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Original article

Synthesis, crystal structure and cytotoxicity of new oxaliplatin analogues indicating that improvement of anticancer activity is still possible

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Dedicated to Prof. Bernt Krebs on the occasion of his 65th birthday

Abstract

Oxaliplatin, (*trans-R,R*-cyclohexane-1,2-diamine)oxalatoplatinum(II), has recently been approved for combination chemotherapy of metastatic colorectal cancer. Oxaliplatin is significantly more active than its *trans-S,S* isomer and the mixture of both enantiomers. New oxaliplatin analogues, (*SP*-4-3)-(4-methyl-*trans*-cyclohexane-1,2-diamine)oxalatoplatinum(II) and (*SP*-4-3)-(4-ethyl-*trans*-cyclohexane-1,2-diamine)oxalatoplatinum(II), have been synthesized, and their cytotoxicity has been tested in comparison to oxaliplatin, its corresponding *trans-S,S* isomer, and the mixture of both enantiomers. In comparison to oxaliplatin, even the *trans-R,R/trans-S,S* mixture of the 4-methyl and 4-ethyl substituted oxaliplatin analogues have shown an equivalent cytotoxicity in ovarian cancer cells (CH1) and superior antiproliferative properties in colon cancer cells (SW480) in the case of a predominantly equatorial position of the substituent at position 4 of the *trans*-cyclohexane-1,2-diamine ligand, whereas an axial substitution results in decreased cytotoxic potency.
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Keywords: Platinum; Anticancer complexes; Oxaliplatin; Synthesis; Crystal structure; Cytotoxic activity

1. Introduction

Platinum-based coordination complexes had first been identified as strong inhibitors of bacterial proliferation by Barnett Rosenberg in the 1960s [1]. Subsequently, their cytostatic activity had been examined in tumor-bearing animals with the encouraging result of a complete inhibition of murine solid sarcoma-180 by cisplatin [2]. Today, cisplatin, *cis*-diamminedichloroplatinum(II), and carboplatin, *cis*-diammine(1,1-cyclobutanedicarboxylato)platinum(II), are routinely used in the clinic as anticancer compounds [3–7]. Apart from the worldwide success of cisplatin and carboplatin, oxaliplatin, (*trans-R,R*-cyclohexane-1,2-diamine)oxalatoplatinum(II), a third generation platinum drug, has been approved for combination chemotherapy of metastatic colorectal cancer in many countries of Asia, Latin America and Europe (Fig. 1).

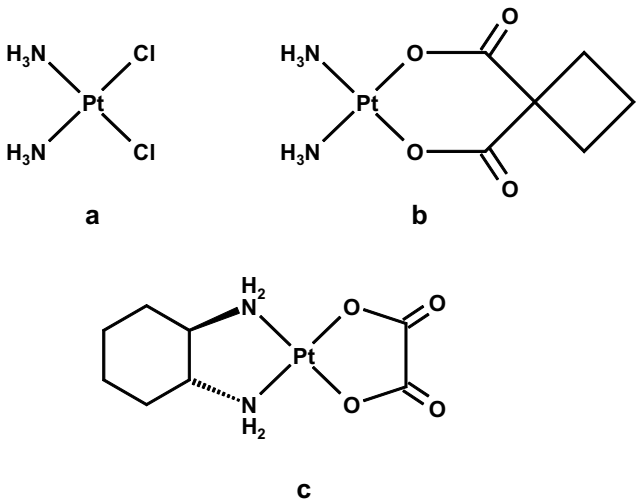

Fig. 1. Platinum(II) complexes in clinical use, cisplatin (a), carboplatin (b) and oxaliplatin (c).

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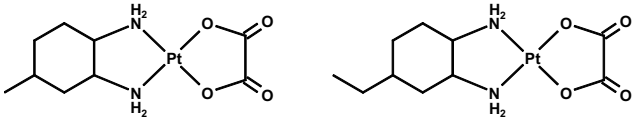
In the United States, oxaliplatin, Eloxatin™ (Sanofi), was approved in 2002, because of its significant benefit in terms of response rate and time to progression, although a long-term benefit such as increased survival time has not yet been demonstrated.[1]

Oxaliplatin, developed by Kidani et al. [8], first attracted interest because of its particularly high activity, even in cisplatin-resistant tumor models. Therefore, it is currently being explored for its potential as a treatment option after failure of cisplatin or carboplatin therapy. However, oxaliplatin is primarily used in colorectal cancer in combination with 5-fluorouracil and leucovorin (5-FU/LV). It shows synergistic effects with 5-FU even in 5-FU-resistant tumors and demonstrates excellent safety with a specific and manageable set of toxicities [9].

The similar or even higher cytotoxicity of oxaliplatin despite lower levels of initial DNA adduct formation and similar magnitudes of adduct removal as compared to cisplatin indicates that the DNA lesions produced by oxaliplatin are on average more lethal to the cell. This correlates with a stronger inhibition of DNA chain elongation in the course of replication [10]. Furthermore, adducts induced by oxaliplatin are not recognized by the damage recognition complex involved in the mismatch repair system, and cells with cisplatin-resistance due to enhanced replicative bypass (accompanied or not by a loss of the futile and eventually fatal mismatch repair activity) consistently retain their sensitivity to oxaliplatin [11].

Oxaliplatin produces adducts on DNA with a similar ratio of GG over AG intrastrand cross-links, similar sequence specificity and similarly low levels of interstrand cross-link formation compared to cisplatin, but with substantially slower kinetics. The effects on DNA structure after coordination are very similar to those of cisplatin with one striking difference, the presence of the cyclohexane ring. Using molecular modeling, it has been proposed that in the region of adduct formation an altered and less polar major groove is being formed by the methylene units of the cyclohexane-1,2-diamine ligand [12], which is in accordance with a different recognition of cisplatin and oxaliplatin DNA adducts by the mismatch repair system [13]. The X-ray crystal structure of the cytotoxic (*trans-R,R*-cyclohexane-1,2-diamine)platinum(II) moiety with a DNA dodecanucleotide duplex [14] was previously reported. Overall geometry and crystal packing were found to be similar to those of the analogous cisplatin structure [15], with formation of a 1,2-intrastrand crosslink, but a novel feature of the oxaliplatin adduct has been found: a hydrogen bond between the pseudoequatorial NH of the *trans-R,R*-cyclohexane-1,2-diamine ligand and the O6 atom of the 3′-G of the platinated d(GpG) lesion, demonstrating the importance of chirality which influences the interaction between oxaliplatin and DNA.

[1] In September 2003, the Food and Drug Administration (FDA) has granted a six-month priority review to eloxatin, for the first line treatment of metastatic colorectal cancer (MCRC).



rac-1(eq/ax) **rac-2(eq/ax)**

Fig. 2. Structure of 4-methyl and 4-ethyl substituted (*trans*-cyclohexane-1,2-diamine)oxalatoplatinum(II) complexes, **rac-1(eq/ax)** and **rac-2(eq/ax)**.

Based on the assumption that the steric demand and/or the hydrophobicity of the cyclohexane ring are structural requirements for the specific pharmacological properties of oxaliplatin, we assumed that derivation of the cyclohexane ring might result in a marked effect on antitumor activity. Following this concept and in order to explore structure–activity relationships, we have synthesized new oxaliplatin analogues, (*SP*-4-3)-(4-methyl-*trans*-cyclohexane-1,2-diamine)oxalatoplatinum(II) and (*SP*-4-3)-(4-ethyl-*trans*-cyclohexane-1,2-diamine)oxalatoplatinum(II) (Fig. 2), and tested their in vitro antitumor activity in comparison to oxaliplatin, its corresponding *trans-S,S*-isomer, (*SP*-4-2)-(*trans-S,S*-cyclohexane-1,2-diamine)oxalatoplatinum(II), and the mixture of both enantiomers, (*SP*-4-2)-(*trans*-cyclohexane-1,2-diamine)oxalatoplatinum(II).

2. Results and discussion

2.1. Syntheses and NMR spectroscopy

The methyl and ethyl substituted *trans*-cyclohexane-1,2-diamine ligands have been synthesized via two synthetic pathways: Method A starts from 4-methyl- and 4-ethylcyclohexanone. After bromination, the 2-bromo-4-alkylcyclohexanones were reacted with hydroxylamine. The vicinale dioximes were then converted into the corresponding *trans*-diamine derivatives by reduction with sodium in ethanol and isolated as the dihydrogensulfates. Method B: Starting from 4-methyl- or 4-ethyl-1-cyclohexene the corresponding *trans*-cyclohexane-1,2-diazides were synthesized by reaction with sodium azide in the presence of $Mn(OAc)_3 \cdot 2H_2O$. Reduction with hydrogen (3.5 bar, 5% $Pd/CaCO_3$) resulted in the *trans*-diamine derivatives, which were also isolated as the dihydrogensulfates. In both cases, the *trans*-cyclohexane-1,2-diamine derivatives are obtained as (1*R*,2*R*/1*S*,2*S*, 1:1) mixtures.

Characterization of the ligands was performed by ^{1}H and ^{13}C NMR spectroscopy as well as elemental analysis. Two sets of resonances were found due to the stereochemistry of the methyl group in *trans*-cyclohexane-1,2-diamine dihydrogensulfates at position 4 (Fig. 3). Synthesis via Method A resulted in 4-methyl-cyclohexane-1,2-diamine with the methyl group being mainly in the equatorial position. As a consequence, the ^{13}C–N resonances C(1) and C(2) (52.4 and 52.5 ppm) were found to be very similar. However, in the case of an axial methyl group (minor isomer), the chemical

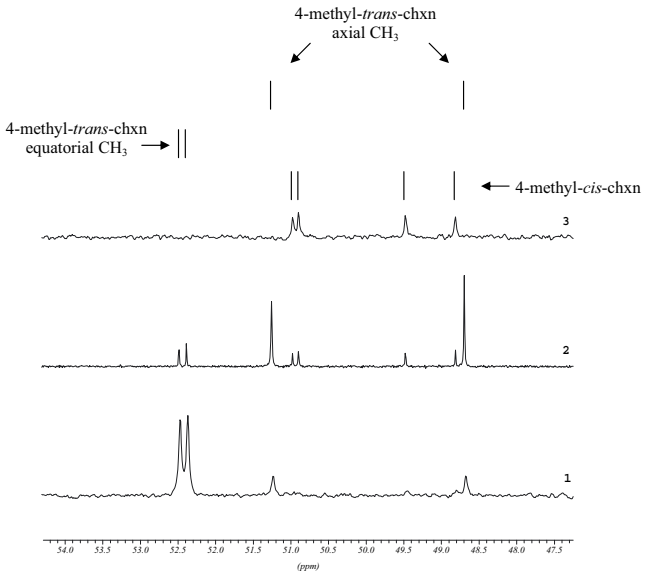


Fig. 3. ^{13}C NMR spectra of 4-methyl *cis*- and *trans*-cyclohexane-1,2-diamine (chxn) dihydrogensulfates, region of the ^{13}CHN carbons is shown. Spectrum 1, 4-methyl-*trans*-chxn·H_2SO_4 obtained via Method A; spectrum 2, 4-methyl-*trans*-chxn·H_2SO_4 obtained via Method B and addition of 4-methyl-*cis*-chxn·H_2SO_4; spectrum 3, 4-methyl-*cis*-chxn·H_2SO_4.

shifts for C(1) and C(2) (48.7 and 51.2 ppm) show a marked splitting.

Following Method B, the relation between the equatorial and axial position of the 4-methyl group is inverted. In order to undoubtedly exclude the presence of a mixture of the *trans*- and *cis*-cyclohexane-1,2-diamine derivatives, the analogous 4-methyl-*cis*-cyclohexane-1,2-diamine dihydrogensulfate has also been synthesized starting from 4-methyl-1,2-cyclohexanedicarboxylic anhydride via hydrolysis and reaction with sodium azide in the presence of H_2SO_4. The resonances of C(1) and C(2) in the pure *cis*-cyclohexane-1,2-diamine derivative and in a mixture with the corresponding *trans*-isomer from Method B were found to be markedly different, proving our hypothesis (synthesis of pure *trans* isomers via Methods A and B).

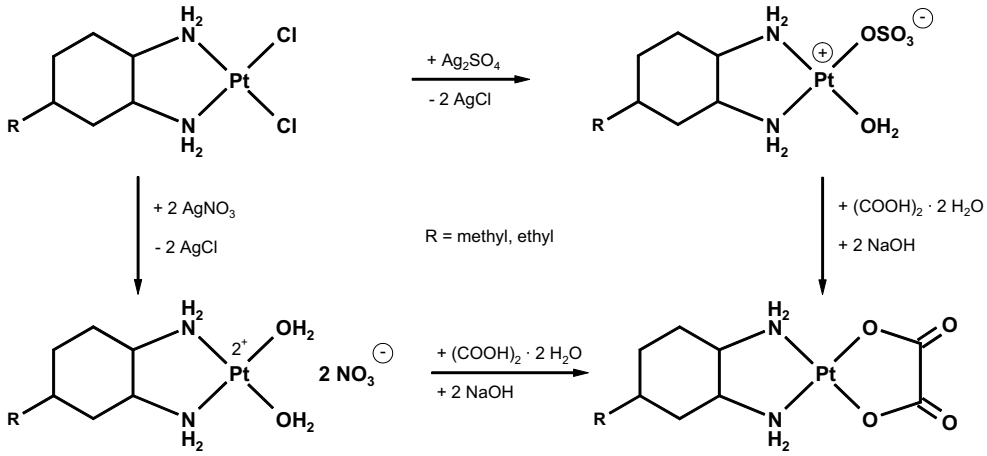
In order to simplify complex spin systems in proton NMR spectra and to get further stereochemical information at the carbon atoms 1, 2 and 4, homonuclear decoupling experiments have been performed. Residual multiplets for protons H(1) and H(2) with two vicinal coupling constants of 11 Hz could be found when the equatorial protons H(3) and H(6) are irradiated. These coupling constants are in accordance with a coupling of two neighboring protons both being in an axial position and can only be found in *trans*-configured cyclohexane-1,2-diamine derivatives (Karplus curve [16]: 180°, 10–16 Hz, typical values for $^3J_{ax/ax}$ in cyclohexane-1,2-diamine derivatives: 7–12 Hz). Irradiation in the methyl resonance H(7) resulted in a broad triplet with two vicinal coupling constants of about 11 Hz, also proving the axial position of H(4) being equivalent with an equatorial methyl substituent at C(4).

The 4-methyl and 4-ethyl substituted (*SP*-4-3)-dichloro(*trans*-cyclohexane-1,2-diamine)platinum(II) complexes were synthesized by direct reaction of the diaminedihydrogen sulfates with potassium tetrachloroplatinate(II), K_2PtCl_4, in the presence of NaOH and obtained as yellow powders with yields of 60–95%. The diaminedichloroplatinum(II) complexes were activated using Ag_2SO_4 or $AgNO_3$ and were brought to reaction with sodium oxalate, which was formed in situ by addition of two equivalents of NaOH to oxalic acid (Scheme 1). The final products precipitated as white solids (yields: 20–50%) and were characterized by NMR spectroscopy and elemental analysis.

Dependent on the synthetic route, the title compounds were obtained as (1*R*,2*R*/1*S*,2*S*) mixtures **rac-1**(**eq**/ax) and **rac-2**(**eq**/ax) with a methyl or ethyl substituent at position 4 being mainly in an equatorial position (ligand synthesis via Method A) whereas **rac-1**(eq/**ax**) and **rac-2**(eq/**ax**) were isolated as (1*R*,2*R*/1*S*,2*S*) mixtures with an excess of an axial substitution at position 4 (ligand synthesis via Method B).

2.2. Molecular structure of *rac-1(ax)*

Crystals suitable for X-ray structure determination were obtained by slow evaporation of an aqueous solution of **rac-1**(eq/**ax**). The structure of **rac-1**(**ax**), a 1:1 mixture of (*SP*-4-3)-(4*R*-methyl-*trans*-1*S*,2*S*-cyclohexane-1,2-diamine)



Scheme 1. Synthesis of the 4-methyl and 4-ethyl substituted (*trans*-cyclohexane-1,2-diamine)oxalatoplatinum(II) complexes.

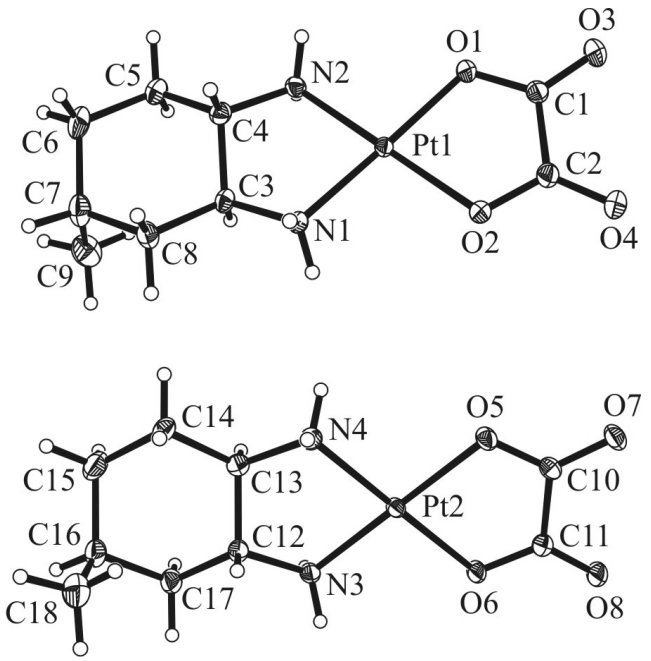


Fig. 4. Structures of the two independent molecules (*SP*-4-3)-(4*R*-methyl-*trans*-1*S*,2*S*-cyclohexane-1,2-diamine)oxalatoplatinum(II) (top) and (*SP*-4-3)-(4*S*-methyl-*trans*-1*R*,2*R*-cyclohexane-1,2-diamine)oxalatoplatinum(II) (bottom) in the crystal in **rac-1**(**ax**). The displacement ellipsoids are drawn at 50% probability level.

oxalatoplatinum(II) and (*SP*-4-3)-(4*S*-methyl-*trans*-1*R*,2*R*-cyclohexane-1,2-diamine)oxalatoplatinum(II), is shown in Fig. 4.

The platinum(II) atoms in both molecules have a square-planar coordination geometry. The 4-methyl-*trans*-cyclohexane-1,2-diamine ligands act as neutral didentate ligands and coordinate to Pt^{II} through the nitrogen atoms [Pt1–N1 2.019(3), Pt1–N2 2.016(2), Pt2–N3 2.030(3), Pt2–N4 2.026(3) Å].

These bond lengths are not significantly shorter compared to Pt–N in oxaliplatin, (*SP*-4-2)-(*trans*-1*R*,2*R*-cyclohexane-1,2-diamine)oxalatoplatinum(II) [Pt–N1 2.06(2), Pt–N2 2.04(2) Å] [17]. The doubly negatively charged oxalate ligands are bound to the platinum(II) ions via oxygen atoms [Pt1–O1 2.027(2), Pt1–O2 2.025(2), Pt2–O5 2.033(2), Pt2–O6 2.022(2) Å]. These bond lengths are comparable with those in (*SP*-4-2)-(*trans*-1*R*,2*R*-cyclohexane-1,2-diamine)oxalatoplatinum(II). The cyclohexane rings in **rac-1**(**ax**) adopt a chair conformation with the two amino groups in equatorial positions. The bond distances and angles are statistically identical between the two molecules within 3σ (Table 1).

2.3. Cytotoxic activity

The concentration–effect curves obtained from MTT assays of the oxalatoplatinum(II) complexes **rac-1**(**eq**/ax) and **rac-2**(**eq**/ax) in comparison with oxaliplatin, its *trans-S,S* enantiomer, and the racemic mixture of oxaliplatin and its *trans-S,S* enantiomer in colon (SW480) and ovarian (CH1) cancer cells after exposure for 96 h are shown in Fig. 5.

Table 1
Selected bond lengths (Å) and angles (°) for (*SP*-4-3)-(4-methyl-*trans*-cyclohexane-1,2-diamine)oxalatoplatinum(II), **rac-1**(**ax**)

Atom 1–Atom 2	Molecule I	Atom 1–Atom 2	Molecule II
N1–C3	1.498(4)	N3–C12	1.509(4)
C3–C4	1.513(4)	C12–C13	1.517(4)
C4–N2	1.488(4)	C13–N4	1.500(4)
C4–C5	1.515(4)	C13–C14	1.520(4)
C5–C6	1.528(5)	C14–C15	1.531(5)
C6–C7	1.536(5)	C15–C16	1.533(5)
C7–C8	1.545(5)	C16–C17	1.548(5)
C7–C9	1.529(5)	C16–C18	1.555(6)
C8–C3	1.530(4)	C17–C12	1.524(4)
O1–C1	1.296(4)	O5–C10	1.288(4)
C1–C2	1.549(4)	C10–C11	1.548(4)
C1–O3	1.223(4)	C10–O7	1.234(4)
C2–O2	1.305(4)	C11–O6	1.291(4)
C2–O4	1.214(4)	C11–O8	1.226(4)
Atom 1–Atom 2–Atom 3	Molecule I	Atom 1–Atom 2–Atom 3	Molecule II
N1–Pt1–N2	83.64(10)	N3–Pt2–N4	83.47(10)
O1–Pt1–O2	82.16(9)	O5–Pt2–O6	82.27(9)

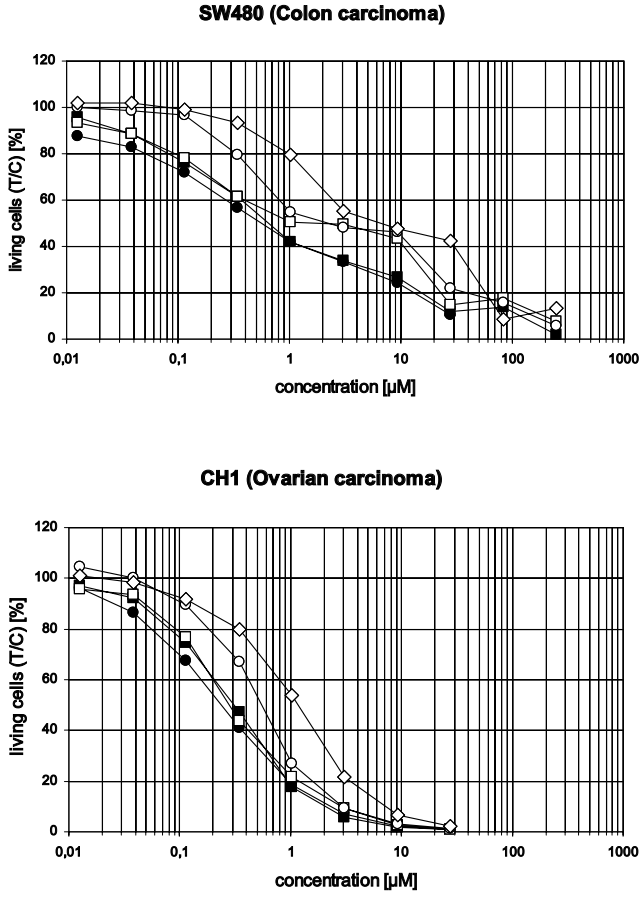


Fig. 5. Concentration–effect curves of the methyl (■) and ethyl (●) substituted oxaliplatin analogues, **rac-1**(**eq**/ax) and **rac-2**(**eq**/ax), compared with oxaliplatin (□), its *trans-S,S* enantiomer (◇), and the racemic mixture of its *trans-R,R-* and *trans-S,S* enantiomer (○), in SW480 and CH1 cells after exposure for 96 h.

Compared to the enantiomerically pure oxaliplatin, the derivatives **rac-1**(**eq**/ax) and **rac-2**(**eq**/ax), prepared as *trans-R,R/trans-S,S* mixtures with a substituent at C(4) being mainly in an equatorial position, show slightly higher cytotoxic potency in SW480 cells and equivalent potency in CH1 cells. This is remarkable, and it should be pointed out that a direct comparison with the racemic mixture of oxaliplatin reveals that the new derivatives display significantly superior activity in both cell lines. Since *trans-R,R*-cyclohexane-1,2-diamine-containing platinum complexes are usually more active than their *trans-R,R/trans-S,S* isomeric mixtures [18,19], it is expected that the antitumor activity should be even more markedly improved by use of the pure *trans-R,R* isomers.

The cytotoxic properties of the analogous oxalatoplatinum(II) complexes **rac-1**(eq/**ax**) and **rac-2**(eq/**ax**), synthesized as *trans-R,R/trans-S,S* mixtures with predominantly axial substitution at position 4, have also been investigated in colon (SW480) and ovarian (CH1) cancer cells. Interestingly, the activity was markedly different in comparison to **rac-1**(**eq**/ax) and **rac-2**(**eq**/ax) and was comparable to the *trans-R,R/trans-S,S*-isomeric mixture of oxaliplatin in the case of complex **rac-1**(eq/**ax**) and comparable to the cytotoxicity of the *trans-S,S* derivative of oxaliplatin in case of compound **rac-2**(eq/**ax**) (data not shown).

The cyclohexane ring is an essential structural requirement for the high cytotoxicity of oxaliplatin and probably also for its different pharmacodynamic properties in comparison to the diammine complexes cisplatin and carboplatin. It seems reasonable to assume that the steric demand of the cyclohexane ring is either directly or indirectly responsible for the stronger replication-inhibiting properties of oxaliplatin. The assumption, that increasing this steric demand is a promising strategy to improve activity, is supported by our findings with derivatives carrying small substituents on position 4 at the cyclohexane-1,2-diamine ligand. However, it could also be demonstrated, that this assumption can only be confirmed by the use of oxaliplatin-derivatives with equatorial substitution at position 4, whereas axial substitution at this position decreases the cytotoxic properties.

In preliminary in vivo experiments in mice bearing L1210 leukemia, even **rac-1**(eq/**ax**) and **rac-2**(eq/**ax**) were found to be more tolerable and displayed a higher anticancer activity in comparison to oxaliplatin at analogous dosage.

3. Conclusions

Even the *trans-R,R/trans-S,S* mixtures of the methyl and ethyl substituted oxaliplatin analogues **rac-1**(**eq**/ax) and **rac-2**(**eq**/ax) with substituents at C(4) being mainly in the equatorial position have demonstrated a superior in vitro antitumor activity in colon cancer cells (SW480) and equivalent potency in CH1 cells in comparison to oxaliplatin. On the other hand, the cytotoxic properties are decreased in the case of complexes **rac-1**(eq/**ax**) and **rac-2**(eq/**ax**) with substituents at C(4) predominantly being in an axial position, indicating a marked dependency of cytotoxicity on the stereochemistry at position 4 of the cyclohexane-1,2-diamine ligand.

At present, we focus on the synthesis of (i) *trans*-cyclohexane-1,2-diamine derivatives bearing exclusively equatorial substituents at position 4 and (ii) on the synthesis of the *trans-R,R* isomers. A marked and positive effect on antitumor activity is expected. Moreover, since oxaliplatin and other cyclohexane-1,2-diamine-containing platinum complexes exhibit a lack of cross-resistance in certain cisplatin-resistant tumors, the new derivatives might not only be more cytotoxic, but also may be endowed with an improved capacity of overcoming this resistance.

4. Experimental

4.1. Syntheses

The new compounds reported in this study are illustrated in Fig. 2. Potassium tetrachloroplatinate 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 methyl substituted *cis*- [20,21] and the methyl and ethyl substituted *trans*-cyclohexane-1,2-diamine ligands (Method A [22,23] and B [24,25]), the corresponding (*SP*-4-3)-dichloro(*trans*-cyclohexane-1,2-diamine)platinum(II) complexes, oxaliplatin, (*SP*-4-2)-(*trans-S,S*-cyclohexane-1,2-diamine)oxalatoplatinum(II) and (*SP*-4-2)-(*trans*-cyclohexane-1,2-diamine) oxalatoplatinum(II) [26] have been synthesized according to standard literature methods. Analyses indicated by the symbols of the elements or functions were within ±0.4%.

4.1.1. Synthesis of trans-cyclohexane-1,2-diamine dihydrogensulfates

4.1.1.1. Method A.

4.1.1.1.1. 4-Methyl-trans-*cyclohexane-1,2-diamine dihydrogensulfate.* ^{1}H NMR in D_2O: δ = 0.85 [d, 3H, H(7), $^3J_{H,H}$ = 6.5 Hz], 0.99 [m, 1H, H(5)], 1.15 [m, 1H, H(3)], 1.42–1.55 [m, 2H, H(4), H(6)], 1.66–1.77 [m, 1H, H(5)], 1.99–2.10 [m, 2H, H(6), H(3)], 3.23–3.39 [m, 2H, H(1), H(2)]; minor isomer: δ = 0.90 [d, 3H, H(7′), $^3J_{H,H}$ = 7.0 Hz]. ^{13}C NMR in D_2O: δ = 20.7 [C(7)], 29.5 [C(6)], 30.2 [C(4)], 31.5 [C(5)], 37.6 [C(3)], 52.4 [C(1) or C(2)], 52.5 [C(1) or C(2)]; minor isomer: δ = 17.9 [C(7′)], 24.0 [C(6′)], 25.8 [C(4′)], 27.8 [C(5′)], 33.8 [C(3′)], 48.7 [C(1′) or C(2′)], 51.2 [C(1′) or C(2′)]. Anal. $C_7H_{16}N_2 \cdot H_2SO_4$ (C, H, N).

4.1.1.1.2. 4-Ethyl-trans-*cyclohexane-1,2-diamine dihydrogensulfate.* ^{1}H NMR in D_2O: δ = 0.77 [t, 3H, H(8), $^3J_{H,H}$ = 7.3 Hz], 0.96 [m, 1H, H(5)], 1.05–1.20 [m, 1H, H(3)], 1.11–1.28 [m, 2H, H(7)], 1.21–1.38 [m, 1H, H(4)], 1.38–

1.56 [m, 1H, H(6)], 1.72–1.84 [m, 1H, H(5)], 2.04–2.13 [m, 2H, H(6), H(3)], 3.23–3.43 [m, 2H, H(1), H(2)]; minor isomer: δ = 0.79 [t, 3H, H(8′), $^3J_{H,H}$ = 6.8 Hz]. ^{13}C NMR in D_2O: δ = 10.8 [C(8)], 28.3 [C(7)], 29.1 [C(5)], 29.5 [C(6)], 35.6 [C(3)], 36.8 [C(4)], 52.5 [C(1) or (2)], 52.7 [C(1) or C(2)]; minor isomer: δ = 11.4 [C(8′)], 24.2 [C(7′)], 24.8 [C(5′)], 25.6 [C(6′)], 31.8 [C(3′)], 32.8 [C(4′)], 48.8 [C(1′) or C(2′)], 51.3 [C(1′) or C(2′)]. Anal. $C_8H_{20}N_2{\cdot}H_2SO_4$ (C, H, N).

4.1.1.2. Method B.

4.1.1.2.1. 4-Methyl-trans-*cyclohexane-1,2-diamine dihydrogensulfate.* ^{1}H NMR in D_2O: δ = 0.88 [d, 3H, H(7), $^3J_{H,H}$ = 7.0 Hz], 1.32–1.44 [m, 1H, H(5)], 1.56 [m, 1H, H(5)], 1.62–1.82 [m, 3H, H(3), H(6)], 1.82–1.98 [m, 2H, H(6), H(4)], 3.37 [m, 1H, H(1)], 3.54 [m, 1H, H(2)]; minor isomer: δ = 0.86 [d, 3H, H(7′), $^3J_{H,H}$ = 6.7 Hz]. ^{13}C NMR in D_2O: δ = 18.0 [C(7)], 24.0 [C(6)], 25.8 [C(4)], 27.8 [C(5)], 33.8 [C(3)], 48.7 [C(1) or C(2)], 51.2 [C(1) or C(2)]; minor isomer: δ = 20.7 [C(7′)], 29.5 [C(6′)], 30.2 [C(4′)], 31.5 [C(5′)], 37.7 [C(3′)], 52.4 [C(1′) or C(2′)], 52.5 [C(1′) or C(2′)]. Anal. $C_7H_{16}N_2{\cdot}H_2SO_4$ (C, H, N).

4.1.1.2.2. 4-Ethyl-trans-*cyclohexane-1,2-diamine dihydrogensulfate.* ^{1}H NMR in D_2O: δ = 0.80 [t, 3H, H(8), $^3J_{H,H}$ = 7.5 Hz], 1.20–1.38 [m, 2H, H(7)], 1.44–1.77 [m, 5H, H(5), H(6), H(3)], 1.80–1.96 [m, 2H, H(6), H(4)], 3.41 [m, 1H, H(1) or H(2)], 3.53 [m, 1H, H(1) or H(2)]; minor isomer: δ = 0.78 [t, 3H, H(8′), $^3J_{H,H}$ = 7.5 Hz]. ^{13}C NMR in D_2O: δ = 11.3 [C(8)], 24.2 [C(7)], 24.9 [C(5)], 25.6 [C(6)], 31.8 [C(3)], 32.8 [C(4)], 48.8 [C(1) or C(2)], 51.3 [C(1) or C(2)]; minor isomer: δ = 10.8 [C(8′)], 28.3 [C(7′)], 29.1 [C(5′)], 29.5 [C(6′)], 35.6 [C(3′)], 36.8 [C(4′)], 52.5 [C(1′) or (2′)], 52.7 [C(1′) or C(2′)]. Anal. $C_8H_{20}N_2{\cdot}H_2SO_4$ (C, H, N).

4.1.2. 4-Methyl-cis-*cyclohexane-1,2-diamine dihydrogensulfate*

^{1}H NMR in D_2O: δ = 0.85 [d, 3H, C*H*$_3$, $^3J_{H,H}$ = 6.5 Hz], 0.90 [d, 3H, C*H*$_3$,$^3J_{H,H}$= 6.0 Hz], 0.97–1.18 [m, 2H, C*H*$_2$], 1.28–1.52 [m, 2H, C*H*$_2$], 1.52–2.08 [m, 10H, C*H*$_2$ and C*H*], 3.47–3.64 [m, 2H, C*H*N], 3.75–3.91 [m, 2H, C*H*N].

^{13}C NMR in D_2O: δ = 20.6 [*C*H$_3$], 21.0 [*C*H$_3$], 24.0 [*C*H$_2$], 24.6 [*C*HCH$_3$], 26.1 [*C*H$_2$], 27.9 [*C*H$_2$], 30.8 [*C*HCH$_3$], 31.4 [*C*H$_2$], 31.9 [*C*H$_2$], 35.8 [*C*H$_2$], 48.8 [*C*HN], 49.5 [*C*HN], 50.9 [*C*HN], 51.0 [*C*HN]. Anal. $C_7H_{16}N_2{\cdot}H_2SO_4$ (C, H, N).

4.1.3. Synthesis of dichloroplatinum complexes

*4.1.3.1. (*SP-*4-3)-Dichloro(4-methyl*-trans-*cyclohexane-1,2-diamine)platinum(II).* To a solution of K_2PtCl_4 (3.944 g, 9.5 mmol) in 50 ml of water, 4-methyl-*trans*-cyclohexane-1,2-diamine dihydrogensulfate (2.15 g, 9.50 mmol, ligand from Method A) 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 3.0 g of [Pt(4-methyl-*trans*-chxn)Cl_2]; yield 80%. Anal. $C_7H_{16}Cl_2N_2Pt$ (C, H, N).

*4.1.3.2. (*SP-*4-3)-Dichloro(4-ethyl*-trans-*cyclohexane-1,2-diamine)platinum(II).* Ligand from Method A was used; yield 95%. Anal. $C_8H_{18}Cl_2N_2Pt$ (C, H, N).

*4.1.3.3. (*SP-*4-3)-Dichloro(4-methyl*-trans-*cyclohexane-1,2-diamine)platinum(II).* Ligand from Method B was used; yield 60%. Anal. $C_7H_{16}Cl_2N_2Pt$ (C, H, N).

*4.1.3.4. (*SP-*4-3)-Dichloro(4-ethyl*-trans-*cyclohexane-1,2-diamine)platinum(II).* Ligand from Method B was used; yield 66%. Anal. $C_8H_{18}Cl_2N_2Pt$ (C, H, N).

4.1.4. Synthesis of oxalatoplatinum complexes

*4.1.4.1. (*SP-*4-3)-(4-Methyl*-trans-*cyclohexane-1,2-diamine)oxalatoplatinum(II),* ***rac-1****(****eq****/ax).* (*SP*-4-3)-Dichloro(4-methyl-*trans*-cyclohexane-1,2-diamine)platinum(II) (803.6 mg, 2.039 mmol, from 4.1.3.1.) was suspended in 30 ml of water and Ag_2SO_4 (643.3 mg, 2.00 mmol) was added in one portion. The mixture was stirred for a period of 2 days at room temperature. Silver chloride precipitated, was filtered off, and the remaining solution was evaporated to dryness. Oxalic acid dihydrate (193 mg, 1.53 mmol), 10 ml of water and NaOH (3 ml 0.5 M, 1.5 mmol) were added to aqua(4-methyl-*trans*-cyclohexane-1,2-diamine)sulfatoplatinum(II) (669 mg, 1.53 mmol) and stirred over night. A white precipitate formed, which was filtered off and dried under reduced pressure over P_2O_5 to obtain 310 mg of [Pt(4-methyl-*trans*-chxn)(ox)] as a white solid; yield 50%.^{1}H NMR in H_2O/D_2O (9/1): δ = 0.75–0.87 [m, 1H, H(5)], 0.83 [d, 3H, H(7), $^3J_{H,H}$ = 6.6 Hz], 0.88–0.99 [m, 1H, H(3)], 1.19–1.40 [m, 2H, H(4), H(6)], 1.41–1.51 [m, 1H, H(5)], 1.86–1.97 [m, 2H, H(6), H(3)], 2.18–2.41 [m, 2H, H(1), H(2)], 5.03 N*H*$_2$, 5.73 N*H*$_2$. ^{13}C NMR in H_2O/D_2O (9/1): δ = 20.4 [C(7)], 31.0 [C(6)], 31.3 [C(4)], 32.6 [C(5)], 39.9 [C(3)], 62.5–62.7 [2C, C(1), C(2)], 168.7 [2C, *C*=O]; minor isomer: δ = 17.1 [C(7′)], 26.9 [C(6′)], 27.4 [C(4′)], 29.7 [C(5′)], 39.8 [C(3′)]. Anal. $C_9H_{16}N_2O_4Pt$ (C, H, N).

*4.1.4.2. (*SP-*4-3)-(4-Ethyl*-trans-*cyclohexane-1,2-diamine) oxalatoplatinum(II),* ***rac-2****(****eq****/ax).* Starting with 805.2 mg (1.972 mmol, from 4.1.3.2.) of (*SP*-4-3)-dichloro(4-ethyl-*trans*-cyclohexane-1,2-diamine)platinum(II), 170 mg of [Pt(4-ethyl-*trans*-chxn)(ox)] were obtained as a white solid; yield 20%. ^{1}H NMR in H_2O/D_2O (9/1): δ = 0.69 – 0.85 [m, 1H, H(5)], 0.75 [t, 3H, H(8), $^3J_{H,H}$ = 7.1 Hz], 0.86–0.99 [m, 1H, H(3)], 1.07–1.31 [m, 4H, H(4), H(6), H(7)], 1.49–1.60 [m, 1H, H(5)], 1.89–2.03 [m, 2H, H(6), H(3)], 2.21–2.41 [m, 2H, H(1), H(2)], 5.75 N*H*$_2$, second N*H*$_2$ group under the water signal. ^{13}C NMR in H_2O/D_2O (9/1): δ = 11.1 [C(8)], 27.9 [C(7)], 30.1 [C(5)], 30.8 [C(6)], 37.5 [C(3)], 37.9 [C(4)], 62.5 [2C, C(1), C(2)], *C*=O could not be detected. Anal. $C_{10}H_{18}N_2O_4Pt$ (C, H, N).

*4.1.4.3. (*SP-*4-3)-(4-Methyl*-trans-*cyclohexane-1,2-diamine) oxalatoplatinum(II),* ***rac-1****(****eq/ax****).* (*SP*-4-3)-Dichloro(4-methyl-*trans*-cyclohexane-1,2-diamine)platinum(II) (1.81 g,

4.60 mmol, from 4.1.3.3.) was suspended in 70 ml of water and $AgNO_3$ (1.48 g, 8.74 mmol) was added in one portion. The mixture was stirred for 24 h at room temperature. Silver chloride precipitated, was filtered off, and the remaining solution was reduced by evaporation to 30 ml. Oxalic acid (550 mg, 4.37 mmol) dissolved in 8.7 ml of 1 N NaOH (8.7 mmol) was added to the diaqua(4-methyl-*trans*-cyclohexane-1,2-diamine)platinum(II) complex and stirred over night. A white precipitate formed which was filtered off and dried under reduced pressure over P_2O_5 to obtain 770 mg of [Pt(4-methyl-*trans*-chxn)(ox)] as a white solid; yield 43%. ^{1}H NMR in D_2O: δ = 0.83 [d, 3H, H(7), $^3J_{H,H}$ = 6.6 Hz], 1.22–1.54 [m, 4H, H(5), H(3), H(6)], 1.72–1.89 [m, 3H, H(6), H(3), H(4)], 2.23 [m, 1H, H(1) or H(2)], 2.49 [m, 1H, H(1) or H(2)]. ^{13}C NMR in D_2O: δ = 17.0 [C(7)], 26.8 [C(6)], 27.4 [C(4)], 29.7 [C(5)], 37.6 [C(3)], 58.2 [C(1) or C(2)], 63.1 [C(1) or C(2)], 168.7 [2C, *C*=O]; minor isomer: δ = 20.4 [C(7′)], 31.0 [C(6′)], 31.2 [C(4′)], 32.5 [C(5′)], 39.1 [C(3′)], 62.3 [C(1′) or C(2′) 62.5 [C(1′) or C(2′)]. Anal. $C_9H_{16}N_2O_4Pt$ (C, H, N).

*4.1.4.4. (*SP*-4-3)-(4-Ethyl-*trans*-cyclohexane-1,2-diamine) oxalatoplatinum(II),* ***rac-2****(eq/****ax****).* Starting with 1.48 g (3.61 mmol, from 4.1.3.4.) of (*SP*-4-3)-dichloro(4-ethyl-*trans*-cyclohexane-1,2-diamine)platinum(II), 650 mg of [Pt(4-ethyl-*trans*-chxn)(ox)] were obtained as a white solid; yield 45%. ^{1}H NMR in D_2O: δ = 0.94 [t, 3H, H(8), $^3J_{H,H}$ = 7.1 Hz], 1.17–2.11 [m, 9H, H(3), H(4), H(5), H(6), H(7)], 2.20–2.52 [m, 2H, H(1), H(2)], 5.31 NH_2, 6.02 NH_2. ^{13}C NMR in D_2O: δ = 11.6 [C(8)], 23.8 [(C7)], 30.7 [C(5)], 31.1 [C(6)], 33.3 [(C4)], 35.6 [C(3)], 58.6 [C(1) or C(2)], 63.4 [C(1) or C(2)], 168.7 [2C, *C*=O]; minor isomer: δ = 10.9 [C(8′)], 27.2 [(C7′)], 27.9 [C(5′)], 28.4 [(C6′)], 37.9 [(C3′)], 38.4 [(C4′)], 62.8 [C(1′) or C(2′)], 63.1 [C(1′) or C(2′)]. 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 H_2O/D_2O (9:1) or D_2O 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 or to external NH_4Cl. Elemental analyses were performed by the microanalytical laboratory at the University of Vienna.

4.3. Structure determination

X-ray diffraction measurements were performed on a Nonius Kappa CCD diffractometer with Mo K_α (λ = 0.71073 Å) radiation. The single crystal was positioned at 30 mm from the detector. The 341 frames were measured, each for 65 s over a 2° ω-scan. The data were processed using the Denzo-SMN software, no empirical absorption correction was applied. Crystal data, data collection parameters, and structure refinement details are given in Table 2. The structure was solved by direct methods and refined by full-matrix least-squares techniques. Non-hydrogen atoms were refined with anisotropic displacement parameters. All hydrogen atoms (except those of four water molecules per asymmetric unit, which were located on difference Fourier maps) were placed at calculated positions and refined as riding atoms in the subsequent least squares model refinement. Their isotropic thermal parameters were estimated to be 1.2 times the value of the equivalent isotropic thermal parameter of the atom to which hydrogen was bound. Computer programs: data reduction, DATAP [27]; structure solution, SHELXS-97 [28]; refinement SHELXL-97 [29], molecular diagrams, ORTEP [30], computer: PENTIUM II; scattering factors [31].

Table 2
Crystal data and details of data collection for (*SP*-4-3)-(4-methyl-*trans*-cyclohexane-1,2-diamine)oxalatoplatinum(II) dihydrate, **rac-1**(**ax**) (synthesis via Method B) [a]

Chemical formula	$C_9H_{16}PtN_2O_4{\cdot}2H_2O$
M (g mol^{-1})	447.36
Temperature (K)	120
Crystal size	0.29 × 0.23 × 0.20
Crystal color, habit	White, block
Crystal system	Monoclinic
Space group	$P2_1/c$
a (Å)	15.809 (3)
b (Å)	12.566 (3)
c (Å)	14.863 (3)
β (°)	102.57 (3)
V (Å^3)	2689.6 (9)
Z	8
D_c (g cm^{-3})	2.210
μ (cm^{-1})	104.55
F (0 0 0)	1712
θ range for data collection (°)	2.15–30.50
h range	–22/22
k range	–17/17
l range	–21/21
Total no. measured reflections	16 385
No. unique measured reflections	8195
No. reflections used in refinement	8195
No. parameters	360
R_{int}	0.024
R_1 (obs.)	0.0219
wR_2 (all data)	0.0537
S	1.208
Largest diff. peak and hole (eÅ^{-3})	1.560 and –1.579

[a] Refinement by full-matrix least-squares (F_o^2) for all reflections, $R_1 = \Sigma||F_o| - |F_c||/\Sigma w|F_o|$, $wR_2 = [\Sigma(F_o^2-F_c^2)^2/\Sigma wF_o^4]^{1/2}$, $w = 1/[(\sigma^2(F_o^2) + (0.0173P)^2 + 5.64P$, with $P = \{[F_o^2 + 2F_c^2)/3\}$. Goodness of fit, $S = \Sigma[(F_o^2-F_c^2)^2/(n-p)]^{1/2}$.

4.4. Cell lines and culture conditions

SW480 cells (adenocarcinoma of the colon) were obtained from the American Type Culture Collection (ATCC) and kindly provided by Brigitte Marian (Institute of Cancer Research, University of Vienna, Austria). The CH1 cell line

has been established from an ascites sample of a patient with a papillary cystadenocarcinoma of the ovary and was kindly provided by Lloyd R. Kelland (CRC Centre for Cancer Therapeutics, Institute of Cancer Research, Sutton, UK). Cells were grown as adherent monolayer cultures in Minimal Essential Medium (MEM) containing 10% heat-inactivated fetal bovine serum, 1 mM sodium pyruvate, 2 mM L-glutamine, 50 U/ml penicillin and 50 μg/ml streptomycin (all purchased from Gibco). Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO_2.

4.5. Cytotocicity 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-2H-tetrazolium bromide). SW480 and CH1 cells were harvested from culture flasks by trypsinization and seeded into 96-well microculture plates. Cell densities of 1.5×10^4 cells/ml (3×10^3 cells/well) and 1.25×10^4 cells/ml (2.5×10^3 cells/well) were chosen for SW480 and CH1 cells, respectively, in order to ensure exponential growth throughout drug exposure. After a 24 h preincubation, cells were exposed to solutions of the test compounds in complete culture medium for 96 h. At the end of exposure, drug solutions were replaced by mixtures of complete culture medium and aqueous MTT solution. After incubation for 4 h the medium/MTT mixtures were removed and the formazan crystals formed by the mitochondrial dehydrogenase activity of living cells were dissolved in DMSO. Optical densities at 550 nm were measured with a microplate reader (Tecan Spectra Classic), and the quantity of living cells was expressed in terms of T/C values by comparison to untreated control microcultures. Evaluation is based on means from at least three independent experiments, each comprising eight microcultures per concentration level.

5. Supplementary material

Crystallographic data for the structural analysis have been deposited with the Cambridge Crystallographic Data Centre, CCDC No. 223975. Copies may be obtained free of charge from the Director Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK (fax (international): +44-1223-336-033; e-mail: deposit@ccdc.cam.ac.uk or www.ccdc.cam.ac.uk/conts/retrieving.html).

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