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Bioorganic & Medicinal Chemistry Letters 17 (2007) 118–122
Bioorganic & Medicinal Chemistry Letters

Estrogen receptor β ligands: Design and synthesis of new 2-phenyl-isoindole-1,3-diones

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Received 3 August 2006; revised 25 September 2006; accepted 27 September 2006
Available online 1 October 2006

Abstract—The design, synthesis, and biological evaluation of the 2-phenyl-isoindole-1,3-diones will be discussed. Detailed modeling studies with X-ray support were used to understand the ligand binding orientation and observed selectivity.

The estrogen receptor (ER) is a ligand-activated transcription factor that belongs to the nuclear hormone receptor superfamily. Other members of this family include the progestin, androgen, glucocorticoid, and mineralocorticoid receptors.[1] Until recently estrogen-mediated events had been thought to be regulated by only one estrogen receptor which now is identified as ERα.[2,3] With the discovery in 1996 of a second estrogen receptor subtype (ERβ), there was an intense interest in elucidating ERβ function.[4,5] The three dimensional structures of ERα[6] and ERβ[7] have been solved by co-crystallization with a variety of ligands. The primary ligand binding domain of these receptors are 58% homologous. However, the well-recognized ligand-binding domain (LBD) cavity varies by only two amino acids[7] (i.e., ERβ Met_{336} is replaced by ERα Leu_{384} and ERβ Ile_{373} is replaced by ERα Met_{421}). As a consequence of these conservative substitutions, it is not surprising that the many ER ligands known prior to the discovery of the ERβ subtype possessed limited selectivity for that subtype. Despite the fact that the design of highly selective subtype ligands appeared to be a daunting task, there have been many reports of high affinity and functionally selective ERα and ERβ ligands.[8–12] Recently, we have disclosed some of our efforts toward identifying possible utilities for ERβ selective ligands.[13]

The phytoestrogen, genistein, has been reported to have modest ERβ selectivity (~40-fold)[14,15] and has been used by our group as a starting point to design novel nonsteroidal ERβ selective ligands (Fig. 1). Some detailed work focused on substitution around simplified tricyclic A-BC scaffolds (i.e., the 6-phenylnaphthalenes[15] and the 2-phenyl quinolines[16]). Further variation on the central ring (ring-B) came about by modification of the benzofuran **1** (obtained from elaboration of an HTS screening hit),[17] with an indenone core (i.e., **2**).[18] The authors hypothesized that these 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 led to two regioisomeric indenone forms, the 6-hydroxy series **2a** and the 5-hydroxy series **2b** (not unlike that of genistein). These compounds, although potent binders to ERβ, have moderate <15-fold ERβ-selectivity. The core template, arylindene-1-one **2** of this series, having the ability to flip 180° (around the *x*- and *y*-axes), allows for multiple binding modes. To simplify this binding paradigm, we explored substitution on a central phthalimide core which has been utilized in the design of a variety of biologically active molecules.[19] The 2-phenyl-isoindole-1,3-dione core (i.e., **3**), bearing two carbonyls, has additional symmetry along the *x*-axis.

Keywords: ERα and ERβ receptor; Estrogen receptor; Nuclear hormone receptor.
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doi:10.1016/j.bmcl.2006.09.088

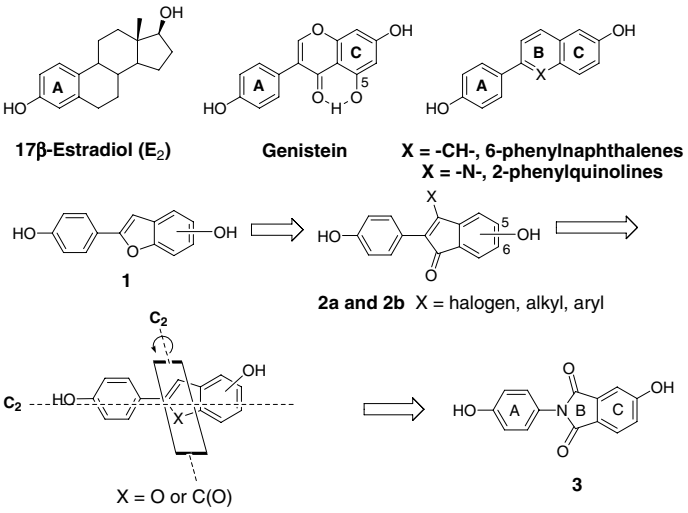


Figure 1. Compounds of interest.

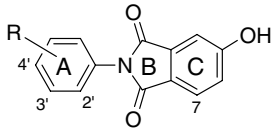
If compound **3** rotates along the *x*-axis the 'B-ring' of this A-BC ring system will not be altered and has the advantage of allowing the template to serve as a vehicle to rapidly evaluate and design ERβ selectivity into the new structural motif.

The synthesis of the phthalimides (i.e., **3**) was straightforward and is outlined in Scheme 1. It was accomplished by reacting a mixture of 4-hydroxyphthalic acid and *N*-methyl-2-pyrrolidine (NMP) in xylenes at reflux temperature with an appropriately substituted amine. The free hydroxyls were obtained by deprotection of the methyl ethers with pyridine–HCl (190 °C) to provide the desired hydroxy phenyl-phthalimides (i.e., **3**). Alternatively, a copper-mediated coupling of an appropriately substituted N–H phthalimide and a suitably substituted iodoanisole may construct the core phthalimide.

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 with variations of the hydroxyl group on the *N*-phenyl ring. The C-4′ placement of the hydroxyl is optimal for binding to the ER.

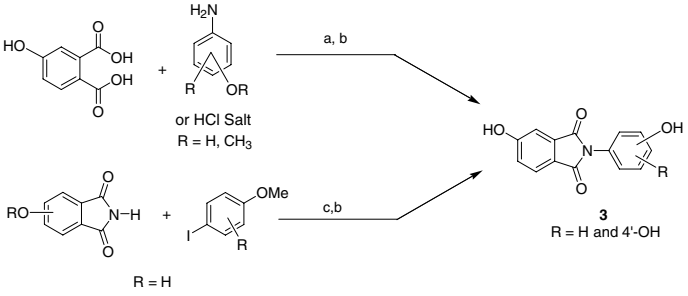
Table 2 shows structural variations on the appended *N*-phenyl ring where we replaced the aryl ring with a saturated six-membered ring (i.e., **10**) and observed a significant decrease in affinity. In addition, a fused aryl system (i.e., **11** and **12**) also decreased affinity. Introduction of a C-2′ electron-withdrawing substituent enhanced binding affinity (i.e., **14** and **16**) which has been previously observed in the literature for ERβ selective ligands.[8,9,12,18] Although an increase in affinity was

Table 1. Binding affinity for 2-phenyl-isoindole-1,3-dione core



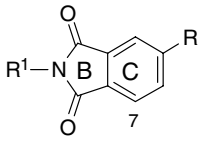
Compound	R	A ring	ERβ IC_{50}[a] (nM)	ERα IC_{50}[a] (nM)
E2	—	—	3.6 ± 1.6	3.2 ± 1.0
Genistein	—	—	9.7 ± 4.3	395 ± 181
3	OH	4′-OH	84 ± 31 (7)	1980 ± 719 (7)
4	OH	3′-OH	2900 ± 346 (3)	>5000 (3)
5	H	4′-OH	>10000	>10000
6	OH	H	2700	>5000
7	OH	4′-OMe	3300	>5000
8	OH	2′,4′-OH	2470	>5000
9	OH	3′,5′-OH	>5000	>5000

[a] Values are means of independent determinations (number in parentheses) ± SD.



Scheme 1. Reagents and conditions: (a) NMP, xylene, 150 °C; (b) Py–HCl, 190 °C; (c) Cu_2O, collidine.

Table 2. Structural variations on 2-phenyl-isoindole-1,3-dione



Compound	R	R^1	ERβ IC_{50}[a] (nM)	ERα IC_{50}[a] (nM)	ERα/ERβ ratio
3	OH	4′-OH-phenyl	84 ± 31 (7)	1980 ± 719 (7)	24
10	OH	4′-OH-C_6H_{11} (trans)	>5000	>5000	n/a
11	OH	5′-OH-1-naphthyl	802	2810	4
12	OH	4′-OH-1-naphthyl	2640	560	0.2
13	OH	2′-NO_2-4′-OMe-phenyl	>5000	>5000	n/a
14	OH	2′-NO_2-4′-OH-phenyl	21	92	4
15	OH	2′-Me 4′-OH-phenyl	802	2810	4
16	OH	2′-F 4′-OH-phenyl	53 ± 13 (2)	339 ± 92 (2)	6
17	OMe, 7-Br	4′-OH-phenyl	>5000	>5000	n/a
18	OH, 7-Br	4′-OH-phenyl	36 ± 2.1 (2)	1625 ± 7.1 (2)	45

[a] Values are means of independent determinations (number in parentheses) ± SD.

observed with substitution at the C-2′ position, the compounds did not exhibit increased ERβ-selectivity when compared to the core template, **3** .

Docking calculations[21] were used to predict the orientation of our novel core phthalimide **3** in the ERβ binding site.[7] Figure 2 shows that the 4′-OH group makes a critical interaction with Arg_{346} and Glu_{305}, while the 5-OH group makes an interaction with the His_{475} residue. This binding mode is consistent with that of other A-B/C ring systems, such as benzofuran and indenone for which ERβ X-ray structures are known.[17,18]

The B ring of this template lies in close proximity to the ERα Leu_{384}/ERβ Met_{336} residue mutation and it appears that the core ERβ selectivity (~24-fold) of this scaffold is due to a favorable interaction of this aromatic ring with ERβ Met_{336}, similar to what has been described for genistein[22] and other compounds.[23]

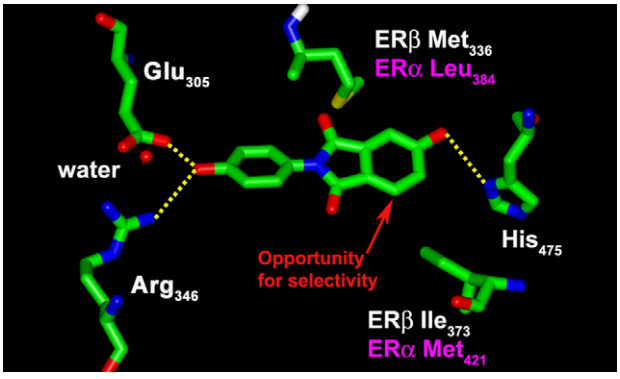
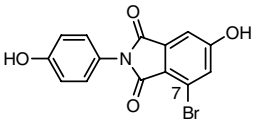


Figure 2. Docked structure of compound **3** in the ERβ/genistein binding site. Only key residues are shown for simplicity. Residue differences within the binding pocket between ERα and ERβ are highlighted. Hydrogen bonds are shown by a yellow dotted line. Arrow indicates opportunity for increasing selectivity by targeting region of residue difference.

Looking closely at the simplified core, there appeared to be an opportunity to increase selectivity by substituting at the 7-position of the phthalimide core. Previous studies in our laboratories[23,24] suggested that substitution at the 7-position may satisfy the binding requirements as well as probe the Met/Ile selectivity pocket. Figure 3 shows the 7-bromo phthalimide **18**[25] docked into the ERβ/genistein pocket.

As discussed earlier, only two residues are different within the ligand binding pockets of ERα and ERβ.



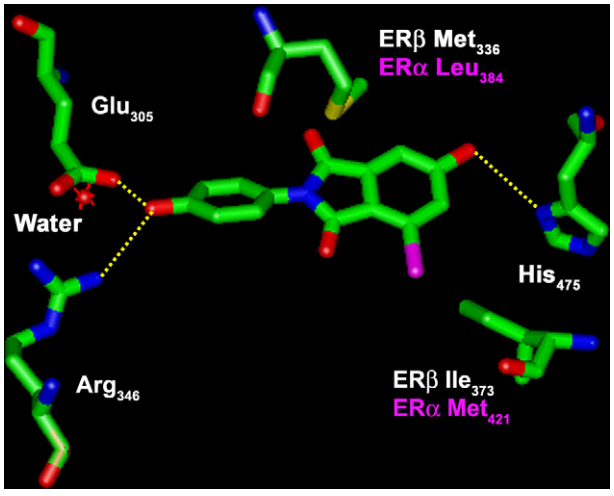


Figure 3. Compound **18** docked into the ERβ/genistein pocket. Only key residues are shown for simplicity. Residue differences within the binding pocket between ERα and ERβ are highlighted. Hydrogen bonds are shown by a yellow dotted line.

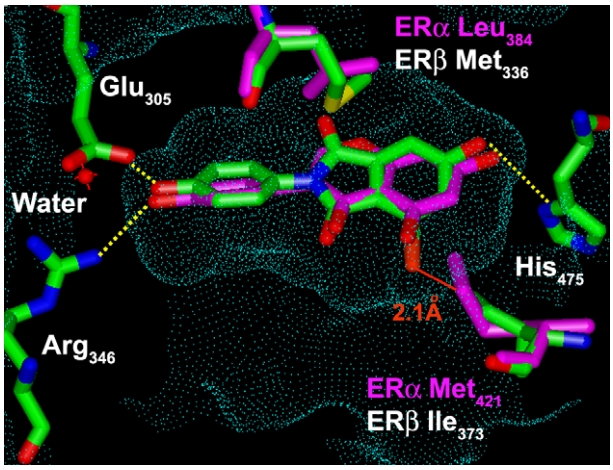


Figure 4. Compound **18** (colored by atom type) docked to the ERβ/genistein pocket (genistein ligand colored by magenta) and overlaid with ERα/DES[26] key residues. Only key residues and a Connolly surface of the ERβ binding site are shown for simplicity. Hydrogen bonds are shown by a yellow dotted line. Distance monitor shows that the 7-Br would be in close proximity to ERα Met_{421} residue.

Docking calculations show that the binding mode of compound **18** remains the same and the 7-bromo group is in close proximity to the residue change (i.e., ERα Met_{421} and ERβ Ile_{373}). To further understand the origin of the ERβ selectivity (~45-fold) of this scaffold, the X-ray structure of ERα/DES[26] was superimposed on the docked structure (see Fig. 4). Only key residues (i.e., Leu_{384} Met_{421}), which are different within the ligand binding pocket, are shown from the ERα/DES structure in magenta. Of particular interest is the 7-bromo group that is in close proximity (2.1 Å) to the sulfur of the ERα Met_{421} residue which would represent a repulsive interaction between ERα Met_{421} and the bromo group. A similar interaction has been suggested between the 5-OH group and the sulfur atom to explain the observed ERβ selectivity of genistein.[22] In contrast, a distance of 3.7 Å between the 7-bromo group and ERβ Ile_{373} ($C^{\gamma 1}$) is not expected to have significant electronic or steric repulsion. The slight increase in ERβ potency for this compound (~2.3-fold) could be attributed to the increased nature of the lipophilic group (i.e., bromine occupying a hydrophobic pocket). This differential interaction may explain the overall increase in ERβ selectivity (~45-fold) observed for the bromo-substituted phthalimide **18**.

In summary, a novel series of 4-hydroxy-*N*-phenyl-substituted phthalimides was prepared. This dihydroxy-substituted core offers a symmetric B-ring that mimics genistein and binds to ERβ with modest ERβ-selectivity (~24-fold) compared to genistein. The binding mode for this series of compounds was consistent with that of other previously reported A-B/C ring systems, such as the benzofuran and indenone series. Based on our previous SAR in this area, the C-7 bromo analog **18** was prepared and found to be slightly more ERβ selective than genistein.

Acknowledgments

We thank the Wyeth Discovery Analytical Chemistry department for physical analysis. Special thanks to Dr. Chris P. Miller for serving as team leader and providing insight into the SERM arena. Thanks to Drs. Jay Wrobel and Gene Trybulski for advice and discussions. Special thanks to Dr. John Yardley.

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