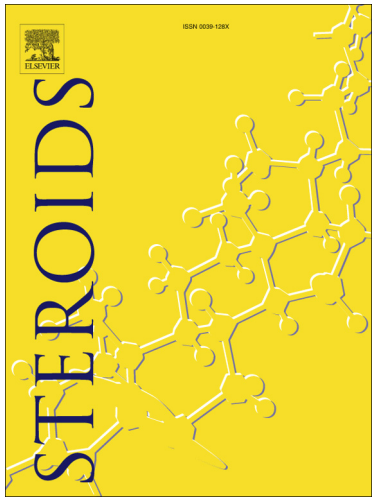

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Steroids (revised)

Chemical Synthesis of C3-oxiranyl/oxiranylmethyl-estrane derivatives targeted by molecular modeling and tested as potential inhibitors of 17β-hydroxysteroid dehydrogenase type 1

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Abstract:

17β-Hydroxysteroid dehydrogenase type 1 (17β-HSD1) is a promising therapeutic target known to play a pivotal role in the progression of estrogen-dependent diseases such as breast cancer, and endometriosis. This enzyme is responsible for the last step in the biosynthesis of the most potent estrogen, estradiol (E2) and its inhibition would prevent the growth of estrogen-sensitive tumors. Based on molecular modeling with docking experiments, we identified two promising C3-oxiranyl/oxiranylmethyl-estrane derivatives that would bind competitively and irreversibly in the catalytic site of 17β-HSD1. They have been synthesized in a short and efficient route and their inhibitory activities over 17β-HSD1 have been assessed by an enzymatic assay. Compound **15**, with an oxiranylmethyl group at position C3, was more likely to bind the catalytic site and showed an interesting, but weak, inhibitory activity with an IC_{50} value of 1.3 µM (for the reduction of estrone into E2 in T-47D cells). Compound **11**, with an oxiranyl at position C3, produced a lower inhibition rate, and the IC_{50} value cannot be determined. When tested in estrogen-sensitive T-47D cells, both compounds were also slightly estrogenic, although much less than the estrogenic hormone E2.

Keywords: Oxirane, Estrane, Chemical synthesis, Inhibitor, 17β-hydroxysteroid dehydrogenase

1. Introduction

The development and differentiation of hormone-sensitive tissues are directly regulated by sexual steroids before menopause. Estrogen-sensitive breast and endometrial tissues are therefore stimulated by the potent estrogen estradiol (E2), which is synthesized in the granulosa cells of the ovaries [1-3]. Other than its physiological effects, E2 is also involved in the onset and progression of estrogen-dependent diseases (EDDs).

17β-Hydroxysteroid dehydrogenase type 1 (17β-HSD1) drives the reduction of estrone (E1) into E2 in the presence of cofactor NADPH [4-6]. For this reason, the inhibition of this enzyme seems to be a promising avenue to treat the EDDs. In fact, some work has been done in the past to inhibit this key enzyme, and many compounds have been synthesized and have shown interesting inhibitory activities [7-14]. As an example, compound CC-156 was synthesized by our research group and was reported as a very potent reversible 17β-HSD1 inhibitor (Figure 1) [15]. Despite its strong inhibitory activity, this compound demonstrated some undesired estrogenic effects, which have been eliminated with the outcome of the inhibitor PBRM (Figure 1) [16-20]. This steroid derivative appears to be the first irreversible non-estrogenic steroidal inhibitor of 17β-HSD1. However, the enzymatic assay demonstrated a lower affinity for the enzyme compared to CC-156.

To design a new generation of irreversible inhibitors that could have better binding for the enzyme catalytic site, the bromoalkyl group of PBRM was replaced by an epoxide function. With that mindset, a relevant example of N-alkylation between the spiro-epoxide inhibitor (fumagillin) and the histidine (His) side chain of methionine aminopeptidase-2 (Met-AP-2) has been reported [21] and supports our hypothesis of a potential alkylation between an epoxide substrate with His residue of 17β-HSD1. Moreover, a docking study of two hypothesized molecules, namely compounds **11** and **15**, using the crystal structure of 17β-HSD1/CC-156 complex, showed orientations and distances to His221 residue that suggest the possible formation of a covalent bond. Both compounds possess an epoxide group, namely an oxiranyl or an oxiranylmethyl group, which is expected to alkylate 17β-HSD1 by the formation of a covalent bond.

To validate the potential of steroidal epoxide derivatives as covalent inhibitors of 17β-HSD1, we synthesized compounds **11** and **15** and evaluated their inhibition potencies. Also, as complement to this study, the compounds **4**, **5, 7** and **8** were included for a better understanding of the structural molecular determinant necessary for enzyme inhibition.

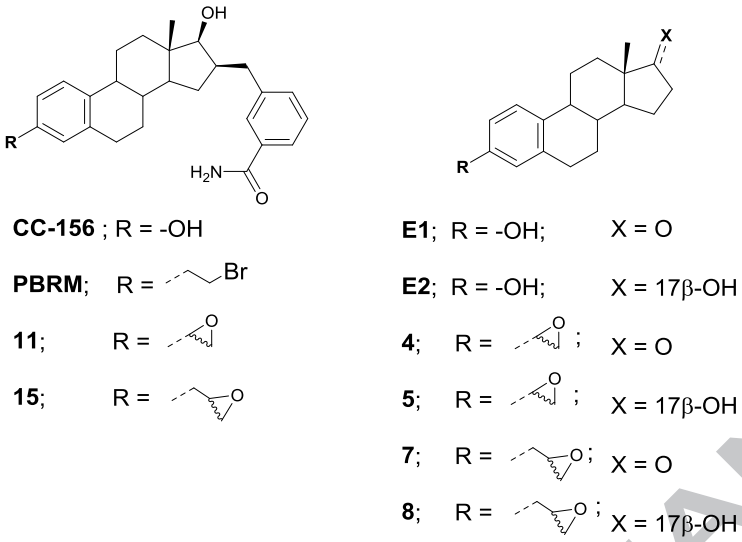


Fig. 1. Structures of known reversible and irreversible 17β-HSD1 inhibitors CC-156 and PBRM, respectively, both having a (*m*-carboxamide)benzyl at position 16β of an estra-1,3,5(10)-triene nucleus, as well as steroid derivatives **4**, **5**, **7**, **8**, **11** and **15** targeted as potential inhibitors of 17β-HSD1.

2. Materials and Methods

2.1. General

Chemical reagents were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada). The usual solvents were obtained from Fisher Scientific (Montréal, QC, Canada) and were used as received. Anhydrous acetonitrile (ACN), anhydrous dichloromethane (DCM), dimethylformamide (DMF), dimethylsulfoxide (DMSO) and tetrahydrofuran (THF) were obtained from Sigma-Aldrich. Thin-layer chromatography (TLC) and flash-column chromatography were performed on 0.20-mm silica gel 60 F254 plates (E. Merck; Darmstadt, Germany) and with 230-400 mesh ASTM silica gel 60 (Silicyle, Québec, QC, Canada), respectively. Microwave experiments were conducted on a Biotage Initiator microwave instrument (Charlotte, NC, USA). Nuclear magnetic resonance (NMR) spectra were recorded at

400 MHz for ^{1}H and 100.6 MHz for ^{13}C on a Bruker Avance 400 digital spectrometer (Billerica, MA, USA). The chemical shifts (δ) were expressed in ppm and referenced to acetone (2.06 and 29.8 ppm) for ^{1}H and ^{13}C NMR, respectively. Low-resolution mass spectra (LRMS) were recorded on a Schimadzu Prominence apparatus (Kyoto, Japan) equipped with a LCMS-2020 mass spectrometer (APCI probe). The purity of the final compounds to be tested was determined with a Shimadzu HPLC apparatus equipped with a SPD-M20A photodiode array detector, a Setima HPC18 reversed-phase column (250 mm x 4.6 mm) and a solvent gradient of MeOH: water. The wavelength of the UV detector was 190 nm.

2.2. Docking

Docking simulations were performed using MOE 2014 with a previously described docking protocol [17]. Briefly, the crystal structure coordinates of 17β-HSD1 complexed with inhibitor CC-156 and/or cofactor NADP, were taken from PDB ID 3HB5 [22]. Solvent was removed, and the protein complex was prepared using the LigX tool, included in MOE, to adjust H and to minimize the energy of the system. His221 was mutated to Ala and Glu282 were moved with the *rotamer explorer* tool to increase its exposure to the solvent. Docking simulations were performed using the *rigid receptor* protocol and default parameters. Validation of the docking protocol was carried out by a self-docking of CC-156, leading to an RMSD of 0.41 Å between the docked and the crystallographic structures. Because all compounds (Figure 1) share their core structure with CC-156, no further optimization of the docking protocol was considered. All compounds were built in MOE based on compound CC-156, hydrogens were readjusted, and molecules were energy-minimized prior to docking, using the same protocol as for compound CC-156.

2.3. Chemistry

*2.3.1. Synthesis of 3-trifluoromethanesulfonyloxyestra-1,3,5(10)-trien-17-one (**2**)*

In an anhydrous round bottom flask was introduced a solution of estrone (20.0 g, 74 mmol) in DCM (1.6 L). The solution was brought to 0 °C under an argon atmosphere before trimethylamine (TEA) (30.0 mL, 222 mmol) and triflic anhydride (14.9 mL, 88.8 mmol) were added, dropwise. The mixture was stirred under argon at 0 °C for 1 h, then poured into water and

extracted with DCM. The organic phase was filtrated on a biotage phase separator and evaporated under reduced pressure. The crude product was purified by flash chromatography with hexanes/EtOAc (8:2) to afford 29.0 g (99%) of compound **2**. NMR data were identical to those found in literature [23].

*2.3.2. Synthesis of **4** from **2***

The oxirane derivative **4** was obtained in two steps from **2**. In the first step, the vinyl derivative **3** was prepared as previously reported by Yasu *et al* [24] in 58% yield after purification by chromatography with hexanes/EtOAc (8:2). In the second step, compound **3** was treated with Oxone, as previously reported by Maltais *et al* [17] to give **4** in 67% yield (8.3 g) after purification chromatography with hexanes/EtOAc (8:2) and 0.5% TEA. NMR data agree with those reported in literature [17]. HPLC purity of 99.9% (retention time = 13.2 min).

*2.3.3. Synthesis of 3-(oxiran-2-yl)-17β-hydroxyestra-1,3,5(10)-triene (**5**)*

To a solution of **4** (28 mg, 0.09 mmol) in MeOH (5 mL) was added $NaBH_4$ (11 mg, 0.29 mmol). The mixture was stirred at 0 °C for 30 min, then evaporated under reduced pressure, poured into water, and extracted with EtOAc. The organic phases were combined, dried over $MgSO_4$, concentrated under reduced pressure, and purified by flash chromatography with hexanes/EtOAc (8:2) to yield 19.8 mg (70%) of compound **5**. ^{1}H NMR (acetone-d_6) δ 7.28 (d, J = 8.0 Hz, 1-CH), 7.04 (d, J = 8.0 Hz, 2-CH), 6.98 (s, 4-CH), 3.78 (dd, J_1 = 3.9 Hz, J_2 = 2.6 Hz, CH_2(O)CH), 3.71 – 3.64 (m, 17α-CH), 3.62 (d, J = 5.1 Hz, 17β-OH), 3.05 (dd, J_1 = 5.6 Hz, J_2 = 4.1 Hz, 1H of CH_2(O)CH), 2.84 – 2.81 (m, 6-CH_2), 2.77-2.73 (m, 1H of CH_2(O)CH), 2.38 – 2.30 (m, 11α-CH), 2.21 (td, J_1 = 11.8 Hz, J_2 = 4.0 Hz, 9α-CH), 2.05 – 1.85 (m, 16β-CH, 12β-CH and 7β-CH), 1.72 – 1.64 (m, 15α-CH), 1.54−1.17 (residual CH and CH_2), 0.78 (s, 18-CH_3). ^{13}C NMR (acetone-d_6) δ 141.2, 137.5, 136.0, 126.9, 126.3, 123.7, 81.8, 52.4, 51.0, 50.9, 45.3, 44.0, 39.6, 37.7, 31.0, 30.1, 27.9, 27.0, 23.8, 11.6. LRMS for $C_{20}H_{25}O$ $[M + H - H_2O]^+$ = 281.3. HPLC purity of 96.7% (retention time = 14.4 min).

*2.3.4. Synthesis of 3-allylestra-1,3,5(10)-trien-17-one (**6**)*

In a microwave biotage vial (2-5 mL) were added **2** (376 mg, 0.9 mmol), 2-propenylboronic acid pinacol ester (872 µL, 4.7 mmol), $Pd(dppf)Cl_2$ (68 mg, 0.09 mmol), K_3PO_4 (988 mg, 4.7 mmol)

and DMF (4 mL). The reaction mixture was stirred at 120 °C for 50 min under microwave radiation. The mixture was then neutralized by the addition of an aqueous $NaHCO_3$ solution and extracted with EtOAc. The organic phases were combined, dried over $MgSO_4$, and concentrated under reduced pressure. The crude product was purified by flash chromatography with hexanes/EtOAc (8:2) to afford 143 mg (52%) of compound **6**. ^{1}H NMR (acetone-d_6) δ 7.22 (d, *J* = 7.9 Hz, 1-CH), 6.96 (d, *J* = 8.0 Hz, 2-CH), 6.91 (s, CH-4), 6.00 – 5.90 (m, CH_2=CH), 5.12 – 4.98 (m, CH_2=CH), 3.31 (d, *J* = 6.7 Hz, CH_2=CHCH_2), 2.94 – 2.84 (m, 6-CH_2), 2.51 – 2.38 (m, 16β-CH and 11α-CH), 2.35 – 2.18 (m, 9α-CH), 2.15 – 1.15 (m, residual CH and CH_2), 0.91 (s, 18-CH_3). ^{13}C NMR (acetone-d_6) δ 219.5, 139.2, 138.9, 138.1, 137.3, 129.8, 126.7, 126.2, 115.6, 51.1, 48.4, 45.0, 40.3, 39.1, 36.0, 32.6, 30.0, 27.2, 26.5, 22.1, 14.1. LRMS for $C_{21}H_{27}O$ $[M + H]^+$ = 295.1.

*2.3.5. Synthesis of 3-(oxiran-2-ylmethyl)estra-1,3,5(10)-trien-17-one (**7**)*

To a solution of **6** (130 mg, 0.44 mmol) in a mixture of acetone and ACN (1:2) (93 mL) was added a saturated aqueous solution of $NaHCO_3$ (62 mL) and Oxone (324 mg, 0.53 mmol). The solution was stirred at 0 °C until it could not process any further (3 h) and the organic solvent was then evaporated under reduced pressure. Water was added, and the mixture was extracted with EtOAc. The organic phases were combined, dried over $MgSO_4$, and concentrated under reduced pressure to afford **7** (51 mg) in 37% as evaluated by ^{1}H NMR. The mixture of **6** and **7** was then treated to completion using the same conditions reported above. The solvent was then evaporated and the mixture purified by flash chromatography with hexanes/EtOAc (9:1) to yield 35 mg (26%) of compound **7**. ^{1}H NMR (acetone-d_6) δ 7.25 (d, *J* = 8.0 Hz, 1-CH), 7.05 (d, *J* = 8.0 Hz, 2-CH), 7.00 (s, 4-CH), 3.09 – 3.03 (m, CH_2(O)CH), 2.91 – 2.86 (m, 6-CH_2), 2.74 (dd, J_1 = 5.6 Hz, J_2 = 3.1 Hz, CH_2(O)CHCH_2), 2.70 (dd, J_1 = 5.2 Hz, J_2 = 3.9 Hz, 1H of CH_2(O)CH), 2.52 (dd, J_1 = 5.2 Hz, J_2 = 2.5 Hz, 1H of CH_2(O)CH), 2.49 – 2.40 (m, 16β-CH and 11α-CH), 2.33 – 2.24 (m, 9α-CH), 2.10 – 1.40 (m, residual CH and CH_2), 0.91 (s, 18-CH_3). ^{13}C NMR (acetone-d_6) δ 219.3, 138.9, 137.3, 135.9, 130.3, 127.2, 126.2, 52.9, 51.2, 48.4, 46.8, 45.2, 39.1, 39.0, 36.0, 32.6, 30.0, 27.3, 26.4, 22.0, 14.1. LRMS for $C_{21}H_{27}O_2$ $[M + H]^+$ = 311.2. HPLC purity as a mixture of two isomers: 97.3% (retention time = 13.6 and 14.4 min).

*2.3.6. Synthesis of 3-(oxiran-2-ylmethyl)-17-hydroxyestra-1,3,5(10)-triene. (**8**)*

To a solution of **7** (35 mg, 0.11 mmol) in MeOH (6 mL) was added $NaBH_4$ (64 mg, 1.69 mmol). The mixture was stirred at 0 °C for 30 min, then evaporated under reduced pressure, poured into water, and extracted with EtOAc. The organic phases were combined, dried over $MgSO_4$, and concentrated under reduced pressure. Purification by flash chromatography with hexanes/EtOAc (8:2) yielded 31 mg (92%) of compound **8**. ^{1}H NMR (acetone-d_6) δ 7.26 (d, *J* = 7.0 Hz, 1-CH), 7.04 (d, *J* = 8.0 Hz, 2-CH), 6.99 (s, 4-CH), 3.68 (m, 17α-CH), 3.62 (d, *J* = 5.1 Hz, 17β-OH), 3.15 – 3.07 (m, CH_2(O)CH), 2.86 – 2.80 (m, 6-CH_2), 2.73 (t, *J* = 6.0 Hz, 1H of CH_2(O)CHCH_2), 2.70 (dd, J_1 = 5.2 Hz, J_2 = 3.9 Hz, 1H of CH_2CH(O)), 2.53 (dd, J_1 = 5.2 Hz, J_2 = 2.5 Hz, 1H of CH_2(O)CH), 2.38 – 2.30 (m, 11α-CH), 2.24 – 2.17 (m, 9α-CH), 2.04 – 1.10 (residual CH and CH_2), 0.90 (s, 18-CH_3). ^{13}C NMR (acetone-d_6) δ 139.3, 137.3, 135.6, 130.3, 127.1, 126.2, 81.8, 52.9, 51.0, 46.8, 45.2, 44.0, 39.8, 39.1, 37.8, 31.0, 30.3, 28.1, 27.0, 23.8, 11.6. LRMS for $C_{21}H_{27}O$ $[M + H - H_2O]^+$ = 295.2. HPLC purity as a mixture of two isomers: 92% (retention time = 24.3 and 24.8 min).

*2.3.7. Synthesis of 3-[(17β-hydroxy-3-ethenylestra-1,3,5(10)-trien-16β-yl)methyl] benzamide (**10**)*

To a solution of PBRM (**9**) [16] (100 mg, 0.20 mmol) in DMSO (20 mL, 0.01M) was added tetrabutylammonium fluoride hydrate (TBAF) (265 mg, 1.01 mmol). The mixture was stirred at rt for 4 h, then quenched with water and extracted with EtOAc. The organic phases were combined, dried over $MgSO_4$, and concentrated under reduced pressure. The crude compound was purified by flash chromatography with DCM/MeOH (96:4) to afford 73.5 mg (88%) of compound **10**. ^{1}H NMR (acetone-d_6) δ 7.84 (s, 2”-CH), 7.76 (d, *J* = 7.5 Hz, 4”-CH), 7.41 (d, *J* = 7.5 Hz, 6”-CH), 7.35 (t, *J* = 7.5 Hz, 5”-CH), 7.26 (d, *J* = 8.2 Hz, 1-CH), 7.21 (d, *J* = 8.4 Hz, 2-CH), 7.12 (s, 4-CH), 6.67 (dd, J_1 = 17.7 Hz, J_2 = 10.9 Hz, CH_2=CH), 5.76 – 5.62 (d, *J* = 17.6 Hz, 1H of CH_2=CH), 5.14 (d, *J* = 11.0 Hz, 1H of CH_2=CH), 3.87 (d, *J* = 9.3 Hz, 17α-CH), 3.22 (d, *J* = 9.6 Hz, 1H of 1’-CH_2), 2.83 – 2.78 (m , 6-CH_2), 2.50 – 2.41 (m, 16α-CH and 1H of 1’-CH_2), 2.38 – 2.29 (m, 11α-CH), 2.26 – 2.20 (m, 9α-CH), 2.04 – 1.12 (m, residual CH and CH_2), 0.91 (s, 18-CH_3). ^{13}C NMR (acetone-d_6) δ 169.1, 144.0, 141.1, 137.8, 137.5, 135.6, 135.2, 132.6, 128.8, 128.9, 127.6, 126.3, 125.5, 124.2, 112.9, 82.0, 49.6, 45.4, 45.1, 43.1, 39.1, 38.6, 38.5, 32.8, 30.0, 28.1, 26.9, 13.2.

*2.3.8. Synthesis of 3-[(17β-hydroxy-3-(oxiran-2-yl)estra-1,3,5(10)-trien-16β-yl)methyl] benzamide (**11**)*

To a solution of **10** (30 mg, 0.07 mmol) in a mixture of acetone and ACN (1:2) (21.6 mL) was added a saturated aqueous solution of $NaHCO_3$ (14.4 mL) and Oxone (54 mg, 0.09 mmol). The solution was stirred at 0 °C until it could not process any further (4 h) and the organic solvent was then evaporated under reduced pressure. Water was added, and the mixture was extracted with EtOAc. The organic phases were combined, dried over $MgSO_4$, and concentrated under reduced pressure to afford **11** in 54% as evaluated by ^{1}H NMR. The mixture of **10** and **11** was then treated using the same conditions reported above to obtain 92% of completion (evaluated by ^{1}H NMR). Purification by flash chromatography with hexanes/acetone (4:6) and LCMS (MeOH/H_2O (7:3), retention time = 13.4 min) yielded 12.2 mg (38%) of compound **11**. ^{1}H NMR (acetone-d_6) δ 7.83 (s, 2''-CH), 7.75 (d, *J* = 7.6 Hz, 4''-CH), 7.41 (d, *J* = 7.7 Hz, 6''-CH), 7.35 (t, *J* = 7.5 Hz, 5''-CH), 7.28 (d, *J* = 8.1 Hz, 1-CH), 7.04 (d, *J* = 7.9 Hz, 2-CH), 6.97 (s, 4-CH), 6.55 (broad, NH_2), 3.86 (d, J_1 = 8.8 Hz 17α-CH), 3.78 (dd, J_1 = 4.0 Hz, J_2 = 2.6 Hz, CH_2(O)CH), 3.22 (dd, J_1 = 12.5 Hz, J_2 = 3.0 Hz, 1H of 1'-CH_2), 3.05 (dd, J_1 = 5.6 Hz, J_2 = 4.1 Hz, 1H of CH_2(O)CH), 2.83– 2.78 (m, 6-CH_2), 2.80 – 2.73 (m, 1H of CH_2(O)CH), 2.55 – 2.41 (m, 16α-CH and 1'-CH_2), 2.37– 2.32 (m, 11α-CH), 2.24 – 2.20 (m, 9α-CH), 2.03 – 1.89 (m, 12β-CH), 1.84 – 1.79 (m, 7β-CH), 1.69 (t, *J* = 7.1 Hz, 15α-CH), 1.54 – 1.22 (m, 11β-CH, 8β-CH, 12α-CH and 7α-CH), 1.19 – 1.11 (m, 14α-CH and 15β-CH), 0.91 (s, 18-CH_3). ^{13}C NMR (acetone-d_6) δ 169.1, 144.0, 141.2, 137.5, 136.1, 135.3, 132.6, 128.9, 128.8, 126.9, 126.3, 125.6, 123.6, 82.1, 52.3, 50.9, 49.6, 45.3, 45.1, 43.0, 39.1, 38.6, 38.5, 32.8, 30.0, 28.1, 27.0, 13.2. LRMS for $C_{28}H_{34}NO_3$ $[M + H]^+$ = 432.3. HPLC purity of 96.4% (retention time = 13.4 min).

*2.3.9. Synthesis of 3-[(17β-hydroxy-3-(trifluoromethanesulfonyloxy)estra-1,3,5(10)-trien-16β-yl)methyl] benzamide (**13**)*

To a solution of CC-156 (**12**) [15] (2.67 g, 6.58 mmol) in DMF (20 mL) was added Cs_2CO_3 (5.36 g, 16.45 mmol), followed by 4-nitrophenyl trifluoromethanesulfonate (2.31 g, 8.55 mmol). The reaction mixture was stirred at rt 1 h, then neutralized by addition of an aqueous $NaHCO_3$ solution and the mixture extracted with EtOAc. The organic phases were combined, dried over $MgSO_4$, and concentrated under reduced pressure. The crude product was purified by flash chromatography hexanes/EtOAc (1:1) to afford 2.75 g (78%) of compound **13**. ^{1}H NMR

(acetone-d_6): δ 7.83 (s, 2''-CH), 7.75 (d, *J* = 7.5 Hz, 4''-CH), 7.49 (d, *J* = 8.8 Hz, 1-CH), 7.41 (d, *J* = 7.6 Hz, 6''-CH), 7.42 (broad, 1H of NH_2), 7.35 (t, *J* = 7.5 Hz, 5''-CH), 7.17 (dd, J_1 = 8.7 Hz, J_2 = 2.7 Hz, 2-CH), 7.12 (d, *J* = 2.7 Hz, 4-CH), 6.55 (broad, 1H of NH_2), 3.90 – 3.86 (m, 17α-CH and 17β-OH), 3.22 (dd, J_1 = 12.7 Hz, J_2 = 3.2 Hz, 1H of 1'-CH_2), 2.92 – 2.88 (m, 6-CH_2), 2.60 – 1.17 (residual CH and CH_2), 0.92 (s, 18-CH_3).

*2.3.10. Synthesis of 3-[(17β-hydroxy-3-allylestra-1,3,5(10)-trien-16β-yl)methyl] benzamide (**14**)*

In a microwave biotage vial (0.5-2 mL) were added **13** (50 mg, 0.09 mmol), 2-propenylboronic acid pinacol ester (87 μL, 0.47 mmol), Pd(dppf)Cl_2 (6.8 mg, 0.01), K_3PO_4 (99 mg, 0.47 mmol) and DMF (0.4 mL). The reaction mixture was stirred at 120 °C for 50 min under microwave radiation. The mixture was then neutralized by the addition of an aqueous $NaHCO_3$ solution and extracted with EtOAc. The organic phases were combined, dried over $MgSO_4$, and concentrated under reduced pressure. The crude product was purified by flash chromatography with hexanes/acetone (7:3) to afford 25 mg (63%) of compound **14**.^{1}H NMR (acetone-d_6) δ 7.84 (s, 2''-CH), 7.76 (d, *J* = 7.5 Hz, 4''-CH), 7.47 (broad, 1H of NH_2), 7.41 (d, *J* = 7.5 Hz, 6''-CH), 7.35 (t, *J* = 7.6 Hz, 5''-CH), 7.21 (d, *J* = 7.9 Hz, 1-CH), 6.93 (d, *J* = 8.0 Hz, 2-CH), 6.86 (s, 4-CH), 6.63 (broad, 1H of NH_2), 5.98 – 5.90 (m, CH_2=CH), 5.12 – 4.96 (m, CH_2=CH), 3.92 (broad, 17β-OH), 3.86 (d, *J* = 9.3 Hz, 17α-CH), 3.29 (d, *J* = 6.8 Hz, CH_2=$CHCH_2$), 3.22 (dd, J_1 = 12.4 Hz, J_2 = 2.8 Hz, 1H of 1'-CH_2), 2.80 – 2.75 (m, 6-CH_2), 2.56 – 2.40 (m, 16α-CH and 1H of 1'-CH_2), 2.38 – 2.28 (m, 11α-CH), 2.24 – 2.17 (m, 9α-CH), 2.05 – 1.10 (m, residual CH and CH_2), 0.91 (s, 18-CH_3). ^{13}C NMR (acetone-d_6) δ 169.2, 144.0, 139.0, 138.9, 137.8, 137.3, 135.2, 132.6, 129.7, 128.9, 128.8, 126.6, 126.1, 125.6, 115.5, 82.1, 49.6, 45.3, 45.2, 43.0, 40.4, 39.2, 38.6, 38.5, 32.8, 30.1, 28.3, 27.0, 13.2.

2.3.11. Synthesis of 3-[(17β-hydroxy-(oxiran-2-ylmethyl)estra-1,3,5(10)-trien-16β-yl)methyl] benzamide (**15**)

To a solution of **14** (25 mg, 0.06 mmol) in a mixture of acetone and ACN (1:2) (12.3 mL) was added a saturated aqueous solution of $NaHCO_3$ (8.2 mL) and Oxone (72 mg, 0.12 mmol). The solution was stirred at 0 °C until it could not process any further, and the solvent was then evaporated under reduced pressure. Water was added, and the mixture was extracted with EtOAc. The organic phases were combined, dried over $MgSO_4$, and concentrated under reduced pressure

to afford **15** in 45% as evaluated by ^{1}H NMR. The mixture of **14** and **15** was then treated to completion using the same conditions as reported above, to obtain 75% (evaluated by ^{1}H NMR). The mixture was next treated with $NaBH_4$ (28 mg, 0.74 mmol) in MeOH (5 mL) to reduce the 17-carbonyl formed using Oxone in excess. Purification by LCMS (MeOH/H_2O (7:3), retention time = 13.6 min) yielded 19.8 mg (76%) of compound **15**. ^{1}H NMR (acetone-d_6) δ 7.84 (s, 2”-CH), 7.75 (d, *J* = 7.6 Hz, 4”-CH), 7.44 (broad, 1H of NH_2), 7.42 (d, *J* = 7.5 Hz, 6”-CH), 7.36 (t, *J* = 7.5 Hz, 5”-CH), 7.23 (d, *J* = 8.0 Hz, 1-CH), 7.03 (d, *J* = 8.1 Hz, 2-CH), 6.96 (s, 4-CH), 6.55 (broad, 1H of NH_2), 3.90 – 3.85 (m, 17α-CH and 17β-OH), 3.22 (dd, J_1 = 12.5 Hz, J_2 = 3.0 Hz, 1H of 1’-CH_2), 3.08 – 3.01 (m, CH_2(O)CH), 2.84 – 2.78 (m, 6-CH_2), 2.72 (t, *J* = 6.7 Hz, CH_2(O)CHCH_2), 2.69 (dd, J_1 = 5.2 Hz, J_2 = 4.0 Hz, 1H of CH_2(O)CH), 2.51 (dd, J_1 = 5.2 Hz, J_2 = 2.5 Hz, 1H of CH_2(O)CH), 2.53 – 2.42 (m, 16α-CH and 1H of 1’-CH_2), 2.37 – 2.34 (m, 11α-CH), 2.23 – 2.20 (m, 9α-CH), 2.01 (dt, J_1 = 12.4 Hz, J_2 = 3.2 Hz, 12β-CH), 1.85 – 1.78 (m, 7β-CH), 1.69 (t, *J* = 7.0 Hz, 15α-CH), 1.52 –1.24 (m, 11β-CH, 8β-CH, 12α-CH and 7α-CH), 1.18 – 1.13 (m, 14α-CH and 15β-CH), 0.92 (s, 18-CH_3). ^{13}C NMR (acetone-d_6) δ 169.1, 144.0, 139.3, 137.3, 135.7, 135.3, 132.6, 130.3, 128.9, 128.8, 127.1, 126.2, 125.6, 82.1, 52.9, 49.6, 46.8, 45.3, 45.2, 43.1, 39.2, 39.1, 38.6, 38.5, 32.8, 30.1, 28.2, 27.0, 13.2. LRMS for $C_{29}H_{36}NO_3$ $[M + H]^+$ = 446.3. HPLC purity of 99.6% (retention time = 13.6 min).

2.4. Biology

2.4.1. 17β-HSD1 inhibition assay

T-47D breast cancer cells were grown in RPMI medium supplemented with 10% (v/v) fetal bovine serum (FBS), L-glutamine (2 nM), penicillin (100 IU/mL), streptomycin (100 µg/mL) and 17β-E2 (1 nM). The cells were seeded in a 24-well plate (3000 cells/well) and suspended in the RPMI medium supplemented with insulin (50 ng/mL). A 5% (v/v) FBS treated with dextran-coated charcoal was used to remove the endogenous steroids. Stock solution of each compound to be tested was previously prepared in DMSO and diluted with culture medium to achieve the appropriate concentrations prior to use. After 24 h of incubation, a diluted solution was added to the cells to obtain the appropriate final concentration (0.1 or 1 µM for screening and ranging from 1 nM to 5 µM for IC_{50} value determination). The final concentration of DMSO in the well was adjusted to 0.1%. Additionally, a solution of [^{14}C]-E1 (American Radiolabeled Chemicals, Inc.,

St. Louis, MO, USA) was added to obtain a final concentration of 60 nM. Cells were incubated for 24 h, and each inhibitor was assessed in triplicate. After incubation, the culture medium was removed and labeled steroids (E1 and E2) were extracted with diethyl ether. The organic phase was evaporated to dryness with nitrogen. Residues were dissolved in DCM, dropped on silica gel thin layer chromatography plates (EMD Chemicals Inc., Gibbstown, NJ, USA), and eluted with toluene/acetone (4:1) as solvent system. Substrate [^{14}C]-E1 and metabolite [^{14}C]-E2 were identified by comparison with reference steroids (E1 and E2) and quantified using the Storm 860 system (Molecular Dynamics, Sunnyvale, CA, USA). The percentage of transformation and the percentage of inhibition were calculated as follow: % transformation = 100[^{14}C]-E2 / ([^{14}C]-E1 + [^{14}C]-E2) and % of inhibition = 100 (% transformation without inhibitor − % transformation with inhibitor) / % transformation without inhibitor.

2.4.2. Cell proliferation assay (estrogenic activity)

Quantification of cell growth was determined using CellTitter 96® Aqueous Solution Cell Proliferation Assay (Promega, Nepean, ON, Canada) following the manufacturer's instructions. T-47D cells were suspended in RPMI supplemented with insulin (50 ng/mL) and 5% (v/v) dextran-coated charcoal to remove the remaining estrogens present in the serum and medium. Aliquots (100 µL) of the cell suspension were seeded in 96-well plates (3000 cells/well). After 48 h, the medium was changed with an appropriate dilution of the different inhibitors and reference compounds in growth medium, and was replaced every 2 days until day 8 of treatment. After the treatments, 20 µL of MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt] solution was added to each well of the plates and incubated at 37 °C for 4 h. The absorbance at 490 nm was then measured with a Thermo max microplate reader (Molecular Devices, Sunnyvale, CA, USA) and the cell growth expressed in percentage (%). The cell proliferation of control (only medium) was fixed as 100%.

3. Results and Discussion

We designed two estrane derivatives (compounds **11** and **15**) as potential competitive covalent inhibitors of 17β-HSD1. Since the C16-benzyl amide group was proven to be helpful to decrease the estrogenic activity of E2-based inhibitors [16-18], we judged important to keep the 16β-

benzyl amide lateral chain in the design of epoxide derivatives **11** and **15**. Also, as a major molecular structural feature, those targeted compounds also possess an oxiranyl or an oxiranylmethyl functionality to potentially generate a covalent bond with a reactive amino acid side chain of 17β-HSD1.

3.1. Docking

A previous study of the crystal structure of 17β-HSD1/CC-156 complex leads to a better knowledge of the inhibitor in the enzyme catalytic site [22]. Due to the strong inhibitory activity of CC-156, these data have therefore been used for docking experiments. To get around the force field limitations that do not allow for a covalent reaction between the epoxide moiety and His221, this latter amino acid was mutated into an alanine, which has a smaller side chain (methyl instead of an imidazolylmethyl group). The docking of CC-156, PBRM, **11**-(*R*), **11**-(*S*), **15**-(*R*) and **15**-(*S*) showed a similar general orientation than the structure of CC-156 complexed with 17β-HSD1 (Figure 2). The RMSD between the four steroidal rings of CC-156 and the docked compounds PBRM, **11**-(*R*), **11**-(*S*), **15**-(*R*) and **15**-(*S*) are 0.22, 0.21, 0.27, 0.32, 0.29 and 0.23 Å, respectively. The distances between the NH of the reconstituted His221 side chain and the reactive CH_2 of the epoxide are 1.2, 2.1, 1.8 and 1.1 Å for **11**-(*R*), **11**-(*S*), **15**-(*R*) and **15**-(*S*), respectively. Except for compound **11**-(*S*), which is not oriented for a nucleophilic attack, the proximity to His221 suggests the possibility of a covalent reaction. These results demonstrate that, without the conformational limitations of His221 and Glu282, the epoxide moieties of compounds **11** and **15** are well positioned in the enzyme pocket, with respect to the crystal structure of CC-156, and at proximity of His221 to potentially form a covalent bond.

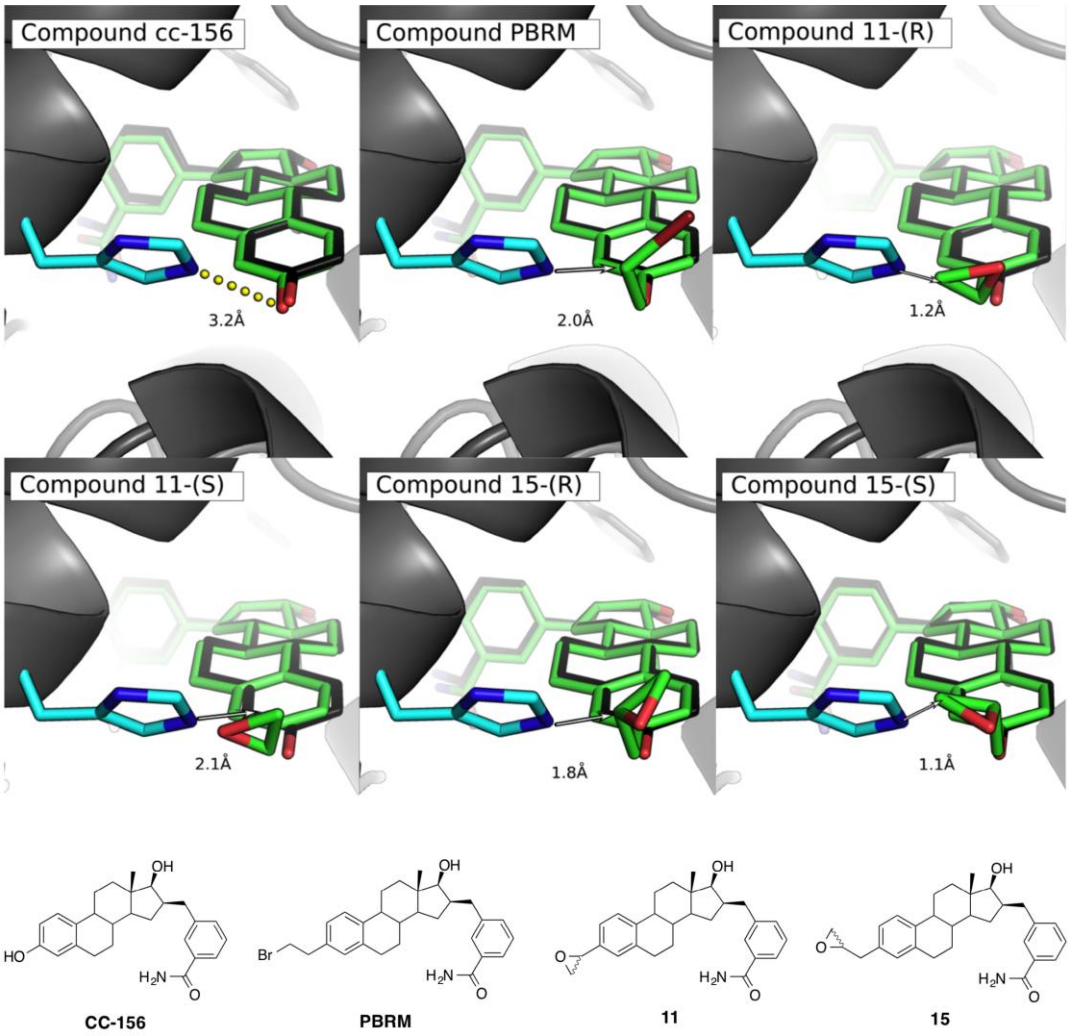
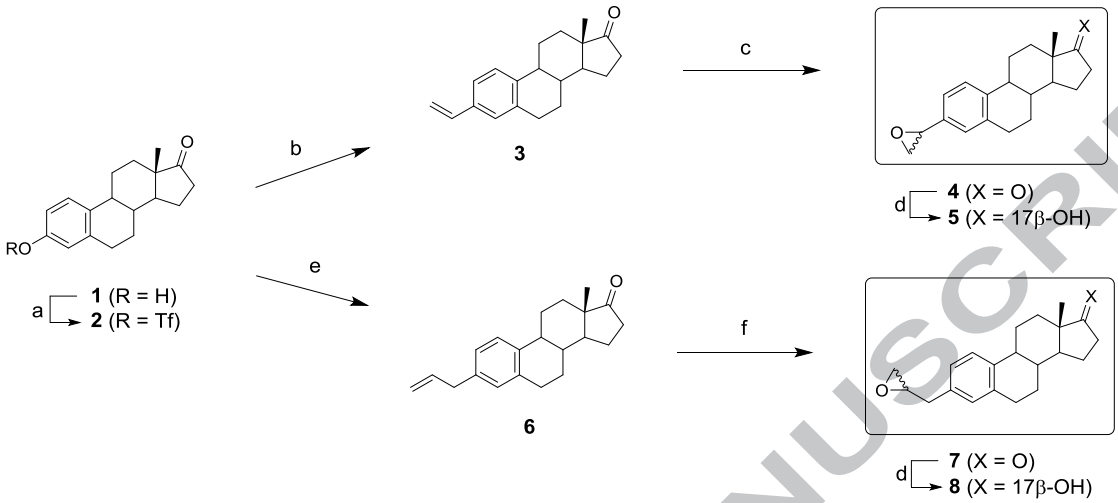


Fig. 2. Docking results of compounds CC-156, PBRM, **11**-(*R*), **11**-(*S*), **15**-(*R*) and **15**-(*S*). Inhibitors represented in green sticks, 17β-HSD1 represented in gray cartoon and His221 in cyan sticks. Crystal structure of CC-156 is shown as reference in black sticks.

3.2. *Chemistry*

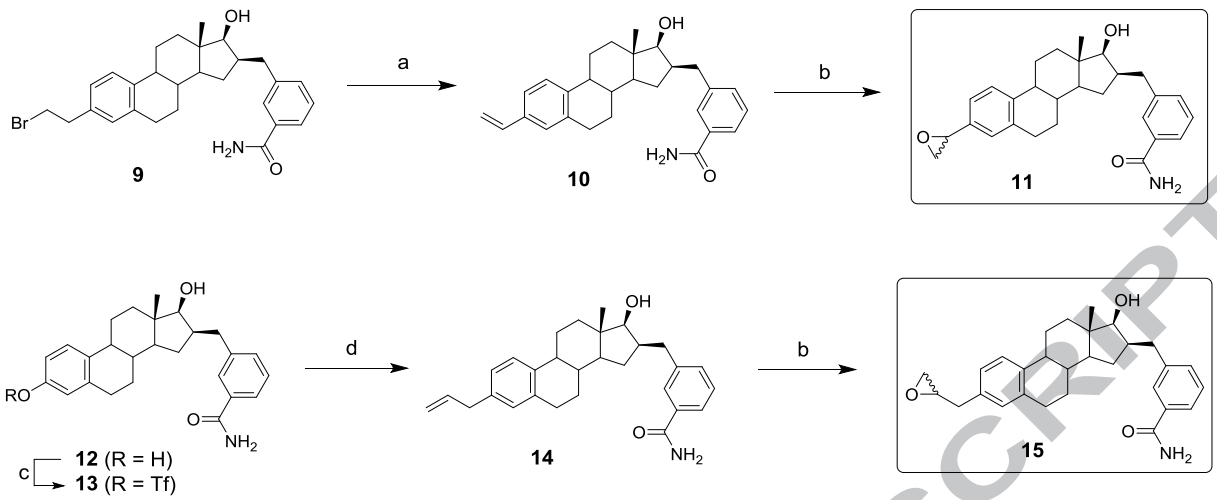
In addition to compounds **11** and **15** studied by docking, we also synthesized the analog epoxides without a 16β-benzylamide side chain (compounds **4**, **5**, **7** and **8**). Their short synthetic routes were reported in Scheme 1, and involved the activation of the C3-hydroxyl group of E1 as the triflate derivative **2**. This latter was then submitted to the conditions of a Suzuki coupling reaction to give the vinyl and allyl derivatives **3** and **6**, respectively. These olefins were next treated with Oxone to afford the corresponding oxiranyl derivative **4** and oxiranylmethyl derivative **7**, which were both obtained as an inseparable mixture of R/S-epoxides. Stereoselective reduction of the

C17-ketone of **4** and **7** was performed with sodium borohydride ($NaBH_4$) to form the 17β-alcohols **5** and **8**.



Scheme 1. Synthesis of epoxides **4**, **5**, **7** and **8**. Reagents and conditions: (a) Triflic anhydride, TEA, DCM, 0 °C, argon; (b) Potassium vinyltrifluoroborate, $PdCl_2$, PPh_3, Cs_2CO_3, THF/H_2O (9:1), 80 °C, argon; (c) Oxone, acetone/ACN (1:2), $NaHCO_3$, rt; (d) $NaBH_4$, MeOH, 0 °C; (e) 2-Propenylboronic acid pinacol ester, Pd(dppf)Cl_2, DMF, K_3PO_4, Microwaves, 120 °C; (f) Oxone, acetone/ACN (1:2), $NaHCO_3$, 0 °C.

Targeted derivatives **11** and **15** were obtained as reported in Scheme 2. The synthesis of **11** used PBRM (**9**) as a starting material. The vinyl intermediate **10** was first obtained from **9** by a treatment with tetrabutylammonium fluoride (TBAF) in DMSO. Reagent and conditions were based on those previously optimized to provide an elimination product and to avoid the fluoride derivative [25]. Next, the epoxidation of the vinylic bond of **10** leads to the desired oxirane **11** as a mixture of diastereoisomers. The synthesis of **15** needs the transformation of the C3-hydroxyl group of **12** into the triflate **13**. The triflate, as a good leaving group, allows the formation of the corresponding allyl derivative **14** through a Suzuki coupling. Afterwards, the epoxidation of the olefinic bond of **14** leads to the desired oxirane **15**, as a mixture of diastereoisomers.



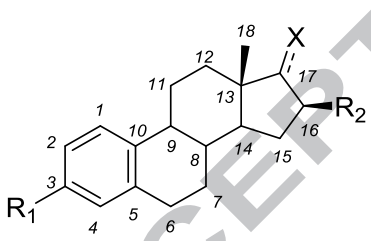
Scheme 2. Synthesis of epoxides **11** and **15**. Reagents and conditions: (a) TBAF, DMSO, rt; (b) Oxone, acetone/ACN (1:2), $NaHCO_3$, 0 °C; (c) 4-nitrophenyl trifluoromethanesulfonate, Cs_2CO_3, DMF, rt; (d) 2-Propenylboronic acid pinacol ester, $Pd(dppf)Cl_2$, DMF, K_3PO_4, Microwaves, 120 °C.

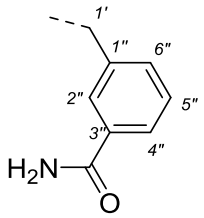
To afford the epoxides and to avoid the formation of side products, all vinyl and allyl derivatives were treated with Oxone instead of *m*-chloroperbenzoic acid or other peracids. Interestingly, we discovered that the yield for the epoxidation can be increased with successive Oxone treatments. In fact, we were unable to force the progression of the reaction over 15 to 20% in both cases, with a first treatment with Oxone in the presence of sodium bicarbonate and an organic solvent. Isolating and resubmitting the compound mixture to a second treatment with Oxone allowed up to 54% yield of the epoxide. At that point, a third treatment did not increase the yield of the epoxide. Although the steroid is fully soluble at that concentration in acetone/ACN (1:2, v/v), we suspected that the reactive mixture formed with Oxone, aqueous sodium bicarbonate and the organic solvents allowed only a small amount of the substrate in solution. Different ratios of reagent and solvents were unsuccessfully tried to solve the issue. In the end, the best results were obtained through two treatments with Oxone, sodium bicarbonate and acetone/ACN (1:2, v/v).

Final epoxides were fully characterized by IR, mass, and NMR spectroscopies while their purity was assessed by HPLC. In the case of epoxides derived from the 3-vinyl-steroids, compounds **4**, **5** and **11**, the presence of an oxiranyl group is well established in ^{13}C NMR by two signals at 50.9 ($CH_2(O)CH$) and 52.3 ($CH_2(O)CH$) ppm. In ^{1}H NMR, three characteristic signals appear as doublet of doublet at 3.78, 3.05 and 2.75 ppm. For epoxides derived from the 3-allyl-steroids,

compounds **7**, **8** and **15**, the presence of an oxiranylmethyl group is also established in ^{13}C NMR by three signals at 46.8, 52.9 and 39.1 ppm, which correspond to the following three carbons $CH_2(O)CHCH_2$, respectively. In ^{1}H NMR, only the CH signal (3.00-3.10 ppm) of the oxiranylmethyl is a key marker because the two CH_2 signals superpose other protons. However, for all epoxides, both ^{1}H and ^{13}C NMR signals were fully assigned (Table 1, Supplementary data) using 2D-NMR experiments, such as HSQC, HMBC, COSY and NOESY, as well as data found in literature for E1, E2 and PBRM [26, 16, 17]. Oxiranyl compounds **4**-**8**, **11** and **15** were all obtained as a mixture of R/S-epoxides since we used the Shi's epoxidation (Oxone in acetone) and considering that the chiral centers of the steroid backbone (C8, C9, C13, etc.) are too far from the vinyl or allyl group at C3 to provide chirality. However, these two R/S-epoxides were non-detectable by TLC or HPLC (only one spot or peak), hardly detectable by 400 MHz ^{1}H NMR (due to the presence of complex signals) and non-detectable by ^{13}C NMR. In this later case, only one signal was observed for each oxiranic carbon ($CH_2(O)CH$) as reported in Table 1. Similarly, as observed for the epoxidation, the chiral centers (C8, C9, C13, etc) seem too far from the racemic oxiranyl group to produce a signal doubling for each oxiranic carbon. For these reasons, all oxirane-estrane compounds were tested as a mixture of two epoxides (R and S).

Table 1. Assignment of ^{13}C NMR signals for R/S-epoxide derivatives **4**, **5**, **11**, **7**, **8** and **15** in acetone-d_6





R_2 = Carbamoylbenzyl

Carbon	**4**	**5**	**11**	**7**	**8**	**15**
	X = O R_1 = 3-oxalyl R_2 = H	X = 17β-OH R_1 = 3-oxalyl R_2 = H	X = 17β-OH R_1 = 3-oxalyl R_2 = 16β-carbamoyl-benzyl	X = O R_1 = 3-oxalylmethyl R_2 = H	X = 17β-OH R_1 = 3-oxalylmethyl R_2 = H	X = 17β-OH R_1 = 3-oxalylmethyl R_2 = 16β-carbamoyl-benzyl
1-CH	126.3	126.3	126.3	126.2	126.2	126.2
2-CH	123.8	123.7	123.6	127.2	127.1	127.1
3-C	140.7	141.2	141.2	135.9	135.6	135.7
4-CH	126.9	126.9	126.9	130.3	130.3	130.3
5-C	137.5	137.5	137.5	137.3	137.3	137.3
6-CH_2	30.0	30.1	30.0	30.0	30.0	30.1
7-CH_2	27.2	27.9	28.1	27.3	28.1	28.2
8-CH	39.0	39.6	39.1	39.1	39.8	39.2

9-CH	51.2	45.3	45.3	51.2	45.2	45.3
10-C	136.3	136.0	136.1	138.9	139.3	139.3
11-CH_2	26.5	27.0	27.0	26.4	27.0	27.0
12-CH_2	32.6	37.7	28.6	32.6	37.8	38.6
13-C	48.4	44.0	45.1	48.4	44.0	45.2
14-CH	45.2	51.0	49.6	45.2	51.0	49.6
15-CH_2	22.1	23.8	32.8	22.0	23.8	32.8
16-CH_2 or CH	36.0	31.0	43.0	36.0	31.0	43.1
17-C or CH	219.3	81.8	82.1	219.3	81.8	82.1
18-CH_3	14.1	11.6	13.2	14.1	11.6	13.2
CH_2(O)CHCH_2	**50.9**	**50.9**	**50.9**	**46.8**	**46.8**	**46.8**
CH_2(O)**C**HCH_2	**52.3**	**52.4**	**52.3**	**52.9**	**52.9**	**52.9**
CH_2(O)CH**C**H_2	**---**	**---**	**---**	**39.0**	**39.1**	**39.1**
1'-CH2	---	---	38.5	---	---	38.5
1''-C	---	---	144.0	---	---	144.0
2''-CH	---	---	128.8	---	---	128.8
3''-C	---	---	135.2	---	---	135.3
4''-CH	---	---	125.6	---	---	125.6
5''-CH	---	---	128.9	---	---	128.9
6''-CH	---	---	132.6	---	---	132.6
CON	---	---	169.1	---	---	169.1

3.3. *Biological activities*

All oxirane-estrane compounds had their biological activities (17β-HSD1 inhibitory activity and estrogenic activity) evaluated *in vitro*. Those assays were carried out on human breast cancer T-47D intact cells, expressing 17β-HSD1 and sensitive to estrogenic compounds. The inhibition percentages of the synthesized compounds are reported in Scheme 1 and showed that epoxide derivatives have a lower inhibitory activity than PBRM or CC-156. At all the concentrations tested, both oxiranes **11** and **15** showed an inhibition percentage at least 4 times lower than PBRM, the analog compound with a 2-bromoethyl side chain instead of an oxiranyl or an oxiranylmethyl group at steroid position C3.

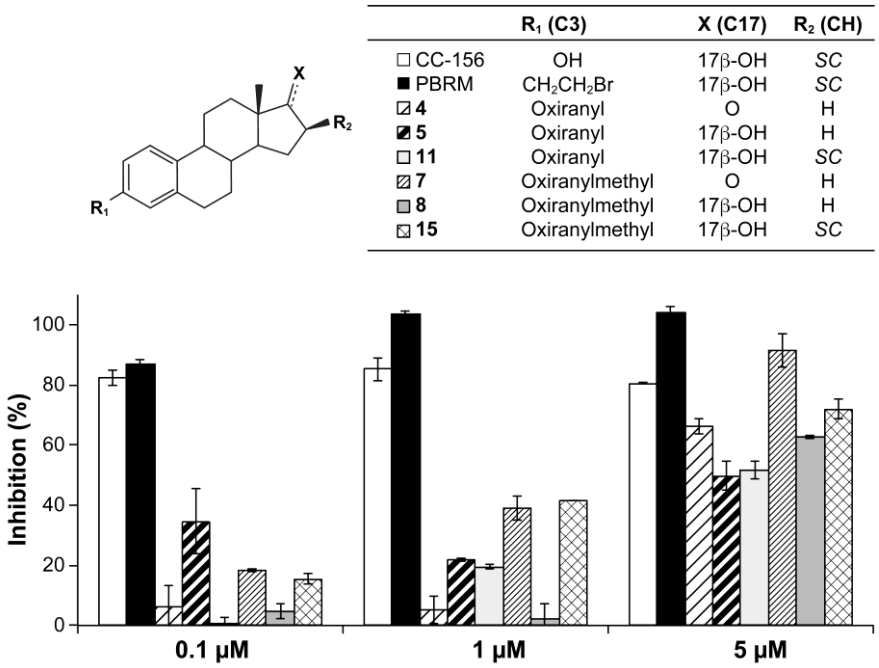


Fig. 3. Inhibition of 17β-HSD1 by a series of oxirane-estrane compounds, reversible inhibitor CC-156 and irreversible inhibitor PBRM. The inhibitory activity was determined for the transformation of [^{14}C]-E1 (60 nM) into [^{14}C]-E2 by 17β-HSD1 in T-47D intact cells incubated 24 h. The experiment was performed in triplicate (±SD). The inhibitors were tested at three concentrations of 0.1, 1 and 5 µM. *SC* = benzylamide side chain.

Compounds **4**, **5**, **7** and **8** were also evaluated to extend our structure-activity relationship study regarding their oxiranyl or oxiranylmethyl group at C3, as well as the functionality (ketone or alcohol) at C17. Although previous results seem to demonstrate that estrane derivatives with a carbonyl at C17 were better inhibitors than the corresponding alcohols [15-18], in our case, this is not relevant. In fact, the inhibition assay did not show any probing pattern that could explain if a C17-ketone, or the corresponding reduced form (alcohol), binds more efficiently to the targeted enzyme site. The results with oxiranes **4** and **5** demonstrated that the hydroxylated compound **5** gives a better 17β-HSD1 inhibition at the lower concentrations of 0.1 µM and 1 µM, but a slightly lower activity at the higher concentration of 5 µM. However, oxiranylmethyl-estrane derivatives **7** and **8** showed a lower activity for hydroxylated compound **8** at the three concentrations tested. We were also interested in determining which of the steroidal oxiranes, those directly linked or spaced to the A-ring by a methylene group, present the best characteristics for 17β-HSD1 inhibition. Compounds **4**, **5** and **11**, with the oxiranyl group, have a

lower inhibitory activity than the corresponding compounds **7**, **8** and **15**, with an oxiranylmethyl group.

We further investigated the inhibitory activity of our targeted compounds **11** and **15** at several increasing concentrations, which allowed us to draw the inhibiting curve and to determine their IC_{50} values, the concentration that inhibited 50% of the enzyme activity (Scheme 2). As we observed in previous work [16-18], PBRM is a weaker inhibitor than CC-156 (IC_{50} value of 0.050 and 0.017 µM, respectively), however, it is important to mention that their mechanisms of action (irreversible or reversible inhibitor, respectively) are different. We therefore tried to increase the inhibitory activity of PBRM by changing its 2-bromoethyl side chain by an oxiranyl or oxiranylmethyl group. Although such electrophilic epoxides were known to provide irreversible inhibition [27, 21], they were not reactive, in our case. For compound **15**, an IC_{50} value of 1.3 µM, which is almost 26-times higher (less potent) than the IC_{50} value of PBRM, suggests a reversible non-covalent binding mode. For compound **11**, the IC_{50} value cannot be determinate, due to its very low inhibitory potency observed at the concentrations used. At this low level of inhibition, we assumed that the affinity of the molecule for the catalytic site was too weak, and thus insufficient to favor a covalent binding with 17β-HSD1. On this basis, we do not proceed to irreversibility kinetic assays since the inhibition levels measured do not fit with the high level of inhibition expected from an irreversible inhibition. Since two relevant examples of His-mediated alkylation with epoxide derivatives have been reported [21, 27], we are intrigued by the inability of the epoxides **11** and **15** to allow alkylation contrary to the lower reactive bromoethyl side chain of PBRM, which was demonstrated as an efficient covalent inhibitor through an His alkylation [19]. The amino acids micro-environment configuration coupled with proximity effect [28] between the electrophilic group of the inhibitor and His side chain of the enzyme could be a key to understanding the N-alkylation event possible with an alkylhalide group and not with an oxiranyl or an oxiranylmethyl group. However, to better understand the key parameters in play to favor the mutual reactivity of a given electrophile and 17β-HSD1, a more in-depth fundamental study on their mutual reactivity would be needed.

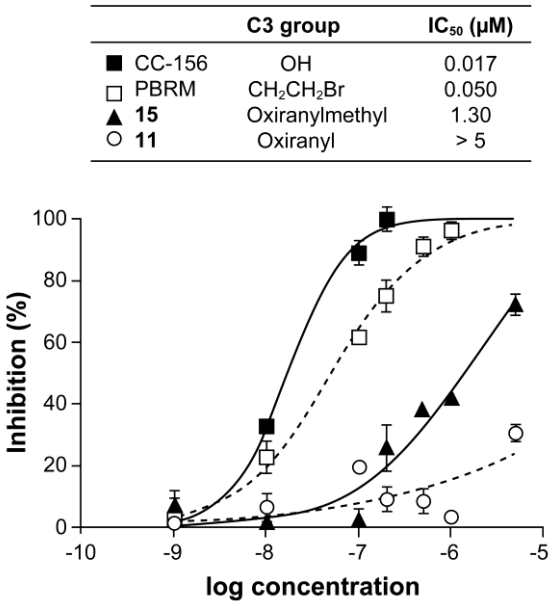


Fig. 4. Inhibition of 17β-HSD1 in T-47D cells at increasing concentrations of oxiranyl derivative **11**, oxiranylmethyl derivative **15** and reference inhibitors CC-156 and PBRM. The inhibitory activity was determined for the transformation of [^{14}C]-E1 (60 nM) into [^{14}C]-E2 in T-47D intact cells incubated 24 h. The experiment was performed in triplicate (±SD). The inhibitors were tested at seven increasing concentrations of 0.001, 0.01, 0.1, 0.2, 0.5, 1 and 5 μM. Curves of 17β-HSD1 inhibition were used for the determination of IC_{50} value, which represents the concentration that inhibited the enzyme activity by 50%.

Even if our epoxide-estrane compounds were not characterized as covalent inhibitors, we were interested in their estrogenic activity profile. As previously mentioned, they should not induce the proliferation of estrogen-sensitive cells, such as breast cancer T-47D cells. In this cell line, both E1 and E2 clearly induced cell proliferation (Figure 5). In fact, the proliferative effect of E1 is the result of its reduction into E2 by 17β-HSD1. Despite being functionalized at position C16, which tends to decrease the proliferation of cells, compounds **11** and **15** possess a slight tendency to induce estrogenic activity. Each compound demonstrated a prozone effect, suggesting a possible cytotoxic effect at the highest concentration of 5 μM. This proliferation profile was already observed for estrane derivatives [29]. Thus, oxirane-estrane compounds **4**, **5**, **7** and **8** are more estrogenic when compared to their benzamide analogs **11** and **15**. From these two estrane compounds, the oxalylmethyl derivative **15** is less estrogenic than the oxalyl derivative **11**.

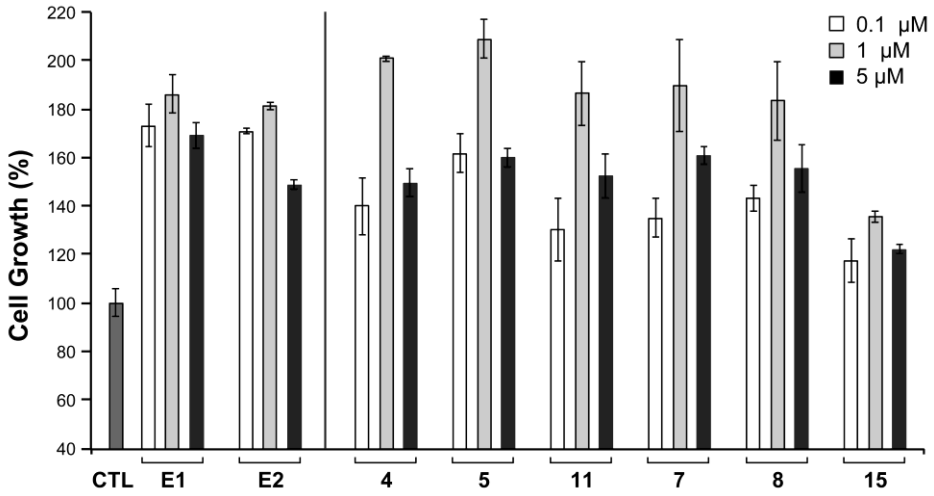


Fig. 5. Effect of inhibitors on the growth of estrogen-sensitive T-47D cells treated 7 days. Control fixed at 100%.

3.4. *Conclusion*

We have successfully synthesized a series of C3-oxiranyl/oxiranylmethyl-estrane derivatives structurally related to the potent irreversible and reversible 17β-HSD1 inhibitors PBRM and CC-156, respectively. Two of these compounds, **11** and **15**, were initially identified as potential irreversible inhibitors, but from the enzymatic assay results, they occur to have a limited inhibitory activity compared to PBRM and CC-156. The oxirane obtained from the allylic compound (oxiranylmethyl derivative **15**) is however a better 17β-HSD1 inhibitor than the oxirane obtained from the vinylic compound (oxiranyl derivative **11**). In fact, it is highly probable that the inhibition observed with compound **15** (IC_{50} = 1.3 µM) is the result of a reversible inhibition instead of an irreversible inhibition. An enzymatic kinetic study will however be necessary to confirm this hypothesis. Additionally, while an epoxide function was used in our inhibitor design, it did not lead to a covalent inhibitor like PBRM. This study demonstrates that for 17β-HSD1, the inclusion of an electrophilic group is not the only parameter to consider in generating a covalent inhibition. These results also highlight the fact that a very particular molecular context seems to be necessary to promote the N-alkylation of a His residue which, in the case of 17β-HSD1, seems very important to favor a covalent inhibition, or not.

Acknowledgments

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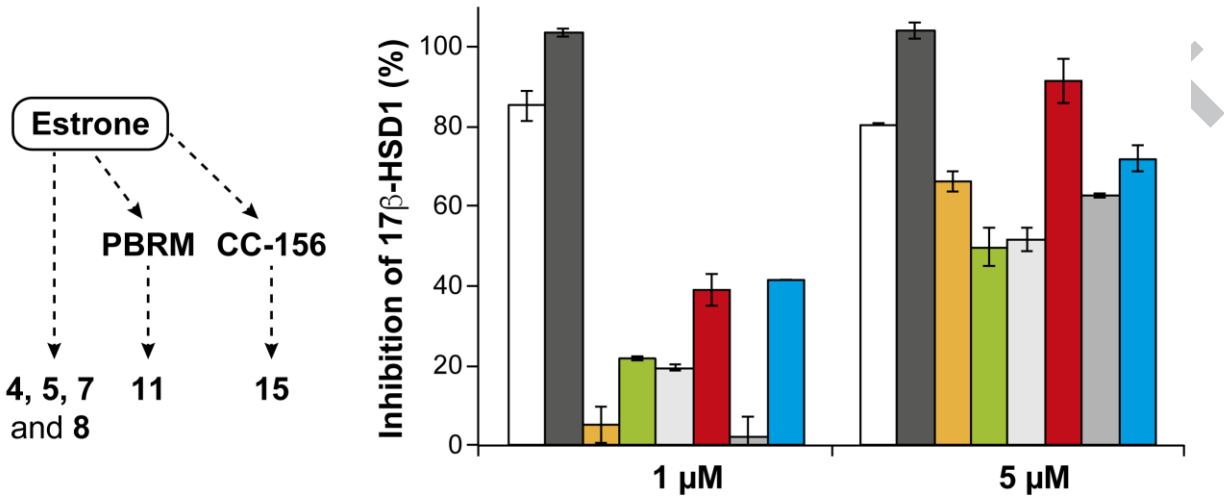
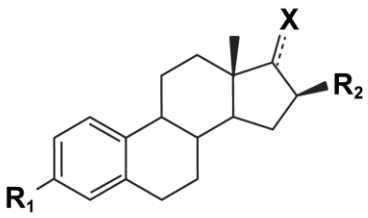
Graphical abstract





	R_1 (C3)	X (C17)	R_2 (CH)
□ CC-156	OH	17β-OH	*Side chain*
■ PBRM	CH_2CH_2Br	17β-OH	*Side chain*
4	Oxiranyl	O	H
5	Oxiranyl	17β-OH	H
11	Oxiranyl	17β-OH	*Side chain*
7	Oxiranylmethyl	O	H
8	Oxiranylmethyl	17β-OH	H
15	Oxiranylmethyl	17β-OH	*Side chain*

Highlights

- C3-oxiranyl/oxiranylmethyl-estrane derivatives **11** and **15** were designed as 17β-HSD1 inhibitors
- Compounds **11** and **15** have been synthesized in a short and efficient route
- A C3-oxiranyl/oxiranylmethyl oxiranyl group did not alkylate the enzyme
- The electrophilic group is not the only parameter to consider in generating a covalent inhibitor