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Impact of structural modifications at positions 13, 16 and 17 of 16β-(*m*-carbamoylbenzyl)-estradiol on 17β-hydroxysteroid dehydrogenase type 1 inhibition and estrogenic activity

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ABSTRACT

The chemical synthesis of four stereoisomers (compounds **5a–d**) of 16β-(*m*-carbamoylbenzyl)-estradiol, a potent reversible inhibitor of 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1), and two intermediates (compounds **3a** and **b**) was performed. Assignment of all nuclear magnetic resonance signals confirmed the stereochemistry at positions 13, 16 and 17. Nuclear overhauser effects showed clear correlations supporting a C-ring chair conformation for **5a** and **b** and a C-ring boat conformation for **5c** and **d**. These compounds were tested as 17β-HSD1 inhibitors and to assess their proliferative activity on estrogen-sensitive breast cancer cells (T-47D) and androgen-sensitive prostate cancer cells (LAPC-4). Steroid derivative **5a** showed the best inhibitory activity for the transformation of estrone to estradiol (95, 82 and 27%, at 10, 1 and 0.1 μM, respectively), but like the other isomers **5c** and **d**, it was found to be estrogenic. The intermediate **3a**, however, was weakly estrogenic at 1 μM, not at all at 0.1 μM, and showed an interesting inhibitory potency on 17β-HSD1 (90, 59 and 22%, at 10, 1 and 0.1 μM, respectively). As expected, no compound showed an androgenic activity. The binding modes for compounds **3a** and **b**, **5a–d** and CC-156 were evaluated from molecular modeling. While the non-polar interactions were conserved for all the inhibitors in their binding to 17β-HSD1, differences in polar interactions and in binding conformational energies correlated to the inhibitory potencies.

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1. Introduction

Estrogen-sensitive diseases affect the health of a significant number of women around the world, from a benign but painful disease such as endometriosis, to fatal diseases such as breast and endometrial cancers. The estrogen hormones, especially estradiol (E2), strongly accelerate tumor growth by stimulating the proliferation of cancer cells through interaction with the estrogen receptor (ER) [1]. Among the different proteins involved in estrogen production and modulation, 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1) has been identified as a promising therapeutic target to block E2 biosynthesis [2–8]. This enzyme transforms the steroid estrone (E1) into E2, the most potent estrogen in women, but also transforms dehydroepiandrosterone (DHEA) into 5-androstene-3β,17β-diol (5-diol), a less potent estrogen found in large concentration in post-menopausal women [9,10]. The blockade of 17β-HSD1 activity has solicited great interest over the past 20 years and many different strategies have been reported to obtain active inhibitors [11–16]. However, despite these intensive efforts, no inhibitor has yet reached the clinical trial step. One of the major causes that delayed the emergence of 17β-HSD1 inhibitors as new drugs was the lack of potent candidates with no estrogenic activity.

In the last few years, we have identified the 16β-(*m*-carbamoylbenzyl) estradiol (CC-156) as a strong inhibitor of 17β-HSD1 (Fig. 1A) [17,18]. Unfortunately this E2 derivative possesses estrogenic activity when tested on estrogen-sensitive breast cancer cell lines (T-47D and MCF-7) and tissues (uterine and vagina) [19]. Different attempts put forth to fix this problem, such as introducing a 2-methoxy group, removing the 3-OH and rigidifying the 16β-side chain (Fig. 1B–D), have met with moderate success [17,20–22]. However, modification of the phenolic moiety of CC-156 by replacing the OH at C3 by an ethylbromide side chain has led to the first non-estrogenic irreversible steroid inhibitor of 17β-HSD1 (Fig. 1E) [19,23,24]. We are now interested in pursuing our modification of the CC-156 scaffold to obtain a non-estrogenic reversible inhibitor. Recent work from our group on the estrogenic activity of E2 epimers has highlighted the potential of the inversion of the configuration at C13 (18-methyl) and/or C17 (OH) to decrease the binding affinity with ERα and consequently the estrogenic activity. Thus, relative binding affinity (RBA) values of 1.6% and 1.2% were obtained for 13-epi-17α-E2 and 13-epi-17β-E2, respectively [16]. Based on these results, we then wanted to validate this strategy in order to decrease the residual estrogenic activity of 17β-HSD1 reversible inhibitor CC-156. However, the consequence of such structural modifications on the inhibitory activity of CC-156 was difficult to predict, and for this reason, the chemical synthesis of each stereoisomeric form of CC-156 at positions 13, 16 and 17 was necessary to assess their potential as a non-estrogenic inhibitor of 17β-HSD1 (Fig. 1F).

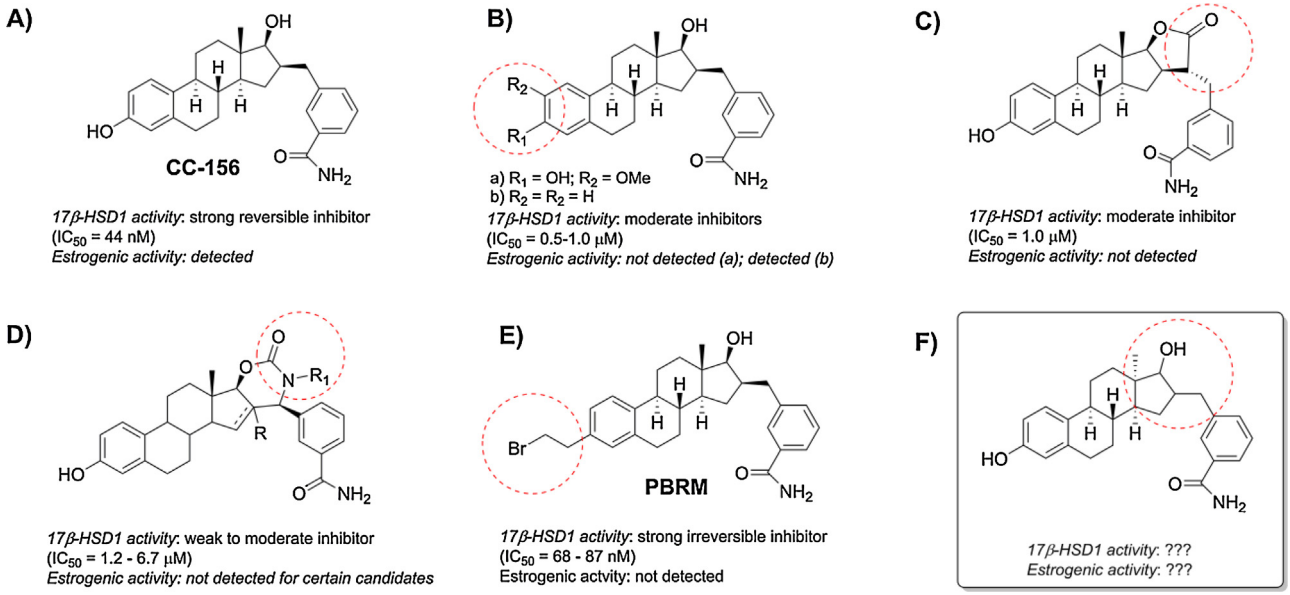

Fig. 1. Chemical structure of 17β-HSD1 inhibitor CC-156 (A) and structures representing the previously reported strategies (B–E) and novel strategy (F) used to reduce the residual estrogenic activity of CC-156. The structural modifications target positions 2, 3, 16 and 17 of CC-156.