JOURNAL OF
MEDICINAL
CHEMISTRY

Volume 40, Number 10 May 9, 1997

Expedited Articles

Discovery and Synthesis of [6-Hydroxy-3-[4-[2-(1-piperidinyl)ethoxy]phenoxy]-2-(4-hydroxyphenyl)]benzo[*b*]thiophene: A Novel, Highly Potent, Selective Estrogen Receptor Modulator

Alan D. Palkowitz,* Andrew L. Glasebrook, K. Jeff Thrasher, Kenneth L. Hauser, Lorri L. Short, D. Lynn Phillips, Brian S. Muehl, Masahiko Sato, Pamela K. Shetler, George J. Cullinan, Tina R. Pell, and Henry U. Bryant

Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285

Received March 13, 1997[⊗]

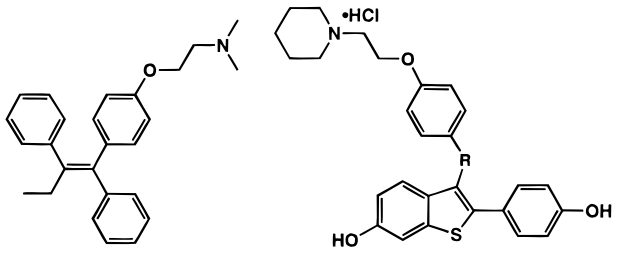
Raloxifene,[2-(4-hydroxyphenyl)-6-hydroxybenzo[*b*]thien-3-yl][4-[2-(1-piperidinyl)ethoxy]phenyl]-methanone hydrochloride (**2**), is representative of a class of compounds known as selective estrogen receptor modulators (SERMs) that possess estrogen agonist-like actions on bone tissues and serum lipids while displaying potent estrogen antagonist properties in the breast and uterus. As part of ongoing SAR studies with raloxifene, we found that replacement of the carbonyl group with oxygen ([6-hydroxy-3-[4-[2-(1-piperidinyl)ethoxy]phenoxy]-2-(4-hydroxyphenyl)]benzo[*b*]thiophene hydrochloride, **4c**) resulted in a substantial (10-fold) increase in estrogen antagonist potency relative to raloxifene in an *in vitro* estrogen dependent cell proliferation assay ($IC_{50} = 0.05$ nM) in which human breast cancer cells (MCF-7) were utilized. *In vivo*, **4c** potently inhibited the uterine proliferative response to exogenous estrogen in immature rats following both sc and oral dosing (ED_{50} of 0.006 and 0.25 mg/kg, respectively). In ovariectomized aged rats, **4c** produced a significant maximal decrease (45%) in total cholesterol at 1.0 mg/kg (po) and showed a protective effect on bone relative to controls with maximal efficacy at 1.0 mg/kg (po). These data identify **4c** as a novel SERM with greater potency to antagonize estrogen in uterine tissue and in human mammary cancer cells compared to raloxifene, tamoxifen or ICI-182,780.

Introduction

Over the past few years, it has become apparent that some compounds previously described as estrogen antagonists also demonstrate estrogen agonist properties in select tissues.[1] This selective estrogen receptor modulator (or SERM) profile has defined a spectrum of mixed agonist/antagonist activities for several structural classes of compounds that include the triphenylethylenes exemplified by tamoxifen (**1**) and benzothiophene derivatives represented by raloxifene (**2**) (Figure 1).[2] By contrast, the steroidal estrogen antagonist ICI-182,780 (**3**) is distinct in that it possesses no estrogen agonist properties.[3] A growing understanding of the pharmacology of these agents has suggested safe clinical uses of optimal compounds for the treatment of estrogen dependent diseases such as osteoporosis, breast cancer, and gynecological disorders.[4] Indeed, raloxifene has been shown in animal models to be an effective antagonist in uterine tissue while potently mimicking the favorable actions of estrogen on bone and lipids.[4a,5] As an extension of this hypothesis to humans, raloxifene is currently undergoing clinical evaluation for the treatment and prevention of osteoporosis as an alternative to hormone replacement therapy.[4b]

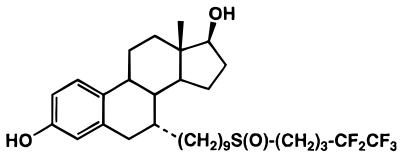
[⊗] Abstract published in *Advance ACS Abstracts,* April 15, 1997.

S0022-2623(97)00167-2 CCC: $14.00



1, tamoxifen

2, R = CO, raloxifene
4a, R = CH_2
4b, R = S
4c, R = O
4d, R = NH



3, ICI-182,780

Figure 1. Chemical structures of tamoxifen, ICI-182,780, raloxifene, and **4a**−**d**.

Recently, there has been considerable speculation as to the mechanism for the tissue selective actions of SERMs.[2a,6] One possibility is that effects of compounds such as raloxifene are mediated through the local expression of TGF-β_3, a multifunctional regulator of cell growth and differentiation. It has been shown that raloxifene upregulates the expression of TGF-β_3 mRNA in bone cells *in vitro* and *in vivo*, as well as stimulates the secretion of TGF-β in human MCF-7 cancer cells and fetal fibroblasts.[7] The distinct tissue actions may be inherent in that the TGF-β_3 promoter contains a sequence that selectively mediates the actions of raloxifene and other SERMs on TGF-β_3 expression, and is not effectively activated by estrogen.[8]

Our interest in the unique actions of raloxifene has prompted an expanded structure−activity relationship (SAR) evaluation of the series.[9] A primary goal of our efforts was to identify novel compounds with enhanced estrogen antagonist activity that maintain the SERM profile. One area of focus for our studies has been examination of point changes directed at the carbonyl region of raloxifene (**4a**−**d**, Figure 1). In this article we summarize the results of this investigation that have led to the discovery of [6-hydroxy-3-[4-[2-(1-piperidinyl)-ethoxy]phenoxy]-2-(4-hydroxyphenyl)]benzo[*b*]thiophene, **4c**. To our knowledge, benzothiophene **4c** is the most potent estrogen antagonist described to date that maintains estrogen agonist actions on bone and lipids.

Chemistry

Compound **4a** was prepared from raloxifene by a two-step reduction process ($LiAlH_4$ followed by Et_3SiH) in 44% overall yield. The syntheses of the thioether and the oxygen-linked derivatives (**4b** and **4c**, respectively) were accomplished via a convergent synthesis employing the known 2-arylbenzothiophene derivative **5** as a key starting material (Scheme 1).[9,10] Bromination of **5** (Br_2, $CHCl_3$) provided exclusively the 3-bromo derivative **6** in quantitative yield. This material was activated for nucleophilic displacement of bromine by oxidation of the sulfur to the sulfoxide **7**. The oxidation was accomplished by treatment of **6** with 1.0 equiv of H_2O_2 in 50% CF_3CO_2H in dichloromethane for 2 h at room temperature.[11] These conditions provided **7** exclusively in 80% yield with no observed overoxidation to the sulfone. Introduction of the basic side chain with construction of the key heteroatom bond was next accomplished by reaction of **7** with **8a**,**b** in DMF in the presence of NaH to give directly sulfoxides **9a** and **9b** in 37% and 89% yield, respectively.[12] Sulfoxide reduction ($LiAlH_4$, THF) and conversion to the hydrochloride salt gave **10a** (73%) and **10b** (96%).[13] Finally, cleavage of the methyl ethers (BBr_3, CH_2Cl_2) and conversion to the hydrochloride salt provided **4b** and **4c** in 60% and 74% yield, respectively.

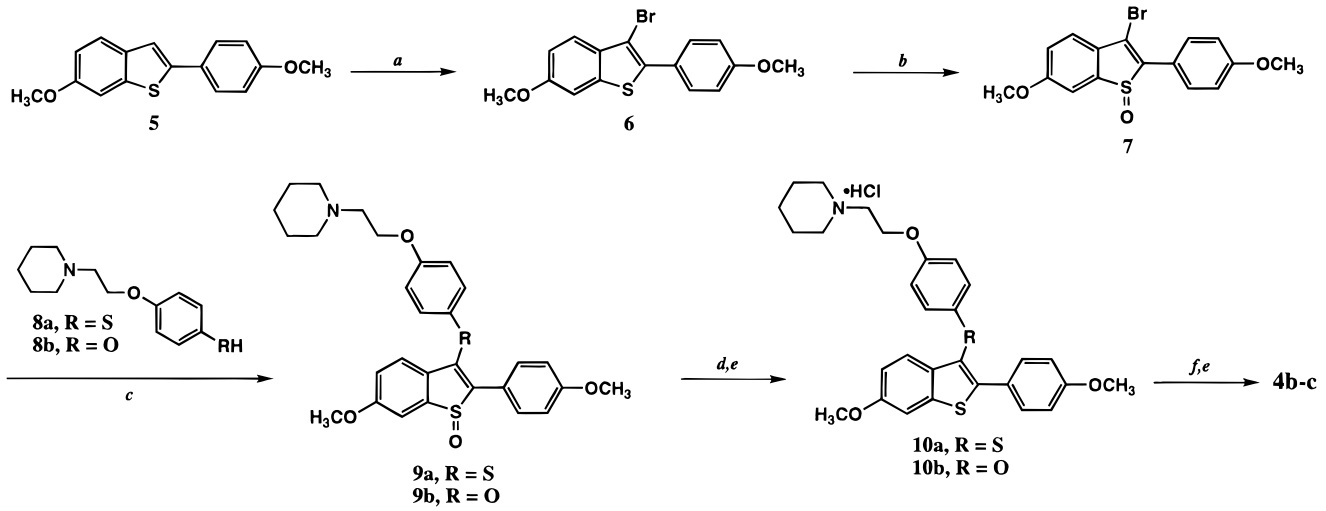
Attempts to extend the sulfoxide mediated chemistry to the synthesis of the N-linked derivative **4d** were unsuccessful. Alternatively, **4d** was prepared from the 3-bromobenzothiophene **6** via an Ullman coupling to form the biarylamine linkage.[14] Thus treatment of **6** with 4-(benzyloxy)acetanilide in refluxing collidine and in the presence of catalytic Cu_2O for 72 h provided coupled derivative **11** in 5% yield following isolation by column chromatography. No attempt was made at this point to optimize the yield of the coupling reaction. Compound **11** was readily transformed to the phenolic derivative **12** by catalytic hydrogenolysis of the benzyl ether protecting group (Scheme 2). The resulting phenol was then alkylated with 2-(chloroethyl)piperidine (Cs_2CO_3, DMF). The crude acetanilide derivative was hydrolyzed with concentrated NaOH to form the diamine that was transformed to the dihydrochloride salt (Et_2O·HCl) **13** in 90% yield from **12**. Dimethyl ether cleavage (BBr_3, CH_2Cl_2), followed by conversion to the hydrochloride salt, provided **4d** in 45% yield from **13**.

Biological Results and Discussion

The testing paradigm employed to guide our SAR studies and characterize the SERM profile consisted of *in vitro* and *in vivo* assays. *In vitro*, intrinsic estrogen antagonist activity (IC_{50}) was determined by measuring the ability of a compound to inhibit estrogen-induced proliferation of human MCF-7 breast cancer cells.[15] *In vivo*, estrogen antagonist activity (ED_{50}) was assessed following either po or sc administration by measuring the ability of a compound to block estrogen-stimulated uterine weight gain in immature rats.[9,16] To complete the evaluation of the SERM profile, agonist actions of compounds were assessed in an ovariectomized (OVX) adult rat model to determine effects on bone, uterine, and lipid parameters.[4a,17]

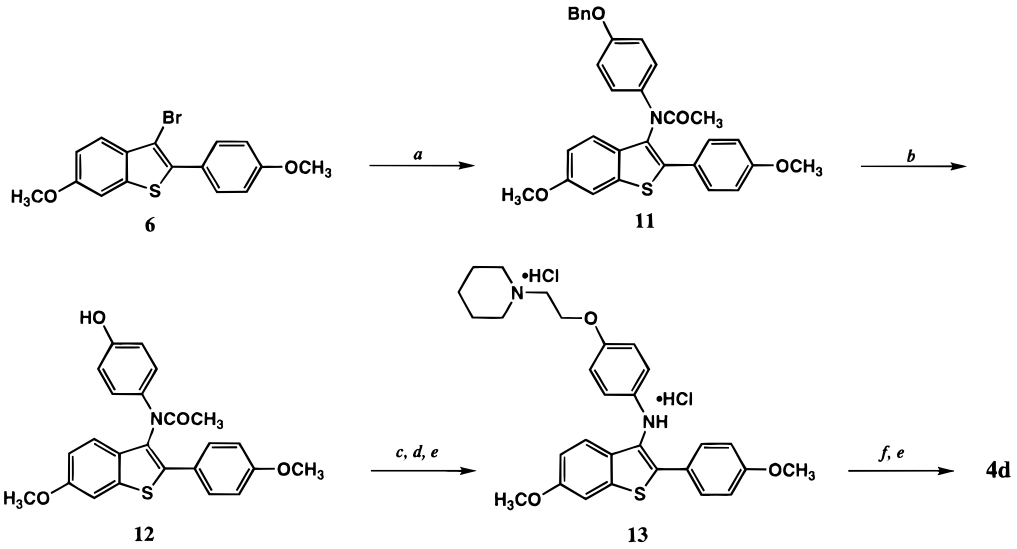
Table 1 shows a comparison of intrinsic estrogen antagonist activity for raloxifene and **4a**−**d** in the MCF-7 cell proliferation assay. Under the conditions of this experiment whereby the cells are stimulated with 10 pM 17β-estradiol (E_2), the IC_{50} value for raloxifene as an inhibitor of proliferation was 0.4 nM. Replacement of the raloxifene carbonyl with S, CH_2, or NH (**4a**,**b**,**d**) provided modest (2−5-fold) increases in antagonist potency. This effect was maximized with the introduction of an oxygen at this site (**4c**). The biaryl ether derivative **4c** produced an IC_{50} of 0.05 nM in this experiment, representing an approximately 10-fold increase in intrinsic estrogen antagonist potency over raloxifene. For comparison, 4-hydroxytamoxifen (the most active metabolite of tamoxifen) and ICI-182,780 under similar conditions yielded IC_{50} values of 1.17 and 0.49 nM, respectively.[18]

Scheme 1[a]



[a] Conditions: (a) Br_2, $CHCl_3$, 60 °C; (b) H_2O_2, CF_3CO_2H, CH_2Cl_2, 2 h; (c) **8a** or **8b**, NaH, DMF, then **7**, 1 h; (d) $LiAlH_4$, THF, 0 °C; (e) Et_2O·HCl, EtOAc; (f) BBr_3, CH_2Cl_2, 0 °C, 1 h.

Scheme 2[a]



[a] Conditions: (a) 4-benzyloxyacetanilide, Cu_2O, collidine, reflux, 72 h; (b) H_2, 10% Pd/C, EtOH/EtOAc, 1% concentrated HCl; (c) 2-(chloroethyl)piperidine hydrochloride, Cs_2CO_3, DMF, 25 °C, 24 h; (d) concentrated NaOH, EtOH, reflux, 18 h; (e) Et_2O·HCl, EtOAc; (f) BBr_3, CH_2Cl_2, −20 to 25 °C, 1−6 h.

Table 1. Physical Properties for **4a**−**d** and *in Vitro* and *in Vivo* Antagonism of Estrogen by **1**−**3** and **4a**−**d**

compound	mp, °C[a]	formula[a,b]	analyses[a]	*in vitro* MCF-7 cell proliferation IC_{50} (nM) ± SE[c]	*in vivo* immature rat uterine assay	
					ED_{50} (mg/kg/po)[c,d,e]	ED_{50} (mg/kg/sc)[c,d,e]
2 (raloxifene)	NA	NA	NA	0.4 ± 0.3 (n = 46)	0.55 ± 0.15 (n = 16)	0.05 (n = 2)
4a	>200	$C_{28}H_{29}NO_3S$·1.0HCl	C, H, N	0.19 ± 0.10 (n = 3)	0.47 ± 0.33 (n = 3)	ND
4b	180−190	$C_{27}H_{27}NO_3S_2$·2.2HCl	C, H, N	0.28 ± 0.15 (n = 4)	1.68 (n = 1)	ND
4c	158−165	$C_{27}H_{27}NO_4S$·1.0HCl	C, H, N	0.05 ± 0.02 (n = 6)	0.25 ± 0.12 (n = 5)	0.006 (n = 2)
4d	140−150	$C_{27}H_{28}N_2O_3S$·1.5HCl	C, H, N	0.10 (n = 2)	0.67 (n = 1)	ND
3 (ICI-182,780)	NA	NA	NA	0.49 ± 0.27 (n = 3)	0.62 (n = 1)	0.17 (n = 1)
1 (tamoxifen)	NA	NA	NA	481 ± 399 (n = 25)	>10 (n = 2)	ND
4-OH tamoxifen	NA	NA	NA	1.17 ± 0.69 (n = 5)	ND	ND

[a] NA = not applicable. [b] All compounds had C, H, and N microanalysis within ±0.4% theoretical value. [c] n = number of individual experiments. [d] ND = not determined. [e] ED_{50} values were detemined by regression analysis using the linear portion of each dose response curve (at least three point dose response) with six animals at each dose. For compounds evaluated in this assay in at least three separate experiments, an average ED_{50} ± SE was calculated. The ethynylestradiol stimulus used in this experiment was 0.1 mg/kg/day (po).

The marked increase in estrogen antagonist activity demonstrated by **4c** suggested that this molecule should bind to the estrogen receptor with enhanced affinity relative to that of raloxifene. This was indeed confirmed by studying the competitive binding of raloxifene and **4c** using an MCF-7 cell lysate preparation. Figure 2 (panel A) demonstrates that under physiologic conditions (37 °C), raloxifene produced a relative binding affinity (IC_{50} 17β-estradiol/IC_{50} test compound) of 0.2−0.3 whereas **4c** bound as efficiently as E_2 to the estrogen receptor. Interestingly, the binding of **4c** to the estrogen receptor was temperature dependent. As shown in

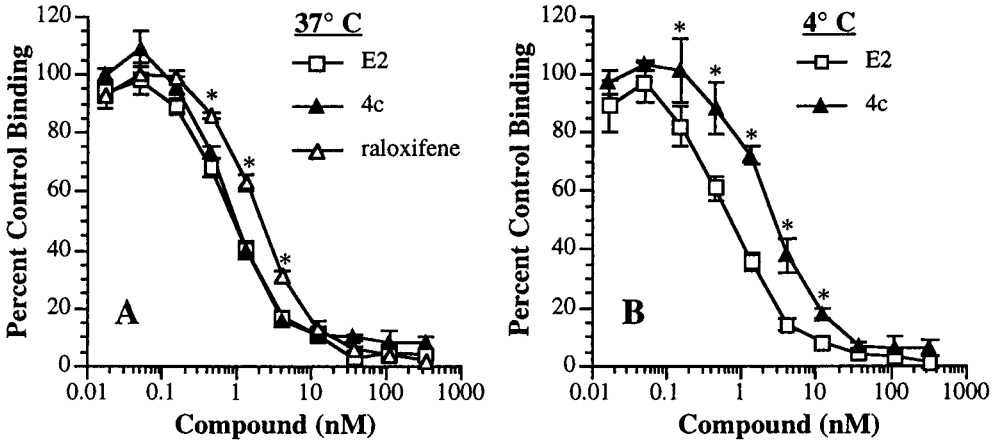


Figure 2. Competitive inhibition of [^{3}H]-17β-estradiol binding to human estrogen receptor. Whole cell lysates containing estrogen receptor were prepared from human MCF-7 cells, mixed with ^{3}H-17β-estradiol in the absence or presence of 17β-estradiol, **4c**, or raloxifene, and incubated at 37 °C (panel A) or 4 °C (panel B) for 20 h. Receptor bound radioactivity was then determined and results expressed as a percent of control binding in the absence of competitor. *$p < 0.05$ vs estrogen control and **4c** (panel A), vs estrogen control (panel B).

panel B of Figure 2, **4c** binds with significantly less affinity at 4 °C than at 37 °C, while the binding of estrogen was thermally unresponsive under the experimental conditions. Not shown was the binding curve for raloxifene at 4 °C, which like estrogen was unchanged by temperature. Studies are currently underway to provide insight into this phenomenon.

The increase in functional antagonism coupled with the higher affinity binding of **4c** was quite intriguing as it represented a profound change in the *in vitro* pharmacological profile with only minor structural modification (i.e. oxygen for carbonyl) of a relatively large and rigid molecule.[19] Studies are currently underway to develop an understanding of the structural and electronic consequences of replacing a carbonyl with oxygen in the raloxifene structure that may influence the interaction of the ligand with the estrogen receptor. For example, it is possible that the introduced change could significantly alter the three-dimensional placement of the basic side chain, which is an important pharmacophore element in conferring antagonist activity to this class of compounds.

The *in vivo* antagonist activities of **4a**−**d** and raloxifene were compared in a 3-day uterine immature rat assay. In young female rats (21-day-old Sprague−Dawley) the uterus is fully responsive to estrogen; however the ovaries do not produce 17β-estradiol. Since the uterus remains unstimulated, this model permits maximal stimulation by exogenous estrogen (ethynyl estradiol (EE) 0.1 mg/kg, po in this case), and hence a ready measure of an antagonist action can be determined. Test compounds were administered daily either po or sc following the estrogen stimulus. Twenty-four hours following the third daily dose, the animals were euthanized and the uteri removed and weighed. The ratio of uterine wet weight to body weight served as the pharmacological end point of this experiment. The results of these studies are summarized in Table 1. As an inhibitor of uterine stimulation, raloxifene demonstrated an oral ED_{50} of 0.55 mg/kg. Of the new derivatives, **4c** was the most potent with an ED_{50} of 0.25 mg/kg (po). The *in vivo* antagonism of **4c** compared to raloxifene was surprisingly low given the large separation of intrinsic estrogen antagonist activity measured *in vitro*. An understanding of this discrepancy was realized when the compounds were compared by an alternate route of administration. When dosed sc, **4c** produced an ED_{50} of 0.006 mg/kg, whereas raloxifene gave an ED_{50} of 0.05 mg/kg. The large separation in activity of oral vs sc suggested poor oral bioavailability of the compounds in this model, presumably due to extensive first pass conjugation (glucuronidation) of the 6 and 4′ phenolic groups. However, the difference in activity between **4c** and raloxifene when dosed subcutaneously was more consistent with the activity difference *in vitro*. Nevertheless, the level of activity observed for **4c** *in vivo* is representative of the most potent estrogen antagonist activity reported to date. For comparison, oral and sc administration of ICI-182,780 (which is in development as a parenteral agent) produced ED_{50} values of 0.62 and 0.17 mg/kg, respectively. Interestingly, tamoxifen produced a partial agonist response in this model. Although tamoxifen did significantly antagonize the estrogen response (ED_{50} estimated at >10.0 mg/kg po), the efficacy was limited to approximately 40% inhibition of the estrogen stimulus (Figure 3). All other agents studied produced maximal inhibition at the higher end of the dose response.

Finally, in order to evaluate the consistency of pharmacological effects of **4c** with the SERM profile established by raloxifene, studies were performed in OVX rats. Of particular interest were effects on bone (where raloxifene, like estrogen, protects against ovariectomy induced osteopenia), cholesterol metabolism (where raloxifene, like estrogen, lowers cholesterol), and the uterus (where raloxifene produces minimal estrogenic effects on uterine weight, eosinophilia, and epithelial cell height).

To measure effects on bone, OVX 6-month-old Sprague−Dawley rats were dosed daily with **4c** for 35 days. As shown in Figure 4, **4c** increased bone mineral density at the proximal tibia as compared to the OVX control (quantified by computed tomography) with significant effects being achieved at 1.0 mg/kg. This compares favorably with raloxifene, which produces comparable effects at 1.0 mg/kg and EE which provides a maximum response in this model at 0.1 mg/kg. In the same experiment, **4c** likewise displayed estrogen like actions on lipid metabolism as measured by a maximum 45% reduction of total serum cholesterol achieved at 1.0 mg/

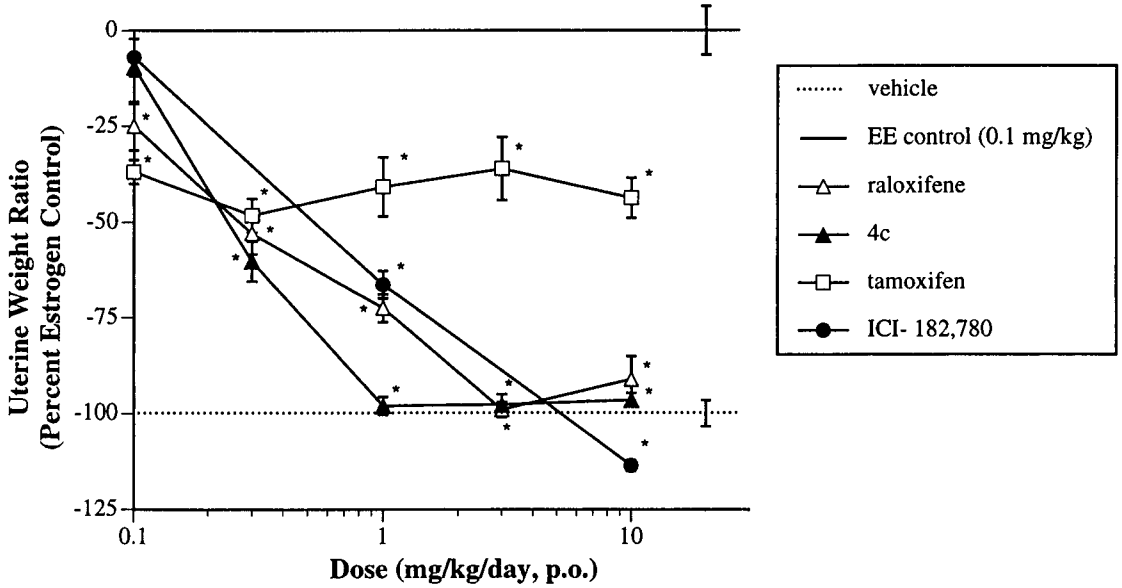


Figure 3. Representative experiment demonstrating estrogen antagonist effects of **1**−**3** and **4c** on the immature rat uterus. Each point represents the mean uterine weight/body weight ratio (as a percentage of the EE positive control) ± SEM for six rats. *p < 0.05 vs estrogen control.

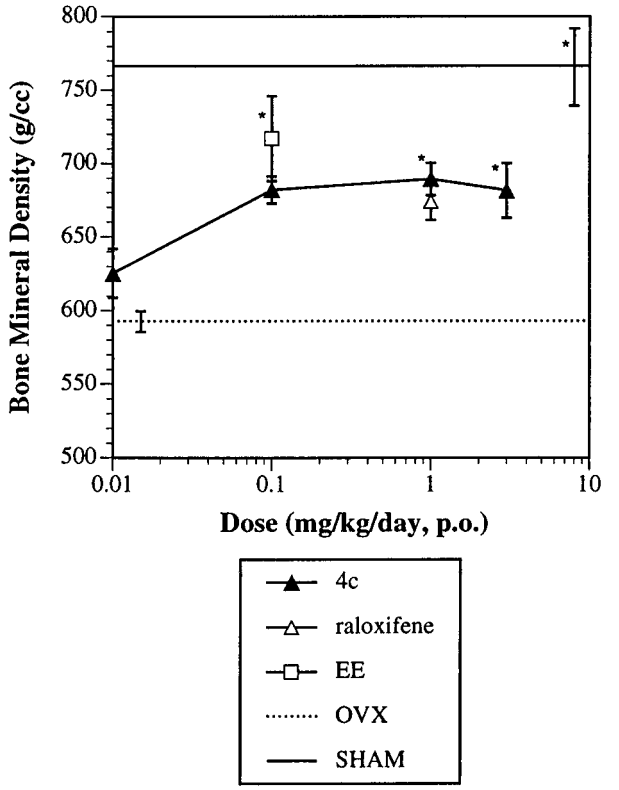


Figure 4. Antiosteopenic effect of **4c**, EE (0.1 mg/kg), and raloxifene (1.0 mg/kg) in OVX rats after 35 days of treatment (*p < 0.05 vs OVX control).

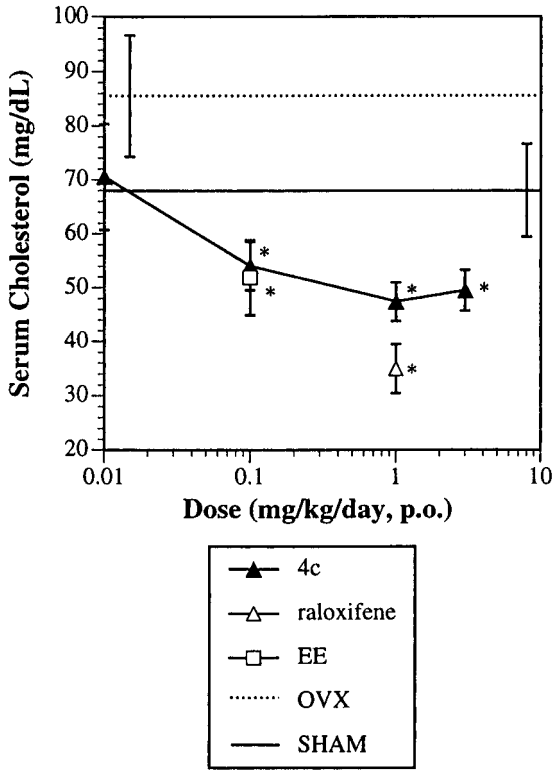


Figure 5. Effects of **4c**, EE (0.1 mg/kg), and raloxifene (1.0 mg/kg) on serum cholesterol in OVX rats after 35 days of treatment (*p < 0.05 vs OVX control).

kg (Figure 5). In addition, no increase in uterine weight was observed relative to the OVX controls for any dose of **4c** tested (Figure 6).

To more rigorously evaluate the effects of **4c** on uterine parameters, a study was conducted in a 4-day version of the OVX model described above. In this assay, **4c** produced no statistically significant effects on uterine weight, epithelial cell height, or eosinophil peroxidase activity relative to control at the doses tested (0.01−10.0 mg/kg), whereas EE produced a marked elevation of these parameters at 0.1 mg/kg.

Conclusion

In summary, as an extension of our SAR studies with raloxifene, we have identified a novel SERM (**4c**) that displays the most potent estrogen antagonist activity *in vitro* of any known agent using a human mammary carcinoma cell line (MCF-7). Furthermore, the superior estrogen antagonism of **4c** *in vivo* is suggestive from experiments in a rat model of EE-induced uterine hypeplasia (po and sc administration). In addition to the antagonist properties of **4c**, this agent retains beneficial estrogen agonist actions on bone and lipid metabolism. Studies ongoing to expand the SAR of the

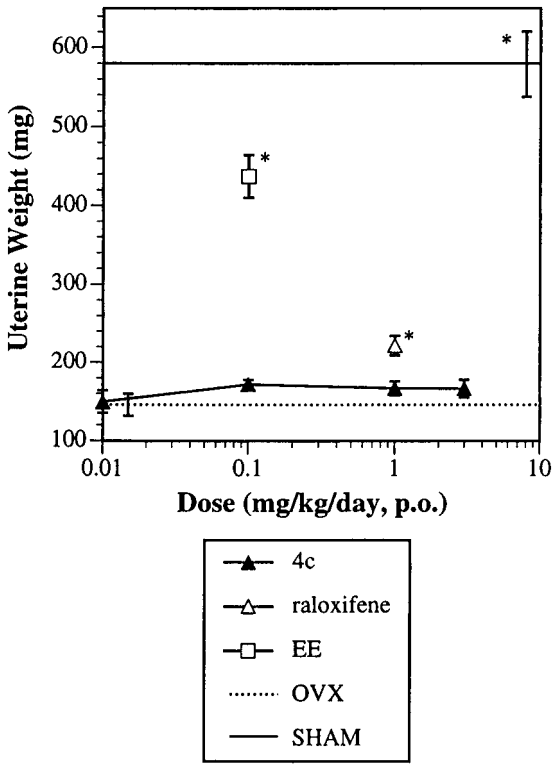


Figure 6. Effects of **4c**, EE (0.1 mg/kg), and raloxifene (1.0 mg/kg) on uterine weight in OVX rats after 35 days of treatment (*p < 0.05 vs OVX control).

biaryl ether benzothiophene series represented by **4c**, with particular emphasis on improving upon the oral bioavailability of this agent, will be reported in due course.

Experimental Section

General. Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. ^{1}H NMR spectra were obtained on a GE QE-300 spectrometer at 300.15 MHz in the solvent indicated. Field desorption (FD) mass spectra were recorded on a VG Analytical ZAB-3F instrument. High-resolution (HR) mass spectra were recorded on a VG Analytical ZAB2-SE instrument. Elemental analyses were determined by the Physical Chemistry Department at Lilly Research Laboratories and are within ±0.4% of the theoretical values unless otherwise indicated.

[2-(4-Hydroxyphenyl)-6-hydroxybenzo[*b*]thien-3-yl][4-[2-(1-piperidinyl)ethoxy]phenyl]methane Hydrochloride (4a). [2-(4-Hydroxyphenyl)-6-hydroxybenzo[*b*]thien-3-yl][4-[2-(1-piperidinyl)ethoxy]phenyl]methanone (**2**, raloxifene free base) (5.40 g, 11.0 mmol) was dissolved in 250 mL of anhydrous THF. The solution was cooled to 0 °C under a nitrogen atmosphere. Over a period of 20 min, 2.0 g (53 mmol) of $LiAlH_4$ was added to the stirring solution. The reaction was allowed to proceed for 6 h while slowly warming to ambient temperature. The solvent was then evaporated *in vacuo*. The residue was suspended in 100 mL of EtOAc, and 50 mL of H_2O was carefully added. The EtOAc layer was separated and extracted with additional H_2O (2×). The organic layer was dried by filtration through anhydrous Na_2SO_4 and the filtrate evaporated *in vacuo*. This provided 4.8 g (91%) of [2-(4-hydroxyphenyl)-6-hydroxybenzo[*b*]thien-3-yl][4-[2-(1-piperidinyl)ethoxy]phenyl]methanol. This material was characterized as the hydrochloride salt, prepared by the following procedure. The alcohol was dissolved in 50 mL of EtOAc, and a saturated solution of Et_2O·HCl was added until no additional precipitate formed. The precipitate was triturated twice with Et_2O. The white precipitate was collected by filtration to provide an amorphous powder that was dried *in vacuo* to yield 4.5 g (88%) of [2-(4-hydroxyphenyl)-6-hydroxybenzo[*b*]thien-3-yl][4-[2-(1-piperidinyl)ethoxy]phenyl]methanol hydrochloride: ^{1}H NMR (DMSO-d_6) δ 9.80 (bs, 1H), 9.55 (bs, 1H), 7.55 (d, J = 3.0 Hz, 1H), 7.40 (d, J = 3.0 Hz, 2H), 7.27 (d, J = 3.0 Hz, 2H), 7.25 (s, 1H), 6.9 (m, 5H), 6.0 (m, 2H), 3.9 (t, J = 4.0 Hz, 2H), 3.50 (m, 4H), 3.05 (t, J = 4.0 Hz, 2H), 1.85 (m, 4H), 1.40 (m, 2H); FD MS 476. Anal. ($C_{28}H_{30}ClNO_4$) C, H, N.

A solution of [2-(4-hydroxyphenyl)-6-hydroxybenzo[*b*]thien-3-yl][4-[2-(1-piperidinyl)ethoxy]phenyl]methanol (710 mg, 1.50 mmol) in 25 mL of CH_2Cl_2 was prepared. To this solution was added 210 mg (1.8 mmol) of Et_3SiH, and the solution was cooled to 0 °C and placed under a nitrogen atmosphere. After several minutes of stirring, 12 mL of CF_3CO_2H was added. The reaction was allowed to proceed for 16 h, while slowly warming to room temperature. The reaction was quenched with the addition of 50 mL of H_2O. The reaction mixture was made basic with the addition of saturated $NaHCO_3$ solution. The reaction mixture was extracted with 50 mL portions of CH_2Cl_2 (2×). The organic layer was separated, washed with water, dried (Na_2SO_4), and evaporated to 500 mg (72%) of the amorphous base. This material was dissolved in 50 mL of EtOAc, and a saturated solution of Et_2O·HCl was added until no more precipitate formed. The solvents were removed by evaporation *in vacuo*. [2-(4-Hydroxyphenyl)-6-hydroxybenzo-[*b*]thien-3-yl][4-[2-(1-piperidinyl)ethoxy]phenyl]methane hydrochloride (**4a**) (330 mg, 48%) was isolated as a white crystalline powder: mp > 200 °C; ^{1}H NMR (DMSO-d_6) δ 9.75 (s, 1H), 9.60 (s, 1H), 7.30 (m, 4H), 7.10 (s, 1H), 7.05 (d, J = 3.0 Hz, 1H), 6.90 (m, 4H), 6.80 (d, J = 6.8 Hz, 1H), 4.30 (t, J = 4.0 Hz, 2H), 4.10 (s, 2H), 3.40 (m, 4H), 2.95 (m, 2H), 1.75 (m, 4H), 1.40 (m, 2H); FD MS 460. Anal. ($C_{28}H_{29}NO_3S$·1.0HCl) C, H, N.

6-Methoxy-2-(4-methoxyphenyl)-3-bromobenzo[*b*]-thiophene (6). To a solution of 6-methoxy-2-(4-methoxyphenyl)benzo[*b*]thiophene (**5**) (27.0 g, 100 mmol) in 1.10 L of $CHCl_3$ at 60 °C was added bromine (15.98 g, 100 mmol) dropwise as a solution in 200 mL of $CHCl_3$. After the addition was complete, the reaction mixture was cooled to room temperature and the solvent removed *in vacuo* to provide 34.2 g (100%) of 6-methoxy-2-(4-methoxyphenyl)-3-bromobenzo[*b*]thiophene (**6**) as a white solid: mp 83−85 °C; ^{1}H NMR (DMSO-d_6) δ 7.70−7.62 (m, 4H), 7.17 (dd, J = 8.6, 2.0 Hz, 1H), 7.09 (d, J = 8.4 Hz, 2H); FD MS 349, 350. Anal. ($C_{16}H_{13}O_2SBr$) C,H.

6-Methoxy-2-(4-methoxyphenyl)-3-bromobenzo[*b*]-thiophene *S*-Oxide (7). To a solution of 6-methoxy-2-(4-methoxyphenyl)-3-bromobenzo[*b*]thiophene (10.0 g, 28.6 mmol) in 50 mL of anhydrous CH_2Cl_2 was added 50 mL of trifluoroacetic acid. After 5 min of stirring, H_2O_2 (4.0 mL, 28.6 mmol, 30% aqueous solution) was added. The resulting mixture was stirred at room temperature for 2 h. Solid sodium bisulfite (1.25 g) was added to the dark solution followed by 15 mL of H_2O. The mixture was stirred vigorously for 15 min and then concentrated *in vacuo*. The residue was partitioned between CH_2Cl_2 and saturated $NaHCO_3$ solution (200 mL each). The layers were separated, and the organic was extracted with saturated $NaHCO_3$ solution. The organic was then dried (Na_2SO_4) and concentrated *in vacuo* to a solid that was triturated from Et_2O/EtOAc. Filtration provided 8.20 g (80%) of 6-methoxy-2-(4-methoxyphenyl)-3-bromobenzo[*b*]thiophene *S*-oxide (**7**) as a yellow solid that can be recrystallized from EtOAc: mp 170−173 °C; ^{1}H NMR (DMSO-d_6) δ 7.24 (d, J = 2.2 Hz, 1H), 7.68 (d, J = 8.8 Hz, 2H), 7.54 (d, J = 8.5 Hz, 1H), 7.26 (dd, J = 8.5, 2.2 Hz, 1H), 7.10 (d, J = 8.8 H, 2H), 3.86 (s, 3H), 3.80 (s, 3H). Anal. ($C_{16}H_{13}O_3SBr$) C, H.

6-Methoxy-3-[4-[2-(1-piperidinyl)ethoxy]thiophenoxy]-2-(4-methoxyphenyl)]benzo[*b*]thiophene *S*-Oxide (9a). To a solution of hydroxyphenyl disulfide (3.3 g, 13.0 mmol) in 100 mL of anhydrous DMF were added 1-(2-chloroethyl)piperidine monohydrochloride (9.7 g, 52.0 mmol) and cesium carbonate (30 g, 91.0 mmol). The mixture was stirred overnight at room temperature and then concentrated *in vacuo*. The resulting residue was partitioned between chloroform and water and extracted with $CHCl_3$ (3×). The combined $CHCl_3$ layers were washed with brine, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The resulting residue was purified by chromatography (SiO_2, $CH_3OH/CHCl_3/NH_3$) to yield 4.6 g (75%) of the disulfide as a yellow oil: ^{1}H NMR (DMSO-d_6) δ 7.37 (d, J = 9

Hz, 4H), 6.94 (d, J = 9 Hz, 4H), 4.04 (t, J = 5.7 Hz, 4H), 2.62 (t, J = 5.7 Hz, 4H), 2.48 (m, 8H), 1.50 (m, 8H), 1.48 (m, 4H); FD MS 243.

To a solution of the disulfide in 20 mL of anhydrous THF was added $LiAlH_4$ (0.48 g, 1.28 mmol). The resulting slurry was heated to reflux for 1 h and then quenched with saturated aqueous sodium bicarbonate and extracted with $CHCl_3$ (2×). The combined organic was dried (Na_2SO_4), filtered, and concentrated *in vacuo* to give **8a** as a crude oil that was not purified. Crude **8a** was then dissolved in 10 mL of anhydrous DMF, and to this solution was added NaH (0.05 g, 1.32 mmol, 60% dispersion in mineral oil) followed by **7** (0.4 g, 1.1 mmol). The resulting slurry was heated to 90 °C for 1 h and then cooled to room temperature. The mixture was concentrated *in vacuo*, and the residue was partitioned between EtOAc and H_2O. The layers were separated and the aqueous layer was extracted with EtOAc (2×). The organic was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The crude product was purified by chromatography (SiO_2, CH_3OH/ $CHCl_3$, gradient) to provide 0.21 g (37% overall) of **9a** product as a yellow oil: ^{1}H NMR (DMSO-d_6) δ 7.68 (d, J = 2.3 Hz, 1H), 7.61 (d, J = 8.7 Hz, 2H), 7.20−7.28 (m, 3H), 7.05−7.15 (m, 3H), 6.87 (d, J = 8.7 Hz, 2H), 4.00 (t, J = 5.84 Hz, 2H), 3.84 (s, 3H), 3.82 (s, 3H), 2.60 (t, J = 5.6 Hz, 2H), 2.35−2.45 (m, 4H), 1.43 (m, 4H), 1.30−1.43 (m, 2H); FD MS 521; HRMS calcd for $C_{29}H_{32}NO_4S_2$ 522.177, found 522.176. Anal. ($C_{29}H_{31}NO_4S_2$) H, N; C: calcd 66.77, found 65.51.

Preparation of 4-[2-(1-Piperidinyl)ethoxy]phenol (8b). To a solution of 4-(benzyloxy)phenol (50.50 g, 0.25 mol) in 350 mL of anhydrous DMF was added 2-(chloroethyl)piperidine (46.30 g, 0.25 mol). After 10 min of stirring, K_2CO_3 (52.0 g, 0.375 mol) and Cs_2CO_3 (85.0 g, 0.25 mol) were added. The resulting heterogeneous mixture was stirred vigorously at room temperature for 48 h. The reaction mixture was then poured into H_2O (500 mL) and extracted with CH_2Cl_2. The organic layer was then extracted with 1 N NaOH several times and finally washed with brine. The organic was then dried (Na_2SO_4) and concentrated *in vacuo* to an oil. Chromatography (SiO_2, 1:1 hexane/EtOAc) provided 60.0 g (77%) of 4-[2-(1-piperidinyl)ethoxy]phenyl benzyl ether as a colorless oil. ^{1}H NMR (DMSO-d_6) δ 7.40−7.27 (m, 5H), 6.84 (q, J_{AB} = 11.5 Hz, 4H), 4.98 (s, 2H), 3.93 (t, J = 6.0 Hz, 2H), 2.56 (t, J = 6.0 Hz, 2H), 2.35−2.37 (m, 4H), 1.48−1.32 (m, 6H); FD MS 311. Anal. ($C_{20}H_{25}NO_2$) C, H, N.

4-[2-(1-Piperidinyl)ethoxy]phenyl benzyl ether (21.40 g, 68.81 mmol) was dissolved in 200 mL of 1:1 EtOH/EtOAc containing 1% concentrated HCl. The solution was transferred to a Parr apparatus, and 5% Pd/C (3.4 g) was added. The mixture was hydrogenated at 40 psi for 2 h. The mixture was then passed through a plug of Celite to remove catalyst. The filtrate was concentrated *in vacuo* to a solid that was slurried in Et_2O and filtered to provide 12.10 g (83%) of 4-[2-(1-piperidinyl)ethoxy]phenol (**8b**): mp 148−150 °C; ^{1}H NMR (DMSO-d_6) δ 8.40 (s, 1H), 6.70 (q, J_{AB} = 11.5 Hz, 4H), 3.93 (t, J = 6.0 Hz, 2H), 2.59 (t, J = 6.0 Hz, 2H), 2.42−2.38 (m, 4H), 1.52−1.32 (m, 6H); FD MS 221. Anal. ($C_{13}H_{19}NO_2$) C, H, N.

6-Methoxy-3-[4-[2-(1-piperidinyl)ethoxy]phenoxy]-2-(4-methoxyphenyl)benzo[*b*]thiophene *S*-oxide (9b). To a solution of 4-[2-(1-piperidinyl)ethoxy]phenol (0.32 g, 1.43 mmol) in 5 mL of anhydrous DMF at room temperature was added NaH (0.57 g, 1.43 mmol, 60% dispersion in mineral oil). After 15 min of stirring, 6-methoxy-2-(4-methoxyphenyl)-3-bromobenzo[*b*]thiophene *S*-oxide (0.50 g, 1.37 mmol) was added in small portions. After 1 h of stirring, the reaction was judged complete by TLC analysis. The solvent was removed *in vacuo*, and the residue was distributed between H_2O and 10% EtOH/EtOAc. The organic was washed several times with H_2O and then dried (Na_2SO_4). Concentration *in vacuo* gave an oil that was triturated from EtOAc/hexane to provide 0.62 g (89%) of 6-methoxy-3-[4-[2-(1-piperidinyl)ethoxy]phenoxy]-2-(4-methoxyphenyl)benzo[*b*]thiophene *S*-oxide as a light yellow solid: mp 97−100 °C; ^{1}H NMR (DMSO-d_6) δ 7.68 (d, J = 2.1 Hz, 1H), 7.62 (d, J = 8.8 Hz, 2H), 7.06−6.92 (m, 6H), 6.85 (d, J = 8.8 Hz, 2H), 3.94 (t, J = 6.0 Hz, 2H), 3.81 (s, 3H), 3.72 (s, 3H), 2.56 (t, J = 6.0 Hz, 2H), 2.39−2.32 (m, 4H), 1.47−1.32 (m, 6H). Anal. ($C_{29}H_{31}NO_5S$) C, H, N.

6-Methoxy-3-[4-[2-(1-piperidinyl)ethoxy]thiophenoxy]-2-(4-methoxyphenyl)benzo[*b*]thiophene (10a). To a solution of the sulfoxide (0.14 g, 0.27 mmol) in 10 mL of anhydrous THF was added $LiAlH_4$ (0.012 g, 0.29 mmol). The resulting slurry was stirred for 90 min at room temperature and was then quenched by the careful addition of H_2O. The solution was diluted with H_2O and then extracted with EtOAc (3×). The combined organic was extracted with brine (2×), dried (Na_2SO_4), filtered, and concentrated *in vacuo* to a residue. The residue was purified by chromatography (SiO_2, $CH_3OH/CHCl_3$, gradient) to yield 0.11 g (79%) of the desired product as a yellow oil. This material was transformed to the HCl salt by treatment with Et_2O·HCl in EtOAc. Isolation by vacuum filtration provided **10a** in 93% yield as a white solid: mp 198−201 °C; ^{1}H NMR (DMSO-d_6) δ 7.63 (d, J = 8.6 Hz, 2H), 7.62 (d, J = 2.0 Hz, 1H), 7.58 (d, J = 8.2 Hz, 1H), 7.07 (d, J = 8.6 Hz, 2H), 7.02 (dd, J = 8.2, 2.0 Hz, 1H), 6.92 (q, J_{AB} = 9.0 Hz, 4H), 4.24 (bt, 2H), 3.82 (s, 3H), 3.80 (s, 3H), 3.49−3.39 (m, 4H), 2.93 (m, 2H), 1.82−1.62 (m, 5H), 1.38 (m, 1H). Anal. ($C_{29}H_{32}NO_3S_2$·1.0HCl) C, H, N.

6-Methoxy-3-[4-[2-(1-piperidinyl)ethoxy]phenoxy]-2-(4-methoxyphenyl)benzo[*b*]thiophene Hydrochloride (10b). To a solution of 6-methoxy-3-[4-[2-(1-piperidinyl)-ethoxy]phenoxy]-2-(4-methoxyphenyl)benzo[*b*]thiophene *S*-oxide (3.00 g, 5.94 mmol) in 200 mL of anhydrous THF under N_2 at 0 °C was added $LiAlH_4$ (0.34 g, 8.91 mmol) in small portions. After 30 min of stirring, the reaction was quenched by the careful addition of 5.0 mL of 2.0 N NaOH. The mixture was stirred vigorously for 30 min, and additional 2.0 N NaOH was added to dissolve salts. The mixture was then distributed between H_2O and 10% EtOH/EtOAc. The layers were separated, and the aqueous layer was extracted several times with 10% EtOH/EtOAc. The organic was dried (Na_2SO_4) and concentrated *in vacuo* to an oil. The crude product was dissolved in 50 mL of 1:1 EtOAc/Et_2O and treated with excess Et_2O·HCl. The resulting precipitate was collected and dried to provide 2.98 g (96%) of 6-methoxy-3-[4-[2-(1-piperidinyl)-ethoxy]phenoxy]-2-(4-methoxyphenyl)benzo[*b*]thiophene hydrochloride (**10b**) as a white solid: mp 216−220 °C; ^{1}H NMR (DMSO-d_6) δ 10.20 (bs, 1H), 7.64 (d, J = 8.7 Hz, 2H), 7.59 (d, J = 1.5 Hz, 1H), 7.18 (d, J = 9.0 Hz, 1H), 7.00 (d, J = 8.7 Hz, 1H), 6.96 (dd, J = 9.0, 1.5 Hz, 1H), 6.92 (q, J_{AB} = 9.0 Hz, 4H), 4.31 (m, 2H), 3.83 (s, 3H), 3.77 (s, 3H), 3.43 (m, 4H), 2.97 (m, 2H), 1.77 (m, 5H), 1.37 (m, 1H); FD MS 489. Anal. ($C_{29}H_{31}NO_4S$·1.0HCl) C, H, N.

6-Hydroxy-3-[4-[2-(1-piperidinyl)ethoxy]thiophenoxy-2-(4-hydroxyphenyl)benzo[*b*]thiophene (4b). To a solution of 6-methoxy-3-[4-[2-(1-piperidinyl)ethoxy]thiophenoxy]-2-(4-methoxyphenyl)benzo[*b*]thiophene hydrochloride (160 mg, 0.29 mmol) in 15 mL of anhydrous CH_2Cl_2 at 0 °C under N_2 was added BBr_3 (0.15 mL). The resulting dark solution was stirred for 1 h at 0 °C and then immediately poured into a stirred solution of EtOAc/saturated $NaHCO_3$ solution (50 mL each). The layers were separated, and the aqueous layer was washed with EtOAc (3 × 30 mL). The organic was dried (Na_2SO_4) and concentrated *in vacuo* to a white solid. Chromatography (SiO_2, 0−5% $CH_3OH/CHCl_3$) provided of 6-hydroxy-3-[4-[2-(1-piperidinyl)ethoxy]thiophenoxy]-2-(4-hydroxyphenyl)benzo[*b*]thiophene that was converted to the hydrochloride salt as described above for **10a**. Isolation provided 95 mg (60%) of **4b** as a white solid: mp 180−190 °C; ^{1}H NMR (DMSO-d_6) δ 9.86 (s, 1H), 9.79 (s, 1H), 7.46 (d, J = 8.5 Hz, 2H), 7.42 (d, J = 8.7 Hz, 1H), 7.29 (d, J = 2.0 Hz, 1H), 6.96 (d, J = 8.7 Hz, 2H), 6.86−6.81 (m, 5H), 4.27 (m, 2H), 3.41−3.37 (m, 4H), 2.96−2.84 (m, 2H), 1.77−1.60 (m, 5H), 1.35−1.28 (m, 1H); FD MS 477. Anal. ($C_{27}H_{27}NO_3S_2$·2.2HCl) C, H, N.

6-Hydroxy-3-[4-[2-(1-piperidinyl)ethoxy]phenoxy]-2-(4-hydroxyphenyl)benzo[*b*]thiophene (4c). 6-Methoxy-3-[4-[2-(1-piperidinyl)ethoxy]phenoxy]-2-(4-methoxyphenyl)benzo-[*b*]thiophene hydrochloride (**10b**) (10.0 g, 19.1 mmol) was dissolved in 500 mL of anhydrous CH_2Cl_2 and cooled to 8 °C. To this solution was added BBr_3 (7.20 mL, 76.2 mmol). The resultant mixture was stirred at 8 °C for 2.5 h. The reaction was quenched by pouring into a stirring solution of saturated $NaHCO_3$ (1 L) and cooled to 0 °C. The CH_2Cl_2 layer was separated, and the remaining solids were dissolved in CH_3-

OH/EtOAc. The aqueous layer was then extracted with 5% CH_3OH/EtOAc (3 × 500 mL). All of the organic extracts (EtOAc and CH_2Cl_2) were combined and dried (Na_2SO_4). Concentration *in vacuo* provided a tan solid that was chromatographed (SiO_2, 1−7% $CH_3OH/CHCl_3$) to provide 7.13 g (81%) of 6-hydroxy-3-[4-[2-(1-piperidinyl)ethoxy]phenoxy]-2-(4-hydroxyphenyl)benzo[*b*]thiophene as a white solid. This material was converted to the hydrochloride salt (**4c**) by treatment with Et_2O·HCl in EtOAc and isolated in 91% yield as a white solid: mp 158−165 °C; ^{1}H NMR (DMSO-d_6) δ 9.79 (s, 1H), 9.74 (s, 1H), 7.40 (d, J = 8.6 Hz, 2H), 7.23 (d, J = 2.0 Hz, 1H), 7.04 (d, J = 8.6 Hz, 1H), 6.86 (q, J_{AB} = 9.3 Hz, 4H), 6.76 (dd, J = 8.6, 2.0 Hz, 1), 6.74 (d, J = 8.6 Hz, 2H), 4.26 (bt, 2H), 3.37 (m, 4H), 2.91 (m, 2H), 1.72 (m, 5 H), 1.25 (m, 1H); FD MS 461. Anal. ($C_{27}H_{27}NO_4S$·1.0HCl) C, H, N.

6-Methoxy-2-(4-methoxyphenyl)-3-[4-(benzyloxy)-*N*-acetamidophenyl]benzo[*b*]thiophene (11). To a solution of 6-methoxy-2-(4-methoxyphenyl)-3-bromobenzo[*b*]thiophene (34.20 g, 0.098 mol) and 4-(benzyloxy)acetanilide (23.7 g, 0.98 mol) in 100 mL of 2,4,6-collidine under N_2 was added Cu_2O (14.0 g, 0.98 mol). The resulting mixture was heated to reflux for 72 h. Upon cooling, the reaction mixture was diluted with CH_2Cl_2 and the precipitated solids were removed by filtration. The filtrate was concentrated *in vacuo*, and the residue was dissolved in EtOAc. The organic was then extracted several times with 5.0 N HCl. The organic was dried (Na_2SO_4) and concentrated *in vacuo* to a solid. Chromatography (SiO_2, $CHCl_3$) provided 2.43 g (5%) of [6-methoxy-2-(4-methoxyphenyl)-3-[4-(benzyloxy)-*N*-acetamidophenyl]benzo[*b*]thiophene (**11**) as an amber foam: ^{1}H NMR (DMSO-d_6) δ (doubling due to amide rotamers) 7.70−6.80 (m, 16H), 4.90 (bs, 2H), 3.78−3.75 (m, 6H), 2.11 and 1.78 (s, 3H); FD MS 509. Anal. ($C_{31}H_{27}NO_4S$) C, H, N.

6-Methoxy-2-(4-methoxyphenyl)-3-(4-hydroxy-*N*-acetamidophenyl)benzo[*b*]thiophene (12). 6-Methoxy-2-(4-methoxyphenyl)-3-[4-(benzyloxy)-*N*-acetamidophenyl]benzo[*b*]thiophene (2.43 g, 4.77 mmol) was dissolved in 50 mL of 1:1 EtOH/EtOAc containing 5% concentrated HCl. To this solution was added 1.0 g of 5% Pd/C. The resulting mixture was hydrogenated at 40 psi for 4 h using a Parr apparatus. The mixture was then filtered through Celite to remove the catalyst. The filtrate was concentrated *in vacuo* to a semisolid. Chromatography (SiO_2, CHl_3) provided 1.32 g (83%) of 6-methoxy-2-(4-methoxyphenyl)-3-(4-hydroxy-*N*-acetamidophenyl)-benzo[*b*]thiophene (**12**) as an amber foam: ^{1}H NMR (DMSO-d_6) δ (doubling due to amide rotamers) 9.53 and 9.36 (s, 1H), 7.62−6.51 (m, 11 H), 3.78 and 3.58 (s, 6H), 2.01 and 1.76 (s, 3H); FD MS 419. Anal. ($C_{24}H_{21}NO_4S$·0.19$CHCl_3$) C, H, N.

6-Methoxy-2-(4-methoxyphenyl)-3-[4-[[2-(1-piperidinyl)-ethoxy]amino]phenyl]benzo[*b*]thiophene Hydrochloride (13). To a solution of 6-methoxy-2-(4-methoxyphenyl)-3-(4-hydroxy-*N*-acetamidophenyl)benzo[*b*]thiophene (1.31 g, 3.13 mmol) and Cs_2CO_3 (4.10 g, 12.50 mmol) in 15 mL of anhydrous DMF was added 2-(chloroethyl)piperidine hydrochloride (1.15 g, 6.26 mmol). The resulting mixture was stirred vigorously at room temperature for 5 h. The reaction mixture was diluted with 200 mL of H_2O and then extracted several times with EtOAc. The combined organic was then washed with H_2O several times and dried (Na_2SO_4). Concentration *in vacuo* provided and oil. Chromatography (1% CH_3OH/$CHCl_3$, SiO_2) provided 1.49 g (90%) of 6-methoxy-2-(4-methoxyphenyl)-3-[4-[2-(1-piperidinyl)ethoxy]-*N*-acetamidophenyl]-benzo[*b*]thiophene as an amber oil: ^{1}H NMR (DMSO-d_6) δ (doubling due to amide rotamers) 7.66−6.70 (m, 11 H), 3.93 (m, 2H) 3.78 and 3.58 (s, 6H), 2.8−2.30 (m, 6H), 2.01 and 1.78 (s, 3H), 1.52−1.11 (m, 6H); FD MS 530. Anal. ($C_{31}H_{34}N_2O_4S$) C, H, N.

To a solution of 6-methoxy-2-(4-methoxyphenyl)-3-[4-[2-(1-piperidinyl))ethoxy)-N-acetamidophenyl)benzo[*b*]thiophene (1.49 g, 2.80 mmol) in 10 mL of absolute EtOH was added 10 mL of 50% NaOH solution. The resulting mixture was heated to reflux for 18 h. Upon cooling, the mixture was diluted with 200 mL of H_2O. The aqueous layer was then extracted with EtOAc (3 × 100 mL). The organic layer was combined, washed with brine, and then dried (Na_2SO_4). Concentration *in vacuo* provided a foam that was chromatographed (SiO_2, $CHCl_3$). The isolated oil was converted to the hydrochloride salt by treatment with Et_2O·HCl in EtOAc. Isolation by filtration provided 1.37 g (100%) of 6-methoxy-2-(4-methoxyphenyl)-3-[4-[[2-(1-piperidinyl))ethoxy]amino]phenyl]benzo[*b*]thiophene hydrochloride (**13**) as a white solid: mp 126−130 °C; ^{1}H NMR (DMSO-d_6) δ 7.54 (d, J = 8.8 Hz, 2H), 7.48 (d, J = 2.2 Hz, 1H), 7.29 (d, J = 8.9 Hz, 1H), 6.92 (d, J = 8.8 Hz, 2H), 6.91 (dd, J = 8.9, 2.2 Hz, 1H), 6.73 (d, J = 8.9 Hz, 2H), 6.50 (d, J = 8.9 Hz, 2H), 4.21 (t, J = 4.7 Hz, 2H), 3.78 (s, 3H), 3.71 (s, 3H), 3.43−3.31 (m, 4H), 2.97−2.90 (m, 2H), 1.80−1.61 (m, 5H), 1.35 (m, 1H); FD MS 488. Anal. ($C_{29}H_{34}N_2O_3S$·2.0HCl) C, H, N.

6-Hydroxy-2-(4-hydroxyphenyl)-3-[4-[[2-(1-piperidinyl)-ethoxy]amino]phenyl]benzo[*b*]thiophene Hydrochloride (4d). To a solution of 6-methoxy-2-(4-methoxyphenyl)-3-[4-[[2-(1-piperidinyl)ethoxy]amino]phenyl]benzo[*b*]-thiophene hydrochloride (0.70 g, 1.25 mmol) in 40 mL of anhydrous CH_2Cl_2 under N_2 10 °C was added BBr_3 (0.36 mL, 3.80 mmol). The solution was allowed to gradually warm to room temperature and stirred for a total of 6 h. The reaction was quenched by pouring the mixture into an excess of saturated $NaHCO_3$ solution. The aqueous layer was then extracted several times with 5% EtOH/EtOAc. The organic layer was combined and dried (Na_2SO_4) and then concentrated *in vacuo* to a foam. The crude product was chromatographed (1−6% $CH_3OH/CHCl_3$) to provide 325 mg (49%) of 6-hydroxy-2-(4-hydroxyphenyl)-3-[4-[[2-(1-piperidinyl)ethoxy]amino]phenyl]benzo[*b*]thiophene as an oil. This material was converted to the hydrochloride salt (**4d**) by treatment with Et_2O·HCl in EtOAc and isolated as a white solid in 92% yield by filtration: mp 140−150 °C; ^{1}H NMR (DMSO-d_6) δ 9.57 (s, 2H), 7.40 (d, J = 8.6 Hz, 2H), 7.19 (d, J = 10.1 Hz, 1H), 7.16 (d, J = 2.0 Hz, 1H), 6.75 (dd, J = 10.1, 2.0 Hz, 1H), 6.70 (d, J = 8.6 Hz, 2H), 6.64 (d, J = 8.8 Hz, 2H), 6.46 (d, J = 8.8 Hz, 2H), 4.21 (m, 2H), 3.50−3.30 (m, 4H), 3.01−2.95 (m, 2H), 1.81−1.63 (m, 5H), 1.38 (m, 1H); FD MS 461. Anal. ($C_{27}H_{28}N_2O_3S$·1.5HCl) C, H, N.

Pharmacological Methods: Estrogen Receptor Binding. Serial dilution of test compounds or 17β-estradiol were mixed with 0.5 nM of [^{3}H]-17β-estradiol, along with 0.5 mg/mL of protein from MCF-7 cell lysates, to a total volume of 0.14 mL. Binding took place for 1 h at 37 °C, followed by addition of 0.07 mL of dextran/charcoal and centrifugation to remove nonbound radioligand. Aliquots of supernatant containing bound radioligand were mixed with scintillant and counted. Relative binding affinity (RBA) was calculated as (IC_{50} 17β-estradiol/IC_{50} test compound) × 100.

MCF-7 Breast Cancer Cell Proliferation. MCF-7 breast adenocarcinoma cells (ATCC HTB 22) were maintained in MEM (Minimal Essential Medium, minus phenol red, Sigma, St. Louis, MO) supplemented with 10 % fetal bovine serum (FBS) (V/V), L-glutamine (2 μM), sodium pyruvate (1 μM), HEPES [*N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid), 10 μM], nonessential amino acids, and bovine insulin (1 mg/mL). Ten days prior to assay, MCF-7 cells were switched to maintenance medium supplemented with 10% dextran-coated FBS in place of 10% FBS to deplete internal stores of steroids. MCF-7 cells were removed from maintenance flasks using cell dissociation medium (Ca^{2+}/Mg^{2+} free HBSS (phenol red-free) supplemented with 10 μM HEPES and 2 nM EDTA). Cells were washed twice with assay medium and adjusted to 80 000 cells/mL. Approximately 100 μL (8000 cells) was added to flat-bottomed microculture wells (Costar 3596) and incubated at 37 °C in a 5% CO_2 humidified incubator for 48 h to allow for cell adherence and equilibration transfer. Serial dilutions of test compounds or DMSO as a diluent control were prepared in assay medium and 50 μL transferred to triplicate microcultures followed by 50 μL assay medium for a final volume of 200 μL. After an additional 48 h incubation, microcultures were pulsed with 1 μCi [^{3}H]thymidine (specific activity 6.7 Ci/mmol; DuPont NEN) for the last 4−6 h of culture and the assay terminated by freezing at −70 °C. Microcultures were then thawed and harvested using a Skatron semiautomatic cell harvester. Samples were counted by liquid scintilation using a Wallace BetaPlate β counter, and

plotting of dpm versus compound concentration was used to determine the mean $IC_{50} \pm$ standar error (SE).

3-Day *in Vivo* Antiestrogen Assay. *In vivo* antiestrogen activity was determined in 21-day-old Sprague−Dawley rats (Charles River Laboratories, Portage, MI). Animals were grouped based on a 12 h light:12 h dark cycle with room temperature set at 22 °C. The animals had *ad libitum* access to both food and tap water. Ethynylestradiol (Sigma Chemical, St. Louis, MO) at a dose of 0.1 mg/kg/d (po) was used as the estrogenic stimulus to increase uterine weight in these animals. Test compounds were administered by oral gavage in a volume of 0.2 mL, 15 min prior to the ethynylestradiol gavage. Dosing with the test compounds was continued for 3 consecutive days. The animals were fasted over the night following the final dose. On the following morning, the animals were weighed and then euthanized (by CO_2 asphyxiation), and uteri were rapidly collected and weighed. All test compounds and ethynylestradiol were dissolved in 20% β-hydroxycyclodextrin (SAF Bulk Chemical). Nonestrogenic controls were given the vehicle (po) only.

Uterine weight/body weight ratios (UWR) were calculated for each animal. The percent inhibition of the estrogen-induced response was then calculated by the following formula: percent inhibition $= 100 \times [(UWR_{estrogen} - UWR_{test\ compound})/(UWR_{estrogen} - UWR_{control})]$. ED_{50} values were derived from a semilog regression analysis of the linear aspect of the dose−response curve.

4-Day OVX Rat Agonist Assay. Virgin, OVX Sprague−Dawley rats (75-day-old) were obtained from Charles River and maintained under similar conditions as described above. Compound administration (dissolved in 20% β-hydroxycyclodextrin) was initiated 14 days after ovariectomy by oral gavage and continued daily for 4 consecutive days. The animals were fasted the evening following the final dose. On the following morning the animals were weighed and then anesthetized (CO_2), and a blood sample was collected by cardiac puncture. The animals were then euthanized by CO_2 asphyxiation, and the uteri were collected and weighed. One horn of the uterus was weighed separately and transferred into Tris buffer for analysis of uterine eosinophil peroxidase activity. Uteri were homogenized on ice in 50 mM Tris buffer (pH = 8.0; 50 μL/mg tissue) containing Triton X-100 (0.05%). Samples were centrifuged at 3000 rpm for 10 min at 4 °C. The resulting supernatant was filtered through a 45 μm filter. Duplicate 50 μL aliquots of the filtered supernatant were measured for eosinophil peroxidase activity as previously described by White et al. (*J. Immunol. Med.* **1991**, *44*, 257−263). Briefly, a calorimetric reaction was initiated by the addition of a substrate solution containing 3.5 nM o-phenylenediamine and H_2O_2 (0.0005%) in 50 mM Tris buffer. The apparent maximal velocity was determined by monitoring the oxidation of *o*-phenylenediamine at 490 nm on a kinetic microplate reader.

Blood samples were allowed to clot at 4 °C for 2 h and then centrifuged at 2000*g* for 10 min. Serum samples were collected and stored at −70 °C for subsequent analytical procedures. Serum cholesterol was determined using a Boehringer Mannheim Diagnostics high-performance cholesterol colorimetric assay.

5-Week OVX Rat Bone Assay. Virgin, OVX Sprague−Dawley rats (6-month-old) were obtained from Charles River and maintained under similar conditions as described above. Compound administration was initiated within 4 days of ovariectomy by oral gavage and continued daily for 5 weeks. The animals were fasted the night prior to sacrifice and euthanized as described above. In addition to collecting blood for cholesterol analysis, the left tibia was removed and frozen (−20 °C) for subsequent bone mineral density (BMD) determination. BMD was measured by quantitative computed tomography using a 960A pQCT (Norland/Stratec, Ft. Atkinson, WI) as previously described (Sato et al., 1995).

Statistics. All treatment groups for *in vivo* studies contained five or six animals. Statistical evaluations were made by one-way analysis of variance (ANOVA) with post-hoc Fisher's PLSD analysis when indicated. Significance was ascribed at $p < 0.05$.

Acknowledgment. The authors thank J. Dodge, J. Sluka, K. Fahey, M. Martin, S. Jones, R. Murff, and K. Marron for the preparation of ICI-182,780 and 4-hydroxytamoxifen.

References

(1) Grese, T. A.; Dodge, J. A. Estrogen receptor modulators: effects in non-traditional target tissues. In *Annual Reports in Medicinal Chemistry*; Bristol, J. A., Ed.; Academic Press: New York, 1996; Vol. 31, pp 181−190.

(2) (a) McDonnell, D. P.; Clemm, D. L.; Hermann, T.; Goldman, M. E.; Pike, J. W. Analysis of estrogen receptor function *in vitro* reveals three distinct classes of antiestrogens. *Mol. Endocrinol.* **1995**, *9*, 659−669. (b) Kauffman, R. F.; Bryant, H. U. Selective estrogen receptor modulators. *Drug News Perspect.* **1995**, *8*, 531−539. (c) Sato, M.; Rippy, M. K.; Bryant, H. U. Raloxifene, tamoxifen, nafoxidine, or estrogen effects on reproductive and nonreproductive tissues in ovariectomized rats. *FASEB J.* **1996**, *10*, 905−912.

(3) (a) Wakeling, A. E.; Dukes, M.; Bowler, J. A potent specific pure antiestrogen with clinical potential. *Cancer Res.* **1991**, *51*, 3867−3873. (b) Gallagher A.; Chambers, T. J.; Tobias, J. H. The estrogen antagonist ICI 182780 reduces cancellous bone volume in female rats. *Endocrinology* **1993**, *133*, 835−842. (c) Bryant, H. U.; Wilson, P. K.; Adrian, M. D.; Cole, H. W.; Phillips, D. L.; Dodge, J. A.; Grese, T. A.; Sluka, J. P.; Glasebrook, A. L. Selective estrogen receptor modulators: pharmacological profile in the rat uterus. *J. Soc. Gynecol. Invest.* **1996**, *3*, 152A.

(4) (a) Black, L. J.; Sato, M.; Rowley, E. R.; Magee, D. E.; Bekele, A.; Williams, D. C.; Cullinan, G. J.; Bendele, R.; Kauffmann, R. F.; Bensch, W. R.; Frolik, C. A.; Termine, J. D.; Bryant, H. U. Raloxifene (LY139481 HCl) prevents bone loss and reduces serum cholesterol without causing uterine hypertrophy in ovariectomized rats. *J Clin. Invest.* **1994**, *93*, 63−69. (b) Draper, M. W.; Flowers, D. E.; Huster, W. J.; Nield, J. A.; Harper, K. D.; Arnaud, C. A controlled trial of raloxifene (LY139481 HCl): Impact on bone turnover and serum lipid profile in healthy postmenopausal women. *J. Bone Mineral Res.* **1996**, *11*, 835−842. (c) Jordan, V. C.; Allen, K. E.; Dix, C. J. Pharmacology of tamoxifen in laboratory animals. *Cancer Treat. Rep.* **1980**, *64*, 745−759. (d) Jordan, V. C. A current view of tamoxifen for the treatment and prevention of breast cancer. *Br. J. Pharmacol.* **1993**, *110*, 507−517. (e) Lindsay, R. Sex steroids in the pathogenesis and prevention of osteoporosis. In *Osteoporosis: Etiology, Diagnosis, and Management*; Riggs, B. L., Melton, L. J., Eds.; Raven: New York, 1988; pp 333−358. (f) Ciocca, D. R.; D. R.; Vargas Roig, L. M. Estrogen receptors in human nontarget tissues: biological and clinical implications. *Endocr. Rev.* **1995**, *16*, 35−62. (g) Session, D. R.; Kelley, A. C.; Jewelewicz, R. Current concepts in estrogen replacement therapy in the menopause. *Fertility Sterility* **1993**, *2*, 277−284. (h) Colditz, G. A.; Hankinson, S. E.; Hunter, D. J.; Willett, W. C.; Manson, J. E.; Stampfer, M. J.; Hennekens, C.; Rosner, B.; Speizer, F. E. The use of estrogens and progestins and the risk of breast cancer in postmenopausal women. *N. Engl. J. Med.* **1995**, *332*, 1589−1593. (i) Porter, K. B.; Tsibris, J. C. M.; Porter, G. W.; Fuchs-Young, R.; O'Brien, W. F.; Knadler, M. P.; Spellacy, W. N. Raloxifene causes rapid regression of abdominal wall leiomyomas in the estrogen-induced guinea pig model. *J. Soc. Gynecol. Invest.* **1996**, *3*, 147A.

(5) Clemens, J. A.; Bennett, D. R.; Black, L. J.; Jones, C. D. Effects of a new antiestrogen, keoxifene (LY156758), on growth of carcinogen-induced mammary tumors on LH and prolactin levels. *Life Sci.* **1983**, *32*, 2869−2875.

(6) Katzenellenbogen, J. A.; O'Malley, B. W.; Katzenellenbogen, B. S. Tripartite steroid hormone receptor pharmacology: Interaction with multiple effector sites as basis for the cell- and promoter-specific action of these hormones. *Mol. Endocrinol.* **1996**, *10*, 119−131.

(7) (a) Knabbe, C.; Lippman, M. E.; Wakefield, L. M.; Flanders, K. C.; Kasid, A.; Derynck, R.; Dickon, R. B. Evidence that transforming growth facotr-beta is a hormonally regulated negative growth factor in human breast cancer cells. *Cell* **1987**, *48*, 417−428. (b) Colletta, A. A.; Wakefield, L. M.; Howell, F. V.; van Roozendaal, K. E. P.; Danielpour, D.; Ebbs, S. R.; Sporn, M. B.; Baum, M. Anti-estrogens induce the secretion of active transforming growth factor beta from human fetal fibroblasts. *Br. J. Cancer* **1990**, *62*, 405−409. (c) Yang, N. N.; Bryant, H. U.; Hardikar, S.; Sato, M.; Galvin, R.; Glasebrook, A. L. Estrogen and raloxifene stimulate transforming growth factor beta-3 (TGF-b3) gene expression in rat bone: a potential mechanism for estrogen and raloxifene mediated bone maintenance. *Endocrinology* **1996**, *137*, 2075−2081.

(8) Yang, N. N.; Venugopalan, M.; Hardikar, S.; Glasebrook, A. L. Identification of an estrogen response element activated by metabolites of 17-β estradiol and raloxifene. *Science* **1996**, *273*, 1222−1225.

(9) (a) Jones, C. D.; Jevnikar, M. G.; Pike, A. J.; Peters, M. K.; Black, L. J.; Thompson, A. R.; Falcone, J. F.; Clemens, J. A. Antiestrogens. 2. Structure-activity studies in a sereis of 3-aroyl-2-arylbenzo[*b*]thiophene derivatives leading to [6-hydroxy-2-(4-hydroxyphenyl)benzo[*b*]thiene-3-yl][4-[2-(1-piperidinyl)-ethoxy]phenyl]methanone hydrochloride (LY156758), a remarkably effective estrogen antagonist with only minimal intrinsic estrogenicity. *J. Med. Chem.* **1984**, *27*, 1057−1066. (b) Grese,T. A.; Cho, S.; Finely, D. R.; Godfrey, A. G.; Jones, C. D.; Lugar, C. W., III; Martin, M. J.; Matsumoto, K.; Pennington, L. D.; Winter, M. A.; Adrian, M. D.; Cole, H. W.; Magee, D. E.; Phillips, D. L.; Rowley, E. R.; Short, L. L.; Glasebrook, A. L.; Bryant, H. U. Structure-activity relationships of selective estrogen receptor modulators (SERMs). Modifications to the 2-arylbenzothiophene core of raloxifene. *J. Med. Chem.*, in press.

(10) Jones, C. D.; Suarez, T. 2-Phenyl-3-aroylbenzothiophenes useful as antifertility agents. U.S. Patent 4,133,814.

(11) Venier, C. G.; Squires, T. G.; Chen, Y.; Hussmann, G. P.; Shei, J. C.; Smith, B. F. Peroxytriflouroacetic acid. A convenient reagent for the preparation of sulfoxides and sulfones. *J. Org. Chem.* **1982**, *47*, 3773−3774.

(12) (a) **8a** was prepared *in situ* by a two-step procedure from hydroxyphenyl disulfide by first alkylation with 2-(chloroethyl)-piperidine (Cs_2CO_3, DMF), followed by reductive ($LiAlH_4$, THF) cleavage of the disulfide. In an analogous manner, **8b** was prepared in 64% yield from 4-(benzyloxy)phenol by alkylation with 2-(chloroethyl)piperidine followed by hydrogenolysis of the benzyl ether (H_2, 10% Pd/C, EtOH/EtOAc). (b) Yield of **9a** is overall from hydroxyphenyl disulfide.

(13) Brown, H. C.; Weissman, P. M.; Min-Yoon, N. Selective reductions IX. Reactions of lithium aluminum hydride with selected organic compounds containing representative functional groups. *J. Am. Chem. Soc.* **1966**, *88*, 1458−1463.

(14) Miller, R. B.; Moock, T. A general synthesis of 6-H-pyrido[4,3-b]carbazole alkaloides. *Tetrahedron Lett.* **1980**, *21*, 3319−3322 and references cited therein.

(15) (a) Scholl, S. M.; Huff, K. K.; Lippman, M. E. Antiestrogenic effects of LY117018 in MCF-7 cells. *Endocrinology* **1983**, *113*, 611−617. (b) Miller, M. A.; Katzenellenbogen, B. S. Characterization and quantitation of antiestrogen binding sites in estrogen receptor-positive and-negative human breast cancer cell lines. *Cancer Res.* **1983**, *43*, 3094−3100. (c) Thompson, E. W.; Reich, R.; Shima, T. B.; Albini, A.; Graf, J.; Martin, G. R.; Dickson, R. B.; Lippman, M. E. Differential growth and invasiveness of MCF-7 breast cancer cells by antiestrogens. *Cancer Res.* **1988**, *48*, 6764−6768.

(16) Black, L. J.; Jones, C. D.; Falcone, J. F. Antagonism of estrogen action with a new benzothiophene derived antiestrogen. *Life Sci.* **1983**, *32*, 1031−1036.

(17) (a) Sato, M.; Kim, J.; Short, L. L.; Slemenda, C. W.; Bryant, H. U. Longitudinal and cross-sectional analysis of raloxifene effects on tibae from ovariectomized aged rats. *J. Pharmacol. Exp. Ther.* **1995**, *272*, 1252−1259. (b) Bryant, H. U.; Bendele, A. M.; Magee, D. E.; Cole, H. W.; Spaethe, S. W. Pharmacologic profile of estrogen induced uterine eosinophilia in the ovariectomized (OVX) rat. *Pharmacologist* **1992**, *34*, 194.

(18) Tamoxifen was purchased from Sigma Chemical Co., St. Louis, MO. 4-Hydroxytamoxifen was prepared at Lilly Research Laboratories according to procedures described in Robertson, D. W.; Katzenellenbogen, J. A. *J. Org. Chem.* **1982**, *47*, 2387−2393. ICI 182,780 was prepared at Lilly Research Laboratories according to the procedures described in Bowler, J.; Tait, B. S. U.S. Patent 4,659,516.

(19) Grese, T. A.; Sluka, J. P.; Bryant, H. U.; Cole, H. W.; Kim, J. R.; Magee, D. E.; Rowley, E. R.; Sato, M. Benzopyran selective estrogen receptor modulators (SERMs): Pharmacological effects and structural correlation with raloxifene. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 903−908.

JM970167B