Design, Synthesis, and Biological Evaluation of (Hydroxyphenyl)naphthalene and -quinoline Derivatives: Potent and Selective Nonsteroidal Inhibitors of 17β-Hydroxysteroid Dehydrogenase Type 1 (17β-HSD1) for the Treatment of Estrogen-Dependent Diseases

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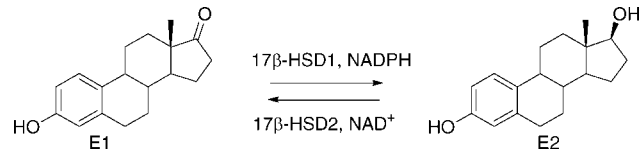
Human 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1) catalyzes the reduction of the weak estrogen estrone (E1) to the highly potent estradiol (E2). This reaction takes place in the target cell where the estrogenic effect is exerted via the estrogen receptor (ER). Estrogens, especially E2, are known to stimulate the proliferation of hormone-dependent diseases. 17β-HSD1 is overexpressed in many breast tumors. Thus, it is an attractive target for the treatment of these diseases. Ligand- and structure-based drug design led to the discovery of novel, selective, and potent inhibitors of 17β-HSD1. Phenyl-substituted bicyclic moieties were synthesized as mimics of the steroidal substrate. Computational methods were used to obtain insight into their interactions with the protein. Compound **5** turned out to be a highly potent inhibitor of 17β-HSD1 showing good selectivity (17β-HSD2, ERα and β), medium cell permeation, reasonable metabolic stability (rat hepatic microsomes), and little inhibition of hepatic CYP enzymes.

Introduction

Estrogens are a family of steroid hormones that play a central role in the growth, development, and maintenance of a diverse range of tissues. They are also known, however, to be involved in hormone-dependent diseases. For instance, they stimulate the estrogen receptor (ER)[a]-mediated proliferation of breast cancer and endometrial cells leading to a progression of cancer and to endometriosis.[1,2] Antagonizing estrogenic effects by selective estrogen receptor modulators (SERMs) or decreasing estrogen levels by aromatase inhibitors are established treatments for these diseases.[3,4] However, these therapies have some limitations: both strategies affect sites where estrogen is required for normal function. SERMs are also known to induce carcinoma in other tissues like endometrium.[3] As estrogenic effects are predominantly caused by the most potent estrogen, estradiol (E2), a novel and promising therapeutic approach could be the inhibition of the last step of E2 biosynthesis which is catalyzed by certain 17β-hydroxysteroid dehydrogenases. In general, this class of dehydrogenases modulates potencies of steroidal sex hormones.

Fourteen members of this enzyme family are known; 11 of them regulate the concentrations of active androgens and estrogens in a tissue-specific manner in humans.[5] This modulation of hormone activities by the target cells themselves is known as intracrinology.[6] 17β-Hydroxysteroid dehydrogenase type 1 (17β-HSD1) is the most important of these 17β-HSD enzymes, which intracellularly activate circulating E1 (estrone) to E2 (Chart 1). In pathologically altered tissues, e.g. breast tumors, it is known that 17β-HSD1 is often overexpressed leading to elevated local E2 levels.[7] Therefore, 17β-HSD1 is being discussed as an attractive target for the treatment and even the prevention[8] of estrogen-dependent diseases such as breast cancer and endometriosis. The advantage of this novel therapeutic approach should be a reduction of side effects compared to established treatments.

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



17β-HSD1 contains 327 amino acids and exists as a homodimer with a subunit mass of 34.9 kDa.[9] The enzymatic reaction is coupled to the consumption of the cosubstrate NAD(P)H.[10] In primates, 17β-HSD1 is mainly expressed in placenta, ovarian granulosa cells, and to a lesser extent in endometrium, prostate, and adipose tissue, but not in adrenals or testes.[11,12] This tissue-specific expression pattern makes 17β-HSD1 an attractive drug target in women's health diseases. Currently, 16 crystal structures of the enzyme are known.[13–21] Only a few inhibitors of 17β-HSD1 have been reported, most of them having a steroidal structure.[22–34] No drug candidate has been described so far. As a biological counterpart, 17β-hydroxysteroid dehydrogenase type 2 (17β-HSD2) catalyzes the oxidation of E2 to E1 (Chart 1), thus deactivating E2 and protecting the cell from excessively high concentrations of active estrogen. This enzyme should not be affected by potential inhibitors of 17β-HSD1. The aim of our work described in the following is the design, synthesis,

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[a] Abbreviations: 17β-HSD1, 17β-hydroxysteroid dehydrogenase type 1; 17β-HSD2, 17β-hydroxysteroid dehydrogenase type 2; E1, estrone; E2, estradiol; ER, estrogen receptor; CYP, cytochrome P450; PDB-ID, protein data bank identification code; P_{app}, apparent permeability coefficient; RBA, relative binding affinity; RMSd, root-mean-square distance; SAR: structure–activity relationship; DME, dimethoxyethane.

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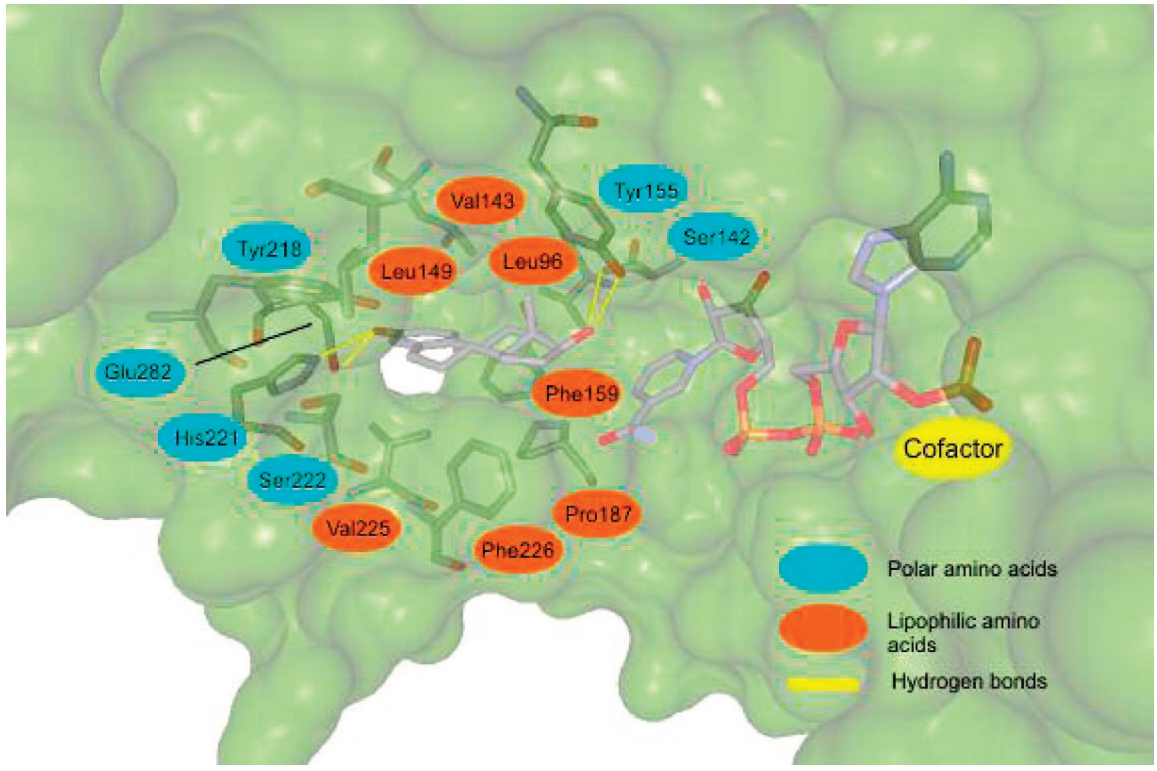


Figure 1. Schematic presentation of the active site of 17β-HSD1 containing E2. Turquoise labels denote polar amino acids and orange labels stand for lipophilic amino acids. Hydrogen bonds are marked in yellow.

and biological evaluation of potent and selective nonsteroidal inhibitors of 17β-HSD1.

Design of Inhibitors

17β-HSD1 Substrate Binding Site. Although 16 crystallographic structures of binary and ternary complexes of 17β-HSD1 have been published,[13–21] there is no structure of the enzyme with the substrate E1 available. Therefore, the ternary complex PDB-ID: 1FDT containing E2 and $NADP^+$ was used to study the three-dimensional architecture of the enzyme. The substrate binding site is a hydrophobic tunnel with polar areas at each end formed by His221/Glu282 on one side and Ser142/Tyr155 (along with Asn114 and Lys159 members of the catalytic tetrad) on the other side. These amino acids establish hydrogen bonds to the two hydroxy groups of E2 OH(3) and OH(17), respectively. Interestingly, there are two additional polar amino acids (Tyr218, Ser222) in this region of hydrophobic amino acids, having no counterpart in the steroid for interaction. Figure 1 shows a schematic presentation of the active site and the binding mode of E2.

In front of the active site a flexible entry loop (amino acids 188−201) is located which is not properly resolved in the crystal structures. This entry loop can adopt two distinct conformations: the loop can close the entrance to either the substrate binding site or the cofactor binding pocket.[35]

Design of Steroidomimetics. In order to reduce the risk of undesired side effects caused by interaction with steroid receptors and to have straightforward access to structural diversity, we focused on the design of nonsteroidal inhibitors. The steroidomimetics should contain two polar groups mimicking the functional groups in the 3- (A-ring) and the 17-positions (D-ring). The distance between them should be approximately 10–13 Å corresponding to the geometry in the steroid. Phenyltetralone, phenylnaphthalene, phenylquinoline, and phenylindole seemed to be appropriate scaffolds as they can adopt a nearly flat conformation similar to the steroid (Chart 2). The synthesis of the heterocyclic compounds seemed to be promising in order to examine whether the heteroatom could establish additional interactions with the polar amino acids Tyr218 and/or Ser222, which are located close to C6 of the steroidal B-ring (Figure 1). For instance, the distance between the Tyr218 oxygen and C6 of the steroid is 3.8 Å (PDB-ID: 1FDT).

Chemistry

The synthesis of the compounds is depicted in Schemes 1–6. Scheme 1 shows the synthesis of compounds **1–3** from 6-hydroxy-1-tetralone via triflation, Suzuki coupling, and aromatization as described in the literature.[36]

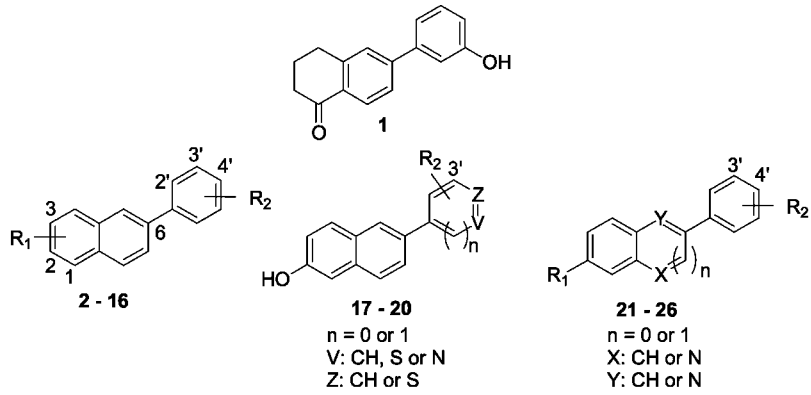
Different quinoline and naphthalene derivatives were coupled to the aromatic/heteroaromatic moiety via the Suzuki reaction using two different methods. Compounds **4a–6a**, **7–9**, **10a**, and **20a−24a** were prepared following method A [$Pd(PPh_3)_4$, sodium carbonate in toluene/water 1/1].[37] Compounds **12–19** were obtained by parallel synthesis using microwave-assisted reactions according to method B [$Pd(OAc)_2$, potassium carbonate in DME/water/ethanol 7/3/2] (Schemes 2 and 4).

Quinoline intermediates **23a** and **24b** were prepared by reaction of 3-methoxyaniline with 2-bromomalonaldehyde or 2-(4-methoxyphenyl)malondialdehyde under acidic conditions (Scheme 3).

All synthesized compounds containing a methoxy substituent were demethylated using aluminum chloride (method C) for compounds **21–24** or boron tribromide (method D) for compounds **3–6**, **11**, and **20** (Schemes 2 and 4).

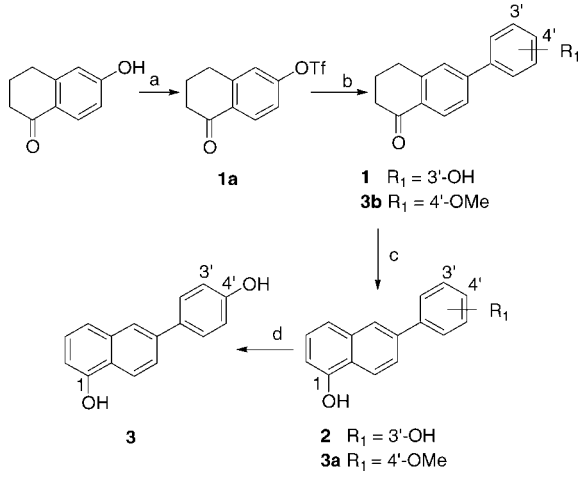
The 2-phenylquinoline derivative **25** was obtained by reaction of 6-methoxyquinoline-*N*-oxide with freshly prepared 3-methoxybenzenemagnesium bromide and subsequent ether cleavage using boron tribromide (method D, Scheme 5).

Chart 2. Designed Compounds



cmpd	R_1	R_2	cmpd	n	V	Z	X	Y	R_1	R_2
2	1-OH	3'-OH	**17**	0	CH	S				H
3	1-OH	4'-OH	**18**	0	S	CH				H
4	2-OH	2'-OH	**19**	1	N	CH				H
5	2-OH	3'-OH	**20**	1	N	CH				3'-OH
6	2-OH	4'-OH	**21**	1			N	CH	H	3'-OH
7	3-OH	3'-OH	**22**	1			N	CH	H	4'-OH
8	2-OH	H	**23**	1			N	CH	OH	4'-OH
9	H	3'-OH	**24**	1			N	CH	OH	3'-OH
10	2-OH	3'-NO_2	**25**	1			CH	N	OH	3'-OH
11	2-OH	3'-NH_2	**26**	0			CH	N	OH	3'-OH
12	2-OH	3'-COOH								
13	2-OH	4'-COOH								
14	2-OH	3'-$NHCOCH_3$								
15	2-OH	3'-CH_2OH								
16	2-OH	4'-CH_2OH								

Scheme 1. Synthesis of Compounds **1–3**[a]



[a] Reagents and conditions: (a) trifluoromethanesulfonic anhydride, CH_2Cl_2, 0°C, 30 min; (b) 3-hydroxy- or 4-methoxybenzeneboronic acid, aq Na_2CO_3, $Pd(PPh_3)_4$, DME, reflux, 4 h; (c) Pd/C, *p*-cymene, reflux, 6–24 h; (d) BBr_3, CH_2Cl_2. −78 °C, overnight.

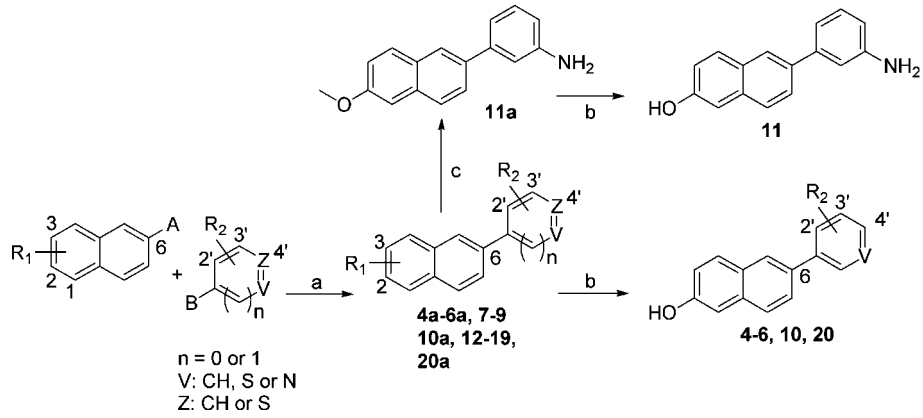
Synthesis of the indole **26** started with commercially available 4-methoxyaniline and 2-bromo-1-(3-methoxyphenyl)ethanone following a procedure described by von Angerer et al.[38] leading to compound **26a**, which was demethylated using boron tribromide (Scheme 6).

Biological Results

Inhibition of Human 17β-HSD1. As source of enzyme, both recombinant as well as placental enzyme were used. The amount of labeled E2 formed from tritiated substrate in the presence of cofactor and inhibitor was determined by HPLC. The percent inhibition values of all hydroxy compounds are shown in Tables 1 and 2, and the IC_{50} values of selected compounds are shown in Table 3. The hybride inhibitor (EM-1745) described by Poirier et al.[19] was used as reference compound and gave similar values as described (IC_{50} = 52 nM). 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).

Phenylnaphthalenes. The tetralone **1** turned out to have weak inhibitory activity. Compounds **2** and **3** bearing a hydroxy substituent at the 1-position of the naphthalene and a 3′-hydroxyphenyl or 4′-hydroxyphenyl substituent also showed weak (**2**) to moderate (**3**) inhibition (Table 1). An interesting structure–activity relationship (SAR) can be observed after shifting the hydroxy group of the naphthalene from the 1 to the 2 position. Three isomers were prepared bearing the second OH group in position 2′ (**4**), 3′ (**5**), or 4′ (**6**) of the phenyl moiety.

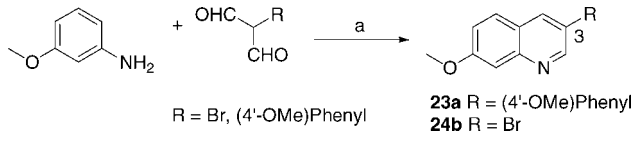
Scheme 2. Synthesis of Compounds **4–20**[a]



cmpd	A	B	R_1	R_2	n	V	Z	Method	cmpd	V	R_2
4a	Br	$B(OH)_2$	2-OMe	2'-OMe	1	CH	CH	A	**4**	CH	2'-OH
5a	Br	$B(OH)_2$	2-OMe	3'-OMe	1	CH	CH	A	**5**	CH	3'-OH
6a	Br	$B(OH)_2$	2-OMe	4'-OMe	1	CH	CH	A	**6**	CH	4'-OH
7	OTf	$B(OH)_2$	3-OH	3'-OH	1	CH	CH	A			
8	Br	$B(OH)_2$	2-OH	H	1	CH	CH	A			
9	Br	$B(OH)_2$	H	3'-OH	1	CH	CH	A			
10a	$B(OH)_2$	Br	2-OMe	3'-NO_2	1	CH	CH	A	**10**	CH	3'-NO_2
12	Br	$B(OH)_2$	2-OH	3'-COOH	1	CH	CH	B			
13	Br	$B(OH)_2$	2-OH	4'-COOH	1	CH	CH	B			
14	Br	$B(OH)_2$	2-OH	3'-$NHCOCH_3$	1	CH	CH	B			
15	Br	$B(OH)_2$	2-OH	3'-CH_2OH	1	CH	CH	B			
16	Br	$B(OH)_2$	2-OH	4'-CH_2OH	1	CH	CH	B			
17	Br	$B(OH)_2$	2-OH	H	0	CH	S	B			
18	Br	$B(OH)_2$	2-OH	H	0	S	CH	B			
19	Br	$B(OH)_2$	2-OH	H	1	N	CH	B			
20a	$B(OH)_2$	Br	2-OMe	3'-OMe	1	N	CH	B	**20**	N	3'-OH

[a] Reagents and conditions: (a) Method A: aq Na_2CO_3, $Pd(PPh_3)_4$, toluene or DME, 80 °C, overnight; Method B: K_2CO_3, Kirschning catalyst, DME/water/ethanol 7/3/2, microwave irradiation 150 °C, 100 W, 300 s; (b) BBr_3, CH_2Cl_2, −78 °C, overnight; (c) Pd/C, H_2, dry THF, rt, overnight.

Scheme 3. Synthesis of Compounds **23a–24b**[a]



[a] Reagents and conditions: (a) ethanol, rt, 18 h and then acetic acid, 100 °C, 10 days or EtOH, concd HCl, 80 °C, 3 days.

Compounds **4** and **6** show no affinity to 17β-HSD1, whereas the isomer with the phenolic OH-group in the meta position (compound **5**) is a highly potent inhibitor of the enzyme. Interestingly, this compound has recently been described to be a weak inhibitor of tyrosinase.[39]

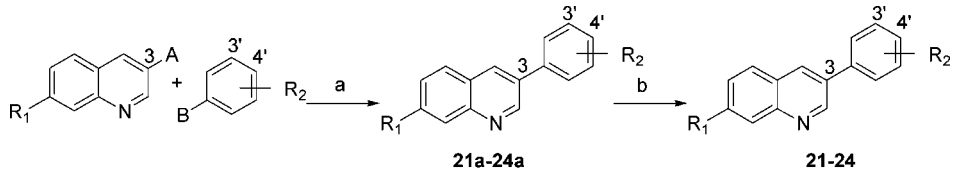
The question whether both hydroxy substituents are necessary for strong inhibition was examined. Starting from the structure of the highly active **5** with hydroxy groups in obviously favorable positions, two compounds **8** and **9** were synthesized, each bearing only one hydroxy substituent. Compound **9** (3′-OH on the phenyl moiety) shows a moderate activity, whereas **8** (2-OH on the naphthalene moiety) is devoid of activity.

Heterocycles and phenyl moieties bearing substituents other than OH, capable of acting as hydrogen-bond donors and/or acceptors, were introduced as potential alternatives for the 3′-hydroxyphenyl substituent of the most active compound **5**. All synthesized compounds **10–20** of this series show no or only moderate inhibition. The most active compound is the benzoic acid derivative **12**, displaying 76% inhibition at a concentration of 1 μM. Compound **20**, a 3′-hydroxypyridyl-substituted naphthol, showed a moderate activity (58% at 1 μM), indicating that a nitrogen atom in this area of the active site is not well tolerated.

Phenylindoles and Phenylquinolines. As discussed above, insertion of a hydrogen-bond acceptor or donor into the nonpolar phenylnaphthalene scaffold might lead to additional interactions with Tyr218 and/or Ser222, thus increasing inhibitory activity for 17β-HSD1. In order to explore this hypothesis, hydroxy-substituted quinoline and indole derivatives **21–26** were synthesized and evaluated for their 17β-HSD1 inhibitory activity (Table 2).

In the class of the quinolines **21–25**, SARs are similar to those observed in the naphthalene series: monohydroxylated phenylquinolines **21** and **22** show little or no inhibition. The 3′-hydroxyphenyl-substituted quinolin-7-ol **24** is a moderate inhibitor (57% inhibition at 1 μM), in contrast to its 4′-hydroxyphenyl-substituted isomer **23**, a weak ER β ligand[40] which is inactive. This again reflects the importance of the substitution pattern at the hydroxyphenyl moiety. On the other

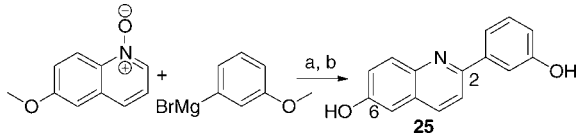
Scheme 4. Synthesis of Compounds **21–24**



cmpd	R_1	A	B	R_2	cmpd	R_1	R_2
21a	H	Br	$B(OH)_2$	3'-OMe	**21**	H	3'-OH
22a	H	Br	$B(OH)_2$	4'-OMe	**22**	H	4'-OH
23a	OMe	Br	$B(OH)_2$	4'-OMe	**23**	OH	4'-OH
24a	OMe	$B(OH)_2$	Br	3'-OMe	**24**	OH	3'-OH

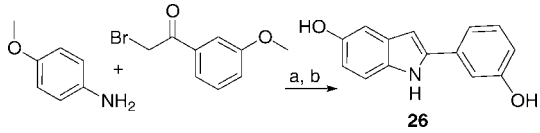
[a] Reagents and conditions: (a) aq Na_2CO_3, $Pd(PPh_3)_4$, toluene or DME, 80 °C, 4 h−3 d; (b) $AlCl_3$, toluene, 90 °C, 2 h.

Scheme 5. Synthesis of Compound **25**[a]



[a] Reagents and conditions: (a) dry toluene, 90 °C, 2 h; (b) BBr_3 CH_2Cl_2, −78 °C, 3 h.

Scheme 6. Synthesis of Compound **26**[a]



[a] Reagents and conditions: (a) *N*,*N*-dimethylaniline, 180 °C, 2 h; (b) BBr_3, CH_2Cl_2, −78 °C, overnight.

hand variations of the position of the nitrogen atom within the scaffold only have little effect on inhibitory potency: both **20** and **25** show moderate inhibition values similar to that of **24**.

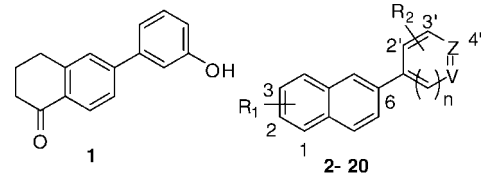
Indole **26**, bearing a hydroxyphenyl substituent at the five-membered ring, shows no activity.

The most active members **24** and **25** in this series of heterocyclic compounds are analogues of the most active naphthalene **5**. The introduction of a nitrogen atom in the scaffold, however, resulted in a reduction of activity (at 100 nM: 24% inhibition for **24** compared to 91% for **5**). This not only indicates that no additional bond is established between the nitrogen atom of the scaffold and the side chains of Tyr218 and Ser222 but it also shows that the polarity brought into the scaffold is not well tolerated by the enzyme.

For selected compounds, IC_{50} values were determined. As shown in Table 3, compound **5** with an IC_{50} value of 116 nM is the most potent inhibitor identified in this series. Moving the OH group on the naphthalene from the 2 position to the 1 position results in a loss of activity (**3**: IC_{50}: 2.4 μM). Insertion of a nitrogen in the phenyl ring also reduces activity by a factor of 10 (**20**).

Selectivity. As 17β-HSD2 deactivates E2 by oxidation to E1, which is beneficial for the treatment of estrogen-dependent diseases, this enzyme should not be affected by inhibitors of the type 1 enzyme. Furthermore, inhibitors of 17β-HSD1 should have low or no affinity at all for the estrogen receptors α and β (ER α and β), since binding to these receptors could counteract the therapeutic concept of 17β-HSD1 inhibition. Compounds

Table 1. Inhibition of Human 17β-HSD1 by Naphthalene Compounds **1–20**



n = 0 or 1
V: CH, S or N
Z: CH or S

						inhibition of 17β-HSD1[a] (%)	
compd	R_1	R_2	*n*	V	Z	100 nM	1 μM
1						n.i.	23
2	1-OH	3′-OH	1	CH	CH	n.i.	23
3	1-OH	4′-OH	1	CH	CH	n.i.	55
4	2-OH	2′-OH	1	CH	CH	n.i.	n.i.
5	2-OH	3′-OH	1	CH	CH	91	94
6	2-OH	4′-OH	1	CH	CH	n.i.	n.i.
7	3-OH	3′-OH	1	CH	CH	16	62
8	2-OH	H	1	CH	CH	n.i.	n.i.
9	H	3′-OH	1	CH	CH	26	61
10	2-OH	3′-NO_2	1	CH	CH	n.i.	n.i.
11	2-OH	3′-NH_2	1	CH	CH	n.i.	n.i.
12	2-OH	3′-COOH	1	CH	CH	36	76
13	2-OH	4′-COOH	1	CH	CH	n.i.	n.i.
14	2-OH	3′-$NHCOCH_3$	1	CH	CH	n.i.	19
15	2-OH	3′-CH_2OH	1	CH	CH	n.i.	n.i.
16	2-OH	4′-CH_2OH	1	CH	CH	n.i.	13
17	2-OH	H	0	CH	S	n.i.	n.i.
18	2-OH	H	0	S	CH	n.i.	n.i.
19	2-OH	H	1	N	CH	n.i.	32
20	2-OH	3′-OH	1	N	CH	30	58

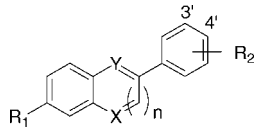
[a] Recombinant 17β-HSD1, substrate [^{3}H]-E1 (30 nM), NADPH (1 mM) procedure A; mean value of two determinations, deviation <20%, n.i. = no inhibition (inhibition <10%).

3, **5**, **9**, and **20**—a selection of the most potent inhibitors of 17β-HSD1 found in this study—were tested for their activities toward 17β-HSD2 and binding affinities to ER α and β.

Briefly, the inhibition assay for 17β-HSD2 was performed by HPLC measurement of the amount of labeled E1 formed from [^{3}H]-E2 in the presence of NAD^+ and inhibitor. IC_{50} values were determined and selectivity factors calculated (Table 3). While compound **3** shows selectivity for 17β-HSD2, the other compounds exhibit selectivity for 17β-HSD1, compound **5** being the most selective (factor 48).

For the ER assays recombinant human protein was used and a competition assay applying [^{3}H]-E2 and hydroxyapatite was run. All tested compounds show very little to moderate affinity to the ERs, respectively. The most promising compound so far,

Table 2. Inhibition of Human 17β-HSD1 by Quinoline and Indole Compounds **21–26**



21 - 26
n = 0 or 1
X: CH or N
Y: CH or N

						inhibition of 17β-HSD1[a] (%)	
compd	*n*	X	Y	R_1	R_2	100 nM	1 μM
21	1	N	CH	H	3′-OH	n.i.	18
22	1	N	CH	H	4′-OH	n.i.	n.i.
23	1	N	CH	OH	4′-OH	n.i.	n.i.
24	1	N	CH	OH	3′-OH	24	57
25	1	CH	N	OH	3′-OH	14	63
26	0	CH	N	OH	3′-OH	n.i.	n.i.

[a] Recombinant 17β-HSD1, substrate [^{3}H]-E1 (30 nM), NADPH (1 mM) procedure A; mean value of two determinations, deviation <20%, n.i. = no inhibition (inhibition <10%).

5, presents low affinities to the ERα (RBA: 0.2%) and the ERβ (RBA: 0.8%, Table 3).

Further Biological Evaluation of Compound 5. Compound **5** was investigated for permeation of Caco-2 cells. These cells exhibit morphological and physiological properties of the human small intestine[41] and are generally accepted to be an appropriate model for the prediction of peroral absorption. Depending on the P_{app} data obtained, compounds can be classified as low (P_{app} ($\times 10^{-6}$ cm/s) < 1), medium (1 < P_{app} < 10), or highly permeable (P_{app} >10). Compound **5** shows medium cell permeation (P_{app}= 8.9 $\pm$ 0.45 $\times 10^{-6}$ cm/s, $n = 3$).

Compound **5** was tested for metabolic stability. It was incubated with rat liver microsomes. Samples were taken at defined time points and the remaining percentage of parent compound was determined by LC-MS/MS. Half-life and intrinsic clearance were evaluated and compared to the two reference compounds diazepam and diphenhydramine. The short half-life $t_{1/2} = 12$ min and the relatively high intrinsic clearance (367 μL/min/mg protein) indicate a moderate metabolic stability for compound **5** (Table 4).

Compound **5** was also investigated for possible drug−drug interaction using six human hepatic enzymes: CYP1A2, 2B6, 2C9, 2C19, 2D6, and 3A4. The latter two enzymes are very crucial for two reasons: they are responsible for approximately 75% of drug metabolism and a genetic polymorphism is described. IC_{50} values of compound **5** and control inhibitors were determined for each enzyme. None of the enzymes was strongly inhibited by compound **5**. This result indicates a low risk of CYP-inhibition induced drug−drug interaction (Table 5).

Molecular Modeling. After having obtained interesting biological data for the synthesized compounds, theoretical studies were performed to develop greater insight into their molecular interactions with the protein. There are two possibilities for our steroidomimetics to superimpose with E2 resulting in similar RMSd values (Figure 2). Each of the two hydroxy groups of compound **5** can mimic the 3-OH or the 17-OH of E2.

In this context, it is striking that taking out one hydroxy function of compound **5**, leading to two monohydroxylated compounds, results in an active (**9**) and an inactive compound (**8**). Obviously, the hydroxy group on the phenyl ring is more important for binding than the one on the naphthyl moiety. This finding favors binding mode B (the hydroxyphenyl group superimposes with the steroidal A ring), as it can be hypothesized that the hydroxy group mimicking the steroidal phenolic 3-OH is more important for binding than the hydroxy group mimicking the 17-oxygen function: the enzymatic product E2 (17β-OH) binds less tightly than the substrate E1 (17-CO) in the binding site.

The surprising result that the introduction of a nitrogen in compound **5** does not increase activity further can be explained by the fact that the nitrogen cannot interact with the amino acids Tyr218 and/or Ser222 and supports our hypothesis that compound **5** binds according to mode B and not to mode A. For a better understanding, we docked the quinoline compound **24** in the active site according to binding mode B using the docking program Gold 3.0 (flexible ligand, rigid protein) and determined the distances to the two polar amino acids Tyr218 and Ser222. The distance $O_{Tyr218}-N_{quinoline}$ is 4.58 Å (Figure 3). The distance $O_{Ser222}-N_{quinoline}$ is 5.88 Å (not shown) but can adopt 3.50 Å due to the conformational flexibility of the CH_2OH group (Figure 3). At this latter distance a hydrogen bond interaction should be possible between the $O_{Ser222}-N_{quinoline}$ and should increase activity.

To obtain further insight, a molecular dynamics simulation of a binary complex of 17β-HSD1 in the conformation shown in Figure 3 and compound **24** was performed in order to identify the preferred position of the CH_2OH group of Ser222. The system was simulated for 500 ps. After 25 ps, the CH_2OH group rotated back to the initial position found in the X-ray structure. This conformation remained stable until the end of the simulation. The formation of an additional hydrogen bond interaction with quinoline **24** could thus be excluded. This means that the quinoline nitrogen has no partner for interaction. The nitrogen consequently being located in a hydrophobic surrounding should

Table 4. Metabolic Stability of Compound **5** Using Rat Liver Microsomes

compd	$t_{1/2}$[a,b] (min)	Cl_{int}[a,c] (μL/min/mg protein)
5	12.6	366.9
diazepam	40.8	113.3
diphenhydramine	6.8	679.6

[a] Mean value of three determinations. [b] $t_{1/2}$: half-life. [c] Cl_{int}: intrinsic body clearance.

Table 3. IC_{50} Values and Selectivity Data for Selected Compounds

compd	17β-HSD1 IC_{50}[a,b] (nM)	17β-HSD2 IC_{50}[a,c] (nM)	selectivity factor[d]	ERα RBA[e] (%)	ERβ RBA[e] (%)
3	2425	302	0.1	5	5
5	116	5641	48	0.2	0.8
9	2257	4007	1.8	0.01 < RBA < 0.1	0.01 < RBA < 0.1
20	1232	>10000	>8	0.01 < RBA < 0.1	1

[a] Mean value of three determinations, standard deviation <20% except **20**: 24% for 17β-HSD1. [b] Human placenta 17β-HSD1, substrate [^{3}H]-E1 (500 nM), NADH (500 μM) procedure B. [c] Human placenta 17β-HSD2, substrate [^{3}H]-E2 (500 nM), NAD^+ (1500 μM). [d] IC_{50} (17β-HSD2)/IC_{50} (17β-HSD1). [e] RBA (relative binding affinity) estradiol: 100%.

Table 5. Inhibition of Selected Hepatic CYP Enzymes by Compound **5** and Control Inhibitors

	IC_{50} (μM) ± SD[a]					
	CYP1A2	CYP2B6	CYP2C9	CYP2C19	CYP2D6	CYP3A4
5	3.99 ± 0.25	13.40 ± 0.45	1.05 ± 0.02	7.54 ± 0.51	32.56 ± 0.72	1.58 ± 0.04
control inhibitor	furafylline	tranylcypromine	sulfenazole	tranylcypromine	quinidine	ketoconazole
	3.04 ± 0.08	6.96 ± 0.025	0.250 ± 0.027	3.04 ± 0.17	0.011 ± 0.001	0.054 ± 0.001

[a] Mean value of three determinations.

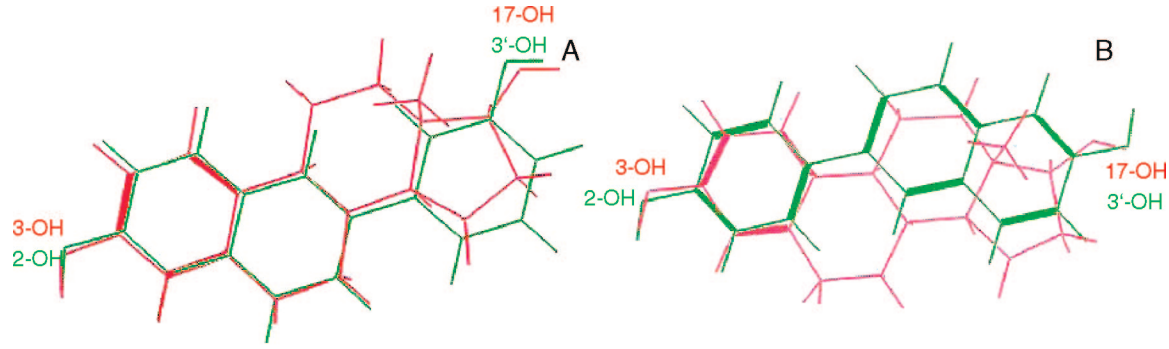


Figure 2. Principal ways of superimposition of **5** (green) and E2 (red). RMSd values: 0.36 Å for A (left) and 0.46 Å for B (right).

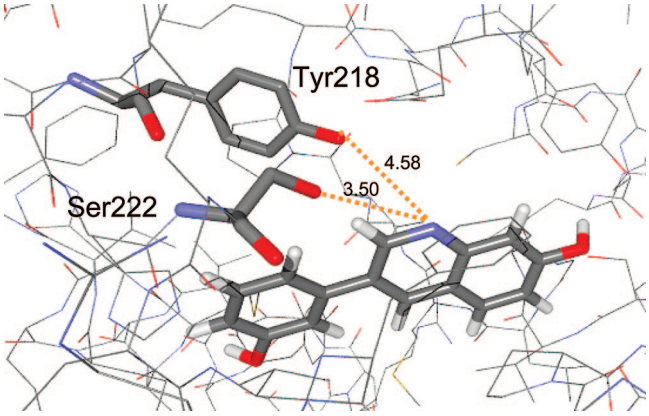


Figure 3. Possible hydrogen bond interactions of the 3-phenylquinoline compound **24** and the polar amino acids Tyr218 and Ser222 within the active site of 17β-HSD1. The CH_2OH group of Ser222 is rotated manually toward the nitrogen atom of **24** resulting in a feasible hydrogen bond. Distances are expressed in Å.

decrease the inhibitory activity of compound **24** strongly (compared to compound **5**) as was seen in the inhibition test.

Discussion and Conclusion

It is striking in this study that the structure–activity relationships encountered in the investigated class of compounds are very sharp: the ortho OH compound **4** and the para OH compound **6** show no affinity for 17β-HSD1, whereas the isomer with the OH group in the meta position of the phenyl ring (**5**) is a highly potent inhibitor of the enzyme. Moving the OH group at the naphthalene moiety of **5** to the 1- or 3-position (compounds **2** and **7**) results in a dramatic loss of activity, again indicating the pivotal role of the hydroxy group positions on the inhibitory activity. This is all the more remarkable because hydroxyphenylnaphthols designed as ligands for the structurally related estrogen receptors do not show this clear-cut SAR.[36] In this context there is an even more interesting aspect: the substitution pattern for optimal 17β-HSD1 inhibition and high affinity for ER are different. Compound **3** is a strong ligand for the estrogen receptors[36] having low 17β-HSD1 inhibitory activity whereas compound **5** displaying a strong inhibition of 17β-HSD1 shows only low affinity for the ERs. For an inhibitor of 17β-HSD1 to be applied clinically this is a prerequisite. There is no debate that the application of an estrogen receptor agonist is detrimental for treating estrogen-dependent diseases. A 17β-HSD1 inhibitor with additional antagonistic activity is also not welcome for our concept of local estrogen deprivation as such a compound would exert systemic effects.

The sharp SAR mentioned above suggests a rather rigid architecture of the substrate binding site of 17β-HSD1. At least as far as the amino acid residues interacting with the hydroxy groups (i.e., His221/Glu282 and Ser142/Tyr155) are concerned, conformational flexibility seems to be marginal. This assumption is in agreement with the fact that the geometry of the steroid binding site is highly conserved in the different X-ray structures available in the protein data bank—independent of the presence or absence of ligands.

The rigid structure of the binding site could also be an explanation for our finding that phenyl naphthalenes very likely bind in one of the two possible binding modes (Figure 2, mode B). This result is important for further structural optimizations in this class of compounds.

In the present study, compound **5** was identified as a highly active inhibitor of 17β-HSD1 showing good selectivity toward 17β-HSD2, ER α and ER β. Furthermore it displays a medium Caco-2 permeability, a reasonable metabolic stability and a low inhibition of the most important hepatic CYP enzymes. This compound will be used as a first lead in the further drug design process.

Experimental Section

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

^{1}H NMR spectra were recorded on a Bruker 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 deuterated 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, dd, m, and b for singlet, doublet, triplet, double–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 unless otherwise mentioned.

Chemical names follow IUPAC nomenclature. Starting materials were purchased from Aldrich, Acros, Lancaster, or Fluka and were used without purification.

Reactions under microwave irradiation were performed using an Emrys Optimizer Workstation apparatus from CMS.

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).

The following compounds were prepared according to previously described procedures: 5-oxo-5,6,7,8-tetrahydronaphthalen-2-yl trifluoromethanesulfonate (**1a**),[36] 6-(3-hydroxyphenyl)-3,4-dihydronaphthalen-1(2*H*)-one (**1**),[36] 6-(3-hydroxyphenyl)-1-naphthol (**2**),[36] 6-(4-methoxyphenyl)-3,4-dihydronaphthalen-1(2*H*)-one (**3b**),[36] 6-(4-hydroxyphenyl)-1-naphthol (**3**),[36] 6-(2-hydroxyphenyl)-2-naphthol (**4**),[36] 2-methoxy-6-(3-methoxyphenyl)naphthalene (**5a**),[36] 6-(3-hydroxyphenyl)-2-naphthol (**5**),[36] 2-methoxy-6-(4-methoxyphenyl)naphthalene (**6a**),[36] 6-(4-hydroxyphenyl)-2-naphthol (**6**),[36]7-(3-hydroxyphenyl)-2-naphthol (**7**),[36] 3-(2-naphthyl)phenol (**9**),[40] 4-(quinolin-3-yl)phenol (**22**),[40] 6-methoxy-2-(3-methoxyphenyl)quinoline **25a**,[43] and 2-(3-hydroxyphenyl)quinolin-6-ol (**25**).[43]

6-(4-Methoxyphenyl)-1-naphthol (3a). A mixture of 6-(4-methoxyphenyl)-3,4-dihydronaphthalen-1(2*H*)-one (510 mg, 2.02 mmol, 1 equiv) in *p*-cymene (7 mL) and palladium on charcoal (528 mg) was heated to reflux for 24 h, cooled, and filtered through Celite. The filtrate was washed twice with 1 M NaOH and twice with water. The aqueous layer was extracted several times with ethyl acetate. The organic layers were combined, dried over $MgSO_4$, and concentrated under reduced pressure. The crude product was purified by column chromatography (dichloromethane/hexane 5/5 and then 7/3) to give 69 mg of the expected product (14% yield): $C_{17}H_{14}O_2$; MW 250; MS (ESI) 251 $(M + H)^+$.

7-Methoxy-3-(4-methoxyphenyl)quinoline (23a).[39] To a solution 2-(4-methoxyphenyl)malondialdehyde (797 mg, 4.48 mmol) in EtOH (20 mL) and 3-methoxyaniline (551 mg, 4.48 mmol, 1.00 equiv) was added concentrated hydrochloric acid (37%; 10 mL). The reaction was heated at 100 °C for 3 days. After the solution was cooled to room temperature, water was added, and the mixture was extracted with dichloromethane. The organic layer was basified, washed with brine, and dried over $MgSO_4$. After evaporation of the solvent and column chromatography (hexane/ethyl acetate 8/2), the pure product was obtained: yield 221 mg (19%); $C_{17}H_{15}NO_2$; MW 265; MS (ESI) 266 (M + H), 251, 223, 208, 180, 152.

3-Bromo-7-methoxyquinoline (24b). To a solution of bromomalonaldehyde (1.80 g, 12.3 mmol) in 30 mL of ethanol was added 3-methoxyaniline (1.25 mL, 11.2 mmol, 0.911 equiv). The mixture was stirred at room temperature overnight. Acetic acid (20 mL) was added, and the reaction mixture was heated to 100 °C for 10 days. After the solution was cooled to room temperature, the solvent was evaporated, and the residue was partitioned between water and ethyl acetate. The organic layer was basified, washed with brine, and dried over $MgSO_4$. After evaporation of the solvent under reduced pressure, the product was purified by column chromatography using hexane/ethyl acetate 8/2 as eluent: yield 700 mg (20%); $C_{10}H_8BrNO$; MW 238.

5-Methoxy-2-(3-methoxyphenyl)-1*H*-indole (26a). To a boiling mixture of 4-methoxyaniline (2.46 g, 19.9 mmol, 6.44 equiv) and *N,N*-dimethylaniline (3.5 mL) was added dropwise 2-bromo-1-(3-methoxyphenyl)ethanone (0.70 g, 3.1 mmol) in solution in ethyl acetate (12 mL). After completion of the addition, the mixture was kept at 180 °C for 2 h. The reaction mixture was cooled to room temperature, and a dark solid was formed. Ethyl acetate was added along with 2 M HCl. The aqueous layer was extracted with ethyl acetate several times. The combined organic layers were washed with brine and dried over $MgSO_4$, filtered, and evaporated. Purification by column chromatography (dichloromethane/hexane 7/3) afforded 760 mg of **26a** (15%): $C_{16}H_{15}NO_2$; MW 253; MS (ESI) 254 $(M + H)^+$.

Suzuki Coupling. Method A. General Procedure for Synthesis of Compounds 4a, 8, 10a, 20a, 21a, 22a, and 24a. 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. The reaction mixture was cooled to room temperature, and water was added. The mixture was extracted with dichloromethane, washed with brine, dried over $MgSO_4$, and concentrated to dryness. The product was purified by column chromatography.

Method B. General Procedure for Parallel Synthesis of Compounds 12–19. Aryl halide (1 equiv), boronic acid (2 equiv), K_2CO_3 (3 equiv), and $Pd(OAc)_2$ were suspended in 5 mL of DME/water/ethanol 7/3/2. The reaction mixture was exposed to microwave irradation 300 s, 100 W, 150 °C. After reaching room temperature, the reaction mixture was filtered, concentrated, and purified by preparative HPLC (Waters fraction lynx autopurification system, Varian Inertsil C18-column 50 × 21 mm, particle size 3 μm, gradient elution, solvents: A: acetonitrile, formic acid (0.01%), B: water, formic acid (0.01%). Flow rate: 25 mL/min. Gradient: 0–100% A within 13 min (linear), then 100% A for 2 min). Purity data are given in the Supporting Information.

2-Methoxy-6-(2-methoxyphenyl)naphthalene (4a). The title compound was prepared by reaction of 2-bromo-6-methoxynaphthalene (300 mg, 1.26 mmol, 1 equiv) with 2-methoxybenzene boronic acid (192 mg, 1.26 mmol, 1 equiv) for 18 h according to method A (quantitative yield). The product was pure enough to be used directly for the subsequent ether cleavage without purification.

6-Phenyl-2-naphthol (8). The title compound was prepared by reaction of 6-bromo-2-naphthol (500 mg, 2.24 mmol) with benzene boronic acid for 22 h according to method A. The product was purified by column chromatography using hexane/ethylacetate 8/2 as eluent: yield 429 mg (87%); $C_{16}H_{12}O$; MW 220.

2-Methoxy-6-(3-nitrophenyl)-naphthalene (10a). The title compound was prepared by reaction of 3-bromonitrobenzene (1.00 g, 4.95 mmol) with 6-methoxynaphthaleneboronic acid for 20 h according to method A. The product was purified by column chromatography using hexane as eluent: yield 557 mg (40%); $C_{18}H_{17}NO_3$; MW 295.

3-(6-Methoxy-2-naphthyl)phenylamine (11a). A mixture of 2-methoxy-6-(3-nitrophenyl)naphthalene (200 mg, 0.71 mmol, 1 equiv) and Pd/C (5%; 50 mg) in dry THF (100 mL) was stirred under hydrogen overnight. After filtration through Celite and evaporation of the solvent, the crude product was purified by preparative chromatography to afford 36 mg of the desired product (20% yield): $C_{17}H_{15}NO$; MW 249.

3-(6-Hydroxy-2-naphthyl)benzoic acid (12). The title compound was prepared by reaction of 6-bromo-2-naphthol (44.6 mg, 0.2 mmol, 1 equiv) with 3-carboxyphenylboronic acid (66.4 mg, 0.4 mmol, 2 equiv) for 5 min according to method B: yield 27.8 mg (53%); $C_{17}H_{12}O_3$; MW 264; MS 265 $(M + H)^+$.

4-(6-Hydroxy-2-naphthyl)benzoic acid (13). The title compound was prepared by reaction of 6-bromo-2-naphthol (44.6 mg, 0.2 mmol, 1 equiv) with 4-carboxyphenylboronic acid (66.4 mg, 0.4 mmol, 2 equiv) for 10 min according to method B: yield 21.1 mg (40%); $C_{17}H_{12}O_3$; MW 264; MS (ESI) 265 $(M + H)^+$.

***N*-[3-(6-Hydroxy-2-naphthyl)phenyl]acetamide (14).** The title compound was prepared by reaction of 6-bromo-2-naphthol (44.6 mg, 0.2 mmol, 1 equiv) with 3-acetanilideboronic acid (71.6 mg, 0.4 mmol, 2 equiv) for 5 min according to method B: yield 22.4 mg (40%); $C_{18}H_{15}NO_2$; MW 277; MS (ESI) 278 $(M + H)^+$.

6-[3-(Hydroxymethyl]phenyl]-2-naphthol (15). The title compound was prepared by reaction of 6-bromo-2-naphthol (44.6 mg, 0.2 mmol, 1 equiv) with 3-(hydroxymethyl)phenylboronic acid (60.8 mg, 0.4 mmol, 2 equiv) for 5 min according to method B: yield 24.6 mg (49%); $C_{17}H_{14}O_2$; MW 250; MS (ESI) 251 $(M + H)^+$.

6-[4-(Hydroxymethyl)phenyl]-2-naphthol (16). The title compound was prepared by reaction of 6-bromo-2-naphthol (44.6 mg, 0.2 mmol, 1 equiv) with 4-(hydroxymethyl)benzeneboronic acid (60.8 mg, 0.4 mmol, 2 equiv) for 5 min according to method B: yield 21.1 mg (40%); $C_{17}H_{14}O_2$; MW 250; MS (ESI) 251 $(M + H)^+$.

6-(3-Thienyl)-2-naphthol (17). The title compound was prepared by reaction of 6-bromo-2-naphthol (44.6 mg, 0.2 mmol, 1 equiv)

with 3-thiopheneboronic acid (51.2 mg, 0.4 mmol, 2 equiv) for 10 min according to method B: yield 45.1 mg (99%); $C_{14}H_{10}S$; MW 226; MS (ESI) 227 $(M + H)^+$.

6-(2-Thienyl)-2-naphthol (18). The title compound was prepared by reaction of 6-bromo-2-naphthol (44.6 mg, 0.2 mmol, 1 equiv) with 2-thiopheneboronic acid (51.2 mg, 0.4 mmol, 2 equiv) for 10 min according to method B: yield 43.7 mg (97%); $C_{14}H_{10}S$; MW 226; MS (ESI) 227 $(M + H)^+$.

6-Pyridin-3-yl-2-naphthol (19). The title compound was prepared by reaction of 6-bromo-2-naphthol (44.6 mg, 0.2 mmol, 1 equiv) with 3-pyridineboronic acid (49.2 mg, 0.4 mmol, 2 equiv) for 5 min according to method B: yield 17.6 mg (40%); $C_{15}H_{11}NO$; MW 221; MS (ESI) 222 $(M + H)^+$.

3-Methoxy-5-(6-methoxy-2-naphthyl)pyridine (20a). The title compound was prepared by reaction of 6-methoxynaphthaleneboronic acid (258 mg, 1.28 mmol) with 3-bromo-5-methoxypyridine for 24 h according to method A. The product was purified by column chromatography using hexane/ethyl acetate 2/1: yield 237 mg (84%); $C_{17}H_{15}NO_2$; MW 265.

3-(3-Methoxyphenyl)quinoline (21a). The title compound was prepared by reaction of 3-bromoquinoline (200 mg, 0.96 mmol) with 3-methoxybenzene boronic acid for 19 h according to method A. The product was purified by column chromatography using hexane/ethyl acetate 75/25: yield 147 mg (70%); $C_{16}H_{13}NO$; MW 235; MS (ESI) 236 $(M + H)^+$, 221, 193, 167, 139.

3-(4-Methoxyphenyl)quinoline (22a). The title compound was prepared by reaction of 3-bromoquinoline (500 mg, 2.40 mmol) with 4-methoxybenzene boronic acid according to method A during 18 h. The product was purified by column chromatography using hexane/ethyl acetate 9/1: yield 504 mg (89%) of a white powder; $C_{16}H_{13}NO$; MW 235; MS(ESI) 236 $(M + H)^+$, 221, 193, 192, 167, 165, 154.

7-Methoxy-3-(3-methoxyphenyl)quinoline (24a). The title compound was prepared by reaction of 3-bromo-7-methoxyquinoline (255 mg, 1.07 mmol) with 3-methoxybenzeneboronic acid for 4.5 h according to method A. The product was purified by column chromatography using hexane/ethyl acetate 9/1: yield 215 mg (76%); $C_{17}H_{15}NO_2$; MW 265; MS (ESI) 266 $(M + H)^+$.

Ether cleavage. Method C. General Procedure. To a solution of methoxy derivative in dry toluene was added aluminum trichloride under N_2. The reaction mixture was heated at 90 °C for 2 h and cooled to room temperature. The reaction was quenched by the addition of 2% Na_2CO_3 and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over $MgSO_4$. and evaporated to dryness.

Method D. General Procedure. To a solution of methoxy derivative in dry dichloromethane (cooled to –78 °C) was added boron tribromide (1 M solution in cyclohexane) under N_2. The reaction mixture was stirred at −78 °C for 1 h, at room temperature 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, dried over $MgSO_4$, filtered, and evaporated.

6-(3-Nitrophenyl)-2-naphthol (10). The title compound was prepared by reaction of 2-methoxy-6-(3-nitrophenyl)naphthalene (200 mg, 0.72 mmol) with boron tribromide (5.6 equiv) according to method D. Purification by column chromatography (hexane/ethyl acetate 9/1) afforded 89.5 mg of the desired product (47%): $C_{16}H_{11}NO_3$; MW 265; MS (ESI) 264 $(M - H)^-$.

6-(3-Aminophenyl)-2-naphthol (11). The title compound was prepared by reaction of 3-(6-methoxynaphthalene-2-yl)phenylamine (87.9 mg, 0.35 mmol, 1 equiv) with boron tribromide (5 equiv) according to method D. Purification by column chromatography (hexane/ethyl acetate 3/1) afforded 12 mg of the desired product (14%): $C_{16}H_{13}NO$; MW 235; MS (ESI) 236 $(M + H)^+$.

5-(6-Hydroxy-2-naphthyl)pyridin-3-ol (20). The title compound was prepared by reaction of 3-methoxy-5-(6-methoxy-2-naphthyl)pyridine (200 mg, 0.754 mmol) with aluminum chloride (804 mg, 6.03 mmol, 8.00 equiv) according to method C. Purification by column chromatography (dichloromethane/methanol 95/5) afforded 13.1 mg (7%) of **20**: $C_{15}H_{11}NO_2$; MW 237; MS (ESI) 238 $(M + H)^+$.

3-(Quinolin-3-yl)phenol (21). The title compound was prepared by reaction of 3-(3-methoxyphenyl)quinoline (101 mg, 0.429 mmol) with aluminum trichloride (343 mg, 2.58 mmol, 6.00 equiv) according to method C. Purification by preparative thin layer chromatography (silica, 1 mm thickness, dichloromethane/methanol 95/5) afforded 81 mg of the desired product (85%): $C_{15}H_{11}NO$; MW 221; MS (ESI) 222 $(M + H)^+$.

3-(4-Hydroxyphenyl)quinolin-7-ol (23).[39] The title compound was prepared by reaction of 7-methoxy-3-(4-methoxyphenyl)quinoline (96 mg, 0.37 mmol, 1 equiv) with aluminum chloride (392 mg, 2.95 mmol, 6.00 equiv) according to method C. Purification by column chromatography (dichloromethane/methanol 96/4) afforded 55 mg of the desired product (63%): $C_{15}H_{11}NO_2$; MW 237; MS (ESI) 238 $(M + H)^+$.

3-(3-Hydroxyphenyl)quinolin-7-ol (24). The title compound was prepared by reaction of 7-methoxy-3-(3-methoxyphenyl)quinoline (108 mg, 0.407 mmol) with aluminum chloride (433 mg, 3.26 mmol, 8.00 equiv) according to method C. Purification by column chromatography (dichloromethane/methanol 95/5) afforded 73 mg of the desired product (76%): $C_{15}H_{11}NO_2$; MW 237; MS (ESI) 238 $(M + H)^+$.

2-(3-Hydroxyphenyl)-1*H*-indol-5-ol (26). The title compound was prepared by reacting 5-methoxy-2-(3-methoxyphenyl)-1*H*-indole (89 mg, 0.35 mmol) with boron tribromide (6.0 equiv) according to method D. Purification by preparative thin-layer chromatography (hexane/ethyl acetate 4/6) afforded 60 mg (76%) of compound **26**: $C_{14}H_{11}NO_2$; MW 225; MS (ESI) 226 $(M + H)^+$.

Biological Methods. [2,4,6,7-^{3}H]-E2 and [2,4,6,7-^{3}H]-E1 were purchased from Perkin-Elmer, Boston. Quickszint Flow 302 scintillator fluid was bought from Zinsser Analytic, Frankfurt. Other chemicals were purchased from Sigma, Roth, or Merck.

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

Procedure A: Percentage of Inhibition Determination. Enzyme Preparation. 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.[44] Aliquots containing 17β-HSD1 were stored frozen until determination of enzymatic activity.

Assay. Recombinant 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 of acetonitrile/water (48:52) as mobile phase. Radioactivity of the eluate was monitored by a Packard Flow Scintillation Analyzer. Total radioactivities for estrone and estradiol were determined in each sample, and percent conversion of estrone to estradiol was calculated.

Procedure B: IC_{50} Value Determination. Enzyme Preparation. 17β-HSD1 and 17β-HSD2 were partially purified from human placenta according to the previously described procedures.[19,45] Fresh human placenta was homogenized and fractionally centrifuged at 1000*g*, 10000*g*, and 150000*g*. The pellet fraction contained the microsomal 17β-HSD2, while 17β-HSD1 was obtained after precipitation with ammonium sulfate from the cytosolic fraction.

Assay. Enzymatic activities were determined as described[45–47] with minor modifications. Briefly, the enzyme preparation was incubated with NADH (500 μM) in the presence of potential inhibitors at 37 °C in a phosphate buffer supplemented with 20% glycerol and EDTA 1 mM. Inhibitor stock solutions were prepared in DMSO. Final concentration of DMSO was adjusted to 1% in all samples. The enzymatic reaction was started by addition of [^{3}H]-E1 (final concentration: 500 nM). After 30 min, the enzymatic reaction was stopped with $HgCl_2$, the steroids were extracted with ether and the solvent evaporated. The steroids were dissolved in acetonitrile. E1 and E2 were separated using an acetonitrile/water mixture (45/55) as the mobile phase on a C18 reversed-phase 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. Each value is given as a mean value of at least three independent experiments. The subsequent IC_{50} values were determined.

2. Inhibition of 17*β*-HSD2. The 17β-HSD2 inhibition assay was performed as previously described for 17β-HSD1 according to procedure B, using [^{3}H]-E2 as substrate (final concentration: 500 nM) and NAD^+ [1500 μM] as cofactor.

3. ER Affinity. The binding affinity of selected compounds to the ER α and ER β was determined according to Zimmermann et al.[48] The human recombinant protein was used. Briefly, 0.25 pmol of ERα or ERβ, respectively, were incubated with [^{3}H]-E2 (10 nM) and inhibitor for 1 h at room temperature. Inhibitor dilutions were made in DMSO (5% final concentration). Nonspecific-binding was determined with diethylstilbestrol (10 μM). After incubation, ligand–receptor complexes were selectively bound to hydroxyapatite (5 g/60 mL TE-buffer). The formed complex was washed and resuspended in ethanol. For radiodetection, scintillator cocktail (Quickszint 212, Zinsser Analytic, Frankfurt, Germany) was added and samples were measured in a liquid scintillation counter (Rack Beta Primo 1209, Wallac, Turku, Finland). For determination of the relative binding affinity (RBA), the inhibitor concentration required to displace 50% of the bound [^{3}H]-E2 was determined. RBA values were calculated according to following equation: RBA (%) = IC_{50}(estradiol)/IC_{50}(compound)100. The RBA value for E2 was arbitrarily set at 100%.

4. Caco-2 Transport Experiments. Caco-2 cell culture and transport experiments were performed according to Yee[49] with small modifications. Cell culture time was reduced from 21 to 10 days by increasing seeding density from 6.3×10^4 to 1.65×10^5 cells per well. Four reference compounds (atenolol, testosterone, ketoprofene, erythromycin) were used in each assay for validation of the transport properties of the Caco-2 cells. The compounds were applied to the cells as mixtures (cassette dosing) to increase the throughput of the cell permeability tests. The initial concentration of the compounds in the donor compartment was 50 μM (for each compound in buffer, containing either 1% ethanol or DMSO). Samples were taken from the acceptor side after 0, 60, 120, and 180 min and from the donor side after 0 and 180 min. Each experiment was run in triplicate. The integrity of the monolayers was checked by measuring the transepithelial electrical resistance (TEER) before the transport experiments and by measuring lucifer yellow permeability after each assay. All samples of the Caco-2 transport experiments were analyzed by LC/MS/MS after dilution with buffer of the opposite transwell chamber (1:1, containing 2% acetic acid). The apparent permeability coefficients (P_{app}) were calculated using equation $P_{app} = (dQ/dtAc_0)$, where dQ/dt is the appearance rate of mass in the acceptor compartment, A the surface area of the transwell membrane, and c_0 the initial concentration in the donor compartment.

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

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

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

6. Inhibition of human hepatic CYPs. The commercially available P450 inhibition kits from BD Gentest (Heidelberg, Germany) were used according to the instructions of the manufacturer. Compound **5** was tested for inhibition of the following enzymes: CYP1A2, 2B6, 2C9, 2C19, 2D6, and 3A4. Inhibitory potencies were determined as IC_{50} values.

Molecular modeling. X-ray structures of 17β-HSD1 were obtained from the Protein Databank (PDB, www.pdb.org; 0). Water molecules, E2 and sulfate ions were removed from the PDB file and missing protein atoms were added.

Close contacts were fixed (Arg37) and correct atom types were set. Finally hydrogen atoms and neutral end groups were added.

Superimpositions of E2 (from PDB ID: 1FDT) and inhibitors of 17β-HSD1 were performed by Schrödinger Maestro/Macromodel.[50]

Docking of inhibitors into the substrate binding site was performed by the automated docking program GOLD 3.0.[51]

The molecular dynamics simulation was performed by the AMBER 8 suite of programs.[52] The simulation system was minimized (5000 steps of steepest descent followed by 10000 steps of conjugate gradient) and equilibrated at 300 K (20000 steps, step size 1 fs). Finally, 500 ps of molecular dynamics simulation (step size 1.0 fs, 100000 steps) at constant temperature (300 K) was performed. Temperature was regulated by coupling to an external bath (Berendsen's method)[53] using a bath coupling constant of 1.0 ps during equilibration and 1.5 ps during production. The generalized Born model[54] was used to account for solvation effects.

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Supporting Information Available: Spectroscopic data (^{1}H NMR, ^{13}C NMR, IR) and purity data of compounds **2**, **3b**, **3**−**5**,

7−**21**, and **23**−**26**. This material is available free of charge via the Internet at http://pubs.acs.org.

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