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Tetrahydrofluorenones with conformationally restricted side chains as selective estrogen receptor beta ligands

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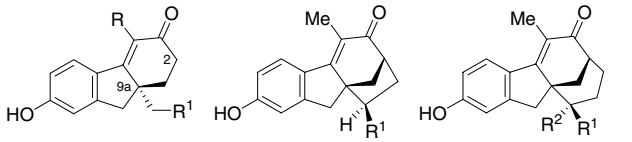
Abstract—A series of 2–9a bridged tetrahydrofluorenone derivatives were prepared which exhibited significant binding affinity for ERβ and were highly selective.

The estrogen receptor (ER) is a member of the superfamily of nuclear hormone receptors which act as ligand activated transcriptional factors.[1] The estrogen receptor was first cloned in 1986 and, at the time, was thought to be the exclusive receptor mediating physiological responses to estradiol.[2,3] In 1996 a second estrogen receptor was discovered and identified as ERβ,[4,5] the original cloned receptor being named ERα. It was found that ERβ is widely expressed in a variety of tissues, including lung, prostate, ovarian granulosa cells, and brain, but is not the dominant ER expressed in the uterus or breast.[6,7] The discovery of ERβ and its differential tissue expression stimulated efforts both to define the relative physiological roles of ERα and ERβ and to identify subtype selective ligands.

Both estrogen receptors (human) show substantial homology in the DNA binding domain (96%) and, to a lesser extent (58%), in the ligand binding domain (LBD).[5] The ligand binding pocket differs in only two amino acids; the ERβ binding pocket has a Met336 replacing a Leu384 in ERα and an Ile373 replacing a Met421.[8] Given the subtle differences in the LBD it is not surprising that estradiol binds equally well to both receptors.

Several groups have disclosed efforts to design ERβ subtype selective ligands working from a variety of diverse structural platforms including tetrahydrochrysenes,[9] 6*H*-benzo[*c*]chromen-6-ones,[10] androstenediols,[11] diarylpropionitriles,[12] benzimidazoles,[13] arylbenzothiophenes,[14] arylbenzoxazines,[15] triazines,[16] biphenyls,[17,18] aryl diphenolic azoles,[19] and 2-phenyl-benzofurans.[20]



1 R=Me, R^1=Me
2 R=Me, R^1=Et
3a R=Me, R^1=Pr
3b R=Br, R^1=Pr

4 R^1=H
5 R^1=Pr

6 R^1=H, R^2=H
7a R^1=Et, R^2=H
7b R^1=H, R^2=Et
8a R^1=Pr, R^2=H
8b R^1=H, R^2=Pr

Our effort in this area has focused on the tetrahydrofluorenone platform. We recently disclosed a new class of tetrahydrofluorenone compounds as potent ERβ selective agonists (e.g. **1**–**3**).[21] Within this series the 9a alkyl substituent of these compounds was shown to be a key determinant of binding potency and selectivity. In addition, the 9a-(*S*) configuration of this substituent was found to be essential for good activity. In this report, we have explored the effect of conformationally restricting this key binding element by incorporating it into a constrained ring system. The derivatives prepared include the simple ethylene and propylene bridged compounds **4** and **6**. These can be viewed as conformation-

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ally restricted extensions of compounds **1** and **2**. Building upon these two derivatives, pendant alkyl chains were incorporated in analogs **5**, **7a**, and **8a**. The comparison of compounds **4** and **6** to the crystallographically determined conformation of **3b** in complex with hERβ[21] (Fig. 1) indicated that the pendant alkyl chains would have to be attached exo to the bridged ring platform in order to map the substituents to the butyl of **3b**.

The synthetic routes to racemic bridged analogs **4** and **6** are described in Scheme 1. With the exception of the first step, the routes to the two analogs are similar. The route to **4** started with the reductive alkylation of 5-methoxy indanone **9** with commercially available glycoaldehyde dimer to give the 2-alkylated indanone **10a**. The route to **6** began from the alkylation of **9** with 3-bromotriethylsilyloxypropane[22] to give **10b**. Robinson annulation conditions employing ethyl vinyl ketone (EVK) with **10a** and **10b**, followed by cyclization under acidic conditions, and deacetylation of the resulting acetates gave the alcohols **11a** and **11b**. Mesylation of **11a** and **11b**, followed by their conversion to iodides and cyclization with LDA gave the products **12a** and **12b**. The final step involved demethylation of the aromatic methyl ethers and was accomplished with aluminum chloride and ethanethiol to give phenols **4** and **6**.

As mentioned above, the preparation of the chiral ethylene bridged compound **5** bearing a pendant propyl chain was guided by the need to establish the two contiguous stereochemical centers. Thus, the stereochemistry of the pendant propyl chain was established at the start with the alkylation of (*S*)-3-propylbutyrolactone[23] with 2-methoxy benzylbromide to give **13** (see Scheme 2). Internal Friedel–Crafts alkylation of **13** in polyphosphoric acid gave indanone **14** in low yield. Michael addition of EVK in the Robinson annulation sequence gave diastereomers **15a** (21%) and **15b** (57%). The desired diastereomer **15a**[24] was processed following chemistry previously described to give **16**. Demethylation of **16** afforded the desired product **5**.

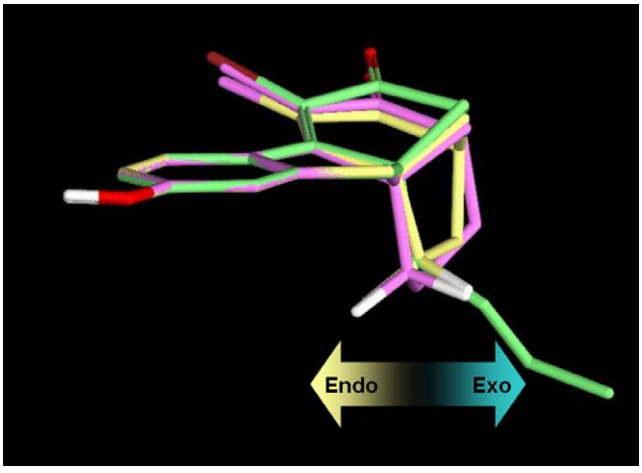
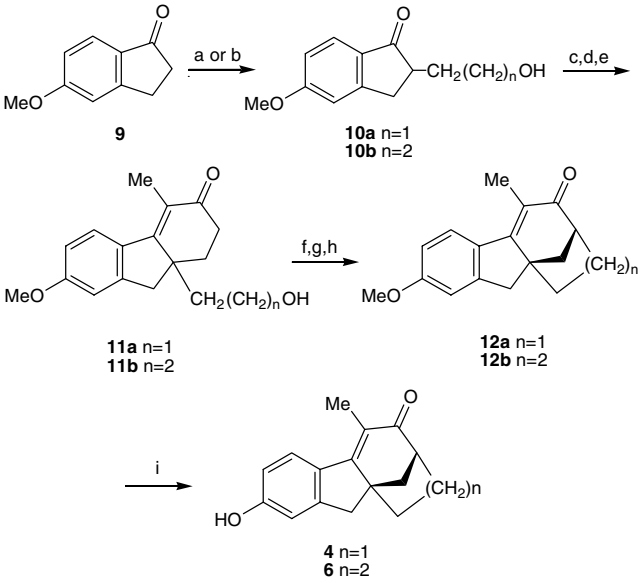
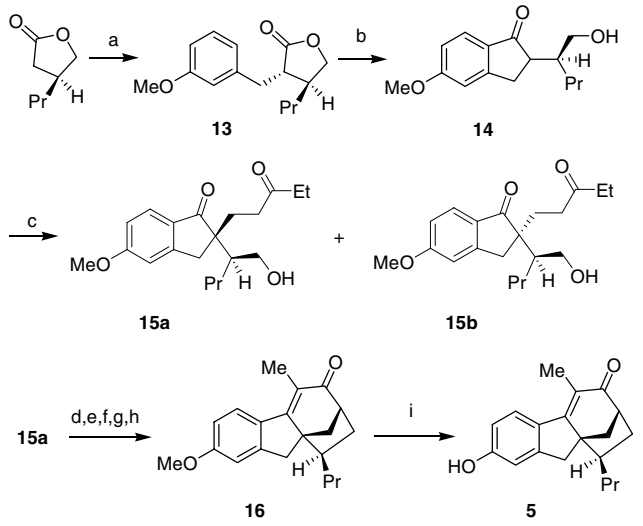


Figure 1. Superposition of the non-bridged **3b** (green) as determined in a crystallographic complex with hERβ and modeled bridged compounds **4** (yellow) and **6** (purple).



Scheme 1. Reagents and conditions: (a) glycoaldehyde dimer, NaOMe, H_2, 10% Pd/C, MeOH, rt; (b) 1-bromo-3-triethylsilyloxypropane, NaH, DMF, rt; (c) EVK, NaOMe, MeOH, rt; (d) 6 N HCl, HOAc, 80 °C; (e) NaOMe, MeOH, rt; (f) MsCl, Et_3N, CH_2Cl_2, 0 °C; (g) NaI, acetone, reflux; (h) LDA, THF, −78 °C to rt; (i) $AlCl_3$, EtSH, CH_2Cl_2, rt.



Scheme 2. Reagents and conditions: (a) LDA, 3-methoxybenzyl bromide, THF, −78 °C; (b) PPA, 100 °C; (c) EVK, NaOMe, MeOH, 60 °C; (d) 6 N HCl, HOAc, 80 °C; (e) 6 N HCl, MeOH, rt; (f) MsCl, Et_3N, CH_2Cl_2, 0 °C; (g) NaI, acetone, reflux; (h) LDA, THF, −78 °C to rt; (i) $AlCl_3$, EtSH, CH_2Cl_2, rt.

The relative stereochemical assignment of **5** was made on the basis of ^1H NMR[25] utilizing COSY and NOESY experiments (see Fig. 2 for key NOESY interactions).[26]

The racemic propylene analogs **7a**, **7b**, **8a**, and **8b** were prepared as described in Scheme 3 following chemistry similar to that previously described. Michael addition of the appropriate acrylate to indanone **9** gave the alkylated indanones **16a** and **16b**. Reduction of the esters

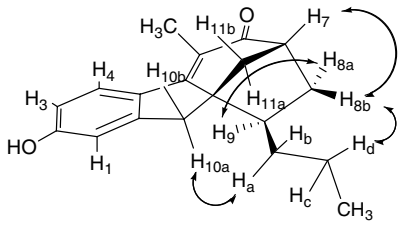
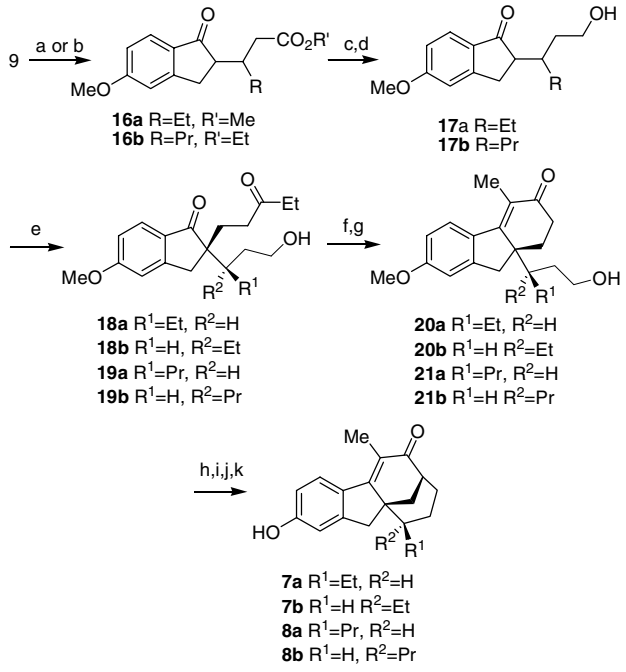


Figure 2. Key ^{1}H NOESY interactions observed.



Scheme 3. Reagents and conditions: (a) LDA, methyl 2-pentenoate, THF, −78 °C; (b) LDA, ethyl 2-hexenoate, THF, −78 °C; (c) LAH, THF, 0 °C; (d) Jones reagent, acetone, −78 °C; (e) EVK, NaOMe, MeOH, 60 °C; (f) 6 N HCl, HOAc, 80 °C; (g) 6 N HCl, MeOH, rt; (h) MsCl, Et_3N, CH_2Cl_2, 0 °C; (i) NaI, acetone, reflux; (j) LDA, THF, −78 °C; (k) $AlCl_3$, EtSH, CH_2Cl_2, rt.

16a and **16b** also resulted in the reduction of the indanone ketone to give diols which were reoxidized to provide the requisite indanones **17a** and **17b**. Robinson annulation conditions using EVK with indanone **17a** gave diastereomers **18a** (31%) and **18b** (44%). Similar reaction of **17b** gave diastereomers **19a** (21%) and **19b** (52%). Cyclization of the alcohols **18a**, **18b**, **19a**, and **19b** gave the tetrahydrofluorenone acetates which were deacetylated to give the alcohols **20a**, **20b**, **21a**, and **21b**, respectively. Following established chemistry these intermediates were converted to **7a** and **7b**, and **8a** and **8b**.

The ER binding results of analogs prepared are shown in Table 1. Comparison of the ethylene bridged analog **4** to the unbridged ethyl analog **1** shows at least equivalent or slightly better ERβ binding with an improvement in selectivity. Comparison of the propylene bridged analog **6** to its unbridged equivalent **2** shows equal ERβ binding and selectivity. Both the ethylene **4** and propylene **6** bridged analogs have similar ERβ binding, although **4** shows better selectivity.

Table 1. Binding affinities[27]

Compound	Human ERα IC_{50} (nM)	Human ERβ IC_{50} (nM)	Selectivity ERα/ERβ
1	1210	28	43
2	455	14	33
3a[a]	567	19	30
4	793	12	66
5[a]	97	1	97
6	414	11	37
7a	146	5	29
7b	310	45	7
8a	84	5	16
8b	605	141	4

[a] Chiral, all others racemates.

Analog **5**, which incorporates a pendant propyl chain in the ethylene bridge, shows significantly more potency in ERβ binding and more selectivity than its parent **4**. Moreover, compound **5** with its conformationally restricted side chain shows a greater than 10-fold enhancement in ERβ potency and more than a doubling of selectivity relative to its unconstrained analog **3a**.

The binding results shown in Table 1 for the pairs of compounds **7a**, **7b**, and **8a**, **8b** clearly confirm the modeling prediction of a preference for exo substitution on the bridge and show the significant effect that orientation of the pendant chain has on binding affinity towards both ERα and ERβ. The more active propylene bridged analogs **7a** and **8a**, incorporating either a pendant ethyl or propyl chain in the exo orientation, showed equal ERβ binding, although the selectivity of **7a** was approximately twice that of **8a**. Both of these analogs were less potent in ERβ binding and significantly less selective than the ethylene analog **5** with a pendant propyl chain.

As has been described previously for the tetrahydrofluorenone lead class,[21] we believe that the ERβ selectivity for the bridged variant arises principally from two sources. The first is the planar nature of the tetrahydrofluorenone core which is maintained in the bridged system. This putative stabilizing interaction between the planar/aromatic surface of the tricyclic platform and Met336 of hERβ is not possible with the analogous Leu384 in hERα (see Fig. 3). This feature appears to be common to other planar ERβ selective molecules including some phytoestrogens such as genistein[28] or other synthetic molecules including some benzisoxazoles,[19] for example.

The second selectivity determinant postulated for the tetrahydrofluorenone class is a favorable hydrophobic interaction of the 9a-alkyl substituent which protrudes orthogonally from the plane of the tricyclic core toward Ile373 in hERβ as depicted in Figure 3. We speculate that Ile373 in hERβ can nicely accommodate the presence of the alkyl substituent into space which is not available in hERα because the side chain of the analogous Met421 fills this space. Fixing the 9a-alkyl substituent into the proper orientation as in compound **5** may enhance this favorable hydrophobic

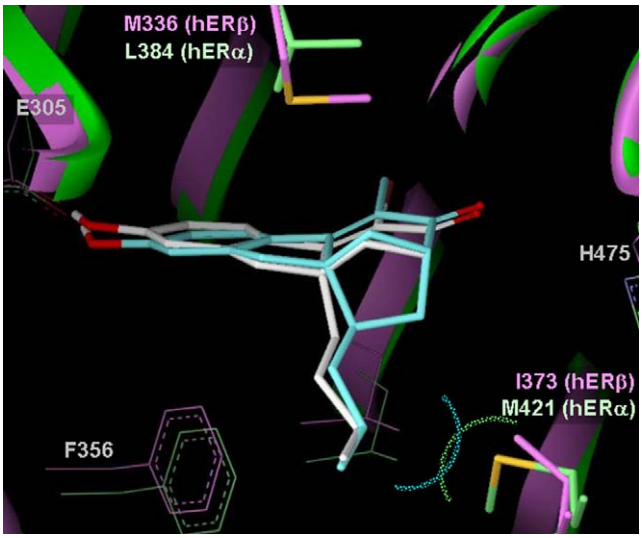


Figure 3. Superposition of the docked model of compound **5** (cyan) with the crystallographic complexes of compound **3b** (white) with hERβ (purple) and hERα (green) (pdb entry: 1ERE). Unless otherwise indicated, residue numbering is that of hERβ.

interaction and thus account for its increased ERβ potency and selectivity relative to the corresponding unconstrained analog **3a**. The reduced potency and selectivity of the propylene bridged analog **8a** may be a consequence of its greater conformational flexibility and/or a less favorable orientation of the pendant propyl group.

In summary, we have designed a series of conformationally constrained 2–9a bridged tetrahydrofluorenones and incorporated pendant alkyl chains to optimize ERβ binding affinity and selectivity. The ethylene bridged analog **5**, which conformationally constrains the butyl side chain of **3a**, exhibits improved ERβ affinity by an order of magnitude while increasing subtype selectivity.

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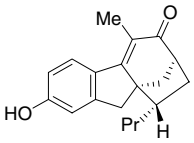
22. Prepared from the reaction of 3-bromopropanol with chlorotriethylsilane.
23. Prepared according to the method of Bode, J. W.; Doyle, M. P.; Protopopova, M. N.; Zhou, Q-L. *J. Org. Chem.* **1996**, *61*, 9146.
24. Stereochemistry determined retrospectively from **5**.
25. ^{1}H NMR (benzene-d_6): δ 0.65–0.72 (1H, m, H_b), 0.70 (3H, t, J = 7.1 Hz, Me), 0.80 (1H, m, H_c), 1.05 (1H, m, H_d), 1.21 (1H, m, H_a), 1.40 (1H, ddd, J = 4.8, 7.1, 13.3 Hz, H_{8b}), 1.48 (1H, dd, J = 4.3, 11.1 Hz, H_{11b}), 1.55–1.60 (1H, m, H_9), 1.59 (1H, d, J = 11.1 Hz, H_{11a}), 1.77 (1H, dd, J = 9.7, 13.3 Hz, H_{8a}), 2.18 (3H, s, Me), 2.49 (1H, d, J = 17.0 Hz, H_{10b}), 2.79 (1H, d, J = 17.0 Hz, H_{10a}), 3.00 (1H, dd, J = 4.3, 7.1 Hz, H_7), 6.54 (1H, d, J = 8.4 Hz, H_3), 6.59 (1H, s, H_1), 7.43 (1H, d, J = 8.4 Hz, H_4).
26. Compound **15b** was also converted by Scheme 2 to give the diastereomer shown below:



A ^{1}H NOESY experiment similar to that done for **5** showed the propyl function to be endo.

27. The IC50 values were generated in an estrogen receptor ligand binding assay. This scintillation proximity assay was conducted in NEN basic flash plates using tritiated estradiol and full length recombinant human ERα and ERβ proteins, with incubation times of 3–22 h. Most compounds are single point determinations.
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