Inhibitors of 17 β -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 17 β -hydroxysteroid dehydrogenase types 1 and 2 (17 β -HSD1 and 17 β -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 17 β -HSD1 and steroid sulfatase (STS) as well as the first inhibitors of 17 β -HSD14 are covered.

Introduction

17 β -Hydroxysteroid dehydrogenase type 1 and type 2 (17 β -HSD1 and 17 β -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 17 β -position of steroidal substrates, under consumption of the natural cosubstrates NADPH (17 β -HSD1) and NAD⁺ (17 β -HSD2). Thus, 17 β -HSD1 (SDR nomenclature: SDR28C1) catalyzes the reduction of the weakly estrogenic estrone (E1) to the most potent estrogen 17 β -estradiol (E2, scheme 1) which plays a central role in the etiology of estrogen dependent diseases (EDD). 17 β -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, 17 β -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 17 β -HSD1, 17 β -HSD2 and 17 β -HSD14

17 β -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. 17 β -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 17 β -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 17 β -HSD1 mRNA are indicative for a crucial role of 17 β -HSD1 e.g. in endometriosis (Šmuc *et al.* 2007;

Huhtinen *et al.* 2012), endometrial hyperplasia (Saloniemi *et al.* 2010), endometrial cancer (Cornel *et al.* 2012, 2017), breast cancer (Jansson 2009), ovarian tumor (Blomquist *et al.* 2002) and uterine leiomyoma (Kasai *et al.* 2004). Inhibition of 17 β -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) 17 β -HSD1 inhibition resulted in a decrease of E2 levels in endometriotic specimens (Delvoux *et al.* 2014); (2) 17 β -HSD1 inhibitors reversed estrogen-induced endometrial hyperplasia in transgenic mice (Saloniemi *et al.* 2010); (3) Inhibition of 17 β -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 17 β -HSD2 is present in osteoblasts (Dong *et al.* 1998) and decreases E2- and T-levels by oxidation to the ketones makes 17 β -HSD2 inhibition a promising approach for the treatment of osteoporosis. In an in vivo monkey model, a 17 β -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 17 β -HSD1 and 17 β -HSD2 should have the advantage to exert little influence on systemic estrogen levels, offering the prospect of fewer side effects.

17 β -HSD14 (SDR47C19; synonyms: retSDR3, DHRS10) is another member of the SDR family and the last 17 β -HSD that has been identified. In vitro, it NAD⁺-dependently oxidizes E2 to E1 (Lukacik *et al.* 2007) - and thus shows analogy to 17 β -HSD2. Moreover, it was found to catalyze the formation of dehydroepiandrosterone and 4-androstene-3,17-dione from 5-androstene-3 β ,17 β -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 17 β -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 17 β -HSDs

The literature covering effects of phytoestrogens on 17 β -HSD enzymes has been reviewed previously (Deluca *et al.* 2005). In a more recent publication, inhibitory activities of different flavones and related compounds on 17 β -HSD enzymes from the fungus *Cochliobolus lunatus* (17 β -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 μ M. Their ability to inhibit the reverse reaction (reduction of estrone) was less pronounced (IC₅₀ values from 1.2 μ M to 7.4 μ M). Coumestrol and kaempferol (figure 1, compounds **6** and **7a**, respectively) inhibited both reactions unselectively (IC₅₀ values between 2.5 μ M and 3.9 μ M).

Vuorinen *et al.* applied a pharmacophore model based on 17 β -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 17 β -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 17 β -HSD1 and 17 β -HSD2 (Engeli *et al.* 2017). All parabens under investigation moderately inhibited 17 β -HSD2. Inhibition of 17 β -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 17 β -HSD1 and 17 β -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 17 β -HSD2 is more likely to occur than through inhibition of 17 β -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*.

Compound		17β-HSD1	17β-HSD2	Reference
		IC ₅₀ (μM), cell-free		
7b	2'-Hydroxygenistein	1.09	2.03	(Vuorinen <i>et al.</i> 2017a)
8	Nordihydroguaiairetic acid	5.5	0.38	
9	(-)-Dihydroguaiairetic 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				
12a	Methylparaben	0	50	(Engeli <i>et al.</i> 2017)
12	Ethylparaben	7	74	
b				
12c	Propylparaben	37	37	
12	Hexylparaben	80	58	
d				
12e	Heptylparaben	83	76	

Figure1

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

Inhibitors of 17 β -HSD1

Steroidal inhibitors of 17 β -HSD1

C16 substituted E2 derivatives. Since the 1990s, the Poirier group has been working on C16 substituted estradiol derivatives as inhibitors of 17 β -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 IC₅₀ 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 (IC₅₀ 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).

Compound d	17β-HSD1 IC ₅₀ , cell-free	Reference
13a	1.3 μM	(Rouillard <i>et al.</i> 2008)
13b	1.2 μM	
14	10.4 μM	(Pelletier & Poirier 1996)
15 ^a	52 nM	(Poirier <i>et al.</i> 2005)
	8 nM	
16a	56 nM	(Bérubé & Poirier 2009)
17a	44 nM in T47D cells	
17b	171 nM in T47D cells	(Laplane <i>et al.</i> 2008)
17c	68 nM in T47D cells	
		(Maltais <i>et al.</i> 2011)

^aDifferent IC₅₀ values reported in the cited references.

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

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 17 β -HSD1 (IC₅₀ 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 16 β -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 16 β positions of E2 and E1, respectively, led to the highly potent compounds **17a** and **17b** (figure 2) with good cellular permeability, displaying IC₅₀ 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 (Laplanche *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 17 β -HSD1 (IC₅₀: 68 nM in intact T47D cells) and it was found to have no effect on 17 β -HSD2, 17 β -HSD7, 17 β -HSD12 and CYP3A4 (Maltais *et al.* 2011, 2014).

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

^a: percent inhibition at 1 μM in T47D cells

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; IC₅₀ 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).

17β-HSD1			
Compound d	IC ₅₀ (nM)		Reference
	cell-free (nM)	cellular (nM)	
19	4	(100% inhibn. at 1 μM)	(Messinger <i>et al.</i> 2009)
20(β)	194	-	
20(α)	353	-	
21a	-	37	(Allan <i>et al.</i> 2006a)
22a	-	110	
22b	-	700	

Figure 4

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

C15 substituted E1 derivatives. Messinger *et al.* introduced another class of 17 β -HSD1 inhibitors by synthesizing C15-substituted estrone derivatives (figure 4, compounds **19** and **20**) (Messinger *et al.*

2009). Compound **19** displayed strong and selective inhibition of the target protein in cell-free assay. At a concentration of 1 μ M, **19** inhibited recombinant human 17 β -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 1 μ M). 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 16 β -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 17 β -HSD1 in transgenic mice expressing human 17 β -HSD1 by 85% and 33% in males and females, respectively (Lamminen *et al.* 2009). Interestingly, the 16 α -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 17 β -HSD1 to discover new, selective and potent inhibitors (figure 4, general structure **21**), with a methyl amide extending from the 16 β -position of E1 (Lawrence *et al.* 2005; Vicker *et al.* 2006). In this series of compounds, **21a** was the most potent inhibitor of 17 β -HSD1 reported, showing selectivity for 17 β -HSD1 over 17 β -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).

Compound	17β-HSD1 IC ₅₀ (nM)		Reference
	Cell-free	Cellular	
23	15	-	(Möller <i>et al.</i> 2009)
24	-	1000	(Ouellet <i>et al.</i> 2014)
25	-	1400	(Maltais <i>et al.</i> 2015)
26	-	180	(Allan <i>et al.</i> 2006a)
27	-	530	
28	-	300	(Fischer <i>et al.</i> 2005)

Figure 5

Steroid 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 IC₅₀ 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 IC₅₀ 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 17 β -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 17 β -HSD1 in T47D cells with IC₅₀ values of 180, 530 and 300 nM, respectively. In addition, they displayed selectivity over 17 β -HSD2 and no estrogenic effects (Fischer *et al.* 2005; Allan *et al.* 2006a, b).

Nonsteroidal inhibitors of 17 β -HSD1

(Hydroxyphenyl)naphthols. Frotscher *et al.* described potent and selective 17 β -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		SF (17β-HSD2/17β-HSD1)	Reference
	IC ₅₀ (nM)			
	Cell-free	Cellular		
29	116	-	48	(Frotscher <i>et al.</i> 2008)
30	20	-	27	(Marchais-Oberwinkler <i>et al.</i> 2008)
31	15	71	27	(Marchais-Oberwinkler <i>et al.</i> 2011b)
32	15	196	27	

33	32	1100	12	(Henn <i>et al.</i> 2012)
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Figure 6

Nonsteroidal inhibitors of 17 β -HSD1: (Hydroxyphenyl)naphthols

In cell-free assays, compound **29** (figure 6) showed inhibitory activity towards 17 β -HSD1, combined with good selectivity over the type 2 enzyme (selectivity factor SF 2/1= IC_{50} (17 β -HSD2) / IC_{50} (17 β -HSD1) = 48), ER α 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 17 β -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 17 β -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*) 17 β -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 17 β -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 17 β -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 17 β -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 17 β -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 *in vitro* in cancer cell lines by influencing the cell cycle (Berenyi *et al.* 2013).

Compound d	X	Y	Z	17 β -HSD1	SF	Reference
				IC ₅₀ (nM), Cell-free	(17 β -HSD2/17 β -HSD1)	
34	N	O	H	310	56	(Bey <i>et al.</i> 2008a)
35	N	S	H	50	80	
36	CH	S	H	69	28	(Bey <i>et al.</i> 2008b)

37a	CH	S	F	8	118	(Bey <i>et al.</i> 2009)
37b	CH	S	CH ₃	46	49	

Figure 7

Nonsteroidal inhibitors of 17 β -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 17 β -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 IC₅₀ values in the low nanomolar range in both cell-free and T47D cellular assays, selectivity over 17 β -HSD2, no significant affinity to ER α and ER β , and metabolic stability in human liver microsomes (phase 1; $t_{1/2} \geq 100$ 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 17 β -HSD2 and metabolic stability ($t_{1/2} = 12.8$ min).

Compound	17β-HSD1		SF (17β-HSD2/17β-HSD1)	Reference
	IC ₅₀ (nM)			
	Cell-free	Cellular		
38a	8	27	48	(Oster <i>et al.</i> 2010a)
38b	19	17	31	
38c	5	126	49	
39	12	78	14	(Oster <i>et al.</i> 2011)
40	2	-	3	(Abdelsamie <i>et al.</i> 2015)
41	0.5	-	82	(Abdelsamie <i>et al.</i> 2017)

Figure 8

Nonsteroidal inhibitors of 17 β -HSD1: Bicyclic substituted hydroxyphenyl methanones

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) 17 β -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 17 β -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

40 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 17 β -HSD1 inhibitor known so far, displaying high selectivity over 17 β -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		SF (17β-HSD2/17β-HSD1)	Reference
	IC ₅₀ (nM)			
	Cell-free	Cellular		
42	44	-	24	(Spadaro <i>et al.</i> 2012a)
43a	27	258	148	
43b	13	37	136	(Spadaro <i>et al.</i> 2012b)
44	13	-	40	(Miralinaghi <i>et al.</i> 2014)

Figure 9

Nonsteroidal inhibitors of 17 β -HSD1: Hydroxybenzothiazoles and hydroxybenzothiophenes

Hydroxybenzothiazole and hydroxybenzothiophene derivatives. The Hartmann group developed another pharmacophore model in an approach to design nonsteroidal 17 β -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 17 β -HSD1, compound **42** (figure 9) displayed an IC₅₀ 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 17 β -HSD1 inhibitors over the type 2 protein. In addition, they were highly potent inhibitors of 17 β -HSD1 in cell-free and T47D cellular assays (IC₅₀: 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 17 β -HSD1 inhibitors bearing a benzothiophene moiety. Compound **44** (figure 9) is more active towards the target enzyme and displayed better selectivity over 17 β -HSD2 than its benzothiazole analog **42** (Miralinaghi *et al.* 2014).

Thiophenepyrimidinones. Messinger *et al.* identified 17 β -HSD1 inhibitors by computer aided drug design starting from a unique nonsteroidal pyrimidinone core (structure **45**, figure 10) by optimizing R₁ and R₂. In protein-based inhibition assays compound **45a** displayed an IC₅₀ value of 5 nM against 17 β -HSD1, and selectivity over the type 2 enzyme (8% inhibition at 100 nM). In intact MCF-7 cells, 17 β -HSD1 was inhibited by 67% when **45** was applied in a concentration of 1 μ M. The compound significantly reduced 17 β -HSD1-dependent tumor growth in a nude mouse model (Messinger *et al.* 2006). Lilienkamp *et al.* also reported a potent 17 β -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	17 β -HSD1	Reference
	IC ₅₀ , cell-free	
45a	5 nM	(Messinger <i>et al.</i> 2006)
46	94 % inhibition at 100 nM	(Lilienkampf <i>et al.</i> 2009)
47	1.7 μ M in T47D cells	(Allan <i>et al.</i> 2008)
48	560 nM	(Oster <i>et al.</i> 2010b)

Figure 10

Nonsteroidal inhibitors of 17 β -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 17 β -HSD1 inhibitors, showing 10-68% inhibition at a concentration of 100 μ M (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 IC₅₀ value of 1.7 μ M in an assay using T47D

cells (Allan *et al.* 2008). Oster *et al.* published heterocyclic substituted biphenylols and their aza-analogues with 17 β -HSD1 inhibitory activity (Oster *et al.* 2010b). The most active compound **48** (figure 10) displayed IC₅₀ values of 560 nM (17 β -HSD1) and IC₅₀ 2370 nM (17 β -HSD2) in cell-free assays, and low affinity for ERs α and β .

Figure 11

Nonsteroidal inhibitors of 17 β -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 17 β -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 μ M, respectively. The same group reported the synthesis of coumarin derivatives as selective nonsteroidal inhibitors of 17 β -HSD1. Compound **51a** (figure 11) was the most potent inhibitor in this series with an IC₅₀ value of 270 nM and selectivity towards 17 β -HSD2 and ERs (Starčević *et al.* 2011a). Recently, Niinivehmas *et al.* reported on a series of 3-phenylcoumarin analogs as inhibitors of recombinant human 17 β -HSD1 (Niinivehmas *et al.* 2018). The molecular basis of inhibition was determined by a docking-based SAR

analysis, and inhibition of 17 β -HSD2 as well as cross-reactivity against ER α , aromatase, CYP1A2 and monoamine oxidases was evaluated. The most active compound in terms of 17 β -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 IC₅₀ value of 45 nM for 17 β -HSD1 inhibition and selectivity over 17 β -HSD2 (Starčević *et al.* 2011b). Schuster *et al.* identified nonsteroidal 17 β -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 IC₅₀ value of 5.7 μ M and selectivity over the related enzymes tested.

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

In addition to 17 β -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 17 β -HSD1. Recently, the first dual inhibitors of both STS and 17 β -HSD1 were reported. Their structures were rationally derived, based on the combination of the SAR for 17 β -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 IC₅₀ values of the dual inhibitor **53** (figure 12) in intact T47D cells were 15 nM and 22 nM for STS and 17 β -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 17 β -HSD1 and steroid sulfatase (STS)

Inhibitors of 17 β -HSD2

Steroidal inhibitors of 17 β -HSD2

Spirolactones. In 1994, Auger *et al.* found that introducing a spiro- γ -lactone at position 17 of E2 provokes a potent inhibition of 17 β -HSD2. The first synthetic inhibitor of this type, compound **54** (figure 13), inhibited 17 β -HSD2 with an IC₅₀ value of 0.27 μ M. 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- γ -lactone analogs containing a steroidal C-18 or C-19 scaffold. Analysis of the inhibitory effect exerted by these compounds on microsomal 17 β -HSD activity indicated that compounds containing the C-18 nucleus (exemplified by compound **55**, figure 13) are more potent 17 β -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- γ -lactone moiety was important for efficient 17 β -HSD2 inhibition.

Figure 13

Steroidal inhibitors of 17β-HSD2: Spirolactones and lactones

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 17 β -HSD2 at a concentration of 0.1 μ M and 19 % inhibition at 0.01 μ M (Bydal *et al.* 2004).

Figure 14

Steroidal inhibitors of 17 β -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 17 β -HSD2 inhibition and the finding that an *N*-butyl-*N*-methylundecanamide moiety in 7 α -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 17 β -HSD2. At a concentration of 1 μ M, 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 h17 β -HSDs. Most of the tested compounds were nonselective inhibitors of

h17 β -HSD1 and 2. Among them, compounds **66** and **67** (figure 14) were shown to be the most potent h17 β -HSD2 inhibitors (Deluca *et al.* 2006).

Nonsteroidal 17 β -HSD2 inhibitors

Disubstituted cis-pyrrolidinones. From a screening effort for novel inhibitors of 17 β -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 17 β -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 11 β -HSD2, 17 β -HSD1 and 17 β -HSD3. Selected enantiomerically pure compounds (**71-74**, figure 15), which showed activity in osteoblast MG63 cells (IC_{50} = 0.03 μ M – 0.48 μ M), 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).

Figure 15

Nonsteroidal inhibitors of 17 β -HSD2: Disubstituted *cis*-pyrrolidinones

Spiro- δ -lactones. Inspired by the molecular structures of steroidal 17 β -HSD2 inhibitors described by the Poirier group, nonsteroidal spiro- δ -lactones were designed and tested for 17 β -HSD2 inhibition. The compounds were either inactive or showed low activity. In addition, they were chemically unstable 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 17 β -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 17 β -HSD1 of this review. Interestingly, the selectivity of these compounds can be shifted in favor of 17 β -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 17 β -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 17 β -HSD2 inhibition. In intact T47D cells, **77** displayed an IC₅₀ value of 31 nM for inhibition of 17 β -HSD2. In addition, the compound was selective over 17 β -HSD4 and 5 (40% and 21% inhibition at 1 μ M, respectively) and had low affinity to ER α : 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 μ M, 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 hydroxyphenylnaphthols, bicyclic substituted hydroxyphenylmethanones were originally designed as inhibitors of 17 β -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 17 β -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 17 β -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 17 β -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 17 β -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 17 β -HSD2 over the type 1 enzyme. In addition, it is one of the rare examples for 17 β -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 17 β -HSD2. Compounds **83** and **84** (figure 17) were the most potent and selective inhibitors in this study, each with an IC_{50} value of 62 nM in a cell-

free assay, similarly strong activities in whole cell assays (T47D) and selectivity over 17 β -HSD1. SAR studies allowed a first characterization of the active site of human 17 β -HSD2. It is quite large and certainly larger than that of 17 β -HSD1. The most potent compounds were investigated regarding their ability to inhibit 17 β -HSD2 from other species (mouse, rat and marmoset). At a concentration of 1 μ M, the hydroxylated compound **82** showed 65 % inhibition of E1 formation in mouse liver preparation.

Figure 18

Nonsteroidal inhibitors of 17 β -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 17 β -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 17 β -HSD2 (IC_{50} = 54 nM) than against the human enzyme (IC_{50} = 235 nM) with a strong selectivity over h17 β -HSD1 ($SF_{1/2}$ = 95) and low affinities to ER α and β (Perspicace *et al.* 2014).

Figure 19

Nonsteroidal inhibitors of 17 β -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 17 β -HSD2 and selectivity over 17 β -HSD1 (SF1/2 = 10). In addition, the compound displayed no affinity for the estrogen receptors (Gargano *et al.* 2014).

Figure 20

Nonsteroidal inhibitors of 17 β -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 17 β -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 17 β -HSD2 inhibitor to show an appropriate profile for the purposes mentioned above, with strong inhibition of both human and murine 17 β -HSD2 (IC_{50} = 300 nM and 140 nM, respectively) and selectivity over the human type 1 enzyme ($SF_{1/2}$ = 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 17 β -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 ($LD_{50} < 6.25 \text{ }\mu\text{M}$). 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 ($LD_{50} \approx 25 \text{ }\mu\text{M}$), improved 17 β -HSD2 inhibitory activity in a cell-free assay and enhanced selectivity over 17 β -HSD1 when compared to **87** (Gargano *et al.* 2016).

Figure 22

Nonsteroidal inhibitors of 17 β -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 17 β -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 17 β -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 17 β -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 17 β -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 17 β -HSD2 inhibitors. Using these models as virtual screening filters, 7 novel 17 β -HSD2 inhibitors were discovered. An additional search for structurally similar compounds resulted in the biological evaluation of 28 small molecules. In total, 13 new 17 β -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, 17 β -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 17 β -HSD1 > 70% at an inhibitor concentration of 20 μ M). Compound **93** showed moderate inhibition of 17 β -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 17 β -HSD2 inhibitory activity. Nine compounds showed IC₅₀-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 17 β -HSD2: Phenylbenzenesulfonamides and-sulfonates

The fact that 17 β -HSD1 and 17 β -HSD2 catalyze the same reaction as 17 β -HSD14 led to the idea that the substrate binding sites of the three enzymes should exhibit a high structural similarity. A small library of 17 β -HSD1 and 17 β -HSD2 inhibitors was tested for 17 β -HSD14 inhibition. Most of the investigated hydroxyphenylnaphthols (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 17 β -HSD14. Active compounds possessing very high potency for 17 β -HSD1 and/or 17 β -HSD2 were neglected for the subsequent optimization as difficulties in achieving high selectivity for 17 β -HSD14 were anticipated. Starting from the hits **94** and **95** (figure 24), approximately thirty compounds were synthesized and tested for 17 β -HSD14 inhibition. In a fluorimetric assay, one of the most active representatives (compound **96**) exhibited a K_i value of 7 nM (Braun *et al.* 2016). The crystal structure of 17 β -HSD14 in complex with NAD⁺ and **96** is the first to be obtained from a human SDR 17 β -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, 17 β -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 17 β -HSD types 1, 2 and 10 as well as ER α . In addition, the compound did not display cytotoxicity, good solubility and auspicious predicted bioavailability.

Figure 24

Inhibitors of 17 β -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 17 β -HSD1 and 17 β -HSD2, respectively, has been pursued intensively within the past 25 years. During this period of time, a considerable number of inhibitors of 17 β -HSD1 and 17 β -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 17 β -HSD1. Such dual inhibitors could reduce intracellular E2 levels more effectively than selective inhibitors of 17 β -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 17 β -HSD1 and 17 β -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 17 β -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 17 β -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 17 β -HSD2 and especially 17 β -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 17 β -HSD14 inhibition, which is an emerging research field. As the native substrates of 17 β -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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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding Sources

We are grateful to the Deutsche Forschungsgemeinschaft for financial support (FR 3002/1-1) of this work.

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