Indolo[2,1-*a*]isoquinolines. Syntheses, Steroid Hormone Receptor Binding Affinities, and Cytostatic Activity

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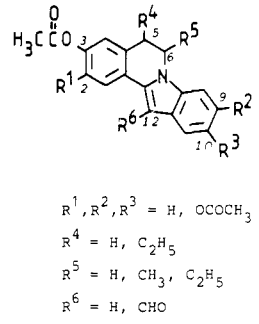
A number of acetoxy-substituted 5,6-dihydroindolo[2,1-*a*]isoquinolines were synthesized and tested for binding affinity for steroid hormone receptors. All of the derivatives bind to the estrogen receptor with RBA values ranging from 1.5 to 17 (17β-estradiol = 100). Some of them show binding affinities for the androgen receptor as well. In the mouse uterine weight test, the tetracycles proved to be weak estrogens with partial antagonistic activity. All of the compounds were tested in vitro for cytostatic activity with hormone-independent MDA-MB 231 and hormone-dependent MCF-7 breast cancer cells. A cytostatic effect was found in both cell lines. The comparison of results exhibited a stronger inhibitory effect on MCF-7 cells only for compounds with high binding affinity for the estrogen receptor. For those derivatives, it can be assumed that the growth inhibition is partly mediated by the estrogen receptor.

The aim of our investigations is the development of new compounds for the treatment of hormone-dependent carcinomas like mammary and prostatic cancer. Since a large fraction of these steroid hormone receptor positive tumors does not respond to antihormonal treatment or has become resistant to it, new agents are needed. In earlier studies,[1] we found that the tetracyclic indoloisoquinoline structure is suitable for the development of drugs binding to the estrogen receptor. The molecular structure is somewhat similar to that of steroidal and nonsteroidal estrogens and might possess the ability to intercalate into DNA.[2] From previous structure–activity studies,[3] we knew that cytostatic activity is associated with the presence of a single acetoxy group in the benzene ring of the indole, whereas the isoquinoline can be substituted with one or two oxygen functions. Studies on 2-phenylindoles have shown that short alkyl chains in position 1 and 3 of the indole improve the receptor affinity.[4] On the basis of these considerations, we have synthesized a number of acetoxy-substituted 5,6-dihydroindolo[2,1-*a*]isoquinolines with alkyl substituents in position 5 and 6 (Chart I) and determined their binding affinities for steroid hormone receptors, their endocrine activity, and their cytostatic effect on hormone-independent MDA-MB 231 and hormone-dependent MCF-7 mammary tumor cells.

Chart I



Chemistry

The substituted 2-phenylethylamines **1** required for the syntheses of the indolo[2,1-*a*]isoquinolines **5** were obtained by modification of a method described previously:[5] nitromethane, nitroethane, or nitropropane was condensed with the respective methoxy-substituted benzaldehyde followed by reduction with $LiAlH_4$. 1-Amino-2-(3-methoxyphenyl)butane (**1d**) was synthesized by ethylation of 2-(3-methoxyphenyl)-1-nitroethane with EtI and 2 equiv of *n*-BuLi and subsequent reduction of the nitro group.

The 2-phenylethylamines **1a–f** were reacted with the methyl bromophenylacetates **2a–c**, which have been described previously,[3] to afford the amides **3a–l** (Scheme I, Table I). Cyclization to the 3,4-dihydroisoquinolines was accomplished by a modified Bischler–Napieralski method with $POCl_3$ in MeCN. The crude products were treated with $NaBH_4$ to give the 1-benzyl-1,2,3,4-tetrahydroisoquinolines **4a–l** (Table II), which were converted to the tetracyclic indolo[2,1-*a*]isoquinolines **5a–l** (Table III) by treatment with NaH in DMSO. The latter reaction must involve a benzyne intermediate[6] because both compounds **2a** and **2b** led to the same structure. The reaction mixture contained the 5,6,12,12a-tetrahydroindoloisoquinolines as byproducts and the 5,6-dihydro compounds **5a–l**, which were separated by column chromatography.

In previous studies we found that a formyl group in position 12 of the tetracycle leads to an additional cytostatic effect in vitro and in vivo.[1] Therefore, this functional group was introduced into **5b** by a Vilsmeier–Haack reaction to give **6** (Scheme II). The methoxy groups in **5a–l** and **6** were cleaved by BBr_3. The resulting phenols were converted into the acetates **7a–l** (Table IV) and **8**, because the free hydroxy derivatives undergo autoxidation when exposed to air. Preliminary experiments have shown that there is no major difference in binding affinities between the hydroxy compounds and their acetates in this series (data not shown). Compound **7c** was oxidized to the aromatic indolo[2,1-a]isoquinoline **9** with Pd/C.

For steric considerations of the interaction of the C-5- and C-6-substituted indoloisoquinolines with steroid hormone receptors, it is of interest whether the substituent is in an axial or equatorial position or whether there is a rapid interconversion of the two possible conformers. The latter possibility was ruled out by NMR studies showing no temperature-dependent alteration of the ^1H NMR spectrum of **5b–d** up to 140 °C. Analysis of their ^1H NMR spectra with respect to the coupling constants for the ABX pattern of the protons at C-5 and C-6 shows that an axial orientation of the substituent is likely. The values for J_{AX} (6 Hz) and J_{BX} (2 Hz) indicate dihedral angles of ap-

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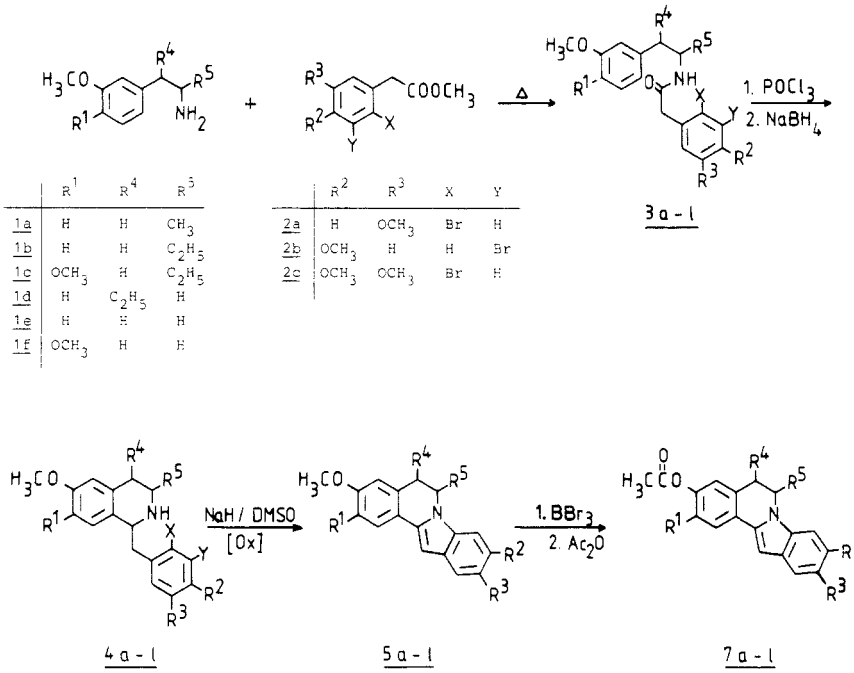
Scheme I



	R^1	R^4	R^5
1a	H	H	CH_3
1b	H	H	C_2H_5
1c	OCH_3	H	C_2H_5
1d	H	C_2H_5	H
1e	H	H	H
1f	OCH_3	H	H

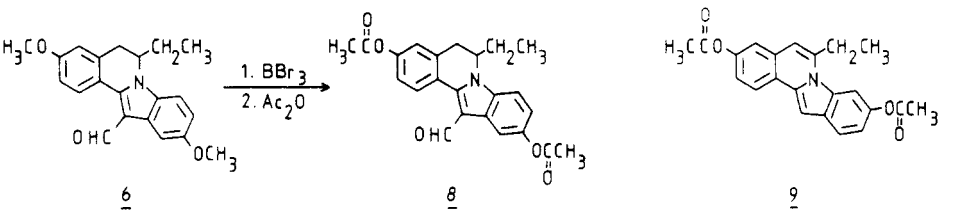
	R^2	R^3	X	Y
2a	H	OCH_3	Br	H
2b	OCH_3	H	H	Br
2c	OCH_3	OCH_3	Br	H

3a-l

4a-l 5a-l 7a-l

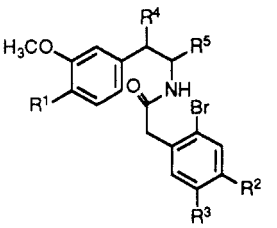
	R^1	R^2	R^3	R^4	R^5
7a	H	H	OAc	H	CH_3
7b	H	H	OAc	H	C_2H_5
7c	H	OAc	H	H	C_2H_5
7d	OAc	H	OAc	H	C_2H_5
7e	H	H	OAc	C_2H_5	H
7f	H	H	OAc	H	H
7g	H	OAc	H	H	H
7h	H	OAc	OAc	H	H
7i	OAc	H	OAc	H	H
7k	OAc	OAc	H	H	H
7l	OAc	OAc	OAc	H	H

Scheme II



6 8 9

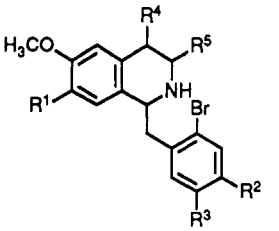
Table I. 2-(Methoxyphenyl)-*N*-[2-(methoxyphenyl)ethyl]acetamides 3



compd	R^1	R^2	R^3	R^4	R^5	% yield	formula[a]	mp,[b] °C
3a	H	H	OCH_3	H	CH_3	46	$C_{19}H_{22}BrNO_3$	111–113
3b	H	H	OCH_3	H	C_2H_5	52	$C_{20}H_{24}BrNO_3$	102
3c[c]	H	OCH_3	H	H	C_2H_5	49	$C_{20}H_{24}BrNO_3$	48
3d	OCH_3	H	OCH_3	H	C_2H_5	45	$C_{21}H_{26}BrNO_4$	114
3e	H	H	OCH_3	C_2H_5	H	55	$C_{20}H_{24}BrNO_3$	74–75

[a] Analyzed for C and H within ±0.40% of the calculated values, except where noted. [b] Recrystallization from EtOAc. [c] Br in the 3-position.

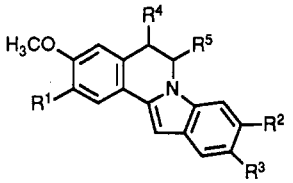
Table II. 1-Benzyl-1,2,3,4-tetrahydroisoquinolines **4**



compd	R^1	R^2	R^3	R^4	R^5	% yield	formula[a]
4a[b]	H	H	OCH_3	H	CH_3	67	$C_{19}H_{22}BrNO_2$
4b	H	H	OCH_3	H	C_2H_5	75	$C_{20}H_{24}BrNO_2$
4c[c]	H	OCH_3	H	H	C_2H_5	69	$C_{20}H_{24}BrNO_2$
4d	OCH_3	H	OCH_3	H	C_2H_5	65	$C_{21}H_{26}BrNO_3$
4e	H	H	OCH_3	C_2H_5	H	80	$C_{20}H_{24}BrNO_2$

[a] All compounds were obtained as oils, except **4a** (mp 58 °C). [b] C: calcd, 60.65; found, 58.62. [c] Br in the 3-position.

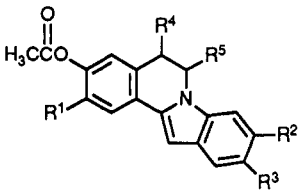
Table III. 5,6-Dihydromethoxyindolo[2,1-*a*]isoquinolines **5**



compd	R^1	R^2	R^3	R^4	R^5	% yield	formula[a]	mp,[b] °C
5a	H	H	OCH_3	H	CH_3	38	$C_{19}H_{19}NO_2$	165
5b	H	H	OCH_3	H	C_2H_5	45	$C_{20}H_{21}NO_2$	100
5c	H	OCH_3	H	H	C_2H_5	41	$C_{20}H_{21}NO_2$	129
5d	OCH_3	H	OCH_3	H	C_2H_5	40	$C_{21}H_{23}NO_3$	132
5e	H	H	OCH_3	C_2H_5	H	30	$C_{20}H_{21}NO_2$	109
5f	H	H	OCH_3	H	H	43	$C_{18}H_{17}NO_2$	208
5g	H	OCH_3	H	H	H	45	$C_{18}H_{17}NO_2$	176
5h	H	OCH_3	OCH_3	H	H	40	$C_{19}H_{19}NO_3$	212
5i	OCH_3	H	OCH_3	H	H	45	$C_{19}H_{19}NO_3$	217
5k	OCH_3	OCH_3	H	H	H	42	$C_{19}H_{19}NO_3$	198
5l[c]	OCH_3	OCH_3	OCH_3	H	H	45	$C_{20}H_{21}NO_4$	209

[a] Analyzed for C and H within ±0.40% of the calculated values, except where noted. [b] Recrystallization from EtOH. [c] Reference 15.

Table IV. Acetoxy-5,6-dihydroindoliso[2,1-*a*]quinolines **7**



compd	R^1	R^2	R^3	R^4	R^5	% yield	formula[a]	mp, °C
7a	H	H	$OCOCH_3$	H	CH_3	63	$C_{21}H_{19}NO_4$	186–187[b]
7b	H	H	$OCOCH_3$	H	C_2H_5	65	$C_{22}H_{21}NO_4$	142[b]
7c	H	$OCOCH_3$	H	H	C_2H_5	74	$C_{22}H_{21}NO_4$	107–108[b]
7d	$OCOCH_3$	H	$OCOCH_3$	H	C_2H_5	52	$C_{24}H_{23}NO_6$	141[b]
7e[d]	H	H	$OCOCH_3$	C_2H_5	H	83	$C_{22}H_{21}NO_4$	158–159[b]
7f	H	H	$OCOCH_3$	H	H	75	$C_{20}H_{17}NO_4$	197–198[c]
7g	H	$OCOCH_3$	H	H	H	80	$C_{20}H_{17}NO_4$	176[c]
7h	H	$OCOCH_3$	$OCOCH_3$	H	H	61	$C_{22}H_{19}NO_6$	182[c]
7i	$OCOCH_3$	H	$OCOCH_3$	H	H	63	$C_{22}H_{19}NO_6$	183–184[c]
7k	$OCOCH_3$	$OCOCH_3$	H	H	H	60	$C_{22}H_{19}NO_6$	170[c]
7l[e]	$OCOCH_3$	$OCOCH_3$	$OCOCH_3$	H	H	60	$C_{24}H_{21}NO_8$	216[c]

[a] Analyzed for C and H within ±0.40% of the calculated values, except where noted. [b] Recrystallization from MeOH. [c] Recrystallization from EtOH. [d] C: calcd, 72.71; found 71.84. [e] Reference 15.

proximately 45° and 75°, which can be rationalized only by an axial position of the substituent.

Because of the chirality of C-6, the substituted compounds are racemic mixtures. The two enantiomers are expected to show different binding affinities for the receptors. Therefore, we separated the enantiomers for on example (**7b**) by HPLC using tribenzoylcellulose as chiral stationary phase and MeOH as eluent. Both enantiomers were obtained and characterized by their melting points.

Binding Affinities for Steroid Hormone Receptors

Because this study was primarily designed to develop agents for treating hormone-dependent mammary tumors, we determined the binding affinities of all compounds with

Table V. Relative Binding Affinities of Acetoxyindolo[2,1-*a*]isoquinolines for the Estrogen (ER), Androgen (AR), and Progesterone (PR) Receptor

compd	ER[a]	AR[a]	PR[a]
7a	12	0.20	0.01
7b	17	0.23	0.13
7c	4.0	2.5	0.05
7d	2.9	<0.01	0.04
7e	2.5	<0.01	<0.01
7f	2.0	<0.01	<0.01
7g	0.16	0.20	0.01
7h	0.01	<0.01	ND[c]
7i	0.15	<0.01	<0.01
7k	0.05	<0.01	ND
7l	<0.01	<0.01	ND
8	4.60	0.03	<0.01
9	1.50	0.01	<0.01

[a] Relative binding affinities for calf uterine receptors = ratio of molar concentrations of unlabeled 17β-estradiol, testosterone or progesterone and inhibitor required to decrease the amount of the respective radioactive hormone (17β-estradiol, mibolerone, or Org 2058) by 50%, × 100. The results represent the mean of two experiments each performed in triplicate. [b] (+)-**7b** and (−)-**7b** show identical RBA values for ER: 17. [c] ND = not determined.

acetoxy groups for the estrogen receptor (ER). The affinities were determined in a competitive binding assay by using [^{3}H]17β-estradiol as tracer and calf uterine cytosol as receptor source.[4] The dextran-coated charcoal (DCC) method was used. The relative binding affinities (RBA) are given as the ratio of the molar concentrations of 17β-estradiol and indoloisoquinoline required to decrease the receptor-bound radioactivity by 50%, multiplied by 100.

All of the indoloisoquinolines without alkyl substituents showed only weak affinities for the estrogen receptor except derivative **7f** (RBA = 2.0), which contains acetoxy groups in position 3 and 10 (Table V). For further structural modifications, only derivatives with substituents leading to RBA values above 0.1 were used. Introduction of a methyl or ethyl group into position 5 or 6 increased the binding affinities considerably. The compound with greatest affinity (RBA = 17) was **7b**, which contains oxygen functions at C-3 and C-10 and an ethyl group at C-6. Therefore, the binding affinities of the enantiomers of **7b** were determined. Both isomers showed identical affinities for the estrogen receptor. The introduction of a formyl group into position 12 (**8**) decreased the binding affinity of **7b** only slightly. The fully aromatic system (**9**) also had affinity for the estrogen receptor.

In order to get more insight in the biological properties of these new compounds, the binding affinities to the androgen (AR) and progesterone receptors (PR) were determined. As a matter of convenience, calf uterine cytosol was used for these assays,[7] since it has been demonstrated that uteri contain similar amounts of AR as prostates.[8] For the determination of the androgen receptor, the progesterone receptor was masked by triamcinolone acetonide,[9] and sodium molybdate[10] was added to stabilize the androgen receptor. Because of the metabolic instability of testosterone, [^{3}H]mibolerone was used as radioligand. In the progesterone receptor assay the synthetic progestin [^{3}H]Org 2058 was used as radioactive tracer.

Usually, nonsteroidal compounds binding to the ER show little affinity for PR[7,11] or AR.[7,12] Thus, it was not surprising that the tested indoloisoquinolines **7a–e**, **8**, and **9** showed little affinity for the progesterone receptor. However, some derivatives (**7a–c**, **7b**) exhibited affinities for the androgen receptor (Table V). A similar observation was made with 17β-estradiol.[11] The highest RBA value was found for **7c** (RBA = 2.5). All compounds possess binding curves parallel to those of the reference hormones, indicating a competitive inhibition.

Table VI. Estrogenic and Antiestrogenic Activity of 5,6-Dihydroindolo[2,1-*a*]isoquinolines in the Mouse Uterine Weight Test

	uterotrophic test		antiuterotrophic test		
compd	dose,[a] μg	effect[b]	dose,[a,c] μg	effect[b]	% inhibn[d]
control		21.9 ± 2.7		21.9 ± 2.7	
7a	2.0	21.2 ± 4.1	2.0	52.6 ± 5.0	−8.0
	10.0	26.3 ± 3.5	10.0	56.0 ± 3.4	−21.0
	50.0	53.3 ± 5.5	50.0	61.2 ± 7.9	−39.0
	250.0	72.2 ± 7.3	250.0	61.1 ± 11.3	−39.0
estrone	0.4	50.2 ± 3.1	0.4	50.2 ± 3.1	
control		14.7 ± 2.7		17.1 ± 2.6	
7b	2.0	18.5 ± 2.9	2.0	52.3 ± 8.9	7.2
	10.0	25.5 ± 5.9	10.0	51.0 ± 6.7	12.2
	50.0	35.8 ± 6.0	50.0	45.7 ± 3.8	25.9[f]
	250.0	53.4 ± 3.4	125.0	39.5 ± 5.1	42.0[f]
			250.0	42.6 ± 5.1	34.1[f]
estrone	0.4	53.4 ± 3.3	0.4	55.8 ± 5.6	
control		15.5 ± 1.9		15.5 ± 1.9	
7c	2.0	11.5 ± 1.6	2.0	49.6 ± 4.5	2.8
	10.0	15.7 ± 1.7	10.0	44.5 ± 5.4	17.4[e]
	50.0	18.5 ± 1.5	50.0	39.9 ± 5.7	31.0[f]
	250.0	40.5 ± 3.6	250.0	48.2 ± 5.4	6.8
estrone	0.4	50.6 ± 5.7	0.4	50.6 ± 5.7	
control		17.2 ± 2.6		17.2 ± 2.6	
7d	2.0	21.2 ± 3.4	2.0	51.3 ± 7.0	11.7
	40.0	42.8 ± 6.9	40.0	49.7 ± 5.6	15.7
	125.0	55.8 ± 4.2	125.0	58.1 ± 3.9	
estrone	0.4	55.8 ± 5.6	0.4	55.8 ± 5.6	
control		14.7 ± 2.7		15.5 ± 1.9	
7e	2.0	16.8 ± 3.2	2.0	50.9 ± 4.4	
	10.0	21.5 ± 4.0	10.0	44.1 ± 4.4	18.7[f]
	50.0	24.1 ± 4.1	50.0	42.5 ± 4.2	23.3[f]
	250.0	46.1 ± 5.3	250.0	42.7 ± 5.9	22.4[f]
estrone	0.4	50.9 ± 7.2	0.4	50.9 ± 7.2	
control		17.1 ± 2.6		17.1 ± 2.6	
8	2.0	14.5 ± 3.6	2.0	48.2 ± 3.9	19.6[f]
	40.0	38.2 ± 7.8	40.0	54.7 ± 5.3	2.6
	125.0	56.0 ± 6.7	125.0	54.7 ± 3.5	2.7
estrone	0.4	55.8 ± 5.6	0.4	55.8 ± 5.6	

[a] Dose per animal, administered on three consecutive days sc. [b] Uterus dry weight (mg)/body weight (g) × 100, determined 24 h after the last injection; mean of 10 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. [e] Significant ($p < 0.05$). [f] Significant ($p < 0.01$).

Endocrine Activity

Six derivatives of acetoxy-5,6-dihydroindoloisoquinolines with relatively high binding affinities for the estrogen receptor (**7a–e**, **8**) were tested for their estrogenicity. In this assay, immature mice received various doses of the drug, and the increase in uterine dry weight was measured.[13] 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. The dose of 0.4 μg was chosen on the basis of the dose–response curve of estrone and is high enough to produce full uterotrophic response.[14]

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Table VII. Inhibitory Effect of Compounds 7a–c on Seminal Vesicle Weight of Intact and Castrated Adult Mice

compd	dose,[a] μg	relative seminal vesicle weight[b]	% inhibn[c]
control[f]		198.5 ± 45.2	
7a	10	195.1 ± 51.6	1
	100	144.6 ± 35.4	27[e]
	1000	45.2 ± 8.0	77[d]
control[f]		170.0 ± 28.3	
7b	10	132.0 ± 49.8	22
	100	54.5 ± 14.8	67[d]
	1000	38.3 ± 5.0	77[d]
DES	100	55.0 ± 11.0	67[d]
control[f]		198.5 ± 45.2	
7c	100	156.1 ± 17.9	21[e]
	1000	121.3 ± 22.4	39[d]
standard[g]		227.6 ± 39.4	
control[f]		211.4 ± 48.4	
7b[g]	1000	187.4 ± 27.6	17
7c[g]	1000	200.0 ± 27.8	12

[a] Dose/animal per day. [b] Organ weight (mg)/body weight (g) × 100. Mean ± SD. [c] % inhibition = [1 − (treated/control)] × 100. [d] Significant ($p < 0.01$). [e] Significant ($p < 0.05$). [f] Intact control: noncastrated animals. [g] Castrated animals supplemented with 0.3 mg of TP/animal per day.

All of the compounds tested exhibited estrogenic activity (Table VI), but except for **7a**, rather high doses (125–250 μg/animal) had to be administered to equal the effect of the reference drug estrone.

In the antiuterotrophic assay, a weak but significant antagonism was found with the derivatives **7b**, **7c**, **7e**, and 8.

Because of their binding affinities for the androgen receptor, the indoloisoquinolines **7a–c** were tested for their ability to decrease the weights of seminal vesicles in intact or castrated, testosterone-supplemented mice. A comparison of results in these two assays allows one to distinguish between a central action on testosterone biosynthesis and a direct antiandrogenic effect in the target organ. A strong reduction of weight was observed only in intact animals, whereas the effect in castrated, testosterone-supplemented animals was neglegible (Table VII). These findings suggest that a central mode of action is likely.

Cytostatic Activity

Because the aim of this study is the development of heterocycles with a selective action on estrogen receptor positive tumors, we determined the activity of a number of indoloisoquinolines with binding affinity for the estrogen receptor on hormone-independent MDA-MB 231 and hormone-dependent MCF-7 mammary tumor cells of human origin. At a concentration of 10^{-5} M, all compounds except **7d** and **7e** showed a marked cytostatic effect in both cell lines. A possible toxic effect is rather unlikely since the number of survivant MDA cells did not drop below the initial cells number during incubation. At 10^{-6} M, some of the derivatives (**7a–c**, 8) inhibited the growth of MCF-7 cells, whereas the activity in MDA-MB 231 cells was low (Table VIII). These compounds possess relatively high binding affinities for the estrogen receptor.

Discussion

The first aim of these investigations was the sythesis of structurally new drugs that bind to the estrogen receptor. We used the tetracyclic indolo[2,1-*a*]isoquinolines as the basic structure and introduced acetoxy groups into various positions of the aromatic rings and alkyl substituents into their heterocycles. Studies of structure–activity relationships revealed that the best conditions for binding to the estrogen receptor are provided by acetoxy groups in position 3 and 10 and an ethyl group at C-6 (**7b**, RBA = 17). The comparison of molecular models shows that the distance between the two oxygen functions in **7b** is close to that of estradiol. The short alkyl chain in position 6 is probably necessary for hydrophobic interaction between the drug and the receptor protein. Interestingly, the two enantiomers show the same binding affinity for the estrogen receptor after chromatographic separation. Since we assume an axial orientation for the ethyl group from NMR studies, the steric requirements above and below the plane of the tetracycle either must be rather similar, or this part of the receptor site is very flexible. The latter assumption is in accordance with results obtained with benzo[*a*]carbazole derivatives.[16] Of course, a rotation of the molecule with the oxygens as pivots cannot be ruled out. Studying the specificity of binding we found that some of the derivatives with affinity for the estrogen receptor bind to the androgen receptor as well. This result was unexpected because the lack of selectivity is rather rare in the case of nonsteroidal estrogens.[17] The binding to the androgen receptor is favored when the acetoxy groups are located in positions 3 and 9 instead of 3 and 10 in the case of the estrogen receptor. Conversion of the derivative **7c** to the aromatic compound **9** led only to a slight decrease in affinity. The same observation was made in the benzo[*a*]carbazole series.[16]

In the mouse uterine weight test, all compounds with relatively high binding affinity for the estrogen receptor produced an estrogenic response at high doses. Some of the derivatives showed weak but significant antiestrogenic effects. The affinity for the androgen receptor of some of the compounds prompted us to look for an antiandrogenic effect. This effect was only observed with intact male mice but not in castrated, testosterone-supplemented animals. Therefore, we assume that the antiandrogenic effect that we observe is due to the antigonatrophic effect generally found with estrogens in male animals.

Since the objective of this work is the development of drugs with a specific cytostatic action on estrogen receptor positive tumors, we compared the effects of the indoloisoquinolines in two different cell lines. One line contains estrogen receptors (MC-7) and the other one lacks them (MDA-MB 231). At a concentration of 10^{-5} M, a strong cytostatic effect was observed for all compounds in both cell lines. We assume that the inhibition of cellular growth at concentrations greater 10^{-6} M is mainly due to a nonspecific effect. However, at 10^{-6} M, some of the derivatives exert a higher effect on MCF-7 cells than on hormone-independent MDA-MB 231 cells. Together with the receptor affinity and endocrine activity of these compounds, this observation suggests that a receptor-mediated mode of action is likely. The mode of the nonspecific cytostatic action can possibly be an intercalation of the indoloisoquinolines into DNA as described for other tetracyclic structures.[2] Further studies will be carried out to elucidate this effect.

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, and

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Table VIII. Effect of **7a–e**, **8**, and **9** on the Growth of MDA-MB 231 and MCF-7 Cells

		MDA-MB 231		MCF-7	
compd	concn, M	cell no.: % T/C[a]	[^{3}H]thymidine incorp: % T/C[a]	cell no.: % T/C[a]	[^{3}H]thymidine incorp: % T/C[a]
7a	1×10^{-5}	5	5	7	2
	5×10^{-6}	10	10	ND[d]	ND
	1×10^{-6}	78	65	55	36
7b	1×10^{-5}	4	4	8	2
	5×10^{-6}	4	4	10	4
	1×10^{-6}	74	45	45	25
	1×10^{-7}	ND	ND	80	63
7c	1×10^{-5}	11	8	6	2
	5×10^{-6}	11	11	39	24
	1×10^{-6}	98[c]	93[c]	67	42
7d	1×10^{-5}	51	38	22	3
	5×10^{-6}	62	35	39	11
	1×10^{-6}	100	77	89[b]	95[c]
7e	1×10^{-5}	45	25	31	7
	5×10^{-6}	88[b]	90[b]	97[c]	95[c]
8	1×10^{-5}	14	7	13	3
	1×10^{-6}	75	65	55	41
9	1×10^{-5}	5	3	5	3
	5×10^{-6}	8	7	ND	ND
	1×10^{-6}	65	80	75	70
tamoxifen	1×10^{-5}	94[c]	97[c]	17	5
	1×10^{-6}	107[c]	100[c]	51	46

[a] % T/C = test compound/control, × 100; mean of three experiments with six dishes. Significant inhibition ($p < 0.01$), except where noted. [b] Significant ($p < 0.05$). [c] Not significant. [d] ND = not determined.

were within ±0.40% of the calculated values except where noted. NMR spectra were obtained on a Varian EM 306L and a Bruker WM250 spectrometer and are consistent with the assigned structures.

2-Amino-1-(3-methoxyphenyl)butane (1b). 3-Methoxybenzaldehyde (82 g, 0.6 mol), nitropropane (53 g, 0.6 mol), anhydrous NaOAc (50 g, 0.6 mol), methylammonium chloride (40 g, 0.6 mol), and trimethyl orthoformate (64 g, 0.6 mol) in 700 mL of dry MeOH were refluxed for 5 h. After cooling, the mixture was poured into ice water and extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4) and the solvent was evaporated. The residue was chromatographed over SiO_2 with CH_2Cl_2 to give yellow crystals (68.3 g, 55%). The 1-(3-methoxyphenyl)-2-nitrobut-1-ene (0.11 mol) was dissolved in dry ether (450 mL) and dry THF (120 mL); $LiAlH_4$ (0.2 mol) was added slowly at 0 °C. The reaction mixture was allowed to come to room temperature and was stirred for 4 h. The excess $LiAlH_4$ was destroyed by the addition of a NH_4Cl solution. The precipitate was filtered and washed with CH_2Cl_2. The organic layer was dried (Na_2SO_4) and the solvent was evaporated. The resulting oil was used without further purification (55%): IR (film) 3380, 3300 (NH) cm^{-1}; NMR ($CDCl_3$) δ 0.97 (t, J = 7 Hz, 3 H, CH_3), 1.36–3.03 (m, 5 H, –CH–, –CH_2–), 1.53 (s, 2 H, NH_2), 3.87 (s, 3 H, OCH_3), 6.77–7.35 (m, 4 H, ArH).

2-Amino-1-(3,4-dimethoxyphenyl)butane (1c). Compound **1c** was prepared in the same way as **1b**: colorless oil; IR (film) 3380, 3300 (NH) cm^{-1}; NMR ($CDCl_3$) δ 0.97 (t, J = Hz, 3 H, CH_3), 1.27–3.23 (m, 7 H, –CH–, –CH_2–, NH_2), 3.91 (s, 6 H, OCH_3), 6.80–7.03 (m, 3 H, ArH).

2-(3-Methoxyphenyl)-1-nitroethane. 3-Methoxy-β-nitrostyrene[5] (20 g, 110 mmol) in 185 mL of dioxane was added to a suspension of $NaBH_4$ (9.4 g, 0.25 mol) in dioxane (185 mL) and EtOH (60 mL) within 20 min at 30 °C. The reaction mixture was stirred for 45 min at room temperature and then treated with 50 mL of ice water and 50% acetic acid. After evaporation of the organic solvent, the mixture was extracted with CH_2Cl_2. The organic layer was washed with water and saline and dried (Na_2SO_4). After evaporation, the residue was chromatographed (SiO_2, CH_2Cl_2) to give a colorless oil (19.5 g, 95%): IR (film) 1550, 1350 cm^{-1}; NMR ($CDCl_3$) δ 3.30 (t, J = 7 Hz, 2 H, –CH_2–), 3.86 (s, 3 H, OCH_3), 4.66 (t, J = 7 Hz, 2 H, –CH_2–), 6.80–6.95 (m, 3 H, ArH), 7.20–7.32 (m, 1 H, ArH). Anal. ($C_{11}H_{15}NO_3$) C, H.

2-(3-Methoxyphenyl)-1-nitrobutane. 2-(3-Methoxyphenyl)-1-nitroethane (11 g, 60 mmol) was dissolved in 360 mL of dry THF and DMPU (*N,N'*-dimethyl-*N,N'*-propyleneurea) (60 mL) was added. *n*-BuLi (52 mL, 120 mmol, 2.3 M in hexane) was added under N_2 at –70 °C within 30 min. The reaction mixture was stirred for 30 min at –78 °C. Then EtI (4.9 mL, 60 mmol) was added and the mixture was allowed to come to –30 °C within 4 h. After cooling to –70 °C, acetic acid (30 mL) was added. The mixture was poured into 500 mL of water, and the aqueous phase was extracted with ether. After drying (Na_2SO_4) and evaporation of the solvent, the residue was chromatographed (SiO_2, hexane/ether 3:1) to give a colorless oil (5.6 g, 42%): NMR ($CDCl_3$) δ 0.87 (t, J = 7 Hz, 3 H, CH_3), 1.71 (quin, J = 7 Hz, 2 H, CH_2CH_3), 3.32 (quin, J = 7 Hz, 1 H, –CH–), 3.80 (s, 3 H OCH_3), 4.55 (d, J = 7 Hz, 2 H, –CH_2–), 6.73–6.90 (m, 3 H, ArH), 7.14–7.38 (m, 1 H, ArH).

1-Amino-2-(3-methoxyphenyl)butane (1d). 2-(3-Methoxyphenyl)-1-nitrobutane (20.8 g, 0.1 mol) in dry ether (100 mL) was added to a suspension of $LiAlH_4$ (7.6 g, 0.2 mol) in refluxing dry ether (170 mL). The reaction mixture was refluxed for 1 h. After cooling and the usual workup, the resulting oil was chromatographed (SiO_2, EtOAc). The yield of the colorless oil was 7.1 g (40%): IR (film) 3380, 3300 (NH) cm^{-1}; NMR ($CDCl_3$) δ 0.83 (t, J = 7 Hz, 3 H, CH_3), 1.40–2.10 (m, 4 H, CH_2CH_3, NH_2), 2.45 (q, J = 7 Hz, 1 H, –CH–), 2.75–3.26 (m, 2 H, –CH_2–), 6.73–6.90 (m, 3 H, ArH), 7.14–7.38 (m, 1 H, ArH).

General Procedure for the Synthesis of the Acetamides 3a–e. A flask containing (methoxyphenyl)ethylamine **1** (0.75 mol) and methyl bromophenylacetate **2** (0.75 mol) was placed into a hot-oil bath. The temperature was kept at 150–155 °C for 10 h. After cooling to 35 °C, 10 mL of EtOAc was added with stirring. The product that crystallized at 4 °C was filtered and washed with ether. Recrystallization from EtOAc yielded colorless crystals. Melting points and yields and reported in Table I.

Compounds **3f–l** have been described previously.[3]

General Procedure for the Synthesis of the 1-Benzyl-1,2,3,4-tetrahydroisoquinolines 4a–e. A mixture of acetamide **3** (55 mmol), $POCl_3$ (20 mL), and dry MeCN (75 mL) was refluxed for 4 h. With cooling, 150 mL of 20% NaOH solution was added. The mixture was poured into ice water and extracted with $CHCl_3$. The $CHCl_3$ solution was extracted with 150 mL of 2 N HCl. The free base was liberated with 20% NaOH and extracted with $CHCl_3$. The organic layer was washed with water and saline and dried (Na_2SO_4). The 3,4-dihydroisoquinolines obtained after evaporation of the solvent were used without further purification.

$NaBH_4$ (4.2 g, 0.11 mol) was added slowly to a solution of the 3,4-dihydroisoquinoline (13.4 mmol) in 100 mL of MeOH and 15 mL of water at 0 °C. The mixture was stirred for 2 h at room temperature. After the solvent had been removed, the residue was treated with 100 mL of water and extracted with $CHCl_3$. The organic layer was washed with water and dried (Na_2SO_4). After evaporation of the solvent, the residue was purified by chromatography (SiO_2, $CHCl_3$/ether 1:1) to give a colorless oil. Melting

points and yields are reported in Table II.

Compounds **4f–l** have been described previously.[3]

General Procedure for the Ring Closure of the Bromotetrahydrobenzylisoquinolines to the 5,6-Dihydroindolo[2,1-a]isoquinolines 5a–e. A solution of bromotetrahydroisoquinoline **4** (10 mmol) in 40 mL of DMSO was added to a solution of sodium methylsulfinylmethanide [prepared from 2.1 g (70 mmol) of NaH (80% in oil dispersion) and 40 mL of DMSO]. After stirring had been continued for 15 h, the mixture was poured into 400 mL of water containing an excess of NH_4Cl, followed by extraction with $CHCl_3$. The organic layer was washed with water and saline. After drying (Na_2SO_4) and evaporation of the solvent, an oil was obtained, which was chromatographed (SiO_2, CH_2Cl_2). The first fraction (R_f 0.7) contained the dihydroindoloisoquinolines. Recrystallization from EtOH yielded colorless crystals. Melting points and yields are reported in Table III.

Compounds **5f–l** have been described previously.[3]

6-Ethyl-12-formyl-5,6-dihydro-3,10-dimethoxyindolo[2,1-a]isoquinoline (6). Dry DMF (0.6 mL) was added slowly to $POCl_3$ (0.9 mL) at 10–20 °C under N_2. The mixture was stirred for 5 min at this temperature. **5b** (1.0 mmol) dissolved in 5 mL of dry DMF, was added slowly to keep the temperature below 35 °C. After stirring for 30 min at 35 °C, the reaction mixture was poured into 30 mL of ice water. The aqueous layer was basified (NaOH) and extracted with $CHCl_3$. After washing with water and drying (Na_2SO_4), the solvent was removed in vacuo. The product was recrystallized from MeOH to give slightly yellow crystals (2.0 g, 58%): mp 201 °C; IR (KBr) 1650 cm^{-1}; NMR ($CDCl_3$) δ 0.80 (t, J = 7 Hz, 3 H, CH_3), 1.59 (mc, 2 H, CH_2CH_3), 3.00 (d,, $J_{1,2}$ = 16, 2 Hz, 1 H, CH*H*), 3.40 (dd, $J_{1,2}$ = 16, 6 Hz, 1 H, C*H*H) 3.90 (s, 3 H, OCH_3), 4.00 (s, 3 H, OCH_3), 4.50 (mc, 1 H, –CH–), 6.90–7.00 (m, 2 H, arH), 7.10 (d, J = 2 Hz, 1 H, ArH), 7.30 (d, J = 9 Hz, 1 H, ArH), 7.90 (d, J = 9 Hz, 1 H, ArH), 8.00 (s, 1 H, ArH), 10.60 (s, 1 H, CHO). Anal. ($C_{21}H_{21}NO_3$) C, H, N.

General Procedure for Ether Cleavage and Acetylation. A solution of the methoxy-substituted indoloisoquinoline **5** (0.03 mol) in dry CH_2Cl_2 (5 mL) was cooled to –15 °C and BBr_3 (0.1 mL) was added under N_2. After 30 min, the cooling bath was removed and the mixture was stirred for 15 h. With cooling, the mixture was poured into 10 mL of an aqueous solution of $NaHCO_3$. Then, 25 mL of EtOAc was added and the mixture was stirred for 15 min. The organic layer was separated, and the aqueous phase was extracted with EtOAc. The combined organic layers were washed with saline and dried (Na_2SO_4). After the solvent was removed, the residue was treated with Ac_2O (6 mL) and pyridine (1 mL). After refluxing for 2 h, the mixture was poured onto ice, stirred for 10 min, and extracted with CH_2Cl_2. The organic layer was washed with water and dried (Na_2SO_4). After evaporation, the remaining residue was chromatographed (SiO_2, CH_2Cl_2). Recrystallization from MeOH yielded **7** as colorless crystals. Melting points and yields are reported in Table IV.

Chromatographic Separation of the Enantiomers of 7b. The enantiomers of **7b** were separated semipreparatively by HPLC on tribenzoylcellulose (5–19 μm)[18] with MeOH as eluent at 22 °C. They were characterized by their capacity factors: k_- = 6.6; k_+ = 10.0. The (–)-enantiomer was characterized by its melting point of 157.5–159 °C, its specific rotations of –320° ± 140° (365 nm) and –57° ± 31° (436 nm) in EtOH, and HPLC analysis giving an enantiomeric purity close to 100%. The amount of the (+)-enantiomer isolated was too small to measure its specific rotation. The melting point was 152–155 °C; the purity was determined by HPLC.

3,10-Diacetoxy-6-ethyl-12-formyl-5,6-dihydroindolo[2,1-a]isoquinoline (8). Compound **8** was prepared from **6** by ether cleavage and acetylation: colorless crystals; mp 168 °C; IR (KBr) 1770 ($OCOCH_3$), 1650 (CO) cm^{-1}; NMR ($CDCl_3$) δ 0.93 (t, J = 7 Hz, 3 H, CH_3), 1.60 (mc, 2 H, CH_2CH_3), 2.34 (s, 6 H, OCH_3), 3.03 (dd, $J_{1,2}$ = 16, 2 Hz, 1 H, C*H*H), 3.40 (dd, $J_{1,2}$ = 16, 6 Hz, 1 H, CH*H*), 4.55 (m, 1 H, –CH–), 7.00–7.25 (m, 3 H, ArH), 7.37 (d, J = 9 Hz, 1 H, ArH), 8.00 (d, J = 9 Hz, 1 H, ArH), 8.17 (d, J = 2 Hz, 1 H, ArH), 10.50 (s, 1 H, CHO). Anal. ($C_{23}H_{21}NO_5 \cdot H_2O$) C, H, N.

3,9-Diacetoxy-6-ethylindolo[2,1-a]isoquinoline (9). Compound **7c** (1.3 mmol) and Pd/C 10% (150 mg) were mixed thoroughly in an agate mortar. This and all of the following operations were carried out under N_2. A flask containing the mixture was placed in an oil bath at 120 °C. The mixture was kept at this temperature for 30 min. After stirring with a spatula, heating was continued for 30 min. After cooling, the mixture was dissolved in CH_2Cl_2 and filtered. The solvent was evaporated and the residue was chromatographed (SiO_2, CH_2Cl_2). Recrystallization from MeOH afforded yellow-green crystals (0.7 g, 15%): mp 154 °C; IR (KBr) 1770 (CO) cm^{-1}; NMR ($CDCl_3$, 250 MHz) δ 1.52 (t, J = 7 Hz, 3 H, CH_3), 2.35 (s, 3 H, $OCOCH_3$), 2.37 (s, 3 H, $OCOCH_3$), 3.31 (q, J = 7 Hz, 2 H, CH_2CH_3), 6.45 (s, 1 H, ArH), 7.10 (dd, $J_{1,2}$ = 8.6, 1.9 Hz, 1 H, ArH), 7.17 (dd, $J_{1,2}$ = 8.6, 2.3 Hz, 1 H, ArH), 7.25 (mc, 2 H, ArH), 7.79 (d, J = 9 Hz, 1 H, ArH), 7.83 (d, J = 1.9 Hz, 1 H, ArH), 8.13 (d, J = 9 Hz, 1 H, ArH). Anal. ($C_{22}H_{19}NO_4$) C, H, N.

Biochemical and Biological Methods. Reagents. [2,4,6,7-^{3}H]Estradiol (110 Ci/mmol) and [^{3}H]thymidine (80 Ci/mmol) were obtained from New England Nuclear, Dreieich, FRG. Hormones and biochemicals were purchased from Sigma, München, FRG. [^{3}H]Mibolerone and [^{3}H]Org 2058 were obtained from Amersham, Braunschweig, FRG. The purity of radiolabeled hormones was determined by HPLC followed by liquid scintillation counting.

Binding Affinities for Steroid Hormone Receptors. ER[4]-, AR[19]- and PR[20]-affinities were determined with calf uterine cytosol as receptor source. Fresh calf uteri, stored in ice-cold saline, were freed of adherent fat and connective tissue at 4 °C. After addition of the relevant buffer (10 mM Tris, 1.5 mM EDTA, 3 mM NaN_3, pH 7.4, for ER; 10 mM Tris, 1.5 mM EDTA, 20 mM Na_2MoO_4, glycerol 10%, pH 7.4, for Ar; 10 mM Tris, 1.5 mM EDTA, 0.25 M sucrose, pH 7.4, for PR) the uteri were homogenized by treatment with a ultraturrax mixer (IKA, FRG) 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 100 min (0 °C). The supernatant (cytosol) was then used for determining the affinity of compounds for the receptor. The protein concentration of the cytosol was ca. 15 mg/mL, leading to a final concentration of 3 mg/mL in the assay. Relative binding affinities were determined by the dextran-coated charcoal method: 100-μL aliquots of the cytosol were incubated with 100 μL (1 nM) of [^{3}H]estradiol (ER), [^{3}H]mibolerone (AR), or [^{3}H]Org 2058 (PR) and different concentrations of the test compounds at 0–4 °C for 16 h (ER) or 2 h (AR, PR). Nonspecific radioligand binding was determined by a parallel incubation containing 2 μM of estradiol (ER), testosterone (AR), or progesterone (PR). After incubation, a dextran-coated-charcoal suspension (0.625% dextran 80.000, 1.25% Norit A in the relevant buffer) was added and the mixture was shaken for 90 min (ER) or 10 min (AR, PR) at 0–4 °C. After centrifugation for 10 min at 800g, the radioactivity of a 100-μL supernatant aliquot was counted. The percentage of bound radioligand was plotted against the concentration of unlabeled test compounds. A standard curve for unlabeled estradiol, testosterone, or progesterone was included in each assay. Four to six concentrations of each competitor were tested. They were chosen to provide a linear portion on a semilogarhythmic plot crossing the point of 50% competition. The RBA was calculated as the ratio of the molar concentrations of hormone and test compound required to decrease the amount of bound radioactivity by 50%, multiplied by 100.

Immature Mice Uterine Weight Test. 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/0.9% saline (1:1; 100 μL/animal) and injected subcutaneously on three consecutive days. Control animals received the vehicle alone. Twenty-four 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 (saturated

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aqueous picric acid/40% formaldehyde/glacial acetic acid 15:5:1 by vol) 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 uterotrophic effect was calculated by the following formula: uterine dry weight (mg)/body weight (g), multiplied by 100.

To determine the antiestrogenic activity, injections contained a standard dose (0.4 μg) of estrone and increasing doses of the compounds. The inhibition (%) of the estrone-stimulated uterine growth was estimated by the following formula: $[100 - [E_{S,T} - E_V)/(E_S - E_V)]] \times 100$ (E_S = effect of estrone standard; $E_{S,T}$ = effect of standard with simultaneous administration of test compound; E_V = effect of vehicle).

Inhibitory Effect on the Seminal Vesicle Weight of Mice. For these experiments, intact or castrated adult male mice (23–25 g at beginning of test, six mice/group, from Ivanovas, Kissleg, FRG) were used.[21] In the experiments with castrated mice, animals were castrated via the scrotal route under ether anaesthesia 5 days before the start of the experiment. A daily dose of 0.3 mg of testosterone propionate (TP) was used to restore accessory sex organ weights in castrated animals to intact levels. Intact and castrated mice were injected with the test compounds dissolved in olive oil for nine consecutive days. Twenty-four hours after the last injection, the mice were killed by cervical dislocation. Seminal vesicles were removed, dissected free from adhering fat and tissue, fixed in Bouin solution, dried overnight at 100 °C, and weighed.

MCF-7 Human Breast Cancer Cells. The MCF-7 cell line was kindly provided by Dr. M. E. Lippman, NCI, Bethesda, MD. Cells were grown in improved minimal essential medium (MEM), as modified by Richter et al.[22] (Biochrom, Berlin, FRG), supplemented with glutamine (0.3 g/L), gentamycin (60 mg/L), and 5% newborn calf serum (NCS) (Gibco) or charcoal-treated NCS (CCS). CCS was prepared by incubation of 500 mL of NCS with a dextran-coated-charcoal pellet[23] for 4 h in a 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 filtered through a 0.20-μm filter (Sartorius, Göttingen, FRG) and stored at −20 °C.

Cells were grown in a humified 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/0.02% EDTA in 0.15 M NaCl. They were syringed gently to prevent clumping, and approximately 2×10^4 cells in 2 mL were plated replicately in six-well dishes (Costar). One day later, cells were switched to a medium containing the substances and 0.1% DMSO in which the compounds had been dissolved. The medium of control wells contained an equal volume of DMSO. On the 4th day, media were changed and substances were added again. Three days later, cells were labeled 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 into two equal parts. One part was counted in a ZM 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 (Sartorius) 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 generously provided by Dr. M. E. Lippman. 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.

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Registry No. **1a**, 17862-91-8; **1b**, 35149-78-1; **1c**, 123406-73-5; **1d**, 123312-25-4; **2a**, 117565-90-9; **2b**, 19626-36-9; **3a**, 123312-26-5; **3b**, 123312-27-6; **3c**, 123312-28-7; **3d**, 123312-29-8; **3e**, 123312-30-1; **4a**, 123312-31-2; **4b**, 123312-32-3; **4c**, 123312-33-4; **4d**, 123312-34-5; **4e**, 123312-35-6; **5a**, 123312-36-7; **5b**, 123312-37-8; **5c**, 123312-38-9; **5d**, 123312-39-0; **5e**, 123312-40-3; **5f**, 117565-94-3; **5g**, 117566-06-0; **5h**, 117566-07-1; **5i**, 117566-08-2; **5k**, 117566-09-3; **6**, 123330-35-8; **7a**, 123312-41-4; **7b**, 123312-42-5; (∓)-**7b**, 123312-43-6; (±)-**7b**, 123312-44-7; **7c**, 123312-45-8; **7d**, 123330-36-9; **7e**, 123312-46-9; **7f**, 123312-47-0; **7g**, 123312-48-1; **7h**, 123312-49-2; **7i**, 123312-50-5; **7k**, 123312-51-6; **7l**, 73053-74-4; **8**, 123330-37-0; **9**, 123330-38-1; 3-methoxybenzaldehyde, 591-31-1; nitropropane, 25322-01-4; 1-(3-methoxyphenyl)-2-nitrobut-1-ene, 23037-57-2; 3,4-dimethoxybenzaldehyde, 120-14-9; 2-(3-methoxyphenyl)-1-nitroethane, 123312-23-2; 3-methoxy-β-nitrostyrene, 3179-09-7; 2-(3-methoxyphenyl)-1-nitrobutane, 123312-24-3.

Supplementary Material Available: ^{1}H NMR data of acetamides **3a–e**, 1-benzyl-1,2,3,4-tetrahydroisoquinolines **4a–e**, 5,6-dihydroindolo[2,1-*a*]isoquinolines **5a–e**, and acetoxy-5,6-dihydroindolo[2,1-*a*]isoquinolines **7a–k** (5 pages). Ordering information is given on any current masthead page.

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