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Original Article

Synthesis and structure-activity relationships of mono- and dialkyl-substituted oxaliplatin derivatives

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Abstract

In order to improve the pharmacological profile of the anticancer drug oxaliplatin, (*trans-R,R*-cyclohexane-1,2-diamine)oxalatoplatinum(II), and to explore activity-structure relationships, new mono- and dialkyl substituted oxaliplatin analogues have been synthesized. Following a new synthetic strategy, racemates with a defined stereochemistry at carbon atoms 1, 2, 4, and 5 of the cyclohexane ring could be prepared, which are the bases for reliable structure-activity relationships and the following enantiomer resolution. The cytotoxicity was evaluated in nine tumor cell lines, indicating that bulky substituents have a negative influence on the cytotoxic potency of the oxaliplatin derivatives. With respect to the antiproliferative properties, the 4-methyl-, *cis*-4,5-dimethyl-, and especially the 4,4-dimethyl-*trans*-cyclohexane-1,2-diamine(oxalato)platinum(II) complexes are the most promising candidates to be further evaluated.

Keywords: Platinum; Anticancer complexes; Oxaliplatin; Cytotoxic activity; Structure-activity relationships (SAR)

1. Introduction

Oxaliplatin, (trans-*R*,R-cyclohexane-1,2-diamine)oxalatoplatinum(II) (Fig. 1), has shown potency in many cancer cell lines and tumors including some that are primarily resistant to cisplatin and carboplatin [1–4].

Oxaliplatin (Eloxatin) received approval for the 1st line treatment of metastatic colorectal cancer (MCRC) in France in 1998 and in major European countries in 1999. Since January 2004, Eloxatin has received marketing approval in the US for the 1st line treatment of MCRC. Eloxatin (Sanofi-Aventis) is currently marketed in more than 60 countries worldwide and reached blockbuster status in 2004 with sales beyond 1 billion (10^9) €.

Eloxatin is used in combination with 5-fluorouracil/leucovorin (5-FU/LV) known as FOLFOX regimen and has shown advantage over the established IFL treatment (irinotecan plus 5-FU/LV) [5]. Oxaliplatin was demonstrated to be more effective and better tolerated with a clear prolongation of the median survival time of patients. The most frequent dose limiting toxicity of Eloxatin is neurotoxicity. Phase III studies of Eloxatin in pancreatic and gastric cancer [6] and phase II studies in non-small-cell lung cancer and breast cancer are ongoing [7]. At present, oxaliplatin is not only the standard treatment option in MCRC, but also for adjuvant therapy in case of complete resection of stage III (Dukes' C) primary colon cancer [8].

At this point it has to be questioned: (i) why is oxaliplatin so efficient in primarily cisplatin and carboplatin resistant cell lines and tumors and (ii) are there possibilities to improve the anticancer properties of oxaliplatin? Two main differences between cisplatin and carboplatin on the one hand and oxaliplatin on the other are obvious. Oxaliplatin is significantly more lipophilic than the two diammineplatinum(II) complexes and the adducts formed with the primary target DNA are processed differently by the cellular machinery.

The lipophilicity of oxaliplatin is a result of the cyclohexane-1,2-diamine (1,2-diaminocyclohexane or DACH) ligand in comparison to cis- and carboplatin, which is expressed by a large volume of distribution and a slower

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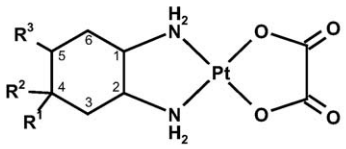

complex	R^1	R^2	R^3
1 = oxaliplatin	H	H	H
2	Me	H	H
3	Et	H	H
4	$C(CH_3)_3$	H	H
5	Me	H	Me
6	Me	Me	H

Fig. 1. Structure of oxaliplatin and new analogues described in this manuscript (the stereochemistry is omitted).

excretion through the kidneys [9]. The lipophilic properties of oxaliplatin may also contribute to the differences in general toxicity as well as to an altered cellular uptake. The diamine ligand as well as its stereochemical features play a crucial role in the cytotoxic profile of oxaliplatin. The platinum complexes with the amino groups in *trans* position display better cytotoxic and anticancer activities than the *cis*-(*R*,*S*) isomer and the *trans*-(*R*,*R*) isomer is a significantly more potent antitumor drug than the *trans*-*S*,*S* congener [10]. The adducts formed with the ultimate target DNA are recognized and processed differently from those of cisplatin and carboplatin [11]. This is explainable by the formation of a certain type of hydrogen bond with the DNA in case of oxaliplatin [12]. Furthermore, due to the methylene units of the cyclohexane ring, an unpolar region is formed at the DNA [13]. Both features contribute to a different recognition and repair of adducts formed between oxaliplatin and the DNA.

Given that the DACH ligand has such a marked influence on the pharmacological profile and on the effectiveness of oxaliplatin in primarily cis- and carboplatin resistant tumors, then derivatization at the *trans*-*R*,*R*-cyclohexane-1,2-diamine moiety could lead to improved anticancer properties.

First results with 4-methyl and 4-ethyl-*trans*-cyclohexane-1,2-diamineoxalatoplatinum(II) analogues have indicated that this goal is feasible [14]. Following the concept described above, also 4-propyl-, 4-*tert*-butyl- and 4-phenyl derivatives have been synthesized, in order to explore structure-activity relationships [15]. It could be demonstrated that best cytotoxicity was achieved with small substituents (methyl or ethyl), but there was one major drawback of the two synthetic procedures used. The oxaliplatin analogues were synthesized as *trans*-*R*,*R*/*S*,*S* 1:1 mixtures with the substituent at position 4 mainly being either in axial or in equatorial position. Pure racemic mixtures, which are the bases for enantiomer resolution, could not be prepared. Moreover, structure-activity relationships deriving from such diastereomeric mixtures are inherently more error prone than those obtained with the pure racemates, since the nature of the substituents as well as the stereochemistry at C(4) of the cyclohexane ring contribute significantly to the cytotoxicity.

Therefore, we have focused on two possible pathways to obtain selectively racemates and not mixtures containing four isomers: (i) a new synthetic route leading exclusively to equatorial substituents at position 4 of the cyclohexane ring, and (ii) design of the cyclohexane-1,2-diamine derivatives with substituents having a fixed configuration, which is not dependent on the chosen synthetic procedure.

2. Results and discussion

2.1. Synthesis and characterization

The mono and dialkyl substituted *trans*-cyclohexane-1,2-diamine derivatives were synthesized via three different pathways, which will be described in detail in a separate publication: In case of the mono alkyl substituted diamine ligands, the corresponding 4-alkyl-cyclohexanones or 4-alkyl-cyclohexanols have to be converted to the 4-alkyl-cyclohexenes if not commercially available (Fig. 2). The following *trans*-dihydroxylation of the alkene using hydrogenperoxide and formic acid is the key step of this procedure [16,17]. The diols were converted to the diazides via mesylation and nucleophilic substitution with sodium azide [18–20]. The diamine ligands were isolated as diaminium sulfates after catalytic hydrogenation over $Pd/CaCO_3$ (Lindlar catalyst) [21,22].

The 4,5-*cis*-dimethyl-*trans*-cyclohexane-1,2-diamine ligand was obtained from *cis*-1,2,3,6-tetrahydrophthalic anhydride, which was converted to *cis*-4,5-dimethylcyclohexene

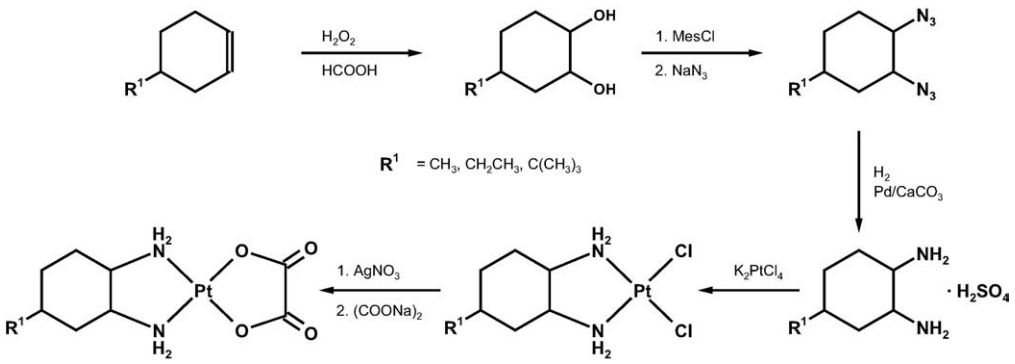

Fig. 2. Synthesis of 4-*trans*-cyclohexane-1,2-diamine)oxalatoplatinum(II) derivatives **2**-**4** (the stereochemistry is omitted).

in four steps as described by Walborsky et al. [23]. Then, direct diazide formation in the presence of $Mn(CH_3COO)_3 \cdot 2H_2O$ was performed as previously reported [14,15]. Reduction with Lindlar catalyst afforded the diamine, which was also isolated as diaminium sulfate.

Synthesis of 4,4-dimethyl-*trans*-cyclohexane-1,2-diamine, starts from 4,4-dimethyl-1-cyclohexanone, which was prepared in analogy to a procedure described recently [24]. In this case, the *trans*-cyclohexane-1,2-diamine derivative was isolated as (L)-mandelate salt.

The ligands were characterized by ^1H and ^{13}C NMR spectroscopy as well as elemental analysis. They were obtained as racemates with the following stereochemical features: In all ligands, the protonated amino groups display a *trans*-configuration and are both in an equatorial position. In case of the 4-methyl-, 4-ethyl-, and 4-*tert*-butyl-*trans*-cyclohexane-1,2-diamine derivatives, the substituents are also found to be equatorial. Contrary, the 4,4-dimethyl-, and the *cis*-4,5-dimethyl-*trans*-cyclohexane-1,2-diamine analogues have one axial and one equatorial methyl group (this will be discussed in more detail in the context of the oxalato complexes).

The diaminedichloroplatinum(II) complexes were synthesized via direct reaction of K_2PtCl_4 with the diaminium salts in the presence of NaOH and obtained as yellow solids with yields in the range of 61 to 95%. The elemental analyses were found to be in good agreement with the calculated values. The chloro ligands were removed with silver nitrate. After reaction of sodium oxalate with the formed diaquaplatinum(II) species, the colorless oxalato complexes **2**-**6** were obtained in yields between 40 and 70%. Characterization of the title compounds was performed by ^1H and ^{13}C NMR spectroscopy as well as elemental analysis. Most indicative for the coordination of the diamine ligands as well as for the stereochemistry at C(4) and C(5) are the chemical shifts of carbon atoms C(1) and C(2).

Coordination of the diamine ligands is accompanied by a downfield shift of the C(1) and C(2) resonances. Chemical shifts of the nitrogen bearing carbon atoms in the ligands are found in the region of 48.7 – 58.3 ppm, whereas coordination to the platinum(II) center is reflected by resonances of C(1) and C(2) between 58.2 and 63.4 ppm (Fig. 3).

Contrary to oxaliplatin, two signals for the methine carbons C(1) and C(2) are detected in complexes **2**-**6**. As discussed previously [14,15], the chemical shift differences ($\Delta\delta$) of C(1) and C(2) can be used to judge the stereochemistry at C(4) and C(5) of the cyclohexane ring. The peak pattern of the mono-substituted 4-alkyl-*trans*-cyclohexane-1,2-diamine derivatives **2**, **3**, and **4** is significantly different from those of the dimethyl derivatives **5** and **6**.

In the case of complexes **2**, **3**, and **4** the resonances of C(1) and C(2) display $\Delta\delta$ values of 0.1 to 0.4 ppm, whereas for **5** and **6** a markedly larger splitting with 4.7 and 3.5 ppm is detected. This fact is explainable taking into account the stereochemistry at C(4) and C(5) of the cyclohexane ring. In **2**-**4** the alkyl substituent is in an equatorial position, thus resonances of the carbon atoms C(1) and C(2) are found in close proximity. Contrary, in **5** and **6** the axial methyl substituent is responsible for significant differences in the chemical shifts of the nitrogen bearing carbon atoms. These results are also in accord to those of complexes **2a**, **3a**, and **4a** (Table 1) [15],

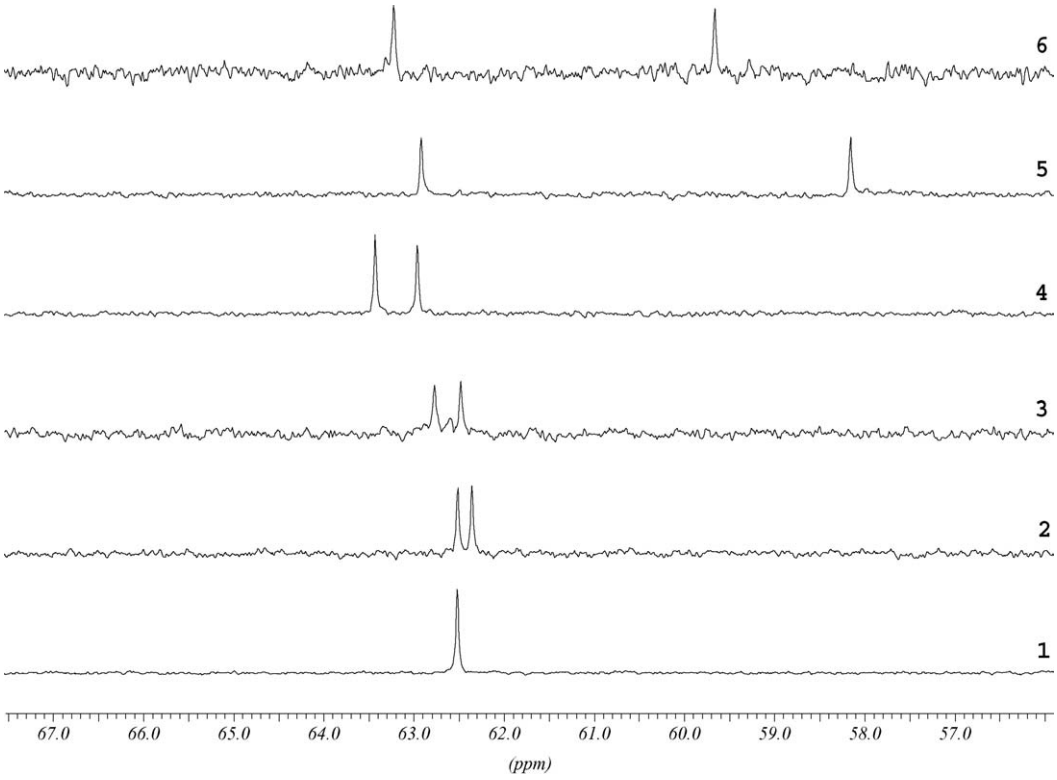

Fig. 3. ^{13}C NMR spectra of oxaliplatin (**1**) and analogues **2**-**6**; the region of the nitrogen bearing carbon atoms C(1) and C(2) is shown.

Table 1
Resonances and chemical shift differences (Δδ) of the nitrogen bearing carbon atoms C(1) and C(2) depending on the stereochemistry at C(4)

complex	Chemical shifts of C(1) and C(2) (ppm)					
	eq. substitution		Δδ	ax. substitution		Δδ
2	62.4	62.5	0.1	---	---	
2a[a]	62.3	62.5	0.2	58.2	63.1	4.9
3	62.5	62.8	0.3	---	---	
3a[a]	62.8	63.1	0.3	58.6	63.4	4.8
4	63.0	63.4	0.4	---	---	
4a[a]	63.1	63.6	0.5	59.9	62.6	2.7

[a] Complexes **2a**, **3a**, and **4a** are analogues to complexes **2**, **3**, and **4** with the substituent at C(4) predominantly being in axial position (**2a**, 85%; **3a**, 82%; **4a**, 81% axial substitution).

which are analogues to complexes **2**, **3**, and **4** with the substituent (methyl, ethyl or 1,1-dimethylethyl) at C(4) mainly being in axial position (**2a**, 85%; **3a**, 82%, and **4a**, 81% axial substitution).

For example, in the case of **2a** the major isomer is the complex with the 4-methyl substituent in axial position (85%). The Δδ of C(1) and C(2) was found to be 4.9 ppm. Contrary, in the minor isomer (15% equatorial substitution) the splitting was significantly smaller with 0.2 ppm and in agreement with the values found for **2** (0.1 ppm).

2.2. Cytotoxicity and structure-activity relationships

The cytotoxicity of oxaliplatin (**1**) and derivatives **2**-**6** was investigated in nine human tumor cell lines originating from colon (SW480, SW620, HCT-15, COLO 205, HT-29) and ovarian carcinoma (NIH-OVCAR-3) as well as from leukemia (MOLT-4, HL-60) and melanoma (SK-MEL-5) by means of the resazurin assay after drug exposure for 48 hours. IC_{50} values are listed in Table 2. At this point, it should be emphasized that the new complexes **2**, **3**, **4**, **5**, and **6** are racemates, and that higher cytotoxic potencies in case of pure enantiomers (*R,R*-configuration at C(1) and C(2)) are expected.

2.2.1. Cytotoxicity of complexes *2, 3, 4, 5, and 6*

In both the ovarian carcinoma cell line NIH-OVCAR-3 and the melanoma cell line SK-MEL-5, the new platinum complexes **2**-**6** display a lower cytotoxic activity than oxaliplatin. The only exception is compound **5** (the *cis*-4,5-dimethyl derivative) with a remarkably low IC_{50} value of 2.63 μM in SK-MEL-5. Contrary, in the leukemia cell lines, **2**, **5**, and **6** are more active (up to a factor of 4) in comparison to oxaliplatin (**1**). The high potency of **1** in the colon carcinoma cell lines is reflected by IC_{50} values in the range of 1.50 to 10.8 μM. The only derivative with comparable potency is the 4,4-dimethyl analogue (**6**, 1.79 – 11.8 μM). The methyl substituted oxalatoplatinum(II) complex **2** is more active in HCT-15 cells than oxaliplatin, comparable to oxaliplatin in SW620 cells, but obviously less potent in the SW480 and especially the COLO 205 and HT-29 colon cell lines. **3**, **4**, and **5** have high IC_{50} values in all colon cell lines. An exceptional case is compound **5** in HCT-15 cells, as it exerts the strongest effect.

2.2.2. Cytotoxicity of previously described complexes *2a, 3a, and 4a*

Complex **4a** with the *tert*-butyl substituent at C(4) mainly being in an axial position shows a low potency in all cell lines under investigation. The methyl- and ethyl analogues **2a** and **3a** are less active in melanoma cells than oxaliplatin, whereas in the ovarian carcinoma cell lines, they show interesting properties. Especially in both leukemia cell lines, complexes **2a** and **3a** display very low IC_{50} values between 1.23 and 1.91 μM in comparison to oxaliplatin and the other derivatives. As to the colon carcinoma cell lines, compounds **2a** and **3a** show a moderate activity in SW480, SW620, and HCT-15 cells, but are much more cytotoxic than the analogous complexes **2** and **3** in the COLO 205 and HT-29 cell line, and their cytotoxicity is roughly comparable to oxaliplatin.

2.2.3. Structure-activity relationships

The following structure-activity relationships can be deduced from the cytotoxicity data. The oxaliplatin analogues **4** and **4a** with a bulky *tert*-butyl substituent at position 4 of the cyclohexane ring show low activities in all cell lines, especially when the substituent is in an axial position. This dependency of cytotoxic activity on the stereochemistry at C(4) in the case of the 4-*tert*-butyl-cyclohexane-1,2-diamine

Table 2
Cytotoxicity of oxaliplatin (**1**) and analogues in nine human tumor cell lines[a]

cell lines		complexes								
		1	**2**	**2a**[b]	**3**	**3a**[b]	**4**	**4a**[b]	**5**	**6**
colon	SW620	2.27	2.10	3.24	5.21	7.53	41.3	136	22.4	2.77
	SW480	2.19	15.0	5.90	6.90	3.67	54.3	180	116	2.83
	HCT-15	10.8	5.74	9.86	16.5	24.3	63.1	117	1.13	11.8
	COLO 205	1.50	73.8	1.03	48.4	3.80	3.60	208	121	1.79
	HT-29	1.93	48.5	2.40	105	1.31	46.6	147	18.1	10.0
ovarian	OVCAR-3	5.57	27.5	5.90	29.6	2.18	49.5	175	56.1	12.4
leukemia	MOLT-4	38.9	8.77	1.42	25.7	1.91	67.3	128	16.6	11.2
	HL-60	4.07	2.69	1.38	4.23	1.23	19.7	19.8	1.22	3.63
melanoma	SK-MEL-5	4.58	36.1	33.9	44.3	15.8	61.0	136	2.63	49.4

[a] IC_{50} values (μM) of oxalatoplatinum(II) complexes after exposure for 48 h, determined by resazurin assay.
[b] Complexes **2a**, **3a**, and **4a** are analogues to complexes **2**, **3**, and **4** with the substituent at C(4) predominantly being in axial position (**2a**, 85%; **3a**, 82%; **4a**, 81% axial substitution).

derivative was observed in all cell lines. In general, all complexes are less cytotoxic in ovarian as well as in melanoma cells than oxaliplatin (exceptions: **3a** in OVCAR-3 and **5** in SK-MEL-5). Contrary, **2**, **2a**, **3**, **3a**, **5**, and **6** exhibit higher cytotoxic potencies up to a factor of nearly 30 in the MOLT-4 and HL-60 leukemia cells than the parent compound **1**.

In the colon carcinoma cell lines, the most interesting oxaliplatin derivatives are **2**, **2a** and **6**. Complex **2** shows a high activity in SW620 and HCT-15 cells, but in COLO 205 and HT-29 the cytotoxic potency is low. Besides a good activity of **2a** and **6** in SW620, SW480, and HCT-15 cell lines, the oxaliplatin analogues display a promising activity in COLO 205 and HT-29 in comparison to the parent compound **1** and **2**.

To summarize, a sterically demanding substituent at C(4) (*tert*-butyl) has a negative influence on the cytotoxic properties of the complex. In this case, axial position causes high IC_{50} values. 4-Methyl substituted oxaliplatin analogues are in many cell lines more active than their ethyl congeners. Therefore, at present, the most interesting substituents seem to be methyl groups.

3. Conclusions

Recently, 4-alkyl substituted oxaliplatin derivatives have been synthesized in our group, and a comparison of their cytotoxicity strongly indicated that improvement of the anticancer activity of oxaliplatin is feasible. The major drawback of these compounds was the fact that only mixtures with substituents at C(4) of the cyclohexane ring with predominantly axial or equatorial position could be isolated. Following a different synthetic strategy, we have now managed to produce oxaliplatin analogues with a defined stereochemistry at the carbon atoms 1, 2 , 4, and 5 resulting in racemates and not in problematical mixtures of 4 isomers. This is not only the basis for reliable structure-activity relationships but also essential for enantiomer resolution. The 4-methyl-, *cis*-4,5-dimethyl-, and 4,4-dimethyl substituted derivatives are the most promising oxaliplatin analogues so far. With respect to the cytotoxic profile as well as to regulatory requirements, compound **6** is being favored, since it displays only two chiral centers. Nevertheless, all methyl derivatives should be included in in vivo experiments.

4. Experimental

4.1. Syntheses

The new compounds reported in this study are illustrated in Fig. 1. Potassium tetrachloroplatinate(II) was obtained from Degussa (Germany). All other chemicals obtained from commercial suppliers were used as received and were of analytical grade. Water was used doubly distilled. The synthetic procedures were carried out in a light protected environment when platinum complexes were involved. The substituted *trans*-cyclohexane-1,2-diaminium salts as well as their dichloro- and oxalatoplatinum(II) complexes have been synthesized according to standard literature methods as discussed in the section synthesis and characterization. Analyses indicated by the symbols of the elements or functions were within ± 0.4%.

4.1.1. Synthesis of dichloroplatinum complexes

4.1.1.1. (SP-4-2)-Dichloro(trans-R,R-cyclohexane-1,2-diamine)platinum(II). A solution of *trans-R,R*-cyclohexane-1,2-diamine (824 mg, 7.22 mmol) and potassium tetrachloroplatinate(II) (3.00 g, 7.23 mmol) in 160 ml of water was stirred at room temperature. A yellow solid formed, which was filtered off and dried under reduced pressure over P_2O_5 to obtain 1.68 g of $[Pt(C_6H_{14}N_2)Cl_2]$; yield 61%. Anal. $C_6H_{14}Cl_2N_2Pt$ (C, H, N).

4.1.1.2. (SP-4-3)-Dichloro(R,R,R/S,S,S-4-methyl-trans-cyclohexane-1,2-diamine)platinum(II). To a solution of K_2PtCl_4 (3.10 g, 7.87 mmol) in 30 ml of water, 4-methyl-*trans*-cyclohexane-1,2-diaminium sulfate (1.78 g, 7.87 mmol) was added. The pH was adjusted to 7 with 0.5 M NaOH and was kept constant during the reaction at this value using 0.1 M NaOH. A yellow precipitate formed which was filtered off and dried under reduced pressure over P_2O_5 to obtain 2.04 g of $[Pt(C_7H_{16}N_2)Cl_2]$; yield 66%. Anal. $C_7H_{16}Cl_2N_2Pt$ (C, H, N).

4.1.1.3. (SP-4-3)-Dichloro(R,R,R/S,S,S-4-ethyl-trans-cyclohexane-1,2-diamine)platinum(II). The synthetic procedure is the same as that for $[Pt(C_7H_{16}N_2)Cl_2]$; yield 92%. Anal. $C_8H_{18}Cl_2N_2Pt$ (C, H, N).

4.1.1.4. (SP-4-3)-Dichloro(R,R,R/S,S,S-4-tert-butyl-trans-cyclohexane-1,2-diamine)platinum(II). The synthetic procedure is the same as that for $[Pt(C_7H_{16}N_2)Cl_2]$; yield 90%. Anal. $C_{10}H_{22}Cl_2N_2Pt$ (H, N), C: calcd, 27.53 found 28.09.

4.1.1.5. (SP-4-3)-Dichloro(R,R,R,S/S,S,R,S-4,5-cis-dimethyl-trans-cyclohexane-1,2-diamine)platinum(II). The synthetic procedure is the same as that for $[Pt(C_7H_{16}N_2)Cl_2]$; yield 65%. Anal. $C_8H_{18}Cl_2N_2Pt$ (C, H, N).

4.1.1.6. (SP-4-3)-Dichloro(4,4-dimethyl-trans-R,R/S,S-cyclohexane-1,2-diamine)platinum(II). The synthetic procedure is the same as that for $[Pt(C_7H_{16}N_2)Cl_2]$; yield 95%; Anal. $C_8H_{18}Cl_2N_2Pt$ (C, H, N).

4.1.2. Synthesis of oxalatoplatinum complexes

4.1.2.1. (SP-4-2)-(trans-R,R-Cyclohexane-1,2-diamine)oxalatoplatinum(II), ***1****.* (*SP*-4-2)-Dichloro(*trans-R,R*-cyclohexane-1,2-diamine)platinum(II) (1.08 g, 2.84 mmol) was suspended in 60 ml of water and $AgNO_3$ (920 mg, 5.40 mmol)

was added in one portion. The mixture was stirred for a period of one day at room temperature. Silver chloride precipitated and was filtered off. Oxalic acid (240 mg, 2.70 mmol) was mixed with NaOH (5.4 ml 1 M, 5.4 mmol), was added to the aqua(*trans*-cyclohexane-1,2-diamine)platinum(II) containing solution and was stirred over night at room temperature. A white precipitate formed which was filtered off and dried under reduced pressure over P_2O_5 to obtain 716 mg of oxaliplatin as a white solid; yield 67%.^{1}H NMR in D_2O: δ = 1.07 [m, 2H, H(4), H(5)], 1.22 [m, 2H, H(3), H(6)], 1.49 [m, 2H, H(4'), H(5')], 1.98 [m, 2H, H(3'), H(6')], 2.28 [m, 2H, H(1), H(2)], 5.06 [m, 2H, N*H*], 5.79 [m, 2H, N*H*]. ^{13}C NMR in D_2O: δ = 24.3 [C(4), C(5)], 32.0 [C(3), C(6)], 62.5 [C(1), C(2)], 168.7 [*C*=O]. Anal. $C_8H_{14}N_2O_4Pt$ (C, H, N).

4.1.2.2. (SP-4-3)-(R,R,R/S,S,S-4-Methyl-trans-cyclohexane-1,2-diamine)oxalatoplatinum(II), ***2****.* The synthetic procedure is the same as that for **1**; yield 40%.^{1}H NMR in D_2O: δ = 0.75 – 0.88 [m, 1H, H(5)], 0.84 [d, 3H, C*H*$_3$, $^3J_{H,H}$ = 6.5 Hz], 0.95 [m, 1H, H(3)], 1.19 – 1.42 [m, 2H, H(4), H(6)], 1.42 – 1.54 [m, 1H, H(5')], 1.86 – 1.99 [m, 2H, H(6'), H(3')], 2.20 – 2.41 [m, 2H, H(1), H(2)]. ^{13}C NMR in D_2O: δ = 20.4 [*C*H$_3$], 31.0 [C(6)], 31.2 [C(4)], 32.6 [C(5)], 39.8 [C(3)], 62.4 [C(1) or C(2)], 62.5 [C(1) or C(2)], 168.7 [*C*=O]. Anal. $C_9H_{16}N_2O_4Pt$ (C, H, N).

4.1.2.3. (SP-4-3)-(R,R,R/S,S,S-4-Ethyl-trans-cyclohexane-1,2-diamine)oxalatoplatinum(II), ***3****.* The synthetic procedure is the same as that for **1**; yield 45%. ^{1}H NMR in D_2O: δ = 0.69 – 0.98 [m, 2H, H(5), H(3)], 0.75 [t, 3H, C*H*$_3$CH$_2$, $^3J_{H,H}$ = 7.6 Hz], 1.07 – 1.32 [m, 4H, H(4), H(6), CH$_3$C*H*$_2$], 1.55 [m, 1H, H(5')], 1.87 – 2.03 [m, 2H, H(6'), H(3')], 2.21 – 2.44 [m, 2H, H(1), H(2)], 5.05 [m, 2H, N*H*], 5.75 [m, 2H, N*H*]. ^{13}C NMR in D_2O: δ = 11.2 [*C*H$_3$CH$_2$], 28.1 [CH$_3$*C*H$_2$], 30.2 [C(5)], 30.9 [C(6)], 37.5 [C(3)], 37.8 [C(4)], 62.5 [C(1) or C(2)], 62.8 [C(1) or C(2)], 168.7 [*C*=O]. Anal. $C_{10}H_{18}N_2O_4Pt$ (C, H, N).

4.1.2.4. (SP-4-3)-(R,R,R/S,S,S-4-tert-Butyl-trans-cyclohexane-1,2-diamine)oxalatoplatinum(II), ***4****.* The synthetic procedure is the same as that for **1**; yield 70%. ^{1}H NMR in DMF-d_7: δ = 0.87 [s, 9H, C*H*$_3$], 1.01 [m, 1H, H(5)], 1.12 [m, 1H, H(4)], 1.28 [m, 1H, H(3)], 1.48 [m, 1H, H(6)], 1.66 [m, 1H, H(5')], 2.05 – 2.19 [m, 2H, H(3'), H(6')], 2.30 – 2.52 [m, 2H, H(1), H(2)], 5.36 [m, 2H N*H*], 6.13 [m, 2H N*H*]. ^{13}C NMR in DMF-d_7: δ = 25.7 [C(5)], 27.6 [C(*C*H$_3$)$_3$], 31.3 [C(6)], 32.1 [*C*(CH$_3$)$_3$], 33.3 [C(3)], 47.0 [C(4)], 63.0 [C(1) or (2)], 63.4 [C(1) or C(2)], 166.8 [*C*=O]. Anal. $C_{12}H_{22}N_2O_4Pt$ (C, H, N).

4.1.2.5. (SP-4-3)-(R,R,R,S/S,S,R,S-4,5-cis-Dimethyl-trans-cyclohexane-1,2-diamine)oxalatoplatinum(II), ***5****.* The synthetic procedure is the same as that for **1**; Yield 48%. ^{1}H-NMR in D_2O: δ = 0.72 [d, 3H, $^3J_{H,H}$ = 7.4 Hz, C*H*$_3$], 0.82 [d, 3H, $^3J_{H,H}$ = 6.8 Hz, C*H*$_3$], 1.24 [m, 1H, H(3) or H(6)], 1.46 [m, 1H, H(3) or H(6)], 1.53 – 1.74 [m, 3H, H(4), H(5) and H(3') or H(6')], 1.82 [m, 1H, H(3') or H(6')], 2.32 [m, H, H(1) or H(2)], 2.49 [m, 1H, H(1) or H(2)]. ^{13}C-NMR in D_2O: δ = 11.5 [*C*H$_3$], 17.9 [*C*H$_3$], 32.6 [C(4) or C(5)], 33.9 [C(4) or C(5)], 33.9 [C(3) or C(6)], 38.3 [C(3) or C(6)], 58.2 [C(1) or C(2)], 62.9 [C(1) or C(2)], 168.7 [*C*=O]. Anal. $C_{10}H_{18}N_2O_4Pt$ (C, H, N).

4.1.2.6. (SP-4-3)-(4,4-Dimethyl-trans-R,R/S,S-cyclohexane-1,2-diamine)oxalatoplatinum(II), ***6****.* The synthetic procedure is the same as that for **1**; Yield 60%^{1}H-NMR in D_2O: δ = 0.94 [s, 3H, C*H*$_3$], 1.00 [s, 3H, C*H*$_3$], 1.20 – 1.34 [m, 3H, H(3), H(5), H(5')], 1.57 [m, 1H, H(6)], 1.75 [m, 1H, H(3')], 1.90 [m, 1H, H(6')], 2.29 [m, 1H, H(2)], 2.56 [m, 1H, H(1)], 5.84 [m, 2H, N*H*], 5.92 [m, 2H, N*H*]. ^{13}C-NMR in D_2O: δ = 24.0 [*C*H$_3$], 27.8 [C(6)], 31.0 [*C*H$_3$], 31.9 [C(4)], 37.3 [C(5)], 44.5 [(C3)], 59.7 [C(1)], 63.2 [C(2)], 168.4 [*C*=O]. Anal. $C_{10}H_{18}N_2O_4Pt$ (C, H, N).

4.2. Physical measurements

^{1}H, ^{13}C{^{1}H}, ^{1}H,^{1}H-COSY, and ^{13}C,^{1}H,-COSY spectra were recorded in D_2O or DMF-d_7 at 298 K (2D in a gradient enhanced mode) using a Bruker Avance DPX 400 instrument (UltraShield™ Magnet) and standard pulse programs at 400.13 (^{1}H) and 100.62 MHz (^{13}C). Chemical shifts were measured relative to the solvent peak. Elemental analyses were performed by the microanalytical laboratory at the University of Vienna.

4.3. Cytotoxicity tests in cancer cell lines

SW480, SW620, HCT-15, COLO 205, HT-29 (all colon carcinoma), NIH-OVCAR-3 (ovarian carcinoma), MOLT-4, HL-60 (both leukemia) and SK-MEL-5 (melanoma) cells were obtained from the American Type Culture Collection (ATCC) and propagated in cell culture medium, i.e. RPMI 1640, except for SW480 and SW620: Iscove's Modified Dulbecco's Medium (IMDM), HT-29 and SK-MEL-5: Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10% heat-inactivated fetal calf serum (FCS) in every case.

Cells were harvested by trypsinization (except for the non-adherent lines MOLT-4 and HL-60 cells), seeded in 100 μl of cell culture medium in defined densities (ranging from 7×10^3 to 2×10^4 living cells per well, depending on the cell line) into 96-well tissue culture plates and incubated at 37 °C in a humidified atmosphere containing 5% CO_2 for 24 hours. Stock solutions of the test substances in water were sterilized by filtration (0.2 μm) and serially diluted (1:2) in cell culture medium. 100 μl of each dilution were added to the cells in quadruples. For a negative control, 100 μl of cell culture medium were added to four wells (100% value). For a positive control, all cells were deadened with sodium selenite (0% value). After drug exposure for 48 hours at 37 °C and 5% CO_2, cells were incubated with resazurin (Sigma-Aldrich) (100 μM in PBS, added in aliquots of 50 μl per well) for further 4 hours at 37 °C and 5% CO_2. Resazurin is metabolized by living cells from its oxidized form (blue) to a fluorescent

intermediate (red). The development of the fluorescent intermediate was quantified in a fluorescence microplate reader (Genios, Tecan) at 590 nm using an excitation of 560 nm. The raw data were normalized to the positive control of deadened cells and set into relation to the metabolic activity of the untreated control cells. IC_{50} values were calculated by four parametric nonlinear regression using Graph Pad Prism 3.0 software.

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