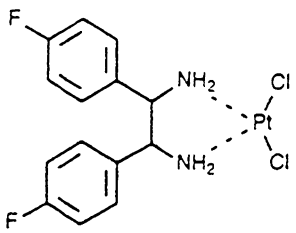
D-17446

Antineoplastic

(±)-(D,L)-[1,2-Bis(4-fluorophenyl)ethylenediamine]dichloroplatinum(II)



$C_{14}H_{14}Cl_2F_2N_2Pt$ Mol wt: 514.27

EN: 160572

Synthesis

The racemic dichloroplatinum(II) complex D-17446 and its *meso* configurated diastereoisomer were synthesized according to Scheme 1 by reacting K_2PtCl_4 with (D,L)- and *meso*-1,2-bis(4-fluorophenyl)ethylenediamine in water at pH 5.5-6.5 at temperatures below 60°. Complexation with K_2PtI_4 led to the diiodoplatinum(II) complexes. The corresponding sulfatoplatinum(II) complexes were obtained by addition of Ag_2SO_4 to the aqueous suspensions of the diiodoplatinum(II) complexes.

The diastereoisomeric 1,2-bis(4-fluorophenyl)ethylenediamines were synthesized according to the method described by Vögtle and Goldschmitt (1). *Meso*-1,2-bis(4-fluorophenyl)ethylenediamine was obtained from 4-fluorobenzaldehyde and *meso*-(2-hydroxyphenyl)ethylenediamine by a stereospecific *meso-meso* diaza-Cope-rearrangement reaction and subsequent hydrolysis of the product with H_2SO_4. The racemic compound was synthesized by *meso*-D,L stereoisomerization of *N,N'*-bis(4-fluorobenzylidene)-*meso*-1,2-bis(4-fluorophenyl) ethylenediamine at temperatures below 200° followed by hydrolysis with H_2SO_4. Separation of the diastereoisomers was carried out by fractional crystallization of the dihydrosulfates yielding the less water soluble D,L-diamine.

Introduction

The search for new antitumor cisplatinum analogs is stimulated by the desire to find a drug for p.o. administration with either less severe side effects or with activity against tumors that exhibit primary or acquired resistance to cisplatin. So far in the clinic no platinum-containing drug has clearly demonstrated therapeutic effect in the treatment of breast cancer.

Investigations on the influence of ring substituents on the antitumor effect of dichloro(1,2-diphenylethylenediamine)platinum(II) complexes uncovered D-17446 (the racemic 4-fluoro-substituted derivative) as the most active compound against the P388 leukemia prescreen *in vivo* and the MDA-MB-231 human breast cancer cell line *in vitro* (2).

Pharmacological Actions

Resolution of D-17446 into its enantiomers failed to enhance the antineoplastic effect (3). The antitumor activity of D-17446 could neither be improved by the synthesis of isomers containing fluoride in the *ortho* and *meta* position (4, 5) nor by the introduction of a second fluorine substituent into the 1,2-diphenylethylenediamine ligand (6, 7).

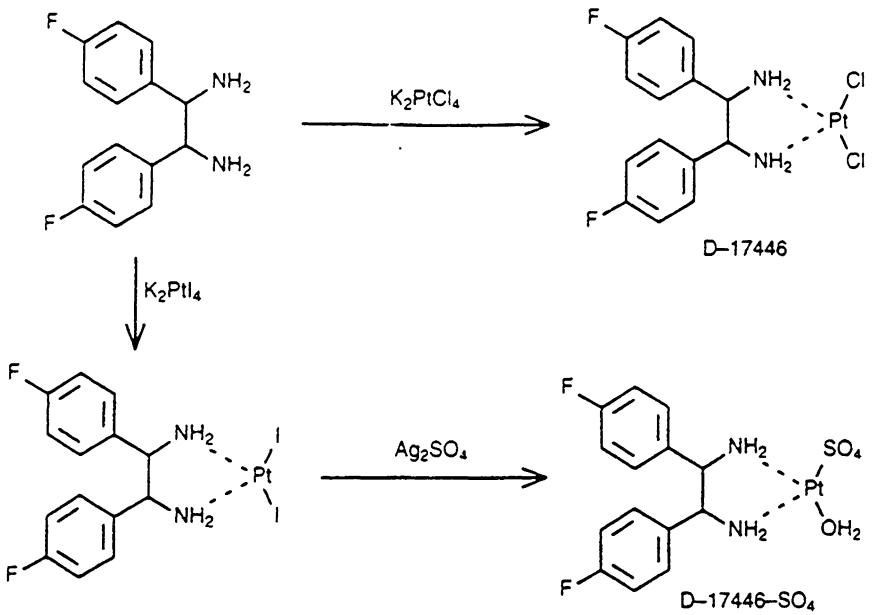
The antileukemic activity of D-17446 and its *meso*-configurated diastereoisomer on the P388 D1 cell line after 48 h of *in vitro* drug exposure were comparable with cisplatin. However, D-17446 produced the half maximal effect much faster ($t_{1/2}$ = 3.3 h) than its *meso*-configurated counterpart. Inhibition of [^{3}H]-thymidine incorporation after short-term contact of the cells with D-17446 was similar to the inhibition observed after permanent drug exposure, thus indicating a rapid uptake of this platinum complex by the tumor cell (5). In addition D-17446 was markedly active on a subline of the L1210 leukemia of the mouse which is about 100-fold resistant to cisplatin (5).

The determination of acute toxicity after i.p. injection of D-17446 resulted in an LD_{50} of > 1000 mg/kg mouse (Asta communication). However, this result is compromised by the extremely low water solubility of the dichloro complex.

Remarkably, (D,L)-[1,2-bis(4-fluorophenyl)ethylenediamine]dichloroplatinum(II) (D-17446) inhibited both the hor-

Günter Bernhardt[1], Herta Reile[1], Thilo Spruss[1], Marion Koch[1], Ronald Gust[1], Helmut Schönenberger[1], Michael Hollstein[2], Franz Lux[2] and Jürgen Engel[3]. [1]Inst. für Pharmazie der Universität Regensburg (SFB234); [2]Inst. für Radiochemie der Technischen Universität München; [3]ASTA Pharma, Frankfurt a.M., Germany.

Scheme 1: Synthesis of D-17446



mone-insensitive MDA-MB-231 (2) and the hormone-sensitive MCF-7 (3) human breast cancer cell line *in vitro*. This mammary tumor inhibiting activity was not restricted to *in vitro* experiments. The more soluble sulfatoplatinum(II) derivative of D-17446 (D-17446-SO_4) was also highly effective *in vivo* on the ovarian-dependent and the hormone-insensitive MXT mammary carcinoma of the B6D2F1 mouse (8).

For the following detailed investigations we chose a representative panel of human mammary carcinoma cell lines (MDA-MB-231, MCF-7, ZR-75-1 and T-47-D) characterized by approximately ten-fold *in vitro* resistance to cisplatin compared with the murine MXT mammary carcinoma cells. All breast cancer models were available in cell culture and as solid tumors implanted subcutaneously into the flank of NMRI nude mice. For *in vitro* chemosensitivity testing we developed a computerized kinetic crystal violet microassay (10, 11) based on the quantitation of the total dye binding capacity of the cells which is directly proportional to the tumor mass. This method allows the convenient collection of large sets of data necessary for the analysis of the proliferation kinetics of the drug-treated cell population and the untreated control, providing results of high statistical significance. By variation of the experimental protocol important data such as drug stability, effective concentration, the time required for net cell kill (a prerequisite for a curative effect) for the establishment of optimal *in vivo* schedules can be easily obtained *in vitro*.

The diastereoisomeric [1,2-bis(4-fluorophenyl)ethylenediamine]dichloroplatinum(II) complexes and cisplatin were extensively tested on the MDA-MB-231, MCF-7, ZR-75-1 and T-47-D human breast cancer cell lines *in vitro*. In all experiments with permanent drug exposure D-17446 was superior to the lead compound cisplatin. Except for minor quantitative differences in chemosensitivity to platinum-containing drugs (cell lines are ranked according to increasing sensitivity: MDA-MB-231 < MCF-7 less than or equal to ZR-75-1 < T-47-D), the results were qualitatively the same. The situation is illustrated for MCF-7 in Figure 1.

MCF-7 cells were treated with D-17446-SO_4 (racemate), the *meso*-configurated analog and cisplatin in three concentrations (0.5, 1 and 5 mcM) (the start of the time axis in Fig. 1). In this experiment the drug (and vehicle) containing culture media were not changed during the entire incubation period.

The extent of dose dependence decreased from the *meso*-configurated compound (Fig. 1B) to the racemate (Fig. 1A) to cisplatin (Fig. 1C), parallelled by an increase in the antiproliferative activity for the 0.5 and 1 mcM doses. At the highest concentration (5 mcM) all three platinum complexes produced complete inhibition of cell proliferation.

Comparative studies with the dichloroplatinum(II) compound D-17446 provided results which were similar to the data obtained from analogous experiments with (D,L)-[1,2-bis(4-fluorophenyl)ethylenediamine]sulfatoplatinum(II).

Unfortunately, these platinum complexes and cisplatin were ineffective on MDA-MB-231, MCF-7 and ZR-75-1 implanted into nude mice (Fig. 2), when the drugs were applied according to conventional schedules (three times a week, s.c. and i.p.).

To find the optimal conditions for an *in vivo* schedule and the time of drug exposure necessary to achieve maximal inhibitory effects, MCF-7 cells were incubated in the presence of 5 mcM of D-17446-SO_4 for 4, 6 and 12 h. After the indicated times of drug exposure the platinum complexes were removed by medium exchange and their antineoplastic ac-

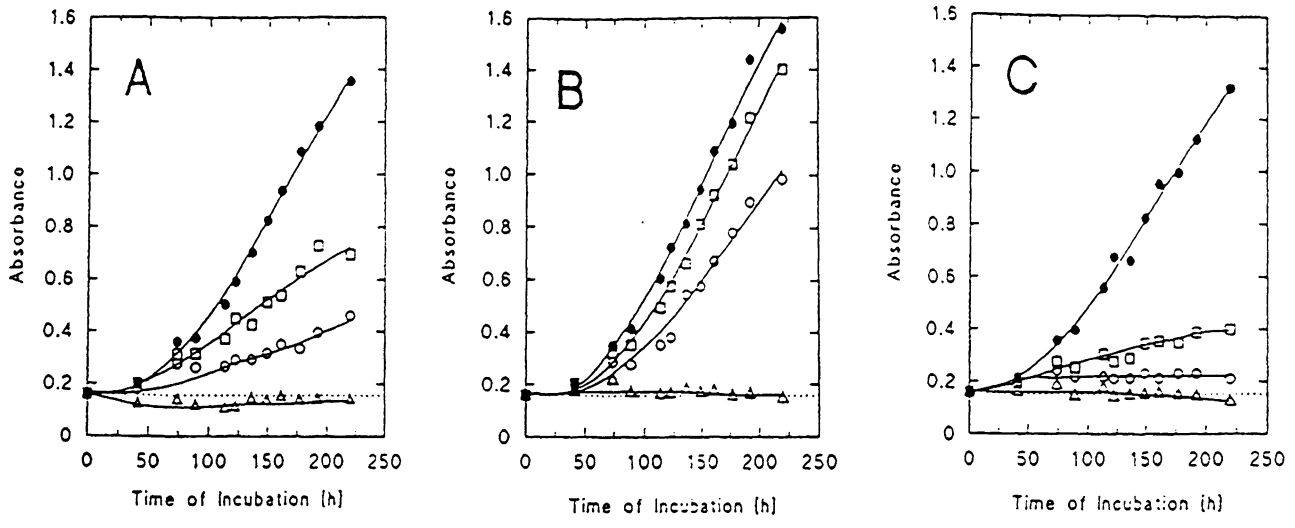


Fig. 1.

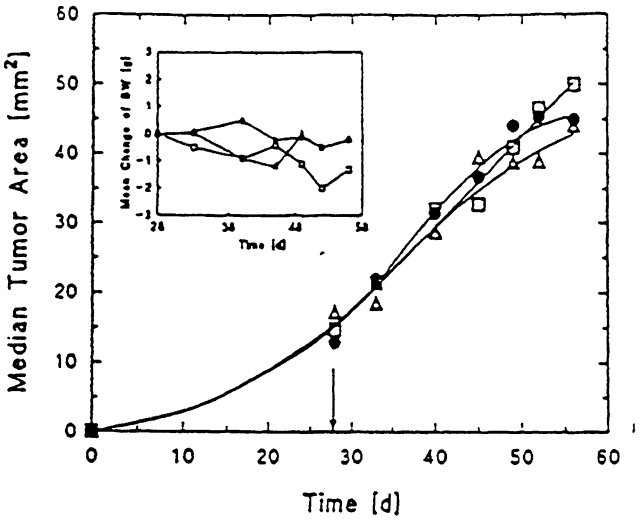


Fig. 2.

tivity was determined after various times of incubation of the cultures in fresh medium. Direct proportionality of the duration of drug exposure and the inhibition of cell proliferation was observed. All three platinum complexes displayed maximum activity after 12 h. Surprisingly, after 6 h of incubation, D-17446-SO_4 and cisplatin produced comparable degrees of inhibition (around 80%), although the (D,L)-[1,2-bis(4-fluorophenyl)ethylenediamine]sulfatoplatinum(II) was definitely less stable than cisplatin in culture medium (12). On the contrary, 12 h of drug exposure were necessary for its *meso*-configurated counterpart to become equiactive at the given concentration. In addition, the onset of action of D-17446-SO_4 was markedly faster in comparison with cisplatin and its *meso*-configurated counterpart.

Becaue of the relatively fast inactivation of D-17446-SO_4 (12), the lesions which finally cause inhibition of cell proliferation and cell death must be produced within the first few hours of the chemosensitivity assay.

MCF-7 cells were incubated with 5 mcM (D,L)-[1,2-bis-(4-fluorophenyl)ethylenediamine]sulfatoplatinum(II), *meso*-[1,2 - bis(4 - fluorophenyl) ethylenediamine] sulfatoplatinum(II) and cisplatin. Platinum uptake was determined by neutron activation analysis (NAA). In contrast to cisplatin, in the case of the diastereoisomeric [1,2-bis(4-fluorophenyl)ethylenediamine]sulfatoplatinum(II) complexes platinum was selectively accumulated with high specificity (Fig. 3). For D-17446-SO_4 accumulation was very rapid, *i.e.*, an intracellular Pt concentration of around 70 mcM was reached within 4 h (12).

Platinum uptake for compound D-17446-SO_4 was highly specific and faster than for its *meso*-configurated counterpart. This discrimination between diastereoisomers supports evidence that the uptake of the diastereoisomeric [1,2 - bis - (4 - fluorophenyl)ethylenediamine]sulfatoplatinum(II) complexes by MCF-7 cells is carrier mediated.

Pharmacokinetics

Measurements of platinum by NAA in plasma and in its ultrafiltrate after application of 10 mcmol/kg of D-17446-SO_4 to NMRI nude mice clearly demonstrated that neither the low water solubility nor the resorption of this platinum complex caused the negative results *in vivo*. Within the first few hours, plasma concentrations of around 10 mcM were reached after a single i.p. injection. However, the [1,2-bis(4-fluorophenyl)ethylenediamine]platinum(II) complexes were extensively bound to plasma protein (Fig. 4). As a consequence, in the nude mouse the effective drug concentration is far too low to produce a therapeutic effect.

In model experiments using human serum albumin (HSA) at concentrations equivalent to the *in vitro* (3.4 mg/ml) and

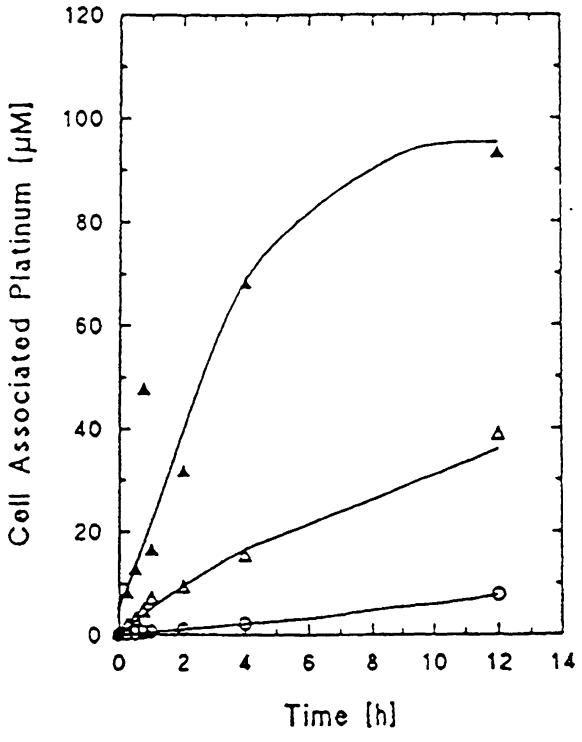


Fig. 3

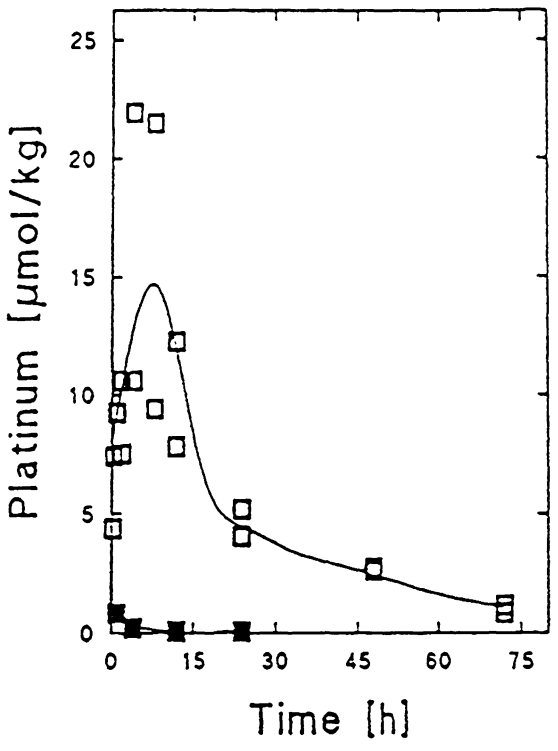


Fig. 4

the *in vivo* (34 mg/ml) situation, we studied the protein binding capacity of selected platinum complexes. Albumin dissolved in PBS was incubated in the presence of 3 mcM platinum complex at 37°. After 4 h, total and free (size of exclusion: 10 Kd) platinum was measured by AAS. The results are summarized in Table I. Compared with carboplatin and cisplatin the [1,2-bis(4-fluorophenyl)ethylenediamine]platinum(II) complexes bound strongly to serum albumin.

Table I: Binding of platinum complexes to 3.4 and 34 mg/ml HSA dissolved in PBS. After 4 h of incubation with 3 mcM platinum complex at 37° ultrafiltrable platinum was determined by AAS.

	Free Platinum in [%]	
	HSA Concentration [mg/ml]	
Compound	3.4	34
racemic-4F-$PtSO_4$ (D-17446-SO_4)	38	3.4
meso-4F-$PtSO_4$	42	2.7
racemic-4F-$PtCl_2$ (D-17446)	51	8.7
Cisplatin	92	43
Carboplatin	>90	>90

The reactivities of the intermediately formed diaquaplatinum(II) species (active drug) were identical, regardless of the differences in stereochemistry. Under conditions simulating both the *in vitro* (3.4 mg/ml) and the *in vivo* situation (34 mg/ml) D-17446 was slightly less reactive than D-17446-SO_4.

Surprisingly, protein binding depended considerably on the serum albumin concentration, although in both experimental series a high molar excess of HSA was used (Table I). Equilibrium dialysis experiments revealed that platinum was bound irreversibly. On this basis the discrepancy between the positive results of the *in vitro* chemosensitivity assays and therapeutic ineffectiveness *in vivo* can easily be explained.

Conclusions

In summary, D-17446 is a promising candidate for further development because of some principal advantages compared with the universe of new cisplatinum analogs investigated in early phases of preclinical trials: 1) activity on tumor models (*e.g.*, L1210) which are resistant to cisplatin; 2) high activity against both hormone-sensitive and hormone-insensitive mammary carcinomas; 3) specific accumulation by human hormone-sensitive and hormone-insensitive mammary carcinomas due to the 1,2-bis(4-fluorophenyl)ethylenediamine ligand. The poor bioavailability caused by the high reactivity of the presently available sulfato and dichloro[1,2 - bis(4 - fluorophenyl) ethylenediamine] platinum(II) complexes seems to be the major limitation of this class of cisplatinum analogs.

Manufacturer

Asta Pharma (Germany).

Acknowledgements

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Legends to the Figures

Fig. 1: Long term incubation of MCF-7 cells with the platinum complexes. Effect of drug concentration (□) 0.5 μM; (○) 1 μM; (△) 5 μM on cell proliferation (●) control.
Panel A: D,L-[1,2-bis(4-fluorophenyl)ethylenediamine]sulfatoplatinum(II)
Panel B: [meso-1,2-bis(4-fluorophenyl)ethylenediamine]sulfatoplatinum(II)
Panel C: cisplatin.

Fig. 2: Effect of D-17446 and its meso-configurated counterpart on the proliferation of the human MCF-7 mammary carcinoma implanted into NMRI nude mice (12 animals per group). The platinum complexes were administered at a dose of 10 μmol/kg i. p. three times a week. The arrow indicates the beginning of treatment.
Inset: Mean changes of body weight. At the maximum tolerated dose (3 μmol/kg i. p.) cisplatin was also ineffective (data not shown).
(●) control
(□) D,L-[1,2-bis(4-fluorophenyl)ethylenediamine]dichloroplatinum(II) (D-17446)
(△) [meso-1,2-bis(4-fluorophenyl)ethylenediamine]dichloroplatinum(II).

Fig. 3: Platinum accumulation by MCF-7 cells. The cultures were treated with 5μM platinum complex in MEM Eagle's medium containing 10% FCS.
The accumulated platinum was measured by neutron activation analysis and is expressed on the basis of mean μM intracellular concentration.
(▲) D,L-[1,2-bis(4-fluorophenyl)ethylenediamine]sulfatoplatinum(II)
(△) [meso-1,2-bis(4-fluorophenyl)ethylenediamine]sulfatoplatinum(II)
(○) cisplatin

Fig. 4: Pharmacokinetics of D-17446-SO^4 after a single i. p. injection (10 μmol/kg, vehicle water) into female NMRI nude mice.
Platinum in plasma (□) and its ultrafiltrate (■) was measured by NAA.