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Synthesis and Biochemical Characterization of a Series of 17α-Perfluoroalkylated Estradiols as Selective Ligands for Estrogen Receptor α

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Despite intensive research efforts, the distinct biological roles of two closely related estrogen receptors, ERα and ERβ, are only partially understood. Therefore, ligands selective for either of two isotypes are useful research tools because they allow for exerting a desired subset of biological effects mediated by only one of the receptors. Here we report on the synthesis of a new class of potent and selective ligands for ERα represented by a series of 17α-substituted estradiols bearing lipophilic perfluoroalkyl chains. These 17α-perfluoroalkylated estradiols were synthesized by Ru-catalyzed cross metathesis reactions of 17α-allyl- or 17α-vinylestradiols with perfluoroalkylpropenes. Compounds were tested in both agonistic and antagonistic modes using a panel of stable steroid receptor reporter cell lines established in U2OS cells and consisting of ERα-LBD, ERβ-LBD, GR-LBD, and MR-LBD reporters. Some of the compounds are potent and selective agonists of ERα, exhibiting weak partial to no detectable agonistic activity on ERβ. Notably, **11c** is the most ERα selective ligand of the prepared compounds because it activates ERα but inhibits ERβ. In addition, some compounds are pure agonists on ERα but show mixed agonistic/antagonistic profile on ERβ which is a typical pattern observed for selective estrogen receptor modulators (SERMs).

Introduction

The estrogen receptor α (ERα,[a] NR3A1), estrogen receptor β (ERβ, NR3A2), glucocorticoid receptor (GR, NR3C1), and mineralocorticoid receptor (MR, NR3C2) belong to a steroid hormone receptor family of ligand inducible transcription factors. These receptors bind to hydrophobic ligands and modulate the transcription of target genes. ERα and ERβ are products of two separate genes and mediate the effect of the main and the most potent natural estrogen: 17β-estradiol (E2). E2 binds to both receptors with a similar affinity.[1] Although ERα and ERβ are very similar proteins, the expression distribution is different in various tissues. ERα mediates the action of estrogens in classical tissues like uterus and mammary gland. ERα is also an important marker and a traditional target for a therapy of breast cancer,[2] and it promotes a proliferation of certain healthy and cancer tissues. On the other hand, the role for ERβ was established in the brain, ovary, cardiovascular system,[3] prostate, and several animal models of inflammation.[4] Numerous studies report about the antiproliferative effect of the increasing expression level of ERβ on the prostatic tissue[5,6] or cell lines derived from different breast[7] or colon cancers.[8,9]

The attachment of highly lipophilic moieties onto the steroid framework has various beneficial effects. A typical example is the synthesis of antiprogestine (ZK 230211, Figure 1) (a potent progesterone receptor antagonist).[10] In case of compounds with the estradiol framework the best known derivatives are fulvestrant[11] and RU58668[12] (steroidal antiestrogen) bearing the pentafluoroethyl moiety. The introduction of perfluoroalkyl chains onto the estrone skeleton has attracted the attention of Blazejewski et al., who synthesized a series of perfluoroalkylated estrone derivatives such as 7α-perfluoroalkylestradiols **I** (perfluorohexyl and trifluoromethyl groups),[13] 11β-perfluoroalkylestradiols **II** (perfluorohexyl,[14] perfluorobutylethyl, and perfluorooctylethyl[15] groups), and 11β isomers of fulvestrant **III**.[16] In general, compounds **I** and **II** retained their affinity for ERs and compound **III** also retained strong antiestrogenic properties. Similarly, we have recently shown that the presence of perfluoroalkylated chain in the brassinosteroid series had a positive effect on the metabolic stability while preserving the biological activity of compounds.[17]

In this regard substituted estradiol derivatives and 17α-arylestradiols have attracted considerable attention because of their interesting activity on ERα and ERβ.[18−24] Poirier et al. reported a synthesis of a new class of reversible inhibitors of steroid sulfatase represented by a series of 17α-alkyl- and 17α-alkenylestradiol derivatives.[18] We have also recently shown that 17α-arylestradiols bearing a relatively bulky lipophilic indanyl moiety surprisingly retain estrogenic properties and affinity to ERs.[25] Moreover, our results show that different substituents at the 17α position can alter the selectivity of the ligand for either ERα or ERβ. In one particular case,

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[a] Abbreviations: ER, estrogen receptor; GR, glucocorticoid receptor; MR, mineralocorticoid receptor; E2, 17β-estradiol; LBD, ligand-binding domain; RTC, relative transactivation capacity; SERM, selective estrogen receptor modulator.

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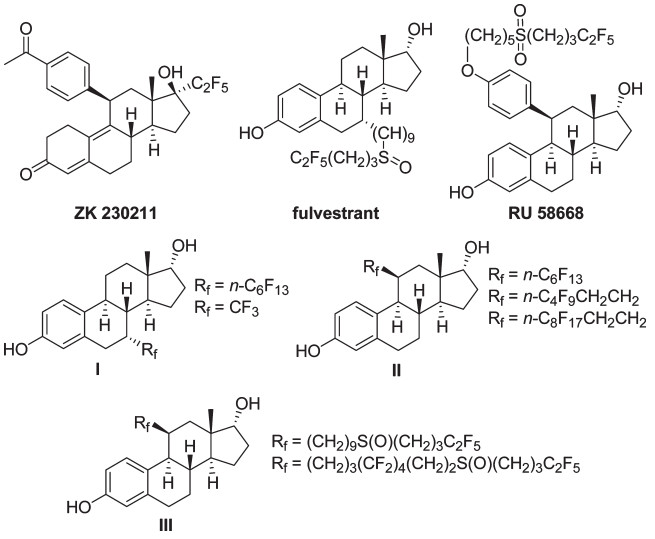
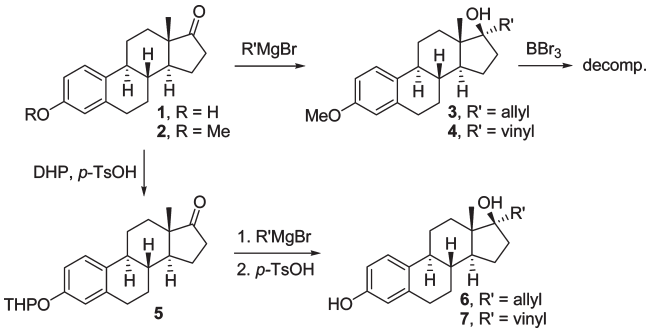


Figure 1. Perfluoroalkylated steroid derivatives.

Scheme 1. Synthesis of Allyl- and Vinylestradiols **3**, **4**, **6**, and **7**



substitution of the hydrogen atom at the 17α position gave rise to a ligand that was 13× more selective for ERα.[25]

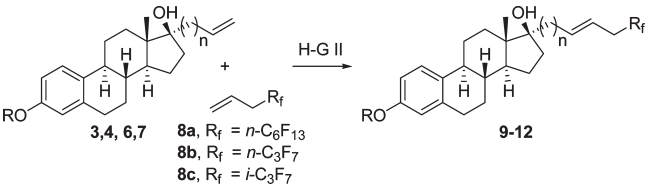
The above-mentioned results, coupled with the fact that adding of perfluoroalkyl chains to the steroid framework introduces interesting and often desired properties to these compounds, sparked our interest in synthesizing a series of 17α-alkylestradiol derivatives bearing highly lipophilic chains represented by perfluoroalkyl moieties.

Results and Discussion

Synthesis of 17α-Perfluoroalkylestradiols. We envisioned that the synthetically easiest and most flexible approach for the introduction of perfluoroalkylated chains would be based on the use of cross-metathesis between 17α-allyl- or 17α-vinylestradiols with perfluoroalkylpropenes. We have recently developed this method[26] and applied it in the synthesis of perfluoroalkylated analogues of brassinosteroids.[17]

The starting 17-alkenyl derivatives were synthesized from the commercial estrone **1** and 3-methoxyestrone **2** via standard procedures using organomagnesium reagents.[27] Since the methyl-18 on the β-face of the steroid directs the nucleophilic attack at the less hindered steroidal α-face, such alkylations of a C17-keto steroid are known to be stereoselective.[28] Starting from 3-methoxyestrone **2**, the 17α-alkylation products were then obtained exclusively (Scheme 1). The first reaction with allylmagnesium bromide proceeded exceptionally cleanly and furnished compound **3** in high 94% isolated yield. The second one using vinylmagnesium

Scheme 2. Cross-Metathesis of Estradiol Derivatives with Perfluoroalkylpropenes



bromide was sluggish and gave rise to the vinyl derivative **4** in a poor yield of 45%. Moreover, the course of the reaction was accompanied by the formation of the nonalkylated product of the reduction and unreacted starting material. The next step, deprotection of **3** and **4** with BBr_3[29] to get unprotected derivatives **6** and **7** with the free hydroxyl group, was not successful. The analysis of reaction mixtures showed extensive decomposition and no formation of any major product. This issue was solved by carrying out the whole alkenylation process with a substrate bearing a different protecting group. The 3-hydroxyl group of estrone **2** was readily converted into the THP ether **5** in high 92% isolated yield. Subsequently, two reactions with Grignard reagents (allyl and vinyl) were carried out and followed by the deprotection under acidic conditions (*p*-TsOH). This procedure gave compounds **7** and **8** in 82% and 47% yields, respectively (Scheme 1).

The attachment of side chains by cross-metathesis was accomplished via our previously reported procedure by using Hoveyda−Grubbs second generation catalyst between the estradiol derivatives **3**, **4**, **6**, and **7** and perfluoroalkylpropenes **8**.[17,26,30] Thus, estrone derivatives **9**−**12** were obtained (Scheme 2), and the results are summarized in Table 1. Initially, a metathesis of the substrate **3** and (perfluorohexyl)propene **8a** afforded the derivative **9a** in acceptable 53% yield. Then the reaction between **8b** and **3** was carried out and compound **9b** was obtained in good 68% yield. Lower efficiency was achieved with the substrate **4**, where products **10a** and **10b** were obtained in low 36% and 29% yields, respectively. This fact could be attributed to a larger steric hindrance of the double bond in the vinyl derivative **4**. The same conditions were also used for metathesis of compound **6** with three (perfluoroalkyl)propenes **8a**, **8b**, and **8c**, and the corresponding metathesis products **11a**−**c** were isolated in reasonable yields of 58−67%. The metathesis of compound **7** with **8a**, **8b**, and **8c** afforded the corresponding products **12a**, **12b**, and **12c** in rather low yields for reasons like in the case of compound **4**. The product **12a** was obtained in 39% yield. The synthesis of **12b** and **12c** was even less effective and gave corresponding compounds in 25% and 12% isolated yields, respectively.

Biological Testing. We tested the ability of newly synthesized compounds to modulate the activity of ERα, ERβ, GR, and MR in the U2OS reporter cell-based assays that we established recently in our laboratory. The agonistic properties are summarized in Table 2 and the antagonistic properties in Table 3. In order to estimate to what extent new ligands activate ERs and to see the relation between efficacy and potency of ligands, Figure 2 shows dose response curves from ERα and ERβ reporter assays. All new compounds activate ERα and ERβ with lower potency than the natural ligand 17β-estradiol. The methoxy group in the position 3 considerably decreases the ability of the compound to activate either of two receptors, which is well illustrated by a comparison of **10b** and **12b** or **9a** and **11a**. Compound **12b** exhibits the highest potency of all new compounds for the

Table 1. Synthesis of 17α-Perfluoroalkylated Estradiols **9**−**12**

Estrone	8	Product	Yield (%)[a]
3	**8a**	**9a**, R = *n*-C_6F_{13}	53
	8b	**9b**, R = *n*-C_3F_7	68
4	**8a**	**10a**, R = *n*-C_6F_{13}	36
	8b	**10b**, R = *n*-C_3F_7	29
6	**8a**	**11a**, R = *n*-C_6F_{13}	62
	8b	**11b**, R = *n*-C_3F_7	58
	8c	**11c**, R = *i*-C_3F_7	67
7	**8a**	**12a**, R = *n*-C_6F_{13}	39
	8b	**12b**, R = *n*-C_3F_7	25
	8c	**12c**, R = *i*-C_3F_7	12

[a] Isolated yields.

Table 2. Agonistic Effect of Compounds on ERα, ERβ, GR, and MR Transactivation in Whole Cells

	log(EC_{50} (M))[a]				RTC (%)[b]		
compd	ERα	ERβ	GR	MR	ERα	ERβ	cLogP[c]
control[d]	−8.557 ± 0.057	−9.339 ± 0.058	−8.172 ± 0.117	−9.471 ± 0.045	100	100	3.78
9a	> −4.8	> −4.902	> −4.8	> −4.8			ND[e]
9b	−5.781 ± 0.045	> −4.817	> −4.8	> −4.8	0.17		ND[e]
10a	> −4.8	> −5.079	> −4.8	> −4.8			ND[e]
10b	−5.395 ± 0.037	−5.285 ± 0.133	> −4.8	> −4.8	0.07	0.01	ND[e]
11a	−5.988 ± 0.050	−7.284 ± 0.178	> −4.8	> −4.8	0.27	0.88	7.46
11b	−6.314 ± 0.069	−5.620 ± 0.144	> −4.8	> −4.8	0.57	0.02	7.34
11c	−6.395 ± 0.069	> −4.8	> −4.8	> −4.8	0.69		6.36
12a	−5.682 ± 0.046	> −5.561	> −4.8	> −4.8	0.13		7.14
12b	−7.450 ± 0.080	−6.888 ± 0.299	> −4.8	> −4.8	7.82	0.35	6.43
12c	−5.002 ± 0.054	> −4.713	> −4.8	> −4.8	0.03		6.04

[a] Transcriptional response of ERα, ERβ, GR and MR to tested compounds was assessed in U2OS reporter cell lines stably expressing fusion of the Gal4 DNA binding domain and the ligand binding domain (LBD) of the corresponding steroid receptor and the reporter vector with 9xUAS response element followed by the coding sequence for the luciferase. Cells were incubated with compounds at the indicated concentrations for 18 h. EC_{50} values were generated by fitting data from the luciferase reporter assay by nonlinear regression function. Data shown are representative of two independent experiments performed in triplicate. Values are reported as the mean ± standard error of the mean (SEM). [b] Relative transactivation capacity (RTC) of each compound was calculated as the ratio of EC_{50} of the tested compound to that of E2. [c] Computed partion coefficient (cLopP) was calculated as described in the Experimental Section. [d] E2 was used as a control ligand for ERα and ERβ, dexamethasone for GR, and aldosterone for MR. [e] ND = not calculated.

ERα and **11a** the highest potency for the ERβ, but the introduction of the methoxy group in position 3 resulting in **10b** and **9a**, respectively, completely eliminates the agonistic property of the compound or it decreases it by more than 2 logs. On the other hand **9b**, which also carries the 3-methoxy group, activates ERα with only slightly higher EC_{50} = 1.66 μM (log EC_{50} = −5.78) than when the methoxy group is replaced by the hydroxy group (**11b**, EC_{50} = 0.49 μM, log EC_{50} = −6.31).

Since the biological effects exerted by ERα and ERβ only partially overlap and given that E2 is a very potent ligand but does not differentiate between two receptors, it has always been essential to prepare new ligands binding and activating ERα or ERβ. Using selective ligands, one can selectively exert a desired set of biological effects mediated by only one of the receptors. Out of the new compounds, **11c** is a highly selective compound for ERα. It activates ERα with EC_{50} = 400 nM (log EC_{50} = −6.40) but is completely inactive on ERβ. Furthermore, this compound antagonizes the effect of E2 on ERβ but not on ERα, which means that it acts as the agonist for ERα and antagonist for ERβ (Table 3, Figure 3).

Similarly **12b** shows high affinity for ERα with EC_{50} = 35.5 nM (log EC_{50} = −7.45) and is 22× selective for ERα over ERβ. It is almost a full ERα agonist reaching 80% of the

Table 3. Antagonistic Effect of Compounds on ERα, ERβ, GR, and MR transactivation in Whole Cells

	log(IC$_{50}$ (M)) [a]			
compd	ERα	ERβ	GR	MR
control [b]	−7.677 ± 0.104	−7.969 ± 0.066	−9.294 ± 0.067	−8.033 ± 0.087
9a	> −4.8	> −4.8	> −4.8	> −4.8
9b	> −4.8	> −4.8	> −4.8	> −4.8
10a	> −4.8	> −4.8	> −4.8	> −4.8
10b	> −4.8	> −4.8	> −5.034	> −4.841
11a	> −4.8	> −4.8	> −4.8	> −5.317
11b	> −4.8	> −4.8	> −5.260	> −4.901
11c	> −4.8	> −5.393	> −4.870	−5.289 ± 0.464
12a	> −4.8	> −4.926	> −4.8	> −5.271
12b	> −4.8	> −4.906	−5.505 ± 0.168	−5.419 ± 0.346
12c	> −4.8	> −4.805	−4.868 ± 0.061	> −5.177

[a] Antagonistic properties of the tested compounds on ERα, ERβ, GR, and MR were tested in U2OS stable reporter cell lines. Cells were incubated with compounds at the indicated concentrations together with 5 nM E2, 5 nM E2, 1 nM dexamethasone, and 1 nM aldosterone for ERα, ERβ, GR, and MR, respectively, for 18 h. IC$_{50}$ values were generated by fitting data from the luciferase reporter assay by nonlinear regression function using GraphPad Prism 5.0 software. Data shown are representative of two independent experiments performed in triplicate. Values are reported as the mean ± standard error of the mean (SEM). [b] ICI 182780 was used as a control for ERα and ERβ, RU-486 for GR, and spironolactone for MR.

full receptor activation by E2 but a weak partial agonist for ERβ reaching only 23% of the receptor activation by E2.

Interestingly, **12a** acts as a partial agonist on both ERα and ERβ, but in addition, it also has an antagonistic effect on ERβ. Similar mixed agonistic/antagonistic patterns can be observed in selective estrogen receptor modulators (SERMs), which are frequently used drugs in the therapy of different cancers and disorders. Such a mixed pattern can be explained by the ability of the liganded receptor to recruit to some extent both coactivators and corepressors. In this particular case, more detailed study would be necessary to clarify the mechanism of the receptor activation by this compound.

Although E2 is natural and the most potent activator of ERα and ERβ, it can also affect the activity of the remaining steroid receptors another two steroid receptors with reduced potency and efficacy. Since the introduction of perfluoroalkylated chains to the 17α position of E2 considerably changes its properties, we speculated that these changes might also change its ability to modulate function of the two remaining steroid hormone receptors GR and MR that are not activated by E2. Therefore, we tested the effect of new compounds for the transactivation by GR and MR in the U2OS reporter assay in both agonist (Table 2, Figure 2) and antagonist mode (Table 3, Figure 3). None of the tested compounds were able to activate any of these receptors. On the other hand, a subset of compounds (**10b**, **11b**, **11c**, **12b**, **12c**) showed antagonistic properties on both receptors with various potencies and **9a**, **9b**, and **10a** were completely inactive in the concentration range used in the experiments. Compound **12b** is the most potent antagonist from the tested compounds of both GR and MR with IC$_{50}$ = 3.13 μM (log IC$_{50}$ = −5.51) and IC$_{50}$ = 3.81 μM (log IC$_{50}$ = −5.42), respectively. At the same time, **12b** is the most potent agonist of ERα and exhibits weak mixed agonistic/antagonistic properties on ERβ.

Conclusion

A ruthenium complex catalyzed cross-metathesis between 17α-vinyl- and 17α-allylestradiols with perfluoroalkylpropenes constitutes a convenient synthetic pathway for the

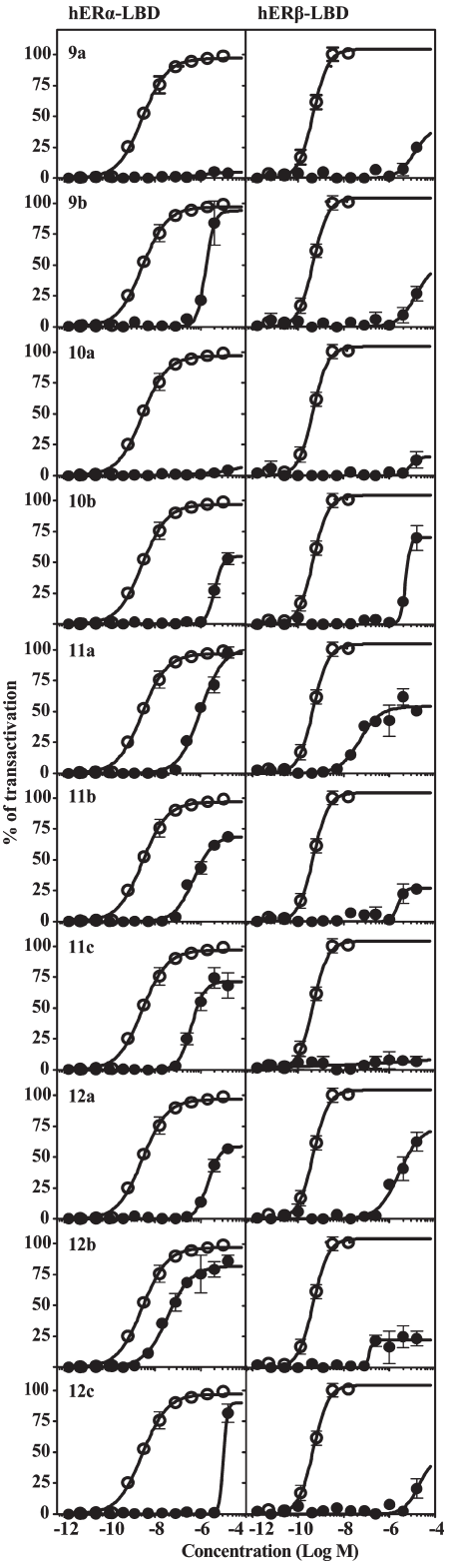


Figure 2. Transactivation by ERα-LBD and ERβ-LBD in response to an increasing concentration of tested compounds (●) and E2 (○). Cells were incubated with compounds at the indicated concentrations for 18 h, and at the end of this time luciferase activity was measured. Maximal stimulation of the receptor by E2 was arbitrarily set to 100%. Data shown are representative of two independent experiments performed in triplicate. Dose response curves were generated by fitting data from the reporter assay by nonlinear regression function using GraphPad Prism 5.0 software. Values are reported as the mean ± standard error of the mean (SEM).

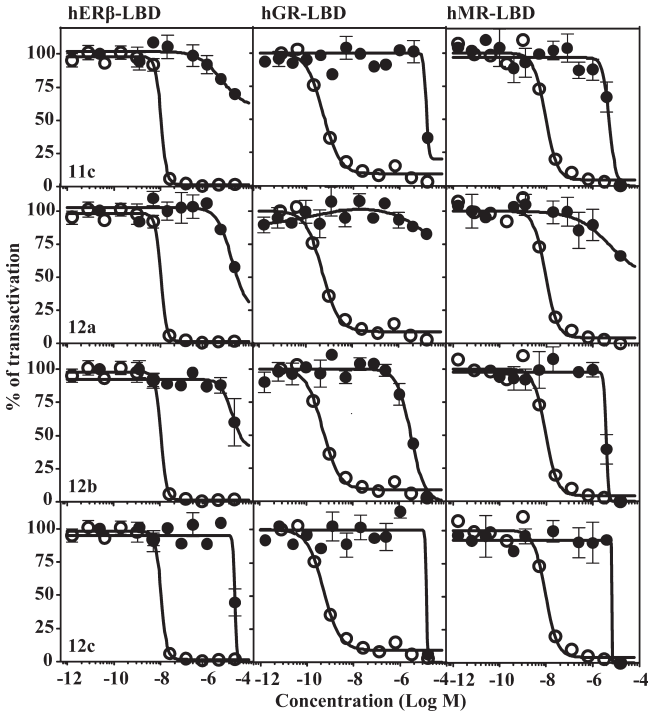


Figure 3. Transactivation by ERβ-LBD, GR-LBD, and MR-LBD in response to an increasing concentration of the selected compounds (●) and control ligand (○) in antagonist mode. Cells were incubated with compounds at the indicated concentrations in the presence of 5 nM E2, 1 nM dexamethasone, and 1 nM aldosterone for ERβ-LBD, GR-LBD, and MR-LBD, respectively, for 18 h, and at the end of this time luciferase activity was measured. Maximal inhibition of the receptor by the control ligand was arbitrarily set to 0%. Data shown are representative of two independent experiments performed in triplicate. Dose response curves were generated by fitting data from the reporter assay by nonlinear regression function using GraphPad Prism 5.0 software. Values are reported as the mean ± standard error of the mean (SEM).

synthesis of 17α-substituted estradiol derivatives bearing perfluoroalkylated side chains in good isolated yields. We subjected compounds to biochemical testing in ERα, ERβ, GR, and MR reporter cell-based assays and measured the ability to induce transactivation or transrepression by these receptors. Some of the derivatives showed activity on ERα and ERβ, and in addition, some compounds were selective for ERα (**11b**, **11c**, **12b**). The most ERα selective compounds were **11c** and **12b** that not only strongly activate ERα but also inhibit ERβ. A few other compounds showing similar properties were described recently; *R*,*R*-THC (*R*,*R*-tetrahydrochrysene)[31] is one well-known example, but in contrast to **11c** and **12b**, *R*,*R*-THC is a partial agonist on ERα and a full antagonist on ERβ. Compounds with similar properties are very useful research tools because they allow for separating biological effects mediated by each of the ERs, given that the natural ligand E2 is unselective.

Furthermore, **12a** and **12b** have unique properties. **12b** has the highest potency from all tested compounds for ERα, activates ERα with almost full efficacy, and at the same time and together with **12a** shows mixed partial agonistic/antagonistic properties on ERβ, which is often observed in SERMs like raloxifene. Interestingly, unlike **12a** and **12b**, classical SERMs only act as both agonists and antagonists on ERα while they are mostly full antagonists on ERβ (data not shown). The mixed agonist/antagonist profile of classical SERMs is often attributed to the involvement of AF-1 in the recruitment of coactivators or corepressors as a result of ligand-induced change in the conformation of ERα. From this perspective, further study of **12b**, **11c**, and related derivatives can bring new light to our understanding of how the specific ligand-induced conformational changes of ERs translate into the transcription of target genes.

Experimental Section

General Procedure for Cross-Metathesis of Terminal Alkenes with (Perfluoroalkyl)propenes. To a mixture of a terminal alkene **4**−**7** (1 equiv) and (perfluoroalkyl)propene **1a**−**c** (2 equiv) in CH_2Cl_2 was added Hoveyda−Grubbs second generation catalyst (10 mol %) under an argon atmosphere. The resulting solution was stirred at 42 °C for 4 h. Removal of the solvent in vacuo gave brown oils, which were purified by flash chromatography.[26]

2′-(*E*)-[17-(5′,5′,6′,6′,7′,7′,7′-Heptafluorohept-2′-en-1′-yl)estra-3,17β-diol] (11b). The reaction was carried out with **6** (130 mg, 0.42 mmol) and (perfluoropropyl)propene **8b** (176 mg, 0.84 mmol) according to the general procedure. Column chromatography on silica gel (1/1 hexane/Et_2O) and on fluorinated silica gel (first elution of 7/3 MeOH/water for washing of the nonfluorinated starting material, second elution of Et_2O for washing of the product) afforded 122 mg (58%) of the title compound **11b** as a white foam: $[\alpha]_D$ +29.7 (*c* 0.20, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.92 (s, 3H, 3 × H-18), 1.88 (m, 1H, H-7b), 1.99 (m, 1H, H-16a), 2.14 (m, 1H, H-9), 2.27 (dd, J_{gem} = 14.1 Hz, $J_{1'a,2'}$ = 8.1 Hz, 1H, H-1′a), 2.32 (m, 1H, H-11b), 2.41 (dd, J_{gem} = 14.2 Hz, $J_{1'b,2'}$ = 6.4 Hz, 1H, H-1′b), 2.83 (m, 2H, 2 × H-6), 2.86 (td, $J_{4',F}$ = 18.4 Hz, $J_{4',3'}$ = 7.0 Hz, 2H, 2 × H-4′), 4.94 (bs, 1H, 3-OH), 5.55 (dm, $J_{3',2'}$ = 15.4 Hz, 1H, H-3′), 5.94 (dm, $J_{2',3'}$ = 15.4 Hz, 1H, H-2′), 6.56 (d, $J_{4,2}$ = 2.7 Hz, 1H, H-4), 6.63 (dd, $J_{2,1}$ = 8.4 Hz, $J_{2,4}$ = 2.8 Hz, 1H, H-2), 7.15 (d, $J_{1,2}$ = 8.6 Hz, 1H, H-1); ^{13}C NMR (150.9 MHz, $CDCl_3$) δ 14.28 (CH_3-18), 23.39 (CH_2-15), 26.25 (CH_2-11), 27.40 (CH_2-7), 29.60 (CH_2-6), 31.68 (CH_2-12), 34.71 (t, $J_{4',F}$ = 22.6 Hz, CH_2-4′), 34.80 (CH_2-16), 39.56 (CH-8), 40.41 (CH_2-1′), 43.76 (CH-9), 46.55 (C-13), 49.59 (CH-14), 82.94 (C-17), 112.67 (CH-2), 115.24 (CH-4), 120.37 (t, $J_{3',F}$ = 4.2 Hz, CH-3′), 126.45 (CH-1), 132.52 (C-10), 135.19 (CH-2′), 138.23 (C-5), 153.40 (C-3); IR ($CHCl_3$) ν 3598, 3388, 1610, 1585, 1499, 1380, 1353, 1228, 975 cm^{-1}; MS (EI, *m*/*z* (rel %)) 494 (M^+, 86), 476 (5), 312 (10), 294 (11), 271 (94), 213 (53), 159 (67); HR-MS (EI) calcd for $C_{25}H_{29}O_2F_7$ [M^+] 494.2056, found 494.2048. R_f(1/1 hexane/Et_2O) = 0.26.

2′-(*E*)-[17-(5′-(Trifluoromethyl)-5′,6′,6′,6′-tetrafluorohex-2′-en-1′-yl)estra-3,17β-diol] (11c). The reaction was carried out with **6** (150 mg, 0.48 mmol) and (perfluoroisopropyl)propene **8c** (210 mg, 1.00 mmol) according to the general procedure. Column chromatography on silica gel (1/1 hexane/Et_2O) and on fluorinated silica gel (first elution of 7/3 MeOH/water for washing of the nonfluorinated starting material, second elution of Et_2O for washing of the product) and crystallization (4/1 hexane/CH_2Cl_2) afforded 158 mg (67%) of the title compound **11c** as white crystals: mp 180−181 °C; $[\alpha]_D$ +32.2 (*c* 0.17, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.92 (s, 3H, 3 × H-18), 1.88 (m, 1H, H-7b), 1.97 (m, 1H, H-16a), 2.14 (m, 1H, H-9), 2.25 (dd, J_{gem} = 14.2 Hz, $J_{1'b,2'}$ = 8.0 Hz, 1H, H-1′b), 2.31 (m, 1H, H-11a), 2.39 (dd, J_{gem} = 14.3 Hz, $J_{1'a,2'}$ = 6.2 Hz, 1H, H-1′a), 2.83 (m, 2H, 2 × H-6), 2.88 (dd, $J_{4',F}$ = 20.0 Hz, $J_{4',3'}$ = 7.0 Hz, 2H, 2 × H-4′), 4.80 (bs, 1H, 3-OH), 5.54 (dm, 1H, $J_{3',2'}$ = 15.1 Hz, H-3′), 5.92 (dm, $J_{2',3'}$ = 15.3 Hz, 1H, H-2′), 6.56 (d, $J_{4,2}$ = 2.8 Hz, 1H, H-4), 6.63 (dd, $J_{2,1}$ = 8.4 Hz, $J_{2,4}$ = 2.8 Hz, 1H, H-2), 7.15 (d, $J_{1,2}$ = 8.4 Hz, 1H, H-1); ^{13}C NMR (150.9 MHz, $CDCl_3$) δ 14.26 (CH_3-18), 23.38 (CH_2-15), 26.25 (CH_2-11), 27.40 (CH_2-7), 29.61 (CH_2-6), 31.67 (CH_2-12), 32.68 (d, $J_{4',F}$ = 20.9 Hz, CH_2-4′), 34.77 (CH_2-16), 39.55 (CH-8), 40.32 (CH_2-1′), 43.76 (CH-9), 46.53 (C-13), 49.59 (CH-14), 82.93 (C-17), 112.65 (CH-2), 115.23 (CH-4), 121.39 (d, $J_{3',F}$ = 5.8 Hz, CH-3′), 126.47 (CH-1), 132.56 (C-10), 134.56 (CH-2′), 138.25 (CH-5), 153.36 (C-3); IR ($CHCl_3$) ν 3598, 3370, 1611, 1585, 1500, 1380, 1353, 1163, 979 cm^{-1}; MS (EI, *m*/*z* (rel %)) 494 (M^+, 89), 476 (7), 271 (100),

253 (61), 228 (24), 213 (82); HR-MS (EI) calcd for $C_{25}H_{29}O_2F_7$ [M^+] 494.2056, found 494.2062. R_f(1/1 hexane/Et_2O) = 0.26.

1′-(*E*)-[17-(5′,5′,6′,6′,7′,7′,8′,8′,9′,9′,10′,10′,10′-Tridecafluorononen-1′-en-1′-yl)estra-3,17*β*-diol] (12a). The reaction was carried out with **7** (100 mg, 0.34 mmol) and (perfluorohexyl)propene **8a** (242 mg, 0.68 mmol) according to the general procedure. Column chromatography on silica gel (1/1 hexane/Et_2O) and on fluorinated silica gel (first elution of 7/3 MeOH/water for washing of the nonfluorinated starting material, second elution of Et_2O for washing of the product) and crystallization (4/1 hexane/CH_2Cl_2) afforded 39 mg (19%) of the title compound **12a** as white crystals: mp 167−168 °C; $[\alpha]_D$ +32.1 (*c* 0.08, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.94 (s, 3H, 3 × H-18), 1.74 (m, 1H, H-15a), 1.89 (m, 2H, H-7a and H-16b), 2.01 (m, 1H, H-16a), 2.11 (m, 1H, H-9), 2.27 (m, 1H, H-11a), 2.83 (m, 2H, 2 × H-6), 2.91 (dt, $J_{3',F}$ = 18.2 Hz, $J_{3',2'}$ = 7.2 Hz, 2H, 2 × H-3′), 4.58 (s, 1H, 3-OH), 5.62 (dt, $J_{2',1'}$ = 15.6 Hz, $J_{2',3'}$ = 7.1 Hz, 1H, H-2′), 6.01 (bd, $J_{1',2'}$ = 15.7 Hz, 1H, H-1′), 6.56 (bd, $J_{4,2}$ = 2.8 Hz, 1H, H-4), 6.62 (dd, $J_{2,1}$ = 8.5 Hz, $J_{2,4}$ = 2.8 Hz, 1H, H-2), 7.13 (d, $J_{1,2}$ = 8.5 Hz, 1H, H-1); ^{13}C NMR (150.9 MHz, $CDCl_3$) δ 14.01 (CH_3-18), 23.22 (CH_2-15), 26.25 (CH_2-11), 27.35 (CH_2-7), 29.58 (CH_2-6), 32.20 (CH_2-12), 34.67 (t, $J_{3',F}$ = 23.1 Hz, CH_2-3′), 36.86 (CH_2-16), 39.40 (CH-8), 43.68 (CH-9), 46.92 (C-13), 49.15 (CH-14), 83.89 (C-17), 112.65 (CH-2), 114.54 (t, $J_{2',F}$ = 4.0 Hz, CH-2′), 115.21 (CH-4), 126.50 (CH-1), 132.60 (C-10), 138.22 (C-5), 143.32 (CH-1′), 153.29 (C-3); IR ($CHCl_3$) ν 3600, 1611, 1585, 1499, 1381, 1357, 1243, 979 cm^{-1}; MS (EI, *m*/*z* (rel %)) 630 (M^+, 38), 612 (33), 597 (17), 437 (9), 387 (10), 213 (100); HR-MS (EI) calcd for $C_{27}H_{27}O_2F_{13}$ [M^+] 630.1803, found 630.1800. R_f(1/1 hexane/Et_2O) = 0.26.

1′-(*E*)-[17-(4′,4′,5′,5′,6′,6′,6′-Heptafluorohex-1′-en-1′-yl)estra-3,17*β*-diol] (12b). The reaction was carried out with **7** (150 mg, 0.50 mmol) and (perfluoropropyl)propene **8b** (210 mg, 1.00 mmol) according to the general procedure. Column chromatography on silica gel (1/1 hexane/Et_2O) and on fluorinated silica gel (first elution of 7/3 MeOH/water for washing of the nonfluorinated starting material, second elution of Et_2O for washing of the product) and crystallization (4/1 hexane/CH_2Cl_2) afforded 61 mg (25%) of the title compound **12b** as white crystals: mp 185−186 °C; $[\alpha]_D$ +26.3 (*c* 0.17, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.94 (s, 3H, 3 × H-18), 1.74 (m, 1H, H-15a), 1.89 (m, 2H, H-7a and H-16b), 2.01 (m, 1H, H-16a), 2.10 (m, 1H, H-9), 2.27 (m, 1H, H-11a), 2.81 (m, 2H, 2 × H-6), 2.89 (td, $J_{3',F}$ = 17.6 Hz, $J_{3',2'}$ = 7.1 Hz, 2H, 2 × H-3′), 4.70 (bs, 1H, 3-OH), 5.62 (dt, $J_{2',1'}$ = 15.5 Hz, $J_{2',3'}$ = 7.2 Hz, 1H, H-2′), 6.00 (bd, $J_{1',2'}$ = 15.6 Hz, 1H, H-1′), 6.56 (d, $J_{4,2}$ = 2.8 Hz, 1H, H-4), 6.62 (dd, $J_{2,1}$ = 8.4 Hz, $J_{2,4}$ = 2.8 Hz, 1H, H-2), 7.13 (bd, $J_{1,2}$ = 8.5 Hz, 1H, H-1); ^{13}C NMR (150.9 MHz, $CDCl_3$) δ 14.01 (CH_3-18), 23.21 (CH_2-15), 26.23 (CH_2-11), 27.35 (CH_2-7), 29.58 (CH_2-6), 32.17 (CH_2-12), 34.40 (t, $J_{3',F}$ = 22.3 Hz, CH_2-3′), 36.79 (CH_2-16), 39.38 (CH-8), 43.68 (CH-9), 46.90 (C-13), 49.10 (CH-14), 83.91 (C-17), 112.65 (CH-2), 114.56 (t, $J_{2',F}$ = 4.4 Hz, CH-2′), 115.22 (CH-4), 126.50 (CH-1), 132.56 (C-10), 138.20 (C-5), 143.26 (CH-1′), 153.31 (C-3); IR ($CHCl_3$) ν 3599, 3399, 1611, 1585, 1499, 1353, 1232, 979 cm^{-1}; MS (FAB, *m*/*z* (rel %)) 680 (M^+, 65), 462 (9), 265 (17), 228 (38), 213 (100); HR-MS (EI) calcd for $C_{24}H_{27}O_2F_7$ [M^+] 480.1899, found 480.1905. R_f(1/1 hexane/Et_2O) = 0.26.

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Supporting Information Available: Additional synthesis information, compound characterization data, and spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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