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# Carboplatin derivatives with superior antitumor activity compared to the parent compound

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**Abstract**

A series of new carboplatin derivatives was synthesized by introducing fluoro, chloro, bromo and hydroxy substituents into the cyclobutane ring. The carboxylic acid groups were used for the complexation with platinum(II) fragments bearing two ammonia and (*RR*/*SS*)-*trans*-1,2-diaminocyclohexane ligands, respectively, as non-leaving groups. The antiproliferative activity of the new carboplatin analogues differing in the cyclobutanedicarboxylate ligands and the type of platinum fragment were studied in tests with J82 bladder cancer cells and SK-OV-3 as well as cisplatin-resistant NIH:OVCAR-3 ovarian cancer cells. The most active compounds were the 3-fluoro, 3-chloro and 3,3-difluoro derivatives of carboplatin. NMR spectroscopy showed that *cis*-diammine(3-chloro-1,1-cyclobutanedicarboxylato)platinum(II) was hydrolyzed much faster than carboplatin explaining its higher cytostatic activity.
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## 1. Introduction

The cytostatic activity of *cis*-diammine(dichloro)platinum(II) (cisplatin) was incidentally discovered in the 1960s by Rosenberg [1]. In 1978 cisplatin was approved for the treatment of testicular cancer and ovarian cancer. It is also used in a number of other human malignancies including cervical, head and neck, esophageal, lung and bladder cancer [2]. The sales of cisplatin were US$ 525 million in 1998 [3,4]. Despite its success cisplatin suffers from limitations due to severe side effects and intrinsic and acquired drug resistance. Cisplatin has a limited solubility in aqueous solutions which is a problem for intravenous application.

These drawbacks gave impetus for the development of new generations of platinum based anticancer drugs. However, only *cis*-diammine(1,1-cyclobutanedicarboxylato)platinum(II) (carboplatin) received worldwide approval. Carboplatin, less toxic than cisplatin, is active against the same tumor entities as cisplatin and is also administered intravenously. In 1989, carboplatin was approved for the treatment of ovarian cancer. Increasing sales (in 1998 of US$ 132 million) indicate that carboplatin is going to substitute cisplatin in many applications [4]. A third important platinum-based anticancer drug is (*R*,*R*-*trans*-1,2-diaminocyclohexane)oxalatoplatinum(II) (oxaliplatin), registered in Europe and Japan. Oxaliplatin has shown potential against cisplatin-resistant tumors and is used to treat colorectal cancer [5].

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We synthesized new carboplatin-like platinum(II) complexes by introducing F, Cl, Br and OH substituents in the 3-position of the cyclobutane ring. In analogy to carboplatin, ammonia ligands were used as non-leaving groups. In order to combine the properties of oxaliplatin with carboplatin we also introduced the (*RR*,*SS*)-*trans*-1,2-diaminocyclohexane ligand as a non-leaving group into our carboplatin derivatives. In the present paper, the synthesis, characterization, and antitumor properties of seven new carboplatin derivatives are described [6].

## 2. Results and discussion

### *2.1. Synthesis of 3-chloro-1,1-cyclobutanedicarboxylic acid (**4**)*

The synthesis of 3-chloro-1,1-cyclobutanedicarboxylic acid **4** starting from 1,1-cyclobutanedicarboxylic acid **1** via its acid chloride **2** was performed according to literature procedures (Scheme 1) [7]. In the radical chlorination of **2** we used AIBN as a radical starter to achieve higher yields instead of commercial dibenzoylperoxide which contained about 20% of water. Hydrolysis of the chlorinated acid chloride **3** afforded **4**.

### *2.2. Synthesis of 3-hydroxy-1,1-cyclobutanedicarboxylic acid (**8**)*

Compound **8** was synthesized in a four step reaction starting from benzylbromide and epibromohydrin. The resulting dibromide **5** was reacted with diethyl malonate and sodium hydride to give the substituted cyclobutane derivative **6**. Hydrogenolysis was used for the cleavage of the benzylether **6** yielding the hydroxy compound **7** as described in the literature [8,9]. The carboxylic acid **8**, which was required for coordination to the platinum(II) moieties, was prepared by hydrolysis of ester **7** with 20% ethanolic KOH solution (Scheme 2).

### *2.3. Synthesis of 3-bromo-1,1-cyclobutanedicarboxylic acid (**11**)*

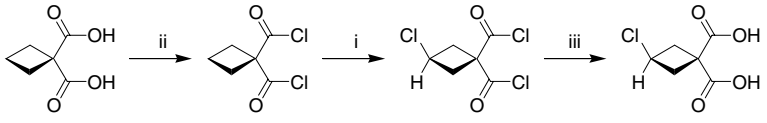
The bromo derivative **11** was prepared starting from compound **7**. The diethyl ester **10** was obtained by reaction of **7** with tosyl chloride to give **9**, followed by treatment with lithium bromide under reflux in DMSO. The resulting diester **10** was hydrolyzed under acidic conditions yielding **11** (Scheme 3).

### *2.4. Synthesis of 3-fluoro-1,1-cyclobutanedicarboxylic acid (**13**)*

Acid **13** had been previously prepared by the reaction of 1-bromo-3-chloro-2-fluoropropane with diethyl malonate [10]. We prepared it by the reaction of compound **7** with fluorinating agents like Deoxo-Fluor [11,12] and DAST [13] giving rise to diethyl ester **12** which on acid hydrolysis afforded **13** as described in the literature [10] (Scheme 4).

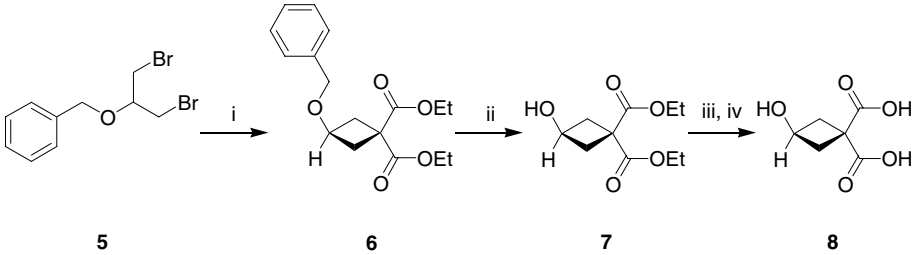
### *2.5. Synthesis of 3,3-difluoro-1,1-cyclobutanedicarboxylic acid (**16**)*

3,3-Difluoro-1,1-cyclobutanedicarboxylic acid **16** was prepared starting from **7**, which on oxidation formed



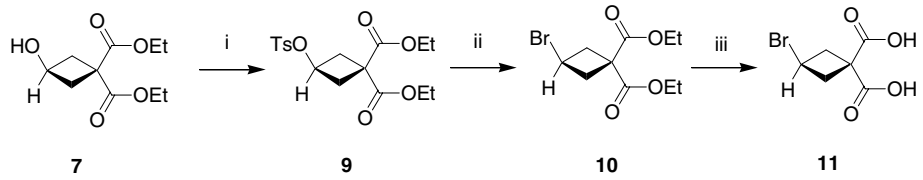

Reagents: (i) excess $SOCl_2$, reflux, 3 h; (ii) $SO_2Cl_2$, AIBN, reflux, 3 h; (iii) $H_2O$.

Scheme 1.



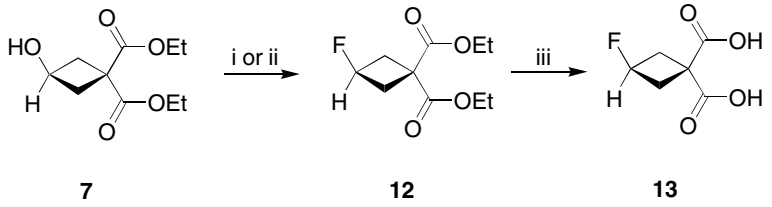

Reagents: (i) diethyl malonate, excess NaH, dioxane, 150 h; (ii) Pd/charcoal (10% Pd), EtOH; (iii) 20% KOH in EtOH, reflux, 4 h; (iv) HCl, $H_2O$.

Scheme 2.



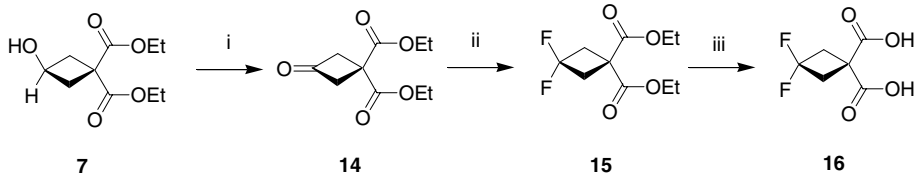

Reagents: (i) 1.1 eq. tosyl chloride, $Et_3N$, $CH_2Cl_2$, rt, 24 h; (ii) 1 eq. LiBr, DMSO, 140 °C, 16 h; (iii) 6M HCl, EtOH, 60 °C, 2 d.

Scheme 3.




Reagents: (i) 1 eq. Deoxo-Fluor, $CH_2Cl_2$, rt, 24 h; (ii) 1 eq. DAST, $CH_2Cl_2$, rt, 18 h; (iii) 6M HCl solution, reflux, 18 h.

Scheme 4.




Reagents: (i) $RuO_4$, $NaIO_4$, $CCl_4$, rt; (ii) 2.2 eq. DAST, $CH_2Cl_2$, rt, 24 h; (iii) 6M HCl, EtOH, reflux, 16 h.

Scheme 5.

diethyl 3-oxocyclobutane-1,1-dicarboxylate **14** as reported in the literature [14]. This oxidized product was reacted with the fluorinating agent DAST following a related literature procedure [15] to obtain **15** which on hydrolysis gave **16** (Scheme 5).

### 2.6. Synthesis of the cis-diammineplatinum(II) complexes

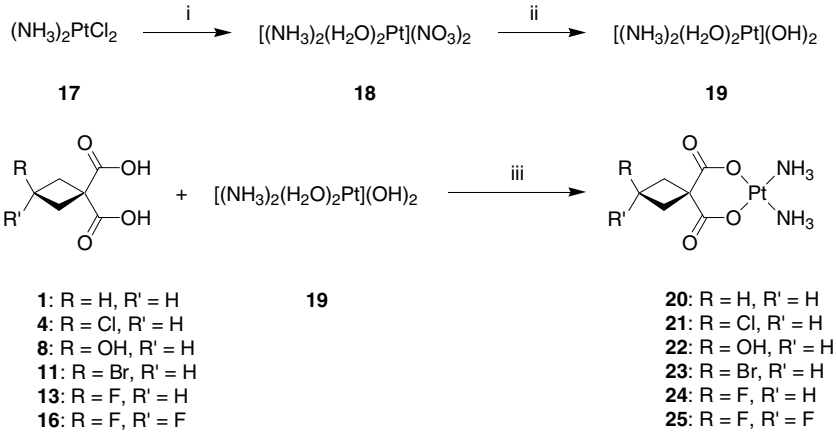
For the reaction with the cyclobutanedicarboxylic acids **4**, **8**, **11**, **13** and **16** it was necessary to transform cisplatin **17** into an active hydrolysis product. cisplatin was stirred with two mol equivalents of silver nitrate in aqueous solution under exclusion of light for one week. The chloro ligands were substituted by aqua ligands and AgCl precipitated. The exchange of the nitrate counter ions in **18** for hydroxide ions was achieved by chromatography on a strongly basic anion exchanger in which the reactive platinum(II) complex **19** was formed (Scheme 6) [16].

Compounds **4**, **8**, **11**, **13** and **16** were dissolved in water before an equimolar amount of an aqueous solution of diammine(diaqua)platinum(II) hydroxide **19** was added. The solution was stirred at room temperature under exclusion of light for 5 d. The grey precipitate was filtered off. After removal of the solvent from the filtrate the residue was recrystallized from water/acetone to give the colorless carboplatin derivatives **20**–**25** (Scheme 6).

For compound **22** the molecular structure was determined by X-ray diffraction. As expected, the coordination geometry around platinum(II) is square planar. The six-membered chelate ring is in a boat conformation. For C5 of the cyclobutane ring a thermal oscillation is observed indicating a dynamic puckering of the cyclobutane ring at a temperature of 173 K. The torsion angle C6–C2–C4–C5 is 20.1(7)°. The C-O bond lengths of the chelate ring are 5 pm longer than those to O2 and O3 (Fig. 1).

### 2.7. Synthesis of the (RR/SS)-trans-1,2-diaminocyclohexaneplatinum(II) complexes

Commercial (*RR*/*SS*)-*trans*-1,2-diaminocyclohexane was used to prepare the corresponding dichloroplatinum(II) complex **26** [17], which had to be activated by




Reagents: (i) 2 eq. $AgNO_3$, $H_2O$, 7 d; (ii) strongly basic anion exchanger; (iii) EtOH, $H_2O$, 5 d.

Scheme 6.

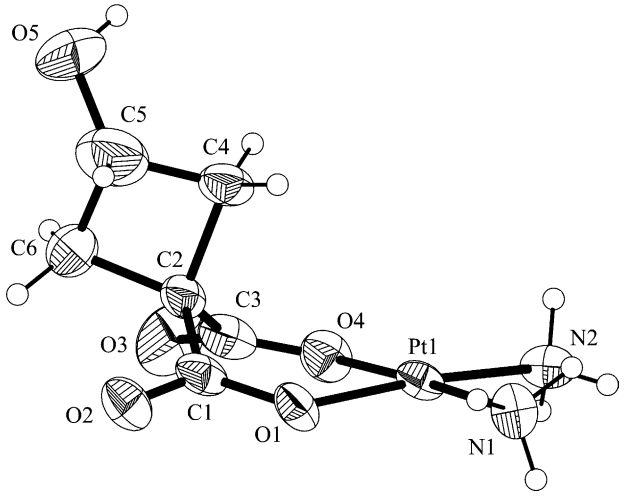



Fig. 1. The molecular structure of **22**.

conversion into the diamine(dihydroxy)platinum(II) hydroxide **27** in analogy to cisplatin (Scheme 7) [16,18].

The activated platinum species **27** was reacted with an equivalent amount of the respective carboxylic acid in a mixture of water and ethanol. The solution was stirred at room temperature under exclusion of light for several days. The grey precipitate was filtered off. After removal of the solvent from the filtrate and recrystallization from water the colorless products **28**–**30** were obtained (Scheme 7).

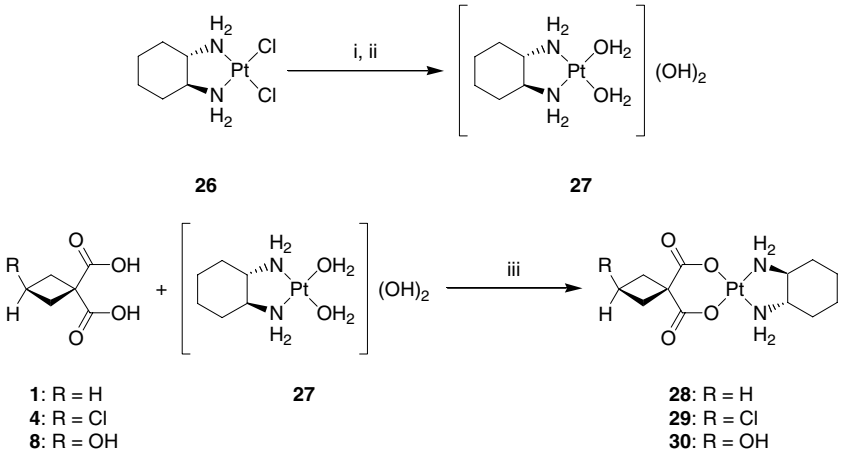
For compound **30** the molecular structure was determined by X-ray diffraction. The local geometry around the platinum atom approximates a square planar arrangement in analogy to the crystal structure of **22**. For the chelate ring again a boat conformation was observed. The torsion angle C6–C3–C4–C5 is 12.6(5)° illustrating the bending of the cyclobutane ring. The cyclohexane ring of the amine ligand takes a chair conformation in which the amine substituents are arranged in equatorial position as expected (Fig. 2).

### 2.8. Spectroscopy

The progress of the complexation of the carboxylic acid ligands with the platinum fragment was monitored by IR spectroscopy. The CO absorptions of the carboxylic acids **4**, **8**, **11**, **13** and **16** appeared in the range 1725–1695 $cm^{-1}$, whereas the platinum(II) complexes **21**–**25** and **28**–**30** afforded two new bands at 1645–1595 and 1395–1355 $cm^{-1}$. The NMR spectra of the carboplatin derivatives **21**–**25** and **28**–**30** revealed sharp signals which were shifted to higher magnetic field compared with the cyclobutanedicarboxylic acids **4**, **8**, **11**, **13** and **16**. Further evidence for the formation of complexes **21**–**25** and **28**–**30** was derived from the mass spectra which showed the respective molecular ions.

### 2.9. Cell lines and general procedure

To determine the antiproliferative activity of the new carboplatin derivatives the bladder cancer cell line J82 was selected as our first in vitro model. The J82 cell line was established from a transitional cell carcinoma of a 58 years old Caucasian male [19]. As in these tests chlorocarboplatin **21** proved to be two times as effective as carboplatin and 3-hydroxycarboplatin **22**, we synthesized the 3-bromo, 3-fluoro and 3,3-difluoro derivatives **23-25**. These compounds were tested with the ovarian cancer cell lines SK-OV-3, a human adenocarcinoma derived from the ascitic fluid of a 64 years old Caucasian [20], and NIH:OVCAR-3, a human adenocarcinoma derived from a 60 years old Caucasian [21]. Of the latter cell line a threefold cisplatin-resistant variant, which




Reagents: (i) 2 eq. $AgNO_3$, $H_2O$, 7 d; (ii) strongly basic anion exchanger; (iii) EtOH, $H_2O$, 5 d.

Scheme 7.

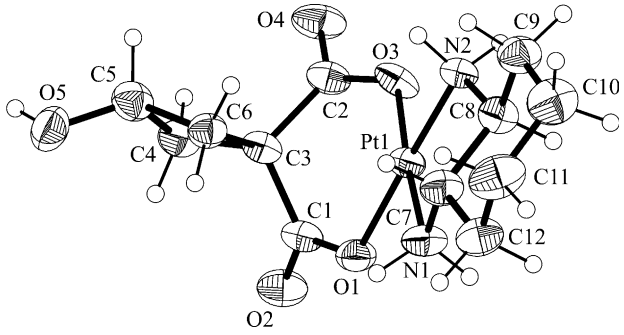



Fig. 2. The molecular structure of **30**.

had been generated by subculturing the wild type cells in the presence of increasing concentrations of cisplatin for 150 days, was used for cytotoxicity testing. We used computerized chemosensitivity assays based on the quantification of biomass by staining viable cells with crystal violet [22,23].

### 2.10. Cytotoxicity testing against J82 cells

J82 cells were incubated for 4 days with 0.5, 1, 5 and 10 μM solutions of the carboplatin derivatives and carboplatin as reference. At a dosage of 0.5 and 1 μM, no statistically significant cytotoxicity was observed for carboplatin and compounds **21** and **22**. At a concentration of 5 μM, the cytotoxic effect of the chloro derivative **21** amounted to a $T/C_{corr.}$ value of approximately 40%, whereas carboplatin and the hydroxy derivative **22** reached only half of the effectiveness of **21**. At 10 μM, **21** gave a $T/C_{corr.}$ value of 4%, significantly lower than carboplatin and **22** (Table 1).

The platinum complexes **28**–**30** with (*RR*/*SS*)-*trans*-1,2-diaminocyclohexane as non-leaving group showed $T/C_{corr.}$ values of about 50–85% at a dosage of 0.5 μM. At higher concentrations, interestingly, it was again the chloro derivative **29** which turned out to be the most effective compound in terms of cytotoxicity. In general, the diaminoplatinum(II) complexes **28**–**30** had a higher cytotoxic potential than the respective diammineplatinum(II) complexes **20**–**22**, the chloro derivative **29** being the most active with $T/C_{corr.}$ values of 33.1% at 1 μM, 10.5% at 5 μM and −1.3% at 10 μM. However, the diaminoplatinum(II) complexes **28**–**30** were only soluble in DMF, in contrast to the water-soluble diammineplatinum(II) complexes **20**–**22** (Table 1).

The antitumor activity of the promising carboplatin derivative **21** was also analyzed with the J82 bladder cancer cell line in a kinetic assay. This procedure provides information concerning activity (cytotoxic, cyto-

Table 1
$T/C_{corr.}$ values or cytocidal effect (underlined) of the carboplatin derivatives **21**, **22** and **28**–**30** at 0.5, 1, 5 and 10 μM concentration with carboplatin 20 as reference for the J82 cell line after the incubation period of 96 h

| $c$ [$10^{-6}$ mol $L^{-1}$] | 0.5 | 1 | 5 | 10 |
|---|---|---|---|---|
| Carboplatin | 94.4 ± 114.0 | 95.5 ± 12.3 | 83.3 ± 13.2 | 49.8±10.6 |
| **21** | 115.3 ± 13.8 | 93.4 ± 16.1 | 44.3 ± 22.7 | 4.1 ± 5.0 |
| **22** | 105.2 ± 11.3 | 110.2 ± 11.8 | 71.4 ± 19.6 | 41.6 ± 21.5 |
| **28** | 87.2 ± 14.6 | 63.4 ± 13.1 | 31.5 ± 11.0 | 14.5 ± 4.5 |
| **29** | 64.7 ± 11.3 | 33.1 ± 10.6 | 10.5 ± 3. 5 | 1.3 ± 3.3 |
| **30** | 52.4 ± 11.8 | 51.6 ± 10.7 | 14.3 ± 4.2 | 7.2 ± 3.9 |

static, or cytocidal) and inactivation as a function of time [22,23].

$T/C_{corr.}$ values or percent cytocidal effect (left ordinate) for the test compounds were plotted together with the absorbance of the untreated solvent control (right ordinate) vs time of drug exposure. In these plots time zero indicates the time at which the drug was added. In these experiments, the drug containing culture media was left unchanged throughout the incubation period.

Fig. 3 shows classical dose–response relationships for carboplatin **20** (A) and its chloro analogue **21** (B). Interestingly, the chloro compound **21** has a much higher cytotoxic potential at the three tested concentrations than the clinically used carboplatin. A clear antiproliferative effect is observed as a function of time for all three concentrations of **21** resulting in $T/C_{corr.}$ values of 34.0 and 0.8% for 5 and 10 μM and a cytocidal effect of 45.4% for 20 μM after an incubation period of 118.5 h. The corresponding $T/C_{corr.}$ values for carboplatin are 64.2, 35.6 and 9.5% for 5, 10 and 20 μM concentration, respectively.

### 2.11. Cytotoxicity testing against SK-OV-3 cells and cisplatin-resistant NIH:OVCAR-3 cells

In a similar kinetic assay the antitumor activity of platinum complexes **21**–**25** was analyzed with the SK-OV-3 ovarian cancer cell line. Four different concentrations (0.5, 1, 2 and 3 μM) of the platinum complexes were administered to the SK-OV-3 cell line along with clinically used carboplatin **20**. Interestingly, the monofluoro derivative **24** had the highest efficacy among all the derivatives tested (Fig. 4). It was followed by the chloro, difluoro, hydroxy and bromo derivatives in that order (Table 2). Remarkably, the differences between the fluoro, chloro and difluoro compounds **24**, **21** and **25** were comparatively small. The improved antiproliferative effect of the fluoro derivative **24** showed up in the $T/C_{corr.}$ values 67.5%, 50.3%, 18.2% and 1.1% for 0.5, 1, 2 and 3 μM concentrations after an incubation period of 264 h. The corresponding $T/C_{corr.}$ values for carboplatin were 88.6, 57.1, 48.3 and 29.8%, respectively.

In the experiments with the cisplatin-resistant NIH:OVCAR-3 subline we used the same four concentrations of carboplatin and its derivatives as described for the SK-OV-3 cell line. In this case too the monofluoro derivative **24** was the most active compound (Fig. 5). It was followed by the difluoro, fluoro, hydroxy and bromo derivatives, although the differences for the fluoro, difluoro and chloro compounds were only small (Table 3). The high antiproliferative effect of the fluoro derivative **24** was evident from the $T/C_{corr.}$ values 74.5% and 65.9% for 0.5 and 1 μM and the cytocidal effect of 8.9% and 12.3% for 2 and 3 μM concentrations after an incubation period of 239 h, respectively. The corresponding $T/C_{corr.}$ values for carboplatin were 100.7%, 78.9%, 63.5% and 25.6%, respectively. In both the cell lines the difluoro derivative **25** started its cytotoxic effect faster and at lower concentrations (0.5 and 1 μM) than the other compounds (Tables 2 and 3). However, later and at higher concentrations (2 and

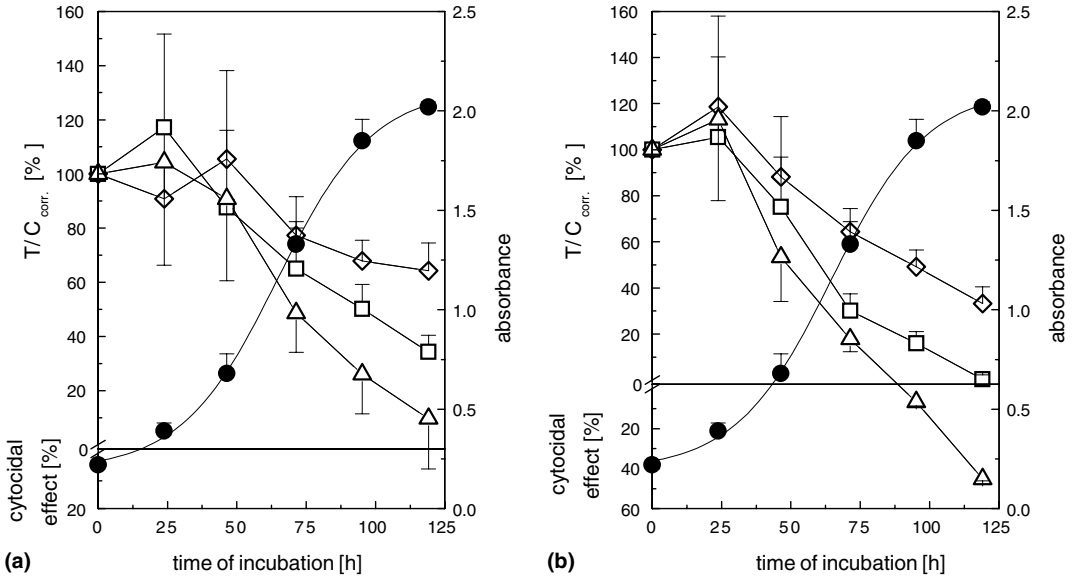



Fig. 3. Dose–response relationship of carboplatin **20** (a) and the chloro derivative **21** (b) on the proliferation of long term incubated J82 cells (in passage 40 from origin) at a concentration of 5 (◇), 10 (□), and 20 μM (△). Proliferation kinetics of the corresponding control (absorbance at 578 nm) (●).

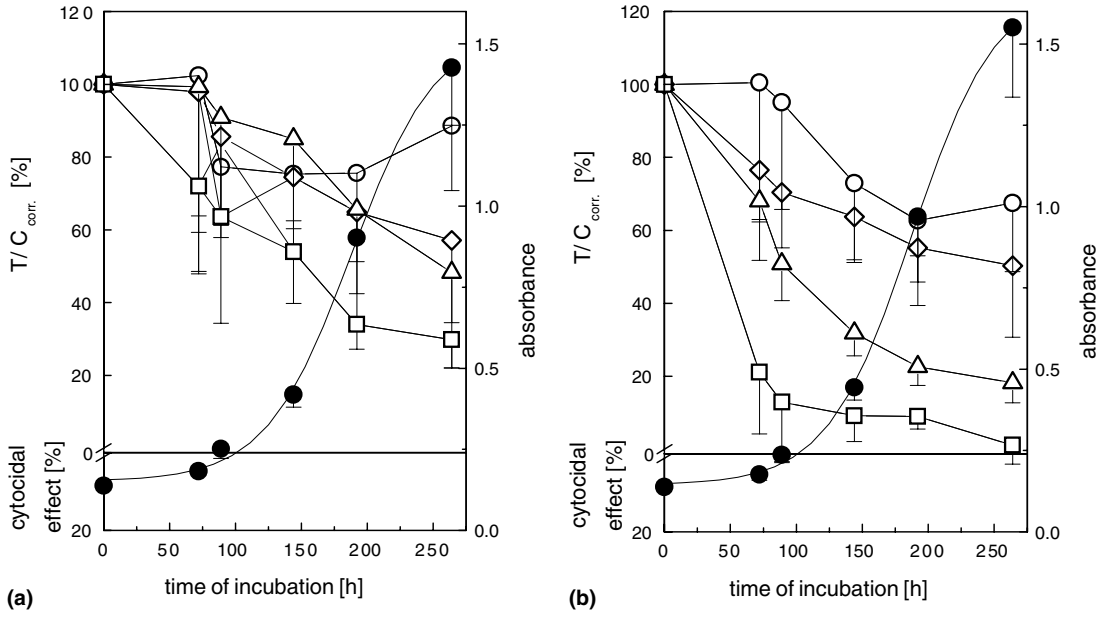



Fig. 4. Dose–response relationship of carboplatin **20** (a) and the fluoro derivative **24** (b) on the proliferation of long term incubated SK-OV-3 cells (in passage 77 from origin) at a concentration of 0.5 (○), 1 μM (◇), 2 μM (△) and 3 μM (□). Proliferation kinetics of the corresponding control (absorbance at 578 nm) (●).

Table 2
$T/C_{corr.}$ values of the carboplatin derivatives **21**–**25** at 0.5, 1, 2 and 3 μm concentration with carboplatin **20** as reference for the SK-OV-3 cell line after the incubation period of 264 h

| $c$ [$10^{-6}$mol$^{-1}$] | 0.5 | 1 | 2 | 3 |
|---|---|---|---|---|
| Carboplatin | 88.6 ± 17.8 | 57.1 ± 22.6 | 48.3 ± 26.2 | 29.8 ± 7.8 |
| **21** | 94.9 ± 18.2 | 55.9 + 16.4 | 28.2 + 17.3 | 6.9 ± 4.9 |
| **22** | 94.5 ± 18.0 | 79.8 ± 19.0 | 47.1 ± 15.2 | 19.0 ± 11.5 |
| **23** | 91.9 + 22.3 | 87.0 ± 21.7 | 48.2 + 17.5 | 20.7 + 9.0 |
| **24** | 67.5 ± 18.8 | 50.3 ± 19.6 | 18.2 + 5.5 | 1.1 ± 2.7 |
| **25** | 58.1 ± 25.1 | 50.3 ± 22.5 | 12.1 ± 8.2 | 7.9 ± 8.9 |

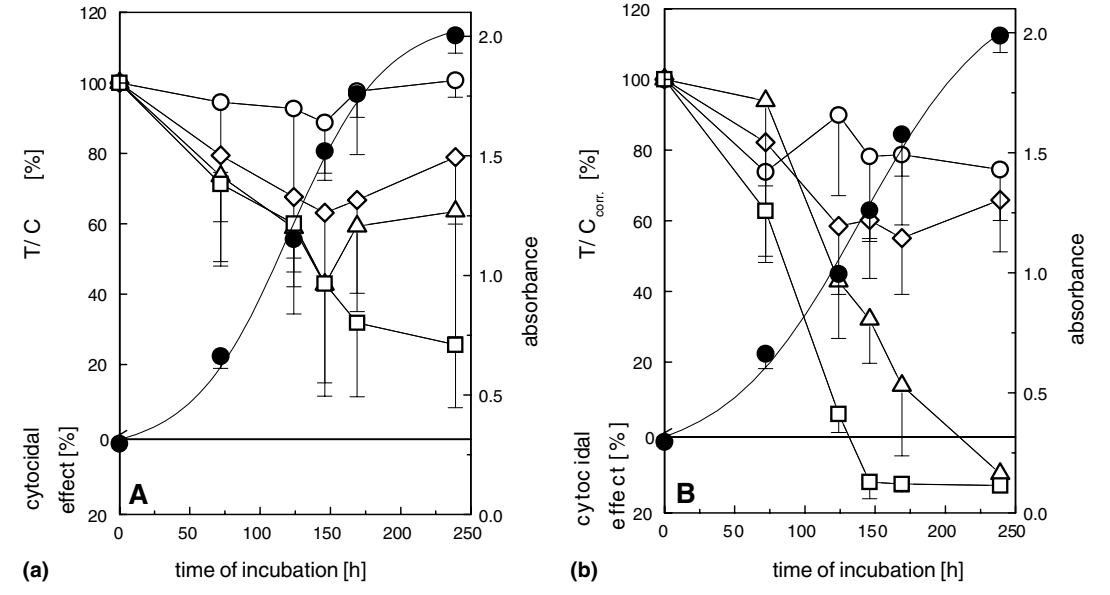



Fig. 5. Dose–response relationship of carboplatin **20** (a) and the fluoro derivative **24** (b) on the proliferation of long term incubated NIH:OVCAR 3 cells (in passage 56 from origin) at a concentration of 0.5 μM (○), 1 μM (◇), 2 μM (△) and 3 μM (□). Proliferation kinetics of the corresponding control (absorbance at 578 nm) (●).

Table 3
$T/C_{corr.}$ values or cytocidal effect (underlined) of the carboplatin derivatives **21**–**25** at 0.5, 1, 2 and 3 μM concentration with carboplatin **20** as reference for the NIH:OVCAR-3 cell line after the incubation period of 239 h

| $c$ [$10^{-6}$mol$^{-1}$] | 0.5 | 1 | 2 | 3 |
|---|---|---|---|---|
| Carboplatin | 100.7 ± 4.8 | 78.9 ± 19.0 | 63.5 ± 35.6 | 25.6 ± 17.9 |
| **21** | 96.1 ± 9.4 | 48.5 ± 25.9 | 1.9 ± 18.2 | 5.4 ± 10.6 |
| **22** | 96.5 ± 6.4 | 89.1 + 13.3 | 70.7 ± 30.3 | 10.1 ± 4.1 |
| **23** | 87.2 ± 14.6 | 63.4 ± 13.1 | 31.5 ± 11.0 | 14.5 ± 4.5 |
| **24** | 74.5 ± 14.4 | 65.9 ± 14.7 | 8.9 ± 3.0 | 12.3 ± 0.5 |
| **25** | 60.3 ± 16.2 | 41.6 ± 16.4 | 8.7 ± 2.0 | 9.3 ± 0.9 |

3 μM) its activity levelled off and it was outperformed by the fluoro compound **24** and in part by the chloro compound **21**.

### 2.12. Investigation of the stability of *21*

The extraordinary activity of the chloro derivative **21** in cell biological experiments prompted us to investigate its stability compared to carboplatin by $^{1}$H NMR spectroscopy. Both compounds (2.5 μM) were dissolved in 1 mL of a 0.9% solution of NaCl in $D_2O$ which is equivalent to a physiological NaCl solution. The NMR tubes were kept in the dark at room temperature.

For carboplatin the signal of the equivalent $CH_2$ groups at C2 and C4 was a triplet at 2.80 ppm. The $CH_2$ group at C3 appeared as a quintet at 1.81 ppm with a coupling constant of $^{3}J$(H,H) = 7.8 Hz. After 1 h a new group of signals emerged at 2.26 ppm which could be assigned to the two equivalent $CH_2$ groups at C2 and C4 of the free cyclobutanedicarboxylic acid **1**. In the course of 168 h the area of this integral increased, whereas the signal at 2.80 ppm decreased (Fig. 6). For the chloro derivative **21** *cis* and *trans* protons could be distinguished for the $CH_2$ groups at C2 and C4 splitting in a symmetrical pattern of signals at 3.52 and 2.97 ppm. After 1 h the $CH_2$ groups of the free cyclobutanedicarboxylic acid **4** can be easily identified because of their highfield shifted signals (Fig. 7).

A quantitative analysis of these kinetic data was based on the time dependent integration of respective $CH_2$ signals. For carboplatin the signals at 2.80 and 2.26 ppm and for **21** the signals at 3.52/2.97 and 2.84/2.53 ppm were compared, respectively. After 48 h 92% of carboplatin but only 76% of complex **21** were left. After 168 h the amount of **21** had dropped to 61% compared with 85% of carboplatin. Thus, the chloro complex **21** was hydrolyzed much faster than carboplatin **20** explaining its higher cytostatic activity.

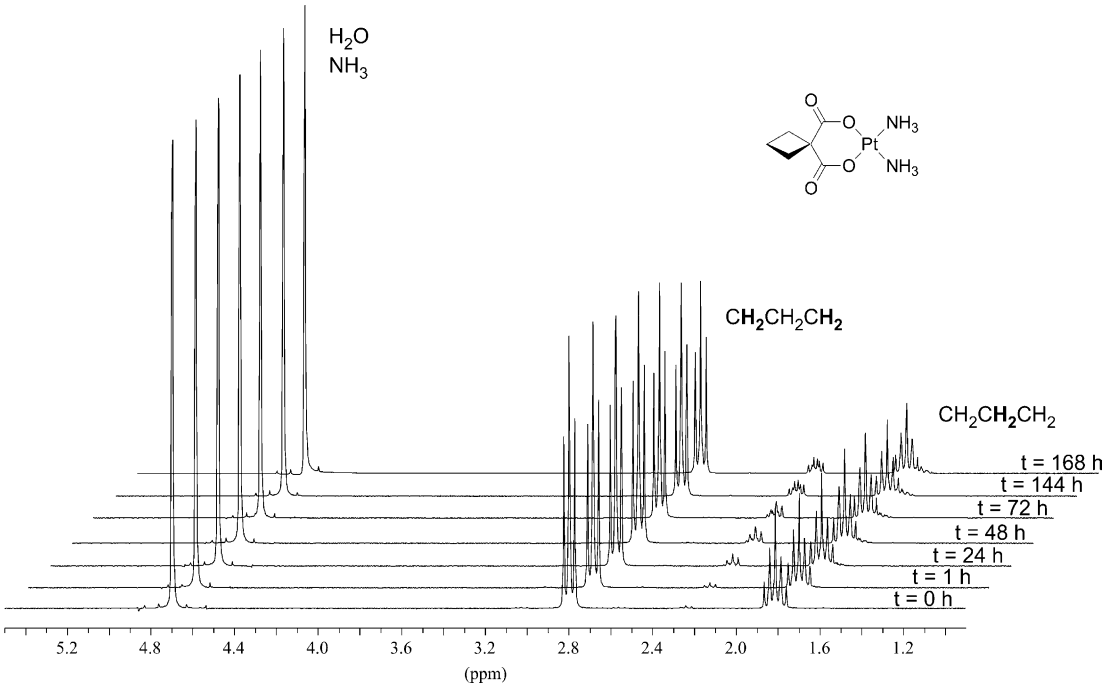



Fig. 6. $^{1}H$ NMR spectra (300 MHz, $D_2O$/0.9% NaCl, 24 °C, TMS external) of carboplatin **20**.

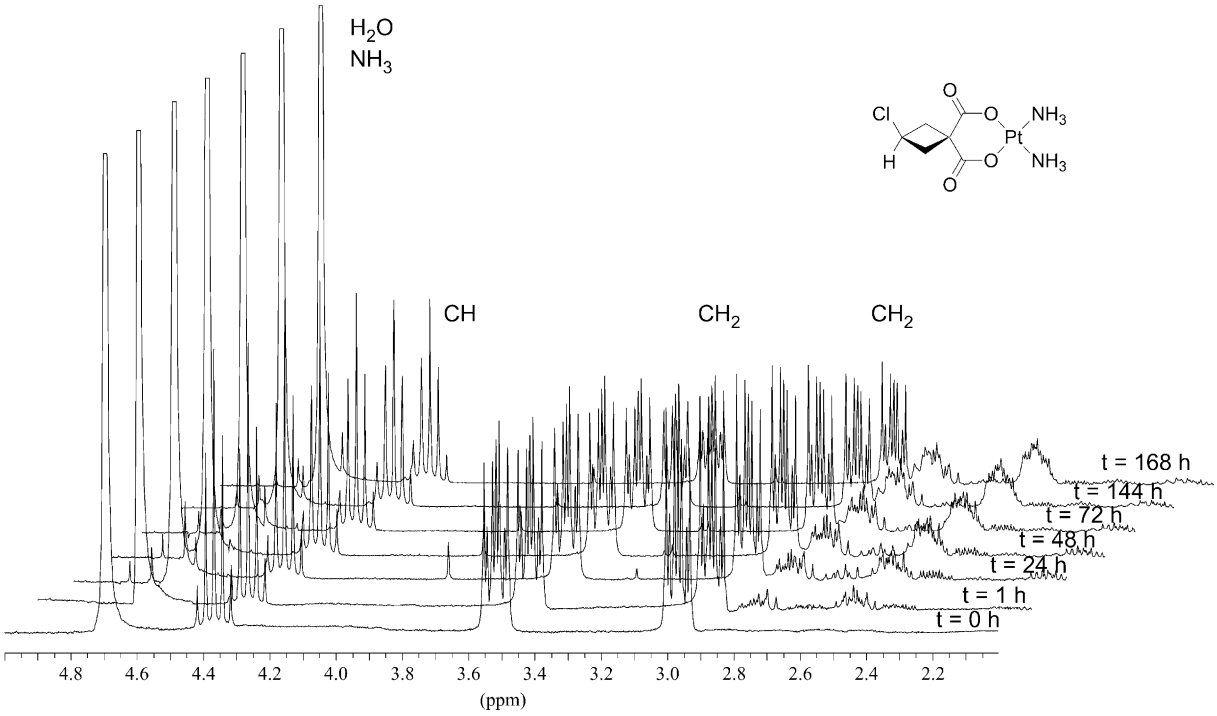



Fig. 7. $^{1}H$ NMR spectra (300 MHz, $D_2O$/0.9% NaCl, 24 °C, TMS external) of **21**.

## 3. Conclusion

We synthesized the hitherto unknown fluoro, chloro, bromo and hydroxy derivatives of the clinically used drug carboplatin. The highest antiproliferative effect was obtained for the fluoro and chloro derivatives **24**, **25**, **21** and **29**. In the series of the *cis*-diammine complexes the fluoro and chloro derivatives **24**, **25** and **21** showed a much higher cytotoxic potential than carboplatin. Moreover, derivatives **24**, **25** and **21** showed a good solubility in water, comparable to carboplatin, in contrast to the (*RR*,*SS*)-*trans*-1,2-diaminocyclohexane complexes **28**–**30** which were only soluble in DMF.

## 4. Experimental

### 4.1. Chemistry

IR: Beckman spectrometer 4240. $^{1}H$ and $^{13}C$ NMR: Bruker WM 250 (250 MHz) and Avance 300 (300 MHz); chemical shifts in ppm; TMS as internal standard. MS: Finnigan MAT 95, MAT 112 S and MAT 311 A, ThermoQuest Finnigan 7000. The molecular ion is designated M; only the most intense peak is specified in an ion cluster. Mp: Büchi SMP 20; not corrected. UV/Vis: Kontron Instruments spectrophotometer UVIKON 922.

Solid reagents were used as obtained from commercial suppliers without further purification; liquids were freshly distilled before use.

### 4.2. 1,1-Cyclobutanedicarboxylic acid dichloride (2)

**2** was prepared according to a literature procedure from 1,1-cyclobutanedicarboxylic acid **1** and thionyl chloride [7]. IR (film): $\nu$ 1790 (C=O) $cm^{-1}$. $^{1}H$ NMR ($CDCl_3$): $\delta$ 2.74 (t, $^{3}J = 7.9$ Hz, 4 H, $CH_2(CH_2)_2$), 2.05 (qi, $^{3}J = 7.9$ Hz, 2 H, $CH_2(CH_2)_2$) ppm. MS (EI): *m*/*z* (rel. int.) 145 (M − Cl, 100), 117 (M − COCl, 60), 89 (M − $(CO)_2Cl$, 80), 54 (M − 2COCl, 36).

### 4.3. 3-Chloro-1,1-cyclobutanedicarboxylic acid dichloride (3)

**3** was prepared in analogy to a literature procedure by reaction of **2** with sulfuryl chloride and AIBN as radical starter [7]. IR (film): $\nu$ 1780 (C=O) $cm^{-1}$. $^{1}H$ NMR ($CDCl_3$): $\delta$ 4.41 (qi, $^{3}J = 7.9$ Hz, 1 H, $CHCl(CH_2)_2$), 3.33, 2.99 (2 m, 4 H, $CHCl(CH_2)_2C(COCl)_2$) ppm. MS (EI): *m*/*z* (rel. int.) 179 (M − Cl, 100).

### 4.4. 3-Chloro-1,1-cyclobutanedicarboxylic acid (4)

**4** was prepared according to a literature procedure from **3** by hydrolysis [7]. IR (KBr): $\nu$ 3000 (OH), 1700 (C=O) $cm^{-1}$. $^{1}H$ NMR (DMSO-$d_6$): $\delta$ 13.05 (s, 2 H, $CO_2H$), 4.41 (qi, $^{3}J = 7.9$ Hz, 1 H, $CHCl(CH_2)_2$), 3.01, 2.64 (2m, 4 H, $CHCl(CH_2)_2C(CO_2H)_2$) ppm. MS (EI): *m*/*z* (rel. int.) 178 (M, 2), 143 (M − Cl, 20), 133 (M − $CO_2H$, 25).

### 4.5. 2-Benzyloxy-1,3-dibromopropane (**5**)

**5** was prepared according to a literature procedure from epibromohydrin and benzylbromide [8]. IR (film): *v* 1590, 1510, 1490 (C=C) $cm^{-1}$. $^1H$ NMR ($C_6D_6$): $\delta$ 7.30–7.00 (m, 5 H, $C_6H_5$), 4.12 (s, 2 H, $C_6H_5CH_2$), 3.24 (qi, $^3J = 4.8$ Hz, 1 H, $CH(CH_2Br)_2$), 3.11 (d, $^3J = 4.8$ Hz, 4 H, $CH(CH_2Br)_2$) ppm. MS (EI): *m/z* (rel. int.) 308 (M, 5), 213 (M – $CH_2Br$, 5), 91 ($C_7H_7$, 100).

### 4.6. Diethyl 3-benzyloxy-1,1-cyclobutanedicarboxylate (**6**)

**6** was prepared according to a literature procedure from **5** and diethyl malonate [8]. IR (film): *v* 1740 (C=O), 1500 (C=C) $cm^{-1}$. $^1H$ NMR ($C_6D_6$): $\delta$ 7.23–7.04 (m, 5 H, $C_6H_5$), 4.14, 4.11, 4.08 (3 m, 3 H, $C_6H_5CH_2OCH$), 3.94, 3.93 (2 q, $^3J = 7.1$ Hz, 4 H, $CH_2CH_3$), 2.90, 2.76 (2 m, 4 H, $CH(CH_2)_2$), 0.89 (t, $^3J = 7.1$Hz, 6 H, $CH_2$ $CH_3$) ppm. MS (EI): *m/z* (rel. int.) 306 (M, 20), 91 ($C_7H_7$, 100).

### 4.7. Diethyl 3-Hydroxy-1,1-cyclobutanedicarboxylate (**7**)

**7** was prepared according to a literature procedure from **6** by hydrogenolysis [8]. IR (film): *v* 1730 (C=O) $cm^{-1}$. $^1H$ NMR ($CDCl_3$): $\delta$ 4.38 (qi, $^3J = 7.5$ Hz, 1 H, $CH(OH)(CH_2)_2$), 4.21 (q, $^3J = 7.1$ Hz, 4 H, $CH_2CH_3$), 2.89, 2.46 (2 m, 4 H, $CH(CH_2)_2$), 2.26 (bs, 1H, OH), 1.26 (t, $^3J = 7.1$ Hz, 6H, $CH_2CH_3$) ppm. MS (EI): *m/z* (rel. int.) 216 (M, 15).

### 4.8. 3-Hydroxy-1,1-cyclobutanedicarboxylic acid (**8**)

**7** (4.32 g, 0.02 mol) was dissolved in a solution of KOH (5.43 g, 0.097 mol) in 13.55 mL of ethanol and refluxed for 4 h. After removal of the solvent the residue was taken up in 15 mL of water. Under ice cooling concentrated HCl was added to pH 1. Subsequent ether extraction, evaporation and recrystallization from ethyl acetate afforded 7. Yield: 1.15 g (36%), colorless solid, m.p. 128 °C. IR (KBr): *v* 3440 (OH), 1720 (C=O) $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): $\delta$ 12.68 (bs, 2 H, COOH), 5.26 (bs, 1 H, $CH(OH)(CH_2)_2$), 4.06 (qi, $^3J = 7.5$ Hz, 1 H, $CH(OH)(CH_2)_2$), 2.58, 2.23 (2 m, 4 H, $CH(CH_2)_2$) ppm. MS (DCI): *m/z* (rel. int.) 178 (M + $NH_4$, 100), 160 (M, 10). *Anal.* Calc. for $C_6H_8O_5$, 160.13: C, 45.01; H, 5.04. Found: C, 44.86; H, 5.19%.

### 4.9. Diethyl 3-(4-toluene sulfonyl)-1,1-cyclobutanedicarboxylate (**9**)

**7** (11.1 g, 51.4 mmol), tosyl chloride (10.7 g, 56.1 mmol) and triethylamine (7.50 mL, 53.8 mmol) were stirred in $CH_2Cl_2$ (50 mL) at room temperature under nitrogen for 24 h and then filtered. The filtrate was evaporated and the residue was purified by chromatography on silica gel using EtOAc/petroleum ether (3:7) as an eluent yielding **9** as a colorless oil. Yield: 16.9 g (89%, purity >90%). IR ($CHCl_3$): *v* 1715 (C=O) $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 7.78 (d, 2 H, $^3J = 8.5$ Hz, ArH), 7.34 (d, 2 H, $^3J = 8.5$ Hz, ArH), 4.89 (qi, 1 H, $^3J = 8.4$ Hz, $CH(CH_2)_2$), 4.18 (q, 4 H, $^3J = 7.1$ Hz, $CH_2CH_3$), 2.80, 2.68 (2 m, 4 H, $CH(CH_2)_2$), 2.45 (s, 3 H, $C_6H_4CH_3$), 1.24 (t, 6 H, $^3J = 7.1$ Hz, $CH_2CH_3$) ppm. $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 13.92 (q), 13.94 (q), 21.62 (q), 37.58 (t, 2C, 2- and 4-C), 46.44 (s, 1-C), 61.66 (t), 61.81 (t), 68.89 (d, 3-C), 127.82 (d, 2C), 129.90 (d, 2C), 133.48 (s), 145.08 (s), 169.90 (s, CO), 170.76 (s, CO) ppm.

### 4.10. Diethyl 3-bromo-1,1-cyclobutanedicarboxylate (**10**)

**9** (3.74 g, 10.1 mmol) and LiBr (0.90 g, 10.4 mmol) were heated in DMSO (15 mL) for 16 h at 140 °C under nitrogen. After addition of water, the reaction mixture was extracted twice with 50 mL of ether. The combined organic layers were washed with water (50 mL), dried over $Na_2SO_4$ and evaporated. The residue was chromatographed on silica gel using EtOAc/petroleum ether (1:49) to give **10** as a colorless oil. Yield: 1.39 g (49%). IR ($CHCl_3$): *v* 1745 (C=O), 1715 (C=O) $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 4.28 (qi, 1 H, $^3J = 9.2$ Hz, $BrCH$), 4.23 (q, 2 H, $^3J = 7.1$ Hz, $CH_2CH_3$), 4.22 (q, 2 H, $^3J = 7.1$ Hz, $CH_2CH_3$), 3.17, 2.99 (2 m, 4 H, $CH(CH_2)_2$), 1.27 (t, 3 H, $^3J = 7.1$ Hz, $CH_2CH_3$), 1.26 (t, 3 H, $^3J = 7.1$ Hz, $CH_2CH_3$) ppm. $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 13.96 (q, 2C), 35.39 (d, 3-C), 41.27 (t, 2C, 2- and 4-C), 50.76 (s, 1-C), 61.78 (t), 61.93 (t), 169.79 (s, CO), 170.69 (s, CO) ppm. MS (CI): *m/z* (rel. int.) 298 [M($^{81}$Br) + $NH_4$, 91], 296 [M($^{79}$Br) + $NH_4$, 100], 281 [M($^{81}$Br) + H, 37], 279 ([M($^{79}$Br) + H, 41]. CI HRMS ($C_{10}H_{16}{}^{79}BrO_4$, M + H) calc. 279.0232; found 279.0230. *Anal.* Calc. for $C_{10}H_{15}BrO_4$, 279.13: C, 43.03; H, 5.42. Found: C, 43.05; H, 5.38%.

### 4.11. 3-Bromo-1,1-cyclobutanedicarboxylic acid (**11**)

**10** (481 mg, 1.73 mmol), 6 M HCl (15 mL) and ethanol (1.0 mL) were stirred at 60 °C for 2 d. Then the reaction mixture was washed twice with $CHCl_3$ (20 mL). The organic layer was extracted with water (20 mL). The combined aqueous layers were evaporated to give colorless crystals of **11** which were recrystallized from acetone/$CHCl_3$. Yield: 208 mg (53%), m.p. 157.5–160.2 °C. IR (KBr): *v* 3700–2400 (COOH), 1720 (C=O), 1700 (C=O) $cm^{-1}$. $^1H$ NMR (300 MHz, acetone-$d_6$): $\delta$ 11.50 (bs, 2 H, COOH), 4.63 (qi, 1 H, $^3J = 8.0$ Hz, BrCH), 3.22, 2.96 (2 m, 4 H, $CH(CH_2)_2$) ppm. $^{13}C$

NMR (75 MHz, acetone-$d_6$): $\delta$ 37.07 (d, 3-C), 42.21 (t, 2C, 2- and 4-C), 51.08 (s, 1-C), 171.30 (s, CO), 171.98 (s, CO) ppm. MS (CIQI): *m/z* (rel. int.) 223 [M($^{81}$Br) – H, 96], 221 [M($^{79}$Br) – H, 100]. *Anal*. Calc. for $C_6H_7BrO_4$, 223.02: C, 32.31; H, 3.16. Found: C, 32.23; H, 3.26%.

### 4.12. Diethyl 3-fluoro-1,1-cyclobutanedicarboxylate (**12**)

#### 4.12.1. Deoxo-fluor variant

Bis(2-methoxyethyl)aminosulfur trifluoride (Deoxo-Fluor®) (3.29 g, 14.9 mmol) was slowly added to a solution of **7** (3.19 g, 14.8 mmol) in $CH_2Cl_2$ (15 mL) under nitrogen at room temperature. The mixture was stirred for 24 h and then evaporated. The yellow oily residue was chromatographed on silica gel using EtOAc/petroleum ether (1:49) as eluent to obtain **12**. Yield: 1.17 g (36%).

#### 4.12.2. DAST variant

To a solution of **7** (3.12 g, 14.4 mmol) in $CH_2Cl_2$ (25 mL) was slowly added diethylaminosulfur trifluoride (DAST) (2.10 mL, 14.9 mmol) in $CH_2Cl_2$ (10 mL) under nitrogen. The mixture was stirred for 18 h at room temperature and then evaporated. The residue was extracted with $CH_2Cl_2$. Chromatography as described above gave **12**. Yield: 831 mg (26%). Spectroscopic data of **12** matched those of the literature [10].

### 4.13. Diethyl 3,3-difluoro-1,1-cyclobutanedicarboxylate (**15**)

Diethylaminosulfur trifluoride (DAST) (2.9 mL, 22.0 mmol) dissolved in $CH_2Cl_2$ (10 mL) was slowly added under nitrogen to $CH_2Cl_2$ (20 mL) solution of diethyl 3-oxacyclobutane-1,1-dicarboxylate **14** [13] (2.15 g, 10.0 mmol) and stirred for 24 h at 20 °C. The reaction mixture was quenched with water (50 mL) and extracted twice with $CH_2Cl_2$ (20 mL). The combined organic layers were dried over $Na_2SO_4$ and evaporated. The residual oil was chromatographed on silica gel using EtOAc/petroleum ether (1:49) to give **15** as a yellow oil. Yield: 1.83 g (77%). IR ($CHCl_3$): $\nu$ 1735 (C=O), 1715 (C=O) cm$^{-1}$. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 4.25 (q, 4 H, $^3J$ = 7.1 Hz, $CH_2CH_3$), 3.14 (t, 4 H, $^3J_{H-F}$ = 11.8 Hz, $CF_2(CH_2)_2$), 1.28 (t, 6 H, $^3J$ = 7.1 Hz, $CH_2CH_3$) ppm. $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 13.91 (q, 2C), 42.47 (st, $J_{H-F}$ = 8.9 Hz, 1-C), 42.81 (tt, $J_{C-F}$ = 25.6 Hz, 2- and 4-C), 62.25 (t, 2C), 113.37 (st, $J_{C-F}$ = 271.0 Hz, 3-C), 169.64 (st, 2C, $J_{C-F}$ = 2.2 Hz, CO) ppm. MS (EI): *m/z* (rel. int.) 236 (M, 6), 209 (47), 191 (80), 164 (18), 143 (18), 135 (46), 87 (36), 54 (28), 29 (100). *Anal*. Calc. for $C_{10}H_{14}F_2O_4$, 236.21: C, 50.85; H, 5.97. Found: C, 50.51; H, 5.59%.

### 4.14. 3,3-Difluoro-1,1-cyclobutanedicarboxylic acid (**16**)

**15** (1.05 g, 4.46 mmol) was refluxed in 6 M HCl (20 mL) for 15 h and washed with $CHCl_3$ (20 mL). The aqueous layer was evaporated and the solid was washed with $CH_2Cl_2$ to yield colorless crystals of **16**. Yield: 337 mg (42%), m.p. 170.2–171.2 °C. IR (KBr): $\nu$ 3800–2700 (COOH), 1725 (C=O), 1695 (C=O) cm$^{-1}$. $^1$H NMR (300 MHz, acetone-$d_6$): $\delta$ 9.80 (bs, 2 H, COOH), 3.17 (t, 4 H, $^3J_{H-F}$ = 12.2 Hz, $CF_2(CH_2)_2$) ppm. $^{13}$C NMR (75 MHz, acetone-$d_6$): $\delta$ 42.60 (st, $J_{C-F}$ = 10.5 Hz, 1-C), 43.24 (tt, 2C, $J_{C-F}$ = 24.8 Hz, 2- and 4-C), 118.96 (st, $J_{C-F}$ = 274.5 Hz, 3-C), 171.16 (st, 2C, $J_{C-F}$ = 2.3 Hz, CO) ppm. MS (CIQI): *m/z* (rel. int.) 179 (M – H, 51), 140 (100). *Anal*. Calc. for $C_6H_6F_2O_4$, 180.11: C, 40.01; H, 3.36. Found: C, 40.17; H, 3.34%.

### 4.15. General procedure 1 (GP 1)

Diammine(diaqua)platinum(II) hydroxide **19** was synthesized from diammine(dichloro)platinum(II) (cisplatin) **17** as previously described [16]. After the solvent had been removed a glassy solid was obtained which was dissolved in a 1:1 mixture of water/ethanol just before reaction with the respective cyclobutanedicarboxylate ligands. Compounds **4**, **8**, **11**, **13** and **16** were dissolved in water before an equimolar amount of a solution of diammine(diaqua)platinum(II) hydroxide **19** was added. The solution was stirred for 5 d at room temperature under exclusion of light. The grey precipitate was filtered off. After removal of the solvent from the filtrate and recrystallization from $H_2O$/acetone at room temperature colorless crystals were obtained.

### 4.16. cis-Diammine(3-chloro-1,1-cyclobutanedicarboxylato)platinum(II) (**21**)

According to GP 1, **4** (71.4 mg, 0.402 mmol) was dissolved in 10 mL of water, combined with 0.4 mmol of the aqueous solution of diammine(diaqua)platinum(II) hydroxide **19** and stirred for 5 d. Yield: 90 mg (50%), colorless needles, m.p. 210 °C. IR (KBr): $\nu$ 3520–3200 ($NH_3$), 1630, 1370 (C=O) cm$^{-1}$. $^1$H NMR ($D_2O$): $\delta$ 4.67 (bs, 6 H, $NH_3$), 4.30 (qi, $^3J$ = 7.6 Hz, 1 H, C*H*-Cl($CH_2$)$_2$), 3.45/2.91 (2 m, 4 H, CHCl($CH_2$)$_2$) ppm. MS (LISI): *m/z* (rel. int.) 407 (MH, 100). *Anal*. Calc. for $C_6H_{11}ClN_2O_4Pt$, 405.70: C, 17.76; H, 2.73; N, 6.93. Found: C, 18.03; H, 2.96; N, 6.58%.

### 4.17. cis-Diammine(3-hydroxy-1,1-cyclobutanedicarboxylato)platinum(II) (**22**)

According to GP 1, **8** (65.1 mg, 0.407 mmol) was dissolved in 10 mL of water, combined with 0.4 mmol of the aqueous solution of diammine(diaqua)platinum(II) hydroxide **19** and stirred for 5 d. Yield: 74 mg (48%),

colorless needles, m.p. 250 °C. IR (KBr): *v* 3520–3200 ($NH_3$), 3280 (OH), 1630, 1380 (C=O) $cm^{-1}$. $^1H$ NMR ($D_2O$): $\delta$ 4.67 (bs, 7 H, $NH_3$, OH), 4.10 (qi, $^3J = 7.6$ Hz, 1 H, $CH(OH)(CH_2)_2$), 3.23, 2.50 (2 m, 4 H, $CH(OH)(CH_2)_2$) ppm. MS (LISI): *m/z* (rel. int.) 388 (MH, 100). *Anal*. Calc. for $C_6H_{12}N_2O_5Pt$, 387.25: C, 18.61; H, 3.12; N, 7.23. Found: C, 18.63; H, 3.30; N, 6.92%.

### 4.18. cis-Diammine(3-bromo-1,1-cyclobutanedicarboxylato)platinum(II) (**23**)

According to GP1, **11** (46.4 mg, 0.208 mmol) was dissolved in water (1 mL), combined with an equimolar aqueous solution of diammine(diaqua)platinum(II) hydroxide **19** and stirred for 5 d in the dark. Yield: 20.6 mg (22%), colorless crystals, m.p. 174–177 °C (decomp.). IR (KBr): *v* 3700–3000 ($NH_3$), 1645 (COO), 1605 (COO), 1390 $cm^{-1}$. $^1H$ NMR (300 MHz, $D_2O$): $\delta$ 4.70 (s, 6 H, $NH_3$), 4.42 (qi, 1 H, $^3J = 7.7$ Hz, $CH(CH_2)_2$), 3.59, 3.12 (2 m, 4 H, $CH(CH_2)_2$) ppm. MS (ESI): *m/z* (rel. int.) 451 [M($^{194}$Pt, $^{81}$Br and $^{196}$Pt, $^{79}$Br) + H, 100]. *Anal*. Calc. for $C_6H_{11}N_2O_4BrPt$, 450.14: C, 16.01; H, 2.46; N, 6.22. Found: C, 15.42; H, 2.67; N, 6.04%.

### 4.19. cis-Diammine(3-fluoro-1,1-cyclobutanedicarboxylato)platinum(II) (**24**)

According to GP1, **13** (33.2 mg, 0.205 mmol) was dissolved in water (1 mL), combined with an equimolar aqueous solution of diammine(diaqua)platinum(II) hydroxide **19** and stirred for 5 d in the dark. Yield: 20.0 mg (25%), colorless crystals, m.p. 226–229 °C (decomp.). IR (KBr): *v* 3700–3000 ($NH_3$), 1640 (C=O), 1595 (C=O), 1370, 1355 $cm^{-1}$. $^1H$ NMR (300 MHz, $D_2O$): $\delta$ 4.99 (d qi, 1H, $^3J_{H-F} = 56.1$ Hz, $^3J = 6.8$ Hz, $C(F)H(CH_2)_2$), 4.70 (s, 6 H, $NH_3$), 3.31, 2.88 (2 m, 4 H, $CHF(CH_2)_2$) ppm. MS (ESI): *m/z* (rel. int.) 390 [M($^{195}$Pt) + H, 100]. *Anal*. Calc. for $C_6H_{11}N_2O_4FPt$, 389.24: C, 18.51; H, 2.85; N, 7.20. Found: C, 18.36; H, 3.27; N, 6.66%.

### 4.20. cis-Diammine(3,3-difluoro-1,1-cyclobutanedicarboxylato)platinum(II) (**25**)

According to GP1, **16** (67.7 mg, 0.376 mmol) was dissolved in water (1 mL), combined with an equimolar aqueous solution of diammine(diaqua)platinum(II) hydroxide **19** and stirred for 5 d in the dark. Yield: 41 mg (27%), colorless crystals, m.p. 198–203 °C (decomp.). IR (KBr): *v* 3700–2900 ($NH_3$), 1645 (C=O), 1605 (C=O), 1395 $cm^{-1}$. $^1H$ NMR (300 MHz, $D_2O$): $\delta$ 4.70 (s, 6 H, $NH_3$), 3.41 (t, 4 H, $^3J = 12.6$ Hz, $CF_2(CH_2)_2$) ppm. MS (ESI): *m/z* (rel. int.) 408 [M($^{195}$Pt) + H, 100]. *Anal*. Calc. for $C_6H_{10}N_2O_4F_2Pt$, 407.23: C, 17.70; H, 2.48; N, 6.88. Found: C, 17.43; H, 2.45; N, 6.62%.

### 4.21. General procedure 2 (GP 2)

(*RR*/*SS*)-*trans*-1,2-Diaminocyclohexane(dichloro)-platinum(II) **26** was synthesized according to a literature procedure [17]. The activation of **26** was performed as previously described [18]. After the solvent had been removed a glassy solid was obtained which was dissolved in a 1:1 mixture of water/ethanol just before reaction with the respective cyclobutanedicarboxylate ligands. Compounds **1**, **4** and **8** were dissolved in water before an equimolar amount of a solution of (*RR*/*SS*)-*trans*-1,2-diaminocyclohexane-(diaqua)platinum(II) dihydroxide **27** was added. The solution was stirred for 5 d at room temperature under exclusion of light. The grey precipitate was filtered off. After removal of the solvent from the filtrate and recrystallization from $H_2O$ at room temperature colorless needles were obtained.

### 4.22. (RR/SS)-trans-1,2-diaminocyclohexane(1,1-cyclobutanedicarboxylato)platinum(II) (**28**)

**28** was synthesized according to a literature procedure [24].

### 4.23. (RR/SS)-trans-1,2-diaminocyclohexane(3-chloro-1,1-cyclobutanedicarboxylato)platinum(II) (**29**)

According to GP 2, **4** (71.4 mg, 0.402 mmol) was dissolved in 20 mL of water, combined with 0.4 mmol of activated **26**, and stirred for 5 d. Yield: 116.8 mg (60%), colorless needles, m.p. > 250 °C. IR (KBr): *v* 3260, 3200, 3110 (NH), 1630, 1360 (C=O) $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): $\delta$ 5.60 (m, 4 H, $NH_2$), 4.31 (qi, $^3J = 7.9$ Hz, 1 H, $CHCl(CH_2)_2$), 3.50, 2.80 (2 m, 4 H, $CHCl(CH_2)_2$), 2.04, 1.81, 1.45, 1.21, 1.02 (5 m, 10 H, $C_6H_{10}$) ppm. MS (ESI): *m/z* (rel. int.) 487 (MH, 100). *Anal*. Calc. for $C_{12}H_{19}ClN_2O_4Pt$, 485.83: C, 29.67; H, 3.94; N, 5.77. Found: C, 29.26; H, 4.07; N, 5.50%.

### 4.24. (RR/SS)-trans-1,2-diaminocyclohexane(3-hydroxy-1,1-cyclobutanedicarboxylato)platinum(II) (**30**)

According to GP 2, **8** (65.1 mg, 0.407 mmol) was dissolved in 20 mL of water, combined with 0.4 mmol of activated **26**, and stirred for 5 d. Yield: 46.2 mg (25%), colorless needles, m.p. 248 °C. IR (KBr): *v* 3260, 3220, 3120 (NH), 1620 (C=O), 1360 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): $\delta$ 5.51 (m, 4 H, $NH_2$), 4.91 (bs, 1 H, OH), 3.84 (qi, $^3J = 7.6$ Hz, 1 H, $CH(OH)(CH_2)_2$), 3.09, 2.31 (2 m, 4 H, $CH(CH_2)_2$), 2.03, 1.80, 1.45, 1.21, 1.01 (5 m, 10 H, $C_6H_{10}$) ppm. MS (ESI): *m/z* (rel. int.) 468 (MH, 100). *Anal*. Calc. for $C_{12}H_{20}N_2O_5Pt$, 467.38: C, 30.84; H, 4.31; N, 5.99. Found: C, 30.72; H, 4.65; N, 5.63%.

### 4.25. Cell cultures

Cell line banking and quality control were performed according to the seed stock concept [25]. Mycoplasma contamination was routinely monitored and only Mycoplasma-free cultures were used. All reagents not specified below (A-grade purity) were purchased from Merck (Darmstadt, Germany).

The human J82 (ATCC No. HTB-1) bladder cancer cell line was obtained from American Type Culture Collection (Rockville, MD, USA). The J82 (ATCC No. HTB-1) cells were maintained in Eagle's Minimum Essential Medium (Sigma–Aldrich Chemie GmbH, Munich, Germany), containing Lglutamine (1% v/v), $NaHCO_3$ (2.2 g/L), sodium pyruvate (110 mg/L) (Serva, Heidelberg, Germany), and 5% fetal calf serum (Biochrom KG, Berlin, Germany) using 75 mL culture flasks (Nunc, Wiesbaden, Germany) in a water-saturated atmosphere (95% air/5% $CO_2$) at 37 °C. The cells were passaged weekly following trypsinization using 0.05% trypsin/0.02% ethylenediaminetetraacetic acid (Roche Diagnostics, Mannheim, Germany).

The SK-OV-3 (ATCC no. HTB-77) cell line [20] was maintained in Mc Coy's 5A (Sigma–Aldrich Chemie GmbH, Munich, Germany) medium containing 15% new born calf serum (Biochrom KG, Berlin, Germany) and 2.2 g/L $NaHCO_3$. In our experiments we used cells from passage 77 [21].

The NIH:OVCAR-3 (ATCC no. HTB-161) wild type cells [26] were cultured in RPMI-1640 medium (Sigma–Aldrich Chemie GmbH, Munich, Germany) containing 10% Basal Medium Supplement (Biochrom KG, Berlin, Germany) and insulin (10 μg/mL) (Sigma–Aldrich Chemie GmbH, Munich, Germany) at 37 °C in a $H_2O$-saturated atmosphere of air and 5% $CO_2$ [27]. From these cells a cisplatin-resistant variant was established by treatment of the cells with increasing concentrations of cisplatin for 21 passages. The cisplatin-resistant variant was established by treatment of the cells with increasing concentrations of cisplatin for 21 passages. The platin-resistant NIH:OVCAR-3 cells were maintained analogous to the wild type cells [26], but in the presence of 0.25 μM cisplatin in the culture medium. The cells were serially passaged following trypnization using trypsin/EDTA (Roche Diagnostics GmbH, Mannheim, Germany), and were used from passage 56.

### 4.26. Drugs

Carboplatin was obtained from Strem Chemicals (Newburyport, USA). Carboplatin and compounds **21**–**25** were dissolved in water. As the diaminocyclohexaneplatinum complexes **28**–**30** were not soluble in water they were dissolved in DMF. For all drugs, 10 mM stock solutions were prepared. Feed solutions were made by appropriate dilution.

### 4.27. Chemosensitivity assays

The cells were seeded (100 μL/well) in 96 well flat-bottomed microtitration plates (Greiner Bio-One GmbH, Frickenhausen, Germany) at an appropriate density of approximately 15 cells/microscopic field (Leitz, Diavert, 320×). After 48 h, the medium was carefully removed by suction and replaced by fresh medium (200 μL/well) containing drugs (feed solutions were diluted 1:1000 with culture medium) or pure solvent. On each plate, 16 wells served as controls and 16 wells were used per drug concentration. After various times of incubation, the culture medium was shaken off and the J82 cells were fixed with 100 μL 1% glutardialdehyde in PBS/well (phosphate-buffered saline) for 25 min. The cells of the SK-OV-3 and NIH:OVCAR-3 cell lines were fixed with 100 μL/well of 3% glutaraldehyde in PBS for 30 min. The fixative was replaced by 180 μL of PBS/well, and the plates were stored in a refrigerator (4 °C). At the end of the experiment, the cells were simultaneously stained with 0.02% aqueous crystal violet solution (100 μL/well) for 20 min. Excess dye was removed by rinsing the microplates with water for 20 min. The stain bound by the cells was redissolved in 70% ethanol (180 μL/well) while shaking the microplates for about 3 h. Absorbance, a parameter proportional to cell mass [28], was measured at 578 nm using a BioTek 309 Autoreader (Winooski).

Drug effects were expressed as corrected $T/C$ values for each group according to

$$T/C_{\text{corr.}}\ [\%] = \frac{T - C_0}{C - C_0} \times 100$$

in which $T$ is the mean absorbance of the treated cells, $C$ is the mean absorbance of the controls, and $C_0$ is the mean absorbance of the cells at the time ($t = 0$) when the drug was added.

When the absorbance of treated cells $T$ is less than that of the culture at $t = 0$ ($C_0$), the extent of cell killing was calculated as

$$\text{cytocidal effect}\ [\%] = \frac{C_0 - T}{C_0} \times 100.$$

The relationship between growth kinetics of a drug-treated cell population and the plot of corrected $T/C$ values vs time is discussed in detail in the literature [13,14].

In the multiple point chemosensitivity assays the cultivation and seeding of the cells, the preparation of the stock solutions and the preparation of the wells of the microplates were carried out as described above. The plates were prepared for each compound in the concentrations indicated for five different periods of incubation. The first time point of the kinetics was chosen 24 or 72 h after drug administration depending on the cell line (Figs. 3–5). In each series an additional plate was used to determine the initial cell density.

*4.28. X-ray structure analysis of cis-diammine(3-hydroxy-1,1-cyclobutanedicarboxylato)platinum(II) (**22**)*

$C_6H_{12}N_2O_5Pt$; MW = 387.25; translucent plates; crystal size [mm]: 0.47 × 0.14 × 0.05; space group $P6_5$; hexagonal; $Z = 6$; *a*/*b*/*c* [Å] = 13.9539(10)/13.9539(10)/9.4556(8); $\alpha/\beta/\gamma$ [°] = 90/90/120; $V = 1594.5(2)$ Å$^3$; $\rho_{calc.} = 2.420$ g cm$^{-3}$; $F(000) = 1080$; $\mu = 13.20$ mm$^{-1}$; T = 173(1) K; $\omega$-scan $2.74 < \theta < 25.80°$; 11 040 reflections collected, 2027 independent, 1919 observed ($I > 2\sigma_I$); $R_{int} = 0.0712$; diffractometer STOE-IPDS (Mo Kα, graphite monochromator); $R(I > 2\sigma_I)$: $R_1 = 0.0233$; $R_2 = 0.0560$; $R$(all data): $R_1 = 0.0260$; $\omega R_2 = 0.0570$; Goof = 1.062. CCDC 203641.

*4.29. X-ray structure analysis of (RR/SS)-trans-1,2-diaminocyclohexane(3-hydroxy-1,1-cyclobutanedicarboxylato)platinum(II) (**30**)*

$C_{12}H_{20}N_2O_5Pt \cdot H_2O$; MW = 485.40; translucent plates; crystal size [mm]: 0.44 × 0.08 × 0.06; space group $P\bar{1}$; triclinic; $Z = 4$; *a*/*b*/*c* [Å] = 11.6676(17)/11.9773(16)/13.1699(17); $\alpha/\beta/\gamma$ [°] = 98.167(16)/103.029(16)/118.268(14); $V = 1511.4(5)$ Å$^3$; $\rho_{calc.} = 2.129$ g cm$^{-3}$; $F(000) = 932$; $\mu = 9.312$ mm$^{-1}$; $T = 173(1)$ K; $\omega$-scan $2.20 < \theta < 25.74°$; 14 866 reflections collected, 5356 independent, 4403 observed ($I > 2\sigma_I$); $R_{int} = 0.0304$; diffractometer STOE-IPDS (Mo Kα, graphite monochromator); $R(I > 2\sigma_I)$: $R_1 = 0.0259$; $\omega R_2 = 0.0624$; $R$(all data): $R_1 = 0.0344$; $\omega R_2 = 0.0647$; Goof = 0.958. CCDC203640.

Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2, UK (fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

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