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Estrogen receptor ligands. Part 5: The SAR of dihydrobenzoxathiins containing modified basic side chains

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Abstract—Dihydrobenzoxathiin analogs (**1**–**11**) with modifications on the basic side chain region were prepared and evaluated for estrogen/anti-estrogen activity in both in vitro and in vivo models. The compounds generally maintained a high degree of selectivity for ERα over ERβ, similar to the original lead compound **I**. Many of the compounds also maintained high potency in the inhibition of human carcinoma MCF-7 cell growth. However, all were less potent in the inhibition of estradiol-triggered uterine growth. This work demonstrates the sensitive nature of modification to the antagonist basic side chain region.

Selective estrogen receptor modulators (SERMs) are unnatural ligands of the estrogen receptor and show tissue-selectivity in regulating the receptor activities, and thus have the potential to effectively treat

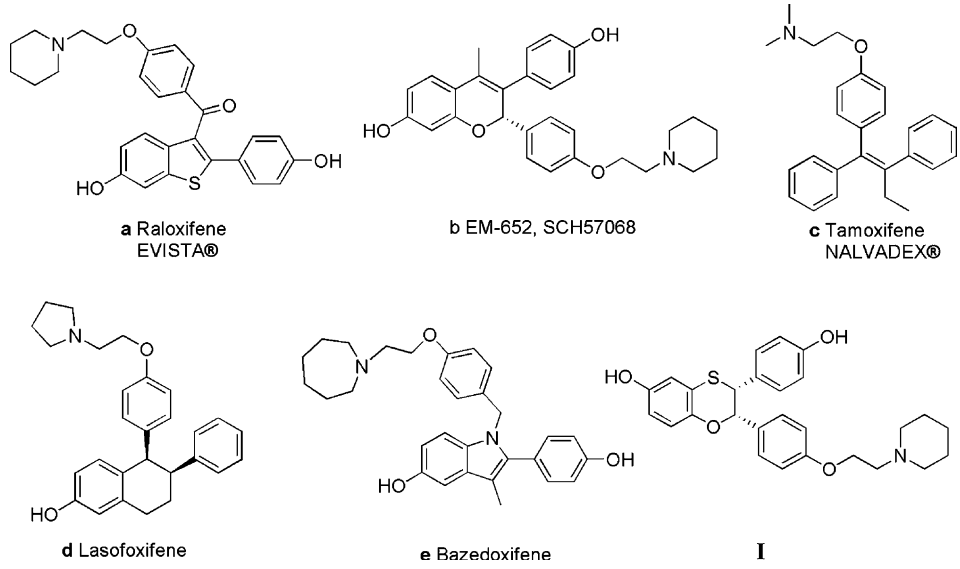

Figure 1. Representative SERMs.

Keywords: Dihyrobenzoxathiin; Estrogen receptor; SERM; SERAM.
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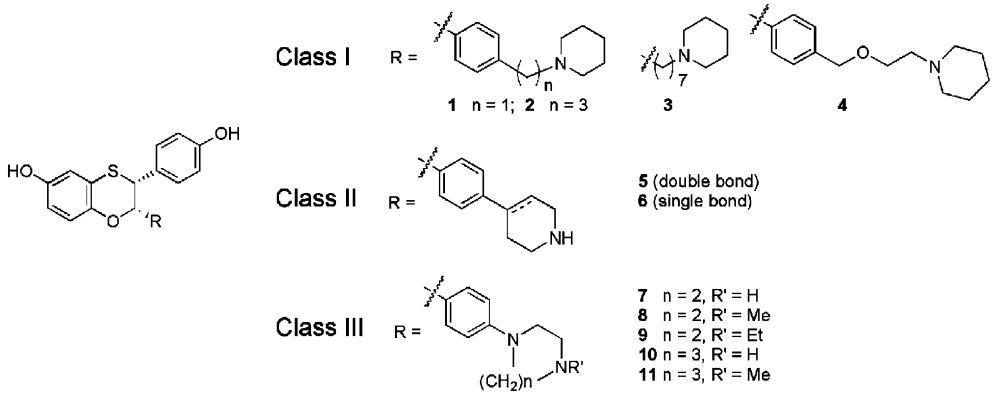
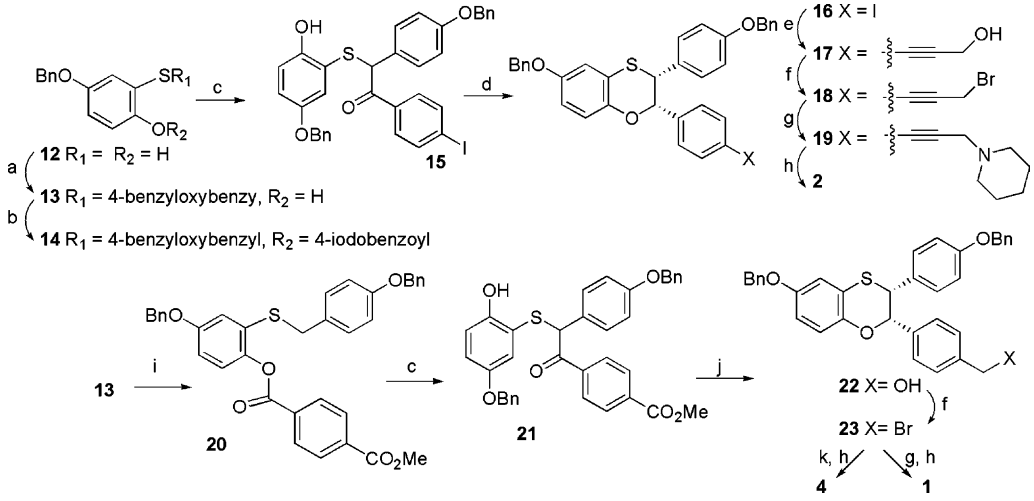


Figure 2. Modified dihydrobenzoxathiins.

estrogen-related disorders while avoiding the adverse side-effects of the natural hormones.[1] SERMs have generated a tremendous amount of interest in pharmaceutical research, in which several entities, **a**–**e**, are currently marketed or in late stage clinical development for the treatment and prevention of osteoporosis or estrogen-sensitive cancers (Fig. 1).[2] All of these compounds exhibit less than 10-fold selectivity in the binding to ERα and ERβ. Our own effort in this area focused on finding SERMs with significant ER binding selectivity, since the ER subtypes are expected to have distinctly different functions. Recently, we identified a new class of dihydrobenzoxathiin-based SERMs, such as **I**, which exhibited greater than 50-fold selectivity favoring ERα, and thus were characterized as SERAMs (Selective Estrogen Receptor Alpha Modulators).[3]

Most second and third generation SERMs, including our benzoxathiin platform, share a common feature in the side chain region: an aminoethyl linkage tethered to a phenolic oxygen atom.[1] Since it is postulated that the interaction between the basic amine side-chain moiety and the acidic amino acid residues in the ER provide a major force in stabilizing the receptor in the inactive state, the precise positioning as well as the electrophysical character of the amine moiety is critical for SERM function. Therefore, it should be valuable to examine various side chain modifications in order to have a clear understanding of the SAR. It is from this perspective that we herein disclose our recent SAR work on the side chain region of the benzoxathiin platform, as studied in compound classes **I**–**III**, wherein compounds **1**–**11** were synthesized (Fig. 2). (For more information on the preparation of relevant structures, see Refs. 3 and 5.)

Scheme 1 depicts the syntheses of compounds **1**, **2** and **4**. Alkylation of thiol **12**[4] followed by *O*-acylation set the stage for an oxygen to carbon acyl transfer process. Under the influence of base, upon warming of the reaction mixture from −78 °C to ice-water temperatures and aging overnight, the acyl group in **14** was efficiently migrated to the adjacent benzylic carbon next to sulfur.



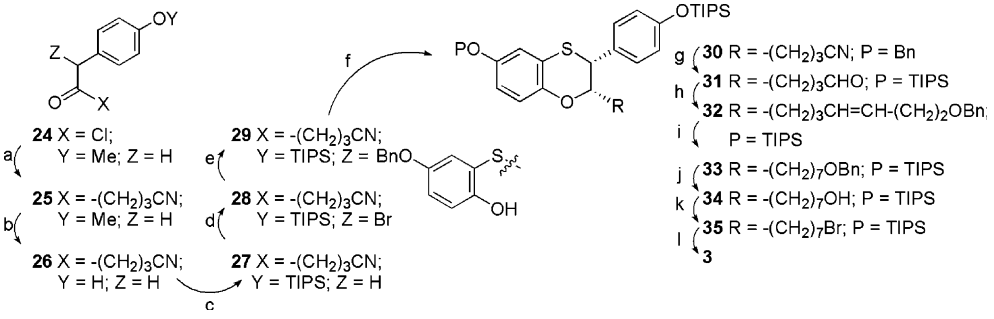
Scheme 1. Synthesis of **1**, **2** and **4**. Reagents and conditions: (a) 4-benzyloxybenzyl chloride, Hunig's base, CH_2Cl_2, 0 °C, 83%. (b) 4-Iodobenzoyl chloride, Hunig's base, DMAP, CH_2Cl_2. (c) 3 equiv LHMDS, THF, −78 °C 1 h, then 0 °C 14 h, two steps 68% for **15** and 31% for **21**. (d) TFA, Et_3SiH, CH_2Cl_2, 86%. (e) Propargyl alcohol, $PdCl_2(PPh_3)_2$, CuI, K_2CO_3, THF, 40%; (f) CBr_4, PPh_3, CH_2Cl_2. (g) Piperidine, 81% two steps for **19**, 41% from **21** toward **1**. (h) H_2, Pd black, ammonium formate, EtOH/EtOAc/H_2O = 7:2:1, 46% for **2**, 44% for **1**, 35% for **4**. (i) Methyl 4-chlorocarbonylbenzoate, Hunig's base, CH_2Cl_2, DMAP. (j) (i) CH_2Cl_2, TFA, Et_3SiH; (ii) DIBAL, CH_2Cl_2. (k) 1-Piperidineethanol, NaH, THF, Bu_4NI, ca. 23% from **21**.

The ketone **15**, thus formed, was treated with TFA–Et_3SiH (TES) to furnish the dehydrative reductive cyclization product **16**, in a stereo-controlled fashion, as previously reported by Kim et al.[5] Sonogashira coupling yielded propargyl alcohol **17**, which in turn was converted to bromide **18**, and subsequently displaced by piperidine to yield **19**. Finally, deprotection of the benzyl groups under transfer-hydrogenation conditions provided target **2**. Similarly, **1** and **4** were prepared.

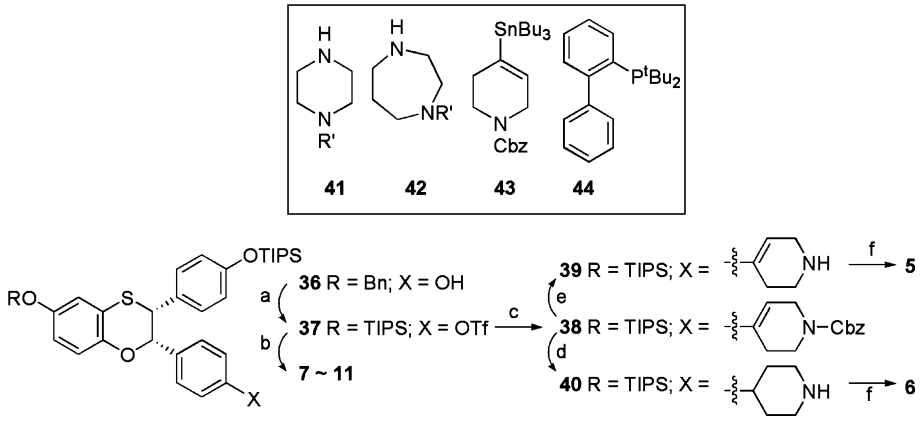
The synthesis of **3** is shown in Scheme 2. A copper(I) mediated coupling reaction[6] between $BrZn(CH_2)_3CN$ and 4-methoxy phenylacetyl chloride gave rise to ketone **25**. Following a protecting group change from methyl to TIPS, the α-position of ketone **27** was brominated and subsequent displacement by mercaptan **12** furnished **29**.[7] Dehydrative, reductive cyclization as before produced **30**. After the benzyl protecting group was replaced by TIPS in a two-step sequence, the nitrile moiety was reduced to aldehyde **31**, and subsequent Wittig olefination gave rise to **32**. The side chain construction was completed by hydrogenation of the double bond, debenzylation to expose the terminal hydroxyl, conversion to bromide **35**, and finally displacement by piperidine. Removal of the two TIPS protecting groups yielded compound **3**.

As shown in Scheme 3, the synthesis of **5**–**11** was achieved using the aryl triflate **37** as a versatile synthon for the installation of various side chains. Thus, the piperazine and homopiperazine side chains **7**–**11** were readily installed via Buchwald coupling reaction,[8] and the piperidine side chains **5** and **6** via the Stille coupling reaction with **43**.[9]

The compounds were evaluated for ER binding affinity, the ability to antagonize the effect of estradiol on the growth of the MCF-7 cancer cell line, and the effect on the stimulation of an immature rat uterus in the presence and absence of estradiol. It is clear from the data in Table 1 that compared to the parent compound **I**, all of the new compounds except **1** and **10** lead to significantly increased agonism in immature rat uterine weight assay, despite the similarity in receptor binding and estradiol antagonism in MCF-7 cells. Compounds **1**–**4** were prepared to evaluate the effect of the linker length. Thus the slightly shorter linker **1** gave rise to an inactive compound, whereas **2** with a slightly longer linker exhibited



Scheme 2. Synthesis of **3**. Reagents and conditions: (a) $BrZn(CH_2)_3CN$, CuCN, LiBr, THF. (b) Pyridinium hydrochloride, 190 °C. (c) TIPSCl, Hunig's base, DMF, 52% three steps. (d) PTAB, 0 °C, CH_2Cl_2. (e) **12**, Et_3N, CH_2Cl_2, 55% two steps. (f) TFA, Et_3SiH, CH_2Cl_2, 59%. (g) (i) Pd black, formic acid, EtOAc; (ii) TIPSCl, Hunig's base, DMF; (iii) DIBAL, toluene, CH_2Cl_2. (h) $BnO(CH_2)_3P^+Ph_3Br^-$, BuLi, THF, 77% from **30**. (i) Pd, EtOH–EtOAc, H_2. (j) Pd black, EtOH–EtOAc, formic acid. (k) CBr_4, CH_2Cl_2, PPh_3. (l) (i) Piperidine, THF; (ii) TBAF, THF; 89% from **32**.



Scheme 3. Synthesis of **5**–**11**. Reagents and conditions: (a) (i) NaH, Tf_2NPh, THF; (ii) Pd black, formic acid, EtOAc, EtOH; (iii) TIPSCl, Hunig's base, DMF; 93% overall based on recovered SM; (b) (i) **41** or **42**, Pd_2dba_3, **44**, K_3PO_4, toluene, 80 °C; (ii) TBAF, THF, 34–90% overall. (c) **43**, Pd_2dba_3, $PdCl_2(PPh_3)_2$, K_3PO_4, NMP, LiCl, 100 °C, 50%. (d) EtOAc, Pd, H_2. (e) Pd black, formic acid, EtOAc, 48%. (f) TBAF, THF, >90% for **5**, 67% from **38** to **6**.

Table 1. Assay[f] data

Compound	Binding affinity,[a] IC_{50} (nM) ERα/ERβ (fold selective for ERα)	MCF-7 Inhibition[b] IC_{50} (nM)	Uterine weight[c] % inhibition/ % control (antagonism/ agonism)
1	155/4372 (28)	—	0/3
2	1.4/186 (133)	6.7	29/73
3	0.7/27 (39)	1.2	41/48
4	2.3/259 (115)	14.6	52/46
5	1.4/31 (22)	0.9	54/45
6	1.7/115 (62)	1.9	54/40
7	4.4/186 (43)	2.8	60/38
8	1.0/59 (59)	3.4	47/40
9	1.0/27 (27)	1.4	66/37
10	3.6/161 (45)	28	43/0
11	3.6/365 (101)	29	44/32
I	0.8/45 (56)[d]	3.0	92/0.4
Estradiol	1.3/1.1 (1)[e]	—	—/100

[a] The single 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, with an incubation time of 3 h. In our experience, this assay provides IC_{50} values that are reproducible to within a factor of 2–3.

[b] The estrogen depleted MCF-7 cells were plated into 96-well cell culture plates at a density of 1000 cells/well in a volume of 180 μL/ well. The test compounds and 3 pmol estradiol were applied to the cells on days 1, 4, and 7. The assay was terminated between days 8 and 10.

[c] 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 (antagonism) activity of compounds was determined by co-administration of the compound with a subcutaneous injection of 17-β-estradiol at 0.004 mpk and reported as % inhibition of uterine growth induced by estradiol. The estrogenic activity (partial agonism) of the compounds was determined by administering the test compound without estradiol and reported as % control.

[d] Average of 36 measurements.

[e] Average of 130 measurements.

[f] More experimental details can be found in Ref. 3.

substantially increased agonism and decreased antagonism. Compounds **3** and **4** with longer tethers exhibited almost equivalent levels of antagonism and agonism. These dramatic results further exemplify the restrictions imposed by the estrogen receptor-alpha on the adoption of an optimal liganded antagonist conformation.

Since raloxifene, when in complex with the ER, is forced to adopt an unnatural s-*cis* conformation in the side chain linker region,[10] we decided to incorporate this structural feature in our linker modifications by using a ring to fix the conformation. Such a design is imbedded in compounds **5**–**11**. Compounds **5** and **6**, with a carbocyclic fixed *cis*-conformation, exhibited a balanced antagonism/agonism response in the uterine weight assay as previously observed, in spite of potent ERα affinity and potent antagonism of estradiol dependent growth of MCF-7 cells. Since the apparent optimal linker found in raloxifene incorporated a hard heteroatom oxygen, we set out to incorporate the hard nitrogen heteroatom into our design which resulted in the piperazines and homopiperazines **7**–**11**. Although these modifications slightly improved the antagonism/agonism profile, as exemplified by **7** and **9**, the uterine weight results for **8** and **10**–**11** once again demonstrated the extreme sensitivity of the receptor in response to subtle changes around the basic amine region.

In conclusion, we have shown that modifications to the basic side chain region of the dihydrobenzoxathiin scaffold had a relatively small influence on the ERα selectivity, but dramatically altered the in vivo antagonism/agonism activity profile of the lead **I**. These modifications included those factors, which may contribute to the optimal antagonist side chain; such as length, the nature of the heteroatom in the chain, and the presence of an s-*cis*-conformation formed between the heteroatom and the cyclic amine. Further work along these lines will be the subject of future publications from our laboratories.

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