Molecular Structures, Conformational Analysis, and Preferential Modes of Binding of 3-Aroyl-2-arylbenzo[b]thiophene Estrogen Receptor Ligands: LY117018 and Aryl Azide Photoaffinity Labeling Analogs

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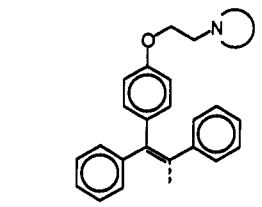
Structural and computational modeling studies were performed on the antiestrogen LY117018 (3) and two photoaffinity labeling analogs, in which an azide replaces the basic ether side chain (methyl ether tetrafluoro azide 7 and its protio analog 8). These studies were undertaken in order to determine the conformational preferences of these compounds and to propose favorable orientational modes for their binding to the estrogen receptor. In the crystallographic studies, we found that, unlike tetrafluoro azide 7, which adopts a face-to-face stacking of the *p*-hydroxyphenyl and benzoyl groups in the solid state, the pendant rings in the corresponding protio analog 8 are found in a predominantly offset π-stacked array. In LY117018, which has an ether on the benzoyl ring, stacking of the pendant rings does not occur in the crystal structure; it assumes a T-shape, with the benzoyl group oriented perpendicular to the benzo[*b*]thiophene nucleus. In modeling studies, analogs of LY117018, 7, and 8 were subjected to a conformational grid search by molecular mechanics, and for each compound, three low-energy conformers (and their atropisomers) were obtained. These conformers were further geometry optimized by semiempirical molecular orbital calculations. For each compound, one of the three minimum-energy conformers is quite similar to the solid-state geometry. The computational structure of the tetrafluoro azide showed the greatest stacking between the benzoyl group and the *p*-methoxyphenyl ring, but less stacking than was observed in the crystallographic structure. The orientational preferences of these benzo[*b*]thiophene ligands with the estrogen receptor were analyzed with the receptor volume mapping technique, a method based on the correspondence of the hydroxyl groups and the volume that the benzo[*b*]thiophene compound shares with a composite molecular volume of high-affinity estradiol-type ligands (the receptor excluded volume, RExV). If the benzo[*b*]thiophene nucleus is overlapped with the steroid AB rings, the best overlap with the RExV is achieved, but there is poor correspondence of the hydroxyl groups. An orientation and conformation in which the benzoyl group of the 3-benzoyl-2-aryl-benzo[*b*]thiophenes occupies a 7α-like position relative to the steroid produces both ample volume overlap with the RExV and close approximation of the hydroxyl groups and is presented as the putative bioactive conformation.

Introduction

Certain triarylethylene and similar compounds bearing a basic ether side chain (1) act as estrogen receptor (ER) antagonists (i.e., antiestrogens).[1] One of these compounds, tamoxifen (2),[2] has been extensively used in the chemotherapy of breast cancer[3] and has elicited interest for the prevention of breast cancer[4] and the treatment of osteoporosis[5] and metastatic melanoma.[6] The spatial dispositions of the aryl rings and the side chain have been investigated in the antiestrogens in an effort to understand the molecular basis of the antagonism.[7] The 3-aroylbenzo[*b*]thiophene LY117018 (3),[8] discovered by investigators at Eli Lilly Co., is a potent antiestrogen that does not conform to the triarylethylene structural motif[9] of tamoxifen (2) and nafoxidine (4).[10]

Recently, the tetrafluoro azido (5) and azido (6) derivatives of LY117018 were employed in a photoaffinity labeling study of the estrogen receptor (ER).[11] Contrary to expectation, the protio compound 6 labeled with greater efficiency than the tetrafluoro analog 5.[11] The crystallographic structure of 7, the dimethyl ether of 5, indicated an apparent intramolecular stacking between the electron-deficient *p*-azidotetrafluorobenzoyl group and the electron-rich methoxyphenyl group.[12] Perhaps in 5, the stacking hydroxyphenyl group may mollify the reactivity of the



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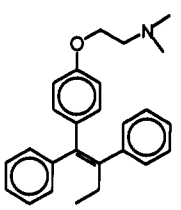
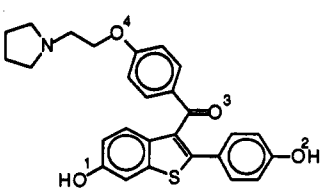
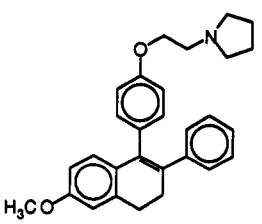


2 (Tamoxifen)



3 (LY117018)



4 (Nafoxidine)

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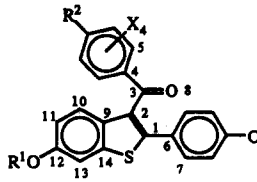
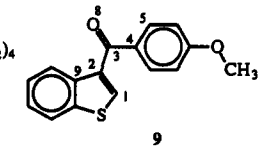
Table I. Torsion Angles in Crystal Structures of 3-Aroyl-2-arylbenzo[*b*]thiophenes (**3**, **7**, **8a**, **8b**) and 3-*p*-anisoylbenzo[*b*]thiophene (**9**)



#	X	R^1	R^2
3	H	H	$O(CH_2)_2N(CH_2)_4$
7	F	CH_3	N_3
8a	H	CH_3	N_3
8b	H	CH_3	N_3



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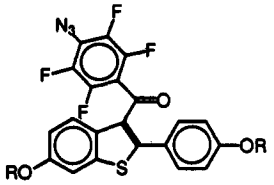
angle	dihedral angle (deg)				
	LY117018 (3)	tetrafluoro azide 7	protio azide 8a	protio azide 8b	9[a]
7-6-1-2[b]	140	114	133	137	-
1-2-3-4	114	-7	-41	-37	-25
2-3-4-5[b]	-14	-67	-26	-31	-38
1-2-3-8	-66	-171	143	142	151
8-3-4-5[b]	-10	-65	-31	-31	-33
9-2-3-8	109	-8	-33	-35	-18

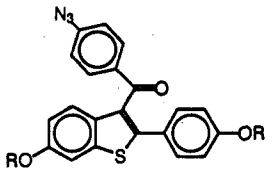
[a] Reference 18. [b] One of the two possible torsion angles that relate to the symmetrical phenyl ring was chosen arbitrarily, but the values are reported in a consistent manner for each compound across the table.

nitrene photochemical intermediate of **5**, or the stacked conformation may alter the orientation of the tetrafluoro azide in the binding site compared to the protio azide. The objective of this study was to investigate the conformational preferences of the benzo[*b*]thiophene derivatives **3**, **5**, and **6** and to propose favorable orientational modes for their interaction with the ER hormone binding domain.

Initially, the X-ray crystallographic structures of LY117018 and the dimethyl ether **8** of protio azide **6** were determined and compared to that of tetrafluoro azide **7**. Conformational analysis was performed on LY117018 and the two aryl azides using molecular mechanics and molecular orbital calculations. Molecular graphics receptor mapping techniques were used to ascertain the most probable bioactive conformations and binding orientations of these compounds in the ER binding cavity.



5, R = H
7, R = CH_3



6, R = H
8, R = CH_3

Molecular Structures

X-ray Crystallography. For protio azide **8**, there are two crystallographically independent molecules in the lattice. These two molecules (**8a** and **8b**) are very similar in conformation, but differ slightly in the torsional angles of the groups appended to positions 2 and 3 of the benzo[*b*]thiophene nucleus (Table I). The presence of multiple conformations of a flexible molecule in a single-crystal lattice indicates that they are of similar energy. Typically, for uncharged organic molecules, the solid-state structure approaches a local minimum-energy conformation.[7d] There are no strong intermolecular interactions in the crystal lattice of protio azide **8**. An ORTEP representation of **8a** is seen in Figure 1, and the crystal data are presented in Table II.

For LY117018 (**3**), a single conformation is observed in the solid state (Figure 1). Two hydrogen bonds are observed in the lattice: [O1···O3 = 2.78 Å, O2H···O3 = 1.91 Å, O3···H–O2 = 161°] and [N···O1 = 2.78 Å, O1H···N = 1.91 Å, N···H–O1 = 162°]. Thus, the *p*-hydroxyl group of the ring at the 2-position acts as a hydrogen bond donor to the carbonyl oxygen, and the benzo[*b*]thiophene phenol donates a hydrogen bond to the nitrogen. The hydrogen bond observed in this case is somewhat longer than the mean H···O distance (1.869 Å) of isolated, noncooperative bonds found in a large series of compounds.[13] However, H···O distances greater than 2 Å have been reported.[14] The mean OH···O bond angle has previously been observed to be 163.1°.[15] The accepted range of oxygen–oxygen distances for hydrogen bonds is 2.5–2.9 Å.[16] The hydrogen bonding pattern in the crystal lattice is significant because similar interactions may be observed in ligand–protein binding.[17]

The dihedral angles of **3**, **7**, **8a**, **8b**, and 3-*p*-anisoylbenzo[*b*]thiophene (**9**),[18] measured from the crystallographic structures, are compared in Table I. In the solid-state structures (Figure 1), cofacial stacking of the 3-benzoyl moiety with the para-oxygenated group at the 2-position occurs only in tetrafluoro azide **7** (Figure 1C). The azidotetrafluorobenzoyl group may act as an electron acceptor in this stacking interaction. In LY117018, which has an electron-donating ether at the para-position of the 3-benzoyl group, stacking would juxtapose two ring positions bearing electron-donating substituents, causing a repulsion.[19] From Figure 1B, this compound adopts a T-like conformation, without stacking, in which the 3-benzoyl moiety is essentially perpendicular to the long axis of the 2-arylbenzo[*b*]thiophene nucleus. In protio azide **8**, the pendant ring systems assume a predominantly offset π-stacked geometry (Figure 1A). Thus, the solid-state structures of **7**, **8**, and LY117018 are consistent with principles governing aromatic π–π donor–acceptor complexation.[19]

Attractive van der Waals forces are considered to be significant in stacking interactions with interplanar separations of greater than 3.4 Å.[19] The centroid-to-centroid distance between the stacked rings of tetrafluoro azide **7** is 3.51 Å.[12] However, each C–F bond in **7** decreases the polarizability 1.2 times relative to a C–H bond.[20] Thus, in **7**, van der Waals attractions in the stacking are lessened, and electrostatic forces may assume more importance.

Another aspect of the crystal structure of tetrafluoro azide **7** is the intermolecular, partially-offset π-stacking of the benzo[*b*]thiophene units in a head-to-tail, top-to-bottom fashion. This arrangement is quite similar to the solid state structure of 1,2-substituted indolizines.[21] The interplanar separation distance in this case is 3.71 Å,

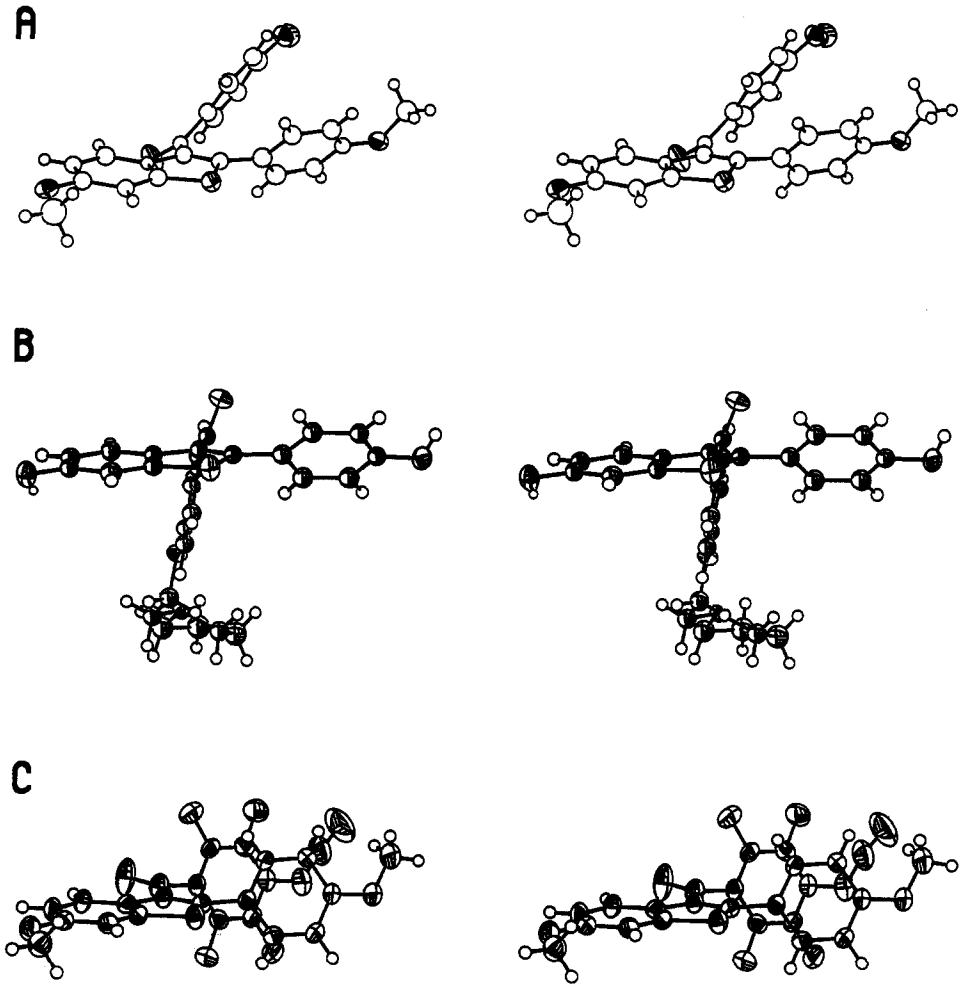


Figure 1. Stereoscopic thermal ellipsoid representations (35% probability level) of **8a**, **3**, and **7** plotted perpendicular to the best-plane normal in A, B, and C, respectively.

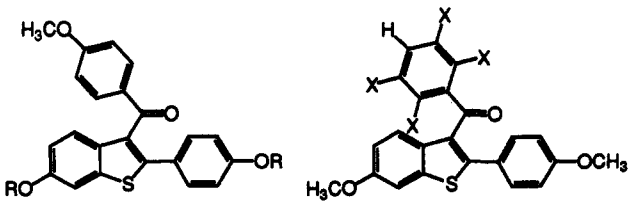
Table II. Crystal Data for **3** and **8**

	3	8
formula	$C_{27}H_{25}NO_4S \cdot CO(CH_3)_2$	$2(C_{23}H_{17}N_3O_3S)$
crystal system	$P2_1/c$	$P\bar{1}$
space group	monoclinic	triclinic
a, Å	15.452(3)	7.832(2)
b, Å	14.705(2)	14.301(4)
c, Å	12.685(3)	18.150(4)
α, deg	90	83.90(2)
β, deg	110.98(1)	85.91(2)
γ, deg	90	82.46(2)
v, Å^3	2691(2)	2001(2)
Z	4	4
density calcd, g/cm^3	1.28	1.38
crystallizing solvent	acetone	ether
crystal habit	platy (yellow)	columnar (yellow)
crystal dimensions, mm	0.1 × 0.3 × 0.4	0.6 × 0.1 × 0.1
μ, cm^{-1}	1.52	1.83
transmission factor range	not applied	0.980–0.966
extinction parameter	5.2 (4) × 10^{-8}	not refined
2θ limit, deg (octants)	50.0 ($+h+k\pm l$)	39.0 ($+h\pm k\pm l$)
intensities (unique, R_i)	5041 (2565, 0.058)	3881 (3459, 0.022)
intensities >2.58$\sigma(I)$	1071	2049
R	0.067	0.109
R_w[for w = $1/(\sigma^2(F_o) + pF\sigma^2)$]	0.083 (p = 0.03)	0.153 (p = 0.03)
max density in ΔF map, e/Å^3	0.29	0.56

compared to 3.4 Å between the planes in layered aromatic hydrocarbons.[22] Benzo[*b*]thiophenes **3** and **8** do not display this behavior.

A dominant feature in the structure of aromatic ketones is the propensity toward coplanarity of the carbonyl group and the adjacent ring. If steric barriers prohibit coplanarity, the dihedral angle between the carbonyl group and the ring is minimized to attain optimal conjugation.[23] In model compound **9**, which lacks the 2-aryl substituent of compounds **3**, **7**, and **8**, the carbonyl group is twisted out of plane −33° and −18° with the *p*-anisyl and thiophene rings, respectively. An empirical index of conjugation can be defined as: Conj = $\cos^2\theta_1 + \cos^2\theta_2 + \cos^2\theta_3$, where Conj = empirical index of conjugation; θ_1, θ_2, and θ_3 = torsional angles 7–6–1–2, 8–3–4–5, and 9–2–3–8, respectively, from Table I; and $\cos^2\theta$ is the relationship between resonance energy and the torsional angle.[24] For LY117018, tetrafluoro azide **7**, and protio azide **8a**, the calculated values of Conj are 1.65, 1.33, and 1.90, respectively. Thus, in tetrafluoro azide **7**, it appears that total conjugation within the molecule is sacrificed to achieve the cofacial stacking arrangement seen in Figure 1C. Cozzi and coworkers have suggested that through-space Colombic stacking interactions between polar aryl rings may predominate over conjugative factors in determining the conformation of conjugated molecules in which stacking is possible.[25]

The basic ether side chain of LY117018 assumes a *gauche* conformation in the solid state, with a O–C–C–N dihedral angle of 59°. However, this moiety is apparently very flexible, based on studies of the conformation of this group in other antiestrogens.[26] Using molecular mechanics,



10 (R = H; analog of 3)
11 (R = CH_3; analog of 3)
12 X = F (analog of 7)
13 X = H (analog of 8)

Figure 2. Structures 11, 12, and 13 are the analogs of 3, 7, and 8 that were used in the initial conformational analysis by the multitorsion grid search method, using the MM2 force field. A hydrogen was substituted for the *p*-azide group in 7 and 8 because of the lack of forcefield parameters for the azide functionality. In 11, the basic ether side chain of LY117018 was truncated to a methyl ether. Structure 10 was used for subsequent receptor volume mapping studies.

Kuroda found that the *gauche*$^+$, *gauche*$^-$, and *trans* conformations differ by only 1 kcal/mol or so, with low-energy barriers between them.[27] Thus, any structure–activity study of the antiestrogen should consider that all these conformations are accessible. The flexible pyrrolidine side chain was replaced by a methyl ether group in the modeling studies. This truncated analog of 3 should allow us to determine the effect alkyl ether substitution has on the preferred conformation of the aroyl benzo[*b*]-thiophene backbone.

Molecular Modeling. Analogs of LY117018, 7, and 8 (Figure 2) were subjected to a conformational grid search by molecular mechanics (details in the Experimental Section). For each compound, three sets of low-energy atropisomers were obtained. One of the atropisomers for each low-energy conformer was further geometry optimized by semiempirical molecular orbital calculations. The dihedral angles that define the three unique conformations for each compound are presented with their relative energies in Table III.

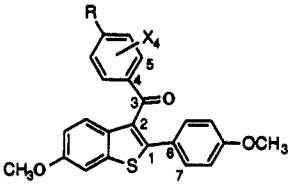
The low-energy conformations of protio azide 8 and the LY117018 analog 11 display remarkable similarity in the three critical backbone torsion angles. Thus, azide substitution for the methyl ether on the benzoyl group has little effect on the conformation of these structures. The low-energy conformations of tetrafluoro-substituted 7 are similar to the low-energy conformations of 8 and 11 in dihedral angles 7–6–1–2 and 1–2–3–4 but are significantly different in dihedral angle 2–3–4–5. This dihedral angle is indicative of the twist out of plane of the carbonyl group relative to the aromatic ring.

The three minimum-energy conformations of each compound differ primarily in the disposition of the pendant *p*-methoxyphenyl ring and of the substituted benzoyl ring (Figure 3). The *p*-methoxyphenyl ring may exist in either of two low energy conformations defined by the torsion angle 7–6–1–2 (130° ± 14° or 58° ± 10°). The benzoyl group also exists in either of two low-energy conformations defined by the torsion angle 1–2–3–4 (−47° ± 10° or 127° ± 7°). The magnitude of out of plane twist of the carbonyl group and the aryl ring of the benzoyl system is related to the dihedral angle 2–3–4–5 and is dependent on the steric character of the aryl ring. Compounds 8 and 11 exhibit a small amount of twist in the nonstacked conformations (−15° ± 3°), but a relatively larger twist in the stacked conformation (−26° ± 2°). Tetrafluoro-substituted 7 has significantly greater twist in all three conformations (−61° ± 1°). In fact, in 2,6-difluoroacetophenone, the acetyl group is reported to be perpendicular to the plane of the ring.[28]

One of the low-energy conformations predicted by molecular modeling for compounds 7, 8, and 11 exhibits close similarity with their crystallographic structures (Figure 4). The offset π-stacking observed between the aromatic rings in the crystal structure of 8 is reproduced in the geometry-optimized computed conformation 1 of the protio azide (Figure 4A). This conformation is 1.41 kcal/mol higher in energy than the lowest-energy conformation predicted by computational methods. The T-shaped conformation observed in the crystal structure of 3 (LY117018) is successfully reproduced in the computer-generated conformation 2 of 11 (Figure 4B). This conformation is 0.49 kcal higher in energy than the lowest-energy conformation.

The face-to-face stacking observed in the crystal structure of the tetrafluoro azide 7 is less successfully reproduced in conformation 1 (stacked) of the geometry-optimized computed structure (Figure 4C), which differs from the crystal structure most notably in the dihedral angle 1–2–3–4 (X-ray, −7°; computer, −38°). The differ-

Table III. Dihedral Angles and Relative Energies of the Conformations of Benzo[*b*]thiophenes 7, 8, and 11 Obtained from Grid Search, Energy Minimization (MM2), and Geometry Optimization (AM1)



#	R	X
7	N_3	F
8	N_3	H
11	CH_3O	H

compd/ conformer no	rel energy (kcal/mol)	dihedral angle (deg) 7–6–1–2	1–2–3–4	2–3–4–5	pendant aryl ring disposition	aryl ring centroid distance (Å)
tetrafluoro azide (7)						
1	0.49	116	−38	−60	stacked, similar to X-ray	3.9
2	0.86	131	132	−61		
3	0.0	53	128	−61		
protio azide (8)						
1	1.41	131	−46	−27	stacked, similar to X-ray	4.1
2	1.29	132	124	−13		
3	0.0	52	122	−13		
LY117018 analog (11)						
1	0.0	134	−57	−24	stacked	4.4
2	0.45	133	123	−13	similar to X-ray	
3	0.49	68	134	−18		

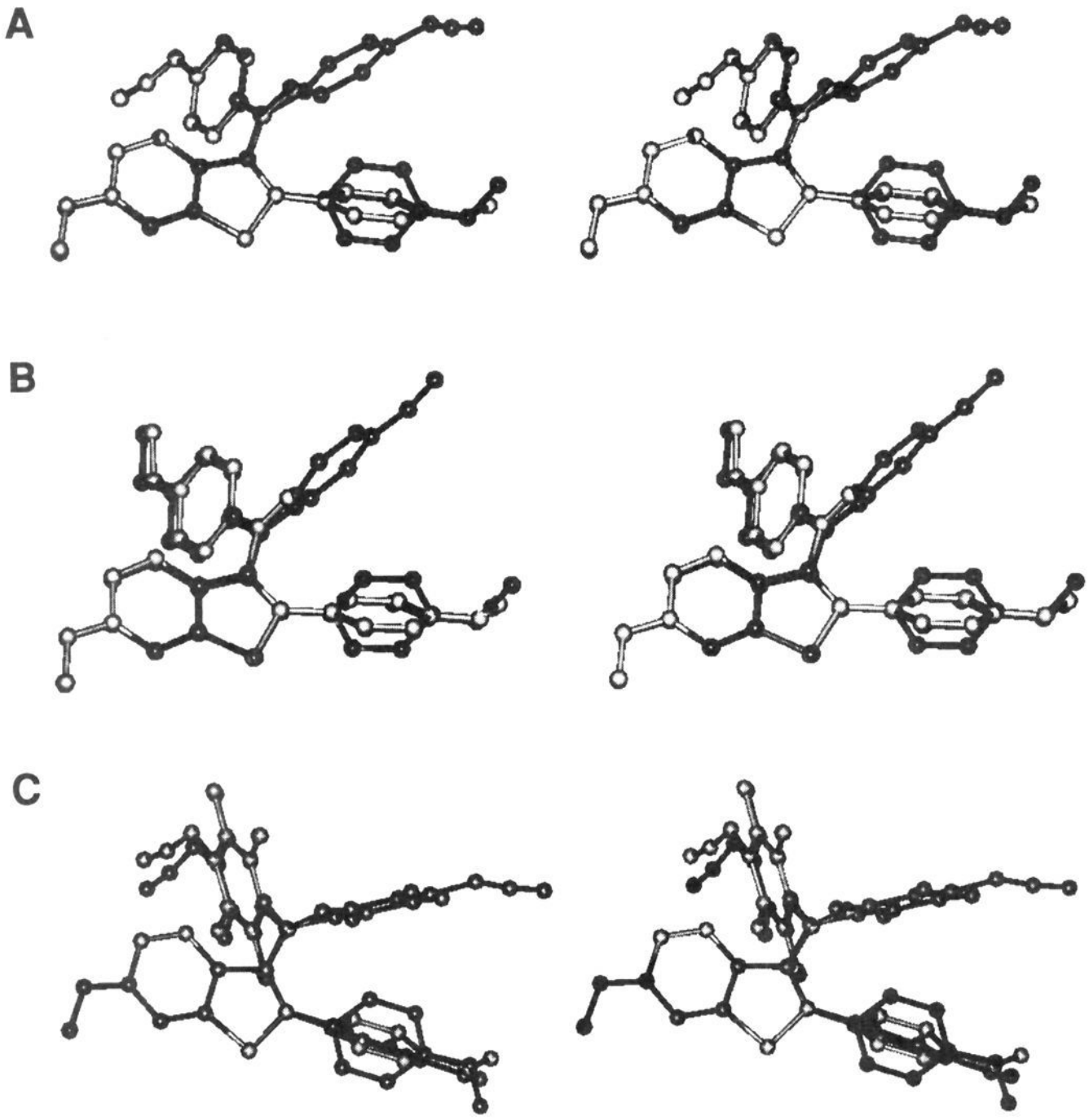


Figure 3. Relaxed stereoviews of the three computed low-energy conformations of protio azide **8** (A), LY117018 analog **11** (B), tetrafluoro azide **7** (C).

ence in this dihedral angle results in an offset stack between the two aromatic rings in the computed structure. The preference for offset stacking of the two interacting aromatic rings in the computer-generated structure may be a consequence of using the AM1 method at the self-consistent field (SCF) level of theory without adding contributions for dispersion interactions.[29] Dispersion forces are important contributors to molecular structure in the solid state but are negligible in solution. Raber has reported that calculations at the SCF level (without consideration of dispersion) may appropriately model the solution-phase structure of a compound.[29]

The degree of stacking observed in conformation 1 of the computer-generated geometry optimized structures of **7**, **8**, and **11** is directly related to the electronic character of the benzoyl ring. The electron-deficient aromatic ring system of the tetrafluoro azide **7** results in a relatively close stacking between the aromatic rings with a centroid distance of 3.9 Å. The protio azide **8** has the aromatic groups disposed in a highly offset stacking arrangement with a centroid distance of 4.1 Å. The electron-rich aromatic rings of the bis-*p*-methoxyphenyl rings of **11** repel each other and force the aromatic groups to move apart to a distance of 4.4 Å.

Orientational Preferences for ER Binding

Estrogen Receptor Binding Affinities and Binding Energies. The estrogen receptor binding affinities (RBA) and binding energies (ΔG) of compounds **3**, **5**, and **6** appear in Table IV. For compounds **3** and **6**, the difference in energy between the three miniumum energy conformations (Table III) is small relative to the binding energy, so the compound may bind to the receptor in any of the three low-energy conformations.[32] Tetrafluoro azide **5** has a lower ΔG than the other two compounds, which suggests poor productive contact with the ER, binding in a relatively strained conformation, or steric interference with the receptor from the more voluminous fluorines.

Orientation of Benzo[*b*]thiophenes Relative to Estradiol: Requirements for ER Binding. The orientation of unsymmetric nonsteroidal ligands in the ER binding site is an area of considerable interest.[17c,33] An accepted pharmacophore for the ER is two hydroxyl groups separated by a hydrophobic spacer.[34] In estradiol, the distance between the two oxygens is 10.9 Å;[35] in diethylstilbestrol (DES) this distance is 12.1 Å.[16] However, in estradiol hemihydrate the *O*3 to O17···OH_2 distance is virtually identical to that of DES.[7d] The O–O distance measured in the crystal structure of LY117018 is 11.7 Å; compounds **7** and **8** are comparable in this regard.

In estradiol, the 3-OH group is more important for ER binding than the 17β-OH,[36] and thus the former is given greater weight in the molecular graphics alignment with a phenol of the 2-arylbenzo[*b*]thiophenes. There are three general orientations of 2-arylbenzo[*b*]thiophenes with respect to estradiol that both optimize skeletal corre-

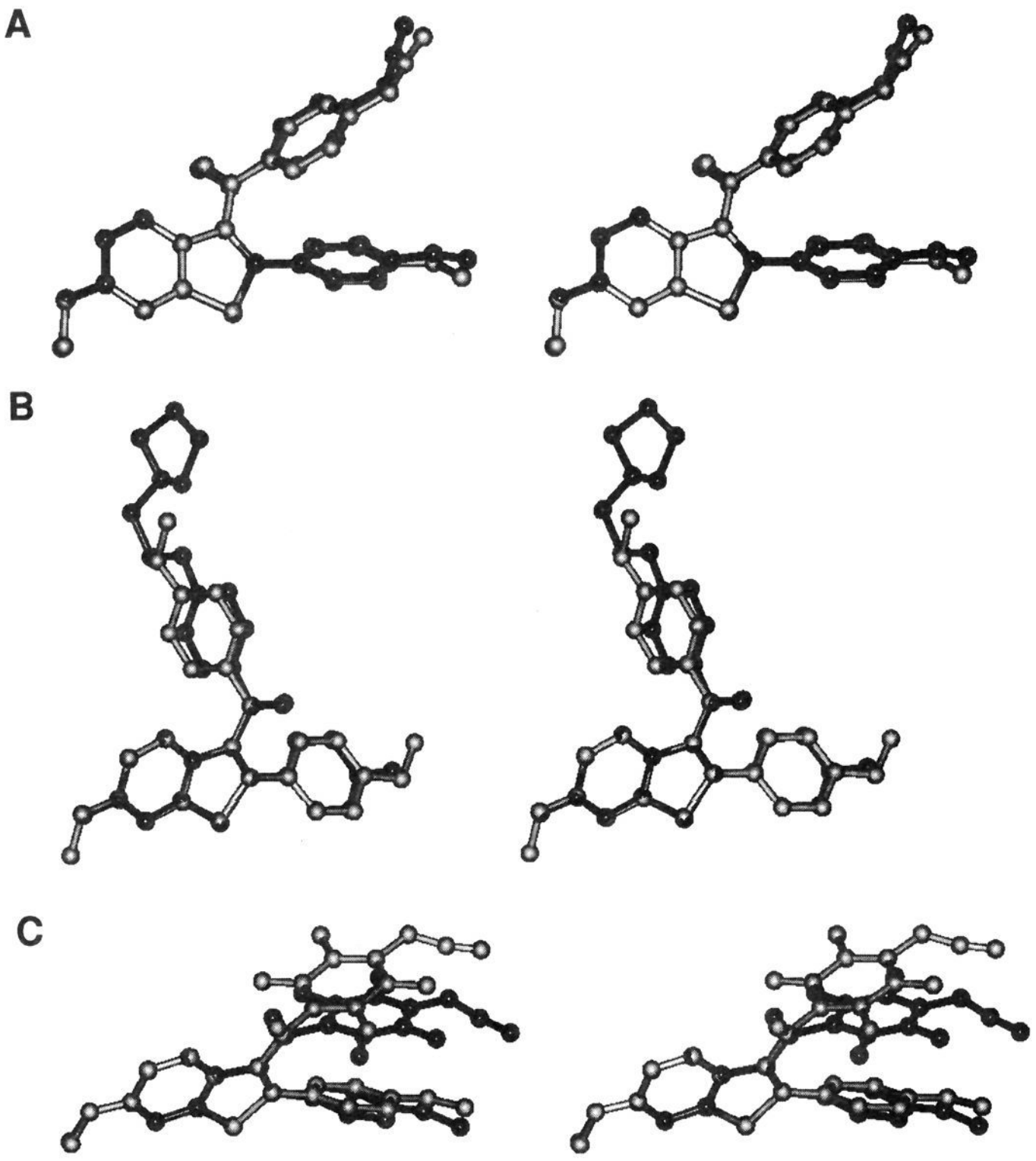


Figure 4. Relaxed stereoview of the overlay of the X-ray crystallographic structures of the protio azide 8, LY117018, and tetrafluoro azide 7 (black) with the best-fit low-energy computational structure (gray).

Table IV. Estrogen Receptor Binding Affinities (RBA) and Receptor Binding Energies

compd	RBA, %[a]	K_{assoc}[b]	binding energy (ΔG), kcal/mol[c]
estradiol	100	3.0×10^9	−11.8
LY117018 (3)	44.7	1.3×10^9	−11.4
tetrafluoro azide 5	5.90	1.7×10^8	−10.3
protio azide 6	35.5	1.0×10^9	−11.3

[a] Relative binding affinity (RBA) determined by competitive radiometric binding assay using lamb uterine cytosol as a receptor source, [^{3}H]estradiol as tracer, and dextran-coated charcoal as adsorbant for free ligand.[11,30] [b] The RBA closely approximates the ratio of association constants (RAC): $K_{assoc,compd}/K_{assoc,estradiol}$. The K_{assoc} is then calculated assuming K_{assoc} (E_2) = 3×10^9 M.[31] [c] Binding energy = $\Delta G = -RT \ln K_{assoc}$.

spondence and overlap of the critical 3-hydroxyl group of estradiol with a phenol of the 2-arylbenzo[*b*]thiophene (Figure 5). In order to judge in a quantitative manner the estrogen receptor interaction of each compound in each conformation/orientation, the molecular graphics receptor mapping approach was adopted.[37] In this technique, the pharmacophores of active analogs of the parent ligand are superimposed, and a composite molecular volume is created. This union of all active analogs and the parent ligand is the receptor-excluded volume (RExV), that region of the receptor binding site available to the ligand and its analogs, and thus not occupied by the receptor. The 11β-ethyl,[38] 17α-ethynyl,[38] 7α-pentenyl,[39] 14α,15α-cyclopropyl,[41] and the 16α-bromo[42] analogs (**16**, **17**, **18**, **19**, and **20**, respectively) of estradiol are known to have higher affinity than estradiol itself. Thus, these six compounds were overlaid through a rigid fit of eight atoms spanning the steroid backbone using molecular graphics to form the RExV for the ER (Figures 6 and 7). Although there are high-affinity nonsteroidal ligands for the ER, such as DES[7d,43] and hexestrol,[44] these were not included in the RExV, because of the complexity of conformational preference in these flexible molecules.

To evaluate the fit of the three benzo[*b*]thiophene ER ligands, three criteria were adopted: (1) intersection of the molecular volume of the ligand with the RExV, (2) lack of interference with the receptor essential volume, and (3) correspondence of the hydroxyl groups of the ligand with those of estradiol. To quantify the first criterion, the intersection of molecular volume of each compound in each conformation/orientation with the RExV was calculated. To determine if the ligand binding site would demonstrate preferential shape selectivity for one of the two atropisomers (enantiomeric rotational isomers) of

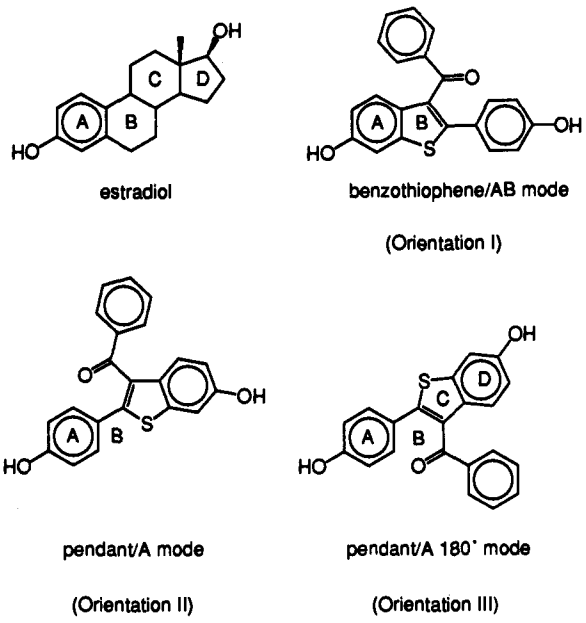


Figure 5. Orientations that benzo[*b*]thiophenes may adopt in the binding site of the estrogen receptor relative to estradiol.

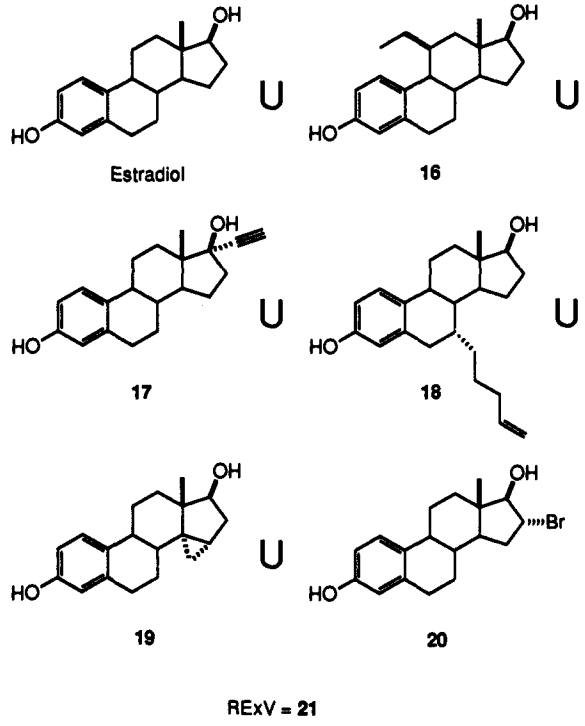


Figure 6. Estradiol and five analogs with RBA > 100% used to construct the receptor excluded volume (RExV). The six compounds were overlaid using a rigid fit of eight atoms in the steroid backbone. The X-ray crystallographic structures of estradiol,[35] 17,[38b] and 18[39] were extracted from the Cambridge Structural Database (CSD).[40] The structure of 16 was obtained from the crystal structure of 11β-ethylestrone,[38a] followed by replacement of the carbonyl group, and minimization with the SYBYL forcefield. The structures of 19 and 20 were obtained by adding the appropriate substituents to the crystal structure of estradiol and subsequent minimization with the SYBYL forcefield.

these compounds, overlaps were calculated for both atropisomers *a* and *b* of 5, 6, and 10. A large volume overlap (intersection) implies a good fit in the ER binding site and a favorable hydrophobic effect (gain in binding energy of 46 cal/Å^2 of surface area).[45] The three benzo[*b*]thiophene ligands 5, 6, and 10 have similar molecular volumes: 289.8, 296.8, and 288.2 Å^3, respectively. (The basic ether side chain of LY117018 was truncated to a methyl ether for the receptor mapping studies because we did not intend to map the area of antiestrogenic influence, which lies further from the steroid nucleus.) For each compound, the conformers shown in Table V differ in molecular volume by no more than 2.7 Å^3.

The second criterion is the location of excess molecular volume beyond the envelope of the RExV. This excess volume may interfere with the receptor essential volume (REsV), that region of space occupied by the receptor itself and not available for ligand binding. Steric interference with the REsV by the ligand represents a "hard" repulsion, which is very energetically unfavorable. Because a formal REsV map for the ER has not yet been assembled, steric interference with the REsV is judged by the location of excess molecular volume in the benzo[*b*]thiophene ligands as compared to substituted estradiols with low RBA. Estrogen derivatives in which substitution of a methyl group for a proton resulted in lowering the affinity for the ER by at least 50% were used to help define areas that may interact with the REsV.[46]

A final criterion used to determine goodness of fit in the ER binding site was the distance between the 17β-OH oxygen of the RExV and the nearby oxygen of the benzo[*b*]thiophene when the two are superimposed. This method of using hydroxyl correspondence to evaluate the fit of ligands with estradiol is commonly reported, with the tacit assumption that the larger this distance is, the poorer the fit in the ER binding cavity. However, the 17β-OH group of estradiol contributes only a meager 0.3 kcal to the binding energy,[36b] and thus is not optimally positioned for hydrogen bonding. Previously, we proposed an *infra*-D-ring hydrogen bonding site in the ER, based on the apparent invariant orientation of hydroxylated 2,3-diarylindenes.[33d] Because the nature of the hydrogen-bonding site in the vicinity of the D ring remains to be delineated, we followed the convention of the literature and calculated the distance between the 17β-OH of estradiol and the second phenol in our 2-arylbenzo[*b*]thiophenes. However, we assign the least weight to this parameter in determining the fit of these ligands in the ER binding cavity.

Results of Overlaying the Low-Energy Conformations of 5, 6, and 10 with the RExV. Analysis of the data in Table V reveals consistent patterns of overlap with the RExV for each of the low-energy conformations of 5, 6, and 10 for both atropisomers. For each compound, there are 18 unique combinations considering the three low-energy conformations in three different orientations for each atropisomer. The systematic evaluation of each unique combination of tetrafluoro azide 5 for goodness of fit with the ER hormone binding domain is described in the flow chart detailed in Figure 8. The results of analogous filtering processes for 6 and 10 are presented in Figure 9.

The first criterion is the intersection of the molecular volume with the RExV, with favorable overlap defined as having an intersection volume >190 Å^3. The atropisomeric pairs of tetrafluoro azide 5 and protio azide 6 have nine combinations that demonstrate favorable overlap with the RExV, while the atropisomeric pair of 10 has six combinations with favorable overlap. For each compound, there are more combinations of favorable overlap for the atropisomers in the b series: six of nine for 5 and 6, and four of six for 10. The overall preferential shape selectivity

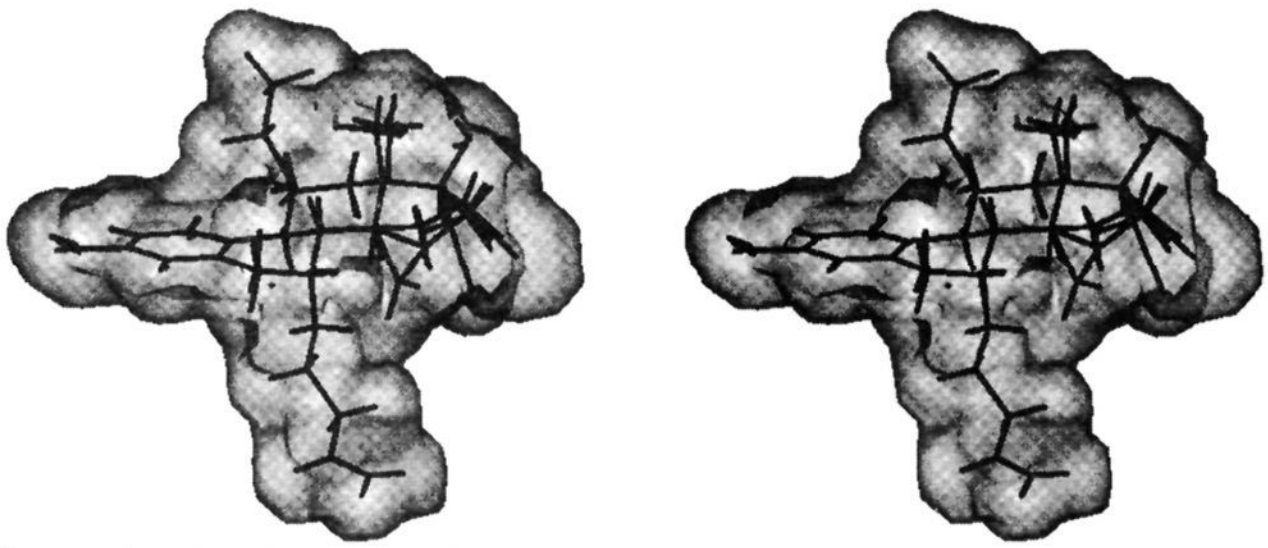


Figure 7. Relaxed stereoview of steric volume of the receptor excluded volume (RExV) for the estrogen receptor.

Table V. Molecular Overlays of Benzo[*b*]thiophene ER Ligands in Specific Conformations and Orientations with the ER-Excluded Volume[a]

orient mode compd/conf	intersection of molecular volumes (Å^3)[b] I	II	III	distance between oxygens (Å)[c] I	II	III
5a/1	*219.1*	183.3	167.5	4.44	3.24	1.69
2	172.2	162.9	179.1	4.28	1.98	3.13
3	177.7	**193.8**	**190.3**	4.28	3.29	1.49
5b/1	*207.6*	*190.1*	180.8	4.33	3.13	1.71
2	202.0	*190.5*	**199.8**	4.08	3.17	1.44
3	206.5	183.1	165.8	4.33	3.12	1.63
6a/1	*215.0*	174.9	161.0	4.54	3.53	1.36
2	173.9	161.3	176.9	4.27	1.94	3.18
3	177.9	**201.5**	**190.7**	4.18	3.42	1.46
6b/1	*191.4*	**194.7**	185.1	4.61	3.19	1.63
2	204.9	*191.9*	**209.5**	4.19	3.29	1.53
3	**197.8**	180.5	164.6	3.91	3.18	1.82
10a/1	*209.6*	176.6	163.4	4.36	3.21	3.27
2	175.9	166.3	176.1	4.27	1.93	3.04
3	179.9	**199.4**	178.4	4.36	3.57	1.35
10b/1	187.7	**196.4**	186.7	4.57	3.19	1.60
2	207.9	185.0	**209.2**	4.43	3.29	1.51
3	198.2	182.0	167.6	4.33	3.18	1.40

[a] A rigid four-atom fit was used. Carbons 1, 3, 5, and 9 of the steroid RExV **21** were aligned with the corresponding atoms of the benzo[*b*]thiophenes oriented as in Figure 5. [b] The common volume shared between the receptor excluded volume (RExV) and the compound in its specified conformation/orientation. Plain text = rejected by criterion I (poor overlap); italics = rejected by criterion II (steric interference with REsV); underline = rejected by criterion III (poor OH correspondence); bold = most favorable fit combinations. [c] Distance between 17β-O of the RExV and the proximal hydroxyl oxygen of the benzo[*b*]thiophene in the indicated conformation/orientation.

shown toward the **b** series of atropisomers is illustrated by examining the average molecular intersection for all possible conformation/orientation combinations with both atropisomers for each compound: **5a** (182.9 Å^3), **5b** (191.8 Å^3); **6a** (181.5 Å^3), **6b** (191.2 Å^3); **10a** (180.6 Å^3), **10b** (191.2 Å^3).

The remaining conformation/orientation combinations were further evaluated based on the location of the electron density that falls outside of the RExV. For both sets of atropisomers of each compound, conformation 1/orientation I (benzo[*b*]thiophene unit mimics the steroid AB system) positions the excessive electron density along the periphery of the steroid backbone in the area of the C and D rings. Estradiol derivatives with substituents in this area (C15 α and β, C16 β, C18) have been shown to have low affinity for the ER.[46] In the atropisomer **b** series, conformation 2/orientation II directs the benzoyl group to the periphery of the steroid backbone in the area of the C ring 11α-position. Substitution of estrogens at this

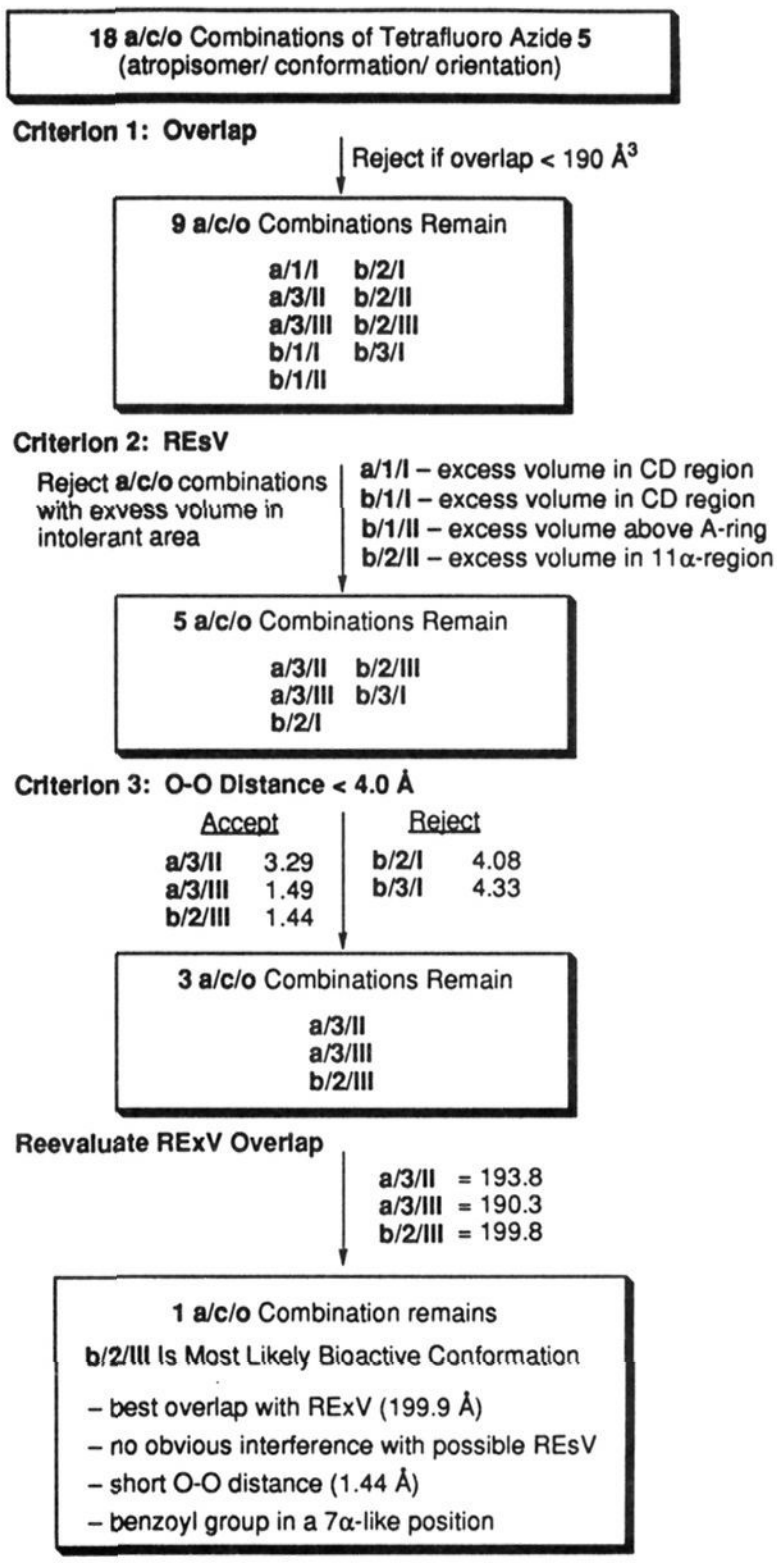


Figure 8. Flow chart describing evaluation of 18 atropisomer/conformation/orientation combinations for tetrafluoro azide **5**.

position has also been shown to reduce binding affinity for the ER.[47] Finally, in the atopisomer **b** series, conformation 1/orientation II is not predicted as a favorable alignment for tetrafluoro azide **5**, because the pendant benzoyl group is oriented directly above the A ring, a region shown to be unfavorable to substitution.[48] This alignment is especially poor for tetrafluoro azide **5**, because of the

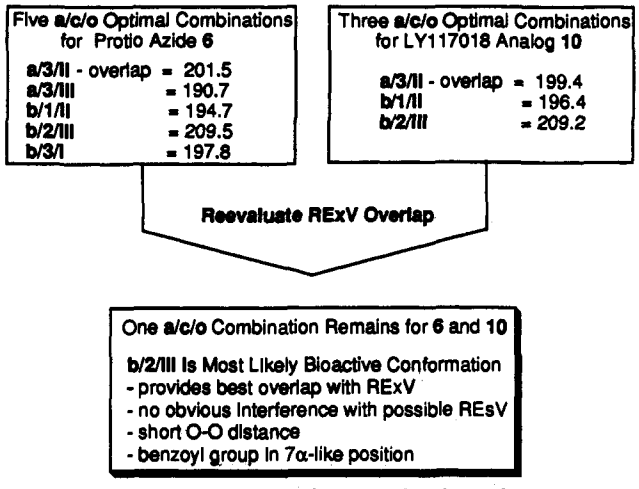


Figure 9. Flow chart describing evaluation of the optimal alignments for protio azide **6** and LY117018 analog **10**.

closer cofacial stacking between the aromatic rings. The alignments that were rejected based on excessive volume in intolerant areas are illustrated for tetrafluoro azide **5** (Figure 10).

Applying these constraints to the structures that demonstrate favorable overlap with the REsV reduces the number of optimum conformation/orientation combinations to five for tetrafluoro azide **5**, six for protio azide **6** and five for LY **10**. The fit combinations that provide the best overlap, combined with the least interference with the REsV, all position the benzoyl side chain in either the 11β- or the 7α- position. The fit combinations that position the benzoyl group in the 11β-position are conformation 3/orientation II in the **a** atropisomer series, and conformation 2/orientation I, conformation 3/orientation I, and conformation 1/orientation II within the **b** atropisomer series. The fit combinations that position the benzoyl group in the 7α-position are conformation 3/orientation III for the **a** atropisomers and conformation 2/orientation III for the **b** atropisomers.

The number of optimum conformation/orientations for each compound can be further reduced based on the distance between the second OH group of the benzo[*b*]-thiophene derivative and the 17β-OH of the REsV. The two fit combinations that direct the benzoyl group toward the 7α-postion (orientation III) place the second OH group within 1.5 Å of the 17β-OH group in the REsV. Fitting the benzo[*b*]thiophene derivatives in orientation II (benzoyl group positioned in the 11β-pocket) places the 17β-OH group within 3.19–3.57 Å of the 17β-OH group in the REsV. Fitting the benzo[*b*]thiophene derivatives in orientation I (benzoyl group positioned in the 11β-pocket) places the second OH group within 3.91–4.43 Å of the 17β-OH group in the REsV. Orientation III results in the most direct correspondence of the two OH groups. In orientation II, the two OH groups are more distant, but may still be able to bind to the same site in the ER. In orientation I, the second OH group is most likely too distant from the 17β-OH of estradiol to bind to the same site in the ER.

Assuming that the alignment of the two hydroxyl groups is important, it is possible to further reduce the number of viable candidates for a putative bioactive conformation/orientation for each compound. Rejecting alignments that position the two hydroxyl groups further than 4.0 Å apart reduces the number of putative bioactive conformation/orientation combinations to three for tetrafluoro azide **5**, five for protio azide **6**, and three for LY117018 analog **10** (Figures 8 and 9). The three optimal alignments for tetrafluoro azide **5** are illustrated in Figure 11.

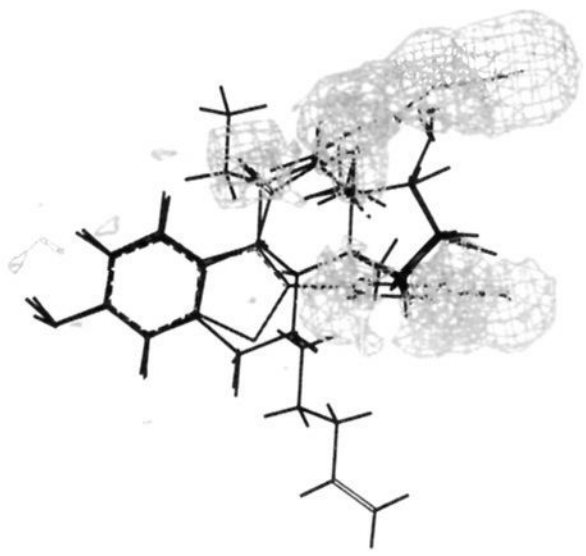
Any of the optimal alignments found for tetrafluoro azide **5**, protio azide **6**, and LY117018 analog **10** could be viable candidates for the bioactive conformation/orientation combinations for these compounds. However, reevaluation of the overlap with the REsV for the optimal alignments reveals one combination that has greater molecular intersection for all three compounds. Fitting conformation 2 of atropisomer **b** in orientation III positions the benzoyl group in the 7α-pocket and results in the highest degree of overlap with absence of interference with the REsV, and closest approximation of the hydroxy groups for all three compounds. This combination of conformation and orientation is therefore postulated as the most likely bioactive alignment for optimal interaction with the ER hormone binding domain.

Receptor mapping studies have led us to propose the same bioactive alignment for each of the benzo[*b*]-thiophene derivatives **3**, **5**, and **6**. The binding affinity of tetrafluoroazide **5**, however, is much lower than **3** or **6** (Table IV). The difference in affinity may be attributed to the two structural differences caused by fluorine substitution of the aroyl ring: increase in volume of the perfluorinated aromatic ring and the increase in torsion angle 2–3–4–5 which defines the out-of-plane twist of the aromatic ring relative to the carbonyl group. These structural differences may result in steric interference with the receptor or binding in a relatively strained conformation to the receptor which is evidenced by the lower binding affinity.

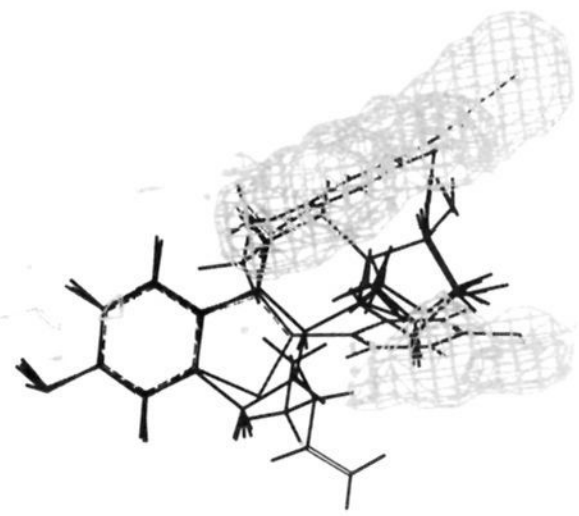
Conclusion

The molecular structures of the antiestrogen LY117018 and two aryl azide photoaffinity labeling analogs **7** and **8** were investigated through X-ray crystallographic and computational methods. From the modeling studies, we found that these benzo[*b*]thiophenes can exist in three low-energy conformations. For each compound, one of the three minimum-energy conformations is quite similar to the conformation determined by X-ray crystallography, in accord with the views expressed by Duax[7d] and Kuntz.[49]

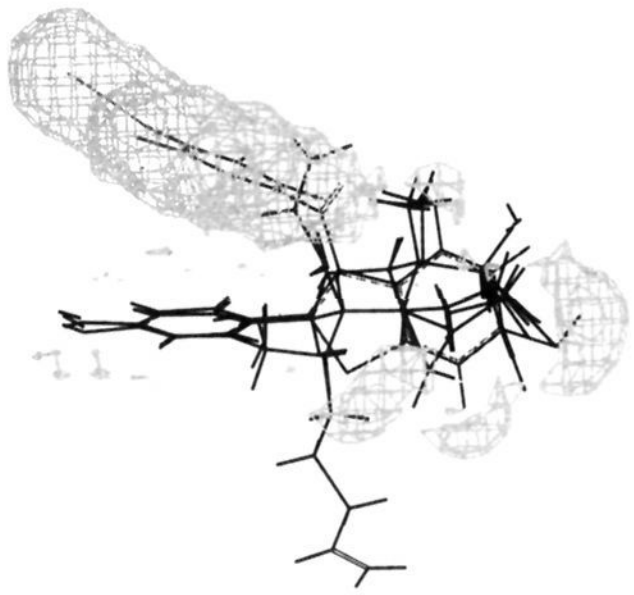
Systematic alignment of the three low-energy conformations in three different orientations with the REsV for both atropisomers of **5**, **6**, and **10** (truncated derivative of **3**) revealed several interesting features about how these benzo[*b*]thiophene derivatives may interact with the ER. First, more efficient overlap was consistently observed for the atropisomer **b** series with the REsV than for the atropisomer **a** series with the REsV. This suggests that the chiral ER hormone binding domain may demonstrate shape selectivity in binding these ligands, based on the direction of rotation of their pendant groups. The significance of atropisomers in determining the biological characteristics of triarylethylene ER ligands has been described elsewhere.[50] Second, the benzoyl group is oriented in positions analogous to the 11β- or 7α-sites of substituted estrogens for optimal alignment with the REsV and minimal contact with the REsV. Finally, we were able to reduce the total number of possible bioactive alignments from 18 for each compound to 3 for tetrafluoro azide **5** and LY117018 analog **10**, and 5 for protio azide **6**



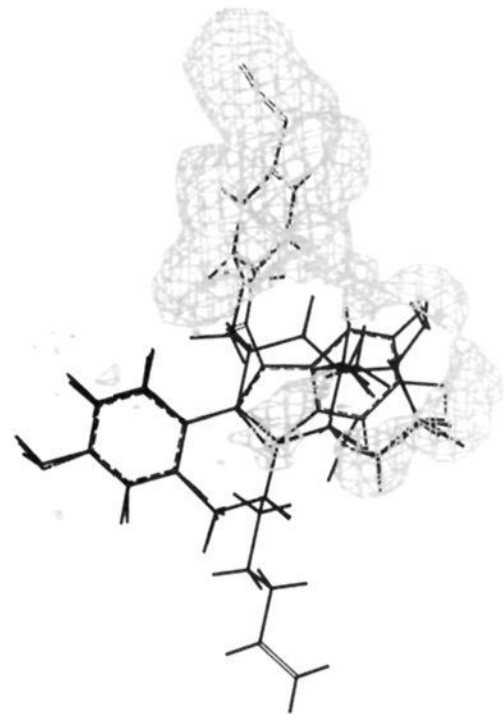
Atropisomer *a*
Conformation 1/Orientation I
Excessive volume in C-D region



Atropisomer *b*
Conformation 1/Orientation I
Excessive volume in C-D region



Atropisomer *b*
Conformation 1/Orientation II
Excessive volume over A-ring



Atropisomer *b*
Conformation 2/Orientation II
Excessive volume in 11α-region

Figure 10. Steric volume that lies outside of the RExV for alignments that position the pendant benzoyl group in positions of steric intolerance for tetrafluoro azide **5**.

based on overlap with the RExV, minimal contact with the REsV, and correspondence of the hydroxyl groups.

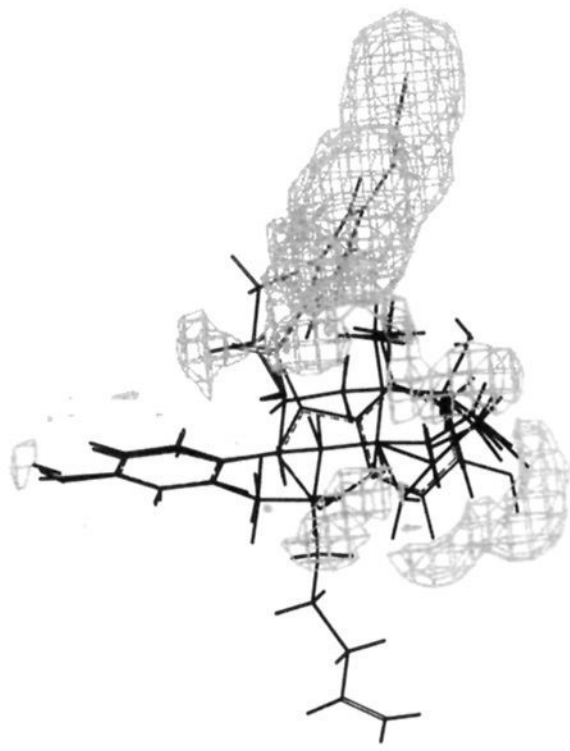
Experimental Section

X-ray Crystallography. Protio azide **8** was prepared as previously described.[12] A sample of LY117018 was generously provided by C. D. Jones of Lilly Research Laboratories. Crystals of LY117018 acetone solvate (**3**) were grown by slow evaporation of an acetone solution at room temperature. Crystals of protio azide **8** were obtained by recrystallization from ether at 0 °C. Diffraction data were measured at room temperature with a Syntex $P2_1$ or an Enraf-Nonius CAD4 diffractometer, using monochromated Mo radiation [λ ($KO(_{\alpha}^{-})$) = 0.710 73 Å]. Final cell dimensions were obtained by a least-squares fit to the automatically centered settings for at least 15 reflections. Three reference reflections were monitored during the experiment; for **3**, no significant variation was observed. For **8**, no problems were encountered in collecting the first two shells of data. After the second shell ($2\theta < 39°$, about 2 days of exposure), standard intensities decreased rapidly to below 50% of their initial values; further measurement was impossible.

Crystal data are listed in Table II. Intensity data were corrected for Lorentz and polarization effects. For **3**, the space group was unambiguously determined under systematic conditions. For **8**, the average values of the normalized structure factors suggested a centric space group; this was confirmed by successful refinement.

The structures were solved by direct methods (SHELXS-86[51]); correct positions for all non-hydrogen atoms were deduced from an E-map. For **3**, a difference Fourier synthesis following several least-squares refinement cycles revealed positions for *H*O1 and *H*O2 atoms. The remaining hydrogen atoms in **3** and those in **8** were included as fixed contributors in idealized positions. In the final least-squares refinement cycle, anisotropic thermal coefficients were refined for sulfur, oxygen, and nitrogen atoms, isotropic thermal coefficients were refined for all carbon atoms (and both hydroxyl hydrogen atoms in **3**), and a common isotropic thermal parameter was varied for the remaining hydrogen atoms. Successful convergence was indicated by maximum shift/error for the last cycle. A final analysis of variance between the observed and calculated structure factors showed a slight dependence on sine (θ).

Atomic scattering factors, mass attenuation coefficients, and anomalous dispersion corrections were taken from ref 52. Protio azide **8** decomposed during the diffraction experiment. Thus, data collection on **8** was terminated prematurely which partly accounts for the rather large agreement factors obtained. *R*-factors in this range suggested a correct overall molecular conformation, but some data may be inaccurate.[53] The estimated standard deviations (esd) of bond lengths are regarded as a better indicator of the accuracy of the determination.[54] For **8**, the esd

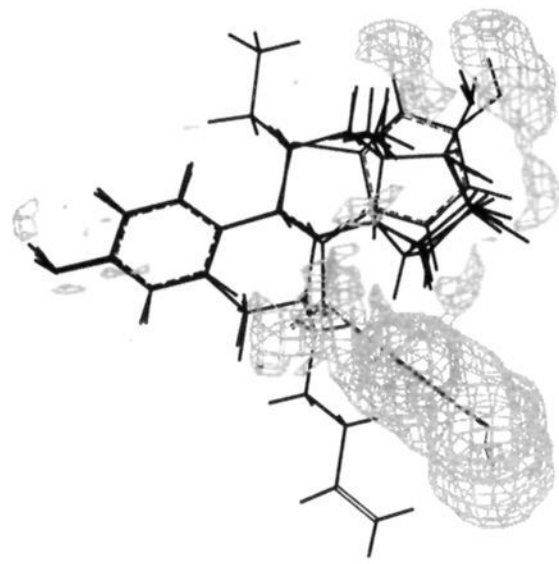


Atropisomer *a*
Conformation 3/Orientation II

Excessive volume in 11β-pocket

Atropisomer *a*
Conformation 3/Orientation III

Excessive volume in 7α-pocket



Atropisomer *b*
Conformation 2/Orientation III

Excessive volume in 7α-pocket

Figure 11. Steric volume that lies outside of the RExV for optimal alignments of tetrafluoro azide **5**.

values were in the range of 0.02–0.03 Å. The C–C and C–O bond lengths and bond angles of the *p*-methoxyphenyl group in **8** were consistent with typical literature values.[55] With these considerations in mind, the structure determination of **8** was deemed satisfactory for this study.

Molecular Modeling. Molecular modeling calculations were performed on a Silicon Graphics Indigo Elan computer. Analogs of compounds **3**, **7**, and **8** (Figure 2) were computer built using Macromodel[56] v. 3.5 and energy minimized to a gradient of <0.05 Å^2 with the MM2[57] force field. The flexible basic ether side chain of **3** was replaced by a methyl ether group in its analog **11**. Because the parameters for the azide group were not available in the MM2 force field, hydrogen was substituted for the azide in the analogs of **7** and **8** (**12** and **13**, respectively). Conformational analysis of each compound was performed by using a multitorsion grid search, rotating the dihedral angles defined by atoms 7–6–1–2, 1–2–3–4, and 2–3-4–5 in 60° increments with MULTIC. Each newly generated conformation was subjected to 500 steps of conjugate gradient minimization. The MULTIC conformational grid search resulted in three low-energy pairs of enantiomeric atropisomers for compounds **11**, **12**, and **13**.

The low-energy conformations were read into the SYBYL Molecular Modeling Package (Version 6.0, Tripos Associates, St. Louis, MO), and the azide functionality was added to the analogs of **7** and **8**. Full geometry optimization of the low-energy conformations of **7**, **8**, and **11** was performed using Dewar's AM1[58] Hamiltonian in AMPAC[59] 3.0 interfaced with the Insight II Molecular Modeling Package (Version 2.1.0, Biosym Technologies, San Diego, CA). The Precise option was used to tighten convergence tolerances. The geometry-optimized low-energy conformations of **7**, **8**, and **11** were read into SYBYL 6.0 for comparison with the crystal structures. The low-energy conformations of each compound were superimposed through a rigid least-squares fit of atoms 1, 2, 10, 12, and 14 in the benzo[*b*]-thiophene system.

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Urbana—Champaign. We thank Vickie Watson for preparation of the manuscript.

Supplementary Material Available: Atomic numbering schemes and tables of atomic coordinates and thermal parameters for compounds **3**, **8a**, and **8b**, torsion angles previously observed for the basic ether side chain of antiestrogens, and crystal packing of tetrafluoro azide **7** present in the unit cell (11 pages). Ordering information is given on any current masthead page.

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