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Synthesis, Binding Affinity, and Transcriptional Activity of Hydroxy- and Methoxy-Substituted 3,4-Diarylsalicylaldoximes on Estrogen Receptors α and β

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Abstract—An effective, unprecedented replacement of the prototypical phenolic 'A-ring' of estrogens with an oxime and a hydroxy-moiety of the salicylaldoxime derivative 3,4-diphenyl-substituted (**1a**) opened the way to study structure–activity relationships of a new class of estrogen receptor (ER)-ligands. Herein, we present a study of the ER binding properties and transcriptional activities of analogues of 3,4-diphenylsalicylaldoxime (**1a**). The introduction of *p*-OH and *p*-OMe groups on the phenyl substituents of **1a**, as in compounds **1b**–**g**, results in unique structure–activity profiles. The preparation of the hetero-disubstituted compounds (**1b**–**e**) was accomplished by a sequential introduction of different 3- and 4-aryl groups, obtained by exploiting the different reactivity of the bromine versus chlorine substituents on the precursor, 2-bromo-3-chloronitrobenzene (**5**), in the palladium-catalyzed cross-coupling reactions. The results of the biological tests show that the introduction of one hydroxy-group on the 3-phenyl substituent of the lead compound **1a** improved the binding affinity on ERβ (**1c**), whereas the introduction of the same group on the 4-phenyl substituent of **1a** gave a compound (**1e**) with better affinity properties on ERα. The introduction of two hydroxyl groups in the *para*-position of both phenyl substituents of **1a**, as in **1g**, lowered the binding on both receptor subtypes. In transcription assays, the ERα agonist character of this class of ligands is enhanced by the presence of a *p*-hydroxy or *p*-methoxy in the 'distal' phenyl ring, whereas substitution on the other phenyl ring does not substantially modify the partial agonist character of **1a**. Thus, results from the binding and transcription assays illustrate that this class of ER ligands has a distinct structure–activity profile on the two ER subtypes, being potent nearly full agonists on ERα and weak, partial antagonists on ERβ.

Introduction

Estrogen receptor (ER), a member of the family of nuclear receptors, is an important pharmaceutical target for prevention and therapy of breast cancer and for hormone replacement treatment in post-menopausal women. Two receptor subtypes are now known, ERα and ERβ,[1,2] and they have different tissue distribution patterns. For example, ERα is predominant in the breast and in reproductive tissues such as the uterus, whereas ERβ is the principal subtype in certain regions of the brain.[3–5] Because of the known importance of ERα as a pharmaceutical target, and the potential importance of ERβ as well,[6] molecules that act as agonists or antagonists on either ER subtypes are currently being investigated by medicinal chemists for their therapeutic potential; those whose activity shows tissue selectivity, termed selective estrogen receptor modulators (SERMs),[7,8] are of particular interest.

In spite of a remarkable variety of molecular structures found in the many classes of non-steroidal ER ligands known so far, a striking chemical feature common to nearly all synthetic ER ligands possessing a good binding affinity is the presence of a phenolic ring (**A**, pharmacophore model, Fig. 1) that seems to mimic the steroid 'A-ring' present in natural estrogens.[9] This phenolic group is thought to be responsible for much of the attractive

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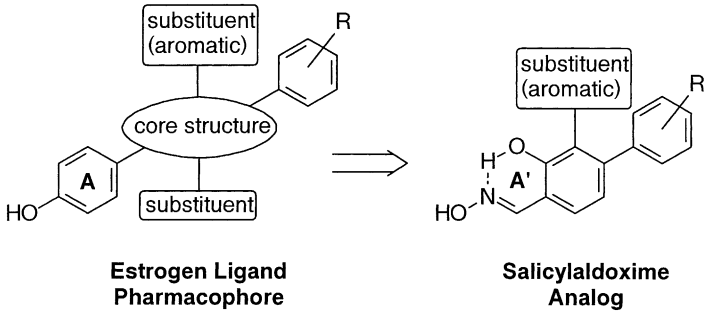
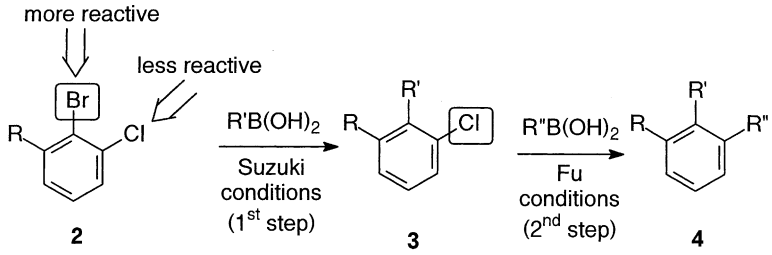


Figure 1. Structural comparison of salicylaldoxime (right) with the estrogen ligand pharmacophore model (left): relationship of the *pseudo*-ring A′ with the phenolic ring A.

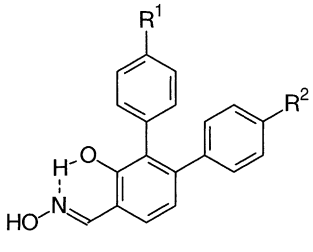


Scheme 1.

interaction between ligand and receptor, because of its participation in a hydrogen-bond network which includes two specific residues of the ER ligand binding domain, Glu353(305) and Arg394(346) of ERα(ERβ).[10,11]

We have recently reported that 3,4-diphenylsalicylaldoxime **1a**,[12] a non-steroidal estrogen of unique structure, has good binding affinity for both ER subtypes. This result was rationalized by assuming that the *pseudo*-ring A′ (Fig. 1) of compound **1a**, formed by the intramolecular hydrogen bond between the phenolic hydroxyl group and the oxime nitrogen atom, was an effective replacement of the phenolic A-ring typically present in natural and synthetic ER ligands,[9] with the oxime hydroxyl being the real mimic of the phenolic OH.

An examination of different classes of synthetic ER ligands, in which, for example, the 'core structure' (Fig. 1) is a pyrazole[13–15] or a furan,[16] shows that the introduction in these molecules of additional peripheral phenolic hydroxy-groups causes an improvement of their binding properties and a general increase in their ERα-selectivity. This fact was explained by the intervention of additional hydrogen-bonds or other types of hydrophilic interactions between these new phenolic groups and other polar functions on residues present nearby in the ligand binding pocket of ERα, namely, His524 and Thr347.



1a-g

	R^1	R^2
a	H	H
b	OMe	H
c	OH	H
d	H	OMe
e	H	OH
f	OMe	OMe
g	OH	OH

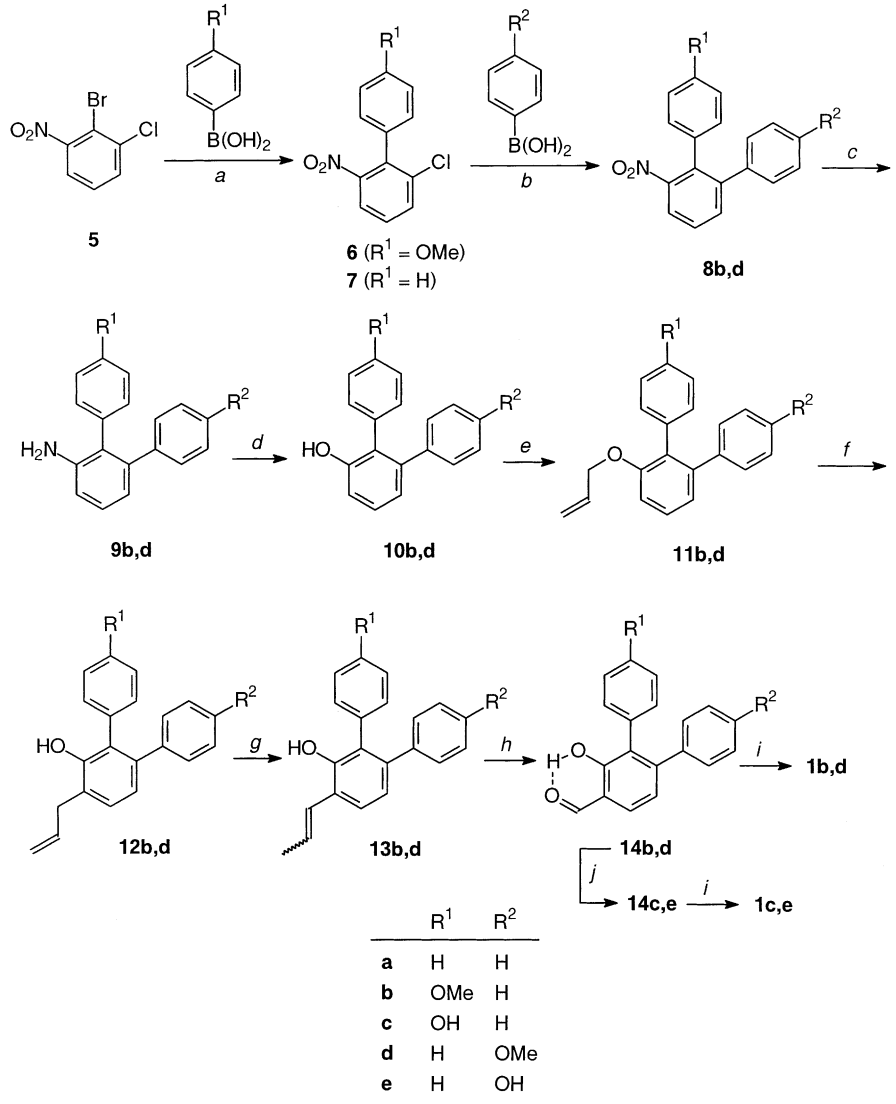
Based on these considerations, compound **1a** may be thought of as the first and simplest member of a new class of ER ligands with 3,4-diarylsalicylaldoxime structure, the members of which might be expanded by introducing various substituents on the 3- and 4-phenyl rings of **1a**. We, therefore, synthesized and tested for their ER-ligand properties and transcriptional activity some analogues of **1a** possessing *p*-hydroxy- and *p*-methoxy-groups in either one of the 3- and 4-aryl substituents (**1b–e**) or in both of them (**1f**,**g**).

Results and Discussion

Chemical synthesis

The synthetic route for preparation of the heterodisubstituted compounds of type **4** (Scheme 1) included a double Pd-catalyzed cross-coupling reaction on a bromo-chloro-disubstituted aryl precursor of type **2**. This approach exploits the different reactivity of aryl-bromides and aryl-chlorides towards the chosen boronic acids under different reaction conditions, Suzuki-type conditions for the bromides[17] and Fu-type conditions for the chlorides,[18] and it provides an efficient way to selectively introduce different substituents (depending on the boronic acid used) in those positions.

The complete synthesis of 3,4-heterodisubstituted compounds **1b–1e** was accomplished as shown in Scheme 2. 2-Bromo-3-chloronitrobenzene (**5**)[19] was first submitted to a 'classical' Suzuki-type cross-coupling reaction,[17] using $Pd(Ph_3P)_4$ as the catalyst, to introduce the first phenyl or *p*-methoxyphenyl group in the place of the bromine atom, affording compounds **6** or **7**. This step did not affect the chlorine atom of **6** and **7**, which was then substituted in the second cross-coupling reaction with phenyl or *p*-methoxyphenyl boronic acid, using the

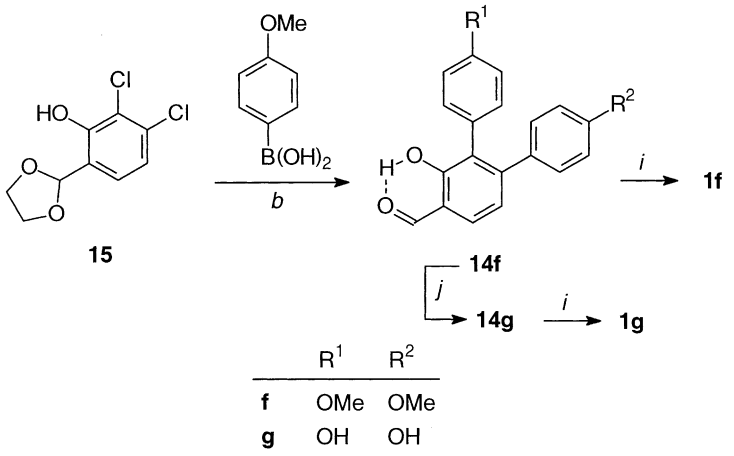


Scheme 2. Reagents and conditions: (a) $Pd(PPh_3)_4$, Na_2CO_3, toluene, EtOH, Δ; (b) $Pd_2(dba)_3$, Cy_3P, Cs_2CO_3, dioxane, Δ; (c) $NH_2NH_2{\cdot}H_2O$, $FeCl_3{\cdot}6H_2O$, activated carbon, MeOH, Δ; (d) $NaNO_2$, H_2SO_4 aq, dioxane, 0 → 45 °C; (e) allyl bromide, K_2CO_3, CH_3CN, 80 °C; (f) *N*-methylaniline, 180 °C; (g) *t*-BuO^-K^+, DMSO, 55 °C; (h) OsO_4 (cat.), $NaIO_4$, dioxane, H_2O, rt; (i) $NH_2OH{\cdot}HCl$, EtOH, Δ; (j) BBr_3, CH_2Cl_2, −78 → 0 °C.

more reactive catalytic system comprised of $Pd_2(dba)_3$ together with tricyclohexylphosphine as the catalyst ligand.[18] Under these conditions it was possible to introduce a second aryl substituent (R^2 = phenyl for **8b** or *p*-methoxyphenyl for **8d**) in place of the aryl-chloride group of **6** and **7**, efficiently assembling the required diaryl-benzene core unit of these systems.

The nitro-group of **8b,d** was then reduced with hydrazine hydrate in the presence of catalytic amounts of ferric chloride and activated carbon to yield **9b,d**.[20] Diazotization of the amine function in **9b,d**, followed by acid hydrolysis of the diazonium group, afforded the corresponding phenolic derivatives **10b,d**.[21] Treatment of **10b,d** with allyl bromide gave allyl ethers **11b,d**, which were directly submitted to a Claisen rearrangement at 180 °C in *N*-methylaniline,[22] affording the corresponding *o*-allyl-substituted phenols **12b,d**. The allylic terminal double bond of **12b,d** was submitted to an alkaline isomerization to give *E*/*Z* diastereoisomeric mixtures of **13b** and **13d**, possessing an internal aryl-conjugated double bond.[23] Salicylaldehydes **14b,d** were then obtained by oxidative cleavage of the double bond of **13b,d**, using sodium periodate in the presence of catalytic amounts of osmium tetroxide,[24] and then were transformed into the corresponding oximes **1b,d** by condensation with hydroxylamine hydrochloride in refluxing ethanol. Transformation of the methoxy groups of **14b,d** into hydroxyls of **14c,e** was achieved by a BBr_3 demethylation.[25] and these hydroxyaryl-substituted salicylaldehydes **14c,e**, thus obtained, were then condensed with hydroxylamine hydrochloride in refluxing ethanol to give oximes **1c,e**.

3,4-Homo-disubstituted compounds **1f** and **1g** were synthesized as indicated in Scheme 3, following the synthetic path previously reported for the preparation of **1a**.[12] Dichloro-substituted cyclic acetal **15**[12] was submitted to a sequential double cross-coupling reaction with *p*-methoxyphenylboronic acid using $Pd_2(dba)_3$ and tricyclohexylphosphine, involving a simultaneous deprotection of the



Scheme 3. Reagents and conditions: see Scheme 2.

aldehyde group, to afford bis(*p*-methoxyphenyl)-salicylaldehyde **14f**. Oxime **1f** was obtained by reaction of aldehyde **14f** and hydroxylamine hydrochloride under the above-mentioned conditions. Double demethylation of **14f** with BBr_3 afforded aldehyde **14g**, which was again treated with hydroxylamine hydrochloride to form oxime **1g**.

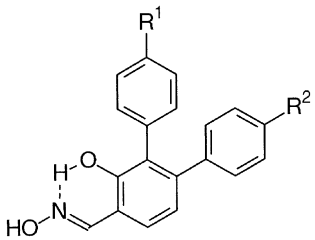
The (*E*)-geometry of the oxime moiety in compounds **1b–g** was assigned based on the chemical shift value of their oxime proton, which is in the δ 8.28–8.45 range. This value is typical for aromatic (*E*)-oximes, whereas the (*Z*)-oximes of the same kind usually have values in the δ 7.3–7.6 range. The downfield chemical shift found with oximes possessing the (*E*)-configuration is due to the greater shielding of the oxime proton when it is on the same side and in a close spatial contact with an electronegative oxygen.[26] The exclusive formation of oxime diastereoisomers of the (*E*)-configuration may be attributed to the stabilization of these diastereoisomers by the intramolecular hydrogen bond between the phenolic OH and the adjacent oxime nitrogen atom.

Estrogen receptor binding assays

The binding affinity of oximes **1b–g**, as well as that of aldehydes **14b–g**, for both ERα and ERβ were determined by a radiometric competitive binding assay, using methods that have been described elsewhere in detail.[27,28] In Table 1 are reported the relative binding affinity (RBA) values determined in a uterine cytosol ER preparation (Uterine ER), and with purified full-length human alpha (hERα) and beta (hERβ) receptor subtypes, together with those previously obtained for **1a** and **14a**.[12] Binding affinity (RBA) values are reported relative to estradiol (E_2), which is set at 100%.

An analysis of the results shows that in uterine cytosol, compound **1e**, which contains a *p*-hydroxy group only on the C(4) phenyl substituent ('distal' phenyl, R^2 = OH), possesses a binding affinity (3.30) that is almost 4 times better than what we have previously reported for its non-substituted counterpart **1a** (0.85).[12] This trend is confirmed, albeit to a smaller extent, on

Table 1. ER relative binding affinities[a] of oximes **1a–g** and aldehydes **14a–g**



Compound	R^1	R^2	Uterine ER	hERα	hERβ
Estradiol	—	—	(100)	(100)	(100)
1a[b]	H	H	0.85 ± 0.21[b]	1.13 ± 0.18[b]	1.71 ± 0.42[b]
14a[b]			< 0.010[b]	0.008 ± 0.001[b]	0.008 ± 0.003[b]
1b	OMe	H	0.108 ± 0.026	0.148 ± 0.043	0.377 ± 0.030
14b			< 0.009	0.014 ± 0.001	0.022 ± 0.006
1c	OH	H	0.992 ± 0.083	0.967 ± 0.047	2.21 ± 0.60
14c			< 0.009	0.013 ± 0.000	0.033 ± 0.001
1d	H	OMe	2.67 ± 0.81	1.35 ± 0.11	0.428 ± 0.103
14d			< 0.010	0.012 ± 0.002	0.035 ± 0.004
1e	H	OH	3.30 ± 1.701	2.59 ± 0.71	1.44 ± 0.37
14e			0.021 ± 0.001	0.014 ± 0.004	0.087 ± 0.026
1f	OMe	OMe	0.172 ± 0.052	0.291 ± 0.057	0.076 ± 0.018
14f			n.d.[c]	n.d.[c]	n.d.[c]
1g	OH	OH	1.37 ± 0.156	0.923 ± 0.045	0.347 ± 0.011
14g			0.027 ± 0.008	0.028 ± 0.011	0.062 ± 0.002

[a]Determined by a competitive radiometric binding assay with [^{3}H]estradiol; cytosol preparations of lamb uterus or full-length human ERα and ERβ (PanVera) were used; see Experimental.[24,25] Values are reported as the mean ± range or SD of 2-3 independent experiments; the K_d for estradiol for uterine ER and ERα is 0.2 nM and for ERβ is 0.5 nM.
[b]See ref 12.
[c]Not determined.

purified human ERs, with an improvement of the affinity on hERα (2.59 of **1e** versus 1.13 of **1a**) and a slight decrease on hERβ (1.44 of **1e** versus 1.71 of **1a**). On the other hand, the introduction of a *p*-hydroxy group on the C(3) phenyl substituent of **1a** ('proximal' phenyl, $R^1 = OH$), as in compound **1c,** did not substantially modify the uterine ER values, but it caused a shift in the α/β preference in favour of the β subtype (β/α ~ 2), due to a slight improvement of the affinity of **1c** on ERβ (RBA = 2.21) with respect to its unsubstituted counterpart **1a** (RBA = 1.71).[12] Compound **1g**, possessing *p*-hydroxy groups in both phenyl substituents ($R^1 = R^2 = OH$), exhibited a decreased binding affinity on both ER subtypes with respect to **1a** (although it showed a slightly better affinity value on uterine ER than **1a**). Among the *p*-methoxy-substituted compounds, only the one where this group is placed on the 'distal' phenyl substituent (**1d**) showed appreciable binding values on uterine ER (2.67) and on hERα (1.35). The same compound did not bind as well on ERβ (0.428). The other mono-methoxy derivative **1b** and the dimethoxy-analogue **1f** exhibited considerably lower affinity than the reference compound **1a**. None of the aldehydic derivatives **14a-g** showed appreciable binding data (< 0.07 in all cases).

These results show that there are improvements in the RBA due to the introduction of only one hydroxyl substituent, with a certain preference for ERα when the *p*-OH group is placed on the 'distal' phenyl (**1e**) or for ERβ when the same group is placed on the 'proximal' phenyl (**1c**). However, when *p*-hydroxy groups are introduced in both phenyl substituents ($R^1 = R^2 = OH$), as in compound **1g**, the binding affinity values for both receptor subtypes were decreased with respect to **1a**. This might be due to the fact that compound **1g** is now too polar to effectively bind the ER receptor.

In principle, the hydroxylated compounds **1c**, **1e** and **1g** could interact with the ligand binding pocket in ER in multiple orientations, since any of the phenolic OH's could potentially replace the oxime OH in serving as the mimic of the phenolic hydroxyl of the A-ring of estradiol and non-steroidal estrogen (Fig. 1). However, the fact that all of the hydroxylated aldehydes (**14c**, **14e** and **14g**) showed very modest binding properties, supports a model in which the peripheral phenolic OH groups in this class of ER ligands play only an 'auxiliary' role [i.e., by interaction with His524(475) of ERα(ERβ)] and that the oxime OH in derivatives **1a–g** continues to function in the 'crucial' role (i.e., by participating in the well-established hydrogen-bond network with Glu353(305) and Arg394(346) of ER). The engagement of the peripheral phenols in auxiliary hydrogen bonds is further supported by the finding that the *p*-methoxy-substituted compounds **1b**, **1d** and **1f** bind less well than the corresponding hydroxy compounds (**1c**, **1e**, and **1g**), and in some cases less well than the unsubstituted parent compound (**1a**).

Transcription assays

Compounds showing the highest affinity values for either one of the two ER's (**1a**, **1c–e**) were assayed for transcriptional activity through both receptor subtypes. These cotransfection assays were conducted in human endometrial (HEC-1) cells, using expression plasmids for either full-length human ERα or ERβ and an estrogen-responsive luciferase reporter gene system.[29] In this initial screen, agonist activity was determined at two concentrations, 10^{-8} and 10^{-6} M, and antagonist activity was assayed at the same two concentrations, but in the presence of 10^{-9} M E_2. In all cases, transcriptional activity is normalized relative to that obtained with 10^{-9} M estradiol, which is set at 100%. These data are summarized Table 2, where the percent efficacy of the compounds tested as agonists (at 10^{-8} and 10^{-6} M) and as antagonists (at 10^{-6} M in the presence of 10^{-9} M E_2) is given. Based on the level of efficacy achieved in the agonist and antagonist modes, the compounds were classified as 'agonists' (nearly full efficacy; > 80%), 'antagonists' (very little efficacy; < 15%), 'partial agonists' (50–80% efficacy), or 'partial antagonists' (15–50% efficacy). In addition, compounds that show equivalent efficacy at both 10^{-8} and 10^{-6} M are classified as 'potent', whereas those that failed to reach a similar level of efficacy at 10^{-6} M in both agonist and antagonist modes were classified as 'weak'. Complete dose-response curves were also obtained for compound **1e**, which has the highest ERα binding affinity (Fig. 2).

Table 2. Transcriptional efficacy of selected salicylaldoxime analogues on ERα and ERβ[a]

Cpd No.	% Efficacy on ERα[b]				% Efficacy on ERβ[b]			
	Agonist[c] (M)		Antag.[c] (M)	Pharmacological character[d]	Agonist[c] (M)		Antag.[c] (M)	Pharmacoligical character[d]
	10^{-8}	10^{-6}	10^{-6}		10^{-8}	10^{-6}	10^{-6}	
1a	13	74	50	Partial agonist	3	23	52	Weak partial antag.
1c	2	41	98	Weak partial antagonist	1	3	41	Weak partial antag.
1d	30	64	83	Agonist	2	12	52	Weak partial antag.
1e	65	72	70	Potent agonist	5	26	32	Partial antag.

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

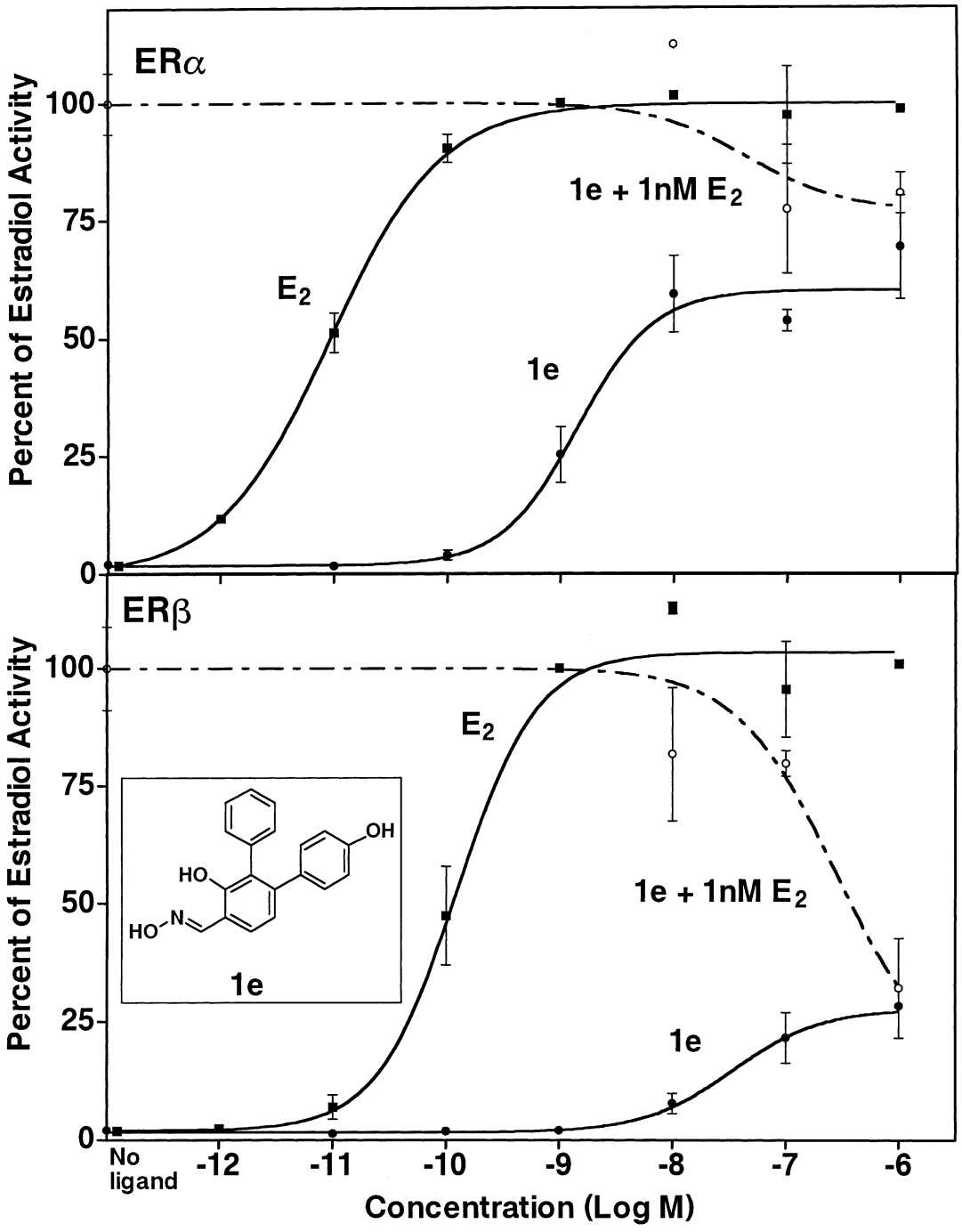


Figure 2. Dose–response curve for transcriptional activation by estradiol (E_2) and the diarylsalicylaldoxime **1e** through ERα (upper panel) and ERβ (lower panel). Assays were done in the agonist mode, with compounds alone (solid lines and symbols), or with compound **1e** in the antagonist mode, i.e., in the presence of 1 nM estradiol (E_2) (dashed line and open symbols). Human endometrial cancer (HEC-1) cells were transfected with expression vectors for ERα?or ERβ and an $(ERE)_2$-pS2-luc reporter gene and were treated with compound **1e** alone, or compound **1e** plus 1 nM estradiol (E_2) for 24 h, at the concentrations indicated. Luciferase activity was expressed relative β-galactosidase activity from an internal control plasmid. The maximal activity with 1 nM E_2 was set at 100. Values are the mean ± SD from three or more separate experiments.

All four compounds (**1a**, **1c–e**) proved to be more potent and more agonistic on ERα than on ERβ (Table 2). In particular, compounds **1d** and **1e**, possessing a *p*-OMe or a *p*-OH group on their 'distal' phenyl substituents, respectively, both proved to be nearly full agonists on ERα and partial antagonists on ERβ. By contrast, the reference unsubstituted compound **1a** and its analogue possessing a *p*-OH on its 'proximal' phenyl substituent (**1c**), showed a partial agonist activity on ERα and a partial antagonism on ERβ that in most cases was rather weak. The full dose response done on the compound with the highest ERα binding affinity and transcriptional potency (**1e**) (Fig. 2) confirms the results of the screening assay (Table 2), showing that this compound is a potent partial agonist on ERα and a weaker partial antagonist on ERβ.

These results indicate that the ERα agonist character of this class of ligands is enhanced by the presence of an oxygen-containing substituent (such as the *p*-hydroxy of **1e** or the *p*-methoxy of **1d**, on the 'distal' phenyl ring), whereas the same kind of substitution on the other phenyl ring (as in **1c**) does not substantially modify the partial agonist character already found in the unsubstituted compound **1a**. Thus, the results from these transcription assays confirm, as do the binding affinity values, that the 3,4-diarylsalicylaldoxime class of ER ligands has a distinct structure–activity profile on the two ER subtypes.

Conclusions

This study was carried out with the aim of determining the effects of introducing hydroxy- and methoxy-groups on the structure of a previously reported estrogen ligand possessing a unique 3,4-diphenylsalicylaldoxime structure (**1a**). The results show that in no case does the introduction of a *p*-OMe groups produce a significant increase in the estrogen receptor affinity, whereas the binding properties are significantly improved by the introduction of one *p*-OH group. In fact, when the *p*-OH is introduced in the C(3)-phenyl ring (proximal), as in ligand **1c**, affinity for the ERβ is increased, whereas when the same group is placed on the C(4)-phenyl ring (distal), as in ligand **1e**, the binding preference for ERα increases. By contrast, the simultaneous introduction of two *p*-OH groups on both phenyl substituents (**1g**) caused a drop in the binding affinities on both receptor subtypes. These initial results provide useful indications towards the identification of an optimal substitution pattern for activity and selectivity, which may be useful in the design of new more effective members of this unique class of estrogen receptor ligands.

Experimental

Chemical methods

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. NMR spectra were obtained with a Varian Gemini 200 MHz spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane and referenced from solvent references. Electron impact (EI) mass spectra were obtained on a HP-5988A mass spectrometer at 70 eV. The elemental compositions of the compounds agreed to within ±0.4% of the calculated value. Chromatographic separations were performed on silica gel columns by flash (Kieselgel 40, 0.040.063 mm; Merck) or gravity column (Kieselgel 60, 0.063.200 mm; Merck) chromatography. Reactions were followed by thin-layer chromatography (TLC) on Merck aluminium silica gel (60 F_{254}) sheets that were visualized under a UV lamp (254 nm). Evaporation was performed in vacuo (rotating evaporator). Sodium sulfate was always used as the drying agent. The toluene solution of tricyclohexylphosphine (20%) was purchased from Strem Chemicals. The other chemicals were purchased from Sigma-Aldrich.

3-Chloro-2-(4-methoxyphenyl)nitrobenzene (6). 2-Bromo-3-chloronitrobenzene (**5**)[19] (2.0 g, 8.5 mmol) was dissolved in 20 mL of a 1:1 mixture of toluene–ethanol and 10 mL of a 2 M aqueous solution of Na_2CO_3. The solution was treated with *p*-methoxyphenylboronic acid (1.5 g, 10 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.29 g, 0.25 mmol). The resulting mixture was refluxed for 16 h. After being cooled to rt, the mixture was diluted with water and extracted with diethyl ether. The combined organic phase were dried and concentrated. The crude product was purified by flash chromatography (*n*-hexane/diethyl ether 9:1) to yield pure **6** (1.32 g, 4.99 mmol, 59% yield) as an oil; ^{1}H NMR ($CDCl_3$) δ 3.85 (s, 3H), 6.97 (AA′XX′, 2H, $J_{AX}=8.8$ Hz, $J_{AA'/XX'}=2.5$ Hz), 7.19 (AA′XX′, 2H, $J_{AX}=8.8$ Hz, $J_{AA'/XX'}=2.5$ Hz), 7.41 (dd, 1H, $J=8.6$, 7.5 Hz), 7.67.71 (m, 2H); MS *m*/*z* 263 (M^+, ^{35}Cl, 100), 265 (M^+, ^{37}Cl, 34). Anal. $C_{13}H_{10}ClNO_3$ (C, H, N).

2-(4-Methoxyphenyl)-3-phenylnitrobenzene (8b). A solution of **6** (0.67 g, 2.5 mmol) in anhydrous dioxane (4 mL) was treated under argon with cesium carbonate (1.4 g, 4.3 mmol), phenylboronic acid (0.57 g, 3.8 mmol), tris(dibenzylideneacetone)dipalladium(0) (0.073 g, 0.080 mmol), and 0.22 mL of a 20% solution of tricyclohexylphosphine in toluene (0.14 mmol). The resulting mixture was heated at 80 °C in a sealed vial for 16 h. After being cooled to rt, the mixture was diluted with diethyl ether, filtered through a Celite pad, and concentrated. The crude product was purified by flash chromatography (*n*-hexane/diethyl ether 9:1) to yield pure **8b** (0.74 g, 2.4 mmol, 96% yield) as yellow crystals: mp 130 °C; ^{1}H NMR ($CDCl_3$) δ 3.76 (s, 3H), 6.74 (AA′XX′, 2H, $J_{AX}=8.8$ Hz, $J_{AA'/XX'}=2.4$ Hz), 6.96 (AA′XX′, 2H, $J_{AX}=8.8$ Hz, $J_{AA'/XX'}=2.4$ Hz), 7.01.06 (m, 2H), 7.19.22 (m, 3H), 7.49 (t, 1H, $J=7.7$ Hz), 7.59 (dd, 1H, $J=7.7$, 1.6 Hz), 7.71 (dd, 1H, $J=7.7$, 1.6 Hz); MS *m*/*z* 305 (M^+). Anal. $C_{19}H_{15}NO_3$ (C, H, N).

2-(4-Methoxyphenyl)-3-phenylaniline (9b). A solution of **8b** (0.74 g, 2.4 mmol) in methanol (25 mL) was treated with ferric chloride hexahydrate (0.0019 g, 0.0070 mmol), and activated carbon (0.13 g). The mixture was heated to reflux for 5 min and added of hydrazine hydrate (1.9 mL, 39 mmol) through a silicone septum. Reflux was continued for 16 h. After being cooled to rt, the mixture was filtered through a paper filter, concentrated under vacuum, and diluted with chloroform. The resulting solution was dried and concentrated to give **9b** (0.56 g, 2.0 mmol, 83% yield) as a yellow solid, which was used in the next step without further purification: mp 118 °C; ^{1}H NMR ($CDCl_3$) δ 3.66 (bs, 2H), 3.77 (s, 3H), 6.76.86 (m, 4H), 7.03.28 (m, 8H); MS *m*/*z* 275 (M^+). Anal. $C_{19}H_{17}NO$ (C, H, N).

2-(4-Methoxyphenyl)-3-phenylphenol (10b). A solution of **9b** (0.56 g, 2.0 mmol) in a 1 M aqueous solution of H_2SO_4 (3 mL) and dioxane (3 mL) was cooled to 0 °C and treated with an aqueous solution (0.3 mL) of sodium nitrite (0.14 g, 2.0 mmol) and stirred at the same temperature for 5 min, after which time 0.3 mL of concentrated H_2SO_4 were added. After 10 min, the mixture was treated with 200 mL of an aqueous solution of

H_2SO_4 and the resulting solution was heated to 45 °C for 18 h. The mixture was then cooled to rt, diluted with satd NaCl, and extracted with ethyl acetate. The combined organic phase were dried over Na_2SO_4 and concd under vacuum. The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 8:2) to yield pure **10b** (0.42 g, 1.5 mmol, 75% yield) as a white solid: mp 92 °C; ^{1}H NMR ($CDCl_3$) δ 3.79 (s, 3H), 5.13 (bs, 1H), 6.85 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.3 Hz), 6.98.20 (m, 9H), 7.31 (t, 1H, J = 7.8 Hz); MS *m*/*z* 276 (M^+, 100), 261 (M^+ –CH_3, 8), 259 (M^+ –OH, 6), 245 (M^+ –OCH_3, 11). Anal. $C_{19}H_{16}O_2$ (C, H).

***O*-Allyl-2-(4-methoxyphenyl)-3-phenylphenol (11b).** A solution of **10b** (0.38 g, 1.4 mmol) in acetonitrile (4 mL) was treated with potassium carbonate (0.35 g, 2.5 mmol), and the mixture was heated to 80 °C. A solution of allyl bromide (0.19 mL, 2.22 mmol) in acetonitrile (1 mL) was then added with a syringe through a silicon septum, and stirring was continued at the same temperature for 3 h. After being cooled to rt, the resulting suspension was filtered, and the filtrate was concentrated to give a crude residue that was purified by flash chromatography (*n*-hexane/ethyl acetate 9:1) to yield pure **11b** (0.35 g, 1.1 mmol, 79% yield) as an oil; ^{1}H NMR ($CDCl_3$) δ 3.76 (s, 3H), 4.51 (dt, 2H, J = 4.8, 1.7 Hz), 5.16 (dq, 1H, J = 10.6, 1.7 Hz), 5.24 (dq, 1H, J = 17.4, 1.7 Hz), 5.93 (ddt, 1H, J = 17.2, 10.6, 1.7 Hz), 6.73 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.5 Hz), 6.97 (dd, 1H, J = 8.1, 0.9 Hz), 6.99.18 (m, 8H), 7.33 (t, 1H, J = 8.0 Hz); MS *m*/*z* 316 (M^+, 100), 275 (M^+ –$CH_2CH{=}CH_2$, 65), 260 (M^+ –$CH_2CH{=}CH_2$ –CH_3, 22), 244 (M^+ –$CH_2CH{=}CH_2$ –OCH_3, 19). Anal. $C_{22}H_{20}O_2$ (C, H).

6-Allyl-2-(4-methoxyphenyl)-3-phenylphenol (12b). A solution of **11b** (0.35 g, 1.1 mol) in *N*-methylaniline (1.6 mL) was heated to 180 °C for 48 h. After being cooled to rt, the mixture was diluted with ethyl acetate and washed with 4 N aqueous HCl. The organic phase was dried and concentrated, to give **12b** (0.26 g, 0.83 mmol, 75% yield) as a solid, which was used in the next step without further purification: mp 97–99 °C; ^{1}H NMR ($CDCl_3$) δ 3.49 (d, 2H, J = 6.6 Hz), 3.76 (s, 3H), 5.10.23 (m, 2H), 5.24 (bs, 1H), 6.10 (ddt, 1H, J = 17.0, 10.0, 6.7 Hz), 6.85 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.4 Hz), 6.96 (d, 1H, J = 7.9 Hz), 7.03.17 (m, 7H), 7.19 (d, 1H, J = 7.9 Hz); MS *m*/*z* 316 (M^+, 100), 301 (M^+ –CH_3, 8), 285 (M^+ –OCH_3, 5), 275 (M^+ –$CH_2CH{=}CH_2$, 7).

***(E/Z)*-2-(4-Methoxyphenyl)-3-phenyl-6-(1-propenyl)phenol (13b).** A solution of **12b** (0.26 g, 0.83 mmol) in dimethylsulfoxide (3 mL) was treated with potassium *tert*-butoxide (0.23 g, 2.1 mmol), and the resulting mixture was heated to 55 °C for 4 h. After being cooled to rt, the mixture was treated with 1 N aqueous HCl and extracted with diethyl ether. The organic phase was dried and concentrated to give a crude residue that was purified by flash chromatography (*n*-hexane/diethyl ether 9:1) to yield **13b** as a 9:1 *E*/*Z* diastereomeric mixture (0.23 g, 0.73 mmol, 88% yield) as a white solid: mp 161–163 °C; ^{1}H NMR ($CDCl_3$) δ (*E*-isomer) 1.94 (dd, 3H, J = 6.6, 1.6 Hz), 3.79 (s, 3H), 5.27 (bs, 1H), 6.32 (dq, 1H, J = 15.8, 6.6 Hz), 6.74 (dq, 1H, J = 15.8, 1.6 Hz), 6.86 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.4 Hz), 6.96 (d, 1H, J = 8.1 Hz), 7.02.18 (m, 7H), 7.43 (d, 1H, J = 8.1 Hz); MS *m*/*z* 316 (M^+, 100), 301 (M^+ –CH_3, 12), 257 (M^+ –CH_3 –$CH_2CH{=}CH_2$, 12), 239 (M^+ –Ph, 14). Anal. $C_{22}H_{20}O_2$ (C, H).

3-(4-Methoxyphenyl)-4-phenylsalicylaldehyde (14b). A solution of **13b** (0.23 g, 0.73 mmol) in dioxane (12 mL) was treated with 3 mL of water, 0.36 g of sodium periodate (1.7 mmol), and 0.02 mL of a 2.5% solution of osmium tetroxide in *tert*-butanol (0.02 mmol), and the mixture was stirred at rt for 30 min. The mixture was then diluted with water and extracted with chloroform. The organic phase was dried and evaporated to afford a crude residue that was purified by flash chromatography (*n*-hexane/diethyl ether 9:1) to yield pure **14b** (0.17 g, 0.55 mmol, 76% yield) as a yellow solid: mp 129–131 °C; ^{1}H NMR ($CDCl_3$) δ 3.78 (s, 3H), 6.79 (AA′XX′, 2H, J_{AX} = 8.6 Hz, $J_{AA'/XX'}$ = 2.4 Hz), 7.06 (AA′XX′, 2H, J_{AX} = 8.6 Hz, $J_{AA'/XX'}$ = 2.3 Hz), 7.09.23 (m, 6H), 7.60 (d, 1H, J = 8.1 Hz), 9.96 (s, 1H), 11.49 (bs, 1H); MS *m*/*z* 304 (M^+, 100), 286 (M^+ –H_2O, 7), 275 (M^+ –CHO, 8), 243 (M^+ –CHO –CH_3 –OH, 16). Anal. $C_{20}H_{16}O_3$ (C, H).

3-(4-Hydroxyphenyl)-4-phenylsalicylaldehyde (14c). A solution of **14b** (0.055 g, 0.18 mmol) in anhydrous dichloromethane (2 mL) was cooled to −78 °C and treated dropwise with a 1 M solution of BBr_3 in dichloromethane (0.6 mL), and the resulting solution was stirred at the same temperature for 5 min and at rt for 2 h. The mixture was then diluted with water and extracted with ethyl acetate. The organic phase was dried and concentrated. The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 1:1) to yield pure **14c** (0.036 g, 0.12 mmol, 67% yield) as a yellow solid: mp 204–205 °C; ^{1}H NMR (acetone-d_6) δ 6.70 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.4 Hz), 6.95 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.5 Hz), 7.13 (d, 1H, J = 8.0 Hz),7.14.25 (m, 5H), 7.82 (d, 1H, J = 8.1 Hz), 8.29 (bs, 1H), 10.08 (s, 1H), 11.51 (bs, 1H); MS *m*/*z* 290 (M^+, 100), 272 (M^+ –H_2O, 15), 261 (M^+ –CHO, 12), 243 (M^+ –H_2O –CHO, 18). Anal. $C_{19}H_{14}O_3$ (C, H).

3-(4-Methoxyphenyl)-4-phenylsalicylaldoxime (1b). A solution of **14b** (0.027 g, 0.088 mmol) in ethanol (1.5 mL) was treated with a solution of hydroxylamine hydrochloride (0.012 g, 0.18 mmol) in water (0.3 mL), and the resulting mixture was heated to 50 °C for 1 h. After being cooled to rt, the solvent was removed under vacuum, and the crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 8:2) to yield pure **1b** (0.024 g, 0.076 mmol, 85% yield) as an off-white solid: mp 180–182 °C; ^{1}H NMR ($CDCl_3$) δ 3.77 (s, 3H), 6.79 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.4 Hz), 7.01 (d, 1H, J = 8.0 Hz), 7.05.19 (m, 7H), 7.23 (d, 1H, J = 8.0 Hz), 8.31 (s, 1H); MS *m*/*z* 319 (M^+, 100), 302 (M^+ –OH, 24), 301 (M^+ –H_2O, 14), 270 (M^+ –H_2O –OCH_3, 14). Anal. $C_{20}H_{17}NO_3$ (C, H, N).

3-(4-Hydroxyphenyl)-4-phenylsalicylaldoxime (1c). Compound **1c** was prepared from **14c** (0.028 g, 0.097 mmol) following the same procedure described

above for **1b**. The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 8:2) to yield pure **1c** (0.028 g, 0.092 mmol, 94% yield) as a pale yellow solid: mp 217—219 °C; ^{1}H NMR (acetone-d_6) δ 6.68 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.4 Hz), 6.94 (AA′XX′, 2H, J_{AX} = 8.7 Hz, $J_{AA'/XX'}$ = 2.4 Hz), 6.98 (d, 1H, J = 8.1 Hz), 7.09.20 (m, 5H), 7.40 (d, 1H, J = 8.1 Hz), 8.20 (bs, 1H), 8.45 (s, 1H), 10.37 (bs, 1H); MS *m*/*z* 305 (M^+, 100), 288 (M^+ –OH, 34), 287 (M^+ –H_2O, 20). Anal. $C_{19}H_{15}NO_3$ (C, H, N).

3-Chloro-2-phenylnitrobenzene (7). Compound **7** was prepared from 2-bromo-3-chloronitrobenzene (**5**)[19] (0.95 g, 4.0 mmol) following the same procedure described above for **6**. The crude product was purified by flash chromatography (*n*-hexane) to yield pure **7** (0.68 g, 2.9 mmol, 72% yield) as an oil; ^{1}H NMR ($CDCl_3$) δ 7.22.28 (m, 2H), 7.41.49 (m, 4H), 7.71 (dd, 1H, J = 6.5, 1.4 Hz), 7.75 (dd, 1H, J = 6.2, 1.3 Hz); MS *m*/*z* 233 (M^+, ^{35}Cl, 10), 152 (M^+ –NO_2 –Cl, 100). Anal. $C_{12}H_8ClNO_2$ (C, H, N).

3-(4-Methoxyphenyl)-2-phenylnitrobenzene (8d). Compound **8d** was prepared from **7** (0.19 g, 0.82 mmol) following the same procedure described above for **8b**. The crude product was purified by flash chromatography (*n*-hexane/diethyl ether 9:1) to yield pure **8d** (0.17 g, 0.56 mmol, 69% yield) as yellow solid: mp 128 °C; ^{1}H NMR ($CDCl_3$) δ 3.75 (s, 3H), 6.71 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.5 Hz), 6.93 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.4 Hz), 7.03.08 (m, 2H), 7.22.25 (m, 3H), 7.50 (t, 1H, J = 7.7 Hz), 7.60 (dd, 1H, J = 7.7, 1.6 Hz), 7.72 (dd, 1H, J = 7.7, 1.6 Hz); MS *m*/*z* 305 (M^+). Anal. $C_{19}H_{15}NO_3$ (C, H, N).

3-(4-Methoxyphenyl)-2-phenylaniline (9d). Compound **9d** was prepared from **8d** (0.12 g, 0.39 mmol) following the same procedure described above for **9b**, to give **9d** (0.072 g, 0.26 mmol, 66% yield) as an oil, which was used in the next step without further purification; ^{1}H NMR ($CDCl_3$) δ 3.73 (s, 3H), 6.67 (AA′XX′, 2H, J_{AX} = 9.0 Hz, $J_{AA'/XX'}$ = 2.7 Hz), 6.97 (AA′XX′, 2H, J_{AX} = 8.9 Hz, $J_{AA'/XX'}$ = 2.6 Hz), 7.02.06 (m, 2H), 7.13.31 (m, 6H); MS *m*/*z* 275 (M^+). Anal. $C_{19}H_{17}NO$ (C, H, N).

3-(4-Methoxyphenyl)-2-phenylphenol (10d). Compound **10d** was prepared from **9d** (0.36 g, 1.3 mmol) following the same procedure described above for **10b**. The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 9:1) to yield pure **10d** (0.33 g, 1.2 mmol, 93% yield) as an oil; ^{1}H NMR ($CDCl_3$) δ 3.74 (s, 3H), 5.09 (bs, 1H), 6.68 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.5 Hz), 6.96.02 (m, 4H), 7.14.19 (m, 2H), 7.27.35 (m, 4H); MS *m*/*z* 276 (M^+). Anal. $C_{19}H_{16}O_2$ (C, H).

***O*-Allyl-3-(4-methoxyphenyl)-2-phenylphenol (11d).** Compound **11d** was prepared from **10d** (0.39 g, 1.4 mmol) following the same procedure described above for **11b**. The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 9:1) to yield pure **11d** (0.30 g, 0.95 mmol, 68% yield) as pale yellow crystals: mp 76 °C; ^{1}H NMR ($CDCl_3$) δ 3.74 (s, 3H), 4.49.51 (m, 2H), 5.10.30 (m, 2H), 5.91 (ddt, 1H, J = 17.2, 10.6, 4.6 Hz), 6.68 (d, 2H, J = 8.2 Hz), 6.93.22 (m, 9H), 7.33 (t, 1H, J = 8.0 Hz); MS *m*/*z* 316 (M^+, 100), 275 (M^+ –$CH_2CH{=}CH_2$, 47), 260 (M^+ –$CH_2CH{=}CH_2$ –CH_3, 89). Anal. $C_{22}H_{20}O_2$ (C, H).

6-Allyl-3-(4-methoxyphenyl)-2-phenylphenol (12d). Compound **12d** was prepared from **11d** (0.24 g, 0.76 mol) following the same procedure described above for **12b**, to give **12d** (0.19 g, 0.59 mmol, 77% yield) as an oil, which was used in the next step without further purification: ^{1}H NMR ($CDCl_3$) δ 3.48 (d, 2H, J = 6.6 Hz), 3.74 (s, 3H), 5.10.22 (m, 2H), 5.20 (bs, 1H), 6.09 (ddt, 1H, J = 17.1, 10.2, 6.7 Hz), 6.67 (d, 2H, J_{AX} = 8.6 Hz), 6.93.99 (m, 3H), 7.14.34 (m, 6H); MS *m*/*z* 316 (M^+).

***(E/Z)*-3-(4-Methoxyphenyl)-2-phenyl-6-(1-propenyl)phenol (13d).** Compound **13d** was prepared from **12d** (0.18 g, 0.57 mmol) following the same procedure described above for **13b**. The crude product was purified by flash chromatography (*n*-hexane/diethyl ether 9:1) to yield **13d** as a *E*/*Z* diastereomeric mixture (0.11 g, 0.34 mmol, 60% yield) as an oil; ^{1}H NMR ($CDCl_3$) δ (*E*-isomer) 1.94 (dd, 3H, J = 6.6, 1.6 Hz), 3.73 (s, 3H), 5.22 (bs, 1H), 6.31 (dq, 1H, J = 15.8, 6.5 Hz), 6.67 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.3 Hz), 6.94.98 (m, 3H), 7.14.19 (m, 3H), 7.29.35 (m, 3H), 7.43 (d, 1H, J = 8.1 Hz); MS *m*/*z* 316 (M^+). Anal. $C_{22}H_{20}O_2$ (C, H).

4-(4-Methoxyphenyl)-3-phenylsalicylaldehyde (14d). Compound **14d** was prepared from **13d** (0.11 g, 0.34 mmol) following the same procedure described above for **14b**. The crude product was purified by flash chromatography (*n*-hexane/diethyl ether, from 9:1 to 8:2) to yield pure **14d** (0.076 g, 0.25 mmol, 73% yield) as a yellow solid: mp 129 °C; ^{1}H NMR ($CDCl_3$) δ 3.76 (s, 3H), 6.72 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.5 Hz), 7.02 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.5 Hz), 7.09.17 (m, 3H), 7.23.29 (m, 3H), 7.60 (d, 1H, J = 8.1 Hz), 9.95 (s, 1H), 11.48 (bs, 1H); MS *m*/*z* 304 (M^+, 100), 275 (M^+ –CHO, 7), 260 (M^+ –CHO –CH_3, 6), 243 (M^+ –CHO –CH_3 –OH, 14), 227 (M^+ –CHO –OCH_3 –OH, 15). Anal. $C_{20}H_{16}O_3$ (C, H).

4-(4-Hydroxyphenyl)-3-phenylsalicylaldehyde (14e). Compound **14e** was prepared from **14d** (0.046 g, 0.15 mmol) following the same procedure described above for **14c**. The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 1:1) to yield pure **14e** (0.027 g, 0.093 mmol, 62% yield) as a yellow solid: mp 190 °C; ^{1}H NMR (acetone-d_6) δ 6.67 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.4 Hz), 6.97 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.4 Hz), 7.11.26 (m, 6H), 7.81 (d, 1H, J = 8.1 Hz), 8.48 (bs, 1H), 10.05 (s, 1H), 11.54 (bs, 1H); MS (EI, 70 eV) *m*/*z* 290 (M^+). Anal. $C_{19}H_{14}O_3$ (C, H).

4-(4-Methoxyphenyl)-3-phenylsalicylaldoxime (1d). Compound **1d** was prepared from **14d** (0.020 g, 0.066 mmol) following the same procedure described above for **1b**. The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 8:2) to yield pure **1d** (0.017 g, 0.054 mmol, 82% yield) as an off-white solid:

mp 188 °C; ^{1}H NMR ($CDCl_3$) δ 3.75 (s, 3H), 6.70 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.4 Hz), 6.98.03 (m, 3H), 7.14.29 (m, 6H), 7.58 (bs, 1H), 8.28 (s, 1H), 10.32 (bs, 1H); MS *m*/*z* 319 (M^+, 100), 302 (M^+ –OH, 23), 285 (M^+ –2OH, 6), 270 (M^+ –2OH –CH_3, 10), 258 (M^+ –OH –CH=NOH, 11). Anal. $C_{20}H_{17}NO_3$ (C, H, N).

4 - (4 - Hydroxyphenyl) - 3 - phenylsalicylaldoxime (1e). Compound **1e** was prepared from **14e** (0.020 g, 0.069 mmol) following the same procedure described above for **1b**. The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 7:3) to yield pure **1e** (0.016 g, 0.052 mmol, 75% yield) as off-white crystals: mp 180 °C; ^{1}H NMR (CD_3OD) δ 6.54 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.5 Hz), 6.86 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.4 Hz), 6.94 (d, 1H, *J* = 7.9 Hz), 7.07.21 (m, 5H), 7.27 (d, 1H, *J* = 8.0 Hz), 8.29 (s, 1H); MS *m*/*z* 305 (M^+, 100), 288 (M^+ –OH, 37). Anal. $C_{19}H_{15}NO_3$ (C, H, N).

3,4-Bis(4-methoxyphenyl)salicylaldehyde (14f). A solution of compound **15**[12] (0.30 g, 1.3 mmol) in dioxane (5 mL) was treated with 0.71 g (2.2 mmol) of cesium carbonate, 0.29 g (1.9 mmol) of *p*-methoxyphenylboronic acid, 0.037 g (0.041 mmol) of tris(dibenzylideneacetone)dipalladium(0), and 0.16 mL of a 20% solution of tricyclohexylphosphine (0.10 mmol) in toluene. The resulting suspension was heated at 80 °C for 16 h under an argon atmosphere. The reaction mixture was then cooled to room temperature, diluted with ethyl acetate and filtered through a Celite pad. The solvent was removed under vacuum, and the resulting crude mixture was submitted again to the same treatment described above. The crude product deriving from the second step was purified by column chromatography, eluting with a 8:2 *n*-hexane/ethyl acetate mixture, to obtain 0.035 g (0.11 mmol, 26% yield, 2 steps) of aldehyde **14f** as a vitreous solid; ^{1}H NMR ($CDCl_3$) δ 3.76 (s, 3H), 3.77 (s, 3H), 6.73.85 (m, 4H), 7.01.27 (m, 5H), 7.57 (d, 1H, *J* = 8.1 Hz), 9.94 (s, 1H), 11.51 (bs, 1H); MS *m*/*z* 334 (M^+). Anal. $C_{21}H_{18}O_4$ (C, H).

3,4-Bis(4-methoxyphenyl)salicylaldoxime (1f). Compound **1f** was prepared from **14f** (0.056 g, 0.17 mmol) following the same procedure described above for **1b**. The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 8:2) to yield pure **1f** (0.042 g, 0.012 mmol, 71% yield) as a white solid: mp 213 °C; ^{1}H NMR ($CDCl_3$) δ 3.76 (s, 3H), 3.78 (s, 3H), 6.71 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.5 Hz), 6.81 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.5 Hz), 6.99 (d, 1H, *J* = 7.9 Hz), 7.01 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.5 Hz), 7.08 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.5 Hz), 7.20 (d, 1H, *J* = 7.9 Hz), 7.53 (bs, 1H), 8.29 (s, 1H), 10.08 (bs, 1H); MS *m*/*z* 349 (M^+, 100), 331 (M^+ –H_2O, 26). Anal. $C_{21}H_{19}NO_4$ (C, H, N).

3,4-Bis(4-hydroxyphenyl)salicylaldehyde (14g). A solution of **14f** (0.057 g, 0.17 mmol) in anhydrous dichloromethane (2.5 mL) was cooled to −78 °C and treated dropwise with a 1 M solution of BBr_3 in dichloromethane (0.8 mL), and the resulting solution was stirred at the same temperature for 5 min and at rt for 2 h. The mixture was then diluted with water and extracted with ethyl acetate. The organic phase was dried and concentrated. The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 1:1) to yield pure **14g** (0.029 g, 0.095 mmol, 56% yield) as a yellow solid: mp 240 °C; ^{1}H NMR (acetone-d_6) δ 6.69 (d, 2H, *J* = 8.8 Hz), 6.73 (d, 2H, *J* = 8.8 Hz), 6.96 (d, 2H, *J* = 8.7 Hz), 6.99 (d, 2H, *J* = 8.8 Hz), 7.12 (d, 1H, *J* = 8.1 Hz), 7.77 (d, 1H, *J* = 8.1 Hz), 8.30 (bs, 1H), 8.43 (bs, 1H), 10.04 (s, 1H), 11.51 (bs, 1H); MS *m*/*z* 306 (M^+). Anal. $C_{19}H_{14}O_4$ (C, H).

3,4-Bis(4-hydroxyphenyl)salicylaldoxime (1g). Compound **1g** was prepared from **14g** (0.014 g, 0.046 mmol) following the same procedure described above for **1b**. The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 1:1) to yield pure **1g** (0.012 g, 0.039 mmol, 84% yield) as a pale yellow solid: mp 234–235 °C; ^{1}H NMR (CD_3OD) δ 6.57 (AA′XX′, 2H, J_{AX} = 8.6 Hz, $J_{AA'/XX'}$ = 2.4 Hz), 6.64 (AA′XX′, 2H, J_{AX} = 8.6 Hz, $J_{AA'/XX'}$ = 2.3 Hz), 6.87 (AA′XX′, 2H, J_{AX} = 8.6 Hz, $J_{AA'/XX'}$ = 2.3 Hz), 6.91 (AA′XX′, 2H, J_{AX} = 8.6 Hz, $J_{AA'/XX'}$ = 2.3 Hz), 6.93 (d, 1H, *J* = 8.1 Hz), 7.23 (d, 1H, *J* = 8.1 Hz), 8.28 (s, 1H); MS *m*/*z* 321 (M^+). Anal. $C_{19}H_{15}NO_4$ (C, H, N).

Biological methods

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

Hormone binding assays. Relative binding affinities were determined by competitive radiometric binding assays using 10 nM [^{3}H]E_2 as tracer, using methods previously described.[27,28] The source of ER was either lamb uterine cytosol or purified full-length human ERα and ERβ purchased from Pan Vera (Madison, WI). Incubations were done at 0 °C for 18–24 h, and charcoal-dextran was used to adsorb the free ligand (uterine cytosol),[27] or hydroxylapatite was used to absorb the purified receptor-ligand complexes (human ERs).[28] The binding affinities are expressed as relative binding affinity (RBA) values, where the RBA of estradiol is 100%; under these conditions, the K_d of estradiol for uterine ER and ERα is ca. 0.2 nM, and for ERβ 0.5 nM. The determination of these RBA values is reproducible in separate experiments with a CV of 0.3 nM, and the values shown represent the average ± range or SD of 2-3 separate determinations.

Cell culture and transient transfections. Human endometrial cancer (HEC-1) cells were maintained in culture as described.[32] Transfection of HEC-1 cells in 24-well plates used a mixture of 0.35 mL of serum-free IMEM medium and 0.15 mL of HBSS containing 5 μL of lipofectin (Life Technologies, Rockville, MD), 1.6 μg of transferrin (Sigma, St. Louis, MO), 0.5 μg of pCMVβ-galactosidase as internal control, 1 μg of the reporter gene plasmid, 100 ng of ER expression vector, and carrier DNA to a total of 3 μg DNA per well. The cells were incubated at 37 °C in a 5% CO_2 containing incubator for 6 h. The medium was then replaced with fresh medium containing 5% charcoal-dextran treated calf serum and the desired concentrations of ligands. Reporter gene activity was assayed at 24 h after ligand addition. Luciferase activity, normalized for the internal control β-galactosidase activity, was assayed as described.[32]

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