European Journal of Medicinal Chemistry 46 (2011) 5978–5990

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Triazole ring-opening leads to the discovery of potent nonsteroidal 17β-hydroxysteroid dehydrogenase type 2 inhibitors

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ARTICLE INFO

Article history:
Received 11 July 2011
Received in revised form
30 September 2011
Accepted 4 October 2011
Available online 10 October 2011

Keywords:
17β-HSD2
Osteoporosis
Achiral inhibitors
Steroidomimetics
Selectivity

ABSTRACT

17β-Hydroxysteroid dehydrogenase type 2 (17β-HSD2) catalyzes the oxidation of the highly potent steroids: the estrogen estradiol (E2) and the androgen testosterone (T) to the less active estrone and androstenedione, respectively. Inhibition of this enzyme may help maintain the local E2 level in bone tissue when the circulating E2 level drops and is therefore a novel and promising approach for the treatment of osteoporosis.

In this work, a series of new nonsteroidal and achiral 17β-HSD2 inhibitors, namely *N*-benzyl-diphenyl-3(or 4)-carboxamide and *N*-benzyl-5-phenyl-thiophene-2-carboxamide was designed and the compounds were synthesized in a two to three steps reaction. A small library was built applying parallel synthesis. Highly potent 17β-HSD2 inhibitors could be identified in the thiophene-2-carboxamide class with IC_{50} in the low nanomolar range. These compounds also showed a good selectivity profile toward 17β-HSD1 and toward the estrogen receptors α and β. The most interesting 17β-HSD2 inhibitor identified in this study is the 5-(2-fluoro-3-methoxyphenyl)-*N*-(3-hydroxybenzyl)-*N*-methylthiophene-2-carboxamide **6w** displaying an IC_{50} of 61 nM and a selectivity factor of 73 toward 17β-HSD1.

1. Introduction

Estrogen deficiency is believed to be responsible for the rapid progression of osteoporosis [1]: *e.g.* high occurrence of this disease has been observed in women after menopause when the estrogen levels drop or after treatment with aromatase inhibitors, which radically prevent estrogen biosynthesis [2]. In addition the osteoprotective action of estrogens is known [3]: they control bone remodeling during reproductive life in both women and men [4,5] and they inhibit osteoclastogenesis leading to a decrease in bone resorption. In addition, an estrogen replacement therapy is efficient in the treatment of bone loss and osteoporotic fractures [6–8]. Furthermore, there is substantial evidence that active androgens like testosterone (T, Fig. 1) and dihydrotestosterone (DHT) are involved in bone formation and might protect bones from osteoporosis [9,10]. Therefore, a controlled increase in active estrogen estradiol (E2) and androgen testosterone (T) in bones of osteoporotic patients will certainly slow down bone loss by lowering bone resorption and raising bone formation and should thus reduce osteoporotic fractures. However, the increase in E2 and T must be restricted to the bones to avoid unwanted side-effect such as induction of breast cancer [11].

17β-Hydroxysteroid dehydrogenase type 2 (17β-HSD2) [12] is a trans-membrane protein localized in the endoplasmic reticulum [13]. It is present in bones [14,15] and catalyzes the transformation of both estrogens and androgens. It shows oxidative as well as reductive activity *in vitro*, depending on the redox state of the cofactor present (NAD^+ or NADH, respectively). However, *in vivo* it has a predominant oxidative activity converting the highly active

Abbreviations: 17β-HSD2, 17β-hydroxysteroid dehydrogenase type 2; 17β-HSD1, 17β-hydroxysteroid dehydrogenase type 1; E1, estrone; E2, 17β-estradiol; T, testosterone; A-dione, 4-androstene-3,17-dione; DHT, dihydrotestosterone; NADP(H), nicotinamide adenine dinucleotide phosphate; NAD(H), nicotinamide adenine dinucleotide; FC, flash chromatography; SF, selectivity factor; RBA, relative binding affinity; ER, estrogen receptor.

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doi:10.1016/j.ejmech.2011.10.010

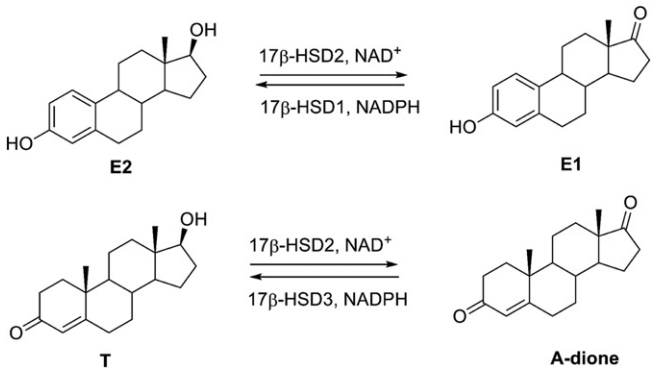


Fig. 1. 17β-HSD1, 2, 3 in sex steroid metabolism.

17β-hydroxysteroids such as E2 and T to the less active forms estrone (E1) and androstenedione (A-dione), using the cofactor NAD^+ (Fig. 1). Thus, 17β-HSD2 is responsible for the inactivation of E2 and T, while the biological counterparts 17β-HSD1 and 17β-HSD3 activate E1 and A-dione respectively (Fig. 1). As intracellular increase of E2 and T in bones can be achieved by inhibition of 17β-HSD2, inhibition of this enzyme provides a novel and promising approach for the treatment of osteoporosis.

There are very few potent and specific 17β-HSD2 inhibitors described [16,17]. They can be divided into three classes (Fig. 2): 1. steroidal spiro-δ-lactone **1** [18–21] (inhibition of 62% at 1 μM [19]). 2. nonsteroidal chiral 4,5-disubstituted *cis*-pyrrolidinone **2** (IC_{50} = 50 nM [22–24]). 3. nonsteroidal hydroxyphenyl naphthol **3** which like **1** shows only moderate inhibitory activity (IC_{50} = 302 nM [25]). A serious drawback of the steroidal inhibitors is the risk to interact with steroid hormone receptors which could result in unwanted severe side effects. The nonsteroidal chiral **2** was evaluated *in vivo* in an osteoporosis model using ovariectomized cynomolgus monkeys [26]. A decrease of bone resorption and maintenance of bone formation was observed. Despite high experimental variations and the little efficacy, this *in vivo* experiment validates the approach of 17β-HSD2 inhibition. However, new nonsteroidal 17β-HSD2 inhibitors with a good pharmacokinetic profile are required for further drug development.

In the present study, we aim at the design, synthesis and *in vitro* evaluation of a novel class of 17β-HSD2 inhibitors: *N*-benzyl-biphenyl-3(or 4)-carboxamides (**4**, **5**, Fig. 3) and *N*-benzyl-5-phenyl-thiophene-2-carboxamides (**6**, Fig. 3) that, in contrast to the existing 17β-HSD2 inhibitors, are easily accessible because they do not contain asymmetric carbons.

2. Inhibitor design

As the 3D-structure of 17β-HSD2 is unknown and there is no homology model available, drug design was based on a ligand-based approach. This strategy was already successfully applied in our group for CYP19 [27–29], CYP11B2 [30–33], CYP11B1 [34,35], CYP17 [36–39] and 5α-reductase inhibitors [40–43].

During the development of 17β-HSD1 inhibitors performed in our group [44–57], new nonsteroidal 17β-HSD2 inhibitors were identified after selectivity screening, including compounds **7** and **8** (Fig. 3) [58]. The latter are weak inhibitors of 17β-HSD2; **7** shows 42% inhibition when tested at a concentration of 1 μM and **8** 30% inhibition. Compounds **7** and **8** share some similarities with the highly potent **2**: they have a biaromatic moiety in common (rings A + B: pyridinethiophene in **2**, biphenyl in **7** and phenylthiophene in **8** (Fig. 3), a phenyl ring (ring C, Fig. 3) as well as a polar group linking rings B and C. Compounds **7**, **8** and **2** differ in the nature of the linker between the B- and C-rings: a flexible hydroxymethylenepyrrolidinone in **2** has been exchanged by a rigid methylated triazole in **7** and **8**, resulting in an overall linear scaffold whereas in case of **2** there is a bent shape. The ring C in **2** and in **7**, **8** covers a different area in the enzyme active site, its position might be critical for inhibitory activity. We hypothesized that the rigidity induced by the triazole moiety in **7** and **8** might be responsible for their weak activity. As an amide (present in the pyrrolidinone of **2**) is a bioisostere of triazole and offers higher flexibility, it was decided to exchange the triazole by an amide (triazole ring-opening). In addition, as the *N*-methyl function present in **2**, **7** and **8** might be important for the activity, *N*-methylated amides **4**, **5** and **6** were designed as potential inhibitors of 17β-HSD2. Varying the nature of the substituents R_1 and R_2 present on the rings A and C, a small library of different *N*-benzyl-*N*-methylbiphenyl-4(or 3)-carboxamides (**4**, **5**) and *N*-benzyl-*N*-methyl-5-phenylthiophene-2-carboxamides (**6**) was synthesized.

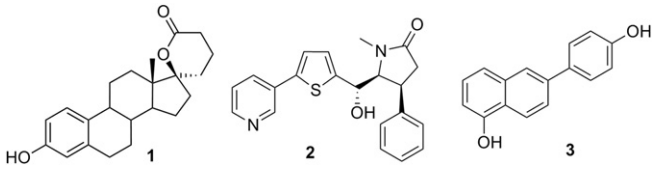


Fig. 2. Structures of known 17β-HSD2 inhibitors.

3. Results

3.1. Chemistry

The synthesis pathway is depicted in Scheme 1. Amidation was first carried out by reaction of the carbonyl chlorides **9**–**11** with the corresponding benzylamines **12** under standard conditions providing the bromophenyl **13**, **14** and bromothiophene **15** derivatives in very good yields (93–99%). In case of the thiophene derivatives **15a**, ether cleavage with borontrifluoride dimethyl sulfide complex afforded the hydroxylated key intermediate **15c**. Subsequently Suzuki cross-coupling was conducted in parallel on the hydroxyphenyl **15c** or methoxyphenyl derivatives **13**, **14**, **15a** or the unsubstituted compound **15b** with the appropriate substituted phenyl boronic acid and afforded the final products **4**–**6** (yield 71–98%). Final hydroxy compounds were obtained after ether cleavage with borontrifluoride dimethyl sulfide complex from the methoxy analogues (yield 82%–98%).

3.2. In vitro biological evaluation

3.2.1. Activity: inhibition of human 17β-HSD2

The synthesized compounds were tested for inhibitory activity on human 17β-HSD2 in a cell-free assay using human placental microsomal enzyme as described earlier on with minor modifications [59]. Briefly, for determination of 17β-HSD2 inhibition the enzyme was incubated with [^{3}H]-E2 (500 nM, 0.135 μCi) and NAD^+ [1500 μM] in presence of potential inhibitors. The amount of labelled E1 formed from tritiated substrate was determined by HPLC. The spiro-δ-lactone **1** described by Poirier et al. [19] was used as reference compound and gave similar values in our test as described in the literature [19] (68% inhibition at 1 μM). IC_{50} values were determined for compounds showing more than 50% inhibition at 1 μM and are shown in Tables 1 and 2. For the less potent compounds, percent inhibition values at 1 μM are given. Compounds showing less than 10% inhibition at 1 μM were considered to be inactive.

Three compound classes have been investigated differing in the nature of the central B-ring: 1,4-disubstituted benzenes (class **4**);

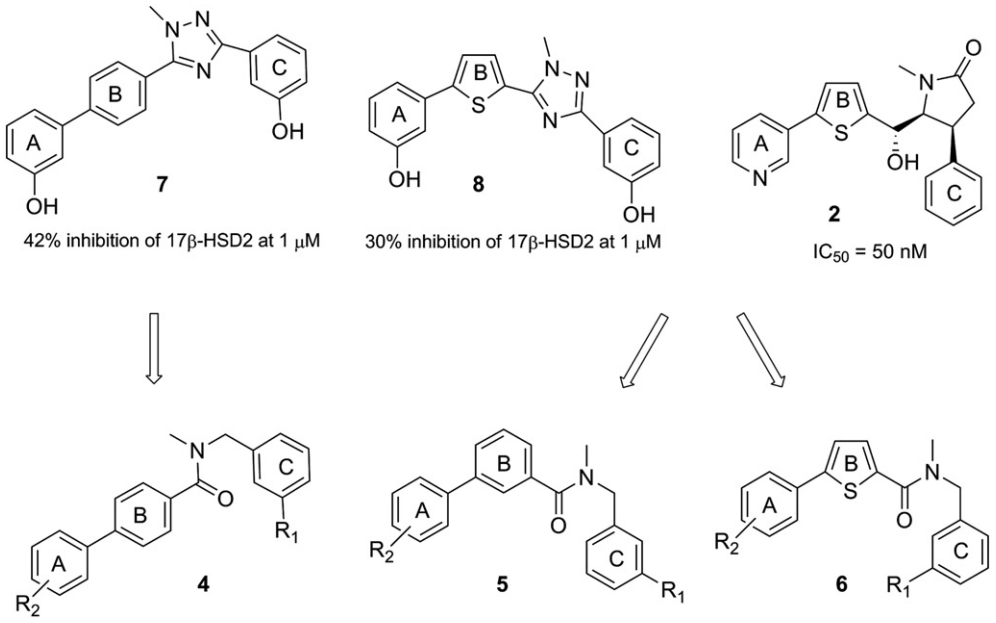
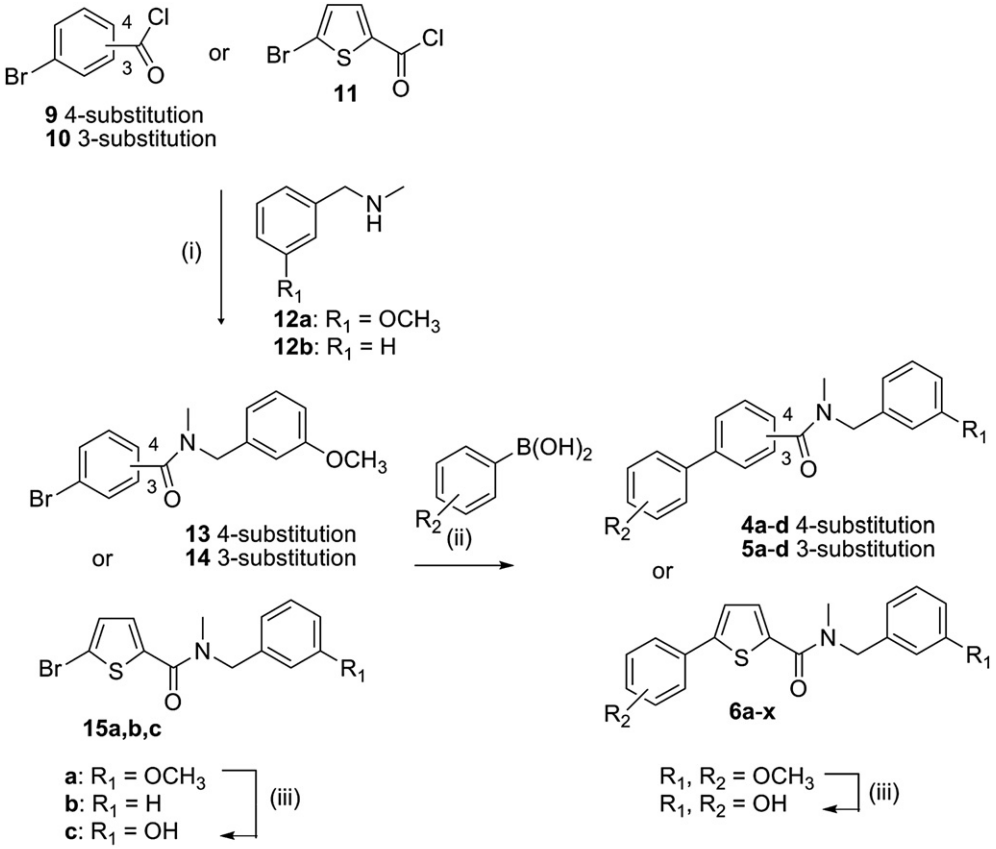


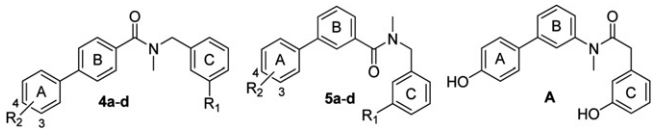
Fig. 3. Designed structures.



Reagents and conditions: (i) NEt_3, CH_2Cl_2, 0 °C, 3 h; Method A
(ii) DME/water, 2N Na_2CO_3, $Pd(PPh_3)_4$, 80 °C, 4-14 h; Method B
(iii) $BF_3{\cdot}S(Me)_2$, CH_2Cl_2, rt, 4-14 h, Method C.

Scheme 1. Synthesis of compounds **4**–**6.**

Table 1
In vitro inhibitory potencies toward 17β-HSD2 and 17β-HSD1 of biphenyl derivatives **1**, **4**, **5** and **A**.



Cmpd	R_2	R_1	IC_{50} (nM)[a]		Selectivity factors[d]
			17β-HSD2[b]	17β-HSD1[c]	
1	–	–	65%[e]	n.d.	
4a	3-OMe	OMe	494	>100,000	>202
4b	3-OH	OH	594	13,009	22
4c	3-Me	OMe	265	>100,000	>375
4d	3-Me	OH	262	64,532	246
5a	4-OMe	OMe	11%[e]	n.i.	n.d.
5b	4-OH	OH	639	9099	14
5c	3-OMe	OMe	13%[e]	n.i.	n. d.
5d	3-OH	OH	482	3801	8
A	–	–	39%[e]	n.i.	n.d.

n.i.: no inhibition; n.d.: not determined.
[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-HSD_1)/IC_{50} (17β-HSD_2).
[e] % inhibition at 1 μM.

1,3-disubstituted benzenes (class **5**, Table 1) and 2,5-thiophenes (class **6,** Table 2).

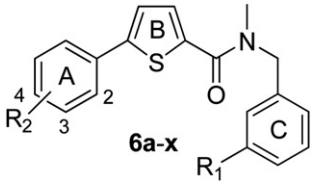
In the first class, the 1,4-disubstituted benzenes (**4a**–**d**, Table 1), the dimethoxy (**4a**) and dihydroxy (**4b**) derivatives showed similar moderate activities with IC_{50} between 500 and 600 nM. The methylated derivatives **4c** (monomethoxy) and **4d** (monohydroxy) are slightly more active with the same IC_{50} of 260 nM. These results point out the fact that only one polar moiety is important, the one present in the C-ring and it should be an H-bond acceptor.

In the class of 1,3-disubstituted benzenes (**5a**–**d**, Table 1), it becomes apparent that the dihydroxy derivatives (**5b**, **5d**) show a better inhibition than the methoxy analogs (**5a**, **5c**), although their potency is still moderate (639 and 482 nM for **5b** and **5d**, respectively). Shifting the hydroxy on the A-ring from the 4- (**5b**) to the 3-position (**5d**) improves the activity slightly. Furthermore, as the exchange of the amide function of **5b** for the retroamide **A** [25] is detrimental for the inhibitory activity, it can be concluded that the polar atoms (O and N) play a role in the binding of the molecule by tight H-bond interactions.

Concerning the 2,5-thiophene compound class (**6**), both dimethoxy and dihydroxy derivatives (**6a**–**d**, Table 2) are moderately potent with IC_{50} values in the same range (between 335 and 496 nM). Exceptions are compounds **6e** and **6f**, substituted with a methoxy (**6e**) and a hydroxy (**6f**) in position 2 on the A-ring: the methoxy derivative **6e** exhibits low 17β-HSD2 inhibition (48% at 1 μM) while the corresponding hydroxy **6f** is highly potent (IC_{50}: 148 nM). Very similar activity profile and potencies can be observed for compounds **6g** and **6h** unsubstituted on the A-ring. This indicates that the presence of the 2-OH group on the A-ring in **6f** does not achieve specific interaction with the protein.

The high potency of the C ring monosubstituted compound **6h** indicates that an OH group should be present in the 3 position of the C-ring and not a methoxy.

Table 2
In vitro inhibitory potencies toward 17β-HSD2 and 17β-HSD1 of phenylthiophene derivatives **6**.



Cmpd	R_2	R_1	IC_{50} (nM)[a]		Selectivity factors[d]
			17β-HSD2[b]	17β-HSD1[c]	
1	–	–	65%[e]	n.d.	
6a	3-OMe	OMe	371	>100,000	>250
6b	3-OH	OH	394	5449	14
6c	4-OMe	OMe	496	>120,000	>240
6d	4-OH	OH	335	5105	15
6e	2-OMe	OMe	49%[e]	n.i.	n.d.
6f	2-OH	OH	149	2982	20
6g	H	OMe	50%[e]	n.i.	n.d.
6h	H	OH	186	3915	21
6i	3-OMe	H	222	>100,000	>450
6j	3-OH	H	42%[e]	10%[e]	n.d.
6k	4-OMe	H	371	>100,000	>265
6l	4-OH	H	36%[e]	n.i.	n.d.
6m	2-OMe	H	1127	55,741	49
6n	2-OH	H	520	>100,000	>190
6o	4-CN	OMe	n.i.	n.i.	n.d.
6p	4-CN	OH	790	43,398	55
6q	4-OMe	OH	161	>40,000	>250
6r	3-OMe	OH	278	23,345	84
6s	3-$N(Me)_2$	OH	246	18,597	75
6t	3-Me	OH	164	10,645	65
6u	3-F	OH	326	3151	10
6v	4-F	OH	430	18,040	42
6w	2-F,3-OMe	OH	61	4452	73
6x	2-F,3-OH	OH	40	202	5

n.i.: no inhibition; n.d.: not determined.
[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-HSD_1)/IC_{50} (17β-HSD_2).
[e] % inhibition at 1 μM.

On the other hand, compounds monosubstituted on the A-ring (**6i**–**6l**) show higher inhibition of the enzyme when R_2 is a methoxy group (**6i**, IC_{50} = 222 nM; **6k**, IC_{50} = 371 nM) compared to the hydroxy compounds analogues (42% for **6j** and 36% for **6l** at 1 μM). The A-ring 2-substituted compounds **6m** and **6n** again behave differently from the 3-and 4-substitued counterparts, the OH derivative **6n** being more potent than the methoxy **6m** with IC_{50} values of 1127 nM for **6m** and 520 nM for **6n**. However, from this study it is clear that the electron donating 3-OMe group leads to the best A-ring monosubstituted derivative.

The disubstituted compound **6p** with an OH group on the C-ring and a cyano group in 4-position on the A-ring shows low inhibition of the enzyme, indicating that an electron withdrawing group as R_2 decreases the activity.

Thus, further compounds with a 3-hydroxy group on the C-ring and electron donating groups as R_2 on the A-ring (compounds **6q**–**t**) were synthesized. They show good to moderate inhibition of the enzyme with IC_{50} values between 161 (**6q**) and 278 nM (**6r**). Substituent like 3-Me or 4-OMe on the A-ring improve the activity slightly compared to the unsubstituted A-ring compound (**6h**). As it is known that a fluorine often enhances inhibitory potency and as

there is space available in position 2 of the A-ring for substitution, a fluorine was also introduced as R_2 instead of the OH group and led to more potent compounds **6u** and **6v** than the OH analogues **6j** and **6l**, $IC_{50} = 326$ nM for **6u** (3-F) *vs*. 42% inhibition at 1 μM for **6j** (3-OH) and 430 nM for **6v** (4-F) *vs*. 36% inhibition at 1 μM for **6l** (4-OH).

Interestingly, a significant increase in activity is observed when the fluorine is combined with a 3-methoxy or a 3-hydroxy group on the A-ring, resulting in the highly potent **6w** ($IC_{50} = 61$ nM) and **6x** ($IC_{50} = 40$ nM), respectively.

3.2.2. Selectivity: inhibition of human 17β-HSD1 and binding affinities for ERα and ERβ

Since 17β-HSD1 is the major enzyme catalyzing the reverse reaction compared to 17β-HSD2, activating E1 to E2, inhibitory activity toward this enzyme must be avoided. 17β-HSD1 inhibition was determined using a similar assay as described for 17β-HSD2 [47]. Briefly, human placental cytosolic 17β-HSD1 was incubated with tritiated E1 in the presence of NADH and the inhibitor. Inhibitory activity was determined as % inhibition or IC_{50} values for the most potent HSD2 inhibitors. A selectivity factor (SF) was calculated as the quotient of the IC_{50} values for 17β-HSD1 and 17β-HSD2 (Tables 1 and 2).

It is apparent that there is a good correlation between the number of methoxy/hydroxy groups and the selectivity toward 17β-HSD1. Derivatives with an OH moiety at both A- and C-rings (**4b**, **5b**, **5d**, **6b**, **6d**, **6f**, **6x**) show a low SF between 5 and 22. However, most compounds with only one OH group at the C-ring (**4d**, **6h**, **6p**–**w**) exhibit a better SF between 21 and 84 except for the fluorinated compound **6u** which has a SF of 10 and the 3-methyl **4d** and the 4-methoxy derivative **6q** with an extraordinarily high SF of 246 or more. Furthermore, most of the compounds with an OMe group on the A-ring (**4a**, **6a**, **6c**, **6i**, **6k**) have SFs of more than 200, with the highest value of more than 450 (**6i**). In summary, most of the compounds are very selective toward 17β-HSD1.

As it is expected that the biological effects of E2 in osteoporotic cells are estrogen receptor (ER) mediated, 17β-HSD2 inhibitors should not bind to the ERs. Binding affinities to ERα and ERβ were determined for the most potent ($IC_{50} < 500$ nM) and selective compounds (SF > 10). The ER assays were performed using recombinant human protein. A competition assay using tritium labelled E2, the protein and the inhibitor was applied. Separation of bound and free E2 was carried out using hydroxyapatite. The relative binding affinity (RBA) of E2 was set up to 100%. All tested compounds showed no binding affinity to the ERα and ERβ with RBA values smaller than 0.1%.

4. Discussion and conclusion

In our design concept we hypothesized that the rigidity induced by the triazole ring in **7** and **8** was responsible for the weak 17β-HSD2 inhibitory activity observed. Replacement of this triazole by the bioisostere amide moiety successfully led to the discovery of new and highly potent 17β-HSD2 inhibitors, especially in the thiophene- 2-carboxamine class (compound **6**). The gain in activity observed might be caused by the fact that the amide derivatives can adopt an energetically more favourable bent geometry similar to the one observed in the pyrrolidinone **2** (while the triazoles **7** and **8** were linear). It seems that to reach a good inhibitory activity, the inhibitors require some flexibility to adopt a "sandwich-like form".

Three different classes of compounds were investigated: 1,4-disubstituted benzenes (class **4**), 1,3-disubstituted benzenes (class **5**) and the 2,5-thiophenes (class **6**). When substituted with dihydroxy or dimethoxy groups (**4a**–**4b**, **5a**–**6d**), the 5- and the 6-membered rings all appear to be moderately potent (IC_{50} values between 335 nM for **6d** and 639 nM for **5b**) except for the dimethoxylated 1,3-benzene (**5a** and **5c**) which are inactive and for dihydroxylated thiophene **6f** which shows a good inhibitory activity. It is striking that the substitution pattern (1,4-, 1,3-benzene or 2,5-thiophene) does not have a great influence on the activity as it can be expected that depending on the substitution pattern the A- or C-ring will cover a completely different region of the enzyme active site.

Because of the interesting activity identified for **6f** and due to the fact that the thiophene moiety was discovered to be a very promising heteroaromatic ring in the design of 17β-HSD1 inhibitors [47], it was decided to focus on this class of compounds for further optimization.

In a step by step optimization process, we wanted to identify whether OH or OMe was optimal for ring A and for ring C. Mono-substituted compounds **6g**–**n** with only one OMe or one OH on either ring A or ring C were evaluated. Concerning substitution on the C-ring, R_1 should be an OH group rather than an OMe group ($IC_{50} = 186$ nM for the OH **6h** *vs*. around 1 μM for the OMe **6g**). Since OH is superior to OMe (**6g** *vs*. **6h**), a hydrogen bond acceptor obviously exists around the C-ring in the enzyme active site. Regarding substitution on the A-ring, the biological data of compounds **6i**–**l** demonstrate that R_2 should be an OCH_3 group rather than an OH group and it should be localized in 3-position.

In a second step, the disubstituted compound **6r** with the best R_1 and R_2 (a 3-OMe on the A-ring and a 3-OH on the C-ring) was prepared and turned out to have a good inhibitory activity, however, slightly less potent than both corresponding mono-substituted analogues.

In the next step, an additional fluorine atom was introduced in **6r** in position 2 of the A-ring because of the positive *ortho* effect observed for **6f**. The fluorine induces an increase in activity, leading to the highly active **6w** and **6x** (IC_{50} values of 61 nM and 40 nM for **6w** and **6x**, respectively). It has been shown in several examples that fluorine may enhance binding efficacy and selectivity in pharmaceuticals because of a variety of multipolar C–F···H–N, C–F···C=O, and C–F···H–C interactions between a fluorinated ligand and protein binding sites [60,61].

As selectivity toward 17β-HSD1 is an important issue for the therapeutic concept, inhibitory activity of this enzyme should be avoided. It was evaluated for the most potent compounds. Interestingly, it can be observed that in all three classes higher selectivity toward HSD1 is always observed for the compounds with an OMe group on the A-ring (compounds **4a**, **6a**, **6c**, **6i**, **6k**, **6q** with a selectivity factor >200) compared to their hydroxy analogues. The biological results indicate that the hydroxy derivatives with low SF are able to bind in both HSD1 and HSD2 active site while the methoxy compounds with high SF cannot bind in the one of HSD1. Providing that the hypothesis that the architecture of HSD1 and HSD2 active sites is very similar, is correct (the product of one catalytic reaction is the substrate of the other), it must be concluded that the methoxy derivatives have a different binding mode compared to the hydroxy analogues. The binding mode investigation of this class of compounds will be the focus of another study.

We developed a novel class of potent 17β-HSD2 inhibitors: *N*-benzyl-5-phenyl-thiophene-2-carboxamides. These nonsteroidal compounds, without asymmetric carbons are the first easily accessible 17β-HSD2 inhibitors (2–3 steps synthesis pathway and an overall yield between 54% and 95%). Parallel synthesis can be applied and allows the fast production of many derivatives in a short time. The inhibitors show a good selectivity profile toward 17β-HSD1 and the estrogen receptors α and β. The 5-(2-fluoro-3-methoxyphenyl)-*N*-(3-hydroxybenzyl)-*N*-methylthiophene-2-carboxamide **6w** is the most promising compound identified in this study (IC_{50} (HSD2) = 61 nM, SF (HSD1) = 73, no binding affinity to the ERs). It will be a good tool for *in vivo* evaluation in a disease-oriented model to validate the concept of 17β-HSD2 inhibition.

5. Experimental section

5.1. Chemical methods

Chemical names follow IUPAC nomenclature. Starting materials were purchased from Aldrich, Acros, Lancaster, Roth, Merck 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). Vizualisation was accomplished with UV light.

^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker AM500 spectrometer (at 500 MHz and 125 MHz, respectively) at 300 K in $CDCl_3$ and CD_3COCD_3. 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 77 ppm (^{13}C NMR); CD_3COCD_3: 2.05 ppm (^{1}H NMR) and 30.8 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) and m (multiplet). All coupling constants (J) are given in Hertz (Hz).

MS (ESI) measurements were executed using a TSQ Quantum (Thermo Finnigan) instrument.

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

Melting points (mp) 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). 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 in was increased from an initial concentration of 5% at 0 min to 100% at 15 min and kept at 100% for 5 min. The injection volume was 20 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 the wave length of 254 nm and in some cases at 360 nm. All tested compounds have >95% chemical purity except compound **6q** which has a purity of 93%.

Compound **A** was prepared according to the previously described procedure [25].

5.1.1. General procedures

5.1.1.1. General procedure for amidation. **Method A.** At 0 °C, a solution of 3(4)-bromobenzoyl chloride or 5-bromothiophene-2-carbonyl chloride (1 eq) in CH_2Cl_2 (2 ml/eq) was added drop wise to a solution of *N*-methylbenzylamine (1 eq) and triethylamine (1.15 eq) in CH_2Cl_2 (2 ml/eq). 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 as eluant.

5.1.1.2. General procedure for Suzuki coupling. **Method B.** A mixture of aryl bromide (1 eq), substituted phenyl boronic acid (1.2 eq), sodium carbonate (2 eq) and tetrakis(triphenylphosphine) palladium (0.1 eq) 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 rt. 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 (6:1-3:1) as eluant.

5.1.1.3. General procedure for ether cleavage. **Method C.** To a solution of methoxyphenyl compounds (1 eq) in dry dichloromethane (5 ml/mmol of reactant), borontrifluoride dimethyl sulfide complex (6 eq/methoxy function) was added drop wise at 0 °C and stirred for 4–14 h. After the reaction was finished, the reaction mixture was diluted with dichloromethane and 5% aqueous $NaHCO_3$ was added until neutral pH. 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 (100:1–50:1) as eluant.

5.1.2. Detailed synthesis procedures

*5.1.2.1. 4-Bromo-N-(3-methoxybenzyl)-N-methylbenzamide (**13**).* The title compound was prepared by reaction of 4-bromobenzoylchloride (329 mg, 1.5 mmol) and 3-methoxybenzylamine (227 mg, 1.5 mmol) in presence of triethylamine (0.24 ml, 1.73 mmol) according to method A. Purification by FC (*n*-hexane/ethyl acetate 6:1) afforded the desired product (468 mg, yield: 93%); $C_{16}H_{16}BrNO_2$; MW: 334; MS (ESI): 334–336 $(M + H)^+$; ^{1}H NMR (CD_3COCD_3): 2.80–2.92 (m, 3H), 3.80 (s, 3H), 4.52–4.69 (m, 2H), 6.79 (br s, 1H), 6.86 (dd, J = 8.5 Hz, 2.2 Hz, 1H), 6.95 (br s, 1H), 7.28 (t, J = 7.9 Hz, 1H), 7.44 (d, J = 7.9 Hz, 2H), 7.62 (br s, 2H); ^{13}C NMR (CD_3COCD_3): 55.3, 112.9, 113.6, 120.0, 123.1, 129.1, 129.4, 130.0, 131.7, 135.7, 139.1, 159.8; IR (cm^{-1}): 2931, 2835, 1630, 1600, 1586, 1488, 1398, 1257, 1070, 1011, 834, 755, 693.

*5.1.2.2. 3-Bromo-N-(3-methoxybenzyl)-N-methylbenzamide (**14**).* The title compound was prepared by reaction of 3-bromobenzoylchloride (217 mg, 1 mmol) and *N*-methyl-3-methoxybenzylamine (151 mg, 1 mmol) in presence of triethylamine (0.16 ml, 1.15 mmol) according to method A. Purification by FC (*n*-hexane/ethyl acetate 6:1) afforded the desired product (320 mg, yield: 98%); $C_{16}H_{16}BrNO_2$; MW: 334; MS (ESI): 334–336 $(M + H)^+$; ^{1}H NMR ($CDCl_3$): 2.86–3.03 (m, 3H), 3.81 (s, 3H), 4.45–4.71 (m, 2H), 6.69–6.74 (m, 1H), 6.85 (d, J = 8.0 Hz, 1H), 6.89–6.92 (m, 1H), 7.24–7.26 (m, 1H), 7.28 (t, J = 7.9 Hz, 1H), 7.37 (d, J = 7.9 Hz, 1H), 7.53 (s, 1H), 7.60 (s, 1H); ^{13}C NMR ($CDCl_3$): 50.8, 55.2, 112.5, 112.9, 118.9, 120.5, 122.6, 125.2, 125.5, 129.7, 130.0, 132.7, 138.2; IR (cm^{-1}): 2934, 1630, 1600, 1562, 1488, 1396, 1255, 1048, 746, 691.

*5.1.2.3. 5-Bromo-N-(3-methoxybenzyl)-N-methylthiophene-2-carboxamide (**15a**).* The title compound was prepared by reaction of 5-bromothiophene-2-carbonyl chloride (225 mg, 1 mmol) and *N*-methyl-3-methoxybenzylamine (151 mg, 1 mmol) in presence of triethylamine (0.16 ml, 1.15 mmol) according to method A. Purification by FC (*n*-hexane/ethyl acetate 6:1) afforded the desired product (335 mg, yield: 99%) as oil; $C_{14}H_{14}BrNO_2S$; MW: 340; ^{1}H NMR ($CDCl_3$): 3.11 (br s, 3H), 3.80 (s, 3H), 4.72 (br s, 2H), 6.80 (s, 1H), 6.83–6.86 (dd, J = 8.0 Hz, 2.0 Hz, 2H), 6.96 (br s, 1H), 7.07 (br s, 1H), 7.29 (t, J = 8.0 Hz, 1H); ^{13}C NMR ($CDCl_3$): 55.2, 112.9, 117.2, 129.4, 129.8, 138.1, 139.5, 153.1, 160.1, 163.3; IR (cm^{-1}): 2938, 2834, 1602, 1530, 1486, 1418, 1396, 1254, 1039, 737, 693.

*5.1.2.4. N-benzyl-5-bromo-N-methylthiophene-2-carboxamide (**15b**).* The title compound was prepared by reaction of 5-bromothiophene-2-carbonyl chloride (225 mg, 1 mmol) and *N*-methylbenzylamine (121 mg, 1 mmol) in presence of triethylamine (0.16 ml, 1.15 mmol) according to method A. Purification by FC (*n*-hexane/ethyl acetate 6:1) afforded the desired compound as oil (298 mg, yield: 96%). $C_{13}H_{12}BrNOS$; MW: 310; MS (ESI): 310–312 $(M + H)^+$; ^{1}H NMR

(CD_3COCD_3): 3.14 (br s, 3H), 4.78 (br s, 2H), 7.14 (d, J = 3.8 Hz, 1H), 7.25–7.40 (m, 6H); ^{13}C NMR (CD_3COCD_3): 117.1, 128.3, 129.6, 131.4, 138.2, 141.9; IR (cm^{-1}): 2923, 1606, 1530, 1483, 1417, 1397, 732, 700.

*5.1.2.5. 5-Bromo-N-(3-hydroxybenzyl)-N-methylthiophene-2-carboxamide (**15c**).* The title compound was prepared by reaction of 5-bromo-*N*-(3-methoxybenzyl)-*N*-methylthiophene-2-carboxamide **15a** (340 mg, 1 mmol) and borontrifluoride dimethyl sulfide complex (0.63 ml, 6 mmol) according to method C for 5 h. Purification by FC (CH_2Cl_2/CH_3OH 100:1) afforded the desired compound as amorph solid (300 mg, yield: 93%). $C_{13}H_{12}BrNO_2S$; MW: 326; ^{1}H NMR (CD_3COCD_3): 3.13 (br s, 3H), 4.71 (br s, 2H), 6.76–6.79 (m, 3H), 7.13–7.14 (m, 1H), 7.20 (t, J = 7.7 Hz, 1H), 7.25 (br s, 1H), 8.69 (s, 1H).

*5.1.2.6. 3′-Methoxy-N-(3-methoxybenzyl)-N-methyl[1,1′-biphenyl]-4-carboxamide (**4a**).* The title compound was prepared by reaction of 4-bromo-*N*-(3-methoxybenzyl)-*N*-methylbenzamide **13** (67 mg, 0.2 mmol) and 3-methoxyphenylboronic acid (36 mg, 0.24 mmol) with tetrakis(triphenylphosphine) palladium (23 mg, 0.02 mmol) according to method B for 4 h. Purification by FC (*n*-hexane/ethyl acetate 6:1→3:1) afforded the desired compound as oil (65 mg, yield: 90%). $C_{23}H_{23}NO_3$; MW: 361; MS (ESI): 362 $(M + H)^+$; ^{1}H NMR (CD_3COCD_3): 2.97 (s, 3H), 3.81 (s, 3H), 3.87 (s, 3H), 4.61–4.72 (m, 2H), 6.85 (dd, J = 8.2 Hz, 2.0 Hz, 2H), 6.96 (dd, J = 8.2 Hz, 2.0 Hz, 2H), 7.23–7.26 (m, 2H), 7.30 (t, J = 7.9 Hz, 1H), 7.38 (t, J = 8.0 Hz, 1H), 7.57 (d, J = 8.5 Hz, 2H), 7.72–7.73 (m, 2H); ^{13}C NMR (CD_3COCD_3): 55.5, 55.6, 113.3, 113.6, 114.2, 120.1, 127.7, 128.5, 130.6, 130.8, 136.8, 142.5, 142.8, 161.1, 161.2; IR (cm^{-1}): 2938, 2835, 1629, 1601, 1583, 1479, 1396, 1259, 1015, 841, 764, 694.

*5.1.2.7. 3′-Hydroxy-N-(3-hydroxybenzyl)-N-methyl[1,1′-biphenyl]-4-carboxamide (**4b**).* The title compound was prepared by reaction of 3′-methoxy-*N*-(3-methoxybenzyl)-*N*-methyl[1,1′-biphenyl]-4-carboxamide **4a** (36 mg, 0.1 mmol) with borontrifluoride dimethyl sulfide complex (0.13 ml, 1.2 mmol) according to method C for 14 h. Purification by FC (CH_2Cl_2/CH_3OH 100:1→50:1) yielded the title compound as amorph solid (28 mg, yield: 84%). $C_{21}H_{19}NO_3$; MW: 333; mp: 134–135 °C; MS (ESI): 334 $(M + H)^+$; ^{1}H NMR (CD_3COCD_3): 2.95 (s, 3H), 4.57–4.60 (m, 2H), 6.75 (dd, J = 8.2 Hz, 2.0 Hz, 2H), 6.85 (dd, J = 8.2 Hz, 2.0 Hz, 2H), 7.14–7.15 (m, 2H), 7.20 (t, J = 8.0 Hz, 1H), 7.29 (t, J = 8.0 Hz, 1H), 7.55 (d, J = 8.2 Hz, 2H), 7.67 (br s, 2H), 8.38 (br s, 1H), 8.48 (s, 1H); ^{13}C NMR (CD_3COCD_3): 114.7, 115.2, 115.7, 119.0, 127.6, 128.4, 130.4, 130.9, 136.7, 142.5, 142.9, 158.8, 158.9; IR (cm^{-1}): 3284, 2976, 1603, 1574, 1486, 1456, 764, 743, 690.

*5.1.2.8. N-(3-methoxybenzyl)-N,3′-dimethyl[1,1′-biphenyl]-4-carboxamide (**4c**).* The title compound was prepared by reaction of 4-bromo-*N*-(3-methoxybenzyl)-*N*-methylbenzamide **13** (53 mg, 0.15 mmol) and 3-methylphenylboronic acid (25 mg, 0.23 mmol) with tetrakis(triphenylphosphine) palladium (17 mg, 0.015 mmol) according to method B for 6 h. Purification by FC (*n*-hexane/ethyl acetate 6:1→3:1) afforded the desired compound as oil (50 mg, yield: 95%). $C_{23}H_{23}NO_2$; MW: 345; MS (ESI): 346 $(M + H)^+$; ^{1}H NMR (CD_3COCD_3): 2.40 (s, 3H), 2.97 (s, 3H), 3.80 (s, 3H), 4.60–4.72 (m, 2H), 6.81–6.98 (m, 3H), 7.20 (d, J = 7.3 Hz, 1H), 7.30 (t, J = 8.0 Hz, 1H), 7.35 (t, J = 7.6 Hz, 1H), 7.47 (d, J = 7.6 Hz, 1H), 7.51 (s, 1H), 7.56 (d, J = 8.5 Hz, 2H), 7.71 (d, J = 7.3 Hz, 2H); ^{13}C NMR (CD_3COCD_3): 21.5, 55.5, 113.6, 124.9, 127.6, 128.5, 129.3, 129.7, 130.6, 136.6, 139.3, 141.0, 143.0, 161.1; IR (cm^{-1}): 2934, 1630, 1606, 1586, 1485, 1396, 1259, 845, 763, 696.

*5.1.2.9. N-(3-hydroxybenzyl)-N,3′-dimethyl[1,1′-biphenyl]-4-carboxamide (**4d**).* The title compound was prepared by reaction of *N*-(3-methoxybenzyl)-*N*,3′-dimethyl[1,1′-biphenyl]-4-carboxamide **4c** (35 mg, 0.1 mmol) with borontrifluoride dimethyl sulfide complex (0.06 ml, 0.6 mmol) according to method C for 6 h. Purification by FC (CH_2Cl_2/CH_3OH 100:1→50:1) yielded the title compound as amorph solid (30 mg, yield: 89%). $C_{22}H_{21}NO_2$; MW: 331; mp: 140–141 °C; MS (ESI): 332 $(M + H)^+$; ^{1}H NMR (CD_3COCD_3): 2.40 (s, 3H), 2.96 (s, 3H), 4.55–4.70 (m, 2H), 6.69–6.89 (m, 3H), 7.20 (t, J = 7.9 Hz, 2H), 7.35 (t, J = 7.6 Hz, 1H), 7.47 (d, J = 7.9 Hz, 1H), 7.51 (s, 1H), 7.56 (d, J = 8.2 Hz, 2H), 7.70–7.71 (m, 2H), 8.36 (br s, 1H); ^{13}C NMR (CD_3COCD_3): 21.5, 115.2, 124.9, 127.6, 128.5, 129.3, 129.7, 136.7, 139.3, 141.0, 143.0; IR (cm^{-1}): 3225, 2977, 1593, 1448, 754, 692.

*5.1.2.10. 4′-Methoxy-N-(3-methoxybenzyl)-N-methyl[1,1′-biphenyl]-3-carboxamide (**5a**).* The title compound was prepared by reaction of 3-bromo-*N*-(3-methoxybenzyl)-*N*-methylbenzamide **14** (67 mg, 2 mmol) and 4-methoxyphenylboronic acid (36 mg, 2.4 mmol) with tetrakis(triphenylphosphine) palladium (17 mg, 0.015 mmol) as catalyst according to method B for 4 h. Purification by FC (*n*-hexane/ethyl acetate 6:1) afforded the desired compound as oil (70 mg, yield: 97%). $C_{23}H_{23}NO_3$; MW: 361; MS (ESI): 362 $(M + H)^+$; ^{1}H NMR (CD_3COCD_3): 2.97 (br s, 3H), 3.79 (s, 3H), 3.84 (s, 3H), 4.57–4.73 (m, 2H), 6.83–6.88 (m, 5H), 7.30 (t, J = 7.9 Hz, 1H), 7.40 (dt, J = 7.9 Hz, 1.3 Hz, 1H), 7.48–7.68 (m, 5H); ^{13}C NMR (CD_3COCD_3): 55.5, 55.6, 113.6, 114.5, 115.2, 125.7, 126.0, 128.1, 128.9, 129.7, 130.7, 133.4, 138.5, 141.6, 160.6; IR (cm^{-1}): 2936, 2836, 1630, 1601, 1584, 1488, 1395, 1246, 1044, 835, 802, 753.

*5.1.2.11. 4′-Hydroxy-N-(3-hydroxybenzyl)-N-methyl[1,1′-biphenyl]-3-carboxamide (**5b**).* The title compound was prepared by reaction of 4′-methoxy-*N*-(3-methoxybenzyl)-*N*-methyl[1,1′-biphenyl]-3-carboxamide **5a** (40 mg, 0.11 mmol) with borontrifluoride dimethyl sulfide complex (0.14 ml, 1.32 mmol) according to method C for 4 h. Purification by FC (CH_2Cl_2/CH_3OH 100:1→50:1) yielded the title compound as amorph solid (30 mg, yield: 82%). $C_{21}H_{19}NO_3$; MW: 333; mp: 128–129 °C; MS (ESI): 334 $(M + H)^+$; ^{1}H NMR (CD_3COCD_3): 2.93–3.01 (m, 3H), 4.53–4.71 (m, 2H), 6.71–6.92 (m, 5H), 7.19 (t, J = 7.9 Hz, 1H), 7.36–7.51 (m, 4H), 7.63 (br s, 1H), 7.66 (s, 1H), 8.51–8.52 (m, 1H), 8.62 (br s, 1H); ^{13}C NMR (CD_3COCD_3): 115.2, 116.7, 125.5, 125.7, 128.1, 128.9, 129.7, 130.7, 132.2, 138.1, 139.9, 141.9, 158.3, 158.7; IR (cm^{-1}): 3060, 1602, 1589, 1460, 752, 705, 687.

*5.1.2.12. 3′-Methoxy-N-(3-methoxybenzyl)-N-methyl[1,1′-biphenyl]-3-carboxamide (**5c**).* The title compound was prepared by reaction of 3-bromo-*N*-(3-methoxybenzyl)-*N*-methylbenzamide **14** (67 mg, 0.2 mmol) and 3-methoxyphenylboronic acid (36 mg, 0.24 mmol) with tetrakis(triphenylphosphine) palladium (23 mg, 0.02 mmol) as catalyst according to method B for 4 h. Purification by FC (*n*-hexane/ethyl acetate 6:1) afforded the desired compound as oil (71 mg, yield: 98%). $C_{23}H_{23}NO_3$; MW: 361; MS (ESI): 362 $(M + H)^+$; ^{1}H NMR (CD_3COCD_3): 2.98 (br s, 3H), 3.79 (s, 3H), 3.85 (s, 3H), 4.58–4.73 (m, 2H), 6.81 (br s, 1H), 6.86 (dd, J = 7.9 Hz, 2.2 Hz, 1H), 6.93–6.99 (m, 2H), 7.13 (br s, 1H), 7.22 (br s, 1H), 7.29 (t, J = 7.9 Hz, 1H), 7.36 (br s, 1H), 7.46 (dt, J = 7.6 Hz, 1.6 Hz, 1H), 7.52 (br s, 1H), 7.71 (br s, 1H), 7.73 (s, 1H); ^{13}C NMR (CD_3COCD_3): 54.9, 55.5, 55.6, 88.6, 113.2, 113.6, 114.2, 120.1, 126.2, 126.8, 128.7, 129.8, 130.5, 130.8, 138.5, 140.1, 141.8, 142.5, 161.1, 161.2; IR (cm^{-1}): 2947, 2836, 1629, 1600, 1585, 1489, 1458, 1257, 1042, 782, 752, 696.

*5.1.2.13. 3′-Hydroxy-N-(3-hydroxybenzyl)-N-methyl[1,1′-biphenyl]-3-carboxamide (**5d**).* The title compound was prepared by reaction of 3′-methoxy-*N*-(3-methoxybenzyl)-*N*-methyl[1,1′-biphenyl]-3-carboxamide **5c** (54 mg, 0.15 mmol) with borontrifluoride dimethyl sulfide complex (0.19 ml, 1.8 mmol) according to method C for 5 h. Purification by FC (CH_2Cl_2/CH_3OH 100:1→50:1) yielded the title compound as oil (46 mg, yield: 92%). $C_{21}H_{19}NO_3$; MW: 333; MS (ESI): 334 $(M + H)^+$; ^{1}H NMR (CD_3COCD_3): 2.96 (br

s, 3H), 4.54–4.70 (m, 2H), 6.74–6.91 (m, 4H), 7.02–7.27 (m, 4H), 7.44 (d, $J = 7.6$ Hz, 1H), 7.51 (br s, 1H), 7.67 (br s, 1H), 7.69 (s, 1H), 8.37 (br s, 1H), 8.45 (s, 1H); ^{13}C NMR (CD_3COCD_3): 114.7, 115.2, 115.6, 119.0, 119.9, 126.2, 126.7, 128.6, 129.7, 130.9, 138.4, 139.9, 142.0, 142.5, 158.7, 158.8; IR (cm^{-1}): 3208, 2985, 1587, 1574, 1454, 752, 693.

*5.1.2.14. N-(3-methoxybenzyl)-5-(3-methoxyphenyl)-N-methylthiophene-2-carboxamide (**6a**).* The title compound was prepared by reaction of 5-bromo-*N*-(3-methoxybenzyl)-*N*-methylthiophene-2-carboxamide **15a** (124 mg, 0.37 mmol) and 3-methoxyphenylboronic acid (67 mg, 0.44 mmol) with tetrakis(triphenylphosphine) palladium (43 mg, 0.04 mmol) as catalyst according to method B for 6 h. Purification by FC (*n*-hexane/ethyl acetate 6:1) afforded the desired compound as oil (130 mg, yield: 96%). $C_{21}H_{21}NO_3S$; MW: 367; MS (ESI): 368 $(M + H)^+$; ^{1}H NMR (CD_3COCD_3): 3.16 (br s, 3H), 3.80 (s, 3H), 3.86 (s, 3H), 4.79 (br s, 2H), 6.86–6.88 (m, 1H), 6.91–6.95 (m, 3H), 7.23 (t, $J = 2.0$ Hz, 1H), 7.25–7.28 (m, 1H), 7.30 (t, $J = 8.0$ Hz, 1H), 7.35 (t, $J = 8.0$ Hz, 1H), 7.41 (br s, 2H); ^{13}C NMR (CD_3COCD_3): 55.5, 55.7, 112.1, 113.5, 114.9, 119.1, 124.3, 130.6, 131.1, 135.7, 138.7, 140.1, 148.1, 161.1, 161.3; IR (cm^{-1}): 2964, 2838, 1593, 1578, 1480, 1400, 1277, 1253, 1158, 1033, 752, 731, 682.

*5.1.2.15. N-(3-hydroxybenzyl)-5-(3-hydroxyphenyl)-N-methylthiophene-2-carboxamide (**6b**).* The title compound was prepared by reaction of *N*-(3-methoxybenzyl)-5-(3-methoxyphenyl)-*N*-methylthiophene-2-carboxamide **6a** (70 mg, 0.19 mmol) with borontrifluoride dimethyl sulfide complex (0.24 ml, 2.28 mmol) according to method C for 4 h. Purification by FC (CH_2Cl_2/CH_3OH 100:1 → 50:1) yielded the title compound as amorph solid (59 mg, yield: 91%). $C_{19}H_{17}NO_3S$; mp: 175–176 °C; MW: 339; MS (ESI): 340 $(M + H)^+$; ^{1}H NMR (CD_3COCD_3): 3.15 (br s, 3H), 4.76 (br s, 2H), 6.77–6.79 (m, 1H), 6.80–6.83 (m, 2H), 6.85 (ddd, $J = 8.0$ Hz, 2.5 Hz, 0.9 Hz, 1H), 7.15–7.18 (m, 2H), 7.21 (t, $J = 8.0$ Hz, 1H), 7.26 (t, $J = 7.9$ Hz, 1H), 7.34 (d, $J = 3.5$ Hz, 1H), 7.39 (br s, 1H), 8.35 (s, 1H), 8.54 (s, 1H); ^{13}C NMR (CD_3COCD_3): 113.5, 115.2, 116.4, 118.0, 124.0, 130.7, 131.1, 135.7, 138.4, 140.0, 148.4, 158.8, 158.9; IR (cm^{-1}): 3111, 1605, 1570, 1481, 752, 685.

*5.1.2.16. N-(3-methoxybenzyl)-5-(4-methoxyphenyl)-N-methylthiophene-2-carboxamide (**6c**).* The title compound was prepared by reaction of 5-bromo-*N*-(3-methoxybenzyl)-*N*-methylthiophene-2-carboxamide **15a** (130 mg, 0.38 mmol) and 4-methoxyphenylboronic acid (70 mg, 0.46 mmol) with tetrakis(triphenylphosphine) palladium (44 mg, 0.04 mmol) as catalyst according to method B for 6 h. Purification by FC (*n*-hexane/ethyl acetate 6:1) afforded the desired compound as amorph solid (138 mg, yield: 98%). $C_{21}H_{21}NO_3S$; MW: 367; mp: 115–116 °C; MS (ESI): 368 $(M + H)^+$; ^{1}H NMR (CD_3COCD_3): 3.17 (br s, 3H), 3.80 (s, 3H), 3.84 (s, 3H), 4.79 (br s, 2H), 6.87 (d, $J = 8.0$ Hz, 1H), 6.91–6.93 (m, 2H), 7.00 (d, $J = 8.8$ Hz, 2H), 7.27–7.32 (m, 2H), 7.38 (br s, 1H), 7.63 (d, $J = 8.8$ Hz, 2H); ^{13}C NMR (CD_3COCD_3): 55.5, 55.7, 113.5, 115.4, 122.9, 127.1, 128.1, 130.6, 137.6, 140.2, 148.5, 161.0, 161.1; IR (cm^{-1}): 2976, 1602, 1573, 1483, 1400, 1266, 736, 698.

*5.1.2.17. N-(3-hydroxybenzyl)-5-(4-hydroxyphenyl)-N-methylthiophene-2-carboxamide (**6d**).* The title compound was prepared by reaction of *N*-(3-methoxybenzyl)-5-(4-methoxyphenyl)-*N*-methylthiophene-2-carboxamide **6c** (100 mg, 0.27 mmol) with borontrifluoride dimethyl sulfide complex (0.34 ml, 3.24 mmol) according to method C for 4 h. Purification by FC (CH_2Cl_2/CH_3OH 100:1 → 50:1) yielded the title compound as amorph solid (85 mg, yield: 92%). $C_{19}H_{17}NO_3S$; MW: 339; mp: 135–136 °C; MS (ESI): 340 $(M + H)^+$; ^{1}H NMR (CD_3COCD_3): 3.15 (br s, 3H), 4.75 (br s, 2H), 6.76–6.80 (m, 3H), 6.90 (d, $J = 8.5$ Hz, 2H), 7.19–7.23 (m, 2H), 7.36 (br s, 1H), 7.54 (d, $J = 8.5$ Hz, 2H), 8.40 (s, 1H), 8.70 (s, 1H); ^{13}C NMR (CD_3COCD_3): 115.2, 116.8, 122.5, 126.1, 128.2, 130.6, 137.1, 140.1, 149.0, 158.8, 158.9; IR (cm^{-1}): 3316, 2971, 1609, 1581, 1442, 1160, 815, 690.

*5.1.2.18. N-(3-methoxybenzyl)-5-(2-methoxyphenyl)-N-methylthiophene-2-carboxamide (**6e**).* The title compound was prepared by reaction of 5-bromo-*N*-(3-methoxybenzyl)-*N*-methylthiophene-2-carboxamide **15a** (90 mg, 0.26 mmol) and 2-methoxyphenylboronic acid (48 mg, 0.32 mmol) with tetrakis(triphenylphosphine) palladium (30 mg, 0.026 mmol) as catalyst according to method B for 4 h. Purification by FC (*n*-hexane/ethyl acetate 6:1) afforded the desired compound as amorph solid (92 mg, yield: 93%). $C_{21}H_{21}NO_3S$; MW: 367; mp: 101–102 °C; MS (ESI): 368 $(M + H)^+$; ^{1}H NMR (CD_3COCD_3): 3.16 (br s, 3H), 3.80 (s, 3H), 3.97 (s, 3H), 4.80 (br s, 2H), 6.87 (dd, $J = 8.0$ Hz, 2.0 Hz, 1H), 6.92–6.94 (m, 2H), 7.03 (td, $J = 7.0$ Hz, 1.0 Hz, 1H), 7.15 (dd, $J = 8.0$ Hz, 1.0 Hz, 1H), 7.29–7.36 (m, 2H), 7.39 (br s, 1H), 7.52 (d, $J = 4.0$ Hz, 1H), 7.76 (dd, $J = 8.0$ Hz, 1.5 Hz, 1H); ^{13}C NMR (CD_3COCD_3): 55.5, 56.0, 112.9, 113.5, 121.8, 123.1, 125.5, 128.8, 129.5, 130.2, 130.6, 132.0, 138.6, 140.3, 143.5, 156.7, 161.1; IR (cm^{-1}): 2948, 1594, 1480, 1255, 1161, 1019, 754, 672.

*5.1.2.19. N-(3-hydroxybenzyl)-5-(2-hydroxyphenyl)-N-methylthiophene-2-carboxamide (**6f**).* The title compound was prepared by reaction of *N*-(3-methoxybenzyl)-5-(2-methoxyphenyl)-*N*-methylthiophene-2-carboxamide **6e** (48 mg, 0.13 mmol) with borontrifluoride dimethyl sulfide complex (0.16 ml, 1.56 mmol) according to method C for 14 h. Purification by FC (CH_2Cl_2/CH_3OH 100:1 → 50:1) yielded the title compound as amorph solid (40 mg, yield: 90%). $C_{19}H_{17}NO_3S$; MW: 339; mp: 177–178 °C; MS (ESI): 340 $(M + H)^+$; ^{1}H NMR (CD_3COCD_3): 3.15 (br s, 3H), 4.76 (br s, 2H), 6.77 (dd, $J = 8.0$ Hz, 2.0 Hz, 1H), 6.81–6.84 (m, 2H), 6.92 (td, $J = 7.6$ Hz, 0.9 Hz, 1H), 7.04 (dd, $J = 8.0$ Hz, 0.9 Hz, 1H), 7.16–7.22 (m, 2H), 7.39 (br s, 1H), 7.55 (d, $J = 3.4$ Hz, 1H), 7.70 (dd, $J = 8.0$ Hz, 0.9 Hz, 1H), 8.40 (s, 1H), 9.34 (s, 1H); ^{13}C NMR (CD_3COCD_3): 115.2, 117.3, 120.9, 121.4, 125.3, 128.9, 130.0, 130.6, 138.1, 140.1, 144.5, 154.6, 158.8; IR (cm^{-1}): 3300, 2941, 1617, 1564, 1477, 749, 693.

*5.1.2.20. N-(3-methoxybenzyl)-N-methyl-5-phenylthiophene-2-carboxamide (**6g**).* The title compound was prepared by reaction of 5-bromo-*N*-(3-methoxybenzyl)-*N*-methylthiophene-2-carboxamide **15a** (90 mg, 0.27 mmol) and phenyl boronic acid (43 mg, 0.35 mmol) with tetrakis(triphenylphosphine) palladium (31 mg, 0.027 mmol) as catalyst according to method B for 14 h. Purification by FC (*n*-hexane/ethyl acetate 6:1) afforded the desired compound as oil (87 mg, yield: 96%). $C_{20}H_{19}NO_2S$; MW: 337; MS (ESI): 338 $(M + H)^+$; ^{1}H NMR (CD_3COCD_3): 3.17 (br s, 3H), 3.80 (s, 3H), 4.80 (br s, 2H), 6.88 (ddd, $J = 8.2$ Hz, 2.5 Hz, 0.9 Hz, 1H), 6.92 (s, 1H), 6.93 (d, $J = 7.9$ Hz, 1H), 7.32 (t, $J = 8.2$ Hz, 1H), 7.36 (t, $J = 7.6$ Hz, 1H), 7.41–7.46 (m, 4H), 7.71 (d, $J = 7.6$ Hz, 2H); ^{13}C NMR (CD_3COCD_3): 55.5, 113.5, 124.1, 126.7, 129.2, 130.0, 130.6, 134.5, 140.1, 148.3, 161.1; IR (cm^{-1}): 2968, 1592, 1481, 1394, 1264, 751, 686.

*5.1.2.21. N-(3-hydroxybenzyl)-N-methyl-5-phenylthiophene-2-carboxamide (**6h**).* The title compound was prepared by reaction of *N*-(3-methoxybenzyl)-*N*-methyl-5-phenylthiophene-2-carboxamide **6g** with borontrifluoride dimethyl sulfide complex (0.13 ml, 1.20 mmol) according to method C for 6 h. Purification by FC (CH_2Cl_2/CH_3OH 100:1 → 50:1) yielded the title compound as amorph solid (63 mg, yield: 98%). $C_{19}H_{17}NO_2S$; MW: 323; mp: 140–141 °C; MS (ESI): 346 $(M + Na)^+$; ^{1}H NMR (CD_3COCD_3): 3.16 (br s, 3H), 4.76 (br s, 2H), 6.78 (ddd, $J = 8.2$ Hz, 2.5 Hz, 0.9 Hz, 1H), 6.81–6.83 (m, 2H), 7.22 (t, $J = 7.8$ Hz, 1H), 7.36 (tt, $J = 8.2$ Hz, 1.3 Hz, 1H), 7.40–7.46 (m, 4H), 7.71 (d, $J = 7.6$ Hz, 2H), 8.37 (s, 1H); ^{13}C NMR (CD_3COCD_3): 115.2, 124.0, 126.7, 129.2, 130.0, 130.7, 134.5, 138.7, 140.0, 148.3, 158.8; IR (cm^{-1}): 3176, 2982, 1599, 1563, 1499, 734, 688.

*5.1.2.22. N-benzyl-5-(3-methoxyphenyl)-N-methylthiophene-2-carboxamide (**6i**).* The title compound was prepared by reaction of *N*-benzyl-5-bromo-*N*-methylthiophene-2-carboxamide **15b** (80 mg, 0.26 mmol) and 3-methoxyphenylboronic acid (47 mg, 0.31 mmol) with tetrakis(triphenylphosphine) palladium (30 mg, 0.026 mmol) as catalyst according to method B for 4 h. Purification by FC (*n*-hexane/ethyl acetate 6:1) afforded the desired compound as amorph solid (75 mg, yield: 86%). $C_{20}H_{19}NO_2S$; MW: 337; MS (ESI): 338 $(M + H)^+$; ^{1}H NMR (CD_3COCD_3): 3.17 (br s, 3H), 3.86 (s, 3H), 4.82 (br s, 2H), 6.94 (ddd, J = 8.2 Hz, 2.5 Hz, 0.9 Hz, 1H), 7.23 (t, J = 2.0 Hz, 1H), 7.27 (ddd, J = 7.9 Hz, 1.6 Hz, 0.9 Hz, 1H), 7.29–7.32 (m, 1H), 7.34–7.41 (m, 7H); ^{13}C NMR (CD_3COCD_3): 55.7, 112.1, 114.9, 119.1, 124.3, 128.2, 129.6, 131.1, 135.7, 138.5, 148.1, 161.3.

*5.1.2.23. N-benzyl-5-(3-hydroxyphenyl)-N-methylthiophene-2-carboxamide (**6j**).* The title compound was prepared by reaction of *N*-benzyl-5-(3-methoxyphenyl)-*N*-methylthiophene-2-carboxamide **6i** (65 mg, 0.19 mmol) with borontrifluoride dimethyl sulfide complex (0.12 ml, 1.14 mmol) according to method C for 4 h. Purification by FC (CH_2Cl_2/CH_3OH 100:1) yielded the title compound as oil (55 mg, yield: 88%). $C_{19}H_{17}NO_2S$, MW: 323; MS (ESI): 324 $(M + H)^+$; ^{1}H NMR (CD_3COCD_3): 3.17 (br s, 3H), 4.82 (br s, 2H), 6.85 (ddd, J = 8.2 Hz, 2.2 Hz, 0.9 Hz, 1H), 7.16–7.18 (m, 2H), 7.26 (t, J = 7.8 Hz, 1H), 7.29–7.33 (m, 1H), 7.34–7.41 (m, 6H), 8.55 (s, 1H); ^{13}C NMR (CD_3COCD_3): 113.5, 116.4, 118.0, 124.0, 128.2, 129.6, 131.1, 135.7, 138.5, 148.4, 158.9; IR (cm^{-1}): 3285, 2967, 1602, 1571, 1488, 744, 691.

*5.1.2.24. N-benzyl-5-(4-methoxyphenyl)-N-methylthiophene-2-carboxamide (**6k**).* The title compound was prepared by reaction of *N*-benzyl-5-bromo-*N*-methylthiophene-2-carboxamide **15b** (72 mg, 0.23 mmol) and 4-methoxyphenylboronic acid (42 mg, 0.27 mmol) with tetrakis(triphenylphosphine) palladium (27 mg, 0.023 mmol) as catalyst according to method B for 5 h. Purification by FC (*n*-hexane/ethyl acetate 6:1) afforded the desired compound as amorph solid (75 mg, yield: 96%). $C_{20}H_{19}NO_2S$; MW: 337; mp: 126–127 °C; MS (ESI): 338 $(M + H)^+$; ^{1}H NMR (CD_3COCD_3): 3.16 (br s, 3H), 3.84 (s, 3H), 4.82 (br s, 2H), 7.00 (d, J = 8.8 Hz, 2H); 7.27 (d, J = 3.8 Hz, 1H), 7.29–7.32 (m, 1H), 7.35–7.41 (m, 5H), 7.63 (d, J = 8.8 Hz, 2H); ^{13}C NMR (CD_3COCD_3): 55.7, 115.4, 122.9, 127.1, 128.1, 128.2, 129.6, 137.6, 138.6, 148.5, 161.0; IR (cm^{-1}): 2937, 1596, 1572, 1486, 1245, 1179, 1024, 732, 695.

*5.1.2.25. N-benzyl-5-(4-hydroxyphenyl)-N-methylthiophene-2-carboxamide (**6l**).* The title compound was prepared by reaction of *N*-benzyl-5-(4-methoxyphenyl)-*N*-methylthiophene-2-carboxamide **6k** (56 mg, 0.17 mmol) with borontrifluoride dimethyl sulfide complex (0.11 ml, 1.02 mmol) according to method C for 6 h. Purification by FC (CH_2Cl_2/CH_3OH 100:1) yielded the title compound as amorph solid (49 mg, yield: 91%). $C_{19}H_{17}NO_2S$; MW: 323; mp: 170–171 °C; MS: 324 $(M + H)^+$; ^{1}H NMR (CD_3COCD_3): 3.16 (br s, 3H), 4.82 (br s, 2H), 6.91 (d, J = 8.8 Hz, 2H), 7.23 (d, J = 3.8 Hz, 1H), 7.28–7.32 (m, 1H), 7.34–7.40 (m, 5H), 7.55 (d, J = 8.8 Hz, 2H), 8.64 (s, 1H); ^{13}C NMR (CD_3COCD_3): 116.8, 122.5, 126.1, 128.2, 129.6, 137.2, 138.6, 149.0, 158.9; IR (cm^{-1}): 3211, 1611, 1573, 1482, 735, 698.

*5.1.2.26. N-benzyl-5-(2-methoxyphenyl)-N-methylthiophene-2-carboxamide (**6m**).* The title compound was prepared by reaction of *N*-benzyl-5-bromo-*N*-methylthiophene-2-carboxamide **15b** (80 mg, 0.26 mmol) and 2-methoxyphenylboronic acid (47 mg, 0.31 mmol) with tetrakis(triphenylphosphine) palladium (30 mg, 0.026 mmol) as catalyst according to method B for 14 h. Purification by FC (*n*-hexane/ethyl acetate 6:1) afforded the desired compound as oil (76 mg, yield: 87%). $C_{20}H_{19}NO_2S$; MW: 337; MS (ESI): 338 $(M + H)^+$; ^{1}H NMR (CD_3COCD_3): 3.16 (br s, 3H), 3.97 (s, 3H), 4.82 (br s, 2H), 7.03 (td, J = 7.6 Hz, 1.3 Hz, 1H), 7.15 (dd, J = 8.2 Hz, 1.3 Hz, 1H), 7.29–7.41 (m, 7H), 7.52 (d, J = 4.0 Hz, 1H), 7.76 (dd, J = 7.8 Hz, 1.6 Hz, 1H); ^{13}C NMR (CD_3COCD_3): 56.0, 112.9, 121.8, 123.1, 125.5, 128.1, 128.8, 129.5, 130.2, 132.0, 138.6, 143.5, 156.7; IR (cm^{-1}): 2965, 2838, 1595, 1480, 1253, 1158, 1033, 771, 682.

*5.1.2.27. N-benzyl-5-(2-hydroxyphenyl)-N-methylthiophene-2-carboxamide (**6n**).* The title compound was prepared by reaction of *N*-benzyl-5-(2-methoxyphenyl)-*N*-methylthiophene-2-carboxamide **6m** (55 mg, 0.16 mmol) with borontrifluoride dimethyl sulfide complex (0.10 ml, 0.96 mmol) according to method C for 14 h. Purification by FC (CH_2Cl_2/CH_3OH 100:1) yielded the title compound as amorph solid (49 mg, yield: 93%). $C_{19}H_{17}NO_2S$; MW: 323; mp: 185–186 °C; MS (ESI): 324 $(M + H)^+$; ^{1}H NMR (CD_3COCD_3): 3.16 (br s, 3H), 4.83 (br s, 2H), 6.93 (ddd, J = 8.5 Hz, 7.3 Hz, 1.3 Hz, 1H), 7.03 (dd, J = 7.8 Hz, 1.3 Hz, 1H), 7.18 (ddd, J = 8.5 Hz, 7.3 Hz, 1.3 Hz, 1H), 7.28–7.32 (m, 1H), 7.35–7.41 (m, 5H), 7.56 (d, J = 4.0 Hz, 1H), 7.70 (dd, J = 7.8 Hz, 1.6 Hz, 1H), 9.23 (s, 1H); ^{13}C NMR (CD_3COCD_3): 117.3, 121.0, 121.5, 125.4, 128.1, 129.0, 129.6, 130.0, 138.2, 138.7, 154.6; IR (cm^{-1}): 2986, 1587, 1455, 752, 697.

*5.1.2.28. 5-(4-Cyanophenyl)-N-(3-methoxybenzyl)-N-methylthiophene-2-carboxamide (**6o**).* The title compound was prepared by reaction of 5-bromo-*N*-(3-methoxybenzyl)-*N*-methylthiophene-2-carboxamide **15a** (80 mg, 0.24 mmol) and 4-cyanophenylboronic acid (42 mg, 0.29 mmol) with tetrakis(triphenylphosphine) palladium (28 mg, 0.024 mmol) as catalyst according to method B for 14 h. Purification by FC (*n*-hexane/ethyl acetate 6:1) afforded the desired compound as amorph solid (80 mg, yield: 92%). $C_{21}H_{18}N_2O_2S$; MW: 362; mp: 137–138 °C; MS (ESI): 363 $(M + H)^+$; ^{1}H NMR (CD_3COCD_3): 3.18 (br s, 3H), 3.80 (s, 3H), 4.80 (br s, 2H), 6.87–6.89 (m, 1H), 6.91–6.93 (m, 2H), 7.31 (t, J = 7.9 Hz, 1H), 7.46 (s, 1H), 7.60 (d, J = 3.3 Hz, 1H), 7.84 (d, J = 8.8 Hz, 2H), 7.92 (d, J = 8.2 Hz, 2H); ^{13}C NMR (CD_3COCD_3): 55.5, 112.3, 113.6, 119.1, 126.3, 127.3, 130.7, 133.9, 138.7, 140.0, 145.7, 161.2; IR (cm^{-1}): 2941, 2223, 1604, 1583, 1486, 1397, 1253, 1041, 750, 697.

*5.1.2.29. 5-(4-Cyanophenyl)-N-(3-hydroxybenzyl)-N-methylthiophene-2-carboxamide (**6p**).* The title compound was prepared by reaction of 5-(4-cyanophenyl)-*N*-(3-methoxybenzyl)-*N*-methylthiophene-2-carboxamide **6o** (60 mg, 0.17 mmol) with borontrifluoride dimethyl sulfide complex (0.11 ml, 1.02 mmol) according to method C for 14 h. Purification by FC (CH_2Cl_2/CH_3OH 100:1 → 50:1) yielded the title compound as amorph solid (55 mg, yield: 95%). $C_{20}H_{16}N_2O_2S$; MW: 348; mp: 166–167 °C; MS (ESI): 349 $(M + H)^+$; ^{1}H NMR (CD_3COCD_3): 3.16 (br s, 3H), 4.76 (br s, 2H), 6.77–6.82 (m, 3H), 7.21 (t, J = 7.7 Hz, 1H), 7.46 (br s, 1H), 7.60 (d, J = 2.8 Hz, 1H), 7.83 (d, J = 8.5 Hz, 2H), 7.92 (d, J = 8.5 Hz, 2H), 8.37 (s, 1H); ^{13}C NMR (CD_3COCD_3): 112.3, 115.2, 115.3, 118.8, 119.1, 126.3, 127.3, 130.7, 133.9, 138.7, 139.9, 145.7, 158.8; IR (cm^{-1}): 3204, 2934, 2227, 1600, 1573, 1483, 751, 696.

*5.1.2.30. N-(3-hydroxybenzyl)-5-(4-methoxyphenyl)-N-methylthiophene-2-carboxamide (**6q**).* The title compound was prepared by reaction of 5-bromo-*N*-(3-hydroxybenzyl)-*N*-methylthiophene-2-carboxamide **15c** (49 mg, 0.15 mmol) and 4-methoxyphenylboronic acid (27 mg, 0.18 mmol) with tetrakis(-triphenylphosphine) palladium (17 mg, 0.015 mmol) as catalyst according to method B for 6 h. Purification by FC (*n*-hexane/ethyl acetate 6:1) afforded the desired compound as oil (48 mg, yield: 90%). $C_{20}H_{19}NO_3S$; MW: 353; MS (ESI): 354 $(M + H)^+$; ^{1}H NMR (CD_3COCD_3): 3.15 (br s, 3H), 3.84 (s, 3H), 4.75 (br s, 2H), 6.77 (dd, J = 8.0 Hz, 2.2 Hz, 1H), 6.80–6.83 (m, 2H), 7.00 (d, J = 8.8 Hz, 2H),

7.21 (t, $J = 8.0$ Hz, 1H), 7.27 (d, $J = 3.5$ Hz, 1H), 7.31 (br s, 1H), 7.63 (d, $J = 8.8$ Hz, 2H), 8.35 (s, 1H); ^{13}C NMR (CD_3COCD_3): 55.7, 115.2, 115.4, 122.9, 127.1, 128.1, 129.6, 130.6, 132.0, 133.4, 140.1, 143.9, 158.8; IR (cm^{-1}): 3150, 2939, 1599, 1482, 1244, 1178, 1034, 745, 699.

*5.1.2.31. N-(3-hydroxybenzyl)-5-(3-methoxyphenyl)-N-methylthiophene-2-carboxamide (**6r**).* The title compound was prepared by reaction of 5-bromo-*N*-(3-hydroxybenzyl)-*N*-methylthiophene-2-carboxamide **15c** (33 mg, 1 mmol) and 3-methoxyphenylboronic acid (18 mg, 0.12 mmol) with tetrakis(triphenylphosphine) palladium (12 mg, 0.01 mmol) as catalyst according to method B for 6 h. Purification by FC (*n*-hexane/ethyl acetate 6:1 →3:1) afforded the desired compound as oil (25 mg, yield: 71%). $C_{20}H_{19}NO_3S$; MW: 353; MS (ESI): 354 $(M + H)^+$; ^{1}H NMR (CD_3COCD_3): 3.16 (br s, 3H), 3.86 (s, 3H), 4.76 (br s, 2H), 6.77–6.82 (m, 3H), 6.94 (ddd, $J = 8.0$ Hz, 2.5 Hz, 0.9 Hz, 1H), 7.21 (t, $J = 8.0$ Hz, 1H), 7.23 (t, $J = 2.0$ Hz, 1H), 7.27 (d, $J = 8.0$ Hz, 1H), 7.35 (t, $J = 8.0$ Hz, 1H), 7.41 (br, s, 2H), 8.36 (s, 1H); ^{13}C NMR (CD_3COCD_3): 55.7, 112.1, 114.9, 115.3, 119.1, 130.6, 131.1, 135.7, 140.0, 148.1, 158.8, 161.2; IR (cm^{-1}): 3193, 2957, 1606, 1583, 1463, 1287, 1240, 732, 686.

*5.1.2.32. 5-(3-(Dimethylamino)phenyl)-N-(3-hydroxybenzyl)-N-methylthiophene-2-carboxamide (**6s**).* The title compound was prepared by reaction of 5-bromo-*N*-(3-hydroxybenzyl)-*N*-methylthiophene-2-carboxamide **15c** (49 mg, 0.15 mmol) and *N,N*-dimethyl-3-aminophenylboronic acid (30 mg, 0.18 mmol) with tetrakis(triphenylphosphine) palladium (17 mg, 0.015 mmol) as catalyst according to method B for 14 h. Purification by FC (*n*-hexane/ethyl acetate 5:1 →3:1) afforded the desired compound as oil (45 mg, yield: 82%). $C_{21}H_{22}N_2O_2S$; MW: 366; MS (ESI): 367 $(M + H)^+$; ^{1}H NMR (CD_3COCD_3): 3.00 (s, 6H), 3.15 (br s, 3H), 4.75 (br s, 2H), 6.75–6.82 (m, 4H), 6.98 (d, $J = 4.0$ Hz, 1H), 7.00 (s, 1H), 7.19–7.26 (m, 2H), 7.36 (s, 1H), 7.39 (br s, 1H), 8.36 (s, 1H); ^{13}C NMR (CD_3COCD_3): 40.5, 110.5, 113.5, 114.9, 115.2, 123.7, 130.5, 135.0, 138.1, 140.1, 149.6, 152.1, 157.8, 158.8; IR (cm^{-1}): 3649, 2977, 2808, 1594, 1571, 1484, 1440, 1232, 742, 687.

*5.1.2.33. N-(3-methoxybenzyl)-N-methyl-5-m-tolylthiophene-2-carboxamide (**6ta**).* The title compound was prepared by reaction of 5-bromo-*N*-(3-methoxybenzyl)-*N*-methylthiophene-2-carboxamide **15a** (53 mg, 0.15 mol) and 3-methylphenylboronic acid (24 mg, 0.18 mmol) with tetrakis(triphenylphosphine) palladium (17 mg, 0.015 mmol) as catalyst according to method B for 6 h. Purification by FC (*n*-hexane/ethyl acetate 6:1) afforded the desired compound as colorless oil (50 mg, yield: 95%). $C_{21}H_{21}NO_2S$; MW: 351; MS (ESI): 352 $(M + H)^+$; ^{1}H NMR (CD_3COCD_3): 2.38 (s, 3H), 3.17 (br s, 3H), 3.80 (s, 3H), 4.80 (br s, 2H), 6.86–6.89 (m, 1H), 6.91–6.93 (m, 2H), 7.19 (d, $J = 7.6$ Hz, 1H), 7.29–7.33 (m, 2H), 7.38–7.42 (m, 2H), 7.49 (d, $J = 7.6$ Hz, 1H), 7.53 (s, 1H); ^{13}C NMR (CD_3COCD_3): 21.3, 55.5, 113.5, 123.8, 123.9, 127.3, 129.9, 130.0, 130.6, 134.4, 138.5, 139.7, 140.1, 148.5, 161.1; IR (cm^{-1}): 3010, 2917, 1592, 1491, 1280, 1262, 1159, 774, 751, 689.

*5.1.2.34. N-(3-hydroxybenzyl)-N-methyl-5-m-tolylthiophene-2-carboxamide (**6t**).* The title compound was prepared by reaction of *N*-(3-methoxybenzyl)-*N*-methyl-5-*m*-tolylthiophene-2-carboxamide **6ta** (35 mg, 0.1 mmol) with borontrifluoride dimethyl sulfide complex (0.06 ml, 0.6 mmol) according to method C for 4 h. Purification by FC (CH_2Cl_2/CH_3OH 100:1) yielded the title compound as amorph solid (30 mg, yield: 89%). $C_{20}H_{19}NO_2S$; MW: 337; mp: 156–157 °C; MS (ESI): 338 $(M + H)^+$; ^{1}H NMR (CD_3COCD_3): 2.37 (s, 3H), 3.15 (br s, 3H), 4.75 (br s, 2H), 6.78 (ddd, $J = 8.2$ Hz, 2.5 Hz, 0.9 Hz, 1H), 6.80–6.83 (m, 2H), 7.17–7.22 (m, 2H), 7.32 (t, $J = 7.8$ Hz, 1H), 7.37–7.43 (m, 2H), 7.49 (d, $J = 7.8$ Hz, 1H), 7.53 (s, 1H), 8.46 (s, 1H); ^{13}C NMR (CD_3COCD_3): 21.3, 115.2, 123.8, 123.9, 127.3, 129.9, 130.0, 130.7, 134.4, 138.5, 139.7, 140.0, 148.5, 158.8; IR (cm^{-1}): 3225, 2932, 1588, 1566, 1489, 740, 688.

*5.1.2.35. 5-(3-Fluorophenyl)-N-(3-hydroxybenzyl)-N-methylthiophene-2-carboxamide (**6u**).* The title compound was prepared by reaction of 5-bromo-*N*-(3-hydroxybenzyl)-*N*-methylthiophene-2-carboxamide **15c** (49 mg, 0.15 mmol) and 3-fluorophenylboronic acid (25 mg, 0.18 mmol) with tetrakis(triphenylphosphine) palladium (17 mg, 0.015 mmol) as catalyst according to method B for 14 h. Purification by FC (*n*-hexane/ethyl acetate 6:1 →3:1) afforded the desired compound as oil (40 mg, yield: 78%). $C_{19}H_{16}FNO_2S$; MW: 341; MS (ESI): 342 $(M + H)^+$; ^{1}H NMR (CD_3COCD_3): 3.16 (br s, 3H), 4.76 (br s, 2H), 6.77–6.82 (m, 3H), 7.11–7.15 (m, 1H), 7.21 (t, $J = 7.7$ Hz, 1H), 7.42 (br s, 1H), 7.46–7.51 (m, 3H), 7.54 (d, $J = 8.2$ Hz, 1H), 8.35 (s, 1H); ^{13}C NMR (CD_3COCD_3): 113.2, 115.3, 115.7, 115.9, 122.7, 125.1, 130.7, 131.9, 132.0, 136.8, 139.2, 140.1, 144.4, 146.5, 148.3, 150.9, 158.8, 160.9, 163.1, 165.1; IR (cm^{-1}): 3660, 2976, 1610, 1576, 1480, 1407, 1276, 1243, 1154, 786, 697.

*5.1.2.36. 5-(4-Fluorophenyl)-N-(3-hydroxybenzyl)-N-methylthiophene-2-carboxamide (**6v**).* The title compound was prepared by reaction of 5-bromo-*N*-(3-hydroxybenzyl)-*N*-methylthiophene-2-carboxamide **15c** (49 mg, 0.15 mmol) and 4-fluorophenylboronic acid (25 mg, 0.18 mmol) with tetrakis(triphenylphosphine) palladium (17 mg, 0.015 mmol) as catalyst according to method B for 14 h. Purification by FC (*n*-hexane/ethyl acetate 5:1 →3:1) afforded the desired compound as oil (43 mg, yield: 84%). $C_{19}H_{16}FNO_2S$; MW: 341; MS (ESI): 342 $(M + H)^+$; ^{1}H NMR (CD_3COCD_3): 3.16 (br s, 3H), 4.75 (br s, 2H), 6.77–6.82 (m, 3H), 7.19–7.24 (m, 3H), 7.37 (d, $J = 3.7$ Hz, 1H), 7.40 (br s, 1H), 7.73–7.76 (m, 2H), 8.35 (br s, 1H); ^{13}C NMR (CD_3COCD_3): 115.3, 116.8, 116.9, 124.3, 127.8, 128.7, 128.8, 130.6, 131.0, 138.9, 140.0, 147.1, 158.8, 162.7, 164.2, 164.6; IR (cm^{-1}): 3168, 2956, 1598, 1561, 1479, 1228, 735, 699.

*5.1.2.37. 5-(2-Fluoro-3-methoxyphenyl)-N-(3-hydroxybenzyl)-N-methylthiophene-2-carboxamide (**6w**).* The title compound was prepared by reaction of 5-bromo-*N*-(3-hydroxybenzyl)-*N*-methylthiophene-2-carboxamide **15c** (49 mg, 0.15 mmol) and 2-fluoro-3-methoxyphenylboronic acid (31 mg, 0.18 mmol) with tetrakis(triphenylphosphine) palladium (17 mg, 0.015 mmol) as catalyst according to method B for 14 h. Purification by FC (*n*-hexane/ethyl acetate 5:1 →3:1) afforded the desired compound as amorph solid (40 mg, yield: 72%). $C_{20}H_{18}FNO_3S$; MW: 371; mp: 169–170 °C; MS (ESI): 372 $(M + H)^+$; ^{1}H NMR (CD_3COCD_3): 3.16 (br s, 3H), 3.93 (s, 3H), 4.76 (br s, 2H), 6.77–6.83 (m, 3H), 7.13–7.23 (m, 3H), 7.31 (td, $J = 7.8$ Hz, 1.6 Hz, 1H), 7.45 (br s, 1H), 7.48 (br s, 1H), 8.35 (s, 1H); ^{13}C NMR (CD_3COCD_3): 56.7, 114.0, 114.1, 115.3, 120.5, 120.6, 122.7, 122.8, 125.6, 127.2, 130.7, 139.9, 140.7, 148.9, 149.6, 150.9, 158.8; IR (cm^{-1}): 2977, 1606, 1584, 1471, 1265.

*5.1.2.38. 5-(2-Fluoro-3-hydroxyphenyl)-N-(3-hydroxybenzyl)-N-methylthiophene-2-carboxamide (**6x**).* The title compound was prepared by reaction of 5-(2-fluoro-3-methoxyphenyl)-*N*-(3-hydroxybenzyl)-*N*-methylthiophene-2-carboxamide **6w** (30 mg, 0.08 mmol) with borontrifluoride dimethyl sulfide complex (0.05 ml, 0.48 mmol) according to method C for 14 h. Purification by FC (CH_2Cl_2/CH_3OH 100:1) yielded the title compound as amorph solid (25 mg, yield: 87%). $C_{19}H_{16}FNO_3S$; MW: 357; mp: 167–168 °C; MS (ESI): 358 $(M + H)^+$; ^{1}H NMR (CD_3COCD_3): 3.16 (br s, 3H), 4.76 (br s, 2H), 6.77–6.99 (m, 3H), 7.01 (td, $J = 8.0$ Hz, 1.6 Hz, 1H), 7.08 (td, $J = 8.0$ Hz, 0.9 Hz, 1H), 7.21 (t, $J = 8.0$ Hz, 2H), 7.44 (br s, 1H), 7.46 (s, 1H), 8.36 (s, 1H), 8.86 (d, $J = 1.6$ Hz, 1H); ^{13}C NMR (CD_3COCD_3): 115.3, 118.4, 119.7, 122.9, 125.6, 126.6, 127.0, 127.1, 130.6, 139.6, 140.0, 141.1, 146.7, 148.3, 150.3, 158.8; IR (cm^{-1}): 3113, 2924, 1599, 1571, 1493, 751, 694.

5.2. Biological methods

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

5.3. 17β-HSD1 and 17β-HSD2 enzyme preparation from human placental enzyme

17β-HSD1 and 17β-HSD2 were obtained from human placenta according to previously described procedures [62,63]. Fresh human placenta was homogenized and the enzymes were separated by fractional centrifugation at 1000 g, 10,000 g and 150,000 g. For the purification of 17β-HSD1, the cytosolic fraction was precipitated with ammonium sulfate. 17β-HSD2 was obtained from the microsomal fraction. Aliquots containing 17β-HSD1 or 17β-HSD2 were stored frozen.

5.4. Inhibition of 17β-HSD2 in cell-free assay

Inhibitory activities were evaluated by an established method with minor modifications [20,64,65]. 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 unlabelled- and [^{3}H]-E2 (final concentration: 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) connected to a HPLC-system (Agilent 1100 Series, Agilent Technologies, Waldbronn). Detection and quantification of the steroids were performed using a radioflow detector (Berthold Technologies, Bad Wildbad). The conversion rate was calculated according to the following equation: $\%conversion = (\%E1/(\%E1+\%E2))\cdot 100$. Each value was calculated from at least three independent experiments.

5.5. Inhibition of 17β-HSD1 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 unlabelled- and [^{3}H]-E1 (final concentration: 500 nM, 0.15 μCi) for 10 min at 37 °C. Further treatment of the samples and HPLC separation was carried out as mentioned above for 17β-HSD2.

5.6. 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. [66] using recombinant human proteins. Briefly, 0.25 pmol of ERα or ERβ, respectively, was incubated with [^{3}H]-E2 (10 nM) and test compound for 1 h at room temperature. The potential inhibitor was dissolved in DMSO (5% final concentration). Nonspecific binding was performed with diethylstilbestrol (10 μM). After incubation, ligand–receptor complexes were selectively bound to hydroxyapatite (5 g/60 ml TE buffer). The formed complex was separated, washed, and resuspended in ethanol. For radiodetection, scintillator cocktail (Quickszint 212, Zinsser Analytic, Frankfurt) was added and samples were measured in a liquid scintillation counter (Rack Beta Primo 1209, Wallac, Turku). For determination of the relative binding affinity (RBA), inhibitor and E2 concentrations required to displace 50% of the receptor bound labelled E2 were determined. $RBA[\%] = IC_{50}(E2)/IC_{50}(compound) \times 100$. The RBA value for E2 was arbitrarily set at 100%.

Acknowledgments

We thank Josef Zapp for the NMR measurement, Ms. Martina Jankowski for the LC-Mass spectra as well as Jannine Ludwig and Jörg Haupenthal for the biological tests. Kuiying Xu and Yaseen Al-Soud are grateful to the Alexander von Humboldt Foundation (AvH) for a fellowship. We acknowledge the Deutsche Forschungsgemeinschaft (DFG) for financial support (HA1315/12-1).

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