Synthesis, Receptor Binding, Molecular Modeling, and Proliferative Assays of a Series of 17α-Arylestradiols

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*A series of new derivatives of estradiol substituted at position 17α by various aryls has been synthesized. This was made possible by efficient activation methods for the addition of aryllithiums to the carbonyl group at position 17 of estrone by using tetramethylethylenediamine (TMEDA) or $BF_3 \cdot OEt_2$. Their relative binding affinity (RBA) for the α and the β forms of the estrogen receptor (ER) have been measured. All except one of the compounds synthesized had an RBA value of around 10% which indicates a level of tolerance towards the bulky substituent at position 17. The lipophilicity values measured for these compounds are higher than that found for estradiol (**E_2**). A study of their proliferative/antiproliferative effects was carried out on hormone-dependent (MCF7) and hormone-independent (MDA-MB231) breast cancer cell lines. It is interesting to note that all the compounds are estrogenic. The possibility of easily attaching an iodine at the end of a phenyl spacer opens up a route to new radiopharmaceuticals for use in radioimaging.*

KEYWORDS:

arylation · estradiol · receptors · steroids · triazenes

Introduction

The recent X-ray crystallographic structural determination of the ligand binding domain (LBD) of estrogen receptors α and β provided a significant advance in the understanding of the mechanism of action of these systems.[1] In particular it provides a molecular view of ligand–receptor binding and a structural approach to the agonist or antagonist effects of various bioligands. Rather than being an end in itself, this structural breakthrough provides the rationale on which further development can be based.

Current sought after advances in the field include more effective and selective estrogen receptor modulators (SERMs) than those currently available, the establishment of affinity chromatography systems on a more solid basis, synthetic approaches to new radiopharmaceuticals, and synthesis of steroids with very local action.[2, 3] It is now widely accepted that estradiol and its derivatives affect a large number of genes in the body.[4] All treatments involving these molecules must take into account the diversity of potential targets and evaluate known and potential risk factors (for example, osteoporosis, heart disease, dyspareunia, breast cancer, stroke).

It is therefore important to seek routes to new derivatives of estradiol that may enable us to target more precisely our response to a multiplicity of current demands. With this in mind one may note that not all possible modifications of the estradiol skeleton have been explored. This is particularly true for the 17α-arylestradiols.

The series of 17α-ethynylestradiols bearing rigid, nonbulky, lipophilic spacers at position 17α has been the subject of several studies.[5–8] It has been clearly established that this type of substitution is compatible with good affinity for the estrogen receptor. Such behavior might be expected with aryl groups attached at this 17α position, which would be expected to permit a complete range of substitutions to the arene. It is surprising to note the weakness of the data available in the literature on this subject.[9, 10] The first 17α-phenylestradiol was not published until 1991 and 1992 and was obtained in modest yield by the action of phenyl lithium on the protected estrone. Reactivity proved to be poor and substituted phenyls were not investigated.

We recently improved this reaction and showed that this approach could be made viable with the aid of in situ activation with boron trifluoride etherate ($BF_3 \cdot OEt_2$) or tetramethylethylenediamine (TMEDA).[11, 12] This preliminary work opened the way for a whole series of substituted aryls attached at position 17α of estradiol. Here we present the full study extended to a biochemical study of the compounds obtained and a rationalization of their behavior by molecular modeling.

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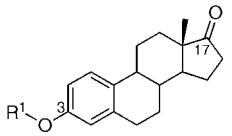
Results and Discussion

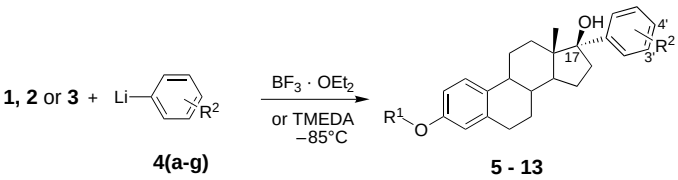
Chemistry

The 17α-arylestradiols described here were prepared in three steps starting from commercially available estrone. The estrone is first protected with the appropriate group (Bn = benzyl, THP = tetrahydropyranyl, TBDMS = *tert*.-butyldimethylsilyl) and various aryllithiums are then added to the carbonyl group at



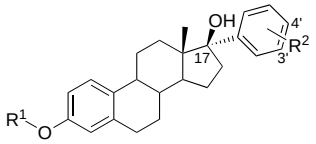
1 : R^1 = Bn
2 : R^1 = THP
3 : R^1 = TBDMS

position 17 (Scheme 1). This addition is carried out at low temperature with activation by $BF_3 \cdot OEt_2$ or TMEDA. A series of 17α-arylestradiols protected at position 3 of the aromatic ring (**5** – **13**) was thus obtained (57 – 80% yields). Structures of the steroids **5** – **13** are detailed in Table 1.



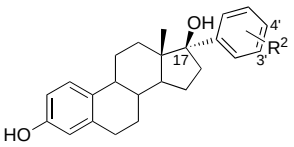
Scheme 1. *Promoted addition of aryllithiums to protected estrones. R^2 = H (**4a**), 4′-Me (**4b**), 4′-OMe (**4c**), 4′-N=N–N(C_4H_8) (**4d**), 4′-NMe_2 (**4e**), 4′-N=N–NEt_2 (**4f**), 3′-N=N–N(C_4H_8) (**4g**).*

Table 1. *Structure of the 3-protected 17α-arylestradiols.*



compound	R^1	R^2
5	Bn	4′-CH_3
6	Bn	4′-OCH_3
7	Bn	4′-N=N–N(C_4H_8)
8	THP	4′-CH_3
9	THP	4′-N=N–N(C_4H_8)
10	THP	4′-OCH_3
11	THP	4′-N(CH_3)$_2$
12	THP	3′-N=N–N(C_4H_8)
13	TBDMS	4′-N=N–NEt_2

The protected estradiols **9** and **12**, which possess a triazene moiety, can be converted directly to iodophenylestradiols **14** and **15**. The protected estradiol **7** can be hydrogenated to aminophenylestradiol **16**. Estradiols **17** – **20** are then obtained by deprotection.



14 : R^2 = 4′-I
15 : R^2 = 3′-I
16 : R^2 = 4′-NH_2
17 : R^2 = 4′-N=N-NEt_2
18 : R^2 = 4′-CH_3
19 : R^2 = 4′-OCH_3
20 : R^2 = 4′-N(CH_3)$_2$
21 : R^2 = H

Activation by $BF_3 \cdot OEt_2$

Addition of phenyllithium to estrone or a protected estrone gave relatively low yields.[9, 10] We attempted to optimize the reaction and to extend it to other aryllithiums (see Scheme 1). The results obtained were described in a preliminary communication whose principal conclusions are summarized below.[12]

Several of the classic methods for the activation of the addition of organometallics to carbonyl compounds, including use of $CeCl_3$[13, 14] or $TiCl_4$,[15, 16] were tested and found to be unsatisfactory. Activation by $LiClO_4$[17] gave moderate results. The best yields for conversion of a protected estrone to the corresponding 17α-arylestradiol were eventually obtained, in the first instance, by using $BF_3 \cdot OEt_2$ as the activation agent. In this way conversions of 60% in THF and 80% in toluene were obtained for the addition of phenyllithium to the estrone benzyl ether at low temperature. This Lewis acid has been used to activate the reactions of organolithiums with various electrophiles. This phenomenon of activation by $BF_3 \cdot OEt_2$ has been interpreted in two ways: either the Lewis acid coordinates to the heteroatom to facilitate the reaction of the organolithium, or there is formation of an ate complex between the lithium and $BF_3 \cdot OEt_2$ at low temperature.[18] This ate complex would be more nucleophilic and less basic than the organolithium.

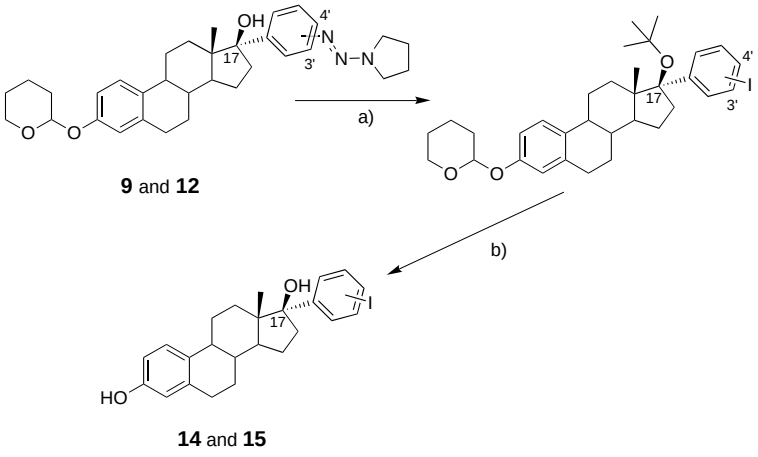
The protected 17α-arylestradiols **5**, **6**, and **7** were prepared by activation with $BF_3 \cdot OEt_2$ in yields of 57 – 80%. The steroid **7** was obtained with 57% conversion by this method; however, problems of reproducibility arose.

Activation by TMEDA

In order to improve the synthesis of compound **7**, we developed a new activation method with TMEDA. Given the low solubility of steroids in most solvents and the structure of aryllithiums in solution, THF appeared the most suitable solvent. In THF, phenyllithium is in monomer – dimer equilibrium. The addition of TMEDA in THF has little if any effect on the monomer/dimer ratio, but forms a series of complexes with phenyllithium.[19] These changes in the structure of the phenyllithium may explain, in this case, the observed increase in reactivity. Thus, with three equivalents of TMEDA per aryllithium, condensation of the aryllithiums **4d** and **4g** onto the carbonyl at position 17 of estrones **1** and **2** occurs in 60% yield to give the arylestradiols **7**, **9**, or **12**.

The aryllithiums **4b** – **g** were all obtained by halogen – metal exchange with *tert*-butyllithium, which was performed at low

temperature to minimize degradation reaction and solvent attack. Once the exchange had occurred, first TMEDA and then the protected estrones **1** – **3** were added to the medium. This method gave the corresponding arylestradiols in yields comparable to those obtained with activation by $BF_3 \cdot OEt_2$. The condensation products were purified by chromatography.



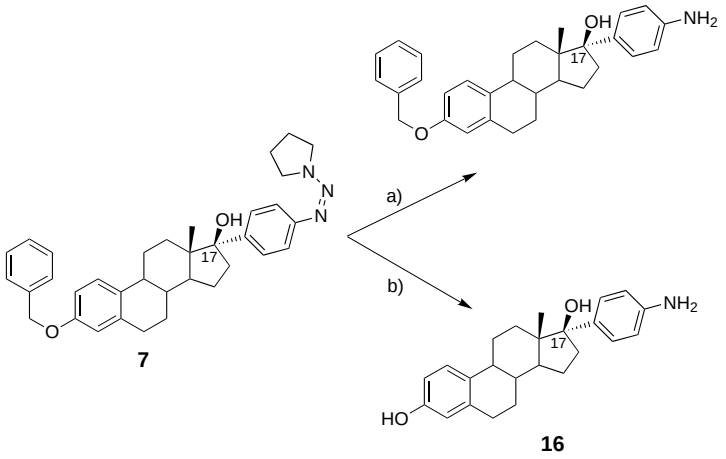
Scheme 2. *Sandmeyer-type reactions. a) NaI, $(CH_3)_3SiCl$, CH_3CN, room temperature, 17 h; b) $NaHCO_3$.*

Sandmeyer-type reactions

Triazene chemistry was employed to transform aryltriazene groups to iodoaryl groups. Barrio described the halogenation of 1-aryl-3,3-dialkyltriazenes either in an acidic medium or in the presence of trimethylsilyl(TMS) halides.[20, 21] We used the latter, milder method on arylestradiol triazenes **7**, **9**, and **12**, by treating them with TMSI to obtain the 4′- and 3′-iodophenyl compounds **14** and **15**. Debenzylation of the iodo compound resulting from **7** did not give convincing results. The choice of the tetrahydropyranyl (THP) protecting group proved judicious, since the deprotection occurs in situ during the halogenation reaction (see below and Scheme 2).

The estradiols formed have a great tendency to dehydrate on heating. The dehydration can be limited at room temperature, and **14** – **15** were obtained in 90% and 80% yield, respectively, after purification by chromatography, while no other steroid was detected.

TMSI was prepared in situ from TMSCl and NaI. This synthesis therefore opens the way for the preparation of radiolabeled derivatives of the estradiols **14** and **15**, which, by using isotopes such as ^{125}I or ^{123}I, could be good candidates as readily accessible radiopharmaceuticals.



Scheme 3. *Reduction of the triazene moiety. a) EtOH, EtOAc, cat. $PdCl_2(CH_3CN)_2$, 15 min; b) MeOH/THF, Pd(10% on charcoal), H_2, 21 h.*

Deprotections

The THP group: we decided to take advantage of the deprotection of THP groups observed during Sandmeyer-type reactions with compounds **9** and **12**. We attempted to deprotect the THP groups of estradiols **8**, **10**, and **11** by treating them with TMSI. The desired deprotection does indeed occur and is quantitative. This recently described method[22] is interesting, since it avoids the dehydration of the tertiary benzyl alcohol that we consistently observed with standard methods. We suppose that the alcohol group at position 17β was silylated in situ, which certainly obviates dehydration. Estradiols **18**, **19**, and **20** were thus obtained in good yields (77 – 90%).

The triazene group: use of a Ni – Al alloy has been proposed for reduction of triazenes to amines.[23] Thus the hydrogenolysis of the triazene **7** in the presence of Ni – Al alloy was tested in various solvents and in every case failed to give a satisfactory result. Similarly, hydrogenation in the presence of $Pd(CH_3CN)_2Cl_2$ only permits conversion of the triazene function to an amine, while the benzyl group resists deprotection (Scheme 3). However, hydrogenation in the presence of palladium on carbon under a hydrogen atmosphere permitted reduction of the triazene to an amine and simultaneous cleavage of the benzyl group. In this case, the use of methanol is necessary to give good hydrogen solubility, while THF was used to dissolve the steroid. The estradiol **16** was obtained by this method in 67% yield. We followed the same procedure starting from the steroid **6** to obtain **19** in 68% yield. Surprisingly, the estradiol **18** could be obtained directly after treatment of the $BF_3 \cdot OEt_2$-activated condensation reaction of tolyllithium on the estrone **2**. The estradiol **13** was successfully deprotected with Bu_4NF in methanol to give **17** in 67% yield.

Biochemical studies

Various biochemical tests were carried out on a series of 17α-4′- or -3′-substituted phenylestradiol derivatives: 1) Measurement of their relative binding affinity (RBA) for the α and β forms of the estrogen receptor (ERα and ERβ), which indicates whether or not these compounds are still recognized by this receptor. These measurements were performed in a competitive radioreceptor binding assay using sheep uterine cytosol as a source of ERα,

purified ERβ purchased from PanVera (USA), and [^{3}H]-estradiol as tracer.[24] 2) Measurement of their lipophilicity (log$P_{o/w}$). This value which is determined by HPLC,[7] is an important indicator of the molecule's ability to penetrate the lipid barrier of the cell. If it is too low the molecule will not be able to enter the cell, but if it is too high, most of the compound will be captured in vivo by the fatty tissues. 3) Study of the proliferative or antiproliferative effects of the compounds on MCF7 cells derived from a hormone-dependent breast cancer cell line. This cell line which contains a high level of estrogen receptors, is considered the standard for estrogen receptor positive (ER(+)) cells. The results obtained are shown in Table 2 and Figures 1 and 2. They should be compared with the values obtained for estradiol (**E_2**), the estrogen of reference.

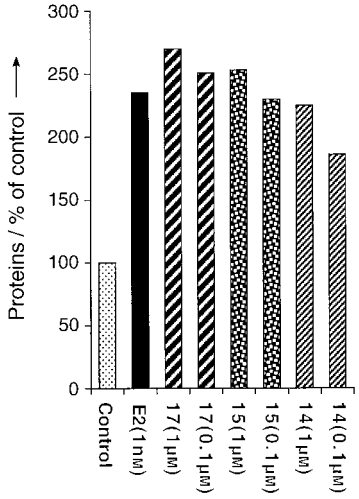


Figure 1. *Effect of estradiol (**E_2**), **17**, **15**, and **14** on the proliferation of MCF7 cells (estrogen receptor-positive breast cancer cells) after six days of culture. The results are from one representative experiment (means of quadruplicate).*

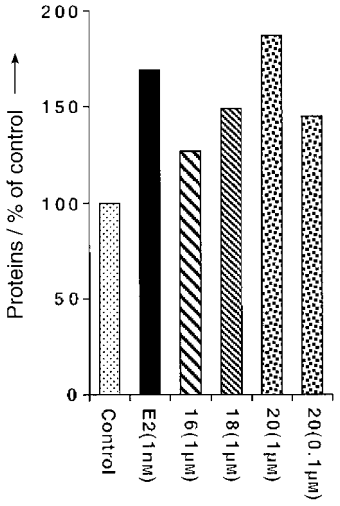
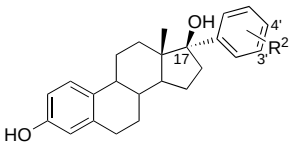


Figure 2. *Effect of estradiol (**E_2**), **16**, **18**, and **20** on the proliferation of MCF7 cells (estrogen receptor-positive breast cancer cells) after six days of culture. The results are the means of two independent experiments.*

Relative binding affinity of the compounds

It is interesting to note that all the compounds synthesized retain affinity for both the α and β form of the estrogen receptor. This affinity is high (around or over 10%) for all the products tested except for **16** (RBA = 3.5). This result confirms the previous observation that the α form of the estrogen receptor possesses a lipophilic pocket that allows it to accommodate fairly bulky substituents at position 17α.[25] This situation seems also to be true for the β form. Still, the lower value found for **16** cannot be ascribed to a steric effect of the substituent. On the other hand, in the series of substituted phenylestradiols, it has the lowest log$P_{o/w}$ value. One may thus suspect that the pocket available to 17α substituents is lipophilic. This view is strength-

Table 2. *RBA values for ERα and ERβ, log$P_{o/w}$ and proliferative effect on hormone-dependent cancer cell line (MCF7) of some 17α-4′- or 17α-3′-substituted phenylestradiol derivatives.*



numbering	R^2	RBA [%] for ERα[a]	RBA [%] for ERβ[b]	Log$P_{o/w}$[c]	proliferative effect of X/E_2 [%][d]
estradiol	–	100[e]	100[e]	3.5	100
14	4′-I	23	nd	5.3	96
15	3′-I	11	14.5	nd	108
16	4′-NH_2	3.5	nd	4.3	75
17	4′-N=N–N(Et)$_2$	11.5	14	5.8	115
18	4′-CH_3	33	16	4.7	88
19	4′-O–CH_3	17	14.5	nd	nd
20	4′-N(CH_3)$_2$	13.5	7.5	4.7	111
21	H	25[f]	nd	nd	nd

[a] RBA measured on lamb uterine cytosol as described in ref. [24] except for **17** and **19** where measurements were performed on purified ERα from PanVera. [b] RBA measured on purified ERβ from PanVera (Michigan, USA). [c] Octanol/water partition coefficients were determined by HPLC as described in ref. [7]. [d] Measured on MCF7 cells in the presence of 1 μM of the compound (X) to be tested or 1 nM of **E_2**. Conditions as described in Figures 1 and 2. [e] Value by definition. [f] Value from ref. [10].

ened by the fact that when the substituent in the 17α position is a cationic arene bearing the organometallic fragment [(Ru^{+}—Cp*)phenyl], the RBA value is zero.[10]

Proliferative effect of the derivatives

The proliferative effect of the compounds on a hormone-dependent breast cancer cell line (MCF7 cell line) was studied at one or two molarities of incubation (0.1 and 1 μM) and compared to that of estradiol (1 nM), the estrogen of reference (Figures 1 and 2). It is interesting to note that all the compounds have a proliferative effect (percentage of protein, number of cells, higher than the control) and all are thus estrogenic. When this value is compared to the effect observed with estradiol (Table 1), three of the compounds, **16**, **17**, and **20**, have at a molarity of 1 μM a greater effect than the standard estradiol (itself at a molarity of 1 nM), and are thus strongly estrogenic. The most effective compound is the triazene derivative **17** which combines a high RBA value with the highest log$P_{o/w}$ value. This result shows that the compound has good stability, as its hydrolysis might have led to the formation of radical species known for their cytotoxic effects.[26] Also, **16** which has the lowest RBA and log$P_{o/w}$ values, has the lowest estrogenic effect.

All the compounds were also tested on MDA-MB231 cells, a cell line derived from a breast cancer that is non-hormone-dependent, that is, without the α form of the estrogen receptor. Our compounds have no effect on this cell line (data not shown). The proliferative effect observed on the MCF7 cells is therefore clearly a hormonal effect mediated by the estrogen receptor.

Molecular modeling

Recent structural determinations have made the first molecular modeling studies on 17α-arylestradiols possible. The structure used is that of the hERα receptor bound to estradiol obtained by Hubbard et al.[27] Only the amino acid molecules that form the wall of the binding cavity have been retained in the model. The estradiol in the cavity was modified successively to model the phenyl bioligands studied. All the heavy (which is, non-hydrogen) atoms of the amino acids of the cavity wall were immobilized. Then the side chains of amino acids His524, Met343, and Met421 were released. This was justified by observations that this part of the cavity is flexible.[28] Energy minimization was then carried out in this configuration by using the Merck molecular force field (MMFF). The ideal positions of the bioligands were calculated in this way. We then determined the affinities of the bioligands for the cavity by using the semi-empirical quantum mechanical method AM1.[29] This involves calculation of the energies of the bioligand cavity, the cavity, and the mediator entities, the last two in the conformations that they had in the full structures. This gives the enthalpy variations $\Delta_r H^\circ$ of the interactions of the bioligands with the cavity. The calculated $\Delta_r H^\circ$ values range from -2.9 to -9.1 kcal mol^{-1} (average $\Delta_r H^\circ = -5.9$ kcal mol^{-1} for an average RBA of around 20%). As discussed above, the $\Delta_r H^\circ$ values calculated clearly show energetically favorable binding, but as before, a precise quantitative correlation with the RBA values was not possible. Obviously not all the factors involved have been taken into account in the calculation. However, the molecular modeling study allows visualization of hormone–receptor binding and appreciation of the conformational changes imposed on the receptor protein by the 17α phenyl substituents.

Figures 3–6 show estradiol, 17α-phenylestradiol (**21**), 17α-tolylestradiol (**18**), and 17α-4′-iodophenylestradiol (**14**) as space-filling models in the estrogen receptor α site (rod model) using the structure obtained for estradiol as a basis.[27]

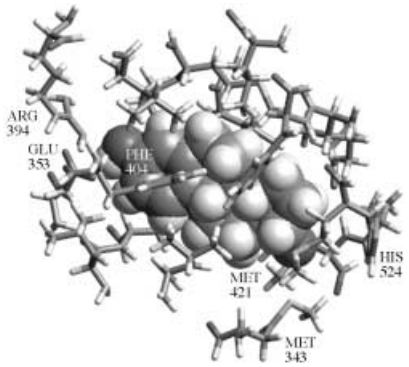


Figure 3. *Estradiol as a space-filling model in the estrogen receptor α site (rod model) using the structure previously described for the agonist binding site as a basis.[27]*

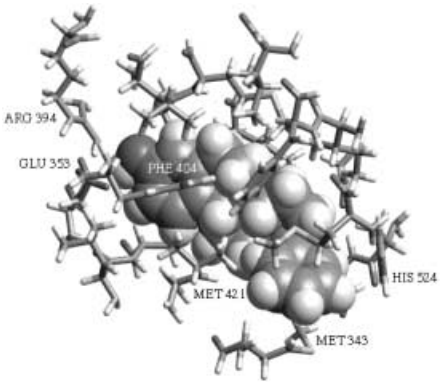


Figure 4. *17α-Phenylestradiol as a space-filling model in the estrogen receptor α site (rod model) using the structure previously described for the agonist binding site as a basis.[27]*

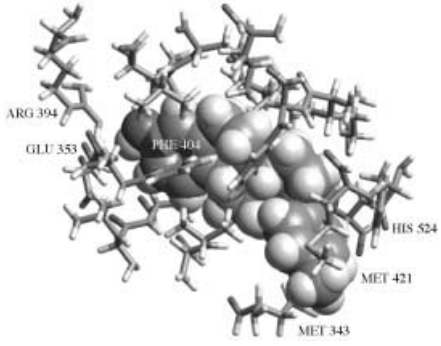


Figure 5. *17α-Tolylestradiol as a space-filling model in the estrogen receptor α site (rod model) using the structure previously described for the agonist binding site as a basis.[27]*

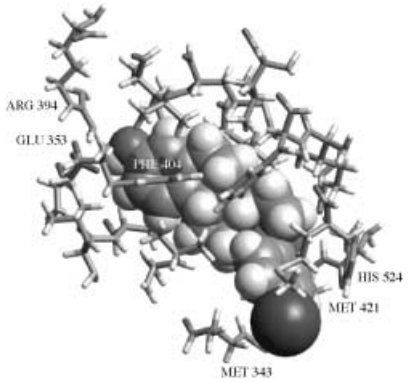


Figure 6. *17α-4′-Iodophenylestradiol as a space-filling model in the estrogen receptor α site (rod model) using the structure previously described for the agonist binding site as a basis.*[27]

Figure 3 is used as the reference. Here we see binding of the phenol group of the estradiol with Glu353 and Arg394, and binding of the 17β hydroxy group with His524. Figure 3 is oriented so that the absence of a clear lipophilic pocket on the 17α side of the estradiol is evident. But this part of the receptor is known to be flexible.[28] Figure 3 shows how a 17α phenyl substituent enters the pocket created by the separation of the two methionines Met421 and Met343. Figures 5 and 6 give more specific views showing the accommodation of the two fairly bulky additional lipophilic methyl and iodo substituents, respectively, by adjustment of the methionine residues. This view would no doubt have been even more precise if other neighboring residues had been freed, but this was beyond our calculation limit. Even at the current level of accuracy, however, it permits rationalization of the results obtained.

The flexible and lipophilic nature of the phenyl pocket provides a good explanation for the global variation of the RBA values depending on the level of lipophilicity of the phenyl substituents. The results as a whole are in agreement with this analysis.

Conclusions

This work shows the viability of preparing estradiol derivatives substituted in position 17α by various functionalized aromatics. This is made possible by efficient activation methods for the addition of phenyllithiums by the use of $BF_3 \cdot OEt_2$ or TMEDA. The reaction occurs in a stereospecific manner, as a result of the control of the angular methyl group in position 13β, and in good to very good yields (57–80%). The functions attached to the arene in this way include $-N(CH_3)_2$, $-NH_2$, $-N{=}N-NEt_2$, and I moieties. The possibility of easily attaching an iodo substituent at the end of the phenyl spacer opens a route to novel pharmaceuticals with applications in imaging, for example, with isotopes ^{125}I (γ emitter) or ^{123}I (usable for SPECT imaging).[2] This goal is all the more realistic, since the 17α-phenylsteroids show very satisfactory recognition for ERα (RBA around 23%). This does not represent an upper limit, since it is easy to increase this value when necessary by the addition of another well-chosen substituent (CH_2Cl, Et) at position 11β.[7]

Studies of the proliferative/antiproliferative effects of these new compounds on hormone-dependent breast cancer cell lines (MCF7 type) show that all the compounds prepared have a marked estrogenic effect comparable to that provided by estradiol. This effect is a little more marked with aryltriazene and 4′-iodophenyl substituents.

Molecular modeling studies, based on structures of the ligand binding domain (LBD) of ERα bound to estradiol, provide a molecular view of the binding factors. The presence of a substituted arene at the 17α position generates a lipophilic pocket around His524, Met343, and Met421. This pocket accommodates the arene well, even when the latter is substituted by a bulky iodine in the *4′* position. This local conformational change, unlike the situation with positions 11β and 7α of estradiol, preserves the agonist properties of the estradiol. This ability to modify the estradiol will be valuable for future applications directed towards retaining agonist effects, especially as potential applications for the chemistry of arenes are many and varied, extending well beyond those explored here.

Experimental Section

CAUTION: Some triazene derivatives are reported to have potent carcinogenic activity.

Chemistry:

General: Solvents were purified by conventional distillation techniques under argon. Anhydrous TMEDA was used as acquired from Aldrich. *tert*-Butyllithium was purchased from Aldrich as a 1.7 M solution in pentane. Other reagents were used as received, unless otherwise noted. Glassware for moisture-sensitive reactions was flame-dried and cooled under vacuum, and reactions were carried out under argon. Analytical thin-layer chromatography (TLC) was performed by using silica gel 60 GF254 and alumina N0.2 mm plates with F-254 indicator (Macherey–Nagel). Visualization was achieved either with a solution of *p*-anisaldehyde in methanol, 50% sulfuric acid solution, or by UV illumination. Flash chromatography was performed following the method of Still[30] on silica gel (Merck Gerudan SI, 40–63 μm). Melting points were determined on either a Kofler apparatus or a Mettler FP61 apparatus and are uncorrected. Nuclear magnetic resonance spectra were obtained on a Bruker AC 200 spectrometer; the solvent was used as an internal standard. EI and DCI mass spectra were obtained on a NERMAG R1010C spectrometer. Elemental analyses were performed by the Microanalytical Service Laboratory at the Université Pierre et Marie Curie (Paris, France). Unless otherwise stated, a general procedure for product isolation was used as follows: The reaction mixture was quenched with water (or aqueous acids or bases as specified) and extracted three times with dichloromethane (or specified solvents). The organic solution was then washed with saturated aqueous $NaHCO_3$, brine (or water), dried over $MgSO_4$, and the solvents were evaporated in vacuo. The starting materials, 4- or 3-bromophenyltriazenes, were prepared according to the published method.[31] The solubility of some estradiols was too weak to allow the acquisition of ^{13}C NMR spectra. For molecular modeling calculations, we used Mac Spartan Pro software (Wavefunction Society, 18401 Von Karman Avenue, Irvine CA92612, USA).

Method A: General procedure for activation with TMEDA or $BF_3 \cdot OEt_2$ in THF: The substituted aryl bromide (10 mmol) was dissolved under argon in anhydrous THF (85 mL) and cooled to −90 °C in an

acetone/N_2 bath. *tert*-Butyllithium (10 mmol) was added dropwise while the temperature was maintained under $-85\,^{\circ}C$, and the resulting mixture was stirred for 3.5 h. TMEDA (30 mmol) or $BF_3 \cdot OEt_2$ (10 mmol) was then added slowly followed by stirring for 5 min. The protected estrones **1** – **3** (3.3 mmol) in THF (85 mL) were added portionwise over 20 min at below $-85\,^{\circ}C$ and the reaction mixture was stirred for an additional 90 min, quenched with water (20 mL) and the THF was evaporated. The product was then extracted with dichloromethane and washed with water, dried over $MgSO_4$, and concentrated to give the crude product. Chromatography over silica gel or neutral alumina gave pure product.

Method B: General procedure for condensation with $BF_3 \cdot OEt_2$ in toluene/ether: The aryllithium (8.8 mmol) in ether (8.5 mL) was cooled to $-90\,^{\circ}C$ in an acetone/N_2 bath. $BF_3 \cdot OEt_2$ (8.8 mmol) was added slowly followed by stirring for 5 min. The protected steroid **1** or **2** (2.8 mmol) in toluene (10 mL) was added portionwise over 20 min while the temperature was maintained between $-80\,^{\circ}C$ and $-85\,^{\circ}C$. The reaction mixture was stirred for an additional 90 min, quenched with water (20 mL), and the solvents were evaporated. The product was extracted with dichloromethane and washed with water, dried over $MgSO_4$, and concentrated to give the crude product. Chromatography on silica gel or neutral alumina gave pure product.

Method C: **General procedure for Sandmeyer-type reactions**: NaI (190 mg, 1.26 mmol) and TMSCl (0.08 mL, 0.63 mmol) were dissolved in acetonitrile (1.5 mL) and stirred under argon at $40\,^{\circ}C$ for 15 min. The reaction medium was allowed to return to room temperature before a suspension of steroids **7**, **10**, or **13** (0.28 mmol) in acetonitrile (9 mL) was added at once. The reaction mixture was stirred for an additional 17 h at room temperature and then hydrolyzed with saturated $NaHCO_3$ (10 mL). The acetonitrile was evaporated and the crude product was extracted with ethyl acetate, washed with water, dried over $MgSO_4$, and concentrated to give the crude product. Flash chromatography gave pure product.

3-Benzyloxyestra-1,3,5(10)-trien-17-one (1): A solution of estrone (4.48 g, 16.5 mmol) and potassium hydroxide (1.46 g, 26 mmol) in dioxane/water (83 mL, 90:10) was stirred at room temperature for 15 min until the potassium hydroxide was dissolved. Benzyl bromide (1.98 mL, 16.6 mmol) was then added, the reaction mixture was stirred at room temperature for 24 h, and the solvent was evaporated. The residues were dissolved in dichloromethane, washed with water, dried over $MgSO_4$, and the solvent was evaporated. The crude solid was crystallized from ether to give **1** as a white solid (4.45 g, 75 %). Melting point (found: 132 °C; lit.: 132 – 134 °C) and other analyses were consistent with the literature.[32]

3-(Tetrahydropyran-2-yloxy)estra-1,3,5(10)-trien-17-one (2): Estrone (4 g, 14.8 mmol), 3,4-dihydropyran (4.6 mL, 76 mmol), *p*-toluenesulfonic acid (34 mg, 176 mmol), and dichloromethane (20 mL) were all mixed together and then warmed until a solution was obtained. The reaction mixture was then allowed to stand at room temperature for 1 h. Pyridine (1 mL) was added, the solution shaken with water (130 mL) containing $NaHCO_3$ (1.2 g), and the dichloromethane extract was washed and dried. After evaporation, the crude solid was recrystallized from methanol to give **2** as a white solid (4.20 g, 80 %). Melting point (found: 144 °C; lit.: 135 – 147 °C) and other analyses were consistent with the literature.[33]

3-(*tert*-Butyldimethylsilanyloxy)estra-1,3,5(10)-trien-17-one (3): A suspension of estrone (1 g, 3,68 mmol) and imidazole (1.46 g, 26 mmol) in DMF (6 mL) was stirred at room temperature and a 1 M solution of TBDMSCl in THF (4 mL) was added dropwise. The mixture was stirred for 15 min and THF (6 mL) was added to dissolve the precipitate. The reaction medium was stirred for an additional 45 min at room temperature and dichloromethane (75 mL) was added. The resulting solution was washed with water, dried over $MgSO_4$, and the solvent was evaporated. Column chromatography on silica gel (dichloromethane) gave **3** (920 mg, 65 %). Melting point (found: 174 °C; lit.: 172 – 174 °C) and other analyses were consistent with the literature.[34]

3-Benzyloxy-17α-(4′-methylphenyl)estra-1,3,5(10)-trien-17β-ol (5): Compound **5** was prepared according to general procedures A and B, and the best result was obtained with the latter. Tolyllithium was prepared in ether from bromotoluene (1.51 g, 8.8 mmol) stirred with lithium (122 mg, 17.6 mmol) for 3 h. The crude product was crystallized in ether to give **5** as white solid in 80 % yield. M.p. 179 °C; ^{1}H NMR ($CDCl_3$): $\delta = 1.09$ (s, 3H, 18-CH_3), 2.37 (s, 3H, CH_3Ph), 5.01 (s, 2H, CH_2Ph), 6.72 (m, 2H, 2-H and 4-H), 7.08 (d, 1H, $J = 8.36$ Hz, 1-H), 7.16 (d, 2H, $J = 7.88$ Hz, 3′-H and 5′-H), 7.28 – 7.45 (m, 5H), 7.39 ppm (d, 2H, $J = 7.88$ Hz); ^{13}C NMR ($CDCl_3$): $\delta = 14.70$, 20.93, 24.03, 26.24, 27.35, 29.78, 33.54, 38.59, 39.45, 43.25, 46.82, 58.06, 69.90, 85.81, 112.12, 114.70, 126.17, 127.24, 127.39, 127.77, 128.00, 128.47, 132.99, 136.37, 137.31, 137.89, 142.95, 156.61 ppm; MS (DCI, NH_3): *m/z* (%): 470 (10) $[M+NH_4]^+$, 452 (12) $[M+NH_4-H_2O]^+$, 435 (100) $[M+H-H_2O]^+$; elemental analysis calcd (%) for $C_{32}H_{36}O_2 \cdot 0.5\,H_2O$: C 83.26, H 8.08; found: C 83.73, H 8.00.

3-Benzyloxy-17α-(4′-methoxyphenyl)estra-1,3,5(10)-trien-17β-ol (6): Compound **6** was prepared according to general procedure B. Anisyllithium was prepared in ether from 4-iodoanisole (2.05 g, 8.8 mmol) stirred at $-65\,^{\circ}C$ with butyllithium (8.8 mmol) for 2 h. The crude product was recrystallized in methanol followed by crystallization in ether to give **6** as white solid in 77 % yield. M.p. 188 °C; ^{1}H NMR ($CDCl_3$): $\delta = 1.08$ (s, 3H, 18-CH_3), 3.83 (s, 3H, CH_3OPh), 5.02 (s, 2H, CH_2Ph), 6.72 (m, 2H, 2-H and 4-H), 6.89 (d, 1H, $J = 8.83$ Hz, 3′-H and 5′-H), 7.09 (d, 2H, $J = 8.31$ Hz, 1-H), 7.33 ppm (d, 2H, $J = 8.83$ Hz, 2′-H and 6′-H); ^{13}C NMR ($CDCl_3$): $\delta = 14.62$, 23.91, 26.18, 27.30, 29.72, 33.44, 39.40, 43.23, 46.80, 48.00, 55.13, 69.84, 85.58, 112.07, 112.54, 126.12, 127.33, 127.71, 128.40, 137.82 ppm; MS (DCI, NH_3): *m/z* (%): 468 (10) $[M+NH_4-H_2O]^+$, 451 (100) $[M+H-H_2O]^+$; elemental analysis calcd (%) for $C_{32}H_{36}O_3$: C 82.01, H 7.74; found: C 81.83, H 7.70.

3-Benzyloxy-17α-[4′-(pyrrolidin-1-ylazo)phenyl]estra-1,3,5(10)-trien-17β-ol (7): Compound **7** was prepared from compound **1** according to general procedure A with TMEDA. Chromatography on neutral alumina with dichloromethane as eluent gave product **7** as a white solid in 60 % yield. M.p. 134 °C; ^{1}H NMR ($CDCl_3$): $\delta = 1.08$ (s, 3H, 18-CH_3), 2.00 (m, 4H, pyrrolidine CH_2CH_2), 3.80 (br s, 4H, CH_2N), 5.02 (s, 2H, CH_2Ph), 6.72 (m, 2H, 2-H and 4-H), 7.06 (d, 1H, $J = 8.22$ Hz, 1-H), 7.40 ppm (m, 9H, H arom); ^{13}C NMR ($CDCl_3$): $\delta = 14.66$, 23.73, 24.01, 26.22, 27.33, 29.74, 33.45, 38.41, 39.42, 43.21, 46.94, 47.96, 69.85, 85.80, 112.07, 114.65, 119.07, 126.16, 127.35, 127.82, 128.43, 133.02, 137.20, 137.85, 142.58, 150.16, 156.56 ppm; MS (DCI, NH_3): *m/z* (%): 536 (100) $[M+H]^+$; elemental analysis calcd (%) for $C_{35}H_{41}N_3O_2 \cdot 0.5\,H_2O$: C 77.17, H 7.77, N 7.71; found: C 77.38, H 7.81, N 7.61.

3-(Tetrahydropyran-2-yloxy)-17α-(4′-methylphenyl)estra-1,3,5(10)-trien-17β-ol (8): Compound **8** was prepared according to general procedure A with TMEDA or $BF_3 \cdot OEt_2$. Chromatography on neutral alumina with dichloromethane as eluent followed by crystallization in ether gave **8** as a white solid in 77 % yield. ^{1}H NMR ($CDCl_3$): $\delta = 1.08$ (s, 3H, 18-CH_3), 2.37 (s, 3H, CH_3Ph), 3.49 – 4.05 (m, 4H), 5.35 (m, 1H, OCHO), 6.79 (m, 2H, 2-H and 4-H), 7.07 (d, 1H, $J =$ 7.86 Hz, 1-H), 7.16 (d, 2H, $J = 8.36$ Hz, 3′-H and 5′-H), 7.30 ppm (d, 2H, $J = 8.36$ Hz, 2′-H and 6′-H); ^{13}C NMR ($CDCl_3$): $\delta = 13.76$, 14.66, 18.69, 20.90, 21.49, 23.97, 25.18, 25.77, 26.13, 26.46, 27.31, 29.53, 29.69, 30.32, 31.49, 33.48, 35.79, 38.21, 38.53, 39.33, 43.23, 46.75, 47.99, 50.33, 85.75, 96.24, 113.75, 113.98, 116.22, 116.38, 126.04, 127.20, 127.95, 132.88, 133.59, 136.31, 137.73, 142.91, 154.71 ppm; MS (DCI, NH_3):

m/z (%): 464 (5) $[M+NH_4]^+$, 447 (5) $[M+H]^+$, 429 (10) $[M+H-H_2O]^+$; elemental analysis calcd (%) for $C_{30}H_{38}O_3 \cdot H_2O$: C 77.55, H 8.68; found: C 77.86, H 8.60.

3-(Tetrahydropyran-2-yloxy)-17α-[4′-(pyrrolidin-1-y1 azo)phenyl]-estra-1,3,5(10)-trien-17β-ol (9): Compound **9** was prepared from estrone **2** according to general procedure A with TMEDA. Chromatography on neutral alumina with dichloromethane as eluent gave compound **9** as a white solid in 57% yield. M.p. 118 °C; ^{1}H NMR ($CDCl_3$): $\delta = 1.08$ (s, 3H, 18-CH_3), 2.03 (m, 4H, pyrrolidine CH_2CH_2), 3.80 (br s, 4H, CH_2N), 3.45 – 4.05 (m, 4H), 5.37 (s, 1H, OCHO), 6.79 (m, 2H, 2-H and 4-H), 7.08 (d, 1H, $J = 8.30$ Hz, 1-H), 7.38 ppm (s, 4H, H arom); ^{13}C NMR ($CDCl_3$): $\delta = 14.64$, 18.69, 23.72, 23.99. 25.18, 26.15, 27.32, 29.68, 30.33, 38.40, 39.35, 43.23, 46.92, 47.96, 61.83, 65.79, 95.26, 113.75, 116.25, 119.04, 126.04, 127.81, 133.68, 137.73, 142.58, 150.13, 154.72 ppm; MS (DCI, NH_3): *m/z* (%): 530 (100) $[M+H]^+$; elemental analysis calcd (%) for $C_{33}H_{43}N_3O_3 \cdot 0.5\,H_2O$; C 73.57, H 8.23, N 7.80; found: C 73.85, H 8.48, N 7.61.

3-(Tetrahydropyran-2-yloxy)-17α-(4′-methoxyphenyl)estra-1,3,5(10)-trien-17β-ol (10): Compound **10** was prepared from 4-bromoanisole according to general procedure A, with TMEDA or $BF_3 \cdot OEt_2$. Chromatography on silica gel with toluene/ethyl acetate (9:1) gave **10** as a white solid in 62% yield. ^{1}H NMR ($CDCl_3$): $\delta = 1.08$ (s, 3H, 18-CH_3), 3.83 (s, 3H, CH_3OPh), 3.50 – 4.95 (m, 4H), 5.37 (m, 1H, OCHO), 6.79 (m, 2H, 2-H and 4-H), 6.89 (d, 2H, $J = 8.83$ Hz, 3′-H and 5′-H), 7.08 (d, 1H, $J = 8.13$ Hz, 1-H), 7.33 ppm (d, 2H, $J = 8.86$ Hz, 2′-H and 6′-H); ^{13}C NMR ($CDCl_3$): $\delta = 14.62$, 18.69, 25.16, 26.13, 30.33, 33.44, 38.56, 39.34, 43.27, 46.78, 48.01, 55.12, 61.85, 85.59, 112.54, 126.03, 128.38, 137.95, 154.75 ppm; MS (DCI, NH_3): *m/z* (%): 445 (15) $[M+H-H_2O]^+$; elemental analysis calcd (%) for $C_{30}H_{38}O_4$: C 77.89, H 8.28; found: C 77.70, H 8.40.

3-(Tetrahydropyran-2-yloxy)-17α-[4′-(*N*,*N*-dimethyl)phenyl]estra-1,3,5(10)-trien-17β-ol (11): Compound **11** was prepared from 4-bromo-*N*,*N*-dimethylaniline and estrone **2** according to general procedure A with TMEDA. Chromatography on neutral alumina with dichloromethane as eluent followed by crystallization in ether gave **11** as a white solid in 58% yield. M.p. 208 °C; ^{1}H NMR ($CDCl_3$) 1.07 (s, 3H, 18-CH_3), 2.97 (m, 6H, CH_3NCH_3), 3.55 – 3.63 (m, 2H), 3.85 – 3.95 (m, 2H), 5.37 (s, 1H, OCHO), 6.72 (d, 2H, $J = 8.94$ Hz, 3′-H and 5′-H), 6.80 (m, 2H, 2-H and 4-H), 7.09 (d, 1H, $J = 8.26$ Hz, 1-H), 7.27 (d, 2H, $J = 8.94$ Hz, 2′-H and 6′-H), 7.46 ppm (s, 1H); ^{13}C NMR ($CDCl_3$): $\delta =$ 14.64, 18.70, 23.90, 25.17, 26.16, 27.29, 29.69, 30.32, 33.45, 38.37, 39.33, 40.45, 43.22, 45.69, 46.80, 48.00, 61.84, 85.51, 96.27, 111.26, 113.74, 126.03, 128.02, 133.74, 137.75, 149.28, 154.71 ppm; MS (DCI, NH_3): *m/z* (%): 476 (100) $[M+H]^+$, 458 (40) $[M+H-H_2O]^+$; elemental analysis calcd (%) for $C_3H_4NO_3 \cdot 0.5\,H_2O$; C 76.82, H 8.73, N 2.89; found: C 77.08, H 8.80, N 2.70.

3-(Tetrahydropyran-2-yloxy)-17α-[3′-(pyrrolidin-1-ylazo)phenyl]-estra-1,3,5(10)-trien-17β-ol (12): Compound **12** was prepared from estrone **2** according to general procedure A with TMEDA. Chromatography on neutral alumina with dichloromethane as eluent gave **12** as a yellow solid in 58% yield. M.p. 210 °C; ^{1}H NMR ($CDCl_3$): $\delta =$ 1.09 (s, 3H, 18-CH_3), 2.03 (m, 4H, pyrrolidine CH_2CH_2), 3.81 (br s, 4H, CH_2N), 3.40 – 4.00 (m, 4H), 5.36 (s, 1H, OCHO), 6.76 (m, 2H, 2-H and 4-H), 7.07 (d, 1H, $J = 7.87$ Hz, 1-H), 7.20 (m, 1H), 7.26 – 7.32 (m, 4H, H arom), 7.46 ppm (s, 1H); ^{13}C NMR ($CDCl_3$): $\delta = 14.68$, 18.70, 23.73, 24.03, 25.17, 26.15, 27.27, 29.69, 30.33, 33.58, 38.46, 39.37, 43.16, 46.88, 47.98, 61.84, 86.03, 96.27, 113.74, 116.37, 118.18, 120.11, 124.26, 126.04, 127.64, 133.71, 137.74, 146.75, 150.63, 154.70 ppm; MS (DCI, NH_3): *m/z* (%): 530 (100) $[M+H]^+$; elemental analysis calcd (%) for $C_{33}H_{43}N_3O_3$: C 74.82, H 8.18, N 7.93; found: C 74.88, H 8.26, N 7.98.

3-(*tert*-Butyldimethylsilanyloxy)-17α-[4′-(3,3-diethyltriazeno)phenyl]estra-1,3,5(10)-trien-17β-ol (13): Compound **13** was prepared from estrone **3** according to general procedure A with TMEDA. Chromatography on neutral alumina with dichloromethane as eluent gave **13** as a yellow solid in 58% yield. ^{1}H NMR ($CDCl_3$) $\delta = 0.17$ (s, 6H, $Si(CH_3)_2$), 0.96 (m, 9H, $SiC(CH_3)_3$), 1.09 (s, 3H, 18-CH_3), 1.29 (t, 6H, $J =$ 7.14 Hz, NCH_2CH_3), 3.79 (q, 4H, $J = 7.12$ Hz, CH_2N), 6.57 (m, 2H, 2-H and 4-H), 7.01 (d, 1H, $J = 7.87$ Hz, 1-H), 7.20 (m, 1H), 7.39 ppm (m, 4H, H arom); ^{13}C NMR ($CDCl_3$): $\delta = 14.66$, 18.04, 23.99, 25.59, 26.15, 27.32, 29.53, 33.46, 39.37, 43.19, 46.93, 48.00, 85.82, 116.9, 119.08, 119.69, 125.91, 127.75, 133.12, 137.65, 142.52, 153.09 ppm; MS (DCI, NH_3): *m/z* (%): 562 (15) $[M+H]^+$, 183 (100); elemental analysis calcd (%) for $C_{34}H_{51}N_3O_2Si \cdot 0.33\,H_2O$; C 71.91, H 9.17, N 7.40; found: C 72.06, H 8.97, N 7.22.

17α-(4′-Iodophenyl)estra-1,3,5(10)-trien-3,17β-diol (14): Estradiol **14** was prepared from compound **9** according to general procedure C. Chromatography on silica gel with toluene/ethyl acetate (4:1) as eluent and crystallization in ether/pentane (1:9) gave **14** as a white solid in 90% yield (with low solubility). M.p. > 300 °C; ^{1}H NMR ([D_6]DMSO): $\delta = 0.95$ (s, 3H, 18-CH_3), 5.17 (s, 1H), 6.40 (m, 2H, 2-H and 4-H), 6.89 (d, 1H, $J = 8.65$ Hz, 1-H), 7.15 (d, 2H, $J = 8.40$ Hz, 2′-H and 6′-H), 7.65 ppm (d, 2H, $J = 8.40$ Hz, 3′-H and 5′-H); MS (EI, 70 eV): *m/z* (%): 474 (20) $[M]^+$, 456 (5) $[M-H_2O]^+$, 348 (10) $[M-I]^+$; elemental analysis calcd (%) for $C_{24}H_{27}IO_2 \cdot 0.5\,H_2O$: C 59.63, H 5.84; found: C 59.43, H 6.24.

17α-(3′-Iodophenyl)estra-1,3,5(10)-trien-3,17β-diol (15): Estradiol **15** was prepared from **12** according to general procedure C. Chromatography on silica gel with cyclohexane/ethyl acetate (4:1) as eluent and crystallization in chloroform/pentane (1:9) gave **15** in 80% yield. M.p. > 300 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): 1.07 (s, 3H, 18-CH_3), 4.89 (s, 1H), 6.52 – 6.58 (m, 2H, 2-H and 4-H), 7.02 (d, 1H, $J =$ 8.29 Hz, 1-H), 7.08 (t, 1H, $J = 7.83$ Hz, 5′-H), 7.33 (d, 1H, $J = 7.83$ Hz, 6′-H), 7.60 (d, 1H, $J = 10.36$ Hz, 4′-H), 7.80 ppm (s, 1H, 2′-H); MS (EI, 70 eV): *m/z* (%): 457 $[M+H-H_2O]^+$, 402 (10), 340 (20), 268 (65), 251 (35), 122 (75), 104 (100); elemental analysis calcd (%) for $C_{24}H_{27}IO_2 \cdot CHCl_3$: C 50.63, H 4.75; found: C 51.00, H 4.94.

17α-(4′-Aminophenyl)estra-1,3,5(10)-trien-3,17β-diol (16): Steroid **7** (130 mg, 0.24 mmol) was dissolved in a mixture of methanol (10 mL) and THF (3 mL). Pd/C (130 mg, 10%) was added and the medium was hydrogenated under atmospheric pressure for 21 h. The solvents were filtered, evaporated, and the residues were crystallized from a THF/pentane mixture (1:9) to give **16** as a white solid in 67% yield (with low solubility in various solvents). M.p. 220 °C; ^{1}H NMR (CD_3OD): $\delta = 1.05$ (s, 3H 18-CH_3), 6.44 (m, 2H, 2-H and 4-H), 6.70 (d, 2H, $J = 8.59$ Hz, 3′-H and 5′-H), 7.00 (d, 1H, $J = 8.65$ Hz, 1-H), 7.14 ppm (d, 2H, $J = 8.59$ Hz, 2′-H and 6′-H); MS (DCI, NH_3): *m/z* (%): 346 (100) $[M-H_2O+H]^+$; MS (EI, 70 eV): *m/z*: 363 $[M]^+$, 345 $[M-H_2O]^+$; elemental analysis calcd (%) for $C_{24}H_{29}NO_2 \cdot H_2O$: C 75.56, H 8.19, N 3.67; found: C 75.63, H 8.44, N 3.88.

17α-[4′-(3,3-Diethyltriazeno)phenyl]estra-1,3,5(10)-trien-3,17β-diol (17): A solution of tetrabutylammonium fluoride in THF (1 M, 0.18 mL) was added to a solution of steroid **13** (68 mg, 0.12 mmol) in THF (4 mL). The reaction mixture was stirred for 4.5 h, and a saturated solution of $NaHCO_3$ was then added. Ether was added and the organic layer was washed with water. The crude product was purified by silica gel column chromatography with toluene/ethyl acetate (4:96) as eluent and crystallized in ether/pentane (1:9) to give **17** as a white solid (32 mg, 76%). ^{1}H NMR ($CDCl_3$): $\delta = 1.08$ (s, 3H, 18-CH_3), 1.27 (t, 6H, $J = 7.10$ Hz, NCH_2CH_3), 3.77 (q, 4H, $J = 7.14$ Hz, CH_2N), 6.54 (m, 2H, 2-H and 4-H), 7.01 (d, 1H, $J = 8.13$ Hz, 1-H), 7.38 ppm (m, 4H, H arom); MS (DCI, NH_3): *m/z* (%): 448 (100) $[M+H]^+$; elemental analysis calcd (%) for $C_{28}H_{37}N_3O_2 \cdot (C_2H_5)_2O$: C 73.67, H 9.08, N 8.05; found: C 73.70, H 8.62, N 8.40.

17α-(4′-Methylphenyl)estra-1,3,5(10)-trien-3,17β-diol (18): Estradiol **18** was prepared from **8** according to general procedure C. Chromatography on silica gel with toluene/ethyl acetate (9:1) as eluent and crystallization from ether gave **18** as a white solid in 67% yield. M.p. 211 °C; ^{1}H NMR ($CDCl_3$): $\delta = 1.09$ (s, 3H, 18-CH_3), 2.36 (s, 3H, CH_3Ph), 4.45 (s, 1H), 6.56 (m, 2H, 2-H and 4-H), 7.03 (d, 1H, $J =$ 8.02 Hz, 1-H), 7.16 (d, 2H, $J = 8.13$ Hz, 3′-H and 5′-H), 7.30 ppm (d, 2H, $J = 8.13$ Hz, 2′-H and 6′-H); ^{13}C NMR ($CDCl_3$): $\delta = 15.18$, 15.44, 20.84, 24.07, 26.30, 27.44, 29.47, 33.54, 37.92, 38.55, 38.97, 39.38, 39.80, 40.22, 40.64, 41.05, 43.42, 46.73, 47.90, 84.49, 112.90, 115.12, 126.20, 127.65, 130.68, 135.13, 137.36, 144.60, 155.14 ppm; MS (EI, 70 eV): *m/z* (%): 380 (5) $[M+NH_4]^+$, 362 (10) $[M+NH_4-H_2O]^+$, 345 (100) $[M+H-H_2O]^+$; elemental analysis calcd (%) for $C_{25}H_{30}O_2$: C 82.83, H 8.34; found: C 82.51, H 8.52.

17α-(4′-Methoxyphenyl)estra-1,3,5(10)-trien-3,17β-diol (19): A mixture of **6** and estrone **1** (155 mg), resulting from preparation of **6**, was dissolved in methanol (15 mL) and THF (4.5 mL) containing Pd/C 10% (25 mg) in suspension. The reaction mixture was stirred under atmospheric pressure of hydrogen for 17 h. After filtration and evaporation of the solvents, the residues were crystallized from dichloromethane to give **19** as a white solid in 68% yield (with low solubility in various solvents). ^{1}H NMR ([D_6]DMSO): $\delta = 0.95$ (s, 3H, 18-CH_3), 3.73 (s, 3H, OCH_3), 4.97 (s, 1H), 6.43 (m, 2H, 2-H and 4-H), 6.86 (d, 2H, $J = 8.76$ Hz, 3′-H and 5′-H), 6.92 (d, 1H, $J = 8.42$ Hz, 1-H), 7.25 ppm (d, 2H, $J = 8.76$ Hz, 2′-H and 6′-H); MS (DCI, NH_3): *m/z* (%): 377 (5) $[M]^+$, 361 (100) $[M+H-H_2O]^+$; elemental analysis calcd (%) for $C_{25}H_{30}O_3 \cdot 0.75\,H_2O$: C 76.60, H 8.10; found: C 76.64, H 7.86.

17α-[4-(*N*,*N*-Dimethyl)phenyl]estra-1,3,5(10)-trien-3,17β-diol (20): Compound **11** was deprotected by using the procedure described in method C in only 1 h, followed by chromatography on silica gel with toluene/ethyl acetate (85:15) and crystallization in toluene to give **20** in 90% yield. M.p. 191 °C; ^{1}H NMR ([D_6]DMSO): $\delta = 0.94$ (s, 3H, 18-CH_3), 2.86 (s, 6H, CH_3NCH_3), 4.82 (s, 1H), 6.39 (m, 2H, 2-H and 4-H), 6.66 (d, 2H, $J = 8.73$ Hz, 3′-H and 5′-H), 6.92 (d, 1H, $J = 8.36$ Hz, 1-H), 7.15 ppm (d, 2H, $J = 8.73$ Hz, 2′-H and 6′-H); MS (DCI, NH_3): *m/z* (%): 392 (100) $[M+H]^+$, 374 (45) $[M-H_2O+H]^+$; elemental analysis calcd (%) for $C_{26}H_{33}NO_2 \cdot H_2O$: C 76.25, H 8.61, N 3.42; found: C 76.60, H 8.41, N 3.13.

Biochemical experiments:

Materials: Dulbecco's modified eagle medium (DMEM) was purchased from Gibco BRL; fetal calf serum from Dutscher, Brumath, France; glutamine, estradiol, and protamine sulfate from Sigma. MCF7 cells were obtained from the Human Tumor Cell Bank. Stock solutions (1×10^{-3} M) of the compounds to be tested were prepared in ethanol and were kept at −20 °C in the dark; under these conditions they are stable for at least two months. Serial dilutions in ethanol were prepared just prior to use.

Determination of the relative binding affinity (RBA) of the compounds for the α and β forms of the estrogen receptor: Lamb uterine cytosol prepared as described in the literature[24] was used as a source of ERα. ERβ produced in a baculovirus-mediated expression system was purchased from PanVera (Madison, Wisconsin, USA). Typically 10 μL of ERβ (3500 pmol mL^{-1}) was added in a silanised flask to 16 mL of the recommended buffer. Aliquots (200 μL) of ERα (in glass tubes) or ERβ (in propylene tubes) were incubated for 3 h at 0 °C with 2×10^{-9} M of [6,7-^{3}H]-estradiol (specific activity 1.96 TBq mmol^{-1}) in the presence of the hormones to be tested in nine different concentrations. At the end of the incubation period, the free and bound fractions of the tracer were separated by protamine sulfate precipitation. The percentage reduction in binding of [^{3}H]-estradiol (Y) was calculated by using the logit transformation of Y ($\text{logit}Y = \ln[Y/1-Y]$) versus the logarithm of the mass of the competing steroid. The concentration of unlabeled steroid required to displace 50% of the bound [^{3}H]-estradiol was calculated for each steroid tested, and the results expressed as RBA. The RBA value of estradiol is by definition equal to 100%.

Culture conditions: Cells were maintained in a monolayer in DMEM with phenol red (Gibco BRL) supplemented with 8–9% fetal calf serum and glutamine (2 mM) at 37 °C in a 5% CO_2/air-humidified incubator. For proliferation assays, cells were plated in 1 mL of DMEM with phenol red, supplemented with 10% decomplemented and hormone-depleted fetal calf serum and 2 mM glutamine and incubated. The following day (D0), 1 mL of the same medium containing the compounds to be tested was added to the plates (final volumes of alcohol: 0.1%; 4 wells for each conditions, one plate per day). After 3 days (D3) the incubation medium was removed and fresh medium containing the compounds was added. After 6 days (D6) the total protein content of the plate was analyzed by methylene blue staining as follows: cell monolayers were fixed for 1 h in methanol, stained for 1 h with methylene blue (1 mg mL^{-1}) in phosphate-buffered saline, then washed thoroughly with water. HCl (0.1 M, 1 mL) was then added and the absorbance of each well was measured at 620 nm with a Biorad spectrophotometer. The results are expressed as the percentage of proteins versus the control.

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[1] a) A. C. Pike, A. M. Brzozowski, J. Walton, R. E. Hubbard, A. G. Thorsell, Y. L. Li, J. A. Gustafsson, M. Carlquist, *Structure* **2001**, *9*, 145–153; b) A. K. Shiau, D. Barstad, P. M. Loria, L. Cheng, P. J. Kushner, D. A. Agard, G. L. Greene, *Cell* **1998**, *95*, 927–937; c) D. M. Tanenbaum, Y. Wang, S. P. Williams, P. B. Sigler, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 5998–6003.
[2] W. J. Welch, J. B. Dowver, J. A. Katzenellenbogen, J. A. in *Current Directions in Radiopharmaceutical Research and Development* (Ed.: S. J. Mather) **1996**, Kluwer, Dordrecht, pp. 137–156.
[3] D. C. Labaree, T. Y. Reynolds, R. B. Hochberg, *J. Med. Chem.* **2001**, *44*, 1802–1814 and references cited therein.
[4] A. H. Charpentier, A. K. Bedmarek, R. L. Daniel, K. A. Hawkins, K. J. Laflin, S. Gaddis, M. C. MacLeod, C. M. Aldaz, *Cancer Res.* **2000**, *60*, 5977–5983.
[5] H. Ali, J. Rousseau, M. A. Ghaffari, E. Van Lier, *J. Med. Chem.* **1991**, *34*, 854–860.
[6] D. L. Garrouj, S. Aliau, A. Aumelas, J. L. Borgna, *J. Med. Chem.* **1995**, *38*, 2339–2348.
[7] S. Top, H. El Hafa, A. Vessières, J. Quivy, J. Vaissermann, D. W. Hughes, M. J. McGlinchey, J. P. Mornon, E. Thoreau, G. Jaouen, *J. Am. Chem. Soc.* **1995**, *117*, 8372–8380.
[8] S. Top, H. El Hafa, A. Vessières, M. Huché, J. Vaissermann, G. Jaouen, *Chem. Eur. J.* **2002**, *8*, 5241–5249.
[9] M. Salman, B. R. Reddy, P. Delgado, P. L. Stotter, L. C. Fulcher, G. C. Chammess, *Steroids* **1991**, *56*, 375–387.
[10] H. El Amouri, A. Vessieres, D. Vichard, S. Top, M. Gruselle, G. Jaouen, *J. Med. Chem.* **1992**, *35*, 3130–3135.
[11] E. Stephan, T. Affergan, P. Weber, G. Jaouen, *Tetrahedron Lett.* **1998**, *39*, 9427–9430.
[12] N. Foy, E. Stephan, G. Jaouen, *Tetrahedron Lett.* **2000**, *41*, 8089–8092.
[13] T. Imamoto, N. Takiyama, K. Nakamura, T. Hatajima, Y. Kamiya, *J. Am. Chem. Soc.* **1989**, *111*, 4392–4398.
[14] X. Li, S. M. Singh, F. Labrie, *Tetrahedron Lett.* **1994**, *35*, 1157–1160.
[15] M. T. Reetz, R. Steinbach, J. Westermann, R. Peter, *Angew. Chem.* **1980**, *92*, 1044–1045; *Angew. Chem. Int. Ed. Engl.* **1980**, *19*, 1011–1012.
[16] M. T. Reetz, J. Westermann, R. Steinbach, B. Wenderoth, R. Peter, R. Ostarek, S. Maus, *Chem. Ber.* **1985**, *118*, 1421–1440.
[17] E. C. Ashby, S. R. Noding, *J. Org. Chem.* **1979**, *44*, 4371–4377.
[18] a) M. Yamaguchi, Y. Nobayashi, I. Hirao, *Tetrahedron* **1984**, *40*, 4261–4266; b) H. C. Brown, U. S. Racherla, S. M. Singh, *Tetrahedron Lett.* **1984**, *25*, 2411–2414; c) Y. Yamamoto, *Angew. Chem.* **1986**, *98*, 945–957; *Angew. Chem. Int. Ed.* **1986**, *25*, 947–1038.

[19] H. J. Reich, D. P. Green, M. A. Medina, W. S. Goldenberg, B. Ö. Gudmundsson, R. Dykstra, N. H. Phillips, *J. Am. Chem. Soc.* **1998**, *120*, 7201 – 7210.
[20] J. R. Barrio, N. Satyamurthy, H. Ku, M. E. Phelps, *J. Chem. Soc. Chem. Commun.* **1983**, 443 – 444.
[21] H. Ku, J. R. Barrio, *J. Org. Chem.* **1981**, *46*, 5239 – 5241.
[22] N. Foy, E. Stephan, G. Jaouen, *J. Chem. Res. Synop.* **2001**, *12*, 518 – 519.
[23] G. Lunn, E. B. Sansone, L. K. Keefer, *Synthesis* **1985**, 1104 – 1108.
[24] A. Vessières, S. Top, A. A. Ismail, I. S. Butler, M. Louer, G. Jaouen, *Biochemistry* **1988**, *27*, 6659 – 6666.
[25] A. Vessières, G. Jaouen, M. Gruselle, J. L. Rossignol, M. Savignac, S. Top, S. Greenfield, *J. Steroid Biochem.* **1988**, *30*, 301 – 305.
[26] R. Preussmann, A. Hodenberg, *Biochem. Pharmacol.* **1970**, *19*, 1505 – 1508; M. B. Kröger-Köpke, R. H. Smith, E. A. Goodnow, J. Brashars, *Chem. Res. Toxicol.* **1991**, *4*, 334 – 340.
[27] a) A. M. Brozozowski, A. Pike, Z. Dauter, R. E. Hubbard, T. Bonn, O. Engström, L. Ohman, G. L. Greene, J. Ä. Gustafsson, M. Carlquist, *Nature* **1997**, *389*, 753 – 758; b) A. C. Pike, A. M. Brozozowski, R. E. Hubbard, T. Bonn, A. G. Thorsell, O. Engström, J. Ljungren, J. Ä. Gustafsson, M. Carlquist, *EMBO J.* **1999**, *18*, 4608 – 4618.
[28] R. N. Hanson, E. Napolitano, R. Fiaschi, *J. Med. Chem.* **1998**, *41*, 4686 – 4692.
[29] a) M. J. Dewar, E. G. Zoebish, E. F. Healy, J. J. Stewart, *J. Am. Chem. Soc.* **1985**, *107*, 3902 – 3909; b) M. J. Dewar, E. G. Zoebish, *THEOCHEM* **1988**, *49*, 1 – 21.
[30] W. C. Still, M. Khan, A. Mitra, *J. Org. Chem.* **1978**, *43*, 2923 – 2925.
[31] M. L. Gross, D. H. Blank, W. M. Welch, *J. Org. Chem.* **1993**, *58*, 2104 – 2109.
[32] P. C. Bulman Page, J. P. G. Moore, I. Mansfield, M. J. McKenzie, W. B. Bowler, J. A. Gallagher, *Tetrahedron* **2001**, *57*, 1837 – 1848.
[33] H. F. VanBrocklin, K. E. Carlson, J. A. Katzenellenbogen, M. J. Welch, *J. Med. Chem.* **1993**, *36*, 1619 – 1629.
[34] T. Arunachalam, E. Caspi, *J. Org. Chem.* **1981**, *46*, 3415 – 3420.

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