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Benzopyrans as selective estrogen receptor β agonists (SERBAs). Part 5: Combined A- and C-ring structure–activity relationship studies

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Abstract—Benzopyrans are selective estrogen receptor (ER) β agonists (SERBAs), which bind the ER subtypes α and β in opposite orientations. Here we describe the synthesis of a late stage intermediate that allowed us to combine A-ring and C-ring modifications and carry out simultaneous SAR studies at both positions. Modification of both positions proved additive, maintaining affinity and improving ERβ selectivity up to 83-fold. An X-ray cocrystal structure confirms the previously observed binding mode in ERβ.

ERα and ERβ are ligand activated transcription factors that belong to the steroid nuclear hormone receptor family. They mediate the activity of estrogen, a hormone important for the development, maintenance, and regulation of the female reproductive system. The classical receptor, now known as ERα, is expressed in nearly all tissues of both sexes. ERβ was discovered in 1996.[1] It is expressed in the ovaries, uterus, and oviduct of the female reproductive tract but not in breast tissue; while in males, it is expressed in the prostate and epididymis but not in the testes.[2] It is possible to develop selective ER modulators (SERMs), such as raloxifene. These compounds demonstrate tissue type functional selectivity with agonist activity in bone, liver, and cardiovascular tissues and antagonist activity in the uterus and breast.[3] This selectivity is most likely due to the interaction of ligand bound receptor with cofactors that either promote the formation of gene transcription complexes or inhibit gene transcription.

Over the last decade several groups have reported ERβ selective ligands.[4] Our own efforts focused on the benzopyran scaffold resulting in the development of benzopyran **1a** as a selective estrogen receptor β agonist (SERBA-1).[5] Recently we reported structural modifications of the C-ring (**2a**)[6] and A-ring (**3a**)[7] of the benzopyran scaffold (Fig. 1). Modifications at either of these positions resulted in significant increases in binding selectivity for ERβ. Data from X-ray crystal structures indicate that benzopyran **1a** binds to both ERα and ERβ with the D-ring phenol interacting with the hydrogen bond network of the Glu-Arg-H_2O triad. The A-ring phenol interacts with His-524 in ERα or the corresponding His-475 in ERβ. Although the two phenols bind in the same places within the binding pocket, the orientation of the benzopyran scaffold is rotated by 180° on the bisphenol axis. In addition the A-ring phenol is found to the left of His-524 in ERα as drawn in Figure 1, while in ERβ the A-ring phenol is shifted to the right of the corresponding His-475. These same rotated and shifted binding orientations were found in crystal structures of both the C-ring and A-ring modified benzopyrans. In all cases the C-ring is pointed toward Leu-384 in ERα, but in ERβ this ring is pointed toward Ile-373 on the other side of the pocket. In a similar fashion, A-ring modifications place functional groups in the vicinity of Met-421 in ERα, while in ERβ these functional groups end up on the opposite side of the binding pocket near Met-336. It was not clear to us if combining C-ring and A-ring modifications to give compounds **4a** would be beneficial. Here we describe the synthesis of a late stage intermediate that allowed us to combine A-ring and C-ring modifications and carry out simultaneous SAR studies in both positions.

Keywords: Estrogen receptor; ERb selective ligands; Benzopyrans.
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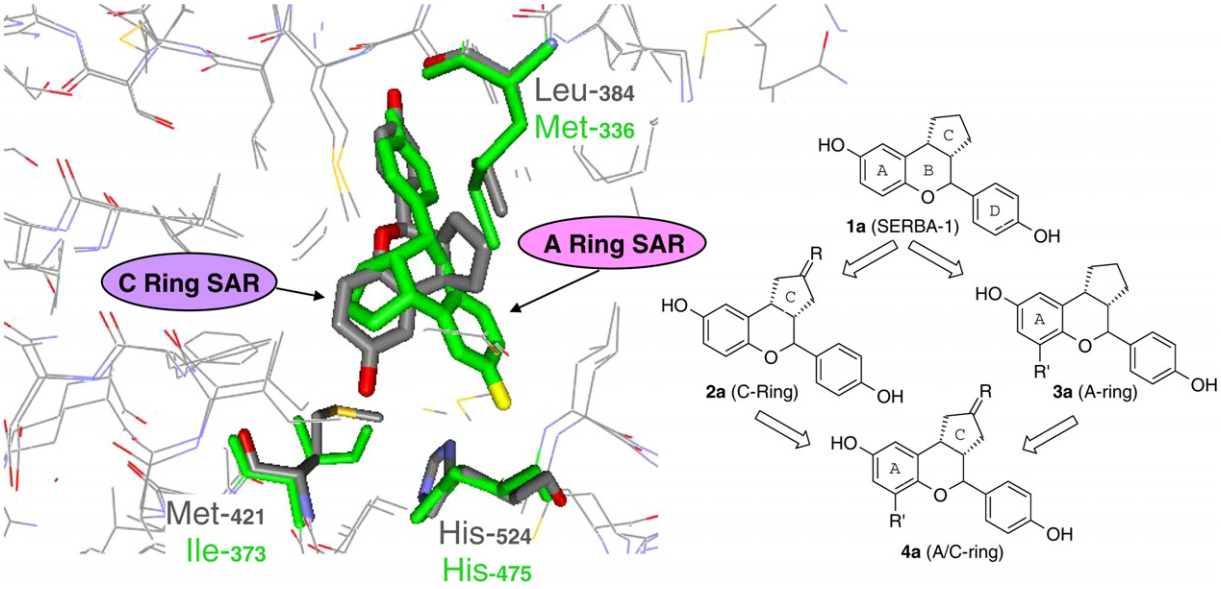
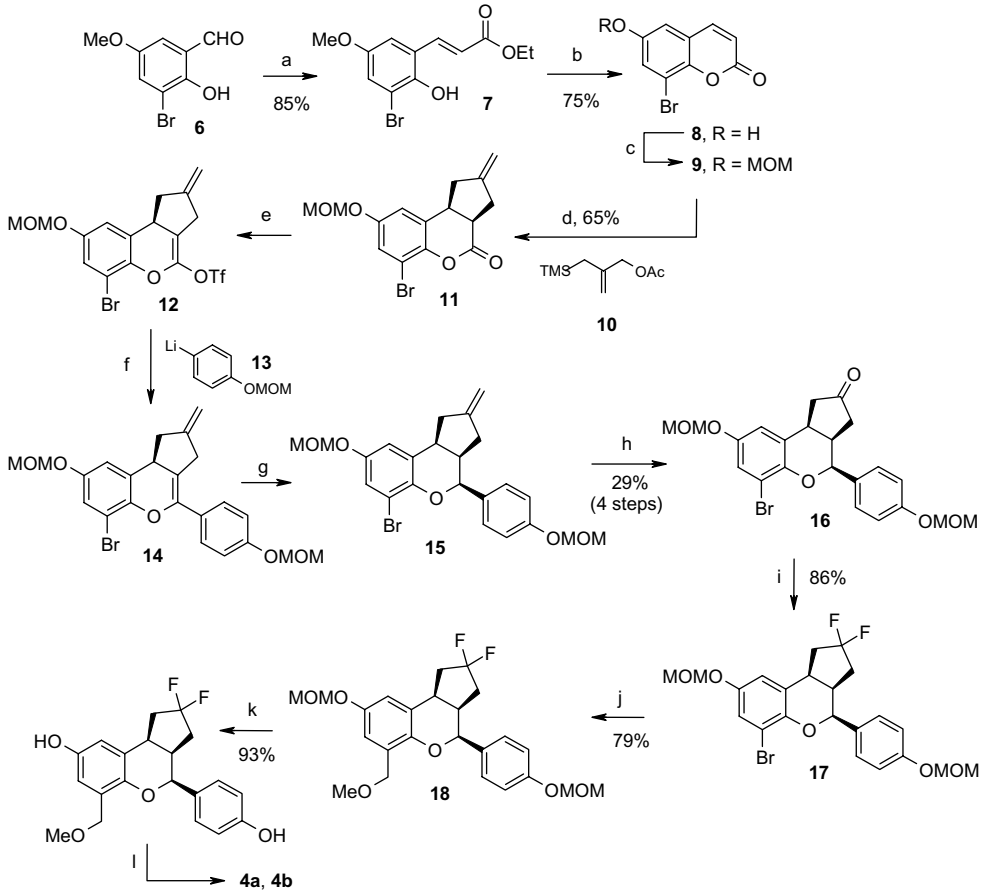


Figure 1. Diagram of the X-ray crystal structures of SERBA-1 **1a** bound to ERβ (green), SERBA-1 **1a** bound ERα (gray), and the C-ring **2a**, A-ring **3a**, and A/C-ring **4a** modifications of SERBA-1.

The synthesis of a common intermediate for combined C-ring and A-ring modifications is described in Scheme 1.[8] The stabilized phosphorus ylide, (carbethoxymethyl-ene)-triphenyl-phosphorane, was used to prepare (*E*)-ethyl cinnamate **7** from 3-bromo-2-hydroxy-5-meth-oxy-benzaldehyde (**6**).[9] Ethyl cinnamate **7** was treated



Scheme 1. Synthesis of **4a** and **4b**. Reagents and conditions: (a) $Ph_3P = CHCO_2Et$, toluene; (b) BBr_3, CH_2Cl_2, 60 °C; (c) K_2CO_3, MOMCl, DMF; (d) **10**, $Pd(OAc)_2$, $P(OEt)_3$, THF, 60 °C; (e) LiHMDS, Comins' reagent, THF, −78 → 0 °C; (f) i—Compound **13**, $ZnCl_2$, THF, 0 °C; ii—Compound **12**, $Pd(PPh_3)_4$, THF, 50 °C; (g) TFA, Et_3SiH, CH_2Cl_2; (h) NMM, NMO, OsO_4, H_2O, THF; $NaIO_4$; (i) DAST, $ClCH_2CH_2Cl$, 40 °C; (j) i—*n*-BuLi, THF, −78 °C; DMF; ii—$NaBH_4$, MeOH, THF; iii—NaH, MeI, THF; (k) HCl, H_2O, THF; (l) Chiralpak AD, heptane–isopropanol.

with boron tribromide to remove the methyl group and promote cyclization to 8-bromo-6-hydroxycoumarin (**8**), which was then protected as its MOM-ether to give **9**. The Trost transition metal-catalyzed [3 + 2] trimethylenemethane (TMM) cycloaddition[10] was used to install a cyclopentane ring containing an exo-methylene group, which served as a latent ketone, to give lactone **11**. The lactone in **11** was deprotonated with LiHMDS and the resulting lactone enolate was reacted with Comins' reagent[11] to give lactone enol triflate **12**. Negishi's palladium catalyzed coupling[12] conditions were used to couple the lactone enol triflate **12** with the aryl zinc reagent generated from aryl lithium **13** to give the MOM-protected 4-(4*H*-chromen-2-yl)phenol **14**. We found that this coupling was completely selective for the enol triflate over the aryl bromide. The enol ether of **14** was reduced with complete stereoselectivity using triethylsilane-trifluoroacetic acid to the all *cis*-stereoisomer of flavanol analog **15**. The latent ketone was revealed by dihydroxylating the exo-methylene of **15** with osmium tetroxide and then cleaving the diol with sodium periodate. We found it was best to carry material from lactone **11** all the way through the oxidation step to provide MOM-protected bromo-cyclopentanone **16**, without extensive purification or handling of intermediates because compound **14** is prone to air oxidation. The overall yield from **11** to **16**, which served as a common, late-stage intermediate for the synthesis of A/C-ring functionalized benzopyrans (**4**), was 29%. Treatment of **16** with DAST[13] gave the difluoromethylene derivative **17**. Lithium halogen exchange was used to convert **17** into an aryl lithium that was reacted with DMF to give an aldehyde. The aldehyde was reduced to the corresponding alcohol using sodium borohydride followed by deprotonation with sodium hydride and then alkylation with methyl iodide to give methyl ether **18**. The penultimate **18** was deprotected under acidic conditions to give the A/C-ring functionalized benzopyran **4**. The enantiomers **4a** and **4b** were separated by chiral chromatography.

The A-ring, C-ring, and A/C-ring derivatives of SERBA-1 (**1a**) were evaluated for their ability to bind estrogen receptors alpha and beta (Table 1). As can be seen, the addition of a methoxymethyl group to the A-ring gives a compound (**2a**) with equal binding affinity to ERβ compared to **1a**, but with significantly increased selectivity (43-fold compared to 14-fold). The difluoromethylene group in the C-ring of compound **3a** also maintains binding affinity to ERβ, but increases selectively by only a marginal amount (19-fold compared to 14-fold). The combination of these two modifications in compound **4a** maintains affinity to ERβ and results in an increase in selectivity (83-fold) to nearly twice that of **2a** and four times that of **3a**. Interestingly the enantiomer of **4a**, compound **4b**, possesses 220-fold less binding affinity for ERβ and is almost completely non-selective. This is significantly different from the enantiomer of **1a**, compound **1b**, which loses only 8-fold binding affinity and maintains 11-fold selectivity.

The cocrystal structure of benzopyran **4a** is shown in Figure 2.[14] As can be seen, the same binding orientation is observed in this structure as was seen in the structures of ERβ with benzopyran **1a**[5] as well as the C-ring[6] and A-ring[7] modified benzopyrans. The D-ring phenol interacts with the hydrogen bond network of the Glu-Arg-H_2O triad, while the A-ring phenol interacts with

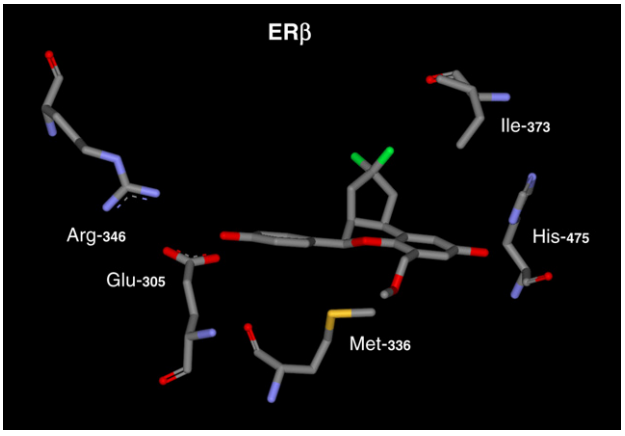
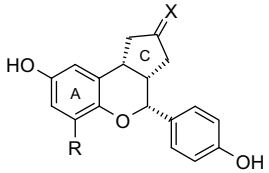
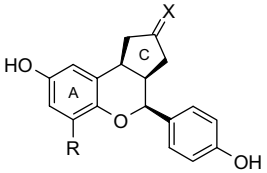


Figure 2. Diagram of the X-ray crystal structure of the A/C-ring functionalized benzopyran **4a** bound to ERβ. The fluorine atoms are colored green while the oxygens are red.

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



1a, 2a, 3a, 4a



1b, 4b

Compound[a]	X	R	ERβ (nM)[b]	ERα (nM)[b]	Ratio
1a	H_2	H	0.19 ± 0.09	2.70 ± 1.5	14
2a	H_2	CH_2OMe	0.28 ± 0.02	11.9 ± 5.6	43
3a	F_2	H	0.44 ± 0.28	8.4 ± 2.3	19
4a	F_2	CH_2OMe	0.53 ± 0.04	43.8 ± 9.1	83
4b	F_2	CH_2OMe	118	198	1.7
1b	H_2	H	1.54 ± 0.45	14.5 ± 6.4	11

[a]All compounds are from the more potent enantiomeric series except **4b** and **1b** which are from the less potent enantiomeric series.
[b] K_i values are means of at least two determinations ±SD.

His-475. The difluoromethylene group of the C-ring is pointed toward Ile 373, while the methymethoxy group is pointed toward Met-336.

In conclusion, we were able to increase the binding selectivity of the benzopyran scaffold by combining modifications of the C- and A-rings of benzopyran **1a**. Fluorination of the C-ring gave a compound with 19-fold selectivity, while addition of a methoxymethyl group to the A-ring gave a compound with 43-fold selectivity. The combination of these two modifications gave a compound that was equipotent at ERβ and 83-fold selective for ERβ over ERα. The cocrystal structures of this A/C-ring analog **4a** in ERβ demonstrated similar binding orientations compared to the C-ring modified, A-ring modified, and unfunctionalized benzopyrans.

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