Synthesis of $PtCl_2$ Complexes of Pyridylmethylamine Ligands and their Activity Against Hormone-dependent Mammary Carcinoma

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Abstract

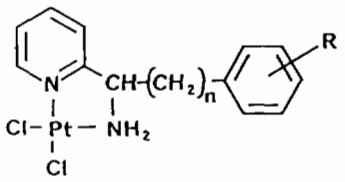
Fifteen ligands of the pyridylmethylamine type and their $PtCl_2$ complexes were prepared, characterized and subjected to a screening system for evaluation of a specific activity against the hormone-dependent mammary carcinoma. To test the estrogenic activity of the ligands, the relative binding affinity (RBA, *in vitro*) and the uterotrophic and antiuterotrophic activities (mouse uterine weight test, *in vivo*) were determined. To study the cytotoxic effects, the $PtCl_2$ complexes were tested *in vitro* towards the hormone-independent human mammary carcinoma cell line MDA-MB 231 and *in vivo* towards the lymphocytic P 388 leukemia. Finally, there was a comparative test of ligands and $PtCl_2$ complexes in the hormone-independent MXT (3.2) Ovex mammary carcinoma and the hormone-dependent MXT M3.2 mammary carcinoma.

Introduction

In a previous paper we reported on the synthesis and *in vitro* antitumor activity of $PtCl_2$ complexes of pyridine- and quinoline-amine and -imine ligands and of carbocyclic ethylenediamine ligands [1]. While continuing this work [2], a Japanese patent on $PtCl_2$ complexes of picolylamine and *N*-methyl-picolylamine appeared [3]. Therefore we specialized on pyridylamine compounds with a specific activity against the hormone-dependent mammary carcinoma. The results of this study are summarized in the present paper [2].

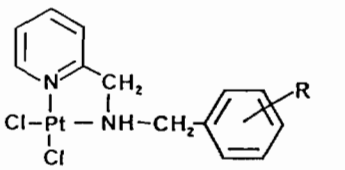
Synthesis of Ligands and Complexes

The $PtCl_2$ complexes prepared in the present study are shown in Schemes 1, 2, and 3.



	R	
1a–$PtCl_2$	4-OH	$n = 0$
1b–$PtCl_2$	4-CH_2-(C_6H_4-4′-OH)	$n = 0$
1c–$PtCl_2$	4-OH	$n = 1$
1d–$PtCl_2$	2.6-Cl_2-4-OH	$n = 1$
1e–$PtCl_2$	2.6-Me_2-4-OH	$n = 1$

Scheme 1.



	R
2a–$PtCl_2$	4-OH
2b–$PtCl_2$	2.6-Cl_2-4-OH

Scheme 2.

The ligands **1a**–**e** were obtained via the route pyridyl ketone, oxime, primary amine [2]. Starting with 2-cyanopyridine, the pyridyl ketones were synthesized either by the normal Grignard procedure or an 'inverse' approach, in which the Grignard was added to the 2-cyanopyridine solution. A third possibility was acylation of benzyl bromides with 2-pyridine-trimethylsiloxyacetonitrile. This readily accessible adduct of 2-pyridinealdehyde and Me_3SiCN was deprotonated by lithium diisopropylamine. The anion was reacted with benzyl bromides. When the protective trimethylsiloxy group was removed, the corresponding ketones were produced in good yields. The ketones were converted into their oximes by reaction with hydroxylammoniumhydrochloride. The

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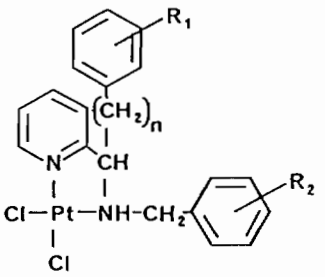

	R_1	R_2	
3a–$PtCl_2$	H	2.6-Cl_2-4-OH	$n = 0$
3b–$PtCl_2$	4-OH	2.6-Cl_2-4-OH	
3c–$PtCl_2$	4-OH	4-OH	$n = 1$
3d–$PtCl_2$	2.6-Cl_2	2.6-Cl_2-4-OH	
3e–$PtCl_2$	4-OH	2.6-Cl_2	
3f–$PtCl_2$	2.6-Cl_2-4-OH	2.6-Cl_2-4-OH	
3g–$PtCl_2$	2.6-Cl_2-4-OH	2.6-Me_2-4-OH	
3h–$PtCl_2$	H	2.6-Cl_2-4-OH	

Scheme 3.

primary amines were obtained by reduction of these oximes with Zn dust in refluxing ethanol/ammonia (25%).

The ligands **2a, b** were prepared by Schiff base condensation of 2-picolylamine with the corresponding carbonyl compounds and consecutive reduction of the imines to the secondary amines by $NaBH_4$ [2].

The ligands **3a–h** were synthesized via pyridyl ketone, oxime, primary amine, imine, secondary amine [2]. Up to the primary amines, the procedures used in the preparation of **1a–e** were applied. The next two steps, the conversion of the primary amines into imines and secondary amines were carried out analogously to **2a, b**. The imines to give **3a, b** were reduced by $LiAlH_4$ using an 'inverse' procedure.

Finally, the *p*-methoxy groups present in all the compounds were converted into hydroxy groups by ether cleavage. BCl_3 was used for the ligands **1a–e** and **2a, b** and BBr_3 for all the others [2]. All the

TABLE 1. Formulas, Melting Points and Elemental Analyses of Ligands

Ligand	Formula (molecular weight)	Melting point (°C)	Analyses: calculated (found) (%)		
			C	H	N
1a	$C_{13}H_{12}N_2O$ (200.24)	136–137	71.92 (71.60)	6.04 (6.11)	13.99 (13.47)
1b	$C_{19}H_{18}N_2O$ (290.35)	172	50.46 (49.97)	4.46 (4.58)	6.20 (6.06)
1c	$C_{13}H_{14}N_2O$ (214.16)	145	72.87 (72.12)	6.59 (6.52)	13.08 (12.85)
1d	$C_{13}H_{12}Cl_2N_2O$ (445.0)	225[a]	35.08 (34.87)	3.17 (3.45)	6.29 (6.15)
1e	$C_{15}H_{18}N_2O$ (242.32)	178	74.34 (73.32)	7.49 (7.41)	11.34 (11.34)
2a	$C_{13}H_{14}N_2O$ (214.26)	74	72.86 (72.60)	6.59 (6.61)	13.07 (13.01)
2b	$C_{13}H_{12}Cl_2N_2O$ (283.16)	115–116	55.14 (55.11)	4.27 (4.25)	9.90 (9.90)
3a	$C_{19}H_{16}Cl_2N_2O$ (359.25)	156–157	63.52 (62.96)	4.49 (4.68)	7.80 (7.54)
3b	$C_{19}H_{16}Cl_2N_2O_2$ (375.25)	182	60.81 (60.82)	4.30 (4.70)	7.47 (7.10)
3c	$C_{20}H_{20}N_2O_2$ (320.38)	90	74.97 (74.96)	6.29 (6.19)	8.74 (8.48)
3d	$C_{20}H_{16}Cl_4N_2O$ (442.16)	149	54.32 (53.81)	3.64 (3.73)	6.34 (6.05)
3e	$C_{20}H_{18}Cl_2N_2O$ (372)	171	65.57 (63.61)	4.87 (4.93)	7.53 (7.21)
3f	$C_{20}H_{16}Cl_4N_2O_2$ (458.18)	152[a]	52.42 (51.77)	3.52 (3.59)	6.15 (5.80)
3g	$C_{22}H_{22}Cl_2N_2O_2$ (579.17)	139[a]	45.62 (44.14)	4.17 (4.41)	4.84 (4.00)
3h	$C_{20}H_{18}Cl_2N_2O$ (535.1)	172[a]	44.88 (44.77)	3.76 (3.94)	5.24 (4.85)

[a] Dihydrobromide.

chiral ligands **1a–e** and **3a–h** were applied as racemic mixtures.

The ligands **1a–e**, **2a, b**, and **3a–h** were transformed into their $PtCl_2$ complexes with K_2PtCl_4 by adaption of known procedures (Schemes 1–3) [2, 4].

The properties and the spectral and analytical characterization of the ligands and complexes are given in Tables 1–3. All the complexes are insoluble in water. **(1a–e)–$PtCl_2$**, **3b–$PtCl_2$** and **3e–$PtCl_2$** are soluble in DMF and **(1a–e)–$PtCl_2$**, **3b–$PtCl_2$**, **3f–$PtCl_2$**, and **3h–$PtCl_2$** in a 1:1 mixture of polyethyleneglycol 400/1.8% aqueous NaCl solution.

Biological Properties

The binding affinities of the ligands and Pt complexes for the estrogen receptor ER were measured by a competitive binding assay using the dextran-coated charcoal technique (competitor: $[^3H]E_2$, E_2 = 17-β-estradiol, ER source: calf uterine cytosol) [5]. The relative binding affinity (*RBA*) was evaluated as the ratio of the molar concentrations of E_2 and inhibitor required to decrease the receptor-bound $[^3H]E_2$ by 50%, multiplied by 100. The most active compounds were the ligands **3b** (1.0), **3f** (1.20) and **3g** (1.15). Indeed, these *RBA* values were low compared to those of real estrogens such as estradiol (*RBA* = 100). The other ligands or complexes showed a still weaker affinity or were completely inactive. Remarkably, appreciable binding affinities for the ER were observed only when at least one of two aromatic rings was 2.6-Cl_2-4-OH substituted (ligands **3a, b, 3d, 3f–h**). Compared to the ligands, the corresponding dichloroplatinum(II) complexes had smaller *RBA* values (Table 4).

In the mouse uterine weight test [6] the ligands **3a** (51%) and **3b** (50%) showed medium estrogenic effects. Whereas the ligands **3d** (92%) and **3f** (75%) almost reached the maximum effect of estrone

TABLE 2. Formulas and Elemental Analysis of $PtCl_2$ Complexes

Complex	Formula (molecular weight)	Analyses: calculated (found) (%)		
		C	H	N
1a–$PtCl_2$	$C_{12}H_{12}Cl_2N_2OPt$ (466.23)	30.91 (30.63)	2.59 (2.88)	6.01 (5.68)
1b–$PtCl_2$	$C_{19}H_{18}Cl_2N_2OPt$ (556.34)	41.02 (41.52)	3.26 (3.81)	5.04 (5.31)
1c–$PtCl_2$	$C_{13}H_{14}Cl_2N_2OPt$ (480.25)	32.51 (32.13)	2.94 (3.16)	5.83 (5.35)
1d–$PtCl_2$	$C_{13}H_{12}Cl_4N_2OPt$ (549.15)	28.43 (28.54)	2.20 (2.35)	5.10 (5.14)
1e–$PtCl_2$	$C_{15}H_{18}Cl_2N_2OPt$ (508.31)	35.44 (35.36)	3.60 (3.61)	5.51 (5.41)
2a–$PtCl_2$	$C_{13}H_{14}Cl_2N_2OPt$ (480.15)	32.52 (32.67)	2.93 (3.01)	5.83 (5.77)
2b–$PtCl_2$	$C_{13}H_{12}Cl_4N_2OPt$ (549.31)	28.42 (28.73)	2.20 (2.34)	5.10 (5.08)
3a–$PtCl_2$	$C_{19}H_{16}Cl_4N_2O_2Pt$ (625.24)	36.50 (36.51)	2.60 (3.09)	4.48 (3.97)
3b–$PtCl_2$	$C_{19}H_{16}Cl_4N_2O_2Pt$ (641.40)	35.57 (35.15)	2.51 (2.91)	4.36 (4.61)
3c–$PtCl_2$	$C_{20}H_{20}Cl_2N_2O_2Pt$ (586.37)	40.69 (40.30)	3.44 (3.48)	4.77 (4.75)
3d–$PtCl_2$	$C_{20}H_{16}Cl_6N_2OPt$ (708.15)	33.91 (33.64)	2.28 (2.52)	3.96 (4.09)
3e–$PtCl_2$	$C_{20}H_{18}Cl_4N_2OPt$ (638.26)	37.63 (37.28)	2.84 (3.05)	4.39 (4.42)
3f–$PtCl_2$	$C_{20}H_{16}Cl_6N_{22}Pt$ (724.32)	33.17 (33.64)	2.23 (2.66)	3.87 (3.62)
3g–$PtCl_2$	$C_{20}H_{22}Cl_4N_2O_2Pt$ (683.32)	38.67 (38.36)	3.24 (3.57)	4.10 (4.28)
3h–$PtCl_2$	$C_{20}H_{18}Cl_4N_2OPt$ (639.19)	37.60 (37.80)	2.83 (2.92)	4.38 (4.33)

TABLE 3. ^{1}H NMR Parameters (δ values in ppm) of Ligands, 250 MHz (DMSO-d_6, i-TMS) and selected $PtCl_2$ Complexes, 250 MHz (DMF-d_7, i-TMS) (for coupling constants see ref. 2)

Compound	Py–H	Py–C*H*	CH–CH_2 | Py	NH–CH_2 | Ph	Aryl–H	Other protons
1a	[a]	5.63(s)			7.29–6.74	3.4(br, NH_2)
1a–$PtCl_2$	[b]	5.65(t)			7.66–6.89	9.9(br, OH) 6.1(br, NH_2)
1b	[a]	5.10(s)			7.27–7.05 6.96–6.64	9.2(br, OH) 3.80(CH_2) 3.4(br, NH_2)
1b–$PtCl_2$	[b]	5.20(t)			7.42–7.10 7.20–6.88	10.2(br, OH) 4.10(s, CH_2)
1c	[a]	4.00(dd)	2.90(AB) 2.67(AB)		6.87–6.58	9.1(br, OH) 1.7(br, NH_2)
1c–$PtCl_2$	[b]	5.35(m)	3.50(m)		7.15–6.78	9.6(br, OH) 6.4(br, NH_2)
1d	[a]	4.16(dd)	3.25(AB) 3.11(AB)		6.79(s)	9.0(br, OH) 6.5(br, NH_2)
1d–$PtCl_2$	[b]	5.60(m)	3.30(m)		7.15(s)	10.5(br, OH) 6.8(br, NH_2)
1e	[a]	4.05(t)	2.78(d)		6.35(s)	8.9(br, OH) 2.05(s, CH_3) 2.0(br, NH_2)
1e–$PtCl_2$[b]	[b]	4.50(m)	3.55(d)		6.48(s)	9.28(s, OH) 6.1(br, NH_2) 1.95(s, CH_3)
2a	[c]	3.95(s)		3.70(s)	7.20–6.48	5.7(br, NH_2, OH)
2a–$PtCl_2$	[d]	4.20(m)		4.26(m)	7.49–6.77	9.72(s, OH) 7.1(br, NH_2)
2b	[c]	4.46(s)		4.37(s)	6.98(s)	9.4(br, OH) 5.4(br, NH_2)
2b–$PtCl_2$	[d]	4.80(m)		4.40(m)	6.95(s)	10.0(br, OH) 6.8(br, NH_2)
3a	[e]	4.91(s)		3.77(s)	7.44–7.19 6.80(s, 2H)	9.2(br, OH)
3a–$PtCl_2$	[f]	5.50(s)		4.70(m)	7.60–7.44 7.05(s, 2H)	11.2(br, OH) 5.4(br, NH_2)
3b	[e]	4.78(s)		3.73(s)	7.20–6.66 6.81(s, 2H)	10.1/9.2 (br, OH) 2.7(br, NH_2)
3b–$PtCl_2$	[f]	5.32(s)		4.60(m)	7.31–6.87 7.06(s, 2H)	10.5/9.7 (br, OH)
3c	[e]	4.66(dd)	3.20(AB) 2.96(AB)	3.93(AB) 3.75(AB)	7.22–6.75 6.70–6.54	9.61(s, OH) 9.22(s, OH) 9.1(br, NH_2)
3d	[e]	4.10(t)	3.30(m)	3.70(m)	7.31–7.18 6.68(s, 2H)	9.5(br, OH)

(continued)

TABLE 3. *(continued)*

Compound	Py–H	Py–C*H*	CH–CH_2 | Py	NH–CH_2 | Ph	Aryl–H	Other protons
3e[g]	e	4.80(dd)	3.49(AB) 3.18(AB)	4.24(AB) 4.08(AB)	7.60–7.29 6.74–6.55	9.5(br, OH) 6.2(br, NH_2)
3e–$PtCl_2$	f	4.45(m)	3.20(m)	4.55(m)	7.12–6.75	9.64(s, OH) 5.1(br, NH_2)
3f	e	4.73(dd)	3.58(AB) 3.40(AB)	4.25(AB) 4.15(AB)	6.92(s) 6.76(s)	9.9(br, OH)
3g	e	4.05(m)	3.48(m)	3.35(m)	6.76(s) 6.50(s)	9.2(br, OH) 2.29(s, CH_3)
3h	e	4.85(dd)	3.66(AB) 3.54(AB)	4.29(AB) 3.95(AB)	7.20–7.08 6.91(s, 2H)	9.4(br, OH) 5.9(br, NH)

[a]8.60–7.10. [b]9.35–6.85. [c]8.75–7.55. [d]9.25–7.50. [e]8.75–7.00. [f]9.45–7.70. [g]7.60–7.29 (3H).

TABLE 4. Estrogenic/Antiestrogenic Activities (*EA*/*AA*) of Ligands and Binding Affinities (*RBA*) of Selected Ligands and Complexes

Ligand	Dose (mol/animal)	*EA* (%)	*AA* (%)	*RBA* value (ligand; ligand–$PtCl_2$)
1c	10^{-6}	16	15	
1d	10^{-6}	19		
	10^{-7}	22		
1e	10^{-6}		18	
	10^{-8}	14		
2a	10^{-6}		16	
2b	10^{-8}	20	16	
3a	10^{-6}	51		0.33; 0.20
	10^{-7}	21		
	10^{-8}	26		
3b	10^{-7}	10		1.0; 0.33
	10^{-8}	50		
3c	10^{-6}	17		
	10^{-7}	15		
	10^{-8}	10		
3d	10^{-6}	92		0.10
	10^{-7}	56		
3e	10^{-6}	15		0.03
	10^{-6}	33		
3f	10^{-6}	75		1.20; 0.40
	10^{-7}	33		
3g	10^{-6}	33		1.15; 0.38
	10^{-7}	37		
	10^{-8}	39		
3h				0.28

(100%). Since it is known from previous studies [7] that low estrogenicity (*EA*) is often accompanied by antiestrogenic activity (*AA*), the antiuterotrophic effect of the compounds was determined by simultaneous administration of 0.4 μg of estrone (E_1). No significant antiestrogenic activity was found. In both tests only the ligands but not the complexes were examined (Table 4).

In the next study, it was investigated whether the platinum complexes possess a cytotoxic effect. For that purpose the complexes were tested against the hormone-independent human mammary carcinoma cell line MDA-MB 231. Cell growth inhibition was monitored by measuring the decreasing optically density [8]. The most active compounds were **1d**–**$PtCl_2$** and **1e**–**$PtCl_2$**. (**3f–h**)–**$PtCl_2$** had lower activities (Table 5).

The lymphocytic leukemia P 388 of the mouse, which is known to be very sensitive to platinum complexes, was used as *in vivo* test model [9]. In this tumor model, **1a**–**$PtCl_2$** and (**1d–e**)–**$PtCl_2$** exhibited similar antitumor activities (*T*/*C*: 155, 170 and 150%), but did not reach the *T*/*C* value of cisplatinum (200%). The complexes (**3c**–h)–**$PtCl_2$** were marginally active and the complexes (**1a, b, 2a, b**, and **3a, b**)–**$PtCl_2$** inactive (Table 5). The tests confirmed other studies in which compounds with primary amine groups were more active than higher substituted ones [10]. A comparative *in vivo* study of all platinum complexes and their ligands was performed with the hormone-dependent and -independent MXT mammary carcinoma of the mouse [11] using an equimolar dosage (2×10^{-5} mol/kg). The studies (Table 6) gave the following results with regard to the hormone-independent model: (i) (**1c–e**)–**$PtCl_2$** and **3c**–**$PtCl_2$** showed activity against tumor growth; (ii)

TABLE 5. Cell Growth Inhibition of MDA-MB 231 Cells (*in vitro*) and Activity Against P 388 Leukemia Cells (*in vivo*)

$PtCl_2$	c (mol/kg) $\times 10^{-6}$	T/C (%)	c (mol/kg) $\times 10^{-5}$	Median survival time (d)	T/C (%)	Changes in weight $d_1 - d_5$
1a–			1	10.0	110	−0.7
			2	10.5	116	−0.7
			4	14.0	155	−0.3
1b–	10	73.1 ± 10.0	1	12.0	120	+0.6
	5	95.5 ± 13.8	2	13.0	130	+0.5
	1	109.3 ± 15.2	4	12.0	120	+0.4
1c–	10	64.0 ± 8.9	1	10.0	100	+0.5
	5	68.6 ± 12.5	2	10.5	105	+0.1
	1	101.8 ± 15.4	4	11.5	115	+0.1
1d–	10	34.2 ± 11.1				
	5	71.1 ± 15.0	2	15.5	163	−0.4
	1	114.5 ± 22.5	4	16.0	170	−0.4
1e–	10	33.8 ± 22	1	12.0	120	−0.8
	5	58.2 ± 21.6	2	11.5	115	−0.6
	1	101.1 ± 28.8	4	15.0	150	−0.1
2a–			1	9.0	100	−1.8
			2	9.5	105	−1.5
			4	10.0	110	−1.5
2b–			1	10.0	111	−0.7
			2	9.5	105	−0.8
			4	10.0	111	−0.9
3a–	10	80.5 ± 23.1	1	10.5	105	+0.8
	5	92.3 ± 16.6	2	11.5	115	+0.9
	1	99.3 ± 18.4	4	11.0	110	+0.5
3b–	10	104.2 ± 23.9	1	9.5	105	−1.6
	5	106.5 ± 16.4	2	10.5	110	−2.0
	1	116.8 ± 19.9	4	9.5	105	−2.2
3c–	10	108.1 ± 26.2				
	5	113.7 ± 24.6	2	11.5	120	−0.6
	1	113.4 ± 24.8	4	13.0	136	−0.8
3d–	10	79.8 ± 11.1				
	5	95.0 ± 16.3	2	11.0	115	−1.1
	1	97.3 ± 16.6	4	13.5	142	−1.4
3e–			2	11.5	120	−0.6
			4	13.0	136	−0.6
3f–	10	58.2 ± 10.4				
	5	70.8 ± 10.9	2	13.5	142	−0.7
	1	88.2 ± 13.6	4	13.5	142	−1.4
3g–	10	53.4 ± 9.0				
	5	80.3 ± 14.8	2	11.0	115	+1.2
	1	104.9 ± 23.6	4	12.5	131	−0.6
3h–	10	49.7 ± 11.0				
	5	95.7 ± 19.3	2	11.0	115	−0.8
	1	117.6 ± 19.6	4	13.5	142	−0.8

within the ligand series only **3a** caused a small inhibition of the tumor growth. The results in view of the hormone-dependent model (Table 6) showed: (i) (**2a**, **3a–d**, **f**, **g**)–$PtCl_2$ and their ligands were able to inhibit the tumor growth; (ii) ligands **1a**, **b** and the corresponding complexes were completely inactive; (iii) (**1c–e**)–$PtCl_2$ and (**2b**)–$PtCl_2$ showed activity against cancer growth, but not their ligands; (iv) tumor inhibition was increased by converting the ligands **3f** and **3g** into their platinum complexes.

TABLE 6. Effect of Ligands and Complexes on Growth of the Hormone-independent MXT Mammary Carcinoma, the Hormone-dependent MXT Mammary Carcinoma and the Uterus of the $B_6D_2F_1$ Mouse

Compound	Tumor area median (range) (mm^2)	*T/C* (%)	Tumor weight median (range) (mg)	*T/C* (%)	Uterotropic effect
1a	248 (25–283)	216	640 (24–1408)	101	90.2 ± 17.8
1a–$PtCl_2$	150 (39–278)	130	620 (170–1300)	98	99.3 ± 21.4
1b					
1b–$PtCl_2$			499 (56–744)	103	117.0 ± 18.1
1c	146 (9–227)	127	270 (15–629)	92	106.7 ± 29.7
1c–$PtCl_2$	66 (4–219)	57	106 (13–1276)	36	84.9 ± 16.5
1d	108 (64–280)	89	378 (60–790)	80	130.0 ± 16.9
1d–$PtCl_2$	65 (9–273)	54	90 (6–419)	19	138.5 ± 15.4
1e	180 (25–308)	147	370 (8–963)	124	110.7 ± 17.7
1e–$PtCl_2$	68 (0–324)	56	53 (4–314)	18	97.8 ± 7.1
2a	116 (4–165)	101	177 (30–605)	60	77.6 ± 16.8
2a–$PtCl_2$	144 (16–189)	125	170 (20–878)	57	84.8 ± 18.4
2b	84 (38–163)	73	150 (188–1600)	182	199.0 ± 33.2
2b–$PtCl_2$	88 (9–284)	76	310 (67–909)	49	120.3 ± 23.2
3a	58 (9–248)	51	70 (8–257)	24	104.7 ± 17.8
3a–$PtCl_2$	137 (58–216)	119	91 (5–526)	30	98.1 ± 26.2
3b	96 (9–245)	83	288 (172–980)	46	112.0 ± 16.9
3b–$PtCl_2$	110 (9–177)	96	310 (67–909)	49	98.7 ± 17.8
3c			199 (31–516)	42	129.1 ± 12.5
3c–$PtCl_2$	69 (9–276)	56	153 (0–607)	32	110.8 ± 21.7
3d	99 (9–276)	95	156 (8–349)	33	155.5 ± 32.1
3d–$PtCl_2$	139 (9–242)	114	119 (6–205)	25	93.1 ± 18.4
3e					
3e–$PtCl_2$			150 (23–330)	30	137.7 ± 19.5
3f	103 (9–256)	84	135 (28–411)	28	144.2 ± 18.7
3f–$PtCl_2$	146 (9–384)	119	72 (6–124)	15	114.2 ± 23.4
3g	146 (9–308)	119	164 (15–825)	34	129.5 ± 18.2
3g–$PtCl_2$	91 (25–190)	74	77 (22–273)	16	102.3 ± 28.5
3h	101 (0–209)	83			
3h–$PtCl_2$	106 (9–264)	87			

Experimental

Synthesis of Precursors

(a) 4-Bromo-4'-methoxybenzophenone

Friedel–Crafts acylation of anisole and *p*-bromobenzoylchloride [12].

(b) 4-Bromo-4'-methoxydiphenylmethane

Reduction of (a) with $LiAlH_4/ACl_3$ in ether [13].

(c) All the substituted benzylbromides

Reaction of the corresponding benzylalcohols with 47% aqueous HBr.

(d) 2,6-Dichloro-4-methoxybenzylalcohol

Chloromethylation of 3,5-dichloroanisole and consecutive hydrolysis with NaOH [14].

(e) 2,6-Dichloro-4-methoxybenzaldehyde

Oxidation of (d) with MnO_2 in benzene [14].

(f) 4-Bromo-3,5-dimethylanisole
Bromination of 3,5-dimethylanisole with Br_2 [15].

(g) 2,6-Dimethyl-4-methoxybenzaldehyde
Reaction of (f) with (1) Mg/EtBr (entrainment) and (2) DMF [16].

(h) 2,6-Dimethyl-4-methoxybenzylalcohol
Reaction of (f) with (1) Mg/EtBr (entrainment), (2) CO_2 and (3) $LiAlH_4$.

(i) Trimethylsilylcyanide
Reaction of KCN with Me_3SiCl [17].

(j) 2-Pyridyl-trimethylsiloxy-acetonitrile
Addition of (i) to 2-pyridinealdehyde [18].

Synthesis of 2-Pyridyl Ketones

(a) Grignard route (normal procedure)
To 3.83 g (0.157 mol) of magnesium turnings in 150 ml of anhydrous THF about one fourth of the calculated quantity of the aryl halogenide (0.140 mol in 120 ml of THF) was added. After the reaction had started, the remaining aryl halogenide was added with stirring. The solution was refluxed for 1 h. Stirring was continued at room temperature while a solution of 13.52 g (0.130 mol) of 2-cyanopyridine in 60 ml of THF was added. The reaction mixture was refluxed for 12 h and at room temperature brought to pH 3 with 3 N HCl. The mixture was made alkaline (pH 10) with dilute NaOH. The organic layer was separated, dried over $NaSO_4$ and evaporated. The ketone was recrystallized from $EtOH/H_2O$ (ketones to ligands **1a** and **1b**).

(b) Grignard route (inverse addition)
To 2.9 g (0.120 mol of magnesium turnings in 25 ml of anhydrous ether about one tenth of the calculated quantity of the benzylhalide (0.100 mol in 200 ml of ether) was added. After the reaction had started the remaining benzylhalide was added dropwise and stirred for 2 h at room temperature. Then the mixture was added dropwise with vigorous stirring to a solution of 15.5 g (0.150 mol) of 2-cyanopyridine in 150 ml of anhydrous ether. The mixture was stirred for 15 h at room temperature. Work-up of the ketone was carried out as in (a) (ketone to ligand **1d**).

(c) Acylation of benzylbromides with 2-pyridyl-trimethylsiloxyacetonitrile
11 mmol of Li-n-butyl (20% in hexane) were added slowly at 0 °C to a solution of 10 mmol of diisopropylamine in 10 ml of absolute THF. After stirring for 15 min, the temperature was lowered to −78 °C. First a solution of 2.05 g (10 mmol) of 2-pyridyl-trimethylsiloxy-acetonitrile in 3 ml of THF was added dropwise, then 10 mmol of benzylbromide in 5 ml of THF. The temperature was maintained at −78 °C for 5 h. Within 10 h, the mixture was allowed to warm up to room temperature, hydrolyzed with a mixture of 20 ml of 2 N HCl and 10 ml of methanol, and stirred for 6 h. The HCl layer was separated. After addition of 20 ml of CH_2Cl_2, the organic layer was extracted with 2 × 20 ml of 2 N HCl. The collected HCl layers were washed with 20 ml of CH_2Cl_2 and then made alkaline with NaOH (pH 10). The oily organic layer was extracted with 3 × 50 ml CH_2Cl_2. After drying over $NaSO_4$ and evaporating the solvent, the ketone was distilled or recrystallized from $EtOH/H_2O$ (ketones to ligands **1c** and **1e**).

Synthesis of Oximes
To a suspension of 0.1 mol of the ketone in 40 ml EtOH a solution of 11 g (0.16 mol) of hydroxylammoniumhydrochloride in 20 ml of H_2O was added and after cooling to 0 °C a solution of 20 g (0.5 mol) of NaOH in 20 ml of H_2O. The mixture was heated under reflux for 3 h, cooled and neutralized with dilute HCl to pH 7. The precipitated oxime was filtered off, washed with water and dried.

Synthesis of Primary Amines
0.01 mol of the oxime and 4.5 g (0.07 mol) of Zn dust were refluxed for 3 h in a solution of 50 ml of NH_3 (25%) and 15 ml EtOH. For the synthesis of the primary amines to the ligands **1d** and **3f** a oxime/Zn dust ratio of 1:4 was used and a temperature of 60 °C. At room temperature, the solution was filtered. The residue was washed with ether and the filtrate with 3 × 50 ml of ether. The combined organic solutions were dried over Na_2SO_4. After evaporation of the solvent the amine was distilled in high vacuum.

Synthesis of Secondary Amines

(a) Imines
11 mmol of the amine, 10 mmol of the aldehyde, and 20 mmol of Na_2SO_4 were refluxed for 3 to 12 h in 100 ml of absolute ether. After filtration of the Na_2SO_4, the solvent was evaporated and the remaining imines were washed with ether and cold ethanol. Sometimes reaction only proceeded in refluxing benzene (imine to ligand **3c**).

(b) Secondary amines
$NaBH_4$. To a solution of 30 mmol of the imine in 50 ml of methanol 2.3 g (60 mmol) $NaBH_4$ were added at −10 °C in small portions. The mixture was allowed to warm to room temperature within 2 h. Most of the solvent was removed and the residue was hydrolyzed with 30 ml of H_2O. After extraction with 3 × 80 ml of ether, the organic layer was dried over

Na_2SO_4. The solvent was evaporated and the amine was distilled in high vacuum (amines to ligands **3c–h**).

$LiAlH_4$. To a solution of 10 mmol of the imine in 100 ml of absolute ether a suspension of 0.35 g (0.9 mol) of $LiAlH_4$ in 20 ml of ether was added slowly at –78 °C. The mixture was stirred at –78 °C for 3 h. After hydrolysis at room temperature with 1 ml of H_2O, the mixture was filtered and dried over Na_2SO_4. The solvent was evaporated and the amine was distilled in high vacuum (amines to ligands **3a, b**).

Ether Cleavage

8.0 mmol of the amine were dissolved in 150 ml of absolute CH_2Cl_2 and cooled to –78 °C. 32 mmol of BBr_3 in 20 ml CH_2Cl_2 were added dropwise and the temperature was maintained at –78 °C for 1 h. The mixture was allowed to warm up to room temperature. The reaction was finished, when the gas formation (MeBr) had ceased. Refluxing was sometimes necessary (ligand **3f**). Subsequently, 5 ml of MeOH were added slowly with cooling and the dihydrobromide precipitated as a white solid. It was filtered off, washed with ether, cold ethanol, and dried.

Synthesis of Platinum Complexes

(1a–e)–$PtCl_2$ and (2a, b)–$PtCl_2$

1 mmol of the ligands was suspended in 20 ml of H_2O and heated to 30–40 °C. Subsequently, the pH was adjusted to 5.0–6.0 by treatment with 0.5 N NaOH. Then 415 mg (1 mmol) of K_2PtCl_4, dissolved in 5 ml of H_2O, were added. The mixture was stirred and adjusted to pH 6 several times. After a period of 3–6 h, the precipitate was collected, washed with 2 N HCl and H_2O, and dried.

(3a–h)–$PtCl_2$

415 mg (1 mmol) of K_2PtCl_4 were dissolved in 5 ml of a 1:1 mixture of DMF/H_2O and a solution of ligand X2 HBr (1 mmol) in 20 ml of DMF was added. The mixture was stirred at 40 °C for three days and the resulting yellow solution was evaporated to dryness. The residue was treated with 20 ml of H_2O and stirred for 6 h. The pale yellow precipitate was collected, washed with 2 N HCl and H_2O, and dried under vacuum.

Biochemical Methods

Reagents

[^{3}H]-Estradiol (110 μCi/mmol in ethanol and [^{3}H]-thymidine were obtained from New England Nuclear, Dreieich; unlabeled estradiol and estrone from Sigma, München; Richter's IMEM medium and the 'newborn' calf serum (NCS) from Biochrom, Berlin; and the (0.05%)/EDTA (0.02%) trypsin solution from Boehringer, Mannheim. The Tris buffer used (0.01 M, pH 7.5) contained EDTA (0.01 M and NaN_3 (0.003 M). The DCC suspension contained 0.8% charcoal Norit A (Merck) and 0.008% dextran (Merck) in Tris/EDTA buffer. Bouin solution consisted of saturated aqueous picric acid, 40% formaldehyde and glacial acetic acid, 15:5:1 by volume. The phosphate buffered saline (PBS) contained NaCl (8 g), KCl (0.2 g), $Na_2HPO_4 \cdot 2H_2O$ (1 g), $NaH_2PO_4 \cdot H_2O$ (0.15 g) and KH_2PO_4 (0.2 g) in 1 l of H_2O. The PBS/EDTA buffer contained 0.02% EDTA.

Estradiol receptor binding assay

The relative binding affinity (*RBA*) of the test compounds was determined by the displacement of [^{3}H]-Estradiol. The described procedure [5] was used with modifications. Calf uterine cytosol was incubated for 18 h at 4 °C with different concentrations of the test compounds and 5×10^{-9} M [^{3}H]-Estradiol. After incubation, dextran-coated charcoal (DCC) was added to adsorb unbound ligand (90 min, 4 °C), and, after centrifugation, radioactivity was determined in the supernatant with use of 100 μl aliquots. Six concentrations of the competitor were chosen to provide values between 10 and 90% bound radioactivity. A semilogarithmic plot of bound radioactivity *versus* concentration was used to determine the relative binding affinity given as the ratio of molar concentrations of estradiol and test compound required to decrease the amount of bound radioactivity by 50%, multiplied by 100.

Immature mice uterine weight test [6]

Immature female NMRI mice (18–20 days old) were randomized into groups of 6–8 animals. To determine the estrogenic activity (*EA*), the compounds were dissolved or suspended in a 1:1 mixture of polyethyleneglycol 400/1.8% aqueous NaCl solution and a 100 μl aliquot was injected sc on three consecutive days. Control animals received the pure solvent, positive control animals an estrone solution (0.4 μg, 1.5 nmol). 24 hours after the last injection, the animals were killed by cervical dislocation and weighed. Uteri were dissected free of fat and fixed in Bouin solution for 20 h. Uteri were freed from connective tissue, washed with ethanol, dried at 100 °C for 16 h, and weighed. The estrogenic activity (*EA*) of a test compound in percent of the standard (estrone) was calculated by the formula: $[(E_T - E_V)/(E_S - E_V)] \times 100$ (E_T = uterotrophic effect of test compounds, E_V = uterotrophic effect of solvent. E_S = uterotrophic effect of standard; the uterotrophic effect was calculated by the formula: uterine dry weight (mg)/body weight (g), multiplied by 100).

To determine the antiestrogenic activity (*AA*), the injections contained a standard dose of estrone (0.4 μg, 1.5 nmol) and increasing doses of the test compounds. The antiestrogenic activity, which is

equivalent to the inhibition of the estrone-stimulated uterine growth, was calculated by the formula: $[(E_S - E_{S,T})/(E_S - E_V)] \times 100$ ($E_{S,T}$ = uterotrophic effect of standard with simultaneous administration of test compound).

P 388 leukemia [9]

The P 388 leukemia was maintained by routine passage in female DBA/2 mice. For the determination of the antitumor activity female CD_2F_1 mice were inoculated ip with 1×10^6 leukemia cells in 0.1 ml PBS buffer (day 0). The animals were assigned randomly to groups of 6 (10 animals to the solvent control) and the compounds were administered ip as suspension or solution in a 1:1 mixture of polyethyleneglycol 400/1.8% aqueous NaCl solution on days 1, 5, and 9. Cisplatinum (0.5×10^{-5} mol/kg) served as positive control. The antitumor activity was evaluated as median day of survival time compared to the untreated control.

Hormone-independent MDA-MB 231 human breast cancer cell line [8]

The MDA-MB 231 cells were grown in a humified incubator in 5% CO_2 at 37 °C. Richter's medium, supplemented with 10% NCS was used as culture medium. The cells were harvested with trypsin/EDTA, diluted with 10% NCS medium in a ratio of 1:20 and syringed gently to prevent clumping. 100 µl of the cell-suspension were inoculated into 96-well plates. The plates were then incubated at 37 °C in a humidified 5% CO_2 atmosphere to ensure growth of the monolayer. Two days later the medium was changed and the platinum complexes were added as freshly prepared 1000-fold concentrated solutions in DMF, leading to a final solvent concentration of 0.1%. After an incubation time of 2 days, the cells were fixed for 15 min with 1% glutaraldehyde in PBS. After this time the cells were stained for 30 min with 1 ml of 0.05% crystal violet solution in deionized water. After this time the plates were submerged in deionized water and destained for 15 min with a continuous stream of water. The plates were then allowed to air-dry. The crystal violet that had absorbed onto the cells was solubilized with 150 µl of 0.2% Triton X-100 (Sigma). The colored Triton solution was taken up directly into a spectrophotometer (BIO TEK 309-Tecnorama) at 25 °C and the absorbance was measured at 590 nm. Control: DMF in Richter's medium 1:1000. *T/C* [%]: (extinction test compound/extinction control) × 100.

Hormone-dependent MXT M3.2 mammary tumor [11]

The MXT tumor used in these studies was the MXT line 3.2 provided by Dr Bogden, EG & Bogden Laboratories, Worcester, U.S.A. Tumors grew for six weeks in the host animals, and 2 mm^2 pieces were transplanted sc into female $B_6D_2F_1$ mice (8–9 weeks old). Animals were assigned randomly to groups of 9 and treatment was started 24 h after transplantation. The ligands and the complexes, respectively, were dissolved or suspended in a 1:1 mixture of polyethyleneglycol 400/1.8% aqueous NaCl solution and administered sc on Monday, Wednesday, and Friday. After a 6-week period of treatment, animals were killed and autopsied. Tumors were removed and weighed at once (wet weight). Uteri were dissected and prepared as described above to serve as an indicator of estrogenic side effects of the compounds.

Hormone-independent MXT (3.2) ovex mammary tumor [11]

The hormone-independent MXT line was also provided by Dr Bogden, Worcester, U.S.A. Tumor propagation was made in ovariectomized $B_6D_2F_1$-mice. After the tumor had reached a diameter of about 1 cm, it was transplanted. The experiment for determining the tumor-inhibiting activity was carried out as described above except that the duration of therapy was shortened and the antitumor activity was evaluated by the tumor area. At the end of a 2-week therapy, the tumor area was calculated from caliper measurements of two perpendicular axes, one across the largest diameter.

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