7β-(Hydrazinocarbonyl)-6,14-*endo*-etheno-6,7,8,14-tetrahydrothebaine (11). The 7β-methyl ester 10 (1.5 g, 3.78 mmol) was heated under reflux with 100% hydrazine hydrate (5 mL ≃ 103 mmol) and 2-ethoxyethanol (3.8 mL) for 8 h. The reaction mixture was cooled and an equal volume of water was added. On scratching, the *7β-hydrazide* 11 crystallized out as colorless needles, which were recrystallized from water containing a small amount of EtOH: 0.54 g, 36%, mp 106 °C; IR (Nujol) ν_{max} 3310 (br, NH and NH_2), 1650 cm^{-1} (C=O); NMR ($CDCl_3$) δ 4.97 (1 H, d, J = 1.5 Hz, $H_{5\beta}$). Anal. ($C_{22}H_{27}N_3O_4 \cdot H_2O$) C, H, N.

Ethyl *N*-(6,14-*endo*-Etheno-6,7,8,14-tetrahydrothebain-7β-ylcarbonyl)formohydrazonate (13). The 7β-hydrazide 11 (0.33 g, 0.83 mmol) was heated under reflux with triethylorthoformate (25 mL) for 24 h. The excess ortho ester was removed in vacuo and the oily residue was dissolved in ethanol (15 mL). The ethanol was evaporated and the residue, by now partly solid, was taken up in hot diethyl ether. The hydrazonate 13 precipitated out as an amorphous solid after several days: 0.1424 g, 38%, mp 134–139 °C; IR (Nujol) ν_{max} 3320 (NH), 1693 (C=O), 1638 cm^{-1} (C=N); NMR ($CDCl_3$) δ 1.41 (3 H, t, J = 7 Hz, CH_3CH_2O), 2.38 (3 H, s, NCH_3), 3.57 (3 H, s, C-6 OCH_3), 3.83 (3 H, s, C-3 OCH_3), 4.18 (2 H, q, J = 7 Hz, CH_3CH_2O), 4.87 (1 H, s, $H_{5\beta}$), 6.59 (3 H, m, H_1, H_2, and N=C*H*OEt); measured mass 453.2255 ($C_{25}H_{31}N_3O_5$ requires 453.2264).

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Registry No. 2b, 83933-13-5; **3**, 24482-20-0; **4a**, 91085-16-4; **4b**, 91085-17-5; **4c**, 91085-18-6; **4d**, 91110-26-8; **4e**, 91085-19-7; **5a**, 91110-54-2; **5b**, 91110-55-3; **6**, 91085-15-3; **7a**, 91085-20-0; **7b**, 91085-21-1; **7c**, 91085-22-2; **8**, 91085-23-3; **9a**, 91085-24-4; **9b**, 91085-25-5; **9c**, 91085-26-6; **9d**, 91085-27-7; 7β-**10**, 91176-83-9; 7α-**10**, 16193-33-2; **11**, 91176-84-0; **13**, 91085-28-8; triethyl orthopropionate, 115-80-0; trimethyl orthobenzoate, 707-07-3; trimethyl ortho-*p*-toluate, 22911-22-4; pyrrolidine, 123-75-1; triethyl orthoformate, 122-51-0; triethyl orthoacetate, 78-39-7; piperidine, 110-89-4; morpholine, 110-91-8; thebaine, 115-37-7; methyl acrylate, 96-33-3.

Dichloro[1,2-bis(4-hydroxyphenyl)ethylenediamine]platinum(II) Complexes: An Approach To Develop Compounds with a Specific Effect on the Hormone-Dependent Mammary Carcinoma

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Stereoisomeric dichloro[1,2-bis(4-hydroxyphenyl)ethylenediamine]platinum(II) complexes (*meso*-**3a**, (±)-**3b**, (+)-**3c**, (−)-**3d**) and their *N,N'*-dibutyl derivatives (*meso*-**4a**, (±)-**4b**, (+)-**4c**, (−)-**4d**) were synthesized and tested on antitumor activity. The most active compound, **3d**, shows a modest inhibition of the [^{3}H]estradiol receptor interaction and causes a marked effect on the growth of the hormone-dependent human MCF 7 breast cancer cell line. It is also active on the hormone-independent human MDA-MB 231 breast cancer cell line, on the ADJ/PC6 plasmacytoma of the Balb/C mouse, and on the L 5222 leukemia of the BD IX rat. Apparently the inhibition of the MCF 7 cell line is not mediated by the estrogen receptor system. Histopathological studies on **3d** revealed very low toxicity.

The resistance of the hormone-dependent mammary carcinoma against cisplatin tempted us to synthesize cytotoxic platinum complexes containing stereoisomeric *N,N'*-dibutyl-1,2-bis(4-hydroxyphenyl)ethylenediamines as ligands, which are able to bind to the estrogen receptor (Scheme I, "estrophilic platinum complexes" **4a–d**).[1] This approach is based on the assumption that these platinum complexes are translocated into the nucleus of the mammary tumor cell by the estrogen receptor system, thereby causing a specific activity against the hormone-dependent breast cancer. Efforts in this direction have also been made by linking cytotoxic agents to estrogens or antiestrogens.[2-8]

Chemistry. The stereoisomeric dichloroplatinum(II) complexes **3a–d** and **4a–d** were synthesized by reacting K_2PtCl_4 with the 1,2-bis(4-hydroxyphenyl)ethylenediamines **1a–d** and the related *N,N'*-dibutyl derivatives **2a–d**. The diamines were applied either as dihydrobromides (method A) or as free bases (method B). The analytical data are listed in Table I. The IR spectra reveal that the N–H stretching vibration has considerably changed upon the formation of the metal–nitrogen bond (free ligand ν(N–H), 3380 cm^{-1}; Pt bond ligand ν(N–H), 3260 cm^{-1}).[9] The weak absorptions in the region of 530 cm^{-1} are characteristic for the metal–nitrogen stretching vibration.[10]

(1) von Angerer, E.; Egginger, G.; Kranzfelder, G.; Bernhauer, H.; Schönenberger, H. *J. Med. Chem.* **1982**, *25*, 832.
(2) Raus, J.; Martens, H.; Leclercq, G. "Cytotoxic Estrogens in Hormone Responsive Tumors"; Academic Press: London, 1980.
(3) Katzenellenbogen, J. A.; McGorrin, R. J.; Tatee, T.; Kempton, R. J.; Carlson, K. E.; Kinder, D. H. *J. Med. Chem.* **1981**, *24*, 435.
(4) Schneider, M. R.; Schönenberger, H.; Michel, R. T.; Fortmeyer, H. P. *Arch. Pharm. (Weinheim, Ger.)* **1982**, *315*, 515.
(5) Schneider, M. R.; Schönenberger, H.; Michel, R. T.; Fortmeyer, H. P. *J. Med. Chem.* **1982**, *25*, 141.
(6) Leclercq, G.; Heuson, J. C.; *Anticancer Res.* **1981**, *1*, 217.
(7) Katzenellenbogen, J. A.; Katzenellenbogen, B. S. *Breast Cancer Res. Treat.* **1982**, *2*, 347.
(8) Fernández G., J. M.; Rubio-Arroyo, M. F.; Rubio-Poo, C.; de la Peña, A. *Monatsh. Chem.* **1983**, *114*, 535.
(9) Saraceno, A. J.; Nakagawa, I.; Mizushima, S.; Curran, C.; Quagliano, J. V. *J. Am. Chem. Soc.* **1958**, *80*, 5018.
(10) Mylonas, St.; Valavanidis, A.; Voukouvalidis, V.; Polyssiou, M. *Inorg. Chem. Acta* **1981**, *55*, 125.

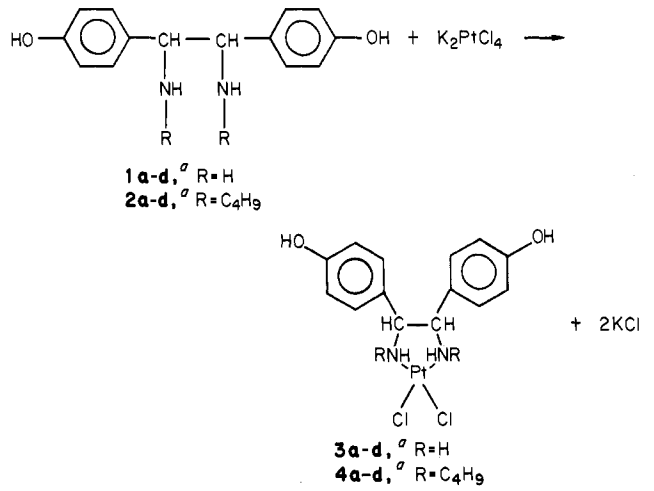
0022-2623/84/1827-1280$01.50/0

Table I. Dichloro[1,2-bis(4-hydroxyphenyl)ethylenediamine]platinum(II)

compd	R (*N*-alkyl chain)	confign	synth method[a]	yield, %	mp, °C dec	formula[b]
3a	H	meso (*R,S*)	B	63	258–260	$C_{14}H_{16}N_2O_2Cl_2Pt$
3b	H	*d,l* (*R,R*/*S,S*)	A	88	355–358	$C_{14}H_{16}N_2O_2Cl_2Pt$
3c[c,g]	H	(+) *R,R*	A	94	350–353	$C_{14}H_{16}N_2O_2Cl_2Pt$
3d[d,g]	H	(–) *S,S*	A	94	350–353	$C_{14}H_{16}N_2O_2Cl_2Pt$
4a	C_4H_9	meso (*R,S*)	A	67	278–281	$C_{22}H_{32}N_2O_2Cl_2Pt$
4b[e,g]	C_4H_9	*d,l* (*R,R*/*S,S*)	A	38	290–293	$C_{22}H_{32}N_2O_2Cl_2Pt$
4c[f,g]	C_4H_9	(+) *R,S*	A	35	288–290	$C_{22}H_{32}N_2O_2Cl_2Pt$
4d	C_4H_9	(–) *S,S*	A	35	305–308	$C_{22}H_{32}N_2O_2Cl_2Pt$

[a] See Experimental Section. [b] All compounds were analyzed for C, H, N, and Cl within ±0.50% of the calculated values. [c] $[\alpha]^{20}_{546}$ 189°. [d] $[\alpha]^{20}_{546}$ –183°. [e] $[\alpha]^{20}_{546}$ 126°. [f] $[\alpha]^{20}_{546}$ –102°. [g] *c* = 0.53 g/100 mL of Me_2SO.

Scheme I



[a] a, meso (*R,S*); b, (±) (*R,R*/*S,S*); c, (+) (*R,R*), d, (–) (*S,S*).

The two absorption bonds in the far-infrared close to 320 cm^{-1} indicate a cis metal–chlorine structure.[10]

The absolute configuration of enantiomeric dichloro-[1,2-bis(4-hydroxyphenyl)ethylenediamine]platinum(II) complexes [(+)-**3c**, (–)-**3d**] was determined by comparing their CD spectra with those of (+)- and (–)-dichloro(1,2-diphenylethylenediamine)platinum(II) [(+),*R,R*; (–), *S,S*] (Figure 1). The structural assignment of the corresponding complexes derives from the known absolute configuration of (+)-1,2-diphenylethylenediamine (*R,R*).[11] According to the CD spectra, the (+)-complex **3c** has *R,R* and the (–)-complex **3d** *S,S* configuration. As the platinum complex (–)-**4d** is obtained from (–)-1,2-bis(4-hydroxyphenyl)ethylenediamine (*S,S* configuration) via the *N,N'*-dibutyl derivative, the following configuration can be assigned to the enantiomeric dichloro[*N,N'*-dibutyl-1,2-bis(4-hydroxyphenyl)ethylenediamine]platinum(II) complexes: **4c**, *R,R*; **4d**, *S,S*.

Biological Properties. In a previous study[1] we found that (±)-, (+)-, and (–)-*N,N'*-dibutyl-1,2-bis(4-hydroxyphenyl)ethylenediamine (**2b–d**) show an appreciable binding affinity to the estrogen receptor. The related meso compound (**2a**) is inactive. Lengthening or shortening of the *N*-alkyl chains leads to a decrease in affinity. By reaction of the diamines **2a–d** with K_2PtCl_4 we obtained the desired "estrophilic platinum complexes" (**4a–d**). However, the receptor affinity of **4b–d** was inferior to that of the corresponding ligands (inhibition of the [^{3}H]estradiol receptor interaction in the presence of 1×10^{-5} M inhibitor and 1×10^{-9} M [^{3}H]estradiol: **4a**, inactive; **4b**, 18.2%; **4c**, 20.1%; **4d**, 20.0%).

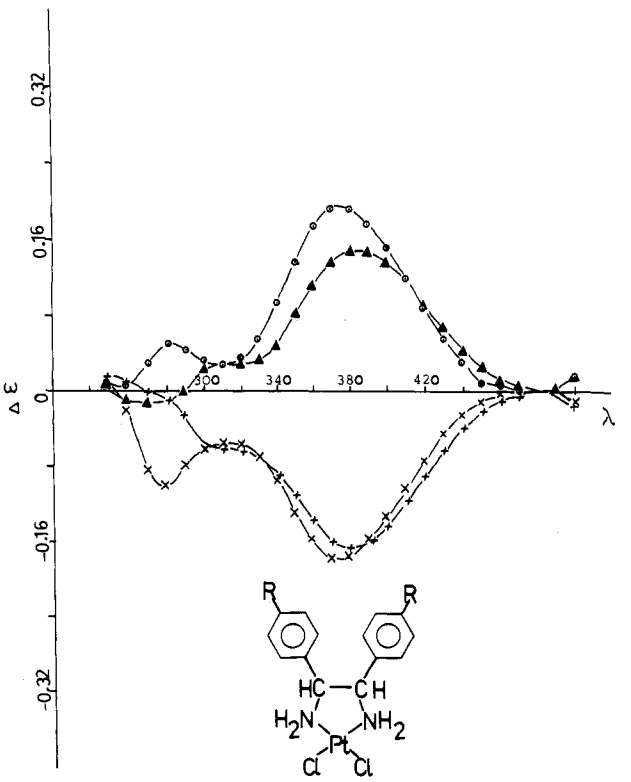


Figure 1. CD spectra of enantiomeric dichloro(1,2-diphenylethylenediamine)platinum(II) and dichloro[1,2-bis(4-hydroxyphenyl)ethylenediamine]platinum(II) complexes: (⊙) R = H (+), (▲) R = OH (+), (+) R = OH (–), (x) R = H (–).

In order to evaluate the cytotoxic activity of complexes **4a–d**, the drugs were tested in vitro on the hormone-independent tumors MDA-MB 231 human breast cancer and ADJ/PC6 plasmacytoma, both known to be very sensitive to platinum complexes. In a concentration of 1×10^{-5} M, compounds **4a–d** display only a negligible growth inhibition. Therefore, compounds **4b–d** can be classified as noncytotoxic, weakly estrophilic platinum complexes. These findings correspond with the observation that *cis*-[PtA_2Cl_2] complexes containing secondary amines (A) are often inferior to those with primary ones regarding antitumor activity.[12]

One of the new estrophilic platinum complexes, **4b**, was examined for its effect on the human MCF-7 mammary carcinoma cell line, which is known to possess estradiol binding sites.[13,14] This experiment is based on the finding that estrophilic drugs inhibit the proliferation of breast

(11) Meric, R.; Vigueron, J. *Tetrahedron Lett.* **1974**, *33*, 2778.
(12) Leh, F. K. V.; Wolf, W. *J. Pharm. Sci.* **1976**, *65*, 315.
(13) Zava, D. T.; McGuire, W. L. *J. Biol. Chem.* **1977**, *252*, 3703.
(14) Brooks, S. C.; Locke, E. R.; Soule, H. D. *J. Biol. Chem.* **1973**, *248*, 6251.

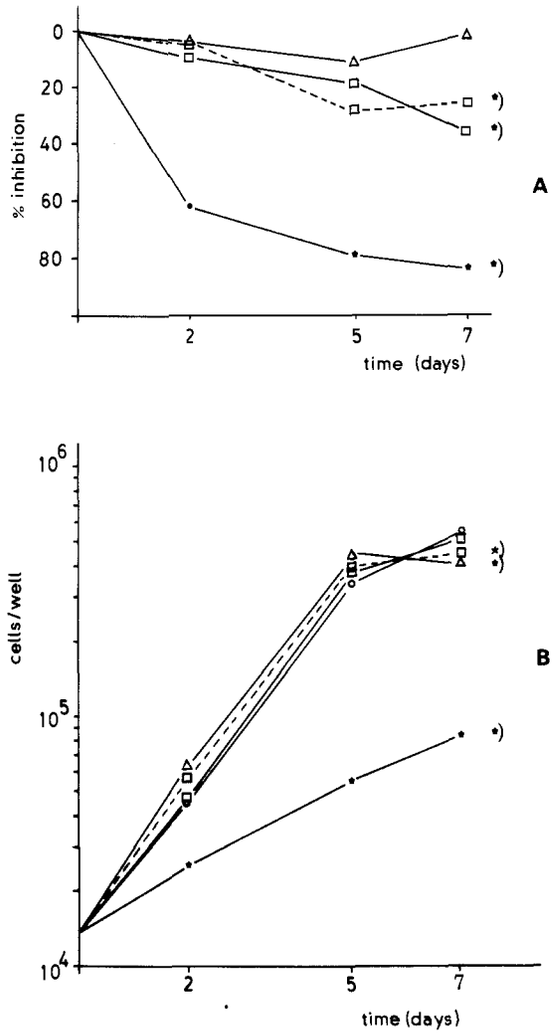


Figure 2. Effect of (±)-dichloro[*N,N'*-dibutyl-1,2-bis(4-hydroxyphenyl)ethylenediamine]platinum(II) (**4b**) on [^{3}H]thymidine incorporation (A) and growth (B) of hormone-dependent MCF-7 breast cancer cells: (○) control, (●) tamoxifen (10^{-6} M), (△) DES (10^{-9} M), (–□–) **4b** (10^{-5} M), (- -□- -) **4b** (10^{-5} M) + DES (10^{-9} M); (*) $p \leq 0.05$ at the end of test. Data points are the mean of triplicate determinations; SD less than 10%.

cancer cells by competing with endogenous estrogens for receptor binding.[15] In accordance with the weak receptor affinity of **4b**, only a modest inhibition of [^{3}H]thymidine incorporation and cell growth is observed (Figure 2). The ligand (**2b**) itself produces a comparable inhibition of precursor incorporation, which, in contrast to the experiment with **4b**, is reversed by simultaneous addition of hexestrol.[1]

In order to find out whether the secondary amino structure of the ligands (**2a–d**) in the platinum complexes **4a–d** is responsible for the lack of cytotoxic activity, complexes (**3a–d**) containing the corresponding ligands with primary amino structure (**1a–d**) were synthesized and evaluated. Though the stereoisomeric 1,2-bis(4-hydroxyphenyl)ethylenediamines (**1a–d**) themselves have no affinity to the estrogen receptor, a weak inhibition of the [^{3}H]estradiol receptor interaction by **3a–d** is observed (**3a**, 11.0%; **3b**, 26.5%; **3c**, 13.7%; **3d**, 9.7% at a concentration of 1×10^{-5} M inhibitor and 1×10^{-9} M [^{3}H]estradiol). This may be explained by the ability of **3a–d** to react with nucleophilic centers of the estradiol receptor.[16] Such an

(15) Hartmann, R. W.; Buchborn, H.; Kranzfelder, G.; Schönenberger, H. *J. Med. Chem.* **1981**, *24*, 1192.

Table II. Effects of Stereoisomeric Dichloro[1,2-bis(4-hydroxyphenyl)ethylenediamine]platinum(II) Complexes on [^{3}H]Thymidine Incorporation into MDA-MB 231 Breast Cancer and ADJ/PC6 Plasmacytoma Cells

compd	ligand confign	concn (M) of compd resulting in 50% inhibn (ID_{50})[a]	
		MDA-MB 231	ADJ/PC6
3a	meso (*R,S*)	7.0×10^{-6}	$>10^{-5}$
3b	(±) (*R,R/S,S*)	2.4×10^{-6}	1.6×10^{-5}
3c	(+) (*R,R*)	2.0×10^{-6}	2.5×10^{-5}
3d	(–) (*S,S*)	8.0×10^{-7}	4.5×10^{-6}
cisplatin		6.5×10^{-7}	9.5×10^{-6}

[a] Mean of triplicate determinations; SD less than 10%.

inactivation of the estradiol receptor by cytotoxic (i.e., reactive) platinum complexes like cisplatin has been described by us.[18]

Compounds **3a–d** produce in vitro a marked inhibition of [^{3}H]thymidine incorporation into ADJ/PC6 plasmacytoma and MDA-MB 231 breast cancer cells (Table II). Encouraged by these results, we decided to determine the antitumor activity of the most promising stereoisomers (**3b–d**) against the ADJ/PC6 plasmacytoma of the Balb/C mouse in vivo (Table III). This tumor model appears to respond better to hydrophobic platinum complexes than the often applied test system leukemia L 1210.[19] Complex **3b** exhibits a dose-dependent strong activity against this tumor. One may conclude from the two different treatment schedules used with compound **3b** that three subsequent doses are better than a single dose. The (+)- and (–)-enantiomers (**3c** and **3d**) proved to be the most active compounds, comparable to cisplatin (Tables II and III). A good correlation between inhibition of [^{3}H]thymidine incorporation in vitro and antitumor effect in vivo was found.

The in vivo experiments with **3d** were extended to further tumor models. This drug (**3d**) proved to be highly active on the rat leukemia L 5222, yielding a 175% ILS, whereas cisplatin causes a 150% ILS at the optimal dosage and treatment schedule (Table IV). Compound **3d** was also thoroughly studied on the hormone-dependent human MCF-7 mammary carcinoma cell line.

[^{3}H]Thymidine incorporation into the estrogen receptor positive MCF-7 human breast cancer cell line was strongly inhibited by **3d** at a concentration of 10^{-5} M, whereas a concentration of 10^{-6} M showed only weak effects (Figure 3). The enantiomer **3c** (10^{-5} M) is less active. Determination of DNA content and cell number led to corresponding results (Figures 3 and 4). The inhibitory effect of compound **3d** was not overcome by 10^{-9} M diethylstilbestrol (DES). On the contrary, the effect of the antiestrogen tamoxifen on MCF-7 cells is strongly decreased by DES (Figures 3 and 4).

The biosynthesis of thymidine monophosphate from thymidine occurs on the so-called "salvage pathway" involving thymidine kinase. Bronzert et al.[20] found that both

(16) An analogous reaction with nucleophilic groups of biomacromolecules is assumed to be the reason of the antitumor activity of *cis*-[PtA_2Cl_2] complexes, DNA being discussed as target molecule.[17]

(17) Prestayko, A. W.; Crooke, St. T.; Carter, St. K.; Alder, N. A. "Cisplatin, Current Status and New Developments"; Academic Press: New York, 1980; pp 9, 21, 213.

(18) Seidenberger, H.; Kranzfelder, G.; Wappes, B.; Schönenberger, H.; Beck, W.; Girnth, M. *Arch. Pharm. (Weinheim, Ger.)* **1981**, *314*, 955.

(19) Wilkinson, R.; Cox, P. J.; Jones, M.; Harrap, K. R. *Biochimie* **1978**, *60*, 851.

(20) Bronzert, D. A.; Monaco, M. E.; Pinkus, L.; Aitken, S.; Lippman, M. E. *Cancer Res.* **1981**, *41*, 604.

Table III. Antitumor Effect of Stereoisomeric Dichloro[1,2-bis(4-hydroxyphenyl)ethylenediamine]platinum(II) Complexes against the ADJ/PC6 Plasmacytoma of the Balb/C Mouse

compd	confign	dose level, mg/kg	total doses (day 1 or 1-3, resp.)	duration of test, days	T/C, %	deaths (day obsd)	animals without tumor at the end of test
3b	(±) (*R,R/S,S*)	10	3	37	39	1/10 (35)	0
		15	3	37	7,8	0/10	3
		20	3	40	4,3	2/10 (15, 28)	4
		30	3	40	1,0	4/10 (21, 23, 28, 30)	4
		30	1	37	44	0/10	0
		50	1	40	31	0/10	0
		60	1	40	12	2/10 (33, 38)	0
3c	(+) (*R,R*)	20	3	38	0,1	0/10	7
3d	(-) (*S,S*)	15	3	38	1,4	1/10 (21)	7
		20	3	37	0	2/10 (35, 37)	8
cisplatin		8	1	37	0	0/10	10

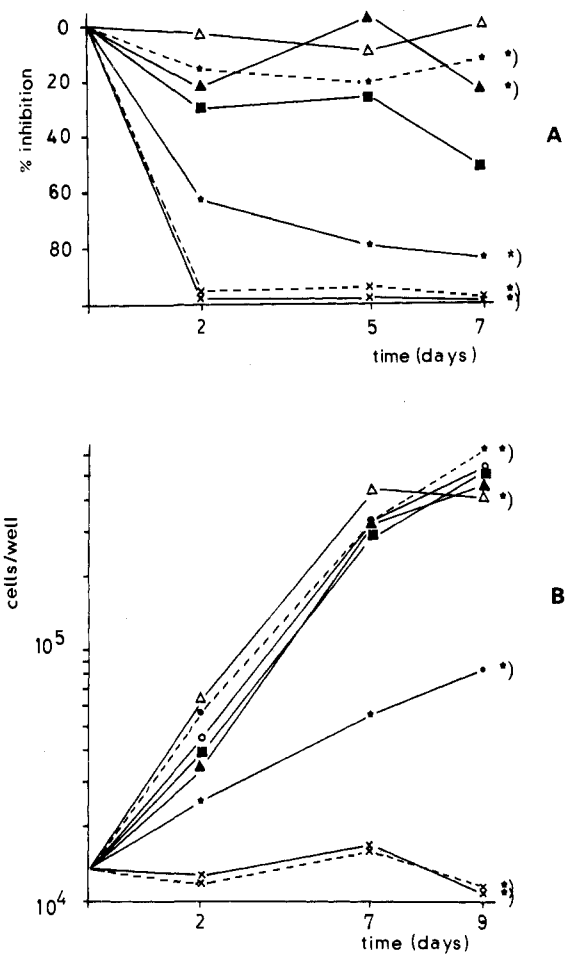


Figure 3. Effect of (-)-dichloro[1,2-bis(4-hydroxyphenyl)-ethylenediamine]platinum(II) (**3d**) on [^{3}H]thymidine incorporation (A) and growth (B) of hormone-dependent MCF-7 breast cancer cells: (○) control, (-●-) tamoxifen (10^{-6} M), (--●--) tamoxifen (10^{-6} M) + DES (10^{-9} M), (△) DES (10^{-9} M), (-x-) **3d** (10^{-5} M), (--×--) **3d** (10^{-5} M) + DES (10^{-9} M), (▲) **3d** (10^{-6} M), (■) **3c** (10^{-5} M); (*) $p \leq 0.05$ at the end of test. Data points are the mean of triplicate determinations. SD less than 10%.

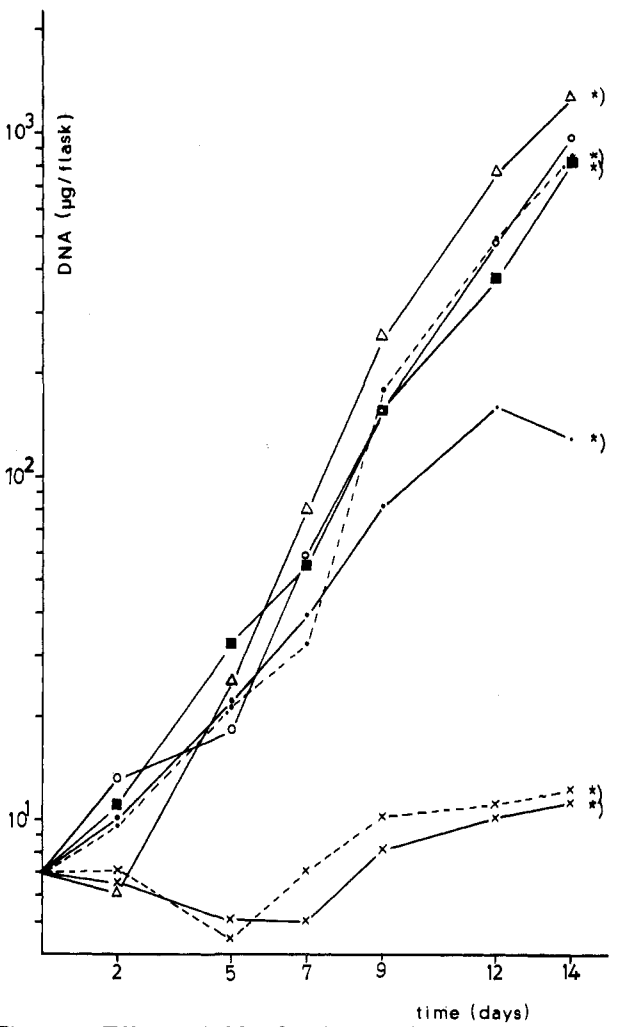


Figure 4. Effect of dichloro[1,2-bis(4-hydroxyphenyl)ethylene-diamine]platinum(II) complexes **3c** and **3d** on DNA content of hormone-dependent MCF-7 breast cancer cells: (○) control, (-●-) tamoxifen (10^{-6} M), (--●--) tamoxifen (10^{-6} M) + DES (10^{-9} M), (△) DES (10^{-9} M), (-x-) **3d** (10^{-5} M), (--×--) **3d** (10^{-5} M) + DES (10^{-9} M), (■) **3c** (10^{-5} M); (*) $p \leq 0.05$ at the end of test. Data points are the mean of triplicate determinations. SD less than 10%.

[^{3}H]thymidine incorporation and thymidine kinase activity of MCF-7 breast cancer cells are inhibited by tamoxifen. These effects can be reversed by simultaneous addition of estrogens. The involvement of thymidine kinase in the hormonal regulation of the mammary carcinoma cell growth is also demonstrated by the fact that incorporation of [^{3}H]thymidine triphosphate ([^{3}H]TTP) into MCF-7 breast cancer cells is far less inhibited by tamoxifen than that of [^{3}H]thymidine (Figures 3 and 5). The weak impairment of [^{3}H]TTP incorporation can also be antagonized by DES but only after prolonged incubation time. On the contrary, compound **3d** inhibits the incorporation of [^{3}H]thymidine and [^{3}H]TTP to the same extent. Both effects cannot be reversed by DES (Figures 3 and 5). The lack of a "rescue effect" of DES when simultaneously added with **3d** as well as the activity of the latter against

Table IV. Chemotherapy of L 5222 Rat Leukemia with (−)-Dichloro[1,2-bis(4-hydroxyphenyl)ethylenediamine]platinum-(II) (**3d**)

compd	single (total) dose, mg/kg	treatment schedule	median day of survival (range)	% ILS
control			7 (6–8)	0
3d	20 (60)	1, 2, 3	18 (17–25)	175[a]
3d	20 (60)	1, 5, 9	18 (11–20)	175[a]
cisplatin	3.5 (10.5)	1, 2, 3	14.5 (7–29)	107[a]
cisplatin	3.5 (10.5)	1, 5, 9	17.5 (12–29)	150[a]

[a] $\alpha \leq 0.01$, determined by multiple comparison according to Dunn.[41]

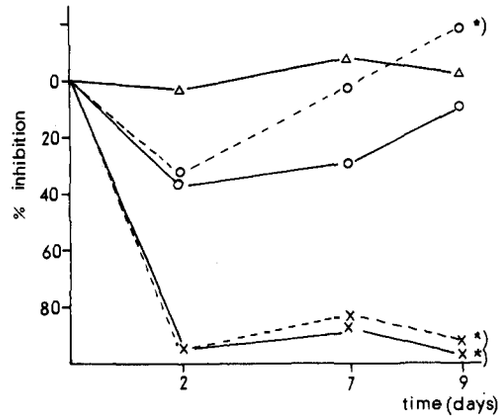


Figure 5. Effect of (−)-dichloro[1,2-bis(4-hydroxyphenyl)-ethylenediamine]platinum(II) (**3d**) on [^{3}H]thymidine triphosphate incorporation into hormone-dependent MCF-7 breast cancer cells: (−O−) tamoxifen (10^{-6} M), (--O--) tamoxifen (10^{-6} M) + DES (10^{-9} M), (△) DES (10^{-9} M), (−x−) **3d** (10^{-5} M), (--x--) **3d** (10^{-5} M) + DES (10^{-9} M); (*) $p \leq 0.05$ at the end of test. Data points are the mean of two determinations. SD less than 10%.

hormone-independent tumors such as the MDA-MB 231 mammary carcinoma and the ADJ/PC6 plasmacytoma points to a reaction with biomacromolecules like DNA rather than an estrogen antagonism as mode of action of **3d** (Table II, Figures 3 and 4).

An interaction of dichloro[1,2-bis(4-hydroxyphenyl)-ethylenediamine]platinum(II) complexes (**3a–d**) with DNA can actually be demonstrated. In the presence of **3a–d** an absorption maximum at 272 nm with a shoulder at 295 nm and a minimum at 248 nm is observed in the UV-difference spectrum of DNA (Figure 6). An identical UV-difference spectrum is obtained under the influence of cisplatin.[21,22] The increment ΔA_{270} is an indication for a substance-mediated loss in "base stacking" accompanied by a change in DNA secondary structure.[22] The ratio $\Delta A_{270}/\Delta A_{295}$ is used as a measure for this conformational disturbance,[22] having a value of approximately 2 in the case of cisplatin.[22] For compounds **3b**, **3c**, and **3d**, the quotients are 2.0, 2.3, and 2.2, respectively. The $\Delta A_{270}/\Delta A_{295}$ value of **3a** (1.5) is in agreement with a somewhat decreased antitumor activity (Table II).

The platinum complexes **3a–d** exist in two conformations (λ and δ, Figure 7). The *R,R*-configurated complex (**3d**) bears its phenyl rings axially located in the δ-conformation, whereas the phenyl rings of the *S,S*-configurated complex (**3c**) are axially located in the λ-conformation. Two axially standing phenyl rings probably impair an approach to DNA. After conversion into λ- and δ-conformation, respectively, an interference with the nu-

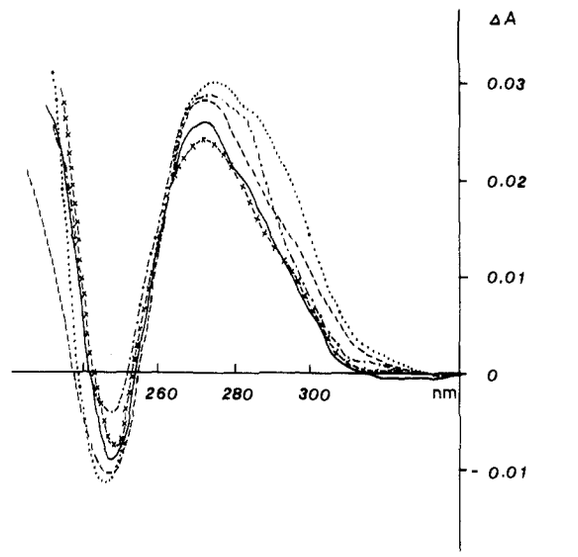


Figure 6. Ultraviolet difference spectra resulting from the interaction of stereoisomeric dichloro[1,2-bis(4-hydroxyphenyl)-ethylenediamine]platinum(II) complexes (Pt/P = 0.1) with DNA: (···) **3a**, (−·−) **3b**, (x) **3c**, (−) **3d**, (−−−) cisplatin.

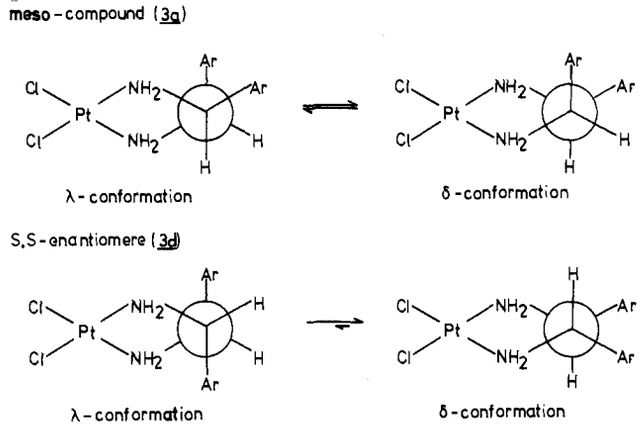


Figure 7. Conformation equilibria of stereoisomeric dichloro-[1,2-bis(4-hydroxyphenyl)ethylenediamine]platinum(II) complexes; Ar = 4-hydroxyphenyl.

cleobases of the DNA can take place, causing a disturbance of the secondary structure. The meso complex (**3a**), however, has an axially standing phenyl ring in each conformation impeding an approach toward DNA. This may be the reason for its slightly weaker antitumor effects.

The toxicology of **3d**, the most active compound, was studied in detail, as it is known that therapy using cisplatin is limited by several severe side effects, especially kidney toxicity, intestinal toxicity, and toxicity to the hemopoetic organs.[23–26] Balb/C mice bearing ADJ/PC6 plasmacytoma were used as test model. On days 5 and 15 after administration of **3d**, the histopathological examination of kidney, ileum, liver, and spleen showed no impairment. In accordance with these findings, the blood urea level was unchanged. The same was found with use of rats (Figure 8), while cisplatin causes a 4-fold increase. A possible myelotoxic effect can be excluded by normal white blood

(21) Inagaki, K.; Kidani, Y.; Suzuki, K.; Tashiro, T. *Chem. Pharm. Bull.* **1980**, *28*, 2286.
(22) Inagaki, K.; Kidani, Y. *Inorg. Chim. Acta* **1980**, *46*, 35.
(23) Walker, E. M.; Gale, G. R. *Ann. Clin. Lab. Sci.* **1981**, *11*, 397.
(24) Cavalli, F.; Jungi, W. F.; Nissen, N. J.; Pajak, T. F.; Coleman, M.; Holland, J. F. *Cancer* **1981**, *48*, 1927.
(25) Schurig, J. E.; Bradner, W. T.; Huftalen, J. B.; Doyle, G. J.; Gylys, J. A. "Cisplatin, Current Status and New developments"; Academic Press: New York, 1980; p 227.
(26) Rose, W. C.; Schurig, J. E.; Huftalen, J. B.; Bradner, W. T. *Cancer Treat. Rep.* **1982**, *66*, 135.

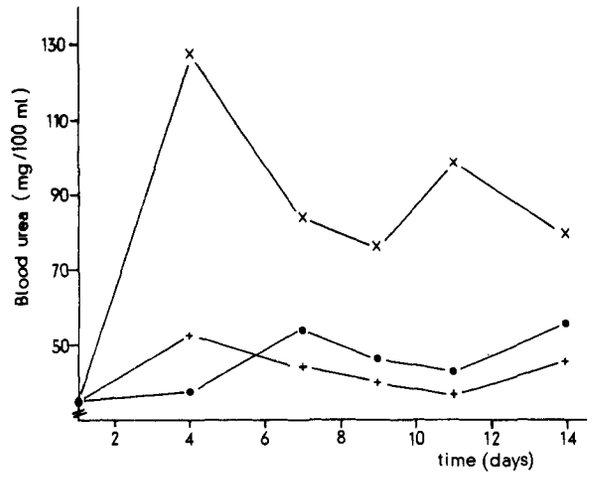


Figure 8. Blood urea level of Sprague–Dawley rats after treatment with (−)-dichloro[1,2-bis(4-hydroxyphenyl)ethylenediamine]platinum(II) (**3d**) and cisplatin: (●) control, (+) **3d** (3 × 10 mg/kg), (x) cisplatin (1 × 7 mg/kg). Data points are the mean of triplicate determinations.

cell counts (control, 4400/μL; day 5, 4500 cells/μL; day 15, 4800 cells/μL; after 5 months, 3800 cells/μL). A further histopathological examination 5 months after administration of **3d** showed an alteration of 20–30% of renal tubules. The damage is not that severe, so a normal function of the kidneys can still be expected.

Our studies show that the synthesis of "estrophilic platinum complexes" is possible. It is of interest that these compounds do not only exhibit marked tumor-inhibiting properties but also very low toxicity. Since the affinity for the estrogen receptor is too low, an accumulation of the platinum complexes by the estrogen receptor system seems to be less probable. Further studies to optimize the estrogen receptor affinity and therewith the mammary tumor inhibiting properties of this type of platinum complexes are in progress.

Experimental Section

General Procedures. Melting points were determined on a Büchi melting point apparatus or an Edmund Bühler melting point apparatus if higher than 220 °C and are uncorrected. The IR data were registrated with a Perkin-Elmer Model 480 A spectrophotometer. Elemental analyses were performed by the Mikroanalytisches Laboratorium, University of Regensburg.

Syntheses. *meso*-1,2-Bis(4-hydroxyphenyl)ethylenediamine (1a).[27] *meso*-1,2-Bis(4-methoxyphenyl)ethylenediamine[27,28] (27.2 g, 100 mmol) was suspended in 600 mL of dry CH_2Cl_2 in a 1000-mL three-necked flask fitted with a reflux condenser and a mechanical stirrer and cooled to −50 °C. With good stirring BBr_3 (38 mL, 400 mmol) was slowly added, stirring was continued for $^1/_2$ h, and finally the mixture was refluxed for 1 h. For hydrolysis the mixture was cooled to 0 °C and treated cautiously with 100 mL of H_2O; after further H_2O (400 mL) was added, the pH was adjusted to 11–12 by 20% NaOH, and the two phases were separated. The H_2O phase was filtered and neutralized with dilute acetic acid. The forming precipitate was collected and washed thoroughly with H_2O. Purification was done by redissolving in dilute acetic acid and carefully precipitating with 2 N NaOH to yield 23.0 g (94%) of the colorless solid **1a**: mp 185–190 °C dec. Anal. Calcd for $C_{14}H_{16}N_2O_2$ (M_r 244.3): C, 68.8; H, 6.60; N, 11.46. Found: C, 69.10; H, 6.70; N, 11.45.

(±)-1,2-Bis(4-hydroxyphenyl)ethylenediamine Dihydrobromide[27] **(1b·2HBr).** (±)-1,2-Bis(4-methoxyphenyl)ethylenediamine[27,28] (2.72 g, 10 mmol) was dissolved in 100 mL of dry CH_2Cl_2 and the solution cooled to −50 °C. With stirring, BBr_3 (3.8 mL, 40 mmol) was added slowly and the mixture then stirred overnight at room temperature. Hydrolysis was achieved with methanol at 0 °C. After evaporation to dryness, the residue was dissolved in MeOH and the product precipitated with ether to yield 2.70 g (66%) of a colorless solid **1b**·2HBr, mp 179–183 °C dec. Anal. Calcd for $C_{14}H_{16}N_2O_2 \cdot 2HBr$ (M_r 406.1): C, 41.40; H, 4.46; N, 6.89; Br, 39.40. Found: C, 42.10; H, 4.61; N, 6.95; Br, 38.20. The two enantiomers (**1c** and **1d**) were obtained by separation of (±)-1,2-bis(4-methoxyphenyl)ethylenediamine with tartaric acid[28] and subsequent ether cleavage by BBr_3.

Method A. (±)-Dichloro[bis(4-hydroxyphenyl)ethylenediamine]platinum(II) (3b).[27] K_2PtCl_4 (415 mg, 1 mmol) was dissolved in 2 mL of H_2O and added to (±)-bis(4-hydroxyphenyl)ethylenediamine dihydrobromide (406 mg, 1 mmol) in 6 mL of H_2O. The pH of the clear solution was adjusted to 6.8–7.0 by addition of 0.1 N NaOH and the mixture kept in the dark for 8 h. During this time the pH was adjusted to neutrality several times. Then the precipitate was collected, washed with H_2O, and dried to yield 450 mg (88%) of a yellow powder (**3b**). The Pt(II) complexes **3c**, **3d**, **4b**, **4c**, and **4d** were prepared in the same manner, whereas **3a** was prepared by method B.

Method B. Dichloro[*meso*-*N*,*N*′-dibutyl-1,2-bis(4-hydroxyphenyl)ethylenediamine]platinum(II) (4a).[27] *meso*-*N*,*N*′-Dibutyl-1,2-bis(4-hydroxyphenyl)ethylenediamine[1] (356 mg, 1 mmol) was dissolved in hot water and added slowly with stirring to a solution of K_2PtCl_4 (415 mg, 1 mmol) in 50 mL of H_2O. After the mixture was stirred for 12 h at 70–80 °C, the precipitate was collected, washed with diluted HCl and water, and dried at 100 °C in vacuo to yield 420 mg (67%) of a pale yellow powder (**4a**).

CD Spectra. The spectra were obtained with a JASCO J-40 A spectropolarimeter (time constant, 16 s; scan speed, 5 nm/min) and recorded in Me_2SO at room temperature in 5-cm quartz cells. The concentrations were 2×10^{-3} M [**3c** and (+)-dichloro(1,2-diphenylethylenediamine)platinum(II)] and 8×10^{-4} M [**3d** and (−)-dichloro(1,2-diphenylethylenediamine)platinum(II)].

UV-Difference Spectroscopy. Calf thymus DNA (type I) was purchased from Sigma Chemicals. The DNA was dissolved in 0.01 M $NaClO_4$ by gentle stirring at 4 °C [concentration, 1×10^{-4} M (P)]. The platinum complexes were dissolved in Me_2SO before use. Aliquots of this solution were added to the DNA solution and to a 0.01 M $NaClO_4$, respectively. The mixture was incubated at room temperature for 4 days. The amount of platinum complex added to the DNA solution is expressed as the molar ratio (r) of platinum to phosphorus in DNA. The ratio r = 0.1 was used in all cases. The UV-difference spectra were recorded on a Uvikon 810 spectrophotometer with a Uvikon LS printer (Kontron) in tandem quartz cells (Hellma). The sample cell contained in one compartment the DNA–platinum solution and in the other a 0.01 M $NaClO_4$ solution with the same amount of Me_2SO; the reference cell contained the $NaClO_4$–platinum solution and a DNA solution with the appropriate amount of Me_2SO.

Biological Methods. Estradiol Receptor Binding Assay. The percent inhibition of the [^{3}H]estradiol receptor binding was evaluated by using a procedure as described previously.[29-31] Calf uterine cytosol (final concentration of protein 5 mg/mL) and the dextran-coated charcoal (DCC) method were applied.

Cell Culture Experiments. 1. ADJ/PC6 Plasmacytoma. ADJ/PC6 plasmacytoma was maintained by routine passage in female Balb/C mice. It was kindly provided to us by Dr. K. R. Harrap, Department of Biochemical Pharmacology, Sutton, Surrey, U.K. For precursor incorporation experiments the tumor was removed aseptically, minced with scissors, and placed into a sterile mortar. The tumor mince was broken up by gentle pressure with a pistil against the bottom of the mortar and suspended in Eagle's minimal essential medium (Flow Laboratories) supplemented with 5% heat-inactivated horse serum (Flow Laboratories), Hepes buffer (5 mg/mL, Sigma), and 0.1% Pluronic F 68 (Serva). The fibrous material was allowed to settle and the supernatant suspension was decanted over sterile gauze. Aliquots

(27) Eicher, N., unpublished results
(28) Vögtle, F.; Goldschmitt, E. *Chem. Ber.* **1976**, *109*, 1.
(29) Hartmann, R. W.; Kranzfelder, G.; von Angerer, E.; Schönenberger, H. *J. Med. Chem.* **1980**, *23*, 841.
(30) Kranzfelder, G.; Schneider, M. R.; von Angerer, E.; Schönenberger, H. *J. Cancer Res. Clin. Oncol.* **1980**, *97*, 167.
(31) Korenman, S. G. *J. Clin. Endocrinol. Metab.* **1968**, *28*, 127.

of 2 mL were then placed into centrifuge tubes. Then the compounds were added as freshly prepared 1000-fold concentrates in Me_2SO, leading to a final solvent concentration of 0.1%. After 3-h incubation in a shaking water bath at 37 °C, 1 μCi of [*methyl*-^{3}H]thymidine (40–60 Ci/mmol, New England Nuclear) was added to each tube. Two hours later incubation was stopped with 3 mL of ice-cold Dulbecco's phosphate-buffered saline. Cells were washed three times with saline and then disrupted by a Branson sonicator. The TCA precipitate was filtered over 0.45-μm filter (Gelman) and counted in a liquid scintillation counter (Beckman LS 8000).

Hormone-Independent MDA-MB 231 Human Breast Cancer Cell Line. This cell line was derived in the laboratory of Dr. R. Cailleau[32] from the pleural effusion of a patient with a poorly differentiated papillary carcinoma. Cells were grown in McCoy 5a medium (Boehringer, Mannheim) supplemented with 10% newborn calf serum (Gibco) and Gentamycin (40 μg/mL, Sigma). [^{3}H]Precursor incorporation into TCA-precipitable material was performed as outlined previously by Lippman.[33,34] One hour prior to the start of the experiment, replicately plated cells were changed to medium supplemented with 5% serum. Compounds were then added as freshly prepared 1000-fold concentrates in Me_2SO, leading to a final solvent concentration of 0.1%.

Hormone-Dependent MCF-7 Human Breast Cancer Cell Line. MCF-7 cell line was established by Dr. H. D. Soule.[35] Cells were grown in Richter's improved minimal essential medium (IMEM, Associated Biomedic Systems, Buffalo, NY) supplemented as above. Both MCF-7 and MDA-MB 231 cell line were generously provided to us by Dr. M. E. Lippman, Medicine Branch National Cancer Institute, Bethesda, MD. To determine antitumor activity, cells growing in log phase were changed to IMEM supplemented with 5% serum stripped twice of endogenous hormones by dextran-coated charcoal (first incubation, 4 h at 4 °C; second incubation, 1 h at 56 °C). Two weeks later cells were harvested in trypsin–EDTA and plated in six-well Linbro dishes (Costar) in IMEM supplemented with 5% stripped serum. The next day medium was changed again, and compounds were added as described above. The incubation time ranged from 2 to 7 days. After 4 days medium and compounds were replenished. Two hours before harvesting, radioactive [^{3}H]thymidine or [^{3}H]thymidine triphosphate was added to each well, and the cells were harvested as previously described by Lippman.[33,34] The amount of total DNA was determined by use of the ethidium bromide technique.[36]

In Vivo Antitumor Activity. 1. ADJ/PC6 Mouse Plasmacytoma.[37] The experiments were conducted on 6–7 weeks old, female Balb/C mice (Zentralinstitut für Versuchstiere Hannover, Federal Republic of Germany), weighing 14–18 g. The animals were inoculated subcutaneously with 5×10^6 cells suspended in 0.1 mL of PBS. The treatment started 1 day after implantation (day 0). The compounds were suspended in olive oil and administered intraperitoneally as single or triple dose (day 1 and days 1, 2, 3, respectively). The duration of the test was about 38 days. Usually groups of 10 animals were used for control and therapy. Cisplatin served as a positive control. Antitumor activity was evaluated by mean tumor volume as percent of untreated control (T/C, percent).

2. Rat Leukemia L 5222.[38] Three to six months old BD IX rats (50% female, 50% male) of own breeding were used, six rats per dosage group. L 5222 cells (1×10^6) in 1.0 mL of physiological saline were injected intraperitoneally. The complex was dissolved in olive oil to a final concentration of 1 mg/mL. Therapy of leukemia L 5222 consisted of a series of three injections given on days 1, 2, 3 or 1, 5, 9 after implantation of leukemia cells (day 0). The experiments were evaluated according to the criterion percent ILS (increased life span).

Histological Procedures. **3d** was administered to female Balb/C mice intraperitoneally on days 1, 2, and 3 after transplantation of ADJ/PC6 plasmacytoma in a dose level of 3×20 mg/kg. On days 5 and 15, respectively, and after 5 months, the animals were first bled by cardiac puncture to determine blood urea levels and white blood cell counts. Kidneys, ileum, liver, and spleen were removed and fixed in Bouin's fluid. Samples were then embedded in paraplast and cut into 5 μm thick sections. They were subjected to the following staining procedures: Masson-Goldner in the modification of Jerusalem[39] and Azan.[40]

Blood Urea Levels in Rats. Female Sprague–Dawley rats weighing 180 g were purchased from Ivanovas, Kisslegg, Federal Republic of Germany. **3d** (3×10 mg/kg) and cisplatin (1×7 mg/kg) were administered intraperitoneally as a suspension in olive oil on days 1, 2, 3 (**3d**) and day 1 (cisplatin), respectively. The animals were bled by cardiac puncture on days 4, 7, 9, 11, and 14. Blood urea levels were determined with the test-set "Urea" from Boehringer, Mannheim, Federal Republic of Germany.

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Registry No. **1a**, 91265-94-0; **1b**·2HBr, 91265-95-1; **3a**, 91265-66-6; **3b**, 91326-61-3; **3c**, 91326-62-4; **3d**, 91326-63-5; **4a**, 91280-57-8; **4b**, 91326-92-0; **4c**, 91326-93-1; **4d**, 91326-94-2; *meso*-1,2-bis(4-methoxyphenyl)ethylenediamine, 58520-45-9; (±)-1,2-bis(4-methoxyphenyl)ethylenediamine, 58519-98-5.

Supplementary Material Available: IR data and elemental analyses for dichloro[1,2-bis(4-hydroxyphenyl)ethylenediamine]platinum(II) complexes (Tables V and VI) (2 pages). Ordering information is given on any current masthead page.

(32) Cailleau, R.; Young, R.; Olivé, M.; Reeves, W. J. *J. Natl. Cancer Inst.* **1974**, *53*, 661.
(33) Lippman, M. E.; Bolan, G. *Nature (London)* **1975**, *256*, 592.
(34) Lippman, M. E.; Monaco, M. E.; Bolan, G. *Cancer Res.* **1977**, *37*, 1901.
(35) Soule, H. D.; Vazquez, J.; Long, A.; Abert, S.; Brennan, M. A. *J. Natl. Cancer Inst.* **1973**, *51*, 1409.
(36) Boer, G. J. *Anal. Biochem.* **1975**, *63*, 433.
(37) Connors, T. A.; Jones, M.; Ross, W. C. J.; Braddock, P. D.; Khokhar, A. R.; Tobe, M. S. *Chem. Biol. Interact.* **1972**, *5*, 415.
(38) Ivankovič, S.; Zeller, B. J. *Blut* **1974**, *28*, 288.
(39) Jerusalem, C. *Z. Wiss. Mikrosk. Mikrosk. Tech.* **1963**, *65*, 320.
(40) Heidenhain, M. *Z. Wiss. Mikrosk. Mikrosk. Tech.* **1915**, *32*, 361.
(41) Dunn, O. J. *Technometrics* **1964**, 6, 241.