Available online at www.sciencedirect.com
ScienceDirect
European Journal of Medicinal Chemistry 44 (2009) 632–644
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Original article

Steroidal lactones as inhibitors of 17β-hydroxysteroid dehydrogenase type 5: Chemical synthesis, enzyme inhibitory activity, and assessment of estrogenic and androgenic activities

Patrick Bydal, Van Luu-The, Fernand Labrie, Donald Poirier*

Medicinal Chemistry Division, Oncology and Molecular Endocrinology Research Center, CHUL Research Center and University Laval, 2705 Laurier Boulevard, Québec, Québec G1V 4G2, Canada

Received 27 August 2007; received in revised form 19 March 2008; accepted 20 March 2008
Available online 8 April 2008

Abstract

Androgens are well known to play a predominant role in prostate cancer and other androgen-dependent diseases. To decrease the level of androgen testosterone in the prostate, we are interested in developing inhibitors of 17β-hydroxysteroid dehydrogenase type 5 (17β-HSD5). This enzyme expressed in the prostate is one of the two enzymes able to convert 4-androstene-3,17-dione into testosterone. From a screening study, it was found that a series of steroid derivatives bearing a lactone on D-ring demonstrated potent inhibition of 17β-HSD5 over-expressed in HEK-293 cells. The results of enzymatic assays using intact cells indicated that a C18-steroid (estradiol or 3-deoxyestradiol) backbone and a spiro-δ-lactone (six-member ring) are important for a strong inhibitory activity. Moreover, the presence of a dimethyl group at the alpha-position of the lactone carbonyl increases the selectivity of the inhibitor toward 17β-HSD5. Compound **26**, a 3-deoxyestradiol derivative with a dimethylated spiro-δ-lactone at position 17, possesses the most potent inhibitory activity for 17β-HSD5 ($IC_{50} = 2.9$ nM). It showed no binding affinity for estrogen, androgen, progestin and glucocorticoid receptors (ER, AR, PR and GR). A weak proliferative effect was, however, observed on ZR-75-1 (ER^+) cells in culture at high concentration (1 μM), but not at 0.03 μM. Interestingly, no significant proliferative effect was detected on Shionogi (AR^+) cells in culture in the presence of 0.1 and 1 μM of lactone **26**.

Keywords: Hydroxysteroid dehydrogenase; Enzyme; Inhibitor; Lactone; Steroid; Androgen; Testosterone; Cancer

1. Introduction

Prostate cancer is the second most frequent cancer for men in North America [1]. Since the prostate is an androgen-dependent organ, most of prostate cancer cells are highly sensitive to androgen deprivation. In fact, among all hormone-sensitive cancers, prostate cancer has the best response to endocrine therapy. Accordingly, for more than 50 years, the exclusive treatment of advanced metastatic disease has been androgen deprivation [2]. Although it is well accepted that the majority of circulating androgens were of testicular origin [3], the virilisation of patients having testicular 17β-HSD (type 3) deficiency in adulthood and the finding of a 17β-HSD5 [4–6] that possesses the ability to convert 4-androstene-3,17-dione (Δ^4-dione) into testosterone (T) strongly suggest the presence of an additional androgen biosynthetic pathway in peripheral tissues [7]. Indeed, in the case of prostate tissue, an important prohormone, namely dehydroepiandrosterone (DHEA), was found to be responsible for nearly 40% of the active androgen concentration in the prostate. Although the presence of DHEA in the circulation of men was reported many years ago [8], it is only recently that its important role in peripheral steroidogenesis has been elucidated [9]. In fact, DHEA-S, the sulfated form of DHEA, is the most abundant steroid in the circulation in men [10]. It is also clearly demonstrated that the prostatic tissue efficiently transforms the inactive steroid precursors DHEA-S and DHEA (Fig. 1), into the active androgens T

* Corresponding author. Tel.: +1 418 654 2296; fax: +1 418 654 2761.
E-mail address: donald.poirier@crchul.ulaval.ca (D. Poirier).

0223-5234/$ - see front matter © 2008 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2008.03.020

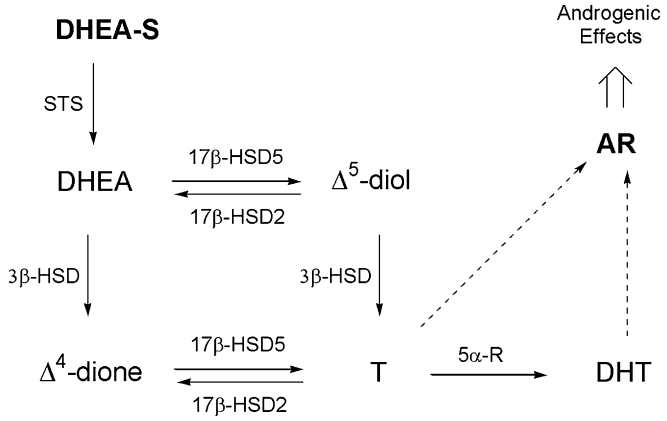


Fig. 1. Illustration of the last biosynthetic steps involved in the formation of the active androgens T and DHT from the inactive precursor DHEA-S in human prostate cells. The new steroid lactones act as inhibitors of 17β-HSD5 and thus prevent agonistic activity of T and DHT on the androgen receptor (AR). DHEA-S: dehydroepiandrosterone-sulfate; DHEA: dehydroepiandrosterone; Δ^5-diol: 5-androstene-3β,17β-diol; Δ^4-dione: 4-androstene-3,17-dione; T: testosterone; DHT: dihydrotestosterone; STS: steroid sulfatase; HSD: hydroxysteroid dehydrogenase; 5α-R: 5α-reductases.

and dihydrotestosterone (DHT) [11]. Therefore, the inhibition of the formation of active steroids in the prostate from inactive precursors is a potential applicable therapy for prostate cancer. Because T is also an active androgen, but less than DHT, the use of 5α-reductase inhibitors is not sufficient for a complete blockade of androgenic effect. The recent discovery of 17β-HSD5 [4–6], which is located in the peripheral tissues such as the prostate [12], gives us a new interesting therapeutic target. The blockade of 17β-HSD5 could thus prevent the formation of androgen T while accumulating Δ^4-dione (Fig. 1), a steroid inactive on the androgen receptor [13].

Few inhibitors of 17β-HSD5 have been reported until now [14]. In addition to the lactones discussed in this manuscript and previously patented [15], we can mention a series of dietary phytoestrogens studied by Krazeisen et al. [16], a series of commercially available cinnamic acids and related compounds synthesized by Brozic et al. [17] and three fluorinated estratriene derivatives identified by Deluca et al. [18] as moderate inhibitors. Some oxirane derivatives of natural enzyme substrates (T, progesterone, 5α-androstane-3,17-dione and androsterone) were also proposed as potential inhibitors by Penning et al. [6]. More recently, the same group also used *N*-phenylanthranilic acid as a template for designing inhibitors of 17β-HSD5 [19]. Herein we present a series of steroid lactones (Fig. 2) and their ability to inhibit the *in vitro* transformation of Δ^4-dione into T by 17β-HSD5 over-expressed in HEK-293 cells. Since it has been reported that similar compounds show inhibitory activity against 17β-HSD2 [14], we decided to verify the specificity of lactones **11**, **13**, **14**, **24–26**, selected from lactones **1–26**, for the inhibition of 17β-HSD5. In addition to their binding affinity for steroid receptors, the proliferative and antiproliferative effects on ZR-75-1 (ER^+) and Shionogi (AR^+) cells have also been determined.

2. Results

2.1. Chemical synthesis of lactones 23–26

The synthesis of lactone **23** reported in Scheme 1 started from 16α-allyl-17α-estradiol (**28**), an intermediate obtained from estrone (**27**). We previously reported the sequence of reactions needed to generate **28** as well as its full characterization of the stereochemistry of C16 and C17-substituents [20]. The key step in this transformation was the inversion of 17β-OH into 17α-OH, which was achieved by a Mitsunobu reaction using triphenylphosphine, diethylazodicarboxylate, and *p*-nitrobenzoic acid in toluene, followed by hydrolysis of the resulting ester with K_2CO_3 in methanol. Diol **28** was protected as the di-TBDMS derivative **29** and its allyl group was converted into a primary alcohol by an oxidative hydroboration using BH_3 in THF followed by a treatment with H_2O_2 and a solution of aqueous 3 N NaOH. Alcohol **30** was oxidized to carboxylic acid with Jones reagent and then treated with $BF_3 \cdot Et_2O$ in CH_2Cl_2 overnight. Under these conditions, the silylated ethers were cleaved with lactonization to yield the target lactone **23**.

The synthesis of lactones **24–26** with a 3-deoxygenated estradiol nucleus was described in Scheme 2. From commercially available estrone (**27**), the hydroxyl group in position 3 was esterified with trifluoromethanesulfonic anhydride and the corresponding estrone-triflate was reduced in the presence of triethylamine, formic acid, triphenylphosphine and palladium diacetate to give **31** [21]. C3-deoxygenated estrone (**31**) was then alkylated with the lithium acetylenide resulting from the reaction of tetrahydro-(butynyl)-2-H-pyran and *n*-BuLi to give **32** exclusively as a 17α-alkyne isomer. The triple bond was hydrogenated in EtOAc with palladium on activated charcoal. Without further purification, the tetrahydropyranyl ether of the side chain was hydrolyzed by a treatment with *p*-TSA in MeOH. Oxidation of this primary alcohol with Jones reagent in acetone gives the carboxylic acid, which, as expected, underwent an intramolecular cyclization with the tertiary 17β-hydroxy to give the corresponding spiro-δ-lactone **24**. Using LDA as a base and methyl iodide in excess, **24** was mono and dimethylated in alpha-position of the carbonyl providing **25** and **26**. In the case of the monomethylated lactone **25**, we obtained a mixture of *R* and *S* isomers, but only the major isomer was characterized and kept for the biological assays.

2.2. Inhibitory activity of lactones 1–26 on human 17β-HSD5

17β-HSD5 catalyzes the conversion of Δ^4-dione into T and its inhibition could be a strategy to lower the level of androgen T, and indirectly to lower the level of potent androgen DHT, in human prostate cells (Fig. 1). Since a C19 steroid such as Δ^4-dione is a good substrate for 17β-HSD5, a series of C19-steroids bearing a spiro-δ-lactone were first tested for their ability to inhibit the reductive activity of 17β-HSD5 transfected in HEK-293 cells (Table 1). We focused on steroidal lactones

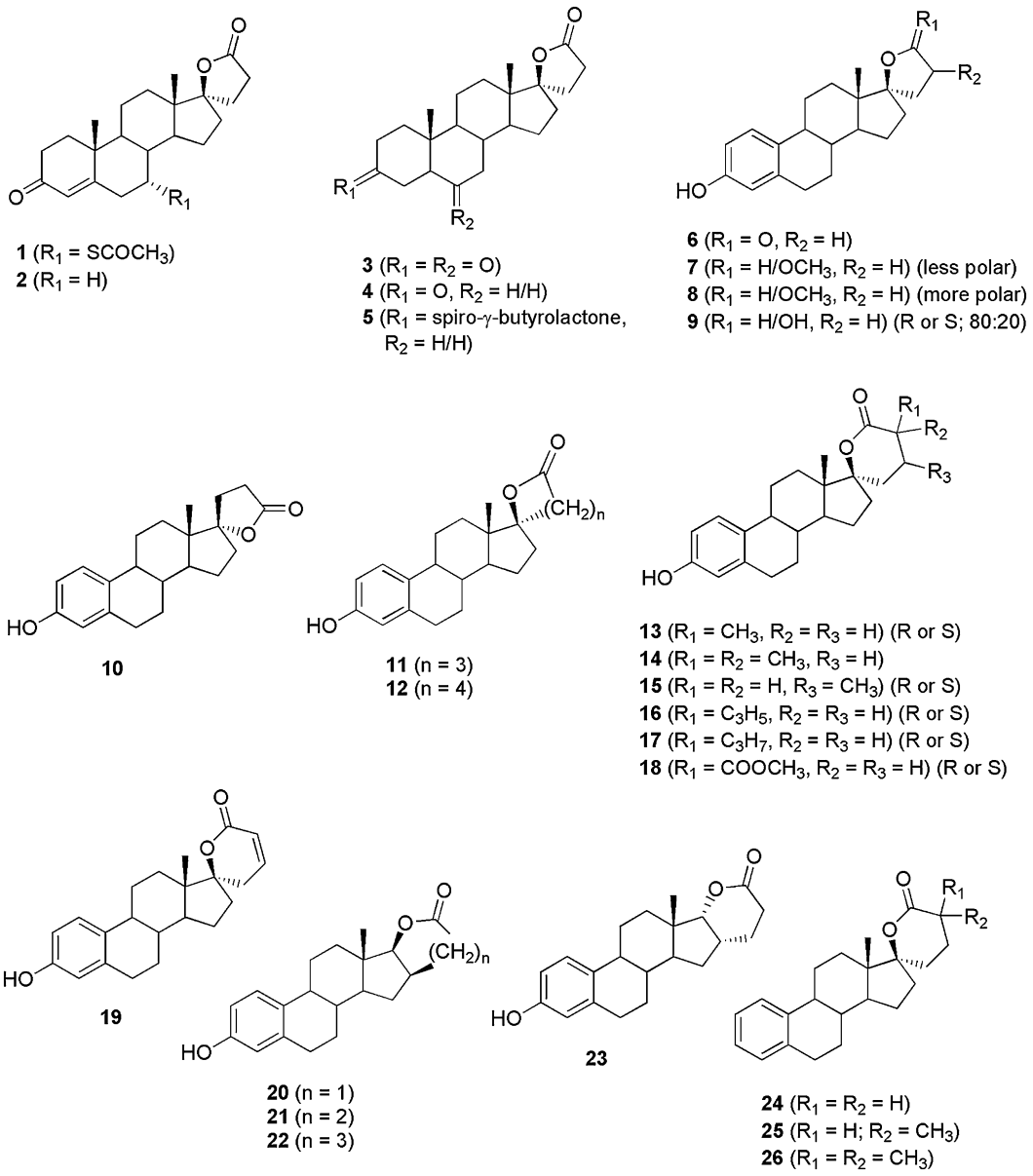
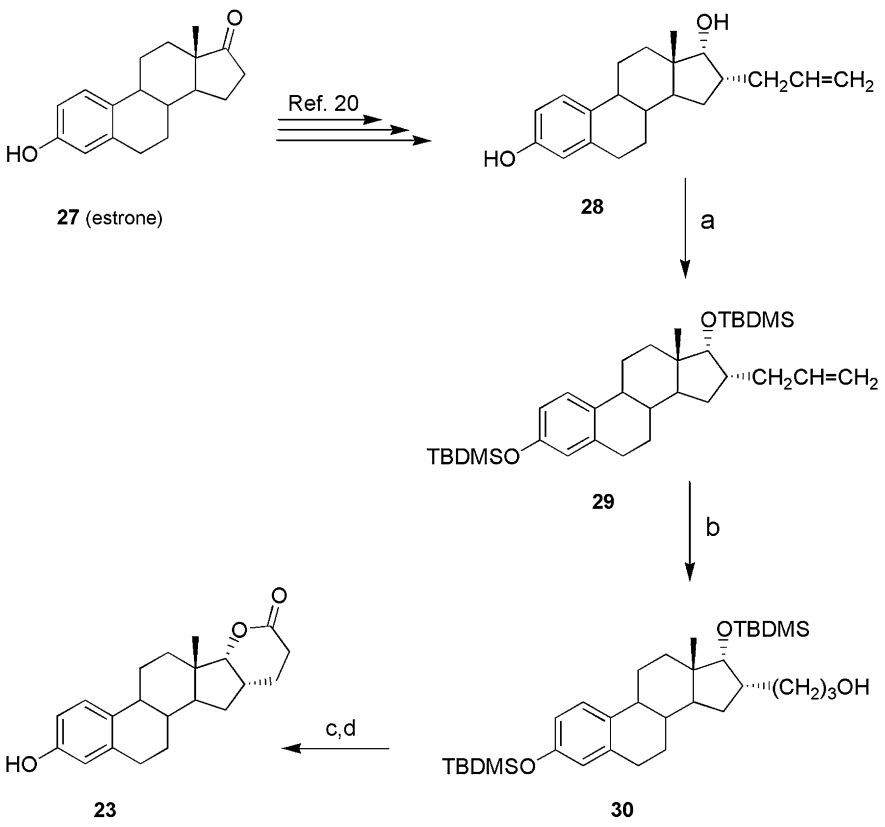


Fig. 2. Chemical structures of C19-steroid lactones **1**–**5** and C18-steroid lactones **6**–**26** tested as inhibitors of human 17β-HSD5.

because they were previously identified as inhibitors of 17β-HSD2 [22,23]. While mono-spiro-γ-lactones **1**–**4** were similarly active (57, 61, 45 and 63% at 3 μM, respectively), bi-spiro-γ-lactone **5** demonstrated a weaker activity with only 29% of inhibition at this concentration.

In order to compare the C19-steroids to C18-steroids, several estradiol (E2) derivatives (mainly as lactone derivatives) were next tested in our assay. At concentrations of 0.3 and 3 μM, spiro-γ-lactone **6** showed higher enzyme inhibition than the C19-steroid analogue (47 and 79% for **6** compared to 7–27 and 29–63% for **1**–**5**), establishing the importance of the C18-steroid backbone. The importance of the carbonyl function was evaluated by testing two *O*-methylated spiro-γ-lactols (compounds **7** and **8**) and spiro-γ-lactol **9**. The lactol derivative demonstrated the best inhibitory activity of this series at 3 μM (61% compared to 4 and 16%, respectively for **7** and **8**) but remains less potent than the corresponding spiro-γ-lactone **6** (79%). We then determined the effect of the orientation of the lactone pharmacophore by testing a 17α-*O*-spiro-γ-lactone (compound **10**). This lactone had less inhibitory activity (22 and 45%) than the corresponding 17β-*O*-spiro-γ-lactone **6** (47 and 79%) at both concentrations of 0.3 and 3 μM. Thus, the optimum orientation of oxygenated counterpart of the 17-spirolactone moiety was found to be in β position (or *S* configuration). The two other C18-steroid spirolactones **11** and **12** were also tested. Thus, while spiro-γ-lactone **6** (five-member ring) demonstrated only 79% of inhibition at 3 μM, spiro-δ-lactone **11** (six-member ring) and spiro-ϵ-lactone **12** (seven-member ring) respectively inhibited 95 and 93% of the enzyme activity at 3 μM. They also keep good inhibitory activities (92 and 90%) at lower concentration of 0.3 μM.



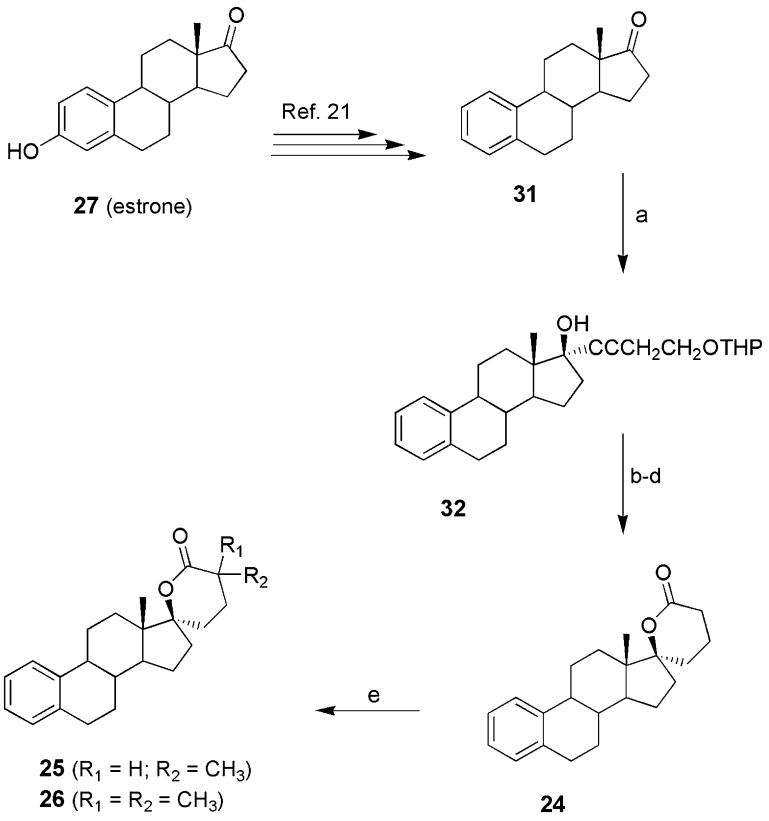
Scheme 1. Synthesis of 16α,17α-lactone-E2 **23**. Reagents and conditions are (a) TBDMS-Cl, imidazole, DMF, rt; (b) (i) BH_3, THF, 0 °C, (ii) H_2O_2, NaOH; (c) Jones reagent (2.7 M), acetone, 0 °C; (d) $BF_3 \cdot Et_2O$, CH_2Cl_2, rt.

If we go over the results so far obtained, we conclude that the presence of a C18-steroid E2 backbone and a spiro-δ- or a spiro-ϵ-lactone are two important requirements for the inhibition of 17β-HSD5. Spiro-δ-lactone was, however, retained over spiro-ϵ-lactone considering its better stability and facility of chemical synthesis. The effect of a substituent on the spiro-δ-lactone moiety was next addressed with lactones **13**–**19**. The methylated lactones **13**–**15** demonstrated good inhibitory activities at 0.3 μM (93, 86 and 88%, respectively). The lactones with more hindered substituents like the allyl derivative **16** and the propyl derivative **17** were less active (68 and 41%, respectively). Two other spiro-δ-lactones were also tested: methoxycarbonylated spiro-δ-lactone **18** and α,β-unsaturated spiro-δ-lactone **19**. The idea with compound **18** was to add another carbonyl function on the pharmacophore since we demonstrated earlier that this function was important for inhibition. Methoxycarbonylated spiro-δ-lactone **18** showed good inhibitory activity with 92% at 3 μM, which is similar to spiro-δ-lactone **11** (95%). α,β-Unsaturated spiro-δ-lactone **19** also demonstrated good inhibitory activity with 92% at 3 μM. Both compounds remain, however, weaker inhibitors than unsubstituted lactone **11** at 0.3 μM.

To verify the importance of the 17α/17β-*O*-spirolactone ring, we tested a series of 16β/17β-*O*-lactones (compounds **20**–**22**) that differ only by the lactone-ring size (5, 6 and 7 members). Lactones **20**–**22** have interesting inhibitory activity (62, 85 and 91%, respectively) at 3 μM, but they are less potent inhibitors than their corresponding 17-spirolactones **6**, **11** and **12** at both concentrations. The inhibitory effect of C16/C17-stereochemistry was also addressed by testing compound **23**. This 16α/17α-*O*-lactone is clearly less active than the 16β/17β-*O*-lactone **21** (6 and 55% of inhibition at 0.3 μM, respectively). The same result has been also obtained for 17-spiro-γ-lactones **10** (17α-*O*) and **6** (17β-*O*).

After we established the better inhibitory activity of 17-spirolactone over 16,17-lactones, the deoxygenated analogues of **11**, **13**, and **14**, compounds **24**–**26**, were synthesized and tested as inhibitors of 17β-HSD5. The removal of C3-OH group as well as mono or dimethylation is not detrimental for the inhibitory activity, since compounds **24**–**26** inhibited more than 90% of the enzyme activity at 0.3 μM.

Because the screening results were very similar for the most potent 17β-HSD5 inhibitors and for a better comparison, a complete curve of inhibition was obtained for each compound that inhibited over 90% of the enzyme activity at a concentration of 3 μM. From these curves, it is possible to obtain the IC_{50} values, which is the concentration of inhibitor that causes 50% of inhibition (Table 1). Spiro-δ-lactone **11** showed an IC_{50} value lower than spiro-ϵ-lactone **12** and 16β/17β-*O*-lactone **22** (20 ± 2, 42 ± 13, and 66 ± 2 nM, respectively). Allyl-spiro-δ-lactone **16** and methoxycarbonylated spiro-δ-lactone **18** (107 ± 8 and 72 ± 3 nM, respectively), which are the more hindered lactones, did not show an IC_{50} value as significant as the methylated products **13**–**15** (16 ± 1, 28 ± 1 and 19 ± 2 nM, respectively). α,β-Unsaturated spiro-δ-lactone **19** gave an intermediate IC_{50} of 49 ± 5 nM. Interestingly,



Scheme 2. Synthesis of 17-spiro-δ-lactone-3-deoxy-E2 **24**–**26**. The reagents and conditions are (a) (i) HC≡C(CH$_2$)$_2$OTHP, THF, *n*-BuLi, 0 °C, (ii) ketone **31**, THF, −78 °C to rt; (b) H_2, Pd/C (10%), EtOAc, rt; (c) *p*-TSA, MeOH, rt; (d) Jones reagent (2.7 M), acetone, 0 °C to rt; (e) LDA, CH_3I, THF, −78 °C to rt.

3-deoxygenated-E2 is a good nucleus for inhibition of 17β-HSD5 giving IC_{50} values of 10, 6.1 and 2.9 nM for **24**–**26**, respectively. As a point of comparison, 3-OH analogue lactones **11**, **13** and **14** gave IC_{50} values of 10, 16, and 28 nM, respectively. With an IC_{50} value of 2.9 ± 0.4 nM, dimethylated compound **26** is the most potent inhibitor obtained in our study.

2.3. Inhibitory activity of lactones on human 17β-HSD2

For a therapeutic use of a 17β-HSD5 inhibitor in the prostate, the candidate should not inhibit 17β-HSD2, an oxidative enzyme involved in the degradation of active 17β-OH steroids as T into Δ^4-dione. Fig. 3 clearly illustrates the selectivity of inhibition for dimethylated lactones **14** and **26** as well as the fact that the removal of the OH group in position 3 of E2 lactone does not affect its inhibitory activity potency on 17β-HSD2. In Fig. 3A, the E2 derivatives **11**, **13** and **14** were tested for their ability to inhibit the reductive transformation of Δ^4-dione into T in homogenated cells. Clearly, dimethylated lactone **14** did not inhibit the 17β-HSD2 activity contrary to monomethylated and unmethylated lactones **13** and **11**, respectively. A similar result was also obtained for the deoxyestradiol derivatives **24**–**26** when using the oxidative transformation of T into Δ^4-dione in intact cells (Fig. 3B). Indeed, no significant inhibition of 17β-HSD2 activity was observed with dimethylated lactone **26**. Thus, the selectivity of inhibition was observed only for lactones **14** and **26** having two methyl groups on the lactone ring. Interestingly, they are among the most active 17β-HSD5 inhibitors. In these cases, the presence of two methyl groups did not reduce the inhibitory potency of lactone on 17β-HSD5, although drastically reducing its ability to inhibit 17β-HSD2. Furthermore, the presence or the absence of a C3-OH did not influence the inhibitory results on both type 2 and type 5 17β-HSDs.

2.4. Inhibitory activity of lactones *11*, *24*–*26* on human 17β-HSD1 and 17β-HSD3

The C18-estrane nucleus of 17β-HSD5 inhibitors discussed above is closely related to the natural substrate estrone (E1) of 17β-HSD1. Although this common feature, spiro-γ-lactone **6** did not inhibit the transformation of E1 into E2 by homogenated HEK-293 cells over-expressing human 17β-HSD1 [24]. The same result was also obtained with spiro-δ-lactone **11** having an E2 nucleus (Fig. 4A). For lactones **24**–**26** having a deoxygenated-E2 nucleus, a weak inhibition (21–27%) was observed for **24** and **25** at both concentrations of 0.1 and 1 μM, but **26** appears to be a better inhibitor with 50% of inhibition at the higher concentration of 1 μM. We next

Table 1
Inhibition of human 17β-HSD5 activity by steroid lactones **1**–**26**[a]

Compound	Inhibition (%) at 0.3 μM[b]	Inhibition (%) at 3 μM[b]	IC_{50} (nM)[b]
1	27	57	–
2	19	61	–
3	14	45	–
4	15	63	–
5	7	29	–
6	47	79	–
7	0	4	–
8	0	16	–
9	19	61	–
10	22	45	–
11	92	95	20 ± 2 (10 ± 2)[c]
12	90	93	42 ± 13
13	93	94	16 ± 1
14	86	94	28 ± 1
15	88	94	19 ± 2
16	68	91	107 ± 8
17	41	83	–
18	78	92	72 ± 3
19	79	92	49 ± 5
20	30	62	–
21	55	85	–
22	70	91	66 ± 2
23	6	52	–
24	93	95	(10 ± 1)[c]
25	91	94	(6.1 ± 0.7)[c]
26	94	95	(2.9 ± 0.4)[c]
Δ^4-dione	21	45	–

[a] See Fig. 2 for the chemical structures of compounds **1**–**26**.
[b] For the transformation of [^{14}C]-Δ^4-dione (100 nM) into [^{14}C]-T in intact HEK-293 cells that over-express human 17β-HSD5.
[c] Data between parentheses were obtained under the same assay conditions but in another experiment.

tested lactones **11**, **24**–**26** as inhibitors of the transformation of Δ^4-dione into T catalyzed by 17β-HSD3 (Fig. 4B). Although spiro-δ-lactone **11** with an E2 nucleus weakly inhibited (25 and 34%) the 17β-HSD3 activity at 0.1 and 1 μM, respectively, the three lactones **24**–**26** with a deoxygenated-E2 nucleus are clearly better inhibitors (25–45 and 64–85% at 0.1 and 1 μM, respectively). Lactones **24**–**26** thus inhibit both 17β-HSD3 and 17β-HSD5, two key enzymes involved in the formation of potent androgen T in testis and prostate.

2.5. Estrogenic and antiestrogenic activities of lactones *14* and *26*

The estrogen-dependent ZR-75-1 cells, which contain the estrogen receptor (ER^+), were selected to assess the estrogenic and antiestrogenic activities of **14** and **26**. In our assay, proliferation was determined by measuring the DNA content of cells after 9 days of treatment. The cell proliferation induced by 0.1 nM of the potent estrogen E2 was fixed to 100% and used as a positive control of cell growth (Fig. 5). On the other hand, the pure antiestrogen EM-139 [25] was added as a reference compound giving no stimulation of

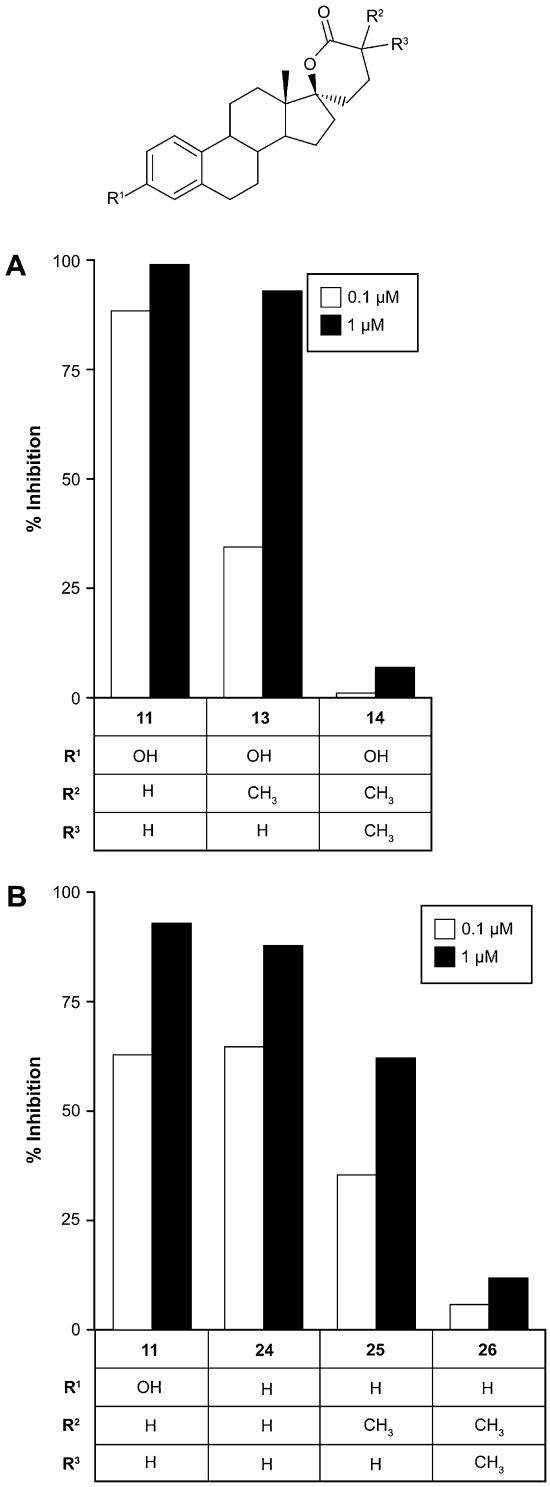


Fig. 3. Inhibition of human 17β-HSD2 over-expressed in HEK-293 cells by spiro-δ-lactones **11**, **13**, **14**, **24**–**26**. The results are reported in percentage (%) of inhibition at two inhibitor concentrations (0.1 and 1 μM). (A) Data obtained from Ref. [23] for the transformation of [^{3}H]-Δ^4-dione (4 nM) into [^{3}H]-T (homogenated HEK-293 transfected cells). (B): Compounds were tested for the transformation of [^{14}C]-T (100 nM) into [^{14}C]-Δ^4-dione (homogenated HEK-293 transfected cells).

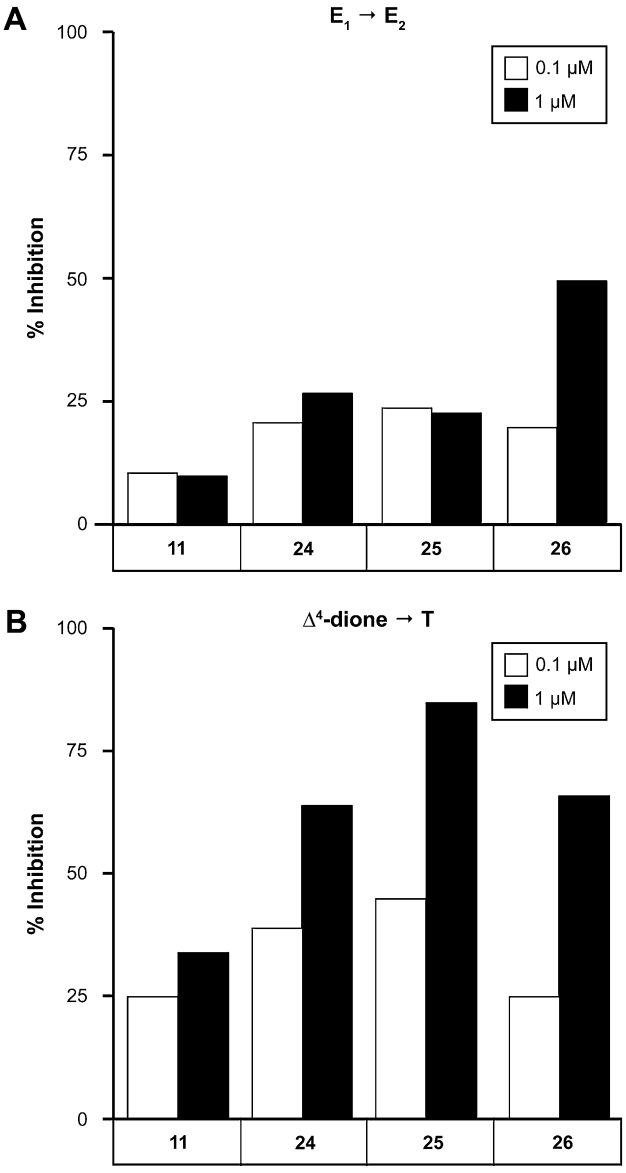


Fig. 4. Inhibition of human 17β-HSD1 (A) and human 17β-HSD3 (B) over-expressed in HEK-293 cells by spiro-δ-lactones **11**, **24**–**26**. The results are reported in percentage (%) of inhibition at two inhibitor concentrations of 0.1 and 1 μM.

cell proliferation. The two 17β-HSD5 inhibitors **14** and **26** follow the same pattern of activity with no significant proliferative (estrogenic) effect observed at 0.03 μM and a moderate effect at 1 μM (30 and 50%). Thus, the addition of a spiro-δ-lactone moiety on the E2 nucleus greatly reduced its estrogenic activity. The ability of these lactones to inhibit the E2 (0.1 nM)-induced stimulation of ZR-75-1 cells was also evaluated as a measure of their antiestrogenic activity. A significant antiestrogenic effect was observed for phenolic lactone **14** at the higher concentration of 1 μM, but not at 0.03 μM. No antiestrogenic effect was observed at both concentrations for lactone **26**. Under the same conditions, the antiestrogen EM-139 fully inhibited the cell proliferation induced by E2 (0.1 nM).

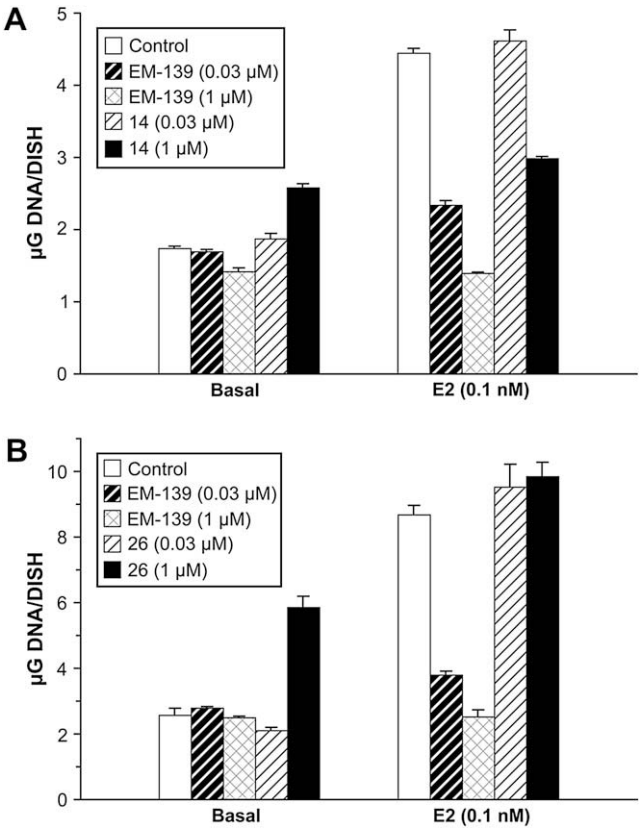


Fig. 5. Effect of two concentrations of pure antiestrogen EM-139, the phenolic dimethylated lactone **14**, and deoxygenated dimethylated lactone **26** on the basal and E2 (0.1 nM)-induced growth of estrogen sensitive (ER^+) ZR-75-1 cell line. Results are expressed as mean ± SEM of triplicates.

*2.6. Androgenic and antiandrogenic activities of dimethylated lactones **14** and **26***

The androgen-dependent Shionogi cell line was selected to assess the androgenic and antiandrogenic activities of **14** and **26**. Proliferation was determined by measuring the DNA content of cells after 10 days of treatment (Fig. 6). These cells contain the androgen receptor (AR^+) and the potent androgen DHT (0.1 nM) stimulated about 2.5-fold the cell growth. This level of cell growth stimulation was fixed at 100%. In the first part of the assay, compounds **14** and **26** showed no proliferative effect (androgenic activity) on Shionogi cells. In the second part, the ability of a compound to reverse the cell proliferation induced by DHT (0.1 nM) was determined. At the two concentrations tested (0.1 and 1 μM), lactones **14** and **26** did not significantly inhibit the proliferation of Shionogi cells induced by DHT indicating that they are not antiandrogenic compounds. As a point of comparison, the antiandrogen hydroxy-flutamide [26] inhibited 94% of the DHT-induced Shionogi cell proliferation at 1 μM but did not induce the cell proliferation at the two concentrations tested.

2.7. Steroid receptor binding affinity

The binding affinities of lactones **14** and **26** on four rat hormonal receptors are reported in Table 2. Although these

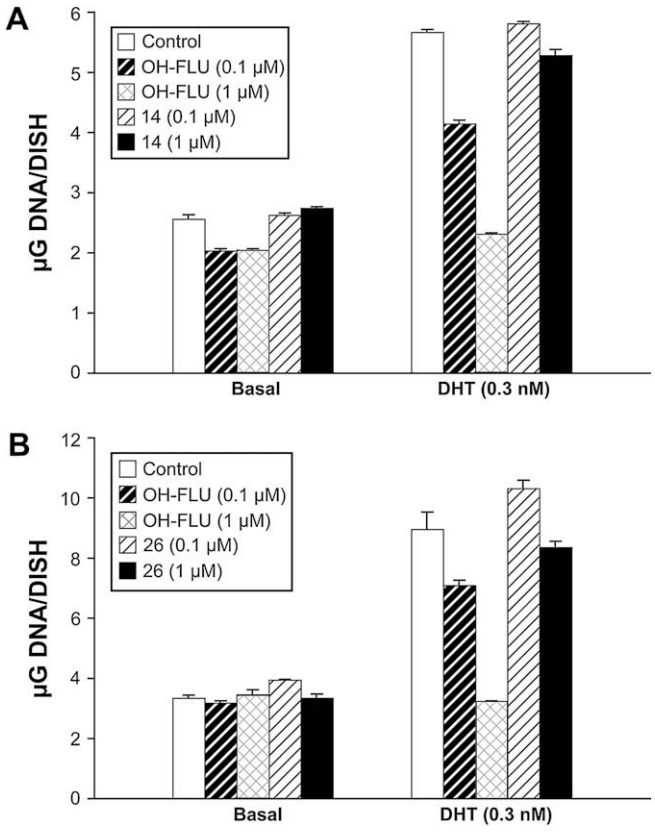


Fig. 6. Effect of two concentrations of pure antiandrogen hydroxyl-flutamide (OH-Flu), the phenolic dimethylated lactone **14**, and deoxygenated dimethylated lactone **26** on the basal and DHT (0.3 nM)-induced growth of androgen sensitive (AR^+) Shionogi cell lines. Results are expressed as mean ± SEM of triplicates.

receptor preparations are not of human origin, it is well documented that they can be used for the study of potential drugs [27–37]. Thus, a rat receptor preparation allows us to easily obtain a good general idea of the binding affinity of tested compounds **14** and **26** in comparison to the binding of a known agonist compound as the natural receptor ligand or and analogue. At the two concentrations tested, no significant binding affinity was observed on ER, AR, PR and GR for lactone **26** having no OH group at position C-3. For lactone **14**, with an OH group at C-3, the weak bindings (~10%) obtained on ER and PR at 0.1 μM increased to 80 and 35% at 10 μM, respectively. In fact, an OH group at position C-3 of an E2 nucleus is well known to promote ER binding by interacting (hydrogen bond) with key amino acids [27].

Table 2
Binding affinity of lactones **14** and **26** for estrogen (ER), androgen (AR), progesterone (PR), and glucocorticoid (GR) rat receptors

Compound	Concentration (μM)	ER binding (%)	AR binding (%)	PR binding (%)	GR binding (%)
14	0.1	10 ± 3	0	11 ± 1	0
14	10	80 ± 4	6 ± 1	35 ± 1	24 ± 2
26	0.01	0	0	0	0
26	1	0	0	5 ± 2	0
E2[a]	0.01	75 ± 1	0	6 ± 3	5 ± 2
DHT[a]	0.01	2 ± 2	70 ± 1	3 ± 2	2 ± 2
R5050[a]	0.01	5 ± 2	1 ± 4	65 ± 2	9 ± 2
DEX[a]	0.01	0	0	0	66 ± 2

[a] Compounds used as controls: natural estrogen estradiol (E2), natural androgen dihydrotestosterone (DHT), synthetic progestin R5050, and synthetic glucocorticoid dexamethasone (DEX).

3. Discussion

Our goal was to obtain a 17β-HSD5 inhibitor that exhibits no estrogenic and no androgenic activities. In fact, a residual estrogenic activity could generate a side effect such as feminization whereas a residual androgenic activity will stimulate the AR^+ prostate cancer cell proliferation. As mentioned above and illustrated in Fig. 1, the enzyme 17β-HSD5 is responsible for the transformation of Δ^4-dione into the active androgen T, which is reduced by 5α-reductase into the most potent androgen DHT. Although numerous isoforms of 17β-HSD are known [38–40], type 5 is a key target since it is mainly expressed in peripheral tissues [41]. Thus, the inhibition of 17β-HSD5 with a specific inhibitor that is not androgenic could be a good strategy to block the production of T, and indirectly DHT, in prostate cancer cells.

The present data show that a series of C19-steroid and C18-steroid derivatives bearing a lactone on D-ring exert inhibitory effects on 17β-HSD5. The C18-steroid backbone is, however, a better nucleus than the C19-steroid backbone. It was established that the carbonyl function was important because *O*-methylated spiro-γ-lactols **7** and **8** and spiro-γ-lactol **9** were less active than the corresponding spiro-γ-lactone **6**. This last statement suggests that a hydrogen bonding occurs between the carbonyl and an amino acid residue of the enzyme. Furthermore, the orientation of the oxygenated counterpart of spirolactone was important for inhibition, and the stereochemistry of the spirolactone giving good inhibition was beta *O*-oriented (as **6**) instead of alpha *O*-oriented (as **10**). We can also conclude that 17-spirolactone showed a better inhibitory activity than 16β/17β-*O* lactone and that the six-member ring spiro-δ-lactone **11** is the most potent inhibitor.

Although the substituted spiro-δ-lactones were not found to be more active inhibitors of 17β-HSD5 than unsubstituted lactone, the dimethylated derivative was found to be a selective inhibitor of 17β-HSD5. In fact, dimethylated spiro-δ-lactone did not inhibit 17β-HSD2, another 17β-HSD isoform that is well known to perform the oxidative transformation of T into Δ^4-dione. Since it was preferable to not inhibit 17β-HSD2, because this isoform deactivates the potent androgen T, the dimethylated strategy appears to be a significant finding. A dimethylated spiro-δ-lactone synthesized from a deoxygenated-E2 nucleus (no OH at position 3) was also clearly less estrogenic than the OH analogue. Thus, dimethylated spiro-δ-lactone **26** with a 3-deoxygenated E2 nucleus represents the most potent inhibitor of 17β-HSD5 ($IC_{50} = 2.9$ nM) in our study. This lactone inhibited the reductive transformation performed by 17β-HSD3 and 17β-HSD5, two key enzymes involved in the formation of potent androgen T, but did not inhibit the oxidative activity of 17β-HSD2. Lactone **26** showed no binding affinity for classical hormonal receptors (ER, AR, PR and GR). No significant proliferative effect

was also observed on AR^+ Shionogi cells for this lactone. However, a weak proliferative effect on ER^+ ZR-75-1 cells was observed at a high concentration (1 μM), but not at 0.03 μM or less.

In addition to the classical steroidogenic tissues, namely the ovaries, testis, adrenals and placenta, a large series of human peripheral tissues possess all the enzymatic systems required for the formation of active androgens from a relatively constant supply of precursor steroids provided by the adrenals [7]. While so far most therapeutic approaches have been aimed to control steroid formation by the classical steroidal tissues or inhibiting androgen receptor response with antagonists, it is clear that efforts should be also turned toward steroid formation in peripheral target tissues (intracrinology) in order to find a complementary therapy that would enhance the success of the therapies that are already known. Additional studies on steroidogenesis enzymes like 17β-HSDs will help us understand the physiological mechanisms controlling local formation and the difference between all the 17β-HSD isoforms. This first series of 17β-HSD5 inhibitors puts us in a position to develop novel therapeutic approaches which take into account the high proportion of steroids made locally and responsible for growth and functions of normal as well as cancerous tissues. In fact, the discovery that a dimethylated 17-spiro-δ-lactone-E2 inhibited the reductive 17β-HSD5 selectively was the starting point for the development of potent inhibitor EM-1404 [15], which was recently crystallized in a binary complex with the enzyme [42]. The availability of such compounds should be also useful as a tool for a better understanding of the role of 17β-HSD5 in the formation and action of androgens in the prostate and other peripheral tissues containing this 17β-HSD isoform.

4. Experimental

4.1. Chemical synthesis

4.1.1. General

Chemical reagents were purchased from Aldrich Chemical Company (Milwaukee, WI, USA) and starting steroid estrone (**27**) from Sigma Chemical Company (St. Louis, MO, USA) or Steraloids (Wilton, NH, USA). The solvents were obtained from Fischer (Montréal, Canada) and BDH (Montréal, Canada). Unless otherwise noted, materials obtained from commercial suppliers were used without further purification. Tetrahydrofuran (THF) was distilled from sodium/benzophenone immediately prior to use. All reactions except those involving water or hydrogen were conducted under argon atmosphere. Thin-layer chromatography (TLC) was performed on 0.20 mm silica gel 60 F254 plates (E. Merck, Darmstadt, GE) while 230–400 Mesh ASTM silica gel 60 (E. Merck, Darmstadt, GE) was used for flash column chromatography. Infrared (IR) spectra were obtained on a Perkin–Elmer 1600 (series FTIR) spectrophotometer and expressed in cm^{-1}. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AC/F300 spectrometer at 300 and 75 MHz, respectively. The chemical shifts (δ in ppm) were referenced to chloroform (7.26 or 77.00) respectively for ^1H and ^{13}C. High resolution mass spectra were provided by the Centre régional de spectrométrie de masse (Université de Montréal, Montréal, Canada). An HPLC system (Waters Associates, Milford, MA, USA) using an UV detector (205 or 210 nm), a reverse phase column and an appropriate mixture of solvents was used to determine the purity of compounds tested (see below for the type of column, solvents and the % of purity).

4.1.2. Compounds *1–22*

Spironolactone (**1**) was purchased from Steraloids (Wilton, NH) whereas C19-steroids **2–5** and C18-steroids **6–22** were available in our laboratory. The chemical syntheses of **2–5** [43] and **6–22** [23] were previously reported.

4.1.3. Synthesis of lactone *23* (Scheme 1)

*4.1.3.1. (16α,17α)-16-Allyl-3,17-bis{[tert-butyl(dimethyl)silyl]oxy}estra-1(10),2,4-triene (**29**).* To a solution of diol **28** [20] (149 mg, 0.48 mmol) in dry DMF were added imidazole (220 mg, 3.23 mmol) and *tert*-butyldimethylsilyl chloride (100 mg, 0.66 mmol). The reaction was stirred 3 days at room temperature under an atmosphere of argon. The mixture was then poured in ice/water and extracted with EtOAc. The organic layer was successively washed with water and brine, dried over $MgSO_4$, filtered, and evaporated under reduced pressure to give **29** (115 mg, 44% yield). The mono TBDMS derivative and the starting diol **28** were not recovered after chromatography. White solid; IR ν (film): 1640 (C=C, alkene), 1608 and 1570 (C=C, aromatic); ^1H NMR δ ($CDCl_3$): 0.06 and 0.07 (2s, $Si(CH_3)_2$ in C17), 0.20 (s, $Si(CH_3)_2$ in C3), 0.77 (s, CH_3-18), 0.96 and 0.99 (2s, $2 \times SiC(CH_3)_3$), 2.80 (m, CH_2-6), 3.66 (d, $J = 5.0$ Hz, CH-17α), 5.01 (m, CH_2-3′), 5.86 (m, CH-2′), 6.56 (s, CH-4), 6.62 (dd, $J_1 = 2.2$ Hz and $J_2 = 8.4$ Hz, CH-2), 7.14 (d, $J = 8.5$ Hz, CH-1); ^{13}C NMR δ ($CDCl_3$): −4.38 (2×), −4.05 (2×), 17.74, 18.18, 18.36, 25.73 (3×), 26.21 (3×), 26.30, 28.04, 29.84, 30.60, 32.69, 35.97, 39.18, 40.63, 43.71, 46.79 (2×), 82.16, 114.51, 117.08, 119.92, 126.08, 133.39, 137.96, 139.33, 153.17.

*4.1.3.2. 3-[(16α,17β)-3,17-Bis{[tert-butyl(dimethyl)silyl]oxy}estra-1(10),2,4-trien-16-yl] propan-1-ol (**30**).* To a stirred solution of **29** (0.115 g, 0.213 mmol) in dry THF (10 mL) at 0 °C was added dropwise a 1 M solution of borane in THF (1.5 mL, 1.5 mmol). The mixture was allowed to react under argon for 3 h, then a 3 N aqueous solution of NaOH (0.5 mL) and H_2O_2 (30% w/v) (0.2 mL) were added. The resulting mixture was stirred at room temperature for 1 h. The reaction was quenched by addition of water and extraction was done with EtOAc. The organic phase was washed with water, washed with brine, and dried over $MgSO_4$. After evaporation of organic solvent under reduced pressure, the crude product was purified by flash chromatography, using a mixture of hexanes and EtOAc (8/2) as eluent to give alcohol **30** as white solid (105 mg) in 88% yield. IR ν (film): 3346 (OH, alcohol); ^1H NMR δ ($CDCl_3$): 0.05 (s, $Si(CH_3)_2$ in C17), 0.19 (s, $Si(CH_3)_2$ in C3), 0.75 (s, CH_3-18), 0.94 (s, $SiC(CH_3)_3$ in C17),

0.99 (s, $SiC(CH_3)_3$ in C3), 2.79 (m, CH_2-6), 3.64 (m, CH-17α and CH_2OH), 6.55 (d, $J = 2.5$ Hz, CH-4), 6.61 (dd, $J_1 = 2.4$ Hz and $J_2 = 8.4$ Hz, CH-2), 7.13 (d, $J = 8.4$ Hz, CH-1); ^{13}C NMR δ ($CDCl_3$): −4.40 (2×), −3.99 (2×), 17.76, 18.15, 18.35, 25.71 (3×), 26.19 (3×), 26.28, 27.51, 28.03, 29.82, 30.92, 32.23, 32.63, 39.16, 41.12, 43.73, 46.68, 46.87, 63.38, 82.30, 117.07, 119.90, 126.05, 133.37, 137.92, 153.16.

*4.1.3.3. (4bS,6aS,6bR,10aR,11aS,11bR)-2-Hydroxy-6a-methyl-5,6,6a,6b,9,10,10a,11,11a,11b, 1,2,13-dodecahydronaphtho [2′,1′:4,5]indeno[1,2-b]pyran-8(4bH)-one (**23**).* Compound **30** (105 mg, 0.19 mmol) was dissolved in acetone (10 mL) and a solution of Jones reagent (2.7 M) (0.75 mL) was added dropwise at 0 °C. After 40 min at room temperature, isopropyl alcohol was added until a persistent green color remained and acetone was removed under reduced pressure. The green concentrate was then dissolved in water, and the extraction was done with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The crude compound (84 mg) was dissolved in dry CH_2Cl_2 and $BF_3 \cdot Et_2O$ (1.06 equiv) was added. The resulting mixture was stirred overnight at room temperature. The reaction was quenched with water, extraction was done with CH_2Cl_2 and the organic layer was washed with brine and dried over $MgSO_4$. After evaporation of the solvent under reduced pressure, the crude product was purified on column chromatography using a mixture of hexanes and EtOAc (7/3) as eluent to give 20 mg (33% yield, two steps) of lactone **23**. IR ν (film): 3350 (OH, phenol), 1716 (C═O, lactone); ^1H NMR δ ($CDCl_3$): 0.82 (s, CH_3-18), 2.70 (m, 1H), 2.80 (m, CH_2-6), 4.24 (d, $J = 6.0$ Hz, CH-17α), 5.35 (broad, OH), 6.54 (d, $J = 2.4$ Hz, CH-4), 6.65 (dd, $J_1 = 2.6$ Hz and $J_2 = 8.5$ Hz, CH-2), 7.13 (d, $J = 8.5$ Hz, CH-1); ^{13}C NMR δ ($CDCl_3$): 17.06 (C-18), 25.55 (C-1′), 25.80 (C-11), 27.97 (C-7), 29.12 (C-2′), 29.60 (C-6), 31.09 (C-12), 32.40 (C-15), 33.95 (C-16), 38.81 (C-8), 43.07 (C-9), 46.30 (C-13), 48.04 (C-14), 88.92 (C-17), 112.96 (C-2), 115.23 (C-4), 126.51 (C-1), 132.18 (C-10), 137.78 (C-5), 153.62 (C-3), 174.30 (C-3′); LRMS: calcd for $C_{21}H_{27}O_3$ $[M + H]^+$ 327.2, found 327.4; HPLC purity: 99.1% (C-18 Nova Pak column, MeCN/H_2O/MeOH (25:50:25)).

4.1.4. Synthesis of lactones **24–26** (Scheme 2)

*4.1.4.1. (17β)-17-[4-(Tetrahydro-2H-pyran-2-yloxy)but-1-yn-1-yl]estra-1(10),2,4-trien-17-ol (**32**).* To a solution of tetrahydro-2-(butynyloxy)-2-H-pyran (0.94 mL, 5.9 mmol) in 30 mL of dry THF was added at 0 °C, 3.53 mL of *n*-BuLi 1.6 M (5.7 mmol), and the mixture was stirred for 40 min. A solution of 3-deoxyestrone (**31**) [21] (500 mg, 1.96 mmol) in 10 mL of THF was then added dropwise at −78 °C and the mixture was stirred for 11 h. After this time, a solution of aqueous $NaHCO_3$ (5%) was added and aqueous phase was extracted with EtOAc. Organic layer was washed with brine and dried over $MgSO_4$. After evaporation of solvent, the crude compound was purified by flash chromatography with hexanes and EtOAc (9:1) as eluent to give 545 mg (68%) of alcohol **32**. Colorless oil; IR ν (film): 3438 (OH, alcohol), 2233 very weak (C≡C); ^1H NMR δ ($CDCl_3$): 0.88 (s, 3H, 18-CH_3), 2.57 (t, $J = 7.0$ Hz, 2H, C≡CCH_2), 2.87 (m, 2H, 6-CH_2), 3.55 and 3.86 (2m, 4H, CH_2O of side chain and CH_2O of THP), 4.68 (sapp, 1H, CH of THP), 7.13 (m, 3H, 1-CH, 2-CH, 3-CH), 7.31 (d, $J = 6.7$ Hz, 1H, 4-CH); ^{13}C NMR δ ($CDCl_3$): 12.72, 19.21, 20.30, 22.76, 25.37, 26.18, 27.13, 29.49, 30.48, 32.87, 38.98, 39.14, 43.07, 47.07, 49.53, 61.95, 65.78, 79.81, 83.07, 84.68, 98.58, 125.25, 125.47, 125.51, 128.91, 136.62, 140.22; LRMS: calcd for $C_{27}H_{40}O_3N$ $[M + NH_4]^+$ 426.3, found 426.5.

*4.1.4.2. (8R,9S,13S,14S,17S)-13-Methyl-4′,5′,6,7,8,9,11,12, 13,14,15,16-dodecahydrospiro [cyclopenta[a]phenanthrene-17,2′-pyran]-6′(3′H)-one (**24**)*

4.1.4.2.1. Reduction of triple bond. To a solution of alkyne **32** (650 mg, 1.6 mmol) in EtOAc was added 40 mg of palladium on activated charcoal (10% w/w). The mixture was stirred at room temperature overnight under an atmosphere of hydrogen and was then filtered through a pad of Celite, washed with EtOAc, and evaporated to dryness to give the corresponding alkane as white foam.

4.1.4.2.2. Hydrolysis of THP group. This crude THP derivative was dissolved in methanol (60 mL) and *p*-TSA (20 mg) was added. After 2 h at room temperature, water was added, the methanol was evaporated, and the resulting mixture was extracted with EtOAc. The organic phase was washed with water, dried over $MgSO_4$, filtered and the solvent evaporated to dryness to give 455 mg of a crude diol.

4.1.4.2.3. Jones oxidation with lactonization. The crude diol was dissolved in acetone (30 mL) and a solution of Jones reagent (2.7 M) (0.9 mL) was added dropwise at 0 °C. After the addition was completed, the reaction mixture was stirred at room temperature for 2 h. Isopropanol (2 mL) was then added and the resulting green solution was evaporated to dryness. The solid was dissolved in water and EtOAc, and the mixture was extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$. After evaporation of solvent, the crude compound was purified by flash chromatography with hexanes and EtOAc (8:2) as eluent to give 360 mg (70%, three steps) of lactone **24**. White solid; IR ν (KBr): 1729 (C═O, lactone); ^1H NMR δ ($CDCl_3$): 1.03 (s, 3H, 18-CH_3), 2.88 (m, 2H, 6-CH_2), 7.12 (m, 3H, 1-CH, 2-CH, 3-CH), 7.29 (d, $J = 5.8$ Hz, 1H, 4-CH); ^{13}C NMR δ ($CDCl_3$): 14.03 (C-18), 15.59 (C-2′), 23.22 (C-15), 25.54 (C-11), 27.13 (C-1′), 27.63 (C-7), 29.17 (C-6 and C-3′), 31.69 (C-12), 33.67 (C-16), 38.56 (C-8), 43.83 (C-9), 46.92 (C-13), 48.64 (C-14), 92.93 (C-17), 124.92 (C-3), 125.39 (C-1 and C-2), 128.70 (C-1), 136.18 (C-10), 139.56 (C-5), 171.70 (C-4′); HRMS: calcd for $C_{22}H_{28}O_2$ 324.20892, found 324.20702; HPLC purity: 99.6 (C-18 Nova Pak column, MeCN/H_2O/MeOH (35:30:35)).

4.1.5. Synthesis of lactones **25** and **26** (methylation of **24**)

A mixture of diisopropylamine (127 μL, 1.0 mmol), *n*-BuLi (1.6 M) (0.5 mL, 0.80 mmol) and dry THF (5 mL) was stirred

at 0 °C for 30 min. The solution was cooled at −78 °C, and a solution of lactone **24** (75 mg, 0.233 mmol) in dry THF (10 mL) was added dropwise. After 1 h, CH_3I (90 μL) was added and the mixture was stirred overnight and let warm to room temperature. Then, the reaction mixture was quenched with water and extracted with EtOAc. The organic phase was washed with water and dried over $MgSO_4$. After evaporation of the solvent, the crude compound was purified by flash chromatography with hexanes and EtOAc (95:5) as eluent to give 28 mg (36%, two steps) of monomethylated lactone **25** and 33 mg (40%, two steps) of dimethylated lactone **26**. Only the more polar and major monomethylated isomer was recovered and analysed.

*4.1.5.1. (8R,9S,13S,14S,17R)-5′,13-Dimethyl-4′,5′,6,7,8,9,11,12,13,14,15,16-dodecahydrospiro [cyclopenta[a]phenanthrene-17,2′-pyran]-6′(3′H)-one (**25**).* [Major isomer only]. White solid; IR *ν* (film): 1734 (C═O, lactone); ^{1}H NMR *δ* ($CDCl_3$): 1.02 (s, 3H, 18-CH_3), 1.25 (d, $J = 6.8$ Hz, 3H, CHCH_3), 2.56 (m, 1H, CHCH$_3$), 2.87 (m, 2H, 6-CH_2), 7.12 (m, 3H, 1-CH, 2-CH, 3-CH), 7.29 (d, $J = 6.1$ Hz, 1H, 4-CH); ^{13}C NMR *δ* ($CDCl_3$): 14.34 (C-18), 17.26 (CHCH_3), 23.52 (C-15), 24.44 (C-2′), 25.78 (C-11), 27.28 (C-1′), 27.40 (C-7), 29.41 (C-6), 32.01 (C-12), 33.51 (C-3′), 34.00 (C-16), 38.84 (C-8), 44.14 (C-9), 47.21 (C-13), 48.76 (C-14), 92.75 (C-17), 125.22 (C-3), 125.56 (C-1 and C-2), 128.97 (C-4), 135.51 (C-10), 139.87 (C-5), 175.84 (C-4′); HRMS: calcd for $C_{23}H_{31}O_2$ $[M]^+$ 339.23239, found 339.23130; HPLC purity: 99.2% (C-18 Nova Pak column, MeCN/H_2O/MeOH (40:35:25)).

*4.1.5.2. (8R,9S,13S,14S,17R)-5′,5′,13-Trimethyl-4′,5′,6,7,8,9,11,12,13,14,15,16-dodecahydrospiro [cyclopenta[a]phenanthrene-17,2′-pyran]-6′(3′H)-one (**26**).* White solid; IR *ν* (film): 1724 (C═O, lactone); ^{1}H NMR *δ* ($CDCl_3$): 1.02 (s, 3H, 18-CH_3), 1.28 (s, 6H, 2 × CH_3), 2.88 (m, 2H, 6-CH_2), 7.13 (m, 3H, 1-CH, 2-CH, 3-CH), 7.30 (d, $J = 6.2$ Hz, 1H, 4-CH); ^{13}C NMR *δ* ($CDCl_3$): 14.41 (C-18), 23.29 (C-15), 25.59 (C-1′), 25.84 (C-11), 27.39 (C-7), 27.54 and 27.76 (2 × CH_3), 29.42 (C-6), 31.51 (C-12), 32.02 (C-16), 34.82 (C-2′), 37.79 (C-3′), 38.87 (C-8), 44.15 (C-9), 47.25 (C-13), 48.84 (C-14), 93.55 (C-17), 125.21 (C-3), 125.52 (C-2), 125.56 (C-1), 128.99 (C-4), 135.52 (C-10), 139.87 (C-5), 177.82 (C-4′); HRMS: calcd for $C_{24}H_{33}O_2$ $[M]^+$ 353.24805, found 353.24660; HPLC purity: 96.1% (C-18 Nova Pak column, MeCN/H_2O/MeOH (40:20:40)).

4.2. Biological assays

4.2.1. Inhibition of 17β-HSD5

The assay was performed using intact human embryonic kidney (HEK)-293 cells stably over-expressing human 17β-HSD5 [4]. Briefly, 0.1 μM of the substrate [^{14}C]-4-androstene-3,17-dione (Dupont Inc. Canada) and 10 μL of an ethanolic solution of inhibitor (or only ethanol as control) were added to freshly changed culture medium in a six-well culture plate. After incubation for 18 h with HEK-293 cells over-expressing 17β-HSD5, the reaction was stopped by adding a solution (10 μM) of unlabeled Δ^4-dione and T before extracting twice with 2 mL of diethyl ether. The organic phases were pooled and evaporated to dryness. The metabolites were solubilized in CH_2Cl_2 (50 μL), applied to Silica gel 60 thin-layer chromatography (TLC) plate (Merck, Darmstad, Germany), and then separated by migration in the toluene-acetone (4:1) solvent system. Substrates and metabolites were identified by comparison with reference steroids and revealed by autoradiography and quantitated using the Phosphoimager System (Molecular Dynamics, Sunnyval, CA, USA). The percentage of transformation (% Transf) and percentage of inhibition (% Inh) were calculated using the following equations: % Transf = 100 × [^{14}C]-T (cpm)/([^{14}C]-T (cpm) + [^{14}C]-Δ^4-dione (cpm)) and % Inh. = 100 × [% Transf (without inhibitor) − % Transf (with inhibitor)]/% Transf (without inhibitor). To avoid the enzyme inhibition by the resulting product of reaction (T), the quantity of enzyme (intact cells) and the incubation time were both selected to give a percentage of transformation below 30%, which is in a linear range.

When several concentrations of an inhibitor were used in the enzymatic assay, an inhibition curve was plotted using the percentage of transformation versus the concentration of inhibitor. From the inhibition curve, the IC_{50} value (the concentration of inhibitor that provokes 50% of enzymatic inhibition) was calculated using DE_{50} program (CHUL Research Center, Quebec, Canada).

4.2.2. Inhibition of 17β-HSDs 1–3

The enzymatic assays for human 17β-HSD1 [44], 17β-HSD2 [22] and 17β-HSD3 [45] were performed as previously reported using homogenated HEK-293 cells over-expressing the appropriate enzyme and by measuring the transformation of the substrate [^{14}C]-E1 (100 nM) into [^{14}C]-E2, [^{14}C]-T (100 nM) into [^{14}C]-Δ^4-dione and [^{14}C]-Δ^4-dione (100 nM) into [^{14}C]-T, respectively. The results reported in Fig. 3A were, however, obtained from published data [23]. In that case, the HEK-293 cells over-expressing human 17β-HSD2 were homogenated and used for the assay evaluating the transformation of [3H]-Δ^4-dione (4 nM) into [3H]-T. To avoid the enzyme inhibition by the resulting product of reaction, the quantity of enzyme (homogenated cells) and the incubation time were both selected to give a percentage of transformation below 30%, which is in a linear range.

4.2.3. Proliferative and antiproliferative ZR-75-1 (ER^+) cell assay

The ZR-75-1 cells in their exponential growth were harvested with 0.05% trypsin, 0.02% EDTA (w/v) and resuspended in RPMI 1640 medium without phenol red supplemented with 2 mM L-glutamine, 1 nM sodium pyruvate, 50 ng insulin/mL, 15 mM HEPES 100 IU penicillin/mL, 100 μg streptomycin sulfate/mL and 5% (v/v) dextran-coated charcoal-treated fetal bovine serum (SD medium). The cells were plated in Falcon 24-well tissue culture plates (2 cm^2/well) with 10,000 cells/dish and allowed to adhere to substrate for 3 days. Estradiol (0.1 nM) and/or tested lactones (0.03 μM

and 1 μM) were added from concentrated stock solutions in 99% redistilled ethanol into fresh SD medium. Cells were then incubated at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air for 9 days with a change of medium every 2 or 3 days. At the end of the incubation period, cell growth was assessed by measurement of DNA content by a modification of the Fiszer-Szafarz method [46], as previously described [47]. Medium was carefully removed from the dishes 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 (Pharmacia) was used as a standard. Standard solutions containing 0.5–20 μg DNA/tube in 1 M NH_4OH were used. 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 dilution with 1.5 mL 1 N HCl. Fluorescence was measured with a LS2B Perkin–Elmer filter fluorimeter and the DNA content was determined (μg DNA/dish) as a measure of cell proliferation.

4.2.4. Proliferative and antiproliferative Shionogi (AR^+) cell assay

A clone of SC115 Shionogi cells was selected for its high sensitivity to DHT. Cells in their exponential growth were harvested with 0.1% pancreatine in HEPES buffer containing 3 mM EDTA, 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 fetal 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 tested lactones were added at concentration of 0.1 μM and 1 μM, with or without 0.3 nM of DHT. Stock solutions of DHT and tested agents were made in ethanol and their concentration was adjusted in order 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 change every 3–4 days. At the end of the incubation period, cell growth was assessed by measurement of DNA content by a modification of the Fiszer-Szafarz method [46], previously described [47]. Medium was 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 for standards (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 dilution with 1.5 mL 1 N HC1. Fluorescence was measured with a LS2B Perkin–Elmer filter fluorimeter and the DNA content determined (μg DNA/dish) as a measure of cell proliferation.

4.2.5. Hormone receptor binding assay

The binding affinity assays on estrogen and progestin receptors from rat uterus were carried out under the standard procedure established in our laboratory by Luo et al. [35] The assay with the androgen receptor from the rat ventral prostate was performed according to the procedure described by Luo et al. [36] In the case of the glucocorticoid receptor from rat liver, the affinity binding assay was done using a slightly modified version of the procedure described by Asselin et al. [37] Herein, a dextran-coated charcoal adsorption, instead of a protamine sulfate precipitation, was used to achieve the separation of bound and free steroids.

Acknowledgments

This work was supported in part by the Canadian Institutes of Health Research, Le Fonds de la recherche en Santé du Québec and Endorecherche. We want to thank the Fondation Georges Phénix for a scholarship (P.B.). We are grateful to Béatrice Tchédam Ngatcha for the synthesis of compound **23**. We also thank Ms. Micheline Harvey for careful reading of the manuscript.

References

[1] American Cancer Society, Cancer Facts and Figures 2006, American Cancer Society, Atlanta, 2006.
[2] C. Huggins, C.V. Hodges, Cancer Res. 1 (1941) 293–307.
[3] R.F. Gittes, New Engl. J. Med. 24 (1991) 236–245.
[4] I. Dufort, P. Rheault, X.F. Huang, P. Soucy, V. Luu-The, Endocrinology 140 (1999) 568–574.
[5] H.K. Lin, J.M. Jez, B.P. Schlegel, D.M. Peehl, J.A. Pachter, T.M. Penning, Mol. Endocrinol. 11 (1997) 1971–1984.
[6] T.M. Penning, M.E. Burczynski, J.M. Jez, H.K. Lin, H. Ma, M. Moore, K. Ratnam, N. Palackal, Mol. Cell. Endocrinol. 171 (2001) 137–149.
[7] F. Labrie, Mol. Cell. Endocrinol. 78 (1991) C113–C118.
[8] E. Baulieu, C. R. Acad. Sci. Paris 251 (1960) 1421–1425.
[9] F. Labrie, A. Dupont, J. Simard, V. Luu-The, A. Bélanger, Eur. Urol. 24 (Suppl. 2) (1993) 94–105.
[10] F. Labrie, J. Clin. Endocrinol. Metab. 80 (1995) 1066–1071.
[11] F. Labrie, A. Bélanger, J. Simard, V. Luu-The, C. Labrie, Ann. N.Y. Acad. Sci. 774 (1995) 16–28.
[12] M. El-Alfy, V. Luu-The, X.F. Huang, L. Berger, F. Labrie, G. Pelletier, Endocrinology 140 (1999) 1481–1491.
[13] Y. Laplante, D. Poirier, Steroids 73 (2008) 266–271.
[14] D. Poirier, Curr. Med. Chem. 10 (2003) 453–477 (an update of this review was recently accepted in Anti-Cancer Agents in Medicinal Chemistry).
[15] F. Labrie, A. Bélanger, S. Gauthier, V. Luu-The, Y. Mérand, D. Poirier, L. Provencher, S.M. Singh, US Patent 6,541,463, 2003.
[16] A. Krazeisen, R. Breitling, G. Moller, J. Adamski, Mol. Cell. Endocrinol. 171 (2001) 151–162.
[17] P. Brozic, B. Golob, N. Gomboc, T. Lanisnik-Rizner, S. Gobec, Mol. Cell. Endocrinol. 248 (2006) 233–235.
[18] D. Deluca, G. Moller, A. Rosinus, W. Elger, A. Hillisch, J. Adamski, Mol. Cell. Endocrinol. 248 (2006) 218–224.
[19] T.M. Penning, S. Steckelbroeck, D.R. Bauman, M.W. Miller, Y. Jin, D.M. Peehl, K.M. Fung, H.K. Lin, Mol. Cell. Endocrinol. 248 (2006) 182–191.
[20] P. Dionne, B. Tchédam Ngatcha, D. Poirier, Steroids 62 (1997) 674–681.
[21] M.R. Tremblay, R.P. Boivin, V. Luu-The, D. Poirier, J. Enzyme Inhib. Med. Chem. 20 (2005) 153–163.
[22] D. Poirier, P. Bydal, M.R. Tremblay, K.M. Sam, V. Luu-The, Mol. Cell. Endocrinol. 171 (2001) 119–128.
[23] P. Bydal, S. Auger, D. Poirier, Steroids 69 (2004) 325–342.

[24] V. Luu-The, Y. Zhang, D. Poirier, F. Labrie, J. Steroid Biochem. Mol. Biol. 55 (1995) 581–587.
[25] C. Lévesque, Y. Mérand, J.M. Dufour, C. Labrie, F. Labrie, J. Med. Chem. 34 (1991) 1624–1630.
[26] P. Poyet, F. Labrie, Mol. Cell. Endocrinol. 42 (1985) 283–288.
[27] J.P. Raynaud, T. Ojasoo, M.M. Bouton, D. Philibert, in: E.J. Ariens (Ed.), Drug Design, vol. 8, Academic Press, New York, 1979, pp. 169–214.
[28] A.E. Wakeling, in: B. Green, R.E. Leake (Eds.), Steroid Hormones: A Practical Approach, IRL Press, Washington, D.C., 1987, pp. 219–236.
[29] E. von Angerer, The Estrogen Receptor as a Target for Rational Drug Design (Molecular Biology Intelligence Unit), R.G. Landes Company, Austin, 1995, pp. 19–30.
[30] J.P. Raynaud, T. Ojasoo, in: A.F. Harms (Ed.), Innovative Approaches in Drug Research, Elsevier Science Publishers B.V., Amsterdam, 1986, pp. 47–72.
[31] J.J. Morris, L.R. Hughes, A.T. Glen, P.J. Taylor, J. Med. Chem. 34 (1991) 447–455.
[32] H. Ali, J. Rousseau, J.E. van Lier, J. Steroid Biochem. Mol. Biol. 49 (1994) 15–29.
[33] P.A. Danhaive, G.G. Rousseau, J. Steroid Biochem. 24 (1986) 481–487.
[34] G. Teutsch, T. Ojasoo, J.P. Raynaud, J. Steroid Biochem. 31 (1988) 549–565.
[35] S. Luo, C. Martel, A. Sourla, S. Gauthier, Y. Mérand, A. Bélanger, C. Labrie, F. Labrie, Int. J. Cancer 73 (1997) 381–391.
[36] S. Luo, C. Martel, G. Leblanc, B. Candas, S.M. Singh, C. Labrie, J. Simard, A. Bélanger, F. Labrie, Endocr. Relat. Cancer 3 (1996) 229–241.
[37] J. Asselin, R. Melançon, G. Moachon, A. Bélanger, Cancer Res. 40 (1980) 1612–1622.
[38] G. Moeller, J. Adamski, Mol. Cell. Endocrinol. 248 (2006) 47–55.
[39] P. Lukacik, K.L. Kavanagh, U. Oppermann, Mol. Cell. Endocrinol. 248 (2006) 61–71.
[40] V. Luu-The, J. Steroid Biochem. Mol. Biol. 76 (2001) 143–151.
[41] G. Pelletier, V. Luu-The, S. Li, F. Labrie, Cell Tissue Res. 320 (2005) 393–398.
[42] W. Qiu, M. Zhou, M. Mazumdar, A. Azzi, D. Ghanmi, V. Luu-The, F. Labrie, S.X. Lin, J. Biol. Chem. 282 (2007) 8368–8379.
[43] K.M. Sam, S. Auger, V. Luu-The, D. Poirier, J. Med. Chem. 38 (1995) 4518–4528.
[44] M.R. Tremblay, S.X. Lin, D. Poirier, Steroids 66 (2001) 821–831.
[45] B. Tchedam-Ngatcha, V. Luu-The, F. Labrie, D. Poirier, J. Med. Chem. 48 (2005) 5257–5268.
[46] B. Fiszer-Szarfarz, D. Skafarz, A. Guevara de Murillo, Anal. Biochem. 110 (1981) 165–170.
[47] J. Simard, S. Dauvois, D.E. Haagensen, C. Lévesque, Y. Mérand, F. Labrie, Endocrinology 126 (1990) 3223–3231.