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Bioorganic & Medicinal Chemistry Letters 14 (2004) 2551–2554
Bioorganic & Medicinal Chemistry Letters

Estrogen receptor ligands. Part 3: The SAR of dihydrobenzoxathiin SERMs

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Received 6 January 2004; revised 19 February 2004; accepted 26 February 2004

Abstract—A series of 3-alkyl, 3-cycloalkyl, and 3-heteroaryl dihydrobenzoxathiin analogs **1** were prepared and evaluated for estrogen/anti-estrogen activity in both in vitro and in vivo models. In general, the compounds were found to exhibit a high degree of selectivity for ERα over ERβ, but were less potent than the original lead compound **1a** in the inhibition of estradiol-driven uterine proliferation.

The decline of estrogen levels in postmenopausal women has been tied to a number of conditions including osteoporosis and coronary heart disease.[1] Although estrogen replacement therapy (ERT) has significantly reduced these risks, the associated risks of uterine and breast cancers have generated tremendous interest in developing alternative treatments, the most promising of which appear to be selective estrogen receptor modulators (SERMs).[2] These agents have the potential to antagonize the detrimental proliferative effects of estrogen on uterine and breast tissue while producing estrogen-like effects on the bone and cardiovascular system. With the discovery of a second estrogen receptor isoform, ERβ, the prospect that subtype selective ligands may offer significant advantages is tantalizing. Currently, none of the marketed SERMs such as Evista®[3] and Nolvadex®[4] for the treatment of osteoporosis and breast cancer, respectively, exhibit any significant selectivity.

Recently, our laboratory reported a structurally distinct series of *selective estrogen receptor alpha modulators* (SERAMs) based on the dihydrobenzoxathiin platform **1**.[5] In particular, **1a** was found to be a potent 50-fold ERα-selective SERM with a demonstrated in vivo activity. As part of our program to further explore the structure–activity relationship of **1**, we examined a series of analogs in which the 4-hydroxyphenyl substituent (**1a**) at C-3 was replaced by an alkyl, cycloalkyl, or heterocyclic moiety (Table 1). Herein, we describe the synthesis of these analogs, and their in vivo and in vitro estrogen/anti-estrogen activity.

The synthetic route to **1b–o** is outlined in Scheme 1. As reported recently by our group,[6] the key step that set the requisite *cis*-stereochemistry at C-2 and C-3 of the dihydrobenzoxathiin involved the reductive cyclization of the hydroxythioketone **5**. To that end, the bromoketones **4**, obtained from treatment of the corresponding ketones **2** with PTAB, were alkylated with either the protected or unprotected **3** under basic conditions to give the hydroxythioketones **5b–o** in good yields.[7] The resulting adducts were cyclized to the *cis*-dihydrobenzoxathiins **6** with TFA and Et_3SiH in CH_2Cl_2 as previously described.[6] Removal of the TIPS protecting group with TBAF in the presence of AcOH followed by installation of the ethyl piperidine side chain by the Mitsunobu reaction afforded the penultimate intermediate. Where P = H, the free phenol of **6** was first

Keywords: Dihydrobenzoxathiin; SERMs; SERAMs.
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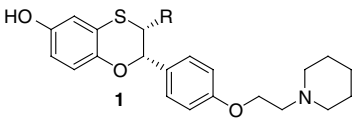
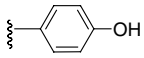
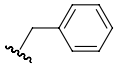
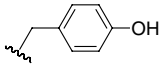
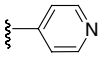
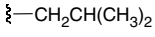
doi:10.1016/j.bmcl.2004.02.084

Table 1. Dihydrobenzoxathiins



Compd	R
a[a]	
b[b]	
c[a]	
d[a]	
e[a]	
f[b]	
g[b]	
h[a]	*i*-Pr
i[b]	*tert*-Butyl
j[b]	
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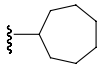
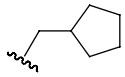
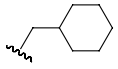
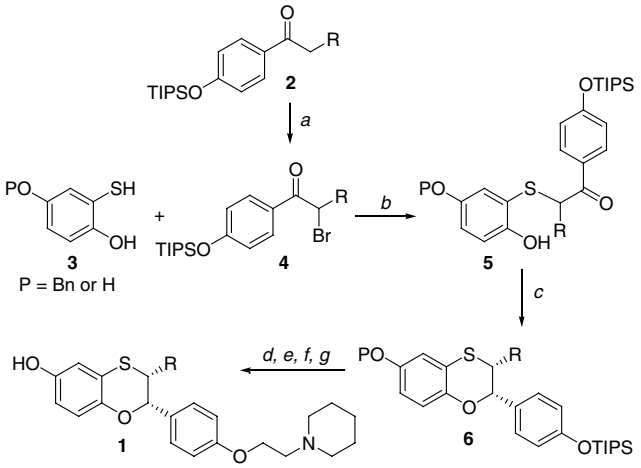
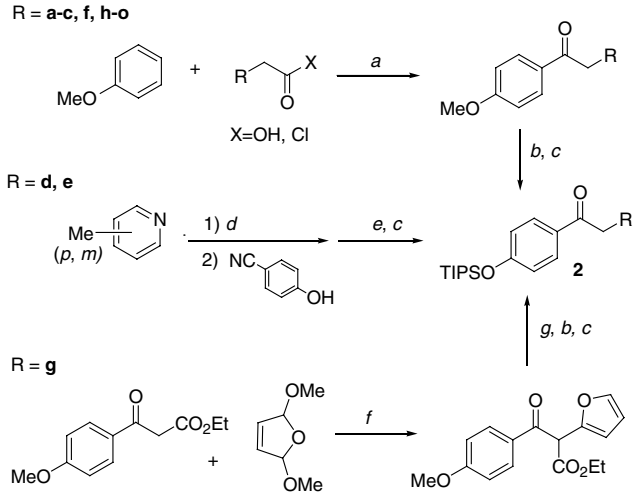
l[a]	
m[b]	
n[b]	
o[b]	

[a] Method A.
[b] Method B.



Scheme 1. Synthesis. Reagents and conditions: (a) $PhN^{+}Me_3Br_3^{-}$ (PTAB), THF, 0 °C, 100%; (b) Hunig's base, DMF, 0 °C, 10–92%; (c) TFA, Et_3SiH, CH_2Cl_2, 43–94%; *Method A, P = Bn*: (d) TBAF, AcOH, THF, 0 °C, 68–79%; (e) 1-(2-chloroethyl)piperidine monohydrochloride, Ph_3P, DIAD, THF, 16–90%; (f) Pd black, ammonium formate, EtOH/EtOAc/H_2O (7:2:1), 80 °C, 4–100%; *Method B, P = H*: (d) NaH, MOMCl, THF, 0 °C; (e) TBAF, AcOH, THF, 0 °C, 61–91% from **6**; (f) 1-(2-chloroethyl)piperidine monohydrochloride, Ph_3P, DIAD, THF, 65–90%; (g) 2 N HCl, MeOH, 80 °C, 55–100%.



Scheme 2. Synthesis of starting ketones **2**. Reagents and conditions: (a) PPA, 80 °C or $AlCl_3$, CH_2Cl_2, 0 °C, 76–100%; (b) pyridine–HCl, 190 °C, 17–100%; (c) TIPSCl, NaH, THF, 0 °C, 52–100%; (d) LDA, HMPA, THF, −78 °C; (e) 2 N HCl, 43–46% from step (d); (f) $ZnCl_2$, 80 °C, 95%; (g) LiCl, AcOH, H_2O, NMP, 130 °C, 82%.

protected with a MOM group before elaboration to the basic side chain. The final racemic *cis*-dihydrobenzoxathiins were obtained after standard debenzylation using ammonium formate and palladium black or removal of the MOM group using 2 N HCl in MeOH.

The preparation of ketones **2** utilized in Scheme 1 is outlined in Scheme 2. The majority of the ketones **2** were prepared by Friedel–Crafts acylation of anisole with the appropriate acid chloride or carboxylic acid using $AlCl_3$ in CH_2Cl_2 at 0 °C or PPA at 80 °C, respectively. Demethylation using pyridine–HCl at 190 °C followed by reprotection with TIPSCl and NaH in THF afforded the desired ketones **2** in good overall yields (53–100% over three steps). Ketones **2d**–**e** were synthesized by addition of the lithium anion of 3- and 4-picoline[8] to 4-cyanophenol followed by acid hydrolysis of the intermediate enamine. The analogous furyl ketone **2g** was prepared from commercially available ethyl 4-methoxy benzoylacetate and 2,5-dimethoxy-2,5-dihydrofuran according to literature procedures with minor modifications.[9]

Compounds **1a**–**o** were tested as racemates for potency and selectivity in an ER competitive binding assay[10] with tritiated 17-β-estradiol. Select compounds were also assessed in a cellular transactivation assay utilizing HEK 293 cells stably co-transfected with either human ERα or ERβ and the alkaline phosphatase reporter.[11] A few representatives were further evaluated in vivo using

Table 2. Binding affinities[10] and in vivo data

Compd	Human ERα IC_{50} [nM]	Human ERβ IC_{50} [nM]	Selectivity $IC_{50}[\beta]/IC_{50}[\alpha]$	HEK 293 cells[11] IC_{50} [nM] ERα/ERβ	Uterine assay[12] (@ 1 mpk, sc[a]) % inhibition/% control
a	3.1 ± 1.4	143 ± 71	46	9.6/52	77/5
b	7.4 ± 2.1	60 ± 12	8	61/416	2/−1
c	2.7	308	114	14/1186	58/32[c]
d	490 ± 9.5	8202 ± 1939	17	ND[b]	ND
e	542 ± 62	8254	15	ND	ND
f	8.8 ± 1.6	162 ± 47	18	ND	27/ND
g	35	361	10	210/ND	ND
h	7.0	105	15	127/3316	ND
i	11	43	4	137/1375	ND
j	3.0	38	13	76/322	5/−2
k	2.5 ± 0.7	31 ± 6.1	12	22/252	ND
l	2.6	16	6	ND	21/27
m	7.5	28	4	21/840	ND
n	3.0	34	11	73/1044	30/27
o	3.9	40	10	67/1213	78/26
Raloxifene	1.8	12	7	4/60	96/8[d]

[a] sc = subcutaneous.
[b] ND = not determined.
[c] po @ 6 mpk.
[d] @ 0.6 mpk.

an immature rat uterine assay to assess estrogen antagonism and agonism activities.[12] The results are shown in Table 2.

In general when R = alkyl or cycloalkyl (**1h–o**), we found that it was relatively easy to obtain compounds that retained affinity for the alpha-receptor. However, most of the modifications increased the binding affinity to ERβ and resulted in a significant loss in the antagonism of estradiol-induced uterine proliferation, as evaluated in the uterine weight model. Increasing the size of the cycloalkyl substituent from cyclopentyl (**1k**) to cycloheptyl (**1m**) led to a corresponding threefold decrease in alpha-selectivity. The same general trend was observed when the isopropyl group (**1h**) was replaced by the larger *tert*-butyl group (**1i**). The accompanying loss of in vivo antagonism was profound as demonstrated by the 72% and 56% decrease for **1j** and **1l**, respectively, as compared with **1a**. Interestingly, this trend did not completely hold true for the extended cycloalkyl series (**1n–o**). In the case of **1n**, both antagonism and alpha-selectivity were significantly reduced relative to **1a**, and its profile was equivalent to its directly attached analog **1k**. However, in the case of **1o**, removing the cyclohexyl ring from C-3 by one carbon translated into a restoration of in vivo antagonism to the same level as **1a** although the transactivation assay would have suggested otherwise (HEK ERα = 67 nM). Selectivity for the alpha-receptor, however, remained compromised.

Replacing the 4-hydroxyphenyl group with other heteroaromatic moieties also proved detrimental to the binding affinities. This was especially pronounced when R = pyridine, as in **1d** and **1e**. Substitution with a basic group transformed a 3.1 nM potent compound into a 500 nM compound. Other substitutions such as thiophene and furan (**1f** and **1g**), were also inferior to the 4-hydroxyphenyl group in every respect.

Extending the 4-hydroxyphenyl group by one carbon to give **1c** resulted in a comparable binding affinity but significantly reduced inhibition of estradiol in the uterine weight assay. Removing the pendant hydroxyl group altogether as in **1b**, reduced selectivity for ERα to eightfold and obliterated all in vivo efficacy.

In summary, the SAR herein, suggests that the 4-hydroxyphenyl group at C-3 of the dihydrobenzoxathiin **1a** is superior to alkyl, cycloalkyl, and heterocyclic replacements with respect to alpha-selectivity and in vivo efficacy, and may represent a nearly optimal C-3 substituent. Further studies at fine-tuning the dihydrobenzoxathiin system will be reported in the near future.

Acknowledgements

We gratefully acknowledge Professor Barry M. Trost and Professor David Evans for their helpful discussions during their scientific consultations.

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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 with a 3 h incubation time. Most compounds are single point determinants unless otherwise indicated by standard deviation where $n = 2–5$.
11. Human embryonic kidney 293 (HEK293) cells were stably transfected with pΔERE2-alkaline phosphatase (ALP) reporter gene and either hERα or hERβ. Cells were seeded into 96 well culture plates (25,000 cells/well) in 100 μL of phenol red-free Coon's/F12-medium supplemented with 10% FBS (charcoal stripped) and 2 mM L-glutamine. The next day, the medium was replaced with Coon's/F12 supplemented with 1% FBS (charcoal stripped), 2 mM L-glutamine, 50 mg/mL gentamicin and different concentrations of test compounds with or without 17-β-estradiol (0.5 nM). After 72 h, an aliquot of medium was removed for determination of ALP activity secreted into the medium. The secreted reporter gene ALP was heat stable, and therefore activity due specifically to transactivation of the reporter gene was determined after heat inactivation of endogenous ALP activity (30 min, incubation at 65 °C). Heat inactivated medium (10 μL) was mixed with 200 μL assay buffer (10 mM diethanolamine; pH 10.0, 1 mM $MgCl_2$, 0.5 mM CSPD [disodium 3-(4-methoxyspirol{1,2-dioxetane-3,2′-(5′-chloro)tricyclo[3.3.1.13,7]decan}-4-yl)phenyl phosphate] in white 96 well plates and product formation was measured using a microplate luminometer. An integral measurement with 1-s reading of each well was used. ALP activity was expressed in light units (LU), which was directly proportional to the level of ALP expression from the cells.
12. Twenty-day old intact Sprague–Dawley rats were treated (sc) with test compounds for 3 days at 1 mpk. The antiestrogenic activity of compounds was determined by co-administration of the compound with a subcutaneous injection of 17-β-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.