European Journal of Medicinal Chemistry 82 (2014) 394–406

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Original article

Inhibition of 17β-HSD1: SAR of bicyclic substituted hydroxyphenylmethanones and discovery of new potent inhibitors with thioether linker

Ahmed S. Abdelsamie [a], Emmanuel Bey [b], Nina Hanke [b], Martin Empting [c], Rolf W. Hartmann [a, c], Martin Frotscher [a, *]

[a] Pharmaceutical and Medicinal Chemistry, Saarland University, Campus C23, D-66123 Saarbrücken, Germany
[b] ElexoPharm GmbH, Campus C11, D-66123 Saarbrücken, Germany
[c] Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Campus C23, D-66123 Saarbrücken, Germany

ARTICLE INFO

Article history:
Received 26 September 2013
Received in revised form
27 May 2014
Accepted 31 May 2014
Available online 3 June 2014

Keywords:
Human 17β-Hydroxysteroid dehydrogenase type 1 (*h*17β-HSD1) inhibitors
*m*17β-HSD1
Bicyclic substituted hydroxyphenylmethanones (BSHs)
Estrogen mimetics
Non-steroidal inhibitors
Estrogen-dependent diseases

ABSTRACT

Estradiol is the most potent estrogen in humans. It is known to be involved in the development and proliferation of estrogen dependent diseases such as breast cancer and endometriosis. The last step of its biosynthesis is catalyzed by 17β-hydroxysteroid dehydrogenase type 1 (17β- HSD1) which consequently is a promising target for the treatment of these diseases. Recently, we reported on bicyclic substituted hydroxyphenylmethanones as potent inhibitors of 17β-HSD1. The present study focuses on rational structural modifications in this compound class with the aim of gaining more insight into its structure–activity relationship (SAR). (4-Hydroxyphenyl)-(5-(3-hydroxyphenylsulfanyl)-thiophen-2-yl)methanone (**25**) was discovered as a member of a novel potent class of human 17β-HSD1 inhibitors. Computational methods were used to elucidate its interactions with the target protein. The compound showed activity also towards the *murine* 17β-HSD1 enzyme and thus is a starting point for the design of compounds suitable for evaluation in an animal disease model.

1. Introduction

The important roles of estrogens and androgens in female and male development and reproduction are well known [1]. They exert their effects by transactivation of the respective nuclear receptors [2], although also non-genomic effects are discussed [3]. However, these steroidal sex hormones are also involved in the genesis and the progression of diseases. Estrogens are known to stimulate the progression of estrogen-dependent diseases (EDD) like endometriosis, the majority of breast cancers, and uterine leiomyoma [4–7]. Besides surgery, chemo- and immunotherapy, the inhibition of estrogen biosynthesis and the blockade of estrogen action, respectively, are today standard therapies for these diseases. These treatments (with aromatase inhibitors, GnRH-analogs, antiestrogens, selective estrogen receptor modulators (SERMs)), however, have a systemic mode of action, i.e. they reduce estrogen effects not only in the diseased tissue. As a result, they may lead to considerable side effects. A novel approach mainly aiming at lowering intracellular estrogen production in the diseased tissue could be a significant improvement for EDD therapy. Such an intracrine approach is presently being pursued using steroid sulfatase inhibitors in the treatment of hormone-dependent cancers [8], and is already successfully applied in the treatment of androgen dependent diseases (ADD) by using 5α-reductase inhibitors [9].

More recently, 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1, SDR28C1) has attracted attention as a potential target for the treatment of EDD. The enzyme catalyses the final step of estradiol (E2) biosynthesis which is the most potent estrogen in humans (Fig. 1).

Abbreviations: 17β-HSD1, 17β-hydroxysteroid dehydrogenase type 1; 17β-HSD2, 17β-hydroxysteroid dehydrogenase type 2; E1, estrone; E2, 17β-estradiol; ER, estrogen receptor; SERM, selective estrogen receptor modulator; GnRH, gonadotropin-releasing hormone; NADP(H), nicotinamide adenine dinucleotide phosphate; NAD(H), nicotinamide adenine dinucleotide; RBA, relative binding affinity; DAST, diethylaminosulfur trifluoride; HPLC, high performance liquid chromatography; CC, column chromatography; TLC, thin layer chromatography; DCM, dichloromethane.

* Corresponding author.
E-mail addresses: m.frotscher@mx.uni-saarland.de, m.frotscher@arcor.de (M. Frotscher).

http://dx.doi.org/10.1016/j.ejmech.2014.05.074
0223-5234/

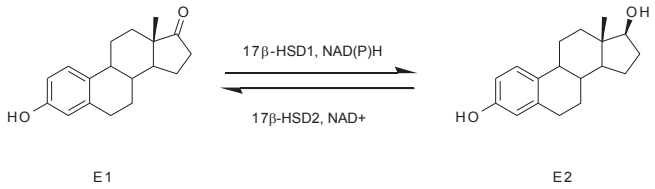


Fig. 1. Interconversion of estrone (E1) and estradiol (E2).

17β-HSD1 is described to be overexpressed at mRNA level in breast cancer tissue [10–12] and endometriotic lesions [13]. Since a more local mode of action can be anticipated compared to existing medical treatments, its selective inhibition is regarded as a promising strategy for the treatment of EDD, with the prospect of less side effects. No 17β-HSD1 inhibitor has entered clinical trials until now, but there is experimental evidence that inhibition of the enzyme is effective against estrone (E1) induced growth of human tumor cells *in vitro* and *in vivo* [14–17]. The availability of compounds not only inhibiting the human enzyme but also 17β-HSD1 of another species would be a prerequisite for a proof of principle study concerning the applicability of 17β-HSD1 inhibitors in the treatment of endometriosis.

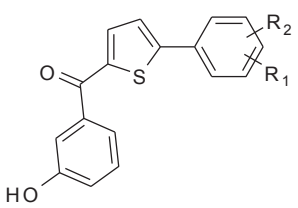
17β-HSD2 can be considered as a functional counterpart of the type 1 enzyme as it de-activates E2 by transforming it to E1. Thus, it plays a protective role against too high E2 concentrations and should therefore not be inhibited by 17β-HSD1 inhibitors.

Both steroidal [18,19] and non-steroidal [20–31] 17β-HSD1 inhibitors have been described in the past. Recently we reported on bicyclic substituted hydroxyphenylmethanones [32] (general structure, Fig. 2) which combine low molecular weight with high inhibitory potency (high ligand efficiency) and show strong intracellular activity. The aim of the present study is to obtain more insight in the structure activity relationships (SARs) of this compound class by rational structural modifications. Moreover, the inhibitory activities of the synthesized compounds are used to verify previous docking results [32] using a broader base of biological data.

2. Design

Starting point for the design of compounds **1**–**28** (Chart 1) were our conceptions concerning the binding mode of bicyclic substituted hydroxyphenylmethanones (Fig. 3) [32]: Previous molecular docking results suggest

a) A hydrogen bond interaction of the hydroxy group of the benzoyl moiety with Asn152
b) A bifurcated H-bond between the carbonyl group and the hydroxyl groups of Ser142 and Tyr155
c) Another bifurcated H-bond between the OH-group of the hydroxyphenyl moiety and the side chains of His221 and Glu282



R_1, R_2: e.g. H, OH, O-alkyl, CN, hetaryl

Fig. 2. Bicyclic substituted hydroxyphenylmethanones: General structure.

d) The close proximity of the side chains of Tyr218 and Ser222 to the inhibitor

In order to evaluate the structure activity relationships, and also to validate the docking results in this compound class, the different structural features mentioned above (a-c) were replaced by possible bioisosteres or other functional groups, see Fig. 4 (modifications a-c). Endeavors to replace the hydroxyl group of the benzoylic part (modification a) were essentially focussed on small, planar substituents which were the basis for subsequent enlargements. This approach reflects previous results which indicate that the introduction of substituents larger than fluorine to the hydroxybenzoyl moiety is detrimental for 17β-HSD1 inhibition [32]. Furthermore, attempts were made to establish additional hydrogen bonding interactions to Tyr218 and Ser222 by introducing an appropriate second linker function between the heterocycle and the hydroxyphenyl moiety (modification d). Due to its ability to act as a hydrogen bond acceptor, the sulfone group was chosen as linker function. The precursors in the syntheses of the prepared sulfones, the corresponding thioethers, have also been tested for activity.

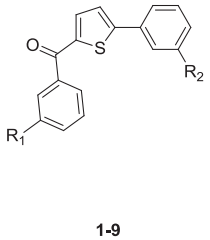
3. Chemistry

The synthesis of compounds **1**–**16** (Scheme 1) started from 2-bromothiophene which – for the preparation of **1**–**5** – was coupled with phenylboronic acid in a Suzuki-reaction [33]. Friedel–Crafts acylation of the resulting intermediate **1a** [34] with the appropriate benzoic acid chloride gave access to compounds **1**–**4** [35]. The intermediate **17c**, which was the starting material for the syntheses according to Scheme 2, was prepared in the same way. Saponification of the ester **4** led to the carbonic acid **5**.

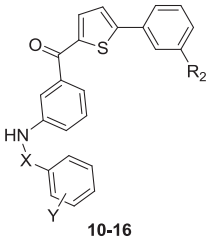
For the synthesis of compounds **6**–**16**, 2-bromothiophene was converted into the ketone **6b** [36] via Friedel–Crafts acylation with 3-nitrobenzoyl chloride. Reduction of the nitro-group with stannous chloride dihydrate yielded amine **6a** which was submitted to a Suzuki reaction with 3-ethoxyphenylboronic acid. The resulting compound **6** was reacted with 2-bromopropane and benzyl bromide to afford the secondary amines **8** and **10**, respectively. In the synthesis of the former, copper(II)-oxide had to be added as a catalyst. The thiourea **9** was obtained by reaction of **6** with methylisothiocyanate. The reaction of **6** with sulfonic acid chlorides at room temperature yielded the corresponding sulfonic acid amides **7** and **11**–**13**. For the analogous preparation of the carbonic acid amides **14** and **15**, higher reaction temperatures had to be applied (pyridine, reflux, overnight). Hydrolysis of the ester **15** under basic conditions resulted in the formation of the carbonic acid **16**.

Starting point for the modifications of the carbonyl group between the aromatic moieties was compound **17c** (Scheme 2). The conversion to the CF_2-group (compound **17a**) with DAST could not be achieved directly but after formation of the thioketone **17b** using Lawesson's reagent [37]. From **17a** and **17b**, the corresponding phenols **17** and **18** could be obtained by ether cleavage (BBr_3, method D) [38]. Reduction of the keto function of compound **17c** with stannous chloride dihydrate yielded the methylene intermediate **19a**, whereas Wittig reaction with methyltriphenylphosphonium bromide afforded the olefin **20a**. Upon treatment with BBr_3 in anhydrous CH_2Cl_2 (method D) both **19a** and **20a** underwent demethylation, resulting in the final compounds **19** and **20**, respectively.

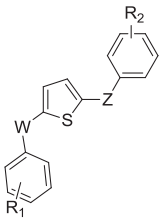
The first step in the synthesis of compounds **21**–**24** was a Friedel–Crafts acylation (method A) of 2-bromothiophene with 3-methoxybenzoylchloride, leading to the benzoylated key intermediate **21b** [32]. The corresponding 4-methoxy isomer **27b**, which was the starting material for one of the syntheses depicted in Scheme 4, was synthesized accordingly. Compounds **21** and **22** were prepared



1-9



10-16



17-28

Cpd	R_1	R_2	X	Y	Cpd	R_1	R_2	W	Z
1	H	H	-	-	**17**	3-OH	H	CF_2	-
2	CH_3	H	-	-	**18**	3-OH	H	C=S	-
3	Cl	H	-	-	**19**	3-OH	H	CH_2	-
4	$COOCH_3$	H	-	-	**20**	3-OH	H	$C=CH_2$	-
5	COOH	H	-	-	**21**	3-OH	3-CH_2OH	C=O	-
6	NH_2	OC_2H_5	-	-	**22**	3-OH	4-CH_2OH	C=O	-
7	$NHSO_2CF_3$	OC_2H_5	-	-	**23**	3-OH	3-$OCH(CH_3)_2$	C=O	-
8	$NHCH(CH_3)_2$	OC_2H_5	-	-	**24**	3-OH	3-$OCH_2CH(CH_3)_2$	C=O	-
9	$NHC(S)NHCH_3$	OC_2H_5	-	-	**25**	3-OH	3-OH	C=O	S
10	-	OC_2H_5	CH_2	H	**26**	3-OH	3-OH	C=O	SO_2
11	-	OC_2H_5	SO_2	H	**27**	4-OH	3-OH	C=O	S
12	-	OC_2H_5	SO_2	3-CN	**28**	4-OH	3-OH	C=O	SO_2
13	-	OC_2H_5	SO_2	4-OCH_3					
14	-	OC_2H_5	CO	H					
15	-	OC_2H_5	CO	CO_2CH_3					
16	-	OC_2H_5	CO	CO_2H					

Chart 1. Synthesized compounds.

from **21b** by Suzuki cross coupling reactions with the appropriate commercially available boronic acids, resulting in compounds **21a** and **22a**, and subsequent demethylation. The synthetic pathway leading from **21b** to the final compounds **23** and **24** required the replacement of the methoxy- by a benzyloxy-group (compound **23c**) which was accomplished via the phenolic intermediate **23d** [32]. A subsequent Suzuki cross coupling reaction with 3-hydroxyphenylboronic acid led to compound **23b** which was alkylated using isopropyl- and isobutyl iodide to afford the ethers **23a** and **24a**, respectively. Selective debenzylation using BCl_3 instead of BBr_3 (method D) yielded the final compounds **23** and **24** (Scheme 3).

The synthesis of sulfides (**25** and **27**) and sulfones (**26** and **28**) is depicted in Scheme 4. The intermediates **21b** and **27b** were transformed into the sulfides **25a** and **27a**, respectively, using 3-methoxybenzenethiol in aqueous dimethylformamide in the presence of potassium hydroxide and copper(I)-oxide as catalyst. The methoxy functions were cleaved with BBr_3 according to method D to yield the hydroxylated compounds **25** and **27**. The sulfones **26** and **28** were obtained from **25** and **27**, respectively, by oxidation with hydrogen peroxide in acetic acid at room temperature.

4. Biological results

4.1. Inhibition of human 17β-HSD1 and selectivity towards human 17β-HSD2

Human placental enzymes were used for both assays and were obtained according to the methods described [39–41]. In the *h*17β-HSD1 assay, incubations were carried out with cytosolic fractions, tritiated E1, cofactor and inhibitor. The separation of substrate and

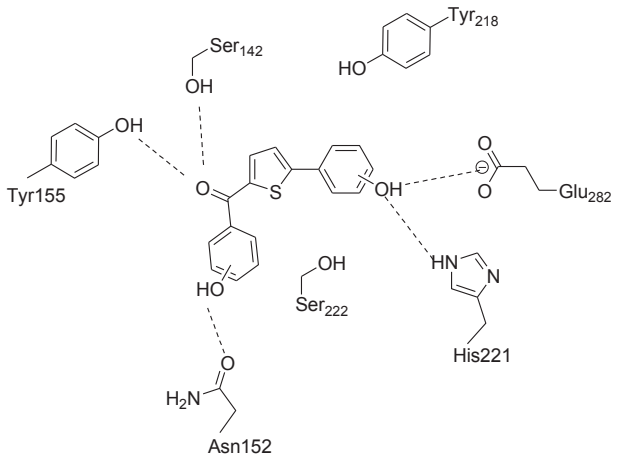


Fig. 3. Schematic H-bond interactions of bicyclic substituted hydroxyphenylmethanones with 17β-HSD1 [32].

product was accomplished by HPLC. The *h*17β-HSD2 assay was performed similarly using tritiated E2 as substrate and a microsomal fraction. Activities are given as percent inhibition at 1 μM (Tables 1–3). For the most active compounds IC_{50} values are reported (Table 3). Compounds **A**–**C** identified in our previous work were used as reference compounds [32]. All methylated intermediates showed no activity towards both *h*17β-HSD1 and 2 (data not shown).

4.1.1. Modification a

In the search for an appropriate replacement of the hydroxy-group at the benzoyl moiety, several structural modifications starting from the reference compounds **A** and **B** were investigated [30]. Simple omission of the OH-group (modification a; Fig. 4), i.e. replacement with H, led to the inactive compound **1** (Table 1). Furthermore, several small and essentially planar functionalities such as Me, Cl, CO_2H, CO_2CH_3, and NH_2 (compounds **2**–**6**, respectively) were introduced. The substituents were chosen considering the Craig plot (variation of the size, lipophilicity, and electronic properties) [42] as well as their different abilities to form hydrogen bond interactions with the target. Compounds **1**–**5** were inactive or showed only marginal inhibition of 17β-HSD1. The introduction of an amino group led to the conservation of a residual activity (compound **6**, 24% inhibition at 1 μM).

Attempts were made to enhance the inhibitory activity of compound **6** by modulating the hydrogen bonding properties of the NH_2-group. For this purpose, several electron-withdrawing or –donating substituents were introduced. These modifications, however, led to a complete loss of activity (compounds **7**–**16**, Chart 1), probably due to the bulkiness of the introduced groups.

4.1.2. Modification b

Modifications of the keto-linker function (modification b; Fig. 4) had a strong impact on biological activity, depending on the nature of the linker group: The thioketo-analog **18** of the reference compound **A** only showed a slightly reduced inhibitory potency (75% vs. 80% inhibn. at 1 μM, Table 2). In contrast, replacement of the keto-group with an olefinic moiety (compound **20**) or saturated groups (compounds **17** and **19**) led to a strong decrease of inhibitory activity. None of the four compounds **17**–**20** showed selectivity over 17β-HSD2.

4.1.3. Modification c

Another structural element under investigation in this study was the phenolic OH-group in ring C which was replaced by a hydroxymethyl-group and different ether functions (modification c; Fig. 4). These structural variations led to highly active

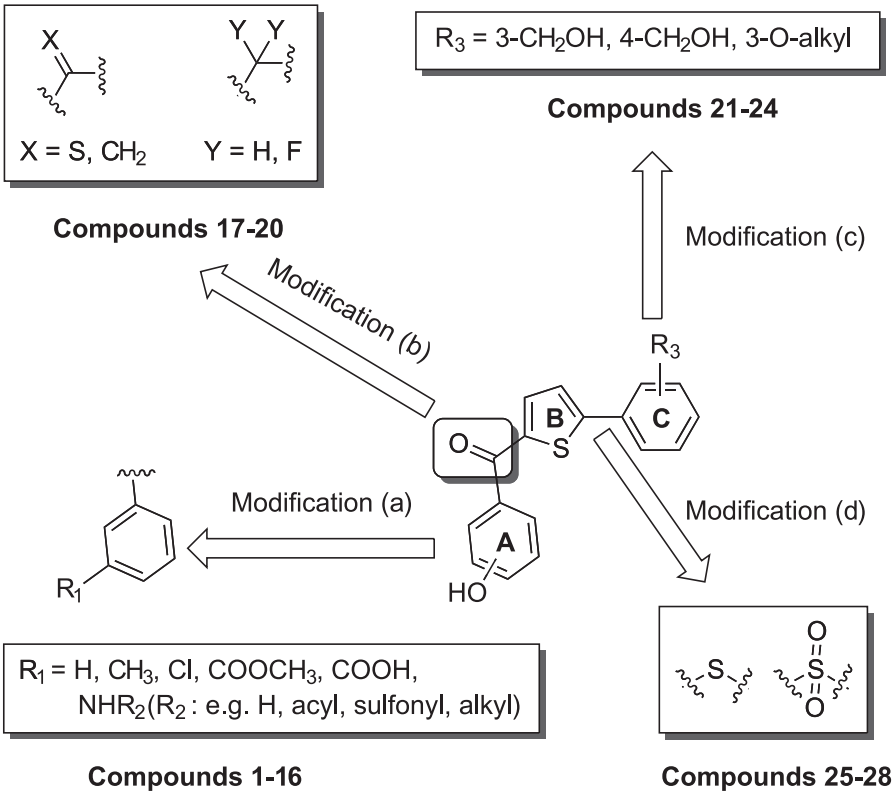
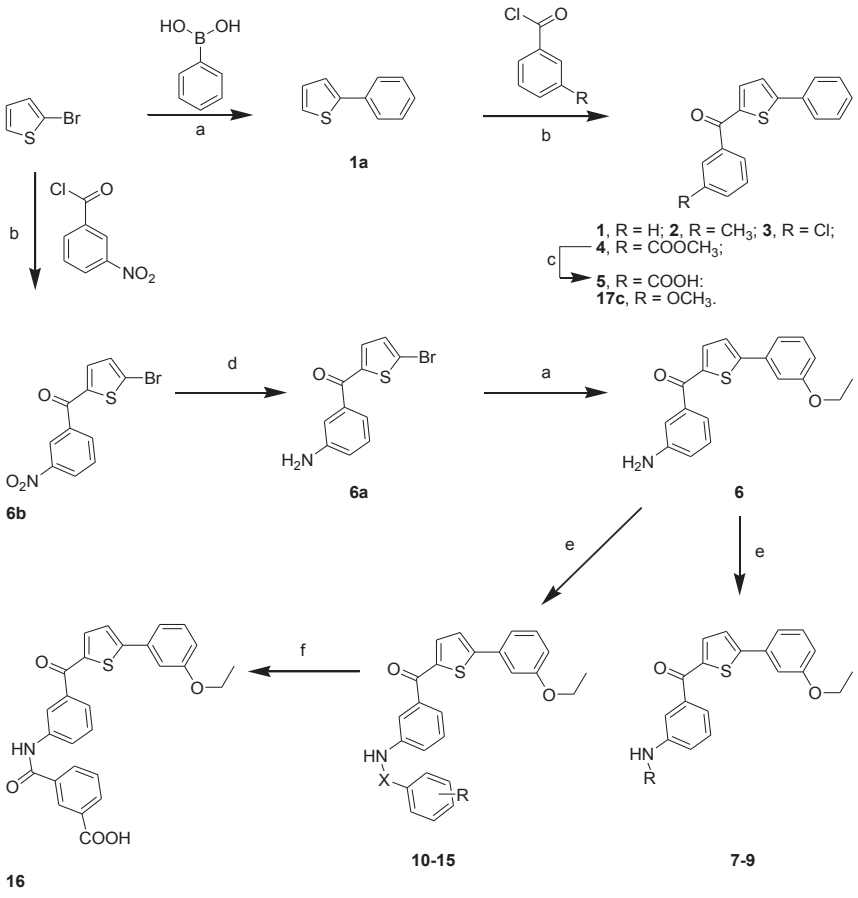


Fig. 4. Design of potential inhibitors **1**–**28**.



cpd	R	cmpd	X	R	cpd	X	R
7	SO_2CF_3	**10**	CH_2	H	**13**	SO_2	4-OMe
8	$CH(CH_3)_2$	**11**	SO_2	H	**14**	CO	H
9	$C(S)NHCH_3$	**12**	SO_2	3-CN	**15**	CO	COOMe

Scheme 1. a) method B, Cs_2CO_3, $Pd(PPh_3)_4$, DME/water (1:1), reflux, 18 h. b) method A, $AlCl_3$, anhydrous CH_2Cl_2, 0 °C, 0.5 h to rt, overnight. c) LiOH, MeOH/H_2O (7:3). d) $SnCl_2 \cdot 2H_2O$, CH_3OH, reflux, 2 h. e) method C, RSO_2Cl or RCOCl, Pyridine, rt or reflux, overnight (compounds **7**, **11**–**15**); 2-bromopropane, CuO, KOH, DMF, reflux, 4 days (compound **8**); CH_3NCS, THF, Et_3N, reflux, overnight (compound **9**); (bromomethyl)benzene, K_2CO_3, acetone, rt, 10 h (compound **10**). f) 10% NaOH, ethanol, reflux, 2 h.

compounds: The formal insertion of CH_2 between the aromatic ring (3- or 4-position) and the OH-group afforded the benzylic alcohols **21** and **22**, showing complete inhibition of *h*17β-HSD1 at a concentration of 1 μM (Table 2). Also the bulky ether derivatives **23** and **24** strongly inhibited the target enzyme. All four compounds displayed similarly low IC_{50}-values in the range of 90 nM–157 nM (Table 3), but no selectivity over the type 2 enzyme with the exception of compound **24** (SF = 5.5).

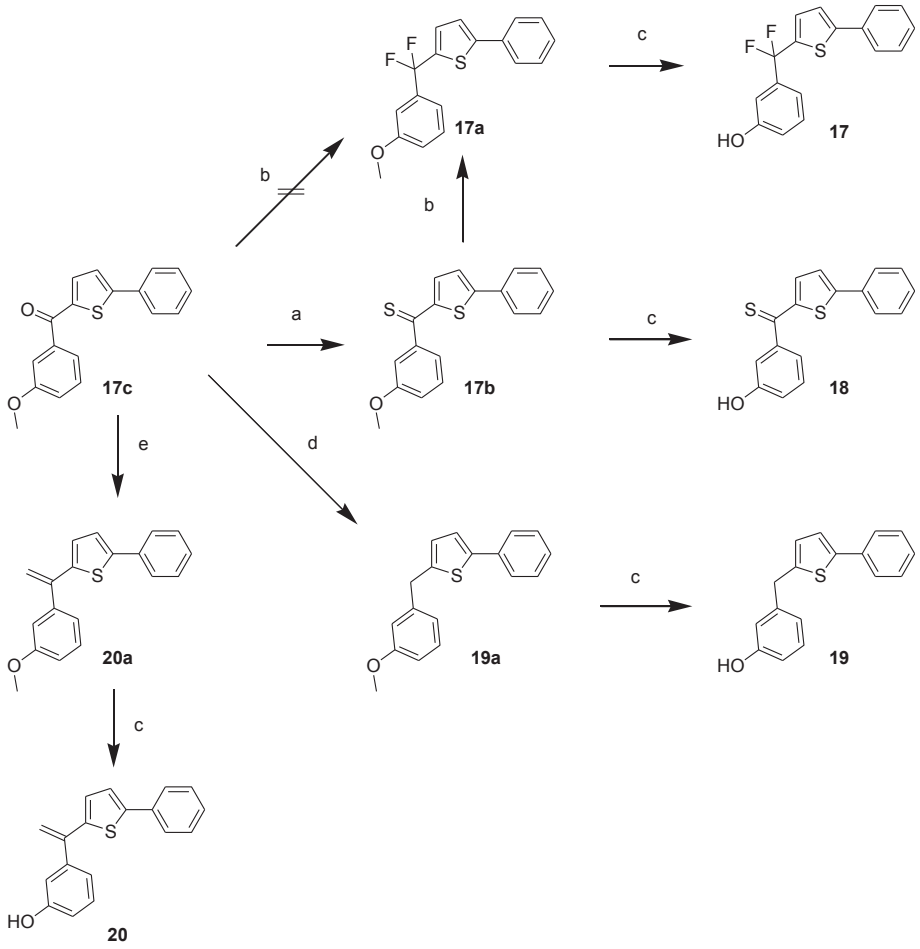
4.1.4. Modification d

Compounds bearing an additional linker group, namely between the thiophene ring and the hydroxyphenyl ring C (modification d; Fig. 4), showed slightly reduced activity towards the target enzyme, compared to the reference **C** (IC_{50} = 22 nM) when the OH-group on the benzoyl moiety was in the 4-position (compounds **27** and **28**, Tables 2 and 3). Moving the OH-group to the 3-position, however, resulted in compounds **25** and **26** with higher activities (IC_{50} = 104 nM and 275 nM, respectively). Thus, regarding the hydroxybenzoyl moiety, the SAR of these novel compounds with two linker functions appears to be similar to that discovered previously for compounds bearing the keto-linker group only [32]. The compounds **25**–**28** showed comparable IC_{50}-values for the inhibition of *h*17β-HSD1 and 2 (Table 3).

4.2. Inhibition of murine 17β-HSD1

The *murine* 17β-HSD1 enzyme was expressed in HEK293 cells. The inhibitory potencies of compound **25** and the reference compound **C** were evaluated in an assay similar to that of the human enzyme.

Compound **25** turned out to be an inhibitor of *murine* 17β-HSD1 (20% inhibn. at 1 μM; **C**: 10%). This is remarkable since the *human* and the *murine* enzymes differ considerably in their primary structure [43] and most of the compound classes described by us to show strong inhibition of *human* 17β-HSD1 are inactive towards the *murine* enzyme (unpublished results). Thus, compound **25** is a



Scheme 2. a) Lawesson's reagent, toluene, reflux, 2 h. b) DAST, abs. CH_2Cl_2, rt, 3 h. c) method D, BBr_3, CH_2Cl_2, −78 °C to rt, overnight. d) $SnCl_2{\cdot}2H_2O$, acetic acid, HCl, reflux, overnight. e) methyltriphenylphosphonium bromide, *n*-BuLi, abs. THF, rt for 2.5 h then 65 °C overnight.

valuable starting point for the design of more potent and selective inhibitors of the *murine* enzymes that allow for *in vivo* evaluation (proof of principle).

5. Molecular modeling

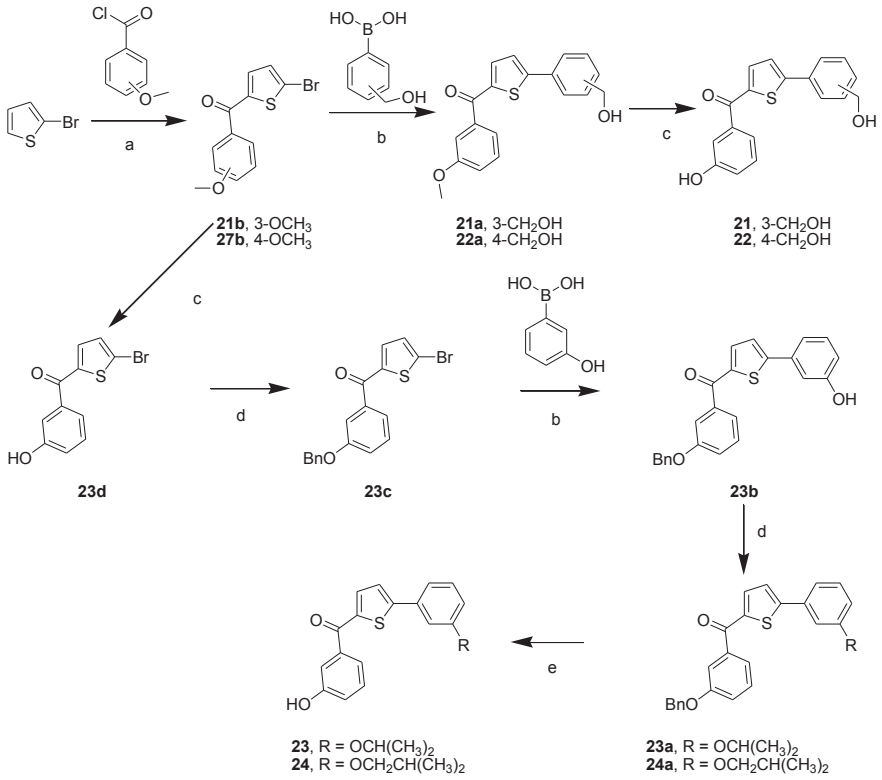
Computational methods were used in order to elucidate the binding modes of the novel inhibitors with two linker functions. Molecular docking results indicate that both thioether **25** and sulfone **26** fit well into the steroid binding pocket of *h*17β-HSD1 (Fig. 5). Interestingly, they dock inversely: The sulfone moiety of compound **26** and the keto-group of **25** are located in the same region of the binding pocket which is distant from Tyr218 and Ser222. An interaction of the sulfone group with these amino acids, which was aimed at, would thus be unlikely.

Ligand-protein interactions can be seen in more detail in Fig. 6: Both compounds form a hydrogen bond with the key amino acid residue, Ser142. Considering compound **25**, this bond is formed via the keto-group of the inhibitor, whereas in case of compound **26** the sulfone moiety is the interaction partner for Ser142 while the keto-group does not appear to play a role in protein binding.

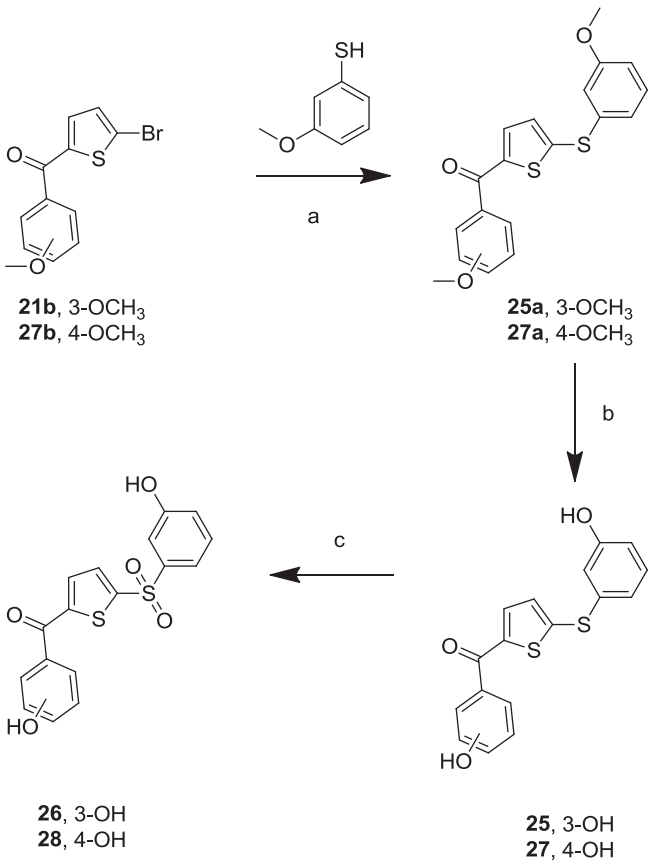
The reversed docking poses of **25** and **26** seem to have an additional effect on ligand–protein interaction: Whereas both phenolic OH-groups of compound **25** are suggested to be involved in hydrogen bonding (with Tyr155 and His221), only one such interaction (with Glu282) can be found for **26**. This is in agreement with the higher inhibitory potency of **25** (IC_{50} = 104 nM; Table 3) compared to **26** (IC_{50} = 275 nM).

6. Discussion and conclusions

The aim of our study was the elucidation of structure activity relationships in the class of bicyclic substituted methanones which have previously been identified as highly active inhibitors of the *h*17β-HSD1 enzyme [30,32]. One of the structural features under investigation was the phenolic OH-group at the benzoyl moiety (ring A) of inhibitors **A** and **B** It was replaced with substituents which covered a broad spectrum of lipophilic and electronic properties and differed in their abilities to form hydrogen bond interactions with the target. The introduction of chlorine appeared interesting to us as it is known that this substituent, due to its σ-hole property, is able to replace classical donors like OH in H-bonding interactions [44]. On the other hand, also hydrogen bond acceptors (such as an ester or a carboxylate function) seemed promising, as it was hypothesized earlier that the OH-group of the inhibitor – as a donor – forms a hydrogen bond with the carbonyl function of the Asn152 side chain [32]. This amino acid residue, however, is in principle also able to act as an H-bond donor via its NH_2-group, allowing for an interaction with an appropriate acceptor function of the inhibitor. Regardless of the properties of the chosen residue, however, a complete loss or a strong reduction of inhibitory activity was observed (compounds **2**–**5**). Similarly



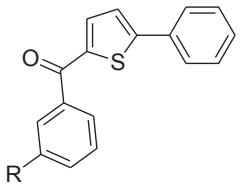
Scheme 3. a) method A, $AlCl_3$, anhydrous CH_2Cl_2, 0 °C, 0.5 h to rt, overnight. b) method B, Cs_2CO_3, $Pd(PPh_3)_4$, DME/water (1:1), reflux, 18 h. c) method D, BBr_3, CH_2Cl_2, −78 °C to rt, overnight. d) R–I, Cs_2CO_3, acetone, reflux, overnight. e) method D, BCl_3, CH_2Cl_2, −78 °C to rt, overnight.



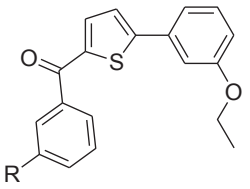
Scheme 4. a) Cu_2O, KOH, DMF, 135 °C, 2 h. b) method D, BBr_3, CH_2Cl_2, −78 °C to rt, overnight. c) H_2O_2 (30%), acetic acid, rt, 4 days.

sharp structure activity relationships have also been reported for other classes of 17β-HSD1 inhibitors [28,45]. At physiological pH, the NH_2-group is the only classical hydrogen bond donor of the substituent selection. Because of its low basicity ($pK_A \approx 3.1$,

Table 1
Inhibition of *human* 17β-HSD1 and 17β-HSD2 by compounds **A**, **B** and **1**–**6**.



A, 1-5

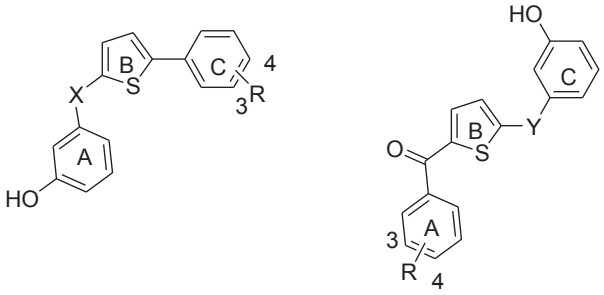


B, 6

Cpd	R	% Inhibition @ 1 μM[a]	
		*h*17β-HSD1[b]	*h*17β-HSD2[c]
A	OH	80	94
1	H	n.i.	n.i.
2	CH_3	14	n.i.
3	Cl	n.i.	n.i.
4	$COOCH_3$	14	11
5	COOH	18	n.i.
B	OH	88	69
6	NH_2	24	n.i.

[a] Mean value of three determinations, standard deviation less than 15%.
[b] Human placenta, cytosolic fraction, substrate [^{3}H]-E1, 500 nM, cofactor NADH, 500 μM.
[c] Human placenta, microsomal fraction, substrate [^{3}H]-E2, 500 nM, cofactor NAD^+, 1500 μM. n.i. = no inhibition (inhibition < 10%).

Table 2
Inhibition of *human* 17β-HSD1 and 17β-HSD2 by compounds **A**, **C** and **17**–**28**.



A, C, 17-24 **25-28**

Cpd	X	R	Y	% Inhibition @ 1 μM[a]	
				*h*17β-HSD1[b]	*h*17β-HSD2[c]
A	C=O	H	–	80	94
17	CF_2	H	–	35	36
18	C=S	H	–	75	85
19	CH_2	H	–	16	29
20	$C=CH_2$	H	–	33	49
C	C=O	3-OH	–	89	89
21	C=O	3-CH_2OH	–	100	100
22	C=O	4-CH_2OH	–	100	81
23	C=O	3-O-isopropyl	–	82	73
24	C=O	3-O-isobutyl	–	85	51
25	–	3-OH	S	84	70
26	–	3-OH	SO_2	70	76
27	–	4-OH	S	66	79
28	–	4-OH	SO_2	62	80

[a] Mean value of three determinations, standard deviation less than 15%.
[b] Human placenta, cytosolic fraction, substrate [^{3}H]-E1, 500 nM, cofactor NADH, 500 μM.
[c] Human placenta, microsomal fraction, substrate [^{3}H]-E2, 500 nM, cofactor NAD^+, 1500 μM. n.i. = no inhibition (inhibition < 10%).

calculated using the PERCEPTA PhysChem software (ACD/Labs, Toronto, Canada)), it should not be protonated and can thus act as a hydrogen bond acceptor as well. The acceptor properties, however, can be expected to be weak, due to delocalization effects. Moreover, also the donor properties of an amino function are generally less pronounced compared to a phenolic OH. Thus, the fact that the amino group (compound **6**) cannot replace the OH-group of compound **B** in a satisfactory manner may be explained by more favorable hydrogen bonding properties of the latter. The biological data are in agreement with docking results that suggest a hydrogen bond interaction, in which the hydroxyl group at the benzoylic moiety acts as a donor.

Table 3
IC_{50} values and selectivity factors for compounds **C** and **21**–**28**.

Cpd	IC_{50} [nM][a]		SF[d]
	*h*17β-HSD1[b]	*h*17β-HSD2[c]	
C	22	109	5.0
21	90	51	0.6
22	157	202	1.3
23	120	224	1.9
24	152	836	5.5
25	104	245	2.4
26	275	283	1.0
27	752	247	0.3
28	630	389	0.6

[a] Mean value of three determinations, standard deviation less than 15%.
[b] Human placenta, cytosolic fraction, substrate [^{3}H]-E1, 500 nM, cofactor NADH, 500 μM.
[c] Human placenta, microsomal fraction, substrate [^{3}H]-E2, 500 nM, cofactor NAD^+, 1500 μM. n.i. = no inhibition (inhibition < 10%).
[d] Selectivity factor: IC_{50}(17β-HSD2)/IC_{50}(17β-HSD1).

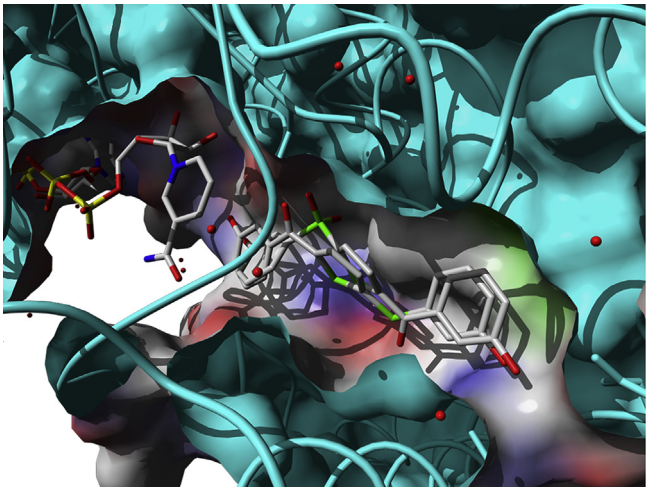


Fig. 5. Compounds **25** and **26**, docked into *human* 17β-HSD1 (PDB: 1FDT). Upper left: Cofactor NADPH.

In contrast to the OH-group at the benzoyl moiety (ring A, cf. Fig. 4) of compound **C**, the hydroxy-function attached to the phenyl-group (ring C) can be replaced or structurally modified to a considerable extent. Substantial attention was paid to the analysis of the role of the keto-group of lead compound **A**. Replacement of the keto- (compound **A**) by the thioketo-group led to a compound with slightly decreased activity (compound **18**, Table 2). This may be explained by the fact that the thioketone retains the geometry of the parent compound **A**, but is a poorer interaction partner for hydrogen bonding. Olefin **20** showed a significant decrease in activity compared to **18** (33% vs. 75% inhibn. at 1 μM), which is in agreement with the lacking hydrogen bonding function. Compounds **17** and **19** were weak inhibitors of 17β-HSD1. Both the difluoromethylene group (compound **17**) and its methylene analog (**19**) show a different geometry compared to compound **A** due to their sp^3-hybridized carbon atoms. The finding that the fluorinated compound **17** is a stronger inhibitor than **19** (35% vs. 16% inhibn. at 1 μM) matches with the fact that fluorine atoms may show weak hydrogen bond accepting capabilities [46]. Thus, the biological data obtained for compounds **17**–**20** is in agreement with former molecular docking studies which suggest a bifurcated H-bond interaction between the carbonyl-group of the inhibitor and Tyr155 and Ser142 [32].

The introduction of a second linker function was triggered by the concept to establish additional hydrogen bond interactions of the inhibitor with the OH groups of Tyr218 and Ser222 (sulfones **26** and **28**). As suggested by molecular docking results of **26** (Fig. 6b), however, they showed a different binding mode compared to the bicyclic substituted hydroxyphenylmethanone class which precludes an establishment of these hydrogen bond interactions.

The structure of the strong inhibitor **25** may be seen as the result of a sequential enlargement of the bis(hydroxyphenyl)thiophene compound class (Fig. 7, D) and can be considered as a starting point for the investigation of a novel and potent class of *h*17β-HSD1 inhibitors bearing two linker groups.

In conclusion, rational structure modifications have been carried out in order to investigate structure–activity relationships of bicyclic substituted hydroxyphenylmethanones (Fig. 7, C) which have recently been described as promising non-steroidal inhibitors of *h*17β-HSD1. The results revealed that inhibitory activity is highly sensitive to changes in the hydroxybenzoyl part of the compounds,

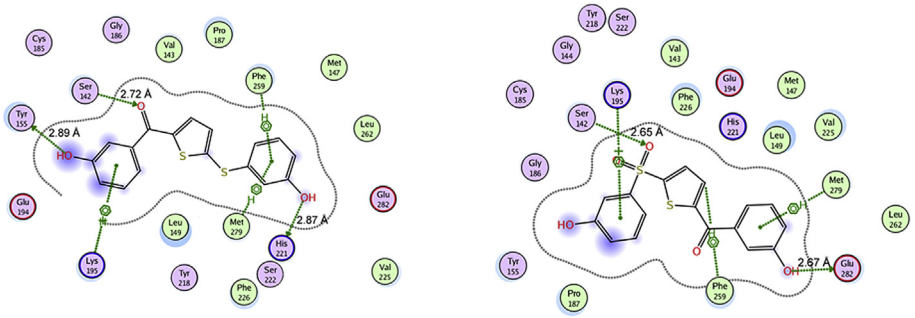
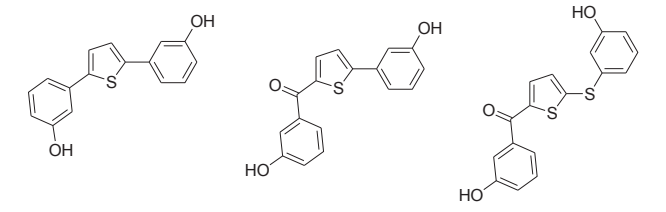


Fig. 6. Suggested binding modes showing three H-bonds to Tyr155, Ser142 and His221 for compound **25** (left) and two H-bonds to Glu282 and Ser142 for **26** (right).



D, (IC_{50} = 173 nM) **C,** (IC_{50} = 22 nM) **25,** (IC_{50} = 104 nM)

Fig. 7. Comparison of potent *h*17β-HSD1 inhibitors: representatives of bis(hydroxyphenyl)thiophenes [31] (D) and bicyclic substituted hydroxyphenylmethanones [32] (C) as well as compound **25**.

whereas in the phenyl moiety, major changes in nature and size of the substituent are well tolerated. The biological data obtained support the conception that the keto linker group is involved in hydrogen-bonding interactions with the target. The introduction of a second linker function led to the discovery of the potent inhibitor (4-hydroxyphenyl)-[5-(3-hydroxyphenylsulfanyl)-thiophen-2-yl] methanone (**25**) which is regarded as a member of a novel inhibitor class and a starting point for further optimization concerning selectivity towards *h*17β-HSD2 and inhibition of *m*17β-HSD1.

7. Experimental section

7.1. Chemical methods

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

Column chromatography (CC) was performed on silica gel (70–200 μm), reaction progress was monitored by thin layer chromatography (TLC) on Alugram SIL G UV_{254} (Macherey–Nagel).

^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker AM500 spectrometer (500 MHz) at 300 K. Chemical shifts are reported in δ (parts per million: ppm), by reference to the hydrogenated residues of deuterated solvent as internal standard (CDCl3: δ = 7.24 ppm (^{1}H NMR) and δ = 77 ppm (^{13}C NMR), CD_3OD: δ = 3.35 ppm (^{1}H NMR) and δ = 49.3 ppm (^{13}C NMR), CD_3COCD_3: δ = 2.05 ppm (^{1}H NMR) and δ = 29.9 ppm (^{13}C NMR), CD_3SOCD_3 δ = 2.50 ppm (^{1}H NMR) and δ = 39.5 ppm (^{13}C NMR)). Signals are described as s, d, t, dd, ddd, m, dt, q, sept for singlet, doublet, triplet, doublet of doublets, doublet of doublets of doublets, multiplet, doublet of triplets, quadruplet and septet, respectively. 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.

A mass spectra ESI was recorded on a TSQ Quantum (Thermo Finnigan) instrument.

Tested compounds are >95% chemical purity as measured by HPLC. The methods for HPLC analysis and a table of data for all tested compounds are provided in the supporting information.

The following compounds were prepared according to previously described procedures: 2-phenylthiophene (**1a**) [34], phenyl-(5-phenylthiophen-2-yl)methanone (**1**) [35], (5-bromo-thiophen-2-yl)-(3-nitrophenyl)methanone (**6b**) [36], (5-bromothiophen-2-yl)(3-methoxy-phenyl)methanone (**21b**) [32] and (5-bromothiophen-2-yl)(3-hydroxyphenyl)methanone (**23d**) [32].

The Supplementary Data section reports the synthesis of compounds **3**–**4**, **11**–**15**, **17c**, **18**–**25**, **21a**–**24a**, **27a**, **23b**, **27b**, **27** and **28**. For each general synthetic procedure, one representative example is given below.

7.2. General procedure for Friedel–Crafts acylation. Method A (*2–4, 6b, 17c, 21b, 27b*)

A mixture of monosubstituted thiophene derivate (1 equiv), arylcarbonyl chloride (0.9 equiv), and aluminum trichloride (1 equiv) in anhydrous dichloromethane was warmed to room temperature and stirred for 3 h. 1 M HCl was used to quench the reaction. The aqueous layer was extracted with dichloromethane. The combined organic layers were washed with brine, dried over magnesium sulfate, filtered, and concentrated to dryness. The product was purified by CC.

7.3. (5-Phenylthiophen-2-yl)(m-tolyl)methanone (*2*)

The product was purified by CC (hexane/ethyl acetate 98:2); yield: 57% (149 mg). ^{1}H NMR (CD_3COCD_3) δ 7.84–7.80 (m, 2H), 7.72 (d, J = 3.8 Hz, 1H), 7.71–7.67 (m, 2H), 7.61 (d, J = 3.8 Hz, 1H), 7.52–7.40 (m, 5H), 2.45 (s, 3H);^{13}C NMR (CD_3COCD_3) δ 186.62, 151.84, 141.70, 137.84, 135.67, 132.62, 132.41, 128.77, 128.63, 127.91, 125.64, 125.52, 124.04, 20.08; MS (ESI): 279.40 $(M + H)^+$.

7.4. Methyl 3-(5-phenylthiophene-2-yl-carbonyl)benzoic acid (*5*)

Methyl 3-(5-phenylthio-phene-2-yl-carbonyl)benzoate (70 mg, 0.22 mmol, 1.00 equiv) (**4**) was dissolved in 5 ml methanol: water (70:30). Lithium hydroxide (15.6 mg, 0.66 mmol, 3.00 equiv) was added and the reaction mixture stirred at 50 °C for 2 h. The mixture was cooled to room temperature, quenched with 1 N NaOH (pH 10–12) and washed two times with ethyl acetate. The aqueous layer was acidified with 1 N HCl to pH 1 and extracted three times with ethyl acetate. The combined organic layers were dried over magnesium sulfate, filtered and evaporated under reduced pressure. No further purification was required; yield: 95% (65 mg; white solid). ^{1}H NMR (CD_3COCD_3) δ 11.46–11.04 (m, 1H), 8.56–8.50 (m, 1H), 8.31 (dt, J = 7.9, 1.1 Hz, 1H), 8.16 (dq, J = 7.6, 1.1 Hz, 1H), 7.86–7.81 (m, 2H), 7.78–7.74 (m, 2H), 7.65 (d, J = 3.8 Hz, 1H),

7.53–7.49 (m, 2H), 7.48–7.43 (m, 1H); ^{13}C NMR (CD_3COCD_3) δ 187.54, 154.02, 142.71, 139.23, 137.47, 134.01, 133.85, 130.74, 130.22, 139.91, 127.10, 125.55; MS (ESI): 309.56 $(M + H)^+$.

7.5. (3-Aminophenyl)(5-bromothiophen-2-yl)methanone (**6a**)

A suspension of **6b** (665 mg, 2 mmol) and tin(II)-chloride dihydrate (2388 mg, 11 mmol) in methanol (10 ml) was refluxed for 2 h. The solvent was removed under vacuum and the residue was diluted with saturated $NaHCO_3$ and water. The suspension was extracted with ethyl acetate. The combined extracts were washed with brine, dried over magnesium sulfate, filtered and evaporated under reduced pressure to give **6a** (450 mg, 75%, brown solid, mp. 102-4 °C). The product was sufficiently pure for use in the subsequent reaction.

7.6. General procedure for Suzuki coupling. Method B (**6, 21a, 22a, 23b**)

A mixture of arylbromide (1 equiv), boronic acid derivative (1.2 equiv), cesium carbonate (4 equiv) and tetrakis(-triphenylphosphine) palladium (0.01 equiv) was suspended in an oxygen-free DME/water (1:1) solution and refluxed under nitrogen for 4 h. The reaction mixture was cooled to room temperature. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, filtered and concentrated to dryness. The product was purified by CC.

7.7. (3-Amino-phenyl)(5-(3-ethoxy-phenyl)-thiophen-2-yl) methanone (**6**)

The product was purified by CC (hexane/ethyl acetate 80:20); yield: 83% (570 mg; yellow solid). ^{1}H NMR ($CDCl_3$) δ 7.54 (d, J = 4.0 Hz, 1H), 7.26–7.22 (m, 2H), 7.18 (dd, J = 1.6, 1.1 Hz, 1H), 7.18–7.14 (m, 2H), 7.13–7.11 (m, 1H), 7.07 (dd, J = 2.1, 1.4 Hz, 1H), 6.83 (ddd, J = 8.2, 2.5, 1.0 Hz, 1H), 6.80 (ddd, J = 7.5, 2.4, 1.6 Hz, 1H), 4.01 (q, J = 7.0 Hz, 2H), 3.78 (s, 2H), 1.37 (t, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 188.17, 159.37, 152.88, 146.62, 142.24, 139.09, 135.78, 135.36, 134.55, 130.10, 129.17, 123.87, 119.37, 118.67, 118.65, 115.06, 112.41, 63.57, 14.75; MS (ESI): 324.00 $(M + H)^+$.

7.8. General procedure for sulfonamide/amide coupling. Method C (**7, 11–15**)

The aminophenyl derivative (1 equiv) was dissolved in absolute pyridine and was spiked with sulfonyl chloride/acid chloride (1.5 equiv). The reaction mixture was stirred overnight at rt (refluxed in case of amide coupling). The reaction was quenched by adding 10 ml of 2 N HCl and extracted with ethyl acetate. The organic layers were washed with saturated $NaHCO_3$ and brine, dried over magnesium sulfate, filtered and concentrated to dryness. The product was purified by CC.

7.9. N-(3-(5-(3-ethoxyphenyl)-thiophene-2-yl-carbonyl)-phenyl)-C,C,C-trifluoro-methanesulfonamide (**7**)

The product was purified by CC (DCM); yield: 28% (60 mg; yellow oil). ^{1}H NMR ($CDCl_3$) δ 7.76–7.72 (m, 1H), 7.67 (d, J = 7.7 Hz, 1H), 7.56–7.49 (m, 2H), 7.41 (t, J = 7.9 Hz, 1H), 7.22 (dd, J = 15.2, 6.0 Hz, 2H), 7.14 (t, J = 3.8 Hz, 1H), 7.11–7.05 (m, 1H), 6.85–6.77 (m, 1H), 3.98 (q, J = 7.0 Hz, 2H), 1.33 (t, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 187.28, 159.43, 154.79, 140.96, 139.04, 136.94, 135.09, 134.16, 130.24, 129.80, 127.58, 126.43, 124.37, 123.62, 121.08, 119.79 (d, J = 323.1 Hz) 118.78, 115.57, 112.50, 63.67, 14.75; MS (ESI): 455.84 $(M + H)^+$.

7.10. (5-(3-Ethoxyphenyl)-thiophen-2-yl)-(3-isopropylamino-phenyl)methanone (**8**)

To a degassed mixture of 2-bromo-propane (123 mg, 1 mmol), cupper II oxide (72 mg, 0.5 mmol) and potassium hydroxide (56 mg, 1 mmol) in DMF (1 ml) was slowly added (3-aminophenyl)-(5-(3-ethoxyphenyl)-thiophen-2-yl)-methanone (**6**) (323 mg, 1 mmol). The resulting mixture was heated at 135 °C for 4 days, allowed to cool to rt and poured into a 0 °C 6 N HCl solution. After 15 min the precipitate was filtered and washed with benzene. The filtrate was extracted with benzene, dried over magnesium sulfate, filtered, and concentrated to dryness. The product was purified by CC (hexane/ethyl acetate 95:5); yield: 46% (168 mg; brownish oil). ^{1}H NMR ($CDCl_3$) δ 7.63 (d, J = 3.9 Hz, 1H), 7.32–7.28 (m, 2H), 7.26–7.22 (m, 2H), 7.20–7.17 (m, 1H), 7.15–7.11 (m, 1H), 7.04–7.01 (m, 1H), 6.89 (ddd, J = 8.2, 2.5, 0.9 Hz, 1H), 6.76 (ddd, J = 8.1, 2.5, 0.9 Hz, 1H), 4.07 (q, J = 7.0 Hz, 2H), 3.67 (sept, J = 12.4, 6.2 Hz, 2H), 1.43 (t, J = 7.0 Hz, 3H), 1.22 (d, J = 6.3 Hz, 6H). ^{13}C NMR ($CDCl_3$) δ 188.75, 159.65, 153.00, 147.81, 142.72, 139.36, 135.92, 134.90, 130.36, 129.34, 124.11, 118.93, 118.10, 117.17, 115.31, 113.20, 112.67, 63.84, 44.43, 23.15, 15.03; MS (ESI): 366.62 $(M + H)^+$.

7.11. 1-(3-(5-(3-Ethoxyphenyl)-thiophene-2-yl-carbonyl)-phenyl)-3-methyl-thiourea (**9**)

A suspension of (3-amino-phenyl)-(5-(3-ethoxyphenyl)-thiophen-2-yl)-methanone (**6**) (205 mg, 0.6 mmol) and CH_3NCS (46 mg, 0.6 mmol) in THF was refluxed overnight. The solution was cooled and the product precipitated with heptane and purified by CC (hexane/ethyl acetate 3:1) to give **9**; yield: 56% (140 mg; yellow solid; mp. 145-6 °C). ^{1}H NMR (CD_3COCD_3) δ 9.14 (br., 1H, NH), 8.14 (d, J = 9.9 Hz, 1H), 7.87 (d, J = 3.9 Hz, 1H), 7.70 (dd, J = 6.2, 3.1 Hz, 1H), 7.64–7.61 (m, 1H), 7.60 (d, J = 4.0 Hz, 1H), 7.51 (t, J = 7.8 Hz, 1H), 7.40–7.36 (m, 1H), 7.35 (dt, J = 7.7, 1.5 Hz, 1H), 7.32–7.30 (m, 1H), 6.98 (ddd, J = 7.8, 2.5, 1.4 Hz, 1H), 4.14 (q, J = 7.0 Hz, 2H), 3.08 (d, J = 4.6 Hz, 3H), 1.40 (t, J = 7.0 Hz, 3H). ^{13}C NMR (CD_3COCD_3) δ 188.17, 175.10, 159.37, 152.88, 146.62, 142.24, 139.09, 135.78, 135.36, 134.55, 130.10, 129.17, 123.87, 119.37, 118.67, 118.65, 115.06, 112.41, 63.57, 35.17, 14.75; MS (ESI): 397.89 $(M + H)^+$.

7.12. (3-Benzylaminophenyl)-(5-(3-ethoxyphenyl)-thiophen-2-yl)-methanone (**10**)

The title compound was prepared by reaction of (3-amino-phenyl)-(5-(3-ethoxyphenyl)-thiophen-2-yl)-methanone (**6**) (135 mg, 0.42 mmol) and (bromomethyl)benzene (108 mg, 0.63 mmol) in acetone (10 ml). The resulting mixture was stirred at rt for 10 h and poured into water. The precipitate was filtered and purified by CC (hexane/ethyl acetate 97:3); yield: 42% (72 mg; yellow oil). ^{1}H NMR ($CDCl_3$) δ 7.35 (d, J = 4.0 Hz, 3H), 7.23 (tdd, J = 10.6, 9.0, 7.2 Hz, 14H), 7.18–7.14 (m, 6H), 7.13–7.11 (m, 7H), 7.08–7.05 (m, 6H), 6.96 (dd, J = 2.2, 1.7 Hz, 3H), 6.78 (ddd, J = 8.2, 2.5, 1.0 Hz, 3H), 6.71 (ddd, J = 8.1, 2.5, 1.0 Hz, 3H), 4.25 (s, 7H), 3.96 (q, J = 7.0 Hz, 6H), 1.32 (t, J = 7.0 Hz, 9H); ^{13}C NMR ($CDCl_3$) δ 188.28, 159.34, 152.70, 147.94, 142.32, 138.91, 138.83, 135.70, 134.56, 130.06, 129.12, 128.66, 127.35, 127.27, 123.86, 118.63, 118.31, 116.67, 114.97, 112.76, 112.41, 63.54, 47.97, 14.74; MS (ESI): 414.81 $(M + H)^+$.

7.13. N-(3-(5-(3-ethoxy-phenyl)-thiophene-2-yl-carbonyl)-phenyl)-isophthalamic acid (**16**)

A reaction of compound **15** (320 mg, 0.66 mmol) in ethanol (5 ml) and 10% sodium hydroxide (15 ml) was refluxed in for 2 h on a water bath. The reaction mixture was cooled, diluted with water and neutralized with acetic acid. The crude product precipitated

and was purified by CC (hexane/ethyl acetate 1:3); yield: 80% (250 mg; greenish-yellow solid; mp. > 280 °C). ^{1}H NMR (CD_3COCD_3) δ 10.06 (s, 1H, COOH), 8.69 (br. s, 1H, NH), 8.46 (s, 1H), 8.21 (dd, J = 37.3, 7.8 Hz, 3H), 7.81 (d, J = 4.0 Hz, 1H), 7.69–7.53 (m, 4H), 7.42–7.30 (m, 3H), 7.02–6.94 (m, 1H), 4.15 (q, J = 7.0 Hz, 2H), 1.40 (t, J = 7.0 Hz, 3H); ^{13}C NMR (CD_3COCD_3) δ 187.63, 165.96, 160.63, 153.48, 143.10, 140.31, 139.25, 137.15, 135.39, 133.71, 133.69, 133.67, 133.66, 132.79, 132.76, 132.72, 131.27, 129.97, 129.65, 125.69, 125.18, 121.73, 119.35, 116.28, 112.89, 64.28, 15.07; MS (ESI): 472.92 $(M + H)^+$.

7.14. (3-Methoxyphenyl)-(5-phenylthiophen-2-yl)-methanethione (**17b**)

(3-methoxyphenyl)(5-phenylthiophen-2-yl)methanone (**17c**) (300 mg, 1.02 mmol, 1.00 equiv) was dissolved under nitrogen in 20 ml abs. toluene and Lawesson's reagent was added. The mixture was stirred for 2 h under reflux and concentrated under reduced pressure. Purification by column chromatography (hexane: ethyl acetate 9:1); yield: 96% (300 mg; dark green oil); Used in the next step without any characterization.

7.15. 2-(Difluoro(3-methoxyphenyl)methyl)-5-phenylthiophene (**17a**)

Under N_2 (3-methoxyphenyl)(5-phenylthiophen-2-yl)methanethione) (**17b**) (100 mg, 0.34 mmol, 1 equiv) was placed in a 100 ml teflon flask and dissolved under nitrogen in 3 ml abs. dichloromethane. Diethylaminosulfur trifluoride (DAST, 167 µl, 1.36 mmol, 3 equiv) to the solution was added slowly at room temperature and the mixture was stirred for 3 h. The reaction was carefully quenched with cold sat. Sodium hydrogen carbonate and extracted three times with diethyl ether. The combined organic layers were washed two times with sat. Sodium hydrogen carbonate, one time with water, one time with brine, dried over magnesium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by CC (hexane/ethyl acetate 85:15); yield: 63% (68 mg, red viscous oil). ^{1}H NMR (CD_3COCD_3) δ 7.71–7.67 (m, 2H), 7.49–7.42 (m, 3H), 7.41–7.35 (m, 2H), 7.23–7.19 (m, 1H), 7.18–7.13 (m, 2H), 7.13–7.10 (m, 1H), 3.87 (s, 3H); ^{13}C NMR (CD_3COCD_3) δ 187.70, 160.70, 153.45, 143.15, 140.35, 137.05, 134.10, 130.55, 130.10, 127.15, 125.50, 122.10, 119.15, 114.50, 55.80; MS (ESI): 317.40 $(M + H)^+$.

7.16. General procedure for ether cleavage. Method D (**17–25, 23d, 27**)

To a solution of ether derivative (1 equiv) in anhydrous dichloromethane at −78 °C (dry ice/acetone bath), boron tribromide in dichloromethane (1 M, 3 equiv per methoxy function) -boron trichloride in dichloromethane (1 M, 2 equiv) in case of **23** and **24**- was added dropwise. The reaction mixture was stirred overnight at room temperature under nitrogen atmosphere. Water was added to quench the reaction, and the aqueous layer was extracted with dichloromethane. The combined organic layers were washed with brine, dried over magnesium sulfate, filtered, and concentrated to dryness. The product was purified by CC.

7.17. 3-(Difluoro(5-phenylthiophen-2-yl)methyl)phenol (**17**)

The product was purified by CC (hexane: ethyl acetate 8:2) followed by preparative TLC (hexane : ethyl acetate 7:3); yield 14% (13 mg; dark green oil). ^{1}H NMR (CD_3COCD_3) δ 7.87–7.85 (m, 1H), 7.52–7.48 (m, 4H), 7.37–7.34 (m, 2H), 7.21–7.17 (m, 1H), 7.15–7.13 (m, 1H), 7.09–7.00 (m, 2H); ^{13}C NMR (CD_3COCD_3) δ 189.55, 168.80, 155.35, 140.30, 135.85, 134.75, 132.40, 128.20, 127.05, 126.40, 121.05, 118.15, 111.00; MS (ESI): 303.35 $(M + H)^+$.

7.18. 2-(3-Methoxybenzyl)-5-phenylthiophene (**19a**)

(3-Methoxyphenyl)(5-phenylthiophen-2-yl)methanone (**17c**) (300 mg, 1.01 mmol, 1 equiv) was dissolved in 20 ml acetic acid, tin (II) chloride dihydrate (1.16 mg, 5.20 mmol, 5.20 equiv) was added in 3.33 ml hydrogen chloride and the mixture was refluxed overnight. The mixture was cooled to room temperature, quenched with water, extracted two times with dichloromethane, dried and evaporated under reduced pressure. The crude product was purified by CC (hexane/ethyl acetate 9:1); yield: 50% (147 mg, white oil). ^{1}H NMR (CD_3COCD_3) δ 7.54–7.61 (m, 2H), 7.32–7.40 (m, 2H), 7.19–7.28 (m, 3H), 6.87–6.92 (m, 3H), 6.75–6.83 (m, 1H), 4.15 (s, 2H), 3.78 (s, 3H).

7.19. 2-(1-(3-Methoxyphenyl)vinyl)-5-phenylthiophene (**20a**)

Methyltriphenylphosphonium bromide (364 mg, 1.01 mmol, 1 equiv) was suspended in 5 ml dry tetrahydrofuran, *n*-butyllithium (400 µl, 2.5 M in hexane) was added dropwise, stirred at room temperature for 90 min, (3-methoxyphenyl)(5-phenylthiophen-2-yl)methanone (**17c**) (300 mg, 1.01 mmol, 1.00 equiv), previously solubilized in 2 ml dry tetrahydrofuran, was added dropwise and stirred at room temperature for 1 h, then at 65 °C overnight. The mixture was cooled to room temperature, quenched with water and extracted two times with ethyl acetate. The combined organic layers were washed one time with water, dried and evaporated under reduced pressure. The crude product was purified by CC (hexane/ethyl acetate 9:1); yield: 35% (105 mg, yellow oil). ^{1}H NMR (CD_3COCD_3) δ 7.68 (d, J = 8.2 Hz, 2H), 7.40–7.45 (m, 2H), 7.38 (d, J = 3.8 Hz, 1H), 7.29–7.36 (m, 2H), 7.01–7.06 (m, 2H), 6.94–7.00 (m, 2H), 5.64 (d, J = 1.5 Hz, 1H), 5.29 (d, J = 1.5 Hz, 1H), 3.83 (s, 3H).

7.20. General procedure for the synthesis of compounds **23c, 23a, 24a**

The intermediates (**23d** or **23b**, 1 equiv) were dissolved in 40 ml acetone under nitrogen. Cesium carbonate (2.10 equiv) and benzyl- or alkyliodide (1.5 equiv) were added and the mixture was stirred at room temperature overnight. The reaction was quenched with water. The combined organic layers were washed one time with water, one time with sat. Sodium hydrogen carbonate, one time with brine, dried over sodium sulfate, filtered and concentrated under vacuum.

7.21. (3-(Benzyloxy)phenyl)(5-bromothiophen-2-yl)methanone (**23c**)

The crude product was recrystallized in hexane/acetone (99:1); yield: 50% (6.9 g, white solid). ^{1}H NMR (CD_3COCD_3) δ 7.46 (t, J = 8.2 Hz, 3H), 7.41–7.36 (m, 5H), 7.33–7.27 (m, 3H), 5.21 (s, 2H); ^{13}C NMR (CD_3COCD_3) δ 186.80, 159.75, 146.15, 139.45, 128.05, 136.50, 132.95, 130.80, 129.45, 128.85, 128.45, 123.00, 122.30, 120.65, 115.55, 70.40; MS (ESI): 373.0 $(M + H)^+$.

7.22. General procedure for the synthesis of compounds **25a, 27a**

3-Methoxybenzenethiol (111 mg, 1.0 mmol) was slowly added to a degassed mixture of the brominated intermediate (**21b** or **27b**; 300 mg, 1.0 mmol), copper(I)-oxide (72 mg, 0.5 mmol) and potassium hydroxide (56 mg, 1.0 mmol) in dimethylformamide (1 ml). The resulting mixture was heated at 135 °C for 3 h, allowed to cool to rt and poured into an ice-cooled 6 N–HCl solution. After 15 min

the precipitate was filtered and washed with benzene. The filtrate was extracted with benzene, dried over magnesium sulfate, filtered, and concentrated under reduced pressure.

7.23. 3-(Methoxyphenyl)-(5-(3-methoxyphenyl)sulfanyl-thiophen-2-yl)-methanone (**25a**)

The product was purified by CC (hexane/ethyl acetate 97:3); yield 75% (270 mg; yellow oil). ^{1}H NMR ($CDCl_3$) δ 7.65 (d, $J = 3.9$ Hz, 1H), 7.54–7.47 (m, 2H), 7.46 (dd, $J = 2.4$, 1.4 Hz, 1H), 7.39–7.36 (m, 1H), 7.26–7.21 (m, 2H), 7.13 (ddd, $J = 7.7$, 1.7, 0.9 Hz, 1H), 7.10–7.07 (m, 1H), 6.95 (ddd, $J = 8.3$, 2.5, 0.8 Hz, 1H), 3.97 (s, 3H), 3.90 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 186.88, 160.17, 159.61, 145.61, 145.27, 138.98, 136.07, 135.10, 131.94, 130.24, 129.40, 122.77, 121.55, 118.61, 116.00, 113.77, 113.66, 55.42, 55.33; MS (ESI): 357.25 $(M + H)^+$.

7.24. General procedure for the synthesis of compounds **26**, **28**

The title compounds were prepared by reaction of the respective thioether (**25** or **27**; 100 mg, 0.30 mmol) and H_2O_2 (30%, 0.1 ml) in acetic acid (2 ml). The solution was allowed to stand at rt for 4 days and was then poured into water. The solid was filtered off, washed with water and dried.

7.25. (5-(3-Hydroxybenzenesulfonyl)-thiophen-2-yl)-(3-hydroxyphenyl)methanone (**26**)

The product was purified by CC (Dichloromethane/methanol 99:1); yield 64% (70 mg, yellow solid, mp. 102 °C). ^{1}H NMR (CD_3OD) δ 7.72 (d, $J = 4.0$ Hz, 1H), 7.61 (d, $J = 4.0$ Hz, 1H), 7.49–7.44 (m, 1H), 7.44–7.38 (m, 2H), 7.33 (t, $J = 7.8$ Hz, 1H), 7.27 (d, $J = 7.7$ Hz, 1H), 7.24–7.20 (m, 1H), 7.07 (dddd, $J = 5.0$, 3.7, 2.5, 0.9 Hz, 2H); ^{13}C NMR (CD_3OD) δ 188.96, 159.83, 159.03, 151.00, 150.83, 143.33, 139.20, 135.32, 134.36, 132.05, 130.97, 122.40, 121.66, 121.56, 119.45, 116.63, 114.92. MS (ESI): 362.23 $(M + H)^+$.

7.26. Biological methods

[2,4,6,7-^{3}H]-E1 and [2,4,6,7-^{3}H]-E2 were bought from Perkin–Elmer, Boston. Quickszint Flow 302 scintillator fluid was bought from Zinsser Analytic, Frankfurt. For a brief description of the biological assays see Supporting Data.

7.27. Molecular modeling

Local docking experiments followed by an energy minimization step were performed with YASARA structure using the AMBER03 force field [47,48]. Protein coordinates were prepared starting from a crystal structure of *human* 17β-HSD1 in complex with estradiol and $NADP^+$ (PDB ID: 1FDT) [49]. After removal of ligand atoms, a grid box of approximately 7.5 nm^3 was set up around the active site of the enzyme. The flexible local docking experiment using the built-in AutoDock 4 algorithm and 999 docking runs was performed with energy-minimized manual models of inhibitors **25** and **26** [50]. Highest-ranked enzyme–inhibitor complexes were subjected to an energy minimization step with fixed protein backbone atoms. Ligand-protein interactions of the yielded structures were analyzed with MOE 2010.

Acknowledgment

We are grateful to the Egyptian Ministry of Higher Education and Scientific Research (MoHESR) and the Deutscher Akademischer Austausch Dienst (DAAD) for financial support of this work (grant number A/09/92319). Thanks are also due to Jessica Hilschmann for her support in the synthetic work.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.05.074.

References

[1] M. Ferin, P.E. Zimmering, S. Lieberman, R.L. Vande Wiele, Inactivation of the biological effects of exogenous and endogenous estrogens by antibodies to 17-beta-estradiol, Endocrinology 83 (1968) 565–571.
[2] J.G. Liehr, Is estradiol a genotoxic mutagenic carcinogen? Endocr. Rev. 21 (2000) 40–54.
[3] J.M. Hall, J.F. Couse, K.S. Korach, The multifaceted mechanisms of estradiol and estrogen receptor signaling, J. Biol. Chem. 276 (2001) 36869–36872.
[4] D.B. Thomas, Do hormones cause breast cancer? Cancer 53 (1984) 595–604.
[5] J. Russo, S.V. Fernandez, P.A. Russo, R. Fernbaugh, F.S. Sheriff, H.M. Lareef, J. Garber, I.H. Russo, 17-Beta-estradiol induces transformation and tumorigenesis in human breast epithelial cells, FASEB J. 20 (2006) 1622–1634.
[6] G.S. Dizerega, D.L. Barber, G.D. Hodgen, Endometriosis: role of ovarian steroids in initiation, maintenance, and suppression, Fertil. Steril. 33 (1980) 649–653.
[7] K. Zeitoun, K. Takayama, H. Sasano, T. Suzuki, N. Moghrabi, S. Andersson, A. Johns, L. Meng, M. Putman, B. Carr, S.E. Bulun, Deficient 17beta-hydroxysteroid dehydrogenase type 2 expression in endometriosis: failure to metabolize 17beta-estradiol, J. Clin. Endocrinol. Metab. 83 (1998) 4474–4480.
[8] A. Purohit, P.A. Foster, Steroid sulfatase inhibitors for estrogen- and androgen-dependent cancers, J. Endocrinol. 212 (2012) 99–110.
[9] F. Picard, E. Baston, W. Reichert, R.W. Hartmann, Synthesis of N-substituted piperidine-4-(benzylidene-4-carboxylic acids) and evaluation as inhibitors of steroid-5alpha-reductase type 1 and 2, Bioorg Med. Chem. 8 (2000) 1479–1487.
[10] C. Gunnarsson, M. Ahnstrom, K. Kirschner, B. Olsson, B. Nordenskjöld, L.E. Rutqvist, L. Skoog, O. Stal, Amplification of HSD17B1 and ERBB2 in primary breast cancer, Oncogene 22 (2003) 34–40.
[11] V. Speirs, A.R. Green, S.L. Atkin, Activity and gene expression of 17beta-hydroxysteroid dehydrogenase type I in primary cultures of epithelial and stromal cells derived from normal and tumourous human breast tissue: the role of IL-8, J. Steroid Biochem Mol. Biol. 67 (1998) 267–274.
[12] T. Suzuki, T. Moriya, N. Ariga, C. Kaneko, M. Kanazawa, H. Sasano, 17betahydroxysteroid dehydrogenase type 1 and type 2 in human breast carcinoma: a correlation to clinicopathological parameters, Br. J. Cancer 82 (2000) 518–523.
[13] T. Šmuc, N. Hevir, M. Ribič-Pucelj, B. Husen, H. Thole, T.L. Rizner, Disturbed estrogen and progesterone action in ovarian endometriosis, Mol. Cell. Endocrinol. 301 (2009) 59–64.
[14] B. Husen, K. Huhtinen, M. Poutanen, L. Kangas, J. Messinger, H. Thole, Evaluation of inhibitors for 17beta-hydroxysteroid dehydrogenase type 1 in vivo in immunodeficient mice inoculated with MCF-7 cells stably expressing the recombinant human enzyme, Mol. Cell. Endocrinol. 248 (2006) 109–113.
[15] B. Husen, K. Huhtinen, T. Saloniemi, J. Messinger, H.H. Thole, M. Poutanen, Human hydroxysteroid (17-beta) dehydrogenase 1 expression enhances estrogen sensitivity of MCF-7 breast cancer cell xenografts, Endocrinology 147 (2006) 5333–5339.
[16] J.M. Day, P.A. Foster, H.J. Tutill, M.F. Parsons, S.P. Newman, S.K. Chander, G.M. Allan, H.R. Lawrence, N. Vicker, B.V. Potter, M.J. Reed, A. Purohit, 17beta-hydroxysteroid dehydrogenase type 1, and not type 12, is a target for endocrine therapy of hormone-dependent breast cancer, Int. J. Cancer 122 (2008) 1931–1940.
[17] P. Kruchten, R. Werth, E. Bey, A. Oster, S. Marchais-Oberwinkler, M. Frotscher, R.W. Hartmann, Selective inhibition of 17beta-hydroxysteroid dehydrogenase type 1 (17betaHSD1) reduces estrogen responsive cell growth of T47-D breast cancer cells, J. Steroid Biochem Mol. Biol. 114 (2009) 200–206.
[18] G. Möller, D. Deluca, C. Gege, A. Rosinus, D. Kowalik, O. Peters, P. Droescher, W. Elger, J. Adamski, A. Hillisch, Structure-based design, synthesis and in vitro characterization of potent 17β-hydroxysteroid dehydrogenase type 1 inhibitors based on 2-substitutions of estrone and D-homo-estrone, Bioorg. Med. Chem. Lett. 19 (2009) 6740–6744.
[19] D. Poirier, Advances in development of inhibitors of 17beta hydroxysteroid dehydrogenases, Anticancer Ag. Med. Chem. 9 (2009) 642–660.
[20] E. Bey, S. Marchais-Oberwinkler, P. Kruchten, M. Frotscher, R. Werth, A. Oster, O. Algül, A. Neugebauer, R.W. Hartmann, Design, synthesis and biological evaluation of bis(hydroxyphenyl) azoles as potent and selective non-steroidal inhibitors of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1) for the treatment of estrogen-dependent diseases, Bioorg Med. Chem. 16 (2008) 6423–6435.
[21] E. Bey, S. Marchais-Oberwinkler, M. Negri, P. Kruchten, A. Oster, T. Klein, A. Spadaro, R. Werth, M. Frotscher, B. Birk, R.W. Hartmann, New insights into the SAR and binding modes of bis(hydroxyphenyl)thiophenes and -benzenes: influence of additional substituents on 17beta-hydroxysteroid dehydrogenase

type 1 (17beta-HSD1) inhibitory activity and selectivity, J. Med. Chem. 52 (2009) 6724–6743.
[22] A. Oster, T. Klein, R. Werth, P. Kruchten, E. Bey, M. Negri, S. Marchais-Oberwinkler, M. Frotscher, R.W. Hartmann, Novel estrone mimetics with high 17beta-HSD1 inhibitory activity, Bioorg Med. Chem. 18 (2010) 3494–3505.
[23] G.M. Allan, N. Vicker, H.R. Lawrence, H.J. Tutill, J.M. Day, M. Huchet, E. Ferrandis, M.J. Reed, A. Purohit, B.V. Potter, Novel inhibitors of 17beta-hydroxysteroid dehydrogenase type 1: templates for design, Bioorg Med. Chem. 16 (2008) 4438–4456.
[24] P. Brožič, P. Kocbek, M. Sova, J. Kristl, S. Martens, J. Adamski, S. Gobec, T. Lanišnik Rižner, Flavonoids and cinnamic acid derivatives as inhibitors of 17beta-hydroxysteroid dehydrogenase type 1, Mol. Cell. Endocrinol. 301 (2009) 229–234.
[25] S. Karkola, A. Lilienkampf, K. Wähälä, A 3D QSAR model of 17beta-HSD1 inhibitors based on a thieno[2,3-d]pyrimidin-4(3H)-one core applying molecular dynamics simulations and ligand-protein docking, ChemMedChem 3 (2008) 461–472.
[26] A. Lilienkampf, S. Karkola, S. Alho-Richmond, P. Koskimies, N. Johansson, K. Huhtinen, K. Vihko, K. Wahala, Synthesis and biological evaluation of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1) inhibitors based on a thieno[2,3-d]pyrimidin-4(3H)-one core, J. Med. Chem. 52 (2009) 6660–6671.
[27] D. Schuster, L.G. Nashev, J. Kirchmair, C. Laggner, G. Wolber, T. Langer, A. Odermatt, Discovery of nonsteroidal 17beta-hydroxysteroid dehydrogenase 1 inhibitors by pharmacophore-based screening of virtual compound libraries, J. Med. Chem. 51 (2008) 4188–4199.
[28] S. Starčević, P. Brožič, S. Turk, J. Cesar, T.L. Rižner, S. Gobec, Synthesis and biological evaluation of (6- and 7-phenyl) coumarin derivatives as selective nonsteroidal inhibitors of 17beta-hydroxysteroid dehydrogenase type 1, J. Med. Chem. 54 (2011) 248–261.
[29] S. Starčević, S. Turk, B. Brus, J. Cesar, T. Lanišnik Rižner, S. Gobec, Discovery of highly potent, nonsteroidal 17beta-hydroxysteroid dehydrogenase type 1 inhibitors by virtual high-throughput screening, J. Steroid Biochem Mol. Biol. 127 (2011) 255–261.
[30] A. Oster, T. Klein, C. Henn, R. Werth, S. Marchais-Oberwinkler, M. Frotscher, R.W. Hartmann, Bicyclic substituted hydroxyphenylmethanone type inhibitors of 17 beta-hydroxysteroid dehydrogenase Type 1 (17 beta-HSD1): the role of the bicyclic moiety, ChemMedChem 6 (2011) 476–487.
[31] E. Bey, S. Marchais-Oberwinkler, R. Werth, M. Negri, Y.A. Al-Soud, P. Kruchten, A. Oster, M. Frotscher, B. Birk, R.W. Hartmann, Design, synthesis, biological evaluation and pharmacokinetics of bis(hydroxyphenyl) substituted azoles, thiophenes, benzenes, and aza-benzenes as potent and selective nonsteroidal inhibitors of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1), J. Med. Chem. 51 (2008) 6725–6739.
[32] A. Oster, S. Hinsberger, R. Werth, S. Marchais-Oberwinkler, M. Frotscher, R.W. Hartmann, Bicyclic substituted hydroxyphenylmethanones as novel inhibitors of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1) for the treatment of estrogen-dependent diseases, J. Med. Chem. 53 (2010) 8176–8186.
[33] N. Miyaura, T. Yanagi, A. Suzuki, The palladium-catalyzed cross-coupling reaction of phenylboronic acid with haloarenes in the presence of bases, Synth. Commun. 11 (1981) 513–519.
[34] L.-C. Liang, P.-S. Chien, M.-H. Huang, Catalytic suzuki coupling reactions by amido phosphine complexes of palladium, Organometallics 24 (2005) 353–357.
[35] J.C. Meslin, Y.T. N'Guessan, H. Quiniou, F. Tonnard, Enchainements heteroatomiques et leurs produits de cyclisationâ–I: Vinylogues de thioamides comme intermediaires de synthese d'acyl-2 thiophenes, thio-1 pyrannones-2 (thiones), dihydro-5,6 dithiinnes-1,2 dioxydes-1,1 et dithiinnes-1,2 dioxydes-1,1 substitues, Tetrahedron 31 (1975) 2679–2684.
[36] C.-M. Liu, B.-H. Chen, W.-Y. Liu, X.-L. Wu, Y.-X. Ma, Conversion of tributylstannylferrocene to a variety of heteroaryl ferrocenes, J. Organomet. Chem. 598 (2000) 348–352.
[37] G.S. Lal, E. Lobach, A. Evans, Fluorination of thiocarbonyl compounds with Bis(2-methoxyethyl)aminosulfur trifluoride (Deoxo-fluor reagent): a facile synthesis of gem-difluorides, J. Org. Chem. 65 (2000) 4830–4832.
[38] B.E. Fink, D.S. Mortensen, S.R. Stauffer, Z.D. Aron, J.A. Katzenellenbogen, Novel structural templates for estrogen-receptor ligands and prospects for combinatorial synthesis of estrogens, Chem. Biol. 6 (1999) 205–219.
[39] P. Kruchten, R. Werth, S. Marchais-Oberwinkler, M. Frotscher, R.W. Hartmann, Development of a biological screening system for the evaluation of highly active and selective 17beta-HSD1-inhibitors as potential therapeutic agents, Mol. Cell. Endocrinol. 301 (2009) 154–157.
[40] W. Qiu, R.L. Campbell, A. Gangloff, P. Dupuis, R.P. Boivin, M.R. Tremblay, D. Poirier, S.X. Lin, A concerted, rational design of type 1 17beta-hydroxysteroid dehydrogenase inhibitors: estradiol-adenosine hybrids with high affinity, FASEB J. 16 (2002) 1829–1831.
[41] K.M. Sam, S. Auger, V. Luu-The, D. Poirier, Steroidal spiro-gamma-lactones that inhibit 17 beta-hydroxysteroid dehydrogenase activity in human placental microsomes, J. Med. Chem. 38 (1995) 4518–4528.
[42] P.N. Craig, Interdependence between physical parameters and selection of substituent groups for correlation studies, J. Med. Chem. 14 (1971) 680–684.
[43] T. Klein, C. Henn, M. Negri, M. Frotscher, Structural basis for species specific inhibition of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1): computational study and biological validation, PLoS One 6 (2011) e22990.
[44] R. Wilcken, M.O. Zimmermann, A. Lange, A.C. Joerger, F.M. Boeckler, Principles and applications of halogen bonding in medicinal chemistry and chemical biology, J. Med. Chem. 56 (2013) 1363–1388.
[45] M. Frotscher, E. Ziegler, S. Marchais-Oberwinkler, P. Kruchten, A. Neugebauer, L. Fetzer, C. Scherer, U. Muller-Vieira, J. Messinger, H. Thole, R.W. Hartmann, Design, synthesis, and biological evaluation of (hydroxyphenyl)naphthalene and -quinoline derivatives: potent and selective nonsteroidal inhibitors of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1) for the treatment of estrogen-dependent diseases, J. Med. Chem. 51 (2008) 2158–2169.
[46] B.E. Smart, Fluorine substituent effects (on bioactivity), J. Fluor. Chem. 109 (2001) 3–11.
[47] Y. Duan, C. Wu, S. Chowdhury, M.C. Lee, G. Xiong, W. Zhang, R. Yang, P. Cieplak, R. Luo, T. Lee, J. Caldwell, J. Wang, P. Kollman, A point-charge force field for molecular mechanics simulations of proteins based on condensed-phase quantum mechanical calculations, J. Comput Chem. 24 (2003) 1999–2012.
[48] E. Krieger, T. Darden, S.B. Nabuurs, A. Finkelstein, G. Vriend, Making optimal use of empirical energy functions: force-field parameterization in crystal space, Proteins 57 (2004) 678–683.
[49] R. Breton, D. Housset, C. Mazza, J.C. Fontecilla-Camps, The structure of a complex of human 17beta-hydroxysteroid dehydrogenase with estradiol and NADP+ identifies two principal targets for the design of inhibitors, Structure 4 (1996) 905–915.
[50] G.M. Morris, R. Huey, W. Lindstrom, M.F. Sanner, R.K. Belew, D.S. Goodsell, A.J. Olson, AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility, J. Comput. Chem. 30 (2009) 2785–2791.