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SYNTHESIS AND PHARMACOLOGY OF 2-ALKYL RALOXIFENE ANALOGS

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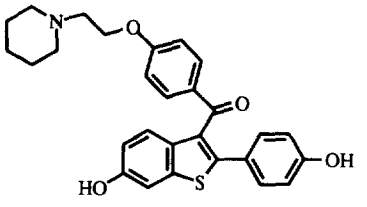
Abstract: A series of 2-alkyl and 2-cycloalkyl raloxifene analogs have been prepared and evaluated in both *in vitro* and *in vivo* models of estrogen/antiestrogen activity. In particular, the 2-cyclohexyl analogs show promise as potent selective estrogen-receptor modulators (SERMs).

The decreased production of ovarian steroids which occurs after the climacteric has been linked to a number of post-menopausal pathologies, particularly osteoporosis and coronary artery disease.[1,2] Estrogen replacement therapy has demonstrated effectiveness in reducing the risks associated with these pathologies, however 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, and the possibility of attenuated cardiovascular benefits have significantly affected patient compliance.[4] Furthermore, recent studies which confirm the increased risk of breast cancer associated with estrogen replacement therapy have led to the search for treatment alternatives.[5]



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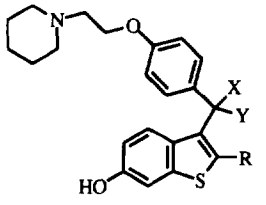
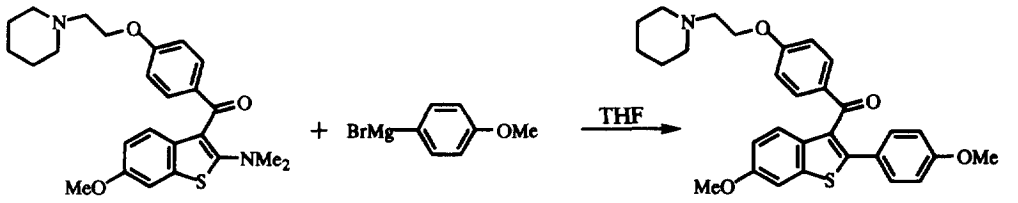


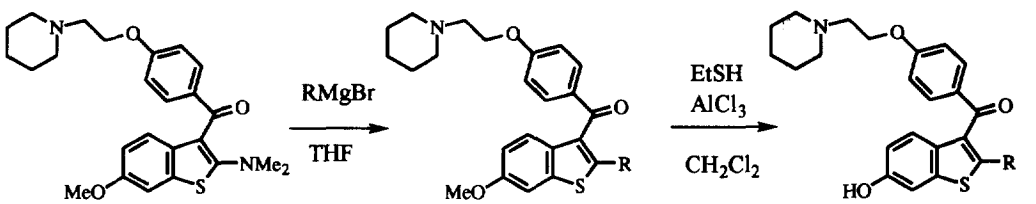
3 X,Y = O, R = alkyl, cycloalkyl
4 X = OH, Y = H, R = alkyl, cycloalkyl
5 X = Y = H, R = alkyl, cycloalkyl

Recently, several groups have described molecules which antagonize the effects of estrogen on uterine and breast tissue, while mimicking the effects of estrogen on bone and the cardiovascular system.[6-8] The term Selective Estrogen Receptor Modulator (SERM)[9] has been coined to describe these agents, and one such compound (raloxifene; LY139481; **1**) is in advanced clinical trials for the prevention and treatment of osteoporosis.[10] As part of our program to further explore structure-activity relationships in the raloxifene series, we have examined a series of analogs in which the 2-aryl substituent has been replaced by an alkyl or cycloalkyl moiety, **3-5**.[11] Herein, we describe the synthesis of these analogs, their *in vitro* effects in receptor binding and cell proliferation assays, and their effects on bone, uterus, and serum lipids in an ovariectomized (OVX) rat model.



6 **Equation 1**

A novel synthesis of raloxifene involving the addition of an aryl Grignard reagent to a 2-amino-3-aroylbenzothiophene **6** has recently appeared in the patent literature (Equation 1).[12] In this example, only 1,4-addition to the enone resulted, even in the presence of excess Grignard reagent. We have extended this methodology to include the addition of alkyl and cycloalkyl Grignard reagents, again with exclusive 1,4-selectivity (Table 1). Conversely, vinylmagnesium bromide gave a mixture of 1,2 and 1,4-addition products and allylmagnesium bromide gave exclusively 1,2-addition. Lewis acid mediated removal of the methyl ether protecting group then provided raloxifene analogs, **3**. Stepwise ketone reduction to the carbinols **4**, and further reduction to the 3-benzylbenzothiophenes **5** provided additional analogs (Scheme 1).[13]



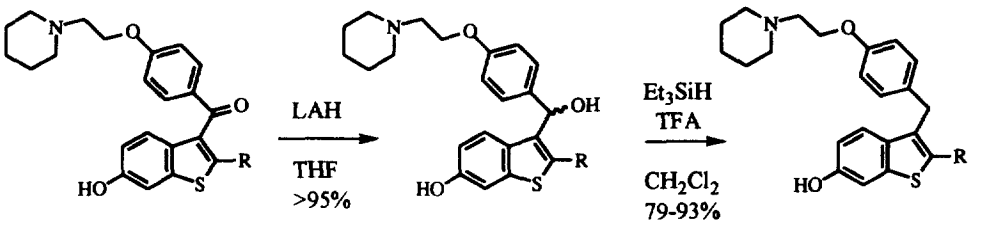
6 2 3

TABLE 1

	R	Yield 2 (%)	Yield 3 (%)
a	Methyl	52	72
b	Ethyl	58	81
c	*i*-Propyl	57	84
d	Cyclopentyl	60	86
e	Cyclohexyl	74	76
f	*trans*-4-Hydroxycyclohexyl	53†	84

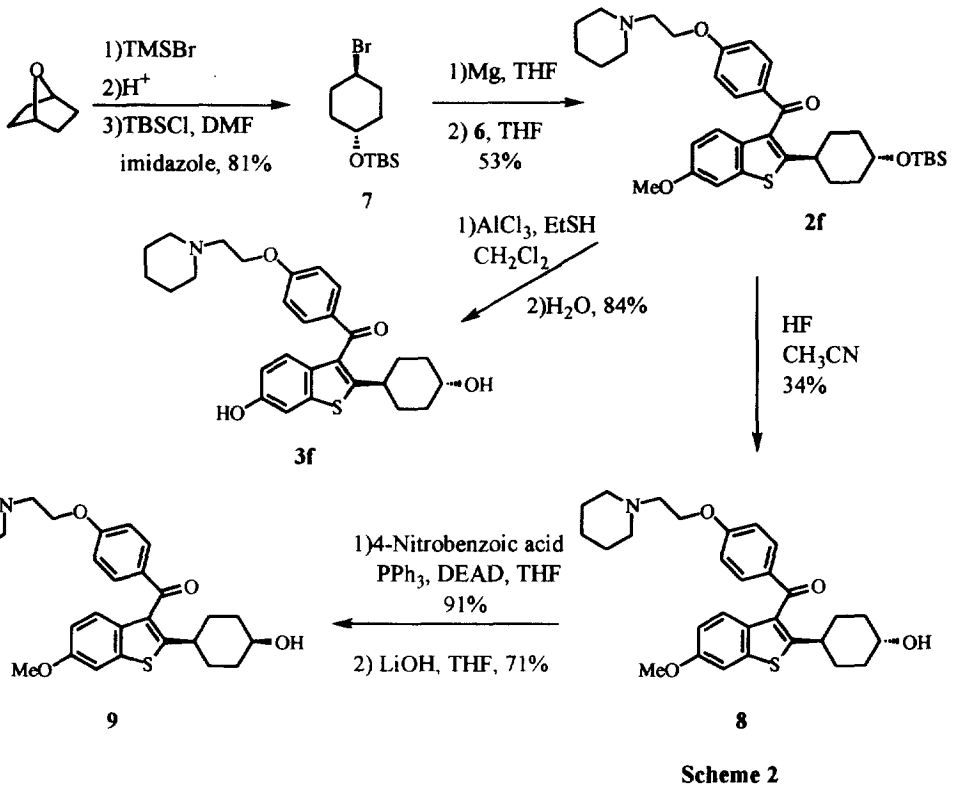
†As the corresponding TBDMS ether.

A 2-(4'-hydroxycyclohexyl) raloxifene analog was prepared in order to explore the effect of the 4'-hydroxyl orientation. Toward that end, oxabicyloheptane underwent ring opening in the presence of bromotrimethylsilane[14] and, following hydrolysis, the resultant alcohol was protected as its TBDMS-ether (Scheme 2). Conversion to the corresponding Grignard reagent and addition to **6** occurred without complication. Demethylation with concomitant desilylation provided the desired hexahydro-raloxifene analog **3f**. The orientation of the hydroxyl group was confirmed by desilylation of **2f**, Mitsunobu inversion, and



Scheme 1

hydrolysis to give **9**. The relative magnitude of the ^{1}H-NMR coupling constants for the methine protons adjacent to the hydroxyl in **8** and **9** (10.2 Hz and < 5 Hz, respectively) confirmed that the stereochemical relationship of **7** was maintained in the Grignard product.



Scheme 2

Compounds were examined for estrogen-receptor binding activity and for displacement of tritiated raloxifene binding in MCF-7 cell lysate (Table 2).[15] Antagonism of estrogen action in breast tissue was assayed via inhibition of MCF-7 cell proliferation stimulated by 10^{-12} M estradiol (Table 2).[16] Interestingly,

although all compounds demonstrated estrogen-receptor affinity similar to that of raloxifene and several had higher affinity for the raloxifene binding site, only **5f** approached raloxifene's ability to inhibit MCF-7 cell-proliferation. In all but the cyclohexyl (**e** and **f**) series cell-proliferation activity was reduced by conversion of the keto **3** analog to the carbinol **4** or methylene **5** analogs.

TABLE 2

	R	RBA* (^{3}H-Estradiol)[a]	RBA* (^{3}H-Raloxifene)[a]	MCF-7 Inhibition 50% R Dose (nM)[b]
	estradiol	1.00	0	na
	raloxifene	0.34	1.00	0.2
3a	Methyl	0.15	1.09	35
3b	Ethyl	0.13	4.65	20
3c	*i*-Propyl	0.15	3.63	3
3d	Cyclopentyl	0.08	3.45	5
3e	Cyclohexyl	0.09	2.19	2.5
3f	*trans*-4-Hydroxycyclohexyl	0.09	0.13	2
4d	Cyclopentyl	0.01	0.078	600
5a	Methyl	0.08	0.42	400
5b	Ethyl	0.14	1.30	100
5c	*i*-Propyl	0.23	2.86	10
5d	Cyclopentyl	0.04	1.59	10
5e	Cyclohexyl	0.11	nd	2
5f	*trans*-4-Hydroxycyclohexyl	0.25	0.13	0.5
9	*cis*-4-Hydroxycyclohexyl[c]	0.01	1.61	100

*RBA = relative binding affinity

[a]Average of at least 2 determinations. Values are ± 10%.

[b]Dose required to give 50% inhibition of a maximally effective (10^{-12} M) dose of estradiol. Average of at least 3 determinations. Values are ± 10%.

[c]Note, tested as the 6-methoxy derivative.

Tissue-specific estrogen agonist effects were examined in an OVX rat model,[6] utilizing uterine weight, uterine eosinophil peroxidase activity (EPO),[17] and serum cholesterol levels as endpoints (Table 3). Specifically, 75-day old OVX Sprague-Dawley rats were dosed daily (oral) for 4-days, commencing 2-weeks after ovariectomy. Vehicle (20% β-hydroxycyclodextrin) treated sham and OVX control groups, OVX-ethinyl estradiol (100 μg/kg) and OVX-raloxifene (0.1 mg/kg) treated control groups were included in each experiment. Each new compound was tested at 3 doses, with n = 5 for all experimental and control groups. A graphical representation of the dose-response for two of the most active compounds is provided in Figure 1a. Selected compounds were further evaluated in a 5-week, ovariectomized rat model in which effects on bone mineral density were also examined (Figure 1b).[6] In this model, 6-month old Sprague-Dawley rats were utilized and daily oral dosing was initiated immediately post-ovariectomy. Bone-mineral density was assessed at the distal metaphysis of the femur by single photon absorptiometry and is shown as percent-protection relative to OVX-controls.

Several trends are apparent from these results. Although many of the compounds induced a non-dose-dependent increase in uterine wet weight, none of the analogs showed any evidence of estrogen-like stimulation as measured by eosinophil infiltration. In general, a modest decrease in biological activity was observed, both *in vitro* and *in vivo* for the reduced (**4** and **5**) series as compared to the keto (**3**) series. In both

TABLE 3

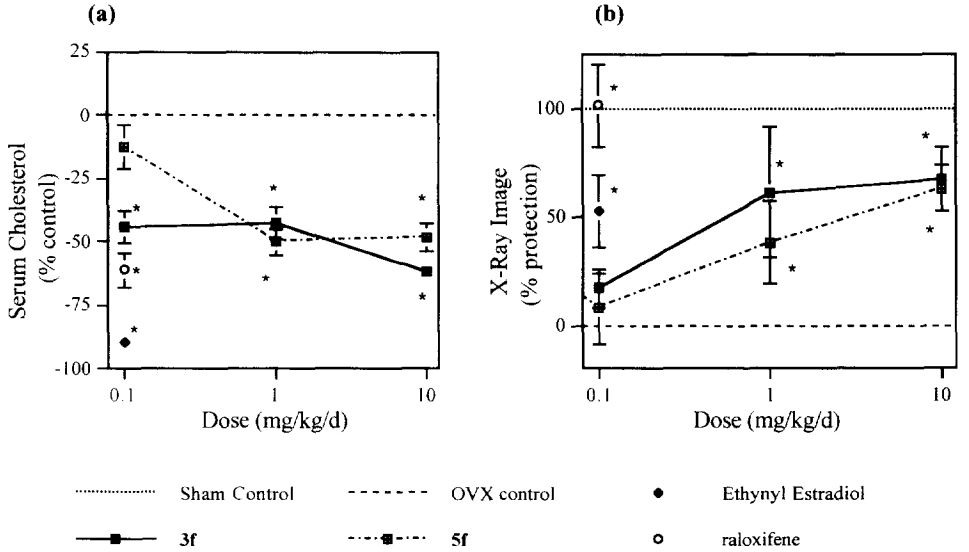
	Uterine Weight		Uterine EPO	Serum Cholesterol	
	Activity[a]	Dose[b]	Activity[a]	Activity[a]	50%Efficacy Dose(mg/kg)[c]
ethinyl estradiol	(+)	0.1	(+) (0.1 mg/kg)	(-)	0.1
raloxifene	(+)	0.1	0	(-)	0.2
3a	0		0	(-)	1.9
3b	0		0	(-)	>10
3c	0		0	(-)	>10
3d	0		0	(-)	1.2
3e	(+)	1	0		1.4
3f	(+)	0.1	0	(-)	1.0
4d	(+)	10	0	0	
5a	0		0	0	
5b	(+)	10	0	(-)	>10
5c	(+)	10	0	(-)	>10
5d	0		0	(-)	>10
5e	(+)	0.1	0	(-)	3.1
5f	0		0	(-)	3.4
9	(+)	1	0	(-)	1.0

[a]Activity: (+) indicates a statistically significant increase, with respect to OVX controls, at a dose of ≤ 10 mg/kg.
0 indicates no significant difference vs. OVX at any dose tested.
(-) indicates a statistically significant decrease, with respect to OVX controls, at a dose of ≤ 10 mg/kg.

[b]Lowest dose which gave a statistically significant increase with respect to OVX controls.

[c]Dose required to reduce serum cholesterol by 50% relative to OVX controls.

Figure 1 Dose-response relationships for **3f** and **5f** in reduction of serum cholesterol (a) and preservation of bone-mineral density (b). Cholesterol values are reported as percent decrease relative to OVX control. Bone mineral density values are determined by single photon absorptiometry and reported as percent protection relative to OVX control, with sham control values defined as 100 percent and OVX controls defined as 0. Raloxifene (0.1 mg/kg) and ethinyl estradiol (0.1 mg/kg) groups are included as comparators. Statistical significance ($p \leq 0.05$) relative to OVX control is denoted by "*".



cases, the compounds which approach raloxifene in potency are those which are most closely related to raloxifene structurally. Also notable is the relative lack of importance of the presence (**3e** vs. **3f**) and orientation (**3f** vs. **9**) of the 4'-hydroxy moiety for *in vivo* biological activity. Although these compounds did not match the potency of raloxifene in this study, **3f** (1.0 mg/kg/d) was as efficacious as ethinyl estradiol (0.1 mg/kg/d) in preventing ovariectomy-induced bone loss and, in contrast to ethinyl estradiol, did not induce significant uterine proliferation.

These studies imply that the 2-(4-hydroxyphenyl) moiety of raloxifene is superior to 2-alkyl and cycloalkyl replacements with respect to biological potency and efficacy, and may represent a nearly optimal 2-substituent. Continuing studies will address the effects of finer modifications to this structural framework and further examine the limitations and requirements of the functionality at the 4'-position. The 2-cyclohexyl analogs, although less potent than the parent compound, may offer a unique pharmacokinetic and/or metabolic profile and merit further evaluation. The condensation of 2-amino-3-aroylbenzothiophenes with Grignard reagents represent a general and efficient methodology for the construction of raloxifene analogs of this type.

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