Carboxylic Acid Analogues of Tamoxifen: (*Z*)-2-[*p*-(1,2-Diphenyl-1-butenyl)phenoxy]-*N*,*N*-dimethylethylamine. Estrogen Receptor Affinity and Estrogen Antagonist Effects in MCF-7 Cells

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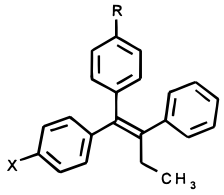
The triarylethylene estrogen mimetic (*E,Z*)-4-[1-(*p*-hydroxyphenyl)-2-phenyl-1-butenyl]phenoxyacetic acid (**4**) represents a novel class of estrogen receptor (ER) ligands which, like tamoxifen (**1**), can elicit estrogen agonist and antagonist effects, in turn, in nonreproductive and reproductive tissues. Analogues of **4**, incorporating structural features shown previously in triarylethylenes to improve ER affinity and estrogen antagonist properties, were prepared with the ultimate aim of identifying substances with improved estrogenicity exclusive of reproductive tissues. Thus, the side chain of **4** was elongated to give oxybutyric acid **13**, which was further altered by (a) repositioning of its *p*-hydroxyl to the neighboring *m*-position (**12**) and (b) ethylenic bond reduction (**14**). Also, the *p*-hydroxyl group and oxyacetic acid groups of **4** were, in turn, shifted to the neighboring *m*-positions, affording **8** and **9**. Oxybutyric acid analogue **13** had about 2 times the affinity for human ERα as **4**, and its antiproliferative effect in MCF-7 cells was greater than that of **1**. Dihydro analogue **14**, which was conformationally similar to *cis*-**13**, had very low ER affinity and antiestrogenicity, and *m*-hydroxy analogue **12** also had reduced ER affinity and potency, but its MCF-7 cell antiproliferative efficacy was retained. Modest ER affinity and antiproliferative potency were seen with **8**, in which phenolic and phenyl rings were *trans* to one another, but **9**, in which these rings were *cis*, was inactive. Our findings indicate that two-carbon side-chain elongation and/or *m*-positioning of the hydroxyl group in **4** affords analogues with dominant estrogen antagonist effects in MCF-7 cells.

Introduction

The triarylethylene antiestrogen tamoxifen (**1**) has become widely used in management and prevention of breast cancer.[1] The mechanistic basis for this medical application is thought primarily to involve interaction of **1** with estrogen receptors (ER) in breast cancer cells, resulting in antagonism of the growth-promoting effects of endogenous estrogens. Interaction of **1** with ER in organs of the reproductive axis also results in antagonism of administered estrogen, but in other tissues such interactions can result in full estrogenic effects. Thus **1**, like 17β-estradiol, was shown to prevent bone density loss in postmenopausal women.[2] These tissue-specific estrogenic responses to **1** are believed to result from increased intrinsic activity of **1**-liganded ER in regard to its interaction with DNA estrogen response elements in bone and other ER-containing tissues, relative to that in reproductive and ER-positive neoplastic cells.[3] Recognition of **1** as an estrogen mimetic in tissues outside of the reproductive system has stimulated subsequent investigations aimed at identification of structurally related ER ligands as tools for investigating the impact of chemical modification on the degree of tissue-specific estrogenicity.

Cultured MCF-7 human breast cancer cells, and other neoplastic human cells naturally endowed with ER, have been used extensively in initial characterization of estrogen agonist (growth stimulatory) and antagonist (growth inhibitory) potency and efficacy of ER ligands.[4] Selection of ER ligands as potential tissue-selective estrogens has generally been based on demonstration of estrogen antagonism in these cell types. Thus, numerous structural variants of **1** bearing basic side chains have been shown to inhibit estrogen-stimulated proliferation of MCF-7 or other estrogen-responsive cell lines and subsequently demonstrated estrogen antagonist effects in uterine tissues and estrogen agonist effects in bone and liver of ovariectomized laboratory rats.[4]

Similarly, an acidic side-chain-substituted analogue (**2**) antagonized 17β-estradiol in vitro and in a uterotrophic assay in immature rats but was an estrogen mimetic on bone maintenance.[5] Its phenolic counterpart (**3**) also



	R	X
1	$OCH_2CH_2N(CH_3)_2$	H
2	CH=CH-COOH	H
3	CH=CH-COOH	OH
4[a]	OCH_2COOH	OH

[a] This compound is a 1:1 mixture of the *trans* isomer (shown) and the *cis* isomer.

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was characterized in vitro as an estrogen antagonist, but replacement of **3**'s acrylic acid side chain with an oxyacetic acid moiety (**4**) resulted in dominant estrogen *agonist* activity in MCF-7 cells.[6] Further structural variation of **4** revealed the importance of the phenolic hydroxyl group, ether oxygen, and side-chain location on ER affinity, and although potency in MCF-7 cells was also affected by these specific structural changes, all such variants were estrogen agonists.[7] Interestingly the dihydrodesethyl analogue of **4**, despite its MCF-7 cell estrogen mimetic effect, was a selective estrogen in the ovariectomized rat: its bone maintenance effects were equivalent to those of **1**, but it had no uterotrophic effect.[8] These findings suggested that oxyalkanoic acids related to **4** were capable of expressing differential estrogenicity by a second mechanism distinct from tissue-specific ER modulation. This mechanism was speculated to involve ER-independent selective distribution to tissues outside the reproductive system.

Accordingly, our attention was directed toward further structural alteration of **4** with the aims of retaining ER affinity and attenuating estrogenic efficacy in MCF-7 cells. This approach was envisioned first to afford antagonists of estrogen-dependent MCF-7 cell proliferation and ultimately to result in characterization of substances expressing more potent ER-mediated agonist effects exclusive of the reproductive tract, due not just to uncharacterized selective distribution processes but also to selective ER modulation.

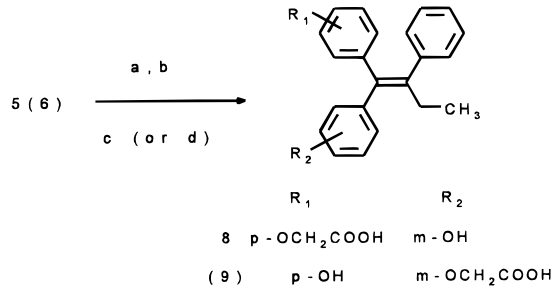
Several types of structural modifications of **4** appeared to be applicable for maximizing antiestrogenic potency and/or efficacy, based on relevant structure−activity studies reported for **1** and its analogues. First, the maximal antiproliferative and antiuterotrophic efficacies of **1** (X = OH) observed in MCF-7 cells and in the immature rat were exceeded by those of its *m*-phenolic analogue.[4b,9] Second, reduction of the ethylenic bond in fused ring analogues of **1** resulted in improvement in ER affinity and estrogen antagonist potency and efficacy.[4d] And, homologation of the basic ether linkage in analogues of **1** from two to four atoms generally resulted in increased ER affinity and/or estrogen antagonist potency and efficacy.[10] Thus we report the syntheses of analogues of **4** in which we evaluated the impact of ethylenic bond reduction, alternate *m*-positioning of aryl substituents (R and X), and elongation of the oxyalkanoic acid side chain on ER affinity and MCF-7 cell proliferation.

Results

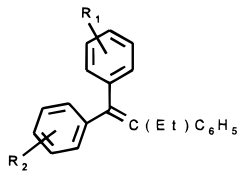
Synthesis and Characterization of 8, 9, and 12−14. Reductive cross-coupling of substituted benzophenones with propiophenone,[11] followed by silica gel column chromatographic separation of monophenolic products from those due to self-coupling reactions, afforded starting *p*-hydroxytriarylethylenes **5** and **7** in high yields. However, the *m*-hydroxy isomer **6** prepared in this manner contained one or more major impurities suggested by proton NMR spectroscopy to be 1,1,2-triarylethane derivatives.

Preparation of the *trans*-3-hydroxy and *cis*-3-oxyacetic acid analogues of **4** is outlined in Scheme 1. Alkylation[7] of **5** and **6** with ethyl bromoacetate followed by saponification of the resultant oxyacetic acid esters gave the

Scheme 1. Synthesis of Hydroxytriarylethylene Oxyacetic Acid Isomers[a]



[a] Reagents: (a) $BrCH_2COOEt$, K_2CO_3, acetone; (b) NaOH, aq dioxane; (c) BBr_3, CH_2Cl_2; (d) H_2, 10% Pd/C, THF.



	R_1	R_2
5	*p*-OH	*m*-$C_6H_5CH_2O$
6	*m*-OH	*p*-$C_6H_5CH_2O$
7	*p*-OH	*p*-$C_6H_5CH_2O$

respective benzyl ethers of **8**† and **9**†. (†The specified triarylethylene oxyacetic acid (cf. Scheme 1) was accompanied by a significant amount of its geometric isomer.) Catalytic debenzylation followed by alkaline equilibration (see below) of the second of these furnished **9**, but **8**† benzyl ether underwent excessive concomitant ethylenic bond hydrogenation during debenzylation. Thus, it was debenzylated noncatalytically.

Relative chemical shifts of side-chain (and benzyl group) *O*-methylene protons in NMR spectra of **8**†, **9**†, and subsequent compounds in this study enabled estimation of geometric isomer ratios and assignment of configurations.[9] Accordingly, the spectrum of **8**† featured *O*-methylene singlets at 4.56 and 4.78 ppm, with an intensity ratio of 3:1. These were assigned in turn to **8** and its *cis* isomer, based on correlation with *O*-methylene chemical shifts of corresponding geometric isomers of **1**.[9,12] Fisher esterification of **8**† with ethanol, followed by preparative thin-layer chromatography and saponification of the more polar, major component, afforded **8**. Equilibration of **9**† in dilute sodium hydroxide resulted in nearly complete conversion to **9**. In its proton NMR spectrum, the ratio of *O*-methylene singlets at 4.73 and 4.39 ppm, indicative of the respective *cis* and *trans* isomers, was 97:3.

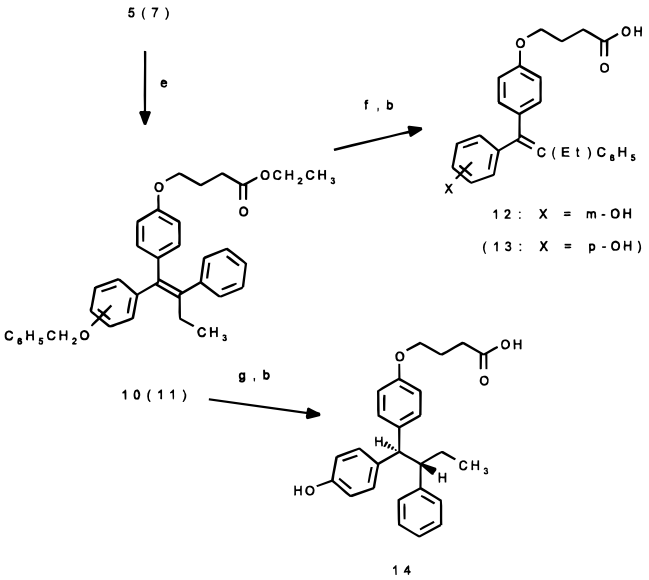
Alkylation of **5** and **7** with ethyl 4-bromobutyrate afforded respective *m*- and *p*-benzyl ethers **10** and **11** (Scheme 2). Attempted isolation of the *cis* isomers of **10** and **11** was not successful. (Excess residual alkylating agent, present prior to or after saponification of *cis* isomer-enriched mixtures of **10** or **11**, could not be removed by preparative TLC or liquid−liquid extraction.) Also, attempted preparation of the oxypropionate homologue of **11** by extension of the specified alkylation method (Scheme 2) afforded only a small amount of the putative alkylation product, accompanied by a large amount of a component which comigrated with starting material on thin layer chromatograms. Hydrolytic de-

Table 1. Affinities of **4** and Selected Analogues for Human ERα

	1	**4**	**8**	**9**	**12**	**13**	**14**	17β-estradiol
IC_{50}, nM[a]	42	500	1440	>11 μM	1700	235	6000	5.9
RBA[b]	14	1.2	0.4	<0.05	0.35	2.5	0.1	100

[a] The concentration of test compound required to displace specifically bound [^{3}H]17β-estradiol by 50%. [b] The relative binding affinity (RBA) of each compound was determined by dividing the IC_{50} of 17β-estradiol by that of the test compound and multiplying the resulting fraction by 100.

Scheme 2. Synthesis of Oxybutyric Acid Analogues **12**−**14**[a]



[a] Reagents: (b) see Scheme 1; (e) $BrCH_2CH_2CH_2COOEt$, K_2CO_3, acetone; (f) HCl, aq ethanol; (g) H_2, 10% Pd/C, 10% aq THF.

benzylation of **10** and **11**, followed by deesterification, gave *m*- and *p*-hydroxylated oxybutyric acids **12** and **13** as 1:1 mixtures of respective geometric isomers. Resolution of constituent geometric isomers of **12** and **13**, using the procedure by which **8** was prepared, was not successful. Reisomerization of the chromatographically separated isomers of **12** and **13** ethyl esters occurred during mild alkaline or acidic hydrolysis, as was the case when preparation of *trans*-**4** from its ethyl ester was attempted.[6] Consequently, biologic evaluation was carried out using **12** and **13** as the indicated (above) isomer mixtures.

Saponification of **11**, followed by catalytic hydrogenolysis/hydrogenation of the intermediate carboxylic acid, appeared to proceed without geometric isomerization. Thus, the proton NMR spectrum of **14** displayed a single *O*-methylene triplet centered at 4.01 ppm. Ethylenic bond isomerization prior to its reduction would have resulted in "pairing" of these signals, based on analogy with diastereoisomers of dihydro-**1**.[13]

Triarylethylenes **8**, **12**, and **13** and triarylethane **14** exhibited contrasting novel mass spectral features. The high-resolution liquid secondary ion (LSI) mass spectra of **8**, **12**, and **13** each exhibited a protonated molecular ion (MH^+) accompanied by an intense peak (70−95% relative intensity) representing the radical cation ($M^{+\bullet}$). The LSI mass spectrum of **14** contained only the expected MH^+ ion and a pronounced fragment ion resulting from the loss of 120 Da (C_6H_5-CH_2-CH_2-CH_3) from the protonated molecular ion. Processes leading to radical cation formation in LSI mass spectra have been studied systematically.[14] In particular, appearance of radical cations has been correlated with relative electron affinities of the sample molecule and liquid matrix used in the experiment.

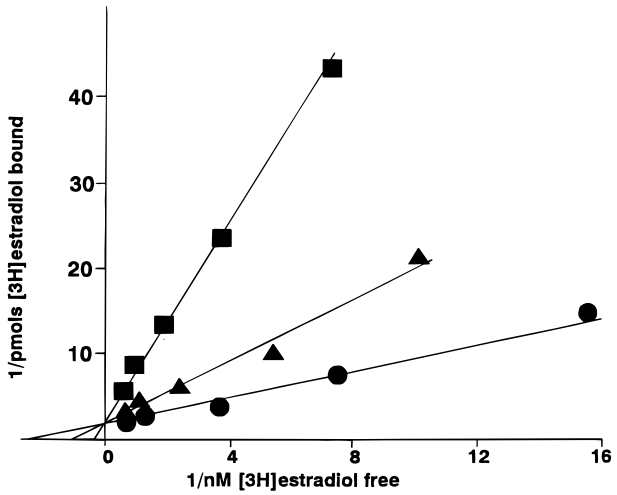


Figure 1. Effect of **13** on the extent of interaction of 17β-estradiol with human ERα. Concentrations of **13** were 0 (●), 70 (▲), and 300 (■) nM. Line slopes and *y*-axis intercepts of each data series were calculated by linear regression analysis.

Estrogen Receptor Affinities of 8, 9, and 12−14. The degree of equilibrium binding of saturating concentrations of [^{3}H]17β-estradiol to human recombinant ERα was determined in turn in the presence of increasing concentrations of unlabeled 17β-estradiol and test compounds. Plots of specifically bound [^{3}H]17β-estradiol as a function of competitor concentration yielded slopes that did not differ substantially from each other, enabling the use of IC_{50} values to calculate affinities of test compounds relative to that of unlabeled 17β-estradiol (Table 1). Affinities of **1** and **4** were determined similarly and are included for comparison.

The oxybutyric acid homologue **13** had an RBA about 2 times that of **4**. Repositioning of **13**'s phenolic hydroxyl (**12**) or saturation of its double bond (**14**) resulted in considerable losses of ER affinity. Regarding the two configurationally defined analogues of **4**, *trans* analogue **8** had an RBA about 1/3 that of **4**, but *cis* analogue **9** was essentially without ER affinity.

The degree to which [^{3}H]17β-estradiol, present at varying concentrations, was displaced from ERα in the presence of set concentrations of **13** was assessed. Data are expressed as a double-reciprocal plot (Figure 1).

Antiproliferative Effects in MCF-7 Cells. The ability of **4**, **8**, **9**, and **12**−**14** to inhibit growth of MCF-7 cells cultured in non-estrogen-depleted medium in a concentration-dependent manner was assessed in comparison with **1**, and results are shown in Table 2. In separate experiments, restoration of cell proliferation to drug-free control values was seen when **1**, **8**, **12**, or **13**, at respective concentrations of ≤1 μM, was coincubated with 1 nM concentrations of 17β-estradiol. In

Table 2. Estrogen Antagonist Effects of Analogues of **4** in MCF-7 Cells

	cell number: percent of control (±SEM)[a]						
concn, nM	**1**	**4**	**8**	**9**	**12**	**13**	**14**
10	97 (6)		99 (8)		89 (7)	74 (4)	
100	72 (5)		82 (4)		91 (5)	25 (5)	99 (7)
1000	31 (3)	96 (5)	78 (2)		50 (4)	20 (3)	102 (4)
10000	24 (5)	102 (4)	52 (3)	100 (5)	19 (5)	18 (5)	68 (6)
estimated IC_{50}, nM[b]	190	NM[c]	1040	NM[c]	720	20	3200

[a] Cell numbers are expressed in terms of viable totals with respect to those found in drug-free control incubations run in parallel. Each value is from at least three separate experiments. In each experiment, the inhibitory effect of 1.0 μM **1** was determined in order to cross-validate results for all compounds. [b] The concentration required for one-half of each compound's "maximal" growth antagonism observed at 10 000 nM. In individual experiments, this was determined from inspection of the plot of cell growth as a percent of control vs log concentration of test compound. [c] No meaningful growth inhibition was observed.

accord with previous studies carried out using different experimental protocols,[6,15] **4** and **9** exhibited no growth inhibitory effects at respective 10 μM concentrations. Additionally **4**, in contrast to **12**−**14**, was shown to reverse the growth suppressive effect of 1 μM **1** in a concentration-dependent manner, with maximal efficacy equal to that of 17β-estradiol. However **8**, the 3-hydroxy regioisomer of **4**, antagonized proliferation with modest potency and efficacy relative to **1**.

Oxybutyric acid analogue **13** compared favorably to **1** in terms of estrogen antagonist potency and maximal effectiveness. Repositioning of its phenolic hydroxyl (**12**) resulted in no change in efficacy, but reduction in potency; hydrogenation of its ethylenic bond (**14**) caused reduction in both.

Discussion

Two-carbon homologation of the acidic side chain of the estrogen mimetic **4** resulted in improvement of ER affinity and reduction in estrogenicity. Thus, **13** was a potent, effective antiestrogen in MCF-7 cells (Table 2). This finding indicates that greater ER affinity and antiestrogenic effectiveness can be achieved by side-chain elongation of triarylethylene carboxylic acids, just as was the case in analogous triarylethylenes with basic side chains.[10]

Previously, binding of **1** and 17β-estradiol to rat uterine ER was shown to be competitive.[16] It has generally been assumed that interactions of newer analogues of **1** with ER are also competitive with 17β-estradiol, but an uncompetitive interaction of a nonsteroidal substance with ER has been reported.[17] The data in Figure 1 indicate that like **1**, interaction of **13** with human ERα was competitive with respect to 17β-estradiol. Together, these results indicate that **1**, **13**, and 17β-estradiol interact with a common site within the hormone binding domain of ER.

In the presence of ERα, **8** exhibited modest affinity but its isomer (**9**) was ineffective. This difference is suggested to be due primarily to the established preference by ER for triarylethylenes endowed with *trans*-hydroxystilbene moieties, such as in **8**, over their *cis* counterparts, such as **9**.[9,18] (However, variance in the position of side-chain attachment (*p* vs *m*) in **8** and **9** might also contribute to observed affinity differences.) Similarly, a *cis*-like orientation of phenolic and phenyl rings in **14** could account for its low ER affinity. The analogous dihydro-4-hydroxytamoxifen produced by hydrogenation of **1** (X = OH) was suggested to resemble its opposite geometric isomer, *cis*-**1** (X = OH), conformationally.[13] Compound **14**, prepared from its *trans* precursor (**11**), featured a large (12 Hz) benzhydryl-benzyl proton coupling constant in its NMR spectrum. This suggested an antiperiplanar orientation of these protons, thus implicating a *gauche* relationship between the phenolic and phenyl rings. The NMR spectrum of **14** also exhibited an *O*-methylene triplet above 4 ppm, typical of triarylethylenes in which the aryl-*O*-methylene and phenyl rings are on opposite sides of the ethylenic bond.

Although the ERα affinity of **13** was 1/5 that of **1**, it exhibited about a 10-fold greater antiestrogenic potency than **1** (Table 2). This discrepancy is unlikely to be a consequence of differential interaction of either ligand with other ER isoforms besides ERα in MCF-7 cells. Although ER heterogeneity, in particular the presence of ERβ and ERβ2, has been demonstrated in reproductive and/or nonreproductive tissues,[19] MCF-7 cells appear to contain only ERα.[19a,20] The overall ER affinities of **1** and **13** (Table 1) do not reveal the specific amino acid residues in the ERα hormone binding domain with which these ligands interact. In particular, the amino acid residue(s) in ERα which interact with the cationic side chain of **1** would presumably differ from those that accommodate the anionic side chain of **13**. This difference in specific amino acid bonding in the ERα hormone binding domain might affect differentially, by one or more of several mechanisms,[21] the ability of respective liganded ERα complexes to interact effectively with DNA estrogen response elements, resulting in greater antagonist potency of **13**.

Another possibility is that differences in permeability of these compounds into MCF-7 cells might account for the observed discrepancy between ER affinity and antagonistic potencies of these ligands. The concentration of **13** available for interaction with nuclear ER might be greater than that of **1** due to more efficient diffusion through cell membranes, a process not involved in interaction of **1** and **13** with isolated ERα.

Isolation of **13**'s geometric isomers was impeded by facile reisomerization of its *cis*- and *trans*-ethyl ester precursors during or after aqueous hydrolysis (see Results). Similar results were obtained with the corresponding ethyl esters of **12**. Consequently, the degree to which the constituent geometric isomers of these compounds contribute to their observed ER affinities and estrogen antagonist effects is not known.

Nevertheless, our studies extend earlier findings suggesting that antiestrogenic activity can be found in hydroxytriarylethylene carboxylic acids endowed with side chains of sufficient length[5] or in which the *p*-phenolic hydroxyl is shifted to the *m*-position in the ring

geminal to the one bearing the side chain.[4b] The antiestrogenic potency and efficacy of **13** were comparable to those of **1** and **2**, which have been shown experimentally to be full estrogen mimetics in bone and/or liver.[5,22] Future efforts will investigate the degree to which **13** is capable of similar tissue-selective estrogenicity.

Experimental Section

Solvents, chemicals, and biochemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI), Sigma Chemical Co. (St. Louis, MO), or the University of Georgia Central Research Stores. Hydroxytriarylethylene **4** was available from previous studies.[7] The tetrahydrofuran (THF) used in moisture-sensitive reactions was distilled from lithium aluminum hydride immediately prior to use. All air-sensitive reactions were performed in dry glassware under a nitrogen atmosphere. Workup of organic extracts of reaction products was carried out by removal of water by addition of magnesium sulfate, followed by filtration and concentration in vacuo. Analytical thin-layer chromatography (TLC), using 5- × 20-cm glass-backed 0.25-mm silica gel GF_{254} plates (Analtech, Inc., Newark, DE) was used to monitor the course of reactions, the progress of column chromatography, and determine the purity of final products. Plates were developed in either solvent 1 [benzene/chloroform (50/50 v/v)] or solvent 2 [chloroform/ 2-propanol/glacial acetic acid (90/10/0.5 v/v/v)] and viewed under light of 254-nm wavelength. Preparative TLC was carried out as described above except using 20- × 20-cm plates coated with 1-mm layers of silica gel GF_{254}, with developing solvents as specified below. Melting points were determined on an Electrothermal 9100 apparatus and are uncorrected. Proton nuclear magnetic resonance (NMR) spectra were obtained using a Bruker AMX 400 spectrometer. The solvent for NMR analyses was acetone-d_6 unless otherwise stated. Chemical shifts (δ) are reported as downfield from the internal standard, tetramethylsilane. Positive ion liquid secondary ion mass spectra were recorded on a Micromass AutoSpec series-M high-resolution magnetic sector mass spectrometer of EBE geometry. The instrument used a cesium ion gun operated at 27 keV at an accelerating voltage of 8 kV. The scan speed was 1.5/decade, and 10 scans were averaged for each mass spectrum. Concentrations of the sample solutions for the neat compounds were about 1 μg/μL using glycerol as the matrix. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA.

Starting Materials. Compound **7** was available as reported;[7] **5** and **6** were prepared in a similar manner. Thus **5** was isolated in 71% yield: TLC (solvent 1) one spot, R_f 0.34; mp 107.6−109.5 °C (initial softening at 87.3−88.7 °C); ^{1}H NMR 7.7−7.0, m, 18H (Ar*H*); {5.5, s and 4.71, s}, 2H (OCH_2Ar); 2.1, m, 2H (CH_2CH$_3$); 0.9, m, 3H, (CH$_2$CH_3). Crude **6** solidified on standing (61% yield): ^{1}H NMR 7.5−6.5, m, 18H (Ar*H*); {5.12, s and 4.95, s}, 2H (OCH_2Ar); 2.47, q, J = 7 Hz, 2H (CH_2CH$_3$); 0.97, t, J = 7 Hz, 3H, (CH$_2$CH_3).

Preparation of 4-[1-(3-Hydroxyphenyl)-2-phenyl-1-butenyl]phenoxyacetic Acid (8). Ethyl bromoacetate (2.55 mL, 22.9 mmol) and potassium carbonate (0.91 g, 6.6 mmol) were added to a solution of **5** (0.80 g, 1.9 mmol) in 25 mL of acetone. The mixture was refluxed for 11 h. The solution was cooled, filtered, and concentrated. The resulting oil was dissolved in 20 mL of dioxane, and 14 mL of 5% aqueous NaOH was added. After stirring for 0.5 h, the reaction mixture was cooled to 0 °C and acidified with 10 mL of 10% aqueous HCl. The product was extracted with 50 mL of ether, washed with two 20-mL portions of water, then dried, and concentrated. Crystallization from acetone and water (2:1) afforded 0.52 g (60%) of **8** benzyl ether as white crystals: TLC (solvent 2) one spot, R_f 0.57; mp 148.4−152.0 °C; ^{1}H NMR 7.7−7.0, m, 18H (Ar*H*); {5.11, s and 4.78, s}, 2H (OCH_2Ar); {4.74 and 4.55, s}, 2H (OCH_2COOH); 2.42, m, 2H (CH_2CH$_3$); 0.85, m, 3H (CH$_2$CH_3).

To a solution of 100 mg (0.22 mmol) of **8** benzyl ether in 30 mL of methylene chloride was added 125 mg (0.50 mmol) of boron tribromide. The orange solution was stirred for 24 h. A cold solution of 5% aqueous sodium bicarbonate was added in portions to the reaction solution until the mixture was pH 8−9 to litmus. The reaction mixture was concentrated in vacuo, and the remaining basic aqueous layer was washed with three 20-mL portions of ether. The aqueous layer was then acidified with 10% aqueous HCl (until pH 1−2 to litmus) and extracted with 35 mL of ether. The organic layer was washed with two 20-mL portions of water, dried, and concentrated, giving a yellow solid. Crystallization of this from chloroform and hexanes (1:1) gave 49 mg (60%) of a 1:1 mixture of **8** and its *cis* isomer: TLC (solvent 2) one spot, R_f 0.39; mp 137.2−140.0 °C; ^{1}H NMR 7.7−7.0, m, 13H (Ar*H*); {4.74 and 4.58, s}, 2H (OCH_2COOH); 2.47, m, 2H (CH_2CH$_3$); 0.91, m, 3H (CH$_2$CH_3).

The above geometric isomer mixture was dissolved in 10 mL of absolute ethanol, and 0.25 mL of concentrated HCl was added. The mixture was stirred for 1 h at room temperature. A solution of 10% aqueous sodium bicarbonate was added, and solvent was removed under a stream of nitrogen. The residual oil was dissolved in 0.25 mL of acetone and subjected to preparative TLC (1.0-mm silica gel GF plate; Analtech, Newark, DE). The plate was developed in benzene−triethylamine (90−10, v/v). Two zones, R_f 0.51 and 0.54, approximate intensity ratio 3:1, were present. The lower of these was removed from the plate and stirred with 5 mL of ethanol for 15 min. The mixture was filtered, and the filtrate was concentrated. The residue was dissolved in 5 mL of 1,4-dioxane, and 1 mL of 10% aqueous NaOH was added. After 0.5 h at room temperature, the solution was acidified with 2 mL of 10% aqueous HCl and extracted with three 10-mL portions of ether. The combined extracts were dried and concentrated to yield a yellow residue, which was dissolved in 5 mL of chloroform, followed by dilution with 3 mL of hexanes. Storage at 8 °C afforded 15.3 mg of **8** as a pale yellow solid: mp 134.4−136.6 °C; ^{1}H NMR {7.25−7.05 and 6.80−6.70, m}, 9H (C$_6H_5$ and HO−C$_6H_4$); {6.82 and 6.61, d, J = 8 Hz}, 4H (CH$_2$C$_6H_4$); 4.58, s, 2H (OCH_2COOH); 2.47, q, J = 7 Hz, 2H (CH_2CH$_3$); 0.89, t, J = 7 Hz, 3H (CH$_2$CH_3); HRMS (LSIMS) m/z calcd for $C_{24}H_{23}O_4$ 375.1596 (MH^+), found 375.1614, rel ion intensity $M^{+\bullet}/MH^+$ = 0.95. Anal. ($C_{24}H_{22}O_4 \cdot 2.25H_2O$) C, H.

Preparation of 3-[1-(4-Hydroxyphenyl)-2-phenyl-1-butenyl]phenoxyacetic Acid (9). Compound **6** (1.03 g, 2.5 mmol) was converted to **9** benzyl ether as described for **8**. The product, a yellow oil, was stored at 8 °C over hexanes. The resulting white powder was filtered and washed with cold hexanes: 481 mg (41%). To a solution of 450 mg of this in 15 mL of tetrahydrofuran was added 50 mg of 10% Pd on carbon. The mixture was shaken under 47 psi of H_2 for 24 h. Filtration and concentration gave 132 mg of light brown oil. This was dissolved in 5 mL of methanol containing HCl gas. After 24 h, solvent was evaporated and the residue was dissolved in 0.6 mL of methanol. The solution was subjected to preparative TLC using benzene−triethylamine (90−10, v/v) as mobile phase. Bands with R_f 0.27−0.31 were cleanly separated from those with R_f 0.20−0.23, characterized separately by proton NMR spectrometry as dihydro-**9** methyl ester. The upper bands were scraped off the plates, combined, and eluted with 25 mL of methanol. The mixture was filtered and concentrated in vacuo. The residue was dissolved in 2 mL of dioxane, and 1 mL of 5% NaOH was added. After 15 min of stirring, the mixture was extracted with 2 mL of ether, acidified with 10% aqueous HCl, and extracted with two 3-mL portions of ether. These last ether extracts were worked up to give a colorless oil. This was dissolved in 0.5 mL of dry chloroform, and the solution was diluted with 0.4 mL of hexanes. Storage at 8 °C gave white crystals, which were filtered and washed with cold chloroform−hexanes (50−50) to give 23 mg of **9**: mp 159.5−165.2 °C (partial), 180.1−181.5 °C (complete); ^{1}H NMR 7.30−7.10, m, 5H (C$_6H_5$); {6.84 and 6.55, d, J = 8.5 Hz}, 4H (C$_6H_4$OH); 6.80−6.60, m, 4H (C$_6H_4$O−CH$_2$); 4.73, s, 2H (CH_2−OAr); 2.45 q, J = 7 Hz, 2H (CH_2CH$_3$); 0.97, t, J = 7 Hz, 3H, (CH$_2$CH_3). Anal. ($C_{24}H_{22}O_4 \cdot 0.5H_2O$) C, H.

Preparation of *trans*-4-{[1-(4-Benzyloxyphenyl)-2-phen-

yl-1-butenyl]phenoxy}-*n*-butanoic Acid Ethyl Ester (11). Ethyl 4-bromobutyrate (4.4 mL, 32.8 mmol) and potassium carbonate (3.22 g, 23.3 mmol) were added to a solution of **7** (1.76 g, 4.33 mmol) in 50 mL of acetone. The mixture was refluxed for 10 h. The solution was cooled, filtered, and concentrated. Hot hexanes was added, and the solution cooled to 4 °C. The resulting white crystals were collected and washed with cold hexanes, affording 0.98 g (44%) of **11** ethyl ester: TLC (solvent 1) one spot, R_f 0.60; ^{1}H NMR ($CDCl_3$) 7.45−7.15, m, 10H (C_6H_5); {7.09 and 6.95, d, J = 8H}, 4H (Bz-O-C_6H_4); {6.76 and, 6.52, J = 8 Hz}, 4H (R−O−C_6H_4); 5.07, s, 2H (OCH_2-Ar); 4.11, q, J = 8 Hz, 2H (COO$CH_2$$CH_3$); 3.86, t, J = 6 Hz, 2H (ArO$CH_2$$CH_2$); 2.55−2.43, m, 4H ($CH_2$COOR and $CH_2$$CH_3$); 2.02, p, J = 6 Hz, 2H ($CH_2CH_2CH_2$); 1.23, t, J = 8 Hz, 3H (COO$CH_2$$CH_3$); 0.93, t, J = 7 Hz, 3H (CH_2CH_3).

Reactivity of 11. A. Saponification and Hydrogenation/Hydrogenolysis: Preparation of 4-{[1-(4-Hydroxyphenyl)-2-phenyl-1-butanyl]phenoxy}-*n*-butanoic Acid (14). To a solution of 0.98 g of **11** in 20 mL of dioxane was added 10 mL of 10% aqueous NaOH. After stirring at room temperature for 1 h, the solution was cooled to 0 °C, acidified with 10% aqueous HCl, and extracted with three 30-mL portions of ether. The organic extracts were combined, dried, and concentrated to give a pale yellow oil. Crystallization from chloroform−hexanes (1:2) afforded 0.42 g (65%) of **11** free acid: TLC (solvent 2) one spot, R_f 0.57; ^{1}H NMR spectrum was identical to that of **11** except signals at 4.11 and 1.23 ppm were absent. To a solution of 262 mg of this (0.53 mmol) in 20 mL of THF were added 2 mL of water and 0.63 g of 10% palladium on carbon. The suspension was shaken under 44 psi of hydrogen gas for 24 h. The reaction mixture was filtered and concentrated to yield a milky white liquid. This was extracted with three 50-mL portions of ether, and the combined extracts were dried and concentrated to afford a yellow oil which solidified on standing. Crystallization from chloroform−hexanes (1:1) yielded 90 mg (42% yield) of **14** as white crystals: TLC (solvent 2) one spot, R_f 0.43; mp 157.2−158.8 °C; ^{1}H NMR {7.39 and 7.24, d, J = 8 Hz}, 4H (R−O−C_6H_4); 7.15−6.95, m, 5H (C_6H_5); {6.88 and 6.50, d, J = 8 Hz}, 4H (HO−C_6H_4); 4.15, d, J = 12 Hz, 1H (Ar_2CH); 4.01, t, J = 6 Hz, 2H (OCH_2); 3.39, ddd, $J_1 = J_2$ = 12 Hz, J_3 = 2.7 Hz, 1H (PhCHEt); 2.49, t, J = 6 Hz, 2H (CH_2COOH); 2.03, p, J = 6 Hz, 2H ($CH_2CH_2CH_2$); {1.62 and 1.42, m}, 2H (CH_2CH_3); 0.60, t, J = 8 Hz, 3H (CH_2CH_3); HRMS (LSIMS) m/z calcd for $C_{26}H_{29}O_4$ 405.2065 (MH^+), found 405.2063, rel ion intensity $MH^+ - 120/MH^+$ = 0.11. Anal. ($C_{26}H_{28}O_4 \cdot 0.25H_2O$) C, H.

B. Hydrolytic Debenzylation: Preparation of 4-{[1-(4-Hydroxyphenyl)-2-phenyl-1-butenyl]phenoxy}-*n*-butanoic Acid (13). To a solution of 360 mg (0.69 mmol) of **11** in 50 mL of ethanol was added 40 mL of concentrated aqueous HCl. The mixture was stirred and refluxed under nitrogen gas for 10 h. The mixture was concentrated to about 1/3 its original volume in vacuo. The supernatant was discarded, and the gummy residue was washed with 20 mL of water and dissolved in 10 mL of dioxane. Then 3 mL each of water and 10% NaOH were added, and the mixture was stirred for 1 h. The mixture was extracted with two 20-mL portions of ether. The aqueous phase was cooled in ice, acidified with 10% HCl, and extracted with two 25-mL portions of ether. These last extracts were combined and worked up to give a gold oil, which solidified on storage at 8 °C yielding 204 mg (73%) of **13**, as a light yellow semisolid after drying for 24 h at 60 °C (0.05 mmHg): TLC (solvent 2) one spot, R_f 0.51; ^{1}H NMR 7.20−7.10, m, 5H + 1H (C_6H_5 + 0.25 O-C_6H_4); {7.07, 6.95, 6.85, 6.78, 6.70, 6.59, 6.49, d, J = 8 Hz}, 7 H (1.75 O-C_6H_4); {4.08 and 3.91, t, J = 6 Hz}, 2 H (OCH_2R); 2.52−2.41, m, 4H (CH_2COOH and CH_2CH_3); 2.06−1.95, m, 2H ($CH_2CH_2CH_2$); 0.91, t, J = 7 Hz, 3H (CH_2CH_3); HRMS (LSIMS) m/z calcd for $C_{26}H_{27}O_4$ 403.1909 (MH^+), found 403.1929, rel ion intensity $M^{+\bullet}/MH^+$ = 0.70. Attempted crystallization of this product was not successful. A 40-mg portion of **13** was dissolved in 2 mL of methanol, and the solution was passaged through a 5-g column of 100−200 mesh Amberlite CG-50 (Na^+). The methanol eluent was concentrated in vacuo, and the sodium salt of **13** (35 mg) was obtained as crystals from water−acetone (1:2): TLC (solvent 2) one spot, R_f 0.51; mp >400 °C. Anal. ($C_{26}H_{25}O_4Na \cdot 2.25H_2O$) C, H.

Preparation of 4-{[1-(3-Hydroxyphenyl)-2-phenyl-1-butenyl]phenoxy}-*n*-butanoic Acid (12). Alkylation of 1.88 g (4.62 mmol) of **5** with ethyl 4-bromobutyrate was carried out as described above for synthesis of **11**. Crystallization of the crude product from hexanes afforded 600 mg (25%) of **10** as a white solid: TLC (solvent 2) one spot, R_f 0.80; mp 108.2−109.6 °C; NMR ($CDCl_3$) 7.40−6.50, m, 18H ArH; 5.04, s, 2H (OCH_2-Ar); 4.11, q, J = 8 Hz, 2H (COOCH_2); 3.86, t, J = 6 Hz, 2H (OCH_2R); 2.47−2.43, m, 4H (CH_2COOR and CH_2CH_3); 2.02, p, J = 6 Hz, 2H ($CH_2CH_2CH_2$); 1.23, t, J = 8 Hz, 3H (COOCH_2CH_3); 0.89, t, J = 7 Hz, 3H (CH_2CH_3). Hydrolytic debenzylation/saponification of 175 mg (0.34 mmol) of **10** was carried out as described in step B above. There was obtained 58 mg (43%) of **12** as an amorphous yellow solid. The sodium salt of **12** obtained in an analogous manner to that described for **13**: TLC (solvent 2) one spot, R_f 0.50; ^{1}H NMR (methanol-d_4) 7.20−6.30, m, 13H (ArH); {4.01, t, 3.84, t, J = 6 Hz}, 2H (OCH_2R); 2.47, m, 2H (CH_2CH_3); {2.35 and 2.26, t, J = 6 Hz}, 2H (CH_2COOH); {2.06 and 1.96, p, J = 6 Hz}, 2H ($CH_2CH_2CH_2$); 0.90, m, 3H (CH_2CH_3); HRMS (LSIMS) m/z calcd for $C_{26}H_{27}O_4$ 403.1909 (MH^+), found 403.1894, rel ion intensity $M^{+\bullet}/MH^+$ = 0.70. Anal. ($C_{26}H_{25}O_4Na \cdot 2H_2O$) C, H.

Estrogen Receptor Binding Affinity. [^{3}H]Estradiol (51 Ci/mmol) was obtained from Amersham Pharmacia Biotech, Inc., Piscataway, NJ. Human estrogen receptor alpha (ERα) from recombinant baculovirus-infected insect cells was obtained from PanVera Corp., Madison, WI. Aliquots (20 μL) of the thawed preparation were stored at −80 °C until use. For preparation of incubation mixtures, an aliquot was warmed to 4 °C and diluted with 16 mL of assay buffer: 10 mM Tris, pH 7.50, 1.5 mM EDTA, 1 mM dithiothreitol, 10% glycerol, 0.1% bovine serum albumin. Incubations were run in triplicate for 20 h at 4 °C in 12- × 75-mm polypropylene tubes and contained 200 μL (0.34 pmol) of diluted ERα, 5.7 nM [^{3}H]-estradiol added in 10 μL of ethanol, and increasing concentrations (1 nM to 10 μM) of test compounds added as solutions in 10 μL of *N*,*N*-dimethylacetamide. Control incubations contained 10 μL of *N*,*N*-dimethylacetamide alone, and nonspecific binding was determined in incubations to which 10 μM estradiol had been added. Unbound [^{3}H]estradiol was removed by addition of 400 μL of a suspension of dextran-coated charcoal (prepared by stirring 100 mg of dextran and 1 g of Norit A in 100 mL of water at 4 °C for 16 h, followed by centrifugation of the mixture at 450*g* for 10 min and resuspension of the pellet in assay buffer), and incubation was continued for 15 min. Tubes were centrifuged at 600*g* for 15 min, and a 400-μL aliquot of each supernatant was shaken for 10 min with 4 mL of scintillation fluid. Specific binding was determined using a single label percent of reference program, with respect to nonspecific and total bound radioactivity (counts/min).

Effects on MCF-7 Cell Growth. Cells (1×10^5) were conditioned at 37 °C in a humidified atmosphere containing 5% carbon dioxide, in 75-cm^2 flasks containing 10 mL of growth medium (phenol red-free Dulbecco's modified Eagle's medium−Ham's nutrient mixture, 50−50) containing 15 mM HEPES buffer, 5 mM L-glutamine, 1.2 g/L sodium bicarbonate, 5 mg/L gentamycin, 1 mg/L insulin, and 10% heat-inactivated newborn calf serum,[15] and then passaged into 25-cm^2 flasks with 5 mL of growth medium. Test compounds, in final concentrations ranging from 10 to 10 000 nM, were added as solutions in dimethyl sulfoxide, without or with similar addition of 17β-estradiol or **1** at respective final concentrations of 1 nM and 1 μM. Medium and test compounds were changed every 3−4 days. After 7 days of incubation, growth medium was aspirated from flasks, and cell numbers/flask were determined as described previously.[15] In each experiment, MCF-7 cell uniformity was validated by assessment of the growth suppressive effect of 1 μM **1** in parallel incubations.

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