Design, Synthesis, Biological Evaluation and Pharmacokinetics of Bis(hydroxyphenyl) substituted Azoles, Thiophenes, Benzenes, and Aza-Benzenes as Potent and Selective Nonsteroidal Inhibitors of 17*β*-Hydroxysteroid Dehydrogenase Type 1 (17*β*-HSD1)

Emmanuel Bey,[†] Sandrine Marchais-Oberwinkler,[†] Ruth Werth,[†] Matthias Negri,[⊥,†] Yaseen A. Al-Soud,[§,†] Patricia Kruchten,[†] Alexander Oster,[†] Martin Frotscher,[†] Barbara Birk,[‡] and Rolf W. Hartmann*,[†]

Pharmaceutical and Medicinal Chemistry, Saarland University, PO Box 15 11 50, D-66041, Saarbrücken, Germany, Pharmacelsus CRO, Science Park 2, D-66123 Saarbrücken, Germany

Received June 6, 2008

17*β*-Estradiol (E2), the most potent female sex hormone, stimulates the growth of mammary tumors and endometriosis via activation of the estrogen receptor α (ERα). 17*β*-Hydroxysteroid dehydrogenase type 1 (17*β*-HSD1), which is responsible for the catalytic reduction of the weakly active estrogen estrone (E1) into E2, is therefore discussed as a novel drug target. Recently, we have discovered a 2,5-bis(hydroxyphenyl) oxazole to be a potent inhibitor of 17*β*-HSD1. In this paper, further structural optimizations were performed: 39 bis(hydroxyphenyl) azoles, thiophenes, benzenes, and aza-benzenes were synthesized and their biological properties were evaluated. The most promising compounds of this study show enhanced IC_{50} values in the low nanomolar range, a high selectivity toward 17*β*-HSD2, a low binding affinity to ERα, a good metabolic stability in rat liver microsomes, and a reasonable pharmacokinetic profile after peroral application. Calculation of the molecular electrostatic potentials revealed a correlation between 17*β*-HSD1 inhibition and the electron density distribution.

Introduction

Estrogens, the most potent one being 17*β*-estradiol (E2[a]), act as female sex hormones and are predominantly produced before menopause by the ovaries. They unfold their activity by stimulation of the estrogen receptors (ERs) α and *β*. Besides their physiological effects, they are, however, also involved in the initiation and progression of estrogen-dependent diseases like mammary tumor[1] and endometriosis.[2] Presently, the three main endocrine therapies for the treatment of breast cancer are:[3,4] inhibition of estrogen biosynthesis by aromatase inhibitors or GnRH agonists or antagonists and interference with the estrogen action at the receptor level by selective estrogen receptor modulators (SERMs) or pure antiestrogens.[5] Besides specific disadvantages of each therapeutic concept, all of these strategies have in common that they reduce estrogen levels systemically, leading to the corresponding side effects.

A softer approach could be inhibition of the enzyme involved in the last step of the E2 biosynthesis: 17*β*-hydroxysteroid dehydrogenase (17*β*-HSD), which is able to convert estrone (E1) into E2. There are three subtypes (1, 7, and 12) described, the most important of which is 17*β*-HSD1. The primary function of 17*β*-HSD7 and 17*β*-HSD12 is supposed to be in the cholesterol synthesis[6] and in the regulation of the lipid biosynthesis,[7] respectively. Moreover, Day et al.[8] showed that 17*β*-HSD12, although highly expressed in breast cancer cell lines, is inefficient in E2 formation.

17*β*-HSD1 is NAD(P)H-dependent and intracellularly converts the weak estrogen E1 into the strong estrogen E2. As it is often overexpressed in breast cancer cells[9−12] and endometriosis,[13] 17*β*-HSD1 is regarded as a promising novel target for the treatment of estrogen-dependent diseases. Appropriate inhibitors of this enzyme should exhibit fewer side effects compared to the current treatments, as they should selectively reduce the concentration of active E2 in the diseased tissues.[14]

As a biological counterpart, 17*β*-hydroxysteroid dehydrogenase type 2 (17*β*-HSD2) catalyzes the deactivation of E2 into E1. It protects the cell from excessively high concentrations of active estrogens[15] and should therefore not be affected by inhibitors of 17*β*-HSD1. In addition, 17*β*-HSD1 inhibitors should not show affinity to the ERs to avoid intrinsic estrogenic effects.

17*β*-HSD1 was crystallized with different steroidal ligands.[16−24] The published X-ray structures provide insight into the active site, a narrow hydrophobic tunnel with polar contacts at each end. On one side His221/Glu282 are located, on the other Ser142/Tyr155 (two members of the catalytic tetrad).[25] Surprisingly, close to the hydrophobic B/C region of the steroid, two polar amino acids, Tyr218 and Ser222, can be found that do not interact with E2.

Several steroidal and nonsteroidal inhibitors of 17*β*-HSD1 have been described. The first report on steroidal compounds by Penning[26] was as early as 1996. In the past few years, several articles of other groups followed.[27] Regarding the nonsteroidal inhibitors, only four compound classes have been described so far, all of them very recently: thienopyrimidinones **A**,[28,29] biphenyl ethanones **B**,[30] and from our group, 6-(hydroxyphenyl) naphthols **C**[31,32] and bis(hydroxyphenyl) azoles **D**[33] (Chart 1).

---

* To whom correspondence should be addressed. Phone: +49 681 302 70302. Fax: +49 681 302 70308. E-mail: rwh@mx.uni-saarland.de. Website: http://www.pharmmedchem.de.

[†] Pharmaceutical and Medicinal Chemistry, Saarland University.

[‡] Pharmacelsus CRO.

[§] Home address: Department of Chemistry, College of Science, University of Al al-Bayt, Al-Mafraq, Jordan

[⊥] Home address: Department of Pharmaceutical Sciences, University of Bologna, Via Belmeloro 6, I-40126 Bologna, Italy

[a] Abbreviations: 17*β*-HSD1, 17*β*-hydroxysteroid dehydrogenase type 1; 17*β*-HSD2, 17*β*-hydroxysteroid dehydrogenase type 2; E1, estrone; E2, 17*β*-estradiol; ER, estrogen receptor; SERM, selective estrogen receptor modulator; NADP(H), nicotinamide adenine dinucleotide phosphate; NAD(H), nicotinamide adenine dinucleotide; RBA, relative binding affinity; ESP, electrostatic potential; P_{app}, apparent permeability coefficient; SAR, structure−activity relationship; CC, column chromatography; MEP, molecular electrostatic potential; AUC, area under the curve; semi-QMAR, semiquantitative MEP−activity relationship. For the sake of clarity, IUPAC nomenclature is not strictly followed except for the Experimental Section, where the correct IUPAC names are given.

10.1021/jm8006917 CCC: $40.75

Chart 1. Nonsteroidal 17*β*-HSD1 Inhibitors

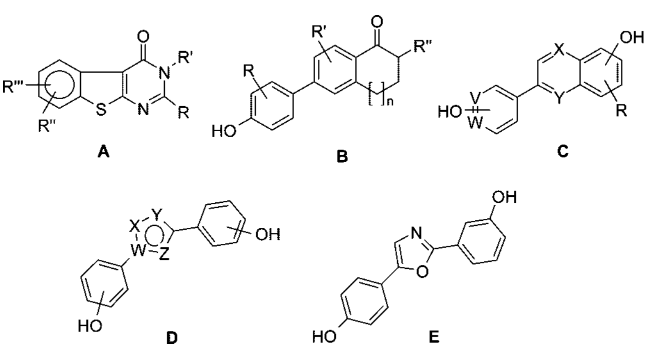
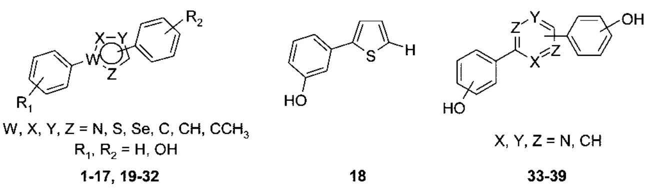


Chart 2. Title Compounds



The most promising compound in the latter series was the 2,5-bis(hydroxyphenyl) oxazole **E**, with an IC_{50} of 310 nM and a selectivity factor of 56 against 17*β*-HSD2. In general, it was discovered that the inhibitory activity of these compounds depends on the existence of hydroxy rather than methoxy groups on the phenyl substituents and on the OH substitution pattern, *meta*−*para* and *para*−*meta* substituted compounds being more active than *para*−*para* substituted ones. Furthermore, it became apparent that inhibition also depends on the nature of the heterocycle. Hydrogen bond donor functions turned out to be inappropriate, whereas in several compounds, H-bond acceptor atoms were favorable. This finding led to the hypothesis that active compounds are capable of interacting with Tyr218 or Ser222 via H-bonds.[33]

To enhance activity and selectivity and to get a better insight into the interaction of these compounds with the active site of 17*β*-HSD1, the significance of the OH groups will be further evaluated and other five-membered heterocycles, especially sulfur containing ones, will be investigated. Furthermore, it will be evaluated whether six-membered rings are also appropriate to connect the two hydroxyphenyl moieties.

In the following, we describe the synthesis of 39 bis(hydroxyphenyl) azoles, thiophenes, benzenes, and aza-benzenes (Chart 2) as well as the determination of their 17*β*-HSD1 inhibitory activity and selectivity toward 17*β*-HSD2, ER*α*, and ER*β*. Furthermore, cell permeability using CaCo-2 cells, metabolic stability in rat liver microsomes, inhibition of the most important hepatic CYP enzymes, and pharmacokinetic properties in the rat of selected compounds were determined. For a better understanding of the SARs, molecular electrostatic potentials (MEPs) were calculated.

Chemistry

Starting from the commercially available dibrominated heterocycles and methoxylated benzene boronic acids, compounds **1**−**8**, **19**−**28**, **31**, and **32** were prepared via two successive Suzuki reactions[34] followed by a demethylation step using borontribromide[35] as reagent. The Suzuki cross-coupling was carried out using three different methods. Intermediates **1ii**−**8ii**, **18ii**−**27ii**, and **1i**−**8i** were prepared following method A (aq sodium carbonate, toluene, $Pd(PPh_3)_4$, reflux, 4 h) and compounds **31ii**, **32ii**, **19i**−**28i**, **31i**, and **32i** were synthesized using method B (sodium carbonate, THF/water (1:1), $Pd(PPh_3)_4$, reflux, 20 h). During the first cross-coupling reaction leading to the mono(methoxyphenyl) substituted derivatives **1ii**−**8ii**, **18ii**−**27ii**, **31ii**, and **32ii**, no disubstituted compounds were obtained, indicating that the (methoxyphenyl)bromo azoles and thiophenes are less reactive than their dibromo aryl precursors. The bromo intermediate **18ii** was treated with *n*-BuLi in anhydrous THF and subsequently hydrolyzed with water to yield the monomethoxylated thiophene **18i**. The methoxy groups of **1i**−**8i**, **18i**−**28i**, **31i**, and **32i** were cleaved with boron tribromide (method E: BBr_3, CH_2Cl_2, −78 °C to rt, 18 h, Scheme 1).

The 1,3,4-thiadiazole **9** was prepared in a three-step synthetic pathway based on the method described by Gierczyk and Zalas.[36] The 3-methoxybenzoyl chloride was treated with hydrazine monohydrate to give the resulting 3-methoxy-*N*′-(3-methoxybenzoyl)benzohydrazide, which was cyclized into the corresponding thiadiazole using Lawesson reagent in DME under microwave assisted conditions. In a last step, the methoxy substituents were cleaved with boron tribromide (method E: BBr_3, CH_2Cl_2, −78 °C to rt, 18 h).

The synthesis of the 1,2,4-thiadiazoles **10** and **11** is shown in Scheme 2. The commercially available 4-methoxybenzonitrile and 3-hydroxybenzonitrile were converted into the thioamide intermediates **10ii** and **11i**, respectively, using aqueous ammonium sulfide under microwave assisted reaction.[37] These thioamides were submitted to strong acidic conditions, resulting in a mixture of the thiadiazoles **10i** and **11**, which were separated by column chromatography. The bis(methoxyphenyl) compound could not be isolated. Compound **10i** was demethylated with boron tribromide (method E: BBr_3, CH_2Cl_2, −78 °C to rt, 18 h, Scheme 2).

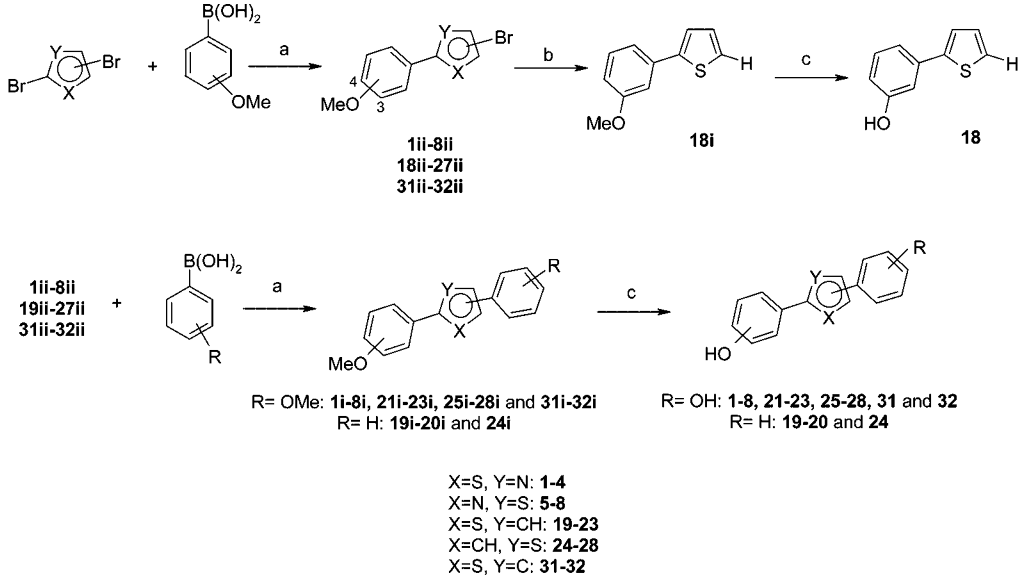
The synthesis of compounds **12**−**15** is presented in Scheme 3. The dimethoxylated-1,2,4-triazoles **12i**−**15i** were synthesized by reaction of the *N*-acylimidates[38] **12ii**−**15ii** with methylhydrazine (method D: $MeNHNH_2$, CH_2Cl_2, 30−40 °C, 4 h). The methoxy groups of compounds **12i**−**15i** were cleaved with borontrifluoride dimethyl sulfide complex[35] (method F: $BF_3 \cdot SMe_2$, CH_2Cl_2, rt, 20 h, Scheme 3).

Compound **16** was prepared according to Sharpless[39] using 3-azidophenol and 3-hydroxyphenyl acetylene.

Compounds **17**, **29**, **30**, **37**, and **38** were obtained, under microwave assisted conditions in a one-pot synthesis (method C: DME/EtOH/water (1:1:1), Cs_2CO_3, $Pd(PPh_3)_4$, MW (150 W, 150 °C, 15 bar, 15 min)) with benzene boronic acid for **17**, hydroxylated benzene boronic acid for **29**, **30**, **37**, and **38** and the corresponding dibrominated heterocycle.

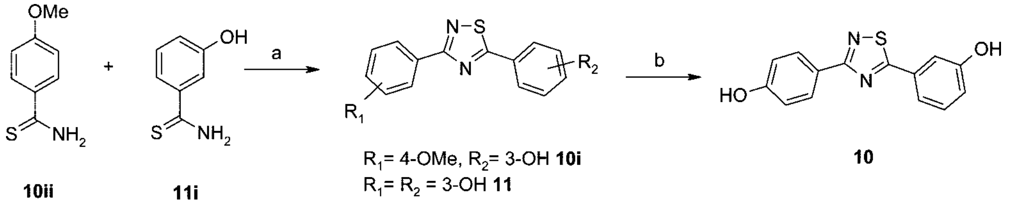
The synthesis of compounds **33**−**36** is depicted in Scheme 4. Starting from the commercially available dibrominated benzene and methoxylated benzene boronic acids, compounds **33**−**36** were prepared via two successive Suzuki reactions following the conditions of method A. In the first reaction, only the monosubstituted compounds **33ii** and **35ii** were obtained due to the fact that the (methoxyphenyl)bromobenzenes are less reactive than the dibromobenzenes. Consequently, longer reaction times were required for the second cross-coupling reaction (20 versus 4 h). The methoxy groups of compounds **33i**−**36i** were cleaved using boron tribromide (method E: BBr_3, CH_2Cl_2, −78 °C to rt, 18 h, Scheme 4). 1,2,4,5-Tetrazine **39** was synthesized following the procedure described by Guither et al.[40] Briefly, 3-hydroxybenzonitrile was refluxed with hydrazine

Scheme 1. Synthesis of Compounds **1**−**8**, **18**−**28**, **31**, and **32**[a]



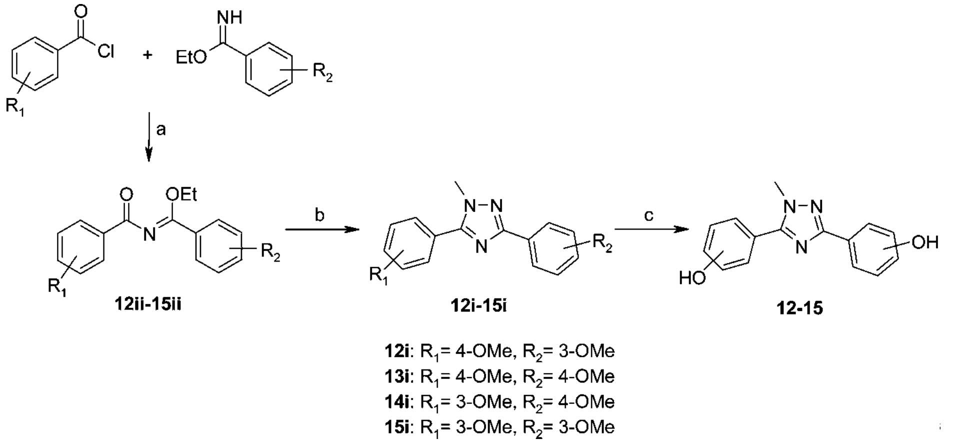
[a] Reagents and conditions: (a) For compounds **1ii**−**8ii**, **18ii**−**27ii**, and **1i**−**8i**: method A: aq Na_2CO_3, toluene, $Pd(PPh_3)_4$, reflux, 4 h; for compounds **31ii**, **31i**, **32i**, **32ii**, and **19i**−**28i**: method B: Na_2CO_3, THF/water (1:1), $Pd(PPh_3)_4$, reflux, 20 h; (b) (1) *n*-BuLi, THF dry, −78 °C, 15 min, (2) water. (c) Method E: BBr_3, CH_2Cl_2, −78 °C to rt, 18 h.

Scheme 2. Synthesis of Compounds **10** and **11**[a]



[a] Reagents and conditions: (a) conc HCl, 38 °C, 8 h; (b) method E: BBr_3, CH_2Cl_2, −78 °C to rt, 18 h.

Scheme 3. Synthesis of Compounds **12**−**15**[a]



[a] Reagents and conditions: (a) CH_2Cl_2, NEt_3, 30-40 °C, 6 h; (b) method D: $MeNHNH_2$, CH_2Cl_2, 30−40 °C, 4 h; (c) method F: $BF_3 \cdot SMe_2$, CH_2Cl_2, rt, 20 h.

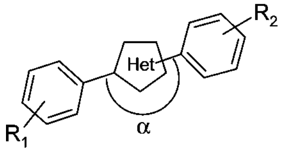
monohydrate and sulfur. Treatment with sodium nitrite led to cyclization resulting in 3,3′-(1,2,4,5-tetrazine-3,6-diyl)diphenol **39**.

Biological Results

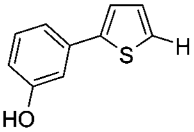
Activity: Inhibition of Human 17*β*-HSD1. Placental enzyme was partially purified following a described procedure.[41] Tritiated E1 was incubated with 17*β*-HSD1, cofactor, and inhibitor. The amount of labeled E2 formed was quantified by HPLC. Compounds showing less than 10% inhibition at 1 *μ*M were considered to be inactive.

The inhibition values of the test compounds are shown in Table 1. It becomes apparent that 11 compounds are more active than the previously described oxazole **E**[33] (IC_{50} = 310 nM). All methoxy compounds (data not shown) and *para*−*para* dihydroxylated derivatives are inactive except phenylene **34**, which is a weak inhibitor (IC_{50} > 1000 nM). The shift of one

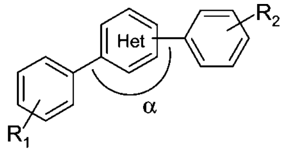
Table 1. Inhibition of Human 17*β*-HSD1 and 17*β*-HSD2 by Compounds **1**−**39**, O−O Distances, and Phenyl-het-phenyl Angles



1-17
19-32



18



33-39

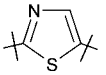
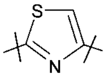
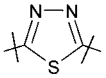
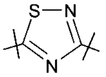
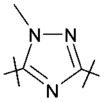
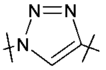
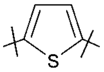
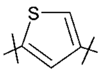
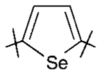
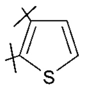
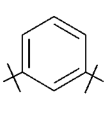
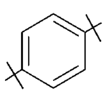
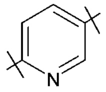
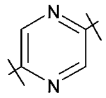
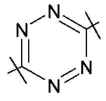
compound	Het	R1	R2	d(Å)[a]	α[b]	IC_{50} (nM)[c]		selectivity factor[f]
						17β-HSD1[d]	17β-HSD2[e]	
1		4-OH	3-OH	11.9< d< 12.7	153	>1000	nt	
2		4-OH	4-OH	13.6		ni	nt	
3		3-OH	4-OH	11.9< d< 12.7		50	4004	80
4		3-OH	3-OH	10.1< d< 11.7		243	2500	10
5		4-OH	3-OH	10.5< d< 12.0	130	>5000	nt	
6		4-OH	4-OH	12.5		ni	nt	
7		3-OH	4-OH	10.5< d< 12.0		>1000	nt	
8		3-OH	3-OH	9.0< d< 11.2		455	2220	5
9		3-OH	3-OH	9.9< d< 11.5	157	336	nt	
10		3-OH	4-OH	9.7< d< 11.9	128	413	2194	5
11		3-OH	3-OH	8.5< d< 11.2		169	602	3
12		4-OH	3-OH	10.2< d< 11.9	145	ni	nt	
13		4-OH	4-OH	12.4		ni	nt	
14		3-OH	4-OH	10.2< d< 11.9		ni	nt	
15		3-OH	3-OH	8.7< d< 11.4		ni	nt	
16		3-OH	3-OH	8.7< d< 11.2	147	>5000	nt	
17		H	H		147	ni	nt	
18						ni	nt	
19		3-OH	H			342	2337	7
20		4-OH	H			ni	nt	
21		4-OH	4-OH	13.5		ni	nt	
22		3-OH	4-OH	11.8< d< 12.8		69	1953	28
23		3-OH	3-OH	9.8< d< 11.8		173	745	4

Table 1. Continued

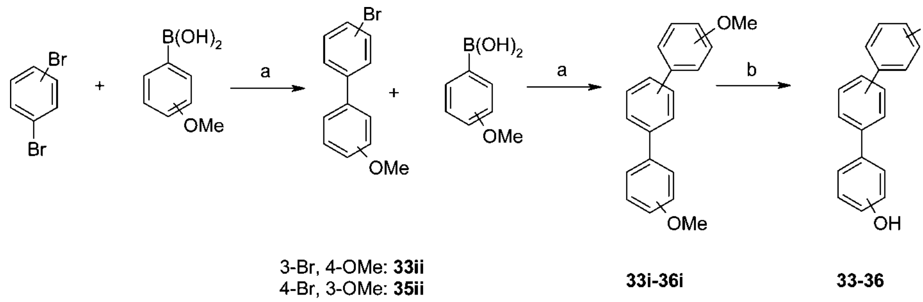
compound	Het	R$_1$	R$_2$	d(Å)[a]	α[b]	IC$_{50}$ (nM)[c]		selectivity factor[f]
						17β-HSD1[d]	17β-HSD2[e]	
24		3-OH	H		136	>5000	nt	
25		4-OH	3-OH	10.4< d< 12.0		151	1690	11
26		4-OH	4-OH	12.3		ni	nt	
27		3-OH	4-OH	10.4< d< 12.0		77	1271	16
28		3-OH	3-OH	8.5< d< 11.4		185	559	3
29		4-OH	4-OH	13.8	162	ni	nt	
30		3-OH	3-OH	10.4< d< 11.3		ni	nt	
31		4-OH	4-OH	8.1	69	ni	nt	
32		3-OH	4-OH	6.2< d< 8.9		>1000	nt	
33		4-OH	3-OH	9.3< d< 12.1	120	>1000	nt	
34		4-OH	4-OH	12.3		>1000	nt	
35		3-OH	3-OH	11.4< d< 12.4	180	173	2259	13
36		3-OH	4-OH	13.1		471	4509	9
37		3-OH	3-OH	11.6< d< 12.3	180	101	3399	34
38		3-OH	3-OH	11.4< d< 12.2	180	1000	5502	5
39		3-OH	3-OH	11.3< d< 12.2	180	201	5102	25

[a] O−O distance between the hydroxy substituents, for E2 $d = 11.0$ Å. [b] Angle between the two phenyl moieties in deg. [c] Mean values of three determinations, standard deviation less than 12% except **3**: 18% for 17β-HSD1. [d] Human placental, cytosolic fraction, substrate [^{3}H]-E1 + E1 [500 nM], cofactor NADH [500 μM]. [e] Human placental, microsomal fraction, substrate [^{3}H]-E2 + E2 [500 nM], cofactor NAD$^+$ [1500 μM]. [f] IC$_{50}$ (17β-HSD2)/IC$_{50}$ (17β-HSD1); ni: no inhibition; nt: not tested.

hydroxy substituent from the *para* into the *meta* position leads to highly active compounds except for thiazoles **1**, **7** (IC$_{50}$ values $>$ 1000 nM) and **5** (IC$_{50}$$>$ 5000 nM), which have a weak inhibitory activity and triazoles **12** and **14**, which are inactive. Moving the second OH substituent also in the *meta* position (*meta*−*meta* derivatives) results in potent compounds except for triazole **16**, which is a weak inhibitor (IC$_{50}$ $>$ 5000 nM), and selenophene **30** and triazole **15**, which are inactive.

The exchange of the *para*-OH group of the highly active thiophene **22** (IC$_{50}$ = 69 nM) with hydrogen reduces activity (compound **19**, IC$_{50}$ = 342 nM). More dramatically, the replacement of the *meta*-OH function of compound **22** with

Scheme 4. Synthesis of Compounds **33**−**36**[a]



[a] Reagents and conditions: (a) method A: Na_2CO_3 10% in water, toluene, $Pd(PPh_3)_4$, reflux, 4 h for **33ii** and **35ii** and 20 h for **33i**–**36i**; (b) method E: BBr_3, CH_2Cl_2, −78 °C to rt, 18 h.

hydrogen results in the inactive compound **20**. Similarly, the exchange of the *meta*-hydroxyphenyl moiety as well as the two hydroxy groups of **22** with hydrogens leads to the inactive compounds **17** and **18**. This exemplifies the importance of the existence of two OH substituents and their positions at the phenyl moieties with at least one being in the *meta* position.

The synthesized thiazoles **1**−**8** show lower activities than the thiophene analogues **17**−**28** except compound **3** (IC_{50} = 50 nM), which exhibits similar inhibition to **22** (IC_{50} = 69 nM) and **27** (IC_{50} = 77 nM). The introduction of a second nitrogen (compounds **9**−**11**) in the heterocyclic scaffold of these potent inhibitors does not strongly reduce activity (**9**, IC_{50} = 336 nM; **11**, IC_{50} = 169 nM). For *meta*−*meta* disubstituted compounds, the introduction of nitrogen in compound **4** decreases activity slightly (**9**, IC_{50} = 336 vs 243 nM), whereas introduction in compound **8** increases activity (**11**, IC_{50} = 169 vs 455 nM), as it is observed for the *meta*−*para* disubstituted compound **7** (**10**, IC_{50} = 413 vs > 1000 nM).

Moving the hydroxyphenyl moiety of the highly active thiophene **22** (IC_{50} = 69 nM) from position 5 to the 3 position decreases activity dramatically (**32**, IC_{50} > 1000 nM). Exchange of the sulfur atom of the potent thiophene **23** (IC_{50} = 173 nM) with selenium results in the inactive compound **30**.

In the case of the six-membered rings, benzene and some of its aza analogues were investigated. The 1,3-bis(hydroxyphenyl) substituted phenylenes were not active, whereas the 1,4-substituted compounds showed some activity, the most active benzene compound being the *meta*−*meta* derivative **35** (IC_{50} = 173 nM). The introduction of one or more nitrogens into the central benzene ring of **35** (compounds **37**−**39**) results in an increase (pyridine **37**, IC_{50} = 101 nM), no change (tetrazine **39**, IC_{50} = 201 nM), and a decrease of inhibitory activity (pyrazine **38**, IC_{50} = 1000 nM).

Furthermore, in Table 1, the angles between the two phenyl moieties are presented as a structural parameter. No correlation to the activities of the corresponding compounds can be observed.

Selectivity: Inhibition of Human 17*β*-HSD2 and Affinities for ERα and ER*β*. Because 17*β*-HSD2 catalyzes the inactivation of E2 to E1, inhibitory activity toward this enzyme must be avoided. The 17*β*-HSD2 inhibition was determined using an assay similar to the 17*β*-HSD1 test. Placental microsomes were incubated with tritiated E2 in the presence of NAD^+ and inhibitor. Separation and quantification of labeled product (E1) was performed by HPLC using radio detection. A selection of the most potent 17*β*-HSD1 inhibitors was tested for inhibition of 17*β*-HSD2. IC_{50} values and selectivity factors (IC_{50} 17*β*-HSD2/IC_{50} 17*β*-HSD1) are presented in Table 1.

It is striking that most *meta*−*meta* bis(hydroxyphenyl) substituted inhibitors present only poor selectivity except for compounds **37** and **39**, which show high selectivity factors of 34 and 35, respectively. The *meta*−*para*-disubstituted inhibitors mostly show higher selectivity, with compound **3** exhibiting the highest selectivity factor of 80.

Table 2. Binding Affinities for the Human Estrogen Receptors α and *β* of Selected Compounds

	RBA[a](%)	
compound	ERα[b]	ER*β*[b]
3	<0.01	0.01< RBA< 0.1
22	0.1< RBA< 1	1.5
25	0.01< RBA< 0.1	0.1 < RBA < 1
27	0.01< RBA< 0.1	0.1 < RBA < 1
35	<0.001	0.01< RBA < 0.1
37	0.01 < RBA< 0.1	<0.01

[a] RBA (relative binding affinity), E2: 100%, mean values of three determinations, standard deviations less than 10%; [b] Human recombinant protein, incubation with 10 nM [^{3}H]-E2 and inhibitor for 1 h.

A further prerequisite for 17*β*-HSD1 inhibitors to be used as potential drugs is that these compounds do not show affinity for ERα and ER*β* or only a marginal one because binding to these receptors could counteract the therapeutic efficacy. The binding affinities of selected compounds were determined using recombinant human protein in a competition assay applying [^{3}H]-E2 and hydroxyapatite (Table 2). All tested compounds show very marginal or marginal affinity to the ERs.

Further Biological Evaluations. The intrinsic estrogenic activity of a representative compound of each class was determined using the ER-positive mammary tumor T-47D cell line. No agonistic, i.e., no stimulatory effect, was observed after application of the inhibitors even at a concentration 1000-fold higher than E2 (data not shown).

A selection of active and selective compounds was investigated for permeation of CaCo-2 cells. These cells exhibit morphological and physiological properties of the human small intestine[42] and are a generally accepted model for the prediction of peroral absorption. Depending on the P_{app} data obtained, compounds can be classified as low (P_{app} (× 10^{-6} cm/s) < 1), medium (1 < P_{app} < 10), or highly permeable (P_{app}> 10)). Thiazole **3** shows medium cell permeation, while thiophenes **22**, **25** and phenylene **35** exhibit high cell permeability (Table 3).

A representative compound of the thiazole, thiophene, and phenylene class (compounds **4**, **25**, and **35**) was evaluated for their phase 1 metabolic stability using 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 (Table 4). All tested compounds show longer half-lives than the antihistaminic drug diphenhydramine (values in the range between 12.6 and 22.7 min vs 6.8 min).

Table 3. CaCo-2 Cell Permeation of Highly Active 17β-HSD1 Inhibitors

compound	P_{app} ($\times 10^{-6}$cm/s)[a,b]	classification
3	7.8	medium
22	22.0	high
25	14.4	high
35	12.5	high
atenolol	0.1	low
testosterone	9.4	medium
ketoprofene	25.7	high

[a] Permeability of reference compounds similar to the values described (atenolol,[71] testosterone,[67] ketoprofene[72]). [b] P_{app}: apparent permeability coefficient, mean values of three determinations, standard deviations less than 10%.

Table 4. Half-Lives and Intrinsic Clearances of Compounds **4**, **25**, and **35** in Rat Liver Microsomes[a]

compound	half-life (min)	CL_{int}[b] (μL /min/mg protein)
4	12.6	367.2
25	18.6	248.5
35	22.7	203.4
diazepam[c]	40.8	113.3
diphenhydramine[c]	6.8	679.6

[a] 0.33 mg/mL protein, NADP$^+$-regenerating system, [inhibitor]: 1 μM, incubation at 37 °C, samples taken at 0, 15, 30, and 60 min, determination of parent compound by MS. [b] CL_{int}: intrinsic body clearance. [c] Values of reference compounds similar to described values.

Table 5. Pharmacokinetic Parameters of Compound **3** and **39** in Male Rats after Oral Application of 10 mg/kg

	compound	
parameters	**3**	**39**
$C_{max\ obs}$ (ng/mL)[a]	7.8	106.0
C_z (ng/mL)[b]	6.6	54.0
$t_{max\ obs}$ (h)[c]	8.0	3.0
t_z (h)[d]	10.0	10.0
$t_{1/2z}$ (h)[e]	1.5	1.2
$AUC_{0\text{-}tz}$ (ng/mL)[f]	99.2	1204.0

[a] $C_{max\ obs}$: maximal measured concentration. [b] C_z: last analytical quantifiable concentration. [c] $t_{max\ obs}$: time to reach the maximum measured concentration. [d] t_z: time of the last sample which has an analytically quantifiable concentration. [e] $t_{1/2z}$: half-life of the terminal slope of a concentration time curve. [f] $AUC_{0\text{-}tz}$: area under the concentration time curve up to the time tz of the last sample.

The same compounds (**4**, **25**, and **35**) were further investigated for inhibition of the six most important human hepatic enzymes: CYP1A2, 2B6, 2C9, 2C19, 2D6, and 3A4. All compounds show very low inhibition of CYP1A2, 2B6, 2C19, and 2D6 ($IC_{50} > 4$ μM). In the case of CYP2C9 and CYP3A4 (**4**: 2.1 and 0.8; **25**: 0.8 and 1.9; **35**: 1.9 and 2.1 μM, respectively), inhibition was higher but still clearly below the IC_{50} values of 17β-HSD1 inhibition. These results indicate a low risk of drug−drug interaction caused by CYP inhibition.

The pharmacokinetic profiles of the most active and selective azole compound **3** and one of the six-membered ring compounds (**39**) were determined in rats after oral administration in a cassette. The most potent six-membered ring compound **37** was not chosen, as it was unstable in buffer over 24 h. Each group consisted of four male rats, and the compounds were administered in doses of 10 mg/kg. Plasma samples were collected over 24 h, and plasma concentrations were determined by HPLC-MS/MS. The pharmacokinetic parameters are presented in Table 5. Maximal plasma concentration (C_{max}) and AUC value are much higher for compound **39**compared to compound **3**.

Computational Chemistry

To obtain an insight into which physicochemical parameter might influence biological activity, the charge density distribution was considered and the molecular electrostatic potentials (MEPs) of selected compounds were determined. The geometry of the compounds had been fully optimized in the gas phase at the B3LYP/6-311++G (d,p) level of density functional theory (DFT). MEPs were plotted for every compound on its electron density with GaussView 3.09. The electrostatic potential distribution of the charge density is presented by a color code ranging from -3.1×10^{-2} to 4.5×10^{-2} Hartree (Figures 1 and 2 and Supporting Information). For better comparison, the MEPs of different compounds were divided into three regions corresponding to each aromatic system.

In Figure 1, the MEPS of five-membered heterocyclic compounds are arranged based on their increasing inhibitory potency. While the positions of the hydroxyphenyl moieties are fixed, the nature of the heterocycle is varied. It becomes apparent that the heterocycle influences the ESP distribution of the whole molecule. To rationalize these MEPs, the ESP distribution ranges (C^1), the mean values of the distribution ranges (C^2), and the Δ of ESP (C^3) were analyzed; negative ESP values (red/orange/yellow) in region I and II and less negative to almost neutral ESP values (green/yellow) in region III are obviously an important factor for high inhibitory potency. Trying to establish a semiquantitative MEP−activity relationship ("semi-QMAR") optimal ESP ranges for areas I, II, and III for potent inhibition were identified (hydrogens and the OH groups were not considered): for region I ESP from -1.7 to -1.2×10^{-2}, for region II -1.6 to -0.9×10^{-2}, and for region III -1.2 to -0.5×10^{-2} Hartree. Similarly, the optimal Δ values of ESP for each region were determined: 0.5, 0.7, and 0.7 Hartree, respectively. Both the shift of a certain ESP distribution range on the scale and the change of the Δ value result in a decrease of inhibitory activity. The combination of these two criteria is substantiated with +, −, and 0 in Figure 1D, indicating favorable, unfavorable, and neutral impact on activity, respectively.

The 2,5-disubstituted imidazloe[33] exhibits a polarization between top and bottom sides of the molecule: the vertex side (NH) has positive ESP values, while on the opposite side (N−C), negative to neutral values are predominant. This polarization ($\Delta = 7.2 \times 10^{-2}$ Hartree) results in an inactive compound, indicating that the ESP of the central heterocycle is crucial for 17β-HSD1 inhibitory activity.

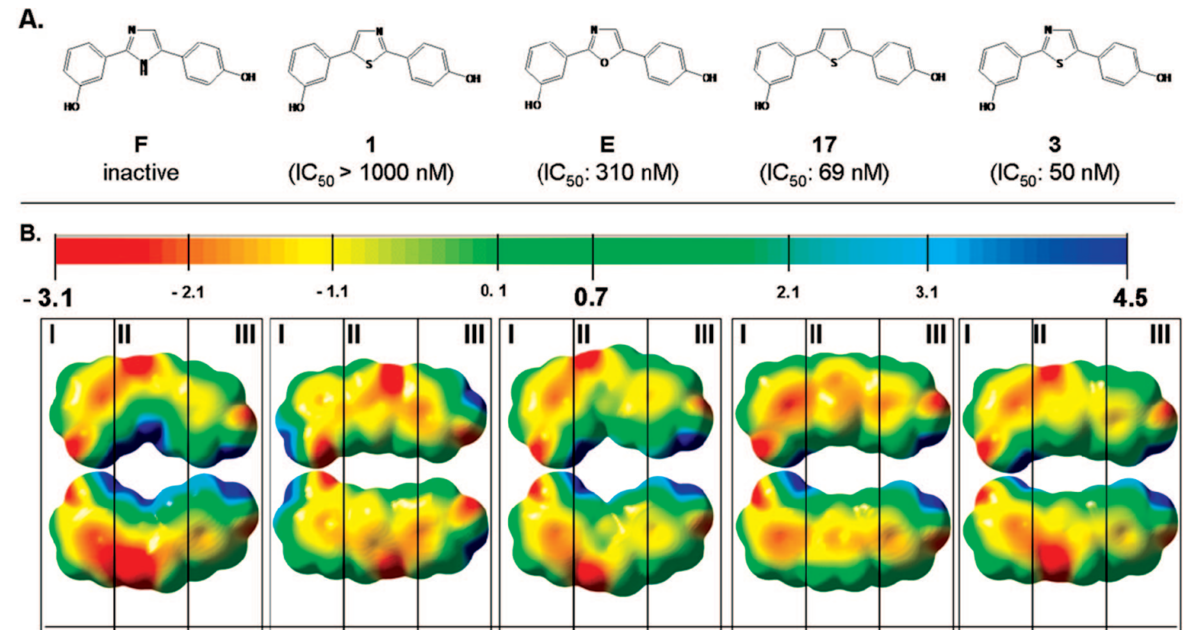
A good example for the change in activity is well demonstrated by the ESP distribution in region III for thiazoles **1** and **3** (Figure 1C^1, C^2). The dramatic loss of activity of compound **1** ($IC_{50} > 1000$ nM) in comparison to **3** ($IC_{50} = 50$ nM) is due to a nonoptimal electron density distribution.

The MEP maps of the six-membered ring compounds are depicted in Figure 2. A similar charge density distribution was observed except for the only planar compound of this series, tetrazine **33**, which showed no polarization between top and bottom side of the molecule. It remains to be clarified whether this is the reason for the reasonable activity of compound **33**.

Discussion and Conclusion

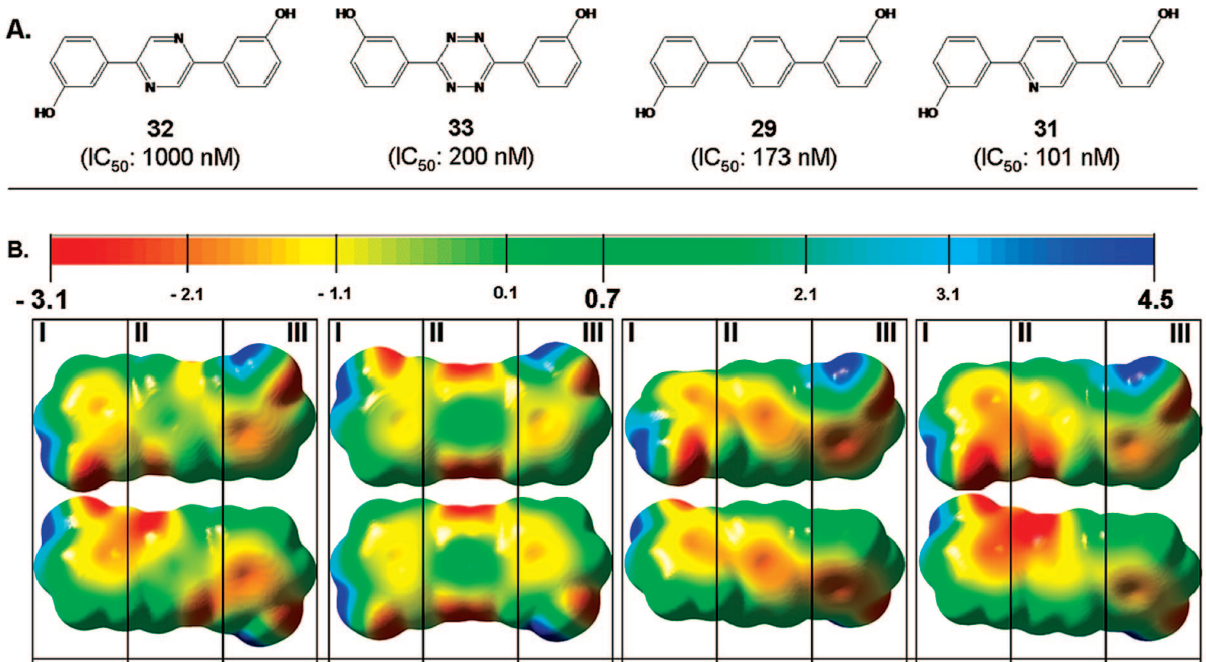
The present paper shows that the 2,5-disubstituted oxazole from a previous study[33] could be optimized. The most active and selective thiazole **3** shows an IC_{50} value of 50 nM and a selectivity factor of 80 (compound **E**, IC_{50}= 310 nM, selectivity factor: 56).

The biological results obtained confirm similar findings described in our previous article:[33] the OH substitution pattern of our compounds is decisive for inhibitory activity. Comparison of the monohydroxylated thiophenes **19** and **20** (*meta-* and *para-*, respectively) shows that the *meta*-hydroxy group is crucial



	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
C1	-2,2 – -1,2	-3,0 – 4.2	-1,3 – 0,1	-1,3 – -1,0	-2,5 – -1,0	-1,7 – -0,9	-1,7 – -1,1	-2,5 – 0,1	-1,1 – -0,1	-1,8 – -1,2	-1,7 – -0,5	-1,5 – -0,8	-1,7 – -1,1	-2,5 – -0,5	-1,2 – -0,5
C2	-1,7	0,6	-0,6	-1,2	-1,75	-1,3	-1,4	-1,2	-0,6	-1,5	-1,1	-1,2	-1,4	-1,5	-0,85
C3	1	7,2	1,4	0,3	1,5	0,8	0,6	2,6	1	0,6	1,2	0,7	0,6	2	0,7
D.	-	- -	0	0	-	-	+	0	0	+	+	0	+	0	+

Figure 1. (A) Structure and 17*β*-HSD1 inhibitory activity of **F**,[33] **E**,[33] and compounds **1**, **3**, and **17** ordered by increasing activity. (B) MEP maps, dorsal and ventral view. (C^1) ESP distribution range (Hartree; × 10^{-2}). (C^2) mean value of distribution range (Hartree; × 10^{-2}). (C^3) Δ of ESP. (D) "semi-QMAR". MEP surfaces were plotted with GaussView 3.09.



	I	II	III	I	II	III	I	II	III	I	II	III
C1	-1,5 – -1,0	-2,5 – 0,1	-1,5 – -1,0	-1,2 – -0,6	-2,5 – 2,1	-1,2 – -0,6	-1,4 – -1,0	-1,5 – -1,0	-1,4 – -1,0	-2,0 – -1,1	-2,5 – -0,6	-1,3 – -0,5
C2	-1,25	-1,3	-1,25	-0,9	-0,2	-0,9	-1,2	-1,25	-1,2	-1,6	-1,6	-0,9
C3	0,5	2,6	0,5	0,3	4,6	0,6	0,4	0,5	0,4	0,6	1,9	0,8
D.	0	-	0	-	0	+	0	+	0	+	0	+

Figure 2. (A) Structure and 17*β*-HSD1 inhibitory activity of compounds **29** and **31**−**33** ordered by increasing activity. (B) MEP maps, dorsal and ventral view. (C^1) ESP distribution range (Hartree; × 10^{-2}). (C^2) Mean value of distribution range (Hartree; × 10^{-2}). (C^3) Δ of ESP. (D) "semi-QMAR". MEP surfaces were plotted with GaussView 3.09.

for activity. The inactivity of compound **18** indicates that the phenyl moiety of the *meta*-hydroxyphenyl thiophene **19** is also important for potency. The replacement of the *meta*-hydroxy group of **19** with hydrogen, leading to the inactive compound **17**, demonstrates the importance of the hydrogen bond interaction for activity.

As observed for the previously described bis(hydroxyphenyl) derivatives,[33] the distance between the two oxygens obviously has to be close to the value observed for the substrate ($d = 11$ Å). The *para*−*para* disubstituted compounds show distances longer than 12.5 Å. They are all inactive. Concerning the *meta*−*para* and *meta*−*meta* disubstituted compounds, which have O−O distances between 8.5 and 12.8 Å, medium to high inhibitory activities are observed for most compounds. They could be able to establish hydrogen bond interactions with His221/Glu282 and Ser142/Tyr155. However, the inactivity of thiazoles **1**, **5**, and **7** and triazoles **12**, **14**−**16**, which are all *meta*−*para* and *meta*−*meta* disubstituted, respectively, indicates that this distance is not the only decisive criterion for activity. The heterocycle also influences the inhibitory potencies of the compounds.

In the five-membered ring series, the potency of 1,2,3-triazoles described previously[33] led us to further investigate this class of compounds. The inactivity of compounds **12**−**15** shows that either the nitrogen distribution in the ring or the methyl group are not tolerated by the enzyme. The replacement of the N-Me moiety of **14** with sulfur (compound **10**), leading to a fairly potent compound, indicates that S in this position has a positive influence. To further investigate the role of the nitrogen in the ring, thiazoles **1**−**8** and thiophenes **21**−**28**, **31**, and **32** were investigated. The fact that thiazole **3** and thiophene **22** exhibit almost identical potencies shows that the nitrogen does not contribute to binding, i.e., that there is obviously no hydrogen bond interaction.

In this report, we could also show that six-membered rings are appropriate for the design of highly active 17β-HSD1 inhibitors. Comparison of the almost equipotent phenylene **35**, pyridine **37**, and tetrazine **39** confirms the hypothesis that nitrogens are tolerated in the ring but do not contribute to a specific interaction.

The role of the angle between the two hydroxyphenyl moieties was also investigated in order to find out whether there is a correlation between this parameter and the inhibitory potency. The broad range of angles calculated (128° for **11** to 180° for **37**, two highly active compounds) could not be correlated with high or low inhibitory potency, indicating that the enzyme presents some flexibility for ligand binding. Interestingly, for smaller angles between the two hydroxyphenyl groups, as in case of compounds **10** and **11** (N atom at the vertex), the *meta*−*meta* substitution results in a higher activity, while for larger angles, in presence of a sulfur atom at the vertex of the five-membered heterocycle (2,5-disubstituted thiazoles and 2,5-disubstituted thiophenes), the optimal substitution pattern is *meta*−*para*/*para*−*meta*. This phenomenon will be further investigated.

Our finding that there exists a correlation between the MEPs and the biological activities of the compounds (semi-QMAR) might be exploited for further structure optimization, underlining the relevance of this descriptor for biological activity. Furthermore, the MEPs could be used to investigate how these inhibitors approach and bind to the enzyme as it is described for genistein and the estrogen receptor.[43]

Interestingly, the exchange of a CH in the thiophene **22** by a N leading to the thiazole **3** increases selectivity toward 17β-HSD2 dramatically (IC_{50} 17β-HSD2/IC_{50} 17β-HSD1, 28 vs 80). In general, the most potent compounds exhibit a really low affinity for the ERα and ERβ and show no stimulation of cell proliferation (agonistic effect) in the ER-positive T-47D cell line. It is worth mentioning that **3**, in spite of its good CaCo-2 permeability, shows a really low bioavailability compared to **39**. Glucuronidation and/or sulfatation might be responsible for the low plasma levels of the parent compound.

In the present report, we described the synthesis of bis(hydroxyphenyl) azoles, thiophenes, benzenes, and aza-benzenes and the evaluation of their biological properties. The most promising compounds of this study, **3**, **22**, and **39**, show a high selectivity toward 17β-HSD2, a low binding affinity to the ERα, a high CaCo-2 permeability, and a reasonable pharmacokinetic profile after peroral application. These new compounds should be useful tools to further investigate in vivo 17β-HSD1 as a target for the treatment of estrogen-dependent diseases.

Experimental Section

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

Column chromatography (CC) was performed on silica gel (70−200 μm) coated with silica, preparative thin layer chromatography (TLC) on 1 mm SIL G-100 UV_{254} glass plates (Macherey-Nagel), and reaction progress was monitored by TLC on Alugram SIL G UV_{254} (Macherey-Nagel).

Melting points were measured on a Mettler FP1 melting point apparatus and are uncorrected.

IR spectra were recorded on a Bruker Vector 33 spectrometer (neat sample).

^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker AM500 spectrometer (500 MHz) at 300 K. Chemical shifts are reported in δ (parts per million: ppm), by reference to the hydrogenated residues of deuteriated solvent as internal standard ($CDCl_3$: $\delta = 7.24$ ppm (^{1}H NMR) and $\delta = 77$ ppm (^{13}C NMR); CD_3OD: $\delta = 3.35$ ppm (^{1}H NMR) and $\delta = 49.3$ ppm (^{13}C NMR); CD_3COCD_3: $\delta = 2.05$ ppm (^{1}H NMR) and $\delta = 29.9$ ppm (^{13}C NMR); CD_3SOCD_3: $\delta = 2.50$ ppm (^{1}H NMR) and $\delta = 39.5$ ppm (^{13}C NMR). Signals are described as s, d, t, dd, m, and dt for singlet, doublet, triplet, doublet of doublets, multiplet, and doublet of triplets, respectively. All coupling constants (*J*) are given in Hertz (Hz).

Mass spectra (ESI) were recorded on a TSQ Quantum (Thermofischer) instrument. Elemental analyses were performed at the Department of Instrumental Analysis and Bioanalysis, Saarland University.

5-Bromo-2-(4-methoxyphenyl)-1,3-thiazole (**1ii**),[44] 4-methoxythiobenzamide (**10ii**),[45] 3-bromo-2-(4-methoxyphenyl)thiophene (**31ii**),[46] 3-bromo-4′-methoxybiphenyl (**33ii**),[47] 4′-bromo-3-methoxybiphenyl (**35ii**),[48] 2-(4-methoxyphenyl)-5-(3-methoxyphenyl)-1,3-thiazole (**1i**),[49] 2,5-bis(4-methoxyphenyl)-1,3-thiazole (**2i**),[50] 2,4-bis(4-methoxyphenyl)-1,3-thiazole (**6i**),[35] 3-hydroxythiobenzamide (**11i**),[16] 2,5-bis(4-methoxyphenyl)thiophene (**21i**),[51] 2,4-bis(4-methoxyphenyl)thiophene (**26i**),[52] 2,3-bis(4-methoxyphenyl)thiophene (**31i**),[53] 3,4″-dimethoxy-1,1′:3′,1″-terphenyl (**33i**),[54] 4,4″-dimethoxy-1,1′:3′,1″-terphenyl (**34i**),[54] 4,4′-(1,3-thiazole-2,5-diyl)diphenol (**2**),[55] 4,4′-(1,3-thiazole-2,4-diyl)diphenol (**6**),[35] 3,3′-(1,2,4-thiadiazole-3,5-diyl)diphenol (**11**),[56] 3,3′-(1*H*-1,2,3-triazole-1,4-diyl)diphenol (**16**),[57] 2,5-diphenylthiophene (**17**),[58] 4,4′-thiene-2,5-diyldiphenol (**21**),[51] 4,4′-thiene-2,4-diyldiphenol (**26**),[55] 1,1′:3′,1″-terphenyl-3,4″-diol (**33**),[59] 1,1′:3′,1″-terphenyl-4,4″-diol (**34**),[60] 1,1′:4′,1″-terphenyl-3,3″-diol (**35**),[59] 1,1′:4′,1″-terphenyl-3,4″-diol (**36**),[59] 3,3′-pyrazine-2,5-diyldiphenol (**38**),[61] and 3,3′-(1,2,4,5-tetrazine-3,6-diyl)diphenol (**39**)[40] were prepared following described procedures.

General Procedure for Suzuki Coupling. Method A. A mixture of arylbromide (1 equiv), methoxybenzene boronic acid (1 equiv), sodium carbonate (2 equiv), and tetrakis(triphenylphosphine) palladium (0.005 equiv) in an oxygen free toluene/water (1:1) solution was stirred at 100 °C for 4 h under nitrogen. The reaction mixture was cooled to rt. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, filtered, and concentrated to dryness. The product was purified by CC.

Method B. A mixture of arylbromide (1 equiv), methoxybenzene boronic acid (1.2 equiv), sodium carbonate (2 equiv), and tetrakis(triphenylphosphine) palladium (0.005 equiv) in an oxygen free tetrahydrofurane/water (1:1) solution was stirred at 100 °C for 20 h under nitrogen. The reaction mixture was cooled to rt. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, filtered, and concentrated to dryness. The product was purified by CC.

Method C. A mixture of aryl dibromide (1 equiv), methoxybenzene boronic acid (2.4 equiv), cesium carbonate (4 equiv), and tetrakis(triphenylphosphine) palladium (0.001 equiv) was suspended in an oxygen-free DME/EtOH/water (1:1:1) solution. The reaction mixture was exposed to microwave irradiation (15 min, 150 W, 150 °C, 15 bar). After reaching rt, water was added and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, filtered, and concentrated to dryness. The product was purified by preparative TLC.

General Procedure for Synthesis of 1,2,4-Triazoles. Method D. A solution of acyl chloride (1 equiv) in dichloromethane was added dropwise to a mixture of ethyl imino ester (1 equiv) and dry triethylamine (1 equiv) in 20 mL dichloromethane and heated to 30−40 °C for 6 h. After cooling to rt, the mixture was poured into 3% $NaHCO_3$ solution (25 mL). The layers were separated and the organic layer was washed with water, dried over sodium sulfate, and evaporated to dryness under reduced pressure. The resulting *N*-acylimino esters **12ii**−**15ii** were heated to 30−40 °C with methyl hydrazine (2 equiv) in CH_2Cl_2 for 4 h. The solvent was removed under reduced pressure and the 1,2,4-triazoles **12i**−**15i** were crystallized from CH_2Cl_2/ Et_2O.

General Procedure for Ether Cleavage. Method E. To a solution of methoxybenzene derivative (1 equiv) in dry dichloromethane at −78 °C (dry ice/acetone bath), boron tribromide in dichloromethane (1 M, 3 equiv per methoxy function) was added dropwise. The reaction mixture was stirred for 20 h at rt under nitrogen. Water was added to quench the reaction, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, evaporated to dryness under reduced pressure, and purified by preparative TLC.

Method F. To a solution of bis(methoxyphenyl) derivative (1 equiv) in dry dichloromethane, borontrifluoride dimethyl sulfide complex (75 equiv) was added dropwise at rt. The reaction mixture was stirred for 20 h. Water was added to quench the reaction, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, evaporated to dryness under reduced pressure, and purified by preparative TLC.

3-[2-(4-Hydroxyphenyl)-1,3-thiazol-5-yl]phenol (1). The title compound was prepared by reaction of 5-(3-methoxyphenyl)-2-(4-methoxyphenyl)-1,3-thiazole (**1i**) (40 mg, 0.13 mmol) and boron tribromide (0.81 mmol) according to method E. The product was purified by preparative TLC (hexane/ethyl acetate 5:5); yield: 77% (28 mg); MS (ESI): 270 $(M + H)^+$; Anal. ($C_{15}H_{11}NO_2S$) C, H, N.

5-Bromo-2-(3-methoxyphenyl)-1,3-thiazole (3ii). The title compound was prepared by reaction of 2,5-dibromo-1,3-thiazole (500 mg, 2.06 mmol), 3-methoxybenzeneboronic acid (376 mg, 2.47 mmol), sodium carbonate (437 mg, 4.12 mmol), and tetrakis(triphenylphosphine) palladium (11 mg, 10 μmol) according to method A. The product was purified by CC (dichloromethane/methanol 95:5); yield: 50% (278 mg).

2-(3-Methoxyphenyl)-5-(4-methoxyphenyl)-1,3-thiazole (3i). The title compound was prepared by reaction of 5-bromo-2-(3-methoxyphenyl)-1,3-thiazole (**3ii**) (250 mg, 0.93 mmol), 4-methoxybenzeneboronic acid (170 mg, 1.11 mmol), sodium carbonate (197 mg, 1.86 mmol), and tetrakis(triphenylphosphine) palladium (5.4 mg, 4.6 μmol) according to method A. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 58% (160 mg).

3-[5-(4-Hydroxyphenyl)-1,3-thiazol-2-yl]phenol (3). The title compound was prepared by reaction of 2-(3-methoxyphenyl)-5-(4-methoxyphenyl)-1,3-thiazole (**3i**) (40 mg, 0.13 mmol) and boron tribromide (0.81 mmol) according to method E. The product was purified by preparative TLC (hexane/ethyl acetate 5:5); yield: 80% (50 mg); MS (ESI): 270 $(M + H)^+$; Anal. ($C_{15}H_{11}NO_2S$) C, H, N.

2,5-Bis(3-methoxyphenyl)-1,3-thiazole (4i). The title compound was prepared by reaction of 5-bromo-2-(3-methoxyphenyl)-1,3-thiazole (**3ii**) (250 mg, 0.93 mmol), 3-methoxybenzeneboronic acid (170 mg, 1.11 mmol), sodium carbonate (197 mg, 1.86 mmol), and tetrakis(triphenylphosphine) palladium (5.4 mg, 4.6 μmol) according to method A. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 40% (111 mg).

3,3′-(1,3-Thiazole-2,5-diyl)diphenol (4). The title compound was prepared by reaction of 2,5-bis(3-methoxyphenyl)-1,3-thiazole (**4i**) (100 mg, 0.36 mmol) and boron tribromide (2.02 mmol) according to method E. The product was purified by preparative TLC (hexane/ethyl acetate 5:5); yield: 85% (82 mg); MS (ESI): 270 $(M + H)^+$; Anal. ($C_{15}H_{11}NO_2S$) C, H, N.

4-Bromo-2-(4-methoxyphenyl)-1,3-thiazole (5ii). The title compound was prepared by reaction of 2,4-dibromo-1,3-thiazole (500 mg, 2.06 mmol), 4-methoxybenzeneboronic acid (376 mg, 2.47 mmol), sodium carbonate (437 mg, 4.12 mmol), and tetrakis(triphenylphosphine) palladium (11 mg, 10 μmol) according to method A. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 55% (305 mg).

2-(4-Methoxyphenyl)-4-(3-methoxyphenyl)-1,3-thiazole (5i). The title compound was prepared by reaction of 4-bromo-2-(4-methoxyphenyl)-1,3-thiazole (**5ii**) (250 mg, 0.93 mmol), 3-methoxybenzeneboronic acid (170 mg, 1.11 mmol), sodium carbonate (197 mg, 1.86 mmol), and tetrakis(triphenylphosphine) palladium (5.4 mg, 4.6 μmol) according to method A. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 52% (143 mg).

3-[2-(4-Hydroxyphenyl)-1,3-thiazol-4-yl]phenol (5). The title compound was prepared by reaction of 2-(4-methoxyphenyl)-4-(3-methoxyphenyl)-1,3-thiazole (**5i**) (70 mg, 0.24 mmol) and boron tribromide (1.44 mmol) according to method E. The product was purified by preparative TLC (hexane/ethyl acetate 5:5); yield: 78% (50 mg); MS (ESI): 268 $(M - H)^-$; Anal. ($C_{15}H_{11}NO_2S$) C, H, N.

4-Bromo-2-(3-methoxyphenyl)-1,3-thiazole (7ii). The title compound was prepared by reaction of 2,4-dibromo-1,3-thiazole (500 mg, 2.06 mmol), 3-methoxybenzeneboronic acid (376 mg, 2.47 mmol), sodium carbonate (437 mg, 4.12 mmol), and tetrakis(triphenylphosphine) palladium (11 mg, 10 μmol) according to method A. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 50% (270 mg).

2-(3-Methoxyphenyl)-4-(4-methoxyphenyl)-1,3-thiazole (7i). The title compound was prepared by reaction of 4-bromo-2-(3-methoxyphenyl)-1,3-thiazole (**7ii**) (250 mg, 0.93 mmol), 4-methoxybenzeneboronic acid (170 mg, 1.11 mmol), sodium carbonate (197 mg, 1.86 mmol), and tetrakis(triphenylphosphine) palladium (5.4 mg, 4.6 μmol) according to method A. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 79% (218 mg).

3-[4-(4-Hydroxyphenyl)-1,3-thiazol-2-yl]phenol (7). The title compound was prepared by reaction of 2-(3-methoxyphenyl)-4-(4-methoxyphenyl)-1,3-thiazole (**7i**) (70 mg, 0.24 mmol) and boron tribromide (1.44 mmol) according to method E. The product was purified by preparative TLC (hexane/ethyl acetate 5:5); yield: 80% (52 mg); MS (ESI): 268 $(M - H)^-$; Anal. ($C_{15}H_{11}NO_2S$) C, H, N.

2,4-Bis(3-methoxyphenyl)-1,3-thiazole (8i). The title compound was prepared by reaction of 4-bromo-2-(3-methoxyphenyl)-1,3-thiazole (**7ii**) (250 mg, 0.93 mmol), 3-methoxybenzeneboronic acid (170 mg, 1.11 mmol), sodium carbonate (197 mg, 1.86 mmol), and tetrakis(triphenylphosphine) palladium (5.4 mg, 4.6 μmol) according to method A. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 18% (50 mg).

3,3′-(1,3-Thiazol-2,4-diyl)diphenol (8). The title compound was prepared by reaction of 2,4-bis(3-methoxyphenyl)-1,3-thiazole (**8i**) (70 mg, 0.24 mmol) and boron tribromide (1.44 mmol) according to method E. The product was purified by preparative TLC (hexane/ethyl acetate 5:5); yield: 78% (50 mg); MS (ESI): 268 (M − H)$^-$; Anal. ($C_{15}H_{11}NO_2S$) C, H, N.

3,3′-(1,2,4-Thiadiazol-2,5-diyl)diphenol (9). A solution of 3-hydroxythiobenzamide (100 mg, 0.17 mmol, 2 equiv.) in DMSO (10 mL) was stirred for 5 h at rt with 3 mL of concentrated chlorhydric acid. The crude mixture was poured into water and the resulting precipitate was filtered, washed with water, and dried overnight in a desiccator; yield: 92% (41 mg); MS (ESI): 269 (M − H)$^-$. Anal. ($C_{14}H_{10}N_2O_2S$) C, H, N.

3-[3-(4-Methoxyphenyl)-1,2,4-thiadiazol-5-yl]phenol (10i). A solution of 4-methoxythiobenzamide (**10ii**) (318 mg, 1.90 mmol, 1 equiv.) in DMSO was heated for 8 h at 38 °C with 3-hydroxythiobenzamide (**11i**) (290 mg, 1.90 mmol, 1 equiv.) and concentrated hydrochloric acid (193 μL, 1.90 mmol, 1 equiv.). After cooling to rt, the crude mixture was poured into water and the resulting precipitate was collected by filtration. The mixture of **10i** and **11** was separated by CC (hexane/ethyl acetate 8:2); yield: 30% (70 mg) for **10i** and 42% (215 mg) for **11**.

3-[3-(4-Hydroxyphenyl)-1,2,4-thiadiazol-5-yl]phenol (10). The title compound was prepared by reaction of 3-[3-(4-methoxyphenyl)-[1,2,4]-thiadiazol-5-yl]-phenol (**10i**) (150 mg, 0.53 mmol) and boron tribromide (1.59 mmol) according to method E. The product was purified by preparative TLC (hexane/ethyl acetate 5:5); yield: 91% (130 mg); MS (ESI): 271 (M + H)$^+$; Anal. ($C_{14}H_{10}N_2O_2S$) C, H, N.

3-(3-Methoxyphenyl)-5-(4-methoxyphenyl)-1-methyl-1*H*-1,2,4-triazole (12i). The title compound was prepared from *para*-anisoyl chloride (170 mg, 1.0 mmol), ethyl 3-methoxybenzimidate (179 mg, 1.0 mmol), and methyl hydrazine (92 mg, 2.0 mmol) according to method D; yield: 85% (250 mg); mp 108−110 °C (CH_2Cl_2/ Et_2O); MS (ESI): 296 (M + H)$^+$.

3-[5-(4-Hydroxyphenyl)-1-methyl-1*H*-1,2,4-triazol-3-yl]phenol (12). The title compound was prepared by reaction of 3-(3-methoxyphenyl)-5-(4-methoxyphenyl)-1-methyl-1*H*-1,2,4-triazole (**12i**) (100 mg, 0.37 mmol) and borontrifluoride dimethyl sulfide complex (27.75 mmol) according to method F. The product was purified by preparative TLC (ethyl acetate); yield: 46% (42 mg); MS (ESI): 268 (M + H)$^+$.

3,5-Bis(4-methoxyphenyl)-1-methyl-1*H*-1,2,4-triazole (13i). The title compound was prepared from *para*-anisoyl chloride (170 mg, 1.0 mmol), ethyl 4-methoxybenzimidate (179 mg, 1.0 mmol), and methyl hydrazine (92 mg, 2.0 mmol) according to method D; yield: 78% (230 mg); mp 141−143 °C (CH_2Cl_2/ Et_2O); MS (ESI): 296 (M + H)$^+$.

4-[5-(4-Hydroxyphenyl)-1-methyl-1*H*-1,2,4-triazol-3-yl]phenol (13). The title compound was prepared by reaction of 3,5-bis(4-methoxyphenyl)-1-methyl-1*H*-1,2,4-triazole (**13i**) (100 mg, 0.37 mmol) and borontrifluoride dimethyl sulfide complex (27.7 mmol) according to method F. The product was purified by preparative TLC (ethyl acetate); yield: 63% (57 mg); MS (ESI): 268 (M + H)$^+$.

3-(4-Methoxyphenyl)-5-(3-methoxyphenyl)-1-methyl-1*H*-1,2,4-triazole (14i). The title compound was prepared from *meta*-anisoyl chloride (170 mg, 1.0 mmol), ethyl 4-methoxybenzimidate (179 mg, 1.0 mmol), and methyl hydrazine (92 mg, 2.0 mmol) according to method D; yield: 77% (227 mg); mp 114−116 °C (CH_2Cl_2/ Et_2O); MS (ESI): 296 (M + H)$^+$.

4-[5-(3-Hydroxyphenyl)-1-methyl-1*H*-1,2,4-triazol-3-yl]phenol (14). The title compound was prepared by reaction of 3-(4-methoxyphenyl)-5-(3-methoxyphenyl)-1-methyl-1*H*-1,2,4-triazole (**14i**) (100 mg, 0.37 mmol) and borontrifluoride dimethyl sulfide complex (27.7 mmol) according to method F. The product was purified by preparative TLC (ethyl acetate); yield: 53% (48 mg); MS (ESI): 268 (M + H)$^+$.

3,5-Bis(3-methoxyphenyl)-1-methyl-1*H*-1,2,4-triazole (15i). The title compound was prepared from *meta*-anisoyl chloride (170 mg, 1.0 mmol), ethyl 3-methoxybenzimidate (179 mg, 1.0 mmol), and methyl hydrazine (92 mg, 2.0 mmol) according to method D; yield: 67% (198 mg); mp 67−69 °C (CH_2Cl_2/ Et_2O); MS (ESI): 296 (M + H)$^+$.

3-[5-(3-Hydroxyphenyl)-1-methyl-1*H*-1,2,4-triazol-3-yl]phenol (15). The title compound was prepared by reaction of 3,5-bis(3-methoxyphenyl)-1-methyl-1*H*-1,2,4-triazole (**15i**) (100 mg, 0.37 mmol) and borontrifluoride dimethyl sulfide complex (27.7 mmol) according to method F. The product was purified by preparative TLC (ethyl acetate); yield: 64% (58 mg); MS (ESI): 268 (M + H)$^+$.

2-Bromo-5-(3-methoxyphenyl)thiophene (18ii). The title compound was prepared by reaction of 2,5-dibromothiophene (465 μL, 4.13 mmol), 3-methoxybenzeneboronic acid (753 mg, 4.95 mmol), sodium carbonate (876 mg, 8.26 mmol), and tetrakis(triphenylphosphine) palladium (24 mg, 20 μmol) according to method A. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 41% (445 mg).

2-(3-Methoxyphenyl)thiophene (18i). To a solution of 2-bromo-5-(3-methoxyphenyl)thiophene (**18ii**) (100 mg, 0.37 mmol, 1 equiv.) in dry THF cooled to −78 °C for 5 min, *n*-BuLi (1.6 M in hexane, 0.28 mL, 0.44 mmol, 1.2 equiv.) was added dropwise and stirred for 15 min at −78 °C. The crude mixture was carefully hydrolyzed by addition of water (10 mL) and layers were separated. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, and evaporated to dryness under reduced pressure; yield: 98% (69 mg).

3-(2-Thienyl)phenol (18). The title compound was prepared by reaction of 2-(3-methoxyphenyl)thiophene (**18i**) (80 mg, 0.42 mmol) and boron tribromide (1.26 mmol) according to method E. The product was purified by preparative TLC (hexane/ethyl acetate 7:3); yield: 85% (63 mg); MS (ESI): 177 (M + H)$^+$; Anal. ($C_{10}H_8OS$) C, H, N.

2-(3-Methoxyphenyl)-5-phenylthiophene (19i). The title compound was prepared by reaction of 2-bromo-5-(3-methoxyphenyl)thiophene (**18ii**) (400 mg, 1.52 mmol), benzeneboronic acid (223 mg, 1.82 mmol), sodium carbonate (322 mg, 3.04 mmol), and tetrakis(triphenylphosphine) palladium (8.8 mg, 7.6 μmol) according to method B. The product was purified by CC (hexane/ethyl acetate 7:3); yield: 70% (283 mg).

3-(5-Phenyl-2-thienyl)phenol (19). The title compound was prepared by reaction of 2-(3-methoxyphenyl)-5-phenylthiophene (**19i**) (100 mg, 0.37 mmol) and boron tribromide (2.22 mmol) according to method E. The product was purified by preparative TLC (hexane/ethyl acetate 5:5); yield: 81% (75 mg); MS (ESI): 253 (M + H)$^+$; Anal. ($C_{16}H_{12}OS$) C, H, N.

2-Bromo-5-(4-methoxyphenyl)thiophene (20ii). The title compound was prepared by reaction of 2,5-dibromothiophene ($V = 465$ μL, 4.13 mmol), 4-methoxybenzeneboronic acid (753 mg, 4.95 mmol), sodium carbonate (876 mg, 8.26 mmol), and tetrakis(triphenylphosphine) palladium (24 mg, 20 μmol) according to method A. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 75% (815 mg).

2-(4-Methoxyphenyl)-5-phenylthiophene (20i). The title compound was prepared by reaction of 2-bromo-5-(4-methoxyphenyl)thiophene (**20ii**) (400 mg, 1.52 mmol), benzeneboronic acid (223 mg, 1.82 mmol), sodium carbonate (322 mg, 3.04 mmol), and tetrakis(triphenylphosphine) palladium (8.8 mg, 7.6 μmol) according to method B. The product was purified by CC (hexane/ethyl acetate 7:3); yield: 75% (303 mg).

4-(5-Phenyl-2-thienyl)phenol (20). The title compound was prepared by reaction of 2-(4-methoxyphenyl)-5-phenylthiophene (**20i**) (100 mg, 0.37 mmol) and boron tribromide (2.22 mmol) according to method E. The product was purified by preparative TLC (dichloromethane/methanol 99:1); yield: 95% (89 mg); MS (ESI): 253 (M + H)$^+$; Anal. ($C_{16}H_{12}OS$) C, H, N.

2-(3-Methoxyphenyl)-5-(4-methoxyphenyl)thiophene (22i). The title compound was prepared by reaction of 2-bromo-5-(3-methoxyphenyl)thiophene (**18ii**) (150 mg, 0.57 mmol), 4-methoxybenzeneboronic acid (223 mg, 0.68 mmol), sodium carbonate (120 mg, 1.14 mmol), and tetrakis(triphenylphosphine) palladium (3.2 mg, 2.8 μmol) according to method B. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 75% (126 mg).

3-[5-(4-Hydroxyphenyl)-2-thienyl]phenol (22). The title compound was prepared by reaction of 2-(3-methoxyphenyl)-5-(4-

methoxyphenyl)thiophene (**22i**) (150 mg, 0.51 mmol) and boron tribromide (3.06 mmol) according to method E. The product was purified by preparative TLC (hexane/ethyl acetate 5:5); yield: 93% (127 mg); MS (ESI): 269 $(M + H)^+$; Anal. ($C_{16}H_{12}O_2S$) C, H, N.

2,5-Bis(3-methoxyphenyl)thiophene (23i). The title compound was prepared by reaction of 2-bromo-5-(3-methoxyphenyl)thiophene (**18ii**) (150 mg, 0.57 mmol), 3-methoxybenzeneboronic acid (223 mg, 0.68 mmol), sodium carbonate (120 mg, 1.14 mmol), and tetrakis(triphenylphosphine) palladium (3.2 mg, 2.8 μmol) according to method B. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 78% (132 mg).

3,3′-Thiene-2,5-diyldiphenol (23). The title compound was prepared by reaction of 2,5-bis(3-methoxyphenyl)thiophene (**23i**) (150 mg, 0.51 mmol) and boron tribromide (3.06 mmol) according to method E. The product was purified by preparative TLC (hexane/ethyl acetate 5:5); yield: 95% (130 mg); MS (ESI): 269 $(M + H)^+$; Anal. ($C_{16}H_{12}O_2S$) C, H, N.

4-Bromo-2-(3-methoxyphenyl)thiophene (24ii). The title compound was prepared by reaction of 2,4-dibromothiophene (1.00 g, 4.13 mmol), 3-methoxybenzeneboronic acid (753 mg, 4.95 mmol), sodium carbonate (876 mg, 8.26 mmol), and tetrakis(triphenylphosphine) palladium (24 mg, 20 μmol) according to method A. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 72% (782 mg).

2-(3-Methoxyphenyl)-4-phenylthiophene (24i). The title compound was prepared by reaction of 4-bromo-2-(3-methoxyphenyl)thiophene (**24ii**) (250 mg, 0.93 mmol), benzeneboronic acid (137 mg, 1.12 mmol), sodium carbonate (197 mg, 1.86 mmol), and tetrakis(triphenylphosphine) palladium (6 mg, 4.64 μmol) according to method B. The product was purified by CC (petroleum ether/ethyl acetate 7:3); yield: 91% (225 mg).

3-(4-Phenyl-2-thienyl)phenol (24). The title compound was prepared by reaction of 2-(3-methoxyphenyl)-4-phenylthiophene (**24i**) (225 mg, 0.85 mmol) and boron tribromide (3.6 mmol) according to method E. The product was purified by preparative TLC (dichloromethane/methanol 99:1); yield: 69% (147 mg); MS (ESI): 253 $(M + H)^+$.

4-Bromo-2-(4-methoxyphenyl)thiophene (25ii). The title compound was prepared by reaction of 2,4-dibromothiophene (1.00 g, 4.13 mmol), 4-methoxybenzeneboronic acid (753 mg, 4.95 mmol), sodium carbonate (876 mg, 8.26 mmol), and tetrakis(triphenylphosphine) palladium (24 mg, 20 μmol) according to method A. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 78% (847 mg).

2-(4-Methoxyphenyl)-4-(3-methoxyphenyl)thiophene (25i). The title compound was prepared by reaction of 4-bromo-2-(4-methoxyphenyl)thiophene (**25ii**) (150 mg, 0.57 mmol), 3-methoxybenzeneboronic acid (223 mg, 0.68 mmol), sodium carbonate (120 mg, 1.14 mmol), and tetrakis(triphenylphosphine) palladium (3.2 mg, 2.8 μmol) according to method B. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 70% (118 mg).

3-[5-(4-Hydroxyphenyl)-3-thienyl]phenol (25). The title compound was prepared by reaction of 2-(4-methoxyphenyl)-4-(3-methoxyphenyl)thiophene (**25i**) (150 mg, 0.51 mmol) and boron tribromide (3.06 mmol) according to method E. The product was purified by preparative TLC (hexane/ethyl acetate 5:5); yield: 80% (109 mg); MS (ESI): 267 $(M - H)^-$; Anal. ($C_{16}H_{12}O_2S$) C, H, N.

2-(3-Methoxyphenyl)-4-(4-methoxyphenyl)thiophene (27i). The title compound was prepared by reaction of 4-bromo-2-(3-methoxyphenyl)thiophene (**24ii**) (150 mg, 0.57 mmol), 4-methoxybenzeneboronic acid (223 mg, 0.68 mmol), sodium carbonate (120 mg, 1.14 mmol), and tetrakis(triphenylphosphine) palladium (3.2 mg, 2.8 μmol) according to method B. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 70% (118 mg).

3-[4-(4-Hydroxyphenyl)-2-thienyl]phenol (27). The title compound was prepared by reaction of 2-(3-methoxyphenyl)-4-(4-methoxyphenyl)thiophene (**27i**) (150 mg, 0.51 mmol) and boron tribromide solution (3.06 mmol) according to method E. The product was purified by preparative TLC (hexane/ethyl acetate 5:5); yield: 85% (116 mg); MS (ESI): 267 $(M - H)^-$; Anal. ($C_{16}H_{12}O_2S$) C, H, N.

2,4-Bis(3-methoxyphenyl)thiophene (28i). The title compound was prepared by reaction of 4-bromo-2-(3-methoxyphenyl)thiophene (**24ii**) (150 mg, 0.57 mmol), 3-methoxybenzeneboronic acid (223 mg, 0.68 mmol), sodium carbonate (120 mg, 1.14 mmol), and tetrakis(triphenylphosphine) palladium (3.2 mg, 2.8 μmol) according to method B. The product was purified by CC (hexane/ethyl acetate 9:1); yield: 72% (121 mg).

3,3′-Thiene-2,4-diyldiphenol (28). The title compound was prepared by reaction of 2,4-bis(3-methoxyphenyl)thiophene (**28i**) (150 mg, 0.51 mmol) and boron tribromide (3.06 mmol) according to method E. The product was purified by preparative TLC (hexane/ethyl acetate 5:5); yield: 88% (120 mg); MS (ESI): 267 $(M - H)^-$; Anal. ($C_{16}H_{12}O_2S$) C, H, N.

4,4′-(Seleniene-2,5-diyl)diphenol (29). The title compound was prepared by reaction of 2,5-dibromoselenophene (150 mg, 0.52 mmol), 4-hydroxybenzeneboronic acid (172 mg, 1.25 mmol), cesium carbonate (679 mg, 2.08 mmol), and tetrakis(triphenylphosphine) palladium (6.0 mg, 5.2 μmol) according to method C. The product was purified by preparative TLC (hexane/ethyl acetate 6:4); yield: 49% (81 mg); MS (ESI): 317 $(M + H)^+$; Anal. ($C_{16}H_{12}O_2Se$) C, H, N.

3,3′-(Seleniene-2,5-diyl)diphenol (30). The title compound was prepared by reaction of 2,5-dibromoselenophene (150 mg, 0.52 mmol), 3-hydroxybenzeneboronic acid (172 mg, 1.25 mmol), cesium carbonate (679 mg, 2.08 mmol), and tetrakis(triphenylphosphine) palladium (6.0 mg, 5.2 μmol) according to method C. The product was purified by preparative TLC (hexane/ethyl acetate 6:4); yield: 42% (69 mg); MS (ESI): 317 $(M + H)^+$; Anal. ($C_{16}H_{12}O_2Se$) C, H, N.

3-Bromo-2-(4-methoxyphenyl)thiophene (31ii). The title compound was prepared by reaction of 2,3-dibromothiophene (234 μL, 2.1 mmol), 4-methoxybenzeneboronic acid (383 mg, 2.52 mmol), sodium carbonate (403 mg, 4.2 mmol), and tetrakis(triphenylphosphine) palladium (12 mg, 10 μmol) according to method B. The product was purified by CC (hexane); yield: 70% (387 mg).

4,4′-(Thiene-2,3-diyl)diphenol (31). The title compound was prepared by reaction of 2,3-bis(4-methoxyphenyl)thiophene (**31i**) (150 mg, 0.51 mmol) and boron tribromide (3.06 mmol) according to method E. The product was purified by preparative TLC (hexane/ethyl acetate 5:5); yield: 70% (95 mg); MS (ESI): 269 $(M + H)^+$; Anal. ($C_{16}H_{12}O_2S$) C, H, N.

3-Bromo-2-(3-methoxyphenyl)thiophene (32ii). The title compound was prepared by reaction of 2,3-dibromothiophene (234 μL, 2.1 mmol), 3-methoxybenzeneboronic acid (383 mg, 2.52 mmol), sodium carbonate (403 mg, 4.2 mmol), and tetrakis(triphenylphosphine) palladium (12 mg, 10 μmol) according to method B. The product was purified by CC (hexane); yield: 58% (320 mg).

2-(3-Methoxyphenyl)-3-(4-methoxyphenyl)-thiophene (32i). The title compound was prepared by reaction of 3-bromo-2-(3-methoxyphenyl)thiophene (**32ii**) (150 mg, 0.57 mmol), 4-methoxybenzeneboronic acid (223 mg, 0.68 mmol), sodium carbonate (120 mg, 1.14 mmol), and tetrakis(triphenylphosphine) palladium (3.2 mg, 2.8 μmol) according to method B. The product was purified by CC (hexane/ethyl acetate 7:3); yield 40% (68 mg).

3-[3-(4-Hydroxyphenyl)-2-thienyl]phenol (32). The title compound was prepared by reaction of 2-(3-methoxyphenyl)-3-(4-methoxyphenyl)thiophene (**32i**) (150 mg, 0.51 mmol) and boron tribromide (3.06 mmol) according to method E. The product was purified by preparative TLC (hexane/ethyl acetate 5:5); yield: 56% (77 mg); MS (ESI): 269 $(M + H)^+$; Anal. ($C_{16}H_{12}O_2S$) C, H, N.

3,3″-Dimethoxy-1,1′:4′,1″-terphenyl (35i). The title compound was prepared by reaction of 4′-bromo-3-methoxybiphenyl (**35ii**) (500 mg, 1.90 mmol), 3-methoxybenzeneboronic acid (346 mg, 2.28 mmol), sodium carbonate (403 mg, 3.80 mmol), and tetrakis(triphenylphosphine) palladium (11 mg, 9.5 μmol) according to method A (heating the mixture 20 h instead of 4 h). The product was purified by CC (hexane/ethyl acetate 95:5); yield: 14% (77 mg).

3,4″-Dimethoxy-1,1′:4′,1″-terphenyl (36i). The title compound was prepared by reaction of 4′-bromo-3-methoxybiphenyl (**35ii**) (500 mg, 1.90 mmol), 4-methoxybenzeneboronic acid (346 mg, 2.28 mmol), sodium carbonate (403 mg, 3.80 mmol), and tetrakis(triphenylphosphine) palladium (11 mg, 9.5 μmol) according to method A (heating the mixture 20 h instead of 4 h). The product was purified by CC (hexane/ethyl acetate 95:5); yield: 90% (496 mg).

3,3′-Pyridine-2,5-diyldiphenol (37). The title compound was prepared by reaction of 2,5-dibromo pyridine (150 mg, 0.63 mmol), 3-hydroxybenzeneboronic acid (231 mg, 1.52 mmol), cesium carbonate (821 mg, 2.52 mmol), and tetrakis(triphenylphosphine) palladium (7.3 mg, 6.3 μmol) according to method C. The product was purified by preparative TLC (dichloromethane/methanol 98:2); yield: 67% (111 mg); MS (ESI): 262 $(M - H)^-$; Anal. ($C_{17}H_{13}NO_2$) C, H, N.

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

17β-HSD1 and 17β-HSD2 were obtained from human placenta according to previously described procedures.[22,62] Fresh human placenta was homogenized and centrifuged. The pellet fraction contains the microsomal 17β-HSD2, while 17β-HSD1 was obtained after precipitation with ammonium sulfate from the cytosolic fraction.

1. Inhibition of 17β-HSD1. Inhibitory activities were evaluated by a well established method with minor modifications.[41,63,64] 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 EDTA 1 mM. Inhibitor stock solutions were prepared in DMSO. Final concentration of DMSO was adjusted to 1% in all samples. The enzymatic reaction was started by addition of a mixture of unlabeled- and [2,4,6,7-^{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.

2. Inhibition of 17β-HSD2. 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 [2,4,6,7-^{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.

3. ER Affinity. The binding affinity of select compounds to the ERα and ERβ was determined according to Zimmermann et al.[65] Briefly, 0.25 pmol of ERα or ERβ, respectively, were incubated with [2,4,6,7-^{3}H]-E2 (10 nM) and test compound for 1 h at rt. The potential inhibitors were dissolved in DMSO (5% final concentration). Nonspecific binding was performed with diethylstilbestrol (10 μM). After incubation, ligand−receptor complexes were selectively bound to hydroxyapatite (5 g/ 60 mL TE-buffer). The formed complex was separated, washed, and resuspended in ethanol. For radiodetection, scintillator cocktail (Quickszint 212, Zinsser Analytic, Frankfurt) was added and samples were measured in a liquid scintillation counter (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%.

4. Evaluation of the Estrogenic Activity Using T-47D Cells. 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% (v/v). RPMI 1640 (without phenol red) was used for the experiments. Cells were grown for 48 h in phenol red-free medium. Compounds **4**, **10**, **22**, **25**, **35**, and **36** 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 two to three days and supplemented with the respective additive. After eight days of incubation, the cell viability was evaluated measuring the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazoliumbromide (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[66] 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

5. Caco-2 Transport Experiments. Caco-2 cell culture and transport experiments were performed according to Yee[67] 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. The compounds were applied to the cells as a mixture (cassette dosing) to increase the throughput. The initial concentration of the compounds in the donor compartment was 50 μM (0.2 M MES, pH: 6.5, containing either 1% ethanol or DMSO). Samples were taken from the acceptor side after 0, 60, 120, and 180 min and from the donor side after 0 and 180 min. Each experiment was run in triplicate. The integrity of the monolayers was checked by measuring the transepithelial electrical resistance (TEER) before the transport experiments and by measuring lucifer yellow permeability after each assay. All samples of the CaCo-2 transport experiments were analyzed by LC/MS/MS 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.

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

The reaction was initiated by the addition of test compound (final concentration 1 μM) 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 μL/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).

7. Inhibition of Human Hepatic CYPs. The commercially available P450 inhibition kits from BD Gentest (Heidelberg, Germany) were used according to the instructions of the manufacturer. Compounds **4**, **25**, and **35** were tested for inhibition of the following enzymes: CYP1A2, 2B6, 2C9, 2C19, 2D6, and 3A4. Inhibitory potencies were determined as IC_{50} values.

8. In Vivo Pharmacokinetics. Male Wistar rats weighing 300−330 g (Janvier France) 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. They were anesthetized with a ketamine (135 mg/kg)/xylyzine (10 mg/kg) mixture and cannulated with silicone tubing via the right jugular vein and attached to the skull with dental cement.[68] 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 **3** and **39** were applied orally in a cassette dosing in 4 rats at the dose of 10 mg/kg body weight by using a feeding needle. The compounds were dissolved in a mixture labrasol/water (1:1) and given at a volume of 5 mL/kg. Blood samples (0.2 mL) were taken at 0, 1, 2, 3, 4, 6, 8, 10, and 24 h postdose and collected in heparinized tubes. They were centrifuged at 3000 *g* for 10 min, and plasma was harvested and kept at −20 °C until analyzed.

HPLC-MS/MS analysis and quantification of the samples was carried out on a Surveyor HPLC-system coupled with a TSQ Quantum (Thermofischer) triple quadrupole mass spectrometer equipped with an electrospray interface (ESI).

Computational Chemistry. 1. Distance and Angle Calculations. All distances and angles were calculated after energy minimization using Hyperchem v. 6.0.

2. MEP. For selected compounds, ab initio geometry optimizations were performed gas phase at the B3LYP/6-311**G (d,p) level of density functional theory (DFT) by means of the Gaussian03 software and the molecular electrostatics potential map (MEP) was plotted using GaussView 3.09, the 3D molecular graphics package of Gaussian.[69] These electrostatic potential surfaces were generated by mapping 6-311G** electrostatic potentials onto surfaces of molecular electron density (isovalue = 0.002 e^-/Å). The MEP maps are color coded, where red stands for negative values (3.1×10^{-2} Hartree) and blue for positive ones (4.5×10^{-2} Hartree).[70]

Acknowledgment. We are grateful to the Deutsche Forschungsgemeinschaft (HA1315/8-1) for financial support. We thank Dr. Christiane Scherer for performing the CaCo-2 cell test, Anja Palusczak and Beate Geiger for their help in performing the enzyme inhibition tests (17β-HSD1, 17β-HSD2, ERα, and ERβ), and Dr. Ursula Müller-Viera, Pharmacelsus CRO, Saarbrücken, Germany, for the stability tests with rat liver microsomes. Patricia Kruchten is grateful to the European Postgraduate School 532 (DFG) for a scholarship. Dr. Yaseen A. Al-Soud is grateful to the Alexander von Humboldt Foundation (AvH) for a fellowship.

Supporting Information Available: Spectroscopic data of all compounds (^{1}H NMR, ^{13}C NMR, IR), purity data of final compounds and MEP maps of compounds **7**, **10**, **14**, **27**, and **G**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Travis, R. C.; Key, T. J. Oestrogen exposure and breast cancer risk. *Breast Cancer Res.* **2003**, *5*, 239–247.

(2) Dizerega, G. S.; Barber, D. L.; Hodgen, G. D. Endometriosis: role of ovarian steroids in initiation, maintenance and suppression. *Fertil. Steril.* **1980**, *33*, 649–653.

(3) Miller, W. R.; Bartlett, J. M.; Canney, P.; Verrill, M. Hormonal therapy for postmenopausal breast cancer: the science of sequencing. *Breast Cancer Res. Treat.* **2007**, *103*, 149–160.

(4) Bush, N. J. Advances in hormonal therapy for breast cancer. *Semin. Oncol. Nurs.* **2007**, *23*, 46–54.

(5) Adamo, V.; Iorfida, M.; Montalto, E.; Festa, V.; Garipoli, C.; Scimone, A.; Zanghi, M.; Caristi, N. Overview and new strategies in metastatic breast cancer (MBC) for treatment of tamoxifen-resistant patients. *Ann. Oncol.* **2007**, *18* (Suppl 6), vi53–57.

(6) Ohnesorg, T.; Keller, B.; Hrabe de Angelis, M.; Adamski, J. Transcriptional regulation of human and murine 17beta-hydroxysteroid dehydrogenase type-7 confers its participation in cholesterol biosynthesis. *J. Mol. Endocrinol.* **2006**, *37*, 185–197.

(7) Sakurai, N.; Miki, Y.; Suzuki, T.; Watanabe, K.; Narita, T.; Ando, K.; Yung, T. M.; Aoki, D.; Sasano, H.; Handa, H. Systemic distribution and tissue localizations of human 17beta-hydroxysteroid dehydrogenase type 12. *J. Steroid Biochem. Mol. Biol.* **2006**, *99*, 174–181.

(8) Day, J. M.; Foster, P. A.; Tutill, H. J.; Parsons, M. F.; Newman, S. P.; Chander, S. K.; Allan, G. M.; Lawrence, H. R.; Vicker, N.; Potter, B. V.; Reed, M. J.; Purohit, A. 17beta-Hydroxysteroid dehydrogenase Type 1, and not Type 12, is a target for endocrine therapy of hormone-dependent breast cancer. *Int. J. Cancer* **2008**, *122*, 1931–1940.

(9) Gunnarsson, C.; Hellqvist, E.; Stål, O. 17beta-Hydroxysteroid dehydrogenases involved in local oestrogen synthesis have prognostic significance in breast cancer. *Br. J. Cancer* **2005**, *92*, 547–552.

(10) Gunnarsson, C.; Olsson, B. M.; Stål, O. Abnormal expression of 17beta-hydroxysteroid dehydrogenases in breast cancer predicts late recurrence. *Cancer Res.* **2001**, *61*, 8448–8451.

(11) Miyoshi, Y.; Ando, A.; Shiba, E.; Taguchi, T.; Tamaki, Y.; Noguchi, S. Involvement of up-regulation of 17beta-hydroxysteroid dehydrogenase type 1 in maintenance of intratumoral high estradiol levels in postmenopausal breast cancers. *Int. J. Cancer* **2001**, *94*, 685–689.

(12) Suzuki, T.; Moriya, T.; Ariga, N.; Kaneko, C.; Kanazawa, M.; Sasano, H. 17beta-Hydroxysteroid dehydrogenase type 1 and type 2 in human breast carcinoma: a correlation to clinicopathological parameters. *Br. J. Cancer* **2000**, *82*, 518–523.

(13) Šmuc, T.; Pucelj Ribič, M.; Šinkovec, J.; Husen, B.; Thole, H.; Lanišnik Rižner, T. Expression analysis of the genes involved in estradiol and progesterone action in human ovarian endometriosis. *Gynecol. Endocrinol.* **2007**, *23*, 105–111.

(14) Labrie, F. Intracrinology. *Mol. Cell. Endocrinol.* **1991**, *78*, C113−C118.

(15) Vihko, P.; Härkönen, P.; Soronen, P.; Törn, S.; Herrala, A.; Kurkela, R.; Pulkka, A.; Oduwole, O.; Isomaa, V. 17beta-Hydroxysteroid dehydrogenases: their role in pathophysiology. *Mol. Cell. Endocrinol.* **2004**, *215*, 83–88.

(16) Azzi, A.; Rehse, P. H.; Zhu, D. W.; Campbell, R. L.; Labrie, F.; Lin, S.-X. Crystal structure of human estrogenic 17beta-hydroxysteroid dehydrogenase complexed with 17beta-estradiol. *Nat. Struct. Biol.* **1996**, *3*, 665–668.

(17) Breton, R.; Housset, D.; Mazza, C.; Fontecilla-Camps, J. C. The structure of a complex of human 17beta-hydroxysteroid dehydrogenase with estradiol and NADP$^+$ identifies two principal targets for the design of inhibitors. *Structure* **1996**, *4*, 905–915.

(18) Gangloff, A.; Shi, R.; Nahoum, V.; Lin, S.-X. Pseudosymmetry of C19 steroids, alternative binding orientations, and multispecificity in human estrogenic 17beta-hydroxysteroid dehydrogenase. *FASEB J.* **2003**, *17*, 274–276.

(19) Ghosh, D.; Pletnev, V. Z.; Zhu, D. W.; Wawrzak, Z.; Duax, W. L.; Pangborn, W.; Labrie, F.; Lin, S.-X. Structure of human estrogenic 17beta-hydroxysteroid dehydrogenase at 2.20 Å resolution. *Structure* **1995**, *3*, 503–513.

(20) Han, Q.; Campbell, R. L.; Gangloff, A.; Huang, Y. W.; Lin, S.-X. Dehydroepiandrosterone and dihydrotestosterone recognition by human estrogenic 17beta-hydroxysteroid dehydrogenase. C-18/C-19 steroid discrimination and enzyme-induced strain. *J. Biol. Chem.* **2000**, *275*, 1105–1111.

(21) Mazza, C.; Breton, R.; Housset, D.; Fontecilla-Camps, J. C. Unusual charge stabilization of NADP$^+$ in 17beta-hydroxysteroid dehydrogenase. *J. Biol. Chem.* **1998**, *273*, 8145–8152.

(22) Qiu, W.; Campbell, R. L.; Gangloff, A.; Dupuis, P.; Boivin, R. P.; Tremblay, M. R.; Poirier, D.; Lin, S.-X. A concerted, rational design of type 1 17beta-hydroxysteroid dehydrogenase inhibitors: estradiol-adenosine hybrids with high affinity. *FASEB J.* **2002**, *16*, 1829–1831.

(23) Sawicki, M. W.; Erman, M.; Puranen, T.; Vihko, P.; Ghosh, D. Structure of the ternary complex of human 17beta-hydroxysteroid dehydrogenase type 1 with 3-hydroxyestra-1,3,5,7-tetraen-17-one (equilin) and NADP$^+$. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 840–845.

(24) Shi, R.; Lin, S.-X. Cofactor hydrogen bonding onto the protein main chain is conserved in the short chain dehydrogenase/reductase family and contributes to nicotinamide orientation. *J. Biol. Chem.* **2004**, *279*, 16778–16785.

(25) Filling, C.; Berndt, K. D.; Benach, J.; Knapp, S.; Prozorovski, T.; Nordling, E.; Ladenstein, R.; Jornvall, H.; Oppermann, U. Critical residues for structure and catalysis in short-chain dehydrogenases/reductases. *J. Biol. Chem.* **2002**, *277*, 25677–25684.

(26) Penning, T. M. 17beta-Hydroxysteroid dehydrogenase: inhibitors and inhibitor design. *Endocr. Relat. Cancer* **1996**, *3*, 41–56.
(27) Brožic, P.; Lanišnik Rižner, T.; Gobec, S. Inhibitors of 17beta-hydroxysteroid dehydrogenase type 1. *Curr. Med. Chem.* **2008**, *15*, 137–150, and references therein.
(28) Messinger, J.; Hirvelä, L.; Husen, B.; Kangas, L.; Koskimies, P.; Pentikäinen, O.; Saarenketo, P.; Thole, H. New inhibitors of 17beta-hydroxysteroid dehydrogenase type 1. *Mol. Cell. Endocrinol.* **2006**, *248*, 192–198.
(29) Karkola, S.; Lilienkampf, A.; Wähälä, K. A 3D QSAR model of 17beta-HSD1 inhibitors based on a thieno[2,3-*d*]pyrimidin-4(3*H*)-one core applying molecular dynamics simulations and ligand−protein docking. *ChemMedChem* **2008**, *3*, 461–472.
(30) Allan, G. M.; Vicker, N.; Lawrence, H. R.; Tutill, H. J.; Day, J. M.; Huchet, M.; Ferrandis, E.; Reed, M. J.; Purohit, A.; Potter, B. V. Novel inhibitors of 17beta-hydroxysteroid dehydrogenase type 1: templates for design. *Bioorg. Med. Chem.* **2008**, *16*, 4438–4456.
(31) Frotscher, M.; Ziegler, E.; Marchais-Oberwinkler, S.; Kruchten, P.; Neugebauer, A.; Fetzer, L.; Scherer, C.; Müller-Vieira, U.; Messinger, J.; Thole, H.; Hartmann, R. W. Design, synthesis and biological evaluation of (hydroxyphenyl)-naphthalene and quinoline derivatives: potent and selective non steroidal inhibitors of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1) for the treatment of estrogen-dependent diseases. *J. Med. Chem.* **2008**, *51*, 2158–2169.
(32) Marchais-Oberwinkler, S.; Kruchten, P.; Frotscher, M.; Ziegler, E.; Neugebauer, A.; Bhoga, U. D.; Bey, E.; Müller-Vieira, U.; Messinger, J.; Thole, H.; Hartmann, R. W. Substituted 6-phenyl-2-naphthols. Potent and selective nonsteroidal inhibitors of 17β-hydroxysteroid dehydrogenase type 1 (17beta-HSD1): design, synthesis, biological evaluation and pharmacokinetics. *J. Med. Chem.* **2008**, *51*, 4685–4698.
(33) Bey, E.; Marchais-Oberwinkler, S.; Kruchten, P.; Frotscher, M.; Werth, R.; Oster, A.; Algül, O.; Neugebauer, A.; Hartmann, R. W. Design, synthesis and biological evaluation of bis(hydroxyphenyl) azoles as potent and selective nonsteroidal inhibitors of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1) for the treatment of estrogen-dependent diseases. *Bioorg. Med. Chem.* **2008**, *16*, 6423–6435.
(34) Miyaura, N.; Suzuki, A. The palladium catalysed cross-coupling reaction of phenylboronic acid with haloarenes in the presence of bases. *Synth. Commun.* **1995**, *11*, 513–519.
(35) Fink, B. E.; Mortensen, D. S.; Stauffer, S. R.; Aron, Z. D.; Katzenellenbogen, J. A. Novel structural templates for estrogen−-receptor ligands and prospects for combinatorial synthesis of estrogens. *Chem. Biol.* **1999**, *6*, 205–219.
(36) Gierczyk, B.; Zalas, M. Synthesis of substituted 1,3,4-thiadiazoles using Lawesson reagents. *Org. Prep. Proc. Int.* **2005**, *37*, 213–222.
(37) Bagley, M. C.; Chapaneri, K.; Glover, C.; Merrit, E. A. Simple microwave-assisted method for the synthesis of primary thioamides from nitriles. *Synlett* **2004**, *14*, 2615–2617.
(38) Kelarev, V. I.; Silin, M. A.; Kobrakov, K. I.; Rybina, I. I.; Korolev, V. K. Synthesis of 1,3,5-trisubstituted 1*H*-1,2,4-triazoles containing hetaryl fragments. *Chem. Heterocycl. Compd.* **2003**, *39*, 736–743.
(39) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. Click chemistry: Diverse chemical function from a few good reactions. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004–2021.
(40) Guither, W. D.; Coburn, M. D.; Castle, R. N. 3,6-Bis-substituted *s*-tetrazines. *Heterocycles* **1979**, *12*, 745–749.
(41) Lin, S.-X.; Yang, F.; Jin, J. Z.; Breton, R.; Zhu, D. W.; Luu-The, V.; Labrie, F. Subunit identity of the dimeric 17beta-hydroxysteroid dehydrogenase from human placenta. *J. Biol. Chem.* **1992**, *267*, 16182–16187.
(42) Hidalgo, I. J. Assessing the absorption of new pharmaceuticals. *Curr. Top. Med. Chem.* **2001**, *1*, 385–401.
(43) Yearley, E. J.; Zhurova, E. A.; Zhurov, V. V.; Pinkerton, A. A. Binding of genistein to the estrogen receptor based on an experimental electron density study. *J. Am. Chem. Soc.* **2007**, *129*, 15013–15021.
(44) White, E. H.; Bursey, M. M.; Roswel, D. F.; Hill, J. H. M. Chemiluminescence of some monoacylhydrazides. *J. Org. Chem.* **1967**, *32*, 1198–1202.
(45) Manaka, A.; Sato, M. Synthesis of aromatic thioamide from nitrile without handling of hydrogen sulfide. *Synth. Commun.* **2005**, *11*, 761–764.
(46) Ando, K.; Kato, T.; Kawai, A.; Nonomura, T. Preparation of heterocyclyl sulfonylbenzene compounds as anti-inflammatory/analgesic agents. Patent WO9964415A1, 1999.
(47) Uozumi, Y.; Kikuchi, M. Controlled monoarylation of dibromoarenes in water with a polymeric palladium catalyst. *Synlett* **2005**, *11*, 1775–1778.
(48) Ewing, W. R.; Becker, M. R.; Pauls, H. W.; Cheney, D. L.; Mason, J. S.; Spada, A. P. Choi-Sledeski, Y. M. Preparation of substituted (sulfinic acid, sulfonic acid, sulfonylamino or sulfinylamino) *N*-[(aminoiminomethyl)phenylalkyl]azaheterocyclamide compounds as factor Xa inhibitors. Patent WO9640679A1, 1996.
(49) Vachal, P.; Toth, L. M. General facile synthesis of 2,5-diarylheteropentalenes. *Tetrahedron Lett.* **2004**, *45*, 7157–7161.
(50) Yokooji, A.; Okazawa, T.; Satoh, T.; Miura, M.; Nomura, M. Palladium-catalyzed direct arylation of thiazoles with aryl bromides. *Tetrahedron* **2003**, *59*, 5685–5689.
(51) Chandra, R.; Kung, M.-P.; Kung, H. F. Design, synthesis, and structure−activity relationship of novel thiophene derivatives for beta-amyloid plaque imaging. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1350–1352.
(52) Sone, T.; Sato, K.; Umetsu, Y.; Yoshino, A.; Takahashi, K. Acid-catalyzed reactions of thiophene nuclei. VIII. Synthesis of unsymmetrical 2,3-diaryl- and 2,4-diarylthiophenes starting from 2,5-dichlorothiophene. *Bull. Chem. Soc. Jpn.* **1994**, *67*, 2187–2194.
(53) Nakano, M.; Satoh, T.; Miura, M. Palladium-catalyzed selective 2,3-diarylation of alpha, alpha-disubstituted 3-thiophenemethanols via cleavage of C-H and C-C bonds. *J. Org. Chem.* **2006**, *71*, 8309–8311.
(54) Liu, L.; Zhang, Y.; Xin, B. Synthesis of biaryls and polyaryls by ligand-free Suzuki reaction in aqueous phase. *J. Org. Chem.* **2006**, *71*, 3994–3997.
(55) Demerseman, P.; Buu-Hoi, N. P.; Royer, R. Thiophene derivatives of biological interest. VII. The synthesis of 2,4-diarylthiophenes and 2,4-diarylselenophenes from anils of alkyl aryl ketones. *J. Chem. Soc.* **1954**, 2720–2722.
(56) Lebrini, M.; Bentiss, F.; Lagrenee, M. Rapid synthesis of 2,5-disubstituted 1,3,4-thiadiazoles under microwave irradiation. *J. Heterocycl. Chem.* **2005**, *42*, 991–994.
(57) Pirali, T.; Gatti, S.; Di Brisco, R.; Tacchi, S.; Zaninetti, R.; Brunelli, E.; Massarotti, A.; Sorba, G.; Canonico, P. L.; Moro, L.; Genazzani, A. A.; Tron, G. C.; Billington, R. A. Estrogenic analogues synthesized by click chemistry. *ChemMedChem* **2007**, *2*, 437–440.
(58) Yas'ko, S. V.; Korchevin, N. A.; Gusarova, N. K.; Kazantseva, T. I.; Chernysheva, N. A.; Klyba, L. V.; Trofimov, B. A. Microwave induced synthesis of diphenylthiophenes from elemental sulfur and styrene. *Chem. Heterocycl. Comp.* **2006**, *42*, 1486–1487.
(59) Lahti, P. M.; Ichimura, A. S. Semiempirical study of electron exchange interaction in organic high-spin pi-systems. Classifying structural effects in organic magnetic molecules. *J. Org. Chem.* **1991**, *56*, 3030–3042.
(60) Tagat, J. R.; McCombie, S. W.; Barton, B. E.; Jackson, J.; Shortall, J. Synthetic inhibitors of interleukin-6. II. 3,5-Diarylpyridines and *m*-terphenyls. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2143–2146.
(61) Muller, G.; Amiard, G.; Mathieu, J. Dihydropyrazines and pyrazines obtained by condensation of some phenacylamines. *Bull. Soc. Chim. Fr.* **1949**, 533–535.
(62) Zhu, D. W.; Lee, X.; Breton, R.; Ghosh, D.; Pangborn, W.; Duax, W. L.; Lin, S.-X. Crystallization and preliminary X-ray diffraction analysis of the complex of human placental 17beta-hydroxysteroid dehydrogenase with NADP$^+$. *J. Mol. Biol.* **1993**, *234*, 242–244.
(63) Sam, K. M.; Auger, S.; Luu-The, V.; Poirier, D. Steroidal spiro-gamma-lactones that inhibit 17beta-hydroxysteroid dehydrogenase activity in human placental microsomes. *J. Med. Chem.* **1995**, *38*, 4518–4528.
(64) Sam, K. M.; Boivin, R. P.; Tremblay, M. R.; Auger, S.; Poirier, D. C16 and C17 derivatives of estradiol as inhibitors of 17beta-hydroxysteroid dehydrogenase type 1: chemical synthesis and structure−activity relationships. *Drug. Des. Discovery* **1998**, *15*, 157–180.
(65) Zimmermann, J.; Liebl, R.; von Angerer, E. 2,5-Diphenylfuran-based pure antiestrogens with selectivity for the estrogen receptor alpha. *J. Steroid Biochem. Mol. Biol.* **2005**, *94*, 57–66.
(66) Denizot, F.; Lang, R. Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. *J. Immunol. Methods* **1986**, *89*, 271–277.
(67) Yee, S. In vitro permeability across Caco-2 cells (colonic) can predict in vivo (small intestinal) absorption in man: fact or myth. *Pharm. Res.* **1997**, *14*, 763–766.
(68) Van Dongen, J. J.; Remie, R.; Rensema, J. W.; Van Wunnik, G. H. J. *Manual of Microsurgery on the Laboratory Rat*; Huston, J. P., Ed.; Elsevier: New York, 1990; p 159.
(69) Dennington, I.; Roy, K. T.; Millam, J.; Eppinnett, K.; Howell, W. L. Gilliland, R.; *GaussView, 3.0*; Semichem Inc.: Shawnee Mission, KS 2003.
(70) Petti, M. A.; Shepodd, T. J.; Barrans, R. E.; Dougherty, D. A. "Hydrophobic" binding of water-soluble guests by high-symmetry, chiral hosts. An electron-rich receptor site with a general affinity for quaternary ammonium compounds and electron-deficient pi-systems. *J. Am. Chem. Soc.* **1988**, *110*, 6825–6840.
(71) Tannergren, C.; Langguth, P.; Hoffmann, K. J. Compound mixtures in CaCo-2 cell permeability screens as a means to increase screening capacity. *Pharmazie* **2001**, *56*, 337–342.
(72) Laitinen, L.; Kangas, H.; Kaukonen, A. M.; Hakala, K.; Kotiaho, T.; Kostiainen, R.; Hirvonen, J. N-in-one permeability studies of heterogeneous sets of compounds across Caco-2 cell monolayers. *Pharm. Res.* **2003**, *20*, 187–197.

JM8006917