Peaks at δ 7.25 and 1.63 were attributed to $CHCl_3$ and $H_2O$, respectively. Computer-assisted determination of the areas of the 8-CH peak and an adjacent upfield small peak before and after the addition of 5% by weight of a 1:1 mixture of diastereomers (see below) indicated that the enantiomerical purity of the [S-(R*,S*)] isomer was greater than 99%. Anal. ($C_{30}H_{30}N_6O_3$·$0.5CHCl_3$·$1.2H_2O$) C, H, N.

The diastereomers (**1c**) were prepared as described above from **1a** (12.5 mg, 0.038 mmol) and (R)-1-(1-naphthyl)ethyl isocyanate (12.5 μL, 14.0 mg, 0.071 mmol): yield, 16.2 mg (76%); mp, gradual decomposition above 220 °C; $^1H$ NMR ($CDCl_3$) δ 1.25 (q, 2-$CH_3$), 1.33 (t, $CH_3CH_2O$), 1.80 (d, $CH_3CHN$), 4.19 (m, $CH_2O$), 4.56 (br s, 1-NH), 4.82 (m, 2-CH), 5.82 (quin, $CH_3CHN$), 6.39 (d, 5-NH), 6.77 (d, 8-CH), 7.87 (m, aromatic CH), 8.15 (7-NH), 9.70 (t, $NHCHCH_3$). Both the 5-NH and 8-CH were observed as two resolved peaks and the latter indicated a 1:1 mixture of diastereomers. Anal. ($C_{30}H_{30}N_6O_3$·$0.27CHCl_3$·$0.4H_2O$) C, H, N.

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# Effect of Triphenylacrylonitrile Derivatives on Estradiol-Receptor Binding and on Human Breast Cancer Cell Growth

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In a study of a series of 26 triphenylacrylonitrile derivatives (TPEs), we investigated the influence of several possibly interrelated factors on the proliferation of human breast cancer cell lines. (1) *Chemical substituents*: the test compounds were for the most part para-hydroxylated with increasingly bulky hydrophobic and/or basic side chains [isopropyloxy or (diethylamino)ethoxy] or standard reference compounds. (2) *Relative binding affinities (RBAs)*: they competed diversely for [$^3H$]estradiol ($E_2$) binding to calf uterus cytosol and little, if at all, for binding to the [$^3H$]tamoxifen-labeled antiestrogen binding site (AEBS) in lower speed supernatant. A multiparametric comparison of RBAs recorded for calf, rat, and mouse uterus cytosol estrogen receptor (ER) revealed a possible influence of species-specific receptor conformation and/or environment on binding. (3) *Estrogen/antiestrogen potency*: their stimulation and inhibition of the proliferation of the ER-positive human breast cancer cell line ($MCF_7$) was measured. Compounds with only hydroxy substituents stimulated proliferation more markedly than methylated derivatives and had a maximum effect at $10^{-11}$–$10^{-6}$ M. Stimulation was related to the RBA for ER. Compounds with isopropyloxy or (diethylamino)ethoxy side chains only weakly stimulated $MCF_7$ cell growth and more powerfully antagonized $E_2$-promoted growth. The extent of inhibition depended upon the bulk of the side chain and could be reversed by $10^{-7}$ M $E_2$. Within the same concentration ranges, the test compounds were without effect on the $BT_{20}$ ER-negative cell line. (4) *Cytostatic and/or cytolytic activity*: most compounds could arrest the proliferation of both $MCF_7$ and $BT_{20}$ cells at concentrations above $3 \times 10^{-6}$ M. This activity was thus independent of ER. Nevertheless, those compounds with a charged hydrophobic side chain, which were the most powerful antagonists of $E_2$-promoted cell growth, were also the most cytotoxic. The overall results for all molecules on all parameters were submitted to a multivariate analysis (correspondence analysis) which revealed the progressive influence of increasing substitution by hydroxy and more bulky groups on the generation of antagonist activity and cytotoxicity.

The growth of benign and neoplastic breast tumor tissue is under the influence of several hormones (insulin, hcG, prolactin, steroid hormones) and of different growth factors such as epidermal, transforming, and insulin growth factors (for a review, see ref 1). Receptors for these hormones and factors have been identified in a much investigated cell line $MCF_7$ derived from a human breast cancer metastasis. Several studies have suggested that the antiproliferative action of triphenylethylene derivatives (TPEs) on the growth of estrogen-dependent tumors might be mediated by the estrogen receptor (ER).[2-6] The observations that have led to this hypothesis are (1) these TPEs can have considerable affinity for ER apart from actions on other molecular targets,[5,7-14] (2) the compounds with the highest affinity for ER are growth inhibitory at the lowest concentrations,[2,13,15-18] (3) this inhibition is abolished in the presence of estradiol,[5,13,17,19-21] and (4) ER-negative cells are less sensitive to TPEs.[6,11,20,22-24]

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Despite the widely reported success of the archetype TPE tamoxifen, the search for a more effective inhibitor of human mammary tumor proliferation continues since many tumors are resistant to this agent. Although several new lead compounds have been experimented with over the last few years, little is known about the specific structural features that determine ER binding, growth inhibition, and cytotoxicity. In the case of tamoxifen, it would be the 4-OH metabolite that is particularly active, the hydroxy group favoring ER binding and the basic side chain promoting growth inhibition and/or cytotoxicity.

In order to identify the structural features that might be implicated in effective antagonism of estrogen-promoted $MCF_7$ cell proliferation, we have undertaken a systematic structure–affinity–activity study of a series of homologous hydroxylated triphenylacrylonitrile derivatives. Some of these compounds have already been studied for their ability to bind to ER[25-29] and to induce progesterone receptors.[28,29] The methylated derivatives also bound to other molecular targets such as prostaglandin synthase[26,29,30] and glutamate dehydrogenase[32] but only at high concentrations; none bound to the antiestrogen binding site (AEBS).[26] Prostaglandins control cell growth[33] and a possible role of AEBS in growth control has been postulated[23,24,34] but recently contested.[35,36]

In the present study, we have investigated the capacity of an enlarged series of test compounds, several with bulky hydrophobic substituents (Table I), to bind to calf uterus ER and AEBS and to stimulate the proliferation of ER-positive $MCF_7$ cells or to inhibit the $E_2$-promoted growth of these cells. We have kept to discrete stepwise modifications in structure in order to preferentially discern effects on proliferation mediated by ER as opposed to other molecular targets. Results have been analyzed by both a classical approach and by a more appropriate multiparametric analysis.

Since cytotoxic agents are a classic therapy of hormone-dependent neoplasia, we have also investigated the high dose cytotoxicity of these compounds in order to establish whether high antagonist and cytotoxic activities are related and/or can be combined to obtain site-directed agents.

## Results and Discussion

**Chemistry.** Compounds **1–9** were prepared as previously described.[31] Isomers **10*E*/*Z*** were the products of partial demethylation of the corresponding bis-ether; **11–14** were obtained by the reaction of alkyl or aminoalkyl halides with the 3,3-bis-phenol in the presence of sodium ethoxide. Compound **15** was similarly obtained from the 2-(*p*-hydroxyphenyl)-3,3-diphenylacrylonitrile. Compounds **16** and **19** were prepared by a published method[39] as was deacetylated cyclofenil.[40] Access to (dimethylamino)methyl compounds **17** and **18** was by reaction of 2-phenyl-3,3-bis(*p*-hydroxyphenyl)acrylonitrile with dimethylamine and formaldehyde according to a known procedure.[41]

**Configuration Assignment and Isomeric Purity.** The configurations of all the TPE isomers were defined according to the classic *E*/*Z* rules as these are unambiguous. The conformation of isomer **7*Z*** has been established by X-ray crystallography.[31] The geometric isomers of compounds **10**, **11**, and **13** were determined by $^1H$ NMR analysis on the basis of the chemical shifts of the $OCH_3$ (**10**), $(CH_3)_2CHO$ (**11**) and (diethylamino)ethoxy (**13**) protons as previously used for the identification of the isomers of **2**, **5**, **7**, and **9**.[26,31] The proton chemical shift for any one group in this TPE series is higher when this group is bound to the $\alpha$-ring than to the $\alpha'$-ring.[18,42,43] The isomers of **10**, **11**, and **13** with the higher chemical shift were assigned the affix *Z*.

The majority of the TPE isomers were 95–98% pure. Before biological testing, small quantities (maximum of 2 mg) of those that were mixtures were purified by HPLC and stored in the dark at 4 °C as $3.10^{-3}$ M stock solutions in ethanol. The $\alpha'$-monohydroxylated isomers were eluted before the $\alpha$-hydroxylated isomers from a silica HPLC


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**Table I.** Physical and Biological Properties of 3,3,2-Triphenylacrylonitriles

DMA: $N(CH_3)_2$
DMAM: $CH_2N(CH_3)_2$
OiPr: $OCH(CH_3)_2$
DMAE: $O(CH_2)_2N(CH_3)_2$
DEAE: $O(CH_2)_2N(C_2H_5)_2$
TAM: tamoxifen ($C_2H_5$ instead of CN)[a]

| | | | | | | | % isomery[l] | | competition for $[^3H]E_2$ binding[m] | | | proliferation of $MCF_7$ cells[n] | | | | cytostatic/cytolytic effects[o] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | agonism | | antagonism vs 0.1 nM $E_2$ | | $MCF_7$ cells | | $BT_{20}$ cells | |
| compd | R(α) | $R_1$(α′) | $R_2$(β) | mp, °C | formula | anal. or ref | *P* | *I* | $RBA_1$ (2 h at 0 °C) | $RBA_2$ (5 h at 25 °C) | $RBA_1$/$RBA_2$ | $EC_{50}$, nM | % | $IC_{50}$, nM | % | $IC_{30}$, μM | % | $IC_{30}$, μM | % |
| **1** | H | H | H | 166–7 | $C_{21}H_{15}N$ | 37 | | | 0.04 ± 0.01 | 0.09 ± 0.04 | – | 28 ± 0.1 | 68 | nm[k] | 1 | nm | 16 | nm | 13 |
| **2*Z*** | OH | H | H | 193–4 | $C_{21}H_{15}NO$ | 38 | 98.5 | 3.5 | 40 ± 8 | 36 ± 11 | 0.9 | 0.19 ± 0.02 | 94 | nm | 13 | nm | 12 | nm | 10 |
| **2*E*** | H | OH | H | 203–5 | $C_{21}H_{15}NO$ | 38 | 99 | 9 | 3.7 ± 0.9 | 2.2 ± 0.8 | 0.59 | 0.40 ± 0.14 | 99 | nm | 5 | 10 | 30 | 8.3 | 36 |
| **3** | H | H | OH | 229 | $C_{21}H_{15}NO$ | 38 | | | 29 ± 4 | 3.3 ± 0.7 | 0.11 | 0.55 ± 0.05 | 90 | nm | 16 | nm | 8 | nm | 17 |
| **4** | OH | OH | H | 249 | $C_{21}H_{15}NO_2$ | 25 | | | 28 ± 4 | 62 ± 11 | 2.21 | 0.059 ± 0.012 | 62 | 3.5 ± 1.3 | 31 | 10 | 30 | 10 | 30 |
| **5*Z*** | OH | H | OH | 242[b] | $C_{21}H_{15}NO_2$ | 38 | 94 | 1 | 27 ± 5 | 74 ± 20 | 2.74 | 0.063 ± 0.003 | 98 | nm | 1 | nm | 17 | 9.4 | 32 |
| **5*E*** | H | OH | OH | 250[c] | $C_{21}H_{15}NO_2$ | 38 | 94 | 1.5 | 19 ± 3 | 6.1 ± 1.5 | 0.32 | 0.091 ± 0.009 | 100 | nm | 8 | 10 | 30 | 7.2 | 43 |
| **6** | OH | OH | OH | 289 | $C_{21}H_{15}NO_3$ | 31 | | | 41 ± 6 | 166 ± 7 | 4.04 | 0.036 ± 0.003 | 85 | 9.5 ± 0.7 | 31 | 8 | 39 | 6.8 | 42 |
| **7*Z*** | OH | $CH_3$ | H | 209 | $C_{22}H_{17}NO$ | 31 | 99 | 1.5 | 29 ± 4 | 28 ± 5 | 0.96 | 0.28 ± 0.08 | 55 | 17 ± 4 | 59 | 8.5 | 38 | 8.4 | 32 |
| **7*E*** | $CH_3$ | OH | H | 192[d] | $C_{22}H_{17}NO$ | 31 | 97 | 15 | 8.1 ± 0.8 | 2.5 ± 0.6 | 0.30 | 0.42 ± 0.04 | 53 | 52 ± 12 | 51 | 5.8 | 60 | 3.4 | 51 |
| **8** | OH | OH | $CH_3$ | 267 | $C_{22}H_{17}NO_2$ | 26 | | | 49 ± 9 | 93 ± 17 | 1.89 | 0.14 ± 0.01 | 71 | 4.8 ± 3.8 | 13 | 8 | 38 | 8.2 | 44 |
| **9*Z*** | OH | $CH_3$ | OH | 250 | $C_{22}H_{17}NO_2$ | 26 | 98.5 | 2.5 | 36 ± 9 | 78 ± 1 | 2.16 | 0.105 ± 0.02 | 69 | 32 ± 16 | 41 | 10 | 35 | 6.5 | 45 |
| **9*E*** | $CH_3$ | OH | OH | 269 | $C_{22}H_{17}NO_2$ | 26 | 97 | 6 | 28 ± 9 | 9.1 ± 1.6 | 0.32 | 0.14 ± 0.02 | 73 | 27 ± 6 | 36 | 6.8 | 54 | 6.3 | 48 |
| **10*Z*** | $OCH_3$ | OH | H | 186 | $C_{22}H_{17}NO_2$ | C,H,N | 100 | 2 | 6.1 ± 0.7 | 0.66 ± 0.13 | 0.10 | 0.20 ± 0.02 | 26 | 149 ± 28 | 80 | 9 | 36 | 2.7 | 47 |
| **10*E*** | OH | $OCH_3$ | H | 190 | $C_{22}H_{17}NO_2$ | C,H,N | 97 | 1 | 25 ± 2 | 17 ± 4 | 0.68 | 0.19 ± 0.02 | 25 | 34 ± 8 | 86 | nm | 13 | 6.8 | 39 |
| **11*Z*** | OiPr | OH | H | | $C_{24}H_{21}NO_2$ | | 99 | 2 | 3.0 ± 0.6 | 0.36 ± 0.09 | 0.12 | 0.12 ± 0.09 | 33 | 178 ± 21 | 81 | 5.2 | 61 | 3.9 | 60 |
| **11*E*** | OH | OiPr | H | 175–9[e] | $C_{24}H_{21}NO_2$ | C,H,N[f] | 99 | 2 | 7.9 ± 1.2 | 6.4 ± 1.3 | 0.81 | 0.18 ± 0.07 | 22 | 34 ± 20 | 98 | 5.2 | 57 | 5.1 | 47 |
| **12** | OiPr | OiPr | H | 138 | $C_{27}H_{27}NO_2$ | C,H,N | | | 0.01 ± 0.01 | 0.01 ± 0.01 | – | 5.0 ± 0.9 | 40 | nm | 1 | 9.5 | 32 | 18 | 19 |
| **13*Z*** | DEAE | OH | H | 166–7 | $C_{27}H_{29}N_2O_2$ | C,H,N | 100 | 2 | 3.6 ± 0.4 | 3.4 ± 0.4 | 0.94 | 0.030 ± 0.003 | 12 | 25 ± 1 | 100 | 3.9 | 89 | 3.5 | 94 |
| **13*E*** | OH | DEAE | H | 194–5 | $C_{27}H_{28}N_2O_2$ | C,H,N | 100 | 9 | 126 ± 8 | 108 ± 14 | 0.85 | 0.020 ± 0.005 | 16 | 4.6 ± 1 | 100 | 4.8 | 77 | 6.4 | 57 |
| **14** | DEAE | DEAE | H | *g* | $C_{33}H_{41}N_3O_2$ | C,H,N | | | 0.42 ± 0.05 | 0.40 ± 0.13 | 0.95 | 0.28 ± 0.01 | 62 | >1000 | 27 | 3.1 | 94 | 3.3 | 87 |
| **15** | H | H | DEAE | 93 | $C_{27}H_{28}N_2O$ | C,H,N | | | 0.17 ± 0.02 | 0.01 ± 0.01 | 0.05 | 25 ± 4 | 71 | nm | 6 | 4.8 | 53 | 9.7 | 38 |
| **16** | DMA | DMA | H | 189 | $C_{25}H_{25}N_3$ | 38 | | | 0.12 ± 0.02 | 0.04 ± 0.01 | 0.33 | 12 ± 10 | 85 | nm | 3 | 5.7 | 48 | 10 | 30 |
| **18** | OH | OH | H | 153 | $C_{27}H_{28}N_3O_2$ | C,H,N | | | 0.30 ± 0.08 | 0.01 ± 0.01 | 0.03 | 44 ± 13 | 31 | nm | 7 | 8.5 | 36 | nm | 15 |
| | 3DMAM | 3DMAM | | | | | | | | | | | | | | | | | |
| **19** | $OCH_3$ | $OCH_3$ | H | 158–9 | $C_{23}H_{19}NO_2$ | 39 | | | 0.17 ± 0.04 | 0.04 ± 0.01 | 0.24 | 102 ± 29 | 34 | nm | 6 | nm | 8 | nm | 10 |
| | | | | | | | | | Reference and Other Products | | | | | | | | | | |
| TAM Z | H | DMAE | H | | $C_{26}H_{23}NO$ | | nd[j] | | 13 ± 2 | 1.1 ± 0.2 | 0.08 | 0.11 ± 0.04 | 21 | 219 ± 55 | 90 | 6.5 | 49 | 5.7 | 56 |
| TAM E | DMAE | H | H | | $C_{26}H_{23}NO$ | | nd | | 0.17 ± 0.05 | 0.02 ± 0.00 | 0.12 | 11 ± 8 | 100 | nm | 1 | 4.3 | 79 | 6.4 | 52 |
| 4-OH-TAM Z | OH | DMAE | H | | $C_{26}H_{29}NO_2$ | | nd | | 123 ± 19 | 243 ± 43 | 1.97 | 0.003 (2) | 10 | 2.5 ± 0.1 | 100 | 4.0 | 81 | 4.8 | 65 |
| 4-OH-TAM E | DMAE | OH | H | | $C_{26}H_{29}NO_2$ | | nd | | 2.65 ± 0.39 | 2.28 ± 0.28 | 0.86 | 0.016 ± 0.006 | 61 | 22 ± 1 | 91 | 5.3 | 74 | 9.3 | 33 |
| DAC | OH | OH | *h* | 195–8 | $C_{20}H_{22}O_2$ | 40 | | | 26 ± 5 | 2.6 ± 0.3 | 0.1 | 4.5 ± 1.2 | 97 | nm | 23 | nm | 12 | nm | 12 |
| E2 | | | | | $C_{18}H_{24}O_2$ | | | | 100 | 100 | 1 | 0.02 ± 0.01 | 100 | – | 0 | nm | 25 | nm | 14 |
| 17 | OH | OH | H | 122[i] | $C_{24}H_{22}N_2O_2$ | C,H,N | 75:25 | | 3.9 (1) | 0.21 (1) | 0.05 | 14 (1) | 28 | >1000 | 28 | 10 | 30 | 10 | 30 |
| | 3DMAM | | | | | | | | | | | | | | | | | | |

[a] The change from CN to $C_2H_5$ leads to a reversal of the *E*/*Z* priority rules. [b] With 70% *Z* isomer, for melting point determination only. [c] With 70% *E* isomer, for melting point determination only. [d] With 68% *E* isomer, for melting point determination only. [e] With 57% *E* isomer, for melting point determination only. [f] Analyzed as an isomer mixture. [g] Bp $E_{0.04}$ 285–290. [h] Bis(*p*-hydroxyphenyl)cyclohexylidenemethane (deacetylated cyclofenil). [i] Isomer mixture (75:25). [j] Nd: not determined. [k] Nm: not measurable. [l] The isomeric purity (*P*), determined by HPLC, is that of the stock solutions used for biological testing. The isomeric interconversion (*I*) is the difference between the initial P and that determined after a 2-day incubation of an aqueous solution (pH 7.4, 37 °C). [m] The relative binding affinities (RBAs), determined under two sets of incubation conditions, are the means (±SEM) of at least four experimental values unless otherwise indicated in parentheses. [n] The effective ($EC_{50}$) and inhibitory ($IC_{50}$) concentrations giving 50% of the maximum response of the TPE were determined in two separate experiments. The percent agonist effect is expressed as a function of the maximum response recorded with $E_2$; the percent antagonist effect is the percent inhibition obtained at $10^{-6}$ M TPE versus $10^{-10}$ M $E_2$. [o] The $IC_{30}$ is the concentration that inhibits cell proliferation by 30%. The percent inhibition is that obtained in the presence of $10^{-5}$ M TPE (the controls (100%) were normal growth for $BT_{20}$ cells and growth in the presence of $10^{-6}$ M $E_2$ for $MCF_7$ cells). The results are the means of two experiments.

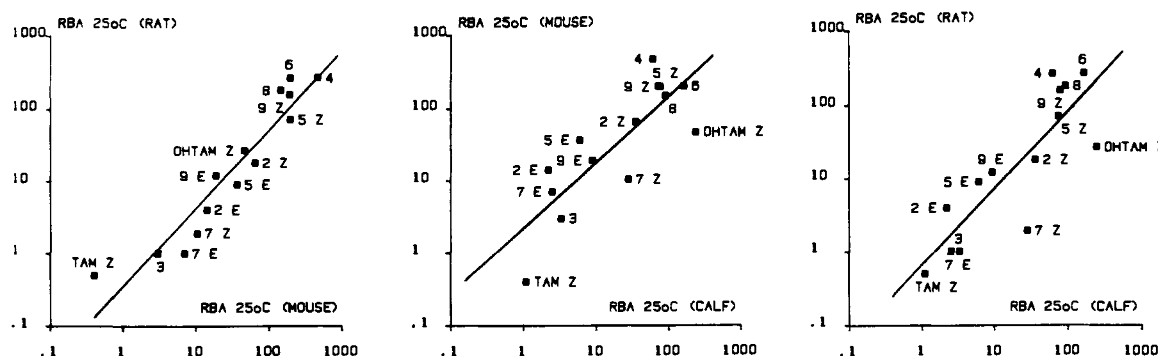


**Figure 1.** Two-by-two correlations by a least-squares regression method between the RBAs for ER of several TPEs (1–9*E*, TAM Z, 4-OH-TAM Z) as measured on mouse, rat, and calf cytosol: (a) slope = 1.08, $r$ = 0.95, (b) slope = 0.90, $r$ = 0.83, (c) slope = 1.05, $r$ = 0.85. In each case, $p < 0.01$.

**Table II.** Optical Properties of TPE Isomers at 280 nm

| | $\lambda_{max}$, nm | $\epsilon_{max}$ | $\epsilon_{280nm}$ |
|---|---|---|---|
| 2*E* | 340 | 16 500 | 13 300 |
| 2*Z* | 340 | 14 700 | 8 100 |
| 5*E* | 350 | 16 200 | 13 570 |
| 5*Z* | 350 | 16 400 | 8 200 |
| 7*E* | 340 | 15 100 | 13 100 |
| 7*Z* | 340 | 16 600 | 9 400 |
| 9*E* | 350 | 14 400 | 13 600 |
| 9*Z* | 350 | 14 200 | 8 900 |
| 10*Z* | 340 | 13 800 | 9 200 |
| 10*E* | 345 | 14 200 | 8 600 |
| 11*Z* | 345 | 15 000 | 9 700 |
| 11*E* | 345 | 15 400 | 9 300 |
| 13*Z* | 345 | 15 900 | 10 100 |
| 13*E* | 345 | 14 800 | 10 300 |

column by the mixture $CHCl_3/CH_3OH/(C_2H_5)_3N$ (95:3:2). On the contrary, the $\alpha,\beta$-dihydroxylated isomers were eluted before the $\alpha',\beta$-isomers by a 91:7:2 mixture. These observations were used to separate **10*E*/*Z*** and **11*E*/*Z***. In these cases, the *Z* isomer, as identified by NMR, was eluted before the *E* isomer.

Isomeric purity was determined by HPLC and UV analysis at 280 nm before or after 48-h incubation at pH 7.4 and 37 °C. Under these conditions, all isomers underwent less than 9% isomerization except for compound **7*E*** (15% isomerization). Compound **17** was an equilibrated 75–25% mixture of isomers. As isomers **2*E***, **5*E***, **7*E***, and **9*E*** have an optical density at 280 nm about 1.5 times higher than the corresponding *Z* isomers (Table II), this discrepancy was taken into account in determining the isomeric purity and the isomerization rate for each of these compounds.

**Interaction with the Estrogen Receptor (ER).** The relative binding affinities (RBAs) of the 26 test TPEs and of several reference compounds for calf uterus cytosol ER are given in Table I. RBAs were measured under two sets of incubation conditions, 2 h at 0 °C and 5 h at 25 °C, in order to obtain an indication of the kinetics of the interaction with ER. An increase in RBA with incubation temperature and time is indicative of slower dissociation kinetics than those observed for the reference hormone $E_2$.[44,45] All the test compounds competed for labeled $E_2$ binding although competition by the unsubstituted TPE **1** and by compounds **12**, **15**, **16**, and **18** was very low but nevertheless meaningful in view of the high sensitivity of the assay (lower limit 0.01). As previously shown[26] and as

**Table III.** Influence of Structural Modifications (a → b) of TPEs on RBAs for ER (Measured at 25 °C)

| structural modification a → b | TPEa | TPEb | RBA of TPEa | RBA of TPEb | RBAb/ RBAa |
|---|---|---|---|---|---|
| | | Hydroxylation | | | |
| α: H → OH | 1 | 2*Z* | 0.09 | 36 | 400 |
| | 2*E* | 4 | 2.2 | 62 | 28 |
| | 5*E* | 6 | 6.1 | 166 | 27 |
| | 3 | 5*Z* | 3.3 | 74 | 22 |
| α′: H → OH | 1 | 2*E* | 0.09 | 2.2 | 24 |
| | 2*Z* | 4 | 36 | 62 | 2 |
| | 5*Z* | 6 | 74 | 166 | 2 |
| | 3 | 5*E* | 3.3 | 6.1 | 2 |
| β: H → OH | 1 | 3 | 0.09 | 3.3 | 37 |
| | 2*Z* | 5*Z* | 36 | 74 | 2 |
| | 2*E* | 5*E* | 2.2 | 6.1 | 3 |
| | 7*Z* | 9*Z* | 28 | 78 | 3 |
| | 7*E* | 9*E* | 2.5 | 9.1 | 4 |
| | 4 | 6 | 62 | 166 | 3 |
| | | Other Substitutions | | | |
| α: H → OMe | 2*E* | 10*Z* | 2.2 | 0.66 | 0.3 |
| α′: H → OMe | 2*Z* | 10*E* | 36 | 17 | 0.5 |
| α: H → OiPr | 2*E* | 11*Z* | 2.2 | 0.36 | 0.2 |
| α′: H → OiPr | 2*Z* | 11*E* | 36 | 6.4 | 0.2 |
| α: H → Me | 2*E* | 7*E* | 2.2 | 2.5 | 1 |
| | 5*E* | 9*E* | 6.1 | 9.1 | 1.5 |
| α′: H → Me | 2*Z* | 7*Z* | 36 | 28 | 0.8 |
| | 5*Z* | 9*Z* | 74 | 78 | 1 |
| α: H → DEAE | 2*E* | 13*Z* | 2.2 | 3.4 | 1.5 |
| α′: H → DEAE | 2*Z* | 13*E* | 36 | 108 | 3 |
| β: H → Me | 4 | 8 | 62 | 93 | 1.5 |
| β: H → DEAE | 1 | 15 | 0.09 | 0.01 | 0.1 |
| | | Isomerization (α-X, α′-OH → α-OH, α′-X) | | | |
| X = | | | | | |
| H | 2*E* | 2*Z* | 2.2 | 36 | 16 |
| H (β-OH) | 5*E* | 5*Z* | 6.1 | 74 | 12 |
| Me | 7*E* | 7*Z* | 2.5 | 28 | 11 |
| Me (β-OH) | 9*E* | 9*Z* | 9.1 | 78 | 9 |
| OMe | 10*Z* | 10*E* | 0.66 | 17 | 26 |
| OiPr | 11*Z* | 11*E* | 0.36 | 6.4 | 18 |
| DEAE | 13*Z* | 13*E* | 3.4 | 108 | 32 |

illustrated in Table III, a hydroxy group in a para position of any phenyl ring of the unsubstituted TPE **1** engendered a considerable increase (24–400-fold) in the RBA at 25 °C. Hydroxylation of an already either mono- or dihydroxylated compound increased the RBAs 20–30-fold when the α-ring was hydroxylated, but only 2–4-fold when it was the α′ or β ring. The introduction of methyl groups had little influence.

The above results on calf uterus cytosol confirm our previous findings on the influence of hydroxy and methyl groups on binding in cytosol from immature rat and mouse uterus.[26,28] Figure 1 shows that the RBAs obtained for the different species are relatively well correlated[28] but, in

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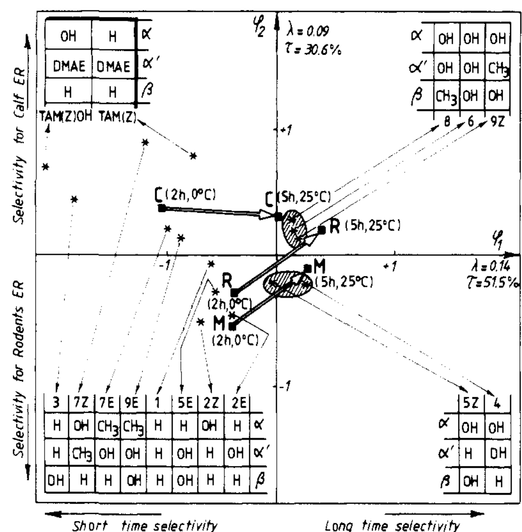


**Figure 2.** $\Phi_1\Phi_2$ distribution map obtained by correspondence analysis of the data in Figure 1. The compounds (*) are identified by their number and their $\alpha,\alpha',\beta$ substituents. The vectors highlight the relative positions of the RBAs (■) measured after long rather than short incubation times and at high rather than low temperatures (C = calf, R = rat, M = mouse). The slashed areas encircle dihydroxylated compounds with an OH-group on ring $\alpha$.

order to analyze the data in finer detail, we performed a multiparametric analysis based on $\chi^2$-metrics (correspondence analysis[46-48]), which has the advantage, over other methods, of depicting the fields of molecules (13 items, **1**–**9***E*) and receptors (ER in three species under two incubation conditions) on a single graph (Figure 2). The position of the items within the two fields is given by projections onto a set of factorial axes accounting for most of the variance of the system. In this instance, the first and second factorial axes, $\Phi_1$ and $\Phi_2$, account for 83% (52% + 31%) of the total variance. The multiparametric analysis confirms the greater similitude between the response profiles of rat and mouse ER, which at short-term incubation conditions are located in closer proximity to each other on the graph than to calf ER (Figure 2, Table IV). On increasing incubation time and temperature, however, all three vectors move in the same direction toward zones containing all dihydroxylated derivatives with a hydroxy group on the $\alpha$-ring, thus highlighting the relative importance of each hydroxy group in binding to ER and the need for two such groups for stable binding. These observations are akin to the known inequivalence of, but also requirement for, both hydroxy groups of $E_2$ in ER binding.[49-51] Several methylated TPEs seem to have a greater propensity for calf ER than rodent ER. This could indicate a slight difference in receptor environment (e.g. in free polyunsaturated fatty acid concentrations) or in receptor specificity as it is not yet known to what extent all four sequences of the cDNAs corresponding to the steroid binding domains of calf, rat[52], and mouse[53] uterine ER and of $MCF_7$[54] ER are homologous. The data on the two reference compounds tamoxifen (TAM Z) and 4-hydroxytamoxifen (4-OH-TAM Z), considered as supplementary variables, were introduced into the system. Both compounds show greatest affinity for the calf receptor.

Further novelty of the data in Table I resides in the study of compounds with bulky substituents. The intro-

**Table IV.** Absolute and Relative Contributions to the Factorial Axes of the Binding Parameters[a]

| | ABSOLUTE CONTRIBUTIONS | RELATIVE CONTRIBUTIONS $\cos^2\theta$ |
|---|---|---|
| $\Phi_1$ $\tau$ = 51.5% | | |
| Calf 2h 0°C | 55.3 % | 0.81 |
| Rat 5h 25°C | 19.8 % | 0.67 |
| Mouse 2h 0°C | 11.3 % | 0.30 |
| Mouse 5h 25°C | 8.5 % | 0.38 |
| Rat 2h 0°C | 5.0 % | 0.36 |
| Calf 5h 25°C | 0.1 % | 0.01 |
| $\Phi_2$ $\tau$ = 30.6% | | |
| Mouse 2h 0°C | 37.2 % | 0.58 |
| Calf 5h 25°C | 19.9 % | 0.54 |
| Calf 2h 0°C | 15.7 % | 0.14 |
| Rat 5h 25°C | 10.7% | 0.22 |
| Mouse 5h 25°C | 10.4 % | 0.28 |
| Rat 2h 0°C | 6.0 % | 0.26 |

[a] I.e., RBAs for ER in three species under two experimental conditions. $\lambda$: eigenvalue obtained by diagonalization of the symmetric matrix (correlation levels, 0–1, between the two fields). $\tau$: percent information (variance) associated with a given factor (total = 100% for all items). Absolute contribution (AC): extent to which an item contributes to the variance explained by a factor (total = 100% for all items of each field). $\cos^2\theta$ (relative contribution (RC)): extent, expressed as a percentage/100, to which an item is dispersed among factors (total = 1 for all factors). +, –: signs of the projection coordinates on the factorial axis.

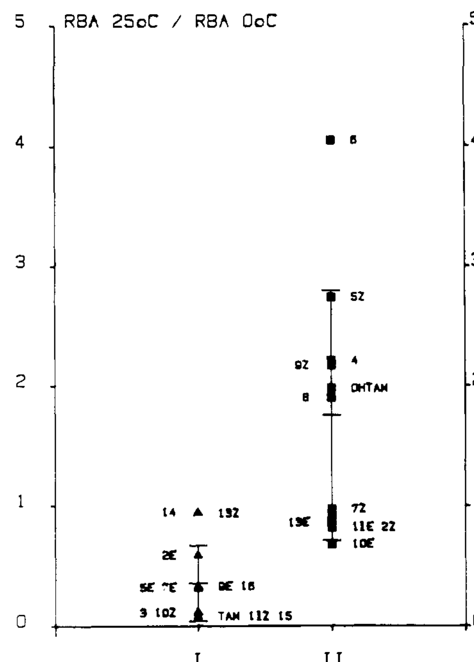


**Figure 3.** Influence of TPE structure on the ratio of the RBAs measured at 25 °C and 0 °C (mean ± SD). The two sets of compounds are distinguished by the absence (I, $n$ = 12) or presence (II, $n$ = 11) of a hydroxy group in position 4 of ring $\alpha$. The ratios for 17 and 18 (<0.05) have not been represented.

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**Table V.** RBAs of TPEs for AEBS of Calf Uterus

| | $\alpha$ | $\alpha'$ | $\beta$ | RBA,[a] 2 h, 0 °C |
|---|---|---|---|---|
| TAM Z[b] | H | DMAE | H | 100 |
| TAM E[b] | DMAE | H | H | 77 ± 28 (4) |
| 4-OH-TAM Z[b] | OH | DMAE | H | 40 ± 10 (4) |
| 4-OH-TAM E[b] | DMAE | OH | H | 20 ± 3 (3) |
| 13*E* | OH | DEAE | H | 27 ± 7 (4) |
| 13*Z* | DEAE | OH | H | 6.9 ± 1.0 (4) |
| 14 | DEAE | DEAE | H | 3.6 ± 0.9 (5) |
| 15 | H | H | DEAE | 41 ± 5 (4) |
| 16 | DMA | DMA | H | <0.1 (4) |
| 18 | OH 3-DMAM | OH 3-DMAM | H | <0.1 (4) |

[a] Mean ± SEM. The number of experiments is in parentheses. [b] $C_2H_5$ instead of CN.

duction of a methoxy or isopropyloxy group invariably decreased RBAs (Table III). Whereas a (diethylamino)ethoxy (DEAE) chain in $\alpha$ (13*Z*) had no significant effect on binding, the same chain in $\alpha'$ (13*E*) noticeably increased binding. This observation can be likened to the results obtained with 4-OH-TAM, which has an even greater RBA for ER than 13*E*. Compounds with no hydroxy group on ring $\alpha$ or $\alpha'$ (1, 12, 14, 15, 16, 19) had very low RBAs (0.01–0.40) (Table I). A (dimethylamino)methyl (DMAM) group introduced into position 3 of the $\alpha$ and/or $\alpha'$ ring of 4 (17, 18) invariably decreased affinity with respect to 4. As already reported in the literature,[9,14,16] $\alpha$-OH isomers had higher RBAs than $\alpha'$-OH isomers (Table III).

On increasing incubation time and temperature, the RBAs of the $\alpha$-hydroxylated compounds increased whereas those of the other compounds decreased (Figure 3). In confirmation of our earlier results, the dihydroxylated compounds with an $\alpha$-hydroxy group were the most stable. Furthermore, whatever the substituents under consideration, the binding of compounds with a hydroxy group on ring $\alpha$ generally formed more stable receptor complexes than those without. Similar results have been obtained by another team[13] on a limited number of compounds. However, in the enclomiphene series,[55] both compounds with or without an $\alpha$-hydroxyl have yielded RBAs that increase between 0 and 25 °C.

**Interaction with the Antiestrogen Binding Site (AEBS).** Among molecular targets for TPEs, a high-affinity antiestrogen specific binding site has been described in estrogen target organs,[56–58] nontarget organs,[59–61] in ER-positive and negative cell lines,[61–64] and in TAM-resistant human breast cancer cells.[65] Of all the test compounds, only those with certain amino side chains competed for [$^3$H]TAM binding to this site in calf uterus supernatant (Table V). 4-OH-TAM Z competed to 40%. Replacement of the $C_2H_5$ by a CN and of the DMAE side chain on ring $\alpha'$ by a DEAE chain decreased the RBA from 40% to 27%, but this decrease was not statistically significant. Permutating this DEAE chain to the $\alpha$-ring further decreased binding (6.9%) as did the introduction of a second DEAE chain (3.6%). However a single DEAE chain on ring $\beta$ gave rise to a compound with an RBA for AEBS similar to that of 4-OH-TAM Z. None of the other compounds competed thus reflecting the importance of the para DEAE side chain, even on ring $\beta$, in binding to AEBS.

**Action on the Proliferation of $MCF_7$ Cells.** Berthois et al.[66] showed that phenol red (used as pH indicator in culture medium) stimulates $MCF_7$ cell proliferation. To clearly distinguish between partial estrogenic and antiestrogenic properties, we performed our experiments in culture medium without phenol red. However, in spite of drastic DCC treatment to remove steroids, control media were nevertheless found to contain a minimal amount of estrogen [$\leq 1 \times 10^{-12}$ M] as measured by a highly sensitive bioluminescence technique.[67] Under these conditions, $E_2$ promoted the growth of our $MCF_7$ cells 5–10-fold over the control, according to a dose–response curve with a maximum at 0.1 nM whereas, without added $E_2$, the cells hardly grew (doubling time = 110–120 h). Over the concentration range 10 pM to 1 $\mu$M, all test compounds could stimulate proliferation to varying extents including the unsubstituted TPE 1, which was an agonist at high concentrations (squares in Figure 4). The monohydroxylated TPEs (2*Z*/*E* and 3) and $\alpha,\beta$- or $\alpha',\beta$-dihydroxylated TPEs (5*Z*/*E*) had a proliferative effect similar to that of $E_2$, but the maximum was reached at a 10–100-fold higher concentration, i.e., 10 nM (1 nM for 5*Z*). The $\alpha,\alpha'$-dihydroxylated (4, 6, 8) and the methylated (7*Z*/*E*, 9*Z*/*E*) compounds were less effective and only gave rise to a partial agonist response. In the concentration range of 10 pM to 1 $\mu$M, the stimulation of proliferation by $\alpha,\alpha'$ disubstituted TPEs hydroxylated on ring $\alpha$ (or $\alpha'$) decreased with the increasing size of the corresponding substituent on $\alpha'$ (or $\alpha$). Stimulation was 100% when this substituent was a hydrogen (2*Z*/*E*), 65% for a hydroxyl (4), 45% for a methyl (7*Z*/*E*), 25% for a methoxy (10*Z*/*E*) and 12.5% for an isopropyloxy (11*Z*/*E*) or a DEAE (13*Z*/*E*) group. On the other hand, certain substitutions on ring $\beta$ tended to increase the agonist response: e.g. a hydroxyl or methyl group introduced into 4 to give either 6 or 8 or into 7*Z*/*E* to give 9*Z*/*E*. A bulky side chain on ring $\beta$ did not reduce the response (15 compared to 1), a result in agreement with the hypothesis of Durani et al.,[68] who consider that the corresponding zone of the receptor has bulk tolerance.

In the presence of 0.1 nM $E_2$ and in the absence of phenol red, most of the TPEs under study decreased $E_2$ stimulation in a concentration-dependent manner (triangles in Figure 4). A significant decrease was observed with 100 nM of TPE. With 1 $\mu$M, stimulation by $E_2$ was abolished, but this effect could be reversed by addition of 100 nM $E_2$ (diamonds in Figure 4). The maximal intensity of the antagonism obtained with 1 $\mu$M of an $\alpha,\alpha'$-substituted TPE hydroxylated on ring $\alpha$ (or $\alpha'$) was inversely related to the intensity of the stimulatory response given by the same TPE in the absence of $E_2$ as shown in Figure 5. It was in the following rank order: DEAE ~ isopropyloxy > methoxy > methyl ~ hydroxyl, showing

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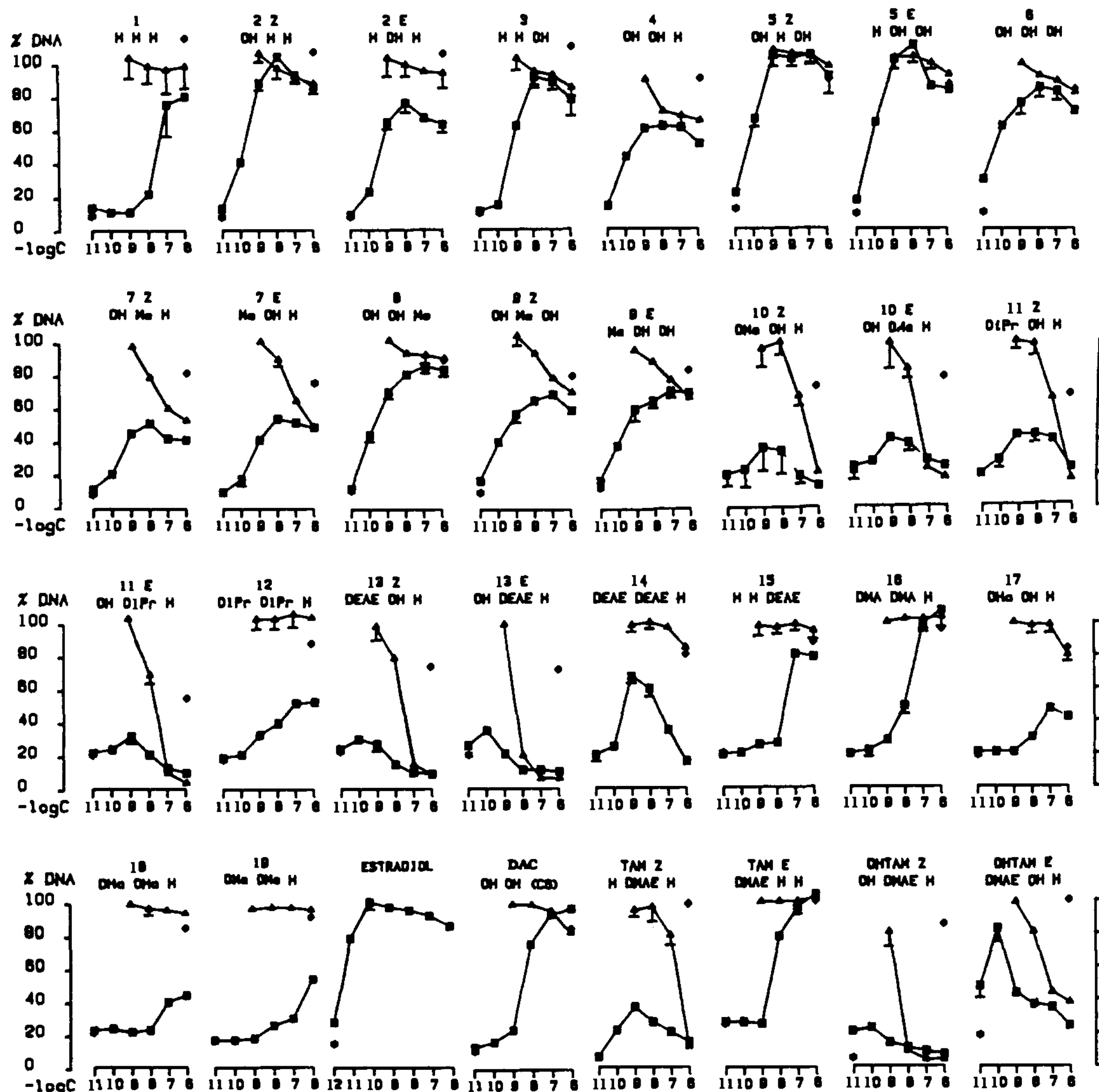

**Figure 4.** Stimulation and inhibition by TPEs of $MCF_7$ cell proliferation in the absence (■) or presence (▲) of 0.1 nM estradiol. Rescue of cells by 100 nM $E_2$ is indicated by the sign ◆. Results (mean ± SD of triplicate wells from a typical experiment) are expressed as percent DNA after 8 days of growth in the presence of 0.1 nM estradiol (Percent DNA = 100 × (μg of DNA TPE/μg of DNA $E_2$). Test compounds are identified by their number and their $\alpha,\alpha',\beta$ substituents (e.g. 2*Z*: OH,H,H). The "a" in compounds 17 and 18 indicates the presence of a *m*-(dimethylamino)methyl substituent. * indicates a control value (vehicle alone).

clearly the influence of substituent size, regardless of hydrophobicity, on the relative agonist/antagonist response. The weak inhibition of $E_2$-promoted cell growth by trisubstituted TPEs (**6**, **8**, **9***Z*/*E*) compared to that obtained with mono- and dihydroxylated derivatives is analogous to the in vivo observation that trisubstituted acetoxy TPEs have weaker antitumor activity in postmenopausal human mammary carcinomas implanted in nude mice.[69] Furthermore, the antagonist response we obtained with the smaller substituents (methyl, methoxy) is analogous to that observed with broparestrol ($\alpha'$ = ethyl) and its $\alpha$-hydroxylated derivative LN2839.[2] However, Murphy and Sutherland[19] have reported a low antiproliferative effect with some TAM derivatives bearing a methoxy or nonbasic side chain on ring $\alpha'$. This discrepancy could be explained by the low affinity of these non-$\alpha$-hydroxylated compounds for ER, necessitating high concentrations to antagonize the action of $E_2$, and/or to different experimental conditions.

**Cytotoxicity: Cytostatic and/or Cytolytic Effects. (a) In the Presence of $E_2$.** Experimental conditions where the effect of the TPE on $MCF_7$ cell proliferation is unlikely to be explained by antagonism of an ER-mediated action were chosen, i.e., the $E_2$ concentration ($10^{-6}$ M) was sufficient to maximally stimulate cell proliferation even in the presence of a 10-fold higher ($10^{-5}$ M) TPE concen-

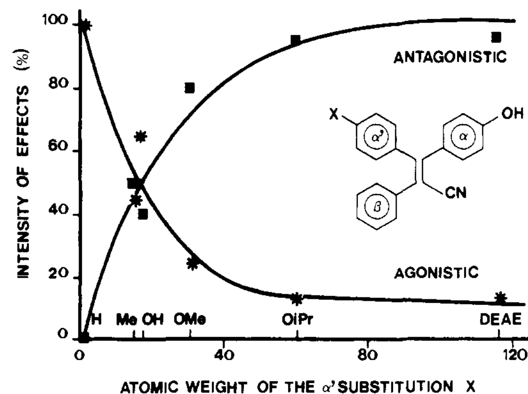


**Figure 5.** Intensity of percent agonist and antagonist response as a function of the $\alpha'$ substituent size. Agonist response, maximum effect at TPE concentrations below $10^{-6}$ M; antagonist reponse, effect of $10^{-6}$ M TPE in the presence of $10^{-10}$ M estradiol. (Percent agonist = [(TPE − C)/($E_2$ − C)] × 100, percent antagonist = [($E_2$ − TPE)/($E_2$ − C)] × 100, where C = control.)

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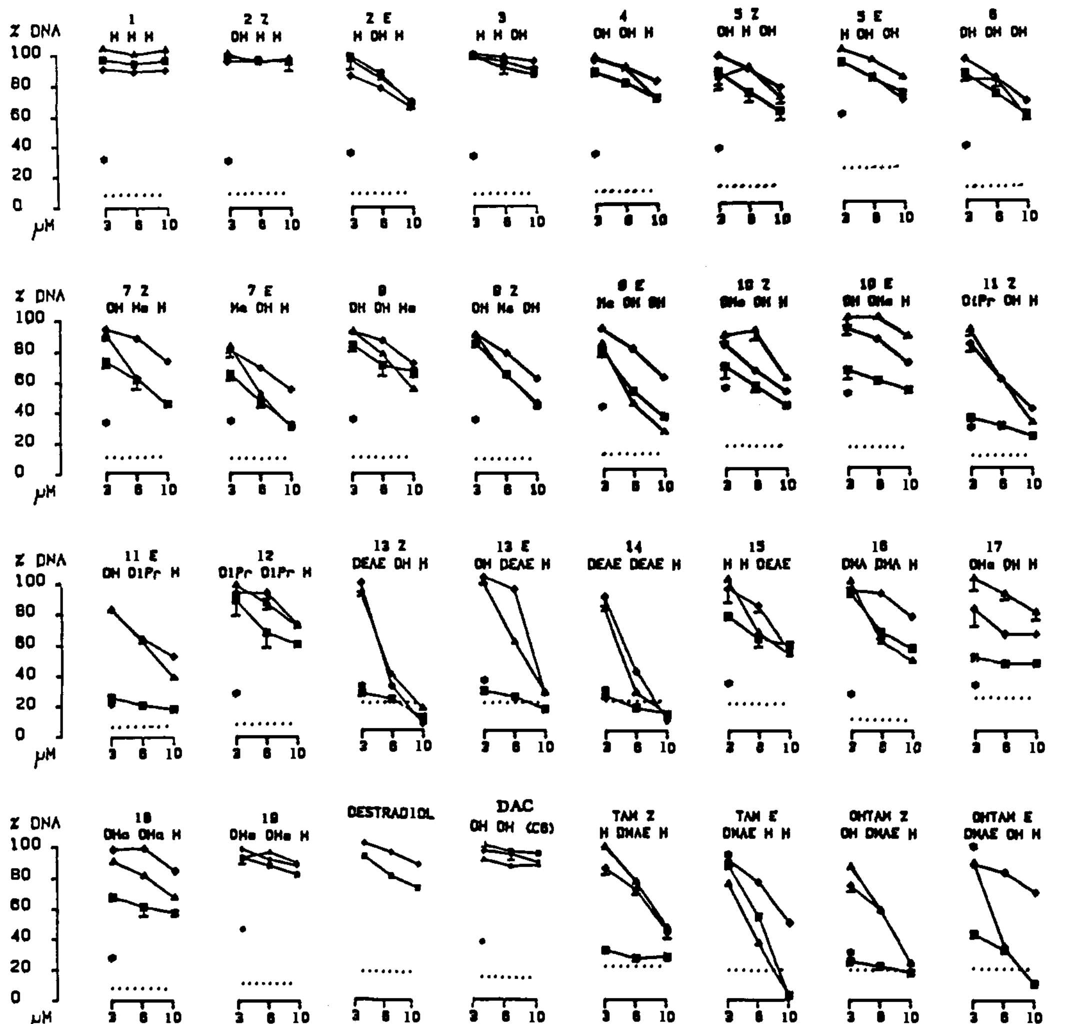

**Figure 6.** Effect of high TPE concentrations (3–10 $\mu$M) on the proliferation of $MCF_7$ and $BT_{20}$ cells. Results for triplicate wells from a typical experiment are expressed as percent DNA after 6 days growth in the presence of 1 $\mu$M $E_2$ for the $MCF_7$ cells (= [($\mu$g of DNA TPE)/($\mu$g of DNA $E_2$)] × 100) and as a function of the control for the $BT_{20}$ cells (= [($\mu$g of DNA TPE)/($\mu$g of DNA control)] × 100): (■) $MCF_7$ + TPE, (▲) $MCF_7$ + 1 $\mu$M $E_2$ + TPE, (★) $MCF_7$ control, (◆) $BT_{20}$ + TPE, (···) level of seeding for $MCF_7$ cells. The seeding level was 20–30% for $BT_{20}$ cells. 100% corresponded to about 7–10 $\mu$g of DNA and to about 3 $\mu$g for $MCF_7$ and $BT_{20}$ cells, respectively. Compounds are identified by their number and their $\alpha$, $\alpha'$, $\beta$ substituents, the "a" in compounds 17 and 18 indicates the presence of a *m*-(dimethylamino)methyl substituent.

tration. Figure 4 shows that, for one and the same concentration ratio, stimulation (>80%) by 0.1 $\mu$M $E_2$ was not inhibited by 1 $\mu$M TPE.

With the exception of derivatives 1, 2*Z*, 3, 19, and deacetylated cyclofenil (DAC), all TPEs inhibited the $MCF_7$ cell proliferation induced by $E_2$ in a dose-dependent fashion (see triangles in Figure 6). The degree of inhibition depended upon the TPE but there was no direct relationship between its RBA for ER and the concentration at which inhibition first appeared. The TPEs with bulky groups in $\alpha$ or $\alpha'$ were the most inhibitory (compound **15** with a bulky substituent on ring $\beta$ had less effect) and those with an $\alpha$ or $\alpha'$ (dialkylamino)ethoxy side chain (13*E*/*Z*, 14, TAM *E*/*Z*, and 4-OH-TAM E/Z) were more active than the isopropyl derivatives. With the exception of TAM Z, they were cytolytic at $10^{-5}$ M (i.e. the cell number fell below the seeding level). Compounds with a hydroxy group on $\alpha'$ and a bulky group on $\alpha$ exerted greater inhibition than their isomers (compare 9*Z*/*E*, 10*Z*/*E*, 11*Z*/*E*, and 13*Z*/*E*) (see levels below seeding in Figure 6). The action of 4-OH-TAM Z was comparable to that of its homologue 13*E* in our triphenylacrylonitrile series. These results support the conclusions of Murphy and Sutherland, who, under different experimental conditions (absence of $E_2$ but presence of phenol red), showed that high levels of zuclomiphene are more growth inhibitory than those of enclomiphene in $MCF_7$ cells.[17]

High TPE concentrations therefore exert a cytolytic action independent of ER in the presence of $E_2$. However, the chemical substituents that favor cytostatic activity are often the same as those that can lead to a parallel reversible growth inhibition at lower concentrations.

**(b) In the Absence of $E_2$.** The non-ER-mediated cytostatic action of TPEs decreases any ER-mediated stimulatory action they may have (see squares in Figure 6). This cytolytic action in the ER-positive $MCF_7$ cell line has been confirmed in ER-negative $BT_{20}$ cells (see diamonds in Figure 6), where the inhibition curves between 3 and 10 $\mu$M were parallel to those obtained on $MCF_7$ cells in the presence of 10 $\mu$M $E_2$ (see triangles in Figure 6). The areas under the curves suggest that the cytostatic actions of these TPEs are highly similar in both cell lines. The TPEs with a nitrogen-bearing $\alpha$ or $\alpha'$ side chain become cytolytic at concentrations of $10^{-5}$ M. These results support the inference that ER is apparently not involved in cytostatic or cytolytic effects.[3,6,20]

**Analysis of the Structural Determinants Governing Binding and Growth Responses.** In order to take simultaneously into account all the above information on the actions of TPEs on cell proliferation with a view to

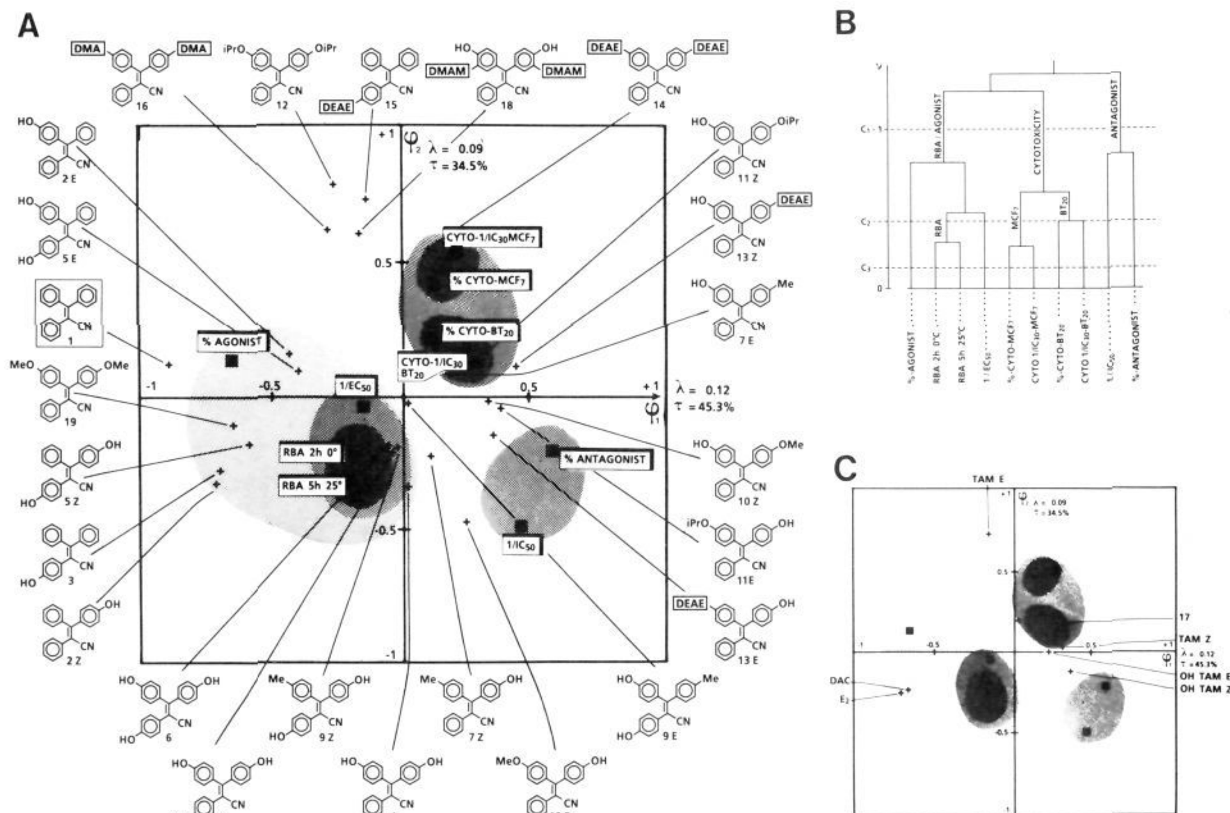


**Figure 7.** (A) $\Phi_1\Phi_2$ distribution map obtained by correspondence analysis of the binding and activity data in Table I after normalization. (B) Hierarchical ascending classification of the response parameters. (The chosen cutoff levels $C_1$, $C_2$, and $C_3$ were used to introduce the shading in A and illustrate different levels of correlation between the biochemical parameters.) (C) Location of reference compounds and of the isomer mixture **17** within the $\Phi_1\Phi_2$ map.

revealing possible relationships among the chosen test parameters and to identifying the relative importance of structural determinants in the various activities, we decided to perform a multivariate analysis on the data of Table I. The analysis on normalized data attempts to relate the different structural features of these molecules to their ability (a) to compete for ER binding under two sets of incubation conditions (RBA, 2 h, 0 °C; RBA, 5 h, 25 °C), (b) to stimulate cell proliferation as given by their $EC_{50}$ or maximum response compared to $E_2$ (% agonist), (c) to antagonize $E_2$-promoted cell growth as given by their $IC_{50}$ or their response at $10^{-6}$ M versus $10^{-10}$ M $E_2$ (% antagonist), and (d) to exert a cytotoxic effect in either ER-positive ($MCF_7$) or ER-negative ($BT_{20}$) cells as expressed by their $IC_{30}$ values and as a percent inhibition at $10^{-5}$ M (% cyto). (A 30% inhibition was chosen in order to be able to take into account the results on a maximum number of test compounds.) In so doing, we also assessed to what extent these effects may be interrelated for this population of molecules.

Calculation of the first three factorial axes $\Phi_1$, $\Phi_2$, and $\Phi_3$ showed that they account for 89.5% (45.3% + 34.5% + 9.7%) of the total variance of the system (Table VI). Factor $\Phi_1$ (45.3%) expresses the fundamental dichotomy between the ability of the test TPEs to stimulate or inhibit cell growth whether this growth is hormone-promoted or not and whether the inhibition is expressed in ER-positive or ER-negative cells. Receptor binding parameters do not greatly influence this axis (<7%), indicating that they are poorly related to percent agonism or percent antagonism. The relationship, albeit poor, is closer with stimulation of cell proliferation than to its inhibition as illustrated by the similarity in sign of the coordinates of the projection on the $\Phi_1$ axis. Factor $\Phi_2$ (34.5%) reveals an opposition between RBAs and cytotoxicity and between cytotoxicity and estrogen antagonism. The third axis, $\Phi_3$ (9.7%), essentially contrasts different expressions of antagonist activity.

The $\Phi_1\Phi_2$ 2-D factorial map in Figure 7A, which accounts for 80% of the total variance, illustrates the relative locations of the response parameters and of the test molecules with respect to these parameters. A hierarchical ascending classification gave the relationships shown in Figure 7B, and the cutoff levels $C_1$, $C_2$, and $C_3$ were used to introduce the different shading in Figure 7A. The factorial map now readily highlights the following conclusions: Measurements of RBAs under activating (5 h, 25 °C) or nonactivating (2 h, 0 °C) conditions are closely related. They appear to be relevant to an appreciation of the TPEs' growth-promoting ability when expressed as an $EC_{50}$ rather than a maximum response and less relevant to an ability to inhibit $E_2$-induced growth. Measurements of cytotoxicity in ER-positive and ER-negative cells are germane for this population of TPE molecules, and for each cell-line, there is a strong correlation between the two parameters used to express this cytotoxicity ($IC_{30}$ and % cyto). Cytotoxicity is anticorrelated to the RBAs but is not related to any antiestrogen activity on cell growth which is clearly opposed to growth stimulation.

In the design of tailor-made drugs, it is necessary to know to what extent a particular class of chemical structure might be associated with a specific property (binding, growth promotion or antagonism, cytotoxicity). This can be deduced from the position of the various test compounds within the factorial map (Figure 7A). This analysis that explores the specificity of the molecules does not however take into account the absolute activity levels. It

Table VI. Absolute and Relative Contributions of the Biochemical Parameters to the Factorial Axes

| | ABSOLUTE CONTRIBUTIONS | RELATIVE CONTRIBUTIONS $\cos^2 \theta$ |
|---|---|---|
| $\Phi_1$ $\tau$ = 45.3% | | |
| % AGONIST | 44.9% | 0.92 |
| % ANTAGONIST | 21.0% | 0.65 |
| $1/IC_{50}$ | 12.2% | 0.32 |
| CYTO-$1/IC_{30}$ ($BT_{20}$) | 6.9% | 0.40 |
| CYTO-$1/IC_{30}$ ($MCF_7$) | 3.7% | 0.12 |
| RBA 2 h 0 °C | 3.7% | 0.28 |
| RBA 5 h 25 °C | 3.1% | 0.16 |
| $1/EC_{50}$ | 2.1% | 0.47 |
| % CYTO-$MCF_7$ | 1.4% | 0.09 |
| % CYTO-$BT_{20}$ | 0.9% | 0.14 |
| $\Phi_2$ $\tau$ = 34.5% | | |
| CYTO-$1/IC_{30}$ ($MCF_7$) | 28.1% | 0.74 |
| $1/IC_{50}$ | 18.2% | 0.37 |
| RBA 5 h 25 °C | 17.9% | 0.73 |
| % CYTO-$MCF_7$ | 16.2% | 0.79 |
| RBA 2 h 0 °C | 7.5% | 0.44 |
| % CYTO-$BT_{20}$ | 4.2% | 0.50 |
| % ANTAGONIST | 3.6% | 0.08 |
| % AGONIST | 2.3% | 0.03 |
| CYTO-$1/IC_{30}$ ($BT_{20}$) | 1.7% | 0.07 |
| $1/EC_{50}$ | 0.2% | 0.04 |
| $\Phi_3$ $\tau$ = 9.7% | | |
| $1/IC_{50}$ | 48 8% | 0.28 |
| % ANTAGONIST | 23.1% | 0.15 |
| CYTO-$1/IC_{30}$ ($MCF_7$) | 11.1% | 0.08 |
| CYTO-$1/IC_{30}$ ($BT_{20}$) | 10.8% | 0.13 |
| % CYTO-$MCF_7$ | 1.9% | 0.02 |
| % CYTO-$BT_{20}$ | 1.6% | 0.05 |
| RBA 2h 0 °C | 1.3% | 0.02 |
| $1/EC_{50}$ | 0.9% | 0.04 |
| % AGONIST | 0.4% | 0.00 |
| RBA 5h 25 °C | 0.0% | 0.00 |

is assumed that increasing the activity of a specific molecule may be as judicious as increasing the specificity of a highly active molecule.

The principal feature of the TPEs down the left-hand side of Figure 7A is their essentially agonist nature, which is more or less marked. Except for the unsubstituted TPE **1** and the $\alpha,\alpha'$-dimethoxy compound **19**, they all bear at least one hydroxyl group (**2*E*/*Z***, **3**, **5*E*/*Z***). These along the bottom are partial agonists/antagonists most often characterized by high ER binding; all are at least $\alpha$-hydroxylated though several possess a second (**8**, **9*Z***, **4**) if not a third (**6**) hydroxy group. Other substituents are small, i.e. either a methyl (**7*Z***, **8**, **9*Z***) or a methoxy (**10*E***) group. Compounds up the right-hand side either are estrogen antagonists that retain the $\alpha$-hydroxy group in the presence, however, of a bulkier $\alpha'$-substituent (e.g. DEAE (**13*E***), isopropyl (**11*E***)) or are $\alpha'$-hydroxylated molecules with a small to bulky $\alpha$-substituent (**7*E***, **10*Z***, **11*Z***, **13*Z***) that display increased cytotoxic properties and lower binding affinity. Replacing both the $\alpha$- and $\alpha'$-hydroxys with bulky groups (**14** and **12**) or introducing meta DMAM substituents into the $\alpha,\alpha'$-dihydroxylated molecule (**18**) leads to a total breakaway from the antagonism pole and reinforces the element of cytotoxicity (see along top of Figure 7A).

The coherence of these results is revealed by the progressive change in biological properties with gradational structural modifications. Moreover, the study clearly shows that the adopted methodology is particularly well-suited to describing structure–activity relationships. This was further confirmed when we used the above factorial map as a mathematical model and introduced the data for seven further molecules into the analysis (Figure 7C). These molecules were either standard reference compounds or a stable mixture of easily interconvertible isomers (**17**). Their location within the factorial map was totally consistent with their known properties. As expected, $E_2$ and DAC were located within the sphere of influence of the RBA/agonism poles. On the other hand, OH-TAM E and OH-TAM Z were characterized by their ability to antagonize estrogen-induced proliferation and by their cytotoxicity. TAM Z was also both antiestrogenic and cytotoxic whereas TAM E was principally cytotoxic. Compound **17** with a meta DMAM substituent had only cytotoxic properties that moreover were weak.

## Conclusions

On the basis of the above analysis, we have been able to highlight, for a given population of TPE molecules characterized by substituents of increasing bulk, the relationship among the classic parameters of biological response that are so often used to describe this chemical class: binding to ER, growth promotion and inhibition, and cytotoxicity. We show that little extra information is obtained by measuring ER binding under both activating and nonactivating conditions and cytotoxicity in both ER-positive and -negative cell lines. Whereas ER binding is relevant to concentrations required ($EC_{50}$) for growth promotion, it has less relevance to the maximum response achieved and even less, if any, to antagonist activity. Paradoxically, it is slightly anticorrelated with cytotoxicity, but this could be due to the very low RBAs of some of the cytotoxic molecules. Increasing and systematic substitution of the TPE skeleton introduces specialized activities: $\alpha$-hydroxylation is associated with ER-binding and agonist activity; introduction of a hydrophobic group can emphasize antagonist activity, whereas bulky and often N-containing substituents introduce an increasing element not only of antagonism but of cytotoxicity. The factorial maps obtained by the study of these TPEs can now be used to evaluate and compare the different components of other novel molecules.

## Experimental Section

Melting points were determined on a Kofler apparatus and are uncorrected. Elemental analyses of all new compounds were performed in the Microanalytical Laboratory of the CNRS (Vernaison, France). Results were within ±0.3% of the theoretical values for those elements shown. $^1$H NMR spectra were recorded ($\delta$ 0) at 90 MHz on a Bruker spectrometer with $Me_4Si$ as internal standard. IR spectra were determined with a Beckman ACCU-LAB IV. Thin-layer chromatography was performed on silica gel 60 $F_{254}$ precoated aluminum sheets. Flash chromatography refers to the method of Still and co-workers.[70] A Jobin Yvon Miniprep 100 equipped with a silica gel 60 15–25-$\mu$m (Lichroprep Merck) column was used for preparative HPLC and a Waters apparatus was used for analytical HPLC.

**Synthetic Procedures. 2-Phenyl-3-(*p*-methoxyphenyl)-3-(*p*-hydroxyphenyl)acrylonitrile (Isomers 10*E* and 10*Z*).** Eight grams (23.5 mmol) of 2-phenyl-3,3-bis(*p*-methoxyphenyl)acrylonitrile (compound **19**), prepared as published[39] (mp 159 °C, yield 49%), and 2.98 g (23.5 mmol) of pyridine hydrochloride were heated between 220 and 230 °C for 1 h. The reaction mixture was cooled, diluted with water, and extracted with $CH_2Cl_2$. The organic phase was treated with 3 × 100 mL of 5% NaOH solution, washed, dried, and concentrated. The residue (3.5 g) contained the starting bis-ether together with small quantities of the desired isomers and of diphenol. The alkaline solution was acidified with 2 N HCl and filtered and the solid that was collected was washed with water and dried (3.6 g). Several flash chromatographies ($CH_2Cl_2$/EtOAc, 95:5) were necessary to eliminate the diphenol (1.90 g) from the isomers, which were then separated by using the Miniprep 100 apparatus ($CH_2Cl_2$/EtOAc, 96:4). **10*E* isomer**: yellow solid; TLC ($CH_2Cl_2$/EtOAc, 95:5) $R_f$ 0.4; mp 190 °C; IR ($CHCl_3$) 3500, 2100, 1600 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 3.79 (s, $OCH_3$, 7% = **10*Z***), 3.70 (s, $OCH_3$, 93% = **10*E***). Anal. ($C_{22}H_{17}NO_2$) C, H, N. **10*Z* isomer**: yellow solid; TLC $R_f$ 0.57; mp 186 °C; $^1$H NMR ($CDCl_3$) $\delta$ 3.79 (s, $OCH_3$, 94% = **10*Z***), 3.70 (s, $OCH_3$, 6% = **10*E***). Anal. ($C_{22}H_{17}NO_2$) C, H, N.

**2-Phenyl-3-(*p*-isopropoxyphenyl)-3-(*p*-hydroxyphenyl)acrylonitrile (Isomers 11*E* and 11*Z*) and 2-Phenyl-3,3-bis-**

(70) Still, W. C.; Khan, M.; Mitra, A. *J. Org. Chem.* 1978, *43*, 2923.

(**p-isopropoxyphenyl)acrylonitrile** (12). 2-Phenyl-3,3-bis(*p*-hydroxyphenyl)acrylonitrile (5 g, 16 mmol), prepared as described,[71] was added to a solution of sodium ethoxide (EtOH, 80 mL; Na, 367 mg, 0.016 atom) with stirring. Isopropyl bromide (3.4 g, 20 mmol) was then slowly introduced and the solution was heated for 5 h. The reaction mixture was concentrated in vacuo, diluted with water (100 mL), and extracted with $CH_2Cl_2$. A small amount of starting bis-hydroxy compound (0.40 g) was collected by filtration. The pH of the aqueous phase was adjusted to 2.0 and the solid was collected (4.95 g). TLC ($CH_2Cl_2$/EtOAc, 95:5) showed four spots: $R_f$ 0.80 (identified later as the diisopropoxy derivative), $R_f$ 0.60 and 0.37 (*Z* and *E* isomers), and $R_f$ 0.14 (starting compound). 1.22 g of the mixture was purified by flash chromatography (elution with $CH_2Cl_2$/EtOAc, 95:5) to give the diisopropoxy derivative (100 mg), a mixture of the two isomers (667 mg), and the starting compound (333 mg). In spite of several successive chromatographies on the mixture of isomers, they have not been isolated in pure crystallized forms because of rapid reequilibration: mp 175–179 °C (2-propanol); $^1$H NMR ($CDCl_3$) $\delta$ 1.30 (d, $(CH_3)_2CH$, 57% = 11*E*), 1.37 (d, $(CH_3)_2CH$, 43% = 11*Z*), 4.35–4.74 (sept, $CH(CH_3)_2$), 5.89 (OH), 6.57–7.46 (13 arom H). Anal. ($C_{24}H_{21}NO_2$) C, H, N. Compound 12 was obtained by treatment of the organic phase, which was washed, dried, concentrated, and chromatographed ($CH_2Cl_2$/EtOAc, 95:5): mp 138 °C (2-propanol); $^1$H NMR ($CDCl_3$) $\delta$ 1.29 (d, 6 H, $(CH_3)_2CH$), 1.37 (d, 6 H, $(CH_3)_2CH$), 4.36–4.74 (2 sept, 2 H, $2(CH_3)_2CH$), 6.60–7.48 (13 arom H). Anal. ($C_{27}H_{27}NO_2$) C, H, N.

**2-Phenyl-3-[4-[2-(diethylamino)ethoxy]phenyl]-3-(4-hydroxyphenyl)acrylonitriles (Isomers 13*Z* and 13*E*) and 2-Phenyl-3,3-bis[4-[2-(diethylamino)ethoxy]phenyl]acrylonitrile** (14). 2-Phenyl-3,3-bis(4-hydroxyphenyl)acrylonitrile (10 g, 32 mmol) was added to a solution of sodium ethoxide (sodium, 2.94 g, 0.128 atom; absolute EtOH, 250 mL) and stirred under nitrogen at 80 °C for 1 h. 2-(Diethylamino)ethyl chloride hydrochloride (11 g, 64 mmol) was then introduced. Stirring and reflux were maintained for 6 h. After cooling, the reaction mixture was filtered (NaCl) and the filtrate was concentrated in vacuo. The residue was triturated with $H_2O$ and extracted with $Et_2O$. The organic phase was treated with 10% NaOH solution (50 mL × 5). The ether solution was washed, dried, and concentrated. The oily residue was distilled, giving a fraction, $bp_{0.04}$ 285–290 °C, identified as compound 14: $^1$H NMR ($CDCl_3$) $\delta$ (chain bound to the $\alpha$-ring) 1.01 (t, 6 H, $CH_3CH_2$), 2.56 (quad, 4 H, $CH_3CH_2$), 2.83 (t, 2 H, $NCH_2CH_2$), 4.02 (t, 2 H, $CH_2O$); (chain bound to the $\alpha'$-ring) 0.94 (t, 6 H, $CH_3CH_2$), 2.53 (quad, 4 H, $CH_3CH_2$), 2.77 (t, 2 H, $NCH_2CH_2$), 3.91 (t, 2 H, $CH_2O$); 6.51–7.60 (m, 13 H, arom). Anal. ($C_{33}H_{41}N_3O_2$) C, H, N. The alkaline solution was acidified with OHAc and filtered and the solid was collected (6 g). A small amount was purified by column chromatography (silica gel 60, 70–200 mesh ASTM) using elution with $CHCl_3$ containing increasing proportions of $CH_3OH$. Compound 13*E* was isolated ($CH_3Cl/CH_3OH$, 75:25): mp 194–195 °C ($CH_3OH$); $R_f$ 0.37 ($CH_3Cl/CH_3OH$, 80:20); $^1$H NMR (DMSO-$d_6$) $\delta$ 0.94 (t, $CH_3CH_2$), 2.53 (quad, $CH_3CH_2$), 2.74 (t, $CH_2N$), 3.96 (t, $CH_2O$), 6.67–7.31 (m. 13 arom H). Anal. ($C_{27}H_{28}N_2O_2$) C, H, N. Compound 13*Z* was also isolated ($CHCl_3/CH_3OH$, 60:40): mp 166–167 °C ($CH_3OH$); $R_f$ 0.27; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.96 (t, $CH_3CH_2$), 2.57 (quad, $CH_3CH_2$), 2.82 (t, $CH_2N$), 4.06 (t, $CH_2O$), 6.53–7.40 (m, 13 H arom). Anal. ($C_{27}H_{28}N_2O_2$) C, H, N.

**2-[4-[2-(Diethylamino)ethoxy]phenyl]-3,3-diphenylacrylonitrile** (15). 2-(4-Hydroxyphenyl)-3,3-diphenylacrylonitrile was prepared by demethylation of the corresponding methyl ether.[38] A mixture of this phenol (4.15 g, 14 mmol), 2-(diethylamino)ethyl chloride hydrochloride (3.8 g, 22 mmol) and anhydrous potassium carbonate (7.73 g, 56 mmol) in anhydrous acetone (200 mL) was stirred and heated for 20 h under nitrogen. A mineral solid was separated by filtration. The solution was concentrated and the residue was diluted with water and extracted with $Et_2O$. The organic phase was washed, dried, and concentrated to an oily residue (3.9 g) that crystallized rapidly. The solid was recrystallized from EtOH: mp 93 °C; yield 70%; $^1$H NMR ($CDCl_3$) $\delta$ 1.08 (t, $CH_3CH_2$), 2.66 (q, $CH_2CH_3$), 2.87 (t, $CH_2N$), 4.04 (t, $CH_2O$), 6.69–7.43 (m, 14 arom H). Anal. ($C_{27}H_{28}N_2O$) C, H, N.

**2-Phenyl-3,3-bis[4-(dimethylamino)phenyl]acrylonitrile** (16). 4,4'-(Dimethylamino)benzophenone (2.68 g, 10 mmol) was added at 80 °C with stirring to a mixture of benzyl cyanide (1.64 g, 14 mmol) and sodium amide (0.78 g, 20 mmol) in anhydrous toluene (100 mL), which was then heated under reflux for 10 h. After cooling, the mixture was triturated with $H_2O$ and the aqueous solution was extracted with toluene. The organic layers were combined, washed with water, dried, and concentrated. The solid residue was recrystallized twice from EtOH (2.5 g): mp 189 °C (lit.[39] mp 186 °C).

**2-Phenyl-3-[4-hydroxy-3-((dimethylamino)methyl)-phenyl]-3-(4-hydroxyphenyl)acrylonitrile** (17) **and 2-Phenyl-3,3-bis[4-hydroxy-3-((dimethylamino)methyl)-phenyl]acrylonitrile** (18). 2-Phenyl-3,3-bis(4-hydroxyphenyl)acrylonitrile (3.13 g, 0.01 mol) was dissolved in 100 mL of EtOH. Solutions of dimethylamine (2.25 mL of a 40% aqueous solution, 0.02 mol) and formaldehyde (1.60 mL of a 37% aqueous solution, 0.02 mol) were added slowly under a nitrogen stream. The reaction mixture was stirred for 1 h at room temperature, refluxed for 10 h, and concentrated in vacuo to a red oil (3.5 g). Three compounds were isolated from this oil by column chromatography on $SiO_2$($CH_2Cl_2/CH_3OH$, 70:30). In the order of elution, they were the starting diphenol (0.80 g), a mixture of the geometric isomers (1.92 g), and the bis-substituted derivative (0.46 g). The best separation of the geometric isomers was obtained with dilute isopropyl alcohol (water 20%): mp 122 °C; IR ($CHCl_3$) 3600, 3500–3150, 2200, 1600 cm$^{-1}$; $^1$H NMR ($CD_3COCD_3$) $\delta$ 2.07 (s, ($CH_3$), 25% = **17a**), 2.19 (s, $CH_3$, 75% = **17b**), 3.27 (s, $CH_2$, 25% = **17a**), 3.54 (s, $CH_2$, 75% = **17b**), 6.31–7.18 (m, 12 arom H). Anal. ($C_{24}H_{22}N_2O_2$) C, H, N. The last fraction of the chromatography gave compound 18: mp 153 °C (hexane); $^1$H NMR ($CD_3COCD_3$) $\delta$ 2.08 (s, $CH_3)_2NCH_2$, in the 3-position on the $\alpha'$-ring), 2.20 (s, $(CH_3)NCH_2$, in the 3-position on the $\alpha$-ring), 3.26 (s, $CH_2$-$\alpha'$-ring), 3.53 (s, $CH_2$-$\alpha$-ring), 6.39–7.20 (m, 11 arom H). Anal. ($C_{27}H_{29}N_3O_2$) C, H, N.

**High-Performance Liquid Chromatography.** For HPLC analysis of the TPE isomers, the Waters system equipped with an UV detector (Waters, Lamda Max Model 480) set at 280 nm was used. The isomers were separated on a 5-mm radial pack silica gel column using chloroform/methanol/triethylamine in the following proportions: 95:3:2 (v/v/v) for monohydroxylated compounds (2*Z*/*E*, 7*Z*/*E*, 10*Z*/*E*, 11*Z*/*E*, and 13*Z*/*E*) and 91:7:2 (v/v/v) for dihydroxylated compounds (5*Z*/*E*, 9*Z*/*E*, and 17). Isomerization rates were studied as follows: 100 $\mu$M of each isomer in Tris buffer, pH 7.4, containing 10% ethanol were left to stand for 48 h at 37 °C. The samples (4 mL) were extracted twice with 5 volumes of chloroform. Extracts were dried under a gentle stream of nitrogen, dissolved in the corresponding HPLC solvent system, and then analyzed by HPLC.

**Biology: Other Chemicals and Materials.** TAM and 4-OH-TAM isomers were kind gifts from Dr. A. H. Todd (ICI, Macclesfield, England). [*N-methyl*-$^3$H]TAM (2.63 TBq/mmol) was obtained from NEN (Boston, MA) and [6,7-$^3$H]$E_2$ (1.85 TBq/mmol) was from the Centre d'Energie Atomique (France). The xylene-based scintillation fluid 299 was from United Technoligies Packard. All media, antibiotics, and the fetal calf serum (FCS) for the $MCF_7$ cell culture were obtained from Seromed (Biopro, Strasbourg, France). Charcoal-treated FCS was prepared by mixing FCS with 1% (w/v) charcoal Norit A and 0.1% (w/v) Dextran T70 for 30 min at 56 °C under virtually sterile conditions and then centrifuging. This procedure was repeated at 37 °C. Serum was sterilized by filtration on 0.22-$\mu$m Millipore Sterivex-GS (yield 95%).

Solutions of all test compounds in 95% alcohol were stored at 4 °C in the dark for no longer than 2 weeks and checked before use by thin-layer chromatography ($CH_2Cl_2$/hexane, 90:10, v/v) or by high-performance liquid chromatography (HPLC) as described above.

**Relative Binding Affinities for the Estrogen Receptor (ER).** Calf uteri (20–40 g) were excised, divided into 1-g fractions, and then stored at −70 °C. The uterine fractions were homogenized in an ice-cooled glass–Teflon Potter in TED buffer (10 mM Tris-HCl, 1.5 mM EDTA, 1 mM dithiothreitol, pH 7.4), and

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the homogenate was centrifuged at 0–4 °C for 1 h at 180000*g* to obtain cytosol. Cytosol aliquots were incubated either for 2 h at 0 °C or for 5 h at 25 °C with 1 nM [6,7-$^3$H]$E_2$ and increasing concentrations (0.3 nM to 10 μM) of unlabeled competitor (final volume 250 μL containing 1% ethanol). The incubated cytosol was stirred for 30 s at 0 °C with 50 μL of DCC (0.6% dextran T70, 6% charcoal Norit A) and then centrifuged for 10 min at 4000*g*. The radioactivity of a 200-μL supernatant sample was measured by liquid scintillation. Relative binding affinities (RBAs) were deduced from competition curves by determining the molar concentrations of unlabeled $E_2$ or competitor that reduced radioligand binding by 50%.

**Relative Binding Affinities for the Antiestrogen Binding Site (AEBS).** The same method as for the determination of the RBAs for ER was used except that the homogenate was centrifuged at 0–4 °C for 1 h at 12000*g*. The supernatant thus obtained was incubated for 30 min at 0 °C with 1 μM $E_2$. Aliquots were then incubated for 2 h at 0 °C with 1.5 nM [$^3$H]TAM and increasing concentrations (0.3 nM to 10 μM) of unlabeled competitor (containing 2.5% dimethylformamide).

**Cell Culture.** $MCF_7$ cells were grown in T-25 flasks in medium A (minimum essential medium without phenol red supplemented with 10 mM HEPES, 2 mM glutamine, 1 nM insulin, nonessential amino acids (1%), 100 units/mL penicillin, 0.1 μg/mL streptomycin, 0.5 g/L sodium hydrogen carbonate] containing 5% FCS. $BT_{20}$ cells were grown in T-25 flasks in medium A supplemented with 10% FCS.

**Cell-Growth Experiments. (a) Stimulation of Cell Growth or Antagonism of $E_2$-Promoted Growth.** $MCF_7$ cells were seeded into one T-75 flask ($10^6$ cells/flask) in medium A containing 5% DCC-treated FCS. The medium was renewed every 2 days for 6 days, after which time the cells were harvested and seeded in 24-well tissue-culture cluster plates (Nunc or Falcon, 10000 cells/well) in medium A containing 5% DCC-treated FCS. One day later, the medium was replaced by fresh medium containing either various concentrations of the test compounds, with or without $E_2$, or the vehicle alone (0.2% ethanol). These media were renewed every two days. Controls with and without $E_2$ (0.1 or 1 nM) and without test compounds were performed for every well plate. After an 8–10-day growth period, triplicate wells of cells were determined for DNA content according to the method of Kissane and Robins.[72] At that time, the cell content in the presence of $E_2$ is not yet at confluence. The amount of fluorogenic group obtained by the reaction of the DNA with diaminobenzoic acid was evaluated with a Perkin-Elmer MPF-3L spectrofluorimeter (excitation 408 nm, emission 508 nm, slits 6 nm).

**(b) Cytotoxic Action.** The procedure was as above, but $MCF_7$ and $BT_{20}$ cells were seeded at a density of 20000 per well for $MCF_7$ cells and 40000 for $BT_{20}$ cells. DNA content was determined after 5 or 6 days in the presence of test compound.

**Correspondence Analysis (CA).**[73] Calculations were performed on a microcomputer (16–32 bits of 655K of central memory, Hewlett-Packard 9836) with a program adapted for BASIC from FORTRAN Anacor software. The factorial maps were drawn directly on a digital plotter with a precision of $^1/_{100}$ in. (but have been redrawn by a professional artist for the purposes of this paper). A simplified version of the program for running on an IBM PC compatible computer is available upon request from J.-C. Doré (Muséum National d'Histoire Naturelle, 63 rue Buffon, 75005 Paris, France).

The values of the biological parameters for the CA were deduced from Figures 4 and 6. The TPEs with little or no antagonist activity were assigned an $IC_{50}$ value of 1000 nM for the multivariate analysis. An $IC_{30}$ of 20 μM was assigned to those compounds that did not inhibit proliferation by 30% in the cytotoxicity experiments. Data transformation prior to analysis involved one to three steps: calculation of the reciprocal, logarithmic transformation, and distribution within a range from 0 to 100 on the basis of the values obtained for the first 25 molecules listed in Table I.

The hierarchical ascending classification was obtained from the matrix of the normalized data. To be in conformity with the CA, we chose $\chi^2$-metrics to define a table of distances between the 10 biological parameters.[74] The aggregation criterion of Lance and Williams[75] was used with $\alpha = 0.625$ and $\beta = -0.25$.

**Registry No.** 1, 6304-33-2; **2z**, 19460-09-4; **2e**, 84836-12-4; **3**, 16143-90-1; **4**, 66422-14-8; **5z**, 16144-07-3; **5e**, 16144-06-2; **6**, 76621-40-4; **7z**, 84836-13-5; **7e**, 84836-14-6; **8**, 90468-83-0; **9z**, 90468-84-1; **9e**, 90468-85-2; **10z**, 118976-11-7; **10e**, 118976-10-6; **11z**, 118976-13-9; **11e**, 118976-12-8; **12**, 118976-14-0; **13z**, 104575-13-5; **13e**, 104575-22-6; **14**, 118976-15-1; **15**, 118976-16-2; **16**, 118976-17-3; **17**, 121425-56-7; **18**, 121425-55-6; **19**, 66422-13-7; isopropyl bromide, 75-26-3; 2-(diethylamino)ethyl chloride, 100-35-6; 4,4'-(dimethylamino)benzophenone, 90-94-8; benzyl cyanide, 140-29-4; sodium amide, 7782-92-5; dimethylamine, 124-40-3.

**Supplementary Material Available:** Elemental analysis of 10*Z*/*E*, 11*Z*/*E*, 12, 13*Z*/*E*, 14, 15, 17, and 18 as well as details of correspondence analysis, i.e., coordinates, relative and absolute contributions, and data transformations (8 pages). Ordering information is given on any current masthead page.

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