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Estrogen receptor ligands. Part 4: The SAR of the *syn*-dihydrobenzoxathiin SERAMs

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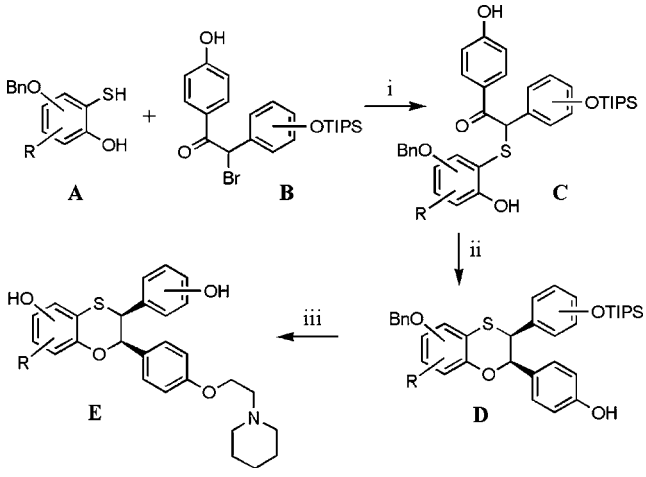
Abstract—A series of estrogen receptor ligands based on a dihydrobenzoxathiin scaffold is described and evaluated for estrogen/*anti*-estrogen activity in both in vitro and in vivo models. The most active analogue, **22**, was found to be 40-fold ERα selective in a competitive binding assay, and **22** demonstrated very potent in vivo antagonism of estradiol driven proliferation in an immature rat uterine weight gain assay.

In our previous communications,[1a,b,c] we described the virtues of SERMs and disclosed the discovery of ERα selective ligands or SERAMs (selective estrogen receptor *alpha* modulators) based on a chromane core with representative types from the flavanone and dihydrobenzoxathiin classes. In particular in the latter series, **11D** was found to be a potent 50-fold selective ligand in a competitive binding assay and 100-fold selective in a transactivation assay in HEK-293 cells. This compound exhibited excellent in vivo efficacy for the suppression of uterine weight increase driven by estradiol, with minimal uterotropic activity. As part of our program to further explore the structure–activity relationships of **E**, we examined a series of derivatives in which the dihydrobenzoxathiin substructure was further modified. Herein, we describe the synthesis and in vivo and in vitro estrogen/*anti*-estrogen activity of these derivatives.

The dihydrobenzoxathiins **E** were prepared following the general method described by this laboratory[1b] and is summarized in Scheme 1. As reported,[2] the key step involved the newly discovered, highly diastereoselective, dehydrative reduction of the keto-sulfides **C** with TFA/Et_3SiH to provide the requisite *cis* stereochemistry at C-2 and C-3 of the dihydrobenzoxathiins **D**. A Mitsunobu alkylation of the phenol **D** with 1-(2-hydroxyethyl)piperidine was utilized for the installation of the basic side chain. Sequential debenzylation, under transfer hydrogenation conditions, and desilyation, with



Scheme 1. Reagents and conditions: (i) Et_3N, DMF; (ii) Et_3SiH, TFA, 0 °C, 50–90%; (iii) (a) PPh_3, DIAD, 1-(2-hydroxyethyl)piperidine, THF, 65–75%, (b) Pd, HCO_2NH_4, EtOH–EtOAc–H_2O, (c) TBAF, HOAc, THF, >80% yield for two steps.

Keywords: SERM; Benzoxathiin; Estrogen.
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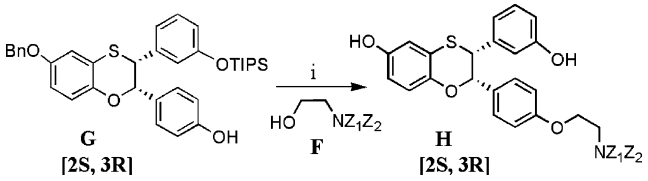


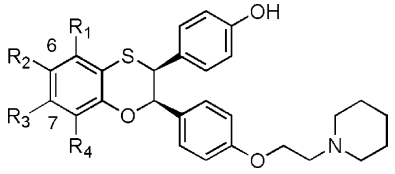
Scheme 2. Reagents and conditions: (i) (a) PPh_3, DIAD, aminoalcohol **F**, THF, 65–75%, (b) Pd, HCO_2NH_4, EtOH–EtOAc–H_2O, (c) TBAF, HOAc, THF, >80% yield for two steps.

TBAF in the presence of HOAc, yielded the desired compounds **E**. Chiral preparative HPLC was relied upon to separate the two enantiomers of **D**.[3] The desired [2*S*,3*R*] enantiomer **G** was converted to **H** (**21D**, **22**–**27**), utilizing the procedures described in Scheme 2.

The compounds were primarily tested for intrinsic activity in an ER binding assay with [^{3}H]-17β-estradiol and full length recombinant human ERα and ERβ proteins. Potency and selectivity of selected compounds were further assessed in a cellular transactivation assay utilizing HEK-293 cells stably co-transfected with either human ERα or ERβ and the alkaline phosphatase reporter gene. The inhibition of the proliferative activity of estradiol along with the estrogenic activity was measured in vivo using an immature rat uterine weight gain assay.

As previously observed,[1b] within the limits of the binding assay, alternating the hydroxyl from position 7 to 6 was without difference (compounds **1** and **11**). This trend was further evidenced by the binding affinity and uterotropic activity of analogues bearing substituents, R_1–R_4 (compounds **1**–**17**). Given the previous proposal[1a,b] that the crucial difference responsible for the *alpha*-selectivity of dihydrobenzoxathiins lay in the interaction of the sulfur atom with the two discriminating residues in the binding pocket of the two receptor isoforms (Leu 384 for ERα, Met 354 for ERβ), it became

Table 1. Binding affinities[a] and in vivo data[b]



Compound[c]	R_1	R_2	R_3	R_4	Binding affinity		HEK 293		Uterine weight assay (*sc*)[b] % inhibition/ % control @ 1 mpk
					ERα	ERβ	ERα	ERβ	
1	H	H	OH	H	1.8	44.4	5.0	238.8	67/1.0[d]
2	Me	H	OH	H	2.0	46.2	5.5	199.8	ND
3	Et	H	OH	H	53.3	185	341.9	1455.0	ND
4	OH	H	OH	H	25	788	ND	ND	64/7.0
5	F	H	OH	H	0.5	13	6.1	516.1	89/−2.0
6	Cl	H	OH	H	0.51	8.0	3.4	121	68/12
7	H	Cl	OH	H	23.1	520	61	3021	ND
8	H	Me	OH	H	34.9	595	ND	ND	ND
9	H	H	OH	Me	1.2	54	9.3	68.3	29/27
10	H	H	OH	Et	4.7	25	ND	ND	ND
11	H	OH	H	H	3.0	143 ($n = 5$)	9.6	52	77/5.0
12	F	OH	H	H	0.8	13.2	2.5	177	76/7.0
13	Cl	OH	H	H	2.4	14	1.0	64	67/27[e]
14	H	OH	Cl	H	5.4	73	14.8	2369	36/38
15	H	OH	F	H	13.5	413.2	ND	ND	53/13
16	H	OH	H	Cl	5.3	105.4	25.4	1013.0	ND
17	H	OH	H	Me	5.3	343.3	22.6	690	−4.0/12
E-2					1.3	1.1 ($n = 132$)			
Ralox					1.8	12 ($n = 7$)			

[a] The IC_{50} values were generated in an estrogen receptor ligand binding assay. This scintillation proximity assay was conducted in NEN basic flash plates using tritiated estradiol and full length recombinant human ERα and ERβ proteins, with incubation times of 3–23 h. In our experience, this assay provides IC_{50} values that are reproducible to within a factor of 2–3. Most compounds are single point determinations. The binding results for **11**,**D**, **22**, and **21D** reflect an average of multiple determinants at 3 h incubation. For estradiol, the binding data reflects an average of over 100 determinants at 3 h of incubation.

[b] 20-Day old intact female Sprague–Dawley rats were treated (*sc*) with test compounds for 3 days at 1 mpk. The uteri wet weights were determined on day 4 and dry weights were determined after air-drying the tissue samples for 3 days. The *anti*-estrogenic activity of the compounds was determined by co-administration of the compound with a subcutaneous injection of 17β-estradiol and reported as % inhibition. The estrogenic activity (partial agonism) of the compounds was determined by administering the test compound without estradiol and reported as % control.

[c] All compounds are racemic.

[d] @ 0.3 mpk.

[e] @ 0.6 mpk.

prudent to explore the effect of the addition of substituents, R_1. As can be seen in Table 1, the presence of a methyl group (compound **2**) was tolerated. However, the simple extension to an ethyl group (compound **3**) not only diminished the affinity to both receptors, but also lowered the selectivity over ERβ. Conversely, a significant increase in binding activity (ERα = 0.5 nM) was observed upon introduction of an electronegative substituent, such as fluorine and chlorine (compounds **5** and **6**); albeit, with reduced selectivity (ca. 3–10-fold). A plausible rationale for the latter maybe, that a reduction in the electron density on sulfur may, in turn, reduce the level of the electrostatic repulsion with the Met 366 residue in ERβ and thereby allow for a greater affinity to it. Interestingly, this remarkable binding affinity to ERα only correlated with the in vivo antagonist/agonist activity profile of compound **5**, as evidenced by the comparison of the uterine weight data (89% inhibition and −2.0% agonism for compound **5** versus 68% inhibition and 12% agonism for compound **6**). A similar trend was observed for the C-6 hydroxylated derivatives **12** and **13**.

On the other hand, substituents R_4 led to comparable binding activities to **1** and **11**, but significantly impacted the ability to inhibit the estradiol stimulated proliferation in the uterus (29% inhibition for **9** and 0% inhibition for **17**). This suggested that subtle conformational differences of the ligand–receptor complex resulting from a minor change in the ligand may alter the nature of the interactions with the transcriptional machinery in the cellular assay and thus the in vivo antagonism in the uterine weight gain assay.[4] In the case of compound **4**, there appears to be an inconsistency in the correlation between the binding activity and anti-uterotropic activity. Thus, compound **4**, with weak binding activity (ERα = 25 nM) and modest *alpha*-selectivity, exhibited an equal level of antagonism in the uterine weight gain assay (64% inhibition) as compared to **1**. Alternative substituent arrangements, as with compounds **7**, **8**, **14**, and **15**, were without improvement.

Having potent SERAMs in hand, the pharmacokinetic profile of selected compounds was assessed in female Sprague–Dawley rats, and the results are depicted in Table 2. Noteworthy are the two sets of structurally close derivatives, which primarily differ in the position of the phenolic oxygen group but exhibit extremely contrasting oral bioavailabilities: $F = 31\%$ and 22% for compounds **11** and **12**, respectively, as compared to $F = 0\%$, 6%, and 4.4% for compounds **1**, **2**, and **5**, respectively. Further, as previously reported by us,[1b] only the [2*S*,3*R*] enantiomer, **11D**, reproduced the activities exhibited by the racemate (**11D** vs **11L**). Likewise compound **12D** was found to possess good oral bioavailability ($F = 37\%$). It is of interest to note that similar observations were denoted in the pharmacokinetics of similarly substituted *cis*-tetrahydronaphthalenes and in particular lasofoxifen,[5] in which the dramatically improved oral bioavailability was attributed to a reduction in intestinal wall, enantioselective glucuronidation. Certainly, the structural model proposed by Rosati et al.,[5] for the resistance to gut wall glucuronidation, which featured nonplanar topology and axial/equatorial disposition of the pendant aryl groups, can be superimposed on the dihydrobenzoxathiin class. However, it also would appear that the topological issues effecting glucuronidation are even more subtle since the remarkable difference between the C-6 and C-7 phenolic dihydrobenzoxathiins transcends the scope of this model.

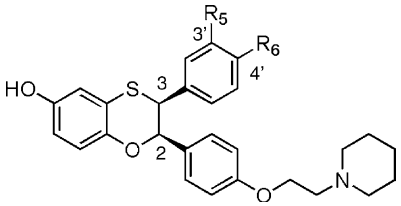
A recent report from this laboratory disclosed that the 4′-hydroxyphenyl group at C-3 of the dihydrobenzoxathiin **11** was superior to alkyl, cycloalkyl, and heterocyclic replacements with respect to *alpha*-selectivity and in vivo efficacy.[1c] The results of extended studies at fine-tuning the C-3 aryl pendant group are described in Table 3. The incorporation of the electronegative substituent fluorine (**20**) maintained the binding activity, as compared to **11**, while the presence of the *p*-OMe functionality in **19** led to a significant decrease in both potency and *alpha*-selectivity (ERα = 41 nM). However, compound **20** failed to inhibit the estradiol mediated proliferation of the uterus, which paralleled the poor activity observed in the functional assay (HEK-293 ERα = 94 nM). Noticeably, the ER binding activity/*alpha*-selectivity was regained by the introduction of a

Table 2. Pharmacokinetic data for selected compounds

Compound	$C_{2,3}$	Binding affinity[a]		Pharmacokinetic parameters[b]			Uterine weight assay (*po*)[e] % inhibition/ % control @ 1 mpk
		ERα	ERβ	*F* (%)	$T_{1/2}$ (h)	Clp (mL/min/kg)	
11	±*cis*[c]	3.0	143 ($n = 5$)	31	3.8	5	77/5.0[f]
11D	[2*S*,3*R*][c]	0.8	45 ($n = 36$)	62	3.4	10	99/9.0
11L	[2*R*,3*S*][c]	23.0	287	10	1.6	31	16/1.0[f]
12	±*cis*	0.8	13.2	22	2.4	16	76/7.0[f]
12D	[2*S*,3*R*][d]	1.0	18	37	3.1	18	100/1.0
1	±*cis*	1.6	44.4	0	2.2	154	ND
2	±*cis*	2.0	46.2	6	2.7	51	ND
5	±*cis*	0.5	13	4.4	5.9	41	ND

[a] IC_{50} (nM), see Table 1.
[b] In female, Sprague–Dawley rats following intravenous dosing at 1 mpk ($n = 2$) and oral dosing at 2 mpk ($n = 3$).
[c] See Ref. [1b]; Absolute stereochemistry of **11D** was determined by X-ray crystallography.
[d] $[\alpha]_D$ +285.8 (*c* 0.875) in MeOH; The absolute stereochemistry of **12D** was assigned based on analogy with **11D** and biological data.
[e] See Table 1.
[f] Dosed *sc* at 1 mpk, see Table 1.

Table 3. Binding affinities[a] and in vivo data[b]



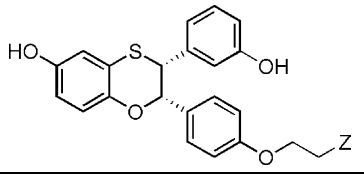
Compound[c]	R_5	R_6	Binding affinity		HEK 293[d]		Uterine weight assay (*sc*)[b] % inhibition/ % control @ 1 mpk
			ERα	ERβ	ERα	ERβ	
18	H	H	11.9	300	ND	ND	ND
11	H	OH	3.0	143 ($n = 5$)	9.6	52	77/5.0
19	H	OMe	41.1	792.0	274	$>10^3$	ND
20	H	F	3.8	66.6	94	1555	9.5/5.0
21	OH	H	3.0	250	3.6	245	90/16[e]

[a] See Table 1.
[b] See Table 1.
[c] All compounds are racemic.
[d] IC_{50} (nM).
[e] @ 0.6 mpk.

3′-OH functionality (**21**), which also resulted in greater potency in the uterine weight gain assay (90% inhibition, 16% agonism at 0.6 mpk), as compared to **11**. These results reconfirmed that the OH functionality in the aryl pendant ring was also required for the maintenance of an optimal antagonist/agonist activity profile in vivo. A similar conclusion was drawn in the 2,3-diaryl-2*H*-1-benzopyran series of phenolic analogues.[6]

To further the SAR of dihydrobenzoxathiins, we next turned our attention to the synthesis of chiral analogues bearing different basic side chains in the quest for optimal *anti*-estrogenic/estrogenic activity in the immature rat uterine weight gain assay; the results of which are shown in Table 4. In general, all of the compounds tested demonstrated good to excellent ERα affinity and *alpha*-selectivity (50–100-fold). However, the relative ability to antagonize the estradiol effect in the uterine assay was dependent upon the nature of basic amine moiety.[7] When orally dosed, at 1 mpk, cyclic derivatives **22** and **21D** suppressed nearly 80% of the estrogen stimulus. However, increasing both the size of the cycloalkylamine (**23** and **24**) and the substitution on the cycloalkylamine (**24**, **25**, and **26**) led to significant decreases in antagonism and increased agonism. A similar trend had been also observed with raloxifene.[7] As

Table 4. Binding affinities[a] and in vivo data[b]



Compound[c]	Z	Binding affinity		HEK 293[d]		Uterine weight assay (*po*)[b] % inhibition/% control @ 1 mpk
		ERα	ERβ	ERα	ERβ	
22	Pyrrolidine	0.9	37 ($n = 106$)	1.7	40.1	77/22[e]
21D	Piperidine	4.1	115 ($n = 4$)	5.4	157.4	78/12
23	Cycloheptylamine	1.2	103	3.5	67.0	57/22
24	Morpholine	3.8	318	4.7	65.3	54/29
25	Methylpiperidine	0.8	109	4.0	131.8	59/29
26	2,6-Dimethylpiperidine	3.4	350	NA	NA	43/30
27	Dimethylamine	1.4	129	2.3	7.3	34/69

[a] See Table 1.
[b] See Table 1.
[c] All compounds are chiral; [2*S*,3*R*] absolute chemistry was assigned based on analogy with **11D** and biological data.
[d] IC_{50} (nM).
[e] $F = 40\%$, $T_{1/2} = 2.8$.

previously seen with tamoxifen,[8] compound **27**, a non-cyclic analogue, exhibited more intrinsic estrogenic activity (69%), and weak estradiol antagonism (34%).

In view of the present SAR of the dihydrobenzoxathiin series, compound **22** was found to be very potent (ER$\alpha = 0.9$ nM), highly ERα-selective (40-fold), and exhibited very potent in vivo antagonism of estradiol (77%) with minimal agonism in the uterine model. In addition, **22** effectively inhibited ovariectomy-induced bone resorption and lowered serum cholesterol levels, in the appropriate rat models. Such a promising profile of activities clearly establish this new class of compounds as potent SERAMs, and compound **22** was selected for further development as a potential agent for osteoporosis, and will be the subject of additional publications from these laboratories.

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