Novel Estrogen Receptor Ligands Based on an Anthranylaldoxime Structure: Role of the Phenol-Type Pseudocycle in the Binding Process

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Received March 13, 2003

The 3,4-diphenylsalicylaldoxime system **1** is an estrogen receptor (ER) ligand of unusual structure, having a hydrogen-bonded pseudocyclic A′-ring in place of the paradigmatic phenolic A-ring that is characteristic of most estrogens. We have investigated the role played by the pseudocycle A′ in binding to the ER by preparing 3,4-diphenylbenzaldoxime (**4**), a compound that completely lacks this ring but still preserves all of the other features of the original molecule **1**, as well as a series of 3,4-diphenylanthranylaldoximes (**5a**−**c**) in which the nature of the heteroatom participating in the formation of pseudoring A′ has been changed from an oxygen (**1**) to a nitrogen that is either unsubstituted (**5a**) or substituted with small alkyl groups (a methyl in **5b**, or an ethyl in **5c**). The importance of hydrogen-bonded pseudocycle A′ in the binding process was confirmed by the fact that benzaldoxime **4** showed a greatly reduced binding affinity compared to salicylaldoxime **1**. Moreover, the binding affinity improved considerably when the A′-ring contained either an unsubstituted nitrogen (**5a**) or an *N*-Me group (**5b**). On the other hand, the *N*-Et-substituted anthranyl derivative **5c** showed a marked drop in binding affinity. Molecular modeling docking studies on ERα confirmed that compounds **5a** and **5b** fit nicely in the ligand binding pocket, with an especially comfortable fit for the *N*-Me group of **5b** in a small hydrophobic pocket surrounded by nonpolar residues. The limited size of this pocket does not allow accommodation of *N*-substituents larger than a methyl group, which is consistent with the low binding affinity of the *N*-Et compound **5c**.

Introduction

The estrogen receptor (ER) is a member of the nuclear receptor superfamily that functions as a ligand-regulated transcription factor.[1] Many physiological processes can be regulated by selectively activating or inhibiting the ER with appropriate agonist or antagonist ligands. Positive effects on the maintenance of bone mineral density,[2] on blood lipid levels, and on vasomotor and central nervous system functions[3] are generally considered to justify the use of estrogen agonists as agents in the treatment of postmenopausal osteoporosis,[4] atherosclerosis,[5] hot flush responses, and Alzheimer's disease.[6] Unfortunately, the activation of the ER also results in an increase in breast and uterine cancer.[7−9] Therefore, molecules, such as tamoxifen and raloxifene, that block the tumor-promoting effects of endogenous estrogens are currently used in the therapy and prevention of breast cancer.[10−12] These drugs, however, are not pure estrogen antagonists, because they promote estrogen-like effects in certain tissues; rather, they are termed selective estrogen receptor modulators (SERMs).[13,14] SERMs are particularly attractive as therapeutic agents because they are able to block estrogen action at those sites where stimulation would be undesirable, such as the breast and uterus, but at the same time stimulate estrogen actions in other tissues where they are desired, such as the bone and liver.[11,15] A great deal of effort has been devoted to the task of understanding the processes by which SERMs are able to exert tissue-specific estrogen agonist and antagonist effects and to improve upon their already rather favorable profile of selectivity.

More recently, a second subtype of the estrogen receptor, named ER*β*, was discovered.[16,17] The two ER subtypes (ERα and ER*β*) show a very high amino acid sequence homology in their DNA-binding domains (DBDs) and considerable homology in their ligand-binding domains (LBDs).[15] The tissue distribution patterns of ERα and ER*β*, however, are rather different,[15,18] as are their biological functions, some of which have been revealed by in vivo studies on receptor subtype-specific knockout mice.[19−21] The tissue-selective pharmacology of SERMs is thought to result from their different action on each ER subtype and/or 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.[22,23]

Nonsteroidal ER ligands known so far encompass a large variety of molecular structures. However, the striking chemical feature common to nearly all synthetic ligands possessing a good ER binding affinity is the presence of a phenolic ring (**A**, ER pharmacophore, Figure 1) that seems to directly mimic the steroid A-ring

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10.1021/jm0308390 CCC: $25.00
Published on Web 08/16/2003

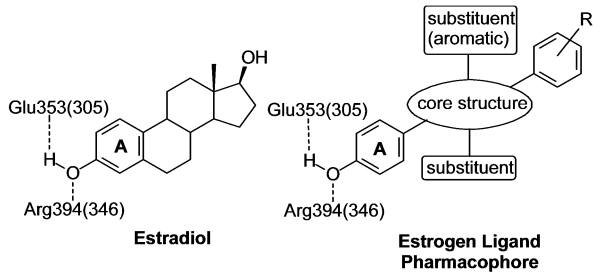


Figure 1. Schematic representation of the H-bond network occurring between the phenolic portions (A-rings) of estradiol or the synthetic ER pharmacophore with the protein residues Glu353(305) and Arg394(346) of ERα(ERβ).

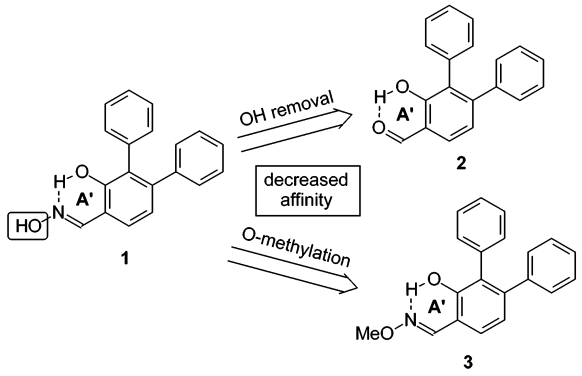


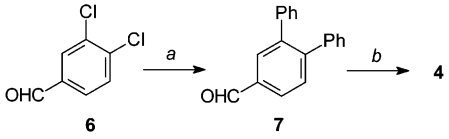
Figure 2. Modifications to salicylaldoxime **1** that caused a decrease in ER binding affinity: OH-removal (**2**) and *O*-methylation (**3**).

present in natural estrogens (e.g., estradiol, Figure 1).[24] This phenolic group was found to participate in a hydrogen-bond network that includes two specific residues of the ER ligand binding domain, Glu353(305) and Arg394(346) of ERα(ERβ), as well as a bound molecule of water.[25,26] This interaction is thought to be responsible for much of the binding affinity between the ligand and the ERs.

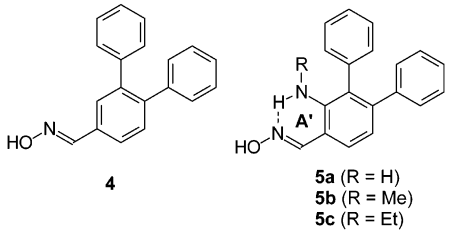
In an investigation of new molecular entities that might bind to the ER and thereby increase the chemical diversity of estrogen ligands, we recently reported that 3,4-diphenylsalicylaldoxime (**1**, Figure 2), a compound that possesses an unprecedented bioisosteric replacement of the phenolic A group, proved to have interesting binding properties for both estrogen receptor subtypes [RBA = 1.13% with ERα and 1.71% with ERβ; RBA (estradiol) = 100%].[27] The good interaction of compound **1** with ERα and ERβ was rationalized by the fact that the six-membered pseudocycle A′, formed by intramolecular hydrogen bond between the phenolic OH and the oxime nitrogen atom, might be functioning as an effective mimic of the phenolic A-ring that is found in most ER ligands.[28] To verify the importance of the OH oxime moiety, we also investigated the binding of aldehyde **2** and *O*-methyloxime **3**, in which the oxime hydroxyl group was either deleted altogether or methylated, respectively. Large reductions—of a 100-fold or greater—were found in the binding affinity of both **2** and **3** for ERα and ERβ.[27] These observations confirmed the crucial role being played by the oxime OH in the binding of the structurally novel salicylaldoxime estrogens to the ERs.

In this report, we have investigated further the structural basis for the phenol mimicry of the pseudocycle A′, to see whether we could improve the ER binding affinity by modifying the stereoelectronic nature of this ring. Therefore, we first synthesized benzaldoxime **4**,

Scheme 1



[a] Key: (a) 2 times: $Pd_2(dba)_3$, Cy_3P, $PhB(OH)_2$, Cs_2CO_3, dioxane, 80 °C; (b) $NH_2OH{\cdot}HCl$, MeOH−H_2O, 50 °C.



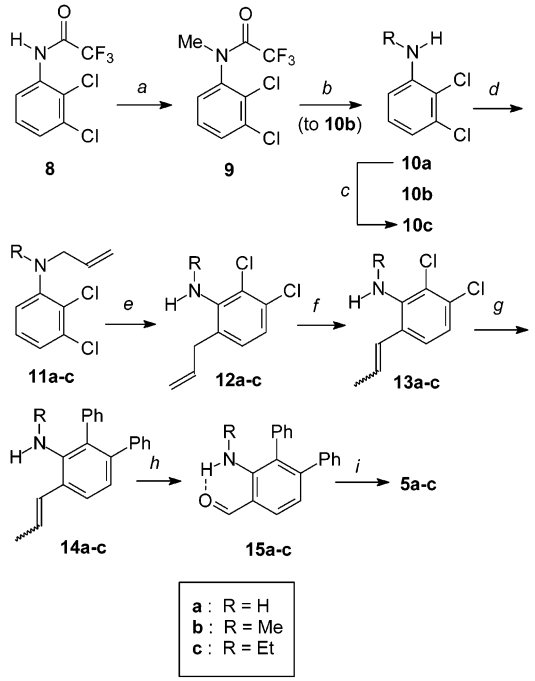
in which the pseudocycle was absent, and then anthranylaldoxime **5a**, in which the oxygen atom of the original compound **1** was replaced by an aniline-type nitrogen. We also prepared anthranyl derivatives possessing small alkyl groups on the nitrogen atom (a methyl in **5b** and an ethyl in **5c**) to see whether these groups would affect the binding properties of this class of molecules.

Results

Synthetic Chemistry. Benzaldoxime **4** was synthesized as shown in Scheme 1. Commercially available 3,4-dichlorobenzaldehyde **6** underwent a double cross-coupling reaction with phenylboronic acid, using a catalytic system containing $Pd_2(dba)_3$ and tricyclohexylphosphine,[29] which efficiently promotes cross-coupling reactions on aryl−chloride bonds (otherwise unreactive under more classical Suzuki conditions[30]). Diphenyl-substituted aldehyde **7** was then treated with hydroxylamine hydrochloride in a 10:1 methanol−water mixture at 50 °C, yielding oxime **4**, which was obtained as the pure (*E*)-diastereomer after column chromatography. The (*E*)-configuration of the oxime moiety of compound **4** was confirmed by the chemical shift value of the oxime proton, which is well below 8 ppm (8.22 ppm). Such a downfield shift is typical for aromatic oximes possessing an (*E*)-configuration, whereas the same group with a (*Z*)-configuration is usually found between 7.3 and 7.6 ppm. This downfield shift occurs when the oxime proton is on the same side and in a close spatial contact with an electronegative heteroatom such as an oxygen atom [(*E*)-configuration of the oxime]; through hydrogen bonding, it is deshielded to a greater extent than when it is positioned on the other side [(*Z*)-configuration].[31]

The synthesis of compounds **5a**−**c** was accomplished as shown in Scheme 2. 2,3-Dichloroaniline (**10a**) was commercially available. *N*-Methylated aniline **10b** was prepared from trifluoroacetamide **8**[32] by an initial methylation with methyl iodide in the presence of sodium carbonate, to give intermediate **9**, which was then submitted to alkaline hydrolysis, yielding **10b**. *N*-Ethylated aniline **10c** was obtained by reductive amination of acetaldehyde with **10a** in the presence of

Scheme 2



[a] Key: (a) MeI, K_2CO_3, DMF; (b) K_2CO_3, H_2O−MeOH; (c) CH_3CHO, $NaBH_3CN$, MeOH; (d) allyl bromide, K_2CO_3, acetonitrile, 80 °C; (e) BF_3·Et_2O, sulfolane, 120 °C; (f) *t*-BuOK, DMSO, 55 °C; (g) 2 times: $Pd_2(dba)_3$, Cy_3P, $PhB(OH)_2$, Cs_2CO_3, dioxane, 80 °C; (h) OsO_4, $NaIO_4$, dioxane−H_2O (1:1); (i) NH_2OH·HCl, MeOH−H_2O, 50 °C.

sodium cyanoborohydride. Treatment of **10a**−**c** with allyl bromide and potassium carbonate in acetonitrile afforded *N*-allyl derivatives **11a**−**c**. Subsequent aza-Claisen transposition of **11a**−**c** with boron trifluoride etherate at 120 °C[33] selectively gave the ortho-allylated anilines **12a**−**c**. Alkaline rearrangement of the terminal double bond of **12a**−**c** to the internal position afforded the styrene derivatives **13a**−**c** as *E*/*Z* diastereomeric mixtures.[34] The two chloro-aryl groups of **13a**−**c** were then submitted to two identical, sequential Pd-catalyzed cross-coupling steps with phenylboronic acid (1.5 equiv each step), using $Pd_2(dba)_3$ as the catalyst, tricyclohexylphosphine as the ligand, Cs_2CO_3 as the base, and dioxane as the solvent.[29] Under these conditions, diphenyl-substituted products **14a**−**c** were obtained. Anthranylaldehydes **15a**−**c** were then obtained by oxidative cleavage of the double bond of **14a**−**c**, using sodium periodate in the presence of catalytic amounts of osmium tetroxide.[35] Final condensation of aldehydes **15a**−**c** with hydroxylamine hydrochloride in refluxing ethanol yielded anthranylaldoximes **5a**−**c**.

In all cases (**5a**−**c**), the (*E*)-form of the oxime was the only diastereoisomer formed, presumably because the intramolecular hydrogen bond, which can only form in the (*E*)-isomer, contributes to the oxime stability. As seen before for oxime **4**, in these cases the chemical shift value of the oxime proton, which is always found below 8 ppm (8.34 for **5a**, 8.37 for **5b**, and 8.54 for **5c**), confirmed the (*E*)-configuration of oximes **5a**−**c**.[31]

Estrogen Receptor Binding Assays. The binding affinity of oximes **4** and **5a**−**c** for both ERα and ER*β* was determined by a radiometric competitive binding assay, using methods that have been described elsewhere in detail.[36,37] In Table 1 are reported the relative

Table 1. Relative Binding Affinities[a] of Compounds **1**−**5** for the Estrogen Receptors α and *β*

ligand	hERα	hER*β*
estradiol	(100)	(100)
1[b]	1.1 ± 0.2[b]	1.7 ± 0.4[b]
4	0.15 ± 0.05	0.27 ± 0.01
5a	2.2 ± 0.2	2.8 ± 0.2
5b	3.7 ± 1.1	5.2 ± 1.2
5c	0.47 ± 0.10	0.048 ± 0.013

[a] Determined by a competitive radiometric binding assay with [^{3}H]estradiol; preparations of purified, full-length human ERα and ER*β* (PanVera) were used (see the 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 ER*β* is 0.5 nM. [b] See ref 27.

binding affinity (RBA) values determined with purified full-length human alpha (hERα) and beta (hER*β*) receptor subtypes, together with those previously obtained for **1**.[27] Binding affinity (RBA) values are reported relative to estradiol (E_2), which is set at 100%.

From the results shown in Table 1, it is evident that benzaldoxime **4** experiences a significant reduction in its binding affinity with respect to the reference compound **1**.[27] In particular, when compared to **1**, the RBA values for compound **4** are 8 times lower with ERα and 6 times lower with ER*β*.

A different trend is found with anthranylaldoxime derivatives **5a**−**c**. Here, the *N*-unsubstituted derivative **5a** proved to possess better binding properties than its salicylic analogue **1**,[27] showing a 2-fold improvement in both receptor subtypes. Similar binding affinities are observed with the *N*-methylated analogue **5b**, which shows RBA values of 3.7 with ERα and 5.2 with ER*β*. These values are three times higher than reference compound **1** with both receptor subtypes, and they correspond to K_i values of 5 and 10 nM with ERα and ER*β*, respectively. On the other hand, the *N*-ethylated derivative **5c** showed a very significant drop in binding properties when compared to its *N*-methylated analogue **5b**, with a nearly 10-fold reduction on ERα and a remarkable >100-fold reduction on ER*β*.

Transcription Assays. Benzaldoxime **4** and anthranylaldoximes **5a**−**c** were assayed for transcriptional activity through both receptor subtypes. These cotransfection assays were conducted in human endometrial (HEC-1) cells, using expression plasmids for either full-length human ERα or ER*β* and an estrogen-responsive luciferase reporter gene system.[38] In this initial screen, agonist activity was determined at two concentrations, 10^{-8} and 10^{-6} M, and antagonist activity was assayed at 10^{-6} M, in the presence of 10^{-9} M E_2. In all cases, transcriptional activity is normalized relative to that obtained with 10^{-9} M estradiol, which is set at 100%. These data are summarized in Table 2, where the percent efficacy of the compounds tested as agonists (at 10^{-8} and 10^{-6} M) and as antagonists (at 10^{-6} M in the presence of 10^{-9} M E_2) is given.[39]

Salicylaldoxime **1** was previously reported to be a partial agonist on ERα and a partial antagonist on ER*β*.[39] "Low-affinity" benzaldoxime derivative **4** showed a marked agonist character on ERα and a weak partial agonist character on ER*β*. The simplest anthranylaldoxime derivative **5a** is a partial agonist on ERα and a partial antagonist on ER*β*. *N*-Methyl-substituted anthranylaldoxime **5b** proved to be a weak partial agonist

Table 2. Transcriptional Efficacy of Selected Salicylaldoxime Analogues on ERα and ERβ[a]

	% efficacy ERα[b]				% efficacy ERβ[b]			
	agonist[c] (M)		antag.[c] (M)		agonist[c] (M)		antag.[c] (M)	
compd	10^{-8}	10^{-6}	10^{-6}	pharmacol. character[d]	10^{-8}	10^{-6}	10^{-6}	pharmacol. character[d]
1	12 ± 0.1	74 ± 8	70 ± 4	part. agon.	2.6 ± 0.2	24 ± 0.3	53 ± 8	wk part. antag.
4	17 ± 2	85 ± 2	98 ± 0.4	full agon.	1.3 ± 0.1	20 ± 1	69 ± 13	wk part. antag.
5a	38 ± 7	71 ± 4	85 ± 5	part. agon.	6.0 ± 0.1	23 ± 1	31 ± 0.3	part. antag.
5b	1.6 ± 0.2	58 ± 1	85 ± 1	wk part. agon.	1.1 ± 0.1	22 ± 1	71 ± 14	wk part. antag.
5c	0.58 ± 0.03	59 ± 1	106 ± 15	wk part. agon.	0.94 ± 0.03	4.9 ± 1.3	85 ± 13	weak

[a] Transcriptional efficacy determined in cotransfection assay in HEC-1 cells using ERα or ERβ expression plasmids and an estrogen regulated reporter gene (see the Experimental Section for details). Values are percent of the transcriptional response of estradiol (E_2) at 10^{-9} M. Abbreviations: "antag." stands for antagonist, "agon." stands for agonist, part. stands for partial, and "wk" stands for weak throughout Table 2. [b] Values are percent of the transcriptional response of estradiol at 10^{-9} M, and they represent the average of triplicate determinations (CV < 0.15). [c] Agonist assays are done with compound alone; antagonist assays are done with compound together with 10^{-9} M estradiol. [d] For a definition of these terms, see ref 39.

on both receptor subtypes. Similarly, its *N*-ethyl analogue **5c** proved to be an even weaker partial agonist on ERα and ERβ.To confirm their pharmacological character in the transcription assays, complete dose−response curves were obtained for compounds **5a** and **5b** (Figure 3), the two compounds that had the highest ERα binding affinities. The dose−response curves obtained with estradiol alone (Figure 3a) serve as a reference point and confirm that this compound is a full agonist on both ERs, with only a slightly higher potency on ERα. The full dose−response curves show that anthranylaldoxime derivative **5a** is a nearly full agonist on ERα and partial antagonist on ERβ, having moderate potency in both cases, although the antagonist effect of **5a** on ERβ is clearly evident (Figure 3b). The *N*-methyl substituted analogue **5b** has a similar pharmacological profile (Figure 3c), being a stronger agonist on ERα than on ERβ. Surprisingly, even though the *N*-methyl analogue (**5b**) has higher ER binding affinity than its unmethylated congener (**5a**), in these transcription assays, it appeared less potent on both ERs (Figure 3c).

Molecular Modeling of Oximes in the Ligand Binding Pockets of the ERs. To better understand the possible binding modes of compounds **1** and **5a**−**c**, we performed molecular docking experiments on ERα. The protein backbone was taken from the X-ray structure of ERα bound to estradiol[25,40] (Figure 4a), where it is clear that the A-ring hydroxyl group makes hydrogen bonds with Arg394 and Glu353. Docking calculations were carried out by replacing the estradiol molecule with salicylaldoxime **1** or anthranylaldoximes **5a**−**c**. We found that the pseudoring A′ of salicylaldoxime **1** could be positioned where estradiol had its phenolic A-ring (Figure 4b), allowing for an efficient participation of the oxime OH in the same hydrogen-bonding network with Arg394 and Glu353. The aromatic central core of **1** replaces the B-ring of estradiol, and the two phenyl substituents protrude toward the relatively hydrophobic empty spaces in the receptor ligand binding pocket. Although **1** binds to ERα in a fashion similar to estradiol, the overall complexes may not be perfectly identical, since **1** exhibits only a partial agonism on this receptor subtype.

Both anthranylaldoximes **5a,b** (Figure 4c) could be positioned in a binding mode which is very similar to **1**, placing their oxime OH and pseudorings A′ in analogous sites. The only significant difference is in the torsional angles of the two phenyl substituents, which are close to 90° in **5a** and **5b**, but only around 70° in the case of **1**. *N*-methyl derivative **5b** places its *N*-methyl group in a small hydrophobic pocket defined by Leu346, Leu349, and Ala350.

By contrast, the higher homologue **5c**, which has an *N*-ethyl substituent, was not able to assume a similar binding mode within the ligand binding pocket as defined in the ERα−E_2 X-ray structure when the pocket was rigidly fixed during the modeling. To examine whether the rigidity of the receptor was responsible for this behavior, we performed molecular dynamics on the ERα structure, fixing **5c** in a binding mode that was similar to the one found for its lower homologues (**5a,b**). Under this molecular dynamics simulation, some amino acid residues underwent spatial shifts to accommodate **5c** (Figure 5), in particular expanding the small hydrophobic pocket that hosts the *N*-alkyl substituent.

The most evident difference between the minimized protein (green, Figure 5) and the X-ray structure (purple, Figure 5) lies in a 0.5 Å shift of the Leu349 side chain, which is the residue closest to the *N*-ethyl substituent of **5c**. We verified that the ERα−**5c** complex obtained after molecular dynamics (green, Figure 5) was of lower energy that the one obtained previously by automated docking in the rigid ERα structure, by minimizing both ERα−**5c** complexes and performing energy calculations after minimization. The structure obtained after molecular dynamics was of lower energy by 5.3 kcal/mol. Because of this increase in ligand stabilization energy, we also submitted the ERα−**1**, ERα−**5a**, and ERα−**5b** complexes to the same minimization procedure, so that we could evaluate the relative stability of all of the modeled ER−ligand complexes on a common basis.

In this manner, we found that the interaction energy between the ligand and the receptor was less favorable with **5c** (4.4 kcal/mol higher) than with **5a** and **5b**, both of which showed practically identical interaction energies. Salicylaldoxime derivative **1** formed a complex with 2.5 kcal/mol higher energy than those with **5a** and **5b**. The van der Waals contribution in the case of **5c** is particularly unfavorable, because the *N*-ethyl group is forced to push against the residue of Leu349.A view of the final docked conformation obtained with the highest affinity compound **5b** (Figure 6) shows the presence of a hydrophobic pocket of limited size fitting tightly around the *N*-Me substituent of **5b**. Both the front view (Figure 6a) and the top view (Figure 6b) show that this pocket is formed by the aliphatic side chains of Leu349 and Ala350, and by the peptide carbonyl portion of

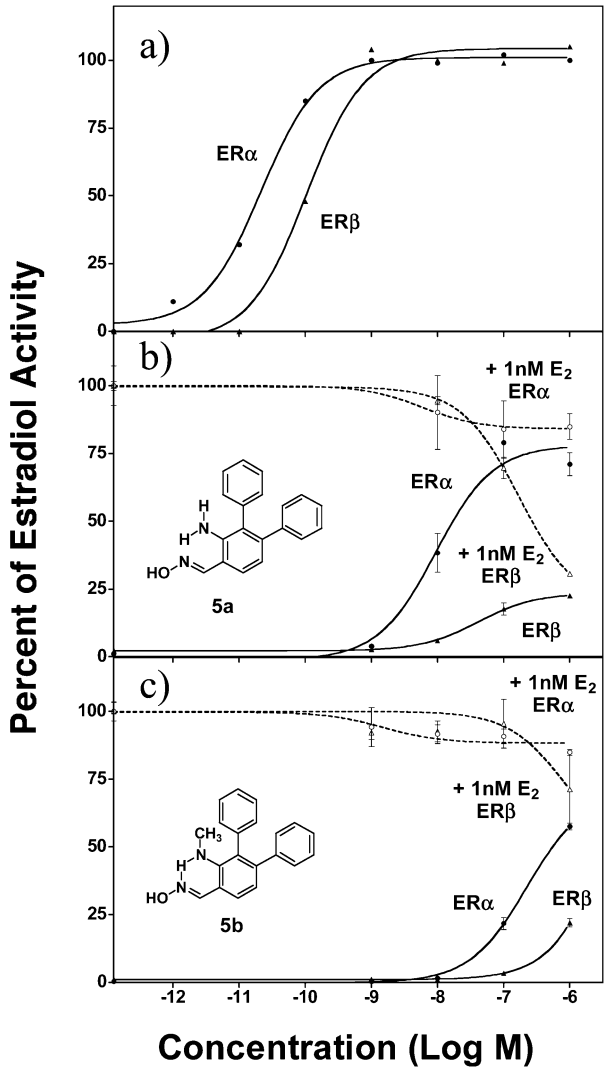


Figure 3. Dose−response curve for transcriptional activation by (a) estradiol (E_2) through ERα (solid circles) and ERβ (solid triangles); (b) **5a** in the agonist mode through ERα (solid circles) and ERβ (solid triangles), or in the antagonist mode, i.e., in the presence of 1 nM estradiol (E_2) through ERα (open circles) and ERβ (open triangles); (c) **5b** in the agonist mode through ERα (solid circles) and ERβ (solid triangles), or in the antagonist mode, i.e., in the presence of 1 nM estradiol (E_2) through ERα (open circles) and ERβ (open triangles). 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 compounds E_2, **5a**, or **5b** alone, or with compounds **5a** or **5b** plus 1 nM estradiol (E_2) for 24 h, 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 (a). Values are the mean ± SD from three or more separate experiments.

Leu346. As a result, there is no room for the extra carbon unit that is present in **5c**, unless the receptor is forced to expand this hydrophobic cavity.

Discussion and Conclusions

Our prior work on the salicylaldoxime estrogens established the fundamental role of the free oxime OH group of compound **1** in determining the high binding affinity of this ligand to the estrogen receptor.[27] In this study, we have shown that it is not just the oxime but the full pseudoring A′ in **1** (formed by intramolecular

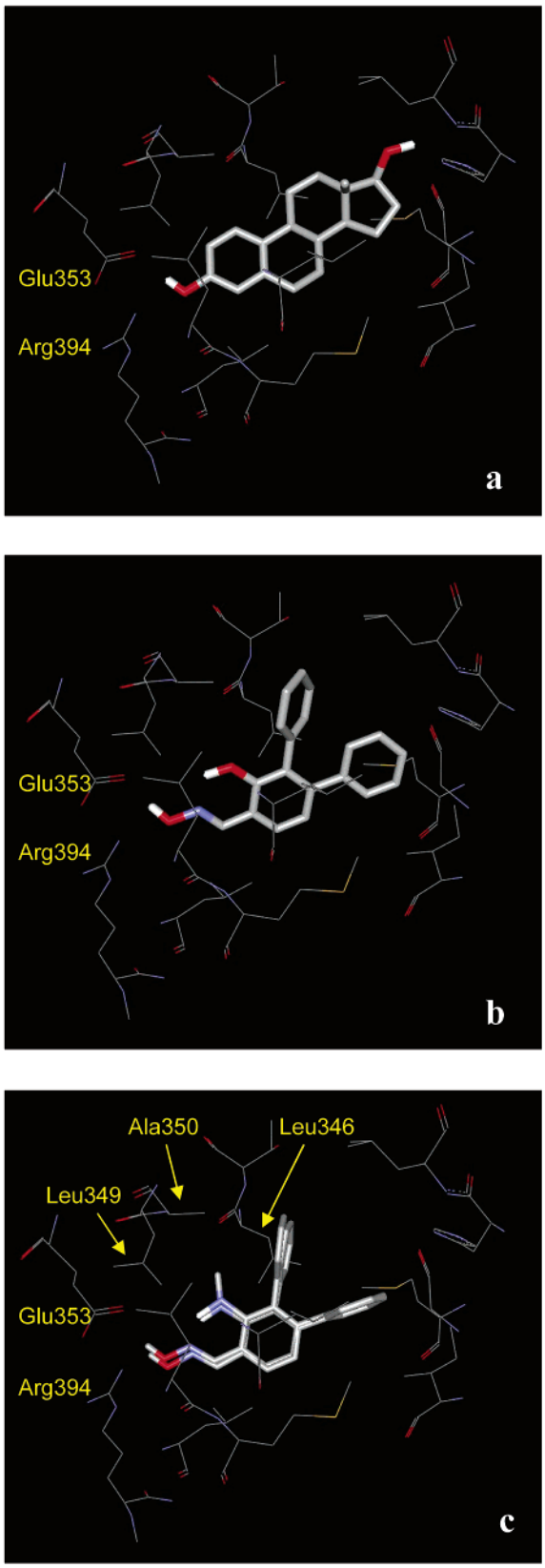


Figure 4. (a) X-ray structure of the complex ERα∠estradiol.[25,40] (b) Docking of compound **1** into ERα. (c) Docking of compounds **5a** and **5b** into ERα. Most H's omitted for clarity.

hydrogen bond between the phenolic OH and the oxime nitrogen atom) that is important in ensuring a high affinity interaction with the ERs. This is evident from that fact that the affinity of benzaldoxime **4** for the ERs

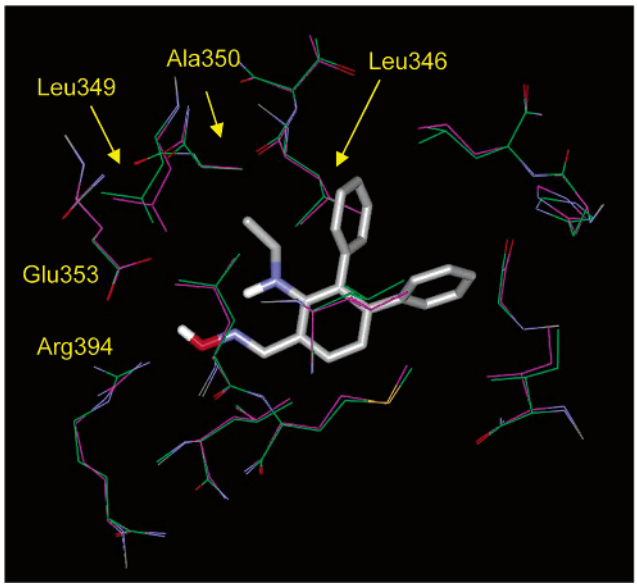


Figure 5. Docking of **5c** into ERα after molecular dynamics and minimization: comparison of the relative spatial position of representative amino acid residues between the receptor X-ray structure[25,40] (purple) and the "relaxed" structure after molecular dynamics (green). Most H's omitted for clarity.

is 6−8 times lower than that of the salicylaldoxime compound **1**.

The difference in binding affinity of the salicylaldoxime (**1**) and the benzaldoxime (**4**) might be due to the fact that in the latter compound [**4**, of (*E*)-configuration], rotation around the single bond between the oxime carbon atom and the aromatic ring is constrained only by resonance overlap with the benzene ring and not by the intramolecular hydrogen bond, so that two low-energy conformations are possible, (s-cisoid and s-transoid; Figure 7). This conformational freedom might enable the oxime OH to populate a "nonpharmacophoric" (i.e., s-transoid) conformation that would be expected to have lower ER binding affinity. This rotation is impeded when the pseudoring A′ is present (as in **1**), because the position of the oximic OH is locked into the s-cisoid conformation by the intramolecular hydrogen bond (Figure 7). Alternatively, the pseudoring A′ might be acting as a real mimic of the phenyl A-ring of estradiol by means of its rather extended π-conjugation, which might engender a greater degree of attractive van der Waals or $\pi-\pi$ interactions between these ligands and the A-ring binding pocket of the receptor.

Once we verified the importance of the pseudoring A′ by comparing the binding affinity of the congeneric benzaldoxime (**4**) and salicylaldoxime (**1**) systems, we improved the binding affinity of the lead compound **1** by replacing the oxygen atom contained in A′ with a nitrogen atom, as in anthranylaldoxime **5a**. The nitrogen atom of **5a** is of the aniline type, and therefore, its hybridization state has a greater sp^2 character than its oxygen counterpart in **1**.[41] We believe that this results in a greater degree of delocalization of the nitrogen lone pair through the aromatic central core to the oxime C−N double bond, giving the pseudocycle a greater degree of π-conjugation. Thus, the A′-rings of the anthranylic derivatives would have greater aromatic character, which would make them better mimics for the phenolic A-ring of estradiol and other classical estrogen ligands.

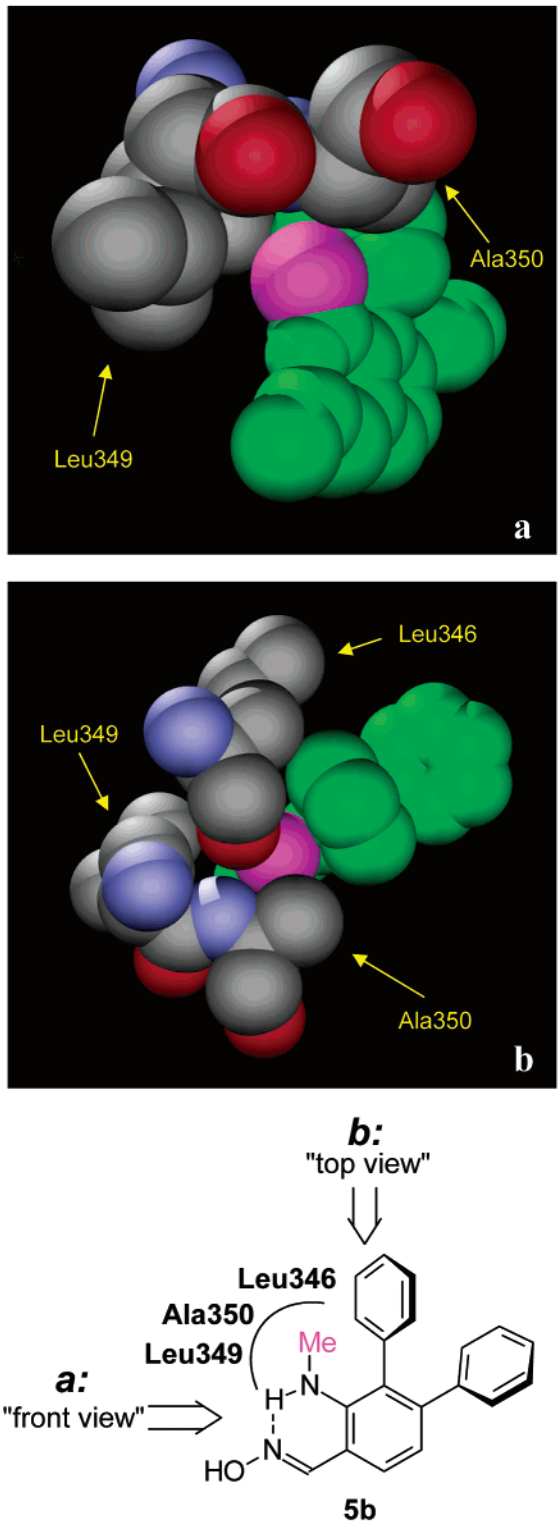


Figure 6. (a) Front view and (b) top view of van der Waals volumes of **5b** (green), docked into ERα−LBD, together with selected amino acid residues that define a small hydrophobic pocket occupied by the *N*-methyl group (purple). Most H's omitted for clarity.

Another factor that might be responsible for the higher affinity of anthranyl derivative **5a** is the considerably higher torsional angles of the phenyl substituents, which were observed to be around 90° in the docking experiments (Figure 4c), as compared to the salicylic derivative **1**, where the same torsionals measure about 70° (Figure 4b). These torsional angle

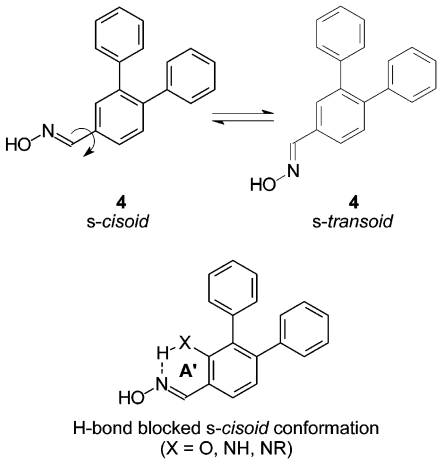


Figure 7. Free rotation of the oxime group in compound **4** and effect of intramolecular H-bond in blocking the s-cis conformation.

differences arise from the presence of the exocyclic N−H bond in **5a**, which sterically hinders the adjacent phenyl substituent in a way not found in the salicylic analogue **1**, where the corresponding atom (oxygen) has only exocyclic lone pairs. To minimize this hindrance, the phenyl ring in position 3 adopts a conformation that is approximately orthogonal to the central aromatic core, a change that also forces the second phenyl substituent to assume a similar, more perpendicular conformation. The greater torsion angles result in an expansion of the molecular volume of these portions of the ligand, so that they more fully fill the empty spaces that surround the ligand in the ligand binding pocket of the receptor (Figure 4c). A similar effect of ligand bulk normal to the plane of the steroid system as a factor that enhances binding affinity has been noted in the tetrahydrochrysene system,[42] in 7α- and 11β-substituted steroids,[24,43] and in the stilbene series, where X-ray crystallography shows that the two ethyl groups of DES are, in fact, filling hydrophobic voids above and below the plane adopted by steroidal ligands, rather than within this plane.[44]

The binding affinity of the anthranylic oxime system turned out to be preserved when a methyl group was placed on the aniline nitrogen atom of the anthranylic system, as in compound **5b**, which showed the best RBA values of this series (Table 1). The docking experiments showed that, as expected, the structure of **5b** in the ERα−LBD perfectly superimposes that found for **5a** (Figure 4c), with the torsional angles of the two phenyl substituents of about 90°. The only structural difference between **5a** and **5b** is the *N*-methyl group, which is found to occupy a small hydrophobic pocket whose boundaries are formed by three residues, Leu346, Leu349, and Ala350 (Figures 4c and 6).

This trend undergoes an early break, however, when an *N*-ethyl substituent is introduced, as in compound **5c**, where an unexpected drop in binding affinity is found. However, our docking experiments showed that **5c** is able to effectively interact with ERα only after the receptor LBD has undergone a certain degree of deformation (Figure 5) and that the resulting complex is less stable than the ERα-**5b** one, basically because of repulsive van der Waals interactions. In fact, the small hydrophobic pocket defined by Leu349 and Ala350 and by the peptide carbonyl portion of Leu346 has limited dimensions; it is able to comfortably host the *N*-Me group of **5b** (Figure 6), but it needs some rearranging to be able to do the same with even the slightly bulkier ethyl moiety, as in **5c**. Thus, the reduced affinity of **5c** effectively probes the spatial dimension of this hydrophobic pocket, which, as visualized in Figure 6, only has space enough to efficiently embrace a methyl group.

The transcriptional efficacy of these compounds confirmed what had been previously found for their salicylaldoxime analogues,[39] that is, a greater level of agonism on ERα than on ERβ (Table 2). A curiosity is that the *N*-methyl anthranyloxime (**5b**), which binds to ER with higher affinity than the unsubstituted analogue (**5a**), has lower potency in the transcription assay. It is not uncommon to find such discrepancies between the rank order of receptor binding and transcriptional potency with ER ligands, and this has been ascribed to differences in the interaction with cellular coregulators, which can act as modulators of ligand potency.[22,23]

In conclusion, we have found in the anthranylaldoximes a new class of estrogen receptor ligands endowed of better binding affinity properties when compared with their oxygenated predecessor salicylaldoximes. Among the anthranyl derivatives, we have recognized in the *N*-Me analogue **5b** the highest affinity member of this class. Docking experiments confirmed the binding affinity data and explained, through consideration of the properties of a small hydrophobic pocket, the highest affinity of the *N*-Me derivative **5b** and the lowest affinity of the *N*-Et derivative **5c**. It will be now interesting to decorate the external aromatic substituents, as we have previously done with related compounds,[39] to explore whether it is possible to find other members of this class of molecules that possess improved biological properties.

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 from solvent references. Electron impact (EI) mass spectra were obtained on a HP-5988A mass spectrometer. 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 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. 3,4-Dichlorobenzaldehyde (**6**) and 2,3-dichloroaniline (**10a**) were purchased from Sigma-Aldrich (St. Louis, MO). 2,3-Dichloro-*N*-(trifluoroacetyl)aniline (**8**) was prepared as previously described.[32]

3,4-Diphenylsalicylaldoxime (1). Preparation of **1** and most characterization data have already been reported.[27] ^{13}C NMR ($CDCl_3$) δ 115.58, 121.86, 126.80, 126.91, 127.76, 127.87, 129.75, 129.80, 131.09, 136.14, 140.82, 144.46, 152.69, 154.71.

3,4-Diphenylbenzaldehyde (7). A solution of 3,4-dichlorobenzaldehyde (**6**) (0.300 g, 1.71 mmol) in dioxane (2 mL) was treated with 0.957 g (2.94 mmol) of cesium carbonate, 0.314 g (2.57 mmol) of phenylboronic acid, 0.050 g (0.055 mmol) of tris(dibenzylideneacetone)dipalladium(0), and 0.21 mL of a 20% solution of tricyclohexylphosphine (0.13 mmol) in toluene. The resulting suspension was heated at 80 °C for 16 h under an argon atmosphere. The reaction mixture was then cooled to room temperature, diluted with diethyl ether, and filtered

through a Celite pad. The solvent was removed under vacuum, and the resulting crude mixture was submitted again to the same treatment described above. The crude product deriving from the second step was purified by column chromatography, eluting with a 9:1 → 7:3 *n*-hexane/ethyl acetate mixture, to obtain 0.352 g (1.36 mmol, 79% yield, two steps) of aldehyde **7** as an oil: ^{1}H NMR ($CDCl_3$) δ 7.12−7.25 (m, 10H), 7.60 (d, 1H, J = 8.4 Hz), 7.90−7.94 (m, 2H), 10.10 (s, 1H). Anal. $C_{19}H_{14}O$ (C, H).

(*E*)-3,4-Diphenylbenzaldoxime (4). A solution of 3,4-diphenylbenzaldehyde (**7**) (0.329 g, 1.28 mmol) in methanol (20 mL) was treated with a solution of hydroxylamine hydrochloride (0.177 g, 2.55 mmol) in water (3 mL), and the resulting mixture was heated to 50 °C for 1 h. After being cooled to room temperature, the solvent was partially removed under vacuum and the residue was extracted with ethyl acetate. The combined organic phase were dried and concentrated under vacuum and the crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 8:2) to yield pure **1b** (0.267 g, 0.980 mmol, 74% yield) as an off-white solid: mp 118−120 °C; ^{1}H NMR ($CDCl_3$) δ 7.11−7.24 (m, 10H), 7.46 (d, 1H, J = 8.4 Hz), 7.63−7.67 (m, 2H), 8.22 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 125.92, 126.89, 128.05, 129.53, 129.82, 129.87, 131.22, 140.82, 140.85, 141.16, 142.35, 150.08; MS *m*/*z* 273 (M^+, 100), 256 ($M^+ - OH$, 16), 255 ($M^+ - H_2O$, 20), 229 ($M^+ - CH{=}NOH$, 59). Anal. $C_{19}H_{15}NO$ (C, H, N).

2,3-Dichloro-*N*-methyl-*N*-(trifluoroacetyl)aniline (9). A solution of 2,3-dichloro-*N*-(trifluoroacetyl)aniline (**8**)[32] (0.472 g, 1.82 mmol) in anhydrous DMF was treated under nitrogen with 0.377 g of potassium carbonate (2.73 mmol) and 0.33 mL of methyl iodide (2.2 mmol) at room temperature for 30 min. The reaction mixture was then quenched with water and extracted with ethyl acetate. The combined organic phase was washed with water, dried, and concentrated under vacuum. The crude residue was purified by flash chromatography (*n*-hexane/ethyl acetate 9:1) to yield pure **9** (0.435 g, 1.60 mmol, 88% yield) as an oil: ^{1}H NMR ($CDCl_3$) δ 3.32 (s, 3H), 7.22−7.34 (m, 2H), 7.56 (dd, 1H, J = 7.2, 2.5 Hz).

2,3-Dichloro-*N*-methylaniline (10b). A solution of 2,3-dichloro-*N*-methyl-*N*-(trifluoroacetyl)aniline (**9**) (0.311 g, 1.14 mmol) in methanol (50 mL) and water (20 mL) was treated with potassium carbonate (1.05 g, 7.60 mmol) and the resulting mixture was stirred at room temperature for 1 h. The solvent was partially removed under vacuum and the residue extracted with ethyl acetate. The combined organic phase was washed with water, dried, and concentrated under vacuum. The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 9:1) to yield pure **10b** (0.162 g, 0.923 mmol, 81% yield) as an oil: ^{1}H NMR ($CDCl_3$) δ 2.91 (s, 3H), 4.29 (br, 1H), 6.53 (dd, 1H, J = 8.1, 1.5 Hz), 6.79 (dd, 1H, J = 8.1, 1.5 Hz), 7.08 (t, 1H, J = 8.1 Hz); MS *m*/*z* 174 (M^+, 100), 139 ($M^+ - Cl$, 10).

2,3-Dichloro-*N*-ethylaniline (10c). A solution of 2,3-dichloroaniline (**10a**) (3.00 g, 18.5 mmol) in methanol (20 mL) was treated with 1.0 mL of acetaldehyde (19 mmol) and 1.4 g of sodium cyanoborohydride (22 mmol). The resulting mixture was stirred at room temperature for 4 h and then diluted with chloroform, washed with brine, dried, and concentrated under vacuum. The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 9:1) to yield pure **10c** (2.16 g, 11.4 mmol, 62% yield) as an oil: ^{1}H NMR ($CDCl_3$) δ 1.31 (t, 3H, J = 7.1 Hz), 3.20 (q, 2H, J = 7.1 Hz), 4.35 (br, 1H), 6.54 (dd, 1H, J = 8.2, 1.5 Hz), 6.77 (dd, 1H, J = 8.2, 1.5 Hz), 7.06 (t, 1H, J = 8.2 Hz); MS *m*/*z* 189 (M^+, 27), 174 ($M^+ - CH_3$, 100).

***N*-Allyl-2,3-dichloroaniline (11a).** A solution of 2,3-dichloroaniline (**10a**) (1.62 g, 10.1 mmol) in acetonitrile (10 mL) was treated with 2.5 g of potassium carbonate (18 mmol). The mixture was then heated to 80 °C and treated, dropwise, with a solution of allyl bromide (1.5 g, 12 mmol) in acetonitrile (6 mL). Heating was continued for 3 h and then the mixture was left under stirring at the same temperature overnight. After cooling to room temperature, the reaction mixture was filtered by suction, with repeated washing of the filter with diethyl ether, and the filtrate was concentrated under vacuum. The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 9:1) to yield pure **11a** (1.39 g, 6.87 mmol, 68% yield) as an oil: ^{1}H NMR ($CDCl_3$) δ 3.81−3.88 (m, 2H), 4.64 (br, 1H, NH), 5.20 (dq, 1H, J = 10.3, 1.5 Hz), 5.29 (dq, 1H, J = 17.4, 1.5 Hz), 5.94 (ddt, 1H, J = 17.2, 10.3, 5.1 Hz), 6.54 (dd, 1H, J = 8.2, 1.1 Hz), 6.79 (dd, 1H, J = 7.9, 1.2 Hz), 7.04 (t, 1H, J = 8.1 Hz); MS *m*/*z* 201 (M^+, 59), 174 ($M^+ - CH{=}CH_2$, 100), 166 ($M^+ - Cl$, 51), 130 ($M^+ - 2Cl$, 55).

***N*-Allyl-*N*-methyl-2,3-dichloroaniline (11b).** Compound **11b** was prepared from **10b** (0.357 g, 2.02 mmol) by following the same procedure described above for **11a**. The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 9:1) to yield pure **11b** (0.345 g, 1.60 mmol, 79% yield) as an oil; ^{1}H NMR ($CDCl_3$) δ 2.76 (s, 3H), 3.64 (d, 2H, J = 6.3 Hz), 5.20 (ddt, 1H, J = 10.1, 1.8, 1.3 Hz), 5.25 (dq, 1H, J = 17.2, 1.6 Hz), 5.91 (ddt, 1H, J = 17.2, 10.2, 6.1 Hz), 6.98−7.01 (m, 1H), 7.12−7.15 (m, 2H); MS *m*/*z* 215 (M^+).

***N*-Allyl-*N*-ethyl-2,3-dichloroaniline (11c).** Compound **11c** was prepared from **10c** (1.79 g, 9.47 mmol) by following the same procedure described above for **11a**. The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 9:1) to yield pure **11c** (1.68 g, 7.32 mmol, 77% yield) as an oil: ^{1}H NMR ($CDCl_3$) δ 1.02 (t, 3H, J = 7.1 Hz), 3.14 (q, 2H, J = 7.0 Hz), 3.67 (d, 2H, J = 6.0 Hz), 5.13 (dq, 1H, J = 10.3, 1.4 Hz), 5.20 (dq, 1H, J = 17.4, 1.5 Hz), 5.83 (ddt, 1H, J = 17.2, 10.1, 6.1 Hz), 6.96 (dd, 1H, J = 7.0, 2.7 Hz), 7.06−7.16 (m, 2H); MS *m*/*z* 229 (M^+).

6-Allyl-2,3-dichloroaniline (12a). Compound **11a** (2.73 g, 13.7 mmol) was dissolved in sulfolane (5 mL) and treated under argon with $BF_3{\cdot}Et_2O$ (5.2 mL, 41.0 mmol). The resulting mixture was heated to 120 °C overnight. After cooling to room temperature, the reaction mixture was poured into water. The resulting aqueous suspension was neutralized with aqueous NaOH (1 N) and extracted with diethyl ether. The organic phase was washed with brine, dried, and concentrated under vacuum. The crude product was purified by flash chromatography (*n*-hexane/dichloromethane 9:1 → 6:4) to yield pure **12a** (1.66 g, 8.22 mmol, 60% yield) as an oil: ^{1}H NMR ($CDCl_3$) δ 3.29 (d, 2H, J = 6.0 Hz), 5.14−5.20 (m, 2H), 5.90 (ddt, 1H, J = 16.5, 10.4, 6.0 Hz), 6.82 (d, 1H, J = 8.2 Hz), 6.89 (d, 1H, J = 8.1 Hz); MS *m*/*z* 201 (M^+).

6-Allyl-*N*-methyl-2,3-dichloroaniline (12b). Compound **12b** was prepared from **11b** (0.260 g, 1.21 mmol) by following the same procedure described above for **12a**. The crude product was purified by flash chromatography (*n*-hexane/dichloromethane 9:1 → 8:2) to yield pure **12b** (0.160 g, 0.738 mmol, 61% yield) as an oil: ^{1}H NMR ($CDCl_3$) δ 2.85 (s, 3H), 3.45 (dt, 2H, J = 6.0, 1.6 Hz), 5.07 (dq, 1H, J = 16.7, 1.6 Hz), 5.14 (dq, 1H, J = 10.1, 1.6 Hz), 5.96 (ddt, 1H, J = 16.8, 10.3, 6.1 Hz), 6.98 (d, 1H, J = 8.4 Hz), 7.06 (d, 1H, J = 8.3 Hz); MS *m*/*z* 215 (M^+).

6-Allyl-*N*-ethyl-2,3-dichloroaniline (12c). Compound **12c** was prepared from **11c** (1.65 g, 7.23 mmol) by following the same procedure described above for **12a**. The crude product was purified by flash chromatography (*n*-hexane/dichloromethane 9:1 → 8:2) to yield pure **12c** (0.993 g, 4.34 mmol, 60% yield) as an oil: ^{1}H NMR ($CDCl_3$) δ 1.20 (t, 3H, J = 7.1 Hz), 3.10 (q, 2H, J = 7.1 Hz), 3.41 (dt, 2H, J = 6.2, 1.5 Hz), 5.07 (dq, 1H, J = 16.8, 1.7 Hz), 5.14 (dq, 1H, J = 10.1, 1.6 Hz), 5.94 (ddt, 1H, J = 16.8, 10.3, 6.2 Hz), 6.97 (d, 1H, J = 8.4 Hz), 7.04 (d, 1H, J = 8.4 Hz); MS *m*/*z* 229 (M^+).

(*E*,*Z*)-2,3-Dichloro-6-(1-propenyl)aniline (13a). Compound **12a** (1.00 g, 4.95 mmol) was dissolved in dimethyl sulfoxide (6 mL) and treated with potassium *tert*-butoxide (1.38 g, 12.3 mmol). The resulting suspension was heated to 55 °C for 4 h. After cooling to room temperature, the mixture was neutralized with a saturated aqueous ammonium chloride solution and extracted with diethyl ether. The organic phase was washed with brine, dried, and concentrated under vacuum. The crude product was purified by flash chromatography (*n*-hexane/dichloromethane 6:4) to yield **13a** as a 9:1 *E*/*Z* diastereomeric mixture (0.690 g, 3.42 mmol, 69% yield) as an oil: ^{1}H NMR ($CDCl_3$, 9:1 *E*/*Z* mixture, asterisk denotes minor

isomer peaks) δ 1.71* (dd, 3H, J = 6.9, 1.3 Hz), 1.90 (dd, 3H, J = 6.4, 1.3 Hz), 6.08 (dq, 1H, J = 15.6, 6.4 Hz), 6.32 (dd, 1H, J = 15.6, 1.2 Hz), 6.81 (d, 1H, J = 8.4 Hz), 7.02 (d, 1H, J = 8.3 Hz); MS m/z 201 (M^+, 100), 186 ($M^+ - CH_3$, 43), 174 ($M^+ - CH{=}CH_2$, 42), 165 ($M^+ - Cl$, 53), 130 ($M^+ - 2Cl$, 84).

(*E,Z*)-2,3-Dichloro-*N*-methyl-6-(1-propenyl)aniline (13b). Compound **13b** was prepared from **12b** (0.402 g, 1.86 mmol) by following the same procedure described above for **13a**. The crude product was purified by flash chromatography (*n*-hexane/diethyl ether 9:1) to yield **13b** as a 9:1 *E*/*Z* diastereomeric mixture (0.309 g, 1.43 mmol, 77% yield) as an oil: ^{1}H NMR ($CDCl_3$, 9:1 *E*/*Z* mixture, asterisk denotes minor isomer peaks) δ 1.76* (dd, 3H, J = 7.0, 1.8 Hz), 1.91 (dd, 3H, J = 6.6, 1.5 Hz), 2.83 (s, 3H), 2.87* (s, 3H), 3.09 (bs, 1H), 5.84* (dq, 1H, J = 11.4, 7.0 Hz), 6.10 (dq, 1H, J = 15.6, 6.6 Hz), 6.38* (dq, 1H, J = 11.4, 1.8 Hz), 6.52 (dq, 1H, J = 15.6, 1.5 Hz), 6.98 (d, 1H, J = 8.6 Hz), 7.15 (d, 1H, J = 8.4 Hz); MS m/z 215 (M^+).

(*E,Z*)-2,3-Dichloro-*N*-ethyl-6-(1-propenyl)aniline (13c). Compound **13c** was prepared from **12c** (2.62 g, 11.4 mmol) by following the same procedure described above for **13a**. The crude product was purified by flash chromatography (*n*-hexane/dichloromethane 8:2) to yield **13c** as a 9:1 *E*/*Z* diastereomeric mixture (1.94 g, 8.44 mmol, 74% yield) as an oil: ^{1}H NMR ($CDCl_3$, 9:1 *E*/*Z* mixture, asterisk denotes minor isomer peaks) δ 0.99* (t, 3H, J = 7.1 Hz), 1.16 (t, 3H, J = 7.1 Hz), 1.90 (dd, 3H, J = 6.6, 1.6 Hz), 3.13 (q, 2H, J = 7.1 Hz), 3.20* (q, 2H, J = 7.1 Hz), 3.83 (br, 1H), 4.19* (br, 1H), 5.83* (dq, 1H, J = 11.2, 6.9 Hz), 6.10 (dq, 1H, J = 15.7, 6.6 Hz), 6.36* (dq, 1H, J = 11.3, 1.8 Hz), 6.50 (dq, 1H, J = 15.7, 1.6 Hz), 6.75* (d, 1H, J = 8.2 Hz), 6.88* (d, 1H, J = 8.4 Hz), 6.98 (d, 1H, J = 8.2 Hz), 7.15 (d, 1H, J = 8.4 Hz); MS m/z 229 (M^+).

(*E,Z*)-2,3-Diphenyl-6-(1-propenyl)aniline (14a). A solution of **13a** (0.300 g, 1.49 mmol) in dioxane (1.5 mL) was treated with 0.834 g (2.56 mmol) of cesium carbonate, 0.273 g (2.24 mmol) of phenylboronic acid, 0.044 g (0.048 mmol) of tris(dibenzylideneacetone)dipalladium(0), and 0.18 mL of a 20% solution of tricyclohexylphosphine (0.11 mmol) in toluene. The resulting suspension was heated at 80 °C for 16 h under an argon atmosphere. The reaction mixture was then cooled to room temperature, diluted with diethyl ether, and filtered through a Celite pad. The solvent was removed under vacuum, and the resulting crude mixture was submitted again to the same treatment described above. The crude product deriving from the second step was purified by column chromatography, eluting with a 9:1 *n*-hexane/ethyl acetate mixture, to obtain **14a** as a 9:1 *E*/*Z* diastereomeric mixture (0.371 g, 1.30 mmol, 87% yield, two steps) as an oil: ^{1}H NMR ($CDCl_3$, 9:1 *E*/*Z* mixture, asterisk denotes minor isomer peaks) δ 1.86* (dd, 3H, J = 6.9, 1.7 Hz), 1.94 (dd, 3H, J = 6.5, 1.6 Hz), 3.71 (br, 1H), 6.17 (dq, 1H, J = 15.6, 6.5 Hz), 6.48 (dq, 1H, J = 15.6, 1.6 Hz), 6.84 (d, 1H, J = 7.9 Hz), 7.03−7.31 (m, 11H); MS m/z 285 (M^+).

(*E,Z*)-2,3-Diphenyl-*N*-methyl-6-(1-propenyl)aniline (14b). Compound **14b** was prepared from **13b** (0.129 g, 0.597 mmol) by following the same procedure described above for **14a**. The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 9:1) to yield **14b** as a 9:1 *E*/*Z* diastereomeric mixture (0.131 g, 0.436 mmol, 73% yield, two steps) as an oil: ^{1}H NMR ($CDCl_3$, 9:1 *E*/*Z* mixture, asterisk denotes minor isomer peaks) δ 1.92* (dd, 3H, J = 6.8, 1.6 Hz), 1.97 (dd, 3H, J = 6.6, 1.6 Hz), 2.65 (d, 3H, J = 5.7 Hz), 2.95* (d, 3H, J = 5.2 Hz), 3.30 (br q, 1H, J = 5.5 Hz), 3.42* (br, 1H), 5.87* (dq, 1H, J = 11.4, 7.1 Hz), 6.23 (dq, 1H, J = 15.7, 6.6 Hz), 6.54* (dq, 1H, J = 11.5, 1.8 Hz), 6.70 (dq, 1H, J = 15.6, 1.5 Hz), 7.02 (d, 1H, J = 8.0 Hz), 7.06−7.14 (m, 6H), 7.21−7.39 (m, 4H), 7.45 (d, 1H, J = 7.9 Hz); MS m/z 299 (M^+).

(*E,Z*)-2,3-Diphenyl-*N*-ethyl-6-(1-propenyl)aniline (14c). Compound **14c** was prepared from **13c** (1.63 g, 7.10 mmol) by following the same procedure described above for **14a**. The crude product was purified by flash chromatography (*n*-hexane/dichloromethane 8:2 → 6:4) to yield **14c** as a 9:1 *E*/*Z* diastereomeric mixture (1.44 g, 4.62 mmol, 65% yield, two steps) as an oil: ^{1}H NMR ($CDCl_3$, 9:1 *E*/*Z* mixture, asterisk denotes minor isomer peaks) δ 0.89* (t, 3H, J = 7.1 Hz), 0.91 (t, 3H, J = 7.1 Hz), 1.93* (dd, 3H, J = 6.8, 1.5 Hz), 1.95 (dd, 3H, J = 6.6, 1.6 Hz), 2.95 (q, 2H, J = 7.1 Hz), 3.10 (br, 1H), 5.85* (dq, 1H, J = 11.4, 7.0 Hz), 6.21 (dq, 1H, J = 15.7, 6.6 Hz), 6.53* (dq, 1H, J = 11.6, 1.4 Hz), 6.69 (dq, 1H, J = 15.6, 1.5 Hz), 6.99−7.25 (m, 11H), 7.44 (d, 1H, J = 8.1 Hz); MS m/z 313 (M^+).

3,4-Diphenylanthranylaldehyde (15a). A solution of **14a** (0.370 g, 1.30 mmol) in dioxane (10 mL) was treated with 5 mL of water, 0.639 g of sodium periodate (2.99 mmol), and 0.05 mL of a 2.5% solution of osmium tetroxide in *tert*-butyl alcohol (0.05 mmol), and the mixture was stirred at room temperature for 4 h. The mixture was then diluted with water and extracted with chloroform. The organic phase was washed with aqueous sodium thiosulfate, dried, and concentrated to afford a crude residue that was purified by flash chromatography (*n*-hexane/ethyl acetate 9:1) to yield **15a** (0.167 g, 0.611 mmol, 47% yield) as a yellow solid: mp 125 °C; ^{1}H NMR ($CDCl_3$) δ 6.84 (d, 1H, J = 8.1 Hz), 7.05−7.17 (m, 8H), 7.23−7.31 (m, 2H), 7.56 (d, 1H, J = 8.2 Hz), 9.95 (s, 1H); MS m/z 273 (M^+, 100), 245 ($M^+ - CO$, 52), 244 ($M^+ - CHO$, 67). Anal. $C_{19}H_{15}NO$ (C, H, N).

3,4-Diphenyl-*N*-methylanthranylaldehyde (15b). Compound **15b** was prepared from **14b** (0.288 g, 0.963 mmol) by following the same procedure described above for **15a**. The crude product was purified by flash chromatography (*n*-hexane/dichloromethane 1:1) to yield **15b** (0.122 g, 0.424 mmol, 44% yield) as a yellow solid: mp 118−120 °C; ^{1}H NMR ($CDCl_3$) δ 2.28 (s, 3H), 6.77 (d, 1H, J = 8.1 Hz), 6.94−6.99 (m, 2H), 7.06−7.19 (m, 8H), 7.51 (d, 1H, J = 7.9 Hz), 8.30 (br, 1H), 9.90 (s, 1H); MS m/z 287 (M^+). Anal. $C_{20}H_{17}NO$ (C, H, N).

3,4-Diphenyl-*N*-ethyl-anthranylaldehyde (15c). Compound **15c** was prepared from **14c** (0.337 g, 1.08 mmol) by following the same procedure described above for **15a**. The crude product was purified by flash chromatography (*n*-hexane/dichloromethane 1:1) to yield **15c** (0.149 g, 0.497 mmol, 46% yield) as a yellow solid: mp 75−77 °C; ^{1}H NMR ($CDCl_3$) δ 0.96 (t, 3H, J = 7.1 Hz), 2.52 (q, 2H, J = 7.1 Hz), 6.89 (d, 1H, J = 8.0 Hz), 6.94−6.99 (m, 2H), 7.09−7.23 (m, 8H), 7.56 (d, 1H, J = 7.9 Hz), 9.94 (s, 1H); MS m/z 301 (M^+). Anal. $C_{21}H_{19}NO$ (C, H, N).

3,4-Diphenylanthranylaldoxime (5a). A solution of **15a** (0.030 g, 0.11 mmol) in methanol (2 mL) was treated with a solution of hydroxylamine hydrochloride (0.016 g, 0.22 mmol) in water (0.5 mL), and the resulting mixture was heated to 50 °C for 3 h. After being cooled to room temperature, the solvent was partially removed under vacuum and the residue was extracted with ethyl acetate. The combined organic phase were dried and concentrated under vacuum, and the crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 8:2) to yield **5a** (0.023 g, 0.079 mmol, 72% yield) as a solid: mp 135−137 °C; ^{1}H NMR ($CDCl_3$) δ 6.81 (d, 1H, J = 7.9 Hz), 7.02−7.32 (m, 11H), 8.34 (s, 1H);^{13}C NMR ($CDCl_3$) δ 113.47, 118.71, 126.47, 127.25, 127.58, 128.84, 129.60, 131.09, 131.57, 137.10, 141.49, 143.38, 153.80; MS m/z 288 (M^+, 100), 271 ($M^+ - OH$, 45), 254 ($M^+ - H_2O - NH_2$, 13), 244 ($M^+ - CH{=}NOH$, 82). Anal. $C_{19}H_{16}N_2O$ (C, H, N).

3,4-Diphenyl-*N*-methylanthranylaldoxime (5b). Compound **5b** was prepared from **15b** (0.033 g, 0.11 mmol) by following the same procedure described above for **5a**. The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 8:2) to yield **5b** (0.026 g, 0.087 mmol, 79% yield) as a solid: mp 130 °C; ^{1}H NMR ($CDCl_3$) δ 2.40 (s, 3H), 6.92 (d, 1H, J = 7.9 Hz), 6.97−7.01 (m, 2H), 7.09−7.22 (m, 8H), 7.39 (d, 1H, J = 8.0 Hz), 8.37 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 36.14, 115.49, 119.98, 122.84, 126.52, 127.11, 127.58, 128.27, 129.60, 130.20, 131.20, 137.78, 141.31, 144.51, 151.81; MS m/z 302 (M^+, 13), 285 ($M^+ - OH$, 100). Anal. $C_{20}H_{18}N_2O$ (C, H, N).

3,4-Diphenyl-*N*-ethylanthranylaldoxime (5c). Compound **5c** was prepared from **15c** (0.116 g, 0.385 mmol) by following the same procedure described above for **5a**. The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 8:2) to yield **5c** (0.093 g, 0.293 mmol, 76% yield) as a solid: mp 103 °C; ^{1}H NMR ($CDCl_3$) δ 1.06 (t, 3H, J = 7.1 Hz),

2.86 (q, 2H, $J = 7.1$ Hz), 7.13 (d, 1H, $J = 7.9$ Hz), 7.15−7.18 (m, 2H), 7.24−7.42 (m, 8H), 7.61 (d, 1H, $J = 8.0$ Hz), 8.54 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 15.45, 44.79, 115.50, 121.02, 123.61, 124.27, 126.54, 127.21, 127.60, 127.94, 128.36, 129.60, 131.04, 137.63, 141.24, 144.22, 151.29; MS *m/z* 316 (M^+, 11), 299 ($M^+ - OH$, 100). Anal. $C_{21}H_{20}N_2O$ (C, H, N).

Biological Methods. Purified human full-length ERα and ER*β* were obtained from PanVera (Madison, WI). 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 luciferase assay system was from Promega (Madison, WI). The expression vectors for human ERα (pCMV5-hERα) and human ER*β* (pCMV5-ER*β*) were constructed previously as described.[45,46] The estrogen responsive reporter plasmid was $(ERE)_2$-pS2-Luc, was constructed by inserting the $(ERE)_2$-pS2 fragment from $(ERE)_2$-pS2-CAT into the *Mlu*I/*Bgl*II sites of pGL3-Basic vector (Promega, Madison, WI). The plasmid pCMV*β* (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 using 10 nM [^{3}H]E_2 as tracer, using methods previously described.[36,37] The source of ER was purified full-length human ERα and ER*β* purchased from Pan Vera (Madison, WI). Incubations were done at 0 °C for 18−24 h, and hydroxylapatite was used to absorb the purified receptor−ligand complexes (human ERs).[37] 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 ca. 0.2 nM and for ER*β* 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 three or more separate determinations, respectively.

Cell Culture and Transient Transfections. Human endometrial cancer (HEC-1) cells were maintained in culture as described.[38] Transfection of HEC-1 cells in 24-well plates used a mixture of 0.35 mL of serum-free IMEM medium and 0.15 mL of HBSS containing 5 *μ*L of lipofectin (Life Technologies, Rockville, MD), 1.6 *μ*g of transferrin (Sigma, St. Louis, MO), 0.5 *μ*g of pCMV*β*-galactosidase as internal control, 1 *μ*g of the reporter gene plasmid, 100 ng of ER expression vector, and carrier DNA to a total of 3 *μ*g DNA per well. The cells were incubated at 37 °C in a 5% CO_2 containing incubator for 6 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.[38]

Docking Methods. ERα/estradiol complex X-ray structure (PDB code 1A52) [40] was taken from the Protein Data Bank.[47] The study of complexes formed by ERα with **3** and **5a**−**c** was performed using the DOCK program by Kuntz and co-workers.[48] The starting point of the calculation was the crystallographic structure of estrogen receptor ligand-binding domain complexed to estradiol.[40] The molecular surface of the binding site was calculated by means of the MS program,[48] generating the Connolly surface with a probe with a radius of 1.4 Å; the points of the surface and the vectors normal to it were used by the SPHGEN program[48] to build a set of spheres, with radii varying from 1.4 to 4 Å, that describe, both from a stereoelectronic point of view, the negative image of the site. The first set of 55 spheres automatically generated and clustered by the program was used to represent the site; around it a grid of 12 × 11 × 13 Å^3 was constructed, with a spacing equal to 0.3 Å, and for each point the GRID program evaluated contact, chemical, and energy score with a cutoff of 10 Å. For each ligand, DOCK calculated 500 orientations; of these, the 10 with the best chemical score were taken into consideration.

A manual docking of the *N*-Et derivative (**5c**) in ERα was performed, imposing the orientation of the ligand in a binding mode that overlapped with the previously found orientation of the other analogues **1**, **5a,b**. A molecular dynamic simulation of 100 ps, $T = 300$ K, time step = 1 fs (Amber force field) was performed on the complex so obtained by the MacroModel program. During the simulation, all the protein backbone atoms were fixed, as were the ligand pseudocycle atoms and the hydrogen bond of the oxime OH with Glu353 and Arg394. All the constraints were relaxed during minimization after the molecular dynamic, performed through the conjugate gradient method, using the derivative convergence criterion at 0.05 kJ/mol. The interaction energy between ligand and receptor was obtained through a MacroModel complete current energy calculation, performed considering the steric and electrostatic terms of all the atoms of the ligand with respect to the protein.

Acknowledgment. This work was partly supported by CNR, Rome. We are grateful for support of this research through grants from the National Institutes of Health [PHS 5R37 DK15556 (J.A.K.) and 5R01 CA19118 (B.S.K.)].

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JM0308390