Investigation of the Conformational Influences on the Estrogenic Activity of 1,2-Bis(2,6-dichloro-4-hydroxyphenyl)-ethylenediamines and of their Platinum(II) Complexes, II[1]:

Synthesis and Studies on the Estrogenic Activity of *cis*- and *trans*[Bis(2,6-dichloro-4-hydroxybenzylamine)]dihaloplatinum(II)-Complexes[+)]

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2,6-Dichloro-4-hydroxybenzylamine (**1**) and its *N*-methyl (**2**) and *N*-ethyl (**3**) derivatives were synthesized and tested for estrogen receptor affinity as well as for estrogenic activity. In contrast to their related highly active 1,2-bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamines (*meso*-**4** - *meso*-**6**) none of the benzylamines showed hormonal activity. The coordination of the benzylamine **1** to platinum did not lead to an estrogenic compound. The reasons for the different activity of [*meso*-1,2(bis-2,6-dichloro-4-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) (*meso*-**4**-$PtCl_2$) and *cis*[bis(2,6-dichloro-4-hydroxybenzylamine)]dichloroplatinum(II) (*cis*-**1**-$PtCl_2$), the latter of which can be considered as a ring-opened counterpart of the highly active *meso*-**4**-$PtCl_2$, are thoroughly discussed under inclusion of conformational facts. The results of this and the preceding work[1] show, that the pharmacophore *meso*-1,2-bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamine (*meso*-**4**) which is exclusively responsible for the estrogenic activity of *meso*-**4**-$PtCl_2$ causes comparable hormonal effects in two different conformations with O-O distances of about 8 Å (complex) and of about 12 Å (diamine). Therefore, we discuss two binding sites for estrogens in their receptor.

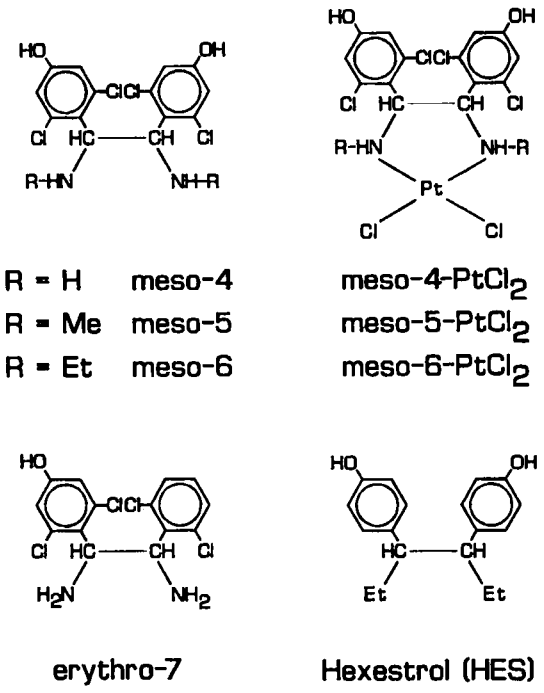
Untersuchungen zum Einfluß der Konformation auf die estrogene Wirkung von 1,2-Bis(2,6-dichlor-4-hydroxyphenyl)ethylendiaminen und deren Platinkomplexe, 2. Mitt.: Synthese und estrogene Wirkung von *cis*- und *trans*[Bis(2,6-dichlor-4-hydroxybenzylamin)dihaloplatin (II)-Komplexen

2,6-Dichlor-4-hydroxybenzylamin (**1**) und sein *N*-Methyl (**2**) und *N*-Ethyl (**3**) Derivat wurden synthetisiert und auf Estrogenrezeptoraffinität und estrogene Wirkung untersucht. Im Gegensatz zu ihren hochaktiven 1,2-Bis(2,6-dichlor-4-hydroxyphenyl)ethylendiamin-Analoga (*meso*-**4** - *meso*-**6**) zeigt keines der Benzylamine estrogene Aktivität. Auch die Koordination von **1** an Platin gibt keine hormonell wirksame Verbindung. Der Grund für die unterschiedliche Wirkung von [*meso*-1,2-Bis(2,6-dichlor-4-hydroxyhenyl)ethylendiamin]dichloroplatin(II) (*meso*-**4**-$PtCl_2$) und *cis*-[Bis(2,6-dichlor-4-hydroxybenztylamin)]dichloroplatin(II) (*cis*-**1**-$PtCl_2$), welches als ringoffenes Gegenstück von *meso*-**4**-$PtCl_2$ betrachtet werden kann, wird ausführlich unter Berücksichtigung der Konformation der Neutralliganden diskutiert. Die Ergebnisse dieser und der vorhergehenden Publikation[1] zeigen, daß das pharmakophore *meso*-1,2-Bis(2,6-dichlor-4-hydroxyphenyl)ethylendiamin (*meso*-**4**), welches ausschließlich für die estrogene Wirkung von *meso*-**4**-$PtCl_2$ verantwortlich ist, in zwei verschiedenen Konformationen mit O-O-Abständen von ca. 8 Å (Komplex) und ca. 12 Å (Diamin) estrogene Wirkungen entfalten kann. Deshalb diskutieren wir zwei Bindungsstellen für Estrogene an deren Rezeptor.

Structure activity studies in the class of [1,2-diphenyl-ethylenediamine]platinum(II) complexes have shown that substituents in the aromatic rings strongly influence their activity against malignant tumors[2-4]. For the development of estrogen receptor (ER)-affinic derivatives, which are thought to be selectively active against ER-containing tumors like breast and prostate cancers (MC and PC) due to an ER-mediated enrichment in the cancer cells, the 2,6-Cl_2, 4-OH-substitution pattern was very favorable. On the R 3327 PC and the DMBA-induced MC of the rat the most interesting compound of this series, [*meso*-1,2-bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) (*meso*-**4**-$PtCl_2$, Scheme 1) is significantly more active than cisplatin[4,5]. Meso-**4**-$PtCl_2$ is even active when given orally[6], which is of great advantage for a long term therapy.

Investigations on the mode of action of meso-**4**-$PtCl_2$ revealed that, beside the cytotoxic activity stemming from the $PtCl_2$-moiety, the estrogenic potency is especially involved in its highly specific MC- and PC-inhibiting properties[4,5,7]. For the binding to the ER, a prerequisite for estrogenic or antiestrogenic potency, the *R/S*-configurated 1,2-bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamine ligand (i.e. *meso*-**4** which itself shows ER-affinity, estrogenic activity, and also MC-inhibiting properties) is the essential pharmacophore[7], while the PtL_2 moiety (L: leaving group, e.g. Cl^-, I^- and SO_4^{2-}) only slightly influences the hormonal activity[4,8]. By N-mono- and *N,N'*-dialkylation the estrogenic activity further increased in the ligand as well as in the complex[1]. The *RR/SS*-configuration proved to be unsuitable for the triggering of marked estrogenic effects[1]. Conformation activity studies in the ligand as well as in the dichloroplatinum(II) series suggest that two binding sites (I and II) exist in the hormone

+) Dedicated to Prof. *H. J. Roth* on the occasion of his 65th birthday



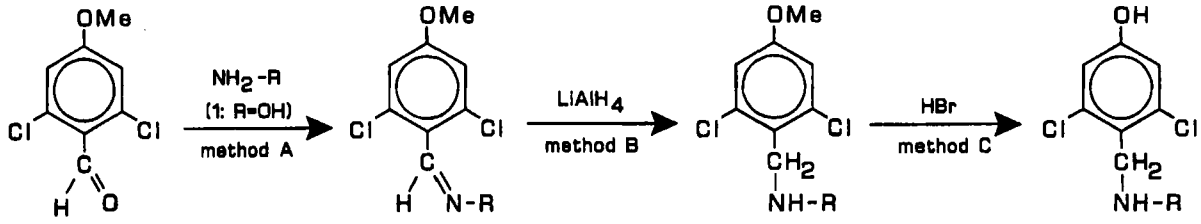
Scheme 1

binding domain of the ER[1,9]. Binding site I (i.e. E2-binding site) accepts estrogens like estradiol (E2), hexestrol (HES), diethylstilbestrol (DES) or *meso*-1,2-bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamine (*meso*-**4**, ligand of *meso*-**4**-$PtCl_2$) whose O-O distances between the two OH groups amount to 11 to 12 Å (E2: 10.9 Å[10]; HES: 12.1 Å[11]; *meso*-**4**: 12.2 Å[1]). For the receptor interaction of estrogens in which the two OH groups are located in markedly shorter O-O distances (*e.g.* *meso*-**4**-$PtCl_2$, O-O distance: about 8 Å*) a binding site different from that of E2, *i.e.* binding site II, was proposed by us.

In addition our structure activity studies in the class of non-steroidal estrogens (*e.g.* 1,2-diphenylethanes[12], 1,2-diphenylethenes[13], and 1,1,2-triphenylethenes[14,15]) show that not only the intensity but also the kind of the pharmacological effect (*e.g.* "true" estrogen or "partial" antiestrogen) can be due to the distance between the two OH groups. For example, the shift of both OH groups in HES to the *m*-positions (yielding *meso*-3,4-bis(3-hydroxyphenyl)hexane = 3,3'-HES) strongly reduced the estrogenicity but led to the appearance of marked antiestrogenic effects. 3,3' HES proved to be a "partial" antiestrogen[12].

In *cis*[bis(2,6-dichloro-4-hydroxybenzylamine)]dichloroplatinum(II) (cis-**1**-$PtCl_2$; Scheme 3), object of this publication, the ethylene bridge of *meso*-**4**-$PtCl_2$, which restricts the conformational flexibility to the δ- and the λ-conformer (O-O distance of both conformers ≈ 8 Å), is absent (cf. ref. 1). Therefore, we assumed that in *cis*-**1**-$PtCl_2$ an adjustment of the O-O distance optimal for the drug receptor interaction and thus for triggering the estrogenic effects was facilitated. A hint that **1** could also be a suitable ligand in ER-affinic platinum(II) complexes follows from studies of *Mueller* and *Kim*[16]. They report that 4-alkylphenols (which can be considered as "half hexestrols") are weakly ER-affinic substances. One of these compounds, *p*-sec-amylphenol, is highly effective at 0°C in displacing [^{3}H]-E2 from the ER.

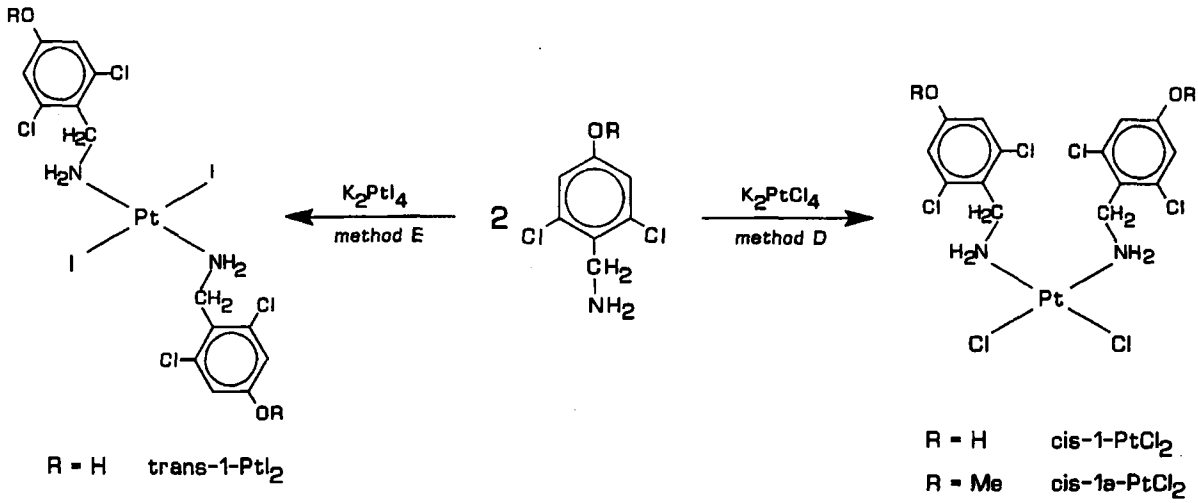
In the following we report on the synthesis of *cis*- and *trans*[bis(2,6-dichloro-4-hydroxybenzylamine)]dihaloplatinum(II) complexes (*cis*-**1**-$PtCl_2$, *trans*-**1**-PtI_2; Scheme 3) as well as on their ER-affinity and estrogenic potency. We also study whether the ligand of *cis*-**1**-$PtCl_2$ (*i.e.* **1**) and its related Me and Et derivatives **2** and **3** bind to the ER and evoke estrogenic effects.



compd.	R
1	H
2	Me
3	Et

Scheme 2

*) Upon coordination to platinum *meso*-**4** changes its phenyl positions from an antiperiplanar to a synclinal phenyl position[1].



Scheme 3

Table 1: ^{1}H-NMR Data of 2,6-Dichloro-4-hydroxybenzylamines and [Bis(2,6-dichloro-4-hydroxybenzylamine)]dihaloplatinum(II) Complexes

compd.[a]	CH_2-N-R	CHbenzylic[b]	Ar-H
1		3.85 (s,2H)	6.52 (s,2H)
2	2.28 (s,-CH_3)	3.77 (s,2H)	6.45 (s,2H)
3	1.13 (t,^{3}J=7Hz,-CH_2-) 2.63 (q,^{3}J=7Hz,-CH_3)	3.92 (s,2H)	6.58 (s,2H)

compd.[b]	CHbenzylic	-NH-	Ar-H	-OH/-OMe
trans-1-PtI_2	4.16 (t,br,4H)	4.45-4.56 (m,4H $^2J_{Pt-H}$=54Hz)	6.93 (s,4H)	10.78 (s,2H)
cis-1a-$PtCl_2$	4.33 (m,4H)	5.28 (m,4H $^2J_{Pt-H}$=64Hz)	7.12 (s,4H)	3.89 (s,6H,-OCH_3)
cis-1-$PtCl_2$	4.29 (t,br,4H)	5.18 (m,4H, $^2J_{Pt-H}$=67Hz)	6.92 (s,4H)	-[c]
cis-1-$PtCl_2$ (N-deuterated)	4.29 (t,br,4H)		6.96 (s,4H)	10.78 (s,2H)

a Spectra at 60 MHz in [D4]methanol/NaOD (**1**, **2**, **3**) or $CDCl_3$ (**1a**), TMS as int. standard, chemical shifts in δ-values

b Spectra at 250 MHz in [D7]DMF, TMS as int. standard, chemical shifts in δ-values

c Exchanged by solvent water

Results

Synthesis

2,6-Dichloro-4-methoxybenzaldehyde[4] was converted to the oxime **1b** and to the benzylimines **2b** and **3b** (Scheme 2, method A).

Reduction of **1b**-**3b** with $LiAlH_4$ resulted in the benzylamines **1a**-**3a** (Scheme 2, method B), which were transformed into the phenols **1**-**3** by HBr (Scheme 2, method C).

The reaction of the *N*-unsubstituted benzylamines **1** and **1a** with K_2PtCl_4 in water led to dichloroplatinum(II) complexes whose amine ligands are situated in *cis*-position (*i.e.* *cis*-**1**-$PtCl_2$ and *cis*-**1a**-$PtCl_2$; Scheme 3, method D). For ^{1}H-NMR spectroscopic investigations we synthesized also cis-[*N*,*N*,*N*',*N*'-tetradeutero-bis(2,6-dichloro-4-hydroxybenzylamine)]dichloroplatinum(II) using D_2O as a solvent. If K_2PtI_4 was used for the coordination of 2,6-dichloro-4-hydroxybenzylamine to platinum, the two amine ligands were exclusively arranged *trans*, presumably due to sterical

reasons (Scheme 3, method E). The analytical data of the benzylamines and their platinum complexes are listed in Table 2 (Experim. Part).

^{1}H-NMR spectroscopy

The assignment of the complexes to the *cis*- or *trans*-series can be achieved by their ^{1}H-NMR spectra. Characteristic for isomeric [bis(alkylamine)]platinum(II) complexes are the ^{195}Pt-H couplings, which amount to $^2J_{Pt,H}$ = 64 ± 2 Hz for *cis*- and to $^2J_{Pt,H}$ = 55 ± 1 Hz for *trans*-isomers[17,18]. In accordance with this we found $^2J_{Pt,H}$-values of 67 Hz for *cis*-**1**-$PtCl_2$ and 64 Hz for *cis*-**1a**-$PtCl_2$ and of 54 Hz for *trans*-**1**-PtI_2 (Table 3). Furthermore, we observed a typical shift of the NH- and CH-resonances of *cis*-complexes downfield from the corresponding *trans*-isomers (ΔNH 0.8 ppm; ΔCH 0.17 ppm; Table 1). Comparable shift differences had been found by *Cherchi et al.*[19,20] for isomeric [pra_2PtX_2] (pra: propane-1-amine, X=Cl^-, Br^-, I^-) with ΔNH = 0.7 to 0.8 ppm and ΔCHα = 0.1 to 0.2 ppm.

Pharmacology

For assessment of estrogenic properties the relative binding affinity to ER (RBA (E2) = 100%) and the estrogenic effect were determined for the hydroxy-substituted compounds. The 2,6-dichloro-4-hydroxybenzylamines **1-3** show only RBAs of 0.01 and 0.03 and no estrogenic activity in the mouse uterine weight test. The coordination of two molecules of the 2,6-dichloro-4-hydroxybenzylamine to platinum does not lead to hormonal potency. *cis*-**1**-$PtCl_2$ possesses a very low ER-affinity (RBA = 0.05) and does not cause any estrogenic effect. Therefore, we excluded *cis*-**1a**-$PtCl_2$ which can be considered as a prodrug of *cis*-**1**-$PtCl_2$ from pharmacological evaluation. These results differ strongly from those of the corresponding highly active 1,2-diphenylethylenediamines meso-**4** - *meso*-**6** and [1,2-diphenylethylenediamine]dichloroplatinum(II) complexes (*meso*-**4**-$PtCl_2$ - *meso*-**6**-$PtCl_2$)[1], showing the importance of the ethane bridge for hormonal potency.

Discussion

Investigations on the estrogenic properties of *R/S*-configurated 1,2-bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamines (*meso*-**4** - *meso*-**6**) and of their dichloroplatinum(II) complexes (*meso*-**4**-$PtCl_2$ - *meso*-**6**-$PtCl_2$; Scheme 1) revealed comparably high activity in both series though with the coordination to platinum a change in the conformation of the diamine ligands takes place. Thorough conformational studies, which showed the existence of only one conformation for the *R/S*-configurated ligands and their dichloroplatinum(II) complexes (occurring in a δ ⇄ λ equilibrium), led to the assumption, that two binding sites existed in the ER (for conformation of *meso*-**4** and *meso*-**4**-$PtCl_2$ see Fig. 1 and ref. 1). Compounds, whose OH groups show an O-O distance of about 12 Å, *e.g.* *meso*-**4**, interact with binding site I, like E2 and the non-steroidal estrogens DES and HES do, while compounds with a shorter O-O distance (≈ 8 Å), *e.g.* *meso*-**4**-$PtCl_2$, combine with binding site II. In these diamines and dichloroplatinum(II) complexes the two 2,6-dichloro-4-hydroxyphenyl residues seem to contribute most to the binding on the ER and, therefore, to the estrogenic potency by hydrogen bridges and *van der Waals* interactions.

Since an optimal fit of these two molecule fragments to their binding site is a prerequisite for strong interaction with the receptor we investigated the RBA-values and the estrogenicity of the *meso*-**4**-$PtCl_2$-related new complex *cis*-[bis(2,6-dichloro-4-hydroxybenzylamine)]dichloroplatinum(II). *cis*-**1**-$PtCl_2$ can be regarded as ring-opened *meso*-**4**-$PtCl_2$ derivative, presumable possessing a greater conformational flexibility than [*meso*-1,2-bis(2,6-dichloro-4-

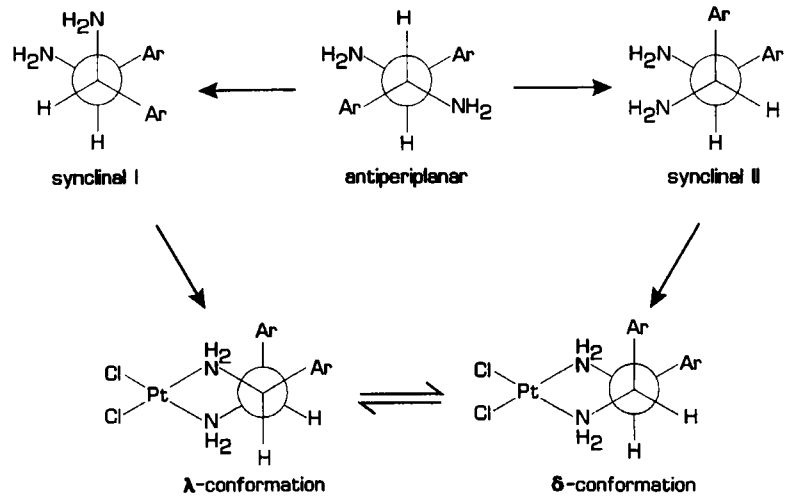


Figure 1: Conformational change of *meso*-**4** by coordination to platinum

hydroxyphenyl)ethylenediamine]dichloroplatinum(II) complexes. In contrast to our original idea, that *cis*-**1**-$PtCl_2$ might have interesting hormonal properties, we found neither a significant ER-affinity nor an appreciable estrogenicity. Therefore, an interaction with binding site II, which we discussed as a prerequisite for the estrogenicity of *meso*-**4**-$PtCl_2$, can be excluded for *cis*-**1**-$PtCl_2$, on account of the following arguments:

The adjustment of *cis*[bis-(2,6-dichloro-4-hydroxybenzylamine)]dichloroplatinum(II) to a conformation similar to that of *meso*-**4**-$PtCl_2$ is unlikely due to the steric hindrance of the necessary approximation of both benzylic C-atoms by their H-atoms as well as to the non-favored arrangement of the space-requiring NH-R- (R = H or alkyl) and Aryl-residues in close vicinity (Fig. 1). This assumption is also supported by: i) the highly stable conformation of *meso*-**4**, in which the two phenyl residues are arranged antiperiplanarly; ii) the (in contrast to D,L-**4**) strongly impeded, time requiring coordination of *meso*-**4** to platinum due to the formation of an energetically unfavorable conformation in the diamine ligand of *meso*-**4**-$PtCl_2$ (Fig. 1 and ref. 1).

From the experiments with *cis*-**1**-$PtCl_2$ and from the results of structure activity studies with [1,2-bis(hydroxyphenyl)ethylenediamine]platinum(II) complexes[1] it can be concluded, that the estrogenic activity in the latter depends not only on the substitution pattern but also very strongly on the spatial structure of the diamine ligand. Moreover, the lack of any estrogenic potency shows that *cis*-**1**-$PtCl_2$ also does not adopt conformations which allow an interaction with another binding site in ER. The same is true for its non-active *trans*-analogues, *trans*-**1**-PtI_2.

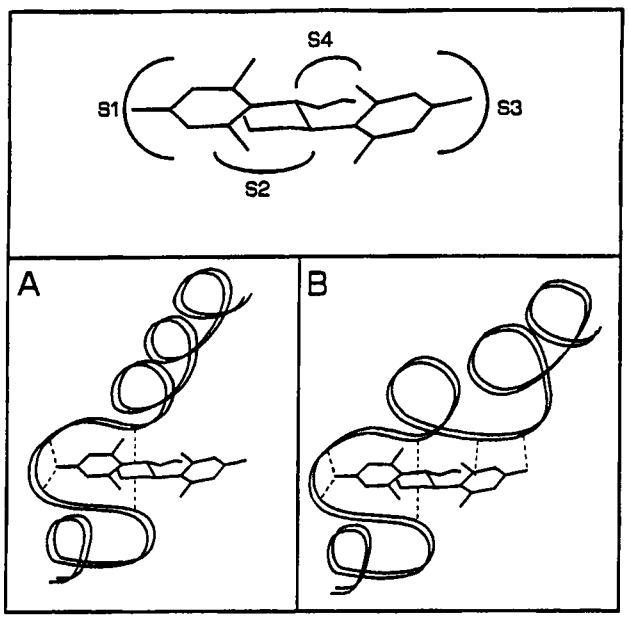


Figure 2: Hypothesis of receptor binding of *meso*-**6** according to *Duax et al.*[23].

A: *meso*-**6** is bound to the S1-spot.

B: conformational change of ER due to interaction of the 2,6-dichloro-4-hydroxyphenyl ring with the S3-spot

The 2,6-dichloro-4-hydroxybenzylamines **1-3** were compared with the *R/S*-configurated 1,2-bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamines *meso*-**4** - *meso*-**6** (antiperiplanarly arranged phenyl residues) in superposition experiments, in order to find out whether in equivalent arrangement two benzylamine molecules can interact with binding site I like a single molecule of the diamines *meso*-**4** - *meso*-**6**. We really achieved a close superposition of two benzylamine molecules over the corresponding diamine. The negative results on estrogenic potency obtained in the mouse uterine weight test, however, suggest that only relatively rigid molecules with two OH groups in an O-O distance of ≈ 12 Å can interact with binding site I in a manner which triggers estrogenic effects*).

The interaction of an estrogen (E) with the hormone binding domain in the ER induces a conformational change, a prerequisite for a dimerisation of the E-ER-complex, which in turn enables the binding to the estrogen responsive element and lastly the transcriptional activation of adjacent estrogen regulated genes**,21).

Corresponding to a model of the estrogen receptor relationship described by *Pons*[22] and *Duax*[23] we assume that in a first step one of the two 2,6-dichloro-4-hydroxyphenyl residues in the diamines *meso*-**4** - *meso*-**6** is bound to the S_1-spot of the receptor by a H-bridge and by *van der Waals* interaction (Fig. 2). The second step, the interaction of the other 2,6-dichloro-4-hydroxyphenyl residue with the S_3-spot in the receptor area seems to demand a conformational flexibility of the ER so that the second H-bridge can be formed and *van der Waals* interaction can take place. From this process results a spatial structure of the ER which can trigger the described reaction cascade culminating in estrogenic effects. In accordance with such a mode of action removal of one of the two OH groups in *meso*-**4** reduces the estrogenic potency drastically (ED_{50} of *meso*-**4**: 1.15 nmol/animal; ED_{50} of *erythro*-**7**: ≫ 1000 nmol/animal; formula see Scheme 1[24]. It is conceivable that the important change of the ER-conformation can only be caused by rigid molecules which contain two OH groups in an appropriate distance and in a distinct spatial structure like in the diamines *meso*-**4** - *meso*-**6**. This also holds true for the complexes *meso*-**4**-$PtCl_2$ - *meso*-**6**-$PtCl_2$.

Technical assistance of *O. Baumann, E. Deml, L. Schneider, D. Götzfried, S. Paulus,* and *F. Wiesenmayer* is gratefully acknowledged. Thanks are also due to the Deutsche Forschungsgemeinschaft (SFB 234), the Matthias Lackas-Stiftung für Krebsforschung, and the Fonds der Chemischen Industrie for financial support.

Experimental Part

General Procedures. Melting points (uncorrected): Büchi 510.- ^{1}H-NMR spectra of intermediates: Varian 360 L, 60 MHz; ^{1}H-NMR spectra of

*) Since benzylamines possess higher pk_a-values than 1,2-diphenylethylenediamines, their much weaker affinity to ER could also result from a higher portion of protonated molecules under physiological conditions (which presumably do not bind to ER).

) Binding of *meso*-4**-$PtCl_2$ to the estrogen responsive element was proved by *Koop*[27] in gel shift experiments.

platinum complexes: Bruker FT-NMR spectrometer WM 250, 250 MHz, TMS as int. standard.- Elemental analyses: Mikroanalytisches Laboratorium der Universität Regensburg.- IR (KBr): Perkin Elmer 580 Spektrophotometer.

2,6-Dichloro-4-methoxybenzaldehyde: [4].

Method A: 2,6-Dichloro-4-methoxybenzaldoxime (**1b**)

Hydroxylamine hydrochloride (60 mmol, 4.17 g) and sodium acetate (48 mmol, 6.53 g) were dissolved in 50 mL of water and warmed to 60°C. 2,6-Dichloro-4-methoxybenzaldehyde (60 mmol, 12.3 g) dissolved in a small amount of ethanol was added and the mixture was stirred for 30 min. Upon cooling (ice bath) the oxime precipitated: colorless powder (90%), mp. 163-164°C from EtOH.- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.81 (s, 3H, O-Me), 6.93 (s, 2H, Ar-H), 8.40 (br, 2H, =C-H and OH).

Method B: 2,6-Dichloro-4-methoxybenzylamine (**1a**)

Oxime **1b** (35 mmol, 7.7 g) dissolved in 40 mL of dry ether was dropped to a suspension of $LiAlH_4$ (35 mmol, 1.35 g) in 50 mL of dry ether. The mixture was heated to reflux for 3-4 h. After cooling to room temp. unreacted $LiAlH_4$ was hydrolyzed, the resulting precipitate was filtered off and the filtrate extracted with 2N HCl. The aqueous solution was alkalized with 10% NaOH and **1a** was extracted with ether. The ethereal solution was dried over $MgSO_4$ and evaporated to dryness: colorless powder (40%), mp. 61-62°C.- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.52 (br, 2H, NH_2), 3.78 (s, 3H, O-Me), 4.04 (s, 2H, CH_2), 6.90 (s, 2H, Ar-H).

Method C: 2,6-Dichloro-4-hydroxybenzylamine (**1**)

1a (7.8 mmol, 1.6 g) dissolved in 50 mL of 47% HBr was refluxed for 24 h. After cooling to 0°C the precipitate was sucked off and dissolved in 5 mL of water. **1** was precipitated by neutralization with 10% NaOH, filtered off and dried over P_2O_5 *in vacuo*: colorless powder (20%) mp. 191-192°C.- ^{1}H-NMR (CD_3OD/NaOD): Table 1.

Method D: cis[Bis(2,6-dichloro-4-hydroxybenzylamine)]-dichloroplatinum(II) (**cis-1-$PtCl_2$**)

K_2PtCl_4 (0.1 mmol) was dissolved in 5 mL of H_2O and added to a solution of **1** (0.2 mmol) in 1 mL of 0.5N HCl. After adjustment of the pH to 6 with 0.5N NaOH the reaction mixture was stirred for 24 h at 40°C with protection from light. Subsequently, 10 mL of 1 N HCl were added, the precipitate was filtered off and dried over P_2O_5: colorless powder (37%).- ^{1}H-NMR ([D_7]DMF): Table 1.

Method E: trans[Bis(2,6-dichloro-4-hydroxybenzylamine)]-diiodoplatinum(II) (**trans-1-PtI_2**)

K_2PtCl_4 (0.5 mmol) and KI (5 mmol) were dissolved in 2 mL of water and stirred for 30 min. The resulting K_2PtI_4 was added to a solution of **1** (1.0 mmol) in 10 mL of 0.5N HCl. After adjustment of the pH to 6 with 0.5N NaOH, the reaction mixture was stirred for 24 h at 40°C with protection from light. Subsequently, 10 mL of 1 N HCl was added and stirring was continued for 1 h. The precipitate was filtered off and dried over P_2O_5: colorless powder (83%).- ^{1}H-NMR ([D_7]DMF): Table 1.

Biological Methods

Estrogen receptor binding assay

The applied method was described by *Hartmann et al.*[25]. The relative binding affinity (RBA) of the test compounds is determined by the displacement of [3H]-17β-estradiol. In brief: At 4°C test compounds are shaken with calf uterine cytosol and [3H]-17β-estradiol for 16 h. To stop the incubation dextran-coated charcoal is added and after centrifugation the radioactivity of 200 µL supernatant aliquot is counted. On a semilog plot the % of bound labeled steroid **vs.** concentration of competitor is plotted. Six concentrations of each compound are chosen to get a linear graph. From this plot the molar concentrations of unlabeled estradiol and of the competitors are determined which reduce the binding of the radioligand by 50%.

Determination of Estrogenic Properties

Estrogenic effects are determined by stimulation of the uterine growth as described[26]: On three consecutive days the compounds, dissolved in polyethylene glycol 400/H_2O, 1:1 (0.1 µL/mouse), are daily administered sc to female, immature NMRI mice (age: 20 days at test beginning; body weight: 10-12 g; 6 mice/group). The uteri are excised 24 h after the last injection, fixed with *Bouin's* solution, dried and weighed.

Table 2: Analytical Data of 2,6-Dichloro-4-hydroxybenzylamines **1**, **2**, **3** and [Bis(2,6-dichloro-4-hydroxybenzylamine)]dihaloplatinum(II) Complexes (*cis*-**1a**-$PtCl_2$, *cis*-**1**-$PtCl_2$ and *trans*-**1**-PtI_2)

Compd.	yield	formula	C % calcd.	C % found	H% calcd.	H% found	N% calcd.	N% found
1	36%	$C_7H_7Cl_2NO$	43.8	43.6	3.67	3.68	7.3	7.1
2	25%	$C_8H_9Cl_2NO$	46.6	46.8	4.37	4.45	6.8	6.5
3	63%	$C_9H_{11}Cl_2NO$	49.1	49.0	5.00	4.77	6.4	6.5
cis-1a-$PtCl_2$	80%	$C_{16}H_{18}Cl_6N_2O_2Pt$	28.3	28.6	2.68	2.52	4.1	4.2
cis-1-$PtCl_2$	37%	$C_{14}H_{14}Cl_6N_2O_2Pt$	26.1	25.9	2.17	2.04	4.3	3.9
trans-1-PtI_2	83%	$C_{14}H_{14}Cl_4I_2N_2O_2Ptx2.5H_2O$	19.1	19.0	2.16	1.86	3.2	2.9

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