2-Phenylindoles. Relationship between Structure, Estrogen Receptor Affinity, and Mammary Tumor Inhibiting Activity in the Rat

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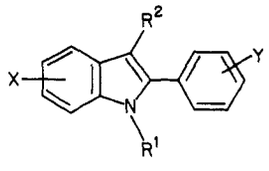
A number of 2-phenylindole derivatives with one hydroxy group in the meta or para position of the phenyl ring and a second one in position 5, 6, or 7 of the indole nucleus were synthesized. In addition, different alkyl groups were introduced into positions 1 and 3 of the heterocycle. The influence of these structural variations on the binding affinity for the calf uterine estrogen receptor was studied. A prerequisite for the binding is the presence of an alkyl group at the nitrogen. Favorable are a hydroxy group located in the para position of the phenyl ring and short alkyl chains both in position 1 and 3 of the indole. The highest relative binding affinity (RBA) values (e.g., 33 for **20b**, 21 for **24b**, 23 for **35b**) are close to that of hexestrol (RBA = 25, estradiol = 100). Depending on the positions of the oxygen functions and size of the alkyl residues, the indole derivatives behaved as strong estrogens (**20c**, **24c**, **35c**) or impeded estrogens with antagonistic activity (**23c**, **29c**, **30c**, **31c**, **40c**, **44c**) in the immature mouse. Some of these derivatives (**20c**, **23c**, **24c**, **29c**, **30c**, **31c**) were tested for their inhibitory effect on dimethylbenzanthracene-induced hormone-dependent mammary tumors of the rat. Both types exhibited a strong growth inhibition with a reduction of the average tumor area at appropriate dosage. A mode of action involving the estrogen receptor system is assumed.

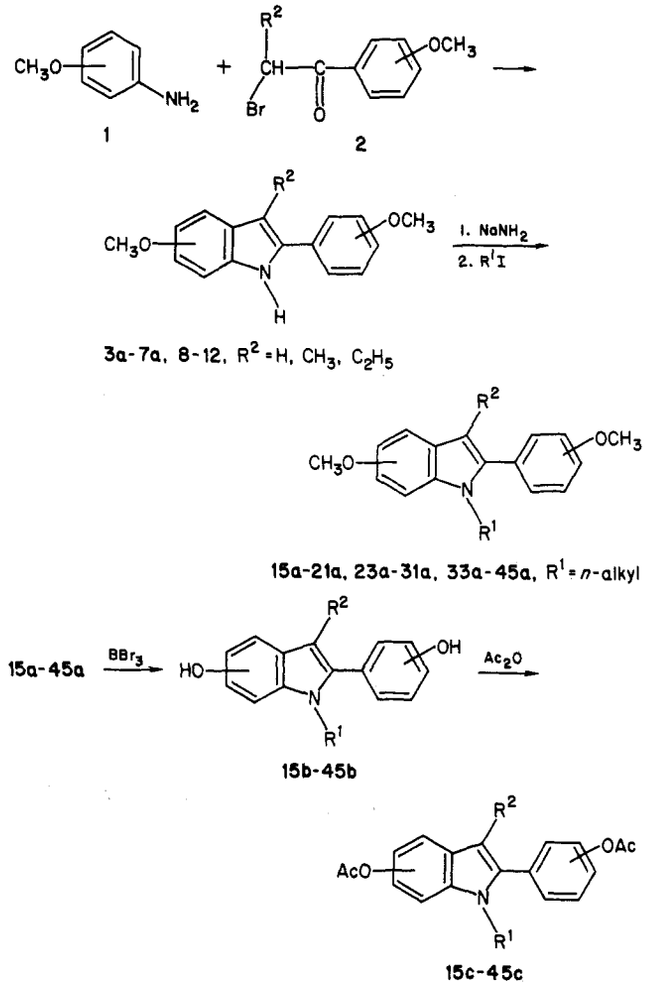
The successful introduction of nonsteroidal estrogen antagonists[1] for treatment of advanced breast cancer[2] has stimulated our interest in the development of new therapeutic agents[3,4] for use in endocrine treatment. In a previous paper,[5] we reported the growth-inhibiting effect of chloro-substituted 2-phenylindole derivatives on the hormone-dependent mammary carcinoma of the rat, the carcinoma having been induced by 7,12-dimethylbenz[*a*]anthracene. These compounds compete with estradiol for the estrogen receptor; the value for the relative binding affinity (RBA) is ca. 1% of the affinity of estradiol. In addition, these indole derivatives possess cytostatic properties in hormone-independent human breast cancer cells. These results prompted us to undertake a systematic study of 2-phenylindoles with respect to their binding affinity for the estrogen receptor, their mammary tumor inhibiting activity, and their estrogenic and antiestrogenic properties.



X = 5-, 6-, or 7-OH
Y = 3- or 4-OH
R^1 = H or alkyl
R^2 = H or alkyl

The structural variations of the 2-phenylindole system concerned the positions of the two oxygen functions in the aromatic rings and the substituents in the 1- and 3-positions of the heterocycle. These substituents are either hydrogen or alkyl groups. From studies of other compounds that bind to the estrogen receptor, we deduced that the existence of two hydroxy groups in the molecule are prerequisite for a strong binding interaction with the receptor site.

Scheme I



Chemistry. For the synthesis of the 2-phenylindole skeleton, the Bischler method[6] was applied. Reaction of the primary aromatic amines **1** with the (α-bromoacyl)-benzenes **2** afforded the 2-phenylindoles **3–12** (Scheme I). Reaction of **2c** ($R^2 = C_2H_5$) with **1a** or **1b** resulted in the unexpected formation of 3-phenylindole derivatives. In the first case, both isomeric indoles **5** and **13** were obtained in equal amounts; in the second, only a small amount of

(1) (a) Hecker, E.; Vegh, J.; Levy, C. M.; Magin, C. A.; Martinez, J. C.; Lowreiro, J.; Garola, J. *Eur. J. Cancer* **1974**, *10*, 747. (b) Heuson, J. C.; Coune, A.; Staquet, M. *Eur. J. Cancer* **1972**, *8*, 387. (c) Manni, A.; Arafah, B.; Pearson, O. H. In "Non-Steroidal Antiestrogens"; Sutherland, R. L., Jordan, V. C., Eds; Academic Press: Sydney, 1981.
(2) Heel, R. C.; Brogden, R. N.; Speight, R. M.; Avery, G. S. *Drugs* **1978**, *16*, 1.
(3) von Angerer, E.; Egginger, G.; Kranzfelder, G.; Bernhauer, H.; Schönenberger, H. *J. Med. Chem.* **1982**, *25*, 832.
(4) von Angerer, E. *J. Med. Chem.* **1982**, *25*, 1374.
(5) von Angerer, E.; Prekajac, J. *J. Med. Chem.* **1983**, *26*, 113.
(6) Mentzer, C.; Molho, D.; Berguer, *J. Bull. Soc. Chim. Fr.* **1950**, *17*, 55.

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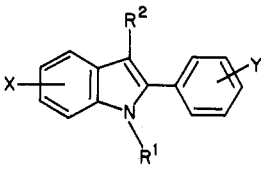
Table I. Methoxy-2-(methoxyphenyl)indoles and Acetoxy-2-(acetoxyphenyl)indoles



X, Y = OMe, OAc

compd[a,b]	R^1	R^2	position of X	position of Y	formula	mp,[c] °C	compd[d]	formula[e]	mp,[c] °C
3a[f]	H	H	6	4	$C_{16}H_{15}NO_2$	227–230	**3c**	$C_{18}H_{15}NO_4$	197–198
4a[g]	H	CH_3	6	4	$C_{17}H_{17}NO_2$	133–135	**4c**	$C_{19}H_{17}NO_4$	161–163
5a[g]	H	C_2H_5	6	4	$C_{18}H_{19}NO_2$	156–158	**5c**	$C_{20}H_{19}NO_4$	127–129
6a[h]	H	H	5	4	$C_{16}H_{15}NO_2$	206–208	**6c**	$C_{18}H_{15}NO_4$	221–223
7a[i]	H	CH_3	5	4	$C_{17}H_{17}NO_2$	135–137	**7c**	$C_{19}H_{17}NO_4$	181–182
15a	CH_3	H	6	4	$C_{17}H_{17}NO_2$	164–166	**15c**	$C_{19}H_{17}NO_4$	118–120
16a	C_2H_3	H	6	4	$C_{18}H_{19}NO_2$	97–99	**16c**	$C_{20}H_{19}NO_4$	153–156
17a	C_3H_7	H	6	4	$C_{19}H_{21}NO_2$	98–100	**17c**	$C_{21}H_{21}NO_4$	111–112
18a	C_4H_9	H	6	4	$C_{20}H_{23}NO_2$	oil	**18c**	$C_{12}H_{13}NO_4$	103–104
19a	CH_3	CH_3	6	4	$C_{18}H_{19}NO_2$	122–124	**19c**	$C_{10}H_{19}NO_4$	119–120
20a	C_2H_5	CH_3	6	4	$C_{19}H_{21}NO_2$	127–128	**20c**	$C_{21}H_{21}NO_4$	150–152
21a	C_3H_7	CH_3	6	4	$C_{20}H_{23}NO_2$	97–99	**21c**	$C_{22}H_{23}NO_4$	108–110
22a	i-C_3H_7	CH_3	6	4	$C_{20}H_{23}NO_2$	152–154	**22c**	$C_{22}H_{23}NO_4$	153–154
23a	CH_3	C_2H_5	6	4	$C_{19}H_{21}NO_2$	117–118	**23c**	$C_{21}H_{21}NO_4$	127–129
24a	C_2H_5	C_2H_5	6	4	$C_{20}H_{23}NO_2$	116–117	**24c**	$C_{22}H_{23}NO_4$	147–148
25a	C_3H_7	C_2H_5	6	4	$C_{21}H_{25}NO_2$	97–98	**25c**	$C_{23}H_{25}NO_4$	115–117
26a	CH_3	H	5	4	$C_{17}H_{17}NO_2$	97–99	**26c**	$C_{19}H_{17}NO_4$	152–154
27a	C_2H_5	H	5	4	$C_{18}H_{19}NO_2$	104–105	**27c**	$C_{20}H_{19}NO_4$	165–167
28a	C_3H_7	H	5	4	$C_{19}H_{21}NO_2$	74–75	**28c**	$C_{21}H_{21}NO_4$	105–106
29a	CH_3	CH_3	5	4	$C_{18}H_{19}NO_2$	139–141	**29c**	$C_{20}H_{19}NO_4$	126–128
30a	C_2H_5	CH_3	5	4	$C_{19}H_{21}NO_2$	111–119	**30c**	$C_{21}H_{21}NO_4$	146–147
31a	C_3H_7	CH_3	5	4	$C_{20}H_{23}NO_2$	119–121	**31c**	$C_{22}H_{23}NO_4$	106–107
32a	i-C_3H_7	CH_3	5	4	$C_{20}H_{23}NO_2$	148–149	**32c**	$C_{22}H_{23}NO_4$	191–193
33a	C_4H_9	CH_3	5	4	$C_{21}H_{25}NO_2$	52–54	**33c**	$C_{23}H_{25}NO_4$	73–75
34a	C_5H_{11}	CH_3	5	4	$C_{22}H_{27}NO_2$	oil	**34c**	$C_{24}H_{27}NO_4$[j]	oil
35a	C_2H_5	C_2H_5	5	4	$C_{20}H_{23}NO_2$	118–119	**35c**	$C_{22}H_{23}NO_4$	164–166
36a	C_3H_7	C_3H_7	5	4	$C_{22}H_{27}NO_2$	99–100	**36c**	$C_{24}H_{27}NO_4$	134–136
37a	C_2H_5	CH_3	7	4	$C_{19}H_{21}NO_2$	78–80	**37c**	$C_{21}H_{21}NO_4$	120–121
38a	C_2H_5	H	6	3	$C_{18}H_{19}NO_2$	61–63	**38c**	$C_{20}H_{19}NO_4$	75–76
39a	CH_3	CH_3	6	3	$C_{18}H_{19}NO_2$	85–87	**39c**	$C_{20}H_{19}NO_4$	103–105
40a	C_2H_5	CH_3	6	3	$C_{19}H_{21}NO_2$	56–58	**40c**	$C_{21}H_{21}NO_4$	106–107
41a	C_3H_7	CH_3	6	3	$C_{20}H_{13}NO_2$	oil	**41c**	$C_{22}H_{23}NO_4$[k]	oil
42a	C_2H_5	H	5	3	$C_{18}H_{19}NO_2$	74–76	**42c**	$C_{20}H_{19}NO_4$	123–124
43a	CH_3	CH_3	5	3	$C_{18}H_{19}NO_2$	73–75	**43c**	$C_{20}H_{19}NO_4$	92–94
44a	C_2H_5	CH_3	5	3	$C_{19}H_{21}NO_2$	oil	**44c**	$C_{21}H_{21}NO_4$	83–85
45a	C_3H_7	CH_3	5	3	$C_{20}H_{23}NO_2$	oil	**45c**	$C_{22}H_{23}NO_4$	118–119

[a] The letter *a* refers to the methoxy derivative (X, Y = OMe) in cases where the hydroxy (*b*) and acetoxy (*c*) derivative are also described. [b] The data for 8–12 are reported in the Experimental Section. [c] Recrystallized from EtOH. [d] The letter *c* refers to the acetates (X, Y = OAc); R^1 and R^2 as in *a*. [e] Analyzed for C and H within ±0.40% of the calculated values. [f] Reference 27. [g] Reference 28. [h] Reference 29. [i] Reference 30. [j] Analyzed as hydroxy derivative. Calcd: C, 77.63; H, 7.49. Found: C, 75.95; H, 7.07. [k] Analyzed as hydroxy derivative. Calcd: C, 76.85; H, 6.81. Found: C, 73.68; H, 6.62.

the 3-phenylindole **14** was isolated from the reaction mixture. The structures of **13** and **14** were confirmed by ^1H NMR and UV spectroscopy. The formation of 3-phenyl derivatives involves a symmetrical intermediate that is formed by the bromo ketone and two molecules of the amine.[7]

The N-alkylated indoles were prepared from the parent indole by treatment with sodium amide followed by addition of *n*-alkyl iodide. In the reaction of **6** with ethyl or propyl iodide, alkylation in the vinylogous 3-position also took place, yielding the disubstituted compounds **35a** and **36a** as byproducts. For the introduction of the isopropyl substituent, it was necessary to treat the indoles with NaH in DMF and to react the anion with isopropyl iodide at room temperature. Demethylation of the methoxy compounds **15a–45a** was readily effected with BBr_3 in CH_2Cl_2.

The hydroxyindoles were separated from oxidation products and boric acid by conversion to the acetates using acetic anhydride in pyridine followed by chromatographic isolation. The free hydroxy compounds were obtained by alkaline hydrolysis of the acetates in methanol under a nitrogen atmosphere (Table I).

Binding Affinity for the Calf Uterine Estrogen Receptor. The binding affinities of the 2-phenylindoles for the estrogen receptor were measured by a competitive binding assay with 17β-[^{3}H]estradiol. Calf uterine cytosol was used as receptor source and dextran coated charcoal (DCC) method was applied.[8] The relative binding affinities (RBA) are given as the ratio of the molar concentrations of 17β-estradiol and indole required to decrease the receptor bound radioactivity by 50%, multiplied by 100. All of the 2-phenylindole derivatives with free hy-

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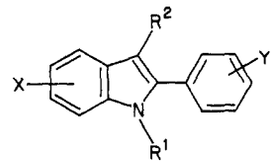
(8) Kranzfelder, G.; Hartmann, R. W.; von Angerer, E.; Schönenberger, H.; Bogden, A. E. *J. Cancer Res. Clin. Oncol.* **1982**, *103*, 165.

Table II. Hydroxy-2-(hydroxyphenyl)indoles and Their Estrogen Receptor Binding Affinity



X, Y = OH

compd	R^1	R^2	position of X	position of Y	formula	mp,[a] °C	RBA[b]
3b	H	H	6	4	$C_{14}H_{11}NO_2$	250–252	0.01
4b	H	CH_3	6	4	$C_{15}H_{13}NO_2$	250–223	0.06
5b	H	C_2H_5	6	4	$C_{16}H_{15}NO_2$	103–106	0.13
6b	H	H	5	4	$C_{14}H_{11}NO_2$	265–268	0.01
7b	H	CH_3	5	4	$C_{15}H_{13}NO_2$	201–204	0.06
15b	CH_3	H	6	4	$C_{15}H_{13}NO_2$	201–203	3.8
16b	C_2H_5	H	6	4	$C_{16}H_{15}NO_2$	133–135	16
17b	C_3H_7	H	6	4	$C_{17}H_{17}NO_2$	138–140	8.5
18b	C_4H_9	H	6	4	$C_{18}H_{19}NO_2$	120–122	4.3
19b	CH_3	CH_3	6	4	$C_{16}H_{15}NO_2$	204–207	10
20b	C_2H_5	CH_3	6	4	$C_{17}H_{17}NO_2$	142–143	33
21b	C_3H_7	CH_3	6	4	$C_{18}H_{19}NO_2$	141–142	13
22b	i-C_3H_7	CH_3	6	4	$C_{18}H_{19}NO_2$	150–161	13
23b	CH_3	C_2H_5	6	4	$C_{17}H_{17}NO_2$	162–163	5.9
24b	C_2H_5	C_2H_5	6	4	$C_{18}H_{19}NO_2$	171–173	21
25b	C_3H_7	C_2H_5	6	4	$C_{19}H_{21}NO_2$	138–139	19
26b	CH_3	H	5	4	$C_{15}H_{13}NO_2$	214–217	0.8
27b	C_2H_5	H	5	4	$C_{16}H_{15}NO_2$	163–165	5.8
28b	C_3H_7	H	5	4	$C_{17}H_{17}NO_2$	154–156	18
29b	CH_3	CH_3	5	4	$C_{16}H_{15}NO_2$	198–200	4.6
30b	C_2H_5	CH_3	5	4	$C_{17}H_{17}NO_2$	179–181	9.5
31b	C_3H_7	CH_3	5	4	$C_{18}H_{19}NO_2$	153–154	16
32b	i-C_3H_7	CH_3	5	4	$C_{18}H_{19}NO_2$	195–198	3.5
33b	C_4H_9	CH_3	5	4	$C_{19}H_{21}NO_2$	64–66	4.6
34b	C_5H_{11}	CH_3	5	4	$C_{20}H_{23}NO_2$	136–138	2.3
35b	C_2H_5	C_2H_5	5	4	$C_{18}H_{19}NO_2$	170–173	23
36b	C_3H_7	C_3H_7	5	4	$C_{20}H_{33}NO_2$	83–85	1.7
37b	C_2H_5	CH_3	7	4	$C_{17}H_{17}NO_2$	86–88	0.02
38b	C_2H_5	H	6	3	$C_{16}H_{15}NO_2$	244–246	1.7
39b	CH_3	CH_3	6	3	$C_{16}H_{15}NO_2$	165–168	0.55
40b	C_2H_5	CH_3	6	3	$C_{17}H_{17}NO_2$	162–163	3.0
41b	C_3H_7	CH_3	6	3	$C_{18}H_{19}NO_2$	140–142	3.5
42b	C_2H_5	H	5	3	$C_{16}H_{15}NO_2$	165–166	1.7
43b	CH_3	CH_3	5	3	$C_{16}H_{15}NO_2$	148–150	0.6
44b	C_2H_5	CH_3	5	3	$C_{17}H_{17}NO_2$	136–138	2.2
45b	C_3H_7	CH_3	5	3	$C_{18}H_{19}NO_2$	139–141	7.4

[a] Crystallized from CH_2Cl_2. [b] Relative binding affinities for the calf uterine estrogen receptor = ratio of molar concentrations of 17β-estradiol (E2) and inhibitor required to decrease the amount of bound [^{3}H]E2 by 50% × 100.

droxy groups were tested for their binding affinity; the RBA values are reported in Table II.

The semilogarithmic plot of bound radioactivity vs. molar concentrations of the 2-phenylindole derivatives exhibited curves parallel to those of 17β-estradiol and hexestrol, suggesting a common binding site for all of the compounds that were tested.

Depending on the substitution pattern, the binding affinities vary considerably, reaching from nearly zero to values that exceed that of the potent synthetic estrogen hexestrol. Derivatives without alkyl substituent at the nitrogen are practically devoid of any binding affinity (**3b–7b**). A prerequisite for a high affinity is a hydroxy group located in the para position of the phenyl ring. The high affinity of **45b** for the receptor has to be considered as an exception. The 5- and 6-hydroxy series possess rather similar RBA values, while the hydroxy group in the 7-position interferes with the binding (**37b**).

The length of the alkyl chains in positions 1 and 3 is important for the receptor binding. Short alkyl groups in these positions cause increased binding, but some of the 3-H derivatives are also very active. A decrease in affinity with compounds containing more than three carbon atoms in the side chain is observed. Replacement of a propyl by an isopropyl group causes little change in binding. In the four series studied, the derivatives possessing the highest affinities for the receptor did not always possess the same arrangement of alkyl substituents. These results indicate a very sensitive dependence on structural alterations. This can be explained by the high specificity that characterizes the interaction between hormone and estrogen receptor proteins.

Estrogenic and Antiestrogenic Activity. On the basis of receptor affinity and certain structural features, a number of phenylindole derivatives were submitted to the uterine weight test in the immature mouse to determine the estrogenic activity. For these and other in vivo experiments, the acetates of the hydroxy indoles were used because of their higher stability and better solubility in olive oil. Since we have not observed any discrepancy in results using either the acetate or the free hydroxy derivative (data not shown), we assume that a rapid hydrolysis by esterases takes place in vivo.

The phenylindole derivatives that were tested can be classified into two groups: strong estrogens with a steep slope of the uterotrophic dose response curve (**20c**, **29c**, **35c**) and impeded estrogens with a slow rise (**23c**, **29c**, **30c**, **31c**, **40c**, **44c**) (Table III). The latter compounds did not

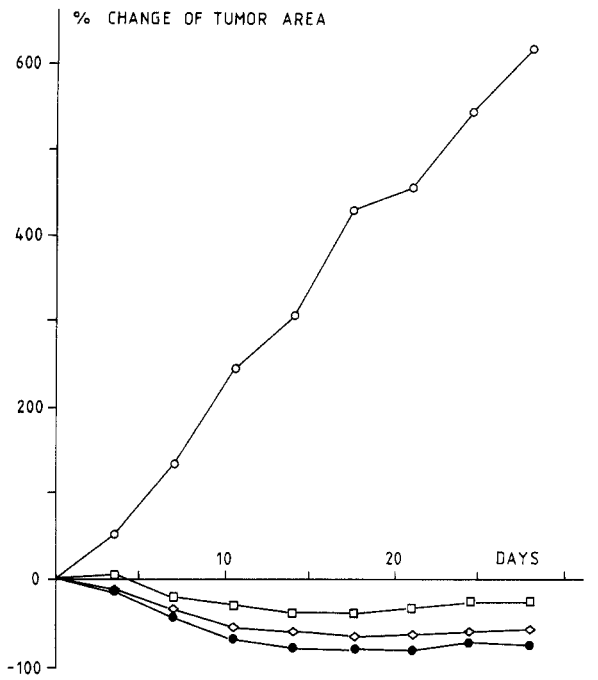


Figure 1. The effect of **20c** and **24c** on the average tumor area of SD rats bearing DMBA-induced mammary carcinoma. Administration scheme: from Monday to Thursday, a single daily dose; on Friday, a double dose sc: control, vehicle alone (○); **20c**, 0.5 mg/kg (□); **20c**, 2.0 mg/kg (◇); **24c**, 2.0 mg/kg (●).

produce full estrogenic responses at doses up to 125 μg per animal. Even by increasing the dose to 1000 μg in some cases, the maximum was not reached (data not shown).

Since we knew from previous experiments that low estrogenicity is often associated with antiestrogenic activity, we determined the antiuerotrophic effect of those compounds by simultaneous administration of 0.4 μg of estrone. We found that all of the phenylindole derivatives that were weak estrogens inhibited the estrone-stimulated uterine growth of immature mice (Table III). The extent of inhibition varied among these compounds and reached a maximum value of 67%. The exact comparison is somewhat hampered by the fact that the results derive from experiments with different values for the estrone standard.

Despite this uncertainty, a significant inhibition can be stated for all compounds studied in this respect. The best effect was seen after administration of doses between 25 and 125 μg. The correlation between molecular structure and biological response—agonist or partial antagonist—showed a simulataneous dependence on the position of the oxygen functions and the kind of alkyl groups in the 1- and 3-positions. Both of (3-acetoxyphenyl)indoles (**40c**, **44c**) that were tested exhibit low estrogenicity. Comparing the 5- with the 6-acetoxy derivatives, the alkyl groups have to be considered as well. The arrangement of the oxygen function and a methyl group on one side of the molecular axis gives rise to an antagonistic effect, while an ethyl substituent located on the same side as the acetoxy group causes a predominantly estrogenic character of the compound (**20c**, **24c**, **35c**). Interestingly, the two closest analogues of diethylstilbestrol with respect to the site of the hydroxy groups and the alkyl substituents belong to the class of weak estrogens (**23c**, **30c**). Preliminary tests using immature rats have shown that there is not much difference in activity (data not shown).

Mammary Tumor Inhibiting Effect. For the evaluation of the antineoplastic activity of some of the phenylindole derivatives, we used 7,12-dimethylbenz[*a*]-

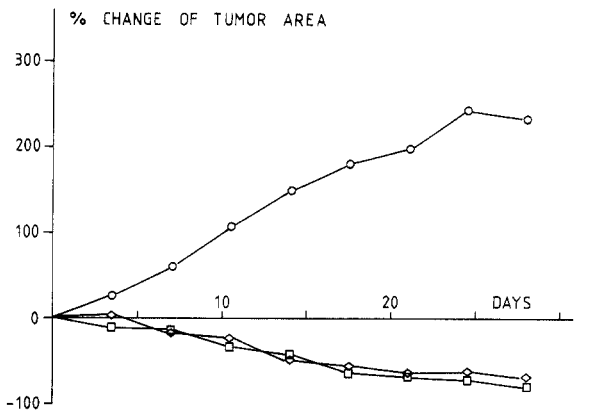


Figure 2. The effect of **23c** and **30c** on the average tumor area of SD rats bearing DMBA-induced mammary carcinoma. Administration scheme: from Monday to Thursday, a single daily dose; on Friday, a double dose sc: control, vehicle alone (○); **23c**, 4.0 mg/kg (□); **30c**, 4.0 mg/kg (◇).

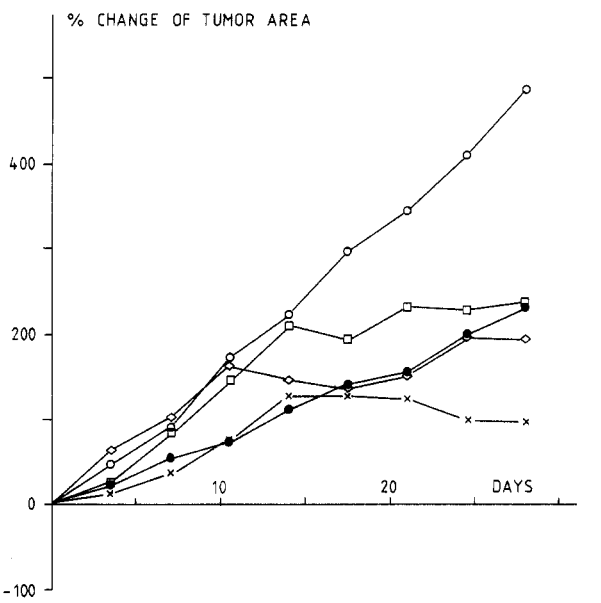


Figure 3. The effect of **29c**, **30c**, **31c**, and tamoxifen citrate on the average tumor area of SD rats bearing DMBA-induced mammary carcinoma. Administration scheme: from Monday to Thursday, a single daily dose; on Friday, a double dose sc: control, vehicle alone (○); **29c**, 5.1 mg/kg (x); **30c**, 1.8 mg/kg (◇); **31c**, 1.8 mg/kg (□); tamoxifen, 2.8 mg/kg (●).

anthracene (DMBA) induced mammary tumors of the Sprague–Dawley rat. This tumor shows a marked sensitivity toward ovarian hormones by regressing following the surgical removal of the ovaries.[5] In this and other respects, the rat tumor resembles the human hormone-dependent mammary tumors.[9] Representatives of both types of biologically active indole derivatives were studied in this breast cancer model: strong estrogens (**20c** and **24c**) and partial antagonists (**23c**, **29c**, **30c**, and **31c**). Tamoxifen served as the reference drug in the antiestrogen therapy.

The average tumor growth was strongly inhibited by all of the compounds tested (Figures 1–3). Only the lower dose of **29c** (1.7 mg) was not effective. The results of three different experiments are reported in detail in Table IV. In the first section, the inhibitory effect of the potent agonists **20c** and **24c** is shown. The marked growth inhibition by these two compounds can be rationalized by

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Table III. Estrogenic and Antiestrogenic Activity of 2-Phenylindole Derivatives 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		15.2 ± 3.9		15.6 ± 1.9	
19c	0.2	18.2 ± 3.4	1	32.3 ± 8.6	31[e]
	1	18.6 ± 3.6	5	38.2 ± 4.4	7
	5	21.4 ± 3.8	25	34.3 ± 6.3	23
	25	24.2 ± 3.9	125	31.7 ± 6.4	33[f]
estrone	1	56.2 ± 9.9	0.4	39.8 ± 8.4	
control		14.3 ± 4.8			
20c	0.2	20.8 ± 3.6	[g]		
	1	31.6 ± 5.0			
	5	41.5 ± 5.2			
	25	44.8 ± 4.6			
	125	42.4 ± 3.2			
estrone	1	44.8 ± 7.2			
control		14.7 ± 2.1		17.3 ± 3.7	
23c	1	13.2 ± 2.4	1	47.6 ± 5.6	17[f]
	5	16.2 ± 3.0	5	48.8 ± 6.2	14
	25	20.4 ± 1.6	25	33.5 ± 4.2	56[e]
	125	30.0 ± 3.8	125	34.7 ± 4.0	52[e]
estrone	0.4	39.8 ± 2.1	0.4	53.8 ± 4.7	
control		14.6 ± 2.0			
24c	0.04	15.1 ± 2.9	[g]		
	0.2	25.5 ± 3.4			
	1	36.2 ± 3.2			
	5	45.6 ± 1.9			
	25	46.4 ± 3.6			
	125	44.7 ± 1.9			
estrone	0.4	43.7 ± 2.6			
control		15.3 ± 2.4		14.4 ± 3.8	
29c	1	13.7 ± 3.1	1	40.0 ± 4.4	33[e]
	5	14.6 ± 2.7	5	39.2 ± 3.6	35[e]
	25	16.1 ± 2.6	25	36.6 ± 3.1	42[e]
	125	20.9 ± 3.6	125	28.8 ± 4.9	62[e]
estrone	1	44.7 ± 3.1	0.4	52.4 ± 5.3	
control		16.3 ± 3.9		17.0 ± 3.5	
30c	1	16.4 ± 3.6	1	51.1 ± 5.1	
	5	19.6 ± 1.7	5	50.4 ± 5.4	
	25	21.0 ± 1.7	25	33.5 ± 3.6	45[e]
	125	30.5 ± 6.5	125	33.0 ± 5.8	46[e]
estrone	1	49.3 ± 6.5	0.4	46.8 ± 7.6	
control		15.2 ± 3.9		17.3 ± 3.7	
31c	0.2	16.7 ± 2.2	1	45.2 ± 6.8	24[e]
	1	21.7 ± 6.0	5	40.3 ± 6.5	37[e]
	5	23.0 ± 3.1	25	29.8 ± 5.2	66[e]
	25	28.5 ± 4.5	125	29.3 ± 2.5	67[e]
estrone	1	56.2 ± 9.9	0.4	53.8 ± 4.7	
control		17.1 ± 2.2			
35c	1	29.5 ± 4.2	[g]		
	5	39.1 ± 6.0			
	25	42.4 ± 3.0			
	125	40.2 ± 3.7			
estrone	0.4	44.7 ± 3.2			
control		16.2 ± 1.8		17.1 ± 2.2	
40c	1	15.2 ± 2.0	1	46.9 ± 4.0	
	5	17.2 ± 1.4	5	41.7 ± 3.5	11[f]
	25	30.2 ± 2.2	25	40.6 ± 3.6	15[e]
	125	39.1 ± 1.8	125	37.4 ± 2.9	26[e]
estrone	0.4	43.8 ± 2.5	0.4	44.7 ± 3.2	
control		16.2 ± 1.8		17.1 ± 2.2	
44c	5	14.1 ± 1.1	1	38.5 ± 3.0	22[e]
	25	17.9 ± 1.2	5	46.3 ± 3.2	
	125	19.5 ± 1.2	25	53.5 ± 4.1	
	625	33.0 ± 2.1	125	37.8 ± 1.7	25[e]
estrone	0.4	43.8 ± 2.5	0.4	44.7 ± 3.2	

[a] Dose per animal, administered at 3 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 per animal and day. [d] The *U* test according to Wilcoxon, Mann, and Whitney was used. [e] Significant ($p < 0.01$). [f] Significant ($p < 0.05$). [g] Antiuterotrophic tests were not performed with strong estrogens.

their strong estrogenic character. Other synthetic estrogens such as diethylstilbestrol produce a similar effect.[10] In the interpretation of this experiment, it has to be considered that the treated animals suffered from weight loss, which is known to cause tumor regression.[11]

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Table IV. Effect of 2-Phenylindole Derivatives on the DMBA-Induced Mammary Carcinoma of the Sprague–Dawley Rat

	dose,[a] mg	no. of animals	no. of tumors[b]	new tumors	complete remission,[c] %	partial remission,[d] %	static tumors,[e] %	progr tumors,[f] %	change of bodyweight,[g] %	change of tumor area[h] median, %	change of tumor area[h] range,[i] %
control[j]		10	32	64	9	3	20	68	+4	525	319–1306
20c	0.5	11	40	6	65	11	15	9	−15	−74[k]	−100–346
20c	2.0	11	34	6	70	23	2	5	−13	−96[k]	−100–55
24c	2.0	11	36	3	74	13	5	8	−13	−95[k]	−100–231
control[j]		10	25	41	3	18	38	41	+5	206	−54–552
23c	4.0	8	18	10	43	29	21	7	−7	−88[k]	−100–47
30c	4.0	8	19	7	35	50	15	0	−3	−84[k]	−100–45
control[j]		10	33	44	0	1	20	79	+2	442	201–689
29c	1.7	9	29	38	9	6	15	70	+2	577	318–1054
29c	5.1	10	37	37	32	10	19	39	−0	18[k]	−44–333
30c	1.8	10	33	25	19	12	14	55	+1	124[k]	−75–656
31c	1.8	10	34	27	7	8	30	55	+2	198[k]	47–510
Tam[l]	2.8	10	34	26	13	7	27	53	+0	210[k]	−39–458

[a] Dose per kilogram of body weight, dissolved in olive oil. The animals received a single dose daily from Monday to Thursday and a double dose on Friday. [b] At the beginning of the test. [c] Tumor not palpable. [d] Reduction of initial tumor size ≤50%. [e] Tumor size 51–150% of the initial size. [f] Tumor size >150% of the initial size. [g] Average on the 7th day of therapy. [h] Median on the 28th day of therapy. The *U* test according to Wilcoxon, Mann, and Whitney was used to determine the significance. [i] Numbers without sign are understood to be positive. [j] Vehicle alone. [k] Significant ($p < 0.01$). [l] Tamoxifen citrate.

In the second experiment, the activity of two partial antiestrogens was studied. These compounds are structurally related to the indole **20c** with an exchange of the alkyl groups in positions 1 and 3 (**23c**) or a replacement of the oxygen function from C-6 to C-5 (**30c**). Because of the low receptor binding affinities, the dose was doubled. We found that these antagonists proved to be as active as the estrogenic indole derivatives. The direct comparison of the results is somewhat hampered by the wide variation in tumor growth in the control animals (206% vs. 525%). The reason for this retardation in tumor growth, which we have also observed previously,[12] is unclear.

In the third experiment the effect of equimolar doses of the homologous 5-acetoxyindole derivatives and of tamoxifen citrate was studied (Table IV). The inactivity of the *N*-methyl compound **29c** at this dosage can be explained by the lower binding affinity of its hydroxy derivative (RBA = 4.8 vs. 9.5 for **30b** and 16 for **31b**). By use of a threefold dose of **29c**, an inhibitory effect was achieved (Figure 3). The fact that tamoxifen, despite its low RBA value of 1.8,[13] is active can be rationalized by its phamacodynamic properties. On the one hand, it has a long residency half-time,[14] and on the other, it can be metabolized to 4-hydroxytamoxifen, an active derivative with a binding affinity close to that of estradiol.[15]

While both tamoxifen and the indole derivative **30c** are equally efficient at this dose, the phenylindoles proved to be superior in higher doses. They are capable of reducing the initial tumor size while tamoxifen only delays the average tumor growth. The reduction of the average tumor size caused by the indoles is due to the large fraction of tumors regressing after administration. Many of them could not be detected by palpation at the end of treatment (Table IV).

Discussion

A number of 2-phenylindole derivatives with one hydroxy function in each carbocycle were synthesized and tested for their affinities for the calf uterine estrogen receptor. The phenylindoles with an alkyl substituent at the nitrogen show, apart from the 7-hydroxy compound **37b**, a moderate to strong binding affinity for the estrogen receptor protein. Derivatives with hydrogen in position 1 of the indole do not bind to the receptor, presumably because they form hydrogen bridges with water molecules.[16] The so-formed polar moieties prevent the necessary hydrophobic interaction with the receptor site.[17] Alterations in both parts of the 2-phenylindole system show the importance of one para-located hydroxy function in the phenyl ring for the receptor binding. This result can be explained by the geometry of the molecule. Stereo models exhibit that the distance between the two oxygen atoms, which is believed to play a major role in the binding to the estrogen receptor,[18] is 1 Å shorter in the (3-hydroxyphenyl)indole series than in the 4-hydroxyphenyl derivatives. The distance between the oxygen atoms in the latter is 11.2–11.6 Å, which is somewhat less than in diethylstilbestrol (12.12 Å[19]).

Aside from the influence of the hydroxy groups on the receptor binding, we studied the effect of different alkyl groups on the receptor affinity. The most favorable structures are those in which a methyl or ethyl group is at position 3 of the indole nucleus and an ethyl or propyl group is present on the nitrogen. This maximum in affinity can be rationalized by two contrary effects: the favorable increase in lipophilicity in the center of the molecule[20] and the steric hindrance by larger groups.

By comparison of the receptor affinities of the 2-phenylindoles with those of carbocyclic analogues,[21,22] it can be demonstrated that an isosteric nitrogen does not

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influence the receptor binding of cyclic diethylstilbestrol analogues provided that the heteroatom carries an alkyl substituent.

Some of the indole derivatives with a high affinity for the estrogen receptor were selected for in vivo tests. Estrogenicity and antiestrogenic activity were estimated in the immature mice uterine weight test. The compounds that were tested can be divided into two categories: strong estrogens and impeded estrogens with antagonistic activity. We found that both the position of the oxygen functions and the size of the alkyl residues influence the character of the phenylindoles. Minute alterations, e.g., replacement of an ethyl by a methyl group, can change the biological profile (**19c**/**20c**). It is not known whether the marked difference in the uterotrophic activity is due to different conformational changes of the drug–receptor complex, higher dissociation rate of the complex, or other mechanism. Differences in pharmacokinetic properties that are reported for estriol and estradiol[23] are rather unlikely.

The mammary tumor inhibiting activity of some of the 2-phenylindole derivatives was examined in the DMBA-induced hormone-dependent rat mammary tumor model. Both strong agonists (**20c**, **24c**) and partial antiestrogens (**23c**, **29c**, **30c**, **31c**) inhibited the growth of established carcinomas and led to the regression of a high percentage of the tumors. This inhibitory effect is probably due to a specific mode of action involving the estrogen receptor system rather than general cytostatic effects, because the indole derivatives bind to the estrogen receptor and evoke biological activity in the murine uterus. The fact that agonists and antagonists are equally active suggests a common mechanism of tumor growth inhibition. This assumption is supported by the following observation. In the dose range where the tumor inhibition of **30c** is enhanced, the antiestrogenic effect remains constant but the uterotrophic activity increases. It has been shown that antiestrogens are capable of producing an estrogenic response at higher dosage especially in ovariectomized animal.[24-26] Bearing in mind that the doses of antiestrogens necessary to inhibit the tumor growth are much higher than those required for estrogens, the agonistic component in these drugs could well be responsible for the antitumor effect. It can be assumed, therefore, that most of the mammary tumor inhibiting estrogens and antiestrogens share the same mode of action that finally leads to an arrest of cell growth.

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 were within ±0.40% of the calculated values except where noted. NMR spectra were obtained on a Varian EM 360A or 390A spectrometer and were consistent with the assigned structures.

General Procedure for the Preparation of Methoxy-2-(methoxyphenyl)-1*H*-indoles (3a–7a, 8–12). A solution of 0.06 mol of (2-bromoacyl)anisole was added slowly to a boiling mixture of methoxyaniline (0.2 mol) and 35 mL of *N*,*N*-dimethylaniline with stirring. After addition, the mixture was kept at 170 °C bath temperature. After cooling, EtOAc was added and the mixture extracted with 2 N HCl. The aqueous layer was extracted several times with EtOAc. After washing with 2 N HCl and water, the organic layer was dried ($MgSO_4$). The solvent was removed in vacuo and the resulting residue chromatographed over SiO_2 with CH_2Cl_2 as eluent. The product was recrystallized from EtOH, affording colorless crystals in 10–50% yield. The compounds **3a**,[27] **4a**,[28] **5a**,[28] **6a**,[29] and **7a**,[30] have been described by other authors.

The reaction of 3-methoxyaniline and 2-bromo-1-(4-methoxyphenyl)butan-1-one led to a mixture of **5a** and **2-ethyl-6-methoxy-3-(4-methoxyphenyl)indole (13)**, which was separated by fractional crystallization. The first fractions contained **5a**, the last ones pure **13**: yield 11%; mp 138–140 °C, ^{1}H NMR ($CDCl_3$) δ 1.27 (t, J = 7 Hz, 3 H, CH_2CH_3), 2.81 (q, J = 7 Hz, 2 H, CCH_2CH_3), 3.84 (s, 3 H, OCH_3), 3.86 (s, 3 H, OCH_3), 6.70–6.85 (7, 2 H, Ar H), 7.00, 7.40 (AB, J = 9 Hz, 4 H, Ar H), 7.50 (d, J = 9 Hz, 1 H, Ar H), 7.86 (s, 1 H, Ar H). Compound **13** is identical with the product obtained by the reaction of (3-methoxyphenyl)hydrazine and 1-(4-methoxyphenyl)butan-2-one.[31]

The reaction of 4-methoxyaniline and 2-bromo-1-(4-methoxyphenyl)butan-1-one yielded **2-ethyl-5-methoxy-3-(4-methoxyphenyl)indole (14)** (2%): mp 113–115 °C; ^{1}H NMR ($CDCl_3$) δ 1.22 (t, J = 7 Hz, 3 H, CH_2CH_3), 2.77 (q, J = 7 Hz, 2 H, CCH_2CH_3), 3.80 (s, 3 H, OCH_3), 3.85 (s, 3 H, OCH_3), 6.82 (dd, J_1 = 9 Hz, J_2 = 2 Hz, 1 H, Ar H), 6.98–7.18 (m, 4 H, Ar H), 7.43 (d, J = 9 Hz, 2 H, Ar H), 7.90 (s, 1 H, NH). Compound **14** is identical with the product obtained by the reaction of (4-methoxyphenyl)hydrazine and 1-(4-methoxyphenyl)butan-2-one.[31]

7-Methoxy-2-(4-methoxyphenyl)-3-methylindole (8): mp 112–113 °C; ^{1}H NMR ($CDCl_3$) δ 2.42 (s, 3 H, CCH_3), 3.82 (s, 3 H, OCH_3), 3.93 (s, 3 H, OCH_3), 6.58–7.22 (m, 5 H, Ar H), 7.50 (d, J = 9 Hz, 2 H, Ar H), 8.33 (s, 1 H, NH). Anal. ($C_{17}H_{17}NO_2$) C, H.

6-Methoxy-2-(3-methoxyphenyl)indole (9): mp 97–98 °C; ^{1}H NMR ($CDCl_3$) δ 3.73 (s, 3 H, OCH_3), 3.78 (s, 3 H, OCH_3), 6.67–7.52 (m, 8 H, Ar H, H-3), 8.23 (s, 1 H, NH). Anal. ($C_{16}H_{15}NO_2$) H; C: calcd, 75.87; found, 75.39.

6-Methoxy-2-(3-methoxyphenyl)-3-methylindole (10): mp 120 °C; ^{1}H NMR ($CDCl_3$) δ 2.38 (s, 3 H, CCH_3), 3.80 (s, 3 H, OCH_3), 3.82 (s, 3 H, OCH_3), 6.70–7.53 (m, 7 H, Ar H), 7.97 (s, 1 H, NH). Anal. ($C_{17}H_{17}NO_2$) C, H.

5-Methoxy-2-(3-methoxyphenyl)indole (11): mp 125–127 °C; ^{1}H NMR ($CDCl_3$) δ 3.83 (s, 6 H, OCH_3), 6.50–7.33 (m, 8 H, Ar H, H-3), 8.33 (s, 1 H, NH). Anal. ($C_{16}H_{15}NO_2$) C, H.

5-Methoxy-2-(3-methoxyphenyl)-3-methylindole (12): mp 110–112 °C; ^{1}H NMR ($CDCl_3$) δ 2.41 (s, 3 H, CCH_3), 3.80 (s, 3 H, OCH_3), 3.85 (s, 3 H, OCH_3), 6.73–7.50 (m, 7 H, Ar H), 7.97 (s, 1 H, NH). Anal. ($C_{17}H_{17}NO_2$) C, H.

General Procedure for the Alkylation of 2-Arylindoles. Sodium (1.44 g, 0.06 mol) was added in portions to 200 mL of liquid ammonia. After the disappearance of the blue color, a solution of 0.035 mol of the indole in 100 mL of dry THF was added at −70 °C. After an additional 30 min of stirring, a solution of the alkyl iodide (0.042 mol) in dry THF was added slowly. After 30 min the cooling bath was removed to allow the ammonia to evaporate. The residue was treated with water and extracted with Et_2O. The organic layer was washed with $NaHSO_3$ solution and water and dried ($MgSO_4$). If the residue obtained after evaporation of the solvent was solid, it was recrystallized from EtOH. Oily products were purified by column chromatography on SiO_2 using CH_2Cl_2 as solvent. The yields ranged from 75% to 90%. Melting points are reported in Table I. Elemental analyses were performed after ether cleavage and acetylation (vide infra). Alkylation of **6a** with EtI gave a mixture of **27a** and **35a**, which was resolved by fractional crystallization from EtOH (**27a** is less soluble) and chromatography (SiO_2; CH_2Cl_2/ligroin, 1:1).

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Reaction of **6a** with *n*-PrI afforded a mixture of **28a** and **36a**, which was separated by chromatography on SiO_2 with CH_2Cl_3/ligroin (1:1) as eluent, yielding **36a** as the first fraction.

General Procedure for the Alkylation with Isopropyl Iodide. A solution of the indole (0.01 mol) in dry DMF (30 mL) was added slowly to a stirred mixture of NaH (0.012 mol of a 80% oil dispersion) in dry DMF (50 mL) with cooling. After stirring for 30 min at 0 °C, isopropyl iodide (0.015 mol) in dry DMF (20 mL) was added at 0 °C. After stirring for 3 h at room temperature, the excess of NaH was destroyed by dropwise addition of water. After addition of Et_2O (300 mL), the organic layer was washed with water and dried ($MgSO_4$). The residue obtained by evaporation of the solvent was chromatographed over SiO_2 with CH_2Cl_2 as eluent. The product was recrystallized; the yield was about 20%. Melting points are reported in Table I.

General Procedure for the Ether Cleavage and Acetylation. A solution of the methoxy-substituted 2-phenylindole (0.008 mol) in dry CH_2Cl_2 (50 mL) was cooled to −60 °C under a nitrogen atmosphere, and then BBr_3 (3.4 mL, 0.035 mol) was added. After 30 min the cooling bath was removed and the mixture stirred over night. With cooling, the mixture was poured into an aqueous solution of $NaHCO_3$. The organic layer was separated, and the aqueous phase was extracted three times with EtOAc. The combined organic layers were washed with water and dried ($MgSO_4$). After the solvent was removed, the dark residue was treated with Ac_2O (6.0 g) and pyridine (6.0 mL). After refluxing for 2 h, the mixture was poured onto ice and extracted with CH_2Cl_2. The organic layer was washed twice with 2 N HCl and water and dried ($MgSO_4$). After evaporation of the solvent, the remaining residue was chromatographed (SiO_2, CH_2Cl_2). The product was usually obtained as a solid and recrystallized from EtOH. The yields were in the range of 50% to 70%. Melting points are reported in Table I.

General Procedure for the Hydrolysis of the Acetates. The acetoxyindole (0.33 g) was suspended in 20 mL of MeOH. Under nitrogen, 2 N NaOH (4 mL) was added, and the mixture was stirred for 2 h at room temperature. The clear solution was acidified with 2 N HCl and the alcohol was removed under reduced pressure. The aqueous mixture was extracted with CH_2Cl_2. After drying ($MgSO_4$), the solvent was evaporated. The residue crystallized after treatment with a small volume of CH_2Cl_2. The yields were between 75% and 90%. Melting points are reported in Table II.

Biochemical and Biological Methods. Reagents. [2,4,6,7-^{3}H]Estradiol (110 Ci/mmol in ethanol) was obtained from New England Nuclear, Dreieich, FRG. Unlabeled estradiol, estrone, hexestrol, and 7,12-dimethylbenz[*a*]anthracene were obtained from Sigma, München, FRG. Tamoxifen citrate was a gift of ICI, Plankstadt, FRG. 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.

Estradiol Receptor Binding Assay. The relative binding affinity (RBA) of the test compounds was determined in a competitive binding assay with [^{3}H]estradiol. The previously described procedure was used with modifications.[8] Calf uterine cytosol was incubated for 18 h at 4 °C with different concentrations of competitor and 5×10^{-9} M [^{3}H]estradiol. After incubation, dextran-coated charcoal 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 competitor were chosen to provide values between 10% and 90% bound radioactivity. A semilogarithmic plot of bound radioactivity vs. 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 Tests. Immature female mice (19 day old, of the NMRI strain) from Ivanovas, Kisslegg, FRG, were randomly divided into groups of 10 animals. To determine estrogenic activity, compounds were dissolved or suspended in olive oil (50 μL per 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 aqueous picric acid –40% formaldehyde–glacial acetic acid, 15:5:1 by volume) for 20 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 uterotrophic effect was calculated by the 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 indole derivatives. The inhibition (percent) of the estrone-stimulated uterine growth was estimated by the 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.

Mammary Tumor Growth Inhibition Test. Female Sprague–Dawley rats (Zentralinstitut Für Versuchstierzucht, Hannover, FRG), 50 days old, were administered by gavage a single dose of 20 mg of DMBA dissolved in 1 mL of olive oil. The rats were examined for tumor masses by palpation twice weekly, beginning 30 days after feeding of DMBA; those without tumors by day 70 were discarded. Animals were assigned randomly to experimental groups when the tumor area per animal exceeded 140 mm^2. The tumor area was determined by caliper measurements of two perpendicular axes, one across the largest diameter. Analysis revealed an approximately equal distribution of tumors of different latencies, tumor number, and total tumor area among each of the treatment and control groups.

Drugs were dissolved or suspended in olive oil (1 mL/kg of body weight) and administered subcutaneously. From Monday to Thursday a single dose was administered and on Friday a double dose. Tumor size and body weight were measured twice weekly. Criteria for determining tumor response to the drug included change in tumor area per animal, change in size of individual tumors > 50% (increased, decreased) or < 50% (static), appearance of new tumors, and proportion of tumors regressing to nonpalpability. Significance of difference was determined by the *U* test according to Wilcoxon, Mann, and Whitney.

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Registry No. *o*-1, 90-04-0; *m*-1, 536-90-3; *p*-1, 104-94-9; *m*-2 (R^2 = H), 2632-13-5; *m*-2 (R^2 = Me), 21086-33-9; *m*-2 (R^2 = Et), 881-43-6; *p*-2 (R^2 = H), 5000-65-7; *p*-2 (R^2 = Me), 21726-71-6; *m*-2 (R^2 = Pr), 36412-64-3; **3a**, 62655-56-5; **3b**, 91444-47-2; **3c**, 91444-48-3; **4a**, 91444-16-5; **4b**, 91444-49-4; **4c**, 91444-50-7; **5a**, 91444-17-6; **5b**, 91444-51-8; **5c**, 91444-52-9; **6a**, 5883-83-0; **6b**, 62613-59-6; **6c**, 91444-53-0; **7a**, 91444-18-7; **7b**, 91444-54-1; **7c**, 91444-55-2; **8**, 91444-10-9; **9**, 91444-11-0; **10**, 91444-12-1; **11**, 91444-13-2; **12**, 91466-47-6; **13**, 91444-14-3; **14**, 91444-15-4; **15a**, 86111-01-5; **15b**, 86111-03-7; **15c**, 86111-02-6; **16a**, 91444-19-8; **16b**, 86111-05-9; **16c**, 86111-20-8; **17a**, 91444-20-1; **17b**, 86111-06-0; **17c**, 86111-21-9; **18a**, 91444-21-2; **18b**, 86111-07-1; **18c**, 86111-22-0; **19a**, 91444-22-3; **19b**, 86111-14-0; **19c**, 86111-29-7; **20a**, 91444-23-4; **20b**, 86111-15-1; **20c**, 86111-30-0; **21a**, 91444-24-5; **21b**, 86111-16-2; **21c**, 86111-31-1; **22a**, 91444-25-6; **22b**, 91444-56-3; **22c**, 91444-57-4; **23a**, 91444-26-7; **23b**, 86111-41-3; **23c**, 86111-35-5; **24a**, 91444-27-8; **24b**, 86111-42-4; **24c**, 86111-36-6; **25a**, 91444-28-9; **25b**, 91444-58-5; **25c**, 91444-59-6; **26a**, 91444-29-0; **26b**, 91444-60-9; **26c**, 91466-50-1; **27a**, 91444-30-3; **27b**, 86111-04-8; **27c**, 86111-19-5; **28a**, 91444-31-4; **28b**, 91444-61-0; **28c**, 91444-62-1; **29a**, 91444-32-5; **29b**, 86111-10-6; **29c**, 86111-25-3; **30a**, 91444-33-6; **30b**, 86111-11-7; **30c**, 86111-26-4; **31a**, 91444-34-7; **31b**, 86111-12-8; **31c**, 86111-27-5; **32a**, 91444-35-8; **32b**, 91444-63-2; **32c**, 91444-64-3; **33a**, 91444-36-9; **33b**, 86111-13-9; **33c**, 86111-28-6; **34a**, 91466-48-7; **34b**, 91444-65-4; **34c**, 91444-66-5; **35a**, 91444-37-0; **35b**, 91444-67-6; **35c**, 91444-68-7; **36a**, 91444-38-1; **36b**, 91444-69-8; **36c**, 91444-70-1; **37a**, 91444-39-2; **37b**, 91444-71-2; **37c**, 91444-72-3; **38a**, 91444-40-5; **38b**, 86111-09-3; **38c**, 86111-24-2; **39a**, 91444-41-6; **39b**, 91444-73-4; **39c**, 91444-74-5; **40a**, 91444-42-7; **40b**, 86111-18-4; **40c**, 86111-33-3; **41a**, 91466-49-8; **41b**, 91444-75-6; **41c**, 91444-76-7; **42a**, 91444-43-8; **42b**, 86111-08-2; **42c**, 86111-23-1; **43a**, 91444-44-9; **43b**, 91444-77-8; **43c**, 91444-78-9; **44a**, 91444-45-0; **44b**, 86111-17-3; **44c**, 86111-32-2; **45a**, 91444-46-1; **45b**, 91444-79-0; **45c**,

91444-80-3; 2-bromo-1-(4-methoxyphenyl)butan-1-one, 881-43-6; ethyl iodide, 75-03-6; propyl iodide, 107-08-4; isopropyl iodide, 75-30-9.

Supplementary Material Available: ^{1}H NMR data of methoxy-2-(methoxyphenyl)indoles (**15a–44a**), hydroxy-2-(hydroxyphenyl)indoles (**3b–45b**), and acetoxy-2-(acetoxyphenyl)-indoles (**3c–45c**) and elemental analysis of acetoxy-2-(acetoxyphenyl)indoles (18 pages). Ordering information is given on any current masthead page.

A Novel Peptide Delivery System Involving Peptidase Activated Prodrugs as Antimicrobial Agents. Synthesis and Biological Activity of Peptidyl Derivatives of 5-Fluorouracil

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As an approach to the development of antimicrobial agents, a novel peptide carrier system was designed, based on the chemical instability of α-substituted glycine analogues, with the explicit intent of actively transporting therapeutically useful compounds into microbial cells. Peptides containing 5-fluorouracil (5-FU) linked to the peptide backbone were selected to test the feasibility of this new delivery system. These peptide conjugates were designed such that they would be substrates for both the microbial peptide permeases and peptidases. After entry into cells, enzymatic hydrolysis of the peptide generates an unstable α-(5-FU)-glycine that spontaneously decomposes to release 5-FU. The 5-FU–peptide conjugates were tested for antifungal (*Candida albicans*) and antibacterial (*Escherichia coli*) activity and were found to have antimicrobial activities comparable to free 5-FU. Noninhibitory peptides antagonized the antimicrobial activities of the 5-FU–peptide conjugates but not of free 5-FU, a result consistent with peptide transport mediated entry of the peptide conjugates into cells. Further support for this conclusion was provided by the finding that biological activities were dependent upon peptide stereochemistry.

Microorganisms have been shown to possess specialized transport systems for the uptake of peptides.[1] In *Escherichia coli*, the most extensively studied bacterium, it has been found that there are separate peptide transport systems for dipeptides and for oligopeptides.[2] *Candida albicans* has also been shown to have peptide transport systems although the multiplicity in this organism has yet to be conclusively defined.[3,4] The factors that determine the recognition of peptides by microbial peptide transport systems have been the subject of numerous investigations.[1,2,5] A significant observation that has emerged from these studies, primarily in bacteria, is that peptide transport systems generally possess little demonstrable side-chain specificity. This is likely an accommodation to the diversity of side-chain combinations that occur in peptides assembled from the naturally occurring amino acids. Early indications that this process could be exploited therapeutically was demonstrated in *E. coli* where normally impermeant amino acid analogues, not recognized by the more selective amino acid transport systems, were shown to enter these cells by a peptide carrier mechanism when incorporated into the backbone of a peptide. Following transport, cytoplasmic peptidases hydrolyze the peptide to release the constituent amino acids.[2] Many natural and synthetic examples involing peptides that contain growth inhibitory amino acids have been reported[6] and considerable interest has been expressed in utilizing this approach as a means of developing novel chemotherapeutic agents.[7,8] The synthesis and development of the wide-spectrum antimicrobial agent alaphosphin[9] [L-alanyl-L-(1-aminoethyl)phosphonic acid] is a powerful example of the peptide transport concept.

In an attempt to broaden the overall scope of the peptide transport approach so that inhibitory agents other than amino acid analogues could be brought into microbial cells, we developed a method that allows the transport of sulfhydryl-containing compounds through their attachment to the cysteine residue of a peptide.[10] The results of these studies encouraged us to examine other approaches of this type.[11] In this paper we describe a more versatile peptide delivery system in which the toxophoric agent is attached to the α-carbon of a glycine residue within a peptide chain. Intracellular cleavage of the peptide by cytoplasmic peptidases results in the formation of an unstable intermediate that decomposes with release of the attached toxophoric group.

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