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PAPER

Mono-carboxylated diaminedichloridoplatinum(IV) complexes – selective synthesis, characterization, and cytotoxicity†

Verena Pichler,[a] Seied M. Valiahdi,[a] Michael A. Jakupec,[a] Vladimir B. Arion,[a] Markus Galanski*[a] and Bernhard K. Keppler*[a,b]

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(OC-6-43)-Dichlorido(*N*,*N*-dimethyl-ethane-1,2-diamine)dihydroxidoplatinum(IV) could selectively be mono-carboxylated with succinic anhydride based on the steric hindrance caused by the two methyl groups of the equatorial ligand. Subsequent esterification of the uncoordinated carboxylic acid with alcohols of different lengths (methanol, butanol, hexanol and octanol) afforded the corresponding esters. The synthesized complexes were characterized in detail by elemental analysis, ESI-MS, multinuclear (^{1}H, ^{13}C, ^{15}N and ^{195}Pt) NMR spectroscopy and in two cases by X-ray crystallography. Cytotoxicity of novel platinum(IV) compounds was investigated in four human cancer cell lines (CH1, A549, SW480 and SK-OV-3). Remarkably, the most lipophilic complexes showed IC_{50} values down to the low micromolar or even nanomolar range.

Introduction

Rosenberg's discovery of the cytotoxic effect of platinum complexes was the start of a successful entry to platinum-based compounds in chemotherapy.[1] Although thousands of platinum agents have been synthesized since then, only three, cisplatin, carboplatin and oxaliplatin, have achieved worldwide approval for anticancer therapy. In fact, square-planar platinum(II) complexes are the only metal-based chemotherapeutics in use against human cancer at present.[2,3] Limitations of these drugs are especially severe side effects, acquired and intrinsic resistance and low selectivity for cancerous cells.

The reasons for the mentioned drawbacks are the kinetic lability of the platinum(II) compounds and their preferential binding to extra- and intracellular sulfur containing biomolecules, like amino acids, glutathione or metallothionein.[4–7]

The investigation of kinetically more inert platinum(IV) complexes was introduced to overcome these problems. Especially as the octahedral configuration permits a more comprehensive spectrum of reactions, due to the fact that there are two additional axial ligands, compared to platinum(II) complexes, that offer the possibility of further derivatization improving *e.g.* drug uptake, stability or reducing general toxicity.

Platinum(IV) complexes act as prodrugs and consequently have to be reduced to the platinum(II) species extra- or preferably intracellularly to unfold their anticancer potential. Therefore, the axial ligands are particularly suitable for coupling with delivery systems or biofunctional molecules, since they are released during the reduction process. Up to date, only a few platinum(IV) compounds have entered clinical trials (Fig. 1).[8,9] Tetraplatin did not succeed in phase I clinical trials due to high neurotoxicity;[10] iproplatin failed in phase III trials owing to a lower activity in untreated advanced ovarian cancer compared to carboplatin.[11] Satraplatin was the most promising compound as it offered the possibility of oral application, although the publication of the SPARC (satraplatin and prednisone against refractory cancer) study, in which satraplatin failed to show a benefit in terms of overall survival, was a setback. At the moment, satraplatin is in different phase I, II and III trials.[12,13]

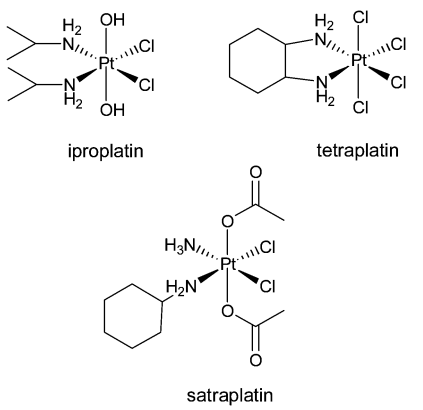

Fig. 1 Platinum(IV) compounds which entered clinical trials.

Platinum(IV) compounds facilitated the expansion of the synthetic spectrum to well known organic chemistry. By now,

[a]*University of Vienna, Institute of Inorganic Chemistry, Waehringer Strasse 42, A-1090, Vienna, Austria. E-mail: markus.galanski@univie.ac.at, bernhard.keppler@univie.ac.at; Fax: +43 1 427752680*
[b]*Research Platform "Translational Cancer Therapy Research" University of Vienna, Waehringer Strasse 42, A-1090, Vienna, Austria*

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carboxylation of *trans* dihydroxidoplatinum(IV) complexes with acyl halides or anhydrides is a well established method. Another method took advantage of the use of cyclic anhydrides,[14–16] producing complexes with two uncoordinated carboxyl groups and offering the possibility of further derivatization to esters or amides, showing promising cytotoxic activity.[17] Targeted coupling of the platinum(IV) species with functionalized biomolecules, like estrogen or with delivery systems, like soluble single-walled carbon nanotubes (SWNT's) attract more and more attention in research.[18,19]

In fact, mainly symmetric dicarboxylation of dihydroxido platinum compounds has been published so far and this double functionality complicates synthetic approaches, as there is always the probability of singly and doubly derivatized products.

Therefore, selective synthesis of asymmetric mono-carboxylated platinum(IV) complexes is a worthwhile way for improving targeted design of novel antitumor platinum compounds. Here, we report for the first time a method for an exclusive mono-carboxylation of a dihydroxidoplatinum complex in the presence of an excess of succinic anhydride. This novel synthetic method is based on sterical hindrance, caused by the equatorial non-leaving *N*,*N*-dimethyl-ethane-1,2-diamine ligand. The remaining free hydroxido group raises the solubility in water and polar organic solvents and thus derivatization of the uncoordinated carboxy group with more lipophilic moieties, as recently described in literature, is now possible. The complexes were characterized by elemental analysis, ESI-MS, multinuclear (^{1}H, ^{13}C, ^{15}N and ^{195}Pt) NMR spectroscopy and in two cases by X-ray crystallography. Cytotoxicity in four human cancer cell lines CH1, SK-OV-3 (ovarian cancer), A549 (non-small cell lung carcinoma), and SW480 (colon carcinoma) was evaluated *via* the MTT assay.

Results and discussion

Synthesis and characterization

The reaction of K_2PtCl_4 with *N*,*N*-dimethyl-ethane-1,2-diamine and subsequent oxidation with 30% hydrogen peroxide in H_2O led to the starting complex (OC-6-43)-dichlorido(*N*,*N*-dimethyl-ethane-1,2-diamine)dihydroxidoplatinum(IV) **1**.[20] Selective carboxylation of exclusively one hydroxido group with 4 equivalents of succinic anhydride took place in DMF at 50 °C to get **2** in a good yield of 73%. (Fig. 2) The doubly carboxylated product was not produced under these conditions. However, it formed only at higher temperatures with concurrent decomposition of **1**. The unreacted hydroxido group led to a higher solubility of the complex in water contrary to symmetric platinum(IV) analogues. Further derivatization with different alcohols resulted in the corresponding esters. Thereby, 1,1-carbonyldiimidazole was used as an activating agent for the carboxyl group. *In situ* addition of sodium alcoholate afforded the ester derivatives **3–6**, which were isolated in moderate yields between 21 and 24% after column chromatography.

Table 1 Selected ^{1}H, ^{13}C, ^{15}N and ^{195}Pt NMR spectroscopic data for asymmetric platinum(IV) complexes **2–6** (values in ppm)

	^{1}H			^{13}C		^{15}N	^{195}Pt
	N*H*5a or N*H*5b		O*H*	C6	C9	*N*H2	Pt
2	7.12	9.39	—	181.3	174.6	−5.9	2497
3	7.16	9.28	1.57	180.9	173.6	−5.9	2497
4	7.13	9.31	1.57	180.9	173.2	−5.4	2497
5	7.13	9.30	1.56	180.9	173.1	−5.4	2499
6	7.12	9.31	1.57	180.9	173.2	−5.4	2495

The platinum(IV) compounds **1–6** were characterized by elemental analysis, multinuclear (^{1}H, ^{13}C, ^{15}N and ^{195}Pt) one- and two-dimensional NMR spectroscopy and ESI mass spectrometry; in case of **1** and **3** additionally by X-ray diffraction.

In fact, ^{195}Pt spectra are highly suitable for examining the coordination sphere and geometry of the complex as well as the oxidation state of the central metal ion, as chemical shifts cover a range of several thousands of ppm (Table 1). The ^{195}Pt signals of all the synthesized compounds are in a narrow range between 2495 and 2499 ppm in DMSO-d_6, these high-frequency shifts are an indication for Pt(IV) complexes with a *cis*,*cis*,*trans*-$PtCl_2N_2O_2$ coordination sphere and in accord with recently published data.[21] Derivatization of the carboxylic acid had no significant effect on the ^{195}Pt, ^{15}N, ^{13}C or even ^{1}H shifts of complex **2**. Remarkably, the ^{1}H-signal of the coordinated hydroxido group, with high field shifts in the range of δ = 1.56–1.59 ppm, could be detected.

Crystal structures

The results of X-ray diffraction studies of **1** and **3** are shown in Fig. 3 and 4, correspondingly. Complex **1** crystallized in the tetragonal space group $I4_1/a$, while **3** in the triclinic centrosymmetric space group $P\bar{1}$. The platinum(IV) atom has an octahedral coordination geometry with a bidentate *N*,*N*-dimethyl-ethane-1,2-diamine ligand and two chlorido ligands bound in the equatorial plane. In **1** and **3**, two hydroxido ligands or one 4-methoxy-4-oxobutanoato ligand and one hydroxido ligand were coordinated in axial positions, respectively.

Upon ligation to platinum(IV) the *N*,*N*-dimethyl-ethane-1,2-diamine ligand forms a five-membered chelate cycle PtN_2C_2. The torsion angle $\Theta_{N1-C1-C2-N2}$, which serves as a measure of the deviation of the chelate ring from planarity, is −55.9(3)° in **3**. The Pt–N and Pt–Cl bond lengths and N1–Pt–N2 angle in **1** (see legend to Fig. 3) are comparable with those in a platinum(II) complex

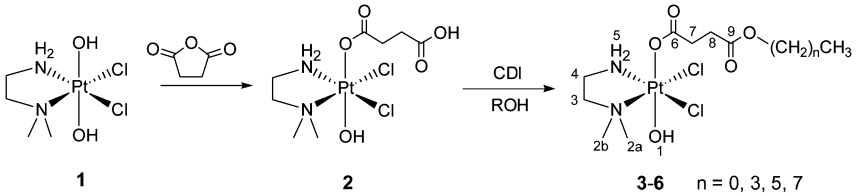

Fig. 2 Synthetic scheme of novel carboxylatohydroxidoplatinum(IV) compounds.

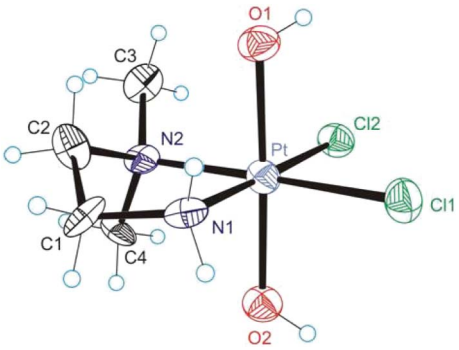

Fig. 3 ORTEP view of **1** with atom labeling scheme; the thermal ellipsoids have been drawn at 50% probability level. Selected bond lengths (Å) and bond angles (°): Pt–N1 2.038(14), Pt–N2 2.106(17), Pt–Cl1 2.300(6), Pt–Cl2 2.351(5), Pt–O1 2.005(10), Pt–O2 1.994(10); O1–Pt–O2 177.1(5), N1–Pt–N2 85.3(6), Cl1–Pt–Cl2 92.12(19).

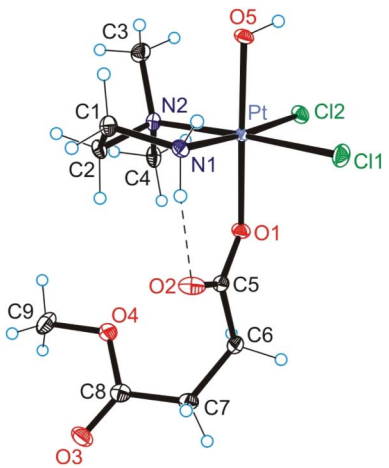

Fig. 4 ORTEP view of **3** with atom labeling scheme showing the intramolecular hydrogen bonding N1–H···O2 [N1···O2 2.684 Å, N2–H···O2 143.0°]; the thermal ellipsoids have been drawn at 50% probability level. Selected bond lengths (Å) and bond angles (°): Pt–N1 2.031(3), Pt–N2 2.103(3), Pt–Cl1 2.3102(8), Pt–Cl2 2.3374(7), Pt–O1 2.039(2), Pt–O5 1.987(2); O1–Pt–O5 177.86(8), N1–Pt–N2 84.95(10), Cl1–Pt–Cl2 92.81(3).

with *N*,*N*-dimethyl-ethane-1,2-diamine [Pt–N1 2.039(14), Pt–N2 2.067(12), Pt–Cl1 2.303(4), Pt–Cl2 2.317(4) Å, N1–Pt–N2 84.0(5)°].[22] The Pt–N1, Pt–Cl2 and Pt–O1 bond lengths in **3** (see legend to Fig. 4) are comparable with those in *cis*,*trans*-[Pt(en)Cl_2($OCOCH_3$)$_2$] [2.040(5), 2.315(1) and 2.017(5) Å][23] and in *cis*,*trans*-[Pt(en)Cl_2(3-carboxy-propanoato)$_2$] [2.054(10), 2.3175(5) and 2.0113(12) Å].[16]

Cytotoxicity in cancer cell lines

The *in vitro* cytotoxicity of complexes **1–5** was evaluated in comparison with cisplatin in four human cancer cell lines (CH1, SK-OV-3, SW480, A549), using the MTT assay (Table 2 and Fig. 5).[24] The octylester derivative **6** could not be tested because of its insufficient solubility.

The cisplatin sensitive cell line CH1 showed the highest response to all asymmetric compounds compared to the intrinsically cisplatin resistant cell lines SK-OV-3, SW480 and A549. The following structure–activity relationships can be deduced from these data. Complexes **1** (two coordinated hydroxido groups) and **2** (one coordinated hydroxido group and one uncoordinated COOH group) featuring a low lipophilicity have a low cytotoxicity. Surprisingly the activity of the dihydroxido compound **1** in the CH1 cell line is in the same micromolar range as that of the methyl ester **3**, although its lipophilicity is distinctly lower; a fact, which can not be explained at the moment. Concerning the ester derivatives **3–5** with different chain lengths, an increase in cytotoxicity in parallel to an enlargement of the alkyl chain can be observed. This behavior is expected and in accord with previously published data.[25]

Table 2 Antiproliferative activity of novel asymmetric platinum(IV) compounds **1–5** compared to cisplatin in four human cancer cell lines

	IC_{50} [μM][a]			
Compound	CH1	A549	SW480	SK-OV-3
1	3.3 ± 1.3	110 ± 15	114 ± 10	80 ± 10
2	8.4 ± 1.0	145 ± 39	75 ± 13	257 ± 11
3	3.2 ± 0.9	48 ± 12	8.4 ± 1.1	38 ± 6
4	0.045 ± 0.006	5.8 ± 0.7	1.2 ± 0.2	3.2 ± 0.4
5	0.032 ± 0.008	4.8 ± 0.4	1.0 ± 0.2	2.3 ± 0.4
Cisplatin	0.16 ± 0.03	1.3 ± 0.4	3.5 ± 0.3	1.9 ± 0.3

[a] 50% inhibitory concentrations in CH1, A549, SW480 and SK-OV-3 cells in the MTT assay (96 h exposure). Values are the means ± standard deviations obtained from at least three independent experiments.

Remarkably, the most potent platinum(IV) complexes **4** and **5** displayed IC_{50} values in the low nanomolar range in the CH1 cell line, which is about a 5-fold enhanced antiproliferative activity compared to cisplatin.

In general, the following rank order of cytotoxicity was observed: ester derivatives (**5** ≥ **4** > **3**) > dihydroxido complex (**1**) ≥ underivatised carboxylic acid derivative (**2**).

Experimental

Materials and methods

All solvents and reagents were obtained from commercial suppliers and were used as received, except for methanol which was dried using standard procedures. K_2PtCl_4 was obtained from Johnson Matthey (Switzerland). Reverse osmosis water was doubly distilled before use. For column chromatography, silica gel 60 (Fluka) was used. The starting compound (OC-6-43)-dichlorido(*N*,*N*-dimethyl-ethane-1,2-diamine)dihydroxido platinum(IV) was synthesized according to standard literature procedures. ^{1}H, ^{13}C, ^{15}N and ^{195}Pt one- and two-dimensional NMR spectra were recorded with a Bruker Avance III 500 MHz instrument at 500.32 (^{1}H), 125.81 (^{13}C), 50.70 (^{15}N) and 107.55 (^{195}Pt) MHz or 500.10 (^{1}H), 125.75 (^{13}C), 50.68 (^{15}N) and 107.51 (^{195}Pt) MHz in DMSO-d_6 at 298 K.

The solvent residual peak for ^{1}H and ^{13}C was used as an internal reference, whereas ^{195}Pt chemical shifts were referenced relative to external K_2PtCl_4 and ^{15}N chemical shifts relative to external NH_4Cl. Electrospray ionization mass spectrometry was carried out with a Bruker esquire$_{3000}$ ion trap using MeOH as solvent. Elemental analyses were performed using a Perkin–Elmer 2400 CHN elemental analyzer by the Microanalytical Laboratory of the University of Vienna.

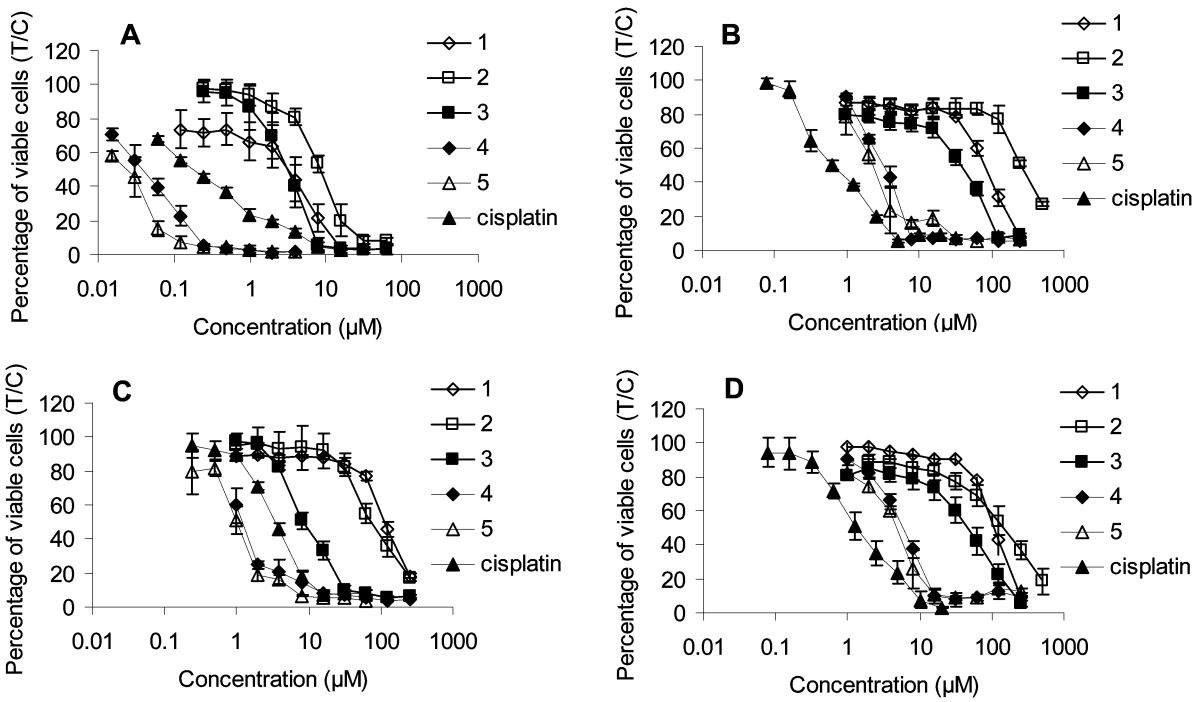

Fig. 5 Concentration-effect curves of complexes **1**, **2**, **3**, **4**, **5** and cisplatin in CH1 (A), SK-OV-3 (B), SW480 (C) and A549 cells (D), obtained by the MTT assay (96 h exposure).

Synthesis

(OC-6-43)-Dichlorido(*N*,*N*-dimethyl-ethane-1,2-diamine)dihydroxidoplatinum(IV) (1). (SP-4-3)-Dichlorido(*N*,*N*-dimethyl-ethane-1,2-diamine)platinum(II) (2.27 g, 6.41 mmol) was suspended in 60 mL of H_2O and 60 mL of 30% H_2O_2 was added. The reaction mixture was stirred at room temperature until a clear solution was observed. The solution was filtered and the solvent was removed under reduced pressure. The yellow product was dried *in vacuo*. Yield: 2.39 g, 96%. Elemental analysis, found C 12.36, H 3.49, N 6.95. Calcd for $C_4H_{14}Cl_2N_2O_2Pt$: C 12.38, H 3.64, N 7.22.

(OC-6-54)-(3-Carboxypropanoato)dichlorido(*N*,*N*-dimethyl-ethane-1,2-diamine)hydroxidoplatinum(IV) (2). A mixture of succinic anhydride (0.846 g, 8.45 mmol) and **1** (0.800 g, 2.06 mmol) in 15 mL of DMF was heated to 60 °C until the solid material dissolved to form a yellow solution. The solvent was removed under reduced pressure and the yellow residue was dissolved in acetone. The 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: 737.7 mg, 73%. Elemental analysis, found C 19.95, H 3.63, N 5.48. Calcd. for $C_8H_{18}Cl_2N_2O_5Pt$: C 19.68, H 3.72, N 5.74. ^{1}H-NMR (DMSO-d_6): δ = 12.09 (br s, 1H, H10); 9.39 (br s, 1H, H5b); 7.12 (br s, 1H, H5a); 2.81 (m, 4H, H3/H4); 2.68 (s+d, 1H, H2a or H2b); 2.60 (s+d, 3H, H2a or H2b); 2.41 (m, 4H, H7/H8) ppm. ^{13}C-NMR (DMSO-d_6): δ = 181.3 (C6); 174.6 (C9); 67.8 (C3); 50.1 (C2a or C2b); 48.6 (C2a or C2b); 45.2 (C4); 32.5 (C7 or C8); 30.4 (C7 or C8) ppm. ^{15}N-NMR (DMSO-d_6): δ = –5.9 (NH_2) ppm. ^{195}Pt-NMR (DMSO-d_6): δ = 2497 ppm. ESI-MS: *m*/*z* 375.2 [M–axial ligands+Na^+]$^+$, 511.0 [M+Na^+]$^+$.

General procedure for the synthesis of complexes 3–6

1,1′-Carbonyldiimidazole in DMF was added to a solution of **2** in DMF and the mixture was heated to 50 °C. After 15 min stirring and simultaneous flushing with argon to remove the formed CO_2, the solution was cooled down to room temperature. Sodium alcoholate in abs. alcohol was added, and the solution was stirred for 24 h at room temperature. The solvents were removed under reduced pressure.

(OC-6-54)-Dichlorido(*N*,*N*-dimethyl-ethane-1,2-diamine)hydroxido(4-methoxy-4-oxobutanoato)platinum(IV) (3). 1,1′-Carbonyldiimidazole (0.136 g, 0.838 mmol) in DMF (8 mL), **2** (0.200 g, 0.398 mmol) in DMF (4 mL), sodium methanolate (4 mg Na in 5 mL of abs. methanol). The crude product was purified by column chromatography (EtOAc–MeOH, 1 : 1) to yield yellow crystals, which were dried *in vacuo*. Yield: 45.6 mg, 22%. Elemental analysis, found C 21.50, H 3.77, N 5.12. Calcd. for $C_9H_{20}Cl_2N_2O_5Pt$: C 21.51, H 4.01, N 5.57. ^{1}H-NMR (DMSO-d_6): δ = 9.28 (br s, 1H, H5b); 7.16 (br s, 1H, H5a); 3.57 (s, 3H, H10); 2.90–2.75 (m, 4H, H3/H4); 2.66 (s+d, 1H, H2a or H2b); 2.59 (s+d, 3H, H2a or H2b); 2.47–2.38 (m, 4H, H7/H8); 1.57 (br s, 1H, H1) ppm. ^{13}C-NMR (DMSO-d_6): δ = 180.9 (C6); 173.6 (C9); 67.8 (C3); 51.8 (C10); 50.0 (C2a or C2b); 48.6 (C2a or C2b); 45.5 (C4); 32.4 (C7 or C8); 30.1 (C7 or C8) ppm. ^{15}N-NMR (DMSO-d_6): δ = –5.9 (NH_2) ppm. ^{195}Pt-NMR (DMSO-d_6): δ = 2497 ppm. ESI-MS: *m*/*z* 375.2 [M–axial ligands+Na^+]$^+$, 525.0 [M+Na^+]$^+$.

(OC-6-54)-(4-Butyloxy-4-oxobutanoato)dichlorido(*N*,*N*-dimethyl-ethane-1,2-diamine)hydroxidoplatinum(IV) (4). 1,1′-Carbonyldiimidazole (0.194 g, 1.197 mmol) in DMF (16 mL), **2** (0.400 g, 0.819 mmol) in DMF (8 mL), sodium butanolate

(4 mg Na in 10 mL of butanol). The crude product was purified by column chromatography (EtOAc–MeOH, 4 : 1) to yield a pale-yellow powder which was dried *in vacuo*. Yield: 106.6 mg, 24%. Elemental analysis, found C 26.32 H 4.55 N 5.01. Calcd. for $C_{12}H_{26}Cl_2N_2O_5Pt$: C 26.48, H 4.81, N 5.15. ^{1}H-NMR (DMSO-d_6): δ = 9.31 (br s, 1H, H5a), 7.13 (br s, 1H, H5b), 4.00 (m, 2H, H10), 2.88–2.79 (m, 4H, H3/H4), 2.67 (s+d, 3H, H2a or H2b), 2.60 (s+d, 3H, H2a or H2b), 2.54–2.40 (m, 4H, H7/H8), 1.57 (s, 1H, H1), 1.54 (m, 2H, H11), 1.33 (m, 2H, H12), 0.89 (t, 3H, H13, $^3J_{H,H}$ = 7.5 Hz) ppm. ^{13}C-NMR (DMSO-d_6): δ = 180.9 (C6), 173.2 (C9), 67.8 (C3), 64.1 (C10), 50.1 (C2a), 48.6 (C2b), 45.2 (C4), 32.4 (C7 or C8), 30.7 (C11), 30.2 (C7 or C8), 19.1 (C12), 14.1 (C13) ppm. ^{15}N-NMR (DMSO-d_6): δ = –5.4 (NH_2) ppm. ^{195}Pt-NMR (DMSO-d_6): δ = 2497 ppm. ESI-MS: *m*/*z* 374.9 [M-axial ligands+Na^+]$^+$, 566.8 [M+Na^+]$^+$, 1109.5 [2M+Na^+]$^+$, 579.6 [M+Cl^-]$^-$, 1120.9 [2M+Cl^-]$^-$.

(OC-6-54)-Dichlorido(*N*,*N*-dimethyl-ethane-1,2-diamine)(4-hexyloxy-4-oxobutanoato)hydroxidoplatinum(IV) (5). 1,1′-Carbonyldiimidazole (0.194 g, 1.197 mmol) in DMF (16 mL), **2** (0.400 g, 0.819 mmol) in DMF (8 mL), sodium hexanolate (4 mg Na in 10 mL of hexanol). The crude product was dissolved in methanol and the solution was filtered. The clear yellow solution was concentrated under reduced pressure and led to precipitation of the product. The white product was dried *in vacuo*. Yield: 102.0 mg, 22%. Elemental analysis, found C 29.40, H 5.13, N 4.78. Calcd. for $C_{14}H_{30}Cl_2N_2O_5Pt$: C 29.38, H 5.28, N 4.89. ^{1}H-NMR (DMSO-d_6): δ = 9.30 (br s, 1H, H5a), 7.13 (br s, 1H, H5b), 3.98 (m, 2H, H10), 2.88–2.78 (m, 4H, H3/H4), 2.66 (s+d, 3H, H2a), 2.60 (s+d), 3H, H2b), 2.43–2.43 (m, 4H, H7/H8), 1.56 (s, 1H, H1), 1.55 (m, 2H, H11), 1.27 (bm, 6H, H12-H14), 0.87 (t, 3H, H15, $^3J_{H,H}$ = 7.0 Hz) ppm. ^{13}C-NMR (DMSO-d_6): δ = 180.9 (C6), 173.1 (C9), 67.7 (C3), 64.4 (C10), 50.0 (C2a), 48.6 (C2b), 45.2 (C4), 32.4 (C7 or C8), 31.3 (C12), 30.3 (C7 or C8), 28.5 (C11), 25.5 (C13), 22.5 (C14), 14.4 (C15) ppm. ^{15}N-NMR (DMSO-d_6): δ = –5.4 (NH_2) ppm. ^{195}Pt-NMR (DMSO-d_6): δ = 2499 ppm. ESI-MS: *m*/*z* 375.0 [M-axial ligands+Na^+]$^+$, 595.0 [M+Na^+]$^+$, 1165.3 [2M+Na^+]$^+$, 571.0 [M–H^+]$^-$.

(OC-6-54)-Dichlorido(*N*,*N*-dimethyl-ethane-1,2-diamine)hydroxido(4-octyloxy-4-oxobutanoato)platinum(IV) (6). 1,1′-Carbonyldiimidazole (0.159 g, 0.983 mmol) in DMF (8 mL), **2** (0.400 g, 0.819 mmol) in DMF (8 mL), sodium octanolate (7 mg Na in 5 mL of octanol). The crude product was purified by column chromatography (EtOAc–MeOH, 7 : 1) to yield a white powder which was dried *in vacuo*. Yield: 105.0 mg, 21%. Elemental analysis, found C 31.93 H 5.49 N 4.58. Calcd. for $C_{16}H_{34}Cl_2N_2O_5Pt$: C 32.00, H 5.71, N 4.67. ^{1}H-NMR (DMSO-d_6): δ = 9.31 (br s, 1H, H5a), 7.12 (br s, 1H, H5b), 3.98 (m, 2H, H10), 2.89–2.78 (m, 4H, H3/H4), 2.66 (s+d, 3H, H2a), 2.60 (s+d, 3H, H2b), 2.50–2.42 (m, 4H, H7/H8), 1.57 (s, 1H, H1), 1.55 (m, 2H, H11), 1.27 (m, 10H, H12-H16), 0.87 (t, 3H, H17, $^3J_{H,H}$ = 7.0 Hz) ppm. ^{13}C-NMR (DMSO-d_6): δ = 180.9 (C6), 173.2 (C9), 67.8 (C3), 64.4 (C10), 50.1 (C2a), 48.6 (C2b), 45.2 (C4), 32.4 (C7 or C8), 31.7 (C12), 30.3 (C7 or C8), 29.1 (C13), 29.0 (C14), 28.6 (C11), 25.8 (C15), 22.5 (C16), 14.4 (C17) ppm. ^{15}N-NMR (DMSO-d_6): δ = –5.4 ppm. ^{195}Pt-NMR (DMSO-d_6): δ = 2495 ppm. ESI-MS: *m*/*z* 374.9 [M-axial ligands+Na^+]$^+$, 623.0 [M+Na^+]$^+$, 1221.8 [2M+Na^+]$^+$, 600.8 [M–H^+]$^-$.

Table 3 Crystal data and details of data collection for **1** and **3**

	1	**3**
Empirical formula	$C_4H_{14}Cl_2N_2O_2Pt$	$C_9H_{20}Cl_2N_2O_5Pt$
Fw	388.16	502.26
Space group	$I4_1/a$	$P\bar{1}$
a (Å)	23.064(5)	8.1295(3)
b (Å)	23.064(5)	8.3664(3)
c (Å)	7.5597(16)	11.0750(4)
α (°)		89.567(3)
β (°)		79.707(2)
γ (°)		84.467(2)
V (Å^3)	4021.4(15)	737.65(5)
Z	16	2
λ (Å)	0.71073	0.71073
ρ_c (g cm^{-3})	2.565	2.261
Crystal size (mm)	0.15 × 0.05 × 0.05	0.20 × 0.20 × 0.15
T (K)	296(2)	100(2)
μ (mm^{-1})	14.449	9.889
Reflections collected/unique	26 869/1805	47 115/4280
$R_{(int)}$	0.3116	0.0623
$R_1{}^a$ [$I > 2\sigma(I)$]	0.0643	0.0206
$wR_2{}^b$ (*all data*)	0.1766	0.0466
GOF^c	1.059	1.019

^{a} $R_1 = \Sigma \| F_o | - | F_c \| / \Sigma | F_o |$. ^{b} $wR_2 = \{\Sigma[w(F_o^2 - F_c^2)^2]/\Sigma[w(F_o^2)^2]\}^{1/2}$. ^{c} GOF = $\{\Sigma[w(F_o^2 - F_c^2)^2]/(n-p)\}^{1/2}$, where *n* is the number of reflections and *p* is the total number of parameters refined.

Crystal structure measurements

X-ray diffraction measurements of **1** and **3** were performed on a Bruker X8 APEXII CCD diffractometer. Single crystals were positioned at 35 and 40 mm from the detector, and 2929 and 1020 frames were measured, each for 10 and 60 s, correspondingly, over a 1° scan width. The data were processed using SAINT software.[26] Crystal data, data collection parameters and structure refinement details, are given in Table 3. The structure was solved by direct methods and refined by full-matrix least-squares techniques. Non-H atoms were refined with anisotropic displacement parameters. H atoms were inserted in calculated positions and refined with a riding model. Structure solution was achieved with SHELXS-97 and refinement with SHELXL-97,[27] and graphics were produced with ORTEP-3.[28]

Cell lines and culture conditions

Human CH1 (ovarian carcinoma) and SK-OV-3 (ovarian carcinoma) cells were kindly provided by Lloyd R. Kelland (CRC Centre for Cancer Therapeutics, Institute of Cancer Research, Sutton, UK) and Evelyn Dittrich (General Hospital, Medical University of Vienna, Austria), respectively, and SW480 (colon carcinoma) as well as A549 (non-small cell lung cancer) cells by Brigitte Marian (Institute of Cancer Research, Department of Medicine I, Medical University of Vienna, Austria). 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% non-essential amino acids (100×) (all purchased from Sigma–Aldrich, Vienna, Austria). Cultures were maintained at 37 °C in a humidified atmosphere containing 95% air and 5% CO_2.

Cytotoxicity tests in cancer cell lines

Cytotoxicity was determined by a colorimetric microculture assay (MTT assay). For this purpose, CH1, A549, SK-OV-3, and SW480 cells were harvested from culture flasks by trypsinization and seeded into 96-well microculture plates (Iwaki/Asahi Technoglass) in densities of 1×10^3, 4×10^3, 3.5×10^3, and 2.5×10^3 viable cells/well, respectively. After a 24 h preincubation, cells were exposed to 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 100 µL/well RPMI 1640 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^{-1}). After incubation for 4 h, medium was removed and the formazan product formed by viable cells was dissolved in DMSO (150 µL/well). Optical densities at 550 nm were measured with a microplate reader (Tecan Spectra Classic), using a reference wavelength of 690 nm to correct for unspecific absorption. The quantity of viable cells was expressed in terms of T/C values by comparison to untreated controls, and 50% inhibitory concentrations (IC_{50}) were calculated from concentration-effect curves by interpolation. Evaluation is based on means from at least three independent experiments, each comprising triplicates for each concentration level.

Conclusions

The two methyl substituents of the equatorial ligand in (OC-6-43)-dichlorido(*N*,*N*-dimethyl-ethane-1,2-diamine)dihydroxido platinum(IV) block one coordinated OH group during the reaction with an excess of cyclic anhydride leading to exclusively the mono-carboxylated derivative. Subsequent esterification led to novel agents with remarkably high cytotoxicity and IC_{50} values down to the nanomolar range depending on the lipophilicity. In this respect it should be stated that this novel synthetic route is the first to produce selectively mono-carboxylated products without laborious purification steps. Coupling to targeting groups and other biologically relevant molecules will be the focus of our future research program.

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