g, 0.0045 mol) was added in portions over a 15-min period. The reaction was removed from the ice bath and stirred for 15 h at room temperature. Water (75 mL) was added and the solution cooled in an ice bath. The precipitated product was collected by vacuum filtration and purified by column chromatography using silica gel with 9:1 toluene/ethyl acetate as the eluent to give 0.7 g (67% yield) of 1-[(*N*-hydroxy-*N*-methylamino)sulfonyl]-4-nitrobenzene: mp 147–149 °C; mass spectrum, *m/e* 232 (M^+). Anal. ($C_7H_8N_2O_5S$) C, H, N.

Synthesis of 4-Chlorobenzenesulfonamide (6). The general method used for the synthesis of the 4-nitro analogue (3) was followed: mp 136–138 °C, lit. 144 °C.[24] Anal. ($C_6H_6NO_2SCl$) C, H, N.

Synthesis of 1-[(*N*-Methylamino)sulfonyl]-4-chlorobenzene (7). The general method used for the synthesis of the 4-nitro analogue 4 was followed: mp 56–58 °C, lit. 59 °C.[25]

Synthesis of 1-[(*N*-Hydroxy-*N*-methylamino)sulfonyl]-4-chlorobenzene (8). The general method used for the synthesis of the 4-nitro analogue 5 was followed: mp 81–83 °C. Anal. ($C_7H_8NO_3SCl$) C, H, N.

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Registry No. 1, 68300-47-0; 3, 6325-93-5; 4, 6319-45-5; 5, 94592-96-8; 6, 98-64-6; 7, 6333-79-5; 8, 94592-95-7; 5-amino-1,3,4-thiadiazole 2-mercaptan, 2349-67-9; 5-acetamido-1,3,4-thiadiazole 2-mercaptan, 32873-56-6; 5-acetamido-1,3,4-thiadiazole-2-methylsulfenamide, 94515-21-6; 4-nitrobenzenesulfonyl chloride, 98-74-8; *N*-methylhydroxylamine hydrochloride, 4229-44-1; 4-chlorobenzenesulfonyl chloride, 98-60-2.

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2-Alkyl-Substituted 1,1-Bis(4-acetoxyphenyl)-2-phenylethenes. Estrogen Receptor Affinity, Estrogenic and Antiestrogenic Properties, and Mammary Tumor Inhibiting Activity

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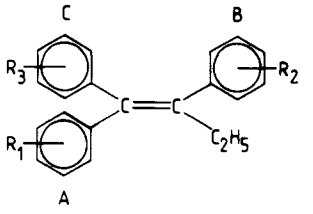
1,1-Bis(4-acetoxyphenyl)-2-phenylethenes that are substituted with H, CH_3, C_2H_5, n-C_3H_7, i-C_3H_7, or CH_2CF_3 in position 2 were synthesized in order to study the influence of the alkyl side chain on estradiol receptor affinity, estrogenic and antiestrogenic properties, and inhibition of the hormone-dependent MXT mammary carcinoma of the mouse. Furthermore, the double bond of 1,1-bis(4-acetoxyphenyl)-2-phenylbut-1-ene was hydrogenated or epoxidated to yield the corresponding ethane and oxirane derivative. Compounds 14 (R = H), 15 (R = CH_3), and 16 (R = C_2H_5) had the best binding affinities. Lengthening the side chain, hydrogenation, or epoxidation decreased the RBA values. In the immature mouse assay, 15 (R = CH_3) and 19 (R = CH_2CF_3) had the highest uterotrophic activity. There was no correlation between receptor affinity and estrogenic properties. Compounds 14 (R = H), 17 (R = n-C_3H_7), the ethane 20, and the oxirane 21 had some antiuterotrophic activity in a low dosage. The MXT tumor was best inhibited by compounds 15 (R = CH_3), 16 (R = C_2H_5), and 18 (R = i-C_3H_7) without significant elevation of the uterine weight determined at the end of the experiment. The antitumor effect of 15, 16, and 18 was significantly better than that of tamoxifen. In this series, a certain estrogenic potency in the immature mouse test seems to be necessary for a good antitumor activity, as all compounds with antiuterotrophic and low uterotrophic properties did not exert any significant tumor-inhibiting effect.

Tamoxifen, a compound of the triarylethylene type, is now routinely used for the treatment of the advanced, hormone-dependent mammary carcinoma.[1] In three former publications,[2-4] we have presented studies on structure–activity relationships in the class of 1,1,2-triphenyl-1-enes, i.e., triphenylethylenes with an ethyl side chain, which are substituted on the three aromatic rings with all possible combinations of 3-acetoxy, 4-acetoxy, and/or 3,4-diacetoxy groups. The pharmacological properties of these compounds with regard to estrogen receptor affinity, estrogenic and antiestrogenic effects, and tumor-inhibiting activities on hormone-dependent tumor models strongly depend on the applied mode of substitution of the phenyl rings (Scheme I).[2-4]

Scheme I



R_{1-3}= 3-$OCOCH_3$, 4-$OCOCH_3$, 3,4-di-$OCOCH_3$

Of major interest are compounds with a substitution in ring A and C, as they had strong tumor inhibiting properties and only low estrogenic side effects.[2-4] A β-aminoethoxy group attached on ring C as in tamoxifen and other triphenylethylene antiestrogens is not absolutely essential for antiestrogenic and tumor-inhibiting activity.[2-4]

Several other molecular modifications of triphenylethylenes have been described. However, no systematic structure–activity study concerning the variation of the substituent in position 2 was carried out in the class of

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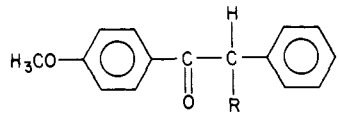
Table I. 1-(4-Methoxyphenyl)-2-phenylethan-1-ones (1–6)



compd	R	yield,[a] %	mp,[b] °C	formula
1[c]	H	61	77	$C_{15}H_{14}O_2$
2[d]	CH_3	64	56	$C_{16}H_{16}O_2$
3[e]	C_2H_5	85	46	$C_{17}H_{18}O_2$
4	n-C_3H_7	74	oil	$C_{18}H_{20}O_2$
5	i-C_3H_7	50	oil	$C_{18}H_{20}O_2$
6	CH_2CF_3	14	76	$C_{17}H_{15}F_3O_2$

[a] Yield of pure product. [b] All solid compounds were crystallized from EtOH. [c] See ref 2 and 5. [d] See ref 5. [e] See ref 3.

triphenylethylenes with regard to the above-mentioned biological properties. Among the molecular variations of this side chain in position 2, which have been already described concerning the chemistry and some biological parameters, are substituents like H, CH_3, C_2H_5, i-C_3H_7, n-C_3H_7,[5,6] CN,[7] NO_2,[1] Cl,[1] and Br.[8,9] A comparison of the influence of these substituents on the biological properties is complicated by the fact that most of these compounds are substituted in a different way in the aromatic rings and that only a few pharmacological properties were evaluated.

Some former studies of our group dealt with the influence of the alkyl substituents in the class of α,β-dialkylstilbenes and 1,2-diphenylethanes.[10,11] Is was shown that the best estrogen receptor affinity and mammary tumor inhibiting activity was exerted by compounds with ethyl side chains and that shortening or lengthening these groups led to a decrease of these effects. Recently, we have described the influence of a CF_3 group instead of CH_3 group[12] in the class of 1,2-dialkylated 1,2-diphenylethanes and -ethenes.[12] The trifluoromethyl substituent generally enhanced the estrogen receptor affinity.

In this paper we present a structure–activity study in the class of 1,1,2-triphenylethylenes with various substituents in position 2, i.e., H, CH_3, C_2H_5, i-C_3H_7, n-C_3H_7, and CH_2CF_3, with regard to estrogen receptor affinity, estrogenic and antiestrogenic properties, and antitumor activity on the MXT mammary tumor.

We chose a 1,1,2-triphenylethylene skeleton with a p-acetoxy substitution in ring A and C, as 1,1-bis(4-acetoxyphenyl)-2-phenylbut-1-ene has a good receptor affinity and a high mammary tumor inhibiting activity.[2,3] Furthermore, E/Z isomerization as described with hydroxytamoxifen[23] is not possible with this type of molecule.

To reveal the importance of the double bond on the biological properties, 1,1-bis(4-acetoxyphenyl)-2-phenylbut-1-ene was converted to its corresponding triphenylethane and -oxirane derivative. In the case of α,β-dialkylstilbenes, we have shown that some of the respective stilbene oxides have even higher receptor affinities than their parent compounds.[13–16]

Scheme II

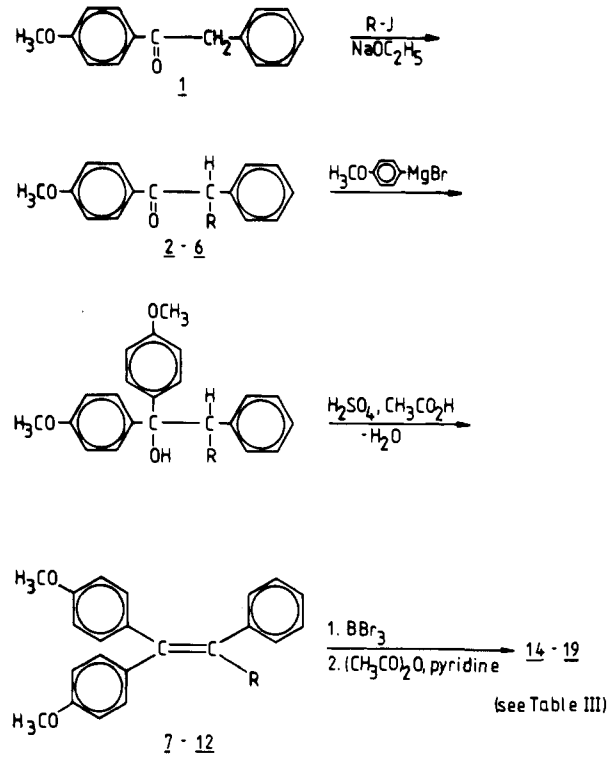
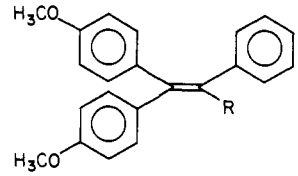


Table II. 1,1-Bis(4-methoxyphenyl)-2-phenylethenes (7–12) and -butane (13)[a]



compd	R	yield, %	mp,[b] °C	formula
7[c]	H	61	63–64	$C_{22}H_{20}O_2$
8	CH_3	51	85–86	$C_{23}H_{22}O_2$
9[d]	C_2H_5	48	115	$C_{24}H_{24}O_2$
10	n-C_3H_7	58	93	$C_{25}H_{26}O_2$
11	i-C_3H_7	55	oil	$C_{25}H_{26}O_2$
12	CH_2CF_3	52	119	$C_{24}H_{21}F_3O_2$
13[e]	C_2H_5	85	oil	$C_{24}H_{26}O_2$

[a] Synthetic method A under Experimental Section. [b] All solid compounds were crystallized from EtOH. [c] See ref 19. [d] See ref 2. [e] See Scheme III; no effort was made to crystallize the crude product.

Chemistry

The 1,1-bis(4-acetoxyphenyl)-2-phenylethene derivatives (**14–19**) were synthesized according to the method of Dodds et al.[17] as previously described by us,[2–4] starting with 1-(4-methoxyphenyl)-2-phenylethanone (**1**).[2] Compound **1** was then alkylated under the influence of sodium ethanolate with the appropriate alkyl iodide to give the alkyl-substituted 1-(4-methoxyphenyl)-2-phenylethanones **2–6** (Scheme II, Table I). Compounds **1–3** and **6** were

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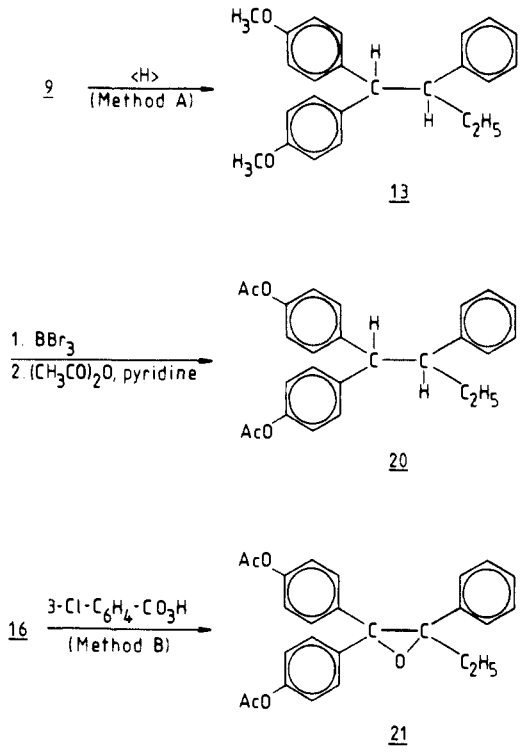
Scheme III



crystallized from the crude product. Compounds **4** and **5** were purified by column chromatography on silica gel with CH_2Cl_2/ligroin (1:1, v:v) as the eluent. Compounds **1–6** were converted in a Grignard reaction with (4-methoxyphenyl)magnesium bromide into the corresponding tertiary carbinols, which were dehydrated by using a mixture of sulfuric acid and acetic acid to give the 2-alkyl-substituted 1,1-bis(4-methoxyphenyl)-2-phenylethenes **7–12** (Scheme II, Table II), which were isolated by crystallization from the crude product.[2-4] 1,1-Bis(4-methoxyphenyl)-2-phenylethane (**13**) was synthesized from its corresponding ethene derivative **9** by catalytic hydrogenation with palladium on charcoal (Scheme III, Table II).[18]

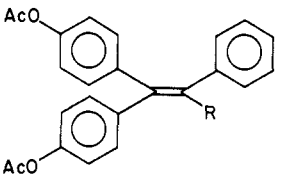
Compounds **7–13** were then converted to the hydroxy derivatives by ether cleavage with BBr_3 and acetylated with acetic anhydride and pyridine to give the 2-alkyl-substituted 1,1-bis(4-acetoxyphenyl)-2-phenylethenes **14–19** and the 2-ethyl-substituted ethane derivative **20** (Scheme II, Table III). 1,1-Bis(4-acetoxyphenyl)-2-phenylethane 1,2-oxide (**21**) was prepared from its corresponding ethene derivative **16** by epoxidation with 3-chloroperbenzoic acid in ethereal solution (Scheme III).[13]

The identity of all compounds was established by ^1H NMR spectroscopy. In the case of compounds **4–6**, **8**, **10–12**, and **19–21**, it was additionally confirmed by mass spectroscopy.

Biological Properties and Discussion

All compounds of this series (**14–21**) showed an affinity for the estrogen receptor from calf uterine cytosol (Table III). As all binding curves were parallel to that of estradiol, a competitive inhibition can be assumed. In this series of compounds, acetylation of the hydroxy groups does not affect the receptor affinity, as **16** and its phenolic analogue (RBA = 5.3)[24] have very similar binding affinities.

Table III. Estrogen Receptor Affinity of 1,1-Bis(4-acetoxyphenyl)-2-phenylethenes (**14–19**), -butane (**20**), and -butane 1,2-Oxide (**21**)



compd	R	yield, %	mp,[a] °C	formula[b]	RBA,[c] %
14	H	85	83	$C_{24}H_{20}O_4$	6.3
15	CH_3	69	109–110	$C_{25}H_{22}O_4$	7.3
16[d]	C_2H_5	72	82	$C_{26}H_{24}O_4$	6.8
17	n-C_3H_7	75	111	$C_{27}H_{26}O_4$	1.4
18	i-C_3H_7	68	144–145	$C_{27}H_{26}O_4$	2.9
19	CH_2CF_3	84	176	$C_{26}H_{21}F_3O_4$	2.9
20[e]	C_2H_5	66	114	$C_{26}H_{26}O_4$	0.4
21[e,f]	C_2H_5	65	125	$C_{26}H_{24}O_5$	2.2

[a] All compounds were crystallized from EtOH. [b] All compounds were analyzed for C and H within 0.40% of the calculated values. [c] RBA = ([E2]/[J]) × 100. [E2] and [J] are the molar concentrations of nonradioactive E2 and inhibitor required to decrease the bound [^3H]E2 by 50%; E2 = 17β-estradiol. [d] See ref 2. [e] See Scheme III. [f] Synthetic method B under Experimental Section.

Since the extensively described antiestrogen tamoxifen has a C_2H_5 group in position 2,[1] compound **16**,[2,3] also having an C_2H_5 alkyl side chain, serves as the standard substance in this series. Surprisingly the abbreviation of the alkyl side chain as in **14** (R = H) or **15** (R = CH_3) did not decrease the binding affinity (Table III). The enlargement of the substituent R as in **17** (R = n-C_3H_7) or in **18** (R = i-C_3H_7), however, led to a decrease of the receptor binding to about 20% (**17**) or 40% (**18**) of that of **16** (Table III). Thus, an i-C_3H_7 group (**18**) fits better to the receptor than a n-C_3H_7 moiety (**17**). As compounds **14–16** have very similar RBA values, the postulated "hydrophobic pocket" of the estrogen receptor[20] does not seem to be important for a good receptor binding in vitro with these triphenylethylenes.

The receptor affinity of compound **19** with a CH_2CF_3 group is identical with that of **18** (R = i-C_{H7}) and lower than that of the C_2H_5-substituted **16**. Thus, the introduction of fluorine atoms did not enhance the binding of these triphenylethylenes in contrast to our results in the class of 1,2-dialkylated 1,2-diphenylethanes and -ethenes.[12] The hydrogenation of the double bond on **16** to give compound **20** led to a tremendous decrease of the RBA value to less than one-tenth of the parent compound. The requirements on the steric configuration for a good receptor binding are thus better fulfilled by a triphenylethene than by a triphenylethane. The differences in receptor affinity between diethylstilbestrol (RBA = 60) and hexestrol (RBA = 27)[18] are not as pronounced as seen above.

Furthermore, epoxidation of the double bond in **16** to give compound **21**, decreased the RBA value, too (Table III). In the case of 3,3′-diacetoxy-α,β-dialkylstilbenes, we have shown that epoxidation even increased the binding affinity.[13,16]

The affinity of a compound to the estrogen receptor in vitro does not directly reflect its biological properties like

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Table IV. Estrogenic and Antiestrogenic Effect of Compounds 14–21 in the Immature Mouse Uterine Weight Test

compd	dose,[a] μg	estrogenic effect[b]	dose,[a] μg	antiestrogenic effect:[c] % inhibn
14	1.0	11	1.0	26
	5.0	13	5.0	0
	25.0	99		
	100	116		
15	1.0	125		
	5.0	108		not tested
	25.0	83		
	100	78		
16	1.0	37	1.0	0
	5.0	72	5.0	0
	25.0	98		
	100	107		
17	1.0	31	1.0	19
	5.0	42	5.0	0
	25.0	90		
	100	114		
18	1.0	60	1.0	0
	5.0	87	5.0	0
	25.0	100		
	100	117		
19	1.0	62	1.0	0
	5.0	140		
	25.0	108		
	100	86		
20	1.0	17	1.0	27
	5.0	42	5.0	0
	25.0	79		
	100	83		
21	1.0	2	1.0	23
	5.0	2	5.0	5
	25.0	33	25.0	0
	100	60		

[a] Dose per animal and day. [b] Estrogenic effect = $[(E_T - E_V)/(E_S - E_V)] \times 100$. Effect = uterus dry weight (milligrams)/body weight (grams) × 100. E_T = effect of test compound; E_V = effect of vehicle; E_S = effect of estrone standard (0.4 μg). Estrone produces a maximum stimulation of the uterine growth at a dose of 0.4 μg/mouse and day.[10] This value was taken as the 100% value. [c] Antiestrogenic effect = % inhibition = $[(E_S - E_{S,T})/(E_S - E_V)] \times 100$. E_S = effect of estrone standard (0.1 μg); $E_{S,T}$ = effect of standard under simultaneous application of test compound.

estrogenic or antiestrogenic activity in vivo.[3] In the case of some compounds, a good correlation between the RBA value and the estrogenic potency was shown.[3] In this series of triphenylethenes it is striking that compounds **14–16** show very different estrogenic properties in spite of their nearly identical RBA values (Table IV).

The strongest uterotrophic activity in the immature mouse uterine weight test[10] was exerted by compound **15** (R = CH_3), which had reached the maximum effect already at a dose of 1 μg/mouse. Compound **16** with a C_2H_5 moiety had a much lower estrogenic activity than **15** but exerted stronger uterotrophic effects than **14** (R = H) at the lower doses tested (Table IV). Comparable results were already stated with similar triphenylethylenes in the literature.[1,8,9] Triphenylethylene itself has only low estrogenic effects, which can be strongly increased by the introduction of a C_2H_5 or a halogen in position 2.[1,8,9]

Whereas the postulated "hydrophobic pocket" of the estrogen receptor[20] had no influence on the binding affinity of compounds **14–16**, it seems to be important according to the estrogenic effects.

Compound **18** (R = i-C_3H_7) had a somewhat stronger uterotrophic activity than **16** in spite of its lower RBA value. The analogue of **18** with a n-propyl side chain (**17**) had lower estrogenic properties than **16** and **18** (Table IV). Whereas the introduction of fluorine atoms into **16** to give compound **19** did not increase the receptor affinity, it had a striking effect on the estrogenic properties as **19** had a much stronger uterotrophic activity than **16**.

Table V. Effects of Compounds 14–21 on the Growth of the Hormone-Dependent MXT Mouse Mammary Carcinoma and on Uterus Growth

compd[a]	tumor[b] T/C, %	uterus[c] T/C, %	compd[a]	tumor[b] T/C, %	uterus[c] T/C, %
14	64.3	54.8[d]	**19**	43.1[d]	128.2
15	0.3[d]	120.4	**20**	69.1	73.7
16	0.4[d]	124.0	**21**	78.9	77.9
17	61.1	93.3	TAM	9.8[d]	87.0
18	0.2[d]	127.8			

[a] All compounds were administered 3 times a week sc for 6 weeks in a dosage equimolar to that of **16** (3 × 8.0 mg/kg). [b] T/C, %: tumor weight of the treated animals/tumor weight of the solvent control × 100 after 6 weeks of therapy (mean of 10 animals). [c] T/C, %: uterotrophic effect of the treated animals/uterotrophic effect of the solvent control × 100 after 6 weeks of therapy (mean of 10 animals). [d] Significant ($\alpha \leq 0.05$). The U test according to Wilcoxon, Mann, and Whitney was used. There were no deaths during the treatment. The body weight of the treated animals was less than ±4% different of that of the control.

The estrogenic effect of **20**, the hydrogenated analogue of **16**, was lower than that of **16** but not as low as was expected according to its very low RBA value of 0.4 (Tables III and IV). Similar results were obtained by Clark and Jordan.[8] The lowest uterotrophic activity in this series was exerted by the oxirane **21**, which did not reach the 100% level even at a dose of 100 μg/mouse. These results clearly demonstrate that in this series of triphenylethenes the degree of the receptor binding does not correlate with the estrogenic potency in vivo. It can be assumed that structural modifications of this kind have different effects on the receptor binding and on the intrinsic activity of such compounds. A further explanation for these differences between in vitro and in vivo results may be a different absorption, distribution, or metabolism of these compounds.

As the parent compound of this study (**16**) had no antiestrogenic activity in the immature mouse uterine weight assay,[10] we did not expect that these structural changes would create strong antiestrogens. It can be easily seen from Table IV that the compounds with low uterotrophic properties, i.e., **14**, **17**, **20**, and **21** exerted some antiestrogenic activity though only at the low dose of 1 μg/mouse, whereas all other compounds had no antagonistic properties (Table IV).

To determine the tumor-inhibiting effect in vivo, we used the transplantable, hormone-dependent MXT mammary tumor of the BDF_1 mouse.[3,4,22] Its growth is inhibited by ovariectomy and also by the administration of antiestrogens like tamoxifen as well as of estrogens like diethylstilbestrol (DES).[3] The antitumor effect of these three modes of therapy is similar. However, whereas the tumor-inhibiting effect of DES is associated by a strong estrogenic side effect, determined by the uterus dry weight at the end of therapy (% T/C = 200), tamoxifen was inhibitory (% T/C = 4) without significantly affecting the uterus weight (% T/C = 79).[3] In this study, we also used tamoxifen as a positive control in the MXT experiment. The inhibitory effect as well as the estrogenic side effect was very similar to the results already published (Table V.)[3]

All compounds were administered in equimolar doses related to **16** (3 × 8 mg/kg per week). Out of the new compounds, **15** (R = CH_3) and **18** (R = i-C_3H_7) as well as **16** (R = C_2H_5)[2,3] showed a very strong tumor inhibiting effect that was significantly ($\alpha \leq 0.05$) better than that of

tamoxifen (Table V). These compounds slightly increased the uterine weight in the MXT tumor assay above the control, but this effect was not significantly different. Compound **19** (R = CH_2CF_3) exerted a similar estrogenic side effect but had a lower (but still significant) antitumor activity compared to that of **15**, **16**, and **18**. All other compounds (**14**, **17**, **20**, and **21**) had no significant tumor-inhibiting effect. Therefore, in this series of triphenylethenes, it can be stated that all compounds with antiestrogenic and relatively low estrogenic properties in the immature mouse assay had no significant tumor-inhibiting effect. On the other hand, all compounds with relatively high estrogenic effects on the immature mouse and without antiestrogenic activity exerted a very good antitumor effect without significant estrogenic side effect. From these results it can be seen that in this series a certain estrogenic potency is necessary for a good antitumor effect. The uterus of the immature mouse is much more sensitive to the action of a compound than the uterus of the mature mouse as can be seen by the comparison of the estrogenic effects of **15** and **16** (Tables IV and V). However, the determination of the estrogenic effect in the MXT assay can be an appropriate method to determine the threshold value of estrogenic potency maximally tolerable for a therapeutical use. Whereas there was no compound in this series that had a tumor-inhibiting activity, antiestrogenic properties, and no estrogenic side effect, we have developed, 1,1,2-triphenylbut-1-enes with the above-named properties.[3,4] From these data (Tables IV and V) it can be additionally seen that the action of a compound on the immature as well as the mature uterus can be different from its effect on tumor growth.

In conclusion, it can be stated that shortening or lengthening the C_2H_5 group as well as hydrogenation or epoxidation of the double bond in **16** does not lead to compounds with more interesting biological properties, as **16** had the best receptor affinity (together with **14** and **15**) and the best tumor inhibiting activity (together with **15** and **18**) and had lower estrogenic effects on the immature mouse compared to **15** and **18**.

Experimental Section

General Procedures. Melting points were determined on a Büchi 510 melting point apparatus and are uncorrected. Elemental analyses were performed by the Mikroanalytisches Laboratorium, University of Regensburg. ^1H NMR spectra were obtained with a Varian EM 390 A (90 MHz) instrument (internal standard Me_4Si, chemical shifts in δ). TLC of each compound was accomplished on Merck F 254 silica gel plates. Mass spectra were performed with a Varian MATCH 5 instrument.

Syntheses. Method A. 1,1-Bis(4-methoxyphenyl)-2-phenylbutane (13). Palladium on charcoal (10%, 0.1 g) was added to a solution of **9** (1.3 g, 0.005 mol) in 150 mL of EtOH. The suspension was shaken under a hydrogen atmosphere at 50 °C until no more H_2 was taken up. The reaction mixture was filtered and the alcohol was removed. The completeness of hydrogenation was proved by ^1H NMR spectroscopy. The crude product was subjected to ether cleavage and acetylation.

Method B. 1,1-Bis(4-acetoxyphenyl)-2-phenylbutane 1,2-Oxide (21). 3-Chloroperbenzoic acid (1.7 g, 0.01 mol) was added to a solution of **16** (2.0 g, 0.005 mol) in 200 mL of dry ether. The solution was stirred under protection from light. The course of the reaction was observed by TLC (CH_2Cl_2). After quantitative conversion to the epoxide, the ethereal solution was washed with saturated $NaHCO_3$ solution and water, and the solvent was removed. The crude product was crystallized from EtOH to give **21**.

Biological Methods. Estradiol Receptor Binding Assay. The method described in ref 2 was used. The relative binding affinity (RBA) of the test compounds was determined by the displacement of [^3H]estradiol. Test compounds were incubated with cytosol from calf uteri and [^3H]estradiol at 4 °C for 16 h. Incubation was stopped by adding dextran-coated charcoal. After centrifugation, the radioactivity of 200 μL of supernatant aliquot was counted. The percentage bound radioligand was plotted vs. the concentration of unlabeled test compounds. Five or 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 properties were determined by stimulation of the uterine growth or by inhibition of the uterine growth stimulated by estrone, respectively, using immature NMRI mice as described previously.[2] Female mice (body weight, 10–12 g; age, 20 days at test beginning, 10 mice per group) were injected sc daily for 3 consecutive days with solutions of the test compounds in olive oil (0.1 mL/mouse). The uteri were removed 24 h after the last injection, fixed with Bouin's solution, dried, and weighed.

Hormone-Dependent, Transplantable MXT Mammary Tumor of the BDF1 Mouse.[22] The applied method was identical with that previously described by us.[3,4] The MXT tumor used in these studies was the MXT line 3.2 kindly provided by Dr. Bogden, Laboratory of Experimental Oncology, EG & G Bogden Laboratories, Worcester, MA. The tumor was transplanted in pieces of about 2 mm^3 (one tumor piece/animal) subcutaneously in female, 8-week-old BDF1 mice (body weight, 20 ± 1.6 g; Charles River Wiga, West Germany). After transplantation, the animals were randomly distributed into groups of 10. Starting with the first day after transplantation, the test compounds were injected sc 3 times a week (Monday, Wednesday, Friday) as olive oil solutions (0.1 mL/mouse). The duration of treatment was 6 weeks. At the end of treatment, the animals were killed by cervical dislocation and weighed. The tumors were removed, washed in 0.9% sodium chloride solution, blotted dry, and weighed, and the average tumor weight was calculated. The uteri were also removed and prepared as described in ref 3 to serve as an indicator of the estrogenic side effects of the compounds.

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Supplementary Material Available: ^1H NMR data (Tables VI–VIII) of compounds **1–21** and mass spectral data (Table IX) of compounds **4–6**, **8**, **10–12**, and **19–21** (4 pages). Ordering information is given on any current masthead page.