# Structural Optimization of 2,5-Thiophene Amides as Highly Potent and Selective 17β-Hydroxysteroid Dehydrogenase Type 2 Inhibitors for the Treatment of Osteoporosis

Sandrine Marchais-Oberwinkler,[†,#] Kuiying Xu,[†,#] Marie Wetzel,[†] Enrico Perspicace,[†] Matthias Negri,[‡] Arne Meyer,[§] Alex Odermatt,[§] Gabriele Möller,[∥] Jerzy Adamski,[∥,⊥] and Rolf W. Hartmann*[,†,‡]

[†]Pharmaceutical and Medicinal Chemistry, Saarland University, D-66041 Saarbrücken, Germany

[‡]Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Campus C2₃, D-66123 Saarbrücken, Germany

[§]Division of Molecular and Systems Toxicology, Department of Pharmaceutical Sciences, University of Basel, Klingelbergstraße 50, CH-4056 Basel, Switzerland

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

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

S *Supporting Information*

ABSTRACT: Inhibition of 17β-HSD2 is an attractive mechanism for the treatment of osteoporosis. We report here the optimization of human 17β-HSD2 inhibitors in the 2,5-thiophene amide class by varying the size of the linker (n equals 0 and 2) between the amide moiety and the phenyl group. While none of the phenethylamides ($n = 2$) were active, most of the anilides ($n = 0$) turned out to moderately or strongly inhibit 17β-HSD2. The four most active compounds showed an IC$_{50}$ of around 60 nM and a very good selectivity toward 17β-HSD1, 17β-HSD4, 17β-HSD5, 11β-HSD1, 11β-HSD2 and the estrogen receptors $α$ and $β$. The investigated compounds inhibited monkey 17β-HSD2 moderately, and one of them showed good inhibitory activity on mouse 17β-HSD2. SAR studies allowed a first characterization of the human 17β-HSD2 active site, which is predicted to be considerably larger than that of 17β-HSD1.
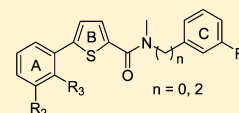
Cmpd	n	R2	R3	R1	Human 17β-HSD2 IC$_{50}$ (nM)	Selectivity Factor (17β-HSD1)
7	1	OMe	F	OH	61	73
31	0	OMe	F	OMe	62	>800
32	0	OMe	F	Me	62	132

INTRODUCTION

17β-Hydroxysteroid dehydrogenase type 2[1] (17β-HSD2) catalyzes the conversion of the highly active 17β-hydroxysteroids into the inactive 17-ketosteroids, i.e., the estrogen estradiol (E2), as well as the androgens testosterone (T) and 5α-dihydrotestosterone (DHT) into their inactive forms estrone (E1), Δ⁴-androstene-3,17-dione (Δ⁴-AD), and 5α-androstanedione, respectively (Chart 1). In addition, it has been described to exhibit a 20α-dehydrogenase activity,[1] transforming 20α-dihydroprogesterone in progesterone, and a 3β-dehydrogenase activity,[2] converting pregnenolone into progesterone and dehydroepiandrosterone (DHEA) in Δ⁴-AD.
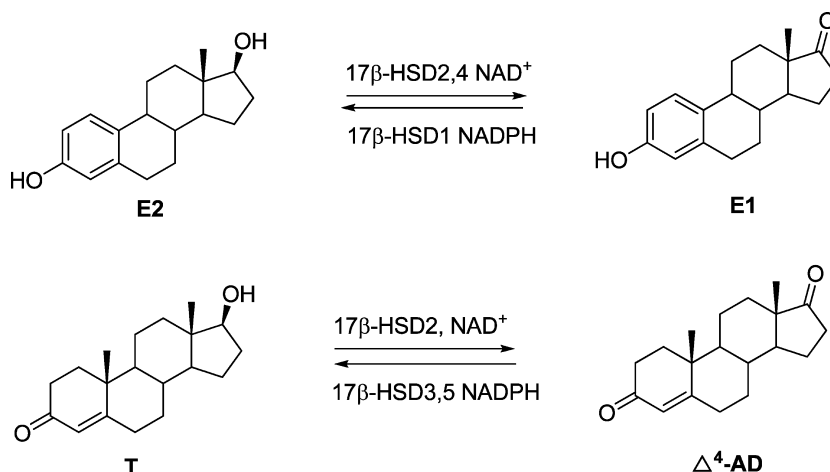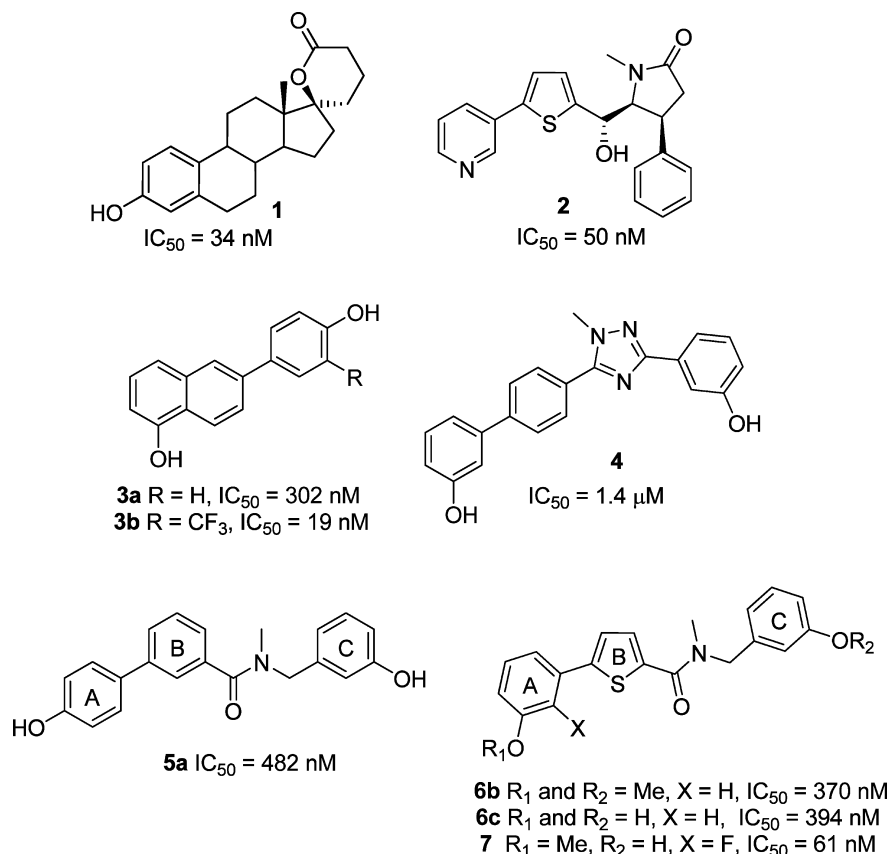
E2 is known to play an important role in the growth, development, and maintenance of a diverse range of tissues (e.g., reproductive tissues, brain). It is also involved in the maintenance of bone balance, inducing bone formation and repressing bone resorption by action on the osteoblasts.[3] There is also evidence that T has beneficial effects on bone formation.[4,5]

Osteoporosis[6] is a systemic disease where rigidity and mechanical stability of the bone decline. Balance between bone formation and bone resorption is disrupted, leading to an increased risk of fractures. High incidence of this disease is observed in women after menopause when the E2 levels drop or following treatment with aromatase inhibitors,[7] which block estrogen biosynthesis. Nowadays two first-line therapies are administered to osteoporotic patients: (1) Bisphosphonates (alendronate) are effective in both postmenopausal women[8] and men;[9,10] however, they lead to reduction of only 50% of fracture risk and are often associated to osteonecrosis of the jaw. (2) Selective estrogen receptor modulators,[11] also called SERMs (raloxifene), are efficient too but are often associated with an increased risk of venous thromboembolism. As the reduction of circulating estrogens induces accelerated bone loss, estrogen replacement therapy (ERT) was given to postmenopausal osteoporosis patients.[12,13] It reduced the risk of fractures but increased the incidence of cardiovascular diseases and breast cancer, which prevented the further use of this therapy.[13–15] All therapies currently available for the treatment of osteoporosis have limitations, and none of them offers a complete cure for the condition. Osteoporosis is an age-

Chart 1. 17β-HSD1, -2, -3, -4, and -5 in Sex Steroid Metabolism

Chart 2. Structures of Known 17β-HSD2 Inhibitors

1
IC$_{50}$ = 34 nM

2
IC$_{50}$ = 50 nM

3a R = H, IC$_{50}$ = 302 nM
3b R = CF$_3$, IC$_{50}$ = 19 nM

4
IC$_{50}$ = 1.4 μM

5a IC$_{50}$ = 482 nM

6b R$_1$ and R$_2$ = Me, X = H, IC$_{50}$ = 370 nM
6c R$_1$ and R$_2$ = H, X = H, IC$_{50}$ = 394 nM
7 R$_1$ = Me, R$_2$ = H, X = F, IC$_{50}$ = 61 nM

dependent disease, and because of the increasing life expectancy and aging population in the industrialized countries, there is a need for development of improved drugs to combat this disease. As 17β-HSD2 is expressed in osteoblastic cells,[16−18] inhibition of 17β-HSD2, which will lead to an increase in E2 and T levels locally in the bones, therefore offers the potential as a novel therapy for osteoporosis.

Ideally, 17β-HSD2 inhibitors should be highly potent and selective. They should not exhibit inhibitory activities on functionally related 17β-HSD subtypes like types 1, 3, 4, 5 (Chart 1). Inhibition of 17β-HSD type 4, which catalyzes the same reaction as type 2, is not desirable because it is ubiquitously expressed and its dysfunction leads to severe

human disorders,[19] e.g., Zellweger syndrome like D-bifunctional protein deficiency. Activity suppression of 17β-HSD1, -3, or -5, which catalyze the reverse reaction (reduction of estrogens or androgens) will thus be counterproductive because it would decrease E2 and T levels in bone and might lead to systemic side effects.

17β-HSD2 inhibitors should not bind to the estrogen receptors (ER) α and β, as it is expected that the E2 effects are ER mediated. In addition, activation upon binding to these receptors might lead to proliferative or antiproliferative effects in steroidogenic tissues, which should be avoided.

Although 17β-HSD2 was already revealed in 1985 by Blomquist[20] and characterized by Wu in 1993,[1] very few 17β-

HSD2 inhibitor classes have been reported to date. Among the steroidal inhibitors, Poirier and colleagues described a series of steroidal spirolactone derivatives;[21−24] the most potent compound is the C17-spiro-δ-lactone **1** (Chart 2, IC$_{50}$ = 34 nM[22]). Wood et al.[25−27] reported about a novel class of *cis*-pyrrolidinones as active and selective nonsteroidal 17β-HSD2 inhibitors, with **2** (Chart 2) being one of the most potent compound (IC$_{50}$ = 50 nM in a cell-free assay). Three further classes of nonsteroidal potent and selective 17β-HSD2 inhibitors were recently published by our group (Chart 2): the hydroxyphenylnaphth-1-ol **3a** and **3b**,[28,29] the hydroxyphenylmethanones[30] derived from the triazole **4**,[31] and the amides **5a**, **6b**, **6c**, and **7**.[32] These amide derivatives are all substituted by a benzyl group that is linked to a biphenylamide **5a** or a phenylthiophene **6b**, **6c**, **7** moiety.

At the time we started this work, a proof of concept for therapeutic efficacy of 17β-HSD2 inhibition had been described using compound **2** in vivo in a monkey model,[33] showing a decrease of bone resorption and maintenance of bone formation. Despite high variations and the moderate effects observed, this in vivo experiment validates this approach and underlines the need for new optimized 17β-HSD2 inhibitors.

In the current report, we describe the optimization of 17β-HSD2 inhibitors in the biphenylamide and phenylthiophene amide classes focusing on the suppression of the methylene from the benzyl group (anilide derivatives) or its replacement by an ethylene linker (phenethylamide derivatives). The synthesis of a small library of achiral derivatives, the biological evaluation, and the structure−activity relationship (SAR) of the new 17β-HSD2 inhibitors will be presented and compared to the benzyl analogues.[32] The selectivity toward further HSD enzymes and the cytotoxicity profile of the best candidates were investigated. Selectivity toward 17β-HSD1 was achieved based on the expertise from the group developing potent and selective inhibitors of 17β-HSD1.[34−45] In order to identify which species could be more suitable to perform a proof of concept in a preclinical model, the most potent and selective compounds (at the human enzymes) identified in this study were further tested for their ability to inhibit 17β-HSD2 from different species (rodents and monkey).

■ DESIGN

In a previous study, it was shown that, starting from the weakly active disubstituted triazole **4**,[31] opening of the triazole central moiety[32] led to the discovery of a new class of biphenylamide **5a** and phenylthiophene amides **6b**, **6c**, and **7** as 17β-HSD2 inhibitors (Chart 2). All the compounds discovered in this class share a methylated amide and two hydroxy/methoxyphenyl moieties differentiated in this study as rings A and C (Chart 2). In addition ring C is attached to the nitrogen of the amide via a methylene linker (n = 1, benzyl group). The compounds differ in their central ring B, which is either a 1,4-phenyl, 1,3-phenyl, or 2,5-thiophene group. Moderately active compounds were identified in the class of the 1,3-phenyl derivatives **5a**, showing an IC$_{50}$ of around 500 nM. Moderate to good active molecules were discovered in the 2,5-thiophene class **6b** and **6c**, with IC$_{50}$ of around 380 nM with the exception of **7**, the most active and promising 17β-HSD2 inhibitor (IC$_{50}$ = 61 nM and selectivity factor of 73 toward 17β-HSD1).

With the hypothesis that the central core B and the hydroxy/methoxyphenyl A ring bind at the same position in the enzyme, variation of the linker size n (n = 0, 1, and 2) will bring ring C into different areas of the binding cavity as seen in Figure 1.
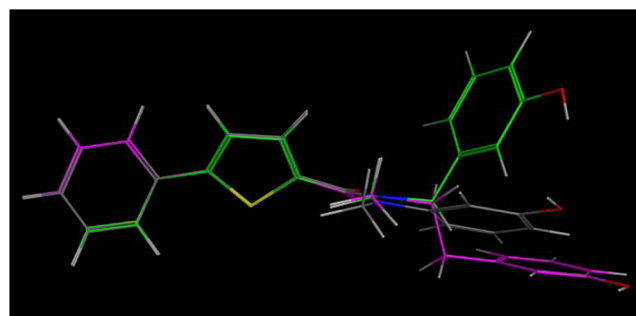

Figure 1. Superimposition of the designed compounds with n = 0 (gray), 1 (green), and 2 (violet). The picture was generated using Moe 2010.10.

Variation of the size of the linker will help the mapping of the enzyme's active site, which is unknown, providing information on the space available there and the global size of the inhibitor accepted by the enzyme as well as on inhibitor rigidity (n = 0)/flexibility (n = 2) tolerated by the enzyme.

In order to investigate more deeply the enzyme's active site and in an attempt to optimize this class of compounds, a small library of 17β-HSD2 inhibitors was synthesized keeping the phenyl C unchanged and varying the size of the linker (n = 0 and n = 2, to be compared with n = 1 previously described[32]) as well as the substituents at ring A in both biphenyl and phenylthiophene amides classes (Chart 3, compounds **8−41**).

Chart 3. Designed Structures
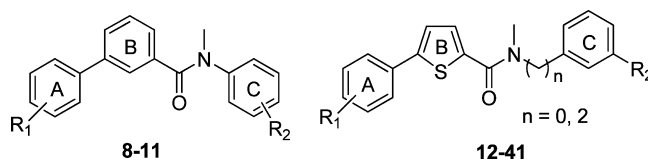
■ RESULTS

Chemistry. The synthesis of the 1,3-phenyl derivatives **8−11**, depicted in Scheme 1, and the synthesis of the 2,5-thiophene derivatives **12−41**, depicted in Scheme 2, were performed following a two- to three-step reaction pathway. First, amidation was carried out by reaction of the commercially

Scheme 1. Synthesis of 1,3-Phenyl Derivatives 8−11[a]
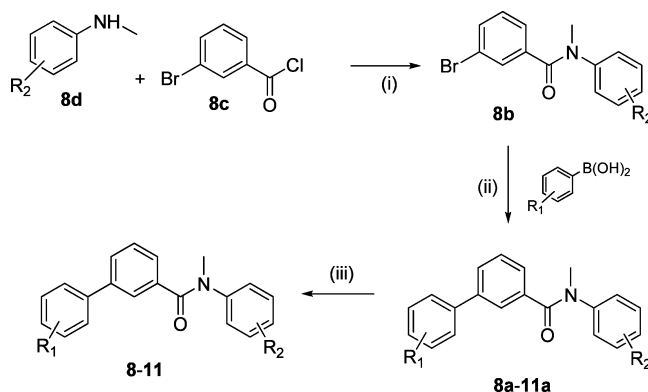
[a]Reagents and conditions: (i) NEt$_3$, CH$_2$Cl$_2$, 0 °C, 3 h, method A; (ii) DME/H$_2$O (1/1), Na$_2$CO$_3$, Pd(PPh$_3$)$_4$, 80 °C, 4−14 h, method B; (iii) BF$_3$·S(Me)$_2$, CH$_2$Cl$_2$, rt, 6−14 h, method C.

Scheme 2. Synthesis of 2,5-Thiophene Derivatives 12−41[a]
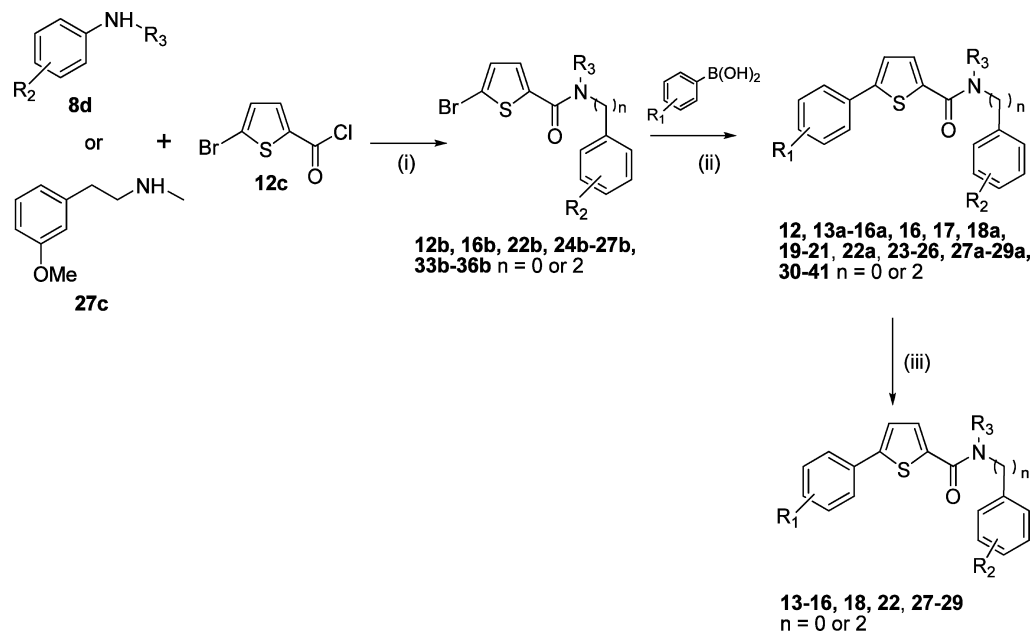
[a]Reagents and conditions: (i) NEt₃, CH₂Cl₂, 0 °C, 3 h, method A; (ii) DME/H₂O (1/1), Na₂CO₃, Pd(PPh₃)₄, 80 °C, 4−14 h, method B; (iii) BF₃·S(Me)₂, CH₂Cl₂, rt, 6−14 h, method C.

Table 1. Inhibition of Human 17β-HSD2 and 17β-HSD1 by Diphenylamide Derivatives 8−11 in Cell-Free System

5a-d, 8-11

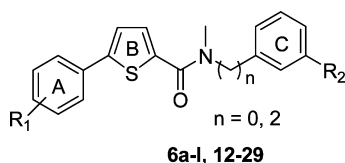|			n = 0			n = 1	
			% inhibition at 1 μM[a,d]			% inhibition at 1 μM[a,d]	
R₁	R₂	compd	17β-HSD2[b]	17β-HSD1[c]	compd	17β-HSD2[b]	17β-HSD1[c]
4-OH	OH	**8**	28	ni	**5a**	70	31
4-OMe	OMe	**8a**	53	ni	**5b**	13	ni
3-OH	OH	**9**	35	13	**5c**	60	10
3-OMe	OMe	**9a**	64	ni	**5d**	11	ni
2-OH	OH	**10**	18	ni			
2-OMe	OMe	**10a**	40	ni			
H	OH	**11**	28	ni			
H	OMe	**11a**	37	ni			

[a]Mean value of three determinations, standard deviation less than 10%. [b]Human placental, microsomal fraction, substrate E2, 500 nM, cofactor NAD⁺, 1500 μM. [c]Human placental, cytosolic fraction, substrate E1, 500 nM, cofactor NADH, 500 μM. [d]ni: no inhibition (inhibition of <10%).

available 5-bromothiophene **12c** or the 3(4)-bromobenzoyl chloride **8c** with substituted anilines **8d** or with the 2-(3-methoxyphenyl)-N-methylethanamine **27c** under standard conditions (method A consisting of triethylamine, dichloromethane at 0 °C for 3 h) providing the brominated intermediates **8b, 12b, 16b, 22b, 24b−27b, 33b−36b** in isolated yields between 57% and 99%. Subsequently, Suzuki coupling using tetrakis(triphenylphosphine)palladium and sodium carbonate in a mixture DME/water, 1:1 (method B), afforded the biphenylamides **8a−11a** or the phenylthiophene amides derivatives **12, 13a−16a, 16, 17, 18a, 19−21, 22a, 23−26, 27a−29a, 30−41** with good yields. Methoxy compounds were submitted to ether cleavage using boron trifluoride−

dimethyl sulfide complex and yielded the hydroxy molecules **8−11, 13−16, 18, 22, 27−29**.

Biological Results. 1. Inhibition of Human 17β-HSD2 in Cell-Free Assay and Cellular Assay. 17β-HSD2 inhibitory activities of the synthesized compounds were first evaluated in a cell-free assay. Human placental enzyme was obtained and used according to described methods.[23,46,47] Briefly, incubations were run with the enzyme microsomal fraction, tritiated E2, cofactor, and inhibitor. The separation of substrate and product was accomplished by HPLC. The percent inhibition values of compounds **8−41** are shown in Tables 1−3. The IC₅₀ values determined for selected compounds are reported in Table 4. Compounds showing less than 10% inhibition when tested at 1 μM were considered to be inactive. The spiro-δ-lactone **1**,

Table 2. Inhibition of Human 17β-HSD2 and 17β-HSD1 by Phenylthiophene Amide Derivatives Monosubstituted on the A-Ring 12−29 in Cell-Free System

6a-l, 12-29

R$_1$	R$_2$	n = 0			n = 1			n = 2		
			% inhibition at 1 μM^{a,d}			% inhibition at 1 μM^{a,d}			% inhibition at 1 μM^{a,d}	
		compd	17β-HSD2^b	17β-HSD1^c	compd	17β-HSD2^b	17β-HSD1^c	compd	17β-HSD2^b	17β-HSD1^c
4-OMe	3-OMe	**12**	89	15	**6a**	61	ni			
3-OMe	3-OMe	**13a**	90	11	**6b**	63	ni	**27a**	31	ni
3-OH	3-OH	**13**	34	33	**6c**	70	21	**27**	37	23
2-OMe	3-OMe	**14a**	66	ni	**6d**	48	ni			
2-OH	3-OH	**14**	69	47	**6e**	83	16			
H	3-OMe	**15a**	82	24	**6f**	49	ni	**28a**	18	11
H	3-OH	**15**	60	50	**6g**	81	15	**28**	11	ni
3-OMe	H	**16a**	67	ni	**6h**	68	ni			
3-OH	H	**16**	31	12	**6i**	42	ni			
H	H	**17**	48	14						
4-CN	3-OMe	**18a**	48	ni	**6j**	ni	ni			
4-CN	3-OH	**18**	28	ni	**6k**	54	7			
3-Me	3-OMe	**19**	95	26						
3-N(Me)$_2$	3-OMe	**20**	82	11						
3-SMe	3-OMe	**21**	88	28						
3-OMe	3-Me	**22a**	87	ni						
3-OH	3-Me	**22**	71	24						
3-F	3-OMe	**23**	67	23				**29a**	ni	ni
3-F	3-OH				**6l**	71	20	**29**	17	12
3-OMe	3-CF$_3$	**24**	75	13						
3-Me	2-F	**25**	68	ni						
3-OMe	3-Ph	**26**	83	50						

^{a}Mean value of three determinations, standard deviation less than 10%. ^{b}Human placental, microsomal fraction, substrate E2, 500 nM, cofactor NAD$^+$, 1500 μM. ^{c}Human placental, cytosolic fraction, substrate E1, 500 nM, cofactor NADH, 500 μM. ^{d}ni: no inhibition (inhibition of <10%).

described by Poirier et al.,[22] was taken as external reference (68% at 1 μM in our test; 62−66% at 1 μM in their assay).

In the 1,3-phenyl class (Table 1), comparison of the biological results indicates that the best 17β-HSD2 inhibitory activities are obtained either when n is 0 and the substituents R1 and R2 are methoxy groups (compounds 8a, 9a, 10a) or when n is 1 and R1 and R2 are hydroxy moieties (compounds 5a and 5c). Taking away R2 (R2 = H; 11 and 11a) is detrimental for the activity, independent of the nature of R1. It indicates that R2 is important for the stabilization of the molecule in the active site. R1 and R2 therefore interact with amino acids from the binding cavity and behave as H-bond acceptors when n is 0 or H-bond donors when n is 1.

In the 2,5-thiophene class (Table 2), the highest inhibition data are observed with the compounds having the linker n = 0 and the substituents R1 and R2 being methoxy (13a) or when the linker n is 1 and R1 and R2 are hydroxy (6e) as observed in the 1,3-phenyl class. The compounds with the linker n = 2 are either inactive (28, 29a) or weakly active (27a, 27), independent of the substituents at rings A and C. With the ethylene linker the compounds might be too long and/or too flexible to fit in the enzyme active site. They were not further investigated.

Focusing on compounds with n = 0, the influence of the central core can also be evaluated. By comparison of the 1,3-phenyl to the 2,5-thiophene derivatives (for 9a compared to

13a, 64% and 90% inh at 1 μM, respectively; for 10a compared to 14a, 40% and 66% inh at 1 μM, respectively; for 11a compared to 15a, 37% and 82% inh at 1 μM, respectively), it is obvious that the 2,5-thiophene is better than the 1,3-phenyl moiety. This preference is difficult to explain, as both aromatic moieties can establish a π-stacking interaction with aromatic amino acids from the active site. However the overall electronic density and the molecular electrostatic potential (MEP) differ depending on the nature of the central scaffold. It is likely that the MEP induced by the 2,5-thiophene leads to a better recognition with the corresponding region in the binding cavity. This property has already been evidence in the discovery of 17β-HSD1 inhibitors.[39,48] Thus, the 2,5-thiophene class only was further investigated in the rest of the study.

Furthermore, the influence of the A and C ring substituents on the activity can be deduced in the 2,5-thiophene class with the linker n = 0. Taking away the methoxy group on the A ring (R2 = H 15a/R2 = OMe 13a, 82%/90% inh at 1 μM, respectively) does not influence the potency of the compound, indicating that this group does not play a critical role in the stabilization of the inhibitor in the active site. Deleting the same group on the C ring (R1 = H 16a/R1 = OMe 13a, 67%/90% inh at 1 μM, respectively) leads to a more consistent decrease in activity, suggesting that this methoxy is involved in specific interactions that stabilize the inhibitor in the binding cavity or

that it affects the electrostatic potential of the C ring which again favors the binding.

The importance of electronic effects is also indicated by the fact that replacement of the methoxy moiety at the A ring (**13a**, 90% inh at 1 μM) by other electron donating groups (3-Me **19**, 3-NMe$_2$ **20**, and 3-SMe **21** giving 95%, 82%, and 88% inh at 1 μM, respectively) is well accepted, whereas replacement by electron withdrawing groups (4-CN **18a** and 3-F **23** giving 48% and 67% inh at 1 μM, respectively) leads to a decrease in activity. The same is also valid for the C ring, where exchange of the methoxy moiety **13a** (90% inh at 1 μM) by electron withdrawing groups like 3-CF$_3$ **24** (75% inh at 1 μM) or 2-F **25** (68% at 1 μM) slightly reduces the 17β-HSD2 inhibitory activity, while in the presence of the electron donating 3-Me **22a** the percentage inhibition does not change. Introduction of the large 3-Ph **26** (83% inh at 1 μM) is also well tolerated by the enzyme, indicating that there is space in the area of the binding site for introduction of bulky substituents.

A fluorine has been introduced at the 3-methoxyphenyl A ring as second substituent in this ring (compounds **30−32**, **36−39**, Table 3) in the 2,5-thiophene class with the linker $n = 0$.

Table 3. Inhibition of Human 17β-HSD2 and 17β-HSD1 by Phenylthiophene Amide Derivatives Di- or Trisubstituted on the A-Ring 30−41 in Cell-Free System

7, 30-41
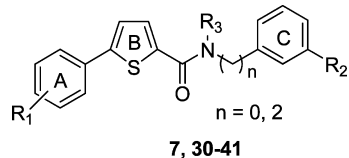
compd	n	R$_1$	R$_2$	R$_3$	17β-HSD2[b]	17β-HSD1[c]
					% inhibition at 1 μM[a,d]	
7	1	2-F,3-OMe	3-OH	Me	89	ni
30	0	2-F,3-OMe	3-OH	Me	76	33
31	0	2-F,3-OMe	3-OMe	Me	93	17
32	0	2-F,3-OMe	3-Me	Me	85	20
33	0	2-F,3-OMe	3-OMe	H	ni	ni
34	0	2-F,3-OMe	3-OMe	Ph	ni	13
35	0	2-F,3-OMe	4-OMe	Me	49	18
36	0	2-F,3-Me	3-OMe	Me	77	ni
37	0	2-F,6-F,3-OMe	3-OMe	Me	94	28
38	0	3-F,4-OMe	3-OMe	Me	72	15
39	0	3-OMe,4-F	3-OMe	Me	66	17
40	0	3-F,4-F	3-OMe	Me	50	ni
41	0	2-OMe,4-OMe	3-OMe	Me	77	30

[a]Mean value of three determinations, standard deviation less than 8% except for **27** in HSD1, 23%. [b]Human placental, microsomal fraction, substrate E2, 500 nM, cofactor NAD$^+$, 1500 μM. [c]Human placental, cytosolic fraction, substrate E1, 500 nM, cofactor NADH, 500 μM. [d]ni: no inhibition (inhibition of <10%).

Introduction at the 2 position led to **31** and **32** (93% and 85% inh at 1 μM, respectively), which have similar activity as the corresponding compounds **13a** and **22a**. This substituent does not achieve any specific interaction with the active site but is also not disturbing the stabilization. Addition of the fluorine at position 4 induces a slight loss in activity, **39** (66% inh at 1 μM). This decrease in activity is consistent with the electronic effect described previously (replacement of a methoxy for an electron withdrawing group, compounds **18a** and **23**).

Addition of a third substituent on the A ring, a 6-F (**37** 94% inh at 1 μM), does not increase the potency of **31**.

Compound **31** (93% inh at 1 μM), differing from **35** (49% inh at 1 μM) in the displacement of the methoxy group on the C ring from the 3 to the 4 position, leads to a decrease in activity and reveals the importance of the interaction achieved by this group, which must have the right orientation.

It was then investigated if the methyl group on the amide function of **31** is necessary for activity: exchange with a hydrogen **33** or a phenyl **34** led to two inactive compounds. The methyl group might be located in a small lipophilic cavity and participate actively in the stabilization of the compound. Loss of this group prevents this interaction, and the phenyl group might be too big to fit into this lipophilic cavity.

For the most active compounds showing more than 70% inhibition at 1 μM, IC$_{50}$ values were determined in the cell-free assay and are shown in Table 4. Four highly active compounds with the linker $n = 0$ (**13a**, **19**, **31**, and **32**) were identified displaying IC$_{50}$ values of around 60 nM. They are equipotent to the previously described **7** carrying a methylene linker. Five other interesting compounds (**12**, **20**, **26**, **36**, and **37**) were discovered with IC$_{50}$ between 100 and 200 nM.

The inhibitory activity of the most potent compounds on 17β-HSD2 was also evaluated in a cellular model system, using the MDA-MB-231 cell line. The compounds' efficiency is expressed as IC$_{50}$ for the most potent compounds or as percentage of inhibition for the others (Table 4). The data obtained lie in the same range as the cell-free inhibition data, with IC$_{50}$ values around 100 nM or below. The results indicate that the compounds can permeate the cell membrane, are stable in the cell, and are not quickly metabolized.

2. Selectivity Aspect. As 17β-HSD1 catalyzes the reduction of E1 to E2, the reversed 17β-HSD2 reaction, it should not be affected by 17β-HSD2 inhibitors. In the 1,3-phenyl class (compounds **8a−11**), the selectivity observed toward this enzyme (Table 1) is very good: no or a very weak inhibition of the 17β-HSD1 enzyme was measured at 1 μM. In the series of the 2,5-thiophenes, independent of the linker size ($n = 0$, 1, or 2; compounds **12−29**), the same results were observed except for the middle active 17β-HSD2 inhibitors **14**, **15**, and **30** (69%, 60%, and 67% 17β-HSD2 inh at 1 μM, respectively), which showed around 50% 17β-HSD1 inhibition at 1 μM. For the most potent 17β-HSD2 inhibitors (Table 4), the selectivity was expressed as selectivity factor (SF) calculated as the ratio of IC$_{50}$ (17β-HSD2) over IC$_{50}$ (17β-HSD1). For the compounds with an IC$_{50}$ (17β-HSD2) below 200 nM, the SF varied between 26 and above 800 except for **26** with a SF of 8. The selectivity toward 17β-HSD1 is good to very good for most of the new 17β-HSD2 inhibitors described. It is even better for the compounds without linker (SF of 112, 116, above 800, and 132 for **13a**, **19**, **31**, and **32**) compared to the one with a methylene linker (SF of 73 for **7**).

Inhibitors of 17β-HSD2 should have no affinity for the estrogen receptors (ER) α and β, as it is expected that most E2 effects are ER mediated. All the compounds with an IC$_{50}$ (17β-HSD2, cell-free assay) below 500 nM were evaluated for their relative binding affinity (RBA) to the ERα and ERβ in a competitive assay using a previously described assay[47,49] and taking E2 as internal reference. All of the tested compounds showed a RBA below 0.1%, compared to the affinity of E2, which was arbitrarily set to 100%.

17β-HSD4 catalyzes the oxidation of E2 into E1 as 17β-HSD2 (Chart 1) and is ubiquitously expressed. 17β-HSD5 is a

Table 4. IC$_{50}$ Values (17β-HSD2 and 17β-HSD1) and Selectivity Factor for Selected Compounds

5a

6c, 7, 12,13a,15a,19-22,
24, 26, 31, 32, 36-38, 41

				cell-free assay				
				IC$_{50}$ (nM)^a			cell test^i	
compd	n	R$_1$	R$_2$	17β-HSD2^b	17β-HSD1^c	selectivity factor^d	IC$_{50}$ (nM)^g HSD2^e	cLogP^f
5a	1	3-OH	OH	482	3801	8	nd	4.04
6c	1	3-OH	OH	394	5449	14	nd	4.08
7	1	2-F,3-OMe	OH	61	4452	73	78	4.50
12	0	4-OMe	OMe	148	6217	42	81%^{h}	4.54
13a	0	3-OMe	OMe	68	7593	112	119	4.54
15a	0	H	OMe	207	4337	21	nd	4.66
19	0	3-Me	OMe	58	6752	116	73	5.15
20	0	3-N(Me)$_2$	OMe	169	10573	63	71%^{h}	4.95
21	0	3-SMe	OMe	242	5306	22	nd	5.10
22a	0	3-OMe	Me	207	11454	55	nd	5.15
22	0	3-OH	Me	645	6800	11	nd	4.89
24	0	3-OMe	CF$_3$	721	12259	17	nd	5.58
26	0	3-OMe	Ph	137	1109	8	nd	6.34
31	0	2-F,3-OMe	OMe	62	>50000	>800	105	4.69
32	0	2-F,3-OMe	Me	62	8209	132	80%^{h}	5.31
36	0	2-F,3-Me	OMe	130	5426	42	83%^{h}	5.79
37	0	2-F,6-F,3-OMe	OMe	184	4812	26	83%^{h}	4.85
38	0	3-F,4-OMe	OMe	242	>40000	>165	nd	4.69
41	0	2-OMe,4-OMe	OMe	313	1927	6	nd	4.41

^{a}Mean value of three determinations, standard deviation less than 15%. ^{b}Human placental, microsomal fraction, substrate E2, 500 nM, cofactor NAD$^+$, 1500 μM. ^{c}Human placental, cytosolic fraction, substrate E1, 500 nM, cofactor NADH, 500 μM. ^{d}IC$_{50}$(17β-HSD1)/IC$_{50}$(17β-HSD2). ^{e}MDA-MB-231 cell line, substrate E2, 200 nM. ^{f}Calculated data. ^{g}Mean value of two determinations, standard deviation less than 15%. ^{h}Inhibition measured at an inhibitor concentration of 1 μM. ^{i}nd: not determined.

reductive enzyme; it converts the inactive DHEA and 4-androstene-3,17-dione into the potent 5-androstene-3β,17β-diol and testosterone, respectively (Chart 1). In order to avoid systemic side effects and not to counteract the effect of 17β-HSD2 inhibition, inhibition of these enzymes should be avoided.

The five most potent compounds 7, 13a, 19, 31, and 32 were evaluated for their ability to inhibit these two enzymes, using recombinant human 17β-HSD4 and 17β-HSD5 expressed in *E. coli* following the described procedure.[50,51] The compounds did not show any inhibition of 17β-HSD4 when tested at 1 μM and inhibited 17β-HSD5 only weakly (inhibition between 17% and 33% at 1 μM, Table 5).

11β-HSDs are involved in the glucocorticoid biosynthesis. 11β-HSD1 catalyzes the transformation of the inactive cortisone into the potent cortisol, and 11β-HSD2 catalyzes the reverse reaction. Some inhibitors of 11β-HSD1[52] have a close structural analogy to the amides identified in this study. In addition, 17β-HSD2 has a relatively high sequence identity with 11β-HSD2 (45%).[53] Therefore, the selectivity profile of the five structurally most relevant compounds 7, 13a, 19, 31, and 32 was thus extended to these two enzymes using the recombinant enzymes 11β-HSD1 and 11β-HSD2 stably transfected in HEK-293 cells following the described procedure.[54] Absence or very low 11β-HSD1 and 11β-HSD2 inhibition was observed at 2 μM except for 7, which showed an IC$_{50}$ of 1 μM for 11β-HSD1. This activity is not negligible, but compared to the IC$_{50}$ of 61

Table 5. Selectivity toward 17β-HSD4, 17β-HSD5, 11β-HSD1, and 11β-HSD2 for Selected Compounds

compd	inhibition of 17β-HSD4, % at 1 μM^{a,b,f}	inhibition of 17β-HSD5, % at 1 μM^{a,c}	inhibition of 11β-HSD1, % at 2 μM (IC$_{50}$)^{a,d,f}	inhibition of 11β-HSD2, % at 2 μM^{a,d,f}
7	ni	33	68 (1 μM)	10
13a	ni	17	ni	ni
19	ni	20	23	14
31	ni	26	9	8
32	ni	29	ni	ni
2–9^e	ni	88	nd	nd

^{a}Mean value of three determinations, standard deviation less than 19% for 17β-HSD5 and less than 9% for 17β-HSD4. ^{b}Enzyme expressed in bacteria (bacterial suspension), substrate [^{3}H]E2, 21 nM, cofactor NAD$^+$, 750 μM. ^{c}Enzyme expressed in bacteria (bacterial lysate), substrate [^{3}H]A-dione, 21 nM, cofactor NADPH, 600 μM. ^{d}Determined in lysate of HEK-293 cells expressing recombinant human enzymes. ^{e}External reference: compounds 2–9 described by Schuster et al.[50] ^{f}ni = no inhibition. nd = not determined.

nM for 17β-HSD2, a selectivity factor of around 16 might be acceptable, especially regarding the fact that 11β-HSD1 activates glucocorticoids and elevated glucocorticoids have been associated with osteoporosis.[55]

3. Further Tests. The lipophilicity profiles of 7, 13a, 19, 31, and 32 were evaluated by calculation of log P (Table 4). For most of the compounds it is between 4 and 5 or slightly above

5, which is still in a good range according to the Lipinski rule of five[56] and which should be correlated to a good permeability.

The cytotoxicity of **7**, **13a**, **19**, **31**, and **32** was evaluated in the MDA-MB-231 cell line based on MTT conversion following the procedure described by Denisot et al.[57] at three different concentrations: 2.5, 10, and 50 μM. No cytotoxicity could be observed even at the highest concentration after 3 h of incubation (data not shown).

In order to identify the appropriate species for demonstration of in vivo efficacy in a disease-oriented model, the five most potent compounds **7**, **13a**, **19**, **31**, and **32** were tested for their ability to inhibit the enzyme responsible for E2 into E1 transformation from three different animals: rat, mouse, and monkey *Callithrix jacchus*. The compounds were evaluated in a cell-free assay using the microsomal fraction of liver preparation from rat and mouse. In the case of the monkey, the microsomal enzyme was gained from placenta. The compounds showed middle activity on the monkey enzyme, between 45% and 53% inh at 1 μM (Table 6). They were inactive to very low active in

Table 6. Inhibition of E1 Formation by Rat, Mouse, and Monkey Enzymes Compared to Human Enzyme for Selected Compounds

compd	human 17β-HSD2[a] inh (%) at 1 μM	rat E1 formation[b] inh (%) at 1 μM	mouse E1 formation[c] inh (%) at 1 μM	monkey E1 formation[d] inh (%) at 1 μM
7	89	25	65	47
13a	90	14	29	45
19	95	ni	30	53
31	93	ni	26	45
32	85	ni	45	49

[a]Human placenta, microsomal fraction, substrate [³H]E2 + E2 [500 nM], NAD⁺ [1500 μM], mean value of three determinations, relative standard deviation of <10%. [b]Rat liver, microsomal fraction, substrate [³H]E2 + E2 [500 nM], NAD⁺ [1500 μM], mean value of three determinations, relative standard deviation of <10%. ni: no inhibition. [c]Mouse liver, microsomal fraction, substrate [³H]E2 + E2 [500 nM], NAD⁺ [1500 μM], mean value of three determinations, relative standard deviation of <10%. [d]Monkey placenta, microsomal fraction, substrate [³H]E2 + E2 [500 nM], NAD⁺ [1500 μM], mean value of three determinations, relative standard deviation of <10%.

the rat, the best one being **7** with 25% inh at 1 μM. In mouse compounds **13a**, **19**, and **31** were also barely active (between 26% and 30% inh at 1 μM) except for **32** and **7** which were middle to good active with 45% and 65% inh at 1 μM, respectively. It is striking that such a difference in activity is observed between the rat (*Rattus norvegicus*) and the mouse (*Mus musculus*) 17β-HSD2 inhibition data, as the protein sequence of both species is highly similar. However from this study, compound **7**, identified as a highly active and selective 17β-HSD2 inhibitor at the human enzyme, exhibits the highest 17β-HSD2 inhibition on the mouse enzyme. This result suggests that the mouse might be a promising species to perform an in vivo experiment using compound **7** and to verify that 17β-HSD2 inhibitors could be effective for the treatment of osteoporosis.

■ DISCUSSION

The aim of this study was the optimization of 17β-HSD2 inhibitors from the amide class by variation of the size of the linker (n) located between the amide function and the C ring. Introduction of an ethylene linker ($n = 2$) is detrimental for the activity, independent of the central moiety 1,3-phenyl or 2,5-thiophene. The compounds might be too long or too flexible. Taking out these two carbons linker ($n = 0$) led to the identification of four promising compounds **13a**, **19**, **31**, and **32** with IC$_{50}$ values of around 60 nM. Interestingly these compounds all bear a methoxy function on the C ring while the equally active **7** with a methylene linker ($n = 1$) is hydroxylated on this ring. High activity is only achieved when $n = 1$ and the C ring is hydroxylated (**7**) or $n = 0$ and the C ring is methoxylated (**13a**, **19**, **31**, and **32**). It is striking that there is no difference in activity between these two series of compounds. These requirements to achieve high activity are also intriguing. These data suggest that these two series of compounds may not interact with the same amino acids in the binding site. Thus, a different binding mode could be expected for these two groups of inhibitors.

From this study it is clear that the 2,5-thiophene central core is superior to the 1,3-phenyl independent of the size of the linker $n = 0$ or 1. This result was already observed developing 17β-HSD1 inhibitors. The molecular electrostatic potential
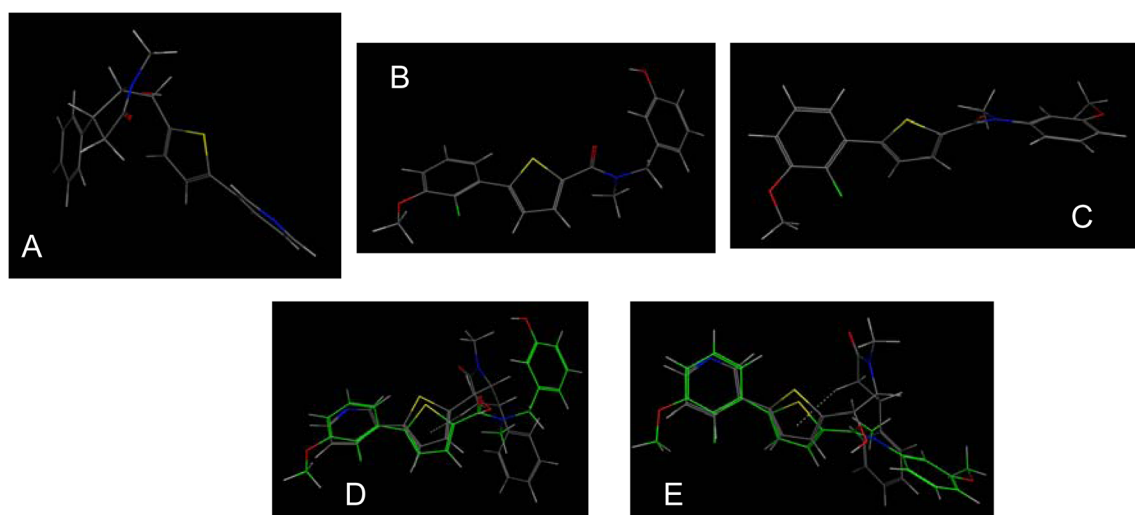

Figure 2. Mapping of 17β-HSD2 active site: (A) 3D-structure of **2**; (B) 3D-structure of **7**; (C) 3D-structure of **31**; (D) superimposition of **2** to **7** ($n = 1$); (E) superimposition of **2** to **31** ($n = 0$). **2** is colored gray, and **7** and **31** are green. The picture was generated using Moe 2010.10.

induced by the thiophene on the whole molecule might lead to better interactions with the enzyme active site. Further, the thiophene differs from the phenyl ring in the presence of d-orbitals on the sulfur. They might allow the thiophene derivatives to undergo specific interactions compared to the phenyl one.

The human 17β-HSD2 accepts ligands with a high structural diversity: steroidal substrates E2, T, 20α-dihydroprogesterone, pregnenolone, and DHEA, steroidal inhibitor spirolactone **1**, nonsteroidal inhibitors *cis*-pyrrolidinone **2**, and amides **7** and **31**. These ligands differ in their shape and volume, but they are all very large. In order to map the enzyme active site, which is unknown, and having the hypothesis that all these compound bind in the enzyme active site, we compared the new nonsteroidal 17β-HSD2 inhibitors with two different linker sizes (**31** for *n* = 0 and **7** for *n* = 1) with the equipotent *cis*-pyrrolidinone **2** described by Wood et al.[25−27] Visualization of the 3D-structure of these three compounds, after energy minimization, highlights the folded shape of compound **2** with the thiophene ring almost parallel to the pyrrolidinone moiety (Figure 2). Thereby the two aromatic groups, phenyl and pyridine, are directed in opposite direction while for **7** and **31** they assume an elongated shape. Superimposition of **7** and **31** to **2** (Figure 2) may indicate that the area occupied by the different compounds varies and sustains the hypothesis that compound **2** may fit into the enzyme's active site with another binding mode compared to **7** and **31**. In addition, the fact that compound **2** does not require any hydroxy or methoxy groups to achieve high activity in contrast to **7** or **31** also suggests a different positioning of these compounds in the binding site. It could therefore be deduced that the 17β-HSD2 active site may be very large. This space could easily be used to achieve selectivity toward other enzymes by introduction of appropriate substituents. It is striking that compounds with a linker *n* = 2 were inactive, although they were expected to fit from the steric hindrance point of view. It might indicate that the flexibility induced by the ethylene linker is not appropriate.

The selectivity profile of the 17β-HSD2 inhibitor is an important issue. Not to counteract the therapeutic concept, the functionally related enzymes like 17β-HSD1, -4, -5 should not be inhibited and no binding affinity to the ERs should be identified. The most potent 17β-HSD2 inhibitors identified in this study, **13a**, **19**, **31**, **32**, **1**, **2**, and **7**, are all selective toward 17β-HSD1 and do not bind to the estrogen receptors. It might indicate that the 17β-HSD2 active site is larger than that of 17β-HSD1 and the binding domains of the ERs. The size difference of the binding sites of these proteins is an interesting property, as it could facilitate the gain in selectivity of the 17β-HSD2 inhibitor toward 17β-HSD1 and ERs. In addition it is notable that the most active compounds with a linker *n* = 0, i.e., **13a**, **19**, **31**, and **32**, have a much higher selectivity factor than the one compound with *n* = 1, i.e., compound **7**. In the case of *n* = 0, the C ring seems to adopt a conformation of higher steric hindrance in the 17β-HSD1 active site than in the case of *n* = 1.

11β-HSD1 and 11β-HSD2 catalyze the oxidoreduction at position 11 of cortisone in cortisol. These two enzymes have an important function in the glucocorticoid biosynthesis and should not be inhibited. On the basis of structural similarities identified between 11β-HSD1 inhibitors and our amides derivatives, it is also important to verify the selectivity of our inhibitors toward these enzymes. None of the new amides discovered in this study inhibit 11β-HSD1 or 11β-HSD2 to an appreciable extend except for compound **7** which shows an IC$_{50}$

of 1 μM for 11β-HSD1 and an IC$_{50}$ of 61 nM for 17β-HSD2, the ratio resulting in a selectivity factor (SF) of 16. On the basis of the fact that elevated glucocorticoids levels have been associated with osteoporosis, moderate 11β-HSD1 inhibition might be still of advantage for the therapeutic concept.

Inhibition of 17β-HSD2 is a completely new approach for the treatment of osteoporosis compared to the existing treatments. A therapeutic effect similar to the one observed with estrogen replacement therapy (ERT), which has already been proven to be efficient in the treatment of osteoporosis, is expected after treatment with 17β-HSD2 inhibitors. ERT is not recommended because of severe side effects caused by the systemic increase in E2 at the necessary high doses. Inhibition of 17β-HSD2 will allow an increase in E2 in lower doses and only in targeted organs where the enzyme is present, i.e., in organs like placenta, brain, bone, breast, and ovaries. In addition after menopause, the ovaries do not function anymore and there is atrophy of the breast and of the uterus connected to a reduction of the metabolism/catabolism of the tissues. The levels in androstenedione and estrone in these organs may be reduced and therefore the amount of E2 and testosterone as well. Consequently treatment with 17β-HSD2 inhibitors may be less susceptible to induce breast cancer compared to ERT. 17β-HSD2 inhibition is thus not expected to induce severe adverse effects. A targeted effect in the bones should result in a superior drug compared to SERMs or bisphosphonates.

The five most potent compounds **7**, **13a**, **19**, **31**, and **32** were investigated regarding their ability to inhibit 17β-HSD2 from other species in order to identify an appropriate species for conducting in vivo experiments. The compounds were tested on the rat, mouse, and monkey 17β-HSD2. Only inhibitor **7** showed a good inhibition on the mouse enzyme. At this stage, the mouse can be considered as potential species to perform the in vivo proof of concept. It has the advantage that it is easily accessible and is well described, as it is often used for the study of bone diseases.[58,59] Metabolic stability and the pharmacokinetic profile of compound **7** have to be evaluated in the mouse to validate this species as adequate model.

In this study, we described the optimization of 17β-HSD2 inhibitors in the 2,5-thiophene and 1,3-phenylamide class by variation of the linker size between the C ring and the amide moiety. It led to the discovery of four new highly active compounds with the C ring directly attached to the amides **13a**, **19**, **31**, and **32** with an IC$_{50}$ of around 60 nM in a cell-free assay, a very good cellular activity in the same range as in the cell-free assay, and a very high selectivity factor toward 17β-HSD1 above 100 and even higher than 800 for **31**. Compounds **13a**, **19**, **31**, and **32** are equipotent to the compound with the methylene linker **7** but show higher selectivity toward 17β-HSD1. SAR studies allowed a first characterization of the 17β-HSD2 active site which must be quite large and certainly larger than the one of 17β-HSD1. The mouse was identified as a potential animal in order to perform an in vivo proof of concept for the demonstration of the efficacy of 17β-HSD2 inhibitors in osteoporosis.

■ EXPERIMENTAL SECTION

1. Chemical Methods. Chemical names follow IUPAC nomenclature.

Starting materials were purchased from Aldrich, Acros, Lancaster, Roth, Merck, Combi-Blocks, or Fluka and were used without purification.

Flash column chromatography (FC) was performed on silica gel (70−200 μm), and reaction progress was monitored by TLC on Alugram SIL G/UV254 (Macherey-Nagel). Visualization was accomplished with UV light.

^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker AM500 spectrometer (at 500 and 125 MHz, respectively) at 300 K in CD_3COCD_3. Chemical shifts are reported in δ (parts per million, ppm) by reference to the hydrogenated residues of deuterated solvent as internal standard: 2.05 ppm (^{1}H NMR) and 30.8 and 206.3 ppm (^{13}C NMR). Signals are described as br (broad), s (singlet), d (doublet), t (triplet), dd (doublet of doublets), ddd (doublet of doublet of doublets), dt (doublet of triplets), or m (multiplet). All coupling constants (J) are given in hertz (Hz).

MS measurements were executed using a TSQ Quantum equipped with an electrospray interface (ESI) or an atmospheric pressure chemical ionization source (APCI) (Thermo Fischer, Dreieich, Germany) instrument. GC/MS spectra were measured on a GCD series G1800A (Hewlett-Packard) instrument with an Optima-5-MS (0.25 μM, 30 m) column (Macherey Nagel).

IR spectra were recorded on a Spectrum 100 FT-IR spectrometer (PerkinElmer) as neat sample.

Melting points were measured in open capillaries on a Stuart Scientific SMP3 apparatus and are uncorrected.

The purity of the compounds was evaluated by LC/MS. The Surveyor-LC-system consisted of a pump, an autosampler, and a PDA detector. Mass spectrometry was performed on a TSQ Quantum (ThermoFisher, Dreieich, Germany). The triple quadrupole mass spectrometer was equipped with an electrospray interface (ESI) or an atmospheric pressure chemical ionization (APCI). The system was operated by the standard software Xcalibur. A RP C18 Nucleodur 100-5 (3 mm) column (Macherey-Nagel GmbH, Dühren, Germany) was used as stationary phase. All solvents were HPLC grade. In a gradient run, the percentage of acetonitrile (containing 0.1% trifluoroacetic acid) in 0.1% trifluoroacetic acid was increased from an initial concentration of 0% at 0 min to 100% at 15 min and kept at 100% for 5 min. The injection volume was 15 μL, and flow rate was set to 800 μL/min. MS analysis was carried out at a needle voltage of 3000 V and a capillary temperature of 350 °C. Mass spectra were acquired in positive mode from 100 to 1000 m/z, and UV spectra were recorded at a wavelength of 254 nm and in some cases at 360 nm. All tested compounds have ≥95% chemical purity except compounds **15a** and **21**, which have a purity of 94% and 90%, respectively.

Compounds **4**,[31] **5a−d**,[32] **6a−l**,[32] and **7**[32] were prepared according to previously described procedures.

General Procedure for Amidation. Method A. At 0 °C, a solution of 3(4)-bromobenzoyl chloride or 5-bromothiophene-2-carbonyl chloride (1 equiv) in CH_2Cl_2 (2 mL/equiv) was added dropwise to a solution of the corresponding amine (1 equiv) and triethylamine (1.15 equiv) in solution in CH_2Cl_2 (2 mL/equiv). The mixture was kept stirred at 0 °C for 3 h and evaporated under reduced pressure. The residue was purified by FC with n-hexane/ethyl acetate or dichloromethane as eluant.

General Procedure for Suzuki Coupling. Method B. A mixture of aryl bromide (1 equiv), substituted phenylboronic acid (1.2 equiv), sodium carbonate (2 equiv), and tetrakis(triphenylphosphine)-palladium (0.1 equiv) in an oxygen free DME/water (1:1) solution was stirred at 80 °C for 4−14 h under nitrogen. The reaction mixture was cooled to room temperature. The aqueous layer was extracted with dichloromethane. The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated to dryness. The product was purified by FC with n-hexane/ethyl acetate, dichloromethane, or dichloromethane/methanol as eluant.

General Procedure for Ether Cleavage. Method C. To a solution of methoxyphenyl compounds (1 equiv) in dry dichloromethane (5 mL/mmol of reactant), boron trifluoride−dimethyl sulfide complex (6 equiv/methoxy function) was added dropwise at 0 °C and stirred for 6−14 h. After the reaction was finished, the reaction mixture was diluted with dichloromethane and 5% aqueous $NaHCO_3$ was added until neutral pH was obtained. The aqueous layer was extracted with dichloromethane. The combined organic layers were washed with

brine, dried over sodium sulfate, evaporated to dryness under reduced pressure. The product was purified by FC, with dichloromethane/methanol as eluant.

Detailed Synthesis Procedures of the Most Interesting Compounds. *N*-(3-Methoxyphenyl)-5-(4-methoxyphenyl)-*N*-methylthiophene-2-carboxamide (12). The title compound was prepared by reaction of 5-bromo-*N*-(3-methoxyphenyl)-*N*-methylthiophene-2-carboxamide **12b** (40 mg, 0.12 mmol) and 4-methoxyphenylboronic acid (22 mg, 0.14 mmol) with tetrakis(triphenylphosphine) palladium (14 mg, 0.012 mmol) according to method B for 6 h. Purification by FC (CH_2Cl_2/CH_3OH, 200:1) afforded the desired compound as a brown solid (40 mg, yield 92%). $C_{20}H_{19}NO_3S$; MW 353; mp 119−120 °C; MS (ESI) 354 (M + H)$^+$; ^{1}H NMR (CD_3COCD_3) 3.37 (s, 3H), 3.81 (s, 3H), 3.82 (s, 3H), 6.55 (d, J = 4.1 Hz, 1H), 6.92−6.96 (m, 3H), 6.97−7.01 (m, 2H), 7.02 (d, J = 4.1 Hz, 1H), 7.37 (td, J = 7.9, 0.6 Hz, 1H), 7.51 (d, J = 9.1 Hz, 2H).

N,5-Bis(3-methoxyphenyl)-*N*-methylthiophene-2-carboxamide (13a). The title compound was prepared by reaction of 5-bromo-*N*-(3-methoxyphenyl)-*N*-methylthiophene-2-carboxamide **12b** (75 mg, 0.23 mmol) and 3-methoxyphenylboronic acid (41 mg, 0.27 mmol) with tetrakis(triphenylphosphine)palladium (27 mg, 0.023 mmol) according to method B for 5 h. Purification by FC (CH_2Cl_2) afforded the desired compound as a yellow solid (80 mg, yield 98%). $C_{20}H_{19}NO_3S$; MW 353; mp 116−117 °C; MS (ESI) 354 (M + H)$^+$; ^{1}H NMR (CD_3COCD_3) 3.38 (s, 3H), 3.82 (s, 3H), 3.83 (s, 3H), 6.58 (d, J = 4.1 Hz, 1H), 6.90 (ddd, J = 8.2, 2.5, 0.9 Hz, 1H), 6.94 (ddd, J = 7.6, 1.6, 0.9 Hz, 1H), 6.98−7.01 (m, 2H), 7.12 (t, J = 2.0 Hz, 1H), 7.15 (ddd, J = 7.6, 1.6, 0.9 Hz, 1H), 7.15 (d, J = 4.1 Hz, 1H), 7.30 (t, J = 8.0 Hz, 1H), 7.38 (td, J = 8.0, 0.9 Hz, 1H).

N-(3-Methoxyphenyl)-*N*-methyl-5-phenylthiophene-2-car-boxamide (15a). The title compound was prepared by reaction of 5-bromo-*N*-(3-methoxyphenyl)-*N*-methylthiophene-2-carboxamide **12b** (75 mg, 0.23 mmol) and phenylboronic acid (33 mg, 0.27 mmol) with tetrakis(triphenylphosphine)palladium (27 mg, 0.023 mmol) according to method B for 4 h. Purification by FC (n-hexane/ethyl acetate, 10:1 → 6:1) afforded the desired compound as a beige solid (70 mg, yield 94%). $C_{19}H_{17}NO_2S$; MW 323; mp 126−127 °C; MS (ESI) 324 (M + H)$^+$; ^{1}H NMR (CD_3COCD_3) 3.38 (s, 3H), 3.82 (s, 3H), 6.60 (d, J = 4.0 Hz, 1H), 6.94 (ddd, J = 8.0, 2.1, 0.9 Hz, 1H), 6.99−7.01 (m, 2H), 7.16 (d, J = 4.0 Hz, 1H), 7.32 (ddt, J = 8.0, 6.3, 1.2 Hz, 1H), 7.36−7.41 (m, 3H), 7.59 (d, J = 8.0 Hz, 2H).

N-(3-Methoxyphenyl)-*N*-methyl-5-*m*-tolylthiophene-2-car-boxamide (19). The title compound was prepared by reaction of 5-bromo-*N*-(3-methoxyphenyl)-*N*-methylthiophene-2-carboxamide **12b** (33 mg, 0.1 mmol) and 3-methylphenylboronic acid (19 mg, 0.14 mmol) with tetrakis(triphenylphosphine)palladium (12 mg, 0.01 mmol) according to method B for 8 h. Purification by FC (n-hexane/ethyl acetate, 10:1 → 6:1) afforded the desired compound as a beige solid (26 mg, yield 77%). $C_{20}H_{19}NO_2S$; MW 337; mp 128−129 °C; MS (ESI) 338 (M + H)$^+$; ^{1}H NMR (CD_3COCD_3) 2.34 (s, 3H), 3.38 (s, 3H), 3.81 (s, 3H), 6.57 (d, J = 4.0 Hz, 1H), 6.94 (ddd, J = 7.9, 2.2, 0.9 Hz, 1H), 6.98−7.01 (m, 2H), 7.13 (d, J = 4.0 Hz, 1H), 7.14−7.16 (m, 1H), 7.27 (t, J = 7.9 Hz, 1H), 7.36−7.39 (m, 2H), 7.41−7.42 (m, 1H).

5-(3-(Dimethylamino)phenyl)-*N*-(3-methoxyphenyl)-*N*-methylthiophene-2-carboxamide (20). The title compound was prepared by reaction of 5-bromo-*N*-(3-methoxyphenyl)-*N*-methylthiophene-2-carboxamide **12b** (49 mg, 0.15 mmol) and 3-(dimethylamino)phenylboronic acid (30 mg, 0.18 mmol) with tetrakis(triphenylphosphine)palladium (17 mg, 0.015 mmol) according to method B for 14 h. Purification by FC (n-hexane/ethyl acetate, 10:1 → 6:1) afforded the desired compound as an orange solid (26 mg, yield 47%). $C_{21}H_{22}N_2O_2S$; MW 366; mp 121−122 °C; MS (ESI) 367 (M + H)$^+$; ^{1}H NMR (CD_3COCD_3) 2.96 (s, 6H), 3.38 (s, 3H), 3.82 (s, 3H), 6.56 (d, J = 4.0 Hz, 1H), 6.72 (dd, J = 8.2, 2.4 Hz, 1H), 6.86 (d, J = 7.6 Hz, 1H), 6.89 (t, J = 2.0 Hz, 1H), 6.94 (d, J = 7.6 Hz, 1H), 6.98−7.01 (m, 2H), 7.10 (d, J = 4.0 Hz, 1H), 7.19 (t, J = 7.9 Hz, 1H), 7.37 (t, J = 7.9 Hz, 1H).

N-(3-Methoxyphenyl)-*N*-methyl-5-(3-(methylthio)phenyl)-thiophene-2-carboxamide (21). The title compound was prepared

by reaction of 5-bromo-*N*-(3-methoxyphenyl)-*N*-methylthiophene-2-carboxamide **12b** (33 mg, 0.1 mmol) and 3-(methylthio)-phenylboronic acid (23 mg, 0.14 mmol) with tetrakis-(triphenylphosphine)palladium (12 mg, 0.01 mmol) according to method B for 8 h. Purification by FC (*n*-hexane/ethyl acetate, 10:1 → 7:1) afforded the desired compound as a yellowish solid (22 mg, yield 59%). C$_{20}$H$_{19}$NO$_2$S$_2$; MW 369; mp 109−110 °C; MS (ESI) 370 (M + H)$^+$; ^{1}H NMR (CD$_3$COCD$_3$) 2.52 (s, 3H), 3.38 (s, 3H), 3.82 (s, 3H), 6.59 (d, *J* = 4.0 Hz, 1H), 6.94 (ddd, *J* = 8.0, 1.9, 0.9 Hz, 1H), 6.98−7.02 (m, 2H), 7.18 (d, *J* = 4.0 Hz, 1H), 7.23 (dt, *J* = 6.9, 1.9 Hz, 1H), 7.30−7.35 (m, 2H), 7.38 (td, *J* = 8.0, 0.9 Hz, 1H), 7.44−7.45 (m, 1H).

5-(2-Fluoro-3-methoxyphenyl)-*N*-(3-methoxyphenyl)-*N*-methylthiophene-2-carboxamide (31). The title compound was prepared by reaction of 5-bromo-*N*-(3-methoxyphenyl)-*N*-methylthiophene-2-carboxamide **12b** (40 mg, 0.12 mmol) and 2-fluoro-3-methoxyphenylboronic acid (25 mg, 0.14 mmol) with tetrakis-(triphenylphosphine)palladium (14 mg, 0.012 mmol) according to method B for 14 h. Purification by FC (*n*-hexane/ethyl acetate, 10:1 → 6:1) afforded the desired compound as a brown solid (30 mg, yield 66%). C$_{20}$H$_{18}$FNO$_3$S; MW 371; mp 159−160 °C; MS (ESI) 372 (M + H)$^+$; ^{1}H NMR (CD$_3$COCD$_3$) 3.38 (s, 3H), 3.82 (s, 3H), 3.90 (s, 3H), 6.65 (dd, *J* = 4.0, 1.0 Hz, 1H), 6.95 (ddd, *J* = 7.6, 1.8, 1.0 Hz, 1H), 6.99−7.01 (m, 2H), 7.09−7.20 (m, 3H), 7.23 (dd, *J* = 4.0, 1.0 Hz, 1H), 7.37 (dd, *J* = 9.1, 7.9 Hz, 1H).

5-(2-Fluoro-3-methylphenyl)-*N*-(3-methoxyphenyl)-*N*-methylthiophene-2-carboxamide (36). The title compound was prepared by reaction of 5-bromo-*N*-(3-methoxyphenyl)-*N*-methylthiophene-2-carboxamide **12b** (49 mg, 0.15 mmol) and 2-fluoro-3-methylphenylboronic acid (28 mg, 0.18 mmol) with tetrakis-(triphenylphosphine)palladium (17 mg, 0.015 mmol) according to method B for 14 h. Purification by FC (*n*-hexane/ethyl acetate, 10:1) afforded the desired compound as a colorless solid (50 mg, yield 94%). C$_{20}$H$_{18}$FNO$_2$S; MW 355; mp 142−143 °C; MS (APCI) 356 (M + H)$^+$; ^{1}H NMR (CD$_3$COCD$_3$) 2.29 (d, *J* = 2.5 Hz, 3H), 3.39 (s, 3H), 3.81 (s, 3H), 6.63 (dd, *J* = 4.1, 0.9 Hz, 1H), 6.94 (ddd, *J* = 7.9, 1.9, 0.9 Hz, 1H), 6.98−7.01 (m, 2H), 7.11 (t, *J* = 7.9 Hz, 1H), 7.22 (dd, *J* = 4.1, 0.9 Hz, 1H), 7.23−7.25 (m, 1H), 7.36−7.39 (m, 1H), 7.45−7.48 (m, 1H).

5-(2,6-Difluoro-3-methoxyphenyl)-*N*-(3-methoxyphenyl)-*N*-methylthiophene-2-carboxamide (37). The title compound was prepared by reaction of 5-bromo-*N*-(3-methoxyphenyl)-*N*-methylthiophene-2-carboxamide **12b** (33 mg, 0.1 mmol) and 2,6-difluoro-3-methoxyphenylboronic acid (26 mg, 0.14 mmol) with tetrakis-(triphenylphosphine)palladium (12 mg, 0.01 mmol) according to method B for 14 h. Purification by FC (*n*-hexane/ethyl acetate, 10:1) afforded the desired compound as an orange solid (10 mg, yield 26%). C$_{20}$H$_{17}$F$_2$NO$_3$S; MW 389; mp 147−148 °C; MS (ESI) 390 (M + H)$^+$; ^{1}H NMR (CD$_3$COCD$_3$) 3.40 (s, 3H), 3.82 (s, 3H), 3.90 (s, 3H), 6,71 (dt, *J* = 4.1, 0.9 Hz, 1H), 6.95 (ddd, *J* = 7.6, 1.9, 0.9 Hz, 1H), 6.99−7.01 (m, 2H), 7.05 (ddd, *J* = 11.4, 9.1, 2.2 Hz, 1H), 7.16 (td, *J* = 9.1, 5.0 Hz, 1H), 7.23 (dt, *J* = 4.1, 1.1 Hz, 1H), 7.36−7.39 (m, 1H).

5-(3-Fluoro-4-methoxyphenyl)-*N*-(3-methoxyphenyl)-*N*-methylthiophene-2-carboxamide (38). The title compound was prepared by reaction of 5-bromo-*N*-(3-methoxyphenyl)-*N*-methylthiophene-2-carboxamide **12b** (40 mg, 0.12 mmol) and 3-fluoro-4-methoxyphenylboronic acid (25 mg, 0.14 mmol) with tetrakis-(triphenylphosphine)palladium (14 mg, 0.015 mmol) according to method B for 6 h. Purification by FC (*n*-hexane/ethyl acetate, 10:1 → 6:1) afforded the desired compound as a yellow solid (40 mg, yield 72%). C$_{20}$H$_{18}$FNO$_3$S; MW 371; mp 157−158 °C; MS (ESI) 372 (M + H)$^+$; ^{1}H NMR (CD$_3$COCD$_3$) 3.38 (s, 3H), 3.82 (s, 3H), 3.91 (s, 3H), 6.55 (d, *J* = 4.0 Hz, 1H), 6.94 (ddd, *J* = 7.9, 1.9, 0.9 Hz, 1H), 6.99−7.01 (m, 2H), 7.08 (d, *J* = 4.0 Hz, 1H), 7.15 (t, *J* = 8.5 Hz, 1H), 7.33−7.39 (m, 3H).

5-(2,4-Dimethoxyphenyl)-*N*-(3-methoxyphenyl)-*N*-methylthiophene-2-carboxamide (41). The title compound was prepared by reaction of 5-bromo-*N*-(3-methoxyphenyl)-*N*-methylthiophene-2-carboxamide **12b** (40 mg, 0.12 mmol) and 2,4-dimethoxyphenylboronic acid (27 mg, 0.14 mmol) with tetrakis(triphenylphosphine)palladium (14 mg, 0.012 mmol) according to method B for 14 h.

Purification by FC (*n*-hexane/ethyl acetate, 10:1 → 6:1) afforded the desired compound as a colorless solid (35 mg, yield 74%). C$_{21}$H$_{21}$NO$_4$S; MW 383; mp 117−118 °C; MS (ESI) 384 (M + H)$^+$; ^{1}H NMR (CD$_3$COCD$_3$) 3.37 (s, 3H), 3.82 (s, 3H), 3.83 (s, 3H), 3.89 (s, 3H), 6.57 (ddd, *J* = 8.5, 2.4, 0.9 Hz, 1H), 6.63−6.64 (m, 2H), 6.92 (ddd, *J* = 7.9, 2.1, 0.9 Hz, 1H), 6.97 (t, *J* = 2.1 Hz, 1H), 7.00 (ddd, *J* = 8.2, 2.7, 0.9 Hz, 1H), 7.15 (d, *J* = 4.3 Hz, 1H), 7.37 (t, *J* = 8.1 Hz, 1H), 7.55 (d, *J* = 8.5 Hz, 1H).

5-(3-Methoxyphenyl)-*N*-methyl-*N*-*m*-tolylthiophene-2-carboxamide (22a). The title compound was prepared by reaction of 5-bromo-*N*-methyl-*N*-*m*-tolylthiophene-2-carboxamide **22b** (78 mg, 0.25 mmol) and 3-methoxyphenylboronic acid (45 mg, 0.3 mmol) with tetrakis(triphenylphosphine)palladium (29 mg, 0.025 mmol) according to method B for 4 h. Purification by FC (*n*-hexane/ethyl acetate, 25:1 → 10:1) afforded the desired compound as a colorless solid (65 mg, yield 77%). C$_{20}$H$_{19}$NO$_2$S; MW 337; mp 97−98 °C; MS (ESI) 338 (M + H)$^+$; ^{1}H NMR (CD$_3$COCD$_3$) 2.36 (s, 3H), 3.37 (s, 3H), 3.83 (s, 3H), 6.51 (d, *J* = 4.1 Hz, 1H), 6.90 (ddd, *J* = 8.2, 2.5, 0.9 Hz, 1H), 7.10−7.11 (m, 1H), 7.13−7.17 (m, 3H), 7.23 (s, 1H), 7.26 (d, *J* = 7.6 Hz, 1H), 7.30 (t, *J* = 7.6 Hz, 1H), 7.36 (t, *J* = 7.7 Hz, 1H).

5-(3-Hydroxyphenyl)-*N*-methyl-*N*-*m*-tolylthiophene-2-carboxamide (22). The title compound was prepared by reaction of 5-(3-methoxyphenyl)-*N*-methyl-*N*-*m*-tolylthiophene-2-carboxamide **22a** (40 mg, 0.12 mmol) with boron trifluoride−dimethyl sulfide complex (0.08 mL, 0.72 mmol) according to method C for 14 h. Purification by FC (CH$_2$Cl$_2$/CH$_3$OH, 100:1 → 50:1) afforded the title compound as a beige solid (30 mg, yield 79%). C$_{19}$H$_{17}$NO$_2$S; MW 323; mp 157−158 °C; MS (ESI) 324 (M + H)$^+$; ^{1}H NMR (CD$_3$COCD$_3$) 2.36 (s, 3H), 3.37 (s, 3H), 6.52 (d, *J* = 4.1 Hz, 1H), 6.81 (ddd, *J* = 8.0, 2.5, 0.9 Hz, 1H), 7.03−7.06 (m, 2H), 7.08 (d, *J* = 4.1 Hz, 1H), 7.16 (d, *J* = 8.0 Hz, 1H), 7.20 (d, *J* = 7.6 Hz, 1H), 7.22 (s, 1H), 7.26 (d, *J* = 7.6 Hz, 1H), 7.36 (t, *J* = 8.0 Hz, 1H), 8.51 (s, 1H).

5-(2-Fluoro-3-methoxyphenyl)-*N*-methyl-*N*-(*m*-tolylthiophene)-2-carboxamide (32). The title compound was prepared by reaction of 5-bromo-*N*-methyl-*N*-*m*-tolylthiophene-2-carboxamide **22b** (46 mg, 0.15 mmol) and 2-fluoro-3-methoxyphenylboronic acid (31 mg, 0.18 mmol) with tetrakis(triphenylphosphine)palladium (17 mg, 0.015 mmol) according to method B for 14 h. Purification by FC (*n*-hexane/ethyl acetate, 10:1) afforded the desired compound as a colorless solid (45 mg, yield 85%). C$_{20}$H$_{18}$FNO$_2$S; MW 355; mp 120−121 °C; MS (ESI) 356 (M + H)$^+$; ^{1}H NMR (CD$_3$COCD$_3$) 2.36 (s, 3H), 3.38 (s, 3H), 3.90 (s, 3H), 6.58 (dd, *J* = 4.0, 1.0 Hz, 1H), 7.08−7.19 (m, 4H), 7.21 (dd, *J* = 4.1, 1.0 Hz, 1H), 7.23−7.27 (m, 2H), 7.36 (t, *J* = 7.7 Hz, 1H).

5-(3-Methoxyphenyl)-*N*-methyl-*N*-(3-(trifluoromethyl)-phenyl)thiophene-2-carboxamide (24). The title compound was prepared by reaction of 5-bromo-*N*-methyl-*N*-(3-(trifluoromethyl)-phenyl)thiophene-2-carboxamide **24b** (36 mg, 0.1 mmol) and 3-methoxyphenylboronic acid (20 mg, 0.13 mmol) with tetrakis-(triphenylphosphine)palladium (12 mg, 0.01 mmol) according to method B for 6 h. Purification by FC (*n*-hexane/ethyl acetate, 10:1 → 5:1) afforded the desired compound as a yellow solid (35 mg, yield 90%). C$_{20}$H$_{16}$F$_3$NO$_2$S; MW 391; mp 97−98 °C; MS (ESI) 392 (M + H)$^+$; ^{1}H NMR (CD$_3$COCD$_3$) 3.47 (s, 3H), 3.83 (s, 3H), 6.57 (d, *J* = 4.1 Hz, 1H), 6.91 (ddd, *J* = 8.2, 2.5, 0.9 Hz, 1H), 7.11 (t, *J* = 2.2 Hz, 1H), 7.14 (ddd, *J* = 7.6, 1.6, 0.9 Hz, 1H), 7.19 (d, *J* = 4.1 Hz, 1H), 7.31 (t, *J* = 8.2 Hz, 1H), 7.69−7.78 (m, 3H), 7.81−7.82 (m, 1H); ^{13}C NMR (CD$_3$COCD$_3$) 39.0, 55.6, 112.0, 115.0, 119.0, 124.1, 125.3, 125.4, 125.8, 125.9, 131.1, 131.7, 132.2, 132.5, 133.0, 133.5, 135.5, 138.2, 146.3, 149.2, 161.2, 162.6; IR (cm^{-1}) 3046, 2963, 1608, 1438, 1330, 1117, 707 .

***N*-(Biphenyl-3-yl)-5-(3-methoxyphenyl)-*N*-methylthiophene-2-carboxamide (26).** The title compound was prepared by reaction of *N*-(biphenyl-3-yl)-5-bromo-*N*-methylthiophene-2-carboxamide **26b** (37 mg, 0.1 mmol) and 3-methoxyphenylboronic acid (18 mg, 0.12 mmol) with tetrakis(triphenylphosphine)palladium (12 mg, 0.01 mmol) according to method B for 14 h. Purification by FC (*n*-hexane/ethyl acetate 8:1) afforded the desired compound as a yellow solid (38 mg, yield 95%). C$_{25}$H$_{21}$NO$_2$S; MW 399; mp 108−109 °C; MS (ESI) 400 (M + H)$^+$; ^{1}H NMR (CD$_3$COCD$_3$) 3.46 (s, 3H), 3.81

(s, 3H), 6.65 (d, J = 4.0 Hz, 1H), 6.89 (dd, J = 8.0, 2.1 Hz, 1H), 7.09 (s, 1H), 7.12 (d, J = 7.6 Hz, 1H), 7.15 (d, J = 4.0 Hz, 1H), 7.28 (t, J = 8.0 Hz, 1H), 7.36−7.39 (m, 2H), 7.46 (t, J = 7.8 Hz, 2H), 7.57 (t, J = 7.8 Hz, 1H), 7.68 (d, J = 8.0 Hz, 2H), 7.71−7.74 (m, 2H).

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

2. Biological Methods. [2,4,6,7-^{3}H]E2, [6,7-^{3}H]E2, [2,4,6,7-^{3}H]E1, and [1,2,6,7-^{3}H]A-dione were bought from Perkin-Elmer, Boston, MA. Quickszint Flow 302 scintillator fluid was bought from Zinsser Analytic, Frankfurt, Germany. ReadyFlow III scintillation fluid was from Beckman. Other chemicals were purchased from Sigma, Serva, Roth, or Merck.

Cytosolic (17β-HSD1) and microsomal (17β-HSD2) fractions were obtained from human and *Callithrix jacchus* placenta according to previously described procedures[46,47,60] and from mouse liver tissues.[61] Fresh tissue was homogenized and centrifuged. The pellet fraction contains the microsomal 17β-HSD2 and was used for the determination of E1 formation, while 17β-HSD1 was obtained after precipitation with ammonium sulfate from the cytosolic fraction for use of testing of E2 formation.

Human 17β-HSD4 and 17β-HSD5 were cloned into the modified pGEX-2T vector.[50] For the multidomain enzyme 17β-HSD4, only the steroid converting SDR domain was subcloned.[50] The human 11β-HSD1 and 11β-HSD2 were stably transfected in HEK cells as described earlier by Odermatt.[62]

Inhibition of 17β-HSD2/E1 Formation in Cell-Free Assay. Inhibitory activities were evaluated by an established method with minor modifications.[23,63,64] Briefly, the enzyme preparation was incubated with NAD$^+$ [1500 μM] in the presence of potential inhibitors at 37 °C in a phosphate buffer (50 mM) supplemented with 20% of glycerol and 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 E2 and [^{3}H]E2 (final concentration of 500 nM, 0.11 μCi). After 20 min, the incubation was stopped with HgCl$_2$ and the mixture was extracted with ether. After evaporation, the steroids were dissolved in acetonitrile/water (45:55). E1 and E2 were separated using acetonitrile/water (45:55) as mobile phase in a C18 RP chromatography column (Nucleodur C18, 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 = [(% E1)/(% E1 + % E2)] × 100. Each value was calculated from at least three independent experiments.

Inhibition of 17β-HSD1/E2 Formation in Cell-Free Assay. The 17β-HSD1 inhibition assay was performed similarly to the 17β-HSD2 test. The microsomal fraction was incubated with NADH (500 μM), test compound, and a mixture of unlabeled E1 and [^{3}H]E1 (final concentration of 500 nM, 0.15 μCi) for 10 min at 37 °C. Further treatment of the samples and HPLC separation were carried out as mentioned above for 17β-HSD2.

Inhibition of Human 17β-HSD4 and 17β-HSD5. Inhibitory activity was assessed as described earlier.[50,51] Briefly, for 17β-HSD4 inhibition, an appropriate amount of bacteria containing recombinantly expressed human 17β-HSD4 (SDR domain)[50] was resuspended in 100 mM sodium phosphate buffer, pH 7.7. Substrate [6,7-^{3}H]E2 and inhibitor (dissolved in DMSO) were added in final concentrations of 21 nM and 1 μM (1% (v/v) DMSO), respectively. Controls contained 1% DMSO without inhibitor. The enzymatic reaction was started with the addition of NAD$^+$ (750 μM final).

For 17β-HSD5 inhibition, an appropriate amount of bacterial lysate containing recombinantly expressed human 17β-HSD556 was dissolved in 100 mM sodium phosphate buffer, pH 6.6. Substrate [1,2,6,7-^{3}H]A-dione and inhibitor (dissolved in DMSO) were added in final concentrations of 21 nM and 1 μM (1% (v/v) DMSO), respectively. Controls contained 1% DMSO without inhibitor. The enzymatic reaction was started with the addition of NADPH (600 μM

final). The incubation at 37 °C was stopped with 0.21 M ascorbic acid in methanol/acetic acid (99:1) after the time needed to convert approximately 30% of the substrate in a control assay without inhibitor. Steroids were extracted from the assay mixture by SPE using Strata C18-E columns (Phenomenex), eluted with methanol, and separated by RP-HPLC (column Luna, 5 μm C18(2), 150 mm; Phenomenex) at a flow rate of 1 mL/min acetonitrile/water (43:57). Radioactivity was detected by online scintillation counting with a Berthold LB506D detector (Berthold Technologies, Bad Wildbad, Germany) after mixing with ReadyFlow III (Beckman). Conversion was calculated from integration of substrate and product peaks. For calculation of inhibitory potential, conversion of the control assays (assays without inhibitor) was set to 0% inhibition. Assays were run in triplicate.

Inhibition of 11β-HSD1 and 11β-HSD2 Using Cell Lysates. HEK-293 cells stably transfected with 11β-HSD1 or 11β-HSD2 were cultured in Dulbecco's modified Eagle medium (DMEM) containing 4.5 g/L glucose, supplemented with 10% fetal bovine serum, MEM nonessential amino acids, 100 U/mL penicillin, 0.1 mg/mL streptomycin, and 10 mM HEPES, pH 7.4. Cells were grown to 90% confluence, washed with PBS, suspended, and centrifuged for 4 min at 150g. Cell pellets were frozen and stored at −80 °C.

Inhibitors were dissolved in DMSO to obtain stock solutions of 10 mM and stored as 100 μL aliquots at −20 °C. [1,2-^{3}H]Cortisone was purchased from American Radiolabeled Chemicals (St. Louis, MO, U.S.), and [1,2,6,7-^{3}H]cortisol was from Amersham Pharmacia (Piscataway, NJ, U.S.). All other chemicals were obtained from Sigma-Aldrich Chemie GmbH (Buchs, Switzerland) of the highest grade available.

Activity assays were performed as described by Kratschmar et al.[54] Briefly, cell lysates were incubated for 10 min at 37 °C in TS2 buffer (100 mM NaCl, 1 mM EGTA, 1 mM EDTA, 1 mM MgCl$_2$, 250 mM sucrose, 20 mM Tris-HCl, pH 7.4) in a final volume of 22 μL, containing either vehicle (0.2% DMSO) or the corresponding inhibitor at 2 and 20 μM. To measure 11β-HSD1 activity, the reaction mixture contained 380 nM unlabeled cortisone, 20 nM [1,2-^{3}H]cortisone, and 500 μM NADPH. 11β-HSD2 activity was determined in a reaction mixture containing 80 nM unlabeled cortisol, 20 nM [1,2,6,7-^{3}H]cortisol, and 500 μM NAD$^+$. Reactions were stopped after 10 min by the addition of an excess of unlabeled cortisone and cortisol (2 mM, in methanol). Steroids were separated by TLC, followed by scintillation counting and calculation of substrate conversion. Data were obtained from three independent experiments.

Inhibition of 17β-HSD2 in a Cellular Assay. Cellular 17β-HSD2 activity is measured using the breast cancer cell-line MDA-MB-231[65] (17β-HSD1 activity negligible). [^{3}H]E2 (200 nM) is taken as substrate and is incubated with the inhibitor for 6 h at 37 °C. After ether extraction, substrate and product are separated by HPLC and detected with a radioflow detector. Potency is evaluated as percentage of inhibition (inhibitor concentration of 1 μM) and as IC$_{50}$ values.

ER Affinity in a Cellular Free Assay. The binding affinity of selected compounds to the ERα and ERβ was determined according to Zimmermann et al.[49] using recombinant human proteins. Briefly, 0.25 pmol of ERα or ERβ was incubated with [^{3}H]E2 (10 nM) and test compound for 1 h at room temperature. The potential inhibitor was dissolved in DMSO (5% final concentration). Nonspecific binding was performed with diethylstilbestrol (10 μM). After incubation, ligand−receptor complexes were selectively bound to hydroxyapatite (5 g/60 mL of 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 (%) = IC$_{50}$(E2)/IC$_{50}$(compound) × 100. The RBA value for E2 was arbitrarily set at 100%.

Cytotoxicity. For evaluation of cytotoxicity, conversion of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) is determined according to Denizot and Lang with minor modifica-

tions.[57] Experiments were performed in 96-well cell culture plates in DMEM supplemented with 10% FCS. MDA-MB-231 cells are incubated with the compounds for 3 h at 37 °C in 5% CO_2 humidified atmosphere. After an MTT incubation of another 3 h the cleavage of MTT to a blue formazane by mitochondrial succinate dehydrogenase was stopped and cell lysis was carried out by addition of sodium dodecyl sulfate (SDS) in 0.01 N HCl (10%). The produced blue formazane was quantified spectrophotometrically at 590 nm.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

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

■ AUTHOR INFORMATION

Corresponding Author
*Phone: +49 681 302 70300. Fax: +49 681 302 70308. E-mail: rwh@mx.uni-saarland.de. Web site: http://www.pharmmedchem.de.

Author Contributions
#S.M.-O. and K.X. contributed equally to this work.

Notes
The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

■ ABBREVIATIONS USED

17β-HSD2, 17β-hydroxysteroid dehydrogenase type 2; 17β-HSD1, 17β-hydroxysteroid dehydrogenase type 1; E1, estrone; E2, 17β-estradiol; T, testosterone; DHT, dihydrotestosterone; DHEA, dehydroepiandrosterone; Δ^4-AD, Δ^4-androstene-3,17-dione; FC, flash chromatography; SF, selectivity factor; RBA, relative binding affinity; ER, estrogen receptor; ERT, estrogen replacement therapy; RBA, relative binding affinity; inh, inhibition; MEP, molecular electrostatic potential

■ ADDITIONAL NOTE

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

■ REFERENCES

(1) Wu, L.; Einstein, M.; Geissler, W. M.; Chan, H. K.; Elliston, K. O.; Andersson, S. Expression cloning and characterization of human 17 beta-hydroxysteroid dehydrogenase type 2, a microsomal enzyme possessing 20 alpha-hydroxysteroid dehydrogenase activity. *J. Biol. Chem.* **1993**, *268*, 12964–12969.

(2) Suzuki, T.; Sasano, H.; Andersson, S.; Mason, J. I. 3β-Hydroxysteroid dehydrogenase/Δ5→4-isomerase activity associated with the human 17β-hydroxysteroid dehydrogenase type 2 isoform. *J. Clin. Endocrinol. Metab.* **2000**, *85*, 3669–3672.

(3) Bodine, P. V. N.; Komm, B. S. Tissue culture models for studies of hormone and vitamin action in bone cells. *Vitam. Horm.* **2002**, *64*, 101–151.

(4) Vanderschueren, D.; Gaytant, J.; Boonen, S.; Venken, K. Androgens and bone. *Curr. Opin. Endocrinol., Diabetes Obes.* **2008**, *15*, 250–254.

(5) Vanderschueren, D.; Vandenput, L.; Boonen, S.; Lindberg, M. K.; Bouillon, R.; Ohlsson, C. Androgens and bone. *Endocr. Rev.* **2004**, *25*, 389–425.

(6) Cree, M.; Soskolne, C. L.; Belseck, E.; Hornig, J.; McElhaney, J. E.; Brant, R.; Suarez-Almazor, M. Mortality and institutionalization following hip fracture. *J. Am. Geriatr. Soc.* **2000**, *48*, 283–288.

(7) McCloskey, E. Effects of third-generation aromatase inhibitors on bone. *Eur. J. Cancer* **2006**, *42*, 1044–1051.

(8) Marcus, R.; Wong, M.; Heath, H., 3rd; Stock, J. L. Antiresorptive treatment of postmenopausal osteoporosis: comparison of study designs and outcomes in large clinical trials with fracture as an endpoint. *Endocr. Rev.* **2002**, *23*, 16–37.

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

(10) Ringe, J. D.; Faber, H.; Farahmand, P.; Dorst, A. Efficacy of risedronate in men with primary and secondary osteoporosis: results of a 1-year study. *Rheumatol. Int.* **2006**, *26*, 427–31.

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

(12) Felson, D. T.; Zhang, Y.; Hannan, M. T.; Kiel, D. P.; Wilson, P. W.; Anderson, J. J. The effect of postmenopausal estrogen therapy on bone density in elderly women. *N. Engl. J. Med.* **1993**, *329*, 1141–1146.

(13) Chen, C.-L.; Weiss, N. S.; Newcomb, P.; Barlow, W.; White, E. Hormone replacement therapy in relation to breast cancer. *JAMA, J. Am. Med. Assoc.* **2002**, *287*, 734–741.

(14) Beresford, S. A.; Weiss, N. S.; Voigt, L. F.; McKnight, B. Risk of endometrial cancer in relation to use of oestrogen combined with cyclic progestagen therapy in postmenopausal women. *Lancet* **1997**, *349*, 458–61.

(15) Grady, D.; Gebretsadik, T.; Kerlikowske, K.; Ernster, V.; Petitti, D. Hormone replacement therapy and endometrial cancer risk: a meta-analysis. *Obstet. Gynecol.* **1995**, *85*, 304–313.

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

(17) Feix, M.; Wolf, L.; Schweikert, H. U. Distribution of 17beta-hydroxysteroid dehydrogenases in human osteoblast-like cells. *Mol. Cell. Endocrinol.* **2001**, *171*, 163–164.

(18) Eyre, L. J.; Bland, R.; Bujalska, I. J.; Sheppard, M. C.; Stewart, P. M.; Hewison, M. Characterization of aromatase and 17 beta-hydroxysteroid dehydrogenase expression in rat osteoblastic cells. *J. Bone Miner. Res.* **1998**, *13*, 996–1004.

(19) van Grunsven, E G; van Berkel, E.; Ijlst, L.; Vreken, P.; de Klerk, J. B.; Adamski, J.; Lemonde, H.; Clayton, P. T.; Cuebas, D. A.; Wanders, R. J. Peroxisomal D-hydroxyacyl-CoA dehydrogenase deficiency: resolution of the enzyme defect and its molecular basis in bifunctional protein deficiency. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 2128–2133.

(20) Blomquist, C. H.; Lindemann, N. J.; Hakanson, E. Y. 17 beta-Hydroxysteroid and 20 alpha-hydroxysteroid dehydrogenase activities of human placental microsomes: kinetic evidence for two enzymes differing in substrate specificity. *Arch. Biochem. Biophys.* **1985**, *239*, 206–215.

(21) Bydal, P.; Auger, S.; Poirier, D. Inhibition of type 2 17β-hydroxysteroid dehydrogenase by estradiol derivatives bearing a lactone on the D-ring: structure–activity relationships. *Steroids* **2004**, *69*, 325–342.

(22) Poirier, D.; Bydal, P.; Tremblay, M. R.; Sam, K. M.; Luu-The, V. Inhibitors of type II 17beta-hydroxysteroid dehydrogenase. *Mol. Cell. Endocrinol.* **2001**, *171*, 119−128.

(23) Sam, K. M.; Auger, S.; Luu-The, V.; Poirier, D. Steroidal spiro-gamma-lactones that inhibit 17 beta-hydroxysteroid dehydrogenase activity in human placental microsomes. *J. Med. Chem.* **1995**, *38*, 4518−4528.

(24) Tremblay, M. R.; Luu-The, V.; Leblanc, G.; Noël, P.; Breton, E.; Labrie, F.; Poirier, D. Spironolactone-related inhibitors of type II 17beta-hydroxysteroid dehydrogenase: chemical synthesis, receptor binding affinities, and proliferative/antiproliferative activities. *Bioorg. Med. Chem.* **1999**, *7*, 1013−1023.

(25) Cook, J. H.; Barzya, J.; Brennan, C.; Lowe, D.; Wang, Y.; Redman, A.; Scott, W. J.; Wood, J. E. 4,5-Disubstituted cis-pyrrolidinones as inhibitors of 17β-hydroxysteroid dehydrogenase II. Part 1: Synthetic approach. *Tetrahedron Lett.* **2005**, *46*, 1525−1528.

(26) Gunn, D.; Akuche, C.; Baryza, J.; Blue, M.-L.; Brennan, C.; Campbell, A.-M.; Choi, S.; Cook, J.; Conrad, P.; Dixon, B.; Dumas, J.; Ehrlich, P.; Gane, T.; Joe, T.; Johnson, J.; Jordan, J.; Kramss, R.; Liu, P.; Levy, J.; Lowe, D.; McAlexander, I.; Natero, R.; Redman, A. M.; Scott, W.; Seng, T.; Sibley, R.; Wang, M.; Wang, Y.; Wood, J.; Zhang, Z. 4,5-Disubstituted cis-pyrrolidinones as inhibitors of type II 17beta-hydroxysteroid dehydrogenase. Part 2. SAR. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3053−3057.

(27) Wood, J.; Bagi, C. M.; Akuche, C.; Bacchiocchi, A.; Baryza, J.; Blue, M.-L.; Brennan, C.; Campbell, A.-M.; Choi, S.; Cook, J. H.; Conrad, P.; Dixon, B. R.; Ehrlich, P. P.; Gane, T.; Gunn, D.; Joe, T.; Johnson, J. S.; Jordan, J.; Kramss, R.; Liu, P.; Levy, J.; Lowe, D. B.; McAlexander, I.; Natero, R.; Redman, A. M.; Scott, W. J.; Town, C.; Wang, M.; Wang, Y.; Zhang, Z. 4,5-Disubstituted cis-pyrrolidinones as inhibitors of type II 17beta-hydroxysteroid dehydrogenase. Part 3. Identification of lead candidate. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4965−4968.

(28) Wetzel, M.; Marchais-Oberwinkler, S.; Hartmann, R. W. 17β-HSD2 inhibitors for the treatment of osteoporosis: identification of a promising scaffold. *Bioorg. Med. Chem.* **2011**, *19*, 807−815.

(29) Wetzel, M.; Marchais-Oberwinkler, S.; Perspicace, E.; Möller, G.; Adamski, J.; Hartmann, R. W. Introduction of an electron withdrawing group on the hydroxyphenylnaphthol scaffold improves the potency of 17β-hydroxysteroid dehydrogenase type 2 (17β-HSD2) inhibitors. *J. Med. Chem.* **2011**, *54*, 7547−7557.

(30) Wetzel, M.; Gargano, E. M.; Hinsberger, S.; Marchais-Oberwinkler, S.; Hartmann, R. W. Discovery of a new class of bicyclic substituted hydroxyphenylmethanones as 17β-hydroxysteroid dehydrogenase type 2 (17β-HSD2) inhibitors for the treatment of osteoporosis. *Eur. J. Med. Chem.* **2012**, *47*, 1−17.

(31) Al-Soud, Y. A.; Marchais-Oberwinkler, S.; Frotscher, M.; Hartmann, R. W. Synthesis and biological evaluation of phenyl substituted 1*H*-1,2,4-triazoles as non-steroidal inhibitors of 17β-hydroxysteroid dehydrogenase type 2. *Arch. Pharm.* **2012**, *345*, 610−621.

(32) Xu, K.; Al-Soud, Y. A.; Wetzel, M.; Hartmann, R. W.; Marchais-Oberwinkler, S. Triazole ring-opening leads to the discovery of potent nonsteroidal 17β-hydroxysteroid dehydrogenase type 2 inhibitors. *Eur. J. Med. Chem.* **2011**, *46*, 5978−5990.

(33) Bagi, C. M.; Wood, J.; Wilkie, D.; Dixon, B. Effect of 17beta-hydroxysteroid dehydrogenase type 2 inhibitor on bone strength in ovariectomized cynomolgus monkeys. *J. Musculoskeletal Neuronal Interact.* **2008**, *8*, 267−280.

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

(35) Marchais-Oberwinkler, S.; Kruchten, P.; Frotscher, M.; Ziegler, E.; Neugebauer, A.; Bhoga, U.; Bey, E.; Müller-Vieira, U.; Messinger, J.; Thole, H.; Hartmann, R. W. Substituted 6-phenyl-2-naphthols. Potent and selective nonsteroidal inhibitors of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1): design, synthesis, biological evaluation, and pharmacokinetics. *J. Med. Chem.* **2008**, *51*, 4685−4698.

(36) Marchais-Oberwinkler, S.; Wetzel, M.; Ziegler, E.; Kruchten, P.; Werth, R.; Henn, C.; Hartmann, R. W.; Frotscher, M. New drug-like hydroxyphenylnaphthol steroidomimetics as potent and selective 17β-hydroxysteroid dehydrogenase type 1 inhibitors for the treatment of estrogen-dependent diseases. *J. Med. Chem.* **2011**, *54*, 534−547.

(37) 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.

(38) Bey, E.; Marchais-Oberwinkler, S.; Werth, R.; Negri, M.; Al-Soud, Y. A.; Kruchten, P.; Oster, A.; Frotscher, M.; Birk, B.; Hartmann, R. W. Design, synthesis, biological evaluation and pharmacokinetics of bis(hydroxyphenyl) substituted azoles, thiophenes, benzenes, and aza-benzenes as potent and selective nonsteroidal inhibitors of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1). *J. Med. Chem.* **2008**, *51*, 6725−6739.

(39) Bey, E.; Marchais-Oberwinkler, S.; Negri, M.; Kruchten, P.; Oster, A.; Klein, T.; Spadaro, A.; Werth, R.; Frotscher, M.; Birk, B.; Hartmann, R. W. New insights into the SAR and binding modes of bis(hydroxyphenyl)thiophenes and -benzenes: influence of additional substituents on 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1) inhibitory activity and selectivity. *J. Med. Chem.* **2009**, *52*, 6724−6743.

(40) Oster, A.; Klein, T.; Werth, R.; Kruchten, P.; Bey, E.; Negri, M.; Marchais-Oberwinkler, S.; Frotscher, M.; Hartmann, R. W. Novel estrone mimetics with high 17beta-HSD1 inhibitory activity. *Bioorg. Med. Chem.* **2010**, *18*, 3494−3505.

(41) Oster, A.; Hinsberger, S.; Werth, R.; Marchais-Oberwinkler, S.; Frotscher, M.; Hartmann, R. W. Bicyclic substituted hydroxyphenyl-methanones as novel inhibitors of 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1) for the treatment of estrogen-dependent diseases. *J. Med. Chem.* **2010**, *53*, 8176−8186.

(42) Oster, A.; Klein, T.; Henn, C.; Werth, R.; Marchais-Oberwinkler, S.; Frotscher, M.; Hartmann, R. W. Bicyclic substituted hydroxyphenylmethanone type inhibitors of 17β-hydroxysteroid dehydrogenase type 1 (17 β-HSD1): the role of the bicyclic moiety. *ChemMedChem* **2011**, *6*, 476−487.

(43) Spadaro, A.; Negri, M.; Marchais-Oberwinkler, S.; Bey, E.; Frotscher, M. Hydroxybenzothiazoles as new nonsteroidal inhibitors of 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1). *PLoS One* **2012**, *7*, e29252.

(44) Spadaro, A.; Frotscher, M.; Hartmann, R. W. Optimization of hydroxybenzothiazoles as novel potent and selective inhibitors of 17β-HSD1. *J. Med. Chem.* **2012**, *55*, 2469−2473.

(45) Henn, C.; Einspanier, A.; Marchais-Oberwinkler, S.; Frotscher, M.; Hartmann, R. W. Lead optimization of 17β-HSD1 inhibitors of the (hydroxyphenyl)naphthol sulfonamide type for the treatment of endometriosis. *J. Med. Chem.* **2012**, *55*, 3307−3318.

(46) 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.

(47) 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.

(48) Negri, M.; Recanatini, M.; Hartmann, R. W. Computational investigation of the binding mode of bis(hydroxylphenyl)arenes in 17β-HSD1: molecular dynamics simulations, MM-PBSA free energy calculations, and molecular electrostatic potential maps. *J. Comput.-Aided Mol. Des.* **2011**, *25*, 795−811.

(49) 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.

(50) Schuster, D.; Kowalik, D.; Kirchmair, J.; Laggner, C.; Markt, P.; Aebischer-Gumy, C.; Ströhle, F.; Möller, G.; Wolber, G.; Wilckens, T.; Langer, T.; Odermatt, A.; Adamski, J. Identification of chemically diverse, novel inhibitors of 17β-hydroxysteroid dehydrogenase type 3 and 5 by pharmacophore-based virtual screening. *J. Steroid Biochem. Mol. Biol.* **2011**, *125*, 148−161.

(51) Möller, G.; Deluca, D.; Gege, C.; Rosinus, A.; Kowalik, D.; Peters, O.; Droescher, P.; Elger, W.; Adamski, J.; Hillisch, A. Structure-based design, synthesis and in vitro characterization of potent 17beta-hydroxysteroid dehydrogenase type 1 inhibitors based on 2-substitutions of estrone and D-homo-estrone. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6740−6744.

(52) Thomas, M. P.; Potter, B. V. L Crystal structures of 11β-hydroxysteroid dehydrogenase type 1 and their use in drug discovery. *Future Med. Chem.* **2011**, *3*, 367−390.

(53) Baker, M. E. Unusual evolution of 11beta- and 17beta-hydroxysteroid and retinol dehydrogenases. *BioEssays* **1996**, *18*, 63−70.

(54) Kratschmar, D. V.; Vuorinen, A.; Da Cunha, T.; Wolber, G.; Classen-Houben, D.; Doblhoff, O.; Schuster, D.; Odermatt, A. Characterization of activity and binding mode of glycyrrhetinic acid derivatives inhibiting 11β-hydroxysteroid dehydrogenase type 2. *J. Steroid Biochem. Mol. Biol.* **2011**, *125*, 129−142.

(55) Ahasan, M. M.; Hardy, R.; Jones, C.; Kaur, K.; Nanus, D.; Juarez, M.; Morgan, S. A.; Hassan-Smith, Z.; Bénézech, C.; Caamaño, J. H.; Hewison, M.; Lavery, G.; Rabbitt, E. H.; Clark, A. R.; Filer, A.; Buckley, C. D.; Raza, K.; Stewart, P. M.; Cooper, M. S. Inflammatory regulation of glucocorticoid metabolism in mesenchymal stromal cells. *Arthritis Rheum.* **2012**, *64*, 2404−2413.

(56) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Delivery Rev.* **2001**, *46*, 3−26.

(57) 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.

(58) Pogoda, P.; Priemel, M.; Schilling, A.; Gebauer, M.; Catalá-Lehnenf, P.; Barvencik, F.; Beil, F.; Münch, C.; Rupprecht, M.; Müldner, C.; Rueger, J.; Schinke, T.; Amling, M.; Pogoda, P.; Priemel, M.; Schilling, A.; Gebauer, M.; Catalá-Lehnen, P.; Barvencik, F.; Beil, F.; Munch, C.; Rupprecht, M.; Miildner, C.; Rueger, J.; Schinke, T.; Amling, M. Mouse models in skeletal physiology and osteoporosis: experiences and data on 14839 cases from the Hamburg Mouse Archives. *J. Bone Miner. Metab.* **2005**, *23*, 97−102.

(59) Joerg, H.; Holstein, P. G. Mouse Models for the Study of Fracture Healing and Bone Regeneration. In *Osteoporosis Research: Animal Models*; Duque, G., Watanabe, K., Eds.; Springer: London, 2011; pp 175−191.

(60) Zhu, D. W.; Lee, X.; Breton, R.; Ghosh, D.; Pangborn, W.; Daux, W. L.; Lin, S. X. Crystallization and preliminary X-ray diffraction analysis of the complex of human placental 17 beta-hydroxysteroid dehydrogenase with NADP. *J. Mol. Biol.* **1993**, *234*, 242−244.

(61) Jazbutyte, V.; Hu, K.; Kruchten, P.; Bey, E.; Maier, S. K. G.; Fritzemeier, K.-H.; Prelle, K.; Hegele-Hartung, C.; Hartmann, R. W.; Neyses, L.; Ertl, G.; Pelzer, T. Aging reduces the efficacy of estrogen substitution to attenuate cardiac hypertrophy in female spontaneously hypertensive rats. *Hypertension* **2006**, *48*, 579−586.

(62) Odermatt, A.; Arnold, P.; Stauffer, A.; Frey, B. M.; Frey, F. J. The N-terminal anchor sequences of 11beta-hydroxysteroid dehydrogenases determine their orientation in the endoplasmic reticulum membrane. *J. Biol. Chem.* **1999**, *274*, 28762−28770.

(63) Lin, S. X.; Yang, F.; Jin, J. Z.; Breton, R.; Zhu, D. W.; Luu-The, V.; Labrie, F. Subunit identity of the dimeric 17 beta-hydroxysteroid dehydrogenase from human placenta. *J. Biol. Chem.* **1992**, *267*, 16182−16187.

(64) Sam, K. M.; Boivin, R. P.; Tremblay, M. R.; Auger, S.; Poirier, D. C16 and C17 derivatives of estradiol as inhibitors of 17 beta-hydroxysteroid dehydrogenase type 1: chemical synthesis and structure−activity relationships. *Drug Des. Discovery* **1998**, *15*, 157−180.

(65) Day, J. M.; Tutill, H. J.; Newman, S. P.; Purohit, A.; Lawrence, H. R.; Vicker, N.; Potter, B. V. L; Reed, M. J. 17Beta-hydroxysteroid dehydrogenase type 1 and type 2: association between mRNA expression and activity in cell lines. *Mol. Cell. Endocrinol.* **2006**, *248*, 246−249.