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Brief Article

Synthesis and Evaluation of 17α-Arylestradiols as Ligands for Estrogen Receptor α and β

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To identify novel estrogen receptor ligands a series of substituted 17α-arylestradiols were synthesized using the catalytic [2 + 2 + 2]cyclotrimerization of 17α-ethynylestradiol with various 1,7-diynes in the presence of Wilkinson's catalysts [$Rh(PPh_3)_3Cl$]. The compounds were subjected to competitive binding assays, cell-based luciferase reporter assays, and proliferation assays. These experiments confirmed their estrogenic character and revealed some interesting properties like mixed partial/full agonism for ERα/ERβ and different selectivity as a result of differing potencies for either ER.

Introduction

Estrogens are steroid hormones responsible for affecting a wide range of biological functions. They predominantly act by binding to their corresponding intracellular receptors, resulting in the downstream modulation of target gene transcription. In 1996, a second gene encoding the estrogen receptor (ER[a]) was identified.[1] This discovery explained how estrogens could be involved in mediating such diverse functions and prompted researchers to define functions specific to ERα or ERβ. While 17β-estradiol (E2) binds to both receptors with a similar affinity,[2] the tissue distribution of each receptor is different. ERα mediates the action of estrogens in classical tissues like the uterus and mammary gland. Given that ERα has been shown to augment the proliferation of both healthy and cancerous cells in a number of tissues, including the mammary gland, ERα has traditionally been targeted in breast cancer therapy.

ERβ functions in the ovary, brain, cardiovascular system, and prostate. Additionally, it has been implicated by several animal models to play a role in inflammation.[3] Intriguingly, it was shown that an increase in ERβ expression could inhibit the growth of some estrogen-dependent cell lines.[4] Moreover, several clinical studies demonstrated an inverse correlation between the level of ERβ expression and advancing grade of breast cancer. Taken together, these findings led to the hypothesis that ERβ may function as a potential tumor suppressor.

The design of new synthetic ligands with modified properties and therefore effects on these receptors was facilitated by the publication of the X-ray structure of ERα LBD in complex with E2 and raloxifene in 1997[5] and ERβ LBD in complex with genistein and raloxifene soon after cloning of this gene in 1999.[6]

17α-Arylestradiols have been shown to possess interesting biological activities.[7−14] Although they can be synthesized via the reaction of estrone with the corresponding organolithiums,[7−14] this method lacks generality, requires the use of protective groups, and often results in low yields of the desired products. Given their interesting biological properties, the development of a simple method for quickly generating a series of variously modified 17α-arylestradiols is highly desirable. Considering the commercial availability of 17α-ethynylestradiol, it is an ideal starting material for the synthesis of 17-arylestradiol derivatives that may later be screened as potential estrogen receptor ligands.

Results and Discussion

From a general point of view, reactions that yield a significant increase in molecular complexity are considered key steps in organic synthesis. In this regard, a union of carbon−carbon π bonds that create a carbocyclic system is especially attractive. One such reaction is the [2 + 2 + 2]-cyclotrimerization of alkynes into benzene derivatives. This reaction is catalyzed by transition metal complexes such as Ni, Co, and Rh under mild reaction conditions that are compatible with the presence of various functional groups.[15] This alkyne cyclotrimerization reaction has previously been used to synthesize many natural products (steroids, alkaloids, terpenes, amino acids, purine derivatives, etc.). Recently, we have successfully used Ni[16−18] and Rh[19,20] based catalysts in the synthesis of various potentially biologically active compounds. Given this success, cyclotrimerization was chosen to synthesize a series of 17-arylestradiol derivatives.

Because organic synthesis should be based on simple reaction conditions that exploit readily available starting materials, we wished to carry out the cyclotrimerization directly with 17α-ethynylestradiol possessing unprotected hydroxyl groups. If successful, we would obtain in one-step the corresponding

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[a] Abbreviations: ER, estrogen receptor; LBD, ligand-binding domain; E2, 17β-estradiol; 17EE, 17α-ethynylestradiol; RBA, relative binding affinity; RTC, relative transactivation capacity; cLogP, computed partition coefficient.

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17α-arylestradiols, allowing us to avoid the use of protection and deprotection protocols. To keep the reaction conditions as simple as possible and circumvent the use of highly air sensitive Ni(0) catalysts, we decided to use the readily available and stable catalyst Wilkinson's complex, $[Rh(PPh_3)_3Cl]$,[21] in cyclotrimerization experiments. This complex was chosen as it is one of the most general cyclotrimerization catalysts that tolerates free OH groups.[22]

The cyclotrimerization of 17α-ethynylestradiol (1 equiv) with diynes (1.2 equiv) was carried out in the presence of $[Rh(PPh_3)_3Cl]$ (10 mol %) in a mixture of toluene and MeCN at 20 °C for 24 h (Scheme 1). The results obtained are summarized in Table 1. Generally, the cyclotrimerization process proceeded with reasonable efficiency giving rise to the corresponding 17-arylestradiols **3a**−**3d** in adequate isolated yields (entries 1−4). Only in the case of diynes **2e** and **2f** were the yields rather low (entries 5 and 6). Interestingly, attempts to catalyze the reaction with [Cp*Ru(cod)Cl],[23] which had recently been shown to efficiently catalyze the cyclotrimerization of alkynes bearing various functional groups failed, and the formation of cyclotrimerization products were not observed.

As we successfully obtained a set of variously substituted 17α-arylestradiols **3**, we decided to subject them to a range of biochemical tests to assess their potential biological activity with respect to ER signaling.

Scheme 1. Cyclotrimerization of **1** with Diynes **2** to **3**

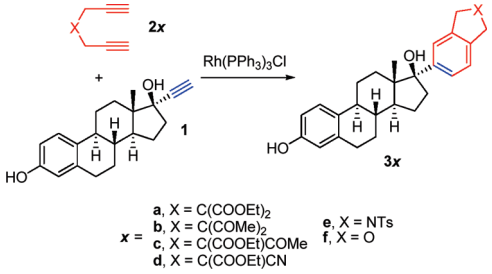

Table 1. Cyclotrimerization of 17α-Ethynylestradiol **1** with Diynes **2** Catalyzed by $[Rh(PPh_3)_3Cl]$

entry	diyne **2**	arylestradiol **3**	yield (%)[a]
1	**2a**	**3a**	56
2	**2b**	**3b**	50
3	**2c**	**3c**	41
4	**2d**	**3d**	45
5	**2e**	**3e**	27
6	**2f**	**3f**	13

[a] Isolated yields.

Initially, the newly prepared compounds were assayed for their ability to specifically bind each ER. In vitro fluorescence polarization-based competitive binding assays were carried out and the resulting data (shown in Table 2) demonstrate that all compounds exhibit a high binding affinity for both ERs with affinities ranging from 1 to 22% of the E2 binding affinity. Substitution of the hydrogen atom at the 17α position of E2 with an aryl group led to a decrease in ER binding affinity. Because the overall lipophilicity of the compound might also have influence on the binding to the receptor, we compared theoretical partition coefficients to the compound structures. Although an increased lipophilicity generally contributes positively to the binding, we saw a decrease in binding affinity in compounds with considerably higher cLogP compared to both E2 and 17EE as probably a result of the bulkiness of the substituent's (**3a**, **3e**, **3c**). This effect was especially pronounced for **3a**, which is the most lipophilic of the tested molecules but binds to both ERs with the lowest affinity. Conversely, 17α-arylestradiols substituted with a smaller aryl group (**3d** and **3f**) bound better to the receptors than compounds with larger and polar substituents.

To determine whether the ER binding affinities of each compound correlated with their transactivation capacity, we used a luciferase reporter assay in HEK293 cells. Dose−response curves representing the activation of ERs in response to each compound are shown in Figure 1 and the corresponding analysis of the obtained data is summarized in Table 3. Satisfyingly, the compounds that demonstrated the highest binding affinities were also the most efficient activators of transcription. For example, in the binding assay, compound **3f** demonstrates a high affinity for both receptors and similarly functions as a good agonist to activate both receptors without preference in the reporter assays. Compound **3d** shows the highest affinity for both ERα (EC_{50} = 6.92 nM, Log EC_{50} = −8.16) and ERβ (EC_{50} = 22.7 nM, Log EC_{50} = −7.65) and similarly exhibits the highest transactivation potency in both receptor reporter assays. Of interest, **3d** is a slightly more potent activator of ERα than ERβ.

Interestingly, compound **3a** is a poor agonist of ERβ (EC_{50} = 174 nM, LogEC_{50} = −6.76), however it preferentially activates ERα. We measured by reporter assay that **3a** is 13× more selective for ERα than ERβ. The decreased potency of **3a** for ERβ can be partially explained by the fact that the binding cavity volume in the ERβ LBD is smaller than in ERα (390 Å^3 versus 450 Å^3) as results from X-ray structures of ER LBD complexes with corresponding ligands.[5,6] Thus, larger side chains at the 17α position of estradiol mitigate efficient binding to ERβ but have less effect on ERα binding.

Table 2. Binding Affinities of Compounds for Human ERα and ERβ

compd	Log IC_{50} ERα (M)[a]	Log IC_{50} ERβ (M)[a]	RBA ERα (%)[b]	RBA ERβ (%)[b]	cLogP[c]
E2	−7.995 ± 0.081	−7.945 ± 0.053	100	100	3.78
17EE	−8.095 ± 0.070	−7.750 ± 0.047	126	63.2	3.86
3a	−6.308 ± 0.033	−5.965 ± 0.218	2.06	1.05	6.36
3b	−6.614 ± 0.094	−6.596 ± 0.102	4.16	4.48	4.77
3c	−6.610 ± 0.095	−6.496 ± 0.063	4.13	3.55	5.57
3d	−7.323 ± 0.121	−7.271 ± 0.199	21.2	21.2	5.20
3e	−6.467 ± 0.131	−6.301 ± 0.078	2.97	2.27	6.84
3f	−7.129 ± 0.227	−7.117 ± 0.191	13.6	14.9	5.01

[a] Log IC_{50} values were determined by testing 14 different concentrations of each compound using the fluorescence polarization-based ER competitor assay and plotting the resultant data using a nonlinear regression plot using GraphPad Prism 5.0 software. The experiment was performed in triplicate and the error is reported as standard error of the mean (SEM). [b] Relative binding affinity (RBA) was calculated as a ratio of E2 and the competitor concentration required to reduce the specific fluorescent ligand binding by 50%. [c] Computed partition coefficient (cLogP) was calculated using ChemBioOffice2010 software suite (CambridgeSoft Corporation).

Three out of the six tested compounds (**3b**, **3e**, **3f**) showed a full agonistic profile for ERα. These compounds generally reached at least 80% of the full activation level of E2, while for ERβ, all compounds except one (**3b**) functioned as full agonists. Moreover, **3d**, **3e**, and **3f** tended to activate ERβ to levels exceeding that of E2, however this supragonist property did not correlate with the transactivation potency of the compounds.

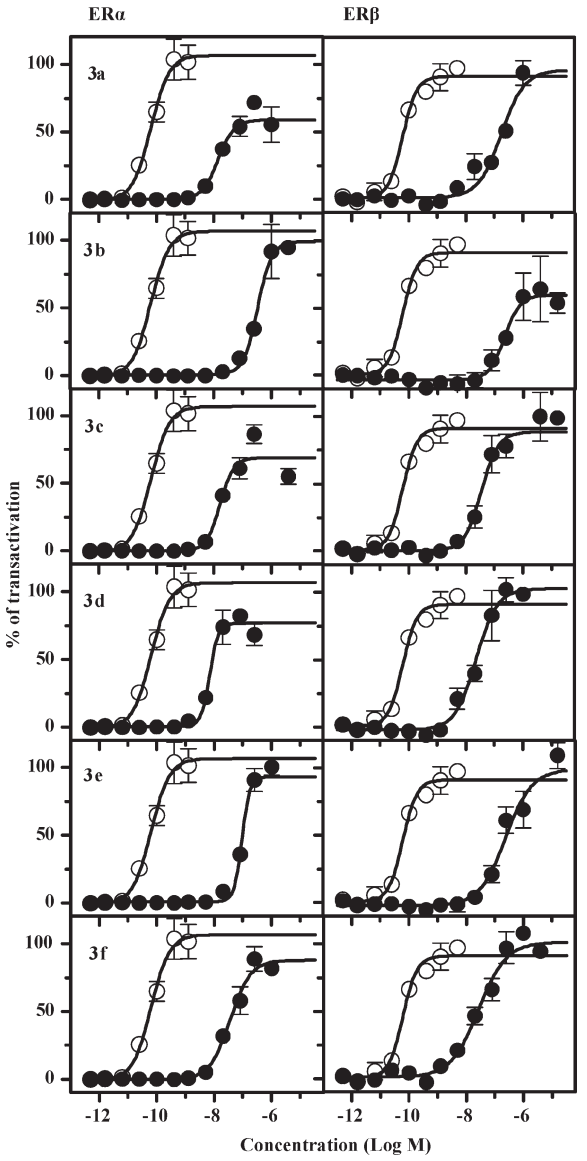

Figure 1. Transactivation of luciferase reporters by ERα and ERβ in response to increasing concentrations of compound (●) or E2 (○) in HEK293 reporter cells. The highest stimulation of the receptor in response to E2 was arbitrarily set as100%. Each value is reported as a mean ± standard error of the mean (SEM) from three experiments.

Steroid hormones exert their biological functions at nano- and subnano-molar concentrations. One of the best established in vitro assays for assessing the estrogenic properties of ER ligands is the MCF-7 proliferation assay.[24,25] For the efficient growth of MCF-7 cells, only a low concentration of estrogens in the medium is required. To analyze the estrogenic properties of our compounds, we cultivated MCF-7 cells in medium depleted of steroid hormones. The growth medium was then reconstituted with varying concentrations of the novel compounds and the cells assayed for proliferation. Data collected from these experiments are summarized in Figure 2. The proliferation rates measured are in agreement with the results obtained from the ERα reporter assays and illustrate clearly that the compounds possess estrogenic properties, albeit with a potency that is approximately 2−3 orders lower than E2. All of the compounds except **3b** stimulated estrogen-dependent growth at concentrations as low as 1 pM and continued to augment proliferation at concentrations ranging between 0.1 and 1 nM. Compound **3b** did not potentiate any estrogenic effect until it reached a concentration of 1 nM. Maximal proliferative effects were observed at 100 nM (data not shown). In agreement, out of all the compounds tested, **3b** also exhibited the weakest ability to function as an ERα agonist in reporter assays.

Conclusion

In conclusion, we have prepared a series of 17α-arylestradiols and tested them using different biochemical assays such as the fluorescence polarization-based competitive binding assay, ERα and ERβ luciferase reporter assays, and the MCF-7 proliferation assay. In all of the assays performed, the compounds clearly showed agonistic properties with one compound displaying a 13× selectivity for ERα. Moreover, we observed a variety of interesting biochemical properties like mixed partial/full agonism in ERα/ERβ reporter assays and differing affinities and potencies for ERα/ERβ as a result of using different substituents at the 17α-position of estradiol.

Experimental Section

All solvents were used as obtained unless otherwise noted. Toluene was distilled from benzophenone/Na under Ar and CH_3CN was purchased from Sigma-Aldrich (St. Louis, MO). Starting diynes **2** were prepared using standard procedures by the reacting propargyl bromide with the corresponding *C*-acids

Table 3. HEK293 Reporter Assay Demonstrating the Effect of Compounds on the Transactivation Ability of ERα and ERβ

compd	Log EC_{50} ERα [M][a]	Log EC_{50} ERβ [M][a]	RTC ERα (%)[b]	RTC ERβ (%)[b]	efficacy ERα (%)[c]	efficacy ERβ (%)[c]	selectivity for ERβ[d]
E2	−10.19 ± 0.119	−10.21 ± 0.063	100	100	100 ± 7.7	100 ± 3.8	1.00
3a	−7.852 ± 0.093	−6.756 ± 0.081	0.45	0.03	55.2 ± 3.4	105 ± 5.1	0.08
3b	−6.474 ± 0.051	−6.655 ± 0.124	0.02	0.03	92.8 ± 4.3	65.5 ± 5.8	1.46
3c	−7.783 ± 0.077	−7.461 ± 0.087	0.39	0.18	64.5 ± 3.3	97.0 ± 5.0	0.46
3d	−8.160 ± 0.071	−7.645 ± 0.142	0.92	0.27	72.0 ± 3.2	112 ± 7.3	0.29
3e	−7.036 ± 0.041	−6.616 ± 0.104	0.07	0.03	87.2 ± 3.5	108 ± 5.1	0.37
3f	−7.444 ± 0.076	−7.574 ± 0.108	0.18	0.23	82.2 ± 4.2	111 ± 6.4	1.29

[a] Log EC_{50} values were generated by fitting data from the ERα and ERβ reporter assays in a nonlinear regression function using GraphPad Prism 5.0 software. The error of the determination is reported as standard error of the mean (SEM). [b] Relative transactivation capacity (RTC) of each compound was calculated as a ratio of EC_{50} for E2 and for the corresponding compound. [c] Efficacy of each compound was calculated as a ratio of the highest luciferase activity induced by the corresponding compound and E2. The highest luciferase activity induced by E2 was arbitrarily set as 100%. [d] Selectivity of the compounds for ERβ was calculated as a ratio of relative transactivation capacity of the tested compound for ERβ and for ERα.

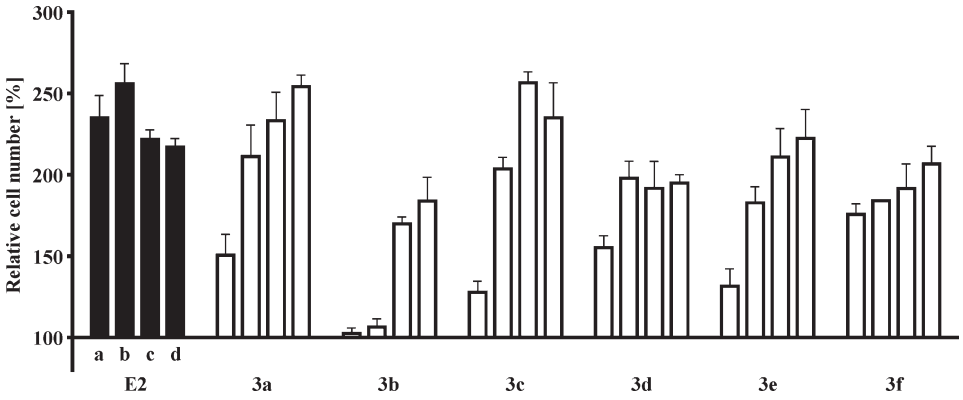

Figure 2. Proliferation of estrogen-growth-dependent MCF-7 cells in response to differing concentrations of either E2 or compounds. Previously starved cells were incubated in the presence of the tested compounds at the following concentrations: (a) 10^{-12} M; (b) 1.3×10^{-10} M; (c) 3.2×10^{-9} M, and (d) 1.6×10^{-8} M. After 6 days of incubation, cell viability was measured as a capacity of cells to convert resazurin to resorufin. Relative cell number was calculated as a ratio of number of viable cells treated with tested compounds and number of viable cells treated with DMSO only. Values are reported as mean ± standard error of the mean (SEM) of three experiments.

under basic conditions.[17] $RhCl(PPh_3)_3$ was prepared according to the previously reported procedures.[26] Cp*RuCl(cod) was purchased from Strem Ltd., and 17α-ethynylestradiol was purchased from Fluka.

NMR spectra were recorded on a Varian UNITY 400 INOVA instrument at 400 MHz (^{1}H) and 100.6 MHz (^{13}C) as solutions in C_6D_6 and are referenced to the residual solvent signal. Infrared spectra were recorded on a Bruker IFS 88 instrument. Mass spectra were recorded on a FINNIGAN MAT INCOS 50 spectrometer. HR mass spectra were recorded on a ZAB-SEQ VG Analytical spectrometer. HPLC analysis was carried out on Supelcosil column (Si: 5 μm; 250 mm × 4.6 mm; heptane/iPrOH 98/2, 1 mL/min; detection: UV−210 nm). Purity of the prepared compounds was determined by a combination of ^{1}H NMR and HLPC techniques and was found to be >95%.

Cyclotrimerization Reaction: General Procedure for $RhCl(PPh_3)_3$ Catalyzed Cyclotrimerization of 17α-Ethynylestradiol with Diynes. 17α-Ethynylestradiol (0.5 mmol, 150 mg) was placed into a solution of dry toluene (6 mL), and CH_3CN (1 mL) was added to $RhCl(PPh_3)_3$ (0.05 mmol, 46 mg) and diyne **2** (0.6 mmol). The reaction mixture was stirred at 20 °C for 48 h or until the consumption of the starting material (TLC). The solvent was then evaporated under reduced pressure, and the residue was subjected to column chromatography.

17α-[2,2-Bis(ethoxycarbonyl)-1,3-dihydro-2*H*-inden-5-yl]-estradiol (3a). 1 (0.25 mmol, 74 mg), **2a** (0.3 mmol, 70.8 mg), $RhCl(PPh_3)_3$ (0.025 mmol, 23 mg). Column chromatography (2/1 hexane/EtOAc) furnished 75 mg (56%) of the title compound as a colorless solid; mp 164 °C; $[\alpha]_D$ = +35 (*c* 0.022 g/mL, acetone). ^{1}H NMR (400 MHz, C_6D_6) δ 0.71−0.79 (m, 1H), 0.87 (t, *J* = 6.8 Hz, 3H), 0.88 (t, *J* = 6.8 Hz, 3H), 1.06 (s, 3H), 1.03−1.12 (m, 1H), 1.29−1.42 (m, 4H), 1.61−1.75 (m, 4H), 1.88−2.00 (m, 2H), 2.18−2.23 (m, 1H), 2.58−2.70 (m, 2H), 3.73−3.85 (m, 4H), 3.91 (q, *J* = 6.8 Hz, 2H), 3.92 (q, *J* = 6.8 Hz, 2H), 5.14 (s, 1H), 6.47 (d, *J* = 2.8 Hz, 1H), 6.57 (dd, *J* = 8, 2.8 Hz, 1H), 6.94 (d, *J* = 8 Hz, 1H), 7.04 (d, *J* = 8 Hz, 1H), 7.164 (s, 1H) signal overlapped, 7.37 (s, 1H). ^{13}C NMR (100 MHz, C_6D_6) δ 14.60 (2×), 15.74, 25.05, 27.33, 28.46, 30.62, 34.67, 39.63, 40.46, 41.37, 41.76, 44.20, 47.83, 49.15, 61.66, 62.35 (2×), 86.64, 113.73, 116.23, 123.90, 124.49, 127.40, 129.23, 133.05, 138.56, 139.50, 140.19, 146.60, 155.06, 172.47 (2×). IR (ATR ZnSe) ν 3398, 2958, 2927, 2870, 1727, 1711, 1610, 1502, 1442, 1283, 1249, 1185, 1068, 1049, 1008 cm^{-1}. MS (EI) 532 (8), 514 (72), 499 (17), 425 (17), 314 (12), 213 (56), 149 (77). HRMS (EI) cacld. for $C_{33}H_{40}O_6$ 532.282489, found 532.283226.

7α-(2,2-Diacetyl-1,3-dihydro-2*H*-inden-5-yl)-estradiol (3b). 1 (0.5 mmol, 150 mg), **2b** (0.6 mmol, 105.6 mg), $RhCl(PPh_3)_3$ (0.05 mmol, 46 mg). Column chromatography (2/1 hexane/EtOAc) furnished 119 mg (50%) of the title compound as a colorless solid; mp 158 °C; $[\alpha]_D$ = +40.9 (*c* 0.0055 g/mL, acetone). ^{1}H NMR (400 MHz, C_6D_6) δ 0.71−0.78 (m, 1H), 1.04 (s, 3H), 1.05−1.19 (m, 1H), 1.30−1.40 (m, 4H), 1.62−1.65 (m, 2H), 1.69 (s, 3H), 1.70 (s, 3H), 1.72−1.77 (m, 2H), 1.90−1.96 (m, 2H), 2.21−2.27 (m, 1H), 2.60−2.70 (m, 2H), 3.22−3.34 (m, 4H), 4.46 (s, 1H), 6.39 (d, *J* = 2.4 Hz, 1H), 6.47 (dd, *J* = 8, 2.4 Hz, 1H), 6.92 (d, *J* = 8 Hz, 1H), 6.99 (d, *J* = 8 Hz, 1H), 7.18 (s, 1H) overlapped, 7.32 (s, 1H). ^{13}C NMR (100 MHz, C_6D_6) δ 15.71, 25.08, 26.60, 26.66, 27.38, 28.50, 30.61, 34.74, 38.01, 38.39, 39.75, 40.52, 44.31, 47.87, 49.20, 75.75, 86.55, 113.69, 116.18, 124.00, 124.59, 127.37, 128.25, 129.24, 133.01, 138.53, 139.30, 140.03, 146.59, 154.96, 204.48 (2×). IR (ATR ZnSe) ν 3427, 2927, 2870, 1692, 1610, 1498, 1359, 1249, 1150 cm^{-1}. MS (EI) 472 (9), 454 (12), 411 (100), 228 (14), 213 (13), 159 (17). HRMS (EI) cacld for $C_{31}H_{36}O_4$ 472.261360, found 472.261119.

Biological Testing: Materials. 17β-Estradiol (E2) was purchased from Sigma-Aldrich (St. Louis, MO), phenol red-free Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serum (FBS) were obtained from Invitrogen (Carlsbad, CA), Hyclone charcoal/dextran treated fetal bovine serum (C/D FBS) was purchased from Thermo Fisher Scientific Inc. (Waltham, MA). One-Glo luciferase assay system and CellTiter-Blue cell viability assay kits were obtained from Promega (Madison, WI). Panvera's fluorescence polarization-based ER competitor assays, RED, for ERα and ERβ were purchased from Invitrogen (Carlsbad, CA).

Fluorescence Polarization-Based Competitive Binding Assay. The binding affinity of each compound for both ERs was performed according to the manufacturer's protocol. The experiment was carried out in black 384-well plates (Corning Inc., NY) in a total volume of 40 μL. Fluorescent data were collected on the EnVision (PerkinElmer, Inc., Waltham, MA) plate reader after a 3 h incubation at room temperature using optimized BODIPY TMR FP Label consisting of 531 nm excitation filter, 595 nm S polarized emission filter, 595 nm P polarized emission filter, and BODIPY TMR FP optical module. Collected data were subsequently analyzed by GraphPad Prism 5.0 statistical software and IC_{50} values were calculated from regression function (dose−response, variable slope).

Reporter Assay. HEK293 cells were maintained in a monolayer in DMEM supplemented with 10% FBS, 2 mM glutamine, and penicillin/streptomycin and incubated in a 5% CO_2 humidified atmosphere at 37 °C. To generate ERα and ERβ responsive reporter cells, HEK293 cells were transfected with either pCDNA3-hERα or pCDNA3-hERβ and the reporter vector pGL4-3ERE-luc (Sedlak et al., unpublished results). Following transfection, cells were maintained in phenol red-free DMEM supplemented with 4% C/D FBS and 2 mM glutamine. Twenty-four hours after transfection, cells were trypsinized, counted,

and seeded at a density of 10^4 cells/well in white opaque cell culture 384-well plates (Corning Inc., NY). Each compound tested was serially diluted in DMSO and stored in 384-well plates before being transferred by the JANUS Automated Workstation (PerkinElmer, Inc.) equipped with a Pin Tool (V&P Scientific, Inc., San Diego, CA). After a further 18 h incubation, luciferase activity was determined using the One-Glo luciferase assay system according to the manufacturer's protocol (Promega Corp.). Luminescence was recorded on the EnVision plate reader using 1s integration and data were analyzed by GraphPad Prism 5.0 statistical software. EC_{50} values were calculated from regression function (dose−response, variable slope).

MCF-7 Cell Proliferation Assay. MCF-7 cells were expanded in a monolayer in DMEM supplemented with 10% FBS, 2 mM glutamine, and penicillin/streptomycin and incubated in a 5% CO_2 humidified atmosphere at 37 °C. One day before each experiment, cells were maintained in phenol red-free DMEM supplemented with 10% C/D FBS and 2 mM glutamine. To determine the proliferative effect of each compound, cells were seeded at a density of 10^3 cells/well in a black cell culture 96-well plate (Corning Inc., NY). Each compound was serially diluted in culture medium, added to cells, and following a 6 day incubation period, the viability of cells was measured using the CellTiter-Blue cell viability assay according to the manufacturer's protocol (Promega Corp.). Fluorescence emitted by the product of the metabolic conversion of resazurin to resorufin was recorded on the EnVision plate reader using the 560 nm excitation filter and 590 nm emission filter. Data were analyzed using the GraphPad Prism 5.0 statistical software.

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Supporting Information Available: Spectral and analytical data for **3a**−**3f**. This material is available free of charge via the Internet at http://pubs.acs.org.

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