Chemical Synthesis and NMR Characterization of Non Steroidal Mimics of an Estradiol Derivative Used as Inhibitor of 17β-Hydroxysteroid Dehydrogenase Type 1

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Correspondence: Donald Poirier, Laboratory of Medicinal Chemistry, CHUQ (CHUL)–Research Center, Québec, QC., G1V 4G2, Canada. Tel: 1-418-654-2296. E-mail: Donald.poirier@crchul.ulaval.ca

Abstract

Inhibiting estradiol (E2) biosynthesis through 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1) inhibitors is a promising strategy for breast cancer therapy. We have designed a non-steroidal template to mimic CC-156, a potent steroidal inhibitor of 17β-HSD1. Starting from tetrahydro-isoquinolinol hydrobromide, two representative compounds were synthesized in six chemical steps: protection of the amino group, protection of the phenol, hydrolysis of the N-protecting group Fmoc, nucleophilic substitution to introduce an ethyloxirane, phenolysis with *meta* or *para* hydroxybenzamide and the hydrolysis of the MOM protecting group. Although the compounds showed a good fit when docked in the catalytic site of the enzyme, conserving the key interactions with amino acids His221 and Ser142, observed from the crystalline structure of inhibitor CC-156 with 17β-HSD1, they weakly inhibited 17β-HSD1. However, they did not show estrogenic activity when tested *in vitro*, suggesting the potential of this non-steroidal template for drug design. Furthermore, the synthetic approach reported here opens a door to the preparation of additional non-steroidal mimics of E2 derivatives, which could be tested on 17β-HSD1 and others biological targets.

Keywords: phenol derivative, tetrahydro-isoquinolinol, chemical synthesis, inhibitor, 17β-hydroxysteroid dehydrogenase

1. Introduction

Breast cancer is the leading cause of death worldwide among all the types of cancers affecting women (Jemal et al., 2011). Estrogenic hormones are important stimulators in most of these breast cancers (Dizerega et al., 1980; Travis & Key, 2003) and estradiol (E2), the most potent natural estrogen, stimulates cancer cell growth through endocrine, paracrine, and intracrine pathways (Labrie, 1991). The last step in E2 biosynthesis requires the enzyme 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1). This enzyme also catalyses the reduction of dehydroepiandrosterone (DHEA) into 5-androstene-3β,17β-diol (5-diol) (Figure 1), a weaker estrogen that becomes more important after menopause (Simard et al., 1988).

Blocking 17β-HSD1 thus appears to be an attractive strategy in breast cancer therapy (Poirier, 2008). To the best of our knowledge, there is still no 17β-HSD1 inhibitor under clinical trials. This is partly explained by the fact that many potent inhibitors of this enzyme have an estrane nucleus that can activate the estrogen receptors (ERs). Of course it is not desirable to use these estrogenic inhibitors in the treatment of hormone-dependent breast cancer, and synthesizing 17β-HSD1 inhibitors without estrogenic activity thus represents a great challenge (Poirier, 2010; Poirier, 2011; Day et al., 2008b). Compound CC-156 (Figure 2A) is a very potent inhibitor of 17β-HSD1 that was synthesized and reported from previous work in our laboratory (Laplante et al., 2008). The crystal structure of the ternary complex of CC-156 with 17β-HSD1 and cofactor NADP has already been described (Figure 2B) (Mazundar et al., 2009) and shown key interactions with amino acids in the enzyme catalytic site. However, this compound was found to stimulate the proliferation of MCF-7 and T-47D estrogen-sensitive (ER$^+$) breast cancer cell lines, thus greatly reducing its therapeutic potential. Three strategies were tried to reduce the proliferative (estrogenic) effect of this steroidal inhibitor: Replacing the hydroxy group at position 3 (C3) by a hydrogen atom; adding a methoxy at C2; or adding an alkylamide chain at C7, but all these methods did not give satisfactory results (Laplante et al., 2008).
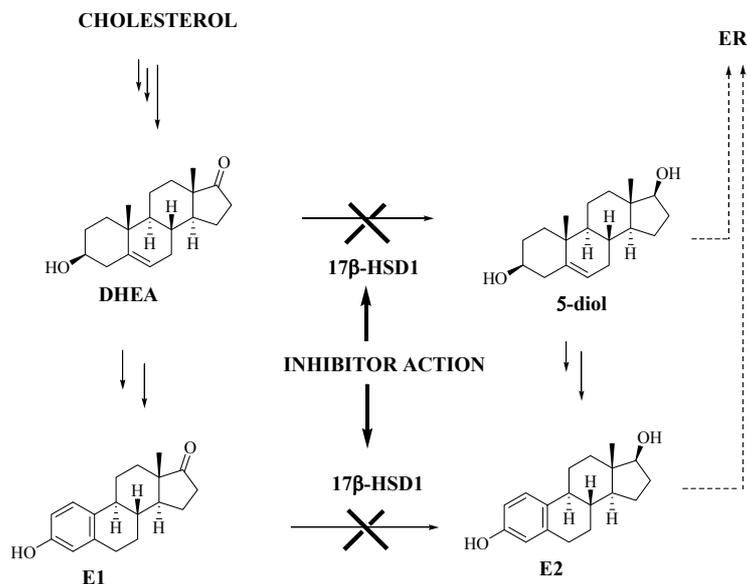
Figure 1. Last steps in the biosynthesis of active estrogens estradiol (E2) and 5-androstene-3β,17β-diol (5-diol), which stimulate the cell proliferation by their action on the estrogen receptor (ER)
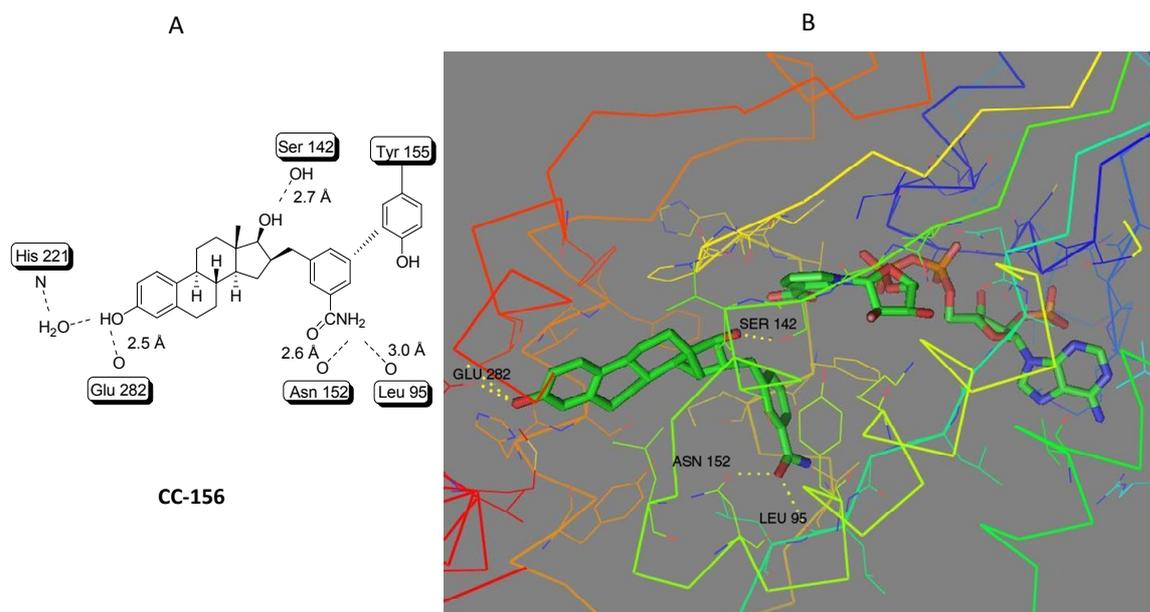

Figure 2. Analysis of the crystalline structure of the ternary complex 17β-HSD1/CC-156/NADP. 2D (A) and 3D (B) representations showing interactions (H-bond and π-π interactions) between the enzyme (17β-HSD1) and the inhibitor (CC-156) (Mazumdar et al., 2009)

Using AutoDock software (http://autodock.scripps.edu/), we found that compound 10 (Figure 3A) fits very well in the enzyme catalytic site of 17β-HSD1 (Figure 3B). We also observed that compound 10 keeps interactions with His221 and Ser142; its phenyl ether moiety interacts with Tyr155 differently (with H-bond) regarding to CC-156 in which it binds via ππ interaction (Mazumdar et al., 2009); and new hydrogen bonds were observed with Val188, Thr190 and Val143. Such non-steroidal compound is interesting because it is expected to be an E2 mimic without residual estrogenic activity. Herein, we report the successful synthesis of 8 and 10, two mimics of CC-156, starting from tetrahydro-6-isoquinolinol hydrobromide (1) (Figure 4). We also report their characterization by IR, ^{1}H NMR, ^{13}C NMR and MS analyses, their assessment as inhibitor of 17β-HSD1, and
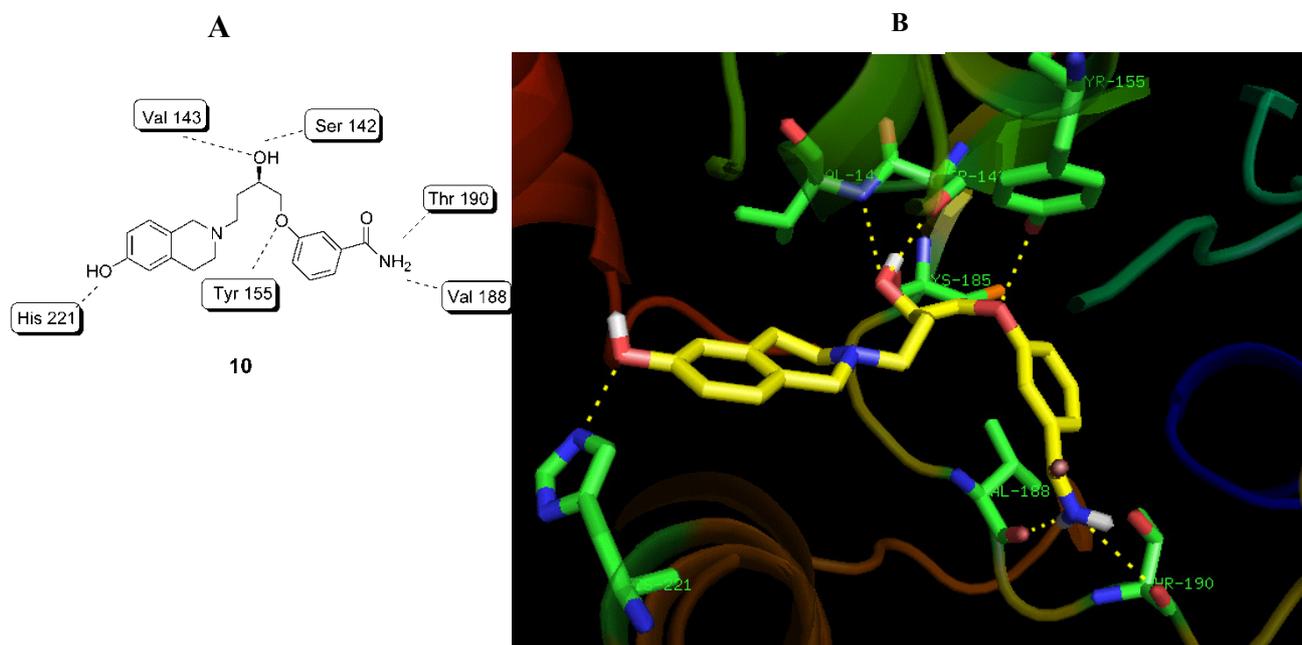
their assessment as estrogenic compounds.

Figure 3. 2D (A) and 3D (B) representations of different interactions observed between the non-steroidal mimic of CC-156 (compound 10) and 17β-HSD1 after a docking experiment (AutoDock v.3). Calculated energy = -11.4 kcal/mol

2. Experimental

2.1 General

1,2,3,4-Tetrahydroisoquinolin-6-ol hydrobromide was purchased from Ark Pharm (Libertyville, IL, USA). 4-Hydroxybenzamide and chemical reagents of highest purity were obtained from Sigma–Aldrich Canada Ltd. (Oakville, ON, Canada), N-(9-fluorenylmethoxycarbonyloxy) succinimide (Fmoc-OSu) was purchased from Novabiochem (Darmstadt, Germany). Solvents were obtained from Fisher Scientific (Montréal, QC, Canada). Reactions were run under an inert (argon) atmosphere in oven-dried glassware. Analytical thin-layer chromatography (TLC) was performed on 0.20 mm silica gel 60 F254 plates (E. Merck, Darmstadt, Germany), and compounds were visualized by using ammonium molybdate/sulfuric acid/water (with heating). Flash column chromatography was performed with Silicycle R10030B 230–400 mesh silica gel (Québec, QC, Canada). Infrared spectra (IR) were obtained from a thin film of compound usually solubilised in CH_2Cl_2 (DCM) and deposited upon a NaCl pellet. They were recorded with a Horizon MB 3000 ABB FTIR spectrometer (ABB, Québec, QC, Canada) and bands are reported in cm^{-1}. Nuclear magnetic resonance (NMR) spectra were recorded with a Bruker Avance 400 digital spectrometer (Billerica, MA, USA) and data reported in ppm. The $CDCl_3$ ^{1}H and ^{13}C NMR signals (7.26 and 77.0 ppm, respectively) and CD_3OD ^{1}H and ^{13}C NMR signals (3.31 and 49.0 ppm, respectively) were used as internal references. Low-resolution mass spectra (LRMS) were recorded on a PE Sciex API-150ex apparatus (Foster City, CA, USA) equipped with a turbo ion-spray source.

2.2 Synthesis of Fmoc-protected Compound 2

1,2,3,4-Tetrahydroisoquinolin-6-ol hydrobromide (1) (9.0 g, 39.1 mmol) was dissolved in a mixture of THF/water (3/1 v/v) and an aqueous 1N sodium bicarbonate solution (117 mL) was added. After the reaction mixture was stirred 5 min, Fmoc-OSu (13.9 g, 41.1 mmol) was added and the mixture stirred for 2 h at room temperature. Water (450 mL) was added and the crude product was extracted with EtOAc. The organic phase was dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. Compound 2 (14.0 g, 96% yield) was obtained as a white powder after purification by chromatography (hexanes/EtOAc; 1/1).

9*H*-Fluoren-9-ylmethyl 6-hydroxy-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (2): Rf = 0.25 (hexanes/EtOAc, 8:2). IR (film) ν 3317 (OH), 2947, 2901, 1674 (NCOO), 1612, 1443, 1221. ^{1}H NMR ($CDCl_3$) δ 2.77 (m,

Ar-$\underline{CH_2}$-CH$_2$), 3.67 (m, CH$_2$-$\underline{CH_2}$-N), 4.28 (t, J = 6.8 Hz, CH of Fmoc), 4.47 (d, J = 6.8 Hz, CH$_2$ of Fmoc), 4.55 (m, Ar-$\underline{CH_2}$-N), 6.63 (d, J = 2.5 Hz, CH of Ar), 6.64 and 6.83 (2m, 2 x CH of Ar), 7.31, 7.40, 7.60 and 7.77 (4m, 8xCH of Fmoc). ^{13}C NMR (CDCl$_3$) δ 28.7 , 41.4 , 45.3 , 47.3 , 67.5 , 114.9, 115.1, 120.0 (2x), 125.0 (2x), 127.0 (2x), 127.4, 127.7 (2x), 133.1, 135.9, 141.3 (2x), 144.0 (2x), 154.2, 155.5. LRMS calculated for C$_{24}$H$_{22}$NO$_3$ (M+H) 372.15, found 372.30.

2.3 Synthesis of MOM and Fmoc-protected Compound 3

To a solution of compound 2 (14.0 g, 37.6 mmol) in anhydrous DCM (1000 mL) under an argon atmosphere was added N,N-diisopropylethylamine (15.7 mL) and the mixture was stirred for 10 min at 0°C (in an ice/water bath). Chloromethyl methyl ether (5.43 g, 67.4 mmol) was then added slowly and the reaction mixture was brought to room temperature after 20 min and stirred for 24 h. Water was added to quench the reaction and the mixture extracted with DCM. The organic phase was dried with anhydrous Na$_2$SO$_4$, filtered and evaporated under reduced pressure. The crude mixture was purified by flash chromatography (hexanes/EtOAc; 3/1) to provide compound 3 (11.0 g, 70% yield).

9*H*-Fluoren-9-ylmethyl 6-(methoxymethoxy)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (3): Rf = 0.38 (hexanes/EtOAc, 8:2). IR (film) ν 2947, 2901, 1697 (NCOO), 1612, 1504, 1427, 1227, 1149, 1095, 1011. ^{1}H NMR (CDCl$_3$) δ 2.81 (m, Ar-$\underline{CH_2}$-CH$_2$), 3.49 (s, CH$_3$O), 3.68 (m, CH$_2$-$\underline{CH_2}$-N), 4.28 (t, J = 6.8 Hz, CH of Fmoc), 4.47 (d, J = 6.8 Hz, CH$_2$ of Fmoc), 4.57 (m, Ar-CH$_2$-N), 5.17 (s, O-CH$_2$-O), 6.84 (d, J = 2.2 Hz, CH of Ar), 6.90 (m, CH of Ar), 7.03 (m, CH of Ar), 7.31, 7.39, 7.59 and 7.75 (4m, 8 x CH of Fmoc). ^{13}C NMR (CDCl$_3$) δ 28.7, 41.2, 45.1, 47.2, 55.8, 67.2, 94.3, 114.6, 115.8, 119.8 (2x), 124.8 (2x), 126.3, 126.9 (2x), 127.1, 127.5 (2x), 135.5, 141.2 (2x), 143.9 (2x), 155.2, 155.6. LRMS calculated for C$_{26}$H$_{26}$NO$_4$ (M+H) 416.18, found 416.30.

2.4 Fmoc Hydrolysis: Synthesis of Compound 4

Compound 3 (11.0 g, 26.4 mmol) was dissolved in anhydrous DCM (220 mL) and piperidine (55 mL). The reaction mixture was stirred for 1 h at room temperature and then quenched with water. The organic phase was washed with water and dried with anhydrous Na$_2$SO$_4$, filtered and evaporated under reduced pressure. The yellowish powder was purified by flash chromatography (DCM/MeOH; 97:3) to provide compound 4 (2.9 g, 55% yield).

6-(Methoxymethoxy)-1,2,3,4-tetrahydroisoquinoline (4): Rf = 0.24 (hexanes/EtOAc, 3:1). IR (film) ν 3317 (NH), 2924, 1612, 1504, 1234, 1149, 1072, 1003. ^{1}H NMR (CD$_3$OD) δ 2.84 (t, J = 6.0 Hz, Ar-$\underline{CH_2}$-CH$_2$), 3.11 (t, J = 6.1 Hz, CH$_2$-$\underline{CH_2}$-N), 3.45 (s, CH$_3$O), 3.95 (s, Ar-$\underline{CH_2}$-N), 5.15 (s, O-CH$_2$-O), 6.80 (s, CH of Ar), 6.83 (d, J = 8.3 Hz, 1H, CH of pseudo A ring), 6.97 (d, J = 8.3 Hz, CH of Ar). ^{13}C NMR (CDCl$_3$) δ 29.3, 43.6, 47.6, 55.9, 94.5, 114.3, 116.5, 127.5, 129.3, 135.5, 155.4. LRMS calculated for C$_{11}$H$_{16}$NO$_2$ (M+H) 194.11, found 194.15.

2.5 Synthesis of Epoxide 5

To a solution of amine 4 (245 mg, 1.3 mmol) and potassium carbonate (261 mg, 1.9 mmol) in acetone (12 mL) was added 4-bromo-1,2-epoxybutane (476 mg, 3.2 mmol) and the mixture was stirred for 16 h at 55°C. The solution was filtered over a büchner and rinsed with DCM, then the collected filtrate was evaporated under reduced pressure and purified by flash chromatography (hexanes/EtOAc/triethylamine; 90/9/1) to yield epoxide 5 (216 mg, 65% yield).

6-(Methoxymethoxy)-2-[2-(oxiran-2-yl)ethyl]-1,2,3,4-tetrahydroisoquinoline (5): Rf = 0.28 (DCM/MeOH, 9:1). IR (film) ν 2924, 1612, 1504, 1242, 1149, 1076, 1011. ^{1}H NMR (CDCl$_3$) δ 1.78 and 1.87 (2m, $\underline{CH_2}$-CH-O), 2.54 and 2.78 (2m, CH$_2$-O), 2.68 (t, J = 7.4 Hz, CH$_2$$\underline{CH_2}$-N), 2.72 (m, Ar-CH$_2$$\underline{CH_2}$-N), 2.88 (m, Ar-$\underline{CH_2}CH_2$-N), 3.03 (m, CH-O), 3.47 (s, CH$_3$O), 3.59 (s, Ar-$\underline{CH_2}$-N), 5.14 (s, O-CH$_2$-O), 6.79 (d, J = 3.0 Hz, CH of Ar), 6.81 (d, J = 8.3 Hz, CH of Ar), 6.94 (d, J = 8.2 Hz, CH of Ar). ^{13}C NMR (CDCl$_3$) δ 29.3, 30.5, 47.1, 50.8, 50.9, 54.7, 55.6, 55.9, 94.5, 114.2, 115.8, 127.5, 128.2, 135.5, 155.5. LRMS calculated for C$_{15}$H$_{22}$NO$_3$ (M+H) 264.15, found 264.15.

2.6 General Method for the Synthesis of Compounds 6 and 7

To a solution of 4-hydroxybenzamide or 3-hydroxybenzamide (104 mg, 0.76 mmol) in DMF (10 mL) was added potassium carbonate (158 mg, 1.14 mmol) and the mixture was stirred for 30 min under argon at 105°C. The epoxide 5 (100 mg, 0.38 mmol) was dissolved in DMF (3 mL) and added to the reaction mixture. After 22 h at 105°C, water (14 mL) and a saturated aqueous sodium bicarbonate solution (10 mL) were added and the mixture extracted with EtOAc. The organic phase was dried with Na$_2$SO$_4$ filtered, evaporated under reduced pressure and purified by flash chromatography (DCM/MeOH; 98:2 to 95:5) to yield the compound 6 (69 mg, 45% yield) or 7 (33 mg, 21% yield).

4-{2-Hydroxy-4-[6-(methoxymethoxy)-3,4-dihydroisoquinolin-2(1*H*)-yl]butoxy}benzamide (6): Rf = 0.40 (DCM/MeOH, 9:1). IR (film) ν 3391 and 3190 (OH and NH$_2$), 2928, 2827, 2762, 1647 (CON, amide), 1612, 1574, 1508, 1423, 1400, 1311, 1261, 1149, 1076, 1022. ^{1}H NMR (CDCl$_3$) δ 1.90 (m, CH$_2$-CH-O), 2.68 and 2.94 (2m, Ar-CH$_2$CH$_2$-N), 2.87 (m, CH$_2$CH$_2$-N and Ar-CH$_2$CH$_2$-N), 3.47 (s, CH$_3$O), 3.62 and 3.74 (2d of AB system, J = 14.5 Hz, Ar-CH$_2$-N), 3.94 (dd, J$_1$ = 5.9 Hz and J$_2$ = 9.3 Hz, 1H of OCH$_2$), 4.04 (dd, J$_1$ = 5.4 Hz and J$_2$ = 9.3 Hz, 1H of OCH$_2$), 4.23 (m, CHO), 5.14 (s, O-CH$_2$-O), 6.79 (d, J = 2.3 Hz, CH of Ar), 6.83 (dd, J$_1$ = 2.5 Hz and J$_2$ = 8.3 Hz, CH of Ar), 6.95 (d, J = 8.7 Hz, CH of Ar), 6.96 (d, J = 8.9 Hz, 2 x CH of benzamide), 7.76 (d, J = 8.8 Hz, 2 x CH of benzamide). ^{13}C NMR (CDCl$_3$) δ 28.5, 29.1, 50.5, 55.7, 55.9, 56.8, 71.5, 71.8, 94.5, 114.3 (2x), 114.4, 115.8, 125.7, 127.4, 127.5, 129.3 (2x), 135.1, 155.7, 161.9, 168.9. LRMS calculated for C$_{22}$H$_{28}$N$_2$O$_5$ (M+H) 401.20, found 401.20.

3-{2-Hydroxy-4-[6-(methoxymethoxy)-3,4-dihydroisoquinolin-2(1*H*)-yl]butoxy}benzamide (7): Rf = 0.30 (DCM/MeOH, 9:1). IR (film) ν 3348 and 3198 (OH and NH$_2$), 2932, 2827, 1666 (CON, amide), 1612, 1582, 1504, 1447, 1381, 1246, 1153, 1076, 1011. ^{1}H NMR (CDCl$_3$) δ 1.88 (m, CH$_2$-CH-O), 2.67 and 3.05 (2m, Ar-CH$_2$CH$_2$-N), 2.87 (m, CH$_2$CH$_2$-N and Ar-CH$_2$CH$_2$-N), 3.47 (s, CH$_3$O), 3.63 and 3.74 (2d of AB system, J = 14.5 Hz, Ar-CH$_2$-N), 3.93 (dd, J$_1$ = 5.6 Hz and J$_2$ = 9.4 Hz, 1H of OCH$_2$), 4.05 (dd, J$_1$ = 5.5 Hz and J$_2$ = 9.4 Hz, 1H of OCH$_2$), 4.23 (m, CH-O), 5.14 (s, O-CH$_2$-O), 6.78 (d, J = 2.3 Hz, CH of Ar), 6.82 (dd, J$_1$ = 2.5 Hz and J$_2$ = 8.4 Hz, CH of Ar), 6.95 (d, J = 8.4 Hz, CH of Ar), 7.10 (m, CH of benzamide), 7.33 (m, 2 x CH of benzamide) 7.41 (s, CH of benzamide). ^{13}C NMR (CDCl$_3$) δ 28.5, 29.0, 50.5, 55.6, 55.9, 56.6, 71.4, 71.9, 94.5, 113.4, 114.5, 115.7, 118.6, 119.5, 127.2, 127.5, 129.6, 134.8, 135.1, 155.7, 159.0, 169.2. LRMS calculated for C$_{22}$H$_{28}$N$_2$O$_5$ (M+H) 401.20, found 401.20.

2.7 Hydrolysis of the MOM Group: Synthesis of 8 and 9

Compound 6 (68 mg, 0.17 mmol) was dissolved in methanol (2 mL), a solution of hydrochloric acid (20% in methanol) (6.2 mL) was added and the resulting mixture was heated 4.5 h at 65°C. Water was then added, the methanol evaporated under reduced pressure, the solution neutralised with a saturated aqueous solution of sodium bicarbonate, and the aqueous phase extracted with DCM. The organic phase was evaporated and the yellowish powder was purified by flash chromatography (DCM/MeOH; 98:2) to yield the expected compound 8 (18 mg, 29% yield) and the ester 9 (14.5 mg, 23% yield).

4-[2-Hydroxy-4-(6-hydroxy-3,4-dihydroisoquinolin-2(1*H*)-yl)butoxy]benzamide (8): Rf = 0.10 (DCM/MeOH, 9:1). IR (film) ν 3344 and 3178 (OH and NH$_2$), 2924, 2851, 1655 (CON, amide), 1605, 1570, 1512, 1458, 1381, 1254, 1180, 1126. ^{1}H NMR (CD$_3$OD) δ 2.00 (m, CH$_2$-CH-O), 2.96 and 3.08 (2m, Ar-CH$_2$CH$_2$-N-CH$_2$), 3.90 (s, Ar-CH$_2$-N), 4.05 (m, Ph-CH$_2$CHO), 6.60 (d, J = 2.3 Hz, CH of Ar), 6.64 (dd, J$_1$ = 2.5 Hz and J$_2$ = 8.3 Hz, CH of Ar), 6.95 (d, J = 8.3 Hz, CH of Ar), 7.04 (d, J = 8.9 Hz, 2 x CH of benzamide), 7.87 (d, J = 8.9 Hz, 2 x CH of benzamide). ^{13}C NMR (CD$_3$OD) δ 28.6, 30.3, 51.7, 55.8, 55.9, 69.7, 73.2, 115.0, 115.3 (2x), 115.7, 123.8, 127.3, 128.8, 130.7 (2x), 135.1, 157.6, 163.3, 172.0. LRMS calculated for C$_{20}$H$_{25}$N$_2$O$_4$ (M+H) 357.17, found 357.15.

Methyl 3-[2-hydroxy-4-(6-hydroxy-3,4-dihydroisoquinolin-2(1*H*)-yl)butoxy]benzoate (9): Rf = 0.40 (DCM/MeOH, 9:1). IR (film) ν 3209 and 3055 (OH phenol and alcohol), 2947, 2831, 1713 (C=O, ester), 1605, 1512, 1435, 1281, 1257, 1173, 1111, 1034. ^{1}H NMR (CDCl$_3$) δ 1.87 (m, CH$_2$-CH-O), 2.65 and 2.85 (2m, Ar-CH$_2$CH$_2$-N-CH$_2$), 3.57 and 3.70 (2d of AB system, J = 14.1 Hz, Ar-CH$_2$-N), 3.88 (s, CH$_3$O), 3.96 (dd, J$_1$ = 5.9 Hz and J$_2$ = 9.4 Hz, 1H of OCH$_2$), 4.06 (dd, J$_1$ = 5.3 Hz and J$_2$ = 9.4 Hz, 1H of Ph-OCH$_2$), 4.28 (m, CH-O), 6.43 (d, J = 2.4 Hz, CH of Ar), 6.56 (dd, J$_1$ = 2.5 Hz and J$_2$ = 10.8 Hz, CH of Ar), 6.79 (d, J = 8.3 Hz, CH of Ar), 6.94 (d, J = 8.9 Hz, 2 x CH of methylbenzoate), 7.98 (d, J = 8.9 Hz, 2 x CH of methylbenzoate). ^{13}C NMR (CDCl$_3$) δ 28.1, 28.6, 50.6, 51.9, 55.5, 56.7, 71.5, 71.6, 113.7, 114.2 (2x), 114.9, 122.7, 124.5, 127.5, 131.6 (2x), 134.6, 155.2 (2x), 162.5, 166.9. LRMS calculated for C$_{21}$H$_{26}$NO$_5$ (M+H) 372.18, found 372.15.

2.8 Hydrolysis of the MOM Group: Synthesis of Compound 10

Compound 7 (27 mg, 0.07 mmol) was dissolved in acetone (3 mL), a solution of 10% hydrochloric acid in acetone (1/1, v/v) (8.4 mL) was added and the resulting mixture was heated for 4 h at 57°C. Water was then added, the acetone evaporated under reduced pressure, the solution neutralised with a saturated aqueous solution of sodium bicarbonate and the aqueous phase extracted with DCM. The organic phase was evaporated and purified by successive trituration in diethyl ether and hexanes to yield compound 10 (20 mg, 83% yield) as a white powder.

Methyl 4-[2-hydroxy-4-(6-hydroxy-3,4-dihydroisoquinolin-2(1*H*)-yl)butoxy]benzoate (10): Rf = 0.10 (DCM/MeOH, 9:1). IR (film) ν 3360 (OH and NH$_2$), 2924, 1666 (CON, amide), 1624, 1582, 1420, 1331, 1288, 1246, 1119. ^{1}H NMR (CD$_3$OD) δ 1.90 (m, CH$_2$-CH-O), 2.68-2.90 (broad m, Ar-CH$_2$CH$_2$-N-CH$_2$), 3.58 (s, Ar-CH$_2$-N), 4.01 (m, OCH$_2$-CH-O), 4.05 (m, OCH$_2$-CH-O), 6.51 (d, J = 2.3 Hz, CH of Ar), 6.55 (dd, J$_1$ = 2.5 Hz

and $J_2 = 8.2$ Hz, CH of Ar), 6.83 (d, $J = 8.3$ Hz, CH of Ar), 7.15 (d, $J = 8.3$ Hz, CH of benzamide), 7.37 (t, $J = 8.2$ Hz, CH of benzamide) 7.44 (m, 2 x CH of benzamide). ^{13}C NMR (CD$_3$OD) δ 29.8, 31.1, 52.1, 56.3, 56.8, 70.3, 73.3, 114.6, 115.1, 116.1, 119.5, 121.0, 125.2, 128.4, 130.6, 135.9, 136.3, 158.5, 160.5, 172.2. LRMS calculated for C$_{20}$H$_{25}$N$_2$O$_4$ (M+H) 357.17, found 357.15.

2.9 17β-HSD1 Inhibition of Compounds 8-10

The enzymatic assay was performed using the T-47D breast cancer cell line as source of 17β-HSD1 activity following a procedure previously published (Ayan et al., 2012).

2.10 Estrogenic Activity of Compounds 8 and 10

Using a procedure we previously published (Ayan et al., 2012), compounds 8 and 10 were tested to determine their potential proliferative (estrogenic) effect on estrogen-sensitive (ER$^+$) breast cancer T-47D cells.

2.11 ER-α Binding Affinity of Compounds 8 and 10

A comparative binding assay using a purified full-length recombinant human estrogen receptor alpha (ER-α) was done as previously described (Ayan et al., 2012).

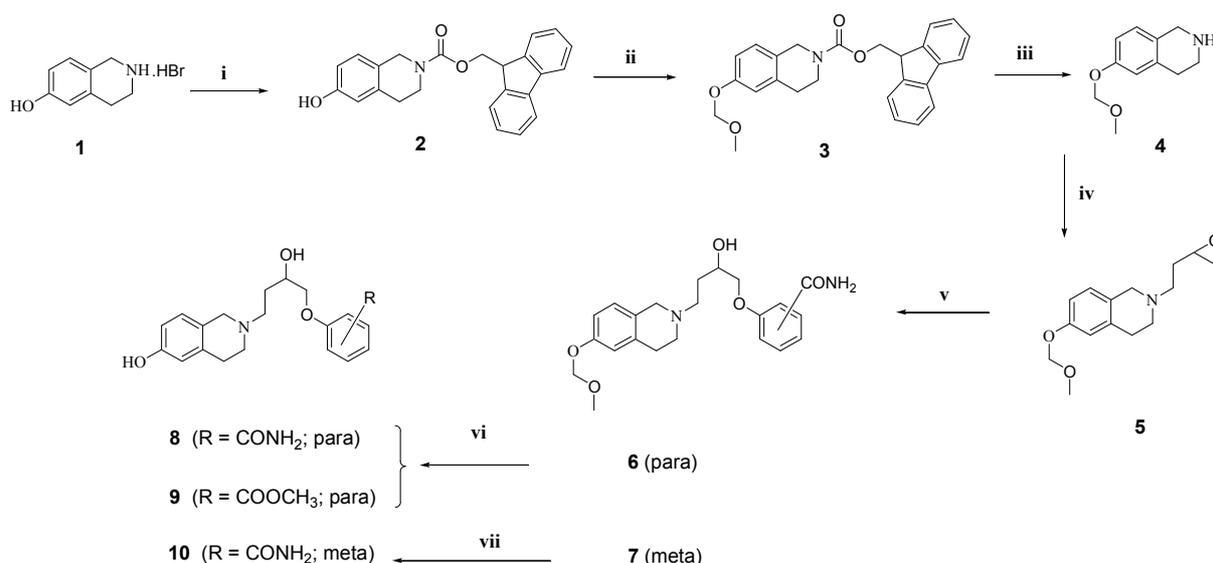Figure 4. Synthesis of targeted compounds 8 and 10. Reagents and conditions: i) THF/H$_2$O, NaHCO$_3$, Fmoc-OSu, rt; ii) CH$_2$Cl$_2$, ClCH$_2$OCH$_3$, DIPEA, 0°C - rt; iii) piperidine; CH$_2$Cl$_2$, rt; iv) 4-bromo-1,2-epoxybutane, K$_2$CO$_3$, acetone, 55°C; v) K$_2$CO$_3$, *para*- or *meta*- hydroxybenzamide, DMF, 105°C; vi) MeOH/HCl, 65°C; vii) acetone/HCl, 57°C

3. Results and Discussion

3.1 Synthesis of Targeted Compounds 8 and 10 (Figure 4)

The free amine function of tetrahydro-6-isoquinolinol hydrobromide (1) was protected with Fmoc-Osu in presence of aqueous NaHCO$_3$. The hydroxyl group of 2 was next protected as a methoxymethyl (MOM) ether group to give compound 3. This compound was found to be unstable at room temperature and has to be used readily to the next step. The Fmoc protecting group of 3 was then hydrolysed by a treatment with a mixture of piperidine in methylene chloride to give compound 4. An undesirable adduct of compound 4 with dibenzofulvene was however observed, thus reducing the yield of the reaction. A nucleophilic substitution of 4-bromo-1,2-epoxybutane by the free amine function of 4 yielded the epoxide 5. *Para* and *meta* hydroxybenzamide were used to open the epoxide and generate the corresponding alcohols 6 and 7. The final compounds 8 and 10 were obtained from acidic hydrolysis of 6 and 7, respectively, but the use of solvent, methanol or acetone, greatly influenced the yield of each compound. In fact, the side product 9 (methyl ester) was isolated from the hydrolysis of 6 when methanol was used instead of acetone, thus reducing the yield of compound 8. A very good yield of compound 10 (83%) was however obtained when we used acetone instead of methanol for the hydrolysis of 7.
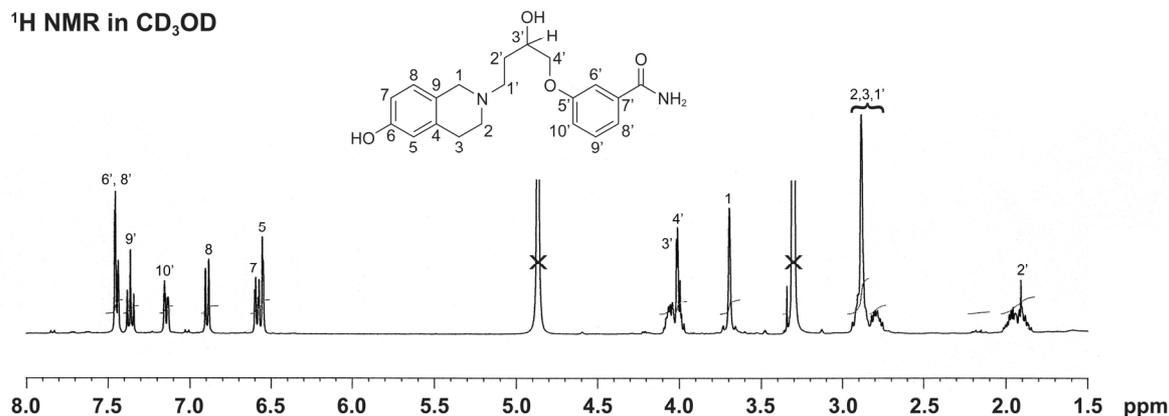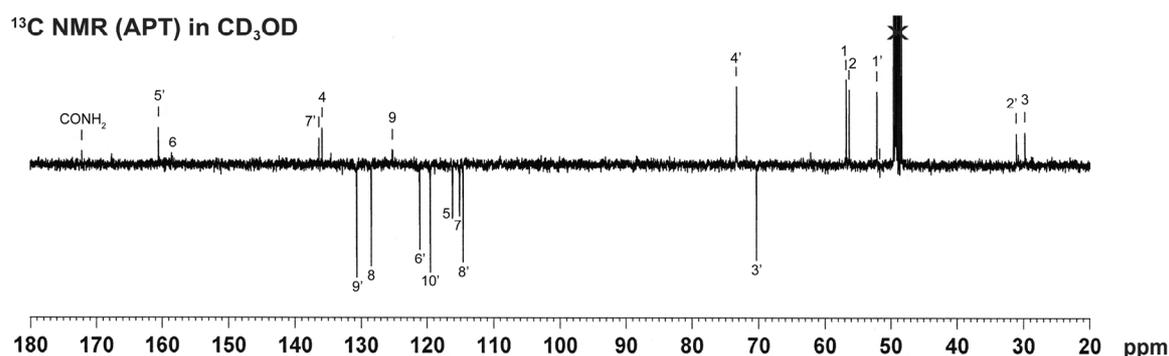
Figure 5. ^{1}H NMR and ^{13}C NMR (APT) spectra of final compound 10 dissolved in methanol-d_4. All protons and carbons were assigned to the appropriate signals

3.2 ^{13}C NMR Characterization of the Intermediate and Final Compounds

The assignment of ^{13}C NMR signals (Table 1) for all compounds was performed using a combination of 2D NMR (APT, HSQC, HMBC, COSY and NOESY) experiments (Claridge, 1999). To illustrate our strategy, we selected the final compound 10 (Figure 5). We started our analysis with the only CH non aromatic signal at 70.3 and 4.05 ppm, which were easily identified as C3' and H3', respectively, from APT and HSQC spectra. From these signals, protons and carbons 1, 2, 3, 1', 2' and 4' were distinctively assigned according to correlations observed in COSY, HSQC or/and HMBC. In fact, from cross-correlation in COSY spectrum, the H3' signal (4.05 ppm) showed two expected correlations: the first attributed to H2' (1.90 ppm) and the other to H4' (4.01 ppm), no more correlation being observed for H4' in COSY spectrum as expected. Proton H2' showed one more cross-correlation with the signal at 2.78 ppm, so we thus assigned this chemical shift value to H1'. Proton H1 (3.58 ppm) is distinguishable from H2 and H3, as it did not show cross-correlation in COSY experiment. However, a correlation appears between the two signals at 2.74 and 2.83 ppm. To differentiate H2 from H3, we next used the results of HMBC experiment. In fact, proton H3 (2.85 ppm) distinctively showed four correlations in the HMBC spectrum (as expected with C2, C4, C5 and C9), and protons H2 (2.74 ppm) did not show long range coupling with C9, only one resolved signal being observed (attributed to C4). From each H signal reported above the corresponding C was easily identified using the HSQC spectrum.

After we identified the CH$_2$ signals and the non-aromatic CH signal, two groups of aromatic CH and C signals remained to be assigned: those in the left part of the molecule (A-ring: C4-C9) and those in the right part of the molecule (C-ring: C5'-C10'). For the former ring system, aromatic protons H5, H7 and H8 are obviously assigned from their multiplicity and COSY experiment. Moreover, a strong cross-correlation was observed in NOESY spectrum between the proton H1 (3.58 ppm) and a proton at 6.83 ppm. This doublet signal (J = 8.3 Hz) was then associated to H8. Using the HSQC data, the C8 was next assigned to the peak at 128.4 ppm. By combining the COSY correlations between H8 and H7 and the HSQC data, we identified H7 (6.55 ppm) and C7 (115.1 ppm). From the NOESY spectrum, a cross-correlation was observed between the known H3 signal and the unknown CH signal at 6.51 ppm, this later was thus assigned to H5. C5 (116.1 ppm) was then identified from

H5 using the HSQC spectrum. In HMBC spectrum, only C4 (135.9 ppm) can correlate with H1, H2 and H3, only C9 (125.2 ppm) can correlate with both H1, H3 and H7 signals, and only C6 (158.5 ppm) correlated with H5, H7 and H8.

Table 1. ^{13}C NMR assignation of intermediates and final compounds

Cpds[a]	2	3	4	5	6	7	8	9	10
R1	H	MOM[b]	MOM	MOM	MOM	MOM	H	H	H
R2	----	----	----	----	p-CONH2	m-CONH2	p-CONH2	p-COOCH3	m-CONH2
Solvent	CDCl3	CDCl3	CDCl3	CDCl3	CDCl3	CDCl3	CD3OD	CDCl3	CD3OD
C1	45.3	45.1	43.6	54.7	55.7	55.6	55.9	55.5	56.8
C2	41.4	41.2	47.6	55.6	56.8	56.6	55.8	56.7	56.3
C3	28.7	28.7	29.3	29.3	29.1	29.0	28.6	28.6	29.8
C4	135.9	135.5	135.5	135.5	135.1	135.1	135.1	134.6	135.9
C5	115.1	115.8	116.5	115.8	115.8	115.7	115.7	114.9	116.1
C6	154.2	155.6	155.4	155.5	155.7	155.7	157.6	155.2	158.5
C7	114.9	114.6	114.3	114.2	114.4	114.5	115.0	113.7	115.1
C8	127.4	127.1	127.5	127.5	127.5	127.5	128.8	127.5	128.4
C9	133.1	126.3	129.3	128.2	127.4	127.2	127.3	124.5	125.2
C1'	155.5	155.2	----	50.8	50.5	50.5	51.7	50.6	52.1
C2'	67.5	67.2	----	30.5	28.5	28.5	30.3	28.1	31.1
C3'	47.3	47.2	----	50.9	71.5	71.4	69.7	71.5	70.3
C4'	144.0	143.9	----	47.1	71.8	71.9	73.2	71.6	73.3
C5'	125.0	124.8	----	----	161.9	159.0	163.3	162.5	160.5
C6'	127.0	126.9	----	----	114.3	119.5	115.3	114.2	121.0
C7'	127.7	127.5	----	----	129.3	134.8	130.7	131.6	136.3
C8'	120.0	119.8	----	----	125.7	113.4	123.8	122.7	114.6
C9'	141.3	141.2	----	----	129.3	129.6	130.7	131.6	130.6
C10'	----	----	----	----	114.3	118.6	115.3	114.2	119.5
COX	----	----	----	----	168.9	169.2	172.0	166.9	172.1
OCH2O	----	94.3	94.5	94.5	94.5	94.5	----	----	----
OCH3	----	55.8	55.9	55.9	55.9	55.9	----	51.9	----

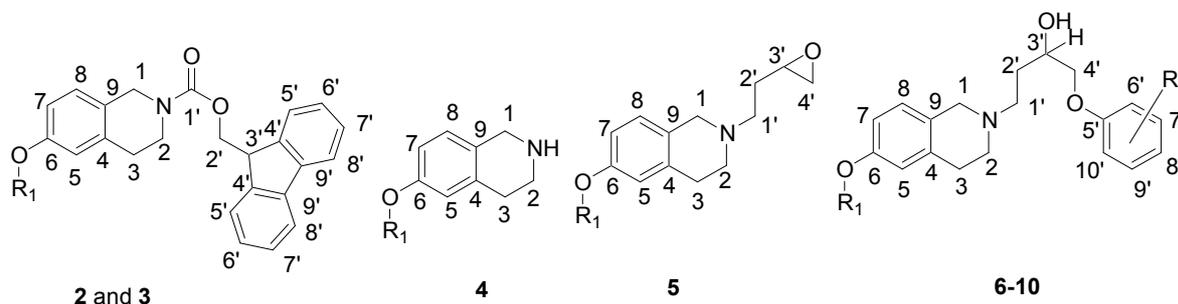a–See Figure 6 for structural formulae and carbon numbering.

b–MOM: OCH_2OCH_3.

Figure 6. Structural formulae and carbon numbering of compounds 2 to 10

For the C-ring system, the C5' (160.5 ppm) was identified by a characteristic HBMC correlation with the previously assigned H4' signal. The carboxamide carbon (172.2 ppm) was also assigned by comparison with literature data for a similar benzamide group. We next identified H6' (7.47 ppm) because it is the only proton producing correlations with both C5' and $CONH_2$. From H6' we also identified C6' (121.0 ppm) with HSQC experiment. The apparent triplet at 7.37 ppm with a large coupling constant (8.2 Hz) was necessarily the H9', which correlated in HSQC experiment with the peak at 130.6 ppm (C9'). The H9' signal showed four cross-correlations in HMBC (as expected with C5', C7', C8', and C10'). Since C5' was previously assigned, the other quaternary carbon in this group is necessary C7' (136.3 ppm). To discriminate the two remaining H8' and H10' signals we used the NOESY experiment which showed a correlation with H10' (7.15 ppm) but not with H8' (7.45 ppm). Using the HSQC spectrum, we then assigned C10' at 119.5 ppm and C8' at 114.6 ppm.

3.3 Biological Evaluation of Compounds 8-10

The inhibitory potency of compounds 8, 9 and 10 on 17β-HSD1 was assessed at 0.1, 1 and 10 μM in intact T-47D cells, which are able to transform E1 to E2 and are known to be a good source of 17β-HSD1 (Day et al., 2008a; Laplante et al., 2009). Cells were co-incubated for 24 h with each compound to be tested and radiolabeled E1 (60 nM). Inhibitory effect of compounds was compared with unlabeled estrone and CC-156, each used as an inhibitor, after separation and quantification of radiolabeled steroids (E1 and E2). Although docking experiments gave us expectation of a good affinity of the target compound 10 with the enzyme (17β-HSD1), we were disappointed by the results of our enzymatic assay because compounds 8, 9 and 10 did not inhibit the transformation of E1 to E2 by 17β-HSD1 found in T-47D cells. This might be due to poor accessibility of these compounds to the cell cytoplasm where 17β-HSD1 is located, but this has to be verified by a biological test on homogenized cells. Moreover, the alkyl side chain between the phenyl rings render the molecules more flexible, with a higher degree of freedom compared to CC-156 where the rigidity is provided by the estrane scaffold. The electronic character of the pseudo-cycle B due to the presence of a nitrogen atom is another possible cause of the weaker affinity of compounds 8-10 with the enzyme.

One important objective of our project was to obtain a compound that is devoid of estrogenic activity. The proliferation of estrogen-sensitive (ER^+) T-47D cells was then carried out to determine the estrogenic activity of compounds 8 and 10. In this assay, an estrogenic compound will stimulate the proliferation of ER^+ cells, as observed when we used E2 as a reference compound. Thus, a treatment with 0.1 nM of E2 during seven days increased the cell proliferation from 100% (control) to 153%. Interestingly, compound 8 (*para*) did not show any proliferative (estrogenic) activity at the six concentrations tested (0.001, 0.01, 0.1, 1, 5 and 10 μM). Compound 10 (*meta*) showed no estrogenic activity at 0.001, 0.01, 0.1 and 1 μM but increased the cell proliferation to 125 and 129% at 5 and 10 μM, respectively. The binding affinity of compounds 8 and 10 was next carried out using the human ERα. In this competition binding assay, an estrogenic compound will displace the tritiated E2 ($[^3H]$-E2), and the remaining bound $[^3H]$-E2 will be measured and used to determine the affinity of this compound for ERα. Thus, when untritiated E2 was used as a reference compound in a range of concentration (10^{-12}-10^{-5} M), an effective concentration of 2.3 ± 0.4 nM was needed to displace 50% of 2.5 nM of $[^3H]$-E2. Interestingly, both compounds 8 and 10 did not show any affinity to ERα in concentrations ranging from 10^{-12} to 10^{-5} M.

4. Conclusion

We have synthesized non-steroidal mimics of CC-156, a potent steroidal inhibitor of 17β-HSD1, but they did not inhibit the transformation of E1 to E2 by 17β-HSD1 found in T-47D cells. The flexibility of compounds 8 and 10,

compared to the rigid steroid backbone of CC-156, is potentially responsible for their lack of enzyme inhibitory activity. It is however interesting to mention that compounds 8 and 10 did not bind to ERα and did not induce the proliferation of estrogen-sensitive (ER[+]) T-47D cells, except compound 10 at high concentrations of 5 and 10 μM, suggesting that they are not estrogenic compounds. We also fully characterized all intermediate and final compounds, and the synthetic approach we developed is now available to generate additional analogs.

Acknowledgements

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