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Furans with Basic Side Chains: Synthesis and Biological Evaluation of a Novel Series of Antagonists with Selectivity for the Estrogen Receptor Alpha

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Abstract—3-Alkyl-2,4,5-triarylfurans with basic side-chain substituents were prepared as ligands for the estrogen receptor. Those analogues having the basic side chain on the C(4) phenol were high-affinity, ERα-selective antagonists. © 2001 Published by Elsevier Science Ltd.

We have recently described the synthesis and biological evaluation of a series of 3-alkyl-2,4,5-triaryl-substituted furans, several of which proved to be ligands for the estrogen receptor (ER) with very high selectivity for ERα over ERβ, both in terms of binding affinity and potency of transcriptional activation.[1] Because there is great interest in the development of *antagonists* as well as *agonists* that are ER subtype-selective,[2,3] we have investigated a way to convert these ERα-selective furan agonists into ERα-selective antagonists.

The antagonist character of the antiestrogens raloxifene and hydroxy-tamoxifen (now more properly designated 'selective estrogen receptor modifiers' or SERMs; cf. Fig. 2)[4] relies on a tertiary amino ethoxy substituent, termed a basic side chain (BSC). When these ligands are bound to ERα, this bulky BSC projects outward from the ligand binding pocket and effects a structural reorganization of the receptor surface, repositioning helix 12 in a manner that blocks the binding of coactivator proteins important in mediating transcriptional activity.[5,6] Thus, we surmised that we might be able to impart antiestrogenic character to the furan series of agonist ligands, by appending a BSC through one of the three phenols of our furan ligands (Fig. 1). It is not immediately evident which attachment site would be preferred, nor that the product BSC furans would retain the ERα selectivity of the parent furans, because neither raloxifene nor tamoxifen demonstrates pronounced ER subtype selective antagonism.[7] We were able to adapt the synthetic route originally used to prepare the tetra-substituted furan systems for the preparation of the BSC analogues,[1] by differential protection of the phenol

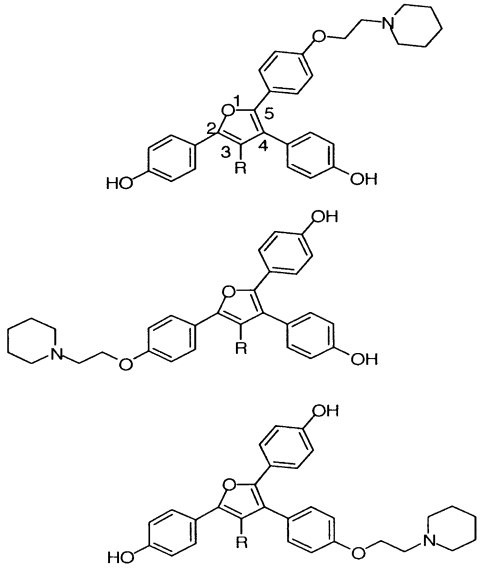

Figure 1. Three potential antiestrogenic furan basic side-chain (BSC) derivatives.

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functions in the starting materials. We recently used a similar approach to attach BSC groups to pyrazoles.[2] Here, we report the results of our efforts to identify the preferred furan position for attachment of the BSC, to obtain ER antagonists that have some selectivity for ERα over ERβ, and to elucidate the orientation with which these furans bind to the receptor.

Treatment of the differentially protected 1,2-diarylethanones (**1**–**2**), ketone (**3**), or desoxyanisoin (**4**) with potassium hexamethyl-disilyl-amide (KHMDS), followed by the addition of an α-bromoketone (**5a**–**e**), provided the desired, differentially protected 1,3,4-triaryl-2-alkyl-butane-1,4-diones (**6a**–**g**) in 66–98% yields (Scheme 1). Although these diones were mixtures of diastereomers, no separation was required, because these stereocenters become trigonal in the furan products.

Diones **6a**–**f** were refluxed in toluene with a catalytic amount of *p*-toluenesulfonic acid (TsOH) to effect furan formation and desilylation, giving mono-deprotected furans **7a**–**f**. The piperidinyl-ethoxy side chain was added using Mitsunobu chemistry,[1] and the intermediates **8a**–**f** were demethylated using $AlBr_3$/EtSH to afford BSC furans **9a**–**f**.[4]

In contrast to the behavior of diones **6a**–**f**, when refluxed with a catalytic amount of TsOH in toluene, dione **6g** cyclized to the furan but did not undergo TIPS deprotection (Scheme 2). However, treatment of furan **10** with tetrabutylammonium fluoride at 0 °C did result in desilylation, giving the mono-deprotected furan **11** that was converted to BSC furan **13**, as described above.

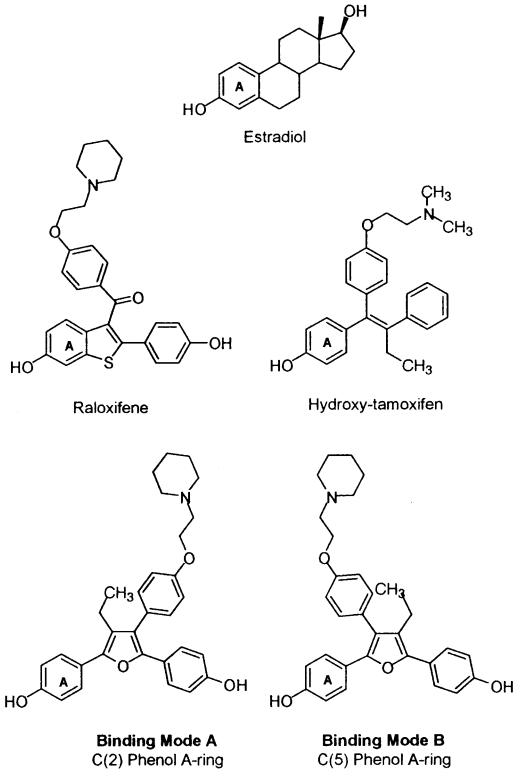
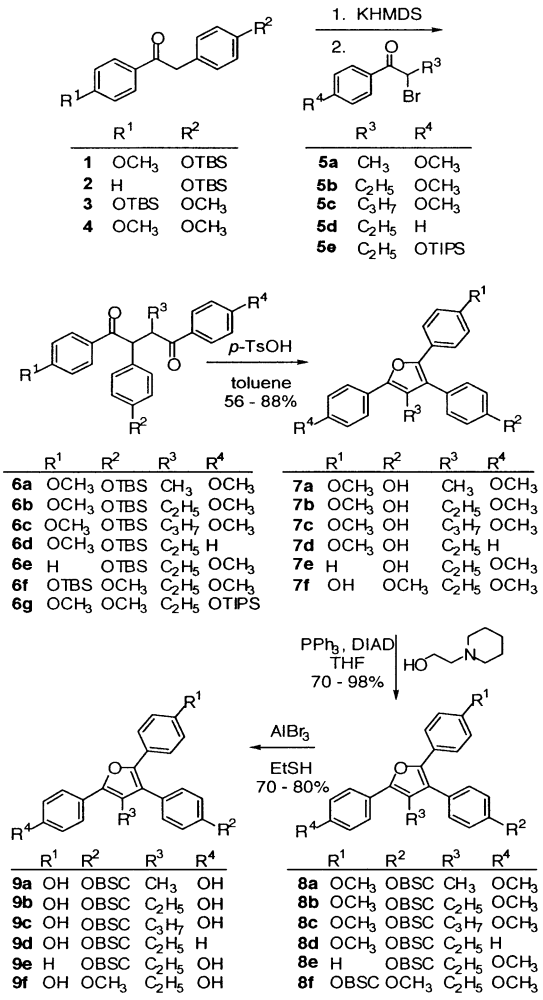


Figure 2. Two possible A-ring mimics of estradiol for furan **9b**.

The affinity of the BSC furans **9a**–**f** and **13** for the estrogen receptor was assayed by a competitive radiometric binding assay, using purified, full-length human ERα and ERβ from recombinant sources.[8,9] The results of these assays are expressed as relative binding affinity (RBA) values, where the affinity of estradiol is considered to be 100%, and they are summarized in Table 1.

By comparison of the ERα affinities for the isomeric series of 3-ethyl-substituted BSC furans **9b**, **9f**, and **13**, it appears that the preferred position for the attachment of the BSC is on the C(4) phenol (**9b**); much lower affinities were observed when it was attached to the C(2) or



	R^1	R^2
1	OCH_3	OTBS
2	H	OTBS
3	OTBS	OCH_3
4	OCH_3	OCH_3

	R^3	R^4
5a	CH_3	OCH_3
5b	C_2H_5	OCH_3
5c	C_3H_7	OCH_3
5d	C_2H_5	H
5e	C_2H_5	OTIPS

	R^1	R^2	R^3	R^4
6a	OCH_3	OTBS	CH_3	OCH_3
6b	OCH_3	OTBS	C_2H_5	OCH_3
6c	OCH_3	OTBS	C_3H_7	OCH_3
6d	OCH_3	OTBS	C_2H_5	H
6e	H	OTBS	C_2H_5	OCH_3
6f	OTBS	OCH_3	C_2H_5	OCH_3
6g	OCH_3	OCH_3	C_2H_5	OTIPS

	R^1	R^2	R^3	R^4
7a	OCH_3	OH	CH_3	OCH_3
7b	OCH_3	OH	C_2H_5	OCH_3
7c	OCH_3	OH	C_3H_7	OCH_3
7d	OCH_3	OH	C_2H_5	H
7e	H	OH	C_2H_5	OCH_3
7f	OH	OCH_3	C_2H_5	OCH_3

	R^1	R^2	R^3	R^4
9a	OH	OBSC	CH_3	OH
9b	OH	OBSC	C_2H_5	OH
9c	OH	OBSC	C_3H_7	OH
9d	OH	OBSC	C_2H_5	H
9e	H	OBSC	C_2H_5	OH
9f	OH	OCH_3	C_2H_5	OH

	R^1	R^2	R^3	R^4
8a	OCH_3	OBSC	CH_3	OCH_3
8b	OCH_3	OBSC	C_2H_5	OCH_3
8c	OCH_3	OBSC	C_3H_7	OCH_3
8d	OCH_3	OBSC	C_2H_5	H
8e	H	OBSC	C_2H_5	OCH_3
8f	OBSC	OCH_3	C_2H_5	OCH_3

Scheme 1. Synthesis of basic side-chain furans **9a**–**f** (BSC = CH_2CH_2–piperidine).

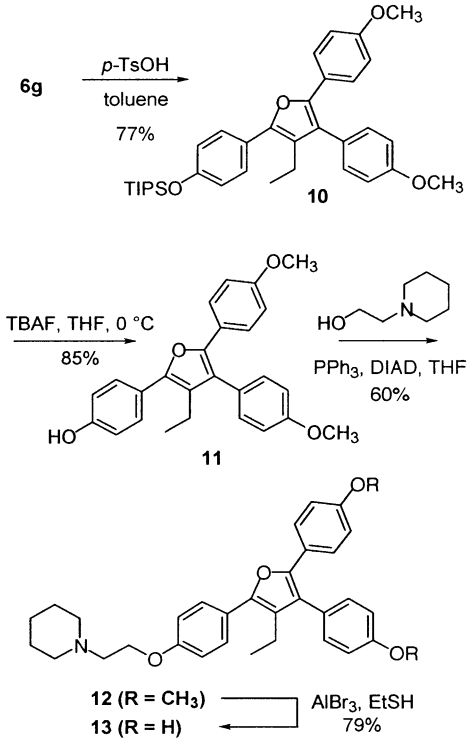
Table 1.

Ligand	ER-α	ER-β	α/β
9a	32±9	0.47±0.1	67.2-fold
9b	75±20	3.1±0.1	24.3-fold
9c	34±2	4.0±1.2	8.5-fold
9d	60±1	20±3.5	3.0-fold
9e	3.8±0.9	0.43±0.04	8.8-fold
9f	2.5±0.3	0.91±0.02	2.7-fold
13	0.36±0.11	0.06±0.02	6.1-fold

Determined by a competitive radiometric binding assay with [^{3}H]estradiol.

C(5) phenols (**13** and **9f**, respectively). The BSC-modified furans did retain much of the selective affinity for ERα shown by their parent furans (which for the triphenol series, with R^3 = Me, Et, and Pr, ranged from 48- to 65-fold),[1] the most selective BSC furan **9a** having a 67-fold ERα/ERβ binding selectivity. Even the analogues with low affinities for ERα maintained some of this selectivity.

The initial series of BSC furans we prepared have two free phenols, either of which could be serving as the analogues of the A-ring of estradiol, a group that functions as a crucial hydrogen-bonding partner in the ligand binding pocket and is essential for the high affinity of the natural in estrogen.[10] We sought to determine which of these phenols in the C(4)-BSC furans was playing this important role in binding by deleting each of them in turn, and then evaluating the effect that this had on their ERα binding affinity.[10]



Scheme 2. Synthesis of basic side-chain furan **13**.

The X-ray crystal structures for ERα complexed with either raloxifene[6] or 4-hydroxy-tamoxifen[5] show that the BSC on these ligands has a common orientation that places it roughly in the 11β direction with respect of a standard steroid core, where a salt bridge can then form between the tertiary amine and aspartate 351 in helix-3. Thus, as indicated in Figure 2, there are likely to be only two binding modes for a C(4)-BSC furan, such as **9b**, mode A, with the C(2) phenol in the A-ring binding pocket, and mode B, with C(5) phenol mimicking the A-ring.

Comparison of the ERα binding affinity of BSC furans **9d** and **9e**, mono-phenol analogues of the high affinity BSC furan diol **9b** (Table 1), clearly shows that the phenol attached at position C(5) serves as the A-ring mimic: there was a 20-fold loss of affinity when this phenol was deleted (**9e**), whereas deletion of the C(2) phenol caused less than a 1.3-fold drop (**9d**). Thus, we conclude that the highest affinity furan with the basic side chain, ligand **9b**, binds to ER in orientation B (Fig. 2), and that in this orientation, having a second phenolic hydroxyl on the C(2) phenyl ring does not seem to be very important for high affinity binding (**9b** vs **9d**); this second phenol does, however, play a significant role in the selective affinity of these BSC furans for ERα.

We selected the BSC furans that combined high affinity with good ERα binding selectivity (namely **9a**–**c**) to be assayed for their capacity to activate transcription through either ERα or ERβ, or to antagonize the transcriptional activity of estradiol through these ERs. These assays were done by cotransfection in human endometrial carcinoma (HEC-1) cells using a luciferase reporter gene system;[11] dose–response studies with the BSC furans alone were conducted to measure agonist activity, and in the presence of 1 nM estradiol to determine antagonist activity. The results are summarized in Figures 3 and 4.

As expected, none of the three BSC furans showed appreciable agonist activity on either ERα or ERβ. The

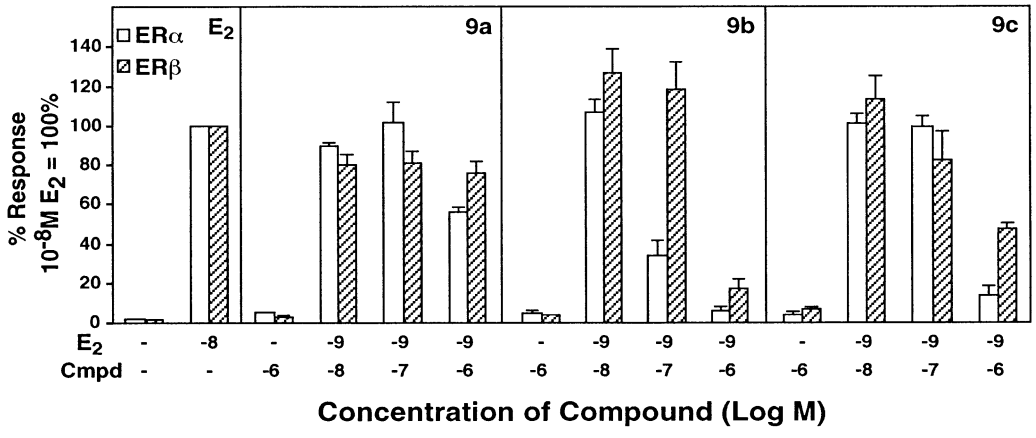


Figure 3. Transcriptional activity of the BSC furans **9a**–**c** and their antagonism of estradiol activity through ERα and ERβ.

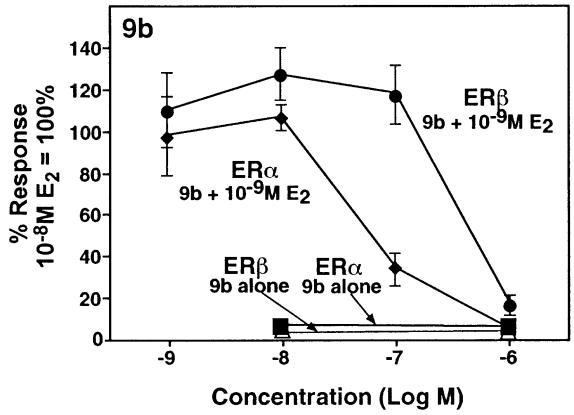


Figure 4. Full dose–response, showing the ERα-selective antagonism of BSC furan **9b**.

methyl analogue (**9a**) has low potency on both ERs, and shows essentially no selectivity; the propyl analogue (**9c**) was somewhat more potent and showed a small degree of selectivity in antagonizing ERα in preference to ERβ. The most potent and selective antagonist was the ethyl BSC furan, **9b**, and a more complete dose–response curve for this compound (Fig. 4) shows that, at 0.1 μM, it almost fully suppresses the transcriptional activity of estradiol through ERα, yet it has no effect on ERβ at this concentration. The IC_{50} values for **9b** on ERα and ERβ are approximately 6.5×10^{-8} and 4.8×10^{-7} M, respectively, which corresponds to a nearly 10-fold antagonist potency selectivity for ERα over ERβ.

We have shown that one can obtain an ERα selective antagonist by adding a BSC to the C(4) phenol of 3-ethyl-2,4,5-tris(4-hydroxylphenyl)furan, a ligand we have previously shown to be very selective agonist for ERα.[1] In the series that we investigated, the BSC furans maintained much of the ERα binding selectivity that was observed with the parent series of ligands.[1] In this regard, the BSC furans are similar to BSC derivatives[12] prepared from the ERα-selective pyrazole ligands.[13] However, the BSC pyrazoles retained a greater degree of ERα-subtype selectivity in terms of both affinity and antagonist potency than did the BSC furans.[12] Through binding affinity correlations, we concluded that these BSC furans bind to ERα with the C(5) phenol playing the role of the important A-ring of estradiol; we had reached a similar conclusion with the BSC pyrazoles.[3] It is of note in both the furan and pyrazole series that the parent ligands appear to bind in a different orientation than do the BSC derivatives, placing the C(2) phenol of the furan (and the corresponding phenol of the pyrazole) in the A-ring binding pocket. The BSC derivatives of ERα-selective ligands should prove to be useful agents to investigate the biological functions of the two ER subtypes.

Acknowledgements

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