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Estrogen receptor ligands. Part 14: Application of novel antagonist side chains to existing platforms

Timothy A. Blizzard,* Jerry D. Morgan, II, Wanda Chan, Elizabeth T. Birzin, Lee-Yuh Pai, Edward C. Hayes, Carolyn A. DaSilva, Ralph T. Mosley, Yi Tien Yang, Susan P. Rohrer, Frank DiNinno and Milton L. Hammond

Merck Research Laboratories, RY800-B116, PO Box 2000, Rahway, NJ 07065, USA

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Abstract—Two novel side chains which had previously been found to enhance antagonist activity in the dihydrobenzoxathiin SERM series were applied to three existing platforms. The novel side chains did not improve the antagonist activity of the existing platforms.
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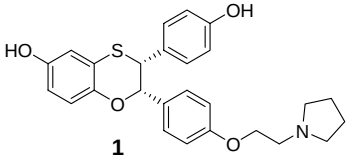
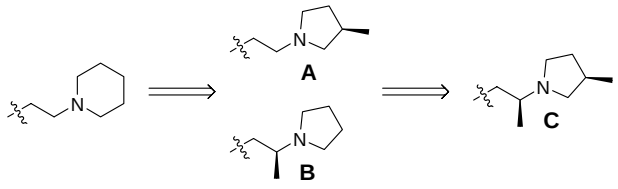
The importance of the selective estrogen receptor modulators (SERMs) is well documented.[1] The discovery of a second ER subtype[2] generated interest in the development of subtype-selective SERMS.[3] Previous publications from this laboratory have reported the discovery of dihydrobenzoxathiins (e.g., **1**) as a novel class of selective estrogen receptor alpha modulators (SERAMs).[4a–e]



1

We have also reported extensive studies on the side chain SAR of this class.[4d–g] These studies resulted in the discovery of two significantly improved antagonist side chains, **A** and **B**,[4f] which were combined to afford an optimized antagonist side chain **C**.[4g]



Since side chains **A**–**C** conferred such excellent antagonist properties on the dihydrobenzoxathiin platform, we wondered if a similar effect would be observed with other platforms or if **A**–**C** were platform-specific. Molecular modeling of **1** versus raloxifene (**2**), bazedoxifene (**3**), and lasofoxifene (**4**) in the hER-α binding site showed considerable overlap of the side chains, suggesting that side chains **A**–**C** might be of interest in these other platforms (Fig. 1).[5] While there have been a few studies on side chain SAR of other platforms,[6] we are unaware of any systematic studies on the portability of side chain SAR between platforms.

We therefore targeted analogs **2a**,**b**, **3a**,**b**, and **4a**,**b** for synthesis (Schemes 1–3 and Table 1). We chose the partially optimized side chains **A** and **B** for these studies, rather than the fully optimized side chain **C**, to afford maximum insight into the applicability of our SAR to other platforms. If substantial improvement was seen in any platform with side chains **A** and **B**, then we would prepare the corresponding analog with side chain **C**.

The raloxifene analogs **2a** (R = **A**) and **2b** (R = **B**) were easily synthesized from fluoride **5**[7] and the appropriate side-chain alcohol[4e] using a modification of the Lilly group's raloxifene analog synthesis (Scheme 1).[7,8]

The bazedoxifene analogs **3a** (R = **A**) and **3b** (R = **B**) were prepared from intermediate **6** and the appropriate side-chain alcohol **7**[4e] using a modification of the Wyeth group's bazedoxifene synthesis (Scheme 2).[9]

Keywords: SERMs; SERAMs; Estrogen.
* Corresponding author. Tel.: +1 732 594 6212; fax: +1 732 594 9556; e-mail: tim_blizzard@merck.com

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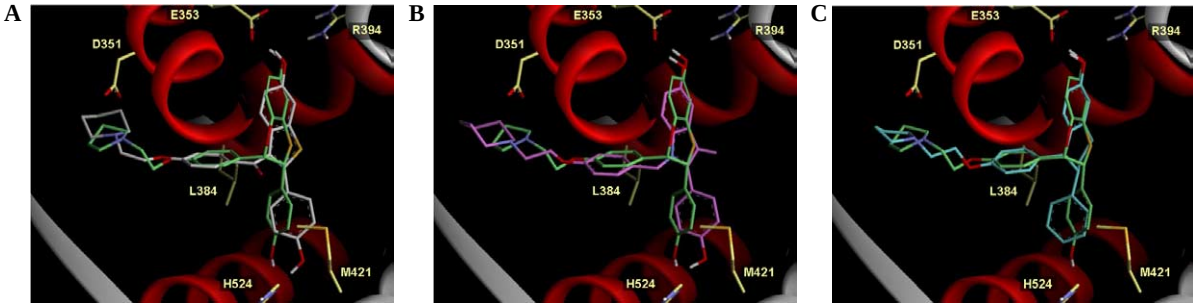
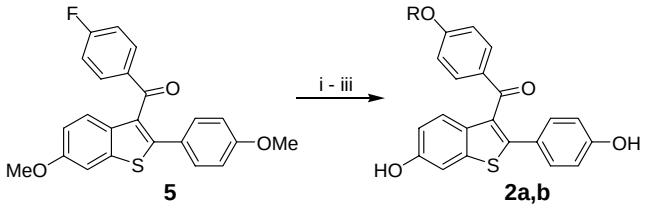
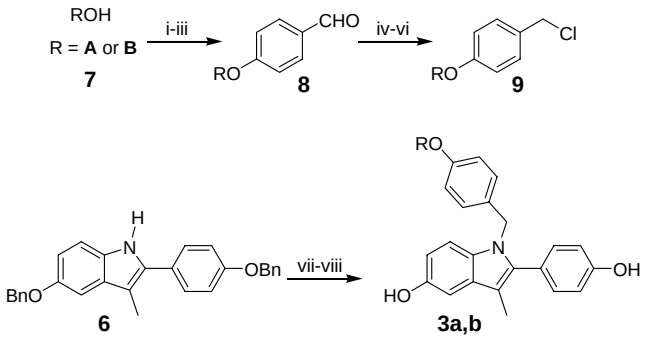


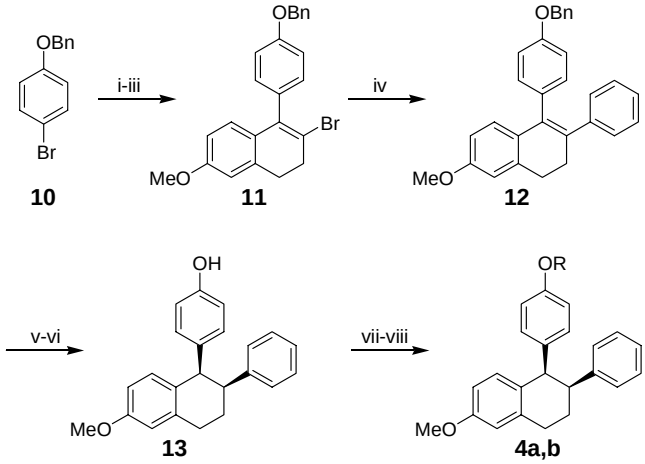
Figure 1. Molecular modeling of compound **1** (green) against models of (A) crystallographically determined raloxifene **2** (white); (B) bazedoxifene **3** (purple); and (C) lasofoxifene **4** (cyan) in the context of hER-α (pdb entry 1ERR).



Scheme 1. Reagents: (i) ROH, NaH, DMF; (ii) HCl, ether; (iii) Br_3, CH_2Cl_2.



Scheme 2. Reagents: (i) 2 N HCl, Et_2O; (ii) $SOCl_2$, THF; (iii) *p*-OH-benzaldehyde, K_2CO_3, DMF (38% from **7**); (iv) $LiAlH_4$, Et_2O, 91%; (v) 2 N HCl, Et_2O; (vi) $SOCl_2$, THF; (vii) **9**, NaH, DMF, 67%; (viii) Pd (black) HCO_2NH_4; EtOH, EtOAc, H_2O, 70%.



Scheme 3. Reagents: (i) Mg, THF, 30%; (ii) tetralone; (iii) pyridinium tribromide, 76–100%; (iv) phenylboronic acid, $(Ph_3P)_4Pd$, THF, 71%; (v) H_2, $Pd(OH)_2/C$, EtOH; (vi) chiral HPLC on Chiralcel AD column eluted with heptane–isopropanol, 4:1; (vii) RCl prepared in situ from ROH and $SOCl_2$, THF, K_2CO_3, DMF; (viii) 2 N HCl, BBr_3, Et_2O, CH_2Cl_2.

Lasofoxifene analogs **4a** (R = **A**) and **4b** (R = **B**) were prepared from commercial **10** and the appropriate side-chain alcohol **7**[4e] using a modification of the Pfizer group's lasofoxifene synthesis (Scheme 3).[10]

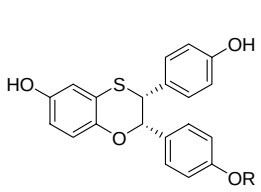
Biodata for the novel analogs and SERAMs **1**–**1c** are summarized in Table 1. All compounds were evaluated in an in vitro estrogen receptor (ER) binding assay.[11] As we had observed in our previous studies with **1**–**1c**, substitution of the novel side chains **A** and **B** for the side-chain of the parent compound did not have a significant effect on ER binding with any platform.

The compounds were also tested in an MCF-7 assay.[12] In the dihydrobenzoxathiin series, the modified side chains resulted in a modest potency enhancement in this assay. However, a similar enhancement was not observed with any of the other platforms. The raloxifene, bazedoxifene, and lasofoxifene analogs all retained potency comparable to or, at most, slightly better than that of the corresponding parent.

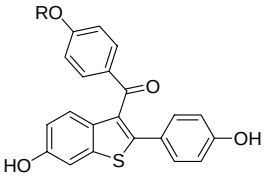
Finally, the novel compounds were evaluated in an immature rat uterine weight assay.[13] This was the assay where the largest impact was observed in the dihydrobenzoxathiin series (compare **1a**–**c** with **1**). In the raloxifene series, substitution of side chains **A** and **B** for piperidine had no effect on the uterine activity of **2**. In the bazedoxifene series, substitution of side chain **A** for homopiperidine of **3** resulted in a slight increase in uterine antagonism but substitution of side chain **B** resulted in a decrease in antagonist activity. In the lasofoxifene series, there was a slight increase in uterine antagonism with a corresponding decrease in agonism upon substitution of side chains **A** and **B** for pyrrolidine in **4** but the effect is clearly not as dramatic as in the dihydrobenzoxathiin series.

The lack of a dramatic effect on the uterine profile upon incorporation of side chains **A** and **B** clearly indicates that the side chain SAR of the dihydrobenzoxathiin

Table 1. ER ligand binding data, rat uterine growth data, and MCF-7 data



1



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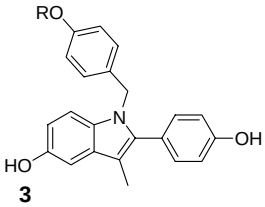
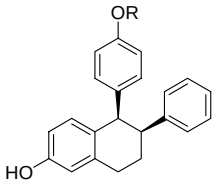
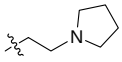
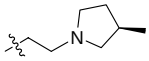
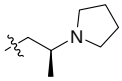
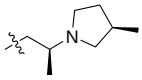
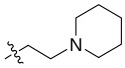
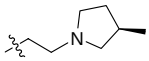
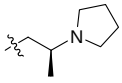
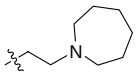
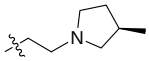
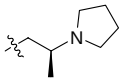
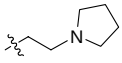
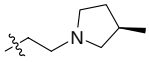
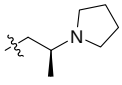


3



4

Compound	R	Human ER binding (IC_{50}, nM)[11]			MCF-7[12] (IC_{50}, nM)	Rat uterine activity[13]	
		hER-α	hER-β	β/α		% Ant.	% Ag.
1		2.6	64	25	3.3	72	34
1a		3.0	161	54	0.9	106	−19
1b		1.3	45	35	0.3	101	0
1c		1.3	52	40	0.2	123	−29
2		1.8	12	7	0.8	81	24
2a		0.6	3.1	5	1.0	79	24
2b		0.2[11b]	2.6[11b]	13	0.2	82	31
3		0.6	3.8	6	0.4	67	15
3a		0.5	2.4	5	0.3	78	−2
3b		0.4	2.3	6	0.1	61	20
4		1.4	1.8	1	0.1	74	26
4a		0.5	0.6	1	0.08	89	10
4b		0.6	0.9	1	0.07	81	18

SERAMs is not transferable to other platforms. Since substantial improvement in the uterine profile was not seen upon introduction of the improved antagonist side chains **A** and **B** in the established platforms, we elected not to prepare the corresponding analogs with the fully optimized dihydrobenzoxathiin antagonist side chain **C**.

In conclusion, we have demonstrated that the side chain SAR of the dihydrobenzoxathiin SERAMs is not applicable to existing platforms. Clearly, it will be necessary to conduct SAR studies for each platform to determine its optimum side chain. This concludes our study of the dihydrobenzoxathiin side chain SAR.

Acknowledgments

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5. (a) Models of the non-crystallographically determined structures were built by modifying close congeners for which crystal structures had been determined Thus, **1** and **3** were derived from the structure of **2**. The models of **4** were more systematically studied and ultimately a conformer was selected which positioned the phenolic OH to interact with Glu353 and Arg394, and the pyrrolidine with Asp351. Energy minimization for all of the models within scontext of the hER-α receptor (derived from pdb entry 1ERR) was accomplished by rigidly fixing all residues, except for side chains which fell within 5 Å of the modeled ligand, which were allowed to minimize in conjunction with the ligand. All minimizations were conducted using the MMFFs forcefield[6b] with a distance-dependent dielectric model of 2r(b) Halgren, T. A. *J. Comp. Chem.* **1999**, *20*, 730.
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8. All new compounds were characterized by LC–MS and 400, 500, or 600 MHz ^{1}H NMR.
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11. (a) The IC_{50} values were generated in an estrogen receptor ligand binding assay. This scintillation proximity assay was conducted in NEN Basic Flashplates using tritiated estradiol and full length recombinant human ERα and ERβ proteins. Compounds were evaluated in duplicate in a single assay. In our experience, this assay provided IC_{50} values that are reproducible to within a factor of 2–3. Dihydrobenzoxathiin **1** ($n = 136$) and estradiol ($n > 100$) were tested in multiple assays; data reported in Table 1 are an average of all determinations; (b) Data for **2b** reflect a 20 h incubation prior to radioactive quantification; all other data obtained with 3 h incubation.
12. This is an in vitro MCF-7 breast cancer cell proliferation assay adapted to a 96-well format. Cells are grown in estrogen-depleted media for six days and then treated with the test compound for seven days. To evaluate the

antagonist activity of a test compound, this treatment occurs in the presence of 0.003 nM (the EC_{70}) estradiol. The amount of cell growth is determined by measuring the protein content of living cells and an IC_{50} for the test compound is determined.

13. (a) The uterine weight assay is an in vivo assay based on a published procedure[13c] that measures estrogen agonism and antagonism in rat uterine tissue. Compounds are dosed orally at 1 mpk. Agonism is reported as percentage of estradiol control; antagonism reported as percentage antagonism of estradiol; (b) Estradiol exhibited 100% agonism at 4 μg/kg; (c) Wakeling, A. E.; Valcaccia, B.; Newboult, E.; Green, L. R. *J. Steroid Biochem.* **1984**, *20*, 111.