28, 91440-43-6; 28·xHCl, 91440-44-7; 29, 91441-01-9; 29·xHCl, 104739-85-7; 30, 91440-37-8; 30·xHCl, 91440-38-9; 31, 91441-00-8; 31·xHCl, 104739-86-8; 32, 91440-30-1; 32·AcOH, 91440-31-2; 33, 91440-39-0; 33·xHCl, 91440-40-3; 34, 91440-35-6; 34·xHCl, 104739-87-9; 35, 91451-21-7; 35·xHCl, 104739-88-0; 36, 104739-89-1; 37, 91440-08-3; 37·xHCl, 104739-90-4; 38, 104740-40-1; 38·xHCl, 104739-91-5; 38 (benzyl deriv.), 104740-57-0; 39, 91451-17-1; 39·xHCl, 104739-92-6; 40, 91440-04-9; 40·xHCl, 104739-93-7; 41, 91451-18-2; 41·xHCl, 104739-94-8; 42, 91451-20-6; 42·xHCl, 104778-44-1; 43, 91451-19-3; 43·xHCl, 104739-95-9; 44, 91470-46-1; 44·xHCl, 104739-96-0; 45, 91440-94-7; 45·xHBr, 91440-14-1; 46, 104740-41-2; 46·xHCl, 104739-97-1; 47, 91440-62-9; 47·xHCl, 91440-65-2; 48, 91441-09-7; 48·xHCl, 104739-98-2; 49, 104740-42-3; 49·xHCl, 104739-99-3; 50, 104740-43-4; 50·xHCl, 104740-00-3; 51, 91441-39-3; 51·xHCl, 104740-01-4; 52, 91450-90-7; 52·xHCl, 91450-91-8; 53, 94059-40-2; 53·xHCl, 91440-74-3; 54, 91441-07-5; 54·xHCl, 91440-59-4; 55, 91440-57-2; 55·xHCl, 104740-02-5; 56, 91441-08-6; 56·xHCl, 91440-61-8; 57, 91440-55-0; 57·xHCl, 91440-56-1; 58, 104740-44-5; 58·xHCl, 91440-60-7; 59, 104740-45-6; 59·xHCl, 91451-08-0; 60, 91441-49-5; 60·xHCl, 91450-93-0; 61, 91441-35-9; 61·xHCl, 91450-86-1; 62, 91441-36-0; 62·xHCl, 91450-87-2; 63, 94035-69-5; 63·xHCl, 104740-03-6; 64, 91441-34-8; 64·xHCl, 104740-04-7; 65, 91441-38-2; 65·xHCl, 91450-89-4; 66, 91441-37-1; 66·xHCl, 91450-88-3; 67, 91451-22-8; 67·xHCl, 104740-05-8; 68, 91441-44-0; 68·xHCl, 104740-06-9; 69, 104740-46-7; 69·xHCl, 104740-07-0; 70, 91441-20-2; 70·xHCl, 104740-08-1; 71, 91441-23-5; 71·xHCl, 91440-86-7; 72, 91441-24-6; 72·xHCl, 104740-09-2; 73, 91441-25-7; 73·xHCl, 91440-88-9; 74, 91441-48-4; 74·xHBr, 91450-82-7; 75, 104740-10-5; 76, 91441-19-9; 76·xHCl, 104740-11-6; 77, 91441-27-9; 77·xHCl, 91440-90-3; 78, 91441-31-5; 78·xHCl, 91450-79-2; 79, 91441-32-6; 79·xHCl, 91450-80-5; 80, 91441-21-3; 80·xHCl, 91440-84-5; 81, 91441-22-4; 81·xHCl, 91440-85-6; 82, 91441-26-8; 82·xHCl, 91440-98-0; 83, 91441-29-1; 83, 91440-92-5; 84, 104740-47-8; 84·xHCl, 104740-12-7; 85, 91441-28-0; 85·xHCl, 91440-91-4; 86, 104740-48-9; 86·xHCl, 104740-13-8; 87, 91441-30-4; 87·xHCl, 91440-93-6; 88, 91441-18-8; 88·xHCl, 104740-14-9; 89, 91440-78-7; 90, 91441-17-7; 90·xHCl, 91440-77-6; 91, 91441-05-3; 91·xHCl, 91440-53-8; 92, 104740-49-0; 92·xHCl, 104740-15-0; 93, 91441-04-2; 93·xHCl, 91440-51-6; 94, 91441-47-3; 94·xHCl, 104740-16-1; 95, 91441-14-4; 95·xHCl, 104740-17-2; 96, 91441-13-3; 96·xHCl, 104740-18-3; 97, 91441-02-0; 97·xHCl, 104740-19-4; 98, 91441-03-1; 98·xHBr, 104740-20-7; 98 (Cbz deriv.), 104778-45-2; 99, 104740-21-8; 100, 91451-14-8; 100·xHCl, 104740-22-9; 101, 91440-47-0; 101·xHCl, 104740-23-0; 102, 91441-51-9; 102·xHCl, 91440-76-5; 103, 91441-12-2; 103-xHCl, 104740-24-1; 104, 91441-10-0; 104-xHCl, 91440-67-4; 105, 104740-50-3; 105·xHCl, 104740-36-2; 106, 104740-26-3; 107, 91441-86-0; 108, 91441-73-5; 109, 104740-27-4; 110, 104740-28-5; 111, 91441-87-1; 112, 91441-45-1; 113, 91441-90-6; 114, 104740-29-6; 115, 104740-30-9; 116, 104740-31-0; 117, 104740-32-1; 118, 104740-33-2; 199, 104740-51-4; 119·xHCl, 104740-34-3; 120, 104740-52-5; 120·xHCl, 104740-35-4; 121, 104740-53-6; 121·xHCl, 104740-36-5; 122, 91450-95-2; 122·xHCl, 104740-37-6; 123, 91441-40-6; 123·xHCl, 104740-38-7; $NH_iCH_2CH_2NEt_2$, 100-36-7; $NH_2(CH_2)_3NH_2$, 109-76-2; $NH_2CH_2CH_2NHCH_2CH_2OH$, 111-41-1; $NH(CH_2)_5CH_3$, 111-26-2; $NH_2CH_2CH_2CO_2^-N^+Bu_4$, 104761-05-9; $NH_2(CH_2)_3Net_2$, 104-78-9; $NH_23(CH_2)_4NEt_2$, 27431-62-5; $NH_2(CH_2)_7NEt_2$, 20526-69-6; $NH_2CH_2CH_2$-c-$N(CH_2CH_2)_2O$, 2038-036-1; NH_2CH_3, 74-89-5; $NH_2CH_2CH_2OH$, 141-43-5; $NH_2CH_2CH_2NH_2$, 107-15-3; $NH_2CH_2CH_2NMe_2$, 108-00-9; $NH_2CH_2CH_2NHMe$, 109-81-9; $NH_2(CH_2)_3NHCH_2CH_2OH$, 4461-39-6; $NH_2(CH_2)_2NH(CvH_2)_2NMe_2$, 24229-53-6; $NH_2(CH_2)_4NH_2$, 110-60-1; $NH_2(CH_2)_5NH_2$, 462-94-2; $NH_2CH_2CH_2N$(Cbz)Me, 19023-94-0; $NH_2CH_2CH_2N(CH_2CH_2OH)_2$, 4439-20-7; $NH_2(CH_2)_3N(CH_2CH_2OH)_2$, 4985-85-7; c-$HN(CH_2CH_2)_1NMe$, 109-01-3; $NH_2CH_2CH_2NHCH_2CH_2NH_2$, 111-40-0; $NH_2CH_2CH_2NHCH_2CH_2NMe_2$, 24229-53-6; $NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$, 71-44-3; $NH(Me)CH_2CH_2NMe_2$, 142-25-6; $NH_2CH_2CH_2N(Me)CH_2Ph$, 14165-18-5; 1-(2-aminoethyl)-piperazine, 140-31-8; benzyl chloroformate, 501-53-1.

2-Phenylindoles. Effect of N-Benzylation on Estrogen Receptor Affinity, Estrogenic Properties, and Mammary Tumor Inhibiting Activity

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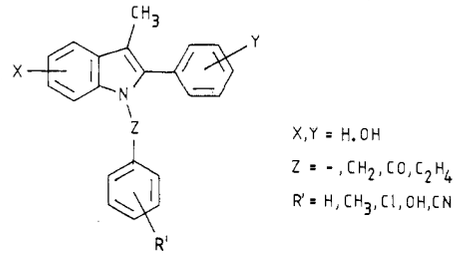
Hydroxy-2-phenylindoles carrying substituted benzyl groups and similar substituents at the nitrogen were synthesized and tested for their ability to displace estradiol from its receptor. All of the derivatives tested exhibited high binding affinities for the calf uterine estrogen receptor, with RBA values ranging from 0.55 to 16 (estradiol 100). The mouse uterine weight tested revealed only low estrogenicity for this class of compounds. Several derivatives showed antiestrogenic activity with a maximum inhibition of estrone-stimulated uterine growth of 40%. Two of the compounds (**6c**, **21c**) were tested for antitumor activity in dimethylbenanthracene- (DMBA-) induced estrogen-dependent rat mammary tumors. Only the 4-cyanobenzyl derivative **21c** was active. After 4 weeks of treatment with 12 mg/kg (6 times/week), the average tumor area was decreased by 57% (control +204%). In vitro, an inhibitory effect of **21b** was only observed with hormone-sensitive MCF-7 breast cancer cells but not with hormone-independent MDA-MB 231 cells. These results make a mode of action involving the estrogen receptor system likely.

In recent years, we paid much attention to the 2-phenylindole system as a new structure for the development of agents for the treatment of estrogen-dependent malignancies.[1-4] In a previous paper,[2] we reported on the influence of structural variations on estrogen receptor affinity, endocrine potency, and antineoplastic activity within a series of *N*-alkyl-2-phenylindoles. Among the compounds studied, several derivatives proved to be very active against experimental hormone-dependent mammary tumors.[1-4] One of these compounds, zindoxifene,[3] is presently undergoing phase I clinical trials.

Chart I



In order to improve the activity in this class of compounds, we introduced aromatic substituents into positions 1 and 3 of the indole nucleus. The latter derivatives have

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(3) von Angerer, E.; Prekajac, J.; Berger, M. R. *Eur. J. Cancer Clin. Oncol.* **1985**, *21*, 531.
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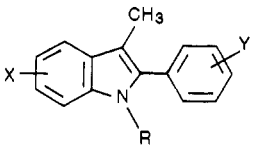
Table I. 1-Substituted Methoxy- and Acetoxy-2-phenylindoles



compd[a]	R	pos of X	pos of Y	formula[b]	mp,[c] °C	compd[d]	formula[b]	mp,[c] °C
6a	$C_6H_5CH_2$	5	4	$C_{24}H_{23}NO_2$	93–94	**6c**	$C_{26}H_{23}NO_4$	142–144
7a	$C_6H_5CH_2$	6	4	$C_{24}H_{23}NO_2$	95–96	**7c**	$C_{26}H_{23}NO_4$	120–122
8a	$C_6H_5CH_2$	5	3	$C_{24}H_{23}NO_2$	120–122	**8c**	$C_{26}H_{23}NO_4$	oil
9a	$C_6H_5CH_2$		4	$C_{23}H_{21}NO_2$	79–80	**9c**	$C_{24}H_{21}NO_4$	133–134
10a	$C_6H_5CH_2$	5		$C_{23}H_{21}NO_2$	92	**10c**	$C_{24}H_{21}NO_2$	114
11a	C_6H_5CO	5	4	$C_{24}H_{21}NO_3$	97–98	**11c**	$C_{26}H_{21}NO_5$	170–172
12a	$C_6H_5CH_2CH_2$	5	4	$C_{25}H_{25}NO_2$	127	**12c**	$C_{27}H_{25}NO_4$	96–97
13a	$C_6H_{11}CH_2$	5	4	$C_{24}H_{29}NO_2$	119–121	**13c**	$C_{26}H_{29}NO_4$	oil
14a	$4\text{-}CH_3C_6H_4CH_2$	5	4	$C_{25}H_{25}NO_2$	74–75	**14c**	$C_{27}H_{25}NO_4$	131–133
15a	$4\text{-}ClC_6H_4CH_2$	5	4	$C_{24}H_{22}ClNO_2$	121–123	**15c**	$C_{26}H_{22}ClNO_4$	102–103
16a	$4\text{-}CH_3OC_6H_4CH_2$	5	4	$C_{25}H_{25}NO_3$	oil	**16c**[e]	$C_{28}H_{25}NO_6$	160–162
17a	$3\text{-}CH_3C_6H_4CH_2$	5	4	$C_{25}H_{25}NO_2$	82–84	**17c**	$C_{27}H_{25}NO_4$	103–105
18a	$3\text{-}ClC_6H_4CH_2$	5	4	$C_{24}H_{22}ClNO_2$	86–87	**18c**	$C_{26}H_{22}ClNO_4$	105–107
19a	$2\text{-}ClC_6H_4CH_2$	5	4	$C_{24}H_{22}ClNO_2$	93–94	**19c**	$C_{26}H_{22}ClNO_4$	122–123
20a	$2,6\text{-}Cl_2C_6H_3CH_2$	5	4	$C_{24}H_{21}Cl_2NO_2$	164–165	**20c**	$C_{26}H_{21}Cl_2NO_4$	167–168
21a	$4\text{-}CNC_6H_4CH_2$	5	4	$C_{25}H_{22}N_2O_2$	127–129	**21c**	$C_{27}H_{22}N_2O_4$	123–124
22a	C_6H_5	5	4	$C_{23}H_{21}NO_2$	149–151	**22c**	$C_{25}H_{21}NO_4$	203–204
23a	C_6H_5	6	4	$C_{23}H_{21}NO_2$	190–191	**23c**	$C_{25}H_{21}NO_4$	170–171

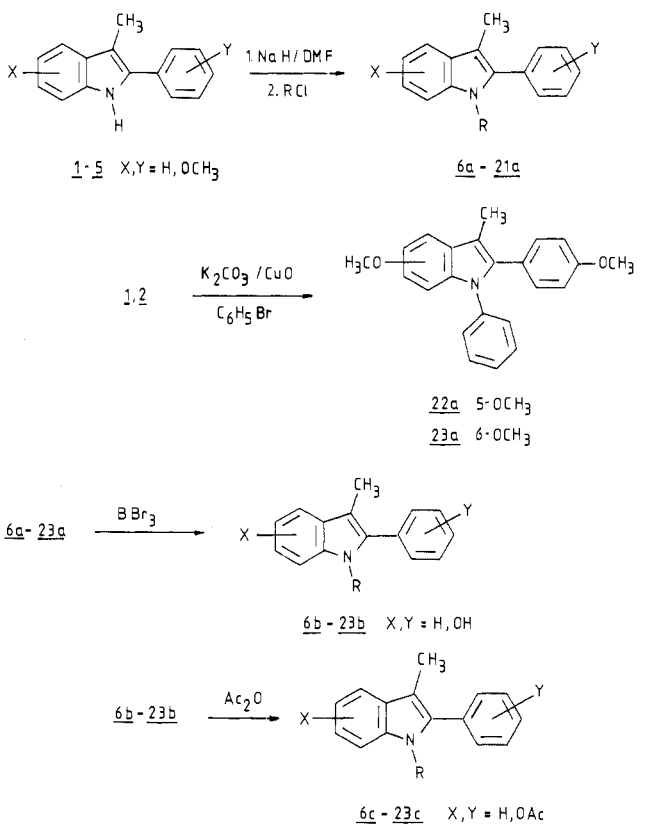
[a] The letter **a** refers to the methoxy derivative (X, Y = OMe). [b] Analyzed for C and H within ±0.40% of the calculated values. [c] Recrystallization from EtOH. [d] The letter **c** refers to the acetoxy derivative (X, Y = OAc); R as in **a**. [e] R = $4\text{-}AcOC_6H_4CH_2$.

turned out to be much less effective than the corresponding 3-alkyl-substituted compounds.[5] The objectives of this paper are the syntheses of a number of 1-benzyl-2-phenylindoles and, for comparison, 1-phenyl, 1-benzoyl, and 1-phenethyl derivatives and studies on their binding affinities for the estrogen receptor, their estrogenic properties in the mouse uterine weight test, and their tumor-inhibiting activity. The structural variations primarily concerned the substituents at the benzyl group, but the hydroxy groups in the 2-phenylindole moiety were also considered.

Chemistry. The starting 1*H*-2-phenylindoles were obtained by the Bischler indole synthesis as described previously.[2] N-Substitution was accomplished by deprotonation with sodium hydride in DMF and subsequent addition of benzyl chloride, benzoyl chloride, or 1-bromo-2-phenylethane (Scheme I). The *N*-phenyl derivatives **22a** and **23a** were synthesized by reacting indole **1** or **2** with bromobenzene in the presence of CuO.[6] Demethylation of the methoxy compounds **6a–23a** 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 **6c–23c** using acetic anhydride in pyridine followed by chromatographic isolation. The free hydroxy derivatives **6b–23b** were obtained by alkaline hydrolysis of the acetates in methanol.

Binding Affinity for the Calf Uterine Estrogen Receptor. The binding affinities of the indole derivatives 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 the dextran-coated charcoal (DCC) method was applied.[7] The relative binding affinities (RBA) are given as the ratio of the molar concentrations of 17β-estradiol and indole required to

Scheme I



decrease the receptor-bound radioactivity by 50%, multiplied by 100. The semilogarithmic plot of bound radioactivity vs. molar concentrations of the indole derivatives exhibited curves parallel to those of 17β-estradiol and hexestrol, suggesting a common binding site for all of the compounds that were tested.

All of the hydroxyindoles in this series exhibit high binding affinities for the calf uterine estrogen receptor. Only the monohydroxy derivative **9b** binds with less than

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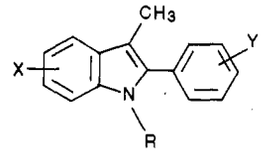
(6) Khan, M. A.; Rocha, E. K. *Chem. Pharm. Bull.* **1977**, *25*, 3110.
(7) Kranzfelder, G.; Hartman, R. W.; von Angerer, E.; Schönenberger, H.; Bogden, A. E. *J. Cancer Res. Clin. Oncol.* **1982**, *103*, 165.

Table II. 1-Substituted 2-Phenylindoles (X, Y = OH) and Their Estrogen Receptor Affinity



compd	R	pos of X	pos of Y	formula	mp,[a] °C	RBA[b]
6b	$C_6H_5CH_2$	5	4	$C_{22}H_{19}NO_2$	179–181	16
7b	$C_6H_5CH_2$	6	4	$C_{22}H_{19}NO_2$	176–178	11
8b	$C_6H_5CH_2$	5	3	$C_{22}H_{19}NO_2$	178–181	3.0
9b	$C_6H_5CH_2$		4	$C_{22}H_{19}NO$	oil	0.55
10b	$C_6H_5CH_2$	5		$C_{22}H_{19}NO$	113–114	2.6
11b	C_6H_5CO	5	4	$C_{22}H_{17}NO_3$	120–123	2.4
12b	$C_6H_5CH_2CH_2$	5	4	$C_{23}H_{21}NO_2$	184–186	2.0
13b	$C_6H_{11}CH_2$	5	4	$C_{22}H_{25}NO_2$	204–206	5.2
14b	$4\text{-}CH_3C_6H_4CH_2$	5	4	$C_{23}H_{21}NO_2$	149–151	11
15b	$4\text{-}ClC_6H_4CH_2$	5	4	$C_{22}H_{18}NO_2$	75–76	8.0
16b	$4\text{-}OHC_6H_4CH_2$	5	4	$C_{22}H_{19}NO_3$	123–125	8.0
17b	$3\text{-}CH_3C_6H_4CH_2$	5	4	$C_{23}H_{21}NO_2$	71–73	6.6
18b	$3\text{-}ClC_6H_4CH_2$	5	4	$C_{22}H_{18}ClNO_2$	113–116	5.1
19b	$2\text{-}ClC_6H_4CH_2$	5	4	$C_{22}H_{18}ClNO_2$	133–134	8.7
20b	$2,6\text{-}Cl_2C_6H_3CH_2$	5	4	$C_{22}H_{17}ClNO_2$	188–190	3.6
21b	$4\text{-}CNC_6H_4CH_2$	5	4	$C_{23}H_{18}N_2O_2$	100–103	14
22b	C_6H_5	5	4	$C_{21}H_{17}NO_2$	195–197	9.0
23b	C_6H_5	6	4	$C_{21}H_{17}NO_2$	190–191	7.5

[a] Crystallized from CH_2Cl_2. [b] Relative binding affinities for the calf uterine estrogen receptor = ratio of molar concentrations of 17β-estradiol (E_2) and inhibitor required to decrease the amount of bound [^{3}H]E2 by 50%, × 100. Values are not corrected for contamination by dehydrogenated derivatives.

1% of the affinity of estradiol (Table II). One objective of this study was to find the right distance between the aromatic ring and the indole nitrogen. When compounds with no (**22b**), one (**6b**), or two (**12b**) CH_2 groups between the phenyl ring and the nitrogen were compared, the highest RBA value was found with one CH_2 fragment. As observed in the *N*-alkyl series,[2] the difference in binding affinities between 5- and 6-hydroxyindoles is not very pronounced, but the displacement of the hydroxy group from the para to the meta position in the 2-phenyl ring weakens the binding to the receptor site. In order to increase the binding affinity of the 1-benzyl-2-phenylindole, various substituents were introduced into the benzyl group. Unexpectedly, both hydrophilic and lipophilic groups led to a decrease of binding affinity.

Estrogenic and Antiestrogenic Activity. Six derivatives of 1-benzyl-2-phenylindole with high binding affinities for the estrogen receptor (**6b**, **7b**, **14b**, **16b**, **21b**, **23b**) were tested for their estrogenicity. For these in vivo experiments, the acetates (**6c**, **7c**, **14c**, **16c**, **21c**, **23c**) were used because of their higher stability and better solubility in olive oil. Preliminary studies on the metabolism of these substances exhibited a rapid hydrolysis of the acetates in vivo. In this assay, immature mice received various doses of the drug, and the increase of uterine dry weight was measured there after.[8] Antagonistic activity was determined by simultaneous administration of test compound and estrone (0.4 μg) and calculation of the inhibition of estrone-stimulated uterine growth. Despite the high binding affinities for the estrogen receptor, none of the compounds tested was a strong estrogen. The dose–response curves in the uterine weight test showed only a slow rise (Table III). Even at the highest doses applied (625 μg), the estrogenic effect of the reference drug estrone was not reached. The least active compound in this assay was the cyano derivative **21c**. In the antiuterotrophic test, partial antagonism was found with all of the derivatives at a dose of 125 μg. Lower doses were usually not effective. The maximum antagonistic effect (ca. 40% inhibition) was observed with the unsubstituted benzyl derivative **6c** and the cyano compound **21c**. Compounds like **6c** and **21c** showing low estrogenicity and antagonistic activity appear to be interesting candidates for further investigations on their effect on hormone-dependent tumors.[3,4]

Table III. Estrogenic and Antiestrogenic Activity of 1-Benzyl-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		13.1 ± 1.3		15.8 ± 5.0	
6c	5	13.3 ± 1.8	1	49.9 ± 6.3	
	25	22.6 ± 3.3	5	48.7 ± 6.8	
	125	30.7 ± 4.8	25	42.8 ± 8.0	17
	625	38.1 ± 4.1	125	34.7 ± 4.5	42[e]
estrone	0.4	47.4 ± 4.7	0.4	48.2 ± 7.3	
control		11.4 ± 1.6		15.8 ± 5.0	
7c	5	12.3 ± 1.2	1	54.2 ± 8.2	
	25	17.1 ± 3.0	5	50.4 ± 6.2	
	125	32.4 ± 2.1	25	52.1 ± 895	
	625	32.6 ± 4.9	125	44.6 ± 6.7	11
estrone	0.4	46.5 ± 3.9	0.4	48.2 ± 7.3	
control		11.4 ± 1.6		13.8 ± 2.0	
14c	5	14.4 ± 2.9	1	49.2 ± 4.2	
	25	20.3 ± 3.8	5	46.3 ± 3.0	
	125	32.9 ± 2.8	25	42.5 ± 3.0	16
	325	40.0 ± 5.7	125	36.0 ± 3.5	35[e]
estrone	0.4	46.5 ± 3.9	0.4	47.8 ± 3.2	
control		12.5 ± 1.7		13.8 ± 2.0	
16c	5	14.3 ± 2.5	1	47.1 ± 3.0	
	25	14.7 ± 2.6	5	47.4 ± 4.4	
	125	30.9 ± 4.6	25	46.8 ± 5.5	
	625	45.0 ± 4.6	125	40.1 ± 4.5	23[e]
estrone	0.4	52.2 ± 8.7	0.4	47.8 ± 3.2	
control		15.8 ± 5.0		11.5 ± 2.3	
21c	5	14.0 ± 2.4	25	37.9 ± 7.6	18
	25	15.1 ± 2.7	75	34.1 ± 7.8	30[e]
	125	20.0 ± 3.7	125	30.5 ± 4.7	41[e]
	625	27.4 ± 4.4	250	30.4 ± 4.9	41[e]
			625	31.5 ± 4.9	38[e]
estrone	0.4	48.2 ± 7.3	0.4	43.7 ± 4.6	
control		13.1 ± 3.1		13.1 ± 3.1	
23c	5	13.4 ± 2.3	1	43.2 ± 5.0	
	25	17.8 ± 3.0	5	41.0 ± 4.1	
	125	26.4 ± 3.2	25	43.5 ± 6.3	
	625	34.8 ± 5.6	125	32.4 ± 4.9	33[e]
estrone	0.4	42.0 ± 6.2	0.4	42.0 ± 6.2	

[a] Dose per animal, administered at three consecutive days sc. [b] Uterus dry weight (mg)/body weight (g) × 100, determined 24 h after the last injection; mean of 10 animals ± SD. [c] Simultaneous administration of 0.4 μg of estrone/animal and day. [d] The U-test according to Wilcoxon, modifed by Mann and Whitney, was used. [e] Significant ($p < 0.01$).

Mammary Tumor Inhibiting Effect. For the evaluation of the antineoplastic activity in vivo, we used the 7,12-dimethylbenz[*a*]anthracene- (DMBA-) induced mammary carcinoma of the Sprague–Dawley rat. This tumor shows a marked sensitivity toward ovarian hormones by regressing following the surgical removal of the ovaries.[1] In this and other respects, the rat tumor resembles the human hormone-dependent mammary tumors.[9]

Treatment of established DMBA-induced tumors with 12 mg of **6c**/kg for 28 days did not inhibit tumor growth but led to a nonsignificant stimulation (Table IV). This

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Table IV. Effect of 6c and 21c on the DMBA-Induced Mammary Carcinoma of the Sprague–Dawley Rat

compd	dose,[a] mg	no. animals	no. tumors[b]	new tumors	complete remission,[c] %	partial remission,[d] %	static tumors,[e] %	progr tumors,[f] %	change tumor area,[g] %
control[h]		8	19	10	14	0	31	55	+204
6c	12	8	16	22	3	3	34	60	+295
21c	12	7	14	4	45	22	22	11	−57[i]

[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 28th day of therapy. The U-test according to Wilcoxon, modified by Mann and Whitney, was used to determine the significance. [h] Vehicle alone. [i] Significant ($p < 0.01$).

Table V. Effect of 6b, 21b, and Tamoxifen on the Growth of the MCF-7 Cells

compd	concn, M	cell no./dish[a] × 10^4 (% T/C)	[^{3}H]thymidine incorp[b] × 10^4 (% T/C)
control		40.1 ± 6.0	6.3 ± 1.1
tamoxifen	10^{-6}	10.5 ± 2.0 (26)[c]	2.2 ± 0.3 (35)[c]
	10^{-7}	26.1 ± 2.1 (65)[c]	4.5 ± 0.4 (71)[c]
21b	10^{-5}	5.9 ± 0.8 (15)[c]	1.3 ± 0.2 (21)[c]
	10^{-6}	25.7 ± 3.9 (64)[c]	4.2 ± 0.3 (67)[c]
control[d]		9.8 ± 1.3	14.2 ± 2.3
tamoxifen	10^{-6}	3.8 ± 0.6 (39)[c]	5.3 ± 0.5 (37)[c]
	10^{-7}	6.7 ± 1.0 (68)[c]	10.8 ± 1.8 (76)[c]
6b	10^{-6}	8.7 ± 1.2 (89)	17.4 ± 2.2 (123)
	10^{-7}	10.1 ± 1.9 (102)	20.3 ± 2.2 (143)
21b	10^{-6}	4.9 ± 0.9 (50)[c]	7.8 ± 1.3 (55)[c]
	10^{-7}	12.1 ± 1.3 (123)	17.3 ± 2.6 (122)

[a] Cell number based on Coulter counts on day 7, mean of six dishes ± SD. [b] Radioactivity/dish; mean of six dishes ± SD. [c] Significant inhibition ($p < 0.01$). [d] Two independent experiments were performed.

result was unexpected since 1-alkylindoles with similar endocrine properties in the mouse are very active in this tumor model.[2] The second compound tested, the (4-cyanobenzyl)indole **21c**, strongly inhibited the growth of DMBA-induced tumors at the same dosage. After 4 weeks of therapy, the average tumor area was reduced by 57% due to a large fraction of regressed tumors.

The striking difference in activity between these two compounds prompted us to study their effect on a cellular level using estrogen sensitive human MCF-7 breast cancer cells in tissue culture. Both compounds were applied as free hydroxy derivatives (**6b**, **21b**). The in vitro experiments also exhibited a difference in activity between these two agents. At 10^{-6} M, only **21b** gave rise to a significant inhibition of cell growth as shown by thymidine labeling and cell count (Table V).

Since general cytostatic effects can possibly be responsible for the inhibition, we tested **21b** for its activity against hormone-independent human MDA-MB 231 breast cancer cells as well. In the same dose range, **21b** did not show any significant inhibition of cell growth (Table VI). This result indicates a specific endocrine mode of action for the cyano derivative **21b**.

Discussion

In previous investigations, we found that the introduction of an aromatic group into position 3 of the 2-phenylindole dramatically decreases the binding affinity for the estrogen receptor and, probably as a consequence, the antitumor activity.[5] Regarding the 2-phenylindole as a pseudosymmetric molecule in relation to the longitudinal axis, a similar decrease in activity had to be expected following N-benzylation of the indole. These considerations proved to be wrong concerning the estrogen receptor affinity of 1-benzyl-2-phenylindoles tested in this study. The RBA values are in the same range as those of the corresponding 1-ethyl- and 1-propyl-2-phenylindole derivatives. Obviously, the additional phenyl ring does not interfere with the binding of the 2-phenylindole moiety to the receptor site. It was not possible to increase the receptor affinity by introduction of substituents into the benzyl group. Neither lipophilic groups like chlorine atoms nor polar substituents (e.g., hydroxy) capable of forming hydrogen bonds did improve the binding of the molecule. That might be the result of the rather tight steric situation at the binding site of the estrogen receptor.

Table VI. Effect of 21b on the Growth of MDA-MB 231 Cells

compd	concn, M	cell no./dish[a] × 10^4 (% T/C)	[^{3}H]thymidine incorp[b] × 10^4 (% T/C)
control		7.3 ± 0.9	9.6 ± 0.9
21b	1×10^{-5}	5.5 ± 0.4 (74)[c]	11.0 ± 1.0 (115)
	5×10^{-6}	6.9 ± 1.0 (94)	10.5 ± 0.8 (109)
	1×10^{-6}	7.0 ± 1.5 (95)	10.5 ± 1.1 (109)

[a] Cell number based on Coulter counts on day 3, mean of six dishes ± SD. [b] Radioactivity/dish; mean of six dishes ± SD. [c] Significant inhibition ($\pi < 0.01$).

The estrogenicity of the 1-benzylindoles is rather similar to that of 1-alkyl-5-hydroxy-2-(4-hydroxyphenyl)-3-methylindoles.[2] The antagonistic activity is somewhat less pronounced. Two compounds (**6c**, **21c**) with high binding affinities and strong antiestrogenic activities were evaluated for their mammary tumor inhibiting effects in DMBA-induced rat mammary tumors. Despite the agreement of results in the preceding assays, there was a striking difference in antitumor activity. The cyano derivative **21c** was a potent inhibitor of tumor growth whereas **6c** led to a slight stimulation. Since the difference might be due to a different metabolic fate of **6c** in the rat (e.g., hydroxylation in the free para position of the benzyl groups), we studied both derivatives in vitro using hormone-dependent mammary tumor cells. Again, we observed cytostatic activity only with the cyano derivative **21b**, not with **6b**. It is not yet clear whether an inhibition of cellular uptake, conformational differences of the receptor complex, or a different intracellular metabolism like sulfatation of the phenolic hydroxy groups[10] accounts for the lack of activity of **6b**. Since **21b** is only effective on hormone-dependent breast cancer cells, a specific mode of action involving the estrogen receptor system is likely for the antitumor effect of **21c**.

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 390 A spectrometer and were consistent with the assigned structures. The starting indoles 5-methoxy-2-(4-methoxyphenyl)-3-methylindole (**1**),[2] 6-methoxy-2-(4-methoxyphenyl)-3-methylindole (**2**),[2] 5-meth-

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oxy-2-(3-methoxyphenyl)-3-methylindole (3),[2] 2-(4-methoxyphenyl)-3-methylindole (4),[11] and 5-methoxy-3-methyl-2-phenylindole (5)[11] have been described previously.

General Procedure for the Synthesis of 1-Benzyl-, 1-Benzoyl-, and 1-(2-Phenylethyl)-2-phenylindoles 6a–21a. Sodium hydride (0.028 mol) was added in portions to an ice-cold solution of 50 mL of dry dimethylformamide (DMF) and the resultant mixture stirred for 15 min. An ice-cold solution of 0.020 mol of the 1*H*-indole in dry DMF was added slowly to this mixture with stirring. After 30 min, a solution of 0.025 mol of the benzyl chloride, benzoyl chloride, or 1-bromo-2-phenylethane, respectively, in 30 mL of DMF was added slowly. Stirring was continued for an additional 30 min at 0 °C and 4 h at room temperature. The excess of sodium hydride was destroyed with a few drops of water. After addition of Et_2O, the organic layer was washed twice with water and dried ($MgSO_4$). After evaporation of the solvent, the residue was purified by column chromatography (SiO_2, CH_2Cl_2) and recrystallized from EtOH. The yields were in the range of 30–70%, except for **12a** (8%). Melting points are reported in Table I.

General Procedure for the Synthesis of 1,2-Diphenylindoles 22a–23a. A mixture of 0.05 mol of 1*H*-2-phenylindole **1** or **2**, 0.05 mol of bromobenzene, 7.0 g of anhydrous K_2CO_3, 0.25 g of CuO, and 10 mL of DMF was refluxed for 24 h. After cooling, 50 mL of CH_2Cl_2 was added and the solution was filtered. After evaporation of the solvent, the residue was chromatographed (SiO_2, CH_2Cl_2). The product was recrystallized from EtOH, affording colorless crystals, yield 7–15%. Melting points are reported in Table I.

General Procedure for the Ether Cleavage and Acetylation. A solution of the methoxy-substituted indole (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 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). Solid products were recrystallized from EtOH. The yields were in the range of 50–70%. 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) and [^{3}H]-thymidine (80 Ci/mmol) were obtained from New England Nuclear, Dreieich, FRG. 7,12-Dimethylbenz[*a*]anthracene (DMBA), hormones, and biochemicals were purchased from Sigma, München, FRG. Tamoxifen citrate was a gift of ICI, Plankstadt, FRG. TEA [Tris buffer (0.01 M, pH 7.5) supplemented with EDTA (0.01 M) and NaN_3 (0.003 M)] was used as buffer.

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

For the determination of the relative binding affinity (RBA), the previously described procedure was applied with modifications.[7] The 500-μL incubation mixture comprised 5 nM 17β-[^{3}H]estradiol (added in 100 μL of TEA), 10^{-9}–10^{-5} M competing ligand (in 100 μL of TEA), 100 μL of uterine cytosol, and TEA. The mixture was incubated for 18 h at 4 °C, then 0.5 mL of dextran-coated charcoal (DCC) slurry (0.8% charcoal Norit A and 0.008 % dextran in TEA) was added to the tubes, and the contents were mixed. The tubes were incubated for 90 min at 4 °C and then centrifuged at 700*g* for 10 min to pellet the charcoal. An aliquot (100 μL) of the supernatant was removed, and the radioactivity was determined by liquid scintillation spectrometry after addition of 2 mL of Quickszint 212 (Zinsser). Nonspecific binding was calculated with 5 μM 17β-estradiol as competing ligand. Six concentrations of competitor (1, 2, 5, and 10×10^{-9} to 10^{-6}) were chosen to provide values between 10% and 90% of specifically bound radioactivity. Radioactivity was plotted as a function of the log concentration of competing ligand in the assay. The RBA was calculated as the ratio of the molar concentrations of estradiol and test compound required to decrease the amount of bound radioactivity by 50%, multiplied by 100.

Immature Mice Uterine Weight Tests. Immature female mice (20 days old, of the NMRI strain) from Ivanovas, Kisslegg, FRG, were randomly divided into groups of 10 animals. To determine estrogenic activity, compounds were dissolved in olive oil (100 μL/animal) by gentle warming and the resultant mixture injected subcutaneously on three consecutive days. Control animals received the vehicle alone. At 24 h after the last injection, the animals were killed by cervical dislocation and weighed. Uteri were dissected free of fat and fixed in Bouin solution (saturated aqueous picric acid–40% formaldehyde–glacial acetic acid, 15:5:1, v/v/v) for 20 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 according to ref 11 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 (%) 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: DMBA-Induced Rat Mammary Tumors. 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 in olive oil (1 mL/kg of body weight) by gentle warming and administered subcutaneously. From Monday to Thursday a single dose and on Friday a double dose were administered. 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 tumor ≥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, modified by Mann and Whitney.[12]

MCF-7 Human Breast Cancer Cells. The MCF-7 cell line was provided by Dr. M. E. Lippman, NCI, Bethesda, MD. Cells were grown in improved minimal essential medium (MEM), as

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modified by Richter et al.[13] (Biochrom, Berlin, FRG), supplemented with glutamine (0.3 g/L), gentamycin (60 mg/L), and 5% newborn calf serum (NCS) (Gibco) or charcoal-treated NCC (CCS). CCS was prepared by incubation of 500 mL of NCS with a dextran-coated charcoal pellet[14] 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 sterilized 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% ethanol in which the compounds had been dissolved. The medium of control wells contained an equal volume of ethanol. At the fourth day, media were changed. Three days later, cells were labeled with 1 μCi of [^{3}H]thymidine/well for 2 h. Cells were washed with cold PBS and harvested in PBS containing 0.02% EDTA. After centrifugation, the cell pellet was resuspended in 1 mL of PBS and divided in two equal parts. One part was counted in a Z I Coulter counter; the other one was sonicated. After addition of 4 mL of 10% trichloroacetic acid (TCA), the acid-insoluble fraction was collected on a 0.45-μm filter (Metricel, Gelman) and counted after addition of 10 mL of scintillation liquid (Quickszint 212, Zinsser) in a LS 8000 Beckman scintillation counter.

MDA-MB 231 Human Breast Cancer Cells. The MDA-MB 231 cell line was also 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 6 to 2 days.

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Registry No. **1**, 91444-18-7; **2**, 91444-16-5; **3**, 91466-47-6; **4**, 5782-15-0; **5**, 64648-65-3; **6a**, 104598-82-5; **6b**, 104599-00-0; **6c**, 104599-18-0; **7a**, 104598-83-6; **7b**, 104599-01-1; **7c**, 104599-19-1; **8a**, 104598-84-7; **8b**, 104599-02-2; **8c**, 104599-20-4; **9a**, 104598-85-8; **9b**, 104599-03-3; **9c**, 104599-21-5; **10a**, 104598-86-9; **10b**, 104599-04-4; **10c**, 104599-22-6; **11a**, 104598-87-0; **11b**, 104599-05-5; **11c**, 104599-23-7; **12a**, 104598-88-1; **12b**, 104599-06-6; **12c**, 104599-24-8; **13a**, 104598-89-2; **13b**, 104599-07-7; **13c**, 104599-25-9; **14a**, 104598-90-5; **14b**, 104599-08-8; **14c**, 104599-26-0; **15a**, 104598-91-6; **15b**, 104599-09-9; **15c**, 104599-27-1; **16a**, 104598-92-7; **16b**, 104599-10-2; **16c**, 104599-28-2; **17a**, 104598-93-8; **17b**, 104599-11-3; **17c**, 104599-29-3; **18a**, 104598-94-9; **18b**, 104599-12-4; **18c**, 104599-30-6; **19a**, 104598-95-0; **19b**, 104599-13-5; **19c**, 104599-31-7; **20a**, 104598-96-1; **20b**, 104599-14-6; **20c**, 104599-32-8; **21a**, 104598-97-2; **21b**, 104599-15-7; **21c**, 104599-33-9; **22a**, 104598-98-3; **22b**, 104599-16-8; **22c**, 104599-34-0; **23a**, 104598-99-4; **23b**, 104599-17-9; **23c**, 104599-35-1; $C_6H_{11}CH_2Br$, 2550-36-9; 4-$CH_3C_6H_4CH_2Br$, 104-81-4; 4-$ClC_6H_4CH_2Br$, 622-95-7; 4-$CH_3OC_6H_4CH_2Br$, 2746-25-0; 3-$CH_3C_6H_4CH_2Br$, 620-13-3; 3-$ClC_6H_4CH_2Br$, 766-80-3; 2-$ClC_6H_4CH_2Br$, 611-17-6; 2,6-$Cl_2C_6H_3CH_2Br$, 20443-98-5; 4-$NCC_6H_4CH_2Br$, 17201-43-3; Ac_2O, 108-24-7; benzyl chloride, 100-44-7; benzoyl chloride, 98-88-4; 1-bromo-2-phenylethane, 103-63-9; bromobenzene, 108-86-1.

Supplementary Material Available: ^{1}H NMR data of 1-substituted methoxy-2-phenylindoles **6a-23a**, hydroxy-2-phenylindoles **6b-23b**, and acetoxy-2-phenylindoles **6c-23c** (6 pages). Ordering information is given on any current masthead page.

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Structure-Activity Relationships of Kadsurenone Analogues

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Kadsurenone, a specific receptor antagonist of platelet-activating factor (PAF), and its analogues were prepared from derivatives of cinnamyl alcohol and (allyloxy)phenol. Racemic kadsurenone, resolvable by a Chiralpak column at low temperatures, has an IC_{50} value of 2×10^{-7} M, which is about 50% of the activity of the natural product ($IC_{50} = 1 \times 10^{-7}$ M). The structural specificity of kadsurenone was further demonstrated by the low PAF-receptor-blocking activities of denudatin B, mirandin A, desallylkadsurenone, and the 2-epimer of kadsurenone.

Platelet-activating factor (PAF), an endogenous phospholipid chemically identified as 1-*O*-alkyl-2-*O*-acetyl-*sn*-glycero-3-phosphorylcholine,[1] has been implicated as a mediator of inflammation and anaphylaxis.[2] It is produced and released by stimulated basophils, neutrophils, platelets, macrophages, endothelial cells, and IgE-sensitized bone marrow mast cells.[3] PAF exerts a myriad of biological actions.[4] It induces smooth-muscle contraction and aggregation and degranulation of platelets and neutrophils.[5] In various animal models, PAF induces bronchoconstriction, hyperalgesia, hypotension, neutropenia, thrombocytopenia, increased cutaneous vascular permeability, increased hematocrit, and lysosomal enzyme secretion.[4,5] PAF may also play a major role in asthma[6,7] and in certain forms of gastric ulceration.[8] With use of a receptor preparation of rabbit platelet membranes to

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