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Review

Optimization of cisplatin for the treatment of hormone-dependent tumoral diseases
Part 2: Use of non-steroidal ligands

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ABSTRACT

Platinum complexes with carrier ligands were synthesized to overcome intrinsic and acquired resistance of the tumors during the therapy. For the treatment of breast and prostate cancer steroidal and non-steroidal ligands are available. This article is a critical review of the attempts made during the last three decades to design platinum complexes with a selective mode of action against hormone-dependent tumors. In part 1 of this paper the use of steroidal carrier ligands is described while in part 2 the view is focussed on derivatives of non-steroidal drugs suitable to coordinate to platinum. Especially the [1,2-diarylethylenediamine]platinum(II) complexes are interesting analogous of cisplatin.

1. Introduction

In part I of this review [1] the state of art in the clinical investigation on Pt-complexes as potential drugs for breast and prostate cancer and the attempts to improve their inhibitory potency by the strategy of selective drug delivery are described. The latter approach is based on the fact that both tumors frequently contain estrogen receptors, ER_α and/or ER_β, and on the assumption that Pt-complexes with ER-affinic ligands are enriched in these tumors making them more active than their parent Pt-complexes.

The cytotoxicity of the Pt-pharmacophore (e.g. by DNA interaction) as well as the estrogenic potency of the carrier ligand (e.g. by interference with processes like angiogenesis important for tumor growth) caused anti-tumor activity. The prevention of untoward side effects of estrogenic Pt-complexes on the vascular system and on the incidence of breast cancer, which is increased in patients after long-term administration of estrogens, by use of specific ER_β agonists or SERMs as ligands is discussed.

Further topics in part I of this review are: (i) the strategy for linking steroidal ER ligands with Pt-complexes under retention of ER-affinity; (ii) a survey of the synthesized and pharmacologically evaluated steroidal Pt-complexes; (iii) the present concepts to their mode of action; (iv) the suggestion of a screening program on the basis of the latter.

In part II of this review studies on platinum complexes with non-steroidal, ER binding ligands are described. This particular chapter in the search for platinum complexes active against hormone-dependent breast and prostate cancer was opened by Schönenberger and his group and continued by Gust and co-workers. They used 1,2-diarylethylenediamines and related diamines (Sections 2–4) and 1,1,2-triarylethylenediamines (Section 5) as chelate ligands in cis-$PtCl_2$ complexes after they had recognized the ER affinity of the free ligands. They discussed a specific inhibitory effect of these Pt-complexes due to the enrichment of the latter in breast cancer cells, a process in which ERs function as carriers ("drug targeting concept") [2,3a,b]. Later on, Schlemmer et al. extended this concept of the mode of action [4a–f] (Section 2.2.3; compare also Section 6, part I of this review). Non-steroidal Pt-complexes in which the Pt-pharmacophore is linked via a spacer to SERMs of the hydroxytamoxifen [5], zindoxifene (ref. to the anti-prostate and anti-breast cancer activity of zindoxifene [2, 6a,b, 7a-c] and of zindoxifene-Pt-complexes [7d-h]) and triarylethylene type [8a–f] as well as related 3,4-diarylbenzopyran derivatives [8g] were also described (see Section 6). Finally it was shown that [1,2-bis(4-fluorophenyl)ethylenediamine]platinum(II) and related complexes fulfil at best the requirements of the drug targeting concept [9a–r] (Section 7).

2. [1,2-Diarylethylenediamine]platinum(II) complexes

2.1. Search for [1,2-bis(4-hydroxyphenyl)ethylenediamine] platinum(II) complexes with estrogen receptor affinity and estrogenic potency

The 1,2-diarylethylenediamines used as ligands in Pt-complexes are structurally related to the non-steroidal estrogen diethylstilbestrol (DES) and to its dihydro derivative hexestrol (HES), which themselves caused marked inhibitory activity on the growth of hormone-dependent breast cancers of rat and mouse [10a–d]. By variation of kind, number and position of the ring substituents and of the 1,2-standing alkyl residues in DES and HES, antiestrogens with marked breast cancer inhibiting properties like metastilbestrol and metahexestrol (3,3′-DES and 3,3′-HES, compounds with OH groups in 3- and 3′-position, formulae see part I of this review) were obtained [11a–d,12a–e,13a–c,14a–c].

However, only some 1,2-diarylethylenediamines derived from these compounds possessed ER affinity, accompanied by estrogenic, but never by antiestrogenic activity [3a,15a,b].

(RS)-1,2-Bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamine (**meso-1**) proved to be the estrogenically most active derivative of the series (established in the mouse uterine weight test) [3a,16a]. Its (RR/SS)-configurated analogue (**racem-1**) showed only marginal estrogenic potency. The ER affinity and estrogenic potency of the diastereomeric diamines could be markedly enhanced by introduction of one alkyl residue into one or both NH_2 groups [16a]. Among these derivatives the diastereomeric N,N′-diethyl-1,2-bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamines, which were tested on the DMBA-induced mammary carcinoma of the rat, produced a marked growth inhibitory effect [7a]. The (RS)-configurated compound showed a stronger tumor-inhibiting and estrogenic potency than the (RR/SS)-configurated counterpart, indicating the importance of ER-agonistic effects for the extent of the anti-breast cancer activity in vivo [7a].

Structure activity studies with **meso-1** revealed that the shift of both OH groups from the 4- into the 3-position and the successive exchange of the four 2,6-standing Cl atoms by F atoms were accompanied by a decrease of the estrogenic potency [16b]. The same was observed, if the substituent pattern of **meso-1** was changed exclusively in one phenyl ring [16b–d]. (RS/SR)-1-(2,6-Dichloro-4-hydroxyphenyl)-2-(2-halo-4-hydroxyphenyl)ethylenediamines with a Cl or F atom in ortho position of the 2-(4-hydroxyphenyl) residue (e.g. **erythro-2**, halo = Cl) caused weak but significant estrogenic effects [16b–d].

The coordination of the diamines to platinum did not weaken but, in some cases, even increased the estrogenic activities

despite of alterations in the spatial structure of the 1,2-diphenylethylenediamine moiety [16a,b].

[(RS)-1,2-bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamine] dichloroplatinum(II) (**meso-1-PtCl$_2$**) proved to be the most active estrogen of the Pt-complex series [3a,16a]. The relative binding affinity (RBA) of **meso-1-PtCl$_2$** to the ER in human ER$_\alpha$-positive MCF-7 breast cancer cells was only 0.35% compared to 17β-estradiol (E_2: RBA = 100%) [16e]. Nevertheless, **meso-1-PtCl$_2$** was able to induce ER processing and to increase the progesterone receptor (PR) level at concentrations of 1–10 nM [16e]. Comparable results were obtained with its ligand [16e].

Several leaving group derivatives **meso-1-PtLL′** (e.g. L = H_2O, L′ = SO_4; LL′ = Cl_2, I_2 or CBDC = cyclobutane-1,1-dicarboxylic acid) were also tested in a thoroughly described in vitro assay [16g,h] on ER$_\alpha$-agonistic activity by use of MCF-7-2a breast cancer cells stably transfected with the reporter plasmid ERE$_{wtc}$luc [16f]. The luciferase expression induced by drug-ER$_\alpha$-dimers bound to the ERE$_{wtc}$luc correlates very well with the estrogenic potency of the drug determined in the frequently used mouse uterine weight test. The study revealed a significant influence of the leaving group in **meso-1-PtLL′** on the ER binding [16f]. It was demonstrated that bulky leaving groups such as CBDC lowered the gene expression in the ER$_\alpha$-positive MCF-7-2a cells.

2.1.1. Stability of [1,2-bis(4-hydroxyphenyl)ethylenediamine] platinum(II) complexes under physiological conditions

Further studies demonstrated the high stability of these Pt-complexes proving an ER binding by the intact [1,2-diarylethylenediamine]platinum(II) chelate [16h]. If the aquasulfatoplatinum(II) complex (**meso-1-PtSO$_4$**) is used, the leaving groups are quickly exchanged by chlorine under test conditions. For the resulting dichloroplatinum(II) complex **meso-1-PtCl$_2$** luciferase formation was only observable for a short time, whereas its estrogenic ligand by itself remained active for longer times. Therefore, liberation of **meso-1** from its Pt-complex is very unlikely and seems not to play an important role in the mode of action of the latter.

Studies on the stability of **meso-1-PtCl$_2$** under cell culture conditions and use of [(RS)-1,2-bis(2,6-dichoro-4-hydroxy[3,5-$^3H_2$]phenyl)ethylenediamine]dichloroplatinum(II) for detection of released diamine were also performed [16i,k]. The results of this work corresponded with those of the former study.

2.1.2. Interaction of [1,2-bis(4-hydroxyphenyl)ethylenediamine] platinum(II) complexes with the ligand binding domain of the estrogen receptor

To understand the ER$_\alpha$-agonistic effect of the new Pt-complexes, Gust and co-workers [16l,m] developed binding models (Figs. 2 and 7) on the example of the ligand (RS/SR)-1-(2-chloro-4-hydroxyphenyl)-2-(2,6-dichloro-4-hydroxyphenyl)ethylenediamine (**erythro-2**, formula see Fig. 1) and of its diiodoplatinum(II) complex (**erythro-2-PtI$_2$**, formula see Fig. 1), which were active in the luciferase assay.

The binding model of the ligand **erythro-2** (Fig. 2) corresponds to that of non-steroidal estrogens, since in solution the predominant antiperiplanar arrangement of the two aryl rings, which is comparable to that of HES, allows an attachment at the ER$_\alpha$ analogously to DES (Fig. 2). The selective hormone recognition of the former non-steroidal estrogens by the ER$_\alpha$ occurs by a combination of H bonding and hydrophobic contacts. One phenolic OH group of these estrogens is bound by H-bridges to the γ-carboxylate of Glu 353, the guanidinium residue of Arg 394, and a water molecule. The second OH group is linked to His 524. Furthermore, hydrophobic interactions with aliphatic residues of amino acids above and below the molecule plain, mainly mediated by the two ethyl residues, are essential for significant ER$_\alpha$-agonistic effects. In the case of **erythro-2**, this function is implemented by the three

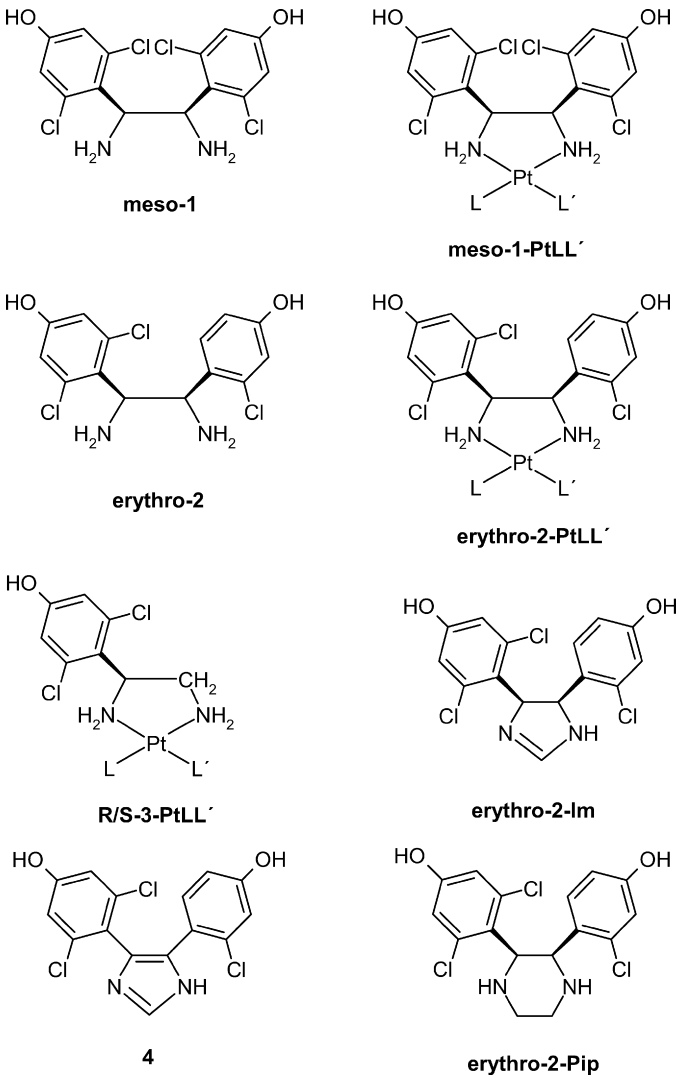


Fig. 1. Estrogenic diamines, their Pt-complexes (LL′ = Cl_2, I_2 or L = H_2O, L′ = SO_4) and related heterocycles [3a,16c,n,l].

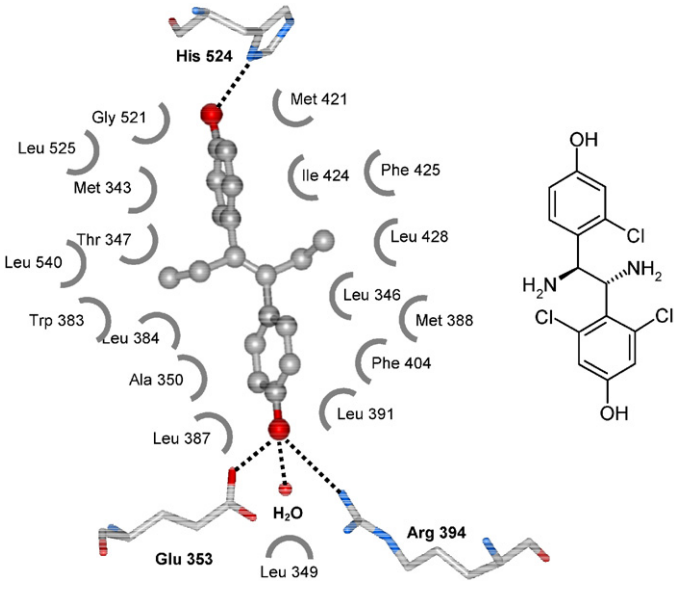


Fig. 2. Interaction of DES in the LBD of ER$_\alpha$ (left), which applies to the ligand **erythro-2** (right).

Table 1
Compounds **erythro-2**, **erythro-2-PtI$_2$**, **erythro-2-Im**, **erythro-2-Pip**, and **4**; estrogen receptor binding and luciferase expression in MCF-7-2a cells.

Compound	Relative binding affinity (% RBA)	Luciferase expression % activation at 10^{-6} M
erythro-2	1.50	98
erythro-2-PtI$_2$	0.43	81
erythro-2-Im	0.08	112
erythro-2-Pip	<0.02	20
4	0.10	27

ortho-standing chlorine atoms, which compensate the hydrophilic character of the two amino groups.

The coordination of **erythro-2** to PtI_2 forces the two phenyl rings into a synclinal position. Despite of this change in the spatial arrangement of the two phenyl rings, the ER_α-agonistic effect of **erythro-2** is preserved in **erythro-2-PtI$_2$** (Table 1).

Erythro-2-PtI$_2$ exists in solution in a relatively stable conformation with the 1-(2,6-dichloro-4-hydroxyphenyl) residue in equatorial and the 2-(2-chloro-4-hydroxyphenyl) residue in axial position (see Fig. 3).

The contribution of the two aryl residues to the ER_α-agonistic activity of **erythro-2-PtI$_2$** is evident from experimental data of the structurally related compound [(R/S)-1-(2,6-dichloro-4-hydroxyphenyl)ethylenediamine]diiodoplatinum(II) (**R/S-3-PtI$_2$**; formula see Fig. 1) which contains only one of the two aryl residues. Thus, the structural comparison of **R/S-3-PtI$_2$** with E_2 shows that the 2,6-dichloro-4-hydroxyphenyl residue can superimpose ring A of the steroid, while the five-membered chelate ring covers ring C and the PtI_2 moiety is positioned at ring D (Fig. 4) [16n].

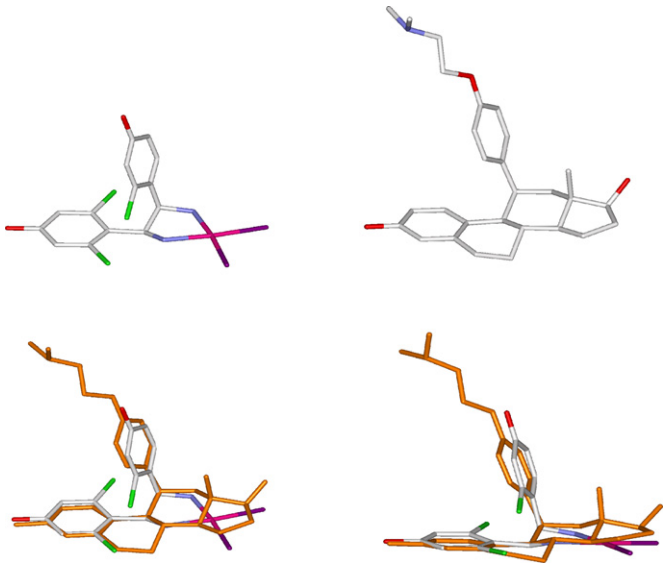


Fig. 3. Superposition of **RU 39411** and **erythro-2-PtI$_2$**.

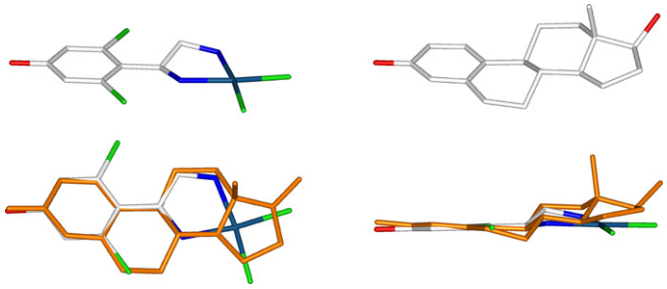


Fig. 4. Superposition of E_2 and **R/S-3-PtI$_2$** [16n].

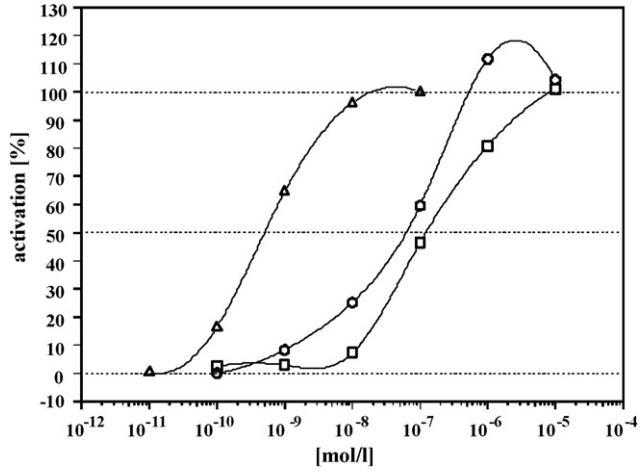


Fig. 5. Activation (%) of luciferase expression in MCF-7-2a cells by E_2 (△), **erythro-2** (□) and **erythro-2-Im** (○) [16l].

This reveals that an interaction of **R/S-3-PtI$_2$** via H-bridges with ER_α is possible as described in Fig. 2, except the binding site His 524. However, the latter H-bond can be formed, if tartronic acid is used as leaving group instead of iodide [16n]. Despite of an ER binding mode comparable with that of E_2, compound **R/S-3-PtMalOH** possesses neither ER_α-affinity nor significant ER_α-agonistic potency [16n]. This gives rise to the assumption that in **erythro-2-PtI$_2$** the axially standing 2-(2-chloro-4-hydroxyphenyl) residue is essential for triggering of ER_α-agonistic effects.

Studies on the strongly ER_α-affinic 11β-4-hydroxyphenyl-estradiol (RBA = 300%) and on its antiestrogenic derivative 11β-(4-dimethylaminoethoxyphenyl) estradiol (**RU 39411**, see formula Fig. 3) give information on the binding mode between **erythro-2-PtI$_2$** and the ER_α, since the two former compounds can superimpose the last mentioned Pt-complex. At the same time, the axially standing 2-(2-chloro-4-hydroxyphenyl) residue in **erythro-2-PtI$_2$** is oriented toward a side pocket close to the ligand binding domain (LBD) of the ER_α as described for the 11β-standing residue in the former E_2 derivatives [16m] (compare Fig. 7).

The influence of the conformation in the 1,2-diarylethane pharmacophore of **erythro-2-PtI$_2$** on the ER_α-agonistic potency was studied on the comparison compounds, 4,5-diaryl-2-imidazoline **erythro-2-Im**, 2,3-diarylpiperazine **erythro-2-Pip** and 4,5-diarylimidazole **4** structurally related to **erythro-2-PtI$_2$**, which differ in their O–O distances (see Fig. 6 [16l,m]). **Erythro-2-PtI$_2$** and the comparison compounds caused only a marginal displacement of E_2 from the receptor due to a different ER_α binding mode. Therefore, the usual test for evaluation of ER_α affinity employing isotope labelled E_2 as competitor yielded only very low RBA values (Table 1).

Nevertheless, significant ER_α-mediated gene activation was observed by these compounds in the luciferase assay on MCF-7-2a cells (Table 1, Fig. 5). As expected, the extent of the latter effect correlated with the O–O distance in compounds: **erythro-2-Im** (5.1 Å) > **erythro-2-PtI$_2$** (7.8 Å) > **4** (8.9 Å) ≥ **erythro-2-Pip** (7.1 Å) (Fig. 6, Table 1).

Presumably, the O–O distance of 5–6 Å is optimal for triggering of an ER_α-agonistic effect in the luciferase assay. A value of 5.1 Å was determined for the most active imidazoline **erythro-2-Im**.

In solution, **erythro-2-PtI$_2$** possessed an O–O distance of 7.8 Å in the λ- as well as in the δ-conformation. However, this distance is presumably reduced to 5.7 Å due to an unhindered λ–δ-conversion of the five-membered chelate ring as observed by Gust and co-workers [16a] on the example of the structurally analogous

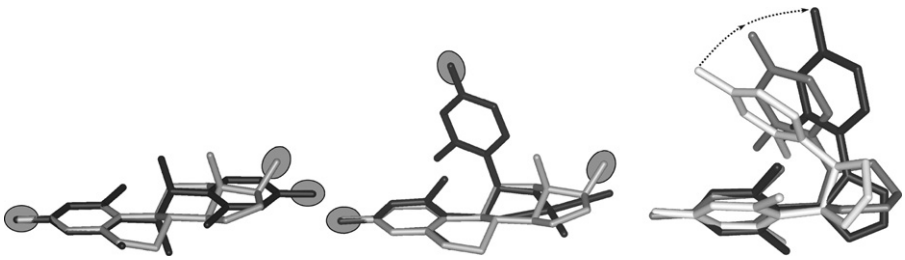


Fig. 6. Superposition of diamine ligand **erythro-2** and E_2 (left), **erythro-2-PtI$_2$** and E_2 (middle), and **erythro-2-Pip**, **erythro-2-Im** and imidazole **4** (right) [16l].

meso-1-PtI$_2$. The effect of this compound is one order of magnitude greater than that of **erythro-2-PtI$_2$** in the luciferase assay.

The new compounds possess different O–O distances giving rise to variable luciferase expressions. Because of these results, the estrogenic Pt-complex **erythro-2-PtI$_2$** and the estrogenic heterocycle **erythro-2-Im** as well as other active compounds of this type were assigned to a second class of hormones (type-II estrogens), whose binding mode to the ER_α is different from that of DES, HES and E_2 (type-I estrogens). In contrast to type-I estrogens, in which one aryl residue is connected to His 524 and the other to Glu 353, Arg 394 and H_2O in the binding site of ER_α, type-II estrogens like **erythro-2-PtI$_2$** and **erythro-2-Im** very likely form an H-bond to Asp 351 or Thr 347 in the hydrophobic side pocket and not to His 524 (Fig. 7) [16o]. Further pharmacological studies with structurally related imidazolines and imidazoles are described in the ref. [16o–r].

2.2. [1,2-Bis(4-hydroxyphenyl)ethylenediamine]platinum(II) complexes; anti-breast cancer activity

The investigation of the relationship between structure and biological properties in the class of [1,2-bis(4-hydroxyphenyl) ethylenediamine]platinum(II) complexes as described in Section 2.1 regarding the ER_α-agonistic effect was extended to the growth inhibiting activity on ER^+ breast cancer. The hormone sensitive ER^+ MXT-M-3.2 breast cancer of the mouse served as model for the simultaneous evaluation of tumor and uterus growth under the influence of the test compounds.

The systematic structural variation of the 3,3′-HES and HES related parent complexes **5-PtLL′** and **6-PtLL′** [17a,b] (formulae see Fig. 8) yielded numerous anti-breast cancer active Pt-complexes. Complexes out of this series acted as ER_α agonists and triggered the uterus growth in the combination experiment [3a,17c,e]. Some other substances caused tumor inhibition but had no effect on the uterus. A third group of compounds was identified which diminished both tumor and uterus development [17d].

According to the obtained results, a classification of the test compounds into three groups of different mode of action is possible [17e].

Mechanism A: A growth inhibiting effect against ER^+ breast cancer without an influence on the uterine weight suggests the binding of the test compound to DNA as described for therapeutically used Pt-complexes. Such a compound is the [(RR/SS)-1-(2-chloro-4-hydroxyphenyl)-2-(2,6-dichloro-4-hydroxyphenyl) ethylenediamine]dichloroplatinum(II) complex (**threo-2-PtCl$_2$**, formula see Fig. 1), while its erythro congener acts according to mechanism C (definition see below) due to its estrogenic properties [17e].

Mechanism B: A twofold effect, an inhibition of tumor growth, and a reduction of uterus weight, points to a contribution of estrogen ablation to the anti-breast cancer activity. For instance, aqua[(RS)-1,2-bis(2,6-difluoro-3-hydroxyphenyl)ethylenediamine]sulfatoplatinum(II) (**meso-7-PtSO$_4$**; formula Fig. 8)

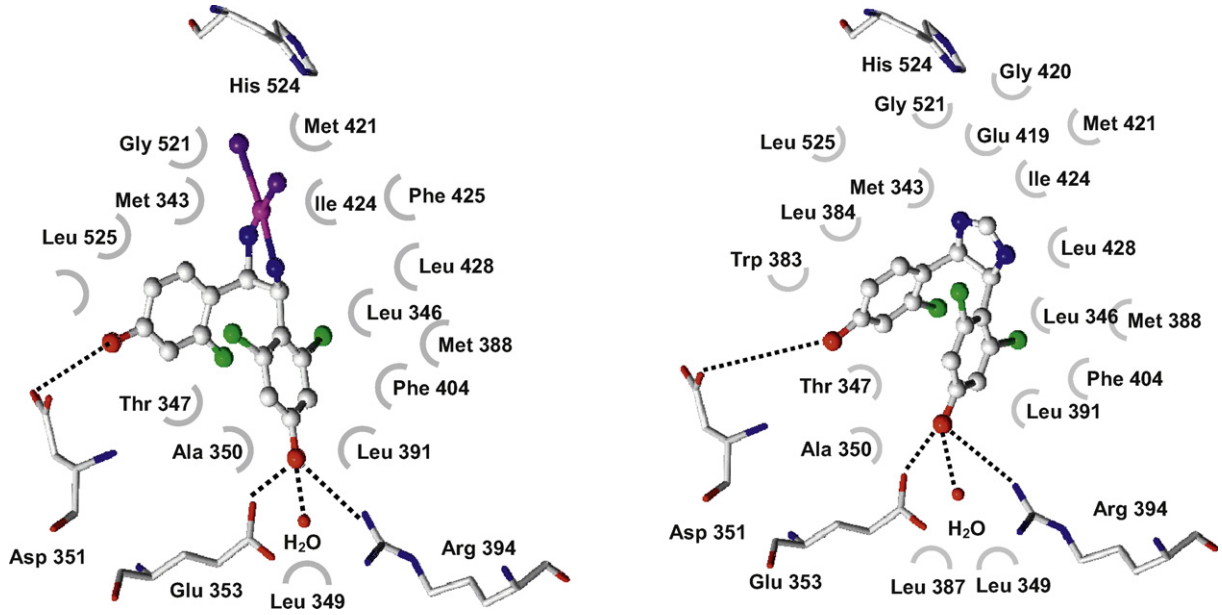
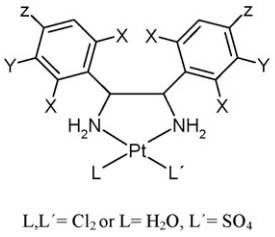


Fig. 7. Interaction of **erythro-2-PtI$_2$** and **erythro-2-Im** in the LBD of the ER_α [16l].



compound	X	Y	Z
meso-5-PtLL´	H	OH	H
racem-5-PtLL´			
meso-6-PtLL´	H	H	OH
racem-6-PtLL´			
meso-7-PtLL´	F	OH	H
racem-7-PtLL´			

Fig. 8. Non-estrogenic, breast cancer inhibiting Pt-complexes [17a,b].

and its racemate were highly active on the murine ER$^+$ MXT-M-3,2 breast cancer and strongly decreased the uterus weight [17f,g,18]. However, they were inactive (**meso-7-PtLL**′) or only moderately active (**racem-7-PtLL**′) in the MXT$^+$ cell culture derived from this tumor at concentrations corresponding to those in animal experiments. This indicates that they mainly caused in vivo effect by a reduction of the endogenous estrogen level in the host animals due to an interference with the ovarian steroid biosynthesis. The relevance of this assumption was proven in an experiment in which a reversal of the breast cancer inhibiting effect of **meso-7-PtLL**′ could be achieved by simultaneous estrone administration [18]. However, especially with the more cytotoxic **racem-7-PtLL**′, a cisplatin-like interaction with DNA contributing to the anti-breast cancer activity cannot be excluded as part of the mode of action. Both effects, estrogen ablation and platination of tumor DNA, entail programmed cell death, which is responsible for the inhibition of tumor development. Further Pt-complexes acting according to mechanism B are the two parent compounds **5-PtLL**′ and **6-PtLL**′ and complexes described in the references [17c,e].

Mechanism C: Another twofold effect, an inhibition of tumor and a stimulation of uterus growth, suggests that the anti-breast cancer activity is at least partially caused by the estrogenic potency of the test compound. [(RS)-1,2-Bis(2,6-dichloro-4-hydroxyphenylethylenediamine]platinum(II) (**meso-1-PtLL**′) proved to be the most active derivative of the studied estrogenic Pt-complexes [2,3a,17c–e,h]. Weaker anti-breast cancer effects were found in the structurally analogous complex types [(RS)-1,2-bis(2,6-dihalo-3-hydroxyphenyl)ethylenediamine]platinum(II) [17c] and [(RS/SR)-1-(2,6-dichloro-4-hydroxyphenyl)-2-phenylethylenediamine]platinum(II), e.g. **erythro-2-PtLL**′ [17e], which also acted according to mechanism C. The present concept to the mode of action of **meso-1-PtLL**′, e.g. ER$_\beta$ agonistic activity as cause of anti-breast cancer potency, is thoroughly discussed in Section 2.2.3. In the following, the preclinical investigation of the top compound **meso-1-PtLL**′ is described in detail.

2.2.1. [(RS)-1,2-Bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamine]platinum(II), test results

In experiments on the DMBA-induced breast cancer of the rat **meso-1-PtSO$_4$** caused a significantly stronger anti-tumor effect and a faster onset of tumor inhibition than the standard cisplatin, if administered in equimolar dosage (Fig. 9, Table 2) [3a].

The advantage of **meso-1-PtSO$_4$** was surprising in view of the fact that the Pt blood level after treatment with this drug reached only one-tenth of that with cisplatin. Evidently, an interaction with DNA leading to an impairment of its function markedly contributed to the anti-tumor activity of **meso-1-PtSO$_4$**, since the free ligand **meso-1** was less active if used in equimolar dosage (Fig. 9, Table 2) [3a].

In fact, a binding of **meso-1-PtSO$_4$** to DNA, though less than that of cisplatin, could be proved in experiments on the human ER$^+$ MCF-7 breast cancer cell line [17h]. Both compounds were not accu-

Table 2
Effect of **meso-1**, **meso-1-PtSO$_4$** and cisplatin on the growth of DMBA-induced, hormone-dependent mammary carcinoma of the SD rat.

Compound	Dose[a] (mg)	No. of animals	No. of tumors		% of tumors with				% change of		
			B[b]	NT[c]	CR[d]	Pr[e]	NC[f]	P[g]	Body wt	Tumor area[h]	
									Day 28	Day 14	Day 28
Control		9	21	23	0	0	38	62	3.0	260	420
cDDP	1.5 (5 μmol/kg)	9	24	4	21	41	25	13	−0.9	51[i,j]	−38[k]
meso-1	1.9 (5 μmol/kg)	8	17	9	0	18	35	47	−4.6	62[k]	129[k]
meso-1-PtSO$_4$	3.5 (5 μmol/kg)	10	25	1	56	24	12	8	−5.4	−86[l]	−84[l]

[a] Dose per kg of body weight and day. The animals received meso-1 three time a week, sc, as solution in polyethylene glycol 400/H_2O, 1:1; cDDP and **meso-1-PtSO$_4$** were dissolved in H_2O; duration of therapy 4 weeks.
[b] At the beginning of the test.
[c] Occuring during the test.
[d] CR: complete remission, tumor not palpable.
[e] PR: partial remission, reduction of the initial tumor size ≥ 50%.
[f] NC: no change; tumor size 51–150% of initial tumor size.
[g] P: progression, tumor size > 150% of initial tumor size.
[h] The *U* test according to Wilcoxon, Mann and Whitney was used.
[i] Significant ($p < 0.025$) compared with the control.
[j] Significant ($p < 0.01$) lower activity compared with **meso-1-PtSO$_4$**.
[k] Significant ($p < 0.01$) compared with the control.
[l] Significant ($p < 0.05$) compared with the control.

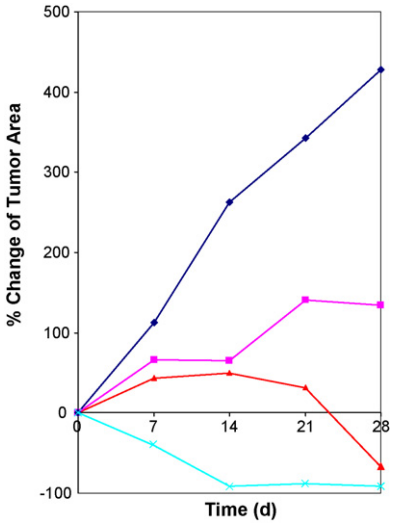


Fig. 9. Activity of **meso-1** (■), **meso-1-PtSO$_4$** (x) and cisplatin (▲) (equimolar dosage: 5 μmol/kg) on the DMBA-induced mammary carcinoma of the SD rat (control: ♦). For experimental conditions, see Table 2 [3a].

mulated by ER$^+$ MCF-7 cells [17h] contradicting pharmacokinetic experiments performed by Lux and co-workers [3a]. They used neutron activation analysis to determine platinum distribution in ER$^+$ MXT-M-3,2 breast cancer bearing mice treated with **meso-1-PtSO$_4$**.

They found a 22-fold Pt concentration in the ER$^+$ tumor compared with that in skeletal muscle. This fact afforded an explanation for the strong tumor growth inhibiting effects of **meso-1-PtSO$_4$**, which matched those of DES, E_2 and cisplatin (Fig. 10, Table 3).

Against the ER-negative variant of the tumor, the ER$^-$ MXT-M-3,2 (ovex) breast cancer, **meso-1-PtSO$_4$** proved to be inactive [4a,b]. The effect of **meso-1-PtSO$_4$** on the proliferation of hormone-dependent and -independent tumor cells was tested in detail on the ER$^+$ MCF-7 and the ER$^-$ MDA-MB-231 human breast cancer cell lines [16e]. Cisplatin and tamoxifen were used for comparison. If the growth inhibitory effect is promoted by accumulation of the Pt-complex via ER, a selective inhibition of ER$^+$ cells is expected.

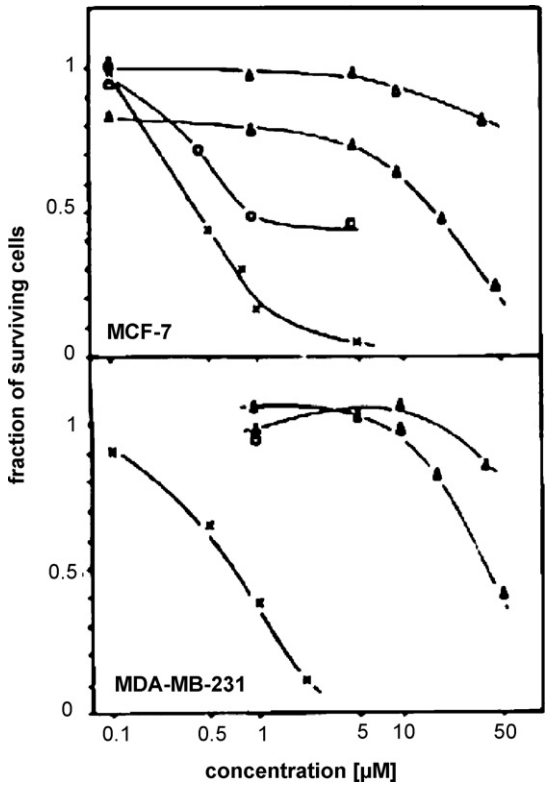


Fig. 11. Concentration-response curves of diamine ligand **meso-1** (△) and **meso-1-PtSO$_4$** (▲) and of the comparison compounds tamoxifen (○) and cisplatin (×) for growth inhibition in ER$^+$ MCF-7 and ER$^-$ MDA-MB-231 cells. Test compounds were added 24 h after plating and the nuclei of viable, attached cells were counted 5 days later [16e].

Fig. 11 shows the concentration response curves for **meso-1** and **meso-1-PtSO$_4$** in experiments on both cell lines [16e]. No significant growth inhibition could be observed for **meso-1-PtSO$_4$** at 1 μM, a concentration at which cisplatin showed a marked inhibitory effect. Instead, a concentration of 20 μM **meso-1-PtSO$_4$** was required to produce 50% inhibition not only in the ER$^+$ MCF-7

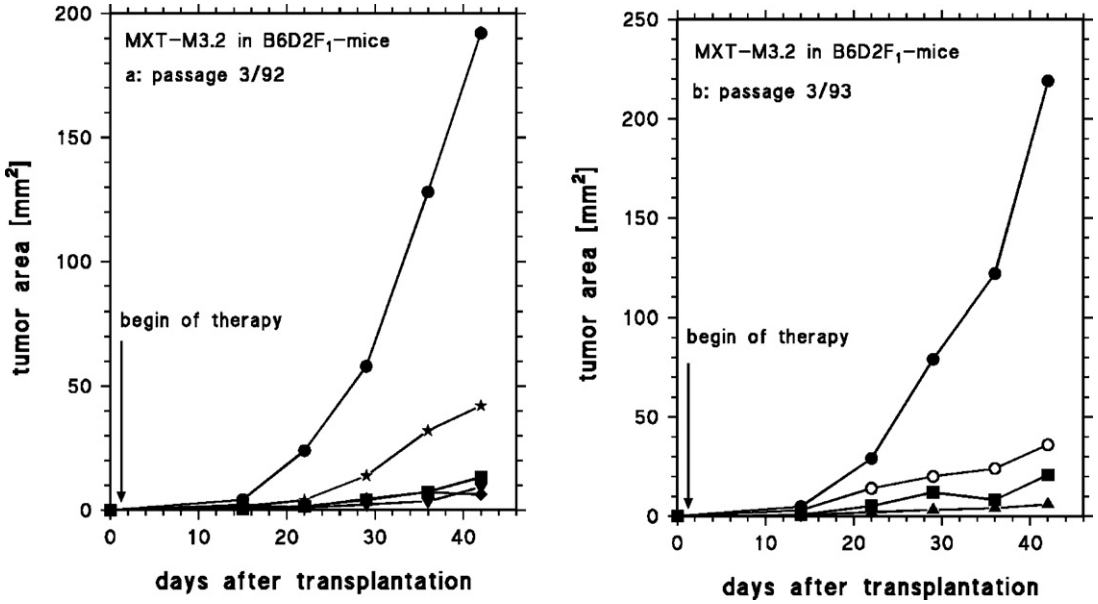


Fig. 10. Growth of the hormone-sensitive ER$^+$ MXT-M-3.2 mammary carcinoma in B6D2F$_1$-mice under the therapy with meso-1-PtCl$_2$ (■), **meso-1** (*) and cDDP (▲), each 5 μmol/kg, i.p., 3 times weekly; DES (▼), 3.7 μmol/kg, s.c., 3 times weekly and E_2 (♦), as depot s.c. from d-5 in comparison to the solvent (●) treated and at d-1 ovariectomized; control (○) [4b].

Table 3
Anti-tumor activity of **meso-1-PtCl$_2$**, **meso-1**, DES, E_2, cDDP and ovariectomy on the hormone-sensitive ER$^+$ MXT-M-3,2 mammary carcinoma of B6D2F$_1$-mice.

Test-group	Number of animals (n)	Dose [μmol/kg]	Mean tumor weight [mg][a]	Standard deviation [mg][b]	Anti-tumor activity T/C (%)[c]	Animal weight [g][d]
Control	11	–	1534	1422	100	+2.6
meso-1-PtCl$_2$[e]	10	5.0	25	24	1.6*	+3.1
meso-1[e]	10	5.0	121	79	7.8*,**	+2.0
DES[f]	10	3.7	16	18	1.0*	+5.3
E_2[g]	10	Depot	8	6	0.5*	+3.3
Control	9	–	1570	1081	100	+1.9
meso-1-PtCl$_2$[e]	9	5.0	45	26	2.8*	+1.7
cDDP[e]	9	5.0	11	16	0.7*	+2.1
Ovariectomy[h]	7	–	117	138	7.5*	+6.0

[a] Mean tumor weight at day 42 after tumor transplantation.
[b] Standard deviation of tumor weight.
[c] T/C = [mean tumor weight of test group/mean tumor weight of control group] × 100%; determined at the end of the 6 week therapy.
[d] [animal weight at day 42 after tumor transplantation] − [animal weight at day 1 after tumor transplantation].
[e] Dissolved in PEG 400/1.8% NaCl 1:1 (vol/vol), 3 times weekly, i.p., duration of therapy: d_1–d_{42}. **Meso-1** was administrated as suspension because of its poor solubility in PEG 400/1.8% NaCl 1:1 (vol/vol).
[f] In Ol. arachidis, 3 times weekly, s.c.
[g] s.c. implantation of 3 mg E_2 placed in a silicon tube (12 mm, ∅ 1.5 mm), 5 days before tumor transplantation.
[h] Ovariectomy on day 1 after tumor transplantation.
* Significant against the control ($p < 0.01$).
** Significant against the therapy with **meso-1-PtCl$_2$** ($p < 0.01$).

but also in the ER$^-$ MDA-MB-231 cell culture. In contrast, tamoxifen was selective in that it inhibited growth of ER$^+$ MCF-7 cells but not of ER$^-$ MDA-MB-231 cells. The ligand **meso-1** inhibited both cell lines only marginally.

In variance with the expectations, **meso-1-PtSO$_4$** did not display a difference in growth inhibition between hormone-dependent and -independent cells. Furthermore, growth inhibition was only observed at concentrations, which were 100-fold higher than those required for occupation of the ERs. The estrogenic Pt-complex **meso-1-PtSO$_4$** and cisplatin showed the same lag phase for manifestation of growth inhibition (about 15 h, a fraction of the doubling time) and a first order inhibition kinetics at equieffective concentrations confirming that both Pt-complexes acted in the cell culture experiments by an identical mechanism. The maximally attainable level of the non-plasma protein bound fraction of a single 10 μmol/kg i.p. dose of **meso-1-PtSO$_4$**, a dosage at which this drug is very active on the murine ER$^+$ MXT-M-3,2 breast cancer, lies at 0.35 μM and thus markedly below the concentration that causes a significant cytotoxic effect on the ER$^+$ MCF-7 cell line (T/C_{corr} = 68% at 5 μM) [17i].

This result contradicts the formerly postulated assumption that cisplatin-like cytotoxic effects are responsible for the anti-breast cancer activity of **meso-1-PtSO$_4$**. The initial working hypothesis predicted that E_2 antagonized the inhibitory effect of **meso-1-PtSO$_4$** by occupying the ER, thereby preventing the binding of the complex to the ER and its accumulation in the nucleus. The results of relevant experiments did not confirm the prediction, since E_2 was not able to counteract the growth inhibition produced by this Pt-complex on ER$^+$ MCF-7 cells [16e].

The estrogen-like and cisplatin-like properties of **meso-1-PtSO$_4$** are most probably expressed independently at the cellular level. The partially inconsistent results show that the earlier supposed plain relation between ER affinity (or estrogenic potency) and activity against the ER$^+$ breast cancer does not exist.

2.2.2. [(RS)-1,2-Bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamine]platinum(II), pharmacokinetic studies

Pharmacokinetic studies with **meso-1-PtSO$_4$** were performed by Bernhardt and co-workers [17i]. In this context, a paper of Spruß and co-workers [19] concerning the anti-tumor activity of po administered **meso-1-PtCl$_2$** is of great interest.

The resorption from the gastrointestinal tract was proved by determining the estrogenic effect of **meso-1-PtCl$_2$** in a dose activity study by means of the immature mouse uterine weight test. In comparison to the sc injection, a 10-fold po dose was administered to achieve identical effects. By po treatment of the ER$^+$ MXT-M-3,2 mammary carcinoma of the mouse with **meso-1-PtCl$_2$** an almost complete inhibition of the tumor growth was obtained. This effect was superior to that of subcutaneously applied cDDP. The strong anti-tumor activity of po-applied **meso-1-PtCl$_2$** was also demonstrated on the hormone-sensitive Noble Nb-R prostate cancer of the rat. Histological examinations showed that the platinum complex **meso-1-PtCl$_2$** did not cause cDDP-like kidney damage or irritations of gastric or intestinal mucosa if given po. The study suggests that in the therapy of breast and prostate cancer with **meso-1-PtCl$_2$** po administration is possible like in that with satraplatin.

2.2.3. [(RS)-1,2-Bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamine]platinum(II), studies on the mode of in vivo action (compare Section 6 of part I of this review)

The selective growth inhibitory effect of **meso-1-PtCl$_2$** in vivo, obvious from the strong activity on the ER$^+$ MXT-M-3,2 breast cancer and the inactivity on the ER$^-$ MXT-M-3,2 (ovex) breast cancer [3a] (see Table 3; Fig. 10a and b; data from ref. [4b]), originally suggested the involvement of endocrinological and/or immunological factors [16e]. Interestingly, the two estrogens E_2 and DES produced the same strong anti-tumor effect like **meso-1-PtCl$_2$** in the test on the ER$^+$ MXT-M-3,2 breast cancer (Fig. 10, Table 3), an indication that the estrogenic potency of **meso-1-PtCl$_2$** might be of importance for its anti-breast cancer activity.

Later studies as well did not support the original drug targeting concept offered as mode of action of **meso-1-PtCl$_2$**:

The marginal inhibitory effect of **meso-1-PtCl$_2$** on the breast cancer cell lines derived from the murine ER$^+$ MXT-M-3,2 and ER$^-$ MXT-M-3,2 (ovex) breast cancers [17k] contradicts a tumor directed cytotoxic effect utilizing the ER as carrier [4b]. Furthermore, the experiment discussed below confirmed that the anti-breast cancer effect of **meso-1-PtCl$_2$** was caused by its estrogenic potency and took place via a complex mode of action under participation of cells of the immune system [4b]. Comparative dose activity studies with **meso-1-PtCl$_2$** and the non-steroidal estrogen DES (earlier used in breast cancer therapy) on ovariectomized mice implanted with ER$^+$ MXT-M-3,2 mammary carcinoma yielded identical results. A maximum stimulation of the tumor growth at low dosage was followed by a regression of the tumor with increasing doses (Fig. 12). The less anti-breast cancer active ligand **meso-1**, too, caused a dose-

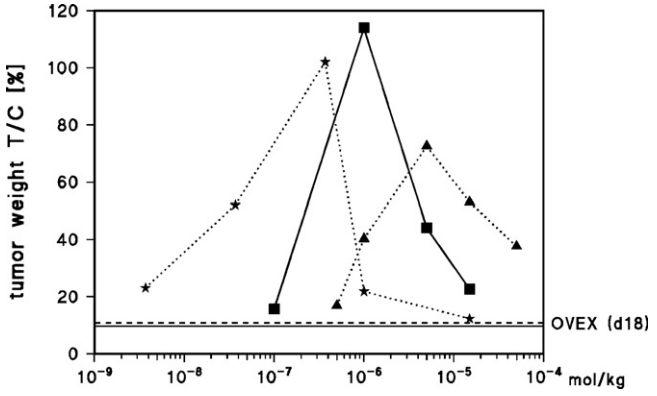


Fig. 12. Dose activity relationship of **meso-1-PtCl$_2$** (■), **meso-1** (▲) and DES (*) on the hormone-sensitive ER$^+$ MXT-M-3.2 mammary carcinoma in ovariectomized B6D2F$_1$-mice [4b].

dependent growth stimulation and inhibition. The latter finding also suggested that a cDDP-like mode of action (damage of DNA function) did not substantially contribute to the inhibition of the tumor by Pt-complex **meso-1-PtCl$_2$**.

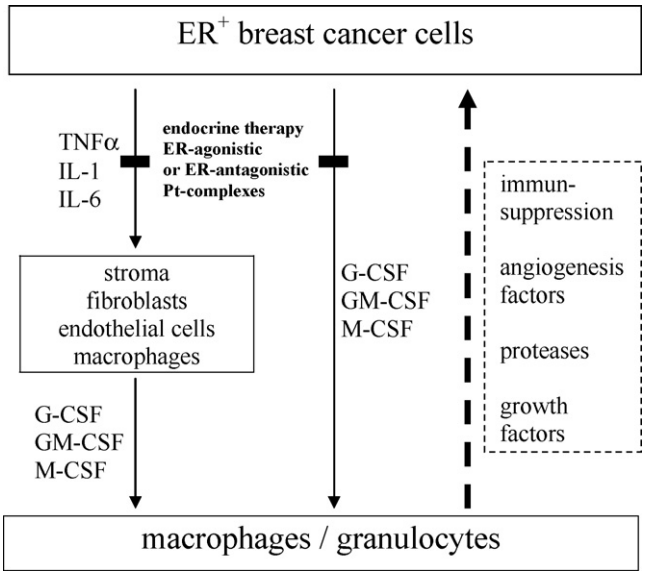
It is conceivable that the biphasic effect of **meso-1-PtCl$_2$**, DES and **meso-1** – dose-dependent tumor growth stimulation followed by inhibition – which was observed in the therapy of the hormone-dependent ER$^+$ MXT-M-3,2 breast cancer (implanted into ovariectomized female mice) [4b], is at first mediated by ER$_\alpha$ (growth stimulation) and than by ER$_\beta$ (growth inhibition). In fact, the hormone sensitive ER$^+$ MXT-M-3,2 breast cancer contains besides the ER$_\alpha$ also the ER$_\beta$, as recently shown by Kunde and Hoffmann [20] by proof of expression of ER$_\beta$-mRNA in this tumor. However, further studies are necessary to fully understand this phenomenon, which can only be observed in animal but not in cell culture experiments under use of the same ER$_\alpha^+$/ER$_\beta^+$ breast cancer cells (see also Section 3.1, part I of this review).

Further studies on the mode of action of **meso-1-PtCl$_2$** [4a–f] give rise to the assumption that in ER$^+$ MXT-M-3,2 breast cancer bearing mice the observed expansion of cells of the Mac/Gra lineage (i.e. macrophages/granulocytes) is due to the secretion of hematopoietic growth factors like GM-CSF directly from tumor cells or indirectly from stroma cells (Scheme 1).

The concomitantly altered Mac/Gra function promotes the tumor development. It is supposed that the secretion of growth factors directly stimulating the tumor cell proliferation, the secretion of proteases and angiogenesis factors supporting the growth, invasion and metastasis of the tumor and the suppression of immune defence are responsible for the tumor growth promoting effects of Mac/Gra in the breast cancer graft bearing mice. An interruption of this vicious circle of mutual growth stimulation of Mac/Gra and breast cancer cells is achieved by administration of the estrogenic Pt-complex **meso-1-PtCl$_2$**. In this process, the attack of the drug aims at the ERs of breast cancer cells leading to an inhibition of the secretion of cytokines and with this to a reduction of the elevated Mac/Gra number and to a restoration of their functions in the natural immunosurveillance. The initiation of apoptosis by **meso-1-PtCl$_2$** also contributes to the tumor regression, since this process is accompanied by a reduction of the number of Mac/Gra promoting the breast cancer development Therefore, estrogenic Pt-complexes can be classified as biological response modifiers (see also Section 6 in part I of this review).

2.2.4. [(RS)-1,2-Bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamine]platinum(II), a drug for the therapy of prostate cancer

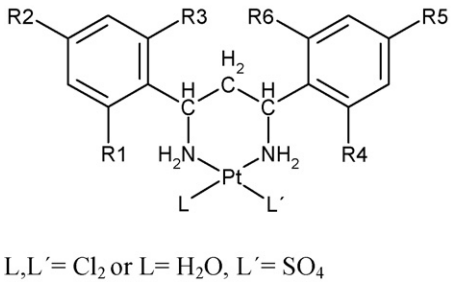
Meso-1-PtLL′ (e.g. LL′ = Cl$_2$) was also successfully tested against prostatic tumors [19,21,22] like the Dunning R 3327 prostatic cancer of the rat. Originally, it was assumed that this Pt-complex acted against the androgen receptor positive (AR$^+$) prostate cancer, which is endowed with ER$_\beta$ as well, on two levels. Besides a direct attack on the DNA after ER$_\beta$-mediated enrichment in the tumor cells, its ER-agonistic potency causes androgen ablation, which itself is a common endocrine therapeutic measurement achieved by orchiectomy or administration of GnRH agonists and antagonists [22]. However, the benefit of the latter interventions is limited in time due to the development of therapy resistant tumors devoid of androgen receptors (AR$^-$). It was supposed that in the case of the estrogenic Pt-complex **meso-1-PtLL′** this process was delayed owing to its Pt-pharmacophore. Indeed, **meso-1-PtLL′** significantly extended the time to disease progression in comparison with orchiectomy in the Dunning R 3327 prostate cancer model [21,22]. Surprisingly, the relapsed tumor, too, responded to **meso-1-PtLL′** as demonstrated in a long-term study on orchiectomized rats bearing Dunning R 3327 prostate cancer [22]. This effect cannot be ascribed to cytotoxic effects of **meso-1-PtLL′** because of the lack of any activity in experiments on prostate cancer cell cultures. Therefore, the beneficial contribution of an additional mechanism to the anti-prostate cancer activity of **meso-1-PtLL′**, presumably owing to an ER$_\beta$ agonistic activity, must be considered.



Scheme 1. Hypothetical mechanism of the anti-breast cancer activity caused by endocrine therapy and by ER-agonistic or ER-antagonistic Pt-complexes. Inhibition of mutual growth stimulation of breast cancer cells and macrophages/granulocytes.

This assumption was supported by results with the non-steroidal estrogen DES on the Dunning R 3327 prostate cancer of the rat, relapsed after orchiectomy, showing a strong inhibitory activity [22]. The supposed mechanism of the anti-prostate cancer activity of **meso-1-PtLL′** is described in Section 6.2 of part I of this review and is supported by studies of Corey and co-workers [23], Joseph and Isaacs [24], Bektic and co-workers [25], and Gustafsson [26] with estrogens, especially with the phytoestrogen genistein which is an ER$_\beta$ selective "partial" agonist [27,28].

For the therapy of prostate cancer by estrogens and estrogenic Pt-complexes like **meso-1-PtLL'** findings of Hedlund and co-workers [29a] and Henriksson and co-workers [29b] are important: The well-known cardiovascular side effects of orally given estrogenic compounds can be reduced or even prevented by parenteral administration.



compound	R1	R2	R3	R4	R5	R6
erythro-8-PtLL´	Cl	OH	Cl	Cl	OH	H
meso-9-PtLL´	F	OH	H	F	OH	H
meso-10-PtLL´	OMe	H	H	OMe	H	H
meso-11-PtLL´	Cl	OH	Cl	Cl	OH	Cl

Fig. 13. [1,3-Diarylpropane-1,3-diamine]platinum(II) complexes [30a-d,g].

3. Studies on [1,3-diarylpropane-1,3-diamine]platinum(II) complexes

Wiegrebe and co-workers [30a–d,g] performed thorough studies on the synthesis of diastereomeric 1,3-diarylpropane-1,3-diamines and their Pt(II)-complexes, which are homologues of the pharmacologically interesting [1,2-diarylethylenediamine]platinum(II) complexes described in Section 2 of this review. Some of Wiegrebe's compounds (e.g. **erythro-8-PtCl$_2$** or **meso-11-PtCl$_2$**, Fig. 13) were endowed with a substituent pattern which had been proved to be essential for estrogenic properties in [1,2-diarylethylenediamine]platinum(II) complexes.

The authors took a non-optimal fit of [1,2-diarylethylenediamine]platinum(II) complexes, e.g. of **meso-1-PtCl$_2$**, to the estrogen receptor into consideration, since the interconversion of the conformers is impeded due to a mutual sterical hindrance of the neighboring aryl residues. They assumed a stronger binding of the [1,3-diarylpropane-1,3-diamine]platinum(II) complexes substituted with 3 or 4 chlorine atoms in the phenyl residues (**erythro-8-PtCl$_2$** and **meso-11-PtCl$_2$**) to the ER compared to their ethane analogues owing to the higher conformational flexibility of the six-membered chelate rings. This would lead to enrichment in ER$^+$ tumor cells and to an increase in inhibitory activity.

The [1,3-diarylpropane-1,3-diamine]platinum(II) complexes were tested for ER$_\alpha$ affinity in competition experiments with E_2 [30e]. Surprisingly, all complexes were only very weakly bound to the receptor. For example, the RBA of **erythro-8-PtCl$_2$**, for which a higher value was expected than for the ethane analogue **erythro-2-PtCl$_2$** (RBA = 0.095%), only amounted to 0.04%. The RBA of **meso-11-PtCl$_2$**, which was considered as top compound of the propane series, was not determined, since the ligand, meso-1,3-bis(2,6-dichloro-4-hydroxyphenyl)propane-1,3-diamine, showed no affinity to the ER and only non-significant estrogenic activity in the luciferase assay [30f]. UV-difference spectroscopy studies revealed that this type of Pt-complexes, e.g. **erythro-8-PtCl$_2$**, an analogue of **erythro-2-PtCl$_2$**, is able to bind to native DNA comparable to cDDP [30e]. Nevertheless, **erythro-8-PtCl$_2$** proved to be inactive in the concentration range between 1 and 10 μM in the test on the ER$^-$ MDA-MB-231 and ER$^+$ MCF-7 breast cancer cell lines [30e]. The three bulky chlorine atoms located in ortho-positions of both phenyl rings in **erythro-8-PtCl$_2$** are most probably responsible for the lack of cytotoxic properties. Modification of the substitution pattern resulting in **meso-9-PtCl$_2$** (2-F,4-OH substitution in both phenyl rings) and **meso-10-PtCl$_2$** (2-OCH_3 substitution in both phenyl rings) caused significant growth inhibitory effects on the ER$^-$ MDA-MB-231 cell line [30e]. Their diastereoisomers, **racem-9-PtCl$_2$** and **racem-10-PtCl$_2$**, were even highly active in the test on this cell line. Both Pt-complexes came up to or even surpassed the activity of the standard tamoxifen on the ER$^+$ MCF-7 breast cancer cell line (tamoxifen: T/C = 61%; **racem-10-PtCl$_2$**: T/C = 27%; 1 μM drug concentration) [30e].

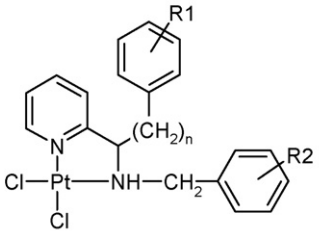
Though the latter compounds are of interest for further preclinical studies due to their marked activity on hormone sensitive as well as insensitive breast cancers, the [1,3-diarylpropane-1,3-diamine]platinum(II) complexes have not yielded the desired strongly ER-affinic drugs.

4. Studies on [1-(2,6-dichloro-4-hydroxybenzylamino)-1-(2-pyridyl)-2-(2,6-dichloro-4-hydroxyphenyl)ethane] dichloroplatinum(II) and related compounds

Synthesis and pharmacological evaluation of [2-(aminomethyl)pyridine]dichloroplatinum(II) derivatives, e.g. those containing multiple substituents in their aryl residues favorable for an interaction with ER, as represented by the title compound **12-PtCl$_2$**, were described by Brunner and co-workers [31a–c].

Compound **12-PtCl$_2$** showed a relative binding affinity to the ER (RBA = 0.4%) comparable with that of [(RS)-1,2-bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) (**meso-1-PtCl$_2$**; RBA = 0.35%), which proved to be very potent on hormone-sensitive breast cancer models of rodents (see Section 2.2.1). Similar data were obtained for **13-PtCl$_2$** (RBA = 0.38%) and **14-PtCl$_2$** (RBA = 0.33%) in a 15 compounds comprising test series. The corresponding ligands were threefold stronger ER-affinic than the three Pt-complexes (formulae see Fig. 14).

In the cytotoxicity test on the ER$^-$ MDA-MB-231 breast cancer cell line a drug concentration of 10 μM was required to attain a 50% inhibition by **12-PtCl$_2$** and **13-PtCl$_2$**, while **14-PtCl$_2$** was



compd	n	R1	R2
12-PtCl$_2$	1	2,6-Cl_2-4-OH	2,6-Cl_2-4-OH
13-PtCl$_2$	1	2,6-Cl_2-4-OH	2,6-Me_2-4-OH
14-PtCl$_2$	0	4-OH	2,6-Cl_2-4-OH

Fig. 14. [1-(2,6-Dichloro-4-hydroxybenzylamino)-1-(2-pyridyl)-2-(2,6-dichloro-4-hydroxyphenyl)ethane]dichloroplatinum(II) and related complexes [31a–c].

without effect on the growth of this cell line. Among the three compounds, only **12-PtCl$_2$** and **13-PtCl$_2$** were able to slightly extend the survival time of mice implanted with P388 leukemia cells if administered in a dosage of three times 20 and 40 μmol/kg, respectively.

A comparative in vivo study on the ER$^-$ MXT-M-3,2 (ovex) and ER$^+$ MXT-M-3,2, carried out in equimolar dosage (20 μmol/kg three times weekly for 6 weeks), revealed inactivity of the three Pt-complexes and their ligands on the hormone-insensitive model and activity on the hormone-sensitive one. In the case of **12-PtCl$_2$** and **13-PtCl$_2$** the inhibitory effect on the ER$^+$ MXT-M-3,2 breast cancer was twice that of their ligands; **14-PtCl$_2$** and its ligand were equipotent. The increased uterus weight observed after administration of the ligand **12** on mice bearing the ER$^+$ MXT-M-3,2 breast cancer, confirmed in the uterus weight test on juvenile mice, pointed to a mode of action of **12** based on ER-agonistic potency thoroughly described in Section 2.2.3. The assumption of an analogous mode of action of **12** is supported by the absence of cytotoxicity proved in tests on the ER$^-$ MDA-MB-231 breast cancer cell line, the P388 leukemia of the mouse and the murine ER$^-$ MXT-M-3,2 (ovex) breast cancer. The stronger inhibitory effect of **12-PtCl$_2$** on the ER$^+$ MXT-M-3,2 breast cancer is possibly caused by the cytotoxic Pt-pharmacophore. However, this presumption must be confirmed in further studies.

Compound **12-PtCl$_2$** is an interesting anti-breast cancer drug for further preclinical and clinical investigations.

5. Studies on [1,1,2-triarylethylenediamine]platinum(II) complexes

1,1,2-Triarylethylenediamines were synthesized as derivatives of acetoxy substituted 1,1,2-triarylbut-1-enes which had proved to be strongly active on an estrogen and progesterone receptor positive, postmenopausal human mammary carcinoma serially implanted into nude mice [32a]. In structure activity studies several 1,1,2-triarylbut-1-enes showed estrogenic and/or antiestrogenic properties, whereby the extent of these effects depended on the number and position of acetoxy groups in the benzene rings, on the length of the alkene fragment and on the configuration [32b–d]. Hitherto it was assumed that in 1,1,2-triarylbut-1-enes a β-dialkylaminoethoxy group in para-position of the cis-standing 1-phenyl residue was necessary for the occurrence of estrogen antagonistic properties like in tamoxifen, a "partial" antiestrogen, which is used in breast cancer prophylaxis and therapy. It is of interest that the breast cancer inhibiting activities of the acetoxy substituted 1,1,2-triarylalkenes rather correlated with their estrogenic than with their antiestrogenic properties [32d].

By transformation of one of these compounds, 1,1-bis(4-hydroxyphenyl)-2-phenylethene, a mammary tumor inhibiting "impeded" estrogen (RBA = 29.1%) into its 1,2-diamino derivative, a ligand with markedly lower ER affinity (RBA = 0.638%) possessing neither estrogenic nor antiestrogenic properties (at the dose of 1 μmol per mouse) was obtained. The related aquasulfatoplatinum(II) complex 15-PtSO4 (Fig. 15) showed significant though moderate antiestrogenic and breast cancer inhibiting properties, presumably due to its changed spatial structure [3b]. In a structure activity, study further anti-breast cancer active 15 analogues were obtained (Gust, unpublished results).

6. Pt-complexes linked via a spacer to non-steroidal "partial" antiestrogens

6.1. 2-Phenylindole derivatives

In contrast to estrogenic 1,2-diarylethylenediamines, the related 2-phenylindoles are "partial" antiestrogens like tamoxifen. This means that they possess estrogenic as well as antiestrogenic properties. Both types of compounds, 1,2-diarylethylenediamines and 2-phenylindoles, are able to inhibit the growth of the hormone-sensitive breast and prostate cancer [2,6a,b,7a-c] cancer [2]. The first antiestrogenically active 2-phenylindoles were obtained by thermolysis of N,N′-dialkyl-1,2-bis(2,6-dichloro-4-methoxyphenyl)ethylenediamine hydrochlorides. The free phenols of the educts proved to be very active on the DMBA-induced breast cancer of the rat [7a], while 1-alkyl-4-chloro-2-(2,6-dichloro-4-hydroxyphenyl)-6-hydroxyindoles not only showed a high activity against this tumor, but also weaker estrogenic effects, a benefit in the therapeutical application (ref. to the synthesis and anti-tumor activity [2,7b,c] and to the reaction mechanism [7c]).

In thorough studies, von Angerer [2] received an optimally active compound, 5-acetoxy-2-(4-acetoxyphenyl)-3-methylindole (zindoxifene, formula Fig. 16). Zindoxifene was introduced into a preliminary phase I/II clinical trial on the hormone-sensitive breast cancer [2]. It also proved to be a potent drug for the treatment of prostate cancer [2,6a,b]. It is remarkable that zindoxifene was able to delay the relapse of the Dunning R 3327-H prostate cancer by 7 weeks in comparison to castration [6b]. von Angerer and co-workers [7d–h] used zindoxifene-related 2-phenylindoles as ER-affinic carriers in Pt-complexes in which 3-alkyl-5-hydroxy-2-(4-hydroxyphenyl)indoles were linked to [1,2-diaminoethane] dichloroplatinum(II), [1,3-diaminopropane]dichloroplatinum(II) or [2-(aminomethyl)pyridine]dichloroplatinum(II) by alkyl spacer groups of varying length (see formula **16-PtCl$_2$** in Fig. 16).

All compounds were endowed with the same pharmacological properties, whereby the extent of activity depended on the length of the spacer. The most active compounds showed moderate ER-affinity in vitro and low estrogenic activity in vivo, but marked effects on ER$^+$ breast cancer models in vitro as well as in vivo. The ER$^-$ breast cancer, however, was only weakly inhibited. The R 3327 Dunning prostatic tumor of the rat, which also contains ERs, was also inhibited as shown on the example of the [1,2-diaminoethane]dichloroplatinum(II) derivative **16-PtCl$_2$**

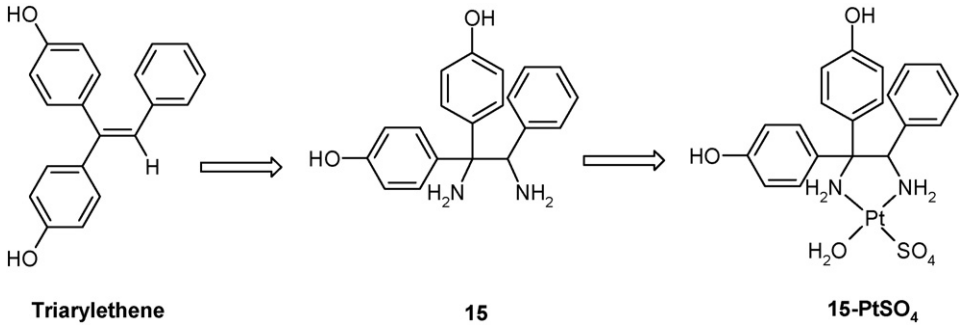


Fig. 15. Transformation of 1,1-bis(4-hydroxyphenyl)-2-phenylethene to aqua[1,1-bis(4-hydroxyphenyl)-2-phenylethylenediamine]sulfatoplatinum(II) [3b].

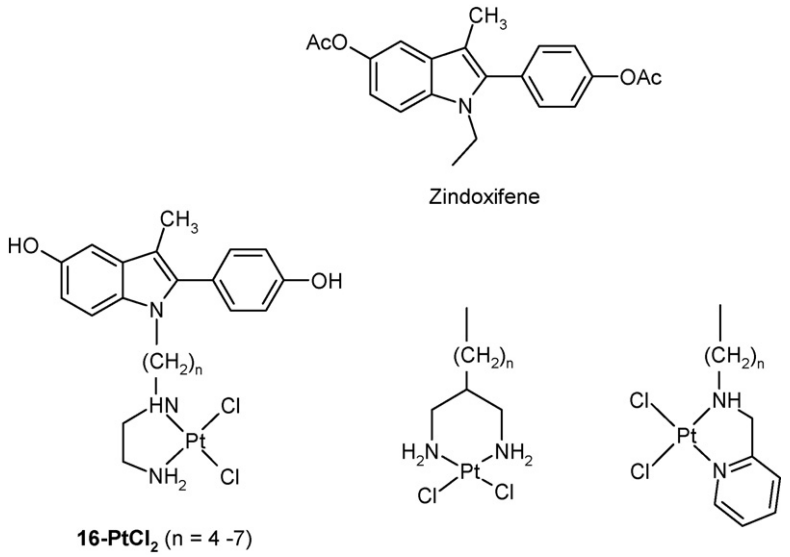


Fig. 16. 2-Phenylindole antiestrogen zindoxifene and related ER-affinic Pt-complexes [2].

($n = 6$) [7e]. A mode of action similar to that of the ligands, which caused comparable effects, was discussed.

The [2-(aminomethyl)pyridine]dichloroplatinum(II) derivative of 5-hydroxy-2-(4-hydroxyphenyl)-3-methylindole (both pharmacophores are linked via a hexane spacer in this compound; see Fig. 17 and formula **17-**$Ptcl_2$) was labelled with platinum 191 and studies of its binding to the ER in vitro and its tissue distribution in immature, female rats in vivo were performed [7h]. The binding affinity of the Pt-complex to the ER was very high (RBA = 32%). However, the ER binding study was complicated by a high degree of irreversible binding to non-ER proteins accompanied by degradation of the Pt-complex under partial release of the diamine ligand. Therefore, it is difficult to be certain, whether the measured binding affinity is due to the Pt-complex or whether it arises from the diamine ligand release. In the tissue distribution studies, much of the ^{191}Pt-diamine complex was deposited in the liver; there was no evidence of selective uptake of this compound by estrogen target like uterus. Thus, it is not clear, whether the observed bioactivity arises from the interaction of the Pt-complex or of the released diamine ligand with the ER.

For the development of stable back up substances in this very interesting class of ER-affinic Pt-complexes, an exchange of the two Pt-standing chlorine atoms by stronger bound dicarboxylic acids like CBDCA seems to be the most promising approach.

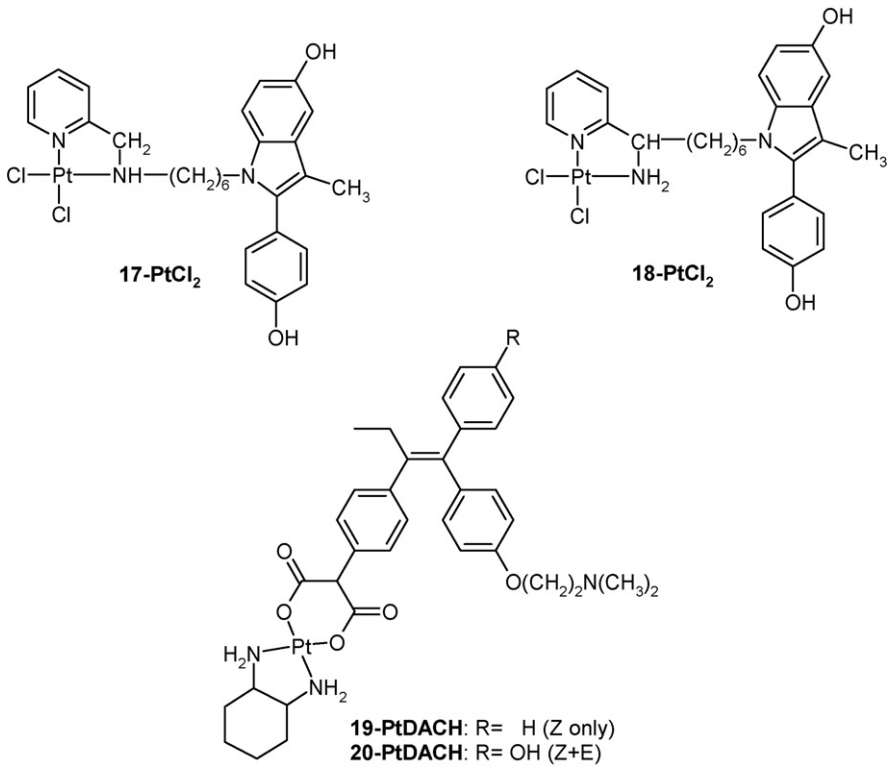


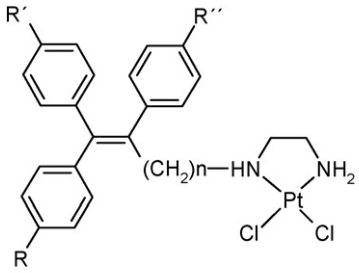
Fig. 17. Platinum complexes of zindoxifene and tamoxifen derivatives [31c].

Brunner and co-workers [31c] synthesized an isomer of **17-PtCl$_2$** (i.e. **18-PtCl$_2$**) which they assumed to possess an enhanced cytotoxicity due to its primary aminomethyl group in the Pt-pharmacophore instead of the secondary one in **17-PtCl$_2$** (compare formula **17-PtCl$_2$** with formula **18-PtCl$_2$**). The complex **18-PtCl$_2$** and its ligand (**18**) showed significant ER affinity (RBA of **18-PtCl$_2$** = 5.3% and of **18** = 10.1%) but no cytotoxicity in tests on the P388 leukemia of the mouse and on the ER$^-$ MDA-MB-231 breast cancer cell line. However, weak anti-tumor effects of **18-PtCl$_2$** were observed on the ER$^+$ MCF-7 breast cancer cell line; they were significantly stronger in its ligand **18**.

6.2. Triarylethylene derivatives

(DACH)Pt(II)malonates containing tamoxifen (**19-PtDACH**) or its metabolite 4-hydroxytamoxifen (**20-PtDACH**) as carrier were described by Vessiéres et al. [5]. In these compounds the Pt-pharmacophore is linked in 2-position of the leaving group malonate to the para position of the 2-standing phenyl residue. The authors obtained the pure Z isomer of **19-PtDACH** and a mixture of the diastereomeric **20-PtDACH** (E/Z ratio of 80/20). The investigation of these compounds on their ER$_\alpha$ affinities yielded, as expected, a lower RBA (0.5%) for **19-PtDACH** than for **20-PtDACH** (6.4%). In the test on the ER$^+$ MCF-7 breast cancer cell line **20-Pt(DACH)** inhibited the DNA synthesis somewhat stronger (IC$_{50}$ = 4.0 μM) than the Pt-pharmacophore **(DACH)PtCl$_2$** (IC$_{50}$ = 6.3 μM), while the effect found for **19-PtDACH** was weaker (IC$_{50}$ = 14.0 μM). These results suggest that the unobtainable pure Z isomer of **20-PtDACH** most likely possesses an anti-tumor activity on the ER$^+$ MCF-7 breast cancer cell line which markedly surpasses that of **(DACH)PtCl$_2$** due to the strong dependence of the ER$_\alpha$ affinity and antiestrogenic potency on the spatial structure of 4-hydroxytamoxifen. Therefore, the synthesis of stable derivatives of the Z-isomer of **20-PtDACH** as potential drugs is recommended.

Berubé and co-workers [8a–g] reported on the synthesis of dichloroplatinum(II) derivatives of a series of ethylenediamines equipped with a triarylethylene group at the end of a $(CH_2)_n$NH-CH_2-CH_2-NH_2 chain for the therapy of the hormone-sensitive breast cancer (see Fig. 18). They evaluated the ER affinity, which pointed to a mode of action according to the "drug targeting concept" as well as to a possible anti-estrogenic potency, and for confirmation, they performed comparative tests on the ER$^+$ MCF-7 and ER$^-$ MDA-MB-231 breast cancer cell line. Compounds with a 4-OH group in two (**22-PtCl$_2$**) or three phenyl residues (**23-PtCl$_2$**) showed weak RBA values between 0.2 and 1.4%, while the non-hydroxyl substituted series **21-PtCl$_2$** did not bind to the ER. The strongest cytotoxic activity, which was comparable with that of cDDP, showed the non-hydroxy substituted complex series **21-PtCl$_2$**. In contrast to the expections, the ER$^-$ MDA-MB-231 breast



21-PtCl$_2$:R = R′= R′′= H
22-PtCl$_2$:R = R′= OCH_3 or OH, R′′=H
23-PtCl$_2$:R = R′= R′′= OCH_3 or OH

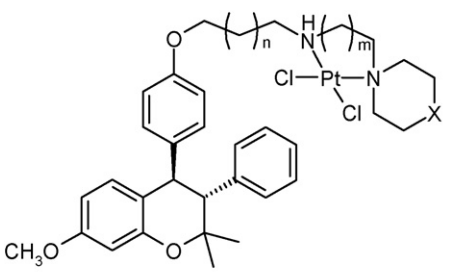
Fig. 18. 1,1,2-Triarylalkene substituted [ethylenediamine]platinum(II) complexes [8a–f].

cancer cell line proved to be more sensitive against the hydroxyl substituted series **22-PtCl$_2$** and **23-PtCl$_2$** than the ER$^+$ MCF-7 cell line. In most cases, the higher homologues were more active. The cytotoxic effects of these drugs seem not to be related to their affinity for the ER. Berubé and co-workers suggested that the addition of a triarylethylene moiety to Pt-complexes increases the hydrophobicity, and consequently the resulting drugs become more permeable to the membrane.

It must be remembered that not only 1,1,2-triarylalkenes in which a (dialkylamino)ethoxy residue is attached to one of the aromatic rings, but also those without this group, cause antiestrogenic effects and therefore show anti-breast cancer activity [32a–d]. The extent of the ER affinity and of the estrogen antagonistic effect of 1,1,2-triarylalkenes depends on the number and position of the OH groups as well as on the configuration [32a–d]. The highest ER affinity and antiestrogenic potency were recorded in C2-alkyl substituted 1,1-bis(4-hydroxyphenyl)-2-phenylethenes and 1,1,2-tris(4-hydroxyphenyl)ethenes with short alkyl chains [32e–g]. Polar terminal groups (e.g. CN or NH_2) at the C2-alkyl chain weakened the activity [32h]. The former results recommend additional experiments which prove, whether a linkage of Pt-pharmacophores to one of the three aryl residues is suitable for obtaining ER-affinic, cytotoxic 1,1,2-triphenylalkenes.

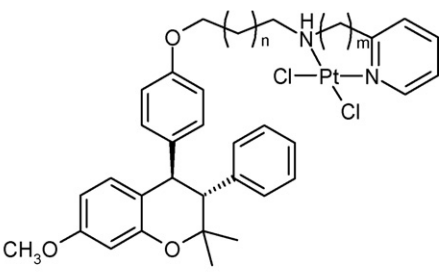
6.3. Benzopyran-based platinum(II) complexes

Berubé and co-workers [8g] synthesised a series of benzopyran-based Pt-complexes and evaluated their cytotoxic efficacy in vitro against different hormone-dependent and -independent breast cancer cell lines. The new compounds of the **24-PtCl$_2$** and **25-PtCl$_2$** types were significantly cytotoxic, whereby complexes from aminoethylpyridine compounds (Fig. 19) showed superior activ-



X = CH_2, O; n= 1,2; m = 4

24-PtCl$_2$



n= 1,2; m = 2,4,6,8,10

25-PtCl$_2$

Fig. 19. Benzopyran-based platinum(II) complexes [8g].

ity to their aminomethylpyridine analogs. Furthermore, complexes from aromatic amines were more potent than their aliphatic amine analogs. However, the length of linker chain has little effect on biological activity. Molecular modeling predicted for the **25-PtCl$_2$** series that the 3S,4S, isomer may be the most active of the two enantiomers. Further, detailed investigation of these compounds on ER binding would be helpful in designing new anticancer drugs.

7. Breast cancer inhibiting [1,2-bis(4-fluorophenyl)ethylenediamine]platinum(II) complexes—compounds acting according to the drug targeting concept

Fluoro-substituted [1,2-diarylethylenediamine]platinum(II) complexes proved to be very active on several in vitro and in vivo tumor models (e.g. leukemia P 388, leukemia L 1210, Ehrlich ascites tumor, breast cancers: ER$^-$ MDA-MB-231, ER$^+$ MCF-7, ER$^+$ MXT-M-3,2, ER$^-$ MXT-M-3,2 (ovex) and others). The efficacy depended on the number and position of the F-atoms in the phenyl rings, the used leaving groups as well as on the configuration (RS or RR/SS) at the five membered chelate ring. The latter (RR/SS-configuration), as a rule, gave rise to stronger tumor growth inhibiting effects [9a–h].

Besides [(RS)- and (RR/SS)-1,2-bis(2,6-difluorophenyl)ethylenediamine]dichloroplatinum(II) [9g,h] the diastereomeric aqua[1,2-bis(4-fluorophenyl)ethylenediamine]sulfatoplatinum(II) complexes (**meso-** and **racem-26-PtSO$_4$**) showed the best effects in the test on the ER$^+$ MXT-M-3,2 mammary carcinoma of the mouse [9a,f]. Therefore, **meso-** and **racem-26-PtSO$_4$** were selected for detailed studies.

7.1. Aqua[1,2-bis(4-fluorophenyl)ethylenediamine]sulfatoplatinum(II)

The anti-breast cancer effect of the diastereoisomeric **26-PtSO$_4$** was accompanied by a strong reduction of the uterine weight indicating an estrogen ablation brought about by interference of these drugs with the steroid biosynthesis (Mechanism B; see Section 2.2). The fact that neither estrogenic nor anti-estrogenic properties could be detected in the uterine weight test on juvenile mice [9f] and that a marked antiproliferative activity was observed on several breast cancer cell lines [9a] confirmed the existence of a mixed mode of action.

An important observation was the 22.3- and 10.3-fold accumulation of **racem-** and **meso-26-PtSO$_4$** in ER$^+$ MCF-7 breast cancer cells after a 24 h treatment at the anti-tumor effective concentration of 5 μM, assessed by neutron activation analysis of platinum by Lux and co-workers [9i,17h]. The accumulation factor of the standard cisplatin was only 2.55. The comparison of DNA-associated platinum revealed a base pair/platinum ratio of 2.1×10^4 for **racem-26-PtSO$_4$**, 3.7×10^4 for **meso-26-PtSO$_4$** and 6.1×10^4 for cisplatin.

The concept of developing Pt-complexes with greater tumor specificity than cisplatin or carboplatin by means of ligands which lend the capability of accumulating in tumor cells to their complexes seemed (in contrast to ER-affinic Pt-complexes like **meso-1-PtLL′**) to be realized with **racem-** and **meso-26-PtSO$_4$**. However, the mechanism of accumulation of **racem-** and **meso-26-PtSO$_4$** is still unknown (compare ref. [33a]).

In comparative in vivo tests on the ER$^+$ MXT-M-3,2 breast cancer of the mouse the anti-tumor effects of **racem-** and **meso-26-PtSO$_4$** were not superior to that of the standard cisplatin [9a,f]. An explanation is the influence of pharmacokinetic properties on their anti-tumor activity in vivo. For instance, hydrolysis sensitive compounds like the aquasulfatoplatinum(II) complexes **racem-** and **meso-26-PtSO$_4$** bind in a fast reaction to bionucleophiles, especially to chloride ions, and irreversibly to nucleophilic centers (especially to S-containing residues) of plasma components. From the latter process insufficient free drug levels can result, which in turn lead to inadequate anti-tumor activities. Therapeutically suitable Pt-complexes must have a hydrolysis behavior, which guarantees an optimal free drug level in plasma as well as a cytotoxic level of aquated metabolites within the tumor cell. The highly reactive nature of the aquasulfatoplatinum(II) moiety of **racem-** and **meso-26-PtSO$_4$** is a disadvantage, not only with respect to their bioavailability, but also to their toxicity.

7.2. [1,2-Bis(4-fluorophenyl)ethylenediamine]dichloroplatinum(II)

Interestingly, the testing of the less reactive and therefore better tolerated dichloroplatinum(II) complexes **meso-** and **racem-26-PtCl$_2$** yielded no significant growth inhibiting effects on tumor and uterus of ER$^+$ MXT-M-3,2 breast cancer bearing mice [9f], presumably due to low water solubility and with this insufficient bioavailability. This assumption was confirmed in comparative pharmacokinetic studies with the diastereoisomeric aquasulfatoplatinum(II) complexes (**26-PtSO$_4$**) which caused plasma levels sufficient for anti-tumor effects [9m]. In contrast to this, **racem-** and **meso-26-PtCl$_2$** were active in the ER$^+$ MCF-7 breast cancer cell line, owing to the lower concentration of S-containing plasma proteins (which inactivate Pt-complexes) in the cell culture medium than in the blood plasma of test animals [9k]. Accordingly, these compounds showed comparable uptake by tumor cells and therefore similar cytotoxicity like the corresponding aquasulfatoplatinum(II) derivatives [9m]. As expected, in this test the (RR/SS)-form of **26-PtCl$_2$** proved to be more active than its (RS)-form [9k]. Resolution of the **racem-26-PtCl$_2$** into its enantiomers failed to enhance the antineoplastic effect [9k,o].

7.3. Galenical formulation of [1,2-bis(4-fluorophenyl)ethylenediamine]dichloroplatinum(II) for parenteral administration

Because of the negative results with the diastereoisomeric **26-PtCl$_2$** in the test on the ER$^+$ MXT-M-3,2 breast cancer of the mouse [9f], a parenterally administrable formulation of the more cytotoxic **racem-26-PtCl$_2$** was developed [9l]. The procedure based on the reaction of **racem-26-PtSO$_4$** with NaCl in water in the presence of pluronic F 68 as stabilizer and results in a sufficiently stable colloidal solution (i.e. hydrosol). In contrast to the poorly water soluble **racem-26-PtCl$_2$**, which was exclusively effective in cell culture experiments, its hydrosol formulation proved to be not only comparably active in vitro in respect to uptake by tumor cells and cytotoxicity [9l,m], but also very potent in vivo towards the ER$^+$ MXT-M-3,2 breast cancer of the mouse [9l]. The **racem-26-PtCl$_2$-hydrosol** inhibited the tumor growth in a dose-dependent manner. At the highest dosage (20 μmol/kg) it reduced the tumor development by 80%. As obvious from the body weight (BW) of the test animals, the hydrosol formulation was tolerated very well (BW = 21.4 ± 1.2 g (control 21.1 ± 1.7 g) on day 34 after administration of 20 μmol/kg **racem-26-PtCl$_2$-hydrosol**). Pharmacokinetic studies showed that a long-lasting blood plasma level of **racem-26-PtCl$_2$** (≥5 μM) could be achieved in mice, if the hydrosol formulation was administered with an intraperitoneally implanted Alzet micro pump [9m]. In this concentration range the hydrosol formulation brought about cytocidal effects on the ER$^+$ MCF-7 as well as on the ER$^-$ MDA-MB-231 breast cancer cell line [9l,m]. Pharmacokinetic studies with **racem-26-PtCl$_2$-hydrosol** formulation revealed moreover that in vivo inactivation of a considerable part of the Pt-complex took place by irreversible binding to plasma proteins [9m].

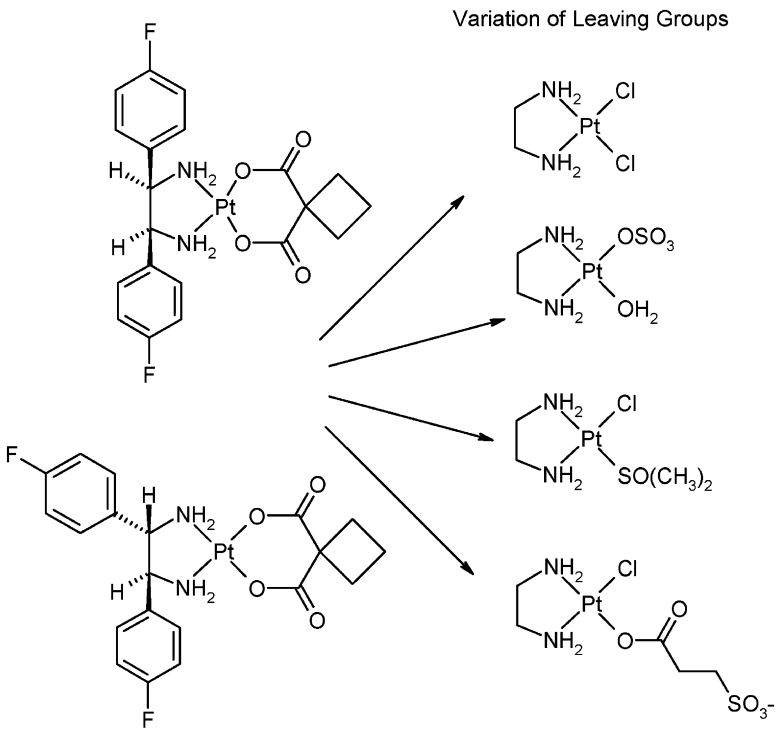


Fig. 20. Leaving group derivatives of [1,2-bis(4-fluorophenyl)ethylenediamine]platinum(II) (**26-PtLL′**): LL′ = aquasulfato-, dichloro-, CBDC-, DMSO- and sulfopropionato [9o,q,r].

7.4. [1,2-Bis(4-fluorophenyl)ethylenediamine][cyclobutane-1,1-dicarboxylato]platinum(II)

Therefore, for improvement of the stability against bionucleophiles and with this of the pharmacokinetic behavior of **racem-26-PtCl$_2$**, thorough studies were performed, in which several mono and dicarboxylic acids were used as leaving groups instead of chlorides [9n–p]. In an assay employing the ER$^+$ MCF-7 breast cancer cell line as test model the activity of **racem-26-PtCBDC** proved to be considerably higher than that of carboplatin (non-significantly active at a concentration of 5 μM; intracellular complex concentration $Pt_{Pt,c}$ = 1.2 μM) and equalled the effect of cisplatin (compare Fig. 21 with Fig. 22) [9o]. In fact, with **racem-26-PtCBDC** an about 20-fold higher intracellular concentration than with the therapeutically used carboplatin containing two NH_3 ligands instead of the 1,2-bis(4-fluorophenyl)ethylenediamine carrier ligand was determined [9o]. However, in comparison to its aquasulfatoplatinum(II) derivative the anti-breast cancer activity of **racem-26-PtCBDC** was reduced (Fig. 21).

The study showed that a marked improvement of the anti-tumor potency of dicarboxylatoplatinum(II) complexes endowed with high stability but low anti-tumor activity, such as carboplatin, can be achieved by using 1,2-bis(4-fluorophenyl)ethylenediamine as carrier ligand.

For estimation of the resistance against attacks of bionucleophiles, which inactivate Pt-complexes by irreversible binding on their way to the tumor, kinetic studies in aqueous solution at 37 °C with **meso-26-PtCBDC** using I^- as nucleophile were performed Scheme 2 [9o]. Carboplatin and **meso-26-PtCl$_2$** were employed for comparison. For technical reasons the better water soluble meso-form of **26-PtCBDC** and **26-PtCl$_2$** was used in these experiments, since no significant differences existed in the rate constants of diastereoisomeric [1,2-diarylethylenediamine]platinum(II) complexes concerning the exchange of the leaving group by nucleophiles like H_2O or I^- [9o]. This pre-screen allows a fast and uncomplicated quantitative determination of the products (formed via direct or indirect nucleophilic substitution; see Scheme 2) by HPLC and gives useful hints as to the pharmacokinetic behavior of the respective Pt-complex. Scheme 2 represents the aquation and the nucleophilic attack at the platinum. From these kinetic studies (data Table 4) it was concluded that the diastereoisomeric **26-PtCBDC** derivatives are relatively resistant against attack by bionucleophiles like S-containing plasma components in vivo, a property which in turn should lead to an elevated non-protein-bound drug level as compared to **26-PtCl$_2$-hydrosol** or **26-PtSO$_4$**.

The results of binding studies to human serum albumin (HSA) confirmed this assumption. HSA, the major protein in plasma and

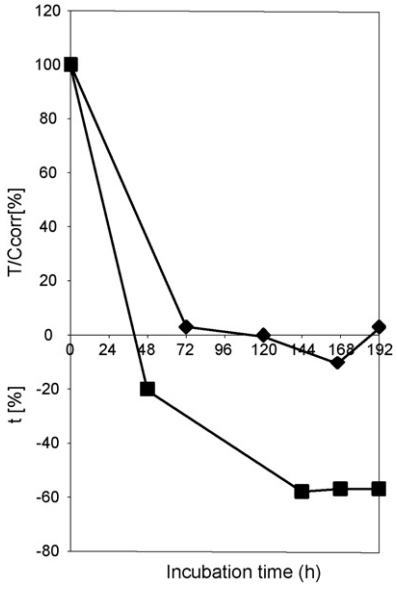


Fig. 21. Proliferation inhibiting effect of **racem-26-PtSO$_4$** (■) and **racem-26-PtCDBC** (♦) on the ER$^+$ MCF-7 breast cancer cell line [9o].

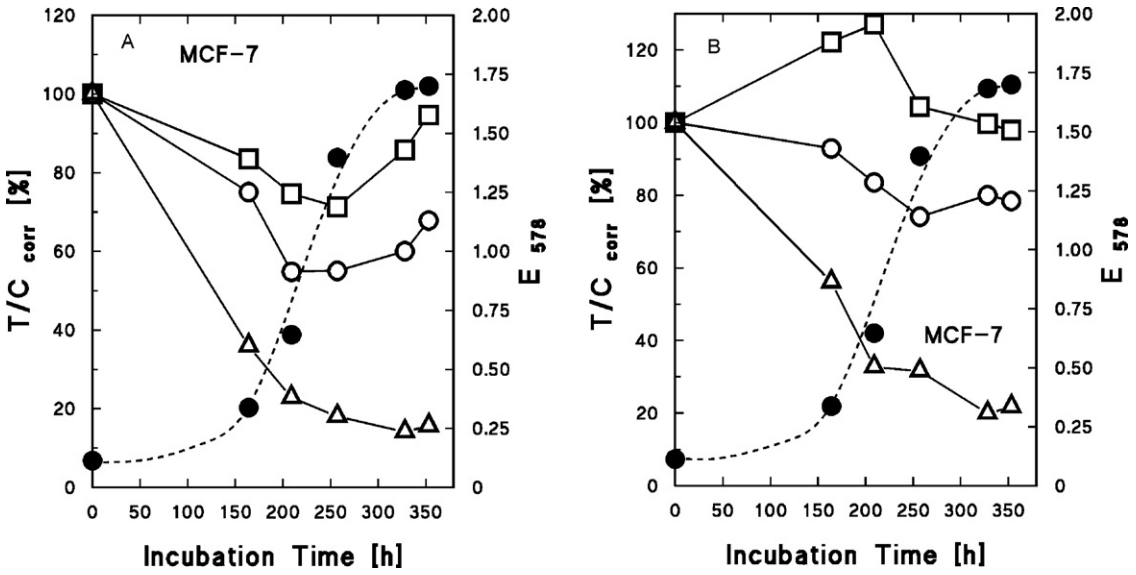
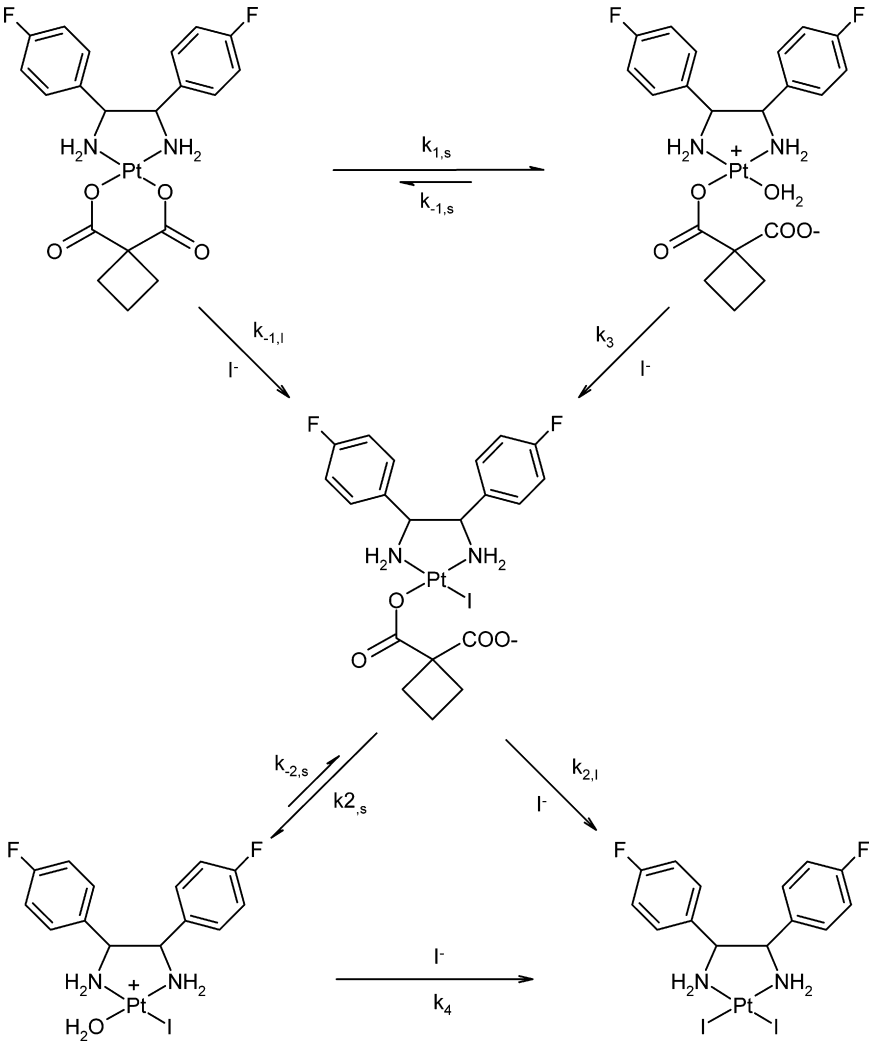


Fig. 22. Proliferation-inhibiting effect of cisplatin (A; 0.5, 1.0 and 5.0 μM) and carboplatin (B; 1.0, 5.0 and 10.0 μM) on the ER+ MCF-7 breast cancer cell line [9o].



Scheme 2. Reaction of **26-PtCBDC** with nucleophiles on the example of iodide [9o].

Table 4
Rate constants[a] for the reaction of **meso-26-PtCl$_2$** and **meso-26-PtCBDC** as well as of carboplatin with several nucleophiles at 37 °C.

Compound	Nucleophile	$k_{1,obs} \times 10^5$ [s^{-1}]	$k_{2,obs} \times 10^5$ [s^{-1}]
meso-26-PtCl$_2$	I^-	15.9	5.6
meso-26-PtCBDC	I^-	0.9	3.1
	Cl^-	0.18	1.27
	H_2O	0.0011	[b]
carboplatin	I^-	0.7	0.83
	Cl^-	0.051	[b]
	H_2O	0.0028	[b]

[a] The Pt-complexes (50.0 μM) were reacted with I^- (5.0 mM) and Cl^- (154 mM; physiological concentration), respectively.
[b] The value is not determinable because of the very high stability of the complex.

mainly responsible for the inactivation of Pt-complexes during their transport to the tumor, is especially suitable as representative bionucleophile for in vitro assays allowing an assessment of the complex stability in vivo. Such experiments were performed with the more anti-tumor active diastereoisomer **racem-26-PtCBDC** (comparison compounds: **racem-26-PtCl$_2$**, carboplatin and cisplatin). To imitate in vivo conditions, an HSA concentration of 500 μM was chosen in this assay. A second experimental series, in which a 50 μM HSA concentration was used, was performed to estimate the complex stability in cell culture experiments (Fig. 23).

The binding kinetic of the complexes to HSA was determined by the measurement of the platinum contents by means of atomic absorption spectroscopy (AAS) in the ultrafiltrates (i. e. the non-albumin bound percentage). The experimental results confirmed the assumption that an exchange of the two chlorine ligands in **racem-26-PtCl$_2$** by the bidentate cyclobutane-1,1-dicarboxylate residue enhanced the stability of this complex type against nucleophilic attack of plasma components (Fig. 23A and B). The percentage of the non-albumin bound **racem-26-PtCBDC** was considerably higher than that of the corresponding dichloroplatinum(II) compound and also superior to that of cisplatin. In comparison to carboplatin, however, the non-albumin bound portion of **racem-26-PtCBDC** was markedly smaller. The order of stability of the test compounds assessed from the 25 h values in Fig. 23A and B is **carboplatin > racem-26-PtCBDC > cisplatin > racem-26-PtCl$_2$** and it is true for the reaction with 50 μM HSA and with 500 μM HSA. The same order of stability was observed in the reaction with the nucleophile I^-.

Though the solubility of **racem-26-PtCBDC** is sufficient to achieve concentrations for optimal activity on the ER$^+$ MCF-7 breast cancer cell line (Fig. 21), it is too low for the preparation of aqueous infusion solutions containing the required drug concentration. For the in vivo testing of this Pt-complex it is necessary to develop formulations by use of appropriate additives. To get better water soluble, stabile analogues [(RR/SS)-1,2-bis(4-fluorophenyl)ethylenediamine]chloro[sulfinyl-bis(methane)-S]platinum(II) chloride **racem-26-PtDMSO** [9q] and [(RR/SS)-1,2-bis(4-fluorophenyl)ethylenediamine](sulfopropionato)platinum(II) **racem-26-Pt-sulfopropionate** [9r] (formulae Fig. 20) were synthesized. The former compound was thoroughly studied. It is endowed with sufficient water solubility for parenteral administration, with a comparable pharmacokinetic behavior and with the same molecular mode of action like carboplatin. Owing to its (RR/SS)-1,2-bis(4-fluorophenyl)ethylenediamine carrier ligand it is markedly more anti-tumor active than carboplatin on the human ER$^+$ MCF-7 breast cancer cell line. The compound is also an interesting candidate for further preclinical and clinical studies.

8. Development of further cytotoxic metal complexes containing a carrier ligand derived from 1,2-bis(4-fluorophenyl)ethylenediamine

A promising development is the polyimine dendrimer complex **26-Pt-Dendrimer** (Fig. 24) in which 4 molecules **meso-26-PtDMSO** are bonded to the linker N,N,N′,N′-tetrakis(3-aminopropyl)butane-1,4-diamine [33b]. The compound showed a 20-fold higher cellular uptake and a ~700-fold higher DNA binding than cisplatin in experiments on ER$^+$ MCF-7 breast cancer cells, but only in serum-free medium, presumably due to a strong inactivation by plasma proteins. Mechanistical studies on the cellular uptake performed with a dinuclear analogue of **26-Pt-Dendrimer** suggested that the drug accumulation was caused by endocytotic processes [33a]. Further structural variations are necessary to get a drug suitable for a clinical application.

Structure activity relationship studies revealed that the exchange of one 4-fluorophenyl residue in **26-PtLL′** by H or a short alkyl group resulted in a marked increase of anti-tumor activity. In particular the (RR/SS)-configurated [1,2-diamino-1-(4-fluorophenyl)alkane]platinum(II) complexes **27-PtCl$_2$** and **27-PtCBDC** proved to be superior to their parent compounds **racem-26-PtCl$_2$** and **racem-26-PtCBDC** and surpassed the standards cisplatin and carboplatin considerably in their activities on the human ER$^+$ MCF-7 breast and LNCaP/FGC prostate cancer cell lines [33c]. However, the existence of a drug accumulation, which can be observed for **racem-26-PtLL′**, must be

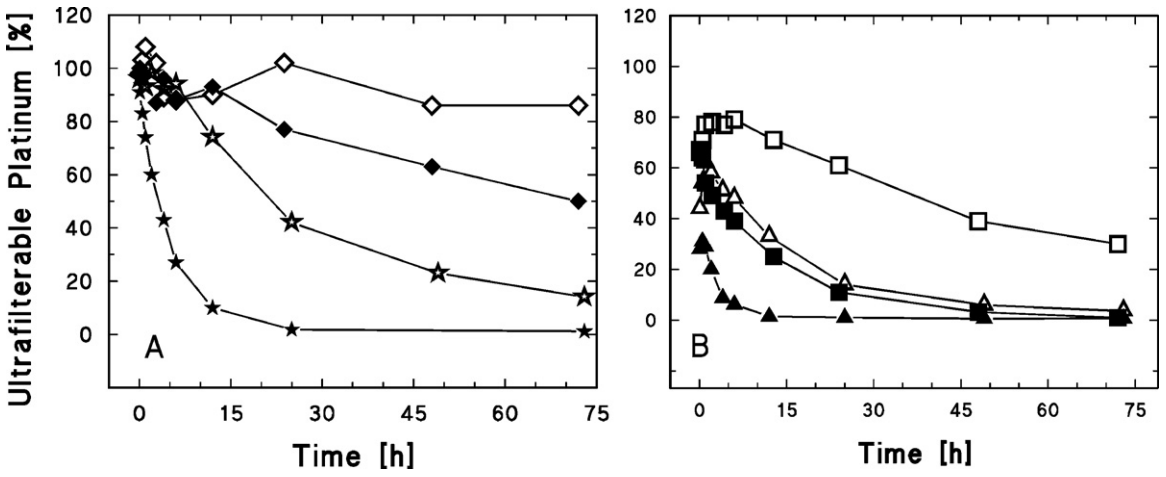


Fig. 23. Binding of cisplatin (A, *), carboplatin (A, ◊), **racem-26-PtCBDC** (B, ■) and **racem-26-PtCl$_2$** (B, ▲) to human serum albumin (HSA); incubation with 50 μM HSA open symbols and with 500 μM HSA solid symbols [9o].

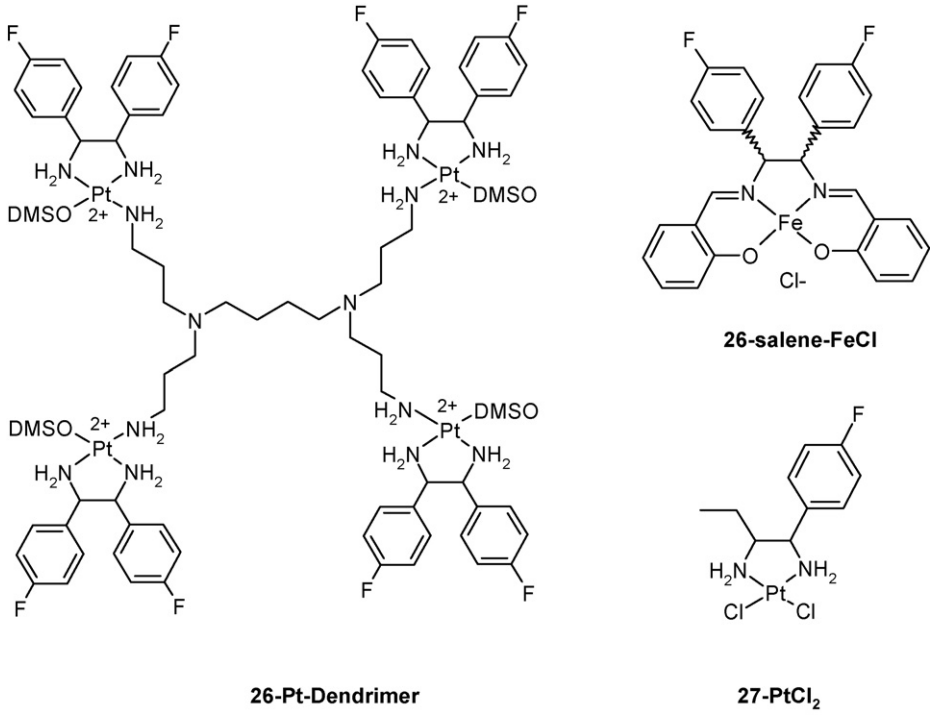


Fig. 24. [1,2-Bis(4-fluorophenyl)ethylenediamine]platinum(II) related polyimine dendrimer complex **26-Pt-Dendrimer**, [1,2-diamino-1-(4-fluorophenyl)butane] platinum(II) complex **27-PtCl$_2$** and [3,4-bis(4-fluorophenyl)-1,6-bis(2-hydroxyphenyl)-2,5-diazahexa-1,5-diene]ferrum(III) chloride (**26-salene-FeCl**) [33b,c,g].

proven. Results of thorough studies with enantiomeric [1,2-diamino-1-phenylalkane]platinum(II) complexes did not support an enantioselectivity of these compounds as suggested by the chiral structure of DNA [33d–f]. In contrast to the diastereoisomeric Pt-complexes the related enantiomers showed at best minimal differences regarding their anti-tumor potency.

Furthermore, Gust and co-workers [33g] studied Fe(III) complexes such as [(RR/SS)-3,4-bis(4-fluorophenyl)-1,6-bis(2-hydroxyphenyl)-2,5-diazahexa-1,5-diene]ferrum(III) chloride **(racem-26-salene-FeCl**, see Fig. 24**)** containing an F-substituted 1,2-diphenylethylenediamine fragment. These complexes proved to be strongly active on breast and prostate cancer cell lines due to its capability to accumulate in these tumor cells (e.g. 22-fold in ER$^+$ MCF-7 breast cancer cells, **racem-26-salene-FeCl** concentration 5 μM). First studies to the mode of action show that **racem-26-salene** can interact with DNA under formation of DNA fragments and induction of apoptosis. Comparable pharmacological properties were observed for cobalt(3,4-diarylsalene) complexes (salene: 1,6-bis(2-hydroxyphenyl)-2,5-diazahexa-1,5-diene) [33h].

9. Conclusion

Studies of numerous scientists show that ER-affinic Pt-complexes are active on estrogen-sensitive (i. e. ER$^+$) tumors like breast and prostate cancer by a complex mode of action in which ER-agonistic or ER-antagonistic properties are involved. ER-mediated accumulation in tumor cells and increased incorporation into their DNA may be contributed to the anti-tumor activity, however, the proof must still be furnished. The functioning of the concept, improvement of anti-tumor activity by use of carrier ligands in Pt-complexes, was shown in studies with [1,2-bis(4-fluorophenyl)ethylenediamine]platinum(II) complexes.

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References

[1] R. Gust, W. Beck, G. Jaouen, H. Schönenberger, Coord. Chem. Rev. in press.
[2] E. von Angerer, The estrogen receptor as a target for cytotoxic agents, in: The Estrogen Receptor as a Target for Rational Drug Design, R. G. Landes Company/Springer Verlag, Austin, TX, USA/Heidelberg, 1995 (Chapter 8).
[3] (a) J. Karl, R. Gust, T. Spruß, M. Schneider, H. Schönenberger, J. Engel, K.H. Wrobel, F. Lux, S. Trebert-Haeberlin, J. Med. Chem. 31 (1988) 72;
(b) R. Gust, Arch. Pharm. (Weinheim) 327 (1994) 49.
[4] (a) R. Schlemmer, Ph.D. Thesis, Universität Regensburg, 1995;
(b) R. Schlemmer, T. Spruß, G. Bernhardt, H. Schönenberger, Arch. Pharm. Pharm. Med. Chem. 332 (1999) 59;
(c) R. Schlemmer, T. Spruß, G. Bernhardt, H. Schönenberger, Arch. Pharm. Pharm. Med. Chem. 333 (2000) 69;
(d) R. Schlemmer, T. Spruß, G. Bernhardt, H. Schönenberger, Arch. Pharm. Pharm. Med. Chem. 333 (2000) 397;
(e) R. Schlemmer, T. Spruß, G. Bernhardt, H. Schönenberger, Arch. Pharm. Pharm. Med. Chem. 333 (2000) 404;
(f) R. Schlemmer, T. Spruß, G. Bernhardt, H. Schönenberger, Arch. Pharm. Pharm. Med. Chem. 334 (2001) 309.
[5] A. Vessière, S. Top, W. Beck, E. Hilllard, G. Jaouen, Dalton Trans. (2006) 529.
[6] (a) M.R. Schneider, E. von Angerer, W. Höhn, F. Sinowatz, Eur. J. Cancer Clin. Oncol. 23 (1987) 1005;
(b) M.R. Schneider, C.D. Schiller, A. Humm, E. von Angerer, J. Cancer Res. Clin. Oncol. 117 (1991) 33.
[7] (a) E. von Angerer, J. Med. Chem. 25 (1982) 1374;
(b) E. von Angerer, J. Prekajac, J. Med. Chem. 26 (1983) 113;
(c) E. von Angerer, A.K. Taneja, R. Ringshandl, H. Schönenberger, Liebigs Ann. Chem. (1980) 409;
(d) N. Knebel, E. von Angerer, J. Med. Chem. 31 (1988) 1675;
(e) N. Knebel, C.D. Schiller, M.R. Schneider, H. Schönenberger, E. von Angerer, Eur. J. Cancer Clin. Oncol. 25 (1989) 293;
(f) E. von Angerer, H. Birnböck, N. Knebel, Anti-Cancer Drug Des. 4 (1989) 21;
(g) N. Knebel, E. von Angerer, J. Med. Chem. 34 (1991) 2145;
(h) J.P. Dizio, K.E. Carlson, C.J. Bannochie, M.J. Welch, E. von Angerer, J.A. Katzenellenbogen, J. Steroid Biochem. Mol. Biol. 42 (1992) 363.
[8] (a) P. Wheeler, G. Berubé, Inorg. Chim. Acta 209 (1993) 97;
(b) G. Berubé, P. Wheeler, C.H.J. Ford, M. Gallant, Z. Tsaltas, Can. J. Chem. 71 (1993) 1327;
(c) Y. He, S. Grolean, R.C. Gaudreault, M. Caron, H.M. Thérien, G. Berubé, Bioorg. Med. Chem. Lett. 5 (1995) 2217;

(d) G. Berubé, Y. He, S. Groleau, A. Séné, H.M. Therién, M. Caron, Inorg. Chim. Acta 262 (1997) 139;
(e) A. Séné, G. Berubé, R.C. Gaudreault, Drug Des. Discov. 15 (1998) 277;
(f) G. Grenier, G. Berubé, C. Gicquaud, Chem. Pharm. Bull. 46 (1998) 1480;
(g) A. Gupta, S.K. Mandal, V. Lebanc, C. Descôteaux, E. Asselin, G. Berubé, Bioorg. Med. Chem. Lett. 18 (2008) 3982.

[9] (a) G. Bernhardt, H. Reile, T. Spruß, M. Koch, R. Gust, H. Schönenberger, M. Hollstein, F. Lux, J. Engel, Drugs Fut. 16 (1991) 899;
(b) M. Jennerwein, B. Wappes, R. Gust, H. Schönenberger, J. Engel, S. Seeber, R. Osieka, J. Cancer Res. Clin. Oncol. 114 (1988) 347;
(c) R. Müller, R. Gust, M. Jennerwein, H. Reile, R. Laske, W. Krischke, G. Bernhardt, T. Spruß, J. Engel, H. Schönenberger, Eur. J. Med. Chem. 24 (1989) 341;
(d) H. Reile, R. Müller, R. Gust, R. Laske, W. Krischke, G. Bernhardt, T. Spruß, M. Jennerwein, J. Engel, S. Seeber, R. Osieka, H. Schönenberger, Arch. Pharm. (Weinheim) 323 (1990) 133;
(e) H. Reile, T. Spruß, G. Bernhardt, R. Müller, R. Gust, H. Schönenberger, Arch. Pharm. (Weinheim) 324 (1991) 405;
(f) H. Reile, T. Spruß, R. Müller, R. Gust, G. Bernhardt, H. Schönenberger, Arch. Pharm. (Weinheim) 323 (1990) 301;
(g) R. Müller, E. Schickaneder, M. Jennerwein, H. Reile, T. Spruß, J. Engel, H. Schönenberger, Arch. Pharm. (Weinheim) 324 (1991) 115;
(h) T. Spruß, G. Bernhardt, E. Schickaneder, H. Schönenberger, J. Cancer Res. Clin. Oncol. 117 (1991) 435;
(i) F. Lux, M. Hollstein, H. Reile, G. Bernhardt, H. Schönenberger, Biol. Trace Elem. Res. 55 (1996) 113;
(k) H.D. vom Orde, H. Reile, R. Müller, R. Gust, G. Bernhardt, T. Spruß, H. Schönenberger, T. Burgemeister, A. Mannschreck, J. Cancer Res. Clin. Oncol. 116 (1990) 434;
(l) R. Gust, G. Bernhardt, T. Spruß, R. Krauser, M. Koch, H. Schönenberger, K.H. Bauer, S. Schertl, Z. Lu, Arch. Pharm. (Weinheim) 328 (1995) 645;
(m) M. Koch, Ph.D. Thesis, Universität Regensburg, 1993;
(n) R. Gust, R. Krauser, B. Schmid, H. Schönenberger, Inorg. Chim. Acta 250 (1996) 203;
(o) R. Gust, B. Schnurr, R. Krauser, G. Bernhardt, M. Koch, B. Schmid, E. Hummel, H. Schönenberger, J. Cancer Res. Clin. Oncol. 124 (1998) 585;
(p) R. Gust, R. Krauser, B. Schmid, H. Schönenberger, Arch. Pharm. Pharm. Med. Chem. 331 (1998) 27;
(q) R. Gust, H. Heinrich, R. Krauser, H. Schönenberger, Inorg. Chim. Acta 285 (1999) 184;
(r) R. Müller, P. Bednarski, H. Schönenberger, Inorg. Chim. Acta 195 (1992) 77.

[10] (a) D.P. Griswold, H.E. Skipper, W.R. Laster, W.S. Wilcox, F.M. Schabel, Cancer Res. 26 (1966) 2169;
(b) M.R. Schneider, H. Schönenberger, Urol. Int. 43 (Suppl. 1) (1988) 10;
(c) R.W. Hartmann, H. Schönenberger, K.H. Wrobel, J. Cancer Res. Clin. Oncol. 103 (1982) 241;
(d) R.W. Hartmann, Eur. J. Cancer Clin. Oncol. 19 (1983) 959.

[11] (a) H. Schönenberger, Berichte aus der Forschung der LMU-München 23 (1978) 3;
(b) G. Kranzfelder, M.R. Schneider, E. von Angerer, H. Schönenberger, J. Cancer Res. Clin. Oncol. 97 (1980) 167;
(c) R.W. Hartmann, H. Buchborn, G. Kranzfelder, H. Schönenberger, J. Med. Chem. 24 (1981) 1192;
(d) J. Engel, R.W. Hartmann, H. Schönenberger, Drugs Fut. 8 (1983) 413.

[12] (a) R.W. Hartmann, W. Schwarz, H. Schönenberger, J. Med. Chem. 26 (1983) 1137;
(b) R.W. Hartmann, A. Heindl, H. Schönenberger, J. Med. Chem. 27 (1984) 577;
(c) R.W. Hartmann, A. Heindl, W. Schwarz, H. Schönenberger, J. Med. Chem. 27 (1984) 819;
(d) R.W. Hartmann, W. Schwarz, A. Heindl, H. Schönenberger, J. Med. Chem. 28 (1985) 1295;
(e) S. Schertl, R.W. Hartmann, C. Batzl-Hartmann, R. Schlemmer, T. Spruß, G. Bernhardt, R. Gust, H. Schönenberger, Arch. Pharm. Pharm. Med. Chem. 334 (2001) 125.

[13] (a) M.R. Schneider, E. von Angerer, G. Kranzfelder, H. Schönenberger, Arch Pharm. (Weinheim) 313 (1980) 919;
(b) R.W. Hartmann, J. Med. Chem. 29 (1986) 1668;
(c) R.W. Hartmann, A. Heindl, M.R. Schneider, H. Schönenberger, J. Med. Chem. 29 (1986) 322.

[14] (a) R.W. Hartmann, Drugs Fut. 10 (1985) 48;
(b) R.W. Hartmann, W. Schwarz, M.R. Schneider, J. Engel, H. Schönenberger, Drugs Fut. 13 (1988) 720;
(c) R.W. Hartmann, G. Kranzfelder, E. von Angerer, H. Schönenberger, J. Med. Chem. 23 (1980) 841.

[15] (a) E. von Angerer, G. Kranzfelder, A.K. Taneja, H. Schönenberger, J. Med. Chem. 23 (1980) 1347;
(b) E. von Angerer, G. Egginger, G. Kranzfelder, H. Bernhauer, H. Schönenberger, J. Med. Chem. 25 (1982) 832.

[16] (a) R. Gust, K. Niebler, H. Schönenberger, J. Med. Chem. 38 (1995) 2070;
(b) R. Gust, H. Schönenberger, Arch. Pharm. (Weinheim) 326 (1993) 405;
(c) R. Gust, T. Burgemeister, A. Mannschreck, H. Schönenberger, J. Med. Chem. 33 (1990) 2535;
(d) R. Gust, H. Schönenberger, U. Klement, K.J. Range, Arch. Pharm. (Weinheim) 326 (1993) 967;
(e) A. Otto, M. Faderl, H. Schönenberger, Cancer Res. 51 (1991) 3217;
(f) R. Gust, R. Keilitz, R. Krauser, K. Schmidt, B. Schnurr, Arch. Pharm. Pharm. Med. Chem. 332 (1999) 261;
(g) F. Hafner, E. Holler, E. von Angerer, J. Steroid Biochem. Mol. Biol. 58 (1996) 385;
(h) T. Meyer, R. Koop, E. von Angerer, H. Schönenberger, E. Holler, J. Cancer Res. Clin. Oncol. 120 (1994) 359;
(i) P. Bednarski, B. Trümbach, N. Kratochwil, H. Schönenberger, J. Med. Chem. 35 (1992) 4479;
(k) A. Otto, P. Bednarski, H. Eggers, B. Trümbach, H. Schönenberger, Pharm. Pharmacol. Lett. 1 (1992) 103;
(l) R. Gust, R. Keilitz, K. Schmidt, J. Med. Chem. 44 (2001) 1963;
(m) R. Gust, R. Keilitz, K. Schmidt, J. Med. Chem. 45 (2002) 2325;
(n) R. Gust, V. Lubczyk, K. Schmidt, U. Shihada, Arch. Pharm. Pharm. Med. Chem. 334 (2001) 93;
(o) R. Gust, R. Keilitz, K. Schmidt, M. von Rauch, J. Med. Chem. 45 (2002) 3356;
(p) M. von Rauch, M. Schlenk, R. Gust, J. Med. Chem. 47 (2004) 915;
(q) M. von Rauch, S. Busch, R. Gust, J. Med. Chem. 48 (2005) 466;
(r) T. Wiglenda, I. Ott, B. Kircher, P. Schumacher, D. Schuster, T. Langer, R. Gust, J. Med. Chem. 48 (2005) 6516.

[17] (a) T. Spruß, R. Gust, R. Müller, J. Engel, H. Schönenberger, Arch. Pharm. (Weinheim) 323 (1990) 99;
(b) S. Schertl, R. Gust, R. Müller, T. Spruß, H. Schönenberger, Arch. Pharm. (Weinheim) 325 (1992) 113;
(c) R. Gust, H. Schönenberger, Eur. J. Med. Chem. 28 (1993) 103;
(d) R. Gust, H. Schönenberger, Eur. J. Med. Chem. 28 (1993) 117;
(e) R. Gust, M. Faderl, H. Schönenberger, J. Cancer Res. Clin. Oncol. 126 (2000) 647;
(f) S. Schertl, R.W. Hartmann, C. Batzl-Hartmann, G. Bernhardt, T. Spruß, K. Beckenlehner, M. Koch, R. Krauser, R. Schlemmer, R. Gust, H. Schönenberger, Arch. Pharm. Pharm. Med. Chem. 337 (2004) 349;
(g) R. Gust, K. Niebler, H. Schönenberger, J. Med. Chem. 48 (2005) 7132;
(h) H. Reile, G. Bernhardt, M. Koch, H. Schönenberger, M. Hollstein, F. Lux, Cancer Chemother. Pharmacol. 30 (1992) 113;
(i) G. Bernhardt, M. Koch, T. Spruß, R. Gust, R. Krauser, R. Schlemmer, M. Hollstein, F. Lux, H. Schönenberger, Arch. Pharm. Pharm. Med. Chem. 332 (1999) 195–200;
(k) G. Bernhardt, K. Beckenlehner, T. Spruß, R. Schlemmer, H. Reile, H. Schönenberger, Arch. Pharm. Pharm. Med. Chem. 335 (2002) 55.

[18] S. Schertl, R.W. Hartmann, C. Batzl-Hartmann, G. Bernhardt, T. Spruß, K. Beckenlehner, M. Koch, R. Krauser, R. Schlemmer, R. Gust, H. Schönenberger, Arch. Pharm. Pharm. Med. Chem. 337 (2004) 335.

[19] T. Spruß, S. Schertl, M.R. Schneider, R. Gust, K. Bauer, H. Schönenberger, J. Cancer Res. Clin. Oncol. 119 (1993) 707.

[20] J. Kunde, J. Hoffmann, Bayer Schering Pharma, unpublished data.

[21] S. Schertl, R.W. Hartmann, C. Batzl-Hartmann, T. Spruß, A. Maucher, E. von Angerer, C.D. Schiller, M.R. Schneider, R. Gust, H. Schönenberger, J. Cancer Res. Clin. Oncol. 133 (2007) 153.

[22] M.R. Schneider, C.D. Schiller, A. Humm, T. Spruß, H. Schönenberger, W. Amselgruber, F. Sinowatz, Prostate 15 (1989) 135.

[23] E. Corey, J.E. Quinn, M.J. Emond, K.R. Buhler, L.G. Brown, R.L. Vesella, Clin. Cancer Res. 8 (2002) 1003.

[24] I.B.J.K. Joseph, J.T. Isaacs, J. Natl. Cancer Inst. 90 (1998) 1648.

[25] J. Bektic, A.P. Berger, K. Pfeil, G. Dobler, G. Bartsch, H. Klocker, Eur. Urol. 45 (2004) 245.

[26] J.Å. Gustafsson, Trends Pharmacol. Sci. 24 (2003) 479.

[27] K.F. Koehler, L.A. Helguero, L.-A. Haldosen, M. Warner, J.Å. Gustafsson, Endocr. Rev. 26 (2005) 465.

[28] F. Roelens, N. Heldring, W. Dhooge, M. Bengtsson, F. Comhaire, J.Å. Gustafsson, E. Treuter, D. De Keukeleire, J. Med. Chem. 49 (2006) 7357.

[29] (a) P.O. Hedlund, P. Henriksson, Urology 55 (2000) 328;
(b) P. Henriksson, K. Carlström, A. Pousette, P.O. Gunnarsson, C.J. Johansson, B. Eriksson, A.K. Altersgard-Brorsson, Ö. Nordle, R. Stege, Prostate 40 (1999) 76.

[30] (a) G.S. Lee, T. Kammermeier, A. Kaiser, E. Eibler, W. Wiegrebe, Arch. Pharm. (Weinheim) 324 (1991) 177;
(b) T. Kammermeier, W. Wiegrebe, Arch. Pharm. (Weinheim) 327 (1994) 547;
(c) T. Kammermeier, W. Wiegrebe, Arch. Pharm. (Weinheim) 327 (1994) 563;
(d) T. Kammermeier, W. Wiegrebe, Arch. Pharm. (Weinheim) 327 (1994) 697;
(e) T. Kammermeier, W. Wiegrebe, Arch. Pharm. (Weinheim) 328 (1995) 409;
(f) P. Bielmeier, A. Kaiser, R. Gust, W. Wiegrebe, Monatsh. Chem. 127 (1996) 1073;
(g) T. Kammermeier, Ph.D. Thesis, Universität Regensburg, 1993.

[31] (a) H. Brunner, W. Dafinger, H. Schönenberger, Inorg. Chim. Acta 156 (1989) 291;
(b) H. Brunner, P. Hankofer, U. Holzinger, B. Treittinger, H. Schönenberger, Eur J. Med. Chem. 25 (1990) 35;
(c) H. Brunner, G. Nerl, E. von Angerer, N. Knebel, Arch. Pharm. (Weinheim) 324 (1991) 779.

[32] (a) M.R. Schneider, E. von Angerer, H. Schönenberger, R.T. Michel, H.P. Fortmeyer, J. Med. Chem. 25 (1982) 1070;
(b) M.R. Schneider, H. Ball, H. Schönenberger, J. Med. Chem. 28 (1985) 1880;
(c) M.R. Schneider, H. Ball, C.D. Schiller, J. Med. Chem. 29 (1986) 1355;
(d) M.R. Schneider, J. Cancer Res. Clin. Oncol. 112 (1986) 119;
(e) M.R. Schneider, J. Med. Chem. 29 (1986) 1494;
(f) V. Lubczyk, H. Bachmann, R. Gust, J. Med. Chem. 45 (2002) 5358;
(g) V. Lubczyk, H. Bachmann, R. Gust, J. Med. Chem. 46 (2003) 1484;
(h) R. Gust, V. Lubczyk, J. Steroid Biochem. Mol. Biol. 86 (2003) 57.

[33] (a) T. Kapp, S. Müller, R. Gust, ChemMedChem. 1 (2006) 560;
(b) T. Kapp, A. Dullin, R. Gust, J. Med. Chem. 49 (2006) 1182;
(c) R. Gust, B. Schnurr, B. Angermaier, H. Bachmann, K. Niebler, A. Maucher, E. von Angerer, H. Schönenberger, in preparation;
(d) F. Dufrasne, M. Gelbcke, B. Schnurr, R. Gust, Arch. Pharm. Pharm. Med. Chem. 5 (2002) 229;
(e) A. Dullin, F. Dufrasne, M. Gelbcke, R. Gust, Arch. Pharm. Pharm. Med. Chem. 337 (2004) 654;
(f) A. Dullin, F. Dufrasne, M. Gelbcke, R. Gust, ChemMedChem. 1 (2006) 644;
(g) D. Posselt, Ph.D. Thesis, Freie Universität, Berlin, 2006;
(h) R. Gust, I. Ott, D. Posselt, K. Sommer, J. Med. Chem. 47 (2004) 5837.