum drugs capable of circumventing resistance to cisplatin (perhaps, particularly, where resistance is mediated through enhanced removal of cisplatin-induced adducts on DNA). This study has investigated the comparative structure–activity relationships for both *in vitro* growth inhibition and *in vivo* antitumor activity for a series of over 25 novel *trans* platinum complexes.

The majority of the novel *trans* complexes investigated exhibited potent *in vitro* growth inhibition against a panel of human ovarian carcinoma cell lines, typically in the low micromolar range as observed for cisplatin. This was in contrast to transplatin which was over 40-fold less potent than cisplatin. Where pairs of *cis* and *trans* isomers were investigated (Table 1), the *trans* platinum(IV) complexes **18**, **21**, and **22** were more potent than their corresponding *cis* isomers and, moreover, appeared to confer some selective antiproliferative effect in two cell lines, HX/62 and SKOV-3, previously described by our studies to be intrinsically resistant to cisplatin.[20] At present, the mechanistic basis of this interesting observation is unclear. In general, the *trans* complexes all showed a similar response against the three examples of acquired cisplatin resistance used in this study; no cross-resistance was observed for 41McisR, a low level of cross-resistance was observed for CH1cisR, and partial to full cross-resistance was observed for A2780cisR. Our previous mechanism-based studies have revealed that resistance to cisplatin in 41McisR is mediated predominantly through reduced platinum accumulation[21,24] and in CH1cisR through enhanced removal of platinum–DNA adducts combined with an increased tolerance to such DNA adducts.[21,25] Acquired

resistance in A2780cisR appears to be multifocal, having been ascribed to reduced drug accumulation and increased levels of glutathione[14] and increased DNA sequence-specific repair of platinum–DNA adducts.[26] Thus, it appears that the *trans* complexes exhibiting potent *in vitro* growth inhibitory properties may be capable of circumventing acquired cisplatin resistance due to reduced drug accumulation and, interestingly, exert some effects against acquired cisplatin resistance due to enhanced DNA repair/enhanced tolerance.

Although all of the novel *trans* complexes except two platinum(II) complexes (**7**, R = diethyl; **12**, R = quinuclidine) were potent inhibitors of at least some human ovarian carcinoma cell lines *in vitro*, clear structure–activity requirements for *in vivo* antitumor activity were apparent. While no Pt(II) or tetrachloro-Pt(IV) complexes were active against the cisplatin-sensitive ADJ/PC6, 13 among a series of 19 dihydroxo-Pt(IV) complexes exhibited *in vivo* antitumor selectivity. The most active (highest therapeutic indices) were those complexes where R = alicyclic substituent; cyclopentyl, (**25**) cyclohexyl (**18**, **36**), or cycloheptyl (**29**). Our preliminary data indicate that these marked differences in *in vivo* antitumor effects may relate to their respective stabilities in tissue culture growth medium at 37 °C. For example, half times (in hours) for the disappearance of parent complex (measured by HPLC) for three of the pairs of isomers in Tables 1 and 3 were as follows: cisplatin, 4.5; transplatin, 1.25; *cis*-[$PtCl_2(NH_3)$(c-C_6H_{11}-NH_2)], 4.5; **1**, 2; *cis,trans,cis*-[$PtCl_2(OH)_2(NH_3)$(c-C_6H_{11}-NH_2)], >96; and **18**, 24. Thus the inactivity of transplatin and the Pt(II) complex **1** may relate to their chemical instability precluding the delivery of active compound to the distant subcutaneous tumor site following *in vivo* ip administration. The additional stability of the dihydroxo-Pt(IV) counterpart **18** appears to be sufficient to confer *in vivo* antitumor effects against a variety of tumor models. Pharmacokinetic and metabolism studies are underway to further support these observations. However, in support of this hypothesis, the platinum(IV) tetrachloro complex, tetraplatin (Ormaplatin; tetrachloro(1,2-diaminocyclohexane)platinum(IV)), has been shown to be rapidly and completely biotransformed to the platinum(II) reduction product within a few minutes in the plasma of drug-treated rats[27] whereas another clinically used platinum(IV) drug, iproplatin (CHIP, *cis*-dichloro-*trans*-dihydroxy-bis(isopropylamine)platinum(IV)), has been shown to be reduced to the platinum(II) species *cis*-dichloro-bis(isopropylamine)platinum(II) but with much slower kinetics.[28]

The 14 *trans* complexes exhibiting antitumor antitumor activity against the ADJ/PC6 [13 dihydroxo-Pt(IV) complexes and the ethylcarbamato-Pt(IV) complex] are the first series of *trans* complexes to demonstrate unequivocal *in vivo* antitumor activity by ip administration to animals bearing distant, advanced stage (20 days following implantation) subcutaneous tumors. While a *trans*-(imino ether)platinum(II) complex has also exhibited *in vivo* antitumor activity,[13] this was achieved using ip administration within one day of ip implantation of P388 leukemia cells. This complex also retained borderline activity against an ascitic P388 leukemia subline possessing acquired resistance to cisplatin (increase in life-span of 133% compared to 170% against the parent tumor). However, of particular note was the observation that three of the *trans* dihydroxo-Pt(IV) complexes studied herein (**18**, R = cyclohexyl; **29**, R = cycloheptyl; and **34**, R = 1-adamantyl), exhibited at least some *in vivo* activity against an acquired cisplatin-resistant subline of the ADJ/PC6 plasmacytoma. While the therapeutic indices for **18** and **29** were lower for animals bearing the acquired resistant subline compared to the parent ADJ/PC6 tumor, notably, the activity of **34** was similar against both tumors. Within a platinum drug discovery program performed over many years and involving >200 novel complexes, these are among very few platinum complexes which have shown activity against this cisplatin refractory tumor model which exhibits complete cross-resistance to carboplatin, iproplatin, and tetraplatin. Our mechanism-based studies with this pair of murine tumors suggest that acquired cisplatin resistance is probably mediated through enhanced removal of DNA–platinum adducts and/or enhanced tolerance to such adducts.[29]

Five of the novel *trans* complexes also exhibited activity against one or more members of our panel of human ovarian carcinoma xenografts, thus providing additional evidence of *in vivo* antitumor efficacy. Somewhat disappointingly, however, activity was generally less than that observed for cisplatin against these tumors; typical growth delays in days for cisplatin (4 mg/kg, q7dx4 schedule) are PXN109T/C, 35; PXN109T/CC, 8; PXN/100, >80; HX/110, 50; OVCAR-3, 40; SKOV-3, 3.[23,30] In addition, where evaluated, the *trans* complexes did not appear to confer marked activity against either the acquired cisplatin resistant counterpart of the CH1cisR cell line (PXN/109T/CC) or the intrinsically cisplatin-resistant SKOV-3. As a weekly ip dosing schedule was used in these experiments (in keeping with our previous evaluations of novel platinum-based drugs) future efforts will investigate whether multiple (i.e., daily) or chronic continuous dosing provides additional antitumor efficacy against these human ovarian carcinoma xenografts. Nonetheless, the five *trans* platinum compounds exhibiting activity (growth delay of >5 days) against these advanced stage subcutaneous xenografts (compounds **18**, **22**, **27**, **28**, and **36**) are the first such *trans* platinum-based complexes to be described.

In summary, contrary to the structure–activity rules established by earlier studies of platinum compounds,[9] a series of *trans* platinum(IV) complexes has been obtained which possesses significant antitumor activity. A number of these complexes have potent *in vitro* growth inhibitory properties and are capable of circumventing some forms of acquired cisplatin resistance *in vitro*. In particular, a series of *trans* platinum(IV) dihydroxo-based complexes exhibit significant *in vivo* antitumor efficacy against a variety of preclinical tumor models. These complexes represent potential leads to a new generation of platinum-based anticancer drugs.

Experimental Section

Chemical Methods. Infrared spectra were recorded as KBr disks using a Perkin-Elmer 1720X spectrometer. Raman spectra were recorded as KBr disks using Innova 70 CRL Ar^+ and 90 CRL Kr^+ lasers with a Spex Ramalog 5 instrument. All organic reagents used in synthesis were obtained from Aldrich and used without further purification. Cisplatin, *cis*-[$PtCl_2(NH_3)_2$], was prepared by the Dhara method[31] and used without recrystallization.

Platinum(II) Complexes. *trans*-Amminedichloro(cyclohexylamine)platinum(II). [*trans*-[$PtCl_2(NH_3)$(c-$C_6H_{11}NH_2$)], 1]. A suspension of *cis*-[$PtCl_2(NH_3)_2$] (5.0 g, 16.7 mmol) in water (50 mL) was treated with cyclohexylamine (3.3 g, 33 mmol). The mixture was stirred and heated at 70 °C until a clear pale yellow solution was obtained and then brought to reflux. After the solution was allowed to cool to room temperature, hydrochloric acid (12 M; 17 mL) was added and the solution heated to reflux for 6 h. After cooling in an ice bath, the product was collected by filtration and washed with water: yield 5.1 g (80%); IR 332 cm^{-1} (Pt–Cl). Anal. ($C_6H_{16}N_2Cl_2Pt$) C, H, N, Cl.

The following compounds were obtained in a similar manner with the indicated variations. ***trans*-amminedichloro(2-propylamine)platinum(II)**[*trans*-[$PtCl_2(NH_3)(Me_2CHNH_2)$], **4**]: yield 56%; IR 331 cm^{-1} (Pt–Cl). Anal. ($C_3H_{12}N_2Cl_2Pt$) C, H, N, Cl. ***trans*-Amminedichloro(2-methyl-2-propylamine)platinum(II)**, [*trans*-[$PtCl_2(NH_3)(Me_3CNH_2)$], **5**]: yield 48%; IR 329 cm^{-1} (Pt–Cl). Anal. ($C_4H_{14}N_2Cl_2Pt$) C, H, N, Cl.

trans-Amminedichloro(cyclopentylamine)platinum(II), [*trans*-[$PtCl_2(NH_3)$(c-$C_5H_9NH_2$)], 8]: yield 82%; IR 331 cm^{-1} (Pt–Cl). Anal. ($C_5H_{14}N_2Cl_2Pt$) C, H, N, Cl.

***trans*-amminedichloro(pyridine)platinum(II), [*trans*-[$PtCl_2(NH_3)(C_5H_5N)$, 9]**: yield 71% IR 338 cm^{-1} (Pt–Cl). Anal. ($C_5H_8N_2Cl_2Pt$) C, H, N, Cl.

***trans*-Amminedichloro(cycloheptylamine)platinum(II), [*trans*-[$PtCl_2(NH_3)$(c-$C_7H_{13}NH_2$)], 11]**. Crude 11 isolated from hydrochloric acid was purified {from [$PtCl(NH_3)$(c-$C_7H_{13}NH_2)_2$]Cl} by extraction with acetone and evaporation of the extract, yield 44%. The complex may also be prepared by heating *cis*-[$Pt(NH_3)_2$(c-$C_7H_{13}NH_2)_2$]Cl_2 in hydrochloric acid (1 M) in *N,N*-dimethylacetamide and precipitating the product by dilution 1:3 with water, IR 330 cm^{-1} (Pt–Cl). Anal. ($C_7H_{18}N_2Cl_2Pt$) C, H, N.

***trans*-Amminedichlorohexylamineplatinum(II)** [*trans*-[$PtCl_2(NH_3)$(*n*-$C_6H_{13}NH_2$)], **10**] was prepared by heating *cis*-[$Pt(NH_3)_2$(*n*-$C_6H_{13}NH_2)_2$]Cl_2 in hydrochloric acid (1 M) in *N,N*-dimethylacetamide and precipitating the product by dilution 1:3 with water: yield 58%; IR 326 cm^{-1} (Pt–Cl). Anal. ($C_6H_{18}N_2Cl_2Pt$) C, H, N, Cl.

***trans*-Amminedichloro(ethylamine)platinum(II)** [*trans*-[$PtCl_2(NH_3)(EtNH_2)$], **3**]. The intermediate *cis*-[$Pt(NH_3)_2$-$(EtNH_2)_2$]Cl_2 was heated to reflux in a saturated sodium chloride/hydrochloric acid (1 M) mixture for 6 h, and the product was precipitated by cooling the reaction mixture with an ice–salt bath: yield 40%; IR 328 cm^{-1} (Pt–Cl). Anal. ($C_2H_{10}N_2Cl_2Pt$) C, H, N, Cl.

The following compounds were prepared in the same manner.

***trans*-Amminedichloro(methylamine)platinum(II) [*trans*-[$PtCl_2(NH_3)(MeNH_2)$], 2]**: yield 75%; IR 343 cm^{-1} (Pt–Cl). Anal. ($CH_8N_2Cl_2Pt$) C, H, N; Cl: calcd, 22.61; found, 23.36.

***trans*-Amminedichloro(morpholine)platinum(II) [*trans*-[$PtCl_2(OH)_2NH_3(NHC_4H_8O)$], 6]**: yield 85%; IR 338, 330 cm^{-1} (Pt–Cl). Anal. ($C_4H_{12}N_2Cl_2Pt$) C, H, N, Cl.

***trans*-Amminedichloro(1,1,3,3-tetramethylbutylamine)platinum(II)**, [*trans*-[$PtCl_2(NH_3)(Me_3CCH_2CMe_2NH_2)$], **14**]. Tetrahydrofuran (30 mL), water (20 mL), 1,1,3,3-tetramethylbutylamine (2 g, 16 mmol), and *cis*-[$PtCl_2(NH_3)_2$] (1.0 g, 3.3 mmol) were mixed, and the suspension was purged with nitrogen. The mixture was heated under reflux for 6 h. The solution was evaporated under reduced pressure until a copious white precipitate formed. The solution volume was made up to 200 mL with aqueous hydrochloric acid (6 M) and the solution heated under reflux for 2 h. The pale yellow product was isolated after cooling: yield 0.49 g (36%); IR 330 cm^{-1} (Pt–Cl). Anal. ($C_8H_{22}N_2Cl_2Pt$) H, N, Cl; C: calcd, 23.30; found, 23.80.

***trans*-(1-Aminoadamantane)amminedichloroplatinum(II) [*trans*-[$PtCl_2(NH_3)(C_{16}H_{15}NH_2)$], 15]**: preparation as for 14; yield 34%; IR 327 cm^{-1} (Pt–Cl). Anal. ($C_{10}H_{20}N_2Cl_2Pt$) C, H, N, Cl.

***trans*-Amminedichloro(diethylamine)platinum(II) [*trans*-[$PtCl_2(NH_3)(Et_2NH)$], 7].** Diethylamine (2.92 g, 40 mmol) and *cis*-[$PtCl_2(NH_3)_2$] (6 g, 20 mmol) were heated in *N,N*-dimethylacetamide (100 mL) at *ca.* 80 °C for 30 min. The dark solution was treated with activated charcoal and filtered. A small amount of pale yellow solid which was deposited on cooling was removed by filtration, and the bulk of the pale yellow product was precipitated from the filtrate by the addition of water (50 mL): yield 4.4 g (62%); IR 339 cm^{-1} (Pt–Cl). Anal. ($C_4H_{14}N_2Cl_2Pt$) C, H, N, Cl.

***trans*-Amminedichloro(*N*-methylcyclohexylamine)platinum(II) *trans*-[$PtCl_2(NH_3)$(c-$C_6H_{11}NHMe$)], 13].** *N*-Methylcyclohexylamine (3.75 g, 33 mmol) and *cis*-[$PtCl_2(NH_3)_2$] (5.0 g, 17 mmol) were heated to boiling in *N,N*-dimethylacetamide (50 mL). The solution was filtered to remove some decomposition products and concentrated HCl (5 mL) was added. The mixture was reheated until a clear solution was obtained and then added to water (150 mL). The mixture was stirred in an ice–salt bath to precipitate the crude product. This was extracted with acetone and the extract evaporated under reduced pressure. The residue was recrystallized from dilute hydrochloric acid (1 M; 250 mL): yield 1.5 g (23%); IR 338 cm^{-1} (Pt–Cl). Anal. ($C_7H_{18}N_2Cl_2Pt$) C, H, N, Cl.

***trans*-Amminedichloro(quinuclidine)platinum(II) [*trans*-[$PtCl_2(NH_3)(NC_7H_{13})$], 12].** The complex *cis*-[$PtCl(NH_3)_2$(quinuclidine)](NO_3) was prepared as described by Hollis.[32] The complex (2.83 g, 6.5 mmol) dissolved in water (50 mL) was passed through a column of ion exchange resin (Dowex 1-X8, 20–50 US mesh, chloride form) to obtain the chloride salt. The eluent was freeze-dried to yield *cis*-[$PtCl(NH_3)_2$(quinuclidine)]Cl as a white solid. This was suspended in hydrochloric acid (6 M, 50 mL) and heated to reflux for 1 1/2 h. The mixture was cooled using an ice bath and filtered and the yellow product washed with water: yield 2.16 g (85%); IR 331 cm^{-1} (Pt–Cl). Anal. ($C_7H_{16}N_2Cl_2Pt$) H, N, Cl; C: calcd, 21.32; found, 20.85.

***trans*-dichlorobis(cyclohexylamine)platinum(II) [*trans*-[$PtCl_2$(c-$C_6H_{11}NH_2)_2$], 16].** Potassium tetrachloroplatinate(II) (8.4 g, 20 mmol) was dissolved in water (100 mL) and cyclohexylamine (7.9 g, 80 mmol) added. The mixture was boiled for 10 min. The pale brown solid formed was collected by filtration and resuspended in *N,N*-dimethylacetamide (100 mL) and further cyclohexylamine added. The mixture was boiled for 10 min. The pale brown solid formed was collected by filtration and resuspended in *N,N*-dimethylacetamide (100 mL) and further cyclohexylamine added. The mixture was heated to reflux, giving a brown solution to which was added hydrochloric acid (12 M; 25 mL). The mixture was heated at reflux for a further 4 h and then allowed to stand overnight. The dark solid precipitated was collected by filtration and extracted with acetone (3 × 50 mL). The extract was treated with water (250 mL) to precipitate the pale yellow product: yield 1.1 g (12%); IR 331 cm^{-1} (Pt–Cl). Anal. ($C_{12}H_{26}N_2Cl_2Pt$) C, H, N, Cl.

***trans*-Amminedibromo(cyclohexylamine)platinum(II) [*trans*-[$PtBr_2(NH_3)$(*c*-$C_6H_{11}NH_2$)], 17].** Lithium bromide (1.16 g, 13.4 mmol) and 1 (1.28 g, 3.35 mmol) were stirred in acetone (100 mL) at ambient temperature for 8 days. After evaporation under reduced pressure, the residue was washed thoroughly with water to remove the lithium salts, yield 1.16 g (74%). Anal. ($C_6H_{16}N_2Br_2Pt$) H, N, Br; C: calcd, 15.29; found, 15.93.

Platinum(IV) Complexes. *a*-Ammine-*b,d*-dichloro-*f*-(cyclohexylamine)-*c,e*-dihydroxoplatinum(IV) [*trans*-[$PtCl_2(OH)_2NH_3$(*c*-$C_6H_{11}NH_2$)], 18]. 1 (8 g, 21 mmol) was suspended in water (130 mL) and hydrogen peroxide (30% w/v; 26.7 mL, 235 mmol) added. The mixture was stirred and heated at 70–80 °C for 2 h. After cooling, the product was collected by filtration as a pale yellow solid and washed with water: yield 7.0 g (80%); IR 3528 (O–H), 552, 537 (Pt–OH), 352 (Pt–Cl) cm^{-1}. Anal. ($C_6H_{18}N_2O_2Cl_2Pt$) C, H, N, Cl.

The following compounds were obtained in a similar manner with the indicated variations. ***a*-ammine-*b,d*-dichloro-*f*-(ethylamine)-*c,e*-dihydroxoplatinum(IV) [*trans*-[$PtCl_2(OH)_2NH_3(EtNH_2)$], 20]**: yield 40%; IR 3480 (O–H), 565 (Pt–OH), 330 (Pt–Cl) cm^{-1}. Anal. ($C_2H_{12}N_2Cl_2O_2Pt$) C, H, N, Cl.

***a*-Ammine*b,d*-dichloro-*c,e*-dihydroxo*f*-(2-propylamine)platinum(IV) *trans*-[$PtCl_2(OH)_2NH_3(Me_2CHNH_2)$], 21]:**

yield 75%; IR 3541, 3521, 3521 (O–H), 558 (Pt–OH), 347 (Pt–Cl) cm^{-1}. Anal. ($C_3H_{14}N_2Cl_2O_2Pt$) C, H, N, Cl.

***a*-Ammine-*b,d*-dichloro-*c,e*-dihydroxo-*f*-(morpholine)-platinum(IV) [*trans*-$[PtCl_2(OH)_2NH_3(NHC_4H_8O)]$, 23]:** yield 75%; IR 3531 (O–H), 559 (Pt–OH), 347 (Pt–Cl) cm^{-1}. Anal. ($C_4H_{14}N_2Cl_2O_3Pt$) C, H, N, Cl.

***a*-Ammine-*b,d*-dichloro-*f*-(cyclopentylamine)-*c,e*-dihydroxoplatinum(IV) [trans-$[PtCl_2(OH)_2NH_3(c\text{-}C_5H_9NH_2)]$, 25]:** yield 75%; IR 3541 (O–H), 557 (Pt–OH), 348 (Pt–Cl) cm^{-1}. Anal. ($C_5H_{16}N_2Cl_2O_2Pt$) C, H, N, Cl.

***a*-Ammine-*f*-(*exo*-2-aminonorbornane)-b,d-dichloro-*c,e*-dihydroxoplatinum(IV) [*trans*-$[PtCl_2(NH_3)(exo\text{-}C_7H_{11}NH_2)]$, 30].** The complex *trans*-$[PtCl_2(NH_3)(exo\text{-}C_7H_{11}NH_2)]$ was prepared by the method described for 1 and oxidized as described above: yield 42% from *cis*-$[PtCl_2(NH_3)_2]$; IR 3528 (O–H), 563 (Pt–OH), 338 (Pt–Cl) cm^{-1}. Anal. ($C_7H_{18}N_2O_2Cl_2Pt$) C, H, N, Cl.

***a*-Ammine-*b,d*-dichloro-*c,e*-dihydroxo-*f*-(*N*-methylcyclohexylamine)platinum(IV) [*trans*-$[PtCl_2(OH)_2NH_3(c\text{-}C_6H_{11}NHMe)$,] 32];** heated at 90 °C for 30 min; yield 46%; IR 3534 (O–H), 557, 547 (Pt–OH), 346 (Pt–Cl) cm^{-1}. Anal. ($C_7H_{20}N_2Cl_2O_2Pt$) C, H, N, Cl.

a-Ammine-*b,d*-dichloro-*c,e*-dihydroxo-*f*-(1,1,3,3-tetramethylbutylamine)platinum(IV) hydrate [*trans*-$[PtCl_2(OH)_2NH_3(Me_3CCH_2CMe_2NH_2)]\cdot H_2O$, 33]: yield 47%; IR 3498 (O–H), 541 (Pt–OH), 344 (Pt–Cl) cm^{-1}. Anal. ($C_8H_{26}N_2Cl_2O_3Pt$) C, H, N, Cl.

***a*-(1-Aminoadamantane)-*f*-ammine-*b,d*-dichloro*c,e*-dihydroxoplatinum(IV) [*trans*-$[PtCl_2(OH)_2NH_3(C_{16}H_{15}NH_2)]$, 34]:** solvent 2:1 water: *N,N*-dimethylacetamide; yield 65%; IR 3528 (O–H), 546 (Pt–OH), 344 (Pt–Cl) cm^{-1}. Anal. ($C_{10}H_{22}N_2Cl_2O_2Pt$) C, H, N, Cl.

***a*-Ammine-*b,d*-dichloro-*c,e*-dihydroxo-*f*-(pyridine)platinum(IV) [*trans*-$[PtCl_2(OH)_2NH_3(C_5H_5N)]$, 27].** **9** (2.0 g, 5.5 mmol) was oxidized with hydrogen peroxide (30% w/v; 6.2 mL, 55 mmol) in *N,N*-dimethylacetamide (20 mL) at ambient temperature over 14 days. The yellow crystalline product was washed with ethanol: yield 11%; IR 3530, 3505 (O–H), 558, 538 (Pt–OH), 348 (Pt–Cl) cm^{-1}. Anal. ($C_5H_{10}N_2Cl_2O_2Pt$) H, N; C: calcd, 15.15; found, 14.68; Cl: calcd, 17.93; found, 18.45.

***a*-Ammine-*b,d*-dichloro-*f*-(cycloheptylamine)-*c,e*-dihydroxoplatinum(IV) [*trans*-$[PtCl_2(OH)_2NH_3(c\text{-}C_7H_{13}NH_2)]$, 29].** 11 (1.7 g, 4.4 mmol) was dissolved in *N,N*-dimethylacetamide (25 mL) and hydrogen peroxide (30% w/v; 5 mL, 44 mmol) added. The solution was heated to 90 °C for 30 min. The product crystallized on cooling: yield 46%; IR 3545 (O–H), 553 (Pt–OH), 349 (Pt–Cl) cm^{-1}. Anal. ($C_7H_{20}N_2Cl_2O_2Pt$) C, H, N, Cl.

***a*-Ammine-*b,d*-dichloro-*f*-(hexylamine)-*c,e*-dihydroxoplatinum(IV) [*trans*-$[PtCl_2(OH)_2NH_3(n\text{-}C_6H_{13}NH_2)]$, 27]:** as described for **29**; yield 46%; IR 3523 (O–H), 559 (Pt–OH), 344 (Pt–Cl) cm^{-1}. Anal. ($C_6H_{20}N_2Cl_2O_2Pt$) C, H, N, Cl.

***a*-Ammine-*b,d*-dichloro-*c,e*-dihydroxo-*f*-(methylamine)platinum(IV) [*trans*-$[PtCl_2(OH)_2(NH_3)(MeNH_2)]$, 19].** **2** (1.5 g, 4.8 mmol) was added to a mixture of hydrogen peroxide (30% w/v; 2.7 mL, 24 mmol) and heptane (10 mL). The mixture was stirred at ambient temperature for 6 days. The yellow product was collected by filtration: yield 1.4g (84%); IR 3519, 3499 (O–H), 554 (Pt–OH), 356 (Pt–Cl) cm^{-1}. Anal. ($C_1H_{10}N_2Cl_2O_2Pt$) C, N, Cl; H: calcd, 2.87; found, 2.12.

***a*-Ammine-*b,d*-dichloro-*c,e*-dihydroxo-*f*-(2-methyl-2-propylamine)platinum(IV) [*trans*-$[PtCl_2(OH)_2NH_3(Me_3CNH_2)]$, 22].** The product crystallized from hydrogen peroxide solution as a hemiperhydrate (confirmed by titration with permanganate and by elemental analysis). The hydrogen peroxide was removed by trituration with acetone: yield 62%; IR 3533 (O–H), 554 (Pt–OH), 346 (Pt–Cl) cm^{-1}. Anal. ($C_4H_{17}N_2Cl_2O_2Pt$) C, H, N, Cl.

***a*-Ammine-*f*-(benzylamine)-*b,d*-dichloro-*c,e*-dihydroxoplatinum(IV) [*trans*-$[PtCl_2(OH)_2NH_3(PhCH_2NH_2)]$, 28].** A suspension of *cis*-$[PtCl_2(NH_3)_2]$ (5 g, 16.7 mmol) in water (50 mL) was reacted with benzylamine (3.93 g, 36.7 mmol) by heating the mixture at reflux for 1 3/4 h. To this mixture were added saturated sodium chloride solution (50 mL) and concentrated HCl (6 mL, 70 mmol). The solution was heated to reflux for a further 4 h. After the mixture was allowed to cool to room temperature, the yellow solid was collected by filtration and washed with water, ethanol, and diethyl ether.

The solid, *trans*-$[PtCl_2(NH_3)(PhCH_2NH_2)]$ (5 g, 12.9 mmol), was suspended in water (7 mL), hydrogen peroxide (30% w/v; 7 mL, 62 mmol), and heptane (15 mL). The mixture was heated to reflux for 2 h. After cooling, the mixture was filtered to yield a yellow solid, which was washed with water and methanol: yield 1.35 g (19%); IR 3549 (O–H), 549 (Pt–OH), 347 (Pt–Cl) cm^{-1}. Anal. ($C_7H_{14}N_2Cl_2O_2Pt$) H, N; C: calcd, 19.81; found, 20.46; Cl: calcd, 16.74; found, 17.37.

***a*-Ammine-*b,d*-dichloro-*c,e*-dihydroxo-*f*-(quinuclidine)platinum(IV) [*trans*-$[PtCl_2(OH)_2(NH_3)(NC_7H_{13})]$, 31].** **12** (2.16 g, 5.5 mmol) was suspended in water (6 mL) and hydrogen peroxide (30% w/v; 6 mL, 53 mmol). The mixture was heated to 70 °C for 2 1/2 h. The mixture was reduced in volume under reduced pressure to obtain the yellow product. This was collected by filtration and washed with water: yield 0.7 g (29%); IR 3576 (O–H), 558 (Pt–OH), 341 (Pt–Cl) cm^{-1}. Anal. ($C_7H_{18}N_2Cl_2O_2Pt$) C, H, N, Cl.

***a,f*-Dichloro-*b,d*-bis(cyclohexylamine)-*c,e*-dihydroxoplatinum(IV) [*trans*-$[PtCl_2(OH)_2(c\text{-}C_6H_{11}NH_2)_2]$, 35].** **16** (0.75 g, 1.6 mmol) was dissolved in *N,N*-dimethylacetamide (20 mL) and hydrogen peroxide (30% w/v; 10 mL, 88 mmol) added. The solution was warmed to *ca.* 70 °C until the solid dissolved to give a pale brown solution. There was significant effervescence, and a yellow solid was deposited. After the mixture was cooled to room temperature, the solid was collected by filtration and washed with water: yield 0.58 g (72%); IR 3532 (O–H), 552, 540 (Pt–OH), 349 (Pt–Cl) cm^{-1}. Anal. ($C_{12}H_{28}N_2Cl_2O_2Pt$) C, H, N.

***a*-Ammine-*b,d*-dichloro-*f*-(diethylamine)-*c,e*-dihydroxoplatinum(IV) [*trans*-$[PtCl_2(OH)_2NH_3(Et_2NH)]$, 24].** **7** (1 g, 2.8 mmol) was stirred in *N,N*-dimethylacetamide (10 mL) and hydrogen peroxide (30% w/v; 10 mL, 88 mmol) at room temperature for 5 days. The yellow solution was extracted with diethyl ether (2 × 100 mL) and dichloromethane (2 × 100 mL) and the remaining aqueous phase diluted with acetone (250 mL). The product was obtained as yellow crystals on cooling to *ca.* 5 °C overnight: yield 0.54 g (48%); IR 3532, 3517 (O–H), 556, 531 (Pt–OH), 345 (Pt–Cl) cm^{-1}. Anal ($C_4H_{16}N_2Cl_2O_2Pt$) C, H, N, Cl.

***a*-Ammine-*b,d*-dibromo-*f*-(cyclohexylamine)-*c,e*-dihydroxoplatinum(IV) [*trans*-$[PtBr_2(OH)_2NH_3(c\text{-}C_6H_{11}NH_2)]$, 36].** The oxidation was carried out as described for **18** with an equal volume of heptane being added to the oxidation mixture to minimize foaming: yield 32%; IR 3549 (O–H), 549 (Pt–OH), 347 (Pt–Cl) cm^{-1}. Anal. ($C_6H_{18}N_2Br_2O_2Pt$) C, H, N, Br.

***trans*-Amminetetrachloro(cyclohexylamine)platinum(IV) [*trans*-$[PtCl_4(NH_3)(c\text{-}C_6H_{11}NH_2)]$, 37].** 1 (1 g, 2.6 mmol) was suspended in water (30 mL) and hydrogen peroxide (30% w/v; 1 mL, 8.8 mmol) added. The mixture was stirred and heated at *ca.* 70 °C for 2 h. To this mixture was added hydrochloric acid (1 M; 10.5 mL, 10.5 mmol). The solution was stirred at ambient temperature for 6 h. The product was collected by filtration as a yellow solid and washed with water: yield 0.3 g (25%); IR 348 (Pt–Cl) cm^{-1}. Anal. ($C_6H_{16}N_2Cl_4Pt$) C, H, N, Cl.

***trans*-Amminetetrachloro(pyridine)platinum(IV) [*trans*-$[PtCl_4(NH_3)(C_5H_5N)]$, 38].** The filtrate remaining from the isolation of **27** was treated with sufficient concentrated HCl to bring the concentration to 1 M HCl and allowed to stand at ambient temperature. Crystals of the product were slowly deposited. Anal. ($C_5H_{10}N_2Cl_2O_2Pt$) C, H, N, Cl.

***trans*-Amminetetrachloro(quinuclidine)platinum(IV) [*trans*-$[PtCl_4(NH_3)(NC_7H_{13})]$, 39].** The complex *cis*-$[PtCl(NH_3)_2(quinuclidine)](NO_3)$ was prepared as described by Hollis.[32] This complex (2.5 g, 5.7 mmol) was stirred in hydrochloric acid (6 M, 30 mL) and heated at 80–90 °C for 4 h. The mixture was cooled using an ice–salt bath. The yellow product was collected by filtration and washed with water: yield 2.26 g (84%); IR 341 (Pt–Cl) cm^{-1}. Anal. ($C_7H_{16}N_2Cl_4Pt$) C, H, N, Cl.

***a,b*-Bis(acetato)-*c*-ammine-*d,f*-dichloro-*e*-(cyclohexylamine)platinum(IV) [*cis,cis,trans*-$[PtCl_2(OAc)_2NH_3(c\text{-}C_6H_{11}NH_2)]$, 40].** **18** **2**.0 g, 4.8 mmol) was stirred in acetic

anhydride (10 mL, 106 mmol) at ambient temperature for 5 days. The cream-colored product was washed with diethyl ether: yield 1.72 g (72%); IR 695 (O_2CCH_3), 358 (symmetric Pt−Cl), 301 (asymmetric Pt−Cl) cm^{-1}; Raman 367, 359 (symmetric Pt−Cl), 301 (asymmetric Pt−Cl) cm^{-1}. Anal. ($C_{10}H_{22}N_2Cl_2O_4Pt$) C, H, N, Cl.

***a*-Ammine-*b,d*-dichloro-*f*-(cyclohexylamine)-*c,e*-bis(ethylcarbamato)platinum(IV) [*cis,cis,trans*-[$PtCl_2(O_2CNHEt)_2NH_3$(c-$C_6H_{11}NH_2$)], 41].** **18** (2.0 g, 4.8 mmol) was stirred with ethyl isocyanate (10 mL) in a stoppered flask at ambient temperature for 8 days. Water was added to precipitate the crude product. This was recrystallized from methanol: yield 0.89 g (33%); IR 353 (Pt-Cl) cm^{-1}. Anal. ($C_{12}H_{28}N_4Cl_2O_4Pt$) C, H, N, Cl.

Biological Methods. *In Vitro* Studies: (A) Cell Lines. Five "parent" human ovarian carcinoma cell lines have been used: SKOV-3, A2780, HX/62, CH1, and 41M as described previously.[2,20] In addition, three pairs of human tumor cell lines (parent line and derived subline with acquired resistance to cisplatin) have been used: 41M/41McisR, CH1/CH1cisR, A2780/A2780cisR, as described previously.[2,14,21] The parent 41M and A2780 lines were derived from previously untreated patients.

All lines grew as monolayers in Dulbecco's Modified Eagle's Medium containing 10% fetal calf serum (Imperial laboratories, Andover, United Kingdom), 50 μg/mL of gentamicin, 2.5 μg/mL of amphotericin B, 2 mM L-glutamine, 10 μg/mL of insulin, and 0.5 μg/mL of hydrocortisone in 10% CO_2/90% air. Cells were periodically checked and found to be free of *Mycoplasma*; parent lines were used from passage 25−60.

(B) Assessment of Cytotoxicity. Platinum drugs were dissolved immediately before use in 0.9% saline at 1 mM or 500 μM (note that not all of the platinum II complexes were evaluated and that some required mixing on a whirlmixer). Cytotoxicity was assessed using the Sulforhodamine B (SRB) assay as described previously.[2,14] Briefly, single viable cells were seeded into 96-well microtiter plates (at concentrations between 5×10^3 and 1×10^4/well in 200 μL growth medium) and allowed to attach overnight. Agents were then added to quadruplicate wells. Agent exposure was for 96 h unless otherwise stated. Basic amino acid content per well was then determined using 0.4% SRB, obtained from Sigma Chemicals (Poole, United Kingdom), dissolved in 1% acetic acid.

***In Vivo* Studies: (A) Tumor Lines.** The murine solid ADJ/PC6 plasmacytoma and its subline selected for acquired resistance to cisplatin have been described previously.[22,29] In addition, six human ovarian carcinoma xenografts have been used: PXN/109T/C (the *in vivo* equivalent of the CH1 cell line), PXN/109T/CC its subline selected (*in vitro*) for acquired resistance to cisplatin, SKOV-3, OVCAR-3, HX/110, and PXN/100.[23,30] These lines (grown subcutaneously in female Balb C nude mice) were chosen to encompass the broad range in responsiveness observed to cisplatin and carboplatin in our xenograft panel; PXN/109T/C and PXN/100 comparably sensitive; SKOV-3 (again derived from the cell line) and PXN/109T/CC, refractory; OVCAR-3 and HX/110 intermediate sensitivity.[23,30]

(B) Assessment of Antitumor Activity: ADJ/PC6. This was performed as described previously.[2,22] Briefly, 20 days postsubcutaneous implantation of 1 mm^3 tumor fragments, drugs were adminstered (at halving doses) by a single intraperitoneal injection as sonicated suspensions in arachis oil. Ten day laters, tumors were dissected out and the weights of control and treated groups compared. Antitumor activity has been defined in terms of a "therapeutic index" (TI), the ratio of the maximum tolerated dose (MTD) to ED_{90} (the dose required to reduce tumor mass by 90%).

Human Ovarian Carcinoma Xenografts. This was performed as described previously.[23,30] Mice bearing comparably-sized tumors (typically of 8 mm largest diameter) were treated with drugs (administered intraperitoneally as a sonicated suspension in arachis oil) at prior-determined maximimum tolerated doses (approximately LD_{10}) on days 0, 7, 14, and 21 (unless otherwise specified). Tumors were measured weekly until they had doubled their starting volume and response assessed as described previously. Growth delays (the difference in time required for control and treated tumors to double in volume) were then determined.

Statistical Analysis. Where appropriate, statistical significance was tested using a two-tailed Student's *t* test.

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