Inhibitors of 17β-Hydroxysteroid Dehydrogenase Type 1, 2 and 14: Structures, Biological Activities and Future Challenges

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Abstract

During the past 25 years, the modulation of estrogen action by inhibition of 17β-hydroxysteroid dehydrogenase types 1 and 2 (17β-HSD1 and 17β-HSD2), respectively, has been pursued intensively. In the search for novel treatment options for estrogen-dependent diseases (EDD) and in order to explore estrogenic signaling pathways, a large number of steroidal and nonsteroidal inhibitors of these enzymes has been described in the literature. The present review gives a survey on the development of inhibitor classes as well as the structural formulas and properties of their most interesting representatives. In addition, rationally designed dual inhibitors of both 17β-HSD1 and steroid sulfatase (STS) as well as the first inhibitors of 17β-HSD14 are covered.

Introduction

17β-Hydroxysteroid dehydrogenase type 1 and type 2 (17β-HSD1 and 17β-HSD2; EC 1.1.1.62) are oxidoreductases belonging to the short chain dehydrogenase-reductase (SDR) family which interconvert ketones and their corresponding secondary alcohols. Their names are derived from the fact that they catalyze oxidoreductions in the 17β-position of steroidal substrates, under consumption of the natural cosubstrates NADPH (17β-HSD1) and NAD⁺ (17β-HSD2). Thus, 17β-HSD1 (SDR nomenclature: SDR28C1) catalyzes the reduction of the weakly estrogenic estrone (E1) to the most potent estrogen 17β-estradiol (E2, scheme 1) which plays a central role in the etiology of estrogen dependent diseases (EDD). 17β-HSD2 (SDR2C9), on the other hand, catalyzes the reverse reaction, namely the inactivation of E2 by oxidation to E1. It can therefore be considered as a physiological adversary of the type 1 enzyme. Besides, 17β-HSD2 is involved in the deactivation of androgens (testosterone, T) and the activation of progestins. Both E1 and E2 exert their physiological effects predominantly by transactivation of the nuclear estrogen receptors (ER) α and β, and the weaker estrogenicity of E1 is due to its lower ER-affinity compared to that of E2.
Scheme 1
Steroid hormone conversions catalyzed by 17β-HSD1, 17β-HSD2 and 17β-HSD14

17β-HSD1 and 2 display an intracrine mode of action, i.e. they interconvert estrogens within the cells where the estrogenic effects are exerted (Labrie 1991). The interplay of these two enzymes modulates estrogen action on a pre-receptor level (Penning 1997; Duax et al. 2000), depending on the requirements of the cell. 17β-HSD1 and 2 may therefore be seen as “molecular switches”, as they increase (switch on) and decrease (switch off) ligand occupancy and concomitant transactivation of the ER, respectively (Labrie et al. 2000; Penning 2003).

In case of EDD, the interplay of 17β-HSD1 and 2 is disrupted, giving rise to an imbalance between estrogen activation and inactivation. Thus, an increased E2/E1 ratio and high levels of 17β-HSD1 mRNA are indicative for a crucial role of 17β-HSD1 e.g. in endometriosis (Šmuc et al. 2007;
Inhibition of 17β-HSD1 is therefore considered as a valuable therapeutic approach for the treatment of these diseases. The validity of this concept is supported by several observations: (1) 17β-HSD1 inhibition resulted in a decrease of E2 levels in endometriotic specimens (Delvoux et al. 2014); (2) 17β-HSD1 inhibitors reversed estrogen-induced endometrial hyperplasia in transgenic mice (Saloniemi et al. 2010); (3) Inhibition of 17β-HSD1 resulted in the reduction of E1-stimulated tumor cell growth in vitro and in animal models, suggesting that this target is eligible for the treatment of breast cancer (Husen et al. 2006; Kruchten et al. 2009). The fact that the enzyme catalyzes the final step in estrogen activation makes this approach particularly interesting as it may offer the prospect of few side effects.

Osteoporosis is an age-related disease which is connected to decreasing amounts of E2 and T (Vanderschueren et al. 2008). Estrogen replacement therapy proved to be an efficient treatment but led to adverse effects and is no longer recommended. The fact that 17β-HSD2 is present in osteoblasts (Dong et al. 1998) and decreases E2- and T-levels by oxidation to the ketones makes 17β-HSD2 inhibition a promising approach for the treatment of osteoporosis. In an in vivo monkey model, a 17β-HSD2 inhibitor led to a decrease of bone resorption and maintenance of bone formation, validating the therapeutic concept (Bagi et al. 2008). As compared to established endocrine treatments of EDD, inhibitors of 17β-HSD1 and 17β-HSD2 should have the advantage to exert little influence on systemic estrogen levels, offering the prospect of fewer side effects.

17β-HSD14 (SDR47C19; synonyms: retSDR3, DHRS10) is another member of the SDR family and the last 17β-HSD that has been identified. In vitro, it NAD⁺-dependently oxidizes E2 to E1 (Lukacik et al. 2007) - and thus shows analogy to 17β-HSD2. Moreover, it was found to catalyze the formation of dehydroepiandrosterone and 4-androstene-3,17-dione from 5-androstene-3β,17β-diol and testosterone, respectively (scheme 1). The physiological role and the native substrate(s) of this enzyme, however, are yet unknown. Therefore, it is still unclear whether this enzyme may serve as a drug target in the future. Recently, the 3D-structures of the cytosolic protein as holo form and as
ternary complexes with E1 and with a nonsteroidal inhibitor have been described (Bertoletti et al. 2016; Braun et al. 2016).

**Inhibitors**

Inhibitors of 17β-HSD1 and 2 have been reviewed previously (Poirier 2003, 2009; Deluca et al. 2005; Brožic et al. 2008; Day et al. 2008, 2010; Marchais-Oberwinkler et al. 2011a). In the following we give a comprehensive overview on inhibitors identified, including recent developments.

**Natural products and parabens as inhibitors of 17β-HSDs**

The literature covering effects of phytoestrogens on 17β-HSD enzymes has been reviewed previously (Deluca et al. 2005). In a more recent publication, inhibitory activities of different flavones and related compounds on 17β-HSD enzymes from the fungus *Cochliobolus lunatus* (17β-HSDcl) were reported (Kristan et al. 2005). Flavones hydroxylated at positions 3, 5 and 7 as well as 5-methoxyflavone (figure 1, compounds 1-5) led to the strongest inhibition of estradiol oxidation, showing IC₅₀ values in the range of 0.4 to 1 µM. Their ability to inhibit the reverse reaction (reduction of estrone) was less pronounced (IC₅₀ values from 1.2 µM to 7.4 µM). Coumestrol and kaempferol (figure 1, compounds 6 and 7a, respectively) inhibited both reactions unselectively (IC₅₀ values between 2.5 µM and 3.9 µM).

Vuorinen et al. applied a pharmacophore model based on 17β-HSD2 inhibitors to a virtual screening of various databases containing natural products in order to discover new lead structures from nature (Vuorinen et al. 2017a). The work resulted in the identification of new 17β-HSD2 inhibitors and provided information on the binding pocket of the enzyme. The structures and inhibitory data of the most potent compounds 7b and 8-11 are given in figure 1.

Parabens (*p*-hydroxybenzoic acid esters) are effective preservatives with high human exposure. In order to investigate possible estrogenic effects exerted by parabens and parabene-like compounds,
Engeli et al. addressed their potential interference with local estrogen metabolism by inhibiting 17β-HSD1 and 17β-HSD2 (Engeli et al. 2017). All parabens under investigation moderately inhibited 17β-HSD2. Inhibition of 17β-HSD1 was size-dependent, whereby hexyl- and heptylparaben (figure 1, compounds \( 12d \) and \( 12e \)) were most active. The results suggest that depending on the tissue expression of 17β-HSD1 and 17β-HSD2, parabens might exert pro-estrogenic or anti-estrogenic effects. As smaller parabens (methyl-, ethyl-, propylparaben; compounds \( 12a-c \)) are more commonly used than larger parabens, disruption of estrogenic effects through inhibition of 17β-HSD2 is more likely to occur than through inhibition of 17β-HSD1. Regarding the rapid metabolism of the compounds to the inactive \( p \)-hydroxybenzoic acid (compound \( 12, R = H \)), it needs to be determined whether the low micromolar paraben concentrations required for effective disturbance of estrogen action can be reached in target cells in vivo.
### Natural products and parabens showing inhibition of 17β-HSD1 and 17β-HSD2

| Compound                  | 17β-HSD1 IC₅₀ (µM), cell-free | 17β-HSD2 IC₅₀ (µM), cell-free | Reference              |
|---------------------------|--------------------------------|--------------------------------|------------------------|
| 7b 2´-Hydroxygenistein     | 1.09                           | 2.03                           | (Vuorinen et al. 2017a) |
| 8 Nordihydroguaiaretic acid| 5.5                            | 0.38                           |                        |
| 9 (-)-Dihydroguaiaretic acid| 7.7                            | 0.94                           |                        |
| 10 Isoliquiritigenin       | 2.83                           | 0.36                           |                        |
| 11 Lupinalbin A            | 0.049                          | 1.52                           |                        |

**percent inhibition at 20 µM, cell-free**

| Compound                  | 17β-HSD1 | 17β-HSD2 |
|---------------------------|----------|----------|
| 12a Methylparaben         | 0        | 50       |
| 12 Ethylparaben           | 7        | 74       |
| b                         |          |          |
| 12c Propylparaben         | 37       | 37       | (Engeli et al. 2017) |
| 12 Hexylparaben           | 80       | 58       |
| d                         |          |          |
| 12e Heptylparaben         | 83       | 76       |

**Figure 1**

Natural products and parabens showing inhibition of 17β-HSD1 and 17β-HSD2

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*Inhibitors of 17β-HSD1*

*Steroidal inhibitors of 17β-HSD1*

*C16 substituted E2 derivatives.* Since the 1990s, the Poirier group has been working on C16 substituted estradiol derivatives as inhibitors of 17β-HSD1. First design approaches consisted in the introduction of bromo-substituted aliphatic chains with various lengths (n= 1-7) and stereochemistry (α or β; figure 2, structure 13) in the 16-position of the steroidal skeleton (Tremblay et al. 1995;
Tremblay & Poirier 1998; Rouillard et al. 2008). In a cell-free assay, the most potent compounds 13a and 13b displayed IC$_{50}$ values of 1.3 µM and 1.2µM, respectively (Rouillard et al. 2008). However, the compounds were found to exert proliferative estrogenic-like effects on the human estrogen dependent breast cancer cell lines ZR-75-1 (Tremblay et al. 1995) and T47D (Rouillard et al. 2008).

Two strategies were pursued to eliminate the estrogenic effect: the first strategy consisted in exchanging the bromine atom with chlorine or hydroxy and the introduction of a methoxy group in position 2 (compounds not shown). These modifications, however, reduced potency, and the compounds displayed estrogenic effects towards T47D cells (Rouillard et al. 2008). The second strategy comprised the introduction of a bromopropyl-linker bearing a bulky alkylamide group in the 16α position of estradiol (figure 2, compound 14). This modification eliminated estrogenic activity on ZR-75-1 cells but on the expense of inhibitory potency (IC$_{50}$ under cell-free conditions: 10.4 µM). Interestingly, compound 14 was found to have antiestrogenic activity as it was able to neutralize the effect of estradiol on ZR-75-1 cells, so it can be considered as a dual-action inhibitor (Pelletier & Poirier 1996).
Figure 2

Steroidal inhibitors of 17β-HSD1: C16 substituted E2 derivatives.

In 2002 the same group successfully enhanced the inhibitory activity of C16-substituted E2 derivatives by designing and synthesizing a bifunctional hybrid inhibitor, combining an E2- and an adenosine

| Compound | IC<sub>50</sub>, cell-free | Reference                  |
|----------|----------------------------|----------------------------|
| 13a      | 1.3 µM                     | (Rouillard et al. 2008)    |
| 13b      | 1.2 µM                     | (Pelletier & Poirier 1996) |
| 14       | 10.4 µM                    | (Poirier et al. 2005)      |
| 15<sup>a</sup> | 52 nM                     | (Bérubé & Poirier 2009)    |
| 16a      | 56 nM                      | (Laplante et al. 2008)     |
| 17a      | 44 nM in T47D cells        | (Maltais et al. 2011)      |
| 17b      | 171 nM in T47D cells       |                            |
| 17c      | 68 nM in T47D cells        |                            |

<sup>a</sup>Different IC<sub>50</sub> values reported in the cited references.
moiety in a single compound (figure 2, EM-1745, compound 15) which allows for simultaneous occupation of both the substrate and the cofactor binding site (Qiu et al. 2002). Compound 15 showed high inhibitory activity against purified 17β-HSD1 (IC$_{50}$ value: 52 nM) (Poirier et al. 2005). However, two major drawbacks were reported for 15 when used in intact cells or in vivo models, namely cell membrane permeation problems and rapid metabolization, most probably at the ester bond site (Fournier et al. 2008). To overcome the stability problem, two strategies were pursued that focused on simplifying the complex structure of 15. The first led to a series of compounds which contain a meta-substituted aniline as a mimic of the adenosine moiety while the ester bond was replaced by a carbon-carbon bond (figure 2, compounds 16) (Bérubé & Poirier 2004). The most active compound of this structurally simplified series contained an alkyl chain with 13 carbon atoms (i.e. n = 13) and was slightly less active than compound 15 (Bérubé & Poirier 2009). The second approach consisted in the parallel solid phase synthesis of diverse libraries of 16β-substituted E2 derivatives (not shown) that could serve as bisubstrate inhibitors, however, none of these libraries resulted in compounds that could reach the strong activity of compound 15 (Bérubé et al. 2010).

The introduction of an m-carbamoylbenzyl substituent at the 16β positions of E2 and E1, respectively, led to the highly potent compounds 17a and 17b (figure 2) with good cellular permeability, displaying IC$_{50}$ values of 44 and 171 nM, respectively, in an assay using intact T47D cells. In cellular proliferation assays (T47D cells), 17a was able to inhibit E1-mediated proliferation to a certain extent, without returning to the basal levels of proliferation as 17a was found to have estrogenic potency itself (Laplante et al. 2008; Mazumdar et al. 2009). Compound 17c, bearing a 2-bromoethyl side chain at position 3, was devoid of estrogenic properties. Moreover, it showed potent irreversible inhibition of 17β-HSD1 (IC$_{50}$: 68 nM in intact T47D cells) and it was found to have no effect on 17β-HSD2, 17β-HSD7, 17β-HSD12 and CYP3A4 (Maltais et al. 2011, 2014).
**Figure 3**

Steroidal inhibitors of 17β-HSD1: C6 and C2 substituted E2 derivatives.

*C6 and C2 substituted E2 derivatives.* Another approach for the design of steroidal 17β-HSD1 inhibitors was reported by the Poirier group. The authors described compounds that bear a butyl methyl alkylamide side chain in the C6 position of E2 (figure 3, general structure 18) (Poirier et al. 1998). The 6β orientation of the thioether linked side chain was found to be essential for activity as compound 18a (figure 3) was 70 times more potent an inhibitor than its 6α-isomer (not shown; IC\textsubscript{50} values 0.17 µM and 12.0 µM, respectively) (Poirier et al. 1998). In an attempt to eliminate the estrogen-like effect of this compound, the C3 hydroxy group was replaced with a hydrogen atom (compound 18b). This modification, however, was not only inadequate to remove estrogenicity but also was clearly unfavorable in terms of 17β-HSD1 inhibition (Tremblay et al. 2005). Replacement of the thioether bond by a C-C bond, as exemplified by compound 18c, led to less estrogenic compounds without affecting inhibition of 17β-HSD1 compared to 18a (Cadot et al. 2007). The introduction of a 2-methoxy group (compound 18d) further decreased inhibitory activity without significantly reducing estrogenic effects (Cadot et al. 2007).

| Compound | X | R\textsubscript{1} | R\textsubscript{2} | 17β-HSD1 IC\textsubscript{50} (µM), cell free | Reference |
|----------|---|----------------|-----------------|---------------------------------|------------|
| 18a      | S | OH            | H               | 0.17                            | (Poirier et al. 1998) |
| 18b      | S | H             | H               | > 1                             | (Cadot et al. 2007) |
| 18c      | CH\textsubscript{2} | OH | H               | 82 %\textsuperscript{a}         | (Tremblay et al. 2005) |
| 18d      | CH\textsubscript{2} | OH | OMe             | 32 %\textsuperscript{a}         | (Cadot et al. 2007) |

\textsuperscript{a}: percent inhibition at 1 µM in T47D cells
### Table 1: IC₅₀ (nM) for 17β-HSD1 Inhibitors

| Compounds | 17β-HSD1 IC₅₀ (nM) | Reference |
|-----------|---------------------|-----------|
|           | cell-free (nM)      | cellular (nM) |
| 19        | 4                   | (100% inhibn. at 1 µM) |
| 20(β)     | 194                 | -         |
| 20(α)     | 353                 | -         |
| 21a       | -                   | 37        |
| 22a       | -                   | 110       |
| 22b       | -                   | 700       |

### Figure 4

Steroidal inhibitors of 17β-HSD1: C2, C6, C15 and C16 substituted E1 derivatives.

**C15 substituted E1 derivatives.** Messinger et al. introduced another class of 17β-HSD1 inhibitors by synthesizing C15-substituted estrone derivatives (figure 4, compounds 19 and 20) (Messinger et al. 2009).
2009). Compound 19 displayed strong and selective inhibition of the target protein in cell-free assay. At a concentration of 1µM, 19 inhibited recombinant human 17β-HSD2 by 10%. The compound was also found to be cell permeable, as evidenced by its cellular inhibition profile on MCF7 cells (100% inhibition at 1µM). Moreover, it showed no estrogen receptor mediated effect (Messinger et al. 2009). Compound 19 was shown to strongly reduce E2 levels in human endometriotic specimens (decrease of E2 by more than 85% in approx. 70% of the specimens) (Delvoux et al. 2014). The 16β-diastereomer of compound 20 which showed an IC₅₀ value of 194 nM in a cell-free assay (Messinger et al. 2009), was found to inhibit human 17β-HSD1 in transgenic mice expressing human 17β-HSD1 by 85% and 33% in males and females, respectively (Lamminen et al. 2009). Interestingly, the 16α-isomer of compound 20 completely reversed estrogen-induced endometrial hyperplasia in these transgenic mice (Saloniemi et al. 2010).

C2, C6 and C16 substituted E1 derivatives. Potter’s group pursued a structure-based drug design strategy using the crystal structure of human 17β-HSD1 to discover new, selective and potent inhibitors (figure 4, general structure 21), with a methyl amide extending from the 16β-position of E1 (Lawrence et al. 2005; Vicker et al. 2006). In this series of compounds, 21a was the most potent inhibitor of 17β-HSD1 reported, showing selectivity for 17β-HSD1 over 17β-HSD2 (Allan et al. 2006a). The same group also investigated modifications at the 6 and/or 16 position of the steroidal scaffold (figure 4, compounds 22a and 22b), but none of the compounds showed higher activity than compound 21a (Allan et al. 2006a).
### Table 1

| Compound | IC<sub>50</sub> (nM) | Reference |
|----------|-----------------------|-----------|
| 23       | Cell-free: 15         | (Möller et al. 2009) |
| 24       | Cell-free: -          | (Ouellet et al. 2014) |
| 25       | Cell-free: -          | (Maltais et al. 2015) |
| 26       | Cell-free: -          | (Allan et al. 2006a) |
| 27       | Cell-free: -          | (Fischer et al. 2005) |
| 28       | Cell-free: -          |           |

### Figure 5

Steroidal inhibitors of 17β-HSD1: D- and E-ring modified estrogens.

**D- and E-ring modified estrogens.** Möller et al. used a computational drug design approach to develop a set of potent 17β-HSD1 inhibitors with a D-homo-E1 scaffold. The 2-phenylethyl derivative (compound 23, figure 5) was the most potent inhibitor of this set with an IC<sub>50</sub> value of 15 nM in a cell-free assay (Möller et al. 2009).

Other groups modified the D-ring by annealing an additional ring (E-ring): Poirier’s group investigated fused substituted lactones and substituted oxazinone. In intact T47D cells the compounds 24 and 25 (figure 5) showed IC<sub>50</sub> values of 1 and 1.4 µM, respectively, and no estrogenic activity. Compound 24 showed a dual action by inhibiting 17β-HSD1 and exerting an antiestrogenic effect on
T47D cells (Ouellet et al. 2014). Compound 25 was selective over the 17β-HSD types 2 and 12 (Maltais et al. 2015).

In an extension of their work on the C16 substituted E1 derivatives, the Potter group explored novel E-ring pyrazole derivatives. Compounds 26-28 (figure 5) inhibited 17β-HSD1 in T47D cells with IC₅₀ values of 180, 530 and 300 nM, respectively. In addition, they displayed selectivity over 17β-HSD2 and no estrogenic effects (Fischer et al. 2005; Allan et al. 2006a, b).

**Nonsteroidal inhibitors of 17β-HSD1**

*(Hydroxyphenyl)naphthols.* Frotscher et al. described potent and selective 17β-HSD1 inhibitors with a (hydroxyphenyl)naphthol scaffold. Starting point for inhibitor design was a pharmacophore model developed by the group. The model mimics the steroidal substrate with two hydroxyl functions at a distance of about 12 Å from each other, mounted on a hydrophobic core structure (Frotscher et al. 2008).

| Compound | 17β-HSD1 IC₅₀ (nM) | SF (17β-HSD2/17β-HSD1) | Reference                              |
|----------|---------------------|-------------------------|---------------------------------------|
| 29       | 116                 | 48                      | (Frotscher et al. 2008)               |
| 30       | 20                  | 27                      | (Marchais-Oberwinkler et al. 2008)    |
| 31       | 15                  | 27                      | (Marchais-Oberwinkler et al. 2011b)   |
| 32       | 15                  | 27                      |                                       |
Nonsteroidal inhibitors of 17β-HSD1: (Hydroxyphenyl)naphthols

In cell-free assays, compound 29 (figure 6) showed inhibitory activity towards 17β-HSD1, combined with good selectivity over the type 2 enzyme (selectivity factor SF 2/1 = IC$_{50}$ (17β-HSD2) / IC$_{50}$ (17β-HSD1) = 48), ERα and β. Thus, it was used as a lead in a further drug design process of this class (Frotscher et al. 2008). The positions of the two hydroxyl-groups were found to be optimum for target inhibition (Marchais-Oberwinkler et al. 2009). Various substituents were introduced on the phenol- and the naphthol-moiety, and only substituents at position 1 of the naphthol system led to more active compounds (e.g. compound 30: IC$_{50}$ = 20 nM in cell-free assay) with good selectivity over the type 2 enzyme and ERs) and good pharmacokinetic profile after oral application in rats (Marchais-Oberwinkler et al. 2008). To obtain more drug-like compounds, further structural optimization was performed at the 1-position of the naphthol moiety by introducing different heteroaromatic rings and substituted phenyl groups, and the introduction of a methyl sulfonamide group (compound 31) was found to be favorable for 17β-HSD1 inhibition in both cell-free and T47D cell assays (Marchais-Oberwinkler et al. 2011b). Further optimization led to the creation of a combinatorial library of substituted sulfonamides. Compound 32 was equiactive to compound 31 with respect to inhibition of human 17β-HSD1 in a cell-free assay, but less potent in a T47D cell assay. With regard to a possible in vivo application, the compound was also tested against marmoset (Callithrix jacchus) 17β-HSD1 and showed an inhibition of 19% at 50 nM. Its rigidified analog 33 was the strongest inhibitor of this series towards both marmoset (79% inhibition at 50 nM) and human 17β-HSD1 in cell-free assays, but displayed poor activity in a T47D whole cell assay (IC$_{50}$ = 1100 nM) (Henn et al. 2012).
Bis(hydroxyphenyl) substituted arenes. Bey et al. introduced bis(hydroxyphenyl) arenes as another inhibitor class based on the above mentioned pharmacophore model (Bey et al. 2008a). Extensive structure activity relationship (SAR) studies (Bey et al. 2008a, b; Al-Soud et al. 2009) revealed that the proper choice of the central ring is crucial for activity: while imidazole and pyrazole resulted in inactive compounds, oxazole, thiazole and thiophene led to strong inhibition of purified human 17β-HSD1 (figure 7, compounds 34-36). In addition, also selectivity could be largely affected by the choice of the central ring: oxazole 34 (figure 7) showed selective inhibition of 17β-HSD1 over the type 2 enzyme, whereas the isomeric isoxazole reversed the selectivity (not shown). Inhibitory potency was also dependent on the positions of the two phenolic OH-groups (Bey et al. 2008a, b). In case of compounds 34-36 the favorable positions of the hydroxyl functions (para/meta) are implemented. Further improvements in the activity and selectivity were achieved when substituents were introduced to the \( p \)-hydroxy substituted ring (compounds 37a and 37b) (Bey et al. 2009). Especially fluorination (compound 37a) proved to be beneficial, leading to strong target inhibition, pronounced selectivity over 17β-HSD2 and excellent pharmacokinetic properties in rats after oral application (Bey et al. 2009). Compounds 36 and 37b were found to neutralize the E1-induced stimulation of proliferation of T47D cells (Kruchten et al. 2009). Some members of this inhibitor class, e.g. compounds 37a and 37b, were reported to exert direct antiproliferative activity \textit{in vitro} in cancer cell lines by influencing the cell cycle (Berenyi et al. 2013).

| Compound | \( X \) | \( Y \) | \( Z \) | 17β-HSD1 IC \textsubscript{50} (nM), Cell-free | SF \((17\beta\text{-HSD2}/17\beta\text{-HSD1})\) | Reference |
|----------|------|------|------|-----------------|-----------------|-----------|
| \( 34 \) | N    | O    | H    | 310             | 56              | (Bey et al. 2008a) |
| \( 35 \) | N    | S    | H    | 50              | 80              | (Bey et al. 2008b) |
| \( 36 \) | CH   | S    | H    | 69              | 28              | (Bey et al. 2008b) |
Figure 7

Nonsteroidal inhibitors of 17β-HSD1: Bis(hydroxyphenyl) substituted arenes.

Bicyclic substituted hydroxyphenyl methanones. A structure- and ligand-based pharmacophore model was introduced by the Hartmann group, resulting in novel 17β-HSD1 inhibitors that were derivatives of bicyclic substituted hydroxyphenylmethanone (figure 8, compounds 38a-c) (Oster et al. 2010a). The influence of changes of the hydroxy-group positions as well as the effects of small substituents introduced at the phenyl ring and of central ring alternatives (thiophene and thiazoles) on inhibitory potency were examined. Compounds 38a-c were the most potent inhibitors with IC$_{50}$ values in the low nanomolar range in both cell-free and T47D cellular assays, selectivity over 17β-HSD2, no significant affinity to ER$_{\alpha}$ and ER$_{\beta}$, and metabolic stability in human liver microsomes (phase 1; t$_{1/2}$ ≥ 100 min) (Oster et al. 2010a). Structural modifications of the bicyclic system (ring A, figure 8) were carried out, paying special attention to the possibility of exchanging the hydroxy function (Oster et al. 2011). This work revealed that the hydroxy function was not essential for activity and could be replaced e.g. with a methylbenzenesulfonamide moiety (compound 39), resulting in high activity in cell-free and cellular assays at the expense, however, of decreased selectivity over 17β-HSD2 and metabolic stability (t$_{1/2}$ = 12.8 min).
More insight into the SAR of this class was gained by Abdelsamie et al., who found that the hydroxyphenyl methanone moiety is important for activity and that its hydroxy function cannot be replaced with possible bioisosteres without complete or significant loss of activity (Abdelsamie et al. 2014). Further structural optimizations were performed to enhance the inhibitory activity against rodent (rat and mouse) 17β-HSD1. This goal was achieved by the introduction of fluorine atoms on the benzoyl moiety. Compound 40 (figure 8) is one of the most potent inhibitors reported of both human and rat 17β-HSD1 enzymes with IC₅₀ values (purified enzymes) of 2 nM and 97 nM, respectively (Abdelsamie et al. 2015). The main drawback of this compound was its low selectivity over the type 2 enzymes of human and rat (SF2/1 = 3 and 0.5, respectively). Nevertheless, compound...
was a lead for further optimizations in order to obtain a suitable candidate for a proof of principle study in a rodent endometriosis model. Thus, compound 41 (figure 8) was discovered which is the most potent 17β-HSD1 inhibitor known so far, displaying high selectivity over 17β-HSD2, ER α and β, and a range of hepatic CYP enzymes. A beneficial pharmacokinetic profile in rats makes the compound eligible for a proof of principle study using xenografted immunodeficient rats (Abdelsamie et al. 2017).

| Compound | 17β-HSD1 IC₅₀ (nM) | SF (17β-HSD2/17β-HSD1) | Reference               |
|----------|---------------------|-------------------------|-------------------------|
| 42       | 44                  | 24                      | (Spadaro et al. 2012a)  |
| 43a      | 27                  | 148                     | (Spadaro et al. 2012b)  |
| 43b      | 13                  | 136                     | (Miralinagli et al. 2014)|
| 44       | 13                  | 40                      |                         |

Figure 9

Nonsteroidal inhibitors of 17β-HSD1: Hydroxybenzothiazoles and hydroxybenzothiophenes

Hydroxybenzothiazole and hydroxybenzothiophene derivatives. The Hartmann group developed another pharmacophore model in an approach to design nonsteroidal 17β-HSD1 inhibitors. The model
was based on crystallographic data of the target protein and was used for the virtual screening of a small library of compounds. The virtual hits were experimentally verified, and subsequent structure modifications and rigidification resulted in inhibitors bearing a benzothiazole ring linked to a phenyl system via a keto or amide linker (Spadaro et al. 2012a). In a functional assay using purified human 17β-HSD1, compound 42 (figure 9) displayed an IC\(_{50}\) value of 44 nM. It was selective over the type 2 enzyme, but showed affinity towards the ERs. Inhibitor optimization in terms of selectivity was achieved by introduction of small substituents on the phenyl ring. The methylated benzoyl derivative 43a and difluoro benzamide derivative 43b (figure 9) were the most selective 17β-HSD1 inhibitors over the type 2 protein. In addition, they were highly potent inhibitors of 17β-HSD1 in cell-free and T47D cellular assays (IC\(_{50}\): 258 and 37 nM, respectively), and showed favorable selectivity profiles towards ERs and hepatic CYP enzymes (Spadaro et al. 2012b). A systemic bioisosteric replacement of benzothiazole by other heterocycles resulted in the identification of potent and selective 17β-HSD1 inhibitors bearing a benzothiophene moiety. Compound 44 (figure 9) is more active towards the target enzyme and displayed better selectivity over 17β-HSD2 than its benzothiazole analog 42 (Miralinaghi et al. 2014).

**Thiophenepyrimidinones.** Messinger et al. identified 17β-HSD1 inhibitors by computer aided drug design starting from a unique nonsteroidal pyrimidinone core (structure 45, figure 10) by optimizing R\(_1\) and R\(_2\). In protein-based inhibition assays compound 45a displayed an IC\(_{50}\) value of 5 nM against 17β-HSD1, and selectivity over the type 2 enzyme (8% inhibition at 100 nM). In intact MCF-7 cells, 17β-HSD1 was inhibited by 67% when 45 was applied in a concentration of 1µM. The compound significantly reduced 17β-HSD1-dependent tumor growth in a nude mouse model (Messinger et al. 2006). Lilienkampf et al. also reported a potent 17β-HSD1 inhibitor class derivative of thienopyrimidinone that was identified by the screening of a small library of commercially available compounds. Compound 46 (figure 10) was the most potent inhibitor of this series with 94% inhibition.
at 100 nM concentration tested in a cell-free assay at low concentration of E1 (30 nM) (Lilienkampf et al. 2009).

| Compound | 17β-HSD1 IC$_{50}$, cell-free | Reference |
|----------|-------------------------------|-----------|
| 45a      | 5 nM                          | (Messinger et al. 2006) |
| 46       | 94% inhibition at 100 nM      | (Lilienkampf et al. 2009) |
| 47       | 1.7 µM in T47D cells          | (Allan et al. 2008) |
| 48       | 560 nM                        | (Oster et al. 2010b) |

**Figure 10**

Nonsteroidal inhibitors of 17β-HSD1: Thiophenepyrimidinones, biphenyl ketones and biphenylols

*Biphenyl ketones, biphenylols and imidazoles*. Several phenylalkyl imidazoles (structures are not shown) and biphenyl ketones were reported as weakly active 17β-HSD1 inhibitors, showing 10-68% inhibition at a concentration of 100 µM (Lota et al. 2007; Olusanjo et al. 2008). Allan et al. represented a promising biphenyl ethanone scaffold as a mimic of the E1 template. Structural optimization led to compound 47 (figure 10), showing an IC$_{50}$ value of 1.7 µM in an assay using T47D
cells (Allan et al. 2008). Oster et al. published heterocyclic substituted biphenylols and their aza-analogues with 17β-HSD1 inhibitory activity (Oster et al. 2010b). The most active compound 48 (figure 10) displayed IC$_{50}$ values of 560 nM (17β-HSD1) and IC$_{50}$ 2370 nM (17β-HSD2) in cell-free assays, and low affinity for ERs α and β.

![Figure 11](image)

**Figure 11**

Nonsteroidal inhibitors of 17β-HSD1: Flavonoids, cinnamic acid, coumarin derivatives and virtual screening candidates 52a and 52b.

*Flavonoids, cinnamic acid and coumarin derivatives.* The inhibitory activity of various flavonoids and cinnamic acid derivatives towards human recombinant 17β-HSD1 was investigated (Brožič et al. 2009). Diosmetin (figure 11, compound 49) was the most active flavonoid and compound 50 was the most active cinnamic acid derivative with more than 90% and 70% inhibition at 6 µM, respectively. The same group reported the synthesis of coumarin derivatives as selective nonsteroidal inhibitors of 17β-HSD1. Compound 51a (figure 11) was the most potent inhibitor in this series with an IC$_{50}$ value of 270 nM and selectivity towards 17β-HSD2 and ERs (Starčević et al. 2011a). Recently, Niinivehmas et al. reported on a series of 3-phenylcoumarin analogs as inhibitors of recombinant human 17β-HSD1 (Niinivehmas et al. 2018). The molecular basis of inhibition was determined by a docking-based SAR
analysis, and inhibition of 17β-HSD2 as well as cross-reactivity against ERα, aromatase, CYP1A2 and monoamine oxidases was evaluated. The most active compound in terms of 17β-HSD1 inhibition was 51b (figure 11).

*Virtual screening candidates.* A structure-based high throughput computational screening of a database containing 13 million drug-like molecules resulted in hits that were based on a 2-benzylidenbenzofuranone scaffold. Compound 52a (figure 11) was the most potent compound identified, showing an IC\(_{50}\) value of 45 nM for 17β-HSD1 inhibition and selectivity over 17β-HSD2 (Starčević et al. 2011b). Schuster et al. identified nonsteroidal 17β-HSD1 inhibitors by a virtual screening using pharmacophore models built from crystal structures containing steroidal compounds (Schuster et al. 2008). After validation of the most promising model by comparing predicted and experimentally determined inhibitory activities of flavonoids, a virtual library of nonsteroidal compounds was screened. 14 virtual hits were selected for biological testing. Compound 52b (figure 11) was the most active one, showing an IC\(_{50}\) value of 5.7 µM and selectivity over the related enzymes tested.

*Dual inhibitors of 17β-HSD1 and steroid sulfatase (STS).*

In addition to 17β-HSD1, the local biosynthesis of estrogen involves steroid sulfatase (STS), a key enzyme which converts the inactive estrone sulfate (E1S) - the main transport and storage form of estrogen - into the weakly active E1. The latter in turn is transformed into E2 via action of 17β-HSD1. Recently, the first dual inhibitors of both STS and 17β-HSD1 were reported. Their structures were rationally derived, based on the combination of the SAR for 17β-HSD1 inhibition in the class of bicyclic substituted hydroxyphenyl methanones (e.g. compound 41, figure 8) and the sulfamate moiety which is the essential feature for STS inhibition (Potter 2018). The IC\(_{50}\) values of the dual inhibitor 53 (figure 12) in intact T47D cells were 15 nM and 22 nM for STS and 17β-HSD1, respectively, and STS inhibition was found to be irreversible. The compound did not interfere with ERs but efficiently
reversed E1S- and E1- induced T47D cell proliferation, without affecting E2-induced proliferation (Salah et al. 2017).

Figure 12
Dual inhibitor of 17β-HSD1 and steroid sulfatase (STS)

Inhibitors of 17β-HSD2

Steroidal inhibitors of 17β-HSD2

Spirolactones. In 1994, Auger et al. found that introducing a spiro-Y-lactone at position 17 of E2 provokes a potent inhibition of 17β-HSD2. The first synthetic inhibitor of this type, compound 54 (figure 13), inhibited 17β-HSD2 with an IC₅₀ value of 0.27 µM. Its IC₅₀ towards the type 1 enzyme was reported to be more than 160 times higher, indicating a distinct selectivity (Auger et al. 1994).

The same group described the synthesis and biological evaluation of eleven spiro-Y-lactone analogs containing a steroidal C-18 or C-19 scaffold. Analysis of the inhibitory effect exerted by these compounds on microsomal 17β-HSD activity indicated that compounds containing the C-18 nucleus (exemplified by compound 55, figure 13) are more potent 17β-HSD2 inhibitors than C-19 analogs such as compounds 56 and 57 (results shown in figure) (Sam et al. 1995). No compound from this latter study, however, was more potent than compound 54, indicating that combination of a phenolic group and a spiro-Y-lactone moiety was important for efficient 17β-HSD2 inhibition.
In a continuation of this work and based on the obtained SAR, further spirolactones and related compounds were described. Starting from compound 54, structural optimizations were carried out. The resulting 17β-HSD2 inhibitors showed good selectivity over type 1 and type 3 enzymes (compounds 58-61, figure 13). Compound 58 was the most potent 17β-HSD2 inhibitor in this series (Poirier et al. 2001).
Starting from compound 58 as a lead, and as an extension of the previous work, Bydal et al. described further E2-based lactone derivatives, aiming at increasing the knowledge on the SAR in this compound class. The most potent compound was the enone 62 (figure 13), displaying 74% inhibition of 17β-HSD2 at a concentration of 0.1 µM and 19% inhibition at 0.01 µM (Bydal et al. 2004).

**Figure 14**

Steroidal inhibitors of 17β-HSD2: C7-substituted E2 derivatives and fluorine-substituted estrogens

*C7-substituted E2 derivatives.* Combining the fact that decorating the E2-scaffold with an appropriate lactone moiety provokes 17β-HSD2 inhibition and the finding that an N-butyl-N-methylundecanamide moiety in 7α-position of E2 leads to anti-estrogenic compounds (63 and 64, figure 14) resulted in the design of compound 65 which displayed a selective inhibition of 17β-HSD2. At a concentration of 1 µM, the compound reversed the estrogenic effect induced by 0.1 nM E2 by 87% (Sam et al. 2000).

*Fluorine-substituted estrogens.* 17-fluorine substituted estratrienes were identified as moderately active inhibitors of various h17β-HSDs. Most of the tested compounds were nonselective inhibitors of
h17β-HSD1 and 2. Among them, compounds 66 and 67 (figure 14) were shown to be the most potent h17β-HSD2 inhibitors (Deluca et al. 2006).

Nonsteroidal 17β-HSD2 inhibitors

Disubstituted cis-pyrrolidinones. From a screening effort for novel inhibitors of 17β-HSD2 as potential therapeutics for osteoporosis the 4,5-disubstituted cis-pyrrolidinones 68 and 69 (figure 15) were identified as lead compounds. Further 4,5-disubstituted cis-pyrrolidinones were designed and investigated for their ability to inhibit 17β-HSD2. It was found that the potency of the initial leads was improved by adding a second aromatic group to the 5-[hydroxy(aryl)methyl] side chain as exemplified by compound 70 (figure 15), which was the most potent inhibitor identified in this work (Gunn et al. 2005). Further structural refinements in this compound class focused on the search for an orally active compound eligible for in vivo studies (Wood et al. 2006). Thus, a series of novel 4,5-disubstituted cis-pyrrolidinones was synthesized and highly active compounds could be identified, and most of them maintained activity in the cell E2 conversion functional assay. In addition, they did not show appreciable binding to the estrogen, androgen or other steroid receptors and no tangible activity against 11β-HSD2, 17β-HSD1 and 17β-HSD3. Selected enantiomerically pure compounds (71-74, figure 15), which showed activity in osteoblast MG63 cells (IC_{50} = 0.03 μM – 0.48 μM), were tested for plasma exposure in rats, and compounds 71-73 were also applied to macaques. Based on its overall in vitro profile and the cynomolgus macaque exposure profile, compound 73 was selected for a non-human primate proof-of-concept study (Wood et al. 2006). Ovariectomized cynomolgus monkeys were used as an osteoporosis model to evaluate the efficacy of 73. After oral application, the compound led to a decrease in bone resorption while bone formation was maintained (Bagi et al. 2008).
Nonsteroidal inhibitors of 17\(\beta\)-HSD2: Disubstituted cis-pyrrolidinones

*Spiro-\(\delta\)-lactones.* Inspired by the molecular structures of steroidal 17\(\beta\)-HSD2 inhibitors described by the Poirier group, nonsteroidal spiro-\(\delta\)-lactones were designed and tested for 17\(\beta\)-HSD2 inhibition. The compounds were either inactive or showed low activity. In addition, they were chemically instable which was confirmed and quantified by determination of their half-lives in buffer, precluding further development. In figure 16 the most potent representative of this inhibitor class, compound 75, is displayed (Xu et al. 2011a).
Figure 16

Nonsteroidal inhibitors of 17β-HSD2: Spiro-δ-lactones, hydroxyphenylnaphthols, bicyclic substituted hydroxyphenylmethanones and (hydroxyphenyl)-1,2,4-triazoles.

Hydroxyphenylnaphthols. This compound class is also described in the section on nonsteroidal inhibitors of 17β-HSD1 of this review. Interestingly, the selectivity of these compounds can be shifted in favor of 17β-HSD2 inhibition, depending on the substitution pattern. Introduction of hydroxynaphthyl, hydroxyphenyl and (hydroxymethyl)phenyl moieties resulted in inactive or moderately active compounds. The most potent and selective member of this series was compound 76 (figure 16) (Wetzel et al. 2011a).
In the further drug optimization process, 21 additional naphthalene-derived compounds were synthesized and evaluated for 17β-HSD2 inhibition and selectivity toward the type 1 enzyme as well as affinity for the estrogen receptors α and β. Compounds with clearly improved properties could be obtained. In cell-free enzyme preparations compound 77 (figure 16) showed strong and selective 17β-HSD2 inhibition. In intact T47D cells, 77 displayed an IC₅₀ value of 31 nM for inhibition of 17β-HSD2. In addition, the compound was selective over 17β-HSD4 and 5 (40% and 21% inhibition at 1µM, respectively) and had low affinity to ERα: its relative binding affinity was less than 0.1 % of that of E2. The compound was tested for its inhibition of E1 and E2 formation in rat, mouse and marmoset (Callithrix jacchus) tissue preparations (liver or placenta). At 1 µM, it inhibited E1 formation in a rat liver preparation by 77 % and E2 formation by 52 %. The respective values for the mouse were 72 % (reduction of E1 formation) and 52 % (reduction of E2 formation) and for the marmoset 99 % and 75 % (reduction of E1 formation vs reduction of E2 formation). The values obtained for marmoset coincided well with those measured using a human placental preparation (98 % and 60 %, respectively) (Wetzel et al. 2011b).

Bicyclic substituted hydroxyphenylmethanones. Like the hydroxyphenynaphthols, bicyclic substituted hydroxyphenylmethanones were originally designed as inhibitors of 17β-HSD1, but could be structurally modified to render them more selective for the type 2 enzyme. This shift of selectivity was achieved by replacing the thiophene moiety (see figure 16) by a six-membered ring (benzene or pyridine). Benzene derivatives were more potent but less selective than their pyridine analogs while the latter showed more favorable combinations of activity and selectivity. The most interesting compound in this work was compound 78 (Wetzel et al. 2012).

(Hydroxyphenyl)-1,2,4-triazoles. Hydroxyphenyl-1,2,4-triazoles constitute a class of 17β-HSD2 inhibitors with moderate activity, displaying selectivity over the type 1 enzyme. Compounds 79 and 80 (figure 16) were the most active inhibitors in this compound class (Al-Soud et al. 2009, 2012).
Figure 17

Nonsteroidal inhibitors of 17β-HSD2: Biphenyl amides and phenylthiophene amides.

Biphenyl amides and phenylthiophene amides. In 2011, Xu et al. discovered N-benzyl-diphenyl-3(or 4)-carboxamides and N-benzyl-thiophene-2-carboxamides as potent nonsteroidal 17β-HSD2 inhibitors (Xu et al. 2011b). Formally, these compounds were derived from the (hydroxyphenyl)-1,2,4-triazoles (see preceding chapter) by replacing the triazole ring with a non-cyclic carboxamide moiety. In the design concept it was hypothesized that the rigidity induced by the triazole ring was responsible for the weak 17β-HSD2 inhibitory activity. In a cell-free assay, compound 81 (figure 17) was the most potent inhibitor in the diphenyl carboxamide class. It is noteworthy that this compound showed a strong selectivity for 17β-HSD2 over the type 1 enzyme. In addition, it is one of the rare examples for 17β-HSD2 inhibitors devoid of a phenolic OH-group. In terms of activity and selectivity, the phenylthiophene amide 82 (figure 17) was the most interesting compound identified in this study. It showed no binding affinity to the estrogen receptors.

Due to favorable biological properties, the phenylthiophene scaffold was used as a lead for drug optimization studies. In a first approach, the length of the linker between the nitrogen atom of the amide group and the adjacent benzene moiety was varied (figure 17, n = 0 and 2) (Marchais-Oberwinkler et al. 2013). While none of the phenethylamides (n=2) were active, most of the anilides (n=0) turned out to be moderate or strong inhibitors of 17β-HSD2. Compounds 83 and 84 (figure 17) were the most potent and selective inhibitors in this study, each with an IC₅₀ value of 62 nM in a cell-
free assay, similarly strong activities in whole cell assays (T47D) and selectivity over 17β-HSD1. SAR studies allowed a first characterization of the active site of human 17β-HSD2. It is quite large and certainly larger than that of 17β-HSD1. The most potent compounds were investigated regarding their ability to inhibit 17β-HSD2 from other species (mouse, rat and marmoset). At a concentration of 1 µM, the hydroxylated compound 82 showed 65 % inhibition of E1 formation in mouse liver preparation.

**Figure 18**

Nonsteroidal inhibitors of 17β-HSD2: Phenylthiazole amides and structurally modified phenylthiophene amides. General formulas (upper part) and compound 85.

*Phenylthiazole amides and structurally modified phenylthiophene amides.* A second approach for the optimization of the phenylthiophene-based carboxamides focused on the variation of substitution pattern of the thiophene ring and its replacement with thiazole (figure 18). Moreover, the effects of substitution of the amide group with a larger moiety as well as the exchange of the \( N \)-methylamide group with possible bioisosteres on the biological properties were investigated. The main aim of this
study was the development of novel active and selective 17β-HSD2 inhibitors with high potency towards both the human protein and its murine ortholog as well as selectivity over the type 1 enzymes of both species, in order to discover compounds eligible for in vivo studies. Very sharp SAR could be observed in this class of compounds. In general, the 2,5-disubstituted thiophene derivatives were less active towards the murine enzyme than towards the human ortholog, except for compound 85 (figure 18), which in protein preparations was four times more potent against murine 17β-HSD2 (IC$_{50}$ = 54 nM) than against the human enzyme (IC$_{50}$ = 235 nM) with a strong selectivity over h17β-HSD1 (SF1/2 = 95) and low affinities to ERα and β (Perspicace et al. 2014).

Figure 19

Nonsteroidal inhibitors of 17β-HSD2: A- and B-ring modifications in the class of carboxamides, part 1. General formula (upper part) and compound 86.
A major drawback of the aforementioned amides featuring a 2,5-disubstituted thiophene moiety, such as 85 (figure 18), was unsatisfactory metabolic stability. Compounds with clearly improved stability towards biotransformation were discovered by structural modifications and changing lipophilicity (figure 19). In addition, an analysis of the main routes of biotransformation of amide-based 2,5-disubstituted thiophenes was performed. Compound 86 (figure 19) showed a half-life greater than 120 minutes in human liver microsomes (S9 fraction), moderate activity towards 17β-HSD2 and selectivity over 17β-HSD1 (SF1/2 = 10). In addition, the compound displayed no affinity for the estrogen receptors (Gargano et al. 2014).

**Figure 20**

Nonsteroidal inhibitors of 17β-HSD2: B-ring modifications in the class of carboxamides, part 2. General formulas (upper part) of the 2,5-thiophene amide (left), 1,3-phenylene amide (middle) and 1,4-phenylene amide classes (right) and compound 87.
Gargano et al. aimed at identifying a compound which can be used for a proof of concept study in a mouse model for osteoporosis beside being a suitable potential therapeutic for treating osteoporosis in humans. For this purpose, 25 previously described inhibitors of human 17β-HSD2, belonging to the 2,5-thiophene amide, 1,3-phenylene amide and 1,4-phenylene amide classes (figure 20), were tested for inhibition of the murine enzyme. From the data obtained, a comparative SAR study was elaborated which was then used to develop a drug optimization strategy. This rationale led to the discovery of compound 87 (figure 20), which was the first 17β-HSD2 inhibitor to show an appropriate profile for the purposes mentioned above, with strong inhibition of both human and murine 17β-HSD2 (IC₅₀ = 300 nM and 140 nM, respectively) and selectivity over the human type 1 enzyme (SF1/2 = 44) and ERs. It also displayed strong inhibitory activity in intact T47D cells (66% inhibition at 250 nM), a half-life of 107 min in human liver microsomes (S9 fraction) and suitable physicochemical parameters (MW = 345 and cLogP = 4.75) (Gargano et al. 2015).
Figure 21

Nonsteroidal inhibitors of 17β-HSD2: Replacing the biphenyl system with non-aromatic or angulate moieties. Structural modifications (upper part) and compound 88.

Unfortunately, compound 87 showed cytotoxic effects in HEK293 cells (LD$_{50}$ < 6.25 µM). It was assumed that this property might be attributed to the biphenyl moiety. A successful minimization of cytotoxicity was achieved by replacing the biphenyl system with non-aromatic or angulate moieties (figure 21, top). Compound 88 (figure 21, bottom) displayed a significantly decreased cytotoxicity (LD$_{50}$ ≈ 25 µM), improved 17β-HSD2 inhibitory activity in a cell-free assay and enhanced selectivity over 17β-HSD1 when compared to 87 (Gargano et al. 2016).
Figure 22

Nonsteroidal inhibitors of 17β-HSD2: Thieno[3,2-d]pyrimidinones and biphenylsulfonamide

Thieno[3,2-d]pyrimidinones and related compounds. Rigidification of inhibitors with amidothiophene scaffold (figure 17, compounds 83 and 84; figure 18, compound 85) led to a series of conformationally restricted thieno[3,2-d]pyrimidinones, thieno[3,2-d]pyrimidines and quinazolinones. The compounds showed no or little activity as 17β-HSD2 inhibitors. However, the two moderately active compounds 89 and 90 (figure 22) were discovered, allowing for conclusions concerning the biologically active conformers of the non-rigidified predecessors (Perspicace et al. 2013a).

Biphenyl- and phenylthiophene-sulfonamides. Starting from 17β-HSD2 inhibitors bearing a biphenylcarboxamide moiety (figure 19, compound 86) another class of biphenyl derivatives was designed by exchanging the carboxamide group with a sulfonamide or a retrosulfonamide group, (Perspicace et al. 2013b). The main goal of this work was to develop new active and selective 17β-HSD2 inhibitors with a good in vitro ADME profile. Inhibitory activity of the retrosulfonamides (with the -SO₂-group linked to the hydroxyphenyl moiety, as seen in compound 91 (figure 22) was superior compared to that of the sulfonamides. This property was attributed to the acidity of the phenolic OH-group which is stronger in case of the retrosulfonamides. There was no correlation between the inhibitory potencies of the carboxamides and the analog sulfonamide classes. This fact was attributed to possible different binding modes of the two compound classes in the active site of the enzyme. The most interesting compound identified in this study, compound 91, showed high inhibitory potency -and selectivity over 17β-HSD1. Moreover, its calculated and experimentally determined ADME parameters (MW = 373 g/mol, experimentally determined log P = 4.42, tPSA = 86 Å², 4 rotatable bonds, 2HD, 4HA) predicted a good bioavailability.
Phenylbenzenesulfonamides and -sulfonates. Vuorinen et al. developed specific ligand-based pharmacophore models for 17β-HSD2 inhibitors. Using these models as virtual screening filters, 7 novel 17β-HSD2 inhibitors were discovered. An additional search for structurally similar compounds resulted in the biological evaluation of 28 small molecules. In total, 13 new 17β-HSD2 inhibitors, from which 10 represented phenylbenzene-sulfonamides and –sulfonates, were discovered (general structure given in figure 23, left). These inhibitors aided in the development of the SAR-model and -rules for this specific scaffold: in general, 17β-HSD2 inhibitors require a hydrogen bond donor functionality on one benzene ring, and hydrophobic substituents on the other. The most potent inhibitor discovered was compound 92 (figure 23). The compound was selective over the type 1 enzyme (remaining activity of 17β-HSD1 > 70% at an inhibitor concentration of 20 µM). Compound 93 showed moderate inhibition of 17β-HSD2 and no inhibition of the type 1 enzyme (Vuorinen et al. 2014). In continuation of this work, twenty novel compounds representing the phenylbenzenesulfonamide and –sulfonate scaffold were synthesized and tested for their 17β-HSD2 inhibitory activity. Nine compounds showed IC_{50}-values in the low nanomolar range. Although none of them was more active than 92 they allowed the derivation of more comprehensive SAR-rules for this scaffold (Vuorinen et al. 2017b).

Figure 23
Nonsteroidal inhibitors of 17β-HSD2: Phenylbenzenesulfonamides and-sulfonates

Inhibitors of 17β-HSD14
The fact that 17β-HSD1 and 17β-HSD2 catalyze the same reaction as 17β-HSD14 led to the idea that the substrate binding sites of the three enzymes should exhibit a high structural similarity. A small library of 17β-HSD1 and 17β-HSD2 inhibitors was tested for 17β-HSD14 inhibition. Most of the investigated hydroxyphenylnapthols (cf. figures 6 and 16) and thiopheneamides (cf. figures 17 and 18) were inactive whereas bis(hydroxyphenylthiophenes and –thiazoles (figure 7) as well as bicyclic substituted hydroxyphenylmethanones containing a pyridine moiety (figure 16, general formula, X = N) showed examples of low to moderate inhibitory activity against 17β-HSD14. Active compounds possessing very high potency for 17β-HSD1 and/or 17β-HSD2 were neglected for the subsequent optimization as difficulties in achieving high selectivity for 17β-HSD14 were anticipated. Starting from the hits 94 and 95 (figure 24), approximately thirty compounds were synthesized and tested for 17β-HSD14 inhibition. In a fluorimetric assay, one of the most active representatives (compound 96) exhibited a Ki value of 7 nM (Braun et al. 2016). The crystal structure of 17β-HSD14 in complex with NAD⁺ and 96 is the first to be obtained from a human SDR 17β-HSD enzyme with a nonsteroidal compound. The inhibitor is embedded in an extended H-bonding network and adopts a V-shaped conformation which corresponds to the active site geometry (Bertoletti et al. 2016).

Very recently, 17β-HSD14 inhibitors containing a quinoline moiety have been published (Braun et al. 2018). Compound 97 (figure 24) is the most interesting inhibitor in this series, showing high affinity to the target protein combined with a good selectivity profile toward the 17β-HSD types 1, 2 and 10 as well as ERα. In addition, the compound did not display cytotoxicity, good solubility and auspicious predicted bioavailability.

Figure 24

Inhibitors of 17β-HSD14
Conclusion and outlook

In search of novel treatment options for EDD and in order to explore estrogenic signaling pathways, the possibility to modulate estrogen action by inhibition of 17β-HSD1 and 17β-HSD2, respectively, has been pursued intensively within the past 25 years. During this period of time, a considerable number of inhibitors of 17β-HSD1 and 17β-HSD2 have been described in the literature. Some of them, such as natural compounds, were discovered in screening approaches. The majority of the inhibitors, however, result from rational drug design approaches, covering both steroidal and nonsteroidal compound classes. An interesting recent evolution is the development of designed multiple ligands which potently inhibit both STS and 17β-HSD1. Such dual inhibitors could reduce intracellular E2 levels more effectively than selective inhibitors of 17β-HSD1 and may thus be superior potential therapeutics, for instance for the treatment of endometriosis where these two proteins are overexpressed.

In spite of the fact that a plethora of potent and selective inhibitors of 17β-HSD1 and 17β-HSD2 have been identified in cell-free and cellular assays, only very few compounds have been applied in vivo in preclinical studies. No compound has been reported to enter the clinical phase. There are several reasons for this poor outcome. Interspecies differences between the human enzymes and their orthologs from other species, especially rodents, are a main factor that hampered preclinical in vivo evaluation. The problem has been neglected for a long time when research was almost exclusively focused on inhibiting the human enzymes. Whereas the search for 17β-HSD2 inhibitors by now has generated compounds which provoke potent and selective inhibition of both the human and the rodent enzyme, and may thus be candidates for in vivo evaluation, the development of inhibitors of 17β-HSD1 was less successful in this respect. In the latter case, transgenic mice expressing the human enzyme have been established to solve this problem. Other approaches relied on primate disease
models or xenograft models using immunodeficient mice or rats. Experiments using primates, however, pose high ethical hurdles and are very expensive. Xenograft models bear the drawback to give no information on possible changes of systemic estrogen levels. Insufficient metabolic stability is another issue potentially impeding (pre-)clinical trials, as many inhibitors bear a phenolic OH-group which could entail phase II biotransformation.

It will be a future task to design novel inhibitors of 17β-HSD2 and especially 17β-HSD1, combining improved pharmacokinetic and selectivity properties with enhanced inhibitory activities towards rodent orthologs in order to facilitate investigations of their effects in vivo and eventually to accelerate the identification of compounds eligible for clinical evaluation as novel therapeutics.

The situation is different when it comes to 17β-HSD14 inhibition, which is an emerging research field. As the native substrates of 17β-HSD14 are unknown, it is presently unclear whether this enzyme may be exploited as a drug target in the future. However, the potent and selective inhibitors which have become available recently could serve as scientific tools, facilitating the elucidation of the physiological role of this protein and allowing conclusions on its druggability.

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