Monoaryl-Substituted Salicylaldoximes as Ligands for Estrogen Receptor β

Filippo Minutolo,*[,‡] Rosalba Bellini,[‡] Simone Bertini,[‡] Isabella Carboni,[‡] Annalina Lapucci,[‡] Letizia Pistolesi,[‡] Giovanni Prota,[‡] Simona Rapposelli,[‡] Francesca Solati,[‡] Tiziano Tuccinardi,[‡] Adriano Martinelli,[‡] Fabio Stossi,[#] Kathryn E. Carlson,[§] Benita S. Katzenellenbogen,[#] John A. Katzenellenbogen,[§] and Marco Macchia[‡]

Dipartimento di Scienze Farmaceutiche, Università di Pisa, Via Bonanno 6, 56126 Pisa, Italy, and Department of Molecular and Integrative Physiology and Department of Chemistry, University of Illinois, 600 S. Goodwin Avenue, Urbana, Illinois 61801

Received November 7, 2007

Salicylaldoximes possess a hydrogen-bonded pseudocyclic A′ ring in place of the typical phenolic A ring that is characteristic of most estrogen receptor (ER) ligands. Monoaryl-substituted salicylaldoximes were obtained by replacing the phenol moiety (ring A) of the ERβ pharmacophore with the pseudocycle A′ ring, which has previously been shown to behave as a bioequivalent of phenols in nonselective ER ligands. In this series, small substituents (CH_3, CN, Cl) were introduced into the central phenyl scaffold. An efficient sequential halogen-selective double cross-coupling reaction was developed for the synthesis of the methyl-substituted ER ligand. The measured ERβ affinity proved to be very sensitive to the effect of central core substituents. The binding affinities of the compounds herein reported were in good agreement with the results of computational docking analysis. The chloro-substituted derivative showed the highest β affinity and selectivity, and it also proved to be an ERβ partial agonist with an EC_{50} of 11 nM.

Introduction

The estrogen receptor (ER)[a] exists in two isoforms, α (ERα) and β (ERβ), that have different distributions in various estrogen target tissues and also have different functions, some of which have not yet been clarified. In fact, although a clear role has been established for ERα in the classical effects of estrogen activity, the physiological role of ERβ is less well understood.[1] In several situations ERβ seems to counteract the actions of the other estrogen receptor subtype (ERα), the form that promotes cancer development in some tissues (uterus, breast glands, ovary). Moreover, the cancer-controlling properties of ERβ seem to extend to colorectal and prostate cancer.[2] Therefore, there is growing evidence that stimulation of ERβ by a β-selective agonist may lead to a chemopreventive or even therapeutic effect in these tumors. Furthermore, highly selective ERβ agonists may present some of the "most wanted" features sought in SERMs (selective estrogen receptor modulators)[3–5] by providing beneficial estrogenic effects in some targets (for example, bone, CNS, liver, adipocytes, and cardiovascular system) while at the same time avoiding estrogen agonist effects on breast and uterus mediated by the ERα-subtype.

Unfortunately, there are only minor structural differences in the ligand binding cavities of ERα and ERβ that can be exploited to obtain highly β-selective ligands. These consist of slightly different pocket volumes, with the ERβ binding cavity being somewhat smaller than that of ERα and with just two amino acid differences: ERβ has a methionine (M336) in the place of the leucine at position 384 of ERα and has an isoleucine (I373) instead of the methionine at position 421 of ERα. There are also some slight differences in the shape of the binding cavities caused by variations at amino acid residues positioned in proximity around the outer side of the cavity borders. This high similarity in the binding pocket of the two ERs constitutes a serious impediment to the development of highly β-selective ligands.

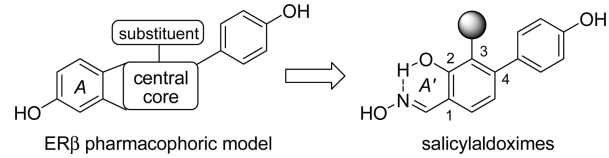


Figure 1. Structural derivation of monoaryl-substituted salicylaldoximes from the ERβ pharmacophore.

In addition to an ERβ-selective binding affinity, compounds with potent transcriptional activity are also sought, since ERβ-selective stimulation is considered to be therapeutically relevant[1,2] and, therefore, would be obtained only with selective ERβ agonists (SERBAs).[6] The silencing/activation process of the ERs is also strongly affected, not only by the ligand structure but also by the different interactions that the ER ligand complex might have with the cellular coregulatory proteins or effector components that vary from tissue to tissue.[7,8] Thus, obtaining selective ERβ agonists with a desirable pharmacological profile is a very challenging goal in this quite complicated framework.

The structural analysis of some examples of either natural (genistein)[9,10] or synthetic nonsteroidal compounds (i.e., DNP,[11] ERB-041,[12] indazoles[13]), showing specific ERβ agonist activity, shows several common features that were collected in a ERβ pharmacophoric model (Figure 1).[13]

In an attempt to enlarge the chemical diversity of estrogen receptor–ligands, we had previously shown that some oxime derivatives, comprising a six-membered pseudocycle formed by an intramolecular H-bond, could isosterically replace one phenol ring in ER ligands. Thus, we have developed *diaryl*-substituted salicylaldoximes[14,15] and anthranylaldoximes[16,17] that could efficiently bind the ERs. Other scientists have also independently reported that some aryl-carbaldehyde oximes can efficiently replace one phenol ring in ERβ-selective ligands.[18]

* To whom correspondence should be addressed. Telephone: +39 050 2219557. Fax: +39 050 2219605. E-mail: minutolo@farm.unipi.it.

‡ Università di Pisa.

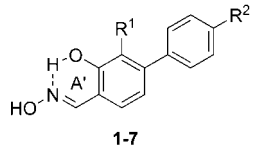
Department of Molecular and Integrative Physiology, University of Illinois.

§ Department of Chemistry, University of Illinois.

[a] Abbreviations: ER, estrogen receptor; SERM, selective estrogen receptor modulator; SERBA, selective estrogen receptor β agonist; SPhos, 2-dicyclohexylphosphino-2′,6′-dimethoxybiphenyl; RBA, relative binding affinity.

10.1021/jm701396g CCC: $40.75
Published on Web 02/13/2008

Chart 1. Monoaryl-Substituted Salicylaldoximes Designed as ERβ-Selective Ligands



1-7

compound	R^1	R^2
1	H	OH
2	H	OCH_3
3	H	H
4	CH_3	OH
5	CH_3	OCH_3
6	Cl	OH
7	CN	OH

On the basis of this evidence we envisaged the possibility of adapting our molecular approach to the newly developed ERβ pharmacophoric model by replacing the phenol A-ring with an A′-pseudocycle formed by an intramolecular H-bond between the oxime N-atom and the *o*-OH group. The design was completed by the insertion of an additional *p*-phenol substituent in position 4 of the central ring (Figure 1). Moreover, we were intrigued by the possibility of exploring the effect that could be caused by the introduction of several small substituents in position 3 of the central ring (gray sphere, Figure 1).

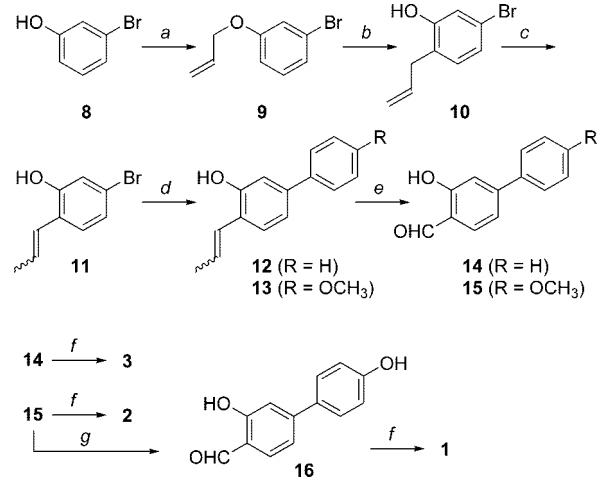
In fact, we were particularly attracted by the effect that was previously observed in an indazole series of ERβ ligands, where the introduction into the central scaffold of a halogen atom (Cl, Br, I), a small alkyl (Et), or a CN group, dramatically increased the ERβ affinities of the resulting molecules and also remarkably enhanced their β selectivities.[13]

We therefore designed a series of new *monoaryl*-substituted salicylaldoximes (**1**−**7**, Chart 1) that could potentially show selective binding for ERβ and a satisfactory potency as ERβ agonists. Initially, we considered the 3-unsubstituted monoarylsalicylaldoxime **1**. Then, among the possible substituents to be introduced into the central ring, the choice of the methyl (**4**), the chloro (**6**), or the cyano (**7**) group was based on the pharmacophoric considerations reported above and on the synthetic accessibilities of the target molecules. For some of them (**1** and **4**), the importance of the additional phenolic *p*-OH group (R^2 = OH) was assessed by preparing their analogues either with a *p*-methoxy moiety (R^2 = OCH_3, **2** and **5**) or completely unsubstituted (R^2 = H, **3**).

Results and Discussion

Synthetic Chemistry. Different synthetic strategies were applied, depending on the kind of substituent to be introduced into the 3-position of the central ring (R^1). The 3-unsubstituted molecules (**1**−**3**) were synthesized as reported in Scheme 1. Commercially available 3-bromophenol (**8**) was treated with allyl bromide to give allyl ether **9**, which was submitted to a Claisen rearrangement at 180 °C in *N*-methylaniline, yielding 2-allyl-5-bromophenol (**10**) together with the other *o*-allyl-substituted derivative (2-allyl-3-bromophenol), from which it was separated by column chromatography. Alkaline isomerization of the terminal double bond of **10** afforded an *E*/*Z* diastereoisomeric mixture of **11**. Palladium-catalyzed cross-coupling reaction of **11** with phenylboronic or 4-methoxyphenylboronic acid afforded biphenyl derivatives **12** or **13**, respectively. Oxidative cleavage of the double bond of **12** and **13**, carried out with sodium periodate in the presence of catalytic amounts of osmium tetroxide, yielded salicylaldehydes **14** and **15**, and their condensation with hydroxylamine hydrochloride respectively afforded oximes **3** and **2**. Treatment of **15** with BBr_3 caused the removal of the *O*-methyl group, and subsequent condensation of the resulting aldehyde (**16**) with hydroxylamine hydrochloride produced 4-hydroxyphenyl-substituted oxime **1**.

Scheme 1. Synthesis of 3-Unsubstituted Salicylaldoximes **1**−**3**[a]



[a] Reagents and conditions: (a) allyl bromide, K_2CO_3, acetonitrile, 80 °C; (b) *N*-methylaniline, 180 °C; (c) *t*-BuOK, DMSO, 55 °C; (d) 4-R-$C_6H_4B(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, toluene, EtOH, Δ; (e) OsO_4, $NaIO_4$, dioxane−H_2O; (f) $NH_2OH \cdot HCl$, MeOH−H_2O, 50 °C; (g) BBr_3, CH_2Cl_2, −78 to 0 °C.

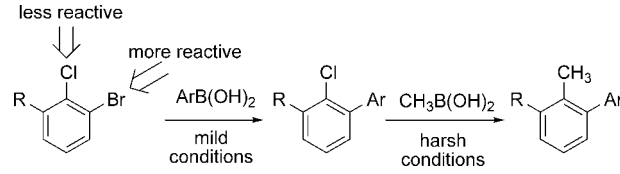


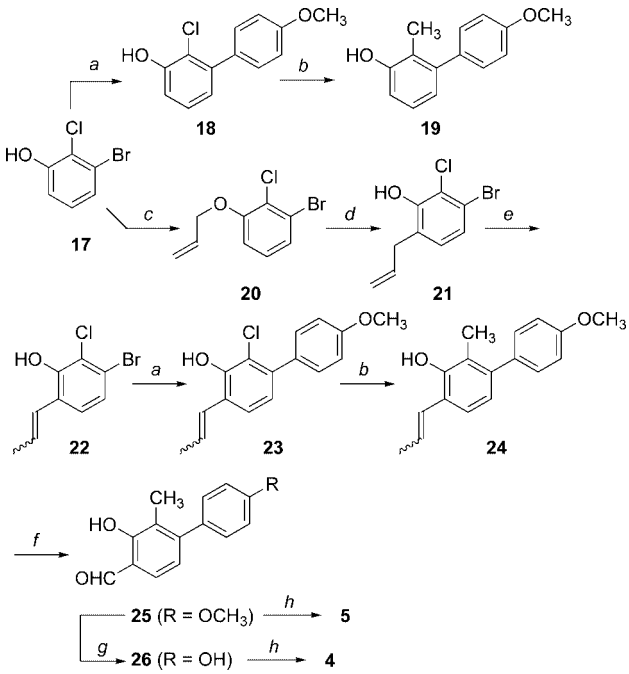
Figure 2. Sequential halogen-selective double cross-coupling reaction.

3-Methyl-substituted oximes **4** and **5** required a slightly different synthetic approach. To this aim we had to introduce a *"sequential halogen-selective double cross-coupling reaction"* into the synthetic plan, as shown in Figure 2.

We had previously exploited a similar approach for the preparation of 3,4-heterodiaryl-substituted salicylaldoximes[15] and *N*-methylanthranylaldoximes.[17] In detail, our idea was to perform this reaction sequence on an appropriate bromochloro disubstituted precursor, where the more reactive Br group could be initially replaced by an aryl under classical Suzuki conditions, using $Pd(PPh_3)_4$ as the catalyst. As a matter of fact, under these "mild" conditions, the chloroaryl group is typically unreactive.[19] Later, the methyl could be introduced by submitting the Cl group to a highly active catalytic system, although this second Pd-catalyzed cross-coupling between an alkylboronic acid and an aryl chloride is generally considered to be quite challenging.[19]

Scheme 2 shows how we applied this strategy. We first verified the suitability of our double cross-coupling sequence by model reactions on 3-bromo-2-chlorophenol (**17**), prepared as previously reported by a remarkably regioselective ortho lithiation, occurring exclusively at the 2-position, of *N*,*N*-diethylcarbamate of 3-bromophenol, followed by quenching with hexachloroethane and hydrolysis.[20] The first step with 4-methoxyphenylboronic acid was carried out in the presence of $Pd(PPh_3)_4$ (Suzuki conditions[19]) and afforded intermediate **18**. The second step was conducted with methylboronic acid in the presence of $Pd(OAc)_2$ as the catalyst and 2-dicyclohexylphosphino-2′,6′-dimethoxybiphenyl (Buchwald Ligand, SPhos) because this catalytic system was known to efficiently promote the cross-coupling reaction even between alkylboronic acids and aryl chlorides.[21] We found that compound **19** could be obtained quite efficiently this way. So these model reactions confirmed that the halogen-selective double cross-coupling was achievable

Scheme 2. Synthesis of 3-Methyl-Substituted Salicylaldoximes **4** and **5**[a]



[a] Reagents and conditions: (a) 4-MeOC$_6$H$_4$B(OH)$_2$, Pd(OAc)$_2$, PPh$_3$, aqueous 2M Na$_2$CO$_3$, 1:1 toluene/EtOH, 100 °C, 16 h; (b) MeB(OH)$_2$, Pd(OAc)$_2$, 2-dicyclohexylphosphine-2′,6′-dimethoxybiphenyl (SPhos), K$_3$PO$_4$, toluene, 100 °C; (c) allyl bromide, K$_2$CO$_3$, acetonitrile, 80 °C; (d) *N*-methylaniline, 180 °C; (e) *t*-BuOK, DMSO, 55 °C; (f) OsO$_4$, NaIO$_4$, dioxane−H$_2$O; (g) BBr$_3$, CH$_2$Cl$_2$, −78 to 0 °C; (h) NH$_2$OH·HCl, MeOH−H$_2$O, 50 °C.

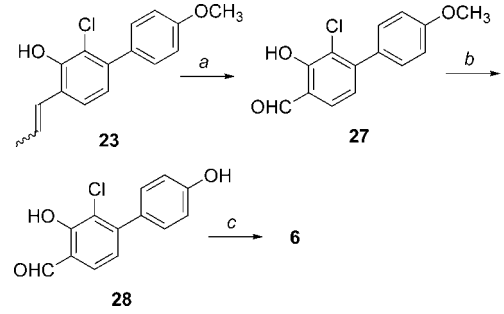
with this kind of compound, and therefore, it was applied to the real synthesis of oximes **4** and **5** (Scheme 2).

3-Bromo-2-chlorophenol (**17**)[20] was submitted to O-allylation (**20**), Claisen rearrangement (**21**), and double bond isomerization (**22**), similar to the synthesis reported above (Scheme 1). At this point, the bromochloro-substituted intermediate **22** was submitted to the halogen-selective double cross-coupling sequence, first introducing the *p*-methoxyphenyl group in the place of the Br atom (**23**) under mild conditions and then replacing the less reactive Cl atom by a methyl group, under harsher Buchwald conditions.[21] Compound **24** so obtained was treated with sodium periodate in the presence of catalytic amounts of osmium tetroxide, yielding salicylaldehyde **25**. Subsequent condensation of **25** with hydroxylamine hydrochloride afforded oxime **5**. Demethylation of **25** by BBr$_3$ afforded aldehyde **26** which, after reaction with hydroxylamine hydrochloride, eventually produced oxime **4**.

The synthesis of chloro-substituted oxime **6** is shown in Scheme 3 and started from intermediate **23**, which was prepared as reported above (Scheme 2). Oxidative cleavage of the styrene-type double bond of **23** was achieved with the catalytic OsO$_4$/NaIO$_4$ system, producing aldehyde **27**, which was then submitted to O-demethylation in the presence of boron tribromide. The final step involved a condensation of **28** with NH$_2$OH·HCl, which yielded salicylaldoxime **6**.

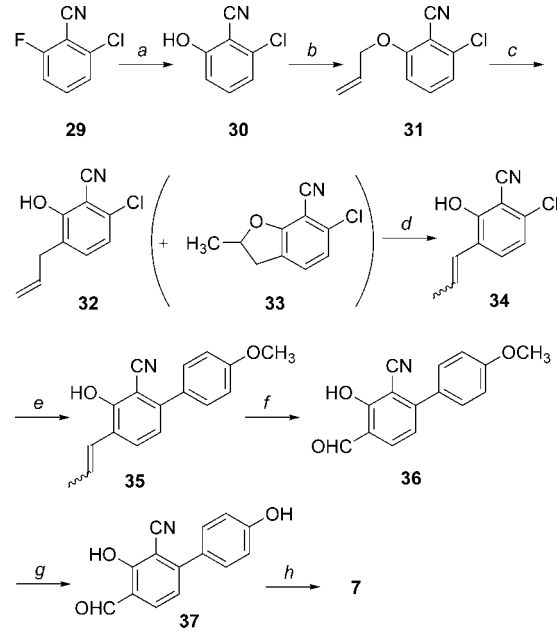
The synthesis of the cyano-substituted oxime followed a slightly different approach in the first steps, which started from commercially available 2-chloro-6-fluorobenzonitrile (**29**), as shown in Scheme 4. Aryl fluoride **29** was submitted to nucleophilic aromatic substitution with potassium acetate, and the resulting aryl acetate was submitted in situ to an alkaline hydrolysis.[22] The resulting phenol **30** was then treated with allyl

Scheme 3. Synthesis of 3-Chloro-Substituted Salicylaldoxime **6**[a]



[a] Reagents and conditions: (a) OsO$_4$, NaIO$_4$, dioxane−H$_2$O; (b) BBr$_3$, CH$_2$Cl$_2$, −78 to 0 °C; (c) NH$_2$OH·HCl, MeOH−H$_2$O, 50 °C.

Scheme 4. Synthesis of 3-Cyano-Substituted Salicylaldoxime **7**[a]



[a] Reagents and conditions: (a) (1) AcOK, 18-crown-6, CH$_3$CN, Δ, (2) aqueous NaOH (10 M); (b) allyl bromide, K$_2$CO$_3$, acetonitrile, 80 °C; (c) microwave, basic Al$_2$O$_3$, 200 °C; (d) *t*-BuOK, DMSO, 55 °C; (e) 4-MeOC$_6$H$_4$B(OH)$_2$, Pd$_2$(dba)$_3$, Cy$_3$P, Cs$_2$CO$_3$, dioxane, microwave, 80 W, 30 min; (f) OsO$_4$, NaIO$_4$, dioxane−H$_2$O; (g) BBr$_3$, CH$_2$Cl$_2$, −78 to 0 °C; (h) NH$_2$OH·HCl, MeOH−H$_2$O, 50 °C.

bromide to give *O*-allylphenol **31**. At this point we observed an unexpected reactivity of **31** in the Claisen rearrangement step. When **31** was submitted to the same reaction conditions reported for Claisen rearrangements in Schemes 1 and 2, we found that the main product obtained was dihydrobenzofuran **33** rather than the ortho-allylated phenol **32**. Since the classical conditions used in Schemes 1 and 2 (180 °C in *N*-methylaniline, 24–48 h) were not suitable for a fast optimization, we tried to run the reaction with neat compound **31** in a microwave synthesizer. In this case, in spite of the very fast conversion (>95% after 20 min), the reaction still produced the cyclized product **33** as the major or exclusive product, regardless of the microwave power used.

In considering why this unexpected side reaction was encountered in this system, we thought that it might be a result of the stronger acidity of cyano-substituted phenol **32** when compared to unsubstituted (**10**, Scheme 1) and chloro-substituted (**21**, Scheme 2) phenols. In fact, the phenol OH group might promote a cyclization reaction by protonating the terminal carbon of the allylic substituent, with the resulting phenoxide then forming a bond with the cation that forms on the internal carbon atom of the allyl double bond (Figure 3). This "acid-

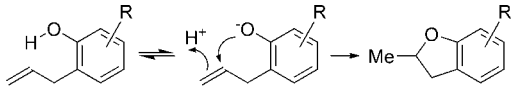


Figure 3. Acid-promoted cyclization process affected by the acid strength of the phenol.

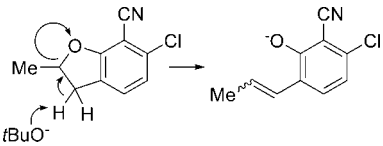


Figure 4. Base-promoted opening reaction of dihydrobenzofuran **33**.

promoted" reaction would then be favored by an increased acidity of the phenol OH group, as in cyano-substituted phenol **32**, whereas it would be less likely when the phenol is less acidic, as in **10** and **21**.

In light of these considerations, we decided to repeat the microwave experiments in the presence of solid supports characterized by different levels of acidity/basicity, such as silica, Celite, and neutral and basic alumina. In particular, we thought that more alkaline conditions due to basic alumina could "quench" the excessive acidity of the CN-substituted phenol, and therefore, the cyclization reaction would not occur significantly. In fact, when the reaction was run in the most acidic media (silica), only the cyclized derivative **33** was recovered, whereas we were pleased to find that basic alumina caused a significant improvement of the reaction outcome, affording a 75:25 ratio of the desired *o*-allylphenol **32** and the benzofuran **33**. Neutral solid supports (Celite and neutral alumina) caused a simple loss of the allyl group with no Claisen rearrangement, producing exclusively phenol **30**.

Fortuitously, while optimizing this step, we found to our surprise that desired compound **34** could be formed not only from *o*-allyl phenol **32** but also from benzofuran **33** upon treatment with potassium *tert*-butoxide in DMSO. We think that the base-promoted ring opening of **33** starts with an initial deprotonation of the benzylic methylene group, followed by formation of the internal double bond and simultaneous ring opening (Figure 4). Therefore, we could use the 75:25 mixture of **32** and **33**obtained in the previous step for the preparation of **34**, which was obtained as a 90:10 mixture of *E*/*Z* diastereoisomers. The final steps follow the same synthetic pathway reported above and involve a Pd-catalyzed cross-coupling with *p*-methoxyphenylboronic acid (**35**), an oxidative cleavage with $OsO_4/NaIO_4$ (**36**), an O-demethylation with BBr_3 (**37**), and a condensation with $NH_2OH \cdot HCl$ to produce oxime **7**.

All the oximes (**1**−**7**) were obtained as single *E*-diastereoisomeric forms. This is probably due to the fact that only these isomers can form intramolecular hydrogen bonds that contribute to the energetic stabilization of the products. This selectivity had already been verified for other oxime analogues previously reported,[14–17] and it was confirmed by the chemical shift value of the oxime proton, which is always found downfield from the 8 ppm value ($\delta > 8$ ppm; see Experimental Section).[23]

Estrogen Receptor Binding Assays. ERα and ERβ binding affinities of oximes **1**–**7** were determined by a radiometric competitive binding assay, using methods that have been previously described.[24,25] In Table 1 are reported the RBA values for the newly reported compounds, determined with purified full-length human α (hERα) and β (hERβ) receptor subtypes. Binding affinity (RBA) values are reported as percentages (%) relative to that of estradiol, which is set at 100% (absolute affinities for estradiol: $K_d = 0.2$ nM on ERα and 0.5 nM on ERβ).

Table 1. Relative Binding Affinities[a] of Compounds **1**–**7** for the Estrogen Receptors α and β

ligand	relative binding affinity (% relative to that of estradiol) hERα	hERβ	β/α ratio
estradiol	(100)	(100)	1
1	0.007 ± 0.001	0.553 ± 0.110	79
2	0.010 ± 0.003	<0.004	
3	<0.004	<0.004	
4	0.249 ± 0.062	4.10 ± 1.00	16
5	0.005 ± 0.001	0.007 ± 0.002	
6	0.065 ± 0.016	4.21 ± 0.66	65
7	0.004 ± 0.001	0.078 ± 0.016	20

[a] Determined by a competitive radiometric binding assay with [^{3}H]estradiol. Preparations of purified, full-length human ERα and ERβ (PanVera/Invitrogen) were used (see Experimental Section). Values are reported as the mean ± SD of three independent experiments. The K_d for estradiol for ERα is 0.2 nM, and for ERβ it is 0.5 nM. K_i values for the new compounds can be readily calculated by using the formula $K_i = (K_d[\text{estradiol}]/\text{RBA}) \times 100$.

As would be expected by an analysis of the ERβ pharmacophoric model (Figure 1), only *p*-hydroxyphenyl-substituted compounds (**1**, **4**, **6**, and **7**; $R^2 = OH$, Chart 1) show significant affinities for the ERs. Among this series, compound **1** is unsubstituted in the central core and shows a remarkable ERβ selectivity, although the affinity for this receptor subtype is quite modest (RBA = 0.553%). The introduction of substituents (R^1, Chart 1) in the central ring have significant effects on binding. The methyl-substituted **4** ($R^1 = CH_3$) shows a very high ERβ affinity (RBA = 4.10%), but its subtype selectivity is not very satisfactory. Better results are obtained with the introduction of a chlorine, as in **6** ($R^1 = Cl$), where high affinity for ERβ is observed, while at the same time, remarkable ERβ selectivity (ERβ/α RBA ratio of 65) is maintained. On the other hand, introduction of a cyano group, as in derivative **7**, causes a loss of affinity for both receptor subtypes. These results establish that in this series chloro-substituted compound **6** is the best ERβ-selective ligand, with an ERβ RBA of 4.21% relative to estradiol, corresponding to an absolute binding (K_i) of 12 nM, whereas its binding on ERα is much weaker (RBA = 0.065%, $K_i = 0.31$ μM).

Transcription Assays. Compound **6**, showing the highest affinity and binding selectivity for ERβ, was assayed for transcriptional activity through both receptor subtypes to determine its pharmacological character. Reporter gene transfection assays were conducted in human endometrial (HEC-1) cells, using expression plasmids for either full-length human ERα or ERβ and an estrogen-responsive luciferase reporter gene system.[26] In these assays, compound **6** proved to be an ERβ partial agonist, reaching a maximum activation of 60% when compared to estradiol, and had an EC_{50} value of 11 nM (Figure 5, solid line). This value is very close to the absolute affinity K_i (12 nM) found in the binding assay (section above).

However, this compound also proved able to stimulate ERα with an EC_{50} of 26 nM (Figure 5, dashed line), thus showing a reduced level of ERβ selectivity in transcriptional potency compared to that of its ERβ and ERα binding. This may be ascribed to differences in the interaction of these receptor–ligand complexes with the various coregulators present in these cellular assays, which can act as modulators of ligand potency.

Molecular Modeling. An automated computational analysis of all the compounds reported herein (**1**−**7**) docking into ERα and ERβ (PDB codes 2I0J and 2I0G, respectively) was performed, using the AUTODOCK 4.0 software.[27] Figure 6A

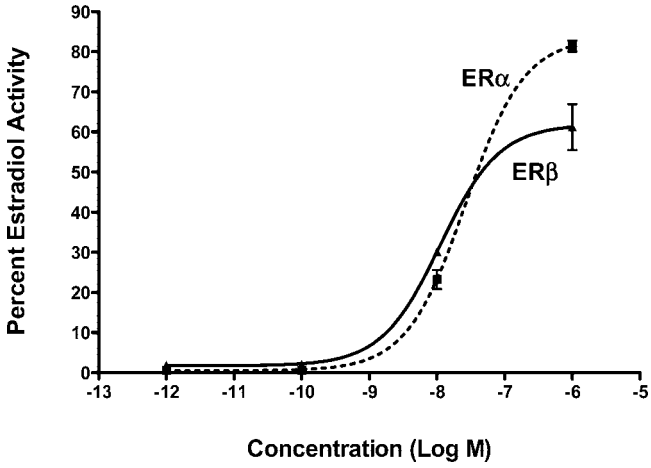


Figure 5. Dose–response curves for transcriptional activation by **6** through ERβ (solid line) and ERα (dashed line). Human endometrial cancer (HEC-1) cells were transfected with expression vectors for ERα or ERβ and an $(ERE)_2$-pS2-luc reporter gene and were treated with compound **6** at the concentrations indicated. Luciferase activity was expressed relative to β-galactosidase activity from an internal control plasmid. The maximal activity with 1 nM E_2 was set at 100%. Values are the mean of duplicate determinations.

displays the docking results for compounds **4** and **6** into ERβ. In agreement with their very similar ERβ affinity levels, they show comparable binding modes. The phenol OH group is involved in a H-bond network, which includes E305, R346, and a water molecule. The pseudocycle/oxime system forms H-bonds with G472 and H475, whereas the methyl substituent of **4** and the chlorine atom of **6** are inserted into the lipophilic pocket delimited by A302, W335, M336, and L339. Compound **1** has an 8-fold lower ERβ affinity than **4** and **6**, and it is characterized by the absence of the substituent on the central ring (R^1 = H). It was found (Figure 6B) in an overturned disposition with the phenol OH group interacting with G472 and H475 and the pseudocycle/oxime system engaged in the H-bond network of the E305−R346−water system. The substitution of the phenolhydroxyl with a methoxy group, as in compounds **2** and **5**, results in a much greater decrease of affinity for both receptors. As shown in Figure 6C, the docking studies confirm that the presence of the methoxy group results in the loss of the H-bond interactions with G472 and H475, thus explaining the dramatic decrease of binding ability. Finally, docking into ERβ of cyano-substituted compound **7** (R^1 = CN), whose ERβ affinity is about 54-fold lower than that of compounds **4** and **6**, shows that the steric hindrance of the CN group prevents its insertion in the lipophilic pocket delimited by A302, W335, M336, and L339 (Figure 6D). This results in a rotation of about 180° along the long molecular axis of **7** docked into ERβ when compared to **4** and **6**, thus resulting in a higher energy situation that explains its lower ERβ affinity. The inability of the lipophilic small pocket delimited by A302, W335, M336, and L339 to host a relatively long and linear substituent, such as the CN sticking out of the 3-position of the central phenyl ring in compound **7**, is also displayed in Figure 7, where the hydrophobic cavity can barely fit the methyl (**4**) or chloro (**6**) substituents (cyan circle).

All the analyzed compounds proved to be weaker ligands with ERα. Docking shows that none of the compounds are able to assume a disposition similar to those assumed in ERβ by the most active ligands **4** and **6**. On the contrary, they assume a disposition similar to that of **1** in ERβ, that is, phenol OH interacting with H524 and the pseudocycle/oxime system forming H-bonds with the E353−R394−water system (see Figure 6E). This overturned disposition of compounds **4** and **6** in ERα, when compared to ERβ, seems to result from different interactions of the two ERs with the R^1 substituents. In fact, in ERβ this group is able to interact with the lipophilic pocket delimited by A302, W335, M336, and L339, whereas in ERα, M336 is replaced by a leucine residue (L384), which restricts the size of this cavity, thus reducing the ability of this pocket to host a substituent and resulting in the different binding disposition of the ligands in ERα. These observations are in agreement with previously reported results,[28,29] which indicate that the leucine/methionine replacement results in differences in the receptor binding cavities that can be exploited for the development of selective ligands.

Compound **4**, which shows the best ERα-binding affinity of the series (and therefore a low ERβ selectivity), differs a bit from the others by arranging its central-ring substituent (CH_3) in the lipophilic pocket delimited by M388, L391, F404, and L428 (Figure 6F), thus benefiting from favorable hydrophobic interactions. This is likely the main cause of its lower ERβ selectivity when compared to the other ERβ ligands of this series.

Conclusions

Monoaryl-substituted salicylaldoximes were designed in agreement with a recently developed ERβ pharmacophoric model and were efficiently synthesized by means of Pd-catalyzed cross-coupling reactions that, in some cases, required an optimization of the catalytic system to have a halogen-selective single reaction or sequential double reactions. This class of compounds generally provided good ERβ ligands. A particularly relevant effect was found with the introduction into the 3-position of a methyl or a chloro substituent, which considerably increased the ERβ binding affinities of the resulting oximes **4** and **6**, respectively, when compared to their 3-unsubstituted analogue **1**. In particular, the 3-Cl substituent gives high ERβ selectivity to **6**, not by increasing its ERβ binding affinity but by decreasing its binding ability for the ERα-subtype, when compared to **4**. Computational docking studies could explain the high ERβ affinity observed with **4** and **6** and the low β-selectivity of **4**, by showing that ERβ has a small side pocket (delimited by A302, W335, M336, and L339) that is able to accommodate both the CH_3 (**4**) and the Cl (**6**) substituents but not a larger one such as CN (**7**). On the other hand, ERα can only efficiently accommodate the methyl group (**4**) in its corresponding lipophilic cavity delimited by M388, L391, F404, and L428. The most interesting ERβ-selective ligand **6** also proved to be an efficient ERβ partial agonist in functional assays, although these tests revealed that the functional activity of **6** is much less ERβ-selective than its binding affinity. Studies are underway in an attempt to produce new monoaryl-subsituted salicylaldoximes that would retain the ERβ binding affinity and selectivity of **6** but simultaneously give improved ERβ-selective agonist character in transcriptional activity assays.

Experimental Section

Chemistry. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. NMR spectra were obtained with a Varian Gemini 200 MHz spectrometer. Chemical shifts (δ) are reported in parts per million downfield from tetramethylsilane and referenced from solvent references. Electron impact (EI, 70 eV) mass spectra were obtained on a HP-5988A mass spectrometer. Where indicated, the elemental compositions of the compounds agreed to within ±0.4% of the calculated value. Chromatographic separations were performed on silica gel columns by flash (Kieselgel

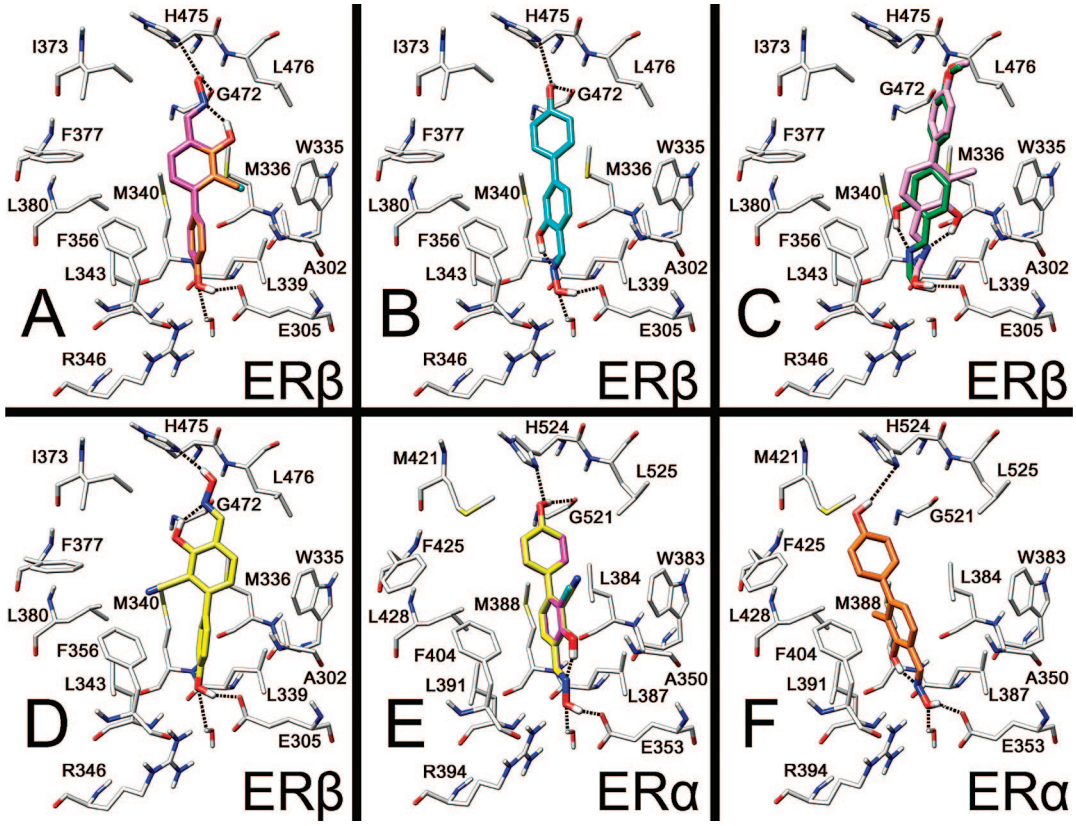


Figure 6. Docking analysis into ERβ and ERα: (A) docking of **4** (orange) and **6** (magenta) into ERβ; (B) docking of **1** (cyan) into ERβ; (C) docking of **2** (green) and **5** (violet) into ERβ; (D) docking of **7** (yellow) into ERβ; (E) docking of **1**, **6**, and **7** into ERα; (F) docking of **4** into ERα.

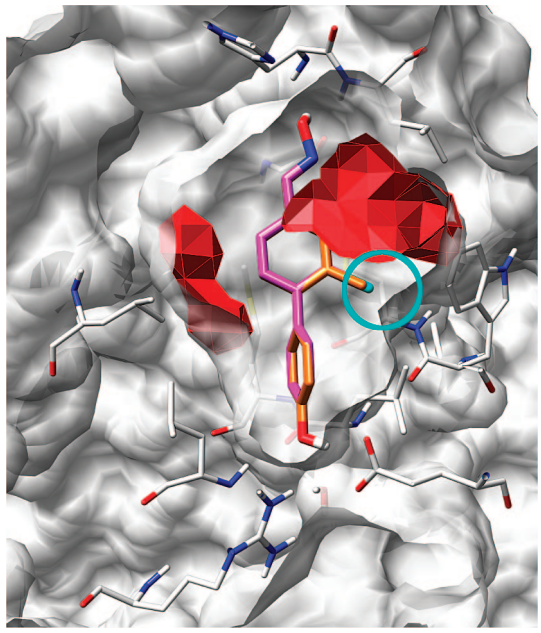


Figure 7. Free space (red polyhedra) around **4** (orange) and **6** (magenta) docked into ERβ. The cyan circle highlights the R^1 substituents (CH_3 and Cl, respectively) and the absence of free extra space around these groups.

40, 0.040–0.063 mm; Merck) or gravity column (Kieselgel 60, 0.063–0.200 mm; Merck) chromatography. Reactions were followed by thin-layer chromatography (TLC) on Merck aluminum silica gel (60 F_{254}) sheets that were visualized under a UV lamp. Evaporation was performed in vacuo (rotating evaporator). Sodium sulfate was always used as the drying agent. Microwave assisted reaction were run in a CEM microwave synthesizer. The preparation procedures and characterization data of intermediate structures have been included in the Supporting Information.

4-Phenylsalicylaldoxime (3). A solution of **14** (200 mg, 1.01 mmol) in ethanol (17 mL) was treated with a solution of hydroxylamine hydrochloride (140 mg, 2.02 mmol) in water (3.5 mL), and the mixture was heated to 50 °C for 1 h. After the mixture was cooled to room temperature, part of the solvent was removed under vacuum, and the mixture was diluted with water and extracted with EtOAc. The organic phase was dried and evaporated to afford a crude residue that was purified by column chromatography (*n*-hexane/ethyl acetate, 90:10) to yield pure **3** (212 mg, 98% yield) as a white solid: mp 130 °C; ^{1}H NMR ($CDCl_3$) δ (ppm) 7.18–7.27 (m, 3H), 7.37–7.49 (m, 3H), 7.59–7.64 (m, 2H), 8.27 (s, 1H), 9.84 (bs, 1H). MS *m*/*z* 213 (M^+). Anal. ($C_{13}H_{11}NO_2$) C, H, N.

4-(4-Methoxyphenyl)salicylaldoxime (2). Compound **14** (40 mg, 0.18 mmol) was submitted to the same procedure described above for the preparation of **3**. The crude product was purified by flash chromatography (*n*-hexane/EtOAc, 70:30) to yield **2** as a white solid (38 mg, 87% yield): mp 153 °C; ^{1}H NMR (acetone-d_6) δ (ppm) 3.85 (s, 3H), 7.03 (AA′XX′, 2H, J_{AX} = 9.0 Hz, $J_{AA'/XX'}$ = 2.5 Hz), 7.14 (d, 1H, J = 1.6 Hz), 7.20 (dd, 1H, J = 7.9, 1.6 Hz), 7.41 (d, 1H, J = 8.1 Hz), 7.64 (AA′XX′, 2H, J_{AX} = 9.2 Hz, $J_{AA'/XX'}$ = 2.6 Hz), 8.39 (s, 1H), 10.71 (bs, 1H). MS *m*/*z* 243 (M^+). Anal. ($C_{14}H_{13}NO_3$) C, H, N.

4-(4-Hydroxyphenyl)salicylaldoxime (1). Compound **16** (53 mg, 0.25 mmol) was submitted to the same procedure described above for the preparation of **3**. The crude product was purified by flash chromatography (*n*-hexane/EtOAc, 6:4) to yield **1** as a white solid (39 mg, 69% yield): mp 138 °C; ^{1}H NMR (acetone-d_6) δ (ppm) 6.93 (AA′XX′, 2H, J_{AX} = 8.4 Hz, $J_{AA'/XX'}$ = 2.5 Hz), 7.12 (d, 1H, J = 1.5 Hz), 7.18 (dd, 1H, J = 8.1, 1.8 Hz), 7.39 (d, 1H, J = 8.2 Hz), 7.55 (AA′XX′, 2H, J_{AX} = 8.6 Hz, $J_{AA'/XX'}$ = 2.6 Hz), 8.38 (s, 1H), 8.56 (bs, 1H), 10.11 (bs, 1H), 10.65 (bs, 1H). MS *m*/*z* 229 (M^+, 100), 212 (M^+ − OH, 43). Anal. ($C_{13}H_{11}NO_3$) H, N. C: calcd, 68.11; found, 67.49.

4-(4-Methoxyphenyl)-3-methylsalicylaldoxime (5). Compound **25** (49 mg, 0.20 mmol) was submitted to the same procedure described above for the preparation of **3**. The crude product was purified by flash chromatography (CH_2Cl_2) to yield **5** as a white solid (36 mg, 71% yield): mp 145 °C; ^{1}H NMR ($CDCl_3$) δ (ppm) 2.20 (s, 3H), 3.86 (s, 3H), 6.83 (d, 1H, J = 7.9 Hz), 6.90 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'XX'}$ = 2.2 Hz), 7.05 (d, 1H, J = 7.9 Hz), 7.10 (bs, 1H), 7.25 (AA′XX′, 2H, J_{AX} = 8.6 Hz, $J_{AA'XX'}$ = 1.8 Hz), 8.25 (s,1H), 10.00 (bs, 1H). MS *m*/*z*: 257 (M^+). Anal. ($C_{15}H_{15}NO_3$) H, N. C: calcd, 70.02; found, 69.25.

4-(4-Hydroxyphenyl)-3-methylsalicylaldoxime (4). Compound **26** (51 mg, 0.22 mmol) was submitted to the same procedure described above for the preparation of **3**. The crude product was purified by flash chromatography (CH_2Cl_2) to yield **4** as a white solid (51 mg, 95% yield): mp 125 °C; ^{1}H NMR ($CDCl_3$) δ (ppm) 2.19 (s, 3H), 4.80 (bs, 1H), 6.82 (d, 1H, J = 8.0 Hz), 6.88 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'XX'}$ = 2.4 Hz), 7.05 (d, 1H, J = 7.8 Hz), 7.20 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'XX'}$ = 2.4 Hz), 8.25 (s, 1H), 10.01 (bs, 1H). MS *m*/*z*: 243 (M^+). Anal. ($C_{14}H_{13}NO_3$) C, H, N.

3-Chloro-4-(4-hydroxyphenyl)salicylaldoxime (6). Compound **28** (39 mg, 0.16 mmol) was submitted to the same procedure described above for the preparation of **3**. The crude product was purified by flash chromatography (*n*-hexane/AcOEt 6:4) to yield **27** as a yellow solid (33 mg, 77% yield): mp 163 °C; ^{1}H NMR (acetone-d_6) δ (ppm): 6.92 (AA′XX′, 2H, J_{AX} = 8.6 Hz, $J_{AA'XX'}$ = 2.5 Hz), 6.95 (d, 1H, J = 7.9 Hz), 7.32 (AA′XX′, 2H, J_{AX} = 8.6 Hz, $J_{AA'XX'}$ = 2.5 Hz), 7.37 (d, 1H, J = 7.9 Hz), 8.43 (s, 1H), 8.56 (bs, 1H), 10.86 (bs, 1H), 10.91 (bs, 1H). MS *m*/*z* 263 (M^+). Anal. ($C_{13}H_{10}ClNO_3$) C, H, N.

3-Cyano-4-(4-hydroxyphenyl)salicylaldoxime (7). Compound **37** (42 mg, 0.18 mmol) was submitted to the same procedure described above for the preparation of **3**. The crude product was purified by flash chromatography ($CHCl_3$/MeOH, 95:5) to produce pure **7** (43 mg, 94% yield) as a white solid: mp 245 °C; ^{1}H NMR (CD_3OD) δ (ppm) 6.89 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'XX'}$ = 2.4 Hz), 7.04 (d, 1H, J = 8.1 Hz), 7.43 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'XX'}$ = 2.5 Hz), 7.55 (d, 1H, J = 8.2 Hz), 8.32 (s, 1H). MS *m*/*z* 254 (M^+). Anal. ($C_{14}H_{10}N_2O_3$) C, H. N: calcd, 11.02; found, 10.50.

Biological Methods. Full-length human ERα and ERβ were obtained from PanVera/Invitrogen (Carlsbad, CA). Cell culture media were purchased from Gibco BRL (Grand Island, NY). Calf serum was obtained from Hyclone Laboratories, Inc. (Logan, UT), and fetal calf serum was purchased from Atlanta Biologicals (Atlanta, GA). The expression vectors for human ERα (pCMV5-hERα) and human ERβ (pCMV5-ERβ) were as described previously.[30,31] The estrogen responsive reporter plasmid, $(ERE)_2$-pS2-Luc, was constructed by inserting the $(ERE)_2$-pS2 fragment from $(ERE)_2$-pS2-CAT into the MluI/BglII sites of pGL3-Basic vector (Promega, Madison, WI). The luciferase assay system was from Promega (Madison, WI). The plasmid pCMVβ-gal (Clontech, Palo Alto, CA), which contains the β-galactosidase gene, was used as an internal control for transfection efficiency.

Estrogen Receptor Binding Assays. Relative binding affinities were determined by competitive radiometric binding assays with 2 nM [^{3}H]E_2 as tracer, as a modification of methods previously described.[24,25] The source of ER was purified full-length human ERα and ERβ purchased from Pan Vera/Invitrogen (Carlsbad, CA). Incubations were done at 0 °C for 18–24 h, and hydroxylapatite was used to absorb the purified receptor–ligand complexes (human ERs).[25] The binding affinities are expressed as relative binding affinity (RBA) values, where the RBA of estradiol is 100%. Under these conditions, the K_d of estradiol for ERα is about 0.2 nM, and for ERβ it is 0.5 nM. The determination of these RBA values is reproducible in separate experiments with a CV of 0.3, and the values shown represent the average ± range or SD of two or more separate determinations, respectively.

Cell Culture and Transient Transfections. Human endometrial cancer (HEC-1) cells were maintained in culture as described.[26] Transfection of HEC-1 cells in 24-well plates used a mixture of 0.35 mL of serum-free MEM medium and 0.15 mL of HBSS containing 5 μL of lipofectin (Life Technologies, Rockville, MD), 20 μL of transferrin (Sigma, St. Louis, MO), 0.2 μg of pCMVβ-galactosidase as internal control, 0.5 μg of the reporter gene plasmid, and 50 ng of ER expression vector. The cells were incubated at 37 °C in a 5% CO_2 containing incubator for 4 h. The medium was then replaced with fresh medium containing 5% charcoal−dextran treated calf serum and the desired concentrations of ligands. Reporter gene activity was assayed at 24 h after ligand addition. Luciferase activity, normalized for the internal control β-galactosidase activity, was assayed as described.[26]

Docking Methods. The crystal structures of ERα (PDB code 2I0J[32]) and ERβ (PDB code 2I0G[32]) were taken from the Protein Data Bank.[33] After adding hydrogen atoms and a water molecule able to interact with E353−R394 in ERα and E305, R346 in ERβ,[17] the two proteins complexed with their reference inhibitor (i.e., benzopyran selective estrogen receptor β agonist-1, SERBA-1[32]) were minimized using Amber 9 software[34] and parm03 force field at 300 K. The complexes were placed in a rectangular parallelepiped water box. An explicit solvent model for water, TIP3P, was used, and the complexes were solvated with a 10 Å water cap. Sodium ions were added as counterions to neutralize the system. Two steps of minimization were then carried out. In the first stage, we kept the protein fixed with a position restraint of 500 (kcal/mol)/Å^2 and we solely minimized the positions of the water molecules. In the second stage, we minimized the entire system through 5000 steps of steepest descent followed by conjugate gradient (CG) until a convergence of 0.05 kcal/(Å·mol).

The ligands were built by means of Maestro[35] and were then minimized in a water environment (using the generalized Born/surface area model) by means of Macromodel.[36] They were minimized using the CG method until a convergence value of 0.05 kcal/(Å·mol) was attained, using the MMFFs force field and a distance-dependent dielectric constant of 1.0.

Automated docking was carried out by means of AUTODOCK 4.0 software.[27] AUTODOCK TOOLS[37] was used to identify the torsion angles in the ligands, add the solvent model, and assign the Kollman and the Gasteiger partial atomic charges to the protein and the ligands, respectively. In order to prevent the loss of the intramolecular H-bond of the pseudocycle/oxime system, during the docking we blocked the torsions involved in this intramolecular bond.[17]

The regions of interest used by AUTODOCK were defined by considering SERBA-1 in both receptors as the central group. In particular, a grid of 50, 40, and 46 points in the *x*, *y*, and *z* directions was constructed centered on the center of mass of this compound. A grid spacing of 0.375 Å and a distance-dependent function of the dielectric constant were used for the energetic map calculations.

By use of the Lamarckian genetic algorithm, all docked compounds were subjected to 100 runs of the AUTODOCK search, in which the default values of the other parameters were used. Cluster analysis was performed on the docked results using an rms tolerance of 1.0 Å, and the best docked conformation was used for the analysis.

All graphic manipulations and visualizations were performed by means of Chimera.[38]

Acknowledgment. We thank Karen J. Kieser for technical assistance. Support of this research from the National Institutes of Health is gratefully acknowledged (Grants R37DK15556, R01CA18119, and P01AG024387 to J.A.K. and B.S.K.). Molecular graphics images were produced using the UCSF Chimera package from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by NIH Grant P41 RR-01081).

Supporting Information Available: Experimental procedures for the intermediate compounds and a table reporting the combustion analysis data of the final products. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Dahlman-Wright, K.; Cavailles, V.; Fuqua, S. A.; Jordan, V. C.; Katzenellenbogen, J. A.; Korach, K. S.; Maggi, A.; Muramatsu, M.; Parker, M. G.; Gustafsson, J.-Å. International Union of Pharmacology. *Pharmacol. Rev.* **2006**, *58*, 773–781.

(2) Deroo, B. J.; Korach, K. S. Estrogen receptors and human disease. *J. Clin. Invest.* **2006**, *116*, 561–570.

(3) Fabian, C. J.; Kimler, B. F. Selective estrogen-receptor modulators for primary prevention of breast cancer. *J. Clin. Oncol.* **2005**, *23*, 1644–1655.

(4) Katzenellenbogen, B. S.; Katzenellebogen, J. K. Defining the ≪S≫ in SERMs. *Science* **2002**, *295*, 2380–2381.

(5) Grese, T. A.; Dodge, J. A. Selective estrogen receptor modulators (SERMs). *Curr. Pharm. Des.* **1998**, *4*, 71–92.

(6) Bryan, H.; Norman, B. A.; Richardson, T. I.; Dodge, J. A.; Pfeifer, L. A.; Durst, G. L.; Wang, Y.; Durbin, J. D.; Krishnan, V.; Dinn, S. R.; Liu, S.; Reilly, J. E.; Ryter, K. T. Benzopyrans as selective estrogen receptor β agonists (SERBAs). Part 4: Functionalization of the benzopyran A-ring. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5082–5085, and references therein.

(7) Katzenellenbogen, J. A.; O'Malley, B. W.; Katzenellenbogen, B. S. Tripartite steroid hormone receptor pharmacology: interaction with multiple effector sites as a basis for the cell- and promoter-specific action of these hormones. *Mol. Endocrinol.* **1996**, *10*, 119–131.

(8) McKenna, N. J.; O'Malley, B. W. Combinatorial control of gene expression by nuclear receptors and coregulators. *Cell* **2002**, *108* (4), 465–474.

(9) Turner, J. V.; Agatonovic-Kustrin, S.; Glass, B. D. Molecular aspects of phytoestrogen selective binding at estrogen receptors. *J. Pharm. Sci.* **2007**, *96*, 1879–1885.

(10) Muthyala, R. S.; Ju, Y. H.; Sheng, S.; Williams, L. D.; Doerge, D. R.; Katzenellenbogen, B. S.; Helferich, W. G.; Katzenellenbogen, J. A. Equol, a natural estrogenic metabolite from soy isoflavones: convenient preparation and resolution of *R*- and *S*-equols and their differing binding and biological activity through estrogen receptors alpha and beta. *Bioorg. Med. Chem.* **2004**, *12*, 1559–1567.

(11) Meyers, M. J.; Sun, J.; Carlson, K. E.; Marriner, G. A.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A. Estrogen receptor-β potency-selective ligands: structure−activity relationship studies of diarylpropionitriles and their acetylene and polar analogues. *J. Med. Chem.* **2001**, *44*, 4230–4251.

(12) Malamas, M. S.; Manas, E. S.; McDevitt, R. E.; Gunawan, I.; Xu, Z. B.; Collini, M. D.; Miller, C. P.; Dinh, T.; Henderson, R. A., Jr.; Harris, H. A. Design and synthesis of aryl diphenolic azoles as potent and selective estrogen receptor-β ligands. *J. Med. Chem.* **2004**, *47*, 5021–5040.

(13) De Angelis, M.; Stossi, F.; Carlson, K. E.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A. Indazole estrogens: highly selective ligands for the estrogen receptor β. *J. Med. Chem.* **2005**, *48*, 1132–1144.

(14) Minutolo, F.; Bertini, S.; Papi, C.; Carlson, K. E.; Katzenellenbogen, J. A.; Macchia, M. Salicylaldoxime moiety as a phenolic "A-ring" substitute in estrogen receptor ligands. *J. Med. Chem.* **2001**, *44*, 4288–4291.

(15) Minutolo, F.; Antonello, M.; Bertini, S.; Rapposelli, S.; Rossello, A.; Sheng, S.; Carlson, K. E.; Katzenellenbogen, J. A.; Macchia, M. Synthesis, binding affinity, and transcriptional activity of hydroxy- and methoxy-substituted 3,4-diarylsalicylaldoximes on estrogen receptors α and β. *Bioorg. Med. Chem.* **2003**, *11*, 1247–1257.

(16) Minutolo, F.; Antonello, M.; Bertini, S.; Ortore, G.; Placanica, G.; Rapposelli, S.; Sheng, S.; Carlson, K. E.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A.; Macchia, M. Novel estrogen receptor ligands based on an anthranylaldoxime structure: role of the phenol-type pseudocycle in the binding process. *J. Med. Chem.* **2003**, *46*, 4032–4042.

(17) Tuccinardi, T.; Bertini, S.; Martinelli, A.; Minutolo, F.; Ortore, G.; Placanica, G.; Prota, G.; Rapposelli, S.; Carlson, K. E.; Katzenellenbogen, J. A.; Macchia, M. Synthesis of anthranylaldoxime derivatives as estrogen receptor ligands and computational prediction of binding modes. *J. Med. Chem.* **2006**, *49*, 5001–5012.

(18) Mewshaw, R. E.; Bowen, S. M.; Harris, H. A.; Xu, Z. B.; Manas, E. S.; Cohn, S. T. ERβ ligands. Part 5: Synthesis and structure−activity relationships of a series of 4′-hydroxyphenyl-aryl-carbaldehyde oxime derivatives. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 902–906, and references therein.

(19) Miyaura, N.; Suzuki, A. Palladium-catalyzed cross-coupling reactions of organoboron compounds. *Chem. Rev.* **1995**, *95*, 2457–2483.

(20) Sanz, R.; Castroviejo, M. P.; Fernández, Y.; Fañanás, F. J. A new and efficient synthesis of 4-functionalized benzo[*b*]furans from 2,3-dihalophenols. *J. Org. Chem.* **2005**, *70*, 6548–6551.

(21) Walker, S. D.; Barder, T. E.; Martinelli, J. R.; Buchwald, S. L. A rationally designed universal catalyst for Suzuki−Miyaura coupling processes. *Angew. Chem., Int. Ed.* **2004**, *43*, 1871–1876.

(22) Kamal, A.; Chouhal, G. Chemoenzymatic synthesis of calcilytic agent NPS-2143 employing a lipase-mediated resolution protocol. *Tetrahedron: Asymmetry* **2005**, *16*, 2784–2789.

(23) Karabatsos, G. J.; Hsi, N. Structural studies by nuclear magnetic resonance. XI: Conformations and configurations of oxime *O*-methyl ethers. *Tetrahedron* **1967**, *23*, 1079–1095.

(24) Katzenellenbogen, J. A., Jr.; Myers, H. N. Photoaffinity labels for estrogen binding proteins of rat uterus. *Biochemistry* **1973**, *12*, 4085–4092.

(25) Carlson, K. E.; Choi, I.; Gee, A.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A. Altered ligand binding properties and enhanced stability of a constitutively active estrogen receptor: evidence that an open-pocket conformation is required for ligand interaction. *Biochemistry* **1997**, *36*, 14897–14905.

(26) Sun, J.; Meyers, M. J.; Fink, B. E.; Rajendran, R.; Katzenellenbogen, J. A.; Katzenellenbogen, B. S. Novel ligands that function as selective estrogens or antiestrogens for estrogen receptor-α or estrogen receptor-β. *Endocrinology* **1999**, *140*, 800–804.

(27) Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. *J. Comput. Chem.* **1998**, *19*, 1639–1662.

(28) Toschi, L.; Hilbig, J.; Wintermantel, T.; Engelhaupt, A.; Walter, A.; Fritzemeier, K. H.; Hillisch, A. Protein-structure-based prediction of animal model suitability for pharmacodynamic studies of subtype-selective estrogens. *ChemMedChem* **2006**, *1*, 1237–1248.

(29) Wilkening, R. R.; Ratcliffe, R. W.; Tynebor, E. C.; Wildonger, K. J.; Fried, A. K.; Hammond, M. L.; Mosley, R. T.; Fitzgerald, P. M.; Sharma, N.; McKeever, B. M.; Nilsson, S.; Carlquist, M.; Thorsell, A.; Locco, L.; Katz, R.; Frisch, K.; Birzin, E. T.; Wilkinson, H. A.; Mitra, S.; Cai, S.; Hayes, E. C.; Schaeffer, J. M.; Rohrer, S. P. The discovery of tetrahydrofluorenones as a new class of estrogen receptor beta-subtype selective ligands. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3489–3494.

(30) Wrenn, C. K.; Katzenellenbogen, B. S. Structure−function analysis of the hormone binding domain of the human estrogen receptor by region-specific mutagenesis and phenotypic screening in yeast. *J. Biol. Chem.* **1993**, *268*, 24089–24098.

(31) McInerney, E. M.; Weis, K. E.; Sun, J.; Mosselman, S.; Katzenellenbogen, B. S. Transcription activation by the human estrogen receptor subtype β (ERβ) studied with ERβ and ERα receptor chimeras. *Endocrinology* **1998**, *139*, 4513–4522.

(32) Norman, B. H.; Dodge, J. A.; Richardson, I.; Borromeo, P. S.; Lugar, C. W.; Jones, S. A.; Chen, K.; Wang, Y.; Durst, G. L.; Barr, R. J.; Montrose-Rafizadeh, C.; Osborne, H. E.; Amos, R. M.; Guo, S.; Boodhoo, A.; Krishnan, V. Benzopyrans are selective estrogen receptor beta agonists with novel activity in models of benign prostatic hyperplasia. *J. Med. Chem.* **2006**, *49*, 6155–6157.

(33) http://www.pdb.org/.

(34) Case, D. A.; Darden, T. A.; Cheatham, T. E., III; Simmerling, C. L.; Wang, J.; Duke, R. E.; Luo, R.; Merz, K. M.; Pearlman, D. A.; Crowley, M.; Walker, R. C.; Zhang, W.; Wang, B.; Hayik, S.; Roitberg, A.; Seabra, G.; Wong, K. F.; Paesani, F.; Wu, X.; Brozell, S.; Tsui, V.; Gohlke, H.; Yang, L.; Tan, C.; Mongan, J.; Hornak, V.; Cui, G.; Beroza, P.; Mathews, D. H.; Schafmeister, C.; Ross, W. S.; Kollman, P. A. *AMBER 9*; University of California: San Francisco, CA, 2006.

(35) *Maestro*, version 7.5; Schrödinger Inc.: Portland, OR, 1999.

(36) *Macromodel*, version 8.5; Schrödinger Inc.: Portland, OR, 1999.

(37) Sanner, M. F. Python: a programming language for software integration and development. *J. Mol. Graphics Modell.* **1999**, *17*, 57–61.

(38) Pettersen, E. F.; Goddard, T. D.; Huang, C. C.; Couch, G. S.; Greenblatt, D. M.; Meng, E. C.; Ferrin, T. E. UCSF Chimera, a visualization system for exploratory research and analysis. *J. Comput. Chem.* **2004**, *25*, 1605–1612.

JM701396G