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Solid-Phase Synthesis and Investigation of Benzofurans as Selective Estrogen Receptor Modulators

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Abstract—A library of benzofurans was prepared by solid-phase synthesis methods, and several analogues were identified as potent ligands for the estrogen receptors ER-α and ER-β, with some compounds having selectivity for ER-α. Analogues designed to more closely mimic Raloxifene were less effective. Certain benzofurans were effective in a bone pit assay, but were characterized as agonists in a MCF-7 breast tumor cell proliferation assay.
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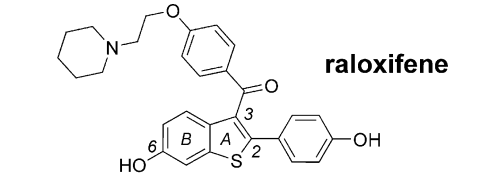
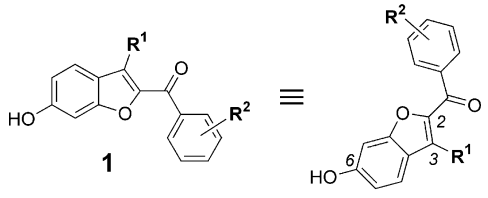
Selective estrogen receptor modulators (SERMs) such as raloxifene (LY139481, LY156758) have emerged as potential therapeutics for the prevention and treatment of osteoporosis.[1] These compounds ideally antagonize the effects of estrogen in uterine and breast tissue, but serve as estrogen mimics in bone tissue. Very recently, the role of coregulator recruitment in the tissue selectivity of certain SERMs has been described.[2] Several aromatic heterocyclic scaffolds have been shown to display activity as SERMs, including benzothiophenes (e.g., raloxifene)[3] and benzopyrans.[4] The hydroxyl group on the B ring of the heterocycle core appears to be essential for activity, and provides a hydrogen-bonding interaction to both the alpha and beta receptor subtypes, based on X-ray crystal structures of raloxifene bound to estrogen receptor-α and-β (ER-α and-β).[5] Interestingly, the piperidinylethoxy side chain of raloxifene appears to extend out of the binding pocket.

Raloxifene is more selective for ER-α than ER-β,[6] despite the fact that the ligand binding domains of the receptor isoforms are highly homologous and very similar in tertiary structure.[5] ER-β is found in equal or greater levels than ER-α in ovary, prostate, and osteoblast-like cells in cancellous bone of rats.[7] It is therefore of interest to identify ligands that are selective for either isoform, towards clarifying the role of these isoforms in the regulation of bone cell function.





A variety of benzofuran derivatives have been investigated by others as estrogen receptor ligands.[8] Computer modeling of benzofurans **1** indicated that this template core would fit the ligand binding site of ER-α[5b] in a 'flipped' orientation relative to that of raloxifene,[5] such that the 6-hydroxy, 2-aroyl and 3-substituent groups in **1** mimic the 6-hydroxy, 3-aroyl and 2-aryl groups, respectively, of raloxifene. 6-Hydroxy-benzofurans **1** were also of interest due to their synthetic accessibility

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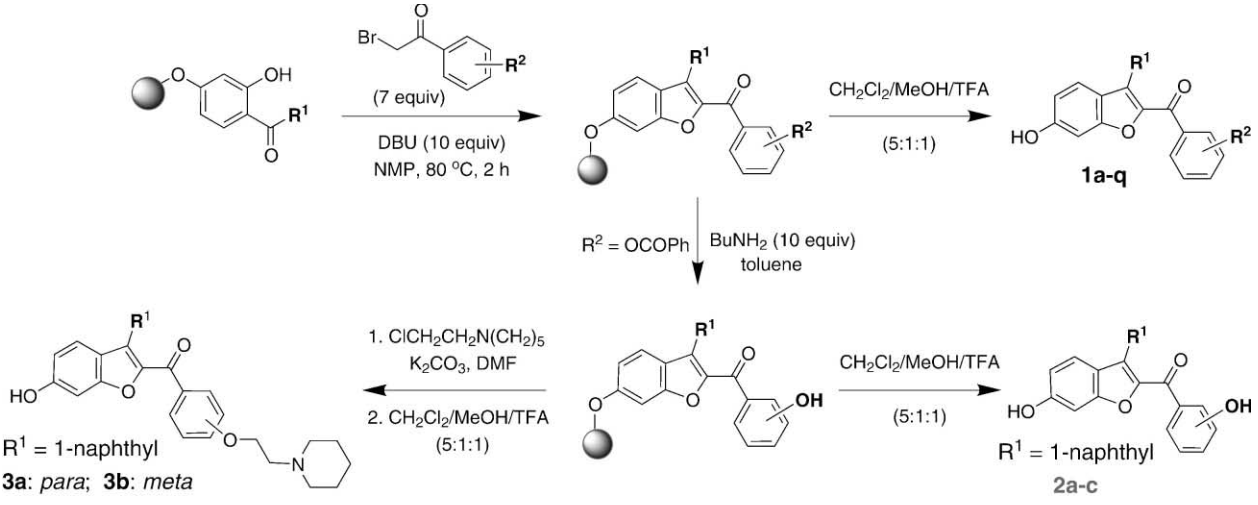
from a variety of commercially-available 2,4-dihydroxyphenyl ketones. We therefore prepared a library of 6-hydroxy-benzofurans **1** to investigate their potential as SERMs and as therapeutics for the treatment of osteoporosis.

The building blocks for this library synthesis were 2,4-dihydroxyphenyl ketones and α-bromoacetophenone derivatives. We exploited the 4-hydroxyl group of the phenyl ketone as our point of attachment to DHP (dihydropyran) resin,[9] and then alkylated the 2-hydroxy moiety with the bromoacetophenones. Modification of Boehm and Showalter's method[10] for the formation of benzofurans allowed the alkylation and cyclization to be accomplished in a single step, using DBU in *N*-methyl-pyrrolidinone. The products were readily cleaved from the resin by treatment with TFA in CH_2Cl_2/MeOH. Overall, eight variations of R^1 and 40 variations of R^2 were recruited to generate a library of 320 compounds (**1**). The average overall yield was 35%, and the desired product was identified by LC/MS as the major component in most cases.[11] Hydroxybenzoyl derivatives **2** were prepared via the corresponding benzoates (Scheme 1, $R^2 = OCOPh$). Selective cleavage of the benzoate ester was readily effected by an excess of butylamine in toluene.[12] In two cases (**3a** and **3b**, Scheme 1), the resultant phenol was alkylated with *N*-(2-chloroethyl)piperidine to incorporate the 2-(*N*-piperidinyl)ethoxy side chain on the 2-aroyl group, to mimic that found in raloxifene on the 3-aroyl substituent (vide supra).[3c]

After preliminary screening of the benzofuran library against both ER-α and ER-β, the most promising actives and related analogues of interest were resynthesized by the same solid-phase methodology, and purified by preparative reverse-phase HPLC. Binding affinities for these compounds are listed in Table 1.[13] The most striking observation is that analogues with the 1-naphthyl fragment at R^1 have generally good affinity for both receptors. Of particular note is **1h**, exhibiting IC_{50} values less than 100 nM for both ER-α and ER-β. Computer modeling[14] of **1h** confirms that the naphthyl group can be accommodated in the ER-α binding pocket that accepts the 2-(4-hydroxyphenyl) group of raloxifene. Other aromatics are accepted as R^1, although the affinity for ER-α decreases considerably as the contact surface is decreased. ER-β affinity is affected to a lesser extent, however. These trends are noted for cases where R^2 is hydrogen (**1a**–**b**), 2-Cl (**1h**–**j**), and 2,4-dimethyl (**1o**–**q**). As well, replacement of the aryl group at R^1 with an aliphatic group (**1k**) gives rise to a significant loss in potency. At the R^2 position, a phenyl ring is accepted with substitution at any ring position, and multiple substituents are also tolerated. A great variety of substituent sizes were found, ranging from halides to fused ring structures. Analogues **1** with quite bulky substituents (**1l**, **1m**, and **1n**) were the only examples to exhibit marked selectivity for ER-α in preference to ER-β, whereas the *para*-hydroxyphenyl compound **2a**[15] was the only compound found to have both nanomolar activity and some apparent selectivity for ER-β. Modeling studies[14] suggest that **2a** may bind to the receptor in a similar orientation as **1b** (or **1h**), with the *para*-OH of **2a** exposed to solvent; the similar binding affinities for **1b** and **2a** are consistent with this model. Not surprisingly, replacement of the R^2-phenyl fragment with a 1-adamantyl group gave a substantial loss in activity (R^1 = 1-naphthyl; ER-α and ER-β $IC_{50} > 10{,}000$ nM).

Analogues **3a** and **3b**, which incorporate the 2-(*N*-piperidinyl)ethoxy side chain found in raloxifene, were evaluated by molecular modeling,[14] and considered to be promising ligands for the estrogen receptor (Fig. 1). From these modeling studies, the 2-(*N*-piperidinyl)ethoxy side chain was expected to be acceptable as an R^2-substituent in either the *para* or *meta* orientation. Unfortunately, both **3a** and **3b** were found to be only weak micromolar ligands (Table 1). In the absence of further analoging and/or X-ray crystal structures, it is difficult to interpret these findings.

Selected compounds were screened for activity in two functional assays: a bone pit assay and a whole cell MCF-7 assay (Table 2). In the bone pit assay,[16] which



Scheme 1. Preparation of benzofurans on solid support.

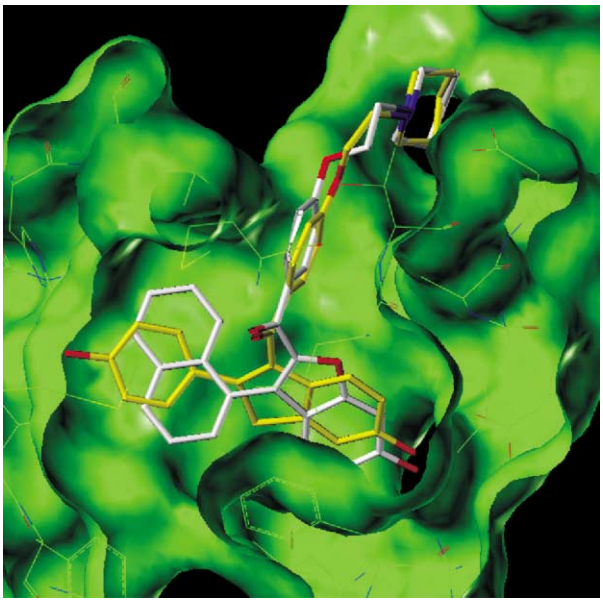


Figure 1. Modeling of benzofuran **3a** (white) in the binding site of a homology model of ER-β, and overlay with raloxifene (yellow; orientation as bound to ER-α).[14]

serves as a model for bone resorption,[17] certain benzofuran analogues were found to be quite active. In particular, **1g** was observed to be ca. 10-fold more effective than raloxifene. However, for the five benzofurans studied, there is no correlation between the bone pit data and the binding affinities for either ER-α or ER-β.

Table 2. Activity in the bone pit assay and MCF-7 cell assay

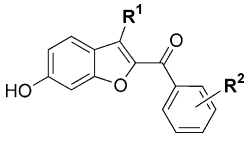
Compd	Bone pit assay IC_{50} (nM)	pS2/MCF-7 assay	
		% Agonist (10 nM)[a]	% Antagonist (10 μM)
1b	760	230	49
1e	350	140	48
1f	780	220	40
1g	210	190	40
1h	530	110	45
2a	n.d.	250	nd
Raloxifene	2000	−33	94[b]

[a]pS2 expression in MCF-7; % stimulation over background; for comparison, 10 nM 17β-estradiol (E2) gives ca. 600%.
[b]Antagonist assay with raloxifene at 10 nM. nd: not determined.

Finally, these benzofurans were evaluated in a breast tumor cell proliferation assay, based on pS2 gene expression in MCF-7 cells (pS2 gene mRNA measured by a branched DNA (bDNA) assay).[18] The pS2 protein is known to be expressed in approximately 50% of breast cancer cell lines, and its expression is driven by the estrogen receptor.[19] Unlike raloxifene, which behaves as a full antagonist of 17-β-estradiol, all benzofuran compounds tested, other than very weak ligands such as **3a** and **3b**, were found to have significant agonistic activity in MCF-7 cells (Table 2).

In summary, through screening of a library of benzofurans prepared by solid-phase synthesis, several compounds were discovered as high affinity ligands for the

Table 1. Binding affinities of benzofurans and other ligands for ER-α and ER-β



Compd	R^1	R^2	IC_{50} (nM)		α-Selectivity $IC_{50}(\beta)/IC_{50}(\alpha)$
			ER-α	ER-β	
1a	Ph	H	1400	2100	1.6
1b	1-Npth	H	150	130	0.87
1c	1-Npth	4-Br	380	1200	3.1
1d	1-Npth	4-$OCHF_2$	300	2300	7.7
1e	1-Npth	4-F	360	300	0.84
1f	1-Npth	3-Br	250	2200	8.8
1g	1-Npth	3-F	200	340	1.7
1h	1-Npth	2-Cl	30	63	2.1
1i	Ph	2-Cl	390	550	1.4
1j	2-MeO-Bn	2-Cl	380	1100	2.9
1k	1-Pentyl	2-Cl	2800	2700	0.97
1l	1-Npth	3,4-$[O(CH_2)_2O]$	140	22,000	160
1m	1-Npth	3,4-$[O(CH_2)_3O]$	190	7700	40
1n	1-Npth	4-Ph	99	8800	88
1o	1-Npth	2,4-Me_2	75	260	3.4
1p	Ph	2,4-Me_2	3400	3100	0.91
1q	2-MeO-Bn	2,4-Me_2	380	830	2.2
2a	1-Npth	4-OH	180	79	0.43
3a	1-Npth	4-$O(CH_2)_2N(CH_2)_5$	4800	64,000	13
3b	1-Npth	3-$O(CH_2)_2N(CH_2)_5$	7300	~100,000	14
Raloxifene			7[a]	470[a]	67
17β-Estradiol			2	2	1

[a]Reported affinities for reporter cell line 293/hER-α and 293/hER-β are IC_{50} = 4 and 58 nM, respectively.[6]

estrogen receptors ER-α and ER-β. Analogues designed to more closely mimic raloxifene (**3a**–**b**) were also prepared, but were found to be relatively ineffective. In a bone pit assay, certain analogues were observed to be significantly more potent than raloxifene. However, these analogues were also characterized as full agonists in a MCF-7 breast tumor cell proliferation assay. As a result, this compound series was not pursued further.

Acknowledgements

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13. (a) The data in Table 1 are the average of two determinations for purified and characterized (^{1}H NMR, LC-MS) materials. Most standard errors are $\leq$10%, and the following are $>$30%: ER-α std errors for **1a** (36%), **1d** (40%), **1h** (58%), **1i** (52%), and the ER-β std error for **1l** (71%). ER-α and ER-β (PanVera Corp.) were assayed using an antibody binding scintillation proximity assay (antimouse SPA).[13b] Tritiated estradiol (Amersham Pharmacia Biotech) was incubated (3 h) in the presence of receptor (0.2–0.3 μg/mL), anti-ER antibody, and SPA beads (2.5 mg/mL) in a standard HEPES buffer (pH 7.4) prior to counting in a Wallac Microbeta scintillation counter (Perkin–Elmer). (b) Udenfriend, S.; Gerber, L.; Nelson, N. *Anal. Biochem.* **1987**, *161*, 494.
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15. The *ortho* (**2b**) and *meta* (**2c**) hydroxy analogues of **2a** were prepared in a similar manner, but were found to have considerably weaker affinity for both ER-α and-β, as compared to **2a**. Thus, these compounds were not pursued further.
16. Rabbit bone cells containing osteoclasts were plated onto bovine cortical bone chips in 96-well plates containing test compounds, in alpha-MEM media plus 10% FBS. After 48 h, the chips were washed (0.1 M cacodylate buffer, 0.25 M NH_4OH, water and acetone), and stained (1% toluidine blue in 1% borax). Highlighted resorption pits were counted under a light microscope.
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18. MCF-7 cells were obtained from the ATCC and grown in Growth Medium [GM: MEM (GIBCO) with 10% HyClone heat inactivated fetal bovine serum (FCS), 1 mM sodium pyruvate (GIBCO), 0.1 mM non-essential amino acid solution (GIBCO), 2 mM L-Glutamine (GIBCO), 100 U/mL penicillin G sodium, 100 μg/mL streptomycin sulfate and 0.25 μg/mL amphotericin B (GIBCO)]. The cells were grown to 80% confluency, trypsinized and washed to remove phenol red, then plated overnight at 10,000 cells/well in Starve Medium [SM: MEM (without Phenol Red, GIBCO) with 5% HyClone charcoal/Dextran Treated Fetal bovine serum (the remainder of the medium is identical to GM)]. Cells were treated with either fresh SM or SM plus test compound (200 μL total volume). The next day the bDNA assay[20] was performed according to manufacturer's protocol from the QuantiGene bDNA kit (Bayer Diagnostics, Emeryville, CA).
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