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Lead Optimization of 17β-HSD1 Inhibitors of the (Hydroxyphenyl)naphthol Sulfonamide Type for the Treatment of Endometriosis

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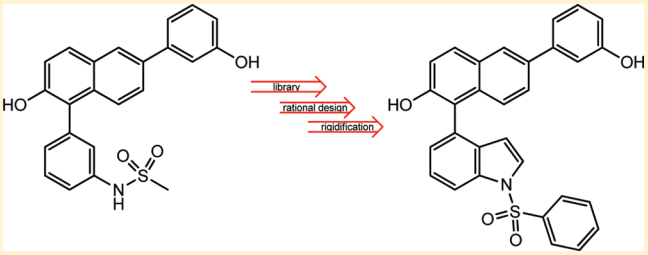
Supporting Information

ABSTRACT: The reduction of estrone to estradiol, the most potent estrogen in human, is catalyzed by 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1). A promising approach for the treatment of estrogen-dependent diseases is the reduction of intracellular estradiol formation by inhibition of 17β-HSD1. For the species-specific optimization of the (hydroxyphenyl)-naphthols, a combinatorial approach was applied and enhanced by a focused synthesis that resulted in the aromatic-substituted (hydroxyphenyl)naphthol sulfonamides. Rigidification of **12** led to the 4-indolylsulfonamide **30**, which is a highly active and selective human 17β-HSD1 inhibitor, as well as a highly potent and selective inhibitor of 17β-HSD1 from *Callithrix jacchus*. It shows no affinity to the estrogen receptors α and β and good intracellular activity (T47D). Thus, compound **30** shows good properties for further ADMET studies and might be a candidate for the in vivo proof of concept in *C. jacchus*.



INTRODUCTION

Estradiol (E2), the most important estrogen in females, is responsible for the development and differentiation of estrogen-sensitive tissues, for example, breast and endometrial tissues. It is mainly produced in the granulosa cells of the ovaries. In a first step, estrone (E1) is formed by aromatization of androstenedione, followed by the local conversion of E1 to E2 by 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1, Figure 1). Besides its physiological effects, E2 is involved in the development and progression of estrogen-dependent diseases (EDDs) like breast cancer,[1,2] ovarian tumor,[3] endometriosis,[4,5] endometrial hyperplasia,[6] and uterine leiomyoma.[7] The conventional treatment for these diseases is therapy with antiestrogens,[8] selective estrogen receptor modulators (SERMs), or aromatase inhibitors[9,10] to block the synthesis of estrogens on the systemic level.

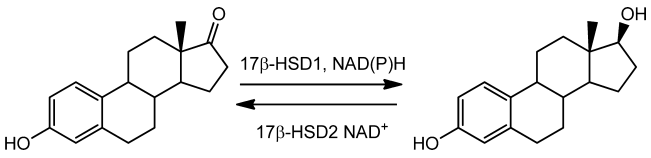


Figure 1. Interconversion of E1 to E2.

Notably, mRNA of 17β-HSD1, which is often used as a prognostic marker, has been found highly expressed in breast cancer[11] and endometriotic tissue.[12] Therefore, the development of 17β-HSD1 inhibitors is an attractive approach to reduce the intracellular E2 levels and to treat EDDs, particularly since such an intracrine concept has already been proven to be successful for the treatment of the androgen-dependent diseases BPH (benign prostatic hyperplasia) and alopecia by using 5α-reductase inhibitors.[13,14]

To decrease undesirable pharmacological effects, the 17β-HSD1 inhibitors should be selective toward 17β-hydroxysteroid dehydrogenase type 2 (17β-HSD2), which catalyzes the deactivation of E2 to E1 (Figure 1) and is downregulated in malignant breast cancer cells. Specific inhibition of 17β-HSD2 was recently published as a novel approach to prevent osteoporosis.[15−19] Furthermore, potential inhibitors should not show any affinity to estrogen receptors α and β (ERα and β) to reduce the risk of estrogenic side effects.

Some 17β-HSD1 inhibitors are already described. Most of them are based on a steroidal skeleton.[20−22] In the last couple of years, selective nonsteroidal inhibitors were published by others[23−26] and our group. Our work is based on a long time experience in steroid mimetics, which led to the development

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of selective inhibitors of CYP19,[27,28] CYP17,[29−31] CYP11B2,[32−34] and CYP11B1[35,36] and resulted in three classes of nonsteroidal 17β-HSD1 inhibitors: the bis(hydroxyphenyl)-heterocycles,[37−42] the hydroxyphenylmethanones,[43−45] and the (hydroxyphenyl)naphthols.[46−48] Recently, we reported on the optimization of the latter class that led to the identification of a highly active and selective sulfonamide substituted derivative **A** with no affinity to ERα and ERβ (Figure 2).[49]

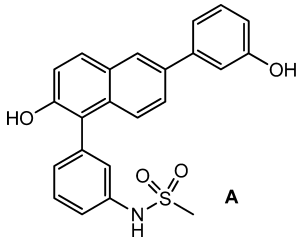
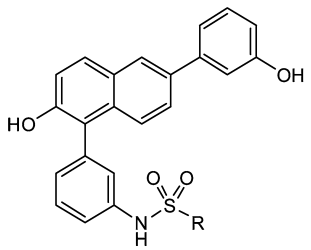


Figure 2. Recently published inhibitor.

As this compound showed good intracellular activity and favorable pharmacokinetics, it should be the starting point for the establishment of an in vivo proof of concept in a disease-oriented animal model. Several animal models for breast cancer and endometriosis using different species are available.[50−54] For an in vivo experiment with rodents, there are either rodent enzyme active inhibitors necessary, or experiments with xenograft models or transgenic mice have to be performed. Previous studies had revealed that representative inhibitors of the aforementioned classes only show low inhibition of E2 formation in the rat and in the mouse.[42,43,45] Therefore, we focus on a species in which 17β-HSD1 shows high sequence identity to the human enzyme to increase the probability to find compounds for in vivo experiments. Because of the high phylogenetic similarity of monkeys and humans, models of the former species are preferable. An appropriate animal should be *Callithrix jacchus* (common marmoset), as there is an endometriosis model described.[54] The high overall sequence identity of 80% between human and *C. jacchus* 17β-HSD1 (*cj*17β-HSD1) increases up to 87%, if only the steroidal binding site is considered, with five major amino acid variations observed in common marmoset: A191P, E194Q, S222N, V225I, and E282N.[55] This high identity makes the animal model very appropriate for in vivo evaluation of designed inhibitors. Two different forms of endometriosis can be induced: developing and established endometriosis. Furthermore, the pathogenesis of endometriosis and the relationship between local and central estrogen metabolism have been studied, which is important for validation of the intracrine concept of 17β-HSD1 inhibitors.

After having established an assay to screen our in-house library for compounds showing good inhibition of *cj*17β-HSD1, the lead compound in the class of the sulfonamide substituted hydroxyphenylnaphthols (Figure 2), however, showed only very low inhibitory potency (16% inhibition at 50 nM) and no selectivity toward *C. jacchus* 17β-hydroxysteroid dehydrogenase type 2 (*cj*17β-HSD2) (23% inhibition at 50nM).[55] Therefore, it is our aim to increase the potency toward *cj*17β-HSD1 without decreasing the activity toward human 17β-hydroxysteroid dehydrogenase type 1 (*h*17β-HSD1) in this class of compounds to identify a potential candidate for the in vivo proof of concept in the common marmoset.

In this study, we report on the optimization of the hydroxyphenylnaphthols at the sulfonamide core. In a previous study, it was found that the exchange of aliphatic sulfonamides by aromatic sulfonamides led to an increase in activity toward



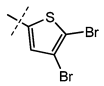
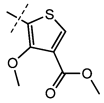
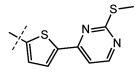
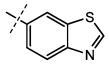
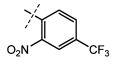
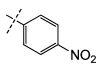
compd	R	compd	R	compd	R	compd	R
1		**2**		**3**		**4**	
5		**6**		**7**		**8**	
9		**10**		**11**		**14**	
15		**17**					

Figure 3. Library compounds.

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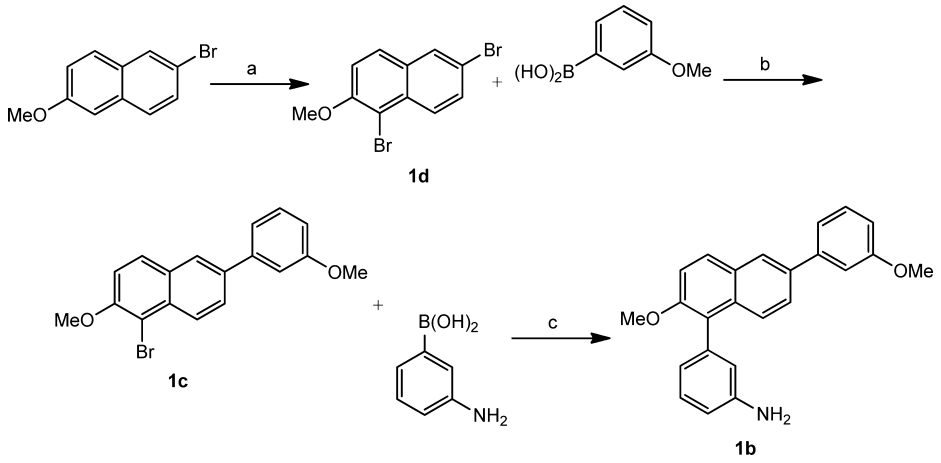
Scheme 1. Synthesis of Compound 1b[a]



[a]Reagents and conditions: (a) NBS, reflux, 2 h. (b) Na_2CO_3, $Pd(PhP_3)_4$, toluene/H_2O/MeOH (3:2:2). (c) Cs_2CO_3, $Pd(PhP_3)_4$, dimethoxyethane/EtOH/H_2O (1:1:1), 15 min microwave irradiation (150 °C, 1.5 bar).

*h*17β-HSD1.[45] Therefore, we designed and synthesized a library (parallel synthesis) of aromatic-substituted sulfonamides (Figure 3), and the compounds were evaluated for inhibition of *h*17β-HSD1 in a cell-free assay. The most promising compounds were resynthesized in a larger amount and were further evaluated biologically. To optimize and to derive a meaningful SAR (structure−activity relationship), substituents in the phenyl moieties were varied regarding their electronic and lipophilic properties (Table 1).

CHEMISTRY

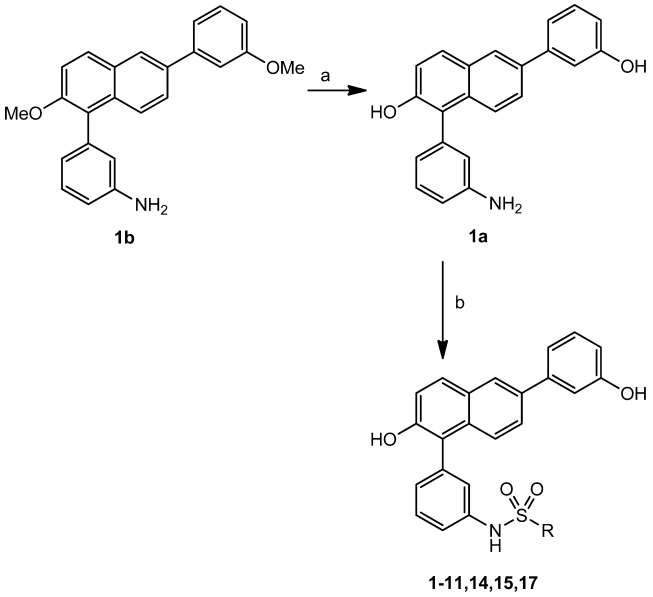
The available 2-bromo-6-methoxynaphthalene was brominated with *N*-bromosuccinimide followed by Suzuki cross-coupling with 3-(methoxyphenyl) boronic acid, sodium carbonate, and $Pd(PhP_3)_4$ in toluene/H_2O/MeOH (3:2:2) to obtain compound **1c**. In a second Suzuki cross-coupling with 3-(aminophenyl) boronic acid, cesium carbonate, and $Pd(Ph_3P)_4$ in dimethoxyethane/EtOH/H_2O (1:1:1), compound **1b** was prepared (Scheme 1).

The library synthesis was started using 3-(2-methoxy-6-(3-methoxyphenyl)naphthalen-1-yl)aniline (**1b**), and an ether cleavage according to method C was performed yielding the demethylated compound **1a**. The diversity was introduced with different commercially available sulfonyl chlorides (**1**−**11**, **14**, **15**, **17**, method A, Scheme 2).

The important intermediate **12b** was synthesized in four steps. After bromination of β-naphthol in glacial acetic acid, the β-hydroxy function was protected with benzylbromide (**12d**). Via two successive Suzuki cross-couplings with 3-(benzoxyphenyl)- and 3-(aminophenyl) boronic acid **12b** was obtained (Scheme 3).

The synthesis of the protected sulfonamides **8a**−**29a** and the deprotection yielding **8**−**29** are shown in Scheme 4. The sulfonamide coupling with compound **1b** or compound **12b** and the commercially available sulfonyl chlorides was performed according to method B (DMAP, pyridine, rt, 6 days). In a last step, compounds **8a**, **10a**, **11a**, **15a**, **17a**, and **18a** were demethylated with boron tribromide (method C; BBr_3, CH_2Cl_2, −78 °C to rt, 18 h, Scheme 4). Compound **9a**, **12a**−**14a**, **16a**, and **19a**−**29a** were debenzylated using the same reagent according to method D (BBr_3, CH_2Cl_2, −25 °C for 1 h, rt for 2 h, Scheme 4).

Scheme 2. Synthesis of Library Compounds 1−11, 14, 15, and 17[a]



[a]Reagents and conditions: (a) BBr_3, CH_2Cl_2, −78 to rt, 18 h, method C. (b) PS-morpholine, PS-DMAP, THF, 48 h, rt, method A.

For the synthesis of the rigidified sulfonamides **30** and **31**, the 4-indolylphenylsulfonamide and the 6-indolylphenylsulfonamide residues were introduced to compound **12c** by a Suzuki coupling with the corresponding indoyl boronic acids. A tetrabutyl ammonium chloride (TBAC)-catalyzed sulfonamide coupling resulted in compounds **30a** and **31a**. Cleavage of the benzyloxy moieties with boron tribromide (method D) afforded **30** and **31** (Scheme 5).

BIOLOGICAL RESULTS

Inhibition of 17β-HSD1 and Selectivity toward 17β-HSD2 in Human and *C. jacchus*. The assays were performed with placental enzymes from human and *C. jacchus* (*cj*). For the 17β-HSD1 assay, the cytosolic fraction, tritiated E1, cofactor, and inhibitor were used. The 17β-HSD2 assay was performed similarly using the microsomal fraction and tritiated E2.

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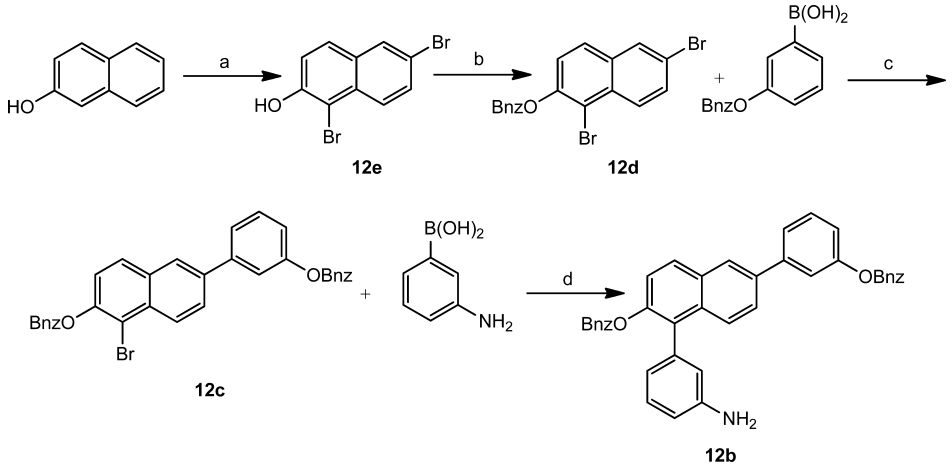
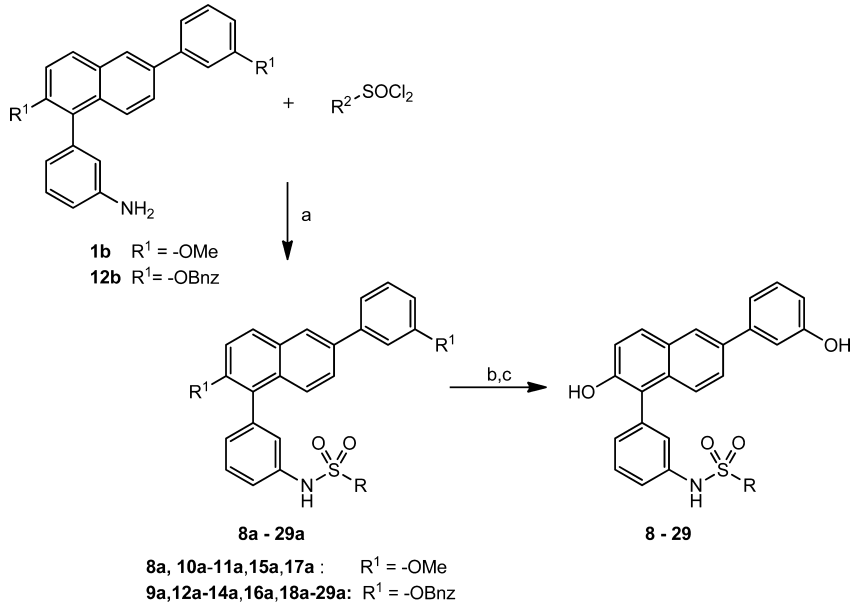
Scheme 3. Synthesis of Compound 12b[a]



[a]Reagents and conditions: (a) Br_2, acetic acid, reflux, 3 h. (b) Benzylbromide, K_2CO_3, EtOH, reflux, 15 h. (c) Cs_2CO_3, $Pd(Ph_3P)_4$, THF/H_2O (1:1), reflux, 4 h. (d) Cs_2CO_3, $Pd(Ph_3P)_4$, $DME/EtOH/H_2O$ (1:1:1), 15 min microwave irradiation (150 °C, 1.5 bar).

Scheme 4. Synthesis of Compound 8−29[a]



[a]Reagents and conditions: (a) DMAP, pyridine, rt, 6 days, method B. (b) BBr_3, CH_2Cl_2, −78 °C to rt, 18 h, method C. (c) BBr_3, CH_2Cl_2, −25 °C for 1 h, rt for 2 h, method D.

In a first step, a synthetic library was designed to introduce aromaticity into the sulfonamide core (Figure 3). Biological evaluation of these compounds revealed that several are highly potent *h*17*β*-HSD1 inhibitiors. Compounds that exhibited more than 30% inhibition of *h*17*β*-HSD1 at 10 nM were resynthesized, and IC_{50} values and selectivity factors (SFs) toward human 17*β*-hydroxysteroid dehydrogenase type 2 (*h*17*β*-HSD2) as well as percentage inhibition values against *cj*17*β*-HSD1 and 17*β*-HSD2 were determined (**8**−**11**, **14**, **15**, and **17**). Furthermore, to elaborate a meaningful SAR, substituents differing in their electronic and lipophilic properties were introduced in the aromatic moiety, and the corresponding compounds were also tested against the human and *cj* enzymes (**12**, **13**, **16**, and **18**−**31**; Table 1)

All compounds showed IC_{50} values toward *h*17*β*-HSD1 in the low nanomolar range (between 12 and 92 nM) as well as selectivity toward *h*17*β*-HSD2 (SF between 2 and 71). The only exception is the dinitro-substituted sulfonamide **16** with 30-fold reduced activity toward *h*17*β*-HSD1. Interestingly, **16** turned out to be a moderate *h*17*β*-HSD2 inhibitor. By introduction of *meta*- and *para*-substituents into the phenyl substituent of **12**, selectivity toward *h*17*β*-HSD2 could be further improved up to a SF of 71 for compound **23**.

To explore whether rigidification leads to an improvement of affinity toward the target, as it has been shown in previous studies,[56] and to get more insights into the binding site, two rigidified conformers were synthesized. The rigidification of **12** resulted in two compounds with different conformations in the sulfonamide moiety, the indoles **30** and **31**. Interestingly, one conformation remained highly active toward *h*17*β*-HSD1 (**30**), while the other conformer lost activity (**31**).

The activity toward *cj*17*β*-HSD1 and also the selectivity toward *cj*17*β*-HSD2 could be slightly optimized for all compounds. Remarkably, the rigidified **12** (compound **30**)

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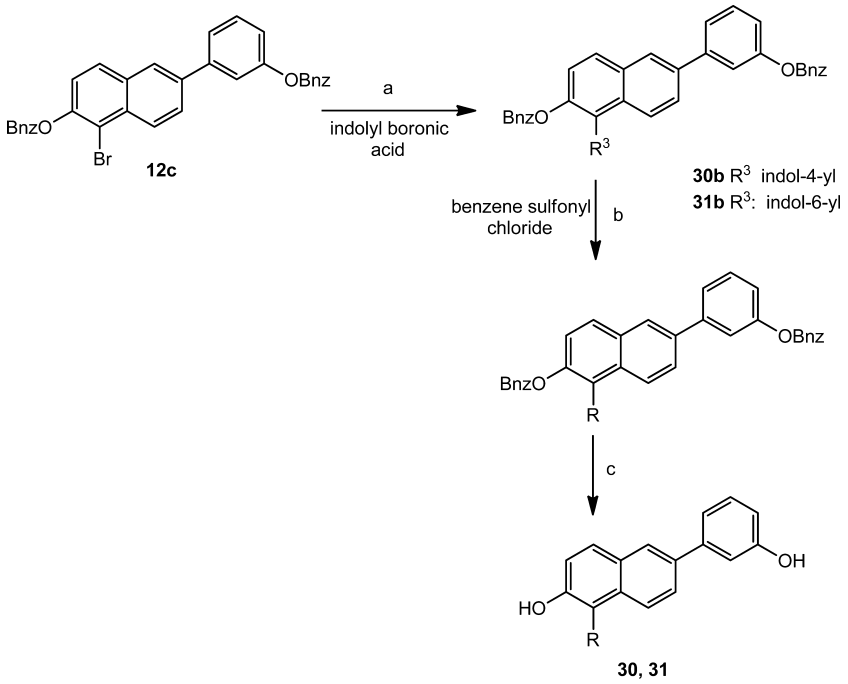
Scheme 5. Synthesis of Compounds 30 and 31[a]



[a]Reagents and conditions: (a) Cs_2CO_3, $Pd(Ph_3P)_4$, DME/EtOH/H_2O (1:1:1), 15 min microwave irradiation (150 °C, 1.5 bar). (b) DCM, TBAC, NaOH, 3 h, 10 °C. (c) BBr_3, CH_2Cl_2, −25 °C for 1 h, rt for 2 h, method D.

turned out to be a highly active and selective inhibitor of *cj*17β-HSD1 (79% inhibiton of *cj*17β-HSD1 and 43% inhibition of *cj*17β-HSD2 at 50 nM).

A selection of the compounds (reference **A**, **12**, **17**, **23**, and **29**) was also tested using the cytosolic and microsomal fraction of mouse liver homogenate, which are described to be responsible for the E1 and E2 conversion, respectively,[57] to examine whether the aromatic sulfonamides show activity in this species. In the cytosolic fraction, E1 conversion was observed, but the compounds did not show inhibitory activity. In the microsomal fraction, E2 oxidation was monitored, and the compounds exhibited moderate inhibiton of E1 formation (Supporting Information, Table 2).

Selectivity toward ERs α and β. Because of the similarity of the compounds to the estrogen scaffold, it cannot be excluded that the phenyl naphthalene sulfonamides show affinity toward the ERs α and β. Agonistic as well as antagonistic effects are not compatible with the therapeutic concept for 17β-HSD1 inhibitors. Agonistic properties would stimulate cell proliferation and thus counteract the effect of these inhibitors. Antagonistic qualities are likely to lead to unwanted pharmacological effects and could reduce the advantages of the new therapeutic intracrine concept as compared to antiestrogens or SERMs. The compounds were tested in an assay using the recombinant human ERs in competition with [^{3}H]-E2. The affinity of E2 was set to 100%. A relative binding affinity (RBA) of 0.1% means that the compound shows a 1000-fold weaker affinity to the receptor than E2 and is considered as satisfactory. All compounds, including reference **A**, showed RBA values lower than 0.1% toward ERα and ERβ and were classified as very low affinity ligands (Supporting Information, Table 3).

Further Biological Evalution. To get insights into cellular activity, the mammary tumor T47D cell line, which expresses *h*17β-HSD1 and *h*17β-HSD2, was used to determine IC_{50} values for selected compounds (Table 2). All tested compounds showed IC_{50} values around or below 1000 nM. Compound **17** exhibiting an IC_{50} value of 157 nM turned out to be the most active one of this series.

To further characterize the inhibitors regarding their biophysical properties, tPSA values were calculated using the commercial ChemDrawUltra 12.0 program and are shown in Table 3. Except for **16** (190.25 Å, Table 3), all compounds showed tPSA values that are in an adequate range for oral bioavailabity[58] (Supporting Information, Table 4).

DISCUSSION AND CONCLUSION

The identification of a highly active *h*17β-HSD1 inhibitor (**A**) with properties suitable for further preclinical studies provided the starting point for the optimization concerning activitiy toward *cj*17β-HSD1. Starting with a parallel synthesis approach, several highly potent *h*17β-HSD1 inhibitors were discovered. With the 4-indolyl-substituted hydroxyphenyl naphthalene sulfonamide **30**, a highly potent *h*- and *cj*17β-HSD1 inhibitor was identified, which might be an appropriate candidate for in vivo proof of concept.

The introduction of aromatic moieties differing in their electronic and lipophilic effects into the sulfonamide core is well tolerated by *h*17βHSD1 but did not resulted in higher activities. This finding cannot exclude additional beneficial interactions in the binding site initiated by the introduced substituents. However, they are obviously compensated by adverse effects, like losing a hydrogen bond from one of the two essential hydroxy groups[46] or by stripping the hydrate sheath for diffusion into the binding pocket

The strongly reduced activity of compound **16** could be explained by the high electron-withdrawing effects of the two bulky nitro groups, disrupting potential $\pi-\pi$ interactions with the protein. A crystal structure will be necessary to shed light on the real binding mode of the synthesized compounds.

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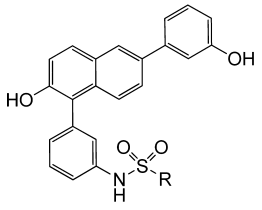
Table 1. IC_{50} Values toward *h*17*β*-HSD1, Selectivity Factor toward *h*17*β*-HSD2, and Inhibition of *cj*17*β*-HSD1 and *cj*17*β*-HSD2 for Compounds 8−31



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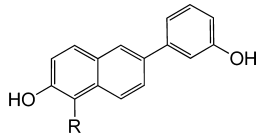
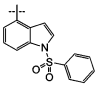
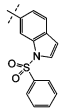


30-31

compd	R	IC_{50} (nM)[a]		SF[d]	Inhibition at 50 nM (%)[a]	
		*h*17βHSD1[b]	*h*17βHSD2[c]		*cj*17β-HSD1[e]	*cj*17β-HSD2[f]
A[g]	-CH_3	15	403	27	16	23
8		46	223	5	14	17
9		74	213	3	n.i.	n.i.
10		92	535	6	38	13
11		28	1104	39	25	18
12		15	403	27	19	n.i.
13		17	238	14	n.i.	21
14		20	374	19	16	17
15		49	1058	22	28	13
16		690	371	0.5	16	36
17		14	439	31	43	35
18		30	607	20	11	n.i.
19		25	825	33	25	30
20		29	749	26	19	n.i.
21		22	396	18	n.i.	33
22		53	834	16	24	20
23		14	999	71	20	n.i.
24		19	352	19	n.i.	30
25		31	188	6	20	n.i.
26		33	446	14	n.i.	11
27		35	367	11	n.i.	11
28		12	408	34	31	n.i.
29	-CH_2CF_3	32	1211	38	17	32
30		32	391	12	79	43
31		519	996	2	14	12

[a]Mean values of three determinations, standard deviation less than 18%. [b]Human placenta, cytosolic fraction, substrate [^{3}H]-E1 + E1 (500 nM), cofactor NADH (500 μM). [c]Human placenta, microsomal fraction, substrate [^{3}H]-E2 + E2 (500 nM), cofactor NAD^+ (1500 μM). [d]IC_{50} (17*β*-HSD2)/IC_{50} (17*β*-HSD1). [e]*C. jacchus* placenta, cytosolic fraction, substrate [^{3}H]-E1 + E1 (500 nM), cofactor NADH (500 μM). [f]*C. jacchus* placenta, microsomal fraction, substrate [^{3}H]-E2 + E2 (500 nM), cofactor NAD^+ (1500 μM). [h]n.i., no inhibition, i.e., inhibition ≤10%. [g]Aliphatic sulfonamide inhibitor **A**[49] is used as a reference.

Rigidification is a common strategy in drug design to minimize entropic costs of binding and thus optimize the interaction. In this study, rigidification of the sulfonamide core led to the discovery of the highly active *h*- and *cj*17*β*-HSD1 inhibitor **30**. The structure of this compound obviously is very close to the biologically active conformation of **12**. Contrarily, for the other conformation, rigidified in compound **31**, a significantly reduced inhibition of *h*17*β*-HSD1 was found. This conformation is obviously not able to form sufficiently strong interactions in the active site of *h*17*β*-HSD1.

Interestingly, the rigidification of **12** leading to **30** has a strong influence on inhibitory activity for the common marmoset enzyme as compared to the human. The discovery of compound **30** as the biologically active conformation of this class of inhibitors is of great importance and can be used for further structural optimization.

Through the introduction of aromatic substituents, the activity toward *cj*17*β*-HSD1 could be increased significantly, and also, selectivity toward *cj*17*β*-HSD2 could be achieved for this class of compounds. H-bond acceptors in the *para*-position of the phenyl moiety seem to be beneficial for selectivity in *C. jacchus*. Considering the fact that the expression of 17*β*-HSD2 is downregulated in EDD tissues, it is nevertheless important to obtain selectivity toward this isozyme to avoid systemic enhancement of E2 levels and the side effects associated with it.

The differences in inhibitory activities between *h*- and *cj*17*β*-HSD1 in this compound class could be indications for different binding modes for the two species. However, an in-depth

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Table 2. IC_{50} Values in the T47D Cell Assay for Selected Compounds

cell assay					
compd	IC_{50} (nM)[a] 17βHSD1[b]	compd	IC_{50} (nM)[a] 17βHSD1[b]	compd	IC_{50} (nM)[a] 17βHSD1[b]
A	71	**17**	157	**25**	525
8	646	**18**	402	**26**	440
10	498	**19**	555	**27**	633
11	316	**20**	429	**28**	240
12	196	**21**	462	**29**	246
13	462	**22**	637	**30**	1100
14	396	**23**	701		
15	753	**24**	1009		

[a]Mean values of three determinations, standard deviation less than 25%. [b]T47D cells, substrate [^{3}H]-E1 + E1 (50 nM).

Table 3. Biophysical Characterization of A, 16, and 30

compd	tPSA (Å)[a]	MW (g/mol)	clog P[a]
A	86.63	405	3.91
16	190.25	557	6.08
30	77.84	491	6.59

[a]Calculated values with ChemDrawUltra 12.0.

investigation of the SAR for further elucidation is hampered by a somewhat blurred relationship.

All aromatic sulfonamides showed a decrease in cellular activity in T47D cells, especially the indolyl derivative **30** (IC_{50} = 1100 nM), which could be explained by the higher molecular weight and the increased clog P value as compared to the reference **A** (491 vs 405 g/mol and clog P 6.59 vs 3.91, respectively; Supporting Information, Table 4). Intracelluar metabolism might also play a role for the decreased cellular activity.

Another important issue in drug development is oral bioavailability. Molecular weight and lipophilicity are not sufficient for prediction of oral bioavailability. The polar surface area and the number of free rotatable bonds are more appropriate parameters[59] and should be considered for the development of potential drugs. The polar surface area of 77.84 Å^2 for compound **30** is in an excellent range (86.63 Å^2 for reference **A**), and also, the more rigid structure is promising.

For mimetics of the steroidal scaffold, selectivity toward the ERs is very important. To avoid estrogenic side effects, neither agonistic nor antagonistic activities can be tolerated. With RBA values below 0.1% for ERα and β, all herein reported compounds are not expected to exert an ER-mediated effect.

In this paper, we described a species-specific optimization of hydroxyphenyl sulfonamide naphthols starting with a combinatorial library approach followed by optimization of discovered hits. The goal of the present study was the identification of *cj*17β-HSD1 inhibitors. Thus, it was successful, as we discovered the rigidified sulfonamide **30**, which is highly potent toward *h*17β-HSD1 as well as toward *cj*17β-HSD1 and shows selectivity toward 17β-HSD2 of both species and the ERs. This *h*- and *cj*17β-HSD1 inhibitor seems to be an appropriate candidate for the proof of concept in the endometriosis common marmoset model. Nevertheless, before performing an in vivo experiment, further parameters like metabolic stability and pharmacokinetic properties will be investigated.

EXPERIMENTAL SECTION

Chemistry. Chemical names follow IUPAC nomenclature. Starting materials were purchased from commercial suppliers and were used without further purification. Column flash chromatography was performed on silica gel (70−200 μm), preparative thin-layer chromatography (PTLC) was performed on 1 mm SIL-G-100 UV_{254} glass plates (Macherey-Nagel), and reaction progress was monitored by TLC on TLC Silica Gel 60 F_{254} (Merck). NMR spectra were measured on a Bruker AM500 spectrometer (500 MHz) at 300 K. Chemical shifts are reported in δ (parts per million: ppm), TMS was used as an internal reference, and hydrogenated residues of deuteriated solvent were used as an internal standard [$CDCl_3$: δ = 7.24 ppm (^{1}H NMR) and δ = 77 ppm (^{13}C NMR); CD_3COCD_3: δ = 2.05 ppm (^{1}H NMR) and δ = 29.8 ppm (^{13}C NMR)]. Signals are described as s, d, t, dd, m, dt, and ddd for singlet, doublet, triplet, doublet of doublets, multiplet, doublet of triplets, and doublet of doublet of doublets, respectively. All coupling constants (J) are given in Hertz (Hz). The purity of final products (≥95%) was confirmed by analytical high-performance liquid chromatography (HPLC). HPLC/MS was performed on a MSQ electrospray mass spectrometer (Thermo Fisher). The system was operated by the standard software Xcalibur. A RP C18 NUCLEODUR 100-5 (125 mm × 3 mm) column was used as the stationary phase with water/acetonitrile mixtures as eluents. Mass spectra (ESI) were recorded on a TSQ Quantum (Thermofischer) instrument. Melting points were measured using melting point apparatus SMP3 (Stuart Scientific). The apparatus is uncorrected.

*General Procedure for Sulfonamide Library (**1**−**11**, **14**, **15**, and **17**): Method A.* A solution of sulfonylchloride (1.2 equiv) in 2 mL of THF was added to a solution of 1-(3-aminophenyl)-6-(3-hydroxyphenyl)naphthalene-2-ol **1a** (1.0 equiv) and PS-morpholine in 2 mL of THF (55 mg). After it was stirred for 15 h at rt, a catalytic amount of PS-DMAP was added, and the mixture was stirred another 15 h at rt. Catalytic amounts of PS-Tris(2-aminoethyl)-amine and PS-isocyanate were added to the mixture of compounds **15** and **17**. After filtration and evaporation, the compounds were purified by preparative HPLC (Varian Inertsil C18 50 mm × 21 mm) (H_2O/CH_3CN + 0.1% TFA) (100:0) → (0:100). The remaining compounds were stirred for 15 h at 60 °C and afterward finished with the same procedure as **15** and **17**. After filtration and evaporation, the compounds were purified by combi-flash chromatography (hexane/ethylacetate (2:1 → 1:1).

General Procedure for the Synthesis of the Protected Phenyl-naphthalenesulfonamides: Method B.[60] 3-(2-methoxy-6-(3-methoxyphenyl)naphthalene-1-yl)aniline (**1b**) or 3-(2-benzyloxy)-6-(3-benzyloxy)phenyl)naphthalene-1-yl)aniline (**12b**) (1 equiv) was dissolved in pyridine abs. and was spiked with the accordant sulfonyl chloride (1.5 equiv) and DMAP as a catalyst. The reaction mixture was stirred for 6 days at rt. The reaction was quenched by adding 10 mL of 2 N HCl and extracted with ethyl acetate. The organic layers were washed with saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated under vacuum. The product was purified by flash chromatography to give the title compounds.

General Procedure for Ether Cleavage: Method C.[61,62] A 3-(2-methoxy)-6-(3-methoxy)phenyl)naphthalene-1-yl)sulfonamide derivative (1 equiv) was dissolved in dichloromethane abs. and was cooled to −78 °C (acetone/dry ice). Boron tribromide (1 M, 3.5 equiv per methoxy group) was added dropwise under stirring. The reaction mixture was stirred for 18 h from −78 °C to rt. The reaction was quenched by adding 10 mL of water and extracted with ethyl acetate. The organic layers were washed with saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated under reduced pressure. The product was purified by flash chromatography to give the title compounds.

General Procedure for Ether Cleavage: Method D.[61,62] A 3-(2-benzyloxy)-6-(3-benzyloxy)phenyl)naphthalene-1-yl)sulfonamide derivative (1 equiv) was dissolved in dichloromethane abs. and was cooled to −25 °C. Boron tribromide (1 M, 10 equiv per benzyloxy group) was added dropwise under stirring. The reaction mixture was stirred for 1 h at −25 °C and then for 3 h at rt. The reaction was quenched by adding 10 mL of water and extracted with ethyl acetate. The organic layers were washed with saturated $NaHCO_3$ and brine,

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dried over $MgSO_4$, and evaporated under reduced pressure. The product was purified by flash chromatography to give the title compounds.

*5-Chloro-N-(3-(2-hydroxy-6-(3-hydroxyphenyl)naphthalen-1-yl)-phenyl)thiophene-2-sulfonamide (**1**).* The title compound was prepared by reaction of 1-(3-aminophenyl)-6-(3-hydroxyphenyl)-naphthalen-2-ol (**1a**) and 5-chlorothiophene-2-sulfonyl chloride according to method A; yield, 35% (8.8 mg). MS (ESI): 508 $(M + H)^+$.

*4,5-Dibromo-N-(3-(2-hydroxy-6-(3-hydroxyphenyl)naphthalen-1-yl)phenyl)thiophene-2-sulfonamide (**2**).* The title compound was prepared by reaction of 1-(3-aminophenyl)-6-(3-hydroxyphenyl)-naphthalen-2-ol (**1a**) and 4,5-dibromothiophene-2-sulfonyl chloride according to method A; yield, 21% (6.6 mg). MS (ESI): 629 $(M + H)^+$.

*Methyl 5-(N-(3-(2-Hydroxy-6-(3-hydroxyphenyl)naphthalen-1-yl)phenyl)sulfamoyl)-4-methoxythiophene-3-carboxylate (**3**).* The title compound was prepared by reaction of 1-(3-aminophenyl)-6-(3-hydroxyphenyl)naphthalen-2-ol (**1a**) and methyl 5-(chlorosulfonyl)-4-methoxythiophene-3-carboxylate according to method A; yield, 19% (5.4 mg). MS (ESI): 562 $(M + H)^+$.

*N-(3-(2-Hydroxy-6-(3-hydroxyphenyl)naphthalen-1-yl)phenyl)-5-(2-(methylthio)pyrimidin-4-yl)thiophene-2-sulfonamide (**4**).* The title compound was prepared by reaction of 1-(3-aminophenyl)-6-(3-hydroxyphenyl)naphthalen-2-ol (**1a**) and 5-(2-(methylthio)-pyrimidin-4-yl)thiophene-2-sulfonyl chloride according to method A; yield, 22% (6.7 mg). MS (ESI): 598 $(M + H)^+$.

*N-(3-(2-Hydroxy-6-(3-hydroxyphenyl)naphthalen-1-yl)phenyl)-benzo[d]thiazole-6-sulfonamide (**5**).* The title compound was prepared by reaction of 1-(3-aminophenyl)-6-(3-hydroxyphenyl)-naphthalen-2-ol (**1a**) and benzo[*d*]thiazole-6-sulfonyl chloride according to method A; yield, 42% (11 mg). MS (ESI): 525 $(M + H)^+$.

*N-(3-(2-Hydroxy-6-(3-hydroxyphenyl)naphthalen-1-yl)phenyl)-2-nitro-4-(trifluoromethyl)benzenesulfonamide (**6**).* The title compound was prepared by reaction of 1-(3-aminophenyl)-6-(3-hydroxyphenyl)naphthalen-2-ol (**1a**) and 2-nitro-4-(trifluoromethyl)-benzene-1-sulfonyl chloride according to method A; yield, 18% (5.1 mg). MS (ESI): 581 $(M + H)^+$.

*5-Chloro-N-(3-(2-hydroxy-6-(3-hydroxyphenyl)naphthalen-1-yl)-phenyl)-6a*H*-pyrrolo[3,2-d]thiazole-6-sulfonamide (**7**).* The title compound was prepared by reaction of 1-(3-aminophenyl)-6-(3-hydroxyphenyl)naphthalen-2-ol (**1a**) and 5-chloro-6a*H*-pyrrolo[3,2-*d*]thiazole-6-sulfonyl chloride according to method A; yield, 24% (6.6 mg). MS (ESI): 548 $(M + H)^+$. Compounds **8**–**11**, **14**, **15**, and **17** were synthesized in the parallel synthetic approach according to method A and afterward resynthesized in a bigger scale according to descriptions below.

*N-(3-(2-Hydroxy-6-(3-hydroxyphenyl)naphthalen-1-yl)phenyl)-4-bromo-2,5-fluorobenzene-sulfonamide (**8**).* The title compound was synthesized by reaction of 4-bromo-2,5-difluoro-*N*-(3-(2-methoxy-6(3-methoxyphenyl)naphthalen-1-yl) phenyl) benzenesulfonamide (**8a**) (70 mg, 0.15 mmol) and boron tribromide (1.05 mmol) according to method C. The product was purified by preparative HPLC (Agilent PrepC18 (H_2O/CH_3CN + 0.1% TFA) (20:80) → (0:100) in 35 min; yield, 63% (52 mg). MS (ESI): 583 $(M + H)^+$.

*N-(3-(2-Hydroxy-6-(3-hydroxyphenyl)naphthalen-1-yl)phenyl)-4-bromo-2-trifluoro-methoxybenzenesulfonamide (**9**).* The title compound was synthesized by reaction of *N*-(3-(2-(benzyloxy)-6-(3-(benzyloxy)phenyl)naphthalen-1-yl)phenyl)-4-bromo-2-(trifluoromethoxy)benzenesulfonamide (**9a**) (280 mg, 0.34 mmol) and boron tribromide (3.40 mmol) according to method D. The product was purified by flash chromatography hexane/ethylacetete (5:3); yield, 84% (180 mg). MS (ESI): 631 $(M + H)^+$.

*N-(3-(2-Hydroxy-6-(3-hydroxyphenyl)naphthalen-1-yl)phenyl)-quinolin-6-sulfonamide (**10**).* The title compound was synthesized by reaction *N*-(3-(2-methoxy-6(3-methoxyphenyl)naphthalen-1-yl) phenyl)quinoline-6-sulfonamide (**10a**) (61 mg, 0.12 mmol) and boron tribromide (0.9 mmol) according to method D. The product was purified by flash chromatography hexane/ethylacetete (1:1); yield, 63% (30 mg). MS (ESI): 519 $(M + H)^+$.

*N-(3-(2-Hydroxy-6-(3-hydroxyphenyl)naphthalen-1-yl)phenyl)-thiophene-2-sulfonamide (**11**).* The title compound was synthesized by reaction *N*-(3-(2-methoxy-6(3-methoxyphenyl)naphthalen-1-yl) phenyl) thiophene-2-sulfonamide (**11a**) (50 mg, 0.1 mmol) and boron tribromide (0.75 mmol) according to method C. The product was purified by flash chromatography hexane/ethylacetete (1:1); yield, 34% (16 mg). MS (ESI): 473 $(M + H)^+$.

*N-(3-(2-Hydroxy-6-(3-hydroxyphenyl)naphthalen-1-yl)phenyl)-benzenesulfonamide (**12**).* The title compound was synthesized by reaction of *N*-(3-(2-(benzyloxy)-6-(3-(benzyloxy)phenyl)naphthalen-1-yl)phenyl)-3-methylbenzene-sulfonamide (**12a**) (215 mg, 0.33 mmol) and boron tribromide (3.32 mmol) according to method D. The product was purified by preparative HPLC [Agilent PrepC18 (H_2O/CH_3CN + 0.1% TFA) (35:65) → (0:100) in 42 min]; yield, 23%. MS (ESI): 468 $(M + H)^+$.

*N-(3-(2-Hydroxy-6-(3-hydroxyphenyl)naphthalen-1-yl)phenyl)-2-nitrobenzene-sulfonamide (**13**).* The title compound was synthesized by reaction of *N*-(3-(2-(benzyloxy)-6-(3-(benzyloxy)phenyl)-naphthalen-1-yl)phenyl)-2-nitrobenzenesulfonamide (**13a**) (190 mg, 0.27 mmol) and boron tribromide (2.70 mmol) according to method D. The product was purified by preparative HPLC [Agilent PrepC18 (H_2O/CH_3CN + 0.1% TFA) (35:65) → (0:100) in 42 min]; yield, 9% (12 mg). MS (ESI): 530 $(M + H_2O)^+$.

*N-(3-(2-Hydroxy-6-(3-hydroxyphenyl)naphthalen-1-yl)phenyl)-3-nitrobenzene-sulfonamide (**14**).* The title compound was synthesized by reaction of *N*-(3-(2-(benzyloxy)-6-(3-(benzyloxy)phenyl)-naphthalen-1-yl)phenyl)-3-nitrobenzene-sulfonamide (**14a**) (150 mg, 0.22 mmol) and boron tribromide (2.20 mmol) according to method D. The product was purified by preparative HPLC [Agilent PrepC18 (H_2O/CH_3CN + 0.1% TFA) (35:65) → (0:100) in 42 min]; yield, 21% (24 mg). MS (ESI): 513 $(M + H)^+$.

*N-(3-(2-Hydroxy-6-(3-hydroxyphenyl)naphthalen-1-yl)phenyl)-4-nitrobenzene-sulfonamide (**15**).* The title compound was synthesized by reaction *N*-(3-(2-methoxy-6(3-methoxyphenyl)naphthalen-1-yl) phenyl)4-nitrobenzene-sulfonamide (**15a**) (150 mg, 0.28 mmol) and boron tribromide (2.8 mmol) according to method C. The product was purified by flash chromatography hexane/ethylacetete (1:1); yield, 43% (62 mg). MS (ESI): 512 $(M + H)^+$.

*N-(3-(2-Hydroxy-6-(3-hydroxyphenyl)naphthalen-1-yl)phenyl)-2,4-dinitrobenzene-sulfonamide (**16**).* The title compound was synthesized by reaction of *N*-(3-(2-(benzyloxy)-6-(3-(benzyloxy)-phenyl)naphthalen-1-yl)phenyl)-2,4-dinitro-benzenesulfonamide (**16a**) (150 mg, 0.26 mmol) and boron tribromide (2.60 mmol) according to method D. The product was purified by flash chromatography hexane/ethyl acetate (5:1); yield, 21% (24 mg). MS (ESI): 558 $(M + H)^+$.

*2-Cyano-N-(3-(2-hydroxy-6-(3-hydroxyphenyl)naphthalen-1-yl)-phenyl)benzene-sulfonamide (**17**).* The title compound was synthesized by reaction of 2-cyano-*N*-(3-(2-methoxy-6(3-methoxyphenyl)-naphthalen-1-yl) phenyl)benzene sulfonamide (**17a**) (226 mg, 0.16 mmol) and boron tribromide (1.12 mmol) according to method C. The product was purified by flash chromatography hexane/ethyl acetate (15:1 → 5:1); yield, 23% (38 mg). MS (ESI): 493 $(M + H)^+$.

*N-(3-(2-Hydroxy-6-(3-hydroxyphenyl)naphthalen-1-yl)phenyl)-3-cyanobenzene-sulfonamide (**18**).* The title compound was synthesized by reaction of *N*-(3-(2-(benzyloxy)-6-(3-(benzyloxy)phenyl)-naphthalen-1-yl)phenyl)-3-cyanobenzene-sulfonamide (**18a**) (200 mg, 0.33 mmol) and boron tribromide (3.30 mmol) according to method D. The product was purified by flash chromatography hexane/ethyl acetate (10:1 → 6:1); yield, 49% (72 mg). MS (ESI): 495 $(M + H)^+$.

*N-(3-(2-Hydroxy-6-(3-hydroxyphenyl)naphthalen-1-yl)phenyl)-4-cyanobenzene-sulfonamide (**19**).* The title compound was synthesized by reaction of *N*-(3-(2-(benzyloxy)-6-(3-(benzyloxy)phenyl)-naphthalen-1-yl)phenyl)-4-cyanobenzene-sulfonamide (**19a**) (170 mg, 0.25 mmol) and boron tribromide (2.50 mmol) according to method D. The product was purified by preparative TLC hexane/ethyl acetate (2:1); yield, 50% (66 mg). MS (ESI): 493 $(M + H)^+$.

*N-(3-(2-Hydroxy-6-(3-hydroxyphenyl)naphthalen-1-yl)phenyl)-2-fluorobenzene-sulfonamide (**20**).* The title compound was synthesized by reaction of *N*-(3-(2-(benzyloxy)-6-(3-(benzyloxy)phenyl)-naphthalen-1-yl)phenyl)-2-fluorobenzene-sulfonamide (**20a**) (190 mg,

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0.29 mmol) and boron tribromide (2.90 mmol) according to method D. The product was purified by preparative TLC hexane/ethyl acetate (3:2); yield, 71% (100 mg). MS (ESI): 508 $(M + Na)^+$.

*N-(3-(2-Hydroxy-6-(3-hydroxyphenyl)naphthalen-1-yl)phenyl)-3-fluorobenzene-sulfonamide (**21**).* The title compound was synthesized by reaction *N*-(3-(2-(benzyloxy)-6-(3-(benzyloxy)phenyl)-naphthalen-1-yl)phenyl)-3-fluorobenzene-sulfonamide (**21a**) (150 mg, 0.23 mmol) and boron tribromide (2.30 mmol) according to method D. The product was purified by preparative TLC hexane/ethyl acetate (2:1); yield, 13% (15 mg). MS (ESI): 508 $(M + Na)^+$.

*N-(3-(2-Hydroxy-6-(3-hydroxyphenyl)naphthalen-1-yl)phenyl)-4-fluorobenzene-sulfonamide (**22**).* The title compound was synthesized by reaction of *N*-(3-(2-(benzyloxy)-6-(3-(benzyloxy)phenyl)-naphthalen-1-yl)phenyl)-4-fluorobenzene-sulfonamide (**22a**) (250 mg, 0.38 mmol) and boron tribromide (3.80 mmol) according to method D. The product was purified by flash chromatography hexane/ethyl acetate (5:3); yield, 44% (80 mg). MS (ESI): 486 $(M + H)^+$.

*N-(3-(2-Hydroxy-6-(3-hydroxyphenyl)naphthalen-1-yl)phenyl)-3-hydroxybenzene-sulfonamide (**23**).* The title compound was synthesized by reaction *N*-(3-(2-(benzyloxy)-6-(3-(benzyloxy)-phenyl)naphthalen-1-yl)phenyl)-3-methoxybenzene-sulfonamide (**23a**) (100 mg, 0.15 mmol) and boron tribromide (2.10 mmol) according to method D. The product was purified by flash chromatography hexane/ethyl acetate (5:3); yield, 72% (52 mg). MS (ESI): 484 $(M + H)^+$.

*N-(3-(2-Hydroxy-6-(3-hydroxyphenyl)naphthalen-1-yl)phenyl)-4-hydroxybenzene-sulfonamide (**24**).* The title compound was synthesized by reaction *N*-(3-(2-(benzyloxy)-6-(3-(benzyloxy)-phenyl)naphthalen-1-yl)phenyl)-4-methoxybenzene-sulfonamide (**24a**) (150 mg, 0.22 mmol) and boron tribromide (2.20 mmol) according to method D. The product was purified by flash chromatography hexane/ethyl acetate (2:1 → 1:1); yield, 10% (11 mg). MS (ESI): 484 $(M + H)^+$.

*N-(3-(2-(Hydroxy)-6-(3-(hydroxy)phenyl)naphthalen-1-yl)-phenyl)-2-methylbenzene-sulfonamide (**25**).* The title compound was synthesized by reaction *N*-(3-(2-(benzyloxy)-6-(3-(benzyloxy)-phenyl)naphthalen-1-yl)phenyl)-2-methylbenzene-sulfonamide (**25a**) (40 mg, 0.08 mmol) and boron tribromide (0.8 mmol) according to method D. The product was purified by HPLC [Agilent PrepC18 (H_2O/CH_3CN + 0.1% TFA) (60:40) → (0:100) in 42 min]; yield, 10% (11 mg). MS (ESI): 482 $(M + H)^+$.

*N-(3-(2-Hydroxy-6-(3-hydroxyphenyl)naphthalen-1-yl)phenyl)-3-methylbenzene-sulfonamide (**26**).* The title compound was synthesized by reaction *N*-(3-(2-(benzyloxy)-6-(3-(benzyloxy)phenyl)-naphthalen-1-yl)phenyl)-3-methylbenzene-sulfonamide (**26a**) (150 mg, 0.23 mmol) and boron tribromide (2.30 mmol) according to method D. The product was purified by HPLC [Agilent PrepC18 (H_2O/CH_3CN + 0.1% TFA) (35:65) → (0:100) in 42 min]; yield, 14% (15 mg). MS (ESI): 499 $(M + H_2O)^+$.

*N-(3-(2-Hydroxy-6-(3-hydroxyphenyl)naphthalen-1-yl)phenyl)-4-methylbenzene-sulfonamide (**27**).* The title compound was synthesized by reaction *N*-(3-(2-(benzyloxy)-6-(3-(benzyloxy)phenyl)-naphthalen-1-yl)phenyl)-4-methylbenzene-sulfonamide (**27a**) (213 mg, 0.32 mmol) and boron tribromide (3.20 mmol) according to method D. The product was purified by HPLC [Agilent PrepC18 (H_2O/CH_3CN + 0.1% TFA) (35:65) → (0:100) in 35 min]; yield, 45% (70 mg). MS (ESI): 499 $(M + H_2O)^+$.

*N-(4-(N-(3-(2-(Hydroxy)-6-(3-(hydroxy)phenyl)naphthalen-1-yl)-phenyl)sulfamoyl)-phenyl)-acetamide (**28**).* The title compound was synthesized by reaction *N*-(4-(*N*-(3-(2-(benzyloxy)-6-(3-(benzyloxy)-phenyl)naphthalen-1-yl)phenyl)sulfamoyl)-phenyl)-acetamide (**28a**) (299 mg, 0.40 mmol) and boron tribromide (4.00 mmol) according to method C. The product was purified by HPLC [Agilent PrepC18 (H_2O/CH_3CN + 0.1% TFA) (55:45) → (0:100) in 42 min]; yield, 37% (70 mg). MS (ESI): 525 $(M + H)^+$.

*N-(3-(2-Hydroxy-6-(3-hydroxyphenyl)naphthalen-1-yl)phenyl)-2,2,2-trifluoro-ethanesulfonamide (**29**).* The title compound was synthesized by reaction *N*-(3-(2-(benzyloxy)-6-(3-(benzyloxy)-phenyl)naphthalen-1-yl)phenyl)-2,2,2-trifluoro-ethanesulfonamide (**29a**) (300 mg, 0.5 mmol) and boron tribromide (5.00 mmol) according to method D. The product was purified by HPLC [Agilent PrepC18 (H_2O/CH_3CN + 0.1% TFA) (35:65) → (0:100) in 38 min]; yield, 25% (60 mg). MS (ESI): 474 $(M + H)^+$.

*6-(3-Hydroxyphenyl)-1-(1-(phenylsulfonyl)-1H-indol-4-yl)-naphthalen-2-ol (**30**).* The title compound was synthesized by reaction of 4-(2-(benzyloxy)-6-(3-(benzyloxy)phenyl)naphthalen-1-yl)-1-(phenylsulfonyl)-1*H*-indole (**30a**) (70 mg, 0.14 mmol) and boron tribromide (1.40 mmol) according to method D. The product was purified by HPLC [Agilent PrepC18 (H_2O/CH_3CN + 0.1% TFA) (75:25) → (0:100) in 55 min]; yield, 10% (5.00 mg). MS (ESI): 493 $(M + H)^+$.

*5-(2-(Benzyloxy)-6-(3-(benzyloxy)phenyl)naphthalen-1-yl)-1-(phenylsulfonyl)-1H-indole (**31a**) and 1-[1-(Benzensulfonyl)indol-6-yl]-6-(3-hydroxyphenyl)naphthalen-2-ol (**31**).* Powdered sodium hydroxide (5 mg, 0.11 mmol) and a catalytic amount of tetra-butyl ammonium chloride were stirred for 10 min in dichloromethane at 0 °C under nitrogen atmosphere. (6-(2-(Benzyloxy)-6-(3-(benzyloxy)-phenyl)naphthalen-1-yl)-1*H*-indole (**31b**) (20 mg, 0.038 mmol) was added without further purfication and stirred for a while. A solution of benzenesulfonyl chloride (7 mg, 0.04 mmol) in dichloromethane was added dropwise during a period of 15 min. The solution was stirred for 3 h under 10 °C. The product was extracted with ethyl acetate, and the combined organic layers were dried over $MgSO_4$ and evaporated under vacuum. The product was used without further purification for the ether cleavage according to method D (3.60 mmol boron tribromide were used). The product was purified by HPLC [Agilent PrepC18 (H_2O/CH_3CN + 0.1% TFA) (75:25) → (0:100) in 40 min]; yield, 11% (2.3 mg). MS (ESI): 493 ($[M + H]$)$^+$.

Biophysical Characterization. The molecular weight, clog *P*, and tPSA values of all compounds were calculated from CambridgeSoft Chem & Bio Draw using the ChemDrawUltra 12.0 program.

Biological Methods. [2,4,6,7-^{3}H]-E2 and [2,4,6,7-^{3}H]-E1 were purchased from Perkin-Elmer (Boston). Quickszint Flow 302 scintillator fluid was bought from Zinsser Analytic (Frankfurt). T47D cells were obtained from ECACC (Salisbury). FCS was purchased from Sigma (Taufkirchen). Cell culture media and dextran-coated charcoal-stripped FCS (DCC-FCS) were ordered from CCPRO (Oberdorla). Other chemicals were purchased from Sigma, Roth, or Merck.

Enzyme Preparation (17βHSD1 and 17βHSD2) of Placenta from Human and *C. jacchus*. 17β-HSD1 and 17β-HSD2 were obtained from human placenta and *C. jacchus* placenta according to previously described procedures.[63,64] Fresh human placenta and frozen *C. jacchus* placenta were homogenized and separated by partial centrifugation. The pellet fraction contained the microsomal 17β-HSD2, while 17β-HSD1 was obtained after precipitation with ammonium sulfate from the cytosolic fraction. Aliquots of 17β-HSD1 and 17β-HSD2 were stored frozen at −80 °C.

Inhibition of 17β-HSD1 in Cell-Free Assay. Inhibitory activities toward human and *C. jacchus* enzymes were evaluated by a well-established method with minor modifications.[65−67] Briefly, the enzyme preparation was incubated with NADH (500 μM) in the presence of potential inhibitors at 37 °C in a phosphate buffer (50 mM) supplemented with 20% glycerol and EDTA. Ten millimolar inhibitor stock solutions were prepared in DMSO. The final concentration of DMSO was adjusted to 1% in all samples. The enzymatic reaction was started by addition of a mixture of unlabeled- and [^{3}H]-E1 (final concentration, 500 nM; 0.15 μCi). After 10 min, the incubation was stopped with $HgCl_2$ (10 mM), and the mixture was extracted with diethylether. After evaporation, the steroids were dissolved in acetonitrile. E1 and E2 were separated using acetonitrile/water (45:55) as the mobile phase in a C18 rp chromatography column (Nucleodur C18 Gravity, 3 μm, Macherey-Nagel, Düren) connected to a HPLC system (Agilent 1200 Series, Agilent Technologies, Waldbronn). Detection and quantification of the steroids were performed using a radioflow detector (Ramona Star, Raytest, Straubenhardt). The conversion rate was calculated according to following equation:

$$\%\ \text{conversion} = \frac{\%\ \text{E2}}{\%\ \text{E2} + \%\ \text{E1}} \times 100$$

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Each value was calculated from at least three independent experiments.

Inhibition of 17β-HSD2. The 17β-HSD2 inhibition assays were performed similarly to the 17β-HSD1 procedure. The microsomal fraction was incubated with NAD^+ (1500 μM), test compound, and a mixture of unlabeled- and [^{3}H]-E2 (final concentration, 500 nM; 0.14 μCi) for 20 min at 37 °C (the reaction was stopped with 1 mM $HgCl_2$). Further treatment of the samples and HPLC separation were carried out as mentioned above.

ER Affinity. The binding affinity of selected compounds to the ERα and ERβ was determined according to Zimmermann et al.[68] Briefly, 0.25 pmol of ERα or ERβ, respectively, was incubated with [2,4,6,7-^{3}H]-E2 (10 nM) and test compound for 1 h at rt. The potential inhibitors were dissolved in DMSO (5% final concentration). Nonspecific binding was performed with diethylstilbestrol (10 μM). After incubation, ligand−receptor complexes were selectively bound to hydroxyapatite (5 g/60 mL TE buffer). The formed complex was separated, washed, and resuspended in ethanol. For radiodetection, scintillator cocktail (Quickszint 212, Zinsser Analytic, Frankfurt) was added, and samples were measured in a liquid scintillation counter (Wallac Micro Beta TriLux, Perkin-Elmer). For determination of the RBA, inhibitor and E2 concentrations required to displace 50% of the receptor bound labeled E2 were determined. RBA values were calculated according to the following equation:

$$\text{RBA}\ (\%) = \frac{\text{IC}_{50}(\text{E2})}{\text{IC}_{50}(\text{compound})} \times 100$$

The RBA value for E2 was arbitrarily set at 100%.

Inhibition of 17β-HSD1 in Cellular Assay Using T47-D. A stock culture of T47D cells was grown in RPMI 1640 medium supplemented with 10% FCS, L-glutamine (2 mM), penicillin (100 IU/mL), streptomycin (100 μg/mL), insulin-zinc-salt (10 μg/mL), and sodium pyruvate (1 mM) at 37 °C under 5% CO_2 humidified atmosphere.

The cells were seeded into a 24-well plate at 5×10^5 cells/well in DMEM medium with FCS and L-glutamine, and the antibiotics were added in the same concentrations as mentioned above. After 24 h, the medium was changed for fresh serum-free DMEM, and a solution of test compound in DMSO was added. The final concentration of DMSO was adjusted to 1% in all samples. After a preincubation of 30 min at 37 °C with 5% CO_2, the incubation was started by addition of a mixture of unlabeled- and [2,4,6,7-^{3}H]-E1 (final concentration, 50 nM; 0.15 μCi). After 30 min of incubation, the enzymatic reaction was stopped by removal of the supernatant medium. The steroids were extracted into diethylether. Further treatment of the samples was carried out as mentioned for the 17β-HSD1 assay.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthesis and characterization of intermediates as well as HPLC purity determination for final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

For the sake of clarity, IUPAC nomenclature is not strictly followed except for the experimental part, where the correct IUPAC names are given.

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■ ABBREVIATIONS USED

*h*17β-HSD1, human 17β-hydroxysteroid dehydrogenase type 1; *cj*17β-HSD1, *Callithrix jacchus* 17β-hydroxysteroid dehydrogenase type 1; *h*17β-HSD2, human 17β-hydroxysteroid dehydrogenase type 2; *cj*17β-HSD2, *Callithrix jacchus* 17β-hydroxysteroid dehydrogenase type 2; BPH, benign prostatic hyperplasia; E1, estrone; E2, 17β-estradiol; NADP(H), nicotinamide adenine dinucleotide phosphate; EDD, estrogen-dependent disease; ER, estrogen receptor; SERM, selective estrogen receptor modulator; SAR, structure−activity relationship; equiv, equivalent; HPLC, high-performance liquid chromatography; RBA, relative binding affinity; rt, room temperature; SF, selectivity factor

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