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Estrogen receptor ligands: design and synthesis of new 2-arylindene-1-ones

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Abstract—The syntheses of a series of 2-arylindene-1-ones as potent ligands of ERβ and ERα are described. Several compounds exhibited high potency and moderate selectivity for the ERβ receptor. X-ray and modeling studies were used to understand ligand binding orientation and observed affinity.

The effects of estrogens in mammalian tissues have been well documented and it is now appreciated that estrogen affects many organ systems.[1] Estrogen can exert effects on tissues in several ways; the most characterized mechanism of action is its interaction with estrogen receptors leading to alterations in gene transcription. Estrogen receptors (ER) are ligand-activated transcription factors belonging to the nuclear hormone receptor superfamily, which also includes the progesterone, androgen, glucocorticoid, and mineralocorticoid receptors.[2]

To date two estrogen receptors have been reported. The first receptor was cloned in 1986 and has been termed ERα.[3] The second form of the receptor, ERβ was found in 1996 by Gustafsson and co-workers.[4] The three dimensional structures of ERα[5] and ERβ[6] have been solved by co-crystallization with various ligands. The ligand binding domain of these receptors are homologous, with 58% amino acid identity when comparing their primary structures. However, the well-recognized ligand-binding cavity differs by only two amino acids.[7] In the ERβ receptor, Met_{336} replaces the ERα Leu_{384} residue as well as Ile_{373} replacing Met_{421}.[6] Early studies of ERβ focused on defining its affinity for a variety of ligands and some differences between that of ERα were observed. The tissue distribution of ERβ has been well mapped in the rodent and it has been shown not to completely overlap with ERα. For example, tissues of the mouse and rat uterus express predominantly ERα, whereas the mouse and rat lung express predominantly ERβ.[8] It has also been reported that within the same organ the distribution of ERα and ERβ can be compartmentalized. As example, in the mouse ovary, ERβ is highly expressed in the granulosa cells and ERα is restricted to the thecal and stromal cells.[9]

Recently it has been reported that the traditional utility of ER ligands for such therapies as hormone replacement and contraception is mediated primarily via ERα.[10] The most potent endogenous ligand for both estrogen receptors is 17β-estradiol (**E_2**).

The limited availability of ERβ selective agents has led to several groups exploring this unmet need. A proliferation of work in this area has been documented recently, and some examples of new ligands include biphenols,[11] aryl benzothiophenes,[12] bicyclo[3.3.1] nonanes,[13] and aryl diphenolic azoles.[14] Herein we report our findings on the activity and selectivity of 2-substituted aryl indenones.

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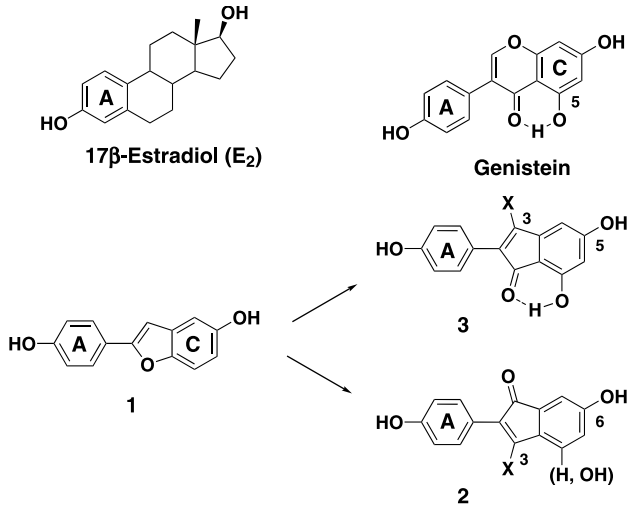
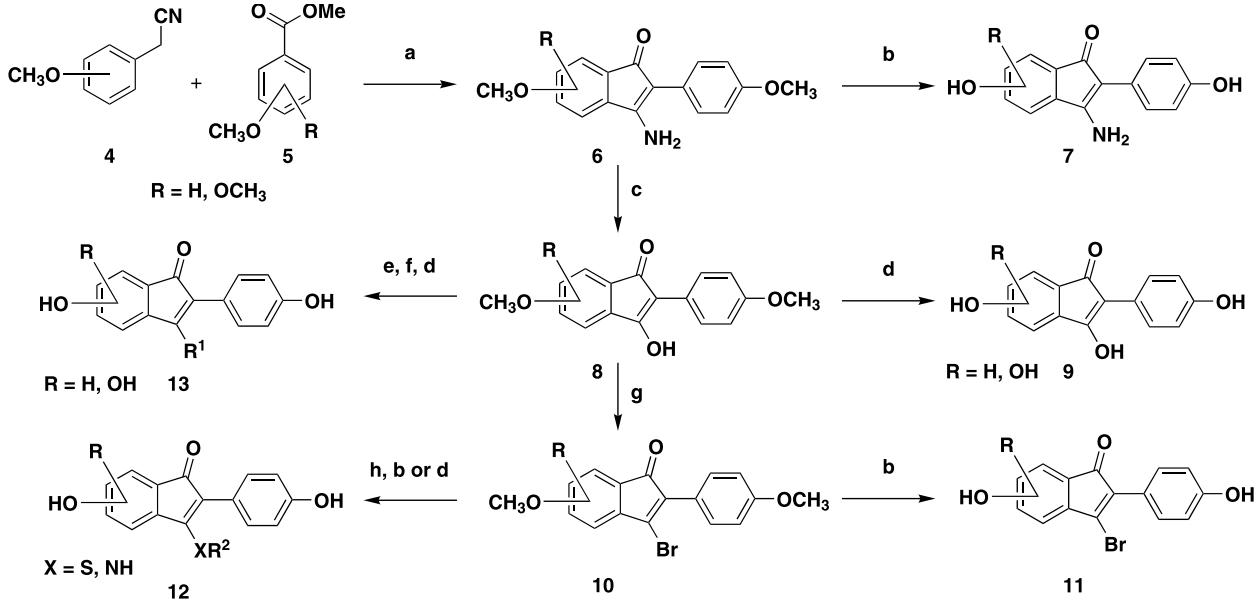


Figure 1.

Our work stems from initial structure–activity relationship (SAR) studies leading to benzofuran **1**,[15] which possesses an ERβ IC_{50} = 6 nM, and 30-fold selectivity over ERα (Fig. 1). As part of our studies we undertook replacing the central core of the benzofuran **1** with an indenone core. With this change it was hypothesized that analogs would mimic the binding activity of the phytoestrogen genistein as well as allowing a handle to incorporate the C-5 hydroxy group of genistein into the indenone core. Maintaining the positions of the A and C ring hydroxyls leads to two regioisomeric indenone forms, the 6-hydroxy series **2** and the 5-hydroxy series **3** (resembling genistein).

Earlier work by Katzenellenbogen and co-workers disclosed 2,3-diaryl-indenes and -indenones[16] as well as 2-aryl-indenes and -indenones as ligands for the estrogen receptor.[17] This work provided insight into the spatial/torsional requirements for binding to the estrogen receptor of indene and indenone systems. Notably, the orientation preference of 2-indene systems was determined through varying a single hydroxyl group on the A and C rings of the core systems. This work suggested that the preferred mode of binding for the 2-aryl-3-ethyl indenone core was that the pendant C-2 *p*-hydroxyphenyl A-ring overlaps with that of the A ring in estradiol. These studies focused on the orientation of the monophenolic compounds and did not discuss selectivity for the ER subtypes. They also found that dihydroxyl-2,3-diarylindene systems bind with greater affinity than their respective monohydroxyl counterparts.[18] We utilized a structure based X-ray approach with the advanced SAR of our program to rapidly develop high affinity estrogen receptor ligands through the 2-arylindenone system.

The indenone core was synthesized quickly and efficiently by the method of Johnson and co-workers[19] Methoxy phenylacetonitrile **4** was treated with commercially available benzoates **5** in the presence of excess lithium diisopropyl amine to produce substituted 3-amino-2-indene-1-ones **6** (Scheme 1). Indenones **6** were then deprotected with boron tribromide to give the diphenolic-3-amino-indenone **7** or converted to the indan-1, 3-diones **8** under acid hydrolysis. The indandiones **8** represented in their enolic forms were converted to the diphenolic derivatives **9** upon treatment with pyridine hydrochloride at 180 °C. Alternatively, conversion of the indandiones **8** to the 3-bromoindenones **10** was effected with carbon tetrabromide and triphenyl phosphine. The regioisomeric forms of the products were separated by column chromatography and the position of the C-5 versus C-6 methoxy group established spectroscopically.



Scheme 1. Reagents and conditions: (a) LDA, THF, −10 °C; (b) BBr_3, CH_2Cl_2; (c) 20% H_2SO_4, 100 °C; (d) pyridine–HCl; 180 °C; (e) NaHMDS, THF, 0 °C; (f) R^1MgBr or R^1Li, THF, 0 °C–rt; R^1 = Me, Ph; (g) CBr_4, PPh_3, $CHCl_3$; (h) R^2–NH_2 or R^2–SNa, DMF, 60–80 °C; R^2 = Me, Et.

The 3-bromo-indenones **10** were doubly deprotected to give **11** or alternatively subjected to nucleophilic displacement with either substituted amines or the sodium salts of alkyl thiols to give the substituted indenones. The indenones were subsequently deprotected with either pyridine hydrochloride or boron tribromide to afford the final diphenolic indenones **12**. In general indandiones **8** were converted to the 3-substituted indenones upon treatment with sodium hexamethyldisilazane followed by an alkyl Grignard or alkyl lithium reagent. Demethylation with concomitant dehydration produced the phenolic indenones **13**. All of the compounds were chemically characterized by melting point, infrared, nuclear magnetic resonance (^{1}H NMR), and elemental analysis or HRMS.

A competitive radioligand binding assay was used to assess the relative binding affinity (IC_{50}) of compounds for the human ligand binding domains (LBD) of ERβ and ERα.[20] Table 1 shows the binding affinities for analogs of the 6-hydroxy series **2**.

One of the more selective derivatives, **14** was co-crystallized with human ERβ (space group $P2_12_12_1$) as described in Ref. 21a. X-ray data (2.0 Å) were collected at 100 K using a Quantum-4 CCD area detector at the Advanced Light Source (ALS, Berkeley, CA), and processed using DENZO and SCALEPACK.[22] The crystal structure was solved by molecular replacement AMORE[23] using the ERβ/genistein complex as a search model. Crystallographic refinement was performed using CNS.[24] The refined models (with no ligand added) were then used to calculate electron density difference maps, which showed clear electron density for the bound compound (see Fig. 2). The final model for the ERβ/**14** complex contained two protein molecules, two ligand molecules, and 167 water molecules. The corresponding refinement values obtained were $R = 22.6\%$ and $R_{free} = 28.6\%$. Atomic coordinates for ERβ complexed with compound **14** has been deposited in the Protein Data Bank, with accession code 1ZAF.

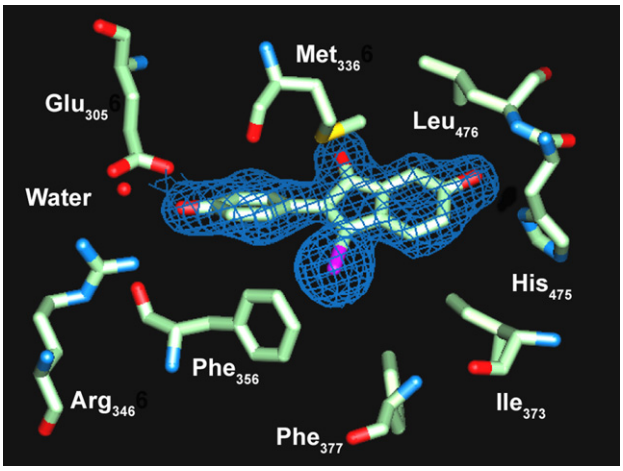
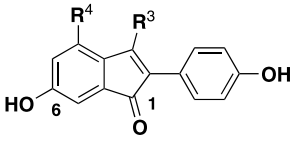


Figure 2. Unbiased $2f_o - f_c$ map contoured at σ, showing the electron density for compound **14** complexed with ERβ.

The crystal structure shows definitive orientation of the phenolic hydroxyl group as the 'A-ring', forming hydrogen bonds to Glu_{305} and Arg_{346} similar to 17β-estradiol,[25] genistein,[26] and other ER ligands.[21a,27] The 6-hydroxy group forms a hydrogen bond with His_{475}.

The remainder of the indenone core fills the rest of the primarily hydrophobic pocket, forming a torsion angle of approximately 39°(averaged over both monomers) with respect to the A-ring.

Table 1. 6-Hydroxy binding affinities (IC_{50}) for human ERα and ERβ ligand binding domain



Compd	R^4	R^3	ERβ IC_{50} (nM)[a]	ERα IC_{50} (nM)[a]	ERβ/ERα ratio
E_2	—	—	3.6 ± 1.6 (144)	3.2 ± 1.0 (144)	1
14	H	Br	20 ± 19 (7)	290 ± 250 (7)	15
15	H	CH_3	11 (1)	55 (1)	5
16	H	SMe	3 (1)	12 (1)	4
17	H	SEt	8 (1)	10 (1)	1
18	H	Ph	9 ± 1 (2)	9 ± 1 (2)	1
19	OMe	Br	150 (1)	200 (1)	1
20	H	OH	2625 (1)	>5000 (1)	—
21	OH	OH	1380 (1)	>5000 (1)	—
22	H	NH_2	1150 (1)	>5000 (1)	—
23	OH	NHEt	2700 (1)	>5000 (1)	—
24	OH	Br	267 ± 108 (5)	710 ± 670 (4)	3
25	OH	SMe	2 (1)	2 ± 1 (2)	1
26	OH	SEt	150 (1)	580 (1)	4
27	OH	Ph	44 (1)	47 (1)	1

[a] Values are the mean of independent determinations (number in parenthesis) ± SD.

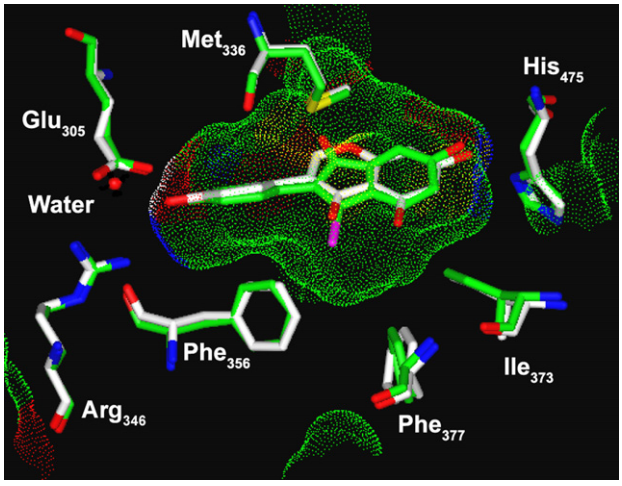


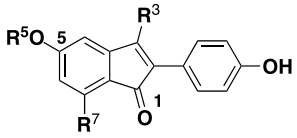
Figure 3. ERβ complexed with **14** (green), overlaid with the ERβ/genistein complex (white). Only key residues and a Connolly surface of the ERβ/**14** binding site are shown for simplicity.

Figure 3 shows an overlay of the ERβ/genistein complex with that of ERβ/**14**. Notice that the A/C rings and the corresponding hydroxyl groups overlap well. One interesting difference is that the carbonyl groups of the two ligands are oppositely oriented in the binding pocket, which appears to be a consequence of interactions made between the C-ring hydroxyl and His_{475}.

This will be discussed further below. Based on the overlay, it appears that the core selectivity of the indenone scaffold is due to interactions of the aromatic B-C ring with ERβ Met_{336}, similar to what has been described for genistein,[21b] and other compounds.[21a] As such, it is likely that variations in the indenone substituents (as well as the substitution pattern) can lead to subtle electronic differences that modulate this methionine–aromatic interaction, leading to slight variations in selectivity. In this series our goal was to exploit the C-3 position by enhancing interaction with the lipophilic pocket in which the bromine resides. Small changes to substituents at the C-3 position (i.e., **15**–**17**), resulted in improved potency at both receptor subtypes. This observation is consistent with the fact that the pocket residues are conserved in the vicinity of the C-3 position. Increasing size at the C-3 position with a bulky phenyl group (i.e., **18**) resulted in similar potency at both LBD's. Given the available space in this region of the pocket, it is unclear whether or not the binding mode shown in Figures 2 and 3 is maintained for the 3-phenyl substituent. Polar groups (i.e., **20**–**23**) at the 3-position resulted in a dramatic loss of potency, most likely due to desolvation of hydrophilic moieties without making any significant compensating interactions. A similar observation was made by Katzenellenbogen and coworkers[13a] with a Troger's base analog in their bicyclo[3.3.1]nonane series. They reasoned that the central core polar bridgehead nitrogen atoms were responsible for the observed low binding affinity. Except for analog **25**, placing a hydroxyl group at the C-4 position of this series also resulted in lower binding affinity (i.e., **24**, **26**, and **27**) versus their non C-4 hydroxylated counterparts (i.e., **14**, **17**, and **18**). This result suggests that a hydroxyl group not intramolecularly hydrogen bonded as in genistein at this position disrupts the lipophilic character required for tight binding to ERβ.

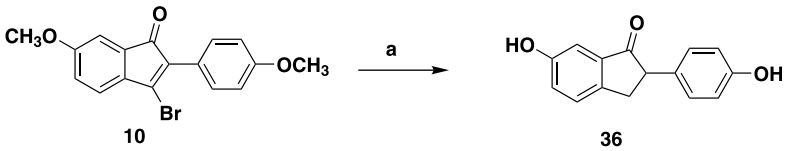
Table 2 shows the binding affinities for the 5-hydroxy analogs (series **3**). Our initial target **28** possessing the C-3 Br substitution had high affinity for the ERβ ligand binding domain (IC_{50} = 1.7 nM). This is slightly more potent than its regioisomer **14**, which has an affinity of 20 nM. Docking studies, performed as previously described[11a,21a] suggest that the 5-hydroxy group in series 3 forms a hydrogen bond to His_{475} and directs the carbonyl group in a similar orientation to that of genistein (in contrast to what we observed for series **2**). Replacing the C-5 hydroxyl with a methoxy group **29** disrupted the hydrogen bonding interaction with His_{475} resulting in a 117-fold decrease in potency, although maintaining a 9-fold selectivity versus ERα. Subsequent C-3 replacement with small alkyl groups (methyl) **30**, or a thiomethyl group **31** led to similar potency (2 nM) but no improvement in selectivity. The C-3 thioethyl analog **32** showed

Table 2. 5-Hydroxy binding affinities (IC_{50}) for human ERα and ERβ ligand binding domain



Compd	R^5	R^7	R^3	ERβ IC_{50} (nM)[a]	ERα IC_{50} (nM)[a]	ERβ/ERα ratio
28	H	H	Br	1.7 ± 0.4 (11)	5.2 ± 0.21 (2)[b]	3
29	Me	H	Br	199 ± 22 (3)	1800 ± 900 (3)	9
30	H	H	Me	2 (1)	6 (1)	3
31	H	H	SMe	1.3 ± 0.5 (2)	3.1 ± 0.1 (2)	3
32	H	H	SEt	150 (1)	480 (1)	3
33	H	H	Ph	10 ± 7 (2)	10 ± 7 (2)	1
34	H	OH	Me	2.4 ± .0.3 (2)	31 ± 15 (2)	13
35	H	OH	Ph	3 (1)	3 (1)	1

[a] Values are the mean of independent determinations (number in parenthesis) ± SD.
[b] Performance of **28** on the ERα receptor was atypical and the value reported are for the best curves.



10 36

Scheme 2. Reagents: (a) HI (57%), AcOH.

decreased affinity for both receptors. As observed in the 6-hydroxy series, placing a phenyl group at the 3-position **33** gave equivalent potency (10 nM) at both ERα and ERβ. The 5,7-dihydroxy analog **34**, being the most structurally similar to genistein, bound tightly to ERβ (2.4 nM) and was 13-fold selective. Docking studies suggest that **34** adopts a binding mode similar to that of genistein.

Finally, we investigated the planarity requirements of the indenone core. As shown in Scheme 2, enone **10** could be simultaneously deprotected and reduced with HI (57%) in acetic acid to afford the indane **36**. This analog displayed a significant decrease in potency at ERβ (700 nM) and ERα (690 nM), with loss of selectivity as compared to **14**. While there may be some contribution to this decrease in affinity due to removal of the bromo group, it is hypothesized that the primary contribution is most likely disruption of the core scaffold shape, preventing optimal orientation of the key hydroxyl groups.

In summary, we have demonstrated that both the 5- and 6-hydroxy indenone cores bind potently to ERβ and ERα and show moderate ERβ selectivity. Compound **14** was co-crystallized with ERβ. The binding mode was found to be similar to that of genistein, with the exception that the direction of the carbonyl group is reversed. This appears to be influenced by the way the 6-hydroxy group is directed toward its hydrogen bonding partner His_{475}. A more genistein-like binding mode, with the carbonyl moiety directed toward a similar region of the binding pocket as that of genistein, is predicted for 5-hydroxy analog **34** based on docking calculations. Analog **34** showed similar binding potency but 2–3-fold less selectivity over its genistein counterpart.

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