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Estrogen receptor ligands. Part 8: Dihydrobenzoxathiin SERAMs with heteroatom-substituted side chains

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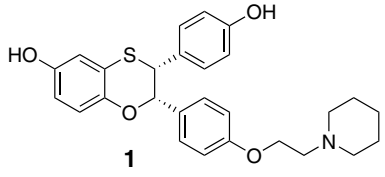
Abstract—A series of benzoxathiin SERAMs with heteroatom-substituted amine side chains was prepared. Minor modifications in the side chain resulted in significant effects on biological activity, especially in uterine tissue.

The clinical significance of the selective estrogen receptor modulators (SERMs) is well documented.[1] The recent discovery of a second estrogen receptor subtype[2] prompted interest in the development of receptor subtype-selective SERMS.[3] Previous reports from this laboratory have reported the discovery of benzoxathiins (e.g., **1**) as a novel class of selective estrogen receptor alpha modulators (SERAMs).[4a,b,c]



1

More recently, we have also described our initial studies on the side chain SAR of **1**, which were aimed at maintaining the potency and selectivity of **1** while reducing oxidative metabolism of the side chain.[4d] We hypothesized that an iminium ion resulting from oxidation of the piperidine residue present in the side chain of **1** might be a significant contributor to the formation of covalent adducts with biological proteins. We therefore examined alternative side chains for **1** with the goal of finding a piperidine replacement that would maintain potency and selectivity for ERα while reducing the formation of covalent protein adducts.

To date, there have been few reports on the systematic exploration of SERM side chains.[5] In our previous communication, we described benzoxathiin analogs with bicyclic amine side chains that should be less readily oxidized than the piperidine residue of **1** due to steric constraints.[4d] Amine side chains with heteroatoms such as those present in compounds **2**–**15** should also be less oxidizable than the piperidine of **1** due to both steric and electronic constraints. In addition, side chains **8a**–**10a** and **14a**–**15a** also contain functional groups that could act as internal iminium ion traps. The latter targets were especially attractive since the internal traps provided a second layer of defense against adduct formation. Although internal trapping would be reversible, the cyclic form should predominate thereby significantly reducing the amount of iminium ion present. We therefore targeted analogs **2**–**15** for synthesis. The requisite amino-alcohol side chain synthons **2a**–**15a** were prepared by a variety of methods as summarized below (Table 1 and Schemes 1–6).[6]

The first method, illustrated by the preparation of thiomorpholine analog **2a**, involved alkylation of the

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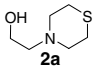
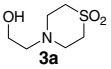
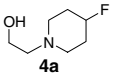
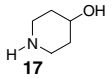
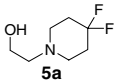
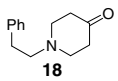
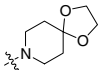
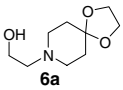
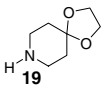
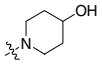
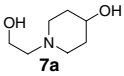
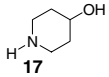
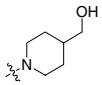
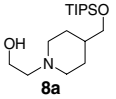
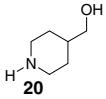
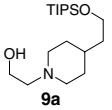
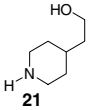
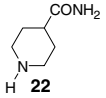
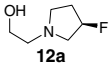
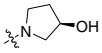
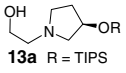
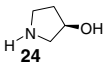
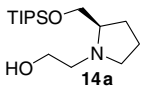
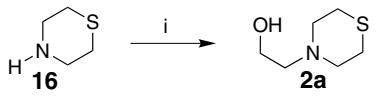
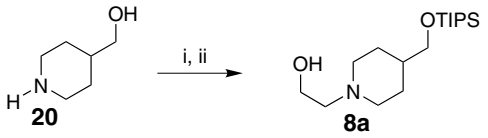
Table 1. Side chain preparation summary and biodata

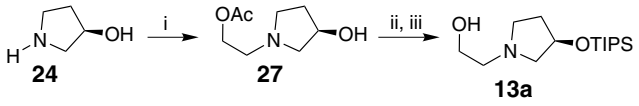
#	R	ER binding (IC_{50}, nM)[8]			Cyanide adduct?[10]	MCF-7[9]	Uterine activity[11]		Side chain	Starting material[6]	Scheme (yield)[6]
		hERα	hERβ	β/α		IC_{50} (nM)	% Antag.	% Ag.			
1		0.8	45	56	Yes	2.8	99	9	 **1a**	Commercial (Aldrich)	
2		1.5	72	48	Yes	15.6	30	17	 **2a**	 **16**	1 (73%)
3		2.0	247	124	No	26.3	1	0	 **3a**	See Ref. 6	
4		2.5	116	46	—	—	56	47	 **4a**	 **17**	4 (32%)
5		16	275	17	No	42	9	2	 **5a**	 **18**	5 (15%)
6		2.0	267	134	No	42.3	28	38	 **6a**	 **19**	1 (71%)
7		1.8	331	184	—	7.5	33	29	 **7a**	 **17**	1 (54%)
8		2.2	436	198	Yes	11.3	20	9	 **8a**	 **20**	2 (49%)
9		7.3	1113	143	No	42.5	—	—	 **9a**	 **21**	2 (65%)
10		2.2	366	166	No	21	0	5	 **10a**	 **22**	1 (61%)
11		2.6	64	25	No	3.3	72	34	 **11a**	Commercial (Aldrich)	
12		0.5	59	118	—	3.8	58	35	 **12a**	 **23**	6 (41%)
13		1.7	81	48	—	0.6	15	32	 **13a** R = TIPS	 **24**	3 (10%)
14		2.0	134	67	No	3.2	22	78	 **14a**	 **25**	2 (67%)
15		3.9	81	21	No	1.9	49	61	**15a**	**26**	2 (75%)
—	Raloxifene	1.8	12	7	No	0.8	81	24	N/A	N/A	N/A
—	17β-Estradiol	1.3	1.1	1	—	—	—	100[11b]	N/A	N/A	N/A



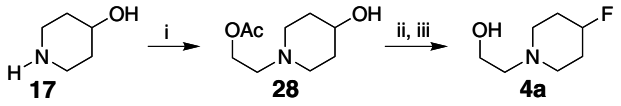
Scheme 1. Reagents and conditions: (i) 2-bromoethanol, K_2CO_3, MeCN, reflux.



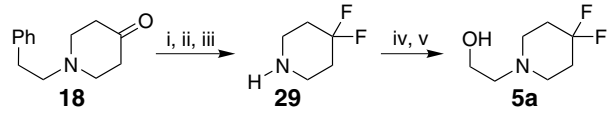
Scheme 2. Reagents and conditions: (i) TIPS-Cl, imidazole, DMAP, CH_2Cl_2; (ii) 2-bromoethanol, K_2CO_3, MeCN, reflux.



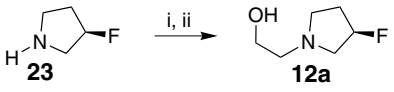
Scheme 3. Reagents and conditions: (i) 2-bromoethyl acetate, K_2CO_3, MeCN, reflux; (ii) TIPS-Cl, imidazole, DMAP, CH_2Cl_2; (iii) $LiAlH_4$, Et_2O.



Scheme 4. Reagents and conditions: (i) 2-bromoethyl acetate, K_2CO_3, MeCN, reflux; (ii) DAST, CH_2Cl_2; (iii) CH_3OH, 40 °C.



Scheme 5. Reagents and conditions: (i) DAST, CH_2Cl_2; (ii) α-chloroacetyl chloride, Et_3N, CH_2Cl_2; (iii) MeOH; (iv) acetoxyacetyl chloride, Et_3N, CH_2Cl_2; (v) $LiAlH_4$, Et_2O.



Scheme 6. Reagents and conditions: (i) acetoxyacetyl chloride, Et_3N, CH_2Cl_2; (ii) $LiAlH_4$, Et_2O.

appropriate secondary amine, for example, **16**, with 2-bromoethanol (Scheme 1). Amines **2a**, **6a**, **7a**, and **10a** were prepared by this route.[6]

The presence of a hydroxyl group in the side chain necessitated a protecting group in some instances. For example, side chain **8a** was prepared by first introducing a tri-isopropylsilyl (TIPS) protecting group onto the primary hydroxyl group of **20**, then introducing the hydroxyethyl group (Scheme 2). Amines **8a**–**9a** and **14a**–**15a** were prepared via this method. It was not necessary to protect the secondary hydroxyl in **7a** since this group did not interfere in the subsequent attachment of the side chain to the benzoxathiin nucleus.

Alternatively, a masked hydroxyethyl group could be installed first by alkylation with bromoethyl acetate (Scheme 3). Subsequent TIPS protection and reduction completed the synthesis of the hydroxypyrrolidine **13a**.

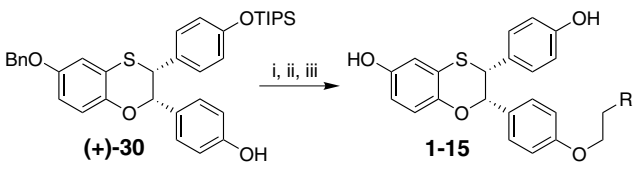
The fluoropiperidine side chain **4a** was synthesized in a similar manner (Scheme 4). Alkylation of commercial 4-hydroxypiperidine **17** with bromoethyl acetate followed by fluorination and deacetylation afforded **4a**.

The difluoropiperidine side chain **5a** was prepared from commercial *N*-benzylpiperidone **18** (Scheme 5).[8] Difluorination with DAST followed by α-chloroethyl chloroformate mediated debenzylation[7] afforded the difluoro intermediate **29**. Acylation of **29** with acetoxyacetyl chloride followed by $LiAlH_4$ reduction gave **5a** in 15% overall yield.

The fluoropyrrolidine side chain **12a** was easily prepared by acylation of 3-(*R*)-fluoropyrrolidine (**23**)[6] with acetoxyacetyl chloride followed by reduction with $LiAlH_4$ (Scheme 6).

Synthesis of the final products proceeded via attachment of the hydroxyethylamine side chains to the benzoxathiin core (+)-**30** using the previously reported procedure[4a,c] followed by deprotection (Scheme 7).

With the exception of the difluoropiperidine **5** and the hydroxyethylpiperidine **9**, all of the novel benzoxathiin analogs (**2**–**15**) retained the excellent ERα potency exhibited by the piperidine analog **1** (Table 1) in an in vitro estrogen receptor binding assay.[8] Although the magnitude of receptor subtype selectivity (ERβ/ERα ratio) varied considerably (from 17X to 198X), all of the novel analogs were alpha selective. Unfortunately, most of the new piperidine analogs suffered a substantial loss of activity in the MCF-7 assay.[9] The pyrrolidine analogs **11**–**15**, however, retained excellent MCF-7 activity. As expected, analogs with strong electron withdrawing substituents (e.g., **3**, **5**, and **6**) were less prone to oxidative metabolism, as measured by their failure to form a detectable cyanide adduct.[10] With the exception of the hydroxymethyl piperidine **8**, all of the analogs with functional groups positioned to act as internal iminium ion traps (**8**–**10** and **14**–**15**) were found to have no detectable cyanide adducts, suggesting that a properly positioned internal trap is a viable method for alleviating the detrimental effects of amine metabolism.



Scheme 7. Reagents and conditions: (i) **1a**–**15a**, DIAD, PPh_3, THF; (ii) Pd, HCO_2NH_4, EtOH, EtOAc, H_2O; (iii) *n*-Bu_4NF, AcOH, THF.

Interestingly, none of the pyrrolidine analogs, even the unsubstituted pyrrolidine **11**, formed detectable cyanide adducts. Unfortunately, with the possible exception of **11**, all of the novel analogs exhibited a less favorable uterine profile than **1**.[11]

Overall, the heteroatom-substituted analogs exhibited a less desirable SERM profile than **1** although many of the novel analogs successfully avoided cyanide adduct formation. However, in conjunction with the results described in the preceding manuscript,[4d] the observations described herein suggested an ultimately successful direction for our piperidine replacement effort. Further results in this area will be reported in future publications from this laboratory.

Acknowledgements

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References and notes

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6. All new compounds were characterized by LC–MS and 400, 500, or 600 MHz ^{1}H NMR. Side chains were prepared using starting materials and methods indicated in Table 1 (yields are the overall yield of the side chain from the indicated starting material). Starting material sources: **1a**, **11a**, **16**–**22**, **25**, and **26**: Aldrich. **3a**: (a) Ford-Moore, A. H.; Lidstone, A. G.; Waters, W. A. *J. Chem. Soc.* **1946**, 819. **23**: (b) Caldwell, C. G.; Chen, P.; He, J.; Parmee, E. R.; Leiting, B.; Marsilio, F.; Patel, R. A.; Wu, J. K.; Eiermann, G. J.; Petrov, A.; He, H.; Lyons, K. A.; Thornberry, N. A.; Weber, A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1265.
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8. 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 provides IC_{50} values that are reproducible to within a factor of 2–3. Benzoxathiin **1** ($n = 36$) and estradiol ($n > 100$) were tested in multiple assays; data reported in Table 1 is an average of all determinations.
9. An in vitro MCF-7 breast cancer cell proliferation assay adapted to a 96-well format. Cells are grown in estrogen-depleted media for 6 days then treated with the test compound for 7 days. To evaluate the antagonist activity of a test compound, this treatment occurs in the presence of low levels of estradiol. The protein content of living cells is then measured and an IC_{50} determined.
10. The cyanide adduct assay was used as a surrogate measure of protein adduct formation subsequent to microsomal oxidation. Compounds were incubated with liver microsomes in the presence of cyanide ion then LC–MS was used to analyze for the presence of cyanide adducts.
11. (a) The uterine weight assay is an in vivo assay that measures estrogen agonism and antagonism in rat uterine tissue. Compounds are dosed orally at 1 mpk. Agonism results are reported as % of estradiol control; antagonism results are reported as % antagonism of estradiol; (b) Estradiol exhibited 100% agonism @ 4 μg/kg.