Eur. J. Med. Chem. 35 (2000) 217–225

S0223523400001240/FLA

Original article

N-Butyl-N-methyl-11-(3'-hydroxy-21',17'-carbolactone-19'-nor-17'α-pregna-1',3',5'(10')-trien-7'α-yl)-undecanamide: an inhibitor of type 2 17β-hydroxysteroid dehydrogenase that does not have oestrogenic or androgenic activity

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Received 30 May 1999; revised 28 July 1999; accepted 5 August 1999

Abstract – It is well known that 17β-hydroxysteroid dehydrogenases (17β-HSDs) play a key role in the formation and inactivation, from circulating precursors, of several active androgens and oestrogens. These enzymes can thus regulate tumoural cell proliferation in androgen- and oestrogen-dependent cancers. Recently, we discovered that adding a spiro-γ-lactone to the oestradiol nucleus results in a novel inhibitor of type 2 17β-HSD, an enzyme that catalyses the interconversions between 4-androstene-3,17-dione and testosterone, and between oestrone and oestradiol. This finding motivated our introducing the spiro-γ-lactone moiety onto an anti-oestrogenic nucleus. The N-butyl-N-methyl-11-(3'-hydroxy-21',17'-carbolactone-19'-nor-17'α-pregna-1',3',5'(10')-trien-7'α-yl)-undecanamide (**4**) was then efficiently synthesized and its biological activity was assessed in vitro. Despite the presence of a bulky alkylamide side chain, the spiro-γ-lactone function conserved its ability to inhibit type 2 17β-HSD (IC_{50} = 0.35 and 0.25 μM, with and without side chain, respectively). Furthermore, the selective inhibition by lactone **4** toward type 2 17β-HSD (microsomal fraction of human placenta) was demonstrated by the absence of inhibitory activity toward type 1 17β-HSD (cytosolic fraction of human placenta). Cell proliferation assays indicated that compound **4** had no oestrogenic activity but did show anti-oestrogenic activity on ER^+ cell line ZR-75-1. No androgenic activity could be detected when assayed on the AR^+ cell line Shionogi either. Based on these facts, we report the synthesis of a new steroidal derivative, one that inhibits type 2 17β-HSD while possessing anti-oestrogenic activity. © 2000 Éditions scientifiques et médicales Elsevier SAS

hydroxysteroid dehydrogenase / enzyme / inhibitor / anti-oestrogen / steroid / lactone

1. Introduction

The 17β-hydroxysteroid dehydrogenases, or 17β-HSDs, play a pivotal role in the formation and inactivation of active androgens and oestrogens from circulating steroid precursors [1–4]. During the past few years we have been developing inhibitors of human placenta cytosolic 17β-HSD (type 1) [5–7] and inhibitors of human placenta microsomal 17β-HSD (type 2) [8, 9], but the C18-steroid nucleus limits the therapeutic use of these inhibitors because of its oestrogenic activity. Our group has concentrated much effort toward the development of new weapons to fight oestrogen- and androgen-dependent diseases [10–15], and one of our challenges is to synthesize inhibitors of 17β-HSD that do not have agonistic activity [7, 16]. In this report, we focus on type 2 17β-HSD, which is the only one of five known human 17β-HSD isoforms [1, 17] that catalyses the transformation of both androgenic (C19-steroids) and oestrogenic (C18-steroids) substrates (*figure 1*) [1–3, 18, 19]. In fact, type 2 17β-HSD catalyses the interconversion of DHEA, Δ^4-dione and E_1 into the Δ^5-diol, T and E_2, respectively. In intact cells, however, it was reported that type 2 prefers the oxidative process, contrary to types 1, 3 and 5, which prefers the reductive one [20–22]. Despite the growing interest in type 2 17β-HSD [16–33], the role of this isoform remains to be clarified and the development of its specific inhibitors should prove useful.

Based on our previous observation that an oestradiol nucleus bearing a spiro-γ-lactone at position 17 (com-

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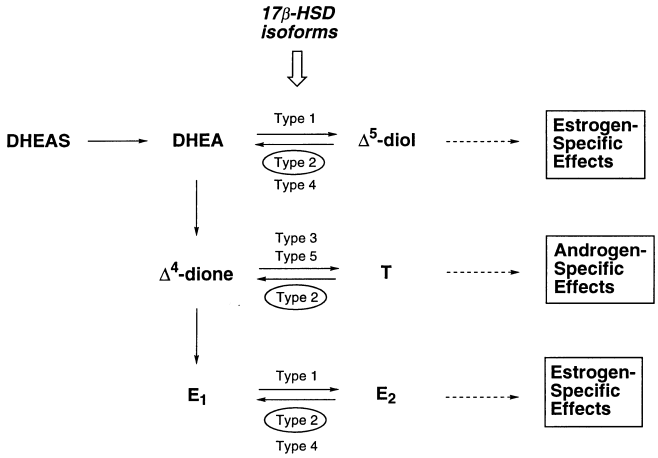

Figure 1. Enzymatic transformations performed by different isoforms (types 1–5) of human 17β-hydroxysteroid dehydrogenase (17β-HSD). DHEAS: dehydroepiandrosterone sulfate; DHEA: dehydroepiandrosterone; Δ^5-diol: 5-andosten-3,17β-diol; Δ^4-dione: 4-androstene-3,17-dione; T: testosterone; E_1: oestrone; E_2: oestradiol.

pound **1**, *figure 2*) inhibits type 2 17β-HSD [8, 9] and that this isoenzyme can accommodate certain types of bulky 7α-side chain [16], we decided to add the same spiro-γ-lactone (the inhibiting group) to an anti-oestrogenic nucleus such as **2**. Indeed, it is well known that the long methyl butyl alkanamide side chain at position 7α of oestradiol plays an essential role in the anti-oestrogenic activity of compounds **2** [34–36] and **3** [12]. Thus, the target compound **4** was suspected to inhibit type 2 17β-HSD, but without residual oestrogenic activity. The spiro-γ-lactone **4** (N-butyl-N-methyl-11-(3'-hydroxy-21',17'-carbolactone-19'-nor-17'α-pregna-1',3',5'(10')-trien-7'α-yl)-undecanamide) was then efficiently synthesized and evaluated using different in vitro assays; namely the inhibition of human placental 17β-HSD type 2 (microsomal fraction) and type 1 (cytosolic fraction), the proliferative and antiproliferative activities on oestrogen-sensitive (ER^+) ZR-75-1 cells, and the proliferative activity on androgen-sensitive (AR^+) Shionogi cells.

2. Chemistry

The six-step synthesis of spiro-γ-lactone **4** is shown in *figure 3*. The starting compound **5**, prepared according to known methodology [36], was oxidized with 2.7 M Jones reagent to give the corresponding carboxylic acid which, upon treatment with tributyl amine, isobutylchloroformate, and N-methyl, N-butyl amine, yielded 73% of amide **6** (two steps). In the process, the 17β-hydroxyl group was oxidized to a ketone. Results of ^{1}H-NMR spectroscopy demonstrated that the methyl of amide group ($CONCH_3$) appeared as two singlets at 2.90 and 2.95 ppm, while the methylene ($CONCH_2$) exhibits two triplets at 3.24 and 3.35 ppm. Moreover, the CH_3 of the N-butyl group (two triplets at 0.92 and 0.94 ppm) as well as other protons surrounding the amide (but masked by steroidal protons) were also duplicated. The duplication of these NMR signals can be explained by the two conformations of the amide bond and is typical of such alkylamide compounds [37]. Further evidence of the formation of amide **6** was observed in ^{13}C-NMR spectroscopy [38].

According to the Salman procedure [39], compound **6** was alkylated at position 17 with tetrahydro-2-(propynyloxy)-2H-pyran and *n*-BuLi to give compound **7** in 75% yield. Under these conditions the benzoate (Bz) group in position 3 was cleaved to generate a phenolic group. In addition to the attack on the ester group the lithium acetylenide derivative attacked the C17 carbonyl by the more accessible steroidal α-face. In this case, the angular CH_3-18 (on steroidal β-face) accounted for the high stereoselectivity of the alkylation that limited synthesis to that of the 17α-side chain derivative. The triple bond of compound **7** was then subjected to catalytic hydrogenation with a 1:1 mixture of 10% Pd/C and 5%

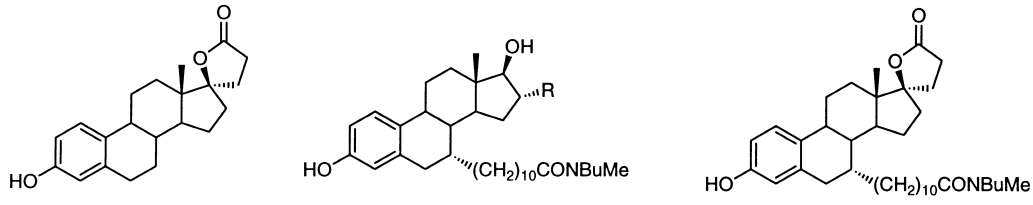

Figure 2. Chemical structures of type 2 17β-HSD inhibitors (**1** and **4**) and pure anti-oestrogens (**2** and **3**).

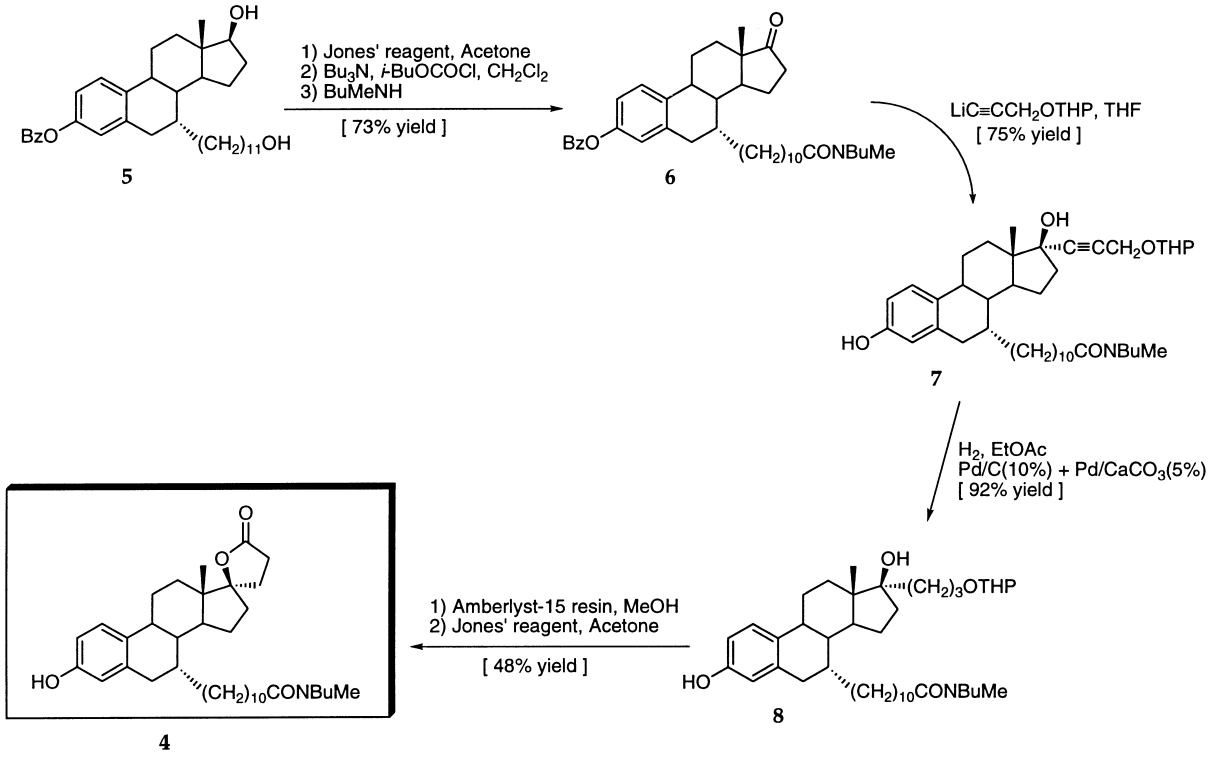

Figure 3. Chemical synthesis of lactone **4** from starting alcohol **5**.

$Pd/CaCO_3$ in ethyl acetate to produce the saturated compound **8** in 92% yield. The amide **8** was then treated with amberlyst 15 ion-exchange acid resin in methanol to cleave the THP group. The crude alcohol was oxidized with 2.7 M Jones reagent to yield the corresponding carboxylic acid, which underwent cyclization with the 17β-OH group, resulting in the more stable γ-lactone function (compound **4**). A 48% yield was obtained for this last sequence of reactions. The IR, ^{1}H-NMR, ^{13}C-NMR, HRMS, and CHN analyses were in agreement with the chemical structure of spiro-γ-lactone **4**.

3. Biological results

3.1. Inhibition of 17β-HSD

The 17β-HSD activity found in the microsomal fraction of human placenta (type 2 17β-HSD) was used to evaluate the ability of lactone **4** to inhibit the transformation of 4-androstenedione (Δ^4-dione) into testosterone (T) [9]. In our enzymatic assay, increasing concentrations of lactone **4**, lactone **1** or Δ^4-dione (non-tritiated substrate) were used to obtain the inhibition curves. As indicated in *figure 4* a total inhibition of type 2 17β-HSD (microsomal fraction) was obtained at a concentration of 10 μM with an IC_{50} value of 0.35 μM for lactone **4**. This IC_{50} value was better than the IC_{50} value of the non-tritiated substrate itself (1.27 μM for Δ^4-dione). Interestingly, the presence of a bulky alkylamide side chain slightly decreased the ability of the spiro-γ-lactone group to inhibit type 2 17β-HSD. Indeed, IC_{50} values of 0.35 and 0.25 μM were obtained, respectively, for lactones **4** and **1** (with and without an alkylamide side chain). The 17β-HSD activity in the cytosolic fraction of human placenta (type 1 17β-HSD) was also used to evaluate the ability of lactone **4** to inhibit the transformation of oestrone to oestradiol. No inhibition of type 1 17β-HSD was observed with lactone **4** at either of the two concentrations used (0.1 and 1 μM). At these same concentrations the previously reported inhibitor of type 1 17β-HSD, 16α-(bromopropyl)-estradiol [7, 40], inhibited 9% and 66% of the enzyme activity, respectively.

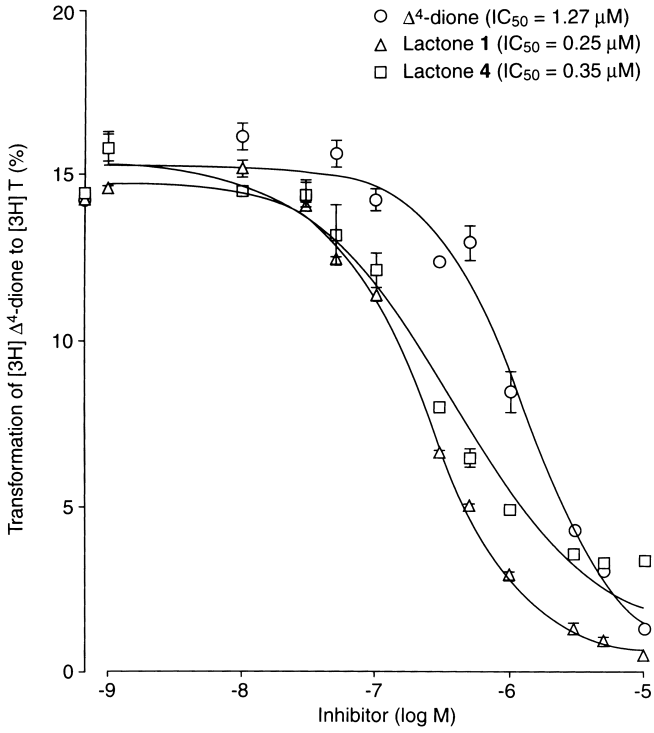

Figure 4. Effect of increasing concentrations of androst-4-en-3,17-dione (Δ^4-dione), lactone **1** and lactone **4** on enzymatic activity of type 2 17β-HSD (human placental microsomes). The enzyme preparation was incubated for 60 min at 37 °C in a pH 7.4 phosphate buffer containing 3.2 nM of [^{3}H]Δ^4-dione, NADH in excess and the indicated concentration of inhibitor. In this assay, the interfering aromatase activity was selectively blocked by an inhibitor as previously described [9]. The results are expressed as a percent of transformation of [^{3}H]Δ^4-dione to [^{3}H]testosterone. The IC_{50} values are 1.27, 0.25 and 0.35 μM, respectively, for Δ^4-dione, lactone **1**, and lactone **4**. Data points represent the mean values ± SEM from triplicate incubations.

3.2. Oestrogenic and anti-oestrogenic activities

The ability of lactone **4** to stimulate the proliferation (oestrogenic effect) and/or to inhibit the 0.1 nM E_2-induced proliferation (anti-oestrogenic effect) of oestrogen-sensitive (ER^+) human breast cancer ZR-75-1 cells was evaluated as previously reported [41]. As shown in *figure 5A*, lactone **4** was tested at two concentrations (0.03 and 1 μM) and no proliferative effect was observed at either concentration when lactone **4** was used alone. We can see that a 0.1 nM concentration of oestradiol provided a 2.4-fold stimulation of basal cell proliferation, while lactone **4** showed no proliferative effect at concentrations 300-fold and even 10 000-fold higher than the oestradiol reference concentration (0.1 nM). The inhibition of the E_2-induced stimulation of ZR-75-1 cells enabled us to assess the anti-oestrogenic activity of lactone **4**. As depicted in *figure 5B*, at a low concentration of lactone **4** (0.03 μM), no significant anti-oestrogenic effect was observed. However, at a higher concentration (1 μM) an important anti-oestrogenic effect was obtained. In fact, at the latter concentration lactone **4** reversed the oestrogenic effect induced by 0.1 nM of oestradiol by 87%. In comparison, the pure anti-oestrogen EM-139 (compound **3**, *figure 2*) [12] fully inhibited the E_2 oestrogenic effect induced at 1 μM.

3.3. Androgenic activity

An androgen-sensitive (AR^+) human cancer cell line (Shionogi) was used to evaluate the ability of lactone **4** to stimulate the proliferation of cells (androgenic activity). As can be seen in *figure 6*, no androgenic or proliferative effect was observed for lactone **4** at either concentration used (0.01 and 1 μM). Similar results were also obtained for hydroxy-flutamide (OH-FLU), the active metabolite of the widely used pure anti-androgen flutamide [42]. Conversely, a 3-fold proliferative effect was obtained when Shionogi cells were stimulated by 0.1 nM of a known androgen, dihydrotestosterone (DHT).

4. Discussion

The successful and efficient synthesis of an oestradiol derivative with a 17-spiro-γ-lactone and a 7α-alkylamide side chain (lactone **4**) was reported in *figure 3*. Starting from alcohol **5**, a six step sequence (Jones oxidation, amidation, alkylation at 17α-position, catalytic hydrogenation of the triple bond, cleavage of THP group and oxidation) provided a good overall yield (24%) of lactone **4**. All synthetic intermediates, in addition to the final lactone **4**, were fully characterized by conventional methods (IR, ^{1}H-NMR, ^{13}C-NMR, and HRMS). This novel spiro-γ-lactone derivative was found to selectively inhibit type 2 17β-HSD (no inhibition was observed toward type 1 17β-HSD) due mostly to the presence of the spiro-γ-lactone group. Furthermore, the bulky alkylamide side chain at position 7α of the steroidal nucleus blocked the oestrogenic activity associated with lactone **1**, thus conferring anti-oestrogenic activity. Inhibition of type 2 17β-HSD by lactone **4** was demonstrated by its IC_{50} value of 0.35 μM. In the absence of the 7α-alkylamide chain, the corresponding lactone **1** had an IC_{50} value of 0.25 μM. Both lactones (**1** and **4**) fit the enzymatic pocket better than did the substrate itself, as indicated by the IC_{50} value of 1.27 μM obtained when non-tritiated Δ^4-dione was used to compete with tritiated Δ^4-dione. We have already

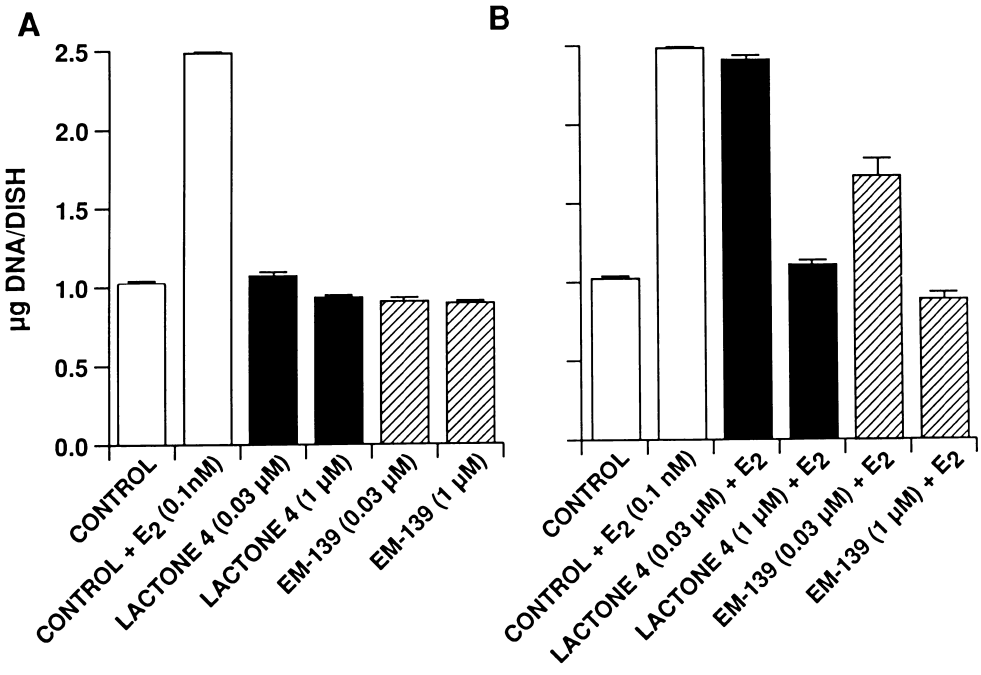

Figure 5. Effects of lactone **4** and pure anti-oestrogen EM-139 [12] on the proliferation (oestrogenic activity) and the inhibition of oestradiol (E_2)-induced proliferation (anti-oestrogenic activity) of oestrogen-sensitive human breast cancer ZR-75-1 cells. Three days after plating, cells were incubated for 9 days with the incubated concentrations of test compound in the absence (**A**) or presence (**B**) of E_2 (0.1 nM). Media were changed every second day. Results were expressed as the mean of DNA content (µg) ± SEM, in triplicate analysis.

reported that lactone **1** acts as a reversible inhibitor [9] and we expect that lactone **4** with its spiro-γ-lactone will share this same mechanism. In addition to its capacity to inhibit the formation of testosterone from Δ^4-dione, lactone **4** did not exhibit androgenic activity.

The data presented here demonstrated that lactone **4** is an inhibitor of type 2 17β-HSD that exerts neither oestrogenic nor androgenic activities and can be considered as an anti-oestrogen. The further development of an inhibitor of human placenta microsomal 17β-HSD (type 2), that is at the same time an anti-oestrogen, will certainly advance the elucidation of the physiological role and action of this 17β-HSD isoform.

5. Experimental

5.1. Chemical synthesis

General methods: unless otherwise noted, chemical solvents and reagents were obtained from commercial suppliers and used without further purification. Thin layer chromatography (TLC) was performed on 0.20 mm Silica Gel 60 F_{254} plates (E. Merck, Darmstadt, Germany) while a 230–400 mesh ASTM Silica Gel 60 (E. Merck) was used for flash column chromatography. Infrared spectra (IR) obtained using Perkin Elmer 1600 (FTIR Series) spectrophotometer were reported in cm^{-1}. Nuclear magnetic resonance spectra (NMR) were obtained at 300 MHz for ^1H and 75 MHz for ^{13}C with a Bruker AC/F300 spectrometer. The chemical shifts (δ in ppm) were referenced to $CDCl_3$ (7.26 ppm for ^1H or 77.00 ppm for ^{13}C). For ^{13}C-NMR spectra, only the important carbons were assigned (between []) while duplication of signals attributed to the amide group [37] were reported between parentheses. High resolution fast atom bombardment mass spectra (HR-FABMS) were provided by the centre régional de spectrométrie de masse (Université de Montréal, Montréal, Canada). The C, H, N analysis of compound **4** was performed by Galbraith Laboratories (Knoxville, TN).

5.1.1. Synthesis of N-butyl-N-methyl-11-(3'-benzyloxy-17'-oxo-1',3',5'(10')-oestratrien-7'α-yl)-undecanamide **6**

To a solution of 10.57 g (19.3 mmol) of alcohol **5** [36] in 30 mL of acetone was added, dropwise, 16.9 mL of a

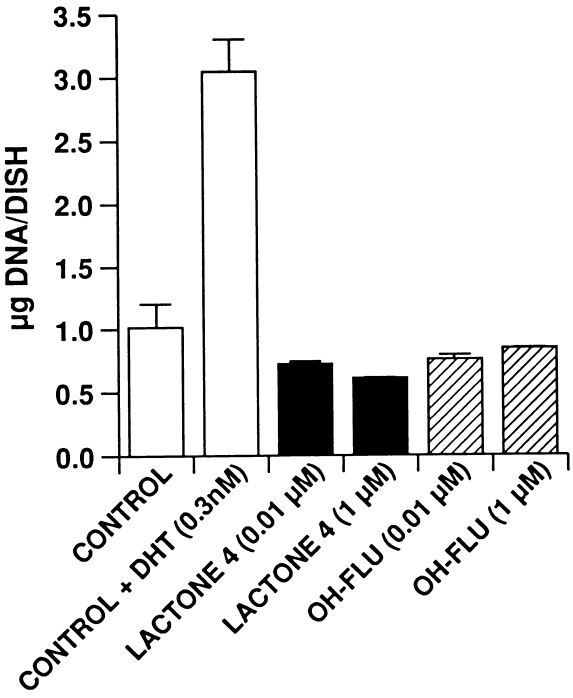

Figure 6. Effect of lactone **4** and pure anti-androgen hydroxyflutamide (OH-FLU) on the proliferation (androgenic activity) of androgen-sensitive human breast cancer Shionogi cells. One day after plating cells were incubated for 10 days with the indicated concentration of test compound. Media were changed every 3–4 days. Results were expressed as the mean of DNA content (µg) ± SEM, in triplicate analysis.

2.7 M Jones reagent at 0 °C and the orange mixture was stirred for 2 h. Then, 1 mL of isopropanol was added and the organic solvent was evaporated under reduced pressure to yield a green solid which was then dissolved in water (50 mL) and EtOAc (30 mL). The aqueous phase was extracted with EtOAc (4 × 100 mL). The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and evaporated under reduced pressure to yield a yellow viscous oil which was used, unpurified, for the next step.

The crude carboxylic acid obtained above was dissolved in 100 mL of dry CH_2Cl_2 and 5.84 mL of tributylamine. After the mixture was cooled to –10 °C, isobutyl chloroformate (3.54 mL) was added dropwise and allowed to react for 80 min. At this time excess N-methylbutylamine (26.8 mL) was added and the cooling bath removed. After 2.5 h, CH_2Cl_2 was added and the organic phase washed with 10% aqueous HCl (v/v), brine and dried over $MgSO_4$. The solvent was removed and the crude amide was purified by flash chromatography (hexane/EtOAc, 85:15) to give 8.83 g (73% yield) of amide **6**. Colourless oil; IR ν (film): 1 738 (C=O, ketone and ester), 1 643 (C=O, amide); ^{1}H-NMR δ ($CDCl_3$): 0.92 (s, CH_3-18'), 0.92 and 0.94 (2t, J = 7.4 Hz, CH_3 of butyl), 2.89 (ABX system, $J_{6,7}$ = 5.0 Hz and $J_{6,6}$ = 16.9 Hz, Δ = 20.5 Hz, CH_2-6'), 2.90 and 2.95 (2s, $CONCH_3$), 3.24 and 3.35 (2t, J = 7.4 Hz, $CONCH_2$), 6.94 (d, J = 2.2 Hz, CH-4'), 6.99 (dd, J_1 = 2.3 Hz and J_2 = 8.5 Hz, CH-2'), 7.33 (d, J = 8.5 Hz, CH-1'), 7.50 (m, 2H, *meta* protons of OBz), 7.63 (m, 1H, *para* proton of OBz), 8.19 (d, J = 8.9 Hz, 2H, *ortho* protons of OBz); ^{13}C-NMR δ ($CDCl_3$): 13.74 [CH_3 of butyl], 14.10 [C-18'], 19.88 (20.00) [$\underline{C}H_2CH_3$ of butyl], 20.92, 25.03 (25.41) [$\underline{C}H_2CH_2CON$], 25.72, 26.68, 28.11, 29.39–29.88 (6×), 29.39 (30.57) [$CONCH_2\underline{C}H_2$], 31.71, 32.61, 32.90 (33.56) [$\underline{C}H_2CON$], 33.21 (35.16) [$CON\underline{C}H_3$], 34.37 [C-6'], 35.69, 38.27, 41.28, 46.98, 47.91, 47.30 (49.65) [$CON\underline{C}H_2$], 118.84 [C-2'], 122.48 [C-4'], 126.95 [C-1'], 128.45 (2×) [OBz], 129.63 [C-10'], 130.02 (2×) [OBz], 133.40 [OBz], 136.65 [OBz], 136.89 [C-5'], 148.83 [C-3'], 165.27 [CO of OBz], 172.73 (172.85) [CON], 220.47 [C-17']; HR-FABMS: calculated for $C_{41}H_{58}O_4N$ (M^+ + H) 628.4366, found 628.4378.

5.1.2. Synthesis of N-butyl-N-methyl-11-{3',17'β-dihydroxy-17'α-[3'-(tetrahydro-2'-H-pyran-2'-yloxy)-propynyl]-1',3',5'(10')-oestratrien-7'α-yl}-undecanamide **7**

To a solution of 2.15 mL (15.3 mmol) of tetrahydro-2-(2-propynyloxy)-2H-pyran (Aldrich, Milwaukee, USA) in 50 mL of dry THF, was added at 0 °C, 9.28 mL of *n*-BuLi 1.6 M in hexane and the mixture was stirred for 40 min. The reaction mixture was then cooled to –78 °C and a solution of ketone **6** (1.75 g, 2.8 mmol) in 30 mL of dry THF was added dropwise. After 3 h of stirring the mixture was poured into a saturated solution of $NaHCO_3$ and the aqueous phase was extracted with EtOAc (3 × 100 mL). The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered and evaporated under reduced pressure. The brownish oil was purified by flash chromatography (hexane/EtOAc, 57:43) to yield 1.59 g (75% yield) of compound **7**. Yellow oil; IR ν (film): 3 374 (OH, alcohol and phenol), 2 242 (C≡C), 1 626 (C=O, amide); ^{1}H-NMR δ ($CDCl_3$): 0.87 (s, CH_3-18'), 0.91 and 0.94 (2t, J = 7.3 Hz, CH_3 of butyl), 2.76 (ABX system, $J_{6,7}$ = 4.8 Hz and $J_{6,6}$ =t16.8 Hz, Δ = 20.2 Hz, CH_2-6'), 2.92 and 2.97 (2s, $CONCH_3$), 3.25 and 3.36 (2t, J = 7.5 Hz, $CONCH_2$), 3.53 and 3.86 (2m, OCH_2 of THP), 4.36 (s, $C\underline{H}_2OTHP$), 4.87 (t, J = 3.1 Hz, CH of THP), 6.59 (d, J = 2.5 Hz, CH-4'), 6.66 (dd, J_1 = 8.4 Hz and J_2 = 2.5 Hz, CH-2'), 7.10 (d, J = 8.5 Hz, CH-1'); ^{13}C-NMR δ ($CDCl_3$): 12.75 [C-18'], 13.78 [CH_3

of butyl], 19.12 [THP], 19.88 (20.00) [$\underline{C}H_2CH_3$ of butyl], 22.31, 25.29 [THP], 25.09 (25.47) [$\underline{C}H_2CH_2CON$], 27.37, 27.76, 29.17–29.49 (6×), 29.49 (30.55) [$CONCH_2\underline{C}H_2$], 30.29 [THP], 32.96 (33.64) [$\underline{C}H_2CON$], 33.07, 33.24, 33.53 (35.42) [$CON\underline{C}H_3$], 34.63 [C-6'], 37.88, 38.76, 42.66, 46.01, 47.44, 47.58 (49.87) [$CON\underline{C}H_2$], 54.39 [C≡$C\underline{C}H_2$OTHP], 62.11 [CH_2O of THP], 79.92 and 81.59 [C≡C], 89.66 [C-17'], 96.59 (96.64) [CH of THP], 113.03 [C-2'], 116.21 [C-4'], 126.78 [C-1'], 130.73 [C-10'], 136.66 [C-5'], 154.36 [C-3'], 173.40 (173.51) [CON]; HR-FABMS: calculated for $C_{42}H_{64}O_5NNa$ (M^+ + Na) 685.4682, found 685.4662.

5.1.3. Synthesis of N-butyl-N-methyl-11-{3',17'β-dihydroxy-17'α-[3'-(tetrahydro-2'-H-pyran-2'-yloxy)-propynyl]-1',3',5'(10')-oestratrien-7'α-yl}-undecanamide **8**

A solution of alkyne **7** (751 mg, 1.13 mmol) in 100 mL of EtOAc containing 113 mg of a 1:1 mixture of Pd/C (10%) and $Pd/CaCO_3$ (5%) was stirred at room temperature under H_2 atmosphere for 64 h. The reaction mixture was then filtered through celite, washed with EtOAc and the solvent was evaporated to dryness. Purification by flash chromatography (hexane/EtOAc, 5:5) afforded 690 mg (92% yield) of saturated compound **8**. Yellow foam; IR ν (film): 3 320 (OH, alcohol and phenol); 1 625 (C=O, amide); ^{1}H-NMR δ ($CDCl_3$): 0.91 (s, CH_3-18'), 0.91 and 0.94 (2t, J = 7.3 Hz, CH_3 of butyl), 2.77 (ABX system, $J_{6,7}$ = 4.8 Hz, $J_{6,6}$ = 16.8 H_z, Δ = Hz, CH_2-6'), 2.92 and 2.97 (2s, $CONCH_3$), 3.25 and 3.36 (2t, J = 7.5 Hz, $CONCH_2$), 3.50 and 3.85 (2m, 4H, OCH_2 of 17α-side chain and OCH_2 of THP), 4.62 (br, CH of THP, 6.59 (d, J = 2.5 Hz, CH-4'), 6.65 (dd, J_1 = 2.5 Hz and J_2 = 8.4 Hz, CH-2'), 7.11 (d, J = 8.4 Hz, CH-1'); ^{13}C-NMR δ ($CDCl_3$): 13.71 [CH_3 of butyl], 14.28 [C-18'], 19.46 [THP], 19.79 (19.89) [$\underline{C}H_2CH_3$ of butyl], 22.79, 23.90, 25.29 [THP], 25.01 (25.29) [$\underline{C}H_2CH_2CON$], 27.20, 27.91, 29.27–29.63 (6×), 29.27 (30.52) [$CONCH_2\underline{C}H_2$], 30.47 [THP], 31.72, 32.85 (33.40) [$\underline{C}H_2CON$], 33.15, 33.18, 33.53 (35.31) [$CON\underline{C}H_3$], 34.33, 34.60 [C-6'], 37.83, 42.71, 45.61, 46.75, 47.47 (49.76) [$CON\underline{C}H_2$], 62.25 [CH_2O of THP], 68.21 [$\underline{C}H_2$OTHP], 83.09 [C-17'], 98.69 (98.78) [CH of THP], 112.91 [C-2'], 116.14 [C-4'], 126.56 [C-1'], 130.57 [C-10'], 136.55 [C-5'], 154.47 [C-3'], 173.26 (173.37) [CON]; HR-FABMS: calculated for $C_{42}H_{69}O_5N$ (M^+) 667.5176, found 667.5211.

5.1.4. Synthesis of N-butyl-N-methyl-11-(3'-hydroxy-21',17'-carbolactone-19'-nor-17'α-pregna-1',3',5'(10')-trien-7'α-yl)-undecanamide **4**

To a solution of compound **8** (290 mg, 0.43 mmol) in 25 mL of methanol, was added 2.5 g of amberlyst 15 ion-exchange resin (Aldrich, Milwaukee, USA) and the mixture was stirred at room temperature for 12 h. The solution was then filtered and evaporated to dryness. The crude alcohol, not having the THP group, was treated with Jones reagent 2.7 M (0.26 mL) in acetone at 0 °C for 25 min. Then, isopropanol (2 mL) was added and the green solution stirred 5 min before the acetone evaporated. The residue was dissolved in water (20 mL) and EtOAc (30 mL). The aqueous phase was extracted with EtOAc (3 × 50 mL). The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered and evaporated under reduced pressure. Purification by flash chromatography (hexane/EtOAc/MeOH, 70:29:1) of the residue yielded 122 mg (48% yield) of lactone **4**. Colourless oil; IR ν (film): 3 272 (OH, phenol), 1 770 (C=O, γ-lactone), 1 620 (C=O, amide); ^{1}H-NMR δ ($CDCl_3$): 0.91 and 0.94 (2t, J = 7.4 Hz, CH_3 of butyl), 0.96 (s, CH_3-18'), 2.77 (ABX system, $J_{6,7}$ = 4.7 Hz and $J_{6,6}$ = 16.6 Hz, Δ = 20.4 Hz, CH_2-6'), 2.93 and 2.97 (2s, $CONCH_3$), 3.26 and 3.37 (2m, $CONCH_2$), 6.60 (d, J = 2.4 Hz, CH-4'), 6.67 (dd, J_1 = 2.5 Hz and J_2 = 8.4 Hz, CH-2'), 7.09 (d, J = 8.5 Hz, CH-1'); ^{13}C-NMR δ ($CDCl_3$): 13.79 [CH_3 of butyl], 14.46 (CH_3-18'), 19.88 (19.99) [$\underline{C}H_2CH_3$ of butyl], 22.12, 25.24, 25.05 (25.40) [$\underline{C}H_2CH_2CON$], 26.98, 27.67, 29.16–29.37 (7×), 29.61 (30.54) [$CONCH_2\underline{C}H_2$], 31.41, 32.05, 32.94 (33.62) [$\underline{C}H_2CON$], 33.08, 33.54 (35.42) [$CON\underline{C}H_3$], 34.59 [C-6'], 35.58, 37.85, 42.25, 45.14, 46.15, 47.59 (49.87) [$CON\underline{C}H_2$], 96.23 [C-17'], 113.15 [C-2'], 116.24 [C-4'], 126.75 [C-1'], 130.11 [C-10'], 136.51 [C-5'], 154.57 [C-3'], 173.41 [CON], 177.04 (CO of γ-lactone); HR-FABMS: calculated for $C_{37}H_{58}O_4N$ (M^+ + H) 580.4366, found 580.4375; Anal. ($C_{37}H_{57}O_4N$): calc. C, 76.64; H, 9.91; N, 2.42, found C, 76.23; H, 9.70; N, 2.40.

5.2. Biological evaluation

5.2.1. Inhibition of human placenta microsomal 17β-hydroxysteroid dehydrogenase (type 2 17β-HSD)

The partially purified 17β-HSD activity obtained from the microsomal fraction of human placenta (type 2 17β-HSD) was used to assess the ability of newly synthesized lactone **4** to inhibit the transformation of [^{3}H]4-androstene-3,17-dione ([^{3}H]Δ^4-dione) to [^{3}H]testosterone. Briefly, this enzymatic assay was performed at pH 7.4 with a low concentration of tritiated Δ^4-dione (3.2 nM) to approximate physiological conditions. NADH was used as cofactor. The time of incubation and temperature were designated as 60 min and 37 °C. Several concentrations of the tested compound (1 nM to 10 μM) were used to obtain the inhibition curve which enabled us to determine the IC_{50} value, or the concentra-

tion of inhibitor that inhibits 50% of target enzyme activity. The procedure of the enzymatic assay has previously been reported [9] and was used without modification.

5.2.2. Inhibition of human placenta cytosolic 17β-hydroxysteroid dehydrogenase (type 1 17β-HSD)

The activity of partially purified 17β-HSD obtained from the cytosolic fraction of human placenta (type 1 17β-HSD) was used to assess the ability of lactone **4** to inhibit the transformation of [^{3}H]oestrone to [^{3}H]oestradiol. Briefly, a low concentration of tritiated oestrone (5 nM) and a pH of 7.4 was used to approximate physiological conditions. The cofactor was NADH and incubation time and temperature were 30 min and 37 °C, respectively. Two concentrations of tested compound (0.1 and 1 μM) were used. A previously reported procedure [40] for the enzymatic assay was implemented without modification.

5.2.3. Oestrogenic and anti-oestrogenic activities

A proliferative assay on oestrogen-sensitive human breast cancer cells (ZR-75-1) was used to assess in vitro oestrogenic and anti-oestrogenic activity. The ability of lactone **4** to stimulate proliferation of ZR-75-1 cells demonstrated oestrogenic activity, while the ability of lactone **4** to inhibit 0.1 nM oestradiol-induced proliferation of ZR-75-1 cells demonstrated anti-oestrogenic activity. The tested compounds were evaluated at two concentrations (0.03 and 1 μM). The procedure of this assay has been previously reported [41] and was used without modification.

5.2.4. Androgenic activity

The androgenic activity was evaluated by testing the ability of lactone **4** to stimulate the proliferation of androgen-sensitive human breast cancer Shionogi cells. Two concentrations of tested compound (0.01 and 1 μM) were used in the assay.

Assay procedure: one clone of SC115 Shionogi cells was selected for its elevated sensitivity to DHT. Cells were harvested at the exponential growth phase with 0.1% pancreatine in HEPES buffer containing 3 mM EDTA, then resuspended in MEM supplemented with non-essential amino acids (1%), penicillin (10 IU/mL), streptomycin sulfate (50 μg/mL) and 2% dextran-coated charcoal-treated foetal calf serum. Cells were plated in Falcon 24-well tissue culture plates (2 cm^2/well) at a density of 20 000 cells/dish. Twenty-four hours after plating, the medium was changed and dihydrotestosterone (DHT) (0.3 nM), lactone **4** (0.01 or 1 μM) or hydroxy-flutamide (0.01 or 1 μM) were added. Stock solutions of DHT and tested compounds were prepared in ethanol and their concentration adjusted to give a final concentration of 0.01% ethanol in the culture medium. Cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air for 10 days with medium changes every 3–4 days. At the end of the incubation period cell growth was assessed by quantifying DNA content using a modified Fiszer-Szafarz method [43], as previously described [44]. The medium was then carefully removed and 150 μL of methanol was added. Plates were then left to dry at room temperature and were either frozen until assayed or processed immediately. Salmon testis DNA was used as a standard (0.5–20 μg/tube). Charcoal-treated 3,5-diaminobenzoic acid (DABA) reagent (200 mg/mL) was added (150 μL) to standards and dishes containing dried, fixed cells. The reaction was carried out for 60 min at 60 °C before cooling on ice and diluting with 1.5 mL of HCl 1.0 N. Fluorescence was measured with an LS2B Perkin-Elmer fluorimeter.

Acknowledgements

We are grateful for the financial support provided through grants from The Medical Research Council of Canada (MRC) and Le Fonds de la Recherche en Santé du Québec (FRSQ). We would like to extend this gratitude to our collaborators: Jacques Simard, who provided the cell proliferation assays, Diane Michaud, who performed the cell culture assays, and Serge Auger, who performed the enzymatic assays.

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