and Mg-ATP were included at the levels specified below. Reactions were started by addition of 10 μL of working enzyme solution and terminated by addition of 10 μL of 4 N $HClO_4$–10 mM-L-methionine with cooling in an ice bath. Each suspension was centrifuged, and 50 μL of supernatent was applied to a 2.3-cm disk of phosphocellulose paper. Disks were washed as described[23] and then immersed in a toluene solution of phosphors and counted in a Packard liquid scintillation spectrometer (Model 2425). Blanks were provided by incubations carried out in the absence of ATP. Reaction velocities were linear for at least 30 min and were proportional to the amount of enzyme added at the levels of enzyme activity employed.

Substrate constants were determined from double-reciprocal plots of velocity vs. substrate concentration; all plots were linear. K_M values reported for methionine were determined at 5 mM ATP for MAT-I (18.8–112.8 μM [^{14}C]methionine) and 2 mM ATP for MAT II (2.1–21 μM [^{14}C]methionine) and MAT-T (10.5–63 μM [^{14}C]methionine). Substrate constants for ATP as well as substrate and inhibition constants for ATP analogues were determined in the presence of 380 μM [^{14}C]methionine for MAT-I and 60 μM [^{14}C]methionine for MAT-II and MAT-T. These methionine levels were noninhibitory. All ATP analogues were tested for substrate activity at a level of 1 mM, using a higher level of MAT (10 μL of stock solution) per assay than in the remaining studies.

Inhibition studies were made with six to eight levels of Mg-ATP in the range 0.5–4.0 × K_M of Mg-ATP for each of two inhibitor levels that were in the range 1–5 × K_i and for control mixtures lacking inhibitor. Inhibitors were tested as their 1:1 complexes formed by admixture of stock solutions with equimolar amounts of $MgCl_2$. Inhibition constants (K_i values) were obtained from replots of inhibitor concentrations vs. slopes of double-reciprocal plots of velocity vs. substrate level. All of the latter plots were linear.

Acknowledgment. This work was supported by Public Health Service Research Grant CA-11196 from the National Cancer Institute, DHHS, and by grants to the Institute for Cancer Research (USPHS Grants CA-06927 and RR-05539 and an appropriation from the Commonwealth of Pennsylvania). We are indebted to Dr. Sidney Weinhouse for providing rat Novikoff hepatoma cells, to Drs. M. C. Liau and J. B. Lombardini for helpful discussions, to Dr. J. L. Hoffman for communicating, prior to publication, the procedure used to obtain MAT-I, and to Dr. Manfred Bayer for assistance with the isolation of MAT-T.

Influence of Alkyl-Chain Fluorination on the Action of Mammary Tumor Inhibiting 2,3-Bis(hydroxyphenyl)butanes and 2,3-Bis(hydroxyphenyl)but-2-enes

Rolf W. Hartmann, Alexander Heindl, Martin R. Schneider, and Helmut Schönenberger*

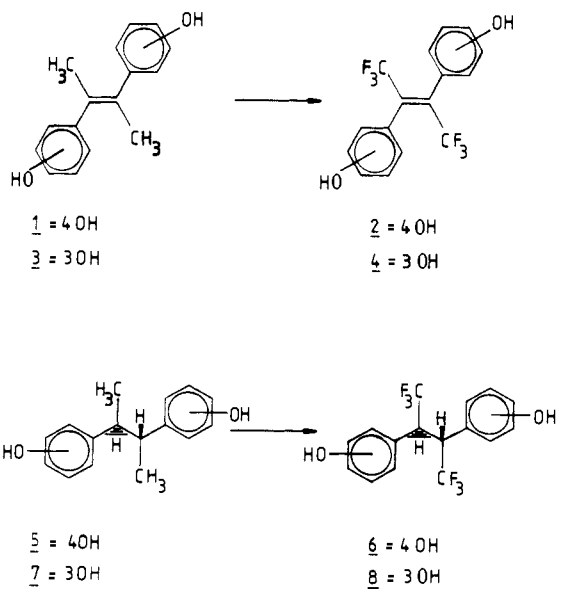
Institute of Pharmacy, Lehrstuhl Pharmazeutische Chemie II, University of Regensburg, Universitätsstrasse 31, 8400 Regensburg, Federal Republic of Germany. *Received February 20, 1985*

trans-1,2-Bis(trifluoromethyl)-1,2-bis(4- and 3-hydroxyphenyl)ethenes **2** and **4** were prepared by reductive coupling ($TiCl_4$/Zn/pyridine) of the methoxy-substituted α,α,α-trifluoroacetophenones, separation of the resulting *cis*- and *trans*-stilbene derivatives, and ether cleavage with BBr_3. The *cis*-stilbenes were catalytically hydrogenated to give *meso*-1,1,1,4,4,4-hexafluoro-2,3-bis(4- and 3-hydroxyphenyl)butanes **6** and **8**. Compounds **2**, **4**, **6**, and **8** showed 2- to 10-fold increased binding affinities for the estradiol receptor (E_2R) and enhanced estrogenicity in the uterine weight test of the immature mouse compared to their unfluorinated analogues. Compound **8** exhibited a 46% inhibition of the estrone-stimulated uterine growth. Antitumor activity was evaluated with use of the transplantable, hormone-dependent MXT mammary tumor of the $BD2F_1$ mouse. All compounds showed tumor growth inhibitory activity corresponding to their RBA values. The most interesting compound **8** led to a significant inhibition of the tumor growth on the DMBA-induced hormone-dependent mammary carcinoma of the Sprague–Dawley rat.

Searching for new mammary tumor inhibiting antiestrogens, we recently found that modification of the synthetic estrogens hexestrol and diethylstilbestrol by displacement of the phenolic hydroxy groups into the 3,3′-positions provided compounds with strongly decreased estrogenicity, marked antiestrogenicity, and strong inhibitory activity on the established 9,10-dimethyl-1,2-benzanthracene (DMBA) induced mammary carcinoma of the Sprague–Dawley (SD) rat.[1-3] Replacement of the ethyl groups by methyl substituents resulted in two compounds totally lacking estrogenic activity (compounds **3** and **7**, Chart I).[1,3,4] Shortening of the alkyl chain, however, led to a reduction of the estradiol receptor (E_2R) affinity[1,3,4] and thus to a decrease of mammary tumor inhibiting activity.[1,3]

In a structure–activity study dealing with the influence of an aromatical disubstitution of 1,2-diphenylethane es-

Chart I

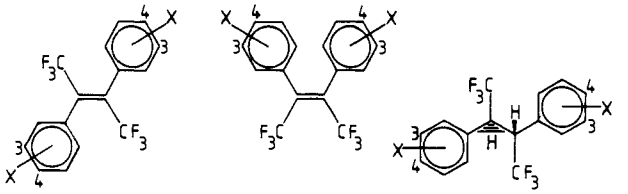


trogens and antiestrogens on their biological properties, we have shown that it is possible to raise the binding affinity and the antitumor activity of 1,2-diphenylethanes by placing substituents ortho to the ethane bridge.[4-7] All

(1) Hartmann, R. W.; Buchborn, H.; Kranzfelder, G.; Schönenberger, H.; Bogden, A. E. *J. Med. Chem.* **1981**, *24*, 1192.
(2) Kranzfelder, G.; Hartmann, R. W.; von Angerer, E.; Schönenberger, H.; Bogden, A. E. *J. Cancer Res. Clin. Oncol.* **1982**, *103*, 165.
(3) Kranzfelder, G.; Schneider, M.; von Angerer, E.; Schönenberger, H. *J. Cancer Res. Clin. Oncol.* **1980**, *97*, 167.
(4) Hartmann, R. W.; Heindl, A.; Schwarz, W.; Schönenberger, H. *J. Med. Chem.* **1984**, *27*, 819.

0022-2623/86/1829-0322$01.50/0

Table I. 1,1,1,4,4,4-Hexafluoro-2,3-diphenylbutanes and -but-2-enes

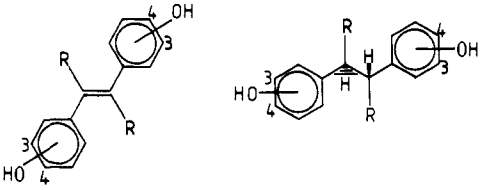


2a, 2, 4a, 4 6b, 8b 6a, 6, 8a, 8

compd	X	synth method[a]	yield,[b] %	mp, °C	formula[c]
2a[d]	4-OCH_3	B	17	174	$C_{18}H_{14}F_6O_2$
2[d]	4-OH	D	84	254	$C_{16}H_{10}F_6O_2$
4a	3-OCH_3	B	22	94	$C_{18}H_{14}F_6O_2$
4	3-OH	D	76	216	$C_{16}H_{10}F_6O_2$
6b	4-OCH_3	B	23	105	$C_{18}H_{14}F_6O_2$
6a	4-OCH_3	C	84	167	$C_{18}H_{16}F_6O_2$
6	4-OH	D	72	258	$C_{16}H_{12}F_6O_2$
8b	3-OCH_3	B	22	44	$C_{18}H_{14}F_6O_2$
8a	3-OCH_3	C	87	106	$C_{18}H_{16}F_6O_2$
8	3-OH	D	81	233	$C_{16}H_{12}F_6O_2$

[a] Capital letters refer to synthetic methods B–D in the Experimental Section. [b] Yield of analytically pure product; no effort was made to optimize yields. [c] All compounds were analyzed for C and H within ±0.40% of the calculated values. [d] See ref 16 and 17.

Table II. Relative Binding Affinity (RBA) of Compounds 1–8 for the Calf Uterine Estrogen Receptor



1–4 5–8

compd		R	RBA value[a]	compd		R	RBA value[a]
1[b]	4-OH	CH_3	14.5	5[e]	4-OH	CH_3	12
2[c]	4-OH	CF_3	30	6	4-OH	CF_3	50
DES	4-OH	C_2H_5	50	HES[f]	4-OH	C_2H_5	27
3[d]	3-OH	CH_3	0.7	7[e]	3-OH	CH_3	1
4	3-OH	CF_3	7	8	3-OH	CF_3	8
MetaDES	3-OH	C_2H_5	7	MetaHES[g]	3-OH	C_2H_5	10

[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. [b] Prepared according to ref 18. [c] See footnote 20. [d] Prepared according to ref 14. [e] Prepared according to ref 4. [f] RBA value taken from ref 5. [g] RBA value taken from ref 6.

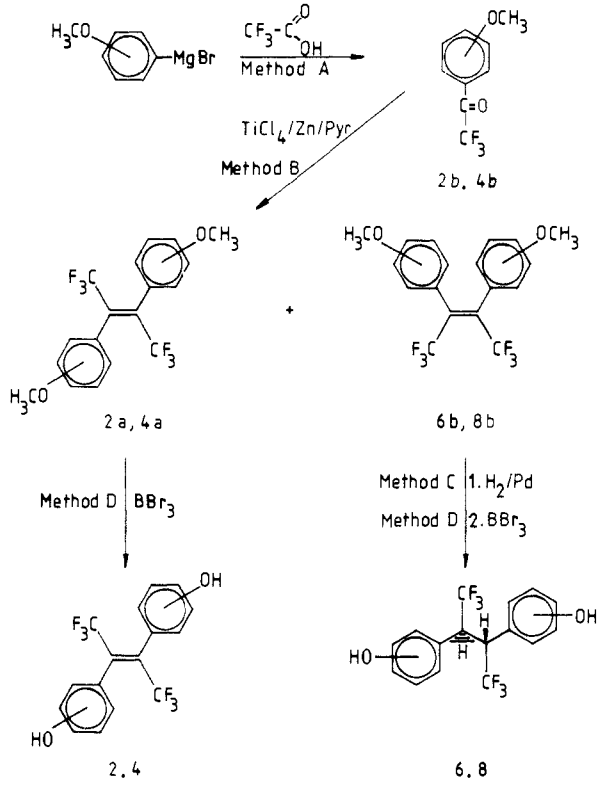
compounds with enhanced binding affinity, however, showed increased estrogenic activity. In the case of the nonestrogenic compound **7**, for example, symmetrical introduction of Cl and CH_3 substituents in the 6- and 6′-positions enhanced the binding affinity by 70% and 420%, respectively, but also gave rise to a weak (CH_3) or moderate (Cl) uterotrophic activity.[4]

In an attempt to enhance the antitumor activity of compounds **3** and **7**, we decided to modify the alkyl chain by synthesizing their 1,1,1,4,4,4-hexafluoro derivatives (compounds **4** and **8**, Chart I). This structural modification would raise the lipophilicity in the center of the molecule strongly, without markedly changing steric properties, as is shown by the corresponding lipophilic parameters and the van der Waals radii: $\pi_{CF_3} = 0.88$, $\pi_{CH_3} = 0.56$, $R_F = 1.35$ Å, $R_H = 1.20$ Å.[8] Fluorination was also performed with the analogous para hydroxy compounds **1** and **5**—compounds that have been shown to be weak estrogens with antiestrogenic activity[4,9,10]—to demonstrate more generally the influence of alkyl-chain fluorination on the biological properties of this class of compounds.

Chemistry. The synthesis of the hexafluorinated compounds **2**, **4**, **6**, and **8** (Table I) started from (3- or 4-methoxyphenyl)magnesium bromide (Chart II). The α,α,α-trifluoroacetophenones **2b** and **4b** (method A)[11] were coupled reductively with $TiCl_4$/Zn and pyridine[12,13] to give a mixture of the *cis*- and *trans*-stilbene derivatives **2a**, **6b** and **4a**, **8b**, respectively (method B, Table I). The isomers were separated by column chromatography. Cis and trans configuration was assigned by ^{1}H NMR spectroscopy. In the case of the trans isomers the signals of the aromatic and OCH_3 protons are shifted downfield compared to the cis isomers as it is described for the corresponding unfluorinated compounds and 3,4-bis(methoxyphenyl)hex-3-enes.[3,5,14,15]

(5) Hartmann, R. W.; Schwarz, W.; Schönenberger, H. *J. Med. Chem.* **1983**, *26*, 1137.
(6) Hartmann, R. W.; Heindl, A.; Schönenberger, H. *J. Med. Chem.* **1984**, *27*, 577.
(7) Hartmann, R. W.; Schwarz, W.; Heindl, A.; Schönenberger, H. *J. Med. Chem.* **1985**, *28*, 1295.
(8) Seydel, J. K.; Schaper, K.-J. "Chemische Struktur und biologische Aktivität von Wirkstoffen"; Verlag Chemie: Weinheim, 1979.
(9) Terenius, L. *Acta Endocrinol.* **1971**, *66*, 431.
(10) Collins, D. J.; Hobbs, J. J. *Aust. J. Chem.* **1967**, *20*, 1413.
(11) Dishart, K. T.; Levine, R. *J. Am. Chem. Soc.* **1956**, *78*, 2268.
(12) Mukaiyama, T.; Sato, T.; Hanna, J. *Chem. Lett.* **1973**, 1041.
(13) Lenoir, D. *Synthesis* **1977**, *8*, 553.

Chart II



The trans isomers **2a** and **4a** were treated with BBr_3 to give compounds **2** and **4** (method D, Table I). The cis isomers **6b** and **8b** were catalytically hydrogenated with use of palladium on carbon to provide the meso compounds **6a** and **8a** (method C, Table I). Subsequent ether cleavage to yield compounds **6** and **8** was also accomplished with BBr_3 (method D, Table I). Compound **2** was prepared previously by reaction of (4-methoxyphenyl)lithium with perfluorobut-2-ene and subsequent ether cleavage with HI.[16,17]

Biological Properties. The compounds were tested for their relative binding affinity (RBA) for the E_2R by a competitive binding assay with calf uterine cytosol, 17β-[^{3}H]estradiol, and the Dextran-coated charcoal technique.[19] Table II shows that alkyl-chain fluorination of the 2,3-bis(hydroxyphenyl)but-2-enes and -butanes raised the RBA values by factors of 2–10.

The binding affinities of compounds **2**, **4**, **6**, and **8** are in the range of the corresponding 3,4-bis(4- and 3-hydroxyphenyl)hex-3-enes (DES and MetaDES, Table II) and -hexanes (HES and MetaHES, Table II). Compounds **2** and **8** showed a slightly decreased RBA value compared to that of DES and MetaHES, whereas compound **6** showed an E_2R binding affinity that was even increased compared to that of HES.

Table III. Estrogenic Activity of Compounds 1–8 in the Mouse Uterine Weight Test

	uterotrophic test	
compd	dose,[a] μg	effect,[b] means ± SD
1	0	16.7 ± 0.9
	1	20.6 ± 3.0
	10	25.4 ± 1.4
	100	35.9 ± 2.7
	1000	41.8 ± 5.4
estrone	0.4	46.4 ± 3.7
2	0	13.7 ± 2.9
	0.1	56.4 ± 5.0
	1	52.6 ± 3.4
	10	38.2 ± 6.7
estrone	0.4	46.6 ± 4.5
3[d]	0	13.3 ± 2.0
	10	12.2 ± 1.7
	100	13.8 ± 2.7
	1000	12.8 ± 1.4
estrone	0.213	39.8 ± 2.3
4	0	11.1 ± 1.8
	0.1	15.7 ± 3.0
	1	25.3 ± 2.7
	10	29.0 ± 3.1
	100	45.5 ± 3.3
	1000	51.8 ± 4.2
estrone	0.4	47.3 ± 2.7
5[e]	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
6	0	10.8 ± 1.2
	0.1	46.9 ± 2.6
	1	42.5 ± 6.2
	10	35.4 ± 2.9
estrone	0.4	46.8 ± 3.4
7[e]	0	11.9 ± 2.1
	1	11.7 ± 2.0
	10	12.4 ± 1.7
	100	12.6 ± 2.3
	1000	13.1 ± 1.0
estrone	0.4	45.6 ± 4.9
8	0	10.8 ± 1.2
	0.1	10.1 ± 2.2
	1	15.4 ± 2.4
	10	19.8 ± 2.1
	100	34.8 ± 3.1
	1000	36.0 ± 2.4
estrone	0.4	46.8 ± 3.4

[a] Dose per animal and day. [b] Uterus dry weight (milligrams)/body weight (grams) × 100. [c] Applied as a suspension. [d] Data taken from ref 3. [e] Data taken from ref 4.

As it has already been described,[9] the binding affinity of DMS (1) is higher than the one of butestrol (**5**) (Table II). This is in accordance with our finding that DES shows a stronger binding affinity than HES. In case of the analogous hexafluorinated compounds, however, the opposite result was obtained (Table II). In contrast to the para hydroxy compounds, both metabutestrol (**7**) and its fluorinated analogue (**8**) exhibited higher binding affinities than the corresponding stilbene derivatives (**3** and **4**).

All compounds were tested for their uterotrophic activity in the immature mouse as a measure of their estrogenicity (Table III). Except for strong estrogens (compounds **2** and **6**), all other compounds were also tested for their antiestrogenicity by inhibition of the uterine growth stimulated by estrone (Table IV).

DMS and butestrol acted like partial agonists as it has been described.[9] Increasing doses led to very gradual increases of the uterine weight reaching the maximum effect of "true" estrogens in high doses. In the antiuterotrophic

(14) Schneider, M.; von Angerer, E.; Kranzfelder, G.; Schönenberger, H. *Arch. Pharm. (Weinheim, Ger.)* **1980**, *313*, 919.
(15) Compared to the signals of the unfluorinated compounds, the signals of the corresponding hexafluorinated derivatives are shifted downfield; in the case of the OCH_3 singlet for ca. 0.1 ppm.
(16) Middleton, W. J. Ger. Offen 1950933, 1970; *Chem. Abstr.* **1970**, *73*, P14425m.
(17) Middleton, W. J. U.S. Patent 3678117, 1972; *Chem. Abstr.* **1972**, *77*, 101125q.
(18) Dodds, E. C.; Goldberg, L.; Grunfeld, E. J.; Lawson, W.; Saffer, C. M.; Robinson, R. *Proc. R. Soc. London, Ser. B* **1944**, *132*, 83.
(19) Hartmann, R. W. *Cancer Treat. Rev. (Suppl. A)* **1984**, *11*, 155.

Table IV. Antiestrogenic Activity of Compounds 1, 3–5, 7, and 8 in the Mouse Uterine Weight Test

		antiuterotrophic test	
compd	dose,[a] μg	effect,[b] means ± SD	% inhibn[c,d]
1	0	14.8 ± 2.3	
	5	39.2 ± 2.8	
	25	32.0 ± 2.8	30.1[e]
	100	39.8 ± 2.8	
	500	48.7 ± 4.4	
estrone	0.1	39.5 ± 1.6	
3[g]	0	12.9 ± 2.1	
	5	37.2 ± 3.1	
	25	42.3 ± 1.3	
	100	43.5 ± 2.6	
	500	33.1 ± 6.0	27.2[e]
estrone	0.1	39.3 ± 3.6	
4	0	14.8 ± 2.3	
	5	43.0 ± 3.2	
	25	47.4 ± 4.8	
	100	47.8 ± 3.1	
	500	52.6 ± 5.2	
estrone	0.1	39.5 ± 1.6	
5	0	14.8 ± 2.3	
	5	33.6 ± 3.5	23.8[e]
	25	36.0 ± 2.2	14.1[f]
	100	45.1 ± 4.1	
	500	56.5 ± 2.7	
estrone	0.1	39.5 ± 1.6	
7[h]	0	11.2 ± 1.5	
	0.1	38.5 ± 2.6	
estrone	0.1	37.5 ± 4.0	
7	0	10.5 ± 1.6	
	5	36.5 ± 5.1	
estrone	0.1	38.4 ± 5.1	
7	0	9.9 ± 1.5	
	50	34.3 ± 2.2	12[f]
	500	29.0 ± 3.5	31[e]
estrone	0.1	37.7 ± 5.5	
8	0	13.3 ± 2.6	
	1	31.4 ± 3.7	36.6[e]
	10	32.0 ± 2.6	34.5[e]
	25	28.8 ± 4.1	45.5[e]
	100	36.7 ± 4.0	18.0[f]
estrone	0.1	41.8 ± 4.3	

[a] Dose per animal and day. [b] Uterus dry weight (milligrams)/body weight (grams) × 100. [c] Percent inhibition = 100 − ($E_{S,T}$ − E_V)/(E_S − E_V) × 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). [g] Data taken from ref 3. [h] Data taken from ref 1.

assay the two compounds exhibited weak antiestrogenic activity. Hexafluorination of both compounds resulted in two strong estrogens (**2** and **6**). At doses of 0.1 μg/animal full uterotrophic responses—comparable to those of DES and HES—were obtained.

The two nonfluorinated meta compounds **3** and **7** showed no estrogenic activity in a dose range of 1–1000 μg and weak antiestrogenic activity. Fluorination of **3** and **7** led to compounds with uterotrophic activity. The stilbene derivative **4** produced a full uterotrophic response at the 100-μg dose whereas compound **8** was less uterotrophic: only 70% of the maximum estrone effect were reached in the 1000-μg dose. A very similar dose–response curve has been obtained from the corresponding ethyl derivative, metahexestrol.[1] In the antiuterotrophic assay compound **4** did not exhibit any antiestrogenic activity in contrast to compound **8**, which—similar to metahexestrol[1]—inhibited the uterine growth already at a dosage of 1 μg by 37% and showed a maximum effect of 46% inhibition.

The evaluation of mammary tumor inhibiting activity of the test compounds was performed with use of the transplantable, hormone-dependent MXT mammary tumor of the $BD2F_1$ mouse. The results in term of tumor weights are shown on Table V. Estrogenic effects were monitored by the determination of the uterine weights.

The fluorinated compounds inhibited the tumor growth more strongly than the parent compounds, the para hydroxy derivatives **2** and **6** being more effective than the meta OH compounds **4** and **8**. The uterine weight stimulating activity of the former reveal them acting as "true" estrogens, which was in accord with the estrogen assay on the immature mouse (Table III).

The fluorinated meta compounds (**4** and **8**) showed no difference in the tumor test. They were highly active compounds with T/C values similar to that of tamoxifen (TAM). Although the stilbene derivative **4** had shown a stronger estrogenic activity in the immature mouse than compound **8**, both compounds showed no difference in the uterine weight assay of the adult tumor-bearing $BD2F_1$ mouse, the values being not significantly different to that of the control group.

The tumor growth inhibiting effects of the nonfluorinated meta compounds **3** and **7** were only weak, even in the high dosages. It is striking that in both experiments the tumor-inhibiting activity of a certain compound corresponds to its RBA value (experiment 1: antitumor activity, 5 = 1 > 3 ≥ 7; RBA, 5 = 1 > 7 ≥ 3; experiment 2: antitumor activity, 6 = 2 > 8 = 4; RBA, 6 > 2 > 8 = 4).

The most interesting compound with regard to antiestrogenic and mammary tumor inhibiting activity on the MXT tumor—compound **8**—was further tested on the DMBA-induced mammary carcinoma of the SD rat. A dose of 2 mg/kg per day of compound **8** resulted in nearly the same effect compared to compound **7** at a dose of 18 mg/kg per day (Table VI). Increasing the dose of compound **8** to 5 mg/kg per day did not improve the antitumor effect.

Discussion

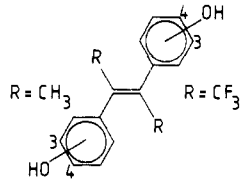
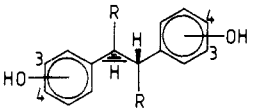
The present paper shows that the replacement of the CH_3 groups by CF_3 substituents results in increased affinity of 2,3-bis(hydroxyphenyl)butanes and -but-2-enes for the E_2R. The RBA values of the fluorinated compounds are in the range of the corresponding 3,4-bis(hydroxyphenyl)hexanes and -hex-3-enes, respectively, suggesting that the CF_3 group might replace the Et group in the class of the 3,4-diphenylhexanes. The increase of binding affinity for the E_2R produced by alkyl-chain fluorination might be due to the enhanced lipophilicity (π_{CH_3} = 0.56, π_{CF_3} = 0.88, $\pi_{C_2H_5}$ = 1.02[8]). The E_2R binding affinity of compound **6** exceeds that of hexestrol; therefore, electronic effects in addition to lipophilicity may be important determinants of binding for this compound.

As could be expected from a previous study,[1] the increase of the RBA values in case of the alkyl-chain fluorination also led to a marked increase of antitumor activity. On the MXT tumor the antitumor activity of the test compounds correlated with their relative binding affinities for the E_2R. An increase of the E_2R affinity of 2,3-bis(3- or 4-hydroxyphenyl)butanes has also been achieved by the introduction of F, Cl, or CH_3 substituents ortho to the ethane bridge.[4] 2,3-Bis(3-hydroxy-6-methylphenyl)butane showed also a strongly enhanced antitumor activity.[4]

It is striking that in the uterotrophic test all fluorinated 1,2-diphenylbutanes and -but-2-enes showed similar estrogenic activities compared to those of the corresponding hexanes and hex-3-enes. This might be a further indication that concerning biological activity the CF_3 group can fully represent the Et group in the class of 3,4-diphenylhexanes.

The phenomenon, however, that increases of E_2R binding affinity and mammary tumor inhibiting activity

Table V. Effects of Compounds 1–8 on Tumor and Uterine Weights of $BD2F_1$ Mice Bearing Transplantable, Hormone-Dependent MXT Tumors

compd	OH	dose,[a] mg	% T/C[b]	uterus effect,[c,f] mean ± SD	compd	OH	dose,[a] mg	% T/C[b]	uterus effect,[c] mean ± SD
									
control			100.0	89.3 ± 19.8	control			100.0	83.4 ± 13.3
TAM[d]		8.00	11.5	69.1 ± 10.0[h]	TAM[d]		8.00	3.5	65.5 ± 13.9[h]
1	4	2.76	69.5	74.7 ± 17.8[h]	2	4	1.33	10.4	182.2 ± 27.2[g]
		13.80	46.2	70.4 ± 15.7[h]			4.00	5.5	179.0 ± 42.4[g]
							8.00	5.2	168.8 ± 18.3[g]
3	3	2.76	88.1	80.7 ± 25.1[h]	4	3	4.00	25.7	74.8 ± 15.2[h]
		13.80	69.9	88.4 ± 16.7[h]			20.00	11.5	82.1 ± 13.0[h]
									
5	4	2.77	68.5	79.9 ± 19.1[h]	6	4	0.80	21.3	135.9 ± 20.0[g]
		13.84[e]	23.1	77.6 ± 11.9[h]			4.00	7.2	188.5 ± 25.7[g]
7	3	2.77	106.0	86.9 ± 17.9[h]	8	3	4.00	23.9	84.0 ± 16.8[h]
		13.84	66.5	73.5 ± 29.7[h]			20.00	10.9	90.1 ± 16.0[h]

[a] Dose per kilogram and application (three times weekly). [b] % T/C = (average tumor wet weight of the treatment group/average tumor wet weight of the control group) × 100. [c] [Uterus dry weight (milligrams)/body weight (grams)] × 100. [d] TAM = Tamoxifen. [e] Applied as a suspension. [f] The *U* test according to Wilcoxon, Mann, and Whitney was used. [g] Significant (α = 0.01). [h] Not significant (α = 0.01).

Table VI. Effect of Compounds 7 and 8 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		17	42	0	2	20	78	4.1	610
7[l]	18	21	16	14	11	24	51	2.4	188[k]
	36	22	12	29	9	38	24	1.6	105[k]
	72	26	22	34	25	16	25	0.9	6[k]
control		22	31	8	8	22	62	2.5	501
8	2	21	19	15	10	28	47	−1.1	165[k]
	5	23	11	21	12	29	38	−0.4	166[k]

[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 (α = 0.05). [l] Data of compound 7 (taken from ref 1) are given for comparative purposes.

were accompanied by enhanced estrogenicity has been noted in the class of the ring-substituted 2,3-bis(hydroxyphenyl)butanes,[4] and this observation gives rise to the question of whether it is possible to enhance the antitumor activity without increasing estrogenic activity. It is striking that there is no potent mammary tumor inhibiting antiestrogen exhibiting little or no estrogenic activity. All strongly active compounds (e.g. tamoxifen and metahexestrol[1,2,6,21]) show at least slight estrogenicity. On the other hand, strong antiestrogens with no or only marginal estrogenic activity (e.g. tetramethyl-HES[7,22,23] and LY 117018[24]) exhibit only moderate or weak antitumor activity. This finding may lead to the conclusion that a certain amount of residual estrogenic activity is essential for a strong antitumor activity of antiestrogens. Furthermore, since it has been known for some time that estrogens may exhibit strong mammary tumor inhibiting activity, it consequently has to be asked whether there is a correlation between estrogenic and mammary tumor inhibiting activity of antiestrogens. The compounds described in this paper indeed show a correlation between estrogenic and antitumor activity. The estrogens **2** and **6** exhibit the strongest antitumor effect, exceeding the activity of compounds **8** and **4**, which have a strongly reduced estrogenicity. The partial estrogens **5** and **1** also show stronger antitumor activity than the nonestrogenic compounds **7** and **3**.

In the class of the strong antiestrogens of the 1,1,2,2-tetramethyl-1,2-bis(3- or 4-hydroxyphenyl)ethane type increases of the binding affinity and antitumor activity

(20) The binding affinity of compound **2** has already been published: Goswami, R.; Harsey, S. G.; Heiman, D. F.; Katzenellenbogen, J. A. *J. Med. Chem.* **1980**, *23*, 1002. The result—the hexafluoro derivative **2** binding more than twice as well as the parent compound—is in accordance with our finding.

(21) For a recent review of the pharmacology of metahexestrol, see: Engel, J.; Hartmann, R. W.; Schönenberger, H. *Drugs Future* **1983**, *8*, 413.

(22) Hartmann, R. W.; Kranzfelder, G.; von Angerer, E.; Schönenberger, H. *J. Med. Chem.* **1980**, *23*, 841.

(23) For a recent review of the pharmacology of tetramethyl-HES, see: Hartmann, R. W. *Drugs Future* **1985**, *10*, 48.

(24) Jones, C. D.; Jevnikar, M. G.; Pike, A. J.; Peters, M. K.; Black, L. J.; Thompson, A. R.; Falcone, J. F.; Clemens, J. A. *J. Med. Chem.* **1984**, *27*, 1057.

were also accompanied by an enhanced estrogenicity.[7] 3,4-Bis(3-hydroxy-6-methylphenyl)hexane, the only compound of a series of 21 derivatives of hexestrol and metahexestrol that showed an increased RBA value compared to the parent compound, also exhibited an increased estrogenicity.[6]

If it will turn out that there is a general correlation between estrogenic effects of antiestrogens and their antitumor activity—this would mean that the antitumor activity of partial antiestrogens is due to their estrogenicity—bounds are set for the development of superpotent tumor-inhibiting antiestrogens. The estrogenic effect should of course be kept at a low level, since although true estrogens show good antitumor activity, they are no longer used therapeutically because of their well-known severe side effects.[25]

The uterus of the immature mouse is a very sensitive target organ, suitable for detecting even weak estrogenic compounds. In contrast to the juvenile uterus, the uterus of the adult MXT tumor mouse can only be stimulated by strong estrogens. The additional determination of the uterine weight in the MXT tumor experiment possibly will turn out to be an appropriate method for the determination of the threshold value of estrogenic activity maximally tolerable for a therapeutical use. This would mean that compounds with strong antitumor activity on the transplantable MXT tumor of the $BD2F_1$ mouse and which also show no stimulation of the uterine weights might be candidates for the therapy of the hormone-dependent breast cancer. Concerning the compounds of the present study, compound **4**, and especially compound **8** because of its additional strong antiestrogenic activity, might be of therapeutic interest.

Experimental Section

General Procedures. TLC of each compound 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. 4-Methoxy-α,α,α-trifluoroacetophenone (2b). Trifluoroacetic acid (11.4 g, 0.1 mol), dissolved in 100 mL of dry ether, was added dropwise over a period of 1 h to a stirred solution of (4-methoxyphenyl)magnesium bromide (0.3 mol) in 300 mL of ether. After refluxing for 2 h and standing overnight, the mixture was poured onto a mixture of ice and excess hydrochloric acid. The ethereal layer was separated and the aqueous phase was extracted with several portions of ether. The combined ether extracts were washed with 5% aqueous Na_2CO_3 solution and dried ($MgSO_4$). The solvent was removed and the residue was processed to give 12.0 g (59.0%) of a colorless oil: bp 68 °C (4 mmHg); ^1H NMR ($CDCl_3$) δ 3.88 (s, 3 H, OCH_3), 6.99, 8.05 (AB, J = 9 Hz, 4 H, Ar H).

3-Methoxy-α,α,α-trifluoroacetophenone (4b): yield 56.0%; oil, bp 90 °C (10 mmHg); ^1H NMR ($CDCl_3$) δ 3.91 (s, 3 H, OCH_3), 7.05–7.83 (m, 4 H, Ar H).

Method B. *cis*- and *trans*-1,1,1,4,4,4-Hexafluoro-2,3-bis(4-methoxyphenyl)but-2-ene (6b and 2a). $TiCl_4$ (2.85 g, 0.015 mol) was added dropwise to 100 mL of cooled, dry dioxane under nitrogen with stirring. A suspension of zinc powder (1.96 g, 0.03 mol) in 20 mL of dry dioxane was added in small portions over a period of 10 min. After the addition of 2 mL of dry pyridine, a solution of 4-methoxy-α,α,α-trifluoroacetophenone (**2b**; 2.04 g, 0.01 mol) in 20 mL of dry dioxane was added and the reaction mixture was heated at reflux. After 4 h at 101 °C, the mixture was cooled, and alkaline hydrolysis was performed with 10% K_2CO_3 solution. After extraction with ether and removal of the solvent, the crude product was purified and separated into the two isomeric compounds by column chromatography [SiO_2; toluene–CCl_4 (4:1)] to give 0.43 g of the cis isomer (**6b**) and 0.32 g of the trans isomer (**2a**).

Method C. *meso*-1,1,1,4,4,4-Hexafluoro-2,3-bis(4-methoxyphenyl)butane (6a). Palladium on charcoal (10%, 0.1 g) was added to a solution of **6b** (3.76 g, 0.01 mol) in 100 mL of EtOH. The suspension was shaken under a hydrogen atmosphere until no more H_2 was accepted. The reaction mixture was filtered. The alcohol was removed, and the crude product was recrystallized from EtOH/H_2O to give 3.18 g of **6a**.

Method D. *trans*-1,1,1,4,4,4-Hexafluoro-2,3-bis(4-hydroxyphenyl)but-2-ene (2). A solution of **2a** (3.76 g, 0.01 mol) in 150 mL of dry CH_2Cl_2 was cooled to −60 °C. 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. Ten milliliters of MeOH was added, and the mixture was shaken with 2 N NaOH. After neutralization of the aqueous layer with 3 N HCl, the solution was extracted with ether. After removal of the solvent, the crude product was recrystallized from EtOH/H_2O to give 2.92 g of **2**.

Biological Methods. Estradiol Receptor Binding Assay. The relative binding affinity (RBA) of the test compounds was determined by the displacement of [^3H]estradiol. A previously described procedure[22] was used with modifications. 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 a 100-μL supernatant aliquot was counted. The percent bound radioligand was plotted vs. the concentration of unlabeled test compound. Six concentrations of each competitor 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 test compounds reducing radioligand binding by 50% were determined.

Estrogen and Antiestrogen Assays. Estrogenic and antiestrogenic activitites 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.[22] 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. Transplantable Hormone-Dependent MXT Tumor of the $BD2F_1$ Mouse.[26] Tumor maintenance is accomplished on syngeneic female $BD2F_1$ mice. At the beginning of the test, tumor-bearing animals were killed and tumors were excised under aseptic conditions. The tumors were minced into 1–2-mm^3 pieces. With the use of a trocar the tumor pieces were serially transplanted sc into syngeneic 8- to 10-week-old female mice (weight 20.0 ± 1.7 g, mean ± SD). After transplantation the animals were randomly distributed into groups of 10. At the next day treatment was started by sc application of the test compounds, dissolved or suspended in olive oil. Application was continued three times weekly for 6 weeks. One control group was treated with tamoxifen. At the end of the test the animals were killed and weighed and their tumors and uteri excised. The wet weights of the tumors and the dry weights of the uteri were determined. The tumor-inhibiting effect was calculated in term of % T/C.

DMBA-Induced, Hormone-Dependent Mammary Carcinoma of the SD Rat. The method used has been described previously.[22] Animals bearing at least one tumor greater than 140 mm^2 were classified in groups of 10. 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.

Acknowledgment. Thanks are due to the Verband der Chemischen Industrie, Fonds der Chemischen Industrie,

(25) Carbone, P. P. In "Cancer Chemotherapy 1983. The EORTC Cancer Chemotherapy Annual 5"; Pinedo, H. M., Chabner, B. A., Eds.; Elsevier: Amsterdam, 1983; p 398.

(26) Watson, C.; Medina, D.; Clark, J. H. *Cancer Res.* **1977**, *37*, 3344.

and to the Deutsche Forschungsgemeinschaft, who supported this work by grants (SFB 234-85). The technical assistance of Maria Beer and Gabi Seidl is gratefully acknowledged. We thank Dr. A. E. Bogden, EG & G Bogden Laboratories, Worcester, MA, for providing us with the MXT tumor and for helpful discussions in setting up the MXT-tumor model.

Registry No. **2**, 28714-19-4; **2a**, 28714-18-3; **2b**, 711-38-6; **3**, 74944-95-9; **4**, 99749-15-2; **4a**, 99749-11-8; **4b**, 30724-22-2; **5**, 2962-14-3; **6**, 99749-16-3; **6a**, 99749-13-0; **6b**, 28714-28-5; **7**, 78682-42-5; **8**, 99749-17-4; **8a**, 99749-14-1; **8b**, 99749-12-9; F_3CCO_2H, 76-05-1; 4-$MeOC_6H_4MgBr$, 13139-86-1; 3-$MeOC_6H_4MgBr$, 36282-40-3.

Supplementary Material Available: ^1H NMR data (Table VII) of compounds **2**, **2a**, **4**, **4a**, **6**, **6a**, **8**, and **8a** (2 pages). Ordering information is given on any current masthead page.

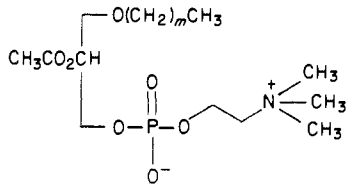
Analogues of Platelet Activating Factor. 4.[1] Some Modifications of the Phosphocholine Moiety

A. Wissner,* R. E. Schaub, P-E. Sum, C. A. Kohler, and B. M. Goldstein

Metabolic Disease Research and Cardiovascular Biological Research Sections, Medical Research Division, American Cyanamid Company, Lederle Laboratories, Pearl River, New York 10965. *Received May 20, 1985*

Racemic analogues of platelet activating factor (PAF) in which the methylene bridge separating the phosphate and trimethylammonium moieties is altered in length (**7a–f**) have been prepared. Increasing the length of this bridge results in a progressive decrease in the hypotensive and platelet aggregation responses. Analogues in which the phosphocholine group is substituted with a methyl group (**7h** and **7i**) or a phenyl group (**5j**) or in which the methylene bridge is replaced with a meta-substituted benzyl group (**5k**) have been prepared. With respect to both the blood pressure and platelet aggregation responses, **7i** and **5k** showed little if any changes in potency compared to racemic C_{16}-PAF (**1a**). While **7h** is more potent than **1a** with respect to both the hypotensive and platelet aggregation properties, **5j** is less potent. Replacement of the phosphate moiety of C_{18}-PAF (**1b**) with a phosphonate group (**7g**) leads to decreased activity in both assays. Analogue **11**, in which the phosphocholine group has been replaced with a 4-(trimethylammonio)butoxy group, exhibited no detectable hypotensive or platelet aggregating activity. None of the analogues exhibited a separation of the blood pressure and platelet aggregation activities.

Platelet activating factor (PAF), a mixture of homologous alkyl ether phospholipids of structure 1, possesses a

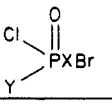


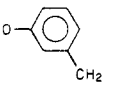
1a, $m = 15$
1b, $m = 17$

variety of biological activities. It is one of the important mediators of anaphylaxis and inflammation and has the ability to activate various inflammatory cells such as basophils, neutrophils, and platelets. PAF is also very potent in its ability to lower blood pressure.[2]

Over the last few years we have been involved in structure–activity studies of this important phospholipid molecule. Previously, we have reported compounds containing modifications of the alkyl ether chain[1a] and glycerine backbone.[1b] We[1c] and others[3] have reported compounds in which the phosphocholine portion of the PAF molecule has been altered. In this report we describe the preparation and structure–activity relationships of

Table I. Phosphorus Reagents Used



no.	X	Y	method	reactn time, h	ref
3a	$O(CH_2)_3$	Cl	A	18	5
3b	$O(CH_2)_4$	Cl	A	18	5
3c	$O(CH_2)_6$	Cl	A	18	5
3d	$O(CH_2)_8$	Cl	A	18	5
3e	$O(CH_2)_{10}$	Cl	A	18	5
3f	$(CH_2)_2$	OH	*a*	18	6
3g	$OCH_2CH(CH_3)$	Cl	A	18	*b*
3h	$OCH(CH_3)CH_2$	Cl	A	18	*b*
3i	$OCH_2CH(C_6H_5)$	Cl	A	76	*b*
3j		Cl	B	20	*b*

[a] See ref 6. [b] This work.

(1) For previous publications in this series see: (a) Wissner, A.; Sum, P.-E.; Schaub, R. E.; Kohler, C. A.; Goldstein, B. M. *J. Med. Chem. 1984, 27*, 1174. (b) Wissner, A.; Sum, P.-E.; Schaub, R. E.; Kohler, C. A.; Goldstein, B. M. *Ibid.* **1985**, *28*, 1181. (c) Wissner, A.; Kohler, C. A.; Goldstein, B. M. *Ibid.* **1985**, *28*, 1365.

(2) For recent reviews of the biological properties of PAF see: (a) Pinckard, R. N.; McManus, L. M.; Hanahan, D. J. *Adv. Inflammation Res.* **1982**, *4*, 147. (b) Snyder, F. *Ann. Rep. Med. Chem.* **1982**, *17*, 243. (c) Vargaftig, B. B.; Chegnord, M.; Benveniste, J.; Lefort, J.; Wal, F. *Ann. N.Y. Acad. Sci.* **1981**, *370*, 119. (d) O'Flaherty, J. T.; Wykle, R. L. *Clin. Rev. Allergy*, **1983**, *1*, 353. (e) Vargaftig, B. B.; Benveniste, J. *Trends Pharmacol. Sci.* **1983**, *4*, 341.

(3) (a) Hanahan, D. J.; Munder, P. G.; Satouchi, K.; McManus, L.; Pinckard, R. N. *Biochem. Biophys. Res. Commun.* **1981**, *99*, 183. (b) Tence, M.; Coeffier, E.; Heymans, F.; Polonsky, J.; Godfroid, J.; Benveniste, J. *Biochim.* **1981**, *63*, 723. (c) Satouchi, K.; Pinckard, R. N.; McManus, L. M.; Hanahan, J. T. *J. Biol. Chem.* **1981**, *256*, 4425. (d) Moschidis, M. C.; Demopoulos, C. A.; Kritikou, L. G. *Chem. Phys. Lipids* **1983**, *33*, 87. (e) Nakomura, N.; Miyazaki, H.; Ohkawa, N.; Koike, H.; Sada, T.; Asai, F.; Kobayoshi, S. *Chem. Pharm. Bull.* **1984**, *32*, 2452. (f) Kasuya, Y.; Masuda, Y.; Shigenobu, K. *Can. J. Physiol. Pharmacol.* **1983**, *62*, 457.

0022-2623/86/1829-0328$01.50/0