Benzopyrans Are Selective Estrogen Receptor β Agonists with Novel Activity in Models of Benign Prostatic Hyperplasia

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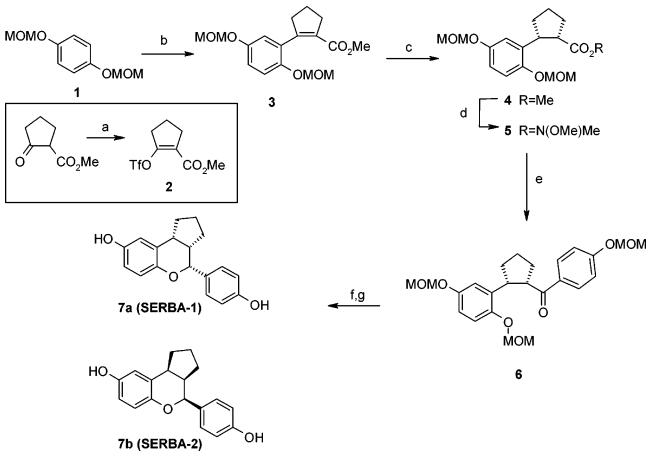
Abstract: Benzopyran selective estrogen receptor beta agonist-1 (SERBA-1) shows potent, selective binding and agonist function in estrogen receptor β (ERβ) in vitro assays. X-ray crystal structures of SERBA-1 in ERα and β help explain observed β-selectivity of this ligand. SERBA-1 in vivo demonstrates involution of the ventral prostate in CD-1 mice (ERβ effect), while having no effect on gonadal hormone levels (ERα effect) at 10× the efficacious dose, consistent with in vitro properties of this molecule.

Estrogen receptors (ERs[a]) are part of a superfamily of nuclear hormone receptors (NHRs) that behave as ligand-activated transcription factors and operate as part of a complex signaling network.[1] The importance of tissue distribution, coactivator/corepressor activity, and agonist/antagonist/modulator function of many NHRs has become an area of current scientific interest and study.[2] Furthermore, with the recent demonstration that selective ER modulators (SERMs) can have beneficial effects in important diseases such as osteoporosis and breast cancer,[2,3] the desire to better understand NHR ligands has increased.

Ligands which bind to the ER have been studied for many decades, and it was generally assumed that these ligands bound to a single ER (now called ERα). In 1996, a second ER subtype was reported (ERβ).[4] Thus, there has been much attention given toward improving our understanding of these receptors, their tissue distribution and biological functions.[5] While biological tools, such as ERα and β knockout animals, have provided some clarity to the physiological roles of both ERs,[6] we and others have focused on the pharmacological effects of subtype specific ligands.[7−9] Toward that end, we report on the characterization of the selective ERβ agonist, selective estrogen receptor beta agonist-1 (SERBA-1).

The benzopyran SERBA-1 was prepared as part of an SAR effort to identify potent and selective agonists of ERβ. The synthesis, which is outlined in Scheme 1, begins with a palladium-mediated Negishi cross coupling reaction between the bis-protected hydroquinone **1**[10] and the cyclopentyl vinyl triflate **2**[11] to give cyclopentene **3**. Hydrogenation of the olefin allowed us to set the critical *cis*-relative stereochemistry in the cyclopentane **4**. The methyl ester was converted to the Weinreb amide **5**, which was used to prepare the aryl ketone **6**, via reaction with the appropriate aryllithium reagent. Deprotection and cyclization of **6** was accomplished in one pot, via treatment with toluenesulfonic acid, followed by the addition of sodium cyanoborohydride under acidic conditions. Careful monitoring of pH (<4, bromocresol green) favored the all-*cis* stereochemistry, as shown. Chiral chromatography (Chiralpak AD, 80% heptane-2-propanol) separated the enantiomers to allow for the isolation of SERBA-1 and SERBA-2, with absolute stereochemistries as drawn.[12]

Scheme 1[a]



[a] Reaction conditions: (a) diisopropylethylamine, Tf_2O (63%); (b) *i.* *t*-BuLi, $ZnCl_2$; *ii.* **2**, $Pd(PPh_3)_4$ (55%); (c) H_2, 60 psi Pd/C (75%); (d) Me(OMe)NMgCl (90%); (e) 4-methoxymethoxyphenyllithium (97%); (f) *i.* TsOH, MeOH; *ii.* $NaBH_3CN$, HCl (45%); (g) chiral chromatography.

Table 1. In Vitro Properties of SERBA-1 and SERBA-2

	SERBA-1	SERBA-2
binding K_i ERα (nM)	2.68 (±0.21)	14.5 (±6.4)
binding K_i ERβ (nM)	0.19 (±0.01)	1.54 (±0.45)
binding selectivity	14-fold	9-fold
PC3/ERα-ERE EC_{50} (nM)	19.4 (±1.13)	32.5 (±18.2)
% rel. efficacy (α agonist)	94 (±1.7)	85 (±14)
PC3/ERβ-ERE EC_{50} (nM)	0.66 (±0.04)	3.61 (±1.51)
% rel. efficacy (β agonist)	101 (±1.3)	100 (±10)
FS	32-fold[a]	11-fold[a]

[a] Functional selectivity (FS) calculation was weighted using relative efficacy (RE), that is, FS = (α EC_{50})·(β RE)/(β EC_{50})·(α RE).

The in vitro properties of SERBA-1 and SERBA-2 are shown in Table 1. The binding K_is were generated using ^{3}H-estradiol and recombinant, full-length, human ERs in a competitive binding assay. The more potent enantiomer, SERBA-1, showed potent binding affinity for both ERs, but was 14-fold selective for the β isoform. The functional activity and selectivity was measured using a transcription assay in the cotransfected human prostate cancer PC3/ER (α or β)-ERE cell line. SERBA-1 was potent (EC_{50} = 0.66 nM) and selective (32-fold) toward ERβ and showed full agonist function in both ERα and ERβ assays (>90% relative efficacy). We felt that, based on the in vitro properties of SERBA-1, this molecule may have unique properties in rodent models for BPH.

Recent studies by Katzenellenbogen et al.,[13] Wyeth,[14] and Schering[15] have shown the utility of X-ray crystallography, molecular modeling, and structure-based drug design (SBDD) in the discovery of ER subtype selective ligands. Although the ligand binding pockets of these two proteins are very similar

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[a] Abbreviations: ER, estrogen receptor; BPH, benign prostatic hyperplasia; NHR, nuclear hormone receptor; SERM, selective estrogen receptor modulator; SERBA, selective estrogen receptor beta agonist; SBDD, structure based drug design; SV, seminal vesicle; T, testosterone; DHT, dihydrotestosterone; DES, diethylstilbestrol.

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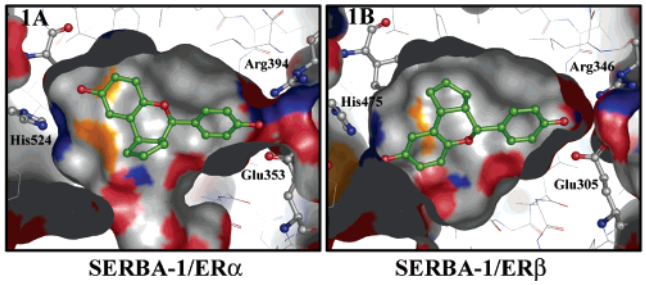

Figure 1. Surface diagram of the X-ray structure of SERBA-1 complexed to ERα (1A) and ERβ (1B).

(only two residue changes), significant binding selectivities have been obtained using SBDD tools. In general, the approach has followed a path of utilizing the conservative residue differences between ERα and ERβ (M421(α) → I373(β) and L384(α) → M336(β)) by targeting molecular design features that can specifically exploit these changes. Inherent in this approach is the fact that most molecules bind similarly in the ERα and ERβ pockets.

We have recently solved SERBA-1/ERα and ERβ X-ray cocrystal structures (Figure 1) and report significant differences in the manner in which these molecules bind within the binding pockets. The phenols in SERBA-1 bind in a predictable manner in both structures. That is, one phenol (D ring phenol) is bound within the glutamic acid-arginine network and the A ring phenol is bound to the appropriate histidine. However, SERBA-1 displays a different orientation in these structures, which corresponds to a (ca. 180°) rotation on its bisphenol axis. Additionally, the A ring phenol, while bound to histidine in both structures, locates to different sides of the imidazole functionality for this interaction. This finding is unique and helps explain the observed selectivity of SERBA-1. It also provides a unique opportunity for SBDD that is not wholly tied to exploiting the differences between two conservative residue changes. We intend to report on our SBDD approach to improved selectivities in due course.

We have recently developed a mouse model for the purpose of evaluating ERβ agonist effects on mouse prostate while, at the same time, measuring ERα-driven risks. It is known that nonselective ER agonists can have indirect effects on prostate involution by lowering serum gonadal hormone levels[16,17] via an ERα pathway. These α effects are also displayed in regression of the testes and seminal vesicle (SV). We felt that a potent SERBA would demonstrate involution of the prostate (β effect) at doses that showed no α-related effects on testes and SV. Additionally, we felt that there should be no effect on circulating levels of the androgens testosterone (T) and dihydrotestosterone (DHT) at these doses. Our mouse prostate model evaluates each of these endpoints after 7 days of oral dosing, using intact vehicle, castrate, and finasteride controls.

The mouse efficacy data for SERBA-1 are shown in Figure 2. It should be noted that this molecule produces the desired effect on prostate weight in a dose−response manner, while having no effect on testes and SV weights in this dose range. Additionally, SERBA-1 had no effect on T and DHT levels, at up to 10× the minimum efficacy dose (0.1 mg/kg). It should be pointed out that the nonselective ER agonist diethylstilbestrol (DES), showed significant regression of prostate, testes, and SV, while also lowering T and DHT (data not shown).[18] There were no doses where the DES effects were displayed only on prostate tissue. Finally, the BPH drug, finasteride, a 5α-reductase inhibitor, is known to exert its prostate regression effects by inhibiting the conversion of T to DHT.[19] Finasteride demonstrated prostate involution in our mouse model, albeit at a significantly higher dose (30 mg/kg).

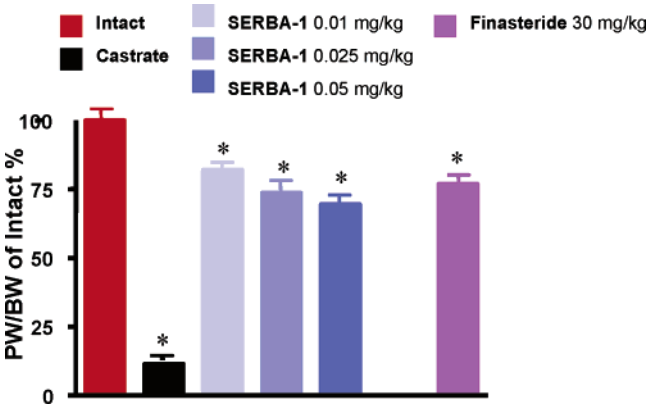

Figure 2. Effect of SERBA-1 on the prostate wet weight in CD-1 mice, measured after 7 days of oral daily doses. Prostate weights (PW) were normalized to body weight (BW) and the quotient PW/BW (as a percentage of intact control) is reported. Castrate and finasteride controls are also shown. *Statistically significant ($P < 0.05$).

In conclusion, we have shown that a potent and selective ERβ agonist, such as SERBA-1, has unique properties in in vitro studies and in rodent prostate models. We feel that these data suggest an opportunity for this class of compound to show efficacy in human BPH. Additionally, we have demonstrated the value of SBDD in this benzopyran series and are optimistic that these tools will allow for significant selectivity improvements, which will be disclosed in subsequent papers.

Acknowledgment. We thank Gregory Stephenson for assistance in the assignments of the absolute stereochemistry of the enantiomers **7a** and **7b**.

Supporting Information Available: Atomic coordinates for ERα and ERβ complexes with SERBA-1 have been deposited in the PDB, with accession codes 2I0J and 2I0G, respectively. Experimental details of the synthesis and characterization of compounds **3**−**7**, in vitro and in vivo assays, and X-ray crystallographic procedures and statistics. This material is available free of charge via the Internet at http://pubs.acs.org.

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