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Research paper

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

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ABSTRACT

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

1. Introduction

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

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

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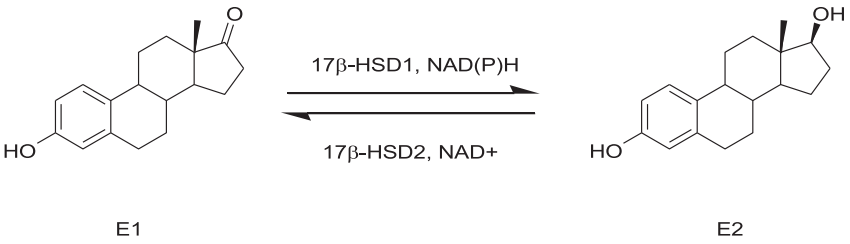


Fig. 1. Interconversion of E1 and E2.

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

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

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

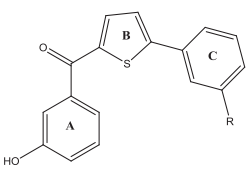
2. Design

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

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

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

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



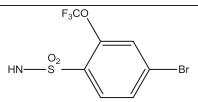
compound	R	IC_{50} (nM) 17β-HSD1	17β-HSD2	M (g/mol)
A		8	199	598
B	OH	22	109	296

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

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