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Original article

Novel, potent and selective 17β-hydroxysteroid dehydrogenase type 2 inhibitors as potential therapeutics for osteoporosis with dual human and mouse activities

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ABSTRACT

17β-Hydroxysteroid dehydrogenase type 2 (17β-HSD2) is responsible for the oxidation of the highly active estradiol (E2) and testosterone (T) into the less potent estrone (E1) and Δ^4-androstene-3,17-dione (Δ^4-AD), respectively. As 17β-HSD2 is present in bones and as estradiol and testosterone are able to induce bone formation and repress bone resorption, inhibition of this enzyme could be a new promising approach for the treatment of osteoporosis. Herein, we describe the design, the synthesis and the biological evaluation of 24 new 17β-HSD2 inhibitors in the 5-substituted thiophene-2-carboxamide class. Structure–activity and structure–selectivity relationships have been explored by variation of the sulfur atom position in the central core, exchange of the thiophene by a thiazole, substitution of the amide group with a larger moiety, exchange of the *N*-methylamide group with bioisosteres like *N*-methylsulfonamide, *N*-methylthioamide and ketone, and substitutions at positions 2 and 3 of the thiophene core with alkyl and phenyl groups leading to 2,3,5-trisubstituted thiophene derivatives. The compounds were evaluated on human and mouse enzymes. From this study, a novel highly potent and selective compound in both human and mouse 17β-HSD2 enzymes was identified, compound **21** (IC_{50}(h17β-HSD2) = 235 nM, selectivity factor toward h17β-HSD1 = 95, IC_{50} (m17β-HSD2) = 54 nM). This new compound **21** could be used for an *in vivo* proof of principle to demonstrate the true therapeutic efficacy of 17β-HSD2 inhibitors in osteoporosis. New structural insights into the active sites of the human and mouse enzymes were gained.

1. Introduction

A healthy skeleton is maintained through life by the constant process of bone remodeling, which is regulated by the balanced activities of bone-resorbing osteoclasts (OC) and bone-forming osteoblasts (OB).

Osteoporosis is a silent bone disease often appearing after the age of 50 in both men and women. It is characterized by a change in bone microarchitecture, leading to a reduction in bone mass density and an increase in the risk of bone fracture [1]. Osteoporosis is caused by the uncoupling of bone resorption from bone formation in a sense that the OC activities by far outweigh those of the OB [2].

There are many factors responsible for the development of osteoporosis such as: vitamin D deficiency, low calcium intake, smoking, alcohol absorption, but also premature ovarian failure, adrenal insufficiency, etc. Furthermore, a decrease in the active sex steroids levels (estradiol, e.g. in women during menopause, or testosterone in aging men) [3,4] is also considered as risk factor. Among the different medications available, two first-line classes of

List of abbreviations: OB, osteoblast; OC, osteoclast; 17β-HSD2, 17β-hydroxysteroid dehydrogenase type 2; 17β-HSD1, 17β-hydroxysteroid dehydrogenase type 1; E1, estrone; E2, estradiol; ER, estrogen receptor; SAR, structure–activity relationship; SSR, structure–selectivity relationship; RBA, relative binding affinity; IR, infrared; DMF, dimethylformamide; DME, dimethoxyethane; THF, tetrahydrofurane; SF, selectivity factor.

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drugs are administered to osteoporotic patients: 1. bisphosphonates (e.g. alendronate), which show good effects on bones in both postmenopausal women [5] and men [6,7] but only lead to a 50% reduction of the fracture risk and are often associated with jaw osteonecrosis; 2. Selective estrogen receptor modulators (SERMs), such as raloxifene [8], that are associated with an increased risk of venous thromboembolism [9], hot flushes [10] and leg cramps [10].

Osteoporosis is an aged-related disease and as the population is getting older, this disease is considered a serious public health problem. Therefore, there is a need to develop new therapeutic strategies to combat this disease.

17β-Hydroxysteroid dehydrogenase type 2 (17β-HSD2, (EC1.1.1.51) [11,12] catalyzes the oxidation of the highly active sex steroids estradiol (E2), as well as testosterone (T), into their less active forms estrone (E1) and Δ^4-androstene-3,17-dione (Δ^4-AD), respectively, using NAD^+ as cofactor. 17β-HSD1 catalyzes the reverse reaction and is regarded as a target for the treatment of breast cancer and endometriosis [13]. 17β-HSD2 and 17β-HSD1 are considered molecular switches, regulating the levels of active E2 and T in the cells. Further activation of T to the most potent androgen DHT is catalyzed by 5α-reductase, inhibitors of which are used for treating benign prostatic hyperplasia [14–16].

17β-HSD2 is expressed in a number of tissues such as placenta, liver, small intestine, endometrium, urinary tract and to a lesser extent in kidney, pancreas, colon, uterus, breast and prostate. This enzyme is also present in osteoblastic cells (OB) [17–20].

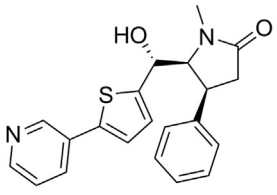
Estrogens are known to play a positive key role in bone physiology, decreasing bone resorption. Their mode of action on bone density is not well understood so far. However, as 17β-HSD2 is expressed in OB, inhibition of this enzyme will lead to an intracellular increase of E2 and T levels in bones, which should be beneficial for osteoporosis patients.

Besides ours [21–29] there are only few non-steroidal inhibitors of the human 17β-HSD2 (h17β-HSD2) described in literature [30,31]. We recently reported [22,28] about substituted 2,5-thiophene carboxamide derivatives (compounds **2a**–**c** and **3a**–**c**, Fig. 1) as new h17β-HSD2 inhibitors. We also assessed the h17β-HSD2 inhibitory activity of bishydroxyphenyl-2,5-thiophene derivatives **4a**–**c** [32]. Bagi and his colleagues developed the *cis*-pyrrolidinone class [4]. They validated the concept of improving bone quality by inhibiting 17β-HSD2 using a monkey osteoporosis model [4]. They could demonstrate that treatment with compound **1** (Fig. 1) resulted in a slight decrease in bone resorption and an increase in bone formation [33-35].

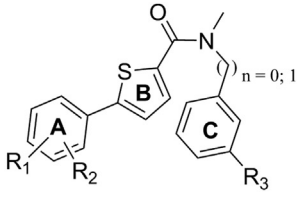
We were interested in gaining deeper insight into the structure–activity and structure–selectivity relationships (SAR and SSR, respectively) of the 2,5-thiophene carboxamide class, which looked very promising, to establish a basis for the rational design of even more potent and selective inhibitors of the human 17β-HSD2. The newly designed compounds should not inhibit 17β-HSD1, the enzyme catalyzing the conversion of E1 into E2, as this would be contra-productive for the increase of estradiol level in bones. Furthermore the new 17β-HSD2 inhibitors should not present affinity to the estrogen receptors alpha and beta (ERα and ERβ) to keep the receptors free for E2 binding. In addition we also wanted to develop 17β-HSD2 inhibitors able to block both the human and rodent enzymes in order to evaluate, later on, the efficacy of the compounds *in vivo* in an appropriate animal model. As the mouse is very well characterized, small and easily available, it was decided to look for compounds able to inhibit both the mouse m17β-HSD2 and the human h17β-HSD2.

Our work is divided into two main parts: 1. Structural modifications in the thiophene carboxamide class to increase our knowledge on the SAR in these series of compounds regarding the human enzyme (h17β-HSD2); 2. identification of compounds with good inhibitory activity on the mouse 17β-HSD2 for species comparison.

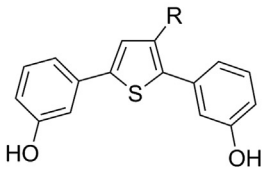
In this study, we synthesized new 17β-HSD2 thiophene carboxamide inhibitors derived from lead compounds **2a**–**c** and **3a**–**c,** which showed very good potency on the mouse enzyme, moderate activity on the human enzyme and a good selectivity toward h17β-HSD1. Starting from **2a**–**c** and **3a**–**c**, several structural modifications were performed such as: variations of the substitution patterns on the central thiophene core, replacement of the central thiophene core by a thiazole ring, change of the amide by a sulfonamide group (as successfully done in a previous study [27]), by a thioamide and a ketone group with a methylene linker, and exchange of the *N*-methyl group on the amide by a bulkier group. In order to investigate the electronic properties role on the activities, a short study using infrared spectroscopy was performed on selected compounds. The synthesized compounds, together with a selection of previously described inhibitors as references (**2a**–**c, 3a**–**c** and **4a**–**c**) were tested for their inhibitory activity on both the human and the mouse 17β-HSD2. The SAR obtained was discussed and a



1 IC_{50} = 50 nM



2a: n = 0, R_1 = 3-OMe, R_2 = H, R_3 = OMe, IC_{50} = 68 nM, SF = 112
2b: n = 0, R_1 = 3-OH, R_2 = H, R_3 = OH, 34% @ 1 μM
2c: n = 0, R_1 = 3-Me, R_2 = H, R_3 = OMe, IC_{50} = 58 nM, SF = 116
3a: n = 1, R_1 = 3-OMe, R_2 = H, R_3 = OMe, IC_{50} = 370 nM, SF >250
3b: n = 1, R_1 = 3-OH, R_2 = H, R_3 = OH, IC_{50} = 394 nM, SF = 143
3c: n = 1, R_1 = 2-F, R_2 = 3-OMe, R_3 = OH, IC_{50} = 61 nM, SF = 73



4a: R = H, IC_{50} = 745 nM, SF = 4
4b: R = Me, IC_{50} = 856 nM, SF = 2
4c: R = 3-OH-Ph, IC_{50} = 188 nM, SF = 2

Fig. 1. Described h17β-HSD2 inhibitors with inhibitory activities expressed as IC_{50} or percent inhibition values when tested at a concentration of 1 μM and selectivity factors (SF) toward h17β-HSD1.

compound with a high inhibitory potency toward m17β-HSD2 was identified.

2. Results

2.1. Chemistry

The synthesis of the thiophene derivatives **5a**–**8a** and **5**–**8** was achieved following a two- or three-step reaction pathway starting from the 5-bromo-thiophene-3-carboxylic acid **9a** or 4-bromo-thiophene-2-carboxylic acid **9b** as shown in Scheme 1. Carbonyl chloride derivatives were freshly prepared from the corresponding carboxylic acid with thionyl chloride. The amide coupling was carried out by reaction of the carbonyl chloride with 3-methoxy-*N*-methylaniline or 3-methoxy-*N*-methylbenzylamine under inert atmosphere and in dry conditions providing the corresponding bromothiophene derivatives **5b**–**8b** in very good yields. Suzuki–Miyaura cross-coupling reaction was performed with 3-methoxyphenylboronic acid and led to the compounds **5a**–**8a** in very good yields. Ether cleavage using boron trifluoride dimethyl sulfide complex $BF_3.SMe_2$ afforded the hydroxy derivatives **5**–**8**.

Thiazoles **10** and **11** were synthesized using the above procedure described for the synthesis of compounds **5**–**8**, starting from 2-bromo-1,3-thiazole-5-carboxylic acid **16a** as shown in Scheme 2. In the case of thiazole derivative **11**, ether cleavage on **11b**, affording the hydroxylated key intermediate **11a,** was performed before Suzuki–Miyaura cross-coupling reaction.

Thiophenes **12** and **13** were prepared as depicted in Scheme 2 following a similar pathway as described above and using the key intermediates **15a** and **15b,** which were synthesized as reported [36]. The amide formation between compounds **15a** and **15b** and freshly prepared 5-bromo-thiophene-2-carbonyl chloride led to the access of *N*-cyclopropyl derivatives **12a** and **13a**. The final compounds **12** and **13** were obtained after Suzuki–Miyaura cross-coupling reaction. For synthesis of compound **13**, ether cleavage on **13b**, leading to the key intermediate **13a,** was performed before the Suzuki reaction.

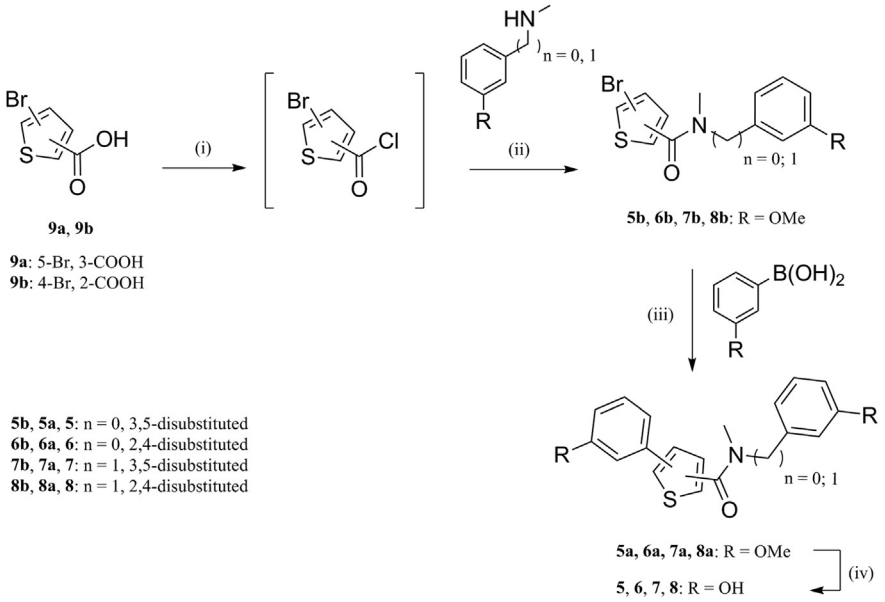
For the synthesis of compound **17** the key intermediate **17b** was synthesized starting from the 5-bromo-1,3-thiazole **18** (Scheme 3) applying a Suzuki–Miyaura cross-coupling reaction with *m*-tolyl boronic acid using microwave irradiation. The acidic hydrogen in position 2 of the thiazole **17b** was easily removed using *n*-butyl lithium as strong base, which reacted immediately with a dry flow of carbon dioxide leading to the corresponding carboxylate **17a**. The isolation of the protonated carboxylic acid derivative failed due to its chemical instability (rapid decarboxylation), while the lithium carboxylate salt could be isolated and stored at room temperature. Consequently, in the last step, the carboxylate **17a** itself was activated as acid chloride using oxalyl chloride at 0 °C and reacted with 3-methoxy-*N*-methylaniline to afford the desired compound **17**.

The synthesis of the 2,3,5-trisubstituted thiophenes **19a** and **19** was performed as shown in Scheme 4 starting from the commercially available thiophene-3-carboxylic acid **20**. First, the dibromination of the thiophene-3-carboxylic acid **20** was performed giving the 2,5-dibromothiophene-3-carboxylic acid **20b**. The acyl chloride, freshly prepared from the corresponding carboxylic acid, was condensed with the 3-methoxy-*N*-methylaniline derivative to give the amide **20a**. Double Suzuki–Miyaura cross-coupling reaction was performed with 3-methoxyphenylboronic acid affording compound **19a** in good yield. Ether cleavage using $BF_3.SMe_2$ resulted in the hydroxy derivative **19**.

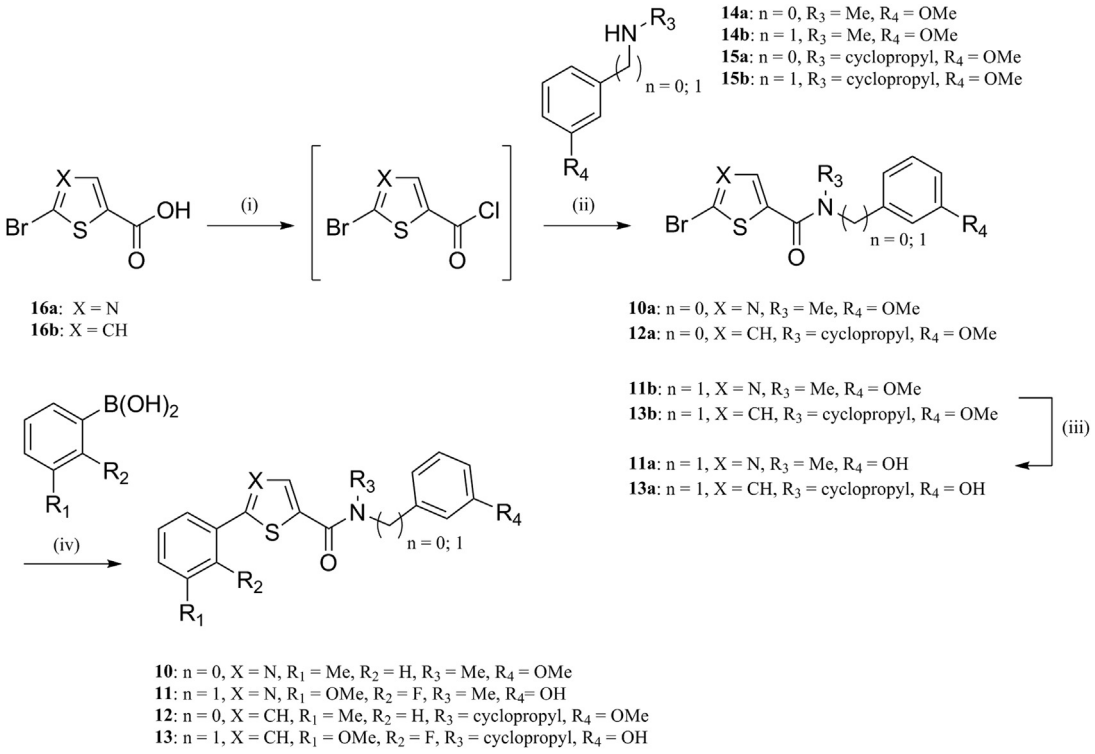
The 2,3,5-trisubstituted thiophene **21** was prepared as depicted on Scheme 5 starting from the 5-bromo-3-methylthiophene-2-carboxylic acid **22** which was obtained following a described procedure [37]. The freshly prepared acyl chloride was condensed with the 3-methoxy-*N*-methylbenzylamine to give compound **22b**. Ether cleavage was performed using $BF_3.SMe_2$ affording the hydroxy derivative **22a** followed by Suzuki–Miyaura cross-coupling reaction to afford the 2,3,5-trisubstituted thiophene **21**.

The synthesis of *N*-methylthioamide derivatives **23a** and **23** was carried out by the reaction of the *N*-methylamide derivative **23b** with Lawesson reagent under dry conditions. Ether cleavage using $BF_3.SMe_2$ afforded the hydroxylated compound **23** as depicted in Scheme 6.

The synthesis of the sulfonamide derivatives **24** and **25** was performed as shown in Scheme 7 starting from the commercially available 5-bromothiophene-2-sulfonyl chloride **28**. It was condensed with 3-methoxy-*N*-methylaniline or 3-methoxy-*N*-



Scheme 1. Synthesis of compounds **5a**–**8a** and **5**–**8**. Reagents and conditions: (i) $SOCl_2$, DMF cat., toluene, reflux 4 h; (ii) Et_3N, CH_2Cl_2, room temperature, overnight; (iii) DME/EtOH/H_2O (1:1:1), Cs_2CO_3, $Pd(PPh_3)_4$, microwave (150 °C, 150 W, 20 min); (iv) $BF_3{\cdot}S(Me)_2$, CH_2Cl_2, room temperature, 3–14 h.

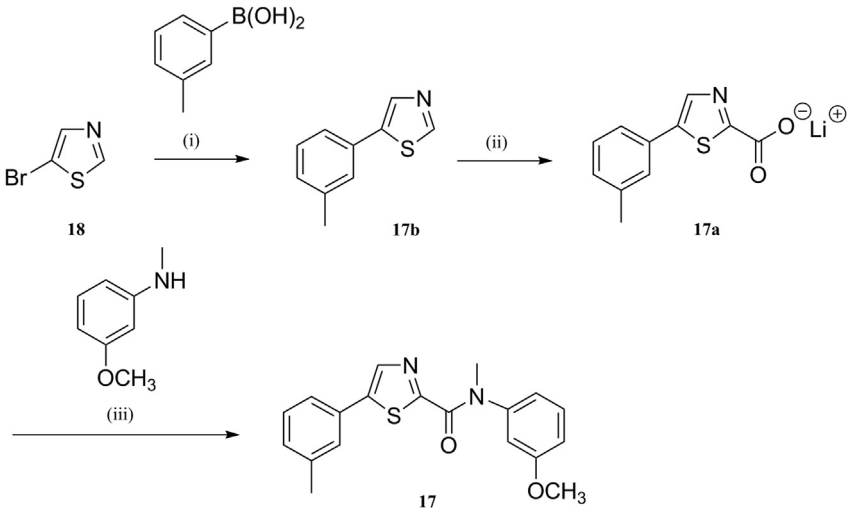


Scheme 2. Synthesis of compounds **10**–**13**. Reagents and conditions: (i) $SOCl_2$, DMF cat., toluene, reflux 4 h; (ii) Et_3N, CH_2Cl_2, room temperature, overnight; (iii) $BF_3{\cdot}S(Me)_2$, CH_2Cl_2, room temperature, 3–14 h; (iv) DME/EtOH/H_2O (1:1:1), Cs_2CO_3, $Pd(PPh_3)_4$, microwave (150 °C, 150 W, 20 min).

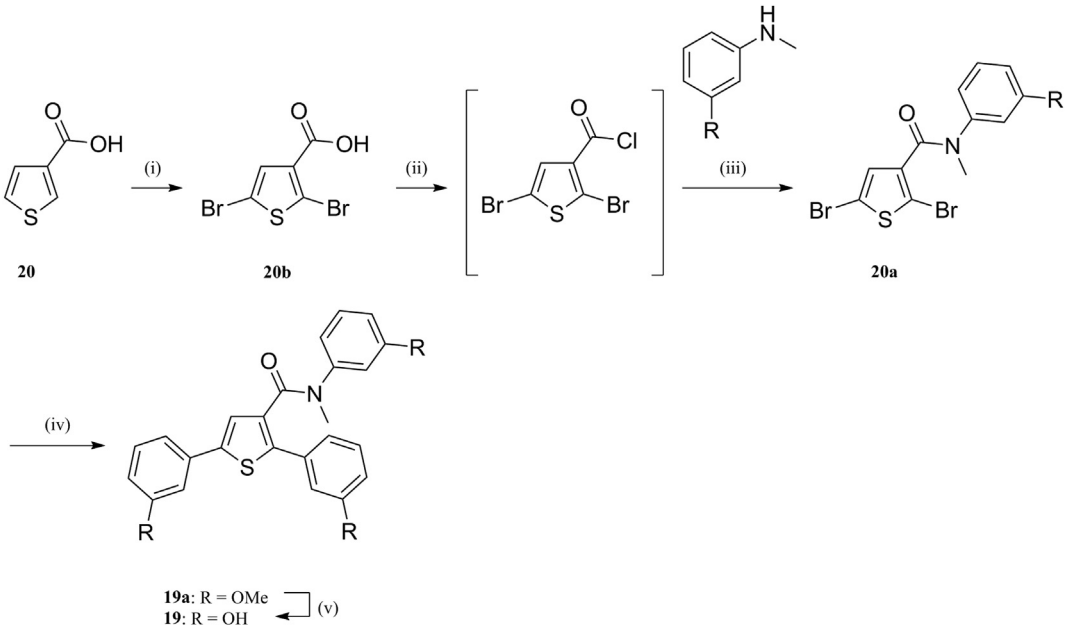
methylbenzylamine using tetrabutylammonium hydrogen sulfate as phase transfer reagent in a non-miscible solvents mixture affording the brominated intermediates **26** and **27b**. Suzuki–Miyaura cross-coupling reaction led to the final compound **24**. For compound **25**, ether cleavage of **27b** into **27a** was performed before the palladium-catalyzed reaction as described above.

The synthesis of the dimethylketone derivatives **29a** and **29** was performed as depicted in Scheme 8. Suzuki–Miyaura cross-coupling reaction led to the key intermediate **30a**. A Friedel–-Crafts acylation was performed between the key intermediate **30a,** the freshly prepared acyl chloride **31a** and aluminum chloride used as Lewis acid to afford the methoxylated derivative **29a**. Ether cleavage using $BF_3.SMe_2$ led to the hydroxylated compound **29** as depicted in Scheme 8. Intermediate **31b** was synthesized as described in literature [38] starting from (3-methoxyphenyl) acetonitrile **31**.

Compounds **32a** and **32** were synthesized as shown in Scheme 9. 3-Methoxyacetophenone **33** was reacted with Vilsmeier-Haack reagent to give the expected β-chloroacrolein **33b**. This key intermediate was used for the preparation of the 2-methylthiophenecarboxylate derivative **33a** as described in the literature [39]. Saponification of **33a** led to the carboxylic acid



Scheme 3. Synthesis of compound **17**. Reagents and conditions: (i) DME/EtOH/H_2O (1:1:1), Cs_2CO_3, $Pd(PPh_3)_4$, microwave (150 °C, 150 W, 20 min); (ii) a) dry THF, *n*-BuLi, −78 °C–0 °C, 4 h, b) dry CO_2 gas, −78 °C, 5 h, room temperature, overnight; (iii) a) oxalyl chloride, DMF cat., CH_2Cl_2, 3 h, b) Et_3N, CH_2Cl_2, room temperature, overnight.



Scheme 4. Synthesis of compounds **19a** and **19**. Reagents and conditions: (i) Br_2, AcOH, room temperature, 1 h; (ii) $SOCl_2$, DMF cat., toluene, reflux 4 h; (iii) Et_3N, CH_2Cl_2, room temperature, overnight; (iv) DME/EtOH/H_2O (1:1:1), Cs_2CO_3, 3-methoxyphenylboronic acid, $Pd(PPh_3)_4$, microwave (150 °C, 150 W, 20 min); (v) $BF_3{\cdot}S(Me)_2$, CH_2Cl_2, room temperature, overnight.

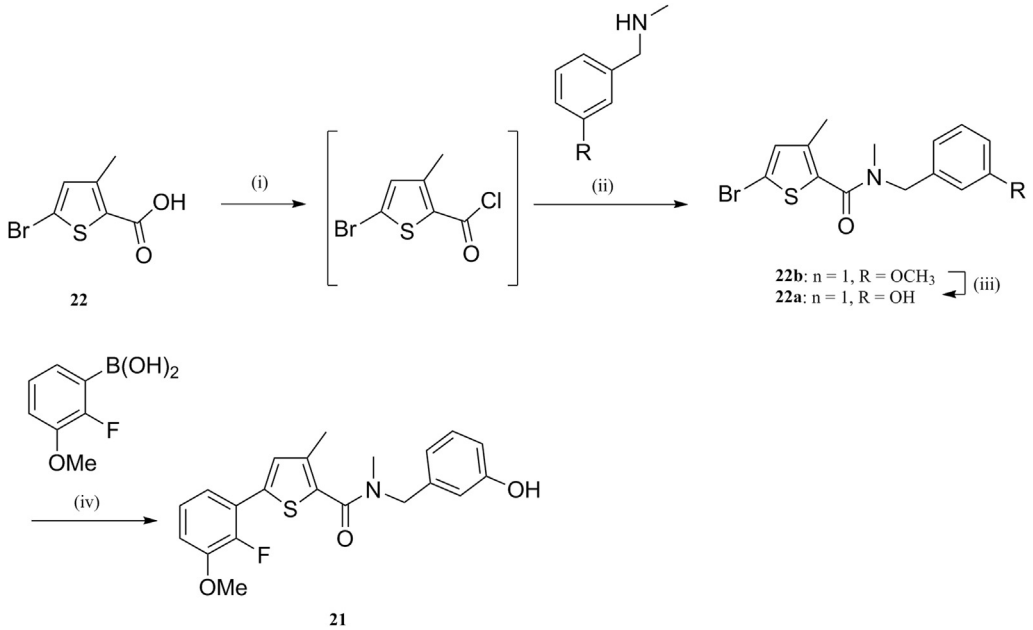
derivative **34**. Chlorination with thionyl chloride followed by the addition of *N*,*O*-dimethylhydroxylamine hydrochloride in presence of triethylamine afforded the intermediate **34a**. Substitution of the Weinreb amide by 3-methoxybenzylmagnesium chloride led to the methoxylated derivative **32a**. Of note, the direct Friedel–Crafts acylation of **30a** with 2-(3-methoxyphenyl)acetyl chloride) did not succeed in this case so the synthesis of derivative **32a** was performed via the Weinreb amide instead. Ether cleavage was performed using $BF_3.SMe_2$ affording the hydroxy derivative **32.**

2.2. Biology

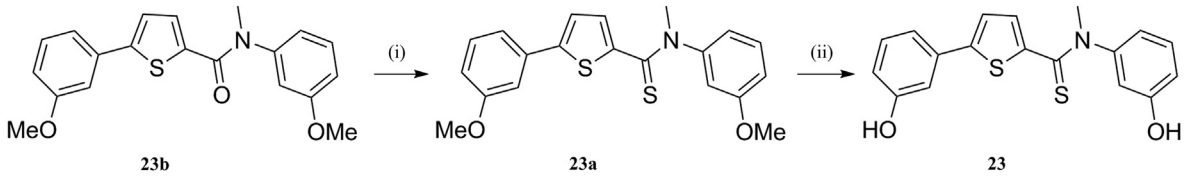
2.2.1. SAR study on h17β-HSD2

The synthesized compounds were tested for their inhibitory activity on h17β-HSD2. The inhibition values of the tested compounds are shown in Table 1.

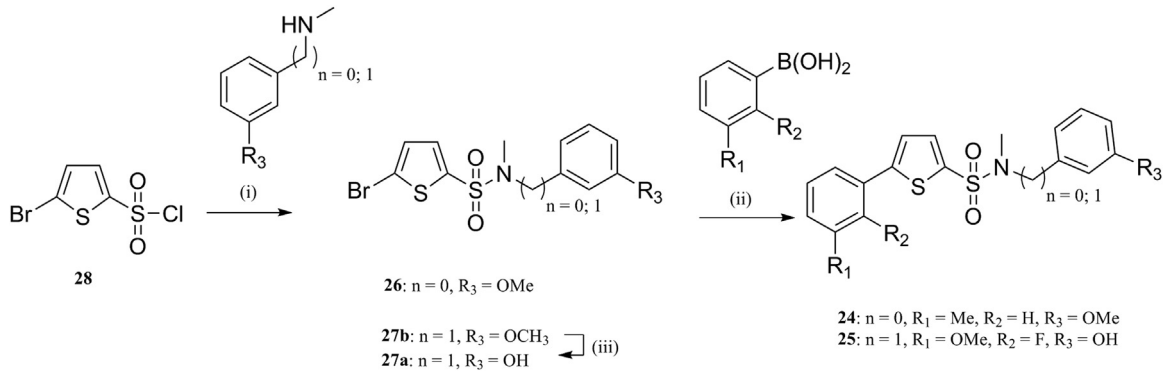
2.2.1.1. Influence of the substitution pattern of the thiophene core. In both the disubstituted 3,5-thiophene and 2,4-thiophene classes the methoxylated derivatives showed a decreased h17β-HSD2



Scheme 5. Synthesis of compound **21**. Reagents and conditions: (i) $SOCl_2$, DMF cat., toluene, reflux 4 h, (ii) Et_3N, CH_2Cl_2, room temperature, overnight; (iii) $BF_3{\cdot}S(Me)_2$, CH_2Cl_2, room temperature, overnight; (iv) DME/EtOH/H_2O (1:1:1), Cs_2CO_3, $Pd(PPh_3)_4$, microwave (150 °C, 150 W, 20 min).

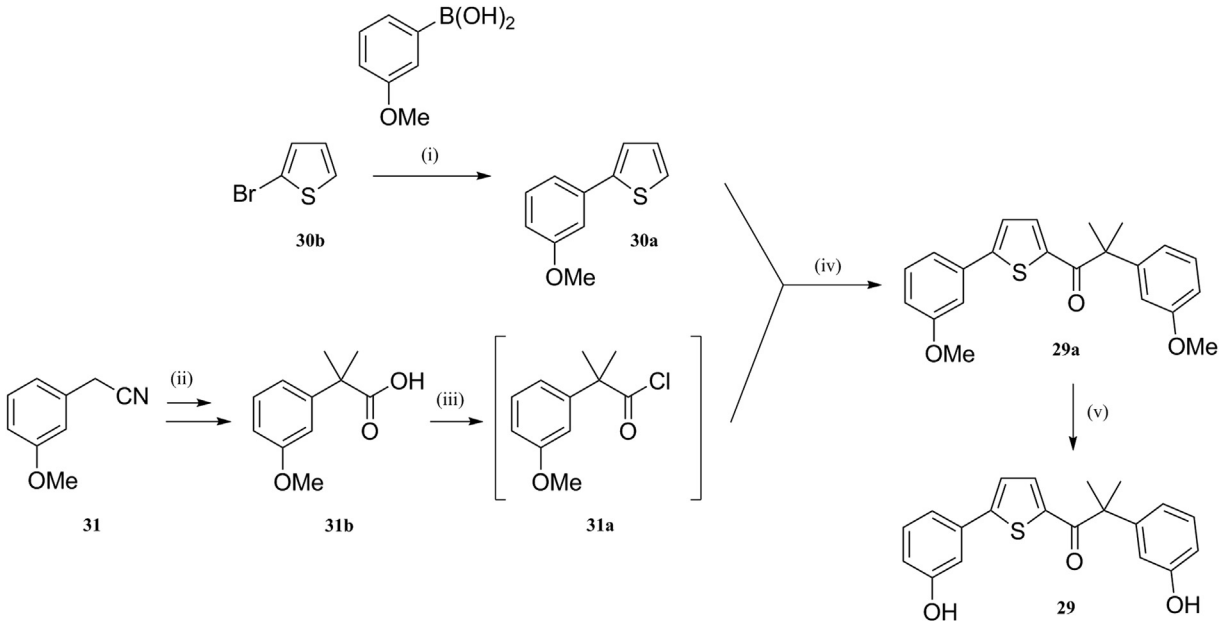


Scheme 6. Synthesis of compounds **23a** and **23**. Reagents and conditions: (i) Lawesson reagent, dry toluene, reflux, 4 h; (ii) $BF_3{\cdot}S(Me)_2$, CH_2Cl_2, room temperature, overnight.

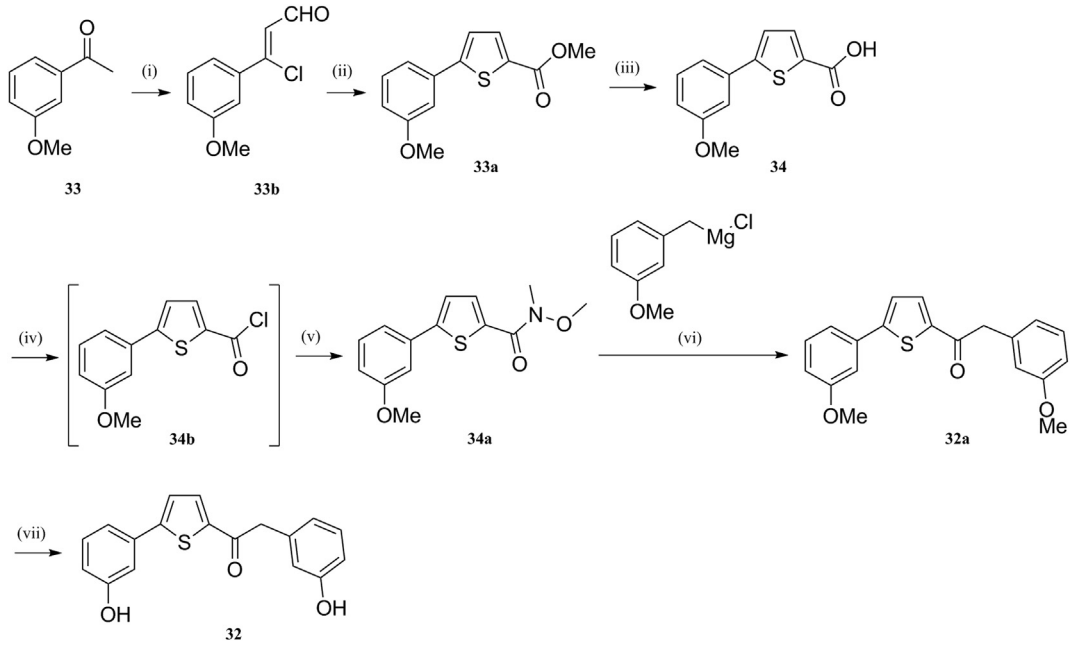


Scheme 7. Synthesis of compounds **24** and **25**. Reagents and conditions: (i) NBu_4HSO_4, NaOH 50%, CH_2Cl_2, room temperature, 5 h; (ii) DME/EtOH/H_2O (1:1:1), Cs_2CO_3, $Pd(PPh_3)_4$, microwave (150 °C, 150 W, 20 min); (iii) $BF_3{\cdot}S(Me)_2$, CH_2Cl_2, room temperature, 3–14 h.

inhibitory activity compared to the 2,5-thiophene. This was observed for compounds without linker between the amide and the phenyl C ring, **5a** and **7a** (621 nM and 487 nM, respectively to be compared to the 2,5-thiophene **2a**: 68 nM) or with linker, **6a** and **8a** (34% and 27% inhibition at 1 μM, respectively to be compared to the 2,5-thiophene **3a**: 63% inhibition). The hydroxylated disubstituted 3,5-thiophene and 2,4-thiophene without linker, **5** and **7**, showed a similar activity compared to the 2,5-thiophene **2b** (for $n = 0$, 44%, 39% and 34% inhibition at 1 μM for compounds **5**, **7** and **2b**, respectively) or a decrease in activity for compounds with linker, **6** and **8**, compared to the 2,5-thiophene **3b** (829 nM, 748 nM and 394 nM for compounds **6**, **8** and **3b**, respectively). As the 3,5- and 2,4-thiophene derivatives differ from the 2,5-thiophenes in the position of the S atom only, these results suggest that this S atom, depending on its place in the ring, might either be able to achieve specific interaction with the protein thereby modulating strongly the inhibitory potency or to modify the angles between the thiophene ring and the two substituents resulting in a slightly different position/orientation of the A and C ring, which impacts the interactions with amino acid side chains of the enzyme.



Scheme 8. Synthesis of compounds **29a** and **29**. Reagents and conditions: (i) DME/EtOH/H_2O (1:1:1), Cs_2CO_3, $Pd(PPh_3)_4$, microwave (150 °C, 150 W, 20 min); (ii) (a) MeI, NaH, dry THF, 0 °C to room temperature, overnight, (b) KOH, ethylene glycol, 150 °C, overnight; (iii) $SOCl_2$, DMF cat., toluene, reflux, 4 h; (iv) $AlCl_3$, dry CH_2Cl_2, room temperature, overnight; (v) $BF_3{\cdot}S(Me)_2$, CH_2Cl_2, room temperature, overnight.



Scheme 9. Synthesis of compounds **32a** and **32**. Reagents and conditions: (i) $POCl_3$/DMF, DMF, 0 °C to room temperature, overnight; (ii) methyl thioglycolate, dry K_2CO_3, dry DMF, 60 °C, overnight; (iii) KOH, MeOH, H_2O, reflux, 4 h; (iv) $SOCl_2$, DMF cat., toluene, reflux, 4 h; (v) *N,O*-dimethylhydroxylamine hydrochloride, Et_3N, CH_2Cl_2, 0 °C to room temperature, overnight; (vi) THF, 0 °C to room temperature, overnight; (vii) $BF_3{\cdot}S(Me)_2$, CH_2Cl_2, room temperature, overnight.

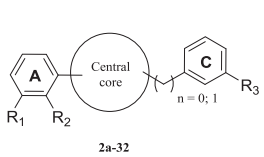
2.2.1.2. Influence of the central heterocyclic core. In order to evaluate the importance of the central core, we investigated the exchange of the 2,5-thiophene by a 2,5-thiazole ring. Keeping the S atom constant in its most favorable position between the 2 substituents, the position of the nitrogen atom on the thiazole core was varied resulting in two symmetrical compounds **10** and **17** with the aim to see if it can achieve an additional H-bond interaction in this area of the binding site. Three thiazole derivatives **10**, **11** and **17** were synthesized and all showed a loss in h17β-HSD2 inhibitory activity compared to their 2,5-thiophene analogues **2c** and **3c** (621 nM, 38% inhibition at 1 μM and 296 nM for the thiazoles **10**, **11** and **17** respectively, in comparison to 58 nM and 61 nM for **2c** and **3c**, respectively). Given the hypothesis that the H-bond interactions made by the OH and OMe groups of phenyl A and C ring are important for the stabilization of the compounds within its binding site, introduction of the nitrogen atom in the central ring might establish either unfavorable electronic and/or hydrophilic interactions.

2.2.1.3. Influence of the substituent on the amide moiety. We previously described [22] that 2,5-disubstituted thiophene carboxamide inhibitors must have an *N*-methylamide group to be active. The primary amide and the amide substituted by a phenyl moiety lose their activity completely. This might suggest that lipophilic interactions, in a limited space, might play a critical role stabilizing the inhibitor in the binding site. In this study a cyclopropyl group was introduced in order to evaluate the volume of this lipophilic cavity. Two *N*-cyclopropylamide analogues **12** and **13** were synthesized and turned out to be less active than the reference *N*-methylamides **2c** and **3c**. The *N*-cyclopropylamide without linker **12** was totally inactive compared to the *N*-methylamide analogue **2c** (IC_{50} = 58 nM) and the *N*-cyclopropylamide with a linker **13** showed a decreased activity compared to the *N*-methylamide analogue **3c** (**13**, IC_{50} = 599 nM vs **3c**, IC_{50} = 61 nM). These results point out two things: 1. The cyclopropyl group in **12** might be too bulky to fit into the lipophilic pocket. 2. Compound **12** without linker (inactive) and **13** with linker (moderately active) might have a different binding mode or might be slightly shifted as the introduction of the cyclopropyl group does not lead to the same decrease in activity.

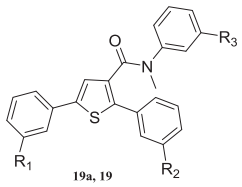
2.2.1.4. Influence of the exchange of the carboxamido group with sulfonamide, thioamide and ketone groups. We recently described [27] novel substituted biphenyl-*N*-methylsulfonamide derivatives as novel inhibitors of h17β-HSD2. Herein, we wanted to exchange the planar *N*-methylcarboxamide group by the tetrahedral *N*-methylsulfonamide moiety with the aim to investigate the enzyme binding site and increase the number of potential H-bond interactions between the inhibitor and the protein. However, exchange of the *N*-methylcarboxamide by the *N*-methylsulfonamide was detrimental for the 17β-HSD2 inhibitory activity in both series without linker (**24**, 21% vs **2c**, 95% inhibition at 1 μM) and with linker (**25**, 48% vs **3c**, 89% inhibition at 1 μM).

Exchange of the amide by a thioamide was then considered to evaluate whether the oxygen of the carbonyl moiety establishes any important interaction with the protein. As S is less electronegative than O, it will be a better lone-pair donor than the oxygen, which means that S in C═S will be a weaker H-bond acceptor compared to the O of the C═O amide, moreover the C═S group is more lipophilic than the C═O group. For the methoxylated compound without linker, replacement of the C═O by C═S induced a dramatic loss in activity (**23a**, 25% vs **2a**, 90% inhibition at 1 μM), while in case of the hydroxylated derivative a slight increase in activity was observed (**23**, 64% vs **2b**, 34% inhibition at 1 μM). Therefore, from these biological results it can be concluded that the oxygen atom of the C═O in the methoxylated 2,5-thiophene carboxamide derivative establishes a stronger H-bond interaction compared to the one of the hydroxylated compound. Taken together these results can be explained by a different binding mode for the methoxylated and the hydroxylated compounds. Moreover, the different sizes of the covalent radii of the S (104 pm) and the O (66 pm), the longer C═S bond compared to the C═O bond (1.6 Å for C═S vs 1.25 Å for C═O) and the higher lipophilicity of the C═S

Table 1
In vitro h17β-HSD2 and h17β-HSD1 inhibitory potencies of compounds **2a**–**32**.



2a-32



19a, 19

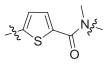
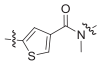
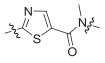
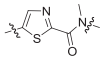
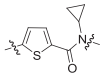
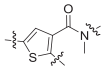
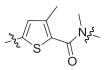
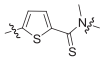
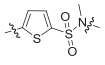
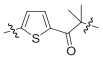
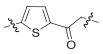
Entry	Central core	*n*	R_1	R_2	R_3	% Inhibition at 1 μM (IC_{50})		SF[d]
						h17β-HSD2[a,b]	h17β-HSD1[a,c]	
2a		0	3-OMe	–	3-OMe	90% (68 nM)	n.i. (7593 nM)	112
2b		0	3-OH	–	3-OH	34%	33%	–
2c		0	3-Me	–	3-OMe	95% (58 nM)	26% (6752 nM)	116
3a		1	3-OMe	–	3-OMe	63% (370 nM)	n.i. (>10000 nM)	>250
3b		1	3-OH	–	3-OH	70% (394 nM)	21% (5449 nM)	14
3c		1	3-OMe	2-F	3-OH	89% (61 nM)	n.i. (4452 nM)	73
5a		0	3-OMe	–	3-OMe	67% (621 nM)	43% (1641 nM)	3
5		0	3-OH	–	3-OH	44%	28%	–
6a		1	3-OMe	–	3-OMe	34%	25%	–
6		1	3-OH	–	3-OH	62% (829 nM)	50% (984 nM)	1
7a		0	3-OMe	–	3-OMe	68% (487 nM)	16% (3071 nM)	6
7		0	3-OH	–	3-OH	39%	28%	–
8a		1	3-OMe	–	3-OMe	27%	15%	–
8		1	3-OH	–	3-OH	70% (748 nM)	21% (3096 nM)	4
10		0	3-Me	–	3-OMe	54% (621 nM)	18% (4821 nM)	8
11		1	3-OMe	2-F	3-OH	38%	17%	–
17		0	3-Me	–	3-OMe	76% (296 nM)	12% (18663 nM)	63
12		0	3-Me	–	3-OMe	n.i.	21	–
13		1	3-OMe	2-F	3-OH	59% (599 nM)	n.i. (85343 nM)	142
19a		0	OMe	OMe	OMe	n.i.	n.i.	–
19		0	OH	OH	OH	74% (362 nM)	29% (2485 nM)	7
21		1	3-OMe	2-F	3-OH	77% (235 nM)	n.i. (22298 nM)	95
23a		0	3-OMe	–	3-OMe	25%	12%	–
23		0	3-OH	–	3-OH	64% (461 nM)	55% (432 nM)	1
24		0	3-Me	–	3-OMe	21%	n.i.	–

Table 1 (*continued*)

Entry	Central core	*n*	R_1	R_2	R_3	% Inhibition at 1 μM (IC_{50})		SF[d]
						h17β-HSD2[a,b]	h17β-HSD1[a,c]	
25		1	3-OMe	2-F	3-OH	48%	23%	–
29a		0	3-OMe	–	3-OMe	28%	21%	–
29		0	3-OH	–	3-OH	73% (357 nM)	54% (336 nM)	1
32a		0	3-OMe	–	3-OMe	17%	n.i.	–
32		0	3-OH	–	3-OH	37%	43%	–

n.i. no inhibition.
[a] Mean value of three determinations, standard deviation less than 20% except for **8** h17β-HSD2: 70% ± 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 [1500 μM].
[d] SF: selectivity factor, ratio IC_{50}(h17β-HSD1)/IC_{50}(h17β-HSD2).

group certainly will contribute to the binding affinities of the tested compounds.

Exchange of *N*-methylamide by a dimethylketo group and by a *N*-methylsulfonamide was undertaken to investigate whether the planarity of the amide is important for binding of the inhibitors to the enzyme. In the case of the methoxylated derivatives both the dimethylketone **29a** and the *N*-methylsulfonamides **24** and **25** resulted in a loss of inhibitory activity (**29a**, 28%, **24**, 21% vs **2a**, 90% inhibition at 1 μM, **25**, 48% vs **3c**, 89% inhibition at 1 μM), indicating that only a rather planar structure is well tolerated by the enzyme. The hydroxylated dimethylketone derivative **29** showed a higher activity (**29**, 73% vs **2b**, 34% inhibition at 1 μM), implying that planarity is not so critical for hydroxylated compounds. These results are also indicative of a different binding mode for the methoxylated and the hydroxylated compounds.

The loss of activity observed for the methoxylated methylene ketone derivative **32a** (**32a**, 17% vs **2a**, 90% inhibition at 1 μM) reveals the importance of the methyl group in alpha position of the carbonyl moiety (already highlighted during the investigation of the amide substituent).

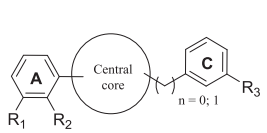
2.2.1.5. 2,3,5-Trisubstituted thiophene vs 2,5-disubstituted thiophene. Three 2,3,5-trisubstituted thiophene derivatives **19a**, **19** and **21** were synthesized in order to further investigate the space available in the binding site and also with the aim to enable additional interactions. In our previous work, we already described 2,3,5-trisubstituted inhibitors in the bis(hydroxyphenyl)thiophene class, compounds **4b** and **4c**, bearing an additional methyl or hydroxyphenyl group, respectively. They showed moderate to good inhibitory activity on h17β-HSD2 (856 nM and 188 nM respectively, Fig. 1) [32,40]. The introduction of a methyl group into position 3 of the thiophene ring did not improve the inhibitory activity toward h17β-HSD2 compared to the unsubstituted **4a** (**4b**, IC_{50} = 856 nM vs **4a**, IC_{50} = 745 nM) suggesting that the small methyl is not hindering nor is it gaining any specific interaction. With the bulky hydroxyphenyl group an increase in activity was observed compared to the unsubstituted derivative **4a** (**4c**, IC_{50} = 188 nM vs **4a**, IC_{50} = 745 nM). This compound might be stabilized in the binding site either via pi-stacking interactions (phenyl ring) or H-bond interactions (OH) or both. We wanted to translate this information into the thiophene carboxamide class, starting from the bishydroxyphenyl thiophene **4a** and adding the *N*-methylamide chain in order to target additional interactions. Interestingly, the inhibitory activity was completely lost for the methoxylated derivative **19a** compared to the unsubstituted 3,5-derivative **5a** while the hydroxylated derivative **19** showed a better activity compared to the unsubstituted 3,5-derivative **5** (**19**, 74% (IC_{50} = 362 nM) vs **5**, 44% inhibition at 1 μM). These results suggest that the 2-hydroxyphenyl group achieves additional interactions with the enzyme either via pi-stacking or H-bond interactions or both, which might partially compensate the unfavorable position of the S atom (see above). The differences in activities between the methoxy and the hydroxy compounds (**19a** and **19**) can be explained by assuming that the compounds bind either in a different or even in the same area in the enzyme, either way, the binding pocket seems to accommodate the OH functional groups of **19** better than the methoxy functional groups of **19a**.

Furthermore, introduction of the methyl group in position 3 (compound **21**) of the 2,5-thiophene **3c** slightly decreases its inhibitory activity on h17β-HSD2 (**21**, IC_{50} = 235 nM vs **3c**, IC_{50} = 61 nM). This suggests that insertion of a small substituent such as a methyl group at the position 3 of the central core might induce a partial steric clash with the enzyme. This result differs from the one observed after introducing a 3-methyl group into compound **4a** leading to **4b** without significant change in inhibitory activity. These observations highlight the different binding modes of compounds **4b** and **21**.

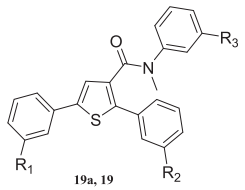
2.2.2. Selectivity: inhibition of h17β-HSD1 and affinities to the estrogen receptors α and β

As 17β-HSD1 is involved in sex steroid metabolism and more precisely in the reduction of E1 to E2 [41], inhibition of this enzyme would decrease the concentration of E2 formed in the target cell and would be detrimental to the effect of 17β-HSD2 inhibition. As a consequence 17β-HSD1 should not interact with the 17β-HSD2 inhibitors. Moreover, 17β-HSD2 inhibitors should not show binding affinity for the estrogen receptors α and β (ERα and ERβ), as it is expected that E2 effects in the treatment of osteoporosis are ER mediated. In addition, agonistic effects would induce undesired side-effects (induction of cell proliferation). Therefore, all compounds were tested on h17β-HSD1 and the most interesting inhibitors were selected and tested for their binding affinity to ERα and ERβ. The disubstituted 2,4- and 3,5-thiophenes, 2,5-thiophene thioamides and 2,5-thiophene dimethylketones were rather unselective exhibiting selectivity factors toward h17β-HSD1 between 1 and 6 (Table 1). However, in addition to the reference compounds

Table 2
In vitro inhibitory activities of compounds **2a**–**32** on m17β-HSD2/E1-formation.



2a-32



19a, 19

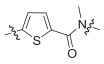
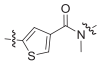
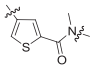
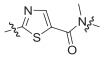
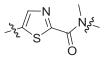
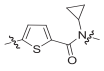
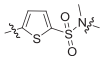
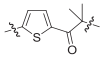
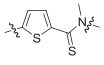
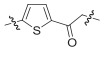
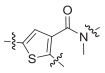
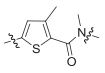
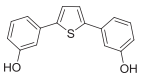
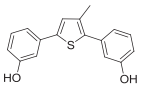
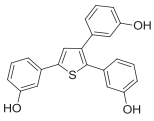
Entry	Central core	*n*	R_1	R_2	R_3	% Inh. at 1 μM (IC_{50}) m17β-HSD2[a,b]
2a		0	3-OMe	–	3-OMe	29%
2b		0	3-OH	–	3-OH	n.i.
2c		0	3-Me	–	3-OMe	30%
3a		1	3-OMe	–	3-OMe	26%
3b		1	3-OH	–	3-OH	16%
3c		1	3-OMe	2-F	3-OH	65%
5a		0	3-OMe	–	3-OMe	33%
5		0	3-OH	–	3-OH	20%
6a		1	3-OMe	–	3-OMe	17%
6		1	3-OH	–	3-OH	58%
7a		0	3-OMe	–	3-OMe	16%
7		0	3-OH	–	3-OH	12%
8a		1	3-OMe	–	3-OMe	17%
8		1	3-OH	–	3-OH	38%
10		0	3-Me	–	3-OMe	14%
11		1	3-OMe	2-F	3-OH	27%
17		0	3-Me	–	3-OMe	13%
12		0	3-Me	–	3-OMe	n.i.
13		1	3-OMe	2-F	3-OH	16%
24		0	3-Me	–	3-OMe	n.i.
25		1	3-OMe	2-F	3-OH	15%
29a		0	3-OMe	–	3-OMe	17%
29		0	3-OH	–	3-OH	42%
23a		0	3-OMe	–	3-OMe	n.i.
23		0	3-OH	–	3-OH	26%
32a		0	3-OMe	–	3-OMe	12%
32		0	3-OH	–	3-OH	n.i.

Table 2 (*continued*)

Entry	Central core	*n*	R_1	R_2	R_3	% Inh. at 1 μM (IC_{50}) m17β-HSD2[a,b]
19a		0	OMe	OMe	OMe	13%
19		0	OH	OH	OH	42%
21		1	3-OMe	2-F	3-OH	95% (54 nM)
4a		–	–	–	–	n.i.
4b		–	–	–	–	n.i.
4c		–	–	–	–	13%

n.i. no inhibition.
[a] Mean value of three determinations, standard deviation less than 25%.
[b] Mouse liver microsomal fraction, substrate E2 [500 nM], cofactor NAD^+ [1500 μM].

2a, **2c**, **3a** and **3c**, three new h17β-HSD2 inhibitors were highly selective: the thiazole derivative **17** (SF: 63), the 2,5-thiophene cyclopropylamide **13** (SF: 142) and the 2,3,5-trisubstituted thiophene **21** (SF: 95). The most potent inhibitors **7a**, **17**, **19**, **21** and **4c** were tested for estrogen receptor binding and showed low binding affinity to both ERα and ERβ (RBA < 0.1% compared to the affinity of E2, which was arbitrarily set to 100%).

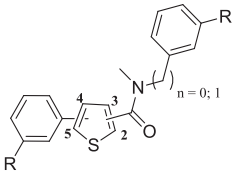
2.2.3. SAR study on mouse m17β-HSD2

In previous studies [23,28], we evaluated several compounds for their 17β-HSD2 inhibition of rat, mouse and monkey enzymes with the aim to identify an animal model suitable for *in vivo* studies. Based on the obtained results we hypothesized that the mouse might be the animal model of choice and we decided to extend our SAR knowledge on the mouse enzyme in order to design inhibitors appropriate for mouse *in vivo* experiments. The inhibitory activities of the newly synthesized compounds together with the ones of their reference analogues (**2a**–**3c**) on m17β-HSD2/E1-formation are reported in Table 2 (expressed as percent inhibition values tested at a concentration of 1 μM).

In the disubstituted 2,5-thiophene class with or without linker, the methoxylated compounds **2a** and **3a** showed a weak inhibition (29% and 26% at 1 μM, respectively), while the hydroxylated derivatives **2b** and **3b** were inactive. Exchange of the methoxy group on the A-ring by a methyl group leads to an equipotent compound (**2a** vs **2c** with percent inhibition values of 29% and 30% at 1 μM, respectively), suggesting that the O of the methoxy moiety is not involved in H-bond interaction. The addition of a fluorine atom next to the 3-OMe in the A-ring (compound **3c)** notably increases the activity on the mouse enzyme (**3c**, 65% of inhibition at 1 μM) as already observed with the human enzyme [28].

Switching the position of the S atom on the thiophene ring (for both hydroxy and methoxy derivatives) in compounds without linker, compounds **5a**, **5**, **7a** and **7**, or with linker, compounds **6a**, **8a**

Table 3
Infrared absorption bands of the carbonyl moieties and inhibitory activities of selected isomeric disubstituted thiophene derivatives.



2a–8

Entry	Substitution pattern on thiophene	*n*	R	ν (C═O) cm^{-1}	IC_{50} in nM or % inh. at 1 μM
					h17β-HSD2
2a	2,5	0	3-OMe	1589	68 nM
5a	3,5	0	3-OMe	1597	621 nM
7a	2,4	0	3-OMe	1597	487 nM
3a	2,5	1	3-OMe	1591	63%
6a	3,5	1	3-OMe	1599	34%
8a	2,4	1	3-OMe	1600	27%
2b	2,5	0	3-OH	1575	34%
5	3,5	0	3-OH	1584	44%
7	2,4	0	3-OH	1582	39%
3b	2,5	1	3-OH	1572	394 nM
6	3,5	1	3-OH	1580	829 nM
8	2,4	1	3-OH	1580	748 nM

and **8**, led to molecules with similarly weak mouse enzyme inhibitory potency as observed in the thiophene 2,5-disubstituted series (**2a** and **3b**): **7a** vs **2a** with 16% and 29% inhibition at 1 μM, respectively, **8** vs **3b** with 16% and 38% inhibition at 1 μM, respectively. Only for the hydroxylated compound with linker **6**, a moderate inhibitory activity was observed (58% inhibition at 1 μM). The introduction of additional nitrogen on the central heterocyclic core (compounds **10**, **11**, **17**) is detrimental for the inhibitory activity independently of the presence or absence of the linker as seen for h17β-HSD2 (without linker: **2c** vs **10** and **17**: 30%, 14% and 13% inhibition at 1 μM, respectively, and with linker: **3c** and **11**: 65% and 27% inhibition at 1 μM, respectively). The exchange of a methyl by a cyclopropyl group on the amide moiety diminishes the m17β-HSD2 inhibitory potency (without linker: **2a** vs **12**: 29% and no inhibition at 1 μM, respectively, and with linker: **3c** vs **13**; 65% and 16% inhibition at 1 μM, respectively).

The exchange of the *N*-methylamide (**2a** and **2c**) by a *N*-methylthioamide (**23a** and **23**) or a keto group (**29a**, **32a**, **29** and **32**) did not improve the potency, neither in the methoxy class nor in the hydroxy series (around 30% inhibition at 1 μM, except for the hydroxy derivative **29**, 42% inhibition). Similar observations could be made when the *N*-methylamide was exchanged by a *N*-methylsulfonamide (**2c** vs **24**: 30% inhibition and no inhibition at 1 μM, respectively, and **3c** vs **25**: 65% and 15% inhibition at 1 μM, respectively). Concerning the 2,3,5-trisubstituted derivatives, the compounds can be divided into two groups: the ones with a 2,5-biphenylthiophene core (**4b**–**4c**, **19a** and **19**) and the others with a 2,5-thiophene carboxamide moiety (**21**). In the group with the 2,5-biphenylthiophene core, all the compounds are inactive independently of the addition in position 3 of a methyl **4b**, a hydroxyphenyl **4c** or a methoxylated carboxamide **19a**. However, for the hydroxylated derivative **19**, a moderate activity was observed (42% inhibition at 1 μM), indicating that the hydroxy groups certainly participate in the stabilization of the enzyme–ligand complex. In the group with the 2,5-thiophene carboxamide core, introduction of a methyl group in position 3 of the thiophene core results in a highly active compound (**21**, $IC_{50} = 54$ nM, 95% inhibition at 1 μM), more active than the corresponding compound without methyl (**3c**, 65% inhibition at 1 μM). The comparison of these 2,3,5-trisubstituted thiophenes shows that the amide group is necessary for a good inhibitory activity, preferably at position 2 in combination with a methyl group at position 3.

From this study it is clear that compounds of the 2,5-disubstituted thiophene class are, in general, less potent on the mouse than on the human enzyme except for the compound with a linker and an additional fluoro substituent on the phenyl A-ring, **3c**, which shows the best inhibitory activity on mouse E1-formation, better than compounds of the thiophene class without linker and the thiazole derivatives. Furthermore, the insertion of a small alkyl group such as methyl in position 3 of the thiophene, **21**, increases the inhibitory activity on m17β-HSD2 suggesting that in position 3 of the central core lipophilic interaction is better tolerated in mouse than in human enzyme.

2.2.4. *Infrared spectroscopy analysis for selected compounds*

A close analysis of the infrared spectra measured for the methoxylated and hydroxylated 2,5-, 3,5- and 2,4-disubstituted thiophene derivatives bearing the same substituents on the A and C rings (compounds **2a**–**b**, **3a**–**b**, **5a**–**8**, Table 3), was undertaken focusing on the frequency of the carbonyl band, with the aim to understand the influence of the electronic properties of these compounds on their activity. A small difference (about 8 cm^{-1}) in the frequency of the C═O moiety was observed comparing compounds having different substitution pattern: 3,5- (**5a**, **6a**, **6**) and 2,4- (**7a**, **8a**, **8**) compared to the corresponding 2,5-disubstituted thiophene (**2a**, **3a**,**3b**, respectively). The IR spectra of the respective compounds reveal a correlation between the frequency of the carbonyl peak of some compounds with their inhibitory activities: the lower the frequency of the carbonyl peak the more active the

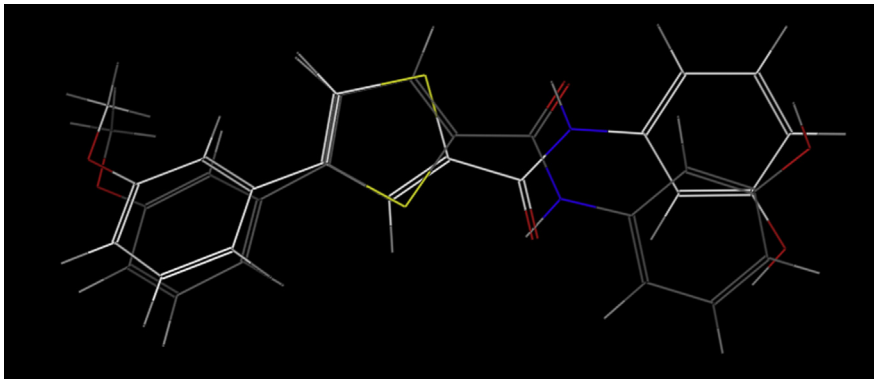


Fig. 2. Superimposition 2,4-thiophene **7a** (white) and 2,5-thiophene **2a** (gray) derivatives.

inhibitor (Table 3). As the frequency depends on the π-electron conjugation state, the IR spectra show that the conjugation state of the compounds influences their activities. In consequence, the different electronic properties, which correlate with different frequencies of the C=O stretch for these compounds, are determinant for the biological activity.

However, the difference in activity between the 2,5- and the 3,5- or 2,4-disubstituted thiophene is certainly mainly coming from their different 3D structures, which induce different positions of the phenyl C ring and therefore different interactions between the ligand functional groups and amino acids in the active site (as seen of the superimposition picture of the 2,4-thiophene **7a** with the 2,5-thiophene **2a**, Fig. 2).

In case of the hydroxylated **2b**, **5 and 7** no correlation between the IR C=O frequency and the biological activity can be found. This result might be explained by a different binding mode as already highlighted in the SAR study.

3. Discussion

The main aim of this study was the development of novel active and selective 17β-HSD2 inhibitors in the thiophene carboxamide class with a good potency on both the human and mouse enzymes and also a good selectivity in both species. Different modifications were made such as variation of the sulfur atom position on the thiophene ring, exchange of the thiophene by a thiazole, substitution of the amide group by a larger moiety (cyclopropyl), exchange of the *N*-methylamide group by bioisosteres such as *N*-methylthioamide, *N*-methylsulfonamide and methylene ketone, and exploration of the positions 2 and 3 of the thiophene ring with alkyl and phenyl substituents leading to the 2,3,5-trisubstituted thiophene derivatives.

In the optimization process, the 2,5-, 3,5- and 2,4-disubstituted thiophene carboxamide regioisomers were explored. From the biological results it became apparent that the h17β-HSD2 inhibitory activity was strongly influenced by the substitution pattern. The position of the S atom with respect to the two substituents has a strong impact on the biological activity due to the modification of the overall geometry conformation of the 2,5-, 3,5- and 2,4-thiophene derivatives. It also has an influence on the electronic distribution all over the molecule, which might modulate biological activity as already described [40]. The higher potency in the 2,5-thiophene class might also indicate that no intramolecular coulombic attraction between the S(thiophene)-O(carbonyl) occurs as already described in thiophene GABA receptor ligands [42] and other thiophenes acting on different targets [43]. All the changes undertaken to modify the central core have been ineffective for the improvement of the potency of the compounds on the human and mouse enzymes (change of the S position in the ring with respect to the two substituents and introduction of an N atom, exchanging the thiophene by a thiazole). However, the binding modes of the differently substituted thiophene rings deserve further investigation. The IR spectra revealed that electronic effects might also participate to the stabilization of the compounds in the enzyme binding site along with hydrogen bonding, pi-stacking and van der Waals interactions.

The structural modifications performed led to a loss in activity on the human enzyme, indicating a very sharp SAR in this class of compounds.

The SAR study deduced from the human enzyme in the 2,5-thiophene class, highlights contradictory biological results for the methoxy- and hydroxy substituted derivatives (by exchanging amide for thioamide or dimethylketone). These results indicate that the methoxylated compounds do not bind exactly in the same area in the binding site as the hydroxylated corresponding analogues. In addition, it is also obvious that the planarity around the amide group is very important for a good potency, at least for the methoxylated compounds (the exchange of amide for sulfonamide or dimethylketone as well as the replacement of the methylamide by a cyclopropylamide are detrimental for the activity), which is indicative of a rather planar surface binding area of the human enzyme. The space in the human enzyme active site seems larger than that of the mouse enzyme, as indicated by the inactivity or low activity of the tri-substituted bishydroxyphenylthiophenes on mouse versus moderate to good activity on human 17β-HSD2. However, the small lipophilic methyl group in position 3 of the central core of **21** fits better into the mouse enzyme binding site than into the human one. The presence of a small lipophilic pocket in the mouse enzyme binding site might explain this result.

In general the 2,5-disubstituted thiophene derivatives are less active on the mouse than on the human enzyme, except for compound **21**, which is four times more potent on m17β-HSD2 (IC_{50} = 54 nM) than on h17β-HSD2 (IC_{50} = 235 nM) indicating clearly different structures of both enzymes. None of the variations undertaken either at the central ring or at the amide function improved this low potency. The geometry of the compounds induced by the substitution pattern at the 5-membered ring might not be optimal for the stabilization of the compounds in the mouse enzyme. Compounds bearing a 6-membered ring as central core or sharing a bulkier lipophilic group on the central ring will be investigated in the future to verify this hypothesis. The SAR in the mouse enzyme did not allow to gain many information on the mouse enzyme active site. However, compounds with a hydroxy group on the C ring and a methylene linker exhibited slightly superior activity. From this study, it is also clear that the human binding site is larger than the one of the mouse.

4. Conclusion

In this study, we described the optimization of h17β-HSD2 and m17β-HSD2 inhibitors in the 5-substituted thiophene-2-carboxamide class by variation of the sulfur atom position in the central core, the exchange of the thiophene by a thiazole, the substitution of the amide group with a larger moiety (cyclopropyl), exchange of the *N*-methylamide group with bioisosteres such as *N*-methylsulfonamide, *N*-methylthioamide and methylene ketone and exploration of the positions 2 and 3 of the thiophene core with alkyl and phenyl moieties leading to the 2,3,5-trisubstituted thiophene derivatives. The biological activities obtained indicate a very sharp SAR in this class of compounds. The geometry of the compounds is also quite restricted allowing only little variation around the thiophene core, the 2,5-disubstituted thiophene derivatives being the best substitution pattern. Our SAR studies lead us to conclude that the active site of the human 17β-HSD2 is quite large, larger than the one of the mouse 17β-HSD2. Furthermore our investigations result in the discovery of the highly active compound **21**, a 2,3,5-trisubstituted thiophene containing a methyl group in position 3 of the thiophene. This compound shows a reasonable IC_{50} of 235 nM in the human enzyme with a good selectivity factor toward h17β-HSD1 (SF = 95), and a very good IC_{50} of 54 nM in mouse 17β-HSD2. Furthermore, as this compound shows very low binding to the ERα and ERβ, it could be used for an *in vivo* proof of principle to demonstrate efficacy of 17β-HSD2 inhibitors in osteoporosis.

5. Experimental section

5.1. Chemical methods

Chemical names follow IUPAC nomenclature, starting materials were purchased from Aldrich, Acros, Combi-Blocks or Fluka and were used without purification.

Column chromatography 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 MHz and 125 MHz, respectively) at 300 K. Chemical shifts are reported in δ (parts per million: ppm), by reference to the hydrogenated residues of deuteriated solvent as internal standard: 2.05 ppm (^{1}H NMR) and 29.80 and 206.30 ppm (^{13}C NMR) for CD_3COCD_3, 2.50 ppm (^{1}H NMR) and 39.50 ppm (^{13}C NMR) for (CD_3SOCD_3), 7.26 ppm (^{1}H NMR) and 77.16 ppm (^{13}C NMR) for $CDCl_3$. 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).

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

IR spectra were recorded neat on a Perkin Elmer Spectrum 100 FTIR spectrophotometer or on a Bruker Tensor 27 (indicated in text).

Mass spectrometry was performed on a TSQ® Quantum (ThermoFisher, Dreieich, Germany). The triple quadrupole mass spectrometer was equipped with an electrospray interface (ESI). The purity of the compounds was assessed by LC/MS. The Surveyor®-LC-system consisted of a pump, an auto sampler, and a PDA detector. The system was operated by the standard software Xcalibur®. An 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) 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 the flow rate was set to 800 μL/min. MS analysis was carried out at the needle voltage of 3000 V and the 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 microwave irradiation experiments were carried out in a CEM-Discover microwave apparatus.

MPLC purifications were performed on Teledyne Isco Combi-Flash Companion.

All tested compounds exhibited ≥95% chemical purity as measured by HPLC/MS.

The following compounds were prepared according to previously described procedures: *N*-cyclopropyl-3-methoxyaniline **15a** [36]; *N*-(3-methoxybenzyl)cyclopropanamine **15b** [36]; 2,5-dibromothiophene-3-carboxylic acid **20b** [44]; 5-bromo-3-methylthiophene-2-carboxylic acid **22** [37]; *N*,5-bis(3-methoxyphenyl)-*N*-methylthiophene-2-carboxamide **23b** [28]; 2-(3-methoxyphenyl)-2-methylpropanoic acid **31b** [38]; 3-chloro-3-(3-methoxyphenyl)prop-2-enal **33b** [45].

5.2. General procedures

5.2.1. General procedure for amide formation (method A)

A solution of bromo heteroaryl carboxylic acid (2 mmol), thionyl chloride (4 mmol) and DMF (5 drops) in toluene (10 mL) was refluxed at 110 °C for 4 h. The reaction mixture was cooled to room temperature and the solvent and the excess of thionyl chloride removed under reduced pressure. To the residue was added at 0 °C the corresponding *N*-methyl amine (2 mmol) and Et_3N (2 mmol) in CH_2Cl_2 (10 mL) under N_2 atmosphere. After 30 min at 0 °C, the ice bath was removed and the solution was warmed up and stirred at room temperature overnight. The reaction mixture was extracted twice with CH_2Cl_2 (2 × 15 mL) and the organic layer dried over $MgSO_4$, filtered and the solution was concentrated under reduced pressure. The residue was purified by silica gel column chromatography using hexanes and EtOAc as eluant or by trituration in a mixture of diethyl ether/petroleum ether to afford the desired compound.

5.2.2. General procedure for Suzuki–Miyaura coupling (method B)

In a sealed tube the previously prepared bromo-*N*-heteroarylcarboxamide derivative (1 eq.) was introduced followed by the corresponding boronic acid (1.5 eq.), cesium carbonate (3 eq.), tetrakis(triphenylphosphine)palladium (0.02 eq.) and a mixture of DME/EtOH/H_2O (1:1:1, v:v:v, 3 mL) as solvent. The reactor was flushed with N_2 and submitted to microwave irradiation (150 °C, 150 W) for 20 min. After cooling to room temperature, a mixture of EtOAc/H_2O (1:1, v:v, 2 mL) was added to stop the reaction. The aqueous layer was extracted with EtOAc (3 × 10 mL). The organic layer was washed once with brine and once with water, dried over $MgSO_4$, filtered and the solution was concentrated under reduced pressure. The residue was purified by column chromatography using hexanes and EtOAc as eluant to afford the desired compound.

5.2.3. General procedure for ether cleavage (method C)

To a solution of methoxy heteroaryl derivative (1 eq.) in CH_2Cl_2 (10 mL) at 0 °C boron trifluoride methyl sulfide complex (6 eq. per methoxy group) was added. The reaction mixture was warmed up to room temperature and stirred overnight. Methanol was added to quench the reaction at 0 °C. After warming up to room temperature for 1 h, the solvent was carefully removed under reduced pressure (temperature of bath was 25 °C). Cold water was added to the residue and the aqueous layer was extracted with CH_2Cl_2 (3 × 15 mL). The organic layer was washed once with water, dried over $MgSO_4$, filtered and evaporated to dryness under reduced pressure. The residue was purified by column chromatography using hexanes and EtOAc as eluant or triturated in the mixture diethyl ether/petroleum ether and filtered off to afford the desired compound.

5.3. Detailed synthesis procedure for the preparation of compounds

5.3.1. 2-Bromo-N-(3-methoxyphenyl)-N-methyl-1,3-thiazole-5-carboxamide (**10a**)

Synthesized according to method A using 2-bromo-1,3-thiazole-5-carboxylic acid (416 mg, 2 mmol) and 3-methoxy-*N*-methylaniline (262 μl, 2 mmol). The residue was purified by silica gel column chromatography (hexanes/EtOAc 70:30) to afford **10a** as pale brown solid (393 mg, 60%). Mp: 106–107 °C. IR (neat): 3098, 3059, 3014, 2966, 2943, 2838, 1633, 1582 cm^{-1}. ^{1}H NMR (CD_3COCD_3) δ 3.67 (s, 3H), 3.85 (s, 3H), 6.99 (ddd, J = 0.9, 2.0, 7.7 Hz, 1H), 7.05 (t, J = 2.3 Hz, 1H), 7.09 (ddd, J = 0.9, 2.5, 8.4 Hz, 1H), 7.18 (s, 1H), 7.44 (t, J = 8.1 Hz, 1H); ^{13}C NMR (CD_3COCD_3) δ 38.6, 56.0, 114.9, 115.8, 121.3, 131.8, 136.8, 144.9, 145.7, 155.6, 159.9, 162.1; LC–MS (ESI): 325.77, 327.45 [M]$^+$.

5.3.2. 2-Bromo-N-(3-methoxybenzyl)-N-methyl-1,3-thiazole-5-carboxamide (**11b**)

Synthesized according to method A using 2-bromo-1,3-thiazole-5-carboxylic acid (418 mg, 2 mmol) and 3-methoxy-*N*-methylbenzylamin (298 μL, 2 mmol). The residue was purified by silica gel column chromatography (hexanes/EtOAc 70:30) to afford **11b** as yellow oil (530 mg, 78%). IR (neat): 2971, 2934, 2835, 1611 cm^{-1}. ^{1}H NMR (CD_3COCD_3) δ 2.98–3.34 (m, 3H), 3.79 (s, 3H), 4.68–4.82 (m, 2H), 6.86–6.93 (m, 3H), 7.26–7.34 (m, 1H), 7.63–8.14 (m, 1H); ^{13}C NMR (CD_3COCD_3) δ 37.1, 52.4, 55.5, 113.7, 114.5, 119.4, 120.9, 130.7, 139.5, 143.2, 154.7, 161.1, 161.5; LC–MS (ESI): 339.70, 341.52 [M]$^+$.

*5.3.3. 2-Bromo-N-(3-hydroxybenzyl)-N-methyl-1,3-thiazole-5-carboxamide (**11a**)*

Synthesized according to method C using **11b** (530 mg, 1.55 mmol) and $BF_3.SMe_2$ (980 μL, 9.32 mmol). The product was purified on MP-LC (hexanes/EtOAc 100:0 to 80:20, 15 min, 80:20 to 60:40, 35 min) afforded the desired product as pale red oil (236 mg, 47%). IR (neat): 3286, 3101, 2969, 2928, 2871, 1731, 1702, 1603 cm^{-1}. ^1H NMR (CD_3COCD_3) δ 2.99–3.34 (m, 3H), 4.61–4.82 (m, 2H), 6.72–6.92 (m, 3H), 7.60–8.13 (m, 1H), 8.47 (br s, 1H); ^{13}C NMR (CD_3COCD_3) δ 36.6, 52.3, 115.3, 119.4, 130.7, 134.6, 139.8, 144.3, 158.7, 162.3, 171.3; LC–MS (ESI): 327.93, 328.94 $[M+H]^+$.

*5.3.4. 5-Bromo-N-cyclopropyl-N-(3-methoxyphenyl)thiophene-2-carboxamide (**12a**)*

Synthesized according to method A using 5-bromothiophene-2-carboxylic acid (414 mg, 2 mmol) and freshly synthesized *N*-cyclopropyl-3-methoxyaniline **15a** (2 mmol). The residue was purified by silica gel column chromatography (hexanes/EtOAc 70:30) to afford **12a** as yellow solid (343 mg, 49%). Mp: 60–61 °C. IR (neat): 3086, 3014, 2946, 2841, 1740, 1630, 1597 cm^{-1}. ^1H NMR (CD_3COCD_3) δ 0.58–0.62 (m, 2H), 0.78–0.82 (m, 2H), 3.28–3.32 (m, 1H), 3.82 (s, 3H), 6.66 (d, $J = 4.1$ Hz, 1H), 6.86 (ddd, $J = 1.0, 1.9, 7.9$ Hz, 1H), 6.91 (t, $J = 2.3$ Hz, 1H), 6.95 (d, $J = 4.1$ Hz, 1H), 7.03 (ddd, $J = 0.9, 2.6, 8.5$ Hz, 1H), 7.38 (t, $J = 8.2$ Hz, 1H); ^{13}C NMR (CD_3COCD_3) δ 7.8, 33.1, 55.9, 115.1, 116.0, 118.5, 122.5, 131.1, 132.9, 142.0, 143.3, 161.6, 162.6; LC–MS (ESI): 351.72, 354.07 $[M+H]^+$.

*5.3.5. 5-Bromo-N-cyclopropyl-N-(3-methoxybenzyl)thiophene-2-carboxamide (**13b**)*

Synthesized according to method A using 5-bromothiophene-2-carbonyl chloride (416 mg, 2 mmol) and freshly synthesized *N*-(3-methoxybenzyl)cyclopropanamine **15b** (2 mmol). The residue was purified by silica gel column chromatography (hexanes/EtOAc 70:30) to afford **13b** as yellow oil (302 mg, 41%). IR (neat): 3089, 3008, 2946, 2838, 1737, 1600 cm^{-1}. ^1H NMR (CD_3COCD_3) δ 0.78–0.81 (m, 2H), 0.88–0.92 (m, 2H), 2.98–3.04 (m, 1H), 3.77 (s, 3H), 4.74 (s, 2H), 6.82–6.85 (m, 1H), 6.88–6.91 (m, 2H), 7.18 (d, $J = 4.1$ Hz, 1H), 7.23–7.27 (m, 1H), 7.61 (d, $J = 4.1$ Hz, 1H); ^{13}C NMR (CD_3COCD_3) δ 11.3, 32.0, 51.9, 55.4, 113.2, 114.1, 117.9, 120.5, 130.4, 131.5, 132.3, 141.0, 142.5, 160.9, 164.1; LC–MS (ESI): 365.66, 367.91 $[M+H]^+$.

*5.3.6. 5-Bromo-N-cyclopropyl-N-(3-hydroxybenzyl)thiophene-2-carboxamide (**13a**)*

Synthesized according to method C using **13b** (278 mg, 0.76 mmol) and $BF_3.SMe_2$ (480 μL, 4.56 mmol). The product as brown solid (270 mg, 100%) was directly obtained analytically pure and used in the next step without further purification. Mp: 128 °C. IR (neat): 3268, 3098, 3002, 2975, 2928, 2429, 1740, 1603, 1570 cm^{-1}. ^1H NMR (CD_3COCD_3) δ 0.77–0.81 (m, 2H), 0.87–0.92 (m, 2H), 2.96–3.02 (m, 1H), 4.70 (s, 2H), 6.73 (ddd, $J = 0.9, 2.7, 7.8$ Hz, 1H), 6.78–6.80 (m, 2H), 7.13–7.17 (m, 1H), 7.18 (d, $J = 4.1$ Hz, 1H), 7.60 (d, $J = 4.1$ Hz, 1H), 8.28 (br s, 1H); ^{13}C NMR (CD_3COCD_3) δ 11.3, 31.9, 51.9, 114.9, 115.1, 117.9, 119.5, 130.4, 131.5, 132.3, 140.9, 142.5, 158.5, 164.1; LC–MS (ESI): 351.75, 353.28 $[M]^+$.

*5.3.7. 5-Bromo-N-(3-methoxyphenyl)-N-methylthiophene-3-carboxamide (**5b**)*

Synthesized according to method A using 5-bromothiophene-3-carboxylic acid (415 mg, 2 mmol) and 3-methoxy-*N*-methylaniline (262 μL, 2 mmol). The residue was purified by silica gel column chromatography (hexanes/EtOAc 70:30) to afford **5b** as pale brown oil (340 mg, 69%). ^1H NMR (CD_3COCD_3) δ 3.36 (s, 3H), 3.77 (s, 3H), 6.80 (ddd, $J = 0.9, 1.9, 7.9$ Hz, 1H), 6.86–6.89 (m, 2H), 6.91 (d, $J = 1.6$ Hz, 1H), 7.26–7.29 (m, 2H); ^{13}C NMR (CD_3COCD_3) δ 38.4, 55.8, 111.4, 113.8, 113.9, 120.3, 131.0, 131.8, 132.0, 139.0, 146.8, 161.5, 163.5; LC–MS (ESI): 325.20, 327.21 $[M]^+$.

*5.3.8. 5-Bromo-N-(3-methoxybenzyl)-N-methylthiophene-3-carboxamide (**7b**)*

Synthesized according to method A using 5-bromothiophene-3-carboxylic acid (414 mg, 2 mmol) and 3-methoxy-*N*-methylbenzylamine (298 μL, 2 mmol). The residue was purified by silica gel column chromatography (hexanes/EtOAc 70:30) to afford **7b** as yellow oil (277 mg, 41%). IR (neat, Bruker Tensor 27): 3094, 2995, 2918, 2833, 1622 cm^{-1}. ^1H NMR (CD_3COCD_3) δ 3.10 (br s, 3H), 3.79 (s, 3H), 4.66 (s, 2H), 6.86 (dd, $J = 1.9, 8.0$ Hz, 1H), 6.88–6.96 (m, 2H), 7.24–7.37 (m, 2H), 7.60–7.84 (m, 1H); ^{13}C NMR (CD_3COCD_3) δ 37.1, 46.7, 55.6, 112.7, 113.6, 114.4, 121.0, 128.8, 129.4, 130.6, 131.4, 138.8, 140.0, 161.1; LC–MS (ESI): 340.21, 342.21 $[M]^+$.

*5.3.9. 4-Bromo-N-(3-methoxyphenyl)-N-methylthiophene-2-carboxamide (**6b**)*

Synthesized according to method A using 4-bromothiophene-2-carboxylic acid (414 mg, 2 mmol) and 3-methoxy-*N*-methylaniline (262 μL, 2 mmol). The residue was purified by silica gel column chromatography (hexanes/EtOAc 70:30) to afford **6b** as pale brown solid (665 mg, 99%). Mp: 79–82 °C. IR (neat, Bruker Tensor 27): 3103, 2997, 2964, 2939, 2935, 2831, 1709, 1624, 1589 cm^{-1}. ^1H NMR (CD_3COCD_3) δ 3.37 (s, 3H), 3.82 (s, 3H), 6.47 (d, $J = 1.5$ Hz, 1H), 6.94 (ddd, $J = 0.9, 2.1, 7.8$ Hz, 1H), 7.00 (t, $J = 2.4$ Hz, 1H), 7.04 (ddd, $J = 0.9, 2.6, 8.3$ Hz, 1H), 7.40 (t, $J = 8.1$ Hz, 1H), 7.58 (d, $J = 1.5$ Hz, 1H). ^{13}C NMR (CD_3COCD_3) δ 39.1, 56.0, 109.2, 114.6, 115.1, 121.1, 129.2, 131.5, 134.0, 141.0, 146.0, 161.2, 161.9; LC–MS (ESI): 325.97, 328.97 $[M]^+$.

*5.3.10. 4-Bromo-N-(3-methoxybenzyl)-N-methylthiophene-2-carboxamide (**8b**)*

Synthesized according to method A using 4-bromothiophene-2-carboxylic acid (413 mg, 2 mmol) and 3-methoxy-*N*-methylbenzylamine (298 μL, 2 mmol). The residue was purified by silica gel column chromatography (hexanes/EtOAc 70:30) to afford **8b** as yellow oil (680 mg, 99%). IR (neat, Bruker Tensor 27): 3109, 3009, 2960, 2939, 2837, 1610, 1591 cm^{-1}. ^1H NMR (CD_3COCD_3) δ 3.17 (br s, 3H), 3.79 (s, 3H), 4.76 (br s, 2H), 6.86–6.92 (m, 3H), 7.30 (t, $J = 7.5$ Hz, 1H), 7.32–7.50 (m, 1H), 7.71 (s, 1H); ^{13}C NMR (CD_3COCD_3) δ 38.9, 46.3, 55.7, 109.5, 113.6, 114.0, 120.5, 128.1, 130.7, 131.7, 139.8, 141.1, 161.1, 163.2; LC–MS (ESI): 340.00, 342.01 $[M]^+$.

*5.3.11. 5-(3-Methylphenyl)-1,3-thiazole (**17b**)*

Synthesized according to method B using 5-bromo-1,3-thiazole (1 g, 6.10 mmol), *m*-tolyl boronic acid (1.24 g, 9.14 mmol), cesium carbonate (5.96 g, 18.30 mmol) and tetrakis(triphenylphosphine) palladium (140 mg, 0.02 eq) in an oxygen free DME/EtOH/water (1:1:1) (6 mL). The residue was purified by silica gel column chromatography (hexanes/EtOAc 70:30) to afford **17b** as colorless oil (1.06 g, 99%). IR (neat): 3086, 3026, 2957, 2922, 2862, 1605 cm^{-1}. ^1H NMR (CD_3COCD_3) δ 2.37 (s, 3H), 7.17–7.20 (m, 1H), 7.32 (t, $J = 7.7$ Hz, 1H), 7.45–7.48 (m, 1H), 7.50–7.52 (m, 1H), 8.19 (s, 1H), 8.93 (s, 1H); ^{13}C NMR (CD_3COCD_3) δ 21.4, 124.8, 128.3, 129.99, 130.01, 132.1, 139.8, 140.1, 153.2; LC–MS (ESI): 176.07 $[M+H]^+$.

*5.3.12. Lithium 5-(3-methylphenyl)-1,3-thiazole-2-carboxylate (**17a**)*

To a solution of **17b** (1.05 g, 6 mmol) in dry THF (50 mL) was added drop wise *n*-BuLi (2.88 mL, 7.21 mmol, 2.5 M in hexane) at −78 °C under N_2 atmosphere. The reaction mixture was kept at −78 °C for 1 h and was warmed up (without removing ice bath) to −5 °C (in about 3 h). Then, the reaction mixture was cooled at −78 °C and dry carbon dioxide gas was bubbled through the

solution for 3 h. The reaction mixture was warmed up to room temperature and the bubbling of dry CO_2 gas was continued overnight. Solvent was removed under reduced pressure (bath temperature of the rotary evaporator at 20 °C). The residue was triturated with diethyl ether and filtered to afford the desired compound as a pale brown solid (977 mg, 72%). IR (neat): 3301, 3095, 3032, 2960, 2922, 2868, 1737, 1618 cm^{-1}. ^1H NMR (CD_3SOCD_3) δ 2.34 (s, 3H), 7.16 (d, $J = 7.2$ Hz, 1H), 7.31 (t, $J = 7.5$ Hz, 1H), 7.46 (d, $J = 7.2$ Hz, 1H), 7.50 (s, 1H), 8.12 (s, 1H); ^{13}C NMR (CD_3SOCD_3) δ 20.9, 123.6, 126.9, 128.99, 129.06, 131.2, 138.5, 141.0, 161.7, 170.3; LC–MS (ESI): 219.95 $[M\text{-}Li + H]^+$.

5.3.13. 5-Bromo-N-(3-methoxyphenyl)-N-methylthiophene-2-sulfonamide (**26**)

To a stirred solution of 3-methoxy-*N*-methylaniline (137 mg, 1 mmol) in CH_2Cl_2 (3 mL) was added NaOH 50% (1 mL) followed by *n*-tetrabutyl ammonium hydrogen sulfate (51 mg, 0.15 mmol). After few minutes, 5-bromothiophene-2-sulfonyl chloride (262 mg, 1 mmol) was added to the reaction mixture. The solution was stirred at room temperature for 5 h. Water (10 mL) was added to quench the reaction followed by EtOAc (10 mL). The aqueous layer was extracted twice with EtOAc (2 × 15 mL). The organic layer was dried over $MgSO_4$, filtered and the solution was concentrated under reduced pressure. The product was purified on silica gel (hexanes/EtOAc 8:2) to afford the desired compound as yellow oil (277 mg, 77%). IR (neat): 3101, 2938, 2835, 1734, 1602, 1354 cm^{-1}. ^1H NMR (CD_3COCD_3) δ 3.27 (s, 3H), 3.77 (s, 3H), 6.78–6.81 (m, 2H), 6.90–6.92 (m, 1H), 7.26–7.30 (m, 2H), 7.31 (d, $J = 4.1$ Hz, 1H); ^{13}C NMR (CD_3COCD_3) δ 38.7, 55.8, 113.5, 114.2, 119.3, 120.1, 130.5, 132.2, 134.3, 138.9, 143.2, 161.0; LC–MS (ESI): 361.07, 362.90 $[M+H]^+$.

5.3.14. 5-Bromo-N-(3-methoxybenzyl)-N-methylthiophene-2-sulfonamide (**27b**)

To a stirred solution of 3-methoxy-*N*-methylbenzylamine (302 mg, 2 mmol) in CH_2Cl_2 (6 mL) was added NaOH 50% (2 mL) followed by *n*-tetrabutyl ammonium hydrogen sulfate (102 mg, 0.30 mmol). After few minutes, 5-bromothiophene-2-sulfonyl chloride (524 mg, 2 mmol) was added to the reaction mixture. The solution was stirred at room temperature for 5 h. Water (10 mL) was added to quench the reaction, followed by EtOAc (10 mL). The aqueous layer was extracted twice with EtOAc (2 × 15 mL). The organic layer was dried over $MgSO_4$, filtered and the solution was concentrated under reduced pressure. The product was purified on silica gel (hexanes/EtOAc 80:20) to afford the desired compound as yellow oil (620 mg, 82%). IR (neat): 3101, 2937, 2835, 1740, 1600, 1345 cm^{-1}. ^1H NMR (CD_3COCD_3) δ 2.70 (s, 3H), 3.80 (s, 3H), 4.21 (s, 2H), 6.88–6.91 (m, 1H), 6.92–6.95 (m, 2H), 7.28–7.31 (m, 1H), 7.38 (d, $J = 4.1$ Hz, 1H), 7.52 (d, $J = 4.1$ Hz, 1H); ^{13}C NMR (CD_3COCD_3) δ 35.0, 54.7, 55.5, 114.2, 114.7, 119.7, 121.3, 130.6, 132.5, 133.7, 138.3, 140.0, 161.0; LC–MS (ESI): 376.22, 378.65 $[M]^+$.

5.3.15. 5-Bromo-N-(3-hydroxybenzyl)-N-methylthiophene-2-sulfonamide (**27a**)

Synthesized according to method C using **27b** (528 mg, 1.40 mmol) and $BF_3.SMe_2$ (880 μL, 8.42 mmol). The precipitate formed was filtered to give the desired product as yellow solid (445 mg, 88%) was used in the next step without any purification. Mp: 105–107 °C. IR (neat): 3438, 3104, 3026, 2981, 2931, 1600, 1337 cm^{-1}. ^1H NMR (CD_3COCD_3) δ 2.69 (s, 3H), 4.16 (s, 2H), 6.80 (ddd, $J = 0.8$, 2.6, 9.0 Hz, 1H), 6.81–6.84 (m, 1H), 6.86–6.88 (m, 1H), 7.19 (t, $J = 7.9$ Hz, 1H), 7.38 (d, $J = 4.0$ Hz, 1H), 7.51 (d, $J = 4.0$ Hz, 1H), 8.38 (s, 1H); ^{13}C NMR (CD_3COCD_3) δ 35.0, 54.7, 115.8, 116.0, 119.6, 120.3, 130.6, 132.4, 133.6, 138.3, 140.0, 158.7; LC–MS (ESI): 361.88, 363.73 $[M+H]^+$.

5.3.16. N-(3-Methoxyphenyl)-N-methyl-2-(3-methylphenyl)-1,3-thiazole-5-carboxamide (**10**)

Synthesized according to method B using **10a** (200 mg, 0.61 mmol), *m*-tolyl boronic acid (105 mg, 0.77 mmol), cesium carbonate (596 mg, 1.83 mmol) and tetrakis(triphenylphosphine) palladium (14 mg, 0.02 eq.). The residue was purified on silica gel (hexanes/EtOAc 70:30) to afford the desired product as beige solid (170 mg, 82%). Mp: 87–90 °C. IR (neat): 3065, 3005, 2924, 1626, 1588 cm^{-1}. ^1H NMR (CD_3COCD_3) δ 2.36 (s, 3H), 3.40 (s, 3H), 3.83 (s, 3H), 6.98–7.00 (m, 1H), 7.04–7.06 (m, 2H), 7.28–7.30 (m, 2H), 7.33 (t, $J = 7.5$ Hz, 1H), 7.42 (dt, $J = 0.7$, 7.8 Hz, 1H), 7.64–7.66 (m, 1H), 7.70–7.71 (m, 1H); ^{13}C NMR (CD_3COCD_3) δ 21.2, 38.7, 55.9, 114.7, 115.2, 121.1, 124.5, 127.7, 129.9, 131.6, 132.4, 134.0, 134.7, 139.8, 145.8, 147.6, 161.2, 161.9, 171.6; LC–MS (ESI): 338.68 $[M]^+$.

5.3.17. 2-(2-Fluoro-3-methoxyphenyl)-N-(3-hydroxybenzyl)-N-methyl-1,3-thiazole-5-carboxamide (**11**)

Synthesized according to method B using **11a** (104 mg, 0.32 mmol), 2-fluoro-3-methoxyphenylboronic acid (81 mg, 0.48 mmol), cesium carbonate (311 mg, 0.95 mmol) and tetrakis(-triphenylphosphine) palladium (7 mg, 0.02 eq.). The residue was purified by MP-LC (hexanes/EtOAc 100:0 to 60:40, 60 min) followed by a trituration cold acetonitrile to afford the desired product as beige solid (83 mg, 70%). Mp: 140–141 °C. IR (neat): 3220, 3029, 2993, 2931, 1591 cm^{-1}. ^1H NMR (CD_3COCD_3) δ 3.20 (br s, 3H), 3.97 (s, 3H), 4.76 (br s, 2H), 6.77–6.85 (m, 3H), 7.22 (t, $J = 7.6$ Hz, 1H), 7.26–7.32 (m, 2H), 7.84 (br s, 1H), 8.36 (br s, 1H); ^{13}C NMR (CD_3COCD_3) δ 56.9, 104.4, 115.3, 115.4, 116.12, 116.14, 120.1, 122.3, 122.4, 125.57, 125.60, 139.7, 149.3, 149.4, 150.0, 152.0, 158.8, 162.9; LC–MS (ESI): 372.70 $[M+H]^+$.

5.3.18. N-(3-Methoxyphenyl)-N-methyl-5-(3-methylphenyl)-1,3-thiazole-2-carboxamide (**17**)

To a solution of **17a** (450 mg, 2 mmol) in CH_2Cl_2 (15 mL) was added drop wise oxalyl chloride (340 μL, 4 mmol) followed by few drops of DMF at 0 °C under N_2 atmosphere. The reaction mixture was stirred at 0 °C for 10 min and at room temperature for 3 h. The solvent was removed under reduced pressure (bath temperature of the rotary evaporator: 20 °C). The residue was diluted in dry CH_2Cl_2 (10 mL) and 3-methoxy-*N*-methylaniline (262 mL, 2 mmol) was added followed by triethylamine (280 μL, 2 mmol). The solution was stirred overnight at room temperature under N_2 atmosphere. The solvent was removed under reduced pressure (bath temperature of rotary evaporator: 20 °C), Na_2CO_3 2 N (15 mL) followed by EtOAc (15 mL) were added. The aqueous layer was extracted once more with EtOAc (15 mL). The organic layer was washed twice with Na_2CO_3 2 N (15 mL), once with water (15 mL), dried over $MgSO_4$, filtered and the solution was concentrated under reduced pressure. Purification on silica gel (hexanes/EtOAc 70:30) to afford the desired product as yellow solid (520 mg, 77%). Mp: 84–86 °C. IR (neat): 3095, 3014, 2975, 2919, 1633, 1588 cm^{-1}. ^1H NMR (CD_3COCD_3) δ 2.36 (s, 3H), 3.54 (br s, 3H), 3.78 (s, 3H), 6.87 (ddd, $J = 0.6$, 2.5, 8.3 Hz, 2H), 6.92 (t, $J = 2.1$ Hz, 1H), 7.20–7.22 (m, 1H), 7.27 (t, $J = 8.1$ Hz, 1H), 7.32 (t, $J = 7.7$ Hz, 1H), 7.43–7.47 (m, 1H), 7.49 (br s, 1H), 7.93 (br s, 1H); ^{13}C NMR (CD_3COCD_3) δ 21.3, 39.5, 55.7, 113.6, 113.8, 120.1, 124.87, 124.93, 128.3, 128.4, 130.1, 130.55, 130.62, 131.5, 139.7, 139.9, 144.3, 146.6, 161.2; LC–MS (ESI): 338.85 $[M+H]^+$.

5.3.19. N-Cyclopropyl-N-(3-methoxyphenyl)-5-(3-methylphenyl) thiophene-2-carboxamide (**12**)

Synthesized according to method B using **12a** (205 mg, 0.58 mmol), *m*-tolyl boronic acid (118 mg, 0.87 mmol), cesium carbonate (570 mg, 1.75 mmol) and tetrakis(triphenylphosphine) palladium (14 mg, 0.02 eq). The residue was purified on MP-LC (hexanes/EtOAc 100:0 to 80:20, 10 min, 80:20 to 60:50, 45 min)

to afford the desired product as yellow solid (181 mg, 86%). Mp: 68–69 °C. IR (neat): 3092, 3014, 2972, 2922, 1627, 1600 cm^{-1}. ^1H NMR (CD_3COCD_3) δ 0.61–0.64 (m, 2H), 0.79–0.83 (m, 2H), 2.33 (s, 3H), 3.31–3.35 (m, 1H), 3.81 (s, 3H), 6.73 (d, J = 4.0 Hz, 1H), 6.88 (ddd, J = 0.9, 2.0, 7.7 Hz, 1H), 6.92 (t, J = 2.3 Hz, 1H), 6.99 (ddd, J = 0.9, 2.6, 8.5 Hz, 1H), 7.13–7.14 (m, 1H), 7.15 (d, J = 4.0 Hz, 1H), 7.26 (t, J = 7.7 Hz, 1H), 7.36 (t, J = 8.1 Hz, 1H), 7.37–7.39 (m, 1H), 7.42–7.43 (m, 1H); ^{13}C NMR (CD_3COCD_3) δ 8.1, 21.3, 33.2, 55.8, 114.5, 115.8, 122.3, 123.7, 123.8, 127.2, 129.8, 129.9, 130.9, 132.9, 134.4, 139.5, 139.6, 144.0, 149.3, 161.5, 163.7; LC–MS (ESI): 363.86 $[M+H]^+$.

5.3.20. N-Cyclopropyl-5-(2-fluoro-3-methoxyphenyl)-N-(3-hydroxybenzyl)thiophene-2-carboxamide (**13**)

Synthesized according to method B using **13a** (104 mg, 0.32 mmol), 2-fluoro-3-methoxyphenylboronic acid (81 mg, 0.48 mmol), cesium carbonate (311 mg, 0.95 mmol) and tetrakis(triphenylphosphine) palladium (7 mg, 0.02 eq). The residue was purified by preparative HPLC (acetonitrile/water 40:60 to 100:0, solvent containing 1 mL of TFA for 1 L of solvent) to afford the desired product as beige solid (41 mg, 37%). Mp: 149–150 °C. IR (neat): 3253, 3008, 2972, 2937, 1600, 1570 cm^{-1}. ^1H NMR (CD_3COCD_3) δ 0.77–0.80 (m, 2H), 0.87–0.92 (m, 2H), 3.00–3.06 (m, 1H), 3.94 (s, 3H), 4.74 (s, 2H), 6.73–6.76 (m, 1H), 6.81–6.84 (m, 2H), 7.13–7.22 (m, 3H), 7.32–7.35 (m, 1H), 7.52 (dd, J = 1.0, 4.1 Hz, 1H), 7.78 (m, 1H), 8.31 (br s, 1H); ^{13}C NMR (CD_3COCD_3) δ 11.2, 32.1, 51.9, 56.7, 114.07, 114.08, 114.9, 115.1, 119.5, 120.57, 120.58, 122.8, 122.9, 125.5, 125.6, 127.2, 127.3, 130.4, 131.8, 140.67, 140.71, 141.1, 141.29, 141.31, 148.9, 149.6, 149.7, 150.9, 158.5, 165.1 (additional peaks due to coupling of carbon with fluorine); LC–MS (ESI): 397.66 $[M+H]^+$.

5.3.21. N-(3-Methoxyphenyl)-N-methyl-5-(3-methylphenyl) thiophene-2-sulfonamide (**24**)

Synthesized according to method B using **26** (142 mg, 0.39 mmol), *m*-tolyl boronic acid (80 mg, 0.59 mmol), cesium carbonate (385 mg, 1.18 mmol) and tetrakis(triphenylphosphine) palladium (10 mg, 0.02 eq). The residue was purified on silica gel (hexanes/EtOAc 8:2) to afford the desired product as yellow oil (147 mg, 99%). IR (neat): 3101, 3008, 2925, 1602 cm^{-1}. ^1H NMR (CD_3COCD_3) δ 2.38 (s, 3H), 3.29 (s, 3H), 3.75 (s, 3H), 6.80 (dd, J = 0.9, 2.2 Hz, 1H), 6.81–6.82 (m, 1H), 6.89 (ddd, J = 0.8, 2.5, 8.4 Hz, 1H), 7.23–7.28 (m, 2H), 7.34 (t, J = 7.7 Hz, 1H), 7.39 (d, J = 4.0 Hz, 1H), 7.48–7.49 (m, 1H), 7.50 (d, J = 4.0 Hz, 1H), 7.53–7.54 (m, 1H); ^{13}C NMR (CD_3COCD_3) δ 21.3, 38.8, 55.7, 113.5, 114.0, 119.3, 124.1, 124.3, 127.6, 130.1, 130.4, 130.8, 133.5, 134.7, 135.9, 140.0, 143.6, 152.0, 160.9; LC–MS (ESI): 373.62 $[M]^+$.

5.3.22. 5-(2-Fluoro-3-methoxyphenyl)-N-(3-hydroxybenzyl)-N-methylthiophene-2-sulfonamide (**25**)

Synthesized according to method B using **27a** (200 mg, 0.55 mmol), 2-fluoro-3-methoxyphenylboronic acid (141 mg, 0.83 mmol), cesium carbonate (540 mg, 1.66 mmol) and tetrakis(triphenylphosphine) palladium (13 mg, 0.02 eq). The residue was purified by preparative HPLC (acetonitrile/water 40:60 to 100:0, solvent containing 1 mL of TFA for 1 L of solvent) to afford the desired product as colorless oil (42 mg, 19%). IR (neat): 3438, 3107, 3029, 2981, 2928, 1600, 1337 cm^{-1}. ^1H NMR (CD_3COCD_3) δ 2.72 (s, 3H), 3.95 (s, 3H), 4.19 (s, 2H), 6.80 (ddd, J = 0.9, 2.5, 8.3 Hz, 1H), 6.83–6.85 (m, 1H), 6.89–6.90 (m, 1H), 7.17–7.27 (m, 3H), 7.37–7.40 (m, 1H), 7.67 (dd, J = 0.6, 4.1 Hz, 1H), 7.71 (dd, J = 1.3, 4.0 Hz, 1H); ^{13}C NMR (CD_3COCD_3) δ 35.1, 54.7, 56.8, 114.8, 115.7, 115.9, 120.3, 120.5, 121.9, 125.8, 127.7, 130.5, 133.3, 138.5, 143.7, 148.9, 149.6, 150.9, 158.6; LC–MS (ESI): 407.61 $[M]^+$.

5.3.23. N,5-bis(3-methoxyphenyl)-N-methylthiophene-3-carboxamide (**5a**)

Synthesized according to method B using **5b** (163 mg, 0.5 mmol) and 3-methoxyphenylboronic acid (91 mg, 0.6 mmol), cesium carbonate (489 mg, 1.5 mmol) and tetrakis(triphenylphosphine) palladium (12 mg, 0.02 eq.). The residue was purified by silica gel column chromatography (hexanes/EtOAc 70:30) to afford **5a** as yellow oil (168 mg, 95%). IR (neat): 3104, 3061, 3001, 2938, 1635, 1597 cm^{-1}. ^1H NMR (CD_3COCD_3) δ 3.40 (s, 3H), 3.76 (s, 3H), 3.82 (s, 3H), 6.83–6.89 (m, 4H), 6.99 (t, J = 1.9 Hz, 1H), 7.05 (ddd, J = 0.9, 1.6, 7.6 Hz, 1H), 7.17 (d, J = 1.4 Hz, 1H), 7.25–7.30 (m, 3H); ^{13}C NMR (CD_3COCD_3) δ 38.3, 55.6, 55.8, 111.7, 113.8, 113.9, 114.5, 118.8, 120.4, 125.5, 129.4, 130.9, 131.0, 135.9, 139.2, 143.5, 147.2, 161.2, 161.5, 164.6; LC–MS (ESI): 353.86 $[M+H]^+$.

5.3.24. N-(3-methoxybenzyl)-5-(3-methoxyphenyl)-N-methylthiophene-3-carboxamide (**7a**)

Synthesized according to method B using **7b** (100 mg, 0.29 mmol) and 3-methoxyphenylboronic acid (53 mg, 0.35 mmol), cesium carbonate (283 mg, 0.87 mmol) and tetrakis(triphenylphosphine) palladium (7 mg, 0.02 eq.). The residue was purified by silica gel column chromatography (hexanes/EtOAc 60:40) to afford **7a** as yellow oil (71 mg, 67%). IR (neat): 3086, 3064, 3001, 2956, 2937, 1626, 1597 cm^{-1}. ^1H NMR (CD_3COCD_3) δ 3.04 (br s, 3H), 3.79 (s, 3H), 3.84 (s, 3H), 4.70 (s, 2H), 6.84–6.97 (m, 4H), 7.16–7.34 (m, 4H), 7.53–7.77 (m, 2H); ^{13}C NMR (CD_3COCD_3) δ 37.3, 51.4, 55.5, 55.7, 112.0, 113.6, 114.6, 119.0, 119.6, 121.0, 124.8, 126.2, 126.8, 130.7, 131.1, 135.9, 139.1, 140.3, 144.9, 161.1, 161.2; LC–MS (ESI): 367.89 $[M+H]^+$.

5.3.25. N,4-bis(3-methoxyphenyl)-N-methylthiophene-2-carboxamide (**6a**)

Synthesized according to method B using **6b** (216 mg, 0.66 mmol) and 3-methoxyphenylboronic acid (151 mg, 0.99 mmol), cesium carbonate (324 mg, 1.98 mmol) and tetrakis(triphenylphosphine) palladium (8 mg, 0.02 eq.). The residue was purified by silica gel column chromatography (hexanes/EtOAc 70:30) to afford **6a** as yellow oil (177 mg, 76%). IR (neat): 3098, 3057, 3001, 2955, 2935, 1624, 1599, 1584 cm^{-1}. ^1H NMR (CD_3COCD_3) δ 3.40 (s, 3H), 3.80 (s, 3H), 3.82 (s, 3H), 6.83 (ddd, J = 0.9, 2.6, 8.3 Hz, 1H), 6.90 (t, J = 2.1 Hz, 1H), 6.92 (d, J = 1.6 Hz, 1H), 6.97–7.00 (m, 2H), 7.03 (q, J = 1.6 Hz, 1H), 7.04 (dd, J = 0.9, 2.6, 1H), 7.25 (t, J = 7.8 Hz, 1H), 7.39–7.43 (m, 1H), 7.77 (d, J = 1.6 Hz, 1H); ^{13}C NMR (CD_3COCD_3) δ 38.8, 55.5, 55.9, 112.0, 114.0, 114.7, 114.8, 119.0, 121.2, 126.2, 130.8, 131.0, 131.4, 137.4, 140.4, 142.2, 146.6, 161.1, 161.9, 162.3; LC–MS (ESI): 354.05 $[M+H]^+$.

5.3.26. N-(3-methoxybenzyl)-4-(3-methoxyphenyl)-N-methylthiophene-2-carboxamide (**8a**)

Synthesized according to method B using **8b** (340 mg, 1 mmol) and 3-methoxyphenylboronic acid (228 mg, 1.5 mmol), cesium carbonate (977 mg, 3 mmol) and tetrakis(triphenylphosphine) palladium (23 mg, 0.02 eq). The residue was purified by silica gel column chromatography (hexanes/EtOAc 70:30) to afford **8a** as yellow oil (312 mg, 85%). IR (neat): 3087, 3061, 3000, 2955, 2935, 1600, 1583 cm^{-1}. ^1H NMR (CD_3COCD_3) δ 3.21 (br s, 3H), 3.80 (s, 3H), 3.82 (br s, 3H), 4.81 (s, 2H), 6.86–6.89 (m, 2H), 6.92–6.96 (m, 2H), 7.16 (m, 2H), 7.28–7.33 (m, 2H), 7.79 (br s, 1H), 7.92 (s, 1H); ^{13}C NMR (CD_3COCD_3) δ 40.2, 55.5, 55.6, 112.6, 113.5, 113.9, 118.0, 119.4, 125.0, 127.1, 128.0, 128.5, 128.6, 130.7, 130.8, 137.4, 140.2, 140.3, 142.6, 161.1, 161.2; LC–MS (ESI): 368.05 $[M+H]^+$.

*5.3.27. N,5-bis(3-methoxyphenyl)-N-methylthiophene-2-carbothioamide (**23a**)*

A solution of *N*,5-bis(3-methoxyphenyl)-*N*-methylthiophene-2-carboxamide **23b** (382 mg, 1.08 mmol) in dry toluene and the Lawesson reagent (437 mg, 1.08 mmol) were heated at reflux under N_2 atmosphere for 3 h. After cooling, water was added to the solution and the aqueous layer was extracted with toluene (3 × 20 mL), washed once with brine and once with water, dried over $MgSO_4$, filtered and the solution was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexanes/EtOAc 80:20) to afford **23a** as yellow solid (252 mg, 63%). Mp: 101–102 °C. IR (neat): 3080, 3004, 2963, 2938, 1591, 1577 cm^{-1}. ^1H NMR (CD_3COCD_3) δ 3.80 (s, 3H), 3.84 (s, 3H), 3.85 (s, 3H), 6.49 (d, J = 4.1 Hz, 1H), 6.89–6.92 (m, 2H), 6.95–6.98 (m, 2H), 7.10 (d, J = 4.1 Hz, 1H), 7.12 (t, J = 2.1 Hz, 1H), 7.16 (ddd, J = 0.9, 1.8, 7.7 Hz, 1H), 7.30 (t, J = 8.1 Hz, 1H), 7.35 (dt, J = 0.7, 7.9 Hz, 1H); ^{13}C NMR (CD_3COCD_3) δ 47.6, 55.7, 55.9, 111.7, 112.2, 114.8, 115.0, 118.8, 119.2, 124.1, 130.4, 131.1, 131.4, 135.6, 146.7, 149.4, 150.0, 161.2, 161.7, 191.0; LC-MS (ESI): 370.12 $[M+H]^+$.

*5.3.28. N,5-bis(3-hydroxyphenyl)-N-methylthiophene-3-carboxamide (**5**)*

Synthesized according to method C using **5a** (161 mg, 0.46 mmol) and $BF_3.SMe_2$ (290 μL, 2.76 mmol). The residue was triturated in the mixture diethyl ether/petroleum ether to afford **5** as green solid (193 mg, 98%). Mp: 164–166 °C. IR (neat): 3212, 2924, 1584 cm^{-1}. ^1H NMR (CD_3COCD_3) δ 3.38 (s, 3H), 6.75–6.80 (m, 4H), 6.94–6.96 (m, 2H), 7.16–7.25 (m, 4H); ^{13}C NMR (CD_3COCD_3) δ 38.4, 113.2, 115.3, 115.9, 117.7, 119.2, 125.2, 129.4, 130.8, 131.0, 135.8, 139.0, 143.8, 147.0, 158.8, 159.2, 164.8; LC-MS (ESI): 325.87 $[M]^+$.

*5.3.29. N-(3-hydroxybenzyl)-5-(3-hydroxyphenyl)-N-methylthiophene-3-carboxamide (**7**)*

Synthesized according to method C using **7a** (48 mg, 0.13 mmol) and $BF_3.SMe_2$ (82 μL, 0.78 mmol). The residue was purified by silica gel column chromatography (hexanes/EtOAc 40:60) to afford **7** as pale brown oil (44 mg, 98%). IR (neat): 3203, 2971, 2925, 1582 cm^{-1}. ^1H NMR (CD_3COCD_3) δ 2.09 (s, 3H), 4.67 (s, 2H), 6.76–6.83 (m, 4H), 7.12–7.25 (m, 4H), 7.50–7.74 (m, 2H); ^{13}C NMR (CD_3COCD_3) δ 37.1, 51.1, 113.4, 115.2, 116.0, 117.9, 124.3, 125.9, 126.6, 130.55, 130.56, 130.7, 131.1, 135.8, 138.9, 140.0, 145.2, 158.8, 158.9; LC–MS (ESI): 339.87 $[M]^+$.

*5.3.30. N,4-bis(3-hydroxyphenyl)-N-methylthiophene-2-carboxamide (**6**)*

Synthesized according to method C using **6a** (164 mg, 0.46 mmol) and $BF_3.SMe_2$ (290 μL, 2.76 mmol). The residue was triturated in the mixture diethyl ether/petroleum ether to afford **6** as pale brown solid (56 mg, 37%). Mp: 187–189 °C. IR (neat): 3232, 3104, 2925, 1580 cm^{-1}. ^1H NMR (CD_3COCD_3) δ 3.38 (s, 3H), 6.74 (ddd, J = 0.9, 2.5, 8.2 Hz, 1H), 6.85 (t, J = 2.2 Hz, 1H), 6.86–6.89 (m, 2H), 6.90 (t, J = 2.2 Hz, 1H), 6.93 (ddd, J = 1.0, 2.5, 8.3 Hz, 1H), 6.98 (d, J = 1.5 Hz, 1H), 7.16 (t, J = 7.9 Hz, 1H), 7.32 (t, J = 7.9 Hz, 1H), 7.69 (d, J = 1.6 Hz, 1H), 8.40 (s, 1H), 8.73 (s, 1H); ^{13}C NMR (CD_3COCD_3) δ 38.9, 113.7, 115.3, 116.1, 116.2, 118.1, 120.0, 126.1, 130.8, 131.1, 131.4, 137.4, 140.3, 142.3, 146.5, 158.7, 159.5, 162.4; LC–MS (ESI): 326.09 $[M+H]^+$.

*5.3.31. N-(3-hydroxybenzyl)-4-(3-hydroxyphenyl)-N-methylthiophene-2-carboxamide (**8**)*

Synthesized according to method C using **8a** (281 mg, 0.76 mmol) and $BF_3.SMe_2$ (480 μL, 4.56 mmol). The residue was purified by silica gel column chromatography (hexanes/EtOAc 40:60) to afford **8** as pale brown solid (296 mg, 97%). Mp: 174–177 °C. IR (neat): 3273, 3097, 2961, 2926, 1580 cm^{-1}. ^1H NMR (CD_3COCD_3) δ 3.19 (br s, 3H), 4.77 (br s, 2H), 6.76–6.86 (m, 4H), 7.12 (br s, 2H), 7.19–7.22 (m, 2H), 7.76 (br s, 1H), 7.82 (s, 1H); ^{13}C NMR (CD_3COCD_3) δ 37.1, 52.7, 114.0, 115.3, 115.0, 115.4, 118.4, 119.4, 124.9, 127.9, 128.7, 130.7, 130.8, 137.4, 139.9, 142.8, 158.7, 164.7; LC–MS (ESI): 340.09 $[M+H]^+$.

*5.3.32. N,5-bis(3-hydroxyphenyl)-N-methylthiophene-2-carbothioamide (**23**)*

Synthesized according to method C using **23a** (120 mg, 0.32 mmol) and $BF_3.SMe_2$ (402 μL, 3.84 mmol). The residue was purified by silica gel column chromatography (hexanes/EtOAc 40:60) to afford **23** as pale brown solid (106 mg, 97%). Mp: 174–177 °C. IR (neat): 3185, 2263, 1584 cm^{-1}. ^1H NMR (CD_3COCD_3) δ 3.82 (s, 3H), 6.53 (d, J = 4.0 Hz, 1H), 6.78–6.84 (m, 3H), 6.85–6.88 (m, 1H), 7.03–7.09 (m, 3H), 7.21 (t, J = 8.0 Hz, 1H), 7.28 (t, J = 8.1 Hz, 1H); ^{13}C NMR (CD_3COCD_3) δ 47.6, 113.1, 114.1, 116.1, 116.4, 117.8, 118.1, 123.8, 130.7, 131.1, 131.5, 135.6, 146.5, 149.3, 150.3, 158.8, 159.4, 190.8; LC–MS (ESI): 341.80 $[M]^+$.

*5.3.33. 2,5-Dibromo-N-(3-methoxyphenyl)-N-methylthiophene-3-carboxamide (**20a**)*

Synthesized according to method A using **20b** (414 mg, 1.45 mmol) and 3-methoxy-*N*-methylaniline (190 μL, 1.45 mmol). The residue was purified by silica gel column chromatography (hexanes/EtOAc 80:20) to afford **20a** as yellow oil (431 mg, 73%). ^1H NMR (CD_3COCD_3) δ 3.39 (s, 3H), 3.76 (s, 3H), 6.81 (ddd, J = 0.8, 2.5, 8.3 Hz, 1H), 6.82–6.85 (m, 2H), 6.95–6.98 (m, 1H), 7.23 (t, J = 8.1 Hz, 1H); ^{13}C NMR (CD_3COCD_3) δ 55.7, 56.1, 113.2, 114.1, 119.7, 129.6, 130.6, 130.8, 131.7, 140.8, 145.3, 161.0, 163.7; LC–MS (ESI): 402.79, 406.80, 404.93 $[M]^+$.

*5.3.34. N-2,5-tris(3-methoxyphenyl)-N-methylthiophene-3-carboxamide (**19a**)*

Synthesized according to method B using **20a** (133 mg, 0.33 mmol) and 3-methoxyphenylboronic acid (75 mg, 0.49 mmol), cesium carbonate (401 mg, 1.23 mmol) and tetrakis(-triphenylphosphine) palladium (1 mg, 0.02 eq). The residue was purified by silica gel column chromatography (hexanes/EtOAc 70:30) to afford **19a** as yellow oil (143 mg, 94%). IR (neat): 3064, 3002, 2955, 2937, 1639, 1596, 1578 cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.35 (br s, 3H), 3.52 (br s, 3H), 3.80 (s, 3H), 3.85 (s, 3H), 5.90–5.93 (m, 1H), 6.09–6.13 (m, 1H), 6.55–6.58 (m, 1H), 6.61–6.64 (m, 1H), 6.78–6.81 (m, 1H), 6.84–6.90 (m, 3H), 7.08–7.09 (m, 1H), 7.14–7.16 (m, 1H), 7.22–7.25 (m, 1H), 7.27 (d, J = 8.0 Hz, 1H), 7.38 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 55.0, 55.28, 55.33, 55.34, 110.7, 111.1, 112.3, 112.7, 113.5, 114.3, 118.15, 118.20, 120.4, 124.6, 128.7, 129.4, 129.9, 134.0, 134.6, 134.8, 141.1, 143.4, 144.2, 159.4, 159.7, 159.9, 167.2; LC–MS (ESI): 460.42 $[M+H]^+$.

*5.3.35. N-2,5-tris(3-hydroxyphenyl)-N-methylthiophene-3-carboxamide (**19**)*

Synthesized according to method C using **19a** (71 mg, 0.15 mmol) and $BF_3.SMe_2$ (283 μL, 2.70 mmol). The residue was triturated in the mixture diethyl ether/petroleum ether to afford **19** as pale green solid (64 mg, 97%). Mp: 139–142 °C. IR (neat): 3253, 2929, 1580 cm^{-1}. ^1H NMR (CD_3SOCD_3) δ 3.25 (br s, 3H), 6.17–6.26 (m, 2H), 6.45–6.43 (m, 2H), 6.72–6.78 (m, 3H), 6.85–7.03 (m, 3H), 7.17–7.33 (m, 3H), 9.37 (br s, 1H), 9.32 (br s, 2H); ^{13}C NMR (CD_3SOCD_3) δ 64.9, 111.8, 113.2, 113.5, 113.9, 115.2, 115.3, 116.0, 116.57, 116.59, 116.7, 117.9, 124.7, 124.8, 129.0, 129.9, 130.2, 133.8, 134.0, 134.3, 144.1, 157.4, 157.6, 157.8; LC–MS (ESI): 417.81 $[M]^+$.

*5.3.36. 5-Bromo-N-(3-methoxybenzyl)-N,3-dimethylthiophene-2-carboxamide (**22b**)*

Synthesized according to method A using 5-bromo-3-methylthiophene-2-carboxylic acid **22** (442 mg, 2 mmol) and 3-methoxy-*N*-methylbenzylamine (298 μL, 2 mmol). The residue was purified by silica gel column chromatography (hexanes/EtOAc 70:30) to afford **22b** as pale brown oil (710 mg, 99%). ^{1}H NMR (CD_3COCD_3) δ 2.22 (s, 3H), 2.95 (s, 3H), 3.79 (s, 3H), 4.65 (s, 2H), 6.85–6.89 (m, 3H), 6.97 (s, 1H), 7.27–7.30 (m, 1H); ^{13}C NMR (CD_3COCD_3) δ 13.5, 33.9, 51.6, 54.1, 111.5, 112.3, 112.6, 119.2, 129.2, 132.2, 132.4, 137.5, 138.2, 159.6, 163.1; LC–MS (ESI): 353.81, 355.88 $[M+H]^+$.

*5.3.37. 5-Bromo-N-(3-hydroxybenzyl)-N,3-dimethylthiophene-2-carboxamide (**22a**)*

Synthesized according to method C using **22b** (625 mg, 1.76 mmol) and $BF_3.SMe_2$ (1.11 mL, 10.59 mmol). The desired product obtained as pale brown oil (598 mg, 99%) was analytically pure and was used in the next step without any purifications. ^{1}H NMR (CD_3COCD_3) δ 2.22 (s, 3H), 2.95 (s, 3H), 4.62 (s, 2H), 6.74–6.79 (m, 3H), 6.97 (s, 1H), 7.16–7.20 (m, 1H); ^{13}C NMR (CD_3COCD_3) δ 14.9, 35.4, 53.1, 113.2, 115.2, 115.4, 119.6, 130.7, 133.4, 133.7, 139.2, 139.4, 158.6, 164.9; LC–MS (ESI): 339.80, 341.81 $[M]^+$.

*5.3.38. 5-(2-Fluoro-3-methoxyphenyl)-N-(3-methoxybenzyl)-N,3-dimethylthiophene-2-carboxamide (**21**)*

Synthesized according to method B using **22a** (326 mg, 0.96 mmol), 2-fluoro-3-methoxyphenylboronic acid (244 mg, 1.44 mmol), cesium carbonate (937 mg, 2.87 mmol) and tetrakis(triphenylphosphine) palladium (22 mg, 0.02 eq.). The residue was purified by silica gel column chromatography (hexanes/EtOAc 60:40) followed by trituration in EtOAc and filtration under reduced pressure to afford **21** as colorless solid (210 mg, 57%). Mp: 123–125 °C. IR (neat): 3162, 2985, 2970, 2935, 1599 cm^{-1}. ^{1}H NMR (CD_3COCD_3) δ 2.29 (s, 3H), 2.97 (s, 3H), 3.92 (s, 3H), 4.66 (s, 2H), 6.76–6.84 (m, 3H), 7.11 (dt, J = 1.6, 8.1 Hz, 1H), 7.16 (dt, J = 1.3, 8.0 Hz, 1H), 7.19 (t, J = 5.8 Hz, 1H), 7.23–7.28 (m, 1H), 7.34 (s, 1H), 8.39 (s, 1H); ^{13}C NMR (CD_3COCD_3) δ 14.9, 16.5, 56.7, 113.71, 113.72, 115.4, 119.7, 120.30, 120.32, 122.7, 122.8, 125.4, 125.5, 129.9, 130.0, 130.6, 132.57, 132.61, 137.1, 137.2, 138.4, 139.8, 148.8, 149.5, 149.6, 150.8, 158.7, 165.5 (additional peaks due to coupling of carbon with fluorine); LC–MS (ESI): 385.89 $[M]^+$.

*5.3.39. 2-(3-Methoxyphenyl)thiophene (**30a**)*

A solution of 2-bromothiophene (326 mg, 8 mmol), 3-methoxyphenylboronic acid (456 mg, 12 mmol), cesium carbonate (1.96 g, 24 mmol) and tetrakis(triphenylphosphine)palladium(0) (46 mg, 0.2 mmol) in O_2-free DME/EtOH/H_2O (3 mL, 1:1:1) was heated under microwave irradiation at 150 °C/150 W for 20 min. After cooling to room temperature water and EtOAc (2 mL, 1:1) were added to quench the reaction. The solution was extracted three times with EtOAc (3 × 10 mL). The organic layer was dried over $MgSO_4$, filtered and the solution was concentrated under reduced pressure. The residue was purified by silica gel column chromatography using hexanes and EtOAc as eluant (95:5) to afford **30a** as pale yellow oil (698 mg, 46%). ^{1}H NMR (CD_3COCD_3) δ 3.85 (s, 3H), 6.88 (ddd, J = 0.7, 2.5, 8.2 Hz, 1H), 7.11 (dd, J = 3.7, 5.1 Hz, 1H), 7.21 (t, J = 1.8 Hz, 1H), 7.23–7.25 (m, 1H), 7.32 (t, J = 8.0 Hz, 1H), 7.44 (dd, J = 1.1, 5.2 Hz, 1H), 7.46 (dd, J = 1.2, 3.7 Hz, 1H); ^{13}C NMR (CD_3COCD_3) δ 55.6, 112.0, 113.9, 119.0, 124.5, 126.0, 129.0, 131.0, 136.6, 144.8, 161.2. LC–MS (ESI): 190.18 $[M]^+$.

*5.3.40. 2-(3-Methoxyphenyl)-1-[5-(3-methoxyphenyl)thiophen-2-yl]-2-methylpropan-1-one (**29a**)*

To a solution of 2-(3-methoxyphenyl)-2-methylpropanoic acid **31b** (510 mg, 2.63 mmol) in dry toluene (10 mL) was added thionyl chloride (760 μL, 10.50 mmol) followed by 5 drops of DMF. The reaction mixture was heated at reflux for 4 h under N_2 atmosphere. After cooling the solvent was removed under reduced pressure to afford the corresponding acyl chloride. To a solution of 2-(3-methoxyphenyl)thiophene **30a** (500 mg, 2.63 mmol) in dry CH_2Cl_2 (10 mL) was added the previously freshly prepared acyl chloride, followed by aluminum chloride (526 mg, 3.94 mmol) at −20 °C and under N_2 atmosphere. After 30 min the ice bath was removed and the reaction mixture was stirred at room temperature overnight. Cold water (10 mL) was added to quench the reaction. The inorganic salts were filtered on Celite and the aqueous layer was extracted three times with CH_2Cl_2. The organic layer was washed with water, dried over $MgSO_4$, filtered and the solution was concentrated under reduce pressure. The residue was purified by silica gel column chromatography using hexanes and EtOAc as eluant (95:5) to afford **29a** as colorless solid (330 mg, 34%). Mp: 109–113 °C. IR (neat): 3018, 2992, 2975, 2951, 2929, 1640, 1605, 1577 cm^{-1}. ^{1}H NMR (CD_3COCD_3) δ 1.63 (s, 6H), 3.79 (s, 3H), 3.84 (s, 3H), 6.85–6.88 (m, 1H), 6.92–6.94 (m, 2H), 6.95 (dd, J = 0.9, 2.6 Hz, 1H), 7.04 (d, J = 4.1 Hz, 1H), 7.18 (t, J = 2.1 Hz, 1H), 7.21 (ddd, J = 0.9, 1.6, 7.8 Hz, 1H), 7.26 (d, J = 4.1 Hz, 1H),7.28–7.34 (m, 2H); ^{13}C NMR (CD_3COCD_3) δ 28.0, 52.0, 55.5, 55.7, 112.3, 112.7, 113.3, 115.4, 119.3, 119.5, 125.2, 130.8, 131.1, 135.3, 135.5, 142.1, 147.9, 151.4, 161.1, 161.2, 196.3. LC–MS (ESI): 367.11 $[M+H]^+$.

*5.3.41. 2-(3-Hydroxyphenyl)-1-[5-(3-hydroxyphenyl)thiophen-2-yl]-2-methylpropan-1-one (**29**)*

Synthesized according to method C using **29a** (250 mg, 0.68 mmol) and $BF_3.SMe_2$ (861 μL, 8.19 mmol). The residue was triturated in the mixture of diethyl ether/petroleum ether to afford **29** as pale green solid (170 mg, 71%). Mp: 209–213 °C. IR (neat): 3363, 3230, 2974, 1621, 1594 cm^{-1}. ^{1}H NMR (CD_3COCD_3) δ 1.60 (s, 6H), 6.77 (ddd, J = 0.9, 2.5, 8.1 Hz, 1H), 6.82 (t, J = 2.0 Hz, 1H), 6.84–6.87 (m, 2H), 7.05 (d, J = 4.1 Hz, 1H), 7.11 (t, J = 2.0 Hz, 1H), 7.13 (ddd, J = 1.0, 1.8, 7.6 Hz, 1H), 7.21 (t, J = 7.9 Hz, 1H), 7.22 (d, J = 4.1 Hz, 1H), 7.24 (t, J = 7.9 Hz, 1H), 8.32 (s, 1H), 8.56 (s, 1H); ^{13}C NMR (CD_3COCD_3) δ 28.0, 51.9, 113.6, 114.2, 114.9, 116.9, 118.16, 118.25, 124.9, 130.8, 131.2, 135.3, 135.4, 141.9, 147.9, 151.5, 158.7, 158.9, 196.4. LC–MS (ESI): 339.10 $[M+H]^+$.

*5.3.42. Methyl 5-(3-methoxyphenyl)thiophene-2-carboxylate (**33a**)*

To a solution of 3-chloro-3-(3-methoxyphenyl)prop-2-enal **33b** (5 g, 25.43 mmol) and dry potassium carbonate (4.22 g, 30.52 mmol) in dry DMF (40 mL) was added drop wise under N_2 atmosphere methyl thioglycolate (2.78 mL, 30.52 mmol) diluted in dry DMF (10 mL). The reaction mixture was heated at 60 °C overnight. After cooling to room temperature the reaction mixture was poured into ice water solution under stirring. The aqueous layer was extracted three times with EtOAc (3 × 30 mL). The organic layer was washed once with water, dried $MgSO_4$, filtered and the solution was concentrated under reduced pressure. The pale brown solid obtained was pure enough to be used in the next step without further purification (1.04 g, 82%). Mp: 38–39 °C. IR (neat, Bruker Tensor 27): 3101, 3071, 3014, 2974, 2943, 2833, 1705, 1601 cm^{-1}. ^{1}H NMR (CD_3COCD_3) δ 3.87 (s, 3H), 3.88 (s, 3H), 6.98 (ddd, J = 0.9, 2.5, 8.2 Hz, 1H), 7.27 (t, J = 2.0 Hz, 1H), 7.31 (ddd, J = 1.0, 1.6, 7.6 Hz, 1H), 7.37 (t, J = 8.1 Hz, 1H), 7.52 (d, J = 4.0 Hz, 1H), 7.76 (d, J = 4.0 Hz, 1H); ^{13}C NMR (CD_3COCD_3) δ 52.5, 55.7, 112.3, 115.5, 119.3, 125.2, 131.2, 133.0, 135.2, 135.4, 151.6, 161.3, 162.8. LC–MS (ESI): 248.94 $[M]^+$.

*5.3.43. 5-(3-Methoxyphenyl)thiophene-2-carboxylic acid (**34**)*

A solution of methyl 5-(3-methoxyphenyl)thiophene-2-carboxylate **33a** (5.15 g, 20.74 mmol) and potassium hydroxide (4.65 g, 82.96 mmol) in a mixture of MeOH/water (100 mL/50 mL) was heated to a slow reflux for 4 h. Methanol was removed under reduced pressure. The aqueous layer was extracted three times with diethyl ether. The aqueous layer was slowly acidified at 0 °C with concentrated hydrochloric acid until pH = 2. The precipitated formed was filtered off and washed twice with water to afford **34** as pale brown solid (519 mg, 53%). Mp: 161–164 °C. IR (neat, Bruker Tensor 27): 2725 (br), 1659, 1601, 1576 cm^{-1}. ^1H NMR (CD_3SOCD_3) δ 3.82 (s, 3H), 6.97 (ddd, J = 0.7, 2.5, 8.2 Hz, 1H), 7.26 (t, J = 1.9 Hz, 1H), 7.28–7.31 (m, 1H), 7.37 (t, J = 8.0 Hz, 1H), 7.59 (d, J = 3.9 Hz, 1H), 7.72 (d, J = 3.9 Hz, 1H), 13.13 (br s, 1H); ^{13}C NMR (CD_3SOCD_3) δ 55.3, 111.2, 114.6, 118.3, 124.9, 130.4, 133.3, 134.1, 134.2, 149.6, 159.8, 162.7. LC–MS (ESI): 234.97 $[M+H]^+$.

*5.3.44. N-methoxy-5-(3-methoxyphenyl)-N-methylthiophene-2-carboxamide (**34a**)*

A solution of 5-(3-methoxyphenyl)thiophene-2-carboxylic acid **34** (4.71 g, 20.12 mmol) and thionyl chloride (5.85 mL, 80.48 mmol) in dry toluene (100 mL) was refluxed for 4 h. The reaction mixture was concentrated to dryness under reduced pressure. CH_2Cl_2 (50 mL) was added and the solution was cooled to 0 °C. *N,O*-dimethylhydroxylamine hydrochloride (2.94 g, 30.18 mmol) was added to the solution followed by Et_3N (4.20 mL, 30.18 mmol). The reaction mixture was warmed up to room temperature and stirred overnight. Water (50 mL) was added to quench the reaction. The aqueous layer was extracted three times with CH_2Cl_2. The organic phase was washed once with HCl 1 N and once with water, before being dried over $MgSO_4$, filtered and the solution was concentrated under reduced pressure. The residue was purified by silica gel column chromatography using hexanes and EtOAc as eluant (70:30) to afford **34a** as orange oil (5.95 g, 97%). IR (neat, Bruker Tensor 27): 3080, 3007, 2974, 2941, 2839, 2372, 1715, 1630, 1607, 1578 cm^{-1}. ^1H NMR (CD_3COCD_3) δ 3.33 (s, 3H), 3.86 (s, 3H), 3.87 (s, 3H), 6.96 (ddd, J = 1.0, 2.6, 8.1 Hz, 1H), 7.28 (t, J = 2.1 Hz, 1H), 7.31 (ddd, J = 1.0, 1.6, 7.6 Hz, 1H), 7.37 (t, J = 8.1 Hz, 1H), 7.49 (d, J = 4.1 Hz, 1H), 7.86 (d, J = 4.1 Hz, 1H); ^{13}C NMR (CD_3COCD_3) δ 33.1, 55.7, 62.1, 112.3, 115.0, 119.2, 124.3, 131.1, 133.7, 135.6, 135.9, 150.9, 161.3, 162.4. LC–MS (ESI): 278.23 $[M+H]^+$.

*5.3.45. 2-(3-Methoxyphenyl)-1-[5-(3-methoxyphenyl)thiophen-2-yl]ethanone (**32a**)*

To the *N*-methoxy-5-(3-methoxyphenyl)-*N*-methylthiophene-2-carboxamide **34a** (2.53 g, 9.11 mmol) in dry THF (20 mL) was added at 0 °C drop wise a solution of 3-methoxybenzylmagnesium chloride 0.25 M in THF (36.4 mL, 9.11 mmol). The reaction mixture was stirred 1 h at 0 °C and warmed up to room temperature overnight. The reaction mixture was quenched at 0 °C by addition of concentrated hydrochloric acid and extracted with EtOAc (4 × 25 mL). The organic layer was washed with water, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using hexanes and EtOAc as eluant (70:30) to afford **32a** as yellow solid (578 mg, 33%). Mp: 61–64 °C. IR (neat): 3076, 3014, 2988, 2957, 2937, 1652, 1578 cm^{-1}. ^1H NMR (CD_3COCD_3) δ 3.77 (s, 3H), 3.86 (s, 3H), 4.25 (s, 2H), 6.82 (dd, J = 2.4, 8.1 Hz, 1H), 6.93–6.99 (m, 3H), 7.23 (t, J = 7.9, 1H), 7.27 (t, J = 1.9, 1H), 7.30–7.32 (m, 1H), 7.37 (t, J = 8.0, 1H), 7.55 (d, J = 3.9 Hz, 1H), 7.99 (d, J = 3.9 Hz, 1H); ^{13}C NMR (CD_3COCD_3) δ 46.1, 55.5, 55.8, 112.4, 112.9, 115.7, 116.2, 119.4, 122.6, 125.7, 130.3, 131.2, 135.2, 135.5, 137.5, 143.7, 153.1, 160.8, 161.3, 190.8. LC–MS (ESI): 338.99 $[M]^+$.

*5.3.46. 2-(3-Hydroxyphenyl)-1-[5-(3-hydroxyphenyl)thiophen-2-yl]ethanone (**32**)*

Synthesized according to method C using **32a** (578 mg, 1.71 mmol) and $BF_3.SMe_2$ (2.16 mL, 20.52 mmol). The residue was recrystallized in a mixture of ethanol/water to afford **32** as yellow solid (251 mg, 48%). Mp: 183–187 °C. IR (neat): 3426, 3151, 3092, 3024, 2971, 1621, 1591 cm^{-1}. ^1H NMR (CD_3COCD_3) δ 4.19 (s, 2H), 6.72 (ddd, J = 0.9, 2.4, 8.0 Hz, 1H), 6.83–6.86 (m, 2H), 6.89 (ddd, J = 1.0, 2.5, 8.1 Hz, 1H), 7.14 (t, J = 7.8, 1H), 7.20 (t, J = 2.1, 1H), 7.22 (ddd, J = 1.1, 1.7, 7.7 Hz, 1H), 7.28 (t, J = 8.1, 1H), 7.49 (d, J = 4.1 Hz, 1H), 7.96 (d, J = 4.1 Hz, 1H), 8.32 (br s, 1H), 8.67 (br s, 1H); ^{13}C NMR (CD_3COCD_3) δ 46.1, 113.7, 114.7, 117.2, 117.3, 118.3, 121.5, 125.4, 130.4, 131.3, 135.2, 135.5, 137.5, 143.6, 153.2, 158.4, 158.9, 190.9. LC–MS (ESI): 310.97 $[M]^+$.

5.4. Biological assays

5.4.1. h17β-HSD2 cell free assay

The placental microsomal enzyme was used for the human 17β-HSD2 cell free assay as described previously [46]. The enzyme h17β-HSD2 was incubated with tritiated E2 (final concentration: 500 nM) in the presence of the cofactor NAD^+ (1500 μM) and potential inhibitor (test concentration: 1 μM) as previously described [46]. Substrate and product were separated by high performance liquid chromatography (HPLC) and the amounts of labeled E2 (substrate) and E1 (product) were quantified. IC_{50} values were determined for compounds showing more than 55% inhibition at 1 μM on h17β-HSD2 as shown in Table 1.

5.4.2. h17β-HSD1 cell free assay

Inhibition of h17β-HSD1 was determined using a similar procedure as for the h17β-HSD2 cell free assay. h17β-HSD1, gained from the placental cytosolic fraction, was incubated with tritiated E1 (final concentration: 500 nM) in the presence of the cofactor NADH (500 μM) and potential inhibitor (test concentration: 1 μM). Separation and quantification of labeled E1 (substrate) and E2 (product) was performed by HPLC using radio flow detection. Selectivity factors were calculated as follows: SF = IC_{50}(h17β-HSD1)/IC_{50}(h17β-HSD2).

5.4.3. ER binding affinity assay

The binding affinity of selected compounds to ERα and ERβ was determined according to the recommendations of the US Environmental Protection Agency (EPA) by their Endocrine Disruptor Screening Program (EDSP) [47] using recombinant human proteins. Briefly, 1 nM of ERα and 4 nM of ERβ, respectively, were incubated with [^{3}H]-E2 (3 nM for ERα and 10 nM for ERβ) and inhibitor. The potential inhibitors were dissolved in DMSO (5% final concentration). Evaluation of non-specific binding was performed with unlabeled E2 at concentrations 100-fold of [^{3}H]-E2 (300 nM for ERα and 1000 nM for ERβ). After incubation, ligand–receptor complexes were selectively bound to hydroxyapatite (83.5 g/L in TE-buffer) and measurements were performed by liquid scintillation counting. From these results the percentage of [^{3}H]-E2 displacement by the compounds was calculated. The plot of percentage displacement versus compound concentration resulted in sigmoidal binding curves. The compound concentrations to displace 50% of the receptor bound [^{3}H]-E2 were determined. Unlabeled E2 IC_{50} values were determined in each experiment and used as reference. The E2 IC_{50} values accepted were 3 nM ± 20% for ERα and 10 nM ± 20% for ERβ. Relative Binding Affinity (RBA) was determined by applying the following equation: RBA[%] = (IC_{50}(E2)/IC_{50}(compound)) × 100 [47]. This results in an RBA value of 100% for E2. Compounds were tested at a concentrations of 1000 × IC_{50}(E2). Compounds with less

than 50% displacement of [^{3}H]-E2 at a concentration of 1000 × IC_{50}(E2) were classified as RBA <0.1%.

5.4.4. m17β-HSD2 cell free assay. Inhibition of E1 formation

According to the potency of novel h17β-HSD2 inhibitors, selected compounds were tested for their ability to inhibit the E1-formation from E2 using the microsomal fraction of mouse liver following a procedure identical to the one described above for h17β-HSD2 [23].

Conflict of interest

Authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.06.036.

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