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# Crucial Role of 3-Bromoethyl in Removing the Estrogenic Activity of 17β-HSD1 Inhibitor 16β-(*m*-Carbamoylbenzyl)estradiol

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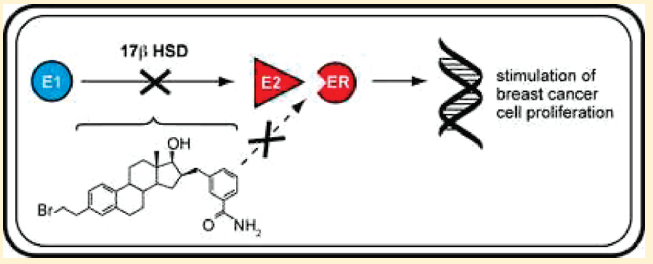
Supporting Information

**ABSTRACT:** 17β-Hydroxysteroid dehydrogenase type 1 (17β-HSD1) represents a promising therapeutic target for breast cancer treatment. To reduce the undesirable estrogenic activity of potent 17β-HSD1 inhibitor 16β-(*m*-carbamoylbenzyl)-estradiol (**1**) ($IC_{50}$ = 27 nM), a series of analogues with a small functionalized side chain at position 3 were synthesized and tested. The 3-(2-bromoethyl)-16β-(*m*-carbamoylbenzyl)-estra-1,3,5(10)-trien-17β-ol (**5**) was found to be a potent inhibitor ($IC_{50}$ = 68 nM) for the transformation of estrone (E1) into estradiol (E2) and, most importantly, did not stimulate the proliferation of estrogen-sensitive MCF-7 cells, suggesting no estrogenic activity. From these results, the crucial role of a bromoalkyl side chain at carbon 3 was identified for the first time. Thus, this new inhibitor represents a good candidate with an interesting profile suitable for further studies including pharmacokinetic and in vivo studies.




**KEYWORDS:** Steroid, estrogen, hormone, enzyme inhibitor, 17β-hydroxysteroid dehydrogenase, cancer

17β-Hydroxysteroid dehydrogenase type 1 (17β-HSD1) transforms estrone (E1) into estradiol (E2),[1,2] the most potent natural ligand for estrogen receptors (ER).[3] This enzyme also catalyzes the reduction of dehydroepiandrosterone (DHEA) into 5-androstene-3β,17β-diol ($\Delta^5$-diol), a weaker estrogen that becomes more important after menopause.[4] Inhibitors of 17β-HSD1 are thus potentially interesting therapeutic agents for the control of estrogen-dependent diseases such as breast cancer and endometriosis.[5−7] During the last 30 years, many efforts were made in designing potent inhibitors of this key steroidogenic enzyme, but it is only recently that lead candidates have been reported with very good inhibitory activity.[8−14] The presence of residual estrogenic activity associated with steroidal inhibitors, which are often built around an estrane nucleus, represented a major drawback in their development and their use as therapeutic agents. From different strategies tested for reducing the estrogenicity of estrane derivatives in the past,[8−14] two approaches gave interesting results. Thus, Sterix Ltd.[15−17] used a combination of an ethyl and alkylamide side chain at positions 2 and 16 of E1, respectively, whereas Solvay Pharmaceuticals[18−20] used another alkylamide side chain at position 15 of E1, with or without a substituent at position 2. Despite of the strong inhibitory potential of 17β-HSD1 to treat estrogen-dependent diseases, the validation of this pharmaceutical target in a clinical perspective remains to be done. New potent inhibitors with a nonestrogenic profile are thus strongly needed to validate the therapeutic approach in vivo and to engage the first clinical trial in humans.

The 16β-(*m*-carbamoylbenzyl)estradiol (**1**; CC-156) has already been reported as a potent inhibitor of 17β-HSD1.[21] Despite its good inhibitory potency, this compound was found to stimulate the MCF-7 and T-47D estrogen-sensitive breast cancer cell lines in vitro, thus greatly reducing its therapeutic potential.[22] To remove the undesirable residual estrogenic activity of E2 derivative **1**, we explored the impact on both 17β-HSD1 inhibition and estrogenicity of a series of small chains differently functionalized in the replacement of the hydroxyl (OH) group at position 3. In fact, this OH is well-known to be very important for ER binding affinity and, consequently, for producing the estrogenic effect.[23] However, replacing the 3-OH group by a hydrogen atom did not allow a full blockade of the estrogenicity as assessed by the proliferation of $ER^+$ cells,[22,24] but additional modifications must be tested at this position. Furthermore, from the X-ray analysis of the crystallized complex of inhibitor **1** with 17β-HSD1, which showed key interactions for inhibitory activity (Figure 1),[25] we noticed that the 3-OH seems less crucial for 17β-HSD1 inhibition. In fact, three major interactions were identified in the binary complex of 17β-HSD1 and inhibitor **1**: The 17β-OH forms a hydrogen bond with the Ser142, the $C(O)NH_2$ group forms a hydrogen bond with the peptide backbone of Phe192, and the phenyl ring at C16 forms a $\pi-\pi$ interaction with Tyr155. However, contrary to E1, the natural substrate of the enzyme, the 3-OH of **1** does not form hydrogen bonding with Glu282 or His221. To reach a third anchoring point with an amino acid in proximity of position 3 of **1**, and thus obtain a better binding with 17β-HSD1, as well as to remove the undesirable estrogenic

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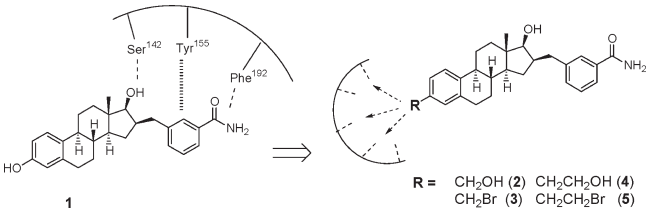
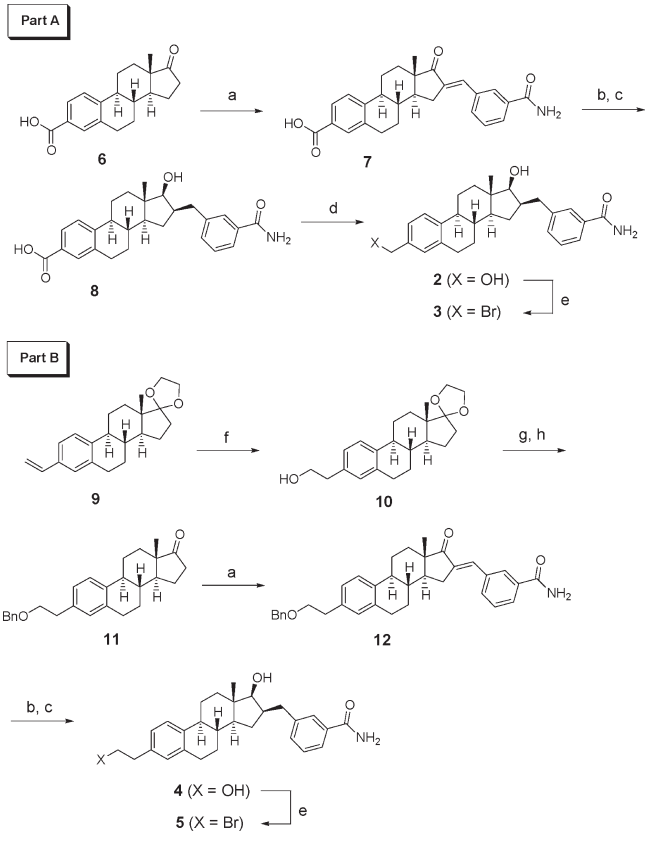



**Figure 1.** Key interactions observed in binary complex (17$\beta$-HSD1/ **1**) and representation of new E2 derivatives modified at position 3 (**2**−**5**). The scope of this side chain is dual, (1) reaching a third interaction with an amino acid and (2) removing the undesirable estrogenic activity of inhibitor **1**.

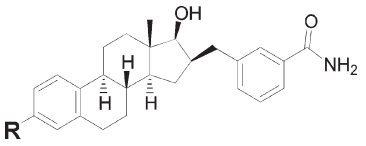
**Scheme 1**[a]




[a] Reagents and conditions: (a) 3-Formyl benzamide, KOH, EtOH, reflux, 0.5 h. (b) $NaBH_4$, MeOH, $CH_2Cl_2$, room temperature, 1 h. (c) $H_2$, 10% Pd/C, MeOH, room temperature, 36 h. (d) (1) BOP, DIPEA, DMF, room temperature; (2) $NaBH_4$, MeOH, room temperature. (e) $PPh_3$, $CBr_4$, $CH_2Cl_2$, 0 °C, 1.7 h. (f) (1) $BH_3$-$(CH_3)_2S$, THF, −78 °C to room temperature; (2) $NaHCO_3$, $H_2O_2$, room temperature, 3 h. (g) NaH, benzylbromide, DMF, 0 °C to room temperature, 18 h. (h) 10% HCl:acetone (1:1), room temperature, 5 h.

activity by disturbing the binding on ER, we chose different functional groups and different side chain lengths that could promote additional hydrogen bonding (alkylalcohol chains) or hydrophobic interactions (alkylbromide chains). From the structure−activity relationship results obtained with 25 derivatives of inhibitor **1**, we have selected compounds **2**−**5** to demonstrate the relevant results highlighted by compound **5**.

**Table 1. Inhibition of 17$\beta$-HSD1 by Compounds 1−5**




| | | inhibition (%) | | |
|---|---|---|---|---|
| compound no. | R | at 0.01 $\mu$M[a] | at 0.1 $\mu$M[a] | $IC_{50}$ (nM)[b] |
| **1** | −OH | 35 | 66 | 27 ± 4 |
| **2** | $-CH_2OH$ | 14 | 37 | ND |
| **3** | $-CH_2Br$ | 20 | 36 | 430 ± 99 |
| **4** | $-CH_2CH_2OH$ | 7 | 17 | ND |
| **5** | $-CH_2CH_2Br$ | 23 | 49 | 68 ± 6 |

[a] Inhibition of the transformation of [$^{14}$C]-E1 (60 nM) into [$^{14}$C]-E2 by 17$\beta$-HSD1 in T-47D intact cells. The experiment was performed in triplicate (SD < ±5%). The inhibitors were tested at two concentrations of 0.01 and 0.1 $\mu$M. [b] Mean ± SD of an experiment performed in triplicate. ND, not determined.

Compounds **2** and **3** were synthesized from 3-carboxy-estrone (**6**)[26] as reported in Scheme 1 (part A). The benzylcarbamide side chain was introduced at position 16 using an aldol condensation reaction with 3-formyl benzamide[27] and potassium hydroxide.[22,28] The C17-ketone of **7** was then stereoselectively reduced with sodium borohydride, and the 16-exo double bound was reduced by a Pd on charcoal-catalyzed hydrogenation. The 3-carboxylic acid of **8** was activated using Castro's reagent (BOP) in the presence of *N*,*N*-diisopropylethylamine (DIPEA) to promote the reduction with sodium borohydride, which give the corresponding methyl alcohol **2**. This later was brominated using triphenylphosphine and carbon tetrabromide to give the bromomethyl derivative **3**.

Compounds **4** and **5** were synthesized from 3-vinyl-17-dioxolane-estrone (**9**) (Scheme 1, part B) obtained from carbonylative vinylation of estrone triflate followed by C17 dioxolane protection.[29] The vinyl group was first transformed to alcohol by an oxidative hydroboration reaction using borane−methylsulfide complex.[30] The alcohol **10** was then protected as benzyl ether followed by dioxolane deprotection in acid conditions to give **11**. The benzylcarbamide side chain was introduced at position 16 using the sequence of reactions reported above for the synthesis of **8**. The cleavage of 3-*O*-benzylether afforded the alcohol **4**, which was brominated using triphenylphosphine and carbon tetrabromide to give the bromoethyl derivative **5**.

During the synthesis of compounds **2**−**5**, two new stereogenic centers were generated at positions 16 and 17. Their C16 $\beta$-R and C17$\beta$-OH configurations were however easily confirmed by NMR spectroscopy. As illustrated with **5**, the chemical shifts for 16α-H and 17α-H (3.17 and 3.84 ppm) in $^1$H NMR are identical to those (3.14 and 3.81 ppm) published for 16$\beta$-*m*-carbamoylbenzyl-estradiol (**1**).[31] Similarly, in $^{13}$C NMR, the signal at 83.0 ppm for **5** is also characteristic of the 16$\beta$-R and 17$\beta$-OH configuration (83.0 ppm for **1**).

Compounds **1**−**5** were tested for their ability to inhibit the transformation of E1 into E2 by 17$\beta$-HSD1 in T-47D cells (Table 1), a cell line well-known to express endogeneous 17 $\beta$-HSD1.[32,21] In the alcohol series, we made the observation that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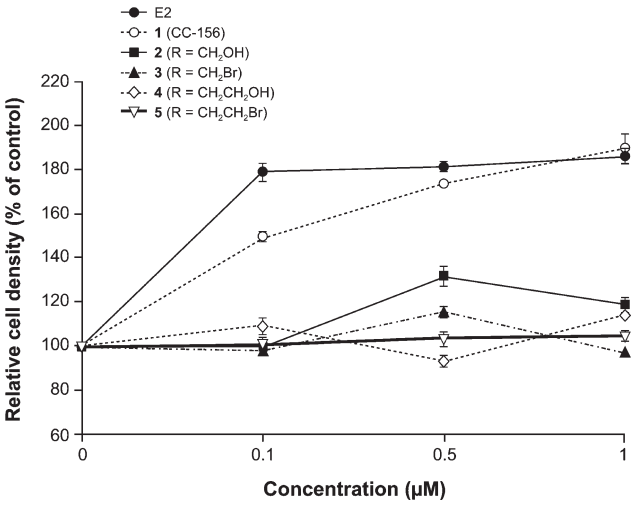


**Figure 2.** Effect of inhibitors **1**−**5** on the growth of estrogen-starved estrogen-sensitive ($ER^+$) MCF-7 cells after 7 days of treatment. The proliferation of control cells is fixed at 100%. Results are expressed as means ± SEMs of triplicate.

moving away the hydroxyl (OH) group from the steroid A ring had a negative effect on 17$\beta$-HSD1 inhibition with % of inhibition ranging from 66% for phenol **1** to 37% for methyl alcohol **2** and 17% for ethylalcohol **4** when tested at 0.1 $\mu$M. These results and those obtained at 0.01 $\mu$M highlight the fact that no additional favorable interaction was obtained with an amino acid of the enzyme. On the other side, the bromoalkyl derivatives **3** and **5** were much more promising. In fact, the inhibitory activity of **5** ($IC_{50}$ = 68 nM) was just 2.5 times lower than that of reference inhibitor **1** ($IC_{50}$ = 27 nM). However, the most important finding was that the estrogenic activity was greatly modulated by the presence of the C3 side chain (Figure 2). Bearing a phenol at position 3, compound **1** clearly stimulated at each concentration tested the proliferation of estrogen-sensitive MCF-7 cells, a breast cancer cell line known to express ER. By adding one or two methylene ($CH_2$) groups between the OH and the steroid A ring, the estrogenicity decreased, but compounds **2** and **4** remained with a significant estrogenic activity. Substituting the OH by a bromide atom also reduced the estrogenicity as illustrated with compounds **2** and **3**. Interestingly, the addition of two $CH_2$ groups and the substitution of the OH by a bromide atom to generate **5** did not stimulate cell proliferation, suggesting no estrogenic effect at the three concentrations tested.

The results presented above identified the importance of a bromoethyl side chain at position 3 in modulating both the 17 $\beta$-HSD1 inhibition activity and the estrogenic activity of our lead compound **1**, but these results also raise the question of the mechanism of action of compound **5**. For the estrogenic activity, because substituting the E2 nucleus generally reduces ER binding, especially when replacing the 3-OH, we expected to obtain a reduction of estrogenic activity for our E2 derivatives. It however remains to be determined whether the presence in position 3 of a bromoethyl group alone is sufficient to block the estrogenicity of E2 nucleus. It is also possible that the combined effect of two chains, a bromoethyl at position 3 and a carbamoylbenzyl at position 16, is necessary to complete blockage of estrogenic effect measured in vitro on $ER^+$ MCF-7 cells.

For inhibition of 17$\beta$-HSD1 activity, the alkylating property of alkyl bromide suggests the possible formation of a covalent bond between a nucleophilic amino acid residue of the enzyme and compound **5** (irreversible inhibitor) by a nucleophilic substitution of the bromide. However, the alkylating property of bromoalkyls is greatly variable according to the environment and the nature of the alkyl moiety, and our bromoethyl inhibitor **5** is much more stable with a less alkylating (electrophilic) potency than a bromobenzyl derivative such as **3**. So, it is also possible that the hydrophobic bromoethyl side chain interacts reversibly with an hydrophobic pocket, such as this identified by Lawrence et al.[15] in the proximity of position 2 of natural enzyme substrate or another hydrophobic pocket that remains to be identified. It can also be highlighted that the X-ray analysis of crystallized binary complex of **1** with 17$\beta$-HSD1 showed no interaction of 3-OH with Glu282 or His221, which can be explained by the presence of three interactions between the 16$\beta$-side chain and the17$\beta$-OH with the enzyme that slightly moves the inhibitor toward the right side. Transposed to the inhibitor **5**, this could free up space to accommodate the bromoethyl group added at position 3. Whatever the case, it is clear that additional experiments such as enzyme kinetic and crystallization studies will be necessary to properly address all of the questions regarding the mechanism of 17$\beta$-HSD1 by new inhibitor **5**.

In conclusion, we have successfully synthesized a series of C3-functionalized derivatives of the potent 17$\beta$-HSD1 inhibitor **1**. The bromoethyl side chain generated the most active inhibitor, compound **5**, thus demonstrating the good tolerance of 17$\beta$-HSD1 for a hydrophobic chain introduced at position 3 of this steroid nucleus. Most importantly, the addition of this small chain removed the residual estrogenic activity of **1**. A more complete investigation of the crucial C3 side chain by the study of different spacers and a larger diversity of chemical groups [$NR_1R_2$, NC(O)R, F, Cl, I, and Ph] is ongoing in our laboratory and will be reported in due course. However, with a very good inhibitory activity ($IC_{50}$ = 68 nM) and no estrogenic effect observed on estrogen-sensitive breast cancer cells, compound **5** represents a good candidate with an interesting profile suitable for further studies including pharmacokinetic and in vivo studies.

## ■ ASSOCIATED CONTENT

**Supporting Information.** Experimental procedures for the synthesis and characterization of new compounds **2**−**5** (IR, $^1$H NMR, $^{13}$C NMR, and HRMS), HPLC chromatogram of compound **5**, and description of biological assays. This material is available free of charge via the Internet at http://pubs.acs.org.

## ■ AUTHOR INFORMATION

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