Benzothiophene Selective Estrogen Receptor Modulators with Modulated Oxidative Activity and Receptor Affinity

Zhihui Qin, Irida Kastrati, R. Esala P. Chandrasena, Hong Liu, Ping Yao, Pavel A. Petukhov, Judy L. Bolton, and Gregory R. J. Thatcher*

Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, 833 South Wood Street, Chicago, Illinois 60612-7231

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The regulation of estrogenic and antiestrogenic effects of selective estrogen receptor modulators (SERMs) is thought to underlie their clinical use. Most SERMs are polyaromatic phenols susceptible to oxidative metabolism to quinoids, which are proposed to be genotoxic. Conversely, the redox reactivity of SERMs may contribute to antioxidant and chemopreventive mechanisms, providing a new approach to improve the therapeutic properties of SERMs. An improved synthetic strategy was developed to generate a family of benzothiophene SERMs. Using computational modeling methods and measurements of antioxidant activity and estrogen receptor (ER) ligand binding, this SERM family was shown to provide both a range of ERα/ERβ selectivity from 1.2- to 67-fold and a range of redox activity. Antioxidant activity was successfully modulated by varying a substituent remote from the OH group; the source of the antioxidant capacity. An efficient synthetic procedure is reported yielding benzothiophene SERMs wherein redox activity and ER affinity are modulated.

Introduction

Tamoxifen is the archetypal selective estrogen receptor modulator (SERM[a]). Despite the demonstrated, increased risk of endometrial cancer, tamoxifen has been the therapy of choice in the endocrine treatment of all stages of hormone-dependent breast cancer and in the primary and secondary chemoprevention of breast cancer.[1] Although the introduction of aromatase inhibitors may change this clinical paradigm, SERMs are likely to be in clinical use for many years.[2] The increased use of SERMs is anticipated on the basis of favorable clinical trial results for the benzothiophene SERMs, raloxifene and arzoxifene, and because SERMs are hoped to provide an alternative to current hormone replacement therapy (HRT) that has been causally linked to breast cancer.[3] Raloxifene is in current clinical use in post-menopausal osteoporosis and is expected to find use in other postmenopausal indications associated with HRT.[4,5] The STAR trial (study of tamoxifen and raloxifene) reported that raloxifene was as effective as tamoxifen in breast cancer chemoprevention in postmenopausal women at high risk and was less likely to cause the potentially dangerous side effects associated with tamoxifen, such as uterine cancer and blood clots. The RUTH trial (raloxifene use for the heart) did not show a significantly increased risk of coronary artery disease, although there is still debate on the potential beneficial or negative effects of raloxifene on other cardiovascular events.[5−7] Arzoxifene, designed to improve upon the therapeutic properties of raloxifene, is in late stage clinical trials with the promise of substantial therapeutic benefits and is likely to find use in cancer chemoprevention.[8,9]

The carcinogenic effects of tamoxifen have been attributed variously to regulation of gene transcription (i.e., hormonal carcinogenesis) and to genotoxicity due to oxidative metabolites (i.e., chemical carcinogenesis). Chemical carcinogenesis can contribute to cancer initiation through damage to DNA and other biomolecules following drug bioactivation to redox-active and electrophilic quinoid metabolites (*o*-quinones, quinone methides, and di-quinone methides).[10,11] Human estrogens and equine estrogens contained in current HRT agents are also proposed to elicit hormonal and chemical carcinogenesis pathways, the latter via *o*-quinone metabolites.[12,13] Interestingly, many SERMs in clinical use and clinical development are also highly susceptible to oxidative metabolism to electrophilic and redox-active quinoids simply because they are based on polyaromatic phenol scaffolds.[14] The SERMs, raloxifene, desmethylarzoxifene (DMA), acolbifene, toremifene, and droloxifene are all oxidatively metabolized to quinoids, which have been shown to form adducts with biomolecules, including glutathione (GSH), proteins, and nucleosides (Scheme 1).[14−20]

Whereas generation of reactive oxygen species (ROS) and covalent modification of biomolecules by redox-active quinoids may contribute to initiation and promotion of carcinogenesis, induction of oxidative stress and oxidation or covalent modification of sensor proteins may trigger cellular stress responses that are cytoprotective.[21,22] This balance between the carcinogenic and the chemopreventive capacity of a drug is determined by the reactivity toward oxidative bioactivation and the chemistry of the reactive metabolite formed and, therefore, can be controlled by structural modification. The contribution of oxidative bioactivation to therapeutic activity versus toxicity is of particular relevance to SERMs, which are designed for chronic use in healthy women who are peri- or postmenopausal or who have known risk factors. The continued development of SERMs based on polyaromatic phenolic scaffolds requires increased understanding of the influence of oxidative bioactivation.

Intensive research is currently directed at discovery of the "ideal SERM": an agent that is antiestrogenic in breast and endometrial tissue, but proestrogenic in the vasculature and brain, which would be of use in cancer chemoprevention and an attractive alternative to HRT. However, there has been little

* To whom correspondence should be addressed. Tel.: 312-355-5282. Fax: 312 996 7107. E-mail: thatcher@uic.edu.

[a] Abbreviations: BDE, bond dissociation energy; DMA, desmethylarzoxifene; X-DMA, 4′-X-4′-desmethoxyarzoxifene; DPPH, diphenylpicrylhydrazyl radical; ER, estrogen receptor; HRT, hormone replacement therapy; LBD, ligand binding domain; SERM, selective estrogen receptor modulator.

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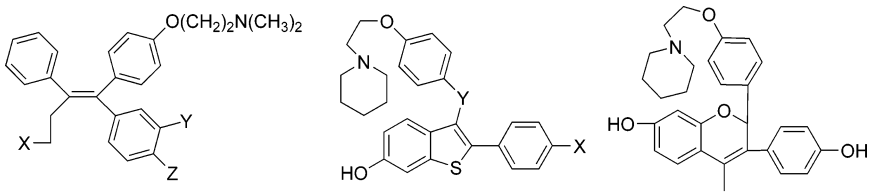
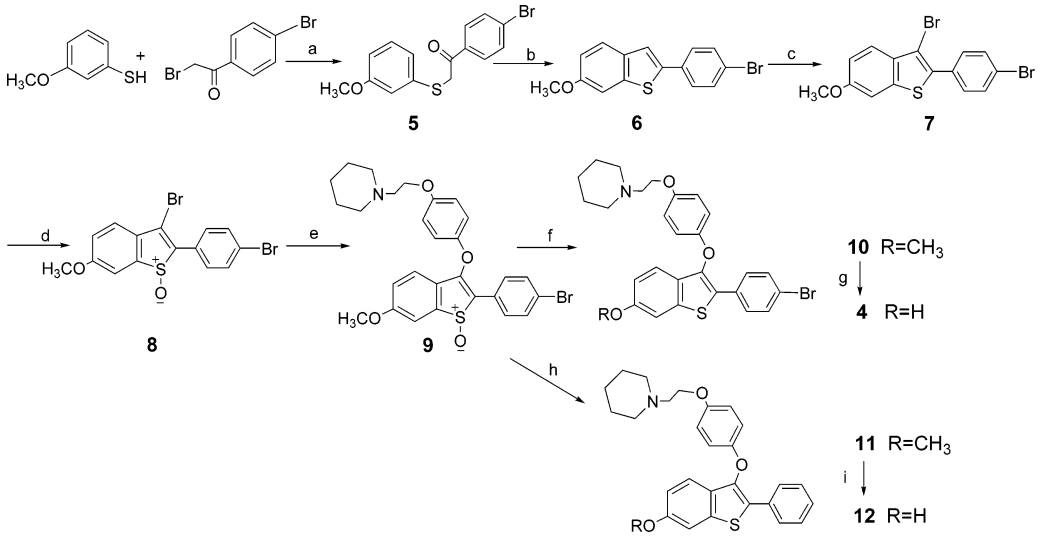
Scheme 1

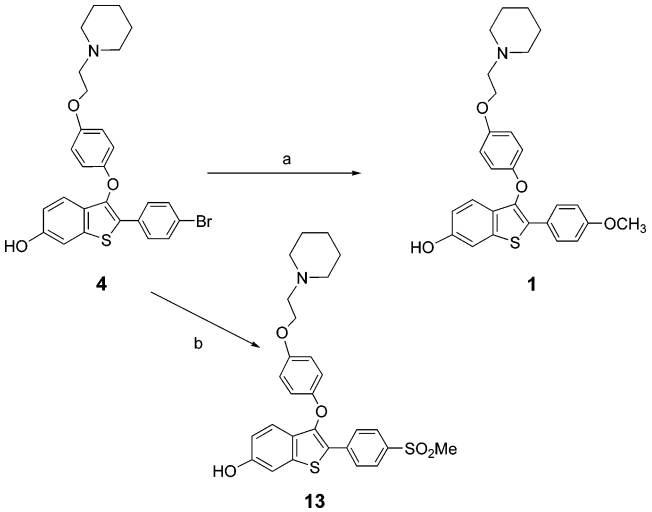


Scheme 2 [a]



[a] Reagents and conditions: (a) KOH, EtOH, EtOAc; (b) PPA, 130 °C; (c) $CH_3C(O)NHBr$; (d) H_2O_2, TFA, DCM; (e) NaH, $C_5H_{10}N(CH_2)_2OC_6H_4OH$, DMF; (f) (i) HCl, ether; (ii) TMSCl, Ph_3P, THF, reflux; (g) (i) HCl, ether; (ii) BF_3SMe_2; (h) $LiAlH_4$, THF, reflux; (i) (i) HCl, ether; (ii) BF_3SMe_2.

Scheme 3 [a]



[a] Reagents and conditions: (a) NaOMe, cat. AcOEt, CuI, DMF, MeOH, reflux; (b) L-proline, NaOH, CH_3SO_2Na, CuI, DMSO.

attention to structural modifications designed to control oxidative bioactivation and thereby enhance therapeutic activity and attenuate toxicity.[23] This approach requires a family of SERMs in which structure is used to modulate both redox reactivity and activity at the estrogen receptor. To that end, a family of benzothiophene SERMs related to arzoxifene has been synthesized, requiring development of a new synthetic methodology for arzoxifene itself.

Results and Discussion

Arzoxifene (**1**; Scheme 1) is a structural analogue of raloxifene in which the carbonyl hinge has been replaced by an ether linkage and the 4′-hydroxy group is methylated. Arzoxifene is in late stage clinical trials as a next generation SERM with promise of substantial therapeutic benefits[24] that are suggested to result from (a) increased antiestrogenic potency and (b) improved bioavailability relative to raloxifene.[25] DMA (**2**; Scheme 1) is an active metabolite of arzoxifene, which has been observed with highly variable steady-state plasma concentrations.[8] In vitro metabolic studies showed that both DMA and raloxifene undergo bioactivation to electrophilic diquinone methides (Figure 1), resulting in potentially cytotoxic actions: depletion of cellular GSH, irreversible inhibition of P450 3A4, and liver protein modification.[15,16,19,20] The desired chemopreventive actions of SERMs will be compromised by the formation of covalent adducts between electrophilic quinoid metabolites and cellular proteins or DNA if these adducts cause genotoxicity or organ toxicity. In an effort to obtain safer benzothiophene SERMs that retain efficacy and have attenuated reactivity toward bioactivation, the arzoxifene analogue, 4′-fluoro-4′-desmethoxyarzoxifene, has been developed (F-DMA, **3**, Scheme 1). F-DMA showed similar antiestrogenic activity to both DMA and raloxifene, and 4′-fluorination was shown to successfully block the formation of an electrophilic diquinone methide (Figure 1) and to suppress phase II metabolism. These properties are predictive of improved bioavailability compared to DMA and raloxifene.[15,16]

Drug Design Rationale. Appropriate structural modifications designed to minimize drug bioactivation are sometimes incor-

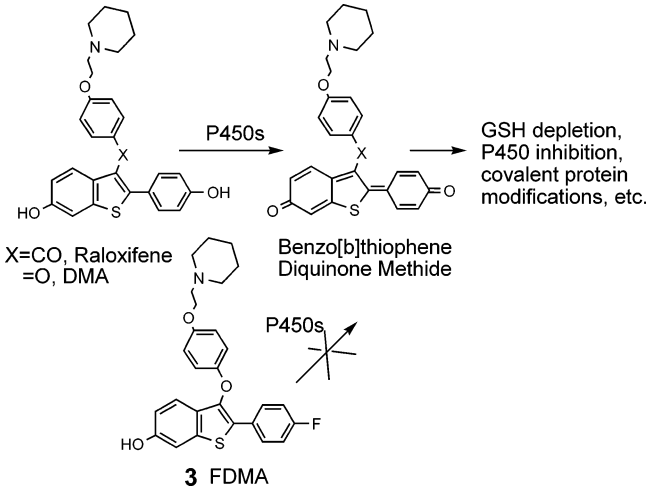


Figure 1. Bioactivation of raloxifene and DMA is blocked for F-DMA.

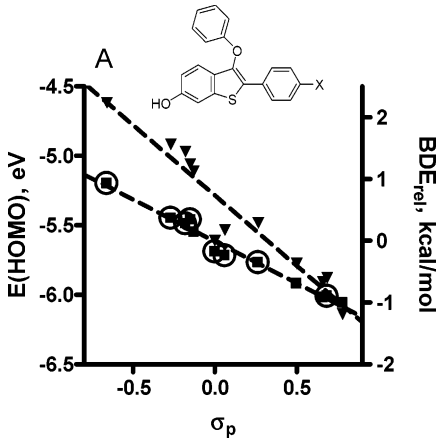


Figure 2. Plots of E_{HOMO} (squares) and relative BDE (triangles) vs Hammett parameter for benzothiophene derivatives from AM1//B3LYP/6-31+G* calculations: open circles represent 4-substituted compounds selected for synthesis.

porated in the lead optimization stage of drug discovery,[23] but most SERMs retain the polyaromatic phenolic core that is susceptible to oxidative bioactiviation. Although minimizing drug bioactivation is often thought to reduce the risks associated with toxic metabolites, bioactivation to a benign redox-active metabolite that induces oxidative stress and oxidation or covalent modification of sensor proteins may trigger cytoprotective cellular responses that contribute to chemoprevention. Keap1 is a notable example of a sensor protein that responds to redox-active compounds to mediate induction of phase II enzymes.[21,22] An additional potential benefit is antioxidant activity, which has been proposed to contribute to SERM biological activity.[26,27] The potential negative outcomes of oxidative bioactivation include modification of liver proteins, drug−drug interactions, and liver dysfunction. Further complicating this picture is the proposal that quinoid metabolites of estrogenic compounds are ligand-independent ER modulators.[28] To explore the influence of bioactivation on SERM activity and toxicity and hence improve SERM design, a homologous family of SERMs was required with modulated ER binding and redox reactivity. A benzothiophene core was selected because of the importance of raloxifene and arzoxifene and on the basis of the promising preliminary studies with F-DMA. Naturally, some, but not all, of the benzothiophene SERMs selected for synthesis have been described in patents but, with the exception of arzoxifene itself, there is little or no data published on biological activity and reactivity.

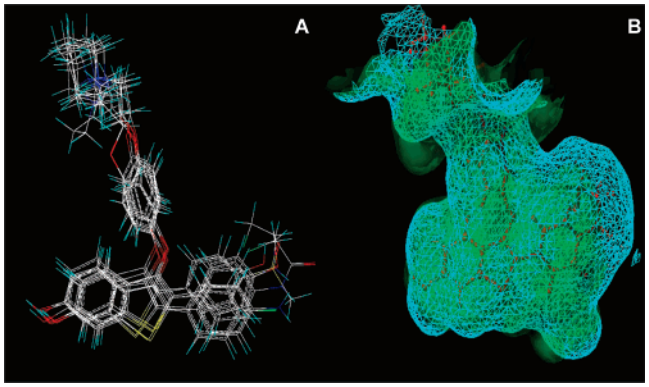


Figure 3. (A) Docking poses for the synthetic analogs in ERα/LBD. (B) Superposition of the rigid (green) vs relaxed (cyan) binding cavity of ERα/LBD with docked 4′-XDMA (X = NH(CH$_2$)$_2$Cl).

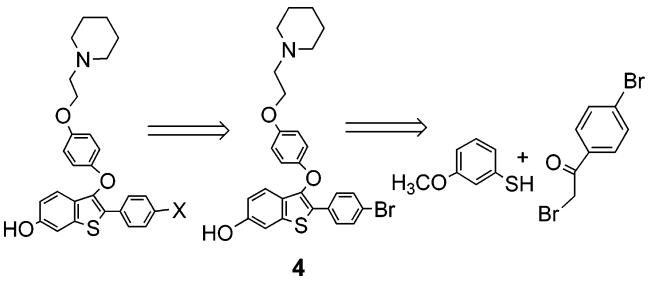
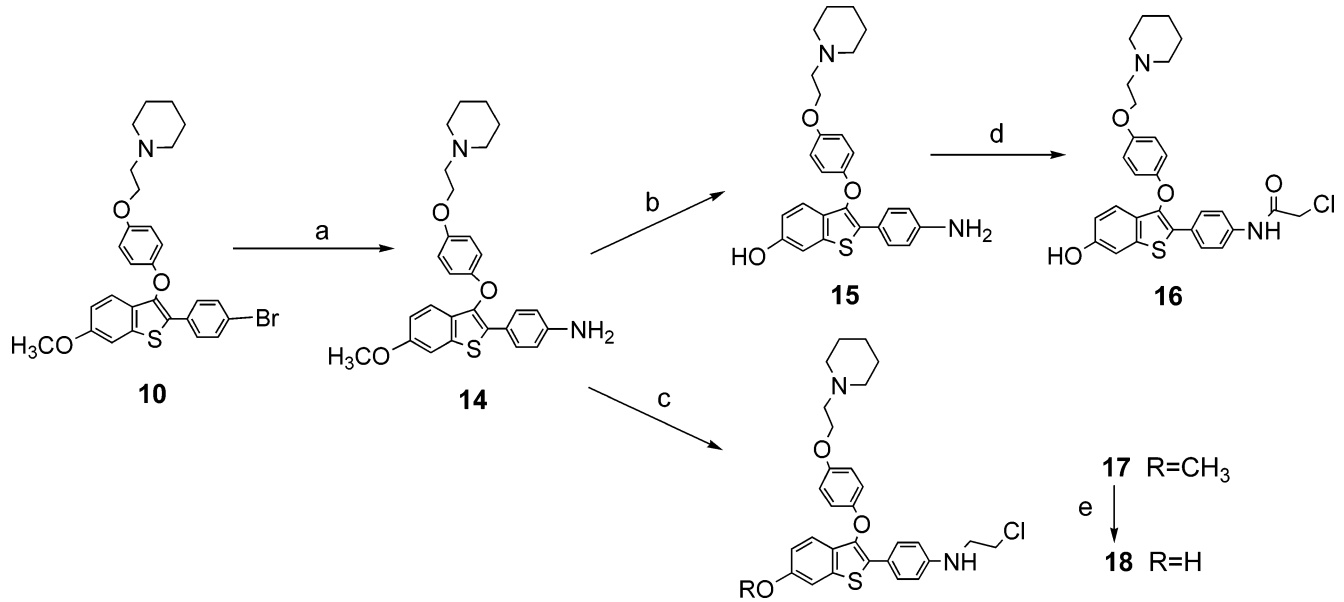


Figure 4. Retro synthetic analysis of 4′-substituted arzoxifene analogues.

I. Redox Activity. Antioxidant capacity can be quantified by the ArO−H bond dissociation energy (BDE), and the relative oxidative capacity for phase II enzyme induction has been correlated with E_{HOMO}.[29] Therefore, calculations were performed to predict antioxidant capacity at the AM1//B3LYP/6-31+G* level on a series of 4′-substituted benzothiophenes (Figure 2; X = NH$_2$, OMe, OH, NHC(O)CH$_3$, Me, H, F, Br, CHO, CN, SO$_2$Me, NO$_2$). Linear correlations were observed between standard Hammett parameters and both E_{HOMO} and BDE (r^2 = 0.98 and 0.92, respectively; Figure 2). On this basis, to provide a range of redox reactivity, the 4′-substituted-4′-desmethoxyarzoxifenes (X-DMA series) selected for synthesis were X = NH$_2$, OMe, OH, H, F, Br, SO$_2$Me, and NHR (R = alkyl, acyl). This family contains, arzoxifene, DMA, and F-DMA, in addition to interesting compounds such as a methylsulfonyl derivative that in simile with the non-CNS penetrating SERM, LY2066948,[30] are predicted to have reduced brain bioavailability and hence diminished ovarian stimulation via actions in the hypothalamus.[31]

II. ER Binding. To assist in drug design, the coordinates for the ERα and ERβ ligand binding domains (LBD) were extracted from the crystal data of the raloxifene−ERα/LBD complex (PDB code: 1ERR) and tamoxifen−ERβ complex (PDB code: 2FSZ), respectively. The ERα/LBD structure indicated that the 4′-OH is involved in a hydrogen-bonding network that includes His-524, with which it forms a hydrogen bond.[32] The simplistic prediction would be that the loss of this hydrogen bond in derivatives such as H-DMA (**12**) would reduce ER affinity. Because His-524 could potentially act as a nucleophile toward an electrophilic 4′-substituent with potential to covalently modify the ERα ligand binding site, for X = NHR, R = (CH$_2$)$_2$Cl (**18**) and C(O)CH$_2$Cl (**16**) were selected. A chloromethyl ketone group is known to alkylate histidine residues,[33] and a nitrogen mustard chloroethylamine group is known to alkylate via an aziridine intermediate.[34]

Scheme 4 [a]



[a] Reagents and conditions: (a) NaN_3, CuI, L-proline, NaOH, DMSO, EtOH; (b) (i) HCl, ether; (ii) BF_3SMe_2; (c) $ClCH_2CHO$, $NaBH_3CN$, HCl, MeOH; (d) $ClCH_2COCl$, pyridine, CH_2Cl_2; (e) (i) HCl, ether; (ii) BF_3SMe_2, 70 °C.

Raloxifene binding in ERα/LBD is achieved through a combination of specific hydrogen bonds and complementarity of the binding cavity with the nonpolar portions of the ligand. Given the similarity between raloxifene and the benzothiophene SERMs described in this study, docking to ERβ/LBD was anticipated to provide good predictive binding data. Whether FlexX was run on its own or with FlexPharm constraints and/or more stringent docking criteria, the bulkier 4′-substituents (X = SO_2CH_3, $NH(CH_2)_2Cl$, $NHC(O)CH_2Cl$, and in some cases OCH_3) failed to position inside the LBD cavity. In FlexX, X-DMA derivatives with smaller 4′-substituents positioned inside the LBD, in simile with the raloxifene−ERα/LBD crystal structure. (Figure 3A). The prediction from the preliminary computational studies was that the selected family of X-DMA derivatives would provide a spectrum of ER affinity and redox reactivity.

Synthesis. It was necessary to develop a new synthetic methodology toward arzoxifene and its analogues. The published synthesis of arzoxifene is multistep and is not readily amenable to adaptation to generate arzoxifene analogs.[35] In addition, the starting material for the published 11-step synthesis of arzoxifene, 1-methanesulfonyloxy-4-bromobenzene, is not commercially available. A new synthesis was designed to provide a versatile common intermediate for preparation of arzoxifene and novel arzoxifene analogs. The 4′-bromo derivative, **4**, provides an ideal synthetic intermediate because it is amenable to copper(I) iodide-catalyzed aryl bromide derivatization (Figure 4).

The synthesis of **4** was proceeded by the adaptation of methods developed by us for F-DMA (Scheme 2).[15] 3-Methoxybenzenethiol and 2,4′-dibromoacetophenone coupling under basic conditions gave the β-ketosulfide **5**, followed by cyclization and rearrangement at 130 °C in polyphosphoric acid (PPA) to yield a mixture of rearranged and isomeric products. The desired rearranged product **6** was readily separated in good yield by simple ethyl acetate extraction and filtration. Recovered unrearranged reactant was subjected to subsequent rearragement to readily provide increased quantities of **6**. Bromination of **6** with bromoacetamide in quantitative yield followed by oxidation of the 3-bromobenzo[*b*]thiophene **7** with H_2O_2 gave the corresponding sulfoxide **8**, in which the bromide was activated by the electron-withdrawing sulfoxide toward S_NAr reaction.[36] The subsequent phenol displacement proceeded smoothly, and compound **9** was obtained in high yield. The desired 4′-bromo synthetic intermediate, **10**, was obtained by reduction with Ph_3P/TMSCl,[37] requiring prior protonation of the piperidine nitrogen by acidification. $LiAlH_4$ reduction at sulfur, employed in the preparation of DMA[25] and 4′-F-DMA,[15] led to substitution of the 4′-bromide with hydride and provided a useful synthetic route to desmethoxyarzoxifene, **12**, from **9**. Methyl ether deprotection of **10** and **11** using BF_3•SMe_2[38] gave the 4′-bromo-4′-desmethoxy and 4′-desmethoxy arzoxifene analogues **4** and **12**, respectively (attenuation of nitrogen basicity by acidification was also required prior to use of the BF_3 complex).

Arzoxifene itself (**1**) was obtained by the single-step methanolysis of **4**, effected by copper(I) iodide-catalyzed aryl bromide substitution with concentrated sodium methoxide solution and a catalytic amount of ethyl acetate that functions to prevent precipitation of the copper species during the reaction (Scheme 3).[39] This modified arzoxifene synthesis reduces the overall number of steps compared to the published 11-step synthesis, uses low-priced commercial starting materials, and is amenable to scale-up.

A sulfone-substituted arzoxifene analogue was readily prepared from **4** by direct introduction of the sulfone at the 4′-position by coupling with sodium methanesulfinate under the catalysis of a copper(I) iodide/L-proline sodium salt, a methodology that has been recently reported (Scheme 3).[40] The Cu(I) iodide/L-proline catalytic system can also be used in the synthesis of aryl azides by the coupling reaction of the aryl halide with sodium azide.[41] This is an attractive route to 4′-amino-4′-desmethoxyarzoxifene, an analogue that is predicted to possess similar estrogenic binding and oxidative bioactivation properties to DMA. Under catalysis of Cu(I) iodide/L-proline, the reaction of compound **10** with sodium azide was achieved by switching the solvent system from the reported EtOH/H_2O to a mixture of DMSO/ethanol heated at around 110 °C (Scheme 4). Instead of the azido product, the reaction directly gave the desired 4′-amino compound **14** as a single product in good yield, which might be due to the instability of the aryl azide at high

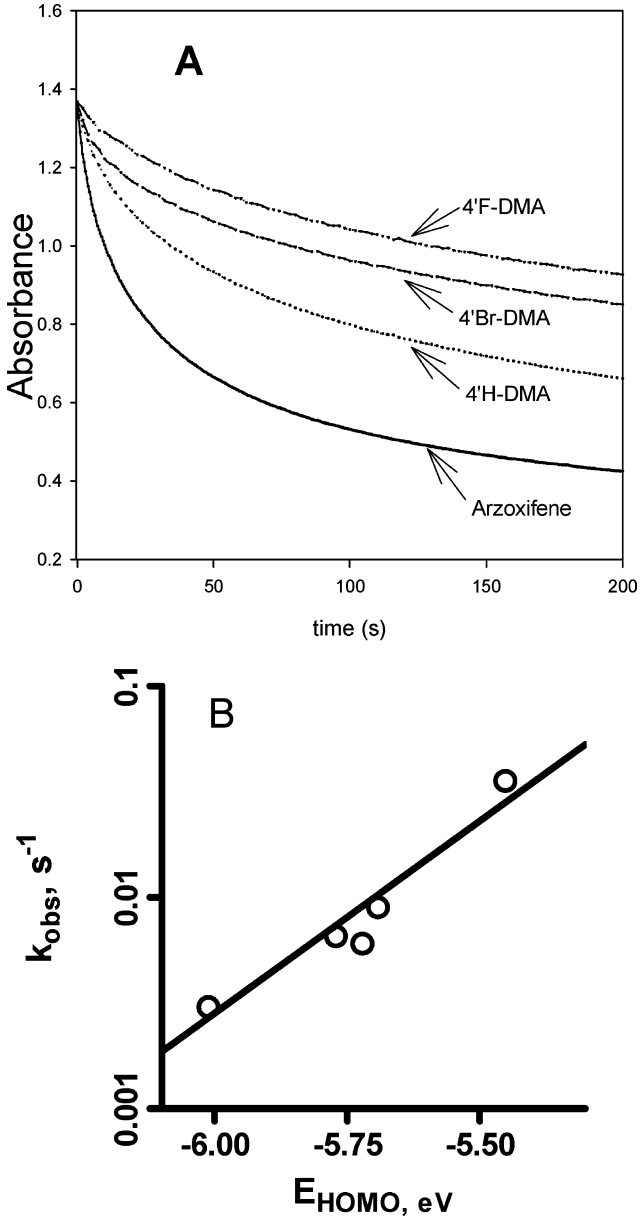


Figure 5. (A) Time plot of relative absorbance (515 nm) showing decay of DPPH radical in the presence of selected X-DMA SERMs. (B) Observed rate constants for quenching of DPPH radical in methanolic solutions by X-DMA derivatives plotted against E_{HOMO}.

temperature (Scheme 4). The subsequent demethylation gave the product **15**. Selective acylation on the introduced amino group gave the α-chloroacetylated product **16**. The aniline mustard **18** was prepared by reductive amination reaction of **14**,[42] and BCl_3•SMe_2 complex[43] was used to avoid the possible halogen exchange that might occur between BF_3 and the chloride of the mustard.

Antioxidant Activity. The simple 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was run to measure the relative antioxidant capacity of the X-DMA series compounds and raloxifene. DPPH is a stable nitrogen radical, the scavenging of which can be measured by its decay at 515 nm (Figure 5). Although scavenging of stable nitrogen radicals is not a major function of antioxidants in vivo, the assay is routinely used to quantify antioxidant capacity and is useful for comparisons within structural families. As shown in Figure 5 for the monophenolic SERMs, an excellent correlation was seen between the rate of radical scavenging and the calculated E_{HOMO} for the X-DMA series, validating the computational predictions. The observed pseudo first-order rate constants are shown in Table 1. DMA (**2**) and NH_2−DMA (**5**) are not expected to follow a similar pattern to the monophenolic SERMs because these are able to form quinoids, a diquinone methide and quinone imine, respectively, and theoretically to quench 2 equiv of DPPH.

Estrogen Receptor Binding Assays. Arzoxifene and its analogues were assayed in the standard ER competitive radioligand binding assay, using full length human recombinant ERα and ERβ and compared to raloxifene.[44] As shown in Table 1, X-DMA ligands bind to ERα with affinities ranging from the most potent ligand **2** (DMA) to **16**, which is a very poor ligand for ERα. All ligands are nonselective or selective for ERα over ERβ, owing to the larger ligand binding cavity of ERα, hence X-DMA ligands **13** and **18** are highly selective for ERα.

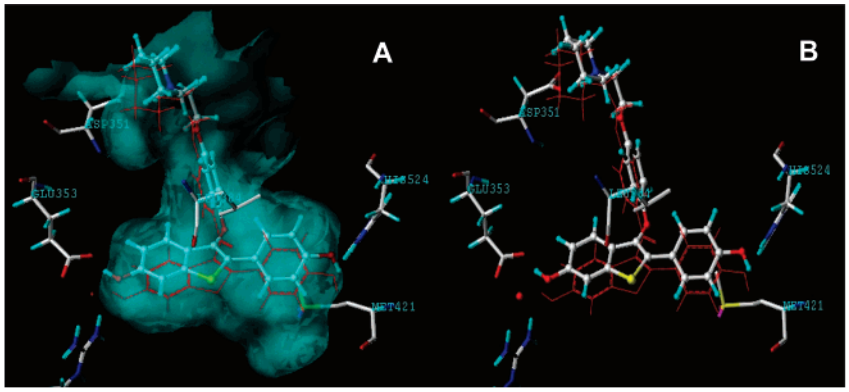
Docking Studies. X-DMA ligands with smaller 4′-substituents docked into the rigid LBD binding sites, using FlexX, giving good correlations between D-Score and the experimental pIC_{50} (exp ΔG; $r^2 = 0.912$ for ERα and $r^2 = 0.744$ for ERβ). Of the scoring methods tested (G_Score, PMF_Score, D_Score, ChemScore, Total-Score), D_Score consistently showed the best correlation for FlexX-derived binding poses with both ERα and ERβ. Ligands with bulkier 4′-substituents (X = SO_2CH_3, $NH(CH_2)_2Cl$, and $NHC(O)CH_2Cl$ and, in some poses, OCH_3) failed to dock inside the rigid LBD cavity, but three of these were observed experimentally to be reasonable ligands for ERα. To account for this observation, docking was achieved using the DMA binding pose (Figure 3) as a starting point, followed by force field minimization of the protein−ligand complex. Using this method to examine the X-DMA ligands, D-Score again provided the best correlation. This scoring function, drawn from the molecular docking program DOCK, is a classical force field energy function, which sums van der Waals and electrostatic interactions in the ligand binding complex.[45] This result is compatible with the dominant contributions to ER/LBD binding from van der Waals interactions with residues, such as Leu384 and Met421, and electrostatic interactions with (i) Arg394/Glu353,[42] His524, and (iii) Asp351(303), leading to displacement of helix12 (Figure 6). Thus, computational docking was able to account for experimental ligand binding using a rigid receptor for ligands with smaller 4′-substituents and using an iteratively relaxed receptor cavity for the more sterically encumbered ligands.

Using the Powell method[46] for energy minimization with the Tripos force field and Gasteiger−Huckel charges,[47,48] relative energies were calculated for binding of raloxifene and the X-DMA ligands to ERα and compared to the experimental binding energies, giving an excellent correlation for the ligands binding to the rigid receptor (Figure 7) but not for those that required relaxation of the receptor residues. This observation demonstrates the limitations of the crystal structure docking protocol for prediction of ligand binding and emphasizes that conformational distortion of the receptor in the region of the 4′-DMA position to accommodate larger ligands does not result in substantial loss of binding affinity. The crystal structure reveals that the 4′-substituent interacts with a less-ordered portion of the LBD. This observation suggests that further modification at the 4′-position can be explored to optimize ERα binding while ablating ERβ affinity. Crystal structures show two distinct conformations of the His-524 imidazole ring.[32] Despite the indicated proximity of His-524 in the relaxed receptor docking and the good ERα affinity of **18**, the present data do not provide any evidence for covalent modification of ERα. Conversely, the good selectivity of **18** for ERα/ERβ

Table 1. ER Binding Data and DPPH Radical Scavenging Rates for X-DMA SERMs Compared to Raloxifene[a]

	ER RBA[b]			ER IC_{50} (nM)		
cmpds (X)	ER-α	ER-β	selectivity α/β	ER-α	ER-β	DPPH scavenging $10^3 \times k_{obs}$, s^{-1}
raloxifene	0.91 ± 0.12	0.03 ± 0.01	30	20.6 ± 2.7	557 ± 146	4.0 ± 0.6
1 (OCH_3) arzoxifene	0.94 ± 0.29	0.25 ± 0.01	3.8	21.5 ± 6.5	66.3 ± 3.1	35 ± 7
2 (OH) DMA	2.51 ± 0.61	1.77 ± 0.35	1.4	7.8 ± 1.9	9.6 ± 1.9	9.3 ± 0. 4
3 (F) F-DMA	1.07 ± 0.04	0.68 ± 0.26	1.6	17.2 ± 0.6	27.9 ± 11	6.0 ± 0.9
4 (Br)	0.70 ± 0.12	0.25 ± 0.03	2.8	27.0 ± 4.6	66.7 ± 8.5	6.5 ± 0.3
12 (H)	1.71 ± 0.23	1.00 ± 0.05	1.7	10.9 ± 1.5	16.3 ± 0.8	8.9 ± 0.9
13 (SO_2CH_3)	0.71 ± 0.12	0.009 ± 0.0005	79	27.0 ± 4.8	1800 ± 100	3.0 ± 0.5
15 (NH_2)	0.96 ± 0.08	0.34 ± 0.01	2.8	19.3 ± 1.7	48.6 ± 1.9	220 ± 0.4
16 ($NHC(O)CH_2Cl$)	0.03 ± 0.01	0.007	4.3	666 ± 219	2360 ± 18	[c]
18 ($NH(CH_2)_2Cl$)	0.56 ± 0.15	<0.01	>50	35.6 ± 10	>1600	[c]

[a] Data shown is the mean ± S.D. for at least triplicate measurements. [b] RBA values calculated relative to IC_{50} assayed for E_2 control (RBA = 1.0). [c] Not measured.



DMA-X	Glu353*	Arg394**	His524†	Asp351‡
1 (OCH_3)	2.53	3.85	(O) 2.79	2.59
2 (OH)	2.51	3.10	2.71	2.61
3 (F)	2.55	3.40	2.98	2.46
4 (Br)	2.48	3.35	3.94	2.60
12 (H)	2.48	3.30	4.27	2.61
13 (SO_2CH_3)	2.52	3.02	(O) 2.69	2.50
15 (NH_2)	2.54	3.48	(N) 3.44	2.46
16 ($NHC(O)CH_2Cl$)	2.55	3.48	(O) 2.58	2.55
18 ($NH(CH_2)_2Cl$)	2.56	3.49	(Cl) 5.45	2.59

*Distance between carboxylate oxygen of Glu353 and benzothiophene OH. **Distance between guanidino nitrogen of Arg394 and benzothiophene OH. †Distance between guanidino nitrogen of Arg394 and benzothiophene OH. ‡Distance between carboxylate oxygen of Asp351 and piperidine nitrogen of ligand side chain.

Figure 6. Raloxifene (red) and DMA docked in the ERα/LBD showing key residues. The MOLCAD multichannel surface was generated in SYBYL. Distances between X-DMA ligands and key ERα/LBD residues, Å, are shown in the table below.

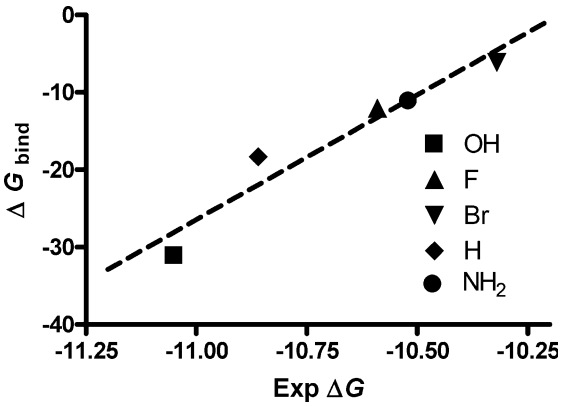


Figure 7. Correlation between experimental pIC_{50} (exp ΔG) and calculated ΔG binding ($r^2 = 0.93$).

further supports modification of the 4′ position of benzothiophene SERMs to enhance selectivity.

Recently, a second low-affinity binding site for tamoxifen has been reported in a crystal structure of 4-hydroxytamoxifen-ERβ/LBD (PBD ID: 2FSZ), therefore, it was of interest to screen the X-DMA ligands for binding.[49] Most interactions at the low affinity binding site are van der Waals contacts provided by the amino acids within a radius of 5.5 Å from tamoxifen, with core subpocket residues including Leu306, Met309, Ile310 Val 328, Leu331, Glu332, and Trp335 (using ERβ 2FSZ reference numbering). The weak, nonspecific ligand association in this hydrophobic grove permits numerous binding poses for the X-DMA ligands, which would require probing by long-range molecular dynamics. The low affinity binding site is not proposed to inhibit estrogen binding to ER, but rather to antagonize coregulator binding, thus, binding of bulkier X-DMA ligands to this site cannot account for the competitive binding assay data shown in Table 1.

Comparisons of X-DMA Activity/Reactivity. Except **16** and **18**, the family of benzothiophene SERMs reported can be subdivided into those that are readily able to form quinoids (**2**, **15**) and the subset that are anticipated to form semiquinones on oxidation (**1**, **3**, **4**, **12**, **13**). For the latter SERMs, two-electron oxidation is blocked by the 4′-modification, thus it might be

anticipated that these will undergo oxidation to *o*-quinones, an alternate pathway that is not blocked. In the latter subset, the redox activity is controlled by the remote 4′-substituent, which is expected to influence the rate of formation of *o*-quinones. For example, **13** is 10-fold less redox active than **1** (Table 1). The range of E_{HOMO} values calculated for the SERM family is similar to that for families of compounds that have shown a wide range in chemopreventive index.[29] However, the anticipated metabolism of both **1** and **12** to yield DMA (**2**) would complicate interpretation in more complex systems. Nevertheless, the data presented herein presents some interesting comparisons. For example, **4** and **13** have similar affinity for ERα, and both have attenuated redox activity because of electron-withdrawing 4′-substituents, but **13** is highly selective for ERα/ER*β*, and **2** and **12** are comparably antiestrogenic, but only **2** can form a diquinone methide. The extrapolation of the spectrum of antiestrogenic and redox activity, measured in simple models herein, to complex cellular and in vivo systems awaits the completion of such studies measuring oxidative metabolism, Phase II enzyme induction, and estrogenic versus antiestrogenic endpoints.

Conclusions

An ideal SERM, antiestrogenic in breast and endometrial tissue, but proestrogenic in the vasculature and brain would be of use in cancer chemoprevention and as an alternative to hormone replacement therapy. The approaches taken have focused largely on amplifying antiestrogenic activity; screening families of SERMs for compounds with increased tissue selectivity and, more recently, isoform selectivity. There has been relatively little research directed at exploring the contribution of the redox reactivity and bioactivation that is common to many SERMs and to test the hypothesis that structural modification of SERMs to modulate bioactivation will lead to an improved SERM. The synthesis presented herein provides access to a structurally conservative family of SERMs related to the clinically important benzothiophene SERMs that are shown to manifest a spectrum of redox reactivity and ER ligand properties.

Experimental Section

Synthesis. ^{1}H and ^{13}C NMR spectra were obtained with Bruker Ultrashield 400 or Advance 300 spectrometer. Chemical shifts are reported as δ values in parts per million (ppm) relative to tetramethylsilane (TMS) for all recorded NMR spectra. Low-resolution mass spectra were recorded on a Agilent 1100 series LC/MSD ion trap instrument, using APCI as ionization method. High-resolution mass spectra were taken on a Micromass QTOF mass spectrometer, using ESI as the ionization method. All reagents and solvents were obtained commercially from Acros, Aldrich, and Fluka and were used without purification.

1-(4-Bromophenyl)-2-(3-methoxylphenylsulfanyl)ethanone (5). KOH (5.2 g, 87%, 80 mmol) was dissolved in ethanol (200 mL), and 3-methoxy benzenethiol (9.7 mL, 79 mmol) was added and stirred for 10 min. Ethyl acetate (80 mL) was added to this solution, then 2,4′-dibromoacetophenone (20 g, 72 mmol) was added in portions. Another 60 mL of ethyl acetate was added to dissolve some precipitate that was produced during the reaction after 3 h. The reaction mixture was stirred overnight at room temperature. Most solvent was removed under reduced pressure, the residue was partitioned between ethyl acetate and brine, and the organic phase was separated and dried over anhydrous $MgSO_4$. Concentration and recrystallization using ethyl acetate gave desired compound **5** as a yellow solid (21.5 g, 89%). ^{1}H NMR (acetone-d_6, 400 MHz): δ 7.96−7.98 (m, 2H), 7.70−7.73 (m, 2H), 7.19−7.23 (m, 1H), 6.93−6.95 (m, 2H), 6.76−6.79 (m, 1H), 4.53 (s, 2H), 3.77 (s, 3H). ^{13}C NMR (acetone-d_6, 100 MHz): δ 193.9, 160.9, 137.5, 135.6, 132.7, 131.3, 130.7, 128.6, 122.1, 115.3, 113.0, 55.5, 40.8. APCI-MS: *m*/*z* 339.0/337.0 (100/98%) $[M + H]^+$.

6-Methoxy-2-(4-bromophenyl)benzo[b]thiophene (6). PPA (70 g) was added to a 250 mL flask and heated to 80 °C with stirring (keep the speed of stirring as fast as possible). Compound **5** (10 g, 29.8 mmol) was added portionwise within 30 min, then the temperature of oil bath was elevated to 130 °C, and the reaction mixture was heated with stirring for 6 h. The reaction mixture was poured into 500 mL of rapidly stirring ice water to allow the PPA to be hydrolyzed. After 2 h, 150 mL of ethyl acetate was added and stirred for 20 min, and the crude product was collected by filtration and washed with 15 mL of H_2O three times. The obtained brown solid was air-dried overnight to get the title compound, which was pure enough for the next step (2.9 g, 30%). The ethyl acetate solution containing unrearranged reactant was washed with H_2O and $NaHCO_3$ solution, concentrated, passed through a short silica gel column, and subjected to another rearrangement reaction to gave more desired product. An analytic sample of **6** was obtained by recrystallization using ethyl acetate. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.81 (s, 1H), 7.73 (d, 1H, J = 8.7 Hz), 7.63−7.68 (m, 4H), 7.56 (d, 1H, J = 2.0 Hz), 7.01 (dd, 1H, J = 8.7 Hz, 2.2 Hz), 3.83 (s, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 157.5, 140.3, 138.9, 134.2, 133.0, 132.0, 127.6, 124.6, 120.9, 120.4, 114.9, 105.2, 55.5. APCI-MS: *m*/*z* 319.1/321.0 (98 /100%) $[M + H]^+$.

6-Methoxy-2-(4-bromophenyl)-3-bromobenzo[*b*]thiophene (7). Compound **6** (3.13 g, 9.87 mmol) was suspended in dried DCM (70 mL), and *N*-bromoacetamide (1.45 g, 10.05 mmol) was added with stirring. The reaction mixture was stirred at room temperature for 2 h, diluted with another 100 mL of DCM, and washed with H_2O and brine, and the organic phase was separated and concentrated. Product was obtained after recrystallization, using ethyl acetate, as slightly brown solid (3.70 g, 95%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.66−7.76 (m, 6H), 7.17 (dd, 1H, J = 8.8 Hz, 2.3 Hz), 3.87 (s, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 158.3, 138.5, 133.6, 132.2, 131.9, 131.6, 131.0, 123.9, 122.3, 115.8, 105.4, 104.3, 55.7. APCI-MS: *m*/*z* 398.8 $[M + H]^+$.

6-Methoxy-2-(4-bromophenyl)-3-bromobenzo[*b*]thiophene S-oxide (8). Compound **7** (2.6 g, 6.53mmol) was dissolved in dichloromethane (30 mL), TFA (25 mL) was added dropwise with stirring, then 1.4 mL of H_2O_2 (30%) was added, and the reaction mixture was stirred at room temperature for 2 h. Sodium bissulfide (500 mg) in 5 mL of H_2O was added and stirred vigorously for 15 min to quench the reaction. Most of the solvent was removed under reduced pressure, and the residue was diluted with dichloromethane and carefully washed with saturated aqueous $NaHCO_3$ solution. After concentration, the crude product was purified by column chromatography, eluting with 6:1 hexanes/ethyl acetate. Product was obtained as slightly yellow solid (1.75 g, 65%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.67−7.80 (m, 5H), 7.62 (d, 1H, J = 8.5 Hz), 7.31 (d, 1H, J = 8.5 Hz), 3.91 (s, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 161.2, 144.4, 143.8, 132.1, 131.0, 129.2, 128.9, 125.6, 123.1, 121.9, 118.7, 112.4, 56.3. APCI-MS: *m*/*z* 414.9 (100%) $[M + H]^+$.

6-Methoxy-3-{4-[2-(1-piperidinyl)ethoxy]phenoxy}-2-(4-bromophenyl) Benzo[*b*]thiophene S-oxide (9). The side chain phenol compound (1.80 g, 8.13 mmol) was dissolved in anhydrous DMF (50 mL), NaH (328 mg, 60%, dispersed in oil) was added in three portions within 20 min, and then bromide **8** (3.3 g, 8.01mmol) was added. The reaction mixture was stirred at room temperature for 1 h and diluted with 200 mL of ethyl acetate, and the resulting solution was washed with water. The aqueous phase was back extracted with 50 mL of DCM, combined with the ethyl acetate solution, and then dried with anhydrous $MgSO_4$. Solvent was removed under high vacuum to get a yellow solid that was pure enough for the next step reaction (4.2 g, 94%). Analytical sample was obtained by PTLC (5:1 DCM/MeOH). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.54 (m, 1H), 7.59−7.70 (m, 4H), 7.06−7.11 (m, 3H), 7.01(d, 1H, J = 8.5 Hz), 6.88 (d, 2H, J = 8.8 Hz), 3.98 (t, 2H, J = 5.6 Hz), 3.86 (s, 3H), 2.59 (t, 2H, J = 5.6 Hz), 2.39 (br s, 4H), 1.42−1.52 (m, 4H), 1.32−1.42 (m, 2H). ^{13}C NMR (DMSO-

d_6, 75 MHz): 160.8, 155.1, 150.1, 148.4, 144.8, 131.9, 129.8, 129.6, 128.8, 125.3, 123.9, 121.6, 118.1, 118.0, 115.7, 112,8, 66.0, 57.3, 56.1, 54.4, 25.6, 23.9. APCI-MS: *m*/*z* 554.2/556.1 (100/98%) $[M + H]^+$.

6-Methoxy-3-{4-[2-(1-piperidinyl) ethoxy]phenoxy}-2-(4-bromophenyl) Benzo[*b*]thiophene (10). Compound **9** (4.0 g, 7.2 mmol) was dissolved in DCM (25 mL), and 1 M HCl/ether solution (15 mL) was added and stirred for 1 h. All solvent was removed under reduced pressure. The residue was dissolved in anhydrous THF (70 mL), TMSCl (9.2 mL, 72 mmol) and Ph_3P (7.0 g, 26.7 mmol) were added, the reaction mixture was refluxed for 8h. Most of the solvent was removed, residue was diluted with 200 mL ethyl acetate, washed with saturated aqueous $NaHCO_3$ solution, concentrated, crude product was purified by column chromatography, eluting with 30:1 DCM/MeOH containing 3‰ HOAc to remove the excess of Ph_3P and Ph_3PO, then eluting with 20:1 DCM/MeOH to get the product (3.3 g, 85%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.61−7.68 (m, 5H), 7.21 (d, 1H, *J*=8.8 Hz), 6.95 (dd, 1H, *J*=8.8 Hz, 2.3 Hz), 6.86−6.89 (m, 4H), 3.95 (t, 3H, *J*= 5.9 Hz), 3.83 (s, 3H), 2.59 (t, 3H, *J*= 5.9 Hz), 2.38 (bs, 4H), 1.43−1.48 (m, 4H), 1.35−1.36 (m, 2H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 158.2 154.0, 150.7 141.0, 136.8 132.1 130.9, 128.7, 126.9, 124.3, 122.0, 121.0, 116.2, 115.6, 115.1, 106.1, 65.9, 57.4, 55.6, 54.4, 25.5, 23.9; HRMS calcd. for $C_{28}H_{29}NO_3SBr$ 538.1052 $[M+H]^+$, found 538.1072.

6-Hydroxy-3-{4-[2-(1-piperidinyl)ethoxy]phenoxy}-2-(4-bromophenyl) benzo[*b*] thio phene (4). Compound **10** (300 mg, 0.56 mmol) was dissolved in DCM (3 mL), 1N HCl in diethyl ether (3 mL) was added, the mixture was stirred at room temperature for 30 min. All solvents was removed and the obtained slightly yellow foam was redissolved in 15 mL DCM, the flask was filled with argon, BF_3 dimethyl sulfide complex (2.2 mL) was added, the resulting mixture was stirred at room temperature for 5h. The reaction was diluted with 30 mL ethyl acetate, and washed with saturated $NaHCO_3$ aqueous solution, organic phase was separated and dried by anhydrous $MgSO_4$. The crude product was purified by column chromatography (15:1 DCM/MeOH), the product was obtained as slight yellow solid (240 mg, 82%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.92 (s, 1H), 7.58−7.67 (m, 4H), 7.30 (d, 1H, *J*=1.9 Hz), 7.14 (d, 1H, *J*=8.7 Hz), 6.85 (bs, 4H), 6.82 (dd, 1H, *J*=8.7 Hz, 1.9 Hz), 3.97 (t, 2H, *J*= 5.9 Hz), 2.59 (t, 2H, *J*= 5.8 Hz), 2.38(m, 4H), 1.43−1.48 (m, 4H), 1.32−1.38 (m, 2H) ; ^{13}C NMR (DMSO-d_6, 100 MHz): δ 156.4, 154.0, 150.7, 141.2, 136.7, 132.0 , 131.1 , 128.6, 125.9, 123.1, 122.2, 120.8, 116.2, 115.6, 115.2, 108.0, 65.8, 57.3, 54.3, 25.4, 23.8 ; HRMS calcd. for $C_{27}H_{27}NO_3SBr$ 524.0895 $[M+H]^+$, found 524.0887.

6-Methoxy-3-{4-[2-(1-piperidinyl) ethoxy]phenoxy}-2-phenyl benzo[*b*] thiophene (11). Compound **9** (270 mg, 0.47 mmol) was dissolved in anhydrous THF (5 mL), $LiAlH_4$ (55 mg, 1.45 mmol) was added, the reaction mixture was refluxed for 6h. The reaction was quenched by adding 2N aqueous NaOH solution (0.5 mL), then diluted with 30 mL ethyl acetate, more NaOH was added until most of the white precipitate dissolved. The organic phase was separated and concentrated, the residue was purified by column chromatography (50:3 DCM/MeOH), product was obtained as white solid (180 mg, 83%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.76 (d, 2H, *J*= 7.4 Hz), 7.25−7.37 (m, 5H), 6.86−6.91 (m, 3H), 6.79−6.81 (m, 2H), 4.05 (t, 2H, *J*= 6.0 Hz), 3.87 (s, 3H), 2.76 (t, 2H, *J*= 6.0 Hz), 2.52 (bs, 4H), 1.59−1.65 (m, 4H), 1.42−1.48 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ158.2, 154.2, 151.9, 141.0, 137.4, 132.6, 128.9, 128.2, 127.8, 127.6, 126.7, 122.7, 116.6, 115.7, 114.6, 105.5, 66.5, 58.1, 55.8, 55.2, 26.0, 24.8; HRMS calcd. for $C_{28}H_{30}NO_3S$ 460.1946 $[M+H]^+$, found 460.1935.

6-Hydroxy-3-{4-[2-(1-piperidinyl) ethoxy]phenoxy}-2-phenyl benzo[*b*] thiophene (12). Compound **11** (150 mg 0.33 mmol) was dissolved in DCM (5 mL), 1N HCl in diethyl ether (1 mL) was added, the mixture was stirred at room temperature for 30 min. All solvents was removed and the residue was redissolved in 8 mL DCM, the flask was filled with argon, BF_3 dimethyl sulfide complex (1.7 mL) was added, the resulting mixture was stirred at room temperature for 5h. The reaction was diluted with 30 mL ethyl acetate, and washed with saturated $NaHCO_3$ aqueous solution, the organic phase was separated and dried by anhydrous $MgSO_4$. The crude product was purified by column chromatography (12:1 to 7:1 DCM/MeOH), the product was obtained as white solid (95 mg, 65%). ^{1}H NMR (Acetone-d_6, 400 MHz): δ 7.75−7.77 (m, 2H), 7.39 (m, 2H), 7.35 (d, 1H, *J*=1.8 Hz), 7.22−7.32 (m, 2H), 6.85−6.92 (m, 5H), 4.07 (t, 3H, *J*= 5.8 Hz), 2.77 (t, 3H, *J*= 5.8 Hz), 2.57 (bs, 4H), 1.54−1.58 (m, 4H), 1.42−1.44 (m,2H); ^{13}C NMR (Acetone-d_6, 75 MHz): δ 157.1, 155.2, 152.5, 141.9, 138.2, 133.4, 129.7, 128.6, 128.0, 127.9, 126.2, 123.2, 117.1, 116.4, 115.7, 108.8, 66.9, 58.5, 55.6, 26.4, 24.7; HRMS calcd. for $C_{27}H_{28}NO_3S$ 446.1790 $[M+H]^+$, found 446.1785.

6-Hydroxy-3-{4-[2-(1-piperidinyl)ethoxy]phenoxy}-2-(4-methoxyphenyl) benzo[*b*] thiophene (1, Arzoxifene). Compound **4** (65 mg, 0.12 mmol), CuI (24 mg, 0.12 mmol) were added into an argon-flushed flask, DMF (0.7 mL), anhydrous MeOH (1.5 mL) and ethyl acetate (40 μL ,0.4 mmol) were added, after the addition of NaOMe (530 mg, 9.9 mmol, concentration 4.5 M), the reaction mixture were heated at 110 °C for 8h. The reaction mixture was diluted with 60 mL ethyl acetate, neutralized with aqueous 5N acetic acid, then washed with brine, the organic phase was separated, after concentration, the residue was purified by column chromatography (12:1 DCM/MeOH), the product was obtained as white solid (48 mg, 85%). ^{1}H NMR (Acetone-d_6, 400 MHz): δ 7.67−7.69 (m, 2H), 7.32 (d, 1H, *J*= 2.0 Hz), 7.21 (d, 1H, *J*=8.6 Hz), 6.94−6.96 (m, 2H), 6.86−6.88 (m, 5H), 4.14 (t, 2H, *J*= 5.8 Hz), 3.80 (s, 3H), 2.92 (t, 2H, *J*=5.8 Hz), 2.73 (m, 4H), 1.56−1.66 (m, 4H), 1.43−1.45 (m, 2H); ^{13}C NMR (Acetone-d_6, 75 MHz): δ 160.3, 156.8, 154.9, 152.7, 140.7, 137.7, 129.4, 128.1, 126.3, 125.8, 122.8, 117.1, 116.5, 115.6, 115.1, 108.9, 66.3, 58.2, 55.6, 55.4, 25.9, 24.3; HRMS calcd. for $C_{28}H_{30}NO_4S$ 476.1896 $[M+H]^+$, found 476.1893.

6-Hydroxy-3-{4-[2-(1-piperidinyl)ethoxy]phenoxy}-2-[(4-methanesulfonyl)phenyl] benzo[***b***] thiophene (13).** Compound **4** (50 mg, 0.095mmol), CH_3SO_2Na (78 mg, 0.76 mmol), L-proline (8.7 mg, 0.076 mmol), NaOH (6 mg, 0.15 mmol), CuI (14.5 mg, 0.076 mmol) were added to a 5 mL flask, then filled with argon, anhydrous DMSO (1.5 mL) was added, the resulting reaction mixture was stirred at 110 °C for 13 h. The reaction mixture was partitioned between ethyl acetate (30 mL) and water (5 mL), the undissolved solid was filtered off, solid was washed with another 10 mL ethyl acetate. The organic phase was combined, separated and concentrated, crude product was purified by column chromatography (DCM/MeOH 20:1), product was obtained as slightly green foam (45 mg, 90%). ^{1}H NMR (Acetone-d_6, 400 MHz): δ 7.93−8.01 (m, 4H), 7.38 (d, 1H, *J*=1.9 Hz), 7.27 (d, 1H, *J*=8.7 Hz), 6.80−6.93(m, 5H), 4.03 (t, 2H, *J*= 5.9 Hz), 3.12 (s, 3H), 2.68 (t, 2H, *J*=5.9 Hz), 2.48 (m, 4H), 1.50−1.54 (m, 4H), 1.39−1.41 (m, 2H) ^{13}C NMR (Acetone-d_6, 75 MHz): δ 157.8, 155.6, 152.1, 144.1, 140.7, 138.9, 138.4, 128.8, 128.3, 127.6, 124.0, 123.9, 117.3, 116.5, 116.2, 108.9, 67.2, 58.6, 55.6, 44.3, 26.7, 24.9. HRMS calcd. for $C_{28}H_{30}NO_5S_2$ 524.1565 $[M+H]^+$, found 524.1575

6-Methoxy-3-{4-[2-(1-piperidinyl) ethoxy]phenoxy}-2-(4-aminophenyl) benzo[*b*] thiophene (14). Compound **10** (150 mg, 0.278 mmol), NaN_3 (217 mg, 3.36 mmol), L-proline (28 mg, 0.25 mmol), NaOH (11.1 mg, 0.27 mmol), CuI (47 mg, 0.25 mmol) were added to a 10 mL flask, then filled with argon, a mixture of 4 mL DMSO/2 mL EtOH was added by syringe, the resulting reaction mixture was stirred at 110 °C for 10h. The reaction was partitioned between ethyl acetate and water, the undissolved solid was filtered off. The organic phase was separated and concentrated, crude product was purified by column chromatography (DCM/MeOH), product was obtained as slightly green syrup (98 mg, 74%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.31 (s,1H), 7.51 (d, 1H, *J*=2.0 Hz), 7.37 (d, 2H, *J*=8.4 Hz), 7.12 (d, 1H, *J*=8.7 Hz), 6.81−6.91 (m, 5H), 6.55 (d, 2H, *J*=8.4 Hz), 5.40 (s, 2H), 3.95 (t, 2H, *J*= 5.7 Hz), 3.80 (s, 3H), 2.59 (t, 2H, *J*=5.6 Hz), 2.39 (bs, 4H), 1.45−1.50 (m, 4H), 1.35−1.40 (m, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 157.3, 153.7, 151.0, 148.9, 137.7, 135.4, 127.9, 127.7, 127.4, 120.9, 118.8, 115.9, 115.5, 114.3, 113.8, 106.0, 65.8, 57.4, 55.5, 54.3, 25.5, 23.9; HRMS calcd. for $C_{28}H_{31}N_2O_3S$ 475.2055 $[M+H]^+$, found 475.2045.

6-Hydroxy-3-{4-[2-(1-piperidinyl) ethoxy]phenoxy}-2-(4-aminophenyl) benzo[*b*] thiophene (15). Compound **14** (250 mg, 0.53

mmol) was dissolved in DCM (10 mL), 1M HCl in ether (3 mL) was added and the mixture was stirred at room temperature for 30 min. All solvents was removed and the residue was redissolved in DCM (12 mL), the flask was filled with argon, BF_3 dimethyl sulfide complex (2.5 mL) was added, the resulting mixture was stirred at room temperature for 5h. The reaction was diluted with 30 mL ethyl acetate, and washed with saturated $NaHCO_3$ aqueous solution, the organic phase was separated and dried by anhydrous $MgSO_4$. The crude product was purified by column chromatography (10:1 DCM/MeOH), the product was obtained as slightly yellow solid (175 mg, 72%). ^{1}H NMR (Acetone-d_6, 400 MHz): δ 7.47 (d, 2H, J=8.68 Hz), 7.28 (d, 1H, J=1.86 Hz), 7.16 (d, 1H, J=8.62 Hz), 6.38−6.91 (m, 5H), 6.66 (d, 2H, J = 6.89 Hz), 4.89 (bs, 2H), 4.21 (t, 2H, J= 5.45 Hz), 3.19 (t, 2H, J= 5.36 Hz), 3.00 (m, 4H), 1.71−1.76 (m, 4H), 1.52−1.56 (m, 2H); ^{13}C NMR (Acetone-d_6, 100 MHz): δ 156.3, 155.1, 152.6, 139.5, 137.1, 129.0, 128.4, 127.7, 122.4, 121.5, 116.9, 116.3, 115.2, 115.1, 108.7, 67.3, 58.7, 55.7, 26.7, 24.9; HRMS calcd. for $C_{27}H_{29}N_2O_3S$ 461.1899 $[M+H]^+$, found 461.1901.

6-Hydroxy-3-{4-[2-(1-piperidinyl)ethoxy]phenoxy}-2-[4-(2-chloroacetamide)phenyl] benzo[*b*] thiophene (16). Compound **15** (46 mg, 0.1 mmol), pyridine (80 μL) were dissolved in anhydrous CH_2Cl_2 (1.5 mL) and cooled in ice bath, chloroacetyl chloride (6 μL) was added by a syringe. The reaction mixture was gradually warmed up to room temperature and stirred overnight. Reaction mixture was diluted with CH_2Cl_2 (25 mL), washed with saturated aqueous $NaHCO_3$ solution. The organic phase was separated and concentrated, crude product was purified by flash column chromatography (AcOEt/CH_2Cl_2/MeOH 30:25:8). The product was obtained as slightly yellow foam (31 mg, 57%). ^{1}H NMR (Acetone-d_6, 400 MHz): δ 9.56 (s, 1H), 7.74−7.69 (m, 4H), 7.33 (d, 1H, J= 2.0 Hz), 7.22 (d, 1H, J= 8.65 Hz), 6.91−6.83 (m, 5H), 4.24 (s,1H), 4.01 (t, 2H, J= 5.96 Hz), 2.66 (t, 2H, J=5.96 Hz), 2.46 (m, 4H), 1.53−1.49 (m, 4H), 1.40−1.35 (m, 2H); ^{13}C NMR (Acetone-d_6, 75 MHz): δ 165.5, 157.1, 155.4, 152.5, 141.7, 139.1, 138.0, 129.2, 128.6, 128.1, 125.9, 123.2, 120.7, 117.2, 116.5, 115.8, 108.9, 67.4, 58.8, 55.8, 44.2, 26.8, 25.1; HRMS calcd. for $C_{29}H_{30}N_2O_4SCl$ 537.1615 $[M+H]^+$, found 537.1595.

6-Methoxy-3-{4-[2-(1-piperidinyl)ethoxy]phenoxy}-2-{4-[(2-chloroethyl) amino] phenyl} benzo[*b*] thiophene (17). Compound **14** (60 mg 0.12 mmol) was dissolved in 2 mL methanol, chloroacetaldehyde (30 μL 45% aqueous solution, 0.16 mmol), $NaBH_3$-CN (15 mg, 0.17 mmol), 6N HCl in methanol (30 μL) were added, the reaction mixture was stirred at room temperature for 3 days, solvent was removed under reduced pressure, residue was purified by column chromatography (DCM/MeOH 20:1 containing 1‰ $NH_3 \cdot H_2O$) to get the product (25 mg, 37.3%). ^{1}H NMR (Acetone-d_6, 400 MHz): δ 7.55(d, 2H, J=8.7 Hz), 7.43(d, 1H, J=2.1 Hz), 7.21 (d, 1H, J=8.7 Hz), 6.84−6.9 3 (m, 5H), 6.69 (d, 2H, J=8.7 Hz), 5.50 (t, 1H, J= 5.96 Hz), 4.01 (t, 2H, J= 5.9 Hz), 3.86 (s, 3H), 3.73 (t, 2H, J=6.2 Hz), 3.52−3.55 (m, 2H), 2.66 (t, 2H, J=6.0 Hz), 2.46 (m, 4H), 1.49−1.55 (m, 4H), 1.38−1.41 (m, 2H); ^{13}C NMR (Acetone-d_6, 75 MHz): δ 158.8, 155.2, 152.6, 148.8, 139.6, 137.2, 129.2, 129.2, 128.3, 122.3, 121.7, 116.9, 116.4, 115.1, 113.4, 106.5, 67.3, 58.7, 55.9, 55.7, 45.8, 43.8, 26.7, 24.9; HRMS calcd. for $C_{30}H_{34}N_2O_3SCl$ 537.1979 $[M+H]^+$, found 537.1983.

6-Hydroxy-3-{4-[2-(1-piperidinyl)ethoxy]phenoxy}-2-{4-[(2-chloroethyl) amino] phenyl} benzo[*b*] thiophene (18). Compound **17** (20 mg, 0.03 7mmol) was dissolved in DCM (1.5 mL), 1N HCl in diethyl ether (0.3 mL) was added, the mixture was stirred at room temperature for 30 min. All solvents was removed and the obtained brown foam was redissolved in DCM (1.5 mL), the flask was filled with argon, BCl_3 dimethyl sulfide complex (0.3 mL) was added, the resulting mixture was stirred at 70 °C for 10 h. The reaction was diluted with 10 mL DCM, and washed with saturated $NaHCO_3$ aqueous solution, the organic phase was separated and dried by anhydrous $MgSO_4$. Crude product was purified by column chromatography (15:1 DCM/MeOH). Product was obtained as slight yellow solid (15 mg, 77%). ^{1}H NMR (Acetone-d_6, 400 MHz): δ 7.54 (d, 2H, J=8.7 Hz), 7.29 (d, 1H, J=1.9 Hz), 7.16 (d, 1H, J=8.6 Hz), 6.83−6.89 (m, 5H), 6.69 (d, 2H, J=8.7 Hz), 5.49 (t, 1H, J= 5.9 Hz), 4.10 (t, 2H, J= 5.8 Hz), 3.73 (t, 2H, J=6.1 Hz), 3.51−3.56 (m, 2H), 2.79 (t, 2H, J=5.7 Hz), 2.60 (m, 4H), 1.56−1.62 (m, 4H), 1.40−1.46 (m, 2H); ^{13}C NMR (Acetone-d_6, 75 MHz): δ 156.5, 155.1, 152.8, 148.8, 139.7, 137.3, 129.3, 128.5, 127.5, 122.5, 121.9, 117.1, 116.5, 115.4, 113.4, 108.9, 66.9, 58.5, 55.5, 45.9, 43.9, 26.3, 24.7; HRMS calcd. for $C_{29}H_{32}N_2O_3SCl$ 523.1822 $[M+H]^+$, found 523.1809.

Estrogen Receptor Binding Assays. ER competitive binding assay with [^{3}H]-estradiol. The assay was slightly modified from the original protocol.[49] Twenty-four hours before the assay, 50% v/v hydroxyapatite (HAP) slurry was prepared using 10 g hydroxylapatite/60 mL of TE buffer (50 mM Tris-Cl, 1 mM EDTA, pH 7.4) and stored at 4 °C. ER binding buffer (10 mM Tris, 10% glycerol, 2 mM dithiothrietol, 1 mg/mL bovine serum albumin, pH 7.5), ERα (40 mM Tris, 100 mM KCl, pH 7.5) and ERβ (40 mM Tris, pH 7.5) wash buffers were prepared subsequently. The reaction mixture consisted of 5μL of test samples in DMSO, 5μL of pure human recombinant diluted ERα or ERβ (0.5pmol) in ER binding buffer, 5μL of "Hot Mix" (400nM, prepared fresh using 3.2μL of 25μM, 83Ci/mM ^{3}H-estradiol, 98.4μL of ER binding buffer), and 85μL ER binding buffer. The incubations were performed at room temperature for 2 h or at 4 °C overnight, then 100μL of 50% HAP slurry was added and the tubes were incubated on ice for 15 min with votexing every 5 min. The appropriate ER wash buffer was added (1 mL), and the tubes were vortexed and then centrifuged at 2000 *g* for 5 min. The supernatant was discarded and this wash step was repeated three times. The HAP pellet, containing the ligand−receptor complex, was then resuspended in 200 μL of ethanol and transferred to scintillation vials. Cytoscint (4 mL/vial) was added, and the samples were counted using a Beckman (Schaumburg, IL) LS 5801 liquid scintillation counter. The percent inhibition of [^{3}H] estradiol binding to each ER was determined as follows: $[1 - (\mathrm{dpm_{sample}} - \mathrm{dpm_{blank}})/(\mathrm{dpm_{DMSO}} - \mathrm{dpm_{blank}})] \times 100$. IC_{50} values were calculated from binding of the sample expressed as a percentage relative to E_2 (50 nM, 100%). Relative binding affinity (RBA; relative to E_2) was calculated from $IC_{50}(E_2)/IC_{50}$(sample). The samples were assayed in triplicate at at least five concentrations.

Antioxidant Activities of SERMs. Antioxidant activities of SERMs were monitored using DPPH assay. The 0.2 mM solution of DPPH in methanol and 1.2 mM of SERMs in DMSO were mixed in a 1 mL spectrophotometer cell. The maximum volume of DMSO used in experiments was less than 4% compare to methanol. Kinetic traces were obtained at 515 nm using a HP8452A diode array spectrometer. Observed rate constants (k_{obs}) were obtained from first-order fitting of the corresponding kinetic traces. Excellent pseudo first-order kinetics were observed for DPPH degradation in the presence of monophenolic SERMs (Figure 5), although for **2** and **15**, the curve shape suggested more complex behavior.

Computational Methods. DFT molecular orbital calculations were performed using Spartan 4.0 for Windows (Wavefunction Inc., CA) using minimum energy conformations obtained at the semiempirical AM1 level. All molecular modeling studies were performed on an SGI computer with the Sybyl 7.2 software packages. The coordinates for the estrogen receptor alpha (ERα) LBD were extracted from the cocrystal structure data of the complex between ERα LBD and raloxifene (PDB code: 1ERR). The coordinates for the estrogen receptor beta (ERβ) LBD were extracted from the cocrystal structure data of the complex between ERβ and tamoxifen (PDB code: 2FSZ). The active site was designated to consist of the amino acid residues within a radius of 4.5 Å from the original ligand raloxifene or tamoxifen. According to ERα (ERβ in parenthesis) reference numbering, Asp351(303), Glu353(305), Arg394(346), and His524(475) were set as a core subpocket. The following FlexX-Pharm settings were used to restrict the binding of the ligands to the raloxifene/tamoxifen binding site: Asp351-(303) is an optional hydrogen-bond acceptor, and a spatial constraint of r = 3 Å is set around carboxylate oxygens of Asp351(303) and the ligand's nitrogen.

Arzoxifene and its 4′-position modified analogs were screened. After ligand docking was performed by the FlexX and Flex-Pharm

modules in Sybyl, the 30 best poses were selected for each ligand and saved for analysis by CScore. We used a combination of several functions and the criterion of consensus ≥4 to select the best pose for each ligand. The binding modes of the docked ligands were found to be consistent with those expected for compounds that are structurally related to raloxifene and tamoxifen. The selection of the binding poses was based on FlexX score. Scoring and ranking of the poses using the CScore scoring function performed reasonably well for the ligands able to dock within the LBD cavity. Bulkier 4′-substituents failed to dock inside the cavity. For these ligands, manual docking was designed based on the top scoring DMA pose. Furthermore, the most meaningful pose or manually docked pose for each ligand was co-minimized with the receptor LBD by employing the Powell method with initial Simplex optimization (20 steps) using the Tripos force field, Gasteiger−Huckel charges, dielectric constant = 1, and nonbonding cutoff of 8 Å, until a convergence of 0.05 kcal/mol·Å was reached. The minimized poses were re-evaluated with CScore functions.

To examine the second, low-affinity reported binding site for tamoxifen in ERβ, a second screening for arzoxifene analogs was devised based on PBD ID: 2FSZ. Because most of the interaction of the low affinity binding site are based on van der Waals contacts, the second active site was designated to consist of amino acids with a radius of 5.5 Å from tamoxifen, with core subpocked residues such as Leu306, Met309, Ile310 Val 328, Leu331, Glu332, and Trp335 according to ERβ 2FSZ reference numbering. The binding resulted in weak, nonspecific association of hydrophobic 4′-substituents, with the hydrophobic groove of the coactivator recognition surface.

Energy Evaluation. To compare the energetic interactions of the arzoxifene series with the estrogen receptor ERα vs raloxifene interaction with ERα, the following equation was used

$$\Delta G_{\text{bind}} = (G_{\text{ER−analog complex}} - G_{\text{analog}}) - (G_{\text{ER−raloxifene complex}} - G_{\text{ER−raloxifene}})$$

Solute energy was evaluated for the lowest energy conformer in the same method as mentioned above for the ERα−analog complex.

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