DOI: 10.1002/cmdc.201200048

Development of Selective Estrogen Receptor Modulator (SERM)-Like Activity Through an Indirect Mechanism of Estrogen Receptor Antagonism: Defining the Binding Mode of 7-Oxabicyclo[2.2.1]hept-5-ene Scaffold Core Ligands

Yangfan Zheng,[a] Manghong Zhu,[a] Sathish Srinivasan,[c] Jerome C. Nwachukwu,[c] Valerie Cavett,[c] Jian Min,[a] Kathryn E. Carlson,[b] Pengcheng Wang,[a] Chune Dong,[a] John A. Katzenellenbogen,*[b] Kendall W. Nettles,[c] and Hai-Bing Zhou*[a]

Previously, we discovered estrogen receptor (ER) ligands with a novel three-dimensional oxabicyclo[2.2.1]heptene core scaffold and good ER binding affinity act as partial agonists via small alkyl ester substitutions on the bicyclic core that indirectly modulate the critical switch helix in the ER ligand binding domain, helix 12, by interactions with helix 11. This contrasts with the mechanism of action of tamoxifen, which directly pushes helix 12 out of the conformation required for gene activation. We now report that a much larger substitution can be tolerated at this position of the bicyclic core scaffold, namely a phenyl sulfonate group, which defines a novel binding epitope for the estrogen receptor. We prepared an array of 14 oxabicycloheptene sulfonates, varying the phenyl sulfonate group. As with the parent compound, 5,6-bis-(4-hydroxyphenyl)-7-oxabicyclo[2.2.1]hept-5-ene-2-sulfonic acid phenyl ester (OBHS), these compounds showed preferential affinity for ERα, and the disposition and size of the phenyl substituents were important determinants of the binding affinity and selectivity of these compounds, with those having *ortho* substituents giving the highest, and *para* substituents the lowest affinities for ERα. A few analogues exhibit ERα binding affinities that are comparable to or, in the case of the *ortho*-chloro analogue, higher than that of OBHS itself. In cell-based studies, we found several compounds with activity profiles comparable to tamoxifen, but acting entirely as indirect antagonists, allosterically interfering with recruitment of coactivator proteins to the receptor. Thus, the OBHS binding epitope represents a novel approach to the development of estrogen receptor antagonists via an indirect mechanism of antagonism.

Introduction

Estrogens, such as the endogenous hormone estradiol (E_2), are important mediators of many physiological functions related to development, growth, and maintenance of reproductive as well as non-reproductive tissues in both men and women.[1] The effects of estrogens are mediated via two estrogen receptor subtypes, ERα and ERβ,[2] which have different tissue distributions and significant differences in their ligand binding preferences.[3] Selective estrogen receptor modulators (SERMs), compounds that show differential levels of agonistic versus antagonistic activity in different estrogen target tissues, as well as ER subtype-selective ligands, have been investigated intensively in recent years in the search for estrogens that have improved patterns of target-tissue selectivity.[4]

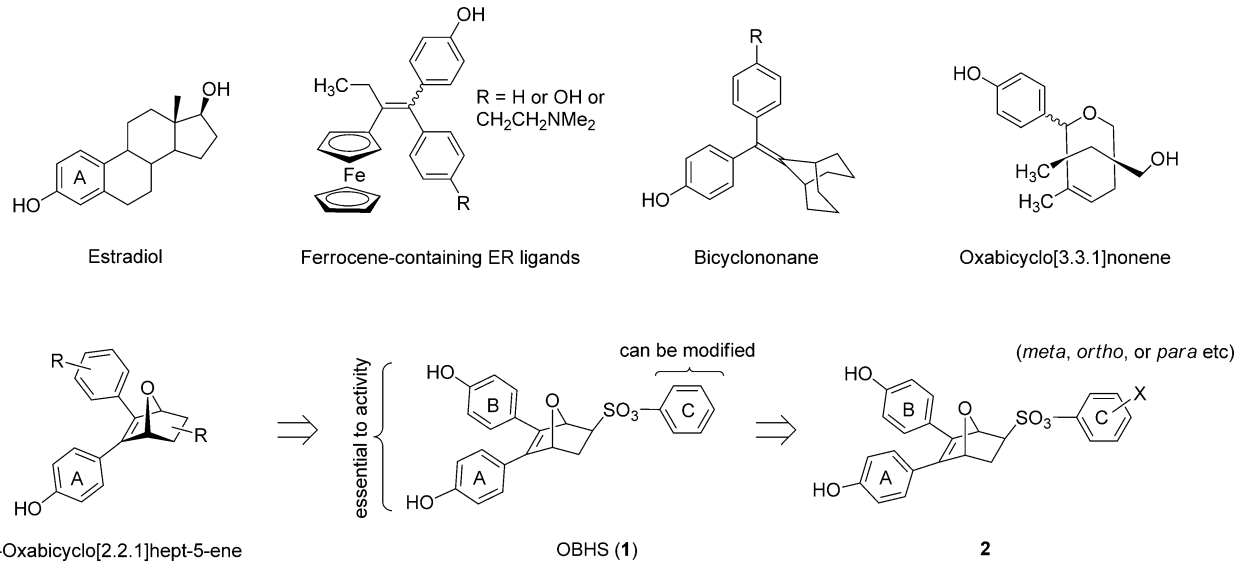
Structural studies of the ligand binding pockets of both ERα and ERβ reveal substantial unoccupied space above and below the mean plane of the endogenous ligand E_2, particularly near the middle of this molecule (namely, below the B ring and above the C ring),[5] as well as considerable flexibility in the shape of the ligand binding pocket.[6] While these characteristics of how different estrogens bind to the ERs have been known for several years,[5,7] there have been only sporadic attempts to exploit this unfilled space and the flexibility of the ER binding pocket as an approach to enhance ligand binding affinity, SERM behavior, or ER-subtype selectivity. Nevertheless, a number of ER ligands with diverse three-dimensional chemical scaffolds have emerged—representative examples include those based on ferrocene,[8] carboranes,[1a,9] bridged poly-

[a] *Y. Zheng, M. Zhu, J. Min, P. Wang, Prof. Dr. C. Dong, Prof. Dr. H.-B. Zhou*
State Key Laboratory of Virology
Laboratory of Combinatorial Biosynthesis and Drug Discovery
Ministry of Education, School of Pharmaceutical Sciences
Wuhan University
185 East Lake Road, Wuhan, 430071 (P.R. China)
E-mail: zhouhb@whu.edu.cn

[b] *K. E. Carlson, Prof. Dr. J. A. Katzenellenbogen*
Department of Chemistry, University of Illinois
600 South Mathews Avenue, Urbana, IL 61801 (USA)
E-mail: jkatzene@uiuc.edu

[c] *Dr. S. Srinivasan, Dr. J. C. Nwachukwu, V. Cavett, Prof. Dr. K. W. Nettles*
Department of Cancer Biology, The Scripps Research Institute
5353 Parkside Drive, Jupiter, FL, 33458 (USA)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cmdc.201200048.



Scheme 1. Structure of estradiol, examples of ER ligands that have three-dimensional elements or core structures, OBHS (**1**) and title compounds **2**.

cyclics,[10] and some other cyclopentadienyl metal tricarbonyl complexes (Scheme 1).[11] We have contributed to this area as well.[7a]

The formula for making ER antagonists is well established: Take an agonist ligand and add a bulky side chain to disrupt helix 12 (Figure 1 a,b). Structure–activity relationship (SAR) studies are then done to optimize for antagonist activity and potency. We previously discovered that certain small substitutions at different sites on agonist ligands could yield partial agonists, which we called passive or indirect antagonists because they modulate helix 12 indirectly by inducing small shifts in helix 11.[12] These partial agonists include a series of oxabicyclic bridged compounds with small alkyl ester substitutions directed towards ER helix 11; these compounds exhibit relatively weak binding affinities, which suggests a limited potential for expanding SAR.[7a] Intriguingly, however, one member of this series, OBHS (**1**) has a much larger, bulky side group, a phenyl sulfonate, yet binds better than those analogues with smaller substituents.[7a] This suggested to us that there might actually be room for much more extensive perturbation of the ligand binding pocket in this region than we had originally envisioned.

The structure of OBHS supports this hypothesis and led us to prepare a series of derivatives targeting this novel binding epitope. Using a series of cell-based assays in liver and breast cancer cells, we identify several compounds with higher potency than OBHS and better activity profiles, matching the level of antagonism seen with tamoxifen. This work thus demonstrates a structure-based design approach to blocking ER activity via a novel binding epitope and an indirect mechanism of antagonism.

Results and Discussion

Structure of OBHS-bound ERα

The previously reported ERα co-crystal structures of complexes of the agonist diethylstilbestrol (DES) and the SERM 4-hydroxytamoxifen (4OHT) illustrate how the bulky side group of tamoxifen directly relocates helix 12, thereby destroying the surface-bound coactivator binding site required for gene activation (Figure 1 a, b).[13] In contrast, the bulky side group of OBHS (**1**) binds between helices 11 and 8 of the receptor, and in doing so significantly shifts helix 11 and His 524 (Figure 1 c). Since OBHS (**1**) profiles as a partial agonist (see below), we prepared a series of modifications on the phenyl group to explore whether this novel epitope can be used to drive antagonist activity to a level comparable to that of tamoxifen.

Synthesis

The synthesis of the oxabicyclic bridged compounds **2** was effectively accomplished, as before, by a Diels–Alder reaction of 3,4-diarylfuran **11** with a variety of dienophiles **5**. The dienophiles (**5**) were prepared according to a modified literature procedure by the reaction of 2-chloroethanesulfonyl chloride (**3**) with substituted phenols **4a**–**m** in the presence of triethylamine and were obtained in generally good yields (Scheme 2).[7a,14]

The synthesis of diarylfuran **11** was accomplished using an improved procedure, as shown in Scheme 3. α-Bromo-4-methoxyacetophenone (**6**) reacted with arylacetic acid **7** in the presence of triethylamine in acetonitrile to give condensation product **8** in 88% yield.[15] In contrast, our previous procedure to produce **8** required condensation of the potassium salts of arylacetic acids **7** with **6** using 18-crown-6 as a catalyst under refluxing conditions.[7a] Treatment of ester **8** with sodium hydride in anhydrous DMSO gave 2(5*H*)-furanone **9**, which was

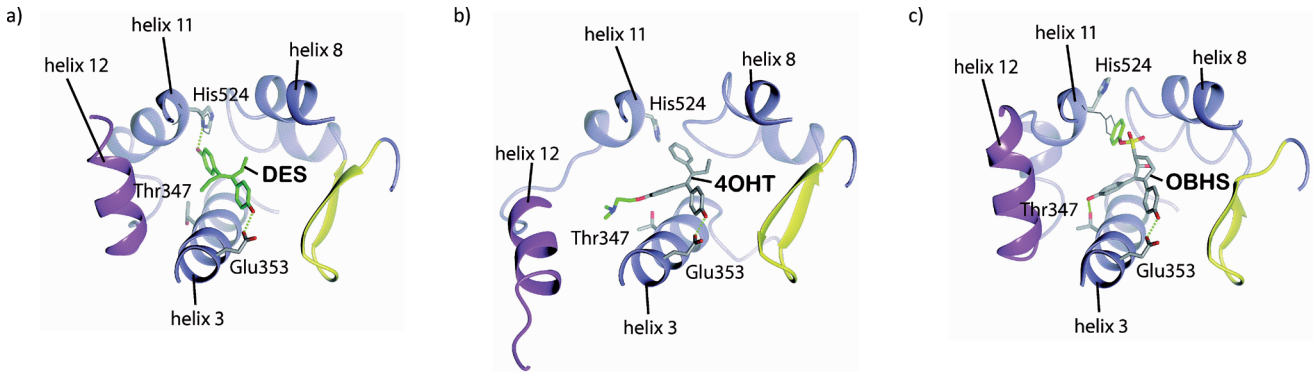
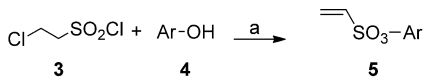
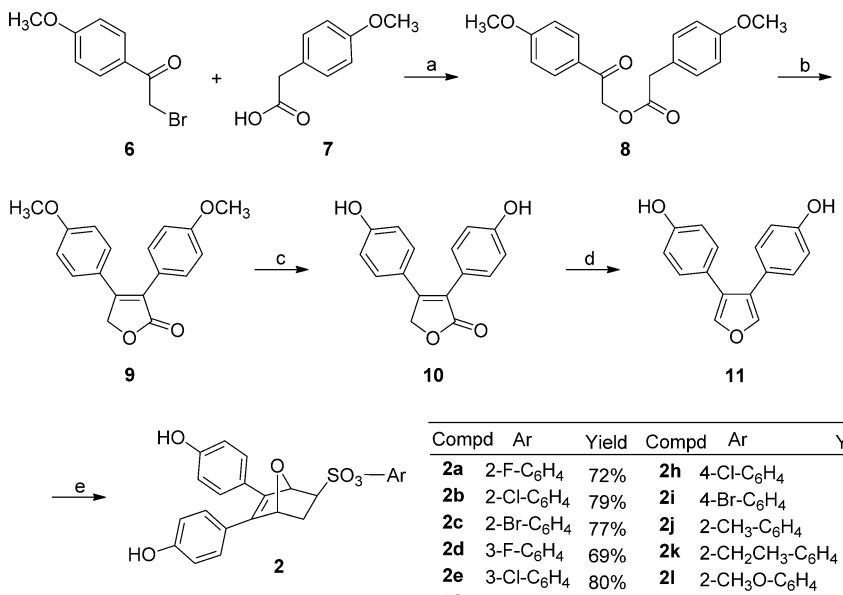


Figure 1. The oxabicycloheptene sulfonate side chain binds in a new epitope between helices 8 and 11. a) Crystal structure of the ERα ligand binding domain (LBD) in complex with diethylstilbestrol (DES) (PDB ID: 3ERD[13]). The side chains of the helix 3 and helix 11 residues, Glu 353 and His 524, both of which engage in hydrogen bonding with DES are shown. b) Crystal structure of the ERα LBD in complex with 4-hydroxytamoxifen (4OHT) (PDB ID: 3ERT[13]). The selective estrogen receptor modulator (SERM) side chain of 4OHT (green) displaces helix 12. c) Crystal structure of the ERα LBD in complex with OBHS (**1**) at 2.1 Å resolution. The position of His 524 in the estradiol-bound structure is shown with faint lines. Notably, the phenylsulfonate moiety (green) of OBHS binds in a new epitope that significantly shifts helix 11.



Compd	Ar	Yield	Compd	Ar	Yield
5a	2-F-C_6H_4	75%	**5h**	4-Cl-C_6H_4	88%
5b	2-Cl-C_6H_4	81%	**5i**	4-Br-C_6H_4	82%
5c	2-Br-C_6H_4	83%	**5j**	2-CH_3-C_6H_4	80%
5d	3-F-C_6H_4	72%	**5k**	2-Et-C_6H_4	78%
5e	3-Cl-C_6H_4	80%	**5l**	2-CH_3O-C_6H_4	87%
5f	3-Br-C_6H_4	86%	**5m**	1-naphthyl	80%
5g	4-F-C_6H_4	77%	**5n**	2-naphthyl	75%

Scheme 2. Synthesis of dienophiles **5a**–**n**. *Reagents and conditions*: a) Et_3N, CH_2Cl_2, 0 °C, 1 h.



Compd	Ar	Yield	Compd	Ar	Yield
2a	2-F-C_6H_4	72%	**2h**	4-Cl-C_6H_4	82%
2b	2-Cl-C_6H_4	79%	**2i**	4-Br-C_6H_4	84%
2c	2-Br-C_6H_4	77%	**2j**	2-CH_3-C_6H_4	87%
2d	3-F-C_6H_4	69%	**2k**	2-CH_2CH_3-C_6H_4	81%
2e	3-Cl-C_6H_4	80%	**2l**	2-CH_3O-C_6H_4	87%
2f	3-Br-C_6H_4	79%	**2m**	1-naphthyl	74%
2g	4-F-C_6H_4	74%	**2n**	2-naphthyl	70%

Scheme 3. Synthesis of oxabicycloheptene sulfonate derivatives **2a**–**n** using an improved procedure. *Reagents and conditions*: a) Et_3N, CH_3CN, RT, 88%; b) NaH, DMSO, 25 °C, 75%; c) BBr_3, CH_2Cl_2, −78 °C→RT, 85%; d) DIBAL-H, THF, −78 °C, 71%; d) **5** (neat), 90 °C.

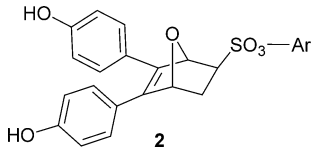
demethylated with boron tribromide to afford butenolide **10** in 85% yield,[16] avoiding our prior use of pyridinium chloride at 220 °C to obtain the free phenols.[7a] Diisobutylaluminum hydride reduction of **10** at −78 °C gave, after a careful, low-temperature acidic workup, 3,4-diphenol furans **11**. Finally, a Diels–Alder reaction of **11** with dienophiles **5** produced compounds **2a**–**n**. It is noteworthy that, similar to our previous study, high stereoselectivity was observed in the cycloaddition reaction of the phenolic furans with the dienophiles, with the *exo* product being obtained nearly exclusively; only traces, if any, of the *endo* products were observed.[7a] This *exo* selectivity is presumed due to the high rate and ready reversibility of the Diels–Alder reaction of furans, which allows the thermodynamically favored *exo* product to become dominant.

Binding affinity and SARs

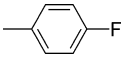
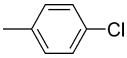
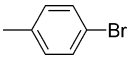
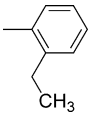
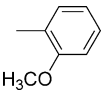
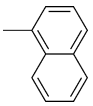
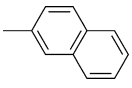
The compounds were assayed for their binding affinity to ERα and ERβ by a competitive radiometric binding assay.[17] These affinities are expressed as relative binding affinity (RBA) values; RBA values are reported as percentages (%) of that of estradiol (E_2), which is set at 100%. All of the 14 newly synthesized compounds were tested, and the results are summarized in Table 1.

It is noteworthy that the disposition and the size of the substituent on the phenyl of the sulfonates prove to be important factors in determining the binding affinity and selectivity of these new compounds. Most of the ligands show moderate-to-

Table 1. Relative binding affinities of compounds **2** for estrogen receptors α and β.[a]



2

Entry	Compd	Ar	ERα	ERβ	Ratio β/α
1	OBHS (**1**)		9.28 ± 0.64	1.71 ± 0.24	5.5
2	**2a**		7.28 ± 0.26	2.24 ± 0.56	3.3
3	**2b**		19.0 ± 4.6	1.76 ± 0.13	10.8
4	**2c**		2.29 ± 0.02	0.419 ± 0.070	5.4
5	**2d**		1.76 ± 0.04	0.39 ± 0.06	4.5
6	**2e**		1.91 ± 0.46	0.36 ± 0.06	5.5
7	**2f**		5.84 ± 0.28	0.707 ± 0.050	8.3
8	**2g**		1.18 ± 0.13	0.494 ± 0.120	2.4
9	**2h**		0.78 ± 0.002	0.284 ± 0.003	2.8
10	**2i**		0.76 ± 0.21	0.404 ± 0.120	1.9
11	**2j**		3.49 ± 0.63	0.294 ± 0.020	11.9
12	**2k**		1.03 ± 0.16	0.096 ± 0.020	10.7
13	**2l**		4.39 ± 0.48	0.210 ± 0.010	20.9
14	**2m**		5.03 ± 1.50	0.478 ± 0.120	10.5
15	**2n**		0.08 ± 0.02	0.019 ± 0.005	2.8

[a] Relative binding affinity (RBA) values were determined by competitive radiometric binding assays and are expressed as $IC_{50}^{estradiol}/IC_{50}^{compound} \times 100 \pm$ the range or standard deviation ($n = 2$–3, RBA, estradiol = 100%). In these assays, the K_d value for estradiol is 0.2 nM on ERα and 0.5 nM on ERβ. K_i values of each compound for each receptor can be obtained from the RBA values by the equation: $K_i = (100/RBA) \times K_d$.

excellent binding affinity, with **2b**, a compound that possesses an *ortho*-chloro substituent on the phenyl group of the sulfonate, showing the highest RBA values of 19.0% and 1.76% for ERα and ERβ, respectively, together with an improved selectivity—now over tenfold—in favor of ERα (Table 1, entry 3). When the chloro substituent was replaced with a fluoro (**2a**) or bromo (**2c**), a progressive decrease in RBA values was observed (Table 1, entries 2 and 4). For the corresponding *meta*-substituted compounds (**2d**–**f**), the fluoro (**2d**) and chloro (**2e**) analogues showed lower binding affinities, but the bromo analogue (**2f**) also gave good RBA values of 5.84% and 0.707% for ERα and ERβ, respectively (Table 1, entries 5–7). At the *para* position, all compounds exhibit rather low binding affinities (Table 1, entries 8–10). Previously, we prepared the *p*-hydroxy and *p*-methoxy analogues of OBHS (**1**), and they too had lower affinities.[7a]

One can conclude that, in terms of binding affinity, *ortho* substitution seems beneficial, while *meta* substitution has a more neutral effect, and *para* substitution is detrimental; at the *ortho* position, a chloro substituent is best, but a bromo is favored at the *meta* position. Additional SAR investigations focused on the most promising class, the *ortho*-substituted series. *Ortho*-methyl analogue **2j** maintained good affinity, and while the *ortho*-ethyl analogue **2k** showed a significant drop in binding affinity, a methoxy group (**2l**) was well tolerated (Table 1, entries 11–13). Interestingly, there was a more than 100-fold difference in binding affinity between the 1- and 2-naphthyl OBHS analogues (**2m** and **2n**) on ERα (Table 1, entries 14 and 15). With respect to a phenyl group, in a way, the 1-naphthyl group combines substitution at the favorable and neutral *ortho* and *meta* positions, while the 2-naphthyl group begins to occupy the same space as the poorly tolerant *para* position.

Transcription activation through ERα and β

The ability of these compounds to regulate ERα- and ERβ-mediated transcription was compared to that of tamoxifen in liver cells, in which tamoxifen displays agonist activity on ERα but not ERβ, due to higher activity of the amino-terminal coactivator binding region in ERα.[18] Cells were transfected with vectors for full-length human ERα or ERβ and a widely used estrogen-responsive element (ERE)-driven luciferase reporter gene.[19] OBHS (**1**) and the 14 analogues exhibited weaker potency than E_2, which activated ERα with an EC_{50} value of 1–2 nM, and also lower efficacy (Table 2, agonist mode). It is important to note that in HepG2 cells, used in the assays reported here, SERMs show higher partial agonist activity than in HEC-1 cells used previously.[7a,18] In fact, 4OHT has an agonist activity that is 35% that of estradiol, although fulvestrandt is still a complete antagonist.[18]

In HepG2 cells, three of the novel compounds, **2a**, **2c** and **2m**, displayed improved EC_{50} values compared with OBHS (**1**) (Table 2). Generally, aryl halides **2a**–**i** showed a similar activity pattern, a trend where fluoro substitution results in higher efficacy than bromo substitution. A similar pattern is evident with methyl substitution (compound **2j**) inducing greater activity than ethyl (**2k**) or methoxy (**2l**). It is noteworthy that the much higher affinity 1-naphthyl isomer (**2m**) has lower activity on ERα than the 2-naphthyl isomer (**2n**), which has low affinity

Table 2. Estrogen receptor-mediated transcriptional activities.

Entry	Compd	Agonist mode[a]				Antagonist mode[b]	
		ERα EC_{50} [nM][c]	ERα Eff. [%]	ERβ Eff. [%]	MCF-7 Eff. [%]	ERβ IC_{50} [nM][c]	ERβ Eff. [%]
1	OBHS (**1**)	95	60±2	0±1	21±4	581	−16±2
2	**2a**	26	61±5	0±3	26±4	–	54±4
3	**2b**	–	29±5	1±1	−7±2	–	86±11
4	**2c**	44	53±5	2±1	23±3	–	11±14
5	**2d**	140	54±2	2±1	n.d.	453	−19±3
6	**2e**	210	34±1	1±2	n.d.	–	−2±5
7	**2f**	300	33±2	0±1	n.d.	248	−26±4
8	**2g**	200	43±6	1±2	n.d.	214	−8±5
9	**2h**	360	36±2	1±5	n.d.	–	−15±4
10	**2i**	160	31±6	4±7	−2±2	–	61±12
11	**2j**	220	52±5	1±4	n.d.	357	−12±2
12	**2k**	390	33±2	0±1	n.d.	3208	−4±4
13	**2l**	560	40±1	0±2	n.d.	1038	17±9
14	**2m**	46	29±1	1±2	n.d.	–	30±3
15	**2n**	–	48±2	3±1	n.d.	–	52±19
16	Fulvestrandt[d]	–	1±1	−1±1	−4±4	1	−23±0
17	4-OHT[d]	1.08	35±3	−1±1	−11±1	1	−20±2

[a] Luciferase activity was measured in HepG2 cells transfected with 3X-ERE-driven luciferase reporter and expression vectors encoding ERα or ERβ, or where indicated in ERα+MCF-7 cells with the ERE reporter, and treated in triplicate with increasing doses (up to 10^{-5} M) of test compound. The EC_{50} values and average efficacy (Eff.), shown as a percentage of 10^{-5} M 17β-estradiol (E_2), were determined; data are presented as the mean±SEM. None of the compounds tested activated ERβ. Not determined (n.d.). [b] Average efficacy (Eff.) of the compounds (10^{-5} M) in combination with 10^{-6} M E_2 (E_2 only=100%). [c] The EC_{50} or IC_{50} value of some compounds was too high to be determined (–). [d] Fulvestrandt (Faslodex) and 4-hydroxytamoxifen (4OHT) were used as reference compounds.

and low potency, but higher efficacy. This highlights the fact that *affinity* and *potency* (i.e., EC_{50}) are independent of the allosteric conformational signals between ligand and the surface transcriptional coactivator binding site, which determine the *activity* or *efficacy* (i.e., *intrinsic activity*) of the compounds. What was unexpected was the low potency of the *ortho*-chloro compound (**2b**), which had the highest affinity on ERα. Beyond the factors noted above, cell permeability and metabolism issues might also be contributing to its low potency, as well as the general difficulty in establishing EC_{50} values for compounds with low intrinsic activity. In this context, it is important to note that the corresponding *ortho*-fluoro and *ortho*-bromo compounds (**2a** and **2c**) have good potency, but only slightly lower efficacy compared with OBHS (**1**).

None of the compounds activated ERβ as agonists in the HepG2 luciferase assay, and so, they were profiled only as antagonists in the presence of 1 μM E_2. Most of the compounds are antagonists selectively on ERβ (Table 2). Because ERβ has considerable basal activity, especially in HepG2 cells, compounds can be found to have inverse agonist activity, that is, an intrinsic activity that is less than that of apo-ERβ; such compounds are registered as having a negative efficacy value. While the size of the substituent predicted the activity on ERα, for ERβ, compounds with *meta* substitution (**2d–f**) were more consistently antagonistic. The naphthyl compounds (**2m**,**n**) showed similar activity profiles on both ER subtypes.

As a final test, a subset of the compounds with the lowest efficacy or potency were assayed for activation of 3XERE-luciferase activity in ERα-positive MCF-7 breast cancer cells, in which tamoxifen acts as an inverse agonist. While OBHS (**1**) showed limited agonist activity, we identified several compounds with inverse agonist activity. Notably, derivative **2m** shows an improved profile in all assays relative to OBHS (**1**), and an antagonist efficacy profile comparable to that of tamoxifen. While the affinity of derivative **2m** is lower than that of 4OHT, these results establish proof-of-principle that indirect antagonists can produce an activity profile comparable to tamoxifen, through by a different structural mechanism.

Conclusions

A small library of OBHS (**1**) analogues, differing in the substituents on the phenyl ring of the sulfonate, were prepared and evaluated as ligands for ERα and ERβ. The substitution on the bulky phenyl sulfonate side group of OBHS (**1**) is well tolerated and can engender a good binding affinity and antagonist efficacy profile. In transcription assays performed in HepG2 cells, some analogues activated ERα only partially and had little or no activity on ERβ, and a few compounds behaved as indirect antagonists exclusively on ERβ. Notably, several of the compounds showed full antagonism (even inverse agonism) on ERβ (e.g., **2d**, **2f**, **2h**, and **2j**), and most derivatives were partial agonist/antagonists on ERα. Importantly, several compounds also act as inverse agonists in MCF-7 breast cancer cells. Because the bulky side group binds the receptor between helices 8 and 11, rather than towards helix 12, compounds such as **2m** represent a novel structural class of SERMs. Interestingly, more than a 100-fold difference in binding affinity was observed between the 1- and 2-naphthyl OBHS derivatives (**2m** and **2n**) on ERα. Thus, despite the apparent constraints of the ER ligand binding pocket noted in crystal structures of related compounds, analogues of OBHS (**1**) with larger sulfonate aryl substituents can actually be more potent and active than OBHS itself. This is, again, a testament to the flexibility of the ligand binding pocket of the ERs and its ability to adapt to bind ligands of diverse structure with good affinity.[6,20]

Experimental Section

Synthesis

General: Synthetic procedures and characterization data for compounds **5**, **8**–**11** and **2** are given in the Supporting Information. All compounds assayed were >95% pure as determined by HPLC analysis. A summary of the HPLC results and HPLC spectra of all final compounds **2** are given in the Supporting Information.

General procedure for the Diels–Alder reaction: Dienophiles **5** and 3,4-bisphenol-furan **11** were synthesized according to the previously reported method with modifications (see Supporting Information for details).[7a,14] The BBr_3 demethylations were performed

according to published procedures.[16] Furan **12** (0.2 mmol) and dienophiles **5** (0.24 mmol) were placed in a round flask, and the mixture was stirred under a N_2 atmosphere at 100 °C for 12–24 h. The crude product was purified by flash chromatography (10–50% EtOAc/petroleum ether), preparative thin-layer chromatography, or recrystallization.

5,6-Bis-(4-hydroxyphenyl)-7-oxabicyclo[2.2.1]hept-5-ene-2-sulfonic acid 2-chlorophenyl ester (2b): Flash chromatography (30% EtOAc/petroleum ether) gave **2b** as a light yellow oil (89%): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.56–7.36 (m, 4H), 7.26–6.92 (m, 6H), 6.87–6.64 (m, 4H), 5.79 (d, J = 0.9 Hz, 1H), 5.40 (dd, J = 12.1, 7.5 Hz, 3H), 3.78 (dd, J = 8.4, 4.4 Hz, 1H), 2.64 (dt, J = 12.3, 4.4 Hz, 1H), 2.22 ppm (dd, J = 12.2, 8.3 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 155.99, 155.95, 153.7, 149.6, 145.0, 141.5, 137.0, 130.9, 129.5, 128.9, 128.2, 128.1, 126.9, 124.6, 124.2, 123.9, 116.1, 115.9, 115.8, 114.9, 84.4, 83.0, 60.8, 30.7 ppm; HRMS (EI): m/z $[M+Na]^+$ calcd for $C_{24}H_{19}ClO_6SNa$: 493.04486, found: 493.04831.

Biological evaluation

Estrogen receptor binding affinity assays: Relative binding affinities were determined by a competitive radiometric binding assay, as previously described,[17b] using 2 nM [^{3}H]estradiol as tracer (Perkin-Elmer Inc., Waltham, MA, USA) and purified full-length human ERα and ERβ (PanVera/InVitrogen, Carlsbad, CA, USA). Incubations were 18–24 h at 0 °C. Then, the receptor–ligand complexes were absorbed onto hydroxyapatite (BioRad, Hercules, CA, USA), and unbound ligand was washed away. The binding affinities are expressed as relative binding affinity (RBA) values, with the RBA value of estradiol set to 100. The values given are the average ± range or standard deviation (SD) of two or more independent determinations. Estradiol binds to ERα with a K_d value of 0.2 nM and to ERβ with a K_d value of 0.5 nM.

Luciferase assay: HepG2 cells cultured in growth media containing Dulbecco's minimum essential medium (DMEM) (Cellgro by Mediatech, Inc. Manassas, VA, USA) supplemented with 10% fetal bovine serum (FBS) (Hyclone by Thermo Scientific, South Logan, UT, USA), 1% nonessential amino acids (Cellgro), penicillin–streptomycin–neomycin antibiotic mixture, and Glutamax (Gibco by Invitrogen Corp., Carlsbad, CA, USA), were maintained at 37 °C and 5% CO_2. Cells in 10 cm dishes were transfected with 10 μg of 3XERE-luciferase reporter plus 1.6 μg of ERα or ERβ expression vector per dish using FugeneHD reagent (Roche Applied Sciences, Indianapolis, IN, USA). The next day, the cells were re-suspended in phenol-red-free growth media supplemented with 10% charcoal-dextran sulfate-stripped FBS, transferred at a density of 20000 cells per well to 384-well plates, incubated overnight at 37 °C and 5% CO_2, and treated in triplicate with the various compounds. After 24 h, luciferase activity was measured using BriteLite reagent (Perkin-Elmer Inc., Shelton, CT, USA) according to manufacturer's protocol.

Abbreviations

Estradiol (E_2); estrogen receptor (ER); estrogen response element (ERE); selective estrogen receptor modulators (SERMs); relative binding affinity (RBA); human liver cancer cells (HepG2); tetrahydrofuran (THF).

Acknowledgements

The authors are grateful to the National Natural Science Foundation of China (NSFC) (20872116, 20972121, 81172935), the Chinese Ministry of Education, New Century Excellent Talents (NCET) in University Program (NCET-10-0625), the Chinese National Mega Project on Major Drug Development (2009ZX09301-014-1), and the Research Fund for the Doctoral Program of Higher Education of China (20100141110021) for support of this research. Research support from the US National Institutes of Health (PHS 5R37 DK015556 to J.A.K. and R01 DK077085 to K.W.N.) is also gratefully acknowledged. The authors thank Dr. Teresa Martin (University of Illinois, Urbana, USA) for help with the binding assays.

Keywords: cycloadditions · estrogen · estrogen receptors · hormones · steroids

[1] a) Y. Endo, T. Yoshimi, K. Ohta, T. Suzuki, S. Ohta, *J. Med. Chem.* **2005**, *48*, 3941–3944; b) J. A. Gustafsson, *Trends Pharmacol. Sci.* **2003**, *24*, 479–485.
[2] G. G. Kuiper, E. Enmark, M. Pelto-Huikko, S. Nilsson, J. A. Gustafsson, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 5925–5930.
[3] K. Pettersson, J. A. Gustafsson, *Annu. Rev. Physiol.* **2001**, *63*, 165–192.
[4] a) M. De Angelis, F. Stossi, K. A. Carlson, B. S. Katzenellenbogen, J. A. Katzenellenbogen, *J. Med. Chem.* **2005**, *48*, 1132–1144; b) C. Gruber, D. Gruber, *Curr. Opin. Investig. Drugs* **2004**, *5*, 1086–1093; c) V. C. Jordan, *J. Med. Chem.* **2003**, *46*, 883–908; d) F. Minutolo, S. Bertini, C. Granchi, T. Marchitiello, G. Prota, S. Rapposelli, T. Tuccinardi, A. Martinelli, J. R. Gunther, K. E. Carlson, J. A. Katzenellenbogen, M. Macchia, *J. Med. Chem.* **2009**, *52*, 858–867; e) F. Minutolo, M. Macchia, B. S. Katzenellenbogen, J. A. Katzenellenbogen, *Med. Res. Rev.* **2011**, *31*, 364–442; f) A. D. Palkowitz, A. L. Glasebrook, K. J. Thrasher, K. L. Hauser, L. L. Short, D. L. Phillips, B. S. Muehl, M. Sato, P. K. Shetler, G. J. Cullinan, T. R. Pell, H. U. Bryant, *J. Med. Chem.* **1997**, *40*, 1407–1416; g) H.-B. Zhou, K. E. Carlson, F. Stossi, B. S. Katzenellenbogen, J. A. Katzenellenbogen, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 108–110.
[5] A. M. Brzozowski, A. C. W. Pike, Z. Dauter, R. E. Hubbard, T. Bonn, O. Engstrom, L. Ohman, G. L. Greene, J.-A. Gustafsson, M. Carlquist, *Nature* **1997**, *389*, 753–758.
[6] K. W. Nettles, J. B. Bruning, G. Gil, E. E. O'Neill, J. Nowak, Y. Guo, Y. Kim, E. R. DeSombre, R. Dilis, R. N. Hanson, A. Joachimiak, G. L. Greene, *EMBO Rep.* **2007**, *8*, 563–568.
[7] a) H. B. Zhou, J. S. Comninos, F. Stossi, B. S. Katzenellenbogen, J. A. Katzenellenbogen, *J. Med. Chem.* **2005**, *48*, 7261–7274; b) Y. L. Wu, X. J. Yang, Z. Ren, D. P. McDonnell, J. D. Norris, T. M. Willson, G. L. Greene, *Mol. Cell* **2005**, *18*, 413–424; c) A. C. W. Pike, A. M. Brzozowski, R. E. Hubbard, T. Bonn, A. G. Thorsell, O. Engstrom, J. Ljunggren, J. K. Gustafsson, M. Carlquist, *EMBO J.* **1999**, *18*, 4608–4618; d) R. W. Hsieh, S. S. Rajan, S. K. Sharma, Y. Guo, E. R. DeSombre, M. Mrksich, G. L. Greene, *J. Biol. Chem.* **2006**, *281*, 17909–17919.
[8] a) A. Nguyen, S. Top, P. Pigeon, A. Vessières, E. A. Hillard, M.-A. Plamont, M. Huché, C. Rigamonti, G. Jaouen, *Chem. Eur. J.* **2009**, *15*, 684–696; b) D. Plaz?uk, A. Vessie'res, E. A. Hillard, O. Buriez, E. Labbe', P. Pigeon, M. A. Plamont, C. Amatore, J. Zakrzewski, G. Jaouen, *J. Med. Chem.* **2009**, *52*, 4964–4967; c) S. Top, A. Vessieres, G. Leclercq, J. Quivy, J. Tang, J. Vaissermann, M. Huche, G. Jaouen, *Chem. Eur. J.* **2003**, *9*, 5223–5236; d) G. Gasser, I. Ott, N. Metzler-Nolte, *J. Med. Chem.* **2011**, *54*, 3–25.
[9] a) Y. Endo, T. Iijima, Y. Yamakoshi, H. Fukasawa, C. Miyaura, M. Inada, A. Kubo, A. Itai, *Chem. Biol.* **2001**, *8*, 341–355; b) J. F. Valliant, P. Schaffer, K. A. Stephenson, J. F. Britten, *J. Org. Chem.* **2002**, *67*, 383–387; c) M. L. Beer, J. Lemon, J. F. Valliant, *J. Med. Chem.* **2010**, *53*, 8012–8020; d) P. W. Causey, T. R. Besanger, J. F. Valliant, *J. Med. Chem.* **2008**, *51*, 2833–2844.
[10] a) R. S. Muthyala, S. B. Sheng, K. E. Carlson, B. S. Katzenellenbogen, J. A. Katzenellenbogen, *J. Med. Chem.* **2003**, *46*, 1589–1602; b) K. J. Kieser,

D. W. Kim, K. E. Carlson, B. S. Katzenellenbogen, J. A. Katzenellenbogen, *J. Med. Chem.* **2010**, *53*, 3320 – 3329; c) L. G. Hamann, J. H. Meyer, D. A. Ruppar, K. B. Marschke, F. J. Lopez, E. A. Allegretto, D. S. Karanewsky, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1463 – 1466.

[11] a) E. S. Mull, V. J. Sattigeri, A. L. Rodriguez, J. A. Katzenellenbogen, *Bioorg. Med. Chem.* **2002**, *10*, 1381 – 1398; b) C. Ramesh, B. Bryant, T. Nayak, C. M. Revankar, T. Anderson, K. E. Carlson, J. A. Katzenellenbogen, L. A. Sklar, J. P. Norenberg, E. R. Prossnitz, J. B. Arterburn, *J. Am. Chem. Soc.* **2006**, *128*, 14476 – 14477.

[12] a) K. W. Nettles, J. B. Bruning, G. Gil, J. Nowak, S. K. Sharma, J. B. Hahm, K. Kulp, R. B. Hochberg, H. Zhou, J. A. Katzenellenbogen, B. S. Katzenellenbogen, Y. Kim, A. Joachmiak, G. L. Greene, *Nat. Chem. Biol.* **2008**, *4*, 241 – 247; b) A. K. Shiau, D. Barstad, J. T. Radek, M. J. Meyers, K. W. Nettles, B. S. Katzenellenbogen, J. A. Katzenellenbogen, D. A. Agard, G. L. Greene, *Nat. Struct. Biol.* **2002**, *9*, 359 – 364; c) H. B. Zhou, S. B. Sheng, D. R. Compton, Y. Kim, A. Joachimiak, S. Sharma, K. E. Carlson, B. S. Katzenellenbogen, K. W. Nettles, G. L. Greene, J. A. Katzenellenbogen, *J. Med. Chem.* **2007**, *50*, 399 – 403; d) H.-B. Zhou, K. W. Nettles, J. B. Bruning, Y. Kim, A. Joachimiak, S. Sharma, K. E. Carlson, F. Stossi, B. S. Katzenellenbogen, G. L. Greene, *Chem. Biol.* **2007**, *14*, 659 – 669.

[13] A. K. Shiau, D. Barstad, P. M. Loria, L. Cheng, P. J. Kushner, D. A. Agard, G. L. Greene, *Cell* **1998**, *95*, 927 – 937.

[14] S. J. Liu, B. Zhou, H. Y. Yang, Y. T. He, Z. X. Jiang, S. Kumar, L. Wu, Z. Y. Zhang, *J. Am. Chem. Soc.* **2008**, *130*, 8251 – 8260.

[15] a) T. Pirali, S. Busacca, L. Beltrami, D. Imovilli, F. Pagliai, G. Miglio, A. Massarotti, L. Verotta, G. C. Tron, G. Sorba, A. A. Genazzani, *J. Med. Chem.* **2006**, *49*, 5372 – 5376; b) A. Zarghi, P. N. P. Rao, E. E. Knaus, *Bioorg. Med. Chem.* **2007**, *15*, 1056 – 1061.

[16] D. S. Mortensen, A. L. Rodriguez, K. E. Carlson, J. Sun, B. S. Katzenellenbogen, J. A. Katzenellenbogen, *J. Med. Chem.* **2001**, *44*, 3838 – 3848.

[17] a) J. A. Katzenellenbogen, H. J. Johnson, H. N. Myers, *Biochemistry* **1973**, *12*, 4085 – 4092; b) K. E. Carlson, I. Choi, A. Gee, B. S. Katzenellenbogen, J. A. Katzenellenbogen, *Biochemistry* **1997**, *36*, 14897 – 14905.

[18] J. C. Gould, L. S. Leonard, S. C. Maness, B. L. Wagner, K. Conner, T. Zacharewski, S. Safe, D. P. McDonnell, K. W. Gaido, *Mol. Cell. Endocrinol.* **1998**, *142*, 203 – 214.

[19] E. M. McInerney, M. J. Tsai, B. W. O'Malley, B. S. Katzenellenbogen, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 10069 – 10073.

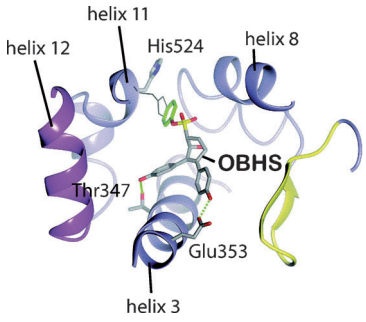
[20] J. A. Katzenellenbogen, *J. Med. Chem.* **2011**, *54*, 5271 – 5282.

Received: January 23, 2012
Published online on ■■ ■■, 0000

Y. Zheng, M. Zhu, S. Srinivasan, J. C. Nwachukwu, V. Cavett, J. Min, K. E. Carlson, P. Wang, C. Dong, J. A. Katzenellenbogen, K. W. Nettles, H.-B. Zhou**

■■ – ■■

Development of Selective Estrogen Receptor Modulator (SERM)-Like Activity Through an Indirect Mechanism of Estrogen Receptor Antagonism: Defining the Binding Mode of 7-Oxabicyclo[2.2.1]hept-5-ene Scaffold Core Ligands



A set of estrogen receptor ligands with a novel phenyl 2,3-diaryl-7-oxabicyclo[2.2.1]hept-7-ene-5-sulfonate core structure with various substituents on the phenyl sulfonate have been prepared (5,6-bis-(4-hydroxyphenyl)-7-oxabicyclo[2.2.1]hept-5-ene-2-sulfonic acid phenyl ester (OBHS), shown). Evaluation of these OBHS derivatives showed a distinctive pattern of affinity and transcriptional activity, and the tolerance for *ortho* substituents can be understood from a new X-ray crystal structure.

