Substituted 6-Phenyl-2-naphthols. Potent and Selective Nonsteroidal Inhibitors of 17β-Hydroxysteroid Dehydrogenase Type 1 (17β-HSD1): Design, Synthesis, Biological Evaluation, and Pharmacokinetics

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17β-Estradiol (E2) is implicated in the genesis and the development of estrogen-dependent diseases. Its concentration is mainly regulated by 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1), which catalyzes the reduction of the weak estrogen estrone (E1) to the highly potent E2. This enzyme is thus an important target for the treatment of hormone-dependent diseases. Thirty-seven novel substituted 6-phenyl-2-naphthols were synthesized and evaluated for 17β-HSD1 inhibition, selectivity toward 17β-HSD2 and the estrogen receptors (ERs) α and β, and pharmacokinetic properties. SAR studies revealed that the compounds most likely bind according to binding mode B to the active site, i.e., the 6-phenyl moiety mimicking the steroidal A-ring. While substitution at the phenyl ring decreased activity, introduction of substituents at the naphthol moiety led to highly active compounds, especially in position 1. The 1-phenyl compound **32** showed a very high inhibitory activity for 17β-HSD1 (IC_{50} = 20 nM) and good selectivity (17β-HSD2 and ERs) and pharmacokinetic properties after peroral application.

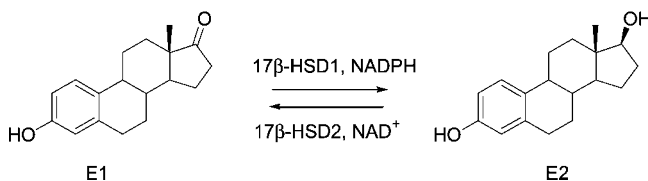
Introduction

It is well recognized that estrogens play a central role in female physiology. 17β-Estradiol (E2[a]), the endogenous ligand of the estrogen receptors, however, is also known to be involved in the development of estrogen-dependent diseases, inducing cell proliferation in breast cancer[1] and playing a critical role in the development and growth of endometriosis.[2]

Until today, two different strategies have been developed for the treatment of hormone-dependent breast cancer:[3,4] (1) reduction of the estrogen biosynthesis either by aromatase inhibitors, which prevent the transformation of androgens into estrogens, or by using GnRH analogues, which inhibit ovarian estrogen formation; (2) blockage of estrogen action at the receptor level via selective estrogen receptor modulators (SERMs).

Another approach to reduce the estrogen action could be achieved by inhibition of an enzyme catalyzing the last step in E2 biosynthesis. Three different 17β-hydroxysteroid dehydrogenase subtypes (1, 7, and 12) are principally able to catalyze the reduction of estrone (E1) to E2. The primary role of 17β-HSD7 and 17β-HSD12 is supposed to be in the cholesterol synthesis[5] and regulation of the lipid biosynthesis,[6] respectively. Moreover, Day et al.[7] showed that 17β-HSD12, although highly expressed in breast cancer cell lines, is inefficient in E2 formation. A pronounced reduction of the estrogen action should be obtained by inhibition of 17β-HSD1.

Chart 1. Interconversion of Estrone (E1) to Estradiol (E2)



17β-HSD1 (EC1.1.1.62) transforms the weak estrogen E1 into the most potent estrogen E2 (Chart 1). 17β-HSD1 is a cytosolic enzyme, which was crystallized with different steroidal ligands.[8–16] The X-ray structures provide information about the active site of the enzyme; it is an elongated hydrophobic tunnel with two polar ends (His221, Glu282 on one side and Ser142, Tyr155 on the other side; the last two amino acids are involved in the catalytic tetrad). Interestingly, another two polar amino acids (Ser222 and Tyr218) are also located in this narrow cavity close to the B/C ring of the steroid but do not interact directly with it.

17β-HSD1 is often overexpressed in breast cancer cells[17–20] resulting in high intracellular E2 concentrations. Inhibition of 17β-HSD1 will prevent the transformation of E1 into E2 and thus will reduce the intracrine[21] effect of E2. In contrast to aromatase inhibition the E1 level will not be affected and a basal estrogenic activity will be maintained. 17β-HSD1 inhibitors therefore might be softer therapeutics for the treatment of estrogen-dependent diseases; i.e., they should lead to fewer side effects. 17β-HSD1 is therefore an attractive target for the design of new drugs for breast cancer and endometriosis.

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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; ER, estrogen receptor; SERM, selective estrogen receptor modulator; SAR, structure−activity relationship; PDB ID, Protein Data Bank identification code; NADP(H), nicotinamide adenine dinucleotide phosphate; RBA, relative binding affinity; DME, dimethoxyethane; DMAP, 4-dimethylaminopyridine; EDCI, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide; HOBt, 1-hydroxybenzotriazole; *m*-CPBA, *m*-chloroperoxybenzoic acid; DMS, dimethyl sulfate. 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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Recently, Laplante et al.[22] showed that potent estradiol type inhibitors were able to reduce the E1 induced T-47D cell proliferation by 62% in vitro. Husen et al.[23,24] and Day et al.[7] have described mouse models to evaluate the efficacy of 17β-HSD1 inhibitors in vivo. Both groups have used ovariectomized immunodeficient mice and implanted MCF-7 cells stably transfected with human 17β-HSD1[23,24] or T-47D cells,[7] respectively. The E1 induced tumor growth could be reduced by 17β-HSD1 inhibitors.

Regarding the therapeutic concept, it is important that inhibitors of 17β-HSD1 are selective toward 17β-HSD2 (which inactivates E2 to E1, thus acting as an adversary to the type 1 enzyme) and toward the estrogen receptors α and β; i.e., they should have no or little affinity to these proteins.

Over the past years, several groups have reported about 17β-HSD1 inhibitors, most of them having a steroidal structure.[22,25,26] The first nonsteroidal inhibitors of 17β-HSD1, the thiophenepyrimidinones, were published by Messinger et al.[27] However, most of the nonsteroidal 17β-HSD1 inhibitors described so far do not appear to be druglike. Recently, on the basis of our experience in the design of steroid mimicking compounds,[28–30] we reported on the discovery of (hydroxyphenyl)naphthalenes as potent and selective inhibitors of 17β-HSD1.[31] The most promising compound of this series of steroidomimetics was the 6-(3-hydroxyphenyl)-2-naphthol **1** (Chart 2).

Compound **1** can be considered as a scaffold for structure optimization. Introduction of additional substituents leading to new interactions with amino acids from the active site might increase potency and selectivity of this class of inhibitors. In this report, the rational design of novel, selective inhibitors of 17β-HSD1 (based on the analysis of possible binding modes of the scaffold in the substrate binding site of the enzyme) will be presented. Subsequently, synthesis and biological evaluation of the derived substituted 6-phenyl-2-naphthols (Chart 2) will be described.

Design

There are two modes for the binding of compound **1** into the substrate binding site (Figure 1). Binding mode A is characterized by interactions of the phenyl-OH with Ser142 and Tyr155 from the catalytic tetrad while the naphthol-OH interacts with His221 and Glu282. Binding mode B is characterized by the formation of hydrogen bonds between the phenyl-OH and His221, Glu282, as well as between the naphthol-OH and Ser142, Tyr155. Docking of compound **1** into the active site of 17β-HSD1 (PDB ID 1FDT with the amino acids of the flexible loop in the B conformation), after having removed E2, shows that the space available for additional substituents on the lipophilic core structure is different in the two binding modes. This is visualized in Figure 1 with colored arrows: green arrows indicate that there is space available for large substituents. In orange, positions are shown with limited space (small substituents), and in red, positions are marked where no space is available for substitution.

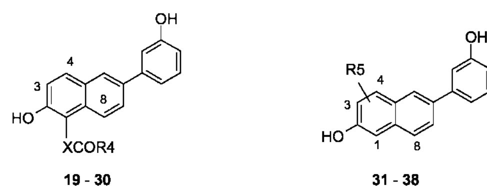
In case compound **1** binds according to mode A, space is available around position 4′ of the phenyl ring and position 1 of the naphthalene moiety to introduce a substituent; a compound substituted at these positions should have some activity. In contrast, introducing a substituent in position 5′, 6′, 3, 4, or 7 should result in an inactive compound. Accordingly, in case compound **1** binds in binding mode B, a substituent in position 2′ and 1 should be tolerated and lead to some activity while substitution in position 4′ and 6′ should result in inactive compounds. These considerations are only valid when the amino acids of the flexible loop (188−201) are in the B conformation, while there is more space available for substitution in the A conformation.

Compounds substituted at positions 4′ and 5′ of the phenyl ring and positions 1, 3, 4, and 8 of the naphthalene moiety were synthesized to get more insight in the binding mode of this class of compounds and to find out about the best position to gain activity.

Chart 2. Synthesized Compounds

1 - 6   7 - 18

Cmpd	R1	Cmpd	R2	X	R3	Cmpd	R2	R3
1	H	7	-OH		CH_3	13	$-NO_2$	CH_3
2	4′- NHCOCH$_3$	8	-OH		phenyl	14	$-NO_2$	phenyl
3	4′- NHCO-phenyl	9	-OH	CH=CH	CH_3	15	$-NO_2$	4-hydroxyphenyl
4	5′- CH_3	10	-OH	CH=CH	phenyl	16	$-NO_2$	3-hydroxyphenyl
5	5′- 4-hydroxyphenyl	11	-OH	CH_2CH_2	CH_3	17	$-NH_2$	CH_3
6	5′- 6-hydroxy-2-naphthyl	12	-OH	CH_2CH_2	phenyl	18	$-NH_2$	phenyl



Cmpd	R4	Cmpd	X	R4	Cmpd	R5
19	NHCH$_3$	25		NH-pyridin-2-yl	31	1- Br
20	NH-phenyl	26		NH-pyrimidin-2-yl	32	1- phenyl
21	NH-3-hydroxyphenyl	27		NH-5-methyl-1,3,4-thiadiazol-2-yl	33	1- SO_2-phenyl
22	piperidin-1-yl	28	CH=CH	NHCH$_3$	34	1- SO_2-4-methylphenyl
23	morpholin-4-yl	29	CH=CH	NH-phenyl	35	3- CONHCH$_3$
24	piperazin-1-yl	30	CH_2CH_2	NHCH$_3$	36	3- CONH-phenyl
					37	4- CN
					38	8- CN

Chemistry

Substitution at the Phenyl Ring. The synthesis of compounds **2**−**18** substituted in positions 4′ and 5′ of the phenyl ring is depicted in Schemes 1–4. Scheme 1 shows the synthesis of compounds **2** and **3** substituted in position 4′ of the phenyl ring with an amide moiety. These compounds were obtained from Suzuki coupling[32] using 4-bromo-2-methoxyaniline **2c** and 6-methoxy-2-naphthalene boronic acid **2d** as reactants. The

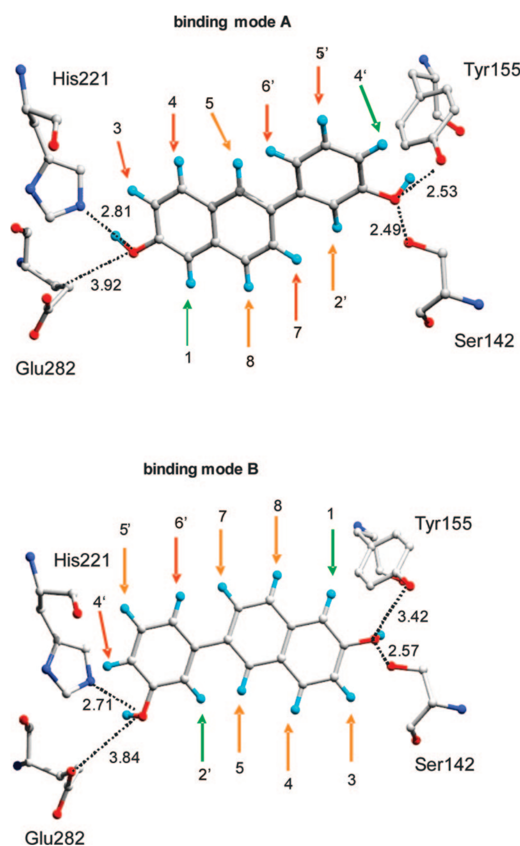
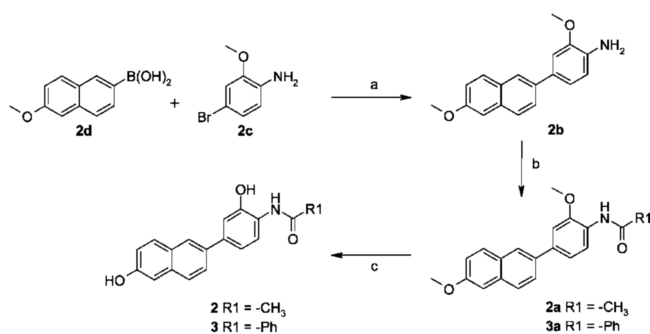


Figure 1. Two possible binding modes for compound **1**. For each binding mode A and B, investigation of the space available around **1** was done for introduction of substituents. Green arrows indicate enough space for large substituents. Orange arrows indicate space for smaller substituents, and red arrows indicate no space for any substituents. Dotted lines represent distances (Å).

Scheme 1. Synthesis of Compounds **2** and **3**[a]



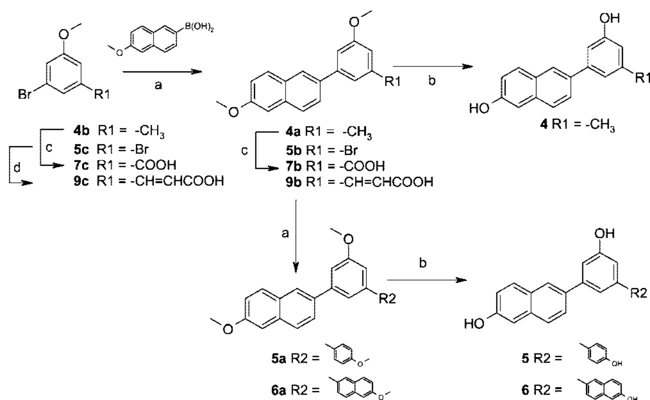
[a] Reagents and conditions: (a) $Pd(PPh_3)_4$, aqueous Na_2CO_3, toluene, 80 °C, overnight; (b) RCOCl, CH_2Cl_2, DMAP, room temp, overnight; (c) BBr_3, CH_2Cl_2, −78 °C, overnight.

aniline moiety was N-acylated with two different acid chlorides. Subsequent ether cleavage with boron tribromide[33] led to **2** and **3**.

Compounds **4**−**6** bearing alkyl or aromatic substituents in position 5′ of the phenyl ring were synthesized according to the route described in Scheme 2. Suzuki coupling between the bromo derivatives (**4b**, **5c**) and the appropriate boronic acid (one Suzuki reaction for **4a** and two successive Suzuki couplings for **5a** and **6a**) followed by a demethylation step using boron tribromide gave compounds **4**−**6**.

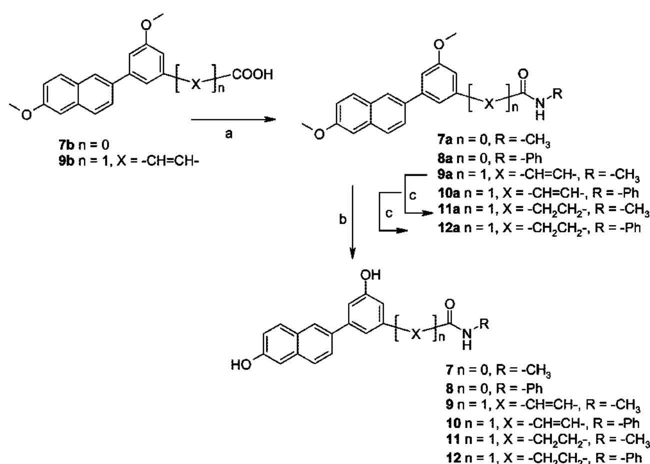
Compounds **7** and **8** with an amide moiety directly linked to position 5′ of the phenyl ring and compounds **9**−**12** bearing the amide function linked via a C2 spacer were synthesized

Scheme 2. Synthesis of Compounds **4**−**6**[a]



[a] Reagents and conditions: (a) $Pd(PPh_3)_4$, aqueous Na_2CO_3, DME, 80°C, 12−26 h; (b) BBr_3, CH_2Cl_2, −78 °C, 4 h; (c) $KMnO_4$, pyridine/H_2O (2:5), 75 °C, 65 h; (d) acrylic acid, PPh_3, $Pd(OAc)_2$, NEt_3, xylene, 100 °C, 11 h.

Scheme 3. Synthesis of Compounds **7**−**12**[a]



[a] Reagents and conditions: (a) RNH_2, EDCI, HOBt, CH_2Cl_2, 0 °C to room temp, overnight; (b) BBr_3, CH_2Cl_2, −78 °C, 4 h; (c) $Pd(OH)_2$, H_2, THF, room temp, 20 h.

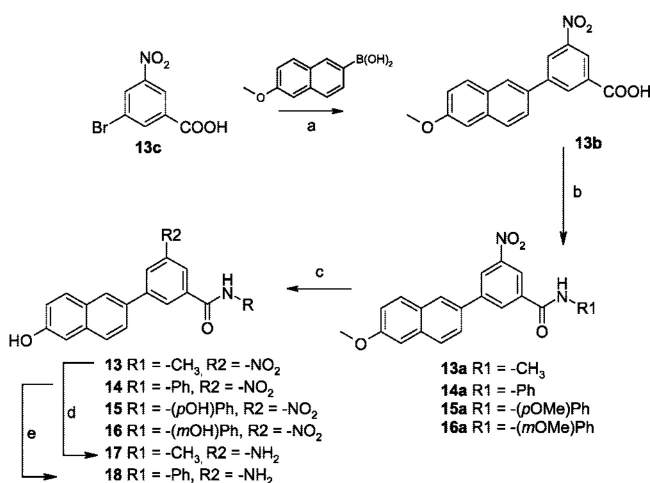
according to the pathway described in Scheme 3. These compounds were obtained after Suzuki coupling, acylation in presence of EDCI and HOBt[34] and ether cleavage (boron tribromide). The intermediates **7b** and **7c** were prepared by oxidation of the methyl derivatives **4a** and **4b**, respectively, with potassium permanganate[35] (Scheme 2). The acrylic acid **9c**, the precursor of **9b**, was prepared from the corresponding bromo derivative **5c** via Heck reaction[36] (Scheme 2). Subsequent catalytic hydrogenation of the double bond (**9a** and **10a**) was performed using Pearlman's catalyst.[37]

Compounds **13**−**16** with a nitro instead of a hydroxy moiety in position 3′ of the phenyl ring and an amide function in position 5′ were also synthesized. The preparation of these compounds **13**−**16** is described in Scheme 4 and is similar to the route leading to (3′-hydroxyphenyl)naphthalenes **7** and **8** mentioned above. Reduction of the nitro groups, either by hydrogenation in presence of palladium on charcoal as catalyst[38] or by action of tin with hydrochloric acid,[39] afforded amines **17** and **18**.

Substitution at the Naphthalene Ring. The synthesis of compounds **19**−**38** substituted in positions 1, 3, 4, and 8 of the naphthalene moiety is depicted in Schemes 5–9.

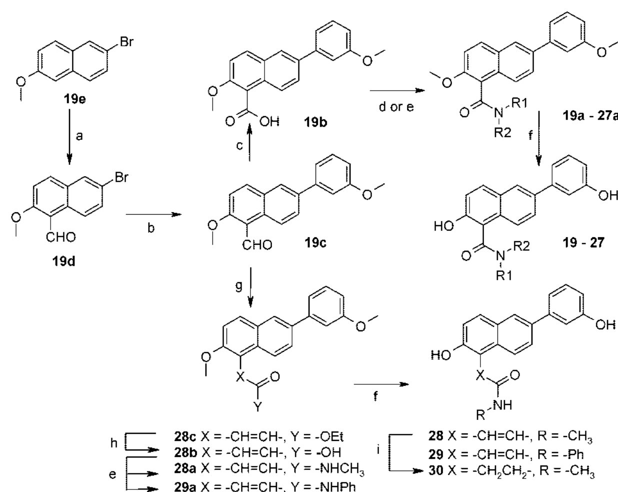
Substituents introduced in position 1 include carboxylic acid amides, bromine, phenyl, and sulfones. Synthesis of the amides

Scheme 4. Synthesis of Compounds **13−18**[a]



[a] Reagents and conditions: (a) $Pd(PPh_3)_4$, aqueous Na_2CO_3, DME, 80 °C, overnight; (b) RNH_2, EDCI, HOBt, CH_2Cl_2, 0 °C to room temp, 48 h; (c) BBr_3, CH_2Cl_2, −78 °C, 4 h; (d) H_2, Pd/C, EtOH, 0 °C to room temp, overnight; (e) Sn, HCl, THF, 50 °C, 1 h.

Scheme 5. Synthesis of Compounds **19−30**[a]

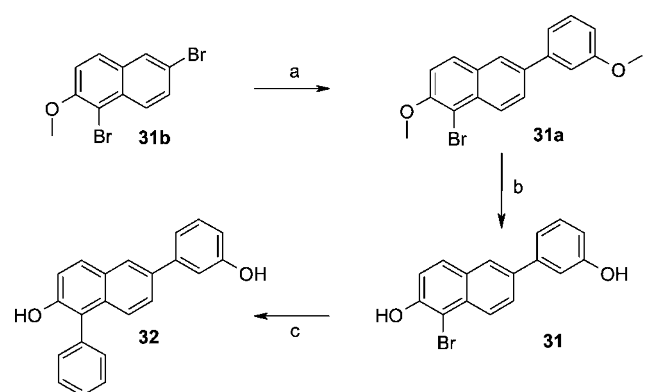


Cmpd	R1	R2
19a / 19	-CH₃	-H
20a / 20	-Ph	-H
21a / 21	-(*m*OMe)Ph/-(*m*OH)Ph	-H
22a / 22	-piperidine-	
23a / 23	-morpholine-	
24a / 24	-piperazine-	
25a / 25	pyridin-2-yl	-H
26a / 26	pyrimidin-2-yl	-H
27a / 27	5-methyl-1,3,4-thiadiazol-2-yl	-H

[a] Reagents and conditions: (a) $TiCl_4$, dichloromethyl methyl ether, CH_2Cl_2, 0 °C to room temp, overnight; (b) 3-methoxybenzeneboronic acid, $Pd(PPh_3)_4$, toluene, aqueous Na_2CO_3, 80 °C, 24 h; (c) H_2NSO_3H, NaOClO, H_2O/acetone (1:2), 0 °C, 30 min; (d) (i) $SOCl_2$, room temp, 30 min; (ii) RNH_2, DME or CH_2Cl_2, DMAP, room temp, overnight; (e) RNH_2, EDCI, HOBt, CH_2Cl_2, room temp, overnight; (f) BBr_3, CH_2Cl_2, −78 °C to room temp, overnight; (g) triethylphosphonoacetate, NaH, dry DME, room temp, 1 h; (h) LiOH, THF/H_2O (3:1), reflux, overnight; (i) $Pd(OH)_2$, H_2, ethanol/THF (2:1), room temp, overnight.

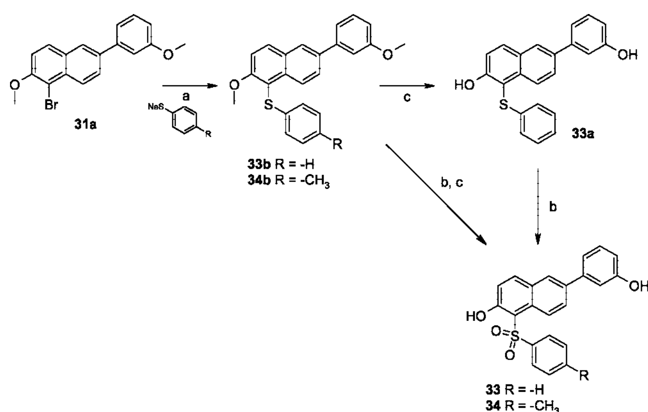
19−30 is shown in Scheme 5. Formylation at position 1 of the naphthalene moiety, performed according to the procedure described by Royer and Buisson,[40] followed by Suzuki cross-coupling, led to the key intermediate, aldehyde **19c**. It was oxidized[41] into the corresponding carboxylic acid **19b**. After

Scheme 6. Synthesis of Compounds **33** and **34**[a]



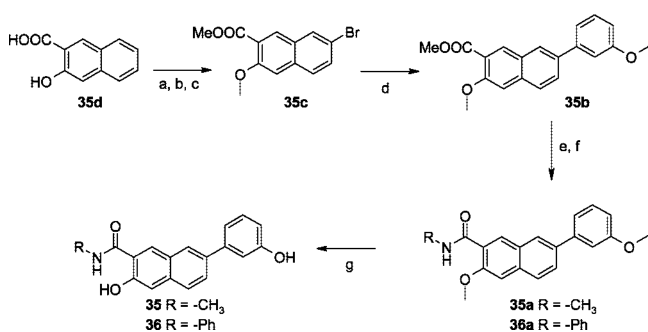
[a] Reagents and conditions: (a) 3-methoxybenzeneboronic acid, $Pd(PPh_3)_4$, toluene, aqueous Na_2CO_3, 80 °C, overnight; (b) BBr_3, CH_2Cl_2, −78 °C to room temp, overnight; (c) benzeneboronic acid, $Pd(PPh_3)_4$, toluene, aqueous Na_2CO_3, 80 °C, 23 h.

Scheme 7. Synthesis of Compounds **31** and **32**[a]



[a] Reagents and conditions: (a) DMF, reflux, 11 h; (b) *m*-CPBA, CH_2Cl_2, 0 °C to room temp, 12 h; (c) BBr_3, CH_2Cl_2, −78 °C to room temp, overnight.

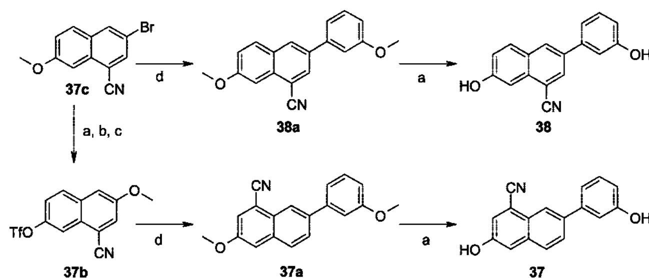
Scheme 8. Synthesis of Compounds **35** and **36**[a]



[a] Reagents and conditions: (a) Br_2, acetic acid, reflux, 2 h; (b) Sn, HCl, acetic acid, reflux, 3 h; (c) DMS, K_2CO_3, acetone, reflux, 3 h; (d) 3-methoxybenzeneboronic acid, $Pd(PPh_3)_4$, toluene, aqueous Na_2CO_3, 80 °C, overnight; (e) LiOH, THF/H_2O (1:1), reflux, 90 min; (f) RNH_2, EDCI, HOBt, CH_2Cl_2, 0 °C to room temp, overnight; (g) BBr_3, CH_2Cl_2, −78 °C to room temp, overnight.

conversion to the amides **19a−27a**, the ether functions were deprotected to give the desired compounds **19−27**. In another synthetic route, the aldehyde **19c** was subjected to the Horner−Wadsworth−Emmons conditions[42] to introduce an acrylic ester moiety (compound **28c**). Hydrolysis of the ester, amide formation, and ether cleavage afforded compounds **28** and **29**. Reduction of the double bond by hydrogenation using Pearlman's catalyst led to **30**.

Scheme 9. Synthesis of Compounds **37** and **38**[a]



[a] Reagents and conditions: (a) pyridinium hydrochloride; 190 °C, 2 h; (b) NaOMe, CuBr, reflux, 3 h; (c) Tf_2O, pyridine, CH_2Cl_2, 0 °C; (d) 3-methoxybenzeneboronic acid, $Pd(PPh_3)_4$, DME, aqueous Na_2CO_3, 80 °C, overnight.

The synthesis of the 1-phenylnaphthalene **32** is depicted in Scheme 6. Regioselective Suzuki reaction of the 1,6-dibromonaphthalene **31b** led to the 1-bromo-6-(3′-methoxyphenyl)-naphthalene **31a**. Subsequent ether cleavage led to the 1-bromonaphthol **31**, which in turn was submitted to a second Suzuki coupling to give compound **32**.

The synthesis of the 1-sulfonylnaphthalenes **33** and **34** was performed according to the route described in Scheme 7 from the key intermediate **31a**. Aromatic nucleophilic substitution of the 1-bromonaphthalene **31a** by sodium benzenethiolate or 4-methylbenzenethiolate[43] led to the thioethers **33b** and **34b**. Hydrolysis of the methoxy groups and oxidation with *m*-CPBA[44] gave the corresponding sulfones **33** and **34**.

Introduction of amide moieties in position 3 of the 2-naphthol core was carried out as depicted in Scheme 8. The intermediate **35c** was synthesized using the route described by Murphy et al.[45] It was submitted to Suzuki coupling, amide formation, and ether cleavage to give the final compounds **35** and **36**.

The synthesis of compounds substituted with a cyano moiety at position 4 (**37**) or 8 (**38**) of the 2-naphthol system started from the common intermediate **37c** (Scheme 9). The latter was obtained using the route described by Mewshaw et al.[46] Starting from **37c** the 4-cyanonaphthalene **37** was prepared in a five-step pathway according to the literature.[46] Suzuki coupling between **37c** and 3-methoxybenzeneboronic acid followed by a subsequent ether cleavage with pyridinium hydrochloride afforded the 8-cyanonaphthalene **38**.

Biological Results

Inhibition of Human 17*β*-HSD1. As source of enzyme, both recombinant and human placental enzymes were used. The incubations were run with tritiated E1, cofactor, and inhibitor and led to comparable results using both enzymes. The separation of substrate and product was performed by HPLC. The percent inhibition values of all hydroxy compounds are shown in Tables 1 and 2 except for compounds **2** and **3** and **5−18**, which are inactive and are not reported. The IC_{50} values of selected compounds are shown in Table 3. Compounds showing less than 10% inhibition at 1 μM were considered to be inactive. All molecules with methoxy groups showed no activity (data not shown).

The unsubstituted compound **1** identified as lead in the previous work[31] was used as reference compound.

Compounds substituted at positions 4′ and 5′ of the phenyl rings of **2−18** were all inactive except compound **4**, with a methyl group in position 5′ which presented a medium activity (42% and 14% inhibition at 1 μM and 100 nM, respectively). Substituents at position 4′ of the phenyl ring as well as at position 5′ with the exception of methyl are not tolerated by the enzyme. A lack of space in this region of the active site might be responsible for the loss of activity compared to compound **1**. In case the compounds bind according to binding mode A, the flexible loop might reduce the space in the area of the catalytic tetrad. It could be expected that the loop also adopts other conformations; space should then be present in the region close to positions 4′ and 5′ of the phenyl ring. The high diversity of substituents introduced at these positions, leading to inactive compounds, shows either that the loop remains located in front of the substrate binding site or that these compounds do not bind according to binding mode A.

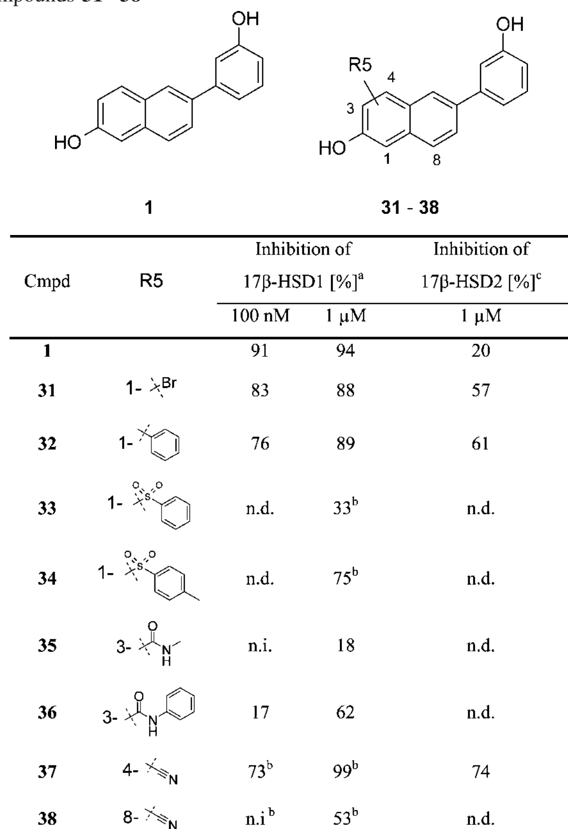
Table 1. Inhibition of Human 17β-HSD1 and 17β-HSD2 by Compounds **19−30**

Cmpd	X	R4	Inhibition of 17β-HSD1 [%][a] 100 nM	Inhibition of 17β-HSD1 [%][a] 1 μM	Inhibition of 17β-HSD2 [%][c] 1 μM
1			91	94	20
19			22	76	18
20			40	80	28
21			21	70	n.d.
22			29	73	n.d.
23			28	62	n.d.
24			n.i.	45	n.d.
25			n.i.[b]	n.i.[b]	n.d.
26			n.i.[b]	n.i.[b]	n.d.
27			n.d.	73[b]	n.d.
28			19	58	n.d.
29			n.i.	60	n.d.
30			n.i.	80	36[d]

[a] Recombinant human 17β-HSD1, substrate [^{3}H]E1 [30 nM], NADPH [1 mM], procedure A, mean value of two determinations. [b] Human placental 17β-HSD1, substrate [^{3}H]E1 [500 nM], NADH [500 μM], procedure B, mean value of three determinations, relative standard deviation of 10% for **27**. [c] Human placental 17β-HSD2, substrate [^{3}H]E2 [500 nM], NAD^+ [1500 μM], procedure D, mean value of three determinations, relative standard deviation of 38% for **19** and 27% for **20**. [d] Recombinant human 17β-HSD2 [^{3}H]E2 [30 nM], NAD^+ [1 mM], procedure C, mean value of two determinations. n.d. = not determined, n.i. = no inhibition (inhibition <10%).

Compounds **19−38** are substituted in positions 1, 3, 4, and 8 of the naphthalene ring. Introduction of a substituent having the ability to be a hydrogen-bond acceptor or donor might enable compounds to additionally interact with Tyr218 and/or Ser222, the two polar amino acids present in the active site, thus increasing 17β-HSD1 inhibitory activity. A compound binding according to binding mode A, substituted in position 1 of the naphthalene, might be able to establish hydrogen bond interac-

Table 2. Inhibition of Human 17β-HSD1 and 17β-HSD2 by Compounds **31**−**38**



Cmpd	R5	Inhibition of 17β-HSD1 [%][a]		Inhibition of 17β-HSD2 [%][c]
		100 nM	1 μM	1 μM
1		91	94	20
31	1-Br	83	88	57
32	1-Ph	76	89	61
33	1-SO_2Ph	n.d.	33[b]	n.d.
34	1-SO_2-tolyl	n.d.	75[b]	n.d.
35	3-C(O)NHCH$_3$	n.i.	18	n.d.
36	3-C(O)NHPh	17	62	n.d.
37	4-CN	73[b]	99[b]	74
38	8-CN	n.i[b]	53[b]	n.d.

[a] Recombinant human 17β-HSD1, substrate [^{3}H]E1 [30 nM], NADPH [1 mM], procedure A, mean value of two determinations. [b] Human placental 17β-HSD1, substrate [^{3}H]E1 [500 nM], NADH [500 μM], procedure B, mean value of three determinations. [c] Human placental 17β-HSD2, substrate [^{3}H]E2 [500 nM], NAD$^+$ [1500 μM], procedure D, mean value of three determinations. n.d. = not determined. n.i. = no inhibition (inhibition <10%).

tions with these amino acids. In order to explore this hypothesis, a number of compounds substituted with an amide in position 1 (**19**−**30**) were synthesized and evaluated for their 17β-HSD1 inhibitory activities (Table 1). The amide moiety was linked directly to the position 1 (**19**−**27**) or linked via a C2 spacer (**28**−**30**) (unsaturated or saturated). Amides **19**−**30** show a moderate activity (between 45% and 80% inhibition at 1 μM) except for the pyridine **25** and the pyrimidine **26**, which are inactive. Introduction of the spacer between the naphthalene and the amide function does not enhance the activity. The sulfones **33** and **34** also turned out to be moderately active (33% and 75% inhibition at 1 μM, respectively) (Table 2). Introduction of an amide or of a sulfone led to a significant drop in activity compared to compound **1**. There is some space available in this position, but the nature of the substituent does not seem to be appropriate. It is unlikely that hydrogen bond interactions are formed with the two targeted amino acids Ser222 and Tyr218.

A bromine and a phenyl ring were also inserted in position 1 of the naphthalene. The corresponding compounds **31** and **32** turned out to be at least as active as the reference compound **1** (Table 2). This shows that there is space available for an aromatic moiety in this region of the active site. The enhanced activity of compound **32** might be indicative of π−π stacking interactions.

Substitution in position 3 of the naphthalene was also investigated. The amides **35** and **36**, however, exhibited only little and moderate activity. Introduction of a cyano function in position 8 of the naphthalene ring (**38**) reduced activity, while the same substituent in position 4 (**37**) was very well tolerated (Table 2).

For selected compounds IC_{50} values were determined (Table 3). Compounds **31** and **32** with an IC_{50} value of 40 and 20 nM, respectively, were the most potent inhibitors identified in this series (3−4 times more potent than the reference compound **1**).

Selectivity. To assess the selectivity of the most interesting compounds, inhibition of 17β-HSD2 and affinity to the estrogen receptors α and β (ER α and β) were determined.

Since 17β-HSD2 catalyzes the oxidative transformation of E2 into E1, thus "inactivating" E2, inhibition of this enzyme is counterproductive for the treatment of estrogen-dependent diseases. Briefly, human placental microsomes as source of 17β-HSD2 were incubated with [^{3}H]E2 in the presence of NAD$^+$ and inhibitor. The amount of labeled E1 formed from substrate was determined after HPLC separation. Inhibition of 17β-HSD2 was measured for compounds showing more than 75% inhibition of 17β-HSD1 at 1 μM. Compounds substituted at the phenyl ring have weak to no inhibitory activity toward 17β-HSD2 (<20%; data not shown). Compounds substituted at the naphthalene ring are also weak to moderate inhibitors of 17β-HSD2 (Tables 1 and 2). By use of the IC_{50} values, selectivity factors ((IC_{50} HSD2)/(IC_{50} HSD1)) were calculated (Table 3). It turned out that compounds **31** and **32** show reasonable selectivity.

Furthermore, inhibitors of 17β-HSD1 should have low or no affinity for ER α and β, since binding to these receptors could counteract the therapeutic concept of 17β-HSD1 inhibition. For the most interesting compounds, binding affinities were determined. The ER assays were performed using recombinant human protein, [^{3}H]E2, and inhibitor. Separation of bound and free E2 was carried out using hydroxyapatite. Compounds **31** and **32** show very little affinity to the ERs (Table 3).

Since the ER binding affinity experiments do not explore the intrinsic activity at the receptor, compounds **1** and **32** were further investigated. By use of the estrogen dependent mammary tumor cell line T-47D, cell proliferation was monitored after incubation with the test compounds. E2 was used as positive control. At an E2 concentration of 0.1 nM, a strong stimulation of cell proliferation was observed after 8 days of incubation. Administered in the same concentration, compounds **1** and **32** did not show any stimulatory effect. The compounds had to be applied in a much higher concentration (100 nM, 1000-fold excess compared to E2) to see a weak stimulation (41% and 39%, respectively, of the E2 effect).

Pharmacokinetic Evaluation of Compounds 1 and 32. To get an idea about the in vivo behavior of compounds **1** and **32**, their pharmacokinetics were determined in the rat. After peroral administration of **1** and **32** to male rats (10 mg/kg) (n = 4) in a cassette dosing approach, plasma samples were collected over 24 h and concentrations determined by LC−MS/MS. The pharmacokinetic parameters of **1** and **32** are presented in Table 4. Each compound first showed a continuous increase in plasma concentration over time for 6 h for **1** and for 4 h for **32** (Figure 2). Maximal plasma concentration ($C_{max\ obs}$) was higher for **1** (2226 ng/mL) than for **32** (860 ng/mL); the time of maximal plasma concentration ($t_{max\ obs}$) was measured 6 and 4 h after administration for **1** and **32**, respectively. Subsequently, plasma concentrations decrease again. Plasma levels were very low 24 h after administration. The mean profile of the plasma concentration of **1** and **32** is depicted in Figure 2. These results indicate that both compounds exhibit a good pharmacokinetic profile in

Table 3. IC_{50} Values, Selectivity Factors, and Binding Affinities for the Estrogen Receptors α and β for Selected Compounds

compd	17β-HSD1 IC_{50} [nM][a,b]	17β-HSD2 IC_{50} [nM][a,c]	selectivity factors[d]	ERα RBA (%)[e]	ERβ RBA (%)[e]
1	116	5641	48	0.2	0.8
31	40	639	16	0.01 < RBA < 0.1	0.01 < RBA < 0.1
32	20	540	27	0.01 < RBA < 0.1	0.01 < RBA < 0.1

[a] Mean value of three determinations. [b] Human placental 17β-HSD1, substrate [^{3}H]E1 [500 nM], NADH [500 μM], procedure B. [c] Human placental 17β-HSD2, substrate [^{3}H]E2 [500 nM], NAD^+ [1500 μM], procedure D. [d] IC_{50}(17β-HSD2)/IC_{50}(17β-HSD1). [e] RBA: relative binding affinity, estradiol: 100%.

Table 4. Pharmacokinetic Parameters of Compounds **1** and **32** in Rats after Oral Application

compd	parameters[a]							
	dose [mg/kg]	$C_{max\ obs}$ [ng/mL]	C_z [ng/mL]	$t_{max\ obs}$ [h]	t_z [h]	$t_{1/2z}$ [h]	$AUC_{0\text{-}tz}$ [ng·h/mL]	$AUC_{0-\infty}$ [ng·h/mL]
1	10	2226	159	6	24	4	29 694	30 698
32	10	860	115	4	24	6	11 701	12 669

[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 that has an analytical 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.

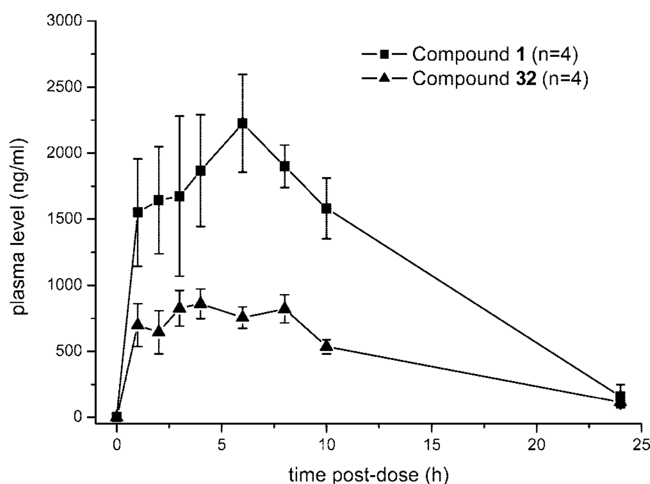


Figure 2. Mean profile (±SEM) of plasma levels (ng/mL) in rat versus time after oral application (10 mg/kg) of compounds **1** and **32** in cassette dosing.

the rat and that they might be good candidates for further experiments in disease-oriented models.

Molecular Modeling

The biological results show that introduction of substituents in position 4′ or 5′ of the phenyl ring is detrimental for activity with the exception of a small group in 5′ (medium activity). Substitution at the naphthalene ring is well tolerated in positions 1 and 4, while a decrease in activity is observed in positions 3 and 8. A correlation between these experimental data and the space available around each position of compound **1** (deduced from the docking poses presented in Figure 1) can be established in the case of binding mode B, which appears to be the most favorite binding mode of this series of substituted (3′-hydroxyphenyl)naphthalenes.

The most potent inhibitor described in this report, the 1-phenylnaphthol **32**, was docked in the enzyme (PDB ID 1FDT) to better understand the favorable interactions achieved by **32** in the active site. The obtained pose is shown in Figure 3. It is located in the substrate binding pocket according to binding mode B. It has a flat geometry, like compound **1**, only the 1-phenyl moiety is turned about 60° away from the plane of the naphthalene.

Compound **32** seems to be stabilized in the active site by hydrogen bond and hydrophobic interactions (van der Waals and arene−arene interactions). The phenyl-OH moiety establishes hydrogen bond interactions with His221 and Glu282, and the naphthalene-OH with Ser142 and Tyr155 as described for compound **1**.[31] Additionally, the phenyl-OH is stabilized via π−π interaction with Tyr218 in a parallel-displaced geometry (distance between the two ring centers, 5.09 Å). The phenyl-naphthalene backbone is stabilized by hydrophobic interactions (van der Waals). The phenyl ring in position 1 of the naphthalene seems to be involved in hydrophobic interactions with the nicotinamide part of the cofactor. The distance of the ring centers (4.72 Å) and the closest contact distance between a carbon of the phenyl ring of **32** and of the nicotinamide (3.63 Å) are in a good range, as it is described for π−π interactions by McGaughey et al.[47] Furthermore, this 1-phenyl ring could additionally establish π−π interactions with Phe226 and with Tyr155 (both T-shape interaction). The distances between the ring centers are 4.23 and 5.42 Å, respectively. From the docking pose, it also becomes apparent that a small hydrophobic pocket is located close to position 4 of the naphthalene. The finding that the 4-cyano compound **37** is active is in agreement with the observation that there is space available for a small substituent.

From the protein structure, steric hindrance and electrostatic repulsion might be the reason for the fact that amide groups in position 3 of the naphthalene (**35** and **36**) decrease activity of the parent compound **1**. However, in the case of the phenylamide **36**, the aromatic moiety might be able to reach the hydrophobic area close to position 4, stabilizing the molecule and explaining a regaining in activity compared to the methylamide **35**.

Discussion and Conclusion

The biological results obtained confirm the hypothesis[31] that substituted (3′-hydroxyphenyl)naphthalenes bind in the active site according to binding mode B; i.e., the phenyl-OH moiety of the compounds mimic the A-ring of E2. In this region, there is no or very little (5′-methylphenyl, compound **4**) space for a substituent. On the other hand, space is available around the naphthalene scaffold in positions 3 and 8 and especially 1 and 4 (region mimicking the C/D ring of E2). These results are in agreement with the activity of substituted steroidal inhibitors of 17β-HSD1 reported in the literature; the most active compounds are substituted with large groups only at the D ring[48,49] (position 15 or 16) and a few at the B ring[50] (position 6 or 7). Only a small substituent like ethyl or methoxy[51] is tolerated in position 2 of the steroid (A-ring), but activity is often reduced. These findings are very important for drug design: the space available for substituents on the scaffold of our compounds is very limited (in the region corresponding to the

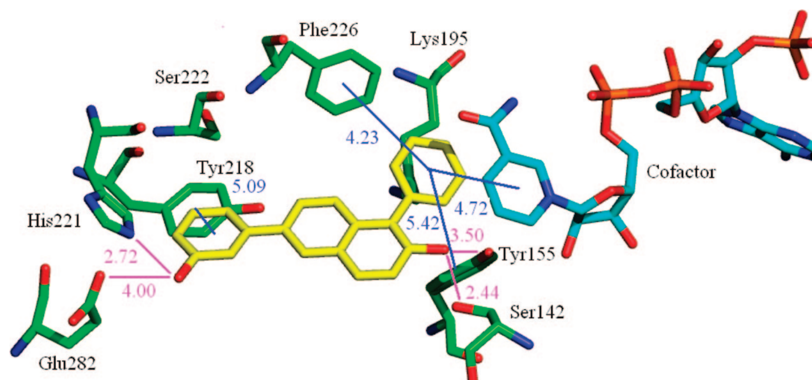


Figure 3. Structure of the 17β-HSD1 binding pocket (green amino acids) with the docked compound **32** (yellow) following binding mode B in the presence of the cofactor. Hydrogen bonding interactions and $\pi-\pi$ stacking interactions are marked by violet lines and blue lines, respectively. All distances are expressed in Å. The figure was generated with Pymol (http://www.pymol.org).

A-ring of E2), while an empty cavity is present in the neighborhood of the catalytic tetrad (around the D-ring of E2).

Furthermore, our results indicate that position 1 of the naphthalene ring is appropriate for the introduction of large substituents and that aromatic groups are better tolerated than amides or sulfones. The latter substituents were introduced with the aim of establishing hydrogen bonds with Ser222 and Tyr218, which might have been adequate partners in case of binding mode A. The fact that all compounds **19**−**30** show a decrease in activity is certainly in disfavor of binding mode A, as in this mode hydrogen bond interactions should have been possible. The low activity of these compounds, however, also demonstrates that these groups are not able to interact with the nicotinamide moiety of the cofactor (binding mode B).

It remains to be elucidated whether it is possible to establish interactions with Ser222 and Tyr218 to improve activity and selectivity. It is striking that these two polar amino acids, which do not interact with the steroid, are present in the active site. However, they might be involved in the stabilization of the three-dimensional structure of the protein and thus might not be free to establish another interaction.

In binding mode B, the phenyl moiety of the most potent inhibitor **32** is located in the catalytic region where space is available. It might undergo $\pi-\pi$ stacking interactions with the nicotinamide part of the cofactor (parallel-displaced geometry). Other $\pi-\pi$ interactions might also occur with the aromatic amino acids Phe226 and Tyr155. It remains unclear why the 1-bromo compound **31** is more active than **1**. It might be due to the electronic effect of the bromine. However, hydrophobic interactions of the bromine with the cofactor cannot be excluded.

Concerning the selectivity of the highly active 17β-HSD1 inhibitors, selected compounds were tested for inhibition of 17β-HSD2 and affinity to ER α and β. Compounds **31** and **32** showed a high selectivity for 17β-HSD2, exhibiting selectivity factors of 16 and 27, respectively. They also showed very little affinity to ER α and β (between 0.01% and 0.1% that of E2). This result is in accordance with the data reported by Mewshaw et al.,[46] who found that the introduction of a phenyl group into the 1 position of 6-(4-hydroxyphenyl)-2-naphthol reduced ER affinity strongly. This shows that there is no or little space in the ERs to introduce a bulky substituent at the 1 position.[46] In contrast to substitution in the 1 position, a gain in ER affinity was described for compounds substituted at the 4 position with a cyano group.[46] Therefore, position 1, and not position 4, is appropriate to achieve selective 17β-HSD1 inhibition. The affinity of **32** to the ERs corresponds to a very small agonistic effect. Tested in 1000-fold higher concentration than E2 using the ER-positive T-47D mammary tumor cell line, it showed 39% of the stimulatory effect of E2.

In this report, we described the synthesis of substituted (3′-hydroxyphenyl)-2-naphthols as inhibitors of 17β-HSD1 and the evaluation of their biological properties. SAR studies using the 17β-HSD1 inhibition data revealed that the compounds most likely bind according to binding mode B into the active site. A new, potent, and selective 17β-HSD1 inhibitor, compound **32**, was discovered. It contains a phenyl group at the 1 position of the naphthalene that may interact with binding partners in the catalytic region. It is highly selective toward 17β-HSD2 and the ER α and β and shows a good pharmacokinetic profile after peroral application. It could be used in an in vivo disease-oriented model to further validate the concept that 17β-HSD1 might be a promising target for the treatment of estrogen-dependent diseases.

Experimental Section

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

^{1}H NMR spectra were recorded on a Bruker AM500 (500 MHz) instrument at 300 K in $CDCl_3$, CD_3OD, DMSO-d_6, or acetone-d_6. Chemical shifts are reported in δ values (ppm). The hydrogenated residues of deuteriated solvent were used as internal standard ($CDCl_3$, $\delta = 7.26$ ppm in ^{1}H NMR and $\delta = 77$ ppm in ^{13}C NMR; CD_3OD, $\delta = 3.35$ ppm in ^{1}H NMR and $\delta = 49.3$ ppm in ^{13}C NMR; DMSO-d_6, $\delta = 2.58$ ppm in ^{1}H NMR and $\delta = 39.7$ ppm in ^{13}C NMR; acetone-d_6, $\delta = 2.05$ ppm in ^{1}H NMR and $\delta = 29.8$ ppm in ^{13}C NMR). Signals are described as s, d, t, q, dd, ddd, m, b for singlet, doublet, triplet, quadruplet, doublet of doublet, doublet of doublet of doublet, multiplet, and broad, respectively. All coupling constants (J) are given in Hz.

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

Chemical names follow IUPAC nomenclature.

Starting materials (compounds **2d**, **5c**, **13c**, **19e**) were purchased from Aldrich, Acros, Lancaster, or Fluka and were used without purification. No attempts were made to optimize yields.

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.

The following compounds were prepared according to previously described procedures: 4-bromo-2-methoxyaniline **2c**,[52] 1-bromo-3-methoxy-5-methylbenzene **4b**,[53] 1,6-dibromo-2-methoxynaphthalene **31b**,[54] methyl 7-bromo-3-methoxy-2-naphthoate **35c**,[45] 8-cyano-6-methoxy-2-naphthyl trifluoromethanesulfonate **37b**,[46] and 3-bromo-7-methoxy-1-naphthonitrile **37c**.[46]

General Procedure for Suzuki Coupling. Method A. A mixture of aryl bromide (1 equiv), boronic acid (1 equiv), 2% aqueous solution of sodium carbonate (2 equiv), and tetrakis(triphenylphosphine)palladium(0) (0.1 equiv) in toluene or DME was stirred at 80 °C under nitrogen for 4−26 h. The reaction mixture was cooled to room temperature, quenched by the addition of 2% HCl, and extracted with dichloromethane. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated to dryness. The product was purified by chromatography.

General Procedures for Amide Bond Formation. Method B. A mixture of carboxylic acid (1 equiv) and amino derivative (1 equiv) dissolved in dichloromethane was added dropwise to a solution of EDCI (1 equiv) and HOBt (1 equiv) in dichloromethane at 0 °C. The reaction mixture was stirred at room temperature overnight. After evaporation of the solvent, the residue was dissolved in ethyl acetate, washed with saturated sodium carbonate solution and brine, dried over $MgSO_4$, filtered, and concentrated. The product was purified by chromatography.

Method C. A mixture of carboxylic acid (1 equiv), EDCI (1 equiv), HOBt (1 equiv), and triethylamine (1 equiv) in dichloromethane cooled at 0 °C was added dropwise to a solution of the amino derivative (1 equiv) in dichloromethane. The reaction mixture was refluxed for 1.5 h and quenched by addition of aqueous HCl (0.1 M). The organic layer was separated, washed with sodium carbonate and brine, dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by chromatography.

Method D. Thionyl chloride (4 equiv) was added to the carboxylic acid (1 equiv) under nitrogen and stirred in the presence of a drop of DMF at room temperature for 30 min. After evaporation of the thionyl chloride, the acid chloride (1 equiv) was dissolved in dry THF or dichloromethane and added to the corresponding amine (1 equiv) in solution in CH_2Cl_2 together with triethylamine (1.2 equiv) and a catalytic amount of DMAP. During the addition, the temperature was kept at 0 °C. The reaction mixture was refluxed overnight, quenched by the addition of water, and extracted with ethyl acetate. The organic phase was dried over $MgSO_4$ and concentrated to dryness. The desired amide was purified by chromatography.

General Procedures for Ether Cleavage. Method E. To a solution of methoxy derivative (1 equiv) in toluene was added aluminum chloride (3−5 equiv per methoxy function) at room temperature under N_2. The reaction mixture was heated at 90 °C for 2 h and then allowed to cool to room temperature. The reaction was quenched by the addition of 2% Na_2CO_3. After extraction with ethyl acetate, the combined organic layers were washed with brine and dried over $MgSO_4$. After evaporation of the solvent, the crude product was purified by chromatography.

Method F. To a solution of methoxy derivative (1 equiv) in dichloromethane cooled at −78 °C under N_2 was slowly added boron tribromide (1 M solution in dichloromethane, 3−5 equiv per methoxy function). The reaction mixture was stirred at −78 °C for 1 h and then allowed to warm to room temperature. 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 $MgSO_4$. After evaporation of the solvent the product was purified by chromatography.

Method G. The methoxy derivative (1 equiv) and pyridinium hydrochloride (12 equiv) were heated at 220 °C for 3 h. The reaction mixture was cooled at room temperature, and 1 N HCl (4 mL) was added. The resulting precipitate was collected and dissolved in a small amount of ethyl acetate. The organic layer was washed with water and dried over Na_2SO_4, and the solvent was evaporated in vacuo.

General Procedure for Reduction of the Double Bond. Method H. A suspension of the olefinic compound (1 equiv) and a catalytic amount of $Pd(OH)_2$ in a mixture ethanol/THF (2:1) was stirred at room temperature for 20 h under hydrogen atmosphere. After completion of the reaction, the crude was filtered and concentrated.

2-Hydroxy-6-(3-hydroxyphenyl)-*N*-methyl-1-naphthamide (19). The title compound was prepared by reaction of 2-methoxy-6-(3-methoxyphenyl)-*N*-methyl-1-naphthamide **19a** (250 mg, 0.78 mmol, 1 equiv) with boron tribromide (3.9 mmol, 5 equiv) according to method F. Purification by column chromatography (dichloromethane/methanol, 98:2) afforded the desired product in 95% yield (217 mg). $C_{18}H_{15}NO_3$, MW 293. MS (ESI): 292 $(M - H)^-$. ^1H NMR (CD_3OD): δ 8.01 (d, J = 1.9 Hz, 1H), 7.90 (dd, J = 2.5 Hz, J = 8.8 Hz, 2H), 7.76 (dd, J = 1.9 Hz, J = 8.8 Hz, 1H), 7.31 (t, J = 7.9 Hz, 1H), 7.22−7.21 (m, 1H), 7.19 (d, J = 8.8 Hz, 1H), 7.17−7.16 (m, 1H), 6.82 (ddd, J = 0.6 Hz, J = 2.2 Hz, J = 7.9 Hz, 1H), 3.06 (s, 3H).

2-Hydroxy-6-(3-hydroxyphenyl)-*N*-phenyl-1-naphthamide (20). The title compound was prepared by reaction of 2-methoxy-6-(3-methoxyphenyl)-*N*-phenyl-1-naphthamide **20a** (217 mg, 0.57 mmol, 1 equiv) with boron tribromide (3.42 mmol, 6 equiv) according to method F. Purification by column chromatography (dichloromethane/methanol, 95:5) afforded the desired product in 23% yield (45 mg). $C_{23}H_{17}NO_3$, MW 355. MS (ESI): 356 $(M + H)^+$. ^1H NMR (CD_3OD): δ 7.99 (d, J = 1.2 Hz, 1H), 7.93 (d, J = 8.8 Hz, 1H), 7.88 (d, J = 8.8 Hz, 1H), 7.77 (d, J = 7.6 Hz, 2H), 7.74 (dd, J = 1.8 Hz, J = 8.8 Hz, 1H), 7.38 (t, J = 7.9 Hz, 2H), 7.27−7.26 (m, 1H), 7.21 (d, J = 8.8 Hz, 1H), 7.17−7.16 (m, 1H), 7.15−7.14 (m, 2H), 6.78 (ddd, J = 0.9 Hz, J = 2.4 Hz, J = 7.9 Hz, 1H).

2-Hydroxy-*N*,6-bis(3-hydroxyphenyl)-1-naphthamide (21). The title compound was prepared by reaction of 2-methoxy-*N*,6-bis(3-methoxyphenyl)-1-naphthamide **21a** (150 mg, 0.36 mmol, 1 equiv) with boron tribromide (2.9 mmol, 8 equiv) according to method F. Purification by column chromatography (dichloromethane/methanol, 93:7) afforded the desired product in 50% yield (66 mg). $C_{23}H_{17}NO_4$, MW 371. MS (ESI): 372 $(M + H)^+$. ^1H NMR (CD_3OD): δ 8.00 (d, J = 1.6 Hz, 1H), 7.92 (d, J = 8.5 Hz, 1H), 7.88 (d, J = 9.1 Hz, 1H), 7.75 (dd, J = 1.9 Hz, J = 8.8 Hz, 1H), 7.44 (t, J = 1.9 Hz, 1H), 7.27 (t, J = 7.9 Hz, 1H), 7.18−7.17 (m, 5H), 6.78−6.77 (m, 1H), 6.61−6.60 (m, 1H).

6-(3-Hydroxyphenyl)-1-(piperidin-1-ylcarbonyl)-2-naphthol (22). The title compound was prepared by reaction of 2-hydroxy-6-(3-hydroxyphenyl)-1-naphthoic acid (160 mg, 0.57 mmol, 1 equiv) with piperidine (113 mL, 97 mg, 1.14 mmol, 2 equiv) according to method D. After purification of the crude product by column chromatography (dichloromethane/methanol, 95:5) compound **22** was obtained in 8% yield (16 mg). $C_{22}H_{21}NO_3$, MW 347. ^1H NMR (CD_3OD): δ 8.04 (d, J = 1.9 Hz, 1H), 7.90 (d, J = 8.8 Hz, 1H), 7.77 (dd, J = 1.9 Hz, J = 8.8 Hz, 1H), 7.66 (d, J = 8.8 Hz, 1H), 7.30 (t, J = 7.9 Hz, 1H), 7.23−7.21 (m, 1H), 7.20 (d, J = 8.8 Hz, 1H), 7.17−7.16 (m, 1H), 6.82 (ddd, J = 0.9 Hz, J = 2.5 Hz, J = 8.2 Hz, 1H), 3.98−3.97 (m, 1H), 3.87−3.83 (m, 1H), 3.69−3.67 (m, 1H), 3.31−3.29 (m, 1H), 1.81−1.79 (m, 3H), 1.75−1.36 (m, 3H).

6-(3-Hydroxyphenyl)-1-(morpholin-4-ylcarbonyl))-2-naphthol (23). The title compound was prepared by reaction of 4-[2-methoxy-(6-(3-methoxyphenyl)-1-naphthyl)]-1-morpholine **23a** (195 mg, 0.52 mmol, 1 equiv) with boron tribromide (2.6 mmol, 5 equiv) according to method F. Purification by column chromatography (dichloromethane/methanol, 95:5) afforded the compound **23** in 93% yield (170 mg). $C_{21}H_{19}NO_4$, MW 349. MS (ESI): 348 $(M - H)^-$. ^1H NMR (CD_3OD): δ 8.04 (d, J = 1.6 Hz, 1H), 7.92 (d, J = 8.8 Hz, 1H), 7.79 (dd, J = 1.9 Hz, J = 8.8 Hz, 1H), 7.70 (d, J = 8.8 Hz, 1H), 7.33−7.30 (m, 1H), 7.23−7.21 (m, 1H), 7.21 (d, J = 8.8 Hz, 1H), 7.18−7.17 (m, 1H), 6.83−6.81 (m, 1H), 3.97−3.96 (m, 2H), 3.88−3.87 (m, 2H), 3.71−3.70 (m, 1H), 3.59−3.57 (m, 1H), 3.41−3.36 (m, 1H), 3.31−3−30 (m, 1H).

6-(3-Hydroxyphenyl)-1-(piperazin-1-ylcarbonyl))-2-naphthol (24). The title compound was prepared by reaction of *tert*-butyl 4-(2-methoxy-6-(3-methoxyphenyl)-1-naphthoyl)piperazine-1-carboxylate **24a** (200 mg, 0.42 mmol, 1 equiv) with boron tribromide (2.52 mmol, 6 equiv) according to method F. The desired compound was obtained in 60% yield (81 mg). $C_{21}H_{20}N_2O_3$, MW 348. MS (ESI): 349 $(M + H)^+$. ^1H NMR (CD_3OD): δ 8.05 (d, J = 1.3 Hz, 1H), 7.93 (d, J = 9.1 Hz, 1H), 7.79 (dd, J = 1.6 Hz, J = 8.5 Hz, 1H), 7.69 (d, J = 8.8 Hz, 1H), 7.31 (t, J = 7.9 Hz, 1H),

7.23 (d, J = 8.8 Hz, 1H), 7.21−7.20 (m, 1H), 7.17−7.16 (m, 1H), 6.84−6.82 (m, 1H), 4.38−4.35 (m, 1H), 4.08−4.05 (m, 1H), 3.68 (m, 1H), 3.59−3.58 (m, 1H), 3.48−3.47 (m, 1H), 3.42−3.41 (m, 1H), 3.40−3.31 (m, 1H), 3.20−3.19 (m 1H).

2-Hydroxy-6-(3-hydroxyphenyl)-*N*-pyridin-2-yl-1-naphthamide (25). The title compound was prepared by reaction of 2-methoxy-6-(3-methoxyphenyl)-*N*-(2-pyridyl)-1-naphthamide **25a** (33 mg, 0.086 mmol, 1 equiv) with boron tribromide (0.52 mmol, 6 equiv) according to method F. Purification by column chromatography (dichloromethane/methanol, 99:1) afforded the desired compound in 36% yield (11 mg). $C_{22}H_{16}N_2O_3$, MW 356. MS (ESI): 357 $(M + H)^+$. ^{1}H NMR (acetone-d_6): δ 9.79 (bs, 1H), 8.48 (d, J = 6.3 Hz, 1H), 8.29−8.28 (m, 1H), 8.25 (d, J = 5.0 Hz, 1H), 8.09−8.08 (m, 1H), 8.00 (d, J = 8.8 Hz, 1H), 7.89−7.88 (m, 1H), 7.82 (dd, J = 1.8 Hz, 6.9 Hz, 1H), 7.33−7.32 (m, 4H), 7.14−7.13 (m, 1H), 6.86−6.85 (m, 1H).

2-Hydroxy-6-(3-hydroxyphenyl)-*N*-(pyrimidin-2-yl)-1-naphthamide (26). The title compound was prepared by reaction of 2-methoxy-6-(3-methoxyphenyl)-*N*-(pyrimidin-2-yl)-1-naphthamide **26a** (117 mg, 0.30 mmol, 1 equiv) with boron tribromide (2.12 mmol, 7 equiv) according to method F. Purification by column chromatography (dichloromethane/methanol, 98:2) afforded the desired compound in 96% yield (99 mg). $C_{21}H_{15}N_3O_3$, MW 357. MS (ESI): 358 $(M + H)^+$. ^{1}H NMR (acetone-d_6): δ 9.90 (bs, 1H), 9.60 (s, 1H), 8.59 (d, J = 5.8 Hz, 2H), 8.40 (s, 1H), 8.13 (d, J = 5.2 Hz, 1H), 8.05−8.04 (m, 1H), 7.95 (d, J = 9.1 Hz, 1H), 7.75 (dd, J = 1.8 Hz, J = 6.9 Hz, 1H), 7.28 (t, J = 8.1 Hz, 1H), 7.20−7.19 (m, 3H), 7.11 (t, J = 5.0 Hz, 1H), 6.80−6.79 (m, 1H).

2-Hydroxy-6-(3-hydroxyphenyl)-*N*-(5-methyl-1,3,4-thiadiazol-2-yl)-1-naphthamide (27). The title compound was prepared by reaction of 2-methoxy-6-(3-methoxyphenyl)-*N*-(5-methyl-1,3,4-thiadiazol-2-yl)-1-naphthamide **27a** (620 mg, 1.53 mmol, 1 equiv) with boron tribromide (12.24 mmol, 8 equiv) according to method F. Purification by column chromatography (dichloromethane/methanol, 98:2) afforded compound **27** in 20% yield (115 mg). $C_{20}H_{15}N_3O_3S$, MW 377. MS (ESI): 378 $(M + H)^+$. ^{1}H NMR (acetone-d_6): δ 12.30 (bs, 1H), 10.03 (s, 1H), 9.07 (s, 1H), 7.66 (s, 1H), 7.55 (d, J = 9.1 Hz, 1H), 7.31 (d, J = 8.8 Hz, 1H), 7.23 (d, J = 7.8 Hz, 1H), 6.84 (t, J = 7.8 Hz, 2H), 6.74 (d, J = 8.1 Hz, 1H), 6.68−6.67 (m, 1H), 6.33−6.32 (m, 1H), 2.22 (s, 3H).

(2*E*)-3-[2-Hydroxy-6-(3-hydroxyphenyl)-1-naphthyl)]-*N*-methylacrylamide (28). The title compound was prepared by reaction of (2*E*)-3-[2-methoxy-6-(3-methoxyphenyl)-1-naphthyl)]-*N*-methylacrylamide **28a** (127 mg, 0.37 mmol, 1 equiv) with boron tribromide (2.31 mmol, 8 equiv) according to method F. Purification by column chromatography (dichloromethane/methanol, 95:5) afforded the desired product in 60% yield (71 mg). $C_{20}H_{17}NO_3$, MW 319. MS (ESI): 320 $(M + H)^+$. ^{1}H NMR (CD$_3$OD): δ 8.29 (d, J = 15.8 Hz, 1H), 8.27 (d, J = 9.1 Hz, 1H), 7.99 (d, J = 1.9 Hz, 1H), 7.81 (d, J = 9.1 Hz, 1H), 7.79 (dd, J = 1.9 Hz, J = 8.8 Hz, 1H), 7.31 (t, J = 7.91 Hz, 1H), 7.24−7.21 (m, 1H), 7.21 (d, J = 8.8 Hz, 1H), 7.20−7.19 (m, 1H), 7.11 (d, J = 15.8 Hz, 1H), 6.82 (ddd, J = 0.9 Hz, J = 2.5 Hz, J = 7.9 Hz, 1H), 2.93 (s, 3H).

(2*E*)-3-[2-Hydroxy-6-(3-hydroxyphenyl)-1-naphthyl)]-*N*-phenylacrylamide (29). The title compound was prepared by reaction of (2*E*)-3-[2-methoxy-6-(3-methoxyphenyl)-1-naphthyl)]-*N*-phenylacrylamide **29a** (70 mg, 0.17 mmol, 1 equiv) with boron tribromide (1.36 mmol, 8 equiv) according to method F. Purification by column chromatography (dichloromethane/methanol, 95:5) afforded the product in 13% yield (8 mg). $C_{25}H_{19}NO_3$, MW 381. MS (ESI): 382 $(M + H)^+$. ^{1}H NMR (CD$_3$OD): δ 8.47 (d, J = 15.8 Hz, 1H), 8.34 (d, J = 8.8 Hz, 1H), 8.03 (d, J = 1.9 Hz, 1H), 7.86 (d, J = 9.1 Hz, 1H), 7.83 (dd, J = 1.9 Hz, J = 8.8 Hz, 1H), 7.76 (d, J = 7.6 Hz, 1H), 7.40−7.36 (m, 3H), 7.34−7.31 (m, 1H), 7.26−7.24 (m, 1H), 7.24 (d, J = 9.1 Hz, 1H), 7.21−7.20 (m, 1H), 7.17−7.14 (m, 1H), 6.83 (ddd, J = 0.9 Hz, J = 2.5 Hz, J = 7.9 Hz, 1H).

3-[2-Hydroxy-6-(3-hydroxyphenyl)-1-naphthyl)]-*N*-methylpropanamide (30). The title compound was prepared by hydrogenation of (2*E*)-3-[2-hydroxy-6-(3-hydroxyphenyl)-1-naphthyl)]-*N*-methylacrylamide **28** (40 mg, 0.13 mmol, 1 equiv) with Pd(OH)$_2$ according to method H. The compound **30** was obtained in quantitative yield (40 mg). $C_{20}H_{19}NO_3$, MW 321. MS (ESI): 322 $(M + H)^+$. ^{1}H NMR (CD$_3$OD): δ 8.04 (d, J = 8.8 Hz, 1H), 7.98 (d, J = 2.2 Hz, 1H), 7.76 (dd, J = 1.9 Hz, J = 8.8 Hz, 1H), 7.72 (d, J = 8.8 Hz, 1H), 7.31 (t, J = 7.91 Hz, 1H), 7.23−7.22 (m, 1H), 7.18−7.17 (m, 1H), 7.16 (d, J = 8.8 Hz, 1H), 6.80 (ddd, J = 0.9 Hz, J = 2.5 Hz, J = 7.9 Hz, 1H), 3.41−3.38 (m, 2H), 2.74 (s, 3H), 2.60−2.57 (m, 2H).

1-Bromo-6-(3-hydroxyphenyl)-2-naphthol (31). The title compound was prepared by reaction of 1-bromo-2-methoxy-6-(3-methoxyphenyl)naphthalene **31a** (500 mg, 1.46 mmol, 1 equiv) with boron tribromide (7.3 mmol, 5 equiv) according to method F. It was obtained in quantitative yield (460 mg). $C_{16}H_{11}BrO_2$, MW 315. MS (ESI): 313−315 $(M - H)^-$. ^{1}H NMR (CD$_3$OD): δ 8.19 (d, J = 8.8 Hz, 1H), 8.02 (d, J = 1.9 Hz, 1H), 7.83 (dd, J = 0.9 Hz, J = 8.5 Hz, 1H), 7.83 (d, J = 8.8 Hz, 1H), 7.32 (t, J = 7.9 Hz, 1H), 7.24 (d, J = 8.8 Hz, 1H), 7.25−7.23 (m, 1H), 7.19−7.18 (m, 1H), 6.83 (ddd, J = 1.3 Hz, J = 2.5 Hz, J = 8.2 Hz, 1H).

6-(3-Hydroxyphenyl)-1-phenyl-2-naphthol (32). The title compound was prepared by reaction of 1-bromo-6-(3-hydroxyphenyl)-2-naphthol **31** (50 mg, 0.16 mmol, 1 equiv) with benzeneboronic acid (19.4 mg, 0.16 mmol, 1 equiv) according to method A. The crude product was purified by column chromatography (hexane/ethyl acetate, 7:3) to give **32** in 30% yield (15 mg). $C_{22}H_{16}O_2$, MW 312. MS (ESI): 311 $(M - H)^-$. ^{1}H NMR (CD$_3$OD): δ 8.01 (d, J = 1.9 Hz, 1H), 7.84 (d, J = 8.8 Hz, 1H), 7.58 (dd, J = 1.9 Hz, J = 8.8 Hz, 1H), 7.56−7.53 (m, 2H), 7.48−7.45 (m, 2H), 7.42−7.40 (m, 2H), 7.30−7.27 (m, 1H), 7.26 (d, J = 8.8 Hz, 1H), 7.21−7.19 (m, 1H), 7.17 (t, J = 1.9 Hz, 1H), 6.80 (ddd, J = 0.9 Hz, J = 2.2 Hz, J = 7.9 Hz, 1H).

6-(3-Hydroxyphenyl)-1-(phenylsulfonyl)-2-naphthol (33). To a solution of 6-(3-hydroxyphenyl)-1-(phenylsulfanyl)-2-naphthol **33a** (71 mg, 0.21 mmol, 1 equiv) in anhydrous dichloromethane (10 mL) at 0 °C was added *m*-CPBA (192 mg, 0.82 mmol, 3.9 equiv) in CH$_2$Cl$_2$ (10 mL). The reaction mixture was stirred at 0 °C for 1 h and at room temperature for 12 h. The mixture was poured into ice−water, and the crude was extracted with ethyl acetate. The combined organic layers were washed with NaHCO$_3$ and brine and dried over Na$_2$SO$_4$. After purification of the crude product by column chromatography compound **33** was obtained in 50% yield (38 mg). $C_{22}H_{16}O_4S$, MW 376. MS (ESI): 375$(M - H)^-$. ^{1}H NMR (acetone-d_6): δ 8.49 (d, J = 9.1 Hz, 1H), 8.23 (d, J = 8.8 Hz, 1H), 8.11 (s, 1H), 8.06 (d, J = 7.9 Hz, 1H), 7.82 (dd, J = 1.8 Hz, J = 8.8 Hz, 1H), 7.70−7.62 (m, 3H), 7.27−7.26 (m, 2H), 7.16−7.15 (m, 2H), 6.85 (dt, J = 1.8 Hz, J = 8.8 Hz, 1H).

6-(3-Hydroxyphenyl)-1-(4-methylphenyl)sulfonyl)-2-naphthol (34). To a solution of 2-methoxy-6-(3-methoxyphenyl)-1-[(4-methylphenyl)sulfanyl]naphthalene **34b** (385 mg, 1.03 mmol, 1 equiv) in anhydrous dichloromethane (10 mL) at 0 °C was added *m*-CPBA (1.42 g, 8.24 mmol, 8.2 equiv) in CH$_2$Cl$_2$ (10 mL). The reaction mixture was stirred at 0 °C for 1 h and at room temperature for 12 h, poured into ice−water, and extracted with ethyl acetate. The combined organic layers were washed with NaHCO$_3$ and brine and dried over Na$_2$SO$_4$. A monodemethylation occurred during the oxidation step. The second methoxy group of the product (105 mg, 0.26 mmol, 1 equiv) was then removed by reaction with boron tribromide (2.1 mmol, 8 equiv) according to method F. The product was obtained in 79% yield (80 mg). $C_{23}H_{18}O_4S$, MW 390. MS (ESI): 389 $(M - H)^-$. ^{1}H NMR (acetone-d_6): δ 8.49 (d, J = 8.8 Hz, 1H), 8.22 (d, J = 8.8 Hz, 1H), 8.12 (d, J = 2.1 Hz, 1H), 7.95 (d, J = 8.5 Hz, 1H), 7.82 (dd, J = 2.1 Hz, J = 8.3 Hz, 1H), 7.43 (d, J = 8.8 Hz, 2H), 7.31−7.26 (m, 2H), 7.19−7.18 (m, 2H), 6.87−6.86 (m, 1H).

3-Hydroxy-7-(3-hydroxyphenyl)-*N*-methyl-2-naphthamide (35). The title compound was prepared by reaction of 3-methoxy-7-(3-methoxyphenyl)-*N*-methyl-2-naphthamide **35a** (93 mg, 0.29 mol, 1 equiv) with boron tribromide (5.5 mmol, 19 equiv) according to method F. The product **35** was obtained in a quantitative yield (87 mg). $C_{18}H_{15}NO_3$, MW 293. MS (ESI): 294 $(M + H)^+$. ^{1}H NMR (CD$_3$OD): δ 8.43 (s, 1H), 8.03 (s, 1H), 7.75 (s, 2H), 7.29 (t, J = 7.9 Hz, 1H), 7.26 (s, 1H), 7.19 (d, J = 7.6 Hz, 1H), 7.15 (s, 1H), 6.80 (dd, J = 2.2 Hz, J = 7.9, 1H), 3.02 (s, 3H).

3-Hydroxy-7-(3-hydroxyphenyl)-*N*-phenyl-2-naphthamide (36). The title compound was prepared by reaction of 3-methoxy-7-(3-methoxyphenyl)-*N*-phenyl-2-naphthamide **36a** (86 mg, 0.22 mol, 1 equiv) with boron tribromide (4.45 mmol, 20 equiv) according to method F. After purification of the crude product by column chromatography (hexane/ethyl acetate, 6:4) compound **36** was obtained in 50% yield (40 mg). $C_{23}H_{17}NO_3$, MW 355. MS (APCI): 355 $(M)^+$. ^{1}H NMR (DMSO-d_6): δ 11.54 (bs, 1H), 10.14 (bs, 1H), 8.73 (s, 1H), 8.47 (bs, 1H), 8.08 (s, 1H), 7.84−7.81 (m, 4H), 7.42 (dd, J = 0.9 Hz, J = 8.5 Hz, 2H), 7.36 (s, 1H), 7.31 (t, J = 8.2 Hz, 1 H), 7.23−7.18 (m, 3H), 6.88−6.86 (m, 1H).

3-Hydroxy-7-(3-hydroxyphenyl)-1-naphthonitrile (37). The title compound was prepared by reaction of 3-methoxy-7-(3-methoxyphenyl)-1-naphthonitrile **37a** (100 mg, 0.35 mmol, 1 equiv) with pyridinium hydrochloride (492 mg, 4.3 mmol, 12 equiv) according to method G. The desired compound was obtained in 64% yield (58 mg). $C_{17}H_{11}NO_2$, MW 261. MS (ESI): 260 $(M - H)^-$. ^{1}H NMR (CD_3OD): δ 8.20 (s, 1H), 7.89 (d, J = 8.5 Hz, 1H), 7.85 (dd, J = 1.9 Hz, J = 6.9 Hz, 1H), 7.61 (d, J = 2.2 Hz, 1H), 7.50 (d, J = 2.5 Hz, 1H), 7.35 (dd, J = 2.2 Hz, J = 7.6 Hz, 1H), 7.24−7.23 (m, 1H), 7.20 (dd, J = 2.2 Hz, J = 1.9 Hz, 1H), 6.87−6.86 (m, 1H).

7-Hydroxy-3-(3-hydroxyphenyl)-1-naphthonitrile (38). The title compound was prepared by reaction of 7-methoxy-3-(3-methoxyphenyl)-1-naphthonitrile **38a** (110 mg, 0.39 mmol, 1 equiv) with pyridinium hydrochloride (547 mg, 4.76 mmol, 12.2 equiv) according to method G. The compound was obtained in 62% yield (63 mg). $C_{17}H_{11}NO_2$, MW 261. MS (ESI): 260 $(M - H)^-$. ^{1}H NMR (CD_3OD): δ 8.30 (bs, 1H), 8.18 (d, J = 1.9 Hz, 1H), 7.98 (d, J = 8.8 Hz, 1H), 7.47−7.46 (m, 1H), 7.36−7.33 (m, 1H), 7.28 (dd, J = 2.2 Hz, J = 8.8 Hz, 1H), 7.24−7.22 (m, 1H), 7.17−7.16 (m, 1H), 6.87−6.85 (m, 1H).

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, Germany. T-47D cells (passage 9) were obtained from ECACC, Salisbury, U.K. FCS was purchased from Sigma, Taufkirchen, Germany. Cell culture media and dextran coated charcoal stripped FCS (DCC-FCS) were 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 (Procedures A and 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.[55] Aliquots containing 17β-HSD1 or 17β-HSD2 were stored frozen until determination of enzymatic activity.

Human Enzyme Enriched from Placental Tissue (Procedures B and D). 17β-HSD1 and 17β-HSD2 were obtained from human placenta according to previously described procedures.[11,56] Fresh human placenta was homogenized, and the enzymes were separated by subcellular fractionation (centrifugation method). 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. The synthesized compounds were tested for their ability to inhibit 17β-HSD1 according to procedure A (recombinant human enzyme) or B (human placental enzyme). For select compounds, IC_{50} values were determined according to procedure B (human placental enzyme). Procedures A and B differ from 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]estrone 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 acetonitrile/water (48:52) as running solvent. 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.[57−59] Briefly, the enzyme preparation was incubated with NADH (500 μM) in the presence of potential inhibitors at 37 °C in a phosphate buffer (50 mM) supplemented with 20% 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 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 Gravity, 3 μm, Macherey-Nagel, Düren) connected to a HPLC system (Agilent 1100 series, Agilent Technologies, Waldbronn). Detection and quantification of the steroids were performed using a radioflow detector (Berthold Technologies, Bad Wildbad). The conversion rate was calculated according to following equation: % conversion = % E2/(% E2 + % E1) × 100. Each value was calculated from at least three independent experiments.

3. Inhibition of 17β-HSD2. The synthesized compounds were tested for their ability to inhibit 17β-HSD2 according to procedure C (recombinant human enzyme) or 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. The microsomal fraction was incubated with NAD^+ (1500 μM), test compound, and a mixture of unlabeled and [^{3}H]E2 (final concentration: 500 nM, 0.11 μCi) for 20 min at 37 °C. Further treatment of the samples and HPLC separation was carried out as mentioned above for 17β-HSD1.

4. ER Affinity

The binding affinity of select compounds to the ERα and ERβ was determined according to Zimmermann et al.[60] using recombinant human proteins. Briefly, 0.25 pmol of ERα or ERβ, respectively, was incubated with [^{3}H]E2 (10 nM) and test compound for 1 h at room temperature. The potential inhibitor was dissolved in DMSO (5% final concentration). Nonspecific binding was performed with diethylstilbestrol (10 μM). After incubation, ligand−receptor complexes were selectively bound to hydroxyapatite (5 g/60 mL TE buffer). The formed complex was separated, washed, and resuspended in ethanol. For radiodetection, scintillator cocktail (Quickszint 212, Zinsser Analytic, Frankfurt) was added and samples were measured in a liquid

scintillation counter (Rack Beta Primo 1209, Wallac, Turku). For determination of the relative binding affinity (RBA), inhibitor and E2 concentrations required to displace 50% of the receptor bound labeled E2 were determined. RBA values were calculated according to the following equation: RBA[%] = IC_{50}(E2)/IC_{50}(compound) × 100. The RBA value for E2 was arbitrarily set at 100%.

5. Proliferation Assay

Cell Culture. Stock culture of T-47D cells (ecacc, U.K.) was maintained in RPMI-1640 supplemented with sodium bicarbonate (2 g/L), streptomycin (100 μg/mL), insuline zinc salt (10 μg/mL), sodium pyruvate (1 mM), penicillin (100 U/mL), and FCS 10% (vol/vol). Cells were cultured at 37 °C under 5% CO_2 humidified atmosphere. Medium was changed every 2−3 days, and cells were subcultured every 4−5 days.

Evaluation of Estrogenic Effects on the Estrogen Dependent Human Breast Cancer Cell Line T-47D. Phenol red-free medium was supplemented with sodium bicarbonate (2 g/L), streptomycin (100 μg/mL), insuline zinc salt (10 μg/mL), sodium pyruvate (1 mM), L-glutamine (2 mM), penicillin (100 U/mL), and DCC-FCS 5% (vol/vol). RPMI 1640 (without phenol red) was used for the experiments. Cells (7500 cells/96-well plate) were grown for 48 h in phenol red-free medium. The compounds tested were added at a final concentration of 100 nM. Inhibitors and E2 were diluted in ethanol (final ethanol concentration was adjusted to 1%). As a positive control E2 was added at a final concentration of 0.1 nM. Ethanol was used as negative control. Medium was changed every 2−3 days and supplemented with the respective additive. After 8 days of incubation, the cell viability was evaluated measuring the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT). The cleavage of MTT to a blue formazan by mitochondrial succinate-dehydrogenase was quantified spectrophotometrically at 590 nm as described by Denizot and Lang[61] with minor modifications. The control proliferation was arbitrarily set at 1, and the stimulation induced by the inhibitor was calculated according to following equation: % stimulation= [proliferation(compound − induced) − 1]/[proliferation(E2 − induced) − 1] × 100%. Each value is calculated as a mean value of at least three independent experiments.

6. Evaluation of Plasma Concentrations of Compounds 1 and 32 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 anesthetized 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. Compounds **1** and**32**, dissolved in labrasol/water (1:1) as vehicle, were administered perorally at doses of 10 mg/kg in a cassette dosing approach. Each application group consisted of four rats. At time 0, compounds **1** and **32** 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 were carried out on a Surveyor HPLC system coupled with a TSQ Quantum (ThermoFisher) triple quadrupole mass spectrometer equipped with an electrospray ion interface (ESI). This test was performed according to the Laws on Animal Care and Use and had been approved by the local Animal Care Committee.

Molecular Modeling. All molecular modeling studies were performed on an Intel(R) P4 CPU, 3.00 GHz, running Linux Suse 9.3. X-ray structure of 17β-HSD1 (PDB ID 1FDT, www.pdb.org using the amino acids 188−201 in the B conformation) was prepared using the BIOPOLYMER module of SYBYL version 7.0 (Sybyl, Tripos Inc., St. Louis, MO). Water molecules, E2, and sulfate ions were stripped from the PDB file, and missing protein atoms were added and correct atom types set. Finally hydrogen atoms and neutral end groups were added. All basic and acidic residues were considered protonated and deprotonated, respectively. Furthermore, the crystal structure was minimized for 200 steps with the steepest descent minimizer as implemented in SYBYL with the backbone atoms kept at fixed positions in order to fix close contacts (Arg37).

Docking of selected inhibitors, built with SYBYL and energy-minimized in MMFF94s force field as implemented in Sybyl, into the substrate binding site was performed by the docking program GOLD version 3.0.1.[62] Since the GOLD docking program allows flexible docking of ligands, no conformational search was employed to the ligand structures. Ligands were docked in 50 independent genetic algorithm (GA) runs. Active-site origin was set at the center of the steroid binding site, while the radius was set equal to 13 Å. The automatic active-site detection was switched on. Further, the CHEMSCORE fitness function was used and genetic algorithm default parameters were set as suggested by the GOLD authors.

The quality of the docked poses was evaluated on the basis of the scoring function, which gives a good measure to discriminate between the found binding modes, and mainly on visual inspection of the putative binding modes of the ligands.

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Supporting Information Available: Additional experimental details on the synthesis and spectroscopic data of **1**−**38** and purity data of compounds **2**, **3**, **5**, **7**, **8**, **10**, **11**, **19**−**24**, **26**, **28**−**32**, **35**, **37**, **38**. This material is available free of charge via the Internet at http://pubs.acs.org.

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