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Ring-Substituted 1,2-Dialkylated 1,2-Bis(hydroxyphenyl)ethanes. 3. Synthesis, Estrogen Receptor Binding Affinity, and Evaluation of Antiestrogenic and Mammary Tumor Inhibiting Activity of 2,2′-Disubstituted Butestrols and 6,6′-Disubstituted Metabutestrols

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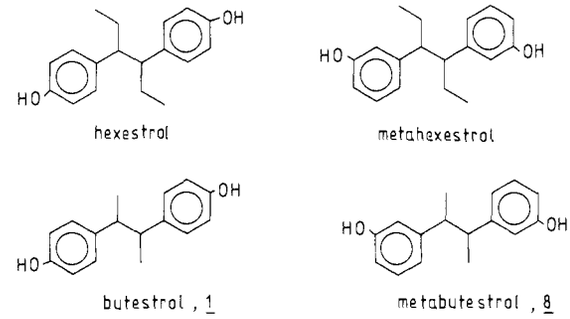
The syntheses of symmetrically 2,2′-disubstituted butestrols [*meso*-2,3-bis(4-hydroxyphenyl)butanes] and of 6,6′-disubstituted metabutestrols [*meso*-2,3-bis(3-hydroxyphenyl)butanes] are described [2,2′-substituents: H (1), OH (2), F (3), Cl (4), Br (5), CH_3 (6), and C_2H_5 (7); 6,6′-substituents: H (8), OH (9), Cl (10), and CH_3 (11)]. Compounds 1–11 were obtained by reductive coupling of the corresponding 1-phenylethanols with $TiCl_3/LiAlH_4$ and separation of the meso diastereomers. The binding affinity of the test compounds to the calf uterine estrogen receptor was measured relative to that of [^{3}H]estradiol by a competitive binding assay. With the exception of **9**, all other compounds showed remarkably high relative binding affinity (RBA) values between 1.0 and 29% that of estradiol. Compounds **3** and **6** (RBA values: 15 and 29), as well as **10** and 11 (1.7 and 5.2), exceeded those of the corresponding unsubstituted compounds 1 and **8** (12 and 1.0). The compounds exhibited strong (**3**, **4**, **6**, and **7**), moderate (1, **2**, and **10**), weak (11), or no (**8**) estrogenic activity in the uterine weight test of the immature mouse. Compounds 1, **2**, **8**, **10**, and 11 showed antiestrogenic activity inhibiting the estrone-stimulated uterine growth (25–35% inhibition). Compound 11 led to a significant inhibition of the tumor growth when tested on the 9,10-dimethyl-1,2-benzanthracene induced, hormone-dependent mammary carcinoma of the Sprague–Dawley rat.

Structural modifications of the synthetic estrogen hexestrol, such as the displacement of the phenolic hydroxy groups into the 3,3′-positions or the replacement of the ethyl groups by methyl substituents, led to compounds with decreased estrogenicity and antiestrogenic properties [metahexestrol and butestrol (1), respectively (Chart I)].[1,2] The combination of these two structure-modification principles resulted in a compound totally lacking estrogenic activity [metabutestrol (**8**; Chart I)].[1]

Since these compounds show a marked inhibitory activity on the established 9,10-dimethyl-1,2-benzanthracene (DMBA) induced mammary carcinoma of the Sprague–Dawley (SD) rat,[1] they are of great interest for the treatment of the hormone-dependent human breast cancer. An increase in the antitumor activity of these compounds might be realized by synthesizing derivatives with a higher affinity for the estradiol receptor (E_2R), since there seems to be a correlation between antitumor activity of antiestrogens and their E_2R association constants.[1]

In the first two parts of a structure–activity study of 1,2-dialkylated or 1,1,2,2-tetraalkylated 1,2-bis(hydroxyphenyl)ethanes dealing with the influence of a symmetrical disubstitution of the two aromatic rings on the E_2R affinity, we have shown that the binding affinity could be increased only in the case of disubstitution in the ortho positions to the ethane bridge.[3,4] For this reason, we

Chart I



describe in this study the effect of a symmetrical disubstitution only in the ortho positions to the alkyl chain of 2,3-bis(hydroxyphenyl)butanes.

In the following paragraphs, the syntheses, the determination of the E_2R affinities, and the evaluation of estrogenic, antiestrogenic, and mammary tumor inhibiting properties of 2,2′-disubstituted butestrols [*meso*-2,3-bis(4-hydroxyphenyl)butanes] and 6,6′-disubstituted metabutestrols [*meso*-2,3-bis(3-hydroxyphenyl)butanes] are described.

Chemistry. Compounds 1–11 (Table I) were obtained by reductive coupling of the 1-(2-substituted-4- or -5-methoxyphenyl)ethanols (**1b–11b**) with $TiCl_3/LiAlH_4$ according to the method of McMurry and Silvestri[6] (method A, Scheme I) and subsequent cleavage of the methoxy

(1) Hartmann, R. W.; Buchborn, H.; Kranzfelder, G.; Schönenberger, H.; Bogden, A. E. *J. Med. Chem.* **1981**, *24*, 1192.
(2) Dodds, E. C.; Goldberg, L.; Grunfeld, E. J.; Lawson, W.; Saffer, C. M.; Robinson, R. *Proc. R. Soc. London, Ser. B* **1944**, *132*, 83.
(3) Hartmann, R. W.; Schwarz, W.; Schönenberger, H. *J. Med. Chem.* **1983**, *26*, 1137.
(4) Hartmann, R. W.; Heindl, A.; Schönenberger, H. *J. Med. Chem.* **1984**, *27*, 577–585.
(5) Bretschneider, H.; Bretschneider, A. de J.; Ajtai, N. *Ber. Dtsch. Chem. Ges.* **1941**, *74*, 571.
(6) McMurry, J. E.; Silvestri, M. *J. Org. Chem.* **1975**, *40*, 2687.

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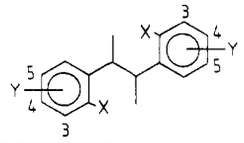
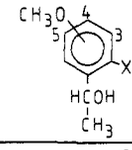
Table I. Substituted *meso*-2,3-Diphenylbutanes



compd	X	Y	synth method[a]	yield,[b] %	mp, °C	recrystn solvent[c]	formula[d]
1a[f]	H	4-OCH_3	A	20	131	H	$C_{18}H_{22}O_2$
1[f]	H	4-OH	B	82	233	J	$C_{16}H_{18}O_2$
2a	OCH_3	4-OCH_3	A	18	156–157	H	$C_{20}H_{26}O_4$
2	OH	4-OH	B[e]	71	233–235	K	$C_{16}H_{18}O_4$
3a	F	4-OCH_3	A	14	108–109	H	$C_{18}H_{20}F_2O_2$
3	F	4-OH	B	88	213–214	J	$C_{16}H_{16}F_2O_2$
4a	Cl	4-OCH_3	A	19	145–146	H	$C_{18}H_{20}Cl_2O_2$
4	Cl	4-OH	B	86	181–183	J	$C_{16}H_{16}Cl_2O_2$
5a	Br	4-OCH_3	A	14	182–184	H	$C_{18}H_{20}Br_2O_2$
5	Br	4-OH	B	81	187–188	J	$C_{16}H_{16}Br_2O_2$
6a[g]	CH_3	4-OCH_3	A	25	140–141	H	$C_{20}H_{26}O_2$
6[g]	CH_3	4-OH	B	85	190–191	J	$C_{18}H_{22}O_2$
7a	C_2H_5	4-OCH_3	A	22	100–102	H	$C_{22}H_{30}O_2$
7	C_2H_5	4-OH	B	83	208–209	J	$C_{20}H_{26}O_2$
8a[h]	H	5-OCH_3	A	23	96	L	$C_{18}H_{22}O_2$
8[h]	H	5-OH	B	87	163	L	$C_{16}H_{18}O_2$
9a	OCH_3	5-OCH_3	A	26	124–125	L	$C_{20}H_{26}O_4$
9	OH	5-OH	B[e]	82	196–197	K	$C_{16}H_{18}O_4$
10a	Cl	5-OCH_3	A	11	159	L	$C_{18}H_{20}Cl_2O_2$
10	Cl	5-OH	B	81	181	L	$C_{16}H_{16}Cl_2O_2$
11a	CH_3	5-OCH_3	A	10	118	L	$C_{20}H_{26}O_2$
11	CH_3	5-OH	B	83	207	L	$C_{18}H_{22}O_2$

[a] A and B refer to synthetic methods A and B; see Experimental Section. [b] Yield of analytically pure product; no effort was made to optimize yields. [c] H = EtOH; J = benzene; K = EtOH/benzene; L = EtOH/H_2O. [d] All compounds were analyzed for C, H, Br, and Cl within ±0.4% of the calculated values. [e] Product was not extracted with NaOH solution. [f] See ref 2. [g] See ref 5. [h] See ref 1.

Table II. Substituted 1-(Methoxyphenyl)ethanols



compd	X	Y	synth method[a]	yield,[b] %	formula
1b	H	4-OCH_3	C	90	$C_9H_{12}O_2$
2b	OCH_3	4-OCH_3	C	88	$C_{10}H_{14}O_3$
3b	F	4-OCH_3	C	92	$C_9H_{11}FO_2$
4b	Cl	4-OCH_3	C	81	$C_9H_{11}ClO_2$
5b	Br	4-OCH_3	C	85	$C_9H_{11}BrO_2$
6b	CH_3	4-OCH_3	C	84	$C_{10}H_{14}O_2$
7b	C_2H_5	4-OCH_3	C	87	$C_{11}H_{16}O_2$
8b	H	5-OCH_3	D	85	$C_9H_{12}O_2$
9b	OCH_3	5-OCH_3	D	91	$C_{10}H_{14}O_3$
10b	Cl	5-OCH_3	D	83	$C_9H_{11}ClO_2$
11b	CH_3	5-OCH_3	D	82	$C_{10}H_{14}O_2$

[a] C and D refer to synthetic methods C and D; see Experimental Section. [b] Yield of analytically pure (TLC) product; no effort was made to optimize yields.

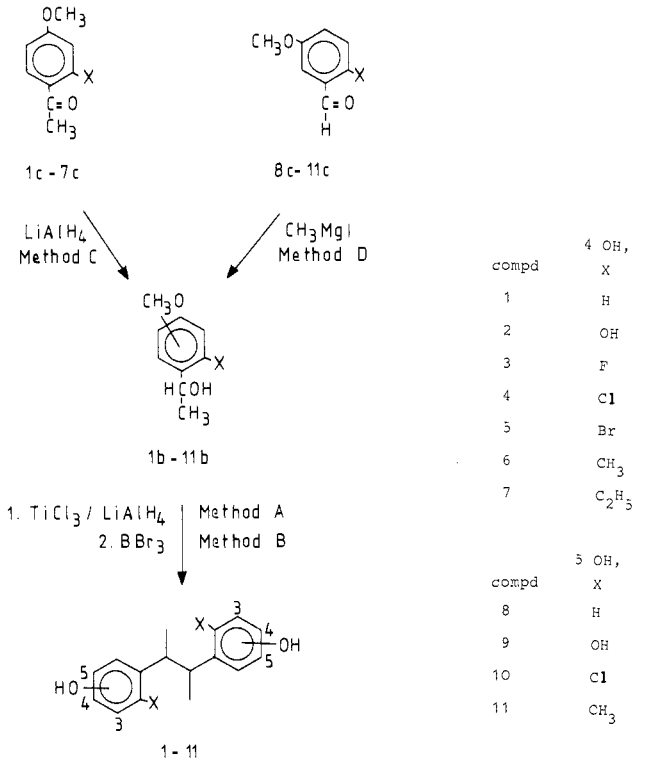
derivatives **1a–11a** (Table I) with BBr_3 (method B, Scheme I).

The coupling resulted in a mixture of the corresponding *meso*- and *d,l*-dibenzyl derivatives, as proven by TLC, HPLC, and ^1H NMR. In accordance with the previously described hexestrol and metahexestrol derivatives, the ^1H NMR spectra of the *d,l* compounds generally showed the OCH_3 signals shifted upfield and the aliphatic CH_3 signals shifted downfield compared to the signals of the corresponding meso compounds. The *meso*-methoxy derivatives were separated by fractional crystallization or by column chromatography with mixtures of CH_2Cl_2/petroleum ether as eluent.

The secondary alcohols **1b–11b** were synthesized either by reduction of the corresponding acetophenones with $LiAlH_4$ (method C, Scheme I, **1b–7b**, Table II) or by Grignard reaction of the corresponding benzaldehydes with CH_3MgI (method D, Scheme I, **8b–11b**, Table II). The synthesis of the acetophenones **2c–7c** was accomplished by Friedel–Crafts acylation of the 3-substituted anisols with $AlCl_3$ and acetic anhydride (method E). The synthesis of 2-chloro-5-methoxybenzaldehyde (**10c**) has been described recently.[4] Compound **11c** was obtained by reduction of 5-methoxy-2-methylbenzoic acid with $LiAlH_4$ (method F) and oxidation of the resulting benzyl alcohol with MnO_2 (method G). The benzoic acid derivative was prepared as described previously.[4]

Biological Properties. The relative binding affinity (RBA) for the E_2R of compounds **1–11** was determined by a competitive binding assay with calf uterine cytosol,

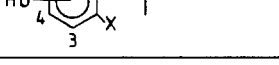
Scheme I



compd	4 OH, X
1	H
2	OH
3	F
4	Cl
5	Br
6	CH_3
7	C_2H_5

compd	3 OH, X
8	H
9	OH
10	Cl
11	CH_3

Table III. Relative Binding Affinity (RBA) of Butestrol (1), Metabutestrol (8), and Compounds 2–7 and 9–11 for the Calf Uterine Estrogen Receptor



compd	4-OH, X	RBA value[a]	compd	5-OH, X	RBA value[a]
1	H	12	8	H	1.0
2	OH	2.1	9	OH	0.01
3	F	15	10	Cl	1.7
4	Cl	4.5	11	CH_3	5.2
5	Br	3.4			
6	CH_3	29			
7	C_2H_5	12			

[a] Relative binding affinity for the calf uterine estrogen receptor = ratio of the molar concentrations of 17β-estradiol (E_2) and inhibitor required to decrease the amount of bound [^{3}H]E_2 by 50% × 100.

17β-[^{3}H]estradiol, and the Dextran-coated charcoal technique. With the exception of the 6,6′-dihydroxylated metabutestrol derivative (**9**), all other test compounds exhibited remarkably high binding affinities, with RBA values between 1.0 (**8**) and 29 (**6**) (Table III).

It is striking that the displacement of the phenolic hydroxy groups from the para (butestrol, **1**) to the meta positions (metabutestrol, **8**) also led to a reduction of the binding affinity, as already observed with hexestrol (RBA = 27[3]) and metahexestrol (RBA = 10[1]). Accordingly, the 6,6′-disubstituted metabutestrols exhibited smaller binding affinities in comparison to the correspondingly 2,2′-disubstituted butestrol derivatives (**9–11** compared to **2**, **4**, and **6**). This has also been demonstrated with the 2,2′-disubstituted hexestrol and the 6,6′-disubstituted metahexestrol derivatives.[4]

In the butestrol and metabutestrol series, some disubstituted derivatives increased the binding affinity of the unsubstituted parent compound. In the case of the butestrol derivatives, the fluorine and methyl substituents led to an enhancement of the E_2R binding affinity (**3** and **6**, respectively). The more lipophilic substituents chlorine and bromine (**4** and **5**) reduced the binding affinity of the parent compound (for lipophilicity parameters of the different substituents, see ref 7). This is probably due to an increased steric hindrance within the center of the molecule. Reduction of the binding affinity of compound **6** by replacement of the aromatic CH_3 groups with C_2H_5 groups is also probably due to this steric effect.

In the metabutestrol series, the chlorine substituents increased the binding affinity slightly (**10**), whereas the CH_3 groups led to a marked enhancement of the binding affinity (**11**), as they did in the butestrol series (**6**).

The hydroxy groups in the 2,2′-positions of butestrol decreased the binding affinity for the E_2R only slightly, whereas the same substituents in the 6,6′-positions of metabutestrol led to a tremendous decrease of binding affinity. The same phenomenon has already been observed with the corresponding hexestrol and metahexestrol derivatives and is presently the subject of further investigations.

The mouse uterine weight test was used to determine the estrogenicity and antiestrogenicity of the most active inhibitors of the E_2R interaction. The parent compound of the butestrol series, **1**, showed only a low uterotrophic activity in small doses; however, in high doses, a stronger uterine growth stimulating effect could be observed without reaching the maximum stimulation of true estrogens like estrone (Table IV). The hydroxy compound **2** exhibited a reduced estrogenic activity compared to the unsubstituted compound. This is probably due to the decreased binding affinity of **2**. The marked uterotrophic activity of the fluoro- and chloro-disubstituted butestrol derivatives (**3** and **4**) demonstrates that halogen substituents increase the estrogenicity of the parent compound very strongly. These compounds stimulated the uterine growth like true estrogens do, reaching their maximum effect at very small doses. In the case of compound **4**, the estrogenicity was increased in spite of a reduced binding affinity compared to the parent compound. In the case of the methyl compound **6**, the enhanced binding affinity led to an increase of uterotrophic activity. Exhibiting the same binding affinity compared to the unsubstituted compound, the ethyl compound **7** also showed stronger uterine growth stimulating effects than the parent compound.

The parent compound of the metahexestrol series, **8**, showed no uterotrophic activity. Introduction of chlorine substituents also led to an increase of estrogenicity (**10**). The latter compound showed similar uterotrophic activity compared to butestrol. The methyl compound **11** was very promising. In spite of a markedly increased binding affinity (factor of 5.2), compound **11**, in a wide dose range, showed, like the unsubstituted compound, no uterotrophic activity, and only with the very high 1000-μg dose could weak uterine growth stimulating effects be observed.

The antiestrogenic activity of the test compounds, which had shown a moderate (**1**, **2**, and **10**), weak (**11**), or no (**8**) estrogenic activity in the uterotrophic test, was determined by inhibition of the uterine growth stimulated by estrone (Table V). All test compounds exhibited moderate antiuterotrophic effects. Regardless of their different uterotrophic activities, they were all similarly effective,

(7) Hansch, C.; Leo, A.; Unger, S. H.; Kim, K. H.; Nikaitani, D.; Lieu, E. J. *J. Med. Chem.* **1973**, *16*, 1207.

Table IV. Estrogenic Activity of Butestrol (1), Metabutestrol (8), and Compounds 2–4, 6, 7, 10, and 11 in the Mouse Uterine Weight Test

compd	dose,[a] μg	uterotrophic test: effect,[b,d] mean ± SD
1	0	11.8 ± 1.2
	0.1	17.7 ± 2.3
	1	22.6 ± 4.2
	10	28.0 ± 3.8
	100	42.2 ± 6.4
	1000[c]	34.8 ± 3.2
estrone	0.4	49.0 ± 2.0
2	0	12.5 ± 1.7
	1	15.4 ± 1.7
	10	20.3 ± 3.2
	100	26.2 ± 2.9
	1000	34.6 ± 4.1
estrone	0.4	52.2 ± 8.7
3	0	11.8 ± 1.2
	1	50.2 ± 4.9
	10	51.4 ± 3.4
estrone	0.4	49.0 ± 2.0
4	0	11.8 ± 1.2
	1	41.6 ± 8.4
	10	40.7 ± 6.3
estrone	0.4	49.0 ± 2.0
6	0	11.8 ± 1.2
	0.1	29.2 ± 2.1
	1	36.5 ± 5.2
	10	39.4 ± 8.5
estrone	0.4	49.0 ± 2.0
7	0	12.5 ± 1.7
	1	38.8 ± 5.8
	10	45.2 ± 6.8
	100	47.0 ± 5.3
estrone	0.4	52.2 ± 8.7
8	0	11.9 ± 2.1
	1	11.7 ± 20[e]
	10	12.4 ± 1.7[e]
	100	12.6 ± 2.3[e]
	1000	13.1 ± 1.0[e]
estrone	0.4	45.6 ± 4.9
10	0	11.1 ± 1.8
	1	13.0 ± 3.0
	10	26.5 ± 3.0
	100	41.8 ± 5.4
estrone	0.4	47.3 ± 2.7
11	0	11.1 ± 1.8
	1	13.7 ± 3.5[e]
	10	14.2 ± 2.2[e]
	100	12.5 ± 1.7[e]
	1000	17.4 ± 3.6[f]
estrone	0.4	47.3 ± 2.7

[a] Dose per animal per day. [b] Uterus dry weight (milligrams)/body weight (grams) × 100. [c] Applied as a suspension (see Experimental Section). [d] The U test according to Wilcoxon, Mann, and Whitney was used. [e] Not significant (α = 0.01). [f] Significant (α = 0.01).

reaching inhibition values of between 25 and 35%. An increase of the dose to 1000 μg per animal per day did not further increase the inhibitory effect (data not given).

Because of its rather high E_2R binding affinity and its antiestrogenic and weak estrogenic properties, compound 11 was tested on the DMBA-induced, hormone-dependent mammary carcinoma of the SD rat. In a dose of 2 mg/(kg day), the test compound led to a significant inhibition of the tumor growth (Table VI). An enhancement of the dose to 5 mg/(kg day) did not improve the antitumor effect. As previously described[1], the unsubstituted parent compound (8) had led to a dose-dependent inhibition on this experimental tumor (change of tumor area in control: 610%; 8: at 18 mg/(kg day), 188%; at 36 mg/(kg day) 105%; at 72 mg/(kg day) 6%).

Table V. Antiestrogenic Activity of Butestrol (1), Metabutestrol (8), and Compounds 2, 10, and 11 in the Mouse Uterine Weight Test

		antiuterotrophic test	
compd	dose,[a] μg	effect,[b] mean ± SD	% inhibn[c,d]
1	0	14.4 ± 2.4	
	5	43.3 ± 4.3	12[f]
	50	38.7 ± 3.6	26[e]
	500	37.8 ± 3.5	28[e]
estrone	0.4	47.1 ± 6.1	
2	0	14.4 ± 2.4	
	5	50.6 ± 5.7	
	50	39.1 ± 6.1	25[f]
	500	38.7 ± 5.5	26[e]
estrone	0.4	47.1 ± 6.1	
8	0	14.4 ± 2.4	
	5	43.0 ± 4.0	13
	50	39.0 ± 3.3	25[e]
	500	36.5 ± 3.3	32[e]
estrone	0.4	47.1 ± 6.1	
10	0	14.4 ± 2.4	
	5	40.1 ± 3.6	21[e]
	50	39.3 ± 1.4	24[e]
	500	37.2 ± 4.1	30[e]
estrone	0.4	47.1 ± 6.1	
11	0	14.4 ± 2.4	
	5	45.4 ± 3.6	5
	50	40.6 ± 2.8	20[e]
	500	35.6 ± 3.5	35[e]
estrone	0.4	47.1 ± 6.1	

[a] Dose per animal and day. [b] Uterus dry weight (milligrams)/body weight (grams) × 100. [c] % inhibn = $100 - (E_{S,T} - E_V)/(E_S - E_V) \times 100$; E_S = effect of estrone standard; $E_{S,T}$ = effect of standard under simultaneous application of test substance; E_V = effect of vehicle. [d] The U test according to Wilcoxon, Mann, and Whitney was used. [e] Significant (α = 0.01). [f] Significant (α = 0.05).

Discussion

The differences in the E_2R binding affinities of the synthesized butestrol and metabutestrol derivatives are apparently caused by the lipophilic and steric parameters of the substituents. To what extent electronic (inductive and resonance) effects are of importance cannot be decided. An increase of the E_2R binding affinity of the parent compounds was achieved by the introduction of groups with smaller van der Waals radii (F and CH_3), which increase the lipophilicity in the center of the molecule. The more lipophilic chlorine and bromine substituents did not further increase the binding affinity for the E_2R significantly (**10**) or even lead to a decrease of the RBA (**4** and **5**). This is probably due to their enhanced van der Waals radii. The bulkier the substituents are, the more marked is the steric hindrance of the formation of the E_2R complex.

The RBA value of the hydroxy-substituted butestrol derivative is surprisingly high, considering the hydrophilicity of this group. This result is in accordance with that of the analogously substituted hexestrol derivative, which even exhibited an E_2R binding affinity slightly higher than that of the parent compound.[4] This result, as well as the fact that *meso*-3,4-bis(2-hydroxyphenyl)-

Table VI. Effect of Compound 11 on the DMBA-Induced, Hormone-Dependent Mammary Carcinoma of the SD Rat

compd	dose,[a] mg	no. of tumors		% of tumors with				% change of	
		B[b]	NT[c]	CR[d]	PR[e]	NC[f]	P[g]	body wt[h,i]	tumor area[j,i]
control		26	26	8	2	13	77	2.5	515
11	2	23	25	11	4	29	56	1.5	245[k]
control		22	31	7	8	23	62	2.5	502
11	5	25	23	15	4	25	56	1.1	283[l]

[a] Dose per kilogram of body weight and day. The animals received a single daily dose from Monday to Thursday and a double dose on Friday. [b] At the beginning of the test. [c] Occurring during the test. [d] CR = complete remission; tumor not palpable. [e] PR = partial remission; reduction of initial tumor size ≥50%. [f] NC = no change; tumor size 51–150% of initial tumor size. [g] P = progression; tumor size >150% of initial tumor size. [h] Average on the 7th day of therapy. [i] The *U* test according to Wilcoxon, Mann, and Whitney was used. [j] Average on the 28th day of therapy. [k] Significant ($\alpha < 0.05$). [l] Not significant ($\alpha > 0.05$).

hexane also exhibits affinity for the E_2R,[8] had led to the discussion that there might be an additional OH group binding area on the receptor.[4]

It is remarkable that aromatic disubstitution with CH_3 in the ortho positions to the ethane bridge could raise the binding affinities of the parent compounds so dramatically. In the case of the butestrol derivative (**6**), an RBA value of 29 was obtained, which is nearly identical with that of hexestrol (27[3]). The metabutestrol derivative (**11**) almost reached the receptor affinity of metahexestrol. (RBA values of 5.2 and 10[4]). Hence, it has to be concluded that methyl substituents in the ortho positions to the ethane bridge can take over the function of the terminal methyl groups of the 3,4-diphenylhexanes with regard to the binding affinity for the E_2R. This phenomenon has already been observed, since *trans*-2-(4-hydroxyphenyl)-3-(2-methyl-4-hydroxyphenyl)pent-2-ene has shown the same receptor affinity as diethylstilbestrol,[9] and since the 6,6′-dimethylmetahexestrol derivative[4] has shown the same receptor affinity as *meso*-3,4-bis(3-hydroxyphenyl)-2,5-dimethylhexane.[8]

The finding that compound **11** did not fully reach the binding affinity of metahexestrol, whereas compound **6** reached that of hexestrol, is a further indication of the validity of our hypothesis that 3,3′-dihydroxylated 1,2-dialkyl-1,2-diphenylethanes may interact with the receptor area in a different conformation compared to the corresponding 4,4′-dihydroxylated compounds (e.g., metahexestrol and hexestrol, respectively).[4] This is why the ortho CH_3 groups of **11** cannot completely take over the function of the terminal methyl groups of the hexane chain of metahexestrol, whereas the methyl groups of **6** can take over that of hexestrol.

In the case of hydroxy- and alkyl-substituted compounds, there seems to be a correlation between uterotrophic properties and E_2R binding affinity, in the sense that an increase of receptor binding leads to an enhancement of estrogenicity and vice versa. However, the halogen-substituted derivatives show, in every case, an increased uterotrophic activity. This phenomenon has already been observed in the metahexestrol series,[4] as well as in other classes of antiestrogens (to be published), and cannot be explained at the present time.

The fact that compound **11**, with antiestrogenic and only weak estrogenic properties (RBA = 5.2), showed a tumor-inhibiting effect comparable to that obtained by metabutestrol (RBA = 1.0) in an approximately 10-fold dose is a further indication that there seems to be a correlation between the antitumor activity and E_2R binding affinities of antiestrogens.[1] Since compound **11** unfolds its antitumor effect in a relatively small dose, this antiestrogen probably acts as a "true" antiestrogen inhibiting tumor growth, i.e., as an estrogen antagonist in the classical sense, preventing the tumor growth stimulating effects of endogenous estrogens. The nonuterotrophic antiestrogen metabutestrol certainly unfolds its tumor growth inhibiting activity in this sense as well. The antitumor activity of partial antiestrogens, on the other hand, might be due to their residual estrogenic activity. The partial antiestrogens tamoxifen and metahexestrol, for example, have been shown to increase the tumor growth stimulating and inhibiting effects of the synthetic estrogen diethylstilbestrol (DES) on DMBA-induced mammary carcinomas of the ovariectomized SD rat, whereas 1,1,2,2-tetramethyl-1,2-bis(4′-hydroxyphenyl)ethane[10] (Me_4-HES) reduced (i.e., antagonized) these DES effects.[11] Me_4-HES has shown endocrinological and tumor-inhibiting data[10] similar to compound **11**. In the next part of our structure–activity study in the diphenylethane class, the effect of a symmetrical disubstitution of the aromatic rings of 1,1,2,2-tetramethyl-1,2-bis(hydroxyphenyl)ethanes will be described.[12]

Experimental Section

TLC was performed on Merck F 254 silica gel plates. Melting points were determined on a Büchi 510 melting point apparatus and are uncorrected. Elemental analyses were performed by the Mikroanalytisches Laboratorium, Universität Regensburg. The structures of all compounds were confirmed by their IR (Beckman AccuLab 3) and ^1H NMR spectra (Varian EM 390, 90 MHz).

Method A. *meso*-2,3-Bis(4-methoxyphenyl)butane (1a). $TiCl_3$ (4.63 g, 0.03 mol) was placed under N_2 in a flask with 60 mL of dry glyme. $LiAlH_4$ (0.38 g, 0.01 mol) was quickly added to the stirred $TiCl_3$ slurry. The resulting black suspension was stirred for 10 min. Compound 1b (1.52 g, 0.01 mol) was dissolved in 10 mL of dry glyme and added dropwise, with stirring, to the solution. The mixture was heated to reflux and kept there for 16 h. After cooling, the reaction mixture was quenched by the addition of 2 N HCl, diluted with water, and extracted with ether. The ether extract was washed ($NaHCO_3$ and H_2O) and dried ($MgSO_4$). The solvent was removed, and the resulting crude product was fractionally crystallized from EtOH to give 0.27 g of **1a**.

Method B. *meso*-2,3-Bis(4-hydroxyphenyl)butane (1). A solution of **1a** (2.70 g, 0.01 mol) in 100 mL of dry CH_2Cl_2 was cooled to -60 °C and BBr_3 (6.26 g, 0.025 mol) was added under nitrogen, with stirring. After 0.5 h, the freezing mixture was removed, and the reaction mixture was kept at room temperature for 4 h. Fifty milliliters of MeOH was added, and the mixture was shaken with 2 N NaOH. After neutralization of the aqueous layer with 3 N H_2SO_4, the solution was extracted with ether. After removal of the ether, the crude product was recrystallized from benzene to give 1.98 g of **1**.

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Method C. 1-(4-Methoxyphenyl)ethanol (1b). A solution of 4-methoxyacetophenone (1.50 g, 0.01 mol) in 20 mL of ether was added dropwise to a stirred suspension of $LiAlH_4$ (0.114 g, 3 mmol) in 40 mL of ether. After stirring for 3 h at room temperature, the mixture was heated to reflux for 1 h. The mixture was cooled and decomposed by the dropwise addition of ice–water, followed by 10% H_2SO_4, to give two clear phases. The ethereal layer was separated, washed (H_2O), and dried ($MgSO_4$). The solvent was removed, and 1.37 g of 1b was obtained.

Method D. 1-(3-Methoxyphenyl)ethanol (8b). Methyl iodide (1.70 g, 12 mmol) was dissolved in ether and added dropwise, with stirring, to magnesium turnings (0.29 g, 12 mmol) in 20 mL of dry ether. The mixture was heated to reflux for 0.5 h. A solution of 3-methoxybenzaldehyde (1.36 g, 0.01 mol) in ether was added dropwise with stirring. After heating to reflux for 2 h, the mixture was cooled and poured onto ice. The resulting precipitate was dissolved by the addition of a NH_4Cl solution. The ethereal layer was separated, washed ($NaHSO_3$, $NaHCO_3$, and H_2O), and dried ($MgSO_4$). The solvent was removed, and the resulting oil was distilled under high vacuum to give 1.29 g of 8b (colorless oil).

Method E. 2-Fluoro-4-methoxyacetophenone (4c). To a stirred mixture of $AlCl_3$ (30.00 g, 0.22 mol) and 3-fluoroanisole (12.60 g, 0.1 mol) in 100 mL of CS_2, acetic anhydride (8.17 g, 0.08 mol) was added dropwise, keeping the internal temperature below 5 °C. After stirring for 1 h at room temperature, the mixture was heated to 30–35 °C, until no more HCl gas was formed. After cooling to room temperature, the solid phase was separated, poured onto ice, acidified with 40 mL of concentrated HCl, and extracted with toluene. The organic layer was washed with 10% NaOH and H_2O and dried ($CaCl_2$). The solvent was removed, and the crude product was recrystallized from petroleum ether to yield 8.48 g (60.7%) of 4c (colorless crystals, mp 53–54 °C).

Method F. 5-Methoxy-2-methylbenzyl Alcohol. Reduction of 3-methoxy-6-methylbenzoic acid (16.6 g, 0.1 mol) was performed with $LiAlH_4$ (3.04 g, 0.08 mol) according to method C to yield 13.9 g (91.5%) of a colorless oil of 3-methoxy-6-methylbenzyl alcohol.

Method G. 5-Methoxy-2-methylbenzaldehyde (11c). A suspension of MnO_2 (43.5 g, 0.5 mol) in 400 mL of benzene was heated to reflux for 2 h, using a water separator. After the addition of 3-methoxy-6-methylbenzyl alcohol (15.2 g, 0.1 mol), dissolved in 50 mL of benzene, heating to reflux was continued overnight, and then the mixture was cooled to room temperature and filtered. After the solvent was removed, the residue was recrystallized from MeOH to give 11.7 g (78 %) of 11c (mp 46 °C).

Biological Methods. Estradiol Receptor Binding Assay. The relative binding affinity (RBA) of the test compounds was determined by the displacement of [^{3}H]estradiol. A previously described procedure[10] was used with modifications. Test compounds were incubated with cytosol from calf uteri and [^{3}H]estradiol at 4 °C for 16 h. Incubation was stopped by adding Dextran-coated charcoal. After centrifugation, the radioactivity of a 100-μL supernatant aliquot was counted. The percent bound radioligand was plotted vs. the concentration of unlabeled test compounds. Six concentrations of the competitors were tested. They were chosen to provide a linear portion on a semilog plot crossing the point of 50% competition. From this plot, the molar concentrations of unlabeled estradiol and of test compounds reducing radioligand binding by 50% were determined.

Estrogen and Antiestrogen Assays. Estrogenic and antiestrogenic activities were determined by stimulation of the uterine growth and the inhibition of the uterine growth stimulated by estrone, respectively, with immature NMRI mice as described previously.[10] Twenty-day-old female mice (weight 14.5 ± 1.2 g, mean ± SD) were randomly distributed into groups of 10 animals. They were subcutaneously (sc) injected daily for 3 days with 0.1 mL of olive oil solutions containing the test compound. The uteri were removed 24 h after the last injection, fixed with Bouin's solution, washed, dried, and weighed.

Mammary Tumor Growth Inhibition Test. The method used has been described previously.[10] The tumor-inhibiting effect was determined by using the DMBA-induced, hormone-dependent mammary adenocarcinoma of the SD rat. Animals bearing at least one tumor greater than 140 mm^2 were classified in groups of ten. Compounds were dissolved in olive oil and applied sc. Measurement of tumor size and determination of body weight were made twice weekly. The therapy was continued for 28 days.

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Registry No. *meso*-1, 2962-14-3; *dl*-1, 5776-76-1; *meso*-**1a**, 65026-51-9; *dl*-**1a**, 65026-54-2; (±)-1b, 43230-31-5; **1c**, 100-06-1; *meso*-**2**, 89691-22-5; *dl*-**2**, 89691-23-6; *meso*-**2a**, 89691-40-7; *dl*-**2a**, 89691-41-8; (±)-**2b**, 89691-57-6; **2c**, 552-41-0; *meso*-**3**, 74457-91-3; *dl*-**3**, 89691-24-7; *meso*-**3a**, 74457-89-9; *dl*-**3a**, 89691-42-9; (±)-**3b**, 89691-58-7; **3c**, 74457-86-6; *meso*-4, 89691-25-8; *dl*-4, 89691-26-9; *meso*-**4a**, 89691-43-0; *dl*-**4a**, 89691-44-1; (±)-4b, 89691-59-8; **4c**, 41068-36-4; *meso*-**5**, 89691-27-0; *dl*-**5**, 89691-28-1; *meso*-**5a**, 89691-45-2; *dl*-**5a**, 89691-46-3; (±)-**5b**, 89691-60-1; **5c**, 89691-67-8; *meso*-**6**, 89691-29-2; *dl*-**6**, 89691-30-5; *meso*-**6a**, 89691-47-4; *dl*-**6a**, 89691-48-5; (±)-**6b**, 89691-61-2; **6c**, 24826-74-2; *meso*-**7**, 89691-31-6; *dl*-**7**, 89691-32-7; *meso*-**7a**, 89691-49-6; *dl*-**7a**, 89691-50-9; (±)-**7b**, 89691-62-3; **7c**, 41068-29-5; *meso*-**8**, 78682-42-5; *dl*-**8**, 89691-33-8; *meso*-**8a**, 78682-49-2; *dl*-**8a**, 89691-51-0; (±)-**8b**, 89691-63-4; **8c**, 586-37-8; *meso*-**9**, 89691-34-9; *dl*-**9**, 89691-35-0; *meso*-**9a**, 89691-52-1; *dl*-**9a**, 89691-53-2; (±)-**9b**, 89691-64-5; **9c**, 705-15-7; *meso*-**10**, 89691-36-1; *dl*-**10**, 89691-37-2; *meso*-**10a**, 89691-54-3; *dl*-**10a**, 89691-55-4; (±)-**10b**, 89691-65-6; **10c**, 77344-69-5; *meso*-11, 89691-38-3; *dl*-11, 89691-39-4; *meso*-**11a**, 89691-56-5; *dl*-**11a**, 89709-53-5; (±)-11b, 89691-66-7; **11c**, 56724-09-5; 4-methoxyacetophenone, 100-06-1; 3-methoxybenzaldehyde, 591-31-1; methyl iodide, 74-88-4; 3-fluoranisole, 456-49-5; 3-methoxy-6-methylbenzoic acid, 3168-59-0; 3-methoxy-6-methylbenzyl alcohol, 73502-04-2.

Supplementary Material Available: ^{1}H NMR data (Table VII) of compounds **1a**–**11a** and 1–11 (4 pages). Ordering information is given on any current masthead page.