

homogenized in a cold Waring blender with two parts of tissues to one part of homogenization buffer consisting of 0.05 M sodium phosphate, 0.25 M sucrose, and 0.04 M nicotinamide, pH 7. The homogenate was centrifuged at 10000g for 30 min. The debris was discarded and the supernatant was centrifuged at 105000g for 1 h. The microsomal pellet obtained was resuspended in 0.1 M sodium phosphate buffer, pH 7, and centrifuged at 105000g for 1 h. The procedure was repeated once again and the resulting pellet was stored at -70 °C until needed. Protein concentrations were determined by the method of Lowry.²⁵

Competitive Inhibition Studies. Aromatase activity in human placental microsomes was assayed by a radiometric method developed by Siiteri and Thompson²¹ in which the tritium from [1β -³H]-4-androstene-3,17-dione was released as ³H₂O and used as an index of estrogen formation. The procedures for evaluation of inhibition are similar to those previously reported by Brueggemeier et al.¹⁴ [1β -³H]-4-Androstene-3,17-dione (300 000 dpm), various concentration of 4-androstene-3,17-dione (60–500 nM), and a concentration of inhibitor (0–600 nM) were preincubated

with propylene glycol, (100 μ L), NADP (1.8 mM), glucose 6-phosphate (2.85 mM) and glucose-6-phosphate dehydrogenase (5 units) at 37 °C for 5 min. Placental microsomes (0.07–0.1 mg) were diluted to 3.0 mL with 0.1 M sodium phosphate buffer, pH 7, and warmed to 37 °C for 5 min. The enzyme assay began with the addition of the microsomal suspension (3.0 mL) to the mixture of steroids and cofactors. The solution was incubated at 37 °C for 15 min in a shaking water bath and was stopped by addition of CHCl₃ (5 mL), followed by vortexing of the samples for 20 s. The samples were then centrifuged for 10 min (1000g). Aliquots of water (200 μ L) were mixed with scintillation cocktail (5 mL) and the radioactivity was counted. Assays were run in duplicate and control samples containing no inhibitor were run simultaneously. Blank samples were obtained by incubating boiled microsomes. Results were analyzed by a weighted regression-analysis computer program.²²

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Registry No. 1, 63-05-8; 2, 4966-70-5; 3, 122623-16-9; 4, 122623-17-0; 5, 122623-18-1; 6, 122623-19-2; 7, 122623-20-5; 8, 122623-21-6; 9, 122623-22-7; 10, 122623-23-8; ethylene glycol, 107-21-1; benzylmagnesium bromide, 1589-82-8; phenethyl lithium, 42808-98-0.

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cis-Diamineplatinum(II) Complexes Containing Phosphono Carboxylate Ligands as Antitumor Agents

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A series of platinum complexes of the form *cis*-M[PtA₂(PC)] (I) has been prepared and tested for antitumor activity in mice. Compounds in this series contain either two monodentate amine ligands (A), such as NH₃ or isopropylamine, or one bidentate diamine (A₂), such as ethylenediamine, 1,2-diaminopropane, or 1,2-diaminocyclohexane. The PC ligand is a bidentate, O-bound, phosphono carboxylate chelate of the form -O₂C(CR₁R₂)_nPO₃⁻, where *n* = 0 or 1 and R₁ and R₂ are chosen from H, methyl, ethyl, propyl, butyl, phenyl, or pentanoic acid substituents. The resulting complexes (I) were prepared as the free acids (M = H) or as sodium salts (M = Na). Members of this series have demonstrated good activity in a number of tumor screens. A total of 18 platinum-phosphono carboxylate (Pt-PC) complexes were tested against Sarcoma 180 ascites (S180a) in CFW mice, with 13 analogues showing activity above the 50% ILS level. Antitumor activity was also observed vs L1210 leukemia in CDF₁ mice, where six of the 12 compounds tested gave ILS values in the 60–160% range, and vs M5076 reticulum cell sarcoma (sc tumor, iv drug), where four of the four compounds tested gave ILS and T-C values comparable to that of cisplatin. Each of the Pt-PC complexes was characterized by NMR (¹⁹⁵Pt, ¹³C, and ³¹P), HPLC, and elemental analysis. These compounds, which are anionic at neutral pH, display excellent solubility and stability in aqueous media, such as phosphate-buffered saline and fetal calf serum. On the basis of a comparative study of BUN and serum creatinine levels in treated mice, representative complexes from this series are also less kidney toxic than cisplatin. The results of these studies demonstrate that the platinum-phosphono carboxylate complexes are a promising new class of antitumor agents.

Cisplatin is an effective anticancer agent that is presently used in the treatment of testicular, ovarian, and bladder carcinomas.^{1,2} In addition, cisplatin is widely used in combination with other antitumor agents, such as VP16, doxorubicin, and bleomycin, in treating small-cell lung carcinoma and head and neck cancers.³ The limited activity that cisplatin displays against such major forms of the disease as breast and colon cancer has stimulated the search for new platinum-based antitumor agents.^{1a} Furthermore, the adverse effects that are observed in patients receiving cisplatin, such as nephrotoxicity, myelosuppression, neurotoxicity, and emesis, have inspired efforts to develop new agents that will display improved toxicological properties.⁴ Research in this field has produced a number of promising new compounds that show good activity and reduced toxicity in a variety of animal tumor screens and

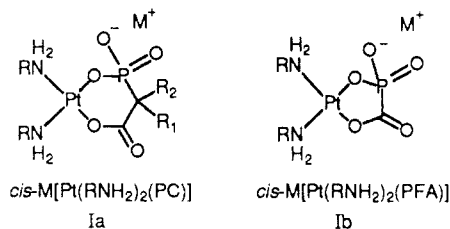
in clinical trials.^{5,6} While several of these second-generation platinum compounds, such as carboplatin, *cis*-[Pt-

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(NH₃)₂(cyclobutane-1,1-dicarboxylate)], iroplatin, *cis*-, *trans*-, *cis*-[Pt(*i*-PrNH₂)(OH)₂Cl₂], and tetraplatin, [Pt-(1,2-diaminocyclohexane)Cl₄], are showing promise as new clinical agents,⁶ the search for new platinum complexes that possess different activity and cross-resistance profiles is continuing.

In an effort to identify new compounds that possess improved therapeutic properties, we have chosen to study a new series of *cis*-diamineplatinum-phosphono carboxylate complexes which are represented by the general structural formulas given below (Ia and Ib). The amine

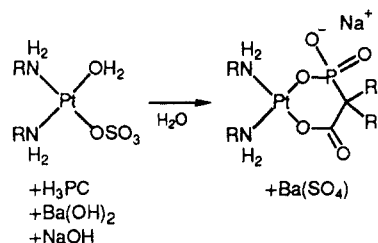


substituents (RNH₂) in this series of compounds can be two monodentate ligands, such as NH₃ or *i*-PrNH₂, or a bidentate ligand such as ethylenediamine (en), or 1,2-diaminocyclohexane (dach). The phosphono carboxylate group can be 2-phosphonoacetate (Ia, where R₁ and R₂ = H) or a substituted 2-phosphonoacetate (Ia, where R₁ and/or R₂ are alkyl, phenyl, or an aliphatic carboxylic acid substituents). Several platinum-phosphonoformate complexes of form Ib were also prepared and screened for antitumor activity. In both cases, the complexes can be isolated as free acids (M = H) or as sodium salts (M = Na).

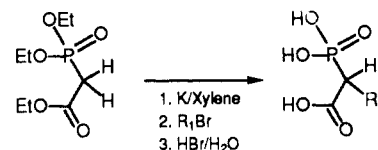
Phosphonic acids and their derivatives are known to display a wide variety of interesting biological properties. A number of phosphonate analogues of naturally occurring phosphate metabolites have demonstrated anticancer, antiviral, and antibacterial activity.⁷ Phosphono carboxylate compounds, such as phosphonoacetic acid (PAA) and phosphonoformic acid (PFA), are known to inhibit viral DNA polymerase and reverse transcriptase activity.⁸ Trisodium phosphonoformate, also known as foscarnet, has demonstrated activity against a variety of viruses, such as

Scheme I

(a) complex synthesis



(b) ligand synthesis



Herpes simplex (types 1 and 2), cytomegalovirus (CMV), and human immunodeficiency virus (HIV).⁸ The recent surge of interest in antiviral therapy has stimulated research in the area of phosphonate chemistry. As a result of the increased activity in this field, two related publications, which describe compounds that are members of the structural classes shown in Ia and Ib, appeared while the work presented in this report was in progress.⁹ In the first report, Speer and Stewart¹⁰ discussed the preparation and antitumor properties of two platinum-phosphonoacetate complexes, H[Pt(R,R-dach)(PAA)] and H[Pt(1-(aminomethyl)cyclooctylamine)(PAA)], and recently Bau et al.¹¹ presented a crystallographic characterization of *cis*-Na[Pt(NH₃)₂(PFA)] and Na[Pt(R,R-dach)(PFA)].

In this report, we present chemical and biological data on a variety of platinum-phosphono carboxylate complexes that demonstrate excellent antitumor activity against murine tumor systems. These compounds also display desirable physical properties, such as high solubility and stability in aqueous media, and improved toxicological properties, such as reduced kidney toxicity relative to cisplatin. In addition, the platinum-phosphono carboxylate complexes represent a potentially broad class of antitumor agents, as a variety of substituents can be placed on the central carbon of the phosphono carboxylate ligand. This combination of favorable physical and biological properties provides these complexes with a number of advantages over existing platinum drugs and establishes them as a promising new class of antitumor agents.

Results and Discussion

Chemical Studies. A number of platinum-phosphono carboxylate complexes containing various amine and phosphono carboxylate ligands were prepared in an attempt to optimize the antitumor properties of compounds in this series. The complexes can be prepared by using the general reaction sequence outlined in Scheme I, part a. This method uses the reaction of Ba(OH)₂ and NaOH with 1 equiv of the chosen phosphono carboxylic acid (H₃PC) and platinum complex (*cis*-[Pt(diamine)-(SO₄)(H₂O)]), to produce the desired product in a one-step process. A second method, which involves the reaction of the diaqua nitrate complex *cis*-[Pt(diamine)(H₂O)₂(NO₃)₂], with the appropriate sodium salt of the phosphono carboxylate ligand, also can be used to prepare these com-

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Table I. ^{195}Pt and ^{31}P NMR Data for Platinum-Phosphono Carboxylate Complexes

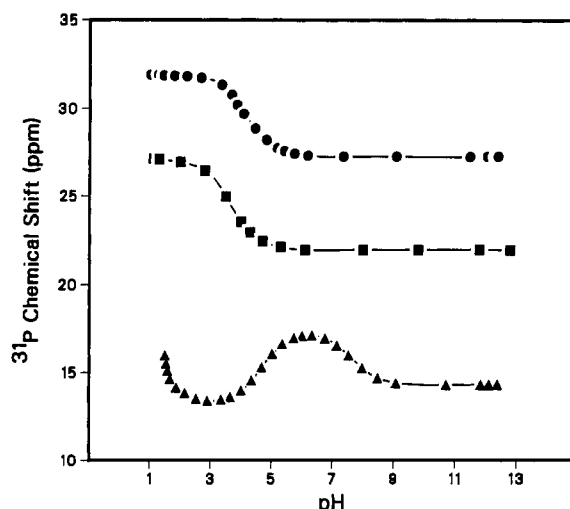
compound ^a	δ	
	^{31}P	^{195}Pt
<i>cis</i> -Na[Pt(NH ₃) ₂ (PAA)] (1)	24.2	-1591
<i>cis</i> -Na[Pt(NH ₃) ₂ (PPA)] (2)	27.6	-1590
<i>cis</i> -Na[Pt(NH ₃) ₂ (PBA)] (3)	27.5	-1585
<i>cis</i> -Na[Pt(NH ₃) ₂ (PVA)] (4)	27.8	-1595
<i>cis</i> -Na[Pt(NH ₃) ₂ (PPHA)] (5)	23.4	-1614
<i>cis</i> -Na[Pt(<i>i</i> -PrNH ₂) ₂ (PAA)] (6)	23.4	-1717
Na[Pt(en)(PFA)] (7)	9.4	-1932
Na[Pt(en)(PAA)] (8)	23.7	-1852
Na[Pt(pn)(PAA)] (9)	23.8	-1803
Na[Pt(pn)(PPA)] (10)	27.1	-1824
Na[Pt(pn)(PBA)] (11)	26.9	-1803
Na[Pt(<i>R,R</i> -dach)(PFA)] (12)	10.2	-1886
Na[Pt(<i>R,R</i> -dach)(PAA)] (13)	26.5	-1822
Na[Pt(<i>R,R</i> -dach)(PBA)] (14)	27.1	-1804
Na[Pt(<i>R,R</i> -dach)(PVA)] (15)	27.3	-1807
Na[Pt(<i>R,R</i> -dach)(PPHA)] (16)	23.1	-1818
Na[Pt(<i>R,R</i> -dach)(MPBA)] (17)	31.6	-1787
Na ₂ [Pt(<i>R,R</i> -dach)(PHD)] (18)	27.5	-1807
<i>cis</i> -[Pt(NH ₃) ₂ (H ₂ O) ₂](NO ₃) ₂		-1582
[Pt(<i>R,R</i> -dach)(H ₂ O) ₂](NO ₃) ₂		-1872

^a See the Experimental Section for abbreviations.

plexes. However, the materials that are obtained by this method are more difficult to purify, as the crude product contains substantial amounts of NaNO₃. The BaSO₄ method provides platinum-phosphono carboxylate (Pt-PC) complexes in fairly good yields with minimal salt contamination. All of the products were purified by recrystallization or chromatography before they were screened for antitumor activity.

A synthetic route to disubstituted and functionalized phosphono carboxylate ligands was developed as part of an effort to examine the relationship between the C2 substituents on the PC ligand and antitumor activity of the resulting Pt-PC complexes. The reaction sequence that was used to prepare the C2-substituted phosphono carboxylate ligands is outlined in Scheme I, part b.¹² In the first step of this procedure, the potassium salt of the chosen triethyl phosphonoacetate anion was alkylated with an alkyl bromide (R₁Br) and the resulting product was purified by vacuum distillation. The free acids were obtained by hydrolyzing the esterified product with aqueous HBr and, after purification, these materials were used directly to prepare the platinum complexes (Scheme I, part a). Both disubstituted and functionalized PC ligands, such as H₃MPBA (R₁ = Me, R₂ = Et) and H₄PHD (R₁ = (CH₂)₄CO₂H, R₂ = H), were prepared in this fashion.

All of the platinum-phosphono carboxylate complexes that were synthesized for evaluation in the tumor screens were characterized by using ^{195}Pt , ^{13}C , and, ^{31}P NMR spectroscopy and elemental analysis. The ^{195}Pt and ^{31}P NMR data for each compound in this series are presented in Table I and the ^{13}C NMR data is given as supplementary material (Table S1). The ^{195}Pt NMR resonances for the various *cis*-Na[Pt(diamine)(PC)] complexes appear within 100 ppm of the corresponding starting materials, *cis*-[Pt(diamine)(H₂O)₂]²⁺. ^{195}Pt chemical shifts in this range are characteristic of *cis*-diamineplatinum(II) complexes bound to oxygen-based donors.¹³ In contrast, the

**Figure 1.** ^{31}P NMR titration curves for the reaction of 1 M NaOH with (▲) phosphonoacetic acid (H₃PAA, 100 mg in 3 mL of H₂O), (■) H[Pt(*R,R*-dach)(PAA)] (13, 100 mg in 3 mL of H₂O), and (●) H₂[Pt(*R,R*-dach)(PHD)] (18, 100 mg in 3 mL of H₂O).

^{31}P resonance for each ligand in this series shows an upfield shift of 10–15 ppm upon coordination to the metal. This change indicates that the ^{31}P nucleus is placed in a more shielded environment upon chelate formation.

NMR titration studies were used to examine the acid properties of phosphonoacetic acid and two of the platinum-phosphono carboxylate complexes, H[Pt(*R,R*-dach)(PAA)] (13) and H₂[Pt(*R,R*-dach)(PHD)] (18). As shown in Figure 1, the ^{31}P chemical shift of phosphonoacetic acid, H₃PAA, is sensitive to the pH of the solution and the position of the resonance shifts direction as each of the three protons are removed during the titration. In a previous study by Popov,¹⁴ the three acidity constants of H₃PAA ($\text{p}K_{\text{a}1} \approx 2$, $\text{p}K_{\text{a}2} = 5.11$, and $\text{p}K_{\text{a}3} = 8.69$) were determined from potentiometric titration data, and NMR studies (^{31}P and ^{13}C) were used to show that the second deprotonation step corresponds to the removal of the carboxylic acid proton. The ^{31}P NMR titration data obtained on H₃PAA (Figure 1) is in good agreement with those reported by Popov.¹⁴

The ^{31}P NMR titration curve for H[Pt(*R,R*-dach)(PAA)] (13) shows one inflection point over the pH range of 1–13 and based on this data, the estimated $\text{p}K_{\text{a}}$ of the phosphonic acid group in this complex is ~ 3.7 . When this value is compared to the third deprotonation step of phosphonoacetic acid ($\text{p}K_{\text{a}3} = 8.69$), which corresponds to the removal of the second phosphonic acid proton ($\text{H}-\text{O}_3\text{PCH}_2\text{CO}_2^-$), the phosphonic acid group in the platinum complex is found to be a stronger acid by ~ 5 $\text{p}K_{\text{a}}$ units. An increase in the acidity of a proton at or near the site of ligand attachment is an effect that is commonly observed with transition-metal complexes.¹⁵ A single inflection point was also observed in a ^{31}P NMR titration study of the diprotic acid H₂[Pt(*R,R*-dach)(PHD)] (18, see Figure 1). In this case, the position of the ^{31}P resonance in 18 appears to be insensitive to the removal of the second proton, which lies on the terminal carboxylate group seven bonds away from the ^{31}P nucleus. The $\text{p}K_{\text{a}}$ of the terminal carboxylic acid in 18, which is expected to lie in the range of 4.6–5.1 (i.e. butyric acid, $\text{p}K_{\text{a}} = 4.9$), should be slightly higher than that of the phosphonic acid group ($\text{p}K_{\text{a}} \approx 4.2$). In a separate potentiometric titration study of 18, both deprotonation steps were found to occur, with a single

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Table II. Summary of S180a Screening Data for the Platinum-Phosphono Carboxylate Complexes in CFW Mice

compound ^a	best %ILS	opt dose, mg/kg	30-day survivors
<i>cis</i> -Na[Pt(NH ₃) ₂ (PAA)] (1)	25	20	0/6
<i>cis</i> -Na[Pt(NH ₃) ₂ (PPA)] (2)	16	10	1/6
<i>cis</i> -Na[Pt(NH ₃) ₂ (PBA)] (3)	38	40	1/6
<i>cis</i> -Na[Pt(NH ₃) ₂ (PVA)] (4)	-74	20	0/6
<i>cis</i> -Na[Pt(NH ₃) ₂ (PPHA)] (5)	71	80	2/6
<i>cis</i> -Na[Pt(<i>i</i> -PrNH ₂) ₂ (PAA)] (6)	15	160	0/6
H[Pt(en)(PFA)] ^b (7)	93	40	5/6
H[Pt(en)(PAA)] ^b (8)	93	80	4/6
H[Pt(pn)(PAA)] ^b (9)	77	20	3/6
Na[Pt(pn)(PPA)] (10)	53	160	1/6
Na[Pt(pn)(PBA)] (11)	95	160	4/6
Na[Pt(<i>R,R</i> -dach)(PFA)] (12)	59	160	0/6
Na[Pt(<i>R,R</i> -dach)(PAA)] (13)	98	40	5/6
Na[Pt(<i>R,R</i> -dach)(PBA)] (14)	76	80	3/6
Na[Pt(<i>R,R</i> -dach)(PVA)] (15)	92	80	5/6
Na[Pt(<i>R,R</i> -dach)(PPHA)] (16)	102	160	5/6
Na[Pt(<i>R,R</i> -dach)(MPBA)] (17)	100	80	6/6
Na ₂ [Pt(<i>R,R</i> -dach)(PHD)] (18)	86	80	4/6
cisplatin	81	8	3/6
carboplatin	95	120	4/6

^a See the Experimental Section for ligand abbreviations.^b Adjusted to pH 7 before administration.

inflection point, over the expected pH range (3–5).

The results of the titration studies indicate that the platinum-phosphono carboxylate complexes are anionic species at neutral or basic pH (>4). Under these conditions, the complexes have high aqueous solubilities (up to 1 g/mL) as a result of their anionic nature. The Pt-PC complexes also have good stability in biological media, such as phosphate-buffered saline (PBS) and fetal calf serum (FCS). By monitoring the ³¹P NMR signals of Na₂[Pt(*R,R*-dach)(PHD)] as a function of time, the half-life of this complex in fetal calf serum was determined to be 60 h at 37 °C. In a related experiment, the half-life of carboplatin, *cis*-[Pt(NH₃)₂(CBDCA)], in fetal calf serum was determined to be 120 h at 37 °C by using ¹⁹⁵Pt NMR. This result is in good agreement with the reported half-life of 123 h for carboplatin in rat plasma, as determined by atomic absorption spectroscopy.¹⁶ These experiments show that the platinum-phosphono carboxylate complex (18) is somewhat more reactive than *cis*-[Pt(NH₃)₂(CBDCA)] but less reactive than cisplatin, which has a half-life of 1.5 h in human plasma at 37 °C.¹⁷ The platinum-phosphono carboxylate complexes also show very little reaction with PBS (<5% decomposition) over a period of 3 days at 37 °C, demonstrating that these complexes are stable in a chloride-containing medium.

Biological Studies

Each of the platinum-phosphono carboxylate complexes was tested against Sarcoma 180 ascites (S180a) in CFW mice. As indicated from the data presented in Table II, activity at levels above 50% ILS was found in 13 of the 18 compounds tested in this screen. With the exception of one analogue (5), all of the complexes containing monodentate amine ligands (ammonia and isopropylamine) were inactive vs the S180a screen. In addition, two analogues, Na[Pt(pn)(PPA)] (10) and Na[Pt(*R,R*-dach)(PFA)] (12), displayed only marginal activity in this screen (ILS = 50–60%). All of the remaining compounds showed excellent activity, with seven analogues giving >90% ILS and a high percentage of 30-day survivors (70–100%). These

Table III. Summary of L1210 Screening Data for the Platinum-Phosphono Carboxylate Complexes in CDF₁ Mice

compound ^a	best %ILS	opt dose, mg/kg	30-day survivors
<i>cis</i> -Na[Pt(NH ₃) ₂ (PAA)] (1)	13	40	0/6
<i>cis</i> -Na[Pt(NH ₃) ₂ (PBA)] (3)	-5	80	0/6
<i>cis</i> -Na[Pt(NH ₃) ₂ (PPHA)] (5)	23	40	0/6
H[Pt(pn)(PAA)] ^b (9)	2	10	0/6
Na[Pt(pn)(PBA)] (11)	16	160	0/6
Na[Pt(<i>R,R</i> -dach)(PFA)] (12)	20	320	0/6
Na[Pt(<i>R,R</i> -dach)(PAA)] (13)	62	160	2/6
Na[Pt(<i>R,R</i> -dach)(PBA)] (14)	106	80	2/6
Na[Pt(<i>R,R</i> -dach)(PVA)] (15)	109	160	0/6
Na[Pt(<i>R,R</i> -dach)(PPHA)] (16)	76	160	0/6
Na[Pt(<i>R,R</i> -dach)(MPBA)] (17)	114	160	3/6
Na ₂ [Pt(<i>R,R</i> -dach)(PHD)] (18)	59	10	0/6
	67	20	0/6
	88	40	0/6
	133	80	3/6
	161	160	3/6
cisplatin	87	8	0/6
carboplatin	90	80	0/6

^a See the Experimental Section for ligand abbreviations.^b Adjusted to pH 7 before administration.**Table IV.** Antitumor Activity of Selected Platinum-Phosphono Carboxylate Complexes against Subcutaneously Implanted M5076 Reticulum Cell Sarcoma

compound ^a	best %ILS	best T-C, days	opt dose, (mg/kg per inj) ^b
Na[Pt(<i>R,R</i> -dach)(PAA)] (13)	31	28	70
Na[Pt(<i>R,R</i> -dach)(PVA)] (15)	33	26	100
Na[Pt(<i>R,R</i> -dach)(MPBA)] (17)	29	19	160
Na ₂ [Pt(<i>R,R</i> -dach)(PHD)] (18)	28	22	100
cisplatin	28	25	5

^a See the Experimental Section for ligand abbreviations.^b Treatments administered on days 5, 9, 13, and 17 after tumor implantation.

analogues were more active than the control compounds cisplatin and carboplatin (see Table II). While the optimum dose in each case was higher than that seen with cisplatin, a number of analogues displayed peak activity at 20–80 mg/kg. These analogues were more potent than carboplatin, which has an optimum dose of 120 mg/kg in this screen.

Antitumor activity also was observed in the L1210 leukemia screen, where six of the 12 compounds tested were active above the 60% ILS level (see Table III). Four of the compounds showed ILS values greater than 100% and two of these analogues gave 3/6 long term survivors (>30 days). Based on these studies, the compounds also performed better than the cisplatin and carboplatin controls, where the best result gave a 90% ILS, with no 30-day survivors. While the compounds that were tested in this screen appear to be less potent than cisplatin and carboplatin, a broad dose response was observed with the Pt-PC complexes. An example of this feature is presented in Table III, where the complete dose-response data is given for Na₂[Pt(*R,R*-dach)(PHD)] (18). In this case, good activity is observed over the 10–160 mg/kg range. On the basis of the data presented in Table III, it appears that the most active complexes are those containing alkylated phosphono carboxylate ligands (14, 15, 17, and 18).

Four of the platinum-phosphono carboxylate complexes (13, 15, 17, and 18) were evaluated for activity against sc implanted M5076 reticulum cell sarcoma (iv treatment).¹⁸

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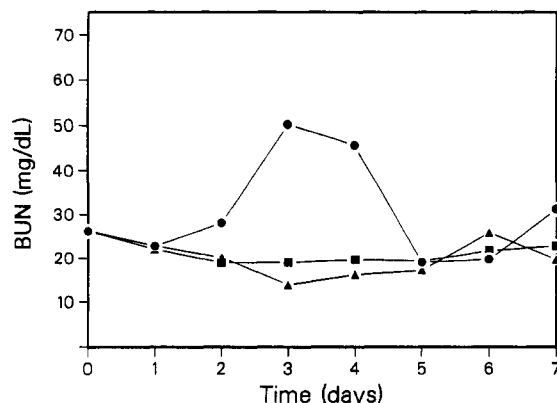


Figure 2. BUN levels in CFW mice as a function of time following treatment with (●) 12 mg/kg cisplatin, (▲) 240 mg/kg Na₂[Pt(R,R-dach)(PHD)] (18), and (■) = 240 mg/kg Na[Pt(R,R-dach)(PVA)] (15). In each case, the administered dose corresponds to an approximate LD₁₀ value.

The results, summarized in Table IV, indicate that these complexes were active in terms of increased lifespan and delay in growth of the primary tumor and that this activity was comparable to that of cisplatin. The results demonstrate that these analogues were active against tumors located distal to the site of drug administration. Further preclinical antitumor studies of these complexes are being conducted against cisplatin-resistant human tumor cell lines.

Preliminary toxicity studies on two of the Pt-PC complexes, Na[Pt(R,R-dach)(PVA)] (15) and Na₂[Pt(R,R-dach)(PHD)] (18), were conducted by using CFW mice. As a measure of kidney toxicity, BUN and serum creatinine levels were monitored after administration of the test compounds at a dose equal to 1.5 times the peak active dose found in the S180a screen (240 mg/kg for both compounds). As a control, cisplatin also was given at 1.5 times the peak active dose (12 mg/kg). In each case, the chosen dose was approximately equal to the LD₁₀ value in CFW mice. A plot of the BUN levels vs time, following the administration of the two test compounds and the cisplatin control, is presented in Figure 2. As indicated from this data, cisplatin produced a significant rise in BUN (>30 mg/dL) on days 3 and 4 following administration, while the two phosphono carboxylate complexes showed no elevation over the 7-day examination period. While there are a number of parameters that are more sensitive indicators of renal toxicity,¹⁹ such as the level of urinary glucose and protein excretion, BUN elevation does correlate well with platinum-induced nephrotoxicity. BUN values in excess of 30 mg/dL in treated mice are generally indicative of a positive nephrotoxic response.^{4b,21}

In addition, these two complexes did not elevate serum creatinine levels over the 7-day posttreatment period, while cisplatin produced a 40% increase in serum creatinine (from 0.40 mg/dL to an average value of 0.56 mg/dL) on days 5–7. Since the two Pt-PC complexes do not increase BUN or serum creatinine, it appears that these complexes are less kidney toxic than cisplatin at a dose which approximates the LD₁₀ level. More detailed toxicological studies of the platinum-phosphono carboxylate complexes are in progress.

Conclusion

It has been shown that a number of platinum-phosphono carboxylate complexes have pronounced activity against S180a, L1210, and M5076 murine tumor systems. These complexes, which are anionic at physiological pH, possess desirable physical properties such as high solubility and stability in aqueous solutions. This series of complexes represents a potentially broad class of antitumor agents, as a variety of substituents can be placed on the central carbon of the phosphono carboxylate ligand. In addition, toxicity studies in CFW mice indicate BUN and serum-creatinine elevation is not observed when two of the Pt-PC complexes are given at 1.5× the optimum dose (~LD₁₀), indicating that these complexes are less kidney toxic than cisplatin in this system. The results of these studies demonstrate that the platinum-phosphono carboxylate complexes show a number of advantages over existing platinum drugs and suggest that they hold considerable promise as a new class of antitumor agents.

Experimental Section

Abbreviations: dach, 1,2-diaminocyclohexane; *R,R*-dach, *trans*-(*R,R*)-dach; en, 1,2-ethylenediamine; pn, 1,2-propylenediamine; PFA, phosphonoformic acid; PAA, 2-phosphonoacetic acid; PPA, 2-phosphonopropionic acid; PBA, 2-phosphonobutyric acid; PVA, 2-phosphonovaleric acid; PPHA, 2-phosphonophenylacetic acid; PHD, 2-phosphono-1,7-heptanedicarboxylic acid; MPBA, 2-methyl-2-phosphonobutyric acid; BUN, blood urea nitrogen.

Physical Methods. NMR spectra were recorded with a Varian XL-200 spectrometer using a 10-mm broadband probe (20–80 MHz). ¹⁹⁵Pt spectra (42.935 MHz) were typically collected by using a 9 μs (70°) pulse, a 0.06 s acquisition time, and a spectral width of 80 kHz (19.6K data points). Spectra were processed by using line broadening (200 Hz) and zero filling (64K). ¹⁹⁵Pt chemical shifts were referenced relative to an external sample of 0.1 M K₂[PtCl₄] in D₂O (at -1624 ppm vs H₂PtCl₆, 1 g/3 mL of D₂O). ¹³C NMR spectra were collected by using a 5-μs pulse (30°), a 12 kHz sweep width and a 1-s acquisition time. ³¹P spectra were collected with a 0.8-s acquisition time, a 10-μs pulse (60°), and 10 kHz sweep width. ³¹P chemical shifts were referenced relative to H₃PO₄ (85%) and ³¹P NMR titrations were monitored with a Corning 145 pH meter equipped with an Ingold combination electrode. A Waters Delta-Prep 5000 HPLC system was used to check sample purity. Samples were typically run in water, water/methanol, or 0.1 M ammonium acetate (pH 6) at a flow rate of 6 mL/min using a Novapack C₁₈ radical compression column (Z module). Peaks were monitored by UV-absorbance at 254 nm. Satisfactory elemental analyses were obtained on all compounds. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN.

Compound Preparation. Starting Materials. All *cis*-[Pt(amine)₂I₂] starting materials were prepared from K₂[PtCl₄] (Engelhard) by using the methods of Dhara.²⁰ The *cis*-[Pt(amine)₂(SO₄)(H₂O)] starting materials were prepared as outlined below for [Pt(*R,R*-dach)(SO₄)(H₂O)]. The PFA, PAA, PPA, and PBA ligands were purchased from commercial sources (Alfa, Fairfield, or Sigma Chemical) either as free acids or as the triethyl esters. The esters were converted to the corresponding free acids by using the general procedure given below for 2-phosphonovaleric acid. Fetal calf serum and phosphate-buffered saline were obtained from Sigma and all other reagents were obtained from commercial sources.

[Pt(*R,R*-dach)(SO₄)(H₂O)]. A suspension of [Pt(*R,R*-dach)I₂] (183 mmol) and Ag₂SO₄ (183 mmol) was stirred in 1 L of water for 18 h at room temperature (in the dark). The AgI precipitate was removed by filtration and the filtrate was evaporated to a volume of ~150 mL. The concentrated solution was refiltered through a 0.45-μm nylon filter and the filtrate was cooled to 10 °C in an ice bath. The resulting solution was poured into cold ethanol and the cream-colored precipitate was collected by filtration. The solid was redissolved in a minimum amount of water and filtered into ethanol and the resulting white precipitate was filtered and dried for 48 h under vacuum. The product was redissolved in water (200 mL) and filtered to remove insoluble

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materials, and the filtrate was evaporated to dryness under vacuum (yield 45 g, white solid). A platinum analysis, by the ash method, indicated that the product is best formulated as $[\text{Pt}(\text{R,R-dach})(\text{SO}_4)(\text{H}_2\text{O})]$.

2-Phosphonovaleric Acid (H_3PVA). A 50-g sample of triethyl 2-phosphonovalerate was heated to 80 °C for 48 h in 250 mL of aqueous HBr (48%). The resulting amber solution was evaporated in a fume hood over a period of 48 h. The remaining oil was dissolved in acetone and filtered through charcoal and the filtrate was evaporated to dryness. Crystals of the free acid were collected, washed with toluene, and dried under vacuum.

2-Methyl-2-phosphonobutyric Acid (H_3MPBA). A solution of triethyl 2-phosphonopropionate in 200 mL of anhydrous xylene was treated with 4.46 g of K metal and the resulting mixture was refluxed for 1 h (under N_2) with stirring. After all of the metal had reacted, 16 mL of ethyl iodide (in 50 mL of xylene) was added dropwise to the solution. The mixture was refluxed for 2 h and then stirred for 18 h at room temperature. After removing the KI precipitate by filtration, the filtrate was evaporated under vacuum. The resulting triethyl ester of MPBA as purified by vacuum distillation (bp 105 °C at 0.1 mmHg). The free acid was obtained by hydrolysis, as described above, and the product was characterized by using NMR.

2-Phosphono-1,7-heptanedicarboxylic Acid (H_4PHD). Triethyl 2-phosphonoacetate (44.6 mmol) was added, with stirring, over a period of 30 min to 1 L of anhydrous xylene containing 46 mmol of K metal. This reaction was determined to be complete when the absence of metal was noted (on some occasions warming was required). The solution was cooled to room temperature and 49 mmol of ethyl 5-bromovalerate was added over a period of 15 min. The mixture was refluxed for 6 h and stirred for 10 h at room temperature. The KBr precipitate was removed by filtration and the filtrate was evaporated under vacuum. The resulting tetraethyl ester of PHD was purified by vacuum distillation (bp 165 °C at 0.1 mmHg). The free acid was obtained by hydrolysis with HBr , as described above.

***cis*- $\text{Na}[\text{Pt}(\text{NH}_3)_2(\text{PAA})]$ (1).** A mixture of 10 mmol of *cis*- $[\text{Pt}(\text{NH}_3)_2(\text{SO}_4)(\text{H}_2\text{O})]$, 10 mmol of $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$, and 10 mmol of 2-phosphonoacetic acid was stirred in 50 mL of water for 2 h at 25 °C. The BaSO_4 precipitate was removed by filtration and the filtrate was evaporated to dryness. The resulting solid was suspended in 2-propanol, filtered, and air dried. The product was suspended in water and the pH was adjusted to 8.0 with 6 M NaOH . A small amount of insoluble material was removed by filtration and the filtrate was evaporated to dryness and washed with 2-propanol (yield 1.8 g, after drying).

***cis*- $\text{Na}[\text{Pt}(\text{NH}_3)_2(\text{PPA})]$ (2).** A solution of 15 mmol of *cis*- $[\text{Pt}(\text{NH}_3)_2(\text{SO}_4)(\text{H}_2\text{O})]$, 15 mmol of NaOH , 15 mmol of $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$, and 15 mmol of 2-phosphonopropionic acid in 300 mL of water was heated to 50 °C for 40 min. Following 4 h of stirring at room temperature, the BaSO_4 precipitate was removed by filtration. The filtrate was evaporated to 15 mL (55 °C, under vacuum) and the solution was cooled to 5 °C for 4 h. The resulting precipitate was filtered and washed with water and ethanol (yield 1.1 g). Two additional crops were obtained from the filtrate. The product was purified from ethanol/water.

***cis*- $\text{Na}[\text{Pt}(\text{NH}_3)_2(\text{PBA})]$ (3).** A mixture of 10 mmol each of *cis*- $[\text{Pt}(\text{NH}_3)_2(\text{SO}_4)(\text{H}_2\text{O})]$, $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$, and 2-phosphonobutyric acid was stirred in 200 mL of water at room temperature. After 10 min, 10 mmol of NaOH was added, and the solution was stirred for an additional 24 h. The BaSO_4 precipitate was removed by filtration and the filtrate was evaporated to dryness (at 55 °C, under vacuum). The resulting solid was stirred in 2-propanol, filtered, and vacuum dried. The product was purified by recrystallization from water and 2-propanol (yield 1.5 g).

***cis*- $\text{Na}[\text{Pt}(\text{NH}_3)_2(\text{PVA})]$ (4).** An aqueous solution (200 mL) of 10 mmol each of *cis*- $[\text{Pt}(\text{NH}_3)_2(\text{SO}_4)(\text{H}_2\text{O})]$, NaOH , $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$, and 2-phosphonovaleric acid was stirred for 24 h at room temperature. The product was isolated and purified as described for compound 3 (yield 3.82 g).

***cis*- $\text{Na}[\text{Pt}(\text{NH}_3)_2(\text{PPHA})]$ (5).** A solution of 11.7 mmol of *cis*- $[\text{Pt}(\text{NH}_3)_2(\text{SO}_4)(\text{H}_2\text{O})]$ and 11.7 mmol of 2-phosphophenylacetic acid in 125 mL of water was treated with 11.7 mmol of $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$ and 11.7 mmol of NaOH . The BaSO_4 precipitate was removed by filtration after 24 h and the resulting product was isolated from the filtrate as described above (yield 0.9 g).

***cis*- $\text{Na}[\text{Pt}(i\text{-PrNH}_2)_2(\text{PAA})]$ (6).** A solution of *cis*- $[\text{Pt}(\text{isopropylamine})_2(\text{SO}_4)(\text{H}_2\text{O})]$ (20 mmol) and phosphonoacetic acid (20 mmol) in 200 mL of water and stirred for 20 min at room temperature. NaOH (20 mmol) and $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$ (20 mmol) were then added to the solution, and after 24 h, the BaSO_4 precipitate was removed by filtration. The filtrate was evaporated to dryness under vacuum at 55 °C and the resulting solid was purified from 2-propanol/water (yield 9.8 g, after drying).

$\text{H}[\text{Pt}(\text{en})(\text{PFA})]$ (7). $[\text{Pt}(\text{en})\text{I}_2]$ (10 mmol) and AgNO_3 (20 mmol) were stirred in 80 mL of water for 1 h at 50 °C. After cooling of the solution to 25 °C, the AgI precipitate was removed by filtration. Trisodium phosphonoformate (10 mmol in 10 mL of water) was added to the filtrate, at which point the pH of the solution increased from 2.3 to 6.4. After 30 min of stirring at room temperature, the pH was readjusted to 5.0 with 0.1 N HNO_3 and the mixture was stirred for an additional 22 h. A yellow precipitate was filtered off and the filtrate was evaporated to dryness. The resulting solid was redissolved in water (10 mL) and filtered. The filtrate was adjusted to pH 1.0 with concentrated HNO_3 and cooled to 5 °C for 1 h. A white precipitate was collected by filtration, washed with water (3 mL) and two portions each of 2-propanol (3 mL) and ethyl ether (3 mL), and vacuum dried (yield 1.6 g). Aqueous solutions of this product were readjusted to pH 5.0 with 6 N NaOH to prepare the sodium salt.

$\text{H}[\text{Pt}(\text{en})(\text{PAA})]$ (8). $[\text{Pt}(\text{en})\text{I}_2]$ (20 mmol) and AgNO_3 (40 mmol) were stirred in 150 mL water at 45 °C for 1 h. The suspension was cooled to 25 °C and the AgI precipitate was removed by filtration. A solution of 2-phosphonoacetic acid (21 mmol in 5 mL water) and sodium hydroxide (20 mmol in 5 mL water) were combined and added to the filtrate. The filtrate was stirred at room temperature for 2 h under N_2 . The volume of the solution was reduced to 5 mL under vacuum and the remaining oil was placed in 100 mL of ethanol for 24 h at 5 °C. The resulting precipitate was collected by filtration (yield 8.3 g). The product was purified by warming the solid in water and adjusting the pH to 4.3 with 6 M NaOH . The solution was filtered and the filtrate was acidified with 2 M HNO_3 (pH 2.5). The resulting precipitate was collected by filtration, washed with methanol, and vacuum dried (yield 4.8 g).

$\text{Na}[\text{Pt}(\text{en})(\text{PAA})]$ (8a). A mixture of $[\text{Pt}(\text{en})(\text{SO}_4)(\text{H}_2\text{O})]$, 2-phosphonoacetic acid, and $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$ (20.24 mmol of each) was stirred at room temperature in 200 mL of water for 18 h. BaSO_4 precipitate was removed by filtration and the solution was evaporated to 50 mL under vacuum and stored at 5 °C for 24 h. The resulting precipitate was isolated from the solution by filtration. The solid was suspended in water and the solution was adjusted to pH 7 with 2 M NaOH . The solution was filtered and the filtrate was evaporated to dryness. The product was purified from water/ethanol and vacuum dried (yield 3.7 g).

$\text{H}[\text{Pt}(\text{pn})(\text{PAA})]$ (9). $[\text{Pt}(\text{pn})(\text{SO}_4)(\text{H}_2\text{O})]$ (20.0 mmol), 2-phosphonoacetic acid (20.0 mmol), and $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$ (20.0 mmol) were dissolved in 200 mL of water and stirred for 18 h at 25 °C. The BaSO_4 precipitate was removed by filtration and the filtrate was evaporated to dryness under vacuum. The resulting solid was purified from methanol/water and dried under vacuum (yield 5.8 g).

$\text{Na}[\text{Pt}(\text{pn})(\text{PAA})]$ (9a). A 2.0-g portion of the free acid (9) was stirred in 5 mL water and adjusted to pH 4.5 with 2 N NaOH . The solution was filtered into absolute ethanol and the resulting precipitate was collected by filtration, washed with methanol, and dried under vacuum (yield 1.2 g).

$\text{Na}[\text{Pt}(\text{pn})(\text{PPA})]$ (10). $[\text{Pt}(\text{pn})(\text{SO}_4)(\text{H}_2\text{O})]$ (10 mmol), 2-phosphonopropionic acid (10 mmol), and $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$ (10 mmol) were stirred in 200 mL of H_2O for 30 min at room temperature. Sodium hydroxide (10 mmol in 5 mL H_2O) was added to the mixture and, after 24 h, the BaSO_4 precipitate was removed by filtration. The filtrate was evaporated to dryness and the solid was purified from 2-propanol/water and vacuum dried (yield 4.3 g).

$\text{Na}[\text{Pt}(\text{pn})(\text{PBA})]$ (11). $[\text{Pt}(\text{pn})(\text{SO}_4)(\text{H}_2\text{O})]$ (10 mmol) and 10 mmol of 2-phosphonobutyric acid were mixed with 10 mmol of $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$ and 10 mmol of NaOH in 200 mL of water. After 24 h of stirring, the BaSO_4 was removed by filtration and the filtrate was evaporated to dryness at 55 °C under vacuum. The resulting solid was suspended in 2-propanol, filtered, and dried under vacuum for 48 h (yield of 3.6 g).

Na[Pt(*R,R*-dach)(PFA)] (12). [Pt(*R,R*-dach) I_2] (10 mmol) and AgNO₃ (20 mmol) were placed in 200 mL of water and the mixture was stirred at 45 °C for 1 h. The AgI precipitate was removed by filtration and 10 mmol of trisodium phosphonofluoridate was added to the filtrate. After stirring of the solution for 24 h, it was evaporated to dryness under vacuum and the residue was stirred in ethanol. The resulting white solid was filtered, washed with ethanol, and air dried for 48 h. The product was recrystallized from acetone/water (yield 1.5 g).

Na[Pt(*R,R*-dach)(PAA)] (13). An aqueous solution (100 mL) containing [Pt(*R,R*-dach)(SO₄)(H₂O)] (10 mmol), 2-phosphonoacetic acid (10 mmol), Ba(OH)₂·8H₂O (10 mmol), and sodium hydroxide (10 mmol) was stirred at room temperature for 24 h. The BaSO₄ precipitate was filtered and the remaining solution was evaporated to dryness under vacuum (50 °C). The resulting solid was stirred in 2-propanol, filtered, and vacuum dried for 24 h. The dried product was dissolved in methanol and refiltered and the filtrate was evaporated to dryness (yield 4.8 g).

Na[Pt(*R,R*-dach)(PBA)] (14). A mixture of 5 mmol each of [Pt(*R,R*-dach)(H₂O)(SO₄)], Ba(OH)₂·8H₂O, NaOH, and 2-phosphonobutyric acid in 200 mL of water was stirred for 18 h at room temperature. The BaSO₄ precipitate was removed by filtration and the solution was evaporated to ~30 mL and re-filtered. The filtrate was evaporated to dryness and the product was purified by recrystallization from 2-propanol/water (yield 2.5 g, after drying).

Na[Pt(*R,R*-dach)(PVA)] (15). [Pt(*R,R*-dach)(SO₄)(H₂O)] (10 mmol), Ba(OH)₂·8H₂O (10 mmol), NaOH (10 mmol), and 2-phosphonovaleic acid (10 mmol) were stirred in 200 mL of water at room temperature for 24 h. The BaSO₄ was removed by filtration and the filtrate was evaporated to dryness under vacuum (55 °C). The product was purified from 2-propanol/water and vacuum dried (yield 3.4 g).

Na[Pt(*R,R*-dach)(PPHA)] (16). A solution of [Pt(*R,R*-dach)(SO₄)(H₂O)] (3.314 mmol) and 2-phosphonophenylacetic acid (3.314 mmol) in 100 mL of water was mixed with 3.314 mmol of Ba(OH)₂·8H₂O and 3.314 mmol of NaOH. After stirring of the solution for 24 h at room temperature, the BaSO₄ precipitate was removed by filtration. The yellow filtrate was evaporated to dryness at 50 °C (yielding a grey-white solid). The solid was stirred in 2-propanol, filtered, and dried under vacuum for 24 h. The dried product was recrystallized from methanol, reprecipitated from water/acetone, and dried under vacuum (yield 0.35 g).

Na[Pt(*R,R*-dach)(MPBA)] (17). A mixture of 3.59 mmol each of [Pt(*R,R*-dach)(SO₄)(H₂O)], Ba(OH)₂·8H₂O, NaOH, and 2-methyl-2-phosphonobutyric acid was stirred in 100 mL of water for 20 h at 45 °C. The BaSO₄ precipitate was removed by filtration and the filtrate was evaporated to dryness under vacuum. The solid was purified by recrystallization from ethanol/water and acetone/water (yield 0.9 g, after drying).

Na[Pt(*R,R*-dach)(PHD)] (18). A mixture of 4.164 mmol each of [Pt(*R,R*-dach)(SO₄)(H₂O)], NaOH, Ba(OH)₂·8H₂O, and 2-phosphono-1,7-heptanedicarboxylic acid was stirred in 100 mL of water for 14 h at 50 °C. The BaSO₄ precipitate was removed by filtration and an off-white solid was isolated by evaporating the filtrate to dryness under vacuum. The product was purified by means of chromatography on Al₂O₃ (methanol eluant).

Antitumor Screening. L1210 Leukemia. L1210 screening was conducted by using previously described methods.²¹ Female CDF₁ mice (16–22 g) were injected ip with a suspension of 1×10^6 tumor cells in 0.5 mL of 0.9 M NaCl on day 0. The mice received the test compound, dissolved in 0.5 mL of water, on day 1. Tests were conducted by using groups of six mice for each dose. Normal controls (six mice) received 1×10^6 tumor cells on day 0 and 0.5 mL of the test medium on day 1. Positive controls (six mice) received tumor cells on day 0 and 8 mg/kg of cisplatin in 0.5 mL of 0.9 M NaCl on day 1. All tests were terminated after 3 times the mean survival time (MST) of the normal control group, and surviving mice were counted as dying on that day. Activity was determined on the basis of the percent increase of the MST

of test mice over the control mice (ILS). An ILS of >25% indicates activity and the maximum possible ILS is 200%. The tumor line was maintained through weekly transfer of 5×10^4 tumor cells in DBA/2 mice.

Sarcoma 180 Ascites. Female CFW mice (18–25 g) were implanted ip with 2×10^6 tumor cells on day 0 and compounds were administered ip in 0.5 mL of water on day 1. Groups of six mice were used for each test dose and control groups were used as defined above. The test was run for twice the MST of the control group, with survivors being counted as dying on that day. An ILS of >50% indicates activity in this screen and the maximum possible ILS is 100%. The tumor line was maintained by weekly transfer of 4×10^6 cells in CFW mice.

M5076 Sarcoma. BDF₁ mice of either sex (6–10 weeks old) were implanted sc with fragments (approximately 25 mg) of M5076 tumor on day 0.¹⁸ The test compounds were administered iv on days 5, 9, 13, and 17 after tumor inoculation. Each treatment and control group consisted of eight mice. The experiments were terminated on day 75 postimplant and mice alive at that time were autopsied and judged to be cured if no tumor was visible. Antitumor activity was determined on the basis of the percent increase in the MST of drug-treated mice relative to the control mice (ILS) and by the relative median time for tumors to reach 1 g in drug-treated groups and the control group (T-C in days). A compound was considered active if it produced an ILS $\geq 25\%$ or T-C > 13 days. The tumor line was maintained as a sc growing tumor in C57BL/6 mice and serially transplanted at 2-week intervals.

Toxicity Testing. Preliminary toxicity testing was performed on Na[Pt(*R,R*-dach)(PVA)] and Na₂[Pt(*R,R*-dach)(PHD)] by monitoring BUN and serum-creatinine levels in treated mice. The initial dosing in CFW mice was established at approximately 1.5 times the peak active dose in the S180a screen, which was 240 mg/kg for both compounds. For comparative purposes, BUN and serum-creatinine measurements were obtained from a separate group of mice that received cisplatin at 1.5 times the peak active dose (12 mg/kg) and from an untreated control group. Each compound was administered ip to a series of mice on day 0 and blood samples were collected from individual groups of three mice at daily intervals for 7 days. The mice were anesthetized and blood samples were collected from the jugular vein into six to eight heparinized microhematocrit tubes. The sample tubes were centrifuged at 13400g for 5 min and the plasma was collected and analyzed with a Du Pont Clinical Analyzer using the Chem-12 Analytical Test Rotors. The BUN and creatinine measurements were used as an indicator of kidney toxicity.^{4b,21}

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Registry No. 1, 119107-28-7; 2, 119141-25-2; 3, 119107-23-2; 4, 119107-27-6; 5, 119107-17-4; 6, 119107-19-6; 7, 119107-22-1; 8, 119107-24-3; 8a, 119107-25-4; 9, 119107-20-9; 9a, 119107-21-0; 10, 119577-70-7; 11, 119107-18-5; 12, 117202-31-0; 13, 123026-22-2; 14, 123051-30-9; 15, 119107-26-5; 16, 119107-16-3; 17, 119107-29-8; 18, 123121-18-6; H₃PVA, 5650-83-9; H₃MPBA, 119143-83-8; H₄PHD, 119143-84-9; Pt(*R,R*-dach) I_2 , 66845-32-7; Pt(*R,R*-dach)(SO₄)(H₂O), 123122-57-6; *cis*-Pt(NH₃)₂(SO₄)(H₂O), 86493-49-4; *cis*-Pt(i-PrNH₂)₂(SO₄)(H₂O), 71361-11-0; Pt(en) I_2 , 23858-10-8; Pt(en)(SO₄)(H₂O), 123026-23-3; Pt(pn)(SO₄)(H₂O), 123026-24-4; [Pt(*R,R*-dach)(H₂O)₂](NO₃)₂, 94042-08-7; *cis*-[Pt(NH₃)₂(H₂O)₂](NO₃)₂, 52241-26-6; Na[Pt(en)(PFA)], 123026-25-5; triethyl 2-phosphonovaleate, 35051-49-1; triethyl 2-phosphonopropionate, 3699-66-9; triethyl 2-phosphonoacetate, 867-13-0; cisplatin, 15663-27-1; carboplatin, 41575-94-4; triethyl 2-methyl-2-phosphonobutyrate, 123004-70-6; tetraethyl 2-phosphono-1,7-heptanedicarboxylate, 66291-46-1.

Supplementary Material Available: ¹³C NMR data for all phosphono carboxylate ligands and complexes (3 pages). Ordering information is given on any current masthead page.