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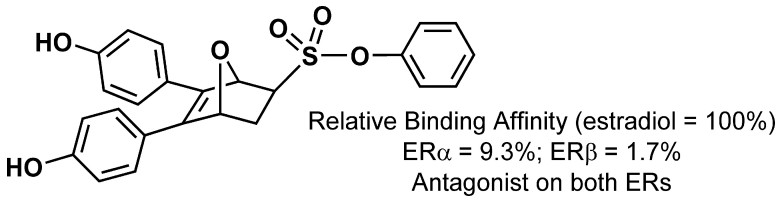

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Synthesis and Evaluation of Estrogen Receptor Ligands with Bridged Oxabicyclic Cores Containing a Diarylethylene Motif: Estrogen Antagonists of Unusual Structure

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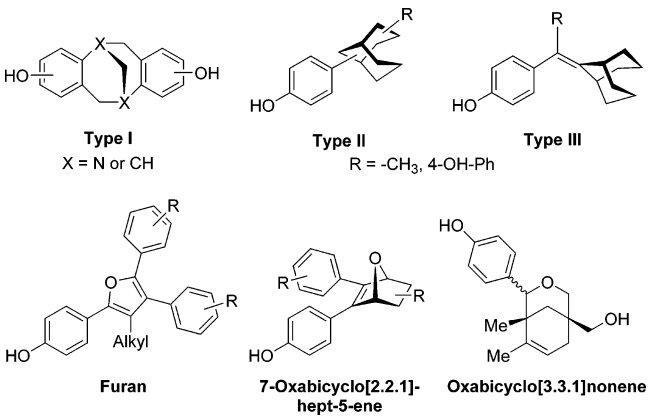
A new series of ligands for the estrogen receptor (ER) based on a three-dimensional structural motif consisting of a bridged oxabicyclic core (7-oxabicyclo[2.2.1]heptene or heptadiene) were synthesized and examined for their receptor binding activity and as regulators of transcription through the two ER subtypes, ERα and ERβ. The prototypical ligands also contain a 1,2-diarylethylene motif, common to many nonsteroidal estrogens, as an embellishment on the oxabicyclic core. Thus, these ligands bear peripheral groups typically found in ER ligands, built here upon an overall three-dimensional core topology that is unusual for these targets. Most of these compounds were conveniently synthesized by a Diels−Alder reaction of various 3,4-diarylfurans with a variety of dienophiles, neat and under mild conditions in the absence of catalysts. Some of the synthesized compounds display good binding affinity for the ER, and in transcription assays, the highest affinity compounds are antagonists on both ERs. Molecular modeling studies suggest a structural basis for the antagonist activity of these compounds. These compounds, based on the bicyclo[2.2.1]core system, expand the structural diversity of ligands that can be antagonists for the estrogen receptors.

Introduction

The estrogen receptors (ERs), members of the family of nuclear receptors, have emerged as attractive pharmaceutical targets for therapeutic intervention in a wide variety of diseases, including osteoporosis and breast cancer. Two receptor subtypes are now known, ERα and ERβ,[1,2] and they have different tissue distribution patterns. For example, ERα predominates in the breast and in reproductive tissues such as the uterus, whereas ERβ is the principal subtype in the ovary and certain regions of the brain.[3−5] Because of the known importance of ERα as a pharmaceutical target and the potential importance of ERβ as well,[6] molecules that act as agonists or antagonists selectively on one or the other of the ER subtypes are currently being investigated for their therapeutic potential; those whose activity also shows tissue selectivity, termed selective estrogen receptor modulators (SERMs),[7,8] are of particular interest.

As part of our long-term interest in ER ligands, we have undertaken exploratory studies aimed at preparing new compounds wherein the central hydrophobic core has, overall, a more *three-dimensional* topology than is typically found in both steroidal and nonsteroidal estrogen ligands. This design strategy was based on structural studies of the ligand binding pockets of both ERα and ERβ that reveal substantial unoccupied space above and below the mean plane of the endogenous ligand, estradiol (E_2), particularly near the middle of this molecule (namely, below the B ring and above the C ring),[9] as well as the apparent flexibility of the ligand binding pocket. By incorporating a hydrophobic bicyclic unit as the core structure of new ligands, we hoped to exploit this unfilled space in the ER binding pocket and thereby, potentially, to enhance binding affinity or ER subtype selectivity. In fact, a number of recent reports have described both steroidal and nonsteroidal estrogens, such as those derived from ferrocene,[10−12] carboranes,[13,14] cyclopentadienyl metal tricarbonyls,[15] and polycyclics,[16] made up of structural elements having a pronounced overall three-dimensional topology. In light of these recent reports and in continuation of our interest in nonsteroidal estrogens, we have taken a novel approach to probe the ligand binding pockets in the two ERs with a new bicyclic core structure.

Scheme 1



In previous studies, we have reported three different structural motifs shown in Scheme 1, each containing

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a bicyclo[3.3.1]nonane core, as possible estradiol mimics, and we have conducted a limited structure−activity relationship (SAR) study of each of these types.[17,18] Type III compounds emerged as the most promising leads for developing high-affinity ER ligands, but they showed little selectivity for either ER subtype. Type II compounds, on the other hand, despite their lower affinity, exhibited significant ERβ binding selectivity. In addition, our examinations of different classes of synthetic heterocyclic ER ligands, in which, for example, the core structure is a simple five-membered ring heterocycle, such as a furan[19] or a pyrazole,[20−22] furnish examples of other compounds with high binding affinity, subtype selectivity, or both. Thus, we wondered whether the introduction of a heteroatom in a bicyclic core (e.g., an oxa- or azabicyclic bridged compound) might provide ER ligands with interesting biological character.

A brief survey of the various available bicyclic systems suggested that a 7-oxabicyclo[2.2.1]hept-5-ene system would be an appropriate three-dimensional hydrophobic core element. In addition, in a ligand of this design, the oxygen group in the heterocyclic moiety would mimic an element of the core of the furan system; furan-core ER ligands are known to have exceptionally high ER binding affinity and ERα subtype selectivity.[19] Recently, ER ligands having a related oxabicyclo[3.3.1]nonene core structure have been reported by investigators at Bayer,[23] and researchers at Ligand Pharmaceuticals have further developed structure−activity relationship (SAR) studies on this class of compounds.[24] However, to the best of our knowledge, there has as yet been no report of the synthesis of 7-oxabicyclo[2.2.1]hept-5-ene compounds as ER ligands.

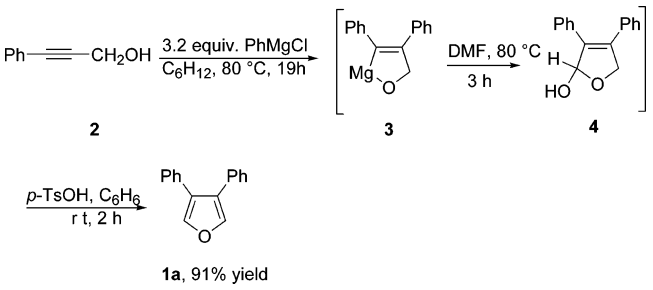
In this report, we describe a novel type of ER ligand constituted of a 7-oxabicyclic[2.2.1]hept-5-ene or hepta-2,5-diene core that can be prepared conveniently by a Diels−Alder reaction of a furan with a suitable dienophile. By starting with appropriately substituted furans, one can produce by this approach bicyclic[2.2.1]heptene systems that are adorned with a 1,2-diarylethylene unit, a motif found in many high-affinity nonsteroidal estrogens. Thus, the bicyclic core ER ligands of this design have the overall three-dimensional topology that we have sought in a form that is adorned with typical ER ligand peripheral elements. These novel bicyclic core compounds present ligands of unusual sizes and geometries that can be used to probe the size, shape, and flexibility of the ERα and ERβ ligand binding pockets and potentially could be further developed as pharmacological agents.

Results and Discussion

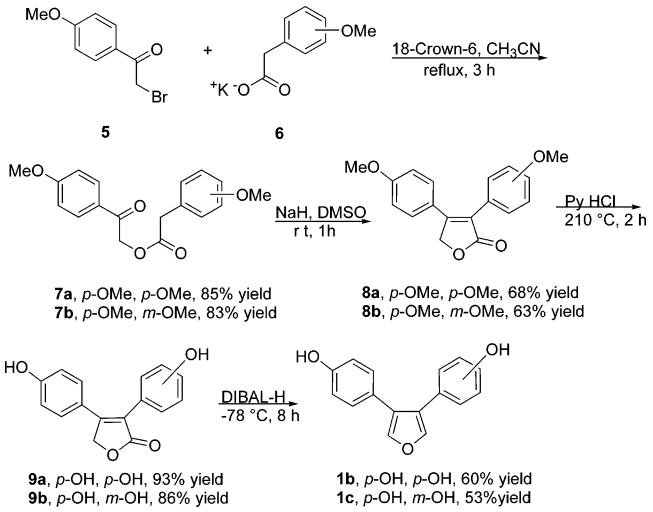
Chemical Synthesis. The synthetic route for the preparation of the oxabicyclic bridged compounds involved a Diels−Alder reaction of aryl-substituted furans with various dienophiles. The synthesis of the basic 3,4-diphenyl furan **1a** was accomplished as shown in Scheme 2. Carbometalation of 3-phenyl-2-propyn-1-ol **2** with phenylmagnesium chloride gave the magnesium chelate **3**, which was allowed to react with DMF; acidification of the intermediate hemiacetal **4** without isolation gave the furan **1a**.[25]

3,4-Bis(4-hydroxyphenyl)furan **1b** and 3-(4-hydroxyphenyl)-4-(3-hydroxyphenyl)furan **1c** were synthesized

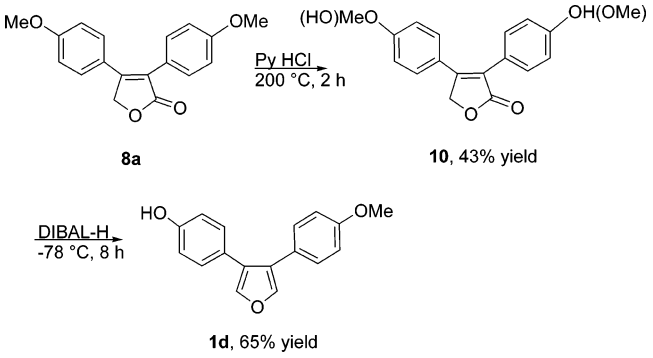
Scheme 2



Scheme 3



Scheme 4



by the general route shown in Scheme 3.[26] Condensation of the potassium salts of arylacetic acids **6** with α-bromo-4-methoxyacetophenone **5** and 18-crown-6 as catalyst gave the corresponding acetates **7**. Treatment of these esters (**7**) with NaH in anhydrous DMSO gave the 2(5*H*)-furanones **8**, which were demethylated with pyridinium chloride at 220 °C to give the phenolic 2(5*H*)-furanones **9**. Diisobutylaluminum hydride reduction of these furanones at −78 °C gave, after acidic workup, the corresponding 3,4-diarylfurans **1b** and **1c**.

When the demethylation reaction of the 2(5*H*)-furanones **8** was carefully controlled at 200 °C, the monodemethylated product **10a** was obtained as a mixture of two isomers, accompanied with almost the same amount of the fully demethylated product **9a**. Diisobutylaluminum hydride reduction of the butenolide **10** at −78 °C gave, after acidic workup, the corresponding monophenolic furan **1d** (Scheme 4).

The synthesis of the oxabicyclic bridged compounds was effectively accomplished by a Diels−Alder reaction

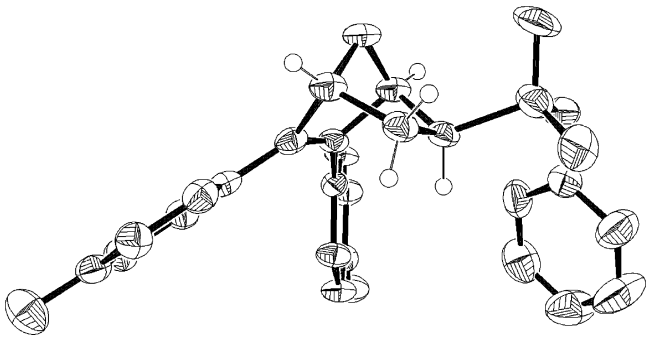
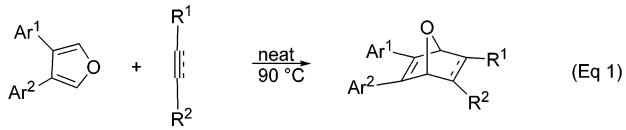


Figure 1. ORTEP of *exo*-**12a**.

of furans **1** and various dienophiles (eq 1). As dienophiles, it proved to be particularly convenient to use vinyl sulfones and sulfonates, acetylene dicarboxylates, maleates, and maleimides. The results from reaction with these dienophiles are summarized in Table 1. While we did explore a range of dienophiles, certain ones, for example, methyl vinyl ketone, gave unstable products; others, especially moderately activated styrene, vinyl ester, and phenylacetylene dienophiles, proved to be rather unreactive, as discussed below.



(Eq 1)

A series of compounds having the 1,2-diphenylene motif were synthesized starting from unprotected phenolic or phenyl furans and the appropriate dienophiles. It is noteworthy that high stereoselectivity was observed in the reaction of phenolic furans with the dienophiles, the *exo* product being obtained nearly exclusively; only traces, if any, of *endo* products were observed. For example, in the reaction of furan **1b** with vinyl phenyl sulfonate **11a**, vinyl phenyl sulfone **11b**, and vinyl ethyl sulfone **11c**, the reaction takes place smoothly at 90 °C, neat (without solvent). The products **12a**−**12c** were obtained exclusively as the *exo* diastereomers in 80%, 81%, and 91% yields, respectively (Table 1, entries 1, 3, and 4).

Endo diastereomers typically predominate as the products of Diels−Alder reactions conducted under conditions of kinetic control because of stabilizing secondary orbital interactions between the diene and dienophile that are only possible in the *endo* transition state. Nevertheless, the predominance of *exo* products in related furan Diels−Alder reactions is precedented.[27] This unexpected *exo* selectivity probably arises because the secondary interactions with a sulfonate-based dienophile are likely to be weaker than those with a carbonyl-based dienophile and also because this facile cycloaddition reaction is probably readily reversible, which results in the rapid accumulation of the thermodynamically more stable *exo* product.

Although the *endo* and *exo* stereoisomers have characteristic ^{1}H NMR signals (see below), to verify our stereoisomeric assignments, we obtained an X-ray crystal structure of compound **12a**, which confirmed that it is *exo*-5,6-bis-(4-hydroxyphenyl)-7-oxabicyclo[2.2.1]hept-5-ene-2-sulfonic acid phenyl ester (Figure 1). As noted below, this compound, in fact, has the highest binding affinity of all of the compounds that we have prepared in this report.

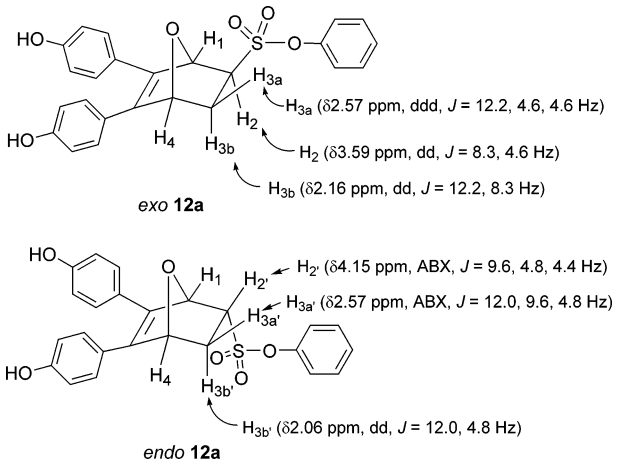
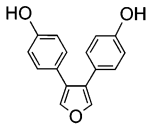
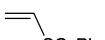
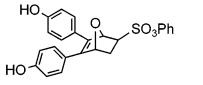
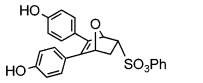
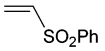
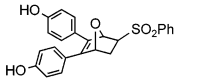
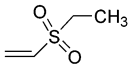
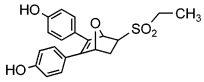
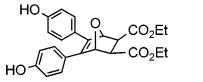
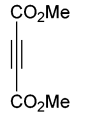
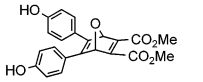
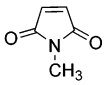
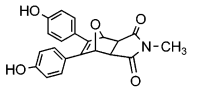
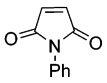
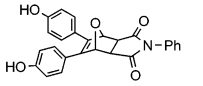
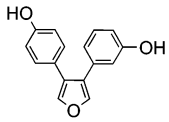
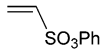
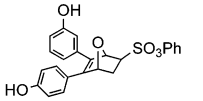
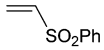
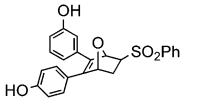
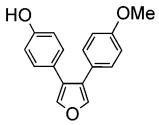
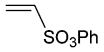
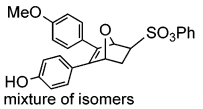
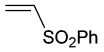
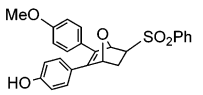
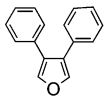
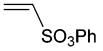
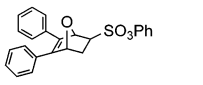
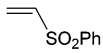
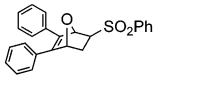
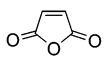
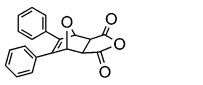


Figure 2. ^{1}H NMR assignments of *exo*- and *endo*-**12a**.

To compare the biological property of the *exo* isomer with the *endo* one, we undertook a brief investigation on the stereoselectivity of the reaction of **1b** with **11a**, and we found that when the reaction was conducted at 35 °C for 24 h, the *endo* isomer of **12a** was formed in 18% yield, in a ratio of 1:3 to the *exo* isomer (Table 1, entry 2). This *endo* isomer could be isolated and characterized spectroscopically. Identifying features in the ^{1}H NMR spectra of *exo* and *endo* **12a** are given in Figure 2.[28] Assignments are facilitated by the fact that the bridgehead protons do not couple with the *endo* protons, because their dihedral angles are very close to 90°; this is evident from the X-ray structure of compound *exo* **12a** (H_1−H_2, 89° and H_{3b}−H_4, 76°). The most characteristic signals are those of the proton on C-2 (H_2): in the case of *exo* **12a**, the H_2 proton at 3.59 ppm is *endo*, appearing as a doublet of doublets having a cis and a gauche coupling of 8.3 and 4.6 Hz, respectively (no coupling to H_1), whereas in *endo* **12a** the $H_{2'}$ proton at 4.15 ppm is *exo*, appearing as an ABX multiplet having one cis and two gauche couplings of 9.6, 4.8, and 4.4 Hz, respectively. The other couplings and chemical shifts are given in Figure 2.

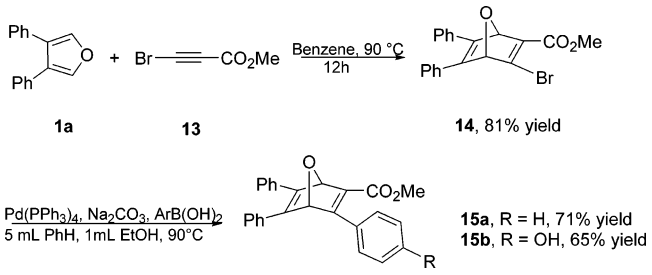
Despite the generally good yields that we obtained with the vinyl sulfone and sulfonyl dienophiles, we found that the reaction of **1b** with diethyl maleate **11d** and dimethyl acetylenedicarboxylate **11e** gave the products **12d** and **12e** in lower yields, 42% and 75%, respectively (Table 1, entries 5 and 6). Part of the reduced yield appears to be the sensitivity of the products to purification by preparative TLC on silica gel. In the reaction of **1b** with **11f** and **11g**, the products **12f** and **12g** were obtained in quantitative yield, and upon completion of the reaction, they were purified simply by filtration and recrystallization from THF (Table 1, entries 7 and 8). Similar to the reactions of furan **1b**, reaction of furans **1c** and **1d** with dienophiles again proceeded with *exo* selectivity but produced a 1:1 mixture of two inseparable regioisomers (Table 1, entries 9−12). In comparison, compounds **12l**, **12m** and **12n**, which have no hydroxyl group on the phenyl ring, were also prepared by the reaction of furan **1a** with **11a**, **11b**, and **11h** in 78%, 81%, and 91% yields, respectively, accompanied with small amounts (∼5%) of *endo* isomers (Table 1, entries 13−15).

Table 1. Diels−Alder Reaction of Furan **1** with Dienophile **11**[a]

Entry	Furan	Dienophile (1.3 equiv.)	Reaction time	Conv.[b] (%)	Product Yield (%)[c]
1	 **1b**	 **11a**	6h	99	 **12a** (*exo*) (80%)
2		**11a**	24h	97[d]	 **12a** (*endo*) (18%)
3		 **11b**	6h	100	 **12b** (81%)
4		 **11c**	6h	100	 **12c** (91%)
5		 **11d**	12h	65	 **12d** (42%)
6		 **11e**	6h	100	 **12e** (75%)
7		 **11f**	6h	100	 **12f** (96%)
8		 **11g**	6h	100	 **12g** (97%)
9	 **1c**	 **11a**	18h	100	 mixture of isomers **12h** (90%)
10		 **11b**	18h	87	 mixture of isomers **12i** (66%)
11	 **1d**	 **11a**	12h	98	 mixture of isomers **12j** (77%)
12		 **11b**	12h	95	 mixture of isomers **12k** (85%)
13	 **1a**	 **11a**	12h	95	 **12l** (78%)
14		 **11b**	12h	100	 **12m** (81%)
15		 **11h**	12h	100	 **12n** (91%)

[a] To a round-bottom flask was added furan (0.2 mmol) and dienophile (0.26 mmol), and the mixture was stirred at the specified temperature and time given in the table. [b] The conversion was calculated based on the crude ^{1}H NMR of the mixture. [c] Isolated yield based on the furan used. [d] The reaction was conducted at 35 °C for 24 h, and 57% of *exo*-**12a** was obtained.

Scheme 5

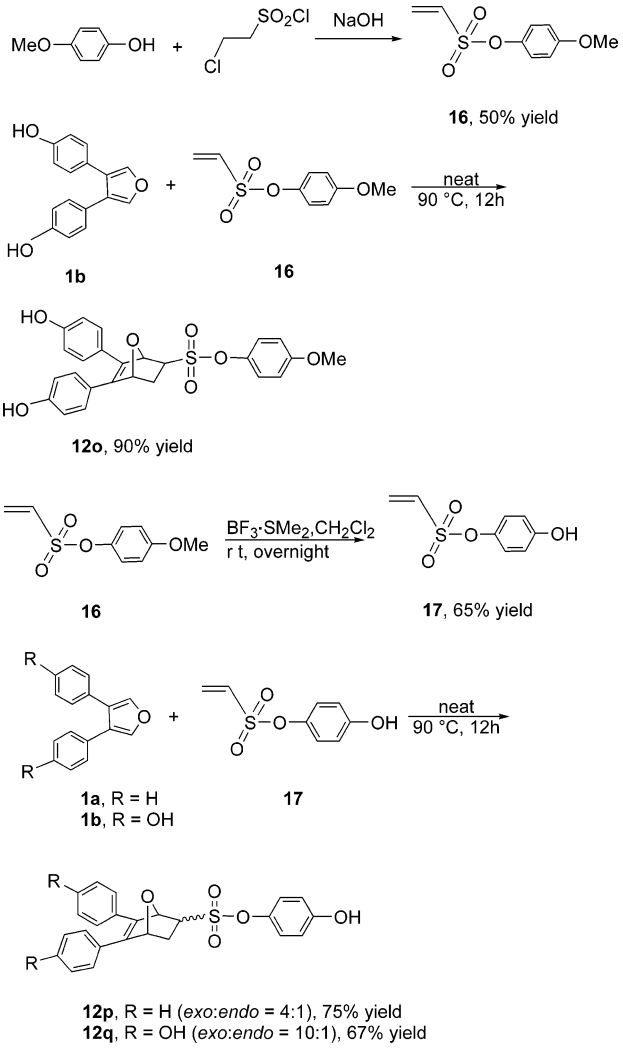


In our previous work on furan-core ER ligands, we found that triaryl, especially trisphenol, furans always show higher binding affinity and subtype selectivity than the corresponding bisphenol analogues.[19] Here, we also wanted to introduce a third phenol ring into the bridged compound, because this design might lead to an ER ligand with interesting biological activity. First, we tried to introduce a third phenol in the bicyclic core as shown in Scheme 5. However, due to the hindrance of the diphenyl groups, attempts to achieve the reaction of furan with dienophiles that bear a phenyl group, for example, methyl 3-phenylpropiolate, diphenylacetylene, styrene, ethyl cinnamate, or vinyl benzoate, failed, even with Lewis acid catalysts. Thus, we took an alternative synthetic strategy.

Methyl 3-bromopropiolate **13**, an active dienophile, was synthesized as described in the literature (Scheme 5).[29] Reaction of **13** with furan **1a** afforded oxabicycloheptene **14** in high isolated yield. The triaryl oxabicyclic bridged compounds **15ab** were prepared by standard Suzuki coupling reactions with the appropriate aryl boronic acids. Unfortunately, the product of the diphenolic furan **1b** with **13** was very unstable, and attempts to isolate the product failed.

In all the compounds that we have prepared, the ligand bearing a phenyl sulfonate in the 2-position of the core always had a higher ER binding affinity (see below). We wondered whether the replacement of the phenyl ring on the sulfonate moiety with a *p*-hydroxyphenyl group might lead to ligands with increased binding affinity. Thus, we investigated the synthesis of compound **12q**, as shown in Scheme 6. It turned out that the synthesis of the ethenesulfonic acid 4-hydroxyphenyl ester (**17**) from the reaction of chloroethanesulfonyl chloride with hydroquinone was unsuccessful. As an alternate approach, 2-chloroethanesulfonic acid 4-methoxyphenyl ester (**16**) was synthesized in 50% yield by the reaction of 2-chloroethanesulfonyl chloride with 4-methoxyphenol in the presence of 25% aqueous NaOH in 1,2-dichloroethane.[30] Reaction of the vinyl sulfone **16** with furan **1b**, using the same reaction conditions as described above, afforded the bicycle **12o** in 90% yield. However, our attempts to demethylate this product (**12o**) gave only products from decomposition. An alternate route was successful however. Demethylation of the vinyl sulfone **16** using 20 equiv of $BF_3{\cdot}SMe_2$ in dichloromethane gave the deprotected vinyl sulfone **17** in 65% yield, and reaction of the latter with furans **1a** and **1b** afforded products **12p** and **12q** in 75% and 67% yields, respectively. At the same time, a significant amount of the *endo* isomer was also observed in these reactions, and the *endo* isomer of **12p** could be isolated in 15% yield.

Scheme 6



Binding Affinity for Estrogen Receptors ERα and ERβ. We examined the binding affinity of these novel bicyclic ligands to both full-length, purified human ERs, ERα and ERβ, as well as ER in uterine cytosol preparations (which contains principally ERα[31] and is included because it is representative of ER in a more natural protein context), by a competitive radiometric binding assay, using methods that have been described elsewhere in detail.[32,33] The binding affinities of all the ligands in this study are expressed as relative binding affinity (RBA) values, where estradiol has an affinity of 100%; the RBA values determined with the three receptor preparations are given in Table 2.

As a global observation, it is noteworthy that the binding affinity of the ligands in this series depends on the position of the hydroxyl group in the phenyl ring and the substituents at the 2 and 3 positions of the 7-oxabicycloheptane core. The compound that has the highest binding affinity in all three ER preparations is *exo*-5,6-bis-(4-hydroxyphenyl)-7-oxabicyclo[2.2.1]hept-5-ene-2-sulfonic acid phenyl ester **12a**, a compound that possesses a *p*-hydroxyl group in both of the core phenyl substituents and a phenyl sulfonate moiety at the 2-position of the bicyclic unit. The RBA values of this compound are 18 on uterine ER and 9.3 and 1.7 for ERα and ERβ, respectively (Table 2, entry 1). Although lamb uterine cytosol consists almost exclusively of ERα,

Table 2. Relative Binding Affinity (RBA) of Oxabicyclic Non-Steroidal Analogues for Estrogen Receptor ERα and ERβ^a

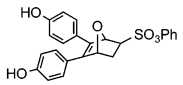
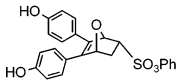
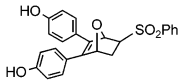
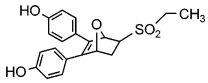
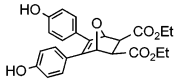
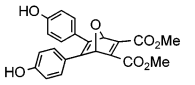
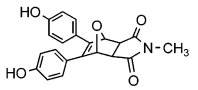
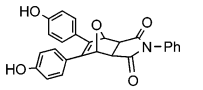
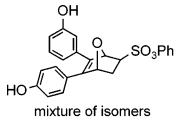
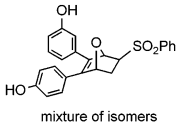
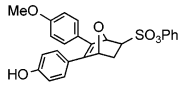
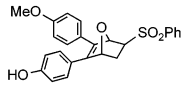
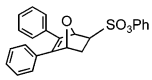
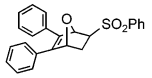
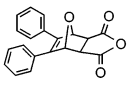
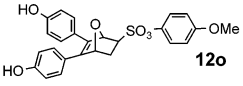
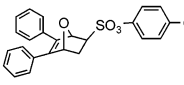
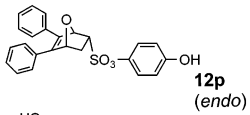
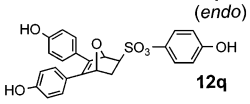
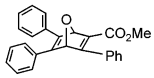
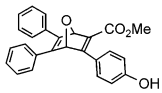
Entry	Compound	Uterine Cytosol	ERα	ERβ	α /β ratio
1	 **12a** (*exo*)	18	9.3 ± 0.6	1.7 ± 0.2	5.4
2	 **12a** (*endo*)	5.6	4.1 ± 0.7	0.15 ± 0.04	27
3	 **12b**	1.69	0.64 ± 0.2	0.059 ± 0.005	11
4	 **12c**	0.041	0.10 ± 0.1	0.077 ± 0.04	1.3
5	 **12d**	0.011	0.021 ± 0.01	0.036 ± 0.004	0.58
6	 **12e**	0.015	~0.009	~0.006	1.5
7	 **12f**	<0.005	0.020 ± 0.02	0.025 ± 0.02	0.8
8	 **12g**	0.03	0.013 ± 0.01	0.017 ± 0.06	0.77
9	 mixture of isomers **12h**	1.5	0.90 ± 0.16	0.21 ± 0.06	4.3
10	 mixture of isomers **12i**	0.18	0.069 ± 0.012	0.037± 0.003	1.9
11	 mixture of isomers **12j**	3.3	0.98 ± 0.13	0.11 ± 0.011	8.9
12	 mixture of isomers **12k**	0.26	0.068± 0.008	0.066 ± 0.018	1.0
13	 **12l**	0.013	0.068 ± 0.03	0.12 ± 0.06	0.57
14	 **12m**	<0.003	0.008	0.013	0.62
15	 **12n**	<0.003	0.014 ± 0.004	0.020 ± 0.002	0.70
16	 **12o**	1.1	0.32 ± 0.1	0.10 ± 0.02	3.3
17	 **12p** (*exo*)	0.047	0.022 ± 0.004	0.027 ± 0	0.82

Table 2. (Continued)

Entry	Compound	Uterine Cytosol	ERα	ERβ	α /β ratio
18	 **12p** (*endo*)	0.045	0.025	0.029	0.86
19	 **12q**	2.8	2.2 ± 0.1	0.16 ± 0.02	14
20	 **15a**	0.006	0.007	0.024	0.29
21	 **15b**	0.03	0.012	0.031	0.39

[a] Relative binding affinitiy (RBA) values are determined by competitive radiometric binding assays and are expressed as $IC_{50}^{estradiol}/IC_{50}^{compound} \times 100 \pm$ the range or the standard deviation (RBA, estradiol = 100%). In these assays, the K_d for estradiol is 0.2 nM on ERα and 0.5 nM on ER*β*. For details, see the Experimental Section.

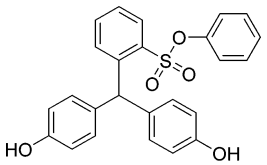
binding affinities to the ER in this complex protein mixture are not always equal to that of purified human ERα, although it is not clear whether this is due to species differences, preferential binding of tracer, [^{3}H]-estradiol, by the other, nonreceptor proteins present in the cytosol preparation, or the influence of endogenous coregulator proteins. Surprisingly, the *endo* isomer of **12a** shows 27-fold selectivity for ERα over ER*β*; although with a decrease in overall binding, particularly for ER*β*, with an RBA of 4.1 and 0.15 for ERα and ER*β*, respectively, it has the highest ERα binding preference in the series (Table 2, entry 2).

The effect of the C-5 and C-6 phenolic functions on binding affinity is evident from a comparison of *exo* **12a** and **12b** with corresponding phenyl sulfonates **12h**−**12k**, which have protected or altered phenol positions. When one of the hydroxyl groups was moved to the meta position of the phenyl ring (e.g., **12h** and **12i**), binding affinity to all receptor preparations decreased 10-fold compared to compounds **12a** and **12b** (Table 2, entries 9 and 10). Compounds having only one hydroxyl group (e.g., **12j** and **12k**) also had substantially lower binding affinities than **12a** and **12b** (Table 2, entries 11 and 12).

A chemical feature common to nearly all synthetic ER ligands having good binding affinity is the presence of a phenolic ring that seems to mimic the steroid "A ring" present in natural estrogens. This phenol forms important hydrogen bonds with residues Glu353 and Arg394 in ERα (or with the corresponding residues in ER*β*), as well as with a water molecule.[9] Consistent with this is the very low affinity of compounds **12l**−**12n**, which have no phenolic hydroxyl groups (Table 2, entries 13−15).

In the ligands studied here, the substituent on the C-2 or C-3 position of the bridged core has a significant effect on the binding affinity and selectivity. The highest affinity ligand, **12a** (*exo*), bears a phenyl sulfonate group at this position. Because many nonsteroidal estrogens are triphenols, it was somewhat surprising that placement of a methoxyl or hydroxyl group at the para position of the sulfonate phenyl ring, as in compounds **12o**−**12q**, caused a decrease, rather than an increase, in affinity for both ERα and ER*β* binding (Table 2, entries 16−19). Even the triol compound **12q** showed a marked drop in binding, particularly for ER*β*, thus giving a compound that has the highest ERα binding preference (14-fold) of all the *exo* ligands in this series (RBA ERα 2.2 and ER*β* 0.16) (Table 2, entry 19). Also, both *exo* and *endo* isomers of compound **12p** gave very low binding affinities on ERα and ER*β* (Table 2, entries 17 and 18). It is of note that replacement of the phenyl sulfonate group with a phenyl sulfone, as the compound **12b**, causes a great decrease in binding, particularly for ER*β*, only giving RBA values of 0.64 and 0.059 for ERα and ER*β*, respectively; thus, the sulfone diphenol **12b** has an ERα binding preference (11-fold) that is comparable to that of the sulfone triphenol **12q** (Table 2, entry 3).

Scheme 7



Phenyl Sulfonate Impurity in Phenol Red Preparations (**18**)

Another high-affinity ER ligand, identified by us earlier, has a phenyl sulfonate substituent, as does the bicycle **12a**. Compound **18** (Scheme 7) is one of several trace impurities that are present in phenol red preparations, the pH indicator dye that is widely used in cell culture, and it is responsible for the estrogenic activity of phenol red-containing cell culture medium.[34] This phenyl sulfonate **18** has an affinity for ER that is about half that of estradiol, yet close analogues in which the sulfonate is replaced by carboxylate or carboxamide links have much lower ER binding affinities,[35,36] as is the case with the 7-oxabicyclo[2.2.1]heptenes.

When the phenyl group on the sulfone in **12b** was replaced with an ethyl group, as compound **12c**, there is a dramatic drop in binding affinity on both ER subtypes, which is probably due to the increased polarity of this compound or a reduced steric effect (Table 2, entry 4). The presence of diethoxyl carbonyl groups at both the C-2 and C-3 positions of the core, as in compound **12d**, gave an RBA value of 0.02% for ERα (Table 2, entry 5). Conversely, replacing the single bond with a rigid ethylene spacer, as in compound **12e**, reduced binding affinities to nondetectable levels. This

might be due in part to the decreased flexibility of the ligand (Table 2, entry 6). However, the compounds **12f** and **12g**, produced by the reaction of furan **1b** with *N*-methyl and phenylmaleimide, also exhibit little or no binding affinity. This suggests that the polar substituent (i.e., the nitrogen atom in these compounds) might be responsible for their immeasurably low binding affinities (Table 2, entries 7 and 8). Also, when steric hindrance is introduced at the C-3 position of the core by an aryl group, as in compounds **15a,b**, one obtains analogues that possess very poor binding properties, presumably because of an unfavorable interaction of the third phenyl ring in **15a,b** with the receptor (Table 2, entries 20 and 21).

Note that all the furans synthesized in this report (**1a**−**d**) exhibit little or no binding affinity (data not shown) except furan **1c**, which has an RBA of 0.087% for ERα and 0.241% for ERβ. This is consistent with our earlier work on furan-core ER ligands where we found that only tetrasubstituted furans had high binding affinities (Scheme 1).[19] Thus, it appears that the other carbocyclic bridge of the oxabicycloheptene analogues is making a strong contribution to the ERα and ERβ binding affinity. This result also indicates that our design for the three-dimensional hydrophobic core element is a reasonable one and that the core bicyclic unit plays an important role in engendering favorable binding to the receptor and for achieving, in some cases, good subtype selectivity.

Overall then, the disposition of the appended phenols, the substituents in the C-2 and C-3 positions of the bicyclic core unit in these ER ligands, and the electron-withdrawing group derived from the dienophile all prove to be factors in determining the affinity and selectivity of these new ligands for the ER subtypes. High affinity is generally found when the bicyclic core is embellished with the diarylethylene unit at the C-5 and C-6 sites, together with an appropriate third substituent, producing a molecule that is, overall, neither excessively crowded nor polar.

Transcription Activation through Estrogen Receptors ERα and ERβ. Two of the compounds showing higher binding affinity values for either one of the two ERs (*exo*-**12a** and -**12b**) were assayed for gene transcriptional activity through ERα and ERβ. These cotransfection assays were conducted in human endometrial cancer (HEC-1) cells, using expression plasmids for full-length human ERα or ERβ and an estrogen-responsive reporter gene construct.[37] In an initial screen, the transcriptional activity of these compounds was tested at 10^{-8} and 10^{-6} M alone (as agonists) or in the presence of 10^{-9} M estradiol (as antagonists). It was clear from this screen that both compounds were essentially devoid of agonist activity (showing stimulatory activity less than 5% that of estradiol) and functioned as antagonists.

To further characterize their activity as ER antagonists, compounds **12a** and **12b** were assayed in an antagonist dose-response mode at four concentrations, 10^{-8} to 10^{-5} M, in the presence of 10^{-9} M E_2. In all cases, transcriptional activity is expressed relative to that obtained with 10^{-9} M estradiol alone, which is set at 100%. The well-known antiestrogen faslodex (fulvestrant; ICI 182,780) was also assayed under these conditions. The dose response curves are shown in Figure 3. From these assays, it is clear that compounds **12a** and **12b** are antagonists on both ERα and ERβ. They have a slight potency preference for the ERβ subtype, similar to that observed with the ICI antiestrogen (data not shown), and overall they are less potent.

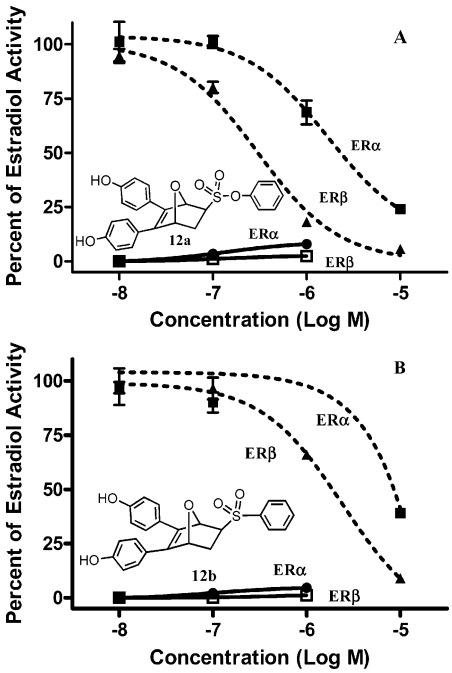


Figure 3. Antagonism (ERα, ■, and ERβ, ▲, shown as dotted lines) and agonism (ERα, ●, and ERβ, □, shown as solid lines) of estradiol-activated transcription activation through ERα and ERβ by compounds **12a** and **12b**. Human endometrial cancer cells (HEC-1) were transfected with expression plamids for ERα or ERβ and the estrogen-responsive reporter gene 2ERE-pS2-Luc and were then incubated with the indicated concentration of ligand, together with 1 nM estradiol (E_2), for 24 h. Values are the mean ± range or SD from two or more experiments and are expressed as a percent of the activity of ERα and ERβ with 10^{-9} M E_2. Further details are given in the Experimental Section and in our prior publication.[37]

Because they lack the typical extended and polar or basic side chain so commonly found in ER antagonists, compounds **12a** and **12b** represent a novel structural class as ER antagonists. It is also curious that their binding affinity preference is for the ERα subtype yet their potency preference as transcriptional antagonists is for the ERβ subtype, although it has long been recognized that binding affinities and transcriptional potencies do not correlate in a strictly quantitative fashion.[38]

Considerations of Ligand Orientation in the Estrogen Receptor Binding Pocket. Because the oxabicyclo[2.2.1]heptene core compounds that we have prepared represent a new structural class of ER ligands, we undertook a molecular modeling study of the highest affinity ligand, the sulfonate **12a** (*exo*). We hoped that this investigation would point to how ligands in this class might be oriented in the ligand binding pockets of the receptors and why they behave as antagonists, rather than agonists.

To build models, we selected the ERα-Ral (raloxifene) X-ray crystal structure (1ERR in the Protein Data Bank) for our ER model, because raloxifene is also an antagonist of ER action and has an ERα/ERβ selectivity similar

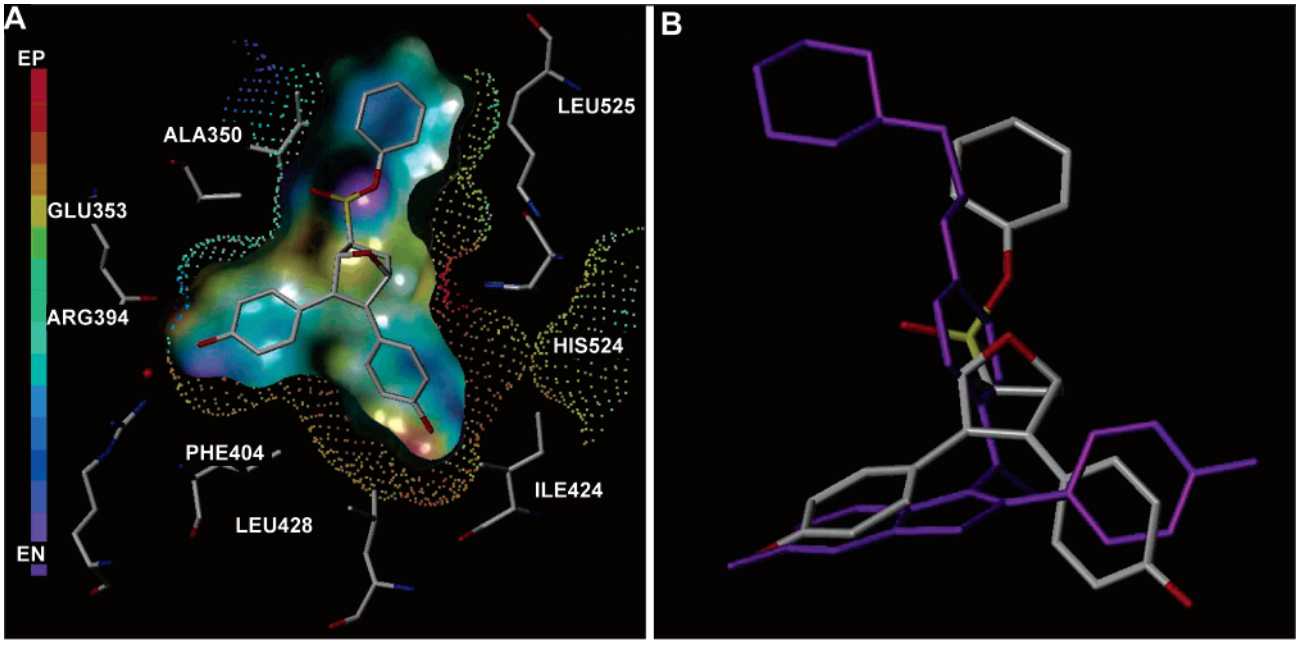


Figure 4. (A) Model of (*1R*,*2S*,*4R*)-oxabicyclo[2.2.1]heptene **12a** in the ERα ligand binding pocket. The surface of the ligand is shown as the electrostatic potential. The ERα pocket is displayed as dots mapped with the electrostatic potential. (B) Comparison of **12a** (element colors) overlaid with raloxifene (purple).

to that of **12a**. Determining the orientation of **12a** within the ligand binding pocket is challenging, because two pendant phenolic rings are attached to the bicyclic core, each of which could serve as the crucial "A ring" mimic of natural estrogens. Also, each phenolic moiety possesses two possible binding orientations of the bicyclic core within the receptor through a 180° rotation around the aryl-oxabicyclic core bond. Binding orientation determination is further complicated in that **12a** was prepared as a racemic mixture of enantiomers.

With eight possible orientations, four each of the two enantiomers of **12a**, we decided to use the FlexX routine for each enantiomer (Sybyl 7.0, Tripos Inc.) instead of individually prepositioning each of the eight possible binding modes as required for use of the FlexiDoc routine. FlexX uses an incremental construction algorithm to build a ligand into a binding pocket and calculate a docking score based on interactions with the binding pocket. We then selected the top-ranked docking orientation and minimized the complex (see Figure 4). Interestingly, only one enantiomer, that possessing the *1R*,*2S*,*4R* absolute configuration, shown in this figure, could be docked effectively into the ligand binding pocket, and a single binding orientation was predicted by the FlexX routine.

The ligand binding pocket of the ERs is largely hydrophobic and rather open, having polar residues only at the regions where the phenol and 17β-hydroxyl group of estradiol are accommodated; the phenol hydrogen bonds with glutamate and arginine residues and a structured water in the rather tight A-ring binding subpocket, while a histidine is the hydrogen bonding partner of the 17β-hydroxyl group.[9] It was encouraging that FlexX predicted a phenol of the bicycle **12a** as an "A ring" mimic, since the phenol moiety is estimated to provide 1.9 kcal/mol of stabilization through its three hydrogen bonds.[39] The second phenol of **12a** was positioned in the 7α region of the ligand binding pocket, a position that is generally tolerant of large substituents.[39] Many ER ligands form a significantly weaker hydrogen bond with His524, which is estimated to provide 0.6 kcal/mol of stabilization energy.[39] The second phenol of **12a**, however, is not oriented properly to hydrogen bond with His524, due to the rigidity of the oxabicyclic core, and it appears to have no hydrogen bonding partner in our model.

The phenylsulfonate ester in our model is positioned into the 11β groove of the ligand binding pocket with the phenyl group extending outward. This could help to explain the antagonistic activity of the **12a**. Antagonists such as raloxifene appear to inhibit coactivator recruitment through displacement of helix 12 with a large substituent that protrudes through this same groove in the 11β-subpocket.[9,40] Analogous to known ER antagonists, the phenylsulfonate ester of **12a** is positioned properly to prevent helix 12 from binding back over the ligand pocket, as it must to form the hydrophobic cleft into which the LXXLL motif of coactivators binds.[40] A portion of the three-dimensional oxabicyclic core also occupies space in the 11β region of the binding pocket that is typically void of substituents with steroidal estrogens.

Molecular modeling studies, of course, are not definitive, so it would prove interesting to perform X-ray crystallographic structure studies in the future to understand in greater detail the molecular basis for the antagonist activity of compounds **12a** and **12b**, because their structures are so unlike that of typical ER antagonists.

Conclusion

In summary, to examine the tolerance of the estrogen receptors ERα and ERβ for "thickness" at the core of a ligand, we have prepared a series of novel ligands for these receptors based on an inherently three-dimensional 7-oxabicyclo[2.2.1]hept-5-ene core. Ligands in this series can be readily prepared by a Diels−Alder reaction

between a 3,4-disubstituted furan and various dienophiles without Lewis acid catalysis; the *exo* stereoisomers predominate. Those that have 4-hydroxyphenyl substituents at C-5 and C-6 and a phenyl sulfonate at C-2 have substantial affinity for both ERα and ER*β*, but with an affinity preference for ERα. Minor changes to this pattern of substitution generally lead to analogues of lower affinity. In cell-based transcription assays, two compounds with high ER binding affinity were found to be antagonists on both ER subtypes, with a modest potency preference for ER*β*. These ER ligands have unusual bicyclic core structures, and although they are antagonists, they do not have substituents that are typically found in ER antagonists; molecular modeling, however, shows that the phenyl sulfonate moiety of the antagonist ligands is capable of stabilizing an ER conformation that is likely not to be able to bind coactivators, and this might explain their antagonist character. The generation of this new series of ER ligands provides important insight into the diversity of structures that can function as ligands for the estrogen receptors and function as antagonists on these important pharmaceutical targets.

Experimental Section

Materials and Methods. All reagents and solvents were obtained from Aldrich. Tetrahydrofuran, diethyl ether, toluene, and dichloromethane were obtained prior to use from a solvent-dispensing system (SDS) built by J. C. Meyer. Glassware was oven-dried, assembled while hot, and cooled under an inert atmosphere. Unless otherwise noted, all reactions were conducted in an inert atmosphere. Reaction progress was monitored using analytical thin-layer chromatography (TLC) on 0.25 mm Merck F-254 silica gel glass plates. Visualization was achieved by either UV light (254 nm) or potassium permanganate indicator. Flash chromatography was performed with Woelm silica gel (0.040−0.063 mm) packing.

^{1}H NMR and ^{13}C NMR spectra were obtained on a 400 or 500 MHz instrument. The chemical shifts are reported in ppm and are referenced to either tetramethylsilane or the solvent. Mass spectra were recorded under electron impact conditions at 70 eV. Melting points were obtained on a Thomas-Hoover MelTemp apparatus and are uncorrected. Elemental analyses were performed by the Microanalytical Service Laboratory of the University of Illinois. Those final components that did not give satisfactory combustion analysis gave satisfactory exact mass determinations and were found to be at least 96% pure by HPLC analysis. Furan **1a**,[25] **1b**, and **1c**[26] and methyl 3-bromopropiolate **13**[29] were synthesized according to published procedures.

General Procedure for the Diels−Alder Reaction. Furan **1** (0.2 mmol) and dienophiles (0.26 mmol) were placed in a round flask, and the mixture was stirred under a N_2 atmosphere at 90 °C for 6−24 h. The crude product was purified by flash chromatography (10−30% EtOAc/hexanes), preparative thin-layer chromatography, or recrystallization. After isolation, the sample was dried under vacuum in an Aberhalden apparatus in refluxing benzene for 24−48 h. Even after this process some samples still contained water in the crystals. This was confirmed by ^{1}H NMR, and suitable corrections are made for this water in evaluation of combustion microanalysis data.

***exo*-5,6-Bis-(4-hydroxyphenyl)-7-oxabicyclo[2.2.1]hept-5-ene-2-sulfonic Acid Phenyl Ester (12a).** *exo*-**12a** was purified by flash chromatography (30% EtOAc/hexanes) to give a light yellow needle (80% yield) that was recrystallized from ethyl acetate/hexanes (mp 142−143 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66−7.36 (m, 9H), 6.77 (d, J = 8.8 Hz, 2H), 6.76 (d, J = 8.6 Hz, 2H), 5.70 (d, J = 1.0 Hz, 1H), 5.40 (dd, J = 4.6, 1.0 Hz, 1H), 5.06 (s, 1H), 5.02 (s, 1H), 3.59 (dd, J = 8.3, 4.6 Hz, 1H), 2.57 (ddd, J = 12.2, 4.6, 4.6 Hz, 1H), 2.16 (dd, J = 12.2, 8.3 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.96, 155.90, 149.48, 141.6, 137.50, 130.16, 129.34, 129.01, 122.37, 116.13, 116.0, 84.56, 83.21, 60.89, 30.81; HRMS (ESI) calcd for $C_{24}H_{20}O_6SH$, 437.1059 (M + H$^+$); found, 437.1063.

***endo*-5,6-Bis-(4-hydroxyphenyl)-7-oxabicyclo[2.2.1]hept-5-ene-2-sulfonic Acid Phenyl Ester (12a).** *endo*-**12a** was purified by preparative thin-layer chromatography (45% EtOAc/hexanes) to give a gray solid (18% yield) (mp 130−132 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39−7.35 (m, 5H), 7.31−7.21 (m, 2H), 7.13 (d, J = 8.8 Hz, 2H), 6.75 (d, J = 8.8 Hz, 2H), 6.70 (d, J = 8.8 Hz, 2H), 5.57 (dd, J = 4.4, 1.0 Hz, 1H), 5.31 (dd, J = 4.8, 1.0 Hz, 1H), 4.87 (s, 1H), 4.80 (s, 1H), 4.15 (ABX, J = 9.6, 4.8, 4.4 Hz, 1H), 2.57 (ABX, J = 12.0, 9.6, 4.8 Hz, 1H), 2.06 (dd, J = 12.0, 4.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.77, 154.29, 148.00, 141.29, 138.27, 136.61, 130.16, 129.88, 129.72, 127.58, 125.78, 122.40, 115.97, 115.41, 84.63, 83.18, 59.56, 29.97; HRMS (ESI) calcd for $C_{24}H_{20}O_6SH$, 437.1059 (M + H$^+$); found, 437.1057.

2-Benzenesulfonyl-5,6-bis-(4-hydroxyphenyl)-7-oxabicyclo[2.2.1]hept-5-ene (12b). Product **12b** was obtained as orange oil that solidified on standing (81% yield). The product was then recrystallized in ethyl acetate/hexanes to give an off-white crystal (mp 174−175 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 7.2 Hz, 2H), 7.66−7.71 (m, 1H), 7.59 (t, J = 7.6 Hz, 2H), 7.12 (d, J = 8.4 Hz, 2H), 7.03 (d, J = 8.4 Hz, 2H), 6.73 (d, J = 8.8 Hz, 2H), 6.69 (d, J = 8.8 Hz, 2H), 5.59 (s, 1H), 5.27 (d, J = 3.6 Hz, 1H), 5.01 (s, 1H), 4.96 (s, 1H), 3.42 (dd, J = 8.0, 4.4 Hz, 1H), 2.38 (ddd, J = 12.4, 3.6, 3.6 Hz, 1H), 1.94 (dd, J = 12.0, 8.4 Hz, 1H); ^{13}C NMR (125 MHz, acetone-d_6) δ 157.60, 157.51, 141.47, 139.86, 137.75, 134.73, 133.86, 129.55, 129.04, 128.91, 128.76, 123.80, 115.70, 115.68, 83.78, 83.13, 65.30, 19.98; HRMS (ESI) calcd for $C_{24}H_{20}O_5SH$, 421.1109 (M + H$^+$); found, 421.1118.

2-Ethylsulfonyl-5,6-bis-(4-hydroxyphenyl)-7-oxabicyclo-[2.2.1]hept-5-ene (12c). A white solid was formed during the reaction, and the pure **12c** was obtained by recrystallization (75%) from THF (mp 178−180 °C); ^{1}H NMR (500 MHz, acetone-d_6) δ 8.60 (s, br, 2H), 7.23 (d, J = 3.0 Hz, 2H), 7.21 (d, J = 3.0 Hz, 2H), 6.82 (d, J = 3.0 Hz, 2H), 6.80 (d, J = 3.0 Hz, 2H), 5.58 (d, J = 4.4 Hz, 1H), 5.36 (dd, J = 4.4, 1.0 Hz, 1H), 3.41 (dd, J = 8.5, 4.5 Hz, 1H), 3.10, (q, J = 7.0 Hz, 2H), 2.37 (ddd, J = 12.0, 4.5, 4.5 Hz, 1H), 2.13 (dd, J = 12.5, 8.5 Hz, 1H), 1.31 (t, J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, acetone-d_6) δ 157.55, 141.40, 137.78, 129.04, 128.97, 124.60, 123.93, 115.77, 115.69 (one carbon missing as a result of overlap), 83.45, 83.11, 62.65, 45.64, 30.11, 5.29; HRMS (ESI) calcd for $C_{20}H_{20}O_5SH$, 373.11096 (M + H$^+$); found, 373.1099.

5,6-Bis-(4-hydroxyphenyl)-7-oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboxylic Acid Diethyl Ester (12d). Compound **12d** was purified by preparative thin-layer chromatography (30% EtOAc/hexanes) to give a light-yellow solid (42% yield) that was recrystallized from ether/hexanes (mp 89−91 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15 (d, J = 8.8 Hz, 4H), 6.75 (d, J = 8.8 Hz, 4H), 5.45 (s, 2H), 5.38 (s, 2H), 4.18 (q, J = 7.0 Hz, 4H), 3.11 (s, 2H), 1.28 (t, J = 7.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.74, 155.87, 139.90, 129.13, 125.09, 115.95, 85.55, 61.51, 48.92, 14.34; HRMS (ESI) calcd for $C_{24}H_{24}O_7H$, 425.16001 (M + H$^+$); found, 425.1598.

5,6-Bis-(4-hydroxyphenyl)-7-oxabicyclo[2.2.1]hepta-2,5-diene-2,3-dicarboxylic Acid Dimethyl Ester (12e). A yellow solid formed during the reaction, and the pure **12e** was obtained as a yellow solid (75%) that was recrystallized from ether (mp 214−216 °C);^{1}H NMR (500 MHz, acetone-d_6) δ 8.64 (s, br, 2H), 7.31 (d, J = 9.0 Hz, 4H), 6.81 (d, J = 9.0 Hz, 4H), 5.93 (s, 2H), 3.77 (s, 6H); ^{13}C NMR (125 MHz, acetone-d_6) δ 176.40, 157.71, 139.37, 129.02, 123.94, 115.82, 85.63, 61.52, 49.10; HRMS (ESI) calcd for $C_{22}H_{18}O_7H$, 395.1131 (M + H$^+$); found, 395.1131.

8,9-Bis-(4-hydroxyphenyl)-4-methyl-10-oxa-4-aza-tricyclo[5.2.1.0^{2,6}]dec-8-ene-3,5-dione (12f). A white solid formed during reaction and was washed by CH_2Cl_2 and ether; pure **12f** was obtained as white solid (96%) yield that was recrystallized from THF (mp 224−226 °C); ^{1}H NMR (400 MHz,

acetone-d_6) δ 8.67 (s, br, 2H), 7.25 (d, J = 8.8 Hz, 4H), 6.84 (d, J = 8.8 Hz, 4H), 5.39 (s, 2H), 3.23 (s, 2H), 2.90 (s, 3H); ^{13}C NMR (100 MHz, acetone-d_6) δ 163.53, 159.22, 152.53, 145.21, 128.73, 125.38, 115.67, 89.32, 51.85; HRMS (ESI) calcd for $C_{21}H_{17}O_5NH$, 364.1185 (M + H$^+$); found, 364.1190.

4,8,9-Triphenyl-10-oxa-4-aza-tricyclo[5.2.1.0^{2,6}]dec-8-ene-3,5-dione (12g). A white solid formed during reaction and was washed by CH_2Cl_2 and ether; the pure **12g** was obtained as white solid (97% yield) that was recrystallized from THF (mp 237−239 °C); ^{1}H NMR (400 MHz, acetone-d_6) δ 8.66 (s, br, 2H), 7.48−7.52 (m, 2H), 7.40−7.44 (m, 1H), 7.29−7.31 (m, 2H), 7.30 (d, J = 8.0 Hz, 4H), 6.85 (d, J = 8.0 Hz, 4H), 5.53 (s, 2H), 3.41 (s, 2H); ^{13}C NMR (100 MHz, acetone-d_6) δ 175.58, 157.87, 139.56, 133.04, 129.06, 129.02, 128.45, 127.04, 123.91, 115.82, 86.21, 49.27; HRMS (ESI) calcd for $C_{26}H_{19}O_5H$, 426.13414 (M + H$^+$); found, 426.1354.

6-(3- or 4-Hydroxyphenyl)-5-(4- or 3-hydroxyphenyl)-7-oxabicyclo[2.2.1]hept-5-ene-2-sulfonic Acid Phenyl Ester (12h, Mixture of 1.2:1 Isomers). Compound **12h** was purified by flash chromatography to give a pale yellow solid (90% yield) that was recrystallized from ether/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16−7.37 (m, 6H), 6.72−6.85 (m, 7H), 5.72 (d, J = 1.2 Hz, 0.45H), 5.69 (d, J = 1.2 Hz, 0.55H), 5.42 (dd, J = 4.4, 0.4 Hz, 0.55H), 5.40 (dd, J = 4.4, 0.4 Hz, 0.45H), 5.11 (m, br, 2 H), 3.64 (dd, J = 8.4, 4.4 Hz, 0.55H), 3.57 (dd, J = 8.4, 4.4 Hz, 0.45H), 2.54−2.59 (m, 1H), 2.19 (dd, J = 12.0, 8.4 Hz, 0.45H), 2.13 (dd, J = 12.4, 8.4 Hz, 0.55H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.22, 156.15, 156.12, 155.97, 149.41, 143.59, 141.53, 139.50, 139.32, 1137.38, 136.36, 135.06, 134.34, 133.55, 130.60, 130.40, 130.20, 129.32, 127.49, 124.60, 123.81, 122.36, 120.09, 119.81, 116.19, 116.05, 115.73, 115.62, 114.17, 113.96, 84.62, 84.51, 83.32, 83.20, 60.95, 60.62, 30.92, 30.53; HRMS (ESI) calcd for $C_{24}H_{20}O_6SH$, 437.10597 (M + H$^+$); found, 437.1064.

2-Benzenesulfonyl-6-(3- or 4-hydroxyphenyl)-5-(4- or 3-hydroxyphenyl)-7-oxabicyclo[2.2.1]hept-5-ene (12i, Mixture of 1.3:1 Isomers). Compound **12i** was purified by flash chromatography to give a pale yellow solid (66% yield) that was recrystallized from ether/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93−7.97 (m, 2H), 7.50−7.68 (m, 3H), 7.00−7.13 (m, 1H), 7.10 (d, J = 8.8 Hz, 2H), 6.73 (d, J = 8.8 Hz, 2H), 6.61−6.75 (m, 3H), 5.70−5.90 (m, br, 2 H), 5.72 (d, J = 1.2 Hz, 0.44H), 5.57 (d, J = 1.2 Hz, 0.56H), 5.27 (dd, J = 4.4, 0.4 Hz, 0.56H), 5.23 (dd, J = 4.4, 0.4 Hz, 0.44H), 3.47 (dd, J = 8.0, 4.4 Hz, 0.56H), 3.39 (dd, J = 8.4, 4.4 Hz, 0.44H), 2.32−2.38 (m, 1H), 1.96 (dd, J = 12.0, 8.4 Hz, 0.44H), 1.89 (dd, J = 12.4, 8.4 Hz, 0.56H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.35, 156.15, 143.66, 141.43, 139.60, 138.63, 138.58, 137.58, 134.45, 134.22, 133.61, 130.33, 129.67, 129.63, 129.06, 128.93, 128.90, 124.44, 123.68, 119.79, 119.32, 116.04, 115.60, 115.51, 114.21, 113.66 (six carbons missing as a result of overlap), 83.92, 83.72, 83.64, 83.49, 65.56, 65.22, 30.48, 30.00; HRMS (ESI) calcd for $C_{24}H_{20}O_5SH$, 421.11096 (M + H$^+$); found, 421.1109.

5(or 6)-(4-Hydroxyphenyl)-6(or 5)-(4-methoxyphenyl)-7-oxabicyclo[2.2.1]hept-5-ene-2-sulfonic Acid Phenyl Ester (12j, Mixture of 1:1 Isomers). Compound **12j** was purified by preparative thin-layer chromatography (30% EtOAc/hexanes) to give a pale yellow solid (77% yield) that was recrystallized from ether/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16−7.36 (m, 9H), 6.84 (d, J = 8.8 Hz, 1H), 6.83 (d, J = 8.8 Hz, 1H), 6.77 (d, J = 8.8 Hz, 1H), 6.77 (d, J = 8.8 Hz, 1H), 5.71,5.70 (d, J = 0.8 Hz, 0.5H), 5.71 (d, J = 0.8 Hz, 0.5H), 5.41 (d, J = 4.4 Hz, 0.5H), 5.40 (d, J = 4.4 Hz, 0.5H), 5.04 (s, br, 0.5 H), 5.01 (s, br, 0.5 H), 3.82 (s, 1.5 H), 3.81 (s, 1.5 H), 3.60 (dd, J = 8.4, 4.8 Hz, 1H), 2.57 (ddd, J = 12.4, 4.6, 4.6 Hz, 1H), 2.16 (dd, J = 12.4, 8.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.85, 159.25, 155.92, 155.86, 149.46, 141.64, 141.48, 137.55, 137.37, 130.15, 129.87, 129.32, 129.13, 129.00, 127.43, 125.23, 124.46, 124.16, 122.37, 116.11, 115.96, 114.61, 114.46, 113.89, 84.58, 83.22, 60.91, 55.56, 55.53, 30.83; HRMS (ESI) calcd for $C_{25}H_{22}O_6SH$, 451.12152 (M + H$^+$); found, 451.1228.

4-[5(or 6)-Benzenesulfonyl-3-(4-methoxyphenyl)-7-oxabicyclo[2.2.1]hept-2-en-2-yl]-phenol (12k, Mixture of 3:2 Isomers). Compound **12k** was purified by preparative thin-layer chromatography (40% EtOAc/hexanes) to give a pale yellow solid (85% yield) that was recrystallized from ether/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96−7.98 (m, 2H), 7.57−7.68 (m, 3 H), 7.17 (d, J = 8.8 Hz, 0.6H), 7.12 (d, J = 8.8 Hz, 0.4H), 7.08 (d, J = 8.8 Hz, 0.4H), 7.02 (d, J = 8.8 Hz, 0.6H), 6.80 (d, J = 8.8 Hz, 0.6H), 6.75 (d, J = 8.8 Hz, 0.4H), 6.73 (d, J = 8.8 Hz, 0.4H), 6.80 (d, J = 8.8 Hz, 0.6H), 6.69 (d, J = 8.8 Hz, 0.6H), 5.60 (d, J = 0.8 Hz, 1H), 5.41 (s, br, 1H), 5.27 (d, J = 4.4 Hz, 1H), 3.79 (s, 3 H), 3.42 (dd, J = 8.4, 4.4 Hz, 1H), 2.38 (ddd, J = 12.4, 4.6, 4.6 Hz, 1H), 1.94 (dd, J = 12.0, 8.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.69, 159.55, 155.93, 155.91, 141.50, 138.92, 138.82, 137.77, 134.11, 130.29, 130.04, 129.59, 129.53, 129.49, 129.43, 129.19, 129.01, 128.94, 128.73, 128.56, 128.11, 125.11, 124.41, 115.95, 115.15, 114.41, 113.82, 83.86, 83.51, 66.13, 65.52, 65.48, 55.5, 30.35; HRMS (ESI) calcd for $C_{25}H_{22}O_5SH$, 435.1266 (M + H$^+$); found, 435.1265.

5,6-Diphenyl-7-oxabicyclo[2.2.1]hept-5-ene-2-sulfonic Acid Phenyl Ester (12l). Compound **12l** was purified by preparative thin-layer chromatography (2% ether/CH_2Cl_2) to give a colorless needle (78% yield) that was recrystallized from ether/hexanes (mp 111−112 °C); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.20−7.35 (m, 15H), 5.77 (d, J = 1.5 Hz, 1H), 5.47 (dd, J = 4.5, 0.8 Hz, 1H), 3.64 (dd, J = 8.5, 4.5 Hz, 1H), 2.61 (ddd, J = 12.0, 4.5, 4.4 Hz, 1H), 2.21 (dd, J = 12.0, 8.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.46, 143.67, 139.56, 132.46, 131.69, 130.18, 129.25, 129.09, 128.85, 128.79, 127.81, 127.49, 127.46, 122.35, 84.62, 83.32, 60.69, 30.64; MS (EI) *m/z* 404 (M$^+$, 28). HRMS (EI) calcd for $C_{24}H_{20}O_4S$, 404.10822; found, 404.1089.

5-Benzenesulfonyl-2,3-diphenyl-7-oxabicyclo[2.2.1]hept-2-ene (12m). Compound **12m** was purified by flash chromatography (30% EtOAc/hexanes) to give a colorless needle (81% yield) that was recrystallized from ether/hexanes (mp 138−139 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98−8.00 (m, 2H), 7.67−7.71 (m, 1H), 7.58−7.62 (m, 2H), 7.21−7.29 (m, 8H), 7.11−7.13 (m, 2H), 5.66 (d, J = 1.2 Hz, 1H), 5.34 (dd, J = 4.4, 0.8 Hz, 1H), 3.47 (dd, J = 8.4, 4.4 Hz, 1H), 2.43 (ddd, J = 12.4, 3.6, 3.6 Hz, 1H), 1.99 (dd, J = 12.0, 8.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.78, 139.80, 138.93, 134.09, 132.65, 131.84, 129.59, 129.05, 129.03, 128.98, 128.69, 128.53, 127.72, 127.25, 83.92, 83.64, 65.32, 30.13; MS (EI) *m/z* 388 (M$^+$, 10). HRMS (EI) calcd for $C_{24}H_{20}O_3S$ 388.11331; found, 388.1125.

8,9-Diphenyl-4,10-dioxa-tricyclo[5.2.1.0^{2,6}]dec-8-ene-3,5-dione (12n). Product **12n** was not stable on the silica gel and was obtained as a pale yellow solid (92%) that was recrystallized from ethyl acetate and hexanes (mp 69−71 °C); ^{1}H NMR (400 MHz, acetone-d_6) δ 7.29−7.35 (m, 10H), 5.72 (s, 2H), 3.50 (s, 2H); ^{13}C NMR (100 MHz, acetone-d_6) δ 170.17, 141.83, 131.33, 129.32, 129.02, 127.55, 87.19, 50.0; MS (EI) *m/z* 318 (M$^+$, 6); HRMS (EI) calcd for $C_{20}H_{14}O_4$, 318.0892; found, 318.0884.

Synthesis of Ethenesulfonic Acid 4-Methoxyphenyl Ester (16). A mixture of 4-methoxyphenol (2 g, 16.5 mmol) dissolved in 3 mL of H_2O and 3 mL of 1,2-dichloroethane was stirred at 0 °C, and 11.4 mL of 25% aqueous NaOH and 2.7 g of chloroethanesulfonyl chloride were added simultaneously and slowly. The mixture was stirred for another 2 h at 0 °C and then extracted with CH_2Cl_2. The combined organics were washed with saturated NaCl, dried over Na_2SO_4, and filtered, and the solvent was evaporated. Distillation under vacuum gave pure **16** as a pale yellow oil in 57% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14 (d, J = 9.2 Hz, 2H), 6.87 (d, J = 9.2 Hz, 2H), 6.65 (dd, J = 16.6, 10.0 Hz, 1H), 6.34 (d, J = 17.2 Hz, 1H), 6.15 (d, J = 10.0 Hz, 1H) 3.8 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.62, 142.99, 132.15, 131.98, 123.53, 114.94, 55.86.

Synthesis of Ethenesulfonic Acid 4-Hydroxyphenyl Ester (17). Boron trifluoride/dimethyl sulfide (15 equiv) was added slowly to a solution of ethenesulfonic acid 4-methoxyphenyl ester (2.34 mmol) in 5 mL of dry CH_2Cl_2 at 0 °C. The solution was allowed to warm to room temperature and was stirred for a total of 18 h. The reaction was quenched by the addition of 10 mL of water and 5 mL of methanol, and the solution was extracted with ethyl acetate (3 × 20 mL). The combined organic phases were washed with saturated NaCl and dried with Na_2SO_4. The crude product was purified by

flash chromatography using 30% EtOAc/hexanes as eluent, which afforded the pure 17 as a pale yellow viscous oil in 75% yield that solidified on standing; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.06 (d, J = 9.0 Hz, 2H), 6.79 (d, J = 9.0 Hz, 2H), 6.64 (dd, J = 16.6, 10.0 Hz, 1H), 6.34 (d, J = 16.6 Hz, 1H), 6.17 (d, J = 10.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.12, 142.75, 132.38, 131.90, 123.68, 116.56.

5,6-Bis-(4-hydroxyphenyl)-7-oxabicyclo[2.2.1]hept-5-ene-2-sulfonic Acid 4-Methoxyphenyl Ester (12o). Compound **12o** was purified by preparative thin-layer chromatography (30% EtOAc/hexanes) to give a pale yellow solid (61% yield) that was recrystallized from ethyl acetate/hexanes (mp 95−97 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17 (d, J = 8.8 Hz, 2H), 7.15 (d, J = 8.4 Hz, 2H), 7.12 (d, J = 9.0 Hz, 2H), 6.82 (d, J = 8.8 Hz, 2H), 6.76 (d, J = 8.4 Hz, 2H), 6.75 (d, J = 8.4 Hz, 2H), 5.68 (d, J = 1.2 Hz, 1H), 5.58 (br, s, 2H), 5.39 (dd, J = 4.5, 1.0 Hz, 1H), 3.78 (s, 3H), 3.56 (dd, J = 8.2, 4.0 Hz, 1H), 2.56 (ddd, J = 12.5, 6.3, 6.3 Hz, 1H), 2.14 (dd, J = 12, 8.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.02, 155.93, 155.88, 155.46, 141.54, 137.49, 129.86, 129.74, 129.37, 128.98, 123.41, 123.37, 116.12, 115.97, 84.62, 83.18, 60.53, 55.86, 30.79; HRMS (ESI) calcd for $C_{25}H_{22}O_7SH$, 467.11644 ($M + H^+$); found, 467.1171.

***exo*-5,6-Diphenyl-7-oxabicyclo[2.2.1]hept-5-ene-2-sulfonic Acid 4-Hydroxyphenyl Ester (12p).** *exo*-**12p** was purified by preparative thin-layer chromatography (30% EtOAc/hexanes) to give a colorless needle (60% yield) that was recrystallized from ether/hexanes (mp 59−60 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (m, 10H), 7. 05 (d, J = 8.8 Hz, 2H), 6.74 (d, J = 8.4 Hz, 2H), 5.76 (d, J = 1.2 Hz, 1H), 5.47 (dd, J = 4.4, 1.2 Hz, 1H), 5.10 (s, br, 1H), 3.61 (dd, J = 8.4, 4.4 Hz, 1H), 2.60 (ddd, J = 12.0, 4.4, 4.4 Hz, 1H), 2.20 (dd, J = 12.0, 8.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.62, 143.62, 142.93, 139.53, 132.43, 131.67, 129.25, 129.09, 128.87, 128.78, 127.84, 127.46, 123.62, 116.55, 84.64, 83.31, 60.35, 30.65; HRMS (ESI) calcd for $C_{24}H_{20}O_5SH$, 421.11096 ($M + H^+$); found, 421.1109.

***endo*-5,6-Diphenyl-7-oxabicyclo[2.2.1]hept-5-ene-2-sulfonic Acid 4-Hydroxyphenyl Ester (12p).** Product **12p** was purified by preparative thin-layer chromatography (30% EtOAc/hexanes) to give a colorless viscous oil (15%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22−7.46 (m, 10H), 6.92 (d, J = 8.8 Hz, 2H), 6.75 (d, J = 9.0 Hz, 2H), 5.65 (dd, J = 4.2, 1.0 Hz, 1H), 5.35 (dd, J = 4.6, 1.0 Hz, 1H), 4.84 (s, br, 1H), 4.16 (ABX, J = 9.2, 4.6, 4.2 Hz, 1H), 2.60 (ABX, J = 12.0, 9.2, 4.6 Hz, 1H), 2.12 (dd, J = 12.0, 4.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 54.63, 149.68, 142.08, 138.33, 132.74, 129.06, 128.65, 128.49, 128.37, 128.22, 128.18, 123.69, 116.51, 116.40, 84.77, 83.15, 59.07, 29.79; HRMS (ESI) calcd for $C_{24}H_{20}O_5SH$, 421.11096 ($M + H^+$); found, 421.1110.

5,6-Bis-(4-hydroxyphenyl)-7-oxabicyclo[2.2.1]hept-5-ene-2-sulfonic Acid 4-hydroxyphenyl Ester (12q). Product **12q** was purified by preparative thin-layer chromatography (45% EtOAc/hexanes) to give a pale yellow solid (67% yield) that was recrystallized from ether/hexanes (mp 110−112 °C); ^{1}H NMR (400 MHz, acetone-d_6) δ 8.66 (s, 1H), 8.64 (s, 1H), 8.60 (s, 1H), 7.24 (d, J = 8.8 Hz, 2H), 7.22 (d, J = 8.4 Hz, 2H), 7.07 (d, J = 9.2 Hz, 2H), 6.84 (d, J = 8.4 Hz, 2H), 6.80 (d, J = 8.8 Hz, 2H), 6.79 (d, J = 8.8 Hz, 2H), 5.62 (d, J = 1.2 Hz, 1H), 5.58 (br, s, 2H), 5.42 (dd, J = 4.4, 0.8 Hz, 1H), 3.70 (dd, J = 8.4, 4.8 Hz, 1H), 2.40 (ddd, J = 12.0, 4.4, 4.4 Hz, 1H), 2.25 (dd, J = 12.0, 8.4 Hz, 1H); ^{13}C NMR (100 MHz, acetone-d_6) δ 157.76, 157.61, 156.45, 142.42, 141.52, 137.30, 129.50, 128.79, 124.41, 123.62, 123.54, 116.19, 115.91, 115.70, 84.61, 82.95, 60.26, 30.76; HRMS (ESI) calcd for $C_{24}H_{20}O_7SH$, 453.1008 ($M + H^+$); found, 453.1008.

3-Bromo-5,6-diphenyl-7-oxabicyclo[2.2.1]hepta-2,5-diene-2-carboxylic Acid Methyl Ester (14). Furan **1a** (0.2 mmol) was dissolved in benzene (5 mL), and methyl 3-bromopropiolate **13** (0.26 mmol) was added. The reaction mixture was stirred at 90 °C for 24 h. The crude product was purified by flash chromatography (10−30% EtOAc/hexanes) to give a pale yellow oil that was essentially pure; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43−7.45 (m, 2H), 7.25−7.38 (m, 8H), 6.01 (d, J = 2.0 Hz, 1H), 5.61 (d, J = 2.0 Hz, 1H), 3.82 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.08, 148.72, 148.47, 146.06, 142.94, 133.54, 133.25, 129.05, 128.87, 128.62, 128.59, 127.50, 127.34, 94.63, 89.29, 52.17.

General Procedure for the Suzuki Coupling Reaction. A 25 mL flask was charged with $Pd(PPh_3)_4$ (0.01 mmol), benzene (5 mL), **14** (0.1 mmol), and an aqueous solution of Na_2CO_3 (0.1 mL of 2 M solution) under nitrogen atmosphere, and then aryl boronic acid (0.15 mmol) in ethanol (1 mL) was added. The mixture was stirred at 90 °C for 12 h. After the reaction was complete, the mixture was extracted with ethyl acetate, washed with saturated NaCl solution, and finally dried over Na_2SO_4. The crude product was purified by preparative thin-layer chromatography (30% EtOAc/hexanes).

3,5,6-Triphenyl-7-oxabicyclo[2.2.1]hepta-2,5-diene-2-carboxylic Acid Methyl Ester (15a). Product **15a** was purified by preparative thin-layer chromatography (20% EtOAc/hexanes) to give a colorless crystal (71% yield) that was recrystallized from ether/hexanes (mp 122−123 °C); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45−7.50 (m, 3H), 7.23−7.32 (m, 12H), 6.09 (d, J = 2.0 Hz, 1H), 5.94 (d, J = 2.0 Hz, 1H), 3.83 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.85, 164.61, 149.70, 146.64, 138.28, 133.97, 133.96, 132.46, 129.94, 129.15, 128.85, 128.73, 128.46, 128.39, 128.14, 127.99, 127.24, 92.97, 89.56, 51.86. MS (EI) *m/z* 380 (M^+, 12). HRMS (EI) calcd for $C_{26}H_{20}O_3$, 380.14123; found, 380.14130.

3-(4-Hydroxyphenyl)-5,6-diphenyl-7-oxabicyclo[2.2.1]-hepta-2,5-diene-2-carboxylic Acid Methyl Ester (15b). Product **15b** was purified by preparative thin-layer chromatography (40% EtOAc/hexanes) to give a light-yellow crystal (65% yield) that was recrystallized from ether/hexanes (mp 80−82 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, J = 8.8 Hz, 2H), 7.45−7.48 (m, 2H), 7.25−7.29 (m, 8H), 6.74 (d, J = 8.8 Hz, 2H), 6.09 (d, J = 2.0 Hz, 1H), 5.96 (d, J = 2.0 Hz, 1H), 3.84 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.74, 164.95, 157.53, 149.77, 146.33, 135.45, 133.97, 134.00, 131.26, 129.13, 128.71, 128.38, 128.10, 127.99, 127.22, 124.95, 115.47, 92.69, 89.47, 51.82. HRMS (ESI) calcd for $C_{26}H_{20}O_4H$, 397.14397 ($M + H^+$); found, 397.1434.

Estrogen Receptor Binding Affinity. Relative binding affinities were determined by a competitive radiometric binding assay as previously described,[32,33] using 10 nM [^{3}H]-estradiol as tracer ([6,7-^{3}H]estra-1,3,5(10)-triene-3,17-β-diol, 51−53 Ci/mmol, Amersham BioSciences, Piscataway, NJ), and purified full-length human ERα and ERβ were purchased from PanVera/Invitrogen (Carlsbad, CA). Incubations were for 18−24 h at 0 °C. Hydroxyapatite (BioRad, Hercules, CA) was used to absorb the receptor−ligand complexes, and free ligand was washed away. Assays with uterine cytosol preparations were done in a related manner, as previously described,[32] but charcoal-coated dextran was used to adsorb free tracer. The binding affinities are expressed as relative binding affinity (RBA) values with the RBA of estradiol set to 100%. The values given are the average ± range or SD of two to three independent determinations. Estradiol binds to ERα and uterine cytosol ER with a K_d of 0.2 nM and to ERβ with a K_d of 0.5 nM.

Gene Transcriptional Activity. Assays were performed as previously described.[37,41] Human endometrial cancer (HEC-1) cells were maintained in minimum essential medium (MEM) plus phenol red supplemented with 5% calf serum and 5% fetal calf serum. Cells were plated in phenol red-free Improved MEM and 5% charcoal dextran-treated calf serum (CDCS) and were given fresh medium 24 h before transfection. Transfection assays were performed in 24-well plates using a mixture of 0.35 mL of serum-free improved MEM medium and 0.15 mL of Hank's balanced salt solution containing 5 μL of lipofectin (Life Technologies, Inc., Gaithersburg, MD), 1.6 μg of transferrin (Sigma, St. Louis, MO), 200 ng of pCMV β-galactosidase as internal control, 1 μg of 2ERE-pS2-Luc, and 100 ng of ER expression vector per well. The cells were incubated at 37 °C in a 5% CO_2-containing incubator for 5 h. The medium was then replaced with fresh Improved MEM supplemented with 5% CDCS plus the desired concentrations

of ligands. Cells were harvested 24 h later. Luciferase and β-galactosidase activity were assayed as described.[41]

Molecular Modeling. The protein structure used in docking simulations was based on the X-ray crystallographic structure of the human estrogen receptor ligand binding domain bound to raloxifene (Protein Data Bank entry 1ERR). The crystal structure contains one homodimer with 222 resolved residues, but only one monomer (chain A) was chosen for modeling purposes. The crystal structure contains several residues with missing atoms, and the flexible loops between helices H9 and H10 (460−469) and H11 and H12 (529−534) are completely missing. We used the molecular modeling program Sybyl 7.0 (Tripos Inc., St. Louis, MO) for all manipulations. Initially, we preformed a loop search of protein fragments from the Protein Data Bank to insert into the missing regions of the original X-ray structure. The optimal loop was selected based on a high RMS fit onto three resolved residues flanking the missing sequence and minimal van der Waals contacts with the rest of the protein. Finally, missing atoms were added to the ligand, protein, and all water molecules within a 4 Å radius of the protein.

The ERα-RAL complex later used for docking studies was minimized by a four-step procedure where each step used the Powell algorithm along with the MMFF94s molecular force field until convergence was reached (RMS < 0.05). First, the inserted loops were minimized, while the rest of the protein was fixed in space. Next, hydrogen atoms on the ligand, protein, and water molecules were allowed to reach a minimum energy. Third, the amino acid side chains were allowed to minimize, except for Glu353 and Arg394, which remained fixed along with the water molecules, the ligand, and the protein backbone. Finally, all atoms were released and allowed to reach a minimum energy. The minimized structure was overlaid with the original X-ray structure and showed very little movement of residues to ensure our model was reasonable.

Raloxifene was deleted from the minimized ERα-RAL complex, and SiteID was used to identify the ligand binding site using a flood-fill solvation technique. FlexX was used incrementally to construct one enantiomer of **12a** in the binding pocket then identify and rank the most favored binding orientations in the ERα complex. The highest-ranked complex was minimized using the last two steps in the minimization procedure mentioned previously (see above).

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Supporting Information Available: Elemental analysis results, HPLC results, HPLC spectra, and X-ray data for compound **12a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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