Cytotoxicity and Antiestrogenicity of a Novel Series of Basic Diphenylethylenes

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On the premise that it is necessary to develop antiestrogens with a higher cytotoxic component in order to reduce the risks of the development of heterogeneous malignant cell populations in breast cancer, we studied a novel series of basic diphenylethylenes, for the most part devoid of estrogenic activity, with low antiestrogenicity but much enhanced cytotoxicity compared to the reference drug tamoxifen. The main structural features associated with cytotoxicity were *E* isomery, substituents of five to eight carbons on the ethylene bond, and dibasicity.

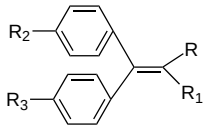
Introduction

Adjuvant chemotherapy or endocrine therapy is commonly administered to patients with breast cancer who have undergone surgery in order to delay reccurrence of the disease. The focus of these two treatments differs. Chemotherapy aims to kill any residual malignant cells but is frequently ill-tolerated, whereas milder endocrine therapy, such as the use of antiestrogens, inhibits hormone-dependent cell growth. Unfortunately, human estrogen receptor (ER)-positive breast cancer cell lines, in the absence of estrogen, lose their estrogen responsiveness over time,[1] and in the clinic, the majority of patients with ER-positive tumors who initially respond to endocrine therapy will eventually relapse. The reasons for progression toward hormone insensitivity are unknown but may be related to the presence of mutated receptors,[2] to the selection of resistant cells due to the malignant process itself, and to the acquisition of drug resistance.[3−6]

A commonly used adjuvant drug is tamoxifen (*trans*-(*Z*)-1-[4-[2-(dimethylamino)ethoxy]phenyl]-1,2-diphenyl-1-butene; Nolvadex, Zeneca). The mechanism underlying its clinical efficacy is still poorly understood. Its effect is attributed in major part to an ER-mediated antiestrogenic action[7] but also to a lesser cytostatic/cytotoxic component, defined as that part of cell growth inhibition that is not reversible by estrogen.[8,9] This cytotoxicity, which is observed at high concentrations but might also occur at lower concentrations, may involve membrane effects,[10] proteins such as the antiestrogen binding protein,[11] calmodulin,[12] or protein kinase C,[13−16] and in general cross-talk between different signaling pathways.

Combination therapy with a cytotoxic and antiestrogen is an option designed to improve treatment efficacy while maximizing tolerance.[17,18] Its aim is to kill all malignant cells before recurrences occur and drug resistance sets in. An alternative to such combination therapy would be to design a single drug that combines both features. Our previous papers[19,20] have described the effects of basic 1,2-diphenyl- and 1,1,2-triphenylethylene derivatives on breast cancer cell proliferation. The present study focuses on the activities of a series of systematically substituted basic 1,1-diphenylethylenes (DPEs) with carbon side chains of different length, linearity (including cyclic substituents), bulk, and symmetry attached to position 2 of the ethylene double bond. Apart from a preliminary investigation of pyrrolidine derivatives of cyclofenil,[21,22] such amino-substituted chemical structures have received little attention so far. To establish whether these new compounds displayed enhanced cytotoxicity while retaining antiestrogenicity, we tested them for their (i) relative binding affinity (RBA) for ER, (ii) estradiol agonist and antagonist activity, and (iii) cytostaticity and cytotoxicity in an estrogen-dependent breast cancer cell-line (MCF7). The correlations between chemical structure and response were analyzed by correspondence factor analysis (CFA) as in previous structure−activity relationship studies on triphenylethylenes[20,23] and steroids.[24−27]

Table 1. Test Compounds



DEAE = $(C_2H_5)_2NCH_2CH_2O$–
DMAE = $(CH_3)_2NCH_2CH_2O$–
DiPAE = $((CH_3)_2CH)_2NCH_2CH_2O$–

compd	R	R_1	R_2	R_3
1Z	H	C_5H_{11}	OH	DEAE
1E	H	C_5H_{11}	DEAE	OH
2Z	H	C_6H_{13}	OH	DEAE
2E	H	C_6H_{13}	DEAE	OH
3Z	H	C_8H_{17}	OH	DEAE
3E	H	C_8H_{17}	DEAE	OH
4Z	H	$C_{14}H_{29}$	OH	DEAE
4E	H	$C_{14}H_{29}$	DEAE	OH
5E	H	C_5H_{11}	cC_4H_8-$N(CH_2)_2O$	OH
6	H	C_5H_{11}	DEAE	DEAE
7	H	$(CH_3)_2$-CH	DiPAE	DiPAE
8		$(CH_2)_5$=	DiPAE	DiPAE
9		$(CH_2)_5$=	DiPAE	OH
10Z	H	$(CH_3)_2CH$	OH	DEAE
10E	H	$(CH_3)_2CH$	DEAE	OH
11Z	H	C_5H_{11}	OH	DMAE
11E	H	C_5H_{11}	DMAE	OH
12Z	H	C_4H_9	OH	DEAE
12E	H	C_4H_9	DEAE	OH
13Z	H	$C_6H_5CH_2$	OH	DEAE
13E	H	$C_6H_5CH_2$	DEAE	OH
14Z	C_2H_5	C_4H_9	OH	DEAE
14E	C_4H_9	C_2H_5	DEAE	OH

Results and Discussion

Chemistry. Compounds **1**−**5** and **9**−**14** (Table 1) were prepared by condensation of the selected *p*-

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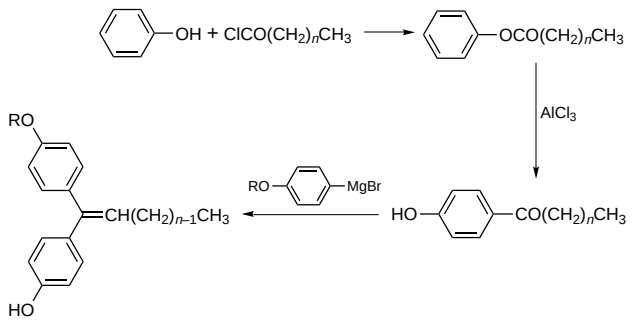
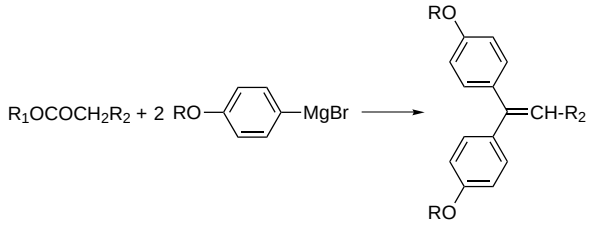
Scheme 1



Scheme 2



hydroxyphenyl alkyl ketone with the appropriate [[2-(dialkylamino)ethoxy]phenyl]magnesium bromide derivative (Scheme 1). Compound **6** was synthesized by condensation of 1 mol of heptanoic acid phenyl ester with 2 mol of [[2-(dialkylamino)ethoxy]phenyl]magnesium bromide (Scheme 2). Compound **7** was obtained in three steps: first, by condensation of isovaleric acid ethyl ester with *p*-anisylmagnesium bromide; second, by action of pyridine hydrochloride on this dimethoxyl derivative to give the diol; and lastly, by condensation of the diol with 2-(diisopropylamino)ethyl chloride under alkaline conditions. The synthesis of **8** was analogous to that of **7**.

X-ray and NMR Analyses. The conformation of the *E* isomer of **1** was determined by X-ray crystallography. With this result in hand, a specific chemical shift could be attributed to the CH_2O group in the basic side chain attached to the phenyl ring of all monohydroxylated compounds in the NMR analyses (4.00 ppm (*Z*), 3.96 ppm (*E*)). The proportion of each isomer within a mixture was calculated either by direct integration or after irradiation of the vicinal CH_2 group. In the case of **14**, the proportion of each isomer was determined with a 300 MHz apparatus only and was calculated on the basis of the CH_3 protons of the ethyl group.

Biochemical Data. Table 2 gives the biochemical results for the 22 test compounds and 3 reference substances (estradiol (E_2), (*Z*)-tamoxifen, and (*Z*)-4-hydroxytamoxifen) as well as data on the isomerization of the compounds under *in vitro* incubation conditions. The extent of isomerization after 3 days incubation in an aqueous medium at pH 7.4 and 37 °C was variable. **10Z** was virtually totally converted into its isomer **10E** whereas the isomerization of the other compounds was less pronounced (**12Z, 11Z, 4Z, 3Z**) or even negligible. The *E* isomers were more stable in solution than the *Z* isomers.

The RBAs of the compounds for lamb cytosol ER were low; only **9** and **14** displayed RBAs above 1%. Cytostatic and cytotoxic effects were given by the extent of inhibition of MCF7 cell proliferation. Cytostatic activity was expressed as the compound concentration inhibiting by 50% (IC_{50}) the cell proliferation induced by 1 μM E_2; cytotoxicity was expressed as the compound concentration leading to cell death in the presence or absence of E_2. Except for **4E** and **4Z**, all compounds displayed greater estrogen-irreversible cytotostatic effects than the reference substances as indicated by their much

Table 2. Isomeric Purity and Biochemical Activity Profiles of the Test Compounds

			response in MCF7 cells[b]			response in MVLN cells[c]		
	isomeric purity[a] (%)		cytostaticity (+1 μM E_2)	cytotoxicity (+1 μM E_2)	cytotoxicity (−1 μM E_2)	antiestrogenicity	estrogenicity	RBA[d]
compd	pre	post	IC_{50} (μM)	(μM)	(μM)	IC_{50} (μM)	% E_2 response	% E_2 affinity
1Z	91	86	1.6	3.2	2.9	0.9	5	0.21
1E	90	89	1.1	3.2	2.7	3.2	0	0.1
2Z	76	69	1.6	3.0	2.9	1.9	5	0.13
2E	94	90	1.2	3.8	2.8	2.5	0	0.02
3Z	70	53	1.7	2.9	5.1	3.5	2	0.025
3E	68	74	1.6	3.0	3.6	4.7	0	0.035
4Z	78	53	7.9	30	30	8.0	0	<0.001
4E	90	90	8.0	30	30	9.5	0	<0.001
5E	95	88	1.6	2.9	2.9	3.3	0	0.08
6			0.7	1.3	2.7	6.0	0	<0.001
7			1.6	4.5	3.6	3.1	0	0.02
8			4.2	8.0	30	2.1	4	0.12
9			1.8	5.0	3.8	0.2	0	3.3
10Z	81	6	2.4	9.4	9.2	0.5	4	1.0
10E	96	97	1.7	8.8	8.4	1.7	3	0.13
11Z	71	50	2.4	9.3	8.5	1.5	1	0.2
11E	63	80	1.7	3.0	2.9	1.8	1	0.07
12Z	58	21	1.7	5.2	4.4	0.5	2	0.75
12E	88	88	1.6	3.0	2.9	1.5	0	0.21
13Z	87	73	1.8	4.3	3.2	1.3	4	0.26
13E	76	81	1.7	3.0	2.9	1.7	0	0.15
14E/Z[e]	50	50	1.0	2.8	2.8	0.15	0	4.25
E2							100	100
TAM Z			8.0	13	9.8	0.3	0	1.1
OH-TAM Z			8.0	9.4	6.0	0.003	0	243

[a] Before and after incubation for 3 days in phosphate-buffered saline (pH 7.4). [b] Cytostaticity is given by the test-substance concentration inhibiting cell proliferation by 50% and cytotoxicity by that leading to cell death (tested concentration range 1−10 μM). [c] Antiestrogenicity is given by the concentration of test substance leading to 50% inhibition (IC_{50}) of the luciferase activity due to 0.1 nM E_2 and estrogenicity by the maximal induction of luciferase activity in the absence of E_2 (% maximum E_2 response). [d] Lamb uterus cytosol; 5 h at 25 °C. [e] 50/50 mixture of isomers.

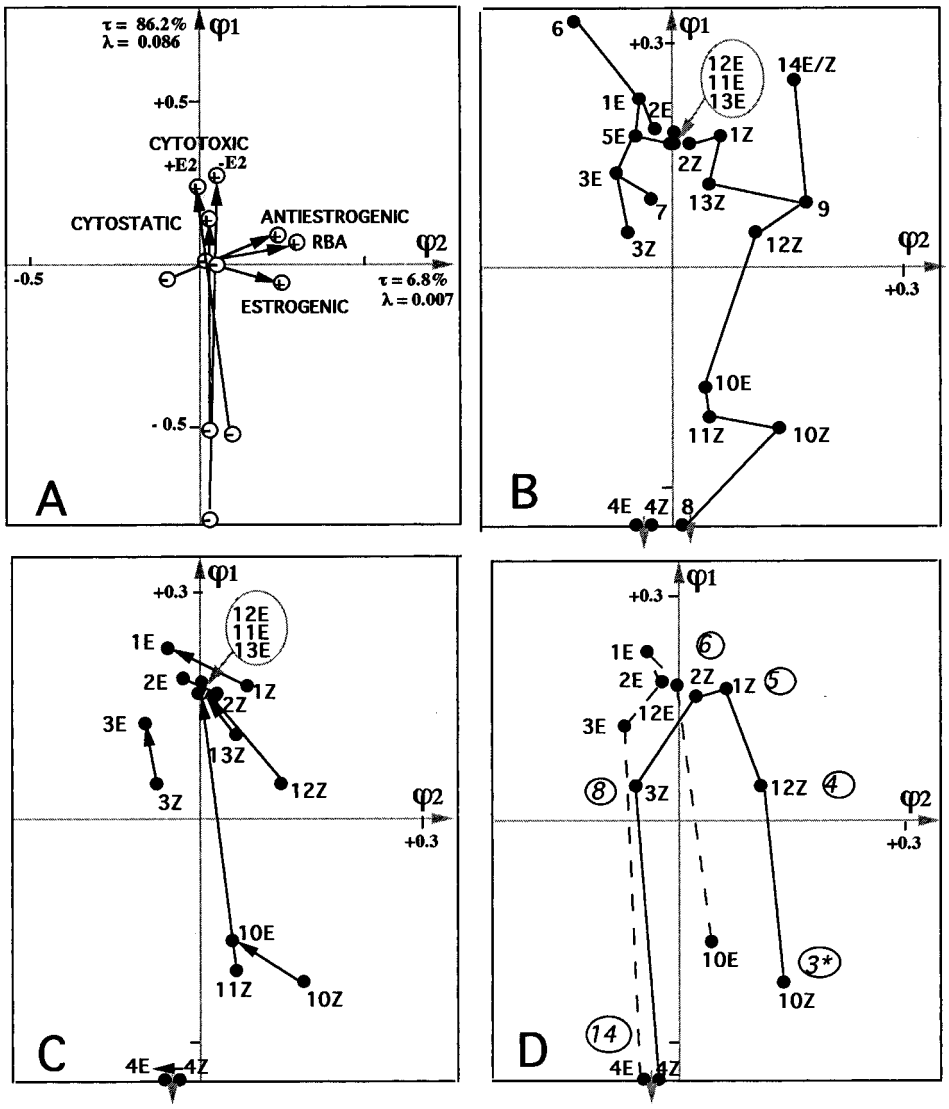


Figure 1. Correspondence factor maps of the data matrix in Table 2 (22 compounds × 6 test conditions) after splitting of the columns. (τ = variance associated with an axis; λ = quality of the representation (If the variables were totally distinct, λ would be equal 1.)) (A) Correlations among the tests represented as vectors (+, high amplitude; −, low amplitude). The basis of the tests was as follows: cytostaticity, concentration giving 50% estrogen-irreversible inhibition of MCF7 cell proliferation; cytotoxicity, concentration leading to cell death; antiestrogenicity in MVLN cells; RBA for the estrogen receptor in lamb uterus cytosol; estrogenicity in MVLN cells. (B) Correlations among the test compounds linked by a minimum spanning tree. (C) Effect of *E* and *Z* isomery. (D) Effect of the length of the side chain attached to the ethylene bond in the *Z* (solid line) and *E* (broken line) series. (The number of carbon atoms of the side chain is encircled).

lower IC_{50} values. The concentrations leading to a cytotoxic effect were also lower, the most cytotoxic molecule being a dibasic compound (**6**).

Because of the very low RBAs, the antiestrogenic potency of the test compounds on cell proliferation was difficult to assess. This antiestrogenicity only manifests itself at high compound concentrations which, after 8−10 days incubation, also induce a marked cytotoxic effect. This is why we decided to use the MVLN cell model (MCF7 cell line obtained by integrating an estrogen-controlled firefly luciferase gene) for the study of antiestrogenic and estrogenic activity. For incubations under 14−16 h, estrogen-regulated luciferase activity accurately reflects estrogenic potency.[28,29] In Table 2, estrogenicity is thus given by the percent maximal reporter response obtained with E_2 and antiestrogenicity by the test-compound concentration needed to inhibit the E_2-induced reporter gene response by 50%. No or very low E_2-type responses were detected; the low responses are probably meaningful, although subject to a relative experimental error of about 50% (for the mean of two experiments involving triplicates). Most compounds were about 10- and 1000-times less effective than (*Z*)-tamoxifen and (*Z*)-4-hydroxytamoxifen, respectively, in antagonising the effect of E_2.

Multivariate Factor Analysis. Structure−activity relationships are displayed in the correspondence factor map of Figure 1 which illustrates the specificity and amplitude of response of each test compound. Figure 1 is a two-dimensional plot of the two main factorial axes which represent a total of 93% (86.2% (φ_1) and 6.8% (φ_2)) of the information content within Table 2. In Figure 1A, the tip of a vector denoting a biochemical test indicates a high response, its origin a low response. The vectors representing cytotoxicity and cytostaticity (with a total absolute contribution to the φ_1 axis of 99%) are parallel to this axis which, as indicated above, embodies 86.2% of the information in Table 2. Both these activities, and in particular cytotoxicity, are thus the primary discriminating features within this population

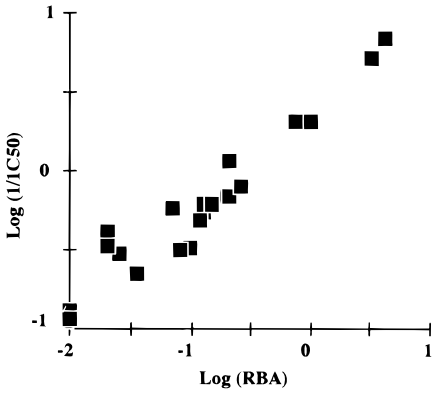


Figure 2. Bivariate plot of antiestrogenicity versus RBA.

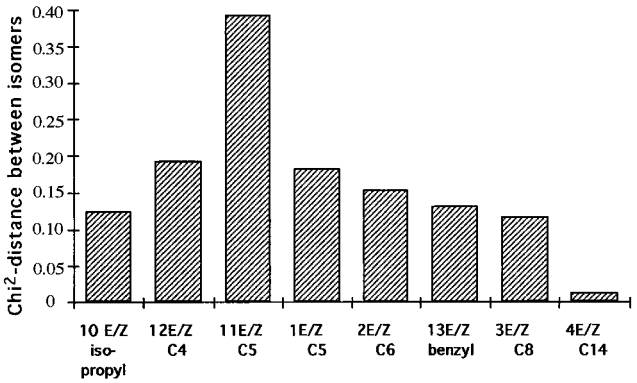


Figure 3. Effect of isomery and side-chain length on specificity of response as given by the χ^2 distance between isomer pairs arranged in the order of increasing chain length.

of molecules. The contiguity of these vectors indicates that they have similar statistical significance and suggests that an increase in test-compound concentration might naturally extend an estrogen-irreversible cytostatic effect into a cytotoxic effect. The secondary discriminating features (along the φ_2 axis) are antiestrogenic potency and relative binding affinity for ER—which are virtually superimposable—and also estrogenicity, although this latter result, albeit coherent, must be viewed with some circumspection because of the very low activity levels that were recorded (see Table 2). The relationship between antiestrogenic potency and RBA was confirmed by a bivariate plot (Figure 2) which disclosed a virtually linear relationship. The near-orthogonality of the cytotoxicity and antiestrogenicity vectors suggests that these two phenomena are not related for this series of molecules.

The positions of the molecules that account for the relationships among the biochemical variables in Figure 1A are given in Figure 1B (the three reference substances were not included in the analysis). Figure 1B can be superimposed on Figure 1A (allowance being made for the change in scale) and depicts the relationships among compounds, among tests, and between compounds and tests. As one moves up the φ_1 axis, one encounters increasingly cytotoxic molecules. **4E** and **4Z** are the least potent molecules of the series; the dibasic compound **6** is the most cytotoxic. Movement along the φ_2 axis primarily describes the antiestrogenic and RBA components of the molecules, **14E/Z, 9, 10Z,** and **12Z** being the most active in this respect. Of these molecules, **10Z** and **12Z** displayed some estrogenic activity. The test compounds in Figure 1B have been linked into a network that is transposed from the results of a minimum spanning tree analysis[19,30] which calculates the shortest distance among all variables.

Structure—Activity Relationships. Panels C and D of Figure 1 are excerpts of Figure 1B. They highlight the influence of isomery (panel C) and of the length of the side chain attached to the ethylene bond (panel D) on the specificity and amplitude of response. In panel 1C, the arrows linking the isomers reflect a general trend toward increasing cytotoxicity (upward) and decreasing antiestrogenicity—RBA (leftward) for the *E* compared to the *Z* isomers. Isomerization of the test compounds during the biochemical assays probably has little influence on RBA and antiestrogenicity results because these tests involve short-term incubation only, but may have a greater impact on cytotoxicity and cytostaticity results. It is, however, unlikely that such isomerization should completely obviate the very clear-cut pattern observed.

In panel 1D, side chains of five, six, or even eight carbons are seen to be conducive to increased cytotoxicity in both the *E* and *Z* series (upward displacement along the φ_1 axis). A long chain of 14 carbons resulted in inactivity (**4E** and **4Z**); shorter chains (three to five carbons) were associated with higher antiestrogenicity—RBA values, especially in the *Z* series. There were, however, exceptions to this tendency. After permutation of the ethyl on the nitrogen atom of the side chain of **1Z** to a methyl (**11Z**), there was a marked decrease in cytotoxicity (Figure 1B). Compound **13Z** with a benzyl side chain also had reduced cytotoxicity and was situated near the origin of the axes, suggesting an average profile. The histograms in Figure 3 give the χ^2 distance between each isomer pair and quantify the effect of isomery and chain length on specificity and amplitude of response.

Although the introduction of a second basic para side chain on a phenyl ring of **1** (**Z** or **E**) yielded the most cytotoxic compound of the series (**6**), a similar modification of the cyclofenil type structure (**9**) decreased cytotoxicity and antagonist activity (**8**) (Figure 1B). Bisubstitution of the ethylene double bond (**14E/Z** compared to **12E** or **-Z**) increased antiestrogenicity—RBA as well as cytotoxicity. Garg et al.[22] have studied an analog of **9** (a pyrrolidine in lieu of diisopropylamino group) and found significantly higher RBAs using rat and mouse cytosol ER (57 and 80%, respectively). However, their RBA results for the disubstituted analog are comparable (0.2 and 0.4%, respectively) to those we found for **8**.

Conclusions

A systematic structure—activity study on a novel series of basic diphenylethylene derivatives has revealed that this is a series of molecules with low antiestrogenic activity, mostly devoid of estrogenic activity, but with a cytotoxicity severalfold higher than that of tamoxifen. The antiestrogenic activity is probably due to an interaction with the estrogen receptor as indicated by the quasi-linear relationship found between antiestrogenic potency and relative binding affinity for the receptor. Within the series, *E* isomery was more conducive to cytotoxicity than *Z* isomery as were side chains on the ethylene bond of five to eight carbons. Similar results have been previously obtained with tamoxifen and 4-hydroxytamoxifen isomers and with triphenylacrylonitrile isomers.[15] Short linear or branched chains

(three carbons) tended to be associated with more antiestrogenic molecules, and a very long side chain of 14 carbons led to inactivity. High cytotoxicity was also associated with a dibasic structure as previously found in a series of triphenylacrylonitriles.[23] The orthogonality of the vectors representing cytostatic/cytotoxic effects and ER-dependent effects (antiestrogenic potency, RBA) in the multivariate correspondence factor analysis revealed that these two types of activity are totally independent for this population of molecules. Consequently, the very high antiestrogenic activity observed for 10 μM concentrations of several test compounds is not accounted for by cytotoxicity; it was reversed by 1 μM E_2 in the MVLN model, giving rise to a 70−80% response.

The relative binding affinity of these DPEs for ER is very low. Their estrogenic activity, as measured with a chimaeric gene, was extremely low—about 1 to 5%—or undetectable. These observations have been confirmed in unpublished experiments on compounds **1**−**8** which have shown that, within a 10−100 nM concentration range, **1Z, 2Z, 4E/Z,** and **8** enhance the proliferation of MCF7 cells with a potency of about 10−12% of that of E_2.

In conclusion, we have designed derivatives with a higher cytostatic/cytotoxic activity than current triphenylethylenes and with some, albeit low, antiestrogenic activity mediated by ER. These first compounds of a new series may not be able to act as a substitute for combination chemotherapy/endocrine therapy in clinical practice. Nevertheless, their dual activity profile suggests that such substitutes might be feasible and that the rationale we have adopted could be interesting from more than just a theoretical point of view.

Experimental Section

Chemical Methods. General Procedures. Melting points were determined on a Mettler FP 61 apparatus and are uncorrected. The results of the elemental analyses, performed in the Microanalytical Laboratory of the CNRS (Vernaison, France), were within ±0.3% of the theoretical values for those elements shown. ^{1}H NMR spectra (Me_4Si internal) were recorded (δ 0) with a Bruker AC 200E instrument at 200 MHz, IR spectra with a Perkin-Elmer 1420 instrument, and mass spectra with a Ribermag (DCI$^+$(NH_3)) instrument. Column chromatography was performed using silica gel 60 Å (35−70 μm). Ether refers to diethyl ether. Petroleum ether refers to the fraction with the boiling range 30−60 °C unless otherwise specified. Anhydrous tetrahydrofuran (THF) was obtained by distillation from potassium and benzophenone.

General Procedure for the Preparation of Alkyl Acid Phenyl Esters. The preparation of the heptanoic acid phenyl ester[31] will illustrate the procedure. Heptanoyl chloride (50 g, 0.336 mol) was added dropwise to phenol (31.9 g, 0.338 mol) at 15 °C under an argon atmosphere, and the mixture was stirred for 4 h at 60 °C. After cooling and addition of water (50 mL), the aqueous layer was extracted with ether. The organic extracts were washed with saturated solutions of NaCl (3 × 15 mL), $NaHCO_3$ (20 mL), and then NaCl (20 mL) again, dried over $MgSO_4$, and evaporated. Distillation of the residue gave a fraction with a $bp_{0.01}$ of 82−86 °C and which was identified as the title compound (yield 98%).

General Procedure for the Preparation of 4-Hydroxyphenyl Alkyl Ketones by Fries Rearrangement. The synthesis of 4-hydroxyhexanophenone[32] will be used as an example. Heptanoic acid phenyl ester (35 g, 0.17 mol) was added dropwise at 30 °C under an argon atmosphere to a solution of anhydrous aluminum chloride (45.5 g, 0.341 mol) in anhydrous nitrobenzene (340 mL) and stirred for 48 h at 40 °C. The mixture was then hydrolyzed with 1 N HCl (100 mL) in an ice bath and steam distilled to eliminate the nitrobenzene. The suspension was extracted with ether, and the organic layer was treated with 5% NaOH (3 × 50 mL) to obtain an alkaline phase which was acidified with 1 N HCl and extracted with ether. The organic layer was washed, dried ($MgSO_4$), and concentrated to a residual oil which was crystallized (73.4%): mp 90 °C.

(*E*)- and (*Z*)-1-[4-[2-(Diethylamino)ethoxy]phenyl]-1-(4-hydroxyphenyl)-1-heptene (1). A solution of *p*-[2-(diethylamino)ethoxy]bromobenzene (13.6 g, 0.05 mol) in anhydrous THF (30 mL) was slowly added under an argon atmosphere to magnesium turnings (1.3 g, 0.053 atom) and iodine crystals in anhydrous ether (15 mL). The Grignard reagent was heated at reflux for 2 h and then allowed to cool. A solution of 4-hydroxyheptanophenone (5.20 g, 0.025 mol) in THF (50 mL) was added in a rapid dropwise fashion. After the mixture was stirred, heated at reflux for 3 h, and cooled, a saturated ammonium chloride solution was added dropwise, and stirring was continued for 2 h. The organic layer was separated, and the aqueous solution was extracted several times with THF. The organic extracts were washed with saturated NaCl (25 mL), dried ($MgSO_4$), and concentrated. The residual oil was dissolved in ether and treated with 2 N HCl to enable recovery of 4-hydroxyheptanophenone (0.6 g, 11.5%) from the ether layer. The acidified aqueous layer was treated with 10% NaOH and extracted with ether. The organic phase was washed with water, dried ($MgSO_4$), and concentrated. The residual oil could be partially crystallized (*ex* petroleum ether) to yield white crystals (3 g, 30%): mp 105 °C (*ex* benzene/petroleum ether, 2/1); R_f 0.27 ($CHCl_3$/CH_3OH, 85/15); IR ($CHCl_3$) ν OH 3580−3080 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.78 (t, $CH_3(CH_2)_4$), 1.01 (t, $(CH_3CH_2)_2N$), 1.17−1.33 (m, $CH_3(CH_2)_3$), 2.01 (q, C=CHCH_2), 2.62 (q, $(CH_3CH_2)_2N$), 2.83 (t, NCH_2-CH_2O), 3.96 (t, CH_2O), 5.83 (t, C=CH), 6.61−7.02 (4d, 8 ArH); MS *m*/*z* 382 [M + 1]$^+$. Anal. ($C_{25}H_{35}NO_2$) C, H, N. According to X-ray crystallography, this is the *E* isomer.

Concentration of the petroleum ether solution that was separated from the crystals gave a crude oil (9.1 g). Purification by chromatography ($CHCl_3$/increasing CH_3OH) yielded the *Z* isomer (30%): mp 68−9 °C (*ex* C_6H_6/ hexane, 5/95); R_f 0.33 ($CHCl_3$/CH_3OH, 85/15); IR ($CHCl_3$) υ OH 3580−3080 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.78 (t, $CH_3(CH_2)_2$), 1.03 (t, $(CH_3CH_2)_2N$), 1.17−1.33 (m, $CH_3(CH_2)_3$), 1.99 (q, C=CHCH_2), 2.65 (q, $(CH_3CH_2)_2N$), 2.86 (t, NCH_2CH_2O), 4.00 (t, CH_2O), 5.82 (t, C=CHCH$_2$), 6.56−7.04 (m, 8 ArH); MS *m*/*z* 382 [M + 1]$^+$. Anal. ($C_{25}H_{35}NO_2$) C, H, N.

(*E*)- and (*Z*)-1-[4-[2-(Diethylamino)ethoxy]phenyl]-1-(4-hydroxyphenyl)-1-octene (2). The above procedure described for **1** was used starting from 4-hydroxyoctanophenone and gave a residual oil from which the *E* isomer (95%) was separated by digestion in petroleum ether: mp 101 °C (*ex* CH_3OH); R_f 0.29 ($CHCl_3$/CH_3OH, 85/15); IR ($CHCl_3$) υ OH 3580−3080 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.78 (t, $CH_3(CH_2)_5$), 1.03 (t, $(CH_3CH_2)_2N$), 1.15−1.32 (m, $CH_3(CH_2)_4$), 2.01 (q, C=CHCH_2), 2.66 (q, $(CH_3CH_2)_2N$), 2.85 (t, NCH_2CH_2O), 3.97 (t, CH_2O), 5.80 (t, C=CH), 6.55−7.00 (4d, 8 ArH); MS *m*/*z* 396 [M + 1]$^+$. Anal. ($C_{26}H_{37}NO_2$) C, H, N.

Concentration of the petroleum ether−oil mixture followed by chromatography yielded the *Z* isomer (75%) as a solid: mp 59 °C (*ex* petroleum ether); ^{1}H NMR ($CDCl_3$) δ 0.78 (t, $CH_3(CH_2)_5$), 1.03 (t, 6H, $(CH_3CH_2)_2N$), 1.20−1.40 (m, 8H, CH_3-$(CH_2)_4$), 2.00 (q, 2H, C=CHCH_2), 2.65 (q, 4H, $(CH_3CH_2)_2N$), 2.86 (t, 2H, NCH_2CH_2O), 4.00 (t, CH_2O), 5.82 (t, C=CH), 6.57−7.04 (m, 8 ArH).

(*E*)- and (Z)-1-[4-[2-(Diethylamino)ethoxy]phenyl]-1-(4-hydroxyphenyl)-1-decene (3). The above procedure using 4-hydroxydecanophenone gave a residual oil which did not precipitate on addition of petroleum ether but which, after chromatography, yielded two solids that were crystallized (*ex* C_6H_6/petroleum ether, 20/80). The proportion of each isomer was determined by ^{1}H NMR. *Z* isomer (72%): mp 63 °C; ^{1}H NMR ($CDCl_3$) δ 0.79 (t, 3H, $CH_3(CH_2)_5$), 1.03 (t, 6H, $(CH_3$-$CH_2)_2N$), 1.07−1.32 (m, 12H, $CH_3(CH_2)_6$), 2.00 (q, C=CHCH_2), 2.64 (q, 4H, $(CH_3CH_2)_2N$), 2.86 (t, NCH_2CH_2O), 4.00 (t, CH_2O), 5.82 (t, C=CH), 6.56−7.04 (m, 8 ArH); MS *m*/*z* 424 [M + 1]$^+$.

Anal. ($C_{28}H_{41}NO_2$) C, H, N. *E* isomer (68%): mp 75 °C; MS *m*/*z* 424 $[M + 1]^+$. Anal. ($C_{28}H_{41}NO_2$) C, H, N.

(*E*)- and (*Z*)-1-[4-[2-(Diethylamino)ethoxy]phenyl]-1-(4-hydroxyphenyl)-1-hexadecene (4). The above procedure using 4-hydroxyhexadecanophenone gave a residual oil that precipitated with petroleum ether. *Z* isomer from a first crop (85%): mp 86−7 °C (*ex* CH_3OH); IR ($CHCl_3$) *v* OH 3580−3080 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.80 (t, 3H, $CH_3(CH_2)_{11}$), 1.03 (t, $(CH_3CH_2)_2N$), 1.17 (br s, $CH_3(CH_2)_{11}$), 1.17−1.40 (m, $CH_2CH_2CH{=}C$), 2.00 (q, $C{=}CHCH_2$), 2.64 (q, $(CH_3CH_2)_2N$), 2.85 (t, NCH_2CH_2O), 4.00 (t, CH_2O), 5.82 (t, $C{=}CH$), 6.56−7.04 (8 ArH); MS *m*/*z* 508 $[M + 1]^+$. Anal. ($C_{34}H_{53}NO_2$) C, H, N. *E* isomer (95%): mp 71−73 °C (*ex* CH_3OH). ^1H NMR ($CDCl_3$) δ 0.80 (t, $CH_3(CH_2)_{11}$), 1.02 (t, $(CH_3CH_2)_2N$), 1.17 (br s, $CH_3(CH_2)_{11}$), 1.18−1.40 (m, $CH_2CH_2CH{=}C$), 2.01 (q, $C{=}CHCH_2$), 2.64 (q, $(CH_3CH_2)_2N$), 2.84 (t, NCH_2CH_2O), 3.97 (t, CH_2O), 5.81 (t, $C{=}CH$), 6.55−7.03 (4d, 8 ArH); MS *m*/*z* 508 $[M + 1]^+$. Anal. ($C_{34}H_{53}NO_2$) C, H, N.

(*E*)- and (*Z*)-1-[4-[2-(Pyrrolidinyl)ethoxy]phenyl]-1-(4-hydroxyphenyl)-1-heptene (5). The above procedure using 4-hydroxyheptanophenone and *p*-[2-(pyrrolidino)ethoxy]bromobenzene yielded a residual oil which crystallized in part from petroleum ether; mp 116 °C (*ex* MeOH). *E* isomer (95%): IR ($CHCl_3$) *v* OH 3580−3080 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.78 (t, $CH_3(CH_2)_4$), 1.10−1.40 (m, 6H, $CH_3(CH_2)_3$), 1.81 (br s, 4H, $CH_2(CH_2)_2CH_2$ (pyrrolidinyl ring)), 2.01 (q, $C{=}CHCH_2$), 2.66 (s, 4H, $(CH_2)_2N$), 2.90 (t, NCH_2CH_2O), 4.00 (t, CH_2O), 5.79 (t, C=CH), 6.44−7.00 (4d, 8 ArH); MS *m*/*z* 380 $[M + 1]^+$. Anal. ($C_{25}H_{33}NO_2$) C, H, N.

Concentration of the petroleum ether filtrate followed by purification by chromatography yielded the *Z* isomer (65%) as a white solid: mp 102−106 °C; ^1H NMR ($CDCl_3$) δ 0.78 (t, CH_3CH_2), 1.13−1.35 (m, 6H, $CH_3(CH_2)_3$), 1.80 (br s, 4H, $CH_2(CH_2)_2CH_2$ (pyrrolidinyl ring)), 1.99 (q, $C{=}CHCH_2$), 2.68 (s, 4H, $(CH_2)_2N$), 2.92 (t, NCH_2CH_2O), 4.05 (t, CH_2O), 5.80 (t, C=CH), 6.47−7.02 (m, 8 ArH).

[4-[2-(Diisopropylamino)ethoxy]phenyl](4-hydroxyphenyl)cyclohexylidenemethane (9). The procedure used 1-(4-hydroxyphenyl)cyclohexyl ketone and *p*-[2-(diisopropylamino)ethoxy]bromobenzene: yield 78%; mp 120 °C (benzene/petroleum ether 40−65 °C, 50/50); ^1H NMR ($CDCl_3$) δ 0.96 (d, 12H, $(CH_3)_2CH$), 1.51 (br s, 6H, $(CH_2)_3$ (3,4,5 of cyclohexyl ring)), 2.15 (br s, 4H, $C{=}C(CH_2)_2$), 1.65 (t, NCH_2CH_2O), 1.43 (sept, 2H, $N(CH)_2$), 3.79 (t, CH_2O), 6.63−7.12 (m, 8 ArH); MS *m*/*z* 408 $[M + 1]^+$. Anal ($C_{27}H_{37}NO_2$) C, H, N.

(*E*)- and (*Z*)-1-[4-[2-(Diethylamino)ethoxy]phenyl]-1-(4-hydroxyphenyl)-3-methyl-1-butene (10). The procedure used 4-hydroxyisobutanophenone. *E* isomer (95%): mp 120 °C (benzene/petroleum ether, 50/50); ^1H NMR ($CDCl_3$) δ 0.91 (d, 6H, $(CH_3)_2CH$), 1.03 (t, 6H, $(CH_3CH_2)_2N$), 2.33−2.44 (m, 1H, $CH(CH_3)_2$), 2.66 (q, 4H, $N(CH_2CH_3)_2$), 2.85 (t, NCH_2CH_2O), 3.97 (t, CH_2O), 5.62 (d, $C{=}CHCH(CH_3)_2$), 6.53−7.06 (m, 8 ArH); MS *m*/*z* 354 $[M + 1]^+$. Anal. ($C_{23}H_{31}NO_2$) C, H, N.

The *Z* isomer (83%) was obtained as above after purification by chromatography: mp 91 °C; ^1H NMR ($CDCl_3$) δ 0.91 (d, 6H, $(CH_3)_2CH$), 1.03 (t, 6H, $(CH_3CH_2)_2N$), 2.37−2.58 (m, 1H, $CH(CH_3)_2$), 2.63 (q, 4H, $N(CH_2CH_3)_2$), 2.85 (t, NCH_2CH_2O), 4.00 (t, CH_2O), 5.65 (d, 1H, $C{=}CHCH(CH_3)_2$), 6.61−7.02 (m, 8 ArH); MS *m*/*z* 354 $[M + 1]^+$. Anal. ($C_{23}H_{31}NO_2$) C, H, N.

(*E*)- and (*Z*)-1-[4-[2-(Dimethylamino)ethoxy]phenyl]-1-(4-hydroxyphenyl)-1-heptene (11). The procedure used 4-hydroxyheptanophenone and *p*-[2-(dimethylamino)ethoxy]-bromobenzene and gave a residual oil that partly crystallized. Repeated recrystallization (*ex* benzene/hexane, 50/50) yielded the *Z* isomer (75%): mp 125 °C; ^1H NMR ($CDCl_3$) δ 0.78 (t, $CH_3(CH_2)_4$), 1.16−1.32 (m, $CH_3(CH_2)_3$), 1.96 (q, $C{=}CHCH_2$), 2.33 (s, $N(CH_3)_2$), 2.74 (t, NCH_2CH_2O), 4.00 (t, CH_2O), 5.80 (t, C=CH), 6.44−7.01 (m, 8 ArH); MS *m*/*z* 354 $[M + 1]^+$. Anal. ($C_{23}H_{31}NO_2$) C, H, N.

A second crop of solid was collected and recrystallized several times (*ex* benzene/hexane in different proportions) to give the *E* isomer (58%): mp 91−3 °C; MS *m*/*z* 354 $[M + 1]^+$. Anal. ($C_{23}H_{31}NO_2$) C, H, N.

(*E*)- and (*Z*)-1-[4-[2-(Dimethylamino)ethoxy]phenyl]-1-(4-hydroxyphenyl)-1-hexene (12). The procedure used 4-hydroxypentanophenone and yielded a mixture of isomers (*E*/*Z*; 55/45%). Pure *E* isomer was obtained after repeated crystallization (*ex* benzene): mp 95 °C; ^1H NMR ($CDCl_3$) δ 0.77 (t, 3H, $CH_3(CH_2)_3$), 1.03 (t, 6H, $N(CH_2CH_3)_2$), 1.16−1.34 (m, 4H, $CH_3(CH_2)_2CH_2$), 2.02 (q, C=CH-CH_2), 2.66 (q, 4H, $N(CH_2CH_3)_2$), 2.85 (t, NCH_2CH_2O), 3.97 (t, CH_2O), 5.80 (t, $C{=}CH$), 6.53−7.02 (m, 8 ArH); MS *m*/*z* 368 $[M + 1]^+$. Anal. ($C_{24}H_{33}NO_2$) C, H, N. *Z* isomer (64%): mp 86 °C. ^1H NMR ($CDCl_3$) δ 0.77 (t, $CH_3(CH_2)_2$), 1.03 (t, 6H, $N(CH_2CH_3)_2$), 1.20−1.27 (m, 4H, $CH_3(CH_2)_2$), 2.01 (q, 2H, $C{=}CHCH_2$), 2.64 (q, 4H, $N(CH_2CH_3)_2$), 2.86 (t, N-CH_2-CH_2O), 4.00 (t, CH_2O), 5.81 (t, $C{=}CH$), 6.56−7.04 (m, 8 ArH); MS *m*/*z* 368 $[M + 1]^+$. Anal. ($C_{24}H_{33}NO_2$) C, H, N.

(*E*)- and (*Z*)-1-[4-[2-(Diethylamino)ethoxy]phenyl]-1-(4-hydroxyphenyl)-3-phenyl-1-propene (13). The procedure used 1-(4-hydroxyphenyl)-2-phenylethyl ketone. *Z* isomer (80%): mp 93−5 °C; ^1H NMR ($CDCl_3$) δ 1.02 (t, 6H, $N(CH_2CH_3)_2$), 2.63 (q, 4H, $N(CH_2CH_3)_2$), 2.85 (t, NCH_2CH_2O), 3.38 (d, CH_2Ar), 4.00 (t, CH_2O), 6.01 (t, C=CH), 6.61−7.13 (m, 8 ArH). MS *m*/*z* 402 $[M + 1]^+$. Anal. ($C_{27}H_{31}NO_2$) C, H, N. *E* isomer (69%): mp 124−5 °C (*ex* benzene); ^1H NMR ($CDCl_3$) δ 1.02 (t, 6H, $N(CH_2CH_3)_2$), 2.64 (q, 4H, $N(CH_2CH_3)_2$), 2.84 (t, NCH_2CH_2O), 3.39 (d, $CH_2C_6H_5$), 3.96 (t, CH_2O), 6.00 (t, C=CH), 6.53−7.20 (m, 8 ArH); MS *m*/*z* 402 $[M + 1]^+$. Anal. ($C_{27}H_{31}NO_2$) C, H, N.

(*E*)- and (*Z*)-1-[4-[2-(Diethylamino)ethoxy]phenyl]-1-(4-hydroxyphenyl)-2-ethyl-1-hexene (14). The procedure used 1-(4-hydroxyphenyl)-2-ethylpentyl ketone. The isomers could not be separated either by solubility differences or by chromatography: mp 80 °C (*ex* CH_3OH/diisopropyl ether, 1/3); ^1H NMR (300 MHz) (CD_3OD) δ 0.82 (t, 3H, $CH_3(CH_2)_3$), 0.977 and 0.980 (2t, $C{=}CH_2CH_3$ (*E*/*Z*, 50/50)), 1.08 (t, 6H, $N(CH_2CH_3)_2$), 1.23 (sext, 2H, $(CH_2)_2CH_2CH_3$), 1.39 (quint, 2H, $CH_2CH_2CH_2CH_3$), 2.08−2.14 (m, 4H, $C{=}C(CH_2)_2$), 2.68 (q, 4H, $N(CH_2CH_3)_2$), 2.89 (t, NCH_2CH_2O), 4.02 (t, CH_2O), 6.68−6.99 (m, 8 ArH); MS *m*/*z* 396 $[M + 1]^+$. Anal. . ($C_{26}H_{37}NO_2$) C, H, N.

1,1-Bis[4-[2-(diethylamino)ethoxy]phenyl]-1-heptene (6). A solution of *p*-[2-(diethylamino)ethoxy]bromobenzene (25 g, 0.092 mol) in anhydrous THF (50 mL) was added slowly under an argon atmosphere to magnesium turnings (2.3 g, 0.094 atom) in anhydrous ether (15 mL). The Grignard reagent was heated at reflux for 2 h and allowed to cool, and a solution of methyl heptanoate (4.40 g, 0.0306 mol) in THF (50 mL) was then added slowly at 40 °C. The reaction mixture was stirred and heated at reflux for 3 h. After cooling, addition of 1 N HCl, and removal of solvent, the aqueous solution was extracted with ether to eliminate the unreacted ester. The aqueous phase was alkalinized with 5% NaOH and extracted with ether. The organic layer was washed, dried ($MgSO_4$), and evaporated to give a crude oil (19.5 g) which was distilled. The 240−245 °C (0.04 mmHg) fraction was isolated (11 g, 74.8%): ^1H NMR ($CDCl_3$) δ 0.78 (t, 3H, $CH_3(CH_2)_4$), 0.98 and 1.01 (2t, 12H, $(CH_3CH_2)N$), 1.04−1.37 (m, 6H, $CH_3(CH_2)_3$), 2.01 (q, 2H, $C{=}CHCH_2$), 2.50−2.64 (m, 8H, $(CH_3CH_2)N$), 2.78 and 2.81 (2t, 4H, NCH_2CH_2O), 3.95 and 3.99 (2t, 4H, CH_2O), 5.85 (t, C=CH), 6.69−7.07 (m, 8 ArH); MS *m*/*z* 481 $[M + 1]^+$. Anal. ($C_{31}H_{48}N_2O_2$) C, H, N.

1,1-Bis[4-[2-(diisopropylamino)ethoxy]phenyl]-3-methyl-1-butene (7). 1,1-Bis(4-hydroxyphenyl)-3-methyl-1-butene was prepared by the action of pyridine hydrochloride for 45 min at 200−220 °C on 1,1-bis(4-methoxyphenyl)-3-methyl-1-butene obtained by reacting ethyl isovalerate with *p*-anisylmagnesium bromide (yield 85%; F 174 °C (diluted EtOH). 2-(Diisopropylamino)ethyl chloride hydrochloride (12.7 g, 0.0635 mol) was added to a mixture of the 1,1-bis(4-hydroxyphenyl)-3-methyl-1-butene (6.5 g, 0.0257 mol) in toluene (100 mL) and NaOH (5.08 g, 0.127 mol/15 mL) under continual stirring. The reaction mixture was heated at 90 °C for 16 h and then poured into water. The organic phase was separated and the aqueous phase extracted with toluene. The extracts were washed with 5% NaOH and water, dried ($MgSO_4$), and concentrated to an oil. Upon distillation, the 210−218 °C (0.01 mmHg) fraction (10.4 g, 80%) was isolated: ^1H NMR ($CDCl_3$) δ 0.90−1.00 (m, 30H, CH_3), 2.32 and 2.45 (oct, 1H, $C{=}CHCH(CH_3)_2$)), 2.72 and 2.76 (2t, 4H, NCH_2CH_2O), 2.97 (sept, 4H, $NCH(CH_3)_2$), 3.79 and 3.83 (2t, 4H, CH_2O), 5.64 (d, 1H, $C{=}CHCH(CH_3)_2$), 6.67−7.07 (m, 8 ArH). Anal. ($C_{33}H_{52}N_2O_2$) C, H, N.

Bis[4-[2-(diisopropylamino)ethoxy]phenyl]cyclohexylidenemethane (8). The procedure was analogous to that used to prepare **7**. Bis(4-hydroxyphenyl)-cyclohexylidenemethane was prepared by the action of pyridine hydrochloride on bis(4-methoxyphenyl)cyclohexylidenemethane previously obtained by condensation of cyclohexanecarboxylic acid ethyl ester with *p*-anisylmagnesium bromide (yield 87%; mp 240 °C). The title compound was prepared by condensation of the dihydroxy derivative with 2-(diisopropylamino)ethyl chloride. Upon distillation of the residual oil, fraction 248−254 °C (0.01 mmHg) was isolated. This oil (77%) crystallized slowly: mp 75 °C; ^{1}H NMR ($CDCl_3$) δ 0.94−0.98 (2s, 24H, CH_3), 1.51 (br s, 6H, $(CH_2)_3$ (3,4,5 of cyclohexyl ring)), 2.16 (s, 4H, C=C-$(CH_2)_2$), 2.72 (2t, 4H, NCH_2), 2.95 (sept, 4H, N$CH(CH_3)_2$), 3.79 (t, 4H, CH_2O), 6.62−6.95 (m, 8 ArH). Anal. ($C_{35}H_{54}N_2O_2$) C, H, N.

X-ray Crystallography. Suitable crystals of **1E** (0.75 × 0.65 × 0.25 cm) were grown from benzene. Data were collected on a Philips PW1100 diffractometer equipped with Mo Kα radiation and a graphite monochromator. The structures were solved by direct methods and refined in a full matrix with the program CRYSTALS. All non-hydrogen atoms were refined anisotropically. A total of 3941 reflections were obtained; of these, 1694 were observed with $I \geq 3\,\sigma(I)$. Abbreviated crystal data are as follows: $C_{25}H_{35}NO_2$, space group $P2_1/a$, a = 25.969(15) Å, b = 13.138(9) Å, c = 6.676(1) Å, V = 2274 Å^3, Z = 4, F_{000} = 832 e$^-$, μ = 0.65 cm^{-1}, density (calc) = 1.12 g/cm^3, 2θ range 1−25°. No absorption corrections were applied. Final conventional and weighted R values were 0.061 and 0.060, respectively.

Storage and Isomerisation of Compounds. Stock 10 mM solutions of the above test compounds and of reference compounds ((*Z*)-4-hydroxytamoxifen and (*Z*)-tamoxifen kindly supplied by Dr. A. E. Wakeling, ICI, Macclesfield, U.K.) in 96% alcohol were stored at 4 °C in the dark and checked before use by thin-layer chromatography (CH_2Cl_2/hexane, 90:10, v/v) or by high-performance liquid chromatography (HPLC) with a Waters system equipped with a UV detector set at 280 nm. The isomers were separated on a 5-mm radial pack silica gel column using chloroform/methanol/ethylamine/water (98:1.5:0.35:0.15, v/v, for **1E/Z** and **2E/Z** and 98.5:1.0:0.35:0.15, v/v, for the remaining isomers).

Isomerization rates under *in vitro* assay conditions were studied as follows: 100 μM of each isomer in phosphate-buffered saline, pH 7.4, were left to stand for 72 h at 37 °C. Samples (4 mL) were extracted twice with chloroform. Extracts were dried under a gentle stream of nitrogen, dissolved in the corresponding HPLC solvent system, and then analyzed by HPLC.

Cell Cultures. MCF_7 human breast cancer cells were routinely cultured at 37 °C in an atmosphere of 95% humidity and 5% CO_2 in DMEM medium (Gibco-BRL, Cergy Pontoise, France) and HEPES buffer supplemented with 1 nM insulin and 5% fetal calf serum. MVLN cells were established by transfecting MCF_7 cells with the pVit-tk-Luc plasmid. In this plasmid, the −331/−87 fragment of the 5′ flanking region of *Xenopus* vitellogenin A2 gene, which contains the estrogen receptor response element, is inserted upstream of the *Herpes simplex* virus thymidine kinase (tk) promoter which drives the firefly luciferase gene (Luc).[29] MVLN cells were routinely cultured in the same medium as MCF7 cells.

Relative Binding Affinities (RBAs) for the Estrogen Receptor (ER). The experiments were performed on immature lamb uterus cytosol. The test compounds were coincubated with 1 nM [6,7-^{3}H]-17β-estradiol for 5 h at 25 °C. The RBAs for ER (RBA of estradiol = 100%) were measured by a competition method as previously described.[23]

MCF_7 Cell Growth. MCF_7 cells were grown for 3−5 days in phenol red-free DMEM medium containing 3% dextran-coated charcoal-treated fetal calf serum in the presence or absence of 10^{-6} M estradiol. Cells were plated in 24-well tissue culture cluster plates at a density of 25 000 to 30 000 cells per well. The test compounds were added at concentrations of 10^{-6}−10^{-5} M (ethanol concentration <0.1%). Triplicate wells for each test-compound concentration were incubated for 9−12 days (the culture medium was replaced every 3 days), after which time DNA content was assayed by the method of DAPI.[33] The cytostatic/cytotoxic effects on cell proliferation were expressed as the compound concentration inhibiting growth by 50% (IC50) or decreasing cell content to below seeding level.

MVLN Cell Luciferase Expression. MVLN cells in phenol red-free DMEM medium containing 2% dextran-coated charcoal-treated fetal calf serum were plated in 24-well tissue culture cluster plates (80 000 cells/well). After 24 or 48 h, 0.5% 4-hydroxytamoxifen was added for 12−24 h in order to decrease the basal level of luciferase expression. The medium was then removed and replaced by a phenol red and serum-free medium containing different concentrations of the test compound either alone (<1% ethanol) to measure estrogenic activity or together with 0.1 nM estradiol to measure antiestrogenic activity. After incubation for 14−16 h, the cells were rinsed, harvested and lyzed with 500 μL of luminescence buffer (15 mM K_2PO_4, 8 mM $MgCl_2$, 2 mM ATP, 1% Triton X-100, pH 7.4). The lyzed cells were stirred for 30 min, and the luminescence emitted during 15 s was measured in a Wallac-LKB 1251 luminometer on a 200 μL aliquot to which 100 μL luciferin and coenzyme A (final concentrations 0.2 μM) had been added.

Correspondence Factor Analysis (CFA). CFA is used to compare specificity profiles and can be likened to a unified 2-fold principal components analysis performed on the compounds, on the one hand, and on the tests, on the other. The method is described in detail in several papers.[23−27] The present study concerns a dual CFA,[26,34] i.e., each column of the 22 × 6 data table (22 compounds (rows) by 6 tests (columns)) was split into two subcolumns giving the experimental values (see Table 2) and antivalues calculated by subtracting each experimental value from the maximal value recorded in the column. This procedure introduces the notion of amplitude of response into the specificity comparison. The data were normalized and then converted into a χ^2 distance semimatrix which was analyzed by a CFA program adapted for BASIC (Microsoft Language) from FORTRAN ANACOR software and written by one of us (J.C.D.). Commercial CFA programs are also available (e.g. SAS/STAT User's Guide, Vol 1; ANOVA-FREQ, Version 6, 1990, SAS Institute Inc., Cary, NC).

A minimum spanning tree, which is the shortest pathway linking the molecules into a network with no backtracking nor loops, was calculated by applying Prim's algorithm[30] to the χ^2 distance matrix derived from the split data table as in previous studies.[19,34]

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Supporting Information Available: Boiling or melting points and yields of intermediate alkyl acid phenyl esters and hydroxyphenyl alkyl ketones are available as well as X-ray crystallography data for **1E**: fractional atomic coordinates, anisotropic thermal parameters, and interatomic bond lengths and angles (5 pages). Ordering information is given on any current masthead page.

References

(1) Darbre, P. D.; Daly, R. J. Transition of human breast cancer cells from an oestrogen responsive to unresponsive state. *J. Steroid Biochem. Mol. Biol.* **1990**, *37*, 753−763.
(2) Murphy, L. C. Estrogen receptor variants in human breast cancer. *Mol. Cell. Endocrinol.* **1990**, *74*, C83−C86.
(3) Horwitz, K. B. Mechanisms of hormone resistance in breast cancer. *Breast Cancer Res. Treat.* **1993,** *26*, 119−130.
(4) Wolf, D. M.; Jordan, V. C. Drug resistance to tamoxifen during breast cancer therapy. *Breast Cancer Res. Treat.* **1993,** *27*, 27−40.
(5) Badia, E.; Duchesne, M. J.; Fournier-Bidoz, S.; Simar-Blanchet, A. E.; Terouanne, B.; Nicolas, J. C.; Pons, M. Hydroxytamoxifen induces a rapid and irreversible inactivation of an estrogenic response in an MCF-7-derived cell line. *Cancer Res.* **1994**, *54*, 5860−5866.

(6) van Agthoven, T.; van Agthoven, T. L. A.; Dekker, A.; Foekens, J. A.; Dorssers, L. C. J. Induction of estrogen independence of ZR-75-1 human breast cancer cells by epigenetic alterations. *Mol. Endocrinol.* **1994**, *8*, 1474−1483.
(7) Bardon, S.; Vignon, F.; Derocq, D.; Rochefort, H. The antiproliferative effect of tamoxifen in breast cancer cells: mediation by the estrogen receptor. *Mol. Cell. Endocrinol.* **1984**, *35* , 89−96.
(8) Taylor, C. M.; Blanchard, B.; Zava, D. T. Estrogen receptor-mediated and cytotoxic effects of the antiestrogens tamoxifen and 4-hydroxytamoxifen. *Cancer Res.* **1984**, *44*, 1409−1414.
(9) Sutherland, R. L.; Watts, C. K. W.; Hall, R. E.; Ruenitz, P. C. Mechanisms of growth inhibition by nonsteroidal antioestrogens in human breast cancer cells. *J. Steroid Biochem.* **1987**, *27*, 891−897.
(10) Clarke, R.; van den Berg, H. W.; Nelson, J.; Murphy R. F. Pharmacological and suprapharmacological concentrations of both 17β-oestradiol and tamoxifen reduce the membrane fluidity of MCF-7 and MDA-MB-436 human breast cancer cells. *Biochem. Soc. Trans.* **1987**, *15*, 243−244.
(11) van der Koedijk, C. D. M. A.; Vis van Heemst, C.; Elsendoorn, G. M.; Thijssen, J. H. H.; Blankenstein, M. A. Comparative affinity of steroidal and non-steroidal antiestrogens, cholesterol derivatives and compounds with a dialkylamino side chain for the rat liver antiestrogen binding site. *Biochem. Pharmacol.* **1992**, *43*, 2511−2518.
(12) Rowlands, M. G.; Parr, I. B.; McCague, R.; Jarman, M.; Goddard, P. Variation of the inhibition of calmodulin dependent cAMP phosphodiesterase amongst analogues of tamoxifen; correlations with cytotoxicity. *Biochem. Pharmacol.* **1990**, *40*, 283−289.
(13) O'Brian, C. A.; Liskamp, R. M.; Solomon, D. H.; Weinstein I. B. Triphenyl ethylenes: a new class of protein kinase C inhibitors. *J. Natl. Cancer Inst.* **1986**, *76*, 1243−1246.
(14) Bignon, E.; Pons, M.; Gilbert, J.; Nishizuka, Y. Multiple mechanisms of protein kinase C inhibition by triphenylacrylonitrile antiestrogens. *FEBS Lett.* **1990**, *271*, 54−58.
(15) Ojasoo, T.; Bignon, E.; Crastes de Paulet, A.; Doré, J. C.; Gilbert, J.; Miquel, J. F.; Pons, M.; Raynaud, J. P. Relative involvement of the estrogen receptor and protein kinase C in the action of a population of triphenylethylenes on MCF7 cell proliferation as revealed by correspondence factorial analysis (CFA). *J. Steroid Biochem. Mol. Biol.* **1993,** *44*, 239−250.
(16) Astruc, M. E.; Chabert, C.; Bali, P.; Gagne, D.; Pons, M. Prolonged treatment of breast cancer cells with antiestrogens increases the AP1-mediated response: involvement of the estrogen receptor. *Endocrinology* **1995,** *136*, 824−832.
(17) Early Breast Cancer Trialists' Collaborative Group. Systemic treatment of early breast cancer by hormonal, cytotoxic or immune therapy. *Lancet* **1992**, *339*, 1−15, 71−85.
(18) Lundgren, S. Prospects in endocrine treatment of breast cancer. *Cancer J.* **1994**, *7*, 220−227.
(19) Doré, J. C.; Gilbert, J.; Bignon, E.; Crastes de Paulet, A.; Ojasoo, T.; Pons, M.; Raynaud, J. P.; Miquel, J. F. Multivariate analysis by the minimum spanning tree method of the structural determinants of diphenylethylenes and triphenylacrylonitriles implicated in estrogen receptor binding, protein kinase C activity, and MCF_7 cell proliferation. *J. Med. Chem.* **1992**, *35*, 573−583.
(20) Gilbert, J.; Doré, J. C.; Bignon, E.; Pons, M.; Ojasoo, T. Study of the effects of basic di- and tri-phenyl derivatives on malignant cell proliferation. An example of the application of correspondence factor analysis to structure-activity relationships. *Quant. Struct. Act. Rel.* **1994**, *13*, 262−274.
(21) Durani, S.; Anand, N. A possible basis for structure-function relationship of estrogens. *Int. J. Quant. Chem.* **1981**, *20*, 71−83.
(22) Garg, S.; Bindal R. D.; Durani, S.; Kapil, R. S. Structure-activity relationship of estrogens: a study involving cyclofenil as the model compound. *J. Steroid Biochem.* **1983,** *18*, 89−95.
(23) Bignon, E.; Pons, M.; Crastes de Paulet, A.; Doré, J. C.; Gilbert, J.; Abecassis, J.; Miquel, J. F.; Ojasoo, T.; Raynaud, J. P. Effect of triphenylacrylonitrile derivatives on estradiol receptor binding and on human breast cancer cell growth. *J. Med. Chem.* **1989**, *32*, 2092−2103.
(24) Doré, J. C.; Gilbert, J.; Ojasoo, T.; Raynaud, J. P. Correspondence analysis applied to steroid receptor binding. *J. Med. Chem.* **1986**, *29*, 54−60.
(25) Ojasoo, T.; Doré, J. C.; Gilbert, J.; Raynaud, J. P. Binding of steroids to the progestin and glucocorticoid receptors analyzed by correspondence analysis. *J. Med. Chem.* **1988**, *31*, 1160−1169.
(26) Doré, J. C.; Ojasoo, T. Molecular taxonomy by Correspondence Factorial Analysis (CFA) In *Advanced Computer-Assisted Techniques in Drug discovery*; van de Waterbeemd, H., Ed.; VCH: Weinheim, 1994; pp 190−227.
(27) Ojasoo, T.; Raynaud, J. P.; Doré, J. C. Correspondence factor analysis of steroid libraries. *Steroids* **1995**, *60*, 458−469.
(28) Pons, M.; Gagne, D.; Nicolas, J. C.; Methali, M. A new cellular model of response to estrogens: a bioluminescent test to characterize (anti)estrogen molecules. *Biotechniques* **1991**, *9*, 450−459.
(29) Demirpence, E.; Balaguer, P.; Trousse, F.; Badia, E.; Gagne, D.; Pons, M. MVLN cells: a bioluminescent MCF-7-derived cell line to study the modulation of estrogenic activity. *J. Steroid Biochem. Mol. Biol.* **1993**, *46*, 355−364.
(30) Prim, R. C. Shortest connection networks and some generalisations. *Bell. Syst. Technol. J.* **1957**, *36*, 1389−1401.
(31) Boissier, J. R.; Ratouis, R.; Dumont, C.; Lesbros, J.; Lavaux J. P. New hypercholesterenic tertiary alcohols. *Arzeim. Forsch.* **1967**, *17*, 1193−1196.
(32) Boissier, J. R.; Ratouis, R. 4-(2-diethylaminoethoxy)phenyl ketones, Pharmaceutical intermediates : French patent no. 1,447,252, July 29, 1966.
(33) Brunck, C. F.; Jones, K. C.; James, T. W. Assay for nanogram quantities of DNA in cellular homogenates. *Anal. Biochem.* **1979**, *92*, 497−500.
(34) Bignon, E.; Pons, M.; Doré, J. C.; Gilbert, J.; Ojasoo, T.; Miquel, J. F.; Raynaud, J. P.; Crastes de Paulet, A. Influence of di- and tri-phenylethylene estrogen/ antiestrogen structure on the mechanisms of protein kinase C inhibition and activation as revealed by a multivariate analysis. *Biochem. Pharmacol.* **1991**, *42*, 1373−1383.

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