were precipitated with an excess (1 mL) of 0.5 M ammonium hexafluorophosphate after acidification with acetic acid (50 μL), isolated, washed with water, and then dessicated; yields were 60%, 50%, 23%, and 49% for **6b**, **6d**, **6f**, and **6h**, respectively (hexafluorophosphate salts).

6b (**6'b**, 90%; **6''b**, 10%): UV–visible (CH_3COOH, H_2O, 50/50) λ 460 nm (ε 1500 M^{-1} cm^{-1}), 370 (8000), 313 (16000), 299 (16500), 274 (25500). Anal. Calcd for $C_{28}H_{27}N_7O_8P_2F_6$: C, 43.92; H, 3.53; N, 12.81. Found: C, 43.21; H, 3.16; N, 11.97.

6d (**6'd**, 90%; **6''d**, 10%): UV–visible (CH_3COOH, H_2O, 50/50) λ 450 nm (ε 2500 M^{-1} cm^{-1}), 366 (11000), 312 (21000), 295 (24500), 274 (38000). Anal. Calcd for $C_{28}H_{27}N_7O_9P_2F_6$: C, 43.02; H, 3.46; N, 12.55. Found: C, 42.54; H, 3.13; N, 11.76.

6f (**6'f**, 80%; **6''f** 20%): UV–visible (CH_3COOH, H_2O, 50/50) λ 375 nm (ε 11100 M^{-1} cm^{-1}), 314 (21500), 277 (44500). Anal. Calcd for $C_{27}H_{27}N_5O_9P_2F_6$: C, 43.72; H, 3.64; N, 9.45. Found: C, 43.14; H, 3.18; N, 8.95.

6h (**6'h**, 80%; **6''h**, 20%): UV–visible (CH_3COOH, H_2O, 50/50) λ 450 nm (ε 4000 M^{-1} cm^{-1}), 373 (17300), 314 (41100), 302 (40000), 276 (54000). Anal. Calcd for $C_{27}H_{26}N_4O_{10}P_2F_6$: C, 43.67; H, 3.50; N, 7.55. Found: C, 42.72; H, 3.12; N, 7.12.

Thioguanine–Elliptinium 7a and Thioguanosine–Elliptinium 7b Adducts. Contrary to the method described above for nucleophiles that are not easily oxidized, thioguanine and thioguanosine were added to an organic solution of 9-oxo-NME (**2**), this quinone imine being generated by HRP/H_2O_2 in the absence of a SH-containing molecule. This procedure was previously used for syntheses of cysteinyl–NMHE adducts.[6] Thus, 1 mL of 0.1 M H_2O_2 was added to a solution of 0.05 mmol of 9-OH-NME and 20 μL of 10^{-4} M HRP in 30 mL of 0.067 M (pH 5) phosphate buffer. After 5 min, the quinone imine was extracted into 100 mL of dichloromethane in the presence of an excess of ammonium hexafluorophosphate (0.5 mmol). A 0.2-mmol sample of thioguanine or thioguanosine in 100 mL of water (acidified with acetic acid to pH 3.5) was then added to the organic extract and the mixture vigorously shaken. The adducts were precipitated from the aqueous phase with an excess of ammonium hexafluorophosphate (0.5 mmol), washed with water, dissolved in acetic acid (10 mL), precipitated again with 50 mL of diethyl ether, and dried under reduced pressure. The adducts (acetate salts) were obtained in 60% and 12% yield for **7a** and **7b**, respectively.

7a: UV–visible (CH_3COOH, H_2O, 50/50) λ 450 nm (ε 3300 M^{-1} cm^{-1}), 386 (7200), 320 (38800). Anal. ($C_{25}H_{23}N_7O_3S$) C, H, N.

7b: UV–visible (CH_3COOH, H_20, 50/50) λ 450 nm (ε 3700 M^{-1} cm^{-1}), 386 (8000), 323 (42400). Anal. Calcd for $C_{30}H_{31}N_7O_7S$: C, 56.87; H, 4.90; N, 15.48. Found: C, 56.22; H, 4.51; N, 14.96.

See Table II for ^1H NMR data of all these elliptinium adducts.

Enzymatic Hydrolysis of 5'-Phosphate Ribonucleotides–Elliptinium Adducts 6b and 6d. The hydrolysis tests were performed under the following conditions: 0.8 unit of alkaline phosphatase was added to 1 mL of 0.127 M of 5'-phosphate ribonucleotide–elliptinium adducts in 20 mM Tris buffer at pH 8.5 and incubated at 37 °C. HPLC conditions were as described above.

Cytotoxicity Experiments. Inhibition of cell growth was determined with L1210 leukemia cells in vitro as previously described (see ref 17). The inhibitory efficiency of the elliptinium adducts is expressed in terms of ID_{50}, the drug concentration that reduces the number of cells by 50% as compared to the control after 48 h (ID = inhibitory dose).

Acknowledgment. We thank Prof. C. Paoletti for fruitful discussions throughout this work and CNRS (PIRMED) and Sanofi Co. for a financial support. R. C. Rao and B. Monsarrat are acknowledged for NMR and mass data and G. Francois and C. Galy for technical assistance in ID_{50} determination.

Registry No. **6'a**·AcO^-, 93033-93-3; **6'b**·PF_6^-, 102420-10-0; **6''b**·PF_6^-, 102420-12-2; **6'c**·AcO^-, 102434-52-6; **6'd**·PF_6^-, 102420-14-4; **6''d**·PF_6^-, 102420-16-6; **6'e**·AcO^-, 102420-00-8; **6'f**·PF_6^-, 102420-18-8; **6''f**·PF_6^-, 102420-20-2; **6'g**·AcO^-, 102420-02-0; **6''g**·AcO^-, 102420-04-2; **6'h**·PF_6^-, 102420-22-4; **6''h**·PF_6^-, 102434-54-8; **6'i**·AcO^-, 102420-06-4; **6''i**·AcO^-, 102420-08-6; **7a**·AcO^-, 102420-24-6; **7b**·AcO^-, 102434-56-0; 9-OH-NME, 58337-35-2; AMP, 61-19-8; GMP, 85-32-5; CMP, 63-37-6; UMP, 58-97-9; adenosine, 58-61-7; guanosine, 118-00-3; cytidine, 65-46-3; uridine, 58-96-8; azauridine, 54-25-1; thioguanine, 154-42-7; thioguanosine, 85-31-4.

(17) Paoletti, C.; Cros, S.; Dat-Xuong, N.; Lecointe, P.; Moisand, A. *Chem. Biol. Interact.* **1979**, *25*, 45.

Catechol Estrogens of the 1,1,2-Triphenylbut-1-ene Type: Relationship between Structure, Estradiol Receptor Affinity, Estrogenic and Antiestrogenic Properties, and Mammary Tumor Inhibiting Activities

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1,1,2-Triphenylbut-1-enes (**26–35**), which are substituted with one or two 3,4-diacetoxy groups or with one 3,4-diacetoxy and one 3- or 4-acetoxy group in two aromatic rings, were synthesized. The occurring *E* and *Z* isomers were isolated, and their identity was established by ^1H NMR spectroscopy. A study on structure–activity relationship was carried out with regard to estradiol receptor affinity in vitro, estrogenic and antiestrogenic properties in the immature mouse, and inhibition of the hormone-dependent MXT mammary tumor of the mouse in vivo. Among the tested compounds, most of the 1,1-disubstituted 1,1,2-triphenylbut-1-enes (**29**, ***Z*-30**, ***Z*,*E*-31**) and (*E*)-1-(3-acetoxyphenyl)-1-phenyl-2-(3,4-diacetoxyphenyl)but-1-ene (***E*-35**) as well as its respective *Z* isomer (***Z*-35**) exerted antiestrogenic properties. Compounds ***Z*-30**, ***Z*,*E*-31**, ***Z*-35**, and ***E*-35** inhibited the growth of the hormone-dependent MXT tumor. The best antitumor effect without estrogenic side effects during therapy was shown by ***E*-35**.

Many compounds of the triarylethylene type have been tested with regard to their mammary tumor inhibiting activity.[1-3] Tamoxifen, one of these compounds, is now routinely used for the treatment of the advanced mammary carcinoma.[1-3] In two former publications,[4,5] we have

(1) Jordan, V. C. In *Hormone Antagonists*; Agarwal, M. K., Ed.; de Gruyter: Berlin, New York, 1982; pp 109–128.
(2) Leclercq, G.; Devleeschouwer, N.; Heuson, J. C. *J. Steroid Biochem.* **1983**, *19*, 75 and ref cited therein.
(3) Paridaens, R. J.; Leclercq, G.; Heuson, J. C. In *The Cancer Pharmacology Annual 2*; Chabner, B. A., Pinedo, H. M., Eds.; *Elsevier*: Amsterdam, The Netherlands, 1984; pp 171–192.
(4) Schneider, M. R.; von Angerer, E.; Schönenberger, H.; Michel, R.-Th.; Fortmeyer, H. P. *J. Med. Chem.* **1982**, *25*, 1070.

0022-2623/86/1829-1355$01.50/0

presented studies on structure–activity relationships in the class of 1,1,2-triphenylbut-1-enes substituted with *m*- and/or *p*-acetoxy groups in different phenyl rings. The first of these two studies, in which compounds with either *p*- or *m*-acetoxy groups on one, two, or three aromatic rings were tested, showed that a 4-acetoxy group in ring A (for the definition of ring A, B and C, see Table I) is essential for a good estradiol receptor affinity, that the introduction of a second 4-acetoxy substituent enhances the binding affinity, and that the para-substituted compounds generally have a higher affinity than the corresponding meta-substituted ones.[4] In this series, a correlation between estrogen receptor affinity and tumor inhibiting activity on the growth of a hormone-dependent, postmenopausal human mammary carcinoma serially implanted in nude mice was observed.[4]

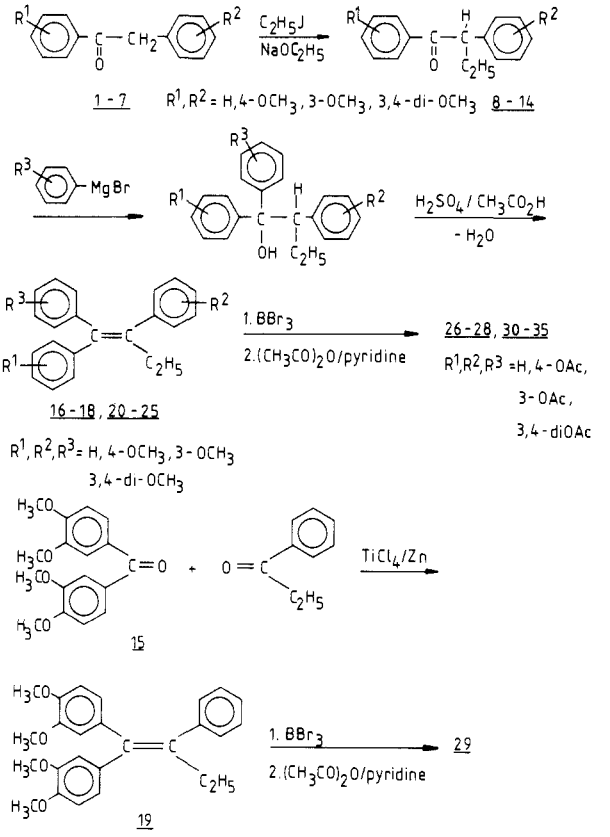
The second study, in which the biological properties of 1,1,2-triphenylbut-1-enes with one *p*- and one *m*-acetoxy group in two different phenyl rings were evaluated, revealed more complex relationships.[5] The best receptor affinity was shown by a compound with a 4-acetoxy group in A and 3-acetoxy group in B, which also proved to be a strong estrogen comparable to estrone.[5] Compounds with a *m*- or *p*-acetoxy group in rings A and C exhibited the most interesting biological properties as they had antiestrogenic activity, but only low estrogenic activity in the mouse uterine weight test, inhibited the growth of the hormone-dependent human MCF-7 breast cancer cell line, and showed a strong antitumor effect in the hormone-dependent MXT mammary tumor of the mouse in vivo without estrogenic side effects on the uterus.[5] In both studies, we have shown that antiestrogenic activity can be exerted by 1,1,2-triphenylbut-1-enes without having a dialkylaminoethoxy side chain like tamoxifen.[4,5]

This study deals with the synthesis and pharmacology of 1,1,2-triphenylbut-1-enes with one or two 3,4-diacetoxy groups or with one 3,4-diacetoxy moiety and one additional 3- or 4-acetoxy group in two phenyl rings concerning their estradiol receptor affinity, antiestrogenic and estrogenic properties, and tumor-inhibiting activity on the hormone-dependent MXT mammary tumor.

We have used a 3,4-diacetoxy moiety for these 1,1,2-triphenylbut-1-enes, as α,β-diethylstilbenes[6] and 3,4-diphenylhexanes[7] with this substitution have a reduced estrogenic activity but a retained antitumor effect compared to their parent compounds diethylstilbestrol or hexestrol.[6,7] One of the metabolites of tamoxifen has a 3,4-dihydroxy substitution in ring A (dihydroxytamoxifen) too.[8] This compound exerts a higher estradiol receptor affinity than tamoxifen.[8] It is only a weak estrogen but an antiestrogen in the rat.[8] Furthermore, endogenous estrogens are hydroxylyzed to yield compounds with a catechol structure.[9] The exact physiological role of these catechol estrogens is at present not well-understood.[9] In some test systems, however, 2-hydroxyestrone seems to have estrogen antagonistic properties.[9]

Chemistry. The methoxy-substituted 1,1,2-triphenylbut-1-enes **16–18** and **20–25** (Scheme I) were synthesized

Scheme I



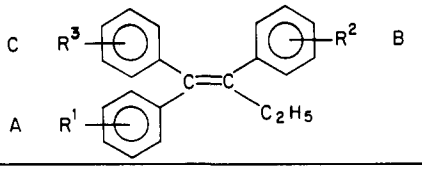
as previously described by us.[4,5] Briefly, the methoxy-substituted 1,2-diphenylethanones **1–7** were prepared by Friedel–Crafts acylation of veratrole or anisole with the corresponding phenylacetyl chlorides, or by a Grignard reaction of 3,4-dimethoxyphenylacetyl chloride with phenyl- or (3-methoxyphenyl)magnesium bromide at low temperature.[4,5] The 1,2-diphenylethanones **1–7** were alkylated with ethyl iodide to give the corresponding 1,2-diphenylbutanones **8–14** (Scheme I).[4,5] Compounds **8–14** were then converted with the respective phenylmagnesium bromide into the corresponding tertiary carbinols, which were dehydrated by use of a mixture of sulfuric acid and acetic acid to give the methoxy-substituted 1,1,2-triphenylbut-1-enes **16–18** and **20–25** as mixtures of the respective *E* and *Z* isomers. These mixtures were not separated at this step, since isomerization can occur during the subsequent ether cleavage,[4] but were purified by column chromatography on silica gel.

Another synthetic route must be used to obtain compound **19** because (3,4-dimethoxyphenyl)magnesium bromide can be prepared only in poor yield.[10] The synthesis of **19** was achieved by a mixed reductive coupling of propiophenone and 3,3′,4,4′-tetramethoxybenzophenone[11] with $TiCl_4/Zn$.[12] Compound **19** was isolated from the reaction mixture by column chromatography on silica gel in a yield of 49% (Scheme I).

Compounds **16–25** were then converted to the corresponding hydroxy derivatives with BBr_3 and acetylated with acetic anhydride and pyridine to give the acetoxy-substituted 1,1,2-triphenylbut-1-enes **26–35** (Tables I–IV).[4] The mixtures of *E* and *Z* isomers were separated either by fractional crystallization or flash chromatography on

(5) Schneider, M. R.; Ball, H.; Schönenberger, H. *J. Med. Chem.* **1985**, *28*, 1880.
(6) Schneider, M. R.; Schönenberger, H.; Michel, R.-Th.; Fortmeyer, H. P. *J. Cancer Res. Clin. Oncol.* **1982**, *104*, 219.
(7) Kranzfelder, G.; Hartmann, R. W.; von Angerer, E.; Schönenberger, H.; Bogden, A. E. *J. Cancer Res. Clin. Oncol.* **1982**, *103*, 165.
(8) Jordan, V. C.; Collins, M. M.; Rowsby, L.; Prestwich, G.; *J. Endocrinol.* **1977**, *75*, 305.
(9) Schneider, J.; Huh, M. M.; Bradlow, H. L.; Fishman, J. *J. Biol. Chem.* **1984**, *259*, 4840.
(10) Kharash, M. S.; Reinmuth, O. *Grignard Reactions of Nonmetallic Substances*; Prentice-Hall: New York, 1954.
(11) Perkin, W. H.; Weizman, C. *J. Chem. Soc.* **1906**, *89*, 1661.
(12) Mukaiyama, T.; Sato, T.; Hanna, J. *Chem. Lett.* **1973**, 1041.

Table I. Biological Properties of 1,1,2-Triphenylbut-1-enes with One or Two 3,4-Diacetoxy Groups[a]



					estrogenic		antiestrogenic		MXT tumor,[f] % T/C	
compd	R^1	R^2	R^3	RBA,[b] %	dose,[c] μg	effect[d]	dose,[c] μg	effect[e]	tumor	uterus
***Z*-26**	H	H	3,4-$(OAc)_2$	0.8	1	5	1	0	19[h]	107
					10	64	5	0		
					100	81	25	0		
					500	77				
***Z*,*E*-26**[i]	3,4-$(OAc)_2$	H	H	4.3	1	31	1	1	17[h]	106
					10	59	5	0		
					100	66	25	0		
					500	66				
27	H	3,4-$(OAc)_2$	H		1	18	1	6	9[h]	115
					10	98	5	0		
					100	70	25	0		
***Z*-28**	H	3,4-$(OAc)_2$	3,4-$(OAc)_2$	0.3	1	7	1	9	75	90
					10	40	5	24[g]		
					100	99	25	1		
					500	77				
***E*-28**	3,4-$(OAc)_2$	3,4-$(OAc)_2$	H	2.3	1	17	1	27[g]	84	99
					10	49	5	0		
					100	76	25	0		
					500	67				
29	3,4-$(OAc)_2$	H	3,4-$(OAc)_2$	2.4	500	13	1	49[g]	134	107
								5		
							25	32[g]		
							100	54[g]		
TAM	H	H	$OCH_2CH_2N(CH_3)_2$	1.8	1	43	1	0	10[h,j]	92[j]
					10	100	5	0		
					100	79	25	11		

[a] OAc, $OCOCH_3$. [b] RBA, % = $[E_2]/[J] \times 100$; $[E_2]$ and $[J]$ are the molar concentrations of nonradioactive E_2 and inhibitor required to decrease the bound radioactivity by 50%; E_2 = 17β-estradiol. [c] Dose per animal and day. [d] 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). [e] 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; the U-test according to Wilcoxon, Mann, and Whitney was used. [f] All compounds were administered in equimolar doses 3 times a week sc. The dose of **27** was 8 mg/kg; T/C, % (tumor): tumor weight of the treated animals/tumor weight of the solvent control × 100 after 6 weeks of therapy (mean of 10 animals); T/C, % (uterus): uterotrophic effect of the treated animals/uterotrophic effect of the solvent control × 100 after 6 weeks of therapy (mean of 10 animals). [g] Significant ($\alpha < 0.01$). [h] Significant ($\alpha < 0.05$). [i] *Z*,*E*, mixture of *Z* and *E* isomer (1:1). [j] Mean of seven independent experiments.

silica gel with CH_2Cl_2 or mixtures of CH_2Cl_2 and ethyl acetate as the eluent. In the case of compound **26**, only the *Z* isomer could be isolated by fractional crystallization. The mixture of the *E* and *Z* isomer of **31** could not be separated, even by preparative HPLC. For the pharmacologial tests, 1:1 mixtures of ***Z*,*E*-26** and ***Z*,*E*-31** were used. The distribution of the isomers was determined by analytical HPLC.

The purity of the *E* and *Z* isomers was proved by HPLC analyses. The identity of the respective isomers was established by ^1H NMR spectroscopy as we have previously described in detail.[4] Briefly, the ^1H NMR spectrum of the unsubstituted 1,1,2-triphenylbut-1-ene shows one singlet for each phenyl group in the order ring in position A (δ 7.31) > B (δ 7.15) > C (δ 6.97). With 1,1-bis(4-acetoxyphenyl)-2-(4-acetoxyphenyl)but-1-ene, the compound with three para-substituted phenyl rings, we observed the same order in the δ values. This order is based on the molecular model and was proved by various compounds with different substitution of the aromatic rings.[4,5] As we therefore know the δ values of the aromatic resonances of the unsubstituted as well as the para-substituted phenyl rings, it was possible to establish the identity of the *E* and *Z* isomers of compounds **26–30** and **32–35**. The isomers of compound **31** could not be separated.

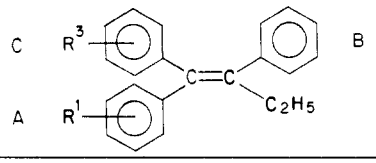
Biological Properties. As the discussion of structure–activity relationships in this class of substances is complex, compounds **26–35** will be arranged in different groups, i.e., compounds with (i) one 3,4-diacetoxy group, (ii) two 3,4-diacetoxy groups, (iii) one 3,4-diacetoxy and one 3- or 4-acetoxy group containing a (a) cyclofenil, (b) *trans*-diethylstilbestrol (DES), or (c) *cis*-DES fragment (Tables I–IV).

All new 1,1,2-triphenylbut-1-enes (**26–35**) showed an affinity to the estrogen receptor from calf uterine cytosol in vitro (Tables I–IV). The inhibition of the interaction of [^3H]estradiol with its receptor was competitive, as the binding curves were parallel to that of unlabeled estradiol.

Compounds with One 3,4-Diacetoxy Group. The estradiol receptor affinity of the new 1,1,2-triphenylbut-1-enes substituted with one 3,4-diOAc group decreases in the order ***Z*,*E*-26 > 27 > *Z*-26**, i.e., substitution in ring A > B > C (Table I). The same order of receptor affinity was also determined by us[4] with 1,1,2-triphenylbut-1-enes with either one 4- or one 3-OAc group in rings A, B, or C and by Pons et al.[13] in the class of triphenylacrylonitriles. The estrogenic potency of ***Z*,*E*-26**, ***Z*-26**, and **27** evaluated in the immature mouse uterine weight test,[14] however, did

(13) Pons, M.; Michel, F.; Crastes de Paulet, A.; Gilbert, J.; Miquel, J.-F.; Precigoux, G.; Hospital, M.; Ojasoo, I.; Raynaud, J.-P. *J. Steroid Biochem.* **1984**, *20*, 137.
(14) Kranzfelder, G.; Schneider, M.; von Angerer, E.; Schönenberger, H. *J. Cancer Res. Clin. Oncol.* **1980**, *97*, 167.

Table II. Biological Properties of Cyclofenil-Fragment-Containing 1,1,2-Triphenylbut-1-enes[a]



compd	R^1	R^3	RBA, %	estrogenic dose, μg	estrogenic effect	antiestrogens dose, μg	antiestrogens effect	MXT tumor, % T/C tumor	MXT tumor, % T/C uterus
***Z*-30**	4-OAc	3,4-$(OAc)_2$	2.1	1	21	0.5	38[g]	23[h]	125
				10	47	1	41[g]		
				100	64	5	29[g]		
***E*-30**	3,4-$(OAc)_2$	4-OAc	1.4	1	1	0.5	0	44[h]	99
				10	70	1	0		
				100	85	5	9		
***Z*,*E*-31**	3-OAc	3,4-$(OAc)_2$	0.5	1	0	25	39[g]	57[h]	102
	3,4-$(OAc)_2$	3-OAc		10	0	100	61[g]		
				100	29				

[a] See footnotes to Table I.

not correlate with the estradiol receptor affinity as we have already shown with some other 1,1,2-triphenylbut-1-enes.[5]

Compound **27** with a substitution in ring B exerted the highest estrogenic effect in this group that reached the maximum effect of estrone, whereas ***Z*,*E*-26** as well as ***Z*-26** did not increase the uterine weight to the 100% level (Table I). ***Z*-26** was more potent than the mixture of the *E* and *Z* isomers of **26**. Surprisingly, the "partial" estrogens ***Z*-26** and ***Z*,*E*-26** did not show any significant antiuterotrophic properties in the immature mouse test (Table I). Thus the good antitumor effect of these two compounds, evaluated on the transplantable, hormone-dependent MXT mammary carcinoma of the BDF_1-mouse,[5,15,16] cannot be due to antiestrogenic effects (Table I). However, the uterus dry weight determined at the end of therapy, which serves as an indicator of estrogenic side effects on the mature mouse in our assay, was not significantly increased above the control value (Table I).

In a former publication we have shown that estrogens like diethylstilbestrol strongly inhibit the hormone-dependent MXT tumor concomitant with an about twofold increase in uterus weight compared to the control.[5]

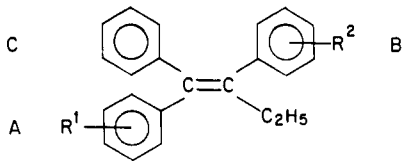
Compounds with Two 3,4-Diacetoxy Groups. The introduction of a second 3,4-$(OAc)_2$ group into the 1,1,2-triphenylbut-1-ene skeleton lowers the estradiol receptor affinity as can be seen by the comparison of the disubstituted compounds ***E*-28** and **29** with the monosubstituted ***Z*,*E*-26** or of ***Z*-28** with ***Z*-26** (Table I). Within this group, the degree of the receptor affinity follows the same order as we have described with 3- or 4-OAc-substituted 1,1,2-triphenylbut-1-enes,[4] i.e., substitution in rings *A*, C ≥ A, B > B, C. The uterotrophic activity in the immature mouse uterine weight assay of **29** with substituents in rings A and C is very low. This result corresponds with the data obtained with 1,1,2-triphenylbut-1-enes, substituted with one 4-OAc and one 3-OAc group in two different aromatic rings.[5] In that publication we have shown that compounds with a 4-OAc and a 3-OAc group in rings A and C have a lower estrogenic potency than their corresponding analogues substituted in A and B. Compound ***E*-28** has also a higher uterotrophic activity than **29**. The estrogenic potencies of ***Z*-28** and ***E*-28** are quite similar though their receptor affinities are very different (Table I). This can be due to a *Z–E* interconversion in vivo, which will be discussed later, or to a metabolic hydroxylation of the para position in ring A similar to the metabolism of tamoxifen.[1,2] An in vivo hydroxylation in ring A can also be the reason for the unusually high uterotrophic activity of compound **27** (Table I).

Due to its low estrogenic properties, compound **29** had a considerable antiuterotrophic activity over a dose range from 1.0 to 100 μg/mouse and day (Table V). The more estrogenic compounds ***Z*-28** and ***E*-28** exerted only an antiuterotrophic potency at low doses. In the MXT tumor assay, ***Z*-28** and ***E*-28** were not able to reduce the tumor weight significantly. The antiestrogen **29** did even stimulate, though not significantly, the tumor growth. Because of this surprising result, compound **29** was also tested in two further mammary tumor assays. In the DMBA-induced hormone-dependent mammary carcinoma,[6,14] **29** was not able to reduce the tumor area in a dosage of 6 times 0.5 and 4.0 mg/kg/week significantly either (data not shown). However, in the hormone-dependent human MCF-7 mammary tumor cell line in vitro,[5] **29** had antitumor activity. In a concentration of 1×10^{-6} M, it reduced the DNA content (% inhibition, 42) as well as the cell number (% inhibition, 47).

Compounds with One 3,4-Diacetoxy and One 3- or 4-Acetoxy Group. Cyclofenil-Fragment-Containing Compounds. The compounds with a cyclofenil fragment (substitution in rings A and C), ***Z*-30**, ***E*-30**, and ***Z*,*E*-31**, exerted affinities to the estrogen receptor that were similar to the affinity of **29** in the case of ***Z*-30** and ***E*-30** and lower with the mixture of ***Z*-** and ***E*-31** (Table II). This is rather surprising as a 4-OAc group in ring A (***Z*-30**) should enhance the affinity.[4,5] It is possible that a 3,4-$(OAc)_2$ group and a further 4-OAc group in ring A and C do not allow the molecule to fit in a proper way to the receptor. The 3-OAc group as in ***Z*,*E*-31** even decreases the RBA value compared to **29** (Table VI). As was expected, the combination of a 3,4-$(OAc)_2$ and a 3-OAc group in rings A and C (***Z*,*E*-31**) led to a compound with very low estrogenic properties, but with the highest antiestrogenic potency of all compounds described in this paper. In spite of its 4-OAc group in ring A and its higher RBA value compared to ***E*-30**, ***Z*-30** had a lower uterotrophic activity than ***E*-30**, and consequently a higher antiestrogenic potency (Table II). In the MXT tumor assay, ***Z*-30**, ***E*-30**, as well as ***Z*,*E*-31** significantly inhibited the tumor growth without a significant influence on the uterus growth. The best tumor-inhibiting effect was shown by ***Z*-30**, which has a good antiestrogenic activity and the best receptor affinity in this group.

(15) Watson, C.; Medina, D.; Clark, J. H. *Cancer Res.* **1977**, *37*, 3344.
(16) Leclercq, G.; Danguy, A.; Devleeschouwer, N.; Heuson, J. C. *Cancer Chemother. Pharmacol. Suppl.* **1982**, *9*, 30.

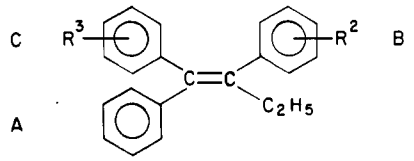
Table III. Biological Properties of *trans*-DES-Fragment-Containing 1,1,2-Triphenylbut-1-enes[a]



compd	R^1	R^2	RBA, %	estrogenic dose, μg	estrogenic effect	antiestrogenic dose, μg	antiestrogenic effect	MXT tumor, % T/C tumor	MXT tumor, % T/C uterus
***E*-32**	3,4-$(OAc)_2$	4-OAc	4.1	1	23	1	0	22[h]	113
				10	73	5	11		
				100	80	25	0		
***E*-33**	3,4-$(OAc)_2$	3-OAc	5.0	1	24	1	0	35[h]	103
				10	74	5	0		
				100	81	25	0		
***E*-34**	4-OAc	3,4-$(OAc)_2$	8.5	1	100	1	0	2[h]	183[h]
				10	93	5	0		
				100	75	25	0		
***E*-35**	3-OAc	3,4-$(OAc)_2$	0.9	10	37	1	31[g]	2[h]	123
				100	71	5	27[g]		
				500	61	25	22[g]		

[a] See footnotes to Table I.

Table IV. Biological Properties of *cis*-DES-Fragment-Containing 1,1,2-Triphenylbut-1-enes[a]



compd	R^2	R^3	RBA, %	estrogenic dose, μg	estrogenic effect	antiestrogenic dose, μg	antiestrogenic effect	MXT tumor, % T/C tumor	MXT tumor, % T/C uterus
***Z*-32**	4-OAc	3,4-$(OAc)_2$	0.4	1	5	1	11	40[h]	113
				10	67	5	0		
				100	81	25	0		
***Z*-33**	3-OAc	3,4-$(OAc)_2$	0.3	1	28	1	19	82	115
				10	53	5	0		
				100	70	25	0		
***Z*-34**	3,4-$(OAc)_2$	4-OAc	2.1	1	60	1	0	1[h]	195[h]
				10	88	5	0		
				100	71	25	0		
***Z*-35**	3,4-$(OAc)_2$	3-OAc	0.3	100	78	1	39[g]	30[h]	87
				500	63	5	53[g]		
						25	35[g]		

[a] See footnotes to Table I.

trans*-DES-Fragment-Containing Compounds.** The 1,1,2-triphenylbut-1-enes of this group are substituted in the phenyl rings A and B (Table III). Compound ***E*-34** with a 4-OAc group in A and a 3,4-$(OAc)_2$ group in B exerted the best estradiol receptor affinity not only in this group but of all compounds in this paper. Its analogue with a 3-OAc substituent in ring A (E*-35**) has a RBA value that is about one-tenth less. The comparison of the 1,1-disubstituted compound ***Z*-30** and its analogue ***E*-34** reveals that the 3,4-$(OAc)_2$ group in ring B is more suitable for good binding to the receptor than the 3,4-$(OAc)_2$ group in ring C (Tables II and III). The RBA values of ***E*-32** and ***E*-33** with a 3,4-$(OAc)_2$ substituent in A and a 3- or 4-OAc group in B are nearly identical and lower than that of ***E*-34**. The difference in the receptor affinity between ***E*-32** (4-OAc in B) and ***E*-33** (3-OAc in B) is not as pronounced as we have found for the 1,1,2-triphenylbut-1-enes with a 4-OAc in A and a 4-OAc group in B (RBA = 5.9), compared to its analogue with a 4-OAc in A and a 3-OAc in B (RBA = 11.1).[4,5]

Corresponding to its high RBA value, compound ***E*-34** has a very high uterotrophic activity that is nearly identical to that of strong estrogens like diethylstilbestrol (Table III).[14] Compound ***E*-35** has only low estrogenic properties, which are due to its 3-OAc group in ring A. This compound is the only one in this group that exerted significant antiestrogenic properties. The uterotrophic activities of ***E*-32** and ***E*-33** are identical and lower than that of ***E*-34**. In this group, a relationship between receptor affinity and estrogenic potency is demonstrable.

All compounds of this group had a strong tumor-inhibiting effect on the hormone-dependent MXT mammary tumor (Table III). The antitumor effect of the strong estrogen ***E*-34** was accompanied by an about twofold increase of the uterine weight compared to the control. The other compounds (***E*-32** and ***E*-33**, and ***E*-35**) did not significantly affect the uterus during therapy. The tumor-inhibiting activity of ***E*-32** and ***E*-33** was similar in the same way as their RBA values and uterotrophic potencies were nearly identical. Whereas the antitumor effect of ***E*-34** is due to its estrogenic activity, the tumor-inhibiting property of ***E*-35**, which was identical to that of ***E*-34**, is due to its antiestrogenic, rather than estrogenic, potency, as this compound has considerable antiuterotrophic but low uterotrophic properties.

***cis*-DES-Fragment-Containing Compounds.** The

compounds of this group are substituted in the phenyl rings B and C (Table IV). Compared to the *trans*-DES-fragment-containing 1,1,2-triphenylbut-1-enes, these compounds generally exerted much lower affinities to the estrogen receptor. In the case of **Z-32** and **Z-33**, the RBA values were only about one-tenth that of their corresponding *E* isomers **E-32** and **E-33**, whereas **Z-34** and **Z-35** exerted about one-fourth or one-third of the affinities of their respective *E* isomers. The best affinity in this class was shown by **Z-34**, and, possibly as a consequence of this, **Z-34** had the highest estrogenic potency in this group. The uterotrophic activities of **Z-32** and **Z-33** were similar and lower than that of **Z-34**. No correlation between the receptor affinity and the uterotrophic potency can be seen by the comparison of **Z-32** and **Z-33** with their corresponding *E* isomers, as the estrogenic potency is quite similar though the RBA values differ about tenfold between the *E* and the *Z* isomers. This may be due to a *Z–E* isomerization in vivo.

In the MXT tumor assay, **Z-34** had the same strong antitumor effect as its respective *E* isomer, accompanied also by high estrogenic side effects (Tables III and IV). **Z-33** was not able to reduce the tumor weight significantly in contrast to **Z-32** and the antiestrogenic compound **Z-35**.

Discussion

Drawing clear structure–activity relationships in this class of compounds is more complex than in our former studies with 1,1,2-triphenylbut-1-enes.[4,5] In the case of compounds with only one or two 3,4-$(OAc)_2$ groups, the estradiol receptor affinity depends on which phenyl ring is substituted in the same way as we have shown before,[4,5] i.e., substitution in A > B > C and A, C ≥ A, B > B, C (Table I). With the 1,1,2-triphenylbut-1-enes containing one 3,4-$(OAc)_2$ and one 3-OAc or 4-OAc group, the situation is more complicated. Here the A,C-disubstituted compounds have lower affinities than their corresponding A,B-substituted analogues. This is due to the bulkiness of the 3,4-$(OAc)_2$ group, and thus the compounds with substituents in A and C cannot fit to the receptor as well as the A,B-substituted ones. The importance of a 4-OAc group in A for a good affinity, as we have shown before,[4,5] is therefore not demonstrable with the A,C-disubstituted compound **Z-30** (Table II), but can clearly be seen with **E-34** (4-OAc in A, 3,4-$(OAc)_2$ in B, Table III). The superiority of the *E* isomers in receptor affinity to their corresponding *Z* isomers is obvious with the compounds of this study (Tables III and IV) and with all other 1,1,2-triphenylbut-1-enes already described.[4,5] A 3,4-$(OAc)_2$ group in ring A or C generally lowers the receptor affinity compared to the compounds of our former studies.[4,5]

The compounds with a low uterotrophic potency in the immature mouse uterine weight test had antiuterotrophic activity (**29**, **Z-30**, **Z,E-31**, **Z-35**, **E-35**). The best antiestrogenic effects were exerted by **29** and **Z,E-31**, i.e., the compounds with an A,C disubstitution (Tables I and II). This mode of substitution is very suitable for an antiuterotrophic potency and for low uterotrophic properties as we have already shown.[5] A further structural element to create estrogen antagonistic properties is a 3-OAc group in ring A (**E-35**) or C (**Z-35**), (Tables III and IV). This is in accordance to our results obtained with 3,3′-diacetoxy- or dihydroxy-α,β-dialkylstilbenes.[14,17] Compounds with a 3,4-diOAc group in A or C have a lower uterotrophic activity than their analogues with a 3,4-$(OAc)_2$ substituent in B (**Z-30** vs. **E-34**, **Z,E-31** vs. **Z-35** or **E-35**, and **E-30** vs. **Z-34**). As shown above, a 3-OAc group in ring A or C leads to antiestrogenic properties and consequently to a low uterotrophic potency (**E-35** vs. **E-34** and **Z-35** vs. **Z-34**).

Compounds **E-34** and **Z-34** strongly inhibited the hormone-dependent MXT tumor together with an about twofold increase of the uterine weight. Thus the antitumor effect of these two compounds is similar to that of strong estrogens like diethylstilbestrol.[5] None of the other compounds of this series increased the uterine weight significantly after the MXT experiment. A comparison of the uterotrophic potency on the immature mouse and on the mature mouse shows that only compounds with a very strong estrogenic effect in the immature mouse assay like **E-34** and **Z-34** also exert estrogenic side effects in the tumor experiment. On the other hand, none of our compounds, as well as tamoxifen (Table I), was able to reduce the uterine weight of the mature mouse significantly.

The best tumor-inhibiting activity without significant estrogenic side effects was exerted by the antiestrogenic compound **E-35**. Its effect was even slightly better than that of tamoxifen (Table I) or of the antiestrogenic 1,1,2-triphenylbut-1-enes recently described by us.[5] **Z-35**, **Z-30**, and **Z,E-31**, all of which have antiestrogenic properties, showed good to reasonable antitumor effects. As they all had only low uterotrophic and good antiuterotrophic activities, their tumor-inhibiting effects may be due to their estrogen antagonistic, rather than agonistic, properties. The stimulation of the tumor growth by the strong antiestrogen **29** and the absence of an antitumor activity of this compound on the DMBA-induced mammary carcinoma of the rat was surprising. Tamoxifen had a good tumor-inhibiting effect on the MXT tumor without significant effect on the uterus (Table I). However, whereas tamoxifen has antiestrogenic activity, but also residual estrogenic properties in the rat,[18,19] it is an almost full agonist in the mouse without any significant antiuterotrophic activity.[20,21] In our assays on the immature mouse, we have determined identical results (Table I). Therefore, the antitumor activity of tamoxifen in the MXT model is probably more due to its agonistic than antagonistic properties.

The mode of action of the mammary tumor inhibiting effect of these compounds can be caused by different mechanisms. The strong estrogens inhibit the MXT tumor concomitant with a significant stimulation of the uterine growth. On the other hand, both partial estrogens without antiestrogenic properties as well as compounds with antiestrogenic and only low estrogenic activity can have an antitumor effect without any influence on the uterus. Whether the mode of action of these antiestrogenic compounds is due to their estrogen antagonistic or agonistic properties is not clear yet. Strong antiestrogens without estrogenic activity, however, like **29** and some other similar compounds tested by us (unpublished results), did not show any antitumor effect in the MXT model. As ovariectomy causes a nearly complete inhibition of tumor growth (% T/C = 2),[5] the lack of antitumor activity of strong estrogen antagonists is difficult to explain. A certain estrogenic potency seems to be necessary for an antitumor effect. From all these results it can be seen that the action

(17) Schneider, M. R.; Schönenberger, H.; Michel, R. T.; Fortmeyer, H. P. *J. Med. Chem.* **1982**, *25*, 141.
(18) Jordan, V. C.; Haldemann, B.; Allen, K. E. *Endocrinology* **1981**, *108*, 1353.
(19) Jones, C. D.; Jevnikar, M. G.; Pike, A. J.; Peter, M. K.; Black, L. J.; Thompson, A. R.; Falcone, J. F.; Clemens, J. A. *J. Med. Chem.* **1984**, *27*, 1057.
(20) Terenius, L. *Acta Endocrinol.* **1971**, *66*, 431.
(21) Black, J. L.; Goode, R. L. *Life Sci.* **1980**, *26*, 1453.

of a compound on the uterus and that on the tumor are not necessarily the same. Therefore, the estrogenic or antiestrogenic activity on the immature mouse can give some insight into the mode of action of a compound, but cannot predict a potency in antitumor activity.

It was shown that tamoxifen and other triphenylethylenes having a basic ether side chain bind to a receptor site different from the estrogen receptor, called antiestrogen receptor binding site.[22,23] A mechanism of action of our compounds through a binding to this site is improbable as triphenylethylenes lacking the basic ether chain, like our compounds, do not bind to this receptor.[22,23] Furthermore, the affinity of antiestrogens to this binding site does not correlate with their antiestrogenic potency.[23] It is not clear yet if this antiestrogen receptor binding site is directly involved in the estrogen antagonism of antiestrogens.[23] Moreover, with the compounds of this study and of our former ones,[4,5] we have shown that triphenylbutenes can have antiestrogenic activity without a basic ether chain.

As already mentioned, a further aspect that can be of importance in the interpretation of structure–activity relationships of *E* and *Z* isomers is the possible isomerization. *cis*- and *trans*-hydroxytamoxifen readily undergo isomeric interconversion at 37 °C as determined in vitro, and therefore show quite similar biological properties in vitro as well as in vivo.[18,24] Concerning the compounds in this study, an isomerization of some compounds in vivo after cleavage of the OAc to an OH group cannot be excluded, as the *E* and *Z* isomers of **28**, **32**, **33**, and **34** have rather similar biological properties in vivo, e.g., in the uterotrophic and MXT tumor assay in spite of their very diffeent RBA values.

In conclusion, it can be stated that the mode of the aromatic substitution in these 1,1,2-triphenylbut-1-enes strongly determines the biological properties. It was possible to obtain compounds ranging from strong estrogens over partial estrogens with or without antiestrogenic properties to strong antiestrogens with almost no agonistic activity. The introduction of the 3,4-diOAc group led to some new mammary tumor inhibiting antiestrogens. Of special interest is compound ***E*-35** with a 3-OAc group in ring A and a 3,4-$(OAc)_2$ moiety in ring B, as it had a strong antitumor activity on the MXT mammary tumor model without estrogenic side effects and as it had antiestrogenic properties on the immature mouse.

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. ^{1}H NMR spectra were obtained with a Varian EM 360 A, 60 MHz, and a EM 390 A, 90 MHz; internal standard, Me_4Si; chemical shifts in δ (ppm). TLC of each compound was accomplished on Merck F 254 silica gel plates. HPLC was performed with an Altex 110 A pump and a Kontron Uvikon 720 LC spectrophotometer, column Lichrosorb Si 60 (5 μm), Merck, West Germany.

Syntheses. Synthetic methods A–E are representatives for compounds reported in Tables I–IV.

Method A. 1-Phenyl-2-(3,4-dimethoxyphenyl)butanone (9). A hot solution of sodium (2.3 g, 0.10 g-atom) in 44 mL of EtOH was added to a mixture of 1-phenyl-2-(3,4-dimethoxyphenyl)ethanone (25.6 g, 0.10 mol) and ethyl iodide (15.6 g, 0.10 mol). The solution was refluxed for 10 min. A further quantity of sodium (1.15 g, 0.05 g-atom) in 22 mL of EtOH and ethyl iodide (7.8 g, 0.05 mol) was added, and the whole was refluxed for 4 h. EtOH was removed under reduced pressure, and water was added. The water layer was extracted with ether. The organic extracts were washed with 0.5 N sodium thiosulfate solution and water and dried over Na_2SO_4. The solvent was removed to give 27.2 g of **7**. The completeness of alkylation was proved by TLC and ^{1}H NMR spectroscopy.

Method B. 1,1-Diphenyl-2-(3,4-dimethoxyphenyl)butan-1-ol. A solution of phenyl-2-(3,4-dimethoxyphenyl)butanone (28.4 g, 0.10 mol) in 60 mL of ether was added dropwise to a solution of phenylmagnesium bromide (54.3 g, 0.30 mol) in 300 mL of ether. The mixture was refluxed for 2 h and then decomposed with ice and 3 N sulfuric acid. The ethereal layer was separated, the water layer extracted with ether, and the organic extracts washed with saturated $NaHCO_3$ solution and water. After the extracts were dried over Na_2SO_4, the solvent was removed to give 1,1-diphenyl-2-(3,4-dimethoxyphenyl)butan-1-ol as crude product. No efforts were made to purify the crude product because spontaneous elimination of water occurred.

Method C. 1,1-Diphenyl-2-(3,4-dimethoxyphenyl)but-1-ene (17). To the crude product of 1,1-diphenyl-2-(3,4-dimethoxyphenyl)butan-1-ol, 30 mL of a mixture of sulfuric acid and acetic acid (8:2 v/v) was added. The whole was heated at 70 °C for 20 min. After the mixture was cooled and water was added, the aqueous layer was extracted with ether. The ethereal extracts were washed with 1 N NaOH and water, dried over Na_2SO_4, and the solvent removed. The crude product was purified by column chromatography on silica gel (CH_2Cl_2 as the eluent). After recrystallization from EtOH, 20.0 g of **17** was obtained.

Method D. 1,1-Bis(3,4-dimethoxyphenyl)-2-phenylbut-1-ene (19). Zinc powder (5.9 g, 0.07 g-atom) was added in portions to a stirred mixture of 3,3',4,4'-tetramethoxybenzophenone (6.7 g, 0.02 mol), propiophenone (3.0 g, 0.02 mol), and $TiCl_4$ (3.8 g, 0.02 mol) in 50 mL of dry dioxane at 0 °C under nitrogen. After warming to room temperature, the whole was refluxed for 5 h. Alkaline hydrolysis was performed with 10% K_2CO_3 solution. After extraction with ether, the ether was removed. The desired product was isolated by column chromatography on silica gel with CH_2Cl_2/ethyl acetate (9:1) as the eluent and crystallized from ethanol to give 4.4 g of **19**.

Method E. 1,1-Diphenyl-2-(3,4-diacetoxyphenyl)but-1-ene (22). A solution of **17** (3.45 g, 0.01 mol) in 250 mL of dry CH_2Cl_2 was cooled to –60 °C. Under nitrogen, BBr_3 (7.52 g, 0.03 mol) was added. After 15 min the freezing mixture was removed, and the reaction mixture was stirred at room temperature for 4 h. Fifty milliliters of MeOH was added with cooling, and the solvents were removed under reduced pressure. Acetic anhydride (4.08 g, 0.04 mol) and pyridine (3.96 g, 0.05 mol) were added. The mixture was refluxed for 1 h. After cooling, 300 mL of ice water was added. The aqueous layer was extracted with ether, and the ethereal extracts were washed with 1 N HCl and saturated $NaHCO_3$ solution and dried over Na_2SO_4. The solvent was removed, and the crude product was crystallized from EtOH to give 3.17 g of **27**.

Biological Methods

Estradiol Receptor Binding Assay. The method described in ref 4 and 14 was used with some modifications. The relative binding affinity (RBA) of the test compounds was determined by the displacement of [^{3}H]estradiol. 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 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. For 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.[5,14] Female mice (body weight, 10–12 g; age, 20 days

(22) Watts, C. K. W.; Murphy, L. C.; Sutherland, R. L. *J. Biol. Chem.* **1984**, *259*, 4223.
(23) Sudo, K.; Monsma, F. J.; Katzenellenbogen, B. S. *Endocrinology* **1983**, *112*, 425.
(24) Katzenellenbogen, B. S.; Norman, M. J.; Eckert, R. L.; Peltz, S. W.; Mangel, W. F. *Cancer Res.* **1984**, *44*, 112.

at test beginning; 10 mice/group) were injected sc daily for three 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 Human MCF-7 Breast Cancer Cells. The applied method was identical with that previously described by us.[5]

Hormone-Dependent, Transplantable MXT Mammary Tumor of the BDF_1 Mouse.[15,16] The applied method was identical with that previously described by us.[5] 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-weeks-old BDF_1 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 14 to serve as an indicator of the estrogenic side effects of the compounds.

Acknowledgment. We thank Dr. H. Schönenberger for helpful discussions, Dr. A. E. Bogden for providing us with the MXT tumor, M. Beer, C. Niel, K. Röhrl, and G. Tondl for skillful technical assistance and the Deutsche Krebshilfe e.V., Dr. Mildred Scheel-Stiftung, and the Deutsche Forschungsgemeinschaft (SFB 234) for financial support.

Registry No. 1, 3141-93-3; **2**, 29955-23-5; **3**, 4927-55-3; **4**, 4927-54-2; **5**, 84675-71-8; **6**, 4927-53-1; **7**, 35989-78-7; **8**, 102520-52-5; **9**, 102520-53-6; **10**, 84675-72-9; **11**, 84675-73-0; **12**, 84675-74-1; **13**, 102520-54-7; **14**, 102520-55-8; **15**, 4131-03-7; (*Z*)-**16**, 102520-56-9; (*E*)-**16**, 102520-57-0; **17**, 102520-58-1; (*Z*)-**18**, 102520-59-2; (*E*)-**18**, 102520-60-5; **19**, 102520-61-6; (*Z*)-**20**, 102520-62-7; (*E*)-**20**, 102520-63-8; (*Z*)-**21**, 102520-64-9; (*E*)-**21**, 102520-65-0; (*Z*)-**22**, 102520-66-1; (*E*)-**22**, 102520-67-2; (*Z*)-**23**, 102520-68-3; (*E*)-**23**, 102520-69-4; (*Z*)-**24**, 102520-70-7; (*E*)-**24**, 102520-71-8; (*Z*)-**25**, 102520-72-9; (*E*)-**25**, 102520-73-0; (*Z*)-**26**, 102520-74-1; (*E*)-**26**, 102520-75-2; **27**, 102520-76-3; (*Z*)-**28**, 102520-77-4; (*E*)-**28**, 102520-78-5; **29**, 102520-79-6; (*Z*)-**30**, 102520-80-9; (*E*)-**30**, 102520-81-0; (*Z*)-**31**, 102520-82-1; (*E*)-**31**, 102520-83-2; (*Z*)-**32**, 102520-84-3; (*E*)-**32**, 102520-85-4; (*Z*)-**33**, 102520-86-5; (*E*)-**33**, 102520-87-6; (*Z*)-**34**, 102520-88-7; (*E*)-**34**, 102520-89-8; (*Z*)-**35**, 102520-90-1; (*E*)-**35**, 102520-91-2; 1,1-diphenyl-2-(3,4-dimethoxyphenyl)butan-1-ol, 102520-92-3; bromobenzene, 108-86-1; 4-bromo-1,2-dimethoxybenzene, 2859-78-1; 1-bromo-4-methoxybenzene, 104-92-7; propiophenone, 93-55-0.

Supplementary Material Available: Tables V–XI giving analytical data of compounds **1–35** (10 pages). Ordering information is given on any current masthead page.

Aromatase Inhibitors. Synthesis and Evaluation of Mammary Tumor Inhibiting Activity of 3-Alkylated 3-(4-Aminophenyl)piperidine-2,6-diones

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The synthesis and biological evaluation of 3-alkyl-substituted 3-(4-aminophenyl)piperidine-2,6-diones as inhibitors of estrogen biosynthesis are described [H (1), methyl (2), ethyl (3), *n*-propyl (4), isopropyl (5), *n*-butyl (6), isobutyl (7), *sec*-butyl (8), *n*-pentyl (9), isopentyl (10), 2-methylbutyl (11), *sec*-pentyl (12), *n*-hexyl (13), *n*-heptyl (14)]. In vitro compounds 4–14 showed a stronger inhibition of human placental aromatase compared to aminoglutethimide (AG, compound 3), which recently has become used for the treatment of hormone-dependent breast cancer. The most active derivative, compound 10, showed a 93-fold stronger inhibition than AG. With the exception of 5, 7, and 8, all other compounds exhibited similar or decreased inhibition of bovine adrenal desmolase compared to AG. Compounds 4 and 6–12 showed a stronger inhibition of the plasma estradiol concentration of pregnant mare serum gonadotropin (PMSG) primed Sprague–Dawley (SD) rats compared to the parent compound. Compounds 4, 6–8, 10, and 12 inhibited the testosterone-stimulated tumor growth of ovariectomized 9,10-dimethyl-1,2-benzanthracene (DMBA) tumor-bearing SD rats more strongly than AG. Being stronger and more selective inhibitors of the estrogen biosynthesis than AG, some of the newly developed derivatives of AG might be better candidates for the treatment of the hormone-dependent human breast cancer.

Recently the use of inhibitors of estrogen biosynthesis has become a new strategy in the treatment of metastatic hormone-dependent breast cancer.[1] As aromatization is the last reaction in the biosynthesis of estrogens, compounds interacting exclusively with the aromatizing enzyme might be very specific drugs. The only nonsteroidal compound clinically used so far, aminoglutethimide (AG, compound 3, 3-(4-aminophenyl)-3-ethylpiperidine-2,6-dione) has shown benefit in many trials with postmenopausal and ovariectomized premenopausal women.[1-5] AG exerts its mammary tumor inhibitory activity principally by reducing the extraglandular peripheral aromatization of the adrenal androgens androstenedione and testosterone into estrone and estradiol (E_2), respectively,[1,6] and patients treated with AG exhibit response rates comparable to

(1) For reviews see: Harvey, H. A.; Lipton, A.; Santen, R. J. *Cancer Res. (Suppl.)* **1982**, *42*, 3261s–3469s.
(2) Santen, R. J.; Samojlik, E.; Worgul, T. J. In *A Comprehensive Guide to the Therapeutic Use of Aminoglutethimide*; Santen, R. J., Henderson, I. C., Eds.; Karger: Basel, München, Paris, London, New York, Sydney, 1982; p 101.
(3) Harris, A. L.; Powles, T. J.; Smith, I. E.; Coombes, R. C.; Ford, H. T.; Gazet, J. C.; Harmer, C. L.; Morgan, M.; White, H.; Parsons, C. A.; McKinna, J. A. *Eur. J. Cancer Clin. Oncol.* **1983**, *19*, 11.
(4) Henderson, I. C.; Canellos, G. P. *N. Engl. J. Med.* **1980**, *302*, 17, 78.
(5) Santen, R. J.; Worgul, T. J.; Samojlik, E.; Interrante, A.; Boucher, A. E.; Lipton, A.; Harvey, H. A.; White, D. S.; Smart, E.; Cox, C.; Wells, S. A. *N. Engl. J. Med.* **1981**, *305*, 545.
(6) Dowsett, M.; Goss, P.; Coombes, R. C.; Brodie, A. M. H.; Hill, M.; Hutchinson, G.; Jeffcoate, S. L. *J. Endocrinol. (Suppl.)* **1985**, *104*, 28.

0022-2623/86/1829-1362$01.50/0