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3β-Sulfamate Derivatives of C19 and C21 Steroids Bearing a *t*-Butylbenzyl or a Benzyl Group: Synthesis and Evaluation as Non-estrogenic and Non-androgenic Steroid Sulfatase Inhibitors

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A series of C19 and C21 steroids bearing one or two inhibiting groups (3β-sulfamate and 17α- or 20(S)-*t*-butylbenzyl or benzyl) were synthesized and tested for inhibition of steroid sulfatase activity. When only a sulfamate group was added to dehydroepiandrosterone, androst-5-ene-3β,17β-diol, pregnenolone and 20-hydroxy-pregnenolone, no significant inhibition of steroid sulfatase occurred at concentrations of 0.3 and 3 μM. With only a *t*-butylbenzyl or a benzyl group, a stronger steroid sulfatase inhibition was obtained in the androst-5-ene than in the pregn-5-ene series. Comparative results from the screening tests and the IC_{50} values have shown that the effect of a sulfamate moiety as a second inhibiting group can be combined to the *t*-butylbenzyl or benzyl effect in the C19 and C21 steroid series. The 3β-sulfamoyloxy-17α-*t*-butylbenzyl-5-androsten-17β-ol (10) was thus found to be the most active compound with IC_{50} values of 46 ± 8 and 14 ± 1 nM, respectively for the transformations of E_1S to E_1 and DHEAS to DHEA. The IC_{50} values of compound 10 are similar to that of 17α-*t*-butylbenzyl-estradiol, which was previously reported by our group as a good steroid sulfatase reversible inhibitor, but remains higher than that of the potent inactivators estrone-3-*O*-sulfamate (EMATE) and 17α-*t*-butylbenzyl-EMATE. However, contrary to these two latter inhibitors, compound 10 did not induce any proliferative effect on estrogen-sensitive ZR-75-1 cells nor on androgen-sensitive Shionogi cells at concentrations tested, suggesting that this steroid sulfatase inhibitor is non estrogenic and non androgenic.

Keywords: C19 steroid; C21 steroid; Sulfatase; Inhibitor; Sulfamate; Estrogen

INTRODUCTION

In steroidogenesis, successive enzymatic reactions are responsible for the formation of sex steroid hormones, androgens (testosterone and dihydrotestosterone) and estrogens (estrone, estradiol, and androst-5-ene-3β,17β-diol), from cholesterol (Figure 1).[1] In androgen synthesis, the 17β-hydroxysteroid dehydrogenases (17β-HSDs) (types 3 and 5) mediate the conversion of 4-androstene-3,17-dione (Δ^4-dione) into testosterone (T).[2–4] Δ^4-dione is directly secreted by the adrenals and results from the transformation of dehydroepiandrosterone (DHEA) under the activity of 3β-HSD enzyme.[2–4] Humans and primates are unique in that their adrenals release in the circulation large amounts of steroid precursors, Δ^4-dione, DHEA and especially dehydroepiandrosterone sulfate (DHEAS).[5] Since DHEAS is the major steroid secreted by adrenals, steroid sulfatase is likely to play an important role in the production of androgens in men. In women the ovaries represent the major site for the production of estrogens during the adult life, through the conversion of Δ^4-dione into estrone (E_1) by aromatase and its conversion to the main female hormone estradiol (E_2) by type 1 17β-HSD. Another active estrogen, the C19 steroid androst-5-ene-3β,17β-diol (Δ^5-diol),[6] is produced in peripheral tissues and originates mainly from DHEAS by successive transformations by the enzymes steroid sulfatase and type 1

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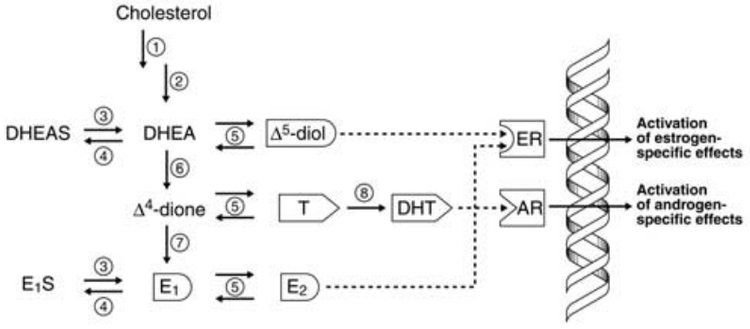


FIGURE 1 Enzymatic steps involved in the biosynthesis of estrogens (E_2, E_1, and Δ^5-diol) and androgens (DHT and T) from cholesterol. ER, estrogen receptor; AR, androgen receptor; DHEAS, dehydroepiandrosterone sulfate; DHEA, dehydroepiandrosterone; Δ^5-diol, androst-5-ene-3β,17β-diol; Δ^4-dione, androst-4-ene-3β,17β-dione; E_1S, estrone sulfate; E_1, estrone; E_2, estradiol. The enzymes are: (1) P450 side-chain cleavage; (2) P450 17α-hydroxylase/P450 17,20-lyase; (3) steroid sulfatase; (4) steroid sulfotransferase; (5) 17β-hydroxysteroid dehydrogenases; (6) 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerases; (7) aromatase; (8) 5α-reductases.

17β-HSD.[7,8] In peripheral tissues, the role of local estrogen production might be to adjust hormone concentration levels in target cells depending on local requirements.[5] In menopausal women, once the aromatase activity of the ovaries has ceased, the desulfation of estrone sulfate (E_1S) and of DHEAS by steroid sulfatase that takes place in peripheral tissues becomes an enzymatic step of major importance in estrogen production.

Steroid sulfatase, the enzyme that catalyses the hydrolysis of steroid sulfates such as DHEAS and E_1S to unconjugated DHEA and E_1, respectively, controls the first step in hormone synthesis at the cellular level (Figure 1). While active hormones are characterized by a specificity of interaction with their corresponding receptors and may act at very low concentrations in target tissues, steroid sulfates are found in high concentrations in circulation, and their local transformation by steroid sulfatase might exert a "long distance" control on the synthesis of androgens and estrogens. Steroid sulfatase inhibition should allow a reduction in substrate amounts for the enzymes involved in the final steps of androgen and estrogen production. Thus, steroid sulfatase inhibitors might serve as valuable therapeutic agents for diseases such as breast and prostate cancers which are stimulated by estrogens and androgens, respectively. In the last ten years, many research groups became interested in developing steroid sulfatase inhibitors.[8,9] C3-sulfate analogues of the natural substrates were first investigated as potential inhibitors. Estrone sulfamate (EMATE), the first irreversible, active-site related, and very potent inhibitor, was developed by Reed and Potter.[10–12] However, as this inhibitor has also shown estrogenic agonist effects,[13,14] steroidal and nonsteroidal analogues with comparable or greater sulfatase inhibition potency, and free of estrogenic activity were developed[8,15–18] to provide new drug candidates for breast cancer therapy.

Our research group previously identified and reported the steroid sulfatase inhibitory effect of a substituent (benzyl, *t*-butylbenzyl, and others) introduced in position 17α of E_2.[19–21] Furthermore, we demonstrated that the combination of a *t*-butylbenzyl and a sulfamate inhibiting groups introduced respectively at positions C17α and C3 on the same E_2 nucleus results in considerably higher inhibition of steroid sulfatase than that obtained with EMATE.[22] As, unfortunately, these compounds were estrogenic, we next investigated strategies to generate non estrogenic inhibitors. In the first one, a *t*-butylbenzyl group was introduced on a nonsteroidal nucleus with good results.[17] In the second strategy, a benzyl group was added in position 17α or 20 of an androstane (C19) and a pregnane (C21) nucleus, respectively.[23] To increase the potency of these latter inhibitors, we then continued our work by studying their 3β-sulfamate analogues (Figure 2). In this article, we report their chemical synthesis, their steroid sulfatase inhibitory activity, and their proliferative activity on estrogen-sensitive ZR-75-1 cells and androgen-sensitive Shionogi cells.

MATERIALS AND METHODS

General

Chemical reagents and anhydrous solvents (except THF) were purchased from Aldrich Chemical Company (Milwaukee, WI, USA). Steroids 3β-hydroxy-5-pregnen-20-one (pregnenolone; PREG), dehydroepiandrosterone (DHEA) and 5-androstene-3β,17β-diol (Δ^5-diol) were purchased from Steraloids (Wilton, NH, USA) while radioactive enzyme substrates [6,7-^{3}H]estrone sulfate (E_1S) ammonium salt (43.10 Ci/mmol) and [1,2,6,7-^{3}H]dehydroepiandrosterone sulfate (DHEAS) sodium salt (60.00 Ci/mmol) were from

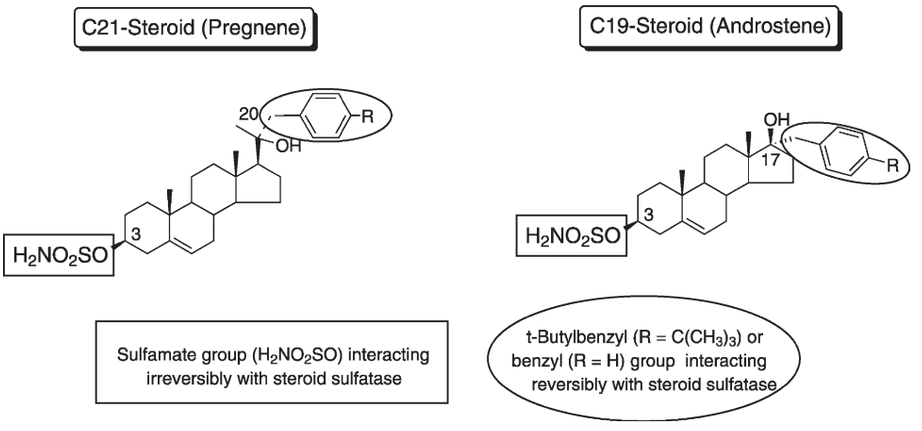


FIGURE 2 Target inhibitors of steroid sulfatase bearing one or two inhibiting group(s) on a C21-steroid nucleus (pregnene) or a C19-steroid nucleus (androstene). The inhibiting group at C3 is a sulfamate whereas the other at C17 or C20 is a benzyl (R = H) or a *t*-butylbenzyl (R = $C(CH_3)_3$). See Schemes 1 and 2 for the exact structure of synthesized compounds.

New England Nuclear (Boston, MA, USA). Other solvents were obtained from BDH Chemicals (Montréal, Qc, Canada) or Fisher Chemicals (Montréal, Qc, Canada). Dry THF was distilled from sodium/benzophenone ketyl prior to use. Sulfamoyl chloride used in reactions was synthesized following the procedure described by Peterson *et al.*[24] Thin-layer chromatography (TLC) was performed on 0.20 mm silica gel 60 F_{254} plates (E. Merck, Darmstadt, Germany), and 230–240 mesh ASTM silica gel 60 (E. Merck) was used for flash column chromatography. Infrared spectra (IR) are expressed in cm^{-1} and were obtained on a Perkin-Elmer 1600 (series FTIR) spectrophotometer (Norwalk, CT, USA). ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded with a Bruker AC/F 300 spectrometer (Billerica, MA, USA), respectively at 300 and 75 MHz. The chemical shifts (δ) are expressed in ppm and referenced to chloroform (7.26 ppm for ^1H and 77.00 ppm for ^{13}C) or to acetone (2.05 ppm for ^1H and 206.26 ppm for ^{13}C). In ^1H NMR, only specific signals were reported from upfield to downfield. Low-Resolution Mass Spectra (LRMS) were recorded on a PE Sciex API-150ex apparatus (Foster City, CA, USA) equipped with a turbo ionspray source. High-Resolution Mass Spectra (HRMS) were provided by Le Centre Régional de Spectrométrie de masse (Université de Montréal, Montréal, Qc, Canada). The purity of synthesized and tested compounds was determined by HPLC (Waters Associates, Milford, MA, USA) by using an ultraviolet detector (205–220 nm).

Chemistry

Protection of PREG as TBDMS Derivative (Synthesis of 1)

To a solution of pregnenolone (317 mg, 1 mmol) in anhydrous CH_2Cl_2 under an argon atmosphere were added successively imidazole (4 mmol), *t*-butyldimethylsilyl chloride (2 mmol) and a catalytic amount of 4-dimethylaminopyridine (DMAP). The reaction mixture was stirred at room temperature overnight, then quenched by addition of water. The crude product was extracted with CH_2Cl_2 and the organic phase was dried over $MgSO_4$ and evaporated under vacuum. Purification by flash column chromatography with hexanes/acetone 95:5 gave 419 mg of the expected TBDMS ether **1**.

3β-*t*-BUTYLDIMETHYLSILYLOXY-5-PREGNEN-20-ONE (**1**)

White solid (97% yield), Rf = 0.39 (hexanes/acetone 95:5); IR ν (KBr) 1701 (C = O, ketone); ^1H NMR ($CDCl_3$) δ 0.08 (s, *t*-BuSi(CH_3)$_2$), 0.65 (s, CH_3-18), 0.91 (s, *t*-*Bu*Si(CH_3)$_2$), 1.02 (s, CH_3-19), 2.15 (s, CH_3-21), 2.56 (t_{app}, CH-17α), 3.50 (m, CH-3α), 5.32 (m, CH-6); ^{13}C NMR ($CDCl_3$) δ −4.62 (2x), 13.19, 18.24, 19.39, 21.03, 22.75, 24.46, 25.89 (3x), 31.55, 31.79 (2x), 31.99, 36.53, 37.34, 38.81, 42.72, 43.99, 50.00, 56.91, 63.68, 72.49, 120.83, 141.48, 209.60; LRMS: calculated for $C_{27}H_{47}O_2Si$ $(MH)^+$ 431.2.

Alkylation of 17- and 20-ketosteroids with t*-butylbenzyl Magnesium Chloride (Synthesis of 2 and 9)*

In a three-neck flask, connected to a refrigerant and under an argon atmosphere, was introduced 10 mmol of magnesium turnings which were activated by heating for 10 min. After normal cooling at room temperature, an iodine crystal was carefully added along with a few drops of 4-*t*-butylbenzyl bromide. Stirring was slowly started as enough anhydrous diethylether was added to cover the magnesium. After initiation of the reaction (disappearance of the iodine characteristic brown–yellow colour), a 0.5 molar solution containing 10 mmol of 4-*t*-butylbenzyl bromide in anhydrous diethyl ether was added dropwise and the temperature

maintained under the reflux, using a cooling bath of ice-water. After 1 h at room temperature, when the magnesium had completely reacted to form the Grignard reagent, the flask was cooled at 0°C and a solution of ketosteroid (**1** or DHEA) in diethyl ether was added dropwise. The reaction mixture was left overnight to rise to room temperature, then quenched with a saturated solution of ammonium chloride, and extracted with diethyl ether (3x). The combined organic phase was dried over $MgSO_4$, evaporated under vacuum and the crude product purified by flash column chromatography using hexanes/acetone as eluent (90:10 for **2**, and 75:25 for **9**).

3β-*t*-BUTYLDIMETHYLSILYLOXY-20(S)-*t*-BUTYLBENZYL-5-PREGNEN-20(S)-OL (**2**)

White solid (90% yield), Rf = 0.37 (hexanes/acetone 80:20); IR ν (KBr) 3580 (OH, alcohol); ^{1}H NMR ($CDCl_3$) δ 0.06 (s, *t*-BuSi(CH_3)$_2$), 0.89 (s, CH_3-18 and *t*-*Bu*Si(CH_3)$_2$), 1.0 (s, CH_3-19), 1.20 (s, CH_3-21), 1.31 (s, C(CH_3)$_3$), 2.57 and 2.79 (2d, J = 13.1 Hz, AB system, CH_2Ph*t*-Bu), 3.48 (m, CH-3α), 5.32 (d, J = 5.1 Hz, CH-6), 7.11 (d, J = 8.1 Hz, CH-3′ and CH-5′), 7.31 (d, J = 8.1 Hz, CH-2′ and CH-6′); ^{13}C NMR ($CDCl_3$) δ −4.60 (2x), 13.50, 18.25, 19.40, 20.92, 22.83, 23.91, 25.93 (3x), 26.33, 31.37 (4x), 31.81, 32.06, 34.36, 36.58, 37.37, 40.13, 42.81, 42.87, 48.54, 50.09, 56.98, 58.71, 72.61, 74.83, 121.06, 124.96 (2x), 130.38 (2x), 134.39, 141.57, 149.08; LRMS: calculated for $C_{38}H_{62}O_2Si$ $(M)^+$ 578.3.

17α-*t*-BUTYLBENZYL-5-ANDROSTENE-3β,17β-DIOL (**9**)

White solid (90% yield), Rf = 0.20 (hexanes/acetone 80:20); IR ν (film) 3350 (OH, alcohols); ^{1}H NMR (acetone-d_6) δ 0.95 (s, CH_3-18), 1.06 (s, CH_3-19), 1.30 (s, C(CH_3)$_3$), 2.63 and 2.83 (2d, J = 13.3 Hz, AB system, CH_2Ph*t*-Bu), 3.40 (m, CH-3α), 5.34 (d, J = 4.9 Hz, CH-6), 7.25 (d, J = 8.5 Hz, CH-3′ and CH-5′), 7.30 (d, J = 8.6 Hz, CH-2′ and CH-6′); ^{13}C NMR (acetone-d_6) δ 15.15, 19.98, 21.75, 24.38, ~30 (masked under solvent peaks), 31.88 (3x), 32.10, 32.66, 33.59, 34.06, 34.93, 37.60, 38.48, 42.87, 43.46, 47.53, 51.47, 51.88, 71.76, 83.68, 121.64, 125.30 (2x), 131.90 (2x), 137.42, 142.56, 149.09; HRMS: calculated for $C_{30}H_{43}O_2$ $(M - H)^+$ 435.32629, found 435.32440; HPLC purity = 96.3% (C-4 YMC Pak column, MeCN/H_2O/MeOH: 35/20/45).

Alkylation of 17- and 20-ketosteroids with Benzyl Magnesium Chloride (Synthesis of 5 and 11)

Compounds **5** and **11** were synthesized as previously reported.[23]

Hydrolysis of TBDMS Ether of 2 (Synthesis of 3)

Under an argon atmosphere, a stirring solution of 1 mmol (578 mg) of **2** in freshly distilled THF (40 mL) was treated at 0°C with 3.5 eq. of tetrabutylammonium fluoride (TBAF) (1.0 *M* in THF). The reaction was completed after 3 h and then quenched by addition of water. The product was extracted with EtOAc, the organic phase dried over $MgSO_4$ and evaporated under vacuum. Purification by flash column chromatography with hexanes/acetone 85:15 gave the expected diol **3**.

20(S)-*t*-BUTYLBENZYL-5-PREGNENE-3β,20(S)-DIOL (**3**)

White solid (85% yield), Rf = 0.27 (hexanes/acetone 80:20); IR ν (film) 3300 (OH, alcohols); ^{1}H NMR ($CDCl_3$) δ 0.89 (s, CH_3-18), 1.01 (s, CH_3-19), 1.20 (s, CH_3-21), 1.31 (s, C(CH_3)$_3$), 2.57 and 2.78 (2d, J = 13.1 Hz, AB system, CH_2Pht-Bu), 3.51 (m, CH-3α), 5.37 (d, J = 5.1 Hz, CH-6), 7.11 (d, J = 8.2 Hz, CH-3′ and CH-5′), 7.30 (d, J = 8.1 Hz, CH-2′ and CH-6′); ^{13}C NMR ($CDCl_3$) δ 13.50, 19.36, 20.92, 22.81, 23.90, 26.33, 31.37 (3x), 31.54, 31.63, 31.77, 34.35, 36.47, 37.22, 40.09, 42.28, 42.85, 48.54, 50.00, 56.94, 58.67, 71.77, 74.82, 121.60, 124.96 (2x), 130.38 (2x), 134.38, 140.76, 149.10; HRMS: calculated for $C_{32}H_{47}O_2$ $(M - H)^+$ 463.35760, found 463.35910; HPLC purity = 99.1% (C-18 Nova Pak column, MeCN/H_2O/MeOH: 45/15/40).

Sulfamoylation of Alcohols 3, 5, 9, PREG and DHEA

SYNTHESIS OF **4**, **7**, **10**, AND **12**

To a stirred solution containing 1 mmol of 3-hydroxy steroid (**3**, PREG, **9**, or DHEA) and 3 mmol of 2,6-di-*t*-butyl-4-methylpyridine (DBMP), in CH_2Cl_2, under an argon atmosphere, was added in portions sulfamoyl chloride (6 mmol). The reaction was monitored by TLC and stopped after 1–2 h by adding water. After extraction with CH_2Cl_2, the organic phase was evaporated, the crude product preabsorbed on silica gel and purified by flash column chromatography with hexanes/acetone (80:20 for **7**, **10**, and **12**, and 85:15 for **4**) containing 2% of triethylamine.

3β-SULFAMOYLOXY-20(S)-*t*-BUTYLBENZYL-5-PREGNEN-20(S)-OL (**4**) White solid (73% yield), Rf = 0.06 (hexanes/acetone 85:15); IR ν (film) 3605, 3400, and 3292 (OH and NH_2); ^{1}H NMR ($CDCl_3$) δ 0.89 (s, CH_3-18), 1.02 (s, CH_3-19), 1.20 (s, CH_3-21), 1.31 (s, C(CH_3)$_3$), 2.52 (m, 2H), 2.58 and 2.78 (2d, J = 13.1 Hz, AB system, CH_2Pht-Bu), 4.43 (m, CH-3α), 4.82 (s, SO_2NH_2), 5.43 (d, J = 4.8 Hz, CH-6), 7.11 (d, J = 8.2 Hz, CH-3′ and CH-5′), 7.31 (d, J = 8.2 Hz, CH-2′ and CH-6′); ^{13}C NMR ($CDCl_3$) δ 13.49, 19.18, 20.88, 22.81, 23.87, 26.33, 28.52, 31.25, 31.37 (3x), 31.75, 34.36, 36.38, 36.90, 38.74, 39.98, 42.84, 48.55, 49.83, 56.83, 58.62, 74.85, 83.19, 123.57, 124.97 (2x), 130.38 (2x), 134.31, 138.84, 149.14; HRMS: calculated for $C_{32}H_{48}O_3NS$ $(MH\text{-}H_2O)^+$ 526.33551, found

526.33740; HPLC purity = 99.1% (C-4 YMC Pak column, MeCN/H_2O/MeOH: 35/20/45).

3β-SULFAMOYLOXY-5-PREGNEN-20-ONE (**7**) White solid (97% yield), Rf = 0.13 (hexanes/acetone/triethylamine 80:20:2); IR ν (film) 3255 (NH_2), 1683 (C = O, ketone); ^{1}H NMR (acetone-d_6) δ 0.62 (s, CH_3-18), 1.06 (s, CH_3-19), 2.08 (s, CH_3-21), 2.48 (m, 2H), 2.61 (t_{app}, CH-17α), 4.35 (m, CH-3α), 5.42 (d, J = 5.2 Hz, CH-6), 6.62 (s, SO_2NH_2); ^{13}C NMR (acetone-d_6) δ 13.45, 19.56, 21.78, 23.41, 25.08, ~30 (masked under solvent peaks), 31.57, 32.63, 32.75, 37.36, 37.95, 39.51, 39.73, 44.52, 51.05, 57.61, 64.08, 81.53, 123.61, 140.61, 205.88; HRMS: calculated for $C_{21}H_{34}O_4NS$ $(M + H)^+$ 396.22086, found 396.22190; HPLC purity = 100% (C-18 Nova Pak column, MeCN/H_2O/MeOH: 35/40/25).

3β-SULFAMOYLOXY-17α-*t*-BUTYLBENZYL-5-ANDROSTEN-17β-OL (**10**) White solid (98% yield), Rf = 0.17 (hexanes/acetone/triethylamine, 75:25:2); IR ν (film) 3368 and 3270 (OH and NH_2); ^{1}H NMR ($CDCl_3$) δ 0.94 (s, CH_3-18), 1.07 (s, CH_3-19), 1.32 (s, $C(CH_3)_3$), 2.55 (m, 2H), 2.58 and 2.83 (2d, J = 13.4 Hz, AB system, CH_2Ph*t*-Bu), 4.45 (m, CH-3α), 4.76 (s, SO_2NH_2), 5.45 (d, J = 4.8 Hz, CH-6), 7.19 (d, J = 8.1 Hz, CH-3′ and CH-5′), 7.33 (d, J = 8.1 Hz, CH-2′ and CH-6′); ^{13}C NMR ($CDCl_3$) δ 14.11, 19.24, 20.74, 23.72, 28.53, 31.25, 31.37 (3x), 31.74, 32.80, 33.77, 34.37, 36.54, 37.00, 38.74, 41.79, 46.27, 50.00, 50.81, 83.00, 83.13, 123.38, 125.09 (2x), 130.65 (2x), 134.98, 138.96, 149.14; HRMS: calculated for $C_{30}H_{44}O_4NS$ $(M - H)^+$ 514.29913, found 514.29670; HPLC purity = 99.8% (C-18 Nova Pak column, MeCN/H_2O/MeOH: 35/25/40).

3β-SULFAMOYLOXY-5-ANDROSTEN-17-ONE(**12**) White solid (90% yield), Rf = 0.08 (hexanes/acetone/triethylamine 80:20:2); IR ν (film) 3224 and 3106, (NH_2), 1724 (C = O, ketone); ^{1}H NMR (acetone-d_6) δ 0.87 (s, CH_3-18), 1.09 (s, CH_3-19), 2.45 (m, 4H), 4.32 (m, CH-3α), 5.46 (d, J = 5.2 Hz, CH-6), 6.60 (s, SO_2NH_2); ^{13}C NMR (acetone-d_6) δ 13.87, 19.65, 21.17, 22.49, ~30 (masked under solvent peaks), 31.57, 32.32, 32.51, 36.11, 37.47, 37.85, 39.71, 48.02, 51.30, 52.52, 81.43, 123.29, 140.75, 219.57; HRMS: calculated for $C_{19}H_{30}O_4NS$ $(M + H)^+$ 368.18954, found 368.18840; HPLC purity = 99.9% (C-18 Nova Pak column, MeCN/H_2O/MeOH: 35/53/12).

SYNTHESIS OF **6**

To a stirred solution of 1 mmol of 3-hydroxysteroid **5**[23] in ethylene glycol dimethyl ether (DME) and under an argon atmosphere, was added excess sodium hydride (10 mmol) and the mixture was stirred for 1 h at room temperature. After the anion was formed, the suspension was cooled at 0°C and sulfamoyl chloride (4 mmol) was added in portions. The reaction mixture was allowed to rise to room temperature overnight, quenched by addition of water, and the aqueous phase extracted with EtOAc. The organic phase was dried over $MgSO_4$, evaporated under vacuum and the crude product was purified by flash column chromatography (hexanes/acetone 85:15 containing 2% of triethylamine) to give the starting alcohol **5** (58%) and sulfamate **6** (28%).

3β-SULFAMOYLOXY-20(S)-BENZYL-5-PREGNEN-20(S)-OL(**6**) White solid, Rf = 0.08 (hexanes/acetone/triethylamine, 85:15:2); IR ν (film) 3542, 3368, and 3270 (OH and NH_2); ^{1}H NMR ($CDCl_3$) δ 0.89 (s, CH_3-18), 1.02 (s, CH_3-19), 1.19 (s, CH_3-21), 2.52 (m, 2H), 2.61 and 2.82 (2d, J = 13.0 Hz, AB system, CH_2Ph), 4.44 (m, CH-3α), 4.68 (s, SO_2NH_2), 5.43 (d, J = 5.0 Hz, CH-6), 7.23 (m, CH_2Ph); ^{13}C NMR (acetone-d_6) δ 14.03, 19.68, 21.76, 23.52, 24.78, 26.90, ~30 (masked under solvent peaks), 32.25, 32.66, 37.32, 37.94, 39.76, 41.11, 43.66, 50.65, 51.09, 57.97, 59.34, 75.11, 81.59, 123.79, 126.87, 128.65 (2x), 131.60 (2x), 139.65, 140.58; LRMS: calculated for $C_{28}H_{40}O_4NS$ $(M - H)^-$ 486.3. HPLC purity = 98.3% (C-18 Nova Pak column, MeCN/H_2O/MeOH: 35/25/40).

Reduction of Ketones 7, 12 and PREG (Synthesis of 8, 13 and 20-OH-PREG)

To a stirred solution of 1 mmol of ketosteroid (**7**, **12** or **PREG**) in MeOH/THF (1:1) or MeOH (for PREG) at 0°C was added 3.3 eq. of sodium borohydride. After 30 min, the MeOH was evaporated, water was added, and the mixture extracted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. Purification by flash chromatography with hexanes/acetone (80:20) containing 2% of triethylamine (for **8** and **13**) gave the desired products.

3β-SULFAMOYLOXY-5-PREGNEN-20(R)-ol (**8**)

White solid (95% yield), Rf = 0.04 (hexanes/acetone 80:20); IR ν (film) 3477 and 3292 (OH and NH_2); ^{1}H NMR ($CDCl_3$) δ 0.77 (s, CH_3-18), 1.03 (s, CH_3-19), 1.14 (d, J = 6.0 Hz, CH_3-21), 2.53 (m, 2H), 3.74 (m, CH-20), 4.44 (m, CH-3α), 4.66 (s, SO_2NH_2), 5.42 (d, J = 5.1 Hz, CH-6); ^{13}C NMR (acetone-d_6) δ 12.60, 19.70, 21.71, 24.33, 25.40, 26.48, ~30 (masked under solvent peaks), 32.69, 32.85, 37.38, 37.98, 39.76, 40.56, 43.24, 51.35, 57.27, 59.23, 70.26, 81.59, 123.73, 140.63; LRMS: calculated for $C_{21}H_{34}O_4NS$ $(M - H)^-$ 396.7. HPLC purity = 99.9% (C-18 Nova Pak column, MeCN/H_2O/MeOH: 35/40/25).

3β-SULFAMOYLOXY-5-ANDROSTEN-17β-OL (**13**)

White solid (92% yield), Rf = 0.10 (hexanes/acetone/triethylamine, 75:25:2); IR ν (film) 3332, (OH and NH_2); ^{1}H NMR (acetone-d_6) δ 0.76 (s, CH_3-18), 1.07 (s, CH_3-19), 2.48 (m, 2H), 3.57 (m, CH-17α), 4.32 (m, CH-3α), 5.41 (d, J = 5.1 Hz, CH-6), 6.62 (s, $SONH_2$); ^{13}C NMR (acetone-d_6) δ 11.60, 19.73, 21.56, 24.22, ~30 (masked under solvent peaks), 31.10,

32.41, 32.91, 37.44, 37.68, 38.01, 39.78, 43.65, 51.42, 52.38, 81.57, 81.88, 123.65, 140.66; LRMS: calculated for $C_{19}H_{31}O_4NS$ $(M)^+$ 369.3; HPLC purity = 99.4% (C-18 Nova Pak column, MeCN/H_2O/MeOH: 30/58/12).

5-PREGNEN-3β,20(R)-DIOL (20-OH-PREG)

White solid (92% yield), Rf = 0.19 (hexanes/acetone 80:20); IR ν (film) 3287 (OH); ^{1}H NMR ($CDCl_3$) δ 0.77 (s, CH_3-18), 1.02 (s, CH_3-19), 1.14 (d, J = 6.3 Hz, CH_3-21), 3.53 (m, CH-3α), 3.74 (m, CH-20), 5.35 (d, J = 5.2 Hz, CH-6); ^{13}C NMR (acetone-d_6) δ 12.65, 19.97, 21.80, 24.38, 25.47, 26.54, ~30 (masked under solvent peaks), 32.71, 32.88, 37.53, 38.44, 40.71, 43.29, 43.54, 51.59, 57.44, 59.31, 70.31, 71.88, 121.68, 142.59; HRMS: calculated for $C_{21}H_{34}O_2$ $(M)^+$ 318.25589, found 318.25740. HPLC purity = 99.7% (C-4 YMC Pak column, MeCN/H_2O/MeOH: 30/45/25).

Steroid Sulfatase Assay

Human embryonic kidney (HEK)-293 cells (American Type Culture Collection, Rockville, MD, USA), transiently transfected with a sulfatase expression vector (pCMV-sulfa), were used as source of steroid sulfatase activity as previously reported.[22] For the assay, the HEK-293 cell homogenate was prepared by repeated freezing (−80°C) and thawing (5 times), and homogenization using a Dounce homogenizer. The reaction was carried out at 37°C in 1.25 mL of 100 mM Tris–acetate buffer (pH 7.4) containing 5 mM of EDTA, 10% glycerol, 100 μM of [^{3}H]E_1S or [^{3}H]DHEAS as substrate, and an ethanolic solution of the compound to be tested (at appropriate concentrations). After 2 h of incubation, the reaction was stopped by adding 1.25 mL of xylene. The tubes were then shaken and centrifuged at 2000g for 10 min to separate the organic and aqueous phases. Radioactivity in 750 μL of each phase (organic: free steroids; aqueous: sulfated steroids) was determined by liquid scintillation counting with a Beckman LS3801 (Irvine, CA, USA). The distribution of steroids in the organic and aqueous phases, respectively, was first determined using radiolabeled E_1 and E_1S as well as DHEA and DHEAS, and we found that the contaminated-distribution was less than 5%. Background contamination was determined using the same incubation conditions, and without enzyme source it was found to be less than 5% and was substracted in the calculation of the enzymatic activity. The results were expressed as percentage (%) of [^{3}H]E_1 or [^{3}H]DHEA produced (100% for the control without inhibitor) and the percentage (%) of inhibition then calculated. The IC_{50} values were determined using the DE_{50} program (CHUL Research Center, Québec, Canada).

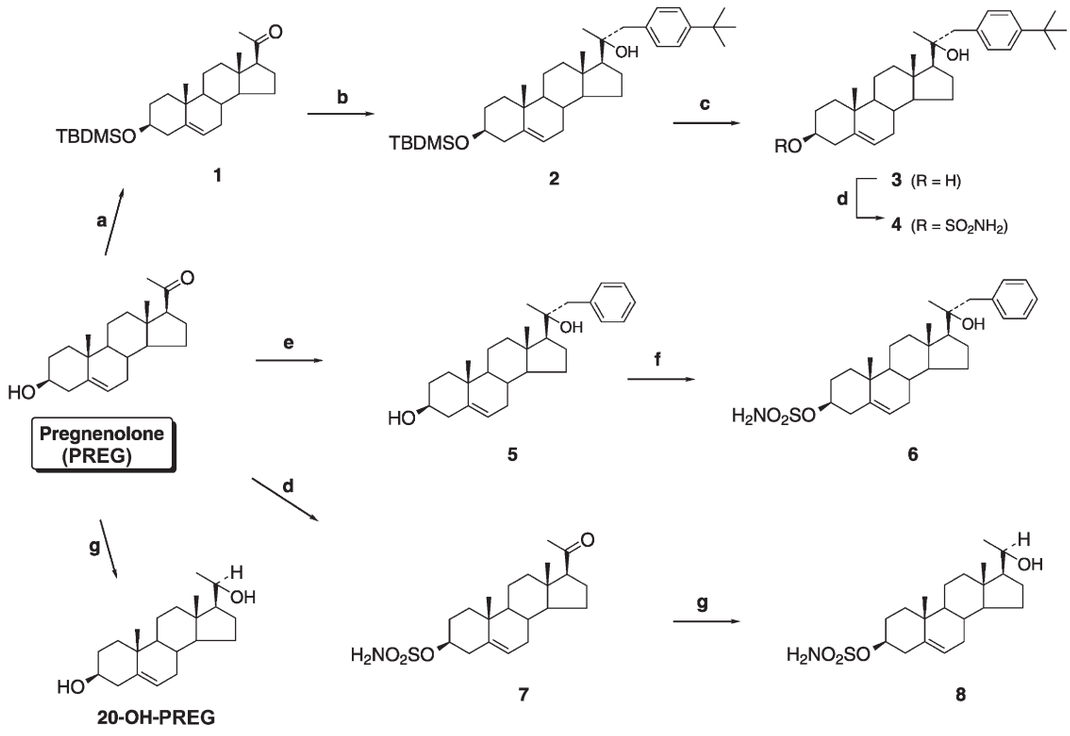
Proliferative Effect on ZR-75-1 (ER^+) Cells and Shionogi (AR^+) Cells

Assays for the proliferation of estrogen-sensitive (ER^+) ZR-75-1 human breast cancer cells were carried out according to the procedure previously described by Poirier *et al.*[25] and the selected compounds were tested at two concentrations, 0.03 and 1 μM. The assays for the proliferation of androgen-sensitive (AR^+) Shionogi mammary carcinoma cells were carried out according to the procedure previously described by Sam *et al.*[26] and the selected compounds were tested at two concentrations, 0.1 and 1 μM. In Table 3, the stimulation of the basal cell proliferation was identified by Yes or No.

RESULTS

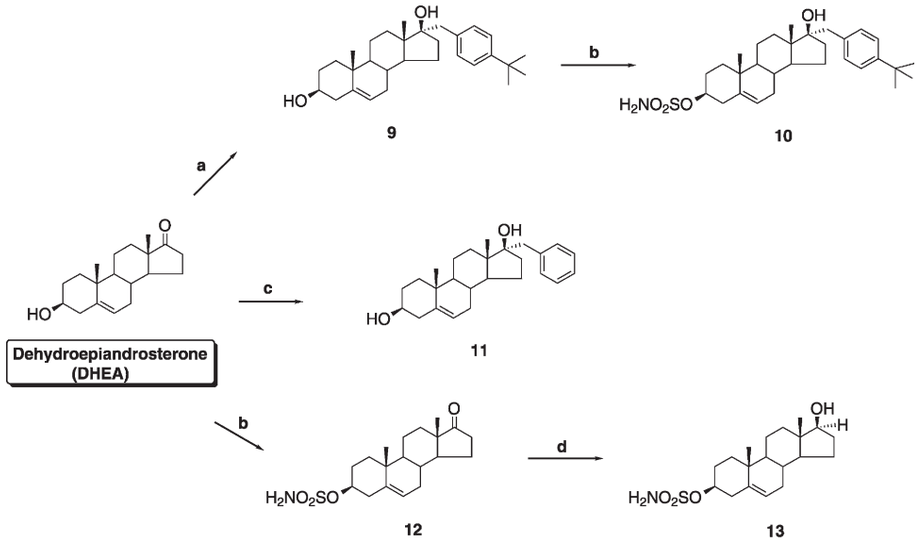
Chemical Synthesis of Compounds 3–13

Following our previous investigation of the potential of both androstane and pregnane derivatives for steroid sulfatase inhibition,[23] we synthesized two corresponding series of compounds, pregnenes and androstenes, bearing one or two inhibiting groups, *t*-butylbenzyl (or benzyl) and sulfamate (Schemes 1 and 2). In the pregnene series, pregnenolone (PREG) was the starting material for the synthesis of compounds **3**–**8** (Scheme 1). Prior to the introduction of *t*-butylbenzyl group in position 20, the 3β-OH of PREG was protected as a *t*-butyldimethylsilyl (TBDMS) ether. Alkylation of compound **1** required the use of an excess of the corresponding Grignard reagent prepared from 4-*t*-butylbenzyl bromide and magnesium turnings and led to the major 20α-OH (or 20S) isomer, in accord with Cram's rule[27] and experimental results that we reported previously for the synthesis of the 20-benzyl analogue.[23] The silyl ether of the alkylated product **2** was then removed at 0°C using a solution of tetrabutylammonium fluoride (TBAF) in THF, to obtain the corresponding diol **3**. Selective sulfamoylation of compound **3** at the 3β-OH was achieved in good yield (73% of **4**) following an improved procedure that used 2,6-di-*t*-butyl-4-methylpyridine (DBMP) as base in CH_2Cl_2.[28] This procedure was used for the synthesis of all the other sulfamoylated derivatives in both steroidal series, except **6**. For the latter, sodium hydride and ethylene glycol dimethyl ether were respectively the base and solvent. Alkylation of PREG with commercially available benzyl magnesium chloride in the presence of cerium chloride as catalyst gave the benzyl derivative **5** in 96% yield as previously reported.[23] The 20-OH (20S) isomer that resulted as major product from this reaction was purified and used for the synthesis of the sulfamoylated derivative **6**. Finally, the C20 ketone group of sulfamate



SCHEME 1 Synthesis of pregnene derivatives **3**–**8**. The reagents and conditions are: (a) TBDMS-Cl, imidazole, CH_2Cl_2, r.t., overnight; (b) Mg, *t*-BuBnBr, diethyl ether, 0°C, 12 h; (c) TBAF, THF, 0°C-r.t., 3 h; (d) sulfamoyl chloride,[24] DBMP, CH_2Cl_2, r.t., 1 h; (e) $PhCH_2MgCl$, $CeCl_3$, THF, −78°C, 5 h; (f) 1. **5**, NaH, DME, r.t., 1 h; 2. sulfamoyl chloride, 0°C; (g) $NaBH_4$, MeOH/THF, 0°C, 30 min.

intermediate **7** prepared from PREG was reduced with a solution of sodium borohydride in THF/methanol (1:1) to give the expected alcohol **8** as the major 20β (20R) isomer, in accord with Cram's rule.[27] Correspondingly, the DHEA derivatives **9**–**13** (Scheme 2) were synthesized from DHEA by following a similar synthetic sequence to that used to obtain the target compounds **3**–**8** of the pregnene series, except that alkylation reactions were performed successfully without protecting the secondary alcohol in position 3 and that the addition of a Grignard reagent to a C19-steroidal



SCHEME 2 Synthesis of androstane derivatives **9**–**13**. The reagents and conditions are: (a) Mg, *t*-BuBnBr, diethyl ether, 0°C, 12 h; (b) sulfamoyl chloride,[24] DBMP, CH_2Cl_2, r.t., 1 h; (c) $PhCH_2MgCl$, $CeCl_3$, THF, −78°C, 5 h; (d) $NaBH_4$, MeOH/THF, 0°C, 30 min.

ketone affords almost exclusively the 17α-alkylated product.[20]

Screening Assay of Sulfatase Inhibition by Compounds 3–13

All the synthesized compounds, including PREG, 20-OH-PREG, DHEA and Δ^5-diol, were first tested in a screening assay for the inhibition of steroid sulfatase activity. For the screening assay, an homogenate of transfected HEK-293 cells served as source of steroid sulfatase to transform the [^{3}H]-labeled E_1S (100 μM) into [^{3}H]-labeled E_1. The inhibitory effect was measured at two inhibitor concentrations, 0.3 and 3 μM. The comparative results regarding the inhibitory potency of each compound of the pregnene and androstene series are listed in Tables I and II, respectively.

In both series of compounds, no significant inhibition was observed for steroids such as PREG, 20-OH-PREG, DHEA, and Δ^5-diol tested as reference, nor for their 3-sulfamoylated derivatives (**7**, **8**, **12** and **13**). These results were in agreement with those previously reported for DHEA sulfamate (**12**) by other researchers.[12] However, in the same test, higher percentages of inhibition were observed for compounds with the hydrophobic substituent, *t*-butylbenzyl or benzyl on the D-ring, as well as for their 3-sulfamate derivatives. Moreover, when tested at 3 μM, compounds **3** and **5** from the pregnene series inhibited respectively only 22 and 34% of the enzyme activity, while at the same concentration the inhibition percentages obtained for their analogues **9** and **11** from the androstene series were correspondingly 79 and 53%. Herein, the inhibiting effect of a *t*-butylbenzyl or benzyl substituent seems more important in the androstene series. The addition of a 3-sulfamate substituent to compounds **5** and **9**, as a second inhibiting group, increases the percentage inhibition. Thus inhibition values increase from 17 to 28% at 0.3 μM, and from 34 to 59% at 3 μM, respectively for **5** and **6** (benzyl derivatives of PREG). Similarly, inhibition values increase from 23 to 64% at 0.3 μM and from 79 to 90% at 3 μM, respectively for **9** and **10** (*t*-butylbenzyl derivative of DHEA).

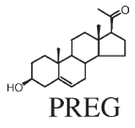
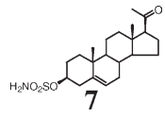
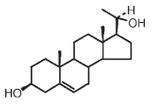
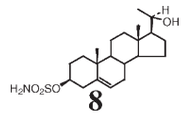
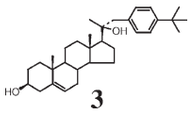
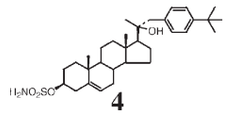
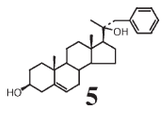
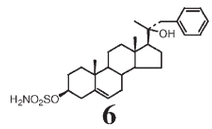
IC_{50} Values

To better discriminate the effect of each inhibiting group, compounds found active in the preliminary screening assay were tested at various concentrations to determine the IC_{50} values (Table III). Potent inhibitors such as 17α-*t*-butylbenzyl-E_2,[20] estrone sulfamate (EMATE)[10,11] and 17α-*t*-butylbenzyl-EMATE[22] were also used as reference compounds. The experimental conditions for measuring IC_{50} were the same as in the screening assay, except that a range of inhibitor concentrations was used and two substrates studied; [^{3}H]-labeled E_1S (100 μM) and [^{3}H]-labeled DHEAS (100 μM). In the pregnene series (C21 steroids), the IC_{50} values determined for compounds **3** and **5** were in the micromolar range suggesting that the *t*-butylbenzyl and benzyl groups have only weak inhibiting effects on the steroid sulfatase activity. However, the corresponding C19-steroid derivatives **9** and **11** were better inhibitors with IC_{50} values of 60 and 360 nM (E_1S as substrate) or 105 and 120 nM (DHEAS as substrate), respectively. In the same test, the IC_{50} values measured for the reference inhibitor 17α-*t*-butylbenzyl-E_2 were 47 nM with E_1S and 13 nM with DHEAS. Thus the efficiency of the hydrophobic group follows roughly this order: C18 > C19 > C21. We were then interested to evaluate the effect of the sulfamate substituent when used as a second inhibiting group on the C21 and C19 steroid nucleus (compounds **4**, **6**, and **10**). In both steroidal series (C21 and C19), the combination of a hydrophobic group and the sulfamate group provided high inhibition of steroid sulfatase. Compounds **4** and **6** were in fact about 3-fold better inhibitors than the corresponding analogues **3** and **5** (without sulfamate). A similar result was obtained when comparing compounds **9** and **10** (IC_{50} = 60 or 105 nM and 46 or 14 nM, respectively). The 3-sulfamoyloxy-17α-*t*-butylbenzyl-5-androsten-17β-ol (**10**) was the most potent inhibitor of our series of C21 and C19-steroids bearing two inhibiting groups. With IC_{50} values of 46 and 14 nM, its inhibitory activities were comparable to that of the reference 17α-*t*-butylbenzyl-E_2 (IC_{50} = 47 and 13 nM), but lower than that of EMATE (IC_{50} = 1.6 and 2.4 nM) or 17α-*t*-butylbenzyl-EMATE (IC_{50} = 0.15 and 1.4 nM).

Estrogenic and Androgenic Activities

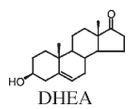
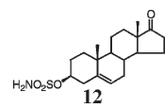
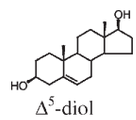
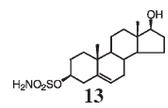
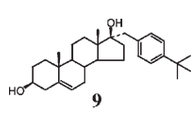
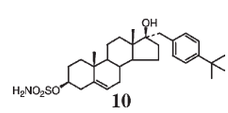
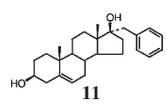
To determine the possible, but unsuitable, estrogenic and androgenic activities of inhibitors **3**–**6** and **9**–**11**, their proliferative effect was determined on two cell lines sensitive to estrogens and androgens respectively. As indicated in Table III, none of the tested compounds showed a proliferative effect on androgen-sensitive Shionogi cells, thus they do not possess any androgenic activity at the higher concentration tested (1 μM). In the same series, only compound **11** with a benzyl substituent at C17β stimulated the proliferation of estrogen-sensitive ZR-75-1 cells. Interestingly, its 17α-*t*-butylbenzyl analogue **10** which is a better inhibitor has no estrogenic activity on ZR-75-1 cells. Since inhibitors **9**–**11** maintain some structural similarities with the estrogenic C19-steroid Δ^5-diol,[6] it is to be expected that they might themselves possess estrogenic properties. However, our results suggest that a C17α-*t*-butylbenzyl substituent used within compounds **9** and **10**

TABLE I Inhibition of steroid sulfatase by pregnene derivatives bearing two (**4** and **6**), one (**3**, **5**, **7**, and **8**), or no (PREG and 20-OH-PREG) substituent (sulfamate, *t*-butylbenzyl, benzyl) in position **3** or/and **20**

	Steroid sulfatase inhibition (%)*			Steroid sulfatase inhibition (%)*	
Pregnenes	0.3 μM	3 μM	3-Sulfamoylated pregnenes	0.3 μM	3 μM
 PREG	3†	8†	 **7**	7	10
 20-OH-PREG	7	10	 **8**	11	23
 **3**	3	22	 **4**	8	60
 **5**	17	34	 **6**	28	59

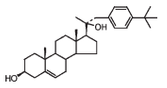
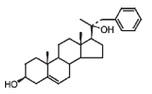
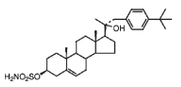
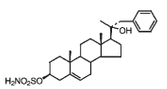
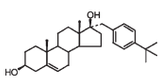
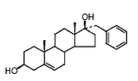
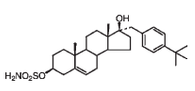
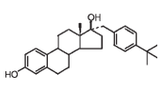
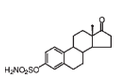
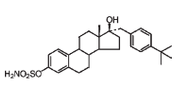
*Data are expressed as percent of inhibition (±10%) for the transformation of [^{3}H]E_1S (100 μM) into [^{3}H]E_1 by homogenates of transfected HEK-293 cells at two concentrations. †Values obtained in another protocol with homogenates of Jeg-3 cells as source of steroid sulfatase activity.

TABLE II Inhibition of steroid sulfatase by androstene derivatives bearing two (**10**), one (**9**, **11**, **12**, and **13**), or no (DHEA and Δ^5-diol) substituent (sulfamate, *t*-butylbenzyl, benzyl) in position **3** or/and **20**

	Steroid sulfatase inhibition (%)*			Steroid sulfatase inhibition (%)*	
Androstenes	0.3 μM	3 μM	3-Sulfamoylated androstenes	0.3 μM	3 μM
 DHEA	15	13	 **12**	3	7
 Δ^5-diol	7	7	 **13**	11	14
 **9**	23	79	 **10**	64	90
 **11**	13†	53†			

*Data are expressed as percent of inhibition (±10%) for the transformation of [^{3}H]E_1S (100 μM) into [^{3}H]E_1 by homogenates of transfected HEK-293 cells at two concentrations of inhibitors. †Values obtained in another protocol with homogenates of Jeg-3 cells as source of steroid sulfatase activity.

TABLE III Inhibitors of steroid sulfatase and their IC_{50} values, estrogenic activity and androgenic activity

Compound (Steroid nucleus)	Chemical structure	Inhibiting group	Steroid sulfatase inhibition (E_1S to E_1) IC_{50} (nM)	Steroid sulfatase inhibition (DHEAS to DHEA) IC_{50} (nM)	ZR-75-1 (ER^+) cells proliferation*	Shionogi (AR^+) cells proliferation†
3 (C21)		*t*-BuBn	>1000	700 ± 170	ND‡	No
5 (C21)		Bn	>1000	715 ± 320	No	No
4 (C21)		*t*-BuBn + Sulfamate	380 ± 90	ND‡	No	No
6 (C21)		Bn + Sulfamate	400 ± 52	250 ± 30	ND‡	No
9 (C19)		*t*-BuBn	60 ± 14	105 ± 260	No	No
11 (C19)		Bn	360 ± 30	120 ± 40	Yes	No
10 (C19)		*t*-BuBn + Sulfamate	46 ± 8	14 ± 1	No	No
17α-*t*-Butylbenzyl-E_2 (C18)		*t*-BuBn	47 ± 1	13 ± 4	Yes	No
EMATE (C18)		Sulfamate	1.6 ± 0.2	2.4 ± 0.2	ND¶	ND‡
17α-*t*-Butylbenzyl-EMATE (C18)		*t*-BuBn + Sulfamate	0.15 ± 0.01§	1.4 ± 0.1§	No¶	No

*Tested at two concentrations of 0.03 and 1 μM. †Tested at two concentrations of 0.1 and 1 μM. ‡ND: not determined. ¶No proliferation observed in ZR-75-1 cells, but the mice uterine and vaginal weights were increased *in vivo* (data not reported). §Data obtained from Ref. [22].

provides not only a better inhibition of steroid sulfatase but also reduced estrogenicity. This can be explained by the fact that a bulky substituent such as *t*-butylbenzyl might efficiently lower ER-binding affinity of a C19 steroidal estrogenic scaffold similar to Δ^5-diol.

DISCUSSION

During our attempts at synthesizing new potent steroid sulfatase inhibitors, we identified the reversible inhibitory effect of a benzyl or *t*-butylbenzyl substituent introduced in C17α-position of estradiol.[19,20] Due to the estrogenic properties of this and other related compounds,[21] we decided to investigate steroid sulfatase inhibitory effect of the same substituent attached in position 17α and 20 of C19 and C21 steroids respectively.[23] Thus, we synthesized and tested for steroid sulfatase inhibition two series of C21- and C19-steroid derivatives bearing alternatively, together or separately, the sulfamate and benzyl pharmacophor (compounds **3**–**8** of pregnene series and **9**–**13** of androstene series). The preliminary screening test inhibition results shown in Tables I and II confirmed our expectations that introduction of the sulfamate group in position 3β of the pregnene or androstene nucleus (compounds **7**, **8** or **12**, **13**) has a far lower inhibition effect than that of EMATE, the 3-*O*-sulfamate of C18-steroid estrone. In fact, DHEA-3-*O*-sulfamate (**12**) was previously reported as a weak inhibitor at an even higher concentration.[12] It was recently pointed out that an aromatic carbon backbone linking the sulfamate moiety better stabilizes the anion released in the hydrolysis reaction, thus resulting in higher inhibition of steroid sulfatase.[9] Indeed, results from our test indicate that aryl sulfamate derivatives from a C18 series are clearly more potent inhibitors than those from C19 and C21 series (alkyl sulfamate).

On the other hand, a hydrophobic group in the C17α- rather than in the C20 position achieves higher inhibition of steroid sulfatase. For example, compound **9** is more potent than the corresponding pregnene analogue **3**. Interestingly, the activity of its sulfamoylated derivative (compound **10**) is comparable to that of 17α-*t*-butylbenzyl-E_2. Thus, as indicated by the percentage of inhibition and proven by the comparative IC_{50} values of compounds **3**, **4**, **5**, **6**, **9** and **10** (Table III), the combination of effects from *t*-butylbenzyl (or benzyl) and sulfamate groups on the same steroid nucleus, results in improved inhibition of steroid sulfatase for both enzymatic transformations studied (E_1S into E_1 and DHEAS into DHEA). These effects might result from interactions with two distinct regions in the enzymatic site. In accord with our previous findings for the C18 steroids,[19–21] the addition of a hydrophobic group like *t*-butylbenzyl, benzyl or others markedly increases the steroid sulfatase inhibitory activity of 3-sulfamoylated steroids from C21 and C19 series and follows this order: C18 > C19 > C21. Further studies will however be necessary to confirm if, as suspected, the alkyl sulfamates reported above are reversible inhibitors. The IC_{50} values of compound **10** are similar to those of 17α-*t*-butylbenzyl-E_2, which was previously reported by our group as a good steroid sulfatase reversible inhibitor, but remain higher than those of potent irreversible inhibitors (inactivators) estrone-3-*O*-sulfamate (EMATE) and 17α-*t*-butylbenzyl-EMATE. However, contrary to these two latter inhibitors, compound **10** did not induce any proliferative effect on estrogen-sensitive ZR-75-1 cells nor on androgen-sensitive Shionogi cells at the concentrations tested, suggesting a non estrogenic and non androgenic steroid sulfatase inhibitor.

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References

[1] Milgrom, E. (1990) "Steroid hormones", In: Baulieu, E.E. and Kelly, P.A., eds, *HORMONES from Molecules to Disease* (Hermann Publishers in Arts and Science, New York), pp. 387–437.
[2] Labrie, F., Luu-The, V., Lin, S.X., Simard, J., Labrie, C., El-Alfy, M., Pelletier, G. and Bélanger, A. (2000) *J. Mol. Endocr.* **25**, 1–16.
[3] Labrie, F., Luu-The, V., Lin, S.X., Labrie, C., Simard, J., Breton, R. and Bélanger, A. (1997) *Steroids* **62**, 148–158.
[4] Luu-The, V. (2001) *J. Steroid Biochem. Mol. Biol.* **76**, 143–151.
[5] Labrie, F. (1991) *Mol. Cell. Endocrinol.* **78**, C113–C118.
[6] Poulin, R. and Labrie, F. (1986) *Cancer Res.* **46**, 4933–4937.
[7] Smith, H.J., Nicholls, P.J., Simons, C. and Le Lain, R. (2001) *Exp. Opin. Ther. Patents* **11**, 789–824.
[8] Poirier, D., Ciobanu, L.C. and Maltais, R. (1999) *Exp. Opin. Ther. Patents* **9**, 1083–1099.
[9] Ahmed, S., Owen, C.P., James, K., Sampson, L. and Patel, C.K. (2002) *Curr. Med. Chem.* **9**, 263–273.
[10] Howarth, N.M., Purohit, A., Reed, M.J. and Potter, B.V.L. (1994) *J. Med. Chem.* **37**, 219–221.
[11] Purohit, A., Williams, G.J., Howarth, N.M., Potter, B.V.L. and Reed, M.J. (1995) *Biochemistry* **34**, 11508–11514.
[12] Reed, M.J., Purohit, A., Woo, L.W.L. and Potter, B.V.L. (1996) *Endocr.-Rel. Cancer* **3**, 9–23.
[13] Purohit, A., Woo, L.W.L., Singh, A., Winterborn, C.J., Potter, B.V.L. and Reed, M.J. (1996) *Cancer Res.* **56**, 4950–4955.
[14] Elger, W., Schwarz, S., Hedden, A., Reddersen, G. and Schneider, B. (1995) *J. Steroid Biochem. Mol. Biol.* **55**, 395–403.
[15] Purohit, A., Woo, L.W.L., Barrow, D., Hejaz, H.A.M., Nicholson, R.I., Potter, B.V.L. and Reed, M.J. (2001) *Mol. Cell. Endocrinol.* **171**, 129–135.

[16] Kolli, A., Chu, G.H., Rhodes, M.E., Inoue, K., Selcer, K.W. and Li, P.K. (1999) *J. Steroid Biochem. Mol. Biol.* **68**, 31–40.
[17] Ciobanu, L.C., Luu-The, V. and Poirier, D. (2002) *J. Steroid Biochem. Mol. Biol.* **80**, 339–353.
[18] Billich, A., Nussbaumer, P. and Lehr, P. (2000) *J. Steroid Biochem. Mol. Biol.* **73**, 225–235.
[19] Poirier, D. and Boivin, R.P. (1998) *Bioorg. Med. Chem. Lett.* **8**, 1891–1896.
[20] Boivin, R.P., Luu-The, V., Lachance, R., Labrie, F. and Poirier, D. (2000) *J. Med. Chem.* **43**, 4465–4478.
[21] Boivin, R.P., Labrie, F. and Poirier, D. (1999) *Steroids* **64**, 825–833.
[22] Ciobanu, L.C., Boivin, R.P., Luu-The, V., Labrie, F. and Poirier, D. (1999) *J. Med. Chem.* **42**, 2280–2286.
[23] Ciobanu, L.C., Boivin, R.P., Luu-The, V. and Poirier, D. (2001) *Eur. J. Med. Chem.* **36**, 659–671.
[24] Peterson, M.E., Brownell, J. and Vince, R. (1992) *J. Med. Chem.* **35**, 3991–4000.
[25] Poirier, D., Auger, S., Mérand, Y., Simard, J. and Labrie, F. (1994) *J. Med. Chem.* **37**, 1115–1125.
[26] Sam, K.M., Labrie, F. and Poirier, D. (2000) *Eur. J. Med. Chem.* **35**, 217–225.
[27] Fieser, L.F., Fieser, M., ed. (1959) *Steroids* (Reinhold Publishing Corp., New York), and 344, p 339.
[28] Schwarz, S., Thieme, I., Richter, M., Undeutsch, B., Henkel, H. and Elger, W. (1996) *Steroids* **61**, 710–717.