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Estrogen receptor ligands. Part 7: Dihydrobenzoxathiin SERAMs with bicyclic amine side chains

Timothy A. Blizzard,* Frank DiNinno, Jerry D. Morgan, II, Helen Y. Chen, Jane Y. Wu, Candido Gude, Seongkon Kim, Wanda Chan, Elizabeth T. Birzin, Yi Tien Yang, Lee-Yuh Pai, Zhoupeng Zhang, Edward C. Hayes, Carolyn A. DaSilva, Wei Tang, Susan P. Rohrer, James M. Schaeffer and Milton L. Hammond

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

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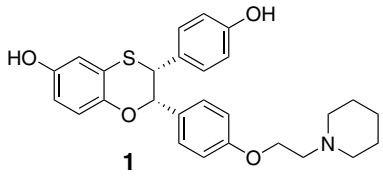
Abstract—A series of benzoxathiin SERAMs with bicyclic amine side chains was prepared. Minor modifications in the side chain resulted in significant effects on biological activity, especially in uterine tissue.

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).[4]



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Although benzoxathiin **1** has excellent potency and selectivity for ERα, it was judged to be unacceptably prone to oxidative metabolism with subsequent formation of covalent protein adducts. 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. 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 are few reports on the systematic exploration of SERM side chain SAR.[5] We decided to target side chains that should be less susceptible to oxidation for mechanistic reasons. Bicyclic amine side chains such as those present in compounds **2**–**15** should be less readily oxidized than the piperidine residue of **1** due to steric constraints. Furthermore, the amines in **2**–**9** and **15**, wherein the nitrogen atom is attached to a bridgehead carbon or is part of an azetidine ring, should be much less susceptible to iminium ion formation upon oxidation. We therefore targeted analogs **2**–**15** for synthesis. The requisite aminoalcohol side chain synthons **2a**–**15a** were prepared by a variety of methods as summarized below (Table 1 and Schemes 1–5).[6]

The first method, illustrated by the preparation of bicyclic amine **2a**, involved α-chloroethyl chloroformate mediated dealkylation[7] of a tertiary amine, such as **16**, followed by acylation of the resulting secondary amine, for example, **29**, with acetoxyacetyl chloride and $LiAlH_4$ reduction to afford the desired hydroxyethyl amine. Amines **2a**–**4a** were prepared by this route although only

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

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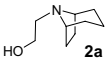
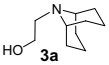
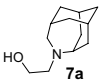
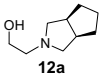
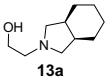
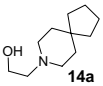
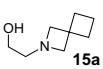
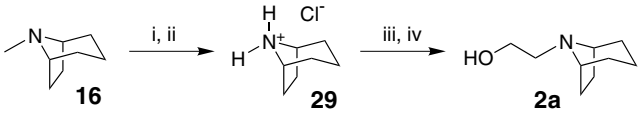
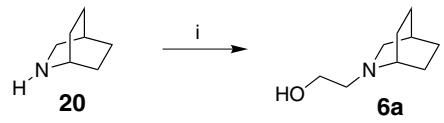
Table 1. Side chain preparation summary and biodata

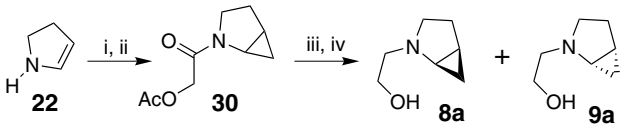
#	R	ER Binding (IC_{50}, nM)[10]			Cyanide adduct?[12]	MCF-7[11] IC_{50} (nM)	Uterine activity[13]		Side chain	Starting material	Scheme (yield)[6]
		hER	HERβ	β/α			%Antag.	%Ag.			
1		0.8	45	56	Yes	2.8	99	9	 **1a**	N/A	N/A
2		0.6	51	85	No	2.2	30	61	 **2a**	**16**	1 (28%)
3		0.6	47	78	No	3.0	47	53	 **3a**	**17**	1 (17%)
4		0.9	118	131	No	3.6	42	53	 **4a**	**18**	1 (47%)
5		0.7	73	104	No	3.3	37	70	 **5a**	**19**	2 (42%)
6		1.3	52	40	No	1.6	33	66	 **6a**	**20**	2 (33%)
7		0.6	40	67	No	5.8	30	61	 **7a**	**21**	2 (71%)
8		0.6	33	55	No	0.4	79	23	 **8a**	**22**	3 (2%)
9		0.5	56	112	No	0.5	43	70	 **9a**	**22**	3 (2%)
10		0.7	136	194	Yes	2.6	86	8	 **10a**	**23**	4 (41%)
11		0.8	85	106	Yes	2.5	76	19	 **11a**	**24**	4 (21%)
12		0.3	18	60	Yes	1.3	—	—	 **12a**	**25**	4 (28%)
13		1.0	33	33	Yes	7.7	—	—	 **13a**	**26**	4 (8%)
14		1.2	39	33	No	29.8	17	1	 **14a**	**27**	4 (30%)
15		0.4	26	65	No	0.3	—	—	 **15a**	**28**	5 (2%)
—	Raloxifene	1.8	12	12	No	0.8	81	24	N/A	N/A	N/A
—	17β-Estradiol	1.3	1.1	1.1	—	—	—	100[13b]	N/A	N/A	N/A



Scheme 1. Reagents and conditions: (i) α-chloroethyl chloroformate, Et_3N, CH_2Cl_2; (ii) MeOH; (iii) acetoxyacetyl chloride, Et_3N, CH_2Cl_2; (iv) $LiAlH_4$, Et_2O.



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



Scheme 3. Reagents and conditions: (i) acetoxyacetyl chloride, Et_3N; (ii) $Pd(OAc)_2$, CH_2N_2; (iii) chiral HPLC (see Ref. 8); (iv) $LiAlH_4$, Et_2O.

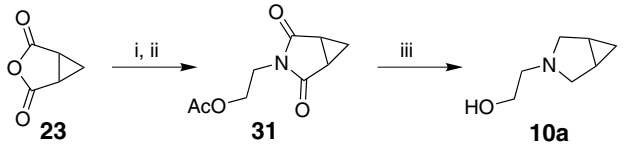
the last two steps were used for **4a** since the des-methyl starting material **18** was prepared by a different route.[6]

An alternative synthesis involved alkylation of the appropriate secondary amine, for example, **20**, with 2-bromoethanol (Scheme 2). Amines **5a**–**7a** were prepared via this method.

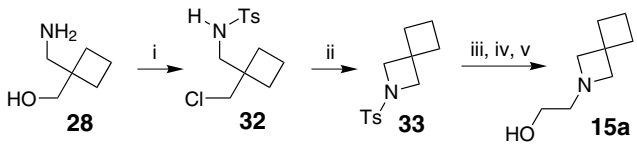
The 2,3-fused cyclopropylpyrrolidine **30** was prepared by acylation of 2,3-dihydropyrrole **22**[6] with acetoxyacetyl chloride (Scheme 3) followed by cyclopropanation. Chiral HPLC separation of the enantiomers of **30**[8] followed by $LiAlH_4$ reduction gave the desired enantiomeric side chains **8a** and **9a**.[9]

Synthesis of several of the fused bicyclic amines began with conversion of a commercially available anhydride, for example, **23**, to an imide, for example, **31**, by sequential reaction with ethanolamine and acetic anhydride (Scheme 4). Subsequent reduction with $LiAlH_4$ afforded the desired side chain, for example, **10a**. Alternatively, hydroxyethylimide formation could be achieved by reaction of the anhydride and ethanolamine with azeotropic removal of water. The resulting hydroxyethylimide was then reduced with $LiAlH_4$ to afford the side chain. Amines **10a**–**14a** were prepared using this method.

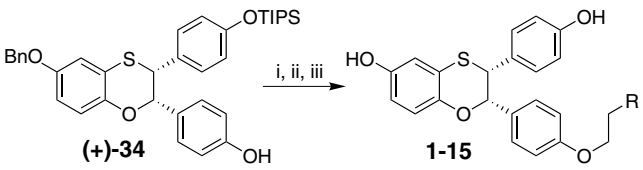
The spiroazetidine **15a** was prepared from the known amino-alcohol **28**[6] (Scheme 5). Reaction of **28** with *p*-toluenesulfonyl chloride to form the chloro-tosylate **32** followed by cyclization afforded spiroazetidine **33**. Deprotection with Red-Al, acylation with acetoxyacetyl chloride, and reduction completed the synthesis of **15a**.



Scheme 4. Reagents and conditions: (i) ethanolamine; (ii) acetic anhydride; (iii) $LiAlH_4$, Et_2O.



Scheme 5. Reagents and conditions: (i) *p*TsCl; (ii) NaH, DMF; (iii) Red-Al; (iv) acetoxyacetyl chloride, Et_3N; (v) $LiAlH_4$, Et_2O.



Scheme 6. Reagents and conditions: (i) **1a**–**15a**, DIAD, PPh_3, THF; (ii) Pd, HCO_2NH_4, 7:2:1 EtOH/EtOAc/H_2O; (iii) *n*-Bu_4NF, AcOH, THF.

Synthesis of the final products proceeded via attachment of the hydroxyethylamine side chains to the benzoxathiin core (+)-**34** using the previously reported procedure[4a,c] followed by deprotection (Scheme 6).

All of the novel benzoxathiin analogs (**2**–**15**) retained the excellent ERα potency exhibited by the monocyclic analog **1** (Table 1) in an in vitro ER binding assay.[10] Although the magnitude of receptor subtype selectivity (ERβ/ERα ratio) varied considerably (from 33 × to 194 ×), all of the novel analogs remained alpha selective. In addition to excellent binding, most of the new analogs (**2**–**12**) retained antagonist activity in the MCF-7 proliferation assay[11] that matched or exceeded the activity of **1**. Only analog **14** showed a substantial decrease in MCF-7 antagonist activity. As expected, analogs with bicyclic amine side chains wherein the nitrogen atom is attached to a bridgehead carbon (**2**–**9**) were less prone to oxidative metabolism, as measured by their failure to form a detectable cyanide adduct.[12] However, analogs **2**–**7** were also surprisingly potent agonists in uterine tissue,[13] in contrast to the uterine antagonism exhibited by **1**. Apparently, the larger bicyclic side chain does not allow the receptor–ligand complex to assume an antagonist conformation. Interestingly, with the exception of **9**, all of the fused bicyclic analogs **8**–**13** that were evaluated in the uterine assay were found to be antagonists. Unfortunately, with the exception of bridgehead nitrogen analogs **8** and **9**, the fused bicyclic analogs all formed cyanide adducts. The differential uterine activity of diastereomers **8** and **9** was especially noteworthy and allowed tentative assignment of their absolute stereochemistry.[9]

Overall, the fused cyclopropyl analog **10** was the most interesting of the novel analogs but was not pursued further due to its cyanide adduct formation. However, its excellent potency, subtype selectivity, and uterine profile encouraged us to continue the search for a piperidine replacement and also suggested a direction for these efforts. Further results in this area will be reported in future publications from this laboratory.

Acknowledgements

The authors thank Dr. Derek Von Langen and Ms. Judy Pisano for the preparation of (+)-**34** and thank Mr. Daniel Kim for performing the preparative chiral HPLC separation of the enantiomers of **30**.

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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 method indicated in Table 1. Starting material sources: **1a**, **16**, **23**, and **26**–**27**: Aldrich. Compound **17**: (a) Nelsen, S. F.; Hollinsed, W. C.; Kessel, C. R.; Calabrese, J. C. *J. Am. Chem. Soc.* **1978**, *100*, 7876; Compound **18**: (b) Fraser, R. R.; Swingle, R. B. *Can. J. Chem.* **1970**, *48*, 2065; Compound **19**: (c) Portoghese, P. S.; Turcotte, J. G. *J. Med. Chem.* **1971**, *14*, 288; Compound **20**: (d) Werner, L. H.; Ricca, S. *J. Am. Chem. Soc.* **1958**, *80*, 2733; Compound **21**: (e) St. Georgiev, V.; Saeva, G. A.; Kinsolving, C. R. *J. Heterocycl. Chem.* **1986**, *23*, 1023; Compound **22**: (f) Karadogan, B.; Parsons, P. J. *Tetrahedron* **2001**, *57*, 8699, Compound **24**: Maybridge. Compound **25**: Rieke Metals Inc.; Compound **28**: (g) Nayer, H.; Giudicelli, R.; Sette, J.; Menin, J. *Bull. Soc. Chim. Fr.* **1965**, 204.
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8. Analytical chiral HPLC performed on a Chiralcel OD 4.6 mm × 250 mm 10 μm column eluted with 60:40 heptane–isopropanol at 0.5 mL/min. Retention times: (−)enantiomer of **30** (converts to **8a**) 16 min; (+)enantiomer (converts to **9a**) 20 min.
9. The absolute stereochemistry of diasteromers **8** and **9** (and thus enantiomers **8a** and **9a**) was assigned by analogy with compounds of known stereochemistry based on uterine assay data. These related compounds will be disclosed in a future publication.
10. 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.
11. 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.
12. 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.
13. (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 reported as % of estradiol control; antagonism reported as % antagonism of estradiol; (b) Estradiol exhibited 100% agonism @ 4 μg/kg.