Novel Di- and Tetracarboxylatoplatinum(IV) Complexes. Synthesis, Characterization, Cytotoxic Activity, and DNA Platination

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Octahedrally configured diaminedichloro- and diamineoxalatoplatinum(IV) complexes with axial hydroxo ligands were carboxylated with succinic or glutaric anhydride. The free, uncoordinated carboxylic acid groups were further derivatized with amines and alcohols to the respective amides and esters and characterized in detail by elemental analysis, mass spectrometry, and multinuclear (^{1}H, ^{13}C, ^{15}N, and ^{195}Pt) NMR spectroscopy. Cytotoxicity of the complexes was studied in four human cancer cell lines derived from ovarian carcinoma (CH1, SK-OV-3), cervical carcinoma (HeLa), and colon carcinoma (SW480) by means of the MTT assay. Structure–activity relationships revealed a low activity for platinum complexes with underivatized carboxylic acid moieties and amide derivatives displaying the hydroxyethylamino residue. Within the series of amides, cyclopentylamino analogues were equipped with the highest cytotoxic potential. However, ester derivatives yielded IC_{50} values mostly in the low micromolar range and comparable to those of cisplatin. DNA platination studies of selected complexes revealed a high DNA platination capacity in parallel to a high cytotoxic potential and vice versa.

Introduction

Almost 40 platinum complexes have been investigated in clinical trials as anticancer agents.[1,2] Of these, only cisplatin, carboplatin, and oxaliplatin (see Figure S1 of Supporting Information for chemical structures) are in worldwide clinical use. In this context, it is worth mentioning that square-planar platinum(II) complexes are at present the only metal-based agents with tumor-inhibiting properties approved in the clinics.[3] Cis-, carbo-, and oxaliplatin are used in every second treatment regime in patients with solid tumors, whereas cisplatin, remarkably active in metastatic germ cell testicular tumors, is one of few anticancer drugs with real curative potential. Anticancer active platinum(II) complexes (especially cisplatin) are kinetically labile agents, which readily react with sulfur-containing biomolecules such as amino acids, glutathione, and proteins.[4–6] Besides deactivation of the platinum(II) agent, these interactions of the soft platinum central ion with the soft S-donor ligands (e.g., thiols or thioethers; HSAB[a] principle) are also responsible at least in part for the side effects observed during platinum-based chemotherapy. Additionally, the labile nature of platinum(II) compounds is also a severe drawback during synthesis, being limited to ligand exchange reactions. Consequently, kinetically more inert platinum(IV) complexes like tetraplatin and iproplatin (Figure 1) with an octahedral coordination sphere were evaluated in phase I clinical trials. Surprisingly, their behavior in vivo was significantly different: tetraplatin was abandoned because of intense side effects (neurotoxicity),[7] whereas iproplatin was abandoned because of lacking activity.[8] This is explainable taking into account that platinum(IV) complexes act as prodrugs and that reduction to the more (re)active platinum(II) counterparts is needed (activation by reduction).[9,10] Tetraplatin displaying two chloro ligands in the axial position is reduced within seconds in the blood stream; in contrast, iproplatin is not easily reduced and excreted to some extend intact.

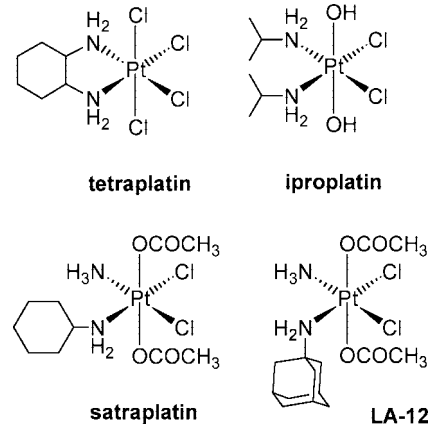

Figure 1. Chemical structures of anticancer platinum(IV) complexes in clinical evaluation: tetraplatin, abandoned (severe side-effects); iproplatin, abandoned (less active); satraplatin, phase III; LA-12, phase I finished.

Considerable progress was achieved with a novel class of platinum(IV) complexes exhibiting axial carboxylato ligands. Besides an intermediate reduction potential, now lying in an optimal window for anticancer treatment in vivo, the new complexes are inert enough to be administered orally offering advantages in terms of patient convenience and reduction of hospitalization costs. At present, the most interesting candidate is satraplatin, (*OC*-6-43)-bis(acetato)amminedichloro(cyclohexylamine)platinum(IV) (formerly JM216, Figure 1), which is in advanced phase III clinical trials showing activity in hormone-refractory prostate cancer in combination with prednisone as second-line chemotherapy.[11,12] A second and very close analogue to satraplatin, LA-12 (Figure 1), with a bulky

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[a] Abbreviations: CDI, 1,1′-carbonyldiimidazole; ESI-MS, electrospray ionization mass spectrometry; HSAB, hard and soft acids and bases; ICP-MS, inductively coupled plasma mass spectrometry; IR, infrared; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide; TBS, Tris-buffered saline.

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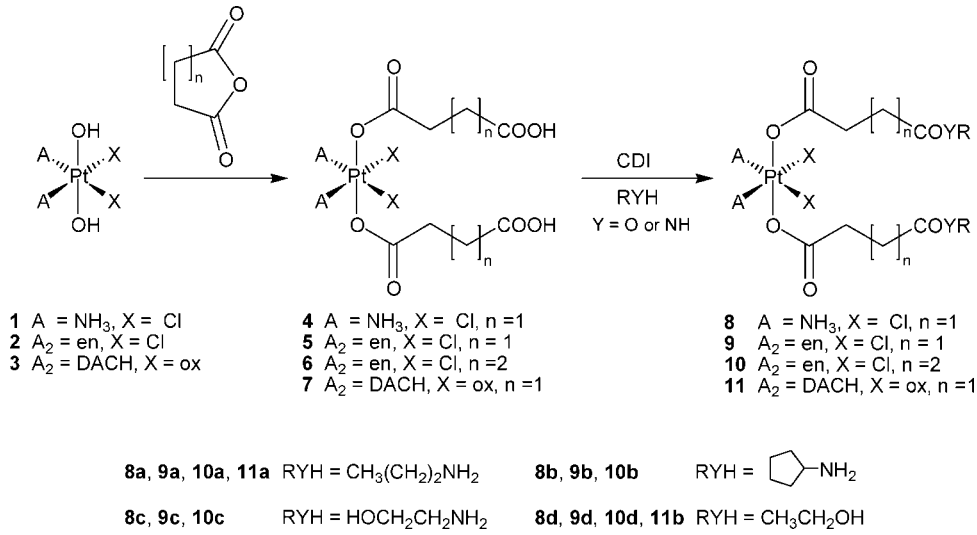

Figure 2. Synthesis of the novel di- and tetracarboxylatoplatinum(IV) complexes **8**−**11** (en = ethane-1,2-diamine, DACH = *trans*-(1*R*,2*R*)-diaminocyclohexane, ox = oxalate).

1-adamantylamine ligand, showed promising oral activity in mice in an ADJ/PC6 plasmacytoma and A2780 ovarian carcinoma tumor model.[13] It has finished phase I clinical trials.

The preparation of satraplatin and analogues is based on the low tendency of ligand exchange reactions in platinum(IV) complexes and therefore a prerequisite for the utilization of interesting and novel synthetic routes in inorganic chemistry. Because of the nucleophilic properties and kinetic inertness of OH coordinated to the platinum(IV) center, it was possible to further derivatize *trans*-dihydroxoplatinum(IV) species via classical carboxylation methods known from organic chemistry.[14] Carboxylation by anhydrides, isocyanates, pyrocarbonates, and also carboxylic acid chlorides in the presence of an excess of pyridine has been described in the literature.[15−18]

Recently, carboxylation of *trans*-dihydroxoplatinum(IV) complexes by cyclic anhydrides such as succinic, maleic, glutaric, and phthalic anhydride has been accomplished.[19−22] The use of cyclic anhydrides is of great interest, since one carbocylic acid group (of the anhydride) is bound to the platinum(IV) center and the second is uncoordinated and therefore an ideal functional group for further derivatization. Till now, only three papers dealing with reactions at the uncoordinated carboxylic acid groups have been published: (i) estrogens displaying an amino substituent were coupled to COOH in the presence of diisopropylcarbodiimide/4-dimethylaminopyridine to form the amide in order to take advantage of the estrogen moiety as sensitizer for platinum complexes in estrogen receptor positive cells;[20] (ii) an amine-functionalized water-soluble single-walled carbon nanotube was coupled to COOH with the aim of producing a prodrug for a carrier-mediated transport into cancer cells;[23] (iii) more elementary oriented, the uncoordinated carboxylic acids of (*OC*-6-33)-bis(3-carboxypropanoato)dichloro(ethane-1,2-diamine)platinum(IV) were derivatized with simple amines and alcohols to form the amides and esters, respectively, via activation of COOH with 1,1′-carbonyl diimidazol.[22]

As delineated above, and besides pharmacological properties, octahedrally coordinated platinum(IV) complexes offer important advantages over their square-planar platinum(II) analogues: (i) they are kinetically inert and can therefore be derivatized more easily, and (ii) in parallel to the increased coordination number (6 versus 4), platinum(IV) complexes display a higher degree of possible variations of the ligand sphere, which will have an influence on the biological (pharmacological) properties.

With the aim to set up and optimize structure–activity relationships, novel di- and tetracarboxylatoplatinum(IV) complexes were synthesized; variations in the ligand sphere were applied to the amine ligands, the equatorial groups, and the axial carboxylato ligands. The complexes were characterized by elemental analysis, ESI-MS, FT-IR, and in depth by multinuclear (^{1}H, ^{13}C, ^{15}N, ^{195}Pt) NMR spectroscopy. Cytotoxic properties of the title compounds were evaluated in four human tumor cell lines originating from ovarian carcinoma (CH1, SK-OV-3), cervical carcinoma (HeLa), and colon carcinoma (SW480) by means of the MTT assay.

Results and Discussion

Synthesis and Characterization. The complexes under investigation have either monodentate (ammine, chloro) or bidentate (ethane-1,2-diamine, trans-(1*R*,2*R*)-diaminocyclohexane, oxalato) ligands in the equatorial position, whereas the axial carboxylato ligands consist of dicarboxylates with two or three methylene groups as spacer (derived from succinic or glutaric acid anhydride). Derivatization of the free, uncoordinated carboxylic acid groups afforded the corresponding esters and amides (Figure 2; see also Figure S2 for a more detailed presentation of the complexes).

Synthesis of the target platinum(IV) compounds started with K_2PtCl_4, from which the diam(m)inedichloro- or diaminedicarboxylatoplatinum(II) complexes were prepared in one or two reaction steps. Oxidation to the platinum(IV) analogues **1**−**3** comprising two axial hydroxo ligands proceeded in aqueous solution in the presence of hydrogen peroxide. Subsequent carboxylation was performed with succinic or glutaric anhydride in DMF at 70 °C (Figure 2). Complexes **4**−**7** were then derivatized at the uncoordinated carboxylic acid group with propylamine, cyclopentylamine, aminoethanol, or ethanol. In order to activate the free COOH groups, 1,1′-carbonyldiimidazol (CDI) was used.

The formed platinum(IV) imidazolide was used in situ; subsequent reaction with the amines resulted in amides **8a**−**11a**, **8b**−**10b**, and **8c**−**10c**, which were isolated after column chromatography in moderate yields of 18–42%. As expected and also as reported recently by us[22] for **9c**, the amides **8c**−**10c** were formed selectively in the case of aminoethanol, whereas the use of ethanol in the presence of catalytic amounts of sodium

afforded the ester derivatives **8d**−**11d** in yields ranging from 17% to 38%.

Complexes **4**−**11** were characterized by elemental analysis (Table S1), ESI-MS, and IR and in detail by multinuclear (^{1}H, ^{13}C, ^{15}N, ^{195}Pt) NMR spectroscopy. IR was found to be a suitable tool to judge the carboxylation as well as the subsequent derivatization of the uncoordinated carboxylic acid group. The intense and exceptional sharp PtO−H stretch of complexes **1**−**3** in the region between 3450 and 3590 cm^{-1} vanishes during the reaction of the dihydroxoplatinum(IV) compounds with the cyclic anhydrides. Thereafter, one C═O stretch of the coordinated carboxylato ligands in the region around 1650 cm^{-1} and one C═O stretch of the uncoordinated COOH groups of compounds **4**−**6** between 1700 and 1740 cm^{-1} were detected. Formation of amides **8a**−**c**, **9a**−**c**, and **10a**−**c** is accompanied by the disappearance of the C(═O)OH absorptions above 1700 cm^{-1}. Instead, two C═O stretches between 1628 and 1675 cm^{-1} were detected. This characteristic feature could not unequivocally be observed in the case of **11a** because of the overlap with the absorption derived from the equatorially coordinated oxalato ligand. In contrast to the amides, the esters **8d**, **9d**, and **10d** gave rise to typical C═O stretches at around 1730 cm^{-1} of the ester C═O absorption. The latter stretches are missing in the case of derivatization with aminoethanol; thus, the C═O stretches between 1630 and 1675 cm^{-1} clearly prove amide and not ester formation for **8c**, **9c**, and **10c**.

Nevertheless, carboxylation and subsequent derivatization can best be judged by multinuclear NMR spectroscopy; compounds **4**−**7** and target complexes **8**−**11** were investigated in deuterated dimethylformamide (dmf-d_7). The geometry as well as the oxidation state of **4**−**11** can unequivocally be judged on the basis of their ^{195}Pt resonances. ^{195}Pt signals in the region between 2620 and 3230 ppm were detected and are in agreement with a *cis*,*cis*,*trans*-$Pt^{IV}Cl_2N_2O_2$[21] and $Pt^{IV}N_2O_4$[24] coordination sphere, respectively. The considerably large range of 600 ppm is not astonishing taking into account a chemical shift span of several thousand ppm for the ^{195}Pt nucleus. Complexes **4** and **8a**–**d**, displaying two ammine and two chloro ligands in the equatorial position, resonated between 2806 and 2812 ppm (see Table S2 displaying the most relevant chemical shifts), whereas ^{195}Pt signals of ethane-1,2-diamine analogues **5**, **6**, **9a**–**10d** were found between 2621 and 2630 ppm. Resonances of the oxaliplatin type complexes **7**, **11a**, and **11b** were detected downfield at 3221−3226 ppm. As expected, these values demonstrate that derivatization at the uncoordinated COOH group had no significant influence on the chemical shift of the ^{195}Pt nucleus. However, the smallest deviations (2 ppm) were found in the case of transformations from **6** to **10a**–**d**; this is explained by the fact that the reaction center is separated by a further methylene group from the platinum nucleus (glutaric versus succinic acid derivatives).

^{15}N NMR spectroscopy is also a powerful tool to study the chemical structure of the synthesized platinum(IV) complexes. Despite the low natural abundance of ^{15}N (spin ½) with 0.37%, the resonances are available via gradient enhanced two-dimensional ^{1}H,^{15}N COSY measurements within a few hours using a 400 MHz NMR spectrometer. Chemical shift differences of *N*-Pt during the transformation of the uncoordinated carboxylic acid group to the respective amides and esters were smaller than 0.6 ppm. In contrast, detection of the directly involved ^{15}N nucleus of the amide resulted in resonances between 90 and 110 ppm, being in good agreement with typical literature values (60–110 ppm). These chemical shifts are significantly different in comparison to the free amines at around 0 ppm. In the case of the aminoethanol derivatives **8c**–**10c**, ^{15}N spectroscopy could verify the selective amide formation. Interestingly, two ^{15}N signals of the coordinated NH_3 ligands were found in complexes **4** and **8a**–**d**. The nonequivalency, which is also observed in the ^{1}H NMR spectra, is explainable due to the formation of intramolecular hydrogen bonds and a hindered rotation of the ammine ligand.

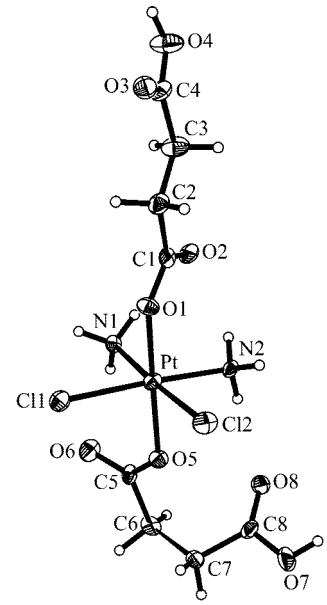

Figure 3. ORTEP view of **4** with atom labeling scheme. The thermal ellipsoids are drawn at the 50% probability level. Selected bond lengths (Å) and bond angles (deg) are as follows: Pt−O1 1.993(4), Pt−O5 2.008(4), Pt−N1 2.050(5), Pt−N2 2.065(5), Pt−Cl1 2.3108(15), Pt−Cl2 2.3194(15), O1−Pt−O5 172.59(18), N1−Pt−N2 90.2(2), Cl1−Pt−Cl2 94.38(6), N1−Pt−Cl1 88.29(14), N2−Pt−Cl2 87.24(14).

Finally, ^{1}H and ^{13}C chemical shifts of compounds **4**–**11** were found in the normal range, proving the chemical structures of the complexes. Proton signals of the coordinated NH_3 and NH_2 groups showed significant different chemical shifts; NH_3 protons of **4** and **8a**–**d** were detected upfield between 6.60 and 7.40 ppm, whereas NH_2 resonances for **5**−**7** and **9**–**11** were observed at around 8.9 ppm. In the case of amides, CON*H* chemical shifts between 7.7 and 8.1 ppm clearly demonstrated the success of the amide formation; values in the range 1–3 ppm would be expected for free amines. Most indicative for following the reactions with ^{13}C NMR are the quaternary C═O resonances. Axially coordinated carboxylato ligands (PtOC═O) gave rise to ^{13}C shifts between 178 and 181 ppm; resonances at 172 ppm for **4**–**7** were assigned to the uncoordinated COOH groups. After derivatization, the quaternary amide and ester carbon atoms (C═O) were detected upfield between 169.9 and 170.8 ppm, whereas the equatorial oxalato ^{13}C═O chemical shifts were observed even more upfield at 162 ppm.

Crystal Structure. The result of the X-ray diffraction study of complex **4**·$(C_2H_5)_2O$ is shown in Figure 3. Crystal data, data collection parameters, and structure refinement details are given in Table S3. The compound crystallizes in the orthorhombic space group *Pna*2$_1$. The platinum(IV) atom has an octahedral coordination geometry with two ammine and two chloro ligands bound in the equatorial plane and two hydrogen succinate ions in axial positions.

Cytotoxicity in Cancer Cell Lines. Cytotoxicity of the platinum(IV) complexes **4**–**7**, **8a**–**d**, **9a**−**d**, **10a**–**d**, and **11a**,**b** was studied by means of a colorimetric microculture assay (MTT

Table 1. Cytotoxicity of Novel Di- and Tetracarboxylatoplatinum(IV) Complexes **4**–**7**, **8a**–**d**, **9a**–**d**, **10a**–**d**, **11a**, and **11b** Compared to Cisplatin in Four Human Cancer Cell Lines

	IC_{50} (μM)[a]			
compd	CH1	HeLa	SW480	SK-OV-3
4	19 ± 1	82 ± 30	136 ± 16	102 ± 45
5	5.5 ± 2.2	14 ± 4	95 ± 5	76 ± 17
6	32 ± 19	87 ± 7	160 ± 10	146 ± 55
7	55 ± 28	71 ± 23	44 ± 9	142 ± 26
8a	12 ± 4	22 ± 4	48 ± 4	84 ± 25
8b	1.9 ± 0.2	6.2 ± 1.6	24 ± 4	33 ± 27
8c	28 ± 2	92 ± 10	183 ± 28	154 ± 39
8d	0.62 ± 0.32	1.5 ± 0.3	3.8 ± 1.0	6.3 ± 2.1
9a	2.3 ± 1.1	9.2 ± 2.0	31 ± 15	66 ± 23
9b	1.9 ± 0.2	2.9 ± 1.4	19 ± 9	29 ± 5
9c	24 ± 3	82 ± 2	142 ± 23	131 ± 16
9d	0.34 ± 0.11	0.68 ± 0.04	4.1 ± 0.5	3.7 ± 1.2
10a	22 ± 12	48 ± 13	43 ± 22	77 ± 23
10b	7.8 ± 1.0	22 ± 5	21 ± 5	46 ± 13
10c	21 ± 8	80 ± 4	90 ± 21	139 ± 22
10d	1.1 ± 0.2	2.1 ± 1.0	3.5 ± 0.1	3.7 ± 1.1
11a	11 ± 2	15 ± 4	12 ± 5	44 ± 10
11b	19 ± 5	19 ± 3	14 ± 3	67 ± 23
cisplatin	0.16 ± 0.03	0.37 ± 0.06	3.5 ± 0.3	1.9 ± 0.3

[a] 50% inhibitory concentrations in CH1, HeLa, SW480, and SK-OV-3 cells in the MTT assay. Values are the mean ± standard deviation obtained from at least three independent experiments.

assay) in four human cancer cell lines representing three tumor entities: ovarian carcinoma (CH1, SK-OV-3), cervical carcinoma (HeLa), and colon carcinoma (SW480), yielding IC_{50} values mostly in the micromolar range (Table 1).

Two of the cell lines are sensitive to cisplatin (CH1, HeLa), while the other two (SK-OV-3, SW480) are intrinsically resistant to cisplatin, yielding IC_{50} values 1 order of magnitude higher than those of the sensitive cells. The most potent of the platinum(IV) complexes (**9d**, **8d**) show the highest degree of conformity with this sensitivity pattern, while differences between the cell lines tend to become less pronounced for the less cytotoxic complexes. In the case of DACH-containing complexes **7**, **11a**, and **11b**, the colon cancer cell line SW480 is not less sensitive than CH1 and HeLa cells, which corresponds to the behavior of other DACH-platinum compounds.[25]

Homologous series of complexes differing only in the carboxylato ligands show similar structure–activity relationships. As can be seen from the concentration−effect curves depicted in Figures S3 and S4, cytotoxicity depends on the distal moiety of the carboxylato ligand in the following rank order, both within the ammine- and the ethane-1,2-diamine-containing series with axial ligands derived from succinic acid: ester derivatives (**8d**, **9d**) > cyclopentylamine derivatives (**8b**, **9b**) > propylamine derivatives (**8a**, **9a**) > underivatized carboxylic acids (**4**, **5**) > hydroxyethylamine derivatives (**8c**, **9c**).

In general, (ethane-1,2-diamine)platinum(IV) complexes (**4**, **8a**–**d**) were significantly more active than their diammine analogues (**5**, **9a**–**d**). In the ethane-1,2-diamine-containing series with axial ligands derived from glutaric acid anhydride, all four derivatives **10a**–**d** yield higher potencies than **6**, with otherwise unaltered rank order of cytotoxicity (Figure S5). In this context, it is worth mentioning that the use of glutaric acid anhydride resulted in complexes with significantly lower cytotoxicity (**6** versus **5**; **10a**–**d** versus **9a**–**d**). Results obtained with the three DACH-containing complexes **7**, **11a**, and **11b** are consistent with the structure–activity relationships described above. Thus, a terminal hydroxyl group (COOH or CH_2OH) is unfavorable in terms of cytotoxicity in any case.

In each homologous series, the ethyl ester derivatives unexpectedly resulted in the most potent compounds. Complex **9d** remarkably approaches the cytotoxicity of cisplatin, with IC_{50} values in submicromolar concentrations in sensitive (0.34 and 0.68 μM) and low micromolar concentrations (4.1 and 3.7 μM) in resistant cell lines. Variation of the chain length of the ethyl ester ligand in the ethylenediamine complexes shows that (4-ethoxy)-4-oxobutanoate is more favorable than (5-ethoxy)-5-oxopentanoate (compare **9d** with **10d** in Figure 4) in terms of cytotoxicity in the cisplatin-sensitive cell lines (yielding about 3 times lower IC_{50} values), whereas no difference is discernible in the inherently cisplatin-resistant cell lines. A comparison of complexes with the (4-ethoxy)-4-oxobutanoato ligand (Figure 4) reveals the following structure–activity relationship with respect to variation of the equatorial ligands: en/Cl (**9d**) ≥ NH_3/Cl (**8d**) > DACH/ox (**11b**).

DNA Platination. The extent of DNA platination was determined in SW480 cells exposed to selected complexes at a concentration of 10 μM for 24 h; r_b values (number of Pt atoms per nucleotide) were calculated by measuring the ratio of platinum to phosphorus. Within the series of novel platinum(IV) complexes, a high DNA platination capacity was found in parallel to a high cytotoxic potential and vice versa. DNA platination by compounds **8d** (r_b = 0.000 87 ± 0.000 21) and **9d** (r_b = 0.000 90 ± 0.000 35) was 1 order of magnitude higher than that by compounds **9b** (r_b = 0.000 09 ± 0.000 03) and **11b** (r_b = 0.000 02 ± 0.000 01), corresponding to a 3–5 times higher cytotoxicity in SW480 cells. In the case of cisplatin (r_b = 0.000 27 ± 0.000 07), a 3 times lower DNA platination seems to be sufficient to obtain a similar cytotoxicity compared to **8d** and **9d**. However, it cannot be deduced from the data that the DNA lesions induced by the platinum(IV) complexes are on average less detrimental to the cells than those of cisplatin, since compound **8d** contains the same equatorial ligands as cisplatin and is therefore expected to give rise to the same DNA adducts after reduction. In this context, it must be born in mind that DNA platination was only determined at one point in time and that kinetics of DNA binding and onset of DNA repair might differ substantially between platinum(II) and platinum(IV) complexes; for the latter, it is, as mentioned above, a prerequisite to be reduced first to the corresponding platinum(II) analogue.

In conclusion, novel octahedrally configured platinum(IV) complexes were synthesized, characterized, and investigated in four human cancer cell lines with respect to their cytotoxic properties. Structure–activity relationships could be drawn from these results. Platinum complexes with underivatized carboxylic acids and amide derivatives displaying the hydroxyethylamino moiety were less active. Cytotoxicity of amides was increased when changing to propylamino and especially to cyclopentylamino analogues. However, the ester derivatives were equipped with the highest cytotoxic potential, being in the range of that for cisplatin. DNA platination was studied for selected complexes; a high DNA platination capacity in parallel to a high cytotoxic potential was found and vice versa. These encouraging results will direct the focus of our future research program.

Experimental Section

All reagents and solvents were obtained from commercial suppliers and were used as received. For column chromatography, silica gel 60 (Fluka) was used. The starting compounds (*OC*-6-33)-diamminedichlorodihydroxoplatinum(IV) (**1**), (*OC*-6-33)-dichloro(ethane-1,2-diamine)dihydroxoplatinum(IV) (**2**), and (*OC*-6-33)-(*trans-R*,*R*-1,2-diaminocyclohexane)dihydroxooxalatoplatinum(IV) (**3**) were synthesized according to standard literature procedures.[26,27] The complexes (*OC*-6-33)-bis(3-carboxypropanoato)dichloro(ethane-1,2-diamine)platinum(IV) (**5**), (*OC*-6-33)-dichloro(ethane-1,2-diamine)bis{(4-propylamino)-4-

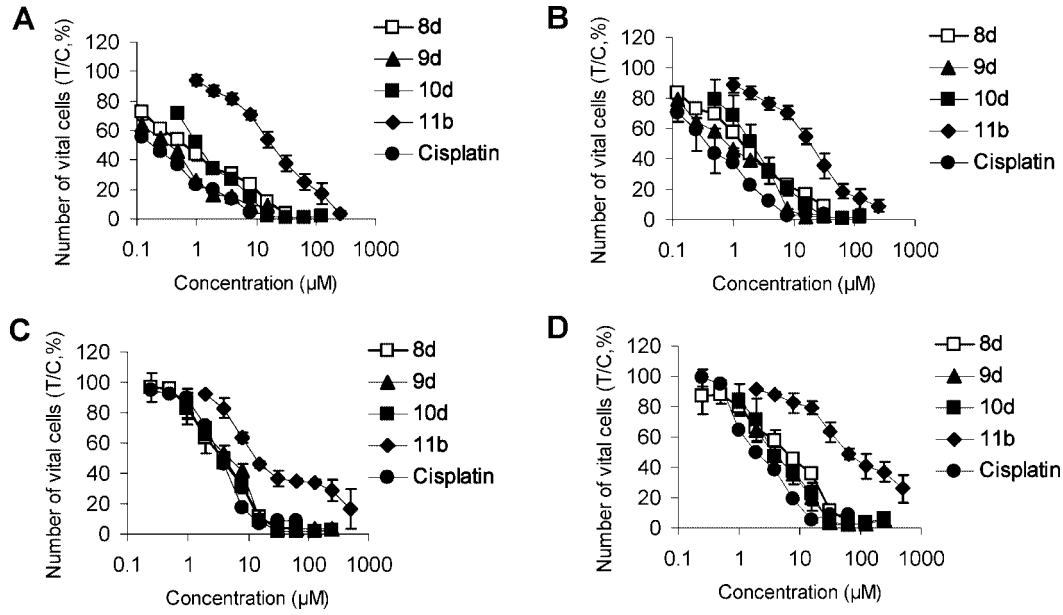

Figure 4. Concentration−effect curves of complexes **8d**, **9d**, **10d**, **11b**, and cisplatin in CH1 (A), HeLa (B), SW480 (C), and SK-OV-3 cells (D), obtained by the MTT assay (96 h exposure).

oxobutanoato}platinum(IV) (**9a**), (*OC*-6-33)-dichlorobis{(4-cyclopentylamino)-4-oxobutanoato}(ethane-1,2-diamine)platinum(IV) (**9b**), (*OC*-6-33)-dichloro(ethane-1,2-diamine)bis{[4-(2-hydroxyethyl)amino]-4-oxobutanoato}platinum(IV) (**9c**), and (OC-6-33)-dichloro(ethane-1,2-diamine)bis{(4-ethoxy)-4-oxobutanoato}platinum(IV) (**9d**) have been prepared as described in the literature.[22] ^{1}H, ^{13}C, ^{195}Pt and two-dimensional ^{1}H,^{13}C and ^{1}H,^{15}N COSY NMR spectra were recorded with a Bruker Avance DPX 400 spectrometer (UltraShield magnet) at 400.13 (^{1}H), 100.62 (^{13}C), 86.11 (^{195}Pt), and 40.55 MHz (^{15}N) in dmf-d_7 at 298 K, using the solvent residual peak for ^{1}H and ^{13}C as internal reference. ^{15}N chemical shifts were referenced relative to external NH_4Cl, whereas ^{195}Pt chemical shifts were referenced relative to external $K_2[PtCl_4]$. Half-height line widths of ^{195}Pt resonances are given in parentheses. All infrared spectra were obtained from a KBr matrix (4000–400 cm^{-1}) using a Bruker Vertex 70 FTIR spectrometer. Electrospray ionization mass spectrometry was carried out with a Bruker Esquire 3000 instrument using MeOH as solvent. Elemental analyses were performed using a Perkin-Elmer 2400 CHN elemental analyzer by the microlaboratory of the Institute of Physical Chemistry, University of Vienna.

Synthesis. (*OC*-6-33)-Diamminebis(3-carboxypropanoato)dichloroplatinum(IV) (4). Succinic anhydride (1.320 g, 13.19 mmol) was added to a suspension of **1** (1.075 g, 3.218 mmol) in DMF (20 mL), and the reaction mixture was stirred at 70 °C for 1.5 h. During this time, the solid material dissolved to form a yellow solution. DMF was then removed under reduced pressure. The residue was dissolved in acetone and filtered to give a clear, yellow solution. This solution was concentrated under reduced pressure, and subsequent addition of diethyl ether led to precipitation of a pale-yellow solid. The product was dried in vacuo. Yield: 1.491 g (87%). ^{1}H NMR: δ = 12.47 (bs, 2 H, COO*H*), 7.26–6.69 (m, 6 H, N*H*$_3$), 2.71 (m, 4 H, 2-H/3-H), 2.63 (m, 4 H, 2-H/3-H) ppm. ^{13}C NMR: δ = 178.7 (C-1), 172.2 (C-4), 29.0 (C-2/C-3), 28.3 (C-2/C-3) ppm. ^{15}N NMR: δ = −40.6, −40.9, ppm. ^{195}Pt NMR: δ = 2812 (567 Hz) ppm. IR: ν = 3523 (ν_{COO-H}), 3248 m, 3184 m, 1706 ($\nu_{as\ C=O}$), 1669 ($\nu_{as\ C=O}$), 1321 s, 1198 s, 675 m cm^{-1}. ESI-MS (positive): *m*/*z* 556.6 [M + Na$^+$]$^+$. Anal. ($C_8H_{16}Cl_2N_2O_8Pt$) C, H, N. Crystals suitable for X-ray data collection were grown via vapor diffusion of ether into a solution of **4** in acetone.

(*OC*-6-33)-Bis(3-carboxybutanoato)dichloro(ethane-1,2-diamine)platinum(IV) (6). The synthesis was carried out as described for **4**. Instead of succinic anhydride, glutaric anhydride was used, and the reaction time was 24 h. Yield: 594.2 mg (73%). ^{1}H NMR: δ = 12.40 (bs, 2 H, COO*H*), 8.99 (bs, 4 H, N*H*$_2$), 3.17 (bs, 4 H, 1-H), 2.51 (m, 4 H, 3-H/5-H), 2.49 (m, 4 H, 3-H/5-H), 1.93 (m, 4 H, 4-H) ppm. ^{13}C NMR: δ = 180.8 (C-2), 172.9 (C-6), 48.1 (C-1), 34.2 (C-3/C-5), 31.5 (C-3/C-5), 19.8 (C-4) ppm. ^{15}N NMR: δ = −3.4 (*N*H$_2$) ppm. ^{195}Pt NMR: δ = 2630 (418 Hz) ppm. IR: ν = 3574 w, 3200 (ν_{N-H}), 1708 ($\nu_{as\ C=O}$), 1644 ($\nu_{as\ C=O}$), 1242 s, 1048 w cm^{-1}. ESI-MS (positive): *m*/*z* 611.0 [M + Na$^+$]$^+$, 627.0 [M + K$^+$]$^+$. ESI-MS (negative): *m*/*z* 587.0 [M − H$^+$]$^-$. Anal. ($C_{12}H_{22}Cl_2N_2O_8Pt$) C, H, N.

(*OC*-6-33)-Bis(3-carboxypropanoato)(*trans*-(*R*,*R*)-1,2-diaminocyclohexane)oxalatoplatinum(IV) (7). Succinic anhydride (285 mg, 2.85 mmol) was added to a suspension of **3** (300 mg, 0.696 mmol) in DMF (5 mL), and the reaction mixture was stirred at 70 °C for 15 h. During this time the solid material dissolved to form a brown solution. DMF was removed under reduced pressure. The residue was dissolved in MeOH and filtered to give a clear, brown solution. The solution was concentrated under reduced pressure, and acetone was added. This procedure was repeated thrice, and precipitation was completed by subsequent addition of diethyl ether to yield a pale-brown powder. Yield: 352.4 mg (80%). ^{1}H NMR: δ = 12.65 (bs, 2 H, COO*H*), 8.97–8.45 (m, 4 H, N*H*$_2$), 3.10 (m, 2 H, 1-H, 2-H), 2.69 (m, 4 H, 9-H/10-H), 2.66 (m, 4 H, 9-H/10-H), 2.46 (m, 2 H, 3-H$_{eq}$, 6-H$_{eq}$), 1.74 (m, 4 H, 3-H$_{ax}$, 6-H$_{ax}$, 4-H$_{eq}$, 5-H$_{eq}$), 1.45 (m, 2 H, 4-H$_{ax}$, 5-H$_{ax}$) ppm. ^{13}C NMR: δ = 179.4 (C-8), 172.5 (C-11), 162.0 (C-7) 60.1 (C-1, C-2), 30.1 (C-3, C-6), 29.4 (C-9/C-10), 28.1 (C-9/C-10), 22.4 (C-4, C-5) ppm. ^{15}N NMR: δ = −6.5 (*N*H$_2$) ppm. ^{195}Pt NMR: δ = 3226 (449 Hz) ppm. IR: ν = 3228 s, 3080 s, 2951 s, 1736 ($\nu_{as\ C=O}$), 1727 ($\nu_{as\ C=O}$), 1656 ($\nu_{as\ C=O}$), 1585 s, 1390 s, 814 s cm^{-1}. ESI-MS (positive): *m*/*z* 631.7 [M + H$^+$]$^+$, 653.7 [M + Na$^+$]$^+$. ESI-MS (negative): *m*/*z* 629.6 [M − H$^+$]$^-$. Anal. ($C_{16}H_{24}N_2O_{12}Pt$) C, H, N.

(*OC*-6-33)-Diamminedichlorobis{(4-propylamino)-4-oxobutanoato}platinum(IV) (8a). 1,1′-Carbonyldiimidazole (CDI; 166 mg, 1.025 mmol) in DMF (12 mL) was added to a solution of **4** (267 mg, 0.5 mmol) in DMF (6 mL), and the mixture was heated to 60 °C. After 10 min of being stirred, the solution was cooled to room temperature and CO_2 was removed by flushing with argon. Propylamine (84.6 μL, 1.025 mmol) in DMF (24 mL) was added to the solution and stirred for 24 h at room temperature. DMF was removed under reduced pressure to form a yellow oil. The crude product was purified by column chromatography (EtOAc/MeOH, 4:1) and recrystallized from acetone to yield a white powder. Yield: 64 mg (21%). ^{1}H NMR: δ = 7.95 (bs, 2 H, N*H*), 7.28–6.70 (m, 6 H, N*H*$_3$), 3.25 (m, 4 H, 5-H), 2.65 (t, $^{3}J_{H,H}$ = 7.0 Hz, 4 H, 2-H/3-H), 2.54 (t, $^{3}J_{H,H}$ = 7.0 Hz, 4 H, 2-H/3-H), 1.62 (m, 4 H, 6-H),

1.03 (t, $^3J_{H,H}$ = 7.4 Hz, 6 H, 7-H) ppm. ^{13}C NMR: δ = 179.3 (C-1), 170.1 (C-4), 39.1 (C-5), 30.2 (C-2/C-3), 30.1 (C-2/C-3), 21.0 (C-6), 9.4 (C-7) ppm. ^{15}N NMR: δ = 95.5 (N*H*), −40.5 (N*H*$_3$), −40.9 (N*H*$_3$) ppm. ^{195}Pt NMR: δ = 2807 (581 Hz) ppm. IR: ν = 3367 m, 3260 m, 1653 ($\nu_{as\ C=O}$), 1636 ($\nu_{as\ C=O}$), 1558 s, 1332 m, 1212 m cm^{-1}. ESI-MS (positive): *m/z* 616.8 [M + H$^+$]$^+$, 638.9 [M + Na$^+$]$^+$, 654.8 [M + K$^+$]$^+$. ESI-MS (negative): *m/z* 614.8 [M − H$^+$]$^-$. Anal. ($C_{14}H_{30}Cl_2N_4O_6Pt$) C, H, N.

(*OC*-6-33)-Diamminebis{(4-cyclopentylamino)-4-oxobutanoato} dichloroplatinum(IV) (8b). The synthesis was carried out as described for **8a**. The crude product was purified by column chromatography (EtOAc/MeOH, 6:1) and recrystallized from MeOH to yield a white powder. Yield: 89.7 mg (18%). ^{1}H NMR: δ = 7.94 (d, $^3J_{H,H}$ = 6.9 Hz, 2 H, N*H*), 7.26–6.71 (m, 6 H, N*H*$_3$), 4.24 (m, 4 H, 5-H), 2.63 (t, $^3J_{H,H}$ = 7.1 Hz, 4 H, 2-H/3-H), 2.52 (t, $^3J_{H,H}$ = 7.1 Hz, 4 H, 2-H/3-H), 1.98 (m, 4 H, 6-H/9-H), 1.82 (m, 4 H, 7-H/8-H), 1.68 (m, 4 H, 7-H/8-H), 1.60 (m, 4 H, 6-H/9-H) ppm. ^{13}C NMR: δ = 179.3 (C-1), 169.7 (C-4), 49.1 (C-5), 30.9 (C-6/C-9), 30.2 (C-2/C-3), 30.1 (C-2/C-3), 21.9 (C-7/C-8) ppm. ^{15}N NMR: δ = 108.7 (N*H*), −40.5 (N*H*$_3$), −40.9 (N*H*$_3$) ppm. ^{195}Pt NMR: δ = 2808 (667 Hz) ppm. IR: ν = 3360 m, 3262 m, 2960 m, 1640 ($\nu_{as\ C=O}$), 1628 ($\nu_{as\ C=O}$), 1542 s, 1337 s cm^{-1}. ESI-MS (positive): *m/z* 690.9 [M + Na$^+$]$^+$, 706.8 [M + K$^+$]$^+$. ESI-MS (negative): *m/z* 666.9 [M − H$^+$]$^-$. Anal. ($C_{18}H_{34}Cl_2N_4O_6Pt$) C, H, N.

(*OC*-6-33)-Diamminedichlorobis{[4-(2-hydroxyethyl)amino]-4-oxobutanoato}platinum(IV) (8c). The synthesis was carried out as described for **8a**. The crude product was purified by column chromatography (EtOAc/MeOH, 1:1) and was dissolved in water and lyophilized to yield a yellow powder. Yield: 81 mg (18%). ^{1}H NMR: δ = 7.98 (t, $^3J_{H,H}$ = 5.4 Hz, 2 H, N*H*), 7.36–6.68 (m, 6 H, N*H*$_3$), 4.87 (bs, 2 H, O*H*), 3.70 (t, $^3J_{H,H}$ = 6.0 Hz, 4 H, 6-H), 3.41 (m, 4 H, 5-H), 2.65 (m, 4 H, 2-H/3-H), 2.56 (m, 4H, 2-H/3-H) ppm. ^{13}C NMR: δ = 179.2 (C-1), 170.5 (C-4), 59.0 (C-6), 40.4 (C-5), 30.2 (C-2/C-3), 30.1 (C-2/C-3) ppm. ^{15}N NMR: δ = 91.1 (*N*H), −40.5 (*N*H$_3$), −40.8 (*N*H$_3$) ppm. ^{195}Pt NMR: δ = 2806 (618 Hz) ppm. IR: ν = 3376 w, 3277 w, 2941 w, 1636 ($\nu_{as\ C=O}$), 1559 s, 1258 m, 1065 m cm^{-1}. ESI-MS (positive): *m/z* 642.8 [M + Na$^+$]$^+$. ESI-MS (negative): *m/z* 618.8 [M − H$^+$]$^-$. Anal. ($C_{12}H_{26}Cl_2N_4O_8Pt$) C, H, N.

(*OC*-6-33)-Diamminedichlorobis{(4-ethoxy)-4-oxobutanoato} platinum(IV) (8d). CDI (249 mg, 1.535 mmol) in absolute DMF (16 mL) was added to a solution of **4** (400 mg, 0.749 mmol) in absolute DMF (8 mL), and the mixture was heated to 60 °C. After 10 min of being stirred, the solution was cooled to room temperature and CO_2 was removed by flushing with argon. Sodium ethanolate (10 mg of Na in 10 mL of absolute EtOH) in absolute EtOH was added to the solution and stirred for 24 h at room temperature. Ethanol and DMF were removed under reduced pressure to form a yellow oil. The crude product was purified by column chromatography (EtOAc/MeOH, 9:1) and recrystallized from EtOAc to yield a white solid. Yield: 110 mg (25%). ^{1}H NMR: δ = 7.26–6.67 (N*H*$_3$), 4.25 (q, $^3J_{H,H}$ = 7.1 Hz, 4 H, 5-H), 2.72 (m, 4 H, 2-H/3-H), 2.64 (m, 4 H, 2-H/3-H), 1.37 (t, $^3J_{H,H}$ = 7.1 Hz, 6 H, 6-H) ppm. ^{13}C NMR: δ = 178.4 (C-1), 170.8 (C-4), 58.3 (C-5), 28.8 (C-2/C-3), 28.4 (C-2/C-3), 12.1 (C-6) ppm. ^{15}N NMR: δ = −40.2 (*N*H$_3$), −40.7 (*N*H$_3$) ppm. ^{195}Pt NMR: δ = 2808 (589 Hz) ppm. IR: ν = 3284 (ν_{N-H}), 3230 (ν_{N-H}), 2986 w, 1735 s, 1707 s, 1616 s, 1559 s, 1319 s, 1187 s cm^{-1}. ESI-MS (positive): *m/z* 612.8 [M + Na$^+$]$^+$, 628.7 [M + K$^+$]$^+$. ESI-MS (negative): *m/z* 588.7 [M − H$^+$]$^-$.

(*OC*-6-33)-Dichloro(ethane-1,2-diamine)bis{(5-propylamino)-5-oxopentanoato}platinum(IV) (10a). The synthesis was carried out as described for **8a**. The crude product was purified by column chromatography (EtOAc/MeOH, 2:1) and recrystallized from MeOH to yield a pale-yellow solid. Yield: 237 mg (42%). ^{1}H NMR: δ = 9.00 (bs, 4 H, N*H*$_2$), 7.81 (bs, 2 H, N*H*), 3.26 (m, 4 H, 8-H), 3.16 (bs, 4 H, 1-H), 2.43 (t, $^3J_{H,H}$ = 7.4 Hz, 4 H, 3-H), 2.34 (t, $^3J_{H,H}$ = 7.4 Hz, 4 H, 5-H), 1.94 (m, 4 H, 4-H), 1.62 (m, 4 H, 7-H), 1.03 (t, $^3J_{H,H}$ = 7.4 Hz, 6 H, 9-H) ppm. ^{13}C NMR: δ = 181.0 (C-2), 170.5 (C-6), 48.1 (C-1), 39.2 (C-8), 34.5 (C-3), 33.7 (C-5), 21.4 (C-7), 20.8 (C-4), 9.7 (C-9) ppm. ^{15}N NMR: δ = 96.0 (*N*H), −3.6 (*N*H$_2$) ppm. ^{195}Pt NMR: δ = 2628 (422 Hz) ppm. IR: ν = 3274(ν_{N-H}), 3204 (ν_{N-H}), 2960 s, 1664($\nu_{as\ C=O}$), 1645 ($\nu_{as\ C=O}$), 1561 s, 1201 s, 1050 m cm^{-1}. ESI-MS (positive): *m/z* 692.8 [M + Na$^+$]$^+$. ESI-MS (negative): *m/z* 668.7 [M − H$^+$]$^-$. Anal. ($C_{18}H_{36}Cl_2N_4O_6Pt$) C, H, N.

(*OC*-6-33)-Dichloro(ethane-1,2-diamine)bis{(5-cyclopentylamino)-5-oxopentanoato}-platinum(IV) (10b). The synthesis was carried out as described for **8a**. The crude product was purified by column chromatography (EtOAc/MeOH, 3:1) and recrystallized from MeOH to yield a pale-yellow solid. Yield: 84.2 mg (14%). ^{1}H NMR: δ = 8.98 (bs, 4 H, N*H*$_2$), 7.76 (d, $^3J_{H,H}$ = 7.1 Hz, 2 H, N*H*), 4.26 (m, 2 H, 7-H), 3.16 (s, 4 H, 1-H), 2.42 (t, $^3J_{H,H}$ = 7,5 Hz, 4 H, 3-H), 2.32 (t, $^3J_{H,H}$ = 7,3 Hz, 4 H, 5-H), 1.99 (m, 2 H, 8-H/11-H), 1.93 (m, 4 H, 4-H), 1,82 (m, 2 H, 9-H/10-H), 1.68 (m, 2 H, 9-H/10-H), 1.59 (m, 2 H, 8-H/11-H) ppm. ^{13}C NMR: δ = 180.8 (C-2), 169.9 (C-6), 49.0 (C-7), 47.8 (C-1), 34.3 (C-3), 33.4 (C-5), 30.9 (C-8/C-11), 21.9 (C-9/C-10), 20.5 (C-4) ppm. ^{15}N NMR: δ = 109.5 (*N*H), −3.7 (*N*H$_2$) ppm. ^{195}Pt NMR: δ = 2629 (371 Hz) ppm. IR: ν = 3322 (ν_{N-H}), 3201 (ν_{N-H}), 2958 s, 1641 ($\nu_{as\ C=O}$), 1531 s, 1247 m, 1052 m cm^{-1}. ESI-MS (positive): *m/z* 722.8 [M + H$^+$]$^+$, 744.8 [M + Na$^+$]$^+$. ESI-MS (negative): *m/z* 720.6 [M − H$^+$]$^-$. Anal. ($C_{22}H_{40}Cl_2N_4O_6Pt$) C, H, N.

(*OC*-6-33)-Dichloro(ethane-1,2-diamine)bis{[5-(2-hydroxyethyl)amino]-5-oxopentanoato}platinum(IV) (10c). The synthesis was carried out as described for **8a**. The crude product was purified by column chromatography (MeOH) to yield a pale-yellow solid. Yield: 107 mg (23%). ^{1}H NMR: δ = 8.99 (bs, 4 H, N*H*$_2$), 7.83 (m, 2 H, N*H*), 4.86 (t, $^3J_{H,H}$ = 5.3 Hz, 2 H, O*H*), 3.70 (m, 4 H, 8-H), 3.42 (m, 4 H, 7-H) 3.16 (s, 4 H, 1-H), 2.43 (t, $^3J_{H,H}$ = 7.4 Hz, 4 H, 3-H), 2.37 (t, $^3J_{H,H}$ = 7.3 Hz, 4 H, 5-H), 1.94 (m, 4 H, 4-H) ppm. ^{13}C NMR: δ = 180.8 (C-2), 170.7 (C-6), 59.1 (C-8), 47.8 (C-1), 40.3 (C-7), 34.2 (C-3), 33.3 (C-5), 20.4 (C-4) ppm. ^{15}N NMR: δ = 91.8 (*N*H), −3.7 (*N*H$_2$) ppm. ^{195}Pt NMR: δ = 2629 (415 Hz) ppm. IR: ν = 3380 m, 3330 m, 3207(ν_{N-H}), 2951 w, 1652 ($\nu_{as\ C=O}$), 1630 ($\nu_{as\ C=O}$), 1531 s, 1199 s, 1077 m cm^{-1}. ESI-MS (positive): *m/z* 674.9 [M + H$^+$]$^+$, 696.8 [M + Na$^+$]$^+$, 712.8 [M + K$^+$]$^+$. ESI-MS (negative): *m/z* 672.7 [M − H$^+$]$^-$. Anal. ($C_{16}H_{32}Cl_2N_4O_8Pt$) C, H, N.

(*OC*-6-33)-Dichloro(ethane-1,2-diamine)bis{(5-ethoxy)-5-oxopentanoato)platinum(IV) (10d). The synthesis was carried out as described for **8d**. The crude product was purified by column chromatography (EtOAc/MeOH, 4:1) to yield a pale-yellow solid. Yield: 90.4 mg (17%). ^{1}H NMR: δ = 8.96 (bs, 4 H, N*H*$_2$), 4.25 (q, $^3J_{H,H}$ = 7.1 Hz, 4 H, 7-H), 3.17 (bs, 4 H, 1-H), 2.53 (t, $^3J_{H,H}$ = 7.5 Hz, 4 H, 5-H), 2.48 (t, $^3J_{H,H}$ = 7.3 Hz, 4 H, 3-H), 1.94 (m, 4 H, 4-H), 1.37 (t, $^3J_{H,H}$ = 7.1 Hz, 6 H, 8-H) ppm. ^{13}C NMR: δ = 180.5 (C-2), 171.3 (C-6), 58.2 (C-7), 47.9 (C-1), 33.8 (C-3), 31.3 (C-5), 19.5 (C-4), 12.2 (C-8) ppm. ^{15}N NMR: δ = −3.9 ppm. ^{195}Pt NMR: δ = 2630 (416 Hz) ppm. IR: ν = 3210 (ν_{N-H}), 2983 m, 1725 ($\nu_{as\ C=O}$), 1648 ($\nu_{as\ C=O}$), 1383 m, 1188 s, 1052 m cm^{-1}. ESI-MS (positive): *m/z* 666.7 [M + Na$^+$]$^+$. ESI-MS (negative): *m/z* 642.6 [M − H$^+$]$^-$. Anal. ($C_{16}H_{30}Cl_2N_2O_8Pt$) C, H, N.

(*OC*-6-33)-*trans*-(1*R*,2*R*)-Diaminocyclohexanebis{(4-propylamino)-4-oxobutanoato}oxalato platinum(IV) (11a). The synthesis was carried out as described for **8a**. The crude product was purified by column chromatography (EtOAc/MeOH, 5:1) and recrystallized from acetone to yield a white powder. Yield: 82.1 mg (18%). ^{1}H NMR: δ = 8.69 (bs, 4 H, N*H*$_2$), 8.01 (m, 2 H, N*H*), 3.26 (m, 4 H, 12-H), 3.21 (m, 2 H, 1-H, 2-H), 2.65 (m, 4 H, 9-H/10-H), 2.56 (m, 4 H, 9-H/10-H), 2.46 (m, 2 H, 3-H$_{eq}$, 6-H$_{eq}$), 1.77 (m, 2 H, 3-H$_{ax}$, 6-H$_{ax}$), 1.76 (m, 2 H, 4-H$_{eq}$, 5-H$_{eq}$), 1.62 (m, 4 H, 13-H), 1.50 (m, 2 H, 4-H$_{ax}$, 5-H$_{ax}$), 1.03 (t, $^3J_{H,H}$ = 7.4 Hz, 6 H, 14-H) ppm. ^{13}C NMR: δ = 179.9 (C-8), 170.1 (C-11), 162.1 (C-7) 59.9 (C1, C-2), 39.4 (C-12), 30.1, 30.0, 29.9 (C-3, C-6, C-9/C-10), 22.5 (C-4, C-5), 21.3 (C-13), 9.7 (C-14) ppm. ^{15}N NMR: δ = 95.1 (*N*H), −5.9 (*N*H$_2$) ppm. ^{195}Pt NMR: δ = 3221 (455 Hz) ppm. IR: ν = 3319 m, 3160 m, 2965 m, 2934 m, 1733 ($\nu_{as\ C=O}$), 1653 ($\nu_{as\ C=O}$), 1550 s, 1358 s, 808 m cm^{-1}. ESI-MS (positive): *m/z* 735.8 [M + Na$^+$]$^+$. ESI-MS (negative): *m/z* 711.7 [M − H$^+$]$^-$. Anal. ($C_{22}H_{38}N_4O_{10}Pt$) C, H, N.

(*OC*-6-33)-(*trans*-(1*R*,2*R*)-Diaminocyclohexane)bis{(4-ethoxy)-4-oxobutanoato}oxalatoplatinum(IV) (11b). The synthesis was carried out as described for **8d**. The crude product was purified by column chromatography (EtOAc/MeOH, 6:1) and recrystallized from acetone to yield a white powder. Yield: 132 mg (30%). ^{1}H NMR: δ = 8.98–8.56 (m, 4 H, NH_2), 4.25 (m, 4 H, 12-H), 3.08 (m, 2 H, 1-H, 2-H), 2.73 (m, 4 H, 9-H/10-H), 2.68 (m, 4 H, 9-H/10-H), 2.48 (m, 2 H, 3-H$_{eq}$, 6-H$_{eq}$), 1.77 (m, 2 H, 4-H$_{eq}$, 5-H$_{eq}$), 1.74 (m, 2 H, 3-H$_{ax}$, 6-H$_{ax}$), 1.45 (m, 2 H, 4-H$_{ax}$, 5-H$_{ax}$), 1.37 (t, $^3J_{H,H}$ = 7.1 Hz, 6 H, 13-H) ppm. ^{13}C NMR: δ = 179.2 (C-8), 171.1 (C-11), 161.9 (C-7), 60.1 (C-1, C-2), 58.7 (C-12), 30.1 (C-3, C-6), 29.2 (C-9/C-10), 28.2 (C-9/C-10), 22.5 (C-4, C-5), 12.4 (C-13) ppm. ^{15}N NMR: δ = −6.4 (NH$_2$) ppm. ^{195}Pt NMR: δ = 3226 (475 Hz) ppm. IR: ν = 3235 m, 3186 m, 2942 w, 1736 ($\nu_{as\ C=O}$), 1653 ($\nu_{as\ C=O}$), 1353 s, 1026 m, 810 m cm^{-1}. ESI-MS (positive): *m*/*z* 688.0 [M + H$^+$]$^+$, 709.9 [M + Na$^+$]$^+$. ESI-MS (negative): *m*/*z* 686.1 [M − H$^+$]$^-$. Anal. ($C_{20}H_{32}N_2O_{12}Pt$) C, H, N.

Crystallographic Structure Determination. X-ray diffraction measurements were performed on a Bruker X8APEX II CCD diffractometer. The single crystal was positioned at 40 mm from the detector, and 3042 frames were measured, each for 10 s over a 1° scan width. The data were processed using SAINT software.[28] Crystal data, data collection parameters, and structure refinement details for **4**·$(C_2H_5)_2O$ are given in Table S2. The structure was solved by direct methods and refined by full-matrix least-squares techniques. Non-hydrogen atoms were refined with anisotropic displacement parameters. H atoms were placed at calculated positions and refined as riding atoms in the subsequent least-squares model refinements. The isotropic thermal parameters were estimated to be 1.2 times the values of the equivalent isotropic thermal parameters of the atoms to which hydrogens were bonded. The following computer programs were used: structure solution, SHELXS-97;[29] refinement, SHELXL-97;[30] molecular diagrams, ORTEP.[31] The computer was a Pentium IV, and scattering factors were used.[32]

Cell Lines and Culture Conditions. Human CH1 (ovarian carcinoma), HeLa (cervical carcinoma), SK-OV-3 (ovarian carcinoma), and SW480 (colon carcinoma) cells were kindly provided by Lloyd R. Kelland (CRC Centre for Cancer Therapeutics, Institute of Cancer Research, Sutton, U.K.), Thomas Czerny (Institute of Genetics, University of Veterinary Medicine Vienna, Austria), Evelyn Dittrich (General Hospital, Medical University of Vienna, Austria), and Brigitte Marian (Institute of Cancer Research, Medical University of Vienna, Austria), respectively. Cells were grown in 75 cm^2 culture flasks (Iwaki/Asahi Technoglass, Gyouda, Japan) as adherent monolayer cultures in complete culture medium, i.e., minimal essential medium (MEM) supplemented with 10% heat-inactivated fetal bovine serum, 1 mM sodium pyruvate, 4 mM L-glutamine, and 1% nonessential amino acids (100×) (all purchased from Sigma-Aldrich, Vienna, Austria). Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO_2.

Cytotoxicity Tests in Cancer Cell Lines. Cytotoxicity was determined by means of a colorimetric microculture assay (MTT assay, MTT = 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide). For this purpose, CH1, HeLa, SK-OV-3, and SW480 cells were harvested from culture flasks by trypsinization and seeded into 96-well microculture plates (Iwaki/Asahi Technoglass, Gyouda, Japan) in cell densities of 2 × 10^3, 1.5 × 10^3, 3.5 × 10^3, and 2.5 × 10^3 cells/well, respectively, in order to ensure exponential growth throughout drug exposure. After a 24 h preincubation, cells were exposed to serial dilutions of the test compounds in 200 μL/well complete culture medium for 96 h. At the end of exposure, drug solutions were replaced by 150 μL/well RPMI 1640 culture medium (supplemented with 10% heat-inactivated fetal bovine serum and 4 mM L-glutamine) plus 20 μL/well MTT solution in phosphate-buffered saline (5 mg/mL). After incubation for 4 h, the medium/MTT mixtures were removed, and the formazan crystals formed by the mitochondrial dehydrogenase activity of vital cells were dissolved in 150 μL of DMSO per well. Optical densities at 550 nm were measured with a microplate reader (Tecan Spectra Classic). The quantity of vital cells was expressed in terms of *T*/*C* values by comparison to untreated control microcultures, and 50% inhibitory concentrations (IC_{50}) were calculated from concentration−effect curves by interpolation. Evaluation is based on mean values from at least three independent experiments, each comprising at least six microcultures per concentration level.

DNA Platination. SW480 cells grown to ∼70% confluency in 90 mm tissue culture plates (Iwaki/Asahi Technoglass, Gyouda, Japan) were exposed to the platinum complexes in complete culture medium for 24 h at 37 °C. Afterward, cells were washed once with TBS (Tris-buffered saline) solution (0.0027 M KCl, 0.137 M NaCl, 0.025 M Tris-base, pH 7.4) and harvested by trypsinization. The number of vital cells was determined with a hemocytometer using trypan blue staining. The (3–6) × 10^6 cells were washed thrice with TBS and centrifuged at 900*g* for 2 min. Cell pellets were resuspended in 500 μL of lysis buffer (10 mM Tris-HCl, pH 7.4, 1 mM EDTA, 20 μg/mL RNase, and 0.2% Triton X-100) and incubated for 30 min at 37 °C. The lysate was centrifuged at 10000*g* for 8 min, and supernatants mixed with the 0.7-fold volume of isopropanol were centrifuged for 30 min at 10000*g* and 4 °C. Pellets were washed with 70% ethanol, again centrifuged for 30 min at 10000*g* and 4 °C, and dried in air. Pellets were then dissolved in Milli-Q water (18.2 MΩ cm) and shaken for 20 min at 65 °C. Because phosphate-containing reagents were strictly avoided, phosphorus was measured simultaneously with platinum by ICP-MS (Agilent 7500ce, Waldbronn, Germany, equipped with a CETAC ASX-520 autosampler and a MicroMist nebulizer at a sample uptake rate of approximately 0.25 mL/min), and this was used for the calculation of r_b values (number of Pt atoms per nucleotide). In order to minimize the impact of $^{15}N^{16}O$ interference with ^{31}P, aliquots (100 μL) were diluted to 5 mL with 2% (w/w) hydrochloric acid. Beryllium and indium were added as internal standards for phosphorus and platinum, respectively, in concentrations of 1 ppb, and concentrations were determined via the isotopes ^{9}Be, ^{31}P, ^{115}In, and ^{195}Pt. All experiments were run in triplicate.

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Supporting Information Available: X-ray crystallographic data in CIF format and figures showing clinically established anticancer platinum(II) complexes, with detailed structures of the di- and tetracarboxylatoplatinum(IV) complexes, NMR numbering scheme, and concentration−effect curves; elemental analysis data and the more relevant chemical shifts for all target complexes; and structure refinement details for **4**. This material is available free of charge via the Internet at http://pubs.acs.org. The crystallographic data have been deposited with the Cambridge Crystallographic Data Center with number CCDC 645537. Copies of data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. (deposit@ccdc.com.ac.uk).

References

(1) Jakupec, M. A.; Galanski, M.; Keppler, B. K. Tumour-inhibiting platinum complexes—state of the art and future perspectives. *Rev. Physiol. Biochem. Pharmacol.* **2003**, *146*, 1–54.

(2) Galanski, M.; Jakupec, M. A.; Keppler, B. K. Update of the preclinical situation of anticancer platinum complexes: novel design strategies and innovative analytical approaches. *Curr. Med. Chem.* **2005**, *12*, 2075–2094.

(3) Galanski, M.; Arion, V. B.; Jakupec, M. A.; Keppler, B. K. Recent developments in the field of tumor-inhibiting metal complexes. *Curr. Pharm. Des.* **2003**, *9*, 2078–2089.

(4) Reedijk, J. Why does cisplatin reach guanine-N7 with competing S-donor ligands available in the cell? *Chem. Rev.* **1999**, *99*, 2499–2510.

(5) Jakupec, M. A.; Galanski, M.; Keppler, B. K. The effect of cytoprotective agents in platinum anticancer therapy. *Met. Ions Biol. Syst.* **2004**, *42*, 179–208.

(6) Wang, X.; Guo, Z. The role of sulfur in platinum anticancer chemotherapy. *Anti-Cancer Agents Med. Chem.* **2007**, *7*, 19–34.
(7) Schilder, R. J.; LaCreta, F. P.; Perez, R. P.; Johnson, S. W.; Brennan, J. M.; Rogatko, A.; Nash, S.; McAleer, C.; Hamilton, T. C.; Roby, D.; Young, R. C.; Ozols, R. F.; O'Dwyer, P. J. Phase I and pharmacokinetic study of ormaplatin (tetraplatin, NSC 363812) administered on a day 1 and day 8 schedule. *Cancer Res.* **1994**, *54*, 709–117.
(8) Trask, C.; Silverstone, A.; Ash, C. M.; Earl, H.; Irwin, C.; Bakker, A.; Tobias, J. S.; Souhami, R. L. A randomized trial of carboplatin versus iproplatin in untreated advanced ovarian cancer. *J. Clin. Oncol.* **1991**, *9*, 1131–1137.
(9) Hall, M. D.; Dolman, R. C.; Hambley, T. W. Platinum(IV) anticancer complexes. *Met. Ions Biol. Syst.* **2004**, *42*, 297–322.
(10) Galanski, M.; Keppler, B. K. Searching for the magic bullet: anticancer platinum drugs which can be accumulated or activated in the tumor tissue. *Anti-Cancer Agents Med. Chem.* **2007**, *7*, 55–73.
(11) Latif, T.; Wood, L.; Connell, C.; Smith, D. C.; Vaughn, D.; Lebwohl, D.; Peereboom, D. Phase II study of oral bis (aceto) ammine dichloro (cyclohexamine) platinum (IV) (JM-216, BMS-182751) given daily × 5 in hormone refractory prostate cancer (HRPC). *Invest. New Drugs* **2005**, *23*, 79–84.
(12) Sternberg, C. N.; Whelan, P.; Hetherington, J.; Paluchowska, B.; Slee, P. H. Th. J.; Vekemans, K.; Van Erps, P.; Theodore, C.; Koriakine, O.; Oliver, T.; Lebwohl, D.; Debois, M.; Zurlo, A.; Collette, L. Phase III trial of satraplatin, an oral platinum plus prednisone vs. prednisone alone in patients with hormone-refractory prostate cancer. *Oncology* **2005**, *68*, 2–9.
(13) Kozubik, A.; Horvath, V.; Svihalkova-Sindlerova, L.; Soucek, K.; Hofmanova, J.; Sova, P.; Kroutil, A.; Zak, F.; Mistr, A.; Turanek, J. High effectiveness of platinum(IV) complex with adamantylamine in overcoming resistance to cisplatin and suppressing proliferation of ovarian cancer cells in vitro. *Biochem. Pharmacol.* **2005**, *69*, 373–383.
(14) Hall, M. D.; Mellor, H. R.; Callaghan, R.; Hambley, T. W. Basis for design and development of platinum(IV) anticancer complexes. *J. Med. Chem.* **2007**, *50*, 3403–3411.
(15) Giandomenico, C. M.; Abrams, M. J.; Murrer, B. A.; Vollano, J. F.; Rheinheimer, M. I.; Wyer, S. B.; Bossard, G. E.; Higgins, J. D., III. Carboxylation of kinetically inert platinum(IV) hydroxy complexes. An entrée into orally active platinum(IV) antitumor agents. *Inorg. Chem.* **1995**, *34*, 1015–1021.
(16) Galanski, M.; Keppler, B. K. Carboxylation of dihydroxoplatinum(IV) complexes via a new synthetic pathway. *Inorg. Chem.* **1996**, *35*, 1709–1711.
(17) Galanski, M.; Keppler, B. K. Carboxylation of dihydroxoplatinum(IV) complexes with acyl chlorides. Crystal structure of the *trans-R,R*- and *trans-S,S*-isomer of (OC-6-33)-bis(1-adamantanecarboxylato)(cyclohexane-1,2-diamine)dichloroplatinum(IV). *Inorg. Chim. Acta* **1997**, *265*, 271–274.
(18) Ang, W. H.; Khalaila, I.; Allardyce, C. S.; Juillerat-Jeanneret, L.; Dyson, P. J. Rational design of platinum(IV) compounds to overcome glutathione-S-transferase mediated drug resistance. *J. Am. Chem. Soc.* **2005**, *127*, 1382–1383.
(19) Alvarez-Valdes, A.; Perez, J. M.; Lopez-Solera, I.; Lannegrand, R.; Continente, J. M.; Amo-Ochoa, P.; Camazon, M. J.; Solans, X.; Font-Bardia, M.; Navarro-Ranninger, C. Preparation and characterization of platinum(II) and (IV) complexes of 1,3-diaminepropane and 1,4-diaminebutane: circumvention of cisplatin resistance and DNA interstrand cross-link formation in CH1cisR ovarian tumor cells. *J. Med. Chem.* **2002**, *45*, 1835–1844.
(20) Barnes, K. R.; Kutikov, A.; Lippard, S. J. Synthesis, characterization, and cytotoxicity of a series of estrogen-tethered platinum(IV) complexes. *Chem. Biol.* **2004**, *11*, 557–564.
(21) Ang, W. H.; Pilet, S.; Scopelliti, R.; Bussy, F.; Juillerat-Jeanneret, L.; Dyson, P. J. Synthesis and characterization of platinum(IV) anticancer drugs with functionalized aromatic carboxylate ligands: influence of the ligands on drug efficacies and uptake. *J. Med. Chem.* **2005**, *48*, 8060–8069.
(22) Reithofer, M.; Galanski, M.; Roller, A.; Keppler, B. K. An entry to novel platinum complexes: carboxylation of dihydroxoplatinum(IV) complexes with succinic anhydride and subsequent derivatization. *Eur. J. Inorg. Chem.*, **2006**, 2612–2617.
(23) Feazell, R. P.; Nakayama-Ratchford, N.; Dai, H.; Lippard, S. J. Soluble single-walled carbon nanotubes as longboat delivery systems for platinum(IV) anticancer drug design. *J. Am. Chem. Soc.* **2007**, *129*, 8438–8439.
(24) Khokhar, A. R.; Al-Baker, S.; Shamsuddin, S.; Siddik, Z. H. Chemical and biological studies on a series of novel (*trans*-(1*R*,2*R*)-, *trans*-(1*S*,2*S*)-, and *cis*-1,2-diaminocyclohexane)platinum(IV) carboxylate complexes. *J. Med. Chem.* **1997**, *40*, 112–116.
(25) Galanski, M.; Yasemi, A.; Slaby, S.; Jakupec, M. A; Arion, V. B; Rausch, M.; Nazarov, A. A; Keppler, B. K. Synthesis, crystal structure and cytotoxicity of new oxaliplatin analogues indicating that improvement of anticancer activity is still possible. *Eur. J. Med. Chem.* **2004**, *39*, 707–714.
(26) Bulten, E. J.; Verbeek, F. Platinum (IV)-diamine Complexes as Antitumor Agents. U.S. Patent 4482569, 1984.
(27) Khokhar, A. R.; al-Baker, S.; Siddik, Z. H. Synthesis and antitumor activity of 1,2-diaminocyclohexane platinum(IV) complexes. *J. Inorg. Biochem.* **1994**, *54*, 39–47.
(28) *SAINT-Plus*, version 7.06a, and *APEX2*; Bruker-Nonius AXS, Inc.: Madison, WI, 2004.
(29) Sheldrick, G. M. *SHELXS-97, Program for Crystal Structure Solution*; University Göttingen: Göttingen, Germany, 1997.
(30) Sheldrick, G. M. *SHELXL-97, Program for Crystal Structure Refinement*; University Göttingen: Göttingen, Germany, 1997.
(31) Johnson, G. K. Report ORNL-5138; Oak Ridge National Laboratory: Oak Ridge, TN, 1976.
(32) *International Tables for X-ray Crystallography*; Kluwer Academic Press: Dodrecht, The Netherlands, 1992; *Vol. C*,Tables 4.2.6.8 and 6.1.1.4.

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