fluid, and counted on a Beckmann LS 5801 liquid scintillation counter at 47% efficiency. Nonspecific binding was determined by co-incubation with 1 μM atropine and amounted to less than 15% of total counts. It was routinely subtracted from the total counts.

Saturation by [$^3$H]NMS before and after treatment of the membranes with the potentially affinity label was measured to assay for irreversible inhibition. Aliquots of brain membranes were incubated with 1.0 mM of freshly dissolved **26** in phosphate-buffered saline (pH 8.0, 10 mM phosphate, 0.15 M NaCl) for 60 min at room temperature and then centrifuged at 15 000 rpm for 10 min. The resulting pellet was resuspended in 25 mL of fresh phosphate-buffered saline (pH 7.2) and centrifuged as above. The pellet was again resuspended in fresh buffer and centrifuged, and aliquots were taken for [$^3$H]NMS saturation binding experiments.

**Acknowledgment.** We thank Dr. Herman Yeh of NIH for assistance with NMR studies and Naftali Malka for technical assistance.

**Registry No.** **1**, 28797-61-7; **3**, 107831-56-1; **4**, 6298-19-7; **5**, 134-20-3; **6**, 885-70-1; **7**, 133727-44-3; **8**, 133727-45-4; **9**, 133727-46-5; **10**, 133727-47-6; **11a**·HCl, 133727-48-7; **11b**, 133727-49-8; **11c**, 133727-50-1; **11c**·oxalate, 133727-51-2; **11d**·oxalate, 133727-53-4; **11e**·oxalate, 133727-55-6; **13**, 133727-56-7; **14**, 133727-57-8; **15**, 133727-58-9; **16**, 133727-59-0; **17a**·HCl, 133727-60-3; **17b**, 133727-61-4; **18**, 63257-31-8; **19a**, 96448-78-1; **19b**·HCl, 133727-62-5; **20**·HCl, 133727-63-6; **21**, 133727-64-7; **22**, 69628-58-6; **23**·oxalate, 133727-65-8; **24a**·oxalate, 133727-67-0; **24b**·oxalate, 133727-69-2; **24c**·oxalate, 133727-71-6; **24d**·oxalate, 133727-73-8; **25**, 28781-43-3; **26**·HCl, 133727-74-9; **27**, 133727-75-0; **28**, 133727-76-1; **29a**, 133727-77-2; **29b**, 133727-78-3; **30a**·TFA, 133727-80-7; **30b**·TFA, 133727-82-9; **31a**, 133727-83-0; **31b**, 133727-84-1; **32a**, 133727-85-2; **32b**, 133727-86-3; **32c**, 133727-87-4; **32d**·oxalate, 133727-89-6; **32e**, 133727-90-9; **32f**, 133727-91-0; **32g**, 133727-92-1; **32h**, 133727-93-2; **33a**, 133727-94-3; **33b**·oxalate, 133727-96-5; **33c**, 133727-97-6; **33d**, 133727-98-7; **33e**, 133727-99-8; **33f**, 133728-00-4; **33g**, 133728-01-5; **33h**, 133728-02-6; **34a**, 133728-03-7; **34b**, 133728-04-8; **34c**, 133728-05-9; **34d**, 133728-06-0; **934e**, 133728-07-1; **34f**, 133728-08-2; **34g**, 133728-09-3; **34h**, 133728-10-6; **35a**, 133728-11-7; **35b**, 133728-12-8; **35c**, 133728-13-9; **35d**, 133728-14-0; **35e**, 133728-15-1; **35f**, 133728-16-2; **35g**·oxalate, 133728-18-4; **35h**, 133728-19-5; **36**·oxalate, 133728-21-9; **37**·oxalate, 133728-23-1; $NH_2(CH_2)_2NH_2$, 107-15-3; $C_6H_5COCl$, 98-88-4; $CH_3SO_2Cl$, 124-63-0; $C_6H_5NCS$, 103-72-0; $BrCH_2C{\equiv}CH$, 106-96-7; $C_6H_5CH_2Br$, 100-39-0; *p*-$BrCH_2C_6H_4CONHCH_3$, 118507-18-9; *p*-$BrCH_2C_6H_4CONH(CH_2)_2NHBOC$, 120131-81-9; *p*-$BrCH_2C_6H_4CONH(CH_2)_4NHBOC$, 120131-82-0; $ICH_2CH_2OH$, 624-76-0; $BOC(CH_2)_5CON(Me)OMe$, 133728-24-2; $Br(CH_2)_3Pht$, 5460-29-7; $Br(CH_2)_4Pht$, 5394-18-3; $Br(CH_2)_5Pht$, 954-81-4; $Br(CH_2)_6Pht$, 24566-79-8; $Br(CH_2)_7Pht$, 52824-42-7; $Br(CH_2)_8Pht$, 17702-83-9; $Br(CH_2)_9Pht$, 93667-91-5; $Br(CH_2)_{10}Pht$, 24566-80-1; $Br(CH_2)_3Br$, 109-64-8; $Br(CH_2)_4Br$, 110-52-1; $Br(CH_2)_5Br$, 111-24-0; $Br(CH_2)_6Br$, 629-03-8; $Br(CH_2)_7Br$, 4549-31-9; $Br(CH_2)_8Br$, 4549-32-0; $Br(CH_2)_8Br$, 4549-33-1; $Br(CH_2)_{10}Br$, 4101-68-2; $CHO(CH_2)_3NHBOC$, 84766-90-5; $CHO(CH_2)_4NHBOC$, 94136-78-4; $CHO(CH_2)_6NHBOC$, 133728-25-3; $CHO(CH_2)_7NHBOC$, 133728-26-4; $CHO(CH_2)_8NHBOC$, 133728-27-5; $CHO(CH_2)_9NHBOC$, 133728-28-6; $CHO(CH_2)_{10}NHBOC$, 133728-29-7; 5,11-dihydro-8-[2-aminoethylsulfenamido]-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one, 133728-30-0; 2-chlorooctyl chloride, 79-04-9; *N*-methylpiperazine, 109-01-3; piperazine, 110-85-0; α-chloroethyl chloroformate, 50893-53-3; methyl 2-amino-4-carbomethoxybenzoate, 5372-81-6; methyl 2-amino-4-(hydroxymethyl)benzoate, 133728-31-1; 5-ethyl-11-hydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one, 4937-79-5; *N*-(2′-chloropyrid-3-yl)-2-amino-4-[[(dimethyl-*tert*-butylsilyl)oxy]methyl]-benzamide, 133728-32-2; *N*-*t*-BOC-2-alanine succinimido ester, 32703-87-0; *N*-*t*-BOC-4-aminobutyric acid, 57294-38-9; 6-(*tert*-butyloxycarbonylamino)hexanoic acid, 6404-29-1; (dimethylamino)pyridine, 1122-58-3; 6-(*tert*-butyloxycarbonylamino)hexanal, 80860-42-0.

# 2-Phenylindole-Linked [2-(Aminoalkyl)pyridine]dichloroplatinum(II): Complexes with a Selective Action on Estrogen Receptor Positive Mammary Tumors

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A number of [2-(aminomethyl)pyridine]dichloroplatinum(II) complexes, linked to 5-hydroxy-2-(4-hydroxyphenyl)indoles by alkyl spacer groups of varying lengths, were synthesized and studied for their binding affinities for the calf uterine estrogen receptor. Their relative binding affinity (RBA) values ranged from 1.0 to 5.2% (estradiol, RBA = 100%). Highest affinities were found with complexes possessing a $(CH_2)_5$- or $(CH_2)_6$-bridge between the pyridine aminomethyl group and the indole nitrogen. Endocrine activities of the complexes and their ligands, determined in the mouse uterine weight test, were low. All compounds entered comparative tests using estrogen receptor positive and negative mammary tumor models. In cell culture, a growth inhibiting effect was only observed in hormone-sensitive MCF-7 cells, but not in hormone-independent MDA-MB 231 cells. In this assay, there was no significant difference between complexes and their ligands. In vivo, the growth of estrogen receptor positive MXT mouse mammary tumors was strongly inhibited by the complexes whereas the hormone-independent MXT mammary tumors showed only a minor response. At a dose of 3 × 20 mg/kg per week, complexes **10d–g** reduced the tumor weight by ca. 80% after 6 weeks of treatment. This effect was generally stronger than that exerted by the ligands. The doses applied were well tolerated. Since the complexes described in this paper require the estrogen receptor for their action, a mechanism similar to that of antiestrogens is assumed.

In our search for new and more effective drugs for the therapy of advanced breast cancer we were interested in exploiting the cytotoxic potential of platinum complexes. Usually, mammary tumors are resistant to cisplatin, the most important platinum based drug. In order to overcome this resistance, we made use of the estrogen receptor, present in the majority of these carcinomas.[1] These receptors which are located predominately in the cell nucleus,[2] should serve as primary target to direct the cytotoxic platinum group toward the DNA. For this purpose, we synthesized a number of different platinum complexes with 5-hydroxy-2-(4-hydroxyphenyl)indole as a receptor binding fragment.[3,4]

A prerequisite for our concept is a sufficiently high binding affinity of the complexes for the estrogen receptor. In previous studies we were able to demonstrate that the

(1) Brodie, A. M. H. *Biochem. Pharmacol.* **1985**, *34*, 3213.

(2) King, W. J.; Greene, G. L. *Nature* **1984**, *307*, 745.

(3) Knebel, N.; von Angerer, E. *J. Med. Chem.* **1988**, *31*, 1675.

(4) von Angerer, E.; Birnböck, H.; Knebel, N. *Anti-Cancer Drug Des.* **1989**, *4*, 21.

0022-2623/91/1834-2145$02.50/0 

**Scheme I**

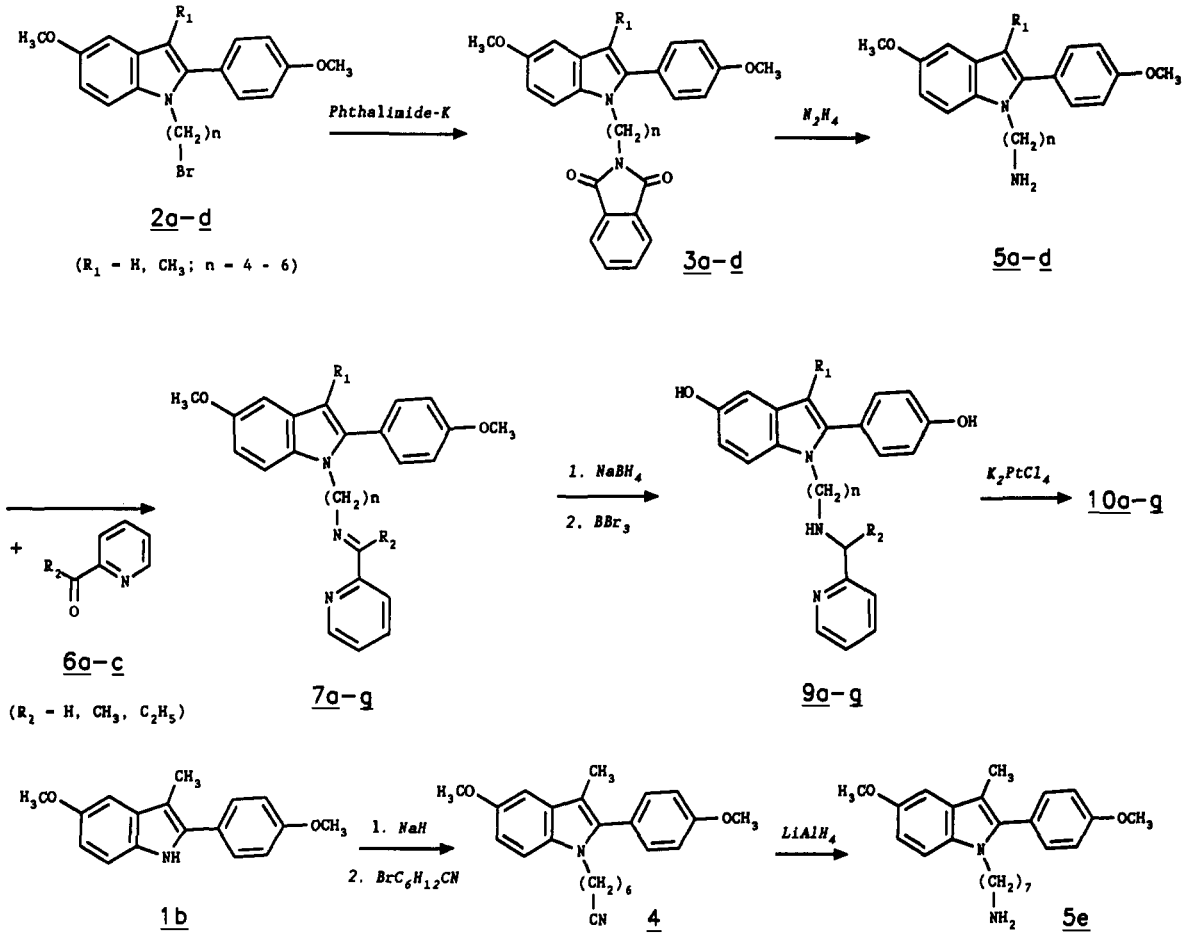
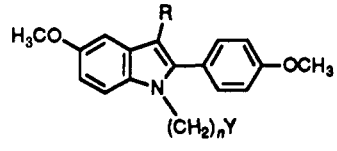



**Table I.** Methoxy-Substituted 1-(Phthalimidoalkyl)-, 1-(6-Cyanohexyl)-, and 1-(Aminoalkyl)-2-phenylindoles **3**, Y = $C_8H_4NO_2$; **4**, Y = CN; and **5**, Y = $NH_2$, respectively)



| compd | n | R | formula[a] | yield, % | mp,[b] °C | compd[c] | formula[d] | yield, % |
|---|---|---|---|---|---|---|---|---|
| **3a**[e] | 4 | $CH_3$ | $C_{29}H_{28}N_2O_4$ | 84 | 89–91 | **5a** | $C_{21}H_{26}N_2O_2$ | 48 |
| **3b**[e] | 5 | $CH_3$ | $C_{30}H_{30}N_2O_4$ | 82 | 130–132 | **5b** | $C_{22}H_{28}N_2O_2$ | 52 |
| **3c**[e] | 6 | H | $C_{30}H_{30}N_2O_4$ | 75 | 143–145 | **5c** | $C_{22}H_{28}N_2O_2$ | 55 |
| **3d**[e,f] | 6 | $CH_3$ | $C_{31}H_{32}N_2O_4$ | 78 | 99 | **5d**[f] | $C_{23}H_{30}N_2O_2$ | 45 |
| **4**[g] | 7 | $CH_3$ | $C_{24}H_{28}N_2O_2$ | 67 | oil | **5e**[g] | $C_{24}H_{32}N_2O_2$ | 45 |

[a] Crystalline products were analyzed for C, H, and N within ±0.4% of the calculated values. [b] Recrystallized from EtOH. [c] Y = $NH_2$. [d] Compounds **5a–e** are obtained as oils and purified by vacuum distillation. [e] Y = $C_8H_4NO_2$. [f] Reference 11. [g] Y = CN ($n$ – 1).

2-phenylindole is a suitable structure when the diaminodichloroplatinum(II) group was linked to it via an alkyl spacer group. In these investigations, we used 1,2-diaminoethane[3] and 1,3-diaminopropane[4] as chelating functions. Lately, Brunner and co-workers showed that 2-(aminomethyl)pyridine and 2-(aminomethyl)quinoline form dichloroplatinum(II) complexes that exhibit cytotoxic activity even if they lack primary amino functions.[5] Their findings prompted us to use this structural element as chelating function.

In this study, the primary nitrogen of 2-(aminomethyl)pyridine was linked to the nitrogen of 2-phenylindole via alkyl spacer groups of varying lengths. In addition, we introduced alkyl substituents onto the methylene group of the pyridine (Chart I). Both the free ligands and the corresponding platinum(II) complexes were tested for binding affinity for the calf uterine estrogen receptor and endocrine activity in the mouse uterine weight test. Selective cytostatic activity was determined in vitro and in vivo with use of estrogen receptor positive and negative mammary tumor cells and MXT mouse mammary tumors respectively.

**Chemistry.** The syntheses of the ligands are outlined in Scheme I. For the preparation of the 2-phenylindoles substituted with primary aminoalkyl groups at N-1 the Gabriel synthesis was applied except for the derivative with a $C_7$-spacer group **5e**. The 1-(bromoalkyl)-2-phenylindoles **2a–d** were obtained from the 2-phenylindoles **1a** and **1b** and the respective dibromoalkane as described previously.[3] They were reacted with potassium phthalimide to form the phthalimines **3a–d** (Table I). The primary amines **5a–d**

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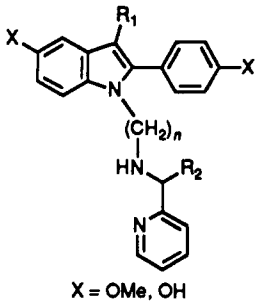
Table II. Dimethoxy- and Dihydroxy-1-[[(2-pyridylmethyl)amino]alkyl]-2-phenylindoles (8 and 9, respectively) and Estrogen Receptor Affinities of 9a–g

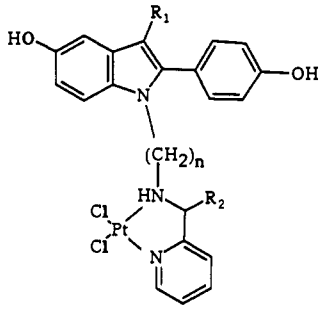



X = OMe, OH

| compd[a] | n | R1 | R2 | formula[b] | compd[c] | formula[d] | mp,[e] °C | RBA,[f] % |
|---|---|---|---|---|---|---|---|---|
| 8a | 4 | $CH_3$ | H | $C_{27}H_{31}N_3O_2$ | 9a | $C_{25}H_{27}N_3O_2$ | 125 | 8 |
| 8b | 5 | $CH_3$ | H | $C_{28}H_{33}N_3O_2$ | 9b | $C_{26}H_{29}N_3O_2$[g] | 80–82 | 22 |
| 8c | 6 | H | H | $C_{28}H_{33}N_3O_2$ | 9c | $C_{26}H_{29}N_3O_2$ | 74 | 18 |
| 8d | 6 | $CH_3$ | H | $C_{29}H_{35}N_3O_2$ | 9d | $C_{27}H_{31}N_3O_2$ | 67–68 | 20 |
| 8e | 7 | $CH_3$ | H | $C_{30}H_{37}N_3O_2$ | 9e | $C_{28}H_{33}N_3O_2$ | 54–56 | 8.5 |
| 8f | 6 | $CH_3$ | $CH_3$ | $C_{30}H_{37}N_3O_2$ | 9f | $C_{28}H_{33}N_3O_2$[h] | 89–91 | 21 |
| 8g | 6 | $CH_3$ | $C_2H_5$ | $C_{31}H_{39}N_3O_2$ | 9g | $C_{29}H_{35}N_3O_2$ | 75–77 | 15 |

[a] X = $OCH_3$. [b] Compounds 8a–g were obtained as oils and purified by vacuum distillation. [c] X = OH. [d] Analyzed for C and H within ±0.4% of the calculated values, except where noted. [e] Crystallized from MeOH. [f] Average of three independent determinations, each performed in triplicate; 17β-estradiol, RBA = 100%. [g] C: calcd, 75.15; found, 74.27. [h] C: calcd, 75.81; found, 75.08.

**Chart I**

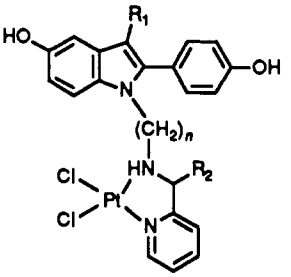



$R_1$ = H, $CH_3$; $R_2$ = H, $CH_3$, $C_2H_5$; n = 4 - 7

were liberated by the reaction with hydrazine hydrate. The amine **5e** was obtained by the $LiAlH_4$-reduction of the 1-(6-cyanohexyl)-2-phenylindole **4**. The primary amines were reacted with pyridine-2-aldehyde (**6a**), 2-acetylpyridine (**6b**), or 2-propionylpyridine (**6c**) to afford the corresponding imines **7a–g**, which were subsequently reduced with $NaBH_4$. Ether cleavage with $BBr_3$ afforded the ligands **9a–g**. They were converted into the complexes **10a–g** by the reaction with $K_2PtCl_4$ in aqueous solution under controlled pH conditions. All of the platinum complexes were characterized by their NMR spectra (250 MHz), IR spectra (300–3600 $cm^{-1}$), and mass spectra (PIFAB or FD).

**Binding Affinity for the Calf Uterine Estrogen Receptor.** The binding affinities of both the free ligands (Table II) and the platinum(II) complexes (Table III) were measured in a competitive binding assay with [$^3$H]17β-estradiol. Calf uterine cytosol was used as receptor source and the dextran-coated charcoal (DCC) method was applied.[6] The relative binding affinities (% RBA) are given as ratio of the molar concentrations of 17β-estradiol and test compound required to decrease the receptor bound radioactivity by 50%, multiplied by 100. The semilogarithmic plot of bound radioactivity vs molar concentrations of competitor exhibited curves parallel to those of 17β-estradiol, suggesting a common binding site for all of compounds that were tested.

Table III. [2-[[[[5-Hydroxy-2-(4-hydroxyphenyl)indol-1-yl]-alkyl]amino]methyl]pyridine]dichloroplatinum(III) Complexes 10 and Their Estrogen Receptor Affinities




| compd | n | R1 | R2 | formula[a] | dec,[b] °C | RBA,[c] % |
|---|---|---|---|---|---|---|
| 10a | 4 | $CH_3$ | H | $C_{25}H_{27}Cl_2N_3O_2Pt$ | 125 | 1.3 |
| 10b | 5 | $CH_3$ | H | $C_{26}H_{29}Cl_2N_3O_2Pt$ | 250–252 | 5.2 |
| 10c | 6 | H | H | $C_{26}H_{29}Cl_2N_3O_2Pt$ | 144–146 | 1.6 |
| 10d | 6 | $CH_3$ | H | $C_{27}H_{31}Cl_2N_3O_2Pt$[d] | 196 | 2.2 |
| 10e | 7 | $CH_3$ | H | $C_{28}H_{33}Cl_2N_3O_2Pt$[e] | 128 | 1.0 |
| 10f | 6 | $CH_3$ | Me | $C_{28}H_{33}Cl_2N_3O_2Pt$ | 142–144 | 3.0 |
| 10g | 6 | $CH_3$ | Et | $C_{29}H_{35}Cl_2N_3O_2Pt$ | 133–135 | 3.0 |
| 17β-estradiol | | | | | | 100 |
| tamoxifen | | | | | | 1.8 |

[a] Analyzed for C, H, and N within ±0.5% of the calculated values, except where noted. [b] Crystallized from water. [c] Average of three independent determinations, each performed in triplicate. [d] H: calcd, 4.49; found, 5.03. N: calcd, 6.04; found, 5.39. [e] C: calcd, 47.39; found, 46.61. H: calcd, 4.68; found, 5.12.

The RBA values for the ligands range from 8 to 22% depending on the length of the alkyl spacer group. The best binding was found with derivatives possessing a penta- or hexamethylene spacer group. However, the differences in affinity are rather small. The values of about 20% are close to that of the potent synthetic estrogen hexestrol (RBA ~25% for calf uterus cytosol) and higher than those of *N*-alkyl-substituted 2-phenylindoles.[6] After complexation, the RBA values are decreased by somewhat less than 1 order of magnitude. The binding affinities of the complexes, ranging from 1 to 5%, can still be considered rather high and are similar to that of tamoxifen (RBA = 1.8%), an antiestrogen that is widely used for the therapy of

(6) von Angerer, E.; Prekajac, J.; Strohmeier, J. *J. Med. Chem.* 1984, *27*, 1439.

hormone-dependent breast cancer.

**Endocrine Activity.** The high binding affinity of both the free ligands and the complexes for the estrogen receptor make a hormonal activity such as an estrogenic or antiestrogenic effect likely. Therefore, all of the compounds were tested for their uterotrophic and antiuterotrophic activity. In this assay, immature mice received doses of 25 and 250 μg of the complex per animal which corresponded to 1.25 and 12.5 mg/kg body weight. The doses of the ligands were reduced to 15 and 150 μg/animal in accord with their lower molecular weight. The increase in uterine dry weight was measured 24 h after the last injection.[4] Antagonistic activity was determined by simultaneous administration of the test compound and estrone (0.4 g) and calculation of the inhibition of estrone-stimulated uterine growth.

Endocrine activity of ligands and complexes was generally very weak at the administered doses which were more than 100-fold higher than that of estrone (Table IV). Significant estrogenic effects were observed with ligands and complexes bearing an alkyl substituent on the methylene group of the pyridine moiety. Some of the ligands exhibited antiestrogenic activity, mainly derivatives **9e–g**, whereas the complexes did not show this effect. For comparison, data of the pure estrogen antagonist ZK119.010[7] are included in Table IV.

**Effect on Human Mammary Tumor Cells in Vitro.** The cytostatic activity of the platinum complexes was evaluated in vitro by using both an estrogen receptor positive (MCF-7) and negative (MDA-MB 231) human mammary carcinoma cell line. The comparison of results should allow one to distinguish between a general cytostatic action and a specific receptor-mediated effect. As parameters for cellular growth we used cell numbers and [$^3$H]-thymidine incorporation. With hormone-independent MDA-MB 231 cells, no significant cytostatic effect was observed up to a concentration of $10^{-5}$ molar of complex or ligand (data not shown).

The number of MCF-7 cells treated with complexes **10c–g** at concentrations between 0.5 and 5.0 μmol was significantly lower than that of control cells (Table V). Maximum inhibition was 69% (**10e**). The effect of the complexes on thymidine incorporation was generally stronger. The ligands of the active complexes also inhibited the growth of MCF-7 cells. There was no significant difference in activity between complexes and ligands. Presumably the amino side chain is responsible for the inhibitory effect of the ligands, as has been shown in the (triphenyl)ethylene series where a basic side chain gives rise to a strong growth-inhibiting effect on MCF-7 cells.[8]

**Effect on Transplanted MXT Mouse Mammary Tumors.** The in vivo antitumor activity was determined in two different lines of the transplantable MXT-mammary tumor of the BDF-1 mouse. Tumors of one line (MXT-M3.2[9]) contain estrogen receptors and respond to endocrine manipulations like ovariectomy, whereas the MXT-ovex[10] tumors, which are propagated in ovariectomized mice, can be considered hormone-independent. Antineoplastic activity is given as ratio of tumor size of treated animals versus that of untreated control animals (% $T/C$). In these experiments with animals bearing solid tumors we did not measure the influence of the complexes on life span. The doses applied (20 mg/kg for the complexes) are well tolerated and correspond to those of previous studies.[4,11]

Table IV. Estrogenic and Antiestrogenic Activity of Ligands 9 and Platinum Complexes 10 in the Mouse Uterine Weight Test

| | | | antiuterotrophic test | |
|---|---|---|---|---|
| compd | dose,[a] μg | uterotrophic test: relative uterus weight[b] | relative uterus weight[b,c] | inhibn,[d] % |
| control | | 14.6 ± 2.7 | | |
| **9a** | 15 | 22.1 ± 3.3 | 52.2 ± 9.1 | 4 |
| **9a** | 150 | 15.0 ± 2.5 | 44.0 ± 9.3 | 25 |
| **10a** | 25 | 21.5 ± 7.2 | 45.8 ± 8.8 | 20 |
| **10a** | 250 | 14.8 ± 1.0 | 51.8 ± 3.9 | 5 |
| estrone | 0.4 | 53.7 ± 12.0 | | |
| control | | 12.3 ± 3.7 | | |
| **9b** | 15 | 16.4 ± 4.0[e] | 42.6 ± 5.4 | 9 |
| **9b** | 150 | 13.3 ± 1.7 | 44.0 ± 5.8 | 5 |
| **10b** | 25 | 16.3 ± 5.1[e] | 40.8 ± 5.4 | 14 |
| **10b** | 250 | 22.1 ± 5.3[e] | 46.6 ± 4.5 | |
| estrone | 0.4 | 45.5 ± 6.0 | | |
| control | | 14.5 ± 2.1 | | |
| **9c** | 15 | 13.6 ± 4.5 | 40.6 ± 9.0 | 18 |
| **9c** | 150 | 22.1 ± 1.0[e] | 46.1 ± 9.0 | 5 |
| **10c** | 25 | 12.7 ± 3.2 | 43.1 ± 5.7 | 12 |
| **10c** | 250 | 16.3 ± 2.1 | 52.7 ± 1.1 | |
| estrone | 0.4 | 48.1 ± 7.3 | | |
| control | | 12.4 ± 3.7 | | |
| **9d** | 15 | 27.9 ± 12.8 | 46.6 ± 7.3 | |
| **9d** | 150 | 24.9 ± 4.3 | 36.9 ± 5.9 | 31 |
| **10d** | 25 | 20.2 ± 3.3 | 56.6 ± 7.7 | |
| **10d** | 250 | 22.5 ± 6.8 | 56.3 ± 7.1 | |
| estrone | 0.4 | 48.0 ± 3.0 | | |
| control | | 12.3 ± 3.7 | | |
| **9e** | 15 | 15.2 ± 3.1 | 41.9 ± 1.1 | 11 |
| **9e** | 150 | 19.0 ± 2.1[e] | 29.4 ± 5.8 | 48[e] |
| **10e** | 25 | 12.8 ± 1.0 | 46.1 ± 7.2 | |
| **10e** | 250 | 21.9 ± 4.4[e] | 48.0 ± 3.5 | |
| estrone | 0.4 | 45.5 ± 6.0 | | |
| control | | 15.4 ± 1.7 | | |
| **9f** | 15 | 14.6 ± 1.6 | 34.2 ± 5.6 | 39[e] |
| **9f** | 150 | 20.2 ± 2.2[e] | 22.4 ± 2.4 | 77[e] |
| **10f** | 25 | 18.8 ± 4.1 | 43.6 ± 7.5 | 8 |
| **10f** | 250 | 34.2 ± 7.9[e] | 47.3 ± 6.3 | |
| **9g** | 15 | 14.7 ± 9.9 | 32.8 ± 6.9 | 42[e] |
| **9g** | 150 | 26.0 ± 3.6[e] | 30.5 ± 9.7 | 51[e] |
| **10g** | 25 | 20.2 ± 4.9 | 39.7 ± 5.5 | 20 |
| **10g** | 250 | 35.7 ± 1.2[e] | 48.0 ± 1.0 | |
| estrone | 0.4 | 46.2 ± 7.5 | | |
| control | | 15.2 ± 2.9 | | |
| ZK119.010[f] | 1 | 12.5 ± 1.7 | 45.8 ± 3.2 | 20 |
| | 5 | 9.0 ± 1.8[e] | 15.4 ± 1.7 | 100[e] |
| | 25 | 13.9 ± 2.8 | 14.4 ± 2.2 | 103[e] |
| | 125 | 14.1 ± 3.8 | 8.9 ± 1.6 | 117[e] |
| estrone | 0.4 | 53.7 ± 3.6 | | |

[a] Dose per animal, administered at three consecutive days sc. [b] Uterus dry weight (mg)/body weight (g) × 100, determined 24 h after the last injection; mean of 6–10 animals ± SD. [c] Simultaneous administration of 0.4 μg of estrone/animal and day. [d] The $U$ test according to Wilcoxon, Mann, and Whitney was used to determine significance. [e] Significant difference ($p < 0.01$). [f] ZK119.010 = 5-hydroxy-2-(4-hydroxyphenyl)-3-methyl-1-(6-pyrrolidinohexyl)indole.[7]

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**Table V.** The Effect of Ligands 9 and Platinum Complexes 10 on Estrogen Receptor Positive MCF-7 Human Breast Cancer Cells

| compd | concn, M | cell no./dish $\times 10^{4}$ [a] | % T/C | [$^3$H]thymidine cpm/dish $\times 10^3$ | incorp[b] % T/C |
|---|---|---|---|---|---|
| control | | 62.3 ± 8.8 | | 43.4 ± 1.8 | |
| **9a** | $5 \times 10^{-6}$ | 45.2 ± 3.8 | 73 | 33.1 ± 1.6 | 76 |
| | $1 \times 10^{-6}$ | 59.8 ± 4.6 | 96 | 37.8 ± 3.4 | 87 |
| **10a** | $5 \times 10^{-6}$ | 48.9 ± 2.6 | 79 | 29.5 ± 1.2 | 68[c] |
| | $1 \times 10^{-6}$ | 55.4 ± 6.0 | 89 | 38.4 ± 2.2 | 88 |
| control | | 66.9 ± 7.0 | | 28.2 ± 3.0 | |
| **9b** | $5 \times 10^{-6}$ | 28.7 ± 4.8 | 43[c] | 18.9 ± 0.4 | 67[c] |
| | $1 \times 10^{-6}$ | 43.0 ± 5.4 | 64 | 21.6 ± 0.8 | 76 |
| **10b** | $5 \times 10^{-6}$ | 57.1 ± 6.7 | 85 | 15.2 ± 0.4 | 54[c] |
| | $1 \times 10^{-6}$ | 67.4 ± 9.2 | 101 | 16.9 ± 2.0 | 60[c] |
| control | | 62.7 ± 8.8 | | 48.6 ± 3.6 | |
| **9c** | $5 \times 10^{-6}$ | 19.5 ± 1.7 | 31[c] | 9.6 ± 1.0 | 20[c] |
| | $1 \times 10^{-6}$ | 33.4 ± 7.5 | 53[c] | 20.0 ± 3.5 | 41[c] |
| | $5 \times 10^{-7}$ | 33.5 ± 10.5 | 54[c] | 22.8 ± 2.8 | 47[c] |
| **10c** | $5 \times 10^{-6}$ | 34.5 ± 6.5 | 55[c] | 16.7 ± 2.6 | 34[c] |
| | $1 \times 10^{-6}$ | 40.1 ± 7.8 | 64 | 27.5 ± 6.0 | 57[c] |
| | $5 \times 10^{-7}$ | 48.2 ± 5.3 | 77 | 33.4 ± 3.4 | 69 |
| control | | 22.3 ± 3.3 | | 7.7 ± 0.8 | |
| **9d** | $5 \times 10^{-6}$ | 5.2 ± 8.4 | 23[c] | 0.4 ± 0.2 | 5[c] |
| | $1 \times 10^{-6}$ | 10.6 ± 8.4 | 48[c] | 1.0 ± 0.6 | 13[c] |
| **10d** | $5 \times 10^{-6}$ | 12.4 ± 8.4 | 56[c] | 0.9 ± 0.4 | 12[c] |
| | $1 \times 10^{-6}$ | 11.6 ± 8.4 | 52[c] | 1.4 ± 0.6 | 18[c] |
| control | | 63.8 ± 9.0 | | 42.6 ± 2.8 | |
| **9e** | $5 \times 10^{-6}$ | 14.6 ± 2.8 | 23[c] | 2.2 ± 0.4 | 5[c] |
| | $1 \times 10^{-6}$ | 26.4 ± 5.9 | 41[c] | 11.3 ± 1.0 | 27[c] |
| | $5 \times 10^{-7}$ | 36.9 ± 6.4 | 58[c] | 14.5 ± 1.6 | 34[c] |
| **10e** | $5 \times 10^{-6}$ | 19.5 ± 10.8 | 31[c] | 7.7 ± 1.6 | 18[c] |
| | $1 \times 10^{-6}$ | 26.1 ± 2.2 | 41[c] | 12.1 ± 1.8 | 28[c] |
| | $5 \times 10^{-7}$ | 26.6 ± 2.2 | 42[c] | 15.4 ± 3.6 | 36[c] |
| control | | 24.4 ± 3.8 | | 10.2 ± 1.4 | |
| **9f** | $5 \times 10^{-6}$ | 9.5 ± 2.1 | 39[c] | 1.2 ± 0.1 | 12[c] |
| | $1 \times 10^{-6}$ | 12.6 ± 2.5 | 52[c] | 4.6 ± 0.8 | 46[c] |
| **10f** | $5 \times 10^{-6}$ | 7.8 ± 1.3 | 32[c] | 1.6 ± 0.2 | 16[c] |
| | $1 \times 10^{-6}$ | 13.3 ± 1.8 | 54[c] | 5.6 ± 0.8 | 55[c] |
| control | | 25.4 ± 3.1 | | 10.6 ± 1.9 | |
| **9g** | $5 \times 10^{-6}$ | 13.7 ± 2.8 | 54[c] | 8.4 ± 0.6 | 79 |
| | $1 \times 10^{-6}$ | 19.5 ± 4.5 | 77 | 10.5 ± 0.4 | 99 |
| **10f** | $5 \times 10^{-6}$ | 14.6 ± 1.3 | 57[c] | 5.8 ± 0.3 | 55[c] |
| | $1 \times 10^{-6}$ | 20.4 ± 3.6 | 80 | 1.3 ± 1.1 | 106 |

[a] Cell number based on Coulter counts on day 5, mean of six dishes ± SD. [b] Radioactivity/dish; mean of six dishes ± SD. [c] Significant inhibition ($p < 0.01$).

With estrogen receptor negative tumors, we did not observe a statistically significant inhibition of tumor growth following the administration of the 2-phenylindole-based complexes and their ligands (data not shown). In estrogen receptor positive tumors, only the complexes exerted a strong antineoplastic effect with one exception: Ligand **9d** at the higher dose inhibited the tumor growth by 69% (Table VI). All of the ligands tested were less effective than the corresponding complexes. Tumor masses were reduced over a six-week period of treatment by 60–87% in comparison to untreated animals. The therapeutic effect was rather similar to that of other treatment modalities like the administration of tamoxifen or cisplatin. The latter drug was applied in a subtoxic dose, whereas animals treated with 2-phenylindole-based complexes did not show toxic symptoms like decrease of body weight. Uterine weights were determined after treatment as a parameter of estrogenic response. There was generally no increase with regard to untreated control animals except for ligand **9d**, indicating an estrogenic effect. The decrease in uterine weight following the administration of tamoxifen and cisplatin is in accord with their antiestrogenic and cytostatic properties, respectively. A similar effect was observed with the most active complex **10f**. As expected the endocrine effects in the adult, tumor-bearing animals were much weaker than those in immature mice in the uterotrophic test (Table IV).

**Discussion.** The first goal of these investigations was the synthesis of *cis*-diaminodichloroplatinum(II) complexes with binding affinity for the estrogen receptor. In this study we accomplished it by connecting 2-(aminoethyl)-pyridine with 5-hydroxy-2-(4-hydroxyphenyl)indoles via an alkyl spacer group and subsequent conversion to the complex. Despite the rather large volume of the 2-(aminomethyl)pyridine group, the ligands bind to the estrogen receptor with RBA values close to that of potent synthetic estrogens like hexestrol. The comparison with analogous 1-alkyl-2-phenylindoles shows that the amino function contributes to the receptor binding.[7] After conversion to the complexes, the receptor affinity decreased, presumably due to the fact that the nitrogen atoms of the chelate have lost their basic character. The RBA values of the complexes were similar to those of 1-alkyl-substituted 2-phenylindoles.

Despite the high binding affinities for the estrogen receptor, the endocrine effects were rather weak. A partial estrogen antagonism was observed for some of the ligands. At higher doses, some of the complexes displayed significant estrogenic activity. These effects have to be considered for the mode of the antitumor action of the complexes. The specific cytostatic activity was determined in vitro and in vivo. In both systems, we used estrogen receptor positive and negative mammary tumor cells in order to distinguish between a receptor-mediated action and a general cytostatic effect. In tissue culture experiments, we recorded no activity in estrogen receptor negative MDA-MB 231 cells. However, hormone-sensitive MCF-7 cells responded to the treatment of both the complexes and

Table VI. Effect of Platinum Complexes 10c–g, Ligands 9c, 9d, and 9f, Cisplatin, Tamoxifen, and Ovariectomy on the Growth of Hormone-Dependent Transplanted MXT Mouse Mammary Tumors and Uterine Weights

| compd | dose,[a] mg | tumor weight,[b] mg | %T/C | uterotrophic effect,[b] %T/C |
|---|---|---|---|---|
| control | | 534 ± 652 | | 100 ± 30 |
| cisplatin | 1.5 | 64 ± 69 | 12[c] | 76 ± 29 |
| **9d** | 6.2 | 378 ± 413 | 71 | 119 ± 41 |
| | 12.4 | 169 ± 234 | 31[c] | 137 ± 35 |
| **10d** | 10.0 | 195 ± 300 | 36[d] | 92 ± 19 |
| | 20.0 | 121 ± 149 | 22[c] | 113 ± 33 |
| control | | 197 ± 130 | | 100 ± 24 |
| **9c** | 12.2 | 158 ± 195 | 80 | 78 ± 10 |
| **10c** | 20.0 | 80 ± 43 | 40[d] | 103 ± 12 |
| **9f** | 12.5 | 148 ± 106 | 75 | 96 ± 6 |
| **10f** | 20.0 | 25 ± 24 | 13[c] | 75 ± 11 |
| control | | 388 ± 269 | | 100 ± 24 |
| ovariectomy | | 48 ± 15 | 12[c] | |
| tamoxifen | 8.8 | 114 ± 100 | 29[c] | 78 ± 11 |
| **10e** | 20.0 | 86 ± 49 | 22[c] | 103 ± 16 |
| **10g** | 20.0 | 80 ± 37 | 21[c] | 104 ± 24 |

[a] Dose/kilogram of body weight, dissolved in PEG 400/1.8% saline (1:1) and administered three times per week sc. Doses of ligands are equimolar to those of the complexes. [b] Mean ± SD after 6 weeks of treatment; %T/C = tumor weights of treated animals/tumor weights of control animals (means) × 100. [c] Significant inhibition of tumor growth ($p < 0.01$). [d] Significant inhibition of tumor growth ($p < 0.05$).

the ligands. Usually the effect of the complexes was not very strong in vitro. Preliminary data of quantitative determinations of platinum by atomic absorption spectroscopy revealed very low levels of complexes inside these cells in comparison to cisplatin.

As in previous studies with complexes possessing 1,2-diaminoethane[11] or 1,3-diaminopropane[4] as the chelating function, the platinum complexes were generally more active in vivo than their ligands. In the case of estrogen receptor positive MXT mouse mammary carcinomas, they were able to compete with other treatment modalities like administration of tamoxifen, an established drug for the therapy of hormone dependent breast cancer. The inhibitory effect on estrogen receptor negative tumors was much weaker and not significant. From these results, we deduce that both the estrogen receptor and the platinum play a role in the antitumor action of these 2-phenylindole-based platinum complexes.

Simple platinum complexes like cisplatin are understood to exert their cytotoxic effect by binding to DNA of tumor cells, followed by a cross-linking reaction. The inability of the 2-phenylindole-linked platinum complexes to inhibit the growth of estrogen receptor negative cells like MDA-MB 231 and MXT-mammary tumor and P388 leukemia cells (data not shown) makes the estrogen receptor as primary target likely, although other hormone-independent cell lines have not yet been studied in this respect. After binding to the hormone binding site of the receptor several alternative ways of action have to be considered: (i) The complex bound to the receptor prevents the receptor from being activated or inhibits the dimerization of the receptor proteins. The latter effect is discussed for the pure estrogen antagonist ICI 164.384.[12] (ii) The platinum chelate interferes with the binding of the receptor to DNA and thereby inhibits the transcription of estrogen responsive elements on DNA. (iii) The platinum reacts with components of the DNA while bound to the receptor. (iv) Due to the 2-phenylindole moiety the complex acts as a hormonally active agent. Since pharmacological doses of both estrogens and antiestrogens inhibit the growth of hormone-dependent mammary tumors, one has to consider two different endocrine mechanisms. An estrogenic action as described for some platinum complexes based on the 1,2-diphenylethylene structure[13] is unlikely because we did not observe strong uterotrophic effects.

We assume that the complexes once they have reached the target cell, bind to the estrogen receptor and displace endogenous estrogens from their binding site. Because of the diaminoplatinum(II) group linked to the carrier, the receptor complex is no longer able to interact with the DNA in a way that induces transcriptional events. From a chemical point of view, this can either be due to the interaction of the platinum with nucleophilic centers of the DNA or to the inability of the receptor to adopt the right conformation for binding to DNA. At last, this would lead to an antiestrogenic effect on the growth of estrogen-dependent mammary tumor cells. Further biochemical studies are in progress to elucidate the effect of these complexes on the regulation of cell proliferation by estrogens.

## Experimental Section

Melting points were determined on a Büchi 510 apparatus and are uncorrected. Elemental analyses were performed by the Mikroanalytisches Laboratorium, University of Regensburg. NMR spectra were obtained on a Varian EM 360L and a Bruker WM 250 spectrometer and are consistent with the assigned structures. Field desorption (FD) mass spectra were recorded on a Varian MAT CH5 spectrometer. Positive ion fast-atom bombardment mass spectra (PIFAB) were determined on a Finnigan MAT 311A spectrometer with glycerol/DMSO used as matrix. The synthesis of the starting indoles 5-methoxy-2-(4-methoxyphenyl)indole (**1a**), 5-methoxy-2-(4-methoxyphenyl)-3-methylindole (**1b**), and 1-(ω-bromoalkyl)-2-phenylindoles (**2a–d**) have been described previously.[3]

**General Procedure for the Synthesis of 1-(Aminoalkyl)-2-phenylindoles 5.** A solution of the 1-(bromoalkyl)-2-phenylindole **2** (8.0 mmol) in 50 mL of dry DMF was added with stirring to a solution of potassium phthalimide (8.8 mmol) in dry DMF. This mixture was heated under reflux for 2 h with stirring. After cooling to room temperature, the mixiture was extracted three times with $CH_2Cl_2$ and water. After the organic layer was dried with $Na_2SO_4$, the solvent was evaporated in vacuo. The residue was purified by column chromatography on $SiO_2$ with $CH_2Cl_2$/EtOAc (10:1) as eluant and recrystallization from EtOH. The phthalimido derivatives **3** were obtained as colorless crystals; their melting points are reported in Table I.

For the preparation of the amines **5**, the phthalimides **3** were dissolved in 50 mL of EtOH and refluxed for 2 h with hydrazine hydrate in 20 mL of EtOH. After cooling to room temperature, the mixture was acidified with 2 N HCl to a pH of 2–3. The precipitate was filtered off. After the solvent was removed in vacuo, the residue was brought to a pH of 9–10 by addition of 2 N NaOH. After extraction with EtOAc, the organic layer was dried with $Na_2SO_4$. The solvent was evaporated and the residue purified by $SiO_2$ column chromatography with $CH_2Cl_2/NEt_3$ (5:1) as eluant. The products are colorless oils.

**1-(6-Cyanohexyl)-5-methoxy-2-(4-methoxyphenyl)-3-methylindole (4).** A solution of the 2-phenylindole (35 mmol) in 100 mL of dry DMA was added with stirring to a mixture of sodium hydride (40 mmol) in 50 mL of dry DMA at 0 °C. After the mixture was stirred for 30 min a solution of 7-bromoheptanonitrile in 30 mL of dry DMA was added and stirring was continued for 3 h at 0 °C. The excess of sodium hydride was

(12) Fawell, S. E.; White, R.; Lees, J. A.; Emmas, C. E.; Parker, M. G. In *Hormones and Cell Regulations*, No. 15; Dumont, J. E., Nunez, J., King, R. J. B., Eds.; John Libbey Eurotext: Paris, 1990; No. 15; pp 59–67 (Colloques INSERM, Vol. 210).

(13) Karl, J.; Gust, R.; Spruss, T.; Schneider, M. R.; Schönenberger, H.; Engel, J.; Wrobel, K.-H.; Lux, F.; Haeberlin, S. T. *J. Med. Chem.* 1988, *31*, 72.

destroyed with the addition of 30 mL of ice/water. After extraction with $CH_2Cl_2$ the organic layer was dried with $Na_2SO_4$. After evaporation of the solvent in vacuo, the residue was purified by $SiO_2$ column chromatography with $CH_2Cl_2$ as eluant. The product was obtained as a yellow oil in a yield of 67%: $^1H$ NMR ($CDCl_3$) $\delta$ 0.83–1.81 (m, 8 H, $(CH_2)_4$), 2.11 (t, $J$ = 7 Hz, 2 H, $CH_2CN$), 2.18 (s, 3 H, $CCH_3$), 3.89 (s, 6 H, $OCH_3$), 3.96 (t, $J$ = Hz, 2 H, $NCH_2$), 6.70–7.41 (m, 7 H, ArH).

**1-(7-Aminoheptyl)-5-methoxy-2-(4-methoxyphenyl)-3-methylindole (5e).** A solution of 4 (23 mmol) in 100 mL dry ether was added slowly with stirring to a suspension of $LiAlH_4$ in 50 mL of dry ether at 0 °C. The mixture was refluxed for 2 h. After the mixture was cooled in an ice bath the excess of $LiAlH_4$ was destroyed cautiously with the addition of 10 mL of ice/water. The ether layer was evaporated in vacuo, an the residue was poured into 50 mL of 2 N NaOH followed by extraction with $CH_2Cl_2$. After drying ($Na_2SO_4$) and evaporation of the solvent the residue was chromatographed on $SiO_2$ with $CH_2Cl_2/NEt_3$ (5:1) as eluant. The amine was obtained as a colorless oil in a yield of 66%.

**2-Propionylpyridine (6c).** A solution of 2-cyanopyridine (0.3 mmol) in 120 mL of dry ether was added slowly to an ice-cold solution of 0.37 mol EtMgBr in 200 mL of dry ether. After refluxing for 1 h and cooling, the mixture was acidified with 2 N HCl to obtain a solution. The aqueous phase was made alkaline (pH 9) with concentrated $NH_3$. After extraction with $CH_2Cl_2$, the organic layer was dried with $Na_2SO_4$. After evaporation of the solvent in vacuo, the residue was purified by fractional distillation (bp 40–45 6C at 0.1–0.01 Torr). The product was obtained as a colorless oil in a yield of 85%: $^1H$ NMR ($CDCl_3$) $\delta$ 1.19 (t, $J$ = 8 Hz, 3 H, $CH_2CH_3$), 3.24 (q, $J$ = 8 Hz, 2 H, $CH_2CH_3$), 7.25–8.17 (m, 3 H, H-5,3,4 (pyridine)), 8.68 (d, $J$ = 8 Hz, 1 H, H-6 (pyridine)).

**General Procedure for the Synthesis of 2-[[[ω-(2-phenylindol-1-yl)alkyl]amino]methyl]pyridines 8.** A solution of pyridine-2-aldehyde (**6a**), 2-acetylpyridine (**6b**), or 2-propionylpyridine (**6c**) (10 mmol) and 1-(aminoalkyl)-2-phenylindole **5** (11 mmol) in 60 mL of dry benzene was refluxed in a Soxhlet apparatus with anhydrous $CaSO_4$ for 24 h. After evaporation of the solvent in vacuo, the product was diluted with ether and dried with $Na_2SO_4$. After microdistillation in vacuo, the respective imines **7** were obtained as yellow oils. Without further purification, they were dissolved in 40 mL of MeOH and treated with $NaBH_4$ (12 mmol) which was added in portions. After 2 h, the reaction mixture was stirred at 50 °C for 12 h. After removal of the solvent in vacuo, the residue was suspended in 20 mL of water and extracted three times with 50 mL of ether and dried with $Na_2SO_4$. Unreacted imine was removed by microdistillation in vacuo. The products were obtained as remaining residues of the distillation and formed yellow oils.

**General Procedure for the Ether Cleavage.** A solution of the methoxy-substituted indole 8 (4.0 mmol) in dry $CH_2Cl_2$ (100 mL) was cooled to −60 °C under a nitrogen atmosphere and $BBr_3$ (10 mmol) in 10 mL of dry $CH_2Cl_2$ was added slowly with vigorous stirring. After removal of the cooling bath, the mixture was stirred overnight, followed by heating at reflux for 2 h. Under a nitrogen atmosphere and cooling with an ice bath, MeOH was added dropwise until the vigorous reaction ceased. After evaporation of the solvent, the residue was treated with a saturated $NaHCO_3$ solution. The crude product was removed by filtration and extracted for 5 h with 200 mL of $NEt_3$ in a Soxhlet apparatus. The hot solution was filtered and the solvent removed in vacuo. The pure product was obtained by resuspension in water and filtration. The yields ranged from 20 to 50%. The melting points are reported in Table II.

**General Procedure for the Preparation of the Dichloroplatinum(II) Complexes 10.** A solution of $K_2PtCl_4$ (1.01 mmol) in 10 mL of a mixture of DMF and water (5:2; v/v) was added slowly to a warm (40 °C) solution of the (aminoalkyl)indole (1.01 mmol) in 20 mL of DMF. The mixture, exhibiting a pH of 9–10, was gently stirred in the dark at 30 °C for 3–5 days until the pH had reached 4–5. After addition of one drop of DMSO, stirring was continued for 2 h, followed by removal of the solvent in vacuo. The oily residue was suspended in a saturated KCl solution, filtered, and washed with EtOH and water. For further purification, the product was dissolved in a small volume of DMF and precipitated with EtOH/water (1:1) or water alone. After filtration the crystalline product was dried for several days. Sometimes this procedure for purification had to be repeated. All of the complexes do not melt but decompose at higher temperature (see Table III).

**Biochemical and Biological Methods. Reagents.** [2,4,6,7-$^3H$]Estradiol (110 Ci/mmol) was obtained from New England Nuclear, Dreieich, FRG. Hormones and biochemicals were purchased from Sigma, München, FRG. TEA [Tris buffer (0.01 M, pH 7.5) supplemented with EDTA (0.01 M) and $NaN_3$ (0.003 M)] was used as buffer.

**Estradiol Receptor Binding Assay.** Fresh calf uteri, stored in ice-cold saline, were freed of adherent fat and connective tissue at 4 °C. After addition of TEA buffer (1 mL/g), the uteri were homogenized by treatment with an ultraturrax mixer (IKA, FRG) and a glass-in-glass homogenizer (Potter S; Braun, FRG) at 4 °C. Lipids were separated by centrifugation at 700$g$ and discarded. The homogenate was centrifuged at 105000$g$ for 1 h (0 °C). The supernatant (cytosol) was then used for determining the affinity of compounds for the estrogen receptor. The protein concentration of the cytosol was ca. 15 mg/mL, leading to a final concentration of 3 mg/mL in the assay.

For the determination of the relative binding affinity (RBA), the previously described procedure was applied with modifications.[6] The 500-μL incubation mixture was comprised of 5 nM [$^3H$]17β-estradiol (added in 100 μL of TEA), $10^{-9}$ to $10^{-5}$ M competing ligand (in 100 μL of TEA), 100 L of uterine cytosol, and TEA. The mixture was incubated for 18 h at 4 °C, and then 0.5 mL of dextran-coated charcoal (DCC) slurry (0.8% charcoal Norit A and 0.008% dextran in TEA) was added to the tubes, and the contents were mixed. The tubes were incubated for 90 min at 4 °C and then centrifuged at 700$g$ for 10 min to pellet the charcoal. An aliquot (100 μL) of the supernatant was removed, and the radioactivity was determined by liquid scintillation spectrometry after addition of 2 mL of Quickszint 212 (Zinsser). Nonspecific binding was calculated by using 4 μM 17β-estradiol as competing ligand. Six concentrations of competitor ((1,2,5 and 10) × $10^{-9}$ to $10^{-6}$) were chosen to provide values between 10 and 90% of specifically bound radioactivity. Radioactivity was plotted as a function of the log concentration of competing ligand in the assay. The RBA was calculated as the ratio of the molar concentrations of estradiol and test compound required to decrease the amount of bound radioactivity by 50%, multiplied by 100.

**Immature Mice Uterine Weight Tests.** Immature female mice (20 days old, of the NMRI strain) from Ivanovas, Kisslegg, FRG, were randomly divided into groups of 6–10 animals. To determine estrogenic activity, compounds were dissolved in polyethylene glycol/1.8% saline (1:1; 100 μL/animal) and injected subcutaneously on three consecutive days. Control animals received the vehicle alone. The animals were killed by cervical dislocation and weighed 24 h after the last injection. Uteri were dissected free of fat and fixed in Bouin solution (saturated aqueous picric acid/40% formaldehyde/glacial acetic acid 15:5:1 by volume) for 2 h. Uteri were freed from connective tissue, washed with saturated alcoholic solution of LiCl, dried at 100 °C for 24 h, and weighed. The relative uterus weight was calculated by the formula: (uterine dry weight (mg)/body weight (g)) × 100.

To determine the antiestrogenic activity, injections contained a standard dose (0.4 μg) of estrone and increasing doses of the complexes or the ligands. The inhibition (%) of the estrone-stimulated uterine growth was estimated by the formula: $100 - [(W_{S,T} - W_V)/(W_S - W_V) \times 100]$ ($W_{S,T}$ = relative uterus weight of animals treated with estrone standard (0.4 g) + test compound; $W_V$ = relative uterus weight of control animals; $W_S$ = relative uterus weight of animals treated with estrone standard).

**MCF-7 Human Breast Cancer Cells.** The MCF-7 cell line was obtained from the American Type Culture Collection (ATCC), Rockville, MD. Cells were grown in improved minimal essential medium (MEM), as modified by Richter et al.[14] (Biochrom, Berlin, FRG), supplemented with glutamine (0.3 g/L), gentamycin (60 mg/L), and 5% newborn calf serum (NCS) (Gibco) or charcoal-treated NCC (CCS). CCS was prepared by incubation of 500 mL of NCS with a dextran-coated charcoal pellet[15] for 4 h in a

(14) Richter, A.; Sandford, K. K.; Evans, V. J. *J. Natl. Canc. Inst.* **1972**, *49*, 1705.

shaker at 0-4 °C. The procedure was repeated with a fresh pellet. After each incubation, the charcoal was removed by centrifugation. The serum was sterilized through a 0.20-μm filter (Sartorius, Göttingen, FRG) and stored at -20 °C. All of the experiments were performed in the presence of phenol red.

Cells were grown in a humidified incubator in 5% $CO_2$ at 37 °C. Two weeks before start of the experiment, cells were switched from NCS to CCS and received two additional media changes before they were harvested with 0.05% trypsin/0.2% EDTA in 0.15 M NaCl. They were syringed gently to prevent clumping, and approximately $2 \times 10^4$ cells in 2 mL were plated replicately in six-well dishes (Costar). Cells were switched 2 days later to a medium containing the substances and 0.1% DMF in which the compounds had been dissolved. The medium of control wells contained an equal volume of DMF. On the 4th day, media were changed and substances added again. Cells were labeled 3 days later with 1 μCi [$^3$H]thymidine per well for 2 h. Cells were washed with cold PBS and harvested in PBS containing 0.02% EDTA. After centrifugation, the cell pellet was resuspended in 1 mL of PBS and divided in two equal parts. One part was counted in a Z I Coulter Counter the other one was sonicated. After addition of 4 mL of 10% trichloroacetic acid (TCA), the acid-insoluble fraction was collected on a 0.45-μm filter (Metricel, Gelman) and counted after addition of 10 mL of scintillation liquid (Quickszint 212, Zinsser) in a LS 1801 Beckman scintillation counter.

**MDA-MB 231 Human Breast Cancer Cells.** The MDA-MB 231 cell line was also obtained from ATCC. Cells were grown in McCoy 5a medium (Boehringer Mannheim, FRG) supplemented with 10% NCS and gentamycin (40 μg/mL). The experiments were performed as described for the MCF-7 cells with one exception: the incubation period was reduced from 5 to 2 days because the cell population doubling time is less than half of that of MCF-7 cells (16 vs 36 h[16]).

**Transplanted MXT-Mammary Tumors of the Mouse.** The MXT-mammary tumors were generously provided by Dr. A. E. Bogden, EG & G Mason Research Institute, Worcester, MA and Dr. G. Leclercq, Institute Jules Bordet, Brussels, Belgium. Hormone-sensitive tumors grew for 4-5 weeks in the host animals before transplantation. Tumor pieces of 1 mm$^2$ were serially transplanted into 8-9-week-old female B6D2F1 mice, obtained from Charles-River-Wiga, Sulzfeld, FRG. Animals were assigned randomly in groups of 10 and treatment was started 24 h after transplantation. Drugs were dissolved or suspended in polyethylene glycol 400/1.8% saline (1:1) and administered subcutaneously on Monday, Wednesday, and Friday. After a 6-week period of treatment, animals were killed and autopsied. Tumors were removed and weighed. The uterine dry weight was determined as described above. The change of body weight between start and end of therapy was recorded in order to detect obvious toxicity.

Hormone-resistant tumors were kept in ovariectomized B2D2F1 mice. Treatment was started 24 h after transplantation and lasted 2 weeks. The administration scheme was the same one as outlined for hormone-sensitive tumors. Since the hormone-resistant tumors can not be dissected free of connective tissue, the tumor area was determined instead of tumor weight. The tumor area was obtained by transdermal caliper measurements of two perpendicular axes, one across the largest diameter.

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**Supplementary Material Available:** $^1$H NMR data of 1-(phthalimidoalkyl)-2-phenylindoles (**3a-d**), 1-(aminoalkyl)-2-phenylindoles (**5a-e**), 1-[ω-[(2-pyridylmethylene)amino]alkyl]-2-phenylindoles (**7a-g**), 5-methoxy-2-(4-methoxyphenyl)-1-[ω-[(2-pyridylmethyl)amino]alkyl]indoles (**8a-g**), 5-hydroxy-2-(4-hydroxyphenyl)-1-[ω-[(2-pyridylmethyl)amino]alkyl]indoles (**9a-g**), and [5-hydroxy-2-(4-hydroxyphenyl)-1-[ω-[(2-pyridylmethyl)amino]alkyl]indole]dichloroplatinum(II) complexes (**10a-g**) (9 pages). Ordering information is given on any current masthead page.

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# 3-*O*-Alkylascorbic Acids as Free-Radical Quenchers: Synthesis and Inhibitory Effect on Lipid Peroxidation

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A novel series of 3-*O*-alkylascorbic acids (3-RASA, **3a-n**) was synthesized to act as radical scavengers for active oxygen species and free radicals, and their redox potentials and inhibitory effects on lipid peroxidation in rat liver microsomes were evaluated. The redox potentials of the 3-RASA compounds were increased by the substituent group to 90-190 mV above the potential for ascorbic acid (i.e., 3-RASA compounds were harder to oxidize). Although 3-*O*-dodecylascorbic acid (**3c**) and 3-*O*-(decylcarbomethyl)ascorbic acid (**3i**) differed in their redox potentials, they both markedly inhibited lipid peroxidation in rat liver microsomes to a similar extent ($IC_{50}$ = 3.1 and 3.3 × $10^{-6}$ M, respectively). Structure-activity relationship studies demonstrated that the anti lipid peroxidation activity of the 3-RASA compounds was markedly dependent upon their hydrophobicity.

## Introduction

It is clear that active oxygen species (AOS, such as superoxide, •OH, •OOH, etc.), as well as the free radicals derived from the biochemical utilization of $O_2$ or the prooxidant stimulation of $O_2$ metabolism, participate in the development or exacerbation of various diseases: e.g., ischemia-reperfusion disturbances in the brain and heart, rheumatism, inflammatory disorders, gastric ulcer, and cancer.[1]

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