vein. Heparinized plasma was prepared, immediately frozen, and stored at -20 °C. Thawed specimens were assayed for the concentrations of **3a** and **21a** by HPLC. Plasma (0.2 mL) was pipetted onto a Bond Elut C2 column (1-mL capacity, Analytichem), followed by the addition of 20 μL of a 10 μg/mL solution of **3l**, which was used as an internal standard. Vacuum was applied, and the columns were washed once with 1.0 mL of water and then once with 1.0 mL of a 20/80 mixture of acetonitrile and water. Compounds **3a**, **3l**, and **21a** were then eluted with 1.0 mL of pure acetonitrile. The acetonitrile eluate was evaporated to dryness with nitrogen gas and the residue was reconstituted to 100 μL of acetonitrile; a 20-μL aliquot was injected onto a Whatman 5μ ODS-3 column (25 cm long × 4.6 mm i.d.). The compounds were eluted isocratically with a mobile phase consisting of 65% acetonitrile and 35% 0.01 M ammonium acetate (pH 7.0) at a flow of 1.0 mL/min, and the UV absorbance was monitored at 335 nm. The ratio of **3a** and **3l** peak heights was directly proportional to the concentration of **3a** in plasma. Similarly, the peak height ratios of **21a** versus **3l** were linearly related to the concentration of **21a**. The recovery of all three substances from plasma was essentially complete.

Biotransformation Studies in Vitro. The test compounds were incubated with rat liver homogenates (1:6 dilution of tissue in buffer) containing the soluble enzyme fraction (100000*g* supernatant) for 1 h at 37 °C at a concentration of 0.1 mmol/L. The concentrations of the test compounds and the hydrolytic products were measured in the incubation mixtures by HPLC. Aliquots from each incubation were diluted with two parts of acetonitrile to precipitate protein and centrifuged at 10000 rpm in an Eppindorf 5413 microcentrifuge. Compounds in the supernatant were separated on a Whatman 5μ ODS 3 column (25 cm length × 4.6 mm i.d.) using a mobile phase consisting of methane/tetrahydrofuran/0.1 M acetic acid, isocratic at 61.7/3.3/35 for 6 min, followed by 6 min gradient up to 76/4/20. The detector was set at 335 nm for **3a**,l and at 280 for **3q**. Pure external standards were injected directly on the column to determine absolute concentrations of each compound.

Registry No. **2a**, 125734-74-9; **2b**, 125734-98-7; **3a**, 128114-29-4; **3b**, 128114-30-7; **3c**, 128114-31-8; **3d**, 128114-32-9; **3e**, 128114-33-0; **3f**, 128114-34-1; **3g**, 128114-35-2; **3h**, 128114-36-3; **3i**, 128114-37-4; **3j**, 128114-38-5; **3k**, 128114-39-6; **3l**, 128114-40-9; **3m**, 128114-41-0; **3n**, 128114-42-1; **3o**, 128114-43-2; **3p**, 128114-44-3; **3q**, 128114-45-4; **3r**, 128114-46-5; **3s**, 128114-47-6; **4**, 125735-01-5; **5**, 125735-02-6; **14**, 128114-48-7; **15**, 128114-49-8; **16a**, 113094-77-2; **16b**, 113116-81-7; **17a**, 128162-86-7; **17b**, 125735-03-7; **18a**, 112770-77-1; **18b**, 112770-80-6; **19a**, 108931-39-1; **19b**, 108931-40-4; **20a**, 128114-50-1; **21a**, 128162-87-8; **21b**, 123371-44-8; (*R*)-(+)-α-methylbenzylamine, 3886-69-9; *p*-methoxy-α-toluenethiol, 6258-60-2; 4-nitrophenyl chloroformate, 7693-46-1.

Supplementary Material Available: Tables of atomic coordinates, bond distances and angles, and thermal parameters (7 pages). Ordering information is given on any current masthead page.

1-(Aminoalkyl)-2-phenylindoles as Novel Pure Estrogen Antagonists

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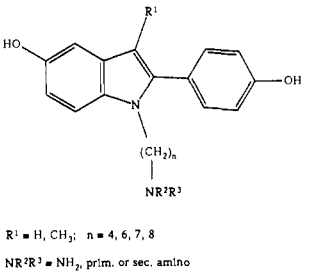
A number of 1-(ω-aminoalkyl)-5-hydroxy-2-(4-hydroxyphenyl)indoles were synthesized and studied for their binding affinities for the calf uterine estrogen receptor and estrogen antagonistic activities. Highest binding affinities were found with derivatives bearing a methyl group in position 3 and a hexamethylene chain between the indole and amino nitrogen atoms. Values for relative binding affinity (RBA) are between 20 and 30 for derivatives **5b**, **5c**, **5f**, and **5h** (17β-estradiol = 100). In the mouse uterine weight test, no significant agonistic (estrogenic) activity was observed up to a daily dose of 125 μg/animal, except for derivatives **5c**, **5j**, and **5l**. 2-Phenylindoles with amino (**5b**), pyrrolidino (**5f**), piperidino (**5h**), and morpholino (**5k**) groups as the amino function completely suppressed estrone-stimulated uterine growth as a dose of 125 μg/animal (100% antagonism). Therefore, these derivatives can be considered as first examples of nonsteroidal pure antiestrogens.

The antiestrogen tamoxifen is widely used in the endocrine therapy of hormone-dependent breast cancer. Unfortunately, about 40% of the patients do not respond to this treatment despite the presence of estrogen receptors in the malignant tissue.[1] The reason for this failure of therapy is not yet known. It might be due to the incomplete antagonism and/or weak agonism of this drug, being only a partial antiestrogen. Tamoxifen exhibits weak estrogenic activity both in man[2] and rat;[3] in the mouse, it acts as pure estrogen.[4] Some of the side effects of tamoxifen during long-term treatment are thought to be a consequence of the agonist (estrogenic) activity of the drug.[5] To our knowledge, all other antiestrogens in clinical use or trials also show estrogenic effects. This observation

Chart I



R^1 = H, CH_3; n = 4, 6, 7, 8

NR^2R^3 = NH_2, prim. or sec. amino

applies not only to triphenylethylene derivatives but also to the 2-phenylindole zindoxifene that we have developed for the treatment of hormone-dependent mammary and prostatic carcinomas.[6,7]

The development of a pure antiestrogen which is entirely free of estrogenic activity should eliminate many of the toxicological problems characteristic of tamoxifen (e.g.

(1) Maass, H.; Jonat, W.; Stolzenbach, G.; Trams, G. *Cancer* **1980**, *46*, 2783.
(2) Furr, B. J. A.; Jordan, V. C. *Pharmacol. Ther.* **1984**, *25*, 127.
(3) Jordan, V. C.; Allen, K. E.; Dix, C. J. *Cancer Treat. Rep.* **1980**, *64*, 745.
(4) Jordan, V. C.; Rowsky, L.; Dix, C. J.; Prestwich, G. *J. Endocr.* **1978**, *78*, 71.
(5) Wakeling, A. E. In *Pharmacology and Clinical Uses of Inhibitors of Hormone Secretion and Action*; Furr, J. A., Wakeling, A. E., Eds.; Bailliere-Tindall, London, 1986; p 1.
(6) von Angerer, E.; Prekajac, J.; Berger, M. *Eur. J. Cancer Clin. Oncol.* **1985**, *21*, 531.
(7) Schneider, M. R.; von Angerer, E.; Höhn, W.; Sinowatz, F. *Eur. J. Cancer Clin. Oncol.* **1987**, *23*, 1005.

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Scheme I

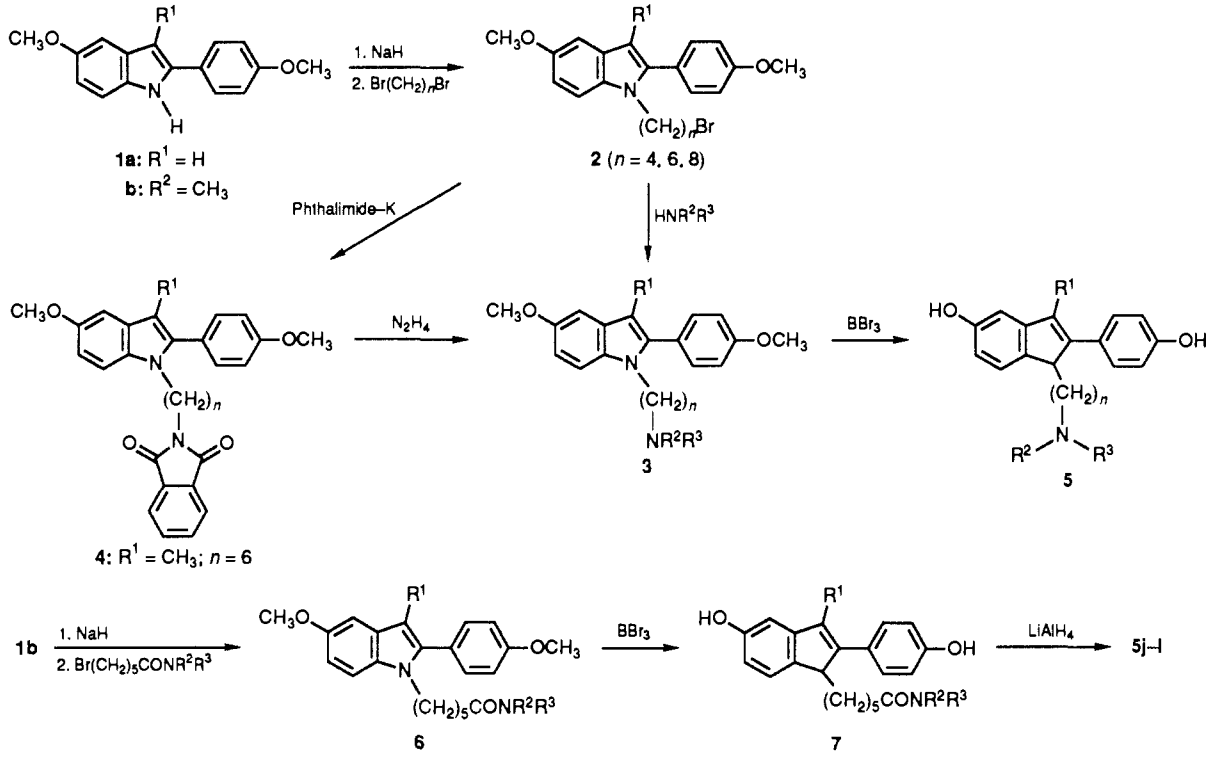
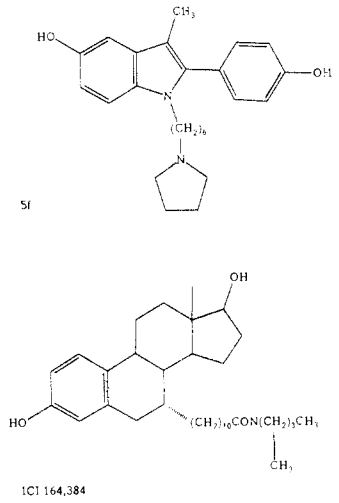


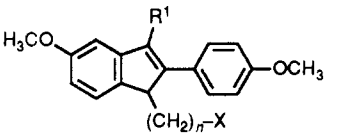
Chart II



tumor flares,[8] vaginal cornification,[2] hypercalciemia[9]) and thus potentially permit their use in nonmalignant, as well as malignant, estrogen-dependent diseases. A pure antagonist would also be an important tool for studies on the mode of action of estrogens and partial antagonists like tamoxifen in breast cancer and other target tissues.

The first pure antiestrogen has recently been described by Wakeling and Bowler.[10] It has an estradiol-based structure with a side chain in position 7 carrying an amide function (Chart II). In this paper, we report on the synthesis and the pharmacological evaluation of the first examples of nonsteroidal pure estrogen antagonists. They derive from 2-phenylindole and possess an aminoalkyl chain at the indole nitrogen (Chart I). Most of the derivatives have the same substitution pattern as zindoxifene.[6]

Table I. 1-Substituted 5-Methoxy-2-(4-methoxyphenyl)indoles



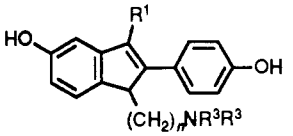
no.	n	R^1	X	formula[a]	mp, °C	% yield
3a	4	CH_3	NC_4H_8	$C_{25}H_{32}N_2O_2$	59–60	51
3b	6	CH_3	NH_2	$C_{22}H_{28}N_2O_2$	oil	55
3c	6	CH_3	$NHCH_3$	$C_{24}H_{32}N_2O_2$	oil	84
3d	6	CH_3	$N(CH_3)_2$	$C_{25}H_{34}N_2O_2$	oil	41
3e	6	H	NC_4H_8	$C_{26}H_{34}N_2O_2$	oil	77
3f	6	CH_3	NC_4H_8	$C_{27}H_{36}N_2O_2$	oil	90
3g	6	H	NC_5H_{10}	$C_{27}H_{36}N_2O_2$	oil	90
3h	6	CH_3	NC_5H_{10}	$C_{28}H_{38}N_2O_2$	oil	89
3i	8	CH_3	NC_4H_8	$C_{29}H_{40}N_2O_2$	oil	65
6a	5	CH_3	$CON(C_2H_5)_2$	$C_{27}H_{36}N_2O_3$	oil	75
6b	5	CH_3	$CONC_4H_8O$	$C_{27}H_{34}N_2O_4$	oil	67
6c	5	CH_3	$CONHCH_2C_6H_5$	$C_{30}H_{34}N_2O_3$	127–129	67

[a] Crystalline compounds were analyzed for C and H within ±0.4% of the calculated values.

Chemistry. The aminoalkyl side chain was introduced into the 2-phenylindole by using three different synthetic methods (Scheme I): In most cases the methoxy-substituted 2-phenyl-1H-indoles **1a** and b were allowed to react with an excess of the respective 1,ω-dibromoalkane in the presence of NaH to afford the ω-bromoalkyl derivatives **2a–d**. Treatment of the bromoalkyl indoles with methylamine, dimethylamine, pyrrolidine, and piperidine respectively yielded the corresponding 1-(aminoalkyl)indoles (Table I). The primary amino group was introduced by the Gabriel synthesis: Compound **2c** was converted into phthalimide **4** which was treated with hydrazine to give amine **3b**. The hydroxy-substituted 1-(aminoalkyl)-2-phenylindoles **5a–h** and **5l** were obtained by ether cleavage of the corresponding methoxy derivatives **3** with BBr_3. For

(8) Legault-Poisson, S.; Jolivet, J.; Poisson, R.; Beretta-Piccoli, M.; Band, P. R. *Cancer Treat. Rep.* **1979**, *63*, 1839.
(9) Villalon, A. H.; Tattersal, M. H. N.; Fox, R. M.; Woods, R. L. *Br. Med. J.* **1979**, 1329.
(10) Wakeling, A. E.; Bowler, J. *J. Steroid Biochem.* **1988**, *31*, 645.

Table II. 1-(Aminoalkyl)-5-hydroxy-2-(4-hydroxyphenyl)indoles and Their Estrogen Receptor Affinities



no.	n	R^1	R^2	R^3	formula[a]	mp, °C	% yield	RBA[b]
5a	4	CH_3	$-(CH_2)_4-$		$C_{23}H_{28}N_2O_2$[c]	148	35	3.6
5b	6	CH_3	H	H	$C_{21}H_{26}N_2O_2$[d]	139	76	25
5c	6	CH_3	CH_3	H	$C_{22}H_{28}N_2O_2$[e]	194	24	27
5d	6	CH_3	CH_3	CH_3	$C_{23}H_{30}N_2O_2$	189	73	12
5e	6	H	$-(CH_2)_4-$		$C_{24}H_{30}N_2O_2$	109–112	50	2.3
5f	6	CH_3	$-(CH_2)_4-$		$C_{25}H_{32}N_2O_2$	148–150	56	21
5g	6	H	$-(CH_2)_5-$		$C_{25}H_{32}N_2O_2$	125–128	80	1.3
5h	6	CH_3	$-(CH_2)_5-$		$C_{26}H_{34}N_2O_2$[f]	102	23	23
5i	8	CH_3	$-(CH_2)_4-$		$C_{27}H_{36}N_2O_2$	166–169	42	7.6
5j	6	CH_3	C_2H_5	C_2H_5	$C_{25}H_{34}N_2O_2$	amorphous	49	14
5k	6	CH_3	$-(CH_2)_2O(CH_2)_2-$		$C_{25}H_{32}N_2O_3$[g]	107–110	43	10
5l	6	CH_3	$CH_2C_6H_5$	H	$C_{28}H_{32}N_2O_2$	amorphous	41	15
5m[h]	7	CH_3	H	H	$C_{22}H_{28}N_2O_2$	167	53	11

[a] Analyzed for C, H, and N within ±0.4% of the calculated values except where noted. [b] Relative binding affinities for the calf uterine estrogen receptor = ratio of molar concentrations of 17β-estradiol (E_2) and inhibitor required to decrease the amount of bound [^{3}H]E_2 by 50%, × 100; mean values of three independent determinations, each performed in triplicate. [c] C: calcd, 75.79; found, 75.13. [d] C: calcd, 74.53; found, 73.68. [e] C: calcd, 74.75; found, 73.96. [f] Calcd: C, 76.81; H, 8.43; N, 6.89. Found: C, 75.17; H, 9.00; N, 5.90. [g] Calcd: C, 73.50; N, 6.86. Found: C, 71.35; N, 6.31. [h] Reference 12.

the synthesis of the amines **5j–l**, a different route was used: The 1*H*-indole was allowed to react with the respective amide of 6-bromohexanoic acid to yield carbamoylalkyl indoles **6a–c**, which were reduced with $LiAlH_4$ after ether cleavage.

Binding Affinity for the Calf Uterine Estrogen Receptor. The binding affinities of the (aminoalkyl)-2-phenylindoles with free hydroxy groups were measured in a competitive binding assay with 17β-[^{3}H]estradiol. Calf uterine cytosol was used as receptor source and the dextran-coated charcoal (DCC) method was applied.[11] The relative binding affinities are given as ratio of the molar concentrations required to decrease the receptor-bound radioactivity by 50%, multiplied by 100. The semilogarithmic plot of bound radioactivity vs molar concentrations of competitors exhibited curves parallel to those of 17β-estradiol, suggesting a common binding site for all of the compounds tested.

The binding affinities provided by derivatives with hexamethylene spacer groups were seen to be better than those of compounds bearing a tetramethylene (**5a**), heptamethylene (**5m**[12]), or an octamethylene (**5i**) group (Table II). A prerequisite for a strong binding interaction is the presence of a methyl group in position 3 of the indole. Compounds lacking this substituent (**5e** and **5g**) display RBA values which are lower by 1 order of magnitude than those of the corresponding methyl derivatives. Comparison with analogous 1-alkyl-2-phenylindoles shows that the amino group contributes considerably to the receptor binding. The RBA values for the corresponding *n*-pentyl, *n*-hexyl, and *n*-octyl are 2.3,[13] 1.0,[14] and 2.7,[14] respectively.

Estrogenic and Antiestrogenic Activity. The aim of the studies described here was the discovery of pure estrogen antagonists. The standard test model for determination of estrogenic and antiestrogenic activity is the uterine weight test, carried out with immature or ovariectomized adult mice or rats.[15] We used immature mice because their uteri appear to be more sensitive to estrogenic effects than rat uteri.[16] In this assay, immature mice received various doses of the drug and the increase of uterine weight was measured there after. Antagonistic activity was determined by simultaneous administration of test compound and a standard dose of estrone (0.4 μg) and calculation of the inhibition of estrone-stimulated uterine growth.

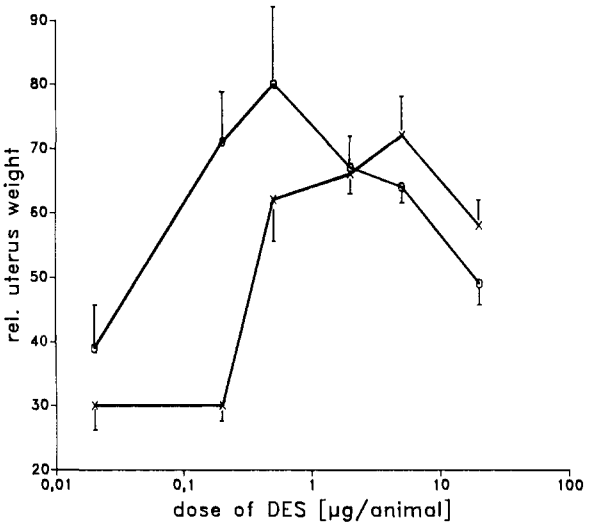


Figure 1. Uterotrophic effect of diethyl stilbestrol (DES), administered alone (○) or together with 125 μg of 5f (×) in immature mice; bars represent SD.

All of the 1-(aminoalkyl)-2-phenylindoles with RBA values greater than 10 except **5d** and **5m** were tested. On the basis of their antagonistic activity, these compounds fall into two categories: derivatives with partial but significant antagonism (**5a**, **5c**, **5j**, and **5l**) and compounds which suppress completely the stimulation of uterine growth (**5b**, **5f**, **5h**, and **5k**) (Table III). As expected, the latter derivatives did not show agonistic activity in the standard dose range between 1 and 125 μg/animal (0.05–6.25 mg/kg) when administered alone. Therefore, these compounds can be considered as pure estrogen an-

(11) von Angerer, E.; Strohmeier, J. *J. Med. Chem.* **1987**, *30*, 131.
(12) Palm, M.; Erber, S.; Eisenbrand, G.; von Angerer, E. *Arch. Pharm. (Weinheim, FRG)*, in press.
(13) von Angerer, E.; Prekajac, J.; Strohmeier, J. *J. Med. Chem.* **1984**, *27*, 1439.
(14) Knebel, N. Doctoral Thesis, Regensburg, 1988.
(15) Rubin, B. L.; Dorfman, A. S.; Black, L.; Dorfman, R. I. *Endocrinology* **1951**, *49*, 429.
(16) Pasqualini, J. R.; Sumida, C.; Giambiagi, N. *J. Steroid Biochem.* **1988**, *31*, 613.

Table III. Estrogenic and Antiestrogenic Activity of 1-(Aminoalkyl)-2-phenylindole Derivatives in the Mouse Uterine Weight Test

compd	dose,[a] μg	uterotrophic test: rel ut weight[b]	antiuterotrophic test[c] rel ut weight[b]	antiuterotrophic test[c] % inhibn[d]
control		7.7 ± 2.5		
5a	1	7.6 ± 2.8	43.7 ± 8.2	5
	5	8.0 ± 3.1	47.6 ± 10.5	
	25	6.4 ± 1.1	16.2 ± 4.6	78[e]
	125	6.4 ± 1.3	25.5 ± 7.9	53[e]
estrone	0.4	45.8 ± 6.2		
control		15.9 ± 3.5		
5b	1	10.9 ± 4.7	65.9 ± 5.3	
	5	11.9 ± 2.1[e]	30.1 ± 8.1	68[e]
	25	12.4 ± 2.7[e]	15.0 ± 1.2	102[e]
	125	16.9 ± 2.6	16.2 ± 2.6	99[e]
5c	1	14.4 ± 2.1		
	5	22.8 ± 8.6[e]	38.1 ± 8.1	49[e]
	125	34.0 ± 6.2[e]	33.7 ± 2.4	59[e]
estrone	0.4	59.8 ± 9.2		
control		15.2 ± 2.9		
5f	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		
control		21.5 ± 3.7		
5h	5	19.7 ± 3.6	28.9 ± 5.4	71[e]
	25	21.4 ± 5.1		
	125	24.7 ± 3.9	22.3 ± 4.0	98[e]
estrone	0.4	69.3 ± 7.5		
control		15.4 ± 2.2		
5j	5	16.1 ± 4.8	46.0 ± 7.6	23[f]
	25	18.2 ± 0.5[f]	52.5 ± 6.7	
	125	22.4 ± 4.9[f]	30.9 ± 3.4	61[e]
estrone	0.4	55.3 ± 5.1		
control		18.8 ± 9.6		
5k	5	19.5 ± 6.9	51.2 ± 7.5	8
	25	21.8 ± 11.3	47.7 ± 8.4	18
	125	17.4 ± 1.7	18.0 ± 4.8	102[e]
estrone	0.4	54.2 ± 12.1	54.2 ± 12.1	
control		18.2 ± 5.5		
5l	5	17.8 ± 4.6	53.2 ± 9.0	25
	25	23.6 ± 2.5[f]	44.6 ± 7.1	43[e]
	125	34.0 ± 7.2[f]	40.8 ± 8.7	51[e]
estrone	0.4	64.7 ± 8.7		

[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 six animals ± SD. [c] Simultaneous administration of 0.4 μg of estrone/animal and day. [d] The *U* test according to Wilcoxon, modified by Mann and Whitney, was used to determine significance. [e] Significant ($p < 0.01$). [f] Significant ($p < 0.05$).

tagonists. With partial estrogen antagonists in this series, weak estrogenic activity was found at higher doses.

In order to demonstrate that the antiuterotrophic effect of these compounds is receptor-mediated and not due to some unknown mechanism, we carried out an experiment in which we kept the dose of the antagonist (**5f**) constant and varied the dose of the estrogen. Diethylstilbestrol (DES) was used as agonist because it serves as reference drug in our studies on antitumor activity which are in progress now. Compound **5f** was chosen for advanced testing because it requires the lowest dose to exert complete antagonism. The pure antiestrogen **5f** (125 μg) shifted the dose–response curve of diethylstilbestrol to 10-fold higher doses without changing the shape of the curve (Figure 1). Obviously, the antiuterotrophic effect of **5f** can be overcome by high doses of estrogen.

Discussion

In previous studies, the 2-phenylindole system proved to be a very versatile structure for the development of compounds active in endocrine systems.[11,13] Depending on the substitution pattern in the indole part, the 1-alkyl derivatives were either pure estrogens or partial estrogen antagonists.[13] In order to increase the antagonistic potency of the 2-phenylindole structure, we modified the substituent at the nitrogen. Introduction of a terminal amino function led to a number of very potent antiestrogens. Among them, some can be considered pure estrogen antagonists. All of the pure antagonists display high binding affinities for the calf uterine estrogen receptors with RBA values of about 20, based on estradiol = 100. From these data, it can be assumed that the aminoalkyl group exerts two effects: It enhances the binding to the receptor and leads to a complete loss of agonistic activity in some cases. The antagonism of these drugs can be rationalized by the complete blockade of the receptors and, consequently, prevention of estrogenic stimulation of the uterine growth. The antagonism is not restricted to uteri as target organ but was also found in MCF-7 human breast cancer cells: Preliminary results with **5f** revealed that the induction of progesterone receptor biosynthesis by 17β-estradiol (10^{-10} M; 175% of control) is completely inhibited by the pure antagonist (10^{-7} M).[17] When given alone, progesterone-receptor levels were lowered below control values.

The main difference to antiestrogens presently in clinical use is the complete lack of agonistic activity. This can be due to some conformational alterations of the receptor protein after binding the aminoalkyl indole. As consequence, the receptor protein is either not any longer capable of recognizing estrogen-responsive elements of the DNA or has lost its ability to bind to them and induce the transcription of estrogen responsive genes. A third alternative would be the induction of synthesis of antiproliferative factors such as TGF-β[18] which would overcome the stimulation by estrogens.

Interestingly, the nonsteroidal pure estrogen antagonists described in this paper display the same biological profile as the steroidal derivative ICI 164,384[10] despite their completely different chemical structure (Chart II). It is not very likely, that the amide group of the steroidal derivative interacts with the same binding site as the amino group of the 2-phenylindole structure, because of the difference in electronic and steric conditions. Our investigations and the studies of Bowler et al.[19] have demonstrated that the pure antagonism or—more precisely—the complete lack of agonistic activity together with high binding affinity is very sensitive to minute structural alterations of the alkyl substituents of the amino group. Obviously, the requirements for a compound being a pure estrogen antagonist are very strict.

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 are consistent with the assigned structures. The syntheses of 1-(4-bromobutyl)-5-methoxy-2-(4-methoxyphenyl)-3-methylindole (**2a**), 1-(6-bromohexyl)-5-methoxy-2-(4-methoxyphenyl)indole (**2b**), and 1-(6-bromohexyl)-5-methoxy-2-(4-methoxyphenyl)-3-methylindole (**2c**) have been described previously.[20] The synthesis of **5m** is published elsewhere.[12]

1-(8-Bromooctyl)-5-methoxy-2-(4-methoxyphenyl)-3-methylindole (2d). A solution of the 2-phenylindole **1b**[13] (7.5 mmol) in 25 mL of dry DMF was added with stirring to a mixture

(17) von Angerer, E.; Knebel, N.; Ganss, B.; Faderl, M. *J. Cancer Res. Clin. Oncol.* **1989**, *115*, S56.
(18) Fernandez-Pol, J. A.; Klos, D. J.; Grant, G. A. *Cancer Res.* **1986**, *46*, 5153.
(19) Bowler, J.; Lilley, T. J.; Pittam, J. D.; Wakeling, A. E. *Steroids* **1989**, *54*, 71.
(20) Knebel, N.; von Angerer, E. *J. Med. Chem.* **1988**, *31*, 1675.

of sodium hydride (12.5 mmol) in 40 mL of dry DMF at 0 °C. After stirring for 30 min at 0 °C, this mixture was added slowly to a solution of the dibromoalkane (11.0 mmol) in 25 mL of dry DMF with stirring and cooling. After stirring for an additional 0.5 h, the excess of sodium hydride was destroyed by adding water. After extraction with CH_2Cl_2, the organic layer was dried with $MgSO_4$. After evaporation of the solvent in vacuo, the residue was purified by column chromatography (SiO_2; CH_2Cl_2) to afford a yellow oil. Yield: 74%. ^1H NMR ($CDCl_3$): δ = 0.91–2.02 (m, 12 H, $(CH_2)_6$), 2.17 (s, 3 H, CCH_3), 3.21–3.50 (mc, 2 H, CH_2Br), 3.85 (s, 6 H, OCH_3), 3.97 (t, J = 7 Hz, 2 H, NCH_2), 6.75–7.38 (m, 7 H, ArH).

General Procedure for the Synthesis of (Pyrrolidinylalkyl)- and (Piperidinylalkyl)-2-phenylindoles (3a, 3e–h, 3l). A solution of the (ω-bromoalkyl)indole **2** (3 mmol) in 100 mL of pyrrolidine or piperidine was heated to 120 °C for 4 h. After cooling, water and CH_2Cl_2 were added, and the organic layer was separated and dried ($MgSO_4$). After evaporation of the solvent, the brown residue was purified by chromatography (SiO_2; MeOH/NEt_3 1:1). The yields were in the range of 50–90%. The products were obtained as colorless or yellow oils, except **3a** (colorless crystals; mp: 59–60 °C). Anal. ($C_{25}H_{32}N_2O_2$): C, H.

General Procedure for the Synthesis of [(Methylamino)alkyl]- and [(Dimethylamino)alkyl]-2-phenylindoles (3c, 3d). A solution of the (ω-bromoalkyl)indole (7 mmol) in 30 mL of EtOH was added to an aqueous solution of methylamine or dimethylamine (40%) and refluxed for 3 h. After cooling, most of the EtOH was removed in vacuo. The residue was dissolved in water and extracted with 100 mL of water. After drying ($MgSO_4$), the solvent was distilled off. The residue was chromatographed (SiO_2; MeOH) to afford a colorless oil. Yields are reported in Table I.

5-Methoxy-2-(4-methoxyphenyl)-3-methyl-1-(6-phthalimidohexyl)indole (4). A mixture of (6-bromoalkyl)indole **2c** (8.0 mmol) and potassium phthalimide (8.8 mmol) in 100 mL of dry DMF was refluxed for 2 h. After cooling, the mixture was extracted with CH_2Cl_2. After washing with water and drying ($MgSO_4$), the solvent was removed. The residue was chromatographed (SiO_2; CH_2Cl_2/EtAC, 10:1) to afford colorless crystals (78%). Mp: 99 °C. ^1H NMR ($CDCl_3$): δ = 0.91–1.89 (m, 8 H, $(CH_2)_4$), 2.20 (s, 3 H, CCH_3), 3.59 (t, J = 7 Hz, 2 H, NCH_2), 3.91 (s, 6 H, OCH_3), 4.01 (t, J = 7 Hz, 2 H, NCH_2), 6.78–8.04 (m, 11 H, ArH). Anal. ($C_{31}H_{32}N_2O_4$): C, H, N.

1-(6-Aminohexyl)-5-methoxy-2-(4-methoxyphenyl)-3-methylindole (3b). Phthalimide 4 (6.0 mmol) was dissolved in 50 mL of EtOH and treated with hydrazine hydrate, dissolved in 20 mL of EtOH. After refluxing for 2 h, the mixture was cooled and then acidified (pH 2–3) by addition of ca. 40 mL of 2 N HCl. The precipitate was filtered off. After evaporation of the EtOH, the aqueous solution was made alkaline (pH 8–9) by addition of 2 N NaOH. The alkaline solution was extracted three times with 50 mL of EtOAC. After drying and evaporation of the solvent, the residue was purified by chromatography (SiO_2; CH_2Cl_2/NEt_3, 5:1). The product was obtained as colorless oil.

General Procedure for the Synthesis of 1-(5-Carbamoylpentyl)-2-phenylindoles (6a–c). A solution of the 2-phenylindole (14 mmol) in 80 mL of dry DMA was added to a mixture of sodium hydride (20 mmol, in paraffin) and 20 mL of dry DMA at 0 °C. With ice cooling, the mixture was sonicated for 20 min, followed by addition of 15 mmol of the respective 6-bromohexanamide in 60 mL of dry DMF. Stirring was continued for 2 h at room temperature. The excess of sodium hydride was destroyed by addition of water. The mixture was extracted several times with EtOAc. The combined organic layers were washed with water and dried (Na_2SO_4). After evaporation of the solvent, the residue was chromatographed (SiO_2) with varying mixtures of CH_2Cl_2 and EtOAc. Yields and melting point (**6c**) are reported in Table I.

General Procedure for Ether Cleavage. A solution of the methoxy-substituted indole (4.0 mmol) in dry CH_2Cl_2 (100 mL) was cooled to −60 °C under a nitrogen atmosphere, and then BBr_3 (10.0 mmol) dissolved in 10 mL of CH_2Cl_2 was added. After removal of the cooling bath, the mixture stirred overnight, followed by boiling under reflux for 2 h. Under a nitrogen atmosphere and cooling with an ice bath, a saturated solution of $NaHCO_3$ was added slowly. The reaction mixture was extracted with EtOAc. The organic layer was washed with $NaHCO_3$ solution and water and dried (Na_2SO_4). After evaporation of the solvent, the residue was purified by chromatography (SiO_2; EtOAc/MeOH 10:1). The products were usually obtained as an amorphous powder or solid foam, which could not be purified by crystallization. Therefore, their elemental analyses were not always satisfactory. Purity was checked by HPLC on a RP-18 column with MeOH/buffer (20 mM KH_2PO_4; 5% pentanesulfonic acid; pH 5.0) (70:30) as eluent. Melting points of amino derivatives **5a–i** are reported in Table II.

1-[5-(Diethylcarbamoyl)pentyl]-5-hydroxy-2-(4-hydroxyphenyl)-3-methylindole (7a). Yield: 67%. Solid foam. ^1H NMR (CD_3COCD_3): δ = 0.82–1.91 (m, 12 H, $-CH_2-$, CH_3), 2.06 (s, 3 H, CCH_3), 2.18 (t, J = 7 Hz, 2 H, $COCH_2$), 3.30 (q, J = 7 Hz, 4 H, $CONCH_2$), 3.97 (t, J = 7 Hz; 2 H, NCH_2), 6.58–7.37 (m, 7 H, ArH), 8.25 (s, 2 H, OH).

5-Hydroxy-2-(4-hydroxyphenyl)-3-methyl-1-[5-(morpholinocarbonyl)pentyl]indole (7b). Yield: 58%. Solid foam. ^1H NMR (CD_3COCD_3): δ = 0.80–1.90 (m, 12 H, CH_2), 2.09 (s, 3 H, CCH_3), 2.20 (t, J = 7 Hz, 2 H, $COCH_2$), 3.08–3.71 (m, 4 H, $CONCH_2$), 4.01 (t, J = 7 Hz, 2 H, NCH_2), 6.69–7.5 (m, 7 H, ArH), 7.95 (s, 1 H, OH), 9.00 (s, 1 H, OH).

1-[5-(Benzylcarbamoyl)pentyl]-5-hydroxy-2-(4-hydroxyphenyl)-3-methylindole (7c). Yield: 43%. Mp: 189–191 °C (EtOH). ^1H NMR (CD_3COCD_3): δ = 0.85–1.88 (m, 6 H, CH_2), 1.90–2.31 (m, 2 H, $COCH_2$), 2.10 (s, 3 H, CCH_3), 2.86 (s, 1 H, NH), 4.03 (t, J = 7 Hz; 2 H, NCH_2), 4.39 (t, J = 3 Hz, 2 H, $NHCH_2$), 6.77–7.49 (m, 12 H, ArH), 7.70 (s, 1 H, OH), 8.74 (s, 1 H, OH). Anal. ($C_{28}H_{30}N_2O_2$): C, H, N.

General Procedure for the Reduction of Carbamoyl Derivatives. A solution of carbamoyl derivative **7** (2.6 mmol) in 80 mL of anhydrous THF was added slowly to a boiling solution of 7.0 mmol of $LiAlH_4$ in 50 mL of anhydrous THF. After refluxing for 1 h, the mixture was cooled. Then, 150 mL of water and 20 mL of saturated $NaHCO_3$ solution were added. After extraction with EtOAc, the organic layer was washed with water and dried (Na_2SO_4). The solvent was removed in vacuo and the residue was treated with 50 mL of hexane to afford an amorphous powder. Purity was checked by HPLC on a RP-18 column with MeOH/buffer (20 mM KH_2PO_4; 5% pentanesulfonic acid; pH 5.0) (70:30) as eluent. Yields and melting points are reported in Table II.

Biochemical and Biological Methods. Reagents. [2,4,6,7-^{3}H]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) and a glass-in-glass homogenizer (Potter S; Braun, FRG) at 4 °C. Lipids were separated by centrifugation at 700g and discarded. The homogenate was centrifuged at 105000g 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.

The 500-μL incubation mixture comprised 5 nM 17β-[^{3}H]estradiol (added in 100 μL TEA), 10^{-9}–10^{-5} M competing ligand (in 100 μL TEA), 100 μL of uterine cytosol, and TEA. The mixture was incubated for 18 h at 4 °C, 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 700g 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 with 5 μM 17β-estradiol as competing ligand. Six concentrations of competitor (1, 2, 5, and 10 × 10^{-9}–10^{-6}) were chosen to provide values between 10 and 90% of specifically bound radioactivity. Radioactivity was plotted as a function of the log of 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 Charles-River-Wiga

were randomly divided into groups of 6–10 animals. To determine estrogenic activity, compounds were dissolved or suspended in olive oil (100 μL/animal) and injected subcutaneously on three consecutive days. Control animals received the vehicle alone. 24 h after the last injection, the animals were killed by cervical dislocation and weighed. Uteri were dissected free of fat and fixed in Bouin solution (saturated aqueous picric acid/40% formaldehyde/glacial acetic acid 15:5:1 by vol) for 2 h. Uteri were freed from connective tissue, washed with a saturated alcoholic solution of LiCl, dried at 100 °C for 24 h, and weighed. The relative uterine weight was calculated with the following formula: uterine dry weight (mg)/body weight (g), multiplied by 100. Agonistic activity (%) was estimated by the following formula: $(W_T - W_V)/(W_S - W_V) \times 100$ (W_S = relative uterine weight of animals treated with a standard dose of estrone (0.4 μg); W_T = relative uterine weight of animals treated with test compound; W_V = relative uterine weight of control animals).

To determine antiestrogenic activity, injections contained a standard dose (0.4 μg) of estrone and increasing doses of test compound. Antagonism (%) was calculated with the following formula: $100 - [(W_{S,T} - W_V)/(W_S - W_V) \times 100]$ ($W_{S,T}$ = relative uterine weight of animals treated with estrone + test compound). Experiments with a constant dose of the antagonist and varying doses of estrogen were performed in an analogous manner with diethylstilbestrol (DES) as agonist.

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Supplementary Material Available: ^{1}H NMR data of 5-methoxy-2-(4-methoxyphenyl)indoles (3a–i, 6a–c) and 1-(aminoalkyl)-5-hydroxy-2-(4-hydroxyphenyl)indoles (5a–l) (4 pages). Ordering information is given on any current masthead page.

Synthesis and Evaluation of a Series of Aryl[*e*]fused Pyrazolo[4,3-*c*]pyridines with Potential Anxiolytic Activity

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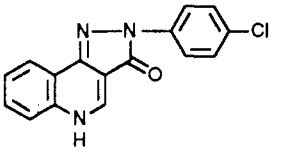
A series of pyrazolo[4,3-*c*]pyridines has been synthesized and evaluated as potential anxiolytic agents. Selected compounds from this series show a pharmacological profile of action different from that of diazepam. A number of the compounds possess higher affinity for central benzodiazepine receptors than diazepam, yet show less anticonvulsant activity and are less sedative. The structure–activity relationships of these potential anxiolytic agents are discussed.

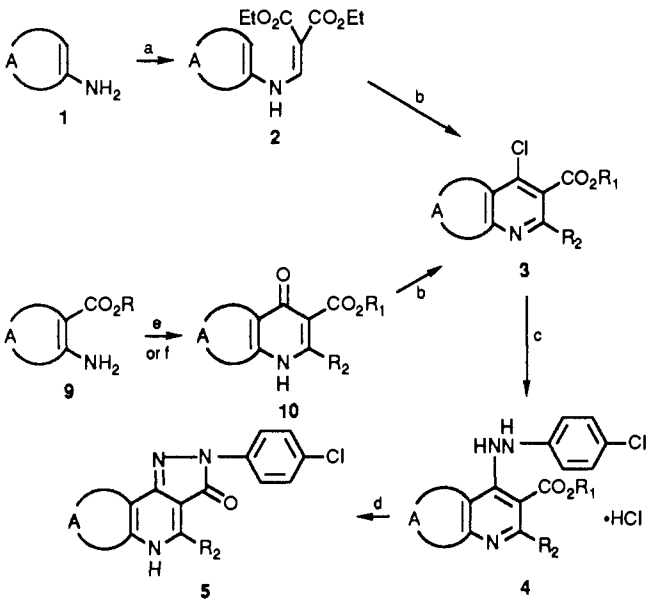
Since the discovery of chlordiazepoxide and diazepam, the 1,4-benzodiazepines (BZs) have been a fruitful source of research.[1] However, anxioselective compounds,[2] which do not possess the undesirable side effects of BZs are needed. Some β-carboline[3] and 2-thienylpyrazoloquinoline[4] derivatives, related to the 2-(4-chlorophenyl)pyrazolo-[4,3-*c*]quinolin-3(5*H*)-one (CGS 9896) (**12**), but with different substituents on the parent skeleton, have a wide spectrum of biological activity. One compound from the



12

former series, 4-(methoxymethyl)-6-(phenylmethoxy)-9*H*-pyrido[3,4-*b*]indole-3-carboxylic acid, ethyl ester (ZK 93423), represents a relatively selective anxiolytic,[5] whereas another, 4-(methoxymethyl)-5-(phenylmethoxy)-9*H*-pyrido[3,4-*b*]indole-3-carboxylic acid, ethyl ester (ZK 91296), is a remarkable anticonvulsant.[6] The selective, non-BZ, pyrazoloquinoline **12**, which is a partial agonist at BZ receptors, is an important structural lead.[7] As part

Scheme I[a]



[a] (a) Diethyl ethoxymethylenemalonate/toluene/reflux. (b) $POCl_3$/reflux. (c) 4-$ClC_6H_4NHNH_2$/EtOH/reflux. (d) Xylene/reflux or K_2CO_3/*sec*-butyl alcohol/reflux. (e) $R_2C(OR_1)$=$CHCO_2R_1$/toluene/reflux followed by $NaOR_1$/ROH/toluene/reflux. (f) Procedure as in ref 13 using dimethyl acetylenedicarboxylate (DMAD).

of a program designed to identify novel compounds with potential anxiolytic activity, molecular modeling of the potential energy surfaces of known anxiolytic agents which act at central BZ receptors was carried out. These studies, which agree with the literature,[8] suggested that the BZ

(1) Sternbach, L. H. *The Benzodiazepines*; Garattini, S., Mussini, E., Randall, L. O., Eds.; Raven Press: New York, 1973; pp 1–25.
(2) Williams, M. *J. Med. Chem.* **1983**, *26*, 619.
(3) Braestrup, C.; Honoré, T.; Nielsen, M.; Petersen, E. N.; Jensen, H. *Biochem. Pharmacol.* **1984**, *33*, 859.
(4) Takada, S.; Shindo, H.; Sasatani, T.; Chomei, N.; Matsushita, A.; Eigyo, M.; Kawasaki, K.; Murata, S.; Takahara, Y.; Shintaku, H. *J. Med. Chem.* **1988**, *31*, 1738.
(5) Stephens, D. N.; Kehr, W.; Wachtel, H.; Schmiechen, R. *Pharmacopsychiatry* **1985**, *18*, 167.
(6) Petersen, E. N.; Jensen, L. H.; Honoré, T.; Braestrup, C.; Kehr, W.; Stephens, D. N.; Wachtel, H.; Seidelman, D.; Schmiechen, R. *Psychopharmacology* **1984**, *83*, 240.
(7) Yokoyama, N.; Ritter, B.; Neubert, A. D. *J. Med. Chem.* **1982**, *25*, 337.

0022-2623/90/1833-2640$02.50/0