Molecular Structures and Conformational Studies of Triarylcyclopropyl and Related Nonsteroidal Antiestrogens

M. Bilayet Hossain, Dick van der Helm,* Francis J. Schmitz, and Eva O. Pordesimo

Department of Chemistry and Biochemistry, University of Oklahoma, Norman, Oklahoma 73019

Robert A. Magarian,* Karen L. Meyer, Lynette B. Overacre, and Billy W. Day†

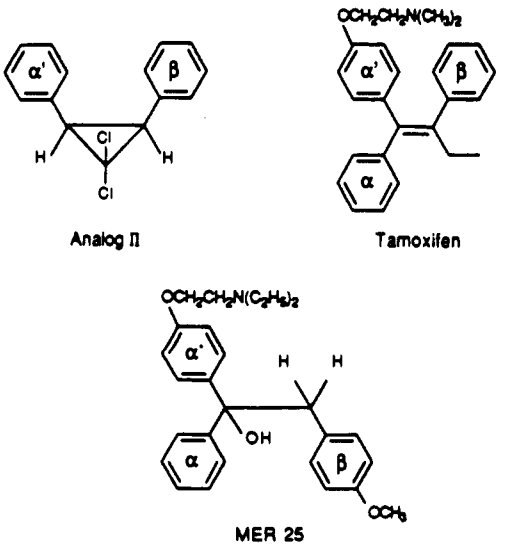
Department of Medicinal Chemistry, College of Pharmacy, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, 73190

Received November 8, 1993®

Molecular structures and conformational characteristics of a series of 1,1-dichloro-2,2,3-triarylcyclopropanes (DTACs), which were reported previously to be distinctly antiestrogenic and inhibitors of the estrogen-receptor-positive MCF-7 human breast cancer cells in culture, are reported. In addition, structural and conformational features of the DTACs were compared to the first-known nonsteroidal antiestrogen, MER25, and the clinically useful antiestrogen Tamoxifen. The molecular structures of four DTAC compounds were determined by X-ray diffraction. Crystallographic structures show that the DTAC molecules have nearly the same relative conformation for the three aryl rings which is designated as a "nonpropeller" conformation in contrast to the observed "propeller" conformation for the three rings in all known triarylethylenes. Systematic conformational searches were performed to find the conformational preferences of DTACs, MER25, and Tamoxifen using idealized model compounds built from their respective crystal structure. Energy-minimization and conformational-search studies demonstrated that all DTAC molecules have a common, single global minimum energy conformer for their central core containing the dichlorotriarylcyclopropyl system, which is similar to that found in their crystal structures. Conformational search of MER25 showed that the molecule can assume a number of low-energy conformers of which two, one *anti* (A1) and one *gauche* (G1A), have about the same energy. The *anti* conformation is similar to the one observed in its crystal structure and resembles the estrogenic *E*-isomer of Tamoxifen, while the lowest energy *gauche* conformer of MER25 resembles more closely the antiestrogenic *Z*-isomer of Tamoxifen. NMR spectroscopic analysis of MER25 showed that the molecule exists predominantly in the *anti* conformation in solution. A comparative review of the structural features and bioactivities of Tamoxifen, DTACs, and MER25 provides a possible explanation for their low estrogen receptor binding affinity which is common to these compounds together with their antiestrogenic activity.

Nonsteroidal antiestrogens which exhibit potent antitumor effects represent a major advance in the management of breast cancer. The representative of this class is Tamoxifen, (*Z*)-1-[*p*-[2-(dimethylamino)ethoxy]phenyl]-1,2-diphenylbut-1-ene (Chart 1), the only antiestrogen clinically available for the adjunctive treatment of primary breast cancer in postmenopausal women.[1,2] Tamoxifen, however, has partial agonist activity which can lead to thromboembolitic events and secondary endometrial tumors, and virtually all patients with metastatic disease develop Tamoxifen resistance.[3-5] MER25, 1-[4-[2-(diethylamino)ethoxy]phenyl]-2-(4-methoxyphenyl)-1-phenylethan-1-ol (Chart 1), the first nonsteroidal antiestrogen reported, was found to be a pure antiestrogen in all species of animals tested, but has a low potency and possesses serious side effects.[6,7] In recent years, the search for nonsteroidal antiestrogens without partial estrogen-like action that would compete effectively with estradiol or would act as effective agents in the treatment of breast tumors devoid of estrogen receptors has led to several interesting experimental compounds.[8-16] Reports from our laboratory[8,9] and others[13,16] have demonstrated that the introduction of a dichlorocyclopropyl or dihydrocyclopropyl moiety in place of the olefinic bridge in estrogenic stilbenes greatly reduces or abolishes their estrogenic action. One compound, analog **II** (Chart 1), has antiestrogenic properties without estrogen agonist activity in the mouse and is comparable to Tamoxifen against the

Chart 1. Nonsteroidal Antiestrogens Analog **II**, Tamoxifen, and MER25

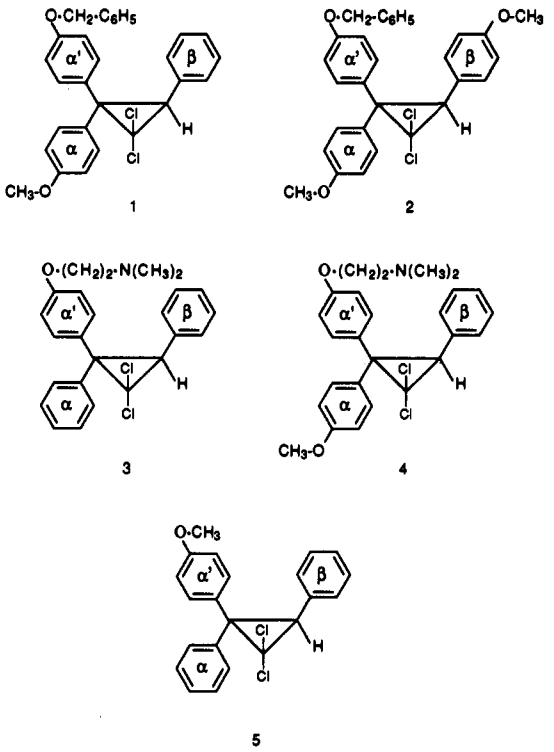


† Present address: Department of Environmental and Occupational Health and Pharmaceutical Sciences, University of Pittsburgh, CEOHT-260 Kappa Drive, Pittsburgh, PA 15238.

® Abstract published in *Advance ACS Abstracts,* April 15, 1994.

0022-2623/94/1837-1670$04.50/0

Chart 2. 1,1-Dichloro-2,2,3-triarylcyclopropanes (DTACs)



hormone-dependent 7,12-dimethylbenz[*a*]anthracene-induced rat mammary tumor model.[17,18] Structure–activity studies of some of the analog **II** derivatives[8-10,19] led us to design more effective cyclopropyl antiestrogens. Starting with analog **II** as the lead compound, we synthesized a series of 1,1-dichloro-2,2,3-triarylcyclopropanes (DTACs) (1–5, Chart 2) by introducing a third phenyl ring and polar substituents in analog **II** so that the compounds possess the structural features of both analog **II** and the clinically useful triarylethylenes. These compounds were found to be antiestrogenic without any estrogenic activity in the mouse and inhibited the growth of estrogen receptor (ER) positive MCF-7 human breast cancer cells in culture.[12]

The stereochemical nature of most of the pharmacologically important receptors involved in estrogenic and antiestrogenic activity has not been characterized at the atomic level. Therefore, the use of the agonists and antagonists to map the size and shape of a receptor binding site using small molecular X-ray structures has proved necessary.

A knowledge of stereochemical similarities and differences in antiestrogens may be useful in the rational development of new agents. If the conformation of a receptor-bound antiestrogen is comparable to the minimum-energy conformation of the free molecule, then it should be possible to compare energy-minimized crystallographic structures of all the compounds that compete for a specific binding site and determine what structural features of the antiestrogens are essential for binding.[20] Although the X-ray structure provides a low-energy conformer, which is generally close to the global minimum energy conformation for the molecule, molecular mechanics calculations can provide information about other local minima for the molecule. The importance of such studies lies in the fact that the pharmacophore conformation of a drug molecule is not necessarily the lowest energy conformer.

Crystal structures of Tamoxifen and several of its derivatives have been reported,[21-26] and these results were used in a number of molecular mechanics and molecular graphic studies[20,27-31] which have provided some insight into the mechanism of action of nonsteroidal antiestrogens. Structural studies of Tamoxifen and its derivatives have shown that the triarylvinyl moiety uniformly exists in a conformation where the three rings are oriented as in a propeller. Although several mechanisms of action have been proposed for Tamoxifen, it is generally assumed that Tamoxifen and its derivatives act mainly by competing with estradiol for its protein receptor (ER). Certain key structural elements including the propeller arrangement of the triaryls are thought to be important for its ability to bind to the ER and block estrogen action.[20,31] The fact that the substitution of a hydroxyl group in the α-ring of Tamoxifen, producing 4-hydroxytamoxifen, a metabolite, greatly increases its binding affinity for the ER and also its antiestrogenic potency led to the hypothesis that the hydroxylated α-ring is responsible for anchoring the ligand tightly to the binding site, while the (alkylamino)ethoxy side chain interferes with subsequent estrogen receptor functions essential for activity.[32,33]

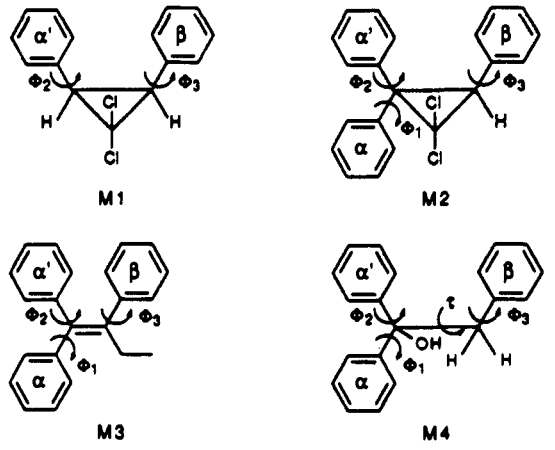
Recently, we have reported the results from the crystal-structure determinations and conformational investigations of analog **II** and its derivatives[19] and the crystal structures of one of the DTACs (5)[34] and the first-known antiestrogen, MER25.[35] Both the DTAC compound **5** and MER25 were shown to assume a nonpropeller arrangement for their three phenyl rings in contrast to the propeller conformation observed in the triarylethylenes.

Crystal-structure determinations of DTACs 1–4 and conformational studies of all DTACs and MER25 were undertaken to gain further insight into their mechanism of action. We present here the results of crystal-structure determinations of four DTAC derivatives (1–4) and a comparative investigation of conformational characteristics of DTACs, MER25, and Tamoxifen.

Methods

For the triaryl antiestrogens, the conformational features of interest include the orientation of the three phenyl rings with respect to the central bridging bond, the geometry of the central core of the molecules, and the relative disposition of the centroids of the three aryl rings. Conformational results were obtained from a complete conformational search using idealized model compounds. Model structures were built for analog **II** (M1), DTACs (M2), Tamoxifen (M3), and MER25 (M4) (Chart 3), which contain the central two atoms and the phenyl rings and other substituents on the central atoms. Phenyl-ring substituents were omitted on the assumption that the conformations of the molecules are not greatly influenced by the para-substituents of the phenyl rings. For all the triaryls, the phenyl rings are designated α, α', and β, with the aminoethoxy side chain on the α'-ring,[36] and their conformations are defined by three dihedral angles, ϕ_1, ϕ_2, and ϕ_3, respectively (Chart 3). All phenyl rings in the model structures were made symmetrical so that a 180° rotation for any phenyl ring would approximately yield the same conformation. All substituents on the phenyl rings were replaced by hydrogen atoms. For cyclopropyl models, the *gem*-dichloro substituents on C(1) were retained. The compression energy in each model is

Chart 3. Model Structures Used for Molecular Mechanics Calculations: Analog **II** (M1), the DTACs (M2), Tamoxifen (M3), and MER25 (M4)



removed by allowing C–H bond distances to change. For each model compound a conformational search was performed for all possible low-energy conformations and this was achieved by using the double-driver facility in the molecular mechanics program MM2/MMP2.[37] For MER25, the central single bond allows for rotation of functional groups around it, leading to three possible low-energy conformers, *anti*, *gauche1*, *gauche2*. Model compounds were built for each of these conformers. For the DTACs and MER25, one of the enantiomeric forms, 2*S*,3*R* for DTAC and *R* for MER25, was used. The diastereomers 2*S*,3*S* and 2*R*,3*R* of the DTACs were not considered because their stereochemistry is similar to that of the estrogenic (*E*)-Tamoxifen.

Results

Crystal Structures of DTACs 1–4. Stereo ORTEP plots of the four structures are shown in Figure 1. All of the DTACs exist as a racemic mixture in the solid state. Selected bond distances and torsion angles for the structures are compared in Table 1.

The bond distances and bond angles in the 1,1-dichlorocyclopropane system observed in the four DTACs 1–4 are generally in agreement with those observed in the crystal structures of **5**[34] and in their diaryl precursors.[19] In all four structures, the cyclopropane ring shows bond-length asymmetry, with the C(2)–C(3) bond being the longest. The bond-length asymmetry observed in the present structures was found to be consistent with the additive scheme of the substituent effects on the cyclopropane ring.[38,19]

The orientation of the three phenyl rings is similar in all the structures (1–4), with the β-ring always in the bisecting position, and both the α′ and α rings are approximately in the perpendicular position with respect to the cyclopropane ring. The dihedral angles ϕ_1, ϕ_2, and ϕ_3 vary within a narrow range: ϕ_1 (α-ring) ranges between 136° in **2** and 148° in **3**, ϕ_2 (α′-ring) ranges between 55° in **4** and 59° in **1** and **3**, and ϕ_3 (β-ring) ranges between 37° in **3** and 46° in **1** and **4**. These dihedral angles represent a nonpropeller conformation as observed in compound **5**.[34] Each structure shows a slight twist about the C(2)–C(3) bond with the C(11)–C(2)–C(3)–C(31) torsion angle varying from −9° in **2** to about −5° in **3**. The side-chain conformations display some flexibility. The benzyloxy side chain in **1** and **2** has a *trans–trans* and *trans–gauche* conformation, respectively. The (dimethylamino)ethoxy side chain in **3** is *trans–trans*, while it is *trans–gauche* in **4** (Table 1).

Energy Minimization of DTAC Structures. The energy minimizations of the five DTAC molecules (1–5) were performed using the MM2 program. The results of these calculations are summarized in Table 2, which provides a comparison of the relevant conformational angles for the crystallographic structures and the energy-minimized structures.

The values of the three dihedral angles ϕ_1, ϕ_2, and ϕ_3 for the five energy-minimized structures are within a few degrees of each other with mean values of 132° (ϕ_1), 59° (ϕ_2), and 31° (ϕ_3), giving the central core of the molecules (the dichlorotriarylcyclopropyl system) an almost identical geometry.

Conformational Search. For each model compound, the calculated relative energy, $E_r = E - E_{min}$, for each conformer was plotted against the torsion angles ϕ_2 and ϕ_3 in the form of a contour map. Each minimum-energy conformer obtained from the energy maps was refined by further energy minimization calculations.

Analog II Model, M1. The results of the conformational search for model M1 are shown in Figure 2a. There are two symmetric low-energy minima having the same energy at around $\phi_2 = 60°$, $\phi_3 = 30°$ (α′-ring is perpendicular and β-ring is in a bisecting position) and $\phi_2 = 150°$, $\phi_3 = 120°$ (α′-ring is bisecting and β-ring is in a perpendicular position). The low-energy region around $\phi_2 = 90°$, $\phi_3 = 90°$ is not a minimum but a saddle point and has about 3 kcal/mol higher energy than the global minimum. These results are almost identical to those reported earlier.[19]

DTAC Model, M2. A survey of the energy matrices for model M2 showed that the low-energy conformers of M2 are confined in the range $\phi_1 = 90°–150°$. Figure 2b shows the energy contours at $\phi_1 = 135°$, which contains the most relevant features of the low-energy conformations of M2. The global minimum is at $\phi_1 = 135°$, $\phi_2 = 60°$, $\phi_3 = 30°$, which closely corresponds to the energy-minimized crystal structure of all the DTAC compounds (Table 2). The local minimum near $\phi_2 = 60°$, $\phi_3 = 105°$ has about 3 kcal/mol higher energy than the global minimum, while a third minimum at $\phi_2 = 150°$, $\phi_3 = 120°$ has about 5 kcal/mol higher energy. The effect of introducing a third ring in the dichlorodiarylcyclopropyl system led to asymmetry and transformation of the saddle point in the diaryl model to a shallow minimum at around (60°, 105°) in the triaryl model compound. It may be noted that the enantiomeric 2*R*,3*S* model of DTAC would give centrosymmetrically related minima.

Tamoxifen Model, M3. Figure 3 shows the two most relevant sections ($\phi_1 = 60°$ and $\phi_1 = 120°$) of the three-dimensional conformational space. Analyses of these maps showed the presence of two minima at around (60°, 50°, 50°) and (120°, 130°, 130°), which have equivalent energy. The two minimum-energy structures are the same unless one restricts free rotation of the phenyl rings, as was assumed in earlier studies.[29,39]

MER25 Model, M4. The τ rotation of model M4 led to the energy profile shown in Figure 4. The profile showed three minima which correspond to three conformers, *anti*, *gauche1*, and *gauche2* represented by Newman projections of the molecule. Analyses of the entire conformational space of each of the three models (*anti*, *gauche1*, and *gauche2*) of MER25 showed the following.

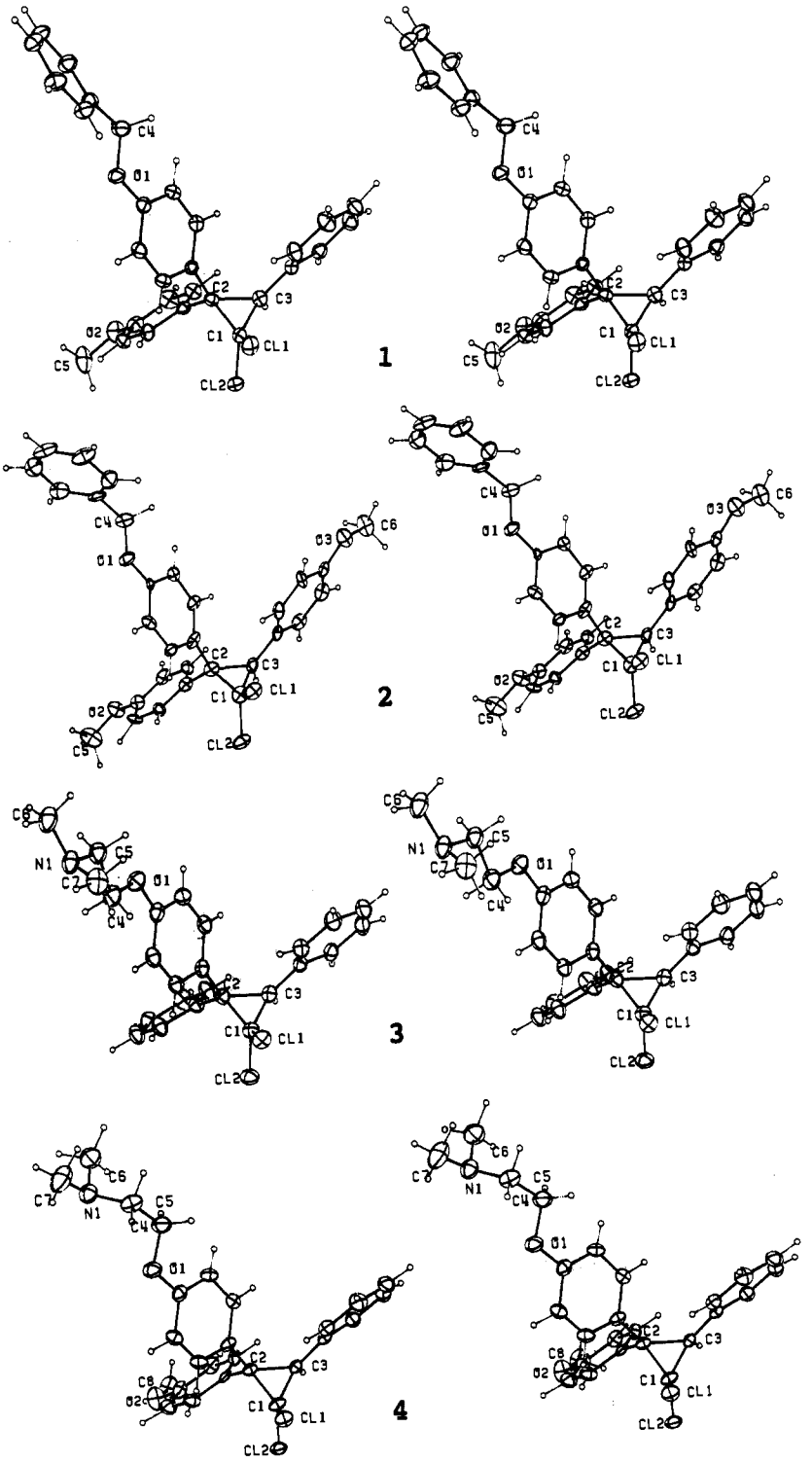


Figure 1. Stereo ORTEP plots of the molecules of compounds 1–4. Only selected atom numbers are shown. Phenyl ring atoms are numbered (not shown) as follows: α'-ring, C11–16; α-ring, C21-26; β-ring, C31-36 and benzyl ring C41-46.

(i) For the *anti* model, there is one prominent low-energy region with a global minimum, A1, at $\phi_1 = 76°$, $\phi_2 = 7°$, $\phi_3 = 84°$ (Figure 5a), which is very close to that found in the crystal structure (82°, 4°, 83°).

(ii) For the *gauche1* model, the two broad low-energy regions, near $\phi_1 = 0°$ (Figure 5b) and $\phi_1 = 75°$ (Figure 5c) revealed three minima with energy difference less than 1.5 kcal/mol between them. The conformer G1A at ($\phi_1 = 5°$, $\phi_2 = 110°$, $\phi_3 = 89°$) has about the same energy as the global minimum in the *anti* model. The energy contours indicate that the phenyl rings (particularly the β-ring) have shallow barriers of rotation.

(iii) For the *gauche2* model, there is a single minimum, G2 (86°, 29°, 88°), with approximately 4 kcal/mol higher energy than the global minimum. Energy contours for this model are not shown. The conformational parameters of the six low-energy conformers of MER25 (A1, A2, G1A, G1B, G1C, G2) are summarized in Table 3. The corresponding molecular skeletons of these six conformers are shown in Figure 6. The enantiomeric MER25(S) would produce equivalent centrosymmetrically related minima.

NMR Spectroscopy of MER25. ^{1}H NMR assignments for MER25 (Chart 4, Table 4) were made via difference decoupling and NOE experiments. The signal for the

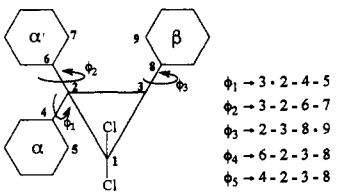
Table 1. Selected Bond Distances (Å) and Torsion Angles (deg) of Compounds 1-4[a]

bond distances	1	2	3	4
Cl(1)-C(1)	1.754(3)	1.770(10)	1.762(2)	1.756(6)
Cl(2)-C(1)	1.763(3)	1.767(10)	1.763(2)	1.771(4)
C(1)-C(2)	1.509(4)	1.522(14)	1.511(3)	1.508(8)
C(1)-C(3)	1.501(4)	1.510(13)	1.502(3)	1.498(7)
C(2)-C(3)	1.540(4)	1.547(13)	1.548(3)	1.538(8)
C(2)-C(11)	1.509(4)	1.486(13)	1.505(3)	1.506(7)
C(2)-C(21)	1.512(4)	1.499(14)	1.518(3)	1.510(6)
C(3)-C(31)	1.491(4)	1.492(14)	1.494(3)	1.484(6)
C(14)-O(1)	1.378(3)	1.369(11)	1.374(3)	1.382(7)
O(1)-C(4)	1.426(4)	1.432(12)	1.435(3)	1.423(6)

torsion angles	1	2	3	4
C(3)-C(2)-C(11)-C(12)	59.4(4)	57.2(12)	59.1(3)	54.6(7)
C(3)-C(2)-C(21)-C(26)	142.3(3)	136.1(10)	147.7(2)	143.1(5)
C(2)-C(3)-C(31)-C(32)	46.1(5)	40.7(15)	37.2(4)	46.2(8)
C(11)-C(2)-C(3)-C(31)	-5.9(5)	-9.3(14)	-4.8(3)	-6.9(8)
C(21)-C(2)-C(3)-C(31)	136.7(3)	134.1(10)	139.8(2)	138.2(5)
C(13)-C(14)-O(1)-C(4)	-1.2(5)	5.9(13)	-173.7(2)	-10.3(8)
C(14)-O(1)-C(4)-C(41)	-177.6(3)	-179.2(9)		
O(1)-C(4)-C(41)-C(42)	-141.1(3)	-98.4(12)		
C(23)-C(24)-O(2)-C(5,8)	174.3(3)	174.4(9)		9.9(8)
C(33)-C(34)-O(3)-C(6)		-176.9(10)		
C(14)-O(1)-C(4)-C(5)			171.7(2)	-173.0(5)
O(1)-C(4)-C(5)-N(1)			179.6(3)	-80.3(7)
C(4)-C(5)-N(1)-C(6)			-166.4(2)	-69.3(6)
C(4)-C(5)-N(1)-C(7)			72.1(3)	171.2(5)

[a] Esd's are within parentheses.

Table 2. Conformational Angles (deg) of the Crystal Structures and Energy-Minimized Structures of Compounds 1-5[a]



dihedral angle	1		2		3		4		5	
	CR	MIN	CR	MIN	CR	MIN	CR	MIN	CR	MIN
ϕ_1	142	131	136	131	148	132	143	131	136	132
ϕ_2	59	58	57	59	59	59	55	57	56	58
ϕ_3	46	32	41	33	37	29	46	31	32	28
ϕ_4	-6	0	-9	2	-5	2	-7	2	1	2
ϕ_5	137	140	134	138	140	142	138	142	142	142

[a] CR = crystal structure; MIN = energy-minimized structure.

protons ortho to the methoxy group was identified from its enhancement upon irradiation at the methoxy resonance. Similarly, irradiation of the two-proton triplet due to the oxygenated methylene group in the $OCH_2CH_2N(Et)_2$ moiety led to identification of the signal for the protons ortho to this group (Table 5). These assignments were further confirmed by the converse NOE experiments. Conventional decoupling then confirmed the assignments of the other protons in the disubstituted aromatic rings. Finally, signals for the protons in the monosubstituted ring were assigned by examination of their integrals and multiplicities.

In CD_3OD the signal for the diastereotopic benzylic protons was a singlet from -60 to 50 °C at 300 MHz and also at 20 °C at 500 MHz. In pyridine-d_5 at 20 °C at 300 MHz this resonance was also a singlet. However, in C_6D_6 at 20 °C at 300 MHz this signal appeared as a very closely spaced AB quartet (Table 4), and at 500 MHz at 20 °C a very distinct AB quartet was evident. Irradiation at the benzylic chemical shift position in either CD_3OD or C_6D_6 produced NOE enhancements of equal intensity on the H4,8 and H16,20 signals, but a noticeably smaller enhancement was noted for the H10,14 proton signal. These results are consistent with a predominance of the *anti* conformer since in this conformation only one of the H2 protons is in a position to relax the H10,14 protons whereas both H2 protons can relax the H16,20 and H4,8 protons. A similar analysis for the *gauche1* conformation predicts a larger NOE interaction between H2 and H10,14 than for H2 versus H16,20, and the opposite is observed. In the

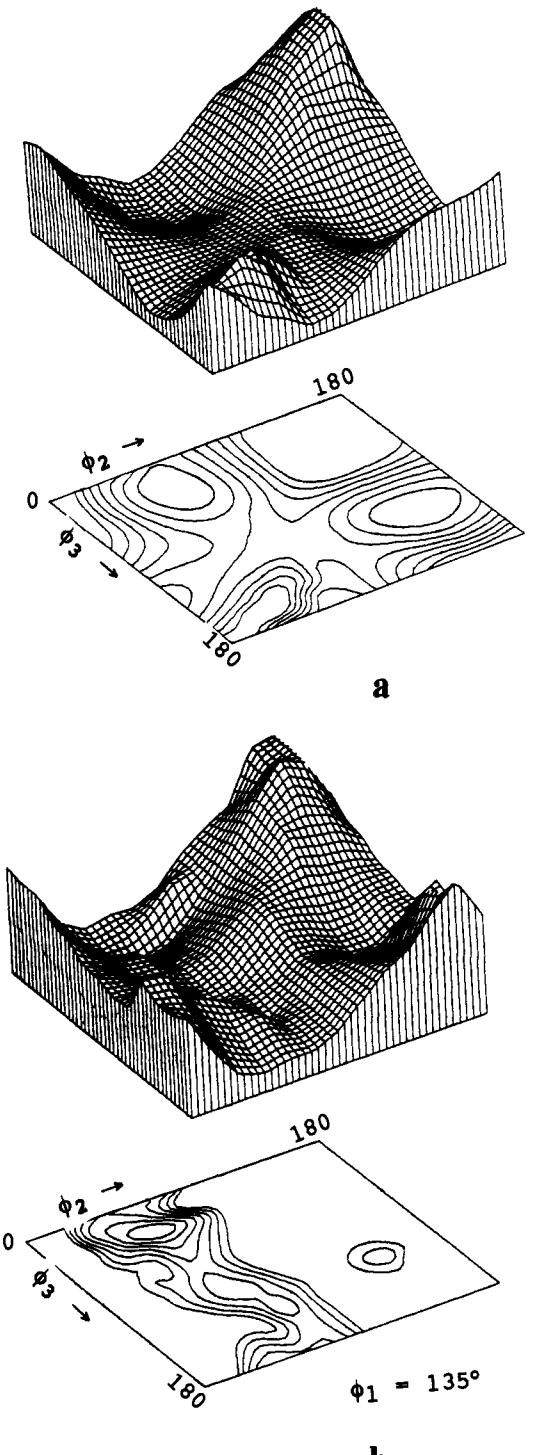


Figure 2. (a) Relative steric energy ($E_r = E - E_{min}$) contour plots for analog II model M1. Contours are drawn from 1 to 7 kcal/mol at intervals of 1 kcal/mol. Corresponding 3-D representation is shown at the top. (b) Section at $\phi_1 = 135°$ of the relative steric energy contour plots for DTAC model M2. Contours are drawn from 1 to 7 kcal/mol at intervals of 1 kcal/mol. Corresponding 3-D representation is shown at the top.

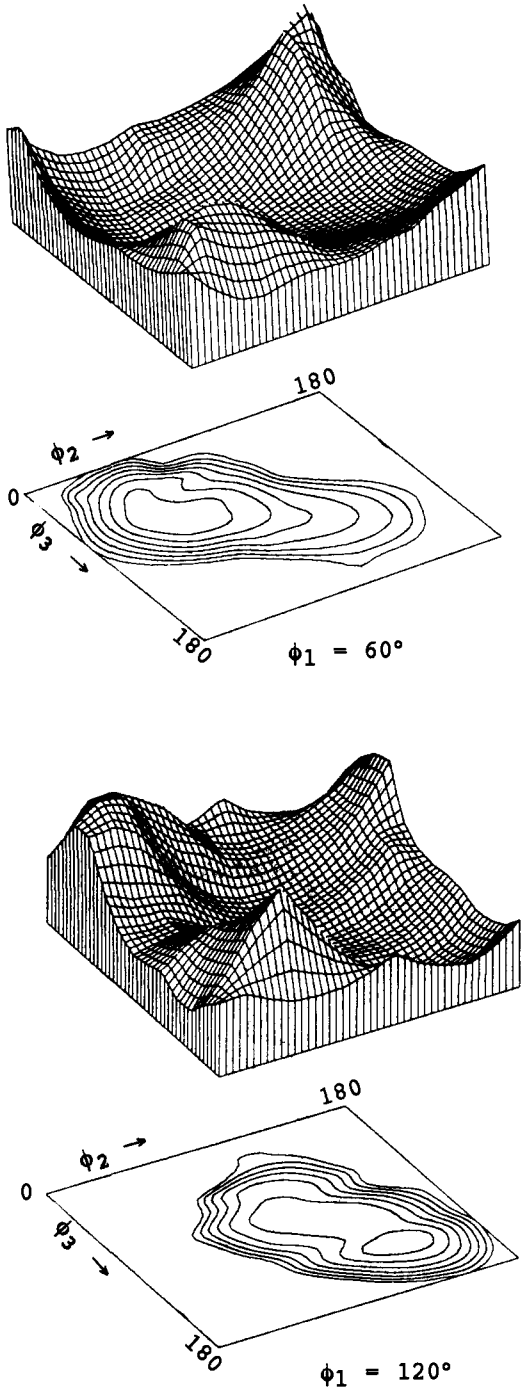


Figure 3. Two sections (at $\phi_1 = 60°$ and $\phi_1 = 120°$) of the relative steric energy contour plots for Tamoxifen model M3. Contours are drawn from 1 to 7 kcal/mol at intervals of 1 kcal/mol. Corresponding 3-D representation is shown above each contour map.

gauche2 conformation, the NOE's between the H2 and the H4,8 or H10,14 protons would be expected to be equal and smaller than for the H2 versus the H4,8 protons. This is not observed. The small NOE observed on the H4,8 signal upon irradiation of the H10,14 resonance is also consistent with a predominance of the *anti* conformation in solution. Likewise the very notable upfield shift of the H4,8 proton signal, 6.78 ppm [cf. 7.1 ppm for the analogous protons of 2-(*p*-methoxyphenyl)ethanol[40]], is consistent with anisotropic shielding of these protons by ring α in the *anti* conformation. The one-dimensional NOE results described above were corroborated by data from NOESY and ROESY experiments in C_6D_6 in which it was found

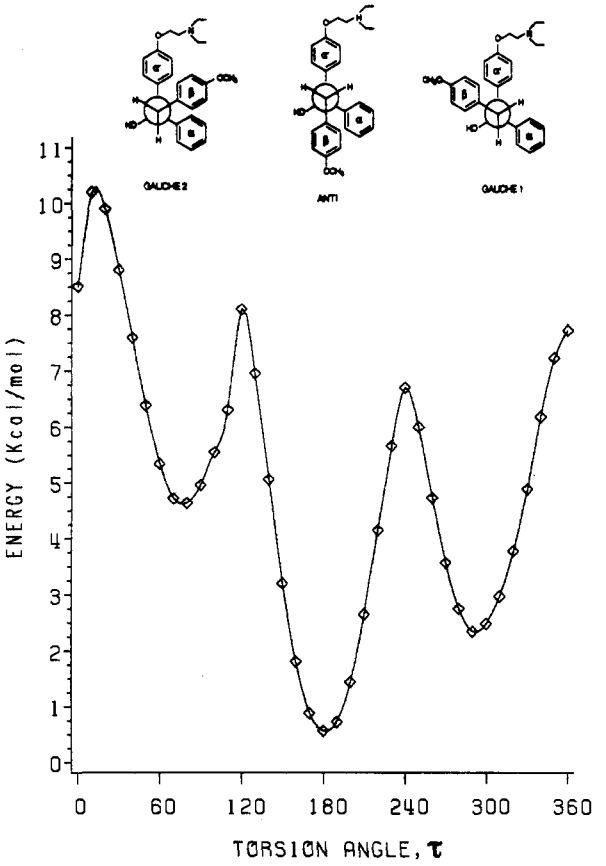


Figure 4. A plot of the relative steric energy against the dihedral angle, τ, obtained by rotation about the central ethylenic single bond in MER25 model M4. The three minima correspond to three Newman's projection of MER25 about the central single bond.

that the ratio of integrals for the crosspeaks for H2 vs H4,8, H16,20, and H10,14 were, respectively, 1:0.7:0.5 (NOESY) and 1:0.6:0.2 (ROESY). Thus all the NMR data suggest that the solution conformation parallels that of the crystal structure.

Discussion

Structures and Conformations. The crystal structure determinations and the energy-minimization calculations for the five DTAC compounds (**1–5**) have shown that the central core, the dichlorotriarylcyclopropane, had a consistent geometry in which the β-ring is always in a bisecting position and α- and α'-rings were in a perpendicular position with respect to the cyclopropane ring. Such a nonpropeller conformation of the three aryl rings in DTAC's is in contrast to the propeller conformation of the triaryls as observed in all known tamoxifen derivatives. Conformational searches of the DTACs have shown that the nonpropeller conformation observed in the crystal also represents the global minimum for each of the DTACs. It appears likely that this geometry is imposed on the molecules by the dichlorocyclopropane system and is not affected by the substituents on the phenyl rings or by the crystal-packing forces. Therefore, it may safely be assumed that all the DTAC molecules retain the same conformation in their biologically active form.

The crystal structure[35] of MER25 showed that in the solid state the drug molecule exists in the *anti* conformation (α'- and β-rings in the *anti* position), which closely

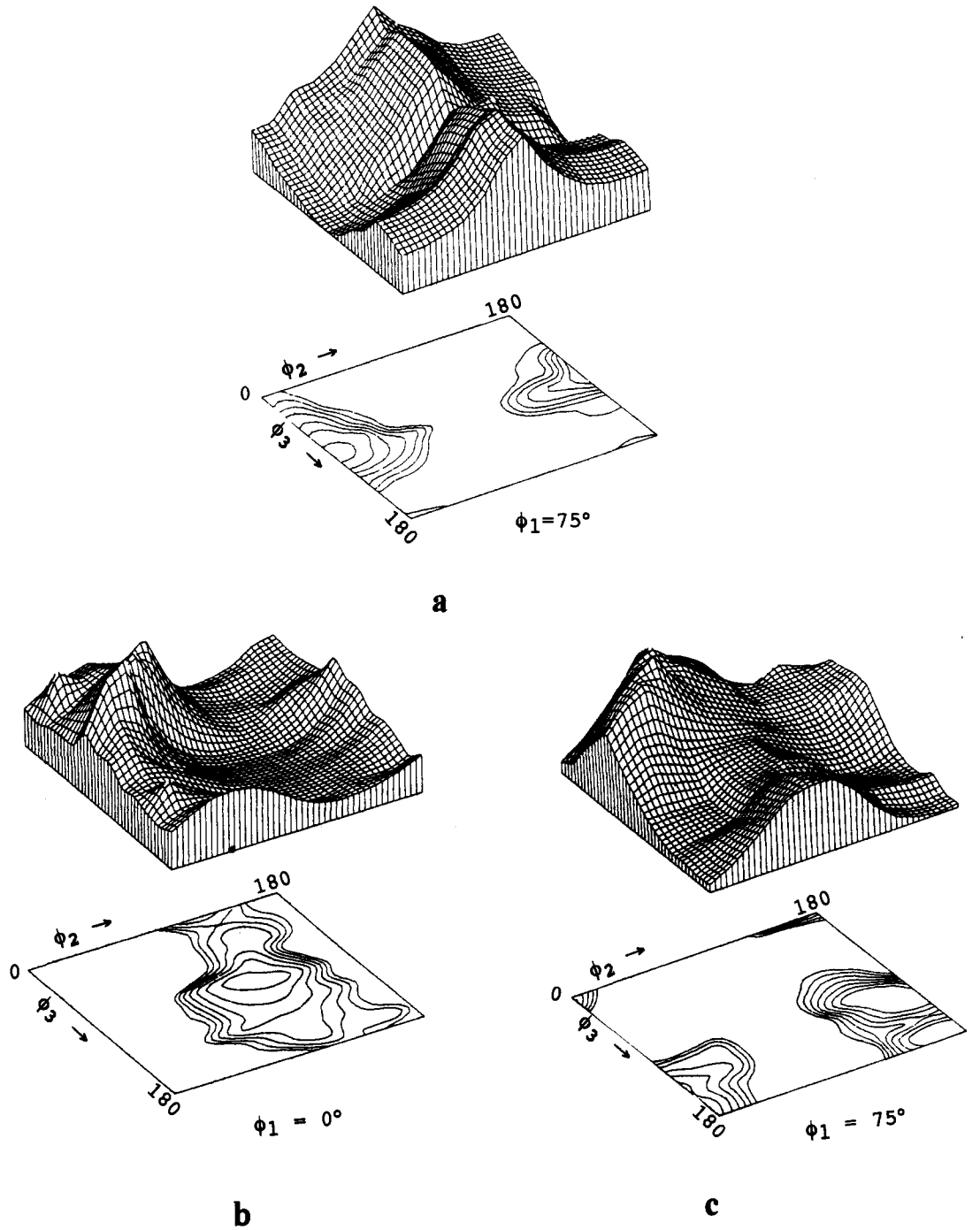
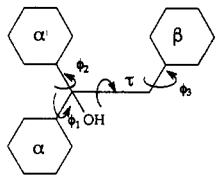


Figure 5. (a) Section at $\phi_1 = 75°$ of the relative steric energy contour plots for MER25 model *anti*. Contours are drawn from 1 to 7 kcal/mol at intervals of 1 kcal/mol. Corresponding 3-D representation is shown at the top. (b) Section at $\phi_1 = 0°$ of the relative steric energy contour plots for MER25 model *gauche I*. Contours are drawn from 1 to 7 kcal/mol at intervals of 1 kcal/mol. Corresponding 3-D representation is shown at the top. (c) Section at $\phi_1 = 75°$ of the relative steric energy contour plots for MER25 model (*gauche I*). Contours are drawn from 1 to 7 kcal/mol at intervals of 1 kcal/mol. Corresponding 3-D representation is shown at the top.

resembles the estrogenic *E*-isomer of Tamoxifen. Our NMR study of MER25 revealed that the molecule is predominantly in the *anti* conformation in solution. This *E*-like conformation was unexpected, particularly when one considers that MER25 is a pure antiestrogen and only the *Z*-isomers of the triarylethylenes are associated with antiestrogenicity.[41] However, our molecular mechanics calculations and steric energy profile searches of MER25 have demonstrated that MER25 can assume a number of possible low-energy conformations (Figure 6) of which two, one *anti* (A1) and one *gauche* (G1A), have about the same energy. One can assume that the *anti* (*E*-like isomer) conformation of MER25 undergoes transformation to the *gauche* (*Z*-like isomer) *in vivo* when attaching to the receptor. However, molecular dynamics and other relevant energetic calculations would be required to elucidate the pathway of such a transformation.

The crystallographic structures of Tamoxifen and other triarylethylenes and a systematic molecular mechanics study of the triarylethylene skeleton have shown that although the three phenyl ring conformations exhibit some degree of flexibility, they consistently assume a propeller conformation with a slightly bent, but rigid, central double bond. Conformational and molecular graphic studies[29,30] have demonstrated that the solid-state conformers of triarylethylenes are also their active forms.

Table 3. Conformational Parameters of Low-Energy Conformers of MER25



		anti		*gauche1*			*gauche2*
conformers	crystal	A1	A2	G1A	G1B	G1C	G2
ϕ_1	82	76	20	5	78	79	86
ϕ_2	4	7	100	110	158	172	29
ϕ_3	83	84	60	89	113	174	88
τ	173	173	176	-59	-59	-58	62
energy (kcal mol^{-1})		16.7	21.1	17.0	18.6	18.5	20.7

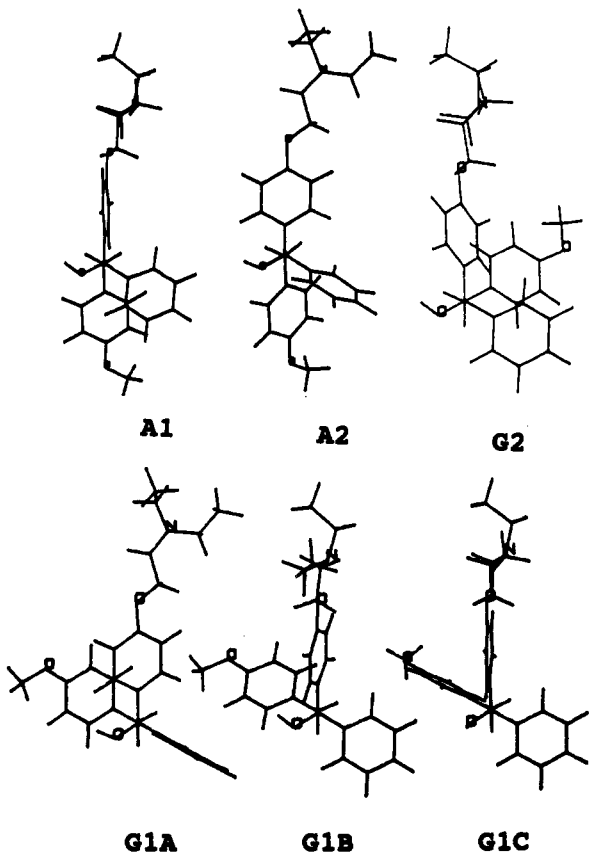
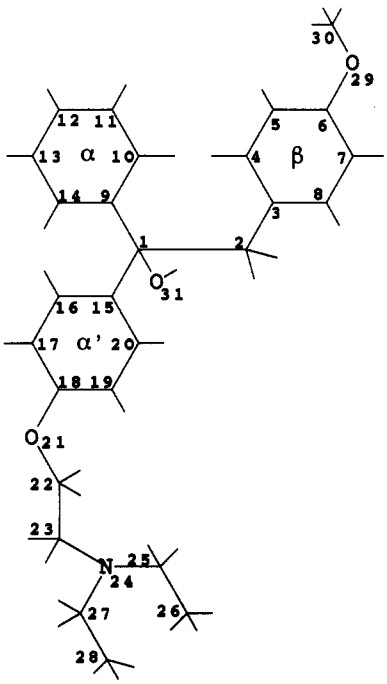


Figure 6. A view down the ethylenic single bond of the six low-energy conformers of MER25: A1, A2, G1A, G1B, G1C, and G2.

It appears that even in their biologically active conformations, the molecules of DTACs, MER25, and Tamoxifen would retain some distinct differences. Figure 7 shows the superimposed molecules of a DTAC (3) and Tamoxifen, a DTAC (3) and MER25 (*gauche1*, conformer G1A), and MER25 (*gauche1*, conformer G1A) and Tamoxifen. The DTACs differ from Tamoxifen and MER25 primarily in the relative disposition and conformation of their α-rings. The matching of compound 3 and Tamoxifen (Figure 7a) shows that there is a closer match of the α'- and β-rings of the two molecules, while their respective α-rings are far apart. Conformational studies have indicated that it would be difficult to achieve a propeller type conformation for the DTACs because of the high barriers of rotation for the α-ring. The *gauche1* (G1A) conformer of MER25, on the other hand, matches better with both Tamoxifen and DTAC (Figure 7b,c). Its superposition with Tamoxifen

Chart 4. Atom Numbering in MER25



(Figure 7c) shows that despite structural differences, the centers of the three aryl rings in the two molecules are very close to each other. And as the conformational studies of MER25 indicated that the aryl rings in MER25 have shallow barriers of rotation, it seems possible for MER25 to achieve a propeller type of conformation as in Tamoxifen; however, MER25 differs from Tamoxifen and DTACs in several ways: (i) the molecule is more flexible and has several low-energy conformers, (ii) in the crystal it exists in an *E*-like isomer, and (iii) it differs also from DTACs and Tamoxifen in having a polar hydroxyl group in the central part of the molecule, while the others have a hydrophobic core.

It should be noted that the DTACs and MER25 exist as racemic mixtures. It has been reported that enantiomeric pairs can exhibit different biological activities,[42] and since there is no difference in their physical properties, the difference in pharmacological activity must be due to their stereochemistry. As there are steric differences (see below) between the enantiomers of a DTAC and of MER25, the separation of enantiomeric pairs may be essential while studying their bioactivity.

The structural element which appears to be consistently similar in all the low-energy conformers of the DTACs, MER25, and Tamoxifen is the *cis*-Ph–C–C–Ph moiety. Even in analog **II**, the conformation of this structural element is very similar to that observed in other molecules, indicating that the *cis*-Ph–C–C–Ph could be part of the pharmacophore for most antiestrogens. Bioactivity studies of triarylethylenes have shown that it is the *cis* arrangement of the α'- and β-rings (*Z*-isomer) which is essential for antiestrogenic activity, while a *trans* arrangement of the α- and β-ring (*E*-isomer) is estrogenic.[41] The *cis*-isomer of analog **II** (*Z*-isomer) is antiestrogenic and is devoid of intrinsic agonist activity, while its *trans*-isomer is without any traceable estrogenic/antiestrogenic action.[10]

Structure–Activity Relationship. Do all of these nonsteroidal antiestrogens, which possess some common structural elements and at the same time show distinct

Table 4. Proton NMR Data for MER25 (Chart 4)

	CD_3OD^a			$C_6D_6^b$			Py-d_5		
ring α									
H12	7.15	t	7.8	7.05	t	6.8	7.22	t	7.8
H11,13	7.23	t	7.8	7.12	t	6.8	7.35	t	7.8
H10,14	7.34	d	7.7	7.43	d	7.7	7.80	d	7.8
ring α'									
H16,20	7.27	d	8.8	7.33	d	8.8	7.74	d	8.7
H17,19	6.81	d	8.6	6.81	d	8.8	7.03	d	8.7
ring β									
H4,8	6.78	d	8.7	6.82	d	8.6	7.22	d	8.6
H5,7	6.61	d	8.7	6.61	d	8.6	6.79	d	8.6
H2	3.49	s		3.41	AB q	11.2	3.80	s	
OCH_3	3.69	s		3.22	s		3.53	s	
H22	4.06	t	5.8	3.82	t	6.5	4.03	t	6.3
H23	2.89	t	5.8	2.70	t	6.5	2.79	t	6.3
H25,27	2.67	q	7.4	2.40	q	7.0	2.50	q	7.1
H26,28	1.09	t	7.4	0.92	t	7.0	0.95	t	7.1
OH				2.13	s		5.0		

[a] 300 MHz. [b] 500 MHz.

Table 5. Nuclear Overhauser Enhancements Observed for MER25 (Chart 4)

CD_3OH^a					C_6D_6					Py-d_5			
proton	irrad	proton	enhanced	(%)	proton	irrad	proton	enhanced	(%)	proton	irrad	proton	enhanced
H2	3.49	H4,8	6.78	(10.4)	H2	3.41		6.82	(10.8)	H2	3.80		7.22
		H16,20	7.27	(10.8)			H16,20	7.33	(10.6)				7.74
		H10,14	7.34	(9.2)				7.43	(7.8)				7.80
CH_3O	3.69	H(5,7)	6.61		CH_3O	3.22		6.61	(10.5)	CH_3O	3.53		6.79
H22	4.06	H17,19	6.81	(9.3)	H22	3.82	H17,19	6.81	(10.3)	H17,19	7.03	H22	4.03
H5,7	6.61	CH_3O	3.69	(3.7)									
H17,19	6.81	H22	4.06	(6.5)									
		H5,7	6.61	(9.0)									
		H16,20	7.27	(15.0)									
H16,20	7.27	H17,19	6.81	(10.3)									
H10,14	7.34	H2	3.49	(2.2)	H10,14	7.43	H11,13	7.12	(9.3)				
							H4,8	6.82	(1.1)				
OH					OH	2.13	H16,20	7.33	(2.6)	OH	5.0	H16,20	7.74
							H10,14	7.43	(2.2)			H10,14	7.80

[a] 300-MHz difference NOE experiments.

structural and conformational differences, have a similar mechanism of action?

Biological studies have shown that analog **II**, DTACs, and MER25 have comparable estrogen-receptor binding affinity, antiestrogenicity, and antiproliferative activity. Selective bioactivity data from an earlier report[12] are listed in Table 6 for a comparative review. Compounds 1–5 and analog **II** exhibited no uterotrophic activity when tested *in vivo* in the absence of estradiol at doses up to 750 μg. Tamoxifen elicited a significant estrogenic response at a dose of 1 μg, and MER25 was slightly estrogenic at 750 μg. In the antiuterotrophic assay, a DTAC molecule with either a methoxy (**5**), benzyloxy (**1**, **2**), or (dimethylamino)ethoxy (**3**, **4**), as the α'-ring side chain, produced significant decreases (30–50%) in the uterine weight at 150 μg, while, as expected, Tamoxifen did not yield any decrease (Tamoxifen is estrogenic/antiestrogenic in the rat and estrogenic in the mouse). Compounds 1–4, Tamoxifen, and analog **II** were active *in vitro* against the estrogen-dependent MCF-7 human breast cancer cell line.

More recently, the antiproliferative activity of the DTACs 1–3 and 5 was examined in the ER-negative MDA-MB-231 human breast cancer cell line and A-549 human lung cancer cells.[43–45] None of the DTACs altered the cell growth in either cell line but were active only in the hormonal-dependent cell line, MCF-7 human breast cancer cells. Further, coadministration of estradiol reversed the antiproliferative activity of the DTACs on the MCF-7 cells. All compounds (10^{-6} M) in a study of cell surface morphology reduced the length and density of microvilli on MCF-7 cells, but not on the ER(-)MDA-MB-231 cells, which were reversed by coadministration of estradiol (10^{-8} M). These results indicate that the pure DTAC antiestrogens are very selective for breast cancer cells, and an ER-dependent mechanism of action is very likely. The relative binding affinity (RBA) for the ER is generally considered an important factor in antiestrogenic activity on the basis of the assumption that antiestrogenic compounds act mainly by competing with estradiol for its receptor.[33] The nonsteroidal antiestrogens listed in Table 6, including Tamoxifen, have, however, a weak affinity for the estrogen receptor, analog **II** and MER25 being the two with lowest RBA. The DTACs **3** and **4** with a (dimethylamino)ethoxy side chain have comparable RBA's to that of Tamoxifen, while the DTACs with methoxy (**5**) or benzyloxy (**1**, **2**) have much lower RBA's, indicating the importance of the aminoethoxy side chain on the α'-ring. On the other hand, MER25 possesses the aminoethoxy side chain and has a very low RBA.

Molecular superpositions of the α-ring of the triaryl antiestrogens with the estradiol A-ring have been used to elucidate structural requirements for antiestrogenic activity.[20,27–28] Similar superpositions are shown for a DTAC (**3**) (Figure 8a), (*Z*)-Tamoxifen (Figure 8b), and the low-energy *gauche* conformer of MER25 (Figure 8c). The bulky substituent on the α'-ring has been hypothesized to prevent activation of the ER.[20,28,31] This structural requirement for antiestrogenic activity is thus a mostly

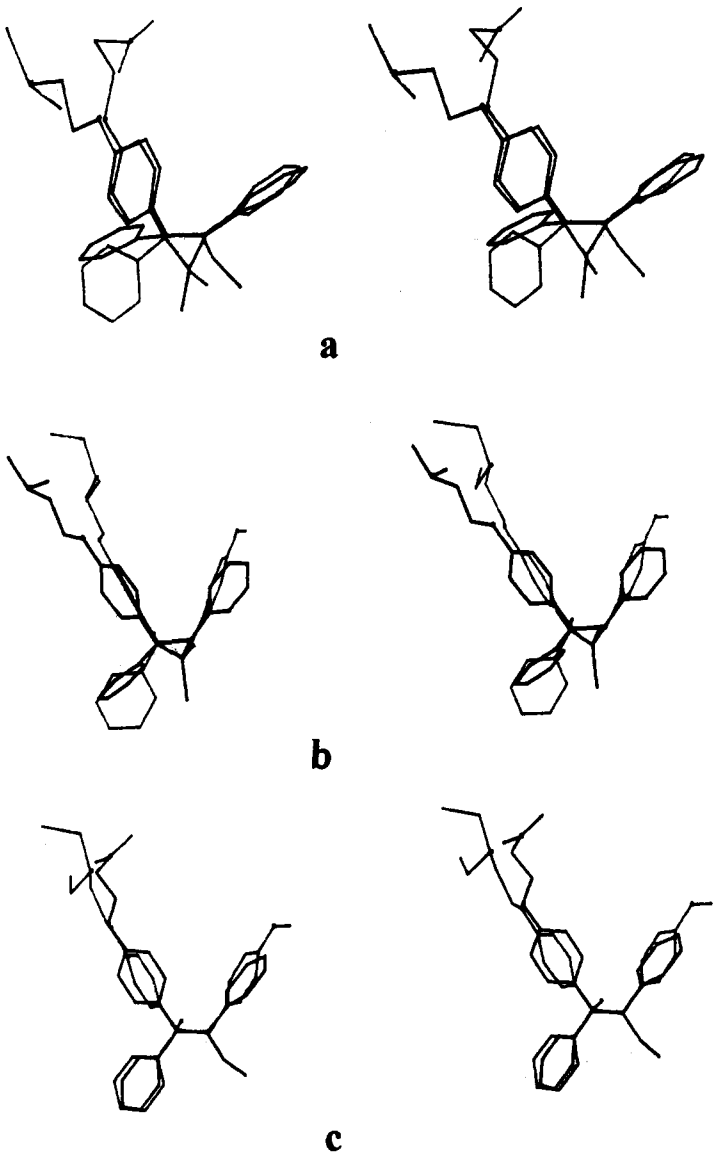


Figure 7. (a) Stereoview of the superposition of Tamoxifen with compound 3. (b) Stereoview of the superposition of compound 3 with MER25 (*gauche* G1A). (c) Stereoview of the superposition of Tamoxifen with MER25 (*gauche* G1A).

Table 6. Comparative Activities of Tamoxifen, MER25, Analog II, and DTACs Antiestrogens[a]

compound	I[b]	II[c]	III[d]	IV[e]
Tamoxifen	0.9	0 ± 10[f]	52 ± 2	weakly estrogenic
analog II	0.009	28 ± 4[g]	60 ± 2	nonestrogenic
MER25	0.002	30 ± 6		nonestrogenic
1	0.1	49 ± 14	46 ± 4	nonestrogenic
2	0.1	47 ± 11	35 ± 2	nonestrogenic
3	0.9	30 ± 10	41 ± 5	nonestrogenic
4	0.7	42 ± 10	35 ± 2	nonestrogenic
5	0.2	49 ± 10	35 ± 8	nonestrogenic
estradiol	100			

[a] Reference 12. [b] RBA (%) relative to estradiol (100%). [c] Percentage reduction in the estradiol-stimulated uterine weight of immature female mice at a dose of 150 μg. [d] Percent inhibition of control cell growth of ER-positive MCF-7 human breast cancer cell line at a concentration of 10^{-7} M. [e] Estrogenic property of the compounds and standard. [f] As expected, Tamoxifen had no antiestrogenic activity in the mouse. [g] Result for a dose of 200 μg.

rigid (phenyl) group and a bulky substituent, i.e. (dialkylamino)ethoxy or benzyloxy group at the para-position of the of the α′-ring, region I (Figure 8a). This requirement is satisfied for Tamoxifen, the DTACs, and MER25. The low RBA values for the compounds may be caused by one of two reasons or a combination of them. One reason, their lack of a *p*-hydroxyl group on the α-ring, has been proposed.[31] The other comes from the superpositions in Figure 8. That is, the rather poor fit in region II (Figure 8a) below the estradiol molecule. Our hypothesis is that a poor fit in region II contributes to the low RBA values of the compounds. We also propose that a certain amount of space is allowed in region II, which can be occupied by the β-ring in MER25, the β-ring and the dichloro group in DTACs, and a β-ring and an ethyl group in Tamoxifen. Supposedly, sufficient bulk in region II would completely prevent binding and eliminate antiestrogenic activity completely. One could test this proposal by probing region II with DTAC compounds bearing different bulky substituents on the β-ring. If one superposes estradiol with the enantiomeric 2*R*,3*S* DTAC 3 (Figure 8d), one interchanges, in principle, only the dichloro group and β-ring. This, therefore, affords another way of probing region II by separating the enantiomers of DTACs and studying their antiestrogenic activity. A similar experiment with MER25 also would yield additional information. The flexible MER25 molecule could have the same conformation in the crystalline state, in solution, and at the receptor, or differ in each state or be alike in two states and not the

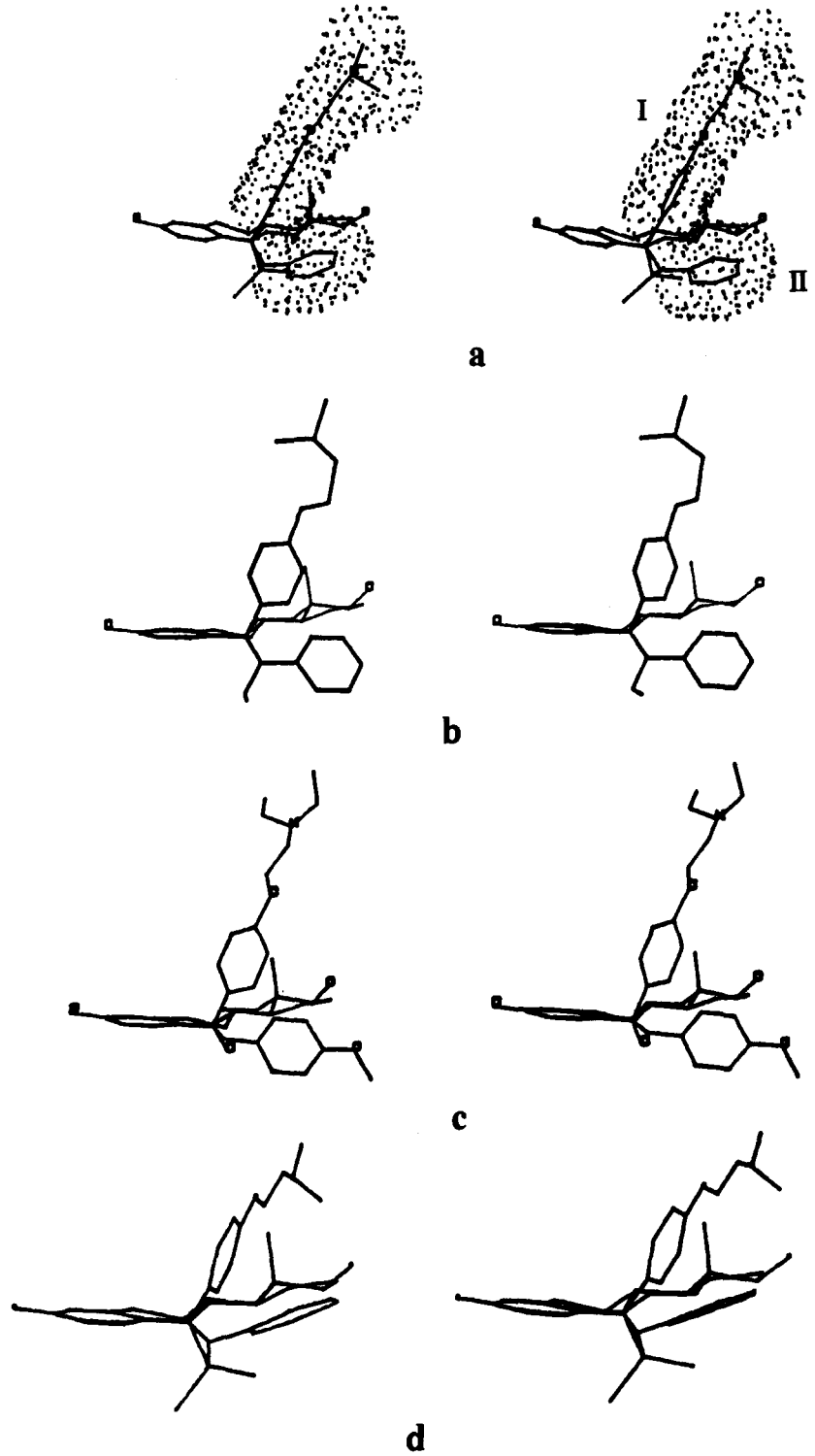


Figure 8. Stereoviews of the superposition with estradiol: (a) Compound 3, (b) Tamoxifen, (c) MER25 (conformer G1A), (d) an enantiomer of 3. In each case the α-ring of the antiestrogen molecule is matched with the A-ring of estradiol. In part a, I and II indicate the two unmatched regions.

other. The synthesis and biological testing of restrictive analogs of MER25 are necessary to find out which conformation of MER25 is needed for antiestrogenic activity.

The structural comparison and superpositions (Figures 7 and 8) do not provide an obvious explanation for the weakly estrogenic activity of (*Z*)-Tamoxifen compared to the pure antiestrogenic activity of the DTAC's. If Tamoxifen is rotated by 180° around the α-ring in the estradiol superposition, the bulk in region I would be eliminated and thus allow activation of ER. On the other hand, this rotation would give a large group (α'-ring and substituent) in region II, possibly preventing binding completely. In addition, other mechanisms besides those involving the ER have been proposed for Tamoxifen, i.e. interactions with calmodulin[46,30] and protein kinase C.[47] Considering the distinct structural and conformational differences between Tamoxifen and DTAC's and between the DTAC's and MER25, combined with the fact that the ER binding affinities are extremely low for these compounds, one can suggest that the modes of action of DTAC's and MER25 may be different from that of Tamoxifen.

Table 7. Crystal Data, Intensity Data Collection, and Refinement Parameters for Compounds 1–4

	1	2	3	4
		Crystal Data		
formula	$C_{29}H_{24}O_2Cl_2$	$C_{30}H_{26}O_3CL_2$	$C_{25}H_{25}ONCL_2$	$C_{26}H_{27}O_2NCl_2$
MW	475.4	505.4	426.3	456.4
no. reflections (for cell)	48	24	40	48
θ-range, deg	12–40	10–15	15–28	11–16
crystal system	monoclinic	monoclinic	monoclinic	monoclinic
space group	$P2_1/c$	$P2_1/c$	$P2_1/n$	$P2_1/a$
a (Å)	13.746(1)	13.021(6)	16.582(2)	11.162(6)
b (Å)	15.7276(6)	16.940(2)	20.156(2)	15.950(6)
c (Å)	11.1811(9)	11.637(7)	6.6687(9)	13.771(8)
β (deg)	105.266(7)	97.69(1)	97.414(8)	108.94(5)
V (Å^3)	2332.0	2543.7	2210.2	2319.0
Z	4	4	4	4
D_x (Mg m^{-1})	1.354	1.319	1.281	1.307
μ (λ) (mm^{-1})	2.58	2.38	2.64	0.26
λ (Å)	1.54178	1.54178	1.54178	0.71069
F(000)	992	1056	896	960
crystal dimensions (mm)	0.24 × 0.10 × 0.04	0.20 × 0.09 × 0.02	0.48 × 0.08 × 0.05	0.18 × 0.10 × 0.05
solvent	ethanol	ethanol/benzene	ethanol	ethanol
		Intensity Data Collection		
$2\theta_{max}$	150°	120°	150°	46°
h	−17 to 17	−14 to 14	−20 to 20	0 to 12
k	0 to 19	0 to 19	0 to 25	0 to 17
l	0 to 14	0 to 13	0 to 8	−15 to 15
scan type	θ–2θ	θ–2θ	θ–2θ	θ–2θ
scan width, deg	0.90 + 0.2 tan θ	0.80 + 0.20 tan θ	0.85 + 0.26 tan θ	1.10 + 0.34 tan θ
monitor/freq	3, 2 h	3, 2 h	3, 2 h	3, 2 h
max variation	3.8%	2.0%	3.5%	4.8%
T_{max} (s)	45	75	80	60
no. unique	4786	3742	4555	4537
no. observed	3204 ($I > 2\sigma(I)$)	1671 ($I > 2\sigma(I)$)	3315 ($I > 2\sigma(I)$)	2336 ($I > 3\sigma(I)$)
		Refinement		
R	0.050	0.076	0.043	0.062
R_w	0.050	0.063	0.044	0.068
S	1.6	1.3	1.4	2.0
(Δ/σ)m	0.05	0.05	0.04	0.06
H-atom	refined	refined[a]	refined	refined
no. parameters	394	394	362	388
$(\Delta\rho)_{max}$ (e/Å^3)	0.50	0.54	0.36	0.40
$(\Delta\rho)_{min}$ (e/Å^3)	−0.25	−0.45	−0.30	−0.50

[a] Refined with bond-length constraints.

In conclusion, it seems that future studies should be directed toward (i) the separation of the enantiomeric pairs of DTACs and MER25 and testing of their bioactivity (ii) synthesis and testing of metabolites of DTACs, (iii) synthesis and testing of restrictive analogues of MER25, and (iv) testing calmodulin-binding affinity for the various cyclopropyl antiestrogens and MER25. Work in all of these areas is in progress.

Experimental Section

X-ray Diffraction. Compounds 1–4 were crystallized by slow evaporation at room temperature. Crystals of compounds 2 and 4 were of poor quality while those of compounds 1 and 3 were of moderate quality. Crystal data, intensity data collection parameters, and refinement results of the four compounds are summarized in Table 7. Cell parameters were determined by least-squares fit to $\pm 2\theta$ of a number of reflections measured at 163 K using Cu Kα_1 radiation. Space groups were determined from systematic absences. All X-ray measurements were carried out on an Enraf-Nonius CAD-4 diffractometer fitted with a liquid-N_2 low-temperature device. In each case intensities were collected employing θ–2θ scan technique using a variable scan width and horizontal aperture. Intensities were corrected for Lorentz and polarization factors but not for absorption. The structures were determined by direct methods using the programs MULTAN80[48] and SHELXS-86[49] and refined by a full-matrix least-squares routine[50] in which the quantity $\sum w(F_o - F_c)^2$ was minimized, $w = 1/\sigma^2(F_o)$. Hydrogen atoms in each case were located from the difference map and they were refined isotropically. For compound 2, the hydrogen atoms were refined with bond-length constraints using "DFIX" instruction in SHELX76 and fixed isotropic temperature factors. The refinement summary for each compound is listed in Table 7.

Molecular Mechanics Studies. Molecular mechanics calculations and energy minimizations were performed by using the programs MM2/MMP2. Interactive molecular modeling, superposition, and other graphic studies were performed by using the program MOGLI (Molecular Graphics Library) on an Evans & Sutherland PS390 color graphics terminal coupled to a MicroVax II computer.

For diaryl model compound M1, a two-dimensional conformational search for all possible low-energy conformations was performed, varying ϕ_2 and ϕ_3 in a step of 15°. Initial exploration was made with only slight relaxation for the whole molecule, which was followed by a full-energy minimization at every grid point.

For DTAC model M2, the same procedure as in M1 was followed except in this case energy values were evaluated for 13 different sections of ϕ_1 ranging from 0 to 180° at a step of 15°. A similar procedure was followed for Tamoxifen model M3. For MER25 model M4, initially angle τ was varied from 0 to 360° at a step of 15° and at each value of τ the rest of the model was allowed to relax and the results of this search led to the building of three new models *anti*, *gauche1*, *gauche2* with τ values of 60°, 180°, and −60°. The aminoethoxy side chain is included in the *anti*, *gauche1*, and *gauche2* models, for in model M4 (without phenyl substituents), the phenyl rings α and α' are indistinguishable and the *anti* model and *gauche1* model are in effect enantiomers of each other. For all three models of MER25, a search procedure similar to that of DTAC and Tamoxifen models was followed.

Normal force-field parameters as given in the MM2/MMP2 program were used along with the following additional parameters for atom types 12 (C1), 22 (cyclopropane C), and 2 (benzene C): (i) torsional parameters, 22–22–22–12 (0.000, –0.250, 0.550), 5–22–22–12 (0.000, 0.000, 0.406), 2–22–22–12 (0.000, 0.000, 0.406); (ii) stretching parameters, 2–2 (6.56, 1.390), 12–22 (3.23, 1.795); (iii) bending parameters, 22–22–12 (0.560, 118.5), 12–22–12 (1.080, 111.7). These parameters were obtained by comparing those given in the MM2 parameter tables for closely related interactions and by trial energy minimization that gave proper geometries for the molecules. For all the compounds only one of the enantiomeric forms was considered, assuming the existence of a mirror-related conformer.

NMR Spectroscopy of MER25. ^{1}H NMR spectra were recorded at 300 and 500 MHz on Varian XL-300 and VXR-500 spectrometers at ambient temperature in the solvents specified in Table 5. Chemical shifts are given relative to tetramethylsilane. Proton assignments were verified by decoupling and NOE experiments (see Discussion). Percent NOE's were determined by difference spectroscopy.

Acknowledgment. This research was supported by NCI (NIH) Grants CA 17562 (DvdH) and CA 40458 (RAM). The authors thank Merrell Dow Research Institute for the MER25 sample. We thank Ms. A. Park for conducting NOESY and ROESY NMR experiments.

Supplementary Material Available: Atomic parameters, bond distances, bond angles, torsion angles, anisotropic thermal parameters, hydrogen atom parameters, hydrogen distances, and atom numbering schemes (19 pages). Ordering information is given on any current masthead page.

References

(1) Lerner, L. J.; Jordan, V. C. Development of antiestrogen and their use in breast cancer. Eighth Cain Memorial Award Lecture. *Cancer Res.* **1990**, *50*, 4177–4189.
(2) Furr, B. J. A.; Jordan, V. C. Pharmacology and clinical use of tamoxifen. *Pharmacol. Ther.*, **1984**, *25*, 127–203.
(3) Fornander, T.; Cedermark, B.; Mattsson, A.; Skoog, L.; Theve, T.; Askergren, J.; Rutqvist, L. E.; Glas, U.; Silfversward, C.; Somell, A.; Wilking, N.; Hjalmar, M. Adjuvant tamoxifen in early breast cancer: Occurrence of new primary cancers. *Lancet*, **1989**, *1*, 117–119.
(4) Malfetano, J. H. Tamoxifen-associated endometrial carcinoma in postmenopausal breast cancer patients. *Gynecol. Oncol.*, **1990**, *39*, 82–84.
(5) Chambon, P.; Dierich, A.; Gaup, M.-P.; Jakowlev, S.; Jongstra, J.; Krust, A.; LeLennec, J.-P.; Oudet, P.; Reudelhuber, T. Promotes elements of genes coding for proteins and modulation of transcription by estrogens and progesterone. *Recent Prog. Horm. Res.*, **1980**, *40*, 1–42.
(6) Kistner, R. W.; Smith, O. W. Observations on the use of a non-steroidal estrogen antagonist: MER25. *Surgical Forum*, **1960**, *10*, 725–729.
(7) Lerner, L. J.; Jordan, V. C. In *The Non-Steroidal Antiestrogen*; Sutherland, R. L., Jordan, V. C., Ed.; Academic Press, Sydney, 1981; pp 1–16.
(8) Magarian, R. A.; Benjamin, E. J. Syntheses of cyclopropyl analogs of stilbene and stilbenediol as possible antiestrogens. *J. Pharm. Sci.*, **1975**, *64*, 1626–1632.
(9) Stobaugh, J. F.; Magarian, R. A.; Pento, J. T. Synthesis and Biological Evaluation of gem-Dichlorocyclopropyl and Cyclopropyl analogs of Stilbene Cogeners as Potential Antiestrogens. *J. Pharm. Sci.*, **1982**, *71*, 1126–1128.
(10) Pento, J. T.; Magarian, R. A.; Wright, R. J.; King, M. M.; Benjamin, E. J. Nonsteroidal Estrogens and Antiestrogens: Biological Activity of Cyclopropyl analogs of stilbene and stilbenediol. *J. Pharm. Sci.*, **1981**, *71*, 399–403.
(11) Griffin, M. T.; Magarian, R. A.; Jain, P.; Pento, J. T.; Mousissian, G. K.; Graves, D. C. Synthesis and Biological Evaluation of a Series of gem-Dichlorocyclopropanes as Antitumor agents. *Anti-Cancer Drug Des.* **1992**, *1*, 49–66.
(12) Day, B. W.; Magarian, R. A.; Jain, P., Pento, J. T.; Mousissian, G. K.; Meyer, K. L. Synthesis and biological evaluation of a series of 1,1-dichloro-2,2,3-triarylcyclopropanes as pure antiestrogens. *J. Med. Chem.*, **1991**, *34*, 842–851.
(13) Bedford, G. R.; Walpole, A. L.; Wright, B. Antiestrogenic Triarylcyclopropanes. *J. Med. Chem.*, **1974**, *17*, 147–149.
(14) Anstead, G. M.; Altenbach, R. J.; Wilson, S. R.; Katzenellenbogen, J. A. 2,3-Diarylindenes and 2,3-Diarylindenones: Synthesis, Molecular Structure, Photochemistry, Estrogen Receptor Binding Affinity and Comparisons with Related Triarylethylenes. *J. Med. Chem.*, **1988**, *31*, 1316–1326.
(15) McCague, R.; Kuroda, R.; Leclercq, G.; Stoessel, S. Synthesis and Estrogen Receptor Binding of 6,7-Dihydro-8-phenyl-9-[4-[2-(dimethylamino)ethoxy]phenyl]-5H-benzocycloheptane, a Non-isomerizable Analogue of Tamoxifen. X-ray Crystallographic Studies. *J. Med. Chem.* **1986**, *29*, 2053–2059.
(16) Schneider, M. R.; Schonenberger, H. Mammary Tumor Inhibiting [(1,2-Diethyl-1,2-cyclopropanedityl)bis(phenyl)]diacetate. *Eur. J. Med. Chem. Chim. Ther.*, **1982**, *17*, 491–495.
(17) Pento, J. T.; Magarian, R. A.; King, M. M. A comparison of the efficacy for antitumor activity of the non-steroidal antiestrogens analog II and tamoxifen in 7,12-dimethylbenz[*a*]*antracine*-induced rat mammary tumors. *Cancer Lett.* **1982**, *15*, 261–269.
(18) King, M. M.; Magarian, R. A.; Terao, J.; Brueggemann, G. L. Effects of nonsteroidal antiestrogens, analog II and tamoxifen, on a metastatic transplantable rat mammary tumor. *J. Natl. Cancer Inst.*, **1985**, *74*, 447–451.
(19) Hossain, M. B.; Wang, J. L.; van der Helm, D.; Magarian, R. A.; Griffin, M. T.; Day, B. W. Structural Comparison of a *gem*-Dichlorodiaryl cyclopropane Antiestrogen and three of its Derivatives. *Acta Cryst.*, **1991**, *B47*, 511–521.
(20) Duax, W. L.; Griffin, J. F.; Weeks, C. M.; Korach, K. S. Molecular Conformation, Receptor binding, and Hormone Action of Natural and Synthetic Estrogens and Antiestrogens. *Environ. Health Persp.* **1985**, *61*, 111–121.
(21) Precigoux, G.; Courseille, C.; Geoffre, S.; Hospital, M. [*p*-(Dimethylamino-2-ethoxy)phenyl]-1-*trans*-Diphenyl-1,2-Butene-1 (Tamoxifen) (ICI-46474). *Acta Crystallogr.* **1979**, *B35*, 3070–3072.
(22) Precigoux, G.; Hospital, M.; Leroy, F.; Delbarre, A.; Roques, B. P. [*p*-(Bromo-2-ethoxy)phenyl]-1-Chloro-2-Diphenyl-1,2-Ethylene. *Acta Crystallogr.* **1982**, *B38*, 312–315.
(23) Hunter, D. H.; Payne, N. C.; Rahman, A.; Richardson, J. F.; Ponce, Y. Z.; The Iodo-Tamoxifens: Molecular Structures and Syntheses of Estrogens for External Imaging of Carcinoma. *Can. J. Chem.*, **1983**, *61*, 421–424.
(24) Kuroda, R.; Cutbush, S. Q.; Neidle, S.; Leung, O.-T. Structural Studies on Some Tamoxifen Derivatives. *J. Med. Chem.*, **1985**, *28*, 1497–1503.
(25) Cutbush, S. Q.; Neidle, S.; Foster, A. B.; LeClercq, F. Structure of 1-(*p*-Methoxyphenyl)-*trans*-1,2-diphenylbut-1-ene. *Acta Crystallogr.* **1982**, *B38*, 1024–1027.
(26) Kaftory, M.; Biali, S. E.; Rappoport, Z. Stable Simple Enols. Solid State Structures and Conformations of Several Simple Enols and Their Keto Tautomers. *J. Am. Chem. Soc.* **1985**, *107*, 1701–1709.
(27) Duax, W. L.; Weeks, C. M.; Molecular Basis of Estrogenicity: X-ray Crystallographic Studies. In *Estrogens in the Environment*. McLachlan, J., Ed.; Elsevier/North Holland: New York, 1980; pp 11–31.
(28) Duax, W. L.; Griffin, J. F.; Weeks, C. M.; Warwrzak, Z. The Mechanism of Action of Steroid Antagonists: Insight from Crystallographic Studies. *J. Steroid Biochem.* **1988**, *31*, 481–492.
(29) Edwards, K. J.; Laughton, C. A.; Neidle, S. A. Note on the Conformational Flexibility of the Antiestrogenic Drug Tamoxifen: Preferred Conformations in the Free State and Bound to the Protein Calmodulin. *Acta Crystallogr.* **1992**, *B48*, 511–514.
(30) Edwards, K. J.; Laughton, C. A.; Neidle, S. A Molecular Modeling Study of the Interactions between the Antiestrogen Drug Tamoxifen and Several Derivatives, and the Calcium-binding Protein Calmodulin. *J. Med. Chem.*, **1992**, *35*, 2753–2761.
(31) Murphy, C. S.; Parker, C. J.; McCague, R.; Jordan, V. C. Structure-Activity Relationships of Non-isomerizable Derivatives of Tamoxifen: Importance of Hydroxyl Group and Side Chain Positioning for Biological Activity. *Mol. Pharm.* **1990**, *39*, 421–428.
(32) Jordan, V. C.; Koch, R.; Lieberman, M. E. Structure-Activity Relationships of Non-Steroidal Estrogens and Antiestrogens. In *Estrogen/Antiestrogen Action and Breast Cancer Therapy*; Jordan, V. C., Ed.; University of Wisconsin Press: Madison, WI, 1986; pp 19–41.
(33) Jordan, V. C. Biochemical Pharmacology of Antiestrogen Action. *Pharmacol. Rev.* **1984**, *36*, 245–276.
(34) Du, L.; Hossain, M. B.; Ji, X.; van der Helm, D.; Magarian, R. A.; Day, B. W. Structure of 1,1-Dichloro-2-(4-methoxyphenyl)-2,3-diphenylcyclopropane. *Acta Crystallogr.* **1992**, *C48*, 887–891.
(35) Hossain, M. B.; Symersky, J.; Neely, S. C.; van der Helm, D.; Magarian, R. A. Structure of 1-{4-[2-(Diethylamino)ethoxy]phenyl}-2-(4-methoxyphenyl)-1-phenylethan-1-ol, the Non-Steroidal Antiestrogen MER25. *Acta Crystallogr.* **1993**, *C49*, 500–504.
(36) There seems to be some confusion about the ring designations in Tamoxifen (see refs 20, 30, 31). We have followed the convention where the ring with the long side chain (aminoethoxy or benzyloxy) is designated α'.
(37) Allinger, N. L. *MM2/MMP2 Program.* Quantum Chemistry Program Exchange, University of Indiana, Bloomington, Indiana, 1985.
(38) Allen, F. H. The Geometry of Small Rings. I. Substituent-Induced Bond-Length Asymmetry in Cyclopropane. *Acta Crystallogr.* **1980**, *B36*, 81–96.
(39) Biali, S. E.; Kaftory, M.; Rappoport, Z. Restricted rotation and torsional isomerism in Tamoxifen derivatives. *J. Org. Chem.* **1989**, *54*, 4959–4962.

(40) Pouchert, D. J.; Campbell, J. R. In *The Aldrich Library of NMR Spectra*. Aldrich Chemicals Co. Inc.: Milwaukee, 1974; spectrum 23.
(41) Robertson, D. W.; Katzenellenbogen, J. A.; Long, D. J.; Rorke, E. A.; Katzenellenbogen, B. S. Tamoxifen and Antiestrogens. A Comparison of the Activity, Pharmacokinetics, and Metabolic Activation of the Cis and Trans Isomers of Tamoxifen. *J. Steroid Biochem.* **1982**, *16*, 1–13.
(42) Bogert, M. G.; Buylaaert, W. A. In: *Stereochemistry and Biological Activity of Drugs*. A. J. Ariens, et al., Ed.; Blackwell Scientific Publications, **1983**, pp 147–148. Jamali, F.; Mehvar, R.; Pasutto, F. M. Enantioselective Aspects of Drug Action and Disposition: Therapeutic Pitfalls. *J. Pharm. Sci.* **1989**, *78*, 695–715.
(43) Jain, P. T.; Pento, J. T.; Magarian, R. A. Antiestrogenic effects of Z-1,1-Dichloro-2,3-diphenyl-2-(4-Methoxyphenyl)cyclopropane (Ia) on human breast cancer cells in culture. *Anticancer Res.* **1992**, *12*, 585–590.
(44) Jain, P. T.; Pento, J. T.; Magarian, R. A. The influence of novel cyclopropyl antiestrogen (compound 7a) in human breast cancer cells in culture. *Breast Cancer Res. Treatment* In press.
(45) Jain, P. T.; Pento, J. T.; Magarian, R. A. A comparison of the antitumor activity of two cyclopropyl antiestrogens (compounds 4d and 5c) in human breast cancer cells in culture. *Endocrinology* In press.
(46) Lam, H. Y. P. Tamoxifen is a calmodulin antagonist in the activation of c-AMP phosphodiesterase. *Biochem. Biophys. Res. Commun.* **1984**, *118*, 27–32.
(47) O'Brien, C. A.; Liskamp, R. M.; Soloman, D. H.; Weinstein, I. B. Triphenylethylenes: A new class of protein kinase C inhibitors. *J. Natl. Cancer Inst.* **1986**, *76*, 1234–1246.
(48) Main, P.; Fiske, S. J.; Hull, S. E.; Lessinger, L.; Germain, G.; Declercq, J. P.; Woolfson, M. M., **1980**. *MULTAN80*. A System of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data. University of York, England and Louvain, Belgium.
(49) Sheldrick, G. M. **1986**. *SHELXS-86*. Program for the Solution of Crystal Structure. University of Gottingen, Gottingen, Germany.
(50) Sheldrick, G. M. **1976**. *SHELX76*. Program for Crystal Structure Determination, University of Cambridge, England.