Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

Introduction of an Electron Withdrawing Group on the Hydroxyphenylnaphthol Scaffold Improves the Potency of 17β-Hydroxysteroid Dehydrogenase Type 2 (17β-HSD2) Inhibitors

Marie Wetzel,[†] Sandrine Marchais-Oberwinkler,[†] Enrico Perspicace,[†] Gabriele Möller,[§] Jerzy Adamski,[§,∥] and Rolf W. Hartmann*[,†,‡]

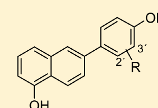
[†]Pharmaceutical and Medicinal Chemistry, Saarland University, and [‡]Helmholtz Institute for Pharmaceutical Sciences Saarland (HIPS), Campus C2$_3$, D-66041 Saarbrücken, Germany

[§]Genome Analysis Center, Institute of Experimental Genetic, Helmholtz Zentrum München, 85764 Neuherberg, Germany

[∥]Lehrstuhl für Experimentelle Genetik, Technische Universität München, 85350 Freising-Weihenstephan, Germany

Supporting Information

ABSTRACT: Estrogen deficiency in postmenopausal women or elderly men is often associated with the skeletal disease osteoporosis. The supplementation of estradiol (E2) in osteoporotic patients is known to prevent bone fracture but cannot be administered because of adverse effect. As 17β-hydroxysteroid dehydrogenase type 2 (17β-HSD2) oxidizes E2 to its inactive form estrone (E1) and has been found in osteoblastic cells, it is an attractive target for the treatment of osteoporosis. Twenty-one novel, naphthalene-derived compounds have been synthesized and evaluated for their 17β-HSD2 inhibition and their selectivity toward 17β-HSD1 and the estrogen receptors (ERs) α and β. Compound **19** turned out to be the most potent and selective inhibitor of 17β-HSD2 in cell-free assays and had a very good cellular activity in MDA-MB-231 cells, expressing naturally 17β-HSD2. It also showed marked inhibition of the E1-formation by the rat and mouse orthologous enzymes and strong inhibition of monkey 17β-HSD2. It is thus an appropriate candidate to be further evaluated in a disease-oriented model.

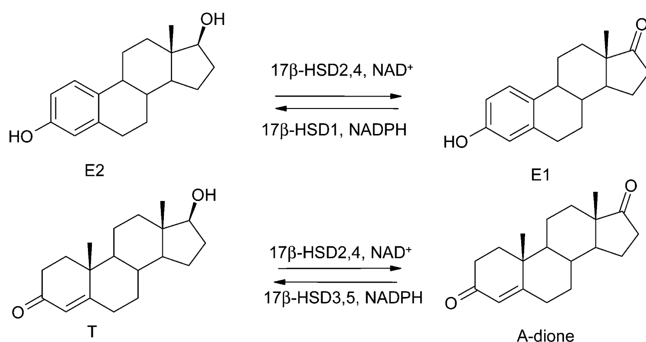


Compd	R	IC_{50} (nM)	Selectivity factor
A	H	302	8
3	3′-CH_3	275	6
11	2′-F	132	17
19	3′-CF_3	19	32

■ INTRODUCTION

17β-Hydroxysteroid dehydrogenase type 2 (17β-HSD2) catalyzes the conversion of the biologically active sex steroids estradiol (E2) and testosterone (T) into their inactive forms using NAD^+ as cofactor (Chart 1). It is a transmembrane protein,[1] and no crystal structure is available yet. This enzyme is abundantly expressed in placenta, liver, small intestine, and endometrium and to a lesser extend also in kidney, pancreas, colon, uterus, breast, and prostate. It was also found in osteoblastic cells.[1−4]

Chart 1. Interconversion of Estradiol (E2) to Estrone (E1) by 17β-HSD2, 17β-HSD4, and 17β-HSD1 and of Testosterone (T) to Androstenedione (A-dione) by 17β-HSD2, 17β-HSD4, 17β-HSD3, and 17β-HSD5



In bone physiology, osteoblastic cells[5] are responsible for bone formation and mineralization and osteoclastic cells (OCs) for bone resorption. The subtle remodeling balance maintained between the OB and OC activities is crucial for bone stability.

Osteoporosis[6] is a systemic skeletal disease characterized by low bone mass and deterioration of bone tissue. Symptoms of this disease include bone fragility[6] and increase in fractures,[6] often at the hips, spine, and wrist. Osteoporosis occurs especially in postmenopausal women and elderly men, when the levels in active sex steroids (E2 and T)[7] drop.

Only few drugs are efficient for the treatment of osteoporosis. Among them, the antiresorptive agent bisphosphonate alendronate[8] is the most potent drug but the risk of fractures in postmenopausal women[8−10] and elderly men[11] is only reduced by 50%. Estrogens stop bone loss in osteoporotic patients but are no longer used because of several nonskeletal effects such as an increased risk of deep vein thrombosis, pulmonary embolism, and breast cancer.[12] Furthermore, low dose of synthetic estrogens, like ethinylestradiol, has no practical

Received: June 28, 2011
Published: October 6, 2011

ACS Publications © 2011 American Chemical Society

application. Raloxifene,[13] a selective estrogen receptor modulator (SERM) is frequently used to treat osteoporosis. Drawbacks of this therapy are the increased risks of thromboembolism,[14] hot flushes,[15] and leg cramps.[15] Therefore, there is a need to develop novel drugs that are more efficient and selective for osteoporosis.

Estrogens are known to play a key role in bone physiology. They act directly on OBs, which regulate the ability of the OC precursors to differentiate to OCs (osteoclastogenesis). The mode of action of estrogens on bone mass is not yet well understood. 17β-HSD2, which is expressed in OBs, can be considered as a molecular switch, as it is involved in the regulation of the level of active E2 and T in the target cell. Therefore, a local intracellular enhancement of E2 and T in bones should be feasible. This strategy has already been successfully applied for several enzymes like aromatase,[16−18] CYP17,[19−22] 17β-HSD1,[23−35] and 5α-reductase.[36−40]

Among the few 17β-HSD2 inhibitors described in the literature[41−50] there is only one class of nonsteroidal compounds:[46−48] the *cis*-pyrrolidinones. By application of a nonsteroidal inhibitor from this class in a monkey osteoporosis model, 17β-HSD2 has been validated as a target for the treatment of osteoporosis by Bagi et al.[51] Treatment with this compound led to a slight decrease in bone resorption and increase in bone formation resulting in maintenance of bone balance and bone strength.[50] As only small effects were observed, there is a need to develop new potent 17β-HSD2 inhibitors with better in vivo efficacy. Additionally, these compounds should show a high selectivity toward 17β-HSD1, the isoenzyme catalyzing the reverse reaction, the reduction of estrone (E1) to E2.

In a previous work,[52] focusing on the design of new nonsteroidal 17β-HSD2 inhibitors, 6-(4′-hydroxyphenyl)-1-naphthol (compound **A**, Table 1) has been identified as a promising scaffold with an IC_{50} of 302 nM for 17β-HSD2 and a selectivity factor (SF) of 8 toward 17β-HSD1. This hit needed to be improved regarding activity and selectivity. According to our hypothesis,[52] the OH substituted benzene ring of the naphthalene moiety in **A** mimics the A-ring of E2 and the OH-phenyl the D-ring, leading to a close binding of the OH-phenyl to the catalytic triad and the cofactor. As the substrate of one enzyme (17β-HSD2 in this case) is the product of the catalytic reaction of the other enzyme (17β-HSD1 in this case), it can be concluded that their active sites must be very similar too. Therefore, with analyzing 17β-HSD1 from which in contrast to 17β-HSD2 the crystal structure is known, it becomes apparent that there is some space available around the OH-phenyl for substituents.

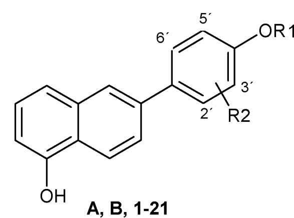
In this work, first the hypothesis concerning the binding mode of hydroxyphenyl naphthol derivatives to 17β-HSD2 was validated. Afterward, the available space for the inhibitors in the active site of the enzyme was explored by synthesis of several derivatives where the size of substituents at positions 2′ and 3′ of the hydroxyphenyl ring was varied. With the expectation that electronic effects play an important role, electron donating (EDG) and electron withdrawing (EWG) groups were also introduced into the OH-phenyl moiety.

■ CHEMISTRY

Synthesis of **1**−**8** and **10**−**20** (Scheme 1) started from the commercially available 6-methoxy-1-tetralone which was triflated and aromatized in **1a** in very good yield according to the method described by us.[52] The cross-coupling reaction[53] of the triflate **1a** with the appropriate methoxyphenylboronic acid led to compounds **1**, **3a**, **4**, **6**, **8**, **10**, **12**, **14**, **16**−**18**, and **20** (method A or B). Ether cleavage was performed using boron tribromide for compounds **2**, **7**, and **13** (method C); boron trifluoride dimethylsulfide complex for compounds **3**, **5**, and **15** (method D); and pyridinium hydrochloride for compounds **11** and **19** (method E).

Compound **9** bearing an additional phenyl ring on the hydroxyphenyl moiety was synthesized according to the route described in Scheme 2. Compound **1a** was first methylated using methyl iodide,[54] and subsequently the triflate group was exchanged by a boronic ester group (intermediate **9c**) with bis(neopentylglycolato)diboron and a crown ether under basic conditions according to the method of Xu et al.[55] In parallel, compound **9b** was synthesized by a Suzuki coupling with 4-bromo-2-iodo-1-methoxybenzene **9e** and phenylboronic acid. Subsequent Suzuki coupling (method B) between compounds **9c** and **9b** led to compound **9a**. Demethylation using boron tribromide (method C) afforded compound **9**.

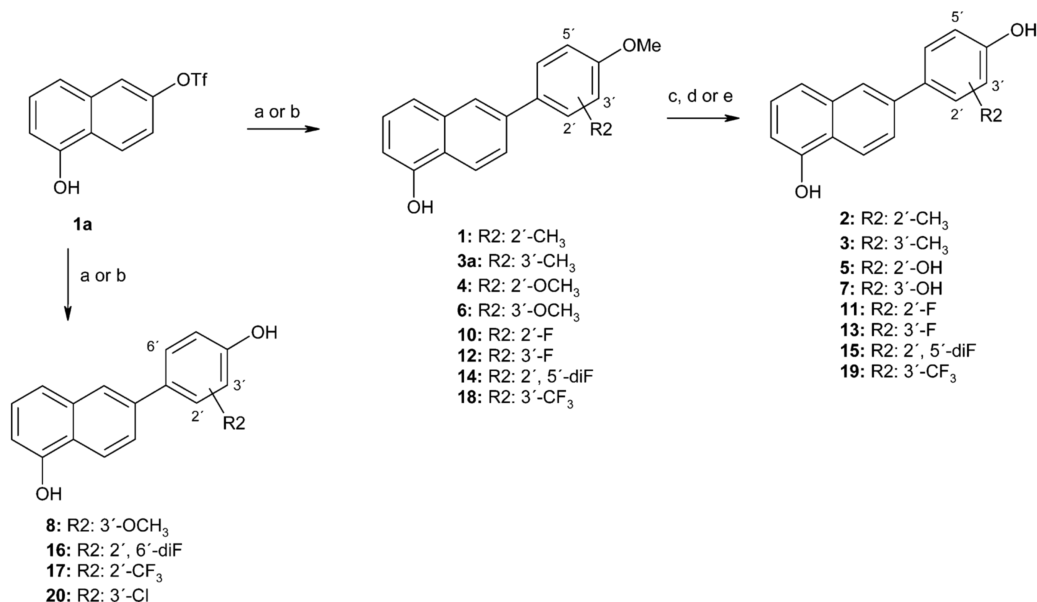
Table 1. Inhibition of Human 17β-HSD2 and 17β-HSD1 by Compounds 1−21



compd	R1	R2	inhibition of 17β-HSD2[a] (%) at 1 μM	inhibition of 17β-HSD1[b,d] (%) at 1 μM	log P[c]
spiro-δ-lactone			68	ni	
A	H	H	74	20	3.93
B	CH_3	H	20	28	
1	CH_3	2′-CH_3	25	20	4.68
2	H	2′-CH_3	65	25	4.41
3	H	3′-CH_3	79	48	4.41
4	CH_3	2′-OCH_3	37	15	4.06
5	H	2′-OH	11	10	3.54
6	CH_3	3′-OCH_3	49	45	4.06
7	H	3′-OCH_3	36	24	3.80
8	H	3′-OH	69	31	3.54
9	H	3′-phenyl	84	47	5.60
10	CH_3	2′-F	32	41	4.35
11	H	2′-F	87	36	4.09
12	CH_3	3′-F	51	66	4.35
13	H	3′-F	78	62	4.09
14	CH_3	2′,5′-diF	26	73	4.51
15	H	2′,5′-diF	83	74	4.24
16	H	2′,6′-diF	69	73	4.24
17	H	2′-CF_3	84	52	4.85
18	CH_3	3′-CF_3	44	69	5.11
19	H	3′-CF_3	98	60	4.85
20	H	3′-Cl	74	43	4.49
21	H	3′-CN	45	ni	3.96

[a]Human placenta, microsomal fraction, substrate [^{3}H]E2 + E2 [500 nM], NAD^+ [1500 μM], mean value of three determinations, relative standard deviation of <10%. [b]Human placenta, cytosolic fraction, substrate [^{3}H]E1 + E1 [500 nM], NADH [500 μM], mean value of three determinations, relative standard deviation of <10%. [c]Calculated log P data. [d]ni: no inhibition (inhibition of <10%).

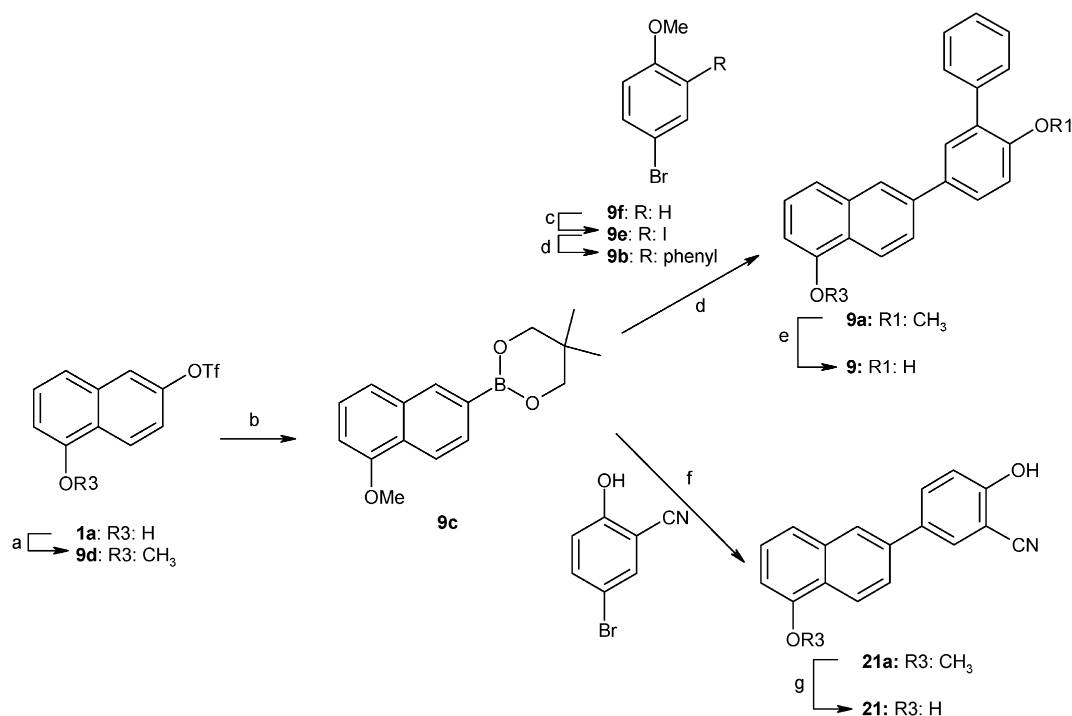
Scheme 1. Synthesis of Compounds 1−8 and 10−20[a]



aReagents and conditions: a. boronic acid, $Pd(PPh_3)_4$, aq. Na_2CO_3, DME/EtOH, 90°C, 2h for **1**, **3a**, **4**, **6**, **12** and **17**, Method A; b. boronic acid, $Pd(PPh_3)_4$, Cs_2CO_3, DME/water, microwave irradiation (150°C, 150 W, 25 min) for **8**, **10**, **14**, **16**, **18** and **20**, Method B; c. BBr_3, CH_2Cl_2, -78°C to rt, overnight, for **2**, **7** and **13**, Method C; d. BF_3.SMe_2, CH_2Cl_2, rt, overnight, for **3**, **5** and **15**, Method D; e. pyridinium hydrochloride, 220°C, 3h for **11** and **19**, Method E.

[a]Reagents and conditions: (a) boronic acid, $Pd(PPh_3)_4$, aq Na_2CO_3, DME/EtOH, 90 °C, 2 h for **1**, **3a**, **4**, **6**, **12**, and **17**, method A; (b) boronic acid, $Pd(PPh_3)_4$, Cs_2CO_3, DME/water, microwave irradiation (150 °C, 150 W, 25 min) for **8**, **10**, **14**, **16**, **18**, and **20**, method B; (c) BBr_3, CH_2Cl_2, −78 °C to room temp, overnight, for **2**, **7**, and **13**, method C; (d) $BF_3{\cdot}SMe_2$, CH_2Cl_2, room temp, overnight, for **3**, **5**, and **15**, method D; (e) pyridinium hydrochloride, 220 °C, 3 h for **11** and **19**, method E.

Scheme 2. Synthesis of Compounds 9 and 21[a]



[a]Reagents and conditions: (a) MeI, K_2CO_3, 18-crown-6, acetone, reflux, overnight; (b) bis(neopentylglycolato)diboron, $PdCl_2(dppf)$, dppf, KOAc, 1,4-dioxane, 85 °C, 4 h; (c) $PhI(OAc)_2$, I_2, EtOAc, 60 °C, dark conditions, 4 h; (d) boronic acid, $Pd(PPh_3)_4$, Cs_2CO_3, DME/water, microwave irradiation (150 °C, 150 W, 25 min), method B; (e) BBr_3, CH_2Cl_2, −78 °C to room temp, overnight, method C; (f) boronic acid, $Pd(PPh_3)_4$, aq Na_2CO_3, DME/EtOH, 90 °C, 2 h, method A; (g) $BF_3{\cdot}SMe_2$, CH_2Cl_2, room temp, overnight, method D.

The synthesis of **21** was carried out by palladium-catalyzed Suzuki coupling reaction using the intermediate **9c** (method A) and the commercially available 5-bromo-2-hydroxybenzonitrile, followed by ether cleavage with boron trifluoride dimethylsulfide complex (method D, Scheme 2).

BIOLOGICAL CHARACTERIZATION

Inhibition of Human 17β-HSD2. 17β-HSD2 inhibitory activity of the synthesized compounds was first evaluated in a cell-free assay. Human placental microsomal enzyme was used as source. The incubation was performed with tritiated E2, cofactor, and inhibitor. The separation of substrate and product was accomplished by HPLC with a subsequent quantification by peak integration.[56] The percent inhibition values of compounds **1−21** are shown in Table 1, and the IC_{50} values of the most active compounds are reported in Table 2.

Table 2. IC_{50}, Selectivity Factor, and Binding Affinities for the Estrogen Receptors α and β for Selected Compounds

	cell-free assay				
compd	17β-HSD2 IC_{50} [a] (nM)	17β-HSD1 IC_{50} [b,e] (nM)	selectivity factor[c,e]	ERα RBA[d,e] (%)	ERβ RBA[d,e] (%)
spiro-δ-lactone	34	nd	nd	nd	nd
A	302	2425	8	5	5
3	275	1748	6	<0.1	0.1−1.0
9	261	919	4	<0.1	<0.1
11	132	2245	17	<0.1	<0.1
13	292	713	2	0.1−1.0	0.1−1.0
15	238	309	1	0.1−1.0	0.1−1.0
17	156	926	6	1.0−10	1.0−10
19	19	611	32	<0.1	0.1−1.0
20	307	1130	4	0.1−1.0	0.1−1.0

[a] Human placenta, microsomal fraction, substrate $[^3H]E2$ + E2 [500 nM], cofactor NAD^+ [1500 μM], mean value of three determinations, relative standard deviation of <10%. [b] Human placenta, cytosolic fraction, substrate $[^3H]E1$ + E1 [500 nM], cofactor NADH [500 μM], mean value of three determinations, relative standard deviation of <20%. [c] IC_{50}(17β-HSD1)/IC_{50} (17β-HSD2). [d] RBA: relative binding affinity. E2: 100%. [e] nd: not determined.

Compounds showing less than 10% inhibition at 1 μM were considered to be inactive. Compound **A**, identified in a previous work,[52] was used as internal reference (74% 17β-HSD2 inhibition vs 20% 17β-HSD1 inhibition at 1 μM), and a spiro-δ-lactone (compound **10** published by Poirier et al.[43]) was taken as external control (68% at 1 μM in our test; 62−66% at 1 μM in their test[43]).

For selected substituents (R2 = 2′-CH_3, 2′-OCH_3, 3′-OCH_3, 2′-F, 3′-F, 2′,5′-diF, 3′-CF_3), both hydroxyphenyl **2**, **5**, **8**, **11**, **13**, **15**, **19** and corresponding methoxyphenyl derivatives **1**, **4**, **6**, **10**, **12**, **14**, **18** were synthesized and tested for 17β-HSD2 inhibitory activity. As observed for the unsubstituted compound **A**, the hydroxylated molecules (**2**, **8**, **11**, **13**, **15**, and **19**) were always more potent than the respective methoxylated analogues except for the compound pair **4** (R2 = 2′-OCH_3) and **5**. For compounds **3**, **9**, **16**, **17**, **20**, and **21**, the hydroxylated derivatives only were therefore tested.

Introduction of a lipophilic group like methyl (compounds **2** and **3**) led to good 17β-HSD2 inhibitory activity (65% inhibition for **2** and 79% for **3** when tested at 1 μM), which was at the level of unsubstituted **A** (74% inhibition at 1 μM). This indicates that there is space around the hydroxyphenyl moiety for introduction of a small substituent in the 2′- or 3′-position, but the methyl is certainly not able to achieve any specific interactions, as no gain in activity is observed compared to the parent hydroxyphenylnaphthol **A**.

Exchange of the methyl group for a hydrophilic hydroxy substituent was detrimental for activity in the 2′-position (**5**, inactive) but tolerated in the 3′-position (**8**, similar activity as the unsubstituted **A**). It can therefore be hypothesized that there is no polar amino acid in the active site close to the 2′-position of the inhibitor. Furthermore, the 3′-OH group of compound **8** did not show specific interactions with the enzyme, as no increase in activity was observed compared to **A**. It is striking that an exchange of hydroxy (**8**) by methoxy (**7**) in the 3′-position decreased the inhibitory activity strongly.

A phenyl ring was also introduced into the 3′-position (**9**). This compound displayed a slight increase in activity (84% inhibition at 1 μM for **9** vs 74% inhibition at 1 μM for **A**), confirming our previous hypothesis that there is space available around the hydroxyphenyl core.

In order to evaluate the influence of electron withdrawing groups (EWGs) or electron donating groups (EDGs) on the 17β-HSD2 inhibitory activity, compounds **10−21** bearing EWGs were synthesized to be compared to compounds with EDGs like methyl (**3**) or phenyl (**9**) groups. All methoxy derivatives (**10**, **12**, **14**, and **18**) showed lower activity than the corresponding hydroxy compounds (**11**, **13**, **15**, and **19**). Most of the hydroxy compounds exhibited identical or better potency than the parent compound **A** except **21**. Compound **11**, substituted with a fluoro group in the 2′- position, turned out to be highly active (87% inhibition at 1 μM, IC_{50} = 132 nM). Disubstituted fluorine compounds (2′,5′-diF **15** and 2′,6′-diF **16**) resulted in highly active inhibitors but were slightly weaker than the monofluoro **11** (IC_{50} of 238 nM for **15** and 132 nM for **11**). Compounds **17** (R2 = 2′-CF_3) and **19** (R2 = 3′-CF_3) showed high (**17**) and very high (**19**) activity (84% and 98% inhibition at 1 μM, respectively), resulting in IC_{50} values of 19 nM for **19** and 156 nM for **17**. However, exchange of the trifluoromethyl group for bioisosteric chloro (**20**) or cyano (**21**) groups was detrimental for the activity (74% and 45% inhibition for **20** and **21**, respectively) when compared with inhibitory efficiency of **19** (98% at 1 μM).

Selectivity and Cellular Activity. 17β-HSD1, as the main enzyme implicated in the reduction of estrone (E1) to E2 in sex steroid metabolism,[57] should not be affected by inhibitors of 17β-HSD2. Furthermore, inhibitors of 17β-HSD2 should have none or little affinity for the estrogen receptors α and β (ERα and ERβ), as most of the E2 effects are ER mediated. Therefore, all compounds were tested for selectivity toward 17β-HSD1 and a selection of the most potent inhibitors for binding affinity to ERα and β.

The assay for 17β-HSD1 inhibition was performed in a similar way as for the 17β-HSD2. Human placental cytosolic 17β-HSD1 was incubated with tritiated E1, cofactor, and inhibitor. The amount of labeled E2 formed was determined after HPLC separation. For the most potent compounds, IC_{50} values were determined and selectivity factors (SFs) calculated (SF = IC_{50}(17β-HSD1)/IC_{50}(17β-HSD2), Table 2). All compounds except **15** showed selectivity toward 17β-HSD1. The most selective one, with a selectivity factor of 32, was compound **19** (R2 = 3′-CF_3), which was also the most potent one exhibiting an IC_{50} of 19 nM. The 2′-F compound **11** also showed a high SF of 17 toward 17β-HSD1.

For the determination of ER binding affinity, a competition assay was used, with tritiated E2. Receptor bound and free E2

were separated by means of hydroxyapatite. Compounds **3**, **9**, **11**, and **19** showed a relative binding affinity (RBA) of less than 0.1% to ERα, compared to the affinity of E2, which is arbitrarily set to 100%. The indicated compounds are therefore classified as low affinity ligands of the ERα.

The log *P* values were calculated using the ChemDrawPro, version 11.0, program in order to get insight into the lipophilicity of this class of compounds and are shown in Table 1. The values for all compounds except **9** and **18** are lower than 5, fulfilling the criteria of Lipinski's rule of five. It therefore can be expected that they are able to easily permeate cell membranes.

The most interesting compounds **11**, **17**, and **19** with IC_{50} values below 200 nM were further investigated in a cellular assay, using the human breast cancer cell line MDA-MB-231,[58] which endogenously expresses 17β-HSD2. Cells were incubated with tritiated E2 and inhibitor for 3.5 h. Separation was performed by HPLC similar as in the cell-free inhibition assays. Compounds **17** and **19** showed very similar inhibitory activity in MDA-MB-231 cells as in the cell-free assay, i.e., IC_{50} of 171 nM for **17** and 31 nM for **19** in cellular assay vs 156 and 19 nM, respectively, in the cell-free assay. Interestingly, compound **11** showed an IC_{50} of 1227 nM in the cellular assay compared to 132 nM in the cell-free assay, indicating that this compound is less appropriate to permeate the cell membrane or is metabolically unstable.

Further in Vitro Assays. In order to refine the profile of the most potent compound **19**, it was further tested for selectivity on other enzymes, namely, 17β-HSD types 4 and 5. 17β-HSD4 catalyzes the same reaction as 17β-HSD2 (Chart 1),[57] and 17β-HSD5 (beside 17β-HSD3) is responsible for the reduction of the androgen A-dione to T.[57] Both enzymes should not be inhibited by 17β-HSD2 inhibitors. To investigate this important issue, experiments were performed with *E. coli* bacteria expressing human 17β-HSD4 and 17β-HSD5.[56,59] These cells were incubated with cofactor, inhibitor, and tritiated E2 in the case of 17β-HSD4 and with A-dione in the case of 17β-HSD5. After RP-HPLC separation of substrate and product, the amount of labeled E1 or T formed was quantified. Compound **19** was found to be selective toward 17β-HSD4 and 17β-HSD5 (40% 17β-HSD4 inhibition and 21% 17β-HSD5 inhibition at 1 μM).

According to the potency of compound **19** in cell-free and cellular assays and its selectivity toward 17β-HSD1, 17β-HSD4, 17β-HSD5, and ERs, it can be considered as potential candidate for further development. Before evaluation in an animal model of the efficacy of this new 17β-HSD2 inhibitor **19**, it is reasonable to determine its profile on orthologous enzymes from different species. Compound **19** was therefore tested for inhibition of 17β-HSD2/E1-formation (activity) and 17β-HSD1/E2-formation (selectivity) using rat, mouse, and monkey enzymes.

In rat and mouse assays, liver enzymes were used, while in monkey assay, placental enzymes were employed. Compound **19** showed good inhibition of the rat and mouse 17β-HSD2 enzymes and very good inhibition on the monkey enzyme. In this case, the efficacy was in the same range as for the human enzyme (Table 3). In addition, selectivity toward 17β-HSD1/E2-formation in monkey seems to be similar to that observed in human.

Table 3. Inhibition of Rat, Mouse, and Monkey E1 and E2-Formation by Compound 19

	inhibition of E1-formation[a,k] (%)		inhibition of E2-formation[b,k] (%)	
compd **19**	1 μM	100 nM	1 μM	100 nM
human	98[c]	87[c]	60[d]	27[d]
rat	77[e]	nd	52[f]	nd
mouse	72[g]	nd	26[h]	nd
monkey	99[i]	75[i]	75[j]	55[j]

[a] Substrate [^{3}H]E2 + E2 [500 nM], NAD^+ [1500 μM], mean value of three determinations, relative standard deviation of <10%. [b] Substrate [^{3}H]E1 + E1 [500 nM], NADH [500 μM], mean value of three determinations, relative standard deviation of <10%. [c] Human placenta, microsomal fraction. [d] Human placenta, cytosolic fraction. [e] Rat liver, microsomal fraction. [f] Rat liver, cytosolic fraction. [g] Mouse liver, microsomal fraction. [h] Mouse liver, cytosolic fraction. [i] Monkey placenta, microsomal fraction. [j] Monkey placenta, cytosolic fraction. [k] nd: not determined.

DISCUSSION AND CONCLUSION

Compound **A**, identified by us in a previous study[52] represents a promising scaffold as 17β-HSD2 inhibitor: substituted by two hydroxy groups (polar), the naphthalene and phenyl cores (hydrophobic) mimick the substrate E2. We have previously shown[52] that an exchange of the hydroxy group on the phenyl moiety by a sulfonamide did not affect the activity toward 17β-HSD2 (74% vs 75% inhibition at 1 μM, respectively), indicating that some space is available around the phenyl core. We hypothesized therefore that the phenyl ring mimics the D-ring of E2 and thus that introduction of additional substituents on this phenyl moiety would be tolerated and would allow an increase of the 17β-HSD2 inhibitory activity. In this paper, we aimed at verifying whether this hypothesis is correct and whether introduction of various substituents on the phenyl ring could improve this activity.

The biological activity of the phenyl-substituted compound **9** confirms that the compound can indeed bind in the active site according to the previous hypothesis (the hydroxynaphthalene mimics the A-ring and the hydroxyphenyl the D-ring). The additional phenyl might be accommodated between cofactor and steroid where space is available.

The electronic effects of the substituents on the 17β-HSD2 inhibitory activity were further elucidated. No matter where they were introduced, EDGs (Me, OH, Ph) in either the 2′- or 3′-position did not lead to significant differences in activity except for the hydroxy group where position 3′ was better (compounds **5** and **8**). None of the synthesized compounds (**2**, **3**, **8**, and **9**) reached a better activity compared to reference **A**, indicating that these substituents are tolerated (there is space available) but not able to establish specific interactions with amino acid residues. The position of the substituent on the phenyl ring will induce either a pronounced rotation of the phenyl toward the naphthalene (position 2′, ortho effect) or a weak rotation (position 3′). In both cases, only the 4′-hydroxy substituent stays at the same position and anchors the molecule into its binding site. Our finding that there is no difference in activity between 2′- and 3′-substituted compounds indicates that the phenyl ring is not able to establish $\pi-\pi$ stacking interactions with amino acid side chains of the protein.

When the substituent was an EWG (F, CF_3, Cl, CN), the highest potency was observed for compound **19** (R2 = 3′-CF_3) in which the additional group was located at the 3′-position. Exchange of the trifluoromethyl group by the bioisosteric groups like chloro (**20**) and cyano (**21**) decreased inhibition. In the case of the CN group, the different activities might be due to the fact that CN is hydrophilic whereas CF_3 is lipophilic. The linear shape of CN giving steric hindrance might also explain the moderate activity of **21**.

As shown in many other cases[5] and also observed for compound **11**, the substitution with F results in an increase in inhibitory activity. However, the introduction of a second fluorine as in **15** or **16** does not further enhance activity, indicating that the effects are not additive.

In our previous study,[52] it was observed that the hydroxy group bound to the phenyl moiety was important for activity (74% at 1 μM for **A** with OH vs 20% inhibition for **B** with OMe). This is confirmed in this study, as all methoxy compounds displayed lower 17β-HSD2 inhibitory activity than their corresponding hydroxy analogues. Contrary to our compounds, the other described nonsteroidal 17β-HSD2 inhibitors,[46−48] the *cis*-pyrrolidinones, do not need any hydrophilic group to be active, allowing us to presume that they might have a different binding mode compared to ours.

As other 17β-HSD enzymes of the short-chain dehydrogenase/reductase (SDR) and the aldo−keto reductases (AKR) family participate in steroid interconversions,[60] the development of enzyme-specific inhibitors is a challenge. We therefore tested the compounds on other 17β-HSDs, like 17β-HSD types 1, 2, 4, and 5. Selectivity toward 17β-HSD1 was low with nearly all of the tested hydroxyphenylnaphthol derivatives; however, the most potent 17β-HSD2 inhibitors **11** and **19** showed the highest selectivity toward 17β-HSD1, exhibiting promising selectivity factors (SFs) of 17 and 32, respectively. Although some inhibition of 17β-HSD4 and -5 was observed with the best inhibitor compound **19**, the substance can be assumed to be selective toward both enzymes. We were surprised by the fact that compound **19** showed some minor inhibition of 17β-HSD4. In our previous projects we have not seen any effect on this particular enzyme with steroidal or nonsteroidal inhibitors.[56,61] Nevertheless, the observed inhibition of compound **19** is fairly low and the impact on the ubiquitously expressed 17β-HSD4 contributing to the peroxisomal β-oxidation of fatty acids[62] is most probably negligible.

Compounds **11** and **19** also displayed very little binding affinity to ERα, which is responsible for cell proliferation. Only compound **19** showed a very good cellular activity (IC_{50} = 31 nM in MDA-MB-231 cells). Thus, it is able to permeate the cell membrane and is not quickly metabolized.

An osteoblastic cell-line expressing 17β-HSD2 and the ERs like SV-HFO should be promising to prove our concept that 17β-HSD2 inhibition is able to induce bone formation and inhibit osteoclastogenesis. For further in vivo experiments, species differences have to be taken into consideration,[63,64] and compound **19** should inhibit the enzyme of the chosen species. In this pursuit, **19** was tested on rat, mouse, and monkey enzymes and revealed a high (72−77% inhibition at 1 μM in mouse and rat enzymes) to very high (99% inhibition at 1 μM in monkey enzyme) inhibitory activity for 17β-HSD2/E1-formation. Showing a better activity and selectivity in marmoset (*Callithrix jacchus*) enzymes, this species should be appropriate for proving our concept.

In the present study, we describe the synthesis of substituted 6-phenyl-1-naphthols as inhibitors of 17β-HSD2 and the evaluation of their biological activities. A new highly potent inhibitor of 17β-HSD2 has been discovered (compound **19**) with very good cellular activity and good selectivity toward 17β-HSD1, 17β-HSD4, 17β-HSD5, and ERs. Compound **19** is also able to inhibit E1-formation in rat, mouse, and monkey. Further biological analyses still need to be investigated to get information on the efficacy of the compound to demonstrate that 17β-HSD2 inhibitors are able to inhibit osteoclastogenesis and induce bone formation.

■ EXPERIMENTAL SECTION

Chemical Methods. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DRX-500 instrument in $CDCl_3$ and acetone-d_6. Chemicals shifts are reported in δ values (ppm). The hydrogenated residues of deuteriated solvent were used as internal standard ($CDCl_3$, δ = 7.26 ppm in ^{1}H NMR and δ = 77.0 ppm in ^{13}C NMR; acetone-d_6, δ = 2.05 ppm in ^{1}H NMR and δ = 30.8 ppm and 206.3 ppm in ^{13}C NMR). Signals are described as s, br, d, t, dd, dt, qt, and m for singlet, broad, doublet, triplet, doublet of doublets, doublet of triplets, quintuplet, and multiplet, respectively. All coupling constants (*J*) are given in Hertz. IR spectra were measured neat on a Bruker Vector 33FT infrared spectrometer. Melting points (mp) were determined in open capillaries on a Stuart Scientific SMP3 apparatus and are uncorrected.

Mass spectra (GC/MS) were measured on a GCD series G1800A (Hewlett-Packard) instrument with an Optima-5-MS (0.25 μM, 30 m) column (Macherey Nagel). All microwave irradiation experiments were carried out in a CEM-Discover monomode microwave apparatus.

Flash chromatography was performed on silica gel 40 (35/40 to 63/70 μM) with hexane/ethyl acetate mixtures as eluents and the reaction progress was determined by thin-layer chromatography analyses on Alugram SIL G/UV254 (Macherey-Nagel). Visualization was accomplished with UV light.

Tested compounds are ≥95% chemical purity as measured by LC/MS. Data for all tested compounds are provided in the Supporting Information. The Surveyor LC system consisted of a pump, an autosampler, and a PDA detector. Mass spectrometry was performed on a MSQ electrospray mass spectrometer (ThermoFisher, Dreieich, Germany). The system was operated by the standard software Xcalibur. A RP-C18 NUCLEODUR 100-5 (125 mm × 3 mm) column (Macherey-Nagel GmbH, Dühren, Germany) was used as stationary phase. All solvents were HPLC grade. In a gradient run the percentage of acetonitrile (containing 0.1% trifluoroacetic acid) in 0.1% trifluoroacetic acid was increased from an initial concentration of 0% at 0 min to 100% at 15 min and kept at 100% for 5 min. The injection volume was 15 μL, and flow rate was set to 800 μL/min. MS analysis was carried out at a spray voltage of 3800 V and a capillary temperature of 350 °C and a source CID of 10 V. Spectra were acquired in positive mode from 100 to 1000 m/z at 254 nm for the UV trace.

Starting materials (different boronic acids and compounds **1e**, **9f**, and **21b**) were purchased from Aldrich, Alfa Aeser, Acros, and Combi-Blocks and were used without further purification. No attempts were made to optimize yields.

The following compounds were prepared according to previously described procedures: 5-hydroxy-2-naphthyl trifluoromethanesulfonate **1a**,[52] 4-bromo-2-iodo-1-methoxybenzene **9e**.[27]

General Procedures for Suzuki Coupling. Method A. To a mixture of aryl bromide (1 equiv) and tetrakis(triphenylphosphine)-palladium(0) (0.02 equiv) in DME was added a 2 N aqueous solution of sodium carbonate (2 equiv), and the mixture (oxygen free) was purged with N_2. The resultant solution was stirred at room temperature for 5 min, and a solution of boronic acid (1.3 equiv) in EtOH was added. The mixture was heated to 90 °C and stirred for 2 h. The reaction mixture was cooled to room temperature, quenched by water, and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, filtered, and concentrated to dryness. The product was purified by column chromatography.

Method B. A mixture of aryl bromide (1 equiv), boronic acid (1 equiv), cesium carbonate (3 equiv), and tetrakis(triphenylphosphine)-palladium(0) (0.02 equiv) was suspended in an oxygen-free DME/water (2:1) solution. The reaction mixture was exposed to microwave irradiation (25 min, 150 W, 150 °C, 15 bar). After the mixture reached room temperature, water was added and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated to dryness. The product was purified by column chromatography.

General Procedures for Ether Cleavage. Method C. To a solution of methoxy derivative (1 equiv) in dry dichloromethane cooled at −78 °C under nitrogen was slowly added boron tribromide (1 M solution in dichloromethane, 5−10 equiv per methoxy function). The reaction mixture was stirred at −78 °C for 1 h and then allowed to warm to room temperature overnight. The reaction was quenched by water, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine and dried over sodium sulfate, evaporated to dryness under reduced pressure, and purified by column chromatography.

Method D. To a solution of methoxy derivative (1 equiv) in dry dichloromethane, boron trifluoride−dimethylsulfide complex (75 equiv per methoxy function) was added dropwise at room temperature. The reaction mixture was stirred at room temperature overnight. Water was added to quench the reaction, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, evaporated to dryness under reduced pressure, and purified by column chromatography.

Method E. To pyridinium hydrochloride (100 equiv) at 190 °C was added methoxy derivative (1 equiv), and the solution was stirred for 3 h. The reaction mixture was cooled to room temperature and was stirred with 1 N HCl. The mixture was dissolved in ethyl acetate, and the combined organic layers were washed with water, dried over sodium sulfate, filtered, evaporated to dryness under reduced pressure, and purified by column chromatography.

6-(4-Hydroxy-3-methylphenyl)-1-naphthol (3). The title compound was prepared by reaction of 6-(4-methoxy-3-methylphenyl)-1-naphthol **3a** (20 mg, 0.08 mmol, 1 equiv) with boron trifluoride−dimethylsulfide complex (6.00 mmol, 75 equiv) according to method D. The product was purified by column chromatography (hexane/ethyl acetate, 1:1) to give 12 mg (60%) of the analytically pure compound as a brown solid. $C_{17}H_{14}O_2$; MW 250; mp 164−166 °C; ^{1}H NMR (acetone-d_6) δ 8.90 (s, br, 1H), 8.26 (s, br, 1H), 8.23 (d, $J \approx$ 8.5 Hz, 1H), 7.96 (d, $J \approx$ 1.9 Hz, 1H), 7.69 (dd, $J \approx$ 2.0 Hz, $J \approx$ 8.5 Hz, 1H), 7.53 (d, $J \approx$ 1.9 Hz, 1H), 7.43 (dd, $J \approx$ 2.0 Hz, $J \approx$ 8.5 Hz, 1H), 7.40−7.37 (m, 1H), 7.28−7.24 (m, 1H), 6.91 (d, $J \approx$ 8.5 Hz, 1H), 6.84 (dd, $J \approx$ 0.8 Hz, $J \approx$ 7.4 Hz, 1H), 2.27 (s, 3H); ^{13}C NMR (acetone-d_6) δ 156.1, 154.0, 139.6, 136.4, 133.0, 130.4, 127.4, 126.4, 125.6, 124.9, 124.7, 123.5, 120.2, 118.2, 116.0, 108.7, 16.3; IR 3337, 2952, 1506, 1274 cm^{-1}; GC/MS m/z 250 (M)$^+$.

5-(5-Hydroxy-2-naphthyl)biphenyl-2-ol (9). The title compound was prepared by reaction of 2-methoxy-5-(5-methoxy-2-naphthyl)-biphenyl **9a** (75 mg, 0.22 mmol, 1 equiv) with boron tribromide (2.2 mmol, 10 equiv) according to method C. The product was purified by column chromatography (hexane/ethyl acetate, 8:2), then by preparative HPLC (gradient acetonitrile/water from 20:80 to 100:0) to give 10 mg (15%) of the analytically pure compound as an orange oil. $C_{22}H_{16}O_2$; MW 312; ^{1}H NMR (acetone-d_6) δ 8.16 (d, $J \approx$ 8.5 Hz, 1H), 7.96 (d, $J \approx$ 2.0 Hz, 1H), 7.67 (dd, $J \approx$ 2.0 Hz, $J \approx$ 8.5 Hz, 1H), 7.61 (d, $J \approx$ 2.0 Hz, 1H), 7.59−7.55 (m, 2H), 7.51 (dd, $J \approx$ 2.0 Hz, $J \approx$ 8.5 Hz, 1H), 7.32−7.28 (m, 3H), 7.22−7.16 (m, 2H), 7.00 (d, $J \approx$ 8.5 Hz, 1H), 6.76 (d, $J \approx$ 7.6 Hz, 1H); ^{13}C NMR (acetone-d_6) δ 154.8, 153.9, 139.7, 139.2, 136.4, 133.7, 130.3, 128.9, 128.1, 127.7, 127.5, 125.2, 124.7, 124.6, 123.6, 120.2, 117.5, 108.7; GC/MS m/z 312 (M)$^+$.

6-(2-Fluoro-4-hydroxyphenyl)-1-naphthol (11). The title compound was prepared by reaction of 6-(2-fluoro-4-methoxyphenyl)-1-naphthol **10** (100 mg, 0.09 mmol, 1 equiv) with pyridinium hydrochloride (9 mmol, 100 equiv) according to method E. The product was purified by column chromatography (hexane/ethyl acetate, 7:3) to afford 48 mg (54%) of the analytically pure product as a beige solid. $C_{16}H_{11}FO_2$; MW 254; mp 145−146 °C; ^{1}H NMR (acetone-d_6) δ 9.15 (s, br, 1H), 9.07 (s, br, 1H), 8.28 (d, $J \approx$ 8.5 Hz, 1H), 7.83 (d, $J \approx$ 8.5 Hz, 2H), 7.62 (dt, $J \approx$ 1.8 Hz, $J \approx$ 8.5 Hz, 1H), 7.50−7.46 (m, 1H), 7.42 (d, $J \approx$ 8.5 Hz, 1H), 7.32−7.31 (m, 1H), 6.92 (dd, $J \approx$ 1.0 Hz, $J \approx$ 7.3 Hz, 1H), 6.84−6.81 (m, 1H); ^{13}C NMR (acetone-d_6) δ 162.7, 154.0, 148.1, 136.0, 132.4, 130.1, 127.8, 127.5, 126.5, 123.1, 120.2, 115.1, 115.0, 112.9, 109.1, 104.2; IR 3225, 1623, 1214 cm^{-1}; GC/MS m/z 254 (M)$^+$.

6-(3-Fluoro-4-hydroxyphenyl)-1-naphthol (13). The title compound was prepared by reaction of 6-(3-fluoro-4-methoxyphenyl)-1-naphthol **12** (20 mg, 0.08 mmol, 1 equiv) with boron tribromide (0.80 mmol, 10 equiv) according to method C. The product was purified by column chromatography (hexane/ethyl acetate, 1:1) to give 8 mg (40%) of the analytically pure compound as a yellow oil. $C_{16}H_{11}O_2F$; MW 254; ^{1}H NMR (acetone-d_6) δ 8.99 (s, br, 1H), 8.74 (s, br, 1H), 8.29 (d, $J \approx$ 8.8 Hz, 1H), 8.05 (d, $J \approx$ 2.0 Hz, 1H), 7.74 (dd, $J \approx$ 2.1 Hz, $J \approx$ 8.7 Hz, 1H), 7.57 (dd, $J \approx$ 2.1 Hz, $J \approx$ 8.7 Hz, 1H), 7.50−7.47 (m, 1H), 7.44 (d, $J \approx$ 8.3 Hz, 1H), 7.34−7.30 (m, 1H), 7.15−7.11 (m, 1H), 6.91 (dd, $J \approx$ 0.9 Hz, $J \approx$ 7.6 Hz, 1H); ^{13}C NMR (acetone-d_6) δ 154.2, 136.3, 127.6, 125.4, 124.4, 124.0, 123.7, 120.3, 119.1, 115.5, 109.0; IR 3247, 1692, 1524, 1277 cm^{-1}; GC/MS m/z 254 (M)$^+$.

6-(2, 5-Difluoro-4-hydroxyphenyl)-1-naphthol (15). The title compound was prepared by reaction of 6-(2,5-difluoro-4-methoxyphenyl)-1-naphthol **14** (40 mg, 0.14 mmol, 1 equiv) with boron trifluoride−dimethylsulfide complex (10.5 mmol, 75 equiv) according to method D. The product was purified by column chromatography (hexane/ethyl acetate, 8:2) to afford 20 mg (50%) of the analytically pure product as a beige solid. $C_{16}H_{10}F_2O_2$; MW 272; mp 178−181 °C; ^{1}H NMR (acetone-d_6) δ 9.22 (s, br, 1H), 9.05 (s, br, 1H), 8.29 (d, $J \approx$ 8.8 Hz, 1H), 7.99−7.97 (m, 1H), 7.63 (dt, $J \approx$ 1.9 Hz, $J \approx$ 8.8 Hz, 1H), 7.44 (d, $J \approx$ 8.4 Hz, 1H), 7.42−7.39 (m, 1H), 7.36−7.32 (m, 1H), 6.96−6.90 (m, 2H); ^{13}C NMR (acetone-d_6) δ 162.4, 159.6, 154.4, 153.2, 135.2, 134.5, 126.6, 125.9, 125.1, 123.5, 122.0, 120.8, 117.9, 117.5, 108.9, 104.6; IR 3427, 1641, 1522, 1268 cm^{-1}; GC/MS m/z 272 (M)$^+$.

6-[4-Hydroxy-2-(trifluoromethyl)phenyl]-1-naphthol (17). The title compound was prepared by reaction of 5-hydroxy-2-naphthyl trifluoromethanesulfonate **1a** (100 mg, 0.34 mmol, 1 equiv) with 4-hydroxy-2-trifluoromethylphenylboronic acid (98 mg, 0.44 mmol, 1.3 equiv) according to method A. The product was purified by column chromatography (hexane/ethyl acetate, 8:2) to give 70 mg (68%) of the analytically pure compound as a brown solid. $C_{17}H_{11}F_3O_2$; MW 304; mp 140−142 °C; ^{1}H NMR (acetone-d_6) δ 9.03 (s, br, 1H), 9.01 (s, br, 1H), 8.25 (d, $J \approx$ 8.8 Hz, 1H), 7.73 (s, 1H), 7.42−7.39 (m, 2H), 7.36−7.31 (m, 2H), 7.28 (d, $J \approx$ 2.5 Hz, 1H), 7.18 (dd, $J \approx$ 2.5 Hz, $J \approx$ 8.6 Hz, 1H), 6.96−6.93 (m, 1H); ^{13}C NMR (acetone-d_6) δ 157.9, 154.3, 138.8, 135.4, 134.9, 128.7, 127.8, 127.2, 125.0, 122.6, 120.4, 119.4, 113.7, 109.5, 60.5; IR 3361, 1691, 1322 cm^{-1}; GC/MS m/z 304 (M)$^+$.

6-[4-Hydroxy-3-(trifluoromethyl)phenyl]-1-naphthol (19). The title compound was prepared by reaction of 6-[4-methoxy-3-(trifluoromethyl)phenyl]-1-naphthol **18** (100 mg, 0.15 mmol, 1 equiv) with pyridinium hydrochloride (15 mmol, 100 equiv) according to method E. The product was purified by column chromatography (hexane/ethyl acetate, 7:3) to afford 40 mg (42%) of the analytically pure product as a brown solid. $C_{17}H_{11}F_3O_2$; MW 304; mp 158−160 °C; ^{1}H NMR (acetone-d_6) δ 8.28 (d, $J \approx$ 8.5 Hz, 1H), 8.04 (d, $J \approx$ 2.0 Hz, 1H), 7.91 (d, $J \approx$ 2.0 Hz, 1H), 7.85 (dd, $J \approx$ 2.0 Hz, $J \approx$ 8.5 Hz, 1H), 7.71 (dd, $J \approx$ 2.0 Hz, $J \approx$ 8.5 Hz, 1H), 7.42 (d, $J \approx$ 8.5 Hz, 1H), 7.29 (m, 1H), 7.22−7.19 (m, 1H), 6.89 (dd, $J \approx$ 0.9 Hz, $J \approx$ 7.3 Hz, 1H); ^{13}C NMR (acetone-d_6) δ 156.3, 154.1, 138.0, 136.3, 133.0, 127.7, 125.5, 124.9, 124.4, 123.9, 120.2, 118.6, 109.1; IR 3229, 3066, 2986, 1619, 1263 cm^{-1}; GC/MS m/z 304 (M)$^+$.

6-(3-Chloro-4-hydroxyphenyl)-1-naphthol (20). The title compound was prepared by reaction of 5-hydroxy-2-naphthyl trifluoromethanesulfonate **1a** (200 mg, 0.68 mmol, 1 equiv) and 3-chloro-4-hydroxyphenylboronic acid (153 mg, 0.89 mmol, 1.3 equiv) according to method B. The product was purified by column chromatography (hexane/ethyl acetate, 7:3) to give 117 mg (64%) of the analytically pure compound as a beige solid. $C_{16}H_{11}ClO_2$; MW 270; mp 112−114 °C; ^{1}H NMR (acetone-d_6) δ 9.10 (s, br, 2H), 8.31 (d, $J \approx$ 8.5 Hz, 1H), 8.07 (d, $J \approx$ 2.0 Hz, 1H), 7.80 (d, $J \approx$ 2.0 Hz, 1H), 7.75 (dd, $J \approx$ 2.0 Hz, $J \approx$ 8.5 Hz, 1H), 7.64 (dd, $J \approx$ 2.0 Hz, $J \approx$ 8.5 Hz, 1H), 7.47 (d, $J \approx$ 8.4 Hz, 1H), 7.36−7.32 (m, 1H), 7.19 (d, $J \approx$ 8.4 Hz, 1H), 6.94 (dd, $J \approx$ 1.0 Hz, $J \approx$ 7.4 Hz, 1H); ^{13}C NMR (acetone-d_6) δ 154.0, 153.5, 137.9, 136.2, 134.5, 129.2, 127.7, 127.6, 125.4, 125.3,

124.4, 123.8, 121.7, 120.2, 118.1, 109.0; IR 3330, 1599, 1283, 1187 cm^{-1}; GC/MS m/z 270–272 $(M)^+$.

Biological Assays. [2,4,6,7-^{3}H]E2 and [2,4,6,7-^{3}H]E1 were bought from Perkin-Elmer, Boston, MA. Quickszint Flow 302 scintillator fluid was bought from Zinsser Analytic, Frankfurt, Germany. Cytosolic (17β-HSD1) and microsomal (17β-HSD2) fractions were obtained from human and *Callithrix jacchus* placenta according to previously described procedures[29,65,66] and from rat and mouse liver tissues.[67] Fresh tissue was homogenized and centrifuged. The pellet fraction contains the microsomal 17β-HSD2 and was used for the determination of E1-formation, while 17β-HSD1 was obtained after precipitation with ammonium sulfate from the cytosolic fraction for use of testing of E2-formation.

Human 17β-HSD4 and 17β-HSD5 were cloned into the modified pGEX-2T vector.[56] For the multidomain enzyme 17β-HSD4, only the steroid converting SDR domain was subcloned.[56]

Inhibition of 17β-HSD2/E1-Formation. Inhibitory activities were evaluated by a well established method with minor modifications.[68−70] Briefly, the enzyme preparation was incubated with NAD^+ [1500 μM] in the presence of potential inhibitors at 37 °C in a phosphate buffer (50 mM) supplemented with 20% of glycerol and 1 mM EDTA. 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 E2 and [2,4,6,7-^{3}H]E2 (final concentration of 500 nM, 0.11 μCi). After 20 min at 37 °C, 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 Gravity, 3 μm, Macherey-Nagel, Düren, Germany) 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 following equation:

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

Each value was calculated from at least three independent experiments.

Inhibition of 17β-HSD1/E2-Formation. The 17β-HSD1 inhibition assay was performed similarly to the 17β-HSD2 procedure. The cytosolic fraction was incubated with NADH [500 μM], test compound, and a mixture of unlabeled E1 and [2,4,6,7-^{3}H]E1 (final concentration of 500 nM, 0.15 μCi) for 10 min. Further treatment of the samples and HPLC separation were carried out as mentioned above.

Inhibition of 17β-HSD4. Inhibitory activity was assessed as originally described[71,72] with minor modifications.[56] Briefly, bacteria containing recombinant 17β-HSD4 were resuspended in PBS and enzymatic assay was performed at pH 7.7. The enzyme preparation was incubated with NAD^+ [7.5 mM]. Inhibitor (dissolved in DMSO) was added in a final concentration of 1 μM. Final concentration of DMSO was adjusted to 1% in all samples. The enzymatic reaction was started with the addition of [6,7-^{3}H]E2 at 21 nM. The incubation at 37 °C was stopped with 0.21 M ascorbic acid in methanol/acetic acid (99:1) after the time needed to convert approximately 30% of the substrate in a control assay without inhibitor. Steroids were extracted from the assay mixture by SPE using Strata C18-E columns (Phenomenex), eluted with methanol, and separated by RP-HPLC (column, Luna, 5 μm C18(2), 150 mm; Phenomenex) at a flow rate of 1 mL/min acetonitrile/water (43:57). Radioactivity was detected by scintillation counting after mixing with ReadyFlowIII (Beckman). Conversion was calculated from integration of substrate and product peaks. Assay was run in triplicate of three independent experiments.

Inhibition of 17β-HSD5. The 17β-HSD5 inhibition assay was performed similarly to the 17β-HSD4 procedure. The recombinant enzyme was incubated with NADPH [6 mM], inhibitor [1 μM], and [1,2,6,7-^{3}H]A-dione at 21 nM. Further treatment of the samples and HPLC separation were carried out as mentioned above.[56]

Inhibition of 17β-HSD2 in a Cellular Assay. Cellular 17β-HSD2 activity is measured using the breast cancer cell-line MDA-MB-231[58] (17β-HSD1 activity negligible). [^{3}H]E2 (200 nM) is taken as substrate and is incubated with the inhibitor for 3.5 h at 37 °C. After ether extraction, substrate and product are separated by HPLC and detected with a radioflow detector. Potency is evaluated as percentage of inhibition (inhibitor concentration of 1 μM) and as IC_{50}.

ER Affinity.[29] The binding affinity of selected compounds to the ERα and ERβ was determined according to Zimmermann et al.[73] using recombinant human proteins. Briefly, 0.25 pM ERα or ERβ was 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 percent displacement versus compound concentration resulted in sigmoidal binding curves. The compound concentration to displace 50% of the receptor bound [^{3}H]E2 was 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:[74]

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

This results in an RBA 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 with 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 with 0.01% < RBA < 0.1%.

log *P* Determination. The log *P* values were calculated from CambridgeSoft Chem & Bio Draw, version 11.0, using the ChemDrawPro, version 11.0, program.

■ ASSOCIATED CONTENT

Supporting Information

Chemical synthesis, characterization of all compounds, and HPLC purity determination. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +49 681 302 70300. Fax: +49 681 302 70308. E-mail: rwh@mx.uni-saarland.de.

■ ACKNOWLEDGMENTS

We thank Jannine Ludwig, Martina Jankowski, Jeannine Jung, and Dr. Jörg Haupenthal for their help in performing the enzyme and cellular inhibition tests (17β-HSD1, 17β-HSD2) and the ER tests. We acknowledge Prof. A. Einspanier for the gift of monkey placentas. We are grateful to the Deutsche Forschungsgemeinschaft for financial support (Grants HA1315/12-1 and AD 127/10-1).

ABBREVIATIONS USED

17β-HSD1, 17β-hydroxysteroid dehydrogenase type 1; 17β-HSD2, 17β-hydroxysteroid dehydrogenase type 2; A-dione, 4-androstene-3,17-dione; AKR, aldo−keto redutase; APCI, atmospheric pressure chemical ionization; DME, dimethoxyethane; DMSO, dimethylsulfoxide; E1, estrone; E2, 17β-estradiol; EDG, electron donating group; equiv, equivalent; EDTA, ethylenediaminetetraacetic acid; ER, estrogen receptor; ESI, electrospray interface; EtOH, ethanol; EWG, electron withdrawing group; HPLC, high pressure liquid chromatography; NAD(P)(H), nicotinamide adenine dinucleotide (phosphate); OB, osteoblast; OC, osteoclast; PBS, phosphate buffered saline; RBA, relative binding affinity; RP, reversed phase; SAR, structure−activity relationship; SDR, short chain dehydrogenase/reductase; SERM, selective estrogen receptor modulator; SF, selectivity factor; T, testosterone

ADDITIONAL NOTE

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

REFERENCES

(1) Lu, M. L.; Huang, Y. W.; Lin, S. X. Purification, reconstitution, and steady-state kinetics of the trans-membrane 17 beta-hydroxysteroid dehydrogenase 2. *J. Biol. Chem.* **2002**, *277* (25), 22123−22130.

(2) Dong, Y.; Qiu, Q. Q.; Debear, J.; Lathrop, W. F.; Bertolini, D. R.; Tamburini, P. P. 17Beta-hydroxysteroid dehydrogenases in human bone cells. *J. Bone Miner. Res.* **1998**, *13* (10), 1539−1546.

(3) Purohit, A.; Flanagan, A. M.; Reed, M. J. Estrogen synthesis by osteoblast cell lines. *Endocrinology* **1992**, *131* (4), 2027−2029.

(4) van der Eerden, B. C.; Lowik, C. W.; Wit, J. M.; Karperien, M. Expression of estrogen receptors and enzymes involved in sex steroid metabolism in the rat tibia during sexual maturation. *J. Endocrinol.* **2004**, *180* (3), 457−467.

(5) Böhm, H. J.; Banner, D.; Bendels, S.; Kansy, M.; Kuhn, B.; Müller, K.; Obst-Sander, U.; Stahl, M. Fluorine in medicinal chemistry. *ChemBioChem* **2004**, *5* (5), 637−643.

(6) Salari Sharif, P.; Abdollahi, M.; Larijani, B. Current, new and future treatments of osteoporosis. *Rheumatol. Int.* **2011**, *31*, 289−300.

(7) Manolagas, S. C. Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. *Endocr. Rev.* **2000**, *21* (2), 115−137.

(8) Liberman, U. A.; Weiss, S. R.; Broll, J.; Minne, H. W.; Quan, H.; Bell, N. H.; Rodriguez-Portales, J.; Downs, R. W. Jr.; Dequeker, J.; Favus, M.; Seeman, E.; Recker, R. R.; Capizzi, T.; Santora, A. C. I.; Lombardi, A.; Shah, R. V.; Hirsch, L. J.; Karpf, D. B. Effect of oral alendronate on bone mineral density and the incidence of fractures in postmenopausal osteoporosis. The alendronate phase III osteoporosis treatment study group. *N. Engl. J. Med.* **1995**, *333* (22), 1437−1443.

(9) Bauer, D. C.; Black, D. M.; Garnero, P.; Hochberg, M.; Ott, S.; Orloff, J.; Thompson, D. E.; Ewing, S. K.; Delmas, P. D. Change in bone turnover and hip, non-spine, and vertebral fracture in alendronate-treated women: the fracture intervention trial. *J. Bone Miner. Res.* **2004**, *19* (8), 1250−1258.

(10) Chesnut, C. H. III; McClung, M. R.; Ensrud, K. E.; Bell, N. H.; Genant, H. K.; Harris, S. T.; Singer, F. R.; Stock, J. L.; Yood, R. A.; Delmas, P. D.; Kher, U.; Pryor-Tillotson, S.; Santora, A. C. I. Alendronate treatment of the postmenopausal osteoporotic woman: effect of multiple dosages on bone mass and bone remodeling. *Am. J. Med.* **1995**, *99* (2), 144−152.

(11) Orwoll, E.; Ettinger, M.; Weiss, S.; Miller, P.; Kendler, D.; Graham, J.; Adami, S.; Weber, K.; Lorenc, R.; Pietschmann, P.; Vandormael, K.; Lombardi, A. Alendronate for the treatment of osteoporosis in men. *N. Engl. J. Med.* **2000**, *343* (9), 604−610.

(12) Delmas, P. D. Treatment of postmenopausal osteoporosis. *Lancet* **2002**, *359* (9322), 2018−2026.

(13) Ettinger, B.; Black, D. M.; Mitlak, B. H.; Knickerbocker, R. K.; Nickelsen, T.; Genant, H. K.; Christiansen, C.; Delmas, P. D.; Zanchetta, J. R.; Stakkestad, J.; Glüer, C. C.; Krueger, K.; Cohen, F. J.; Eckert, S.; Ensrud, K. E.; Avioli, L. V.; Lips, P.; Cummings, S. R. Reduction of vertebral fracture risk in postmenopausal women with osteoporosis treated with raloxifene: results from a 3-year randomized clinical trial. Multiple Outcomes of Raloxifene Evaluation (MORE) Investigators. *J. Am. Med. Assoc.* **1999**, *282* (7), 637−645.

(14) Barrett-Connor, E.; Mosca, L.; Collins, P.; Geiger, M. J.; Grady, D.; Kornitzer, M.; McNabb, M. A.; Wenger, N. K. Effects of raloxifene on cardiovascular events and breast cancer in postmenopausal women. *N. Engl. J. Med.* **2006**, *355* (2), 125−137.

(15) Draper, M. W. An update on raloxifene. *Int. J. Gynecol. Cancer* **2006**, *16*, 502−503.

(16) Cavalli, A.; Bisi, A.; Bertucci, C.; Rosini, C.; Paluszcak, A.; Gobbi, S.; Giorgio, E.; Rampa, A.; Belluti, F.; Piazzi, L.; Valenti, P.; Hartmann, R. W.; Recanatini, M. Enantioselective nonsteroidal aromatase inhibitors identified through a multidisciplinary medicinal chemistry approach. *J. Med. Chem.* **2005**, *48* (23), 7282−7289.

(17) Leze, M. P.; Le Borgne, M.; Pinson, P.; Palusczak, A.; Duflos, M.; Le Baut, G.; Hartmann, R. W. Synthesis and biological evaluation of 5-[(aryl)(1*H*-imidazol-1-yl)methyl]-1*H*-indoles: potent and selective aromatase inhibitors. *Bioorg. Med. Chem. Lett.* **2006**, *16* (5), 1134−1137.

(18) Gobbi, S.; Cavalli, A.; Negri, M.; Schewe, K. E.; Belluti, F.; Piazzi, L.; Hartmann, R. W.; Recanatini, M.; Bisi, A. Imidazolylmethylbenzophenones as highly potent aromatase inhibitors. *J. Med. Chem.* **2007**, *50* (15), 3420−3422.

(19) Haidar, S.; Ehmer, P. B.; Barassin, S.; Batzl-Hartmann, C.; Hartmann, R. W. Effects of novel 17alpha-hydroxylase/C17,20-lyase (P450 17, CYP 17) inhibitors on androgen biosynthesis in vitro and in vivo. *J. Steroid Biochem. Mol. Biol.* **2003**, *84* (5), 555−562.

(20) Jagusch, C.; Negri, M.; Hille, U. E.; Hu, Q.; Bartels, M.; Jahn-Hoffmann, K.; Pinto-Bazurco Mendieta, M. A.; Rodenwaldt, B.; Müller-Vieira, U.; Schmidt, D.; Lauterbach, T.; Recanatini, M.; Cavalli, A.; Hartmann, R. W. Synthesis, biological evaluation and molecular modelling studies of methyleneimidazole substituted biaryls as inhibitors of human 17alpha-hydroxylase-17,20-lyase (CYP17). Part I: Heterocyclic modifications of the core structure. *Bioorg. Med. Chem.* **2008**, *16* (4), 1992−2010.

(21) Hu, Q.; Negri, M.; Jahn-Hoffmann, K.; Zhuang, Y.; Olgen, S.; Bartels, M.; Müller-Vieira, U.; Lauterbach, T.; Hartmann, R. W. Synthesis, biological evaluation, and molecular modeling studies of methylene imidazole substituted biaryls as inhibitors of human 17alpha-hydroxylase-17,20-lyase (CYP17). Part II: Core rigidification and influence of substituents at the methylene bridge. *Bioorg. Med. Chem.* **2008**, *16* (16), 7715−7727.

(22) Hille, U. E.; Hu, Q.; Vock, C.; Negri, M.; Bartels, M.; Müller-Vieira, U.; Lauterbach, T.; Hartmann, R. W. Novel CYP17 inhibitors: synthesis, biological evaluation, structure−activity relationships and modelling of methoxy- and hydroxy-substituted methyleneimidazolyl biphenyls. *Eur. J. Med. Chem.* **2009**, *44* (7), 2765−2775.

(23) Frotscher, M.; Ziegler, E.; Marchais-Oberwinkler, S.; Kruchten, P.; Neugebauer, A.; Fetzer, L.; Scherer, C.; Müller-Vieira, U.; Messinger, J.; Thole, H.; Hartmann, R. W. Design, synthesis, and biological evaluation of (hydroxyphenyl)naphthalene and -quinoline derivatives: potent and selective nonsteroidal inhibitors of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1) for the treatment of estrogen-dependent diseases. *J. Med. Chem.* **2008**, *51* (7), 2158−2169.

(24) Bey, E.; Marchais-Oberwinkler, S.; Kruchten, P.; Frotscher, M.; Werth, R.; Oster, A.; Algul, O.; Neugebauer, A.; Hartmann, R. W. Design, synthesis and biological evaluation of bis(hydroxyphenyl) azoles as potent and selective non-steroidal inhibitors of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1) for the treatment of estrogen-dependent diseases. *Bioorg. Med. Chem.* **2008**, *16* (12), 6423−6435.

(25) Marchais-Oberwinkler, S.; Kruchten, P.; Frotscher, M.; Ziegler, E.; Neugebauer, A.; Bhoga, U.; Bey, E.; Müller-Vieira, U.; Messinger, J.; Thole, H.; Hartmann, R. W. Substituted 6-phenyl-2-naphthols. Potent and selective nonsteroidal inhibitors of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1): design, synthesis, biological evaluation, and pharmacokinetics. *J. Med. Chem.* **2008**, *51* (15), 4685–4698.
(26) Bey, E.; Marchais-Oberwinkler, S.; Werth, R.; Negri, M.; Al-Soud, Y. A.; Kruchten, P.; Oster, A.; Frotscher, M.; Birk, B.; Hartmann, R. W. Design, synthesis, biological evaluation and pharmacokinetics of bis(hydroxyphenyl) substituted azoles, thiophenes, benzenes, and aza-benzenes as potent and selective nonsteroidal inhibitors of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1). *J. Med. Chem.* **2008**, *51* (21), 6725–6739.
(27) Bey, E.; Marchais-Oberwinkler, S.; Negri, M.; Kruchten, P.; Oster, A.; Klein, T.; Spadaro, A.; Werth, R.; Frotscher, M.; Birk, B.; Hartmann, R. W. New insights into the SAR and binding modes of bis(hydroxyphenyl)thiophenes and -benzenes: influence of additional substituents on 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1) inhibitory activity and selectivity. *J. Med. Chem.* **2009**, *52* (21), 6724–6743.
(28) Al-Soud, Y. A.; Bey, E.; Oster, A.; Marchais-Oberwinkler, S.; Werth, R.; Kruchten, P.; Frotscher, M.; Hartmann, R. W. The role of the heterocycle in bis(hydroxyphenyl)triazoles for inhibition of 17beta-hydroxysteroid dehydrogenase (17beta-HSD) type 1 and type 2. *Mol. Cell. Endocrinol.* **2009**, *301* (1–2), 212–215.
(29) Kruchten, P.; Werth, R.; Marchais-Oberwinkler, S.; Frotscher, M.; Hartmann, R. W. Development of a biological screening system for the evaluation of highly active and selective 17beta-HSD1-inhibitors as potential therapeutic agents. *Mol. Cell. Endocrinol.* **2009**, *301* (1–2), 154–157.
(30) Marchais-Oberwinkler, S.; Frotscher, M.; Ziegler, E.; Werth, R.; Kruchten, P.; Messinger, J.; Thole, H.; Hartmann, R. W. Structure–activity study in the class of 6-(3′-hydroxyphenyl)naphthalenes leading to an optimization of a pharmacophore model for 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1) inhibitors. *Mol. Cell. Endocrinol.* **2009**, *301* (1–2), 205–211.
(31) Kruchten, P.; Werth, R.; Bey, E.; Oster, A.; Marchais-Oberwinkler, S.; Frotscher, M.; Hartmann, R. W. Selective inhibition of 17beta-hydroxysteroid dehydrogenase type 1 (17betaHSD1) reduces estrogen responsive cell growth of T47-D breast cancer cells. *J. Steroid Biochem. Mol. Biol.* **2009**, *114* (3–5), 200–206.
(32) Oster, A.; Klein, T.; Werth, R.; Kruchten, P.; Bey, E.; Negri, M.; Marchais-Oberwinkler, S.; Frotscher, M.; Hartmann, R. W. Novel estrone mimetics with high 17beta-HSD1 inhibitory activity. *Bioorg. Med. Chem.* **2010**, *18* (10), 3494–3505.
(33) Oster, A.; Hinsberger, S.; Werth, R.; Marchais-Oberwinkler, S.; Frotscher, M.; Hartmann, R. W. Bicyclic substituted hydroxyphenyl-methanones as novel inhibitors of 17beta-hydroxysteroid dehydrogen-ase type 1 (17beta-HSD1) for the treatment of estrogen-dependent diseases. *J. Med. Chem.* **2010**, *53* (22), 8176–8186.
(34) Marchais-Oberwinkler, S.; Wetzel, M.; Ziegler, E.; Kruchten, P.; Werth, R.; Henn, C.; Hartmann, R. W.; Frotscher, M. New drug-like hydroxyphenylnaphthol steroidomimetics as potent and selective 17beta-hydroxysteroid dehydrogenase type 1 inhibitors for the treatment of estrogen-dependent diseases. *J. Med. Chem.* **2011**, *54* (2), 534–547.
(35) Oster, A.; Klein, T.; Henn, C.; Werth, R.; Marchais-Oberwinkler, S.; Frotscher, M.; Hartmann, R. W. Bicyclic substituted hydroxyphenylmethanone type inhibitors of 17 beta-hydroxysteroid dehydrogenase type 1 (17 beta-HSD1): the role of the bicyclic moiety. *ChemMedChem* **2011**, *6* (3), 476–487.
(36) Baston, E.; Hartmann, R. W. N-Substituted 4-(5-indolyl)-benzoic acids. Synthesis and evaluation of steroid 5alpha-reductase type I and II inhibitory activity. *Bioorg. Med. Chem. Lett.* **1999**, *9* (11), 1601–1606.
(37) Picard, F.; Baston, E.; Reichert, W.; Hartmann, R. W. Synthesis of N-substituted piperidine-4-(benzylidene-4-carboxylic acids) and evaluation as inhibitors of steroid-5alpha-reductase type 1 and 2. *Bioorg. Med. Chem.* **2000**, *8* (6), 1479–1487.
(38) Hartmann, R. W.; Reichert, M. New nonsteroidal steroid 5 alpha-reductase inhibitors. Syntheses and structure–activity studies on carboxamide phenylalkyl-substituted pyridones and piperidones. *Arch. Pharm.* **2000**, *333* (5), 145–153.
(39) Picard, F.; Schulz, T.; Hartmann, R. W. 5-Phenyl substituted 1-methyl-2-pyridones and 4′-substituted biphenyl-4-carboxylic acids. Synthesis and evaluation as inhibitors of steroid-5alpha-reductase type 1 and 2. *Bioorg. Med. Chem.* **2002**, *10* (2), 437–448.
(40) Picard, F.; Barassin, S.; Mokhtarian, A.; Hartmann, R. W. Synthesis and evaluation of 2′-substituted 4-(4′-carboxy- or 4′-carboxymethylbenzylidene)-*N*-acylpiperidines: highly potent and in vivo active steroid 5alpha-reductase type 2 inhibitors. *J. Med. Chem.* **2002**, *45* (16), 3406–3417.
(41) Tremblay, M. R.; Luu-The, V.; Leblanc, G.; Noel, P.; Breton, E.; Labrie, F.; Poirier, D. Spironolactone-related inhibitors of type II 17beta-hydroxysteroid dehydrogenase: chemical synthesis, receptor binding affinities, and proliferative/antiproliferative activities. *Bioorg. Med. Chem.* **1999**, *7* (6), 1013–1023.
(42) Sam, K.; Labrie, F.; Poirier, D. *N*-Butyl-*N*-methyl-11-(3′-hydroxy-21′,17′-carbolactone-19′-nor-17′alpha-pregna-1′,3′, 5′(10′)-trien-7′alpha-yl)-undecanamide: an inhibitor of type 2 17beta-hydroxysteroid dehydrogenase that does not have oestrogenic or androgenic activity. *Eur. J. Med. Chem.* **2000**, *35* (2), 217–225.
(43) Poirier, D.; Bydal, P.; Tremblay, M. R.; Sam, K. M.; Luu-The, V. Inhibitors of type II 17beta-hydroxysteroid dehydrogenase. *Mol. Cell. Endocrinol.* **2001**, *171* (1–2), 119–128.
(44) Bydal, P.; Auger, S.; Poirier, D. Inhibition of type 2 17beta-hydroxysteroid dehydrogenase by estradiol derivatives bearing a lactone on the D-ring: structure–activity relationships. *Steroids* **2004**, *69* (5), 325–342.
(45) Bydal, P.; Luu-The, V.; Labrie, F.; Poirier, D. Steroidal lactones as inhibitors of 17beta-hydroxysteroid dehydrogenase type 5: chemical synthesis, enzyme inhibitory activity, and assessment of estrogenic and androgenic activities. *Eur. J. Med. Chem.* **2009**, *44* (2), 632–644.
(46) Cook, J. H.; Barzya, J.; Brennan, C.; Lowe, D.; Wang, Y.; Redman, A.; Scott, W. J.; Wood, J. E. 4,5-Disubstituted cis-pyrrolidinones as inhibitors of 17beta-hydroxysteroid dehydrogenase II. Part 1: Synthetic approach. *Tetrahedron Lett.* **2005**, *46*, 1525–1528.
(47) Gunn, D.; Akuche, C.; Baryza, J.; Blue, M. L.; Brennan, C.; Campbell, A. M.; Choi, S.; Cook, J.; Conrad, P.; Dixon, B.; Dumas, J.; Ehrlich, P.; Gane, T.; Joe, T.; Johnson, J.; Jordan, J.; Kramss, R.; Liu, P.; Levy, J.; Lowe, D.; McAlexander, I.; Natero, R.; Redman, A. M.; Scott, W.; Seng, T.; Sibley, R.; Wang, M.; Wang, Y.; Wood, J.; Zhang, Z. 4,5-Disubstituted cis-pyrrolidinones as inhibitors of type II 17beta-hydroxysteroid dehydrogenase. Part 2. SAR. *Bioorg. Med. Chem. Lett.* **2005**, *15* (12), 3053–3057.
(48) Wood, J.; Bagi, C. M.; Akuche, C.; Bacchiocchi, A.; Baryza, J.; Blue, M. L.; Brennan, C.; Campbell, A. M.; Choi, S.; Cook, J. H.; Conrad, P.; Dixon, B. R.; Ehrlich, P. P.; Gane, T.; Gunn, D.; Joe, T.; Johnson, J. S.; Jordan, J.; Kramss, R.; Liu, P.; Levy, J.; Lowe, D. B.; McAlexander, I.; Natero, R.; Redman, A. M.; Scott, W. J.; Town, C.; Wang, M.; Wang, Y.; Zhang, Z. 4,5-Disubstituted cis-pyrrolidinones as inhibitors of type II 17beta-hydroxysteroid dehydrogenase. Part 3. Identification of lead candidate. *Bioorg. Med. Chem. Lett.* **2006**, *16* (18), 4965–4968.
(49) Poirier, D. Inhibitors of 17 beta-hydroxysteroid dehydrogenases. *Curr. Med. Chem.* **2003**, *10* (6), 453–477.
(50) Poirier, D. Advances in development of inhibitors of 17beta-hydroxysteroid dehydrogenases. *Anti-Cancer Agents Med. Chem.* **2009**, *9* (6), 642–660.
(51) Bagi, C. M.; Wood, J.; Wilkie, D.; Dixon, B. Effect of 17beta-hydroxysteroid dehydrogenase type 2 inhibitor on bone strength in ovariectomized cynomolgus monkeys. *J. Musculoskeletal Neuronal Interact.* **2008**, *8* (3), 267–280.
(52) Wetzel, M.; Marchais-Oberwinkler, S.; Hartmann, R. W. 17beta-HSD2 inhibitors for the treatment of osteoporosis: identification of a promising scaffold. *Bioorg. Med. Chem.* **2011**, *19*, 807–815.

(53) Miyaura, N.; Suzuki, A. Palladium-catalyzed cross-coupling reactions of organoboron compounds. *Chem Rev* **1995**, *95*, 2457−2483.
(54) Lucas, S.; Heim, R.; Negri, M.; Antes, I.; Ries, C.; Schewe, K. E.; Bisi, A.; Gobbi, S.; Hartmann, R. W. Novel aldosterone synthase inhibitors with extended carbocyclic skeleton by a combined ligand-based and structure-based drug design approach. *J. Med. Chem.* **2008**, *51* (19), 6138−6149.
(55) Xu, D.; Penning, T. M.; Blair, I. A.; Harvey, R. G. Synthesis of phenol and quinone metabolites of benzo[*a*]pyrene, a carcinogenic component of tobacco smoke implicated in lung cancer. *J. Org. Chem.* **2009**, *74* (2), 597−604.
(56) Schuster, D.; Kowalik, D.; Kirchmair, J.; Laggner, C.; Markt, P.; Aebischer-Gumy, C.; Ströhle, F.; Möller, G.; Wolber, G.; Wilckens, T.; Langer, T.; Odermatt, A.; Adamski, J. Identification of chemically diverse, novel inhibitors of 17beta-hydroxysteroid dehydrogenase type 3 and 5 by pharmacophore-based virtual screening. *J. Steroid Biochem. Mol. Biol.* **2011**, *125* (1−2), 148−161.
(57) Marchais-Oberwinkler, S.; Henn, C.; Möller, G.; Klein, T.; Negri, M.; Oster, A.; Spadaro, A.; Werth, R.; Wetzel, M.; Xu, K.; Frotscher, M.; Hartmann, R. W.; Adamski, J. 17beta-Hydroxysteroid dehydrogenases (17beta-HSDs) as therapeutic targets: protein structures, functions, and recent progress in inhibitor development. *J. Steroid Biochem. Mol. Biol.* **2011**, *125*, 66−82.
(58) Day, J. M.; Tutill, H. J.; Newman, S. P.; Purohit, A.; Lawrence, H. R.; Vicker, N.; Potter, B. V.; Reed, M. J. 17Beta-hydroxysteroid dehydrogenase type 1 and type 2: association between mRNA expression and activity in cell lines. *Mol. Cell. Endocrinol.* **2006**, *248* (1−2), 246−249.
(59) Leenders, F.; Tesdorpf, J. G.; Markus, M.; Engel, T.; Seedorf, U.; Adamski, J. Porcine 80-kDa protein reveals intrinsic 17 beta-hydroxysteroid dehydrogenase, fatty acyl-CoA-hydratase/dehydrogenase, and sterol transfer activities. *J. Biol. Chem.* **1996**, *271* (10), 5438−5442.
(60) Möller, G.; Adamski, J. Integrated view on 17beta-hydroxysteroid dehydrogenases. *Mol. Cell. Endocrinol.* **2009**, *301* (1−2), 7−19.
(61) Möller, G.; Deluca, D.; Gege, C.; Rosinus, A.; Kowalik, D.; Peters, O.; Droescher, P.; Elger, W.; Adamski, J.; Hillisch, A. Structure-based design, synthesis and in vitro characterization of potent 17beta-hydroxysteroid dehydrogenase type 1 inhibitors based on 2-substitutions of estrone and D-homo-estrone. *Bioorg. Med. Chem. Lett.* **2009**, *19* (23), 6740−6744.
(62) van Grunsven, E. G.; van Berkel, E.; Ijlst, L.; Vreken, P.; de Klerk, J. B.; Adamski, J.; Lemonde, H.; Clayton, P. T.; Cuebas, D. A.; Wanders, R. J. Peroxisomal D-hydroxyacyl-CoA dehydrogenase deficiency: resolution of the enzyme defect and its molecular basis in bifunctional protein deficiency. *Proc. Natl. Acad. Sci. U S A* **1998**, *95* (5), 2128−2133.
(63) Möller, G.; Husen, B.; Kowalik, D.; Hirvela, L.; Plewczynski, D.; Rychlewski, L.; Messinger, J.; Thole, H.; Adamski, J. Species used for drug testing reveal different inhibition susceptibility for 17beta-hydroxysteroid dehydrogenase type 1. *PLoS One* **2010**, *5* (6), No. e10969.
(64) Klein, T.; Henn, C.; Negri, M.; Frotscher, M. Structural basis for species specific inhibition of 17b-hydroxysteroid dehydrogenase type 1 (17b-HSD1): computational study and biological validation *PLoS One* **2011**, *6* (8), No. e22990.
(65) Qiu, W.; Campbell, R. L.; Gangloff, A.; Dupuis, P.; Boivin, R. P.; Tremblay, M. R.; Poirier, D.; Lin, S. X. A concerted, rational design of type 1 17beta-hydroxysteroid dehydrogenase inhibitors: estradiol−adenosine hybrids with high affinity. *FASEB J.* **2002**, *16* (13), 1829−1831.
(66) Zhu, D. W.; Lee, X.; Breton, R.; Ghosh, D.; Pangborn, W.; Duax, W. L.; Lin, S. X. Crystallization and preliminary X-ray diffraction analysis of the complex of human placental 17beta-hydroxysteroid dehydrogenase with $NADP^+$. *J. Mol. Biol.* **1993**, *234* (1), 242−244.
(67) Jazbutyte, V.; Hu, K.; Kruchten, P.; Bey, E.; Maier, S. K.; Fritzemeier, K. H.; Prelle, K.; Hegele-Hartung, C.; Hartmann, R. W.; Neyses, L.; Ertl, G.; Pelzer, T. Aging reduces the efficacy of estrogen substitution to attenuate cardiac hypertrophy in female spontaneously hypertensive rats. *Hypertension* **2006**, *48* (4), 579−586.
(68) Lin, S. X.; Yang, F.; Jin, J. Z.; Breton, R.; Zhu, D. W.; Luu-The, V.; Labrie, F. Subunit identity of the dimeric 17 beta-hydroxysteroid dehydrogenase from human placenta. *J. Biol. Chem.* **1992**, *267* (23), 16182−16187.
(69) Sam, K. M.; Auger, S.; Luu-The, V.; Poirier, D. Steroidal spiro-gamma-lactones that inhibit 17beta-hydroxysteroid dehydrogenase activity in human placental microsomes. *J. Med. Chem.* **1995**, *38* (22), 4518−4528.
(70) Sam, K. M.; Boivin, R. P.; Tremblay, M. R.; Auger, S.; Poirier, D. C16 and C17 derivatives of estradiol as inhibitors of 17beta-hydroxysteroid dehydrogenase type 1: chemical synthesis and structure−activity relationships. *Drug Des. Discovery* **1998**, *15* (3), 157−180.
(71) Deluca, D.; Möller, G.; Rosinus, A.; Elger, W.; Hillisch, A.; Adamski, J. Inhibitory effects of fluorine-substituted estrogens on the activity of 17beta-hydroxysteroid dehydrogenases. *Mol. Cell. Endocrinol.* **2006**, *248* (1−2), 218−224.
(72) Adamski, J. Isolation of vesicles mediating the conversion of 17 beta-estradiol to estrone. *Eur. J. Cell Biol.* **1991**, *54* (1), 166−170.
(73) Zimmermann, J.; Liebl, R.; von Angerer, E. 2,5-Diphenylfuran-based pure antiestrogens with selectivity for the estrogen receptor alpha. *J. Steroid Biochem. Mol. Biol.* **2005**, *94* (1−3), 57−66.
(74) Hartmann, R. W. Influence of alkyl chain ramification on estradiol receptor binding affinity and intrinsic activity of 1,2-dialkylated 1,2-bis(4- or 3-hydroxyphenyl)ethane estrogens and antiestrogens. *J. Med. Chem.* **1986**, *29* (9), 1668−1674.