Estrogenic Triarylethylene Acetic Acids: Effect of Structural Variation on Estrogen Receptor Affinity and Estrogenic Potency and Efficacy in MCF-7 Cells

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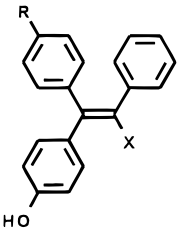
Triarylethylenecarboxylic acids exemplified by (*E,Z*)-2-{4-[1-(*p*-hydroxyphenyl)-2-phenyl]-1-butenyl}phenoxyacetic acid (**8**) are a new class of estrogen receptor (ER) ligands capable of tissue selective estrogen agonist and antagonist effects. We report the syntheses of **8** and of analogues incorporating structural features known or anticipated to facilitate ER affinity in triarylethylenes. These studies revealed that the *p*-hydroxyphenyl moiety, ethylenic bond, and ether oxygen of **8** were all critical for high ER affinity. Although a 1,1-bisphenolic analogue bearing the *p*-(oxyacetic acid) moiety on its 2-phenyl ring, **12**, had low ER affinity, it exhibited estrogenic potency approaching that of **8** in MCF-7 cells. Unlike **8** which was a partial agonist with weak antagonist potency, **12** was a full agonist. A similar profile of potency/efficacy in MCF-7 cells was seen in **9**, an ethylenic bond saturated analogue of **8**. Growth-promoting effects of **8**, **9**, and **12** were fully antagonized by the antiestrogen tamoxifen, suggesting that such effects were mediated solely via ER. Thus, our studies in MCF-7 cells have confirmed the estrogenicity of **8** and have enabled identification of two analogues with favorable estrogenic potency and full estrogen efficacy. On this basis, these three (triarylethylene)acetic acids have been selected for more intensive animal studies of their extrareproductive tract estrogenic effects.

Effects of estrogens have conventionally been associated with the female reproductive organs (uterus, ovary), and are mediated primarily if not solely through estrogen receptors (ER). But estrogens have direct effects on other tissues as well. The number of tissues shown to contain significant levels of ER is growing due to use of more sensitive detection methods. Thus, ER is now known to be present in specialized cells of the skeletal[1] and cardiovascular systems.[2] The molecular basis for estrogen replacement therapy involves interactions of ligands with ER in extrareproductive as well as reproductive tissues.[3]

Being flexible regarding binding of ligands, ER accommodates not only estradiol and other steroidal compounds but also a diverse array of aromatic nonsteroidal structural types, exemplified by mono- and dihydroxylated triarylethylenes **1**.[4] As examples, **1a**−**d** had respective ER relative binding affinities (RBA values) of 38, 55, 95, and 160% (estradiol = 100%) in rat uterine cytosol.



	R	X
1a	H	CN
b	OH	CN
c	H	C_2H_5
d	OH	C_2H_5

Cultured human and animal cells naturally endowed with ER have been used extensively in characterizing effects of steroidal and nonsteroidal ER ligands. In particular, MCF-7 cells, a clonally durable line derived from a human breast cancer, have been well established with regard to assessment of growth suppressive effects of steroidal and nonsteroidal antiestrogens.[5] But MCF-7 cells have also been used to evaluate estrogenic (growth stimulatory) potency and efficacy of ER ligands.[4a,6] Accordingly, **1a,b** and estradiol had half-maximal growth stimulatory potencies in MCF-7 cells of 0.19, 0.06, and 0.02 nM, respectively, and estrogenic efficacies of **1a,b** were in turn 94% and 62% that of estradiol.[4a] Other lines of ER positive cancer cells have been used in bioassays of ER ligands.[7] Estrogenicity in such cells is due to interaction of liganded ER with composite estrogen response elements (ERE) in DNA, rather than with only classical palindromic ERE as appears to be the case in ER-transfected cell lines.[8]

Formal etherification of **1d** with a single acetic acid moiety afforded a 1:1 mixture of isomeric (hydroxytriarylethylene)oxyacetic acids which had an ER RBA 20% that of estradiol and exhibited half-maximal estrogenic potency at a concentration of 35 nM in MCF-7 cells.[9] As a major metabolite of the antiestrogen tamoxifen in the female rat, this oxyacetic acid was not detected in reproductive tissues, unlike the parent drug and its basic metabolites.[10] On parenteral administration to the ovariectomized rat, it exhibited an effect on trabecular bone maintenance that was qualitatively similar to that of estradiol. However it had no observable uterotrophic effect, a finding consistent with results of the biotransformation studies but which could also signify a differential lack of reproductive tract estrogenicity. Agents displaying such selective estrogenicity are of interest due to their potential for reduced reproductive tract toxicity compared with conventional estrogens.[11]

An immediate aim of structural studies was to compare, in MCF-7 cells, the effects of this oxyacetic acid derivative of **1d** with analogues modified structurally in ways consistent with preserving ER affinity. The goals were to identify structural features critical for ER affinity and potency and to provide a basis for selection

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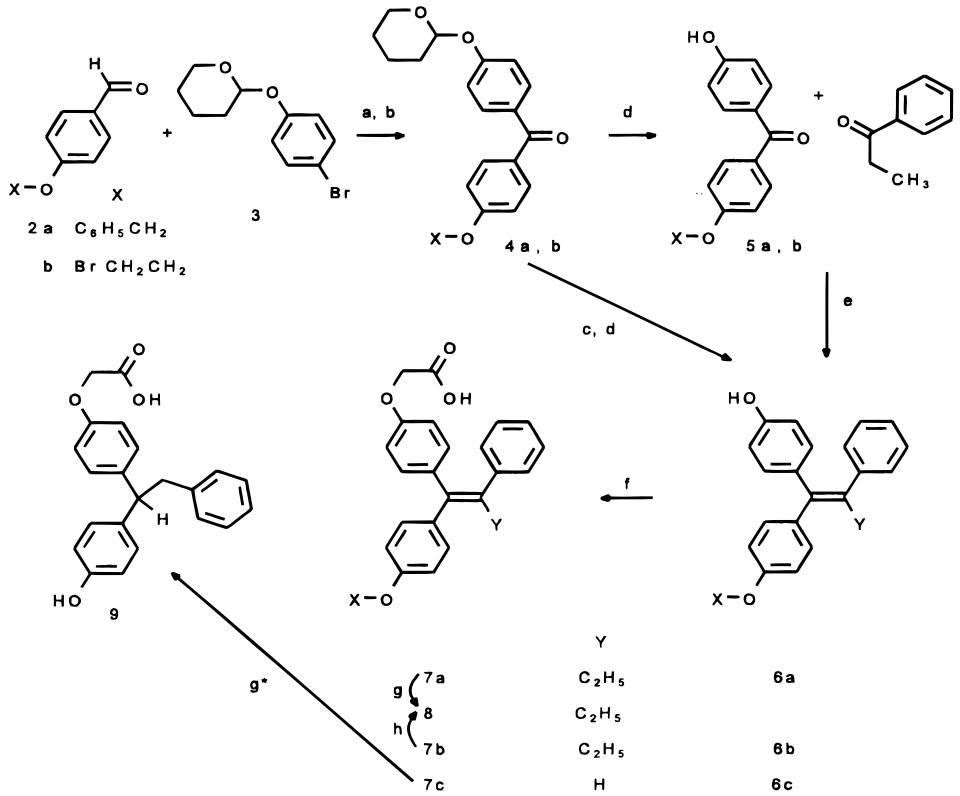
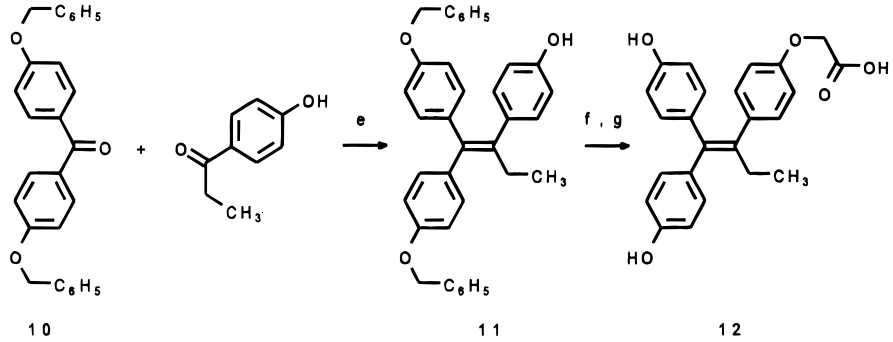
Scheme 1. Synthesis of Hydroxytriarylethylene **8** and Hydroxytriarylethane **9**[a]



[a] Reagents: (a) Mg, THF; (b) $(C_5H_4N)_2Cr_2O_7$, acetone; (c) $C_6H_5CH_2MgCl$, Et_2O; (d) HCl, EtOH; (e) Ti, THF; (f) $BrCH_2COOC_2H_5$, K_2CO_3, acetone, then NaOH, aqueous dioxane; (g) H_2, 10% Pd/C, THF (*MeOH); (h) NaCN, DMF.

Scheme 2. Synthesis of Bisphenolic Oxyacetic Acid **12**[a]



[a] Reagents e, f, and g are specified in Scheme 1.

of compounds for in vivo studies. We also wished to establish the degree to which effects on MCF-7 cells were mediated by ER in these structural types.

Results

Synthesis. Schemes 1−3 illustrate the approaches used to prepare the aforementioned oxyacetic acid **8**, as well as **9**, **12**, **15**, and **17**.[12] A central element in our synthetic strategy was application of the McMurry reaction in reductive cross-coupling of substituted benzophenones with propiophenone or hydroxypropiophenone.[13] The intermediate monophenols **6a,b**, **11**, and **14b** were readily separated from products of self-coupling by column chromatography.

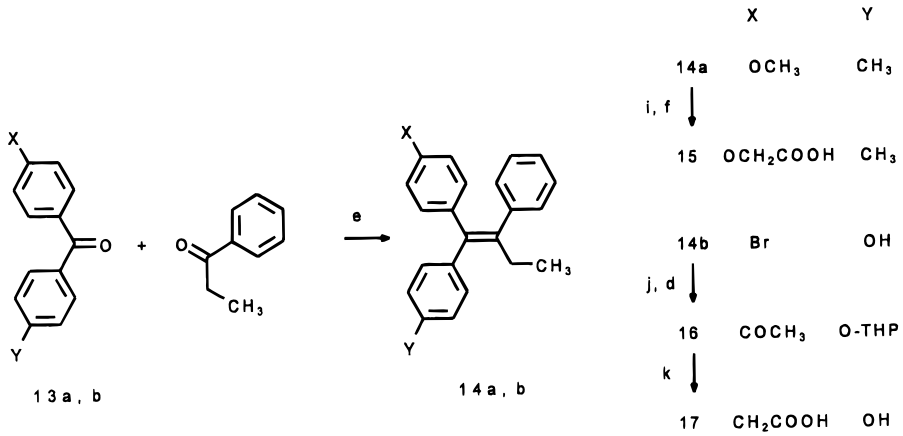
Catalytic debenzylation of **7a** sometimes proceeded slowly in tetrahydrofuran. Use of methanol as solvent increased the reaction rate, but resulted in ethylenic bond reduction as well, an observation applied to the preparation of **9** from **7c**. These observations prompted use of the β-bromoethyl protecting group[14] in the reaction sequence leading to **8** (Scheme 1, h).

Deoxy analogue **17** was prepared by Willgerodt−Kindler rearrangement of protected aryl methyl ketone **16** (Scheme 3, k),[15] and this intermediate was in turn prepared by acetylation of the aryllithium produced by reaction of **14b** tetrahydropyran-2-yl ether with *n*-butyllithium.[16]

Interaction of 8 and Analogues with ER. Interaction of "target" compounds with cell-free rat ER as well as intracellular human ER was determined, in turn, in rat uterine cytosol at 0 °C as previously described[17] and in MCF-7 cells as described below. As derived from Table 1, ER affinities of analogues of **8** were 0.2−2% that of **8** itself for rat ER and were 0.9−36% that of **8** for human ER.

Effects of 8 and Analogues on MCF-7 Cell Proliferation. Saturated analogue **9** and bisphenol **12** each exhibited greater proliferative potencies (Table 2)

Scheme 3. Synthesis of **15** and **17**[a]



[a] Reagents d, e, and f: Scheme 1; (i) BBr_3, $CHCl_3$; (j) DHP, p-TsOH; n-BuLi in Et_2O−hexanes; $CH_3CON(CH_3)_2$; (k) S, morpholine, then H_2SO_4.

Table 1. Affinity of (Triarylethylene)acetic Acids for Rat and Human ER

compd	IC_{50}, nM[a](RBA)	
	Rat uterine cytosol	MCF-7 cells
8	9 (20)	21 (1.54)
9	900 (0.20)	660 (0.05)
12	750 (0.24)	2390 (0.02)
15 (Y = H)	4500 (0.04)	320 (0.10)
15	1060 (0.17)	97 (0.33)
17	450 (0.40)	58 (0.56)
estradiol	1.8 (100)	0.32 (100)

[a] The concentration required to displace by 50% specifically bound [^{3}H]estradiol in rat uterine cytosol or MCF-7 cells.

Table 2. Stimulatory and Inhibitory Effects of (Triarylethylene)acetic Acids on MCF-7 Cells[a]

compd	EC_{50}, nM[b] (eff[c])	IC_{30}, μM[d] (eff[e])
8	5.3 ± 0.3 (79)[f]	30 ± 5 (22)[g]
9	16 ± 10 (102)[f]	*h*
12	46 ± 15 (100)[f]	*h*
15 (Y = H)	300 ± 50 (67)	20 ± 5 (22)[g]
15	350 ± 50 (61)	13 ± 5 (26)[i]
17	42 ± 10 (83)[f]	2.5 ± 0.5 (51)[i]
estradiol	0.7 ± 0.3 pM (100)	

[a] Values are expressed in terms of effects of compounds on observed cell growth rates. Each value is the average ± standard deviation for 3−6 separate experiments. [b] The concentration required for half-maximal growth stimulation. [c] Maximal growth-stimulatory effect, as a percent of that of estradiol. [d] The concentration required for 30% maximal inhibition of growth seen in the presence of 10 pM estradiol. [e] The percent by which the proliferative effect of 10 pM estradiol was maximally inhibited at 10 μM of the test compound. [f] This effect was diminished or eliminated when 1 μM tamoxifen was present. [g] This effect was not seen when 0.1 μM estradiol was present. [h] No inhibitory effect was seen. [i] This effect was partially prevented when 0.1 μM estradiol was present.

than expected based on their ER affinities. Furthermore, their proliferative effects were completely prevented in the presence of 1 μM tamoxifen (data not shown). Although not evident from the data in Table 2, all compounds exhibited concentration-dependent growth stimulatory effects which were maximal at 1−10 μM concentrations.

Estradiol-stimulated MCF-7 cell proliferation was not inhibited by **9** or **12**, but weak inhibition was seen with the other compounds. This inhibition was at least partly reversible when increased concentrations of estradiol were present. Efficacy of **9** and **12** was similar to that of estradiol, presumably due to inability of these analogues to antagonize estradiol.

Discussion

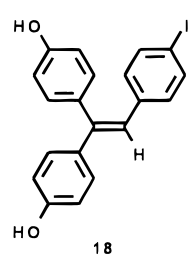
Our studies suggest the ER affinity of **8** is dependent on several structural characteristics in addition to the hydroxytriarylethylene nucleus. Removal of the ether oxygen as in **17**, repositioning of the oxyacetic acid moiety as in **12**, or saturation of the ethylenic bond as in **9** resulted in significant reductions in ER affinity (Table 1). These findings underscore the contribution each of these features makes to ER affinity.

The ER affinity of bisphenol **12** was much lower than expected based on its structural similarity to high-affinity ligand **1d**[4b] and to **18**, which had a RBA of 173 for rat ER.[18] Because the bulky, lipophilic iodine substituent of **18** clearly did not impede affinity, polarity of the oxyacetic acid side chain of **12**, rather than its bulk, might account for low affinity. Using TLC R_f values as a measure of comparative lipophilicity, the R_f value of **12** was one-fourth that of **8**, which had much higher ER affinity.

Despite relatively low MCF-7 cell ER affinities, **12** and triarylethane **9** were full estrogens with potencies approaching that of **8**. In general, ER RBA values in substituted triarylethylenes have been shown to correlate well with MCF-7 cell growth stimulating potencies.[4a] Regardless of the underlying mechanism(s) accounting for our observations, these results clearly illustrate the need to examine pharmacodynamic properties of substances displaying "unpromising" ER affinities.

Estrogenic potency of these compounds was clearly enhanced by the presence of a 4-hydroxy group. Thus the EC_{50} values (Table 2) of **15** and its desmethyl analogue were about one order of magnitude higher than those of the phenolic analogues.

The dominant estrogen-mimicking effect of (aryloxy)-acetic acid **8** is in contrast to that of arylacrylic acid **19**, which exhibited lower estrogen efficacy than the partial agonist tamoxifen in Ishikawa cells and in immature rat uterus.[19] These results infer that the lack of uterotrophic effects of **8**, referred in the introduction, are not accounted for by low intrinsic activity. Generally, effects of ER ligands in estrogen responsive cells and in the immature rat (uterus) are at least qualitatively comparable.



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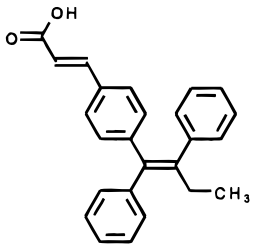

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The lack of proliferative effects of the phenolic triarylethylenes in the presence of "excess" concentrations of the nonsteroidal antiestrogen tamoxifen suggests that these proliferative effects are mediated solely by ER. Tamoxifen (1 μM) similarly antagonized fully the growth-promoting effect of 1 pM estradiol.

In conclusion, our studies indicate that incorporation of a polar (oxy)acetic acid moiety into these structural types results in ER ligands expressing MCF-7 cell estrogenicity accompanied by little or no estrogen antagonism, unlike triarylethylenes substituted with basic or neutral polar side chains, which tend to exhibit antiestrogenicity in such cells. Extrapolation of these results to possible therapeutic applications of (triarylethylene)acetic acids is not justified at this time, because effects of compounds on MCF-7 cell growth are not always consistent with effects seen in animals or humans. Thus, genistein, a flavone with ER affinity and MCF-7 cell growth stimulatory potency/efficacy similar to that of **8** and its analogues, is considered to be a cancer preventative agent.[20] In contrast, *N*-[(*p*-benzylphenoxy)ethyl]diethylamine was an inhibitor of MCF-7 cell growth, but was a cancer-promoting agent in the rat.[21] Therefore, our results require confirmation using in vivo models of extrareproductive tract estrogenicity.

Experimental Section

Infrared (IR) and 400 MHz ^{1}H NMR spectra were recorded in turn on Nicollet 510P FT-IR and Bruker AMX 400 spectrometers. NMR chemical shifts (δ) were determined using tetramethylsilane as standard. Mass spectra were obtained using a Perkin-Elmer Sciex API 1plus mass spectrometer. Melting points were determined using an Electrothermal 9100 apparatus. Reaction progress, column chromatographic fractions, and purity of products were analyzed qualitatively by analytical TLC using 0.25 mm Analtech silica gel GF254 plates. Plates were developed with solvent 1 [benzene−chloroform (50/50, v/v)], solvent 2 [chloroform−methanol−28% aqueous ammonia (90/10/0.5, v/v)], or solvent 3 [chloroform−2-propanol−glacial acetic acid (90/10/0.5, v/v)]. Developed plates were viewed under light of 254 nm wavelength. Reactions involving air-sensitive reagents were run under dry nitrogen gas. Chromatographic mobilities of compounds are expressed as R_f values. Reaction mixture solutions in ether were generally worked up by removal of excess water with anhydrous sodium sulfate, followed by filtration and concentration in vacuo. In some cases redissolution of product residues in benzene followed by reconcentration in vacuo was carried out to remove residual water.

Starting Materials. Benzyl ether **2a**, propiophenone, and 4-hydroxypropiophenone were obtained from Aldrich Chemical Co. β-Bromoethyl ether **2b** was prepared by refluxing a stirred mixture of 5 g (41 mmol) of *p*-hydroxybenzaldehyde, 50 mL of 10% aqueous NaOH, 1 g (2.9 mmol) of tetra-*n*-butylammonium hydrogen sulfate, and 8.5 mL (18.5 g, 98 mmol) of 1,2-dibromoethane for 2 h. After cooling, the mixture was extracted with two 60 mL portions of ether. The combined extracts were washed with 50 mL of water and worked up to give 3.1 g (40%) of **2b**. Tetrahydropyran-2-yl (THP) ether **3** was prepared by stirring a mixture of 1.73 g (10 mmol) of *p*-bromophenol with 10 mL of dihydropyran to which was added a small crystal of *p*-toluenesulfonic acid. After 1 h, 50 mL of ether was added, and the solution was washed with two 20 mL portions of 5% aqueous NaOH and then 20 mL of water. After 25 μL of triethylamine was added, workup gave 2.5 g (97%) of a colorless syrup which crystallized on storage at 8 °C. Dibenzyl ether **10** was prepared by dropwise addition of 2.77 g (22 mmol) of benzyl chloride to an ice-cold stirred solution of 2.14 g (10 mmol) of 4,4′-dihydroxybenzophenone in 45 mL of dimethylformamide which initially contained 0.52 g (22 mmol) of NaH. The mixture was heated for 3.5 h at 50 °C, then cooled, and poured into 200 mL of ice−water. The resulting solid was collected and mixed with 50 mL of 5% NaOH. The mixture was extracted with four 60 mL portions of chloroform. Workup of the combined extracts gave 4.25 g (100%) of **10** as a yellow powder: TLC (solvent 2) one spot, R_f 0.81. *p*-Methoxy-*p*′-methylbenzophenone (**13a**) was prepared by addition, in portions, of 2.67 g (20 mmol of aluminum chloride to an ice-cooled solution of 3.1 g (20 mmol) of *p*-toluoyl chloride in 5 mL of anisole over a period of 5−10 min with vigorous swirling. The orange solution was allowed to warm to room temperature. After 45 min, 10 mL of ice and water was added. The mixture was extracted with 20 mL of benzene. The organic phase was washed with 20 mL each of dilute aqueous HCl and water. Workup gave a colorless oil which was dissolved in 5 mL of benzene. Addition of 25 mL of petroleum ether resulted in immediate formation of white crystals which were collected and washed with cold petroleum ether: 3.1 g (68%); IR (CCl_4) 2870 (O−CH stretch), 1660 cm^{-1} (C═O). Similarly, *p*-bromo-*p*′-methoxybenzophenone was prepared by reaction of *p*-bromobenzoyl chloride with anisole; the product (14.2 mmol) was O-demethylated by treatment with 57 mmol of aluminum chloride in 100 mL of refluxing benzene for 8 h. The reaction mixture was poured into 100 mL of ice/water, and the product was extracted by addition of 40 mL of ether and worked up. The phenolic benzophenone **13b** crystallized from benzene: TLC (solvent 2) one spot, R_f 0.52; IR (KBr) 3300 (broad, OH stretch) 1640 cm^{-1} (C═O).

***p*-[(Tetrahydropyran-2-yl)oxy]-*p*′-(benzyloxy)benzophenone (4a).** A small (0.5−1 mL) portion of a solution of 5 g (19.6 mmol) of **3** in 17 mL of tetrahydrofuran was added to 0.54 g (22.5 mmol) of magnesium turnings, to which a few drops of dibromoethane and an iodine crystal had been added. After ca. 1 h of intermittent stirring, a mild exothermic reaction started. The remainder of the solution of **3** was added dropwise to the stirred mixture at a rate so as to maintain reaction momentum. Then the mixture was heated at reflux for 1 h. The clear gray solution was cooled to 0 °C, and a solution of 3.74 g (17.6 mmol) of **2a** in 15 mL of tetrahydrofuran was added dropwise. The mixture was refluxed for 2 h. The reaction solution was cooled in ice, and 4.0 mL of 30% NH_4Cl (9.2 mmol) was added slowly. The supernatant was decanted from precipitated salts and was concentrated in vacuo to give a yellow syrup which solidified on storage at 8 °C. This was mixed with petroleum ether, filtered, and washed with this solvent to give 6.91 g (100%) of a light orange powder: TLC (solvent 1) one spot, R_f 0.14. This intermediate (17.8 mmol) was dissolved in 70 mL of methylene chloride, and 5.13 g (13.6 mmol) of pyridinium dichromate was added. The mixture was stirred for 32.5 h. The mixture was concentrated in vacuo, and the residue was extracted with four 30 mL portions of ether. The combined extracts were filtered and concentrated in vacuo to give 6.81 g (99%) of **4a** as a gold oil: IR (neat, NaCl) 1650 cm^{-1} (C═O).

For conversion to *p*-(benzyloxy)-*p*′-hydroxybenzophenone **(5a)**, **4a** was dissolved in 30 mL of acetone. The solution was cooled in ice, and 3 mL of 10% HCl was added dropwise. After 1 h at room temperature, the reaction solution was concentrated in vacuo and the residue was shaken with 100 mL of ether and 50 mL of 1% aqueous HCl. The organic layer was washed with 50 mL of water and worked up routinely after addition of 10 mL of benzene. The residue was stirred for 2 h with 25 mL of 10% ethyl acetate in hexanes. The resulting orange solid was filtered and washed with this solvent to give 4.03 g (75%) of an orange powder: TLC (solvent 2) one spot

R_f 0.60. A small sample was crystallized from ethyl acetate−hexanes as shiny yellow plates: mp 136−137.5 °C. Anal. ($C_{20}H_{16}O_3$) C, H.

Application of these methods gave benzophenone **5b**, mp 135−139 °C (lit.[14] mp 139−142 °C) in 81% yield.

General Method for Olefination of 5, 10, and 13. The synthesis of 1-[(4-(benzyloxy)phenyl]-1-(4-hydroxyphenyl)-2-phenylbut-1-ene (**6a**) is typical. To a cold (−15 °C), well-stirred suspension of 5.07 g (79 mmol) of zinc powder in 60 mL of tetrahydrofuran was added slowly by syringe 4.4 mL (39 mmol) of titanium tetrachloride. The mixture was heated at reflux for 2 h and then cooled to ca. 40 °C. A solution of 4 g (13.2 mmol) of **5a** and 1.77 g (13.2 mmol) of propiophenone in 24 mL of tetrahydrofuran was added dropwise via syringe to the stirred suspension. The mixture was refluxed for 4 h, then cooled, and poured into a solution of 7 g of potassium carbonate in 70 mL of water. After standing overnight, the mixture was filtered and the collected precipitate was washed with tetrahydrofuran. The combined filtrate and washings were concentrated in vacuo to give a mixture of oil and water. This was shaken with 50 mL of benzene. The organic layer was dried and concentrated in vacuo to give 8.05 g of a gold oil. This was chromatographed on 50 g of silica gel using benzene as eluting solvent. The first 170 mL of eluate was discarded. The next 240 mL was collected and concentrated to give 4.4 g (82%) of a gold oil which solidified on standing at 8 °C. A portion of this was crystallized from petroleum ether, giving **6a**: mp 107.5−111 °C; ^{1}H NMR (acetone−d_6) δ 0.91 (t, J = 7 Hz, 3, CH_3), 2.48 (q, J = 7 Hz, 2, CH_2), 4.97 and 5.14 (s, 2, OCH_2Ar), 6.48−7.04 (m, 18, ArH). Anal. ($C_{29}H_{26}O_2$) C, H.

By this procedure the following triarylethylenes were prepared. β-Bromoethyl ether **6b** (40%): TLC (solvent 1) one spot, R_f 0.30; ^{1}H NMR (acetone-d_6) δ 0.92 (t, J = 7 Hz, 3, CH_3), 2.51 (q, J = 7 Hz, 2, CH_2), 3.78 and 3.80 (t, J = 6 Hz, 2, CH_2Br), 4.22 and 4.41 (t, J = 6 Hz, 2, OCH_2), 6.50−7.22 (m, 13, ArH).

Dibenzyl ether **11** (42%): mp 146−148 °C; TLC (solvent 1) one spot, R_f 0.21; ^{1}H NMR ($CDCl_3$) δ 0.95 (t, J = 7 Hz, 3, CH_3), 2.42 (q, J = 7 Hz, 2, CH_2), 4.63 (s, 1, OH), 4.94 and 5.09 (s, 2 each, CH_2Ar), 6.59−7.44 (m, 22, ArH). Anal. ($C_{36}H_{32}O_3$) C, H. [Note: addition of the starting ketones as a solution required 50 mL of warm (50 °C) tetrahydrofuran.]

Methyl ether **14a** crystallized on standing, and was recrystallized from ethanol in 55% yield, thus obviating chromatography: TLC (solvent 1) one spot, R_f 0.67; mp 94.5−95.5 °C (sublimed at 90 °C, 0.05 mmHg); ^{1}H NMR ($CDCl_3$) δ 0.92 (t, J = 7.6 Hz, 3, CH_2CH_3), 2.37 (s, 3, $ArCH_3$), 2.47 (q, J = 7.6 Hz, 2, CH_2CH_3), 3.67 (s, 3, OCH_3), 6.54 (d, J = 8.7 Hz, 2, ArH *ortho* to −O−), 6.78 (d, J = 8.7 Hz, 2, ArH meta to −O−), 7.09−7.16 (m, 9, remaining ArH). Anal. ($C_{24}H_{24}O$) H; C: calcd, 87.76; found, 86.92.

Bromophenol **14b** crystallized from ethanol in 70% yield after chromatography: TLC (solvent 1) one spot, R_f 0.24; ^{1}H NMR ($CDCl_3$) δ 0.91 and 0.93 (t, J = 7.4 Hz, 3, CCH_3), 2.45 and 2.49 (q, J = 7.4 Hz, 2, CH_2), 4.59 and 4.82 (s, 1, OH), 6.47 and 6.70 (d, J = 8.5 Hz, ca. 2, *ortho* and *meta* ArH to OH in *E*-isomer), 6.73 and 6.81 (d, J = 8.4 Hz, ca. 2, ArH *ortho* and *meta* to OH in *Z*-isomer), 7.47 (d, J = 8.2 Hz, 2, ArH *ortho* to Br in *E*-isomer), 7.07−7.18 (m, 7, remaining ArH).

Preparation of 6c. Reaction of 4.62 g (12 mmol) of **4a** with a 1.5 molar excess of benzylmagnesium chloride was carried out by a standard procedure.[5c] The resulting carbinol was dissolved in 15 mL of ethanol, and 2.5 mL of 5% HCl was added. After 1.5 h, 20 mL of ether and 3 mL of 10% aqueous sodium carbonate was added, and the mixture was worked up, affording 5.45 g of a brown oil. This was chromatographed on 38 g of 60−200 mesh silica gel, with benzene as eluent. The first 125 mL of eluate was discarded. The next 170 mL was collected and concentrated in vacuo to give 5.04 g (112%) of **6c** as a light yellow oil which solidified on storage at 8 °C. A 200 mg sample of this was further purified by washing with two 5 mL portions of hexanes, followed by trituration with 5 mL of methanol. The precipitate was collected and washed with cold methanol to give 57 mg of **6c** as a white powder: TLC (serial development two times with solvent 1) one spot, R_f 0.39; mp 129−132 °C. Anal. ($C_{27}H_{22}O_2$) C, H.

O-Demethylation of 14a. A stirred solution of 1.5 g (4.5 mmol) of **14a** in 30 mL of methylene chloride was cooled in ice-water, and a solution of 3.4 g (13.6 mmol) of boron tribromide in 3.4 mL of methylene chloride was added dropwise. The mixture was allowed to warm to room temperature, and after 3.5 h, a mixture of 5 mL of 28% aqueous ammonia and 5 g of ice was added. The mixture was shaken with 35 mL of ether, and the aqueous layer was discarded. The organic extract was washed with two 15 mL portions of water and worked up. The residue was crystallized from petroleum ether to give two crops of white crystals, 1.03 g (73%): IR (KBr) 3610 cm^{-1} (s, OH).

General Method for Conversion of (Triarylethylene)-monophenols to Oxyacetic Acids. The synthesis of **12** dibenzyl ether is typical. To a solution of 1.14 g (2.22 mmol) of **11** in 25 mL of acetone was added 1.86 g (1.25 mL, 11.13 mmol) of ethyl bromoacetate and 0.78 g (5.56 mmol) of potassium carbonate. The mixture was stirred and refluxed for 6 h, after which time TLC (solvent 1) indicated a major component, R_f 0.56, and the absence of **11**. The cooled mixture was filtered and concentrated. The resulting yellow syrup was dissolved in 19 mL of dioxane, and 10 mL of 5% NaOH was added. After 0.5 h, the solution was cooled in ice and 7 mL of 10% HCl was added. The resulting suspension was extracted with three 40 mL portions of ether. Workup left a white solid. In order to remove residual bromoacetic acid, this was mixed with 10 mL of acetone and then 20 mL of water was added. The mixture was filtered and washed with cold acetone−water (10/20, v/v). Drying (60 °C, 0.05 mmHg, 4 h) gave 1.03 g (81%) of halogen-free white powder: TLC (solvent 3) one spot, R_f 0.51.

By this procedure the following (triarylethylene)oxyacetic acids were prepared. Benzyl ether **7a** crystallized from chloroform−hexanes in 48% yield: mp 115−117 °C; TLC (solvent 3) one spot, R_f 0.42; ^{1}H NMR (acetone-d_6) δ 0.90 (t, J = 7.3 Hz, 3, CCH_3), 2.48 (q, J = 7.3 Hz, 2, CH_2), 4.55 and 4.72 (s, 1.06 and 0.94, $OCH_2C{=}O$), 4.96 and 5.13 (s, 0.96 and 1.04, OCH_2Ar), 6.60−7.40 (m, 18, ArH). Its desethyl counterpart **7c** was obtained as white crystals from chloroform (25%): mp 143−147 °C; TLC (solvent 3) two components of equal intensity, R_f 0.38 and 0.44; ^{1}H NMR (acetone-d_6) δ 4.57 and 4.73 (s, ca. 1 each, $OCH_2C{=}O$), 4.93 and 5.13 (s, ca. 1 each, OCH_2-Ar), 6.55−7.38 (m, 18, ArH). β-Bromoethyl ether **7b** (75%): TLC (solvent 3) one spot, R_f 0.48. *p*-Methyl analogue **15** was prepared from *O*-desmethyl **14a** in 94% yield after crystallization from acetone-water: mp 150−151 °C; ^{1}H NMR (acetone-d_6) δ 0.93 (t, J = 7.3 Hz, 3, CCH_3), 2.15 and 2.34 (s, 1.5 each, $ArCH_3$), 2.44 (q, J = 7.3 Hz, 2, CH_2), 4.58 and 4.64 (s, 1 each, $OCH_2C{=}O$), 6.98−7.71 (m, 13, ArH). Anal. ($C_{25}H_{24}O_3{\cdot}0.25H_2O$) C, H.

Catalytic Hydrogenolysis of 7a,c and 11-Benzyl Ether. To a solution of 1.15 g (2.48 mmol) of **7a** in 20 mL of tetrahydrofuran was added 155 mg of 10% palladium on carbon. The mixture was shaken for 11.5 h under 44 psi of H_2. TLC (solvent 3) suggested the reaction to contain 90−95% **8** (R_f 0.16) accompanied by 5−10% of **7a**. The mixture was filtered, and the collected catalyst was washed well with tetrahydrofuran. The combined filtrate and washings were concentrated in vacuo to give 1.14 g of a light yellow oil which solidified on storage at 8 °C. This was stirred with 10 mL of dry $CHCl_3$−hexanes (50/50) at room temperature for 1 h. The suspended product was filtered and washed with two 5 mL portions of the above solvent to give 676 mg (73%) of **8** as a white powder, after drying at 60 °C (0.05 mmHg) for 7 h: TLC (solvent 3) one spot, R_f 0.16; mp 215−219 °C; ^{1}H NMR (acetone-d_6) δ 0.92 (t, J = 7 Hz, 3, CCH_3), 2.49 (q, J = 7 Hz, 2, CH_2), 4.47 and 4.74 (s, ca. 1 each, $OCH_2C{=}O$), 6.51−7.11 (m, 13, ArH); EIMS m/z (rel intensity) 374 (M, 52), 315 (14, M − CH_2COOH), 44 (100). Anal. ($C_{24}H_{22}O_4{\cdot}1.5H_2O$) C, H.

By this same procedure was prepared bisphenol oxyacetic acid **12** from 1.03 g (1.81 mmol) of its dibenzyl ether, using 1 mL of acetic acid in 80 mL of tetrahydrofuran as solvent and 590 mg of 10% palladium on carbon. The crude product, from which residual solvent was removed by lyophilization, was crystallized from chloroform: 440 mg (63%) of beige crystals: mp 82−85 °C; TLC (solvent 3) one spot, R_f 0.04; ^{1}H NMR

(acetone-d_6) δ 0.90 (t, J = 7 Hz, 3, CCH_3), 2.42 (q, J = 7 Hz, 2, CCH_2), 4.62 (s, 2, OCH_2), 6.49 (d, J = 8 Hz, 2, ArH *ortho* to one phenolic OH), 6.6−6.9 (m, 8, unassigned ArH), 7.07 (d, J = 7.5 Hz, 2, *meta* ArH in Ar−O−C); EIMS m/z (rel intensity) 390 (M, 100), 331 (7, M − CH_2COOH), 44 (41). Anal. ($C_{24}H_{22}O_5 \cdot 0.25H_2O$) C, H.

Hydrogenolysis/Hydrogenation of 7c. A solution of 1.46 g (3.34 mmol) of **7c** in 100 mL of methanol was shaken with 0.16 g of 10% palladium on carbon for 2 h under 44 psi of H_2. The mixture was filtered. The filtrate was concentrated in vacuo, and the residue was dissolved in 10 mL of dioxane. The solution was diluted with 4 mL of 5% NaOH. After 0.5 h, the mixture was cooled in ice, acidified with 4 mL of 10% HCl, and extracted with 50 mL of ether. The extract was washed with 30 mL of water. Addition of 10 mL of benzene, followed by workup, gave 1.83 g of a gold oil. This was dissolved in 1 mL of dry chloroform, and the solution was diluted with 25 mL of hexanes. The resulting precipitate solidified on storage at 8 °C. This was crystallized from 3 mL of alcohol-free chloroform to give a total of 0.78 g (67%) of **9** as beige crystals after drying at room temperature for 16 h (0.05 mmHg): mp 143−148 °C (sublimed); TLC (solvent 3) one spot, R_f 0.23; ^{1}H NMR (acetone-d_6) δ 3.30 (d, J = 8 Hz, 2, CH_2Ph), 4.21 (t, J = 8 Hz, 1, $CHAr_2$), 4.62 (s, 2, OCH_2), 6.75 ("t", J = 9 Hz, 4, C_6H_4-OH), 7.05−7.25 (m, 9, remaining ArH), 8.30 (s, 1, ArOH). Anal. ($C_{22}H_{20}O_4 \cdot 0.5\ H_2O$) C, H.

Preparation of 8 via Cyanolysis of 7b. To a solution of 1.0 g (2.09 mmol) of **7b** in 60 mL of *N*,*N*-dimethylformamide was added 2.4 g (49 mmol) of powdered NaCN. The mixture was stirred for 48 h. The mixture was concentrated in vacuo. The residue was shaken with 70 mL each of ether and 5% aqueous $NaHCO_3$. The organic phase was discarded, and the aqueous phase was washed with two 50 mL portions of ether. Then it was acidified to pH <2 by addition of 10% aqueous H_2SO_4. The resulting suspension was extracted with two 60 mL portions of methylene chloride. Workup gave an oily residue which crystallized from chloroform−hexanes: 0.51 g (64%). Its TLC R_f value, mp, and ^{1}H NMR and mass spectra were nearly identical to that of the product of debenzylation of **7a**.

Conversion of 14b to 16 THP ether. The THP ether of **14b** was prepared from 2.31 g (6.09 mmol) of **14b** as described for **3**. This was dissolved in 25 mL of ether. The solution was cooled to −13 °C, and to the stirred suspension was added slowly by syringe 7.6 mL of a 1.6 M solution of *n*-BuLi in hexane (12.18 mmol). After 0.5 h under these conditions, a solution of 0.65 mL (6.5 mmol) of neat *N*,*N*-dimethylacetamide (stored over KOH pellets) was added dropwise via syringe to the orange solution. After being warmed to room temperature, the light yellow reaction suspension was cooled in ice and 1.1 mL of 30% aqueous NH_4Cl was added slowly. The mixture was separated, and the upper layer was washed with water and worked up to give 3.71 g of yellow syrup. Chromatography on 30 g of 60−200 mesh silica gel (Baker) using benzene as eluent gave fractions containing a single component, R_f 0.23 (solvent 1), which eluted after an unwanted component, R_f 0.46. Evaporation of solvent left 2.08 g (80%) of **16** THP ether. Preparative TLC of 160 mg of this on two Analtech silica gel GF254 TaperPlates gave 45 mg (28%) of **16** THP ether as a glassy white solid: IR (CCl_4) 1685 cm^{-1} Ar−C═O; ^{1}H NMR ($CDCl_3$) δ 0.93 (t, J = 7.4 Hz, 3, CH_2CH_3), 1.50−2.00 (m, 6, aliph ring CH_2), 2.44 (q, J = 7.4 Hz, 2, CH_2CH_3), 2.61 (s, 3, CH_3C═O), 3.54 and 3.84 (m, 1 each, OCH_2), 5.26 (t, J = 3.2 Hz, 1, O−CH−O), 6.70 (dd, J_1 = 6.5 Hz, J_2 = 2.7 Hz, 2, arom H *ortho* to −O−), 6.74 (dd, J_1 = 6.5 Hz, J_2 = 2.7 Hz, 2, arom H *meta* to −O−), 7.09−7.18 (m, 5, Ph), 7.35 (d, J = 8 Hz, 2, arom *meta* to C═O), 7.95 (d, J = 8 Hz, 2, arom *ortho* to C═O).

Synthesis of 17. A mixture of 570 mg (1.33 mmol) of **16** THP ether, 64 mg (2 mmol) of sulfur, and 7 mL of morpholine was stirred at 120 °C for 15 h. The brown mixture was mixed with 20 mL of ether and 10 mL of water. The ether was washed with 10 mL of 1% HCl and concentrated to give 0.54 g of yellow foam. This was dissolved in 7 mL of acetic acid, and 0.8 mL of water and 0.64 mL of sulfuric acid were added. The solution was heated at 120 °C for 5 h. The mixture was shaken with 30 mL of ether and 10−15 mL of water. The organic phase was washed with 15 mL of water and then shaken with 20 mL of 10% aqueous sodium carbonate. The aqueous extract was washed with 20 mL of ether, acidified with 10% HCl, and extracted twice with 25 mL portions of ether. Workup of the combined extracts gave 95 mg (19%) of a brown gum. Storage over benzene for several weeks resulted in crystallization of a tan solid, which was filtered and washed with cold benzene to give 28 mg of tan crystals: mp 189.5−192 °C (uncharacterized transition at 160 °C); TLC (solvent 3) one component, R_f 0.40; ^{1}H NMR (acetone-d_6) δ 0.91 (t, J = 7 Hz, 3, CH_3), 2.46 and 2.48 (q, J = 7 Hz, 2, allylic CH_2), 3.46 and 3.66 (s, 1 each, $ArCH_2$−CO−), 6.50−7.35 (13, ArH); EIMS m/z (rel intensity) 358 (M, 14), 299 (4, M − CH_2COOH), 44 (100). Anal.($C_{24}H_{22}O_3 \cdot 0.25\ H_2O$) C, H.

Estrogen Receptor Binding Affinity. Cell-free competitive assays were conducted using aliquots (0.2 mL) of cytosol prepared from immature rat uterus. Incubations were run in triplicate at 0 °C for 4 h and contained 5 nM [^{3}H]estradiol (56 Ci/mmol) and concentrations of test compound ranging from 1 nM to 10 μM. The extent of specific ^{3}H bound was plotted as a function of test compound concentration.[17]

Competitive binding in MCF-7 cells was determined as previously described,[22] with the following modifications. Incubation wells contained ca. 2×10^5 attached cells. To each well was added 3 mL of serum-free growth medium which contained 0.35 nM (56 nCi) of [^{3}H]estradiol. Medium also contained test compounds in varying concentrations, from 1 nM to 10 μM. Incubations were run in triplicate at 37 °C for 60 min. Medium and unbound ligands were removed by aspiration. Suspensions of cell nuclei were prepared by adaptation of methods to be described elsewhere.[23] Radioactivity in aliquots of lysed cells with suspended cell nuclei was determined in Ecolite liquid scintillation fluid. Specific ^{3}H bound was calculated as a function of test compound concentration by subtracting nonspecific binding found in incubations containing 10 μM estradiol from total ^{3}H bound at each test compound concentration.

Effects on MCF-7 Cell Growth. Cells ($0.5-5 \times 10^5$) were conditioned at 37 °C in a humidified atmosphere containing 5% carbon dioxide, in 75 cm^2 flasks containing 5 mL of complete growth medium (phenol red free Dulbecco's modified Eagle's medium-Ham's nutrient mixture, 50/50) containing 5% newborn calf serum, then passaged into 25 cm^2 flasks using low-estrogen medium.[23] Then, varying concentrations (1−10000 nM) of test compound without or with specified concentrations of estradiol or tamoxifen (see Table 2) were added. [Up to nine flasks were prepared this way for each compound concentration.] This was repeated on days 4, 7, and 9. At each of these time intervals, cells in two or three flasks were trypsinized and lysed to release cell nuclei. Aliquots of suspended nuclei were counted as described.[24] For sets of identically prepared flasks, ranges of cell numbers were within 5% of averages. Data were plotted as the log of the number of cell nuclei per flask as a function of incubation time. From such plots, observed generation (doubling) times (g) were found. These were used to calculate first-order growth rates (μ) by the formula $\mu = 0.693/g$. Calculated growth rates were plotted as a function of test compound concentration in order to determine potencies (EC_{50}, IC_{30}) and efficacies.

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