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New Drug-Like Hydroxyphenylnaphthol Steroidomimetics As Potent and Selective 17β-Hydroxysteroid Dehydrogenase Type 1 Inhibitors for the Treatment of Estrogen-Dependent Diseases

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Inhibition of 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1) is a novel and attractive approach to reduce the local levels of the active estrogen 17β-estradiol in patients with estrogen-dependent diseases like breast cancer or endometriosis. With the aim of optimizing the biological profile of 17β-HSD1 inhibitors from the hydroxyphenylnaphthol class, structural optimizations were performed at the 1-position of the naphthalene by introduction of different heteroaromatic rings as well as substituted phenyl groups. In the latter class of compounds, which were synthesized applying Suzuki-cross coupling, the 3-methanesulfonamide **15** turned out to be a highly potent 17β-HSD1 inhibitor (IC_{50} = 15 nM in a cell-free assay). It was also very active in the cellular assay (T47D cells, IC_{50} = 71 nM) and selective toward 17β-HSD2 and the estrogen receptors α and β. It showed a good membrane permeation and metabolic stability and was orally available in the rat.

Introduction

The great importance of 17β-estradiol (E2[a]),[1] the most potent estrogen, in the development and progression of estrogen-dependent diseases (EDD) like breast cancer[2,3] and endometriosis[4,5] is now well established. In case of estrogen-dependent tumors, current hormonal therapies are aimed either at blocking the action of estrogens (with antiestrogens or selective estrogen receptor modulators (SERMs)) or at lowering the levels of both circulating and tissue E2 by inhibiting estrogen synthesis (by use of aromatase inhibitors (AI)[6−8] or GnRH analogues). Although the emergence of these therapies has led to major advances in the treatment of breast cancer, the tumor often develops resistance mechanisms during the treatment, leading to failure of these medications. Search for alternative therapies is therefore necessary. Restricting the estrogen decrease to the target cells only is an attractive approach for the treatment of EDD which should be associated with fewer side effects. Such a therapeutic strategy (intracrine concept) had been shown to be successful in androgen dependent diseases like benign prostate hyperplasia by using 5α-reductase inhibitors.[9−12] Such an approach could be implemented for EDD applying inhibitors of 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1), the enzyme which catalyzes the last step of estrogens biosynthesis, transforming estrone (E1) to E2. These inhibitors, decreasing the E2 levels in the target tissues only, should thus be superior to AI, which influence the systemic levels of E1 and E2, leading to severe side effects like osteoporosis.

High expression of 17β-HSD1 mRNA has been found in breast cancer tissues[13,14] and in endometriosis.[15] In addition, 17β-HSD1 is highly expressed in breast cancer cells while 17β-HSD2, the enzyme catalyzing the reverse reaction (deactivating E2 into E1), is frequently downregulated in malignant cells. This disrupted 17β-HSD1/17β-HSD2 ratio suggests the important role of 17β-HSD1 in the production of E2 in breast tumors compared to normal breast tissue (E2 concentration is up to 2.3 times higher in malignant tissues[16,17]). Blocking the last step of estrogen biosynthesis by inhibition of 17β-HSD1 is therefore an attractive approach for the treatment of these diseases.

Recently, in vivo efficacy of 17β-HSD1 inhibitors has been reported in two animal models. Immunodeficient mice were inoculated either with MCF-7 cells overexpressing the human recombinant 17β-HSD1 enzyme[18,19] or with T47D cells naturally expressing 17β-HSD1.[20] In both models, the E1 induced tumor growth was reduced by 17β-HSD1 inhibitors. This effect was also demonstrated by Kruchten et al.[21] using an in vitro proliferation assay: in a control experiment, equivalent tumor growth stimulation could be reached at equal concentrations of E1 and E2 because of the very fast reduction of the weakly active EDD stimulator E1 to the highly potent E2 catalyzed by 17β-HSD1. Application of a 17β-HSD1 inhibitor could reduce significantly the cell proliferation induced by E1 only. The combination of these in vitro and in vivo experiments validates 17β-HSD1 as a novel target for the treatment of EDD. Up to now, however, no 17β-HSD1 inhibitor has entered clinical trials.

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[a] Abbreviations: 17β-HSD1, 17β-hydroxysteroid dehydrogenase type 1; 17β-HSD2, 17β-hydroxysteroid dehydrogenase type 2; E1, estrone; E2, 17β-estradiol; NADP(H), nicotinamide adenine dinucleotide phosphate; EDD, estrogen-dependent disease; ER, estrogen receptor; AI, aromatase inhibitor; SERM, selective estrogen receptor modulator; SAR, structure−activity relationship; PDB-ID, protein data bank identification code; equiv, equivalent; HPLC, high performance liquid chromatography; RBA, relative binding affinity; SF, selectivity factor; AUC, area under the curve. 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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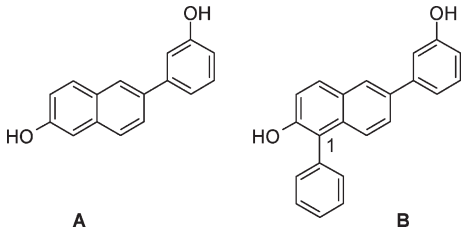
Chart 1. Previously Identified Hits as 17β-HSD1 Inhibitors



17β-HSD1 belongs to the 17β-hydroxysteroid dehydrogenase (HSD) family. Together with type 7 and 12, the subtype 1 is able to catalyze the NADPH dependent reduction of E1 into E2. However, only type 1 is considered to be efficient in the transformation of the sex steroid, type 7 and 12 are involved in cholesterol synthesis[22] and in lipid biosynthesis,[20] respectively.

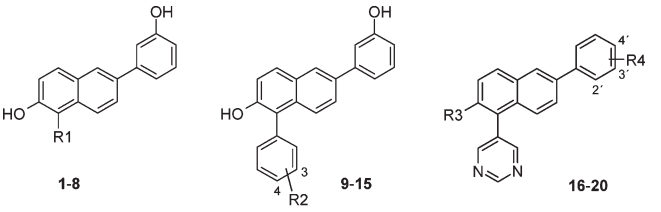
17β-HSD1 is a soluble cytosolic enzyme; it has been crystallized with different steroidal ligands.[23−31] The X-ray structures provided useful information about the enzyme active site for the design of potent inhibitors.

Several classes of 17β-HSD1 inhibitors have been described in the last years,[32−38] most of them having a steroidal core. Within the nonsteroidal inhibitors, only five different templates revealed interesting activities: flavones and isoflavones,[39] thienopyrimidinones,[40,41] bishydroxyphenyl arenes,[42−45] heterocyclic substituted biphenylols,[46] and hydroxyphenylnaphthols,[47−49] the three latter classes were described by our group. In the series of hydroxyphenylnaphthols, the steroidomimetic **A**[47] (Chart 1) was previously identified as a new scaffold for 17β-HSD1 inhibitors. The biological profile of **A** was optimized and the hit compound **B**[48,49] (Chart 1), bearing a phenyl ring at the 1-position of the naphthalene ring, was discovered as the most promising in this series of inhibitors with a good inhibitory activity (IC_{50} = 20 nM), a good selectivity toward 17β-HSD2 (selectivity factor (SF): 27), and a weak affinity to the estrogen receptors (ER) α and β. After introduction of various substituents at different positions on the hydroxyphenyl and/or naphthalene moiety, we identified a plausible binding mode for **B** in the active site: the hydroxyphenyl part mimics the A-ring of E2 and the naphthol-OH interacts with the catalytic tetrad. The 1-phenyl group is stabilized by $\pi-\pi$ interactions with the amino acids Phe226 and Tyr155 and the nicotinamide ring of the cofactor.[48]

As our goal is to develop new compounds demonstrating therapeutic efficacy, they should not only show good inhibitory activity but they should also have a good ADME/Tox profile and therefore should not be as lipophilic as compound **B** (logP 5.6). Considering the sharp structure−activity relationship (SAR) observed in this class of compounds[47] and in order to improve the in vivo activity of this compound class, we decided to perform structure modifications at the 1-position of the naphthalene. Two strategies were followed: (a) exchange of the 1-phenyl moiety of **B** for different heterocycles (compounds **1**−**8**, Chart 2) and (b) introduction of substituents on the 1-phenyl group (compounds **9**−**15**, Chart 2). These substituents should on the one hand be able to establish H-bond interactions with amino acids from the active site located around the catalytic tetrad and on the other increase the hydrophilicity of the inhibitors.

Herein, we report about our efforts to improve the therapeutic properties of this class of compounds starting from **B**, developing new, potent, and selective 17β-HSD1 inhibitors.

Chart 2. Synthesized Compounds



Cmpd	R1	Cmpd	R2	Cmpd	R3	R4
1		**9**	3-OH	**16**	H	2′-OH
2		**10**	3-COOH	**17**	H	3′-OH
3		**11**	3-NH_2	**18**	OH	H
4		**12**		**19**	OH	2′-OH
5		**13**		**20**	OH	4′-OH
6		**14**				
7		**15**				
8						

Chemistry

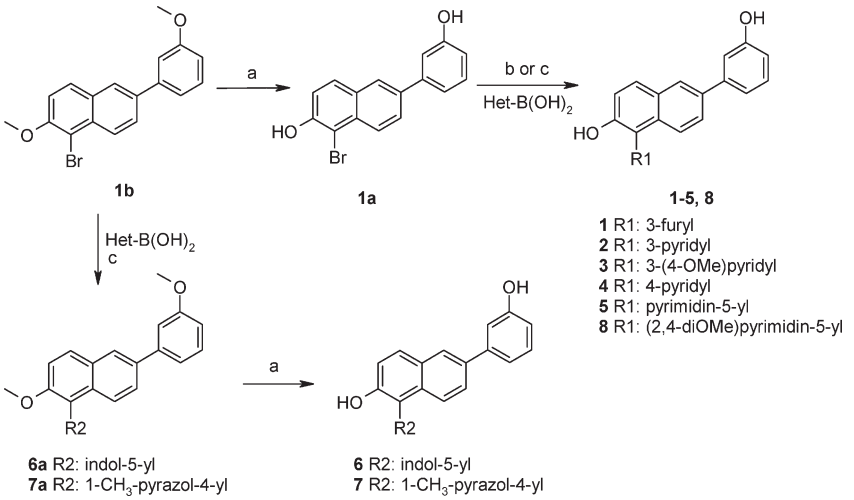
Introduction of Aromatic Heterocycles: Compounds 1−8. Compounds **1**−**8** were prepared in two steps from 1-bromo-2-methoxy-6-(3-methoxyphenyl)naphthalene **1b** (Scheme 1). Demethylation using boron tribromide[50] (Method A) led to **1a**, which was subsequently submitted to Suzuki coupling using the appropriate boronic acid[51] (Methods B or C) to afford **1**−**5** and **8**. Compounds **6** and **7** were obtained from **1b** via a cross-coupling under microwave assisted conditions (Method C) and subsequent ether cleavage.

Introduction of Substituted Aryls: Compounds 9−15. Compound **9** was obtained by cross coupling reaction of 1,6-dibromo-2-naphthol **9a** with an excess of 3-hydroxybenzene boronic acid following Method B (Scheme 2). Compounds **10** and **12**−**15** were prepared by coupling **1a** either to a boronic acid to give **10** and **14** or to a pinacolato ester in case of the amides **12**, **13**, and the sulfonamide **15** (Method B, Scheme 2).

The synthesis of compound **11** is depicted in Scheme 3. The dimethoxylated aniline derivative **11a** was prepared from **1b** by cross-coupling reaction. Cleavage of the methoxy groups with boron tribromide (Method A) afforded **11** directly.

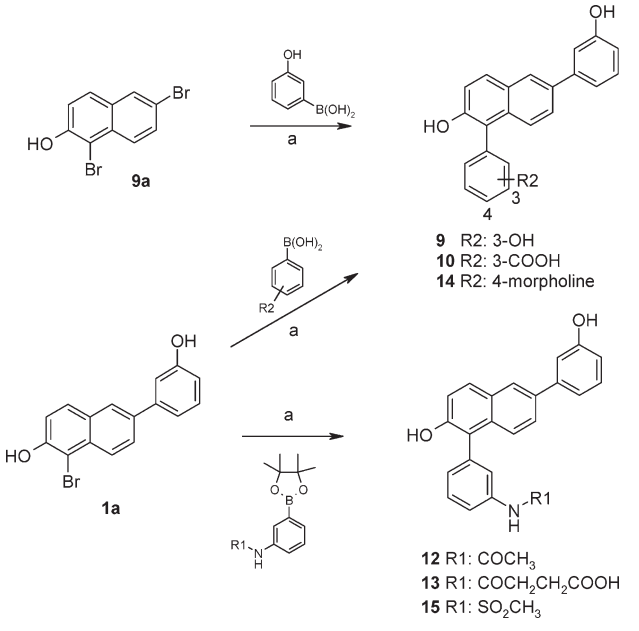
Investigation of the Hydroxy Group Substitution Pattern of the Pyrimidine Derivative: Compounds 16−20. Three monohydroxylated derivatives of the pyrimidine **5** (compounds **16**−**18**) were synthesized (Schemes 4 and 5). Compounds **16** and **17** with the hydroxy group on the phenyl ring were prepared from the tetralone **16f**, which was converted to **16e**. A Suzuki reaction of the triflate **16e** and the corresponding methoxybenzeneboronic acid (Method B) led to the tetralones **16d** and **17d**,[47,52] which were aromatized in the presence of

Scheme 1. Synthesis of Compounds **1**−**8**[a]



[a] Reagents and conditions: (a) BBr_3, CH_2Cl_2, −78 °C to RT, overnight, Method A; (b) $Pd(PPh_3)_4$, aq Na_2CO_3, toluene/ethanol 5:1 for **1**, **3**, and **4**, 2:1 for **2**, 5:3 for **5**, 80 °C for **1**−**3** and **5**, 100 °C for **4**, 1 h for **2** and **4**, 2 h for **1**, 3 h for **3**, 15 h for **5**, Method B; (c) $Pd(PPh_3)_4$, aq Cs_2CO_3, DME/EtOH/H_2O 1:1:1 microwave irradiation, 150 W, 150 °C, 15 bar, 25 min for **8**, **6a**, and **7a**, Method C.

Scheme 2. Synthesis of Compounds **9**−**10**, **12**−**15**[a]



[a] Reagents and conditions: (a) $Pd(PPh_3)_4$, aq Na_2CO_3, toluene for **9**, toluene/ethanol 2:1 for **10** and **13**, 5:3 for **14**, and 5:1 for **12** and **15**, 80 °C, 2 h for **10**, **12**, and **15**, 10 min for **13**, 1 h for **9** and 21 h for **14**, Method B.

palladium on charcoal as catalyst. Subsequent triflation resulted in **16b** and **17b**.[52] Cross-coupling reaction with pyrimidine-5-boronic acid (Method B[52]), followed by ether cleavage with pyridinium hydrochloride or boron tribromide, afforded compounds **16**[52] and **17**, respectively.

The synthesis of the monohydroxylated naphthalene **18** started from 6-bromo-2-methoxy-naphthalene **18c** (Scheme 5). Bromination with *N*-bromosuccinimide followed by regioselective Suzuki coupling with 1 equiv of benzeneboronic acid (Method C) gave compound **18b**,[53] which after coupling with pyrimidine-5-boronic acid (Method D) and subsequent ether cleavage with boron tribromide (Method A) afforded **18**. For the synthesis of the dihydroxylated derivatives **19** and **20**, the hydroxylated phenyl group was introduced on 6-bromo-2-naphthol **19d** by Suzuki reaction (Method C). The resulting compounds **19c** and **20b**[52] were further brominated with *N*-bromosuccinimide to afford **19b** and **20a**,[54] respectively. A second Suzuki coupling reaction with pyrimidine-5-boronic acid (Method D) gave compounds **19a** and **20**.[53] The ether group in **19a** was cleaved using boron tribromide (Method A) to yield **19**.[52]

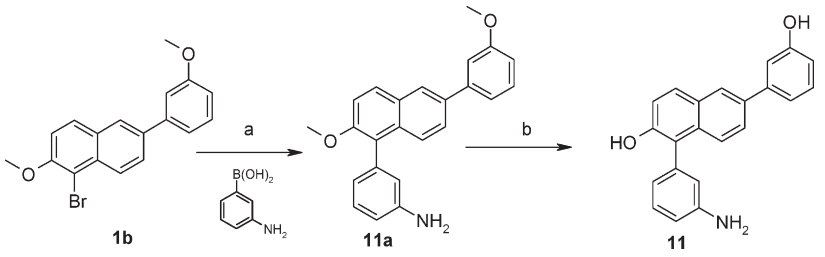
Results

Inhibition of Human 17β-HSD1 and Selectivity toward 17β-HSD2. 17β-HSD1 and 17β-HSD2 inhibitory activities of the synthesized compounds were first evaluated in cell-free assays. 17β-HSD2 catalyzes the oxidation of E2 into E1 and thus deactivates E2. It should not be affected by 17β-HSD1 inhibitors.

In the assays, both human recombinant and placental enzymes were used. In the 17β-HSD1 assay, incubations were run with tritiated E1, cofactor, and inhibitor. The hybrid inhibitor (EM-1745) described by Poirier et al.[55] was used as reference compound and gave similar values as described (IC_{50} = 52 nM). The 17β-HSD2 assay was performed similarly using tritiated E2 as substrate. The percent inhibition values of all hydroxy compounds are shown in Tables 1−3. Compounds showing less than 10% inhibition at 1 μM were considered to be inactive. IC_{50} values were determined for compounds showing more than 70% inhibition at 100 nM in the 17β-HSD1 assay and are shown in Table 4. All molecules with methoxy groups showed activity neither in the 17β-HSD1 nor in the 17β-HSD2 assays (data not shown). The 1-phenyl substituted naphthol **B** identified in a previous work[48] was used as internal reference.

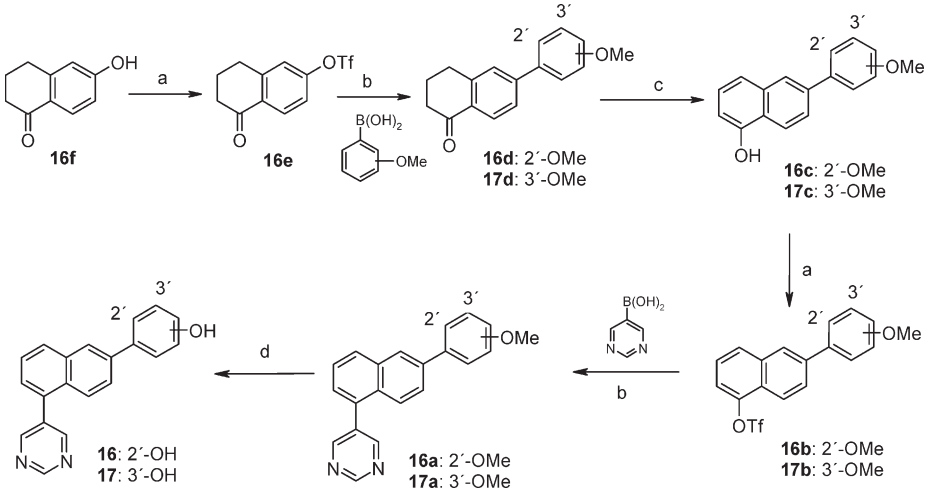
In an attempt to decrease the lipophilicity of **B** and to strengthen interactions with the enzyme, the 1-phenyl moiety was replaced by different six- and five-membered heteroaromatic cycles (**1**−**8**, Table 1). Introduction of a nitrogen in the phenyl ring (3-pyridine **2** and 4-pyridine **4**) was well tolerated by the enzyme, showing activity in the same range as the reference **B** (87%, 74%, and 76% inhibition at 100 nM for **2**, **4**, and **B**, respectively), indicating that it is unlikely that supplementary interactions are achieved by the N. Addition of the electron donating methoxy group on the pyridine ring

Scheme 3. Synthesis of Compound **11**[a]



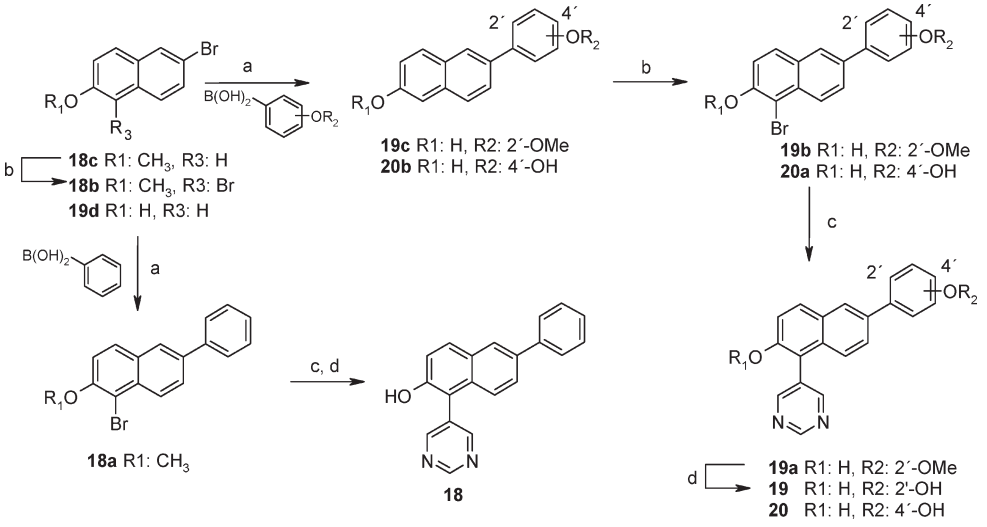
[a] Reagents and conditions: (a) $Pd(OAc)_2$, Na_2CO_3, TBAB, water, 150 °C, 1 h; (b) BBr_3, CH_2Cl_2, −78 °C to RT, overnight, Method A.

Scheme 4. Synthesis of Compounds **16** and **17**[a]



[a] Reagents and conditions: (a) triflic anhydride, pyridine, CH_2Cl_2, 0 °C, 30 min; (b) $Pd(PPh_3)_4$, aq Na_2CO_3, toluene/ethanol 5:1, 80 °C, 11 h for **16a** and 8 h for **17a**, Method B; (c) 10% Pd/C, *p*-cymene, reflux, overnight; (d) pyridinium hydrochloride, 220 °C, 3 h for **16**, BBr_3, CH_2Cl_2, −78 °C to RT, overnight for **17**, Method A.

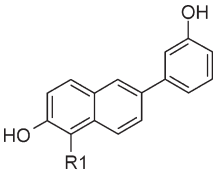
Scheme 5. Synthesis of Compounds **18**−**20**[a]



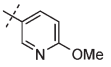
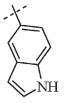
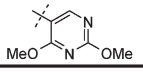
[a] Reagents and conditions: (a) $Pd(PPh_3)_4$, aq Cs_2CO_3, DME/EtOH/H_2O 1:1:1, microwave irradiation 150 W, 150 °C, 15 bar, 25 min, Method C; (b) NBS, MeCN, RT, 5 min for **19b** and 1 h for **18b** and **20a**; (c) pyrimidine-5-boronic acid, $Pd(OAc)_2$, PPh_3, K_2CO_3, 1,4-dioxane/EtOH 2:1, microwave irradiation 150 W, 150 °C, 15 bar, 15 min, Method D; (d) BBr_3, CH_2Cl_2, −78 °C to RT, overnight, Method A.

(**3**) is also tolerated; it shows that there is some space around the heterocycle for introduction of a small substituent. Interestingly, introduction of a second nitrogen into the pyridine (resulting in pyrimidine **5**) led to an inactive compound (at 100 nM). The position of this N in the active site or the change of electronic distribution might be unfavorable, preventing **5** from integrating into the binding pocket. A moderate regain of activity (31% inhibition at 100 nM)

Table 1. Inhibition of Human 17β-HSD1 and 17β-HSD2 by Compounds **1**−**8**



1-8

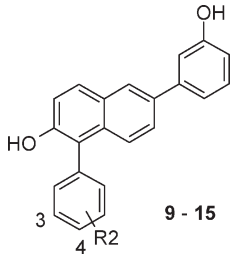
Cmpd	R1	Inhibition of 17β-HSD1 [%]		Inhibition of 17β-HSD2 [%]		LogP[e]
		100 nM	1 μM	100 nM	1 μM	
B		76[a]	89[a]	22[b]	77[b]	5.6
1		83[a]	89[a]	61[b]	92[b]	4.2
2		87[a]	92[a]	66[b]	90[b]	4.3
3		86[a]	89[a]	24[b]	67[b]	4.9
4		74[a]	86[a]	12[b]	49[b]	4.3
5		< 10[c]	41[c]	nd	85[d]	3.7
6		50[c]	87[c]	nd	19[d]	5.1
7		28[c]	71[c]	nd	24[d]	3.9
8		31[c]	79[c]	nd	9[d]	4.6

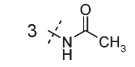
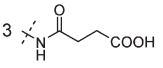
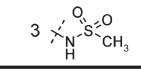
[a] Recombinant human 17β-HSD1, substrate [^{3}H]-E1 + E1 [30 nM], cofactor NADPH [1 mM], procedure A; mean value of two determinations, relative standard deviation < 20%. [b] Recombinant human 17β-HSD2, substrate [^{3}H]-E2 + E2 [30 nM], cofactor NAD^+ [1 mM], procedure C, mean value of two determinations, relative standard deviation < 20%. [c] Human placenta, cytosolic fraction, substrate [^{3}H]-E1 + E1 [500 nM], cofactor NADH [0.5 mM], procedure B, mean value of three determinations, relative standard deviation < 10% except for **7** 20%. [d] Human placenta, microsomal fraction, substrate [^{3}H]-E2 + E2 [500 nM], cofactor NAD^+ [1.5 mM] procedure D, mean value of three determinations, relative standard deviation < 10%, nd: not determined. [e] Calculated logP data.

was achieved by introduction of two methoxy groups into the pyrimidine core (**8**). The 2-OMe group of **8** certainly induces an ortho effect, constraining the pyrimidine to adopt a different geometry compared to **5** and restraining its free rotation. The *N*-methylpyrazole **7** also containing two nitrogens showed a weak inhibition (28% at 100 nM), indicating that only one nitrogen is tolerated in this area. Conversely, the furane **1** is a highly potent inhibitor (83% inhibition at 100 nM). The moderate activity of the indole **6** (50% inhibition at 100 nM) confirms that there is space available in this area for introduction of substituents on the phenyl ring as seen with **3**. A good (**3**, **4**, **6**, **7**, **8**) to moderate (**1**, **2**) selectivity toward 17β-HSD2 was observed for all compounds with the exception of **5**, which was more potent for 17β-HSD2 (85% HSD2 inhibition vs 41% HSD1 inhibition at 1 μM).

The logP values were calculated using the commercial ChemDrawPro 11.0 program and are indicated in Table 1 and 2. Compared to **B** the calculated logP suggested for all

Table 2. Inhibition of Human 17β-HSD1 and 17β-HSD2 by Compounds **9**−**15**



Cmpd	R2	Inhibition of 17β-HSD1 [%][a]		Inhibition of 17β-HSD2 [%][b]		LogP[c]
		100 nM	1 μM	100 nM	1 μM	
B	H	76	89	22	77	5.6
9	3-OH	83	93	< 10	40	5.2
10	3-COOH	45	80	< 10	34	5.2
11	3-NH_2	74	87	< 10	39	4.8
12		35	82	24	64	4.5
13		< 10	62	24	68	4.2
14		32	65	12	32	5.5
15		88	91	31	71	3.8

[a] Recombinant human 17β-HSD1, substrate [^{3}H]-E1 + E1 [30 nM], cofactor NADPH [1 mM], procedure A; mean value of two determinations, relative standard deviation < 20%. [b] Recombinant human 17β-HSD2, substrate [^{3}H]-E2 + E2 [30 nM], cofactor NAD^+ [1 mM], procedure C, mean value of two determinations, relative standard deviation < 20%. [c] Calculated logP data.

the compounds a better hydrophilicity, which should facilitate membrane permeation.

Various substituents were also introduced in the phenyl ring (Table 2). We focused on substituents which could establish interactions with the protein and which could increase the hydrophilicity of the lipophilic parent compound **B**. Introduction of a hydroxy (**9**) or an amino group (**11**) in position 3 did not dramatically change the inhibition compared to the naked **B** (83%, 74%, and 76% inhibition at 100 nM for **9**, **11** and **B**, respectively). The unsubstituted carboxylic groups (compounds **10** and **13**) led to a decrease in activity (45% and no inhibition at 100 nM, respectively), which might be due to the absence of protonated amino acids in this area of the active site. The amides **12** and **13** bearing different substituents showed reasonable logP but only low or no activity. The most interesting compound in this series was the sulfonamide **15**, which exhibited the lowest logP (3.8) and a high potency. The most potent compounds **9**, **11**, and **15** showed no to low inhibition of 17β-HSD2 (inhibition at 100 nM < 31% for **15**, Table 2) and therefore a good selectivity toward this enzyme.

Intrigued by the selectivity shown by pyrimidine **5** in favor of 17β-HSD2, a few pyrimidine derivatives (Table 3) lacking one OH group (**16**−**18**) or showing a changed hydroxysubstitution pattern (**19** and **20**) were examined with the goal to simplify and identify a new template for the further development of 17β-HSD2 inhibitors. Derivatives bearing one hydroxy group (**16**−**18**) turned out to be inactive on both 17β-HSD1 and 17β-HSD2. The potency could not be improved with derivatives bearing two hydroxy moieties (**19**−**20**). The distance between the two hydroxy groups might be either too long (**20**) or too short (**19**) to establish H-bond interactions.

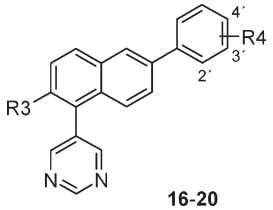
We focused therefore our efforts on the novel 17β-HSD1 inhibitors and measured IC_{50} values for the most potent ones (Table 4) and determined their selectivity factor (SF). All tested compounds showed IC_{50} values in the low nanomolar range between 15 and 70 nM. The selectivity toward 17β-HSD2 could also be optimized with a SF up to 52.

As cell membrane permeation is an important criterion in the development of a drug, the potencies of the compounds were evaluated in a cellular assay using T47D cells (Table 4).

All tested compounds, except the methoxy pyridine **3**, showed a better activity compared to the reference **B** (IC_{50} = 281 nM), the sulfonamide **15** being the best one with an IC_{50} of 71 nM. This finding also indicates, that **15** is metabolically stable in these cells.

Selectivity toward the ERα and ERβ. Designed as steroidomimetics, the synthesized compounds might show some affinity to the ERα and ERβ. Agonistic properties would counteract the therapeutic concept of 17β-HSD1 inhibition and cannot be tolerated. This is also true for antagonistic effects, which could lead to unwanted drug action similar to the ones observed for the antiestrogens or SERMs. The most potent compounds were tested for their binding affinity to the ERs using the recombinant human proteins and applying a competition assay with [^{3}H]-E2. All tested compounds show a relative binding affinity of less than 0.1% to the estrogen receptors except for the reference compound **A**, which is included for comparison and is considered as weak ligand of the ERs (Table 4). A RBA range between 0.01 and 0.1 means that the compound shows an affinity to the ERs between 0.01% and 0.1% compared to the affinity of E2 (RBA = 100%). Because of these results, the compounds are classified as low to very low affinity ligands of the ERs and are not expected to evoke ER-mediated effects under in vivo conditions.

Table 3. Inhibition of Human 17β-HSD1 and 17β-HSD2 by Compounds **16**−**20**



16-20

compd	R3	R4	inhibition of 17β-HSD1 [%][a,b] at 1 μM	inhibition of 17β-HSD2 [%][b,c] at 1 μM
5	OH	3′-OH	41	85
16	H	2′-OH	< 10	< 10
17	H	3′-OH	< 10	< 10
18	OH	H	22	< 10
19	OH	2′-OH	< 10	< 10
20	OH	4′-OH	35	22

[a] Human placenta, cytosolic fraction, substrate [^{3}H]-E1 + E1 [500 nM], cofactor NADH [0.5 mM], procedure B. [b] Mean value of three determinations, standard deviation < 10%. [c] Human placenta, microsomal fraction, substrate [^{3}H]-E2 + E2 [500 nM], cofactor NAD^+ [1.5 mM], procedure D.

Further in Vitro Assays. According to their potency (cell-free and cellular assays) and their selectivity toward 17β-HSD2 and ERs, compounds **2**, **3**, **9**, **11**, and **15** were the best compounds identified in this study. They were further evaluated in our screening system.[56]

Metabolic stability of **2**, **3**, and **11** was determined using rat liver microsomes and human liver microsomes for **15** (Table 5). For **2**, **3**, **11**, and **15**, half-life and intrinsic body clearance were evaluated and compared to the references diazepam (high metabolic stability, $t_{1/2}$ = 41 min) and diphenhydramine (low metabolic stability, $t_{1/2}$ = 7 min). The short half-life of **2** ($t_{1/2}$ = 14 min) and the fast clearance of **11** (Cl_{int} = 93 μL/min/mg protein) indicate a moderate metabolic stability. In the case of **15**, an exceptional metabolic stability was observed: 100% of the compound is still intact after 45 min of incubation.

Subsequently, the compounds were investigated for permeation of Caco-2 cells (Table 5), which are generally accepted to be an appropriate model for the prediction of peroral absorption.[57] All compounds showed medium to high cell permeation.

For the evaluation of potential drug−drug interactions, compounds **2**, **9**, and **11** were tested for inhibition of the most important hepatic CYP enzymes (six human hepatic enzymes: CYP1A2, 2B6, 2C9, 2C19, 2D6, 3A4, Table 6). Compounds **2** and **9** show high inhibition of CYP3A4 (IC_{50} = 0.22 and 0.04 μM, respectively), which is responsible for 50% of drug metabolism. In the case of compound **11**, only CYP2C19 (IC_{50} = 0.68 μM) was slightly inhibited, indicating a rather low risk of drug−drug interactions.

In Vivo Pharmacokinetics in the Rat. The pharmacokinetics of **2**, **9**, **11**, and **15** were determined in Wistar male rats. Compounds **B**, **9**, **11**, and **15** were administered (10 mg/kg, n = 4) in a cassette dosing approach. Compound **11** as well as compound **2** were also administered in a single dose (50 mg/kg, n = 5). Plasma samples were collected over 24 h, and concentrations of each compound were independently measured by LC-MS/MS quantification. The pharmacokinetic profiles are presented in Table 7.

Beside the advantage that data can be generated for more compounds and that fewer animals are used, cassette dosing is mainly used to rank compounds. The data shown in Table 7 indicates that compound **B** has the best area under the

Table 4. IC_{50} Values, Selectivity Factor, and Binding Affinities for the ERα and ERβ for Selected Compounds

	cell-free assay			cell assay		
compd	17β-HSD1 IC_{50} [nM][a,b]	17β-HSD2 IC_{50} [nM][a,c]	SF[d]	17β-HSD1 IC_{50} [nM][a,e]	ERα RBA (%)[a,f]	ERβ RBA (%)[a,f]
A	116	5641	49	229	0.1 < RBA < 1	0.1 < RBA < 1
B	20	540	27	281	0.01 < RBA < 0.1	0.01 < RBA < 0.1
1	70	527	8	nd	0.01 < RBA < 0.1	0.01 < RBA < 0.1
2	26	1157	45	165	0.01 < RBA < 0.1	0.01 < RBA < 0.1
3	33	530	16	670	< 0.01	< 0.01
4	64	3340	52	nd	< 0.01	< 0.01
9	36	959	27	115	< 0.01	0.01 < RBA < 0.1
11	53	1757	33	nd	< 0.01	< 0.01
15	15	403	27	71	0.01 < RBA < 0.1	< 0.01

[a] Mean value of three determinations except IC_{50} (17β-HSD2) for **4** two determinations. [b] Human placenta, cytosolic fraction, substrate [^{3}H]-E1 + E1 [500 nM], cofactor NADH [0.5 mM], procedure B, relative standard deviation < 10%. [c] Human placenta, microsomal fraction, substrate [^{3}H]-E2 + E2 [500 nM], cofactor NAD^+ [1.5 mM], procedure D, relative standard deviation < 8%. [d] SF: selectivity factor = IC_{50} (17β-HSD2)/IC_{50} (17β-HSD1). [e] T47D cells, substrate [^{3}H]-E1 + E1 [50 nM], relative standard deviation < 13%. [f] RBA, relative binding affinity, E2 = 100%, nd, not determined.

Table 5. Metabolic Stability Using Liver Microsomes and Caco-2 Cell Permeation

compd	$t_{1/2}$ [min][a,b]	Cl_{int}[a,c] [μL/min/mg protein]	P_{app}[a,d] [$\times 10^{-6}$ cm/s]	permeability[e]
B	nd	nd	9.3	medium
2	14[f]	340[f]	14	high
3	289[f]	nd[f]	8	medium
11	50[f]	93[f]	16	high
15	nc[g,h]	nc[g,h]	19	high
diazepam	41[f]	113[f]	nd	nd
diphenhydramine	7[f]	680[f]	nd	nd

[a] Mean value of three determinations. [b] $t_{1/2}$: half-life. [c] Cl_{int}: intrinsic body clearance. [d] P_{app}: apparent permeability, relative standard deviation < 9%. [e] Permeability of the investigated molecules was classified according to Yee.[68] [f] Rat liver microsomes. [g] Human liver microsomes. [h] Not calculated as **15** was metabolically stable over 45 min; nd, not determined; nc, not calculated.

Table 6. Inhibition of Selected Hepatic CYP Enzymes by Compounds **2**, **9**, and **11** and Control Inhibitors

	IC_{50} [μM][a]					
	CYP1A2	CYP2B6	CYP2C9	CYP2C19	CYP2D6	CYP3A4
B	7.16	7.96	1.64	1.41	15.49	2.77
2	0.01	5.34	0.15	0.88	22.90	0.22
9	19.04	11.85	0.27	6.03	24.80	0.04
11	10.45	10.4	1.25	0.68	40.00	1.07
control inhibitors	furafylline	tranylcypromine	sulphenazole	tranylcypromine	quinidine	ketoconazole
	3.04	6.96	0.25	3.04	0.01	0.05

[a] Mean value of three determinations, standard deviation < 10% except for **2**: 20%

Table 7. Pharmacokinetic Parameters of Compounds **B**, **2**, **9**, **11**, and **15** in Rats after Oral Application in Cassette Dosing (10 mg/kg) and/or in Single Dose Application (50 mg/kg)

	parameters[a]						
compd	dose [mg/kg]	$C_{max\ obs}$ [ng/mL]	C_z [ng/mL]	$t_{max\ obs}$ [h]	t_z [h]	$t_{1/2z}$ [h]	AUC_{0-tz} [ng·h/mL]
			Cassette Dosing				
B	10	861	115	4	24	5.9	11702
9	10	844	557	6	10	0.9	10681
11	10	43	0.4	2	24	2.4	539
15	10	110	67	2	10	1.1	1332
			Single Dose				
2	50	16	18	2	8	1.5	203
11	50	44	14	2	8	1.6	337

[a] $C_{max\ obs}$, maximal measured concentration; C_z, last analytical quantifiable concentration; $t_{max\ obs}$, time to reach the maximum measured concentration; t_z, time of the last sample which has an analytically quantifiable concentration; $t_{1/2z}$, half-life of the terminal slope of a concentration−time curve; AUC_{0-tz}, area under the concentration−time curve up to the time t_z of the last sample; $AUC_{0-\infty}$, area under the concentration−time curve extrapolated to infinity.

curve (AUC) and C_{max}, followed by **9**, **15**, and **11**. In single dose application, compounds **2** and **11** (AUC = 203 and 337 ng·h/mL, respectively) showed insufficient pharmacokinetics. Accordingly, **2** and **11** could not be considered as potential candidates for further development. Even tested at a 5 times higher dose, a poorer AUC value (337 vs. 539 ng·h/mL for single dose and cassette dosing, respectively) is observed for **11** in the single dose application compared to the one in the cassette dosing experiment. This might be explained by a high metabolism of **11** in the case of the single dose application, while in the cassette experiment, metabolism might be lower because of the competition between the test compounds and the metabolizing enzymes.

Considering that the half-life of the compounds in plasma ($t_{1/2}$) and oral delivery (C_{max} and AUC) are the most important criteria, the hydroxyphenyl compound **9** presents the most favorable profile (AUC = 10681 ng·h/mL, C_{max} = 843 ng/mL, $t_{1/2}$ = 0.9 h). Compound **15** also showed acceptable pharmacokinetics (AUC = 1332 ng·h/mL, C_{max} = 110 ng/mL, $t_{1/2}$ = 1.1 h).

Regarding all biological data, compound **15** can be considered as a good drug candidate while compound **9** inhibits hepatic CYP enzymes and shows faster metabolism.

Discussion and Conclusion

In this study, starting from the hit candidate **B**, we have developed new highly active inhibitors of 17β-HSD1. Exchange of the phenyl moiety by different heteroaromatics is well tolerated but does not improve the activity. Introduction of substituents like carboxylic acid, amide, or morpholine into the phenyl ring are detrimental for the activity. However, in this study, a very interesting steroidomimetic[47] was identified as an inhibitor of 17β-HSD1: the sulfonamide **15**. It is highly potent (cell-free assay IC_{50} = 15 nM) and selective toward 17β-HSD2 and the ERs. In addition, its cellular activity (T47D cells, IC_{50} = 71 nM) was improved compared to **B** (T47D cells, IC_{50} = 281 nM). Such a good cellular activity, which is identified for the first time in this class of inhibitors, indicates that it can permeate membranes easily. This finding demonstrates that **15** is clearly superior to the hybrid inhibitor (EM-1745) and slightly better than **B**. Its favorable pharmacokinetic profile in the rat validates **15**, together with **B**, as two promising drug candidates.

To be appropriate as a drug, a compound should have a favorable ADME/Tox profile as well as no or low toxicity. Hepatic CYP interactions and metabolic stability have been evaluated for the most interesting compounds. Compound **15** showed favorable properties. As toxicity remains a major cause of attrition during development, further toxicology studies should be undertaken for compound **15**. Off-target effects should be investigated by performing metabolomic

studies, and reactive metabolites should be identified and tested as they often cause toxicity.

Sulfonamides are often considered as key polar replacement groups for amides. Interestingly, in this study, the exchange amide (**12**)/sulfonamide (**15**) led to an important increase in activity. This might be explained by the different geometry of the two groups: the amide is planar while the sulfonamide exhibits a tetrahedral shape, allowing these functional groups to undergo H-bond interactions with partners located in different areas of the binding site. Additionally, the presence of the second oxygen in the sulfonamide group is responsible for an increased acidity.

The selectivity demonstrated by the compounds toward 17β-HSD2 and the ERs is an important aspect of this study. Selectivity toward 17β-HSD2 is important because this enzyme catalyzes the reverse reaction compared to 17β-HSD1. 17β-HSD2 inhibitory activity was measured for all compounds, and for the most potent ones a SF was determined. Considering that the expression of 17β-HSD2 is downregulated in EDD tissues, selectivity should be achieved to mainly avoid systemic effects. 17β-HSD2 is present in several organs (liver, small intestine, endometrium, adrenals, bones). It can be expected that inhibition of this enzyme would mainly lead to side effects in estrogen sensitive tissues like endometrium. However, it is difficult to estimate how high the SF should be to minimize potential side effects.

Selectivity toward the ERs is also an important issue. Neither agonistic nor antagonistic effects can be tolerated for ERs to avoid activation of the receptor or systemic effects as observed with the use of antiestrogens. After analysis of the ERs X-ray structures, it became apparent that designing large compounds would be beneficial to achieve selectivity toward the ERs, as the ER binding site is smaller than that of 17β-HSD1 (no cofactor binding pocket present). For the evaluation of the ER affinity, RBA values were determined for all compounds showing a SF (toward 17β-HSD2) higher than 10. All newly reported inhibitors exhibit less than 0.1% relative binding affinity and are therefore considered not to bind to the ERs under in vivo conditions (T47D cell line). This assumption is based on the comparison of the compound's binding affinity with the one of E1. Under 17β-HSD1 inhibitor treatment, E2 levels are decreased and E1 levels are maintained or slightly increased. At an E1 concentration of 50 nM, 71 nM of **15** are needed to suppress E2 formation by 50% (Table 4). E1 itself is a ligand of the ERs with a relative binding affinity of about 10%.[58,59] Thus, the inhibitors compete with E1 for the ER binding. Because of their RBA values of less than 0.1%, they will be displaced by E1 from the ER binding site and are unlikely to exert an ER mediated effect in vivo.

In this study, the pyrimidine **5** was identified as a 17β-HSD2 inhibitor (IC_{50} = 249 and 1387 nM for 17β-HSD2 and 17β-HSD1, respectively, with a SF of 6 in favor of 17β-HSD2). Contrarily, the pyridines **2** and **4** showed a high potency for 17β-HSD1 (IC_{50} = 26 and 64 nM, respectively), indicating that the position of the N is not critical for the inhibition. It is striking that introduction of one additional nitrogen in **2** induced a dramatic loss in 17β-HSD1 inhibitory activity. This might be due to a direct effect of the nitrogen but also to an unfavorable electronic distribution increasing the electron deficiency in the heterocycle. As a consequence the acidity of the naphthol-OH located in ortho position to the heteroring is increased and prevents tight interactions with amino acids in the binding pocket as observed for **2** or **B**. In the case of the dimethoxypyrimidine **8**, a regain in activity was observed (79% inhibition at 1 μM for **8**). This might be due to the increase of electronic density caused by the dimethoxy substituents or to the *ortho*-effect forcing the second nitrogen in a different area.

Attempts to identify a new template for 17β-HSD2 inhibitors with only one OH group failed. Change in the dihydroxy-substitution pattern led to a complete loss of activity. Obviously, this protein is rather inflexible, i.e., it cannot adopt a favorable conformation to bind the ligand. A similar phenomenon has been observed for 17β-HSD1,[47] where sharp SARs were also proposed as a result of enzyme rigidity.

In this paper, we reported on the synthesis and biological evaluation of new 1-substituted hydroxyphenyl-2-naphthols as 17β-HSD1 inhibitors. The goal of the study was the identification of a drug-like steroidomimetic with a good in vivo profile and ADME/Tox properties. We discovered the highly potent methyl sulfonamide **15** (with a very good cellular activity), which proved to be selective toward 17β-HSD2 and ERs. It shows good membrane permeation, low metabolism, and rather good pharmacokinetics after peroral application. The efficacy of this compound still needs to be demonstrated in an appropriate disease oriented animal model. The identification of the species, in which this compound will show the best 17β-HSD1 inhibitory activity, will be the goal of another study.

Experimental Section

Chemical Methods. IR spectra were measured neat on a Bruker Vector 33FT-infrared spectrometer.

^{1}H NMR spectra were recorded on a Bruker DRX-500 (500 MHz) instrument at 300 K in $CDCl_3$, CD_3OD, DMSO-d_6, and acetone-d_6. Chemical shifts are reported in δ values (ppm), and the hydrogenated residues of deuteriated solvents were used as internal standard ($CDCl_3$: δ = 7.26 ppm in ^{1}H NMR and δ = 77 ppm in ^{13}C NMR, CD_3OD: δ = 3.35 ppm in ^{1}H NMR and δ = 49.3 ppm in ^{13}C NMR, DMSO-d_6: δ = 2.50 ppm in ^{1}H NMR and δ = 39.5 ppm in ^{13}C NMR, acetone-d_6: δ = 2.05 ppm in ^{1}H NMR and δ = 29.8 ppm and 206.3 ppm in ^{13}C NMR). Signals are described as s, d, t, dd, ddd, m, b for singlet, doublet, triplet, doublet of doublet, doublet of doublet of doublet, multiplet, and broad, respectively. All coupling constants (J) are given in Hz.

Mass spectra (ESI) were measured on a TSQ Quantum instrument (ThermoFisher).

Chemical names follow IUPAC nomenclature.

All microwave irradiation experiments were carried out in a CEM-Discover microwave apparatus.

Column chromatography was performed using silica gel (70−200 μm), and the reaction progress was determined by TLC analyses on ALUGRAM SIL G/UV_{254} (Macherey-Nagel). Preparative chromatography was performed on glass plate SIL G-100/UV_{254} (TLC, silica, 1 mm thick) from Macherey-Nagel. Visualization was accomplished with UV light. Purifications with preparative HPLC were carried out on a Agilent 1200 series HPLC system from Agilent Technologies, using a RP C18 Nucleodur 100−5 column (30 mm × 100 mm/50 μm from Macherey-Nagel GmbH) as stationary phase, with acetonitrile/water as solvent in a gradient from 20:80 to 100:0.

Tested compounds are ≥95% chemical purity as measured by HPLC. The methods for HPLC analysis and a table of purity for all tested compounds are provided in the Supporting Information.

Starting materials (different boronic acids and compounds **9a**, **16f**, **18c**, and **19d**) were purchased from Aldrich, Acros, Lancaster, or Fluka and were used without purification. No attempts were made to optimize yields.

The following compounds were prepared according to previously described procedures: 6-(3-hydroxyphenyl)-2-naphthol **A**,[47] 6-(3-hydroxyphenyl)-1-phenyl-2-naphthol **B**,[48] 1-bromo-6-(3-hydroxyphenyl)-2-naphthol **1a**,[48] 1-bromo-2-methoxy-6-(3-methoxyphenyl)naphthalene **1b**,[48] 5-oxo-5,6,7,8-tetrahydronaphthalen-2-yl trifluoromethanesulfonate **16e**,[60] 6-(3-methoxyphenyl)-3,4-dihydronaphthalen-1(2*H*)-one **17d**,[61] 6-(4-hydroxyphenyl)-2-naphthol **20b**.[52]

General Procedure for Ether Cleavage. Method A. To a solution of methoxy derivative (1 equivalent (equiv)) in dichloromethane cooled at −78 °C boron tribromide (1 M solution in dichloromethane, 3−5 equiv per methoxy function) was slowly added under N_2. The reaction mixture was stirred at −78 °C for 1 h and at room temperature overnight. The reaction was quenched by the addition of 2% Na_2CO_3 and extracted with dichloromethane. The combined organic layers were washed with brine and dried over magnesium sulfate and concentrated to dryness.

General Procedures for Suzuki Coupling. Method B. A mixture of arylbromide (1 equiv), boronic acid (1 equiv), 10% aqueous solution of sodium carbonate (2 equiv), and tetrakis(triphenylphosphine) palladium(0) (0.05 equiv) in toluene/ethanol mixture (oxygen free) was stirred at 80 °C under nitrogen for several hours. The reaction mixture was cooled to room temperature, quenched by the addition of 2% HCl, and extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate, and concentrated to dryness.

Method C. A mixture of arylbromide (1 equiv), boronic acid (1.3 equiv), cesium carbonate (2 equiv), and tetrakis(triphenylphosphine) palladium(0) (0.05 equiv) was suspended in a DME/EtOH/water (1:1:1) solution. The reaction mixture was exposed to microwave irradiation (25 min, 150 W, 150 °C, 15 bar). After reaching room temperature, 1N NH_4Cl was added and the aqueous layer was extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, filtered, and concentrated to dryness.

Method D. A mixture of arylbromide (1 equiv), boronic acid (1.2 equiv), potassium carbonate (2 equiv), palladium acetate (0.1 equiv), and triphenylphosphine (0.2 equiv) was dissolved in a 1,4-dioxane/EtOH (2:1) solution. The reaction mixture was exposed to microwave irradiation (15 min, 150 W, 150 °C, 15 bar). After reaching room temperature, the solution was filtered off and water and dichloromethane were added to the filtrate. The aqueous layer was extracted with dichloromethane, and the combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated to dryness.

1-(3-Furyl)-6-(3-hydroxyphenyl)-2-naphthol (1). The title compound was prepared by reaction of 1-bromo-6-(3-hydroxyphenyl)-2-naphthol **1a** (80 mg, 0.25 mmol, 1 equiv) with furan-3-boronic acid (28 mg, 0.25 mmol, 1 equiv) in toluene/ethanol 5:1 at 80 °C for 2 h according to Method B. The analytically pure compound was obtained after purification by column chromatography under pressure (dichloromethane/methanol 98:2) in 46% yield (35 mg); $C_{20}H_{14}O_3$; MW 302. ^{1}H NMR (CD_3OD + 3 drops $CDCl_3$): δ 7.98 (d, J = 1.9 Hz, 1H), 7.86 (d, J = 8.8 Hz, 1H), 7.79 (d, J = 8.8 Hz, 1H), 7.71−7.70 (m, 1H), 7.68−7.67 (m, 1H), 7.64 (dd, J = 2.2 Hz, J = 8.8 Hz, 1H), 7.31−7.28 (m, 1H), 7.23 (d, J = 8.8 Hz, 1H), 7.20−7.19 (m, 1H), 7.18−7.17 (m, 1H), 6.81 (ddd, J = 0.9 Hz, J = 2.5 Hz, J = 7.9 Hz, 1H), 6.65 (dd, J = 0.9 Hz, J = 1.9 Hz, 1H). ^{13}C NMR (CD_3OD + 3 drops $CDCl_3$): δ 161.3, 156.1, 146.6, 146.3, 145.8, 139.4, 137.2, 133.5, 133.0, 132.8, 129.4, 128.7, 122.5, 122.1, 121.9, 117.7, 117.5, 116.9, 116.1. IR: 3340, 1601, 1493 cm^{-1}. MS (ESI): 301 $(M - H)^-$.

6-(3-Hydroxyphenyl)-1-(pyridin-3-yl)-2-naphthol (2). The title compound was prepared by reaction of 1-bromo-6-(3-hydroxyphenyl)-2-naphthol **1a** (100 mg, 0.32 mmol, 1 equiv) with pyridine-3-boronic acid (39 mg, 0.32 mmol, 1 equiv) in toluene/ethanol 2:1 at 80 °C for 1 h according to Method B. The analytically pure compound was obtained after purification by column chromatography under pressure (gradient hexane/ethyl acetate 3:2 to 0:1) in 59% yield (59 mg); $C_{21}H_{15}NO_2$; MW 313. ^{1}H NMR (CD_3OD): δ 9.03−9.02 (m, 1H), 8.95 (d, J = 5.7 Hz, 1H), 8.81−8.79 (m, 1H), 8.30 (dd, J = 5.7 Hz, J = 7.9 Hz, 1H), 8.12 (d, J = 1.9 Hz, 1H), 8.04 (d, J = 8.8 Hz, 1H), 7.75 (dd, J = 1.9 Hz, J = 8.8 Hz, 1H), 7.55 (d, J = 8.5 Hz, 1H), 7.35 (d, J = 9.1 Hz, 1H), 7.32 (t, J = 7.9 Hz, 1H), 7.23−7.21 (m, 1H), 7.18−7.17 (m, 1H), 6.83 (ddd, J = 0.9 Hz, J = 2.5 Hz, J = 7.9 Hz, 1H). ^{13}C NMR (CD_3OD): δ 159.1, 154.1, 151.0, 144.8, 143.2, 140.8, 133.3, 133.1, 131.0, 130.3, 128.4, 128.2, 127.2, 124.1, 119.3, 119.1, 115.4, 114.8. IR: 3091, 1581, 1493, 1276, 1209, 1180 cm^{-1}. MS (ESI): 314 $(M + H)^+$.

6-(3-Hydroxyphenyl)-1-(4-methoxypyridin-3-yl)-2-naphthol (3). The title compound was prepared by reaction of 1-bromo-6-(3-hydroxyphenyl)-2-naphthol **1a** (100 mg, 0.32 mmol, 1 equiv) with 4-methoxypyridin-3-boronic acid (49 mg, 0.32 mmol, 1 equiv) in toluene/ethanol 5:1 at 80 °C for 3 h according to Method B. The analytically pure compound was obtained after purification by column chromatography under pressure (hexane/ethyl acetate 8:2) in 84% yield (92 mg); $C_{22}H_{17}NO_3$; MW 343. ^{1}H NMR (CD_3OD): δ 8.15 (d, J = 2.5 Hz, 1H), 8.01 (d, J = 1.9 Hz, 1H), 7.84 (d, J = 8.8 Hz, 1H), 7.72 (dd, J = 2.5 Hz, J = 8.5 Hz, 1H), 7.61 (dd, J = 1.9 Hz, J = 8.8 Hz, 1H), 7.49 (d, J = 8.8 Hz, 1H), 7.29−7.26 (m, 1H), 7.26 (d, J = 8.8 Hz, 1H), 7.20−7.19 (m, 1H), 7.18−7.17 (m, 1H), 6.98 (dd, J = 0.6 Hz, J = 8.5 Hz, 1H), 6.81 (ddd, J = 0.9 Hz, J = 2.5 Hz, J = 8.2 Hz, 1H), 4.02 (s, 3H). ^{13}C NMR (CD_3OD): δ 164.8, 158.9, 153.6, 149.5, 143.7, 143.5, 136.8, 134.7, 130.9, 130.3, 126.9, 126.8, 125.5, 119.4, 118.9, 115.1, 114.8, 111.2, 54.2. IR: 3357, 2917, 2849, 1586, 1493 cm^{-1}. MS (ESI): 344 $(M + H)^+$.

6-(3-Hydroxyphenyl)-1-(pyridin-4-yl)-2-naphthol (4). The title compound was prepared by reaction of 1-bromo-6-(3-hydroxyphenyl)-2-naphthol **1a** (100 mg, 0.32 mmol, 1 equiv) with pyridine-4-boronic acid (39 mg, 0.32 mmol, 1 equiv) in toluene/ethanol 5:1 at 100 °C for 1 h according to Method B. The analytically pure compound was obtained after purification by column chromatography under pressure (hexane/ethyl acetate 8:2) in 44% yield (44 mg); $C_{21}H_{15}NO_2$; MW 313. ^{1}H NMR (CD_3OD): δ 8.95 (d, J = 6.9 Hz, 2H), 8.26 (d, J = 2.6 Hz, 2H), 8.13 (d, J = 1.9 Hz, 1H), 8.06 (d, J = 8.8 Hz, 1H), 7.77 (dd, J = 1.9 Hz, J = 8.8 Hz, 1H), 7.65 (d, J = 8.8 Hz, 1H), 7.34 (d, J = 8.8 Hz, 1H), 7.34−7.31 (m, 1H), 7.24−7.22 (m, 1H), 7.18−7.17 (m, 1H), 6.84 (ddd, J = 0.9 Hz, J = 2.2 Hz, J = 7.9 Hz, 1H). ^{13}C NMR (CD_3OD): δ 159.0, 153.0, 149.9, 148.1, 143.5, 137.1, 133.8, 133.1, 133.0, 131.6, 130.9, 130.0, 129.9, 128.4, 127.2, 126.8, 125.2, 119.4, 119.3, 115.2, 114.8. IR: 3080, 1631, 1596, 1580, 1359, 1276, 1201, 1179 cm^{-1}. MS (ESI): 314 $(M + H)^+$.

1,6-Bis(3-hydroxyphenyl)-2-naphthol (9). The title compound was prepared by reaction of 1,6-dibromo-2-naphthol **9a** (200 mg, 0.66 mmol, 1 equiv) with 3-hydroxyphenylboronic acid (366 mg, 2.64 mmol, 4 equiv) in toluene for 15 h according to Method B. The analytically pure compound was obtained after purification by column chromatography (gradient dichloromethane/methanol 99:1 to 90:10) in 20% yield (43 mg); $C_{22}H_{16}O_3$; MW 328. ^{1}H NMR ($CDCl_3$ + 3 drops CD_3OD): δ 7.92 (d, J = 1.6 Hz, 1H), 7.76 (d, J = 8.8 Hz, 1H), 7.53 (dd, J = 1.9 Hz, J = 8.8 Hz, 1H), 7.48 (d, J = 8.8 Hz, 1H), 7.39−7.36 (m, 1H), 7.26−7.23 (m, 1H), 7.20 (d, J = 8.8 Hz, 1H), 7.16−7.14 (m, 1H), 7.11−7.10 (m, 1H), 6.92 (ddd, J = 0.9 Hz, J = 2.5 Hz, J = 8.2 Hz, 1H), 6.89−6.87 (m, 1H), 6.85−6.84 (m, 1H), 6.78 (ddd, J = 0.9 Hz, J = 2.5 Hz, J = 7.9 Hz, 1H). ^{13}C NMR ($CDCl_3$ + 3 drops CD_3OD): 190.7, 157.5, 156.9, 150.3, 142.6, 135.9, 135.8, 132.5, 130.5, 129.8, 129.5, 125.7, 125.2, 122.4, 121.0, 118.9, 117.9, 117.8, 115.3, 114.0. IR: 3355, 1702, 1581, 1494, 1447, 1203, 1154 cm^{-1}. MS (ESI): 327 $(M - H)^-$.

1-(3-Aminophenyl)-6-(3-hydroxyphenyl)-2-naphthol (11). The title compound was prepared by reaction of 3-[2-methoxy-6-(3-methoxyphenyl)-1-naphthyl]aniline **11a** (1.0 g, 2.82 mmol, 1 equiv) with boron tribromide (14.1 mL, 14.1 mmol, 5 equiv) according to Method A. The title compound was obtained pure in a quantitative yield (922 mg); $C_{22}H_{17}NO_2$; MW 327. ^{1}H NMR

(CD_3OD): δ 8.00 (d, J = 1.6 Hz, 1H), 7.82 (d, J = 8.8 Hz, 1H), 7.58 (dd, J = 1.9 Hz, J = 8.8 Hz, 1H), 7.54 (d, J = 8.8 Hz, 1H), 7.32−7.29 (m, 1H), 7.29 (t, J = 7.9 Hz, 1H), 7.23 (d, J = 9.1 Hz, 1H), 7.22−7.20 (m, 1H), 7.17−7.16 (m, 1H), 6.87−6.85 (m, 1H), 6.81−6.79 (m, 2H), 6.75−6.73 (m, 1H). ^{13}C NMR (CD_3OD): δ 158.9, 152.4, 148.9, 143.9, 136.6, 134.6, 130.8, 130.2, 130.0, 126.5, 126.4, 126.3, 123.5, 122.1, 119.5, 119.4, 119.3, 115.7, 115.0, 114.8. IR: 3387, 3282 cm^{-1}. MS (ESI): 326 $(M - H)^-$.

***N*-{3-[2-Hydroxy-6-(3-hydroxyphenyl)-1-naphthyl]phenyl}-methanesulfonamide (15).** The title compound was prepared by reaction of 1-bromo-6-(3-hydroxyphenyl)-2-naphthol **1a** (100 mg, 0.32 mmol, 1 equiv) with *N*-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]methanesulfonamide (94 mg, 0.32 mmol, 1 equiv) in toluene/ethanol 5:1 for 2 h according to Method B. The analytically pure compound was obtained after purification by column chromatography under pressure (hexane/ethyl acetate 3:2) in 64% yield (83 mg); $C_{23}H_{19}NO_4S$; MW 405. ^{1}H NMR (CD_3OD + 3 drops $CDCl_3$): δ 8.01 (d, J = 1.6 Hz, 1H), 7.85 (d, J = 9.1 Hz, 1H), 7.60 (dd, J = 1.9 Hz, J = 8.8 Hz, 1H), 7.53−7.50 (m, 2H), 7.39−7.37 (m, 1H), 7.32−7.31 (m, 1H), 7.29 (t, J = 7.9 Hz, 1H), 7.26 (d, J = 8.8 Hz, 1H), 7.23−7.20 (m, 2H), 7.17−7.16 (m, 1H), 6.82−6.80 (m, 1H), 3.07 (s, 3H). ^{13}C NMR (CD_3OD + 3 drops $CDCl_3$): δ 161.4, 155.3, 146.3, 142.1, 141.7, 139.3, 136.8, 133.4, 133.1, 133.0, 132.8, 131.2, 129.3, 129.2, 128.6, 127.0, 124.9, 123.0, 122.0, 121.9, 117.6, 117.4, 64.1. IR: 3406, 1704, 1600, 1585, 1323, 1268 cm^{-1}. MS (ESI): 404 $(M - H)^-$.

LogP Determination. The LogP values of compounds **B** and **1**−**15** were calculated from CambridgeSoft Chem & Bio Draw 11.0 using the ChemDrawPro 11.0 program.

Biological Methods. [2,4,6,7-^{3}H]-E1 and [2,4,6,7-^{3}H]-E2 were purchased from Perkin-Elmer, Boston, MA. Quickszint Flow 302 scintillator fluid was bought from Zinsser Analytic, Frankfurt. T47D cells (passage 9) were obtained from ECACC, Salisbury, UK. FCS was purchased from Pan Biotech GmbH. Cell culture media was bought from CCPRO, Oberdorla, Germany. Other chemicals were purchased from Sigma, Roth, or Merck.

1. 17β-HSD1 and 17β-HSD2 Enzyme Preparation. Recombinant Human Enzyme (17β-HSD1, Procedure A; 17β-HSD2, Procedure C). Recombinant baculovirus was produced by the "Bac to Bac Expression System" (Invitrogen). Recombinant bacmid was transfected to Sf9 insect cells using "Cellfectin Reagent" (Invitrogen). Sixty hours later, cells were harvested; the microsomal fraction was isolated as described by Puranen.[62] Aliquots containing 17β-HSD1 or 17β-HSD2 were stored frozen until determination of enzymatic activity.

Human Placental Enzyme (17β-HSD1, Procedure B; 17β-HSD2, Procedure D). 17β-HSD1 and 17β-HSD2 were obtained from human placenta according to previously described procedures.[56,63] Fresh human placenta was homogenized and the enzymes were separated by fractional centrifugation at 1000g, 10000g, and 150000g. For the purification of 17β-HSD1, the cytosolic fraction was precipitated with ammonium sulfate. 17β-HSD2 was obtained from the microsomal fraction. Aliquots containing 17β-HSD1 or 17β-HSD2 were stored frozen.

2. Inhibition of 17β-HSD1 in Cell-Free Assay. The synthesized compounds were tested for their ability to inhibit 17β-HSD1 according to Procedure A (recombinant human enzyme, percentage of inhibition determination) or Procedure B (human placental enzyme). For selected compounds, IC_{50} values were determined according to Procedure B (human placental enzyme). Procedures A and B differ in enzyme source and substrate concentration. The two procedures have been compared and give similar results.

Procedure A Using Recombinant Human Enzyme. Assay: Recombinant human protein (0.1 μg/mL) was incubated in 20 mM KH_2PO_4 pH 7.4 with 30 nM [^{3}H]-E1 and 1 mM NADPH for 30 min at room temperature, in the presence of potential inhibitors at concentrations of 1 μM or 100 nM. Inhibitor stock solutions were prepared in DMSO. Final concentration of DMSO was adjusted to 1% in all samples. The enzyme reaction was stopped by addition of 10% trichloroacetic acid (final concentration). Samples were centrifuged in a microtiter plate at 4000 rpm for 10 min. Supernatants were applied to reverse phase HPLC on a Waters Symmetry C18 column, equipped with a Waters Sentry Guard column. Isocratic HPLC runs were performed at room temperature at a flow rate of 1 mL/min of acetonitrile/water (48:52) as eluent. Radioactivity of the eluate was monitored by a Packard flow scintillation analyzer. Total radioactivities for E1 and E2 were determined in each sample. The conversion rate was calculated according to the following formula: % conversion = 100 [(cpm E2 in sample with inhibitor)/(cpm E1 in sample with inhibitor + cpm E2 in sample with inhibitor)]/[(cpm E2 in sample without inhibitor)/(cpm E1 in sample without inhibitor + cpm E2 in sample without inhibitor)]. Percentage of inhibition was calculated according to the following equation: % inhibition = 100 − % conversion. Each value was calculated from two independent experiments.

Procedure B Using Human Placental Enzyme. Assay: Inhibitory activities were evaluated by an established method with minor modifications.[56,63−65] Briefly, the enzyme preparation was incubated with NADH [0.5 mM] in the presence of potential inhibitors at 37 °C in a phosphate buffer (50 mM) supplemented with 20% of glycerol and EDTA 1 mM. Inhibitor stock solutions were prepared in DMSO. 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 $HgCl_2$ and the mixture was extracted with ether. After evaporation, the steroids were dissolved in acetonitrile. E1 and E2 were separated using acetonitrile/water (45:55) as mobile phase in a C18 RP chromatography column (Nucleodur C18, 3 μm, Macherey-Nagel, Düren) connected to a HPLC-system (Agilent 1100 series, Agilent Technologies, Waldbronn, Germany). Detection and quantification of the steroids were performed using a radioflow detector (Berthold Technologies, Bad Wildbad, Germany). The conversion rate was calculated according to the following equation: % conversion = [(%E2)/(% E2 + %E1)] × 100. Each value was calculated from at least three independent experiments. Compounds showing < 10% inhibition were considered to be inactive.

3. Inhibition of 17β-HSD2 in Cell-Free Assay. The synthesized compounds were tested for their ability to inhibit 17β-HSD2 according to Procedure C (recombinant human enzyme) or Procedure D (human placental enzyme). For select compounds, IC_{50} values were determined according to Procedure D (human placental enzyme). Procedures C and D differ from enzyme source and substrate concentration. The two procedures have been compared and give similar results.

Procedure C Using Recombinant Human Enzyme. The 17β-HSD2 inhibition assay was performed as previously described for 17β-HSD1 according to Procedure A from the recombinant human protein, using [^{3}H]-E2 as substrate [30 nM] and NAD^+ [1 mM] as cofactor.

Procedure D Using Human Placental Enzyme. The 17β-HSD2 inhibition assay was performed similarly to the 17β-HSD1 procedure B. The microsomal fraction was incubated with NAD^+ [1.5 mM], test compound, and a mixture of unlabeled- and [^{3}H]-E2 (final concentration: 500 nM, 0.11 μCi) for 20 min at 37 °C. Further treatment of the samples and HPLC separation was carried out as mentioned above for 17β-HSD1. Compounds showing < 10% inhibition were considered to be inactive.

4. Inhibition of 17β-HSD1 in Cellular Assay. Cellular 17β-HSD1 activity was measured using the breast cancer cell line T47D. 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 1 × 10^6 cells/well in DMEM medium with FCS, L-glutamine and the antibiotics

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. 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 incubation, the enzymatic reaction was stopped by removing of the supernatant medium. The steroids were extracted with ether. Further treatment of the samples was carried out as mentioned for the 17β-HSD1 assay (Procedure B).

5. ER Affinity. The binding affinity of selected compounds to the ERα and ERβ was determined according to Zimmermann et al.[66] using recombinant human proteins. Briefly, 0.25 pM of ERα or ERβ, respectively, were incubated with [^{3}H]-E2 (10 nM) and test compound for 1 h at room temperature. The potential inhibitors were dissolved in DMSO (5% final concentration). Evaluation of 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, Germany) was added and the samples were measured in a liquid scintillation counter (Rack Beta Primo 1209, Wallac, Turku, Finland). From these results, the percentage of [^{3}H]-E2 displacement by the compounds was calculated. The plot of % displacement versus compound concentration resulted in sigmoidal binding curves. The compound concentration to displace 50% of the receptor bound [^{3}H]-E2 were determined. Unlabeled E2 was used as a reference. For determination of the relative binding affinity (RBA), the ratio was calculated according to the following equation: RBA-[%] = [(IC_{50}(E2))/(IC_{50}(compound))] × 100.[67] This results in an RBA value of 100% for E2. After the assay was established and validated, a modification was made to increase throughput. Compounds were tested at concentrations of 1000 × IC_{50}(E2) and 10000 × IC_{50}(E2). Results were reported as RBA ranges. Compounds with less than 50% displacement of [^{3}H]-E2 at a concentration of 10000 × IC_{50}(E2) were classified as RBA < 0.01%, compounds that displace more than 50% at 10000 × IC_{50}(E2) but less than 50% at 1000 × IC_{50}(E2) were classified as 0.01% < RBA < 0.1%.

6. Caco-2 Assay. Caco-2 cell culture and transport experiments were performed according to Yee[68] with small modifications. Cell culture time was reduced from 21 to 10 days by increasing seeding density from 6.3×10^4 to 1.65×10^5 cells per well. Four reference compounds (atenolol, testosterone, ketoprofene, erythromycin) were used in each assay for validation of the transport properties of the Caco-2 cells. The compounds were applied to the cells as a mixture (cassette dosing) to increase the throughput of the cell permeability tests. The initial concentration of the compounds in the donor compartment was 50 μM (for each compound, in buffer 0.2 M MES, pH 6.5, containing either 1% ethanol or DMSO). Samples were taken from the acceptor side after 0, 60, 120, and 180 min and from the donor side after 0 and 180 min. Each experiment was run in triplicate. The integrity of the monolayers was checked by measuring the transepithelial electrical resistance (TEER) before the transport experiments and by measuring lucifer yellow permeability after each assay. All samples of the CaCo-2 transport experiments were analyzed by LC/MS/MS. The apparent permeability coefficients (P_{app}) were calculated using equation $P_{app} = (dQ/dtAc_0)$, where dQ/dt is the appearance rate of mass in the acceptor compartment, A the surface area of the transwell membrane, and c_0 the initial concentration in the donor compartment.

7. Metabolic Stability Assay. The assay was performed with liver microsomes from male Sprague−Dawley rats (BD Gentest, Heidelberg, Germany). Stock solutions (10 mM in acetonitrile) were diluted to give working solutions in 20% acetonitrile. The incubation solutions consisted of a microsomal suspension of 0.33 mg/mL of protein in phosphate buffer 100 mM pH 7.4 and 90 μL NADP-regenerating system ($NADP^+$ 1 mM, glucose-6-phosphate 5 mM, glucose-6-phosphate dehydrogenase 5 U/mL, $MgCl_2$ 5 mM).

The reaction was initiated by the addition of test compound to the preincubated microsomes/buffer mix at 37 °C. The samples were removed from the incubations after 0, 15, 30, and 60 min and processed for acetonitrile precipitation. The samples were analyzed by LC-MS/MS. Two control groups were run in parallel: positive controls (PC; $n = 1$) using 7-ethoxycoumarin as reference compound to prove the quality of the microsomal enzymatic activity and negative controls (NC; $n = 1$), using boiled microsomes (boiling water bath, 25 min) without regenerating system to ensure that the potential apparent loss of parent compound in the assay incubation is due to metabolism. The amount of compound in the samples was expressed in percentage of remaining compound compared to time point zero (= 100%). These percentages were plotted against the corresponding time points, and the half-life time was derived by a standard fit of the data.

Intrinsic clearance (Cl_{int}) estimates were determined using the rate of parent disappearance. The slope ($-k$) of the linear regression from log [test compound] versus time plot was determined as well as the elimination rate constant: $k = \ln 2/t_{1/2}$. The equation expressing the microsomal Cl_{int} can be derived: $Cl_{int} = k \cdot V \cdot f_u$ [μL/min/mg protein], where f_u is the unbound fraction. V gives a term for the volume of the incubation expressed in microliters per mg protein. As f_u is not known for the tested compound, the calculation was performed with $f_u = 1$ (V = incubation volume [μL]/microsomal protein [mg] = 6667).

8. Inhibition of Human Hepatic CYPs. The commercially available P450 inhibition kits from BD Gentest (Heidelberg, Germany) were used according to the instructions of the manufacturer. Compounds were tested for inhibition of the following enzymes: CYP1A2, 2B6, 2C9, 2C19, 2D6, and 3A4. Inhibitory potencies were determined as IC_{50} values.

9. Evaluation of Plasma Concentrations of Compounds B, 9, 11, and 15 after Peroral Application to Adult Male Rats in Cassette Dosing. Four adult male Wistar rats (Janvier, France) were used. Animals were housed in a temperature-controlled room (20−22 °C) and maintained in a 12 h light/12 h dark cycle. Food and water were available ad libitum. Rats were anaesthetized with a ketamine (135 mg/kg)/xylazine (10 mg/kg) mixture and cannulated with silicone tubing via the right jugular vein. Prior to the first blood sampling, animals were connected to a counterbalanced system and tubing to perform blood sampling in the freely moving rat. The tested compounds, dissolved in labrasol/water (1:1) as vehicle, were administered in peroral doses of 10 mg/kg in a cassette dosing approach. At time 0, the tested compounds were applied and blood samples (200 μL) were taken at 1, 2, 3, 4, 6, 8, 10, and 24 h postdose, collected in heparinized tubes and stored on ice. Plasma was harvested and kept at −20 °C until being assayed. HPLC-MS/MS analysis and quantification of the samples was carried out on a Surveyor HPLC system coupled with a TSQ Quantum (ThermoFisher) triple quadrupole mass spectrometer equipped with an electrospray ioninterface (ESI).

10. Evaluation of Plasma Concentrations of Compounds 2 and 11 after Peroral Application to Adult Male Rats in Single Dose Application. Five adult male Wistar rats (Janvier, France) were used in similar conditions as described in the cassette dosing experiment. The tested compounds, dissolved in labrasol/water (1:1) as vehicle, were administered in peroral doses of 50 mg/kg. At time 0, the tested compounds were applied and blood samples (200 μL) were taken at 1, 2, 3, 4, 6, 8, 10, and 24 h postdose and treated the same way as described in the cassette dosing experiment.

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Supporting Information Available: Chemical synthesis and characterization of compounds **5**−**8**, **10**, **11a**, **12**−**14**, and **16**−**20** as well as HPLC purity determination. This material is available free of charge via the Internet at http://pubs.acs.org.

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