Tumor Inhibiting [1,2-Bis(fluorophenyl)ethylenediamine]platinum(II) Complexes, III[1]:

Evaluation of the Mammary Tumor Inhibiting Properties

Herta Reile, Thilo Spruß, Richard Müller, Ronald Gust, Günther Bernhardt, and Helmut Schönenberger*

Institut für Pharmazie, Lehrstuhl für Pharmazeutische Chemie II der Universität Regensburg, Sonderforschungsbereich 234, Universitätstraße 31, D-8400 Regensburg, FRG

Jürgen Engel

Asta Pharma AG, Postfach 10 01 05, D-6000 Frankfurt/Main 1, FRG

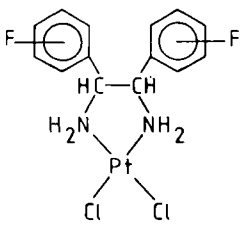
Received May 8, 1989.

Diastereomeric diaqua[1,2-bis(4-fluorophenyl)ethylenediamine]platinum(II) sulfates and nitrates produce a strong inhibition of the hormone-dependent MXT-M 3.2 mammary carcinoma of the B6D2F1 mouse. Besides an interference in the DNA synthesis in analogy to cisplatin a lowering of the estrogen level due to an interference in steroid biosynthesis is suggested as the mode of action. In contrast to the R,R/S,S configurated diaqua[1,2-bis(4-fluorophenyl)ethylenediamine]platinum(II) salts the corresponding R,S configurated compounds are also markedly active on the hormone-independent MXT-Ovex mammary carcinoma of the B6D2F1 mouse.

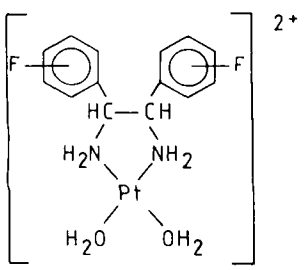
Tumorhemmende [1,2-Bis(fluorphenyl)ethylendiamin]platin(II)-Komplexe, 3. Mitt.[1]:
Untersuchung auf mammatumorhemmende Eigenschaften

Diastereomere Diaqua[1,2-bis(4-fluorphenyl)ethylendiamin]platin(II)sulfate and -nitrate rufen eine starke Hemmung des hormonabhängigen MXT-M 3.2 Mammacarcinoms der B6D2F1 Maus hervor. Neben einem dem Cisplatin analogen Eingriff in die DNA-Synthese wird die Absenkung des Östrogenspiegels durch Eingriff in die Steroidbiosynthese für die Wirkung verantwortlich gemacht. Im Gegensatz zu den R,R/S,S-konfigurierten Diaqua[1,2-bis(4-fluorphenyl)ethylendiamin]platin(II)salzen sind die entspr. R,S-konfigurierten Verbindungen auch am hormonunabhängigen MXT-Ovex-Mammacarcinom der B6D2F1 Maus deutlich wirksam.

The isomeric [1,2-bis(fluorophenyl)ethylenediamine]platinum(II) complexes proved to be comparably active antitumor compounds in long term experiments (48 h) on the P 388 D1 leukemia cell line, irrespective of the position of the fluorine atoms (ortho, meta or para) and the nature of the "leaving group" (Cl^- or H_2O)[1]. However, the compounds of the R,R/S,S series (**13** to **15**, **25** to **27**, **31**) are more active than those with R,S configuration (**16** to **18**, **28** to **30**, **32**) and comparable to cisplatin[1]. In this publication we show that isomeric [1,2-bis(fluorophenyl)ethylenediamine]platinum(II) complexes also possess a marked effect on several breast cancer models.



Compd.	Config.	F-Position	Abbreviation
13	D,L	2	D,L-2F-$PtCl_2$
14	D,L	3	D,L-3F-$PtCl_2$
15	D,L	4	D,L-4F-$PtCl_2$
16	Meso	2	Meso-2F-$PtCl_2$
17	Meso	3	Meso-3F-$PtCl_2$
18	Meso	4	Meso-4F-$PtCl_2$



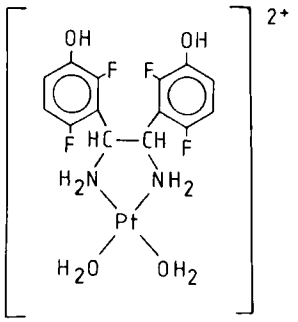
Compd.	Config.	F-Position	Counter Ion	Abbreviation
25	D,L	2	SO_4	D,L-2F-$PtSO_4$
26	D,L	3	SO_4	D,L-3F-$PtSO_4$
27	D,L	4	SO_4	D,L-4F-$PtSO_4$
28	Meso	2	SO_4	Meso-2F-$PtSO_4$
29	Meso	3	SO_4	Meso-3F-$PtSO_4$
30	Meso	4	SO_4	Meso-4F-$PtSO_4$
31	D,L	4	NO_3	D,L-4F-$Pt(NO_3)_2$
32	Meso	4	NO_3	Meso-4F-$Pt(NO_3)_2$

Results and discussion

Hormone-independent human MDA-MB 231 breast cancer cell line

On the MDA-MB 231 breast cancer cell line as well, an influence of the fluorine position (ortho, meta or para) on

  0365-6233/90/0505-0301 $ 02.50/0



Compd.	Config.	Counter Ion
33	Meso	SO_4

the antitumor activity was not detectable. The R,R/S,S configurated compounds inhibited the tumor growth to a larger extent than their R,S analogues. This study was performed with the isomeric diaqua[1,2-bis(fluorophenyl)ethylenediamine]platinum(II) sulfates (R,R/S,S series: **25** to **27**; R,S series: **28** to **30**; table 1). The same results were obtained with the analogous dichloroplatinum(II) complexes[2].

Hormone-dependent MXT-M 3.2 mammary carcinoma of the B6D2F1 mouse[3]

For the testing of the isomeric [1,2-bis(fluorophenyl)ethylenediamine]platinum(II) complexes we used the transplantable MXT-M 3.2 mammary tumor of the mouse (MXT-MC, ER^+) as a model for the hormone-dependent breast cancer. This tumor was induced by urethane treatment in C57BL x DBAfF1 mice by *Watson* and coworkers[3]. It is described as a ductal papillary carcinoma, which contains estrogen receptors (ER: 8 to 9 fmoles/mg wet weight)[3] and is strongly inhibited by ovariectomy, administration of antiestrogens like tamoxifen, or by pharmacological doses of estrogens [4,5]. Thus, it is similar to the human mammary carcinoma. For the testing of new compounds the tumor was implanted subcutaneously in fragments using female B6D2F1 mice as test animals.

One day later, treatment was started lasting 6 weeks. The substances were applied three times per week. At the end of

Table 1: Effect of diastereomeric diaqua[1,2-bis(fluorophenyl)ethylenediamine]platinum(II) sulfates on [^{3}H]-thymidine incorporation and cell proliferation of the hormone-independent human MDA-MB 231 breast cancer cell line, 48 h drug incubation

		Cell number		^{3}H-Thymidine Incorp.	
Compd.	Abbreviation	% T/C (at $1 \cdot 10^{-6}$M)	ED_{50}^a [M]	% T/C (at $1 \cdot 10^{-6}$ M)	ED_{50}^a [M]
25	D,L-2F-$PtSO_4$	20	$3.3 \cdot 10^{-7}$	6	$2.1 \cdot 10^{-7}$
26	D,L-3F-$PtSO_4$	28	$5.0 \cdot 10^{-7}$	4	$1.7 \cdot 10^{-7}$
27	D,L-4F-$PtSO_4$	20	$2.0 \cdot 10^{-7}$	21	$3.3 \cdot 10^{-7}$
28	Meso-2F-$PtSO_4$	78	$2.2 \cdot 10^{-6}$	31	$6.5 \cdot 10^{-7}$
29	Meso-3F-$PtSO_4$	66	$1.1 \cdot 10^{-6}$	23	$6.1 \cdot 10^{-7}$
30	Meso-4F-$PtSO_4$	42	$6.1 \cdot 10^{-7}$	30	$5.5 \cdot 10^{-7}$

[a] ED_{50} = the effective dose which decreases the tumor growth by 50%; mean of two to four tests.

Table 2: Effect of [1,2-bis(fluorophenyl)ethylenediamine]platinum(II) complexes on the hormone-dependent MXT mammary carcinoma of the B6D2F1 mouse

Compd.	Abbreviation	dose[a] x 10^{-5} (mol/kg)	Median tumor weight (mg)	% T/C[b]	Change in body weight (g)[g]	Uterotrophic effect[b,c]
13	D,L-2F-$PtCl_2$	2	1462 (257-2735)	123	-0.3	72[e]
14	D,L-3F-$PtCl_2$	2	1360 (175-2408)	114	-0.4	81
15	D,L-4F-$PtCl_2$	2	1299 (414-3102)	109	±0.0	87
16	Meso-2F-$PtCl_2$	2	1017 (18-1803)	85	-0.1	96
17	Meso-3F-$PtCl_2$	2	1257 (706-2978)	105	-0.5	73[f]
18	Meso-4F-$PtCl_2$	2	700 (161-1868)	59	-0.8	74[f]
27	D,L-4F-$PtSO_4$	2	103 (0- 439)	8[d]	-0.9	34[d]
30	Meso-4F-$PtSO_4$	2	64 (0- 423)	5[d]	-0.3	35[d]
31	D,L-4F-$Pt(NO_3)_2$	2	154 (29- 826)	13[d]	-0.2	39[d]
32	Meso-4F-$Pt(NO_3)_2$	2	81 (0- 851)	7[d]	-0.2	46[d]
Cisplatin		0.5	82 (0- 530)	7[d]	-0.3	85

[a] Compounds were administered three times a week (Monday, Wednesday, and Friday), sc. as solution or suspension in polyethyleneglycol 400/H_2O, 1:1

[b] Determined at the end of the 6-week therapy; the U test according to *Wilcoxon, Mann,* and *Whitney* was used

[c] Uterotrophic effect = [uterus dry weight (mg)/body weight (g)] · 100

[d] Significant ($p < 0.01$)

[e] Significant ($p < 0.025$)

[f] Significant ($p < 0.05$)

[g] (body weight day 1) - (body weight day 6)

therapy we estimated the tumor weight as well as the uterine dry weight, which gives additional hints on the mode of action or on side effects (e.g. estrogenic or antiestrogenic properties). Among the isomeric [1,2-bis(fluorophenyl)ethylenediamine]dichloroplatinum(II) complexes **13** to **18** only the R,S configurated 4-fluorine derivative **18** showed a moderate inhibition of tumor growth (% T/C = 59; not significant). This effect was accompanied by a weak but significant reduction of the uterine weight.

To confirm these results the related R,S configurated diaqua[1,2-bis(4-fluorophenyl)ethylenediamine]platinum(II) sulfate (**30**) and nitrate **32** as well as - for comparison - the R,R/S,S configurated diaqua[1,2-bis(4-fluorophenyl)ethylenediamine]platinum(II) sulfate (**27**) and nitrate **31** and also cisplatin were tested. Under physiological conditions these diaquaplatinum(II) compounds are formed from the non-reactive prodrug **15** and **18**. They inhibit the in vitro DNA synthesis catalysed by *E. coli*-DNA-polymerase I as a result of coordination reaction with DNA, presumably in N-7 position of guanine. The kinetics of inhibition is faster in the case of the R,R/S,S configurated compounds **27** and **31** than of their analogues with R,S configuration **30** and **32**[6]. It is supposed that these more water soluble compounds **27, 30** to **32** yield higher blood levels and thus stronger antitumor effects than the corresponding dichloroplatinum(II) complexes **15** and **18**. In fact the diastereomeric diaqua[1,2-bis(4-fluorophenyl)ethylenediamine]platinum(II) sulfates **27** - R,R/S,S; **30** - R,S and nitrates **31** - R,R/S,S; **32** - R,S produce strong effects on the hormone-dependent MXT-MC, while the related dichloroplatinum(II) complexes are weakly active (**18**) or inactive (**15**). The effect of diaqua[1,2-bis(4-fluorophenyl)ethylenediamine]platinum(II) salts on the MXT-MC, ER^+ is independent of the ligand configuration and identical with that of cisplatin. In contrast to cisplatin, however, the diaqua[1,2-bis(4-fluorophenyl)ethylenediamine]platinum(II) salts **27** and **30** to **32** cause a strong decrease in uterine weight (Table 2). To find out, whether the effect of these compounds on the MXT-MC, ER^+ is due to antiestrogenic properties, their estrone-antagonistic activity was evaluated in the uterine weight test using juvenile mice.

Evaluation of estrogenic and antiestrogenic properties[7]

The administration of estrogens causes a stimulation of uterine growth in juvenile mice. This effect is used as a

Table 3: Studies on estrogenic and antiestrogenic properties of diastereomeric [1,2-bis(fluorophenyl)ethylenediamine]platinum(II) complexes and their ligands in the mouse uterine weight test

Compd.	Abbreviation	dose[a] (nmol)	Estrogenic activity[b] effect	UE, %	Antiestrogenic activity[c] effect	AUE, %
15	D,L-4F-$PtCl_2$	1000	12.1 ± 1.7	0.0	37.3 ± 3.0	9.6
		100	12.6 ± 4.7	1.8	42.8 ± 5.7	-9.3
		10	12.7 ± 3.3	2.2	38.6 ± 5.0	5.2
18	Meso-4F-$PtCl_2$	1000	13.7 ± 1.7	5.7	39.9 ± 6.1	0.7
		100	15.0 ± 2.8	10.4	42.0 ± 7.9	-6.5
		10	13.4 ± 4.0	4.7	37.1 ± 5.9	10.3
27	D,L-4F-$PtSO_4$	1000	9.4 ± 0.8[g]	-9.7	48.2 ± 12.4	-27.8
		100	11.5 ± 1.5	-2.2	39.9 ± 1.9	0.7
		10	11.6 ± 1.9	-1.8	36.1 ± 4.1	13.7
30	Meso-4F-$PtSO_4$	1000	16.9 ± 2.4[e]	17.2	44.8 ± 6.4	-16.2
		100	11.1 ± 2.1	-3.6	38.6 ± 8.6	5.2
		10	13.7 ± 3.3	5.7	32.1 ± 6.2[f]	27.5
Ligand	D,L	1000	13.2 ± 1.4	3.9	36.5 ± 2.8	12.4
		100	13.9 ± 2.8	6.5	41.0 ± 3.4	-3.1
		10	11.5 ± 3.0	-2.2	37.7 ± 5.6	8.2
Ligand	Meso	1000	12.4 ± 2.5	1.1	39.9 ± 3.6	0.7
		100	14.8 ± 1.6[f]	9.7	40.1 ± 6.7	0.0
		10	14.2 ± 2.3	2.3	41.7 ± 4.2	-5.5
Control		-	12.1 ± 2.5	0	11.0 ± 1.8	-
Estrone		0.4 (μg)	40.0 ± 6.9	100	40.1 ± 7.5	0.0

[a] Dose/animal per day

[b] Estrogenic activity (UE, %) = $(E_T-E_V)/(E_S-E_V) \cdot 100$. Effect = uterus dry weight (mg)/body weight (g) x 100. E_T = effect of test compound. E_V = effect of vehicle; E_S = effect of estrone standard (0.4 μg). Estrone produces a maximum stimulation of the uterine growth at a dose of 0.4 μg/mouse per day.

[c] Antiestrogenic activity (AUE, %) = $(E_S-E_{ST})/(E_S-E_V) \cdot 100$. E_S = effect of estrone standard (0.4 μg). E_{ST} = effect of standard under simultaneous application of test compound.

[d] The U-test according to Wilcoxon, Mann and Whitney was used

[e] Significant ($p < 0.01$)

[f] Significant ($p < 0.025$)

[g] Significant ($p < 0.05$)

parameter for the estrogenic potency of new compounds. In this experiment the drug is given on three subsequent days. The uterine dry weight is determined on day 4 and the per cent uterotrophic effect (UE, %) is calculated. Upon simultaneous administration of the drug and estrone (E1) the uterine growth stimulating effect of E1 is reduced if the drug possesses antiestrogenic properties. From the uterine dry weights the per cent antiuterotrophic effect (AUE, %) is calculated.

Except the R,S configurated diaqua[1,2-bis(4-fluorophenyl)ethylenediamine]platinum(II) sulfate (**30**) and its ligand, which produced a small but significant uterotrophic activity, none of the tested compounds showed any effect (Table 3). Complex **30** was the only compound which brought about a minor antiuterotrophic effect. These results are in contrast to the strong inhibition of uterine weight caused by the diaquaplatinum(II) complexes **27** and **30** to **32** in experiments on the MXT-MC, ER^+ (Table 2). We assume that this antiuterotrophic effect is due to a decrease of the estrogen level as the result of an interference in the steroid biosynthesis of the adult female mice[15]. [1,2-Bis(4-fluorophenyl)ethylenediamine]platinum(II) complexes, whose ligand is structurally similar to the nonsteroidal estrogen hexestrol, seem to have an affinity to enzymes of the estrogen biosynthesis since cisplatin itself produces no significant reduction of the uterine weight (cf. Table 3). Indeed we were able to prove a lowering of the estrogen level in rats by the structurally related compound **33** (Formula 3)[8]. The mode of action of the [1,2-bis(4-fluorophenyl)ethylenediamine]platinum(II) complexes corresponds with that of aromatase inhibitors, which influence the hormone-dependent mammary carcinoma by lowering the estrogen level.

However, these complexes are also markedly active on the hormone-independent mammary carcinoma depending on the ligand configuration (cf. Table 1 and 4). Therefore, it cannot be excluded that [1,2-bis(4-fluorophenyl)ethylenediamine]platinum(II) complexes exert their mammary tumor inhibiting properties mainly by inhibition of the estrogen biosynthesis as well as by interference with processes of the DNA synthesis (concerning the mode of action of cisplatin, cf. ref. 1 and 9).

Hormone-independent, MXT-Ovex mammary carcinoma of the B6D2F1 mouse[10]

Hormone-dependent, human breast cancers often show a transition to a gradually decreasing cellular differentiation and autonomous growth. This phenomenon (tumor progression) is thought to be a consequence of either clonal selection or epigenetic changes[11].

Molecular events of this depelopment of resistance to drugs which act via ER system are[11]:

1. Inactive receptor system, e.g. reduced ER concentration or dysfunctional ER.
2. Altered estrogen response of chromatin acceptor sites, e.g. nonfunctional or defective acceptor sites, or increased concentration of acceptor sites.
3. Development of alternative pathways for growth regulation.

Such tumors are resistant against endocrine therapy (e.g. ovariectomy, antiestrogens, aromatase inhibitors). Therefore, tumor models like the hormone-independent MXT-Ovex mammary carcinoma of the mouse (MXT-MC, ER^-) are important for the evaluation of new drugs. They show whether autonomous MC clones, which are responsible for the development of resistance, can be influenced by the test compound. This hormone-independent mouse mammary tumor line was derived by transplantation of the hormone-dependent line MXT-3590 into ovariectomized-adrenalectomized animals and subsequent transplantation of surviving tumors[10].

In the experiments on the hormone-independent MXT-MC the platinum(II) complexes were administered beginning one day after transplantation of the tumor in female B6D2F1 mice 3 times weekly for two weeks. Among the

Table 4: Effect of [1,2-bis(fluorophenyl)ethylenediamine]platinum(II) complexes on the hormone-independent MXT mammary carcinoma of the B6D2F1 mouse

Compd.	Abbreviation	dose[a] · 10^{-5} (mol/kg)	Median tumor area (mm^2)	% T/C[b]	Change in body weight (g)[f]
13	D,L-2F-$PtCl_2$	2	45 (2-130)	57	-0.8
14	D,L-3F-$PtCl_2$	2	70 (2-177)	89	-0.2
15	D,L-4F-$PtCl_2$	2	23 (6-214)	30	-0.1
16	Meso-2F-$PtCl_2$	2	76 (4-116)	97	-0.7
17	Meso-3F-$PtCl_2$	2	70 (1-145)	89	-0.5
18	Meso-4F-$PtCl_2$	2	21 (1- 90)	27[d]	-0.6
27	D,L-4F-$PtSO_4$	2	102 (57-175)	129	0.2
30	Meso-4F-$PtSO_4$	2	24 (3-151)	30[e]	1.2
31	D,L-4F-$Pt(NO_3)_2$	2	70 (1-104)	88	0.1
32	Meso-4F-$Pt(NO_3)_2$	2	21 (0- 80)	27[c]	0.9
Cisplatin		0.5	14 (0-150)	9[c]	0.3

[a] Compounds were administered three times a week (Monday, Wednesday, and Friday), sc. as solution or suspension in polyethyleneglycol 400/H_2O, 1:1

[b] Determined at the end of the 2-week therapy; the U test according to *Wilcoxon, Mann,* and *Whitney* was used

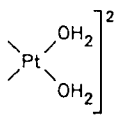
[c] Significant ($p < 0.01$)

[d] Significant ($p < 0.025$)

[e] Significant ($p < 0.05$)

[f] (body weight day 1) - (body weight day 6)

isomeric [1,2-bis(fluorophenyl)ethylenediamine]dichloroplatinum(II) complexes **13** to **18** only the R,S configurated 4-fluorine substituted compound **18** (% T/C = 27) showed a marked activity, which was significant versus control. For the R,R/S,S configurated analogue **15** we found a comparable but non-significant inhibition (% T/C = 30). Upon transformation of [(R,R/S,S)-1,2-bis(4-fluorophenyl)ethylenediamine]dichloroplatinum(II) (**15**) into the diaquaplatinum(II) sulfate **27** or nitrate (**31**) a considerable loss of activity is observed. By contrast the change from the R,S configurated dichloroplatinum(II) complex **18** into the diaquaplatinum(II) sulfate **30** or nitrate **32** does not result in a change of activity. The striking differences in activity of the diastereomeric diaquaplatinum(II) salts (**27** and **31** - R,R/S,S-configurated: inactive; **30** and **32** - R,S configurated: active) can bee explained as follows:

The active center  of the R,S configurated diaquaplatinum(II) complexes **30** and **32** is much more shielded than that of the R,R/S,S configurated analogues **27** and **31**. This gives rise to a delayed reaction with plasma proteins and other bionucleophiles to form inactive products during the transport to the tumor site, resulting in a higher drug level in the tumor and a better effect compared to the more reactive diastereomer (cf. ref. 1). In cell culture studies these inactivation processes are much less important, since the tumor cell faces a high drug concentration, at least at the beginning of the experiment. Thus a stronger effect of the more reactive R,R/S,S configurated diaquaplatinum(II) complex **27** is observed in cell culture experiments on the MDA-MB 231 breast cancer cell line.

The diastereomeric dichloroplatinum(II) complexes **15** and **18** are prodrugs, which yield the reactive tumor inhibiting diaquaplatinum(II) complexes **27** and **30** only after exchange of Cl by H_2O. Contrary to **30** the more reactive R,R/S,S configurated diaquaplatinum(II) complex **27** is inactivated during its transport to the tumor. Due to the prior formation of **27** from **15** the inactivation process is delayed. This affords a drug level (**15** and **27**) in the tumor which is sufficient for a therapeutic effect.

For the therapy of the hormone-dependent breast cancer the R,S configurated diaqua[1,2-bis(4-fluorophenyl)ethylenediamine]platinum(II) salts **30** and **32** are of special interest, since they inhibitit ER-positive and -negative mammary tumor cells as well. The latter are thought to be responsible for the development of resistance of the breast cancer during endocrine therapy.

In further investigations we will study whether the diaquaplatinum(II) salts **30** and **32** produce longer lasting remissions than the currently used endocrine therapies.

The technical assistance of F. Birk, S. Dehen, B. Hofmann, P. Pistor, and P. Richthammer is gratefully acknowledged. Thanks are also due to the Deutsche Forschungsgemeinschaft (SFB 234), the Matthias Lackas-Stiftung für Krebsforschung, and the Fonds der chemischen Industrie for financial support.

Experimental Part

MDA-MB 231 human breast cancer cell line [12,13]

Cells were grown in a humified incubator in 5% CO_2, at 37°C. *McCoy* 5a, supplemented with gentamycin (40 µg/ml), 10% NCS, and $NaHCO_3$ (11 g/5 l) was used as culture medium. The cells were harvested with trypsin/EDTA, diluted with 5% NCS containing medium, and gently syringed to prevent clumping. Approximately, $4 \cdot 10^4$ cells in 2 ml were plated in duplicate in 6-well dishes (Costar). Then 2 days later the medium was changed and the Pt complexes were added as freshly prepared 1000-fold concentrated solutions in dimethyl formamide or H_2O, leading to a final solvent concentration of 0.1%. The cells of control wells contained an equal volume of dimethyl formamide or H_2O. After an incubation time of 2 days, which complied with a triple duplication time, the cells were labeled with 0.5 mCi ^{3}H-thymidine/well for 2 h. Cells were washed with ice-cold PBS and harvested with PBS/EDTA buffer. After centrifugation, the cell pellet was resuspended in 1 ml of PBS and divided in two 0.5 ml aliquots. One part was counted in a ZI Coulter counter, the other one was sonicated. After addition of 4 ml of 10% trichloroacetic acid, the acid-insoluble fraction was collected on a 0.45 µm Filter (Metricel, Gelman) and counted after addition of 10 ml scintillation liquid.

Hormone-dependent, transplantable MXT-M 3.2 mammary tumor of the B6D2F1 mouse [1]

The applied method was identical with that described by us [14]. The tumor was transplanted in pieces of about 2 mm^3 (one tumor piece/animal) subcutaneously in female, 8-weeks-old B6D2F1 mice (body weight: 20 g, Charles River Wiga, West Germany). After transplantation, the animals were randomly distributed into groups of 10. Starting with the first day after transplantation, the test compounds were injected s.c. 3 times a week (Monday, Wednesday, Friday) as solution or suspension in polyethylene glycol 400/H_2O, 1:1 (0.1 ml/mouse). The duration of therapy was 6 weeks. At the end of treatment, the animals were killed by cervical dislocation and weighed. The tumors were removed, washed in 0.9% NaCl-solution, blotted dry, and weighed, and the average tumor weight was calculated. The uteri were also removed and prepared as described [14] to serve as an indicator of the estrogenic or antiestrogenic effects of the compounds.

Hormone-Independent, transplantable MXT-Ovex mammary tumor of the B6D2F1 mouse [10]

This tumor model was developed from MXT-M 3.2 tumors, which showed growth in ovariectomized B6D2F1 mice, and was propagated in ovariectomized B6D2F1 mice. Testing was performed in intact female B6D2F1 mice (Charles River Wiga, West Germany, 10 mice/group, age: 8 weeks at beginning of test, body weight: 20 g) as described in the preceding section. The duration of therapy was 14 days. On day 14 the tumor size (length x width/2) was determined by caliper measurements.

Estrogen and antiestrogen assays

Estrogenic and antiestrogenic properties were determined by stimulation of the uterine growth or by inhibition of the uterine growth stimulated by estrone, respectively, using immature NMRI mice as described[7]. Female mice (body weight: 10-12 g; age: 20 days at test beginning, 7 mice/group) were injected sc. daily for 3 consecutive days with solutions or suspensions of the test compounds in polyethylene glycol 400/H_2O, 1:1 (0.1 ml/mouse). The uteri were removed 24 h after the last injection, fixed with *Bouin's* solution, dried, and weighed.

References

1 Part II: H. Reile, R. Müller, R. Gust, R. Laske, W. Krischke, G. Bernhardt, Th. Spruß, M. Jennerwein, J. Engel, S. Seeber, R. Osieka, and H. Schönenberger, Arch. Pharm. (Weinheim) *323*, 133 (1990).

2 H. Reile, Diplomarbeit, Naturwissenschaftliche Fakultät IV - Chemie und Pharmazie, Universität Regensburg 1987.

3 Ch. Watson, D. Medina, and J.H. Clark, Cancer Res. *37*, 3344 (1977).

4 M.R. Schneider, E. von Angerer, J. Prekajac, and W.P. Brade, J. Cancer Res. Clin. Oncol. *111*, 110 (1986).

5 M.R. Schneider and E. Schönenberger, Urol. Int. *43* (suppl. 1), 10 (1988).

6 W. Schaller, H. Reisner, and E. Holler, Biochemistry *26*, 943 (1987).

7 M.R. Schneider, E. von Angerer, H. Schönenberger, R.-Th. Michel, and H.P. Fortmeyer, J. Med. Chem. *25*, 1070 (1982).

8 H. Schönenberger, R. Gust, J. Karl, Th. Spruß, M.R. Schneider, R.W. Hartmann, Ch. Batzl, S. Schertl, J. Engel, F. Lux, and S. Trebert-Haeberlin: "Rezeptorgebundene Chemotherapie" in "Antiöstrogene in Forschung und Klinik", Reihe Aktuelle Onkologie 46, Zuckschwerdt-Verlag, München 1989.

9 M. Ch. Sorenson and A. Eastman, Cancer Res. *48*, 6703 (1988).

10 Ch. Watson, D. Medina, and J.H. Clark, Endocrinology *107*, 1432 (1980).

11 P.S. Rennie in "Drug and Hormone Resistance in Neoplasia", Vol. I, Basic Concepts; N. Bruchovsky, J.H. Goldie, Eds. CRC-Press, Inc. Boca Raton, Florida 1982, p. 95.

12 R. Cailleau, R. Young, M. Olivé, and W.J. Reeves, Natl. Cancer Inst. 53, 661 (1974).

13 M.E. Lippman, M.E. Monaco, and G. Bolan, Cancer Res *37*, 1901 (1977).

14 M.R. Schneider, H. Ball, and H. Schönenberger, J. Med. Chem. *28*, 1880 (1985).

15 A strong inhibition of the MXT-MC, ER^+ accompanied by a significant reduction of the uterine dry weight was also seen in the case of [(S,S-1, 2-bis(4-hydroxyphenyl)ethylenediamine]-dichloroplatinum(II)[16].

16 Th. Spruß, R. Gust, R. Müller, J. Engel, and H. Schönenberger, Arch. Pharm. (Weinheim) *323*, 99 (1990).

[Ph675]