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New Insights into the SAR and Binding Modes of Bis(hydroxyphenyl)thiophenes and -benzenes: Influence of Additional Substituents on 17β-Hydroxysteroid Dehydrogenase Type 1 (17β-HSD1) Inhibitory Activity and Selectivity

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17β-Hydroxysteroid dehydrogenase type 1 (17β-HSD1) is responsible for the catalytic reduction of weakly active E1 to highly potent E2. E2 stimulates the proliferation of hormone-dependent diseases via activation of the estrogen receptor α (ERα). Because of the overexpression of 17β-HSD1 in mammary tumors, this enzyme should be an attractive target for the treatment of estrogen-dependent pathologies. Recently, we have reported on a series of potent 17β-HSD1 inhibitors: bis(hydroxyphenyl) azoles, thiophenes, and benzenes. In this paper, different substituents are introduced into the core structure and the biological properties of the corresponding inhibitors are evaluated. Computational methods and analysis of different X-rays of 17β-HSD1 lead to identification of two different binding modes for these inhibitors. The fluorine compound **23** exhibits an IC_{50} of 8 nM and is the most potent nonsteroidal inhibitor described so far. It also shows a high selectivity (17β-HSD2, ERα) and excellent pharmacokinetic properties after peroral application to rats.

Introduction

Estrogens are involved in the regulation of the female reproduction system. However, it is also well-known that 17β-estradiol (E2[a]), the natural ligand of the estrogen receptors (ERs) α and β, plays a critical role in the development of several estrogen-dependent pathologies like breast cancer[1] and endometriosis.[2]

Until now, hormone-dependent breast cancers are treated using three different endocrine therapies:[3,4] aromatase inhibitors and GnRH analogues disrupt the estrogen biosynthesis, while selective estrogen receptor modulators (SERMs) or pure antiestrogens[5] prevent E2 to unfold its action at the receptor level. Besides specific disadvantages of each therapeutic approach, all of these strategies have in common a rather radical reduction of estrogen levels in the whole body leading to significant side effects.

A softer approach could be the inhibition of an enzyme of the 17β-hydroxysteroid dehydrogenase (17β-HSD) family, especially one that is responsible for the E2 formation from estrone (E1). Until now, three subtypes (1, 7, and 12) are able to catalyze this reaction, the most important being 17β-HSD1. The primary physiological role of 17β-HSD7 and 17β-HSD12 is supposed to be in the cholesterol synthesis[6,7] and in the regulation of the lipid biosynthesis,[8] respectively. In addition, Day et al.[9] recently showed that 17β-HSD12, although highly expressed in breast cancer cell lines, is inefficient in E2 formation.

17β-HSD1, which is responsible for the intracellular NAD(P)H-dependent conversion of the weak E1 into the highly potent estrogen E2, is often overexpressed in breast cancer cells[10−13] and endometriosis.[14] Inhibition of this enzyme is therefore regarded as a promising novel target for the treatment of estrogen-dependent diseases.

Recently, two groups[9,15,16] reported on the in vivo efficacy of 17β-HSD1 inhibitors to reduce E1 induced tumor growth using two different mouse models and indicating that the 17β-HSD1 enzyme is a suitable target for the treatment of breast cancer.

In order to not counteract the therapeutic efficacy of 17β-HSD1 inhibitors, it is very important that the compounds are selective toward 17β-HSD2, the enzyme that catalyzes the deactivation of E2 into E1. Additionally, to avoid intrinsic estrogenic effects, the inhibitors should not show affinity to the estrogen receptors α and β.

During the past decade, several groups reported on 17β-HSD1 inhibitors, most of them having steroidal structures.[17−20] Recently nonsteroidal cores have been published too. Until today four classes of compounds were described: thienopyrimidinones,[21,22] biphenyl ethanones,[23] and from our group (hydroxyphenyl)naphthalenes[24−26] and bis(hydroxyphenyl)azoles, thiophenes, benzenes, and azabenzenes.[27−32] The most promising compounds of the

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[a] Abbreviations: 17β-HSD1, 17β-hydroxysteroid dehydrogenase type 1; 17β-HSD2, 17β-hydroxysteroid dehydrogenase type 2; E1, estrone; E2, 17β-estradiol; ER, estrogen receptor; SERM, selective estrogen receptor modulator; NADP(H), nicotinamide adenine dinucleotide phosphate; NAD(H), nicotinamide adenine dinucleotide; SUB, substrate binding site; COF, cofactor binding site; RBA, relative binding affinity; ESP, electrostatic potential; P_{app}, apparent permeability coefficient; SAR, structure−activity relationship; PDB, Protein Data Bank; CC, column chromatography; MEP, molecular electrostatic potential; HYC, hybrid inhibitor O5′-[9-(3,17β-dihydroxy-1,3,5(10)-estratrien-16β-yl)nonanoyl]-adenosin; AUC, area under the curve; HOBt, 1-hydroxybenzotriazole; EDCI, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride.

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Chart 1. Described Bis(hydroxyphenyl)azoles, Thiophenes and Benzenes

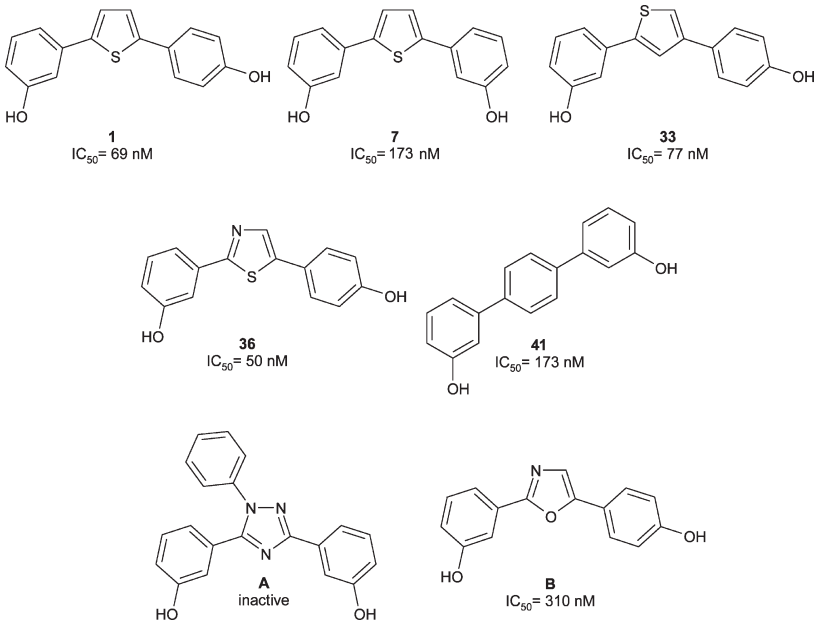
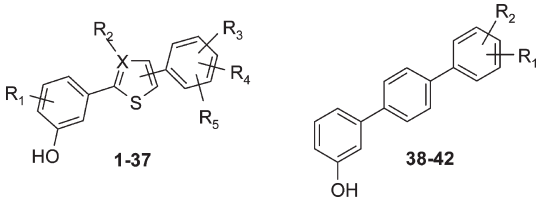


Chart 2. Title Compounds



last series are thiophenes **1**, **7**, and **33**, thiazole **36**, and phenylene **41**, exhibiting IC_{50} values toward 17β-HSD1 in the nanomolar range and high selectivity toward 17β-HSD2 and the ERs (Chart 1).

In the following, we will report on structural optimizations that led to the discovery of new highly potent and selective 17β-HSD1 inhibitors (Chart 2).

Design

Up to now, several crystal structures of human 17β-HSD1 were resolved: as apoenzyme (i.e., PDB code 1BHS[33]), as binary complex (enzyme−E2, i.e., PDB code 1IOL[34]), or as ternary complex (enzyme−E2−$NADP^+$, i.e., PDB codes 1FDT[35] and 1A27[36] and enzyme−HYC (hybride inhibitor), PDB code 1I5R[37]).

The analysis of the ternary complexes available from 17β-HSD1 provides useful knowledge about the architecture of the enzyme and important hints for structure based drug design: a substrate binding site (SUB) and a cofactor binding pocket (COF) are present as well as the most important amino acids responsible for substrate and cofactor anchoring. The SUB is a narrow hydrophobic tunnel containing two polar regions at each end: His221/Glu282 on the one side and Ser142/Tyr155 on the other side, corresponding to the binding oxygens in 3- and 17-hydroxy group of E2. Additionally a flexible loop can be identified that is not well resolved in almost all the structures.

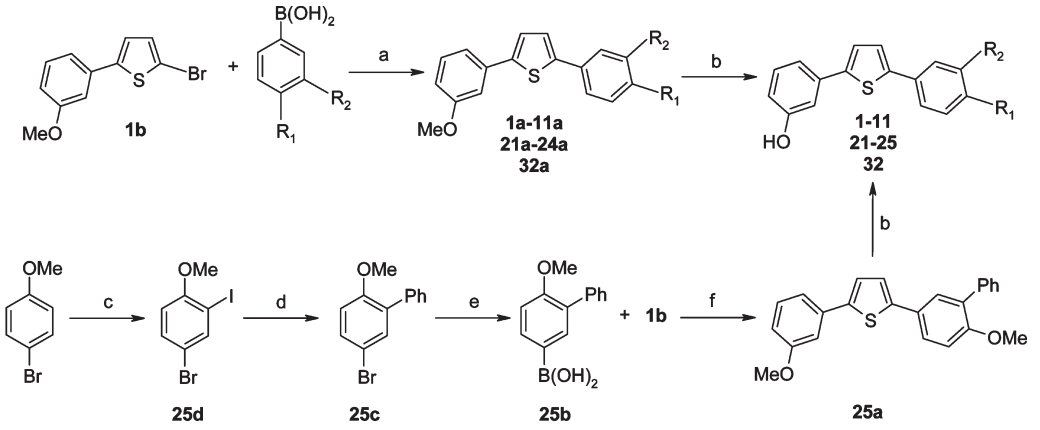
From previous results obtained in the class of bis-(hydroxyphenyl)azoles, thiophenes, benzenes, and azabenzenes,[27,28] a SAR study highlighted four structural features that are important for high 17β-HSD1 inhibitory activity: (1) one hydroxyphenyl moiety on the core structure is not sufficient for a high potency; (2) only the meta−para and meta−meta dihydroxy substitution pattern (O−O distance in the same range as observed for the steroid, d = 11 Å) are active; (3) the presence of the *m*-hydroxy group is more important for inhibitory activity than the *p*-hydroxy group; (4) only central aromatic rings without hydrogen-bond-donor function like thiophene, thiazole, benzene exhibit high inhibitory activity. It was also shown that a correlation seems to exist between the activity of the compounds and the electrostatic potential distribution of the molecules;[28] to be active the ESP values of the different regions of the inhibitor have to be in an appropriate range.

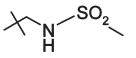
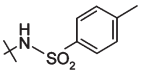
In the present report, we will present the structure optimization of this class of compounds leading to an increase in activity and in selectivity of these inhibitors. First, the influence of the bioisosteric exchange of one OH group on the enzyme activity will be determined. Second, the space availability around the inhibitors and the nature of the most appropriate substituents will be investigated by substitutions, either on the heterocycle or on the hydroxyphenyl moieties. The nature of the substituents will be varied in order to investigate the possible interactions between the inhibitor and the enzyme. Third, computational studies (docking studies and ESP calculations) will be performed in order to identify the most plausible binding mode for this class of compounds. Furthermore, the selectivity toward 17β-HSD2 and the ERs α and β will be determined as well as the potency of the compounds in T-47D cells and inhibition of three hepatic CYP enzymes. Finally, the pharmacokinetic profile of the two most promising candidates will be evaluated in rats after oral administration.

Chemistry

The synthesis of compounds **1**−**11**, **21**−**25**, and **32** is presented in Scheme 1. Starting from the monobrominated key intermediate **1b** and the appropriate commercially

Scheme 1. Synthesis of Compounds **1**−**11**, **21**−**25**, and **32**[a]



cmpd	R_1	R_2	cmpd	R_1	R_2
1	OH	H	**9**	H	
2	H	H	**10**	H	
3	F	H	**11**	H	
4	NH_2	H	**21**	OH	CH_3
5	SH	H	**22**	OH	OH
6	CN	H	**23**	OH	F
7	H	OH	**24**	OH	CF_3
8	H	F	**25**	OH	Ph
			32	F	F

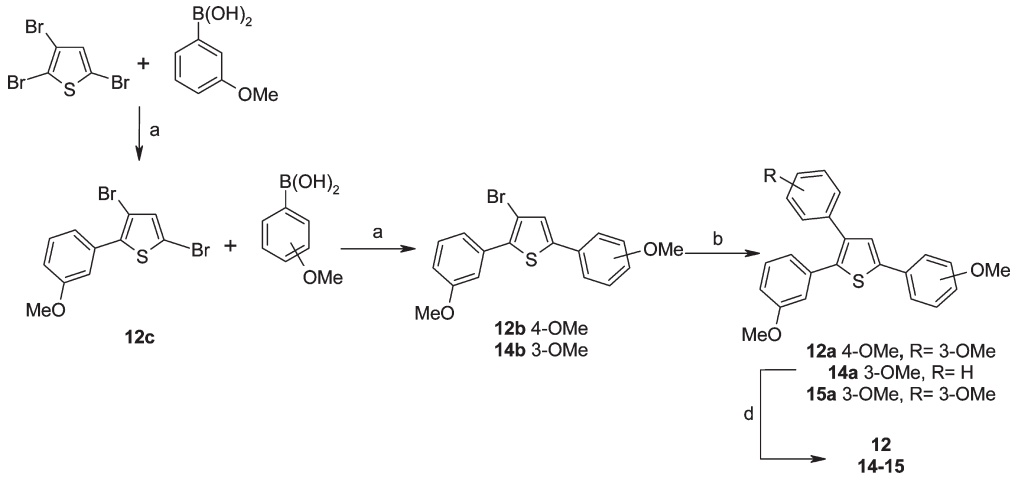
[a] Reagents and conditions: (a) method A, Cs_2CO_3, DME/EtOH/water (1:1:1), $Pd(PPh_3)_4$, microwave (150 W, 150 °C, 15 bar), 15 min; (b) BBr_3, CH_2Cl_2, −78 °C to room temp, 18 h; (c) $PhI(OAc)_2$, I_2, AcOEt, 60 °C, 5 h; (d) Ph-$B(OH)_2$; method B, Na_2CO_3, toluene/water (1:1), $Pd(PPh_3)_4$, reflux, 20 h; (e) (1) *n*-BuLi, dry THF, 5 min, −78 °C, (2) $B(OEt)_3$, 2 h −78 °C to room temp, (3) 1 N HCl, room temp; (f) method B, Na_2CO_3, toluene/water (1:1), $Pd(PPh_3)_4$, reflux, 20 h.

available boronic acids, the preparation of compounds **1a**−**11a**, **21a**−**25a**, and **32a** was accomplished via Suzuki cross-coupling reaction[38] under microwave assisted conditions (method A: Cs_2CO_3, DME/EtOH/water (1:1:1), $Pd(PPh_3)_4$, microwave (150 W, 150 °C, 15 bar), 15 min). The resulting disubstituted thiophenes were subsequently submitted to ether cleavage with boron tribromide[28] (method C: BBr_3, CH_2Cl_2, −78 °C to room temp, 18 h) leading to compounds **1**−**11**, **21**−**25**, and **32** (Scheme 1). In the case of intermediate **25a**, the boronic acid **25b** was prepared in a three-step synthesis pathway. First, an iodine substituent was selectively introduced in position 2 of the *p*-bromoanisole (compound **25d**) using (diacetoxyiodo)benzene.[39] Then a selective Suzuki reaction on the iodo position of **25d** under method B (Na_2CO_3, toluene/water (1:1), $Pd(PPh_3)_4$, reflux, 20 h) led to the intermediate **25c**, and the corresponding boronic acid **25b** was prepared using *n*-butyllithium and triethyl borate followed by hydrolysis with diluted hydrochloric acid.

The preparation of compounds **31** and **33**−**42** is similar to the synthetic pathway presented in Scheme 1 for compounds **1**−**11**. The first Suzuki coupling was carried out according to method B with the corresponding dibrominated heterocycle and the methoxylated benzene boronic acid. The resulting monosubstituted compounds **31b** and **33b**−**42b** were submitted to a second cross-coupling reaction under microwave assisted conditions following method A. The compounds were subsequently demethylated with boron tribromide to yield compounds **31** and **33**−**42**.

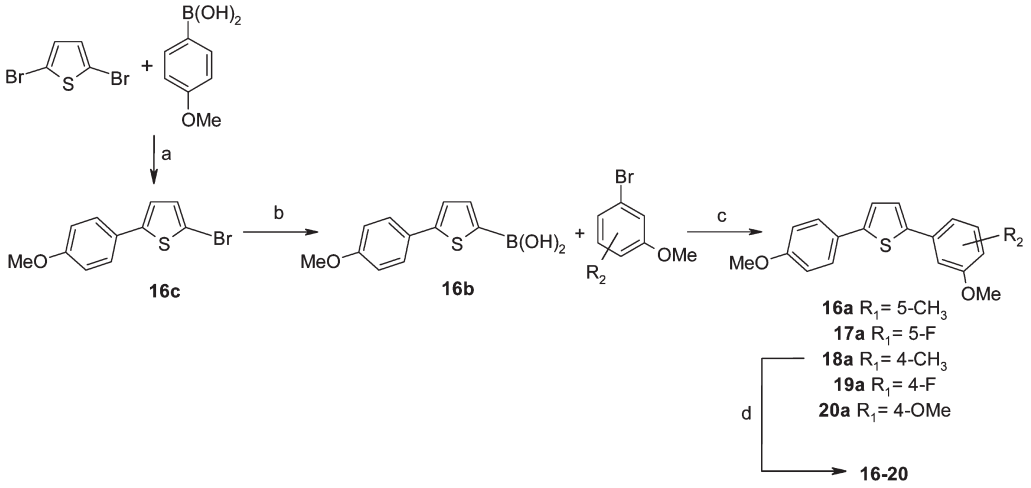
The synthesis of compounds **12**, **14**, and **15** is depicted in Scheme 2. The key intermediate dimethoxylated bromothiophene **12b** was prepared following two successive

Scheme 2. Synthesis of Compounds **12**, **14**, and **15**[a]



[a] Reagents and conditions: (a) method B, Na_2CO_3, toluene/water (1:1), $Pd(PPh_3)_4$, reflux, 4 h; (h) boronic acid; method B, Na_2CO_3, toluene/water (1:1), $Pd(PPh_3)_4$, reflux, 20 h; (d) BBr_3, CH_2Cl_2, −78 °C to room temp, 18 h.

Scheme 3. Synthesis of Compounds **16**−**20**[a]



[a] Reagents and conditions: (a) method B, Na_2CO_3, toluene/water (1:1), $Pd(PPh_3)_4$, reflux, 4 h; (b) (1) *n*-BuLi, anhydrous THF, −78 °C, 15 min, (2) $B(OEt)_3$, THF, −78 °C to room temp, 2 h, (3) 1 N HCl; (c) method A for **17a**−**20a** (Cs_2CO_3, DME/EtOH/water (1:1:1), $Pd(PPh_3)_4$, microwave (150 W, 150 °C, 15 bar), 15 min); method B for **16a** (Na_2CO_3, toluene/water (1:1), $Pd(PPh_3)_4$, reflux, 20 h); (d) BBr_3, CH_2Cl_2, −78 °C to room temp, 18 h.

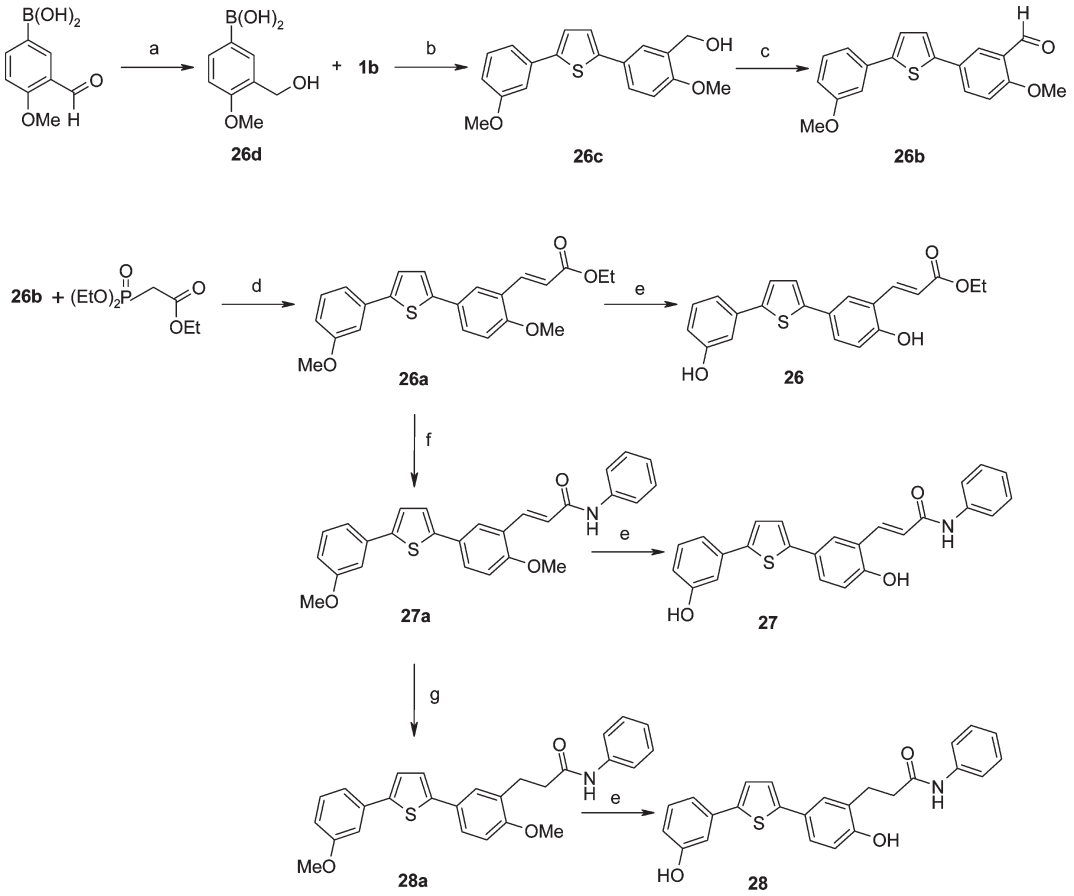
Suzuki coupling reactions according to method B (Na_2CO_3, toluene/water (1:1), $Pd(PPh_3)_4$, reflux, 4 h) from 2,3,5-tribromothiophene and methoxybenzene boronic acid. The reaction time of both cross-couplings was carefully controlled (restricted to 4 h) in order to get a selective bromine replacement each time. Intermediates **12a**, **14a**, and **15a** were obtained via a third Suzuki coupling using method B. The methoxy substituents were cleaved in the last step, using boron tribromide (method C: BBr_3, CH_2Cl_2, −78 °C to room temp, 18 h).

Compound **13** was synthesized under microwave assisted conditions in a one pot reaction using 2,5-dibromo-3-methylthiophene and 3-hydroxyphenyl boronic acid following method A (Cs_2CO_3, DME/EtOH/water (1:1:1), $Pd(PPh_3)_4$, microwave (150 W, 150 °C, 15 bar)) for 15 min.

The synthesis of the molecules bearing an additional substituent on the *m*-hydroxyphenyl moiety of thiophene **1** (compounds **16**−**20**) is shown in Scheme 3. Intermediate **16c** was prepared via Suzuki reaction from the *p*-methoxylated benzene boronic acid and the 2,5-dibromothiophene following method B, heating the reaction 4 h instead of 20 h in order to avoid any dicoupling reaction. Treatment of **16c** with *n*-butyllithium and triethyl borate afforded after hydrolysis with diluted hydrochloric acid the corresponding boronic acid **16b**. The resulting compound was subjected to an additional cross-coupling reaction which was carried out with the appropriate bromine derivative following method A for compounds **17a**−**20a** and method B for compound **16a**. The hydrolysis of the methoxy groups with boron tribromide (method C) led to compounds **16**−**20**.

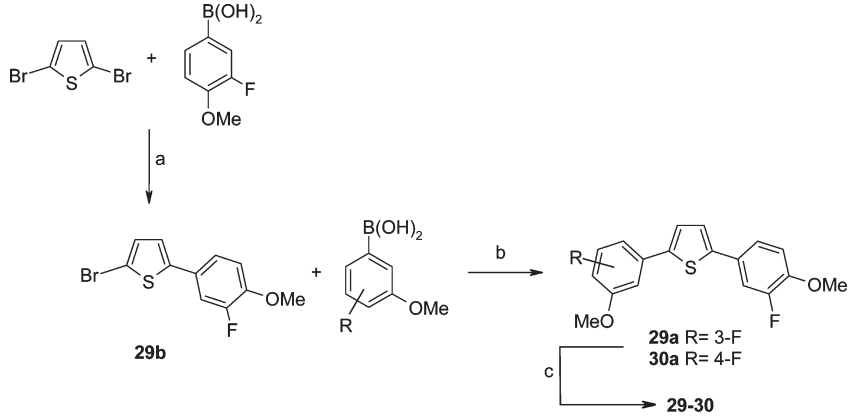
The synthesis of compounds **26**−**28** substituted in ortho-position of the *p*-OH group is depicted in Scheme 4. The preparation of the key intermediate **26b** started from the commercially available 3-formyl-4-methoxyphenylboronic acid. Reduction of the aldehyde function using sodium borohydride followed by a cross-coupling reaction with **1b** under microwave irradiation according to method A (Cs_2CO_3, DME/EtOH/water (1:1:1), $Pd(PPh_3)_4$, microwave (150 W, 150 °C, 15 bar), 15 min) afforded the disubstituted thiophene **26c**. The alcohol function of **26c** was subsequently oxidized with pyridinium chlorochromate to yield to the key aldehyde **26b**. It was subjected to the Horner−Wadworths−Emmons

Scheme 4. Synthesis of Compounds **26**−**28**[a]



[a] Reagents and conditions: (a) $NaBH_4$, THF/EtOH (1:1), 0 °C to room temp, 2 h; (b) method A, Cs_2CO_3, DME/EtOH/water (1:1:1), $Pd(PPh_3)_4$, microwave (150 W, 150 °C, 15 bar), 15 min; (c) PCC, CH_2Cl_2, room temp, 30 min; (d) NaH, THF dry, room temp, 4 h; (e) BBr_3, CH_2Cl_2, −78 °C to room temp, 18 h; (f) (1) LiOH, THF/H_2O (2:1), reflux, 20 h, (2) aniline, EDCI, HOBt, CH_2Cl_2, reflux, 20 h; (g) $Pd(OH)_2$, THF/EtOH (1:1), H_2, room temp, 20 h.

Scheme 5. Synthesis of Compounds **29**−**30**[a]

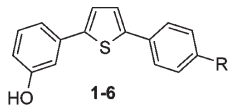


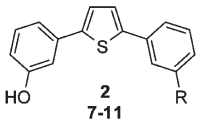
[a] Reagents and conditions: (a) method B, Na_2CO_3, toluene/water (1:1), $Pd(PPh_3)_4$, reflux, 20 h; (b) method A, Cs_2CO_3, DME/EtOH/water (1:1:1), $Pd(PPh_3)_4$, microwave (150 W, 150 °C, 15 bar), 15 min; (c) BBr_3, CH_2Cl_2, −78 °C to room temp, 18 h.

conditions[40] to introduce the acrylic ester moiety (intermediate **26a**). Hydrolysis of the ester function using lithium hydroxide,[25] amide bond formation with aniline, EDCI, and HOBt[41] afforded compound **27a**. The catalytic double bond hydrogenation of **27a** was performed using Perlman's catalyst.[42] The ether functions of **26a**, **27a**, and **28a** were deprotected using boron tribromide (method C) to give the desired compounds **26**−**28**.

The synthesis of the difluorinated thiophenes **29** and **30** is presented in Scheme 5. These compounds were obtained after two successive cross-coupling reactions. In the first step 2,5-dibromothiophene reacted with 3-fluoro-4-methoxyphenylboronic acid following method B (Na_2CO_3, toluene/water (1:1), $Pd(PPh_3)_4$, reflux, 20 h). In the second step, the resulting monosubstituted thiophene **29b** was subsequently submitted to a second cross-coupling reaction under microwave

Table 1. Effect of the Exchange of One OH Substituent for Other Functional Groups on Human 17β-HSD1 and 17β-HSD2 Inhibitory Activities[e]





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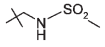
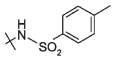
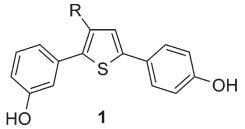
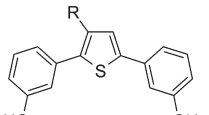
cmpd	R	IC$_{50}$ (nM)[a] 17β-HSD1[b]	IC$_{50}$ (nM)[a] 17β-HSD2[c]	selectivity factor[d]	cmpd	R	IC$_{50}$ (nM)[a] 17β-HSD1[b]	IC$_{50}$ (nM)[a] 17β-HSD2[c]	selectivity factor[d]
1	OH	69	1950	28	**7**	OH	173	745	4
2	H	342	2337	7	**2**	H	342	2337	7
3	F	717	3655	5	**8**	F	535	1824	3
4	NH_2	>5000	nt		**9**		523	1575	3
5	SH	>5000	nt		**10**		>1000	nt	
6	CN	>1000	nt		**11**		350	276	1

[a] Mean values of three determinations, standard deviation less than 10%. [b] Human placenta, cytosolic fraction, substrate [^{3}H]E1 + E1 [500 nM], cofactor NADH [500 μM]. [c] Human placenta, microsomal fraction, substrate [^{3}H]E2 + E2 [500 nM], cofactor NAD^+ [1500 μM]. [d] IC$_{50}$(17β-HSD2)/IC$_{50}$(17β-HSD1). [e] ni: no inhibition. nt: not tested.

Table 2. Inhibition of Human 17β-HSD1 and 17β-HSD2 by Compounds Bearing a Supplementary Substituent on the Thiophene Core Structure[f]





cmpd	R	IC$_{50}$ (nM)[a] 17β-HSD1[b]	IC$_{50}$ (nM)[a] 17β-HSD2[c]	selectivity factor[d]	cmpd	R	IC$_{50}$ (nM)[a] 17β-HSD1[b]	IC$_{50}$ (nM)[a] 17β-HSD2[c]	selectivity factor[d]
1	H	69	1950	28	**7**	H	173	745	4
					13	CH_3	567	856	1
					14		493[e]	nt	
12		>1000	nt		**15**		119	188	2

[a] Mean values of three determinations, standard deviation less than 10%. [b] Human placenta, cytosolic fraction, substrate [^{3}H]E1 + E1 [500 nM], cofactor NADH [500 μM]. [c] Human placenta, microsomal fraction, substrate [^{3}H]E2 + E2 [500 nM], cofactor NAD^+ [1500 μM]. [d] IC$_{50}$(17β-HSD2)/IC$_{50}$(17β-HSD1). [e] Predicted value, obtained with the LOGIT transformed. [f] ni: no inhibition. nt: not tested.

irradiation (method A: Cs_2CO_3, DME/EtOH/water (1:1:1), $Pd(PPh_3)_4$, microwave (150 W, 150 °C, 15 bar), 15 min) to yield the intermediates **29a** and **30a**. Ether cleavage with boron tribromide led to the final compounds **29** and **30**.

Biological Results

Activity: Inhibition of Human 17β-HSD1. Placental enzyme was partially purified following a described procedure.[27,28] Tritiated E1 was incubated with 17β-HSD1, cofactor, and inhibitor. After HPLC separation of substrate and product, the amount of labeled E2 formed was quantified. The inhibition values of the test compounds are shown in Tables 1−5. Thiophenes **1**, **2**, **7**, and **33**, thiazole **36**, and phenylenes **38** and **41**, identified in our previous article,[28] were used as reference compounds.

It was first investigated whether one of the two hydrophenyl moieties could be exchanged by another functional group having similar properties. Recent results[28] showed that the *m*-hydroxy group is highly important for activity and was therefore maintained in the core structure.

The exchange of the *p*-hydroxy group on the meta−para disubstituted thiophene (**1**, IC_{50} = 69 nM) by a bioisosteric function (F, NH_2, SH) resulted in moderate (**3**, IC_{50} = 717 nM) or weak inhibitors (**4** and **5**, IC_{50} > 5000 nM) of 17*β*-HSD1 (Table 1). Moving the F atom from the para- (compound **3**) to the meta-position (compound **8**) led to a small increase in activity (**8**, IC_{50} = 535 nM vs **3**, IC_{50} = 717 nM). Replacement of the *m*-fluorine for a methylsulfonamide moiety (**9**) did not improve the activity (**9**, IC_{50} = 523 nM vs **8**, IC_{50} = 535 nM), while a compound bearing a bulky substituent like tolylsulfonamide (**11**, IC_{50} = 350 nM) showed comparable activity to the monohydroxylated thiophene (**2**, IC_{50} = 342 nM), indicating that there is some space in this region of the enzyme for substitution but it is unlikely that specific interactions between the tolylsulfonamide moiety and amino acids of the active site take place. The insertion of a C1-linker between the phenyl moiety and the methylsulfonamide group was detrimental for the activity (**9**, IC_{50} = 523 nM vs **10**, IC_{50} > 1000 nM). It can therefore be concluded that the two hydroxy functions are necessary for high activity and the *p*-hydroxy group cannot be replaced by a bioisoteric group.

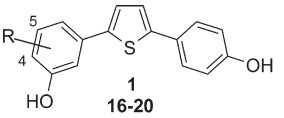
In order to improve the activity and the selectivity of our inhibitors, substituents capable of establishing further interactions with the enzyme were added either on the heterocycle or on the hydroxyphenyl moieties. Additional functional groups were introduced in both of the meta−para 2,5-bis(hydroxyphenyl)thiophene **1** (IC_{50} = 69 nM) and the meta−meta 2,5-disubstituted derivative **7** (IC_{50} = 173 nM).

Concerning substitution on the heterocycle, two kinds of hydrophobic substituents (Me, Ph) were introduced in position 3 on the thiophene ring to investigate the space availability around the core (Table 2). The meta−meta thiophenes bearing a methyl (compound **13**) or phenyl (compound **14**) showed a reduction of activity compared to the reference compound **7** (IC_{50} = 173 nM vs IC_{50}, 567, and 493 nM for **13**, and **14**, respectively). It is striking that in only the case of the meta−meta disubstituted series the insertion of a polar *m*-hydroxyphenyl substituent leads to an increase in activity (**15**, IC_{50} = 119 nM vs **12**, IC_{50} > 1000 nM). This exemplifies that there is space available for further substitution around the heterocycle only in the case of the meta−meta bis(hydroxyphenyl) substitution pattern and that the third *m*-OH group is certainly at an appropriate distance to establish supplementary hydrogen bond interactions with the active site.

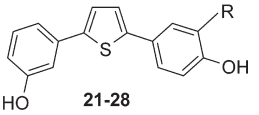
Concerning the substitution of the hydroxyphenyl rings, different groups were introduced either on the *m*-hydroxyphenyl ring (compounds **16**−**20**) or on the *p*-hydroxyphenyl moiety (compounds **21**−**28**, Table 3). The synthesis of compounds bearing ortho (to the heterocycle) substituents at the hydroxyphenyl moieties was not considered, as the conformational constraints induced by the ortho effect will not allow the compound to adopt a planar geometry. Planarity of our structures was considered necessary to mimic the steroidal substrate.[27,28]

Introduction of a substituent in position 5 on the *m*-hydroxyphenyl moiety resulted in the case of a methyl group in a reduction of activity (**16**, IC_{50} = 629 nM vs **1**, IC_{50} = 69 nM). The introduction of a fluorine atom led to a slight increase in activity in comparison to the unsubstituted compound **1** (**17**, IC_{50} = 42 nM vs **1**, IC_{50} = 69 nM). Moving these functional groups to position 4 gave an active fluorinated compound **19** (IC_{50} = 113 nM) and a very weak methylated inhibitor **18** (IC_{50} > 5000 nM). Substituents have also been introduced in position 5 on the *p*-hydroxyphenyl ring: a polar group like hydroxy (compound **22**) or a bulky substituent like the phenyl (compound **25**) in *o*- of the *p*-OH induced a decrease in activity compared to thiophene compound **1** (IC_{50} = 69 nM vs IC_{50} = 402 and > 5000 nM for **22** and **25**, respectively). The introduction of a fluorine substituent into the same position led to the highly potent compound **23** (IC_{50} = 8 nM), while substituents like methyl or trifluoromethyl showed similar or slightly better activities compared to the reference compound **1** (IC_{50} = 69 nM vs IC_{50} = 46 and 38 nM for **21** and **24**, respectively). Other functional groups showing a higher flexibility like ethyl acrylate (compound **26**), phenylacrylamide (compound **27**), or phenylpropaneamide (compound **28**) were also synthesized, and the resulting compounds **26**, **27**, and **28** turned out to have weaker inhibitory activity compared to the unsubstituted thiophene **1** (IC_{50} = 69 nM vs 130, 427, and 620 nM

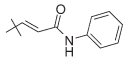
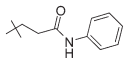
Table 3. Effect of a Supplementary Substituent on the Hydroxyphenyl Moieties on the Inhibition of the Human 17*β*-HSD1 and 17*β*-HSD2[e]



1
16-20

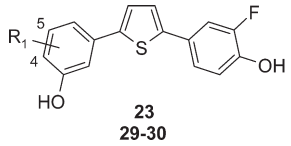


21-28

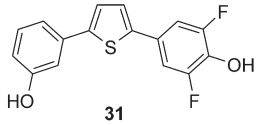
cmpd	R	IC_{50} (nM)[a] 17β-HSD1[b]	IC_{50} (nM)[a] 17β-HSD2[c]	selectivity factor[d]
1	H	69	1950	28
16	5-CH_3	629	2584	4
17	5-F	42	463	11
18	4-CH_3	>5000	nt	
19	4-F	113	183	2
20	4-OH	>5000	nt	
21	CH_3	46	1971	49
22	OH	402	1636	4
23	F	8	940	118
24	CF_3	38	97	3
25	Ph	>5000	nt	
26		130	502	4
27		427	468	1
28		620	982	2

[a] Mean values of three determinations, standard deviation less than 15%. [b] Human placenta, cytosolic fraction, substrate [^{3}H]E1 + E1 [500 nM], cofactor NADH [500 μM]. [c] Human placenta, microsomal fraction, substrate [^{3}H]E2 + E2 [500 nM], cofactor NAD^+ [1500 μM]. [d] IC_{50}(17*β*-HSD2)/IC_{50}(17*β*-HSD1). [e] ni: no inhibition. nt: not tested.

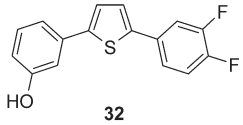
Table 4. Effect of Two Additional Fluorine Atoms on the 17β-HSD1 and 17β-HSD2 Inhibitory Activity



23
29-30



31



32

compd	R_1	IC_{50} (nM)[a] 17β-HSD1[b]	17β-HSD2[c]	selectivity factor[d]
23	H	8	940	118
29	5-F	29	227	8
30	4-F	17	218	13
31		56	312	6
32		780	2640	3

[a] Mean values of three determinations, standard deviation less than 10%. [b] Human placenta, cytosolic fraction, substrate [^{3}H]E1 + E1 [500 nM], cofactor NADH [500 μM]. [c] Human placenta, microsomal fraction, substrate [^{3}H]E2 + E2 [500 nM], cofactor NAD^+ [1500 μM]. [d] IC_{50}(17β-HSD2)/IC_{50}(17β-HSD1).

for **26**, **27**, and **28**, respectively). The low activity of the unconjugated compound **28** indicates that an overall distributed electronic density is an important parameter for activity. These results also indicate that there is space available in this area for substituents but the nature of the substituents is probably not yet optimal (Table 3).

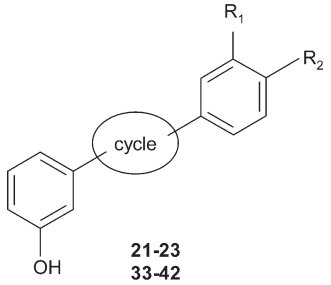
The influence of the introduction of a second fluorine on the highly active thiophene **23** (IC_{50} = 8 nM), either one F on each hydroxyphenyl ring or two F atoms on the same hydroxyphenyl moiety, was also examined (Table 4). When the two F atoms are located on each hydroxyphenyl moiety, the 4-substituted fluoro derivative (compound **30**) is slightly more potent than the one with the fluorine in the 5-position (**29** IC_{50} = 29 nM vs **30** IC_{50} = 17 nM). A small decrease in activity was observed when the two fluorine substituents were present at the same hydroxyphenyl ring (compound **31**, IC_{50} = 56 nM). The exchange of the *p*-OH function of **23** by a fluorine atom (compound **32**) confirmed the essential role of this *p*-hydroxy moiety as previously observed.

Methyl and fluorine substituents have been identified as functional groups able to increase the inhibitory activity of the 2,5-bis(hydroxyphenyl)thiophene **1**. Previously[28] we reported that other central core structures like 2,4-thiophene, 2,5-thiazole, and 1,4-benzene lead to highly active compounds. The influence of an additional methyl or fluorine substituent at these structures was therefore also investigated (Table 5). Introduction of CH_3 or F into the *p*-hydroxyphenyl ring of **33**, **36**, and **38** resulting in compounds **34**, **35**, **37**, **39**, and **40** led to similarly active derivatives in the cases of **34** and **35** (IC_{50} = 64 nM vs **21** IC_{50} = 46 nM). A decrease in inhibitory activity in the thiazole and in the benzene classes of compounds was observed compared to the thiophene family (**37** IC_{50} = 143 nM vs **21** IC_{50} = 46 nM; **40** and **42** IC_{50} = 123 and 51 nM, respectively, vs **23** IC_{50} = 8 nM). Among the investigated molecules, introduction of a methyl or fluorine substituent led to an increase in activity only in the class of the bis(hydroxyphenyl)thiophenes.

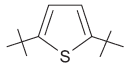
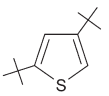
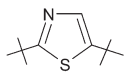
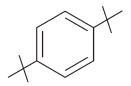
Selectivity: Inhibition of 17β-HSD2 and Affinities to the Estrogen Receptors α and β. In order to gain insight into the selectivity of the most active compounds, inhibition of 17β-HSD2 and the relative binding affinities to the estrogen receptors α and β were determined.

Since 17β-HSD2 catalyzes the inactivation of E2 into E1, inhibitory activity toward this enzyme must be avoided. The 17β-HSD2 inhibition was determined using an assay similar to the 17β-HSD1 test. Placental microsomes were incubated with tritiated E2 in the presence of NAD^+ and inhibitor.

Table 5. Influence of the Core and a Supplementary Substituent on the Inhibition of the Human 17β-HSD1 and 17β-HSD2



21-23
33-42

cmpd	cycle	R_1	R_2	IC_{50} (nM)[a] 17β-HSD1[b]	17β-HSD2[c]	selectivity factor[d]
21		CH_3	OH	46	1971	49
23		F	OH	8	940	118
33		H	OH	77	1270	16
34		CH_3	OH	64	869	14
35		F	OH	64	510	8
36		H	OH	50	4000	80
37		CH_3	OH	143	2023	14
38		H	OH	471	4509	10
39		CH_3	OH	171	1248	7
40		F	OH	123	872	7
41		OH	H	173	2259	21
42		OH	F	51	253	5

[a] Mean values of three determinations, standard deviation less than 13%. [b] Human placenta, cytosolic fraction, substrate [^{3}H]E1 + E1 [500 nM], cofactor NADH [500 μM]. [c] Human placenta, microsomal fraction, substrate [^{3}H]E2 + E2 [500 nM], cofactor NAD^+ [1500 μM]. [d] IC_{50}(17β-HSD2)/IC_{50}(17β-HSD1).

Table 6. Binding Affinities for the Human Estrogen Receptors α and β of Selected Compounds

	RBA[a] (%)	
compd	ERα[b]	ERβ[b]
1	0.1 < RBA < 1	1.5
17	0.1 < RBA < 1	0.1 < RBA < 1
21	< 0.01	< 0.01
23	0.01 < RBA < 0.1	1
30	0.1	0.01 < RBA < 0.1
34	0.01 < RBA < 0.1	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.

Separation and quantification of labeled product (E1) was performed by HPLC using radiodetection. A selection of the most potent 17β-HSD1 inhibitors was tested for inhibition of 17β-HSD2. IC_{50} values and selectivity factors (IC_{50}(HSD2)/IC_{50}(HSD1)) are presented in Tables 1−5.

Monohydroxylated compounds (Table 1) exhibited a poor selectivity regarding 17β-HSD2, the most selective one being compound **3** with a selectivity factor of 5. This finding suggests that the *p*-OH is important for activity as well as for selectivity (selectivity of the para−meta derivative **1** is 28). Introduction of further substituents (Tables 2−5) into the highly active bis(hydroxyphenyl) scaffold induced a loss of selectivity against 17β-HSD2 except in case of compounds **21** and **23**, which exhibit excellent selectivity factors of 49 and 118, respectively.

A further prerequisite for 17β-HSD1 inhibitors to be used as potential drugs is that they do not show affinity for ERα and ERβ, since binding to these receptors could counteract the therapeutic concept of selective 17β-HSD1 inhibition. The binding affinities of the most selective compounds of this study were determined using recombinant human protein in a competition assay applying [^{3}H]E2 and hydroxyapatite (Table 6). All tested compounds show very marginal to marginal affinity to the ERs except compound **23**, which binds weakly to ERβ (RBA = 1%). Compound **21** was evaluated for estrogenic effects on the ER-positive, mammary tumor T47D cell line. No agonistic, i.e., stimulatory effect was observed after application of compound **21** even at a concentration 1000-fold higher compared to E2.

Further Biological Evaluations. Additionally, the intracellular potency of compounds **21**, **23**, and **34** on E2 formation was evaluated using a cell line that expresses both 17β-HSD1 and 17β-HSD2 (T47D cells). The compounds inhibited the formation of E2 after incubation with labeled E1 showing IC_{50} values of 426, 282, and 362 nM for **21**, **23**, and **34**, respectively. These results indicate that the compounds are able to permeate the cell membrane and inhibit the transformation of E1 into E2.

The same compounds (**21**, **23**, and **34**) were further investigated for inhibition of three human hepatic enzymes: CYP3A4, CYP2D6, and CYP2C19. The three compounds showed very little to weak inhibition of the hepatic CYP enzymes (10 μM > IC_{50} > 1.50 μM) except for CYP3A4 (IC_{50} = 0.50 and 0.82 μM for **21** and **23**, respectively) and CYP2C19 (IC_{50} = 0.94 μM for **21**) for which an enhanced inhibition was observed.

The relatively high inhibition of CYP3A4 by **21** and **23** has to be taken into consideration for the further optimization process but should not have an impact on the proof of concept in vitro.

Table 7. Pharmacokinetic Parameters of Compounds **21** and **23** in Male Rats after Oral Application (10 mg/kg)

	compd	
parameters[a]	**21**	**23**
$C_{max\,obs}$ (ng/mL)	905.0	1388.2
C_z (ng/mL)	43.3	24.9
$t_{max\,obs}$ (h)	4.0	8.0
t_z (h)	24.0	24.0
$t_{1/2z}$ (h)	3.8	2.7
$AUC_{0-\infty}$ (ng/mL)	12275	19407

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

The pharmacokinetic profiles of the two most active and selective compounds of this study (**21** and **23**) were determined in rats after oral administration in a cassette dosing approach. 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 7. The maximal concentration ($C_{max\,obs}$) as well as the AUC value is higher for compound **23** (C_{max} = 1388.2 ng/mL, AUC = 19407 ng/mL) than for compound **21** (C_{max} = 905.0 ng/mL, AUC = 12275 ng/mL). The maximal plasma concentration ($t_{max\,obs}$) for compounds **21** and **23** was reached after 4.0 and 8.0 h, respectively. These data show that both compounds exhibit excellent pharmacokinetic properties in the rat and might therefore be good candidates for further experiments in disease-oriented rat models.

Computational Chemistry

Molecular Modeling. From the biological results it became apparent that introduction of a fluorine atom in the position ortho to the *p*-OH phenylthiophene (compound **23**) led to a significant increase in the 17β-HSD1 inhibitory activity. To get an insight into the binding mode of this compound and to better understand the favorable interactions achieved by this inhibitor in the active site, computational studies were performed by means of the docking software GOLD, version 3.2, and Autodock 4.1.

The choice of the 3D-structure of the enzyme, i.e., crystal structure, and the oxidation state of the cofactor, i.e., NADPH or $NADP^+$, used for the docking studies are crucial for obtaining reliable results.

We focused on X-ray structures of 17β-HSD1 having a high resolution and showing a ternary complex (to get closer to the in vivo conditions). Three structures fulfilled these criteria: 1FDT and 1A27 both describing the ternary complex; enzyme−E2−$NADP^+$ and 1I5R, describing the binary complex; enzyme−steroidal hybride inhibitor (HYC), which is an adenosine moiety linked to an E2 core via a C9-linker. These three crystal structures differ mainly in the location of the amino acids belonging to the flexible loop αG′βF (Pro187-Pro200). Since this loop borders both the SUB and the COF, its conformational variations strongly influence the size of both binding cavities. It is therefore important to take care of the position of this loop in the structures used for the docking studies.

In the X-ray structure 1FDT, the residues 187−200 are not well resolved but two plausible conformations for the loop (noted 1FDT-A and 1FDT-B) have been described.[35] The backbones of these two loops are similar (rmsd of ~1 Å), while the main difference is given by the orientation of the side chains, mainly concerning the four amino acids Phe192, Met193, Glu194, and Lys195. In 1FDT-A, Phe192 and Met193 are turned toward the outer part of the enzyme while Glu194 and Lys195 are oriented toward the substrate and the cofactor (extending the substrate binding site = open conformation). On the other hand, in 1FDT-B these two pairs of residues show a reversed orientation limiting length and volume of the steroid binding site compared to 1FDT-A (=closed conformation). Although others[22] have only considered 1FDT-B, we decided to investigate both conformations of this loop.

Interestingly, the flexible loop in 1A27 shows a comparable geometry as observed in 1FDT-B, with Phe192 and Met193 oriented toward the nicotinamide moiety, also restricting the space in the substrate binding site. In the case of 1I5R, the loop is shifted in the direction of the cofactor, resulting in a different conformation compared to both 1FDT-A and 1FDT-B, although, like for 1FDT-A, it extends the SUB.

The docking studies were performed with NADPH as cofactor, as it is described that NADPH is present in a 500-fold higher concentration than its oxidized form $NADP^+$ in living cells.[43,44]

Compound **23** was docked with NADPH into four different X-ray structures: 1FDT-A, 1FDT-B, 1A27, and 1I5R. Two different binding modes were observed for compound **23**. In the cases of 1FDT-B and 1A27, the inhibitor is located exclusively in the steroid binding site (Figure 1), adopting a similar orientation as previously described for the bis-(hydroxyphenyl)oxazole **B**[27] (Chart 1), while for 1FDT-A and 1I5R, the inhibitor is located between the steroid and the cofactor binding sites, interacting with the nicotinamide moiety. In the following, this binding mode will be named as alternative binding mode (Figure 2).

In the case of the steroidal binding mode (1FDT-B and 1A27, Figure 1) the following specific interactions can be observed: hydrogen bond interactions between the *m*-hydroxy group of **23** and Ser142/Tyr155 (d_{O-O} = 2.6 Å for both amino acids) and between the *p*-OH group and His221/Glu282 (d_{O-N} = 2.8 Å and d_{O-O} = 3.8 Å, Figure 1). Additionally, hydrophobic interactions and $\pi-\pi$ stackings (Phe226, Phe259) are also involved.

In the alternative binding mode obtained using 1FDT-A and 1I5R (Figure 2), compound **23** is also stabilized by hydrogen bond interactions: the *m*-OH group forms a strong H-bond with the phosphate group of the cofactor (d_{O-O} = 2.9 Å). The fluorine atom could establish halogen bonds with the backbone −NH− of Val143 and Gly144 (d_{F-N} = 3.2 and 3.8 Å, respectively), in addition to a halogen bond with the OH-group of Ser142 (d_{F-O} = 3.5 Å) which is involved in the catalytic process. Further, the *p*-OH points perpendicular toward Phe259 ($d_{O\text{-centroid}}$ = 4.5 Å), indicating a possible OH−π interaction. This could explain the importance of this group observed in the SARs. Moreover, strong $\pi-\pi$ stacking interactions seem to stabilize the inhibitor in this binding mode: between the *m*-OH-phenylthiophene moiety and the nicotinamide part of the cofactor (parallel-displaced configuration; distance between the two ring centers, 4.3 Å) and between the *p*-OH-phenylthiophene moiety and Phe226 (T-shape conformation; closest C−C contact distance, 3.7 Å). Moreover, electrostatic interactions between the sulfur atom of the heterocycle with the surrounding amino acids like Tyr155 and Ser142 might also play a role as described.[45]

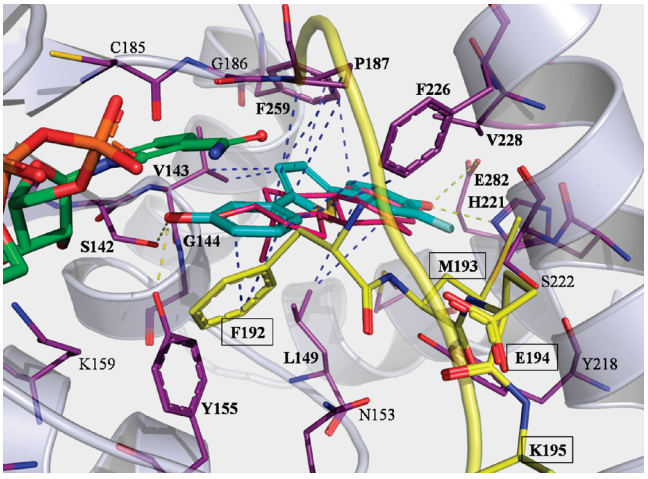


Figure 1. Docking complex between 17β-HSD1 (X-ray 1FDT-B) and compound **23** (blue, SUB binding mode). NADPH, interacting residues, and ribbon rendered tertiary structure of the active site are shown. Residues of the flexible loop are rendered in sticks and colored in yellow. Hydrogen bonds and $\pi-\pi$ stackings (and hydrophobic interactions) are drawn in yellow and blue dashed lines, respectively. For comparison, E2 is depicted in magenta lines. Figures were generated with Pymol (http://www.pymol.org).

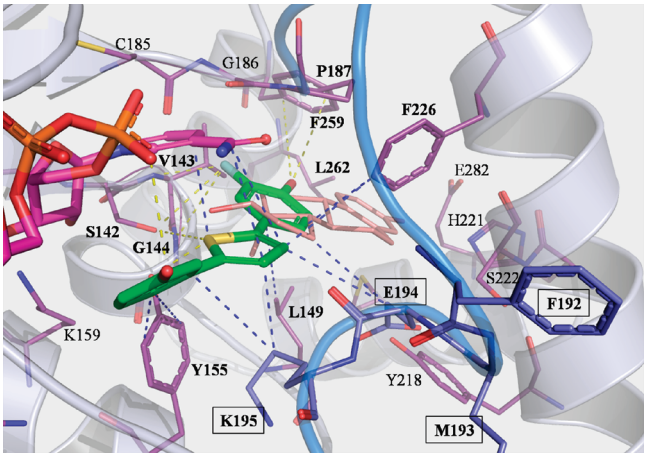


Figure 2. Docking complex between 17β-HSD1 (X-ray 1FDT-A) and compound **23** (green, alternative binding mode). NADPH, interacting residues, and ribbon rendered tertiary structure of the active site are shown. Residues of the flexible loop are rendered in sticks and colored in blue. Hydrogen bonds and $\pi-\pi$ stackings (and hydrophobic interactions) are drawn in yellow and blue dashed lines, respectively. For comparison, E2 is depicted in magenta lines.

The results presented so far suggest that both binding modes have to be considered as possible for this class of inhibitors. They depend mainly on the orientation of the flexible loop. There is only one conformation of the loop leading to a steroidal binding mode (1FDT-B/1A27). In the case of 1FDT-A/1I5R the pose showing the alternative binding mode is obtained using two X-ray structures having two different conformations of the loop. Unfortunately, because of the almost identical scoring function values observed for both poses with the docking programs (Gold and Autodock), it was not possible to determine which

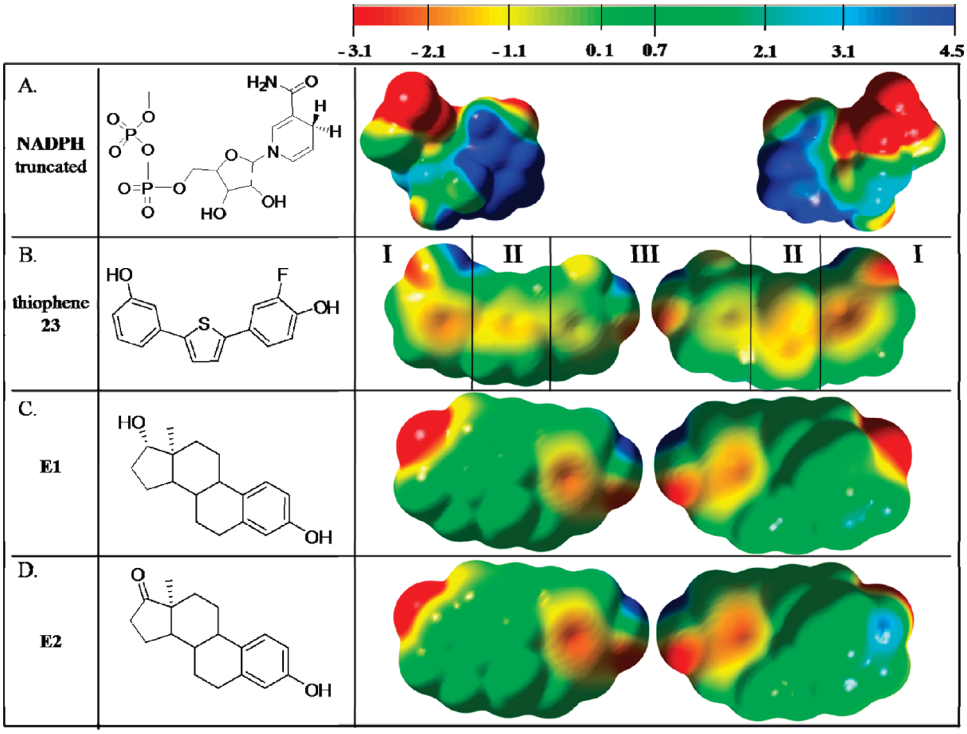


Figure 3. Structures and MEP maps of both ventral (steroidal α-side) and dorsal (steroidal β-side) views of truncated NADPH (A), thiophene **23** (B), E2 (C), and E1 (D). MEP surfaces were plotted with GaussView, version 3.0.

model (1FDT-A/1I5R or 1FDT-B/1A27) is the most appropriate to describe the interactions between the inhibitor and the enzyme and therefore which is the most plausible binding mode.

Comparing both poses obtained by docking of **23** in 1FDT-A and 1FDT-B shows that there is a common area in the neighborhood of the catalytic tetrade that corresponds to the D-ring of the enzyme−substrate complex (figure in Supporting Information).

Molecular Electrostatic Potential (MEP). Recently we reported on the influence of the electronic density (MEP maps, "semi-QMAR") on the potency of the inhibitors in this class of compounds.[28] The 3D-structures of the inhibitors were virtually divided into three areas, and a given optimal range of ESP values (in hartree) was determined for each region (-1.7×10^{-2} to -1.2×10^{-2} for I, -1.6×10^{-2} to -0.9×10^{-2} for II, and -1.2×10^{-2} to -0.5×10^{-2} for III). The MEPs of compound **23** were calculated as shown in Figure 3. The molecular ESP distribution observed (-1.8×10^{-2} to -1.2×10^{-2} for I, -1.6×10^{-2} to -0.8×10^{-2} for II, and -1.1×10^{-2} to -0.4×10^{-2} for III) fitted well to the optimal ranges identified previously, confirming the correlation between the ESP range and the potency of the compounds. The MEP maps of the natural substrate E1 and of E2 were also calculated (Figure 3) and compared to the one of **23**. The finding that the ESP distribution of **23** and E2/E1 is very different might be an indication that compound **23** does not bind in the same way as the steroid.

According to the alternative binding mode, the *m*-hydroxyphenylthiophene part of **23** overlaps with the nicotinamide part of the cofactor and forms stabilizing $\pi-\pi$ interactions. The ESP distribution of these two entities should therefore show complementarity. To get an insight into this, the MEP map of a truncated NADPH, the counterpart of the *m*-OH-phenylthiophene moiety, was calculated by ab initio methods. As can be seen in Figure 3, a certain complementarity was observed. The NADPH MEP maps give the explanation for the observation[28] that a strong polarization between the vertex and the base of the central ring of the inhibitors is negative for binding. Positive ESP values on the vertex side lead to repulsion effects with the nicotinamide and therefore reduce the inhibitory activity. This finding indicates that this class of compound might bind according to the alternative binding mode (Figure 2). However, this hypothesis needs to be further investigated.

Discussion and Conclusion

Structural optimizations of compound **1** led to the discovery of new substituted 2,5-bis(hydroxyphenyl)thiophene derivatives, the fluorinated **23** and the methylated **21** being the most active and selective inhibitors identified.

From a previous work in this class of compounds,[28] it was demonstrated that removal of one of the two hydroxyphenyl moieties is detrimental for the activity. In this paper it was shown that replacement of the *p*-OH function by a bioisoteric group like F, NH_2, SH, CN leads to a reduction of activity. The lack of hydrogen donating properties of the fluoro and cyano substituents might not be the only reason for this decrease in activity, as the amino and the thiol derivatives are also less active than the parent compound **1**. Interestingly, the omission of a C1-linker between the methylsulfonamide moiety and the phenyl ring (compound **9**) resulted in an increase of potency. Deprivation of electrons from the phenyl ring obviously is necessary for good inhibition. The relatively high activity observed for compound **11** (IC_{50} = 350 nM) especially compared to compound **9** (IC_{50} = 523 nM) demonstrates that in the protein there is some space available in this position for a bulky substituent. Furthermore, the tolyl

group might also be involved in the stabilization of the inhibitor in the binding site, establishing $\pi-\pi$ stacking interactions with appropriate amino acid residues present in this region.

With the aim to increase the activity and the selectivity in this class of compounds, substituents were introduced on the 2,5-bis(hydroxyphenyl)thiophenes **1** and **7**. This was successful for compound **15** (IC_{50} = 119 nM vs **7**, IC_{50} = 173 nM). Apparently, the formation of an additional hydrogen bond is responsible for this increase in inhibitory activity, while a pure $\pi-\pi$ stacking interaction as supposed for compound **14** is not sufficient. The 2,5-disubstituted thiophenes **12** and **15** differ only in the position of one hydroxy group (para, compound **12**; meta, compound **15**). The fact that compound **15** shows a much higher activity (IC_{50} = 119 nM vs **12**, IC_{50} > 1000 nM) indicates that only in case of **15** is the geometry of the OH groups is acceptable for a reasonable interaction. It demonstrates, as observed already,[24,27] a sharp SAR and a reduced flexibility in this region of the active site.

The trisubstituted compound **14** bearing a phenyl substituent at the thiophene differs from the triazole **A**[32] (Chart 1) only in the nature of the heterocycle. The following comparisons highlight the importance of the heterocycle for the potency of the molecules: inactive compound **A** vs thiophene **14** (IC_{50} = 493 nM), thiophene **21** (IC_{50} = 46 nM) vs thiazole **37** (IC_{50} = 143 nM), and thiophene **23** (IC_{50} = 8 nM) vs benzene **40** (IC_{50} = 123 nM). It becomes apparent that the thiophene ring is the most appropriate heterocycle for high inhibitory activity. Provided that all compounds bind according to the same binding mode, there are different explanations for these results: (1) The presence and the position of one or several nitrogens in the heterocycle modulate the MEP distribution of the compound and in particular the electronic distribution of the *m*-hydroxyphenyl moiety[28] (in the case of the compounds under consideration, resulting in inadequate electronic properties for binding). (2) Similarly the replacement of the sulfur leads to an inadequate repartition of the electron density in the heterocycle. (3) A reduced flexibility in the binding site is responsible for the enzyme not being able to adjust its geometry to the different hydroxyphenyl moieties (depending on the heterocycle, the angles between the phenyl-OHs are different).

A high increase in activity and selectivity could be reached by introduction of substituents into the hydroxyphenyl moiety, especially when the substituent is located ortho of the *p*-hydroxyphenyl group (compounds **21**−**24**). Not all substituents are equally well tolerated: there is no space available for a phenyl group (compound **25**). An additional OH group (compound **22**) is obviously not able to establish specific interactions, while small lipophilic substituents (methyl, **21**; fluorine, **23**; trifluoromethyl, **24**) are enhancing the activity. There is enough space in this region of the enzyme to introduce a flexible chain (**26**), but conjugation seems to be necessary to achieve a higher activity as already observed with the tolylsulfonamide substituent (compound **11**).

The positive influence of the fluorine atom has often been demonstrated in medicinal chemistry[46−48] and was also proven in this study with compound **23** (IC_{50} = 8 nM). The position of the fluorine is decisive for an increase in activity: it has to be in the meta-position (**17** and **23**). Highest activity was achieved at the position ortho of the hydroxy group (compound **23**). This indicates that either direct interactions of the fluorine with amino acid residues in this region of the active site or the increase of acidity of the neighboring OH groups might be responsible for this effect.

Introduction of a second fluorine atom into this fluorohydroxyphenyl ring (compound **31**) does not enhance the activity, suggesting that the effects of the fluorine are not additive. A second fluorine was also added to the other hydroxyphenyl moiety (in ortho and meta of the *m*-OH group), leading to compounds **29** and **30**. However, no enhancement of the activity compared to the monofluorinated **23** was observed, indicating that there are no specific interactions of the second fluoro substituent.

A close look at the X-ray structures of 17β-HSD1 crystallized in the presence of different steroidal ligands showed that the flexible loop (amino acids 187−200) can adopt different geometries depending on the nature of the ligand and on the absence or presence of the cofactor in the catalytic region. It indicates that some parts of the enzyme can adapt their geometry to the molecule present in the active site in order to stabilize it. However, other parts are rigid, explaining the sharp SAR observed in this paper and previously.[27,28]

Two plausible conformations of the loop in the ternary complex enzyme−E2−$NADP^+$, PDB code 1FDT, have been described (1FDT-A, 1FDT-B).We have shown that both can be used for docking studies. In the case of 1FDT-A the substrate binding pocket is extended, enhancing the volume of the active site. It is therefore a good model to evaluate an alternative binding mode for inhibitors that are larger than the steroid. A binding mode as observed for steroids in the X-ray structures was found when the loop closes the SUB (1FDT-B). Surprisingly, when the inhibitors were docked to the protein with the loop in the open conformation, they interact with the nicotinamide part of the cofactor. MEP calculations showed a certain complementarity between the electronic density of **23** and of the nicotinamide moiety of the cofactor, indicating that this alternative binding mode is not only plausible but also might be the one that is more likely.

Up to now, in the design of compounds as potential 17β-HSD1 inhibitors, one group[37,49−51] tried to mimic the cofactor. Our finding of the above-mentioned alternative binding mode makes another strategy very promising: the cofactor, which is likely to be present in the active site when the inhibitor is entering, could be used as partner to achieve additional interactions rather than trying to displace it.

The most potent 17β-HSD1 inhibitors **21** and **23** exhibit a higher selectivity toward 17β-HSD2 compared to parent compound (selectivity factors 49 and 118, respectively, vs 28 for **1**). This indicates that the amino acids close to the CH_3 or F substituents must have different properties in the two 17β-HSD enzymes, which could be further exploited to increase selectivity.

The most potent inhibitors show only marginal to very little affinity to the ERα, and no stimulation of cell proliferation (agonistic effect) in the ER-positive T47D cell line could be observed. The weak affinity of compound **23** for ERβ may not be critical, as it is reported that ERβ exhibits antiproliferative effects in breast cancer cells.[52]

Compound **23** might be used in an appropriate animal model to prove the concept of 17β-HSD1 inhibition with nonsteroidal inhibitors. This compound shows a good pharmacokinetic profile in rats.

In this paper, we described the synthesis of substituted bis(hydroxyphenyl)thiophenes, thiazoles, and benzenes as inhibitors of 17β-HSD1 and the evaluation of their biological properties. The most promising compounds of this study, **21** and **23**, exhibit high selectivity toward 17β-HSD2, marginal binding to ERα, and excellent pharmacokinetic profiles in

rats after peroral application. These new compounds provide useful tools to validate 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) was performed 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).

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 ($CDCl_3$, δ = 7.24 ppm (^{1}H NMR) and δ = 77 ppm (^{13}C NMR); CD_3OD, δ = 3.35 ppm (^{1}H NMR) and δ = 49.3 ppm (^{13}C NMR); CD_3COCD_3, δ = 2.05 ppm (^{1}H NMR) and δ = 29.9 ppm (^{13}C NMR); CD_3SOCD_3, δ = 2.50 ppm (^{1}H NMR) and δ = 39.5 ppm (^{13}C NMR)). Signals are described as s, d, t, q, dd, m, dt for singlet, doublet, triplet, quadruplet, 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 (C, H, N) were performed at the Department of Instrumental Analysis and Bioanalysis, Saarland University, Germany. The purity of the compounds, which in all cases was higher than 95%, was determined using two different methods: elemental analysis for compounds **3**−**6**, **8**−**32**, **34**, and **37** and LC/MS for compounds **35**, **39**, **41**, and **42**.

Compounds 2-bromo-5-(3-methoxyphenyl)thiophene (**1b**),[28] 2-(3-methoxyphenyl)-5-(4-methoxyphenyl)thiophene (**1a**),[28] 3-[5-(4-hydroxyphenyl)-2-thienyl]phenol (**1**),[28] 2-(3-methoxyphenyl)-5-phenylthiophene (**2a**),[28] 3-(5-phenyl-2-thienyl)phenol (**2**),[28] 2,5-bis-(3-methoxyphenyl)thiophene (**7a**),[28] 3,3′-thiene-2,5-diyldiphenol (**7**),[28] 2-bromo-5-(4-methoxyphenyl)thiophene (**16c**),[28] 4-bromo-2-iodo-1-methoxybenzene (**25d**),[53] 5-bromo-2-methoxybiphenyl (**25c**),[54] [6-methoxy-1,1′-biphenyl-3-yl]boronic acid (**25b**),[54] [3-(hydroxymethyl)-4-methoxyphenyl]boronic acid (**26d**),[55] 4-bromo-2-(3-methoxyphenyl)thiophene (**33b**),[28] 2-(3-methoxyphenyl)-4-(4-methoxyphenyl)thiophene (**33a**),[28] 3-[4-(4-hydroxyphenyl)-2-thienyl]phenol (**29**),[28] 5-bromo-2-(3-methoxyphenyl)-1,3-thiazole (**36b**),[28] 2-(3-methoxyphenyl)-5-(4-methoxyphenyl)-1,3-thiazole (**36a**),[28] 3-[5-(4-hydroxyphenyl)-1,3-thiazol-2-yl]phenol (**36**),[28] 4′-bromo-3-methoxybiphenyl (**38b**),[28] 3,4″-dimethoxy-1,1′:4′,1″-terphenyl (**38a**),[28] 1,1′:4′,1″-terphenyl-3,4″-diol (**38**),[28] 3,3″-dimethoxy-1,1′:4′,1″-terphenyl (**40a**),[28] and 1,1′:4′,1″-terphenyl-3,3″-diol (**40**)[28] were prepared following described procedures.

General Procedure for Suzuki Coupling. Method A. A mixture of aryl bromide (1 equiv), arylboronic acid (1.2 equiv), cesium carbonate (2.2 equiv), and tetrakis(triphenylphosphine)palladium (0.01 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 cooling to room temperature, 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 column chromatography (CC).

Method B. A mixture of aryl bromide (1 equiv), arylboronic acid (1 equiv), sodium carbonate (2 equiv), and tetrakis-(triphenylphosphine)palladium (0.05 equiv) in an oxygen-free toluene/water (1:1) solution was stirred at 100 °C for 20 h under nitrogen atmosphere. The reaction mixture was cooled to room temperature. 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.

General Procedure for Ether Cleavage. Method C. To a solution of methoxyphenyl 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 room temperature under nitrogen atmosphere. 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, filtered, and concentrated to dryness. The product was purified by preparative thin layer chromatography (TLC).

2-(4-Fluorophenyl)-5-(3-methoxyphenyl)thiophene (3a). The title compound was prepared by reaction of 2-bromo-5-(3-methoxyphenyl)thiophene (**1b**) (150 mg, 0.56 mmol), 4-fluorophenylboronic acid (94 mg, 0.67 mmol), cesium carbonate (383 mg, 1.24 mmol), and tetrakis(triphenylphosphine)palladium (6.4 mg, 5.6 μmol) according to method A. The product was purified by CC (hexane/ethyl acetate 9:1). Yield: 75% (120 mg).

3-[5-(4-Fluorophenyl)-2-thienyl]phenol (3). The title compound was prepared by reaction of 2-(4-fluorophenyl)-5-(3-methoxyphenyl)thiophene (**3a**) (80 mg, 0.28 mmol) and boron tribromide (0.84 mmol) according to method C. The product was purified by preparative TLC (hexane/ethyl acetate 1:1). Yield: 68% (52 mg). MS (ESI): 270 $(M + H)^+$. Anal. ($C_{16}H_{11}FOS$) C, H, N.

4-[5-(3-Methoxyphenyl)-2-thienyl]aniline (4a). The title compound was prepared by reaction of 2-bromo-5-(3-methoxyphenyl)thiophene (**1b**) (150 mg, 0.56 mmol), 4-aminophenylboronic acid (92 mg, 0.67 mmol), cesium carbonate (383 mg, 1.24 mmol), and tetrakis(triphenylphosphine)palladium (6.4 mg, 5.6 μmol) according to method A. The product was purified by CC (hexane/ethyl acetate 7:3). Yield: 63% (100 mg).

3-[5-(4-Aminophenyl)-2-thienyl]phenol (4). The title compound was prepared by reaction of 4-[5-(3-methoxyphenyl)-2-thienyl]aniline (**4a**) (100 mg, 0.37 mmol) and boron tribromide (1.11 mmol) according to method C. The product was purified by preparative TLC (hexane/ethyl acetate 1:1). Yield: 82% (82 mg). MS (ESI): 268 $(M + H)^+$. Anal. ($C_{16}H_{13}NOS$) C, H, N.

4-[5-(3-Methoxyphenyl)-2-thienyl]benzenethiol (5a). The title compound was prepared by reaction of 2-bromo-5-(3-methoxyphenyl)thiophene (**1b**) (250 mg, 0.93 mmol), 4-mercaptophenylboronic acid (172 mg, 1.12 mmol), cesium carbonate (636 mg, 2.05 mmol), and tetrakis(triphenylphosphine)palladium (10.8 mg, 9.3 μmol) according to method A. The product was purified by CC (hexane/ethyl acetate 7:3). Yield: 61% (160 mg).

3-[5-(4-Sulfanylphenyl)-2-thienyl]phenol (5). The title compound was prepared by reaction of 4-[5-(3-methoxyphenyl)-2-thienyl]benzenethiol (**5a**) (150 mg, 0.50 mmol) and boron tribromide (1.50 mmol) according to method C. The product was purified by preparative TLC (hexane/ethyl acetate 1:1). Yield: 81% (115 mg). MS (ESI): 285 $(M + H)^+$. Anal. ($C_{16}H_{12}OS_2$) C, H, N.

4-[5-(3-Methoxyphenyl)-2-thienyl]benzonitrile (6a). The title compound was prepared by reaction of 2-bromo-5-(3-methoxyphenyl)thiophene (**1b**) (200 mg, 0.74 mmol), 4-cyanophenylboronic acid (131 mg, 0.89 mmol), cesium carbonate (508 mg, 1.64 mmol), and tetrakis(triphenylphosphine)palladium (8.5 mg, 7.4 μmol) according to method A. The product was purified by CC (hexane/ethyl acetate 7:3). Yield: 27% (60 mg).

4-[5-(3-Hydroxyphenyl)-2-thienyl]benzonitrile (6). The title compound was prepared by reaction of 4-[5-(3-methoxyphenyl)-2-thienyl]benzonitrile (**6a**) (42 mg, 0.14 mmol) and boron tribromide (0.42 mmol) according to method C. The product was purified by preparative TLC (hexane/ethyl acetate 6:4). Yield: 62% (25 mg). MS (APCI): 277 $(M)^+$. Anal. ($C_{17}H_{11}NOS$) C, H, N.

2-(3-Fluorophenyl)-5-(3-methoxyphenyl)thiophene (8a). The title compound was prepared by reaction of 2-bromo-5-(3-methoxyphenyl)thiophene (**1b**) (150 mg, 0.56 mmol), 3-fluorophenylboronic acid (94 mg, 0.67 mmol), cesium carbonate (381 mg, 1.22 mmol), and tetrakis(triphenylphosphine)-palladium (6.5 mg, 5.6 μmol) according to method A. The product was purified by CC (hexane/ethyl acetate 9:1). Yield: 82% (130 mg).

3-[5-(3-Fluorophenyl)-2-thienyl]phenol (8). The title compound was prepared by reaction of 2-(3-fluorophenyl)-5-(3-methoxyphenyl)thiophene (**8a**) (130 mg, 0.45 mmol) and boron tribromide (1.35 mmol) according to method C. The product was purified by preparative TLC (dichloromethane/methanol 99:1). Yield: 66% (82 mg). MS (ESI): 271 $(M + H)^+$. Anal. ($C_{16}H_{11}FOS$) C, H, N.

***N*-(3-[5-(3-Methoxyphenyl)-2-thienyl]phenyl)methanesulfonamide (9a).** The title compound was prepared by reaction of 2-bromo-5-(3-methoxyphenyl)thiophene (**1b**) (160 mg, 0.60 mmol), 3-methylsulfonylaminophenylboronic acid (155 mg, 0.72 mmol), cesium carbonate (410 mg, 1.32 mmol), and tetrakis(triphenylphosphine)palladium (6.9 mg, 6.0 μmol) according to method A. The product was purified by CC (petroleum ether/ethyl acetate 6:4). Yield: 75% (150 mg).

***N*-(3-[5-(3-Hydroxyphenyl)-2-thienyl]phenyl)methanesulfonamide (9).** The title compound was prepared by reaction of *N*-(3-[5-(3-methoxyphenyl)-2-thienyl]phenyl)methanesulfonamide (**9a**) (150 mg, 0.44 mmol) and boron tribromide (1.32 mmol) according to method C. The product was purified by preparative TLC (hexane/ethyl acetate 1:1). Yield: 61% (92 mg). MS (ESI): 346 $(M + H)^+$. Anal. ($C_{17}H_{15}NO_3S_2$) C, H, N

***N*-(3-[5-(3-Methoxyphenyl)-2-thienyl]benzyl)methanesulfonamide (10a).** The title compound was prepared by reaction of 2-bromo-5-(3-methoxyphenyl)thiophene (**1b**) (150 mg, 0.56 mmol), 3-[(methylsulfonylamino)methyl]benzeneboronic acid (153 mg, 0.67 mmol), cesium carbonate (382 mg, 1.23 mmol), and tetrakis-(triphenylphosphine)palladium (6.5 mg, 5.6 μmol) according to method A. The product was purified by CC (petroleum ether/ethyl acetate 8:2). Yield: 58% (122 mg).

***N*-(3-[5-(3-Hydroxyphenyl)-2-thienyl]benzyl)methanesulfonamide (10).** The title compound was prepared by reaction of *N*-(3-[5-(3-methoxyphenyl)-2-thienyl]benzyl)methanesulfonamide (**10a**) (122 mg, 0.37 mmol) and boron tribromide (1.11 mmol) according to method C. The product was purified by preparative TLC (hexane/ethyl acetate 1:1). Yield: 33% (44 mg). MS (ESI): 360 $(M + H)^+$. Anal. ($C_{18}H_{17}NO_3S_2$) C, H, N.

***N*-(3-[5-(3-Methoxyphenyl)-2-thienyl]phenyl)-4-methylbenzenesulfonamide (11a).** The title compound was prepared by reaction of 2-bromo-5-(3-methoxyphenyl)thiophene (**1b**) (150 mg, 0.56 mmol), [3-[[(4-methylphenyl)sulfonyl]amino]phenyl]boronic acid (195 mg, 0.67 mmol), cesium carbonate (383 mg, 1.23 mmol), and tetrakis(triphenylphosphine)palladium (6.5 mg, 5.6 μmol) according to method A. The product was purified by CC (petroleum ether/ethyl acetate 9:1). Yield: 88% (214 mg).

***N*-(3-[5-(3-Hydroxyphenyl)-2-thienyl]phenyl)-4-methylbenzenesulfonamide (11).** The title compound was prepared by reaction of *N*-(3-[5-(3-methoxyphenyl)-2-thienyl]phenyl)-4-methylbenzenesulfonamide (**11a**) (214 mg, 0.49 mmol) and boron tribromide (1.47 mmol) according to method C. The product was purified by preparative TLC (hexane/ethyl acetate 1:1). Yield: 75% (156 mg). MS (APCI): 421 $(M)^+$. Anal. ($C_{23}H_{19}NO_3S_2$) C, H, N.

3,5-Dibromo-2-(3-methoxyphenyl)thiophene (12c). The title compound was prepared by reaction of 2,3,5-tribromothiophene (100 mg, 0.31 mmol), 3-methoxybenzeneboronic acid (46 mg, 0.31 mmol), sodium carbonate (67 mg, 0.62 mmol), and tetrakis(triphenylphosphine)palladium (17.9 mg, 15.5 μmol) according to method B, heating the mixture 4 h instead of 20 h. The product was purified by CC (petroleum ether/ethyl acetate 9:1). Yield: 23% (25 mg).

3-Bromo-2-(3-methoxyphenyl)-5-(4-methoxyphenyl)thiophene (12b). The title compound was prepared by reaction of 3,5-dibromo-2-(3-methoxyphenyl)thiophene (**12c**) (500 mg, 1.43 mmol), 4-methoxybenzeneboronic acid (268 mg, 1.72 mmol), sodium carbonate (333 mg, 3.15 mmol), and tetrakis-(triphenylphosphine)palladium (82.6 mg, 71.5 μmol) according to method B. The product was purified by CC (petroleum ether/ethyl acetate 9:1). Yield: 52% (278 mg).

2,3-Bis(3-methoxyphenyl)-5-(4-methoxyphenyl)thiophene (12a). The title compound was prepared by reaction of 3-bromo-2-(3-methoxyphenyl)-5-(4-methoxyphenyl)thiophene (**12b**) (250 mg, 0.67 mmol), 3-methoxybenzeneboronic acid (124 mg, 0.80 mmol), sodium carbonate (142 mg, 1.34 mmol), and tetrakis-(triphenylphosphine)palladium (38.7 mg, 33.5 μmol) according to method B. The product was purified by CC (petroleum ether/ethyl acetate 9:1). Yield: 72% (194 mg).

3,3′-[5-(4-Hydroxyphenyl)thiene-2,3-diyl]diphenol (12). The title compound was prepared by reaction of 2,3-bis(3-methoxyphenyl)-5-(4-methoxyphenyl)thiophene (**12a**) (100 mg, 0.24 mmol) and boron tribromide (2.16 mmol) according to method C. The product was purified by preparative TLC (hexane/ethyl acetate 1:1). Yield: 92% (79 mg). MS (ESI): 359 $(M - H)^+$. Anal. ($C_{22}H_{16}O_3S$) C, H, N.

3,3′-(3-Methylthiene-2,5-diyl]diphenol (13). The title compound was prepared by reaction of 2,5-dibromo-3-methylthiophene (150 mg, 0.58 mmol), 3-hydroxybenzeneboronic acid (179 mg, 1.27 mmol), cesium carbonate (868 mg, 2.79 mmol), and tetrakis(triphenylphosphine)palladium (6.7 mg, 5.8 μmol) according to method A. The product was purified by CC (hexane/ethyl acetate 4:6). Yield: 45% (73 mg). MS (ESI): 281 $(M - H)^+$. Anal. ($C_{17}H_{14}O_2S$) C, H, N.

3-Bromo-2,5-bis(3-methoxyphenyl)thiophene (14b). The title compound was prepared by reaction of 3,5-dibromo-2-(3-methoxyphenyl)thiophene (**12c**) (250 mg, 0.72 mmol), 3-methoxybenzeneboronic acid (134 mg, 0.86 mmol), sodium carbonate (148 mg, 1.44 mmol), and tetrakis(triphenylphosphine)palladium (41.6 mg, 36.0 μmol) according to method B. The product was purified by CC (petroleum ether/ethyl acetate 9:1). Yield: 72% (194 mg).

2,5-Bis(3-methoxyphenyl)-3-phenylthiophene (14a). The title compound was prepared by reaction of 3-bromo-2,5-bis(3-methoxyphenyl)thiophene (**14b**) (102 mg, 0.27 mmol), benzeneboronic acid (38 mg, 0.27 mmol), sodium carbonate (58 mg, 0.54 mmol), and tetrakis(triphenylphosphine)palladium (15.6 mg, 13.5 μmol) according to method B. The product was purified by CC (petroleum ether/ethyl acetate 9:1). Yield: 54% (51 mg).

3,3′-(3-Phenylthiene-2,5-diyl)diphenol (14). The title compound was prepared by reaction of 2,5-bis(3-methoxyphenyl)-3-phenylthiophene (**14a**) (50 mg, 0.13 mmol) and boron tribromide (0.78 mmol) according to method C. The product was purified by preparative TLC (hexane/ethyl acetate 1:1). Yield: 53% (49 mg). MS (ESI): 345 $(M + H)^+$. Anal. ($C_{22}H_{16}O_2S$) C, H, N.

2,3,5-Tris(3-methoxyphenyl)thiophene (15a). The title compound was prepared by reaction of 3-bromo-2,5-bis(3-methoxyphenyl)thiophene (**14b**) (102 mg, 0.27 mmol), 3-methoxybenzene boronic acid (42 mg, 0.27 mmol), sodium carbonate (58 mg, 0.54 mmol), and tetrakis(triphenylphosphine)palladium (15.6 mg, 13.5 μmol) according to method B. The product was purified by CC (petroleum ether/ethyl acetate 9:1). Yield: 34% (37 mg).

3,3′,3″-Thiene-2,3,5-triyltriphenol (15). The title compound was prepared by reaction of 2,3,5-tris(3-methoxyphenyl)-thiophene (**15a**) (37 mg, 0.09 mmol) and boron tribromide (0.81 mmol) according to method C. The product was purified by preparative TLC (hexane/ethyl acetate 1:1). Yield: 67% (21 mg). MS (ESI): 361 $(M + H)^+$. Anal. ($C_{22}H_{16}O_3S$) C, H, N.

5-(4-Methoxyphenyl)-2-(boronic acid)thiophene (16b). To a solution of 2-bromo-5-(4-methoxyphenyl)thiophene (**16c**) (100 mg, 0.37 mmol, 1 equiv) in anhydrous 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 at −78 °C. After 15 min, triethyl

borate (0.37 mL, 2.22 mmol, 6 equiv) was added at −78 °C and the mixture was stirred for 2 h. After warming to room temperature, the crude material was acidified with 20 mL of a 1 N hydrochloric acid solution. The aqueous layer was washed with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered, and evaporated under reduced pressure. The title compound was not characterized and used without further purification.

2-(3-Methoxyphenyl-5-methylphenyl)-5-(4-methoxyphenyl)-thiophene (16a). The title compound was prepared by reaction of 1-bromo-3-methoxy-5-methylbenzene (150 mg, 0.74 mmol), [5-(4-methoxyphenyl)-2-thienyl]boronic acid (**16b**) (206 mg, 0.88 mmol), sodium carbonate (181 mg, 1.76 mmol), and tetrakis(triphenylphosphine)palladium (42.7 mg, 37.0 μmol) according to method B. The product was purified by CC (hexane/ethyl acetate 7:3). Yield: 22% (50 mg).

3-[5-(4-Hydroxyphenyl)-2-thienyl]-5-methylphenol (16). The title compound was prepared by reaction of 2-(3-methoxyphenyl-5-methylphenyl)-5-(4-methoxyphenyl)thiophene (**16a**) (50 mg, 0.16 mmol) and boron tribromide (0.96 mmol) according to method C. The product was purified by preparative TLC (hexane/ethyl acetate 1:1). Yield: 90% (41 mg). MS (ESI): 281 $(M - H)^+$. Anal. ($C_{17}H_{14}O_2S$) C, H, N.

2-(3-Fluoro-5-methoxyphenyl)-5-(4-methoxyphenyl)thiophene (17a). The title compound was prepared by reaction of 2-bromo-5-(4-methoxyphenyl)thiophene (**16c**) (200 mg, 0.75 mmol), 3-fluoro-5-methoxybenzeneboronic acid (152 mg, 0.89 mmol), cesium carbonate (513 mg, 1.65 mmol), and tetrakis(triphenylphosphine)palladium (8.7 mg, 7.5 μmol) according to method A. The product was purified by CC (hexane/ethyl acetate 7:3). Yield: 43% (122 mg).

3-Fluoro-5-[5-(4-hydroxyphenyl)-2-thienyl]phenol (17). The title compound was prepared by reaction of 2-(3-fluoro-5-methoxyphenyl)-5-(4-methoxyphenyl)thiophene (**17a**) (100 mg, 0.32 mmol) and boron tribromide (1.92 mmol) according to method C. The product was purified by preparative TLC (hexane/ethyl acetate 7:3). Yield: 88% (80 mg). MS (APCI): 286 M^+. Anal. ($C_{16}H_{11}FO_2S$) C, H, N.

5-[5-(4-Methoxyphenyl)-2-thienyl]-2-methylphenol (18a). The title compound was prepared by reaction of 5-bromo-2-methylphenol (250 mg, 1.34 mmol), [5-(4-methoxyphenyl)-2-thienyl]boronic acid (**16b**) (690 mg, 2.95 mmol), cesium carbonate (914 mg, 2.94 mmol), and tetrakis(triphenylphosphine)palladium (15.5 mg, 13.4 μmol) according to method A. The product was purified by CC (hexane/ethyl acetate 7:3). Yield: 47% (193 mg).

5-[5-(4-Hydroxyphenyl)-2-thienyl]-2-methylphenol (18). The title compound was prepared by reaction of 5-[5-(4-methoxyphenyl)-2-thienyl]-2-methylphenol (**18a**) (161 mg, 0.54 mmol) and boron tribromide (3.24 mmol) according to method C. The product was purified by preparative TLC (hexane/ethyl acetate 7:3). Yield: 27% (42 mg). MS (ESI): 283 $(M + H)^+$. Anal. ($C_{17}H_{14}O_2S$) C, H, N.

2-(4-Fluoro-3-methoxyphenyl)-5-(4-methoxyphenyl)thiophene (19a). The title compound was prepared by reaction of 2-bromo-5-(4-methoxyphenyl)thiophene (**16c**) (200 mg, 0.75 mmol), 4-fluoro-3-methoxybenzeneboronic acid (152 mg, 0.89 mmol), cesium carbonate (553 mg, 1.78 mmol), and tetrakis(triphenylphosphine)palladium (8.7 mg, 7.5 μmol) according to method A. The product was purified by CC (hexane/ethyl acetate 7:3). Yield: 49% (149 mg).

2-Fluoro-5-[5-(4-hydroxyphenyl)-2-thienyl]phenol (19). The title compound was prepared by reaction of 2-(4-fluoro-3-methoxyphenyl)-5-(4-methoxyphenyl)thiophene (**19a**) (100 mg, 0.32 mmol) and boron tribromide (1.92 mmol) according to method C. The product was purified by preparative TLC (hexane/ethyl acetate 7:3). Yield: 88% (80 mg). MS (ESI): 287 $(M + H)^+$. Anal. ($C_{16}H_{11}FO_2S$) C, H, N.

2-(3,4-Dimethoxyphenyl)-5-(4-methoxyphenyl)thiophene (20a). The title compound was prepared by reaction of 2-bromo-5-(4-methoxyphenyl)thiophene (**16c**) (195 mg, 0.73 mmol), 3,4-dimethoxybenzeneboronic acid (160 mg, 0.88 mmol), cesium carbonate (500 mg, 1.61 mmol), and tetrakis(triphenylphosphine)palladium (8.4 mg, 7.3 μmol) according to method A. The product was purified by CC (dichloromethane/methanol 99:1). Yield: 46% (119 mg).

4-[5-(4-Hydroxyphenyl)-2-thienyl]benzene-1,2-diol (20). The title compound was prepared by reaction of 2-(3,4-dimethoxyphenyl)-5-(4-methoxyphenyl)thiophene (**20a**) (100 mg, 0.31 mmol) and boron tribromide (2.79 mmol) according to method C. The product was purified by preparative TLC (hexane/ethyl acetate 1:1). Yield: 17% (49 mg). MS (ESI): 285 $(M + H)^+$. Anal. ($C_{16}H_{12}O_3S$) C, H, N.

2-(4-Methoxy-3-methylphenyl)-5-(3-methoxyphenyl)thiophene (21a). The title compound was prepared by reaction of 2-bromo-5-(3-methoxyphenyl)thiophene (**1b**) (250 mg, 0.92 mmol), 3-methyl-4-methoxybenzeneboronic acid (152.8 mg, 0.92 mmol), sodium carbonate (243 mg, 2.36 mmol), and tetrakis(triphenylphosphine)palladium (53.1 mg, 46.0 μmol) according to method B. The product was purified by CC (hexane/ethyl acetate 7:3). Yield: 54% (154 mg).

4-[5-(3-Hydroxyphenyl)-2-thienyl)-2-methyl]phenol (21). The title compound was prepared by reaction of 2-(4-methoxy-3-methylphenyl)-5-(3-methoxyphenyl)thiophene (**21a**) (100 mg, 0.32 mmol) and boron tribromide (1.92 mmol) according to method C. The product was purified by preparative TLC (hexane/ethyl acetate 6:4). Yield: 79% (72 mg). MS (ESI): 281 $(M - H)^+$. Anal. ($C_{17}H_{14}O_2S$) C, H, N.

2-(3,4-Dimethoxyphenyl)-5-(3-methoxyphenyl)thiophene (22a). The title compound was prepared by reaction of 2-bromo-5-(3-methoxyphenyl)thiophene (**1b**) (206 mg, 1.14 mmol), 3,4-dimethoxybenzeneboronic acid (247 mg, 1.36 mmol), cesium carbonate (779 mg, 2.51 mmol), and tetrakis(triphenylphosphine)palladium (13.2 mg, 11.4 μmol) according to method A. The product was purified by CC (dichloromethane/methanol 99:1). Yield: 34% (126 mg).

4-[5-(3-Hydroxyphenyl)-2-thienyl]benzene-1,2-diol (22). The title compound was prepared by reaction of 2-(3,4-dimethoxyphenyl)-5-(3-methoxyphenyl)thiophene (**22a**) (100 mg, 0.32 mmol) and boron tribromide (2.88 mmol) according to method C. The product was purified by preparative TLC (hexane/ethyl acetate 6:4). Yield: 61% (56 mg). MS (ESI): 283 $(M - H)^+$. Anal. ($C_{16}H_{12}O_3S$) C, H, N.

2-(3-Fluoro-4-methoxyphenyl)-5-(3-methoxyphenyl)thiophene (23a). The title compound was prepared by reaction of 2-bromo-5-(3-methoxyphenyl)thiophene (**1b**) (370 mg, 1.37 mmol), 3-fluoro-4-methoxybenzeneboronic acid (255 mg, 1.50 mmol), cesium carbonate (717 mg, 3.01 mmol), and tetrakis(triphenylphosphine)palladium (15.8 mg, 13.7 μmol) according to method A. The product was purified by CC (hexane/ethyl acetate 7:3). Yield: 98% (421 mg).

2-Fluoro-4-[5-(3-hydroxyphenyl)-2-thienyl]phenol (23). The title compound was prepared by reaction of 2-(3-fluoro-4-methoxyphenyl)-5-(3-methoxyphenyl)thiophene (**23a**) (240 mg, 0.76 mmol) and boron tribromide (4.56 mmol) according to method C. The product was purified by preparative TLC (hexane/ethyl acetate 1:1). Yield: 90% (195 mg). MS (ESI): 285 $(M - H)^+$. Anal. ($C_{16}H_{11}FO_2S$) C, H, N.

2-(3-Methoxyphenyl)-5-[4-methoxy-3-(trifluoromethyl)-phenyl]thiophene (24a). The title compound was prepared by reaction of 2-bromo-5-(3-methoxyphenyl)thiophene (**1b**) (408 mg, 1.51 mmol), 3-trifluoromethyl-4-methoxybenzeneboronic acid (398 mg, 1.81 mmol), cesium carbonate (1033 mg, 3.32 mmol), and tetrakis(triphenylphosphine)palladium (17.5 mg, 15.1 μmol) according to method A. The product was purified by CC (hexane/ethyl acetate 7:3). Yield: 75% (412 mg).

4-[5-(3-Hydroxyphenyl)-2-thienyl]-2-(trifluoromethyl)phenol (24). The title compound was prepared by reaction of 2-(3-methoxyphenyl)-5-[4-methoxy-3-(trifluoromethyl)phenyl]-thiophene (**24a**) (300 mg, 0.82 mmol) and boron tribromide

(4.95 mmol) according to method C. The product was purified by preparative TLC (hexane/ethyl acetate 1:1). Yield: 98% (272 mg). MS (ESI): 285 $(M - H)^+$. Anal. ($C_{17}H_{11}F_3O_2S$) C, H, N.

2-(6-Methoxybiphenyl-3-yl)-5-(3-methoxyphenyl)thiophene (25a). The title compound was prepared by reaction of 2-bromo-5-(3-methoxyphenyl)thiophene (**1b**) (287 mg, 1.07 mmol), [6-methoxy-1,1′-biphenyl-3-yl]boronic acid (**25b**) (338 mg, 1.29 mmol), sodium carbonate (250 mg, 2.35 mmol), and tetrakis-(triphenylphosphine)palladium (61.8 mg, 53.5 μmol) according to method B. The product was purified by CC (hexane/ethyl acetate 7:3). Yield: 35% (135 mg).

5-[5-(3-Hydroxyphenyl)-2-thienyl]biphenyl-2-ol (25). The title compound was prepared by reaction of 2-(6-methoxybiphenyl-3-yl)-5-(3-methoxyphenyl)thiophene (**25a**) (100 mg, 0.26 mmol) and boron tribromide (1.56 mmol) according to method C. The product was purified by preparative TLC (hexane/ethyl acetate 1:1). Yield: 88% (81 mg). MS (ESI): 343 $(M - H)^+$. Anal. ($C_{22}H_{16}O_2S$) C, H, N.

[2-Methoxy-5-[5-(3-methoxyphenyl)-2-thienyl]phenyl]methanol (26c). The title compound was prepared by reaction of 2-bromo-5-(3-methoxyphenyl)thiophene (**1b**) (408 mg, 1.51 mmol), [3-(hydroxymethyl)-4-methoxyphenyl]boronic acid (**26d**) (329 mg, 1.81 mmol), cesium carbonate (1032 mg, 3.32 mmol), and tetrakis(triphenylphosphine)palladium (17.5 mg, 15.1 μmol) according to method A. The product was purified by CC (hexane/ethyl acetate 8:2). Yield: 12% (59 mg).

2-Methoxy-5-[5-(3-methoxyphenyl)-2-thienyl]benzaldehyde (26b). To a solution of [2-methoxy-5-[5-(3-methoxyphenyl)-2-thienyl]phenyl]methanol (**26c**) (100 mg, 0.31 mmol, 1 equiv) in dichloromethane, pyridium chlorochromate (66 mg, 0.31 mmol, 1 equiv) was added in small portions over 5 min and stirred at room temperature. After 30 min, the reaction was quenched with water. The resulting organic layer was dried over sodium sulfate, filtered, and concentrated to dryness. The title compound was not characterized and used in the next step without purification.

Ethyl (2*E*)-3-[2-Methoxy-5-[5-(3-methoxyphenyl)-2-thienyl]-phenyl]acrylate (26a). To a solution of sodium hydride (10.4 mg, 0.43 mmol, 1 equiv) in anhydrous THF, triethyl phosphonoacetate (93 μL, 0.46 mmol, 1.1 equiv) was added dropwise and stirred at room temperature. After 15 min, 2-methoxy-5-[5-(3-methoxyphenyl)-2-thienyl]benzaldehyde (**26b**) (100 mg, 0.31 mmol, 0.6 equiv) was added and stirred for 4 h at room temperature. To quench the reaction water was added and the resulting organic layer was washed with brine, dried over sodium sulfate, filtered, evaporated, and purified by CC (hexane/ethyl acetate 7:3). Yield: 98% (120 mg).

Ethyl (2*E*)-3-[2-Hydroxy-5-[5-(3-hydroxyphenyl)-2-thienyl]-phenyl]acrylate (26). The title compound was prepared by reaction of ethyl (2*E*)-3-[2-methoxy-5-[5-(3-methoxyphenyl)-2-thienyl]phenyl]acrylate (**26a**) (60 mg, 0.15 mmol) and boron tribromide (0.90 mmol) according to method C. The product was purified by preparative TLC (hexane/ethyl acetate 1:1). Yield: 17% (10 mg). MS (APCI): 366 $(M)^+$. Anal. ($C_{21}H_{18}O_4S$) C, H, N.

(2*E*)-3-[2-Methoxy-5-[5-(3-methoxyphenyl)-2-thienyl]phenyl]-*N*-phenylacrylamide (27a). Ethyl (2*E*)-3-[2-methoxy-5-[5-(3-methoxyphenyl)-2-thienyl]phenyl]acrylate (**26a**) (720 mg, 2.22 mmol, 1 equiv) in a solution of THF/water (2:1) was refluxed for 20 h together with lithium hydroxide (320 mg, 13.33 mmol, 6 equiv). After the mixture was cooled to room temperature, ether was added, the aqueous layer was acidified with 1 N hydrochloric acid and washed with dichloromethane. The combined organic layers were dried over sodium sulfate, filtered, and evaporated under reduced pressure. The resulting carboxylic acid was solubilized in dichloromethane (180 mg, 0.53 mmol, 1 equiv) and refluxed for 20 h with EDCI (102 mg, 0.53 mmol, 1 equiv) and HOBt (72 mg, 0.53 mmol, 1 equiv). After the mixture was cooled to room temperature, the organic layer was washed with a 1.5 M sodium hydrogenocarbonate solution, brine, dried over sodium sulfate, evaporated under reduced pressure, and purified by CC (hexane/ethyl acetate 7:3). Yield: 51% (120 mg). MS (ESI): 442 $(M + H)^+$.

(2*E*)-3-[2-Hydroxy-5-[5-(3-hydroxyphenyl)-2-thienyl]phenyl]-*N*-phenylacrylamide (27). The title compound was prepared by reaction of (2*E*)-3-[2-methoxy-5-[5-(3-methoxyphenyl)-2-thienyl]-phenyl]-*N*-phenylacrylamide (**27a**) (55 mg, 0.13 mmol) and boron tribromide (0.78 mmol) according to method C. The product was purified by preparative TLC (hexane/ethyl acetate 4:6). Yield: 31% (17 mg). MS (ESI): 414 $(M + H)^+$. Anal. ($C_{25}H_{19}NO_3S$) C, H, N.

3-[2-Methoxy-5-[5-(3-methoxyphenyl)-2-thienyl]phenyl]-*N*-phenylpropanamide (28a). (2*E*)-3-[2-Methoxy-5-[5-(3-methoxyphenyl)-2-thienyl]phenyl]-*N*-phenylacrylamide (**27a**) (50 mg, 0.11 mmol, 1 equiv) was solubilized in a mixture of THF/EtOH (1:1). After addition of palladium hydroxide (1.7 mg, 0.01 mmol, 0.1 equiv) the mixture was stirred at room temperature under nitrogen atmosphere for 20 h. The crude mixture was filtered, and the organic layer was evaporated under reduced pressure. Yield: quantitative.

3-[2-Hydroxy-5-[5-(3-hydroxyphenyl)-2-thienyl]phenyl]-*N*-phenylpropanamide (28). The title compound was prepared by reaction of 3-[2-methoxy-5-[5-(3-methoxyphenyl)-2-thienyl]-phenyl]-*N*-phenylpropanamide (**28a**) (55 mg, 0.13 mmol) and boron tribromide (0.78 mmol) according to method C. The product was purified by preparative TLC (hexane/ethyl acetate 1:1). Yield: 20% (10 mg). MS (ESI): 416 $(M + H)^+$. Anal. ($C_{25}H_{21}NO_3S$) C, H, N.

2-Bromo-5-(3-fluoro-4-methoxyphenyl)thiophene (29b). The title compound was prepared by reaction of 2,5-dibromothiophene (500 mg, 2.10 mmol), 3-fluoro-4-methoxybenzeneboronic acid (357 mg, 2.10 mmol), sodium carbonate (432 mg, 4.20 mmol), and tetrakis(triphenylphosphine)palladium (121 mg, 1.05 mmol) according to method B. The product was purified by CC (hexane/ethyl acetate 95:5). Yield: 85% (427 mg).

2-(3-Fluoro-4-methoxyphenyl)-5-(3-fluoro-5-methoxyphenyl)thiophene (29a). The title compound was prepared by reaction of 2-bromo-5-(3-fluoro-4-methoxyphenyl)thiophene (**29b**) (100 mg, 0.41 mmol), 3-fluoro-5-methoxybenzeneboronic acid (85 mg, 0.50 mmol), cesium carbonate (280 mg, 0.90 mmol), and tetrakis-(triphenylphosphine)palladium (4.7 mg, 4.1 μmol) according to method A. The product was purified by CC (hexane/ethyl acetate 9:1). Yield: 82% (113 mg).

2-Fluoro-4-[5-(3-fluoro-5-hydroxyphenyl)thien-2-yl]phenol (29). The title compound was prepared by reaction of 2-(3-fluoro-4-methoxyphenyl)-5-(3-fluoro-5-methoxyphenyl)thiophene (**30a**) (100 mg, 0.30 mmol) and boron tribromide (1.80 mmol) according to method C. The product was purified by preparative TLC (hexane/ethyl acetate 6:4). Yield: 38% (35 mg). MS (APCI): 304 $(M)^+$. Anal. ($C_{16}H_{10}F_2O_2S$) C, H, N.

2-(3-Fluoro-4-methoxyphenyl)-5-(4-fluoro-3-methoxyphenyl)-thiophene (30a). The title compound was prepared by reaction of 2-bromo-5-(3-fluoro-4-methoxyphenyl)thiophene (**29b**) (100 mg, 0.41 mmol), 4-fluoro-3-methoxybenzeneboronic acid (85 mg, 0.50 mmol), cesium carbonate (279 mg, 0.90 mmol), and tetrakis(triphenylphosphine)palladium (4.7 mg, 4.1 μmol) according to method A. The product was purified by CC (hexane/ethyl acetate 9:1). Yield: 72% (100 mg).

2-Fluoro-4-[5-(4-fluoro-3-hydroxyphenyl)thien-2-yl]phenol (30). The title compound was prepared by reaction of 2-(3-fluoro-4-methoxyphenyl)-5-(3-fluoro-5-methoxyphenyl)thiophene (**30a**) (100 mg, 0.30 mmol) and boron tribromide (1.80 mmol) according to method C. The product was purified by preparative TLC (hexane/ethyl acetate 6:4). Yield: 75% (69 mg). MS (APCI): 304 $(M)^+$. Anal. ($C_{16}H_{10}F_2O_2S$) C, H, N.

2-(3,5-Difluoro-4-methoxyphenyl)-5-(3-methoxyphenyl)thiophene (31a). The title compound was prepared by reaction of 2-bromo-5-(3-methoxyphenyl)thiophene (**1b**) (430 mg, 1.60 mmol), 3,5-difluoro-4-methoxybenzeneboronic acid (357 mg, 1.92 mmol), cesium carbonate (1094 mg, 3.52 mmol), and

tetrakis(triphenylphosphine)palladium (18.5 mg, 16.0 μmol) according to method A. The product was purified by CC (petroleum ether/ethyl acetate 9:1). Yield: 42% (223 mg).

2,6-Difluoro-4-[5-(3-hydroxyphenyl)-2-thienyl]phenol (31). The title compound was prepared by reaction of 2-(3,5-difluoro-4-methoxyphenyl)-5-(3-methoxyphenyl)thiophene (**31a**) (220 mg, 0.62 mmol) and boron tribromide (3.72 mmol) according to method C. The product was purified by preparative TLC (hexane/ethyl acetate 5:5). Yield: 10% (18 mg). MS (ESI): 305 $(M + H)^+$. Anal. ($C_{16}H_{10}F_2O_2S$) C, H, N.

2-(3,4-Difluorophenyl)-5-(3-methoxyphenyl)thiophene (32a). The title compound was prepared by reaction of 2-bromo-5-(3-methoxyphenyl)thiophene (**1b**) (150 mg, 0.56 mmol), 3,4-difluorobenzeneboronic acid (105 mg, 0.67 mmol), cesium carbonate (383 mg, 1.23 mmol), and tetrakis(triphenylphosphine)palladium (6.5 mg, 5.6 μmol) according to method A. The product was purified by CC (hexane/ethyl acetate 9:1). Yield: 90% (152 mg).

3-[5-(3,4-Difluorophenyl)-2-thienyl]phenol (32). The title compound was prepared by reaction of 2-(3,4-difluorophenyl)-5-(3-methoxyphenyl)thiophene (**32a**) (120 mg, 0.40 mmol) and boron tribromide (1.20 mmol) according to method C. The product was purified by CC (dichloromethane/methanol 99:1). Yield: 85% (98 mg). MS (ESI): 289 $(M + H)^+$. Anal. ($C_{16}H_9F_2OS$) C, H, N.

4-(4-Methoxy-3-methylphenyl)-2-(3-methoxyphenyl)thiophene (34a). The title compound was prepared by reaction of 4-bromo-2-(3-methoxyphenyl)thiophene (**33b**) (400 mg, 1.49 mmol), 3-methyl-4-methoxybenzeneboronic acid (296 mg, 1.79 mmol), cesium carbonate (1019 mg, 3.27 mmol), and tetrakis-(triphenylphosphine)palladium (17.2 mg, 14.9 μmol) according to method A. The product was purified by CC (hexane/ethyl acetate 7:3). Yield: 69% (320 mg).

4-[5-(3-Hydroxyphenyl)-3-thienyl]-2-methylphenol (34). The title compound was prepared by reaction of 4-(4-methoxy-3-methylphenyl)-2-(3-methoxyphenyl)thiophene (**34a**) (180 mg, 0.58 mmol) and boron tribromide (3.48 mmol) according to method C. The product was purified by preparative TLC (hexane/ethyl acetate 1:1). Yield: 54% (88 mg). MS (ESI): 281 $(M - H)^+$. Anal. ($C_{17}H_{14}O_2S$) C, H, N.

4-(3-Fluoro-4-methoxyphenyl)-2-(3-methoxyphenyl)thiophene (35a). The title compound was prepared by reaction of 4-bromo-2-(3-methoxyphenyl)thiophene (**33b**) (400 mg, 1.49 mmol), 3-fluoro-4-methoxybenzeneboronic acid (303 mg, 1.78 mmol), cesium carbonate (1019 mg, 3.30 mmol), and tetrakis-(triphenylphosphine)palladium (17.2 mg, 14.9 μmol) according to method A. The product was purified by CC (petroleum ether/ethyl acetate 9:1). Yield: 80% (403 mg).

2-Fluoro-4-[5-(3-hydroxyphenyl)-3-thienyl]phenol (35). The title compound was prepared by reaction of 4-(3-fluoro-4-methoxyphenyl)-2-(3-methoxyphenyl)thiophene (**35a**) (400 mg, 1.27 mmol) and boron tribromide (7.63 mmol) according to method C. The product was purified by CC (dichloromethane/methanol 98:2). Yield: 22% (88 mg). MS (ESI): 287 $(M - H)^-$

4-(4-Methoxy-3-methylphenyl)-2-(3-methoxyphenyl)-1,3-thiazole (37a). The title compound was prepared by reaction of 5-bromo-2-(3-methoxyphenyl)-1,3-thiazole (**36b**) (402 mg, 1.49 mmol), 3-methyl-4-methoxybenzeneboronic acid (247 mg, 1.79 mmol), cesium carbonate (1019 mg, 3.27 mmol), and tetrakis-(triphenylphosphine)palladium (17.2 mg, 14.9 μmol) according to method A. The product was purified by CC (hexane/ethyl acetate 7:3). Yield: 69% (320 mg).

4-[2-(3-Hydroxyphenyl)-1,3-thiazol-5-yl]-2-methylphenol (37). The title compound was prepared by reaction of 4-(4-methoxy-3-methylphenyl)-2-(3-methoxyphenyl)-1,3-thiazole (**37a**) (80 mg, 0.26 mmol) and boron tribromide (1.56 mmol) according to method C. The product was purified by preparative TLC (hexane/ethyl acetate 3:7). Yield: 16% (11 mg), MS (ESI): 274 $(M + H)^+$. Anal. ($C_{16}H_{13}NO_2S$) C, H, N.

3,4″-Dimethoxy-4-methyl-1,1′:4′,1″-terphenyl (39a). The title compound was prepared by reaction of 4′-bromo-3-methoxybiphenyl (**38b**) (230 mg, 0.87 mmol), 4-methoxy-3-methylbenzeneboronic acid (172 mg, 1.04 mmol), cesium carbonate (595 mg, 1.91 mmol), and tetrakis(triphenylphosphine)palladium (10.1 mg, 8.7 μmol) according to method A. The product was purified by CC (hexane/ethyl acetate 98:2). Yield: 53% (140 mg).

4-Methyl-1,1′:4′,1″-terphenyl-3,4″-diol (39). The title compound was prepared by reaction of 3,3″-dimethoxy-4-methyl-1,1′:4′,1″-terphenyl (**41a**) (120 mg, 0.39 mmol) and boron tribromide (2.34 mmol) according to method C. The product was purified by preparative TLC (dichloromethane/methanol 97:3). Yield: 38% (42 mg). MS (ESI): 277 $(M + H)^+$.

3-Fluoro-3″,4-dimethoxy-1,1′:4′,1″-terphenyl (40a). The title compound was prepared by reaction of 4′-bromo-3-methoxybiphenyl (**38b**) (175 mg, 0.67 mmol), 3-fluoro-4-methoxybenzeneboronic acid (136.7 mg, 0.88 mmol), cesium carbonate (457 mg, 1.47 mmol), and tetrakis(triphenylphosphine)palladium (7.7 mg, 6.7 μmol) according to method A. The product was purified by CC (hexane/ethyl acetate 98:2). Yield: 58% (117 mg).

3″-Fluoro-1,1′:4′,1″-terphenyl-3,4″-diol (40). The title compound was prepared by reaction of 3-fluoro-3″,4-dimethoxy-1,1′:4′,1″-terphenyl (**40a**) (115 mg, 0.37 mmol) and boron tribromide (2.22 mmol) according to method C. The product was purified by preparative TLC (dichloromethane/methanol 99:1). Yield: 62% (65 mg). MS (ESI): 281 $(M + H)^+$.

4-Fluoro-3,3″-dimethoxy-1,1′:4′,1″-terphenyl (42a). The title compound was prepared by reaction of 4′-bromo-3-methoxybiphenyl (**38b**) (200 mg, 0.76 mmol), 4-fluoro-3-methoxybenzeneboronic acid (154 mg, 0.91 mmol), cesium carbonate (520 mg, 1.67 mmol), and tetrakis(triphenylphosphine)palladium (8.8 mg, 7.6 μmol) according to method A. The product was purified by CC (hexane/ethyl acetate 9:1). Yield: 75% (175 mg).

4-Fluoro-1,1′:4′,1″-terphenyl-3,3″-diol (42). The title compound was prepared by reaction of 4-fluoro-3,3″-dimethoxy-1,1′:4′,1″-terphenyl (**42a**) (175 mg, 0.57 mmol) and boron tribromide solution (3.42 mmol) according to method C. The product was purified by preparative TLC (hexane/ethyl acetate 1:1). Yield: 63% (100 mg). MS (ESI): 281 $(M + H)^+$.

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

17β-HSD1 and 17β-HSD2 were obtained from human placenta according to previously described procedures.[31,37,56] 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.[57−59] Briefly, the enzyme preparation was incubated with NADH [500 μM] in the presence of potential inhibitors at 37 °C in a phosphate buffer (50 mM) supplemented with 20% of glycerol and 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 E1 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, Germany) connected to a HPLC-system (Agilent 1100 Series, Agilent Technologies, Waldbronn, Germany). Detection and quantification of the steroids were performed using a radioflow detector (Berthold Technologies, Bad Wildbad, Germany). The conversion rate was calculated according to the following equation:% conversion $= \frac{\%\ E2}{\%\ E2 + \%\ E1} \times 100$. 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 E2 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 were carried out as mentioned above.

3. ER Affinity. The binding affinity of selected compounds to the ERα and ERβ was determined according to Zimmermann et al.[60] Briefly, 0.25 pmol of ERα or ERβ, respectively, was incubated with [2,4,6,7-^{3}H]E2 (10 nM) and test compound for 1 h at room temperature. 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, Germany) was added and samples were measured in a liquid scintillation counter (Rack Beta Primo 1209, Wallac, Turku, Finland). For determination of the relative binding affinity (RBA), 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: $\mathrm{RBA}\ [\%] = \frac{\mathrm{IC_{50}(E2)}}{\mathrm{IC_{50}(compound)}} \times 100$. 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. Compound **21** was added at a final concentration of 100 nM. Inhibitors and E2 were diluted in ethanol (final ethanol concentration was adjusted to 1%). As a positive control, E2 was added at a final concentration of 0.1 nM. Ethanol was used as negative control. Medium was changed every 2−3 days and supplemented with the respective additive. After 8 days of incubation, the cell viability was evaluated measuring the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT). The cleavage of MTT to a blue formazan by mitochondrial succinate dehydrogenase was quantified spectrophotometrically at 590 nm as described by Denizot and Lang[61] with minor modifications. The control proliferation was arbitrarily set at 1 and the stimulation induced by the inhibitor was calculated according to following equation: $\%\ \mathrm{stimulation} = \frac{[\mathrm{proliferation(compound\text{-}induced)} - 1]}{[\mathrm{proliferation(E2\text{-}induced)} - 1]} \times 100$. Each value is calculated as the mean value of at least three independent experiments

5. 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 **21**, **23**, and **34** were tested for inhibition of the following enzymes: CYP3A4, 2D6, and 2C19. Inhibitory potencies were determined as IC_{50} values.

6. 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. 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 **21** and **23** were applied orally in a cassette dosing in four rats at a 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 heparinised 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 (Thermo/Fisher) triple quadrupole mass spectrometer equipped with an electrospray interface (ESI).

Computational Chemistry. Molecular Modeling. All molecular modeling studies were performed on Intel(R) P4 CPU 3.00 GHz running Linux CentOS 5.2. The X-ray structures of 17β-HSD1 (PDB codes 1A27, 1FDT, and 1I5R) were obtained from the Protein Data Bank[62] and further prepared using the BIOPOLYMER module of SYBYL version 8.0 (Sybyl, Tripos Inc., St. Louis, MO). Water molecules, E2 (or HYC for 1I5R), and sulfate ions were stripped from the PDB files, and missing protein atoms were added and correct atom types set. Finally hydrogen atoms and neutral end groups were added. All basic and acidic residues were considered protonated and deprotonated, respectively. Since almost all histidines are oriented toward the outer part of the enzyme, accessible for the surface, they were considered as protonated (HIP) after a prediction run made by MolProbity.[63] For 1I5R the cofactor NADPH was merged into the enzyme after an accurate overlay with the hybrid inhibitor HYC and the X-rays 1A27 and 1FDT. Further, every crystal structure was minimized for 500 steps with the steepest descent minimizer as implemented in SYBYL with the backbone atoms kept at fixed positions in order to fix close contacts, followed by 2000 steps conjugate gradient minimization requested for an overall better starting structure.

Inhibitor **23** was built with SYBYL and energy-minimized in a MMFF94s force field as implemented in Sybyl. Subsequently an ab initio geometry optimizations was performed in the gas phase at the B3LYP/6-311++G (d,p) level of density functional theory (DFT) by means of the Gaussian 03 software,[64,65] in order to obtain the RESP charges of compound **23**.

Two different softwares were used for docking studies: GOLD, version 3.2,[66] and Autodock4,[67,68] using the graphical user interface AutoDockTools (ADT 1.5.2). Since both allow flexible docking of ligands, no conformational search was employed to the ligand structure. For both programs the compound **23** was docked in 50 independent genetic algorithm (GA) runs.

GOLD, Version 3.2. Active-site origin was set at the center of the steroid binding site, while the radius was set equal to 13 Å. The automatic active-site detection was switched on. Further, a slightly modified GOLDSCORE fitness function (increased scaling for hydrophobic contacts) was used and genetic algorithm default parameters were set as suggested by the GOLD authors.

Autodock4. The docking area has been defined by a box, centered on the mass center of the CD-rings of the cocrystallized E2. Grids points of 60 × 70 × 74 with 0.375 Å spacing were calculated around the docking area for all the ligand atom types using AutoGrid4. The Lamarckian genetic algorithm local search (GALS) method was used. Each docking run was performed with a population size of 200. A mutation rate of 0.02 and a crossover rate of 0.8 were used to generate new docking trials for subsequent generations. The GALS method evaluates a population of possible docking solutions and propagates the most successful individuals from each generation into the next one.

Both programs performed in a similar way, supporting the herein suggested binding modes. The quality of the docked poses was evaluated based mainly on visual inspection of the putative binding modes of the ligand and on the scoring functions, which

give a good measure of the discrimination between the found binding modes for one single X-ray conformation but do not help us to compare the poses of different X-rays.

MEP. For selected compounds ab initio geometry optimizations were performed for the gas phase at the B3LYP/6-311++G (d,p) level of density functional theory (DFT) by means of the Gaussian 03 software, and the molecular electrostatics potential map (MEP) was plotted using GaussView, version 3.0, the 3D molecular graphics package of Gaussian.[69] These electrostatic potential surfaces were generated by mapping 6-311++G electrostatic potentials onto surfaces of molecular electron density (isovalue of 0.0002 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).

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Supporting Information Available: NMR spectroscopic data for all compounds, elemental analysis results of compounds **3**−**6**, **8**−**32**, **34**, and **37**, purity determination of compounds **35**, **39**, **41**, and **42** using LC/MS, and overlay of the binding poses of compound **23** obtained with the two 1FDT X-rays. 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–vi57.
(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) Shehu, A.; Mao, J.; Gibori, G. B.; Halperin, J.; Le, J.; Devi, Y. S.; Merrill, B.; Kiyokawa, H.; Gibori, G. Prolactin receptor-associated protein/17beta-hydroxysteroid dehydrogenase type 7 gene (Hsd17b7) plays a crucial role in embryonic development and fetal survival. *Mol. Endocrinol.* **2008**, *22*, 2268–2277.
(8) 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.
(9) 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.
(10) Gunnarsson, C.; Hellqvist, E.; Ståal, O. 17Beta-hydroxysteroid dehydrogenases involved in local oestrogen synthesis have prognostic significance in breast cancer. *Br. J. Cancer* **2005**, *92*, 547–552.
(11) Gunnarsson, C.; Olsson, B. M.; Ståal, O. Abnormal expression of 17beta-hydroxysteroid dehydrogenases in breast cancer predicts late recurrence. *Cancer Res.* **2001**, *61*, 8448–8451.
(12) 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.
(13) 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.
(14) Šmuc, T.; Ribič Pucelj, 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.
(15) Husen, B.; Huhtinen, K.; Poutanen, M.; Kangas, L.; Messinger, J.; Thole, H. Evaluation of inhibitors for 17beta-hydroxysteroid dehydrogenase type 1 in vivo in immunodeficient mice inoculated with MCF-7 cells stably expressing the recombinant human enzyme. *Mol. Cell. Endocrinol.* **2006**, *248*, 109–113.
(16) Husen, B.; Huhtinen, K.; Saloniemi, T.; Messinger, J.; Thole, H.; Poutanen, M. Human hydroxysteroid (17-beta) dehydrogenase 1 expression enhances estrogen sensitivity of MCF-7 breast cancer cell xenografts. *Endocrinology* **2006**, *147*, 5333–5339.
(17) Penning, T. M. 17Beta-hydroxysteroid dehydrogenase: inhibitors and inhibitor design. *Endocr. Relat. Cancer* **1996**, *3*, 41–56.
(18) Poirier, D. Inhibitors of 17 beta-hydroxysteroid dehydrogenases. *Curr. Med. Chem.* **2003**, *10*, 453–477.
(19) Brožic, P.; Lanišnik Rižner, T.; Gobec, S. Inhibitors of 17beta-hydroxysteroid dehydrogenase type 1. *Curr. Med. Chem.* **2008**, *15*, 137–150 and references cited therein .
(20) Poirier, D. Advances in development of inhibitors of 17beta hydroxysteroid dehydrogenases. *Anti-Cancer Agents Med. Chem.* **2009**, *9*, 642–660.
(21) 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.
(22) 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.
(23) 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.
(24) 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.
(25) 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 non-steroidal inhibitors of 17β-hydroxysteroid dehydrogenase type 1 (17beta-HSD1): design, synthesis, biological evaluation and pharmacokinetics. *J. Med. Chem.* **2008**, *51*, 4685–4698.
(26) Marchais-Oberwinkler, S.; Frotscher, M.; Ziegler, E.; Werth, R.; Kruchten, P.; Messinger, J.; Thole, H.; Hartmann, R. W. Structure−activity study in the class of 6-(3′-hydroxyphenyl)-naphthalenes leading to an optimization of a pharmacophore model for 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1) inhibitors. *Mol. Cell. Endocrinol.* **2009**, *301*, 205–211.
(27) 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 non-steroidal inhibitors of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1) for the treatment of estrogen-dependent diseases. *Bioorg. Med. Chem.* **2008**, *16*, 6423–6435.
(28) Bey, E.; Marchais-Oberwinkler, S.; Werth, R.; Negri, M.; Al-Soud, Y. A.; Kruchten, P.; Oster, A.; Frotscher, M.; Birk, B.; Hartmann, R. W. Design, synthesis, biological evaluation and pharmacokinetics of bis(hydroxyphenyl) substituted azoles, thiophenes, benzenes and aza-benzenes as potent and selective non-steroidal inhibitors of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1). *J. Med. Chem.* **2008**, *51*, 6725–6739.
(29) Kruchten, P.; Werth, R.; Bey, E.; Oster, A.; Marchais-Oberwinkler, S.; Frotscher, M.; Hartmann, R. W. Selective inhibition of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1) reduces estrogen responsive cell growth of T-47D breast cancer cells. *J. Steroid Biochem. Mol. Biol.* **2009**, *114*, 200–206.

(30) Kruchten, P.; Werth, R.; Marchais-Oberwinkler, S.; Bey, E.; Ziegler, E.; Oster, A.; Frotscher, M.; Hartmann, R. W. Development of biological assays for the identification of selective inhibitors of estradiol formation from estrone in rat liver preparations. *C. R. Chim.* **2009**, DOI: 10.1016/j.crci.2009.05.005.
(31) Kruchten, P.; Werth, R.; Marchais-Oberwinkler, S.; Frotscher, M.; Hartmann, R. W. Development of a biological screening system for the evaluation of highly active and selective 17beta-HSD1-inhibitors as potential therapeutic agents. *Mol. Cell. Endocrinol.* **2009**, *301*, 154–157.
(32) Al-Soud, Y. A.; Bey, E.; Oster, A.; Marchais-Oberwinkler, S.; Werth, R.; Kruchten, P.; Frotscher, M.; Hartmann, R. W. The role of the heterocycle in bis(hydroxyphenyl) triazoles for inhibition of 17beta-hydroxysteroid dehydrogenase (17beta-HSD) type 1 and type 2. *Mol. Cell. Endocrinol.* **2009**, *301*, 212–215.
(33) 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.
(34) 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 17 beta-estradiol. *Nat. Struct. Biol.* **1996**, *3*, 665–668.
(35) 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.
(36) Mazza, C. Human Type I 17Beta-hydroxysteroid Dehydrogenase: Site Directed Mutagenesis and X-ray Crystallography Structure−Function Analysis. Ph.D. Thesis, Université Joseph Fourier, Grenoble, France, **1997**.
(37) 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.
(38) 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.
(39) Togo, H.; Nogami, G.; Yokoyama, M. Synthetic application of poly[styrene(iodoso diacetate)]. *Synlett* **1998**, *5*, 534–536.
(40) Emmons, W. D. The utility of phosphonate carbanions in olefin synthesis. *J. Am. Chem. Soc.* **1961**, *83*, 1733–1738.
(41) Pascal, C.; Dubois, J.; Guénard, D.; Guéritte, F. Synthesis of biphenyls mimicking the structure of the antimitotic rhazinilam. *J. Org. Chem.* **1998**, *63*, 6414–6420.
(42) Hwang, K.-J.; O'Neil, J. P.; Katzenellenbogen, J. A. 5,6,11,12-Tetrahydrochrysenes: synthesis of rigid stilbene systems designed to be fluorescent ligands for the estrogen receptor. *J. Org. Chem.* **1992**, *57*, 1262–1271.
(43) Sherbet, D. P.; Guryev, O. L.; Papari-Zareei, M.; Mizrachi, D.; Rambally, S.; Akbar, S.; Auchus, R. J. Biochemical factors governing the steady-state estrone/estradiol ratios catalyzed by human 17beta-hydroxysteroid dehydrogenases types 1 and 2 in HEK-293 cells. *Endocrinology* **2009**, *150*, 4154–4162.
(44) Sherbet, D. P.; Papari-Zareei, M.; Khan, N.; Sharma, K. K.; Brandmaier, A.; Rambally, S.; Chattopadhyay, A.; Andersson, S.; Agarwal, A. K.; Auchus, R. J. Cofactors, redox state, and directional preferences of hydroxysteroid dehydrogenases. *Mol. Cell. Endocrinol.* **2007**, *265−266*, 83–88.
(45) Bruno, I. J.; Cole, J. C.; Lommerse, J. P. M.; Rowland, R. S.; Taylor, T.; Verdonk, M. L. Isostar: a library of information about non-bonded interactions. *J. Comput. Chem.* **1997**, *11*, 523–537.
(46) Hagmann, W. K. The many roles for fluorine in medicinal chemistry. *J. Med. Chem.* **2008**, *51*, 4359–4369.
(47) Bohm, H. J.; Banner, D.; Bendels, S.; Kansy, M.; Kuhn, B.; Muller, K.; Obst-Sander, U.; Stahl, M. Fluorine in medicinal chemistry. *ChemBioChem* **2004**, *5*, 637–643.
(48) Muller, K.; Faeh, C.; Diederich, F. Fluorine in pharmaceuticals: looking beyond intuition. *Science* **2007**, *317*, 1881–1886.
(49) Poirier, D.; Boivin, R. P.; Tremblay, M. R.; Bérubé, M.; Qiu, W.; Lin, S.-X. Estradiol−adenosine hybrid compounds designed to inhibit type 1 17beta-hydroxysteroid dehydrogenase. *J. Med. Chem.* **2005**, *48*, 8134–8147.
(50) Bérubé, M.; Poirier, D. Synthesis of simplified hybrid inhibitors of type 1 17beta-hydroxysteroid dehydrogenase via cross-metathesis and Sonogashira coupling reactions. *Org. Lett.* **2004**, *6*, 3127–3130.
(51) Fournier, D.; Poirier, D.; Mazumdar, M.; Lin, S.-X. Design and synthesis of bisubstrate inhibitors of type 1 17beta-hydroxysteroid dehydrogenase: overview and perspectives. *Eur. J. Med. Chem.* **2008**, *43*, 2298–2306.
(52) Hartman, J.; Lindberg, K.; Morani, A.; Inzunza, J.; Strom, A.; Gustafsson, J. A. Estrogen receptor beta inhibits angiogenesis and growth of T47D breast cancer xenografts. *Cancer Res.* **2006**, *66*, 11207–11213.
(53) Smith, G.; Mikkelsen, G.; Eskildsen, J.; Bundgaard, C. The synthesis and SAR of 2-arylsulfanylphenyl-1-oxyalkylamino acids as GlyT-1 inhibitors. *Bioorg. Med. Chem.* **2006**, *16*, 3981–3884.
(54) Pfahl, M.; Al-Shamma, H. A.; Fanjul, A.; Pleynet, D. P. M.; Spruce, L. W.; Wiemann, T. R.; Ibarra, J. B.; Tachdjian, C. Preparation of benzylidenethiazolidenediones and analogs as antitumor agents. WO 2002072009 A2 20020919, 2002.
(55) Duggan, H. M. E.; Leroux, F. G. M.; Malagu, K.; Martin, N. M. B.; Menear, K. A.; Smith, G. C. M. 2-Methylmorpholine-substituted pyrido-, pyrazo- and pyrimidopyrimidine derivatives as mTOR inhibitors and their preparation, pharmaceutical compositions and use in the treatment of cancer. WO 2008023161 A1 20080228, 2008.
(56) 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.
(57) 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.
(58) Sam, K. M.; Auger, S.; Luu-The, V.; Poirier, D. Steroidal spirogamma-lactones that inhibit 17beta-hydroxysteroid dehydrogenase activity in human placental microsomes. *J. Med. Chem.* **1995**, *38*, 4518–4528.
(59) 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.
(60) 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.
(61) 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.
(62) Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N.; Weissig, H.; Shindyalov, I. N.; Bourne, P. E. The Protein Data Bank. *Nucleic Acids Res.* **2000**, *28*, 235–242.
(63) Davis, I. W.; Leaver-Fay, A.; Chen, V. B.; Block, J. N.; Kapral, G. J.; Wang, X.; Murray, L. W.; Arendall, W. B.; Snoeyink, J.; Richardson, J. S.; Richardson, D. C. MolProbity: all-atom contacts and structure validation for proteins and nucleic acids. *Nucleic Acids Res.* **2007**, *35*, W375–W383.
(64) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Lyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03*; Gaussian, Inc.: Pittsburgh, PA, 2004.
(65) Bayly, C. I.; Cieplak, P.; Cornell, W. D.; Kollman, P. A. A well behaved electrostatic potential based method using charge restraints for determining atom-centered charges: the RESP model. *J. Phys. Chem.* **1993**, *97*, 10269–10280.
(66) Jones, G.; Willett, P.; Glen, R. C.; Leach, A. R.; Taylor, R. Development and validation of a genetic algorithm for flexible docking. *J. Mol. Biol.* **1997**, *267*, 727–748.
(67) Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. Automated docking using a Lamarckian genetic algorithm and empirical binding free energy function. *J. Comput. Chem.* **1998**, *19*, 1639–1662.
(68) Huey, R.; Morris, G. M.; Olson, A. J.; Goodsell, D. S. A semi-empirical free energy force field with charge-based desolvation. *J. Comput. Chem.* **2007**, *28*, 1145–1152.
(69) Dennington, I.; Roy, K. T.; Millam, J.; Eppinnett, K.; Howell, W. L.; Gilliland, R. *GaussView*, version 3.0; Semichem Inc.: Shawnee Mission, KS, 2003.