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Research paper

Towards the evaluation in an animal disease model: Fluorinated 17β-HSD1 inhibitors showing strong activity towards both the human and the rat enzyme

Ahmed S. Abdelsamie [a], Emmanuel Bey [b], Emanuele M. Gargano [a], Chris J. van Koppen [b], Martin Empting [c], Martin Frotscher [a, *]

[a] Pharmaceutical and Medicinal Chemistry, Saarland University, Campus C23, D-66123 Saarbrücken, Germany
[b] ElexoPharm GmbH, Campus A12, D-66123 Saarbrücken, Germany
[c] Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Campus C23, D-66123 Saarbrücken, Germany

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ABSTRACT

17β-Estradiol (E2), the most potent human estrogen, is known to be involved in the etiology of estrogen-dependent diseases (EDD) like breast cancer and endometriosis. 17β-Hydroxysteroid dehydrogenase type 1 (17β-HSD1) catalyses the last step of E2 biosynthesis and is thus a promising target for the treatment of EDD. The previously described bicyclic substituted hydroxyphenylmethanones (BSHs) display high inhibitory potency towards human 17β-HSD1, but marginal activity towards rodent 17β-HSD1, precluding a proof of principle study in an animal endometriosis model. The aim of this work was to perform structural optimizations in the BSHs class to enhance inhibitory activity against rodent (mouse and rat) 17β-HSD1 while maintaining activity against the human enzyme. The introduction of fluorine atoms on the benzoyl moiety resulted in compounds with the desired properties. Molecular docking and homology modeling were applied to elucidate the binding mode and interspecies differences in activity. Compound **33** is the most potent inhibitor of both *human* and *rat* 17β-HSD1 up to date (IC_{50} = 2 nM and 97 nM, respectively).

1. Introduction

17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1) catalyses the NAD(P)H dependent transformation of estrone (E1) to 17β-estradiol (E2) which represents the most potent estrogen in humans (Fig. 1) [1]. E2 is known to play a crucial role in the development and progression of several estrogen dependent diseases (EDD) [2–5]. Increased E2/E1 ratios as well as elevated 17β-HSD1 mRNA levels are indicators of the involvement of 17β-HSD1 in breast cancer [6,7], ovarian tumor [8], endometriosis [9], endometrial hyperplasia [10] and other EDD [11]. Consequently, the selective inhibition of this enzyme is considered as a valuable treatment option. This intracrine concept has recently been supported by the observation that an 17β-HSD1 inhibitor tool compound can effectively block the increased E2 synthesis in endometriotic tissue specimens [12]. Due to the tissue-selective expression and the intracrine mode of action of 17β-HSD1 [13], its inhibition should be associated with less side effects compared to established treatments using GnRH-analogs, aromatase inhibitors, anti-estrogens or selective estrogen receptor modulators (SERMs) [14–16]. 17β-HSD2 catalyses the reverse reaction (inactivation of E2 to E1, Fig. 1). Therefore, inhibitors of 17β-HSD1 should be selective over the type 2 enzyme.

Therapeutics interfering with intracrine pathways of hormone

Abbreviations: 17β-HSD1, 17β-hydroxysteroid dehydrogenase type 1; 17β-HSD2, 17β-hydroxysteroid dehydrogenase type 2; E1, estrone; E2, 17β-estradiol; EDD, estrogen-dependent disease; PoP, proof of principle; SERM, selective estrogen receptor modulator; GnRH, gonadotropin-releasing hormone; NADP(H), nicotinamide adenine dinucleotide phosphate; NAD(H), nicotinamide adenine dinucleotide; ADME, absorption, distribution, metabolism, and excretion; *h*17β-HSD1, *human* 17β-hydroxysteroid dehydrogenase type 1; *m*17β-HSD1, *mouse* 17β-hydroxysteroid dehydrogenase type 1; *r*17β-HSD1, *rat* 17β-hydroxysteroid dehydrogenase type 1; BSHs, bicyclic substituted hydroxyphenylmethanones; HPLC, high performance liquid chromatography; CC, column chromatography; Cpd., compound; TLC, thin layer chromatography; rt, room temperature; DCM, dichloromethane; BBr_3, boron tribromide; DME, dimethoxyethane; CCl_4, carbon tetrachloride; NaH, sodium hydride; MeI, methyl iodide; DMF, *N*,*N*-dimethyl formamide; $Pd(dppf)Cl_2$, [1,1′-Bis(-diphenylphosphino)ferrocene]palladium(II) dichloride; $Pd(PPh_3)_4$, Tetrakis(triphenylphosphine)palladium(0); HEK 293 cells, human embryonic kidney 293 cells; PDB, Protein Data Bank.

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

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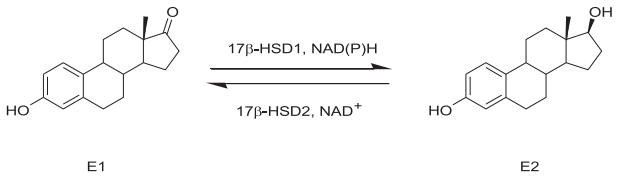

Fig. 1. Interconversion of estrone (E1) and 17β-Estradiol (E2).

biosynthesis are already in clinical use, e.g. 5α-reductase inhibitors [17–20]. Although a number of steroidal and non-steroidal inhibitors of 17β-HSD1 [21–23] are described and there is experimental evidence for the effectiveness of 17β-HSD1 inhibition against human tumor cell lines *in vitro* and in animal models, there is no inhibitor under clinical evaluation. Moreover, no proof of principle study in an animal disease model has been conducted for the indication endometriosis, although this is a widespread disease for which no adequate medical treatment is available.

A reason for this are interspecies differences between human 17β-HSD1 and animal orthologs which impede corresponding *in vivo* experiments, for instance in rodents [24]. Thus, human and mouse 17β-HSD1 are 83% similar in the first 287 amino acids and show significant differences concerning the topology of the substrate binding site [25,26]. As a consequence, compounds highly active towards the human enzyme are often weakly active or inactive towards rodent 17β-HSD1.

The aim of this study was the design of compounds inhibiting both human and rodent 17β-HSD1. Such a compound may be used in a proof of principle study in an animal endometriosis model and may serve as potential candidate for clinical development afterward. Starting point for the design was the class of bicyclic substituted hydroxyphenylmethanones (BSHs) [27–29]. In contrast to most other classes of 17β-HSD1 inhibitors described by us [30–38], it contains members which not only are strong inhibitors of the human enzyme but also show inhibition of the murine ortholog (previously unpublished results, Fig. 2). Examples are compounds **D** and **E** (Fig. 2) [27,28]. Drug design was focused on compound **E** as a lead and included structural variations with the aim of enhancing inhibitory activity towards rodent 17β-HSD1 while maintaining activity towards the human enzyme.

2. Design

In a screening across all compound classes of our in-house compound library, the BSH **E** (Fig. 2) showed the highest inhibitory activity against murine 17β-HSD1 (for data of other compound classes *cf.* Supplementary Table S3). Compounds from the same class lacking the aromatic sulfonamide moiety displayed slightly inferior activity (compare compounds **D** and **E**, Fig. 2). Thus, the aromatic sulfonamide moiety in combination with the BSH scaffold not only leads to a favorable activity profile against human 17β-HSD1 and 2 [28], but it also appears to be attractive in terms of activity against murine 17β-HSD1.

The fact that **E** was the only BSH bearing an aromatic sulfonamide group prompted us to investigate this structural element in more detail (Chart 1). Its optimal position on the phenyl ring C was determined (compounds **1**–**10**), and analogous amides were investigated as possible bioisosteres (compounds **11** and **12**). Moreover, compounds **13**–**29** with diverse substituents on ring D were prepared in a parallel synthesis approach in order to explore the chemical space more thoroughly.

Judging from previous data, fluorination of ring A could be beneficial. However, the introduction of a single fluorine atom was not expected to lead to a pronounced enhancement of activity [27,39–41]. Therefore, a structural optimiziation was performed which focused on the introduction of two fluorine atoms into the benzoylic moiety (ring A; compounds **30**–**33**), considering the following structure activity relationships obtained in previous studies: [27,28].

- the OH-group on the benzoylic moiety (ring A) is very important for activity
- only minor structural variations in this part are tolerated by the human target enzyme

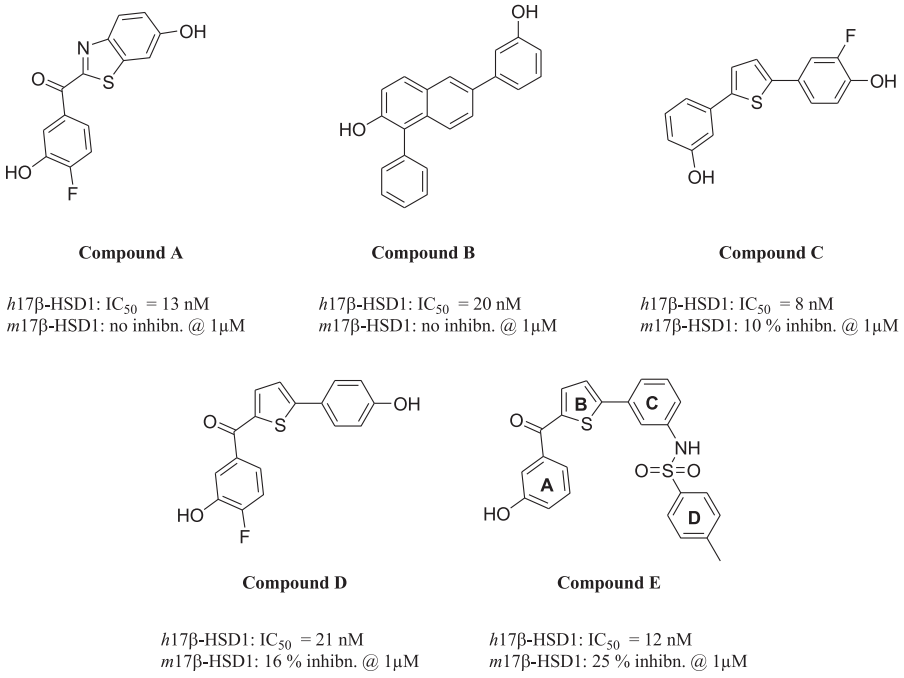

Fig. 2. Different in-house classes of highly potent 17β-HSD1 inhibitors: Representative members and their activities against the human and the murine enzyme.