Synthesis and Evaluation of 17α-20*E*-21-(4-Substituted phenyl)-19-norpregna-1,3,5(10),20-tetraene-3,17*β*-diols as Probes for the Estrogen Receptor α Hormone Binding Domain

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As part of our program to develop probes for the hormone binding domain of the estrogen receptor α (ERα), we prepared a series of 4-para-substituted phenylvinyl estradiol derivatives using a combination of solution and solid-phase Pd(0)-catalyzed methods. The compounds **5a**−**j** were evaluated for their binding affinity using the ERα hormone binding domain (HDB) isolated from transfected BL21 cells. The results indicated that although the new compounds were somewhat lower in relative binding affinity (RBA at 25 °C is 1−60%) than estradiol (100%), most had higher affinity than the unsubstituted parent phenylvinyl estradiol (RBA = 9%). Because the substituents did not generate a structure−activity relationship directly based on physicochemical properties, the series was evaluated using molecular modeling and molecular dynamics to determine key interactions between the ligand, especially the para substituent, and the protein. The results suggest that the observed relative binding affinities are directly related to the calculated binding energies and that amino acids juxtaposed to the para position play a significant but not dominant role in binding. In conclusion, we have identified the 17α-*E*-(4-substituted phenyl)vinyl estradiols as a class of ligands that retain significant affinity for the ERα-HBD. In particular, 4-substitution tends to increase receptor affinity compared to the unsubstituted analogue, as exemplified by **5e** (4-$COCH_3$), which had the highest RBA value (60%) of the series. Palladium(0)-catalyzed coupling reactions on solid support or in solution using suitably substituted iodo arenes and 17α-*E*-tributylstannylvinyl estradiols offer a flexible approach to their preparation. Molecular modeling studies of the receptor suggest that there exists additional ligand accessible regions within the ERα-HBD to generate interactions that may enhance receptor affinity or modify efficacy in developing new therapeutic agents. Studies to undertake modification in the properties and/or position of the aryl substituents in subsequent series to further define that role are in progress.

Introduction

Breast cancer is the most common cancer diagnosis among women, with an estimated 181 000 new cases per year in the U.S.[1] Approximately 60% of these newly diagnosed patients have hormone-responsive breast cancer, defined as containing estrogen receptor (ER) and requiring the presence of circulating estrogens for maintenance of tumor growth.[2] This relationship has generated considerable interest both for understanding the mechanism of the hormone receptor interactions and for targeting the ER in therapeutic breast cancer drug development. Recent publications of the crystal structure of the liganded ER-HBD have suggested that the key interaction may involve the N-terminal region (helix-12) of the receptor.[3−6] Antagonists apparently cause this helical region of the ER-HBD to occupy a different binding mode compared to that produced by agonists, thereby disrupting the interaction between the receptor and the coactivator proteins that initiate the agonist response.[7−10] Because the orientation of the helix-12 of the ER-HBD may be affected differently by various ligands, a variety of approaches can be used to generate compounds that can bind effectively to the receptor protein and subsequently produce the desired pharmacological response. Most strategies have involved modifications of the nonsteroidal antagonists tamoxifen and raloxifene[11−20] (Figure 1); however, other groups who have used a heterocyclic moiety to replace the ethylene bridge have also been successful in preparing interesting ER ligands.[21−24]

As part of our program to develop new probes for the estrogen receptor, we have focused on the preparation and evaluation of novel steroidal derivatives. Our approach involved the introduction of substituents at the 17α-position of estradiol as a means to enhance receptor binding and/or alter receptor response. Our initial studies described the synthesis and evaluation of several series of 17α-phenylvinyl estradiols (Figure 2). These studies, conducted prior to the publication of the first ER-HBD crystal structures, suggested that there was significant tolerance for large functional groups at this site.[25−29] Later reviews of the structure−activity rela-

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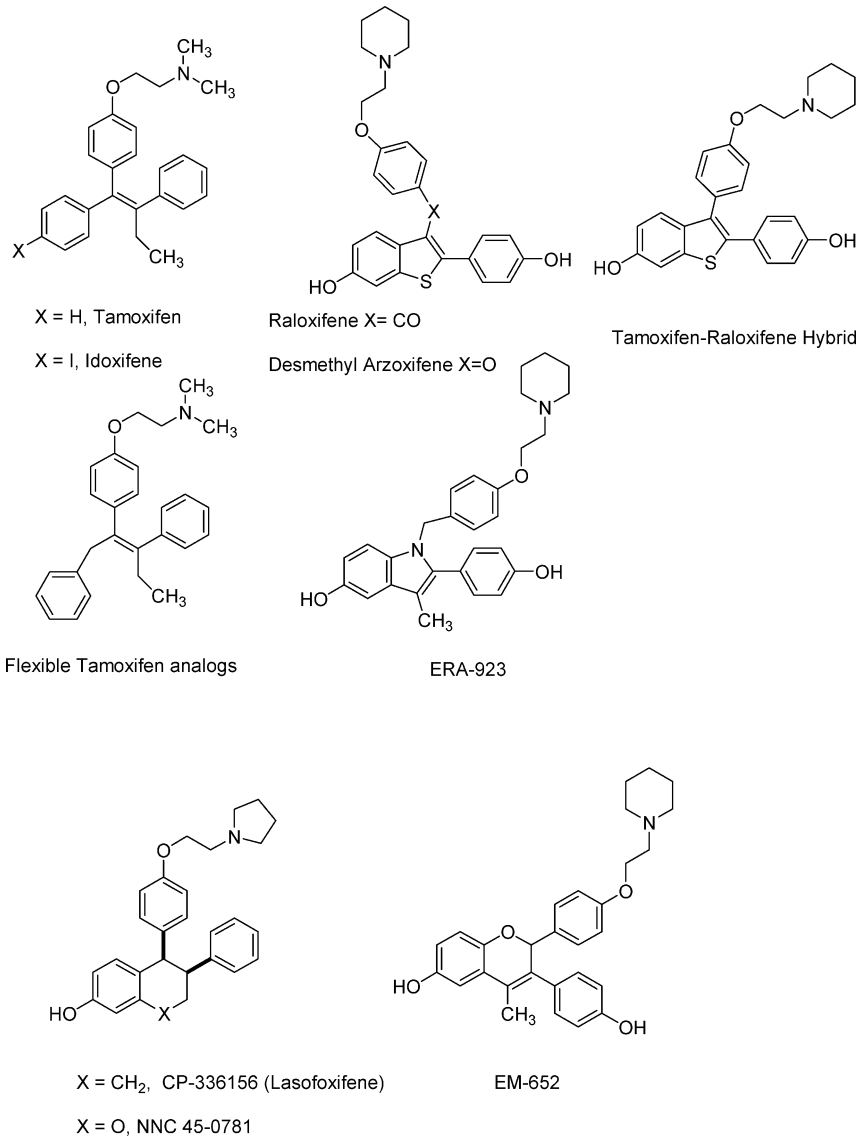


Figure 1. Structures of nonsteroidal antiestrogens developed or evaluated as selective estrogen receptor modulators (SERMs).

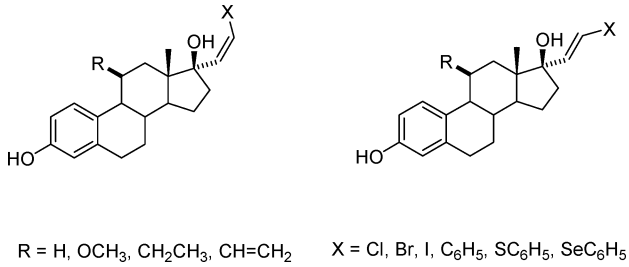


Figure 2. Structures of 11β-substituted 17α-(*Z*- and *E*-)-X-vinyl estradiols prepared and evaluated as estrogen receptor ligands.

tionships for ER ligands supported these observations and provided a rationale for the orientation of the 17α-substituent within the ER-HBD.[30−32] To appreciate these observations, we used molecular modeling to dock our initial 17α-*E*-phenylvinyl estradiol with the ER-HBD and performed energy minimization to identify potential interactions. This model, in which we have oriented the steroid nucleus in the same manner as that found for the estradiol−ER complex, provided two significant points. The 17α-group was accommodated within the outer portion of the domain and relatively close to the hinge between helices 11 and 12. The substituent was also close to Met-421, a residue that is one of two amino acids that are different from that found in the binding region of the estrogen receptor β (ERβ) isoform.[9,33−35] Therefore, we proposed that the introduction of substituents onto the 17α-phenylvinyl group would provide information regarding the interactions between ligands and the estrogen receptor isoforms. However, the model of the interaction between the ligand and the receptor could not predict either the magnitude of the effects of additional substituents on the terminal aromatic ring or the effect on the orientation of the helix 12 and, by extension, the biological response.

The strategy that we utilized in our program involved the preparation of new 17α-substituted phenylvinyl estradiol derivatives in which the substituents would probe the receptor surface. The phenylvinyl group provides six degrees of variation, i.e., *E* vs *Z* stereochemistry around the C−C double bond as well as 2-, 3-, or 4-substitution on the phenyl ring (Figure 3). In this report, we describe the synthesis, receptor binding, and computational analysis of a series of 4-substituted 17α-*E*-phenylvinyl estradiol derivatives. The reasons for

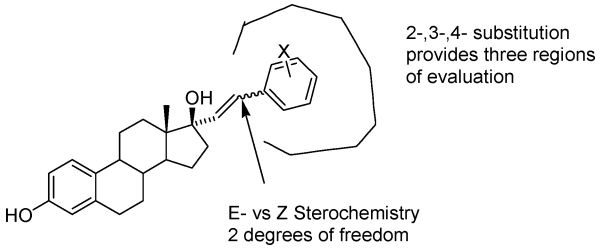


Figure 3. Rationale for selection of 17α-(*Z*- and *E*-)-(substituted phenyl)vinyl estradiols as probes for the hormone binding site of the estrogen receptor.

this selection included synthetic concerns as well as conformational issues. Our experience with the Stille coupling reaction indicated that our synthetic approach via the vinylstannanes and the commercially available (or readily accessible) para-substituted aryl halides would easily generate a series of compounds with a variety of functional groups.[36,37] Ultimately we intended to extend the solution-phase chemistry to our solid-phase organic synthesis strategy for combinatorial chemistry.[38] Of equal importance was the recognition that para substitution would yield products that would be symmetrical along the aryl axis. This would reduce the number of potential conformational isomers in which the compound could exist and simplify modeling of the interactions between the ligand and the receptor. Our preliminary NMR studies with the substituted phenylvinyl estradiols (*E* and *Z* isomers) indicated that the compounds existed in a conformational equilibrium with a relatively low-energy barrier between them.[39,40] Therefore, incorporation of the conformational mobility of the ligand into the docking interaction with the receptor would be simplified by the use of the para substitution. As our results suggest, the presence of the substituent and its properties had a significant effect on the binding of the ligand to the ER-HBD.

Results

Synthesis of Estrogenic Ligands. The target compounds in this series were prepared as part of a larger program to probe ligand−receptor interactions and to develop potential theraputic agents. As a result, we utilized several methods to obtain the compounds. The synthesis of most of the 17α-*E*-(4-substituted phenyl)-vinyl estradiols (**5a**−**g**) was achieved using the solution-phase Stille coupling approach developed in our laboratories (Scheme 1). The commercially available ethynyl estradiol **1** was acetylated to give the 3-acetyl intermediate **2**,[41] which was then hydrostannated with tri-*n*-butyltin hydride and triethylborane to give predominantly the *E*-stannylvinyl estradiol **3.** The acetylated intermediate was then coupled with the 4-substituted aryl halides (Br/I) using standard Stille coupling conditions to yield the intermediates **4a**−**f**. Hydrolysis with sodium methoxide in methanol provided the target 17α-*E*-(4-substituted phenyl)vinyl estradiols **5a**−**f**, while saponification of **5f** provided the carboxy derivative **5g**.

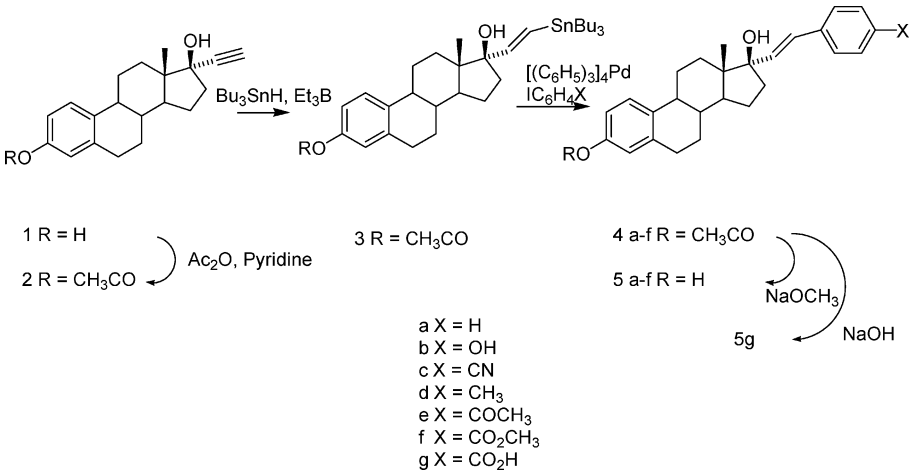
Alternatively, as part of our combinatorial chemistry approach, ethynyl estradiol **1** was hydrostannated to give predominately the *E*-stannylvinyl estradiol **6**, which was coupled to a carboxylated polystyrene resin to give the intermediate **7**. Stille coupling with the appropriate aryl halide followed by cleavage from the resin gave the target estradiol derivatives **5h**−**i** (Scheme 2).

A third approach utilized the Suzuki coupling reaction.[42,43] This involved first performing iododestannylation of **3** to give the iodovinyl estradiol **8**, which underwent facile Suzuki coupling with 4-fluorophenylboronic acid to give, after hydrolysis, the product **5j** (Scheme 3). The products were purified by column chromatography, recrystallized, and characterized by NMR and elemental analysis. Because the objective of the study was to generate the target compounds and demonstrate synthetic feasibility, the yields of the compounds were not optimized (Table 1). Stereochemistry of the products was established by the coupling constant for the vinylic protons, which was $J = 16-18$ Hz, consistent with the previously synthesized *E*-trans derivatives.[27]

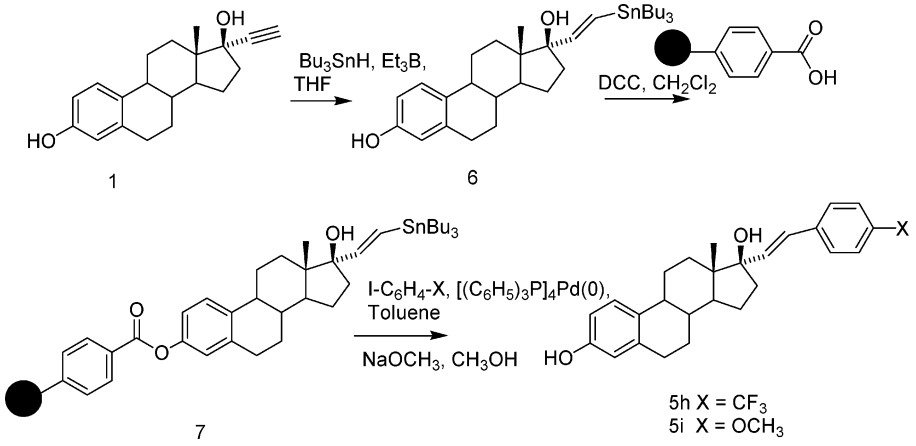
Biological Studies. Relative Binding Affinities. The new compounds were evaluated for their relative binding affinities (RBA) at 2 and 25 °C using the ERα-HBD isolated from the transfected BL21 cells. The RBA values for the ligands were determined using a competitive radiometric receptor binding assay and were compared to both estradiol and the unsubstituted phenylvinyl estradiol. The results are summarized in Table 2 where the RBA of estradiol is 100%.

Although none of the members of this bound as potently as estradiol, most of the compounds retained

Scheme 1. Solution-Phase Synthesis of Estrogens



Scheme 2. Solid-Phase Synthesis of Estrogens



Scheme 3. Suzuki Synthesis of Estrogen

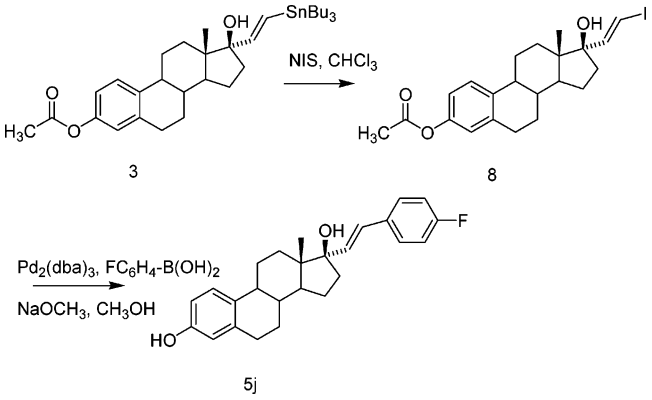
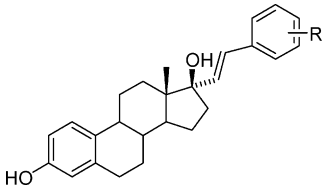


Table 1. Yields of 17α-*E*-(4-Substituted phenyl)vinyl Estradiols

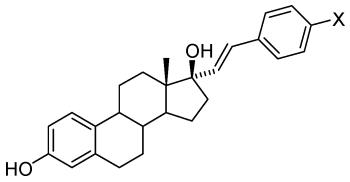


compd	R	yield of **4** (%)	yield of **5** (%)	method[a]
5a	H	25	92	I
5b	OH	21	89	I
5c	CN	50	80	I
5d	CH_3	59	60	I
5e	$COCH_3$	89	83	I
5f	CO_2CH_3	65	70	I
5g	CO_2H		91	I
5h	CF_3		49	II
5i	OCH_3		36	II
5j	F	37	97	III

[a] Method I: two-step solution Stille sequence of **3** → **4** → **5**. Yields are for each step. Method II: solid-phase Stille sequence of **7** → **5**. Yields are for the combined sequence. Method III: Suzuki reaction sequence of **8** → **4** → **5**. Yields are for each step.

significant affinity for the estrogen receptor. The range of relative binding affinities straddled that of the unsubstituted phenylvinyl estradiol (RBA = 16% at 2 °C; RBA = 9% at 25 °C). At 2 °C, the derivatives with the highest affinity were the 4-acetyl (RBA = 53%), 4-methoxy (RBA = 36%), 4-hydroxy (RBA = 21%), and 4-fluoro (RBA = 20%) phenylvinyl estradiols. At 25 °C, the ligands demostrating the highest RBA values were the 4-acetyl (RBA = 60%), 4-methoxy (RBA = 32%),

Table 2. Relative Binding to the ERα Ligand Binding Domain



compd	X	RBA[a] at 2 °C (%)	RBA[a] at 25 °C (%)
5a	H	16	9
5b	OH	21	25
5c	CN	9	27
5d	CH_3	10	18
5e	$COCH_3$	53	60
5f	CO_2CH_3	18	26
5g	CO_2H	1	1
5h	CF_3	5	8
5i	OCH_3	36	32
5j	F	24	22

[a] RBA = 100 × [E]/[C], where [E] is the concentration of unlabeled estradiol necessary to reduce the specific binding of tritiated estradiol to the ERα-LBD by 50% and [C] is the concentration of competitive ligand necessary to reduce specific binding by 50%. The RBA of estradiol is 100% at each incubation temperature. The ERα-LBD was extracted from BL21 cells overexpressing the 33 kDa pET-23d-ERG vector.[58] Curves for ligand and estradiol had correlation coefficients of >95%.

4-fluoro (RBA = 28%), 4-cyano (RBA = 27%), 4-methoxycarbonyl (RBA = 26%), and 4-hydroxy (RBA = 25%) phenylvinyl estradiols. The only compound with RBA values significantly less than that of phenylvinyl estradiol at either temperature was the polar 4-carboxy derivative **5g** (RBA = 1−2%).

Molecular Modeling Studies. Initially we examined the structure−activity relationships for this series using the parameters described by Gao et al.[32] for the 17α-substituted estradiols; however, a strong, direct correlation could not be generated between the substituent properties and the observed RBA values. Therefore, molecular modeling of the ligands and the ligand−ERα-HBD complexes was undertaken to interpret the relationship between the structure of the compounds and their receptor binding affinity. Earlier studies with estrogenic ligands[44,45] focused on compounds that were either substituted directly on the A- or D-rings or were nonsteroidal analogues of estrogens. As such, the results were not directly applicable to our work, even though the approaches were similar.

The results of our molecular modeling/dynamics study are depicted in Figures 4 and 5. In this study, we

utilized the X-ray crystal structure of ERα-HBD in which estradiol was the estrogenic ligand. The ligands were docked with the complex in a manner in which the two phenolic A-rings were superimposed. Estradiol was removed, and the subsequent complex was subjected to molecular dynamics and simulated annealing manipulations. The docking experiments indicated two low-energy modes, as previously noted;[44] however, only the complexes similar to the crystal forms, i.e., with the A-ring orientation toward the Arg-394-Glu-353 site, were evaluated in this study. Docking with the unsubstituted phenylvinyl estradiol gave a complex in which the 17α-substituent generated new potential interactions with a region of the ER-HBD formed by the junction of the β-sheet, 2−3 loop, helices 3, 6, 7, and the 6−7 loop. Critical amino acids that line this pocket include Met-421, Met-342, Met-343, Leu-410, Leu-346, Phe-425, and Val-418. It is of interest to note that of these residues, Met-421 is replaced by Ile in the ERβ-HBD.

In our proposed binding model, the two edges of the phenyl ring interact with a different set of residues; however, conformational mobility around the phenyl−vinyl axis would allow an ortho or meta substituent to select its individual low-energy conformation. Para subsituents, on the other hand, are independent of the rotation of the phenyl group around the double bond and would interact with a common set of residues. As Figure 4A indicates, this set consists of several methionine residues, notably Met-342, -348, and -421, plus Phe-425. This is consistent with recent evaluations of ligand−ER-LBD complexes.[46] The other amino acids associated with the ligand−receptor binding have been identified from earlier studies, i.e., Phe-404, Glu-353, and Arg- 394, and interact similarly to the other ligands. The steroidal skeleton interacts with a lipophilic surface, while the 17α-moiety induces a pocket surrounded by methionine and phenylalanine residues. The volume of the induced pocket clearly accommodates the phenylvinyl goup and orients the para substituents toward the hinge between helices 11 and 12 (Figure 4B). Overall, the ligands are displaced approximately 1 Å toward the Glu−Arg interface with a concomitant weakening of the hydrogen bond interaction between the 17β-hydroxyl and His-524.

The methylthio groups of the Met-342, -343, and -421 form a cage around the phenyl ring with the para position now oriented toward the junction of Met-342 and Met-421. The introduction of substituents at this position has relatively little effect on the torsion angle between the phenyl ring and the C−C double bond (Figure 5). On the other hand, the conformation of the substituent is primarily affected by the local environment of the HBD adjacent to the para position of the phenyl ring. As the model demonstrates, small groups such as the 4-F, CN, methyl, trifluoromethyl, and hydroxy are easily accommodated within the space and establish few interactions. Larger groups such as the 4-acetyl, methoxycarbonyl, carboxy, and methoxy are required to undergo torsional motion to establish a low-energy conformation within the HBD. This equilibration is reflected not only in the final orientation of the substituent but also in the translational motion of amino acid side chains in the vicinity of the ligand. These movements are ultimately reflected in the calculated binding energies for the complexes (Figure 6).

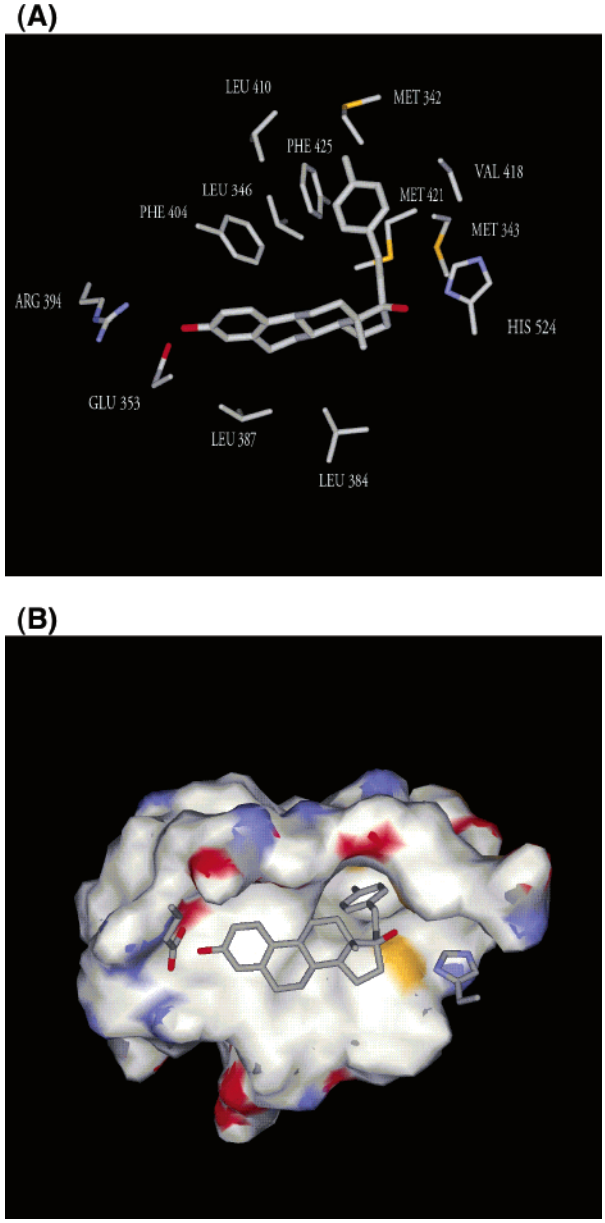


Figure 4. (A) Representation of **5d** (X = CH_3, RBA(25 °C) = 18%) docked in lowest energy conformation of the ERα-HBD complex. View is from above the β-face of the steroid looking toward the junction of helices 11 and 12. Side chains of key amino acids that are within 5 Å of the ligand are identified. In particular, the phenylvinyl group is bounded by three methionines (Met-342, -343, and -421), a phenylalanine (Phe-425), and the hydrophobic residues (Leu-346, Leu-410, and Val-418). (B) Representation of **5d** and the ERα-HBD from directly above the C-18 methyl group. The surface of the HBD is in white (hydrophobic) and shaded to indicate the position of other functional groups, yellow for sulfur (methionine), red for oxygen (tyrosine), and blue for nitrogen. The figure clearly delineates the pocket induced by the binding of the ligand to the region of the HBD. Also clear are the methionine and phenylalanine residues that line the binding pocket. The dimensions easily accommodate the phenyl ring and direct the para substituent toward Met-342.

Using the parameters described in the Experimental Section, we calculated the binding energies for each of the complexes and compared those values to the observed RBA (25 °C) values. Except for two compounds,

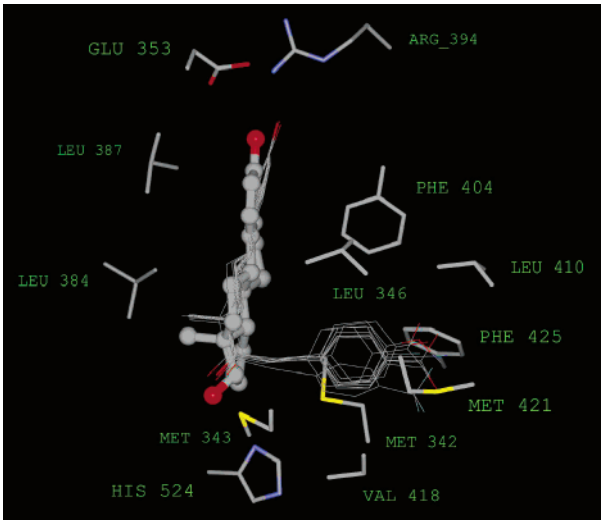


Figure 5. Binding of the 17α-(4-substituted phenyl)vinyl estradiols **5a**−**j** in the ERα-HBD. Estradiol is represented in the ball-and-stick format, and the new ligands are represented as the shaded lines. Compounds **5a**−**j** are displaced approximately 1 Å toward the Glu-353, Arg-394 binding locus. The 4-substituents are closely flanked by Met-421 and Phe-425.

5g and **5h**, which have the lowest RBA, a strong correlation, $R^2 = 0.94$, was observed. In addition, binding energies between the docked ligand and selected amino acids were evaluated using the same method. The highest contributions to the binding energy were derived from Phe-404 and Leu-387, which interact respectively with the α- and *β*-faces of the steroidal A-ring. The second highest contribution arises from Leu-346, which interacts with both the C-ring and the vinyl segment of the 17α-phenylvinyl moiety. The three methionine residues that interact with the phenyl group and the para substituent also provide significantly to the binding energy. These results suggested the proposed interactions between the ERα-HBD and this homologous set of ligands represented a reasonable model and a basis for interpreting the binding of our series of compounds.

Discussion

In this study, we prepared a series of 4-substituted phenylvinyl estradiols and evaluated them as probes for the ERα−ligand binding domain. The methods used for the synthesis of the target compounds were chosen to demonstrate the feasibility of each approach and did not represent the optimal conditions. The synthesis of the target compounds **5a**−**j**, which were obtained in reasonable yields and in high purity by a combination of solution- and solid-phase palladium-catalyzed reactions, illustrated the versatility and flexibility of this strategy. Given the ability to prepare the requisite stannylvinyl estradiol precursors, either **3** or **7**, and the appropriate coupling partner, it is possible to prepare any number of functionalized phenylvinyl estradiol derivatives.

We screened the new compounds with the ERα-HBD and most of the 4-substituted derivatives displayed high relative binding affinity (RBA) for the ERα-HBD with values in the range 25−60%, exceeding that of the unsubstituted parent. Docking the new ligands in the ERα-HBD using molecular modeling suggested that the substituted phenylvinyl group was accommodated by the outer portion of the ligand binding pocket. The results of those studies provide the basis for evaluating the relationship between the 4-substituent and the binding (RBA) data.

Structure−Activity Relationships in the 4-Substituted Phenylvinyl Series. Previous studies in our laboratories have shown that the estrogen receptor tolerated the introduction of 17α-X-vinyl substituents. Although the highest affinity was observed for the halovinyl estradiols, phenyl- and phenylthio/selenovinyl estradiols also were good ligands. Our topographical studies of the ER-HBD using the halovinyl estrogens as probes were limited by the small number of substituted ligands available. The use of phenylthio/selenovinyl estrogens was hampered as well by the limited availability of substituted reagents for electrophilic destannylation and by rotation around the S/Se−vinyl bond. Introduction of substituents on the phenylvinyl group via the versatile Pd-catalyzed Stille or Suzuki reactions made the phenylvinyl estrogens a more promising method for investigating the ligand−receptor interactions. The substituents that we have introduced at the 4-position possess a variety of physicochemical properties. These included electron-withdrawing as well as electron-donating, hydrophilic as well as hydrophobic, and small as well as large groups. Virtually all of the

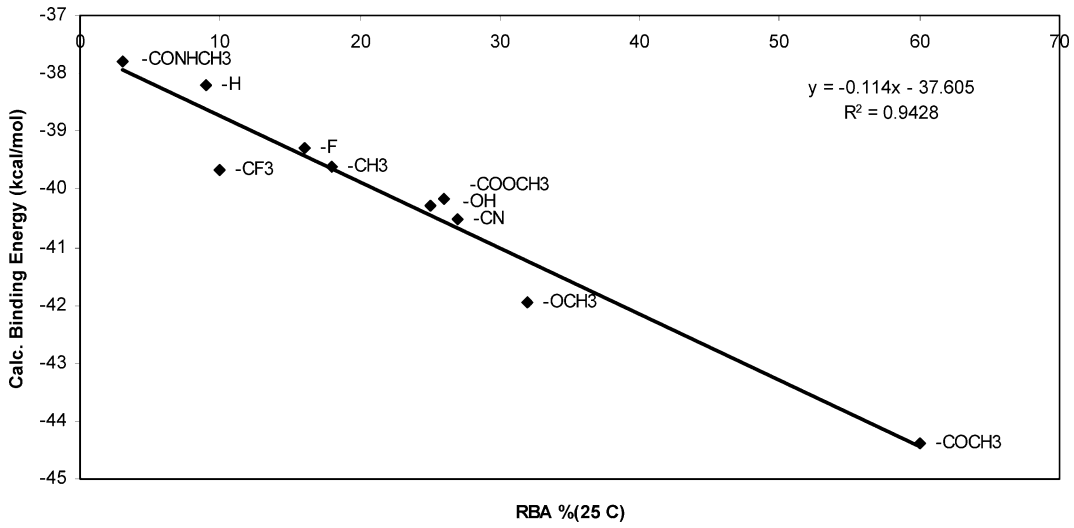


Figure 6. Relationship between the observed RBA (25 °C) values and the calculated binding energy.

new compounds are equal to or better in their ER affinity relative to the unsubstituted parent compound except for the 4-trifluoromethyl and 4-carboxy compounds (RBA at 25 °C is 5% and 1%, respectively). The rest had RBA values that were roughly 20−60% that of estradiol (RBA = 100%) and significantly higher than the unsubstituted compound **5a** (RBA = 9%). This increase in binding was essentially independent of the properties of the substituent. For example, the 4-hydroxy compound was virtually identical to the 4-cyano and 4-methoxycarbonyl derivatives (RBA = 25% vs 27% vs 26%), and 4- fluoro was similar to 4-methyl (RBA = 22% vs 18%). The highest affinity was observed for the 4-acetyl derivative (RBA = 60%), although its properties are similar to those of the methyl ester (RBA = 26%) or the methyl ether (RBA = 32%). The lack of a clear relationship between physicochemical properties of the substituents and their RBA values suggested to us that in the process of binding, both the ligand and receptor were undergoing structural adjustments to reach a binding energy minimum for the resultant complex. We concluded that an analysis of this type of interaction would best be achieved using molecular modeling and docking studies.

Investigation of the Interactions of the 17α-4-Substituted Phenylvinyl Estradiols with the ER-HBD. Molecular Modeling Studies. We used molecular modeling and molecular dynamics to investigate the interactions between the phenylvinyl substituent of our ligands and the amino acid side chains of the ERα-HBD. We chose the coordinates of the estradiol−ERα-HBD complex because of the steroidal nature of our compounds and because preliminary biological data indicated that the compounds behaved as agonists in the immature rat uterotrophic assay. Therefore, the orientation of the critical helix 12, associated with coactivator binding, was probably the agonist orientation. Using the modeling program with the Insight II package,[47] we docked the 4-unsubstituted phenylvinyl estradiol into the estradiol binding site, overlaying the aromatic rings. Employing molecular mechanics and energy minimization routines, approximately 20 low-energy conformers were obtained for each complex. In each case, the17α-substituent was oriented toward the external surface of the receptor. The translations of the internal amino acids associated with the A−B−C ring interactions were relatively small, consistent with the crystal structures obtained with the other estrogen receptor agonists and with the steroidal and nonsteroidal androgens at the androgen receptor.[48,49] This effect has also been observed with the vitamin D analogue−vitamin D receptor−HBD crystal structures where the internal structure remains relatively rigid while the side chain of the analogue tends to undergo the conformational deformations.[50−52] In our model, the phenylvinyl substituent occupied a region bounded by three methionines (Met-342, -343, -421), a phenylalanine (Phe-425) as well as two leucines (Leu-346, -410) and a valine (Val-418). While relatively lipophilic in character, these residues also can interact through the electron pairs of the thioether and/or through the π-cloud of the phenyl ring. Therefore, substituents present at the para position of the phenylvinyl group can experience multiple effects. Analysis of individual amino acids indicated that the highest contribution to binding energy was derived from Phe-404 and Leu-387 via direct interactions with the α- and β-faces of the A-ring. The second highest contribution arose from Leu-346, which interacts directly with both the steroidal C-ring and the phenylvinyl group. Met-421 is closest to the 17α-phenyl group, while Met-342 and Met-343 juxtapose the para and vinyl groups, respectively. If one includes the consideration that steric factors could influence translational or torsional responses on these side chains, then the interpretation of the individual effects becomes more complex. As shown in Figure 5, the overlap of the ligands (deleting the ERα-HBD) shows that the substituents occupy a reasonably small volume in which electronegativity is not as critical as the conformation of the substituent. As a result, the methionines tolerate a polar substituent (fluoro-, carbonyl-) adjacent to the phenyl ring as long as the next group is lipophilic (-methoxy, -methyl). The most significant deviations were observed for the 4-trifluoromethyl compound **5h**, which had a greater torsional distortion between the vinyl group and the phenyl ring, and the 4-carboxy compound **5g**, which may undergo ionization under binding conditions. These effects may account, at least in part, for reduction in binding affinity and/or lack of correlation with the calculated binding energy.

There are several conclusions that can be drawn from this study. First, ERα-HBD can accommodate the presence of a significant variety of substituents at the para position of the phenylvinyl estradiols without a serious reduction in overall receptor binding affinity. This finding had not been previously observed and leads to the possibility that other functional groups can be introduced to impart higher receptor affinity, selectivity, or altered efficacy. Second, molecular modeling and molecular dynamics have provided a method for not only evaluating the interactions between ligands and the receptor hormone binding domain but, at least within a homologous (para-substituted) series, possibly predicting the affinity of putative ligands. Last, we have demonstrated the feasibility of Pd(0) coupling methods to prepare the diverse members of such a series as may be required to identify a potential clinical candidate. Subsequent publications in this project will describe those efforts to extend these methods to the preparation of other variations in the phenylvinyl estradiol series.

Experimental Section

General Methods. All reagents and solvents were purchased from Aldrich or Fisher Scientific. THF and toluene were distilled from sodium/benzophenone. Reactions were monitored by TLC, performed on 0.2 mm silica gel plastic backed sheets containing F-254 indicator. Visualization on TLC was achieved using UV light, iodine vapor, and/or phosphomolybdic acid reagent. Column chromatography was performed with 32−63 μm silica gel packing. Melting points were determined using an Electrotherm capillary melting point apparatus and are uncorrected. NMR spectral chemical shifts are reported in parts per million downfield from TMS and referenced either to TMS internal standard for deuteriochloroform or deuterioacetone solvent peak. Coupling constants are reported in hertz. All compounds gave satisfactory elemental analyses, ±0.4% (Atlantic Microchemical Laboratories, Inc., Norcross, GA), unless otherwise stated.

Solution-Phase Synthesis. 17α-*E*-(Tri-*n*-butylstannyl)-vinyl Estradiol 3-Acetate (3). To a solution of 3-acetoxy-17α-ethynyl estradiol **2**[41] (1.5 g, 4.4 mmol) in THF (5 mL) were

added 1.7 mL (6.3 mmol) of tri-*n*-butyltin hydride and 3 mL (26 mmol) of triethylborane. The reaction mixture was stirred magnetically for 5 h at 60 °C[46] and then purified by column chromatography on silica gel using hexanes−ethyl acetate (5:1) as the eluent. The reaction afforded 0.5 g (0.79 mmol) of 3-acetoxy-17α-*Z*-(tri-*n*-butylstannyl)vinyl estradiol and 1.89 g (3 mmol) of 3-acetoxy-17α-*E*-(tri-*n*-butylstannyl)vinyl estradiol in a combined yield of 86%. R_f (*Z* isomer) = 0.58 (hexanes−ethyl acetate, 5:1), R_f (*E* isomer) = 0.43 (hexanes−ethyl acetate, 5:1), amorphous. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (s, 3H, C_{18}−C*H*$_3$), 1.2−2.4 (steroid envelope), 2.28 (s, 3H, C*H*$_3$C=O), 2.7−2.9 (m, 2H, $C_{6\alpha}$−*H* and $C_{6\beta}$−*H*), 6.06 (d, 1H, *J* = 19.4 Hz, CH=C_{21}*H*), 6.21 (d, 1H, *J* = 19.4 Hz, C_{20}*H*=CH), 6.79 (d, 1H, *J* = 2.4 Hz, C_4−H), 6.84 (dd, 1H, *J* = 2.6, 8.4 Hz, C_2−H), 7.28 (d, 1H, *J* = 8.8 Hz, C_1−H). ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 9.64 (C_{22}, 4C), 13.78 (C24, 4C), 14.18 (C_{18}), 21.13 (*C*H$_3$C=O), 23.43 (C_{15}), 26.15 (C_{11}), 27.28 (C_{25}, 4C), 27.37 (C_7), 29.20 (C_{23}, 4C), 29.59 (C_6), 32.35 (C_{12}), 35.87 (C_{16}), 39.05 (C_8), 44.06 (C_9), 46.61 (C_{13}), 49.06 (C_{14}), 85.47 (C_{17}), 118.54 (C_2), 121.48 (C_4), 124.68 (C_{21}), 126.39 (C_1), 138.05 (C_{10}), 138.27 (C_5), 148.38 (C_{20}), 152.40 (C_3), 169.89 (CH$_3$*C*=O).

Method I. General Procedures for the Synthesis of 4a−g. Stille Coupling. To a solution 3-acetoxy-17α-*E*-(tri-*n*-butylstannyl)vinyl estradiol **3** (0.5 mmol) in dry toluene (5 mL) were added the aryl halide (Br/I) (0.6−0.7 mmol) and a catalytic amount (5.0 mg) of tetrakis(triphenylphosphine)-palladium(0) and three crystals of 3,5-di-*tert*-butyl-4-hydroxytoluene. The reaction mixture was stirred for 10 h at 90−100 °C under a nitrogen atmosphere. The reaction mixture was cooled to ambient temperature and filtered to remove the catalyst. The filtrate was concentrated by rotary evaporation, dissolved in ethyl acetate (50 mL), and washed sequentially with saturated ammonium chloride, saturated potassium fluoride, and brine. The organic layer was dried over magnesium sulfate (anhydrous), filtered, and evaporated to dryness. The residue was purified by column chromatography on silica gel using hexanes−ethyl acetate or chloroform−methanol as the eluent.

General Procedure for Deacetylation. Synthesis of 5a−g,j. The purified 3-acetoxy-17α-(4-substituted phenyl)vinyl estradiols were dissolved in methanol (5 mL) containing 0.4 mL of 10 N sodium hydroxide (or sodium methoxide for **5f**). The solution was stirred for 2 h and then acidified with dilute acetic acid (4%) and partitioned between ethyl acetate and water. The organic phase was washed with 10% sodium bicarbonate, dried over magnesium sulfate, filtered, and evaporated to dryness. The crude product was purified by column chromatography on silica gel using hexanes−ethyl acetate. The final compounds were crystallized from hexanes−acetone (ethyl acetate) to provide analytical samples for the binding studies.

Method II. General Procedure for Solid-Phase Synthesis of 5h,i. Preparation of the Resin-Bound 17α-Tri-*n*-butylstannylvinyl Estradiol (7). The 17α-ethynyl estradiol **1** (3 g, 10 mmol) was dissolved in THF in a flask and treated with triethylborane (2 mL, 17 mmol) and tributyltin hydride (3 g, 11 mmol). The mixture was stirred at 40 °C for 10 h. The reaction mixture was evaporated to dryness, dissolved in CH_2Cl_2, and then transferred to the preswollen carboxy resin (5 g) in CH_2Cl_2 (20 mL) in the presence of DCC (2.3 g, 11 mmol). A catalytic amount of DMAP was added to the mixture, and the reaction mixture was allowed to stand for 24 h. The total loading yield for the mixture of *E* and *Z* isomers was 50% (0.59 mmol/g), comprising 47% (0.56 mmol/g) *E* isomer and 3% *Z* isomer (0.03 mmol/g).

The stannylated resin (1.0 g, 0.6 mmol) was placed in the reaction vessel and swelled with dichloromethane. The solvent was removed by evacuation, and the resin was treated with dry toluene (10 mL). To the slurry were added the appropriate aryl halide (Br/I), two to three crystals of 3,5-di-*tert*-butyl-4-hydroxytoluene, and a small amount (5 mg) of the Pd(0) catalyst. The reaction mixture was heated at 80−90 °C overnight under nitrogen. The reaction mixture was agitated to maintain dispersal of the materials. After cooling to ambient temperature, the resin was washed three times each with dichloromethane, methanol, tetrahydrofuran, and warm dimethyl formamide, dried in vacuo, and characterized by FTIR. The resin was swelled in dichloromethane (10 mL) containing 3 mL of 5 N sodium hydroxide in methanol, and the mixture was stirred for 1 h. The cleavage step was repeated three times. The solutions were combined, acidified with dilute acetic acid, and partitioned between ethyl acetate and water. The organic phase was washed with 10% sodium bicarbonate and brine, dried over magnesium sulfate, filtered, and evaporated to dryness. The crude product was purified by column chromatography on silica gel using hexanes−ethyl acetate as the eluent. The final product was crystallized from hexanes−acetone/ethyl acetate to obtain analytical samples for the binding studies.

17α-20*E*-21-Phenyl-19-norpregna-1,3,5(10),20-tetraene-3,17β-diol 3-Acetate (4a). 25% yield, R_f = 0.23 (hexanes−ethyl acetate, 5:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (s, 3H, C_{18}−C*H*$_3$), 1.2−2.4 (m, steroid envelope), 2.28 (s, 3H, C*H*$_3$C=O-), 2.7−2.9 (m, 2H, $C_{6\alpha}$−*H* and $C_{6\beta}$−*H*), 6.48 (d, 1H, *J* = 16.1 Hz, CH=C_{21}*H*), 6.60 (d, 1H, *J* = 16.1 Hz, C_{20}H=CH), 6.80 (d, 1H, *J* = 2.3 Hz, C_4−H), 6.83 (dd, 1H, *J* = 2.4, 8.4 Hz, C_2−H), 7.26 (d, 2H, *J* = 7.4 Hz, C_1 and C_{25}−H), 7.34 (t, 2H, *J* = 7.8 Hz, C_{24} and C_{26}−H), 7.44 (d, 2H, *J* = 7.1 Hz, C_{23} and C_{27}−H). ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 14.10 (C_{18}), 21.08 (*C*H$_3$C=O), 23.35 (C_{15}), 26.09 (C_{11}), 27.17 (C_7), 29.50(C_6), 32.43 (C_{12}), 36.85 (C_{16}), 39.08 (C_8), 43.77 (C_9), 47.36 (C_{13}), 49.34 (C_{14}), 84.03 (C_{17}), 118.49 (C_2), 121.41 (C_4), 126.31 (C_{25}), 126.40 (C_{24}, C_{26}), 127.32 (C_1), 127.50 (C_{21}), 128.55 (C_{23}, C_{27}), 134.82 (C_{10}), 137.10 (C_{20}), 137.94 (C_5), 138.14 (C_{22}), 148.33 (C_3), 169.80 (CH$_3$*C*=O).

17α-20*E*-21-Phenyl-19-norpregna-1,3,5(10),20-tetraene-3,17β-diol (5a). Hydrolysis of the 3-acetate group afforded the product in 92% yield. R_f = 0.18 (hexanes−ethyl acetate, 4:1), mp 176−177 °C, R_f = 0.17 (hexane-acetone, 4:1), elemental analysis $C_{26}H_{30}O_2 \cdot 0.5CH_3CO_2C_2H_5$. ^{1}H NMR (300MHz, acetone-d_6): δ 1.01 (s, 3H, C_{18}−C*H*$_3$), 1.2−2.4 (m, steroid envelope), 2.7−2.9 (m, 2H, $C_{6\alpha}$−*H* and $C_{6\beta}$−*H*), 3.77 (s, 1H, $C_{17\beta}$−O*H*), 6.52 (d, 1H, *J* = 2.6 Hz, C4−H), 6.58 (dd, 1H, *J* = 2.7, 8.4 Hz, C_2−H), 6.63 (s, 2H, C_{20}*H*=C_{21}*H*), 7.07 (d, 1H, *J* = 8.3 Hz, C_1−H), 7.20 (t, 1H, *J* = 7.2 Hz, C_{25}−H), 7.31 (t, 2H, *J* = 7.7 Hz, C_{24}, C_{26}−H), 7.46 (d, 2H, *J* = 7.1 Hz, C23, C27−H). ^{13}C NMR (75.4 MHz, acetone-d_6): δ 14.73 (C_{18}), 24.09 (C_{15}), 27.28 (C_{11}), 28.31 (C_7), 33.46 (C_{12}), 37.41 (C_{16}), 40.71 (C_8), 44.62 (C_9), 48.29 (C_{13}), 50.06 (C_{14}), 84.10 (C_{17}), 113.52 (C_2), 115.89 (C_4), 126.98 (C_{25}), 127.13 ($C_{24}$and C_{26}), 127.38 (C_1), 127.70 (C_{21}), 129.31 (C_{23} and C_{27}), 132.06 (C_{10}), 137.24 (C_{20}), 138.39 (C_5), 138.71(C_{22}), 155.87 (C_3).

17α-20*E*-21-(4-Hydroxyphenyl)-19-norpregna-1,3,5(10),-20-tetraene-3,17β-diol 3-Acetate (4b). To a solution of 3-acetoxy-17α-*E*-(tri-*n*-butylstannyl)vinyl estradiol (0.35 g, 0.56 mmol) in toluene (5 mL) were added 4-iodophenol (0.15 g, 0.68 mmol), three crystals of 3,5-di-*tert*-butyl-4-hydroxytoluene, and a catalytic amount (15 mg) of $Pd(PPh_3)_4$ to afford 50 mg of the product in 21% yield as an amorphous solid.

17α-20*E*-21-(4-Hydroxyphenyl)-19-norpregna-1,3,5(10),-20-tetraene-3,17β-diol (5b). Evaporation followed by silica gel column chromatography with 2% methanol in chloroform afforded the amorphous product in 89% yield (0.04 g). Elemental analysis $C_{26}H_{30}O_3 \cdot 0.5CH_3CO_2C_2H_5$. ^{1}H NMR (300 MHz, acetone-d_6): δ 0.86 (s, 3H, C_{18}−C*H*$_3$), 1.2−2.4 (m, steroid envelope), 2.7−2.9 (m, 2H, $C_{6\alpha}$−*H* and $C_{6\beta}$−*H*), 3.67 (s, 1H, $C_{17\beta}$−O*H*), 6.28 (d, 1H, *J* = 16 Hz, CH=C_{21}*H*), 6.39 (d, 1H, *J* = 2.7 Hz, C_4−H), 6.40 (d, 1H, *J* = 16.1 Hz, C_{20}*H*=CH), 6.44 (dd, 1H, *J* = 2.6, 8.4 Hz, C_2−H), 6.66 (d, 2H, *J* = 8.7 Hz, C_{24}, C_{26}−H), 6.94 (d, 1H, *J* = 8.3 Hz, C_1−H), 7.17 (d, 1H, *J* = 8.6 Hz, C_{23}−H and C_{27}−H), 7.96 (s, 1H, C3−O*H*), 8.37 (s, 1H, C_{25}−O*H*). ^{13}C NMR (75.4 MHz, acetone-d_6): δ 14.72 (C_{18}), 24.05 (C_{15}), 27.29 (C_{11}), 28.32 (C_7), 33.40 (C_{12}), 37.26 (C_{16}), 40.71 (C_8), 44.66(C_9), 48.16 (C_{13}), 49.94 (C_{14}), 84.02 (C_{17}), 113.43 (C_2), 115.80 (C_4), 116.07 (C_{26}), 116.15 (C_{24}), 126.99 (C_{21}), 127.21 (C_1), 128.33 (C_{23} and C_{27}), 130.27 (C_{22}), 132.08 (C_{10}), 134.02 (C_{20}), 138.40 (C_5), 155.78 (C_3), 157.45 (C_{25}).

17α-20*E*-21-(4-cyanophenyl)-19-norpregna-1,3,5(10),20-tetraene-3,17β-diol 3-Acetate (4c). The purification by silica

gel column chromatography using a hexanes−ethyl acetate gradient (5/1 → 3/1) afforded the product in 50% yield. R_f = 0.21 (hexanes−ethyl acetate, 3:1). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.98 (s, 3H, C_{18}−CH_3), 1.2−2.4 (m, steroid envelope), 2.28 (s, 3H, CH_3C═O), 2.7−2.9 (m, 2H, $C_{6\alpha}$−*H* and $C_{6\beta}$−*H*), 6.63 (t, 2H, *J* = 16.6 Hz, C_{20}*H*═C_{21}*H*), 6.79 (d, 1H, *J* = 2.3 Hz, C_4−H), 6.83 (dd, 1H, *J* = 2.5, 8.4 Hz, C_2−H), 7.24 (d, 1H, *J* = 8.3Hz, C_1−H), 7.49 (d, 2H, *J* = 8.3 Hz, C_{23}−H and C_{27}−H), 7.60 (d, 2H, *J* = 8.3 Hz, C_{24}−H and C_{26}−H). ^{13}C NMR (75.4 MHz, acetone-d_6): δ 14.05 (C_{18}), 20.99 (*C*H$_3$C═O), 23.31 (C_{15}), 25.98 (C_{11}), 27.10 (C_7), 29.37 (C_6), 32.53 (C_{12}), 37.12 (C_{16}), 38.99 (C_8), 43.69 (C_9), 47.54 (C_{13}), 49.59 (C_{14}), 84.03 (C_{17}), 110.22 (C_{25}), 118.47 (C_2), 118.90 (*C*≡N), 121.38 (C_4), 125.73 (C_1), 126.19 (C_{21}), 126.81 (C_{23}, C_{27}), 132.27 (C_{24}, C_{26}), 137.62 (C_{10}), 137.98 (C_5), 138.98 (C_{20}), 141.68 (C_{22}), 148.30 (C_3), 169.74 (CH$_3$*C*═O).

17α-20*E*-21-(4-Cyanophenyl)-19-norpregna-1,3,5(10),20-tetraene-3,17β-diol (5c). The product was purified by silica gel column chromatography with a hexanes−ethyl acetate (4:1) eluent. Recrystallization (hexane−acetone) afforded the pure product (0.11 g, 0.26 mmol) in 80% yield. R_f = 0.08 (hexanes−ethyl acetate, 4:1), mp 139−140 °C, elemental analysis $C_{27}H_{29}O_2N_1$·0.5$CH_3CO_2C_2H_5$. ^{1}H NMR (300 MHz, acetone-d_6): δ 1.01 (s, 3H, C_{18}−CH_3), 1.2−2.4 (m, steroid envelope), 2.7−2.9 (m, 2H, $C_{6\alpha}$−*H* and $C_{6\beta}$−*H*), 3.92 (s, 1H, $C_{17\beta}$−O*H*), 6.52 (s, 1H, C_4−H), 6.58 (dd, 1H, *J* = 2.7, 8.7 Hz, C_2−H), 6.73 (d, 1H, *J* = 16 Hz, CH═C_{21}*H*), 6.90 (d, 1H, *J* = 16 Hz, C_{20}*H*═CH), 7.07 (d, 1H, *J* = 8.3 Hz, C_1−H), 7.67 (d, 2H, *J* = 8.9 Hz, C_{23}−H and C_{27}−H), 7.71 (d, 2H, *J* = 8.7 Hz, C_{24}−H and C_{26}−H), 7.97 (s, 1H, C_3−O*H*). ^{13}C NMR (75.4 MHz, acetone-d_6,): δ 14.72 (C_{18}), 24.13 (C_{15}), 27.26 (C_{11}), 28.30 (C_7), (C_6), 33.57(C_{12}), 37.65 (C_{16}), 40.69 (C_8), 44.55 (C_9), 48.54 (C_{13}), 50.18 (C_{14}), 84.29 (C_{17}), 110.76 (C_{25}), 113.53 (C_2), 115.90 (C_4), 119.51 (*C*≡N), 125.90 (C_{21}), 126.97 (C_1), 127.88 (C_{23}, C_{27}), 131.95 (C_{10}), 133.18 (C_{24}, C_{26}), 138.36 (C_5), 141.73 (C_{20}), 143.39 (C_{22}), 155.89 (C_3).

17α-20*E*-21-(4-Methylphenyl)-19-norpregna-1,3,5(10),-20-tetraene-3,17β-diol 3-Acetate (4d). The product was purified by silica gel column chromatography using a hexanes−ethyl acetate (4:1) eluent. 59% yield, R_f = 0.26 (hexanes−ethyl acetate, 4:1), amorphous. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90 (s, 3H, C_{18}−CH_3), 1.2−2.4 (m, steroid envelope), 2.19 (s, 3H, C_{28}−CH_3), 2.28 (s, 3H, CH_3C═O), 2.7−2.9 (m, 2H, $C_{6\alpha}$−*H* and $C_{6\beta}$−*H*), 6.34 (d, 1H, *J* = 16.1 Hz, CH═C_{21}*H*), 6.46 (d, 1H, *J* = 16 Hz, C_{20}*H*═CH), 6.71 (s, 1H, C_4−H), 6.74 (dd, 1H, *J* = 2.6, 8.3 Hz, C_2−H), 7.06 (d, 1H, *J* = 7.8 Hz, C_{24}−H and C_{26}−H), 7.16 (d, 1H, *J* = 8.5 Hz, C_1−H), 7.25 (d, 2H, *J* = 8.2 Hz, C_{23}−H and C_{27}−H).

17α-20*E*-21-(4-Methylphenyl)-19-norpregna-1,3,5(10),-20-tetraene-3,17β-diol (5d). The recrystallization (hexane−acetone) step afforded the pure product (0.09 g). 60% yield, R_f = 0.19 (hexanes−ethyl acetate, 4:1), mp 169−170 °C, elemental analysis $C_{27}H_{32}O_2$·0.5$CH_3CO_2C_2H_5$. ^{1}H NMR (300 MHz, acetone-d_6): δ 1.00 (s, 3H, C_{18}−CH_3), 1.2−2.4 (m, steroid envelope), 2.30 (s, 3H, C_{28}−CH_3), 2.7−2.9 (m, 2H, $C_{6\alpha}$−*H* and $C_{6\beta}$−*H*), 3.72 (s, 1H, $C_{17\beta}$−OH), 6.52−6.63 (m, 4H, C_2−H, C_4−H, C_{20}*H*═CH and CH═C_{21}*H*), 7.07 (d, 1H, *J* = 8.8 Hz, C_1−H), 7.13 (d, 1H, *J* = 7.8 Hz, C_{24}−H and C_{26}−H), 7.35 (d, 2H, *J* = 8.1 Hz, C_{23}−H and C_{27}−H), 7.95 (s, 1H, C_3−O*H*). ^{13}C NMR (75.4 MHz, acetone-d_6): δ 14.73 (C_{18}), 21.06 (C_{28}), 24.09 (C_{15}), 27.29 (C_{11}), 28.32 (C_7, C_6), 33.45 (C_{12}), 37.35 (C_{16}), 40.73 (C_8), 44.65 (C_9), 48.26 (C_{13}), 50.04 (C_{14}), 84.07 (C_{17}), 113.53 (C_2), 115.91 (C_4), 126.98 (C_1), 127.08 (C_{24}, C_{26}), 127.30 (C_{20}), 129.94 (C_{23}, C_{27}), 132.06 (C_{10}), 135.94 (C_{22}), 136.15 (C_{21}), 137.27 (C_{25}), 138.40 (C_5), 155.90 (C_3).

17α-20*E*-21-(4-acetylphenyl)-19-norpregna-1, 3, 5, (10), 20-tetraene-3,17β-diol 3-Acetate (4e). The product was purified by silica gel column chromatography using a hexanes−ethyl acetate gradient (8/1 → 1/1). 89% yield, R_f =0.18 (hexanes−ethyl acetate, 3:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.02 (s, 3H, C_{18}−CH_3), 1.2−2.4 (m, steroid envelope), 2.28 (s, 3H, C_3, CH_3−C═O), 2.56 (s, 3H, C_{29}, C═OCH_3), 2.7−2.9 (m, 2H, $C_{6\alpha}$−*H* and $C_{6\beta}$−*H*), 6.60 (d, 1H, *J* = 16.1 Hz, CH═C_{21}*H*), 6.67 (d, 1H, *J* = 16.1 Hz, C_{20}*H*═CH), 6.79 (d, 1H, *J* = 2.3 Hz, C_4−H), 6.82 (dd, 1H, *J* = 2.6, 8.4 Hz, C_2−H), 7.24 (d, 1H, *J* = 8.4 Hz, C_1−H), 7.50 (d, 2H, *J* = 8.4 Hz, C_{23}−H and C_{27}−H), 7.92 (d, 2H, *J* = 8.4 Hz, C_{24}−H and C_{26}−H).

17α-20*E*-21-(4-Acetylphenyl)-19-norpregna-1,3,5(10),20-tetraene-3, 17β-diol (5e). The product was purified by recrystallization (hexanes−ethyl acetate) to afford 0.27 g (0.65 mmol). 83% yield, mp149−150 °C, R_f = 0.07 (hexanes−ethyl acetate, 4:1), elemental analysis $C_{28}H_{32}O_3$·0.5$CH_3CO_2C_2H_5$. ^{1}H NMR (300 MHz, acetone-d_6): δ 1.02 (s, 3H, C_{18}−CH_3), 1.2−2.4 (m, steroid envelope), 2.56 (s, 3H, C═OCH_3), 2.7−2.9 (m, 2H, $C_{6\alpha}$−*H* and $C_{6\beta}$−*H*), 3.88 (s, 1H, $C_{17\beta}$−O*H*), 6.52 (d, 1H, *J* = 2.6 Hz, C_4−H), 6.58 (dd, 1H, *J* = 2.7, 8.3 Hz, C_2−H), 6.72 (d, 1H, *J* = 16 Hz, CH═C_{21}*H*), 6.85 (d, 1H, *J* = 16 Hz, C_{20}*H*═CH), 7.07 (d, 1H, *J* = 8.3 Hz, C_1−H), 7.60 (d, 2H, *J* = 8.5 Hz, C_{23}−H and C_{27}−H), 7.94 (d, 2H, *J* = 8.5 Hz, C_{24}−H and C_{26}−H). ^{13}C NMR (75.4 MHz, acetone-d_6): δ 14.75 (C_{18}), 24.15 (C_{15}), 26.57 (C═O*C*H$_3$), 27.29 (C_{11}), 28.33 (C_7), 33.57 (C_{12}), 37.51 (C_{16}), 40.72 (C_8), 44.61 (C_9), 48.49 (C_{13}), 50.20 (C_{14}), 84.27 (C_{17}), 113.52 (C_2), 115.88 (C_4), 126.52 (C_1), 126.98 (C_{21}), 127.19 (C_{24}, C_{26}), 129.46 (C_{23}, C_{27}), 132.0 (C_{10}), 136.61 (C_{25}), 138.39 (C_5), 140.49 (C_{20}), 143.28 (C_{22}), 165.46 (C_3), 197.20 (*C*═OCH$_3$).

17α-20*E*-21-(4-Methoxycarbonylphenyl)-19-norpregna-1,3,5(10),20-tetraene-3,17β-diol 3-Acetate (4f). The product was purified by silica gel column chromatography using a hexanes−ethyl acetate gradient (4/1 → 3/1) in 65% yield. R_f = 0.28 (hexanes−ethyl acetate, 3:1), amorphous. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.99 (s, 3H, C_{18}−CH_3), 1.2−2.4 (m, steroid envelope), 2.28 (s, 3H, CH_3C═O), 2.7−2.9 (m, 2H, $C_{6\alpha}$−*H* and $C_{6\beta}$−*H*), 3.91 (s, 3H, C═OOCH_3), 6.59 (d, 1H, *J* = 16.1 Hz, CH═C_{21}*H*), 6.66 (d, 1H, *J* = 16.1 Hz, C_{20}*H*═CH), 6.79 (s, 1H, *J* = 2.4 Hz, C_4−H), 6.82 (dd, 1H, *J* = 2.6, 8.4 Hz, C_2−H), 7.24 (d, 1H, *J* = 8.7 Hz, C_1−H), 7.48 (d, 2H, *J* = 8.4 Hz, C_{23}−H and C_{27}−H), 8.0 (d, 2H, *J* = 8.5 Hz, C_{24}−H and C_{26}−H). ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 14.14 (C_{18}), 21.10 (*C*H$_3$C═O), 23.41 (C_{15}), 26.10 (C_{11}), 27.20 (C_7), 29.51 (C_6), 32.58 (C_{12}), 37.14 (C_{16}), 39.12 (C_8), 43.80 (C_9), 47.56 (C_{13}), 49.58 (C_{14}), 52.03 (C═O-OCH_3), 84.16 (C_{17}), 118.55 (C_2), 121.46 (C_4), 126.30 (C_{24}, C_{25}, C_{26}), 126.61 (C_1), 128.75 (C_{21}), 129.93 (C_{23}, C_{27}), 137.67 (C_{10}), 137.84 (C_{20}), 138.14 (C_5), 141.67 (C_{22}), 148.38 (C_3), 166.88 (*C*═OOCH$_3$), 169.83 (CH$_3$*C*═O).

17α-20*E*-21-(4-Methoxycarbonylyphenyl)-19-norpregna-1,3,5(10),20-tetraene-3,17β-diol (5f). The product was purified by silica gel column chromatography using a hexane-acetone system (3:1). Recrystallization using hexanes−ethyl acetate afforded the pure product in a 25% yield. R_f = 0.19 (hexane−acetone, 3:1), mp 144−145 °C, elemental analysis $C_{28}H_{32}O_4$. ^{1}H NMR (300 MHz, acetone-d_6): δ 1.01 (s, 3H, C_{18}−CH_3), 1.2−2.4 (m, steroid envelope), 2.28 (s, 3H, CH_3C═O), 2.7−2.9 (m, 2H, $C_{6\alpha}$−*H* and $C_{6\beta}$−*H*), 3.87 (s, 3H, C═OOCH_3), 6.53 (s, 1H, C_4−H), 6.58 (dd, 1H, *J* = 2.2, 8.4 Hz, C_2−H), 6.72 (d, 1H, *J* = 16 Hz, CH═C_{21}*H*), 6.85 (d, 1H, *J* = 15.9 Hz, C_{20}*H*═CH), 7.07 (d, 1H, *J* = 8.6 Hz, C_1−H), 7.60 (d, 2H, *J* = 8.3 Hz, C_{23}−H and C_{27}−H), 7.93 (s, 1H, C3−O*H*), 7.95 (d, 2H, *J* = 8.3 Hz, C_{24}−H and C_{26}−H). ^{13}C NMR (75.4 MHz, acetone-d_6): δ 14.75 (C_{18}), 24.15 (C_{15}), 27.29 (C_{11}), 28.32 (C_7), 33.57 (C_{12}), 37.59 (C_{16}), 40.73 (C_8), 44.60 (C_9), 48.49 (C_{13}), 50.20 (C_{14}), 52.17 (C═OO*C*H$_3$), 84.28 (C_{17}), 113.55 (C_2), 115.92 (C_4), 126.48 (C_{25}), 126.99 (C_1), 127.16 (C_{24}, C_{26}), 129.33 (C_{21}), 130.51 (C_{23}, C_{27}), 132.03 (C_{10}), 138.40 (C_5), 140.50 (C_{20}), 143.41 (C_{22}), 155.80 (C_3), 167.01 (*C*═OOCH$_3$).

17α-20*E*-21-(4-Carboxyphenyl)-19-norpregna-1,3,5(10),-20-tetraene-3,17β-diol (5g). Compound **5g** was prepared by the same method as compound **5f**. 91% yield, mp 157−158 °C, R_f = 0.24 ($CHCl_3$−CH_3OH, 95:5); elemental analysis $C_{27}H_{32}O_4$. ^{1}H NMR (300 MHz, acetone-d_6): δ 1.02 (s, 3H, C_{18}−CH_3), 1.2−2.4 (m, steroid envelope), 2.7−2.9 (m, 2H, $C_{6\alpha}$−*H* and $C_{6\beta}$−*H*), 6.54 (d, 1H, *J* = 2.5 Hz, C_4−H), 6.59 (dd, 1H, *J* = 2.6, 8.5 Hz, C_2−H), 6.73 (d, 1H, *J* = 16 Hz, CH═C_{21}*H*), 6.84 (d, 1H, *J* = 16.1Hz, C_{20}*H*═CH), 7.06 (d, 1H, *J* = 8.8 Hz, C_1−H), 7.59 (d, 2H, *J* = 8.3 Hz, C_{23}−H and C_{27}−H), 8.0 (d, 2H, *J* = 8.3 Hz, C_{24}−H and C_{26}−H). ^{13}C NMR (75.4 MHz, acetone-d_6): δ 14.75 (C_{18}), 24.11 (C_{15}), 27.23 (C_{11}), 28.26 (C_7, C_6), 33.50 (C_{12}), 37.50 (C_{16}), 40.65 (C_8), 44.52 (C_9), 48.45 (C_{13}), 50.17 (C_{14}), 84.31 (C_{17}), 113.51 (C_2), 115.89 (C_4), 126.57 (C_{21}), 126.94 (C_1), 127.08 (C_{24},

C_{26}), 129.59 (C_{25}), 130.79 (C_{23}, C_{27}), 132.01 (C_{10}), 138.36 (C_5), 140.22 (C_{20}), 143.26 (C_{22}), 155.78 (C_3), 167.58 (*C*=OOH).

17α-20*E*-21-(4-Trifluoromethylphenyl)-19-norpregna-1,3,5(10),20-tetraene-3,17*β*-diol (5h). The product was cleaved, and the resulting mixture was purified by silica gel column chromatography using chloroform to afford 0.12 g of the *E* isomer product and 1 mg of the *Z* isomer product. 49% yield, R_f = 0.15 (hexanes−ethyl acetate, 4:1), mp 215−217 °C, elemental analysis $C_{27}H_{29}O_2F_3$. ^{1}H NMR (300 MHz, acetone-d_6): δ 1.02 (s, 3H, C_{18}−C*H*$_3$), 1.2−2.4 (m, steroid envelope), 2.7−2.9 (m, 2H, $C_{6\alpha}$−H and $C_{6\beta}$−H), 3.90 (s, 1H, $C_{17\beta}$−O*H*), 6.53 (d, 1H, *J* = 2.6 Hz, C_4−H), 6.58 (dd, 1H, *J* = 2.6, 8.4 Hz, C_2−H), 6.73 (d, 1H, *J* = 16 Hz, CH=C_{21}*H*), 6.85 (d, 1H, *J* = 16 Hz, C_{20}*H*=CH), 7.07 (d, 1H, *J* = 8.3 Hz, C_1−H), 7.64 (d, 2H, *J* = 8.7 Hz, C_{23}−H and C_{27}−H), 7.70 (d, 2H, *J* = 8.6 Hz, C_{24}−H and C_{26}−H), 8.0 (s, C_3−OH). ^{13}C NMR (75.4 MHz, acetone-d_6): δ 14.73 (C_{18}), 24.13 (C_{15}), 27.26 (C_{11}), 28.31 (C_7), (C_6), 33.54 (C_{12}), 37.58 (C_{16}), 40.69 (C_8), 44.58 (C_9), 48.46 (C_{13}), 50.16 (C_{14}), 84.23 (C_{17}), 113.53 (C_2), 115.90 (C_4), 125.44 (q, *J* = 270.6 Hz, *C*F$_3$), 125.97(C_{21}), 126.21 (q, *J* = 3.5 Hz, C_{26}), 126.22 (q, *J* = 3.5 Hz, C_{24}), 126.98 (C_1), 127.62 (C_{23}, C_{27}), 128.85 (q, *J* = 32 Hz, C_{25}), 131.98 (C_{10}), 138.38 (C_5), 140.64 (C_{20}), 142.75 (C_{22}), 155.88 (C_3).

17α-20*E*-21-(4-Methoxyphenyl)-19-norpregna-1,3,5(10),-20-tetraene-3,17*β*-diol (5i). 36% yield, R_f = 0.23 ($CHCl_3$−CH_3OH, 99:1), elemental analysis $C_{27}H_{32}O_3$·0.5$CH_3CO_2C_2H_5$. ^{1}H NMR (300 MHz, acetone-d_6): δ 0.99 (s, 3H, C_{18}−C*H*$_3$), 1.2−2.4 (m, steroid envelope), 2.7−2.9 (m, 2H, $C_{6\alpha}$−H and $C_{6\beta}$−H), 3.68 (s, 1H, $C_{17\beta}$−OH), 3.78 (s, 3H, OC*H*$_3$), 6.46 (d, 1H, *J* = 16.1 Hz, CH=C_{21}*H*), 6.51−6.59 (m, 3H, C_2−H, C_4−H, and C_{20}−H), 6.88 (d, 2H, *J* = 8.8 Hz, C_{24}−H and C_{26}−H), 7.07 (d, 1H, *J* = 8.3 Hz, C_1−H), 7.39 (d, 2H, *J* = 8.8 Hz, C_{23}−H and C_{27}−H), 7.95 (s, 1H, C_3−O*H*). ^{13}C NMR (75.4 MHz, acetone-d_6): δ 14.74 (C_{18}), 24.07 (C_{15}), 27.30 (C_{11}), 28.33 (C_7, C_6), 33.43 (C_{12}), 37.32 (C_{16}), 40.73 (C_8), 44.67 (C_9), 48.21 (C_{13}), 49.98 (C_{14}), 55.49 (O*C*H$_3$), 84.05 (C_{17}), 113.54 (C_2), 114.73 (C_{24}, C_{26}), 115.91 (C_4), 126.95 (C_1), 126.98 (C_{21}), 128.26 (C_{23}, C_{27}), 131.35 (C_{22}), 132.07 (C_{10}), 134.87 (C_{20}), 138.40 (C_5), 155.91 (C_3), 159.89 (C_{25}).

17α,20*E*-21-Iodo-19-norpregna-1,3,5(10),20-tetraene-3,-17*β*-diol 3-Acetate (8). To a solution of **3** (2.36 g, 3.75 mmol) in chloroform−methylene chloride (1:1, 30 mL) was added a slurry of *N*-iodosuccinimide (1.0 g, 4.4 mmol) in the same solvent solution. The reaction mixture was stirred, under aluminum foil, at 0 °C for 24 h. The reaction was followed by TLC for the conversion of **3** (R_f = 0.4, hexane−ethyl acetate, 5:1) to **8** (R_f = 0.2, same solvent system). The reaction mixture was washed with saturated sodium bicarbonate/water (50 mL). Aqueous and organic layers were separated. Aqueous layer was extracted with chloroform (50 mL × 2). Organic layers were combined, washed with water (50 mL × 2) and brine (50 mL × 2), dried over magnesium sulfate, and concentrated. The yellow oil was separated on a silica gel column (60 g) and covered with aluminum foil, using chloroform−methanol (98:2) as the eluting solvent to give **8** as a pure white powder (1.62 g, 93%). R_f = 0.2 (hexane−ethyl acetate, 5:1). ^{1}H NMR ($CDCl_3$): δ 0.96 (s, 3H, 18-CH_3), 1.2−2.9 (m, 15H, steroid nucleus), 6.32 (d, 1H, J_{21-20} = 14.34 Hz, 21-H), 6.78 (d, 1H, J_{4-2} = 2.46 Hz, 4-H), 6.84 (dd, 1H, J_{2-4} = 2.58 Hz, J_{2-1} = 8.04 Hz, 2-H), 6.88 (d, 1H, J_{20-21} = 14.22 Hz, 20-H), 7.29 (d, 1H and $CDCl_3$ peak, J_{1-2} = 8.28 Hz, 1-H). ^{13}C NMR ($CDCl_3$): δ 14.16 (C-18), 21.17 (−OCO*C*H$_3$), 22.67 (C-15), 26.08 (C-11), 27.18 (C-7), 29.51 (C-6), 32.47 (C-12), 36.65 (C-16), 39.07 (C-8), 43.77 (C-9), 47.07 (C-13), 49.35 (C-14), 74.72 (C-21), 87.10 (C-17), 118.62 (C-2), 121.52 (C-4), 126.40 (C-1), 137.80 (C-10), 138.15 (C-5), 150.46 (C-3), 148.43 (C-20), 169.92 (−O*C*OCH$_3$).

Method III. Suzuki Coupling. Synthesis of 17α,20*E*-21-(4-Fluorophenyl)-19-norpregna-1,3,5(10),20-tetraene-3,17*β*-diol 3-Acetate (4j). To a solution of **8** (1.34 g, 2.9 mmol) in THF (10 mL) was added tris(dibenzylideneacetone)dipalladium (0.25 g, 0.27 mmol), sodium bicarbonate (1.28 g, 12.08 mmols, 4 equiv, in 5 mL of water), and 4-fluorobenzeneboronic acid (0.86 g, 6.1 mmol). The reaction mixture was protected from light and stirred at room temperature for 12 h. The mixture was extracted with ethyl acetate (4 × 100 mL), washed with water (5 × 100 mL) and brine (200 mL), dried over magnesium sulfate, filtered, and concentrated to yield a yellow powder. The residue was chromatographed on a silica gel column (50 g) using 98:2 chloroform−methanol as the eluting solvent to give **4j** (0.46 g, 37%). R_f = 0.2 (hexane−ethyl acetate, 4:1). ^{1}H NMR ($CDCl_3$): δ 0.97 (s, 3H, 18-CH_3), 1.2−2.9 (m, b, 15H, steroid nucleus), 6.37 (d, 1H, J_{20-21} = 15.99 Hz, 20-H), 6.54 (d, 1H, J_{21-20} = 16.11 Hz, 21-H), 6.78 (d, 1H, J_{4-2} = 2.3 Hz, 4-H), 6.83 (dd, 1H, J_{2-4} = 2.5 Hz, J_{2-1} = 8.4 Hz, 2-H), 6.9 (∼t, 2H, J_{24-F} and J_{26-F} = 8.4 Hz, J_{24-23} and J_{26-27} = 6.7 Hz, J_{24-27} and J_{26-23} = 2 Hz, 24-H and 26-H), 7.24 (d, 1H and $CDCl_3$ peak, J_{1-2} = 8.3 Hz, 1-H), 7.37 (m, 2H, 25-H and 27-H)

17α,20*E*-21-(4-Fluorophenyl)-19-norpregna-1,3,5(10),-20-tetraene-3,17*β*-diol (5j). Our standard deprotection method of **4j** (0.34 g, 0.79 mmol) yielded **5j** (0.31 g, 100%). Recrystallization in hexane−acetone, 3:1, produced a fine white powder (0.31 g, 97%). R_f = 0.17 (hexane−ethyl acetate, 4:1), mp 189−191 °C, elemental analysis $C_{26}H_{29}FO_2$·0.5$CH_3CO_2C_2H_5$. ^{1}H NMR (acetone-d_6): δ 1.0 (s, 3H, 18-CH_3), 1.2−2.9 (m, 15H, steroid nucleus), 6.39 (d, 1H, J_{21-20} = 16.08 Hz, 21-H), 6.55 (d, 1H, J_{20-21} = 16.02, 20-H), 6.56 (d, 1H, J_{4-2} = 2.79 Hz, 4-H), 6.61 (dd, 1H, J_{2-1} = 8.4 Hz, J_{2-4} = 2.82 Hz, 2-H), 7.02 (ddd, 2H, 24-H and 26-H), 7.12 (d, 1H, J_{1-2} = 8.31 Hz, 1-H), 7.38 (dd, 2H, 23-H and 27-H). ^{13}C NMR (acetone-d_6): δ 15.07 (C-18), 24.42 (C-15), 27.60 (C-11), 28.64 (C-7), ∼29 under acetone peak (C-6), 33.79 (C-12), 37.78 (C-16), 41.02 (C-8), 44.92 (C-9), 48.61 (C-13), 50.34 (C-14), 84.44 (C-17), 113.86 (C-2), 116.24 (C-4), 116.30 (d, J_{CCF} = 21 Hz, C-24 and C-26), 126.50 (C-21), 127.30 (C-1), 129.15 (d, J_{CCCF} = 7.9 Hz, C-23 and C-27), 132.31 (C-10), 135.50 (C-22), 137.52 (C-20), 138.71 (C-5), 156.18 (C-3), 163.06 (d, J_{C-F} = 243 Hz, C-25).

Molecular Modeling and Dynamics. We initially evaluated the conformations of our ligands **5a**−**j** using the Builder module from Insight II. Potentials for each atom were assigned automatically or manually when necessary. Low-energy conformations were generated using the molecular mechanics method (Discover program, 100 steps, 0.001 final convergence) and compared to solution conformations determined by NMR.[39] The ER-HBD used in our study was obtained from the Protein Data Bank (PDB code 1QKU, wild-type ER_α-HBD cocrystallized with estradiol). Monomer C from the homodimer B/C was selected for the docking and molecular dynamics studies. All water molecules were deleted except for the one positioned near ARG-394 and GLU-353 that is present in all crystal structures. The monomer C contains all the amino acid residues between ASN-304 and HIS-550. All manipulations were performed using the Builder module in Insight II. The complex of ER-LBD monomer and estradiol bound within the binding cavity was minimized using the molecular mechanics method (Discover_3 module, CVFF force field, conjugate gradient minimization 10 000 steps, 0.001 final convergence).

Docking of the ligands with the ERα-HBD was performed using the Docking module in InsightII.[47] The ligand was superimposed on the estradiol molecule (A-ring over A-ring), and the estradiol was then deleted. During the docking procedure, both the ligand and the protein residues within the ligand binding cavity (amino acids within 15 Å of the ligand as well as all amino acids in helix 12, loops 11−12, 1−3, 6−7) were allowed to flex. In addition, the phenylvinyl side chain of the ligand was rotated with 30° increments in order to more fully explore the potential binding modes of the conformational choices of the ligand. After each docking procedure, structures within 10 kcal/mol of the lowest energy structure and the rms distance of more than 0.125 Å were selected and used in simulated annealing studies. In this procedure, the structures were subjected to short molecular dynamics runs (100 fs per stage, total of 50 stages, initial temperature of 500 K, final temperature of 300 K, 1000 steps). CVFF force field and default values for all other parameters were used.

Binding energies were calculated for each of several structures generated during the docking studies. Values of the binding energy $\Delta E_{\text{binding}}$ were calculated as the difference between the potential energy of the complex (E_{complex}) and the

potential energy of the ligand (E_{ligand}) and receptor ($E_{receptor}$).[52,53] Binding energy calculations were performed using the Energy Analysis macro within the Discover_3 module.

Receptor Binding Studies. In Vitro Competitive Binding Assay. The compounds were screened for their affinity for the ERα-LBD isolated from BL 21 cells that overexpressed the 33 kDa PER-23d ERG vector. The cells were induced with 0.6 mM isopropyl-*β*-thiogalactopyranoside for 3 h at room temp, pelleted by centrifugation, frozen, and stored at −75 °C. The cells were thawed and lysed by sonication (4 × 20 s) in four volumes of lysis buffer (50 mM Tris, 50 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 1 M urea, pH 7.4) several times. Clarified fractions, obtained at 30000*g* for 30 min were pooled, assayed for receptor binding, and diluted to 50 nM in ER, and 100 μL aliquots were frozen and stored at −75 °C until ready for use. Then 80 μL of the ERα-LBD-containing extract was incubated with 10 μL of 10 nM 6,7-[H-3]-estradiol (specific activity of 51 Ci/mmol) and 10 μL of either buffer, unlabeled estradiol, or test ligand in 100 μL total volume. The final concentrations were 1 nM 6,7-[H-3]-estradiol, 2 nM unlabeled estradiol (using 200 nM estradiol to define specific binding), and 0.5−5000 nM of the test ligand. In all cases, 10 μL of each incubation solution was removed for assay of the actual initial concentration of [H-3]-estradiol and the remainder was incubated at 2 or 25° C for 18 h. After incubation, 100 μL of dextran-coated charcoal suspension (fines removed) was added to adsorb the unbound [H-3]-estradiol, the mixture was incubated for 10 min and centrifuged, and 100 μL samples were taken from the supernatant fraction for assay of radioactivity. The results were calculated and plotted as the percent specific binding as a function of the log of the competitor concentration using the best fit equation for the binding inhibition to define the 50% inhibition level. The relative binding affinity was calculated as 100 × [E]/[C], where [E] was the concentration of unlabeled estradiol needed to reduce the specific binding of [H-3]-estradiol by 50% and [C] was the concentration of test ligand needed to reduce the specific binding by 50%.

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