Rationally Designed Analogues of Tamoxifen with Improved Calmodulin Antagonism

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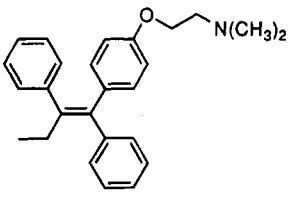
Computerized molecular modeling studies on the interactions of the antiestrogen tamoxifen (**1**) and its analogues bound to the calcium-binding protein calmodulin have guided the rational design of more potent antagonists. Compounds with either three or four methylene units in the basic side chain or slim lipophilic 4-substituents were expected to be more potent. All compounds were tested for antagonism of the calmodulin-dependent activity of cAMP phosphodiesterase and for binding affinity to the estrogen receptor from rat uteri. Some compounds were assayed for cytotoxicity against MCF-7 breast tumor cells *in vitro*. Introduction of lipophilic 4-substituents was accomplished by using palladium(0)-catalyzed coupling reactions with a 4-iodinated precursor. Both the 4-ethynyl (**16** and **17**) and 4-butyl (**18** and **19**) compounds were more potent calmodulin antagonists than tamoxifen. Extension of the basic aminoethoxy side chain of 4-iodotamoxifen (**3**) and idoxifene (**2**) ((*E*)-1-[4-[2-(*N*-pyrrolidino)ethoxy]phenyl]-1-(4-iodophenyl)-2-phenyl-1-butene) by one or two methylene units resulted in modest gains in calmodulin antagonism (**10**–**13**). All the compounds assayed retained estrogen receptor binding characteristics. The compound possessing the optimal combination of calmodulin antagonism and estrogen receptor binding was **12** ((*E*)-1-[4-[3-(*N*-pyrrolidino)propoxy]phenyl]-1-(4-iodophenyl)-2-phenyl-1-butene) (IC_{50} = 1.1 μM, RBA = 23). Correlation between calmodulin antagonism and cytotoxicity was demonstrated for selected compounds.

The nonsteroidal antiestrogen tamoxifen (**1**, *trans*-1-[4-[2-(dimethylamino)ethoxy]phenyl]-1,2-diphenyl-1-butene; Zeneca-Nolvadex) is widely used in the treatment of hormone-responsive breast cancer.[1,2] Despite the low incidence of side effects, the use of tamoxifen and other antiestrogens suffers from serious limitations in that (a) not all estrogen receptor-positive tumors respond and (b) responding patients invariably relapse after an average duration of response of about 18 months. The principal mode of action of **1** is thought to



Tamoxifen (1)

be displacement of the growth-promoting hormone estradiol from its protein receptor.[3] In addition the drug exerts other effects that cannot be reversed by estrogen, including inhibition of protein kinase C,[4,5] antagonism of the calcium-binding protein calmodulin,[6,7] and binding to antiestrogen binding sites.[8,9] The inhibition of breast cancer cell growth by some steroidal and nonsteroidal antiestrogens appears to involve the antagonism of calmodulin function,[10,12] but for some compounds this mechanism has been discounted.[13] Other reports suggest that the cytotoxicity of the triphenylethylene class of antiestrogens toward MCF-7 cells correlates with protein kinase C inhibition.[4,14] Recently, **1** has been shown to antagonize the formation of a complex between calmodulin and the estrogen receptor,[15,16] and an estradiol-dependent protein tyrosine kinase which is responsible for phosphorylation of the estrogen receptor (ER) has been isolated and was demonstrated to be stimulated by Ca^{2+}–calmodulin.[17] Therefore our attention has focused on the role of calmodulin, and previously we synthesized a variety of analogues of **1** to explore the effect of variations in the basic side chain and of various 4-substituents on the calmodulin antagonistic properties of these analogues. There appeared to be a relationship between cytotoxicity toward ER-positive cells and antagonism of calmodulin-dependent cAMP phosphodiesterase.[18] The most potent calmodulin antagonist was idoxifene (**2**) which was developed in our laboratories as a new antiestrogen[19] and has completed a phase I clinical trial.[20]

Calmodulin is a small (16.7 kD, 148 residue) calcium-binding protein that is responsible for the regulation of a wide range of cellular processes in eukaryotic systems. Its sequence is highly conserved throughout the animal kingdom. X-ray crystallographic studies have been reported on the protein from bovine brain,[21] *Drosophila melanogaster*,[22] a vertebrate sequence recombinant source,[23] and *Paramecium tetraurelia*.[24] These show the native protein the crystalline state to have a highly conserved three-dimensional structure comprising N- and C-terminal globular domains separated by a 28-residue α helix. Each domain contains a characteristic helix–loop–helix calcium-binding motif. The solution conformation of calmodulin, as revealed by NMR studies,[25] by contrast, shows that the central α helix is

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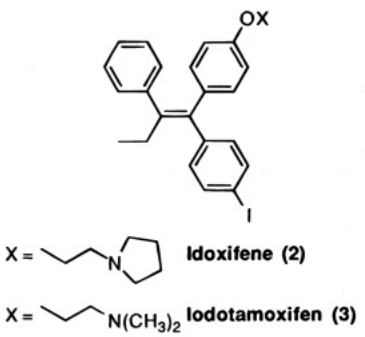
flexible and can be highly bent around its midpoint, resulting in an altogether more compact and globular structure for the protein. Structures of calmodulin with peptides representative of the calmodulin-binding domain of smooth muscle myosin light-chain kinase[26,27] and protein kinase IIa[26] have shown that the protein folds around these peptides with unwinding of the central helix being common to both structures.

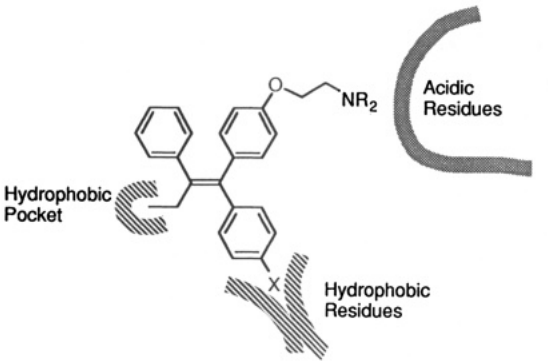
The fact that the calmodulin structure *per se* is now well established makes it an attractive target for drug design; we have previously used molecular modeling methods to examine possible modes of interaction between calmodulin and tamoxifen, together with several of its analogues.[28] This study located a hydrophobic cavity in the C-terminal domain as the most likely binding site for the parent drug, and it was possible to demonstrate a structure–activity relationship for the analogues. Analysis of these models shows the importance of the ethyl group's interaction with a small hydrophobic pocket, the interaction of the basic side chain, which is protonated at physiological pH, with a region of acidic residues, and the presence of a hydrophobic region close to the 4-position of the drug (Figure 1). Extension of the molecular model for the calmodulin–tamoxifen complex has assisted the design of new analogues of **1**, **2** and **3**, with enhanced calmodulin-recognition features that have either (a) increased length of the basic side chain while retaining the 4-iodo substituent or (b) slim lipophilic 4-substituents (ethynyl and *n*-butyl). Here we report the design and synthesis



of such derivatives, their antagonism of calmodulin-dependent cAMP phosphodiesterase, their estrogen receptor binding affinities, and for selected analogues their *in vitro* cytotoxicities against MCF-7 breast cancer cells.

Results and Discussion

Molecular Modeling. The basic side chain of **1** extends toward a region of acidic residues in calmodulin, at the start of the α helix connecting the two domains. The closest approach by the protonated nitrogen atom of the drug is to Glu84, with a distance of 4.6 Å to the carbonyl oxygen atom of this residue (Figure 2). Extension of the tamoxifen side chain by one -(CH_2)- unit (compound **10**), together with torsion angle changes in the Glu84 residue (primarily around the Cβ-Cγ bond), results in the drug nitrogen atom and the Glu84 carbonyl atom now being ca. 2.8 Å apart. The tamoxifen derivative with a further -(CH_2)- (compound **11**) also has a 2.8 Å N···O separation (Figure 3); in this instance further torsion angle changes in the Glu84 residue were required in order to avoid bad contacts between it and the ligand. Calculation of binding energies for **1**, **10**, and **11** interacting with calmodulin shows (Table 1) a systematic increase in ΔE as the length of the side chain is extended.



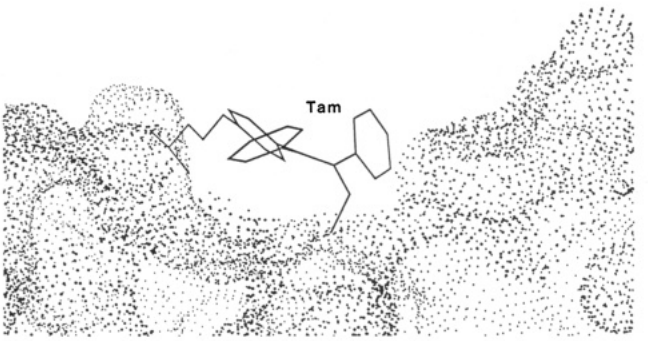


Figure 1. (top) Schematic of the interactions of tamoxifen and its derivatives with calmodulin. (bottom) Plot of tamoxifen (Tam) bound in the high-affinity hydrophobic cavity of calmodulin (shown as solvent accessible surface), from the molecular modeling study.[28]

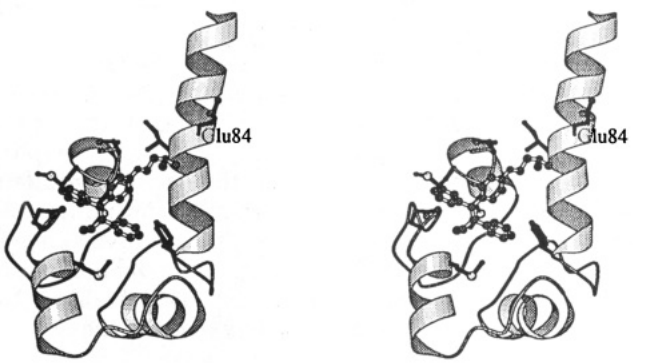


Figure 2. Molscript plot showing the spatial relationship between tamoxifen (Tam) and Glu84.

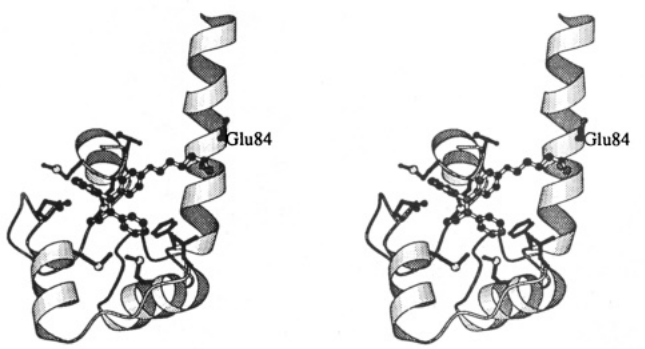


Figure 3. Molscript plot showing compound **11** and its relationship to Glu84, with the charged amino group of **11** in close contact with atom OE2 of Glu84.

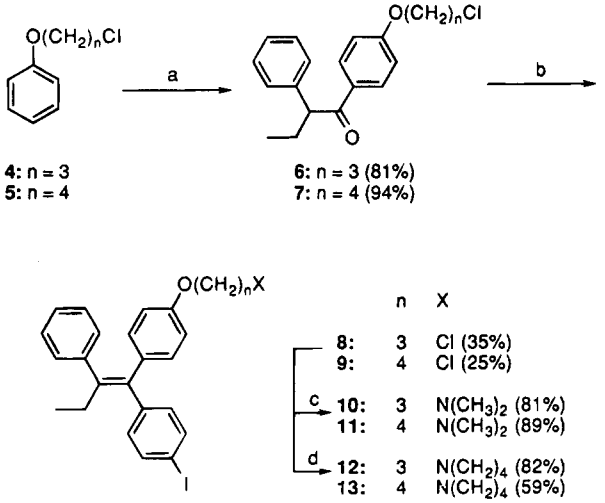
The hydrogen atom at the 4-position in **1** (and its phenyl ring) is close to one orientation of the terminal

Table 1. Calculated Eneriges (kJ mol^{-1}) for **1** and Selected Derivatives Alone and Bound to Calmodulin

compd	in isolated state	bound to calmodulin[a]	binding energy	log P^b
1	106.2	490.9	−122.8	5.19
10	103.7	485.4	−125.8	5.24
11	106.7	479.5	−134.6	5.69

[a] The total minimized energy of calmodulin alone was 507.5 kJ mol^{-1}. [b] log *P* values were calculated with the Chemplus module of HYPERCHEM.

Scheme 1. Preparation of Homologues of **2** and **3**[a]



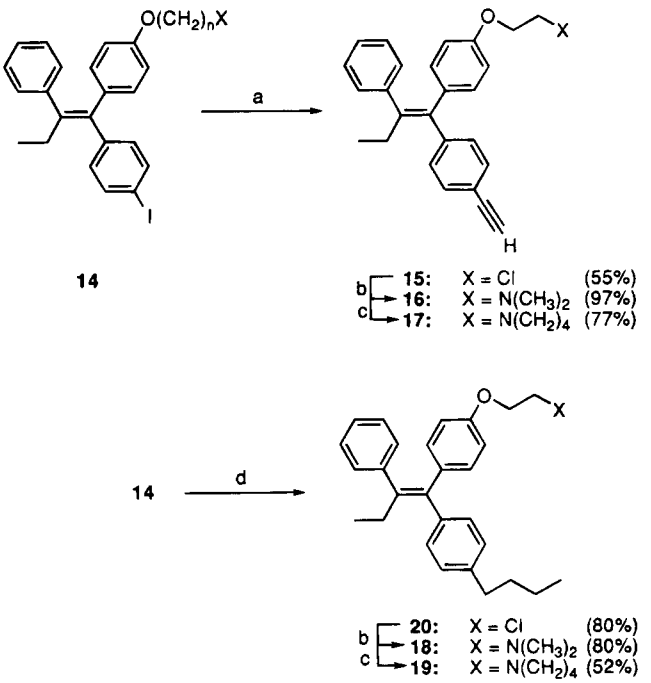
[a] Reagents: (a) 2-phenylbutyric acid, trifluoroacetic acid; (b) (i) 4-I-C_6H_4-I, *n*-BuLi (1 equiv), THF, −78−20 °C; (ii) HCl (aq), EtOH, 80 °C; (c) $(CH_3)_2NH$, EtOH, 80 °C; (d) pyrrolidine, EtOH, 80 °C.

methyl group of residues Met144 in the calmodulin complex. Replacement of this hydrogen atom with an ethynyl group (compound **16**) would result in some close van der Waals contacts between it and the terminal methyl group of Met144. The 4-*n*-butyl analogue (compound **18**) is able to make rather more close contacts with this methyl group on account of the flexibility of this alkyl group. Alternative orientations of the terminal methyl group of Met144 leave the 4-position free, and thus a group such as hydroxyl could readily be accommodated.

Synthesis. Homologues of Idoxifene (2) and 4-Iodotamoxifen (3). The synthesis of the 4-iodo homologues is outlined in Scheme 1. The procedure essentially follows that reported for the synthesis of **2** and 4-iodotamoxifen (**3**),[19] substituting (2-chloroethoxy)-benzene with the appropriate [(ω-chloroalkyl)oxy]-benzene **4** or **5** in the Friedel−Crafts acylation of 2-phenylbutanoic acid to give the 1,2-diarylbutanones **6** and **7**, respectively. Reaction of the ketone **6** or **7** with (4-iodophenyl)lithium, readily generated by treatment of 1,4-diiodobenzene with 1 equiv of *n*-butyllithium, and subsequent dehydration of the resulting tertiary alcohol gave the triarylbutenes **8** and **9**, respectively, as a mixture of *E* and *Z* isomers which were separable by fractional crystallization. (*Trans* and *cis* are used in this paper to designate the relative positions of the ethyl group and the ring bearing the basic side chain.) The desired *E* (*trans*) isomers were then treated with dimethylamine or pyrrolidine to give the dimethylamino compounds **10** and **11** or pyrrolidino compounds **12** and **13**, respectively.

4-Substituted Tamoxifens. The preparation of analogues of **1** bearing lipophilic 4-substituents is outlined in Scheme 2. The application of palladium-catalyzed cross-coupling reactions allowed the convenient preparation of the 4-ethynyl and 4-*n*-butyl analogues from the 4-iodinated compound **14**. Alkynylzinc reagents are effective nucleophiles in palladium-catalyzed cross-coupling reactions with alkenyl and aryl iodides.[29,30] An attractive feature of this approach is that protection of the ethynyl terminus is not required despite this containing an acidic hydrogen. Ethynylzinc chloride was prepared *in situ* by transmetalation of lithium acetylide ethylenediamine complex with anhydrous zinc chloride in THF. This reagent was then reacted with the 4-iodinated compound **14** with tetrakis-(triphenylphosphine)palladium(0) as catalyst to afford **15** in moderate yield. Subsequent reaction with dimethylamine or pyrrolidine gave the dimethylamino compounds **16** or pyrrolidino compounds **17**, respectively.

Scheme 2. Preparation of 4-Substituted Analogues of **1**[a]



[a] Reagents: (a) lithium acetylide ethylenediamine complex, $ZnCl_2$, THF, $(Ph_3P)_4Pd(0)$; (b) $(CH_3)_2NH$, EtOH, 80 °C; (c) pyrrolidine, EtOH, 80 °C; (d) *n*-BuLi, $ZnCl_2$, (dppf)$PdCl_2$, Et_2O, 0 °C.

The palladium-catalyzed cross-coupling of primary alkyl reagents with aryl halides usually fails due to the undesired β-hydride elimination pathway being favored. This problem has been solved by the use of dichloro-[1,1′-bis(diphenylphosphino)ferrocene]palladium(0) (Pd-(dppf)Cl_2) as catalyst allowing alkylzinc reagents to be successfully coupled with aryl halides.[31] The bisphosphinoferrocene ligand facilitates the reductive elimination of the oxidative addition intermediate over competing β-hydride elimination allowing the desired coupling reaction to proceed efficiently. This catalyst was used in the synthesis of the 4-*n*-butyl compounds **18** and **19**. The required *n*-butylzinc reagent was prepared *in situ* by transmetalation of *n*-butyllithium with zinc chloride in ether. Reaction of this with the 4-iodinated compound **14** with Pd(dppf)Cl_2 as catalyst gave the 4-n-butyl product **20**, subsequently isolated in good yield. Reaction of **20** with dimethylamine or

Table 2. Antagonism of Calmodulin-Dependent cAMP Phosphodiesterase, Receptor Binding Affinities (RBA), and Short-Term *in Vitro* Cytotoxicity Assay Results

compd	*n*	4-substituent	X	antagonism of CaM-dependent cAMP,[a] IC_{50} (μM) ± SE	binding affinity for ER (RBA)	short-term *in vitro* cytotoxicity (MCF-7), IC_{50} (μM) ± SD
1	2	H	$N(CH_3)_2$	6.8 ± 1.1	4	14.0 ± 1.0
2	2	I	$N(CH_2)_4$	1.5 ± 0.1	17	7.3 ± 0.4
3	2	I	$N(CH_3)_2$	2.3 ± 0.4	10	nd
10	3	I	$N(CH_3)_2$	1.4 ± 0.5	8	nd
11	4	I	$N(CH_3)_2$	1.3 ± 0.4	25	nd
12	3	I	$N(CH_2)_4$	1.1 ± 0.1	23	4.5 ± 0.1
13	4	I	$N(CH_2)_4$	1.0 ± 0.1	9	4.0 ± 0.6
16	2	C≡CH	$N(CH_3)_2$	4.6 ± 1.2	6	nd
17	2	C≡CH	$N(CH_2)_4$	3.3 ± 0.3	11	14.7 ± 1.5
18	2	*n*-Bu	$N(CH_3)_2$	3.7 ± 0.7	2	nd
19	2	*n*-Bu	$N(CH_2)_4$	1.8 ± 0.3	2	nd
21	2	OH	$N(CH_3)_2$	19.0 ± 2.9	100	15.5 ± 0.7

[a] None of the compounds gave any significant inhibition of the calmodulin-independent activity of cAMP PDE when assayed at final concentrations of 10 and 20 μM. nd = not determined.

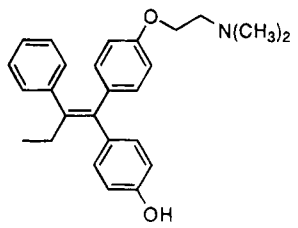
pyrrolidine gave the dimethylamino compounds **18** or pyrrolidino compounds **19**, respectively.

Biological Evaluation and General Discussion. Compounds were assayed for the inhibition of calmodulin-dependent cyclic AMP phosphodiesterase (cAMP → AMP)[18] (none of the compounds inhibited the calmodulin-independent component of cAMP phosphodiesterase when assayed at the final concentrations of 10 and 20 μM), and the binding affinities of the compounds toward rat uterine cytosolic estrogen receptor were determined relative to estradiol (RBA = 100).[32] The results are summarized in Table 2. All the derivatives were more potent antagonists of calmodulin-dependent cAMP phosphodiesterase than **1**.

The model suggests that the compounds with extended basic side chains with $-(CH_2)_4-$ or possibly with $-(CH_2)_3-$ can form a geometrically acceptable strong hydrogen bond with Glu84 of calmodulin. This can take place when the nitrogen atom of the drug is part of either a dimethylamino or pyrrolidino substituent. Table 2 shows that compounds **10**–**13** exhibited modest increases in potency as regards their antagonism of calmodulin-dependent cAMP phosphodiesterase, in comparison with **2** and **3**, respectively. There are only modest changes in log *P* values for these compounds, suggesting that these effects are not due to the small changes in lipophilicity on progressing from $n = 2$ to $n = 4$ (Table 1). For example, as shown in Table 2, the IC_{50} values for **2** and its three- and four-carbon homologues, **10** and **11**, are 2.3, 1.4, and 1.3 μM, respectively. All the pyrrolidino compounds showed increased potency when compared with their dimethylamino counterparts, e.g., IC_{50} for **3** = 2.3 μM and for **2** = 1.5 μM. The increased potency of the pyrrolidino compounds may be attributed to their increased basicity compared to their dimethylamino counterparts and is in accord with the increased strength of the proposed hydrogen bonding.

The derivatives with hydrophobic substituents at the 4-position (compounds **16**–**19**) also show some increase in calmodulin potency compared to **1**, though to a lesser extent than all the 4-iodinated derivates tested (**2, 3, 10**–**13**). These results are consistent with the qualitative modeling which indicates only a modest increase in van der Waals contacts between these substituents and the protein, especially as such contacts can only occur with one orientation of the Met144 side chain.

The structure of the hormone binding domain of the estrogen receptor has not been defined. However, structure–activity relationships have established that the presence of a polar 4-substituent results in enhanced binding affinity,[19] e.g., 4-OH-tamoxifen (**21**), RBA = 100. The 4-substituents are proposed to interact with the same region of the binding site to which the 3-phenolic group of estrogen binds.



(21)

4-Iodo derivatives have previously been identified as being more potent antiestrogens than **1**.[19] This has been attributed the ability of iodine to form relatively strong interactions with nucleophilic sites.[33,34] The chain-extended 4-iodo derivatives all possess binding affinities greater than or equal to that of **1**, the most potent being the (dimethylamino)butoxy derivative **11** (RBA = 25) and the pyrrolidinopropoxy derivative **12** (RBA = 23). The results in the pyrrolidino series may be contrasted with that of Robertson et al.[35] who demonstrated that the 4-unsubstituted three-carbon side-chain homologue of **1** displayed a lower binding affinity in comparison to the parent compound, and it is surprising that the trend is different for the 4-iodo series observed here.

The 4-ethynyl derivatives **16** and **17** displayed greater binding affinities than **1**, with the pyrrolidino compound being the more potent. The ability of an acetylenic hydrogen to participate in a C–H···X hydrogen bond has been well documented.[36] This suggests that the increased binding affinity may be due to such interactions between the terminal ethynyl hydrogen and polar residues of the receptor.

The 4-butyl derivatives **18** and **19** showed a modest binding affinity compared with the 4-methyl derivative of **1** which has an RBA similar to that of tamoxifen.[37] The reduction in binding affinity may be due to the increased steric bulk conferred by the butyl group and/or the presence of the hydrophobic group in the polar region of the receptor. These further variations of the 4-substituent fail to supplant iodine as the substituent conferring optimal characteristics for binding to both

calmodulin and the estrogen receptor and indicate the requirement for a 4-substituent which is able to form polar interactions with the estrogen receptor.[19,33,34]

Some of the compounds were assayed in short-term cytotoxicity tests against the hormone-responsive MCF-7 cell line and compared with previous results (Table 2).[18] The presence of a 4-iodo substituent produces a halving of the IC_{50} value in comparison with **1**, while the analogues with an extended basic side chain are the most potent with IC_{50} values of around 4 μM. The least cytotoxic was **20** which has the greatest binding affinity to the estrogen receptor. Therefore, the cytotoxicity toward MCF-7 cells appears to correlate with the degree of calmodulin antagonism rather than the binding to the estrogen receptor as has been reported by other workers.[10,11]

In conclusion, our previous molecular modeling study of calmodulin interactions with several established tamoxifen derivatives has shown that modeling can rationalize their binding behavior, at least in a semi-quantitative manner.[28] The present study has extended this approach to the design of new tamoxifen analogues. We have used molecular mechanics calculations to predict the relative binding affinities of several new tamoxifen analogues, together with some more quantitative modeling to maximize either hydrogen-bonding or van der Waals nonbonded interactions, factors that tend to increase the strength of ligand–protein interactions. This approach has proved to be useful in the design of analogues of **1** which are more potent antagonists of calmodulin.

Extension of the basic side chain produced only marginal improvements in calmodulin antagonism and estrogen receptor binding affinity compared with **2** which remains in clinical development. However, these results suggest that further analogues of **1** combining improved calmodulin antagonism with greater ER binding affinity may be developed which might have greater therapeutic effect against breast cancer. To this end, new triphenylethylene derivatives are being synthesized in our laboratories to extend these structure–activity relationships and shed further light on the role of calmodulin action in antiestrogens.

Experimental Section

Chemical Methods. General Procedures. ^{1}H NMR spectra (internal Me_4Si) were obtained with a Bruker AC250 instrument. Mass spectra were obtained with a VB 7070H spectrometer and VG 2235 data station. Melting points were obtained on a Reichert hotstage and are uncorrected. Chromatography refers to flash column chromatography on silica gel (Merck 15111) with the eluant indicated applied at a positive pressure of 0.5 atm. All reactions performed under an inert atmosphere were carried out in oven-dried glassware (110 °C, 24 h). Ether refers to diethyl ether. Petrol refers to the fraction with the boiling range 60–80 °C. Anhydrous tetrahydrofuran (THF) was obtained by distillation from potassium and benzophenone. Purification of *E* and *Z* geometrical isomers was monitored by ^{1}H NMR spectroscopy and carried out until none of the undesired isomer could be detected.

General Procedure for Preparation of (ω-Chloroalkoxy)benzenes. A two-phase mixture of phenol (5 g, 53 mmol), α,ω-dichloroalkane (30 mL), tetrabutylammonium hydrogen sulfate (0.3 g, 1 mmol), and 3 M NaOH (25 mL) was heated to reflux for 3 h. The organic layer was separated, dried ($MgSO_4$), and concentrated *in vacuo*.

(3-Chloropropoxy)benzene (4). The general procedure was followed using 1,3-dichloropropane. Distillation gave **4** as a colorless, viscous oil (8.0 g, 88%): bp 110 °C (0.1 mmHg) (lit.[38] bp 133–136 °C (15 mmHg); ^{1}H NMR ($CDCl_3$) δ_H 2.26 (q, J = 6 Hz, 2, $CH_2CH_2CH_2$), 3.77 (t, J = 6 Hz, 2, CH_2Cl), 4.13 (t, J = 6 Hz, 2, CH_2OPh), 6.90–7.00 (m, 3, Ar*H*), 7.26–7.34 (m, 2, Ar*H ortho* to OCH_2).

(4-Chlorobutoxy)benzene (5). The general procedure was followed using 1,4-dichlorobutane. Chromatography (petrol) gave **5** as a colorless, viscous oil (7.57 g, 77%): bp 70 °C (0.2 mmHg) (lit.[39] bp 79 °C (0.3 mmHg); ^{1}H NMR ($CDCl_3$) δ_H 1.89–1.98 (m, 4, $CH_2CH_2CH_2$), 3.60 (t, J = 11.75 Hz, 2, CH_2Cl), 3.98 (t, J = 11.75 Hz, 2, CH_2OAr), 6.85–6.95 (m, 3, Ar*H*), 7.23–7.29 (m, 2, Ar*H ortho* to OCH_2).

1-[4-(3-Chloropropoxy)phenyl]-2-phenyl-1-butanone (6). To a stirred solution of 2-phenylbutyric acid (8.5 g, 52 mmol) in trifluoroacetic anhydride (7.5 mL, 52 mmol) was added (3-chloropropoxy)benzene (**4**; 8 g, 47 mmol), and stirring continued for 16 h. The mixture was poured into saturated aqueous $NaHCO_3$ (100 mL), neutralized by addition of solid $NaHCO_3$, and extracted with ethyl acetate (2 × 100 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated *in vacuo*. Distillation gave **6** as a white waxy solid (12.01 g, 81%): bp 200 °C (0.1 mmHg); ν_{max} (film from CH_2Cl_2) 2966, 2934, 1736, 1600 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ_H 0.87 (t, J = 7.25 Hz, 3, CH_3CH_2), 1.87–2.26 (m, 4, $CH_2CH_2CH_2$ and CH_2CH_3), 3.70 (t, J = 6 Hz, 2, CH_2Cl), 4.11 (t, J = 6 Hz, 2, CH_2OAr), 4.37 (t, J = 7.25 Hz, 1, C*H*(Ph)Et), 6.61 (d, J = 10 Hz, 2, Ar*H meta* to OCH_2), 7.10–7.14 (m, 1, Ar*H para* to CHEt), 7.20–7.28 (m, 3, Ar*H ortho* and *Meta* to CHEt), 7.91 (d, J = 10 Hz, 2, Ar*H ortho* to OCH_2); MS (FAB) *m/z* 317 (M^+ + 1); accurate mass ($C_{19}H_{21}O_2Cl$) found 316.1245, required 316.1230.

1-[4-(4-Chlorobutoxy)phenyl]-2-phenyl-1-butanone (7). To a stirred solution of 2-phenylbutyric acid (5.6 g, 34 mmol) in trifluoroacetic anhydride (20 mL) was added (4-chlorobutoxy)benzene (**5**; 7.5 g, 41 mmol). After 16 h the mixture was worked up as for **6**. Chromatography (petrol/ethyl acetate, 10/3) gave **7** as an orange oil (10.6 g, 94%): ν_{max} (film) 2961, 1671, 1599, 1574 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ_H 0.88 (t, J = 7.35 Hz, 3, CH_3CH_2), 1.80–1.91 (m, 5, CH_2CH_2 and CH_3CH_2), 2.12–2.24 (m, 1, CH_3CH_2), 3.56–3.61 (m, 2, CH_2Cl), 3.98–4.02 (m, 2, CH_2OAr), 4.38 (t, J = 7.35 Hz, 1, C*H*(Ph)Et), 6.83 (d, J = 9 Hz, 2, Ar*H meta* to OCH_2), 7.12–7.17 (m, 1, ArH *para* to CHEt), 7.23–7.29 (m, 4, Ar*H ortho* and *meta* to CHEt), 7.93 (d, J = 9 Hz, 2, Ar*H ortho* to OCH_2): MS (EI) *m/z* 330 (M^+, 1); accurate mass ($C_{20}H_{23}O_2Cl$) found 330.140, required 330.1368.

(*E*)-1-[4-(3-Chloropropoxy)phenyl]-1-(4-iodophenyl)-2-phenyl-1-butene (8). To a stirred solution of 1,4-diiodobenzene (1.34 g, 4 mmol) in anhydrous tetrahydrofuran (5 mL) was added *n*-butyllithium (1.6 M, 2 mL, 4 mmol) in hexanes under N_2 at −78 °C, and stirring was continued for 1 h. A solution of the ketone **6** (2.01 g, 4 mmol) in tetrahydrofuran (10 mL) was added, and the mixture was allowed to attain ambient temperature. After 16 h the mixture was poured into ethyl acetate (50 mL) and washed with brine (50 mL) and water (2 × 50 mL). The organic phase was dried ($MgSO_4$) and concentrated *in vacuo*. The residues were dissolved in EtOH (20 mL), and concentrated hydrochloric acid (5 mL) was added. The mixture was heated at reflux for 3 h and then poured into aqueous $NaHCO_3$ (50 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were washed with water (2 × 50 mL), dried ($MgSO_4$), and concentrated *in vacuo*. Chromatography (petrol/CH_2Cl_2, 10/1) gave the crude product as a mixture of *E* and *Z* isomers. Recrystallization (*ex* EtOH) gave the pure *E* isomer **8** as white crystals (0.698 g, 35%): mp 98–100 °C; ν_{max} (KBr) 2965, 2929, 2871, 1605, 1508 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ_H 0.92 (t, J = 7.26 Hz, 3, CH_3CH_2), 2.16 (quintet, J = 6 Hz, 2, $CH_2CH_2CH_2$), 2.45 (q, J = 7.25 Hz, 2, CH_2CH_3), 3.69 (t, J = 6 Hz, 2, CH_2Cl), 3.98 (t, J = 6 Hz, 2, CH_2OAr), 6.55 (d, J = 10 Hz, 2, Ar*H ortho* to OCH_2), 6.745 (d, J = 10 Hz, 2, Ar*H meta* to OCH_2), 6.99 (d, J = 10 Hz, 2, Ar*H meta* to I), 7.09–7.20 (m, 5, Ph), 7.675 (d, J = 10 Hz, 2, Ar*H ortho* to I); MS (EI) *m/z* 502 (M^+ − 1), 197 (M^+ − 306). Anal. ($C_{25}H_{24}ClIO$) C,H,Cl,I.

(*E*)-1-[4-(4-Chlorobutoxy)phenyl]-1-(4-iodophenyl)-2-phenyl-1-butene (9). 1,4-Diiodobenzene (3.63 g, 11 mmol)

in anhydrous tetrahydrofuran (30 mL), *n*-butyllithium (1.6 M, 6.9 mL, 11 mmol) in hexanes, and the ketone **7** (3.3 g, 10 mmol) in tetrahydrofuran (20 mL) were treated as above. Chromatography (petrol/CH_2Cl_2, 10/1) gave the crude product as a mixture of *E* and *Z* isomers. Recrystallization (*ex* EtOH) gave the pure *E* isomer **9** as white crystals (1.316 g, 25%): mp 85–87 °C; ^1H NMR ($CDCl_3$) δ_H 0.84 (t, J = 7.5 Hz, 3, CH_3CH_2), 1.86–1.91 (m, 4, $CH_2(CH_2)_2CH_2$), 2.425 (q, J = 7.5 Hz, 2, CH_3CH_2), 3.56 (t, J = 6 Hz, 2, CH_2Cl), 3.84 (t, J = 6 Hz, 2, CH_2OAr), 6.50 (t, J = 6.73 Hz, 2, Ar*H ortho* to OCH_2), 6.71 (d, J = 6.73 Hz, 2, Ar*H meta* to OCH_2), 6.965 (d, J = 6.73 Hz, 2, Ar*H meta* to I), 7.07–7.19 (m, 5, Ph), 7.65 (d, J = 6.73 Hz, 2, Ar*H ortho* to I); MS (EI) *m/z* 516 (M^+, 100). Anal. ($C_{26}H_{26}OClI$) C,H,Cl,I.

(*E*)-1-[4-[3-(Dimethylamino)propoxy]phenyl]-1-(4-iodophenyl)-2-phenyl-1-butene (10). A mixture of the chloropropoxy compound **8** (0.302 g, 0.6 mmol) and dimethylamine solution (30%, 20 mL) in EtOH was heated in a sealed bomb at 100 °C for 2 h and then poured into ether (100 mL) and washed with brine (100 mL) and water (2 × 100 mL). The organic layer was dried (Na_2SO_4) and concentrated *in vacuo*. Chromatography (ether) gave **10** as an off-white solid (0.245 g, 81%): mp 103–106 °C (*ex* EtOH); ^1H NMR ($CDCl_3$) δ_H 0.89 (t, J = 7.3 Hz, 3, CH_3CH_2), 1.8–1.92 (m, 2, $CH_2CH_2CH_2$), 2.2 (s, 6, CH_3N), 2.32–2.50 (m, 4, CH_2N and CH_3CH_2), 3.85 (t, J = 6.4 Hz, 2, CH_2OAr), 6.515 (d, J = 8.7 Hz, 2, Ar*H ortho* to OCH_2), 6.70 (d, J = 8.7 Hz, 2, Ar*H meta* to OCH_2), 6.86 (d, J = 8.7 Hz, 2, Ar*H meta* to I), 7.05–7.20 (m, 5, Ph), 7.64 (d, J = 8.7 Hz, 2, Ar*H ortho* to I): MS (EI) *m/z* 511 (M^+, 30). Anal. ($C_{27}H_{30}NOI$), C,H,N; I: found, 24.81; calcd, 24.31.

(*E*)-1-[4-[3-(*N*-Pyrrolidino)propoxy]phenyl]-1-(4-iodophenyl)-2-phenyl-1-butene (12). A mixture of chloropropoxy compound **8** (0.25 g, 0.5 mmol), pyrrolidine (1 mL), and EtOH (5 mL) was heated at reflux for 4 h and then concentrated *in vacuo*. Chromatography (ether) gave **12** as an off-white solid (0.219 g, 82%): mp 86–87 °C (*ex* EtOH); ^1H NMR ($CDCl_3$) δ_H 0.90 (t, J = 7 Hz, 3, CH_3CH_2), 1.72–1.81 (m, 4), 1.95–2.0 (m, 2), 2.40–2.60 (m, 8), 3.88 (t, J = 6 Hz, 2, CH_2OAr), 6.535 (d, J = 9 Hz, 2, Ar*H ortho* to OCH_2), 6.72 (d, J = 9 Hz, 2, Ar*H meta* to OCH_2), 6.975 (d, J = 9 Hz, 2, Ar*H meta* to I), 7.05–7.10 (m, 5, Ph), 7.66 (d, J = 9 Hz, 2, Ar*H ortho* to I)l; MS (EI) *m/z* 537 (M^+, 45). Anal. ($C_{29}H_{32}NOI$) C,H,N,I.

(*E*)-1-[4-[3-(Dimethylamino)butoxy]phenyl]-1-(4-iodophenyl)-2-phenyl-1-butene (11). A mixture of the chlorobutoxy compound **9** (0.429 g, 0.83 mmol) and dimethylamine solution (30%, 30 mL) in EtOH was heated in a sealed bomb at 100 °C for 2 h and then worked up as above. Chromatography (ether/MeOH, 10/1) gave **11** as a white solid (0.391 g, 89%): mp 78–80 °C (*ex* EtOH); ^1H NMR ($CDCl_3$) δ_H 0.89 (t, J = 7.3 Hz, 3, CH_3CH_2), 1.50–1.66 (m, 2, $CH_2(CH_2)_2CH_2$), 1.66–1.79 (m, 2, $CH_2(CH_2)_2CH_2$), 2.20 (s, 6, CH_3N), 2.27 (t, J = 7.3 Hz, 2, $CH_2N(CH_3)_2$), 2.415 (q, J = 7.3 Hz, 2, CH_3CH_2), 3.81 (t, J = 6.1 Hz, 2, CH_2OAr), 6.505 (d, J = 8.7 Hz, 2, Ar*H ortho* to OCH_2), 6.605 (d, J = 8.7 Hz, 2, Ar*H meta* to OCH_2), 6.965 (d, J = 8.7 Hz, 2, Ar*H meta* to I), 7.05–7.2 (m, 5, Ph), 7.635 (d, J = 8.7 Hz, 2, Ar*H ortho* to I); MS (EI) *m/z* 525 (M^+, 1). Anal. ($C_{28}H_{32}NOI$), C,H,N,I.

(*E*)-1-[4-[4-(*N*-Pyrrolidino)butoxy]phenyl]-1-(4-iodophenyl)-2-phenylbutene (13). A mixture of the chlorobutoxy compound **9** (0.2 g, 0.4 mmol), pyrrolidine (2.5 mL), and EtOH (10 mL) was heated at reflux for 3 h and then worked up as for **11**. Chromatography (ether) gave **13** as a colorless oil (0.131 g, 59%): ^1H NMR ($CDCl_3$) δ_H 0.89 (t, J = 7.33, Hz, 3, CH_3CH_2), 1.57–1.80 (m, 8), 2.37–2.46 (m, 6), 3.81 (t, J = 6 Hz, 2, CH_2OAr), 6.51 (d, J = 9 Hz, 2, Ar*H ortho* to OCH_2), 6.70 (d, J = 9 Hz, 2, Ar*H meta* to OCH_2), 6.96 (d, J = 9 Hz, 2, Ar*H meta* to I), 7.06–7.18 (m, 5, Ph), 7.645 (d, J = 9 Hz, 2, Ar*H ortho* to I); MS (EI) *m/z* 550 ($M^+ - 1$, 5), 126 ($M^+ - 425$, 100), 84 ($M^+ - 467$, 100). Anal. ($C_{30}H_{34}NOI$) C,H,N; I: found, 23.01; calcd, 22.51.

(*E*)-1-[4-(2-Chloroethoxy)phenyl]-1-(4-ethynylphenyl)-2-phenyl-1-butene (15). To a suspension of lithium acetylide ethylenediamine complex (0.88 g, 8.8 mmol) in THF (50 mL) was added zinc chloride (anhydrous, 1.2 g, 8.8 mmol), and the mixture was stirred under N_2 for 48 h. The iodo compound **14** (2.0 g, 4 mmol) and tetrakis(triphenylphosphine)palladium (0.24 g, 0.2 mmol) were added, and stirring continued for 24 h. The mixture was poured into ether (100 mL), washed with water (3 × 100 mL), dried ($MgSO_4$), and concentrated *in vacuo*. Chromatography (petrol/CH_2Cl_2, 10/1) gave **15** as a white solid (0.84 g, 55%): mp 94–96 °C (*ex* EtOH/H_2O); ^1H NMR ($CDCl_3$) δ_H 0.92 (t, J = 7.4 Hz, 3, CH_3CH_2), 2.44 (q, J = 7.4 Hz, 2, CH_3CH_2), 3.09 (s, 1, C≡CH), 3.73 (t, J = 6.0 Hz, 2, CH_2Cl), 4.10 (t, J = 6.0 Hz, 2, CH_2OAr), 6.55 (d, J = 8.8 Hz, 2, Ar*H ortho* to OCH_2), 6.75 (d, J = 8.8 Hz, 2, Ar*H meta* to OCH_2), 7.07–7.21 (m, 7, Ph and Ar*H meta* to C≡CH), 7.47 (d, J = 7.8 Hz, 2, Ar*H ortho* to C≡CH); MS (EI) *m/z* 386 (M^+, 20). Anal. ($C_{20}H_{23}OCl$) C,H,N,Cl.

(*E*)-1-[4-[2-(Dimethylamino)ethoxy]phenyl]-1-(4-ethynylphenyl)-2-phenyl-1-butene (16). A mixture of 4-ethynyl compound **15** (0.50 g, 1.3 mmol) and dimethylamine (33%, 20 mL) in EtOH was heated in a sealed bomb at 100 °C for 90 min and then allowed to cool, poured into ether (100 mL), washed with water (3 × 100 mL), dried ($MgSO_4$), and concentrated *in vacuo*. Chromatography (ether) gave **16** as an off-white solid (0.5 g, 97%): mp 99–104 °C; ^1H NMR ($CDCl_3$) δ_H 0.91 (t, J = 7.4 Hz, 3, CH_3CH_2), 2.27 (s, 6, $N(CH_3)_2$), 2.43 (q, J = 7.4 Hz, 2, CH_3CH_2), 2.63 (t, J = 5.8 Hz, 2, $CH_2N(CH_3)_2$), 3.07 (s, 1, C≡CH), 3.91 (t, J = 5.8 Hz, 2, CH_2OAr), 6.55 (d, J = 8.8 Hz, 2, Ar*H ortho* to OCH_2), 6.72 (d, J = 8.8 Hz, 2, Ar*H meta* to OCH_2), 7.07–7.20 (m, 7, Ph and Ar*H meta* to C≡CH), 7.46 (d, J = 7.8 Hz, 2, Ar*H ortho* to C≡CH); MS (EI) *m/z* 395 (M^+, 12); accurate mass ($C_{28}H_{29}NO$) found 395.2260, expected 395.2249.

(*E*)-1-[4-[2-(*N*-Pyrrolidino)ethoxy]phenyl]-1-(4-ethynylphenyl)-2-phenyl-1-butene (17). A mixture of ethynyl compound **15** (0.455 g, 1.2 mmol), pyrrolidine (5 mL), and EtOH (15 mL) was heated to reflux for 4 h and then poured into ether (50 mL), washed with water (5 × 30 mL), dried (Na_2SO_4), and concentrated *in vacuo*. Chromatography (petrol/ether/triethylamine, 100/20/1) gave **17** (0.391 g, 77%): mp 82–85 °C (*ex* EtOH); ^1H NMR ($CDCl_3$) δ_H 0.92 (t, J = 7.4 Hz, 3, CH_3CH_2), 1.8–1.9 (m, 4, $NCH_2(CH_2)_2$), 2.45 (q, 2, J = 7.4 Hz, CH_3CH_2), 2.62–2.72 (m, 4, $N(CH_2)_2$), 2.85–2.95 (m, 2, CH_2N), 3.09 (s, 1, C≡CH), 4.03 (t, J = 5.8 Hz, 2, CH_2OAr), 6.55 (d, J = 8.8 Hz, 2, Ar*H ortho* to OCH_2), 6.74 (d, J = 8.7 Hz, 2, Ar*H meta* to OCH_2), 7.05–7.2 (m, 7, Ph and Ar*H meta* to C≡CH), 7.47 (d, J = 8.1 Hz, 2, Ar*H ortho* to C≡CH); MS (EI) *m/z* 421 (M^+, 1). Anal. ($C_{30}H_{31}NO$) C,H,N.

(*E*)-1-[4-(2-Chloroethoxy)phenyl]-1-(4-*n*-butylphenyl)-2-phenyl-1-butene (20). A solution of zinc chloride (1 M, 16 mL, 16 mmol) in ether was diluted with ether (10 mL), and to this was added *n*-butyllithium (1.6 M, 10 mL, 16 mmol) in hexanes at 0 °C under N_2. After 10 min a degassed solution of the iodo compound **14** (3.97 g, 8.12 mmol) and [(1,1′-diphenylphosphinyl)ferrocene]palladium dichloride (0.08 g, 0.1 mmol) in ether (40 mL) was added, and stirring continued at 0 °C for 4 h and at ambient temperature for 16 h. The mixture was poured into ether (75 mL), filtered through a plug of silica gel, and concentrated *in vacuo*. Chromatography (petrol/CH_2Cl_2, 5/1) followed by crystallization (*ex* EtOH) gave **20** as fine white needles (2.734 g, 80%): mp 87–88 °C; ^1H NMR ($CDCl_3$) δ_H 0.88–0.96 (m, 6, CH_3CH_2 and $CH_3(CH_2)_3$), 1.29–1.42 (m, 1.75), 1.51–1.68 (m, 1.75), 2.40–2.51 (m, 2.5), 2.60 (t, J = 7.6 Hz, 2, $ArCH_2$), 3.71 (t, J = 5.9 Hz, 2, CH_2Cl), 4.08 (t, J = 5.9 Hz, 2, CH_2OAr), 6.53 (d, J = 8.8 Hz, 2, Ar*H ortho* to OCH_2), 6.77 (d, J = 8.9 Hz, 2, Ar*H meta* to OCH_2), 7.01–7.15 (m, 9, 4-Bu-Ar*H* and Ph): MS *m/z* 418 ($M^+ - 1$, 1). Anal. ($C_{28}H_{31}OCl$) C,H,N,Cl.

(*E*)-1-[4-[2-(Dimethylamino)ethoxy]phenyl]-1-(4-*n*-butylphenyl)-2-phenyl-1-butene (18). A mixture of chloro compound **20** (0.42 g, 1 mmol) and diethylamine solution (33%, 20 mL) in EtOH was heated to 100 °C in a sealed bomb for 16 h and then allowed to cool and concentrated *in vacuo*. Chromatography (ether) followed by recrystallization (*ex* MeOH/H_2O) gave **18** as white crystals (0.324 g, 80%): mp 63–65 °C; ^1H NMR ($CDCl_3$) δ_H 0.87–0.95 (m, 6, CH_3CH_2 and $CH_3(CH_2)_3$), 1.16–1.66 (m, 4, $CH_3CH_2CH_2$), 2.35 (s, 6, $N(CH_3)_2$), 2.45 (q, J = 7.4 Hz, 2, CH_3CH_2), 2.60 (t, J = 7.9 Hz, 2, $ArCH_2C_3H_7$), 2.73 (t, J = 5.6 Hz, 2, $CH_2N(CH_3)_2$), 3.97 (t, J = 5.6 Hz, 2, CH_2OAr), 6.53 (d, J = 8.78 Hz, 2, Ar*H ortho* to OCH_2), 6.75 (d, J

= 8.75 Hz, 2, Ar*H meta* to OCH_2), 7.07–7.10 (m, 9, 4-Bu-Ar*H* and Ph). Anal. ($C_{28}H_{37}NO$) C,H,N.

(*E*)-1-[4-[2-(*N*-Pyrrolidino)ethoxy]phenyl]-1-(4-*n*-butylphenyl)-2-phenyl-1-butene (19). A mixture of chloro compound **20** (0.42 g, 1 mmol), pyrrolidine (5 mL), and EtOH (15 mL) was heated to 80 °C in a sealed bomb for 3 h and then allowed to cool and concentrated *in vacuo*. Chromatography (ether) followed by crystallization (*ex* MeOH, H_2O) gave **19** as off-white crystals (0.236 g, 52%): mp 49–52 °C; ^{1}H NMR ($CDCl_3$) δ_H 0.88–0.95 (m, 6, CH_3CH_2 and $CH_3(CH_2)_3$), 1.32–1.63 (m, 6, $CH_3CH_2CH_2$ and pyrrolidine), 1.91 (brs, 4, pyrrolidine), 2.44 (q, J = 7.4 Hz, 2, CH_3CH_2), 2.60 (t, J = 7.5 Hz, 2, $ArCH_2C_3H_7$), 2.65–3.16 (m, 4, CH_2CH_2N and pyrrolidine), 4.12 (brs, 2, CH_2OAr), 6.52 (d, J = 8.7 Hz, 2, Ar*H ortho* to OCH_2), 6.75 (d, J = 8.7 Hz, 2, Ar*H meta* to OCH_2), 7.07–7.15 (m, 9, 4-Bu-Ar*H* and Ph). Anal. ($C_{32}H_{39}NO$) C,H,N.

Estrogen Receptor Binding Assay. The affinity of the antiestrogens for the ER was measured using a competitive binding assay as described by Wakeling.[32] Immature rat cytosol was incubated at 4 °C for 16 h with 5 nM 17β-[2,4,6,7-^{3}H]-estradiol in the presence of increasing amounts (0.1–100 000 nM) of test compounds or unlabeled estradiol (control). The nonspecific binding was quantified by a parallel set of tubes containing 200-fold excess (with respect to [^{3}H]estradiol) of diethylstilboestrol. Unbound compounds were removed with dextran-coated charcoal, and the receptor-bound [^{3}H]estradiol was determined. The relative concentrations of estradiol and test compound required to achieve 50% inhibition of [^{3}H]estradiol binding give the relative binding affinity (RBA) which is ([I_{50}] estradiol/[I_{50}] test compound) × 100.

Calmodulin Antagonism. This was determined using the calmodulin-dependent cyclic AMP phosphodiesterase as previously described.[18,40] The enzyme was assayed using cyclic [8-^{3}H] AMP as substrate. The tritiated AMP formed during the incubation was converted into tritiated adenosine by the 5′-nucleotidase in snake venom. Product nucleosides were separated from unreacted substrate by batch elution with Dowex anion exchange resin with 3 mM acetic acid. The basal activity of cAMP phosphodiesterase (calmodulin independent) was determined by adding 1 mM EGTA to the assay medium. Assays were carried out in the presence and absence of different concentrations of the compounds dissolved in dimethyl sulfoxide. The results are expressed as the concentration of inhibitor giving 50% inhibition of the calmodulin-dependent cAMP phosphodiesterase and are the mean of triplicate determinations ± standard error.

Cytotoxicity against MCF-7 Cells. The cells were originally obtained from Michigan Cancer Foundation (Detroit, MI). They were maintained in routine culture in Dulbecco's modified Eagles medium (DMEM) supplimented with L-glutamine (2 mM), gentamycin (1 mg mL^{-1}), fungisone (2.5 μg mL^{-1}), insulin (10 μg mL^{-1}), and 5% fetal bovine serum (FBS). MCF-7 cells were plated in 96-multiwell plates, 5 × 10^3 cells/well, in 200 μL of DMEM, containing 2% carbon-stripped FBS. After 24 h at 37 °C in a humidified atmosphere, the various compounds were added in a fresh medium and the plates incubated for a further 24 h. Control wells not containing test compounds were treated similarly. To estimate the number of live cells present after 24 h contact with the test compounds, the MTT test was used.[41,42] Triplicate wells were analyzed for each test compound concentration, and four concentrations were used to generate the IC_{50} values.

Molecular Modeling Studies. Initial coordinates for a calmodulin–tamoxifen model were taken from our previous molecular modeling study,[28] where a number of models were derived in both N- and C-terminal domains of the protein, with distinct orientations for the drug. One model, with the drug bound in the C-terminus hydrophobic pocket of calmodulin, has a significantly superior calculated binding energy compared to all others. We have used this model in the present study. The initial coordinates for calmodulin were taken from the 2.2 Å X-ray analysis[21,22] of the bovine protein, as deposited in the Brookhaven Databank. Those for tamoxifen were derived from X-ray and molecular modeling analyses.[43,44]

The structures of a number of tamoxifen derivatives were built by computer modeling using the parent drug as a starting point, with varying numbers of -(CH_2)- units in the basic side chain or with the hydrophobic groups *n*-butyl and ethynyl at the 4-position. The HYPERCHEM package (Autodesk Inc. version 2.0) running on a Silicon Graphics Indigo workstation was used for structure building, with the geometry of each new derivative being optimized by molecular mechanics using the MM2(85) force field.

Each derivative was in turn overlaid on the position of tamoxifen in the structure of its calmodulin complex, using the MIDAS program (University of California, San Francisco) on the Indigo workstation. In several instances, small changes were made to selected calmodulin side-chain and/or tamoxifen derivative substituent torsion angles in order to optimize particular nonbonded interactions.

The two analogues **10** and **11**, with respectively one and two extra -CH_2- units in the side chain, were, together with **1** itself, selected for empirical energy calculations. The MacroModel version 4.5 package[45] was employed for these calculations, with a modified all-atom AMBER force field[46] and a distance-dependent dielectric constant. Atom-centered point charges were generated within MacroModel by means of a combination of bond dipoles and united atom charges held within the charge library of the program. The manually optimized structures were energy-minimized using a constrained refinement strategy that ensured the structural integrity of the C-terminal domain of the protein. The minimizations were judged to be complete when gradients in successive cycles were less than 0.2 kJ Å^{-1} mol^{-1}. The global energy minima for **1** and the two analogues **10** and **11** were located by systematic search procedures within the MacroModel program, followed by molecular mechanics optimizations. Binding eneriges (ΔE) were calculated as: $\Delta E = E_{COMPLEX} - (E_{CALMODULIN} - E_{LIGAND})$ Figures were drawn with the aid of the Molscript program.[46]

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