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Benzopyrans as selective estrogen receptor β agonists (SERBAs). Part 3: Synthesis of cyclopentanone and cyclohexanone intermediates for C-ring modification

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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 syntheses of cyclopentanone and cyclohexanone intermediates for SAR studies of the C-ring on the benzopyran scaffold. Modification of the C-ring disrupts binding to ERα, thus improving ERβ selectivity up to 100-fold. X-ray cocrystal structures confirm previously observed binding modes.

The estrogen receptors are members of the steroid nuclear hormone receptor family. ERα is important for development and regulation of the female reproductive system as well as maintenance of the skeletal and cardiovascular systems. ERβ was discovered in 1996 in the rat prostate gland and adds another layer of complexity to our understanding of estrogen physiology.[1] 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.[2] Selective ER modulators (SERMs) demonstrate tissue type functional selectivity with agonist activity in bone, liver, and cardiovascular tissues, and antagonist activity in the uterus and breast.[3] This tissue type functional selectivity is most likely due to the interaction of ligand bound receptor with coactivators that combine to form gene transcription complexes or interaction with corepressors that 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 structure activity relationship (SAR) studies focused on the size of the 3,4-fused ring labeled C in Figure 1.[6] The addition of a fused cyclopentane ring at the 3,4-carbon positions of the benzopyran scaffold resulted in a dramatic increase in binding affinity, 9-fold selectivity for ERβ over ERα, and provided us with a ligand architecture containing structural elements that take advantage of the space available above and below the plane marked by the two phenol rings. As can be seen in Figure 1, the cyclopropane is projected down from the plane marked

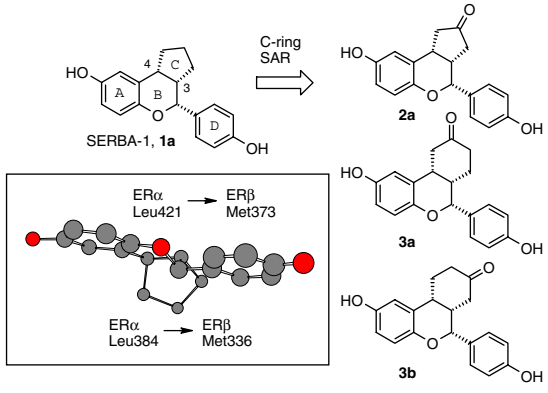

Figure 1. Unique structural features of the benzopyran platform.

Keywords: Estrogen; Estrogen receptor β; ERb; ERβ; Selective estrogen receptor β agonist; SERBA.
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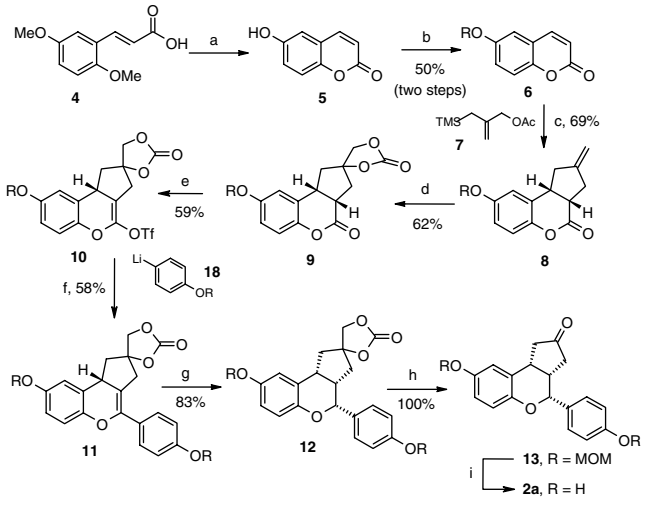
by the phenol rings. This unique structural feature allowed us to take advantage of the Met-421 → Ile-373 and the Leu-384 → Met-336 differences between the binding pockets of ERα → ERβ. Here we describe the syntheses of cyclopentanone **2a** and cyclohexanones **3a** and **3b**. The ketone functional groups of these late stage intermediates served as handles to perform SAR studies of the C-ring on the benzopyran scaffold.

Cyclopentanone **2a** was prepared as described in Scheme 1.[7] Commercially available 2,5-dimethoxycinnamic acid (**4**) was treated with boron tribromide to remove the methyl groups and promote cyclization to 6-hydroxycoumarin (**5**), which was then protected as its MOM-ether to give **6**. The Trost transition metal-catalyzed[8] trimethylenemethane (TMM) cycloaddition[8] was used to install a cyclopentane ring containing an *exo*-methylene group to give lactone **8**. The *exo*-methylene group, which serves as a latent ketone, was dihydroxylated with osmium tetroxide and the resulting diol was protected as its cyclic carbonate using phosgene to give **9**. The lactone **9** was deprotonated with LiHMDS and the resulting lactone enolate was reacted with *N*-phenyl triflamide[9] to give lactone enol triflate **10**. Negishi's palladium-catalyzed coupling[10] conditions were used to couple the lactone enol triflate **10** with the aryl zinc reagent generated from aryl lithium **18** to give the MOM-protected 4-(4*H*-chromen-2-yl)phenol **11**. The enol ether of **11** was easily reduced by hydrogenation over Pd/C to give exclusively the all *cis*-stereoisomer of flavanol analog **12**. At this point the latent ketone could be revealed by hydrolyzing the cyclic carbonate of **12** with LiOH and then cleaving the diol to the ketone with sodium periodate in the same pot. This procedure gave MOM-protected cyclopentanone **13**, which served as a late stage intermediate for the synthesis of C-ring functionalized derivatives of SERBA-1 (**1a**). The MOM-protecting groups of **13** could be removed under mildly acidic aqueous conditions to give cyclopentanone **2a**.

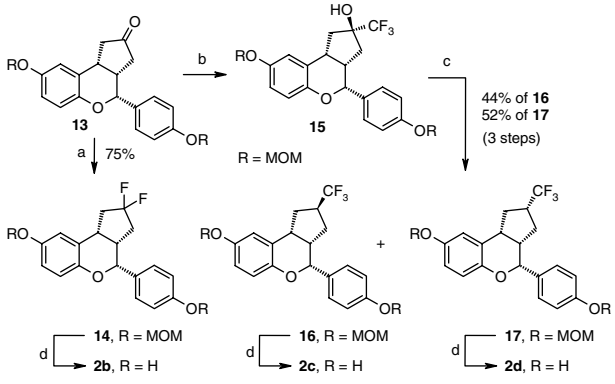


Scheme 1. Coumarin route to cyclopentanone intermediate **13** for C-ring SAR. R = MOM; Reagents and conditions: (a) BBr_3, CH_2Cl_2, rt–82 °C; (b) MOMCl, *i*-Pr_2EtN, CH_3CN; (c) **7**, $Pd(OAc)_2$, $P(OEt)_3$, THF, 60 °C; (d) i—OsO_4, NMO, *t*-BuOH, H_2O, THF; ii—$COCl_2$, Et_3N, CH_2Cl_2, 0 °C; (e) LiHMDS, THF, −78 °C; $PhNTf_2$, HMPA; (f) i—**18**, $ZnCl_2$, THF, 0 °C; ii—**10**, $Pd(PPh_3)_4$, THF, 50 °C; (g) 60 psi H_2, Pd/C, MeOH; (h) LiOH, H_2O, THF; $NaIO_4$; (i) HCl, H_2O, THF.

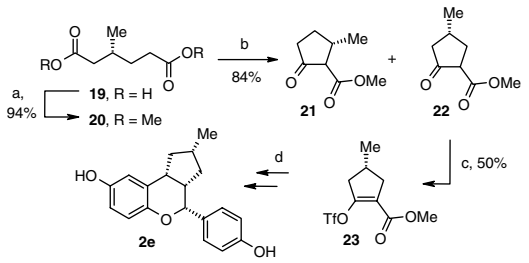
The use of cyclopentanone **13** as a late stage intermediate for C-ring SAR studies is described in Scheme 2. Treatment of **13** with DAST[11] gave the difluoromethylene derivative **14**, which was deprotected with aqueous HCl to give difluoromethylene cyclopentane **2b**. Ruppert's reagent[12] was added to the ketone of **13** to give trifluoromethyl cyclopentanol **15**. The tertiary alcohol of **15** was removed using Dolan and MacMillan's[13] modified Barton–McCombie[14] radical deoxygenation conditions. The methyl oxalyl ester of **15** was prepared using methyl oxalyl chloride and DMAP, and was then subjected to radical reduction initiated by AIBN and heat in the presence of triphenyl silane. This procedure gave a nearly 1:1 mixture of trifluoromethyl cyclopentane diastereomers **16** and **17**. The diastereomers were easily separated by silica gel chromatography and then deprotected to give the trifluoromethyl cyclopentanes **2c** and **2d**.[15]

We wanted to compare the trifluoromethyl cyclopentane derivatives described in Scheme 2 to the simple methyl analog. This compound was prepared using the reductive cyclization route reported earlier[5] and is described in Scheme 3. The bis-methyl ester of commercially available (+)-3-methylhexanedioic acid (**19**) was prepared using sulfuric acid and methanol. Treatment of **20** with sodium methoxide in methanol and toluene effected a Dieckmann cyclization to give a mixture of the 3- and 4-methyl substituted isomers of methyl 2-oxocyclopentanecarboxylate (**21** and **22**). This mixture could not be separated; however, when treated with triflic anhydride in the presence of Hunig's base, the 4-methyl isomer was preferentially converted to enol triflate **23**, which could be easily purified by silica gel chromatography. Enol triflate **23** was carried through the reductive cyclization route to provide methyl cyclopentane **2e**.

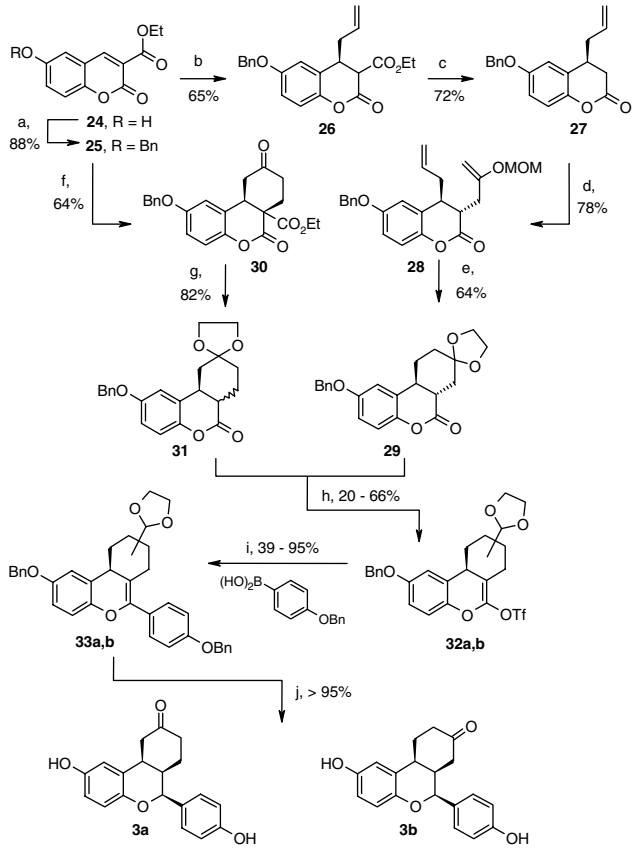
The synthesis route that led to cyclohexanones **3a** and **3b** is described in Scheme 4. The approach to cyclohexanone **3a** utilized a Diels–Alder reaction as the key step to install the C-ring. Beginning with hydroxy-coumarin



Scheme 2. Benzopyrans prepared from ketone intermediate **13**. Reagents and conditions: (a) DAST, $ClCH_2CH_2Cl$, 40 °C; (b) $TMSCF_3$, TBAF, THF; (c) i—$ClCOCOOCH_3$, DMAP, Et_3N, CH_2Cl_2; ii—Ph_3SiH, AIBN, toluene, 80 °C; (d) HCl, H_2O, THF.



Scheme 3. Synthesis of methyl analog **2e**. Reagents and conditions: (a) H_2SO_4, MeOH, 60 °C; (b) NaOMe, MeOH, toluene, 70–110 °C; (c) Tf_2O, *i*-Pr_2EtN, CH_2Cl_2, −78 °C; (d) reductive cyclization route.



Scheme 4. Olefin metathesis and Diels–Alder routes to cyclohexanones **3a** and **3b**. Reagents and conditions: (a) NaH, BnBr, DMF; (b) allylmagnesium bromide, THF, 0 °C; (c) i—LiOH, THF/H_2O/MeOH; ii—*o*-xylenes, reflux; (d) LDA, THF, −78 °C, then 2-*O*-methoxymethylallyl iodide; (e) i—Grubbs' catalyst second gen (0.3 equiv), toluene; ii—HCl, THF/H_2O; iii—ethylene glycol, TsOH, toluene; (f) 2-trimethylsilyloxy butadiene, *o*-xylenes, 135 °C, then pour into TBAF/AcOH; (g) i—LiOH, THF/H_2O/MeOH; ii—*o*-xylenes, reflux; iii—ethylene glycol, TsOH, toluene; (h) KHMDS (**3a**) or LDA (**3b**), PhN_2Tf, HMPA/THF; (i) boronic acid, $Pd(PPh_3)_4$, LiCl, Na_2CO_3, DME, reflux; (j) i—H_2, Pd/C, MeOH; ii—HCl, THF/H_2O.

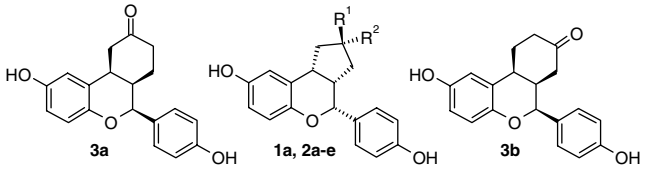
24,[16] benzyl protection of the phenol afforded benzyl ether **25**. Diels–Alder reaction with 2-trimethylsilyloxybutadiene under thermal conditions afforded tricycle **30**. The β-keto ester was decarboxylated via a two-step protocol, and the ketone moiety of the resultant intermediate was protected as a ketal to afford **31**. Conversion of ester **31** to an enol triflate **32a** followed by Suzuki coupling with 4-benzyloxyphenyl boronic acid yielded the fully elaborated tetracycle **33a**. Finally, joint reduction of the enol and benzyl ethers followed by deprotection of the ketal group afforded cyclohexanone **3a** in good yield.

The key step in forming the cyclohexanone ring of **3b** involved ring-closing metathesis (RCM) of an intermediate enol ether. To this end, Michael addition of allyl magnesium bromide to **25** afforded the desired β-keto lactone **26** containing the first RCM tether as an inconsequential mixture of diastereomers. A two-step saponification protocol afforded the β-allyl lactone **27**. The enol ether portion of the RCM substrate was installed under basic conditions, alkylating with 2-*O*-methoxymethylallyl iodide[17] to yield the RCM substrate **28**, which was subjected to the second generation Grubb's catalyst[18] to affect ring closure. Hydrolysis of the intermediate enol ether followed by ketal protection of the resultant ketone afforded tricycle **29** in good yield. Conversion of **29** to **3b** followed the same route described for **3a**.

The C-ring derivatives described above were separated by chiral chromatography (Chiralpak AD, heptane-isopropanol) and evaluated for their ability to bind estrogen receptors α and β. SAR for the more potent enantiomeric series is presented in Table 1.[19] As can be seen, structural variation in the C-ring of SERBA-1 (**1a**) had a profound impact on binding affinity and selectivity. Although addition of a ketone at the apical position of the cyclopentane ring (**2a**) resulted in a 36-fold loss in affinity to ERβ, it caused a much greater 260-fold loss in affinity to ERα. As a result, cyclopentanone **2a** is 100-fold selective for ERβ over ERα. Cyclohexanones **3a** and **3b** demonstrate that the position of the ketone functional group is important. Cyclohexanone **3a** was almost devoid of affinity for either receptor while its regioisomer **3b** was nearly identical in affinity and selectivity to cyclopentanone **2a**. Although the selectivities of the ketone analogs **2a**, **3a**, and **3b** were encouraging, their affinities were not competitive with SERBA-1 (**1a**). We were able to improve affinity by preparing fluorinated analogs **2b**–**d**. The difluoro analog **2b** was 19-fold selective for ERβ with affinity nearly identical to **1a** (0.44 nM vs 0.19 nM). Both diastereomers of the trifluoromethyl cyclopentanes **2c** and **2d** possessed nearly the same selectivity for ERβ as the difluoro analog **2b** with the *endo*-diastereomer **2d** being slightly less potent at both receptors. The corresponding *endo*-methyl diastereomer **2e** possessed affinity and selectivity closer to SERBA-1 (**1a**).

The cocrystal structures of benzopyran **2b** with ERα and ERβ are shown in Figure 2. As can be seen, the same binding orientations are observed in these structures as was seen in the structures of benzopyran **1a** bound to ERα and ERβ, which were reported previously.[5] The D-ring phenols interact with the hydrogen bond network of the Glu-Arg–H_2O triad, while the A-ring phenols interact 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,

Table 1. C-ring modifications: ERα and ERβ binding data[a]



Compound	R^1	R^2	ERβ[b] (nM)	ERα[b] (nM)	Ratio
1a	H	H	0.19 ± 0.09	2.70 ± 1.5	14
2a	=O		6.92 ± 0.62	700 ± 170	101
3a			375 ± 46	>1000	⩾2.7
3b			10.2	410	40
2b	F	F	0.44 ± 0.26	8.3 ± 2.7	19
2c	CF_3	H	0.41 ± 0.07	7.2 ± 0.8	17
2d	H	CF_3	0.70 ± 0.45	12.4 ± 4.9	18
2e	H	CH_3	0.31 ± 0.19	3.3 ± 2.4	11

[a] All compounds are from the more potent enantiomeric series as drawn except **3a** and **3b** which are racemic.
[b] K_i values are means of at least two determinations ±SD.

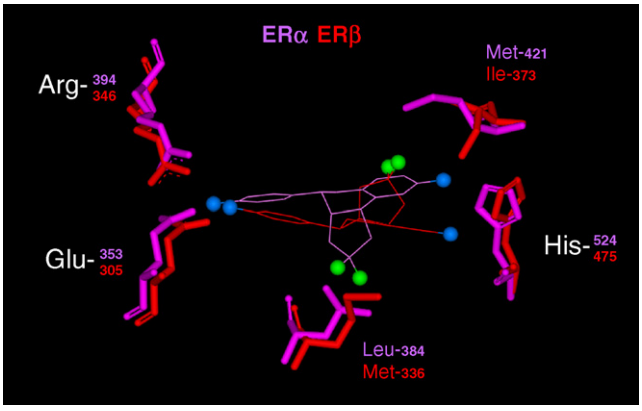


Figure 2. Diagram of the X-ray structure of the difluoro analog **2b** bound to ERα (purple) and ERβ (red). The fluorine atoms are marked with green spheres while the phenol oxygens are colored blue.

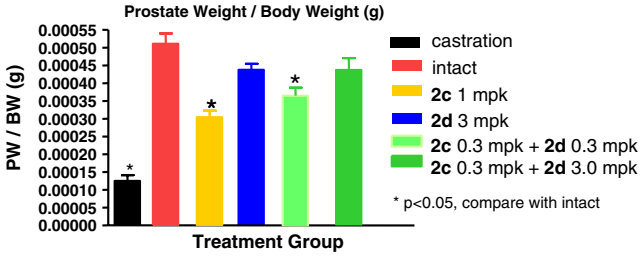


Figure 3. Effect of compounds **2c** and **2d** on prostate wet weight in CD-1 mice, measured after 7 days of oral daily doses (mpk = mg/kg). Prostate weights (PW) were normalized to body weight (BW) and the quotient PW/BW is reported. Castrate and intact controls are also shown. *Statistically significant ($P < 0.05$).

the benzopyran scaffold is rotated by 180° on the bisphenol axis. As a result, the difluoromethylene substituted 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 addition, the A-ring phenol is found to be above His-524 in ERα (as drawn in Fig. 2), while in ERβ the A-ring phenol is shifted down so that it is below the corresponding His-475.

Although the two trifluoromethyl cyclopentane diastereomers **2c** and **2d** possessed nearly the same in vitro properties, their in vivo activities were quite different (Fig. 3). We tested these compounds in a mouse model designed to evaluate ERβ agonist effects on the mouse prostate.[5] After 7 days of oral dosing at 1 mpk (mg/kg), the *exo*-diastereomer of the trifluoromethyl analog (**2c**) caused a significant reduction in prostate weight compared to intact control animals. The effect of the *endo*-diastereomer **2d** was not statistically significant at 3 mpk. Interestingly, when the inactive diastereomer was co-dosed with the active diastereomer, both at 0.3 mpk, a statistically significant reduction of prostate weight was observed, but when the dose of **2d** was increased to 10× the dose of **2c** (3.0 and 0.3 mpk, respectively) the effect on prostate weight was eliminated. This result suggests that compound **2d** is able to antagonize the effects of **2c**.

In summary, we were able to increase the binding selectivity of the benzopyran scaffold by modifying the cyclopentane C-ring of benzopyran **1a**. The addition of a ketone functional group at the apical position of the ring significantly increased selectivity at the expense of affinity. We were able to gain the affinity back and maintain good selectivity up to 19-fold with fluorinated analogs. The cocrystal structures of the difluoromethylene analog **2b** demonstrated similar binding orientations compared to the unfunctionalized benzopyran **1a**. Although the *endo*- and *exo*-diastereomers of the trifluoromethyl analog possessed nearly identical in vitro binding profiles, their activities in vivo were markedly different. Further attempts to increase the selectivity of the benzopyran scaffold will be disclosed in subsequent papers.

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19. The absolute stereochemical assignments of benzopyrans **2a**, **2b**, and **2c** are based on cocrystal X-ray structure data with either ERα or ERβ. The absolute stereochemistry of **2e** is defined by the starting material (+)-3-methylhexanedioic acid (**19**). The absolute stereochemistry of **2d** is assumed by analogy. The X-ray data for **2b** bound to ERα and ERβ (Fig. 2) have been deposited into the Protein Data Bank with accession codes 2Q70 and 2Z4B, respectively.