European Journal of Medicinal Chemistry xxx (2016) 1–14

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Research paper

Treatment of estrogen-dependent diseases: Design, synthesis and profiling of a selective 17β-HSD1 inhibitor with sub-nanomolar IC_{50} for a proof-of-principle study

Ahmed S. Abdelsamie [a, b], Chris J. van Koppen [c], Emmanuel Bey [c], Mohamed Salah [a], Carsten Börger [d], Lorenz Siebenbürger [d], Matthias W. Laschke [e], Michael D. Menger [e], Martin Frotscher [a, *]

[a] Pharmaceutical and Medicinal Chemistry, Saarland University, Campus C23, D-66123 Saarbrücken, Germany
[b] Chemistry of Natural and Microbial Products Department, National Research Centre, Dokki, 12622 Cairo, Egypt
[c] ElexoPharm GmbH, Campus A12, D-66123 Saarbrücken, Germany
[d] PharmBioTec GmbH, Science Park 1, D-66123 Saarbrücken, Germany
[e] Institute for Clinical and Experimental Surgery, Saarland University, D-66421, Homburg/Saar, Germany

ARTICLE INFO

Article history:
Received 22 September 2016
Received in revised form
2 November 2016
Accepted 3 November 2016
Available online xxx

Keywords:
17β-Hydroxysteroid dehydrogenase type 1
Enzyme inhibitor
Steroidogenic enzymes
Estrogen-dependent disease
Intracrinology

ABSTRACT

Current endocrine therapeutics for the estrogen-dependent disease endometriosis often lead to considerable side-effects as they act by reducing estrogen action systemically. A more recent approach takes advantage of the fact that the weak estrogen estrone (E1) which is abundant in the plasma, is activated in the target cell to the highly estrogenic estradiol (E2) by 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1). 17β-HSD1 is overexpressed in endometriosis and thus a promising target for the treatment of this disease, with the prospect of less target-associated side-effects. Potent inhibitors from the class of bicyclic substituted hydroxyphenylmethanones with sulfonamide moiety recently described by us suffered from high molecular weight and low selectivity over 17βHSD2, the physiological adversary of 17β-HSD1. We describe the structural optimizations leading to the discovery of (5-(3,5-dichloro-4-methoxyphenyl)thiophen-2-yl)(2,6-difluoro-3-hydroxyphenyl)methanone **20**, which displayed a sub-nanomolar IC_{50} towards 17β-HSD1 as well as high selectivity over the type 2 enzyme, the estrogen receptors α and β and a range of hepatic CYP enzymes. The compound did neither show cellular toxicity, nor PXR activation nor mutagenicity in the AMES II assay. Additional favourable pharmacokinetic properties (rat) make **20** a suitable candidate for proof-of-principle studies using xenotransplanted immunodeficient rats.

1. Introduction

17β-Hydroxysteroid-dehydrogenase type 1 (17β-HSD1) is a member of the NADPH/NAD^+-dependent oxidoreductases. It catalyses the activation of estrone (E1) to the most potent estrogen estradiol (E2; Fig. 1) within the target cell. Besides its beneficial physiological effects, E2 is also known to play crucial roles in the development of estrogen-dependent diseases (EDD). Thus, endometriosis [1], breast cancer [2,3], ovarian tumor [4] and other EDD are typically attended by locally increased E2/E1-ratios and high levels of 17β-HSD1 mRNA in the diseased tissue. Therefore, inhibition of 17β-HSD1 is considered to be a valuable treatment option for EDD: The tissue-selective expression of 17β-HSD1 and its intracrine mode of action [5] offer the prospect of a therapy which

Abbreviations: (*h*)17β-HSD1, (human) 17β-hydroxysteroid dehydrogenase type 1; (*h*)17β-HSD2, (human) 17β-hydroxysteroid dehydrogenase type 2; ADME, absorption, distribution, metabolism, and excretion; BSHs, bicyclic substituted hydroxyphenylmethanones; CC, column chromatography; DBPO, dibenzoyl peroxide; DCM, dichloromethane; DME, dimethoxyethane; E1, estrone; E2, 17β-estradiol; EDD, estrogen-dependent disease; ER, estrogen receptor; GnRH, gonadotropin-releasing hormone; HPLC, high performance liquid chromatography; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NAD(H), nicotinamide adenine dinucleotide; NADP(H), nicotinamide adenine dinucleotide phosphate; NBS, *N*-bromosuccinimide; PXR, pregnane X receptor; RBA, relative binding affinity; SEM, standard error of the mean; SERM, selective estrogen receptor modulator; SF, selectivity factor over 17β-HSD2; TLC, thin layer chromatography.

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

http://dx.doi.org/10.1016/j.ejmech.2016.11.004
0223-5234/

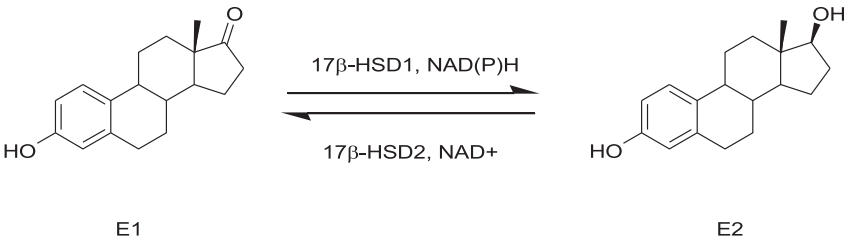


Fig. 1. Interconversion of E1 and E2.

is associated with less side-effects compared to established (but unsatisfactory) treatments with GnRH-analogues, [6,7], aromatase-inhibitors [8–13], anti-estrogens [14] and selective estrogen-receptor modulators (SERMs) [14]. The validity of this concept is supported by the observation that a 17β-HSD1 inhibitor led to a decrease of E2-levels in endometriotic specimens [15]. In addition, 17β-HSD1 inhibitors were shown to reduce the E1-stimulated tumor cell growth *in vitro* and in animal models, suggesting the suitability of this target for the treatment of breast cancer [16–18]. 17β-HSD1 inhibitors should be selective over 17β-HSD2, the physiological adversary of 17β-HSD1 which inactivates E2 by oxidation to E1 (Fig. 1). Moreover, they should not bind to estrogen receptors in order to keep systemic interference with estrogenic pathways to a minimum.

A number of 17β-HSD1 inhibitors are described in the literature, many of them with a steroidal scaffold [19–25]. Our group reported on several classes of non-steroidal 17β-HSD1 inhibitors, [26–30], among them the bicyclic substituted hydroxyphenylmethanones (BSHs) which displayed very strong inhibition of the target protein [26,29]. Previous studies in this compound class revealed that inhibition of the target enzyme strongly depends on the substitution pattern of the benzoyl ring (Fig. 2, ring A) [26,29]. Here, already minor structural modifications were found to induce dramatic changes in activity. Thus, bulky substituents led to a loss of activity whereas the introduction of fluorine atoms resulted in considerably more active compounds [26,29]. In contrast, it was rather selectivity (towards 17β-HSD2) than inhibitory potency which was influenced by substituents at the phenyl ring (ring C) [26].

The majority of these SAR data was derived from inhibitors bearing a bulky aromatic sulfonamide moiety. There was evidence, however, that - in terms of biological activities towards human 17β-HSD1 and 2 - the sulfonamides do not have substantial advantages compared to compounds devoid of the sulfonamide group (Fig. 2) [29]. Consequently, the design and synthesis of novel potential inhibitors lacking this group was aimed at, thus lowering molecular weight while preserving or increasing inhibitory potency and selectivity.

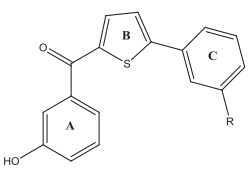
2. Design

As a starting point, structural modifications of lead compound **A** (Chart 1) were carried out focussing on the substitution patterns of rings A and C (compounds **1**–**25**, Chart 1), taking into account the following previous SAR data:

- The possibilities for variations of ring A-substitution are very restricted: An OH-group in position 3 of ring A is important for inhibitory activity towards 17β-HSD1 and should be retained. In addition, the target enzyme only tolerates small additional substituents on ring A.
- Much more flexibility exists concerning the substitution pattern of ring C: here, even bulky substituents should be tolerated, and the OH-group can be omitted – albeit its presence can be expected to lead to increased selectivity over 17β-HSD2.

First, based upon a simplified analogue of lead **A** (compound **1**, Chart 1), the synthesis of a couple of compounds bearing small substituents, especially fluorine, on ring A was envisaged in order to identify a beneficial substitution pattern (compounds **2**–**5**) which should be maintained in the further design process. Subsequent structural modifications aimed at the optimization of ring C and its substitution pattern. To this purpose, electron-donating and –withdrawing groups of different sizes were introduced. In addition, the effect of a replacement of benzene with pyridine was evaluated (compounds **6**–**25**).

Moreover, compounds **26** and **27** as non-fluorinated analogues of **20** and **21** were synthesized to re-evaluate the effect of the fluorine atoms on ring A on activity and selectivity. Finally, the phenyl ring C of the most interesting compounds **20** and **21** was decorated with heterocyclic moieties in order to increase solubility (compounds **28**–**32**, Chart 2).



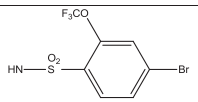
compound	R	IC_{50} (nM) 17β-HSD1	17β-HSD2	M (g/mol)
A		8	199	598
B	OH	22	109	296

Fig. 2. Comparison of compound **A** with sulfonamide moiety and compound **B**, lacking this group.

Please cite this article in press as: A.S. Abdelsamie, et al., Treatment of estrogen-dependent diseases: Design, synthesis and profiling of a selective 17β-HSD1 inhibitor with sub-nanomolar IC_{50} for a proof-of-principle study, European Journal of Medicinal Chemistry (2016), http://dx.doi.org/10.1016/j.ejmech.2016.11.004

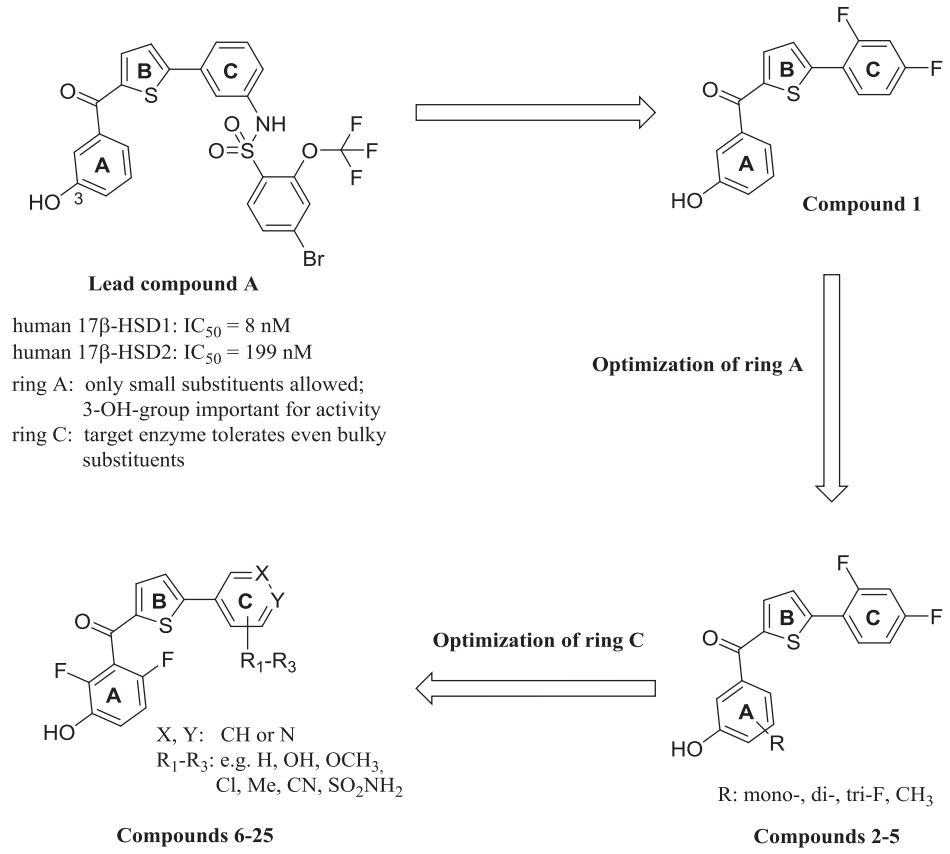


Chart 1. Design of synthesized compounds **1**–**25**.

3. Chemistry

The key intermediates **I**–**V** were synthesized in good yield from the appropriate benzoyl chlorides and 2-bromothiophene using Friedel-Crafts conditions (method A). Subsequent ether cleavage of intermediates **I** and **IV** (method B) yielded **VI** and **VII**, respectively, in a quantitative yield (Scheme 1).

For the preparation of compounds **1**–**7** the key intermediates

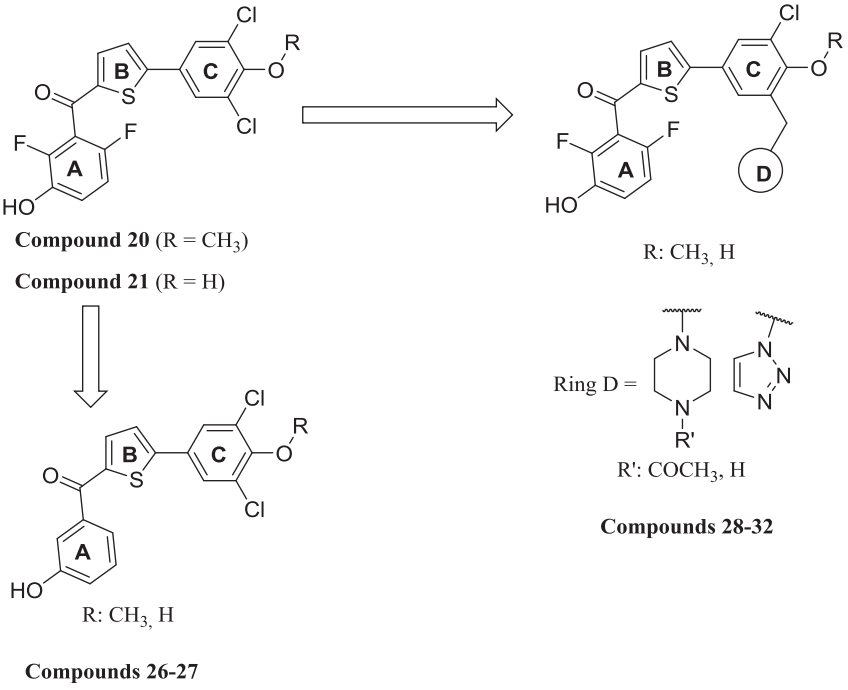
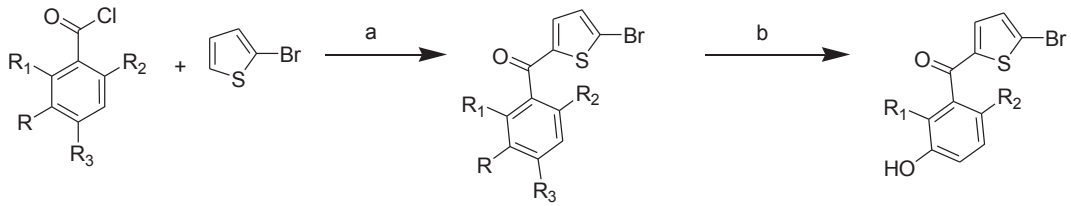


Chart 2. Design of synthesized compounds **26**–**32**.

Please cite this article in press as: A.S. Abdelsamie, et al., Treatment of estrogen-dependent diseases: Design, synthesis and profiling of a selective 17β-HSD1 inhibitor with sub-nanomolar IC_{50} for a proof-of-principle study, European Journal of Medicinal Chemistry (2016), http://dx.doi.org/10.1016/j.ejmech.2016.11.004



Scheme 1. Synthesis of intermediates **I-VII**. a) method A, $AlCl_3$, anhydrous CH_2Cl_2, 0 °C, 0.5 h and then rt, 3 h. b) method B, BBr_3, CH_2Cl_2, -78 °C to rt, overnight.

I–**V** were submitted to a Suzuki reaction (method C1 or C2) with the appropriate boronic acid to give **1a-7a**. The latter were submitted to ether cleavage with BBr_3 in dichloromethane to afford the final compounds (**1, 3**–**7**) or hydrolysis using 10% NaOH in ethanol to give compound **2**, respectively (Schemes 2 and 3).

The compounds **8**–**21** and **24**–**27** were obtained from the intermediates **VI** or **VII** by standard methods (either Suzuki reaction (method C1) alone or followed by ether cleavage (method B)) (Scheme 4).

The synthesis of compound **23** was accomplished as shown in Scheme 5: Bromination of 2-chloro-6-methylphenol with NBS in acetic acid selectively gave the ring-brominated compound **22c**, which underwent methylation upon treatment with methyl iodide. The resulting compound **22b** was transformed to the boronic acid ester **22a**. Finally, Suzuki cross coupling reaction of the latter with **VII** yielded compound **22**, which was submitted to ether cleavage with BBr_3, resulting in **23**.

The boronic acid ester **22a** was also used as starting material for the syntheses leading to compounds **28**–**30** (Scheme 5). By using NBS and DBPO in CCl_4, **22a** was converted to the brominated intermediate **28b**. Nucleophilic substitution of the bromine atom with 1-piperazine-1-ylethanone yielded **28a**. The latter was reacted with **VII** in a Suzuki reaction (method C1) to afford compound **28**, which gave access to **29** (via ether cleavage) and **30** (via amide hydrolysis under acidic conditions).

The azide intermediate **31c** was prepared by reaction of **28b** with NaN_3 in DMF (Scheme 6). The subsequent Suzuki cross-coupling reaction with **VII** yielded **31b**, which was submitted to a cycloaddition reaction with acetic acid vinyl ester [31] to give the 1,2,3-triazole substituted compound. Interestingly, the latter was exclusively isolated as acetic acid ester **31a**. Saponification with 2M-NaOH gave the phenol **31**, which was transformed to the diol **32** by reaction with BBr_3 (method B).

4. Biological results and discussion

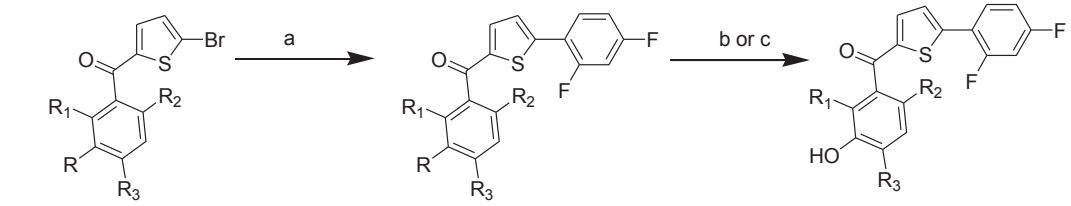
4.1. Inhibition of human 17β-HSD1 and selectivity towards 17β-HSD2, ERα, and ERβ

Human placental enzymes were used for both 17β-HSD assays and were obtained according to described methods [32–34]. In the 17β-HSD1 assay, incubations were run with cytosolic fractions, tritiated E1, cofactor and inhibitor. The separation of substrate and product was accomplished by HPLC. The 17β-HSD2 assay was performed similarly using tritiated E2 as substrate and the microsomal fraction. Activities are given as IC_{50}-values (Tables 1–4).

As expected, 17β-HSD1 inhibition of the synthesized compounds was strongly dependent on the substitution pattern of the benzoyl moiety (ring A, see Chart 1): Whereas compound **1** showed moderate activity (IC_{50} = 105 nM, Table 1), the presence of a methyl group was detrimental for inhibitory potency (compound **2**). A very strong inhibition of the target enzyme, however, could be achieved by the introduction of one or more fluorine atoms (compounds **3**–**5**). The most interesting properties in terms of activity towards 17β-HSD1 and selectivity over 17β-HSD2 was detected for the 2,6-difluorinated compound **4**. Therefore, this substitution pattern of the benzoyl moiety was maintained in the subsequent optimization of ring C.

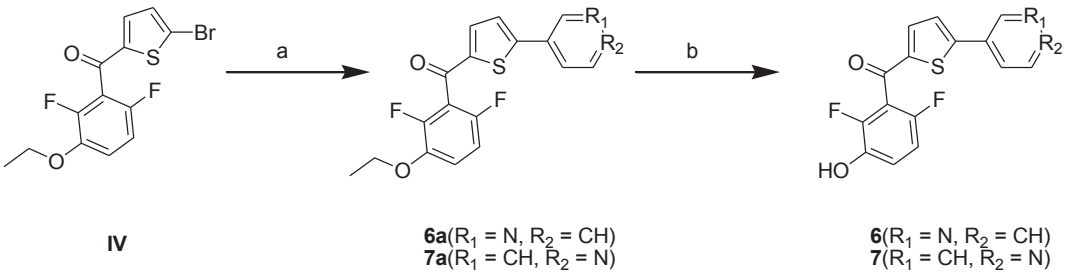
The replacement of the phenyl ring C with pyridine (compounds **6** and **7**) or omission of the fluorine substituents (**8**) led to a more or less pronounced drop in activity, compared to compound **4** (Table 2). For the sulfonamides **9**–**11** this effect was also observed. The introduction of a nitrile group proved to be beneficial for activity (compounds **12** and **13**), but led to low selectivity over h17β-HSD2.

Starting from **13,** the nitrile group was omitted or replaced by chlorine while maintaining an oxygen-function of ring C, in order to



Scheme 2. Synthesis of compounds **1**–**5**. a) method C1, 2,4-difluorophenylboronic acid, Cs_2CO_3, $Pd(PPh_3)_4$, DME/water (1:1), reflux, 4 h. b) method B, BBr_3, CH_2Cl_2, -78 °C to rt, overnight. c) 10% NaOH, ethanol, reflux, 2 h.

Please cite this article in press as: A.S. Abdelsamie, et al., Treatment of estrogen-dependent diseases: Design, synthesis and profiling of a selective 17β-HSD1 inhibitor with sub-nanomolar IC_{50} for a proof-of-principle study, European Journal of Medicinal Chemistry (2016), http://dx.doi.org/10.1016/j.ejmech.2016.11.004



Scheme 3. Synthesis of compounds **6** and **7**. a) method C2, 3- pyridinylboronic acid pinacol ester or 4-pyridinylboronic acid for **6a** and **7a**, respectively, Na_2CO_3, $Pd(PPh_3)_4$, toluene/ethanol (1:1), reflux, overnight. b) method B, BBr_3, CH_2Cl_2, -78 °C to rt, overnight.

enhance selectivity (compounds **14**, **15**, **17**–**19**). Compound **16** lacks this oxygen function and was synthesized for comparison reasons. An OH-group in 4-position of ring C proved to be beneficial (see e.g. compound **15**: IC_{50} = 3 nM, SF = 24 and compound **18**: IC_{50} = 0.5 nM, SF = 40) whereas the removal of the OH-group (compound **16**) or shifting its position from *para* to *ortho* (compound **19**), led to a decrease or a complete loss of selectivity, respectively (Table 2). From this optimization step, compound **18** emerged as the most active and selective inhibitor.

It was striking that compounds bearing an additional substituent on ring C in *ortho*-position to the oxygen-functionality (hydroxy or methoxy) stand out in terms activity and selectivity (compounds **13**, **17** and **18**, Table 2). This prompted us to investigate compounds with two *ortho*-substituents (compounds **20**–**25**, Table 3). In fact, this structural modification resulted in highly active compounds, generally displaying sub-nanomolar IC_{50}-values for h17β-HSD1 inhibition. Interestingly, compounds bearing chloro- and methyl-substituents showed similarly strong inhibition of the target enzyme. In terms of selectivity towards the type 2 enzyme it is noteworthy that generally the phenols were superior compared to their methoxy-analogs. An exception was compound **20** which was not only one of the most active but also the most selective compound of its class (IC_{50}(h17β-HSD1) = 0.5 nM; SF = 82). To get an evidence for the importance of fluorine atoms on the benzoyl part on the inhibitory activity issue of this compound class, compounds **26** and **27** were synthesized. Comparison with the inhibition data of compound **20** and its analog non-fluorinated compound **26** highlights again the beneficial impact of the fluorine atoms at the benzoyl moiety. The same dependence of activity on benzoyl fluorination can be seen when comparing compounds **21** and **27** (Table 3).

The dichlorinated inhibitor **20** showed favourable properties concerning activity and selectivity, but exhibited high lipophilicity (clogD (pH = 7.4): 4.9, as determined using the ACD-Labs *Percepta* software). In order to render the compound more hydrophilic, one of the chlorine atoms was replaced by heterocyclic substituents (compounds **28**–**32**, Table 4). The synthesized compounds showed greatly reduced lipophilicities (clogD range (pH = 7.4): 1.7 (**30**) – 3.3 (**31**)) and were highly active towards the target enzyme: apart from the dihydroxy compound **32**, all compounds displayed IC_{50}-values in the one-digit nanomolar range (Table 4). Selectivity over the type 2 enzyme, however, was in most cases less favourable than for compounds **20**–**25**.

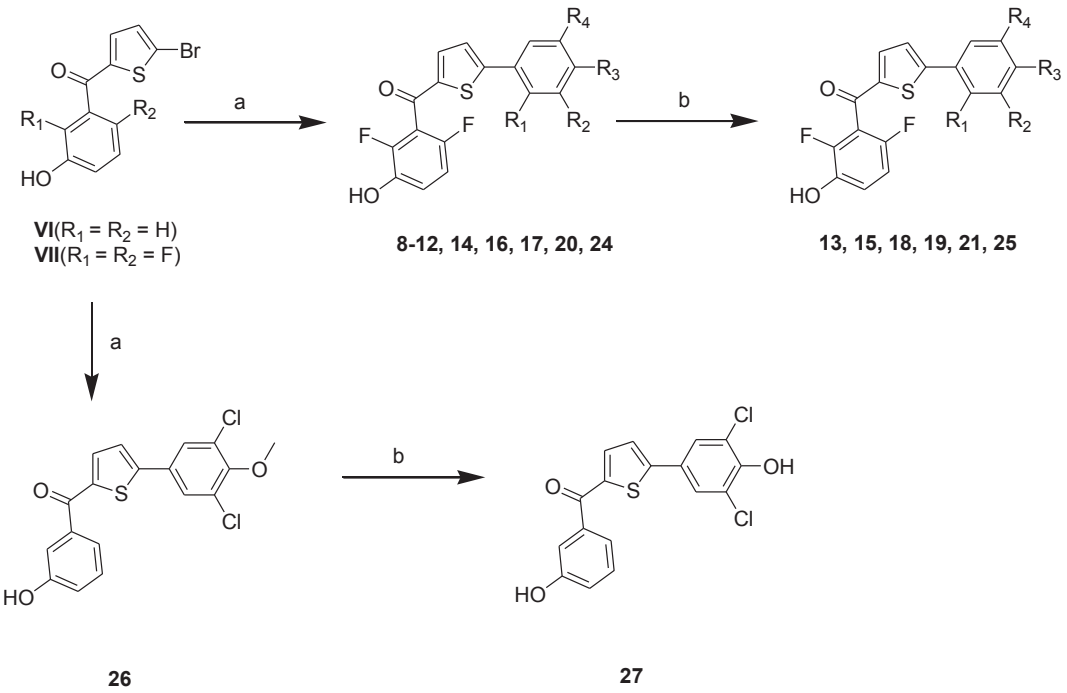
The affinities of selected compounds to the recombinant human estrogen receptors α and β were determined by incubation of tritiated E2 with the respective receptor and compound in 1000-fold excess, based on E2 concentration. Receptor affinities are expressed as the percentage of E2 replaced by a compound. ER-binding of 50% is equal to a relative binding affinity (RBA) of 0.1% of that of E2 [35].

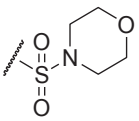
The determined estrogen receptor affinities are given in Table 5. The data suggest that fluorination of the benzoyl moiety increases ER-affinity (cf. compound **20** vs. **26**). Increasing the number or size of substituents adjacent to the oxygen-function of the phenyl ring (ring C) decreases ER-affinity (cf. compound **18** vs. **11**, **13**, **20**, **21**, **24**, and **25**).

Compounds **20** and **25**, which were highly active towards the target enzyme and selective over 17β-HSD2, displayed very low affinities towards ER α and β, in particular in view of their sub-nanomolar activity towards 17β-HSD1.

We next profiled the most interesting compound **20** in several relevant toxicity assays. First, compound **20** did not affect cell viability up to a concentration of 5.6 μM, as measured in the MTT cytotoxicity assay in HEK293 cells after an incubation period of 66 h, yielding a safety margin of 11,160-fold (i.e., IC_{20} in the MTT assay divided by the IC_{50} value in the human 17β-HSD1 assay). Secondly, for determination of the mutagenic potential of compound **20,** an AMES II test was performed using TA98 (frameshift mutation) or TAMix (base-pair substitution, TA7001-TA7006) strains of *Salmonella typhimurium* in the presence or absence of rat liver S9 fraction. Compound **20** did not show mutagenic potential up to the highest tested concentration of 100 μM in the absence or presence of rat liver S9 fraction. We next investigated whether compound **20** has the potential of CYP3A4 induction. For this, compound **20** was tested for activation of the pregnane X receptor (PXR), which is responsible for the induction of CYP3A4, and other members of the CYP3 subfamily of hepatic CYP450 enzymes, respectively. Compound **20** tested at a concentration of 3.16 μM did not stimulate the PXR receptor (effect < 5% activation, data not shown). Compound **20** was also profiled for inhibition of several hepatic CYP450 enzymes at a compound concentration of 1 μM, which is 2000-fold higher than the IC_{50} value for 17β-HSD1 inhibition. Compound **20** only moderately inhibited CYP1A2, 2B6, 2C9, 2C19, 2D6, and 3A4 by 11%, 4%, 0%, 27%, 0% and 36%, respectively. We next investigated whether our best current compound **20** can be administered to rodents to allow *proof-of-principle* testing of a potent 17β-HSD1 inhibitor in a rodent model of endometriosis. For this, we subcutaneously administered compound **20** at a dose of 150 μmol/kg body weight once-a-day for four consecutive days, during which we determined plasma levels of **20**, and measured also the plasma level of compound **20** four days after the last dosing. As shown in Fig. 3, compound **20** subcutaneously administered as suspension in 0.5% gelatin/5% mannitol in water led to a gradual increase in the plasma concentration from 24 nM measured at day 2, 42 nM at day 4, and to 72 nM at day 8, 4 days after the last dosing at day 4. These *in vivo* plasma concentrations are 10-fold higher than the concentrations required *in vitro* in the 17β-HSD1 assay to block 17β-HSD1by 80–100% (data not shown).

Please cite this article in press as: A.S. Abdelsamie, et al., Treatment of estrogen-dependent diseases: Design, synthesis and profiling of a selective 17β-HSD1 inhibitor with sub-nanomolar IC_{50} for a proof-of-principle study, European Journal of Medicinal Chemistry (2016), http://dx.doi.org/10.1016/j.ejmech.2016.11.004



Compd	R_1	R_2	R_3	R_4	Comd	R_1	R_2	R_3	R_4
8	H	H	H	H	**16**	H	Cl	H	H
9	H	SO_2NH_2	H	H	**17**	H	Cl	OCH_3	H
10	H	H	SO_2NH_2	H	**18**	H	Cl	OH	H
11	H		OCH_3	H	**19**	OH	Cl	H	H
12	H	CN		H	**20**	H	Cl	OCH_3	Cl
13	H	CN	OH	H	**21**	H	Cl	OH	Cl
14	H	H	OCH_3	H	**24**	H	CH_3	OCH_3	CH_3
15	H	H	OH	H	**25**	H	CH_3	OH	CH_3

Scheme 4. Synthesis of compounds **8**–**21** and **24**–**27**. a) method C1, corresponding boronic acid, Cs_2CO_3, $Pd(PPh_3)_4$, DME/water (1:1), reflux, 4 h. b) method B, BBr_3, CH_2Cl_2, -78 °C to rt, overnight.

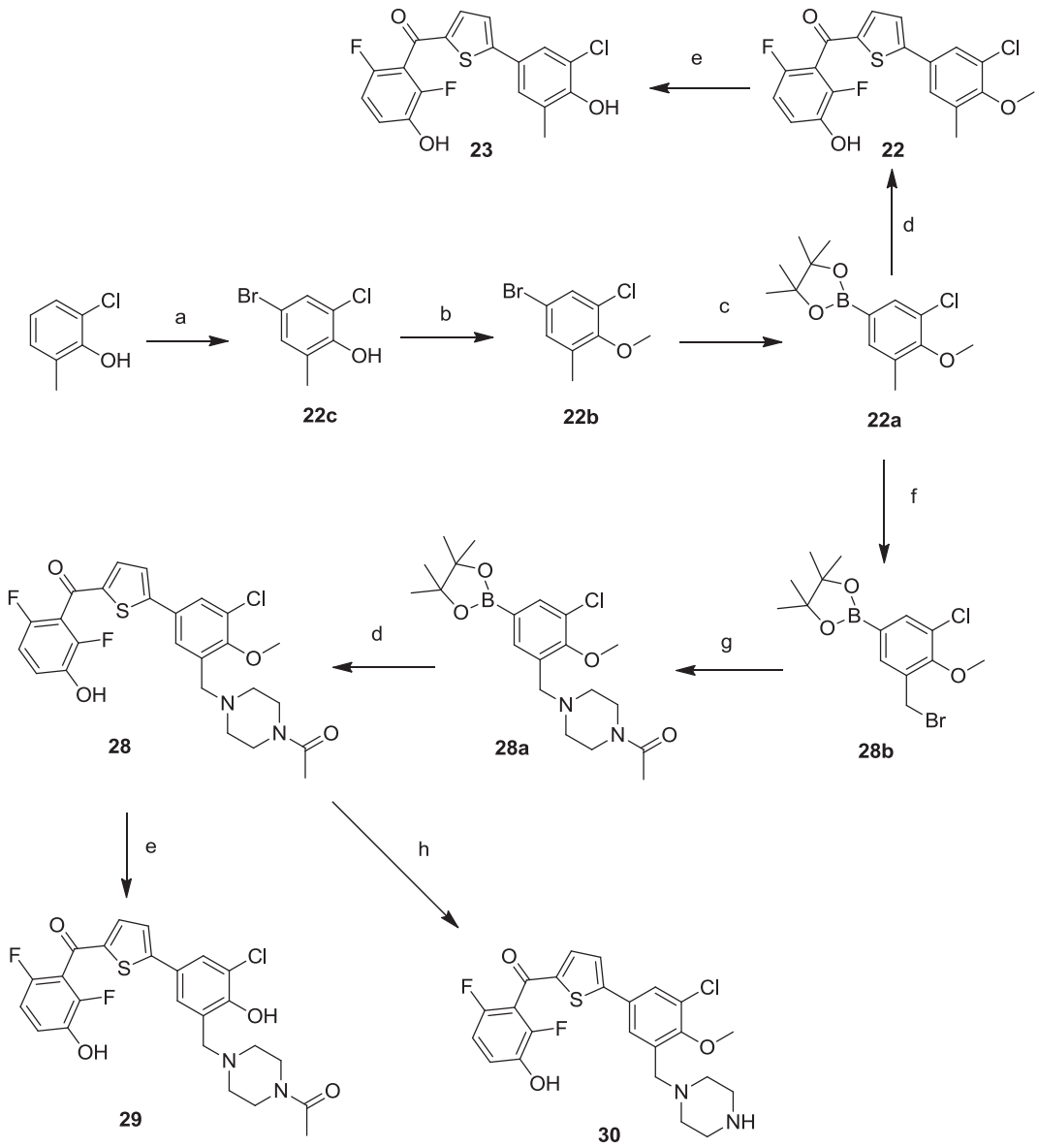
5. Conclusions

The aim of the present study was the design of potent and selective, low molecular weight inhibitors of human 17β-HSD1, which can be used to conduct a proof of principle study in an animal disease model for EDD. Starting point were sulfonamide-substituted bicyclic substituted hydroxyphenylmethanones previously described by us. Structural modifications focused on the substitution patterns of the benzoyl- and the phenyl-moieties.

To our knowledge, the 17β-HSD1 inhibitors identified in this study are the most active ones ever described for this enzyme. The most interesting compounds were **20**, **21**, and **25** as their high potency towards the target protein was accompanied by good selectivities over the human type 2 enzyme and the estrogen receptors α and β.

We profiled compound **20** in several ADME and toxicity assays. Compound **20** did not activate PXR at the tested concentration of 3.16 μM and did not show mutagenicity in the AMES II assay up to

Please cite this article in press as: A.S. Abdelsamie, et al., Treatment of estrogen-dependent diseases: Design, synthesis and profiling of a selective 17β-HSD1 inhibitor with sub-nanomolar IC_{50} for a proof-of-principle study, European Journal of Medicinal Chemistry (2016), http://dx.doi.org/10.1016/j.ejmech.2016.11.004



Scheme 5. Synthesis of compounds **22**–**23**, and **28**–**30**. a) NBS, AcOH, rt, overnight. b) CH_3I, K_2CO_3, DMF, rt, overnight. c) bispinacolato diborane, $Pd(dppf)Cl_2$, KOAc/DMSO, 2 h. d) method C1, **VII**, Cs_2CO_3, $Pd(PPh_3)_4$, DME/water (1:1), reflux, overnight. e) method B, BBr_3, CH_2Cl_2, -78 °C to rt, overnight. f) NBS, DBPO, CCl_4, reflux, 2 h. g) NEt_3, 1-piperazin-1-yl-ethanone. h) HCl 3 M, reflux, 3 h.

the highest tested concentration of 100 μM. Compound **20** also did not show cellular toxicity up to a concentration of 5.6 μM, as measured after 66 h of incubation in the MTT cell viability assay. Given the very potency of compound **20** towards 17β-HSD1 (IC_{50} of 0.5 nM), it is therefore to be expected that no cytotoxic effects will be observed in therapeutic doses. In addition, compound **20** showed only moderate CYP450 enzyme inhibition at the relatively high concentration of 1 μM. Immunocompromised rodents such as athymic nude and severe-compromised immunodeficient (SCID) mice and rats are frequently utilized to determine *in vivo* efficacy of preclinical drug candidates towards implanted human cells, including breast cancer cells and human endometriotic tissue samples. The very promising pharmacokinetic profile of compound **20** in rat after repetitive subcutaneous administration will allow for the first time *proof-of-principle* studies in rodent models of breast cancer and endometriosis, in which inhibition of 17β-HSD1 is considered to be therapeutically highly beneficial.

6. Experimental section

6.1. Chemical methods

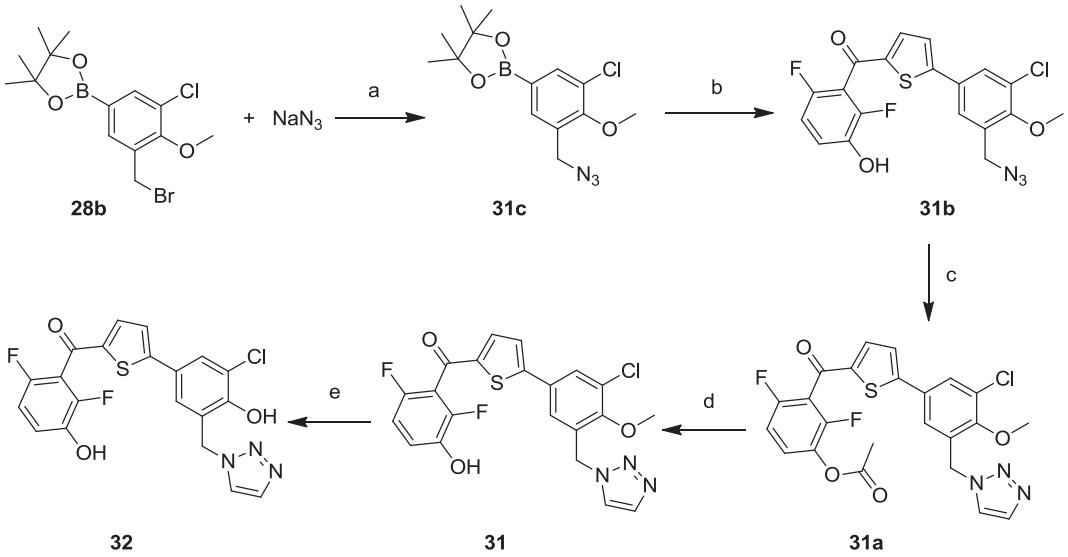
Chemical names follow IUPAC nomenclature. Starting materials were purchased from Aldrich, Acros, Lancaster, Maybridge, Combi Blocks, Merck, or Fluka and were used without purification.

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).

All microwave irradiation experiments were carried out in a CEM-Discover monomode microwave apparatus.

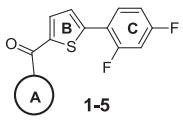
^1H NMR and ^{13}C NMR spectra were measured on a Bruker AM500 spectrometer (500 MHz) at 300 K. Chemical shifts are reported in δ (parts per million: ppm), by reference to the hydrogenated residues of deuteriated solvent as internal standard ($CDCl_3$: δ = 7.24 ppm (^1H NMR) and δ = 77 ppm (^{13}C NMR), CD_3OD:

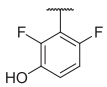
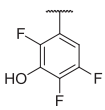
Please cite this article in press as: A.S. Abdelsamie, et al., Treatment of estrogen-dependent diseases: Design, synthesis and profiling of a selective 17β-HSD1 inhibitor with sub-nanomolar IC_{50} for a proof-of-principle study, European Journal of Medicinal Chemistry (2016), http://dx.doi.org/10.1016/j.ejmech.2016.11.004



Scheme 6. Synthesis of compounds **31** and **32**. a) DMF, rt, overnight. b) method C1, **VII**, Cs_2CO_3, $Pd(PPh_3)_4$, DME/water (1:1), reflux, overnight. c) $CH_3COOCH{=}CH_2$, microwave, 120 °C, 10 h.d) 2 M NaOH, THF, rt, 2 h. e) method B, BBr_3, CH_2Cl_2, -78 °C to rt, overnight.

Table 1
Optimization of A-ring-substitution: Activities of compounds **1**–**5** towards *h*17β-HSD1 and 2.



Cmpd	Ring A	IC50 [nM][a]		SF[d]	Cmpd	Ring A	IC50 [nM][a]		SF[d]
		*h*17β-HSD Type					*h*17β-HSD Type		
		1[b]	2[c]				1[b]	2[c]	
1		110	140	1.3	**2**		3400	300	0.1
3		47	55	1.1	**4**		3.5	9.0	2.5
5		7.2	1.4	0.2					

[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.
[d] SF (selectivity factor): IC_{50}(17β-HSD2)/IC_{50}(17β-HSD1).

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, sep, br. for singlet, doublet, triplet, doublet of doublets, doublet of doublets of doublets, multiplet, doublet of triplets, quadruplet, septet, and broad, respectively. All coupling constants (*J*) are given in hertz (Hz).

Mass spectra (ESI) were recorded on a TSQ Quantum (Thermo Finnigan) instrument.

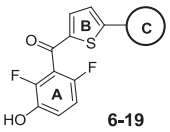
Tested compounds are >95% chemical purity as measured by HPLC. The methods for HPLC analysis and a table of data for all tested compounds are provided in the supporting information.

The following compounds were prepared according to previously described procedures: (5-bromo-thiophen-2-yl)(3-methoxyphenyl)methanone **(I)**, [26], (5-bromo-thiophen-2-yl)(3-hydroxy-phenyl)methanone **(IV)**, [29], (5-bromo-thiophen-2-yl)(3-hydroxy-phenyl)methanone **(VI)**, [26], (5-Bromo-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone **(VII)**, [29], 4-bromo-2-chloro-6-methyl-phenol **(22c)**, [36], 5-bromo-1-chloro-2-methoxy-3-methyl-benzene **(22b)** [37].

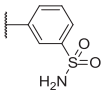
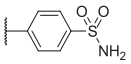
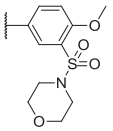
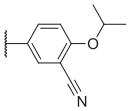
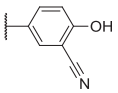
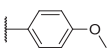
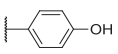
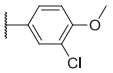
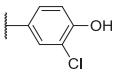
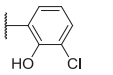
The Supplementary Data section reports the synthesis of compounds **II**, **V**, **1a-5a, 7a**, **3**–**7**, **9**–**29**, **31b** and **32**. For each general synthetic procedure, one representative example is given below.

Please cite this article in press as: A.S. Abdelsamie, et al., Treatment of estrogen-dependent diseases: Design, synthesis and profiling of a selective 17β-HSD1 inhibitor with sub-nanomolar IC_{50} for a proof-of-principle study, European Journal of Medicinal Chemistry (2016), http://dx.doi.org/10.1016/j.ejmech.2016.11.004

Table 2
Optimization of C-ring-substitution: Activities of compounds **6**–**19** towards *h*17β-HSD1 and 2.



6-19

Cmpd	Ring C	IC$_{50}$ [nM][a]		SF[d]	Cmpd	Ring C	IC$_{50}$ [nM][a]		SF[d]
		*h*17β-HSD Type					*h*17β-HSD Type		
		1[b]	2[c]				1[b]	2[c]	
6		27	47	1.7	**7**		150	39	0.7
8		11	35	3.2	**9**		55	86	1.6
10		150	85	0.6	**11**		130	180	1.4
12		8.0	19	2.4	**13**		3.3	29	8.8
14		18	38	2.1	**15**		3	71	24
16		1.2	10	8.3	**17**		1.3	11	8.5
18		0.5	20	40	**19**		27	29	1

[a-d] See Table 1.

*6.1.1. General procedure for Friedel-Crafts acylation. Method A. (**I**–**V**)*

An ice-cooled mixture of monosubstituted thiophene derivative (1.5 equiv), arylcarbonyl chloride (1 equiv), and aluminumtrichloride (1 equiv) in anhydrous dichloromethane was warmed to room temperature and stirred for 2–4 h 1 M HCl was used to quench the reaction. 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.

*6.1.2. General procedure for ether cleavage. Method B. **(VI**, **VII**, **1,3**–**7**, **13**, **15**, **18**, **19**, **21**, **23**, **25**, **27**, **29**, and **32)***

To a solution of methoxybenzene derivative (1 equiv) in anhydrous dichloromethane at −78 °C (dry ice/acetone bath), boron tribromide in dichloromethane (1 M, 5 equiv per methoxy function) 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 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.

6.1.3. General procedure for Suzuki coupling

*6.1.3.1. Method C1. **(1a-5a, 8**–**12, 14, 16, 17, 20, 22, 24, 26, 28, and 31b)**.* A mixture of arylbromide (1 equiv), boronic acid derivative (1.2 equiv), cesium carbonate (4 equiv) and tetrakis(-triphenylphosphine) palladium (0.05 equiv) was suspended in an oxygen-free DME/water (1:1) solution and refluxed under nitrogen atmosphere. The reaction mixture was cooled to room temperature. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, filtered and concentrated to dryness. The product was purified by CC.

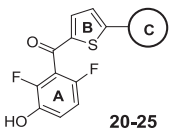
*6.1.3.2. Method C2. **(6a and 7a)**.* A mixture of arylbromide (1 equiv), boronic acid derivative (1.2 equiv), Na_2CO_3 (2 equiv) and tetrakis(triphenylphosphine) palladium (0.05 equiv) was suspended in an oxygen-free toluene/ethanol (1:1) solution was refluxed overnight under nitrogen atmosphere. The 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.

*6.1.4. (5-Bromo-thiophen-2-yl)-(2-fluoro-3-methoxy-phenyl)-methanone **(III)***

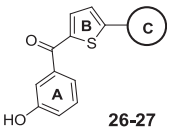
The title compound was prepared by reaction of 2-bromothiophene (1297 mg, 7.95 mmol), 2-fluoro-4-methoxybenzoyl chloride (1000 mg, 5.30 mmol) and aluminum chloride (707 mg, 5.30 mmol) according to method A. The product was purified by CC (hexane/ethyl acetate 96:4); yield: 57% (1000 mg). ^{1}H NMR (500 MHz, acetone-d_6) δ 7.32 (d, J = 7.4 Hz, 1H),

Please cite this article in press as: A.S. Abdelsamie, et al., Treatment of estrogen-dependent diseases: Design, synthesis and profiling of a selective 17β-HSD1 inhibitor with sub-nanomolar IC$_{50}$ for a proof-of-principle study, European Journal of Medicinal Chemistry (2016), http://dx.doi.org/10.1016/j.ejmech.2016.11.004

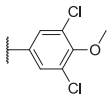
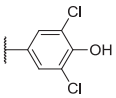
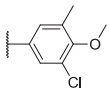
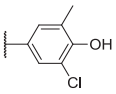
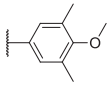
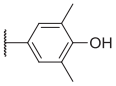
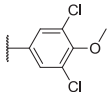
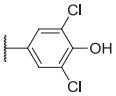
Table 3
Optimization of C-ring-substitution: Activities of compounds **20**–**27** towards *h*17β-HSD1 and 2.



20-25

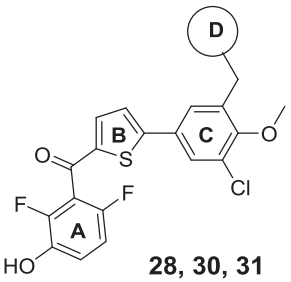


26-27

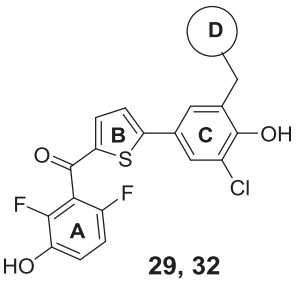
Cmpd	Ring C	IC_{50} [nM][a]		SF[d]	Cmpd	Ring C	IC_{50} [nM][a]		SF[d]
		*h*17β-HSD Type					*h*17β-HSD Type		
		1[b]	2[c]				1[b]	2[c]	
20		0.5	41	82	**21**		2.4	39	16
22		0.3	7.2	21	**23**		0.2	6.5	30
24		0.9	18	20	**25**		0.4	7.0	18
26		6.0	250	42	**27**		73	540	7.4

[a-d] See Table 1.

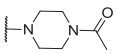
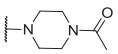
Table 4
Introduction of hydrophilic moieties: Activities of compounds **28**–**32** towards *h*17β-HSD1 and 2.



28, 30, 31



29, 32

Cmpd	Ring D	IC_{50} [nM][a]		SF[d]	Cmpd	Ring D	IC_{50} [nM][a]		SF[d]
		*h*17β-HSD Type					*h*17β-HSD Type		
		1[b]	2[c]				1[b]	2[c]	
28		1.4	21	15	**29**		2.1	11	5
30		4.7	26	6	**31**		2.1	9	4
32		18	26	1					

[a-d] See Table 1.

7.33 (ddd, J = 7.3, 4.9, 1.4 Hz, 1H), 7.12 (d, J = 7.4 Hz, 1H), 7.09 (t, J = 7.4 Hz, 1H), 6.92–6.87 (m, 1H), 3.82 (s, 3H); ^{13}C NMR (125 MHz, acetone-d_6) δ 184.05, 151.12 (d, J = 246.0 Hz), 149.54, 143.29, 136.87, 128.24 (dd, J = 9.9, 4.4 Hz), 126.67 (d, J = 13.0 Hz), 122.18 (d, J = 4.3 Hz), 117.46 (d, J = 3.0 Hz), 116.52 (d, J = 1.3 Hz), 115.94 (dd, J = 12.8, 4.1 Hz), 54.21; MS (ESI): 316.41 $(M+H)^+$.

*6.1.5. (5-(2,4-Difluoro-phenyl)-thiophen-2-yl)-(3-hydroxy-phenyl)-methanone **(1)***

The title compound was prepared by reaction of (5-(2,4-difluoro-phenyl)-thiophen-2-yl)-(3-methoxy-phenyl)-methanone **(1a)** (417 mg, 1.26 mmol) and boron tribromide (3.8 mmol) according to method B. The product was purified by CC

Please cite this article in press as: A.S. Abdelsamie, et al., Treatment of estrogen-dependent diseases: Design, synthesis and profiling of a selective 17β-HSD1 inhibitor with sub-nanomolar IC_{50} for a proof-of-principle study, European Journal of Medicinal Chemistry (2016), http://dx.doi.org/10.1016/j.ejmech.2016.11.004

Table 5
Binding affinities of selected compounds for the estrogen receptors α and β.

Cmpd.	% Binding[a]		Cmpd.	% Binding[a]	
	ERα[b]	ERβ[c]		ERα[b]	ERβ[c]
11	0	4	**21**	37	11
13	44	6	**24**	51	69
18	73	55	**25**	17	22
20	27	8	**26**	8	0

[a] Mean value of three determinations, standard deviation less than 15%.
[b] c(ERα) = 1 nM, c(E2) = 3 nM, c(test compound) = 3 μM.
[c] c(ERβ) = 4 nM, c(E2) = 10 nM, c(test compound) = 10 μM.

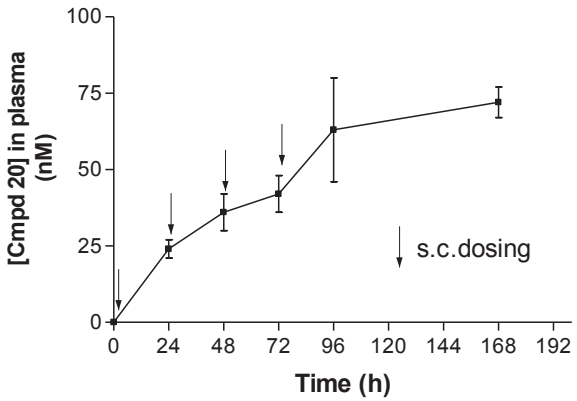


Fig. 3. Pharmacokinetic profile of compound 20 in Sprague-Dawley rats following repetitive subcutaneous administration. Compound **20** was subcutaneously administered once-a-day at a dose of 150 μmol/kg body weight in 0.5% gelatin/5% mannitol in water to three female Sprague-Dawley rats. Plasma samples were taken at 24 h, 48 h, 72 h and 168 h. Arrows indicate the administration of compound **20** during the first four days, immediately after plasma sampling. Data shown are the mean ± SEM of three animals.

(dichloromethane/methanol 99.75:0.25); yield: 41% (330 mg). ^{1}H NMR (500 MHz, acetone-d_6) δ 8.77 (s, 1H), 7.94 (td, J = 8.8, 6.3 Hz, 1H), 7.75 (dd, J = 4.0, 1.1 Hz, 1H), 7.63 (dd, J = 4.0, 1.0 Hz, 1H), 7.43–7.40 (m, 1H), 7.39–7.37 (m, 1H), 7.35 (ddd, J = 2.5, 1.5, 0.5 Hz, 1H), 7.27–7.21 (m, 1H), 7.20–7.15 (m, 1H), 7.14 (ddd, J = 7.7, 2.6, 1.5 Hz, 1H); ^{13}C NMR (125 MHz, acetone-d_6) δ 187.84, 163.90 (dd, J = 250.2, 12.1 Hz), 160.35 (dd, J = 254.0, 13.3 Hz), 158.45, 144.41 (dd, J = 91.1, 3.7 Hz), 140.16, 136.06, 131.44 (dd, J = 9.9, 4.4 Hz), 130.63, 128.25–127.77 (m), 121.15, 120.39, 118.70 (dd, J = 12.9, 4.1 Hz), 116.32, 113.36 (dd, J = 21.8, 3.6 Hz), 105.70 (t, J = 26.4 Hz); MS (ESI): 317.63 $(M+H)^+$.

6.1.6. (5-(2,4-Difluoro-phenyl)-thiophen-2-yl)-(3-hydroxy-2-methyl-phenyl)-methanone **(2)**

Compound **2a** (430 mg, 1.16 mmol) in ethanol (5 ml) was refluxed in 10% NaOH (15 mL) for 2 h on a water bath. The reaction mixture was cooled, diluted with water and neutralized with acetic acid. The product was purified by CC (dichloromethane/methanol 99.5:0.5); yield: 58% (220 mg). ^{1}H NMR (500 MHz, acetone-d_6) δ 9.51 (s, 1H, OH), 8.83 (dd, J = 15.1, 8.5 Hz, 1H), 8.49 (d, J = 3.4 Hz, 1H), 8.37 (d, J = 3.4 Hz, 1H), 8.24–8.02 (m, 3H), 7.95 (d, J = 7.9 Hz, 1H), 7.89 (d, J = 7.3 Hz, 1H), 3.11 (s, 3H); ^{13}C NMR (125 MHz, acetone-d_6) δ 189.56, 163.02 (dd, J = 251.3, 12.8 Hz), 159.45 (dd, J = 253.4, 12.4 Hz), 155.85, 144.44 (dd, J = 10.1, 4.1 Hz), 140.21, 135.70, 130.55 (dd, J = 9.9, 4.4 Hz), 127.34, 127.28, 126.11, 122.33, 118.97, 117.80 (dd, J = 12.8, 4.0 Hz), 116.62, 112.45 (dd, J = 21.8, 3.6 Hz), 104.79 (t, J = 26.4 Hz), 11.99; MS (ESI): 331.04 $(M+H)^+$.

6.1.7. (3-Ethoxy-2,6-difluoro-phenyl)-(5-pyridin-4-yl-thiophen-2-yl)-methanone **(6a)**

The title compound was prepared by reaction of (5-bromo-thiophen-2-yl)-(3-ethoxy-2,6-difluoro-phenyl)-methanone **(III)** (300 mg, 0.86 mmol), pyridine-3-boronic acid pinacol ester (355 mg, 1.73 mmol), sodium carbonate (2.5 mL, 2 M) and tetrakis(triphenylphosphine) palladium (5 μmol) according to method C2. The product was purified by CC (dichloromethane/methanol 99.5:0.5); yield: 91% (450 mg). The product was used in the next step without any characterization.

6.1.8. (5-(3-Chloro-phenyl)-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone **(8)**

The title compound was prepared by reaction of (5-bromo-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone **(VII)** (430 mg, 1.35 mmol), 3-chlorophenylboronic acid (253 mg, 1.62 mmol), cesium carbonate (1756 mg, 5.39 mmol) and tetrakis(triphenylphosphine) palladium (5 μmol) according to method C1. The product was purified by CC (hexane/ethyl acetate 90:10); yield: 74% (350 mg). ^{1}H NMR (500 MHz, acetone-d_6) δ 9.02 (s, 1H, OH), 7.84 (tt, J = 2.4, 1.2 Hz, 1H), 7.78–7.76 (m, 1H), 7.69 (d, J = 4.1 Hz, 1H), 7.66 (dt, J = 4.1, 0.9 Hz, 1H), 7.52 (td, J = 7.9, 0.5 Hz, 1H), 7.47 (ddd, J = 8.0, 2.0, 1.1 Hz, 1H), 7.23–7.21 (m, 1H), 7.06–7.04 (m, 1H); ^{13}C NMR (125 MHz, acetone-d_6) δ 180.77, 153.31, 152.56 (dd, J = 240.6, 5.8 Hz), 148.44 (dd, J = 245.9, 7.7 Hz), 143.92, 142.68 (dd, J = 12.9, 3.2 Hz), 138.12, 135.80, 135.69, 131.92, 130.20, 126.96, 126.90, 125.81, 120.42 (dd, J = 9.1, 3.9 Hz), 117.97 (dd, J = 23.9, 19.7 Hz), 112.47 (d, J = 22.8 Hz); MS (ESI): 351.63 $(M+H)^+$.

6.1.9. 2-(3-Chloro-4-methoxy-5-methyl-phenyl)-4,4,5,5-tetramethyl-(1,3,2)dioxaborolane **(22a)**

5-Bromo-1-chloro-2-methoxy-3-methylbenzene **(22b)** (5,00 g, 20,2 mmol, 1,00 equiv), bis(pinacolato)diboron (8,09 g, 31,8 mmol, 1,50 equiv), potassium acetate (5,95 g, 60,6 mmol, 3,00 equiv) and 1,1′-Bis(diphenylphosphino)ferrocene-palladium(II)dichloride (739 mg, 1,01 mmol, 0,05 equiv) were dissolved under N_2 in 40 ml dry DMSO and the mixture was stirred at 80 °C for 2 h. The reaction was quenched with water, diluted with diethyl ether and filtered over celite. The phases were separated and the aqueous layer was extracted two times with diethyl ether. The combined organic layers were washed three times with water; one time with brine, dried over $MgSO_4$, filtered and concentrated under reduces pressure. The crude product was purified by CC (hexane/ethyl acetate 85:15); yield: 88% (4.71 g). ^{1}H NMR (500 MHz, acetone-d_6) δ 7.54–7.56 (m, 1H), 7.48–7.50 (m, 1H), 3.82 (s, 3H), 2.31 (t, J = 0.6 Hz, 3H), 1.33 (s, 12H).

6.1.10. 2-(3-Bromomethyl-5-chloro-4-methoxy-phenyl)-4,4,5,5-tetramethyl-(1,3,2)dioxaborolane **(28b)**

2-(3-Chloro-4-methoxy-5-methylphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane **(22a)** (200 mg, 0,71 mmol, 1,00 equiv) and *N*-bromosuccinimide (113 mg, 0,63 mmol, 0,90 equiv) were dissolved under N_2 in 17 ml CCl_4, followed by a catalytic amount of dibenzoyl peroxide. The mixture was stirred under reflux for 1 h. The reaction was quenched with water and extracted three times with dichloromethane. The combined organic layers were washed two times with water, one time with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by CC (hexane/ethyl acetate 85:15); yield: 70% (180 mg). ^{1}H NMR (500 MHz, acetone-d_6) δ 7.75 (d, J = 1.5 Hz, 1H), 7.68 (d, J = 1.5 Hz, 1H), 4.71 (s, 2H), 4.00 (s, 3H), 1.33–1.35 (m, 12H).

6.1.11. 1-(4-(3-Chloro-2-methoxy-5-(4,4,5,5-tetramethyl-(1,3,2)dioxaborolan-2-yl)-benzyl)-piperazin-1-yl)-ethanone **(28a)**

2-(3-(Bromomethyl)-5-chloro-4-methoxyphenyl)-4,4,5,5-

Please cite this article in press as: A.S. Abdelsamie, et al., Treatment of estrogen-dependent diseases: Design, synthesis and profiling of a selective 17β-HSD1 inhibitor with sub-nanomolar IC_{50} for a proof-of-principle study, European Journal of Medicinal Chemistry (2016), http://dx.doi.org/10.1016/j.ejmech.2016.11.004

tetramethyl-1,3,2-dioxaborolane **(28b)** (180 mg, 0,50 mmo, 1,00 equiv) was dissolved under N_2 in 1 ml dry THF and 1-acetylpiperazine was added. The mixture was stirred for 1 h under reflux. The reaction was quenched with water and extracted three times with ethyl acetate. The combined organic layers were washed two times with water, one time with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by CC (ethyl acetate/ethanol 8:2), to give the desired product as yellow solid; yield: 70% (198 mg). ^{1}H NMR (500 MHz, acetone-d_6) δ 7.69 (d, J = 1.5 Hz, 1H), 7.65 (d, J = 1.5 Hz, 1H), 3.90 (s, 3H), 3.57 (s, 3H), 2.77 (t, J = 5.2 Hz, 2H), 2.70 (t, J = 5.2 Hz, 2H), 2.47 (t, J = 5.2 Hz, 2H), 2.42 (t, J = 5.2 Hz, 2H), 2.00 (s, 3H), 1.34 (s, 12H).

6.1.12. (5-(3-Chloro-4-methoxy-5-piperazin-1-ylmethyl-phenyl)-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone **(30)**

1-(4-(3-Chloro-5-(5-(2,6-difluoro-3-hydroxybenzoyl)-thiophen-2-yl)-2-methoxybenzyl)piperazin-1-yl)ethanone **(28)** (150 mg, 0.29 mmol, 1.00 equiv) was dissolved in 20 ml 3 M aqueous HCl and heated to 80 °C for 3 h. The reaction was washed two times with ethyl acetate, the aqueous layer was basified to pH 10 with 2 M NaOH and washed two times with ethyl acetate. The aqueous layer was neutralized with 2 M HCl and extracted three times with ethyl acetate. The combined organic layers were washed one time with water, one time with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure to give the desired pure product in 40% yield as pale yellow solid. ^{1}H NMR (500 MHz, acetone-d_6) δ 7.83–7.79 (m, 2H), 7.67–7.60 (m, 2H), 7.19 (m, 1H), 7.00 (td, J = 9.0, 1.9 Hz, 1H), 3.92 (s, 3H), 3.59 (s, 2H), 2.89–2.81 (m, 4H), 2.53–2.45 (m, 4H); ^{13}C NMR (125 MHz, acetone-d_6) δ 180.8, 156.8, 153.3, 151.5, 149.4, 147.5, 143.6, 142.9, 142.7, 138.2, 135.4, 130.7, 129.6, 128.4, 127.9, 126.7, 120.5, 118.1, 112.3, 112.2, 62.0, 56.9, 50.4, 44.3; MS (ESI): 479.22 $(M+H)^+$.

6.1.13. 2-(3-Azidomethyl-5-chloro-4-methoxy-phenyl)-4,4,5,5-tetramethyl-(1,3,2)dioxaborolane **(31c)**

2-(3-(Bromomethyl)-5-chloro-4-methoxyphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane **(28b)** (800 mg, 2.19 mmol, 1.00 equiv) was dissolved under N_2 in 8 ml dry DMF and sodium azide (143 mg, 2.19 mmol, 1.00 equiv) was added. The mixture was stirred at room temperature overnight. The mixture was quenched with water and extracted three times with ethyl acetate. The combined organic layers were washed two times with water, one time with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by CC (hexane/ethyl acetate 8:2); yield: 71% (503 mg). ^{1}H NMR (500 MHz, acetone-d_6) δ 7.72 (d, J = 1.6 Hz, 1H), 7.69 (d, J = 1.6 Hz, 1H), 4.55 (s, 2H), 3.93 (s, 3H), 1.34 (s, 12H).

6.1.14. Acetic acid 3-(5-(3-chloro-4-methoxy-5-(1,2,3)triazol-1-ylmethyl-phenyl)-thiophene-2-carbonyl)-2,4-difluoro-phenylester **(31a)**

(5-(3-(Azidomethyl)-5-chloro-4-methoxyphenyl)-thiophen-2-yl)(2,6-difluoro-3-hydroxyphenyl)methanone **(31b)** (50 mg, 0.11 mmol, 1.00 equiv), was dissolved in 106 μl vinyl acetate and the reaction was heated at 120 °C under microwave for 10 h. The reaction was concentrated under reduced pressure and purified by CC using ethyl acetate as eluent; yield: 27% (15 mg). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.11 (d, J = 0.9 Hz, 1H), 7.92 (d, J = 2.5 Hz, 1H), 7.70 (d, J = 0.9 Hz, 1H), 7.68–7.66 (m, 1H), 7.64–7.61 (m, 2H), 7.53 (td, J = 8.9, 5.5 Hz, 1H), 7.26 (td, J = 8.8, 1.9 Hz, 1H), 5.78 (s, 2H), 3.89 (s, 3H), 2.34 (s, 3H); MS (ESI): 504.12 $(M+H)^+$.

6.1.15. (5-(3-Chloro-4-methoxy-5-(1,2,3)triazol-1-ylmethyl-phenyl)-thiophen-2-yl)-(2,6-difluoro-3-hydroxy-phenyl)-methanone **(31)**

Acetic acid 3-(5-(3-chloro-4-methoxy-5-(1,2,3)triazol-1-ylmethyl-phenyl)-thiophene-2-carbonyl)-2,4-difluoro-phenylester **(31a)** (110 mg, 0.24 mmol, 1.00 equiv) was dissolved under N_2 in a degased mixture of 5 ml THF and 600 μl of 2 M NaOH. The mixture was stirred for 2 h at room temperature. The reaction was acidified to pH 6 with 1 M HCl and extracted three times with ethyl acetate. The combined organic layers were washed two times with water, one time with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure to give the desired product; yield: 95% (90 mg). ^{1}H NMR (500 MHz, acetone-d_6) δ 9.05 (br. s, 1H), 8.11 (d, J = 0.9 Hz, 1H), 7.91 (d, J = 2.2 Hz, 1H), 7.70 (d, J = 0.9 Hz, 1H), 7.67 (d, J = 2.2 Hz, 1H), 7.65–7.62 (m, 1H), 7.61–7.58 (m, 1H), 7.21 (td, J = 8.9, 5.5 Hz, 1H), 7.03 (td, J = 8.8, 1.9 Hz, 1H), 5.78 (s, 2H), 3.89 (s, 3H); MS (ESI): 462.15 $(M+H)^+$.

6.2. 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.

6.2.1. Preparation of human 17β-HSD1 and 17β-HSD2

Human 17β-HSD1 and 17β-HSD2 were obtained from human placenta according to previously described procedures [32]. Fresh human placenta was homogenized, and cytosolic fraction and microsomes were separated by fractional centrifugation. For the partial purification of 17β-HSD1, the cytosolic fraction was precipitated with ammonium sulfate. 17β-HSD2 was obtained from the microsomal fraction.

6.2.2. Inhibition of human 17β-HSD1

Inhibitory activities were evaluated by an established method with minor modifications [32]. Briefly, the enzyme preparation was incubated with NADPH (500 μM) in the presence of potential inhibitors at 37 °C in a phosphate buffer (50 mM) supplemented with 20% of glycerol and EDTA (1 mM). Inhibitor stock solutions were prepared in DMSO. The final concentration of DMSO was adjusted to 1% in all samples. The enzymatic reaction was started by addition of a mixture of unlabeled- and [2, 4, 6, 7-^{3}H]-E1 (final concentration: 500 nM, 0.15 μCi). After 10 min, the incubation was stopped with $HgCl_2$ and the mixture was extracted with diethylether. After evaporation, the steroids were dissolved in acetonitrile. E1 and E2 were separated using acetonitrile/water (45:55) as mobile phase in a C18 reverse phase chromatography column (Nucleodur C18 125/3100-5, Macherey-Nagel) connected to a HPLC-system (Agilent 1200 Series, Agilent Technologies). Detection and quantification of the steroids were performed using a radioflow detector (Ramona, raytest). The conversion rate was calculated after analysis of the resulting chromatograms according to the following equation:

$$\%\text{conversion} = [\%E2/(\%E2 + \%E1)] \times 100$$

Each value was calculated from at least three independent experiments.

6.2.3. Inhibition of human 17β-HSD2

The *h*17β-HSD2 inhibition assay was performed similarly to the *h*17β-HSD1 procedure. The microsomal fraction was incubated with NAD^+ [1500 μM], test compound and a mixture of unlabeled- and [2,4,6,7-^{3}H]-E2 (final concentration: 500 nM, 0.11 μCi) for 20 min at 37 °C. Further treatment of the samples and HPLC separation was carried out as mentioned above.

Please cite this article in press as: A.S. Abdelsamie, et al., Treatment of estrogen-dependent diseases: Design, synthesis and profiling of a selective 17β-HSD1 inhibitor with sub-nanomolar IC_{50} for a proof-of-principle study, European Journal of Medicinal Chemistry (2016), http://dx.doi.org/10.1016/j.ejmech.2016.11.004

The conversion rate was calculated after analysis of the resulting chromatograms according to the following equation:

$$\%\text{conversion} = [\%E1/(\%E1 + \%E2)] \times 100$$

6.2.4. ER affinity

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) 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 test compound for 16–20 h at 4 °C.

The inhibitors were dissolved in DMSO (5% final concentration). Evaluation of non-specific [^{3}H]-E2 binding was performed with unlabeled E2 at concentrations 100-fold of [3H]-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). The bound complex was washed three times and resuspended in ethanol. For radiodetection, scintillator cocktail (Quickszint 212, Zinsser Analytic, Frankfurt) was added and samples were measured in a liquid scintillation counter (1450 LSC & Luminescence Counter, Perkin Elmer).

From these results the percentage of [^{3}H]-E2 displacement by the compounds and unlabeled E2 was calculated. The plot of % displacement versus unlabeled E2 concentration resulted in sigmoidal binding curves. The E2 concentrations to displace 50% of the receptor bound [^{3}H]-E2 were determined in each experiment and E2 IC50 values were used as reference. The E2 IC_{50} values accepted were 3 ± 20% nM for ERα and 10 ± 20% nM for ERβ.

Compounds were tested at 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%.

6.2.5. MTT cell viability assay

Evidence of cell viability by the MTT assay is based on the reduction of the yellow, water-soluble dye 3-(4,5-dimethyl-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to a blue-violet, water insoluble formazane. HEK293 cells (2 × 105 cells per well) were seeded in 24-well flat-bottom plates. Culturing of cells, incubations and absorbance measurements were performed as described previously [38] with minor modifications. 3 h after seeding the cells in Dulbecco's minimal essential medium with 5% fetal calf serum and penicillin G (100 units/ml)/streptomycin (100 μg/ml), the incubation was started by the addition of compound **20** at 10, 5, 2.5, 1.25 and 0.625 μM in a total volume of 1 ml with a final DMSO concentration of 1%. After 66 h, 50 μl of a 5 mg/ml MTT in phosphate-buffered saline, pH 7.4 (PBS) was added to the medium, and incubated in a CO_2 incubator at 37 °C. After 30 min, medium was removed and 250 μl of DMSO containing 10% SDS and 0.5% acetic acid were added to dissolve the cells and MTT crystals. Absorbance of formazane was measured at 570 nm in an Omega plate reader spectrometer. The decrease in fluorescence at 570 nm after incubation in the presence of compound **20** compared with the fluorescence measured in the presence of the vehicle control alone (1% DMSO) was determined, followed by the calculation of the IC_{20} value using GraphpadPrism 3. Arbitrarily, below IC_{20} cell viability is not considered to be affected.

6.2.6. Mutagenicity testing

Mutagenic potential of compound **20** was evaluated at 100, 33, 11, 3.7, 1.2 and 0.4 μM using Xenometrix AMES II mutagenicity assay kit with TA98 and TA7001-TA7006 strains of *Salmonella typhimurium* in the presence or absence of rat liver S9 fraction according to the manufacturer's instructions.

6.2.7. Hepatic CYP450 inhibition and PXR assays

The inhibition of hepatic CYP enzymes CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 by 1 μM of compound **20** was determined in microsomes of baculovirus-infected insect cells expressing the recombinant human enzyme according to the manufacturer's instruction (BD Gentest/Corning). Agonist activity of compound **20** on the pregnane X receptor (PXR) was performed at CEREP (now Eurofins) in a cofactor recruitment cell-free assay.

6.2.8. Rat pharmacokinetics

All animal procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals. Experiments were conducted on female Sprague-Dawley rats (body weight 255–274 g) purchased from Charles River Laboratory (Sulzfeld, Germany). After an acclimatization period of 1 week, compound **20** was subcutaneously administered at a dose of 150 μmol/kg (66 mg/kg) body weight (n = 3) per animal. Suspensions of compound **20** in 0.5% porcine gelatine/5% mannitol (w/w) in demineralized water were prepared every day following 10 min in an ultrasonic bath, 30 min before administration. Before application of suspension (4 mL/kg body weight) at 0 h, 24 h, 48 h and 72 h, rats were anesthetized with 2% isoflurane. At 24 h, 48 h, 72 h, 96 h and 168 h, blood samples of 50 μl were taken from the tail vein (always before renewed subcutaneous administration of compound **20**) and collected in 0.2 ml Eppendorf tubes containing 5 μl of 106 mM sodium citrate buffer. After centrifugation at 5000 rpm at 4 °C, the plasma samples were first frozen at −20 °C and within 24 h, stored at −80 °C. For bioanalysis, plasma samples were thawed and 10 μL of plasma were added to 50 μL acetonitrile containing diphenhydramine (750 nM) as internal standard. Samples and calibration standards (in rat plasma) were centrifuged at 15,000 rpm for 5 min at 4 °C. The solutions were transferred to fresh vials for HPLC-MS/MS analysis (Accucore RP-MS, TSQ Quantum triple quadrupole mass spectrometer, APCI interface). After injection of 10 μL (performed in duplicate), data were analyzed based on the ratio of the peak areas of compound **20** and internal standard. Detection limit of compound **20** in plasma was 10 nM.

Author contributions

A.S.A., E.B., M.S. and M.F. are responsible for design, synthesis and chemical characterization of the compounds. C.J.v.K., M.S., C.B., L.S., M.F., M.W.L. and M.D.M. are responsible for the biological assays. A.S.A., C.J.v.K. and M.F. wrote the manuscript.

Notes

The authors declare no competing financial interest.

Acknowledgments

We are grateful to the Egyptian Ministry of Higher Education and Scientific Research (MoHESR) and the Deutscher Akademischer Austauschdienst (DAAD) for financial support of this work (A/09/92319). Thanks are due to Professor Rolf W. Hartmann for valuable discussions. We thank Dr. Claudia Scheuer and Julia Parakenings for their technical assistance in performing the pharmacokinetic profile experiments.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://

dx.doi.org/10.1016/j.ejmech.2016.11.004.

References

[1] T. Šmuc, M.R. Pucelj, J. Šinkovec, B. Husen, H. Thole, T. Lanišnik Rižner, Expression analysis of the genes involved in estradiol and progesterone action in human ovarian endometriosis, Gynecol. Endocrinol. 23 (2007) 105–111.
[2] Y. Miyoshi, A. Ando, E. Shiba, T. Taguchi, Y. Tamaki, S. Noguchi, Involvement of up-regulation of 17β-hydroxysteroid dehydrogenase type 1 in maintenance of intratumoral high estradiol levels in postmenopausal breast cancers, Int. J. Cancer 94 (2001) 685–689.
[3] A. Jansson, 17β-hydroxysteroid dehydrogenase enzymes and breast cancer, J. Steroid Biochem. Mol. Biol. 114 (2009) 64–67.
[4] C.H. Blomquist, M. Bonenfant, D.M. McGinley, Z. Posalaky, D.J. Lakatua, S. Tuli-Puri, D.G. Bealka, Y. Tremblay, Androgenic and estrogenic 17β-hydroxysteroid dehydrogenase/17-ketosteroid reductase in human ovarian epithelial tumors: evidence for the type 1, 2 and 5 isoforms, J. Steroid Biochem. Mol. Biol. 81 (2002) 343–351.
[5] T.M. Penning, Molecular endocrinology of hydroxysteroid dehydrogenases, Endocr. Rev. 18 (1997) 281–305.
[6] W.R. Miller, J.M. Bartlett, P. Canney, M. Verrill, Hormonal therapy for post-menopausal breast cancer: the science of sequencing, Breast Cancer Res. Treat. 103 (2007) 149–160.
[7] N.J. Bush, Advances in hormonal therapy for breast cancer, Semin. Oncol. Nurs. 23 (2007) 46–54.
[8] A. Cavalli, A. Bisi, C. Bertucci, C. Rosini, A. Paluszcak, S. Gobbi, E. Giorgio, A. Rampa, F. Belluti, L. Piazzi, P. Valenti, R.W. Hartmann, M. Recanatini, Enantioselective nonsteroidal aromatase inhibitors identified through a multidisciplinary medicinal chemistry approach, J. Med. Chem. 48 (2005) 7282–7289.
[9] M. Le Borgne, P. Marchand, M. Duflos, B. Delevoye-Seiller, S. Piessard-Robert, G. Le Baut, R.W. Hartmann, M. Palzer, Synthesis and in vitro evaluation of 3-(1-azolylmethyl)-1H-indoles and 3-(1-azolyl-1-phenylmethyl)-1H-indoles as inhibitors of P450 arom, Arch. Pharm. Weinh. 330 (1997) 141–145.
[10] C. Jacobs, M. Frotscher, G. Dannhardt, R.W. Hartmann, 1-imidazolyl(alkyl)-substituted di- and tetrahydroquinolines and analogues: syntheses and evaluation of dual inhibitors of thromboxane A(2) synthase and aromatase, J. Med. Chem. 43 (2000) 1841–1851.
[11] M. Le Borgne, P. Marchand, B. Delevoye-Seiller, J.M. Robert, G. Le Baut, R.W. Hartmann, M. Palzer, New selective nonsteroidal aromatase inhibitors: synthesis and inhibitory activity of 2,3 or 5-(alpha-azolylbenzyl)-1H-indoles, Bioorg Med. Chem. Lett. 9 (1999) 333–336.
[12] M.P. Lézé, M. Le Borgne, P. Pinson, A. Palusczak, M. Duflos, G. Le Baut, R.W. Hartmann, Synthesis and biological evaluation of 5-[(aryl)(1H-imidazol-1-yl)methyl]-1H-indoles: potent and selective aromatase inhibitors, Bioorg Med. Chem. Lett. 16 (2006) 1134–1137.
[13] R. Maltais, A. Trottier, A. Delhomme, X. Barbeau, P. Lagüe, D. Poirier, Identification of fused 16beta,17beta-oxazinone-estradiol derivatives as a new family of non-estrogenic 17β-hydroxysteroid dehydrogenase type 1 inhibitors, Eur. J. Med. Chem. 93 (2015) 470–480.
[14] V. Adamo, M. Iorfida, E. Montalto, V. Festa, C. Garipoli, A. Scimone, M. Zanghi, N. Caristi, Overview and new strategies in metastatic breast cancer (MBC) for treatment of tamoxifen-resistant patients, Ann. Oncol. 18 (Suppl 6) (2007) vi53–57.
[15] B. Delvoux, T. D'Hooghe, C. Kyama, P. Koskimies, R.J. Hermans, G.A. Dunselman, A. Romano, Inhibition of type 1 17β-hydroxysteroid dehydrogenase impairs the synthesis of 17β-estradiol in endometriosis lesions, J. Clin. Endocrinol. Metab. 99 (2014) 276–284.
[16] B. Husen, K. Huhtinen, M. Poutanen, L. Kangas, J. Messinger, H. Thole, Evaluation of inhibitors for 17β-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.
[17] B. Husen, K. Huhtinen, T. Saloniemi, J. Messinger, H.H. Thole, M. Poutanen, Human hydroxysteroid (17-β) dehydrogenase 1 expression enhances estrogen sensitivity of MCF-7 breast cancer cell xenografts, Endocrinology 147 (2006) 5333–5339.
[18] 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, 17β-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.
[19] D. Poirier, Advances in development of inhibitors of 17β hydroxysteroid dehydrogenases, Anticancer Agents Med. Chem. 9 (2009) 642–660.
[20] 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 17β-hydroxysteroid dehydrogenase type 1, J. Med. Chem. 54 (2011) 248–261.
[21] D. Poirier, Inhibitors of 17β-hydroxysteroid dehydrogenases, Curr. Med. Chem. 10 (2003) 453–477.
[22] D. Poirier, 17β-Hydroxysteroid dehydrogenase inhibitors: a patent review, Expert Opin. Ther. Pat. 20 (2010) 1123–1145.
[23] J.M. Day, H.J. Tutill, A. Purohit, M.J. Reed, Design and validation of specific inhibitors of 17β-hydroxysteroid dehydrogenases for therapeutic application in breast and prostate cancer, and in endometriosis, Endocr. Relat. Cancer 15 (2008) 665–692.
[24] J.M. Day, H.J. Tutill, A. Purohit, 17β-hydroxysteroid dehydrogenase inhibitors, Minerva Endocrinol. 35 (2010) 87–108.
[25] P. Brožič, T. Lanišnik Rižner, S. Gobec, Inhibitors of 17β-hydroxysteroid dehydrogenase type 1, Curr. Med. Chem. 15 (2008) 137–150.
[26] A. Oster, S. Hinsberger, R. Werth, S. Marchais-Oberwinkler, M. Frotscher, R.W. Hartmann, Bicyclic substituted hydroxyphenylmethanones as novel inhibitors of 17β-hydroxysteroid dehydrogenase type 1 (17β -HSD1) for the treatment of estrogen-dependent diseases, J. Med. Chem. 53 (2010) 8176–8186.
[27] A. Spadaro, M. Frotscher, R.W. Hartmann, Optimization of hydroxybenzothiazoles as novel potent and selective inhibitors of 17β-HSD1, J. Med. Chem. 55 (2012) 2469–2473.
[28] A.S. Abdelsamie, E. Bey, N. Hanke, M. Empting, R.W. Hartmann, M. Frotscher, Inhibition of 17β-HSD1: SAR of bicyclic substituted hydroxyphenylmethanones and discovery of new potent inhibitors with thioether linker, Eur. J. Med. Chem. 82 (2014) 394–406.
[29] A.S. Abdelsamie, E. Bey, E.M. Gargano, C.J. van Koppen, M. Empting, M. Frotscher, Towards the evaluation in an animal disease model: fluorinated 17β-HSD1 inhibitors showing strong activity towards both the human and the rat enzyme, Eur. J. Med. Chem. 103 (2015) 56–68.
[30] S. Marchais-Oberwinkler, C. Henn, G. Möller, T. Klein, M. Negri, A. Oster, A. Spadaro, R. Werth, M. Wetzel, K. Xu, M. Frotscher, R.W. Hartmann, J. Adamski, 17β-Hydroxysteroid dehydrogenases (17beta-HSDs) as therapeutic targets: protein structures, functions, and recent progress in inhibitor development, J. Steroid Biochem. Mol. Biol. 125 (2011) 66–82.
[31] S.G. Hansen, H.H. Jensen, Microwave irradiation as an effective means of synthesizing unsubstituted N-linked 1,2,3-triazoles from vinyl acetate and azides, Synlett 20 (2009) 3275–3276.
[32] 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 17β-HSD1-inhibitors as potential therapeutic agents, Mol. Cell Endocrinol. 301 (2009) 154–157.
[33] 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 17β-hydroxysteroid dehydrogenase inhibitors: estradiol-adenosine hybrids with high affinity, FASEB J. 16 (2002) 1829–1831.
[34] K.M. Sam, S. Auger, V. Luu-The, D. Poirier, Steroidal spiro-gamma-lactones that inhibit 17 β-hydroxysteroid dehydrogenase activity in human placental microsomes, J. Med. Chem. 38 (1995) 4518–4528.
[35] J. Moreno-Farre, P. Workman, F.I. Raynaud, Analysis of potential drug-drug interactions for anticancer agents in human liver microsomes by high throughput liquid chromatography/mass spectrometry assay, Recent Adv. Res. Updat. 7 (2006) 207–224.
[36] D.J. Kertesz, C. Brotherton-Pleiss, M. Yang, Z. Wang, X. Lin, Z. Qiu, D.R. Hirschfeld, S. Gleason, T. Mirzadegan, P.W. Dunten, S.F. Harris, A.G. Villasenor, J.Q. Hang, G.M. Heilek, K. Klumpp, Discovery of piperidin-4-yl-aminopyrimidines as HIV-1 reverse transcriptase inhibitors. *N*-benzyl derivatives with broad potency against resistant mutant viruses, Bioorg Med. Chem. Lett. 20 (2010) 4215–4218.
[37] M. Iwao, H. Takehara, S. Furukawa, M. Watanabe, A regiospecific synthesis of carbazoles via consecutive palladium-catalyzed cross-coupling and aryne-mediated cyclization, Heterocycles 36 (1993) 1483–1488.
[38] J. Haupenthal, C. Baehr, S. Zeuzem, A. Piiper, RNAse A-like enzymes in serum inhibit the anti-neoplastic activity of siRNA targeting polo-like kinase 1, Int. J. Cancer 121 (2007) 206–210.

Please cite this article in press as: A.S. Abdelsamie, et al., Treatment of estrogen-dependent diseases: Design, synthesis and profiling of a selective 17β-HSD1 inhibitor with sub-nanomolar IC_{50} for a proof-of-principle study, European Journal of Medicinal Chemistry (2016), http://dx.doi.org/10.1016/j.ejmech.2016.11.004