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Estrogen receptor ligands. Part 13: Dihydrobenzoxathiin SERAMs with an optimized antagonist side chain

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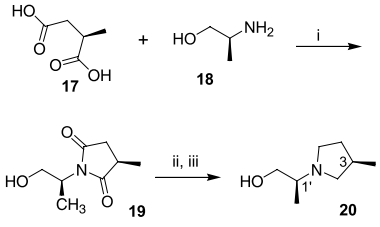
Abstract—An optimized side chain for dihydrobenzoxathiin SERAMs was discovered and attached to four dihydrobenzoxathiin platforms. The novel SERAMs show exceptional estrogen antagonist activity in uterine tissue and an MCF-7 breast cancer cell assay.

The importance of the selective estrogen receptor modulators (SERMs) is well-documented.[1] The discovery[2a,b] of a second estrogen receptor, ERβ, has generated considerable interest in the development of receptor sub-type-selective SERMs.[2c–m] Previous reports from this laboratory have reported the discovery of dihydrobenzoxathiins (e.g., **1** and **2**) as a novel class of selective estrogen receptor alpha modulators (SERAMs).[3a–e] We have also described studies on the side chain SAR of this class of SERAMs.[3d–f] These studies resulted in the discovery that addition of a methyl group to the side chain at the appropriate position and with the right stereochemistry, either on the pyrrolidine ring (as in **3**) or on the linker (as in **5**), substantially increased estrogen antagonist activity in uterine tissue. These compounds were also more potent inhibitors in an MCF-7 breast cancer cell assay.

Compounds **3** and **5**, had what we considered to be, an excellent ER antagonist profile.[3f] However, we felt that it might be possible to find a compound with an even better profile. Since introduction of a single methyl group at either the 1′ or the 3 position of the side chain of **2** resulted in such a significant increase in uterine antagonism and MCF-7 activity, we prepared and report herein the 'combo' compounds **7**–**10**, which incorporate methyl groups at both positions in the side chain.

Although our previous studies[3f] strongly suggested that the optimum stereochemistry for these methyl groups would be as in side chain **20** (compare compound **3** with **4** and **5** with **6**), we also prepared the other three diastereomers of **20** (**21**–**23**) to be sure that we had the best combination and to probe the relative contribution of the two side chain methyl groups to the uterine antagonist properties of the final compounds. Once evaluation of the corresponding dihydrobenzoxathiins **7**–**10** confirmed that the orientation of the methyl groups in **20** was optimal, we proceeded to append this side chain to three additional dihydrobenzoxathiin platforms, affording analogs **11**–**13** for evaluation.

Side chains **20**–**23** were prepared by a significantly improved modification of our previously reported synthesis of mono-alkylated pyrrolidine side chains (Scheme 1).[3f] Condensation of 2-(*R*)-methylsuccinic acid **17**[4] with L-alaninol **18**[4] in refluxing toluene with azeotropic



Scheme 1. Reagents and conditions: (i) toluene, reflux, 24 h, 75–85%; (ii) chiral HPLC; (iii) $LiAlH_4$, ether, 63–69%.

Keywords: SERMs; SERAMs; Estrogen.
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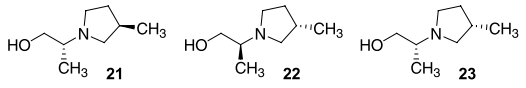
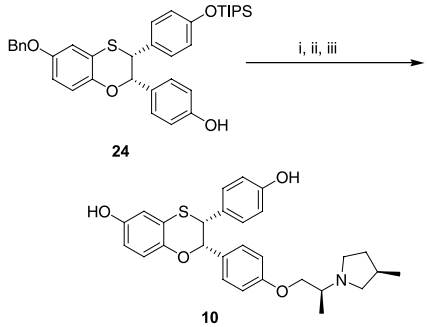
removal of water afforded imide **19**[5] as a 9:1 mixture of diastereomers in 75–85% yield on multigram scale. This improved condensation procedure eliminated some reagents (no acetyl chloride or acetic anhydride) and afforded the desired product in higher yields than the previous procedure. Chiral HPLC[6] removed the minor diastereomer resulting from partial epimerization at C-3. Reduction of **19** with $LiAlH_4$ afforded side chain **20** in 63–69% yield on multigram scale. As we expected based on our experience with an earlier sequence[3f] there was no epimerization at C-3 in the reduction step.[7]

The diastereomeric side chains **21**–**23** were prepared by substitution of 2-(*S*)-methylsuccinic acid[4] for the 2-(*R*)-acid and/or substitution of D-alaninol[4] for L-alaninol, as appropriate, in the synthetic scheme outlined above.



The dihydrobenzoxathiin core structures (e.g., **24**) were synthesized using the procedure previously reported by Kim et al. (Scheme 2).[3a] Attachment of the side chain to the core using a previously described procedure[3a], followed by deprotection, afforded the novel SERAMs **7**–**13**. As previously noted during the synthesis of **5** and **6**, [3f] the presence of a methyl group in the linker resulted in the formation of varying amounts of a rearranged by-product. However, **7**–**13** were the major products.

The novel bis-methylated pyrrolidine analogs **7**–**10** retained an excellent ERα potency exhibited by previously reported compounds **1**–**6** in an in vitro estrogen receptor binding assay (Table 1).[8] Although all of the novel analogs were alpha selective, **7** and **8** were particularly selective (note that a longer incubation time was used in the evaluation of these two analogs in the binding assay; we do not believe that a shorter incubation time would result in lower selectivity but this has not been proven). As we had anticipated, the 1′-(*S*),3-(*R*) configuration present in side chain **20** (analog **10**) proved to be optimal for uterine antagonism as measured in a highly sensitive immature rat assay.[9] Interestingly, the methyl group at C-1′ in the linker appears to be considerably more



Scheme 2. Reagents: (i) **20**, DIAD, PPh_3, THF; (ii) Pd, HCO_2NH_4, EtOH; (iii) nBu_4NF, AcOH, THF.

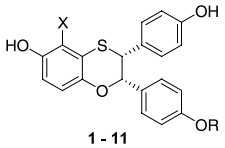
important than the methyl at C-3 in the ring as **9** ('right' stereochemistry at C-1′, 'wrong' stereochemistry at C-3) has a much better uterine profile than **8** ('wrong' stereochemistry at C-1′, 'right' stereochemistry at C-3). Analog **7**, which has the 'wrong' stereochemistry at both sites, is clearly the worst of the four diastereomers.

We were encouraged by the finding that the beneficial effect of adding a methyl group to the pyrrolidine ring or to the linker appeared to be additive so that the 'combo' compound **10** did have an improved uterine profile and enhanced MCF-7 activity relative to the mono-methylated compounds **3** and **5**. Although the improvement was small, we felt it to be sufficient to warrant additional investigation. We therefore prepared and evaluated three additional analogs wherein the optimized antagonist side chain **20** was appended to three modified dihydrobenzoxathiin platforms. All three of these new analogs (**11**–**13**) exhibited a substantially improved uterine profile, relative to the mono-methylated analogs **3** and **5**, which were in turn significantly better than the unmethylated analog **2**. The uterine profiles of compounds **10**–**13** were clearly superior to raloxifene (**14**) and were comparable to the known pure antagonist fulvestrant (**16**).[10] Note, however, that compounds **1**–**13** are orally active, whereas fulvestrant must be dosed parenterally. In addition to their excellent uterine activity, all of the new analogs showed improved potency in an MCF-7 assay.[11] The fluorinated analogs **11** and **13** were especially noteworthy in this regard. In contrast to the analogous methylated chromane system,[12] wherein, the introduction of the fluorine atom does not effect selectivity, we speculate that a decrease in the selectivity of fluoro-dihydrobenzoxathiins may arise from a reduction of the electron density on sulfur, and in turn, a reduction of the electronic repulsion with the Met366 residue of ERβ.

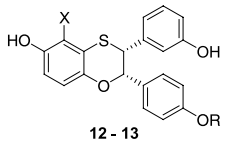
Although it was not possible to obtain X-ray quality crystals of the ligand-bound ER complexes of compounds **10**–**13**, we were able to obtain and have reported X-ray data for **3** and **5**.[3f] The antagonist activity of **3** and **5** can be rationalized as resulting from the positive interaction of the 3-(*R*)-configured ring methyl of **3** and the 1′-(*S*)-linker methyl group of **5** with residues in the antagonist-arm region of the ligand binding domain of ERα.[3f] The apparent additivity of the uterine antagonist effects of **3** and **5** observed for the combo compounds **10**–**13** prompts us to speculate that the exceptional antagonism exhibited by **10**–**13** arises from a sum of positive interactions previously demonstrated for **3** and **5**, thereby further enhancing the stabilization of the antagonist configuration.

Because of their excellent activity in the MCF-7 and rat uterine assays, compounds **10**–**13** were evaluated for anti-tumor activity against MCF-7 human breast carcinoma xenografts in athymic mice. All of the compounds were found to be superior to the clinically approved therapies, tamoxifen and fulvestrant, in that both tumor growth rates and final tumor burdens were significantly lower.[13] In this regard, compound **13** was most efficacious. In a mode similar to the known SERD (selective estrogen receptor down-regulator) fulvestrant,[14] the

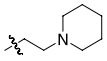
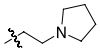
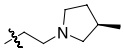
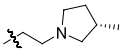
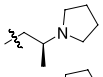
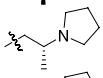
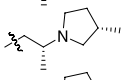
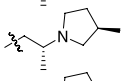
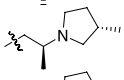
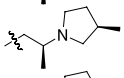
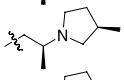
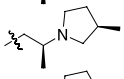
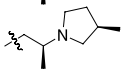
Table 1. ER ligand binding data, rat uterine growth data, and MCF-7 data



1 - 11



12 - 13

Compound	X	R	Human ER binding (IC_{50}, nM)[8]			Immature rat uterine growth assay[9]				MCF-7[10]
			hERα	hERβ	β/α	% Antagonism		% Agonism		IC_{50} (nM)
						0.3 mpk	1 mpk	0.3 mpk	1 mpk	
1	H		0.9	43	48	80	99	11	9	3.0
2	H		2.6	64	25	55	73	38	34	3.3
3	H		1.6	87	54	98	109	−1	−14	0.3
4	H		1.0	50	50	70	85	14	8	1.0
5	H		1.0	45	45	85	102	9	−8	0.3
6	H		1.7	71	42	40	18	59	71	0.5
7	H		0.8	87	109	34	30	54	69	2.1
8	H		0.6	90	150	57	72	33	27	0.9
9	H		2.5	45	18	100	118	−7	−9	0.4
10	H		1.3	52	40	109	123	−18	−29	0.1
11	F		0.8	5.3	7	108	118	−12	−17	0.03
12	H		1.2	69	58	93	118	−8	−17	0.1
13	F		0.7	9.1	13	102	122	−19	−20	0.03
14	Raloxifene		0.7	1.9	3	39	81	28	24	0.8
15	Estradiol		1.3	1.1	1	—	—	—	100	—
16	Fulvestrant		8.0	8.4	1	104	—	−24	—	0.1

exceptional activity of **10**–**13** against breast cancer cells may be due, in part, to their ability to down-regulate the estrogen receptor.[15]

To our knowledge, compounds **10**–**13** represent the first reported examples wherein a relatively small structural modification of an existing SERM (i.e., addition of two methyl groups as in **2** vs **10**) results in conversion of the SERM to a SERD. By analogy to our previous classification of the ERα sub-type-selective SERMs as SERAMs, the ERα sub-type-selective SERDs **10**–**13**, especially the more selective analogs **10** and **12**, may reasonably be described as SERADs (selective estrogen receptor alpha down-regulators).

In conclusion, our effort to delineate the side-chain SAR of the dihydrobenzoxathiin SERAMs has led to the discovery of an optimized antagonist side chain for this platform. This side chain has been attached to four dihydrobenzoxathiin platforms to prepare four novel α-selective estrogen receptor ligands (**10**–**13**) with exceptional antagonist activity. These orally active optimized SERAMs are comparable or superior to previously reported SERMs with regard to estrogen antagonism in uterine tissue and breast cancer cells. A future communication from this laboratory will discuss the applicability of the optimized antagonist side chain **20** to known SERM platforms.

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the dihydrobenzoxathiin cores. We also thank Dr. Derek Von Langen, Mr. Daniel Kim, Mr. Steve Young, and Mr. Jerry Morgan for assistance in the large-scale preparation of side chain **20**. We thank Dr. Sudha Warrier Mitra and Mr. Joel Yudkovitz for the estrogen receptor down-regulation experiments.

References and notes

1. (a) Jordan, V. C. *J. Med. Chem.* **2003**, *46*, 883; (b) Jordan, V. C. *J. Med. Chem.* **2003**, *46*, 1081.
2. (a) Mosselman, S.; Polman, J.; Dijkema, R. *FEBS Lett.* **1996**, *392*, 49; (b) Kuiper, G. G.; Enmark, E.; Pelto-Huikko, M.; Nilsson, S.; Gustafsson, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 5925; (c) Malamas, M. S.; Manas, E. S.; McDevitt, R. E.; Gunawan, I.; Xu, Z.; Collini, M. D.; Miller, C. P.; Dinh, T.; Henderson, R. A.; Keith, J. C.; Harris, H. A. *J. Med. Chem.* **2004**, *47*, 5021; (d) Collini, M. D.; Kaufman, D. H.; Manas, E. S.; Harris, H. A.; Henderson, R. A.; Xu, Z. B.; Unwalla, R. J.; Miller, C. P. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4925; (e) Yang, W.; Wang, Y.; Ma, Z.; Golla, R.; Stouch, T.; Seethala, R.; Johnson, S.; Zhou, R.; Gungor, T.; Feyen, J. H. M.; Dickson, J. K. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2327; (f) Yang, C.; Edsall, R. J.; Harris, H. A.; Zhang, X.; Manas, E. S.; Mewshaw, R. E. *Bioorg. Med. Chem.* **2004**, *12*, 2553; (g) Chesworth, R.; Zawistoski, M. P.; Lefker, B. A.; Cameron, K. O.; Day, R. F.; Mangano, F. M.; Rosati, R. L.; Colella, S.; Petersen, D. N.; Brault, A.; Lu, B.; Pan, L. C.; Perry, P.; Ng, O.; Castleberry, T. A.; Owen, T. A.; Brown, T. A.; Thompson, D. D.; DaSilva-Jardine, P. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2729; (h) Meyers, M. J.; Sun, J.; Carlson, K. E.; Marriner, G. A.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A. *J. Med. Chem.* **2001**, *44*, 4230; (i) Mortensen, D. S.; Rodriguez, A. L.; Carlson, K. E.; Sun, J.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A. *J. Med. Chem.* **2001**, *44*, 3838; (j) Muthyala, R. S.; Carlson, K. E.; Katzenellenbogen, J. A. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4485; (k) Henke, B. R.; Consler, T. G.; Go, N.; Hale, R. L.; Hohman, D. R.; Jones, S. A.; Lu, A. T.; Moore, L. B.; Moore, J. T.; Orband-Miller, L. A.; Robinett, R. G.; Shearin, J.; Spearing, P. K.; Stewart, E. L.; Turnbull, P. S.; Weaver, S. L.; Williams, S. P.; Wisely, G. B.; Lambert, M. H. *J. Med. Chem.* **2002**, *45*, 5492; (l) Schopfer, U.; Schoefter, P.; Bischoff, S. F.; Nozulak, J.; Feuerbach, D.; Floersheim, P. *J. Med. Chem.* **2002**, *45*, 1399; (m) Manas, E. S.; Unwalla, R. J.; Xu, Z. B.; Malamas, M. S.; Miller, C. P.; Harris, H. A.; Hsiao, C.; Akopian, T.; Hum, W. T.; Malakian, K.; Wolfrom, S.; Bapat, A.; Bhat, R. A.; Stahl, M. L.; Somers, W. S.; Alvarez, J. C. *J. Am. Chem. Soc.* **2004**, *126*, 15106.
3. (a) Kim, S.; Wu, J. Y.; Birzin, E. T.; Frisch, K.; Chan, W.; Pai, L.; Yang, Y. T.; Mosley, R. T.; Fitzgerald, P. M. D.; Sharma, N.; DiNinno, F.; Rohrer, S.; Schaeffer, J. M.; Hammond, M. L. *J. Med. Chem.* **2004**, *47*, 2171; (b) Chen, H. Y.; Kim, S.; Wu, J. Y.; Birzin, E. T.; Chan, W.; Yang, Y. T.; DiNinno, F.; Rohrer, S.; Schaeffer, J. M.; Hammond, M. L. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2551; (c) Kim, S.; Wu, J.; Chen, H. Y.; Birzin, E. T.; Chan, W.; Yang, Y. T.; Colwell, L.; Li, S.; DiNinno, F.; Rohrer, S.; Schaeffer, J. M.; Hammond, M. L. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2741; (d) Blizzard, T. A.; DiNinno, F.; Morgan, J. D., II; Chen, H. Y.; Wu, J. Y.; Gude, C.; Kim, S.; Chan, W.; Birzin, E. T.; Yang, Y.; Pai, L.; Zhang, Z.; Hayes, E. C.; DaSilva, C. A.; Tang, W.; Rohrer, S. P.; Schaeffer, J. M.; Hammond, M. L. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3861; (e) Blizzard, T. A.; DiNinno, F.; Morgan, J. D.; Wu, J. Y.; Chen, H. Y.; Kim, S.; Chan, W.; Birzin, E. T.; Yang, Y.; Pai, L.; Zhang, Z.; Hayes, E. C.; DaSilva, C. A.; Tang, W.; Rohrer, S. P.; Schaeffer, J. M.; Hammond, M. L. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3865; (f) Blizzard, T. A.; DiNinno, F.; Morgan, J. D., II; Chen, H. Y.; Wu, J. Y.; Kim, S.; Chan, W.; Birzin, E. T.; Yang, Y.; Pai, L.; Li, Y.; Zhang, Z.; Hayes, E. C.; DaSilva, C. A.; Fitzgerald, P. M. D.; Sharma, N.; Tang, W.; Rohrer, S. P.; Schaeffer, J. M.; Hammond, M. L. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 107.
4. Aldrich Chemical Company.
5. All new compounds were characterized by LC–MS and 400, 500, or 600 MHz ^{1}H NMR. Selected compounds were characterized further by elemental analysis.
6. Analytical chiral HPLC analysis was performed on a Chiralpak AD 4.6 × 250 mm 10 μm column eluted with 3:1 heptane/isopropanol at 0.4 mL/min. Under these conditions, the major (*R*,*S*)-diastereomer of **19** has a retention time of 23.7 min, while the minor (*S*,*S*)-diastereomer elutes at 15.1 min.
7. The optical purity of **20** was confirmed by NMR analysis of the corresponding Mosher ester. The 600 MHz ^{1}H NMR of the Mosher ester clearly showed the presence of only one diastereomer. By contrast, the corresponding NMR spectrum of the Mosher ester of the 1:1 diastereomeric mixture generated using racemic acid as the starting material clearly showed both diastereomers. Similarly, when the reaction sequence was run with racemic acid and racemic amine as starting materials, the NMR spectrum of the Mosher ester of the product showed $\geqslant$3 diastereomers.
8. (a) The IC_{50} values were generated in an estrogen receptor ligand binding assay. This scintillation proximity assay was conducted in NEN Basic Flashplates using tritiated estradiol and full-length recombinant human ERα and ERβ proteins. Compounds were evaluated in duplicate in a single assay. In our experience, this assay provided IC_{50} values that are reproducible to within a factor of 2–3. Dihydrobenzoxathiin **1** ($n = 36$) and estradiol ($n > 100$) were tested in multiple assays; data reported in Table 1 are an average of all determinations; (b) Data for **7** and **8** reflect a 20 h incubation prior to radioactive quantification; all other data obtained with 3 h incubation.
9. (a) The uterine weight assay is an in vivo assay based on a published procedure[9c] that measures estrogen agonism and antagonism in rat uterine tissue. Compounds are dosed orally at the indicated doses, except for fulvestrant, which was dosed subcutaneously. 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.
10. 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 6 days and then treated with the test compound for 7 days. To evaluate the antagonist activity of a test compound, this treatment is given 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.
11. Levin, M.; D'Souza, N.; Castaner, J. *Drugs Future* **2001**, *26*, 841.
12. Tan, Q.; Blizzard, T. A.; Morgan, J. D., II; Birzin, E. T.; Chan, W.; Yang, Y.; Pai, L.; Hayes, E. C.; DaSilva, C. A.; Mitra, S. W.; Yudkovitz, J.; Wilkinson, H. A.; Sharma, N.; Fitzgerald, P. M. D.; Li, S.; Colwell, L.; Fisher, J. E.; Adamski, S.; Reszka, A. A.; Kimmel, D.; DiNinno, F.; Rohrer, S. P.; Freedman, L. P.; Schaeffer, J. M.;

Hammond, M. L. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1675.
13. Hayes, E. C. et al., manuscript in preparation.
14. Osborne, C. K.; Wakeling, A.; Nicholson, R. I. *Br. J. Cancer* **2004**, *90*, S2.
15. Warrier, S. et al., manuscript in preparation.