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Synthesis and preliminary evaluation of a modified estradiol-core bearing a fused γ-lactone as non-estrogenic inhibitor of 17β-hydroxysteroid dehydrogenase type 1

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ABSTRACT

A non-estrogenic inhibitor of 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1) was designed based on a modified 3-hydroxy-estra-1,3,5(10)-triene core having an additional five-member lactone ring and a benzamide group. The inhibitor was synthesized, fully characterized and tested for its ability to inhibit the enzyme activity. Estrogenicity was also investigated and tested on estrogen-dependent T-47D cell line. Interestingly, this steroid derivative showed inhibitory potency towards 17β-HSD1 and did not present residual unwanted estrogenic activity.

17β-Hydroxysteroid dehydrogenase type 1 (17β-HSD1) is a steroidogenic enzyme that catalyzes the reduction of estrone (E1) into estradiol (E2), the most potent estrogen. Therefore, inhibition of 17β-HSD1 is a suitable approach for the treatment of estrogen-dependent diseases such as breast cancer (Fig. 1).[1–6] Moreover, this enzyme intervenes at the very last step in the production of E2, meaning that its inhibition does not interfere with the production of other essential steroids (i.e., corticosteroid and mineralocorticoid).[7] The development of therapeutic inhibitors of this enzyme is mainly lacking because the majority of inhibitors have residual estrogenic activity. Recently, our research group developed a potent inhibitor for 17β-HSD1; namely CC-156 (Fig. 2).[8,9] Unfortunately, this compound revealed to be slightly estrogenic when tested on estrogen-dependent cell lines. In fact, due to its E2 core, activation of estrogen receptor occurs, thereby causing cell proliferation. Nevertheless, the great potency of this inhibitor has led researchers to investigate the interactions and bonding, which resulted in crystallization of CC-156 in the active site of 17β-HSD1 by Mazumdar et al.[10] Thereby, they highlighted various significant interactions responsible for the high affinity between the inhibitor and the enzyme. More precisely, the crystallized binary and ternary complexes showed strong interactions between the 3-phenol of the inhibitor and amino acids His221 and Glu282 of the enzyme, between 17-OH and Ser142 and, more importantly, between the benzamide moiety and Phe192 and Asn152.

In order to develop a novel inhibitor of 17β-HSD1 based on E2 nucleus deprived of residual estrogenic activity, we considered using a modified E2 core. The new scaffold employed consists of a 3-hydroxy-estra-1,3,5(10)-triene nucleus with an additional substituted E-ring lactone fused at positions 16 and 17 (Fig. 2). The addition of a substituted five-member lactone moiety is more likely to eliminate or reduce estrogenicity while keeping the crucial interactions with 17β-HSD1. To validate our strategy we targeted compound **1**.

The synthetic methodology for the preparation of compound **1** is outlined in Scheme 1 starting from steroidal lactone **2**. The

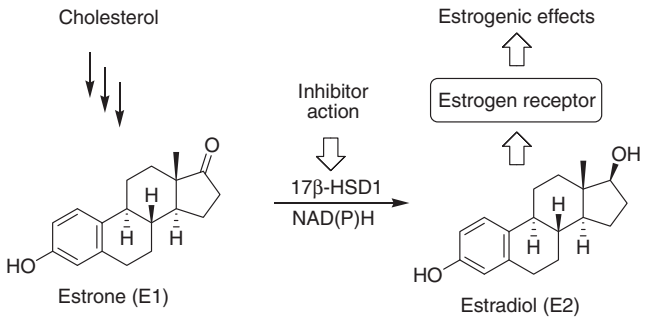

Figure 1. Key role of 17β-HSD1 in the synthesis of potent estrogen E2.

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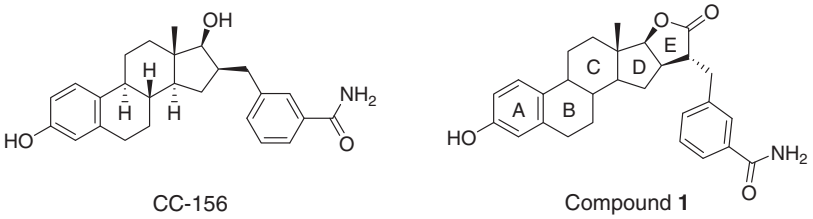
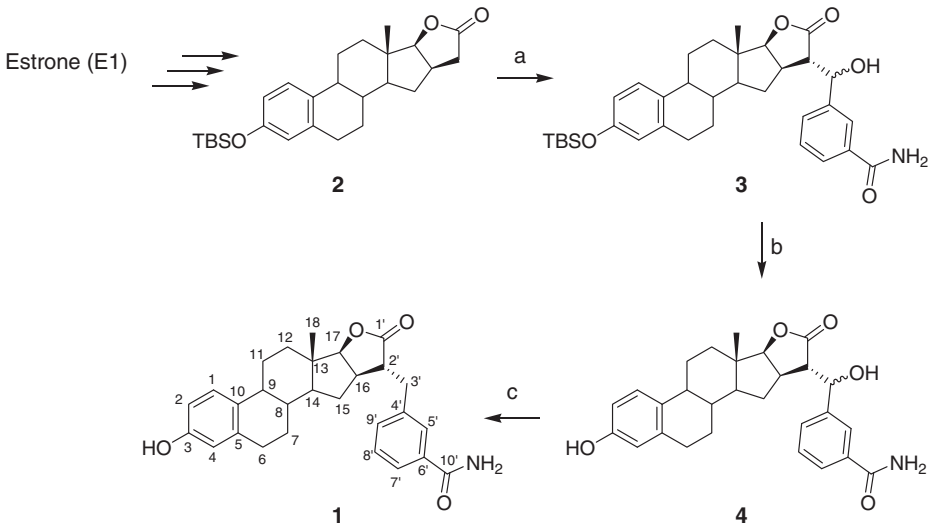


Figure 2. Structures of 17β-HSD1 inhibitors **1** and CC-156 (compound **4m** in Ref. 8). The stereogenic centers at C-8, C-9 and C-14 are illustrated only for CC-156, but they are the same for all other steroid derivatives reported in this paper. See Scheme 1 for the carbon numbering of **1**.



Scheme 1. Chemical synthesis of compound **1**. Reagents and conditions: (a) NaHMDS (1.0 M/THF), *meta*-amidobenzaldehyde, THF, −78 °C, 2 h; (b) 2% HCl/MeOH, rt, 3.5 h; (c) 1 atm H_2, 20% $Pd(OH)_2/C$, THF/AcOH (1:1), rt, 18 h. Carbon numbering used for ^1H and ^{13}C NMR signal attribution is showed for compound **1**.

synthesis of **2** has already been reported in literature[11] and necessitates three steps: (1) the 3-phenol protection of E1 using a *t*-butyldimethylsilyl (TBS) ether, (2) the alkylation in position 16 using methyl bromoacetate, (3) the side chain isomerisation from 16α- to 16β-configuration, and (4) the reduction of the 17-ketone with lactonisation. From the key intermediate **2**, three steps are needed to obtain the target compound **1**.

In the first step, an aldol addition using *meta*-amidobenzaldehyde afforded **3** in 70% yield. Different bases, temperatures and reaction times were investigated in order to find the right conditions. Fine-tuning for this reaction was needed because alkylation in alpha position of a lactone is usually less efficient than alkylation in alpha position of a ketone. It is important to notice that alkylation occurs only from one side of the steroidal core, thus yielding one isomer only, as is represented in Scheme 1. This stereoselectivity is due to the orientation of the lactone in beta position and the presence of the axial methyl in position 18. The stereochemistry of the side chain (position 2′) was determined by a series of NMR experiments. The first step was to determine the signal of the proton 2′, which was done using correlation spectroscopy (COSY). Clear interaction between proton 2′ and the characteristic benzylic proton (3′) made it obvious for the attribution. Then, the NOESY experiment permitted to see the interactions between the characteristic C-18 methyl and the proton 2′ (Fig. 3a). The two visible interactions are explained by the fact that two isomers of the benzylic alcohol are generated during the addition, thus duplicating the signals. This nuclear Overhauser effect demonstrates that the side chain is indeed in alpha position (*S* configuration).

The stereochemistry of **3** being confirmed, we next proceeded with the second step which is the hydrolysis of the protecting TBS group. This reaction took place in 2% HCl/MeOH to yield the phenol derivative **4** in a quantitative yield. The third step is the removal of the benzylic alcohol by means of hydrogenolysis using 20% $Pd(OH)_2/C$ to afford compound **1** (50% yield). The synthesis of **1** from the precursor **2** is achieved in an overall yield of 35% for three steps. What keeps us from having better yield is the reaction of hydrogenolysis of the benzyl alcohol, because it appears that only one isomer is able to react. One hypothesis to explain the difference in reactivity is a possible hydrogen bonding between the benzylic alcohol and the lactone carbonyl, which stabilizes the structure. Such hydrogen bonding is not favorable with the other isomer due to steric hindrance between the benzamide substituent and the lactonic steroidal core. Thereby, the hydrogen bond extends the C–OH bond, which facilitates its hydrogenolysis. The stereochemistry of **1** at the new generated chiral center (position 2′) was kept from intermediate **3** and no isomerization happened during the hydrogenolysis reaction. In fact, the nuclear Overhauser effect can be observed between protons of methyl 18 and H2′ (Fig. 3b). This informs us that the benzamide chain is still in alpha position (*R* configuration).

Once compound **1** was fully characterized,[12] it was tested for its ability to inhibit the transformation of [^{14}C]-E1 (60 nM) into [^{14}C]-E2 catalyzed by 17β-HSD1 in intact T-47D cells. This cell line is well recognized to exert a strong endogenous 17β-HSD1 activity.[13,14] Compound **1** was tested at five concentrations (0.1, 0.3, 1, 3 and 5 μM) and the results are reported in Figure 4. It showed 75% of inhibition at a concentration of 5 μM compared to 90% for CC-156. The difference of inhibition at this concentration already shows us that the lead compound is more potent than compound **1**. This gap is accentuated at lower concentrations. Indeed, at 0.3 μM, compound **1** displayed 20% of inhibition while the potent CC-156 inhibited 85% of transformation. The difference of

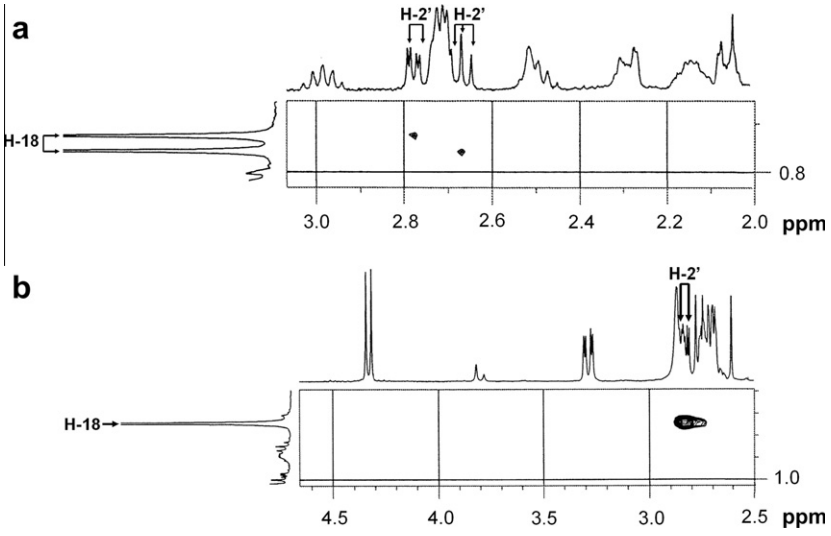


Figure 3. Partial 2D NOESY spectrum of compounds **3** (a) and **1** (b).

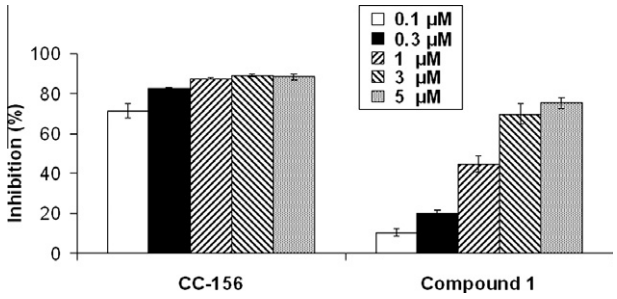


Figure 4. Inhibition of 17β-HSD1 by CC-156 and compound **1** at five concentrations (0.1, 0.3, 1, 3 and 5 μM. Results are expressed as mean ± SEM of triplicate.

inhibition strongly suggests that compound **1** did not keep all of the identified crucial interactions with 17β-HSD1, thus reducing its IC_{50} value to 1.0 μM compared to 44 nM for CC-156, as previously determined under the same conditions.[8]

The next step was to determine the presence or absence of estrogenic activity. To do so, cell proliferative assays were carried out on intact T-47D cells. This cell line is known to express the estrogen receptor (ER).[15] This means that molecules possessing estrogenic activity will stimulate cell growth. Proliferative activity of compound **1** and CC-156 was evaluated at 0.1 and 1 μM (Fig. 5). It is clear that compound **1** did not stimulate proliferation at both concentrations, contrary to CC-156 that did stimulate the cell growth by 48% at 0.1 μM and 83% at 1 μM. The absence of

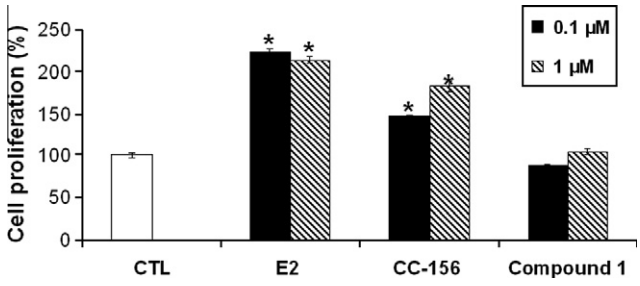


Figure 5. Effect of E2, CC-156 and compound **1** on the growth of estrogen-starved T-47D cells (ER^+) after 7 days of treatment. Control is fixed at 100% cell proliferation. Results are expressed as mean ± SEM of triplicate. $^*P \leqslant 0.01$ versus control.

estrogenicity is explained to be due to the fused lactonic cycle in combination with the benzamide moiety, which clearly reduces the ability of compound **1** to bind to the estrogen receptor, thus depriving it of any estrogenic activity.

In summary, we have designed and synthesized a steroidal 17β-HSD1 inhibitor (compound **1**) deprived of estrogenic activity by using a new E2-derived scaffold. However, it showed less inhibitory activity towards the enzyme than estrogenic lead compound CC-156, but its potency could be increased by a judicious lactone diversification. Further work reporting full details of the diversification of the benzyl ring, including the chemistry and SAR study, will be presented in a full paper in due course.

Acknowledgments

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Supplementary data

^{13}C NMR spectrum, ^{1}H NMR spectrum and HPLC analysis report of compound **1**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.06.110.

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12. Data for compound **1**: White amorphous solid; IR (film) ν in cm^{-1}: 3352 (OH and NH_2), 1753 (C=O, lactone), 1666 (C=O, amide); ^1H NMR (400 MHz, acetone-d_6) δ in ppm: 0.74 (s, 18-CH_3), 1.10–2.80 (unassigned CH and CH_2), 2.81 (m, 2′-CH), 3.28 (dd, J_2 = 13.1 Hz, J_1 = 3.7 Hz, 1H of 3′-CH_2), 4.33 (d, J = 10.0 Hz, 17α-CH), 6.50 (d, J = 2.5 Hz, 4-CH), 6.58 (dd, J_2 = 8.4 Hz, J_1 = 2.6 Hz, 2-CH), 6.67 (br s, 1H of NH_2), 7.07 (d, J = 8.4 Hz, 1-CH), 7.41–7.51 (m, 8′-CH, 9′-CH and 1H of NH_2), 7.83 (d, J = 7.7 Hz, 7′-CH), 7.90 (s, 5′-CH), 7.99 (br s, 3-OH); ^{13}C NMR (400 MHz, acetone-d_6) δ in ppm: 11.9 (C-18), 26.2 (C-11), 27.4 (C-7), 29.5 (C-6), 32.8 (C-15), 37.2 (C-12), 37.4 (C-3′), 38.2 (C-8), 41.5 (C-16), 43.6 (C-9), 43.7 (C-13), 49.3 (C-2′), 51.0 (C-14), 89.6 (C-17), 112.8 (C-2), 115.0 (C-4), 125.7 (C-7′), 126.2 (C-1), 128.2 (C-5′), 128.5 (C-8′), 130.6 (C-10), 131.9 (C-9′), 134.7 (C-6′), 137.3 (C-5), 139.4 (C-4′), 155.1 (C-3), 168.0 (C-10′), 178.9 (C-1′); HRMS: calcd for $C_{28}H_{32}NO_4$ $[M+H]^+$ 446.2326, found 446.2331. HPLC purity = 94.3%, rt = 17.2 min.
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