Synthesis and Pharmacology of Conformationally Restricted Raloxifene Analogues: Highly Potent Selective Estrogen Receptor Modulators

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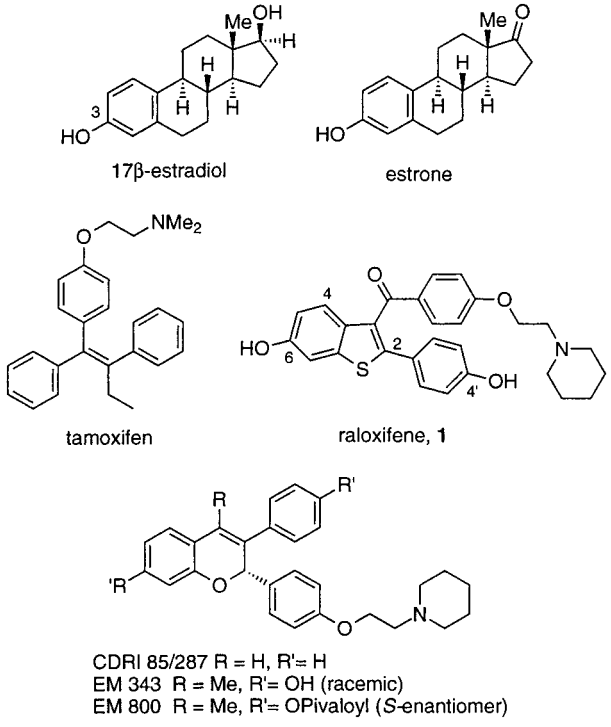
Received October 9, 1997

The 2-arylbenzothiophene raloxifene, **1**, is a selective estrogen receptor modulator (SERM) which is currently under clinical evaluation for the prevention and treatment of postmenopausal osteoporosis. In vivo structure−activity relationships and molecular modeling studies have indicated that the orientation of the basic amine-containing side chain of **1**, relative to the stilbene plane, is an important discriminating factor for the maintenance of tissue selectivity. We have constructed a series of analogues of **1** in which this side chain is held in an orientation which is orthogonal to the stilbene plane, similar to the low-energy conformation predicted for raloxifene. Herein, we report on the synthesis of these compounds and on their activity in a series of in vitro and in vivo biological assays reflective of the SERM profile. In particular, we describe their ability to (1) bind the estrogen receptor, (2) antagonize estrogen-stimulated proliferation of MCF-7 cells in vitro, (3) stimulate TGF-β3 gene expression in cell culture, (4) inhibit the uterine effects of ethynyl estradiol in immature rats, and (5) potently reduce serum cholesterol and protect against osteopenia in ovariectomized (OVX) rats without estrogen-like stimulation of uterine tissue. These data demonstrate that one of these compounds, LY357489, **4**, is among the most potent SERMs described to date with in vivo efficacy on bone and cholesterol metabolism in OVX rats at doses as low as 0.01 mg/kg/d.

Introduction

The decreased production of ovarian steroids which occurs after the climacteric has been linked to a number of postmenopausal pathologies, particularly osteoporosis and coronary artery disease.[1,2] Although estrogen replacement therapy (ERT) reduces the risks associated with these pathologies, concerns relating to the increased risk of endometrial cancer have necessitated the development of therapeutic regimens in which the uterine effects of estrogen are opposed by progestin treatment.[3] Side effects of progestin treatment, such as resumption of menses, central nervous system disturbances, and the possibility of attenuated cardiovascular benefits, have significantly reduced patient compliance.[4] Furthermore, recent studies which suggest an increased risk of breast and uterine cancer associated with estrogen replacement therapy have stimulated the search for treatment alternatives.[5,6]

Historically, a number of nonsteroidal compounds which interact with the estrogen receptor (ER) have been investigated as contraceptives and for the treatment of breast cancer, uterine dysfunction, and other disorders of the female reproductive system.[7] Tamoxifen, originally developed as an estrogen antagonist and widely utilized for the treatment of breast cancer, has paradoxically been found to act as a partial estrogen agonist in the uterus and to display estrogen agonist effects in bone and in the cardiovascular system.[8] More recently, selective estrogen receptor modulators (SERMs) which fully antagonize the effects of estrogen on uterine and mammary tissue, while mimicking the effects of estrogen on bone and the cardiovascular system, have been investigated as a possible alternative to ERT.[9] One such compound, raloxifene (LY139481 HCl; **1**), is in advanced clinical trials for the prevention and treatment of osteoporosis.[10,11]

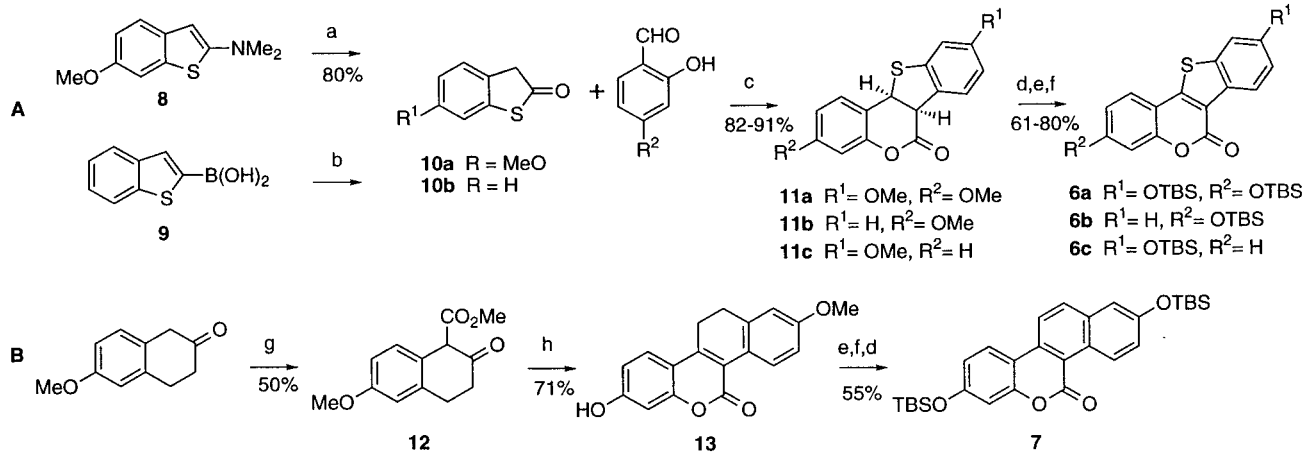


Pharmacologically, raloxifene is distinguished from tamoxifen by its lack of proliferative effects in uterine

S0022-2623(97)00688-2 CCC: $15.00 © 1998 American Chemical Society
Published on Web 03/12/1998

Figure 1. Overlay of minimized structures of raloxifene (green carbons) and **3a** (gray carbons).

Scheme 1[a]



[a] Reagents: (a) HCl, H_2O, THF, reflux; (b) H_2O_2, Na_2CO_3 (see ref 24); (c) Et_3N, EtOH, CH_2Cl_2; (d) DDQ, dichloroethane, 80 °C; (e) $AlCl_3$, EtSH, CH_2Cl_2; (f) TBDMSCl, Et_3N, CH_2Cl_2; (g) NaH, $(MeO)_2CO$, MeOH (see ref 25); (h) resorcinol, $P(O)Cl_3$, toluene, 80 °C.

tissue.[12,13] Other researchers have developed a series of benzopyran-containing molecules (e.g. EM-800,[14] CDRI-85/287[15]) which also exhibit this tissue specificity.[16] We have recently speculated that this difference in biological activity may be related to a specific structural property, that is, the orientation of the basic amine-containing side chain with respect to the stilbene plane.[16] While in tamoxifen this side chain is coplanar with the stilbene moiety, both molecular modeling and X-ray crystallographic studies predict an orthogonal orientation for the side chain in raloxifene (Figure 1).[17] We have demonstrated that raloxifene analogues which are forced to adopt the tamoxifen orientation exhibit a tamoxifen-like biological profile, while analogues which are conformationally locked in a raloxifene-like orientation maintain the raloxifene profile.[18] Herein, we describe the synthesis and biological activity of a series of conformationally locked ER modulators which incorporate structural elements of both raloxifene and the aforementioned benzopyrans. In particular, we disclose LY357489, **4**, an extremely potent SERM with in vivo efficacy on bone and cholesterol metabolism at doses as low as 0.01 mg/kg.

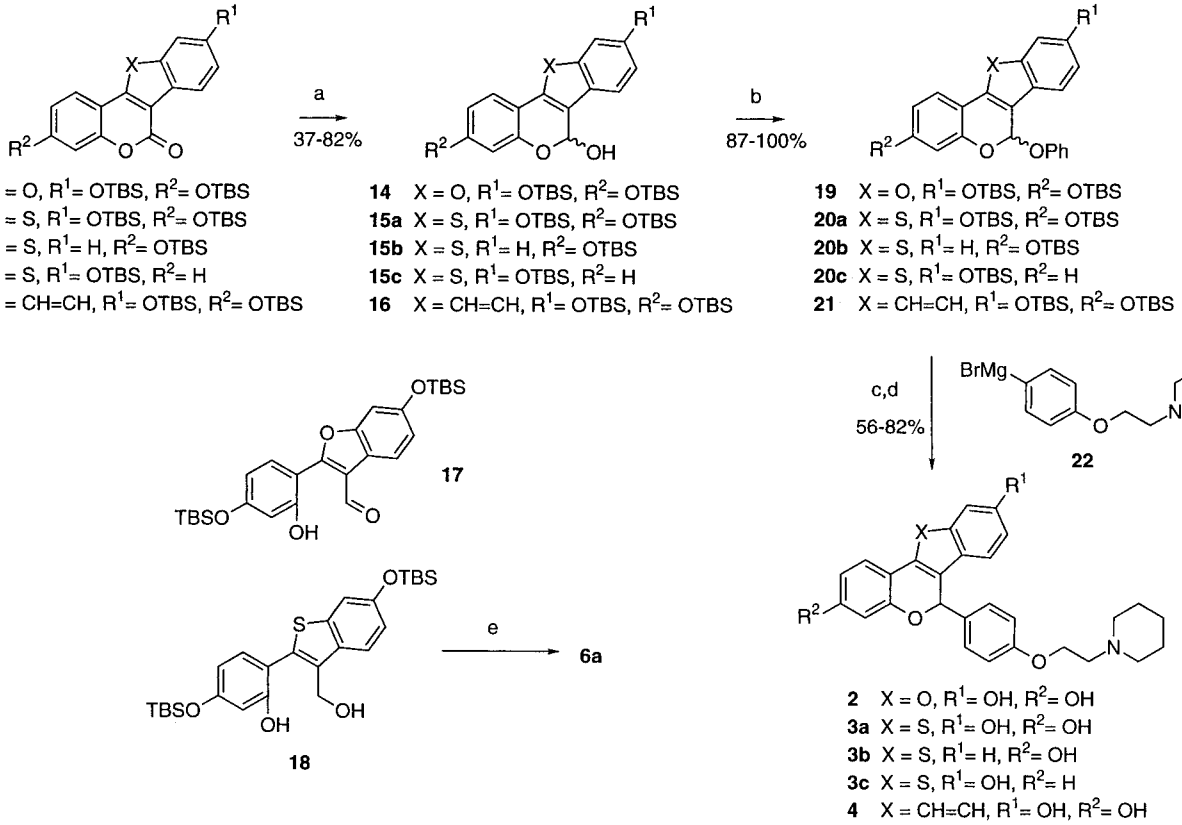
Chemistry

Compounds **2**−**4** were designed on the basis of molecular modeling studies of raloxifene and benzopyran-containing SERMs which predicted an orthogonal orientation of their respective side chains relative to the stilbene plane.[16] Overlay of the minimized structure of compound **3** with raloxifene (Figure 1) indeed predicted a similar topology.[19,20]

A retrosynthetic disconnection of the side chain of compounds **2**−**4**, leading to the tetracyclic coumarins **5**−**7** and a functionalized aryl Grignard reagent, **22**, allowed the utilization of methodology previously developed for the synthesis of 2,3-diarylisoflav-3-enes.[21] Compound **5** was readily available by the silylation of commercially available coumestrol.[22] Thiacoumestans **6a**−**c** were prepared by the condensation of a salicyl aldehyde with thianaphthen-2-ones **10a,b** followed by oxidation, demethylation, and silylation (Scheme 1a).[23] This protecting group interchange was necessary, both for ease of removal and to provide improved solubility in later stages of the synthesis (vide supra). Compounds **10a**,**b** were in turn prepared either by hydrolysis of the 2-(dimethylamino)benzothiophene, **8**,[24,25] or from benzothiophene via oxidation of the corresponding boronic acid, **9**.[26] Naphthocoumarin **7** was prepared from carbomethoxytetralone **12**[27] via a von Pechman condensation,[28] followed by protecting group interchange and dehydrogenation (Scheme 1b).

Reduction of the tetracyclic coumarins **5**−**7** with diisobutylaluminum hydride (DIBAL-H) then provided the corresponding lactols **14**−**16** (Scheme 2).[21] The silyl protecting groups were required at this stage, since the reduction was best carried out in toluene or toluene/dichloromethane mixtures at low temperatures and the corresponding methoxy-substituted coumarins had limited solubility under these conditions. In the benzofuran-containing system, the lactol was isolated as a mixture with the corresponding aldehyde tautomer **17**, and the yield of the reduction was significantly lower. Presumably, the ring strain engendered by the incorporation of the smaller furan ring leads to a larger proportion of the open form, which is prone to overreduction under the reaction conditions. For the benzothiophene-containing system, significant amounts of

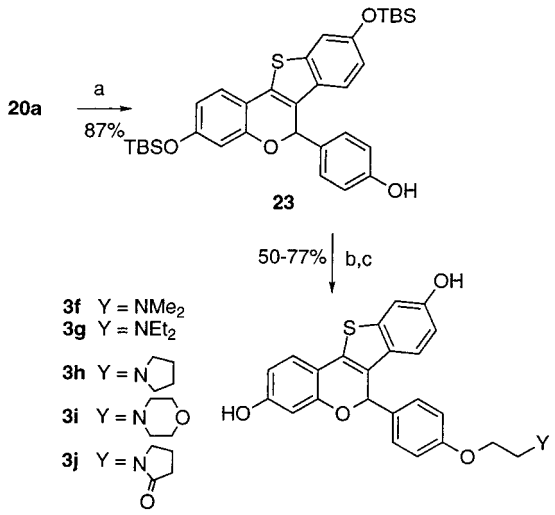
Scheme 2[a]



[a] Reagents: (a) DIBAL-H, toluene, −100 to −70 °C; (b) phenol, ($MgSO_4$), chlorobenzene or CH_2Cl_2, room temperature to reflux; (c) THF, 0 °C to room temperature; (d) TBAF, THF; (e) PCC, Celite, CH_2Cl_2.

starting material and overreduced product, **18**, were also obtained. Diol **18** could be recycled to **6** by oxidation with PCC; however, partial oxidation to the lactol **16** was not feasible. Conversion of the lactols to the corresponding phenyl acetals **19**−**21** and displacement with the aryl Grignard reagent **22** proceeded as expected, and desilylation then provided the desired compounds **2**−**4** in racemic form.[21] Alternatively, condensation of **20a** with (4-((trimethylsilyl)oxy)phenyl)-magnesium bromide[29] followed by selective removal of the trimethylsilyl moiety provided monophenol **23** (Scheme 3). Mitsunobu alkylation with a variety of *N*-hydroxyethylamines followed by desilylation then provided analogues **3f**−**j** containing modified side chains. Finally, deshydroxy analogue **3d** was prepared from **3a** by conversion to the bistriflate and palladium-catalyzed transfer hydrogenation, and dimethoxy analogue **3e** was prepared by methylation of **3a** with diazomethane (Scheme 4).

Scheme 3[a]



[a] Reagents: (a) (i) [4-[(trimethylsilyl)oxy]phenyl]magnesium bromide, THF; (ii) K_2CO_3, MeOH; (b) $HOCH_2CH_2Y$, PPh_3, DEAD, toluene; (c) TBAF, THF.

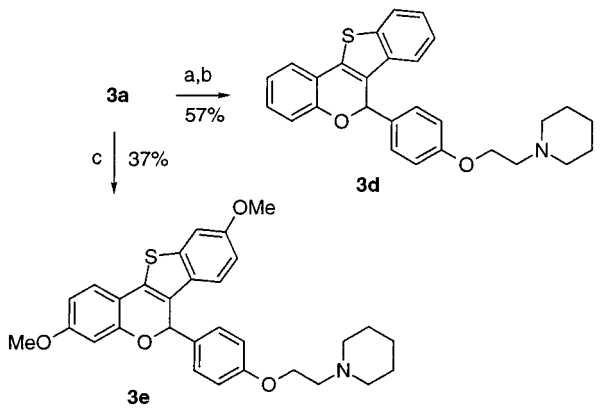
Biological Testing

In Vitro. ER binding affinities were determined by displacement of bound [^{3}H]-17β-estradiol from MCF-7 cell lysate for compounds **2**−**4** and are reported in Table 1.[30] Antagonism of estrogen action in a mammary tumor cell line was assayed via inhibition of MCF-7 cell proliferation stimulated by 10^{-11} M 17β-estradiol and IC_{50} values are also included in Table 1.[31]

In Vivo. Tissue-specific estrogen agonist effects were examined in OVX rats,[13] utilizing uterine weight, uterine eosinophil peroxidase (EPO) activity,[32] and serum cholesterol levels as endpoints after 4 days of treatment (Table 1, Figure 2). Selected compounds were further evaluated in a 5-week, OVX rat model in which effects on bone mineral density (BMD) were also examined.[33] Bone-mineral density was assessed at the proximal aspect of the tibia by quantitative computed tomography (QCT) and is reported as percent protection relative to sham-operated and OVX controls (Figure 3).

In vivo estrogen antagonist activity in the uterus was determined in 21-day-old female Sprague−Dawley rats dosed with a maximally stimulatory dose of 17α-

Scheme 4[a]



[a] Reagents: (a) $PhN(Tf)_2$, Et_3N, DMF; (b) $Pd(PPh_3)_4$, dppp, HCO_2H, Et_3N, DMF; (c) CH_2N_2, Et_2O, MeOH.

ethynylestradiol (0.1 mg/kg) for 3 consecutive days.[34] Test groups were also administered various doses of compounds **2**−**4** 15 min prior to ethynylestradiol dosing for 3 days. Uterine weight/body weight ratios were calculated for each animal and are reported as percent inhibition (Figure 4).

Results

In Vitro. The in vitro results in Table 1 indicate the importance of the hydroxy functionality of **2**−**4** for optimal ER binding and inhibition of MCF-7 cell proliferation. Comparison of the binding activities of **3b**−**e** with that of **3a** reveals 6−20-fold decreases in relative binding affinity (RBA) when either or both of the hydroxy groups are replaced with a proton or converted to methyl ethers. In parallel fashion, MCF-7 inhibitory potency is decreased by 30−500-fold for the same compounds. A similar importance has previously been reported in the raloxifene series; however, unlike the raloxifene series in which the 6-hydroxy functionality is clearly more important for in vitro biological activity, compounds **3b** and **3c** show only minor differentiation.[35]

The importance of the nature of the amine substituents with respect to in vitro estrogen antagonism is demonstrated by the compounds **3f**−**j** and is similar to effects which have been reported in the raloxifene series, with the piperidine base providing the optimum activity.[18] Interestingly, the amine substituent has little effect on the receptor binding observed for these compounds and yet decreases MCF-7 inhibitory potency 3−50-fold relative to that observed for **3a**.

Finally, the replacement of the benzothiophene substructure of **3a** with a benzofuran (**2**) or a naphthalene (**4**) produces relatively minor effects on the in vitro biological activity. The lower binding affinity observed with **2** may be ascribed to the general intolerance of the ER toward polar substitution.[36] The replacement of the benzothiophene core of raloxifene with a naphthalene has previously been shown to provide compounds with a similar pharmacological profile.[37]

In Vivo. The in vivo data from the 4-day OVX rat assay (Table 1, Figure 2) provide additional insights into the effects of structural modifications on the complex biological activities of SERMs. For example, tamoxifen stimulates a dose dependent increase in uterine wet weight as well as increases in other measures of uterine stimulation such as epithelial cell height.[12a] Although raloxifene also stimulates a modest increase in uterine wet weight, this increase is not dose related and is not coincident with increases in other measures of uterine hypertrophy such as epithelial cell height and total estrogenicity.[12a] This uterine weight increase has therefore been attributed to water retention.[12b] The inconsistent correlation between elevation of uterine weight and stimulation of other uterine parameters has led to reliance on uterine EPO activity, a very sensitive indicator of estrogen action in the uterus.[32] Although ethynylestradiol and tamoxifen both potently reduce serum cholesterol, they also induce significant increases in uterine EPO while raloxifene does not. All three compounds induce significant increases in uterine weight, although the effect of raloxifene is relatively modest and is not dose related. We have used uterine EPO, therefore, to discriminate compounds which demonstrate a tamoxifen-like profile from those whose activity parallels that of raloxifene.[34,35]

As shown in Table 1, the conformationally restricted SERMs generally mimic the effects of raloxifene on uterine parameters and serum cholesterol. In contrast to original the raloxifene series, in which the deshydroxy congeners showed increased in vivo potency, changes in the hydroxylation pattern of the benzothienobenzopyran (**3a**−**d**) resulted in a reduction in potency.[33] This distinction may reflect different efficiency or selectivity of metabolic hydroxylation for the two series. Similar to the raloxifene series,[18] changing the base from piperidine to other cyclic or acyclic amine functionality reduced both potency and selectivity, with **3f**−**j** all showing increased evidence of uterine stimulation relative to **3a**.[38] The piperidine-containing compounds **2**, **3a**−**c**, and **4** produce no significant increase in uterine EPO or, unlike raloxifene, uterine weight. A similar lack of uterine weight effects has previously been reported for another series of benzopyran-containing SERMs, but the relevance of this difference in comparison with raloxifene is unknown.[16]

Figure 2 demonstrates the surprising increase in in vivo potency which was observed across the benzofuran (**2**), benzothiophene (**3a**), naphthalene (**4**) series. Although similar to **3a** and raloxifene in vitro, compound **4** (LY357489) demonstrated remarkable potency (ED_{50} = 0.02 mg/kg/d) for lowering serum cholesterol in the OVX rat. Although the reasons for this increased potency relative to **3a** are unclear, other highly potent, naphthalene-containing SERMs have been described previously.[37,39]

Compounds **3a** and **4** were further evaluated in an OVX rat model of estrogen-deficiency-induced osteopenia. As shown in Figure 3, both **3a** and **4** maintain the ability of raloxifene and estrogen to prevent ovariectomy-induced bone loss. In particular, LY357489, **4**, showed increased potency relative to raloxifene, with significant effects at doses as low as 0.01 mg/kg/d.

Compounds **2**, **3a**, and **4** were also evaluated for uterine estrogen antagonist activity in the immature rat model as shown in Figure 4. In this model also, LY357489, **4**, showed improved potency (ED_{50} = 0.05 mg/kg/d, raloxifene ED_{50} = 0.55 mg/kg/d) and was even able to reduce uterine weight below the level observed for untreated controls. Compound **3a** again showed

Table 1. In Vitro and in Vivo Biological Activity of Conformationally Restricted SERMs

no.	X	Y	R^1	R^2	ER RBA[a,b]	MCF-7 Inhib. IC_{50} (nM)[c]	Uterine Weight MED(%incr OVX)[d]	Uterine EPO MED(V_{max})[e]	Serum Cholesterol (% decr OVX)[f]		ED_{50}[g] (mg/kg)
									0.01 mg/kg	0.1 mg/kg	
	17α-ethynyl estradiol				1.00	NA[h]	0.01(41.5 ± 13.7)	0.1(281.7 ± 6)	57.1*± 4.9	84.5*± 1.7	0.005
									0.1 mg/kg	1.0 mg/kg	
	4-OH-tamoxifen[i]				0.36	0.5	ND[j]	ND[j]	ND[j]	ND[j]	ND[j]
	tamoxifen				ND[j]	ND[j]	0.1(62.4 ± 3.0)	0.1(57.1 ± 3.3)	39.2*± 4.5	71.1*± 4.3	0.2
	raloxifene				0.34	0.2	0.1(23.5 ± 10.0)	>10	44.9*± 8.0	66.7*± 4.1	0.2
2	O		-OH	-OH	0.07	0.7	>10	>10	17.4 ± 8.5	30.9*± 12.2	>10
3a	S		-OH	-OH	0.23	0.2	>10	>10	62.2*± 4.3	47.6*± 9.5	0.15
3b	S		-H	-OH	0.03	20	>10	>10	46.5*± 10.7	53.7*± 6.1	0.5
3c	S		-OH	-H	0.04	6	>10	>10	25.4*± 14.6	51.5*± 18.1	1.0
3d	S		-H	-H	0.01	80	0.1(28.9 ± 3.0)	>10	39.8*± 7.9	65.5*± 4.5	0.7
3e	S		-OMe	-OMe	0.01	100	ND[j]	ND[j]	ND[j]	ND[j]	ND[j]
3f	S	$-NMe_2$	-OH	-OH	0.16	2	0.1(77.8 ± 13.3)	0.1 (52.2 ± 0.4)	40.1*± 16.9	54.7*± 8.3	0.5
3g	S	$-NEt_2$	-OH	-OH	0.29	8	0.1(46.6 ± 6.1)	0.1(108.0 ± 1.9)	20.6 ± 13.2	38.7*± 7.6	>10
3h[k]	S		-OH	-OH	0.21	0.6	0.1(27.4 ± 7.7)	>1.0	38.5*± 11.7	68.0*± 5.2	0.2
3i	S		-OH	-OH	0.28	6	1.0(43.0 ± 10.5)	1.0(192.3 ± 3.7)	-48.7 ± 28.7	14.3 ± 21.4	>10
3j[l]	S		-OH	-OH	0.28	9	3.0(47.9 ± 14.5)	>3.0	-15.2 ± 14.8	4.4 ± 14.3	NA[h]
4	CH=CH		-OH	-OH	0.22	0.4	>10	>10	57.2*± 5.3	65.7*± 6.6	0.02

[a] RBA = relative binding affinity by competition with [^{3}H]-17β-estradiol. [b] Average of at least two determinations. Values are ±10%. [c] Dose required to give 50% inhibition of a maximally effective (10^{-11}) dose of 17β-estradiol. Average of at least three determinations. Values are ±10%. [d] MED = minimally effective dose (mg/kg body weight) at which a statistically significant ($p \leq 0.05$) increase in uterine weight/body weight was observed. Activity at the MED is expressed as percent increase relative to OVX controls ± standard error. [e] MED at which a significant (>5-fold increase relative to OVX control and value of $V_{max} \geq 10$) increase in EPO activity was observed. Activity at the MED is expressed as V_{max} ± standard error. [f] Percent decrease in serum cholesterol relative to OVX controls ± standard error. Statistically significant ($p \leq 0.05$) differences are denoted by an asterisk (*). [g] Dose required to reduce serum cholesterol by 50% relative to OVX controls. [h] NA = not active at the doses tested. [i] 4-Hydroxytamoxifen, the primary biologically active metabolite of tamoxifen. [j] Not determined. [k] Maximum dose tested = 1.0 mg/kg. [l] Maximum dose tested = 3.0 mg/kg.

potency similar to that of raloxifene, while compound **2** was somewhat less effective.

Discussion

As part of our program to explore structure−activity relationships in the raloxifene series, we discovered a unique dependence upon the orientation of the basic amine-containing side chain for the maintenance of full estrogen antagonist activity in the uterus.[16,18] Herein, we have exploited this finding in the design of a new series of SERMs in which the side chain is rigidly held in an orthogonal orientation relative to a tetracyclic, steroid-like core. Indeed, as described above, these compounds function as full antagonists in the OVX rat uterus without any evidence of intrinsic agonist activity in that tissue. Nevertheless, as with raloxifene, they maintain the ability to reduce serum cholesterol and protect against ovariectomy-induced osteopenia. The structure−activity relationship observed in the benzothiophene-containing series, **3**, nicely parallels that reported for raloxifene,[18,35] although there is increased dependence upon the degree of hydroxylation for in vivo, as well as in vitro, potency.

Surprisingly, substitution of oxygen or ethylene for the benzothiophene sulfur revealed a marked dependence of in vivo potency upon the nature of X (Scheme 1), with **4** (X = CH_2=CH_2) > **3a** (X = S) > **2** (X = O), roughly correlated with increasing hydrophobicity of the steroid-like nucleus. Most notably, shifting from the benzothiophene to the naphthalene-containing nucleus of **4** resulted in a significant boost in in vivo potency of both agonist and antagonist activities, without a corresponding increase in in vitro effects. This shift may reflect improved bioavailability for **4**, although the reasons for this improvement are unclear. Compound **4** maintains the hydroxylation pattern of **3a** and ral-

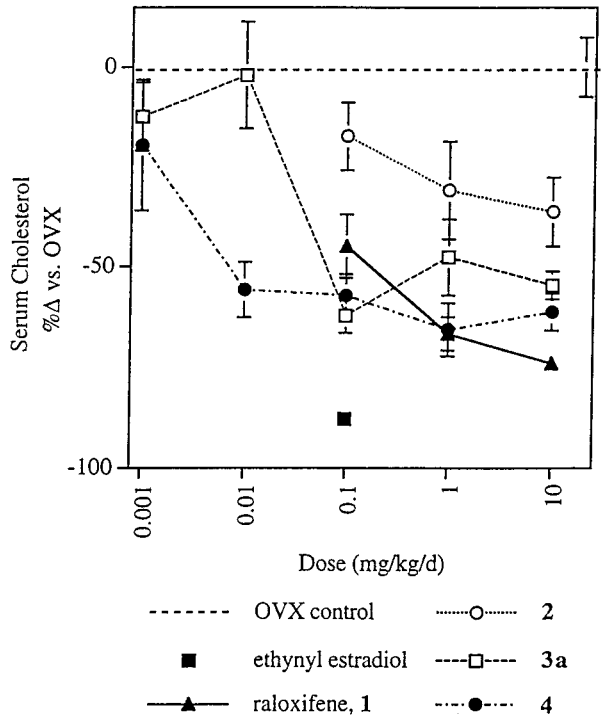


Figure 2. Effects of **2**, **3a**, and **4** on serum cholesterol levels in OVX rats. Cholesterol values are reported as the mean percent decrease relative to OVX control (±SEM) with $n \geq 5$ for all groups. Raloxifene- and ethynylestradiol-treated groups are included as internal standards. Observed effects at doses of **2** ≥ 1.0 mg/kg/d, **3a** ≥ 0.1 mg/kg/d, **4** ≥ 0.01 mg/kg/d and all doses of raloxifene or ethynyl estradiol differ significantly from OVX control at $P \leq 0.05$.

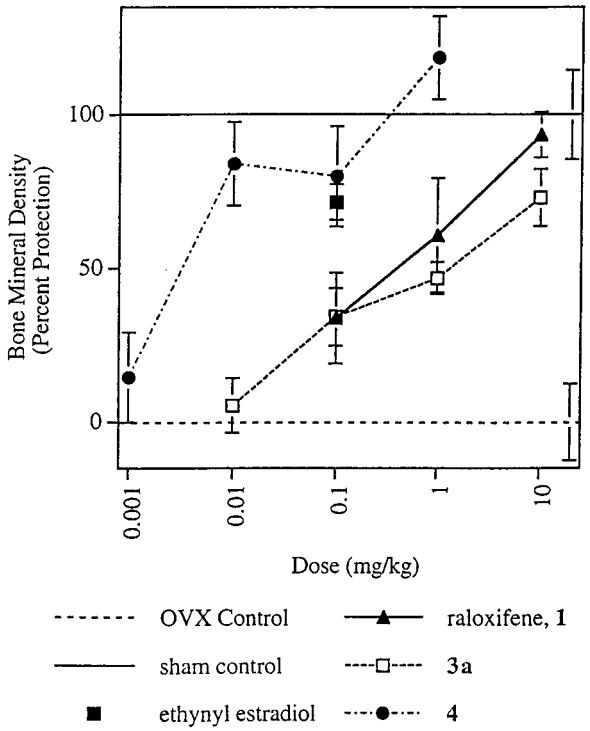


Figure 3. Effects of raloxifene, **3a**, and **4** on bone mineral density (BMD) in the OVX rat. BMD values are reported as percent protection (±SEM) relative to OVX controls, with sham control values defined as 100% and OVX controls defined as 0 and $n \geq 6$ for each group. Observed effects at doses of **3a** or raloxifene ≥ 1.0 mg/kg/d and doses of **4** or ethynyl estradiol ≥ 0.01 mg/kg/d differ significantly from OVX control at $P \leq 0.05$.

oxifene, and glucuronidation of the phenolic moieties is thought to be the primary factor limiting the bioavailability of raloxifene.[40] Nevertheless, improved bioavailability has been recently claimed for another series of hydroxylated SERMs which are built upon a tetrahydronaphthalene core unit.[39] Further experimentation will be necessary to determine if pharmacokinetics are indeed responsible for this effect.

One alternative explanation involves the mechanisms by which the ER controls gene transcription. It has recently been proposed that the conformation of the ER−ligand complex is dependent upon the nature of the interacting ligand and that the distinct conformation induced by a particular ligand affects differentially which genes are transcriptionally activated.[41,42] To the extent that structural modification of the ligand disrupts or alters this receptor−ligand conformation, different sets of estrogen responsive genes may be influenced positively or negatively, resulting in an altered biological profile. We have explored the effects of LY357489, **4**, on TGF-β3 promoter activity in vitro, a candidate gene which may be involved in the bone effects of raloxifene, and found no significant increase in potency (Figure 5).[43] Nevertheless, further evaluation of the effects **4** at the transcriptional level in other promoter systems may be warranted.

The compounds examined within this study were prepared in racemic form, and therefore the biological results reported herein must be interpreted with caution. The possibility that the individual enantiomers may exert differential biological activities, functioning synergistically in some tissues and antagonistically in others, cannot be excluded. Studies with other chiral, nonsteroidal estrogen receptor modulators have indicated that although the majority of the biological effects reside in a single enantiomer, the racemates exhibit the same qualitative profile of activity.[14,44] Further studies elucidating the biological effects of individual enantiomers of **4** will be reported in due course.

In conclusion, we have prepared a series of novel raloxifene analogues in which the side chain is constrained in an orientation which approximates the low-energy conformation predicted for the parent molecule. We have demonstrated that this series of compounds maintain the SERM profile of biological activity which has previously been described for raloxifene, including ER binding, inhibition of MCF-7 cell proliferation, estrogen antagonist effects in the immature rat uterus, and tissue-specific estrogen agonist effects on serum cholesterol and bone in the OVX rat.[12b,34] Finally, we have demonstrated that one of these compounds, LY357489, **4**, is among the most potent SERMs described to date with in vivo efficacy on bone and cholesterol metabolism in OVX rats at doses as low as 0.01 mg/kg/d.

Experimental Section

Chemistry. General experimental procedures have been recently published.[35] The abbreviations THF, DMF, DCE, DMSO, DDQ, TBAF, and DEAD refer to tetrahydrofuran, dimethylformamide, 1,2-dichloroethane, dimethyl sulfoxide, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, tetra-*n*-butylammonium fluoride, and diethyl azodicarboxylate, respectively. All spectra were recorded in acetone-d_6 unless otherwise indicated. Elemental analyses were carried out by the Physi-

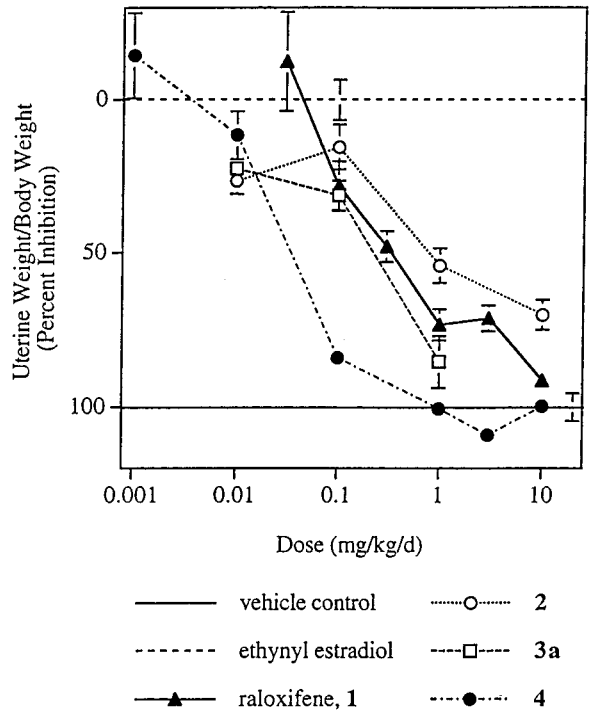


Figure 4. Effect of raloxifene, **2**, **3a**, and **4** on ethynylestradiol (EE)-induced uterine weight increase in immature rats. Data are reported as percent inhibition of uterine weight/body weight (±SEM) relative to EE-treated controls, with vehicle control values defined as 100% and EE-treated controls as 0 and $n \geq 6$ for each group. Observed effects at doses of **2** ≥ 1.0 mg/kg/d and doses of **3a**, **4**, and raloxifene ≥ 0.1 mg/kg/d differ significantly from EE-treated controls at $P \leq 0.05$.

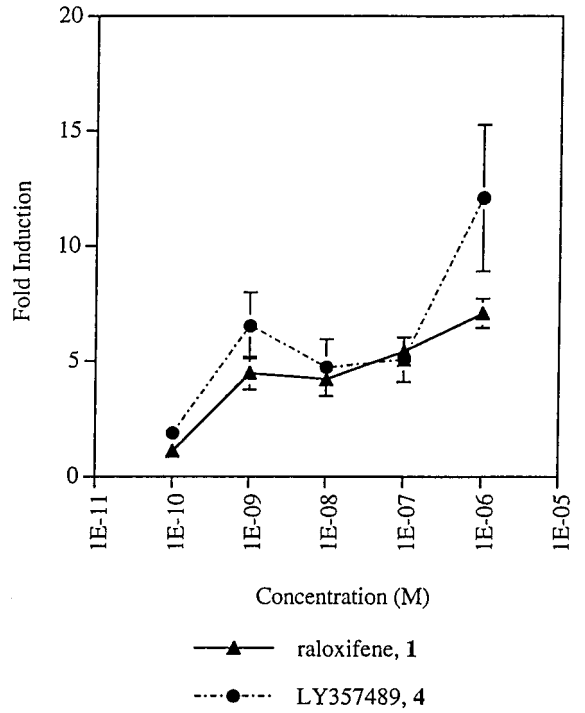


Figure 5. Effects of raloxifene, **1**, and LY357489, **4**, on TGF-β3 promoter activity. Data are reported as the mean fold-induction (±SEM) versus matched controls with $n = 3$ for all groups.

cal Chemistry Department of Lilly Research Laboratories and are within ±0.4% of theory unless otherwise noted.

6-Methoxythianaphthen-2-one (10a).[25] A solution of **8**[24] (75.1 g, 360 mmol) in THF (600 mL) was treated with 1 N HCl (615 mL) and heated at reflux for 12 h. The mixture was neutralized with saturated $NaHCO_3$, diluted with ether (1 L), washed with saturated $NaHCO_3$ (2 × 1 L) and brine (1 L), dried ($MgSO_4$), and concentrated. The residue was recrystallized from ether to provide 52.7 g (81%) of the title compound: ^{1}H NMR ($CDCl_3$) δ 7.16 (d, $J = 9.7$ Hz, 1H), 6.87 (d, $J = 2.9$ Hz, 1H), 6.73 (dd, $J = 9.7$, 2.9 Hz, 1H), 3.88 (s, 2H), 3.79 (s, 3H).

(±)-6a,11a-Dihydro-3,9-dimethoxy-6*H*-[1]benzothieno-[3,2-*c*][1]benzopyran-6-one (11a). To a stirred solution of **10a** (20 g, 111 mmol) in a mixture of EtOH (100 mL) and CH_2Cl_2 (50 mL) was added 4-methoxysalicyl aldehyde (17.5 g, 115 mmol) followed by Et_3N (567 mg, 784 mL, 5.6 mmol) at room temperature. After 30 min, a solid began to precipitate, and stirring was continued overnight. The mixture was then diluted with cold hexane (1 L) and filtered to yield 28.7 g (82%) of the title product as an off-white solid, pure by ^{1}H NMR analysis. An analytical sample was obtained by recrystallization from toluene as light yellow crystals, mp 157−165 °C (dec): ^{1}H NMR ($CDCl_3$) δ 7.33 (d, $J = 8.6$ Hz, 1H), 7.31 (d, $J = 8.1$ Hz, 1H), 6.75 (d, $J = 2.4$ Hz, 1H), 6.70 (m, 2H), 6.60 (d, $J = 2.5$ Hz, 1H), 5.22 (d, $J = 7.2$ Hz, 1H), 4.33 (d, $J = 7.2$ Hz, 1H), 3.79 (s, 3H), 3.76 (s, 3H); IR ($CHCl_3$) 1759 cm^{-1}; MS (FD) *m/e* 314 (M^+). Anal. ($C_{17}H_{14}O_4S$) C, H.

(±)-6a,11a-Dihydro-9-methoxy-6*H*-[1]benzothieno[3,2-*c*][1]benzopyran-6-one (11c). The title compound was prepared in 83% yield from **10a** and salicyl aldehyde by a method similar to that described for **11a**: ^{1}H NMR ($CDCl_3$) δ 7.45 (d, $J = 7.5$ Hz, 1H), 7.33 (m, 2H), 7.17 (m, 1H), 7.07 (d, $J = 8.1$ Hz, 1H), 6.77 (d, $J = 2.4$ Hz, 1H), 6.69 (dd, $J = 2.4$, 8.5 Hz, 1H), 5.25 (d, $J = 7.4$ Hz, 1H), 4.37 (d, $J = 7.3$ Hz, 1H), 3.77 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 166.4, 160.7, 150.8, 141.4, 130.1, 129.0, 127.7, 127.0, 124.9, 119.5, 117.2, 111.2, 108.3, 55.5, 50.6, 49.6; IR ($CHCl_3$) 1766 cm^{-1}; MS (FD) *m/e* 284 (M^+). Anal. ($C_{16}H_{12}O_3S$) C, H.

3,9-Bis[(*tert*-butyldimethylsilyl)oxy]-6*H*-[1]benzothieno-[3,2-*c*][1]benzopyran-6-one (6a). A mixture of **11a** (4.5 g, 14.3 mmol) and DDQ (3.4 g, 15 mmol) in DCE (100 mL) was heated briefly to 80 °C, inducing the formation of a precipitate. The mixture was filtered hot; the precipitate was rinsed with CH_2Cl_2 and the mother liquor concentrated in vacuo. The remnant was dissolved in hot CH_2Cl_2, filtered to remove residual hydroquinone, and reconcentrated. The product was recrystallized from toluene to provide 3.94 g (88%) of 9-methoxy-6*H*-[1]benzothieno[3,2-*c*][1]benzopyran-6-one as white needles, mp 220−221 °C: ^{1}H NMR (acetone-d_6/DMSO-d_6) δ 8.41 (d, $J = 8.9$ Hz, 1H), 7.82 (d, $J = 8.7$ Hz, 1H), 7.74 (d, $J = 2.3$ Hz, 1H), 7.20 (dd, $J = 8.9$, 2.4 Hz, 1H), 7.09 (d, $J = 2.4$ Hz, 1H), 7.03 (dd, $J = 8.6$, 2.4 Hz, 1H), 3.94 (s, 3H), 3.91 (s, 3H); IR (KBr) 1717 cm^{-1}; MS (FD+) *m/e* 312 (M^+).

To a mechanically stirred slurry of the product prepared above (12 g, 38.4 mmol) in CH_2Cl_2 (220 mL) was added ethanethiol (11.9 g, 13.4 mL, 192 mmol) followed by aluminum chloride (38.4 g, 288 mmol), portionwise. The reaction mixture was stirred at ambient temperature for 5 h, cooled to 0 °C, and quenched cautiously with THF (250 mL) followed by saturated $NaHC_3$ (250 mL). The mixture was diluted with THF (1 L), the layers were separated, and the aqueous layer was washed with THF (200 mL). The combined organic layers were dried (Na_2SO_4) and concentrated to yield 11.1 g of crude diphenol as a yellow solid, which was used without further purification.

The crude product was slurried in CH_2Cl_2 (220 mL) and treated with Et_3N (20.2 g, 28 mL, 200 mmol) and *tert*-butyldimethylsilyl chloride (20.3 g, 134.4 mmol). The mixture was stirred at ambient temperature for 5 h, during which it slowly became homogeneous. After dilution with hexane (600 mL), the mixture was washed with brine (600 mL) and the aqueous layer was extracted with hexane (300 mL). The combined organic layers were dried (Na_2SO_4) and concentrated, and the residue was recrystallized from hexane to provide 18.0 g (91%) of the title compound as a fluffy white solid, mp 142−144 °C: ^{1}H NMR ($CDCl_3$) δ 8.50 (d, $J = 8.7$ Hz, 1H), 7.58 (d, $J = 8.5$ Hz, 1H), 7.32 (d, $J = 2.1$ Hz, 1H),

7.05 (dd, J = 8.7, 2.1 Hz, 1H), 6.92 (d, J = 2.2 Hz, 1H), 6.84 (dd, J = 8.4, 2.2 Hz, 1H), 1.01 (s, 9H), 1.00 (s, 9H), 0.27 (s, 6H), 0.25 (s, 6H); ^{13}C NMR (DMSO-d_6) δ 158.4, 157.0, 154.6, 152.7, 149.6, 138.5, 130.5, 125.2, 124.6, 120.0, 117.6, 116.3, 112.7, 111.3, 108.0, 25.6, 25.6, 18.2, 18.2, −4.4, −4.4; IR ($CHCl_3$) 1717 cm^{-1}; MS (FD) *m/e* 512 (M^+). Anal. ($C_{27}H_{36}O_4SSi_2$) C, H.

3-[(*tert*-Butyldimethylsilyl)oxy]-6*H*-[1]benzothieno-[3,2-*c*][1]benzopyran-6-one (6b). The title compound was prepared from 6a,11a-dihydro-3-methoxy-6*H*-[1]benzothieno-[3,2-*c*][1]benzopyran-6-one (**11b**)[23] in three steps and 61% overall yield by a method similar to that described for **6a**: ^{1}H NMR δ 8.57 (d, J = 7.8 Hz, 1H), 8.10 (d, J = 8.0 Hz, 1H), 7.81 (d, J = 8.2 Hz, 1H), 7.5−7.7 (m, 2H), 6.99 (m, 2H), 1.03 (s, 9H), 0.33 (s, 6H); IR ($CHCl_3$) 1716 cm^{-1}; MS (FD) *m/e* 382 (M^+). Anal. ($C_{21}H_{22}O_3SSi$) C, H.

9-[(*tert*-Butyldimethylsilyl)oxy]-6*H*-[1]benzothieno-[3,2-*c*][1]benzopyran-6-one (6c). The title compound was prepared from **11c** in three steps and 73% overall yield by a method similar to that described for **6a**: ^{1}H NMR ($CDCl_3$) δ 8.55 (d, J = 8.5 Hz, 1H), 7.72 (d, J = 8.7 Hz, 1H), 7.4−7.6 (m, 2H), 7.34 (m, 2H), 7.08 (dd, J = 2.2, 8.8 Hz, 1H), 1.02 (s, 9H), 0.26 (s, 6H); IR ($CHCl_3$) 1717 cm^{-1}; MS (FD) *m/e* 382 (M^+). Anal. ($C_{21}H_{22}O_3SSi$) C, H.

(±)-3,9-Bis[(*tert*-butyldimethylsilyl)oxy]-6*H*-[1]benzothieno[3,2-*c*][1]benzopyran-6-ol (15a). A solution of **6a** (2.0 g, 3.9 mmol) in toluene (200 mL) was cooled to −92 °C and treated dropwise with a 1.0 M toluene solution of diisobutylaluminum hydride (4.7 mL, 4.7 mmol) at a rate that maintained the internal temperature below −89 °C. The mixture was stirred for approximately 3 h as the temperature gradually rose to −77 °C and then was quenched with MeOH (5 mL) and 10% aqueous citric acid (50 mL). After dilution with CH_2Cl_2 (200 mL), the mixture was washed with saturated potassium sodium tartrate (100 mL), and the aqueous layer was extracted with CH_2Cl_2 (2 × 200 mL). The combined organic layers were washed with brine (300 mL), and the brine wash was further extracted with CH_2Cl_2 (100 mL). The organic layers were dried (Na_2SO_4) and concentrated, and the remnant was chromatographed (silica gel, 1−15% EtOAc/hexane) to yield 360 mg (18%) of starting material, 1.21 g (60%, 74% based on recovered starting material), of the titled compound as a white crystalline solid (analytical sample recrystallized from hexane/EtOAc, mp 162−164 °C) [^{1}H NMR δ 7.70 (d, J = 9.0 Hz, 1H), 7.46 (d, J = 2.1 Hz, 1H), 7.29 (d, J = 8.1 Hz, 1H), 7.00 (dd, J = 8.5, 1.8 Hz, 1H), 6.85 (br s, 1H), 6.6 (m, 2H), 6.39 (br s, 1H), 1.01 (s, 9H), 1.00 (s, 9H), 0.25 (s, 6H), 0.25 (s, 6H); ^{13}C NMR δ 156.9, 153.3, 151.7, 139.9, 131.9, 124.5, 123.9, 121.7, 118.8, 113.7, 113.1, 112.7, 108.7, 90.7, 25.1, 25.1, 17.9, −5.2, −5.2; IR ($CHCl_3$) 3540 cm^{-1}; MS (FD+) *m/e* 514 (M^+). Anal. ($C_{27}H_{38}O_4SSi_2$) C, H] and 260 mg (13%, 16% based on recovered starting material) of diol **18** as an amorphous solid: ^{1}H NMR ($CDCl_3$) δ 7.73 (d, J = 8.7 Hz, 1H), 7.29 (d, J = 2.0 Hz, 1H), 7.16 (d, J = 8.2 Hz, 1H), 6.98 (dd, J = 8.7, 2.0 Hz, 1H), 6.5 (m, 3H), 6.30 (br s, 1H), 4.71 (s, 2H), 1.01 (s, 9H), 1.00 (s, 9H), 0.22 (s, 6H), 0.19 (s, 6H); IR ($CHCl_3$) 3600, 3510 cm^{-1}; MS (FD+) *m/e* 516 (M^+).

(±)-3-[(*tert*-Butyldimethylsilyl)oxy]-6*H*-[1]benzothieno-[3,2-*c*][1]benzopyran-6-ol (15b). The title compound was prepared in 45% yield from **6b** by a method similar to that described for **15a**: ^{1}H NMR δ 7.96 (d, J = 7.7 Hz, 1H), 7.83 (d, J = 6.7 Hz, 1H), 7.3−7.8 (m, 3H), 6.91 (m, 1H), 6.62 (m, 2H), 1.00 (s, 9H), 0.26 (s, 6H); IR ($CHCl_3$) 2958, 2932, 2861, 1616, 1594 cm^{-1}; MS (FD) *m/e* 384 (M^+). Anal. ($C_{21}H_{24}O_3SSi$) C, H.

(±)-9-[(*tert*-Butyldimethylsilyl)oxy]-6*H*-[1]benzothieno-[3,2-*c*][1]benzopyran-6-ol (15c). The title compound was prepared in 49% yield from **6c** by a method similar to that described for **15a**: ^{1}H NMR δ 7.77 (d, J = 8.6 Hz, 1H), 7.50 (d, J = 2.1 Hz, 1H), 7.42 (m, 1H), 7.28 (m, 1H), 7.05 (m, 2H), 6.89 (m, 1H), 6.46 (m, 1H), 1.02 (s, 9H), 0.26 (s, 6H); IR ($CHCl_3$) 2959, 2932, 2861, 1612, 1598 cm^{-1}; MS (FD) *m/e* 384 (M^+). Anal. ($C_{21}H_{24}O_3SSi$) C, H.

(±)-3,9-Bis[(*tert*-butyldimethylsilyl)oxy]-6-phenoxy-6*H*-[1]benzothieno[3,2-*c*][1]benzopyran (20a). To a solution of **15a** (4.52 g, 8.78 mmol) and phenol (4.13 g, 43.9 mmol) in CH_2Cl_2 (100 mL) was added anhydrous $MgSO_4$ (4.5 g), and the resultant slurry was stirred for 4 h at ambient temperature. The mixture was filtered and concentrated and the residue dissolved in chlorobenzene and reconcentrated in vacuo at approximately 70 °C. The residue was then dissolved in CH_2Cl_2 (300 mL), washed with saturated Na_2CO_3 (3 × 300 mL) and water (2 × 300 mL), dried (Na_2SO_4), and concentrated to yield 5.16 g (99%) of the title compound as an amorphous solid which was used without further purification: ^{1}H NMR δ 7.67 (d, J = 8.7 Hz, 1H), 7.50 (d, J = 2.1 Hz, 1H), 7.4−7.5 (m, 4H), 7.23 (d, J = 8.4 Hz, 1H), 7.08 (t, J = 7.2 Hz, 1H), 6.99 (dd, J = 8.7, 2.1 Hz, 1H), 6.67 (dd, J = 8.4, 2.3 Hz, 1H), 6.62 (d, J = 2.3 Hz, 1H), 0.99 (s, 9H), 0.96 (s, 9H), 0.24 (s, 6H), 0.21 (s, 6H); ^{13}C NMR δ 156.9, 153.3, 151.7, 139.9, 131.9, 124.5, 123.9, 121.7, 118.8, 113.7, 113.1, 112.7, 108.7, 90.7, 25.1, 25.1, 17.9, −5.2, −5.2; MS (FD+) *m/e* 590 (M^+). Anal. ($C_{33}H_{42}O_4SSi_2$) C, H.

(±)-3-[(*tert*-Butyldimethylsilyl)oxy]-6-phenoxy-6*H*-[1]-benzothieno[3,2-*c*][1]benzopyran (20b). A solution of **15b** (1.59 g, 4.1 mmol) and phenol (5.29 g, 56 mmol) in chlorobenzene (50 mL) was heated at reflux for 2.5 h. The mixture was concentrated in vacuo at approximately 70 °C, dissolved in additional chlorobenzene (50 mL), and reconcentrated to yield 1.9 g (100%) of the title compound as a pink solid which was used without further purification: ^{1}H NMR δ 8.05 (d, J = 8.7 Hz, 1H), 7.85 (d, J = 8.7 Hz, 1H), 7.3−7.6 (m, 8H), 7.14 (t, J = 8.2 Hz, 1H), 6.75 (dd, J = 8.7, 2.1 Hz, 1H), 6.69 (d, J = 2.1 Hz, 1H), 1.00 (s, 9H), 0.26 (s, 6H).

(±)-9-(*tert*-Butyldimethylsilyl)oxy-6-phenoxy-6*H*-[1]-benzothieno[3,2-*c*][1]benzopyran (20c). The title compound was prepared in 93% yield from **15c** by a method similar to that described for **20b**: ^{1}H NMR δ 7.76 (d, J = 8.7 Hz, 1H), 7.60 (d, J = 2.1 Hz, 1H), 7.56 (d, J = 8.7 Hz, 1H), 7.1−7.5 (m, 10H), 1.04 (s, 9H), 0.30 (s, 6H).

(±)-3,9-Dihydroxy-6-[4-[2-(1-piperidinyl)ethoxy]phenyl]-6*H*-[1]benzothieno[3,2-*c*][1]benzopyran (3a). To a solution of **20a** (4.0 g, 6.7 mmol) in toluene (100 mL) at 0 °C was added a 0.5 M THF solution of [4-[2-(1-piperidinyl)ethoxy]phenyl]-magnesium bromide (prepared from the corresponding bromobenzene and magnesium turnings, catalyzed by iodine in THF, 27 mL, 13.5 mmol). The mixture was allowed to warm to room temperature and stirred for 1.5 h. After being quenched with water (300 mL), the mixture was extracted with EtOAc (2 × 300 mL), and the organic layer was dried (Na_2SO_4) and concentrated. The residue was purified via chromatography (silica gel, 3:1 hexane:EtOAc, 0.1% NH_4OH) to give 3.85 g (82%) of the title compound as a colorless, gummy solid: ^{1}H NMR δ 7.45 (s, 1H), 7.2−7.3 (m, 4H), 6.8−6.9 (m, 3H), 6.68 (s, 1H), 6.51 (d, J = 8.1 Hz, 1 H), 6.36 (s, 1H), 4.01 (t, J = 6.0 Hz, 2H), 2.62 (t, J = 6.0 Hz, 2H), 2.41 (m, 4H), 1.4−1.6 (m, 4H), 1.3−1.4 (m, 2H), 0.99 (s, 9H), 0.96 (s, 9H), 0.22 (s, 6H), 0.20 (s, 6H); MS (FD) *m/e* 702 (M^+).

To a solution of the product of the above reaction (3.85 g, 5.5 mmol) in THF (150 mL) was added a 1.0 M THF solution of TBAF (27.4 mL, 27.4 mmol). The solution was stirred at ambient temperature for 2 h, diluted with EtOAc (300 mL), and washed with saturated NH_4Cl (300 mL). The aqueous layer was washed with EtOAc (150 mL), and the combined organic layers were washed with saturated $NaHCO_3$ (300 mL), dried (Na_2SO_4), and concentrated. The remnant was purified by chromatography (silica gel, 1:1 hexane:EtOAc, 10% MeOH, 0.1% NH_4OH) to give 2.35 g (90%) of the titled product as a red foam. Crystallization from MeOH gave an off-white powder, mp 242−245 °C (dec): ^{1}H NMR δ 8.58 (br s, 2H), 7.35 (d, J = 2.1 Hz, 1H), 7.26 (d, J = 8.6 Hz, 2H), 7.17 (d, J = 8.2 Hz, 1H), 7.16 (d, J = 8.6 Hz, 1H), 6.84 (d, J = 8.5 Hz, 2H), 6.80 (m, J = 2.2 Hz, 1H), 6.63 (s, 1H), 6.46 (dd, J = 8.2, 2.2 Hz, 1H), 6.35 (d, J = 2.3 Hz, 1H), 4.02 (t, J = 6.0 Hz, 2H), 2.63 (t, J = 6.0 Hz, 2H), 2.42 (m, 4H), 1.4−1.6 (m, 4H), 1.3−1.4 (m, 2H); ^{13}C NMR (DMF-d_7) δ 160.2, 159.9, 156.4, 153.2, 140.8, 132.8, 130.9, 129.6, 125.3, 124.6, 122.5, 115.2, 115.1,

112.2, 109.6, 108.8, 108.7, 104.5, 77.6, 66.6, 58.3, 55.3, 26.5, 24.8; HRMS (FAB+) *m/e* calcd for $C_{28}H_{28}NO_4S$ 474.1739 (MH^+), found 474.1726. Anal. ($C_{28}H_{27}NO_4S \cdot 0.8H_2O$) C, H, N.

(±)-3-Hydroxy-6-[4-[2-(1-piperidinyl)ethoxy]phenyl]-6*H*-[1]benzothieno[3,2-*c*][1]benzopyran (3b). The title compound was prepared in two steps and 82% overall yield from **20b** by a method similar to that described for **3a**. The product was slurried in MeOH, trifluoroacetic acid was added dropwise until all the material went into solution, the insoluble material was filtered away, and the mother liquor was concentrated in vacuo to yield 0.28 g (80%) of the TFA salt as a fluffy orange solid: ^{1}H NMR δ 7.95 (m, 1H), 7.38 (m, 1H), 7.2−7.3 (m, 5H), 6.90 (d, J = 8.6 Hz, 2H), 6.76 (s, 1H), 6.51 (dd, J = 2.2, 8.2 Hz, 1H), 6.38 (d, J = 2.3 Hz, 1H), 4.44 (t, J = 4.9 Hz, 2H), 3.5−3.7 (m, 4H), 3.0−3.2 (m, 2H), 1.7−2.0 (m, 5H), 1.4−1.7 (m, 1H); IR ($CHCl_3$) 3271, 3022, 3009, 1670, 1610 cm^{-1}; HRMS (FAB) *m/e* calcd for $C_{28}H_{28}NO_3S$ (MH^+) 458.1790, found 458.1781. Anal. ($C_{28}H_2NO_3S \cdot CF_3COOH \cdot 1.2H_2O$) C, H, N.

(±)-9-Hydroxy-6-[4-[2-(1-piperidinyl)ethoxy]phenyl]-6*H*-[1]benzothieno[3,2-*c*][1]benzopyran (3c). The title compound was prepared in two steps and 56% overall yield from **20c** by a method similar to that described for **3a**: ^{1}H NMR δ 6.7−7.4 (m, 12H), 4.02 (t, J = 6.0 Hz, 2H), 2.63 (t, J = 6.0 Hz, 2H), 2.41 (m, 4H), 1.49 (m, 4H), 1.37 (m, 2H); IR (KBr) 2934, 1609 cm^{-1}; HRMS (FAB) *m/e* calcd for $C_{28}H_{28}NO_3S$ (MH^+) 458.1790, found 458.1798. Anal. ($C_{28}H_{27}NO_3S \cdot 0.5H_2O$) C, H, N.

(±)-6-[4-[2-(1-Piperidinyl)ethoxy]phenyl]-6*H*-[1]benzothieno[3,2-*c*][1]benzopyran (3d). To a solution of the **3a** (500 mg, 1.06 mmol) in THF (25 mL) containing DMF (2 mL) at room temperature was added Et_3N (0.64 g, 0.69 mL, 6.36 mmol) followed by *N*-phenyltrifluoromethanesulfonimide (0.83 g, 2.33 mmol). The reaction mixture was stirred for 12 h and warmed to 60 °C, and additional *N*-phenyltrifluoromethanesulfonimide (0.30 g, 0.85 mmol) was added. After 30 min, the reaction mixture was cooled to ambient temperature and concentrated, and the residue was purified by radial chromatography (silica gel, 1:1 hexane:EtOAc, 1% MeOH under an NH_3 atmosphere) to afford 780 mg (100%) of the bistriflate as a white foam: ^{1}H NMR (MeOH-d_4) δ 7.63 (d, J = 8.8 Hz, 1H), 7.56 (d, J = 2.5 Hz, 1H), 7.13 (dd, J = 8.8, 2.7 Hz, 1H), 7.03 (d, J = 8.8 Hz, 2H), 6.80 (d, J = 8.9 Hz, 2H), 6.49 (d, J = 2.5 Hz, 1H), 6.39 (d, J = 8.7 Hz, 1H), 6.05 (dd, J = 8.7, 2.6 Hz, 1H), 4.00 (t, J = 5.5 Hz, 2H), 2.67 (m, 2H), 2.45 (m, 4H), 1.4−1.6 (m, 4H), 1.3−1.4 (m, 2H); MS (FD) *m/e* 737 (M^+).

A solution of the product of the above reaction (780 mg, 1.06 mmol), $Pd(OAc)_2$ (42 mg, 0.19 mmol), 1,2-bis(diphenylphosphino)propane (149 mg, 0.36 mmol), formic acid (0.6 mL), and Et_3N (3.0 mL) in anhydrous DMF (40 mL) was stirred at ambient temperature for 4 d. After concentration, the residue was subjected chromatography (silica gel, 1:1 hexane:EtOAc, 2−10% MeOH, 0.1% NH_4OH). Product-containing fractions were concentrated and partitioned between CH_2Cl_2 (100 mL) and saturated $NaHCO_3$ (100 mL), and the aqueous layer was extracted with CH_2Cl_2 (50 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated, and the residue was purified by chromatography (silica gel, 1:1 hexane:EtOAc, 2−10% MeOH, 0.1% NH_4OH) to give 267 mg (57%) of the title compound as an oil which gave a white, crystalline solid, mp 107 °C, upon trituration with ether/hexane: ^{1}H NMR ($CDCl_3$) δ 7.86 (d, J = 7.4 Hz, 1H), 7.40 (m, 1H), 7.1−7.3 (m, 6H), 6.97 (t, J = 7.3 Hz, 1H), 6.89 (d, J = 8.1 Hz, 1H), 6.84 (d, J = 8.6 Hz, 2H), 6.66 (s, 1H), 4.07 (t, J = 6.1 Hz, 2H), 2.75 (t, J = 6.1 Hz, 2H), 2.49 (m, 4H), 1.5−1.7 (m, 4H), 1.4−1.5 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 159.1, 151.6, 139.3, 137.1, 133.0, 131.5, 129.8, 129.1, 126.8, 124.6, 124.4, 123.7, 122.6, 121.5, 121.5, 119.3, 116.9, 114.6, 77.6, 65.7, 57.7, 54.9, 25.8, 24.0; MS (FD+) *m/e* 441 (M^+). Anal. ($C_{28}H_{27}NO_2S$) C, H, N.

(±)-3,9-Dimethoxy-6-[4-[2-(1-piperidinyl)ethoxy]phenyl]-6*H*-[1]benzothieno[3,2-*c*][1]benzopyran (3e). A solution of **3a** (300 mg, 0.633 mmol) in MeOH (75 mL) was treated with a solution of diazomethane in ether/EtOH (approximately 16 mmol) at ambient temperature. The mixture was stirred until gas evolution ceased, N_2 was bubbled through for 10 min to remove excess diazomethane, and the mixture was concentrated. Radial chromatography of the residue (1:1 hexane:EtOAc, 2% MeOH, under an NH_3 atmosphere) provided 118 mg (37%) of the title compound as white crystals, mp 134−36 °C: ^{1}H NMR δ 7.51 (d, J = 2.2 Hz, 1H), 7.2−7.4 (m, J = 8.6 Hz, 4H), 6.86 (m, 3H), 6.70 (s, 1H), 6.55 (dd, J = 8.4, 2.3 Hz, 1H), 6.41 (d, J = 2.4 Hz, 1H), 4.01 (t, J = 6.0 Hz, 2H), 3.83 (s, 3H), 3.74 (s, 3H), 2.62 (t, J = 6.0 Hz, 2H), 2.40 (m, 4H), 1.4−1.6 (m, 4H), 1.3−1.4 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 160.8, 159.2, 157.1, 152.6, 140.3, 131.6, 131.3, 130.5, 129.1, 124.3, 124.0, 121.7, 114.6, 114.0, 112.8, 107.6, 105.7, 102.3, 77.9, 65.8, 57.8, 55.5, 55.2, 54.9, 25.9, 24.1; MS (FD+) *m/e* 501 (M^+). Anal. ($C_{30}H_{31}NO_4S$) C, H, N.

(±)-3,9-Bis[(*tert*-butyldimethylsilyl)oxy]-6-[4-hydroxyphenyl]-6*H*-[1]benzothieno[3,2-*c*][1]benzopyran (23). To a solution of **20a** (3.0 g, 5.08 mmol) in toluene (150 mL) at 0 °C was added a 0.4 M THF solution of [4-(trimethylsilyloxy)-phenyl]magnesium bromide (prepared from the corresponding bromobenzene and magnesium turnings, catalyzed by iodine, in THF, 25.4 mL, 10.16 mmol).[27] The mixture was allowed to warm to room temperature and stirred for 1.5 h. After dilution with ether (250 mL), the mixture was quenched with saturated NH_4Cl (250 mL), and the organic layer was dried (Na_2SO_4) and concentrated. The residue was slurried in MeOH (100 mL), and ether was added until the mixture was homogeneous. The solution was cooled to 0 °C and treated with anhydrous K_2CO_3 (3 g) for 15 min. After dilution with ether (250 mL), the mixture was filtered through Celite and washed with saturated NH_4Cl, and the aqueous layer was extracted with additional ether (100 mL). The combined organic layers were dried (Na_2SO_4) and concentrated, and the residue was purified by chromatography (silica gel, 10:1 hexane:EtOAc). Recrystallization from hexane provided 2.6 g (87%) of the title compound as a light pink solid, mp 174−175 °C: ^{1}H NMR δ 8.49 (s, 1H), 7.44 (d, J = 2.2 Hz, 1H), 7.3−7.4 (m, 4H), 6.83 (dd, J = 8.7, 2.2 Hz, 1H), 6.76 (d, J = 8.5 Hz, 2H), 6.65 (s, 1H), 6.50 (dd, J = 8.2, 2.3 Hz, 1H), 6.36 (d, J = 2.3 Hz, 1H), 0.98 (s, 9H), 0.95 (s, 9H), 0.21 (s, 6H), 0.20 (s, 6H); ^{13}C NMR δ 158.7, 157.8, 153.9, 153.5, 140.9, 132.7, 131.6, 131.1, 129.9, 126.1, 124.7, 122.7, 119.3, 116.1, 114.4, 114.0, 113.9, 109.3, 78.3, 26.0, 25.9, 18.7, −4.3; IR ($CHCl_3$) 3590, 3310 cm^{-1}; MS (FD+) *m/e* 590 (M^+). Anal. ($C_{33}H_{42}O_4SSi_2$) C, H.

(±)-3,9-Dihydroxy-6-[4-(2-dimethylaminoethoxy)-phenyl]-6*H*-[1]benzothieno[3,2-*c*][1]benzopyran (3f). A toluene (30 mL) solution of **23** (450 mg, 0.76 mmol), triphenylphosphine (799 mg, 3.1 mmol), and *N*,*N*-dimethylethanolamine (340 mg, 383 mL, 3.81 mmol) was treated with DEAD (531 mg, 480 μL, 3.1 mmol) at ambient temperature, and the mixture was stirred for 2 h. The mixture was then diluted with ether (100 mL) and washed with saturated NH_4Cl (100 mL), and the aqueous layer was washed with additional ether (50 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. Chromatography (silica gel, 1:1 hexane:EtOAc, 3% MeOH, 0.1% NH_4OH) provided 357 mg (71%) of a white foam: ^{1}H NMR δ 7.45 (d, J = 2.1 Hz, 1H), 7.27 (d, J = 8.6 Hz, 2H), 7.24 (m, 2H), 6.83 (m, 3H), 6.67 (s, 1H), 6.50 (dd, J = 8.2, 2.2 Hz, 1H), 6.36 (d, 2.2 Hz, 1H), 4.00 (t, J = 5.9 Hz, 2H), 2.60 (t, J = 5.9 Hz, 2H), 2.20 (s, 6H), 0.98 (s, 9H), 0.95 (s, 9H), 0.21 (s, 6H), 0.20 (s, 6H); MS (FD+) *m/e* 661.5 (M^+).

A solution of the above product (328 mg, 0.50 mmol) in THF (10 mL) was treated with 1.0 M TBAF in THF (2.5 mmol) for 2 h, diluted with saturated NH_4Cl (100 mL), and extracted with EtOAc (100 mL). The aqueous layer was washed with EtOAc (50 mL), and the combined organic layers were washed with saturated $NaHCO_3$ (2 × 100 mL), dried (Na_2SO_4), and concentrated. Radial chromatography (silica gel, 1:1 hexane:EtOAc, 30% MeOH, under an NH_3 atmosphere) provided 212 mg (99%) of the title compound, which crystallized from CCl_4/acetone as a pink solid, mp 130−140 °C (dec): ^{1}H NMR δ 7.34 (d, J = 2.1 Hz, 1H), 7.25 (d, J = 8.6 Hz, 2H), 7.15 (m, 2H), 6.9−7.0 (m, 3H), 6.62 (s, 1H), 6.45 (dd, J = 8.3, 2.3 Hz, 1H), 6.34 (d, J = 2.2 Hz, 1H), 4.01 (t, J = 5.8 Hz, 2H), 2.64 (t, J = 5.8 Hz, 2H), 2.30 (s, 6H); ^{13}C NMR δ 160.0, 159.8, 156.0, 153.5,

141.1, 133.0, 131.4, 130.2, 129.8, 125.3, 124.8, 122.6, 115.3, 115.2, 112.7, 109.7, 109.0, 104.8, 78.0, 66.6, 58.6, 45.8; IR (KBr) 3300 cm^{-1}; HRMS (FAB+) *m/e* calcd for $C_{25}H_{24}NO_4S$ 434.1426 (MH^+), found 434.1440. Anal. ($C_{25}H_{23}NO_4S \cdot H_2O$) C, H, N.

(±)-3,9-Dihydroxy-6-[4-[2-(diethylamino)ethoxy]phenyl]-6*H*-[1]benzothieno[3,2-*c*][1]benzopyran (3g). The title compound was prepared in two steps and 66% overall yield from **23** and *N*,*N*-diethylethanolamine by a method similar to that described for **3f** as a red solid, mp 118−123 °C (dec) (acetone/ether): ^{1}H NMR δ 7.35 (d, J = 2.1 Hz, 1H), 7.26 (d, J = 8.6 Hz, 2H), 7.16 (d, J = 8.1 Hz, 1H), 7.13 (d, J = 8.5 Hz, 1H), 6.81 (m, 3H), 6.62 (s, 1H), 6.45 (dd, J = 8.3, 2.3 Hz, 1H), 6.35 (d, J = 2.2 Hz, 1H), 3.98 (t, J = 6.2 Hz, 2H), 2.78 (t, J = 6.2 Hz, 2H), 2.56 (q, J = 7.1 Hz, 4H), 0.97 (t, J = 7.1 Hz, 6H); ^{13}C NMR δ 160.2, 159.7, 155.9, 153.5, 141.1, 132.9, 131.5, 130.2, 129.9, 125.4, 124.8, 122.7, 115.2, 112.8, 109.6, 108.9, 104.8, 78.1, 67.5, 52.6, 48.3, 12.4; IR (KBr) 3311 cm^{-1}; MS (FD+) *m/e* 462 (MH^+). Anal. ($C_{27}H_{27}NO_4S$) C, H, N.

(±)-3,9-Dihydroxy-6-[4-[2-(1-pyrrolidinyl)ethoxy]-phenyl]-6*H*-[1]benzothieno[3,2-*c*][1]benzopyran (3h). The title compound was prepared in two steps and 70% overall yield from **23** and 1-(2-hydroxyethyl)pyrrolidine by a method similar to that described for **3f** as a white solid, mp 237−240 °C (dec) (hexane/EtOAc): ^{1}H NMR (DMF-d_7) δ 10.05 (br, 2H), 7.45 (d, J = 2.0 Hz, 1H), 7.30 (d, J = 8.4 Hz, 2H), 7.23 (d, J = 8.6 Hz, 1H), 7.19 (d, J = 8.2 Hz, 1H), 6.91 (d, J = 8.7 Hz, 2H), 6.86 (dd, J = 8.9, 2.2 Hz, 1H), 6.74 (s, 1H), 6.51 (dd, J = 8.2, 2.1 Hz, 1H), 6.40 (d, J = 2.1 Hz, 1H), 4.14 (t, J = 5.6 Hz, 2H), 2.96 (t, J = 5.5 Hz, 2H), 2.6−2.8 (m, 4H), 1.6−1.8 (m, 4H); ^{13}C NMR (DMF-d_7) δ 160.2, 159.6, 156.4, 153.2, 140.8, 133.0, 130.8, 129.6, 125.2, 124.6, 122.4, 115.3, 115.1, 112.2, 109.7, 108.8, 104.5, 77.6, 66.8, 54.8, 54.7, 23.8; IR (KBr) 3286 cm^{-1}; HRMS (FAB+) *m/e* calcd for $C_{27}H_{26}NO_4S$ 460.1583 (MH^+), found 460.1572. Anal. ($C_{27}H_{25}NO_4S \cdot 0.5H_2O$) C, H, N.

(±)-3,9-Dihydroxy-6-[4-[2-(1-morpholinyl)ethoxy]-phenyl]-6*H*-[1]benzothieno[3,2-*c*][1]benzopyran (3i). The title compound was prepared in two steps and 77% overall yield from **23** and 1-(2-hydroxyethyl)morpholine by a method similar to that described for **3f** as a red solid, mp 147−153 °C (dec) (acetone/ether): ^{1}H NMR δ 7.35 (d, J = 2.1 Hz, 1H), 7.25 (d, J = 8.6 Hz, 2H), 7.16 (d, J = 8.3 Hz, 1H), 7.14 (d, J = 8.6 Hz, 1H), 6.81 (m, 3H), 6.62 (s, 1H), 6.46 (dd, J = 8.2, 2.2 Hz, 1H), 6.35 (d, J = 2.2 Hz, 1H), 4.04 (t, J = 5.7 Hz, 2H), 3.56 (t, J = 4.7 Hz, 4H), 2.68 (t, J = 5.7 Hz, 2H), 2.47 (t, J = 4.3 Hz, 4H); ^{13}C NMR δ 160.0, 159.7, 155.9, 153.5, 141.1, 132.9, 131.4, 129.8, 129.7, 125.4, 124.8, 122.6, 115.2, 112.8, 109.6, 108.9, 104.7, 78.0, 67.2, 66.5, 58.1, 54.7; IR (KBr) 3471 cm^{-1}; MS (FD+) *m/e* 475 (MH^+). Anal. ($C_{27}H_{25}NO_5S \cdot 0.25H_2O$) C, H, N.

(±)-3,9-Dihydroxy-6-[4-[2-(2-oxo-1-pyrrolidinyl)ethoxy]-phenyl]-6*H*-[1]benzothieno[3,2-*c*][1]benzopyran (3j). The title compound was prepared in two steps and 50% overall yield from **23** and 1-(2-hydroxyethyl)-2-pyrrolidinone by a method similar to that described for **3f** as a red solid, mp 150−160 °C (dec) (acetone solvate): ^{1}H NMR (MeOH-d_4) δ 7.21 (m, 3H), 7.11 (d, J = 8.3 Hz, 1H), 7.02 (d, J = 8.7 Hz, 1H), 6.80 (d, J = 8.6 Hz, 2H), 6.71 (dd, J = 8.6, 2.2 Hz, 1H), 6.51 (s, 1H), 6.37 (dd, J = 8.2, 2.3 Hz, 1H), 6.25 (d, J = 2.3 Hz, 1H), 4.04 (t, J = 5.2 Hz, 2H), 3.57 (t, J = 5.2 Hz, 2H), 3.48 (t, J = 7.1 Hz, 2H), 2.28 (t, J = 8.1 Hz, 2H), 2.14 (s, 6H, CH_3CO) 1.91 (quintet, J = 7.5 Hz, 2H); ^{13}C NMR (MeOH-d_4) δ 178.0, 160.2, 160.0, 156.2, 153.9, 141.7, 133.7, 131.9, 130.9, 130.3, 125.6, 125.0, 122.7, 115.6, 115.4, 113.4, 109.8, 109.0, 104.9, 78.6, 66.6, 49.7, 43.4, 31.8, 30.7, 18.9; IR ($CHCl_3$) 1680 cm^{-1}; MS (FD+) *m/e* 474 (MH^+). Anal. ($C_{27}H_{23}NO_5S \cdot (CH_3)_2CO$) C, H, N.

3,9-Bis[(*tert*-butyldimethylsilyl)oxy]-6*H*-benzofuro[3,2-*c*][1]benzopyran-6-one (5). Coumestrol (10 g, 37.3 mmol) was slurried in CH_2Cl_2 (600 mL), cooled to 0 °C, and treated with Et_3N (24.9 g, 34.3 mL, 246 mmol) and *tert*-butyldimethylsilyl chloride (24.7 g, 164 mmol). The mixture was warmed to ambient temperature and stirred overnight, during which it slowly became homogeneous. After dilution with ether (800 mL), the mixture was washed with brine (800 mL) and the aqueous layer was extracted with ether (500 mL). The combined organic layers were dried (Na_2SO_4) and concentrated, and the residue was recrystallized from hexane to provide 14.2 g (77%) of the title compound as a white powder, mp 118−119 °C: ^{1}H NMR ($CDCl_3$) δ 7.90 (d, J = 8.5 Hz, 1H), 7.82 (d, J = 8.5 Hz, 1H), 7.10 (d, J = 2.0 Hz, 1H), 6.97 (m, 2H), 6.90 (dd, J = 8.5, 2.0 Hz, 1H), 1.03 (s, 9H), 1.02 (s, 9H), 0.28 (s, 6H), 0.25 (s, 6H); IR ($CHCl_3$) 1733 cm^{-1}; MS (FD+) *m/e* 496 (M^+). Anal. ($C_{27}H_{36}O_5Si_2$) C, H.

(±)-3,9-Bis[(*tert*-butyldimethylsilyl)oxy]-6*H*-benzofuro-[3,2-*c*][1]benzopyran-6-ol (14). The title compound was prepared from **5** in 37% yield as a mixture with its aldehyde tautomer (**17**) by a method similar to that described for **15a**: ^{1}H NMR δ 10.15 (s, 0.5H), 9.2 (br s, 0.5H), 7.99 (d, J = 8.4 Hz, 1H), 7.56 (d, J = 8.4 Hz, 1H), 7.50 (m, 0.5H), 7.09 (d, J = 2.0 Hz, 1H), 6.96 (m, 1H), 6.6−6.7 (m, 2H), 6.55 (m, 0.5H), 1.01 (s, 9H), 1.00 (s, 9H), 0.27 (s, 6H), 0.25 (s, 6H); IR ($CHCl_3$) 3550, 1663 cm^{-1}; MS (FD) *m/e* 499 (MH^+). Anal. ($C_{27}H_{38}O_5Si_2$) C, H.

(±)-3,9-Bis[(*tert*-butyldimethylsilyl)oxy]-6-phenoxy-6*H*-benzofuro[3,2-*c*][1]benzopyran (19). The title compound was prepared in 87% yield from **14** by a method similar to that described for **20a**: ^{1}H NMR δ 7.1−7.7 (m, 8H) 6.6−6.9 (m, 3H), 1.01 (s, 9H), 1.00 (s, 9H), 0.28 (s, 6H), 0.25 (s, 6H).

(±)-3,9-Dihydroxy-6-[4-[2-(1-piperidinyl)ethoxy]phenyl]-6*H*-benzofuro[3,2-*c*][1]benzopyran (2). The title compound was prepared in two steps and 65% overall yield from **19** by a method similar to that described for **3a** as a bright red foam, which crystallized from CCl_4: ^{1}H NMR (DMF-d_7) δ 10.00 (br s, 1H), 9.83 (br s, 1H), 7.3−7.5 (m, 3H), 7.10 (s, 1H), 6.98 (d, J = 8.5 Hz, 2H), 6.7−6.9 (m, 3H), 6.54 (dd, J = 8.1, 1.8 Hz, 1H), 6.42 (s, 1H), 4.10 (t, J = 5.8 Hz, 2H), 2.67 (m, 2H), 2.44 (m, 4H), 1.4−1.6 (m, 4H), 1.3−1.4 (m, 2H); ^{13}C NMR (DMF-d_7) δ 160.2, 159.9, 156.8, 156.4, 155.1, 147.2, 133.0, 129.5, 121.4, 119.5, 118.9, 115.1, 112.9, 109.2, 108.9, 108.3, 104.3, 98.7, 78.5, 66.5, 58.2, 55.3, 26.4, 24.6; IR (KBr) 3220 cm^{-1}; HRMS (FAB+) *m/e* calcd for $C_{28}H_{28}NO_5$ 458.1967 (MH^+), found 458.1974. Anal. ($C_{28}H_{27}NO_4S \cdot 1.75H_2O$) C, N; H: calcd, 6.30; found, 5.81.

(±)-2-Methoxy-8-hydroxy-11,12-dihydro-5*H*-benzo[*b*]-naphtho[2,1-*d*]pyran-5-one (13). To a solution of 1-carbomethoxy-6-methoxy-2-tetralone (**12**)[27] (18.0 g, 76.8 mmol) and resorcinol (8.9 g, 80.7 mmol) being stirred at ambient temperature in toluene (450 mL) was added $POCl_3$ (12.0 g, 7.3 mL, 18.3 mmol) dropwise, and the mixture was warmed to 80 °C for 12 h. After being cooled to room temperature, the mixture was poured into water (500 mL) and filtered, and the precipitate was rinsed with ether. The filtrate layers were separated, the aqueous layer was extracted with EtOAc (3 × 500 mL), and the combined organic layers were dried (Na_2SO_4) and concentrated. Recrystallization of the residue from MeOH provided 16.0 g (71%) of the title compound as a yellow solid, mp 244−249 °C (dec): ^{1}H NMR (DMSO-d_6) δ 10.55 (br s, 1H), 8.24 (d, J = 8.6 Hz, 1H), 7.75 (d, J = 8.7 Hz, 1H), 6.6−7.0 (m, 4H), 3.76 (s, 3H), 2.8−3.0 (m, 4H); ^{13}C NMR (DMSO-d_6) δ 170.3, 160.3, 158.3, 158.1, 153.3, 147.7, 137.5, 127.4, 125.8, 122.5, 114.5, 112.6, 112.4, 110.9, 101.4, 54.6, 26.3, 22.7; IR (KBr) 3250, 1676, 1618 cm^{-1}; MS (FD+) *m/e* 294 (M^+). Anal. ($C_{18}H_{14}O_4$) C, H.

2,8-Bis[(*tert*-butyldimethylsilyl)oxy]-5*H*-benzo[*b*]naphtho[2,1-*d*]pyran-5-one (7). To a mechanically stirred slurry of **13** (4.17 g, 14.2 mmol) in CH_2Cl_2 (100 mL) was added ethanethiol (3.53 g, 3.95 mL, 56.8 mmol) followed by aluminum chloride (9.0 g, 67.5 mmol), portionwise. The reaction mixture was stirred at ambient temperature for 5 h, cooled to 0 °C, and quenched cautiously with THF (100 mL) followed by saturated $NaHCO_3$ (100 mL). The mixture was diluted with THF (100 mL), the layers were separated, and the aqueous layer was washed with THF (100 mL). The combined organic layers were dried (Na_2SO_4) and concentrated to yield 3.6 g of crude diphenol as a yellow solid, which was used without further purification.

The crude product was slurried in CH_2Cl_2 (100 mL) and treated with Et_3N (8.6 g, 11.9 mL, 85.2 mmol) and *tert*-butyldimethylsilyl chloride (8.6 g, 56.8 mmol). The mixture was stirred at ambient temperature for 5 h, during which it

slowly became homogeneous. After dilution with ether (250 mL), the mixture was washed with brine (250 mL) and the aqueous layer was extracted with ether (150 mL). The combined organic layers were dried (Na_2SO_4) and concentrated, and the residue was recrystallized from hexane to provide 5.3 g (74%) of the title compound as a yellow solid, mp 145−147 °C: ^{1}H NMR ($CDCl_3$) δ 8.37 (d, J = 8.6 Hz, 1H), 7.58 (d, J = 9.3 Hz, 1H), 6.83 (s, 1H), 6.81 (m, 2H), 6.72 (d, J = 2.4 Hz, 1H), 2.8−3.0 (m, 4H), 1.00 (s, 18H), 0.27 (s, 6H), 0.24 (s, 6H); MS (FD) m/e 508 (M^+).

A sample of the above product (7.0 g, 13.8 mmol) and DDQ (3.3 g, 14.5 mmol) in DCE (100 mL) was heated at reflux overnight, inducing the formation of a precipitate. The mixture was cooled to room temperature, diluted with hexane (300 mL), filtered through Celite, and concentrated. The remnant was partitioned between hexane:ether (1:1, 200 mL) and water (200 mL), and the organic layer was dried ($MgSO_4$), concentrated, and recrystallized from hexane to provide 5.5 g (79%) of the title product as a tan solid, mp 154−156 °C: ^{1}H NMR ($CDCl_3$) δ 9.69 (d, J = 9.4 Hz), 8.80 (s, 2H), 8.01 (d, J = 9.5 Hz), 7.34 (dd, J = 9.3,2.5 Hz, 1H), 7.28 (d, J = 5.4 Hz, 1H), 6.90 (m, 2H), 1.05 (s, 9H), 1.03 (s, 9H), 0.29 (s, 12H); IR ($CHCl_3$) 1711, 1622, 1604 cm^{-1}; MS (FD) m/e 506 (M^+). Anal. ($C_{29}H_{38}O_4Si_2$) C, H.

(±)-2,8-Bis[(*tert*-butyldimethylsilyl)oxy]-5*H*-benzo[*b*]naphtho[2,1-*d*]pyran-5-ol (16). The title compound was prepared from **7** in 82% yield by a method similar to that described for **15a** as a white solid, mp 188−190 °C (hexane/ether): ^{1}H NMR ($CDCl_3$) δ 7.98 (d, J = 9.1 Hz, 1H), 7.86 (d, J = 8.8 Hz, 1H), 7.79 (d, J = 8.6 Hz, 1H), 7.75 (d, J = 8.2 Hz, 1H), 7.23 (d, J = 2.3 Hz, 1H), 7.18 (dd, J = 9.0, 2.4 Hz, 1H), 7.06 (d, J = 7.4 Hz, 1H), 6.67 (m 2H), 3.24 (d, J = 7.7 Hz, 1H), 1.04 (s, 9H), 1.02 (s, 9H), 0.27 (s, 6H), 0.27 (s, 6H); IR ($CHCl_3$) 3574 cm^{-1}; MS (FD) m/e 508 (M^+). Anal. ($C_{29}H_{40}O_4Si_2$) Calcd: C, 68.44; H, 7.94. Found: C, 68.63; H, 8.11.

(±)-2,8-Bis[(*tert*-butyldimethylsilyl)oxy]-5-phenoxy-5*H*-benzo[*b*]naphtho[2,1-*d*]pyran (21). The title compound was prepared in 95% yield from **16** by a method similar to that described for **20a**: ^{1}H NMR δ 7.9−8.1 (m, 4H), 7.61 (s, 1H), 7.3−7.5 (m, 3H), 7.2−7.3 (m, 3H), 7.11 (t, J = 7.3 Hz, 1H), 6.74 (dd, J = 8.5, 2.3 Hz, 1H), 6.62 (d, J = 2.4 Hz, 1H), 1.03 (s, 9H), 0.99 (s, 9H), 0.29 (s, 6H), 0.24 (s, 6H); MS (FD) m/e 584 (M^+).

(±)-2,8-Dihydroxy-5-[4-[2-(1-piperidinyl)ethoxy]phenyl]-5*H*-benzo[*b*]naphtho[2,1-*d*]pyran (4). The title compound was prepared in two steps and 81% overall yield from **21** by a method similar to that described for **3a** as a white solid, mp 158−161 °C (ether): ^{1}H NMR δ 7.89 (d, J = 8.7 Hz, 1H), 7.77 (d, J = 8.7 Hz, 1H), 7.68 (m, 2H), 7.23 (d, J = 2.4 Hz, 1H), 7.09 (m, 3H), 6.97 (s, 1H), 6.71 (d, J = 8.7 Hz, 2H), 6.49 (dd, J = 8.3, 2.4 Hz, 1H), 6.36 (d, J = 2.3 Hz, 1H), 3.95 (t, J = 6.0 Hz, 2H), 2.60 (t, J = 6.0 Hz, 2H), 2.40 (m, 4H), 1.4−1.5 (m, 4H), 1.3−1.4 (m, 2H); ^{13}C NMR δ 159.5, 159.5, 155.8, 153.9, 135.0, 132.4, 130.1, 128.0, 126.7, 125.6, 125.4, 125.3, 124.7, 121.4, 119.9, 116.1, 114.8, 111.0, 110.1, 105.5, 75.8, 66.1, 58.3, 55.3, 26.3, 24.7; IR (KBr) 2934 cm^{-1}; MS (FD) m/e 468 (MH^+). Anal. ($C_{30}H_{29}NO_4 \cdot H_2O$) C, H, N.

Biological Assays. Methods utilized for the ER binding, MCF-7 proliferation, 4-day and 5-week OVX rat assays have been recently described.[35] Procedures for the immature rat assay have also been published elsewhere.[45] Stimulation of TGF-β3 promoter activity was assayed in HeLa cells which had been cotransfected with a TGF-β3/luciferase reporter plasmid and an expression plasmid (pCMVER) encoding the human ER by the published procedure.[43]

Acknowledgment. The authors thank Dr. Chris Schmid for helpful discussions and suggestions. The authors also thank Kennan Fahey, Dr. David Mitchell, and Robert Murff for the preparation of starting materials and intermediates. The authors also acknowledge the contributions of the Physical Chemistry Department of Lilly Research Laboratories (analytical and spectral data and support).

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JM970688Z