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Benzopyrans as selective estrogen receptor β agonists (SERBAs). Part 2: Structure–activity relationship studies on the benzopyran scaffold

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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 structure–activity relationship studies that led to the discovery of bezopyran **5b**. X-ray crystal structures of **5b** and a non-selective analog **5c** in ERα help explain the observed selectivity of the benzopyran platform.
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Estrogen activity is mediated by two members of the steroid nuclear hormone receptor family, ERα and ERβ. They function as ligand-activated gene transcription factors and mediate a number of important physiological processes with the classical receptor, ERα, being most important for development and regulation of the female reproductive system as well as maintenance of the skeletal and cardiovascular systems. It is possible to develop synthetic estrogens that have tissue type selectivity.[1] These selective ER modulators (SERMs), such as raloxifene, act as agonists in some tissues such as bone, liver, and cardiovascular, while behaving as antagonists in others such as uterus and breast. The origin of tissue selectivity is complex and not completely understood, but is most likely due to the interaction of ligand bound receptor with a wide array of cofactors, coactivators and corepressors, which in concert with the receptor mediate gene transcription. ERβ, which was discovered in 1996, adds another layer of complexity to our understanding of estrogen physiology.[2] While ERα is expressed in nearly all tissues of both sexes, ERβ is expressed in the ovaries, uterus, and oviduct of the female reproductive tract but not in breast tissue; while in males, ERβ is expressed in the prostate and epididymis but not in the testes.[3]

Over the last decade several groups have developed ERβ selective ligands using structure based design methods along with inspiration from the well-known ERβ-selective isoflavone phytoestrogens daidzein (**1**) and genistein (**2**).[4] All of these ligands have in common two phenols which bind to the Glu-Arg-water triad at one end of the ERβ receptor binding pocket and His475 at the other end (Fig. 1).[5] These two critical features of the ERβ pharmacophore (**3**) require a separation of the phenol oxygen atoms by a distance of about 10–12 Å (roughly 10 atoms). The benzopyran scaffold (**4**) served as an excellent starting point to build ERβ selective ligands because the benzopyran substructure provides a rigid platform with a distance between the phenol oxygens of 11.9 Å. This distance positions the phenol oxygen atoms between the genistein distance of 12.2 Å and the estradiol distance of 10.9 Å. Herein we describe structure–activity relationship studies that led to the discovery of benzopyran **5b** as a selective estrogen receptor β agonist (SERBA).[6]

The unadorned benzopyran **4a** was prepared as described in Scheme 1. Base catalyzed condensation of 4-(methoxymethoxy)-benzaldehyde (**7**) and 2-hydroxy-5-(methoxymethoxy)-acetophenone (**6**) gave 4′,6-dihydroxyflavanone **8**. The ketone of **8** was reduced by catalytic hydrogenation over Pd/C to give the corresponding MOM-protected 4′,6-flavandiol **9**, which was then deprotected under acidic conditions to give the desired benzopyran **4a**.

Keywords: Estrogen receptor; ERβ selective ligands; Benzopyrans.
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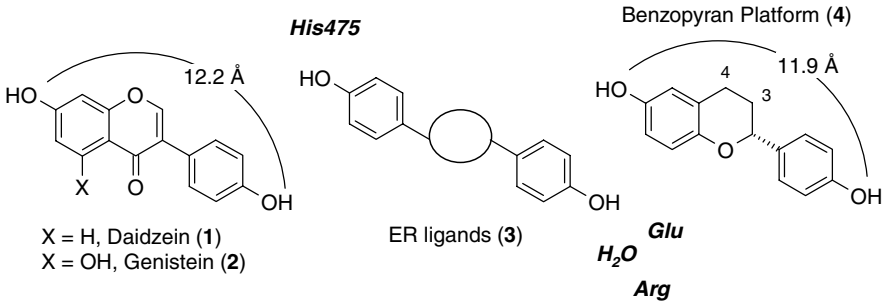
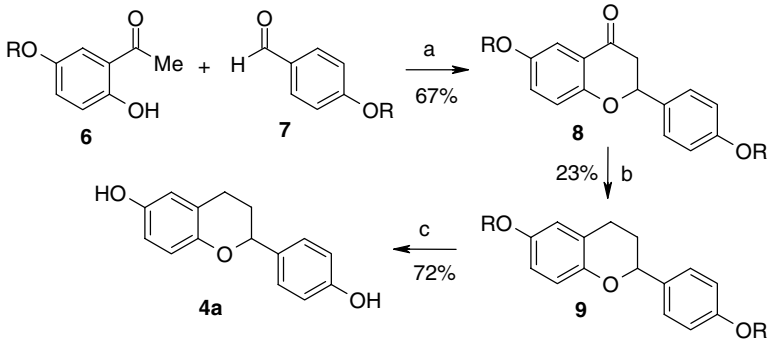


Figure 1. ER ligands: genistein, daidzein, and the benzopyran platform.



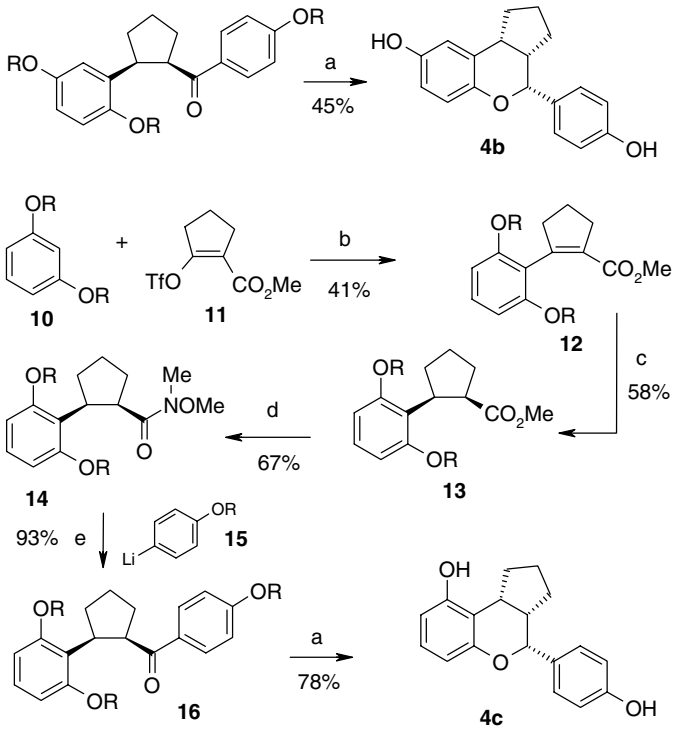
Scheme 1. Synthesis of benzopyran **4a**. R = MOM; Reagents and conditions: (a) NaOH, H_2O, EtOH, 0 °C → rt; (b) H_2, Pd/C, AcOH, EtOAc; (c) HCl, H_2O, THF.

The synthesis of the cyclopentane analog **5b** was described in part 1 of this series.[6] The key step of the synthesis was a reductive cyclization, which provided the corresponding racemate **4b** with excellent diastereoselectivity for the all syn diastereomer (Reaction a, Scheme 2). This same route was used to prepare analogs of **4a** with cycloalkyl rings fused at the C3 and C4 positions of the benzopyran scaffold. The synthesis of the 5-OH analog **4c** followed the same route and is described in Scheme 2. MOM-protected resorcinol **10** was lithiated, followed by transmetallization to the corresponding aryl zinc, which was subjected to Palladium-mediated Negishi cross coupling with the cyclopentyl vinyl triflate **11**[6] to give cyclopentene **12**. Palladium catalyzed hydrogenation of the olefin in **12** gave exclusively the *cis*-stereochemistry in cyclopentane **13**. The methyl ester of **13** was converted to the Weinreb amide **14**. Addition of aryllithium **15** to Weinreb amide **14** provided aryl ketone **16**. Deprotection and cyclization of **16** was accomplished in a one pot sequence: treatment with toluenesulfonic acid, followed by the addition of sodium cyanoborohydride under acidic conditions (monitoring pH with bromocresol green) favored benzopyran **4c** with the all *cis*-stereochemistry.

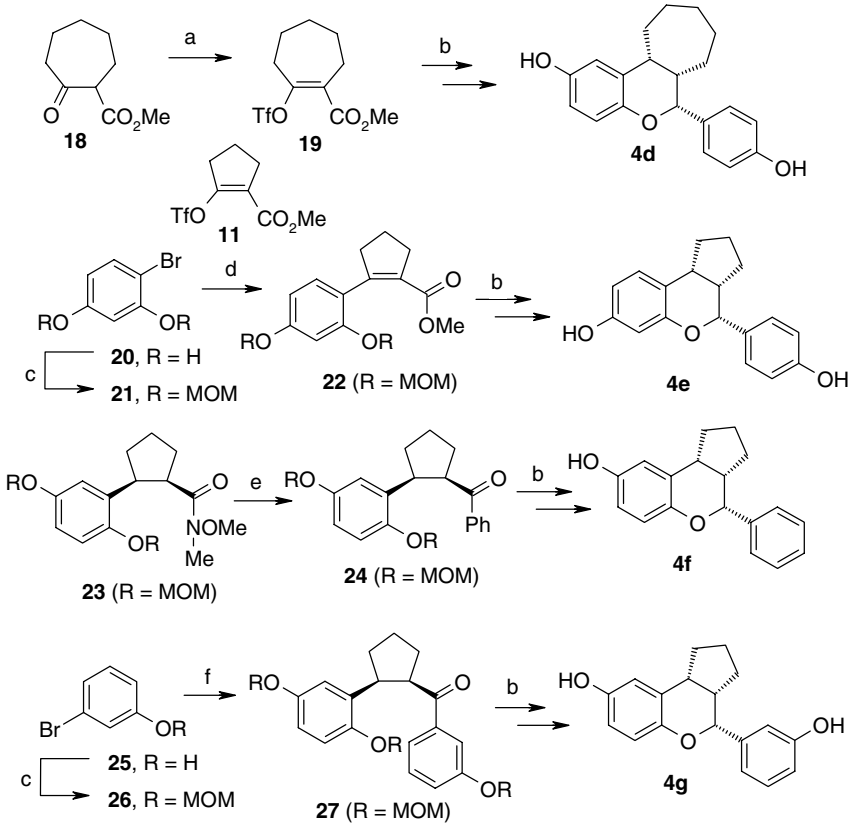
The reductive cyclization route described in Scheme 2 supported the syntheses of the 3,4-cycloheptane-fused benzopyran **4d** as well as several other phenol isomers of **4b** (Scheme 3). Enol triflate **19** was prepared from commercially available methyl 2-oxo-1-cycloheptanecarboxylate (**18**) and then carried through the reductive cyclization route to provide cycloheptane-fused benzopyran **4d**. MOM-protected 4-bromoresorcinol **21** was lithiated and then coupled to cyclopentyl vinyl triflate **11** to give cyclopentene **22**. Cyclopentene **22** was then carried through the reductive cyclization route to provide 4′,7-flavandiol **4e**. Phenyl lithium was added to Weinreb amide **23**[6] to give aryl ketone **24**. In like manner, lithium–halogen exchange on MOM-protected 3-bromophenol **26** followed by addition to Weinreb amide **23** gave aryl ketone **27**. Both of these aryl ketones were then carried through the reductive cyclization route to provide 6-flavanol **4f** and 3′,6-flavandiol **4g**.

The 3,4-cyclohexane-fused benzopyran **4h** and the 4′-flavanol analog **4i** were prepared using the Pechmann Condensation Route described in Scheme 4. Hydroquinone (**29**) was condensed with ethyl 2-cyclohexanonecarboxylate (**30**) in 80% H_2SO_4 to give 6-hydroxycoumarin **31**. The hydroxyl group of **31** was protected as its TBS-ether to give **32**. The δ-lactone of **32** was reduced with DIBAL-H to the corresponding lactol, which was then opened with aryllithium **33** to give the styryl-benzyl alcohol **34**. The TBS-protecting groups on **34** were removed using TBAF. When this deprotection reaction was acidified with TFA, the styryl-benzyl alcohol was displaced by the proximal phenol to give 2H-benzopyran **35**. The alkene of **35** was reduced with hydrogen over Pd/C to give 3,4-cyclohexane-fused benzopyran **4h**. In like manner methyl 2-oxocyclopentanecarboxylate (**36**) was condensed with hydroquinone (**29**) in 80% H_2SO_4 and then carried through the same reaction sequence to give 4′-flavanol analog **4i**.

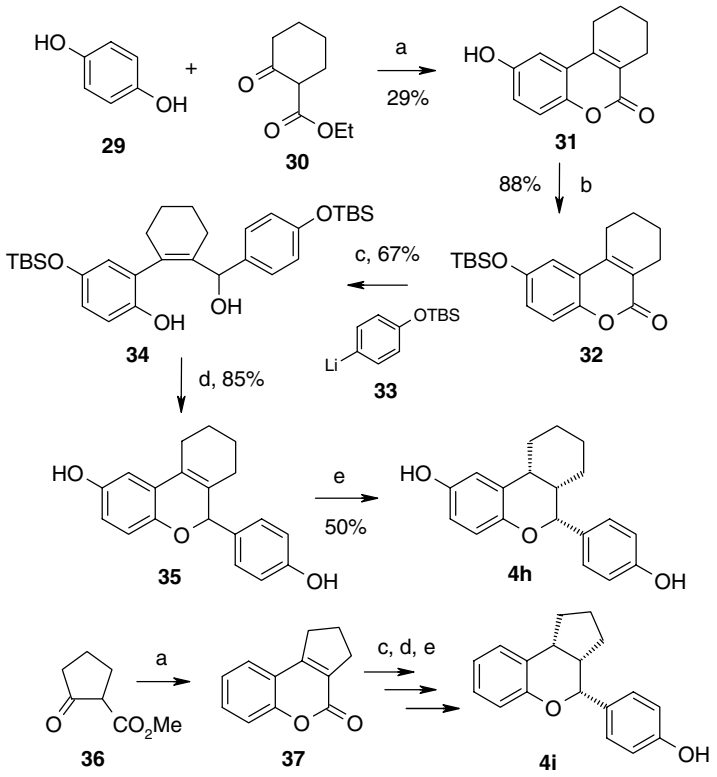
As shown in Tables 1 and 2, benzopyrans **4a**–**i** were evaluated for their ability to bind estrogen receptors



Scheme 2. Reductive cyclization route to 3,4-cycloalkyl fused benzopyrans. R = MOM; Reagents: (a) i—*p*-TsOH, MeOH, 50 °C; ii—HCl, $Na(CN)BH_3$, rt; (b) i—**10**, *t*-BuLi, THF, −78 → 0 °C; ii—$ZnCl_2$, 0 °C → rt; iii—**11**, $Pd(PPh_3)_4$, rt → 50 °C; (c) 5% Pd/C, MeOH, 60 psi H_2; (d) HN(OMe)Me·HCl, iPrMgCl, THF, −10 °C; (e) **15**, THF, −0 °C.

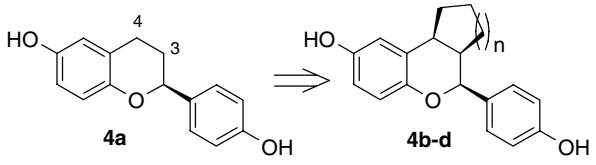


Scheme 3. Benzopyrans prepared via the reductive cyclization route. Reagents and conditions: (a) Tf_2O, *i*-Pr_2EtN, CH_2Cl_2, −78 °C; (b) reductive cyclization route, Scheme 2; (c) NaH, MOMCl, DMF, 0 °C; (d) i—*s*-BuLi, THF, 0 °C; ii—$ZnCl_2$, 0 °C → rt; iii—**11**, $Pd(PPh_3)_4$, rt → 50 °C; (e) PhLi, THF, 0 °C; (f) i—*t*-BuLi, THF, −78 °C; ii—**23**, THF, −78 °C.



Scheme 4. Pechmann condensation route to 3,4-cycloalkyl fused benzopyrans. R = MOM; Reagents and conditions: (a) 80% H_2SO_4; (b) TBSCl, imidazole, DMF; (c) i—DIBAL-H, THF, −78 °C; ii—35, THF, −78 °C; (d) TBAF, THF, TFA; (e) 5% Pd/C, MeOH, H_2.

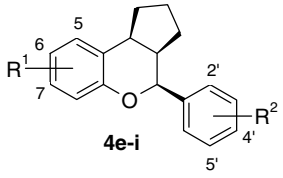
Table 1. Cycloalkyl ring SAR: ERα and ERβ binding data



Compound	*n*	ERβ[b] (nM)	ERα[b] (nM)	Ratio
4a		20.8 ± 4.3	126 ± 82	6
4b	1	0.47 ± 0.18	4.34 ± 3.1	9
4h	2	0.63 ± 0.46	2.88 ± 2.9	5
4d	3	0.88 ± 0.38	6.11 ± 2.8	7

[a]All compounds are racemic.
[b] K_i values are means of at least two determinations ±SD.

Table 2. Phenol isomer SAR: ERα and ERβ binding data



Compound	R^1	R^2	ERβ[b] (nM)	ERα[b] (nM)	Ratio
4b	6-OH	4′-OH	0.47 ± 0.18	4.34 ± 3.1	9
4f	6-OH	H	51 ± 11	267 ± 112	5
4g	6-OH	3′-OH	18.5 ± 7.4	65 ± 31	4
4i	H	4′-OH	22.1 ± 7.3	40 ± 27	2
4c	5-OH	4′-OH	0.66 ± 0.08	0.35 ± 0.04	0.5
4e	7-OH	4′-OH	18.9 ± 1.6	41 ± 17	2

[a]All compounds are racemic.
[b] K_i values are means of at least two determinations ±SD.

alpha and beta. As can be seen in Table 1, fusing a cyclopentane ring to the 3,4-positions of benzopyran **4a** dramatically improved binding affinity and afforded a compound (**4b**) with 9-fold selectivity for ERβ over ERα. Increasing the ring size to cyclohexane (**4h**) or cycloheptane (**4d**) did not improve ERβ binding potency over **4b**. Since these two larger ring derivatives were slightly less selective for ERβ we chose to carry out phenol isomer SAR studies with the cyclopentane ring in place (Table 2). Removal of the hydoxyl group from the pendant 4′-aryl ring (**4f**) resulted in a 60-fold and a 100-fold loss in binding affinity for ERα and ERβ, respectively, demonstrating the importance of this phenol in the ER pharmacophore.[7] Compounds **4b** and **4g** demonstrate that the 4′-position is optimal because when moved to the 3′-position there is a 39-fold loss in binding affinity for ERβ. Removal of the hydroxyl group from the 6-position on the benzopyran ring (**4i**) also reduced binding affinity but not as dramatically (47-fold vs 100-fold) as compared to the 4′-position. Moving the benzopyran hydroxyl group from the 6-position (**4b**) to the 5-position (**4c**) maintained potency at ERβ but increased potency at ERα, resulting in complete loss of selectivity. Placing the hydroxyl group in the 7-position resulted in a loss of affinity at both receptors and gave a compound (**4e**) that was only 2-fold selective for ERβ.

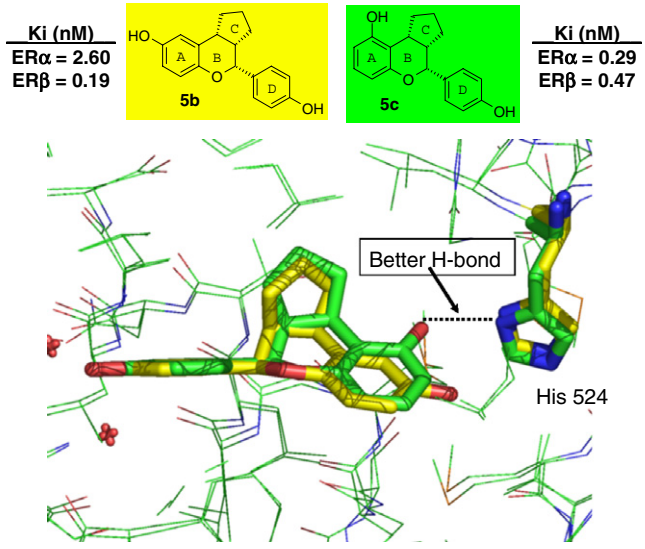


Figure 2. Diagram of the X-ray structure of SERBA-1 (**5b** in yellow) and the non-selective benzopyran **5e** (green) bound to ERα.

In order to better understand the basis for binding selectivity we investigated the binding interactions that the non-selective benzopyran **4c** forms with ERα (Fig. 2). We have already reported the cocrystal structures of SERBA-1 (**5b**), the more potent enantiomer of benzopyran **4b**, with both ERα and ERβ.[6] The data indicate that SERBA-1 binds to both receptors with its D-ring phenol interacting with the hydrogen bond network of the Glu-Arg-H_2O triad, while the A-ring phenol interacts with His-524 in ERα or the corresponding His-475 in ERβ. Although the phenols bind in the same places within the binding pocket, the orientation of the benzopyran scaffold is rotated by 180° on the bisphenol axis. The positions of the A-ring phenol are also shifted with respect to the histidines. The rotated binding orientations and shifted phenol positions result in slightly disrupted binding interactions within the ERα pocket compared to ERβ and thus conferring selectivity for ERβ. The enantiomers of **4c** were separated by chiral chromatography (Chiralpak AD, 80% heptane-isopropanol). The more potent enantiomer **5c** was cocrystallized with ERα. Figure 2 shows an overlay of this structure with that of **5b** and ERα. As can be seen, moving the phenol from the 6-position (**5b**) to the 5-position (**5c**) compensates for the shift in position of the A-ring phenol with respect to His-524. As a result benzopyran **5c** is able to form a better hydrogen bond to the histidine compared to benzopyran **5b**. Thus, the affinity to ERα is increased and selectivity for ERβ is diminished. Atomic coordinates for ERa complexed with benzopyran **5c** have been deposited in the Protein Data Bank with accession code 2POG.

In conclusion, we were able to increase the binding affinity and selectivity of the benzopyran scaffold by fusing a fourth ring at the 3,4-carbon positions of **4a**. A cyclopentane ring was marginally optimal compared to the cyclohexane and cycloheptane rings. We demonstrated that both phenols were critical for affinity. The positioning of the phenols was important for both affinity and selectivity. The 5-OH isomer **4c** was equipotent compared to the 6-OH isomer **4b** at ERβ but was non-selective. Cocrystal structures of **5b** and **5c**, the more potent enantiomers of **4b** and **4c**, revealed that moving the hydroxyl to the 5-position compensates for the shift in the position of the A-ring with respect to His-524 and allows the formation of a better hydrogen bond. Attempts to increase the selectivity of the benzopyran scaffold will be disclosed in subsequent papers.

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