(s, 3 H), 1.93 (m, 1 H), 0.50 (m, 4 H).

2-[*n*-Butyl(2-hydroxyethyl)amino]ethyl acetate (9): yield 10.4%; TLC (EtOAc + 5% MeOH), single spot (R_f 0.89); NMR ($CDCl_3$) δ 4.14 (t, 2 H), 3.55 (t, 2 H), 2.92–2.30 (m, 7 H), 2.10 (s, 3 H), 1.37 (m, 4 H), 0.93 (m, 3 H).

2-[Isobutyl(2-hydroxyethyl)amino]ethyl acetate (10): yield 8.2%; TLC (EtOAc + 5% MeOH), single spot (R_f 0.87); NMR ($CDCl_3$) δ 4.12 (t, 2 H), 3.53 (t, 2 H), 3.10–2.40 (m, 5 H), 2.24 (d, 2 H), 2.06 (s, 3 H), 1.74 (m, 1 H), 0.88 (d, 6 H).

Preparation of 2-[*n*-Propyl(2-chloroethyl)amino]ethyl Acetate Hydrochloride (11) (General Procedure: Step d). To a magnetically stirred and ice-cooled solution of 460 mg (2.4×10^{-3} mol) of **6** in 2.0 mL of $CHCl_3$ was added 382 mg (3.2×10^{-3} mol) of $SOCl_2$ dropwise with a hypodermic syringe, under an N_2 atmosphere. After the completion of addition of $SOCl_2$ (10 min), the ice bath was removed and the reaction mixture was stirred for 2.5 h at room temperature under an N_2 atmosphere to complete the reaction. Removal of $CHCl_3$ and $SOCl_2$ under high vacuum gave a thick yellow oil. The oil was dissolved in 3.0 mL of dry THF and cooled with stirring. Addition of 5.0 mL of dry diethyl ether to the stirred solution under N_2 resulted in the precipitation of a white solid, which was filtered, washed with dry diethyl ether, and then dried in an Abderhalden pistol (CH_2Cl_2 as solvent and 0.5 mmHg pressure) to give 460 mg of a white solid (78.6% yield) as the hydrochloride salt **11**: mp 96–98 °C; NMR ($CDCl_3$) δ 4.62 (t, 2 H), 4.10 (t, 2 H), 3.70–2.90 (m, 6 H), 2.60 (s, 3 H), 1.93 (m, 2 H), 1.05 (t, 3 H); MS, *m/e* (relative intensity), 207 (0.31, M^+ - HCl), 136 (34.82, M^+ - HCl - $C_3H_5O_2$), 134 (91.23, M^+ - HCl - $C_3H_5O_2$), 87 (100.00, $C_4H_7O_2$). Anal. ($C_9H_{19}NO_2Cl_2$) C, H, N, Cl.

In the same manner, other 2-[alkyl(2-chloroethyl)amino]ethyl acetate hydrochlorides were prepared from compounds **7–10** by route (d).

2-[Isopropyl(2-chloroethyl)amino]ethyl acetate hydrochloride (12): mp 72–74 °C; yield 71.9%; NMR ($CDCl_3$) δ 4.45 (t, 2 H), 3.94 (m, 3 H), 3.66 (m, 4 H), 2.15 (s, 3 H), 1.37 (d, 6 H); MS, *m/e* (relative intensity) 209 (0.29, M^+ - HCl), 207 (1.28, M^+ - HCl), 136 (31.13, M^+ - HCl - $C_3H_5O_2$), 134 (85.62, M^+ - HCl - $C_3H_5O_2$), 94 (32.87, C_3H_7NCl), 92 (100.00, C_3H_7NCl). Anal. ($C_9H_{19}NO_2Cl_2$) C, H, N, Cl; C: calcd, 44.44; found, 43.95.

2-[Cyclopropyl(2-chloroethyl)amino]ethyl acetate hydrochloride (13): mp 67–69 °C; yield 62.47%; NMR ($CDCl_3$) δ 4.64 (t, 2 H), 4.22 (t, 2 H), 3.64 (m, 4 H), 2.72 (m, 1 H), 2.16 (s, 3 H), 1.61 (m, 2 H), 1.00 (m, 2 H); MS, *m/e* (relative intensity) 205 (1.72, M^+ - HCl), 132 (47.65, M^+ - HCl - $C_3H_5O_2$, 87 (100.00, $C_4H_7O_2$). Anal. ($C_9H_{17}NO_2Cl_2$) C, H, N, Cl.

2-[*n*-Butyl(2-chloroethyl)amino]ethyl acetate hydrochloride (14): mp 110–112 °C; yield 85.5%; NMR ($CDCl_3$) δ 4.62 (t, 2 H), 4.12 (t, 2 H), 3.75–2.95 (m, 6 H), 2.14 (s, 3 H), 2.10–1.15 (m, 4 H), 1.00 (t, 3 H); MS, *m/e* (relative intensity) 223 (0.43, M^+ - HCl), 221 (1.29, M^+ - HCl), 180 (6.30, M^+ - HCl - C_3H_7), 178 (18.78, M^+ - HCl - C_3H_7), 150 (36.57, M^+ - HCl - $C_3H_5O_2$, 148 (100.00, M^+ - HCl - $C_3H_5O_2$), 108 (24.67, C_4H_9NCl), 106 (74.67, C_4H_9NCl), 87 (78.75, $C_4H_7O_2$). Anal. ($C_{10}H_{21}NO_2Cl_2$) C, H, N, Cl.

2-[Isobutyl(2-chloroethyl)amino]ethyl acetate hydrochloride (15): mp 94–96 °C; yield 88.3%; NMR ($CDCl_3$) δ 4.63 (t, 2 H), 4.15 (t, 2 H), 3.57 (t, 4 H), 3.12 (d, 2 H), 2.28 (m, 1 H), 2.15 (s, 3 H), 1.16 (d, 6 H); MS, *m/e* (relative intensity) 221 (0.49, M^+ - HCl), 180 (2.87, M^+ - HCl - C_3H_7), 178 (8.52, M^+ - HCl - C_3H_7), 87 (100.00, $C_4H_7O_2$). Anal. ($C_{10}H_{21}NO_2Cl_2$) C, H, N, Cl.

Preparation of 10 mM Solution of 1-Alkyl-1-(2-hydroxyethyl)aziridinium Chloride for Biological Assays. The hydrochloride salt **11–15** was accurately weighed and stirred in sufficient distilled H_2O to dissolve the salt. At this point, the pH was usually between 3.5 and 4.0. A sufficient amount of 5 N NaOH was added to bring the pH to 11.3–11.6. The solution was then stirred for another 30 min at room temperature while the pH was maintained between 11.3 and 11.6. Next, the pH was adjusted to 7.4 by first adding concentrated HCl to bring the pH to slightly acidic (about 6) and then adding the required amount of $NaHCO_3$. Finally, the required amount of distilled H_2O was added to make up a final solution of 10 mM concentration for use in biological assays.

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Registry No. 1, 63918-37-6; **2**, 6735-35-9; **3**, 121-93-7; **4**, 17259-20-0; **5**, 30769-76-7; **6**, 99748-79-5; **7**, 99748-80-8; **8**, 99748-81-9; **9**, 78818-89-0; **10**, 99748-82-0; 11, 99748-83-1; **12**, 99748-84-2; **13**, 99748-85-3; **14**, 78818-91-4; **15**, 99748-86-4; diethanolamine, 111-42-2; *n*-propyl bromide, 106-94-5; isopropylamine, 75-31-0; ethylene oxide, 75-21-8; cyclopropylamine, 765-30-0; isobutylamine, 78-81-9; 1-propyl-1-(2-hydroxyethyl)aziridinium chloride, 99748-87-5; 1-isopropyl-1-(2-hydroxyethyl)-aziridinium chloride, 99748-88-6; 1-cyclopropyl-1-(2-hydroxyethyl)aziridinium chloride, 99748-89-7; 1-butyl-1-(2-hydroxyethyl)aziridinium chloride, 99748-90-0; 1-isobutyl-1-(2-hydroxyethyl)aziridinium chloride, 99748-91-1; *N,N*-bis(2-hydroxyethyl)butylamine, 102-79-4.

Benzo[*a*]carbazole Derivatives. Synthesis, Estrogen Receptor Binding Affinities, and Mammary Tumor Inhibiting Activity

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A number of 11-alkylbenzo[*a*]carbazoles and their 5,6-dihydro derivatives with one or two hydroxy groups in the aromatic rings were synthesized and studied for their binding affinities for the estrogen receptor. Best conditions for the receptor binding are provided by one hydroxy group at C-3 and a second one at position 8 or 9. The binding affinities of the benzo[*a*]carbazoles are somewhat lower than those of the dihydro derivatives but still high regarding the planar structure of these molecules. The highest relative binding affinity (RBA) values (e.g., 30 for **13b**, 13 for **16b**, 20 for **25a**; estradiol = 100) are close to those of the corresponding 2-phenylindole derivatives. Depending on the positions of the oxygen functions, the benzo[*a*]carbazoles behaved as strong estrogens (**13c**, **25a**) or impeded estrogens (**16c**, **28a**) in the immature mouse. Derivative **16c** inhibited the growth of dimethylbenzanthracene-induced hormone-dependent mammary tumors of the rat at a dose of 6×1 mg/kg per week. In vitro, **16b** and **28b** showed inhibitory activity on estrogen receptor positive MCF-7 breast cancer cells. A mode of action involving the estrogen receptor system is assumed.

Pharmacotherapy with antiestrogens has become an established method in the management of advanced breast cancer.[1-3] The advantages of this treatment are because of the greatly reduced toxicity compared to cytostatic

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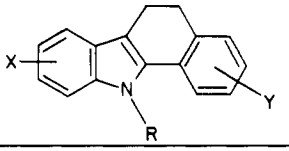
Table I. 11-Alkyl-6,11-dihydromethoxy-5*H*-benzo[*a*]carbazoles and Acetoxy-11-alkyl-6,11-dihydro-5*H*-benzo[*a*]carbazoles

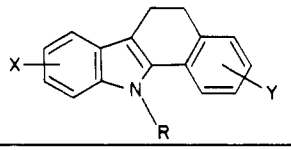


X, Y = OCH_3. OAc

compd[a]	R	position of X	position of Y	formula	mp,[b] °C	compd[c]	formula[d]	mp,[b] °C
12a	CH_3	3	9	$C_{19}H_{19}NO_2$	125–126	**12c**	$C_{21}H_{19}NO_4$	197–198
13a	C_2H_5	3	9	$C_{20}H_{21}NO_2$	141–143	**13c**	$C_{22}H_{21}NO_4$	183–185
14a	C_3H_7	3	9	$C_{21}H_{23}NO_2$	111–112	**14c**	$C_{23}H_{23}NO_4$	160–162
15a	C_4H_9	3	9	$C_{22}H_{25}NO_2$	92–94	**15c**	$C_{24}H_{25}NO_4$	125–127
16a	C_2H_5	3	8	$C_{20}H_{21}NO_2$	140–141	**16c**	$C_{22}H_{21}NO_4$	165–167
17a	C_2H_5	4	9	$C_{20}H_{21}NO_2$	134–135	**17c**	$C_{22}H_{21}NO_4$	195–196
18a	C_2H_5	4	8	$C_{20}H_{21}NO_2$	96–98	**18c**	$C_{22}H_{21}NO_4$	139–141
19a	C_2H_5	2	9	$C_{20}H_{21}NO_2$	136–138	**19c**	$C_{22}H_{21}NO_4$	104–105
20a	C_2H_5	2	8	$C_{20}H_{21}NO_2$	98–99	**20c**	$C_{22}H_{21}NO_4$	129–130
21a	C_2H_5	3		$C_{19}H_{19}NO$	133–134	**21c**	$C_{20}H_{19}NO_2$	148–149
22a	C_2H_5		8	$C_{19}H_{19}NO$	115–116	**22c**	$C_{20}H_{19}NO_2$	96–97
23a	C_2H_5		9	$C_{19}H_{19}NO$	97–98	**23c**	$C_{20}H_{19}NO_2$	177–178

[a] X, Y = OMe. [b] Recrystallized from EtOH. [c] X, Y = OAc, R as in a. [d] Analyzed for C and H within ±0.40% of the calculated values.

Table II. Estrogen Receptor Binding Affinities of 11-Alkyl-6,11-dihydrohydroxy-5*H*-benzo[*a*]carbazoles



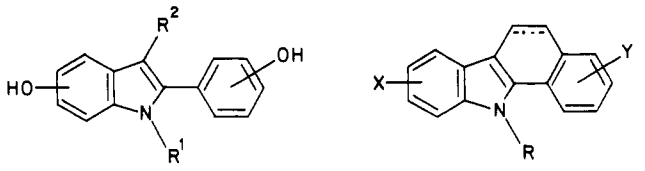
X, Y = OH

compd	R	position of X	position of Y	formula	mp,[a] °C	RBA[b]
12b	CH_3	3	9	$C_{17}H_{15}NO_2$	240–242	9.6
13b	C_2H_5	3	9	$C_{18}H_{17}NO_2$	207–209	30
14b	C_3H_7	3	9	$C_{19}H_{19}NO_2$	142–144	38
15b	C_4H_9	3	9	$C_{20}H_{21}NO_2$	167–170	9.5
16b	C_2H_5	3	8	$C_{18}H_{17}NO_2$	182–184	13
17b	C_2H_5	4	9	$C_{18}H_{17}NO_2$	171–173	1.3
18b	C_2H_5	4	8	$C_{18}H_{17}NO_2$	182–185	1.9
19b	C_2H_5	2	9	$C_{18}H_{17}NO_2$	180–185	9.7
20b	C_2H_5	2	8	$C_{18}H_{17}NO_2$	179–180	0.7
21b	C_2H_5	3		$C_{18}H_{17}NO$	98–100	1.8
22b	C_2H_5		8	$C_{18}H_{17}NO$	157–159	0.06
23b	C_2H_5		9	$C_{18}H_{17}NO$	122–124	0.8

[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.

agents. A general limitation met within all antiestrogens so far available is the therapeutic failure in more than one-third of the patients with estrogen receptors in the malignant tissue.[4] One possible way to overcome this problem is the development of new structures with a higher efficacy. Hydroxy-substituted 2-phenylindoles have proved to be an active class of compounds in this respect. They show high binding affinities for the estrogen receptor and exert an inhibitory effect on hormone-dependent experimental mammary tumors.[5-7] These results prompted us to extend our studies in this class of heterocycles. Linkage of one ortho position of the phenyl ring with C-3 of the indole nucleus by an ethylene or ethane bridge leads to benzo[*a*]carbazole derivatives (Scheme I). These tetracycles are more rigid than the 2-phenylindoles and might resemble the natural estrogens in respect to binding affinity for the estrogen receptor. In addition, benzo[*a*]carbazoles are planar polycycles that have the potential of intercalating into the DNA.[8,9]

Scheme I



X = H; 8- or 9-OH
Y = H; 2-, 3-, or 4-OH
R = CH_3, C_2H_5, C_3H_7, C_4H_9

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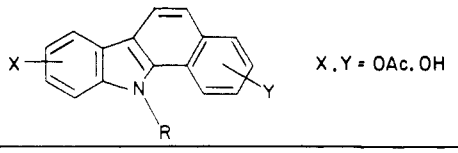
Table III. Acetoxy-11-alkyl-11*H*-benzo[*a*]carbazoles, Hydroxy-11-alkyl-11*H*-benzo[*a*]carbazoles, and Estrogen Receptor Binding Affinities



X, Y = OAc, OH

compd[a]	R	position of X	position of Y	formula[b]	mp,[c] °C	compd[d]	formula	mp,[e] °C	RBA[f]
24a	CH_3	3	9	$C_{21}H_{17}NO_4$	207–208	**24b**	$C_{17}H_{13}NO_2$	256–258	9.5
25a	C_2H_5	3	9	$C_{22}H_{19}NO_4$	176–178	**25b**	$C_{18}H_{15}NO_2$	226–227	20
26a	C_3H_7	3	9	$C_{23}H_{21}NO_4$	166–167	**26b**	$C_{19}H_{17}NO_2$	198–202	13
27a	C_4H_9	3	9	$C_{24}H_{23}NO_4$	152–153	**27b**	$C_{20}H_{19}NO_2$	225–227	2.3
28a	C_2H_5	3	8	$C_{22}H_{19}NO_4$	193–194	**28b**	$C_{18}H_{15}NO_2$	223–224	6.5
29a	C_2H_5	4	9	$C_{22}H_{19}NO_4$	184–186	**29b**	$C_{18}H_{15}NO_2$	222–223	0.65
30a	C_2H_5	4	8	$C_{22}H_{19}NO_4$	164–165	**30b**	$C_{18}H_{15}NO_2$	210–211	0.10
31a	C_2H_5	2	9	$C_{22}H_{19}NO_4$	159–160	**31b**	$C_{18}H_{15}NO_2$	176–178	3.6
32a	C_2H_5	2	8	$C_{22}H_{19}NO_4$	149–150	**32b**	$C_{18}H_{15}NO_2$	197–198	0.08
33a	C_2H_5	3		$C_{20}H_{17}NO_2$	146–147	**33b**	$C_{18}H_{15}NO$	188–190	0.33
34a	C_2H_5		8	$C_{20}H_{17}NO_2$	177–179	**34b**	$C_{18}H_{15}NO$	185–187	<0.01
35a	C_2H_5		9	$C_{20}H_{17}NO_2$	181–183	**35b**	$C_{18}H_{15}NO$	167–170	0.01

[a] X, Y = OAc. [b] Analyzed for C and H within ±0.40% of the calculated values. [c] Recrystallized from EtOH. [d] X, Y = OH; R as in a. [e] Crystallized from CH_2Cl_2. [f] 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.

A number of 11-alkylbenzo[*a*]carbazoles and their dihydro analogues with oxygen functions were synthesized and investigated for their binding affinity for the estrogen receptor, their estrogenic and antiestrogenic effects in the immature mouse, and their mammary tumor inhibiting activity.

Chemistry. 6,11-Dihydro-5*H*-benzo[*a*]carbazoles were obtained by the same procedure as applied for the Fischer indole synthesis:[10] Reaction of (methoxyphenyl)-hydrazines (**1**) with methoxy-1-tetralones (**2**) afforded the methoxy-substituted 6,11-dihydro-5*H*-benzo[*a*]carbazoles **3–11** (Scheme II). The substituents at the nitrogen were introduced by deprotonation with sodium amide in liquid ammonia and subsequent addition of alkyl iodide. Demethylation of the methoxy compounds **12a–23a** was readily effected with BBr_3 in CH_2Cl_2 (Table I). The hydroxydihydrobenzocarbazoles **12b–23b** (Table II) were purified by conversion to the acetates **12c–23c** with use of acetic anhydride in pyridine followed by chromatographic isolation. The free hydroxy compounds were obtained by alkaline hydrolysis of the acetates in methanol. Chromatographic analysis by HPLC and NMR spectroscopy showed that the 6,11-dihydro-5*H*-benzo[*a*]carbazole **12b,c–23b,c** are contaminated by 5–10% of the corresponding benzo[*a*]carbazoles that are formed by dehydrogenation in position 5 and 6. This oxidation reaction took place although ether cleavage, acetylation, and hydrolysis were performed under nitrogen atmosphere.

The acetoxy-substituted 11H-benzo[*a*]carbazoles **24a–35a** were obtained by oxidation of the acetates **12c–23c** with 2,3-dichloro-4,6-dicyanobenzoquinone (DDQ) in CH_2Cl_2 in high yield. Alkaline hydrolysis afforded the hydroxy benzo[*a*]carbazoles **24b–35b** (Table III).

Binding Affinity for the Calf Uterine Estrogen Receptor. All of the hydroxy-substituted benzo[*a*]carbazole derivatives were tested for their estrogen receptor affinity. Binding affinities were measured by a competitive binding assay with 17β-[^{3}H]estradiol as described previously[11] and by other authors.[12,13] Calf uterine cytosol was used as receptor source and the dextran-coated charcoal (DCC) method was applied. The incubation period was

Scheme II. General Synthesis of 11-Alkyl-11*H*-benzo[*a*]carbazoles

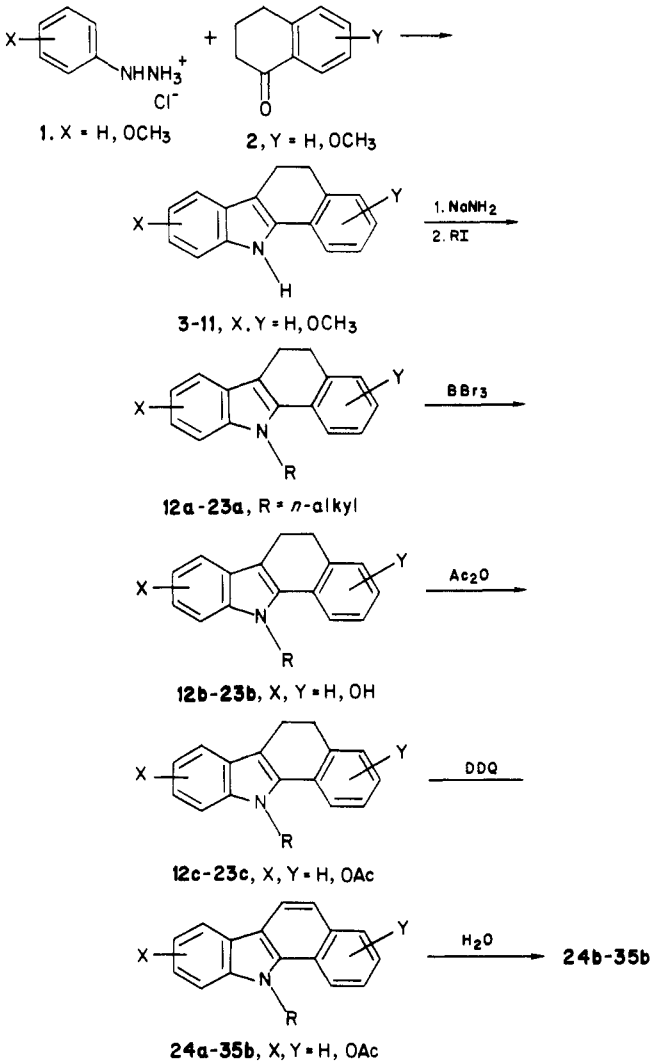


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Table IV. Uterotrophic Activity of Benzo[a]carbazole Derivatives in the Mouse

compd	dose,[a] μg	effect[b]	compd	dose,[a] μg	effect[b]
control		14.4 ± 2.0	control		12.8 ± 2.0
13c	0.2	20.3 ± 4.7	**25a**	0.2	21.6 ± 2.1
	1	29.3 ± 3.1		1	28.5 ± 3.5
	5	45.8 ± 4.2		5	38.5 ± 3.9
	25	43.9 ± 4.3		25	38.7 ± 3.2
				125	46.0 ± 2.4
estrone	1	42.5 ± 3.7	estrone	1	46.9 ± 3.8
control		12.8 ± 2.0	control		9.6 ± 2.5
16c	0.2	15.3 ± 1.7	**28a**	0.2	12.0 ± 2.5
	1	19.6 ± 2.5		1	13.7 ± 1.8
	5	18.4 ± 2.4		5	14.4 ± 2.5
	25	23.6 ± 1.7		25	15.1 ± 2.4
	125	25.4 ± 1.9		125	18.1 ± 4.2
estrone	1	46.9 ± 3.8	estrone	0.4	48.6 ± 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.

18 h to guarantee equilibrium conditions for the binding assay and exclude the possible influence of different dissociation rates of the ligands. According to Leclercq et al.,[14] the concentration of [^{3}H]estradiol used (5 mM) is sufficient to reach nearly saturation of the specific binding sites. The relative binding affinities (RBA) are given as the ratio of the molar concentrations of 17β-estradiol and carbazole required to decrease receptor bound radioactivity by 50%, multiplied by 100. The RBA values are reported in Tables II and III. The semilogarithmic plot of bound radioactivity vs. molar concentrations of the benzo[a]carbazole derivatives exhibited curves parallel to those of 17β-estradiol and hexestrol, suggesting a common binding site for all of the compounds that were tested.

Several dihydroxy-substituted benzo[a]carbazoles show strong binding affinities for the calf uterine estrogen receptor (Tables II and III). The highest RBA values (**13b**, **14b**) exceed the affinity of the potent synthetic estrogen hexestrol (RBA = 25). As expected, the receptor affinity decreases considerably if one hydroxy group is missing. Conversion of the dihydro derivatives to the planar aromatic system does not affect very much the binding of the benzo[a]carbazoles to the receptor site. The contamination of the dihydro compounds by a small amount of oxidation products can be neglected for these considerations because its effect is within the experimental error. Generally, the affinity decreases by this structural alteration. The best conditions for the binding to the estrogen receptor are provided by two hydroxy groups located in positions 3 and 9.

Estrogenic and Antiestrogenic Activity. The acetates of the two dihydro benzo[a]carbazoles with high binding affinities (**13c**, **16c**) and their oxidized analogues (**25a**, **28a**) were evaluated for their estrogenicity in the mouse uterus. The assay was performed according to the procedure described by Rubin et al.[15] Estrone (1.0 and 0.4 μg, respectively) was used as reference for maximum stimulation of uterine weight gain. In these and other in vivo experiments the acetates were used because of their higher stability and better solubility in olive oil. The acetates are readily cleaved in vivo to afford the free hydroxy derivatives as active metabolites (data not shown). Compounds **13c** and **25a** with oxygen functions in positions 3 and 9 produced the same estrogenic response like the reference drug but at higher doses (Table IV). Already at low doses, uterotrophic activity was observed. Displacement of one acetoxy group from C-9 to C-8 (**16c**, **28a**) diminishes the estrogenicity considerably. A daily dose of 125 μg/animal is required for doubling the uterine weight. Since we knew from previous experiments that low estrogenicity is often associated with antiestrogenic activity, we determined the antiuterotrophic effect of compounds **16c** and **28a** by simultaneous administration of 0.4 μg of estrone. Only compound **16c** showed significant antiestrogenic effects (inhibition: 14% at 5 μg and 27% at 125 μg) whereas **28a** was devoid of antagonistic activity.

Mammary Tumor Inhibition. Two representatives of the benzo[a]carbazoles were tested for their inhibitory activity against the 7,12-dimethylbenz[a]anthracene (DMBA) induced mammary carcinoma of the Sprague–Dawley rat. This tumor is widely used as in vivo model for estrogen-sensitive carcinomas because it shows many similarities to human hormone-dependent breast cancer.[16] Regarding a potential clinical application we are mainly interested in compounds with low estrogenicity. Therefore, two of the 8-hydroxy derivatives (**16c**, **28a**) were tested for their mammary tumor inhibiting effect. Tamoxifen, a drug that is used for treatment of estrogen receptor positive breast cancer, served as standard in this experiment. Benzo[a]carbazole **28a** in both dosages was inactive. The slight increase of tumor area that was observed after 4 weeks of treatment was not significant. The administered dose of 1 and 4 mg/kg were chosen following the experience with structurally related 2-phenylindoles. The dihydro analogue **16c** inhibited the growth of DMBA-induced tumors, but only the lower dose gave rise to a significant effect ($p < 0.01$). The inhibitory activity of **16c** was similar to that of tamoxifen. Change of body weight during therapy was less than 3% (Table V).

In addition to the in vivo experiments, the deacetylated benzo[a]carbazole derivatives **16b** and **28b** were tested for their in vitro antitumor activity against hormone-dependent and -independent human breast cancer cells. Both compounds inhibited the growth of estrogen-receptor positive MCF-7 cells at concentrations of 10^{-5} and 10^{-6} M (Table VI). The inhibition was nearly complete at 10^{-5} M. At the lower concentration the cell number was clearly reduced, but the inhibitory effect on thymidine incorporation was rather weak. All of these compounds failed to inhibit the growth of hormone-independent MDA-MB 231 breast cancer cells (data not shown).

Discussion

A number of benzo[a]carbazoles and their dihydro derivatives with one or two hydroxy functions were synthesized in order to establish structure–activity relationships. The first objective was to study the influence of structural variations on estrogen receptor binding. Like in the 2-phenylindole series, the arrangement of two hydroxy groups in positions 3 and 8 or 9 provide the most favorable conditions for the receptor affinity.[6] The low affinity of derivatives bearing a hydroxy function at C-4 can be explained by a steric effect of the adjacent C_2 bridge that impedes the formation of a hydrogen bond between the hydroxy group and the receptor site. The reason for

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Table V. Effect of 16c, 28c, and Tamoxifen Citrate on the DMBA-Induced Mammary Carcinoma of the Sprague–Dawley Rat

compd	dose,[a] mg	no. of animals	no. of tumors[b]	no. of new tumors	complete remission,[c] %	partial remission,[d] %	static tumors,[e] %	progr tumors,[f] %	change of tumor area[g] median, %	change of tumor area[g] range,[h] %
control[i]		10	31	51	7	5	21	67	460	224–620
16c	1.0	8	22	21	9	7	35	49	179[k]	49–492
16c	4.0	8	24	24	10	6	23	61	230	−94–691
28a	1.0	8	22	41	3	8	19	70	493	−29–2088
28a	4.0	8	23	45	6	10	25	59	561	111–988
tamoxifen	3.0	10	31	24	22	7	27	44	136[k]	−57–600

[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] Median on the 28th day of therapy. The *U* test according to Wilcoxon, Mann, and Whitney was used to determine the significance. [h] Numbers without sign are understood to be positive. [i] Vehicle alone. [k] Significant ($p < 0.01$).

Table VI. Effect of 16b, 28b, and Tamoxifen on the Growth of MCF-7 Cells

compd	concn, M	(cell no./dish)[a] × 10^4 (% T/C)	[^{3}H]thymidine incorp[b] × 10^3 (% T/C)
control		11.65 ± 0.61	37.31 ± 2.30
16b	10^{-5}	0.46 ± 0.13 (4)[c]	0.76 ± 0.16 (2)[c]
28b	10^{-5}	0.52 ± 0.09 (4)[c]	1.04 ± 0.17 (3)[c]
tamoxifen	10^{-5}	0.20 ± 0.05 (2)[c]	0.08 ± 0.01 (0)[c]
control		4.05 ± 0.40	30.58 ± 1.86
16b	10^{-6}	2.82 ± 0.14 (70)[c]	25.84 ± 2.61 (85)[c]
28b	10^{-6}	2.41 ± 0.21 (59)[c]	26.46 ± 3.13 (87)
tamoxifen	10^{-6}	0.48 ± 0.10 (12)[c]	1.85 ± 0.46 (6)[c]

[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 ($p < 0.05$).

the unexpectedly weak binding of the 2,8-disubstituted compounds is unclear. The main difference between the 2-phenylindole system and the benzo[*a*]carbazoles is based on the planar structure of the tetracyclic compounds. The high estrogen receptor affinity of some of the benzo[*a*]-carbazole derivatives is contrary to the hypothesis of other authors that a molecular thickness of 4.5 Å is required for a strong binding interaction with the receptor site.[17] Geometric similarities of the benzo[*a*]carbazoles with the synthetic estrogen diethylstilbestrol make an interaction with the receptor site similar to that of estrogens likely.

Two representatives both of the benzo[*a*]carbazoles and the dihydro derivatives were tested for their endocrine activity. According to the results that were obtained in the 2-phenylindole series, the 3,9-disubstituted compounds behave as strong estrogens whereas the 3,8-dihydroxy compounds exert only low agonistic activity despite their high binding affinities.

Since the aim of these investigations is the development of mammary tumor inhibiting agents with low estrogenicity, we studied the antitumor activity of the latter compounds (**16c**, **28a**) in vivo and in vitro. The dihydrobenzo[*a*]carbazole **16c** showed a significant inhibitory effect on DMBA-induced hormone-dependent rat mammary tumors at a dose of 1 mg/kg. Unfortunately, it was not possible to enhance this effect by increasing the dose. These findings are different from those obtained in the related 2-phenylindole series where compounds with similar receptor affinities were very active in this tumor model.[5-7] Hoping to find an explanation for these results, we studied the effect of both compounds on hormone-sensitive MCF-7 breast cancer cells. A marked growth inhibition was only found at a concentration of 10^{-5} M. We assume that this comparatively high concentration of the drug cannot be reached in the tumor tissue and, therefore, the antitumor effect is not as strong in vivo as expected. The lack of activity in hormone-independent MDA-MB 231 cells make an additional cytostatic action via DNA intercalation unlikely.[18] Therefore, we assume that the benzo[*a*]carbazole derivatives act via the cellular estrogen receptor system.

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 390A spectrometer and were consistent with the assigned structures.

General Procedure for the Preparation of 6,11-Dihydro-5*H*-benzo[*a*]carbazoles 3–11. A solution of 1-tetralone or methoxy-1-tetralone (0.023 mol) in 50 mL of EtOH was added to a boiling mixture of phenyl- or (methoxyphenyl)hydrazine hydrochloride (0.023 mol) and concentrated HCl (2.0 mL) in 75 mL of EtOH within 30 min. Boiling was continued for 5 h. After addition of hot water (150 mL), the solution was allowed to cool to room temperature. The product was separated by filtration and recrystallized from EtOH.

6,11-Dihydro-3,9-dimethoxy-5*H*-benzo[*a*]carbazole (3) has been described previously.[10]

6,11-Dihydro-3,8-dimethoxy-5*H*-benzo[*a*]carbazole (4): yield 75%; mp 189–191 °C (lit.[19] mp 190 °C); ^{1}H NMR ($CDCl_3$) δ 2.95 (mc, 4 H, CH_2CH_2), 3.78 (s, 3 H, OCH_3), 3.87 (s, 3 H, OCH_3), 6.57–7.25 (m, 6 H, Ar H), 8.00 (s, 1 H, NH).

6,11-Dihydro-4,9-dimethoxy-5*H*-benzo[*a*]carbazole (5): yield 58%; mp 222–223 °C; ^{1}H NMR ($CDCl_3$/CF_3COOH) δ 3.00 (m, 4 H, CH_2CH_2), 3.87 (s, 3 H, OCH_3), 3.95 (s, 3 H, OCH_3), 7.00–7.87 (m, 6 H, Ar H). Anal. ($C_{18}H_{17}NO_2$) C, H.

6,11-Dihydro-4,8-dimethoxy-5*H*-benzo[*a*]carbazole (6): yield 64%; mp 207–208 °C; ^{1}H NMR ($CDCl_3$) δ 3.00 (mc, 4 H, CH_2CH_2), 3.87 (s, 6 H, OCH_3), 6.67–7.30 (m, 6 H, Ar H), 8.07 (s, 1 H, NH). Anal. ($C_{18}H_{17}NO_2$) C, H.

6,11-Dihydro-2,9-dimethoxy-5*H*-benzo[*a*]carbazole (7): yield 68%; mp 178–181 °C; ^{1}H NMR ($CDCl_3$) δ 2.93 (s, 4 H, CH_2CH_2), 3.83 (s, 6 H, OCH_3), 6.50 (dd, J_1 = 9 Hz, J_2 = 2 Hz, 1 H, Ar H), 6.63–7.13 (m, 4 H, Ar H), 7.43 (d, J = 9 Hz, 1 H, Ar H), 8.13 (s, 1 H, NH). Anal. ($C_{18}H_{17}NO_2$) C, H.

6,11-Dihydro-2,8-dimethoxy-5*H*-benzo[*a*]carbazole (8): yield 75%; mp 152–153 °C; ^{1}H NMR ($CDCl_3$) δ 2.93 (s, 4 H, CH_2CH_2), 3.82 (s, 3 H, OCH_3), 3.88 (s, 3 H, OCH_3), 6.70 (dd, J_1 = 9 Hz, J_2 = 2 Hz, 1 H, Ar H), 6.76–7.30 (m, 5 H, Ar H), 8.17 (s, 1 H, NH). Anal. ($C_{18}H_{17}NO_2$) C, H.

6,11-Dhydro-3-methoxy-5*H*-benzo[*a*]carbazole (9): yield 80%; mp 167–168 °C (lit.[19] mp 168 °C); ^{1}H NMR ($CDCl_3$) δ 2.97 (s, 4 H, CH_2CH_2), 3.78 (s, 3 H, OCH_3), 6.63–7.57 (m, 7 H, Ar H), 8.03 (s, 1 H, NH).

6,11-Dihydro-8-methoxy-5*H*-benzo[*a*]carbazole (10): yield 74%; mp 186–187 °C; ^{1}H NMR ($CDCl_3$) δ 3.00 (mc, 4 H, CH_2H_2),

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3.87 (s, 3 H, OCH_3), 6.82 (dd, J_1 = 9 Hz, J_2 = 2 Hz, 1 H, Ar H), 7.00 (d, J = 2 Hz, 1 H, Ar H), 7.13–7.30 (m, 5 H, Ar H). Anal. ($C_{17}H_{15}NO$) C, H.

6,11-Dihydro-9-methoxy-5*H*-benzo[*a*]carbazole (11): yield 66%; mp 173–174 °C; ^1H NMR (CD_3COCD_3) δ 2.93 (mc, 4 H, CH_2CH_2), 3.77 (s, 3 H, OCH_3), 6.67 (dd, J_1 = 9 Hz, J_2 = 2 Hz, 1 H, Ar H), 6.90 (d, J = 2 Hz, 1 H, Ar H), 7.02–7.57 (m, 5 H, Ar H). Anal. ($C_{17}H_{15}NO$) H; C: calcd, 81.90; found, 81.30.

General Procedure for the Alkylation of 6,11-Dihydro-5*H*-benzo[*a*]carbazoles. Sodium (1.44 g, 0.06 mol) was added in portions to 200 mL of liquid ammonia. After disappearance of the blue color, a solution of 0.035 mol of the benzocarbazole in 100 mL of dry THF was added at −70 °C. After the mixture was stirred for an additional 30 min, 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 ($MgSO_4$). After evaporation of the solvent the product was recrystallized from EtOH. Yields ranged between 80% and 90%. Melting points are reported in Table I. Elemental analyses were performed after ether cleavage and acetylation (vide infra).

General Procedure for the Ether Cleavage and Acetylation. A solution of the methoxy-substituted dihydrobenzo[a]carbazole (0.008 mol) in dry CH_2Cl_2 (50 mL) was cooled to −60 °C under 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) under nitrogen. 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 obtained as a solid and 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 (0.03 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 treating with a small volume of CH_2Cl_2. The yields were between 75% and 90%. Melting points are reported in Table II.

General Procedure for the Oxidation of Dihydrobenzo[*a*]carbazoles. 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone was added in portions to a stirred solution of acetoxy-11-alkyl-6,11-dihydro-5*H*-benzo[a]carbazole (1.0 mmol) in 10 mL of CH_2Cl_2 until the green color persisted. The mixture was stirred for an additional 30 min. Without removal of the solvent, the mixture was transferred onto a silica gel column and chromatographed with CH_2Cl_2. The product was isolated in a yield of 90–95% and was recrystallized from EtOH. Hydroxy derivatives were obtained after alkaline hydrolysis as described above. Melting points both of the acetates and the hydroxy compounds are reported in Table III.

Biochemical and Biological Methods. Reagents. [2,4,6,7-^3H]Estradiol (110 Ci/mmol) and [^3H]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 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.[11] The 500-μL incubation mixture comprised 5 nM 17β-[^3H]estradiol (added in 100 μL of TEA), 10^{-9}–10^{-4} 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, and 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 in it was quantified by liquid scintillation spectrometry after addition of 2 mL of Quickszint 212 (Zinsser). Nonspecific binding was calculated with use of 5 μM 17β-estradiol as competing ligand. Six concentrations of competitor (1, 2, 5, and 10×10^{-9} to 10^{-5}) were chosen to provide values between 10% and 90% of specificly 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 (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 in olive oil (50 μL/animal) by gentle warming and injected subcutaneously on 3 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 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 carbazole 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. A single dose was administered from Monday to Thursday and on Friday a double dose was 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, Mann, and Whitney.[20]

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),

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as modified by Richter et al.[21] (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[22] 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 clumbing, 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 [^{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 and the other one was sonicated. After addition of 4 mL of 10% trichloroacetic acid, 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.

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Supplementary Material Available: ^{1}H NMR data of 11-alkyl-6,11-dihydromethoxy-5*H*-benzo[*a*]carbazoles (**12a–23a**), 11-alkyl-6,11-dihydrohydroxy-5*H*-benzo[*a*]carbazoles (**12b–23b**), acetoxy-11-alkyl-6,11-dihydro-5*H*-benzo[*a*]carbazoles (**12c–23c**), acetoxy-11-alkyl-11*H*-benzo[*a*]carbazoles (**24a–35a**), and 11-alkyl-hydroxy-11*H*-benzo[*a*]carbazoles (**24b–35b**) (12 pages). Ordering information is given on any current masthead page.

Imidazo[2,1-*b*]benzothiazoles. 2.[1] New Immunosuppressive Agents

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A series of 2-phenylimidazo[2,1-*b*]benzothiazole derivatives was prepared and tested for immunological activities. Some of the compounds showed significant suppressive activity of delayed type hypersensitivity (DTH) without inhibition of humoral immunity in mice by oral administration. The most active compound was 2-(*m*-hydroxyphenyl)imidazo[2,1-*b*]benzothiazole (**20**).

Immunosuppressive agents have been used for the treatment of some autoimmune diseases, such as systemic lupus erythematosus, rheumatoid arthritis, and glomerulonephritis,[2] and in organ transplantation.[2] However, these drugs, for example, alkylating agents, such as cyclophosphamide, and antimetabolites, such as azathioprine, are cytotoxic agents, inhibit both cell-mediated and humoral immunity, and are associated with a number of side effects, such as bone marrow depression, infection, hepatic toxicity, and rash.[3] Therefore, they are not used widely. In recent years, several immunosuppressive agents, such as cyclosporin A,[4] niridazole,[5] and oxisuran[6] have been reported to suppress selectively cell-mediated immunity. Recently, cyclosporin A[7] has been widely used in organ transplantation. However, this agent also has side effects, such as hepatic and renal toxicities.[7] In these aspects, less toxic immunosuppressive agents are awaited.

In a search for new compounds that alter the immune

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