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6*H*-Benzo[*c*]chromen-6-one derivatives as selective ERβ agonists

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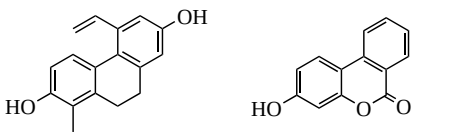
Abstract—A series of 6*H*-benzo[*c*]chromen-6-one and 6*H*-benzo[*c*]chromene derivatives were prepared, and the affinity and selectivity for ERα and ERβ was measured. Many of the analogs were found to be potent and selective ERβ agonists. Bis hydroxyl at positions 3 and 8 is essential for activity in a HTRF coactivator recruitment assay. Additional modifications at both phenyl rings led to compounds with ERβ < 10 nM potency and >100-fold selectivity over ERα.

Until 1996, estrogens were assumed to mediate their effects through a single nuclear receptor (now called ERα). The discovery of a new estrogen receptor named ERβ, in 1996,[1] followed by tissue distribution studies[2] which showed that the expression of the two ERs is not coincident in tissues, has led to renewed interest in estrogen receptor modulators, especially compounds that are selective for the two subtypes ERα and ERβ. X-ray crystal structures of receptor–ligand complexes of ERα and ERβ[3] show that the binding pockets of the two receptors are very similar and differ by only two amino acids. The leucine present at position 384 in ERα is replaced by a methionine in ERβ (Met 336) and the methionine in position 421 is replaced by an isoleucine in ERβ (Ile373). In light of this it is not surprising that most previously known estrogens and SERMs bind fairly non-selectively to the two receptors. However, recent reports have described compounds that bind selectively to ERα and ERβ.[4]

Our search for ERβ agonists started with the natural product Effusol which has a simple dihydrophenanthrene structure (Fig. 1). This compound bound to ERβ with an IC_{50} = 12 nM and was 20× selective.[5] However, the dihydrophenanthrene ring system, because of its easy oxidation to the phenanthrene analog was considered unsuitable. Substituting the dihydrophenanthrene by a 6*H*-benzo[*c*]chromen-6-one retained the geometry of the dihydrophenanthrene without the possibility of its oxidation to a phenanthrene. This publication describes the synthesis and SAR of such 6*H*-benzo[*c*]chromen-6-one derivatives as selective ERβ agonists. Compounds with a similar core structure with SERM activity have been reported recently.[6]



Effusol — 3-Hydroxy-6H-benzochromen-6-one

Figure 1.

Most of the benzo[*c*]chromenone analogs were prepared by a procedure of Bruggink and McKillop[7] as shown in Scheme 1.[8]

Treatment of a resorcinol (2 equiv) with a substituted bromobenzoic acid, catalyzed by $CuSO_4$ in the presence of 2 equiv of NaOH in water at 100 °C, gave the desired product in most cases as a solid, which was isolated by filtration, washing with water, and drying to give the clean product. The yields varied from 10% to 60% depending on the substitution of the bromobenzoic acid.

Keywords: Selective estrogen receptor modulator; ERβ agonist; 6*H*-Benzo[*c*]chromen-6-one.
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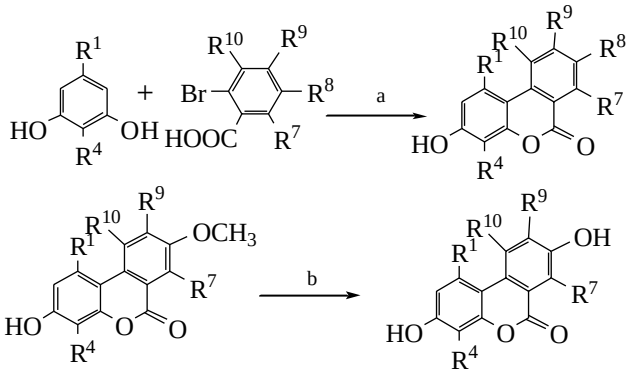
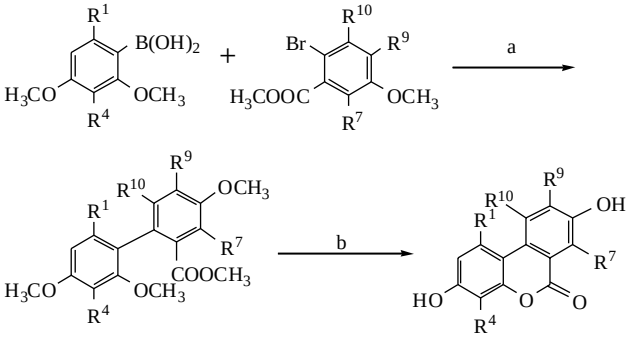


Scheme 1. Synthesis of 6*H*-benzo[*c*]chromen-6-one Reagents and conditions: (a) 2 equiv, 5 N NaOH, 10% aq $CuSO_4$, 100 °C; (b) BBr_3, CH_2Cl_2, rt.

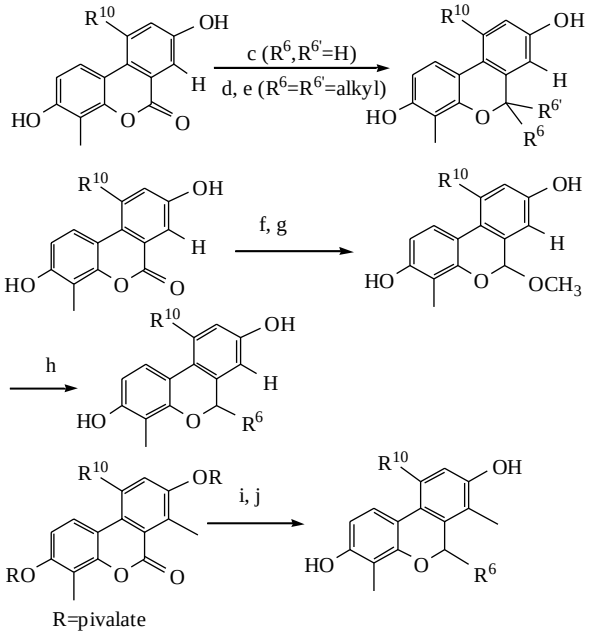
8-Hydroxy analogs were prepared by treatment of the corresponding 8-methoxy compounds with BBr_3.

Alternatively (Scheme 2) a suitably substituted aryl boronic acid was coupled to a 2-bromo-benzoic acid ester in the presence of $(Ph_3P)_4Pd$ catalyst and Na_2CO_3 in EtOH/DME to give the corresponding biphenyl. Removal of the methyl-protecting groups with BBr_3 gave the desired products.

As shown in Scheme 3, when R^7 was H, 6,6-unsubstituted 6*H*-benzo[*c*]chromenes were prepared by reduction of 6*H*-benzo[*c*]chromene-6-ones with boron trifluoride etherate–sodium borohydride.[9] 6-Monosubstituted 6*H*-benzo[*c*]chromenes were synthesized by reducing the lactones to lactols with DIBAL in THF followed by treatment with MeOH and aq HCl to give the 6-methoxy-6*H*-benzo[*c*]chromenes. Reaction of this intermediate with various Grignard reagents in benzene gave the corresponding 6-monosubstituted 6*H*-benzo[*c*]-chromenes.[10] 6,6-Disubstituted 6*H*-benzo[*c*]chromenes were prepared by treatment of the lactone with excess of corresponding Grignard reagents followed by treatment with boron trifluoride etherate to effect dehydrative cyclization of the intermediate carbinol.[9] When R^7 was CH_3, the dipivalate of the chromenone was reacted with excess Grignard reagent and gave the product of mono



Scheme 2. Alternative synthesis of 6*H*-benzo[*c*]chromene-6-ones. Reagents and conditions: (a) $(Ph_3P)_4Pd$, Na_2CO_3, EtOH, DME, 80 °C, 2–24 h; (b) BBr_3, CH_2Cl_2, 0°–rt, 1–3 h.



Scheme 3. Synthesis of 6*H*-benzo[*c*]chromenes. Reagents and conditions: (c) BF_3–Et_2O, $NaBH_4$, THF, Rfx, 1 h; (d) 10 equiv R^6MgX, benzene, 80 °C, 2 h; (e) BF_3–Et_2O, benzene; (f) 1.3 equiv DIBAL, THF −78 °C; (g) MeOH, 2 N HCl; (h) 4 equiv R^6MgX, benzene, 80 °C, 2 h; (i) 10 equiv R^6MgX, C_6H_6, 80 °C, overnight; (j) 5 equiv Et_3SiH, 10 equiv TFA, CH_2Cl_2, rt, 1 h.

addition only. The resulting 6-carbinol was reduced to the desired product with TFA and Et_3SiH.

The ERβ affinities and selectivity over ERα of the analogs were measured by a competitive binding assay.[11] The assay results are depicted in Table 1.

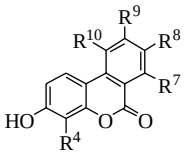
Compounds **23** and **25**, close analogs of Effusol, which have the methyl and the vinyl groups in positions 4 and 10, and a substituent on position 8, show binding affinity and selectivity similar, or better than that of Effusol, showing that the 6*H*-benzo[*c*]chromen-6-one is an excellent substitute for the dihydrophenanthrene ring system.

The importance of the 4-methyl group is shown by comparison of **6** versus **20** (H vs Me) and **3** versus **7** (Me vs Et). The Me group at position 4 appears to be extremely important for affinity and selectivity.

We discovered that position 7 needs a substituent such as Me, Et or bromo to improve the ERβ-binding affinities as well as the selectivity. Compounds **1** and **4**; **11** and **2**; **15** and **8**; **28** and **27**; **29** and **30** are five pairs of compounds that show more potency and ERβ selectivity when Me or Br replaces H on position 7. An ethyl group is only slightly poorer than methyl at this position (**30** vs **36**).

SAR study of position 8 revealed that along with the hydroxy at position 3, a hydroxy substituent here improves activity (**1** vs **29**). However, a different polar group, amino, lowers the binding affinity and selectivity (**1** vs **12**).

Table 1. Substituted 3-hydroxy-6*H*-benzo[*c*]chromene-6-ones



Compound	R^4	R^7	R^8	R^9	R^{10}	IC_{50} (nM)		ERα/ERβ
						ERβ	ERα	
Estradiol						1.2	1.35	1.1
Effusol						12	240	20
1	Me	Me	H	H	H	93	>10,000	107
2	Me	H	OMe	H	H	308	>10,000	32
3	Me	H	Br	H	H	128	>10,000	78
4	Me	H	H	H	H	1450	>10,000	7
5	Me	H	Vinyl	H	H	310	4590	15
6	H	H	Me	H	Me	90	585	6.5
7	Et	H	Br	H	H	2340	5460	2.3
8	Me	H	H	H	Me	326	>10,000	30
9	Me	H	H	H	vinyl	208	>10,000	48
10	Me	H	H	H	Propen-1-yl	116	3030	26
11	Me	Me	OMe	H	H	117	1000	85
12	Me	Me	NH_2	H	H	279	14,300	51
13	Me	Me	$NHSO_2CH_3$	H	H	433	4258	9.8
14	Me	Br	H	Br	H	24	597	25
15	Me	Me	H	H	Me	80	3074	38
16	Me	Me	NHCHO	H	H	957	>10,000	10
17	Me	H	Br	Me	H	215	6850	32
18	Me	H	OMe	Br	H	77	10,000	129
19	Me	H	Br	Buten-1-yl	H	26	1090	42
20	Me	H	Me	H	Me	20	3710	184
21	Me	H	Me	H	Br	44	3120	71
22	Me	H	Me	H	Allyl	35	10,000	285
23	Me	H	Me	H	Vinyl	10	10,000	1000
24	Me	H	OMe	H	Et	69	9830	142
25	Me	H	OMe	H	Vinyl	53	>10,000	188
26	Me	H	Me	Br	Me	57	>10,000	175
27	Me	H	Me	OMe	Me	16	4160	258
28	Me	Br	Me	OMe	Me	1.15	225	150

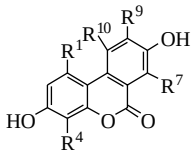
Increasing the acidity of the NH by substitution with electron-withdrawing groups (**13** and **16**) gave poorly active compounds because that position is unable to sustain a group much larger than methyl (**3**, **5**, and **11**). Compound **29** with a hydroxyl group showed very potent affinity (IC_{50} = 4.1 nM) with good selectivity.

Positions 9 and 10 seem to have enough room for at least a four-carbon chain: **19** and **22** have IC_{50} = 26 and 35 nM. Some of the most selective compounds in Table 1 (**21**–**28**) are substituted at the 10-position. Compound **23** with a vinyl at position 10 gave quite potent binding affinity (IC_{50} = 10 nM) with very high selectivity (1000-fold). Compound **28** with five substituents on the aromatic rings also has excellent binding and selectivity.

In general, it is noticeable that increasing the number of small hydrophobic substituents on the phenyl rings gives better ERβ binding affinities with significant increase in selectivity. Among the more substituted compounds, **23**, **27**, and **28** have outstanding binding and selectivity. However, most of the compounds in Table 1 showed large serum binding, exemplified by **23** (ERβ IC_{50} = 1276 nM, in the presence of serum, 142-fold-increase in IC_{50}), and were inactive in a HTRF (homogeneous time-resolved fluorescence) coactivator recruitment assay[12] (**23**, EC_{50} > 1000 nM). In contrast, **29** with two hydroxyl groups showed much less serum binding (IC_{50} = 78 nM in the presence of serum, 20-fold increase) and has an EC_{50} of 7.2 nM in the coactivator recruitment assay.

Table 2 presents the binding affinities for a series of compounds with OH at positions 3 and 8. There is pseudo-symmetry in these molecules which could allow either of the 2-hydroxy groups to bind at the same site as the phenolic group of estradiol and the only difference would be the way the lactone is oriented, Figure 2. Thus the SAR of these compounds has to be considered with this in mind. Three pairs (compounds **30** and **31**, **33** and **42**, **43** and **44**), which show this pseudo-symmetry, have comparable binding and selectivity. Compound **29** is an internally symmetric compound with good activity, while compounds **36** and **37** in which the positions of Me and Et are interchanged have similar activity and selectivity. Substitution at 4 and 7 is essential for good

Table 2. 3,8-Dihydroxy-6*H*-benzo[*c*]chromene-6-ones



Compound	R^1	R^4	R^7	R^9	R^{10}	IC_{50} (nM)		ERα/ERβ
						ERβ	ERα	
29	H	Me	Me	H	H	4.1	159	38.5
30	H	Me	H	H	H	46	654	14
31	H	H	Me	H	H	49	1170	24
32	Me	Me	H	H	H	27	488	18
33	Me	H	Me	H	H	67	1860	28
34	H	Cl	Cl	H	Me	18	2521	143
35	H	Me	OH	H	H	82	1280	16
36	H	Me	Et	H	H	14	484	35
37	H	Et	Me	H	H	7.8	229	29
38	H	F	Me	H	H	54	1580	29
39	H	F	Et	H	H	21	228	11
40	H	Br	Me	H	H	67	1920	29
41	H	Me	H	H	Et	91	1780	20
42	H	Me	H	H	Me	54	583	11
43	Me	Me	Me	H	H	6.7	785	115
44	H	Me	Me	H	Me	5.7	716	124
45	H	Cl	Cl	F	H	180	11,600	65
46	H	Cl	Me	Cl	H	19	524	28
47	H	Cl	Me	Cl	Me	22	597	27

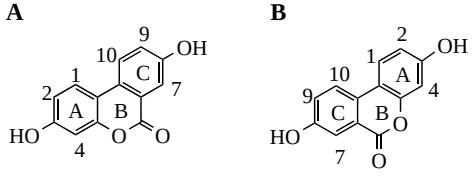


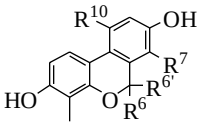
Figure 2.

binding and selectivity (**29**, **36**, and **37**). Again additional substitution at the 1 or 10 positions gives better selectivity (**43** and **44**). Analogs **43** and **44** possess high ERβ binding affinity and selectivity. Molecular modeling studies described below are in accord with these observations and provide a rationale for the high selectivity of **43** and **44**.

Analogs with halogen substituents in this series have less binding affinities than their corresponding methyl analogs (**44** vs **34**, **29** vs **38**, **36** vs **39**, and **29** vs **40**). Halogens at the 9-position reduce activity (**45** vs **34** and **46** vs **29**). A hydroxy group at position 7 as in **35** has poorer binding affinity its corresponding methyl analog compound **29**.

Table 3 presents the binding affinities for 6*H*-benzo[*c*]chromenes. In general, 6-monosubstituted analogs are more active than the 6-unsubstituted and 6-di-substituted derivatives (compare **48**, **51** and **58**). Among the 6-monosubstituted compounds **50**–**57**, the ethyl group gave the best binding affinities and selectivity. Compound **56**, **53** and **52** are better than their close methyl analogs **55**, **50** and **51**. The 6*H*-benzo[*c*]chromene

Table 3. Substituted 3-hydroxy-4-methyl 6*H*-benzo[*c*]chromenes



Compound	R^6,$R^{6'}$	R^7	R^{10}	IC_{50} (nM)		ERα/ERβ
				ERβ	ERα	
48	H, H	Me	H	88	4610	52
49	H, H	H	Me	74	1030	14
50	Me, H	H	Me	24	1210	50
51	Me, H	Me	H	8	335	42
52	Et, H	Me	H	3.2	101	32
53	Et, H	H	Me	8	435	54
54	Pr, H	H	Me	22	626	29
55	Me, H	Me	Me	9	237	26
56	Et, H	Me	Me	2.3	129	57
57	Isobutyl, H	H	Me	49	627	13
58	Me, Me	Me	H	45	468	10
59	Me, allyl	Me	Me	13	157	12

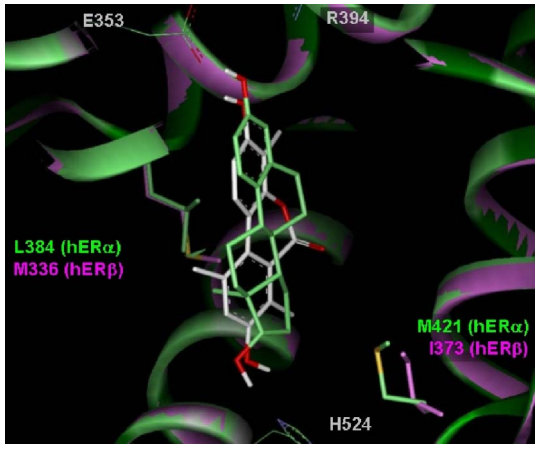


Figure 3. Molecular modeling of compound **44** (white) against estradiol (green). hERα is depicted in green and hERβ in purple. Residue numbering is hERα unless otherwise indicated.

(**56**) showed comparable binding activity and selectivity to 6*H*-benzo[*c*]chromene-6-one (**29**).

The docking and energy minimization approach[13], which will be described elsewhere, identified a binding mode for compounds **29**, **43**, and **44** (Fig. 3) consistent between the crystallographically determined ligand binding domains of human ERα (1ERE)[14] and ERβ (1QKM)[14] in which the tricyclic core of the chromenone derivatives spans the length of the steroid estradiol and the lactone ring is oriented in the binding pocket to map to the 'B' ring of the steroid away from helix 12. The high selectivity of these compounds for hERβ is presumably due, in part, to the ability of the aromatic ring at the 'C' ring position to better interact with the Met 336 side chain in hERβ. In the case of compound **29** in which the phenols are symmetrically substituted and either can mimic the estradiol 'A' ring position, energetics between each receptor and the two different binding

orientations slightly favor (by ~0.5 kcal/mol) that in which the 3-phenol is equivalent to the estradiol phenol (**Structure A**, Fig. 2) and the lactone carbonyl is directed toward His 524 (hERα numbering). For compounds **43** and **44**, substituted by a CH_3 at the 1 or the 10 position, respectively, the energetics between each receptor and the two different binding orientations clearly indicate (by ~15 kcal/mol) that the phenol which is least substituted interacts with the Glu/Arg residue pair at the top of the binding cavity. This appears to be primarily due to a steric clash between the added CH_3 and residues in helix 3 particularly Ala 350.

The additional CH_3 has another impact on the structure as well. The energy minimized structures of compounds not substituted at the 1 or 10 positions are essentially planar; the dihedral angle C1–C–C–C10 (where C–C is the biphenyl bond) in these is 0.4°. Conversely, substitution at positions 1 or 10 results in a twist about this same central bond with a concomitant pucker of the lactone ring presumably due to a steric clash between the CH_3 and the proton on the adjacent aromatic. For CH_3 substitution at position 1 (compound **43**), the dihedral angle about C1–C–C–C10 is ~11.5° and at position 10 (compound **44**) ~13.5°. In the models of the compounds **43** and **44** docked into hERα and hERβ, it appears that the twist and pucker of the lactone results in a closer and presumably repulsive interaction between the lactone and Leu 384 in hERα than is seen with Met 336 in hERβ.

We have shown that properly substituted 6*H*-benzo[*c*]chromenenones and 6*H*-benzo[*c*]chromenes yield very selective ERβ ligands which are active in a HTRF coactivator recruitment assay. Small hydrophobic groups, methyl and ethyl in positions 4, 7, and 10 are required for optimum binding and selectivity. Hydroxy groups at 3 and 8 positions are essential for activity in the HTRF coactivator recruitment assay. 6*H*-Benzo[*c*]chromenes are also active if they bear a methyl or ethyl group at the 6-position. The best compounds (**29**, **37**, **43**, **44**, **51**, **52**, **53** and **56**) have an $IC_{50} < 10$ nM and a selectivity over ERα ranging from 30 to 124×.

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