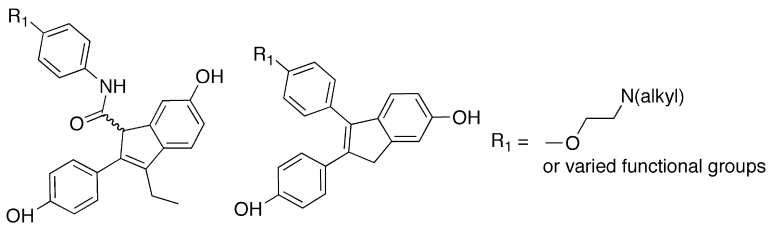
Journal of Medicinal Chemistry

Article

Differential Response of Estrogen Receptor Subtypes to 1,3-Diarylindene and 2,3-Diarylindene Ligands

Nicola J. Clegg, Sreenivasan Paruthiyil, Dale C. Leitman, and Thomas S. Scanlan

J. Med. Chem., **2005**, 48 (19), 5989-6003• DOI: 10.1021/jm050226i • Publication Date (Web): 19 August 2005



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 2 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Differential Response of Estrogen Receptor Subtypes to 1,3-Diarylindene and 2,3-Diarylindene Ligands

Nicola J. Clegg,[†,‡] Sreenivasan Paruthiyil,[||,⊥] Dale C. Leitman,[||,⊥] and Thomas S. Scanlan[†,‡,§,*]

Chemistry and Chemical Biology Graduate Program, Department of Pharmaceutical Chemistry, Department of Cellular and Molecular Pharmacology, Department of Obstetrics, Gynecology, and Reproductive Sciences, and Center for Reproductive Sciences, University of California, San Francisco, California 94143

Received March 11, 2005

Estrogen receptors (ERs) control transcription of genes important for normal human development and reproduction. The signaling networks are complex, and there is a need for a molecular level understanding of the roles of receptor subtypes ERα and ERβ in normal physiology and as therapeutic targets. We synthesized two series of ER ligands, based on a common indene scaffold, in an attempt to develop compounds that can selectively modulate ER-mediated transcription. The 3-ethyl-1,2-diarylindenes, utilizing an amide linker for the 1-aryl extension, bind weakly to the ERs. The 2,3-diarylindenes bind with high affinity to the ER subtypes and demonstrate a range of different biological activities, both in transcriptional reporter gene assays and inhibition of estradiol-stimulated proliferation of MCF-7 cells. Several ligands differentiate between ERα and ERβ subtypes at an estrogen response element (ERE), displaying various levels of partial to full agonist activity at ERα, while antagonizing estradiol action at ERβ.

Introduction

The estrogen receptor (ER) controls the transcription of genes important for developmental, reproductive, neural, skeletal, and cardiovascular processes. The natural ligand, estradiol, modulates ER activity at specific DNA response elements by binding to the two ER subtypes, ERα and ERβ, which results in recruitment of coregulatory complexes.[1] Several compounds have been developed as therapeutic agents to modulate ER transcriptional activity for treatment of diseases such as breast cancer and osteoporosis.[2] Unlike estradiol, selective ER modulators (SERMs) display both agonist and antagonist properties, depending on intrinsic cellular differences in processes or factors, such as cell-signaling pathways, accessory cofactors, and transcription factor modulatory proteins,[3] as well as on the DNA response element;[4] however, the molecular details for these selective ER modulating effects remains unclear.

There is evidence that SERMs regulate ER-mediated transcription by several different mechanisms,[5] which may involve the recruitment of distinct cofactor complexes to the response element.[6,7] The triphenylethylene scaffold of tamoxifen is capable of producing a range of tissue-specific effects that warrant further investigation at a biological level.[5,8] However, facile *E*/*Z* isomerization via a tautomeric quinoid intermediate is encountered in high-affinity derivatives containing the 4-hydroxylated triphenylethylene structure, such as 4-hydroxytamoxifen (OHT) (Figure 1a).[9] The associated difficulties in compound synthesis, purification, and biocharacterization,[5,10,11] prompted our search for a new scaffold that was synthetically amenable to derivatization, for development of novel SERMs.

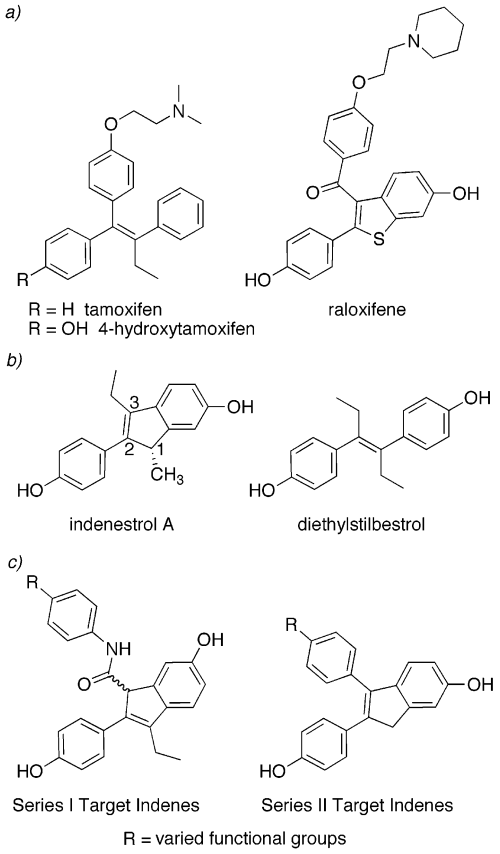


Figure 1. (a) Structures of prototypic SERMs. (b) Indenestrol A is a metabolite of diethylstilbestrol. (c) Structures of the Series I and Series II target indenes.

* To whom correspondence should be addressed: Department of Pharmaceutical Chemistry, University of California San Francisco, Genentech Hall, 600 16th Street Box 2280, San Francisco, CA 94143-2280. Telephone: (415) 476-3620. Fax: (415) 502-7220. E-mail: scanlan@cgl.ucsf.edu.

† Chemistry and Chemical Biology Graduate Program.

‡ Department of Pharmaceutical Chemistry.

§ Department of Cellular and Molecular Pharmacology.

|| Department of Obstetrics, Gynecology, and Reproductive Sciences.

⊥ Center for Reproductive Sciences.

10.1021/jm050226i CCC: $30.25
Published on Web 08/19/2005

We desired a scaffold with a high likelihood of ER activity, for which the pharmacology had not yet been extensively explored, thereby maximizing the likelihood of discovering compounds with novel properties. Several derivatives have already been investigated to circumvent the problems with the 4-hydroxylated triphenylethylene scaffold.[12−14] Precedence for our selection of an indene-based scaffold arises from reports of indenestrol A,[15−18] a diethylstilbestrol (DES) metabolite which is poorly uterotrophic,[19] but has strong binding affinity for ERα and acts as an agonist at an ERE.[20] Limited SAR studies of 1,3-dialkyl-2-arylindenes have highlighted the importance of the C-3 stereochemistry and the phenolic hydroxyls for binding and activity[21−25] and demonstrated a preference for an ethyl substituent at the 3-position of the indene ring.[26] A limited series of basic 2,3-diarylindenes was examined for antifertility properties in rats during the 1960s,[27,28] followed by analysis of the binding orientation and fluorescent properties of the 2,3-diarylindenes[21,29−33] and limited SAR studies.[34] Recently, basic 2-aryl-3-benzylindenes and -benzylindanes have been investigated for their SERM effects in bone and the cardiovascular system.[35]

The currently known SERMs, along with the indene SAR data described above, provided a starting point for synthesis of pathway-specific probes based on the unifying diarylindene structure (Figure 1c). Aryl extensions were built off the core indene scaffold at either the 1- or the 3-position, analogous to the extensions of the prototypical SERMs (Figure 1a). These extensions are known to disrupt the conformation of the ERs by displacing helix 12, thus preventing interaction with coactivator proteins.[36−38] The Series I indenes, incorporating an amide-linked aryl extension off carbon-1 of the core scaffold, bind only weakly to the receptor, whereas the 2,3-diarylindenes of Series II bind tightly to the ERs. Subtle changes to the functional R-group of Series II (Figure 1c) produce different biological activities, both in transcriptional reporter gene assays, and inhibition of estradiol-stimulated proliferation of MCF-7 cells. Several of the Series II ligands can differentiate between the ERα and ERβ subtypes at an ERE and display various levels of partial to full agonist activity at ERα, while antagonizing estradiol action at ERβ.

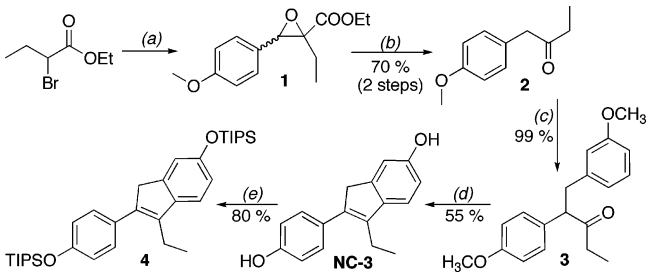
Results and Discussion

Synthesis. The first series of indene ligands (Series I) are based on the 3-ethyl-1,2-diarylindene structure (Figure 1c). The 1-position of indenestrol A (Figure 1b) was already known to accommodate an alkyl extension of up to at least four carbons, without loss in binding affinity,[26,39] indicating a prime target site for derivatization with aryl-based extensions. Because the target indene structure contains a stereocenter at C-1, all compounds were prepared as racemic mixtures.[38,40−42] The core 3-ethyl-2-arylindene scaffold (**4**) was synthesized by Lewis-acid induced cyclodehydration of a ketone intermediate (**3**), following adaptations to a published synthetic route[43,44] (Scheme 1). Boron tribromide was used for the cyclodehydration of **3**, because of its chelating effect, which sterically prevented formation of undesirable isomers obtained using polyphosphoric acid.

We initially envisaged the use of organometallic coupling methods to synthesize target compounds having either a ketone or a direct carbon−carbon linker between the aryl extension and the 1-position of the core indene scaffold. Synthetic difficulties associated with the high stability of the aromatic indenyl anion,[45,46] prevented derivatization of **4** to the requisite indenyl bromide or boronic acid for Suzuki couplings. Attempts at reaction of the indenyllithium with benzyl bromide or an aryl Weinreb's amide were also unsuccessful, and coupling with benzaldehyde derivatives gave mixed products. Changes to temperature or solvent polarity, use of alternate metal cations, and addition of HMPA had no effect on reactivity. However, conversion of indene **4** to the indenyl carboxylate **6** was successful by direct reaction of the anion with carbon dioxide (Scheme 2). Our plans for conversion of the indenyl carboxylate **6** to a Weinreb's amide, for reaction with aryllithium or arylmagnesium bromide derivatives, again proved fruitless, due to unreactivity of the indenyl Weinreb's amide derivative. However, the ease of formation of the amide by means of peptide coupling methods, convinced us to redesign the compounds to target the amide-linked diarylindenes of Series I.

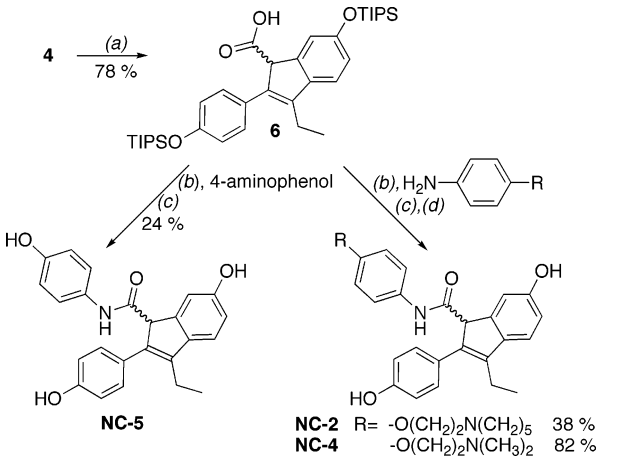
Aniline derivatives were coupled to the indenyl carboxylate (**6**) using HBTU with catalytic DMAP (Scheme 2). Deprotection of the silyl ethers to give the target indenes of Series I was accomplished with triethylamine−trihydrogen fluoride, rather than tetrabutylammonium fluoride, which resulted in degradation. In the case of the indenes with the basic side-chains (**NC-2**, **NC-4**), this meant that the final products were

Scheme 1[a]



[a] Reagents and conditions: (a) Lin(TMS)$_2$, THF, −78 °C, *p*-methoxybenzaldehyde; (b) (i) NaOH, EtOH, reflux, (ii) heat; (c) (i) NaH, THF, (ii) 3-methoxybenzyle bromide, 50 °C; (d) BBr_3, DCM, −78 → 25 °C; (e) TlPSCl, imidazole, CH_2Cl_2.

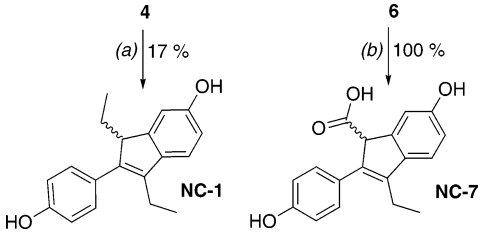
Scheme 2[a]



[a] Reagents and conditions: (a) (i) *n*-BuLi, THF, −78 → 25 °C, (ii) CO_2(s), (b) (i) HBTU, DMAP, CH_2Cl_2, (ii) (i-Pr$_2$)EtN, 4-aminophenol derivative, (c) Et_3N-3HF, THF, (d) HCl, ether.

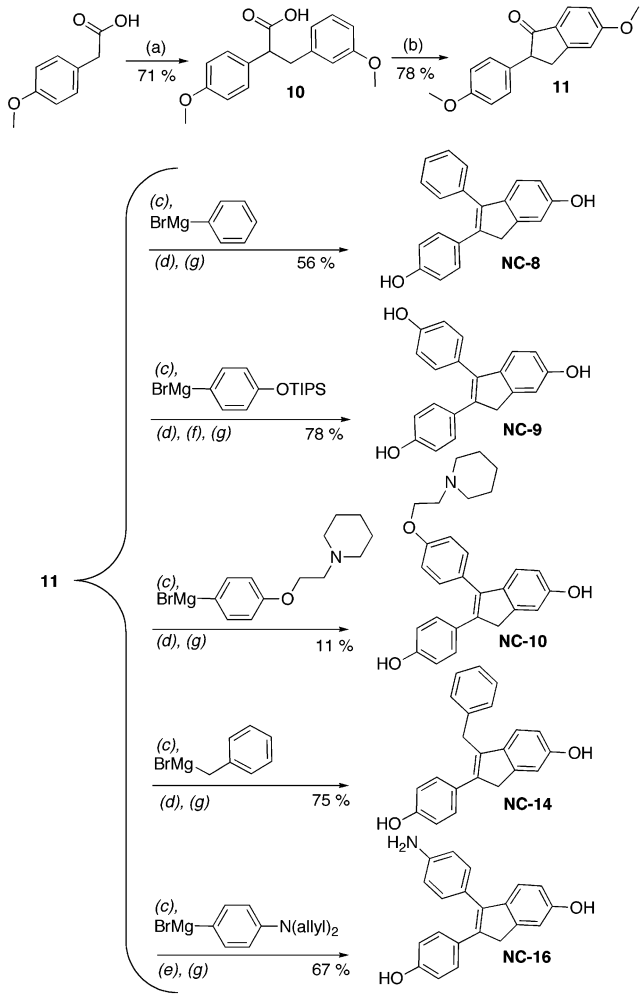
Aniline derivatives were coupled to the indenyl carboxylate (**6**) using HBTU with catalytic DMAP (Scheme 2). Deprotection of the silyl ethers to give the target indenes of Series I was accomplished with triethylamine−trihydrogen fluoride, rather than tetrabutylammonium fluoride, which resulted in degradation. In the case of the indenes with the basic side-chains (**NC-2**, **NC-4**), this meant that the final products were

Scheme 3[a]



[a] Reagents and conditions: (a) (i) *n*-BuLi, ether, −78 °C → 0 °C, (ii) EtI, (iii) TBAF, THF, (b) $Et_3N{\cdot}3HF$, THF.

Scheme 4[a]

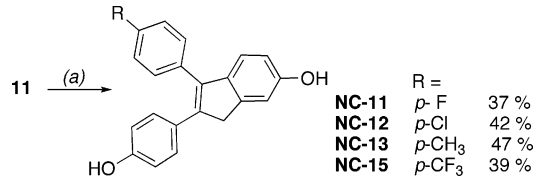


[a] Reagents and conditions: (a) KHMDS, THF, 3-methoxybenzyl bromide, (b) (i) oxalyl chloride, CH_2Cl_2, (ii) $AlCl_3$, CH_2Cl_2, (c) THF, Grignard reagent, (d) HCl, (e) NDMBA, $Pd(PPh_3)_4$, CH_2Cl_2, (f) $Et_3N{\cdot}3HF$, THF, (g) BBr_3, DCM.

isolated as the HCl salts. In addition to **NC-3**, which was prepared during the synthesis of the core indene scaffold, several other indenes lacking a bulky aryl extension were prepared from intermediates in the synthetic pathway (Scheme 3).

Series II indenes consist of a 2,3-diarylindene structure, in which the aryl extension is directly attached to carbon-3 of the core scaffold (Figure 1c). The general synthesis via an indanone intermediate **11** is based on previously developed routes[27,28] (Scheme 4). Literature methods cite various Lewis acids as effective in the cyclization of the acid;[27,28,47,48] however, we found the most effective route to be conversion of carboxylate **10**

Scheme 5[a]



[a] Reagents and conditions: (a) MgX-PhR (X = Br or Cl), THF, (b) HCl, (c) BBr_3, DCM.

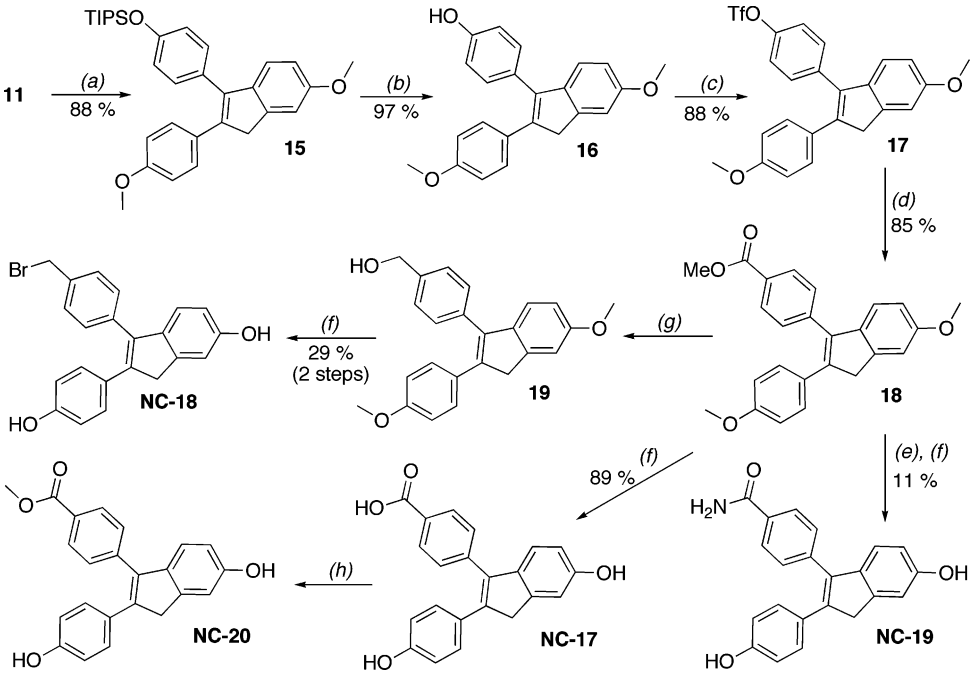
to the acid chloride, followed by Friedel−Crafts acylation to give indanone **11**. Because the fused aryl ring contains an activating group, ring closure is directed to the 6-position, resulting in formation of only the desired isomer. Indanone **11** can be derivatized using a variety of aryl Grignard reagents (Schemes 4−6). Subsequent acid workup and deprotection with boron tribromide yields the target Series II indenes, which display a variety of functional groups, differing in the charge, electron-withdrawing effects, geometry, and polarity. The indene products must be protected from air, humidity, and light, due to facile air-oxidation resulting in formation of 2,3-diarylindenones.[29]

The synthesis of **NC-8** through **NC-16** is depicted in Schemes 4 and 5. The synthesis of **NC-17**, **N-18**, **N-19**, and **NC-20** proceeded via a methyl ester intermediate (**18**), which was synthesized from triflate **17** via palladium-catalyzed alkoxycarbonylation[49] (Scheme 6). Reaction of ester **18** with boron tribromide resulted in saponification and deprotection of the methyl ethers to give the carboxylic acid derivative **NC-17**. Reduction of the ester **18** with DIBAL-H produced the benzyl alcohol **19**. Treatment of **19** with boron tribromide not only deprotected the methyl ethers, but also converted the alcohol to a bromide (**NC-18**), presumably as the result of excess hydrogen bromide. We were unable to effect selective deprotection of the phenolic methyl ethers of ester **18**, to produce **NC-20**. However, addition of methanol to a solution of the acid (**NC-17**) and lithium aluminum hydride resulted in formation of the methyl ester (**NC-20**). The amide **NC-19** was produced by amination of ester **18** with trimethylaluminum-ammonium chloride.

Estrogen Receptor Binding Affinity. All target compounds were tested for binding affinity to full-length ERα and ERβ in a competition assay with a fixed concentration of fluorescent estradiol ligand, using fluorescence polarization as a read-out. Results are shown in Table 1. Both indenes **NC-1** (ethyl indenestrol A) and **NC-3** (indenestrol A), which lack an aryl extension, show binding affinities for ERα and ERβ that are comparable to, or better than, OHT. This is in general agreement with previous studies for ERα.[26] Although **NC-7** also lacks an aryl extension, it contains a charged carboxylate group, which may explain its reduced affinity for both ER subtypes (only 10% of the affinity of OHT).

Of note, is that the 3-ethyl-1,3-diarylindenes of Series I that contain the amide-linked 1-aryl extension (**NC-2**, **-4**, and **-5**), all show poor binding affinity for both ER subtypes, less than 1% of the affinity of OHT. In contrast, the 2,3-diarylindenes of Series II (**NC-8** through **NC-20**) show fair binding to both ERα and ERβ, on the order of the affinity of raloxifene, and on average about

Scheme 6[a]



[a] Reagents and conditions: (a) 4-(triisopropylsiloxy)phenylmagnesium bromide, THF, (b) $Et_3N{\cdot}3HF$, THF, (c) Tf_2O, 2,6-lutidine, CH_2Cl_2, 0 → 25 °C, (d) CO, $Pd(OAc)_2$/dppp. DMF, MeOH, Et_3N, (e) $AlMe_3$-NH_4Cl, benzene, (f) BBr_3, CH_2Cl_2, −78 → 25 °C, (g) DIBAL-H, THF, (h) $LiAlH_4$, THF, MeOH.

10-fold weaker than OHT. The major exception is **NC-17** that has a negatively charged carboxylate group, and therefore very poor affinity for the ERs as a result of unfavorable interactions with the receptor (1000-fold worse than OHT).

The weak affinity of the Series I indenes was unanticipated and suggests that the amide linkage may interact unfavorably with residues in the ligand binding pocket. Interestingly, research on the related spiroindene ligand scaffold has also found that a carbonyl linker was detrimental to binding affinity, despite modeling predictions to the contrary.[50]

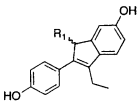
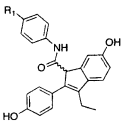
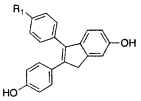
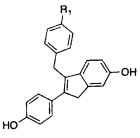
With the exception of **NC-3**, none of the indene ligands showed significant binding preference for either of the ER subtypes. **NC-3** has a 9-fold preference in binding affinity for ERβ in this assay, only slightly higher than the 5-fold ERβ preference shown by estradiol. This increased affinity of **NC-3** for ERβ may be due to its lack of a functional group at the 1-position of the indene ring, the absence of which may avoid potentially unfavorable steric interactions with the Met354 residue in the ERβ binding pocket. However, modeling studies are necessary to further validate this hypothesis.

ER Subtype-Selective Ligand Activity at an ERE. All the indene ligands were tested for their effects on ER-mediated transcription in U2OS cells using high throughput reporter gene assays. A luciferase reporter gene construct regulated by a consensus ERE (the vitellogenin A2 ERE-tk promoter) was used. On the basis of the level of efficacy achieved in the dose−response assays, the ligands were classified as 'full agonists' (efficacy > 60%) or 'partial agonists' (10−59% efficacy). Ligands that were able to abolish the agonist activity of estradiol in competition assays, were termed 'full antagonists' (inhibit to basal activity levels). Ligands defined as partial agonists can, in competition assays with estradiol, display some partial antagonist properties (inhibit down to the levels of partial activation).

In dose−response assays, the ligands without an aryl extension, **NC-1** and **NC-3**, were potent agonists with both ER subtypes at an ERE (EC_{50}s in the range of 0.1 to 0.3 nM) (Table 2). **NC-7** showed lower potency (EC_{50} = 50−160 nM), as a result of its weaker binding affinity. The poor affinity of the Series I indenes (**NC-2**, **-4**, and **-5**), translated into very weak agonist activity at ERα/ERE in dose−response assays (EC_{50} = 100−430 nM). Despite the bulky aryl extensions of these ligands, the weak interactions with the receptor must be insufficient to induce either an optimal agonist conformation, or an optimal antagonist conformation with full displacement of helix 12.[36−38] **NC-4**, with the dimethylamino extension analogous to OHT, is actually a full agonist of both ERα and of ERβ, albeit at very high concentrations (EC_{50} = 150 nM for ERα and 500 nM for ERβ). **NC-2** and **NC-5** have little or no effect on ERβ, although high concentrations of **NC-2** do result in weak antagonism of estradiol in competition assays (K_i = 147 nM).

The most interesting activities are seen with the Series II 2,3-diarylindenes. These ligands display a wide range of activities at both ERα and ERβ and in several cases are able to differentiate between the receptor subtypes, acting as agonists at one subtype and antagonists at the other (Table 2). In agreement with the binding data, the agonist potencies or EC_{50}s of most of the Series II ligands are on the order of 4−17 nM, which is 100-fold less potent than E_2, and the antagonist potencies or K_is, range from 0.2 to15 nM which are 10−100 fold weaker than OHT. However, these potencies are sufficient for the ligands to be effective ER agonists and antagonists in vitro. **NC-17** is the only Series II indene ligand that is virtually inactive for both ERs, due to its weak affinity for the receptors.

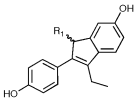
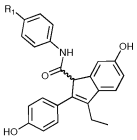
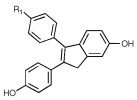
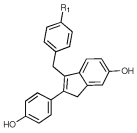
Table 1. Relative Binding Affinities of the Series I and Series II Indene Ligands for Full Length ERα and ERβ, Determined by a Competitive Binding Assay Kit with Fluorescently Labeled Estradiol, Using Methods Described in the Experimental Section

Ligand	R_1 =	Relative Binding Affinity[a] ERα	ERβ
estradiol		380	2100
4-hydroxytamoxifen		100	100
raloxifene		18	4
Indenes lacking an aryl extension			
 NC-1 (Et indenestrol A)	Et	28	118
NC-3 (indenestrol A)	H	209	1772
NC-7	COOH	12	8
Series I indenes			
 NC-2	$O(CH_2)_2N(C_5H_{10})$	1	0.7
NC-4	$O(CH_2)_2N(CH_3)_2$	0.4	0.4
NC-5	OH	0.1	0.1
Series II indenes			
 NC-8	H	9	5
NC-9	OH	4	4
NC-10	$O(CH_2)_2N(C_5H_{10})$	10	4
NC-11	F	20	14
NC-12	Cl	20	15
NC-13	CH_3	14	7
NC-15	CF_3	7	3
NC-16	NH_2	13	7
NC-17	COOH	0.2	0.4
NC-18	CH_2Br	2	1
NC-19	$C(O)NH_2$	12	18
NC-20	$C(O)CH_3$	1	1
 NC-14	H	15	5

[a] Relative binding affinities are expressed as a percentage of the potency of 4-hydroxytamoxifen, found to have a K_i of 5.7 nM for ERα and 1.7 nM for ERβ.

Dose−response assays determine **NC-14** to be a full agonist at both ERα and ERβ. This is the only ligand with a methylene-linked phenyl extension. The directly linked phenyl equivalent, **NC-8**, displays full agonist activity at ERα and partial agonist activity at ERβ. All the other Series II indenes show virtually no agonist activity at ERβ, and competition experiments versus a fixed estradiol concentration show them to be partial to full antagonists at ERβ. The activity of these same ligands at ERα ranges from full agonism (**NC-11**, **-13**), to partial agonism (**NC-9**, **-12**, **-15**, **-16**, **-18**), to full antagonism (**NC-10**, **-19**, **-20**). This suggests

Table 2. Transcriptional Activity of Series I and Series II Indenes on ERα and ERβ at a Classical ERE in U2OS Cells. Transient Transfection Assays Were Performed as Described in the Experimental Section Using ERα and ERβ Expression Vectors and an Estrogen-Regulated Luciferase Reporter Gene Plasmid

Ligand		Agonism[a]				Antagonism[a]	
		ERα		ERβ		ERα	ERβ
	R_1 =	EC_{50} (nM)	efficacy	EC_{50} (nM)	efficacy	K_i (nM)	K_i (nM)
estradiol		0.019±0.004	100%	0.098±0.024	100%	-	-
OHT		-	-	-	-	0.04±0.010	0.014±0.003
raloxifene		-	-	-	-	0.03±0.004	4.5 ± 2.0
Indenes lacking an aryl extension							
 NC-1 (EIA)	Et	0.059±0.012	102±13%	0.30 ± 0.13	75 ± 6%	-	-
NC-3 (IA)	H	0.31 ± 0.13	69 ±10%	0.30 ± 0.083	87 ± 13%	-	-
NC-7	COOH	158 ± 6	110±12%	52 ± 18	90 ± 7%	-	-
Series I indenes							
 NC-2	$O(CH_2)_2N(C_5H_{10})$	100	32 ± 3%	-	-	184 ± 29	147 ± 99
NC-4	$O(CH_2)_2N(CH_3)_2$	152 ± 25	96 ± 9%	512 ± 182	83 ± 13%	-	-
NC-5	OH	427	16 ±16%	-	-	-	-
Series II indenes							
 NC-8	H	4.3 ± 1.6	90 ±12%	54 ± 17	30 ± 3%	-	-
NC-9	OH	7.0	11 ± 6%	-	-	8.5 ± 3.3	1.3 ± 0.4
NC-10	$O(CH_2)_2N(C_5H_{10})$	-	-	-	-	1.7 ± 0.5	0.19 ± 0.0
NC-11	F	3.9 ± 1.7	77 ± 4%	10 ± 0.3	13 ± 1%	-	2.8 ± 1.0
NC-12	Cl	17 ± 8	57 ± 3%	-	-	24	0.49 ± 0.18
NC-13	CH_3	17 ± 7	73 ± 3%	-	-	-	1.3 ± 0.3
NC-15	CF_3	15 ± 5	50 ± 8%	-	-	2.1	0.91 ± 0.27
NC-16	NH_2	6.7 ± 1.8	54 ± 5%	-	-	0.67	2.2 ± 0.73
NC-17	COOH	-	-	-	-	-	421 ± 304
NC-18	CH_2Br	109 ± 31	25 ± 6%	-	-	59 ± 17	15 ± 7
NC-19	$C(O)NH_2$	-	-	-	-	4.2 ± 1.3	0.46 ± 0.16
NC-20	$C(O)CH_3$	-	-	-	-	7.3 ± 1.7	3.8 ± 1.3
 NC-14	H	14 ± 3	77 ± 3%	37 ± 9	64 ± 7%	-	-

[a] Values represent the average of multiple experiments for which all data points were determined in triplicate. Agonist assays are done with test compound alone; antagonist assays are done with compound together with 0.1 nM estradiol. EC_{50}s and K_is were calculated with Prism software analysis tools from dose−response and competition curves, as described in the Experimental Section. Compounds are classified as either full agonists (efficacy > 60%), full antagonists versus 0.1 nM estradiol (inhibit to basal activity levels), or partial agonists (10−59% efficacy) that can also display partial antagonist properties (inhibit down to the levels of partial activation in competition assays). Refer to text for details.

that ERβ is more sensitive to ligand structure than ERα, and that it can convert from an agonist to antagonist conformation more readily.[51] For example, the addition of a phenolic hydroxyl to **NC-8** converts it from an ERα full agonist/ERβ partial agonist into an ERα partial agonist/ERβ antagonist (**NC-9**). Interestingly, a similar conversion is seen with 3-ethyl-2,4,5-tris(4-hydroxyphenyl)furan, an ERα-selective furan ligand that is inactive at ERβ, but converts to an ERβ agonist merely by removal of the 5-phenolic hydroxyl.[52]

The above results demonstrate that very small changes to the indene ligand structure can have drastic effects on the transcriptional activity of the ER subtypes at an ERE. The indene ligand scaffold has provided access to a range of subtype-selective ER ligands. There is no distinct correlation between the properties of the

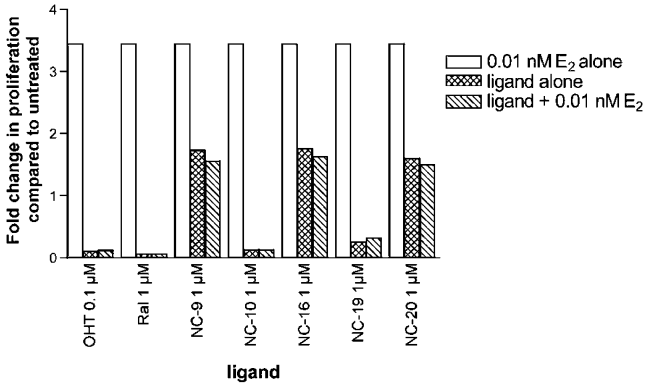


Figure 2. Antiproliferative effect of Series II indene ligands on estradiol-stimulated MCF-7 cells. Cells were treated with 0.01 nM estradiol alone (open bars), with test compound alone (double cross-hatched bars), or with both 0.01 nM estradiol and test compound, and incubated for 7 days, with replacement of media and hormones after day 3. The extent of proliferation was quantified by incorporation of tritiated thymidine, and results were reported as fold proliferation compared to the no hormone control.

functional groups and the efficacies or potencies of activation at ERα. For example, the methyl (**NC-15**) and fluoride (**NC-11**) derivatives are more efficacious than the chloride (**NC-12**) and trifluoromethyl derivatives (**NC-15**). The ethyl bromide (**NC-18**) shows lower overall potencies, which may be due to covalent modification of lysines or other electron-donating residues. In addition, there may be differences in the cell-permeability of the indenes, which may also contribute to apparent differences in potency. Indeed, **NC-8**, **-9**, **-10**, **-16**, **-18**, **-19**, and **-20** were soluble only to 10 mM in ethanol stock solution (**NC-16** and **NC-18** were sparingly so), whereas other ligands of Series II were soluble at 100 mM.

Antiproliferative Effects in MCF-7 Cells. To study the pharmacological activity of the Series II indene ligands in MCF-7 breast cancer cells, we screened a representative subset of ligands, to determine whether, at saturating concentrations, they could block the 4-fold proliferative effect induced by 0.01 nM estradiol. The extent of proliferation was quantified by incorporation of tritiated thymidine into the newly synthesized DNA of the dividing cells. MCF-7 cells express ERα at high levels; thus it follows that the ligands that show most potent antagonism at ERα/ERE in reporter gene assays, namely **NC-9**, **-16**, and **-20**, are likely to be most effective at preventing E_2-stimulated proliferation in these cells. As expected, both prototypic SERMs, OHT and raloxifene, inhibited proliferation completely at saturating levels of ligand (Figure 2). Saturating levels of **NC-10** and **NC-19** also resulted in close to complete inhibition. **NC-9**, **-16**, and **-20** induced 2-fold proliferation of cells upon ligand treatment in the absence of E_2. Therefore, in the presence of E_2, the 4-fold proliferative effect of E_2 was inhibited by 2-fold. The remaining indene ligands demonstrated no antiproliferative effects. These results indicate that the data from reporter gene assays do correlate to ligand effects in a more physiological context using breast cancer cells that express endogenous receptors.

The selective ERα agonist and ERβ antagonist properties of the Series II indenes (**NC-11**, **-13**, **-9**, **-12**, **-15**, **-16**, and **-18**) are similar to the *R*,*R*-tetrahydrochrysene ligand (*R*,*R*-THC), which acts as an agonist on ERα at ERE but an antagonist on ERβ.[53] SAR data exploring the role of substituent size and stereochemistry in determining agonist versus antagonist activity of the tetrahydrochrysenes, determined that all the THC derivatives were agonists on ERα, and those with small substituents were also agonists on ERβ.[38] Of note is that the induction of an antagonist conformation in ERβ was achieved with less steric perturbation than in ERα, as is also the case with the Series II indene ligands.

There are three Series II indene ligands that are full antagonists of E_2 activity at both ERα and ERβ. **NC-10** has an *N*-piperidinyl extension group analogous to raloxifene and is likely to function in the same way as the prototypical SERMs, by displacing helix 12 as a result of the bulky extension group, thereby disrupting the coactivator binding interface.[36−38] On the other hand, **NC-19** and **NC-20** have much smaller aryl extensions, containing an amide and a methyl ester, respectively. In this respect, they are similar to *R*,*R*-THC, which functions as an antagonist on ERβ but has a structure that is very different from the bulky side-chain extensions of the typical antiestrogens tamoxifen and raloxifene.[53] The crystal structures of *R*,*R*-THC bound to the ERα and ERβ ligand binding domains provide an interesting insight into its novel mechanisms for selectivity.[54] In the case of ERα, *R*,*R*-THC is buried within the ligand binding pocket, however, for ERβ, helix 12 is unable to close over the opening to the pocket and adopts an antiestrogenic conformation, despite the lack of a bulky side-chain as is the case for the other SERMs. It appears that *R*,*R*-THC stabilizes a nonproductive conformation of key residues within the binding pocket, that allosterically act to prevent progression to an active agonist complex. This has been termed 'passive antagonism'.[54] We believe that the interactions in the receptor binding pocket with the carbonyl functionality of the extensions of **NC-19** and **NC-20** may result in a similar allosteric effect on the receptor conformation, which translates into a form of 'passive antagonism'.[54,55] We hypothesize that the subtype selective effects of the Series II ligands on ER transcriptional activity, result from differences between the interactions of the ligand functional groups with residues within the ligand binding pocket. This in turn leads to differences in the allosteric communication with distal secondary structural interactions, both within the ER, and with other interacting proteins, thereby influencing cofactor recruitment.[55]

Conclusion

Using a common indene scaffold, we have developed two series of indene ligands with a range of selective activities at the ER subtypes. This subtype selectivity is particularly striking, because it results from only very small changes in the ligand structure. Several functional groups were explored in the side-chain of this series in order to refine their selectivity and to gain insight into how the chemical structure of a ligand relates to the effect on ER mediated signaling. Previously characterized SERMs such as raloxifene possess a bulky basic side-chain which displaces helix 12 of the receptor, disrupting the interaction of coactivators necessary for transcriptional activation. The indene ligands do not

have the same large bulky side-chain and would appear to disrupt the receptor conformation in a more subtle way, by means of 'passive antagonism'.

Several of the Series II indenes show various levels of agonist activity at ERα, while possessing full antagonist activity at ERβ. In particular, **NC-13** is a full agonist at ERα, whereas it fully antagonizes activation by estradiol at ERβ. These indenes can be used to investigate the mechanisms of ligand-mediated transcriptional activation or repression of ER target genes and to study the biological roles of ERα and ERβ. Understanding the molecular events that convert a drug from being predominantly antiestrogenic to being estrogenic could potentially open the door to a better understanding of the molecular mechanism of SERM action, and ultimately, the use of our Series II selective indene ligands in a combination of structural, biochemical, cellular, and physiological studies could enable a greater understanding of how ligand−receptor conformations relate to estrogen agonist or antagonist behavior. This has significant implication for the design of more effective drugs in the treatment of diseases such as breast cancer and osteoporosis.

Experimental Section

Materials and Methods: Molecular Biology. The construction of the expression vectors for both hERα (HEG0) and hERβ have been previously described.[4,56] The wild-type ERα (HEG0) and the longer form of ERβ (ERβ1) with 149 residues in the N-terminal domain were used in transfection experiments. The ERE-driven luciferase reporter gene consists of two repeats of the upstream region of the vitellogenin ERE promoter from −331 to −289, followed by region −109 to +45 upstream of the thymidilate kinase, followed by the luciferase gene.[4,5,56]

ER Binding Assays. The relative binding affinities of compounds for full length ERα and ERβ were determined using ERα and ERβ competitor assay kits (green), according to the manufacturer's instructions (PanVera Corp, Madison, WI). Fluorescence polarization was used as a read-out, and the experiments were performed in 96-well plates (Costar black polystyrene round-bottom assay plates #3792) and read (five readings per well; 0.1 s integration time; entire plate read eight times; G factor = 0.91) using the Analyst AD plate-reader (LJL Biosystems) with fluorescein excitation (485 nM) and emission (530 nM) filters. Each hormone dose was performed in triplicate, and the relative error was determined by calculating the standard error of the three values from the mean. In all cases we controlled for minimal competition (ethanol vehicle alone), for no ER, for no fluorescent ligand, and for maximal competition (10^{-5} M E_2). The binding curves were fit using a single binding site competition model, with the Prism statistical analysis software package. The R^2 values in all cases were greater than 0.8. Experiments were conducted multiple times to ensure reproducibility of the results, and the standard error of the mean (SEM) was less than 0.2 log units from the average logIC_{50} value in all cases. K_i values were reported as averages across experiments along with SEM. Percent relative binding affinity (RBA) was then determined by dividing the K_i determined for unlabeled 4-hydroxytamoxifen by the test ligand K_i and then multiplying the result by 100.

Tissue Culture, Transfection and Luciferase Assays. U2OS human osteosarcoma cells were grown in 0.1 μm filtered DME/H21 growth medium, supplemented with 4.5 g/L glucose, 0.876 g/L glutamine, 100 mg/L streptomycin sulfate, 100 units/mL of penicillin G, and 10% newborn calf serum. Cells were grown to a confluence of no more than 80%. For transient transfection assays, cells were trypsinized, washed with PBS, and resuspended in 0.5 mLof electroporation buffer in 0.4 cm gap electroporation cuvettes at approximately 1.8×10^6 cells per cuvette, together with 5 μg of the reporter plasmid and 2.5 μg of the ERα or ERβ expression vector, determined as optimal for the ERE response. The electroporation buffer consisted of 0.2 μm filtered PBS and 0.1% glucose. Cells were transfected by electroporation at a potential of 0.25 kV and a capacitance of 960 mF. Transfected cells were pooled and immediately resuspended in assay medium. The assay medium was prepared by the UCSF Cell Culture facility and consisted of 1 μm-filtered DME/F12 Ham's media 1:1 without phenol red, with 15 mM HEPES and L-glutamine (Sigma #D-2906), supplemented with 1.338 g/L $NaHCO_3$, as well as with 100 mg/L streptomycin sulfate, 100 units/mL of penicillin G, and 10% heat-inactivated and hormone-depleted newborn calf serum. This is serum that had been incubated at 56 °C for 30 min to inactivate complement, followed by treatment with dextran-coated charcoal as described previously.[4] Cells were plated into 96-well Costar assay plates (#3917, opaque flat bottom with lid, tissue culture treated) at 100 μL assay medium per well, and at a density of approximately 1.5×10^4 cells per well for ERα, and 1.75×10^4 cells per well for ERβ. After 6 to 8 h of incubation at 37 °C, to allow for adherence of the cells, the media was removed, and fresh assay media was added to the plates, along with 10-fold serial dilutions of hormones, prepared in triplicate. The maximum concentration of ethanol vehicle in all cases was 1% of the total volume. In the case of the dose−response experiments, the serial dilutions were done in the tissue culture plates, taking care not to disturb the cells. In the case of the competition experiments, a constant concentration of estradiol was required, so the serial dilutions of test ligand were done on a separate plate and transferred over to the wells of plated cells. Controls were performed in all cases for no hormone response (ethanol vehicle alone) and for maximal activation (10^{-7} M estradiol). In the case of competition experiments, additional controls were performed for maximal antagonism of estradiol (10^{-7} M 4-hydroxytamoxifen).

After 12 to 24 h of incubation at 37 °C, the cells were lysed by first removing the media from the wells, washing with 100 μL/well of PBS (Ca^{2+}- and Mg^{2+}-free) and then adding 40 μL/well of passive lysis buffer (Promega #E194A) at room temperature. The plates were incubated for 20 to 30 min on a shaker and then combined with reconstituted luciferase assay buffer (Promega Luciferase Assay System #E1501) at room temperature. Luminescence was immediately measured for 0.1 s/well on an Analyst AD plate-reader (LJL Biosystems), with prior shaking of the plate (5 s) to ensure mixing of the reagents.

In dose−response experiments, the transcriptional activation with increasing amounts of added ligand yielded an EC_{50} value, indicative of the potency of ER activation at the ERE. Antagonist properties were measured in competition assays, where an increasing amount of ligand is added to compete away a constant level of 10^{-10} M E_2 agonist, to calculate an IC_{50} value, which is then converted to a K_i. Each hormone dose was performed in triplicate, and the relative error was determined by calculating the standard error of the three values from the mean. The curves were fit with the Prism statistical analysis software package, using either the sigmoidal dose−response curve for dose−response experiments, or the single binding site competition model for competition experiments. The R^2 values in all cases were greater than 0.8. Experiments were conducted multiple times to ensure reproducibility of the results, and the standard error of the mean (SEM) was less than 0.2 log units from the average log EC_{50} or log IC_{50} value in all cases. EC_{50} or K_i values were reported as averages across experiments along with SEM.

Cell Proliferation Assays. Assays to determine the antiproliferative effects of selected indene test ligands on estradiol-stimulated MCF-7 human breast cancer cells were performed according to published procedures.[57] Briefly, cells were treated with both 10^{-11} M estradiol and a saturating concentration of test ligand and incubated for 7 days, with replacement of media and hormones after day 3. The extent of proliferation was quantified by incorporation of tritiated thymidine into the

newly synthesized DNA of the dividing cells, and results were reported as fold proliferation compared to the no hormone control.

Materials and Methods: Chemistry. ^{1}H and ^{13}C NMR spectra were obtained on a Varian Model AS 400 (400 MHz) instrument. ^{1}H NMR chemical shifts are reported as δ values in ppm downfield from internal tetramethylsilane or downfield from residual H_2O peak in CD_3OD. ^{13}C NMR chemical shifts are reported as δ values with reference to the solvent peak. High-resolution mass spectrometry (HRMS) using electrospray ionization (EI) was performed by the National Bio-Organic, Biomedical Mass Spectrometry Resource at the University of California, San Francisco. In instances that fast atom bombardment (FAB) was required, HRMS was performed by the Mass Spectrometry Facility at the University of California, Berkeley. Flash column chromatography on crude products was performed using 230−400 mesh silica gel (Aldrich Chemical Co.) or 40−63 μm EMD Geduran Silica Gel 60 (VWR International). Preparative TLC (pTLC) was run using glass precoated silica gel plates (250 μm, particle size 5 to 17 μm, pore size 60 Å, 20 × 20 cm; Aldrich Chemical Co. Z12272-6). Purity of all compounds was determined by TLC using commercial silica gel plates (Alltech, Alugram Sil B/UV 254) and by ^{1}H NMR and HRMS. Developed TLC plates were visualized using short wave UV light (254 nm) and were typically stained using a *p*-anisaldehyde solution followed by heating with a heat gun. For final compounds, purity was assessed as 95% pure or greater unless otherwise noted, by analytical reverse phase high-pressure liquid chromatography (RP-HPLC). This was done using an Alliance 2695 Separations Module with a Waters 2996 photodiode array detector, together with two different columns: a XTerra RP18 Column (3.5 μm; 4.6 × 50 mm) and a YMC-Pack Pro C4 Column (5−3 μm, 12 nm; 4.6 × 50 mm). The following solvent gradient was used with a 1 mL/min flow rate: water:acetonitrile (0.05% trifluoroacetic acid) 90:10 to 5:95 over 10 min. Retention times for the two different column conditions differed at least by approximately 1 min, for each compound analyzed (see Supporting Information).

Glassware was oven or flame-dried prior to use, and reactions were performed under argon inert atmosphere that was passed through a Drierite drying tube. CH_2Cl_2, diethyl ether, DMF, CH_3OH, THF, and toluene were dried using the procedure recommended by Grubbs, using the solvent purification system manufactured by Glass Contour, Inc. (Laguna Beach, CA).[58] Prior to the installation of this system, anhydrous solvents were purchased from Aldrich Chemical Co. and in some instances, THF (anhydrous) was additionally purified by distillation from sodium metal. All other reagents were purchased from Aldrich Chemical Co. and were used without further purification. The term 'solvent was removed under reduced pressure' generally implies rotary evaporation, followed by use of a high-vacuum pump. *N*,*N*-Diallyl-4-bromobenzenamine (**14**) was prepared according to the published procedure,[59] and 2-(4-hydroxyphenyl)-1-phenyl-3*H*-inden-5-ol (**NC-8**) was prepared from ketone **11** following published methods.[27,28] Improved syntheses of the core indene scaffold (**NC-3**),[33,43,47,60,61] and indanone **11** [62−64] were achieved via adaptations to published procedures (see Supporting Information). Syntheses of the final test compounds are described below, whereas preparation and characterization of intermediates is provided in the online supplement (see Supporting Information).

(±)-3-Ethyl-6-hydroxy-*N*,2-bis(4-hydroxyphenyl)-1*H*-indene-1-carboxamide (NC-5). Coupling. To a solution of carboxylate **6** (150 mg, 0.246 mmol) in CH_2Cl_2 (2 mL) at 0 °C were added HBTU (102.76 mg, 0.271 mmol) and 4-(dimethylamino)pyridine (1.5 mg, 0.0123 mmol). The reaction was stirred at 0 °C for 50 min, followed by 25 °C for 30 min. 4-Aminophenol (29.57 mg, 0.271 mmol) and diisopropylethylamine (128.7 μL, 0.739 mmol) were added dropwise, and the solution became a dark red which dissipated over time. The reaction was stirred at 25 °C for 17 h, poured into a 5% HCl solution, extracted with CH_2Cl_2, washed with saturated $NaHCO_3$ followed by dilute NaCl solution, and dried over anhydrous $MgSO_4$, and solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (silica, 0%-25% EtOAc−hexanes) to give (±)-3-ethyl-*N*-(4-hydroxyphenyl)-6-triisopropylsiloxy-2-(4-triisopropylsiloxyphenyl)-1*H*-indene-1-carboxamide as a white solid (66.8 mg, 0.095 mmol) in 39% yield. R_f 0.47 (15% EtOAc−hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (d, J = 8.79 Hz, 2 H), 7.17 (m, 3 H), 6.86 (d, J = 8.79 Hz, 2 H), 6.80 (d, J = 8.79 Hz, 2 H), 6.61 (s, 1 H), 6.52 (d, J = 8.79 Hz, 2 H), 5.52 (s, 1 H), 4.66 (s, 1 H), 2.71 (m, 2 H), 1.22 (m, 9 H), 1.03 (s, 18 H), 1.01 (s, 18 H); ^{13}C NMR (100 MHz, $CDCl_3$) 169.10, 155.48, 155.03, 153.06, 143.54, 143.06, 138.17, 135.81, 129.74, 129.11, 128.29, 122.98, 120.46, 120.11, 119.19, 115.94, 115.50, 30.31, 19.70, 17.94, 17.89. 13.58, 12.68, 12.64 ppm; HRMS (FAB) exact mass calculated for $C_{42}H_{61}NO_4Si_2$: 699.4139, found: 699.4126.

Deprotection. To a solution of (±)-3-ethyl-*N*-(4-hydroxyphenyl)-6-triisopropylsiloxy-2-(4-triisopropylsiloxyphenyl)-1*H*-indene-1-carboxamide (60.5 mg, 0.086 mmol) in THF (3 mL) at 25 °C was added triethylamine trihydrofluoride (42.26 μL, 0.259 mmol), and the reaction was stirred for 4 h 30 min. The solution was poured into a 1 M sodium fluoride, extracted with diethyl ether, washed with saturated $NaHCO_3$, and dried over anhydrous $MgSO_4$, and solvent was removed under reduced pressure. The crude yellowish-white solid was purified by pTLC (silica, 15% $CH_3OH-CHCl_3$) and eluted off silica with 85% 2-propanol−CH_2Cl_2 to give **NC-5** as a solid (26.5 mg, 0.053 mmol) in 62% yield. R_f 0.45 (10% $CH_3OH-CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) δ 9.51 (s, 1 H), 7.22 (d, J = 8.79 Hz, 2 H), 7.14 (d, J = 8.30 Hz, 1 H), 7.08 (d, J = 8.79 Hz, 2 H), 6.92 (s, 1 H), 6.74 (d, J = 8.79 Hz, 2 H), 6.71 (d, J = 5.86 Hz, 1 H), 6.60 (d, J = 8.79 Hz, 2 H), 4.63 (s, 1 H), 2.61 (m, 2 H), 1.21 (m, 3 H); ^{13}C NMR (100 MHz, CD_3OD) 171.85, 157.61, 156.91, 155.63, 145.54, 143.36, 139.43, 138.17, 131.27, 130.63, 128.93, 123.81, 121.18, 116.30, 116.15, 115.24, 111.69, 60.77, 20.37, 13.83 ppm; HRMS (FAB) exact mass calculated for $C_{24}H_{21}NO_4$: 387.1471, found: 387.1467.

(±)-*N*-(4-(2-(Piperidinyl)ethoxy)phenyl)-3-ethyl-6-hydroxy-2-(4-hydroxyphenyl)-1*H*-indene-1-carboxamide Monohydrochloride (NC-2). Coupling. To a solution of carboxylate **6** (231.5 mg, 0.380 mmol) in CH_2Cl_2 (7.5 mL) at 0 °C were added HBTU (158.6 mg, 0.418 mmol) and 4-(dimethylamino)pyridine (2.32 mg, 0.019 mmol). The reaction was stirred at 0 °C for 30 min, followed by 25 °C for 30 min. Aniline **8** (92.12 mg, 0.418 mmol) and diisopropylethylamine (132 μL, 0.76 mmol) were added, and the reaction was stirred at 25 °C for 1 h. The deep orange-red solution was poured into a 5% HCl solution, extracted with CH_2Cl_2, washed with saturated $NaHCO_3$, and dried over anhydrous $MgSO_4$, and solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (silica, 0%-10% $CH_3OH-CHCl_3$) to give (±)-*N*-(4-(2-(piperidinyl)ethoxy)phenyl)-3-ethyl-6-triisopropylsiloxy-2-(4-triisopropylsiloxyphenyl)-1*H*-indene-1-carboxamide monohydrochloride as a pale yellowish-white solid (273.7 mg, 0.34 mmol) in 89% yield. R_f 0.67 (20% $CH_3OH-CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (d, J = 8.30 Hz, 2 H), 7.29 (s, 1 H), 7.23 (d, J = 8.30 Hz, 1 H), 7.02 (d, J = 8.79 Hz, 2 H), 6.94 (d, J = 8.30 Hz, 2 H), 6.90 (d, J = 8.30 Hz, 1 H), 6.77 (s, 1 H), 6.72 (d, J = 8.79 Hz, 2 H), 4.76 (s, 1 H), 3.99 (m, 2 H), 2.78 (m, 2 H), 2.68 (m, 2 H), 2.45 (s, 4 H), 1.57 (m, 4 H), 1.39 (m, 2 H), 1.35 (m, 3 H), 1.27 (m, 6 H), 1.11 (s, 18 H), 1.10 (s, 18 H); ^{13}C NMR (100 MHz, $CDCl_3$) 168.21, 155.47, 155.26, 154.80, 143.18, 143.01, 137.98, 135.74, 130.34, 128.95, 128.22, 121.93, 120.24, 119.89, 118.93, 115.77, 114.46, 65.98, 60.17, 57.66, 54.81, 25.71, 23.98, 19.52, 17.78, 17.72, 13.43, 12.51, 12.46 ppm; LRMS (EI) exact mass calculated for $C_{49}H_{75}N_2O_4Si_2$: 811.5265, found: 811.666.

Deprotection. To a solution of (±)-*N*-(4-(2-(piperidinyl)-ethoxy)phenyl)-3-ethyl-6-triisopropylsiloxy-2-(4-triisopropylsiloxyphenyl)-1*H*-indene-1-carboxamide monohydrochloride (273.7 mg, 0.337 mmol) in THF (7.5 mL) at 25 °C was added triethylamine trihydrofluoride (549.9 μL, 3.37 mmol), and the reaction was stirred for 3 h. The reaction was poured into a

separatory funnel, and the two organic layers were separated. The lower layer was saved, solvent was removed under reduced pressure, and the residue was resuspended in diethyl ether (2.5 mL). Excess HCl (2 M in diethyl ether) (3.7 mL) was added, and the solution was stirred at 25 °C for 2 h, to form the HCl salt, whereupon solvent was removed under reduced pressure. The crude product was redissolved in CH_3OH, purified by repeated recrystallization ($CH_3OH-EtOAc$), filtered, and washed with cold EtOAc to give a 1:12 ratio of product:triethylamine as a yellow crystalline solid (313.5 mg, 0.143 mmol) corresponding to a 43% yield of pure product **NC-2** (71.4 mg, 0.143 mmol): R_f 0.37 (40% $CH_3OH-CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) δ 10.20 (s, 1 H), 7.42 (d, J = 7.33 Hz, 2 H), 7.33 (d, J = 8.30 Hz, 2 H), 7.20 (d, J = 7.81 Hz, 1 H), 7.01 (s, 1 H), 6.91 (d, J = 8.30 Hz, 2 H), 6.82 (d, J = 8.30 Hz, 2 H), 6.80 (m, 1 H), 4.93 (s, 1 H), 4.32 (m, 2 H), 3.53 (m, 4 H), 2.67 (m, 4 H), 1.80 (m, 6 H), 1.46 (m, 3 H); ^{13}C NMR (100 MHz, CD_3OD) 171.61, 157.61, 156.91, 155.85, 145.66, 142.88, 139.44, 138.40, 133.68, 130.60, 128.80, 123.20, 121.15, 116.33, 115.93, 115.24, 111.80, 63.65, 60.38, 57.12, 54.87, 23.96, 22.49, 20.38, 13.93 ppm; HRMS (FAB) exact mass calculated for $C_{31}H_{35}N_2O_4$: 499.2597, found: 499.2607.

(±)-*N*-(4-(2-(Dimethylamino)ethoxy)phenyl)-3-ethyl-6-hydroxy-2-(4-hydroxyphenyl)-1*H*-indene-1-carboxamide monohydrochloride (NC-4). Coupling. To a solution of carboxylate **6** (231.5 mg, 0.380 mmol) in CH_2Cl_2 (7.5 mL) at 0 °C were added HBTU (158.6 mg, 0.418 mmol) and 4-(dimethylamino)pyridine (2.32 mg, 0.019 mmol). The reaction was stirred at 0 °C for 30 min, followed by 25 °C for 45 min. Aniline **9** (75.34 mg, 0.418 mmol) and diisopropylethylamine (132 μL, 0.76 mmol) were added, and the reaction was stirred at 25 °C for 1 h. The solution was poured into a 5% HCl solution, extracted with CH_2Cl_2, washed with saturated $NaHCO_3$, and dried over anhydrous $MgSO_4$, and solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (silica, 0%-5% $CH_3OH-CHCl_3$) to give the pure (±)-*N*-(4-(2-(dimethylamino)ethoxy)phenyl)-3-ethyl-6-triisopropylsiloxy-2-(4-triisopropylsiloxyphenyl)-1*H*-indene-1-carboxamide monohydrochloride product (238.9 mg, 0.309 mmol) in 81% yield. R_f 0.71 (20% $CH_3OH-CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (d, J = 8.79 Hz, 2 H), 7.29 (s, 1 H), 7.23 (d, J = 8.30 Hz, 1 H), 7.04 (d, J = 8.79 Hz, 2 H), 6.94 (d, J = 8.79 Hz, 2 H), 6.90 (d, J = 8.30 Hz, 1 H), 6.73 (d, J = 8.79 Hz, 2 H), 4.76 (s, 1 H), 3.95 (m, 2 H), 2.77 (m, 2 H), 2.65 (t, J = 5.86 Hz, 2 H), 2.28 (s, 6 H), 1.35 (m, 3 H), 1.26 (m, 6 H), 1.11 (s, 18 H), 1.10 (s, 18 H); ^{13}C NMR (100 MHz, $CDCl_3$) 168.17, 155.39, 155.19, 154.71, 143.10, 143.01, 137.98, 135.75, 130.41, 128.91, 128.22, 121.84, 120.16, 119.84, 118.85, 115.68, 114.38, 65.91, 57.95, 45.55, 38.31, 19.46, 17.73, 17.67, 13.37, 12.46, 12.41 ppm; HRMS (FAB) exact mass calculated for $C_{46}H_{71}N_2O_4Si_2$: 771.4952, found: 771.4955.

Deprotection. To a solution of (±)-*N*-(4-(2-(dimethylamino)ethoxy)phenyl)-3-ethyl-6-triisopropylsiloxy-2-(4-triisopropylsiloxyphenyl)-1*H*-indene-1-carboxamide (87.5 mg, 0.113 mmol) in THF (4 mL) at 25 °C was added triethylamine trihydrofluoride (73.97 μL, 0.45 mmol), and the reaction was stirred for 3 h. The solvent was removed under reduced pressure, and the residue was redissolved in CH_3OH (1.5 mL). Excess HCl (2 M in diethyl ether) (1.5 mL) was added and the solution stirred at 25 °C for 2 h to form the HCl salt. The crude product was purified by repeated trituration ($CH_3OH-EtOAc$) to give a 1:2 ratio of product:triethylamine as a yellowish-white precipitate (87.1 mg, 0.113 mmol) corresponding to a 100% yield of pure **NC-4** product (61.1 mg, 0.113 mmol): R_f 0.16 (20% $CH_3OH-CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) δ??concentration dependent? 6.79 (d, J = 8.30 Hz, 2 H), 6.73 (d, J = 8.30 Hz, 2 H), 6.63 (d, J = 8.30 Hz, 1 H), 6.43 (s, 1 H), 6.30 (d, J = 8.30 Hz, 2 H), 6.25 (d, J = 7.81 Hz, 2 H), 6.24 (m, 1 H), 4.20 (s, 1 H), 3.64 (m, 2 H), 2.85 (m, 2 H), 2.28 (m, 6 H), 2.09 (m, 2 H), 0.70 (m, 3 H); ^{13}C NMR (100 MHz, CD_3OD) 171.95, 157.61, 156.90, 155.84, 145.41, 143.36, 139.42, 138.11, 133.73, 130.60, 128.83, 123.47, 121.19, 116.25, 115.90, 115.22, 111.68, 63.40, 60.73, 57.83, 44.00, 20.34, 13.80 ppm; HRMS (FAB) exact mass calculated for $C_{28}H_{31}N_2O_4$: 459.2284, found: 459.2282.

(±)-1,3-Diethyl-2-(4-hydroxyphenyl)-3*H*-inden-5-ol (ethyl indenestrol A, NC-1). Coupling. To a solution of indene (**4**) (50 mg, 0.088 mmol) in diethyl ether (1.2 mL) at −78 °C was added *n*-BuLi (2.5 M in hexanes) (38.9 μL, 0.0973 mmol) dropwise. The clear yellow solution was stirred at −78 °C for 10 min, followed by 0 °C for 10 min, and the color became orange, then bright yellow. The solution was then cooled to −78 °C, iodoethane (7.8 μL, 0.973 mmol) was added dropwise, and the solution was stirred at −78 °C for 1 h and then allowed to warm slowly to 25 °C and stirred 24 h. The solution was poured into water, extracted with diethyl ether, and dried over anhydrous $MgSO_4$, and solvent was removed under reduced pressure. The crude product was purified by pTLC (silica, 5% EtOAc−hexanes) to give a light orange oil (29.9 mg, 0.123 mmol) containing a 2:1 mixture of starting material to (±)-1,3-diethyl-2-(4-triisopropylsiloxyphenyl)-5-triisopropylsiloxy-3*H*-indene product, which corresponds to a 38% yield of product (29.9 mg, 0.033 mmol), deemed 87% pure by HPLC. R_f 0.63 (5% EtOAc−hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.03 (m, 7 H), 3.80 (m, 1 H), 2.70 (m, 2 H), 1.87 (m, 1 H), 1.63 (m, 1 H), 1.23 (m, 9 H), 1.13 (s, 18 H), 1.11 (s, 18 H), 0.42 (t, J = 7.32 Hz, 3 H); the sample was too dilute for adequate analysis by ^{13}C NMR; HRMS (EI) exact mass calculated for $C_{37}H_{60}O_2Si_2$: 592.4132, found: 592.4127.

Deprotection. To a partly pure solution of (±)-1,3-diethyl-2-(4-triisopropylsiloxyphenyl)-5-triisopropylsiloxy-3*H*-indene (19.8 mg, 0.033 mmol) in THF (1.5 mL) at 25 °C was added tetrabutylammonium fluoride (1 M in THF) (66 μL, 0.066 mmol). The solution immediately turned yellow, blue, and then maroon and was stirred for 15 min and then quenched with water. The organics were extracted with diethyl ether, washed with saturated $NaHCO_3$, and dried over anhydrous $MgSO_4$, and solvent was removed under reduced pressure. The crude product was purified by pTLC (silica, 2.5% $CH_3OH-CHCl_3$) to give **NC-1** as a light yellow oil (2.0 mg, 0.0071 mmol) in 22% yield. R_f 0.27 (5% $CH_3OH-CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) δ 7.09 (d, J = 8.79 Hz, 2 H), 7.06 (d, J = 7.81 Hz, 1 H), 6.83 (s, 1 H), 6.76 (d, J = 8.79 Hz, 2 H), 6.64 (d, J = 7.81 Hz, 1 H), 3.72 (m, 1 H), 2.54 (m, 2 H), 1.82 (m, 1 H), 1.55 (m, 1 H), 1.16 (m, 3 H), 0.32 (t, J = 7.32 Hz, 3 H); ^{13}C NMR (100 MHz, CD_3OD) 157.22, 156.16, 149.73, 142.60, 140.03, 139.40, 130.88, 129.66, 120.33, 116.18, 114.05, 111.67, 52.23, 24.16, 20.16, 14.44, 8.24 ppm; HRMS (EI) exact mass calculated for $C_{19}H_{20}O_2$: 280.1463, found: 280.1470.

(±)-3-Ethyl-6-hydroxy-2-(4-hydroxyphenyl)-1*H*-indene-1-carboxylic Acid (NC-7). To a solution of carboxylate **6** (83.0 mg, 0.136 mmol) in THF (3 mL) at 25 °C was added triethylamine trihydrofluoride (222 μL, 1.362 mmol), and the reaction was stirred for 3 h 45 min. The solution was poured into a 1 M sodium fluoride, acidified with 1 N HCl, extracted with diethyl ether, washed with 0.5 M HCl, and dried over anhydrous $MgSO_4$, and solvent was removed under reduced pressure. The crude brown solid was purified by pTLC (silica, 10% $CH_3OH-CHCl_3$) and eluted off silica with diethyl ether, 85% 2-propanol-CH_2Cl_2, and 5% $CH_3OH-CHCl_3$ to give a solid (40.3 mg, 0.136 mmol) in 100% yield, deemed 61% pure by HPLC. R_f 0.54 (20% $CH_3OH-CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) δ 7.18 (d, J = 7.81 Hz, 2 H), 7.04 (d, J = 8.30 Hz, 1 H), 6.92 (s, 1 H), 6.68 (d, J = 7.81 Hz, 2 H), 6.63 (d, J = 8.30 Hz, 1 H), 4.52 (s, 1 H), 2.55 (m, 2 H), 1.15 (m, 3 H); ^{13}C NMR (100 MHz, CD_3OD) 157.28, 156.50, 145.85, 141.81, 139.53, 139.03, 130.61, 129.69, 120.62, 120.62, 116.05, 114.74, 111.86, 49.64, 20.32, 13.80 ppm; HRMS (FAB) exact mass calculated for $C_{18}H_{16}O_4$: 296.1049, found: 296.1051.

1,2-Bis(4-hydroxyphenyl)-3*H*-inden-5-ol (NC-9). To a solution of methyl 4-(6-methoxy-2-(4-methoxyphenyl)-1*H*-inden-3-yl)phenol [27,28] (31.3 mg, 0.091 mmol) in CH_2Cl_2 (3 mL) at −78 °C was added boron tribromide (1 M in CH_2Cl_2) (300 μL, 0.3 mmol). The reaction mixture was stirred at −78 °C for 1 h, followed by further addition of boron tribromide (1 M in CH_2Cl_2) (1 mL, 1 mmol). The solution was then stirred at 25

°C for 3 h. The solution was poured into water, stirred 10 min, and extracted with CH_2Cl_2. The aqueous layer was basified with 1 N NaOH and extracted with CH_2Cl_2. The combined organic layers were washed with saturated $NaHCO_3$, and EtOAc was used to dissolve the UV-active precipitate off the sides of the glassware. The organic layers were combined, and dried over anhydrous $MgSO_4$, and solvent was removed under reduced pressure. The crude oil was purified by pTLC (silica, 20% $CH_3OH-CH_2Cl_2$) and eluted off silica with 10% $CH_3OH-CH_2Cl_2$ to give **NC-9** as an orange oil (24.2 mg, 0.076 mmol) in 84% yield, and deemed 81% pure by HPLC. R_f 0.48 (15% $CH_3OH-CH_2Cl_2$); ^{1}H NMR (400 MHz, CD_3OD) δ 7.04 (m, 4 H), 6.88 (s, 1 H), 6.84 (d, J = 8.30 Hz, 1 H), 6.77 (d, J = 8.30 Hz, 2 H), 6.59 (dd, J = 8.30, 1.95 Hz, 1 H), 6.53 (d, J = 8.79 Hz, 2 H), 3.66 (s, 2 H); ^{13}C NMR (100 MHz, CD_3OD) 157.71, 157.14, 156.38, 145.33, 140.94, 138.68, 131.53, 130.23, 129.96, 129.14, 121.18, 121.18, 116.59, 115.84, 114.11, 112.20, 41.62 ppm; HRMS (EI) exact mass calculated for $C_{21}H_{16}O_3$: 316.1099, found: 316.1096.

1-(4-(2-(Piperidin-1-yl)ethoxy)phenyl)-2-(4-hydroxyphenyl)-*3H*-inden-5-ol (NC-10). Coupling. To a solution of bromide **12** (498 mg, 1.753 mmol) in THF (4 mL) were added Mg turnings (39.4 mg, 1.753 mmol) and a catalytic amount of iodine. The reaction mixture was refluxed for 4 h, until disappearance of the Mg metal indicated formation of the Grignard reagent. The solution was allowed to cool and was added to a solution of ketone **11** (150 mg, 0.559 mmol) in THF (2 mL). The reaction mixture was refluxed for 20 h overnight. The solution was poured into 1.5N HCl, extracted with EtOAc and then CH_2Cl_2, and washed with water. The aqueous layer was basified with 1 N NaOH, extracted with EtOAc and CH_2Cl_2, and washed with water. The organic layers were combined and dried over anhydrous $MgSO_4$, and solvent was removed under reduced pressure. The crude oil was purified by flash column chromatography (silica, 0−2% $CH_3OH-CHCl_3$) to give 1-(2-(4-(6-methoxy-2-(4-methoxyphenyl)-1*H*-inden-3-yl)phenoxy)-ethyl)piperidine as a red semisolid (181.5 mg, 0.398 mmol) in 71% yield. R_f 0.48 (15% $CH_3OH-CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (d, J = 7.81 Hz, 2 H), 7.18 (d, J = 8.30 Hz, 2 H), 7.07 (d, J = 11.72 Hz, 1 H), 7.06 (s, 1 H), 6.93 (d, J = 7.81 Hz, 2 H), 6.78 (d, J = 8.79 Hz, 1 H), 6.71 (d, J = 8.79 Hz, 2 H), 4.12 (t, J = 5.86 Hz, 2 H), 3.79 (s, 3 H), 3.76 (s, 2 H), 3.72 (s, 3 H), 2.78 (m, 2 H), 2.51 (m, J = 4.88 Hz, 4 H), 1.60 (m, 4 H), 1.44 (m, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$) 158.07, 157.80, 143.74, 140.41, 137.85, 137.40, 130.27, 129.24, 128.96, 128.53, 120.22, 114.74, 114.41, 113.42, 111.77, 109.94, 65.76, 57.88, 55.40, 54.95, 54.87, 40.81, 25.79, 24.07 ppm; HRMS (EI) exact mass calculated for $C_{30}H_{33}NO_3$: 455.2460, found: 455.2476.

Deprotection. To a solution of 1-(2-(4-(6-methoxy-2-(4-methoxyphenyl)-1*H*-inden-3-yl)phenoxy)ethyl)piperidine (181.5 mg, 0.398 mmol) in CH_2Cl_2 (4 mL) at −78 °C was added boron tribromide (1 M in CH_2Cl_2) (876 μL, 0.876 mmol). The reaction mixture was stirred at −78 °C for 2 h and then at 25 °C for 1 h 30 min, followed by further addition of boron tribromide (1 M in CH_2Cl_2) (1.4 mL, 1.4 mmol). The solution was then stirred at 25 °C for 1 h. The solution was poured into water (resulting in precipitation of a brown residue), extracted with CH_2Cl_2, washed with saturated $NaHCO_3$, and dried over anhydrous $MgSO_4$. The residue from the glassware was redissolved in acetone, the organics were combined, and solvent was removed under reduced pressure. The crude oil was purified by flash column chromatography (silica, 0%-5% $CH_3OH-CH_2Cl_2$), followed by pTLC (silica, 20% $CH_3OH-CH_2Cl_2$), and eluted off silica with 10% $CH_3OH-CH_2Cl_2$, and solvent was removed under reduced pressure. The residue was redissolved in 5% $CH_3OH-CH_2Cl_2$ followed by filtration through a fine silica frit to give **NC-10** as a purple oil (25.1 mg, 0.059 mmol) in 15% yield and deemed as 61% pure by HPLC. Additionally 1,2-bis-(4-hydroxyphenyl)-3*H*-inden-5-ol (122.3 mg, 0.387 mmol) was retrieved as a side-product in 52% yield. R_f 0.29 (15% $CH_3OH-CH_2Cl_2$); ^{1}H NMR (400 MHz, CD_3OD) δ 6.88 (m, 11 H), 4.07 (m, 2 H), 3.64 (s, 2 H), 2.74 (m, 2 H), 2.51 (m, 4 H), 1.56 (m, 4 H), 1.41 (m, 2 H); ^{13}C NMR (100 MHz, CD_3OD) 159.29, 157.23, 156.45, 145.35, 140.75, 139.08, 138.32, 132.24, 131.62, 130.27, 129.81, 123.40, 121.13, 115.89, 114.15, 112.27, 66.22, 58.84, 55.91, 41.70, 26.35, 24.93 ppm; HRMS (EI) exact mass calculated for $C_{28}H_{29}NO_3$: 427.2147, found: 427.2149.

1-(4-Fluorophenyl)-2-(4-hydroxyphenyl)-3*H*-inden-5-ol (NC-11). Coupling. To a solution of ketone **11** (150 mg, 0.559 mmol) in THF (4 mL) at 25 °C was added 4-fluorophenylmagnesium bromide (1 M in THF) (615 μL, 0.615 mmol). The yellow solution was stirred at 25 °C for 2 h, poured into 1.5 N HCl, and extracted with diethyl ether, followed by CH_2Cl_2, the organic layers were washed with water, combined, and dried over anhydrous $MgSO_4$, and solvent was removed under reduced pressure. The crude oil was purified by flash column chromatography (silica, 0%-10% EtOAc−hexanes) to give 3-(4-fluorophenyl)-6-methoxy-2-(4-methoxyphenyl)-1*H*-indene as a pinkish orange crystalline solid (144.5 mg, 0.417 mmol) in 75% yield. R_f 0.63 (40% EtOAc−hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (dd, J = 8.30, 5.86 Hz, 2 H), 7.15 (d, J = 8.79 Hz, 2 H), 7.09 (m, 2 H), 7.03 (d, J = 8.30 Hz, 1 H), 7.03 (d, J = 8.30 Hz, 1 H), 6.80 (dd, J = 8.30, 2.44 Hz, 1 H), 6.73 (d, J = 8.79 Hz, 2 H), 3.82 (s, 3 H), 3.80 (s, 2 H), 3.75 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) 158.39, 158.05, 143.82, 140.16, 138.85, 136.84, 132.34, 131.03, 130.95, 129.12, 120.16, 115.89, 115.68, 113.63, 111.96, 110.17, 55.55, 55.12, 41.03 ppm; HRMS (EI) exact mass calculated for $C_{23}H_{19}FO_2$: 346.1369, found: 346.1353.

Deprotection. To a solution of 3-(4-fluorophenyl)-6-methoxy-2-(4-methoxyphenyl)-1*H*-indene (144.5 mg, 0.398 mmol) in CH_2Cl_2 (4 mL) at −78 °C was added boron tribromide (1 M in CH_2Cl_2) (834 μL, 0.834 mmol). The reaction mixture was stirred at −78 °C for 2 h and then at 25 °C for a further 2 h, followed by further addition of CH_2Cl_2 (4 mL) and boron tribromide (1 M in CH_2Cl_2) (1 mL, 1 mmol). The semisolid mixture was stirred at 25 °C for a further 26 h overnight. The solution was poured into water, sonicated extensively to dissolve the solids, extracted with CH_2Cl_2, and washed with saturated $NaHCO_3$, and solvent was removed under reduced pressure. The crude oil was purified by flash column chromatography (silica, 0%-40% EtOAc−hexanes), followed by pTLC (40% EtOAc−hexanes), and eluted off silica with EtOAc to give the pure **NC-11** (62.1 mg, 0.195 mmol) in 49% yield. R_f 0.33 (40% EtOAc−hexanes); ^{1}H NMR (400 MHz, CD_3OD) δ 7.10 (m, 2 H), 6.96 (m, 2 H), 6.89 (d, J = 8.79 Hz, 2 H), 6.82 (d, J = 2.44 Hz, 1 H), 6.72 (d, J = 8.30 Hz, 1 H), 6.54 (dd, J = 8.30, 1.95 Hz, 1 H), 6.47 (d, J = 8.30 Hz, 2 H), 3.55 (s, 2 H); ^{13}C NMR (100 MHz, CD_3OD) 157.33, 156.48, 145.32, 140.32, 139.91, 137.52, 132.33, 132.25, 130.32, 129.38, 120.94, 116.62, 116.41, 115.93, 114.18, 112.34, 41.71 ppm; HRMS (EI) exact mass calculated for $C_{21}H_{15}FO_2$: 318.1056, found: 318.1056.

1-(4-Chlorophenyl)-2-(4-hydroxyphenyl)-3*H*-inden-5-ol (NC-12). Coupling. To a solution of ketone **11** (150 mg, 0.559 mmol) in THF (4 mL) at 25 °C was added 4-chlorophenylmagnesium bromide (1 M in diethyl ether) (615 μL, 0.615 mmol). The orange solution was stirred at 25 °C for 2 h, poured into 1.5 N HCl, and extracted with diethyl ether, followed by CH_2Cl_2, the organic layers were washed with water, combined, and dried over anhydrous $MgSO_4$, and solvent was removed under reduced pressure. The crude oil was purified by flash column chromatography (silica, 0%-5% EtOAc−hexanes) to give 3-(4-chlorophenyl)-6-methoxy-2-(4-methoxyphenyl)-1*H*-indene as a pinkish white solid (131.3 mg, 0.3626 mmol) in 65% yield. R_f 0.57 (40% EtOAc−hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (m, 2 H), 7.26 (d, J = 8.30 Hz, 2 H), 7.14 (d, J = 8.79 Hz, 2 H), 7.07 (s, 1 H), 7.02 (d, J = 8.30 Hz, 1 H), 6.79 (dd, J = 8.30, 1.95 Hz, 1 H), 6.73 (d, J = 8.79 Hz, 2 H), 3.81 (s, 3 H), 3.78 (s, 2 H), 3.73 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) 158.44, 158.04, 143.81, 139.84, 139.11, 136.58, 134.90, 132.94, 130.73, 129.12, 129.01, 128.91, 120.10, 113.65, 111.93, 110.17, 55.51, 55.09, 41.07 ppm; HRMS (EI) exact mass calculated for $C_{23}H_{19}ClO_2$: 362.1074, found: 362.1053.

Deprotection. To a solution of 3-(4-chlorophenyl)-6-methoxy-2-(4-methoxyphenyl)-1*H*-indene (131.3 mg, 0.362 mmol) in CH_2Cl_2 (4 mL) at −78 °C was added boron tribromide (1 M in CH_2Cl_2) (876 μL, 0.876 mmol). The reaction mixture was stirred at −78 °C for 2 h and then at 25 °C for a further 2 h,

after which additional CH_2Cl_2 (4 mL) and boron tribromide (1 M in CH_2Cl_2) (1 mL, 1 mmol) were added. The semisolid mixture was stirred at 25 °C for a further 26 h overnight. The solution was poured into water, sonicated extensively to dissolve the solids, extracted with CH_2Cl_2, and washed with saturated $NaHCO_3$, and solvent was removed under reduced pressure. The crude oil was purified by flash column chromatography (silica, 0%-40% EtOAc−hexanes) to give **NC-12** as an orange oil (79 mg, 0.236 mmol) in 65% yield. R_f 0.33 (40% EtOAc−hexanes); ^{1}H NMR (400 MHz, CD_3OD) δ 7.16 (d, J = 8.79 Hz, 2 H), 7.01 (d, J = 8.30 Hz, 2 H), 6.84 (d, J = 8.79 Hz, 2 H), 6.79 (s, 1 H), 6.69 (d, J = 8.30 Hz, 1 H), 6.52 (dd, J = 8.30, 2.44 Hz, 1 H), 6.45 (d, J = 8.30 Hz, 2 H), 3.48 (s, 2 H); ^{13}C NMR (100 MHz, CD_3OD) 157.33, 156.43, 145.32, 140.19, 140.01, 137.23, 136.74, 133.78, 132.04, 130.35, 129.84, 129.22, 120.90, 115.94, 114.19, 112.36, 41.75 ppm; HRMS (EI) exact mass calculated for $C_{21}H_{15}ClO_2$: 334.0761, found: 334.0772.

2-(4-Hydroxyphenyl)-1-*p*-tolyl-3*H*-inden-5-ol (NC-13). Coupling. To a solution of ketone **11** (150 mg, 0.559 mmol) in THF (4 mL) at 25 °C was added *p*-tolylmagnesium bromide (1 M in diethyl ether) (615 μL, 0.615 mmol). The yellow solution was stirred at 25 °C for 2 h, poured into 1.5 N HCl, and extracted with diethyl ether, followed by CH_2Cl_2, the organic layers were washed with water, combined and dried over anhydrous $MgSO_4$, and solvent was removed under reduced pressure. The crude orange oil was purified by flash column chromatography (silica, 0%-10% EtOAc−hexanes) to give 6-methoxy-2-(4-methoxyphenyl)-3-*p*-tolyl-1*H*-indene as an orange semisolid (149.2 mg, 0.436 mmol) in 78% yield. R_f 0.61 (40% EtOAc−hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (m, 6 H), 7.07 (m, 2 H), 6.78 (dd, J = 8.30, 2.44 Hz, 1 H), 6.71 (d, J = 8.30 Hz, 2 H), 3.79 (s, 3 H), 3.77 (s, 2 H), 3.71 (s, 3 H), 2.38 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) 158.16, 157.86, 143.83, 140.44, 138.06, 137.81, 136.70, 133.35, 129.45, 129.38, 129.11, 129.03, 120.34, 113.46, 111.80, 109.99, 55.46, 55.02, 40.91, 21.29 ppm; HRMS (EI) exact mass calculated for $C_{24}H_{22}O_2$: 342.1620, found: 342.1624.

Deprotection. To a solution of 6-methoxy-2-(4-methoxyphenyl)-3-*p*-tolyl-1*H*-indene (149.2 mg, 0.436 mmol) in CH_2Cl_2 (4 mL) at −78 °C was added boron tribromide (1 M in CH_2Cl_2) (959 μL, 0.959 mmol). The reaction mixture was stirred at −78 °C for 1 h 30 min, allowing the dry ice bath to warm to 25 °C, after which the solution was stirred a further 2 h. Additional boron tribromide (1 M in CH_2Cl_2) (1 mL, 1 mmol) was added, and the solution was stirred 24 h at 25 °C. The solution was poured into a dilute $NaHCO_3$ solution, extracted with CH_2Cl_2, washed with saturated $NaHCO_3$, and again extracted with CH_2Cl_2, and solvent from the combined organic layers were removed under reduced pressure. The crude brown oil was purified by flash column chromatography (silica, 0%-40% EtOAc−hexanes), followed by pTLC (40% EtOAc−hexanes), and eluted off silica with EtOAc to give the pure **NC-13** (81.9 mg, 0.260 mmol) in 60% yield. R_f 0.32 (40% EtOAc−hexanes); ^{1}H NMR (400 MHz, CD_3OD) δ 7.00 (m, 4 H), 6.91 (d, J = 8.79 Hz, 2 H), 6.81 (d, J = 2.44 Hz, 1 H), 6.73 (d, J = 8.30 Hz, 1 H), 6.53 (dd, J = 8.30, 2.44 Hz, 1 H), 6.45 (d, J = 8.79 Hz, 2 H), 3.53 (s, 2 H), 2.19 (s, 3 H); ^{13}C NMR (100 MHz, CD_3OD) 157.04, 156.23, 145.31, 140.74, 139.05, 138.58, 137.77, 135.08, 130.35, 130.25, 130.25, 129.71, 121.13, 115.80, 114.08, 112.23, 41.62, 21.36 ppm; HRMS (EI) exact mass calculated for $C_{22}H_{18}O_2$: 314.1307, found: 314.1310.

1-Benzyl-2-(4-hydroxyphenyl)-3*H*-inden-5-ol (NC-14). Coupling. To a solution of ketone **11** (150 mg, 0.559 mmol) in THF (4 mL) at 25 °C was added benzylmagnesium chloride (2 M in diethyl ether) (307.5 μL, 0.615 mmol). The light yellow solution was stirred at 25 °C for 1 h 30 min and then refluxed for 2 h 30 min. Additional benzylmagnesium chloride (2 M in diethyl ether) (307.5 μL, 0.615 mmol) was added, and the solution was refluxed overnight for 16 h. The solution was cooled, poured into 3 N HCl, extracted with diethyl ether, washed with water, and dried over anhydrous $MgSO_4$, and solvent was removed under reduced pressure. The crude oil was purified by flash column chromatography (silica, 0%-10% EtOAc−hexanes) to give 3-benzyl-6-methoxy-2-(4-methoxyphenyl)-1*H*-indene as a red-orange oil (150.5 mg, 0.440 mmol) in 79% yield. R_f 0.65 (40% EtOAc−hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (d, J = 8.79 Hz, 2 H), 7.14 (m, 3 H), 7.09 (m, 2 H), 6.95 (d, J = 1.95 Hz, 1 H), 6.89 (d, J = 8.30 Hz, 1 H), 6.76 (d, J = 8.79 Hz, 2 H), 6.63 (dd, J = 8.30, 2.44 Hz, 1 H), 3.98 (s, 2 H), 3.66 (s, 3 H), 3.65 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) 158.47, 157.70, 144.08, 140.13, 139.83, 139.56, 134.71, 129.72, 128.79, 128.49, 128.16, 125.95, 120.14, 113.87, 111.74, 109.94, 55.40, 55.14, 41.17, 32.19 ppm; HRMS (EI) exact mass calculated for $C_{24}H_{22}O_2$: 342.1620, found: 342.1623.

Deprotection. To a solution of 3-benzyl-6-methoxy-2-(4-methoxyphenyl)-1*H*-indene (150.5 mg, 0.440 mmol) in CH_2Cl_2 (4 mL) at −78 °C was added boron tribromide (1 M in CH_2Cl_2) (1.73 mL, 1.73 mmol). The reaction mixture was stirred at −78 °C for 1 h 30 min, allowing the dry ice bath to warm to 25 °C, after which the solution was stirred a further 2 h. Additional boron tribromide (1 M in CH_2Cl_2) (1.73 mL, 1.73 mmol) was added, and the solution was stirred 1 h at 25 °C. The solution was poured into water, stirred 30 min, extracted with CH_2Cl_2, washed with saturated $NaHCO_3$, and dried over anhydrous $MgSO_4$, and solvent was removed under reduced pressure. The crude oil was purified by flash column chromatography (silica, 0%-40% EtOAc−hexanes) to give the pure **NC-14** (130.8 mg, 0.416 mmol) in 95% yield. R_f 0.40 (40% EtOAc−hexanes); ^{1}H NMR (400 MHz, CD_3OD) δ 7.04 (m, 7 H), 6.80 (d, J = 2.44 Hz, 1 H), 6.71 (d, J = 7.81 Hz, 1 H), 6.62 (d, J = 8.79 Hz, 2 H), 6.47 (dd, J = 8.30, 2.44 Hz, 1 H), 3.85 (s, 2 H), 3.52 (s, 2 H); ^{13}C NMR (100 MHz, CD_3OD) 157.41, 156.12, 145.56, 141.19, 140.93, 140.21, 135.55, 130.10, 129.95, 129.41, 129.23, 126.90, 121.09, 116.21, 113.99, 112.11, 41.89, 32.98 ppm; HRMS (EI) exact mass calculated for $C_{22}H_{18}O_2$: 314.1307, found: 314.1324.

1-(4-(Trifluoromethyl)phenyl)-2-(4-hydroxyphenyl)-3*H*-inden-5-ol (NC-15). Coupling. To a solution of 4-bromobenzotrifluoride (236 μL, 1.709 mmol) in THF (6 mL) were added Mg turnings (41.55 mg, 1.709 mmol) and a catalytic amount of iodine. The reaction mixture was refluxed for 50 min, until disappearance of the Mg metal indicated formation of the Grignard reagent. The dark orange solution was allowed to cool and added to a second flask, containing a solution of ketone **11** (139 mg, 0.518 mmol) in THF (4 mL). The reaction mixture was stirred at reflux for 1 h 30 min. The solution was poured into 3 N HCl and stirred for 30 min, extracted with diethyl ether, washed with water, and dried over anhydrous $MgSO_4$, and solvent was removed under reduced pressure. The crude oil was purified by flash column chromatography (silica, 0%-10% EtOAc−hexanes) to give 3-(4-(trifluoromethyl)phenyl)-6-methoxy-2-(4-methoxyphenyl)-1*H*-indene as a whitish crystalline solid (164.4 mg, 0.415 mmol) in 80% yield. R_f 0.64 (40% EtOAc−hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (d, J = 7.81 Hz, 2 H), 7.32 (d, J = 8.30 Hz, 2 H), 6.98 (m, 3 H), 6.91 (d, J = 8.30 Hz, 1 H), 6.67 (d, J = 8.79 Hz, 1 H), 6.61 (d, J = 8.30 Hz, 2 H), 3.68 (s, 3 H), 3.67 (s, 2 H), 3.61 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) 158.57, 158.11, 143.84, 140.40, 139.85, 139.52, 136.39, 129.73, 129.31, 129.18, 128.62, 125.70, 125.67, 120.04, 113.69, 111.95, 110.19, 55.44, 55.05, 41.17 ppm; HRMS (EI) exact mass calculated for $C_{24}H_{19}F_3O_2$: 396.1337, found: 396.1331.

Deprotection. To a solution of 3-(4-(trifluoromethyl)phenyl)-6-methoxy-2-(4-methoxyphenyl)-1*H*-indene (164.4 mg, 0.415 mmol) in CH_2Cl_2 (4 mL) at −78 °C was added boron tribromide (1 M in CH_2Cl_2) (913 μL, 0.913 mmol). The reaction mixture was stirred at −78 °C for 1 h 30 min, allowing the dry ice bath to warm to 25 °C, after which the solution was stirred a further 2 h. Additional boron tribromide (1 M in CH_2Cl_2) (913 μL, 0.913 mmol) was added, and the solution was stirred 1 h at 25 °C. The solution was poured into water, stirred 30 min, extracted with CH_2Cl_2, washed with saturated $NaHCO_3$, and dried over anhydrous $MgSO_4$, and solvent was removed under reduced pressure. The crude oil was purified by flash column chromatography (silica, 0%-30% EtOAc−hexanes) to give **NC-15** as an orange oil (75.2 mg, 0.204 mmol) in 49% yield, and deemed as 78% pure by HPLC. R_f 0.38 (40% EtOAc−hexanes); ^{1}H NMR (400 MHz, CD_3OD) δ 7.52 (d, J = 7.81 Hz, 2 H), 7.28 (d, J =

8.30 Hz, 2 H), 6.87 (d, J = 8.79 Hz, 2 H), 6.83 (d, J = 1.95 Hz, 1 H), 6.73 (d, J = 8.30 Hz, 1 H), 6.54 (dd, J = 8.30, 2.44 Hz, 1 H), 6.48 (d, J = 8.79 Hz, 2 H), 3.58 (s, 2 H); ^{13}C NMR (100 MHz, CD_3OD) 157.66, 156.70, 145.41, 142.48, 141.10, 139.67, 137.14, 131.19, 130.45, 130.21, 128.98, 126.63, 126.59, 120.86, 116.05, 114.29, 112.44, 41.98 ppm; HRMS (EI) exact mass calculated for $C_{22}H_{15}F_3O_2$: 368.1024, found: 368.1026.

1-(4-Aminophenyl)-2-(4-hydroxyphenyl)-3*H*-inden-5-ol (NC-16). Coupling. To a solution of bromide **14** (577.5 mg, 2.29 mmol) in THF (4 mL) were added Mg turnings (56 mg, 2.29 mmol) and a catalytic amount of iodine. The reaction mixture was refluxed for 2 h 30 min, until disappearance of the Mg metal indicated formation of the Grignard reagent. The solution was allowed to cool, and ketone **11** (409.7 mg, 1.53 mmol) was added. The yellow reaction mixture was stirred at 25 °C for 2 h. The solution was poured into 3 N HCl, and extracted with EtOAc and then CH_2Cl_2, and washed with saturated $NaHCO_3$. The aqueous layer was basified with 1 N NaOH, extracted with CH_2Cl_2, and washed with saturated $NaHCO_3$. The organic layers were combined and dried over anhydrous $MgSO_4$, and solvent was removed under reduced pressure. The bright purple oil was purified by flash column chromatography (silica, 0%-20% EtOAc−hexanes) to give *N*,*N*-diallyl-4-(6-methoxy-2-(4-methoxyphenyl)-1*H*-inden-3-yl)benzenamine as an orange oil (487.4 mg, 1.151 mmol) in 75% yield. R_f 0.31 (40% EtOAc−hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14 (d, J = 8.79 Hz, 2 H), 7.08 (m, J = 8.30, 6.84 Hz, 3 H), 6.96 (d, J = 1.95 Hz, 1 H), 6.69 (dd, J = 8.30, 1.95 Hz, 1 H), 6.63 (d, J = 8.79 Hz, 4 H), 5.78 (m, 2 H), 5.09 (m, 4 H), 3.84 (m, J = 5.37 Hz, 4 H), 3.70 (s, 3 H), 3.66 (s, 2 H), 3.63 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) 157.96, 157.73, 147.80, 143.92, 140.73, 137.93, 137.03, 134.03, 129.98, 129.04, 123.84, 120.55, 116.13, 113.42, 112.42, 112.24, 111.77, 109.89, 55.47, 55.04, 52.66, 40.91 ppm; HRMS (EI) exact mass calculated for $C_{29}H_{29}NO_2$: 423.2198, found: 423.2207.

Allyl Deprotection. Palladium tetrakis triphenylphosphine catalyst was prepared by adding tris(dibenzylideneacetone)dipalladium(0)−choloroform adduct (23.8 mg, 0.023 mmol) to a solution of triphenylphosphine (36.2 mg, 0.138 mmol) in CH_2Cl_2 (2 mL) and stirring 10 min until the solution turned clear orange. The catalyst was transferred via cannula to a solution of *N*,*N*-diallyl-4-(6-methoxy-2-(4-methoxyphenyl)-1*H*-inden-3-yl)benzenamine (487.4 mg, 1.151 mmol) in CH_2Cl_2 (2 mL). *N*,*N*-1,3-Dimethylbarbituric acid (1.797 g, 11.51 mmol) was added, and the orange-brown reaction mixture was stirred at 25 °C for 6 h.[65] The solvent was removed under reduced pressure, the residue was redissolved in diethyl ether, washed with saturated $NaHCO_3$, washed with water, and dried over anhydrous $MgSO_4$, and solvent was removed under reduced pressure. The crude oil was purified by flash column chromatography (silica, 0%-40% EtOAc−hexanes) to give 4-(6-methoxy-2-(4-methoxyphenyl)-1*H*-inden-3-yl)benzenamine (353.1 mg, 1.028 mmol) in 89% yield. R_f 0.41 (40% EtOAc−hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15 (d, J = 8.79 Hz, 2 H), 7.07 (d, J = 8.30 Hz, 2 H), 7.04 (d, J = 8.79 Hz, 1 H), 7.00 (d, J = 2.44 Hz, 1 H), 6.72 (dd, J = 8.30, 2.44 Hz, 1 H), 6.65 (m, 4 H), 3.75 (s, 3 H), 3.71 (s, 2 H), 3.68 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) 158.08, 157.82, 145.39, 143.90, 140.68, 137.89, 137.48, 130.24, 129.73, 129.06, 126.40, 120.41, 115.45, 113.49, 111.83, 110.02, 55.57, 55.11, 40.89 ppm; HRMS (EI) exact mass calculated for $C_{23}H_21NO_2$: 343.1572, found: 343.1575.

Deprotection of Methyl Ether. To a solution of 4-(6-methoxy-2-(4-methoxyphenyl)-1*H*-inden-3-yl)benzenamine (353.1 mg, 1.028 mmol) in CH_2Cl_2 (5 mL), at −78 °C was added boron tribromide (1 M in CH_2Cl_2) (4.524 mL, 4.524 mmol). The reaction mixture was stirred at −78 °C for 2 h and then at 25 °C for 2 h 30 min. The solution was poured into water and stirred 30 min, and then the organic layer was separated from the aqueous layer. Any remaining precipitate in the organic or aqueous layer was collected via filtration, redissolved in acetone, and added to the organic layer, and solvent was removed under reduced pressure. The crude oil was purified by flash column chromatography (silica, 0%-10% $CH_3OH-CH_2Cl_2$) to give **NC-16** as a white solid (324 mg, 1.028 mmol) in 100% yield. R_f 0.45 (10% $CH_3OH-CH_2Cl_2$); ^{1}H NMR (400 MHz, CD_3OD) δ 7.25 (d, J = 8.30 Hz, 2 H), 7.13 (d, J = 8.79 Hz, 2 H), 7.01 (d, J = 8.79 Hz, 2 H), 6.91 (d, J = 1.95 Hz, 1 H), 6.83 (d, J = 8.30 Hz, 1 H), 6.60 (dd, J = 8.30, 2.44 Hz, 1 H), 6.52 (d, J = 8.79 Hz, 2 H), 3.70 (s, 2 H); ^{13}C NMR (100 MHz, CD_3OD) 157.02, 156.29, 147.77, 145.36, 141.06, 138.96, 138.28, 131.14, 130.21, 130.13, 127.74, 121.22, 116.90, 115.78, 114.06, 112.17, 41.58 ppm; HRMS (EI) exact mass calculated for $C_{21}H_{17}NO_2$: 315.1259, found: 315.1251.

4-(6-Hydroxy-2-(4-hydroxyphenyl)-1*H*-inden-3-yl)benzoic Acid (NC-17). To a solution of methyl ester **18** (20 mg, 0.052 mmol) in CH_2Cl_2 (2 mL) at −78 °C was added boron tribromide (1 M in CH_2Cl_2) (228 μL, 0.228 mmol). The reaction mixture was stirred at −78 °C for 1 h 30 min, additional boron tribromide (1 M in CH_2Cl_2) (456 μL, 0.456 mmol) was added, and the solution was stirred at 25 °C for 20 h. There was no further reaction progress, so the solution was poured into water, extracted with CH_2Cl_2, and then with diethyl ether, after which the organic layers were combined and dried over anhydrous $MgSO_4$, and solvent was removed under reduced pressure. The crude oil was purified by pTLC (silica, 15% $CH_3OH-CH_2Cl_2$) and eluted off silica with diethyl ether to give **NC-17** as a yellow solid (15.9 mg, 0.046 mmol) in 89% yield, deemed as 73% pure by HPLC. R_f 0.18 (10% $CH_3OH-CH_2Cl_2$); ^{1}H NMR (400 MHz, CD_3OD) δ 8.00 (d, J = 8.30 Hz, 2 H), 7.34 (d, J = 8.30 Hz, 2 H), 6.99 (d, J = 8.30 Hz, 2 H), 6.92 (d, J = 1.95 Hz, 1 H), 6.84 (m, 1 H), 6.61 (dd, J = 8.30, 2.44 Hz, 1 H), 6.54 (d, J = 8.79 Hz, 2 H), 3.73 (s, 2 H); ^{13}C NMR (100 MHz, CD_3OD) 169.78, 157.67, 156.73, 145.45, 143.47, 140.90, 139.87, 137.78, 131.24, 130.65, 130.45, 129.21, 126.12, 120.99, 116.05, 114.31, 112.41, 42.02 ppm; HRMS (EI) exact mass calculated for $C_{22}H_{16}O_4$: 344.1049, found: 344.1424.

1-(4-(Bromomethyl)phenyl)-2-(4-hydroxyphenyl)-3*H*-inden-5-ol (NC-18). To a solution of alcohol **19** (18.6 mg, 0.052 mmol) in CH_2Cl_2 (4 mL) at −78 °C was added boron tribromide (1 M in CH_2Cl_2) (229 μL, 0.229 mmol). The reaction mixture was stirred at −78 °C for 30 min, allowing the dry ice bath to warm to 25 °C, after which the solution was stirred a further 2 h 30 min. The solution was poured into water, stirred 30 min, extracted with CH_2Cl_2, washed with saturated $NaHCO_3$, and dried over anhydrous $MgSO_4$, and solvent was removed under reduced pressure. The crude oil was purified by pTLC (50% EtOAc−hexanes) to give **NC-18** as a yellowish solid (5.9 mg, 0.015 mmol) in 29% yield. Purity could not be ascertained by HPLC, due to instability of the compound on the columns. R_f 0.30 (30% EtOAc−hexanes); ^{1}H NMR (400 MHz, CD_3OD) δ 7.39 (d, J = 8.30 Hz, 2 H), 7.20 (d, J = 7.81 Hz, 2 H), 6.99 (d, J = 8.79 Hz, 2 H), 6.89 (s, 1 H), 6.82 (d, J = 8.30 Hz, 1 H), 6.59 (m, 1 H), 6.52 (d, J = 8.79 Hz, 2 H), 4.55 (s, 2 H), 3.69 (s, 2 H); ^{13}C NMR (100 MHz, CD_3OD) 157.45, 156.58, 145.39, 140.26, 140.07, 138.55, 138.44, 138.07, 130.84, 130.61, 130.36, 129.47, 121.07, 115.94, 114.24, 112.32, 41.88, 34.00 ppm; HRMS (EI) exact mass calculated for $C_{22}H_{18}O_3$: 392.0412, found: 392.2022.

4-(6-Hydroxy-2-(4-hydroxyphenyl)-1*H*-inden-3-yl)-benzamide (NC-19). Coupling. To a solution of flame-dried ammonium chloride (43.1 mg, 0.806 mmol) in benzene (3 mL) at °0 C was slowly added trimethylaluminum (2 M in hexanes) (403 μL, 0.366 mmol). The reaction was stirred for 1 h 15 min at 25 °C, until the evolution of bubbles ended. This trimethylaluminum-ammonium chloride complex[49] was transferred via cannula to a solution of methyl ester **18** (141.5 mg, 0.366 mmol) in benzene (2 mL), and the fluorescent yellow solution was stirred at 60 °C for 4 h 30 min. Further reaction time did not result in more than 50% conversion as monitored by TLC, so the solution was cooled to 25 °C, poured into water, and extracted with diethyl ether and then with dicholoromethane. The organic layers were washed with saturated NaCl, combined, and dried over $MgSO_4$, and solvent was removed under reduced pressure. The crude oil was purified by flash column chromatography (silica, 0%-2% $CH_3OH-CHCl_3$), followed by pTLC (silica, 5% $CH_3OH-CH_2Cl_2$) and eluted off silica with EtOAc and 5% $CH_3OH-CHCl_3$ to give 4-(6-methoxy-2-(4-methoxyphenyl)-1*H*-inden-3-yl)benzamide as a whitish solid

(71.0 mg, 0.191 mmol) in 52% yield. R_f 0.23 (5% $CH_3OH-CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, J = 8.42 Hz, 2 H), 7.52 (d, J = 8.61 Hz, 2 H), 7.25 (d, J = 8.97 Hz, 2 H), 7.22 (d, J = 2.01 Hz, 1 H), 7.12 (d, J = 8.42 Hz, 1 H), 6.90 (dd, J = 8.42, 2.38 Hz, 1 H), 6.82 (d, J = 8.97 Hz, 2 H), 3.95 (s, 2 H), 3.93 (s, 3 H), 3.85 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) 171.62, 159.39, 158.90, 144.74, 141.41, 140.56, 140.45, 137.63, 132.88, 130.23, 129.93, 129.68, 128.85, 120.71, 114.29, 112.70, 110.81, 55.92, 55.51, 41.81 ppm; HRMS (EI) exact mass calculated for $C_{24}H_{21}NO_3$: 371.1521, found: 371.1534.

Deprotection. To a solution of methyl 4-(6-methoxy-2-(4-methoxyphenyl)-1*H*-inden-3-yl)benzoate (74.3 mg, 0.200 mmol) in CH_2Cl_2 (2 mL) at −78 °C was added boron tribromide (1 M in CH_2Cl_2) (880 μL, 0.880 mmol). The reaction mixture was stirred at −78 °C for 45 min and then at 25 °C for 2 h 30 min. The solution was poured into water and extracted with CH_2Cl_2 and then with EtOAc, after which the organic layers were combined and dried over anhydrous $MgSO_4$, and solvent was removed under reduced pressure. The crude oil was purified by pTLC (silica, 25% $CH_3OH-CH_2Cl_2$) and eluted off silica with EtOAc to give **NC-19** as a yellow oil (45.5 mg, 0.133 mmol) in 66% yield. R_f 0.28 (10% $CH_3OH-CH_2Cl_2$); ^1H NMR (400 MHz, CD_3OD) δ 7.86 (d, J = 8.79 Hz, 2 H), 7.34 (d, J = 8.30 Hz, 2 H), 7.00 (d, J = 8.79 Hz, 2 H), 6.92 (d, J = 1.95 Hz, 1 H), 6.84 (d, J = 8.30 Hz, 1 H), 6.61 (m, 1 H), 6.53 (d, J = 8.79 Hz, 2 H), 3.73 (s, 2 H); ^{13}C NMR (100 MHz, CD_3OD) 172.26, 157.64, 156.72, 145.45, 142.32, 140.78, 139.94, 137.76, 133.60, 130.68, 130.45, 129.25, 129.18, 120.97, 116.03, 114.29, 112.41, 42.00 ppm; HRMS (EI) exact mass calculated for $C_{22}H_{17}NO_3$: 343.1208, found: 343.1214.

Methyl 4-(6-Hydroxy-2-(4-hydroxyphenyl)-1*H*-inden-3-yl)benzoate (NC-20). To a slurry of **NC-17** (39.1 mg, 0.114 mmol) in THF (3 mL) was added CH_3OH (2 mL). The solution was cooled to 0 °C, and lithium aluminum hydride (4.74 mg, 0.125 mmol) was added. The orange suspension was stirred for 2 h at 0 °C and then overnight at 25 °C, upon which it became brown. The reaction was poured into saturated sodium potassium tartarate, and the CH_3OH was removed under reduced pressure. The solution was acidified to pH 3 with 3 N HCl, extracted with ether, washed with water, and dried over anhydrous $MgSO_4$, and solvent was removed under reduced pressure. The crude oil was purified by pTLC (10% $CH_3OH-CHCl_3$) to give retrieved acid starting material (9 mg, 0.026 mmol) in 23% yield, and **NC-20** product (7.6 mg, 0.0212 mmol) in 19% yield, deemed as 72% pure by HPLC. R_f 0.40 (10% $CH_3OH-CHCl_3$); ^1H NMR (400 MHz, CD_3OD) δ 8.00 (d, J = 8.30 Hz, 2 H), 7.36 (d, J = 8.79 Hz, 2 H), 6.99 (d, J = 8.79 Hz, 2 H), 6.92 (d, J = 2.44 Hz, 1 H), 6.83 (d, J = 8.30 Hz, 1 H), 6.61 (dd, J = 8.30, 2.44 Hz, 1 H), 6.54 (d, J = 8.79 Hz, 2 H), 3.87 (s, 3 H), 3.74 (s, 2 H); the sample was too dilute for adequate analysis by ^{13}C NMR; HRMS (EI) exact mass calculated for $C_{23}H_{18}O_4$: 358.1205, found: 358.1206.

Acknowledgment. We are grateful for support of this research through a grant from the National Institutes of Health (DK-57574) (T.S.S). N.J.C. was supported by a dissertation fellowship from the California Breast Cancer Research Program (grant #8GB-0025) and by a University of California Regents Fellowship. Mass spectra were obtained on instruments supported by the NIH (NCRR RR01614). We thank the laboratory of Dr. R. Kip Guy for assistance with HPLC analysis, and members of the Scanlan laboratory for helpful discussions.

Supporting Information Available: Preparation of synthetic intermediates and HPLC purity data for all final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Weatherman, R. V.; Fletterick, R. J.; Scanlan, T. S. Nuclear-receptor ligands and ligand-binding domains. *Annu. Rev. Biochem.* **1999**, *68*, 559−581.

(2) Jordan, V. C. Antiestrogens and selective estrogen receptor modulators as multifunctional medicines. 1. Receptor interactions. *J. Med. Chem.* **2003**, *46*, 883−908.

(3) Smith, C. L.; O'Malley, B. W. Coregulator function: a key to understanding tissue specificity of selective receptor modulators. *Endocr. Rev.* **2004**, *25*, 45−71.

(4) Paech, K.; Webb, P.; Kuiper, G. G.; Nilsson, S.; Gustafsson, J. et al. Differential ligand activation of estrogen receptors ERalpha and ERbeta at AP1 sites. *Science* **1997**, *277*, 1508−1510.

(5) Weatherman, R. V.; Clegg, N. J.; Scanlan, T. S. Differential SERM activation of the estrogen receptors (ERalpha and ERbeta) at AP-1 sites. *Chem. Biol.* **2001**, *8*, 427−436.

(6) Rosenfeld, M. G.; Glass, C. K. Coregulator codes of transcriptional regulation by nuclear receptors. *J. Biol. Chem.* **2001**, *276*, 36865−36868.

(7) Glass, C. K.; Rosenfeld, M. G. The coregulator exchange in transcriptional functions of nuclear receptors. *Genes Dev.* **2000**, *14*, 121−141.

(8) Qiu, J.; Bosch, M. A.; Tobias, S. C.; Grandy, D. K.; Scanlan, T. S. et al. Rapid signaling of estrogen in hypothalamic neurons involves a novel G-protein-coupled estrogen receptor that activates protein kinase C. *J. Neurosci.* **2003**, *23*, 9529−9540.

(9) Katzenellenbogen, J. A.; Carlson, K. E.; Katzenellenbogen, B. S. Facile geometric isomerization of phenolic nonsteroidal estrogens and antiestrogens: limitations to the interpretation of experiments characterizing the activity of individual isomers. *J. Steroid Biochem.* **1985**, *22*, 589−596.

(10) Katzenellenbogen, B. S.; Norman, M. J.; Eckert, R. L.; Peltz, S. W.; Mangel, W. F. Bioactivities, estrogen receptor interactions, and plasminogen activator-inducing activities of tamoxifen and hydroxy-tamoxifen isomers in MCF-7 human breast cancer cells. *Cancer Res.* **1984**, *44*, 112−119.

(11) Robertson, D. W.; Katzenellenbogen, J. A.; Long, D. J.; Rorke, E. A.; Katzenellenbogen, B. S. Tamoxifen antiestrogens. A comparison of the activity, pharmacokinetics, and metabolic activation of the cis and trans isomers of tamoxifen. *J. Steroid Biochem.* **1982**, *16*, 1−13.

(12) Lednicer, D.; Lyster, S. C.; Aspergren, B. D.; Duncan, G. W. Mammalian antifertility agents. 3. 1-Aryl-2-phenyl-1,2,3,4-tetrahydro-1-naphthols, 1-aryl-2-phenyl-3,4-dihydronaphthalenes, and their derivatives. *J. Med. Chem.* **1966**, *9*, 172−176.

(13) Lednicer, D.; Lyster, S. C.; Duncan, G. W. Mammalian antifertility agents. IV. Basic 3,4-dihydronaphthalenes and 1,2,3,4-tetrahydro-1-naphthols. *J. Med. Chem.* **1967**, *10*, 78−84.

(14) McCague, R.; Kuroda, R.; Leclercq, G.; Stoessel, S. Synthesis and estrogen receptor binding of 6,7-dihydro-8-phenyl-9-[4-[2-(dimethylamino)ethoxy] phenyl]-5H-benzocycloheptene, a non-isomerizable analogue of tamoxifen. X-ray crystallographic studies. *J. Med. Chem.* **1986**, *29*, 2053−2059.

(15) Adler, E.; Hagglund, B. *Ark Kemi Mineral Geol.* **1945**, *19A*, 1.

(16) Korach, K. S.; Metzler, M.; McLachlan, J. A. Estrogenic activity in vivo and in vitro of some diethylstilbestrol metabolites and analogs. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 468−471.

(17) Korach, K. S.; Metzler, M.; McLachlan, J. A. Diethylstilbestrol metabolites and analogues. New probes for the study of hormone action. *J. Biol. Chem.* **1979**, *254*, 8963−8968.

(18) Korach, K. S.; Fox-Davies, C.; Quarmby, V. E.; Swaisgood, M. H. Diethylstilbestrol metabolites and analogs. Biochemical probes for differential stimulation of uterine estrogen responses. *J. Biol. Chem.* **1985**, *260*, 15420−15426.

(19) Korach, K. S.; Levy, L. A.; Sarver, P. J. Estrogen receptor stereochemistry: receptor binding and hormonal responses. *J. Steroid Biochem.* **1987**, *27*, 281−290.

(20) Curtis, S. W.; Shi, H.; Teng, C.; Korach, K. S. Promoter and species specific differential estrogen-mediated gene transcription in the uterus and cultured cells using structurally altered agonists. *J. Mol. Endocrinol.* **1997**, *18*, 203-211.

(21) Chae, K.; Gibson, M. K.; Korach, K. S. Estrogen Receptor Stereochemistry − Ligand Binding Orientation and Influence on Biological Activity. *Mol. Pharmacol.* **1991**, *40*, 806−811.

(22) Kohno, H.; Bocchinfuso, W. P.; Gandini, O.; Curtis, S. W.; Korach, K. S. Mutational analysis of the estrogen receptor ligand-binding domain: influence of ligand structure and stereochemistry on transactivation. *J. Mol. Endocrinol.* **1996**, *16*, 277−285.

(23) Mueller, S. O.; Hall, J. M.; Swope, D. L.; Pedersen, L. C.; Korach, K. S. Molecular determinants of the stereoselectivity of agonist activity of estrogen receptors (ER) alpha and beta. *J. Biol. Chem.* **2003**, *278*, 12255−12262.

(24) Korach, K. S.; Chae, K.; Levy, L. A.; Duax, W. L.; Sarver, P. J. Diethylstilbestrol metabolites and analogs. Stereochemical probes for the estrogen receptor binding site. *J. Biol. Chem.* **1989**, *264*, 5642−5647.

(25) Chae, K.; Johnston, S. H.; Korach, K. S. Multiple estrogen binding sites in the uterus: stereochemistry of receptor and nonreceptor binding of diethylstilbestrol and its metabolites. *J. Steroid Biochem. Mol. Biol.* **1991**, *38*, 35−42.

(26) Sadler, B. R.; Cho, S. J.; Ishaq, K. S.; Chae, K.; Korach, K. S. Three-dimensional quantitative structure−activity relationship study of nonsteroidal estrogen receptor ligands using the comparative molecular field analysis/cross-validated r2-guided region selection approach. *J. Med. Chem.* **1998**, *41*, 2261−2267.
(27) Lednicer, D.; Babcock, J. C.; Marlatt, P. E.; Lyster, S. C.; Duncan, G. W. Mammalian Antifertility Agents. I. Derivatives of 2,3-Diphenylindenes. *J. Med. Chem.* **1965**, *39*, 52−57.
(28) Duncan, G. W.; Lednicer, D. *Method for Controlling Birds and Rodents*; US Patent Office, 1967; p 46.
(29) Anstead, G. M.; Altenbach, R. J.; Wilson, S. R.; Katzenellenbogen, J. A. 2,3-Diarylindenes and 2,3-diarylindenones: synthesis, molecular structure, photochemistry, estrogen receptor binding affinity, and comparisons with related triarylethylenes. *J. Med. Chem.* **1988**, *31*, 1316−1326.
(30) Anstead, G. M.; Katzenellenbogen, J. A. Fluorescence Properties of 2,3-Diarylindenes. *J. Phys. Chem.* **1988**, *92*, 6249−6258.
(31) Anstead, G. M.; Peterson, C. S.; Pinney, K. G.; Wilson, S. R.; Katzenellenbogen, J. A. Torsionally and hydrophobically modified 2,3-diarylindenes as estrogen receptor ligands. *J. Med. Chem.* **1990**, 33, 2726−2734.
(32) Anstead, G. M.; Wilson, S. R.; Katzenellenbogen, J. A. 2-Arylindenes and 2-arylindenones: molecular structures and considerations in the binding orientation of unsymmetrical nonsteroidal ligands to the estrogen receptor. *J. Med. Chem.* **1989**, *32*, 2163-2171.
(33) Anstead, G. M.; Peterson, C. S.; Katzenellenbogen, J. A. Hydroxylated 2,3-diarylindenes: synthesis, estrogen receptor binding affinity, and binding orientation considerations. *J. Steroid Biochem.* **1989**, *33*, 877−887.
(34) Kirkiacharian, S.; Koutsourakis, P. G.; Philibert, D.; Bouchoux, F.; Van de Velde, P. Synthesis and relative binding affinity to steroid receptors and antiproliferative activity on MCF-7 cells of 2,3-disubstituted indenes. *Farmaco* **1999**, *54*, 678−683.
(35) Bryant, H. U.; Jones, C. D.; Pennington, P. A. *Indene compounds having activity as SERMs*; European Patent Office: London, Great Britain, 1998; p 33.
(36) Shiau, A. K.; Barstad, D.; Loria, P. M.; Cheng, L.; Kushner, P. J. et al. The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. *Cell* **1998**, *95*, 927−937.
(37) Pike, A. C.; Brzozowski, A. M.; Hubbard, R. E.; Bonn, T.; Thorsell, A. G. et al. Structure of the ligand-binding domain of oestrogen receptor beta in the presence of a partial agonist and a full antagonist. *EMBO J.* **1999**, *18*, 4608−4618.
(38) Brzozowski, A. M.; Pike, A. C.; Dauter, Z.; Hubbard, R. E.; Bonn, T. et al. Molecular basis of agonism and antagonism in the oestrogen receptor. *Nature* **1997**, *389*, 753−758.
(39) Korach, K. S.; Chae, K.; Gibson, M.; Curtis, S. Estrogen Receptor Stereochemistry − Ligand Binding and Hormonal Responsiveness. *Steroids* **1991**, *56*, 263−270.
(40) Chae, K.; Levy, L. A.; Korach, K. S. Chromatographic separation and isolation of the enantiomers of diethylstilbestrol metabolites. *J. Chromatogr.* **1988**, *439*, 484−487.
(41) Parker, C. E.; Levy, L. A.; Smith, R. W.; Yamaguchi, K.; Gaskell, S. J. et al. Separation and detection of enantiomers of stilbestrol analogues by combined high-performance liquid chromatography-thermospray mass spectrometry. *J. Chromatogr.* **1985**, *344*, 378−384.
(42) Sadler, B. R.; Chae, K.; Ishaq, K. S.; Korach, K. S. Separation of indenestrol A and B isomers and enantiomers by high-performance liquid chromatography. *J. Chromatogr. A* **1998**, *799*, 117−124.
(43) Anstead, G. M.; Ensign, J. L.; Peterson, C. S.; Katzenellenbogen, J. A. 2-Arylindenes and 2-arylindenones: synthesis of probes to study the binding orientation of unsymmetrical nonsteroidal ligands to the estrogen receptor. *J. Org. Chem.* **1989**, *54*, 1495−1491.
(44) Plentl, A. A.; Bogert, M. T. The Synthesis of Tricyclic Hydrocarbons Related to Stilbestrol. *J. Am. Chem. Soc*. **1941**, *63*, 989−995.
(45) Meurling, L. Alkylation of Indenyllithium and Alkylindenyllithium Compounds. *Acta Chem. Scand., Ser. B*, *Org. Chem. Biochem.* **1974**, *B28*, 295−300.
(46) Cedheim, L.; Eberson, L. Simple Procedure for Preparation of 1-Alkylindenes. *Synthesis (Stuttgart)* **1973**, 159−159.
(47) Silverman, T.; Bogert, M. T. The Synthesis of Some Indene and Dihydronaphthalene Derivatives Related to Stilbestrol. *J. Org. Chem.* **1948**, *11*, 34−49.
(48) Brown, D. W.; Denman, C.; O'Donnell, H. The Chemistry of Indanones. Part 1. The Ring Closure of 2-(4-Methoxyphenyl)-3-(3-Methoxyphenyl)propionic Acid. *J. Chem. Soc. C* **1971**, *19*, 3195−3198.
(49) Hwang, K. J.; Oneil, J. P.; Katzenellenbogen, J. A. 5, 6, 11, 12-Tetrahydrochrysenes − Synthesis of Rigid Stilbene Systems Designed to Be Fluorescent Ligands for the Estrogen-Receptor. *J. Org. Chem.* **1992**, *57*, 1262−1271.
(50) Blizzard, T. A.; Mosley, R. T.; Birzin, E. T.; Chan, W.; Hammond, M. L. Comparison of 2-phenylspiroindenes and 2-phenylspiroindenediones as estrogen receptor ligands—modeling and binding data don't agree! *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1317−1321.
(51) Muthyala, R. S.; Sheng, S.; Carlson, K. E.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A. Bridged bicyclic cores containing a 1,1-diarylethylene motif are high-affinity subtype-selective ligands for the estrogen receptor. *J. Med. Chem.* **2003**, *46*, 1589−1602.
(52) Mortensen, D. S.; Rodriguez, A. L.; Carlson, K. E.; Sun, J.; Katzenellenbogen, B. S. et al. Synthesis and biological evaluation of a novel series of furans: ligands selective for estrogen receptor alpha. *J. Med. Chem.* **2001**, *44*, 3838−3848.
(53) Sun, J.; Meyers, M. J.; Fink, B. E.; Rajendran, R.; Katzenellenbogen, J. A. et al. Novel ligands that function as selective estrogens or antiestrogens for estrogen receptor-alpha or estrogen receptor-beta. *Endocrinology* **1999**, *140*, 800−804.
(54) Shiau, A. K.; Barstad, D.; Radek, J. T.; Meyers, M. J.; Nettles, K. W. et al. Structural characterization of a subtype-selective ligand reveals a novel mode of estrogen receptor antagonism. *Nat. Struct. Biol.* **2002**, *9*, 359−364.
(55) Nettles, K. W.; Sun, J.; Radek, J. T.; Sheng, S.; Rodriguez, A. L. et al. Allosteric control of ligand selectivity between estrogen receptors alpha and beta: implications for other nuclear receptors. *Mol. Cell* **2004**, *13*, 317−327.
(56) Webb, P.; Lopez, G. N.; Uht, R. M.; Kushner, P. J. Tamoxifen Activation of the Estrogen Receptor/AP-1 Pathway − Potential Origin For the Cell-Specific Estrogen-Like Effects of Antiestrogens. *Mol. Endocrinol.* **1995**, *9*, 443−456.
(57) Paruthiyil, S.; Parmar, H.; Kerekatte, V.; Cunha, G. R.; Firestone, G. L. et al. Estrogen receptor beta inhibits human breast cancer cell proliferation and tumor formation by causing a G2 cell cycle arrest. *Cancer Res.* **2004**, *64*, 423−428.
(58) Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. Safe and convenient procedure for solvent purification. *Organometallics* **1996**, *15*, 1518−1520.
(59) Hone, N. D.; Baxter, A. D. *Organosilicon Compounds and Their Use in Combinatorial Chemistry*; European Patent Office: London, Great Britain, 1998.
(60) Myers, T. C.; Pratt, R. J.; Morgan, R. L.; O'Donnell, J.; Jensen, E. V. Hormone Analogues. I. Preparation of Intermediate Ketones for the Synthesis of Stilbestrol Analogues. *J. Am. Chem. Soc.* **1955**, *77*, 5655−5657.
(61) Skaddan, M. B.; Katzenellenbogen, J. A. Integrated "3+1" oxorhenium(V) complexes as estrogen mimics. *Bioconjugate Chem.* **1999**, *10*, 119−129.
(62) Matsuo, J.; Koga, K. Enantioselective alpha-alkylation of phenylacetic acid using a chiral bidentate lithium amide as a chiral auxiliary. *Chem. Pharm. Bull.* **1997**, *45*, 2122−2124.
(63) Hauser, C. R.; Chambers, W. J. Alkylations of the α-Carbon of Phenylacetic and Diphenylacetic Acids with Benzyl Chloride and Related Halides by Alkali Amides. *J. Am. Chem. Soc.* **1956**, *78*, 4942−4944.
(64) Carpino, L. A.; Lin, Y. Z. Benz[*f*]Indene. *J. Org. Chem.* **1990**, *55*, 247−250.
(65) Garro-Helion, F.; Merzouk, A.; Guibe, F. Mild and selective palladium(0)-catalyzed deallylation of allylic amines. Allylamine and diallylamine as very convenient ammonia equivalents for the synthesis of primary amines. *J. Org. Chem.* **1993**, *58*, 6109−6113.

JM050226I