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Benzopyrans as selective estrogen receptor β agonists (SERBAs). Part 4: Functionalization of the benzopyran A-ring

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Abstract—Benzopyrans are selective estrogen receptor (ER) β agonists (SERBAs), which bind the ER receptor subtypes α and β in opposite orientations. We have used structure based drug design to show that this unique phenomena can be exploited via substitution at the 8-position of the benzopyran A-ring to disrupt binding to ERα, thus improving ERβ subtype selectivity. X-ray cocrystal structures with ERα and ERβ are supportive of this approach to improve selectivity in this structural class.

Estrogen receptors (ERs, subtypes α and β) are nuclear hormone receptors with diverse tissue distribution and biological function.[1] The understanding of ER ligands, agonists and antagonists, is a fairly mature science, dating back over the past several decades.[2] More recently, the development of selective estrogen receptor modulators (SERMs) have impacted several disease targets, primarily in women's health.[3] From a historical perspective, most of the ER pharmacology to date has focused on ERα. However, since its discovery in 1996,[4] much effort has gone into understanding the relative importance of ERβ.[5,6] One important ERβ responsive tissue is the prostate.[7] We have recently described novel benzopyrans which are selective ERβ agonists (SERBAs) displaying striking effects on rodent prostates in models of benign prostatic hyperplasia (BPH).[8] As part of this program, we have tried to improve ERβ selectivity through the use of structure based drug design.[9] In this paper, we describe our approaches to selectivity improvements in the benzopyran A-ring.[10]

In recent years, we and others have utilized structure based drug design (SBDD) tools to assist our understanding of ER-ligand binding, with the hopes of improving subtype specificity.[11,12] Specifically, protein crystallography has been instrumental in providing information regarding the binding modes of ligands within the ligand binding pockets (LBP) of ERα and ERβ. As we have already shown, benzopyrans, such as **1**, are unique, in that they bind to ERα and ERβ with different orientations (a 180° rotation around the bisphenol axis). This is illustrated in Figure 1, which shows an overlay of the X-ray cocrystal structures of **1**/ERα (blue) and **1**/ERβ (green). The residues lining the LBP are also shown in the overlay, with phenol-binding residues and α versus β residue changes highlighted. It should be noted that the D-ring phenol binds within the Arg–Glu network in both ERα (Arg394–Glu353) and ERβ (Arg346–Glu305). Similarly, the A-ring phenol forms a hydrogen bond to the imidazole ring in His525 in ERα (His475 in ERβ). We and others have shown that these interactions are critical to the overall binding affinity of ER ligands. Structure modifications which compromise either interaction are expected to significantly diminish ER binding affinity.

The 'ligand rotation' phenomenon noted in Figure 1 provides a unique opportunity to improve ERβ specificity, which does not focus completely on exploiting the conservative residue changes within the LBP (M421(α) → I373(β); L384(α) → M336(β)). Since the benzopyran scaffold already possesses very high affinity

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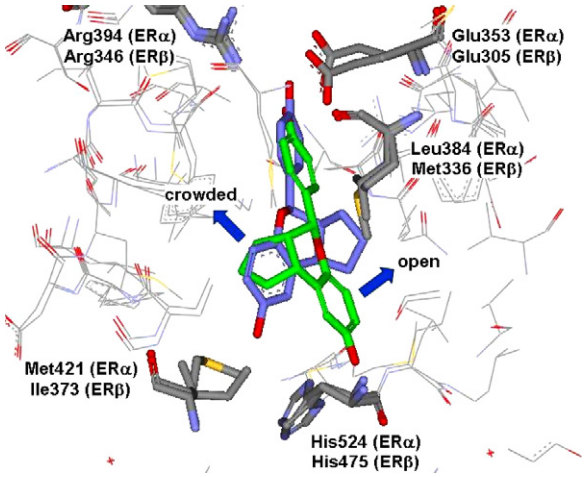
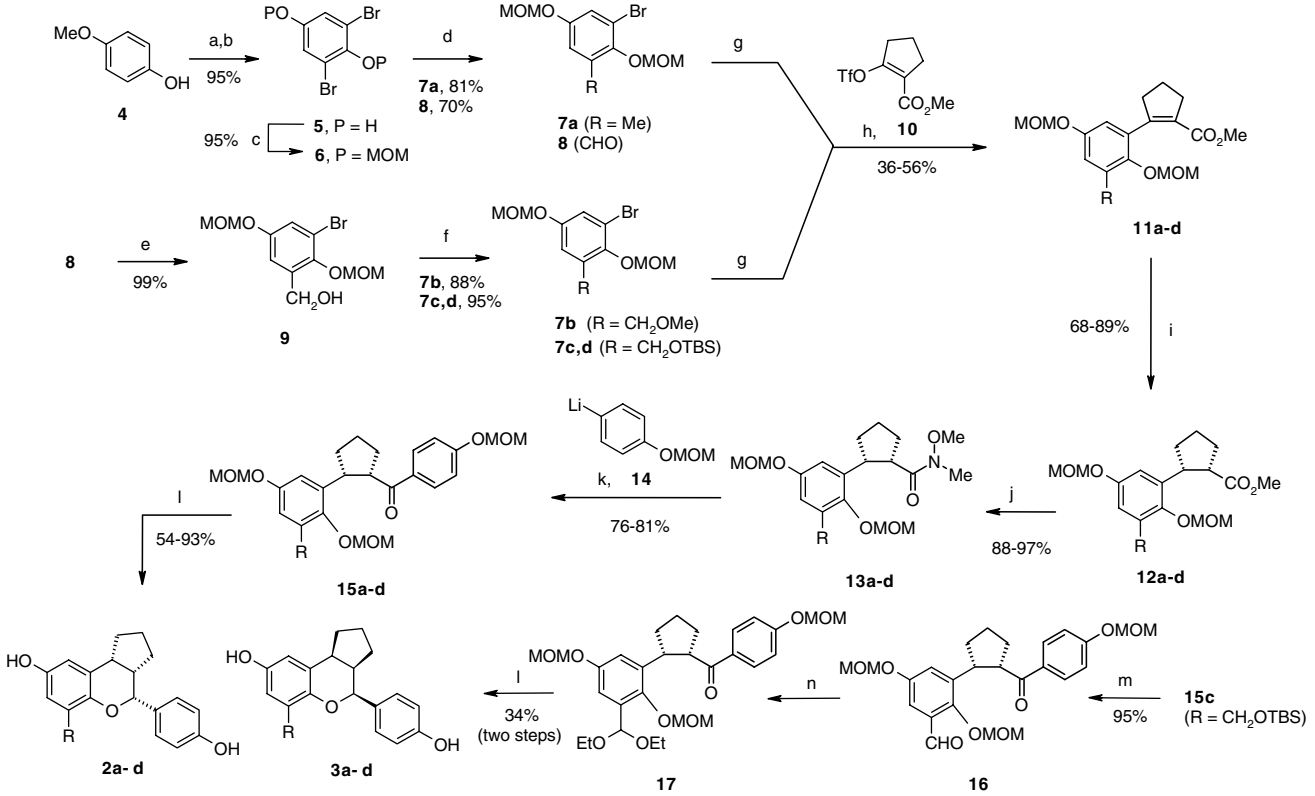


Figure 1. Overlay of 1 with ERα and ERβ.

for ERβ, the task of improving ERβ selectivity becomes an exercise in selectively disrupting ERα binding. Figure 1 shows that substitution at the 8 position of the benzopyran should have minimal effect on ERβ binding (note the 'open' area), as compared to the 8 position of the benzopyran in the ERα structure (crowded). When we prepared the 8-methyl substituted analog of **1**, we observed no effect on binding affinity to either receptor subtype. Therefore, steric interactions alone are insufficient and it is always possible that the binding modes are altered when substitution at this position is made. Here we describe the effect of substitutions at the 8 position that do disrupt ERα binding to a greater extent than ERβ binding, thereby improving ERβ selectivity.

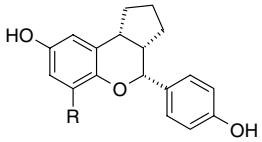
The synthesis of the 8-methyl substituted analogs took advantage of the reductive cyclization route reported earlier and is described in Scheme 1.[8,13] 4-Methoxy phenol was treated with benzyltrimethylammonium tribromide to provide the dibromide. Demethylation with trimethylsilyl iodide gave hydroquinone **5**, which was protected as the bis-methoxymethyl ether **6** using sodium hydride and chloromethyl methyl ether. Ortho mono-lithiation of protected hydroquinone **6** with sBu-li for phenyllithium for **8** followed by quenching of the anion with iodomethane or DMF provided **7a** and **8**, respectively. **8** could be elaborated to **7b** and **7c** via reduction to the benzyl alcohol **9**, followed by alkylation with iodomethane (for **7b**) or protection as the TBS ether (for **7c**). A second ortho lithiation followed



Scheme 1. Reagents and conditions: (a) Benzyltrimethylammonium tribromide, CH_2Cl_2, MeOH; (b) TMSI, CH_3CN; (c) NaH, DMF, MOMCl; (d) sBuLi, THF; MeI for **7a** or PhLi, THF; DMF for **8**; (e) $NaBH_4$, EtOH; (f) NaH, DMF, MeI for **7b** or TBSCl, DMF, imidazole for **7c**; (g) i—PhLi, THF, ii—$ZnCl_2$; (h) **10**, $Pd(PPh_3)_4$; (i) H_2, Pd/C, EtOH; (j) NH(OMe)Me, THF, *i*-PrMgCl; (k) **14**, THF; (l) i—HCl, MeOH, $NaBH_3CN$, ii—Chiral separation; (m) i—TBAF, THF; ii—NaOCl, Bu_4NBr; (n) H_2, PtO_2, EtOH.

by coupling with enol triflate **10** using Negeshi conditions gave the unsaturated esters **11a–c**. Unsaturated esters **11a–c** were hydrogenated over palladium on carbon to give **12a–c**, and then transformed into racemic Weinreb amides **13a–c**. The Weinreb amides **13** were reacted with lithiated *p*-bromophenyl methoxymethyl ether **14** to give racemic ketones **15a–c**. Deprotection and cyclization of ketones **15** under acidic conditions was followed by reduction with sodium cyanoborohydride under acidic conditions to give racemic benzopyrans **2** and **3** (a, b, and d analogs), which were separated into their individual enantiomers using chiral preparative HPLC. Benzopyrans **2c** and **3c** were prepared from **15c** under slightly different conditions. Specifically, **15c** was deprotected using TBAF and oxidized with bleach to give benzaldehyde **16**. This material was subjected to hydrogenation conditions in an attempt to selectively reduce the aldehyde in the presence of the ketone. However, these conditions did not result in reduction, but merely generated diethyl acetal **17**. Reductive cyclization of **17** using conditions described above gave ethoxymethyl benzopyrans **2c** and **3c**, which were separated into their individual enantiomers using chiral preparative HPLC. The 2*R*,3*R*,4*R* enantiomer (isomer **2**, as drawn) was found to have the higher binding affinity for all analogs.

Table 1. A-ring modifications: ERα and ERβ binding data



2a-e

Compound	R	ERα (nM) K_i[a]	ERβ (nM) K_i[a]	Ratio
1	H	2.29 (±1.52)	0.17 (±0.094)	14
2a	Me	2.82 (±2.01)	0.26 (±0.091)	11
2b	CH_2OMe	11.0 (±5.64)	0.28 (±0.02)	43
2c	CH_2OEt	27.2	0.745	36
2d	CH_2OH	147	5.97	25

[a] Standard errors shown in parentheses. Others not tested in duplicate. Assay conditions described in Ref. 8.

The A-ring, position 8 effects on ERα and ERβ binding are shown in Table 1. The addition of a methyl group at the 8-position caused no change in binding affinity to either receptor subtype; however, when larger, more polar groups were added, the ERα binding affinity decreased significantly. We attribute this finding to the limited space available (steric effect) and the nature of the surrounding residues in this area of the LBP, which are very hydrophobic (polarity effect). Perhaps ligands such as **2a** maintain good ERα binding due to the plasticity of the ER LBPs,[14] which may accommodate hydrophobic substituents residing in the highly hydrophobic pocket. Alternatively, a larger, more polar group, such as alkoxymethyl or hydroxymethyl is tolerated in the hydrophobic LBP only when adequate space exists between the ligand polar groups and the hydrophobic residues. In this series, it appears that **2b** (methoxymethyl substitution) provides the best mix of polarity and steric bulk to disrupt ERα binding, while maintaining very good binding to ERβ, thus improving ERβ selectivity to 43-fold.

With these results in hand, we sought to test our hypothesis with X-ray crystal structures of **2b** with both ERα and ERβ (Fig. 2).[15] The similarity of binding modes, relative to **1**, is demonstrated by overlaying the structures of **2b** with the structures of **1** in both ERα and ERβ. This result clearly shows (b) that **2b** binds to ERβ in an identical manner, relative to **1**/ERβ. However, as shown in Figure 2a, the methoxymethyl group in **2b** forces the A-ring out of its optimal position, thereby disrupting the important A-ring phenol-His524 hydrogen bonding interaction. We believe that this is responsible for the ~4-fold decrease in binding affinity of **2b**, relative to **1**.

In conclusion, we have shown that the introduction of A-ring substitution to the 8 position of benzopyrans results in diminished ERα binding, while maintaining strong ERβ binding. We have shown that this is due to the fact that compounds in this class bind to the ER subtypes with different poses, and that substitution at the 8 position is less tolerated in the ERα LBP, relative to ERβ. Finally, the 8-methoxymethyl shows improved binding selectivity (43-fold) in this class of selective ERβ agonists.

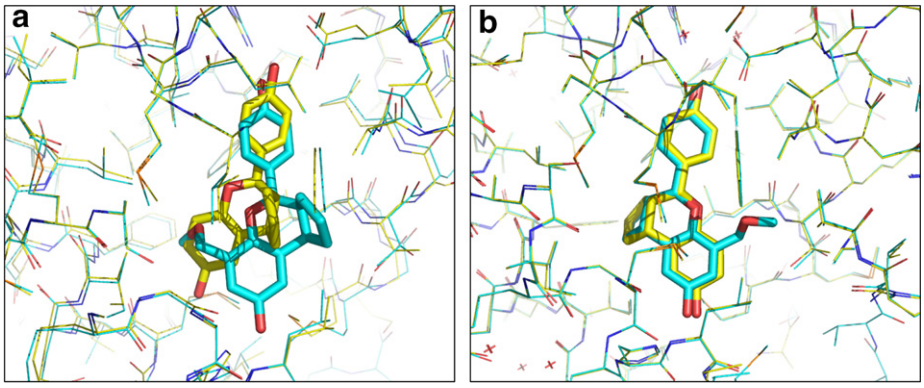


Figure 2. Overlay of **1** and **2b** with ERα (a) and ERβ (b).

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