Anticancer Activity in Murine and Human Tumor Cell Lines of Bis(platinum) Complexes Incorporating Straight-Chain Aliphatic Diamine Linker Groups

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The biological activity of a series of dinuclear bis(platinum) complexes of formula [{*cis*-$PtX_2(NH_3)$}$_2(NH_2(CH_2)_nNH_2)$] (X = Cl, n = 4–9, compounds **6–11**; X_2 = malonate, n = 5 or 6, compounds **12** and **13**) is described in selected murine leukemia, murine solid tumor, and human tumor cell lines and in murine leukemia cell lines rendered resistant to cisplatin (*cis*-$[Pt(NH_3)_2Cl_2]$). The bis(platinum) compounds showed greater activity in vitro against murine tumor cell lines resistant to either cisplatin or DACH ($[Pt(DACH)Cl_2]$). The resistance factor is dependent on chain length of the diamine, and the structural feature of a dinuclear complex is of general use in reducing cross-resistance with cisplatin. In vivo [{*cis*-$PtCl_2(NH_3)$}$_2(NH_2(CH_2)_5NH_2)$] (**7**) showed a % T/C of 204 against murine L1210 leukemia resistant to cisplatin compared to a % T/C of 104 for cisplatin itself at optimal doses. The complex [{$Pt(mal)(NH_3)$}$_2(NH_2(CH_2)_6NH_2)$] (**13**) was highly active in the colon 26 tumor line with 3/10 tumor-free survivors (dose of 186 mg/kg, ip D1,5,9); however, **13** was subject to substantial cross-resistance in the cisplatin resistant L1210 leukemia (% T/C 139 versus % T/C of 223 in the sensitive line). In four selected human tumor lines in vitro, compounds **6–11** were uniformly more potent than cisplatin. In the corresponding xenografts, compound **7** showed greater activity in the HCT-8 (coloadenocarcinoma) and H23 (nonsmall cell lung), but diminished potency in AH125 and H520 (both nonsmall cell lung) lines in comparison to cisplatin. Retention of activity against cisplatin-resistant cell lines and a different spectrum of activity compared to cisplatin in some human tumor cell lines suggest that this class of complexes is mechanistically different from mononuclear complexes and worthy of further development toward clinical trials.

The clinical utility of the presently used anticancer agents cisplatin, *cis*-$[Pt(NH_3)_2Cl_2]$, and carboplatin, Pt-$(NH_3)_2$(1,1-cyclobutanedicarboxylato), is well established,[1,2] but their more widespread use is limited by inherent resistance (limited activity against many common human cancers), by acquired drug resistance (reduced efficacy upon repeated treatment), and by their relative toxicities. While the mechanisms of cellular resistance to cisplatin are multifactorial, enhanced DNA repair is implicated in many cell lines.[3-5] Dinuclear bis(platinum) complexes of the general formula [{$PtCl_m(NH_3)_{3-m}$}$_2$-(diamine)]$^{2(2-m)+}$, where m = 0–3 and the diamine usually is $H_2N(CH_2)_nNH_2$, represent a unique class of potent anticancer agents with activity in cisplatin-resistant model systems.[6-8] The dinuclear bis(platinum) complexes produce an array of structurally distinct Pt–DNA lesions in comparison to those produced by cisplatin.[9,10] Studies of bis(platinum) complexes have led to the development of the hypothesis that altered DNA binding and conformational changes in comparison to those produced by cisplatin may result in more difficult repair of the drug–DNA lesions, especially in cisplatin-resistant cells. Complexes capable of undergoing molecular interactions not possible by monomeric complexes or acting by different mechanisms might also display a broader spectrum of clinical activity. Thus, there appears to be a basis for the development of an approach to overcome cisplatin resistance.

The preliminary biological properties in L1210 cell lines of the parent series, [{*cis*-$PtCl_2(NH_3)$}$_2(H_2N(CH_2)_nNH_2)$] have been reported, where in the above general formula

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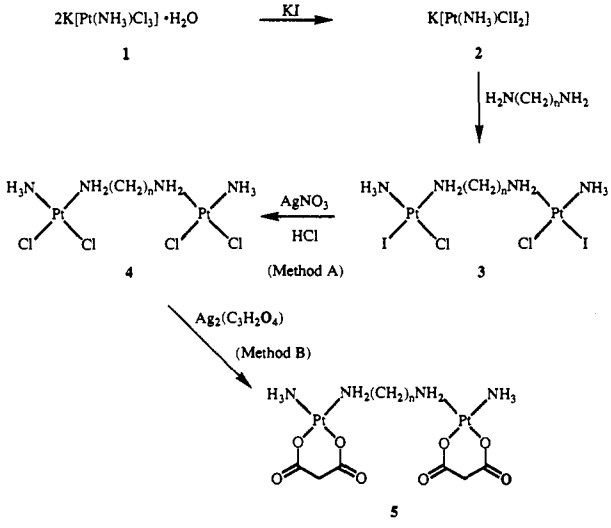
Scheme I. Synthetic Scheme for Preparation of Bis(platinum) Complexes



both platinum(II) coordination spheres are bidentate and n = 4–6.[11] Cytotoxicity studies against a wider range of cell lines than L1210 leukemia are necessary to evaluate fully the clinical potential of new platinum complexes, since testing against a broader range of tumors might be of greater predictive value.[12] The basic dinuclear bis(platinum) structure lends itself to systematic modification in a number of ways including the nature of the leaving group and bridging diamine, and, in particular, the length and functionalization of the linker group. This paper presents our findings with representative complexes incorporating straight-chain diamines, $NH_2(CH_2)_nNH_2$, wherein n = 4–9.

Chemistry

The complexes described in this paper were prepared by routes shown in Scheme I and are listed in Table I. An adaptation of the well-known Dhara method for the synthesis of cisplatin[13] was devised to prepare the chlorides **6**–**11**.[7] In the Dhara preparation, the Pt–amine complex is precipitated first as *cis*-$[Pt(NH_3)_2I_2]$ from the reaction of K_2PtCl_4 in the presence of an excess of KI with aqueous NH_3. The iodide is subsequently converted to the chloride. Detailed examination of the reaction of $K[Pt(NH_3)Cl_3]$ with 4 equiv of KI showed that the principal species formed in solution is $[Pt(NH_3)I_2Cl]$, in which the chloride ligand is trans to the ammine group, and not the expected $K[Pt(NH_3)I_3]$.[14] Subsequent reaction with a diamine displaces iodide and produces the mixed chloride/iodide compounds **3** wherein the Cl must be trans to the NH_3 since no isomerization is observed by either ^{195}Pt or ^{15}N NMR.[14] The mixed chloride/iodide is then converted to the target chloride (**4**) by reaction with 4 equiv of Ag^+ followed by addition of HCl to precipitate the desired chloride. If necessary, the complexes may be purified by recrystallization from *N*,*N*-dimethylacetamide/HCl. The $K[Pt(NH_3)Cl_3]$ starting material (**1**) was synthesized via a newly developed practical and efficient process starting from cisplatin.[15] Its preparation is given in detail in the Experimental Section. The malonate complexes (**12** and **13**) were prepared by treatment of the chloride with silver(I) malonate followed by recrystallization from aqueous ethanol. An alternate route to reach the malonate complexes (**12** and **13**) consists of reacting the chloride/iodide (**3**) directly with Ag(I) malonate. However, the yield and purity of the products are inferior to the products obtained in method B. The spectroscopic data for all compounds are fully consistent with the proposed structures, as previously reported.[11,16]

Biological Properties

In Vitro Growth Inhibition. The effect of each bis(platinum) compound on the in vitro growth inhibition of murine leukemia cells sensitive and resistant to cisplatin and $Pt(DACH)Cl_2$ is shown in Table II. The resistance factor for each bis(platinum) chloride complex in comparison with those for cisplatin, $Pt(DACH)Cl_2$, and the newer platinum agent, CI-973, a compound currently in clinical trials,[17] is defined by the ratio of the ID_{50} of the resistant line divided by the ID_{50} of the sensitive line and is shown in Figure 1. The four resistant cell lines demonstrated lower resistance to each of the bis(platinum) chloride compounds than to the reference compounds used to generate the resistance, suggesting that these compounds retain activity in the resistant systems. For example, in the L1210PtR4 and L1210DACH cell lines, the resistance factors for all of the bis(platinum) chloride complexes were substantially less than that for the compound to which the cell lines were primarily resistant. The data from Figure 1 suggest that only the nine-carbon complex (**11**) displays lowered activity in the resistant cell lines.

Replacement of the chloride ligands by malonate groups (compounds **12** and **13**) resulted in about a 10-fold loss of potency compared to the analogous bis(platinum) chloride complexes (Table II). The diminished potency for malonate in comparison to chloride complexes in in vitro screens is influenced in part by the slower displacement of the chelating dicarboxylate to give the purported active aqua species.[18,19]

In the L1210 resistant cell lines, the compounds **6**–**10** (n = 4–8) were both more potent and generally showed less cross-resistance than CI-973 (Table II and Figure 1). The cross-resistance of the P388PtR4 cell line to the bis(platinum) chloride compounds was substantial, although

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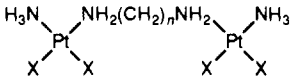
Table I. Structural Data for Straight-Chain Alkyl Bis(platinum) Complexes



compd	n	X (or X_2)	method	yield,[a] %	$\delta(^1H)$[b,c]	$\delta(^{195}Pt)$[d]	molecular formula[e]
6	4	Cl	A	76	1.8, 4.2	-2154	$C_4H_{18}Cl_4N_4Pt_2$
7	5	Cl	A	69	1.4, 1.78, 4.35	-2161	$C_5H_{20}Cl_4N_4Pt_2$
8	6	Cl	A	86	1.32, 1.7, 4.2	-2158	$C_6H_{22}Cl_4N_4Pt_2$
9	7	Cl	A	64	1.3, 1.7, 4.2, 4.8	-2157	$C_7H_{24}Cl_4N_4Pt_2$
10	8	Cl	A	65	1.3, 1.7, 4.23, 4.85	-2157	$C_8H_{26}Cl_4N_4Pt_2$
11	9	Cl	A	73	1.3, 1.7, 4.25	-2156	$C_9H_{28}Cl_4N_4Pt_2$
12	5	mal[f]	B	85	1.23, 1.83, 2.64, 3.73	-1811	$C_{11}H_{24}N_4O_8Pt_2$
13	6	mal[f]	B	79	1.42, 1.77, 2.6, 3.68	-1804	$C_{12}H_{26}N_4O_8Pt_2$

[a] Yields were not optimized. Yields of chloride complexes based on overall conversion from $K[PtCl_3(NH_3)]$ (1). [b] In ppm relative to TMS. [c] The chemical shifts of the CH_2NH_2 protons occur at 2.7–3.0 ppm and are therefore obscured by the solvent peaks. In all complexes two sets of resonances due to the "central" CH_2 groups are clearly observed at 1.3–1.4 and 1.7–1.8 ppm. The relative integrations of these two peaks vary depending on the diamine and no attempt was made to assign individual resonances. In favorable cases, and especially in the absence of any water of solvation, the NH_3 and NH_2 resonances are clearly observed at 4.2–4.3 and 4.8–4.9 ppm, respectively. [d] In ppm relative to Na_2PtCl_6. [e] Analyses are within ±0.4% of the theoretical values. [f] Mal = malonate.

Table II. In Vitro Growth Inhibition by Bis(platinum) Complexes in Cisplatin-Resistant Murine Leukemia Cell Lines

	ID_{50} (μM)[a,b]					
compd	L1210S	L1210PtR4	L1210DDP5	L1210DACH	P388S	P388PtR4
cisplatin	0.92 ± 0.09	19.1 ± 0.88	16.1 ± 1.3	2.1 ± 0.12	0.70 ± 0.13	23.3 ± 1.4
$Pt(DACH)Cl_2$[c]	0.21 ± 0.04	0.19 ± 0.01	0.38 ± 0.09	9.50 ± 1.3	0.21 ± 0.01	2.26 ± 0.28
CI-973[d]	1.4 ± 0.3	5.4 ± 2	8.6 ± 2	3.6 ± 0.5	0.22	3.7 ± 1
6[e]	0.55	1.5	0.93	0.76	0.15	1.3
7	0.38	1.57	2.1	0.98	0.30	1.9
8	0.45	1.45	2.21	1.2	0.17	1.57
9	0.22	0.73	1.23	0.50	0.14	0.91
10	0.33	1.04	1.35	0.97	0.27	1.12
11	0.29	2.2	2.8	1.6	0.21	3.2
12	3.83	13.0	17.2	9.49	2.06	16.0
13[e]	3.82	6.31	8.12	5.22	1.47	6.46

[a] Values are means ± standard error of the mean from at least three and up to 17 separate experiments in which values were determined as average of duplicates. All other values are averages of at least two separate experiments in which values were determined as average of duplicates. [b] L1210S and P388S are murine cell lines sensitive to cisplatin. L1210DDP5 and L1210DACH were made resistant to cisplatin and DACH chloride, respectively, and were provided by Dr. A. Eastman. L1210PtR4 and P388PtR4 were made resistant to cisplatin in vivo and adapted to in vitro culture in our laboratory.[20] [c] DACH chloride, [*S,P*-4-2-(1*R*,2*R*)]-(1,2-cyclohexanediamine-*N,N'*)dichloroplatinum(II). [d] CI-973, [*S,P*-4-3-(*R*)]-[1,1-cyclobutanedicarboxylato(2-)](2-methyl-1,4-butanediamine-*N,N'*)platinum. [e] Single experiment, average of duplicate determinations.

still less than its resistance to cisplatin. The explanation for this relative insensitivity may lie in the multiple mechanisms of resistance possessed by this cell line.[20,21] The various resistant murine cell lines demonstrated lower cross-resistance to the n = 6 malonate (**13**) than to the corresponding n = 6 chloride (**8**) (Table II).

The potency of compounds **6**–**11** in human tumor cell lines in vitro was greater than that of either cisplatin or CI-973 (Table III). Within the series, the greatest potency resided in compound **9**, containing a seven-carbon linker. This same compound was also the most potent in the murine leukemia lines although the resistance factor was not appreciably different from that of the other bis(platinum) compounds (vide supra). As was the case in the resistant murine leukemia lines, the malonate complexes (**12** and **13**) were markedly less potent than the chlorides in the in vitro human tumor cell lines. The same reason can be used to explain the reduced potency of the malonate complexes in the human and L1210 leukemia lines. Supporting this reasoning, compounds **12** and **13** were at least as active as CI-973, which also contains a malonate leaving ligand, in the human tumor cell lines.

In Vivo Antitumor Activity

The in vivo activity of compound **7** (n = 5) in sensitive and resistant leukemia showed the same trend seen in vitro (Table IV), i.e., **7** remained active to a varying degree in cisplatin-resistant cell lines in which cisplatin displayed no activity. The data for this complex showed substantial cell kill in mice bearing the cisplatin-resistant L1210 leukemia tumor cell line from which the in vitro line was derived. The difference in net cell kill observed in the resistant line compared to the sensitive line was only 1 log for **7** as opposed to an 8 log decrease for cisplatin. In the in vivo resistant P388 leukemia line, **7** was somewhat less active but retained greater activity than cisplatin (2.5 logs of tumor burden reduction versus 1.5 logs of net tumor growth, respectively, over the course of the 9-day treatment). In contrast to the activity retained by **7** in the L1210 cisplatin-resistant leukemia line, the n = 5 and n = 6 malonate complex (**12** and **13**, respectively) were ineffective against this tumor at the highest doses evaluated. It is possible that the malonate complexes would show greater antitumor activity at higher tolerated doses.

Against a panel of solid murine tumors comprised of colon, breast, sarcoma, and melanoma, **7** (n = 5) showed shorter growth delays and less net cell kill than cisplatin (Table V). The effect of a malonate leaving ligand on in vivo activity was evaluated in murine colon 26 tumor. When tested at 186 mg/kg per injection on days 3, 7, and 11,

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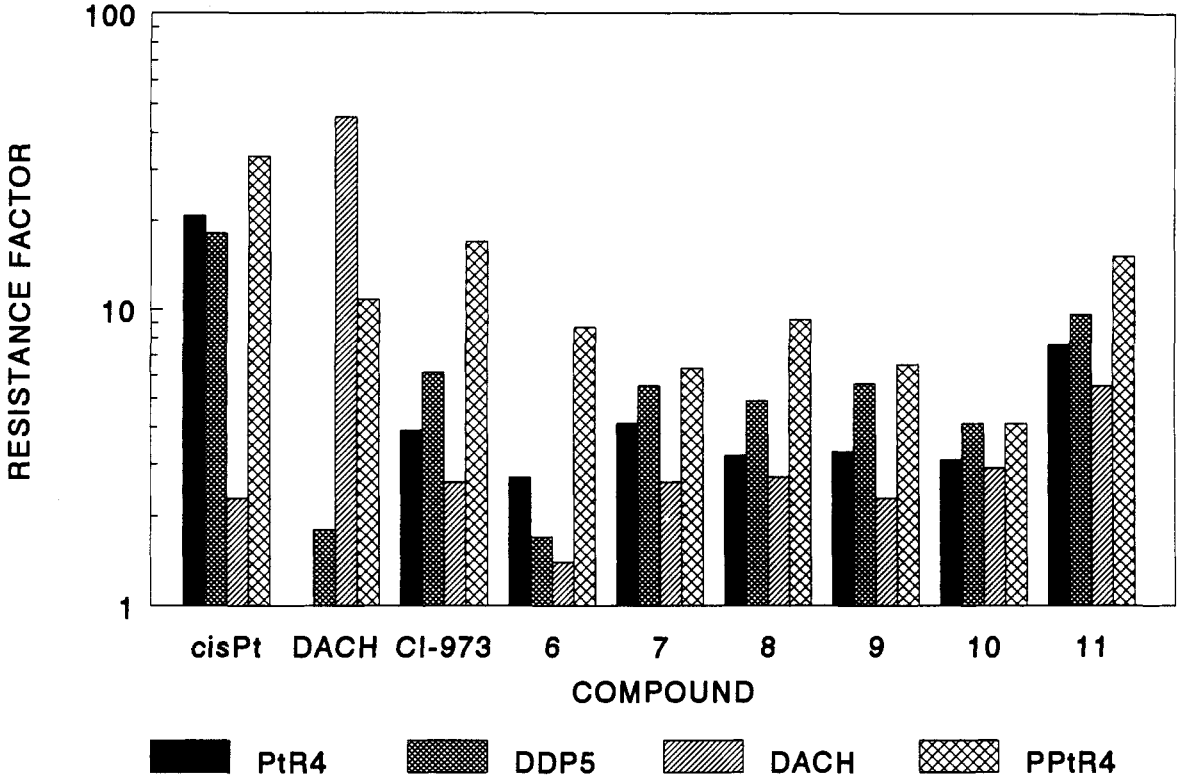

Figure 1. In vitro resistance of murine leukemia to bis(platinum) dichloride complexes. Growth inhibition of cells was assessed as described.[20] Resistance factor is ID_{50} resistant cell line/ID_{50} sensitive cell line. PtR4, L1210PtR4; DDP5, L1210DDP5; DACH, L1210DACH; PPtR4, P388PtR4. Values presented are means of at least three separate determinations done in duplicate except for 6, which was a single test done in duplicate; CisPt, cisplatin; DACH, $Pt(DACH)Cl_2$.

Table III. In Vitro Growth Inhibition by Bis(platinum) Complexes in Human Tumor Cell Lines

	ID_{50} (μM)[a]			
compd	HCT-8[b]	H23	AH125	H520
cisplatin	1.3 ± 0.12	0.31 ± 0.03	0.70 ± 0.17	0.47 ± 0.03
CI-973[c]	4.2 ± 0.77	3.9 ± 0.28	ND[d]	3.9 ± 1.1
6	0.25	0.07	0.46	0.20
7	0.31 ± 0.04	0.11 ± 0.01	0.42 ± 0.09	0.36 ± 0.07
8	0.17	0.06	0.28	0.16
9	0.10	0.03	0.12	ND
10	0.13	0.04	0.21	0.15
11	0.19	0.056	0.26	0.14
12	4.42	1.48	3.68	1.83
13	3.79	1.61	3.13	1.50

[a] Values are means ± standard error of the mean for at least four experiments. Other values are averages of at least two separate experiments. [b] HCT-8 is a human coloadenocarcinoma; H23, AH125, and H520 are human nonsmall cell lung carcinoma cell lines. [c] CI-973, as in Table II. [d] ND = not determined.

compound 13 (n = 6) showed a 24.9-day growth delay, three of ten cures, and 1.8 net log cell kill. By comparison, cisplatin and compound 7 were considerably less active.

In two of four human tumor cell lines grown in athymic nude mice, the chloride complex 7 (n = 5) produced marginally greater growth delay, and therefore marginally greater net cell kill, than cisplatin (Table VI).

Summary and Conclusions

In a number of preclinical model systems, the bis(platinum) complexes described herein demonstrate desirable antitumor activity for agents that might be further developed toward clinical trials, especially the retention of significant activity in cisplatin-resistant systems and differential activity in human tumor systems in comparison to cisplatin although this activity has not been universally apparent. In addition, the retention of activity against a cell line resistant to a (diaminocyclohexane)platinum(II) complex may become more important as such compounds enter clinical trials.[22] The resistance factor (ID_{50} resistant/ID_{50} sensitive) for the series of compounds with n = 4–9 carbon linkers remained similar within a given cell line until n = 9 (compound 11) at which point the resistance factors generally increased. Whether the lower activity of 11 in resistant cell lines can be related to the previously described[9,10] structural modes of binding to DNA by the bis(platinum) complexes remains unclear. Perhaps a nine-carbon linker between the two platinum centers imparts structural characteristics different enough to prohibit the same mode of binding to DNA shown by the other bis(platinum) complexes in this series. It is possible that other factors contributing to the development of resistance such as cellular uptake (association) become more important for this derivative.

A comparison of the effect of malonate vs chloride leaving ligand suggests that although the potency of the malonate compounds is lower, they show better in vivo antitumor activity in selected tumor model systems such as murine colon 26 (Table V). However, in cisplatin-resistant L1210 murine leukemia, both potency and antitumor activity are decreased compared to the chloride complexes, pointing to a cell line/tumor type dependence for activity in the malonate complexes (Table IV).

The availability of in vitro and in vivo data for a series of bis(platinum) compounds with n = 4–9 carbon linkers allows for an examination of proposals for the mode of binding of this class of complexes to DNA.[9,11] In this series,

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Table IV. In Vivo Activity of Bis(platinum) Complexes against Cisplatin-Resistant Murine Leukemia[a]

	L1210[b]			L1210/DDPt[b]			P388[c]			P388/DDPt[c]		
compd	dose (mg/kg per inj)	wt chg (g)	% T/C	net kill (log)	wt chg (g)	% T/C	net kill (log)	dose (mg/kg per inj)	wt chg (g)	% T/C	net kill (log)	wt chg (g)	% T/C	net kill (log)
cisplatin	10	-4.9	434(2/6)	6.3	-3.0	102	-2.3	5.0	+0.2	261	6.8	+1.0	132	-1.5
7	4	-5.1	218(1/6)	4.2	-1.3	204	3.2	7.4	-6.7	296	6.8	-4.8	183	2.5
12	210[e]	-2.7	187	1.0	-4.1	123	-2.0							
13	210[e]	-4.5	223(2/6)	4.7	-2.7	139	-1.7							

[a] The optimal dose from a complete dose response is presented, unless noted otherwise. [b] Mice were inoculated ip with 1×10^4 cells on day 0. Treatment was ip on days 3, 7, and 11. Figures in parentheses denote cures/total mice. [c] Six mice per treatment group were inoculated ip with 1×10^6 cells on day 0. Treatment was ip on days 1, 5, and 9. [e] Highest dose tested.

Table V. In Vivo Activity of Selected Bis(platinum) Complexes against Murine Solid Tumors[a]

tumor	compd	treatment	dose (mg/kg per inj)	wt chg (g)	T - C (days)	net kill
B16	7	ip D1, 5, 9	4	-4.0	2.5	-1.0
	cisplatin	ip D1, 5, 9	9	-4.5	5.1	-0.4
colon 26	7	ip D3, 7, 11	4	-4.1	8.8	0.1
	12	ip D3, 7, 11	50[b]	+	12.8	0.5
	13	ip D3, 7, 11	186	-2.9	24.9 (3/10)	1.8
	cisplatin	ip D3, 7, 11	10	-2.3	13.6	0.9
colon 51	7	ip D1, 5, 9	4	-5.6	6.8	-0.2
	cisplatin	ip D1, 5, 9	10	-5.2	9.9	0.2
M5076	7	ip D1, 5, 9	4	-2.8	10.2	0.2
	cisplatin	ip D1, 5, 9	9	-5.1	15.9	0.8
mammary 25	7	ip D3, 7, 11	4	0	6.0	-0.2
	cisplatin	ip D3, 7, 11	6	-2.1	10.7 (1/10)	0.2

[a] All solid tumors were implanted subcutaneously (sc) as trocar fragments. Each treatment group consisted of 10 mice. Numbers in parentheses denote cures over total mice in that group. Dose units are milligrams/kilogram per injection. The optimal dose, $\leq LD_{10}$, from a complete dose response is presented. [b] Highest dose tested.

Table VI. In Vivo Activity of Bis(platinum) Complex 7 against Human Tumor Xenografts[a]

tumor	compd	treatment	dose (mg/kg per inj)	wt chg (g)	T - C (days)	net kill
HCT-8	7	ip D9, 13, 17	8	-5.4	8.1	0
	cisplatin	ip D9, 13, 17	8	-4.6	5.8	-0.2
H23	7	ip D15, 19, 23	5	-3.5	13.2	0.3
	cisplatin	ip D15, 19, 23	3	-0.8	0.5	-0.4
AH125	7	ip D21, 25, 29	5	-3.3	8.7	0.1
	cisplatin	ip D21, 25, 29	12	-2.9	26.5	1.3
H520	7	ip D15, 19, 23	8	-1.3	7.0	-0.1
	cisplatin	ip D15, 19, 23	12	-2.0	32.6	1.8

[a] All tumors were implanted subcutaneously (sc) as trocar fragments. Treatment was initiated on the indicated schedules when tumors reached approximately 100–150 mg. The optimal dose, $\leq LD_{10}$, from a complete dose response is presented.

the extension of the distance between the platinum centers by the successive addition of methylene carbons did not have an appreciable effect on activity in vitro until the linker length was nine carbon atoms at which point cross-resistance to cisplatin increased. This suggests that whatever the mode of molecular action is, a linker length of four to eight carbons results in basically an equivalent effect (Table II). Upon initial monodentate binding to DNA, the tetrafunctional complexes discussed here may form an array of structurally distinct lesions in comparison to cisplatin. These include (Pt,Pt) interstrand cross-links and (Pt,Pt) intrastrand cross-links by binding of both Pt atoms to the same strand as well as cisplatin-like adducts by initial binding of one bidentate coordination sphere of the bis(platinum) complex.[6,23] The flexibility of the diamine chains used in this study suggests that a similar array of adducts may be possible for all the complexes. The contributions of these individual lesions to retention of activity in cisplatin-resistant cell lines remains to be tested.[24]

Roberts et al. characterized the DNA–DNA interstrand cross-linking by $[\{Pt(mal)(NH_3)\}_2((NH_2(CH_2)_nNH_2)]$, ($n$ = 4, 5, 6) on a plasmid DNA fragment and found an apparent decrease in cross-linking with an increase in the number of linker carbons.[9] The absence of the same trend in the in vitro growth inhibition and in vivo antitumor activity suggests that the results of the isolated DNA binding experiments may not translate to the cellular environment to the same extent. Intracellular uptake and distribution also affect the cytotoxic potency of a drug. Thus, the rigid comparison of cytotoxicity and DNA binding must take into account such pharmacokinetic factors.

Based upon examination of molecular models, interstrand cross-link formation has been cited as a possible primary difference between bis(platinum) complexes and monomeric platinum compounds.[9,10] Interestingly, analysis of the cytotoxicity of 7 and 12 in the NCI Human Tumor Screening Panel using the COMPARE program showed no correlation with any monomeric platinum compounds, but high correlation with standard alkylating agents such as Thio-TEPA, AZQ, and melphalan.[8] However, a comparison of cross-resistance to bifunctional alkylators and bis(platinum) complexes for the cell lines used here suggests that the bis(platinum) complexes are

(23) Qu, Y.; Farrell, N. Interaction of Bis(platinum) Complexes with the Mononucleotide 5'-Guanosine Monophosphate. Effect of Diamine Linker and the Nature of the Bis(platinum) Complex on Product Formation. *J. Am. Chem. Soc.* **1991**, *113*, 4851–4857.

(24) Roberts, J. D.; Lafayette, A. R.; Johnson, A. L.; Farrell, N. Cellular Pharmacology of Bis(platinum) Complexes. *Proc. Am. Assoc. Cancer Res.* **1992**, *33*, 3218.

not just interstrand bifunctional alkyators in the same sense that melphalan or BCNU are. The resistance factors of melphalan and BCNU were 3.4 or less in the four resistant murine leukemia cell lines[20] as opposed to those shown in Figure 1. Thus, the resistance patterns suggest that the bis(platinum) compounds are not wholly functionally similar to the mustards. The resistance patterns do show that the bis(platinum) complexes are distinctly different from the monomeric cisplatin and Pt(DACH) complexes. These findings, plus the enhanced activity in some human tumor cell lines in comparison to cisplatin, indicate an antitumor activity profile for the bis(platinum) complexes which is different from that of cisplatin and warrants further investigation.[25]

Experimental Section

General Chemical Procedures. NMR spectra were run on the chloride complexes in d_7-DMF and on the malonate complexes in D_2O at sample concentrations of 1 mM and 10 mg/mL, respectively. ^1H NMR spectra were recorded on Varian XL-200 and Bruker AM250 spectrometers with chemical shifts reported as δ units in ppm downfield from internal tetramethylsilane. The malonate complexes were recorded with a pulse width of 8 μs and a sweep width of 3501 Hz, and the chloride complexes with a pulse width of 4 μs and a sweep width of 5000 Hz. ^{195}Pt NMR spectra were recorded on a Bruker WM250 spectrometer with a 0.1 M Na_2PtCl_6 solution in D_2O serving as external reference. Samples were run with a pulse width of 15 μs and a sweep width of 30 KHz. Combustion analyses were performed on a Perkin-Elmer Model 240, Control Equipment Corporation Model 240XA, or Carlo-Erba Model 1106 elemental analyzer. Halogens were determined by silver nitrate titration. Purity for malonate complexes was assessed by HPLC on a Beckmann Ultrasphere CN, 5 μ column. The mobile phase utilized was 9:1 H_2O:acetonitrile, with peak monitoring at 205 nm. All diamines were purchased from Aldrich Chemical Co. as their free bases and used without further purification.

Improved Preparation of Potassium Amminetrichloroplatinate(II) Monohydrate (1). The following is a fully optimized procedure incorporating in part the procedure of Elleman et al.[15] A mixture of 12.0 g (40 mmol) of cisplatin, 780 mg (6.5% w/w) of platinum powder, and 250 mL of 6 N aqueous HCl was heated at reflux for 2.25 h. The mixture was quickly chilled to 0 °C and maintained there overnight to crystallize unreacted cisplatin. The mixture was filtered and the filtrate was concentrated to a slush that was redissolved in 1 N aqueous HCl. The resultant solution was loaded onto an anion-exchange column containing Sephadex DEAE(A-25) resin preconditioned with H_2O. The column was eluted with 1 N aqueous HCl and a composite fraction containing $H[Pt(NH_3)Cl_3]$ was assayed by UV spectroscopy (ϵ_{345nm} for $Pt(NH_3)Cl_3^-$ is 115 M^{-1} cm^{-1}). The combined fraction containing the $H[Pt(NH_3)Cl_3]$ was treated with an equimolar amount of KCl, and the solution was concentrated to a slush. After chilling to <0 °C, the solids were collected by filtration, washed immediately with cold (0 °C) absolute ethanol, and air-dried to yield 9.0 g (60%) of an analytically pure product. Anal. ($KPtNH_3Cl_3 \cdot H_2O$) H, N.

Synthesis of Bis(platinum) Chloride Complexes (Method A). μ-(1,7-Heptanediamine-*N,N'*)bis[*cis*-amminedichloroplatinum(II)] (9). A solution of 4.98 g (30.0 mmol) of KI in 40 mL of H_2O was added to a solution of 3.576 g (9.5 mmol) of $K[Pt(NH_3)Cl_3]$ monohydrate in 50 mL of H_2O, previously adjusted to pH 7 with 0.1 N NaOH. The aqueous solution was stirred for 15 min, filtered to remove a slight haze, and then treated by the slow addition (peristaltic pump) of a solution of 685 mg (5.25 mmol) of 1,7-diaminoheptane in 20 mL of H_2O. The mixture was stirred for 3 h, and the solids were filtered, washed successively with ice-cold 0.01 M KI solution (2 × 30 mL) and then cold H_2O (2 × 30 mL), and dried under high vacuum at 50 °C for 60 h to leave 3.89 g of chloride/iodide as a yellow solid. The chloride/iodide was suspended into 60 mL of H_2O by sonication and then treated with a solution of 2.80 g (16.5 mmol) of $AgNO_3$ in 18 mL of H_2O. The mixture was sonicated for 10 min, stirred for 20 h, and then filtered through a 0.2-μm frit. The filtrate was treated with a solution of 1.02 g of $AgNO_3$ in 10 mL of H_2O, and the resultant solution was stirred for 2 h and filtered. The filtrate was treated with 5.9 mL of 1.0 M HCl, and the resultant mixture was stirred for 1.5 h and then filtered. The pale yellow filtrate was treated with 3.75 mL of 12 M HCl and then refrigerated overnight. The solids were collected by filtration, washed successively with cold H_2O (2 × 15 mL) and cold ethanol (2 × 15 mL), and dried to give 2.12 g of **9** as a pale yellow solid. Anal. ($C_7H_{24}N_4Cl_4Pt_2$) C, H, N, Cl.

Synthesis of Bis(platinum) Malonate Complexes (Method B). μ-(1,5-Pentanediamine-*N,N'*)bis[ammine(propanedioato(2-)-*O,O'*)platinum(II)] (12). A slurry of 3.0 g (4.49 mmol) of $[\{cis\text{-}PtCl_2(NH_3)\}_2H_2N(CH_2)_5NH_2]$ (**7**) in 150 mL H_2O was treated with 2.853 g (8.98 mmol) of solid silver(I) malonate followed by a 50 mL H_2O rinse. The reaction mixture was protected from light and stirred (occasional sonication) for 3 d at 35 °C. The mixture was filtered over a 0.2-μm frit, and the filtrate was concentrated to 50 mL and then cooled at 5 °C, leading to the precipitation of a white, fluffy solid. The solids were collected by filtration, washed with ethanol, and vacuum dried to give 590 mg of **12** as a white solid, 99.4% pure by HPLC. The AgCl collected from the above filtration was digested with 500 mL of H_2O at 35 °C, the suspension was filtered, and the filtrate was concentrated to ca. 100 mL. Cooling at 0 °C followed by collection of precipitate and drying as above afforded 1.53 g of a second crop of **12**, 99.9% by HPLC. The AgCl precipitate was digested a second time with 200 mL of H_2O followed by concentration to dryness. The solids were triturated in cold ethanol, filtered, and dried to leave 670 mg of a third crop, 96.3% pure by HPLC. Anal. ($C_5H_{24}N_4O_8Pt_2$) C, H, N.

Mice and Tumor Passage. Mice used for in vivo testing at the Parke-Davis Laboratories were from Charles River Breeding Laboratories and housed in barrier facilities. Immunocompetent mice were housed on automatic flush racks while immunodeficient mice were housed in microisolator cages. Food and water were provided ad libitum. Mice were maintained on a 12-h light/dark cycle. Tumors of murine origin were maintained in the inbred strain of tumor origin. Human tumors were maintained in NCr-nu mice. The H23 lung adenocarcinoma and H520 squamous cell carcinoma (lung) tumors were provided by Dr. Adi Gazdar. The AH125 lung adenocarcinoma was provided by Dr. Thomas Corbett.

Experimental Chemotherapy. In vitro growth inhibition in cisplatin-resistant murine leukemia cell lines was carried out by a published procedure.[20] In vivo anticancer activity was evaluated in Balb/c × DBA/2 F_1 mice ($CD2F_1$) for all leukemias, colon 26, colon 51, and mammary 25, and in C57BL/6 × C3H F_1 mice ($B6C3F_1$) for B16 melanoma and M5076 sarcoma. Activity against human tumor xenografts was evaluated in athymic NCr-nu mice. For all tests mice (18–22 g) were randomized and then inoculated with counted numbers of tumor cells or trocar fragments on day 0. Mice were then randomized again and distributed into treatment cages. Treatment was on the basis of average cage weight. Test compounds were dissolved in saline or 0.5% methylcellulose in saline. Methylcellulose in saline was used when there was a question concerning the uniformity of a test agent solution in saline.

Host body weight change data are reported as the difference between mean group weight on the last and first days of treatment for life span assays, or as the maximum treatment related weight loss for growth delay assays. In all tests an assessment of the probable cause of death of each mouse was made at necropsy. Calculation of the median day of death, percent T/C (leukemia models), T – C (solid tumor models), and net log cell kill were performed as described previously.[26-28] Net kill provides a measure of tumor response over duration of therapy. A positive

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net kill indicates that the tumor burden at the end of therapy was less than at the beginning of therapy. A negative net log cell kill indicates that the tumor grew during treatment. In growth delay assays, any mouse surviving tumor free for a period sufficient for a single surviving tumor cell to grow to a mass of 500 mg in a growth delay assay was considered cured. Likewise, in a life span assay any mouse surviving 60 days following treatment was considered cured. Cures were excluded from the calculation of % T/C, T - C, and net logs of tumor cell kill.

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