Aqua[1,1-bis(4-hydroxyphenyl)-1,2-diamino-2-phenylethane]-sulfatoplatinum(II), a New Compound for the Treatment of the Mammary Carcinoma

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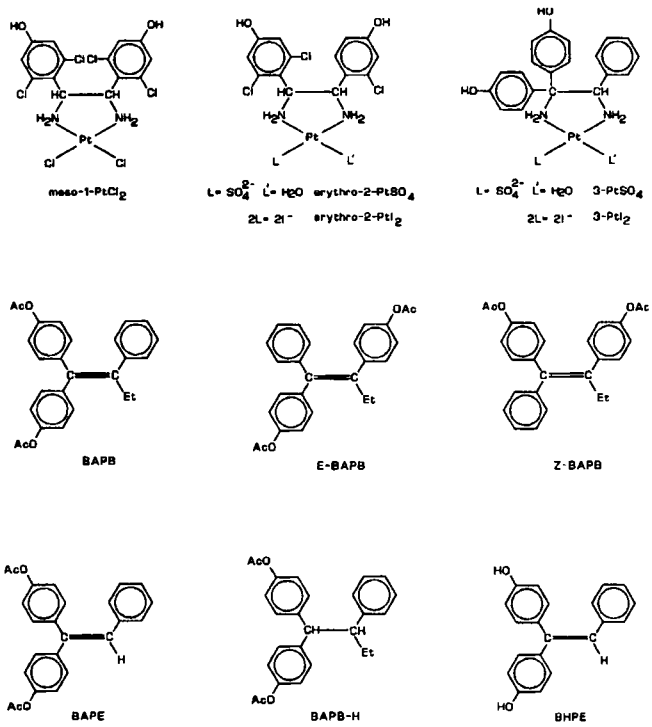
The highly estrogen receptor(ER)-affinic compound 1,1-bis(4-hydroxyphenyl)-2-phenylethene (BHPE) was used as carrier ligand for a new cisplatin derivative, aqua[1,1-bis(4-hydroxyphenyl)-1,2-diamino-2-phenylethane]sulfatoplatinum(II) (**3**-$PtSO_4$) for the therapy of the ER-positive (*i.e.* hormone sensitive) breast cancer. The diamine ligand 1,1-bis(4-hydroxyphenyl)-1,2-diamino-2-phenylethane (**3**) was synthesized by transformation of the *O*-methyl ether derivative of BHPE into the 1,2-bisazido compound **3b** with IN_3/NaN_3, reduction of both N_3 residues into NH_2 groups [**3a**] and subsequent ether cleavage with BBr_3. From the diamine ligand **3** and K_2PtI_4 a five-membered chelate **3**-PtI_2 was obtained which was transformed into **3**-$PtSO_4$ with Ag_2SO_4. In comparison to BHPE the ER-affinity of **3**-$PtSO_4$ is markedly reduced. **3**-$PtSO_4$ possesses, however, weak cytotoxic activity (P388) and antiestrogenic, but no estrogenic properties. **3**-$PtSO_4$ is comparable with BHPE in terms of its activity on the hormone sensitive MXT-M 3.2 breast cancer of the mouse.

Aqua[1,1-bis(4-hydroxyphenyl)-1,2-diamin-2-phenylethan]sulfatoplatin(II), eine neue Verbindung zur Behandlung des Brustkrebses

Die stark Östrogenrezeptor(ER)-affine Verbindung 1,1-Bis(4-hydroxyphenyl)-2-phenylethen (BHPE) wurde als Carrierligand für ein neues, für die Therapie des ER-positiven (d.h. hormonsensitiven) Mammacarcinoms vorgesehenen Cisplatin-Derivats, des Aqua[1,1-bis(4-hydroxyphenyl)-1,2-diamin-2-phenylethan]sulfatoplatin(II) (**3**-$PtSO_4$) benutzt. Der Diaminligand 1,1-Bis(4-hydroxyphenyl)-1,2-diamin-2-phenylethan (**3**) wurde durch Überführung des *O*-Methylether Derivats von BHPE mit IN_3/NaN_3 in das 1,2-Diazid-Derivat **3b**, Reduktion der N_3-Reste von **3b** in NH_2-Gruppierungen [**3a**] und anschließende Etherspaltung mit BBr_3 hergestellt. Aus dem Diamin-liganden **3** und K_2PtI_4 wurde das Fünfringchelat **3**-PtI_2 erhalten, das mit Ag_2SO_4 in **3**-$PtSO_4$ übergeführt wurde. Im Vergleich zu BHPE ist die ER-Affinität von **3**-$PtSO_4$ merklich reduziert. **3**-$PtSO_4$ besitzt jedoch schwache cytotoxische Aktivität (P388) und antiöstrogene, aber nicht östrogene Eigenschaften. Verbindung **3**-$PtSO_4$ ist bezüglich ihrer Wirkung am hormonsensitiven MXT-M 3.2 Brustkrebs der Maus mit BHPE vergleichbar.

By linking of the two ammine ligands of cisplatin to the estrogen 1,2-bis(2,6-dichloro-4-hydroxyphenyl)ethene in positions 1 and 2 a new drug, [*meso*-1,2-bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) (*meso*-**1**-$PtCl_2$, Scheme 1) was obtained, which is very active on several estrogen receptor-positive breast and prostate cancer models[1]. The mode of action of *meso*-**1**-$PtCl_2$ is very complicated and includes: 1. the estrogen receptor-mediated transport of the cytotoxic drug into the nucleus of the cancer cell leading to a more selective antitumor effect, 2. the reduction of the level of tumor growth stimulating hormones, especially of testosterone in the case of prostate cancer, 3. the triggering of an autophagic mechanism resulting in an involution of the tumor, as it is known in the therapy of breast cancer with "true" and also with "partial" estrogens[1].

In order to reduce the strong estrogenic potency of meso-**1**-$PtCl_2$, (which could cause cardiovascular side effects[1)2)]) the ring substituents pattern of the 1,2-diphenylethylenediamine ligand in this compound has been systematically modified[3)7)]. The most interesting derivative of this series, [*erythro*-1-(2-chloro-4-hydroxyphenyl)-2-(2,6-dichloro-4-hydroxyphenyl)ethylenediamine]sulfatoplatinum(II) (*erythro*-**2**-$PtSO_4$, Scheme 1) was indeed markedly less estrogenic but also only somewhat less antitumor active on the MXT M3.2 breast cancer of the mouse (MXT,ER^+) than the parent compound *meso*-**1**-$PtCl_2$. X-ray analysis and ^{1}H-NMR-spectroscopic studies showed that the arrangement of the two phenyl rings (important for the degree of estrogenicity[1]) in *erythro*-**2**-PtL_2 (synclinal position; O-O-distance 7.8 Å)) is quite different from that in the strong non-steroidal estrogen hexestrol (antiperiplanar position O-O-distance 12.2 Å). However, the structural comparison of *erythro*-**2**-PtL_2 with different estrogenic 1,1,2-triphenylbut-1-enes which possess two 4-standing AcO-groups in



Scheme 1

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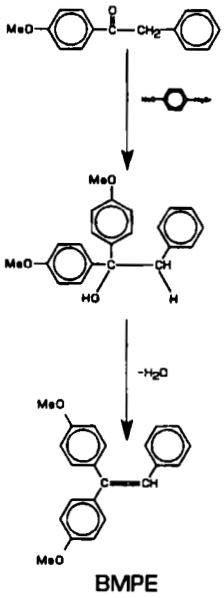
two of the phenyl rings (BAPB, *E*- and *Z*-BAPB, Scheme 1), revealed a high degree of spatial correspondence of the complex with *Z*-BAPB[1]. An almost complete congruence of *erythro*-**2**-PtL_2 and Z-BHPB, especially regarding the 1,2-bis(4-hydroxyphenyl)ethane- and the 1,2-bis(4-hydroxyphenyl)ethene-moieties with O-O-distances of 7.8 Å and 8.4 Å, respectively, is remarkable.

It is of interest that the three 1,1,2-triphenylbut-1-enes BAPB, *E*- and *Z*-BAPB (Scheme 1) themselves produce a strong inhibition of the hormone-sensitive MXT-M 3.2 breast cancer of the mouse[4]. In this test *E*- and *Z*-BAPB proved to be strong estrogens (as evident by the elevated uterus weight of the test animals), while 1,1-bis(4-acetoxyphenyl)-2-phenylbut-1-ene (BAPB) led only to weak, non-significant estrogenic side effects[4]. The transformation of BAPB into its dihydro derivative (BAPB-H) or the formal replacement of the ethyl group by hydrogen (BAPE) reduces the estrogenic potency only slightly[5,6]. However, the antitumor activity (MXT,ER$^+$) of these compounds is markedly reduced in comparison to the parent compound[5].



Scheme 2

Starting from these interesting results we used 1,1-bis(4-hydroxyphenyl)-2-phenylethene (BHPE) as an estrogen receptor-affinic carrier ligand for the design of new cisplatin derivatives for the therapy of breast cancer.

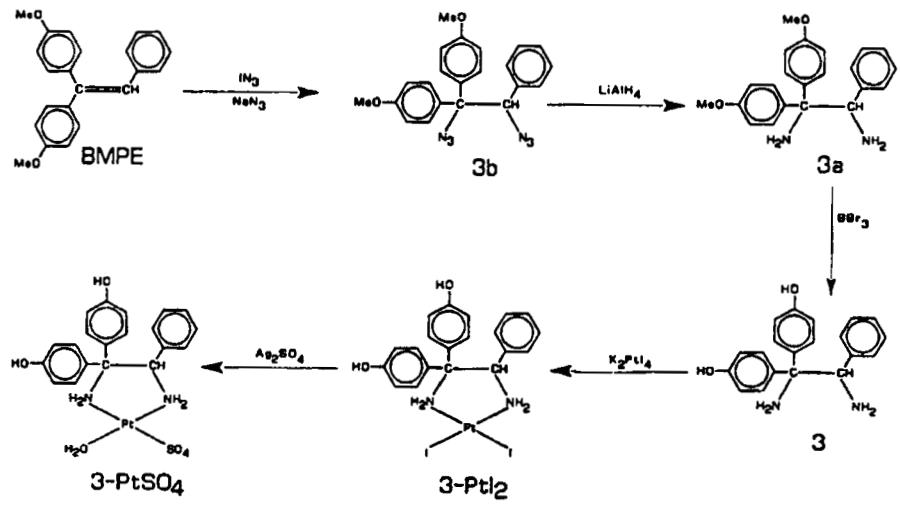
Here we report on the synthesis of 1,1-bis(4-hydroxyphenyl)-1,2-diamino-2-phenylethane (**3**) and the related sulfatoplatinum(II) complex (**3**-$PtSO_4$) as well as on the evaluation of their estrogen receptor-affinities and of their hormonal and mammary tumor inhibiting activities. In addition we discuss their pharmacological properites in relation to the activities and spatial structure of 1,1-bis(4-hydroxyphenyl)-2-phenylethene.

Chemistry

1,1-Bis(4-hydroxyphenyl)-1,2-diamino-2-phenylethane **3** was obtained in a four step reaction which we had applied earlier for the synthesis of a great number of unequal ring-substituted 1,2-diphenylethylenediamines[7]. As educt 1,1-bis(4-methoxyphenyl)-2-phenylethene (BMPE), synthesized by *Grignard* reaction of 4-methoxyphenylbenzylketone and 4-methoxyphenylmagnesium bromide and by subsequent elimination of water from the intermediate was used (Scheme 2).

BMPE was treated with IN_3 in acetonitrile solution at -60°C[8], to give the intermediate 2-azido-1,1-bis(4-methoxyphenyl)-1-iodo-2-phenylethane. By subsequent heating the reaction mixture to reflux I^- was substituted by N_3^- (Scheme 3). Reduction of the diazido compound **3b** with $LiAlH_4$ led to 1,1-bis(4-methoxyphenyl)-1,2-diamino-2-phenylethane **3a**, which was converted into 1,1-bis(4-hydroxyphenyl)-1,2-diamino-2-phenylethane **3** by BBr_3 (Scheme 3).

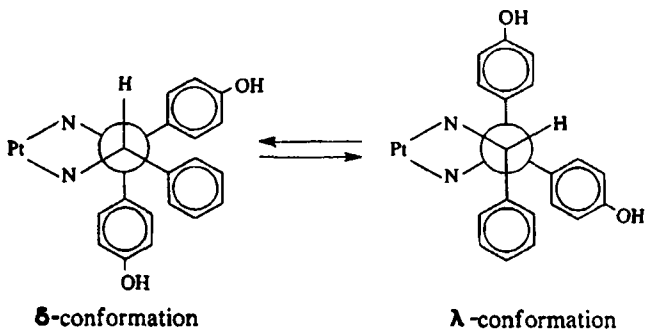
[1,1-Bis(4-hydroxyphenyl)-1,2-diamino-2-phenylethane]-diiodoplatinum(II) (**3**-PtI_2) was synthesized by reacting the diamine **3** with K_2PtI_4 at room temp. Subsequent reaction of **3**-PtI_2 with Ag_2SO_4 in water (Scheme 3) resulted in the sulfatoplatinum(II) complex **3**-$PtSO_4$.



Scheme 3

The new complexes were characterized by elemental analyses, ^{1}H-NMR- and IR-spectroscopy.

The ^{1}H-NMR spectrum of **3**-PtI_2 confirms a stable Pt-bound 1,1,2-triphenyl-1,2-diaminoethane ligand. After coordination to Pt(II) the rotation around the C-N-axis is blocked, whereby the protons of the amino groups become magnetically non-equivalent. Therefore, (beside the CH-benzylic-absorption) four individual NH signal groups appear between 4 and 7 ppm. The benzylic H can be identified by use of the *N,N'*-tetradeuterated Pt-complex obtained by coordination of **3** to Pt(II) in D_2O solution. The spectrum of this complex exhibits a signal for the benzylic H (δ = 5.10), which is broadened by Pt-satellites. From $^3J_{195Pt\text{-}H}$ = 40 Hz predictions of the spatial structure of the five-membered chelate rings are possible, since a *Karplus*-like dependence of the coupling constant on the dihedral angle between the C-H and N-Pt-bonds exists. As can be deduced from *Dreiding* models only, a C-H-bond of an equatorially arranged benzylic CH (λ-conformation) forms an 180° angle to the N-Pt-bond giving rise to $^3J_{195Pt\text{-}H} \approx 80$ Hz. On the other hand, a C-H-bond of an axially standing proton (δ-conformation) forms an angle with the Pt-bond of about 90°, which results in $^3J_{195Pt\text{-}H} = 0$ Hz. The coupling constant of 40 Hz found for **3**-PtI_2 represents the mean value of both, so a rapid interconversion of the five-membered chelate ring can be assumed (δ-λ-interconversion, Scheme 4).



Scheme 4

The same conformational behavior must be assumed for the sulfatoplatinum(II) derivative **3**-$PtSO_4$ (cf.[3]).

While the diiodoplatinum(II) complex exists in solid state in a well defined form with both iodide-residues coordinated to Pt, the sulfate ion in **3**-$PtSO_4$, however, can be bound in different ways. Unidentate as well as bidentate coordination to Pt(II) or the presence as counter ion to the $Pt(OH_2)_2$-moiety are conceivable.

Since the elemental analysis indicates the presence of only one water molecule the latter structure can be excluded. Whether the sulfate ion is monofunctionally or bifunctionally bound to Pt(II) can be decided by the S-O stretching vibrations. The $\tilde{\nu}$(SO) bands at 1150; 1130, and 625 cm^{-1} of **3**-$PtSO_4$ are in agreement with that of a sulfate ion coordinated to Pt(II) through one oxygen[3)9)].

Biological Properties

The hormonal properties of the ligand 1,1-bis(4-hydroxyphenyl)-1,2-diamino-2-phenylethane (**3**), of its sulfatoplatinum(II) complex (**3**-$PtSO_4$), and of the parent compound 1,1-bis(4-hydroxyphenyl-2-phenylethene (BHPH) were determined in the mouse uterine weight test (Table 1).

Table 1: Estrogenic and Antiestrogenic Effects of 1,1-Bis(4-hydroxyphenyl)-2-phenylethene (BHPE), 1,1-Bis(4-hydroxyphenyl)-1,2-diamino-2-phenylethane (**3**), Aqua[1,1-bis(4-hydroxyphenyl)-1,2-diamino-2-phenylethane]sulfatoplatinum(II) (**3**-$PtSO_4$)

compd.	RBA[a]	dose[b] [nmol]	estrogenic effect[c]	antiestrogenic effect[d]
BHPE	29.1	1	4	-
		10	12	25
		100	60	25
		1000	100	-23
3	0.638	10	6	0
		100	7	-8
		1000	15	6
3-$PtSO_4$	0.066	10	0	31
		100	1	-14
		1000	10	-40

a RBA, % = $[E_2]/[I] \times 100$; $[E_2]$ and [I] are the molar concentrations of nonradioactive E_2 and inhibitor required to decrease the bound radioactivity by 50%; E_2 = 17β-estradiol.

b Dose per animal and day.

c Estrogenic effect = $[(E_T\text{-}E_V)/(E_S\text{-}E_V)] \times 100$. Effect = uterus dry weight (mg)/body weight (g) x 100. E_T = effect of test compound; E_V = effect of vehicle; E_S = estrone standarad (0.1 µg).

d Antiestrogenic effect = % inhibition = $100\text{-}[(E_S\text{-}E_{S,T})]/(E_S\text{-}E_V)] \times 100$; E_S = estrone standard (0.1 µg); $E_{S,T}$ = effect of standard under simultaneous administration of test compound.

Compared to BHPE, which proved to be a weak "impeded" estrogen, **3** showed neither estrogenic nor antiestrogenic potency. Nevertheless, **3** possesses a relative binding affinity to the estrogen receptor (RBA) of 0.638 (BHPE:RBA = 29.1). Though coordination of **3** to Pt(II) reduces the receptor affinity even more (RBA = 0.066), it gives rise to antiestrogenic potency. 10 nmol/animal **3**-$PtSO_4$ produced a 31% inhibition of the maximum effect of estrone, while BHPE caused a 25% inhibition. Besides

Table 2: Effects of Aqua[1,1-bis(4-hydroxyphenyl)-1,2-diamino-2-phenylethane]sulfatoplatinum(II) (**3**-$PtSO_4$) on the Hormone Dependent MXT,ER^+ and Hormone Independent MXT,ER^- Mammary Tumor of the Mouse

	MXT,ER^+				MXT,ER^-		
compd.[a]	median tumor weight [mg](range)	%T/C[c]	uterotrophic effect[b] %T/C	change of body weight [g] d5-d1	median tumor area [mm^2] (range)	%T/C[d]	change of body weight [g] d5-d1
control	545 (120 - 693)	100	100	2.2	325 (243 - 697)	100	1.2
3-$PtSO_4$	190 (0 - 525)	45	89	1.6	319 (77 - 364)	98	0.7
cisplatin	10 (10 -50)	2	46	1.8	72 (0 - 319)	22	1.4
BAPE[e]		64.3	54.8				

a) Compounds were administered three times a week (Monday, Wednesday, Friday), sc as a solution in water (**3**-$PtSO_4$) or 0.9% NaCl solution (cisplatin); **3**-$PtSO_4$ was used in dose of 15 µmol/kg; cisplatin: 5 µmol/kg; BAPE: 20 µmol/kg.

b) Uterotrophic effect = [uterine dry weight (mg)/body weight (g)] x 100; T/C = (uterotr. effect of test group/uterotr. effect of control group) x 100.

c) T/C = (median tumor weight of test group)/(median tumor weight of control group) x 100, determined at the end of the 5-week therapy.

d) T/C = (median tumor area of test group)/(median tumor area of control group) x 100, determined at the end of the 2-week therapy.

e) Data from ref.[5]

these properties **3**-$PtSO_4$ possesses cytotoxic activity, too, as found in the test on the P388 leukemia of the mouse. At a dose of 40 µmol/kg **3**-$PtSO_4$ exceeded a T/C-value of 125%, which is regarded as a proof of cytotoxic properties (Table 3).

For assessment of mammary tumor inhibiting effects **3**-$PtSO_4$ was tested on the hormone sensitive MXT,ER^+ as well as on the hormone insensitive MXT,ER^- mammary tumor of the mouse (Table 2). At a dose of 15 µmol/kg **3**-$PtSO_4$ the growth of the MXT,ER^+ was reduced to 45% of that of the control (100%). The hormone insensitive variation of this tumor (MXT,ER^-) proved to be resistent against **3**-$PtSO_4$. The parent compound BHPE itself was not tested on the MXT,ER^+, but its acetoxy derivative BAPE which is converted into BHPE under *in vivo* conditions, was evaluated. BAPE caused a somewhat weaker effect (T/C = 64.3%) on the MXT,ER^+ though applied at a higher dose (20 µmol/kg) than **3**-$PtSO_4$ (15 µmol/kg). Presumably this effect was the result of the antiestrogenic potency of BAPE, since the uterine weight of the tumor bearing animals was reduced to T/C = 54.8% (control: T/C = 100%). In comparison to this **3**-$PtSO_4$ influenced the uterine weight only marginally.

In all tests **3**-$PtSO_4$ did not influence the body weights of the animals indicating its good tolerance.

Table 3: Effects of Aqua[1,1-bis(4-hydroxyphenyl)-1,2-diamino-2-phenylethane]sulfatoplatinum(II) (**3**-$PtSO_4$) on the P388 Leukemia of the Mouse

compd[a]	single dose [µmol/kg]	median day of survival (range)	animal weight change d5-d1 [g]	%T/C[b]
control	-	10 (9- 11)	0.5	100
3-$PtSO_4$	10	11 (10-11)	2.2	110
	20	12 (12)	2.1	120
	40	12 (12-13)	1.6	130

a) **3**-$PtSO_4$ was administered at day 1; 5; 9 *ip* as a solution in water

b) Ratio of mean survival time of treated and untreated leukemic mice in percent.

Discussion

Aqua[1,1-bis(4-hydroxyphenyl)-1,2-diamino-2-phenylethane]sulfatoplatinum(II), **3**-$PtSO_4$, represents a new type of ER-affinic, cytotoxic Pt(II) complexes, which is distinguished by an antiestrogen-like activity pattern (Table 1). **3**-$PtSO_4$ produces a weak but significant inhibition of the hormone sensitive MXT-M 3.2 mammary carcinoma of the mouse (Table 2). For therapeutic use in breast cancer, however, derivatives with higher ER-affinity and stronger antiestrogenicity and cytotoxicity (finding expression in a higher activity against MXT,ER^- and P388 - Tables 2 and 3) are mandatory. The development of such compounds is object of an ongoing structure-activity-study.

Supposedly, **3**-$PtSO_4$ owes its antiestrogenic properties to the 1,1-bis(4-hydroxyphenyl)-2-phenylethane-residue, in which the O-O-distance seems to be of fundamental importance. The antiestrogenic potency of the parent compound BHPE, a weak "impeded" estrogen, is comparable to that of **3**-$PtSO_4$ (Table 1). In BHPE the two 1,1-standing 4-hydroxyphenyl-residues are linked to an sp^2-hybridized C-atom forming an angle of nearly 120° and an O-O-distance of 9.6 Å (cf. ref.[13]: X-ray analysis of BHPE). By conversion of BHPE into the diamine ligand **3** the $(4\text{-HO-}C_6H_4)_2C$-moiety changes its planar trigonal arrangement into a tetrahedral one. In **3** the angle between the geminal aromatic rings is diminished (109°), but the O-O-distance is only reduced by

0.2 Å to 9.4 Å (estimation by molecular modeling, program: Alchemy III, Fa. Tripos).

In contrast to **3**-$PtSO_4$ its diamine ligand **3** does not produce an antiestrogenic effect of any kind (Table 1). BHPE, too, loses its feature of an "impeded" estrogen upon transformation into **3**. As a consequence of this structural alteration the affinity to the ER is also strongly reduced (from RBA_{BHPE} = 29.1 to RBA_3 = 0.638).

It must be assumed that the two NH_2 groups are responsible for the loss in activity, since they cause a profound weakening of the hydrophobic interaction between **3** and the ER. In **3**-$PtSO_4$ the hydrophilic character of **3** is somewhat reduced by the coordination to Pt(II), which can give an explanation for the appearance of antiestrogenic properties.

The assumption, that the NH_2 groups exert a negative influence on the estrogenic/antiestrogenic properties, is also supported by the results of a comparative testing of 1,1-bis(4-acetoxyphenyl)-2-phenylbut-1-ene (BAPB) and its dihydro derivative (BAPB-H)[5]. These experiments reveal that the estrogenic potency of BAPB-H is only somewhat weaker than that of BAPB, though the steps from BAPB to BAPB-H (active) and from BMPE to **3** (inactive) involve the same alteration in the spatial structures. BAPB-H shows antiestrogenic properties comparable with those of **3**-$PtSO_4$. Presumably the three aromatic rings in BAPB-H can adapt to positions, which are similar to those in BAPB, enabling a strong hydrophobic interaction with the ER, a prerequisite for estrogenic/antiestrogenic potency and also for activity on the MXT,ER^+.

Presumably not only the potency to antagonize the interaction of endogenous, tumor growth-stimulating estrogens with the ER (*i.e.* the mechanism of action of antiestrogens like tamoxifen and BAPB), but also its ER-mediated cytotoxic properties are responsible for the activity of **3**-$PtSO_4$ on MXT,ER^+. In the binding of **3**-$PtSO_4$ to the ER one of the two possible conformers, defined by the position of the 2-standing phenyl ring, may be preferred, due to differences in the strength of hydrophobic interactions.

The five-membered chelate ring of **3**-$PtSO_4$ is puckered and can exist in two conformations, δ or λ (Scheme 4). In the λ-conformation the 2-standing phenyl ring is in both an antiperiplanar and a synclinical position to the two 4-hydroxyphenyl rings. This grouping resembles that of BHPE. In the δ-conformation the 2-standing phenyl ring is synclinally arranged to both 4-hydroxyphenyl rings. ^{1}H-NMR spectroscopy indicates an interconversion between both conformers at room temp. Hence it can be assumed that **3**-$PtSO_4$ adopts a conformation which is optimal for the interaction with the ER.

The goal of further studies is the development of derivatives of the parent compound **3**-$PtSO_4$, which are capable of a stronger hydrophobic interaction with the ER and of which superior antiestrogenic and mammary tumor-inhibiting properties can be expected.

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Experimental Part

General Procedures

Melting points (uncorrected): Büchi 510.- ^{1}H-NMR: Varian 360 L, 60 MHz; ^{1}H-NMR spectra of platinum complexes: Bruker FT-NMR spectrometer WM 250, 250 MHz, internal standard TMS.- Elemental analyses: Mikroanalytisches Laboratorium der Universität Regensburg.- IR (KBr): Perkin Elmer 580 spectrophotometer.

Syntheses

BMPE has been synthesized according to *Schneider*[5].

1,1-Bis(4-methoxyphenyl)-1,2-diazido-2-phenylethane (**3b**)

To a stirred slurry of NaN_3 (2.6 g, 10 mmol) in 60 mL of dry acetonitrile was added slowly ICl (3.24 g, 20 mmol). After the mixture was cooled in an isopropanol-ice bath BMPE (3.16 g, 10 mmol) was added. The suspension was allowed to warm to room temp. and was finally refluxed for 45 min. The red-brown slurry was poured into 250 mL of water, and the mixture was extracted several times with ether. The combined org. layers were washed with 150 mL of 5% $Na_2S_2O_3$ and subsequent with 1000 mL of water in 4 portions and dried over Mg_2SO_4. Removal of ether *in vacuo* left a colorless oil from which the diazidoethane **3b** was separated by column chromatography (SiO_2, petrolether/ether 2:1) (90%, colorless oil).- ^{1}H-NMR ($CDCl_3$): δ(ppm) = 3.75 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 5.48 (s, 1H, CHbenzylic), 6.60-7.50 (m, 13 H aromat.).

1,1-Bis(4-methoxyphenyl)-1,2-diamino-2-phenylethane (**3a**)

A solution of **3b** (1.6 g, 4 mmol) in 30 mL of dry ether was added dropwise to a stirred suspension of $LiAlH_4$ (314 mg, 8 mmol) in dry ether at ice bath temp. After heating to reflux for 1 h 5 mL of water were added with cooling. The precipitate was filtered off and the filtrate was evaporated *in vacuo* after drying over Mg_2SO_4 (63%, pale yellow powder mp. 121-123°C).- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.75 (br, 4H, NH), 3.73 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 5.03 (s, 1H, CHbenzylic), 6.57-7.83 (m, 13 H aromat.).

1,1-Bis(4-hydroxyphenyl)-1,2-diamino-2-phenylethane (**3**)

A solution of **3a** (540 mg, 1.55 mmol) in 50 mL of dry $CHCl_3$ was cooled to -60°C. At this temp. BBr_3 (1.55 g, 6.20 mmol) was added. The reaction mixture was brought to room temp. and refluxed for 2 h. Subsequently 20 mL of methanol were added with cooling, and the solvent was evaporated. The residue was dissolved in 5 mL of water, filtered and the filtrate brought to pH 8 with 2N HCl. The formed precipitate was collected by suction, washed with water and dried over P_2O_5 to obtain 220 mg of **3** as a colorless powder (44%, mp. 110-114°C).- ^{1}H-NMR (MeOD): δ(ppm) = 4.90 (s, 1H, CHbenzylic), 6.50-7.73 (m, 13 H aromat.).- $C_{20}H_{20}N_2O_2$ (392.9) Calcd. C 74.9 H 6.24 N 8.7 Found C 74.5 H 6.49 N 8.5.

[1,1-Bis(4-hydroxyphenyl)-1,2-diamino-2-phenylethane]diiodoplatinum(II) (**3**-PtI_2)

K_2PtCl_4 (208 mg, 0.5 mmol) and KI (830 mg, 5 mmol) were dissolved in 10 mL of water and stirred for 30 min. The resulting black reaction mixture was added to a suspension of the diamine **3** (160 mg, 0.5 mmol) in 10 mL of water. Subsequently the mixture was warmed to 50-60°C for 8 h with stirring. During this time the pH was adjusted several times to 8. After this 10 mL of 2 N HCl were added and stirring was continued for an additional h. The precipitate was isolated by suction, washed with water and dried over P_2O_5 (94%, yellow powder).- ^{1}H-NMR ([D7]-DMF): δ (ppm) = 5.06-5.18 (m, 1H, CHbenzylic), 5.18-5.29 (m, 1H, NH), 5.66-6.06

(m, 3H, NH), 6.70 (d, ^3J = 8.8 Hz, 2H aromat.), 6.84 (d, ^3J = 8.8 Hz, 2H aromat.), 7.18-7.25 (m, 3H aromat.), 7.40 (d, ^3J = 8.8 Hz, 2H aromat.), 7.55-7.58 (m, 2H aromat.), 7.62 (d, ^3J = 8.8 Hz, 2H aromat.), 9.67 (br, 1H, OH), 9.76 (br, 1H, OH).- $C_{20}H_{20}I_2N_2O_2Pt$ (769.2) Calcd. C 31.2 H 2.60 N 3.6 Found C 30.8 H 2.84 N 3.8.

Aqua[1,1-bis(4-hydroxyphenyl)-1,2-diamino-2-phenylethane]sulfatoplatinum(II) (3-PtSO$_4$)

Solid Ag_2SO_4 (118 mg, 0.38 mmol) was added to 50 mL of an aqueous suspension of **3**-PtI_2 (308 mg, 0.4 mmol). The mixture was stirred for 24 h at 40°C with protection from light. Precipitated AgI was filtered off, and the clear filtrate was lyophilized (58%, colorless powder).- IR: 1150s, 1130s, 625m cm^{-1}.- $C_{20}H_{20}N_2O_7SPt$ (629.4) Calcd. C 38.1 H 3.50 N 4.4 Found C 37.8 H 3.68 N 4.2.

Biological Methods

Estrogen Receptor Binding Assay

The applied method was described by *Hartmann et al.*[12]. The relative binding affinity (RBA) of the test compounds is determined by the displacement of 17β-[^{3}H]estradiol. At 4°C the test compound is shaken with calf uterine cytosol and 17β-[^{3}H]estradiol for 16 h. To stop the incubation dextran-coated charcoal is added and after centrifugation the radioactivity of a 200 µL supernatant aliquot is counted. On a semilog plot the percentage of bound labeled steroid *vs.* concentration of the competitor is plotted. Six concentrations of the compound are chosen to get a linear graph. From the plot the molar concentrations of unlabeled estradiol and of the competitor are determined which reduce the binding of the radioligand by 50%.

Determination of Estrogenic and Antiestrogenic Properties

Estrogenic and antiestrogenic effects are determined by stimulation of the uterine growth or by inhibition of the uterine growth stimulated by estrone, respectively, as described[6]. On three consecutive days the compounds, dissolved in polyethylene glycol 400/H_2O 1:1 (ligand) or water (complex), are daily administered sc (0.1 µL/mouse) 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.

P388 Leukemia

The P388 leukemia cells were kindly provided by Dr. *A.E. Bodgen*, EG & G Bogden Laboratories, Worcester, USA. This tumor was maintained by routine passage in female DBA/2 mice (Ivanovas, Kissleg, FRG). For determination of the antitumor activity, female CD_2F_1 mice (18-22 g, Zentralinstitut für Versuchstierzucht, Hannover, Germany) were inoculated ip with 10^6 leukemia cells in 0.1 ml of PBS buffer (day 0). The animals were randomly assigned to groups of 6 (10 animals to the solvent control) and the compound was administered ip as a solution in water on days 1; 5; 9. Cisplatin served as positive control. The antitumor activity was evaluated as median day of survival time compared to the untreated control.

Hormone-dependent, Transplantable MXT-M 3.2 Mammary Tumor of the BDF_1 Mouse (MXT,ER$^+$)

The applied method was identical with that described[10]. In female BDF_1-mice (age: 8 weeks at test beginning; body weight about 20 g; Charles River Wiga, Germany) the tumor was transplanted subcutaneously in pieces of about 2 mm^3 (one piece/mouse). Then the mice were randomly assigned to test groups of 9 animals each. On the first day after transplantation the treatment with the test compound, dissolved in water, was started. Three times a week (Monday, Wednesday, Friday) 0.1 mL/mouse was injected sc for 6 weeks. On day 36 the animals were killed by cervical dislocation and weighed. The tumors were removed and after washing in 0.9% NaCl solution dabbed dry and weighed. Then the median tumor weight was calculated. To assess the estrogenic or antiestrogenic side effects the uteri were excised, too, fixed, dried and weighed as described[10].

Hormone-independent, Transplantable MXT-Ovex Mammary Tumor of the BDF_1-Mouse (MXT,ER$^-$)[11]

The MXT-Ovex tumor was maintained by routine passage in ovariectomized BDF_1-mice. The tumor was transplanted in pieces of about 2 mm^3 (one piece/mouse) in intact female BDF_1-mice (age: 8 weeks at test beginning; body weight: 20 g; Charles River Wiga, Germany; 9 animals/group) and then the animals were treated with the solution of the compound as described above, but only for 14 days. On day 15 the tumor area (the product of two perpendicular diameters; one across the longest side) was measured.

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