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Estrogen receptor β selective ligands: Discovery and SAR of novel heterocyclic ligands

Richard Chesworth,[a,*] Matthew D. Wessel,[b] Lisa Heyden,[a] F. Michael Mangano,[a] Michael Zawistoski,[a] Laura Gegnas,[a] David Galluzzo,[a] Bruce Lefker,[a] Kimberly O. Cameron,[a] Jeanne Tickner,[a] Bihong Lu,[c] Tessa A. Castleberry,[c] Donna N. Petersen,[c] Amy Brault,[c] Pia Perry,[c] Oicheng Ng,[c,†] Thomas A. Owen,[c] Lydia Pan,[c] Hua-Zhu Ke,[c] Thomas A. Brown,[c] David D. Thompson[c] and Paul DaSilva-Jardine[a]

[a]*Cardiovascular and Metabolic Diseases, Medicinal Chemistry, Pfizer Global Research and Development, Groton Laboratories, Eastern Point Road, Groton, CT 06340, USA*
[b]*Computational Chemistry, Pfizer Global Research and Development, Groton Laboratories, Eastern Point Road, Groton, CT 06340, USA*
[c]*Cardiovascular and Metabolic Diseases, Biology, Pfizer Global Research and Development, Groton Laboratories, Eastern Point Road, Groton, CT 06340, USA*

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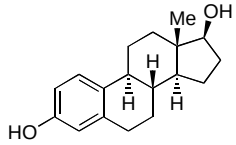
Abstract—A series of ligands with varying heterocyclic cores and substituents that display a range of selectivity's (up to >100x) for ER-β over ER-α are reported.

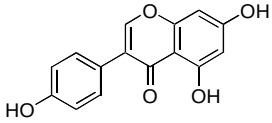
The estrogen receptor (ER) is a member of the nuclear hormone superfamily of receptors.[1] The ER mediates the regulation of a number of important physiological processes, including bone mineral density (BMD) and lipid levels.[2] Post-menopausal women are often treated with hormone (estrogen) replacement therapy (HRT) to maintain BMD and relieve post-menopausal symptoms, such as hot flashes and night sweats.[3] HRT though, is not without its drawbacks, such as an increased risk in both breast and uterine cancers. For this reason, compliance is poor and many women stop taking HRT after less than one year of treatment.[4] Selective estrogen receptor modulators (SERMs), such as raloxifene,[5] have overcome the increased risk of breast and uterine cancers, but unfortunately do not provide full bone protection and do not ameliorate hot flashes.[6]

Until the recent discovery of a second estrogen receptor (designated ER-β),[7,8] it was believed that only a single estrogen receptor existed (now known as ER-α). This discovery has led to an intensive effort to define the roles that the individual receptors play in the hope of designing an improved SERM/HRT.

Both ER-α and ER-β bind the endogenous ligand 17-β estradiol **1**[9] with high potency (see Fig. 1). The tissue distribution of the receptors suggests that their roles may not be redundant.[10] Various hypotheses for the function of each receptor have been generated.[11–14] To



1
17-β Estradiol



2
Genistein

Figure 1. Estrogen receptor ligands.

Keywords: Estrogen receptor; ER beta selective ligands.
* Corresponding author at present address: En Vivo Pharmaceuticals, 480 Arsenal Street, Watertown, MA 02472, USA. Tel.: +1 617 247 1652; fax: +1 617 746 8877; e-mail: rchesworth@gmail.com
† Deceased May 19, 2002.

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test these hypotheses and determine the function of each receptor, selective compounds for each receptor are sought after, in particular ligands for ER-β.

ER-α is a larger protein than ER-β. ER-α contains 595 amino acids, whereas ER-β has only 485. Although the two receptors have overall about 56% sequence homology, in the ligand binding domain only two of the 23 amino acids that make direct contact to the encapsulated ligand are different (L384M and M421I), both of which are highly conservative substitutions.[15] The net effect of these differences is that ER-β has a slightly smaller ligand binding pocket than ER-α[16] and it has been found that sterically demanding ligands can display selectivity for ER-α.[17] Compounds with some selectivity for ER β include genistein **2**, tetra-hydrochrysenes,[18] diarylpropionitriles,[19] 2-aryl benzothiophenes,[20] 1,3,5 triazines,[21] and most recently, the benzoxazole ERB-041.[22] In this paper we describe our own efforts toward the discovery of ligands that are selective for ER-β (Fig. 2).

A series of benzimidazoles was prepared via standard procedures as follows: 2-Fluoro nitrobenzene was treated with *para*-anisdine in the presence of K_2CO_3 at 160 °C to give the nitro aniline **3**, which upon reduction with hydrogen over Pd/C gave the bis-aniline **4**. This compound was condensed with various carboxylic acids to give the amides **5a**–**e**, which upon treatment with hot acetic acid underwent acid-catalyzed dehydration to form the benzimidazoles **6a**–**e**. The methyl ethers were removed using BBr_3 to give the desired target compounds **7a**–**e**. This sequence allowed the rapid synthesis of a large number of C-2-substituted benzimidazole analogs from the common intermediate **4** (see Scheme 1).

Isoxazole **8** was prepared by acylating the dianion of the oxime derived from 1-(4-methoxy-phenyl)-2-phenylethanone **9** with trifluoroacetic acid ethyl ester. The resultant hemiacetal **10** was dehydrated with *p*-TsOH, followed by O-demethylation with BBr_3, to give the desired isoxazole (**8**). The synthesis of the imidazole[23] **11** and the indole[24,25] **12**–**15** compounds has been previously described (see Scheme 2).

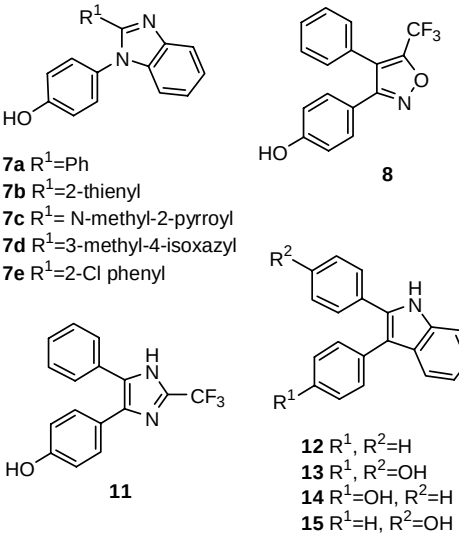
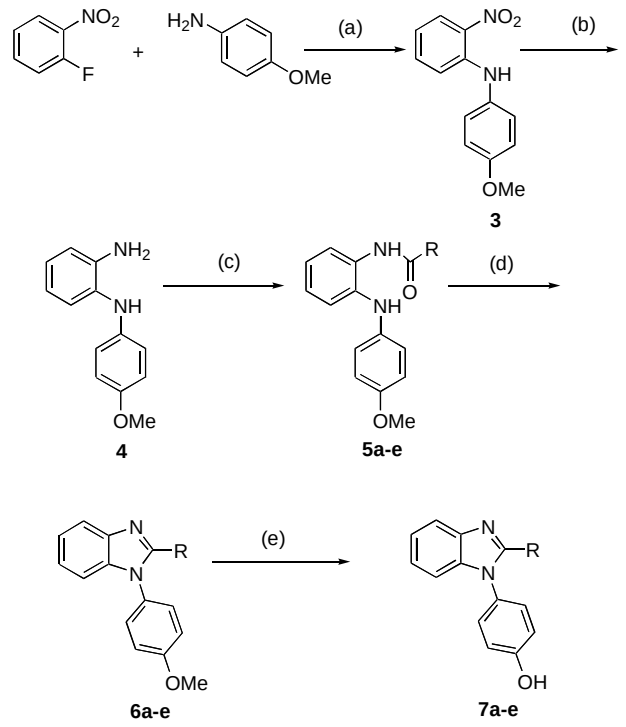


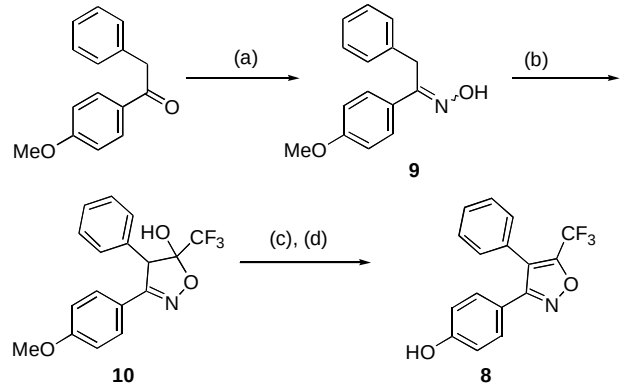
Figure 2. Heterocyclic ligands.



Scheme 1. Synthesis of benzimidazole derivatives. Reagents and conditions: (a) K_2CO_3, neat, 160 °C; (b) H_2, Pd/C, MeOH, dioxane, HCl; (c) RCO_2H, 1-propane phosphonic cyclic anhydride, Et_3N, solvent; (d) AcOH, 65 °C; (e) BBr_3, CH_2Cl_2, −78 °C to rt.

To screen compounds for their affinity for ER-β versus ER-α, an in vitro ligand binding assay using recombinant ER-α and ER-β using [^{3}H] 17β-estradiol as the radioligand was employed[26].

A number of standard compounds were evaluated in the binding assay and their affinities for both receptors were found to be in agreement with previously reported values.[9] As shown in Table 1, 17-β-estradiol **1** demonstrat-



Scheme 2. Synthesis of isoxazole (**8**). Reagents and conditions: (a) NH_2OH, EtOH, pyridine; (b) *n*-BuLi, CF_3CO_2Et; (c) TsOH, toluene, Δ; (d) BBr_3 0 °C to rt.

Table 1. IC_{50}s and binding selectivities of ligands[26]

Compound	ER-α (nM)[a]	ER-β (nM)[a]	Selectivity (for ER-β)
1	0.75 ± 0.60	1.18 ± 1.04	0.63
2	29.4 ± 4.33	1.30 ± 0.69	22.5
7a	1800 ± 569	137 ± 42.4	13.1
7b	>6260 ± 3330	49.7 ± 6.06	>126
7c	2940 ± 1400	28.1 ± 0.05	105
7d	1160 ± 271	14.3 ± 1.20	81.1
7e	1730[b]	149[b]	11.6
8	8.5 ± 4.9	2.4 ± 0.69	3.5
11	403 ± 207	15.1 ± 3.04	26.7
12	6950[b]	171[b]	40.6
13	94.8 ± 21.5	11.9 ± 3.01	8.0
14	24.0 ± 13.9	1.12 ± 0.73	21.8
15	8310 ± 2397	355 ± 224	23.4

[a] ±Value represents the standard deviation.
[b] Only one independent experiment was run.

ed an approximately equal affinity for each receptor, while genistein **2** showed a slight bias for ER β (14.3×). During the course of high-throughput screening, 2,3-diphenyl indole **12**[27] emerged as a compound showing reasonable selectivity for ER-β (ca. 40×), although potency for this receptor is modest. Although this compound is commercially available, it has not been previously characterized as an ER ligand. The majority of ER ligands contain a phenol on the A-ring biostere (cf. to 17β-estradiol). This hydroxyl group of the phenol is responsible for ca. 1.9 kcal/mol interaction energy with ER α.[28] We anticipated that the introduction of appropriately positioned phenol(s) into 2,3-diphenyl indole **12** would improve the potency, while retaining a reasonable degree of selectivity. A systematic SAR survey of the indole **12** found that the introduction of a phenolic group into the *para* position of each of the pendant phenyl rings gave **13**, which demonstrated improved potency at ER-β by an order of magnitude, though its selectivity for ER-β was slightly diminished. The individual removal of each phenol clearly indicated which of these was more important for potency. As can be seen in Table 1, the mono-phenolic indole **14** has a much improved potency compared to indole **15**. We undertook a systematic investigation of **14** in the hope of improving the selectivity for ER-β. Initial efforts focused on varying the central core, as different cores allow for the different spatial arrangement of substituents. This led to the identification of the isoxazole **8**, imidazole **11**, and the benzimidazoles **7a-d**. The isoxazole **8** retains potency for ER-β, but unfortunately loses most of the selectivity that is seen in the indole series. The trifluoromethyl imidazole **11** however, maintains selectivity for ER-β but loses an order of magnitude in potency for this receptor compared to the indole **14**. This may be due in part to the energetic penalty paid for the desolvation of the acidic (pK_a ca. 9)[23] NH group of the imidazole and the general increase in the polarity of the central core. Replacement of the indole core with a benzimidazole ring system produced the analog **7a**, which, although maintaining a degree of selectivity, lost two orders of magnitude in potency compared to **14**. Despite the loss of potency, switching to the benzimidazole core allowed for a more rapid synthesis of analogs compared to the other templates, and in particular, allowed for a rapid exploration of the vector defined by the C-2 substituent.

The potency and selectivity could be increased by replacement of the C-2 phenyl group of **7a** with select 5-membered heterocycles. Introduction of a 2-thienyl group **7b** results in an analog that is over 100× selective for ER-β over ER-α. Analogs containing an *ortho* substituent on the 5-membered heterocycle, such as N-methyl-2-pyrrolyl **7c** or 3-methyl-4-isoxazyl group **7d**, have improved potency at ER-β, whilst retaining the selectivity for ER β. It is interesting to speculate on the origin of improved potency and selectivity versus the prototypical phenyl analog **7a**. One of the two differences in the ligand binding site between ER α and ER-β is a Met 421:Ile 373 residue change, giving rise to a small pocket in the ER-β structure (see Fig. 3). As suggested from docking the benzimidazole **7c** into the ER crystal structures, it may take advantage of this difference by positioning its N-methyl group into the small pocket in ER-β. It is interesting to note that the *ortho*-chloro phenyl analog **7e** displays essentially the same selectivity and potency as the unsubstituted phenyl analog **7a** as based on this model, it too may have been expected to take advantage of this small pocket in ER-β. To fully understand the factors responsible for the observed selectivity for ER β, crystal structures of one of the selective agents bound to both of the ERs would be highly useful.

A number of compounds were profiled further for estrogenic activity in whole cell functional assays. Cell lines were chosen that expressed predominantly ER-α or ER-β, respectively. MCF-7 cells are derived from human breast cancer tissue and these cells predominantly contain ER-α.[30] Conversely, primary granulosa cells, which are derived from rat ovaries, do not contain ER-α but contain endogenous ER β.[31] Each cell line was independently transiently transfected with an estrogen responsive luciferase reporter vector (ERE3-TK-lux)[32] (see Table 2).

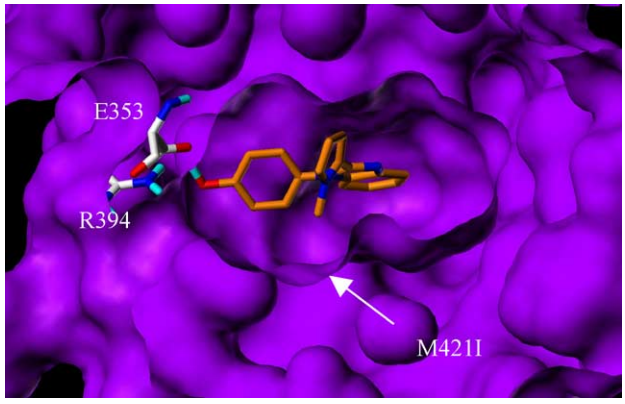


Figure 3. Binding mode of benzimidazole **7c** (orange) when docked into the X-ray crystal structure of ER-β.[16,29]

Table 2. Estrogenic activity of selected compounds in whole cells

Compound	MCF-7 EC_{50} (nM)	Activation[a] %	Granulosa EC_{50} (nM)	Activation[b] %
Estradiol **1**	0.01	100	0.14	100
7c	>1000	21	40.8	101
11	243	87	6.70	93
14	38.5	83	1.09	78

[a] Percent activation observed at 1 μM in MCF-7 cells.
[b] Percent activation observed at 1 μM in granulosa cells.

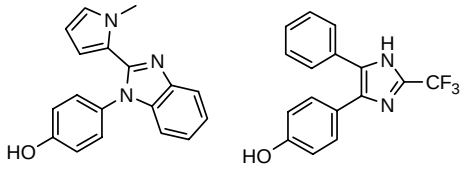


Figure 4. Structures of the ER-β selective compounds **7c** and **11**.

The synthetic analogs **11** and **14** displayed partial to full agonism in both the MCF-7 and granulosa cell lines. These analogs, though, were considerably more potent in the granulosa cells, presumably due to the higher affinity of the analogs for ER-β. The highly selective benzimidazole **7c** appears to be a potent full agonist in the granulosa cells (ER-β), while displaying little activity in the MCF-7 cells presumably due to its weak binding activity at ER-α . Due to the differing nature of the two cell lines (both background and species), only limited conclusions can be drawn about the functional selectivity of the compounds, though it does seem that binding selectivity is reflected in the potency of the functional assays.

In summary, we have found compounds that have displayed up to 100x selectivity for ER-β in competition binding assays that are also functional agonists in whole cells, as exemplified by the analogs **7c** and **11** (Fig. 4). We have also demonstrated that the ER receptors are tolerant of various heterocyclic cores and that, despite the high homology between the two receptors, ligands can be made to exploit the small differences between these two receptors to achieve binding selectivity. We anticipate that these compounds, upon further characterization, will become useful pharmacological tools for determining the role ER-β plays in ER mediated physiology.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2005.08.010.

References and notes

1. Manglesdorf, D. J.; Thummel, C.; Beato, M.; Herrlich, P.; Schutz, G.; Umesono, K.; Blumberg, B.; Kastner, P.; Mark, M.; Chambon, P.; Evans, R. M. *Cell* **1995**, *83*, 835.
2. Nasr, A.; Breckwoldt, M. *Gynecol. Endocrinol.* **1998**, *12*, 43.
3. Ulmsten, U. *Proc. Soc. Exp. Biol. Med.* **1998**, *217*, 2.
4. Cauley, J. A.; Cummings, S. R.; Black, D. M.; Mascioli, S. R.; Seeley, D. G. D. *Am. J. Obstet. Gynecol.* **1990**, *163*, 438.
5. Scott, J. A.; Da Camara, C. C.; Early, J. E. *Am. Fam. Physician* **1999**, *60*, 1131.
6. Miller, C. *Curr. Pharm. Des.* **2002**, *8*, 2089.
7. Kuiper, G. C.; Carlsson, B.; Enmark, E.; Pelto-Huikko; Nilsson, S.; Gustafsson, J. Å. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 5925.
8. Mosselmann, S.; Polman, J.; Dijkema, R. *FEBS Lett.* **1996**, *392*, 49.
9. Kuiper, G. C.; Carlsson, B.; Grandien, K.; Enmark, E.; Haggblad, J.; Nilsson, S.; Gustafsson, J. Å. *Endocrinology* **1997**, *138*, 863.
10. Couse, J. F.; Lindzey, J.; Grandien, K.; Gustafsson, J. Å.; Korach, K. S. *Endocrinology* **1997**, *138*, 4613.
11. Katzenellenbogen, B. S.; Korach, K. S. *Endocrinology* **1997**, *138*, 861.
12. Shughrue, P. J.; Komm, B.; Merchenthaler, I. *Steroids* **1996**, *61*, 678.
13. Wang, L.; Andersson, S.; Warner, M.; Gustafsson, J. Å. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 2792.
14. Tremblay, G. B.; Trembaly, A.; Copeland, N. G.; Gilbert, D. J.; Jenkins, N. A.; Labrie, F.; Giguere, V. *Mol. Endocrinol.* **1997**, *11*, 353.
15. Wessel, M. Unpublished data.
16. (a) Pike, A. C. W.; Brzozowski, A. M.; Hubbard, R. E.; Bonn, T.; Thorsell, A. G.; Engstrom, O.; Ljunggren, J.; Gustafsson, J. Å.; Carlquist, M. *EMBO* **1999**, *18*, 4608; (b) Brzozowski, A. M.; Pike, A. C.; Dauter, Z.; Hubbard, R. E.; Bonn, T.; Engstrom, O.; Ohman, L.; Greene, G. L.; Gustafsson, J. Å. *Nature* **1997**, *389*, 753.
17. (a) Chesworth, R.; Zawistoski, M. P.; Lefker, B. A.; Cameron, K. O.; Day, R. F.; Mangano, F. M.; Rosati, R. L.; Colella, S.; Petersen, D. N.; Brault, A.; Lu, B.; Pan, L. C.; Perry, P.; Ng, O.; Castleberry, T. A.; Owen, T. A.; Brown, T. A.; Thompson, D. D.; DaSilva-Jardine, P. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2729; (b) Renaud, J.; Bischoff, S. F.; Buhl, T.; Floersheim, P.; Fournier, B.; Halleux, C.; Kallen, J.; Keller, H.; Schlaeppi, J. M.; Stark, W. *J. Med. Chem.* **2003**, *46*, 2945.
18. Meyers, M. J.; Sun, J.; Carlson, K. E.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A. *J. Med. Chem.* **1999**, *42*, 2456.
19. Meyers, M. J.; Sun, J.; Carlson, K. E.; Marriner, G. A.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A. *J. Med. Chem.* **2001**, *44*, 4230.
20. Schopfer, U.; Schoeffter, P.; Bischoff, S. F.; Nozulak, J.; Feuerbach, D.; Floersheim, P. *J. Med. Chem.* **2002**, *45*, 1399.
21. Henke, B. R.; Consler, T. G.; Go, N.; Hale, R. L.; Hohman, D. R., et al. *J. Med. Chem.* **2002**, *45*, 5492.

22. Harris, H. A.; Albert, L. M.; Leathbury, Y.; Malamas, M. S.; Mewshaw, R. E.; Miller, C. P.; Kharode, Y. P.; Marzolf, J.; Komm, B. S.; Winneker, R. C.; Frail, D. E.; Henderson, R. A.; Zhu, Y.; Keith, J. C., Jr. *Endocrinology* **2003**, *144*, 4241.
23. Lombardino, J. G.; Wiseman, E. H. *J. Med. Chem.* **1974**, *17*, 1182.
24. Iyer, R. N.; Gopalchari, R. *Indian J. Chem.* **1966**, *4*, 520.
25. Strohmeier, J.; von Angerer, E. *Arch. Pharm. (Weinheim)* **1987**, *320*, 407.
26. Competition binding assay. All compounds, except for **7e** and **12**, were run $n \geqslant 2$ and each experiment was measured in triplicate. The ability of various compounds to inhibit [^{3}H]estradiol binding was measured by a competition binding assay using dextran-coated charcoal, as has been described by Leake, R. E.; Habib, F. In *Steroid Hormone Receptors a Practical Approach;* Green B.; Leake, R. E., Roe, Eds.; IRL Press: Oxford, 1990; pp 67–92. 293T cell extracts expressing either hER.α or hER.β were incubated in the presence of increasing concentrations of competitor and a fixed concentration of [^{3}H]estradiol (141 Ci/mmol, New England Nuclear, Boston, MA) in 50 mM Tris–HCl, pH 7.4, 1.5 mM EDTA, 50 mM NaCl, 10% glycerol, 5 mM DTT, and 0.5 mg/mL. β-Lactoglobulin in a final volume of 0.2 mL. All competitors were dissolved in dimethylsulfoxide. Final concentration of the receptor was 50 pM with 0.5 nM [^{3}H]estradiol. After 16 h at 4 °C, dextran-coated charcoal (20 μL) was added. After 15 min at room temperature, the charcoal was removed by centrifugation and the radioactive ligand present in the supernatant was measured by scintillation counting. All reagents were obtained from Sigma (St. Louis, MO)..
27. 2,3-Diphenyl Indole CAS # 3469-20-3. MP BioMedicals 15 Morgan, Irvine, CA 92618-2005.
28. Anstead, G. M.; Carlson, K. E.; Katzenellenbogen, J. A. *Steroids* **1997**, *62*, 268.
29. We used the Sybyl program (Tripos Associates, St. Louis, MO.) for docking structures **7c** manually into the active sites of ER-α and ER-β crystal structures (pdb codes 1L2I,1QKM, respectively). The compounds were manually overlapped with the co-crystallized ligands and then allowed to relax in the active site of the protein using the Sybyl force field, Gasteiger–Huckel charges on the ligands, and Kollman All Atom charges on the protein. The phenolic substituents of each structure were held in place to mimic the phenolic substituents of the co-crystallized ligands. The resulting conformations were then used to analyze contact points between the protein and the ligand for the purpose of explaining potential selectivity differences in the active sites of ER-α and ER-β based on our predicated binding modes.
30. Mor, G.; Sapi, E.; Abrahams, V. M.; Rutherford, T.; Song, J.; Hao, X.-Y.; Muzaffer, S.; Kohen, F. *J. Immunol.* **2003**, *170*, 114.
31. (a) O'Brien, M. L.; Park, K.; In, Y.; Park-Sarge, O.-K. *Endocrinology* **1999**, *140*, 4530; (b) del Rio, B.; Pedrero, J. M. G.; Martinez-Campa, C.; Zuazuo, P.; Lazo, P. S.; Ramos, S. *J. Biol. Chem.* **2004**, *279*, 38294.
32. Whole cell assays. Estrogenic activity in human breast cancer MCF7 cells and primary rat granulosa cells was assessed by transient transfection of an estrogen responsive ERE3-TK-lux luciferase reporter vector essentially as we have described previously in other cell backgrounds (Petersen et al.). The MCF7 cell activity was considered to be mediated through ER-α and the granulosa activity was considered to be mediated through ER-β. MCF7 cells were obtained from ATTC (Manassas, VA) and transfected with Lipofectamine Plus (Gibco/BRL, Rockville, MD), as described by the manufacturers. Luciferase activity was measured 24 h after the addition of compounds. Petersen, D. N.; Tkalcevic, G. T.; Koza-Taylor, P. H.; Turi, T. G.; Brown, T. A. *Endocrinology* **1998**, *139*, 1082.