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ERβ ligands. Part 6: 6*H*-Chromeno[4,3-*b*]quinolines as a new series of estrogen receptor β-selective ligands

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Abstract—A new class of estrogen receptor beta (ERβ) ligands based on the 6*H*-chromeno[4,3-*b*]quinoline scaffold has been prepared. Several C7-substituted analogues displayed high affinity and modest selectivity for ERβ.

Estrogens are known to play a crucial role in mammalian reproductive system and also in many other non-reproductive organs such as skeletal, cardiovascular, and central nervous systems.[1] Estrogens exert their effects mainly by interactions with the estrogen receptor (ER), which is ligand-activated transcription factor and belongs to a superfamily of nuclear hormone receptors. The unexpected discovery of a second subtype of estrogen receptor, estrogen receptor beta (ERβ)[2], in 1996 necessitated renaming of the first estrogen receptor ERα. Because ERβ possesses unique tissue distribution patterns and transcriptional properties from those of ERα,[3] its discovery has prompted intense research to investigate ERβ as a potential new drug target[4] as well as to develop novel, tissue and cell-selective estrogens.[5] Recently, studies from Wyeth laboratories have demonstrated certain therapeutic potentials of ERβ-selective ligands.[6]

Although the ligand binding domains (LBD) of ERα and ERβ share only modest homology (58% identity), their ligand binding cavities are highly conserved, differing by only two amino acid residues (ERα Leu_{384} is replaced by ERβ Met_{336}, and ERα Met_{421} is replaced by ERβ Ile_{373}).[7] While this slight difference in the binding cavities poses a great challenge in developing ER subtype-selective ligands, medicinal chemistry efforts from our laboratories and others in recent years have yielded a number of structural motifs with impressive ERβ selectivity.[8]

We recently reported a series of 2-phenylquinoline ERβ-selective ligands,[9] which was developed based on the 6-phenylnapthalene scaffold.[10] The best compound in this report was **4** (Scheme 1), which exhibited ERβ binding IC_{50} value of 3.4 nM and 83-fold selectivity for ERβ. Docking of **4** into the ligand binding domain of the ERβ LBD/6-phenylnapthalene **3** cocrystal structure[10] placed the bromo group in close proximity to the ERβ Ile_{373} → ERα Met_{421} residue substitution (Fig. 1A), suggesting that the observed ERβ selectivity of **4** may be due to a differential interaction of the bromo group with these amino acid residues.[9,12] The docked structure of **4** also revealed a 16° dihedral angle between the pendant phenyl ring and the quinoline core. We anticipated that locking this dihedral angle by introducing a ring would restrict the rotational freedom about the bond connecting the two aryl planes, and generate a rigid quinoline framework that would mimic the docked conformation of **4**. This anticipation led us to investigate the 6*H*-chromeno[4,3-*b*]quinoline scaffold (Scheme 1). To retain similar geometrical arrangement as that of the 2-phenylquinoline, a hydroxyl group will be placed at positions 3 and 9, which are known to be essential for binding to ER. Docking of 6*H*-chromeno[4,3-*b*]quinoline-3,9-diol **7** (R = Br) into the ERβ LBD/**3** binding pocket reveals a 13° torsion angle between the phenyl ring and the

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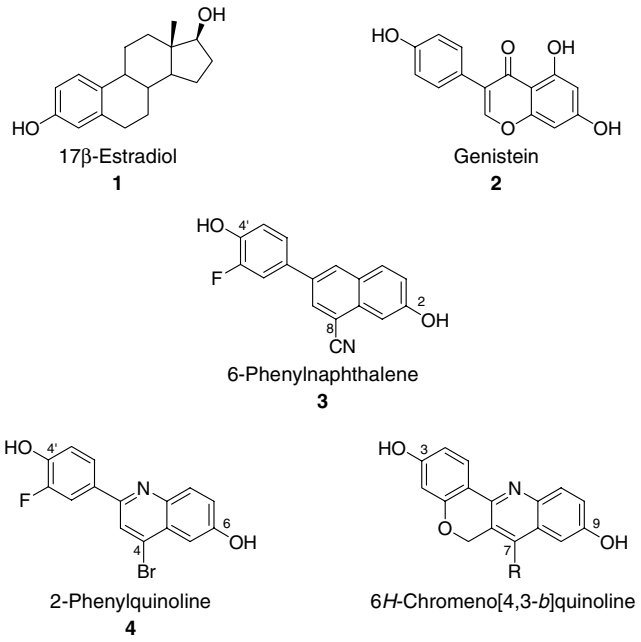


Scheme 1. Compounds of interest.

quinoline core (Fig. 1B), which is similar to the dihedral angle of the docked **4**. Overlaying docked structures of **4** and **7** shows a near perfect alignment (Fig. 1B). In addition, a variety of functional groups including electronegative, aliphatic, and aromatic substituents will also be introduced at position 7, which corresponds to position 4 of the 2-phenylquinoline scaffold and has been shown to be the preferred position to gain ERβ selectivity and/or binding affinity (Scheme 1 and Fig. 1B).[9] Herein, we describe the synthesis and structure–activity relationships of this novel series of 6*H*-chromeno[4,3-*b*]quinolines.

All 6*H*-chromeno[4,3-*b*]quinolines in Table 1 were synthesized as shown in Schemes 2 and 3. The synthesis began with alkylation[13] of 3-methoxyphenol and subsequent intramolecular Friedel–Crafts acylation[14] of the resulting 3-(3-methoxyphenyl)-propionic acid to give 7-methoxychroman-4-one (Scheme 2).[15] The construction of the 6*H*-chromeno[4,3-*b*]quinoline core was carried out using the Niementowski modification of the Freidländer synthesis.[16] Thus, condensation of 7-methoxychroman-4-one and 5-methoxyanthranilic acid gave **5**. Subsequent reaction with phosphorus oxychloride, followed by removal of the methyl protecting group, afforded the 7-chlorochromenoquinoline **6**. Treatment of **5** with phosphorus oxybromide, followed by demethylation furnished the bromo analogue **7**.

Compound **7** was also the common intermediate from which a number of 7-substituted 6*H*-chromeno[4,3-*b*]quinolines could be prepared using various transition metal-mediated cross-coupling reactions (Scheme 3). Thus, heating **7** with copper cyanide gave the cyano analogue **8**, whereas Stille coupling of **7** with tributyl(vinyl)tin afforded the vinyl derivative **9**. Similarly, reaction of **7** with (trimethylsilylethynyl)tributyltin[17] followed by desilylation yielded the alkynyl compound **10**, which upon reduction furnished the ethyl analogue **11**. Suzuki reaction of **7** with substituted phenylboronic acids provided targets **12**–**18**.

The 7-substituted 6*H*-chromeno[4,3-*b*]quinolines were evaluated in a competitive radioligand binding assay measuring the relative binding affinity (IC_{50}) of the compounds for the human ERα and ERβ LBD.[18] Results are presented in Table 1. As expected, endogenous ligand 17β-estradiol bound equally well to both ER isoforms in this assay.

The chloro analogue **6** exhibited high ERβ binding affinity (3.3 nM IC_{50}) comparable to that of 17β-estradiol, and a respectable ERβ selectivity (27-fold). Larger electronegative bromo substituent of **7** and electron-withdrawing cyano group of **8** also showed strong affinity for ERβ (nanomolar ERβ IC_{50} values), but with slightly lowered selectivity. Small aliphatic groups such as vinyl (**9**), alkynyl (**10**), and ethyl (**11**) all showed some ERβ selectivity, although with considerably weaker binding affinities. This affinity reduction for both ER isoforms may be attributed to a greater desolvation penalty caused by the increased basicity of the quinoline core in the absence of an electron-withdrawing substituent of analogues **9**–**11** (relative to derivatives **6**–**8**). Phenyl groups of analogues **12**–**18** bearing either electron-rich

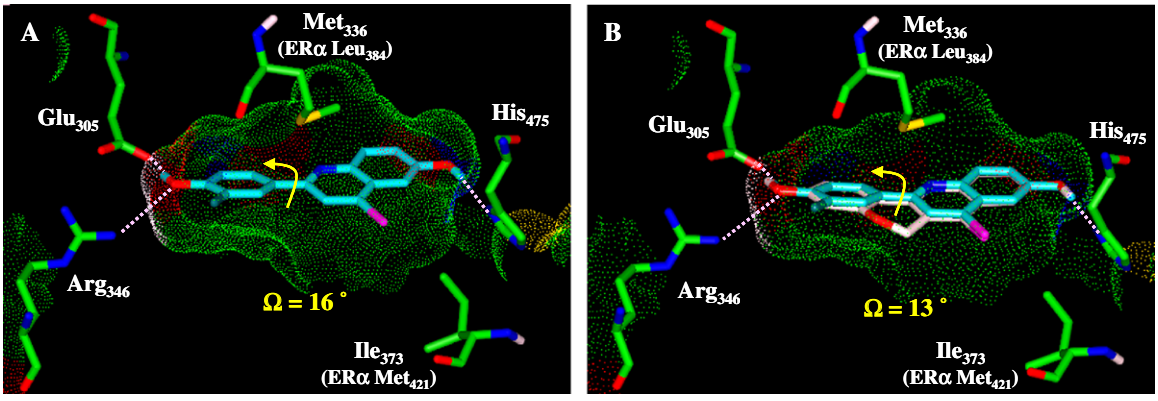
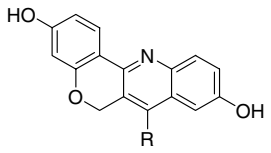
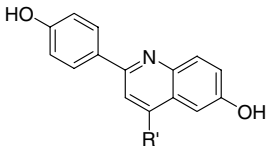


Figure 1. (A) Compound **4** docked to ERβ LBD/**3** complex; (B) Overlaying of docked **4** (cyan) with docked 6*H*-chromeno[4,3-*b*]quinoline **7** (white). Calculations were performed as described in Ref. 11. Only key residues and a Connolly surface of the ERβ binding site are shown for simplicity.

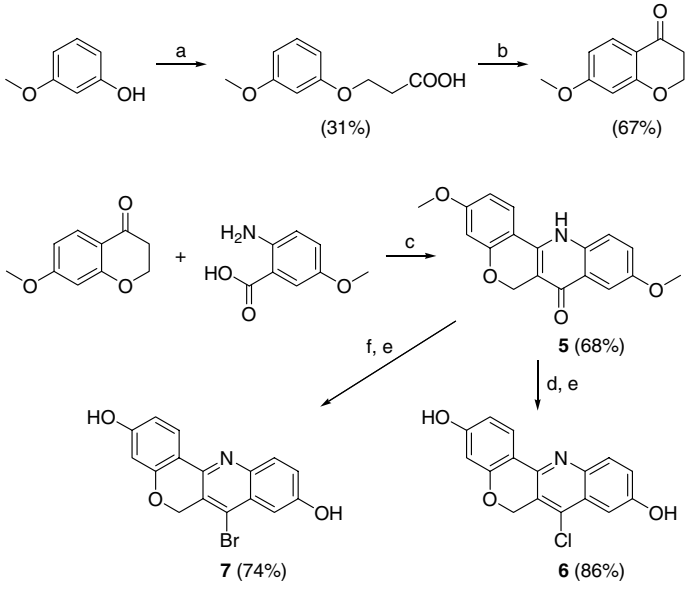
Table 1. Binding affinity for human ERα and ERβ ligand binding domain





Compound	R	ERβ IC_{50}[a] (nM)	ERα IC_{50}[a] (nM)	α/β[b]	Compound[c]	R′	ERβ IC_{50}[a] (nM)	ERα IC_{50}[a] (nM)	α/β[b]
1	17β-Estradiol	3.6 ± 1.6	3.2 ± 1.0	1					
2	Genistein	10 ± 4	395 ± 181	41					
6	Cl	3.3 ± 3.2	88 ± 19	27	**19**	Cl	4.6 ± 2.1	213 ± 38	46
7	Br	3.6 ± 3.5	56 ± 20	16	**20**	Br	4.3 ± 2.3	212 ± 87	50
8	CN	6.1 ± 0.7	98 ± 82	16	**21**	CN	28 ± 7	455 ± 107	16
9	CH_2=CH	22 ± 8.6	526 ± 31	24	**22**	CH_2=CH	60	504	8
10	HC≡C	52 ± 18	624 ± 510	12	**23**	HC≡C	75 ± 15	1500 ± 113	20
11	Et	46 ± 5	641 ± 27	14	**24**	Et	52 ± 13	634 ± 287	12
12	4-Cl–Ph	160 ± 0	2345 ± 1054	15					
13	4-CN–Ph	65 ± 24	1017 ± 570	16					
14	4-CF_3–Ph	319 ± 257	5235 ± 1209	16					
15	4-MeO–Ph	94 ± 47	549 ± 272	6					
16	3-Cl–Ph	144 ± 115	504 ± 117	4					
17	3-CN–Ph	150	1130	8					
18	3-MeO–Ph	86 ± 18	820	10					

[a] IC_{50} values are the means of at least two experiments ± STD, determined from eight concentrations (performed in triplicate). Values without STDs are for a single determination only.
[b] ERβ selectivity is expressed as ERα IC_{50}/ERβ IC_{50} ratio.
[c] See Ref. 9 for the 2-phenylquinolines **19**–**24**.

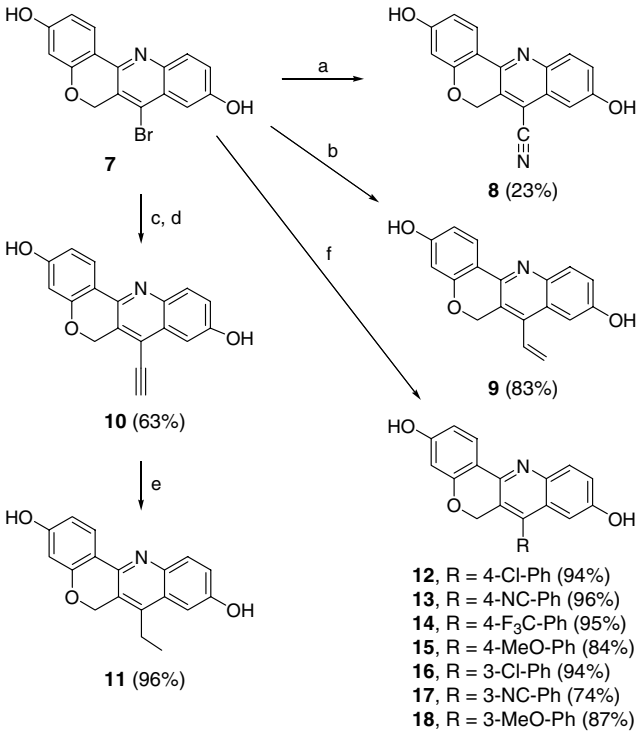


Scheme 2. Reagents and conditions: (a) $BrCH_2CH_2COOH$, $NaHCO_3$, NaOH, H_2O, 100 °C, 3 h; (b) TfOH, 0 °C–rt, 3 h; (c) Ph_2O, 170 °C, 1 h, then 200 °C, 7 h; (d) $POCl_3$, reflux, 1 h; (e) BBr_3, cyclohexene, $ClCH_2CH_2Cl$, 35 °C, 12 h; (f) $POBr_3$, DMF, 70 °C, 30 min.

or electron-deficient substituents did not offer any further improvement in either binding affinity or selectivity.

Compared to the corresponding 2-phenylquinolines (**19**–**24**, Table 1),[9] the chromenoquinolines (**6**–**11**) generally showed a similar SAR trend. However, they appeared to exhibit slightly stronger affinity for both ER isoforms. This affinity enhancement may be attributed to a favorable hydrophobic effect and van der Waals interactions between the methylene moiety of the chromane ring and surrounding hydrophobic residues.

In summary, we have identified the 6*H*-chromeno[4,3-*b*] quinolines as a new series of ERβ selective ligands, which was developed by rigidifying the 2-phenylquinoline framework. Analogues with halogen or cyano substitution at the C7 position displayed high binding affinities (comparable to that of 17β-estradiol) and were moderately selective toward the ERβ receptor. Our



Scheme 3. Reagents and conditions: (a) CuCN, DMF, 200 °C, sealed tube, 5 h; (b) $Bu_3SnCH{=}CH_2$, $Pd(PPh_3)_4$, tol., reflux, 1 h; (c) $Bu_3SnC{\equiv}C{-}TMS$, $Pd(PPh_3)_4$, tol., reflux, 1 h; (d) K_2CO_3, MeOH, 30 min; (e) H_2, 1 atm, Pd/C, EtOAc, 1 h; (f) substituted $Ph{-}B(OH)_2$, $Pd(PPh_3)_4$, DME, aq Na_2CO_3, reflux, 1 h.

current efforts focus on multiple substitution strategy to maximize ERβ binding affinity and/or selectivity.

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