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Spironolactone-related Inhibitors of Type II 17β-Hydroxysteroid Dehydrogenase: Chemical Synthesis, Receptor Binding Affinities, and Proliferative/Antiproliferative Activities

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Abstract—The family of 17β-hydroxysteroid dehydrogenases (17β-HSDs) catalyzes the formation and inactivation of testosterone (T), dihydrotestosterone (DHT), and estradiol (E_2), thus playing a crucial role in the regulation of active steroid hormones in target tissues. Among the five known 17β-HSD enzymes, type II catalyzes the oxidation of E_2 into estrone (E_1), T into androstenedione, DHT into androstanedione, and 20α-dihydroprogesterone into progesterone. Specific inhibitors are thus an interesting means to study the regulation and to probe the structure of type II 17β-HSD. In this context, we have efficiently synthesized a series of 7α-thioalkyl and 7α-thioaryl derivatives of spironolactone that inhibit type II 17β-HSD. These new C19-steroidal inhibitors possess two important pharmacophores, namely 17-spiro-γ-lactone and a bulky side-chain at the 7α-position. It was found that a *para*-substituted benzylthio group at the 7α-position enhances the inhibitory potency of spironolactone derivatives on type II 17β-HSD. In fact, the compound with a *para*-hydroxy-benzylthio group showed an IC_{50} value of 0.5 μM against type II 17β-HSD, whereas the compound with a *para*-[2-(1-piperidinyl)-ethoxy]-benzylthio group inhibited this enzyme with an IC_{50} value of 0.7 μM. The latter inhibitor is more selective than the former because it did not show any inhibitory potency against P450 aromatase as well as any affinity towards four steroid receptors (AR, PR, GR, ER). As a result, this inhibitor did not show any proliferative effect on androgen-sensitive Shionogi cells and estrogen-sensitive ZR-75-1 cells. These findings contribute to a better knowledge of the structure of type II 17β-HSD and offer an interesting tool to study the regulation of this enzyme in several biological systems.

Introduction

Over the last decade, the involvement of steroid hormones in the development and proliferation of hormone-sensitive tumors (e.g. breast and prostate) has been clearly demonstrated.[1,2] Steroid receptors and steroidogenic enzymes are thus attractive targets for drugs designed to treat hormone-sensitive diseases.[3,4] Among the steroidogenic enzymes, the 17β-hydroxysteroid dehydrogenase (17β-HSD) family exerts an important role in the regulation of active hormone levels in extraglandular tissues (Fig. 1).[5,6] These peripheral tissues contribute to a large proportion of steroid hormone formation from the adrenal precursor dehydroepiandrosterone (DHEA) and its conjugated sulfate (DHEAS).[7]

Although five types of 17β-HSD cDNAs and genes have been cloned from human tissues,[6,8–14] our group has mainly focused on the first two types. Type I is the best known 17β-HSD: its role and its structure have been elucidated. It has a high substrate affinity for estrone (E_1), a C18 steroid, and its preferred reaction is reduction using NADPH as cofactor. Clearly, the cytosolic type I 17β-HSD is involved in estradiol (E_2) biosynthesis from several E_2-producing tissues, including normal and neoplastic breast.[15,16] The 3D structures of the type I enzyme alone as well as the complexed enzyme with E_2 and cofactor NAD^+ were reported.[17–19] A consensus sequence Tyr-X-X-X-Lys was clearly identified as a key feature of the active site. During the past few years, first and second generation inhibitors against type I 17β-HSD have been reported by our group and others.[20–25]

The presence of a microsomal enzyme, named type II 17β-HSD, was first revealed by Blomquist and coworkers.[26] After isolation and characterization, it was shown that type II 17β-HSD is a protein of 387 amino acids containing an amino-terminal signal-anchor motif and a carboxy-terminal endoplasmic reticulum retention

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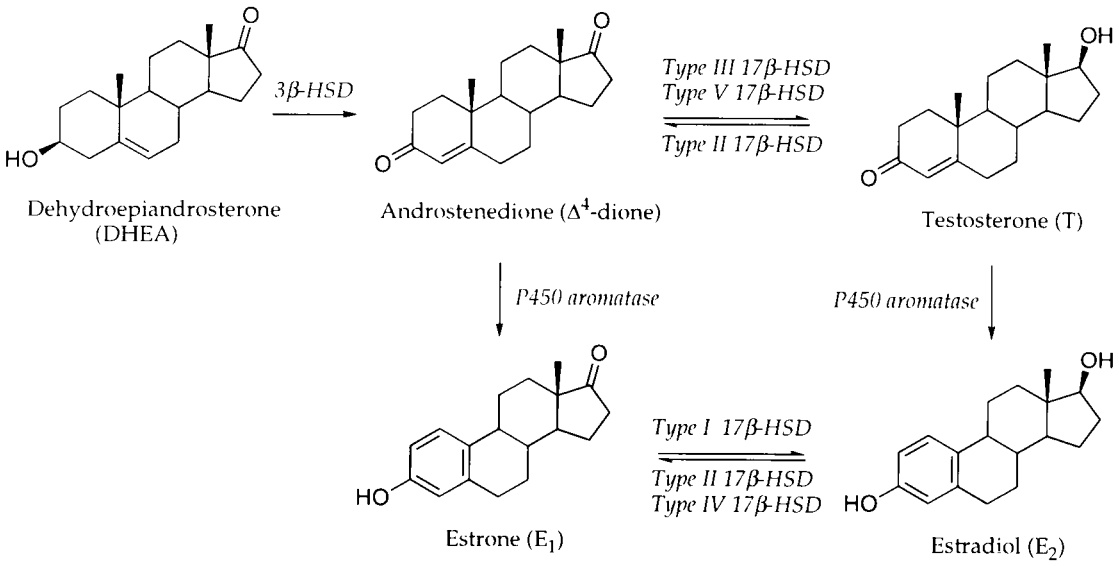


Figure 1. Peripheral biosynthesis of active estrogens and androgens from the adrenal precursor dehydroepiandrosterone (DHEA).

motif. The type II cDNA sequence showed only 20% homology with type I 17β-HSD.[11] Although the 3D structure of cytosolic type I 17β-HSD has been elucidated, the structure of microsomal type II remains unknown. Therefore, synthetic inhibitors should help to probe the active site as well as allosteric sites of type II 17β-HSD, and could possibly clarify the role of type II 17β-HSD, which is not fully understood. Type II 17β-HSD catalyzes the interconversion of testosterone (T) and androstenedione (Δ^4-dione), dihydrotestosterone (DHT) and androstanedione as well as E_2 and E_1.[11,27] This isoenzyme also catalyzes the interconversion of 20α-dihydroprogesterone and progesterone to a lower extent. Experiments with cellular models revealed that the type II prefers an oxidative process using NAD^+ as cofactor.[27] The in vitro kinetic data showed that the substrate binding affinity is between 0.2 and 0.4 μM for all of the reduced substrates reported above.[11] Type II 17β-HSD is expressed mainly in the placenta, liver, small intestine, endometrium, kidney, pancreas, and colon.[12] It has also been reported that benign and malignant prostate tissue as well as human meningioma tumors possess significant levels of type II 17β-HSD activity.[28,29] These data suggest that type II 17β-HSD inactivates the reduced steroidal hormone by oxidation in peripheral tissues. In classical endocrine tissues such as endometrium and placenta, it has been hypothesized that the type II isoenzyme together with the type I may control the intracellular ratio of active C18 and C19 steroid hormones during pregnancy.[30,31] Moreover, the 20α-HSD activity may provide high progesterone levels during pregnancy. Our specific inhibitors of both type I and type II 17β-HSDs have been used to further investigate this fine regulation and to clarify the role of type II 17β-HSD.[27,32] Thus, inhibitors of type II 17β-HSD are useful tools to elucidate the role of this enzyme in particular biological systems and they are also helpful for improving our knowledge of its 3-D structure. In this view, we are interested in developing various kinds of steroidal inhibitors of type II 17β-HSD.

Recently, we reported a spiro-γ-lactone derivative of E_2 (compound **1**) as the first C18-steroidal inhibitor of type II 17β-HSD (Fig. 2).[33] Concurrently, we also observed that spironolactone (**2**) was the most potent inhibitor of type II 17β-HSD among a series of tested C19-steroids.[34] They both possess the spiro-γ-lactone that is now established as an important pharmacophore for the inhibition of type II 17β-HSD. Moreover, the 7α-thioacetyl group was shown to enhance the inhibitory

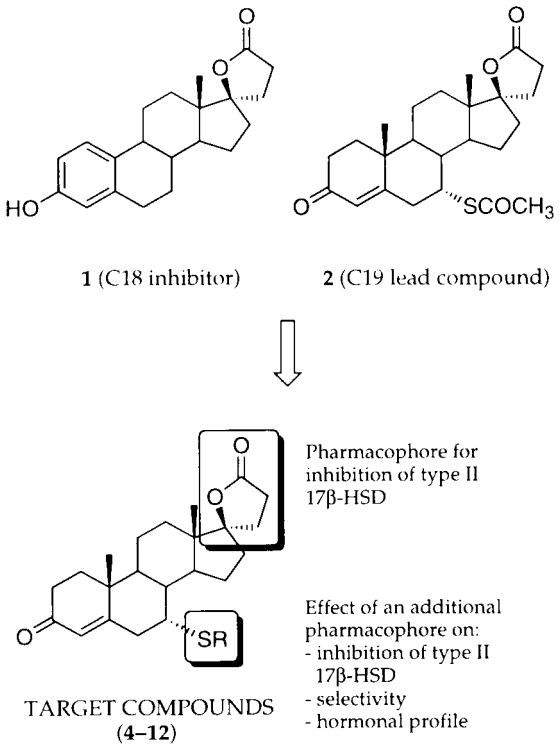


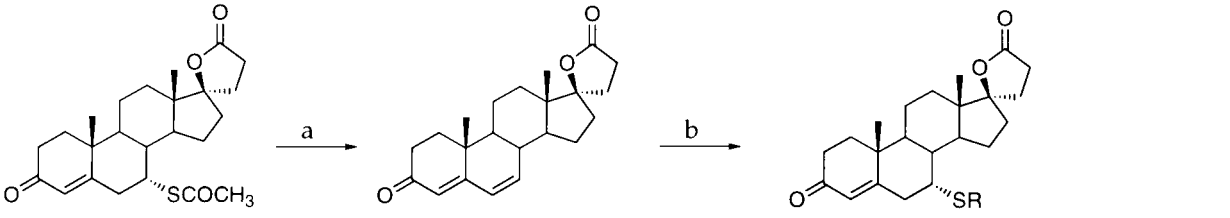
Figure 2. Chemical structure of the reported C18-steroidal inhibitor of type II 17β-HSD (**1**) and the lead compound, spironolactone (**2**). The strategy for lead optimization is the substitution of position **7α**.

potency of C19-steroid derivatives bearing a spiro-γ-lactone.[34] Thus, we wanted to develop a potent C19-steroid inhibitor of type II 17β-HSD to modify the endocrine profile of our first estrogen-like inhibitor (compound **1**) because the latter may have limited uses due to its estrogenic activity. Therefore, position 7α of spironolactone was substituted with various thioalkyl and thioaryl side chains. Most importantly, a 2-(1-piperidinyl)-ethoxybenzyl group was introduced at the position 7α to assess the ability of type II 17β-HSD to tolerate this pharmacophore. It was shown that closely related chemical groups provide interesting properties to both steroidal and non steroidal derivatives toward estrogen receptors.[35–38] Herein, we report the synthesis and biological activities of novel spironolactone derivatives that represent another class of type II 17β-HSD inhibitors.

Chemistry

A simple and straightforward synthesis was used to generate the 7α-analogues of spironolactone (Scheme 1). First, the thioacetyl group of spironolactone (**2**) was removed by a retro-Michael elimination to yield canrenone (**3**). Then, a sodium-mediated 1,6-conjugate addition of the appropriate thioalkyl side chain was performed following the conditions described by Brueggemeier and co-workers.[39] For the synthesis of compounds **4–6** and **8–9**, canrenone was simply solubilized in commercially available thioalkyls or thioaryls with metallic sodium (1 equiv). Thiobenzoyl **7** was prepared by adding thiobenzoic acid to canrenone using a standard procedure.[40–42] Compounds **10–12** were synthesized by convergent approaches using synthetic thiols **17** and **18** (Scheme 2). The starting material 4-hydroxybenzaldehyde was protected as a *t*-butyldimethylsilyl ether or *O*-alkylated with 1-(2-chloroethyl)-piperidine hydrochloride according to standard procedures to yield compounds **13** and **14**, respectively. Subsequent reduction of the aldehydes using sodium borohydride and thioacetylation according to the Mitsunobu procedure[43] yielded compounds **15** and **16**. Reduction of the thioacetyl group by lithium aluminum hydride gave the thiols **17** and **18**. This synthetic sequence allowed us to generate a gram-scale of both **17** and **18** in a short period of time. The efficiency of this sequence was necessary because of the special need of the subsequent 1,6-conjugate addition (Scheme 1). In fact, the yield of this addition was unsatisfactory (< 20%) when canrenone (**3**) was solubilized in 1,4-dioxane with only 3–10 equivalents of thiol **17**. However, target product **10** was obtained in a substantially improved yield when the two components were mixed neat with a catalytic amount of metallic sodium. Afterward, the cleavage of the TBDMS group of compound **10** using TBAF at a low temperature (−78 °C) gave the phenol **11**. It is noteworthy that the fluoride ion is basic enough to undergo the retro-Michael elimination when the reaction is carried out at higher temperatures (0 °C and 25 °C). Direct *O*-alkylation of **11** with 1-(2-chloroethyl)-piperidine hydrochloride required drastic conditions giving mainly the retro-Michael elimination. We thus alternatively used with success the same procedure as for the synthesis of **10** to produce compound **12** (vide infra).

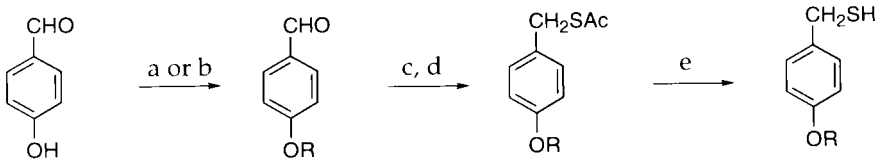
The synthesis of nine analogues of spironolactone was achieved by a short-step and efficient sequence. The 1,6-conjugate addition proceeded smoothly without affecting other functionalities on the key intermediate **3**. Indeed, the formation of the thioether bond is regio- and stereoselective. The regiospecificity could be attributed to the soft character of both the sulfur atom and the carbon atom at position 7, which is the most electropositive carbon. The stereoselectivity is mainly due to the steric hindrance provoked by 19-CH_3 and 18-CH_3 on the β face of the substrate. The assignment of the position as well as the configuration of the alkylthio substituents was made using NMR spectroscopy.



2 **3**

4 (R = CH_2CH_3)
5 (R = $CH_2CH_2CH_3$)
6 (R = $CH_2(CH_2)_4CH_3$)
7 (R = COPh)
8 (R = Ph)
9 (R = CH_2Ph)
10 (R = CH_2Ph-*p*-OTBDMS)
11 (R = CH_2Ph-*p*-OH)
12 (R = CH_2Ph-*p*-$OCH_2CH_2N(CH_2)_5$

Scheme 1. Reagents and conditions: (a) NaOMe, THF, rt (79%); (b) Na(s), commercial or synthetic thiols (neat), 60 °C (49–93%); (c) TBAF, THF, −78 °C (88%).



13 (R = TBDMS) **15** (R = TBDMS) **17** (R = TBDMS)
14 (R = $CH_2CH_2N(CH_2)_5$) **16** (R = $CH_2CH_2N(CH_2)_5$) **18** (R = $CH_2CH_2N(CH_2)_5$)

Scheme 2. Reagents and conditions: (a) TBDMS–Cl, imidazole, DMF, rt (83%); (b) K_2CO_3, $ClCH_2CH_2N(CH_2)_5$, DMF; (c) $NaBH_4$, MeOH, 0 °C; (d) DEAD, PPh_3, CH_3COSH, THF, 0 °C (63–82%, two steps); (e) $LiAlH_4$, THF, 0 °C (43–76%).

Heteronuclear shift correlation (HSC) experiments made on compound **10** allowed us to clearly identify 7-H signal. Afterward, the 8β-H and 6β-H signals were localized by combination of HSC and nuclear Overhauser effect (nOe) experiments. The absence of axial–axial coupling constant ($J \sim 10$ Hz) between all these protons was easily revealed by J-resolve experiments and confirmed that the proton at position 7 is equatorial (β). Moreover, all spectroscopic data (^1H and ^{13}C NMR) were in agreements with studies reported by Brueggemeier and co-workers.[39]

Biological Results

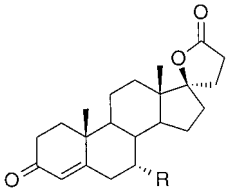
In a previous study on the reductive activity of type II 17β-HSD, we observed that spironolactone (**2**), bearing a 7α-thioacetyl group on a Δ^4-dione nucleus, was a more potent inhibitor than the analogue without a 7α-substituent.[34] We aimed to further characterize the importance of the 7α-substituent on this C19-steroid nucleus in relation to the inhibition of type II 17β-HSD. We first changed the thioacetyl group for thioalkyls (**4–6**), thiobenzoyl (**7**), thioaryl (**8**), and thiobenzyl (**9**) groups (Scheme 1). To avoid contamination by aromatase, which is also expressed in microsomes, we chose a system that is free of endogenous aromatase activity. Transformed human embryonic kidney (293) cells were transfected with an expression vector encoding type II 17β-HSD. Assays were performed using subcellular fractions by a previously reported method.[27]

A screening test was performed on our first series of 7α-derivatives of spironolactone (Table 1). The reductive activity was initially tested to make the connection with our previously published work that was reported before the preferred activity of the enzyme was established.[34] The ability of the compound to inhibit the reduction of radiolabelled Δ^4-dione into testosterone was assessed. It can be seen that neither compound **3** without substitution at position 7α nor compounds bearing a thioester group (**2** and **7**) are very potent against type II 17β-HSD. On the other hand, thioalkyl (**4–6**) and particularly thioaryl compounds (**8** and **9**) displayed a more potent inhibitory effect. As shown in Table 1, the 7α-thiobenzyl analogue **9** exhibited the highest inhibitory effect with an IC_{50} value of 0.42 μM, while other compounds showed IC_{50} values close to micromolar. Moreover, analogue **9** had a better affinity (threefold) than the natural oxidized substrate Δ^4-dione (1.5 μM). We further investigated the inhibitory effect provoked by the thiobenzyl group. Thus, we synthesized the phenolic derivative **11** and its substituted analogue **12**. As can be seen in Table 2, the phenolic derivative **11** is better than its substituted analogue **12**, which is more potent than the lead compound **9**. It is interesting to note that compounds **11** and **12** displayed inhibitory potency very similar to our previously reported C18-steroid inhibitor **1**. Moreover, compound **12** did not show any activity against types I, III, and V of 17β-HSD when tested at 3 μM.

We decided to test these type II 17β-HSD inhibitors on the human P450 aromatase because the 7α-derivatives of Δ^4-dione are well known as inhibitors of this enzyme.[39,44,45] Results shown in Table 3 reveal that only compounds **8** and **11** weakly inhibited P450 aromatase activity compared with the established inhibitor 4-OH-androstenedione,[46] while other newly synthesized compounds were totally inactive toward this enzyme.

Afterward, we were interested in studying the hormonal profile of our type II 17β-HSD inhibitors. Thus, binding affinities on the steroid receptors were determined using

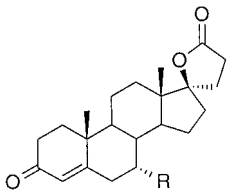
Table 1. Inhibitory effects of 7α-derivatives of spironolactone on the activity of transfected type II 17β-HSD



No.	R	Inhibition of reductase activity Type II 17β-HSD*		
		% at 0.1 μM	% at 1.0 μM	IC_{50} (μM)
2	$SCOCH_3$	3	22	nd
3	$\Delta^{6\text{-}7}$	7	22	nd
4	SCH_2CH_3	6	47	0.9 ± 0.3
5	$S(CH_2)_2CH_3$	9	42	1.1 ± 0.4
6	$S(CH_2)_5CH_3$	12	41	1.4 ± 0.3
7	SCOPh	0	4	nd
8	SPh	17	56	1.0 ± 0.4
9	SCH_2Ph	25	58	0.42 ± 0.07
Δ^4-dione	substrate	—	—	1.5 ± 0.4

*For the reduction of [^{3}H]-Δ^4-dione (5 nM) to [^{3}H]-T catalysed by transfected type II 17β-HSD.

Table 2. Inhibitory effects of 7α-derivatives of spironolactone on the activity of transfected type II 17β-HSD

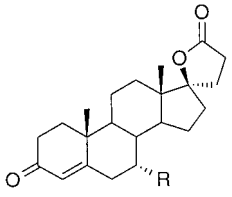


No.	R	Inhibition of oxidative activity* Type II 17β-HSD IC_{50} (μM)
2	$SCOCH_3$	1.1 ± 0.4
9	SCH_2Ph	1.0 ± 0.2
11	$SCH_2Ph(4\text{-}OH)$	0.53 ± 0.03
12	$SCH_2Ph(4\text{-}OCH_2CH_2N(CH_2)_5)$	0.7 ± 0.3
1	C18 inhibitor	0.7 ± 0.1

*For the oxidation of [^{14}C]-T (0.1 μM) into [^{14}C]-Δ^4-dione catalysed by transfected type II 17β-HSD.

standard conditions. Percentages of binding for two concentrations of the tested compound are shown in Table 4. The firstly reported inhibitor **1** efficiently bound to the estrogen receptor, while none of the newly synthesized inhibitors had any affinity for this receptor. However, several 7α-derivatives of spironolactone bound to progestin or glucocorticoid receptors and, to a lesser extent, to androgen receptor. Among the tested compounds, only **12** was devoid of any affinity for all of the tested steroid receptors. This compound did not display significant proliferative/antiproliferative activity on estrogen-sensitive ZR-75-1 cells and showed weak antiproliferative effect on androgen-sensitive Shionogi cells (Fig. 3).

Table 3. Inhibitory effects of 7α-derivatives of spironolactone on the activity of transfected P450 aromatase



No.	R	Inhibition of P450 aromatase*	
		% at 0.3 μM	% at 3.0 μM
2	$SCOCH_3$	12	13
3	$\Delta^{6\text{-}7}$	20	—
4	SCH_2CH_3	17	—
5	$S(CH_2)_2CH_3$	14	22
6	$S(CH_2)_5CH_3$	16	17
7	SCOPh	12	11
8	SPh	8	40
9	SCH_2Ph	14	—
11	$SCH_2Ph(4\text{-}OH)$	38	52
12	$SCH_2Ph(4\text{-}OCH_2CH_2N(CH_2)_5)$	12	14
—	4-OH-androstenedione**	88	93

*For the aromatization of [^{14}C]-Δ^4-dione to [^{14}C]-E_1 catalyzed by transfected P450 aromatase.
**Standard inhibitor of P450 aromatase.

Discussion

We have described an efficient synthesis of 7α-derivatives of spironolactone. Target compounds **4–9** were synthesized within two chemical steps from spironolactone when the thiols introduced at position 7α were commercially available. A convergent synthesis of six chemical steps was performed to generate the two other inhibitors (**11** and **12**). The first series of inhibitors (**2** to **9**) was tested against the reductive activity of type II 17β-HSD (transformation of Δ^4-dione (5 nM) into testosterone). We then established the 7α-thiobenzyl analogue as an interesting lead compound. During optimization of this lead compound, it was reported that type II 17β-HSD prefers oxidation over reduction.[27] Consequently, our newly synthesized spironolactone derivatives (**11** and **12**) as well as the compounds used as references (**1**, **2**, and **9**) were evaluated for the inhibition of the oxidation of [^{14}C]-testosterone into [^{14}C]-Δ^4-dione catalyzed by type II 17β-HSD. These compounds that inhibit type II 17β-HSD with IC_{50} values between 0.5 and 1.0 μM have two pharmacophores: a 17-spiro-γ-lactone group and a 7α-thioaryl group. Our results from previous reports[33,34] together with the present paper clearly demonstrate that the 17-spiro-γ-lactone group is a very important pharmacophore that provides to both C18 and C19 steroids an inhibitory effect on type II 17β-HSD. In addition, we have shown that a thiobenzyl group at position 7α can enhance the inhibitory potency of spironolactone. More importantly, we have suitably substituted the spironolactone with a 7α-(*para*-hydroxybenzyl sulfide) group to obtain a C19-steroid inhibitor (compound **11**) that has similar inhibitory potency to the first reported C18-steroid inhibitor **1**. Although the combination effect of these two pharmacophores is significant (twofold), it is too weak to conclude that the 7α-substitution leads to a specific and strong binding to type II 17β-HSD. However, these results have demonstrated that a bulky pharmacophore such as a 2-(1-piperidinyl)-ethoxybenzylthio group (compound **12**) is well tolerated by the type II enzyme. On the other hand, it is known that the type I isoform does not allow strong binding with E_2 bearing a bulky side chain at position 7α.[25,47] Although type I 17β-HSD has a strong preference for C18 over C19 steroids, we reported that the spiro-γ-lactone derivative of E_2 (compound **1**) does not inhibit this enzyme.[27] The spiro-γ-lactone group thus provides selectivity toward the type I isoenzyme even in the C18-steroid series. Consequently, it is logical that the newly synthesized compound **12** with the spiro-γ-lactone group placed on a 7α-substituted C19-steroid nucleus is selective for type II over type I 17β-HSD. However, the reasons why compound **12** does not inhibit type III or type V 17β-HSD are less clear because no SAR studies concerning these enzymes are reported in the literature so far.

In addition to the inhibitory potency for type II 17β-HSD, we have evaluated several other biological parameters that are related to this type of steroidal compound. According to previous reports from Brueggemeier and co-workers,[39,44,45] the *para*-substituted thiobenzyl groups at position 7α also provoke inhibition of P450 aromatase

Table 4. Percentages of binding affinity of compounds **2–12** on four steroid receptors

No.	Androgen receptor		Progestin receptor		Glucocorticoid receptor		Estrogen receptor	
	10 nM	1 μM	10nM	1 μM	10 nM	1 μM	10 nM	1 μM
1*	0±1	29±1	15±2	90±1	0±1	74±1	33±1	98±1
2	5±2	53±1	0±2	23±5	2±4	17±3	0±1	2±2
3	1±1	8±2	0±2	26±2	0±3	1±4	0±5	0±2
4	0±4	28±1	0±1	29±2	3±3	59±2	0±3	0±1
5	0±2	41±3	0±2	59±2	0±3	53±3	1±1	0±2
6	5±3	12±3	7±4	87±1	6±3	57±3	0±2	0±2
7	5±1	53±2	3±2	62±2	0±3	10±3	0±2	0±2
8	1±3	35±1	0±2	30±1	0±2	3±3	0±1	0±2
9	5±1	2±2	0±2	42±1	1±2	12±3	0±1	0±1
11	0±3	9±2	0±2	31±4	0±3	0±4	0±1	0±1
12	0±2	0±2	0±1	0±2	4±2	4±2	0±2	0±2
DHT	70±1	100±1	3±2	40±2	2±2	6±2	2±2	4±1
R5050	1±4	28±2	65±2	99±2	9±2	85±2	5±2	5±2
DEX	0±1	2±1	0±3	1±2	66±2	99±1	0±3	0±1
E_2	0±2	34±1	6±3	25±2	5±2	12±2	75±1	100±1

*Concentrations are 100 nM and 10 μM instead of 10 nM and 1 μM. Abbreviations of steroids used as standard: DHT: dihydrotestosterone; R5050: synthetic progestin; DEX: dexamethasone; E_2: estradiol.

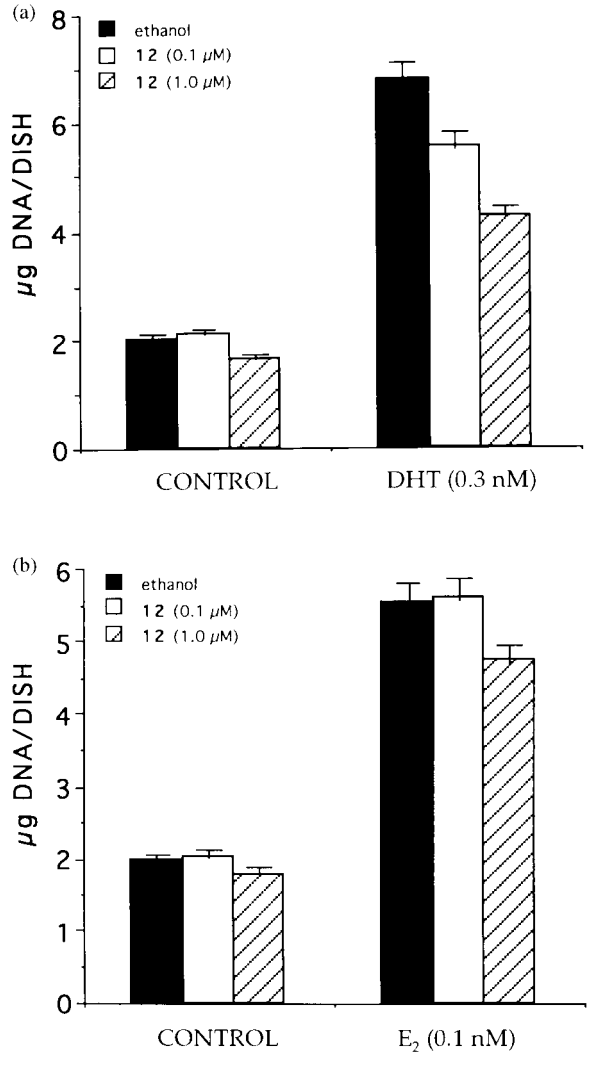


Figure 3. (**A**) Effect of compound **12** on the growth of androgen-sensitive Shionogi mouse mammary carcinoma cells in absence and in presence of dihydrotestosterone (DHT). (**B**) Effect of compound **12** on the growth of estrogen-sensitive human breast cancer ZR-75-1 cells in absence and in presence of estradiol (E_2).

if they are linked to a Δ^4-dione nucleus. In our series, we found that only the phenolic derivative **11** displays a weak activity on P450 aromatase. The inhibitory potency is clearly abolished when the phenol is alkylated or when the 7α position was substituted with a thioalkyl instead of a thiobenzyl group.

We also evaluated the steroid receptor binding affinity of our series of inhibitors. We found that our synthetic spironolactone derivatives with 7α-thioalkyl and 7α-thioaryl bound to glucocorticoid, progestin, and androgen receptors. The spironolactone template should provide the affinity for the androgen receptor because spirolactone itself efficiently binds to this receptor. The affinity for glucocorticoid and progestin receptors displayed by some of our inhibitors may be attributable to the 7α-substitution. It has been identified that substituents, particularly a 4-dimethylamino phenyl group, at C11β or C7α of an appropriate steroidal template leads to progesterone antagonists and some of them retain affinity for the glucocorticoid receptor.[48–50] However, the compound **12**, which has a 2-(1-piperidinyl)-ethoxybenzylthio group, was totally devoid of such affinity for all tested steroid receptors (AR, PR, GR, ER).

Finally, we conclude that compound **12** is a new potent selective inhibitor of type II 17β-HSD that does not bind to the estrogen receptor as well as the three other tested receptors. This interesting hormonal profile makes it suitable for further studies to establish the regulation of steroid hormone biosynthesis by 17β-HSDs in several biological systems. It can also be used to study the structure of type II 17β-HSD.

Experimental

General method for chemical synthesis

Chemical reagents were purchased from the Aldrich Chemical Company (Milwaukee, WI) and spironolactone was purchased from Steraloids Inc. (Wilton,

NH). Solvents were obtained from BDH Chemicals (Montréal, Canada) or Fischer Chemicals (Montréal, Canada). Tetrahydrofuran (THF) used in anhydrous conditions was distilled from sodium benzophenone ketyl; other commercially available dry solvents were stored under argon. Glassware used in anhydrous conditions was baked for 1 h at 80 °C, assembled hot and filled with argon before use. Standard inert-atmosphere techniques were used for solvents transfers by syringe. Flash-column chromatographies were performed using 230–400 mesh ASTM silica gel 60 (E. Merck, Darmstadt, GE). Infrared spectra (IR) were recorded on a Perkin–Elmer series 1600 FT-IR spectrometer and are reported in cm^{-1}. High-resolution mass spectra (HRMS) obtained from fast atom bombardment (FAB) were provided by the Centre Régional de Spectrométrie de Masse (Université de Montréal, Canada). The NMR spectra were recorded at 300 MHz for ^1H and 75 MHz for ^{13}C on a Bruker AC/F300 spectrometer. The chemical shifts (δ in ppm) were referenced to $CHCl_3$ (δ 7.26 ppm and 77.00 ppm for ^1H and ^{13}C, respectively). The structures shown below are given to help clarify the assignment of carbon and related proton signals.

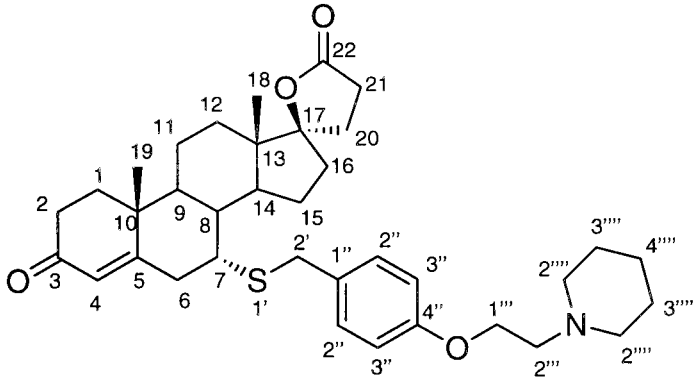
Synthesis of spironolactone derivatives 3–12 (Scheme 1)

3-Oxo-17α-pregna-4, 6-diene 21, 17-carbolactone (canrenone, 3). To a solution of spironolactone (**2**) (2.0 g, 4.8 mmol) in dry THF (150 mL) was added sodium methoxide (1.6 g, 28 mmol) at room temperature under argon atmosphere. The mixture was allowed to stir 18 h. Then, water was added and THF was removed in vacuo. The aqueous phase was acidified to pH 2 and extraction of the crude product was performed with CH_2Cl_2. The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. Purification by column chromatography (CH_2Cl_2/EtOAc, 65/35) gave 1.2 g (72% yield) of canrenone **3**: White amorphous solid; IR ν (KBr) 1770 (C=O, lactone), 1660 (C=O, conjugated ketone), 1617 (C=C); ^1H NMR ($CDCl_3$) δ 1.03 (s, 3H, 18-CH_3), 1.12 (s, 3H, 19-CH_3), 5.68 (s, 1H, 4-CH), 6.07 (d, J = 10.7 Hz, 6-CH), 6.12 (dd, J_1 = 10.7 Hz and J_2 = 2.2 Hz, 1H, 7-CH); ^{13}C NMR ($CDCl_3$) δ 14.42 (C-18), 16.33 (C-19), 20.11 (C-15), 22.46 (C-11), 29.22 (C-21), 31.17 (C-20), 31.65 (C-12), 33.90 (C-2), 33.96 (C-1), 35.49 (C-16), 36.05 (C-10), 37.80 (C-8), 46.44 (C-13), 47.05 (C-9), 50.46 (C-14), 95.31 (C-17), 124.00 (C-4), 128.36 (C-6), 139.34 (C-7), 162.98 (C-5), 176.48 (C-22), 199.30 (C-3); HRMS-FAB calcd for $C_{22}H_{29}O_3$ (MH^+), 341.2117. Found, 341.2111.

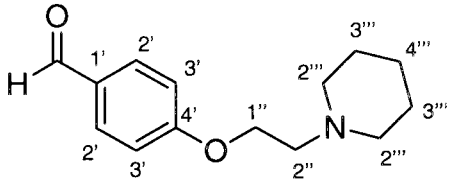
Synthesis of 7α-thioalkyl and 7α-thioaryl derivatives of spironolactone (4–6 and 8–9) (general procedure). Canrenone (**3**) was dissolved in the appropriated thiol (15–20 equiv) under argon and the mixture was gently heated at 45 °C. Then, sodium metal (1 equiv) was added to the solution which was allowed to stir at 60 °C for 45 min to 24 h. Afterward, saturated aqueous NH_4Cl was added until the neutrality was obtained and the crude product was extracted with diethyl ether (**4–6**) or CH_2Cl_2 (**8–9**). The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. Purification of the crude compounds was performed by column chromatography (**4**: hexane/EtOAc, 1/1; **5**: hexane/EtOAc, 6/4; **6**: hexane/EtOAc, 4/6; **8**: hexane/EtOAc, 1/1; **9**: hexane/EtOAc, 1/1).

3-Oxo-17α-pregna-4-ene-7α-(ethylthia)-21, 17-carbolactone (4). White solid (59% yield); IR ν (film) 1775 (C=O, lactone), 1667 (C=O, conjugated ketone), 1616 (C=C); ^1H NMR ($CDCl_3$) δ 0.97 (s, 3H, 18-CH_3), 1.20 (s, 3H, 19-CH_3), 1.22 (t, J = 7.3 Hz, 3H, SCH_2CH_3), 3.06 (m, 1H, 7β-CH), 5.76 (s, 1H, 4-CH); ^{13}C NMR δ ($CDCl_3$) 14.47 (C-18), 14.68 (SCH_2CH_3), 17.94 (C-19), 20.48 (C-15), 22.23 (C-11), 24.68 (SCH_2CH_3), 29.23 (C-21), 31.18 (C-12 and C-21), 33.90 (C-2), 35.25 (C-16), 35.47 (C-1), 38.40 (C-6 and C-10), 39.87 (C-8), 44.92 (C-7), 45.22 (C-14), 45.38 (C-13), 46.85 (C-9), 95.70 (C-17), 126.92 (C-4), 166.90 (C-5), 176.36 (C-22), 198.59 (C-3); HRMS-FAB calcd for $C_{24}H_{35}O_3S$ (MH^+), 403.2307. Found, 403.2291.

3-Oxo-17α-pregna-4-ene-7α-(propylthia)-21, 17-carbolactone (5). White solid (72% yield); IR ν (film) 1770 (C=O, lactone), 1672 (C=O, conjugated ketone), 1620 (C=C); ^1H NMR ($CDCl_3$) δ 0.98 (s, 3H, 18-CH_3), 0.98 (t, J = 7.3 Hz, 3H, $SCH_2CH_2CH_3$), 1.21 (s, 3H, 19-CH_3), 3.03 (m, 1H, 7β-CH), 5.78 (s, 1H, 4-CH); ^{13}C NMR ($CDCl_3$) δ 13.55 ($SCH_2CH_2CH_3$), 14.47 (C-18), 17.94 (C-19), 20.48 (C-15), 22.28 (C-11), 22.91 (CH_2CH_3), 29.23 (C-21), 31.18 (C-12 and C-20), 33.03 (SCH_2), 33.90



compound **12**



compound **14**

(C-2), 35.26 (C-16), 35.46 (C-1), 38.39 (C-10), 38.54 (C-6), 39.94 (C-8), 45.23 (C-7), 45.39 (C-13 and C-14), 46.81 (C-9), 95.71 (C-17), 126.91 (C-4), 166.97 (C-5), 176.68 (C-22), 198.64 (C-3); HRMS-FAB calcd for $C_{25}H_{37}O_3S$ (MH^+), 417.2463. Found, 417.2451.

3-Oxo-17α-pregna-4-ene-7α-(hexylthia)-21, 17-carbolactone (6). White solid (62% yield); IR ν (film) 1771 (C=O, lactone), 1670 (C=O, conjugated ketone), 1618 (C=C); ^{1}H NMR ($CDCl_3$) δ 0.87 (t, J = 6.9 Hz, 3H, $S(CH_2)_5CH_3$), 0.97 (s, 3H, 18-CH_3), 1.20 (s, 3H, 19-CH_3), 3.03 (m, 1H, 7β-CH), 5.76 (s, 1H, 4-CH); ^{13}C NMR ($CDCl_3$) δ 14.01 (CH_2CH_3), 14.52 (C-18), 17.97 (C-19), 20.51 (C-15), 22.34 (C-11), 22.52 (CH_2CH_3), 28.63 (CH_2), 29.26 (C-21), 29.56 (CH_2), 31.02 (CH_2), 31.23 (C-12 and C-20), 31.39 (SCH_2), 33.93 (C-2), 35.31 (C-16), 35.49 (C-1), 38.43 (C-10), 38.55 (C-6), 39.98 (C-8), 45.28 (C-7), 45.41 (C-13), 45.47 (C-14), 46.85 (C-9), 95.75 (C-17), 126.97 (C-4), 166.97 (C-5), 176.71 (C-22), 198.65 (C-3); HRMS-FAB calcd for $C_{28}H_{43}O_3S$ (MH^+), 459.2933. Found, 459.2952.

3-Oxo-17α-pregna-4-ene-7α-(phenylthia)-21, 17-carbolactone (8). White solid (59% yield); IR ν (film) 1770 (C=O, lactone), 1665 (C=O, conjugated ketone), 1618 (C=C); ^{1}H NMR ($CDCl_3$) δ 0.99 (s, 3H, 18-CH_3), 1.20 (s, 3H, 19-CH_3), 3.46 (m, 1H, 7β-CH), 5.68 (s, 1H, 4-CH), 7.28 (m, 3H, 4″-CH and 2″-CH), 7.38 (m, 2H, 3″-CH); ^{13}C NMR ($CDCl_3$) δ 14.46 (C-18), 17.81 (C-19), 20.44 (C-15), 22.19 (C-11), 29.18 (C-21), 31.14 (C-12), 31.19 (C-20), 33.88 (C-2), 35.25 (C-16), 35.48 (C-1), 38.03 (C-6), 38.44 (C-10), 39.92 (C-8), 45.44 (C-13 and C-14), 46.81 (C-9), 50.15 (C-7), 95.56 (C-17), 127.15 (C-4), 127.62 (C-4″), 129.14 (2C, C-3″), 133.32 (2C, C-2″), 133.94 (C-1″), 166.41 (C-5), 176.56 (C-22), 198.60 (C-3); HRMS-FAB calcd for $C_{28}H_{35}O_3S$ (MH^+), 451.2307. Found, 451.2296.

3-Oxo-17α-pregna-4-ene-7α-(benzylthia)-21, 17-carbolactone (9). White solid (74% yield); IR ν (film) 1769 (C=O, lactone), 1665 (C=O, conjugated ketone), 1618 (C=C); ^{1}H NMR ($CDCl_3$) δ 0.90 (s, 3H, 18-CH_3), 1.16 (s, 3H, 19-CH_3), 2.86 (m, 1H, 7β-CH), 3.63 (q_{app}, ABX system, 2H, CH_2Ph), 5.72 (s, 1H, 4-CH), 7.27 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$) δ 14.37 (C-18), 17.82 (C-19), 20.43 (C-15), 21.75 (C-11), 29.15 (C-21), 31.11 (C-12 and C-20), 33.84 (C-2), 35.11 (SCH_2), 35.21 (C-16), 35.40 (C-1), 38.13 (C-6), 38.34 (C-10), 39.72 (C-8), 44.72 (C-7), 45.07 (C-14), 45.25 (C-13), 47.19 (C-9), 95.61 (C-17), 126.88 (C-4), 127.12 (C-4″), 128.41 (2C, C-3″), 128.81 (2C, C-2″), 137.99 (C-1″), 166.75 (C-5), 176.59 (C-22), 198.54 (C-3); HRMS-FAB calcd for $C_{29}H_{36}O_3S$ (MH^+), 465.2463. Found, 465.2479.

3-Oxo-17α-pregna-4-ene-7α-(benzoylthia)-21, 17-carbolactone (7). According to the procedure described by Cella and Tweit,[40–42] canrenone (**3**) (52 mg, 0.15 mmol) was dissolved in 1 mL of thiobenzoic acid. The mixture was allowed to stir at 60 °C for 3 h. Then, water was added and the crude product was extracted with diethyl ether. The organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. Purification by silica gel chromatography (hexane/EtOAc, 4/6) gave 29 mg (40% yield) of **7** as an amorphous white solid: IR ν (film) 1770 (C=O, lactone), 1663 (C=O, conjugated ketone and thioester), 1616 (C=C); ^{1}H NMR ($CDCl_3$) δ 1.01 (s, 3H, 18-CH_3), 1.26 (s, 3H, 19-CH_3), 2.91 and 2.95 (2m, 1H), 4.25 (m, 1H, 7β-CH), 5.71 (s, 1H, 4-CH), 7.45 (m, 2H, 3″-CH), 7.58 (m, 1H, 4″-CH), 7.94 (d, J = 7.4 Hz, 2H, 2″-CH); ^{13}C NMR ($CDCl_3$) δ 14.57 (C-18), 17.78 (C-19), 20.54 (C-15), 22.35 (C-11), 29.13 (C-21), 31.05, 31.17, 33.90 (C-2), 35.15 (C-16), 35.63 (C-1), 38.54 (C-10), 39.11 (C-7), 40.07 (C-6), 45.01 (C-8), 45.46 (C-16), 46.07 (C-14), 49.68 (C-9), 95.51 (C-17), 126.99 (C-4), 127.35 (2C, C-3″), 128.64 (C-4″), 133.64 (2C, C-2″), 136.94 (C-1″), 165.69 (C-5), 176.51 (C-22), 190.26 (SC=O), 198.61 (C-3); HRMS-FAB calcd for $C_{29}H_{35}O_4S$ (MH^+), 479.2256. Found, 479.2248.

Synthesis of 7α-(4′-*O*-alkylated-thiobenzyl) derivatives of spironolactone (10 and 12) (general procedure). Canrenone (**3**) was dissolved in the appropriated synthetic thiols **17** or **18** (15–20 equiv) under argon and the mixture was gently heated at 45 °C. Then, sodium metal (1 equiv) was added to the solution which was allowed to stir for 45 min (**10**) or for 60 min (**12**). Afterward, saturated aq NH_4Cl was added to the cooled reaction mixture until the neutrality was obtained and the crude product was extracted with diethyl ether. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. Purifications of the crude compounds were performed by column chromatography (hexane/EtOAc, 95/5 for **10**; CH_2Cl_2/MeOH, 95/5 for **12**).

3-Oxo-17α-pregna-4-ene-7α-[(4-t-butyldimethylsilyloxy)-benzylthia]-21, 17-carbolactone (10). White amorphous solid (91% yield): IR ν (film) 1774 (C=O, lactone), 1672 (C=O, conjugated ketone), 1609 (C=C); ^{1}H NMR ($CDCl_3$) δ 0.17 (s, 6H, $Si(CH_3)_2$), 0.92 (s, 3H, 18-CH_3), 0.96 (s, 9H, $SiC(CH_3)_3$), 1.18 (s, 3H, 19-CH_3), 2.84 (m, 1H, 7β-CH), 3.58 (q_{app}, ABX system, 2H, CH_2Ph), 5.74 (s, 1H, 4-CH), 6.76 (d, J = 8.6 Hz, 2H, 3″-CH), 7.12 (d, J = 8.5 Hz, 2H, 2″-CH); ^{13}C NMR ($CDCl_3$) δ −4.47 ($Si(CH_3)_2$), 14.45 (C-18), 17.87 (C-19), 18.20 ($SiC(CH_3)_3$), 20.49 (C-15), 21.78 (C-11), 25.63 ($SiC(CH_3)_3$), 29.23 (C-21), 31.18 (C-12 and C-20), 33.90 (C-2), 34.50 (SCH_2), 35.27 (C-16), 35.46 (C-1), 38.14 (C-6), 38.39 (C-10), 39.73 (C-8), 44.35 (C-7), 45.15 (C-14), 45.28 (C-13), 47.32 (C-9), 95.70 (C-17), 120.07 (2C, C-3″), 126.96 (C-4), 129.92 (2C, C-2″), 130.59 (C-1″), 154.82 (C-4″), 166.92 (C-5), 176.69 (C-22), 198.65 (C-3); HRMS-FAB calcd for $C_{35}H_{51}O_3SSi$ (MH^+), 595.3278. Found, 595.3262.

3-Oxo-17α-pregna-4-ene-7α-{4-[2-(1-piperidinyl)-ethoxy]-benzylthia}-21, 17-carbolactone (12). Off-white solid (82% yield): IR ν (film) 1772 (C=O, lactone) 1664 (C=O, conjugated ketone), 1610 (C=C); ^{1}H NMR ($CDCl_3$) δ 0.89 (s, 3H, 18-CH_3), 1.15 (s, 3H, 19-CH_3), 2.78 (t, J = 5.9 Hz, 2H, 2‴-CH_2N), 2.79 (m, 1H, 7β-CH), 3.57 (q_{app}, ABX system, 2H, CH_2Ph), 4.08 (t, J = 5.9 Hz, 2H, 1‴-CH_2O), 5.70 (s, 1H, 4-CH), 6.80 (d, J = 8.4 Hz, 2H, 3″-CH), 7.15 (d, J = 8.5 Hz, 2H, 2″-CH); ^{13}C NMR ($CDCl_3$) δ 14.34 (C-18), 17.78 (C-19), 20.38 (C-15), 21.75 (C-11), 23.82 (C-4‴′), 25.47 (2C, C-3‴′;), 29.14 (C-21), 31.07 (C-12 and C-20), 33.80 (C-2),

34.36 (SCH_2), 35.15 (C-16), 35.33 (C-1), 38.03 (C-6), 38.27 (C-10), 39.61 (C-8), 44.44 (C-7), 45.01 (C-14), 45.19 (C-13), 47.16 (C-9), 54.83 (2C, 2‴′-CH_2N), 57.62 (2‴-CH_2N), 65.58 (1‴-CH_2O), 95.65 (C-17), 114.44 (2C, C-3″), 126.77 (C-4), 129.81 (2C, C-2″), 129.89 (C-1″), 157.70 (C-4″), 166.98 (C-5), 176.68 (C-22), 198.64 (C-3); HRMS-FAB calcd for $C_{36}H_{50}O_4NS$ (MH^+), 592.3461. Found, 592.3471.

3-Oxo-17α-pregna-4-ene-7α-(4-hydroxy-benzylthia)-21, 17-carbolactone (11). To a solution of **10** (237 mg, 0.40 mmol) in 30 mL of dry THF at −78 °C was added 0.6 mL of tetrabutylammonium fluoride (1 M solution in THF). The resulting mixture was allowed to stir 45 min at −78 °C under argon. Then, water was added and extraction of the crude product was performed using CH_2Cl_2. The combined organic layers were washed with brine, dried over $MgSO_4$ and evaporated in vacuo. Silica gel chromatography (CH_2Cl_2/acetone, 95/5) gave 159 mg (88% yield) of **11** as a white amorphous solid: IR ν (film) 3325 (OH, phenol), 1762 (C=O, lactone), 1678 (C=O, conjugated ketone), 1619 (C=C); ^{1}H NMR ($CDCl_3$) δ 0.93 (s, 3H, 18-CH_3), 1.18 (s, 3H, 19-CH_3), 2.89 (m, 1H, 7β-CH), 3.60 (q_{app}, ABX system, 2H, CH_2Ph), 5.72 (s, 1H, 4-CH), 6.78 (d, J = 8.6 Hz, 2H, 3″-CH), 7.15 (d, J = 8.3 Hz, 2H, 2″-CH); ^{13}C NMR ($CDCl_3$) δ 14.46 (C-18), 17.89 (C-19), 20.49 (C-15), 21.90 (C-11), 29.27 (C-21), 31.18 (C-12 and C-20), 33.84 (C-2), 34.72 (SCH_2), 35.27 (C-16), 35.38 (C-1), 38.31 (C-10), 38.43 (C-6), 39.81 (C-8), 44.84 (C-7), 45.14 (C-14), 45.32 (C-13), 47.29 (C-9), 95.91 (C-17), 115.42 (2C, C-3″), 126.81 (C-4), 129.55 (C-1″), 130.06 (2C, C-2″), 155.31 (C-4″), 167.85 (C-5), 177.00 (C-22), 199.33 (C-3); HRMS-FAB calcd for $C_{29}H_{37}O_3S$ (MH^+), 481.2413. Found, 481.2428.

Preparation of synthetic thiols 17 and 18 (Scheme 2)

4-(*t*-Butyldimethylsilyloxy)-benzaldehyde (13). To a solution of *p*-hydroxybenzaldehyde (20.0 g, 160 mmol) in 150 mL of dry DMF was added imidazole (55.7 g, 800 mmol) and TBDMS–Cl (48.2 g, 320 mmol) under argon atmosphere. The resulting mixture was stirred for 4 h at room temperature. Then, water was added and the crude compound was extracted twice with ether and twice with CH_2Cl_2. Organic solvents were dried over $MgSO_4$ and removed under reduced pressure. The crude oil was purified by filtration on silica gel (hexane/EtOAc, 95/5) to give 31.5 g (83% yield) of **13** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.23 (s, 6H, Si(CH_3)$_2$), 0.97 (s, 9H, SiC(CH_3)$_3$), 6.92 (d, J = 8.6 Hz, 2H, 3′-CH), 7.77 (d, J = 8.6 Hz, 2H, 2′-CH), 9.86 (s, 1H, CHO); ^{13}C NMR ($CDCl_3$) δ −4.44 (Si(CH_3)$_2$), 18.17 (SiC(CH_3)$_3$), 25.48 (SiC(CH_3)$_3$), 120.40 (2C, C-3′), 130.32 (C-4′), 131.91 (2C, C-2′), 161.44 (C-1′), 190.80 (CHO).

4-[2-(1-Piperidinyl)-ethoxy]-benzaldehyde (14). To a solution of *p*-hydroxybenzaldehyde (0.5 g, 4.1 mmol) in 500 mL of dry acetonitrile was added K_2CO_3 (8.49 g, 61.5 mmol) and 1-(2-chloroethyl)piperidine hydrochloride (7.53 g, 41.0 mmol) under argon atmosphere. The resulting mixture was gently refluxed overnight. Then, an aqueous solution of 5% HCl was added until a neutral pH was obtained. The crude compound was extracted three times with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, and removed under reduced pressure. The crude oil was purified by chromatography on silica gel ($CHCl_3$/MeOH, 95/5) to give 512 mg (54% yield) of **14** as a yellow liquid: ^{1}H NMR ($CDCl_3$) δ 1.43 (m, 2H, 4‴-CH_2), 1.58 (m, 4H, 3‴-CH_2), 2.50 (m, 4H, 2‴-CH_2N), 2.78 (t, J = 6.0 Hz, 2H, 2″-CH_2N), 4.17 (t, J = 6.0 Hz, 2H, 1″-CH_2O), 6.98 (d, J = 8.7 Hz, 2H, 3′-CH), 7.80 (d, J = 8.3 Hz, 2H, 2′-CH), 9.85 (s, 1H, CHO); ^{13}C NMR ($CDCl_3$) δ 24.01 (C-4‴), 25.80 (2C, C-3‴), 54.96 (2C, 2‴-CH_2N), 57.55 (2″-CH_2N), 66.31 (1″-CH_2O), 114.74 (2C, C-3′), 129.80 (2C, C-2′), 131.82 (C-4′), 163.78 (C-1′), 190.64 (CHO).

Synthesis of thioacetyl derivatives 15 and 16. To a solution of the aldehyde **13** (31.5 g, 130 mmol) or **14** (7.3 g, 27 mmol) in MeOH (240 mL or 60 mL, respectively) at 0 °C was added sodium borohydride (7.6 g, 200 mmol or 1.6 g, 41 mmol) and the reaction was stirred at 0 °C for 45 min (**13**) and for 3 h (**14**). Water was then added and MeOH removed under reduced pressure. The crude alcohol was extracted twice with diethyl ether and twice with EtOAc. Organic solvents were dried over $MgSO_4$ and removed in vacuo. The crude alcohols were used without purification for the next step. To an efficiently stirred solution of triphenylphosphine (52.5 g, 200 mmol or 7.1 g, 27 mmol) in THF (200 mL or 50 mL) was added diethyl azodicarboxylate (31.5 mL, 200 mmol or 4.3 mL, 27 mmol) at 0 °C. The mixture was stirred for 30 min, after which a thick white precipitate was obtained. Then, the crude alcohols dissolved in THF and thiolacetic acid (21.4 mL, 300 mmol or 5.8 mL, 81 mmol) were added dropwise while the temperature was maintained below 0 °C. The reaction was stirred for 2 h at 0 °C. Solvent was removed in vacuo. The residue was purified by silica gel chromatography (hexane/EtOAc, 95/5 for **15**; CH_2Cl_2/MeOH, 98/2 for **16**).

4-(*t*-Butyldimethylsilyloxy)-α-thioacetyl-toluene (15). Colorless oil (63% yield); ^{1}H NMR ($CDCl_3$) δ 0.18 (s, 6H, Si(CH_3)$_2$), 0.97 (s, 9H, SiC(CH_3)$_3$), 2.34 (s, 3H, $COCH_3$), 4.07 (s, 2H, CH_2Ph), 6.75 (d, J = 8.5 Hz, 2H, 3′-CH), 7.13 (d, J = 8.3 Hz, 2H, 2′-CH); ^{13}C NMR ($CDCl_3$) δ −4.48 (Si(CH_3)$_2$), 18.11 (SiC(CH_3)$_3$), 25.60 (SiC(CH$_3$)$_3$), 30.25 and 32.94 (SCH_2 and COCH_3), 120.05 (2C, C-3′), 129.81 (2C, C-2′), 131.82 (C-1′), 154.82 (C-4′), 195.14 (COCH$_3$).

4-[2-(1-Piperidinyl)-ethoxy)]-α-thioacetyl-toluene (16). White wax (89% yield); ^{1}H NMR ($CDCl_3$) δ 1.46 (m, 2H, 4‴-CH_2), 1.66 (m, 4H, 3‴-CH_2), 2.32 (s, 3H, $COCH_3$), 2.63 (m, 4H, 2‴-CH_2N), 2.88 (t, J = 5.8 Hz, 2H, 2″-CH_2N), 4.05 (s, 2H, SCH_2Ph), 4.15 (t, J = 5.8 Hz, 2H, 1″-CH_2O), 6.80 (d, J = 8.7 Hz, 2H, 3′-CH), 7.17 (d, J = 8.7 Hz, 2H, 2′-CH); ^{13}C NMR ($CDCl_3$) δ 23.36 (C-4‴), 24.78 (2C, C-3‴), 30.29 and 32.81 (SCH_2 and COCH_3), 54.51 (2C, 2‴-CH_2N), 57.18 (2″-CH_2N), 64.91 (1″-CH_2O), 114.56 (2C, C-3′), 129.91 (2C, C-2′), 131.88 (C-1′), 154.47 (C-4′), 195.27 (COCH$_3$).

Synthesis of thiols 17 and 18. To a stirred solution of **15** or **16** (5 g, 17 mmol or 6.2 g, 21 mmol) in dry THF (200

or 120 mL) was added lithium aluminum hydride (960 mg, 25 mmol or 1.2 g, 32 mmol) at 0 °C. The reaction was stirred for 3 h at 0 °C. Then, EtOAc and water were added. The pH was brought to 5 with an aqueous solution of 10 % HCl and the resulting slurry was filtered. The crude product was extracted with EtOAc and the combined organic layers were washed successively with Rochelle's salt, brine, dried over $MgSO_4$ and evaporated under reduced pressure. Purifications were performed by silica gel chromatography (hexane/EtOAc, 98/2 for **17**; CH_2Cl_2/acetone/MeOH, 80/15/5 for **18**).

4-(*t*-Butyldimethylsilyloxy)-benzyl mercaptan (17). Colorless liquid (76% yield); ^{1}H NMR ($CDCl_3$) δ 0.19 (s, 6H, Si(CH_3)$_2$), 0.98 (s, 9H, SiC(CH_3)$_3$), 1.73 (t, J = 7.3 Hz, 1H, SH), 3.70 (d, J = 7.3 Hz, 2H, CH_2S), 6.78 (d, J = 8.5 Hz, 2H, 3′-CH), 7.18 (d, J = 8.5 Hz, 2H, 2′-CH); ^{13}C NMR ($CDCl_3$) δ −4.44 (Si(CH_3)$_2$), 18.23 (Si*C*(CH_3)$_3$), 25.66 (SiC(*C*H_3)$_3$), 28.43 (CH_2SH), 120.16 (2C, C-3′), 129.06 (2C, C-2′), 133.30 (C-1′), 155.00 (C-4′).

4-[2-(1-Piperidinyl)-ethoxy]-benzyl mercaptan (18). Colorless liquid (43% yield); ^{1}H NMR ($CDCl_3$) δ 1.47 (m, 2H, 4‴-CH_2), 1.63 (m, 4H, 3‴-CH_2), 1.73 (t, J = 7.3 Hz, 1H, SH), 2.54 (m, 4H, 2‴-CH_2N), 2.80 (t, J = 6.0 Hz, 2H, 2″-CH_2N), 3.70 (d, J = 7.3 Hz, 2H, SCH_2Ph), 4.11 (t, J = 5.8 Hz, 2H, 1″-CH_2O), 6.85 (d, J = 8.5 Hz, 2H, 3′-CH), 7.22 (d, J = 8.3 2H, 2′-CH); ^{13}C NMR ($CDCl_3$) δ 24.07 (C-4‴), 25.81 (2C, C-3‴), 28.32 (CH_2SH), 54.95 (2C, 2‴-CH_2N), 57.82 (2″-CH_2N), 65.85 (1″-CH_2O), 114.65 (2C, C-3′), 129.03 (2C, C-2′), 133.22 (C-1′), 157.75 (C-4′).

Biology

Inhibition of type II 17β-HSD (Table 1). Transfected 293 cells with cDNA encoding for type II 17β-HSD were sonicated to liberate the crude enzyme that was used as the enzymatic pool without further purification.[27] The enzymatic assay was performed as follows: a stock solution was first prepared containing the radiolabelled substrate [^{3}H]-Δ^4-dione (5 nM), NADH (1 mM) in a phosphate buffer (pH 7.4, 50 mM KH_2PO_4, EDTA 1 mM, 20% glycerol). For the assay, 890 μL of the stock solution and 10 μL of a solution of inhibitor (EtOH or EtOH/DMSO, 95/5) were added in a tube. The reaction was started by adding 100 μL of a solution of crude enzyme (0.4 μg protein/tube) prepared as above. The mixture was incubated for 1 h at 37 °C, and the reaction was stopped by adding an excess of unlabeled Δ^4-dione and T. Steroids were extracted with diethyl ether and solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2, spotted on a silica gel plate (TLC, 20×20×0.2 cm, Kieselgel 60 F_{254}) and eluted with CH_2Cl_2/EtOAc (9/1). Less polar Δ^4-dione and more polar T were identified on TLC as two rows of visible spots under UV light. Each spot was cut from the plate and stored in a vial with 1 mL EtOH and 10 mL of scintillating solution. Radioactivity was measured in a β-counter. The percent of transformation of [^{3}H]-Δ^4-dione into [^{3}H]-T was calculated as follows: % trans. = 100 X {[^{3}H]-T(cpm)/([^{3}H]-Δ^4-dione(cpm) + [^{3}H]-T(cpm))}. Subsequently, percent of inhibition = [(% trans. of control − % trans. of compound)/(% trans. of control)]×100. 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 this inhibition curve, the IC_{50} value was calculated by a computer using an unweighted iterative least-squares method for 4-parameters logistic curve fitting.

Inhibition of type II 17β-HSD (Table 2). The same enzymatic source as reported above was used to catalyze the oxidation of 0.1 μM of [^{14}C]-testosterone to [^{14}C]-Δ^4-dione, using NAD^+ as cofactor. The enzymatic assay was performed exactly as described in our earlier report.[27]

Inhibition of P450 aromatase (Table 3). Aromatase activity was obtained from 293 cells transfected with the cDNAs encoding P450 aromatase and NADPH P450 reductase. The cells were sonicated in 50 mM sodium phosphate buffer (pH 7.4), containing 20% glycerol and 1 mM EDTA, and were used as the enzymatic pool without further purification (3.6 μg protein/tube). The transformation of 0.1 μM of [^{14}C]-Δ^4-dione to [^{14}C]-estrone was evaluated. The enzymatic reaction was carried out at 37 °C in 1 mL 50 mM sodium phosphate buffer (pH 7.4), containing 20% glycerol and 1 mM EDTA, in the presence of the indicated concentrations of inhibitor (0.3 μM and 3.0 μM in EtOH or EtOH/DMSO (95/5)) for 1 h. After incubation, steroids were extracted twice with diethyl ether and separated by TLC (toluene/acetone, 4/1). Radioactivity signals associated to [^{14}C]-Δ_4-dione and [^{14}C]-estrone were detected and quantified using a Phosphor Imager (Sunny Vale, CA). Data were calculated as mentioned above.

Steroid receptor binding affinity screening (Table 4). The affinity binding assays on estrogen and progestin receptors from rat uterine were carried out under the standard procedure established in our laboratory.[51] Assays for androgen receptor from rat ventral prostate were performed according to the procedure described by Luo and co-workers.[52] For binding assay on glucocorticoid receptor from rat liver, a slightly modified version of the procedure described by Asselin and co-workers was used.[53] In this case, separation of bound and free steroids was achieved with dextran-coated charcoal adsorption instead of protamine sulfate precipitation.

Proliferative/antiproliferative activities (Fig. 3). The in vitro evaluation of compound **12** on the proliferation of estrogen-sensitive human breast cancer ZR-75-1 cells as well as its ability to inhibit the 0.1 nM E_2-induced proliferation was performed according to the procedure described by our laboratory.[54] Assays on the proliferation of Shionogi mammary carcinoma cells as well as on the inhibition of 0.3 nM DHT-induced proliferation were carried out using a previously described method.[52]

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References and Notes

1. Baum, M. *J. Steroid Biochem.* **1990**, *36*, 187.
2. Labrie, F. *Endocrinology and Metabolism Clinics of North America* **1991**, *20*, 845.
3. von Angerer, E. In *The Estrogen Receptor as a Target for Rational Drug Design*; Springer-Verlag; R.G. Landes Company: Austin, Texas, USA, 1995.
4. Dowsett, M. *J. Steroid Biochem. Molec. Biol.* **1991**, *39*, 805.
5. Labrie, F. *Molecular and Cellular Endocrinology* **1991**, *78*, C113.
6. Labrie, F.; Luu-The, V.; Lin, S.-X.; Labrie, C.; Simard, J.; Breton, R.; Bélanger, A. *Steroids* **1997**, *62*, 148.
7. Labrie, F.; Simard, J.; Bélanger, A.; Lin, S.-X.; Luu-The, V.; Labrie, C. In *Cancer: what are the roles and the sources of steroid hormones and the possible role of adipose tissue*; Angel, A.; Anderson, H.; Bouchard, C.; Lau, D.; Leiter, L.; Mendelson, R., Eds.; John Libbey: London, 1996; Vol. 7, pp 559–567.
8. Peltoketo, H.; Isomaa, V.; Mäentausta, O.; Vihko, R. *FEBS Lett.* **1988**, *239*, 73.
9. Luu-The, V.; Labrie, C.; Zhao, H.-F.; Couët, J.; Lachance, Y.; Simard, J.; Leblanc, G.; Côté, J.; Bérubé, D.; Gagné, R.; Labrie F. *Mol. Endocrinol.* **1989**, *3*, 1301.
10. Luu-The, V.; Labrie, C.; Simard, J.; Lachance, Y.; Zhao, H.-F.; Couët, J.; Leblanc, G.; Labrie, F. *Mol. Endocrinol.* **1990**, *4*, 268.
11. Wu, L.; Einstein, M.; Geissler, W. M.; Chan, H. K.; Elliston, K. O.; Andersson, S. *J. Biol. Chem.* **1993**, *268*, 12964.
12. Casey, M. L.; MacDonald, P. C.; Andersson, S. *J. Clin. Invest.* **1994**, *94*, 2135.
13. Geissler, W. M.; Davis, D. L.; Wu, L.; Bradshaw, K. D.; Sushma, P.; Mendonca, B. B.; Elliston, K. O.; Wilson, J. D.; Russel, D. W.; Andersson, S. *Nature Genetics* **1994**, *7*, 34.
14. Adamski, J.; Normand, T.; Leenders, F.; Monté, D.; Begue, A.; Stéhelin, D.; Jungblut, P. W.; de Launoit, Y. *Biochem. J.* **1995**, *311*, 437.
15. Bonney, R. C.; Reed, M. J.; Davidson, K.; Beranek, P. A.; James, V. H. T. *Clinical Endocrinology* **1983**, *19*, 727.
16. Martel, C.; Rhéaume, E.; Takahashi, M.; Trudel, C.; Couët, J.; Luu-The, V.; Simard, J.; Labrie, F. *J. Steroid Biochem. Molec. Biol.* **1992**, *41*, 597.
17. Ghosh, D.; Pletnev, V. Z.; Zhu, D.-W.; Wawrzak, Z.; Duax, W. L.; Pangborn, W.; Labrie, F.; Lin, S.-X. *Structure* **1995**, *3*, 503.
18. Azzi, A.; Rehse, P.; Zhu, D.-W.; Campbell, R. L.; Labrie, F.; Lin, S.-X. *Nature Structural Biology* **1996**, *3*, 665.
19. Breton, R.; Housset, D.; Mazza, C.; Fontecilla-Camps, J. C. *Structure* **1996**, *4*, 905.
20. Penning, T. M. *Endocrine-Related Cancer* **1996**, *3*, 41.
21. Sam, K.-M.; Boivin, R. P.; Auger, S.; Poirier, D. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2129.
22. Pelletier, J. D.; Poirier, D. *Bioorg. Med. Chem.* **1996**, *4*, 1617.
23. Tremblay, M. R.; Auger, S.; Poirier, D. *Bioorg. Med. Chem.* **1995**, *3*, 505.
24. Poirier, D.; Dionne, P.; Auger, S. *J. Steroid Biochem. Molec. Biol.* **1998**, *64*, 83.
25. Tremblay, M. R.; Poirier, D. *J. Steroid Biochem. Molec. Biol.* **1998**, *66*, 179.
26. Blomquist, C. H.; Lindemann, N. J.; Hakanson, E. Y. *Arch. Biochem. Biophys.* **1985**, *239*, 206.
27. Luu-The, V.; Zhang, Y.; Poirier, D.; Labrie, F. *J. Steroid Biochem. Molec. Biol.* **1995**, *55*, 581.
28. Elo, J. P.; Akinola, L. A.; Poutanen, M.; Vihko, P.; Kyllönen, A. P.; Lukkarineen, O.; Vihko, R. *Int. J. Cancer* **1996**, *66*, 37.
29. Carsol, J.-L.; Martin, P.-M.; de Launoit, Y. *Neuroendocrinology* **1994**, *60*, 445.
30. Blomquist, C. H.; D'Ascoli, P. T. *Human Reproduction* **1995**, *10*, 2685.
31. Beaudoin, C.; Blomquist, C. H.; Tremblay, Y. *Endocrinology* **1995**, *136*, 3807.
32. Blomquist, C. H.; Leung, B. S.; Beaudoin, C.; Poirier, D.; Tremblay, Y. *J. Endocrinology* **1997**, *153*, 453.
33. Auger, S.; Luu-The, V.; Sam, K.-M.; Poirier, D. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2045.
34. Sam, K.-M.; Auger, S.; Luu-The, V.; Poirier, D. *J. Med. Chem.* **1995**, *38*, 4518.
35. Claussner, A.; Nedelec, L.; Nique, F.; Philibert, D.; Teutsch, G.; Van de Velde, P. *J. Steroid Biochem. Molec. Biol.* **1992**, *41*, 609.
36. Gauthier, S.; Caron, B.; Cloutier, J.; Dory, Y. L.; Favre, A.; Larouche, D.; Mailhot, J.; Ouellet, C.; Schwerdtfeger, A.; Leblanc, G.; Martel, C.; Simard, J.; Mérand, Y.; Bélanger, A.; Labrie, C.; Labrie, F. *J. Med. Chem.* **1997**, *40*, 2117.
37. Palkowitz, A. D.; Glasebrook, A. L.; Thrasher, K. J.; Hauser, K. L.; Short, L. L.; Phillips, D. L.; Muehl, B. S.; Sato, M.; Shetler, P. K.; Cullinan, G. J.; Pell, T. R.; Bryant, H. U. *J. Med. Chem.* **1997**, *40*, 1407.
38. Grese, T. A.; Sluka, J. P.; Bryant, H. U.; Cullinan, G. J.; Glasebrook, A. L.; Jones, C. D.; Matsumoto, K.; Palkowitz, A. D.; Sato, M.; Termine, J. D.; Winter, M. A.; Yang, N. N.; Dodge, J. A. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 14105.
39. Brueggemeier, R. W.; Floyd, E. E.; Counsell, R. E. *J. Med. Chem.* **1978**, *21*, 1007.
40. Cella, J. A.; Tweit, R. C. *J. Org. Chem.* **1959**, *24*, 1109.
41. Dodson, R. M.; Tweit, R. C. *J. Am. Chem. Soc.* **1959**, *81*, 1224.
42. Schaub, R. E.; Weiss, M. J. *J. Org. Chem.* **1961**, *26*, 3915.
43. Oehlschlager, A. L.; Singh, S. M.; Sharma, S. *J. Org. Chem.* **1991**, *56*, 3856.
44. O'Reilly, J. M.; Li, N.; Duax, W. L.; Brueggemeier, R. W. *J. Med. Chem.* **1995**, *38*, 2842.
45. Darby, M. V.; Lovett, J. A.; Brueggemeier, R. W.; Groziak, M. P.; Counsell, R. E. *J. Med. Chem.* **1985**, *28*, 803.
46. Brodie, A. M. H. *J. Steroid Biochem. Molec. Biol.* **1994**, *48*, 281.
47. Santner, S. J.; Santen, R. J. *J. Steroid Biochem. Molec. Biol.* **1993**, *45*, 383.
48. Ray, S.; Sharma, I. *Pharmazie* **1987**, *42*, 656.
49. Dwivedy, I.; Ray, S.; Grover, A. *Prog. Drug Res.* **1993**, *40*, 239.
50. Grunwell, J. F.; Benson, H. D.; Johnston, J. O. N.; Petrow, V. *Steroids* **1976**, *27*, 759.
51. Luo, S.; Martel, C.; Gauthier, S.; Mérand, Y.; Bélanger, A.; Labrie, C.; Labrie, F. *Int. J. Cancer* **1997**, *73*, 735.
52. Luo, S.; Martel, C.; Leblanc, G.; Candas, B.; Singh, S. M.; Labrie, C.; Simard, J.; Bélanger, A.; Labrie, F. *Endocrine-Related Cancer* **1996**, *3*, 229.
53. Asselin, J.; Melanon, R.; Moachon, G.; Bélanger, A. *Cancer Res.* **1980**, *40*, 1612.
54. Poirier, D.; Auger, S.; Mérand, Y.; Simard, J.; Labrie, F. *J. Med. Chem.* **1994**, *37*, 1115.