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A- and D-ring structural modifications of an androsterone derivative inhibiting 17β-hydroxysteroid dehydrogenase type 3: Chemical synthesis and structure-activity relationships

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ABSTRACT: Decreasing the intratumoral androgen biosynthesis by using an inhibitor of 17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3) is a strategy to treat prostate cancer. The androsterone (ADT) derivative **1** (RM-532-105) has shown strong inhibitory activity on 17β-HSD3, but needs to be improved. Herein, we describe the chemical synthesis and characterization of two series of analogs to address the impact of A- and D-ring modifications on 17β-HSD3 inhibitory activity, androgenic effect, and metabolic stability. Structure-activity relationships were generated by adding different groups at C16/C17 (D-ring diversification) or replacing the ADT backbone by a nor-androstane or an estrane backbone (A-ring diversification). D-ring derivatives were less potent inhibitors than lead compound **1**, whereas steroidal backbone (A-ring) change led to identifying promising novel estrane derivatives. This culminated with potent 17β-HSD3 inhibitors **23**, **27**, **31**, and **33** (IC_{50} = 0.10, 0.02, 0.13, and 0.17 μM, respectively), which did not stimulated LAPC-4 cell proliferation and displayed higher plasma concentration in mice than lead compound **1**.

***Keywords*:** Hydroxysteroid dehydrogenase, Steroid, Ring modification, Enzyme inhibitor, Prostate cancer, Chemical synthesis.

INTRODUCTION

Prostate Cancer (PCa) is the most common cancer found in men. For instance, 174,650 American men were diagnosed with PCa and 31,620 men died from it in 2019.[1] Initially, the development and progression of PCa requires the androgen receptor (AR) signaling, as well as a supply of androgens, particularly testosterone (T) and dihydrotestosterone (DHT) (Fig. 1), which play a critical role in promoting the prostate tumor.[2-4] In fact, it has been demonstrated that the AR pathway drives PCa progression in most patients.[5] Early detection of PCa, as well as the arrival of new potent and selective treatments, could be two of the key aspects to improve the survival and quality of life for patients with an androgen-sensitive PCa. With that mindset, decreasing *de novo* biosynthesis of androgens in tumors with inhibitors targeting key steroidogenesis enzymes represents an active and promising research field for the development of innovative PCa treatments.[6-10]

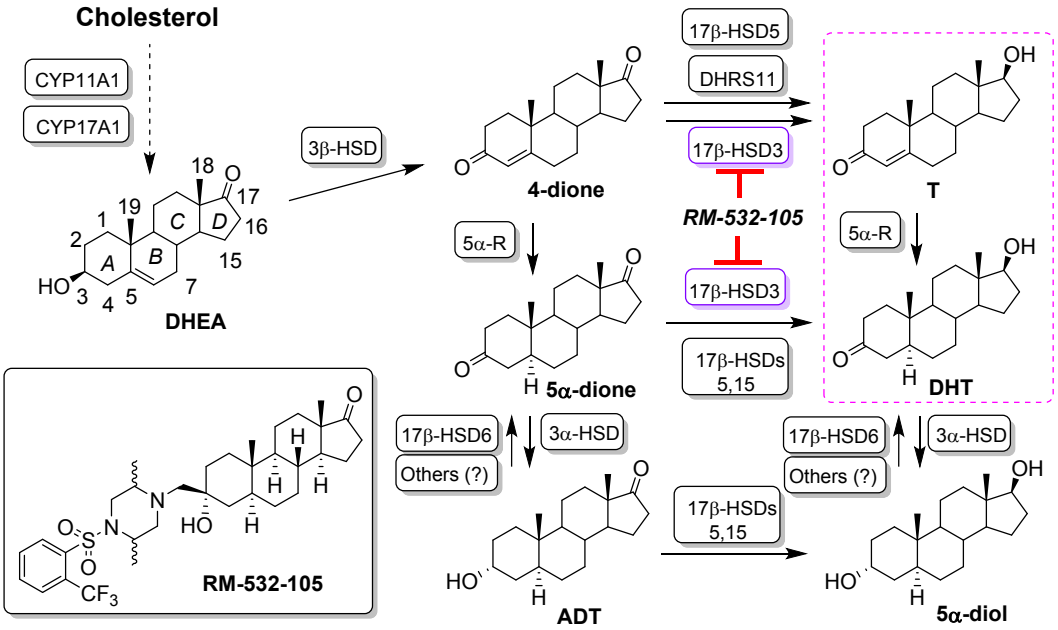


Figure 1. Contribution of 17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3) to the biosynthesis of the androgens testosterone (T) and 5α-dihydrotestosterone (DHT) from 4-androstene-3,17-dione (4-dione)

and 5α-androstene-3,17-dione (5α-dione), respectively. RM-532-105 is a 3β-substituted derivative of androsterone (ADT) that inhibits the steroidogenic enzyme 17β-HSD3. Steroid (A-D) ring identification and partial carbon numbering are reported for DHEA (also see Figure 5 for a full carbon numbering of RM-532-105 and its analogs).

17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3) is a member of the 17β-HSD family, which is constituted of several types (*1-15*) that catalyze the reduction of a carbonyl group at position 17 or the oxidation of a 17β-hydroxy group for different steroids.[11-19] This key enzyme also catalyzes the transformation of the weak androgen 4-androstene-3,17-dione (4-dione) into T, which is further converted to the most potent androgen DHT (Fig. 1). T and DHT eventually interact with AR to promote the growth and proliferation of PCa cells. Even though 17β-HSD3 is expressed mainly in the microsomal fraction of testes,[20] Pfeiffer *et al*.[21] have reported that in castrate-resistant prostate cancer (CRPC) biopsies, the expression of 17β-HSD3 is up-regulated, even after long-term hormone depletion. Although extensive researches have been carried out on the enzyme inhibitors mentioned above, there are no approved drugs to date for 17β-HSD3 inhibitors. Thus, this enzyme has been and remains an interesting target for PCa hormonal therapy and its inhibition should be able to maximize the androgen depletion in androgen-dependent PCa tumors.

During our efforts to discover novel 17β-HSD inhibitors, we have reported the synthesis and biological activity of RM-532-105.[22] This compound is an androsterone (ADT) derivative having a 4-(2,5-dimethyl-4-((2-(trifluoromethyl)phenyl)sulfonyl)piperazin-1-yl)methyl side chain at position 3β (Fig. 1). RM-532-105 has shown potent inhibitory activity on 17β-HSD3 in HEK-293[17β-HSD3] cells, LNCaP[17β-HSD3] cells[22-24] as well as in microsomal fraction of

rat testes.[23] Moreover, this lead compound significantly decreased T and DHT levels when administered subcutaneously in rats.[25] Although RM-532-105 significantly accumulated inside the tumor of LAPC-4 xenografts, it was not able to reduce the growth of tumor.[26]

Recently, we reported the synthesis and biological activity of two RM-532-105 stereoisomers,[27] the first one possessing an inversed hydrogen on carbon 5 (5β-H instead of 5α-H) while the second one bears an inversed methyl carbon attached to C13 (13α-CH_3 instead of 13β-CH_3). Both modifications were carried out to explore the impact on 17β-HSD3 inhibitory activity, androgenic profile and metabolic stability by structural modifications on the A- and D-ring shape, respectively. From this study, we first found that RM-532-105 has a relatively low metabolic stability, leading to unfavorable pharmacokinetics. Conversely, A-ring inversion in a 5β configuration was well-tolerated by the 17β-HSD3 enzyme, but did not improve its metabolic stability. On the contrary, D-ring inversion significantly decreased the anti-17β-HSD3 activity while increasing its metabolic stability.

Following this preliminary structure-activity relationship (SAR) study,[27] we are now interested in further studying the impact of additional modifications on 17β-HSD3 inhibition and on their metabolic stability. Since no crystal structure of 17β-HSD3 is available to date, and considering the low predictive capacity of these homology models, we decided to focus, in the present study, on a classical approach by using systematic structure modifications and SAR study to progress toward compounds with improved activities. Thus, we describe herein the chemical synthesis of several RM-532-105 analogs (Fig. 2) and show the impacts of A- and D-ring modifications on their 17β-HSD3 inhibitory activity, cell proliferative effect, and metabolic stability. This study will expand our current understanding of the optimal pharmacophore requirements for 17β-HSD3 inhibitory activity and their ability to block androgen biosynthesis

for therapeutic purposes.

RESULTS AND DISCUSSION

Design Strategy. RM-532-105 is a metabolically labile steroid derivative showing 16% of remaining compound after 1 hour in a microsomal preparation of human liver.[27] Such low metabolic stability could generate problems of low bioavailability or adverse drug reactions due to off-target interactions at the high doses needed to obtain the desired pharmacological action. Thus, we first considered synthetic modifications of RM-532-105 at D-ring because we suspected that this compound may be hydroxylated or reduced at position C16 or C17, respectively, by metabolic enzymatic reactions. In fact, it is well-known that those two reactions are involved in the formation of steroidal metabolites.[28] Therefore, the addition of a substituent at C16 or C17 can decrease their reactivity over cytochrome P450 enzymes. We also considered modifications at A-ring by using an estrane backbone, instead of the androstane backbone, and consequently eliminated the presence of a tertiary alcohol at C3.

Our first attempt to reduce the metabolism was initially focused on the A-ring shape of RM-532-105, but was ineffective, since the inversion of configuration (5α-H for 5β-H) altered neither 17β-HSD3 inhibitory activity nor metabolic stability.[27] However, we recently found that the replacement of the C19-steroid backbone of ADT by the C18-steroid backbone of estrone, significantly enhanced the metabolic stability for a series of anti-cancer aminosteroids.[29] Therefore, this strategy could also be applied to 17β-HSD3 inhibitors, but remains to be validated, since no attempt of estrane mimics has been reported to date for 17β-HSD3 inhibitors. Based on these rational considerations, we thus proposed two series of systematic modifications, keeping the 3β-optimized side chain intact, to obtain a RM-532-105 analog with low hepatic

clearance and good inhibitory activity against 17β-HSD3 (Fig. 2).

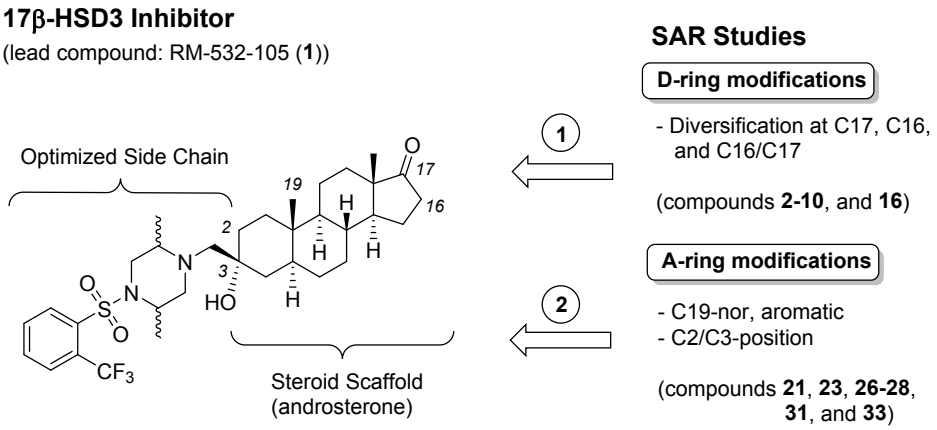
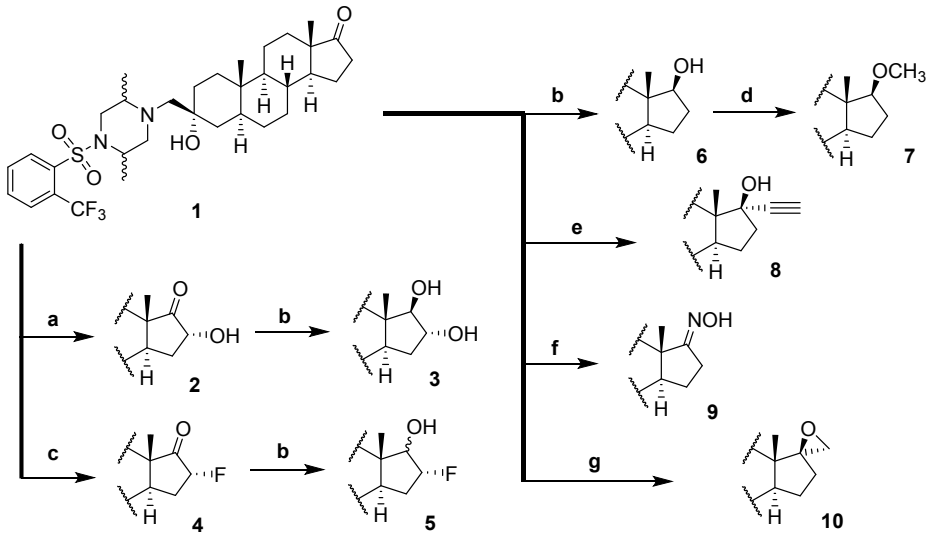


Figure 2. Structure of the 17β-HSD3 inhibitor RM-532-105 (**1**) and two strategies (*# 1-2*) to optimize its inhibitory activity and metabolic stability.

To explore the SAR of D-ring modifications and to determine whether blocking C16 and/or C17 positions by adding several substituents could improve metabolic stability, we synthetized RM-532-105 analogs **2-10** and **16** with varied groups such as hydroxyl, fluorine, methoxy, ethinyl, oxime, oxirane, and acetyl (Schemes 1 and 2).

Changing the androstane A-ring by an estrane has been a successful strategy for the discovery of steroidal compounds with better affinities and selectivity, as well as to improve physicochemical and ADMET properties.[30] We thus planned the synthesis of A-ring analogs of RM-532-105 like nor-androstane derivative **21** (Scheme 2) and estrane derivatives **23**, **26-28**, **31**, and **33** (Scheme 3). In addition to avoiding the presence of a tertiary alcohol at C3, an aromatic A-ring could facilitate the addition of a substituent at positions that are difficult to access by chemical modifications of the androstane A-ring.

Chemistry. *D-ring modifications.* D-ring analogs at position C16 (compounds **2**-**5**) and at position C17 (compounds **6**-**10**) were prepared from the lead inhibitor **1** (Scheme 1) while another analog at C17 (compound **16**) was obtained from 3β-hydroxy-5α-pregnan-20-one (Scheme 2). Briefly, compound **1** was refluxed with $CuBr_2$ to yield the brominated intermediate which was later reacted with aqueous NaOH to obtain the corresponding hydroxyl derivative **2**, whereas fluorinated compound **4** was readily synthesized using Selectfluor™ according to the methodology reported by Liu *et al*.[31] The ^{1}H NMR spectra for **2** and **4** presented characteristic signals corresponding to the 16-H proton at 4.35 ppm and 5.08 ppm, respectively. Moreover, in NOESY experiments, these signals correlated through the space with 18β-CH_3, confirming that the 16-H proton is oriented to the β-face of the steroid. Conversely, the carbonyl group at C17 for compounds **1**, **2**, and **4** was reduced to its corresponding alcohol, yielding **3**, **5**, and **6**, respectively. In the ^{13}C NMR spectra, we observed that the 17-CHOH signal appeared at 80-90 ppm, which signal is also characteristic of the expected C17-stereochemistry.[32] Surprisingly, compound **5** was obtained as a stereoisomeric mixture according to its NMR data and several attempts to separate the two stereoisomers were unsuccessful.

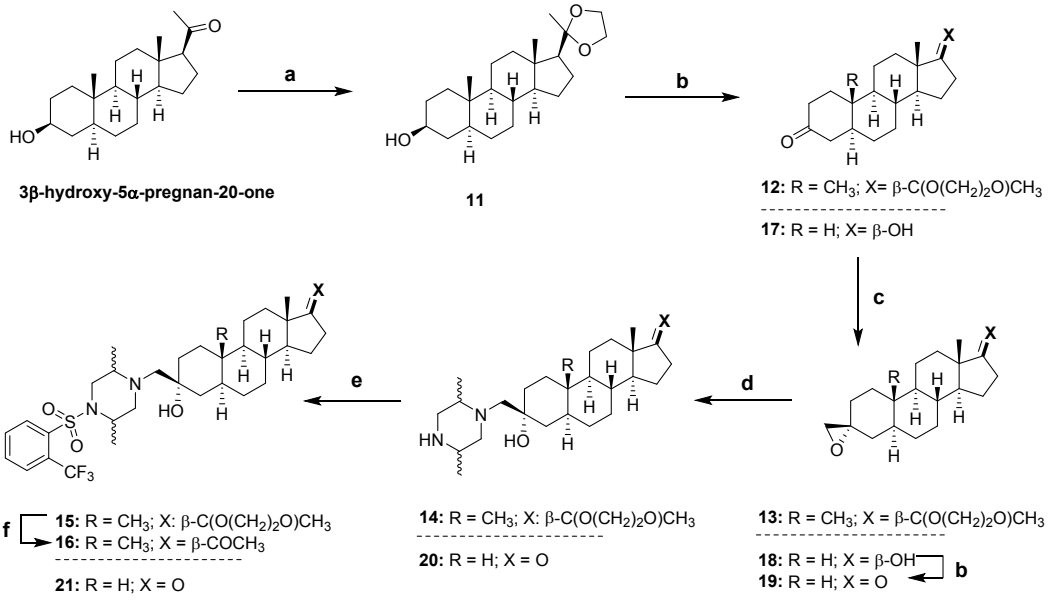


Scheme 1. Synthesis of compounds **2-10**. Reagents and conditions: (a) *1*. $CuBr_2$, MeOH, 65 °C, overnight, *2*. NaOH 1 M, DMF, DCM, rt, 3 h; (b) $NaBH_4$, MeOH, 0 °C, 1 h; (c) Selectfluor™, H_2SO_4 6 M, MeOH, 60 °C, 72 h; (d) NaH, CH_3I, DMF, rt, overnight; (e) *1*. trimethylsilylacetylene, MeLi, THF/diethyl ether (1:1), 0 °C to rt, overnight, *2*. 5% K_2CO_3 in MeOH, rt, 3 h; (f) hydroxylamine hydrochloride, sodium acetate, EtOH, 60 °C, 1 h; (g) $(CH_3)_3SI$, NaH, DMSO, THF, rt, 4.5 h.

Compounds **7** (17β-OCH_3) was prepared from **6** by a selective *O*-methylation and .the 17-OCH_3 was clearly identified as a single signal at δ_H = 3.31 ppm and δ_C = 57.8 ppm as well. The compound **8** was synthesized by reacting **1** with lithium trimethysilylacetylide to lead a TMS-acetylenic intermediate, which was then treated with K_2CO_3 in MeOH to give the corresponding 17α-C≡CH derivative. For this final compound, the characteristic 17-CHOH signal in the ^{13}C NMR spectra appeared at 79.9 ppm. Moreover, the 17α-C≡CH group was associated to signals at δ_H = 2.56 ppm, δ_C = 87.6 ppm (17α-C≡CH) and δ_C = 73.8 ppm (17α-C≡CH). NOESY experiments and literature data [33] confirmed the stereoselective attack by the α-face of the steroid.

The C17-oxime **9** was readily generated in high yield by treating compound **1** with hydroxylamine hydrochloride and sodium acetate in ethanol at 60 °C. The NOH proton was clearly identified in the ^1H NMR as a broad singlet at 8.41 ppm. Furthermore, the C17 signal in the ^{13}C NMR spectra was shifted from 221.5 ppm to 171.2 ppm. Conversely, the ketone of **1** was efficiently reacted under Corey-Chaykovsky epoxidation conditions,[34] using trimethyl-sulfonium iodide and sodium hydride, to obtain the oxirane **10**. The characteristic diastereotopic protons of the methylene group from epoxide were observed as two signals at δ_H = 2.59 and 2.89 ppm. These signals, and those for 18-CH_3 (δ_H = 0.86 ppm and δ_C = 14.4 ppm), are characteristic of the

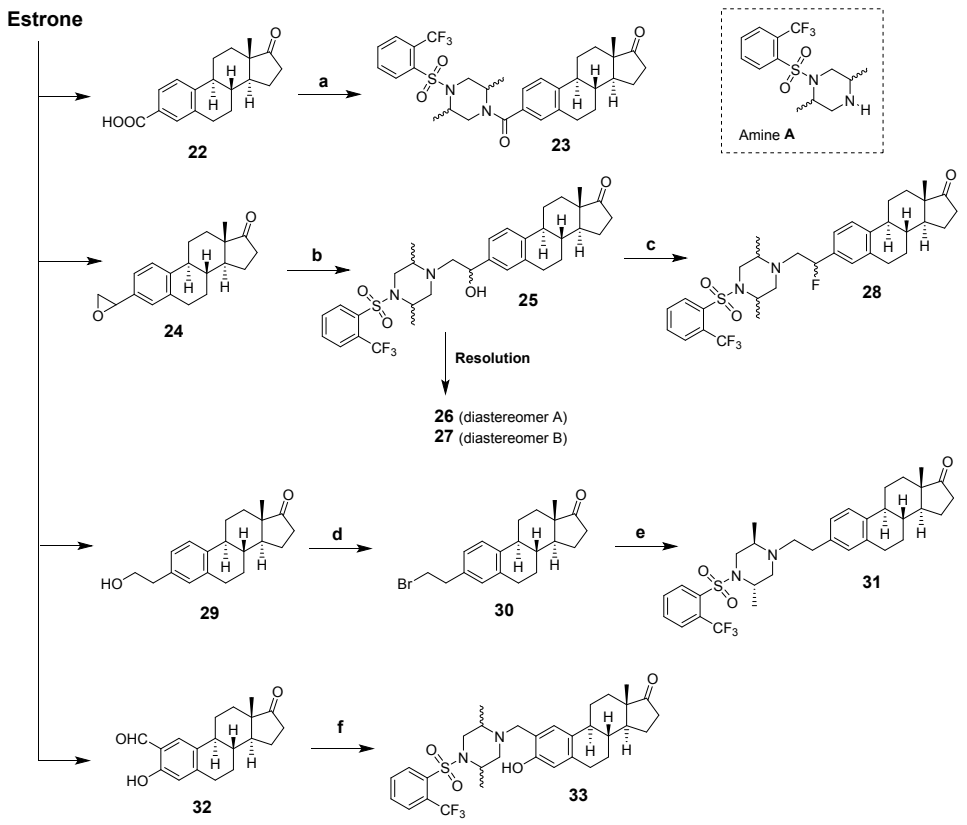
expected stereochemistry (17β-O).[35]



Scheme 2. Synthesis of D-ring derivative **16** and A-ring derivative **21**. Reagents and conditions: a) ethylene glycol, *p*-TSA, toluene, reflux, 12 h; b) Dess-Martin, DCM, rt, 1 h; c) $(CH_3)_3SOI$, NaH, DMSO, THF, rt, 5 h; d) *trans*-2,5-dimethylpiperazine, ethanol, 70 °C, 12 h; e) 2-(trifluoromethyl)benzenesulfonyl chloride, TEA, DCM, rt, 4 h; f) $HClO_4$, acetone, 0 °C, 30 min.

The pregnane derivative **16** (X = $COCH_3$) was synthesized from commercially available 3β-hydroxy-5α-pregnan-20-one, which was first protected at C20 as the acetal **11** (Scheme 2). After subsequent oxidation of the 3β-OH of **11** using the Dess-Martin reagent, the resulting ketone **12** was then submitted to the epoxidation of Corey-Chaykovsky, using trimethylsulfoxonium iodide and sodium hydride, to give **13**. The oxirane of **13** was then opened using *trans*-2,5,-dimethyl-piperazine in refluxing ethanol, and the resulting secondary amine **14** was reacted with 2-(trifluoromethyl)benzenesulfonyl chloride and TEA to give **15**. Finally, hydrolysis of the acetal group in acidic conditions leads to pregnane derivative **16**.

A-ring modifications. The next strategy consisted in replacing the ADT backbone of **1** by a C19-nor-ADT core (**21**; Scheme 2) or a C18-estra-1,3,5(10)-triene core (**23**, **26-28**, **31**, and **33**; Scheme 3). Compound **21** was prepared from 19-nor-DHT (**17**) as starting material using the same chemical route reported in Scheme 2 for the synthesis of **16**. The NMR spectra of **21** showed all characteristic signals related to compound **1**, except the 19-CH_3 signal that is lacking. For the synthesis of estrane series (Scheme 3), the amine **A** was first obtained by a condensation of 2-(trifluoromethyl)benzenesulfonyl chloride with *trans*-2,5-dimethylpiperazine as previously reported [27] and next introduced at position C3 or C2. We thus prepared the amide **23** by means of a coupling between the carboxylic acid **22** [37] and the amine **A** using HBTU as a coupling agent. Conversely, the oxirane **24** [38, 39] served as an intermediate for the synthesis of **26**-**28**. First, this oxirane was reacted with amine **A** in ethanol at 70 °C to yield the diastereomeric mixture **25**, which was thereafter purified by HPLC to afford **26** and **27**. Interestingly, for these two isomers, the chiral carbinol can be clearly differentiated by their NMR data. For instance, this proton appears as two double signals at δ_H = 4.66 and 4.54 ppm for **26** and **27**, respectively.



Scheme 3. Synthesis of compounds **23**, **26-28**, **31**, and **33**. Reagents and conditions: (a) amine **A**, HBTU, DIPEA, DMF, rt, 2 h; (b) amine **A**, EtOH, 70 °C, 48 h; (c) DAST, DCM, rt, 3 h; (d) PPh_3, CBr_4, DCM, 0 °C, 1.7 h; (e) amine **A**, KI, DIPEA, CH_3CN, 65 °C, 72 h; (f) amine **A**, $NaBH_3CN$, AcOH, molecular sieves, MeOH/DCM (8:2), rt, 4 h.

The different inhibitory activities observed with alcohols **26** and **27**, the latter being the most active, encouraged us to address their composition. After the proton and carbon assignment was established using a combination of HSQC, HMBC and COSY experiments, and literature data, different chemical shifts were clearly observed for CH-22, CH-24, CH_2-27, and CH_3-28. In ^{1}H NMR, chemical shifts are close for **26** and **27**, but small differences are observed for H-22 (4.66 and 4.54 ppm), H-24 (3.06 and 2.92 ppm), 1H of CH_2-27 (3.06 and 2.92 ppm), and CH_3-28 (0.95 and 0.86 ppm). More significant effects were however observed in ^{13}C NMR for two

carbons (Table S1; Supporting Information). Thus, the CH-24 signals appear at 50.2 and 55.0 ppm, while the CH_2-27 signals appear at 52.6 and 48.1 ppm, for **26** and **27**, respectively.

From the NMR analysis, we noticed that the CH_2-25 appears as two well differentiated signals, one (Ha) producing an NOE correlation with CH_3-29 (# *1*) and the other (Hb) with CH_3-28 (# *2*) (Fig. 3). Since the two nitrogens of piperazine are differently substituted, there are however two possible *trans*-diaxial configurations (Fig. 3A-D). The ^{1}H NMR spectra of the Mosher ester of the mixture of alcohols **25** (R-OCO-C(CF_3)(OCH_3)Ph) showed the presence of four OCH_3 singlet signals, suggesting the presence of four alcohols with 22R,24R,26S; 22R,24S,26R; 22S,24R,26S; and 22S,24S,26R configurations (Fig. S1, Supporting Information). We next hypothesized that a hydrogen bond was formed between the alcohol (OH) and the free nitrogen electron doublet, then producing a five-member ring that fixed the orientation of the two different C22 substituents (H and steroid A-ring (Ar)), as shown in Fig. 3A-D. The NOESY spectra of **26** and **27**, which compounds were obtained by chromatography of the mixture of alcohols **25** generated from the opening of oxirane **24**, then provided interesting results (Fig. 3E,F). In addition to the expected H-22/H-23 correlation, we observed in the NOESY spectrum of **26** a correlation between H-22 and a multiplet at 3.1 ppm, which corresponds to H-24 and 1H of CH_2-27 (H-27a), but not with the other H-27 (H-27b) at 2.2 ppm. On the other hand, the NOESY spectrum of **27** showed a correlation between H-22 and H-23 and 1H of CH_2-27 at 2.5 ppm (H-27b), but not with the multiplet at 3.0 ppm, which encompasses the other H-27 (H-27a) and H-24. These NOESY results suggest that **26** is a mixture of two alcohols (22R,24R,26S (Fig. 3A) and 22S,24S,26R (Fig. 3D)) while **27** is a mixture of two alcohols (22R,24S,26R (Fig. 3B) and 22S,24R,26S (Fig. 3C)). The proximity of H-22 to H-27b that is responsible for the nuclear overhauser (NOE) effect is clearly observed in the optimized 3D structure of **27** (Fig. 3G).

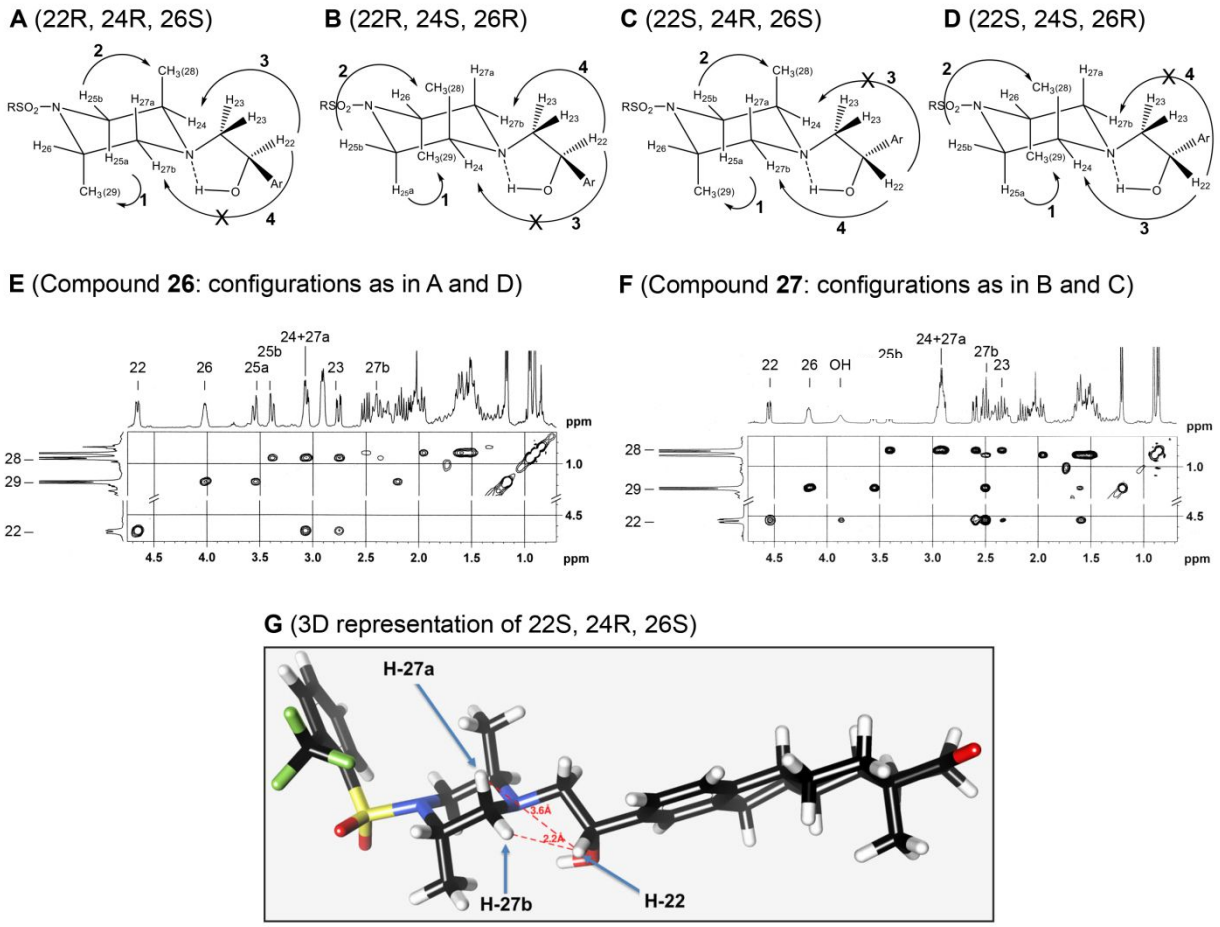


Figure 3. Important NOE correlations observed for compounds **26** (A, D, and E) and **27** (B, C, and F) as well as an optimized structure showing the proximity of H-22 to H-27b (G). Figure G was built by assembling the side chain to the C3-position of estrone (E1). The structure of E1 was retrieved from the ZINC database.[40] The geometry of the resulting 22S,24R,26S-compound was first minimized by a UFF force field and then optimized by the semiempirical PM6 method using Gaussian 09 software.[41] The figures A-D were produced with ChemDraw 14.0 and figure G with UCSF Chimera program.[42]

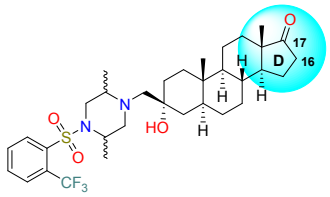
Diastereomeric mixture of alcohols **25** was also reacted with DAST to give fluorinated derivative **28** (Scheme 3). This derivative was prepared to address the bioisosteric replacement of OH by a fluorine atom. Compound **31**, having an amine linked at C3 by an ethyl group, was designed to see the impact of the absence of an OH group close to the steroid A-ring. Thus, the Appel reaction was carried out on the intermediate **29**,[39] by using CBr_4 and PPh_3, giving **30** in good yield. This bromoethyl steroid was then reacted with amine **A** combined with a catalytic amount of potassium iodide to obtain the desired product **31**. Finally, compound **33** was readily prepared *via* a reductive amination between the amine **A** and 2-formyl-estrone (**32**), readily obtained from estrone.[43] In NMR, the new methylene group formed at C2 appears at δ_H = 3.61-3.69 and δ_C = 57.8 ppm. Interestingly, the OH attached at C3 is shifted downfield to δ_H = 10.23 ppm, suggesting an intramolecular H-bond with the tertiary amine in the piperazine moiety, which may be favored since a six-member ring is formed.

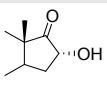
17β-HSD3 inhibition and LAPC-4 (AR^+) cell proliferative activity (Structure-activity relationships). The inhibition of 17β-HSD3 activity was carried out using transfected LNCaP cells overexpressing 17β-HSD3 (LNCaP[17β-HSD3] cells) as a source of enzyme activity. In this study, we measured the amount of labeled T formed from labeled 4-dione in the presence of the designed inhibitors at three concentrations (0.1, 0.5, and 1.0 μM). IC_{50} values were then determined for the best compounds (Table 1). To explore whether the synthesized compounds exhibit an androgenic profile, which is an undesired effect in the context of prostate cancer therapy, we assessed their proliferative activities on androgen-sensitive prostate cancer LAPC-4 (AR^+) cells at three inhibitor concentrations (0.1, 0.5, and 1.0 μM). The basal cell proliferation being fixed at 100%, a proliferative value (in %) over 100% will be associated with

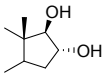
an androgenic activity whereas a value (in %) under 100% will be associated with a cytotoxic effect. RM-532-105 (**1**) and DHT were used as reference compounds in the enzymatic and androgen-sensitive cell proliferation assays, respectively.

D-ring modifications. Initially, we prepared and tested compounds **2-5** to assess the effect of adding a group, such as OH or F. As results, small modifications at this position significantly decreased the anti-17β-HSD3 activity. The 16α-fluorine derivative **4** is 2-fold less potent than **1**, whereas **2** lack inhibitory activity. Surprisingly, altering C16 position clearly increases the proliferation of LAPC-4 cells, but this effect is not dose dependent. For instance, the presence of a fluorine atom exhibited an androgenic activity at 0.1 µM (147.7%). In addition, **3** and **5**, which are the C17-OH counterparts of **2** and **4**, did not exert inhibitory activity on 17β-HSD3, whereas **5** enhanced the proliferative capability of LAPC-4 cells to a greater extent than **4**.

Table 1. 17β-HSD3 inhibition and LAPC-4 (AR^+) cell proliferation of D-ring modified compounds **2-10** and **16**



Cpd	D-ring group	17β-HSD3 inhibition (%)[a] 0.1 µM / 0.5 µM / 1.0 µM	IC_{50}[b] (µM)	LAPC-4 cell proliferation (%)[c] 0.1 µM / 0.5 µM / 1.0 µM
1		60 / 96 / 100	0.09[d]	96.0 / 47.6 / 32.9
2		0 / 60 / 80	---	114.0 / 83.1 / 69.3

3		20 / 48 / 55	---	90.8 / 54.1 / 43.8
4		22 / 75 / 90	0.20	147.7 / 134.2 / 126.6
5		12 / 58 / 70	---	153.6 / 149.7 / 142.4
6		38 / 78 / 94	0.19	116.4 / 88.1 / 31.3
7		10 / 18 / 30	---	---
8		5 / 30 / 40	---	130.9 / 95.8 / 81.2
9		40 / 80 / 90	0.22	107.8 / 58.9 / 44.0
10		5 / 10 / 20	---	92.6 / 61.2 / 49.3
16		10 / 30 / 42	2.6	45.9 / 26.4 / 27.1

[a] Inhibition at three concentrations of the transformation of [^{14}C]-4-dione (15 nM) to [^{14}C]-T by 17β-HSD3 in LNCaP cells overexpressing 17β-HSD3. [b] The concentration that inhibits 50% of 17β-HSD3 activity (IC_{50}). The assay was performed using seven concentrations (0.001 – 5.0 μM). IC_{50} values were calculated using GraphPad Prism 6 software. Values represent the average of two independent experiments performed in triplicate. [c] Proliferation of androgen-sensitive LAPC-4 cells at three concentrations. At the end of the assay, the proliferation of the cells not treated with a drug (negative control) was fixed to 100% while the proliferation of cells treated with the potent androgen DHT at 10 nM (positive control) was 190%. [d] Mean value of 0.06, 0.067, and 0.13 μM.

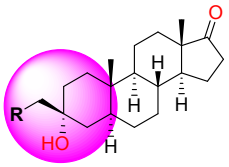
On the other hand, we also explored the impact of a modification at C17 on the inhibitory

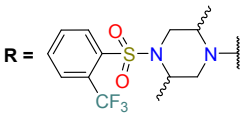
activity. Thus, the C17-ketone was reduced to the corresponding alcohol (17β-OH, **6**), but this compound showed a 2-fold less inhibitory activity on 17β-HSD3 when compared to **1**. The *O*-methylation of **6** (compound **7**) also produced a detrimental effect on 17β-HSD3 inhibition. Another finding emerged when a substituent was added to the α-face in the presence of a 17β-OH. We noted that **8**, having a 17α-C≡CH, was inactive against 17β-HSD3. Even worse, **8** caused a significant proliferation of LAPC-4 cells (130.9%) at low concentrations of 0.1 μM. Interestingly, when 17-C=O was changed to 17-C=NOH, the resulting oxime **9** was only 2-fold less effective as an inhibitor than **1** on 17β-HSD3 (IC_{50} = 0.22 μM) and it did not stimulate the cell proliferation. The introduction of an oxirane at C17 (compound **10**) greatly reduced the inhibition of 17β-HSD3, but this compound did not induce cell proliferation. Pregnane derivative **16**, with an acyl group at C17β, also weakly inhibited 17β-HSD3 and produced an antiproliferative (cytotoxic) effect on LAPC-4 cells.

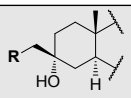
These SAR data suggest that altering positions C16 and C17 will have a moderate to negative impact on the 17β-HSD3 inhibitory activity and a mixed effect on LAPC-4 cell proliferation. Regarding 17β-HSD3, we observed that the presence of a hydroxyl group at the C17 position is relatively well tolerated since compounds **6** (17β-OH) and **9** (17-NOH) were the most active inhibitors in the 17-substituted series. It is however difficult at first sight to assign the exact nature of the molecular interaction of the hydroxyl group, since that functional group can act as an H-bond donor group (HBD) or as an H-bond acceptor group (HBA) depending on the molecular context. However, the excellent activity of the C17 ketone, as an HBA group, let us suppose that the HBA capacity of the OH is in play. On the other hand, compounds **7** (OCH_3) and **16** ($COCH_3$) were inactive, despite their HBA capacity. The steric hindrance could be a major factor to explain this loss of inhibition potency by preventing the HBA interaction. These

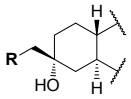
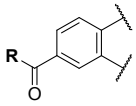
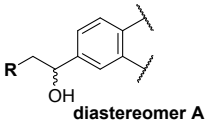
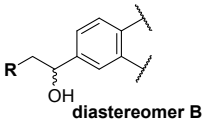
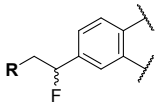
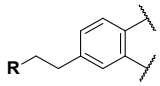
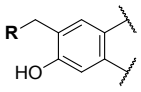
results point toward a weak tolerance for a bulky group in this area of 17β-HSD3. In this way, it is also important to note that attaching a group on the α-face of C17 (e.g. **8** and **10**) has a negative impact for the inhibition of 17β-HSD3. Thus, we believe that a substituent at C17 prevents an efficient hydrogen bonding with a residue in the binding pocket of 17β-HSD3. This observation reinforced our finding that a very precise position of C17 oxygen is necessary to favor H-bonding with 17β-HSD3. Regarding the proliferative activity in LAPC-4 (AR^+) cells, this series of compounds gave mixed proliferative and antiproliferative results. Among the most active inhibitors of the series, the 17β-OH and 17-NOH derivatives **6** and **9**, respectively gave a very slight proliferative activity at 0.1 µM and an antiproliferative activity at 0.5 and 1.0 µM. Conversely, the addition of a fluoride atom at C16α resulted in clear proliferative activity (see compounds **4** and **5**). Despite its acyl group at C17β, pregnane derivative **16** produced the higher antiproliferative activity.

Table 2. 17β-HSD3 inhibition and LAPC-4 (AR^+) cell proliferation of A-ring modified compounds **21**, **23**, **26-28**, **31**, and **33**





Cpd	A-ring group	17β-HSD3 inhibition (%)[a] 0.1 µM / 0.5 µM / 1.0 µM	IC_{50}[a,b] (µM)	LAPC-4 cell proliferation (%)[c] 0.1 µM / 0.5 µM / 1.0 µM
1		60 / 96 / 100	0.09[d][0.08][e]	96.0 / 47.6 / 32.9

21		20 / 82 / 94	0.22	92.8 / 66.3 / 63.1
23		45 / 78 / 90	0.10 [0.11][e]	60.0 / 45.6 / 36.7
26	 diastereomer A	50 / 95 / 98	0.14	115.0 / 94.9 / 81.9
27	 diastereomer B	84 / 98 / 100	0.02 [0.06][e]	103.2 / 68.0 / 56.4
28	 diastereomeric mixture	~30 / 80 / 90	~0.25	---
31		38 / 60 / 70	0.13 [0.10][e]	51.9 / 19.6 / 21.4
33		50 / 76 / 84	0.17 [0.12][e]	99.6 / 92.8 / 93.6

[a] Inhibition at three concentrations of the transformation of [^{14}C]-4-dione (15 nM) to [^{14}C]-T by 17β-HSD3 in LNCaP cells overexpressing 17β-HSD3. [b] The assay was performed using seven concentrations (0.001 – 5.0 μM). IC_{50} values were calculated using GraphPad Prism 6 software. Values represent the average of two independent experiments performed in triplicate. [c] Proliferation of androgen-sensitive LAPC-4 cells at three concentrations. At the end of the assay, the proliferation of the cells not treated with a drug (negative control) was fixed to 100% while the proliferation of cells treated with the potent androgen DHT at 10 nM (positive control) was 190%. [d] Mean value of 0.06, 0.067, and 0.13 μM. [e] IC_{50} values for the transformation of [^{14}C]-4-dione (50 nM) into [^{14}C]-T by 17β-HSD3 from homogenized rat

testes.

A-ring modifications. To better understand the impact of the nature of the steroid A-ring, we next assessed the biological activities of compounds **21**, **23**, **26**-**28**, **31**, and **33** (Table 2). These analogs of compound **1** are potent 17β-HSD3 inhibitors and did not display a proliferative activity in LAPC-4 cells. Precisely, the order of potency for this new series of 17β-HSD3 inhibitors was **27** > **23** > **31** = **26** > **33** > **21** > **28**. First, we observed that the absence of 19-CH_3 in compound **21** (or 19-*nor*-RM-532-105) had a negative impact and caused a drop of the inhibitor potency (~2.5 fold less active than **1**). The most striking aspect of the data was displayed by replacing the saturated A-ring of **1** for an aromatic ring. This modification yielded potent 17β-HSD3 inhibitors with similar or better activities compared to **1**. Moreover, we observed that either the side chain is attached to C2 or C3, the potency is almost retained, while being slightly better when this substituent is linked to position C3. In this way **33** (C2 substituted, IC_{50} = 0.17 μM) was ~2-fold less potent than **1**, whereas the amide **23** (C3 substituted, IC_{50} = 0.10 μM) was equipotent. Additionally, it is of interest to note that the presence of an OH near to the aromatic A-ring as well as its orientation through the space is an important factor. For example, compound **31**, having an ethyl spacer between the A-ring and the side chain, exhibited an IC_{50} of 0.13 μM. However, introducing an OH group on this ethyl spacer resulted in an inhibitory activity comparable to that of **31**, as depicted by compound **26** (IC_{50} = 0.14 μM), while the simple inversion of the OH in **26** to give **27** (IC_{50} = 0.02 μM) led to the most potent inhibitor of the series (4.5-fold more potent than **1**).

For a more in-depth investigation the role of OH in **27**, we prepared its fluorinated bioisoster **28**. As expected, this modification resulted in a significant loss of 17β-HSD3 inhibitory activity with an IC_{50} of 0.25 μM. Thus, OH is suspected to act as an HBD group with a residue from an amino acid in the binding pocket of 17β-HSD3 and provide a beneficial

molecular interaction. Interestingly, the HBA carbonyl group of amide **23** is well-tolerated, even if its potency is about 4 times weaker than **27**.

We then evaluated the ability of these new analogs to stimulate the proliferation of LAPC-4 cells. A non-significant proliferative effect was only observed at 0.1 μM for the isomer **26** (115%), but not for the most potent 17β-HSD3 inhibitor **27** (103.2%). On the other hand, when LAPC-4 cells were treated with **23** and **31**, we observed a strong reduction of cell proliferation when compared to the control at 0.1 μM (60.0 and 51.9%, respectively) suggesting an anti-proliferative action on this cell line potentially due to an anti-androgenic or cytotoxic activity. Further research will thus be necessary to determine the main cause of this unexpected antiproliferative activity. Interestingly, compound **33** did not display proliferation or anti-proliferation of androgen-sensitive LAPC-4 cells.

Inhibition of rat testis 17β-HSD3. The more potent inhibitors on LNCaP transfected cells, compounds **23**, **27**, **31**, and **33**, were also tested for the inhibition of 17β-HSD3 contained in a microsomal fraction of homogenized rat testes. We thus measured the amount of [^{14}C]-T formed from natural substrate [^{14}C]-4-dione in the presence of NADPH as cofactor at six concentrations of inhibitor. The results were expressed as a percentage of the inhibitory activity, and the resulting curves used to determine the IC_{50} values (Table 2). Interestingly, the same results obtained with both sources of 17β-HSD3, human transfected cells and rat testis preparation, suggests that it will be possible to use rat for preclinical model studies.

Metabolic stability in vivo. A series of enzyme inhibitors were selected to determine their potential in the metabolic stability assay. Compounds **1**, **4**, **9**, **16**, **21**, **23**, **26**, **27**, **31**, and **33**

were thus treated in the presence of NADPH as cofactor for 1 hour with the human liver S9 fraction. In this assay, a compound is transformed by phase-I reactions (oxidation, reduction, and hydrolysis) and phase-II reactions (glucuronidation, sulfatation, and acetylation). The remaining compound is measured at the end of the incubation period, and expressed in %. As depicted in Figure 4, blocking C16 position by introducing a fluorine atom gave a better metabolic stability as showed by compound **4** and similarly when converting the 17-C=O in **1** to a 17-oxime in **9**. The metabolic stability was also significantly improved with the presence of a 17-acyl group, such as for C21-steroid derivative **16**. As expected, the absence of 19-CH_3 in **21** caused no impact on the stability when compared to **1** (with a 19-CH_3). Regarding the estra-1,3,5(10)-triene series (compounds **23**, **26**, **27**, **31**, and **33**) the order of metabolic stability was **23** > **33** > **26** = **27** = **31**.

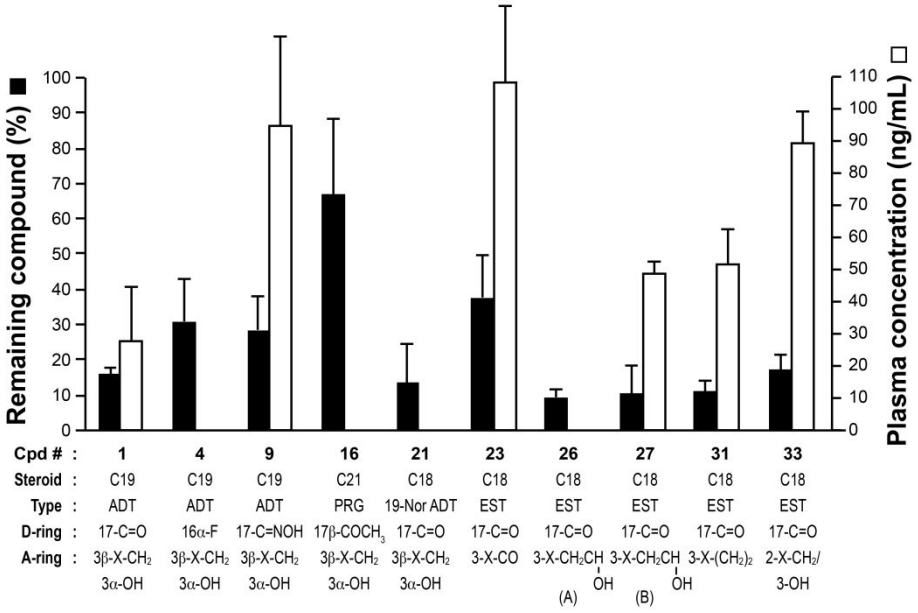


Figure 4. Human hepatic metabolic stability and plasma concentration in mice of lead 17β-HSD3 inhibitor **1** and selected analog inhibitors. Results represent the % of remaining quantity of compound treated with the human liver S9 fraction (black columns) or the plasma concentration in ng/mL of a single subcutaneous injection of an inhibitor in mice (white columns). Data are the average ± SD of four

experiments. ADT: androsterone; PRG: 3α-hydroxy-5α-pregnan-20-one; EST: estrane and X: 2,5-dimethyl-4-((2-(trifluoromethyl)phenyl) sulfonyl)piperazinyl.

Results with D-ring derivatives suggest that the formation of metabolites can be blocked or modulated by adding a group at C16 or C17. This conclusion is in agreement with a previous report showing that 13-epi-RM-532-105, with an inverted D-ring, was more stable than **1** in a microsomal preparation.[27] For A-ring derivatives, compound **23** unexpectedly showed the best metabolic stability despite lacking modifications on the D-ring. Its rigid and planar structure (due to the amide at C3) may prevent, in a certain way, a favorable interaction with the cytochrome P450 enzyme family. Compound **23** is thus an interesting derivative, since it has as good enzymatic inhibition as lead candidate **1**, with better metabolic stability and non-androgenic activity on LAPC-4 as well.

Plasma concentration of inhibitors in mice. In addition to the metabolic stability assessed in vitro (human liver S9 fraction), we also tested the stability of a selection of 17β-HSD3 inhibitors in vivo (Figure 4). A single dose of an inhibitor was then injected subcutaneously in mice and its plasma concentration determined at 3 h. Interestingly, the selected new inhibitors reached higher plasma concentrations (48.7 – 108.2 ng/mL) than the lead inhibitor **1** (27.7 ng/mL). The N-oxime D-ring derivative **9**, which is the only representative of the androstane-based series of inhibitors, is clearly more stable with a plasma concentration 3.4 fold higher (94.7 ng/mL) than its corresponding ketone **1**. Although bearing all a C17-carbonyl group, the A-ring derivatives **23**, **27**, **31**, and **33** of the estrane series of inhibitors were found more stable than androstane derivative **1**. In fact, closely related compounds **27** (alcohol; CH_2CHOH at C3) and **31** (alkyl; CH_2CH_2 at C3) produced the same plasma concentrations (48.7

and 51.6 ng/mL, respectively), whereas for **23** the presence of an amide (NCO) at C3 seems limited the metabolism around this position which resulted in the higher plasma concentration observed (108.2 ng/mL; 3.9 fold compound **1** value). The last estrane representative, compound **33**, has a side chain that is moved from position 3 to 2 and a phenolic group. This estrone derivative generated a plasma concentration of 89.3 ng/mL although the presence of both C17-ketone and C3-OH.

Proliferative androgenic/estrogenic effect of inhibitors. A proliferative androgenic activity is not suitable for a therapeutic agent targeting PCa; consequently, the cell proliferation of androgen-dependent LAPC-4 cells was one of the criteria we used to discriminate our 17β-HSD3 inhibitors. Easy to use, this assay is however not ideal because it is possible for a molecule to be an androgen receptor agonist although it does increase the cell proliferation, as it displays a cytotoxic activity by a hormone-independent mechanism of action. Thus, the lack of androgenic effect should be addressed using other methods such as yeast-based reporter gene assay,[44] or AR transcriptional amplification system.[26] Using this later transcriptional activity assay, we thus previously observed no androgenic activity for the lead compound **1**, but a cytotoxic activity was observed at the higher concentration tested of 2 μM.[26] However, a compound that can reduce the proliferation of cancer cells is however interesting in itself and, for this reason, we considered the proliferation of cancer cells as an assay providing interesting information. Furthermore, since some of the new inhibitors are based on an estrane backbone, instead of the androstane backbone of **1**, we also addressed their potential estrogenic activity.

Based on their 17β-HSD3 inhibitory activity and bioavailability expressed *vs* the lead

compound **1**, we selected compounds **9**, **23**, **27**, **31**, and **33** to evaluate their androgenic and estrogenic effects (Table 3). Similarly to compound **1**, except for estrone derivative **33**, the proliferation results on androgen-dependent LAPC-4 cells showed a dose-dependent reduction of cell proliferation (increasing % of cell inhibition) suggesting an antiproliferative activity as for RM-581, a cytotoxic agent.[29] However, this effect does not seem to be mediated by the AR, since these compounds did not bind this receptor as estimated on the basis of their negative Goldscore values (Table 3). As a point of comparison, the potent androgen DHT, when docked on the AR binding domain (PDB: 2PNU), produced a positive Goldscore value of 62. Using the androgen-independent PC-3 cells, we next determined that all selected inhibitors, contrary to the cytotoxic agent RM-581, did not affect cell proliferation (no significant % of cell inhibition) at the three concentrations tested independently of the backbone (androstane or estrane). Finally, the binding affinity of selected inhibitors for ER was also addressed by a docking study with the ER binding domain (PDB: 1ERE). The negative Goldscore values generated for the selected inhibitors (Table 3), contrary to the positive value of 53.7 generated for the potent estrogen estradiol, suggest no estrogenic activity for these compounds although the presence of an estrane backbone. Furthermore, compounds **31** and **33** did not stimulate or inhibited the cell proliferation of estrogen-dependent breast cancer T-47D cells, thus supporting the docking results.

Table 3. 17β-HSD3 inhibition, plasma concentration in mice, prostate cancer cell proliferative/antiproliferative/inhibition activities, and AR/ER binding of selected compounds **1**, **9**, **23**, **27**, **31** and **33**

Cpd	17β-HSD3 inhibition (fold *vs* 1)[a]	Plasma concentration (fold *vs* 1)[b]	LAPC-4 (AR^+) cell Proliferation	AR binding (Goldscore) [d]	PC-3 (AR^-) cell Proliferation	ER binding (Goldscore) [e]

			(%)[c]		(%)[c]	
1	1.0	1.0	96 / 48 / 33 *[4 / 52 / 67]*	-342	91 / 94 / 89 *[9 / 6 / 11]*	-367
9	0.41	3.4	108 / 59 / 44 *[-8 / 41 / 56]*	-338	90 / 94 / 96 *[10 / 6 / 4]*	-424
23	0.90	3.9	60 / 46 / 37 *[40 / 54 / 63]*	-152	93 /100 / 94 *[7 / 0 / 6]*	-200
27	4.5	1.8	103 / 68 / 56 *[-3 / 32 / 44]*	-95	92 / 94 / 95 *[8 / 6 / 5]*	-292
31	0.69	1.9	52 / 20 / 21 *[48 / 80 / 79]*	-160	99 / 95 / 99 *[1 / 5 / 1]*	-253[f]
33	0.53	3.2	100 / 93 / 94 *[0 / 7 / 6]*	-153	96 / 95 / 93 *[4 / 5 / 7]*	-234[f]
RM-581 **(cytotoxic)** *g*	--	--	81 / 38 / 31 *[19 / 62 / 69]*	--	97 / 80 / 61 *[3 / 20 / 39]*	--
DHT **(androgen)**	--	--	190 (at 0.01 μM)	62	--	--
E2 **(estrogen)**	--	--	--	--	--	53.7

[a] Ratio (fold more active): IC_{50} (lead compound **1**) / IC_{50} (new compound). [b] Ratio (fold more active): plasma concentration (new compound) / plasma concentration (lead compound **1**). [c] Proliferation of androgen-dependent LAPC-4 cells or androgen-independent PC-3 cells at 3 concentrations (0.1 μM / 0.5 μM / 1.0 μM). Data are expressed in % of cell proliferation related to the control of untreated cells (fixed at

100%) and expressed as the % of inhibition of cell proliferation (between square brackets). [d] Goldscore as an estimation of androgen-receptor (AR) binding affinity. [e] Goldscore as an estimation of estrogen-receptor (ER) binding affinity. [f] No proliferation of T-47D (ER^+) cells at 1.0 and 5 μM. [g] See reference 29.

CONCLUSION

Several A- and D-ring steroid derivatives were designed and synthetized by systematic modifications of the lead compound RM-532-105 (**1**). Substituents at C16 and C17 of the D-ring generally reduced the enzymatic inhibitory effect on 17β-HSD3. The 17-substituted ADT derivatives **7** and **9** were the most potent inhibitors, suggesting that the presence of HBA groups as well as their orientation in space at this position is crucial for enzymatic inhibition. Interestingly, adding a N-oxime at C17 position to generate **9** improved the metabolic stability in vitro and in vivo. Nevertheless, it is worthwhile to mention that, in some cases (compounds **4** and **5**), D-ring modifications yielded compounds stimulating the proliferation of LAPC-4 (AR^+) cells.

On the other hand, the inhibitory effect on 17β-HSD3 is almost retained by replacing the saturated steroid A-ring of **1** with a benzene ring (ADT *vs* estrane backbone). This approach allowed us to discover a series of estrane derivatives as new 17β-HSD3 inhibitors, not reported to date. In addition to the ease of synthetizing these derivatives, the scaffold replacement yields active compounds even though the optimized side chain is attached to the C2 or C3 position. These analogs induced no cell proliferation, and produced a higher plasma concentration in mice than **1**. Thus, changing the ADT backbone by an estra-1,3,5(10)-triene backbone led to identify four-interesting 17β-HSD3 inhibitors (Table 3): **23** that is equally potent as **1** (0.9 fold), **27** as the

most potent inhibitor (4.5 fold *vs* **1**), and **31**/**33** just slightly less potent than **1** (0.69/0.53 fold). Furthermore, while compounds **23**, **27**, and **31** disclosed an anti-proliferative effect against LAPC-4 cells, **33** disclosed no stimulation or inhibition of cell proliferation. These four 17β-HSD3 inhibitors also displayed a plasma concentration in mice 1.8 to 3.9-fold higher than lead compound **1**. In the light of these results, further research could be conducted to establish the potential of **9**, **23**, **27**, **31**, and **33** to shrink or slow the growth of PCa tumors (mouse xenografts) by lowering the intratumoral T and DHT or/and by exerting a direct antiproliferative (cytotoxic) effect.

EXPERIMENTAL SECTION

General. Compound **1** was prepared according to the method described by our group.[22] Epi-androsterone acetate and 3β-hydroxy-5α-pregnan-20-one were obtained from Steraloids (Newport, RI, USA). The reagents for chemical synthesis were purchased from Sigma–Aldrich Canada Ltd (Oakville, ON, Canada). The usual solvents were obtained from Fisher Scientific (Montréal, QC, Canada) and were used as received. Anhydrous tetrahydrofuran (THF) and anhydrous dichloromethane (DCM) were from Sigma–Aldrich. Thin-layer chromatography (TLC) and flash-column chromatography were performed on 0.20-mm Silica Gel 60 F254 plates and with Silicycle R10030B 230–400 mesh silica gel (Québec, QC, Canada). Infrared spectra (IR) were recorded on a Horizon MB 3000 ABB FTIR spectrometer (Québec, QC, Canada), and only the significant bands were reported in cm^{-1}. Samples were prepared as KBr pellets. Nuclear magnetic resonance (NMR) spectra were recorded at 400 MHz for ^{1}H and 100.6 MHz for ^{13}C with a Bruker Avance 400 digital spectrometer (Billerica, MA, USA). The chemical shifts (δ) are

expressed in ppm and referenced to chloroform (7.26 ppm for ^{1}H and 77.0 ppm for ^{13}C). ^{1}H NMR signals were reported as singlet (s), doublet (d), triplet (t), quartet (q), and multiplet (m). Carbon numbering used for ^{1}H- and ^{13}C-NMR signal assignment was reported in Figure 5. Low-resolution mass spectra (LRMS) were recorded on a Shimadzu apparatus (Kyoto, Japan) equipped with a turbo ion-spray source and expressed in m/z. High-resolution mass spectra (HRMS) were provided by Pierre Audet in the Laval University Chemistry Department (Québec, QC, Canada). The purity of final compounds to be tested was determined with a Shimadzu HPLC apparatus (Kyoto, Japan) using a Shimadzu SPD-M20A photodiode array detector, an Alltima HP C18 column (250 mm x 4.6 mm, 5 µm) (Grace, Columbia, MD, USA), and a solvent gradient of MeOH: water (70:30) to MeOH (100%). The wavelength of the UV detector was selected between 190 and 205 nm. Final compounds showed purities ≥95% (95.7-99.9%) except for compounds **3**, **4**, **10**, and **26** (93.2%-94.9%). The chemical names of steroid derivatives were generated with ACD/Laboratories (Chemist' version) software (Toronto, ON, Canada) which uses the IUPAC nomenclature.

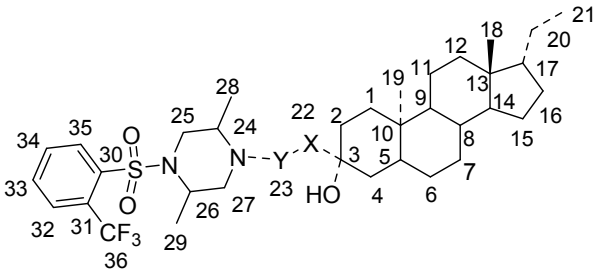


Figure 5. Numbering used for NMR assignment.

Synthesis of (3α,16α)-3-[(*trans*-2,5-dimethyl-4-{[2-(trifluoromethyl)phenyl]sulfonyl} piperazin-1-yl)methyl]-3,16-dihydroxyandrostan-17-one (2). To a solution of **1** (100 mg, 0.16

mmol) in MeOH (5 mL) was added $CuBr_2$ (107 mg, 0.48 mmol) and the reaction mixture was stirred at 65 °C overnight. After evaporation of the solvent, the gummy product was dissolved in DCM (10 mL) and an aqueous solution of NH_4Cl 2.6 M (10 mL) was added to remove the copper salts. The mixture was stirred at room temperature (rt) for 3 h and the organic layer was separated, washed with brine, dried with Na_2SO_4, filtered, and evaporated under reduced pressure. The resulting solid was dissolved in a mixture of DMF (2 mL), DCM (0.5 mL) and an aqueous solution of NaOH 1 M (0.6 mL). The solution was stirred for 3 h and concentrated under vacuum. The oily residue was purified by flash column chromatography using hexanes/EtOAc (gradient elution from 80:10 to 30:70) to afford 24 mg (23%) of **2** as a white solid. IR (KBr) ν_{max}: 3472 (OH), 2932, 2854 (C-H, aliphatic), 1751 (C=O), 1157 (S=O). ^{1}H NMR ($CDCl_3$): δ_H 0.74 (s, 19-CH_3), 0.82 (m, 9-CH), 0.87 (d, J = 6.5 Hz, 28-CH_3), 0.93 (s, 18-CH_3), 0.98 (m, 1 H of 7-CH_2), 1.18 (d, J = 5.4 Hz, 29-CH_3), 1.10-1.80 (m, 1-CH_2, 2-CH_2, 4-CH_2, 5-CH, 6-CH_2, 1H of 7-CH_2, 8-CH, 11-CH_2, 12-CH_2, 14-CH, 1H of 15-CH), 1.90 (m, 1H of 15-CH_2), 2.12 (d, J = 13.9 Hz, 1 H of 22-CH_2), 2.30 (m, 1H of 22-CH_2 and 1H of 27-CH_2), 2.45 (broad, OH), 2.88 (m, 24-CH), 3.08 (d, J = 10.2 Hz, 1H of 27-CH_2), 3.35 and 3.49 (2m, 25-CH_2), 4.05 (m, 26-CH), 4.35 (d, J = 6.6 Hz, 16β-CH), 7.68 (m, 33-CH and 34-CH), 7.87 (m, 32-CH), 8.17 (m, 35-CH). ^{13}C NMR ($CDCl_3$): δ_C 8.7 (C28), 11.2 (C19), 14.1 (C18), 15.6 (C29), 19.8 (C11), 28.5 (C6), 30.4 (C7), 30.5 (C15), 31.3 (C12), 32.0 (C2), 33.7 (C1), 35.0 (C8), 35.9 (C10), 39.3 (C4), 40.6 (C5), 46.0 (C25), 47.6 (C13), 48.2 (C14), 49.5 (C26), 52.4 (C27), 54.1 (C9), 54.7 (C24), 65.7 (C22), 70.9 (C3), 71.3 (C16), 122.0 (q, $J_{C\text{-}F}$ = 274.5 Hz, C36), 127.5 (q, $J_{C\text{-}C\text{-}F}$ = 33.5 Hz, C31), 128.5 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 6.4 Hz, C32), 131.9 (C35), 132.1 (C34), 132.5 (C33), 139.3 (C30), 219.6 (C17). HRMS for $C_{33}H_{49}F_3N_2O_5S$ $[M+H]^+$: 641.3231 (calc.), 641.3216 (found). HPLC purity of 100% (retention time = 16.8 min).

Synthesis of (3α,16α,17β)-3-[(*trans*-2,5-dimethyl-4-{[2-(trifluoromethyl)phenyl]sulfonyl}piperazin-1-yl)methyl]androstane-3,16,17-triol (3). To a solution of **2** (26 mg, 0.04 mmol) in MeOH (3 mL) was added $NaBH_4$ (4 eq). The solution was stirred into an ice bath for 1 h. The resulting solution was evaporated and the solid was extracted with EtOAc, washed with brine, dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude compound was purified by flash column chromatography using hexanes/EtOAc (gradient elution from 70:30 to 20:80) to afford 15 mg (58%) of **3**. IR (KBr) ν_{max}: 3418 (OH), 2924, 2854 (C-H, aliphatic), 1157 (S=O). ^{1}H NMR ($CDCl_3$): δ_H 0.72 (s, 19β-CH_3), 0.74 (s, 18-CH_3), 0.80 (m, 9-CH), 0.87 (d, J = 6.5 Hz, 28-CH_3), 1.18 (d, J = 6.8 Hz, 29-CH_3), 1.10-1.80 (m, 1-CH_2, 2-CH_2, 4-CH_2, 5-CH, 6-CH_2, 7-CH_2, 8-CH, 11-CH_2, 12-CH_2, 14-CH, 15-CH_2) 1.95 (broad, OH), 2.12 (d, J = 13.9 Hz, 1H of 22-CH_2), 2.30 (m, 1H of 22-CH_2 and 1H of 27-CH_2), 2.89 (m, 24-CH), 3.08 (d, J = 10.2 Hz, 1H of 27-CH_2), 3.35 (m, 1H of 25-CH_2), 3.49 (m, 1H of 25-$CH_2$1and 17α-H), 4.05 (m, 26-CH), 4.09 (m, 16β-CH), 7.68 (m, 33-CH and 34-CH), 7.87 (m, 32-CH), 8.17 (m, 35-CH). ^{13}C NMR ($CDCl_3$): δ_C 8.6 (C28), 11.2 (C19), 12.3 (C18), 15.7 (C29), 20.0 (C11), 28.3 (C6), 31.4 (C7), 32.4 (C2), 33.7 (C1), 33.9 (C15), 35.0 (C8), 35.8 (C10), 39.5 (C4), 36.6 (C12), 40.7 (C5), 43.6 (C13), 46.0 (C25), 48.7 (C14), 49.6 (C26), 52.4 (C27), 54.1 (C9), 54.7 (C24), 65.7 (C22), 71.0 (C3), 78.7 (C16), 90.0 (C17), 122.6 (q, J_{C-F} = 274.0 Hz, C36), 127.5 (q, J_{C-C-F} = 33.5 Hz, C31), 128.5 (q, $J_{C-C-C-F}$ = 6.5 Hz, C32), 131.9 (C35), 132.1 (C34), 132.5 (C33), 139.3 (C30). HRMS for $C_{33}H_{50}F_3N_2O_5S$ $[M+H]^+$: 643.3387 (calc.), 643.3353 (found). HPLC purity of 94.9% (retention time = 16.3 min).

Synthesis of (3α,16α)-3-[(*trans*-2,5-dimethyl-4-{[2-(trifluoromethyl)phenyl]sulfonyl} piperazin-1-yl)methyl]-16-fluoro-3-hydroxyandrostan-17-one (4). Under an argon atmosphere, ketone **1** (200 mg, 0.32 mmol) and Selectfluor™ (320 mg, 0.90 mmol) were

suspended in anhydrous MeOH (3 mL) and H_2SO_4 6 M (0.06 mmol) was added. The reaction mixture was stirred at 60 °C for 72 h and the resulting white suspension was evaporated under vacuum. The solid was extracted with EtOAc and washed with brine, dried over Na_2SO_4, filtered and evaporated under reduced pressure. The oily residue was purified by flash column chromatography using hexanes/EtOAc (gradient elution from 95:5 to 60:40) to afford 30 mg (14%) of **4** as a white solid. IR (KBr) ν_{max}: 3433 (OH), 2924, 2854 (C-H, aliphatic), 1759 (C=O), 1157 (S=O). ^{1}H NMR ($CDCl_3$): δ_H 0.74 (s, 19-CH_3), 0.85 (m, 9-CH), 0.88 (d, J = 7.0 Hz, 28-CH_3), 0.89 (s, 18-CH_3), 1.04 (m, 1H of 7-CH_2), 1.19 (d, J = 6.8 Hz, 29-CH_3), 1.10-1.85 (m, 1-CH_2, 2-CH_2, 4-CH_2, 5α-CH, 6-CH_2, 1H of 7-CH_2, 8-CH, 11-CH_2, 12-CH_2, 14-CH), 1.86-2.10 (m, 15-CH_2), 2.12 (d, J = 13.9 Hz, 1H of 22-CH_2), 2.30 (m, 1H of 22-CH_2 and 1H of 27-CH_2), 2.81 (broad, OH), 2.89 (m, 24-CH), 3.08 (d, J = 10.2 Hz, 1H of 27-CH_2), 3.35 and 3.49 (2m, 25-CH_2), 4.05 (m, 26-CH), 5.08 (dd, $J_{H,F}$ = 50.7 Hz, $J_{H,H}$ = 7.4 Hz, 16β-CH), 7.68 (m, 33-CH and 34-CH), 7.87 (m, 32-CH), 8.17 (m, 35-CH). ^{13}C NMR ($CDCl_3$): δ_C 8.7 (C28), 11.2 (C19), 14.0 (C18), 15.6 (C29), 19.8 (C11), 28.2 (C6), 29.6 (d, J_{C-F} = 12.1 Hz, C15), 30.4 (C7), 31.2 (C12), 32.4 (C2), 33.7 (C1), 34.9 (C8), 35.9 (C10), 39.4 (C4), 40.6 (C5), 46.0 (C25), 47.8 (C13), 48.3 (C14), 49.5 (C26), 52.4 (C27), 54.0 (C9), 54.7 (C24), 65.7 (C22), 70.9 (C3), 90.2 (d, J_{C-F} = 186.7 Hz, C16), 122.4 (q, J_{C-F} = 274.5 Hz, C36), 127.5 (q, J_{C-C-F} = 33.5 Hz, C31), 128.5 (q, $J_{C-C-C-F}$ = 6.3 Hz, C32), 131.9 (C35), 132.1 (C34), 132.5 (C33), 139.3 (C30), 213.7 (C17). HRMS for $C_{33}H_{47}F_4N_2O_4S$ $[M+H]^+$: 643.3187 (calc.), 643.3157 (found). HPLC purity of 93.2% (retention time = 26.9 min).

Synthesis of (3*α*,16*α*)-3-[(*trans*-2,5-dimethyl-4-{[2-(trifluoromethyl)phenyl]sulfonyl} piperazin-1-yl)methyl]-16-fluoroandrostane-3,17-diol (5). To a solution of **4** (21 mg, 0.03 mmol) in MeOH (3 mL) was added $NaBH_4$ (4 eq). The solution was stirred into an ice bath for 1

h. The resulting solution was evaporated and the solid was extracted with EtOAc, washed with brine, dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude compound was purified by flash column chromatography using hexanes/EtOAc (gradient elution from 70:30 to 20:80) to afford 17 mg (82%) of **5**. IR (KBr) ν_{max}: 3433 (OH), 2924, 2854 (C-H, aliphatic), 1157 (S=O). ^{1}H NMR ($CDCl_3$): δ_H 0.65 and 0.71 (2s, 18-CH_3), 0.72 and 0.73 (2s, 19-CH_3), 0.82 (m, 9-CH), 0.88 (d, J = 6.5 Hz, 28-CH_3), 0.95 (m, 1H of 7-CH_2), 1.18 (d, J = 6.8 Hz, 29-CH_3), 1.10-1.85 (m, 1-CH_2, 2-CH_2, 4-CH_2, 5-CH, 6-CH_2, 1H of 7-CH_2, 8-CH, 11-CH_2, 12-CH_2, 14-CH, 15-CH_2), 2.12 (d, J = 13.9 Hz, 1H of 22-CH_2), 2.30 (m, 1H of 22-CH_2 and 1H of 27-CH_2), 2.80 (m, OH), 2.89 (m, 24-CH), 3.08 (d, J = 10.2 Hz, 1H of 27-CH_2), 3.35 and 3.49 (2m, 25-CH_2), 3.70 and 3.75 (2m, 17-CH), 4.05 (m, 26-CH), 4.90 (d, J = 54.4 Hz, 16β-CH), 5.21 (d, J = 52.9 Hz, 16β-CH), 7.68 (m, 33-CH and 34-CH), 7.87 (m, 32-CH), 8.17 (m, 35-CH). ^{13}C NMR ($CDCl_3$): δ_C 8.6 (C28), 11.2 (C19), 12.3/17.1 (C18), 15.6 (C29), 20.0 (C11), 28.4 (C6), 30.5 (C7), 31.1/31.9 (C15), 32.4 (C2), 33.7 (C1), 34.8 (C8), 35.8 (C10), 36.5 (C12), 39.4 (C4), 40.6 (C5), 43.7/45.2 (C13), 46.0 (C25), 47.0/48.7 (C14), 49.5 (C26), 52.4 (C27), 54.0 (C9), 54.6 (C24), 65.7 (C22), 71.0 (C3), 77.9/87.5(2d) (C17), 94.6/101.3 (2d) (C16), 122.5 (q, $J_{C\text{-}F}$ = 274.1 Hz, C36), 127.5 (q, $J_{C\text{-}C\text{-}F}$ = 33.0 Hz, C31), 128.5 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 6.5 Hz, C32), 131.9 (C35), 132.1 (C34), 132.5 (C33), 139.3 (C30). HRMS for $C_{33}H_{49}F_4N_2O_4S$ $[M+H]^+$: 645.3344 (calc.), 645.3309 (found). HPLC purity of epimeric mixture = 54.7:43.3% (retention time = 17.2 and 18.6 min, respectively).

Synthesis of (3α,17β)-3-[(*trans*-2,5-dimethyl-4-{[2-(trifluoromethyl)phenyl]sulfonyl} piperazin-1-yl)methyl]androstane-3,17-diol (6). To a solution of **1** (100 mg, 0.16 mmol) in MeOH (5 mL) was added $NaBH_4$ (24 mg, 0.64 mmol). The solution was stirred into an ice bath for 1 h. The resulting solution was evaporated and the solid was extracted with EtOAc, washed

with brine, dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude compound was purified by flash column chromatography using hexanes/EtOAc (gradient elution from 90:10 to 50:50) to give 91 mg (90%) of **6** as a white solid. IR (KBr) ν_{max}: 3441 (OH), 2932, 2854 (C-H, aliphatic), 1157 (S=O). ^{1}H NMR ($CDCl_3$): δ_H 0.71 (s, 18-CH_3), 0.73 (s, 19-CH_3), 0.78 (m, 9-CH), 0.87 (d, J = 6.3 Hz, 28-CH_3), 0.90 (m, 1H of 7-CH_2 and 14-CH), 1.03 (m, 1H of 12-CH_2), 1.10-1.70 (m, 1-CH_2, 2-CH_2, 4-CH_2, 5-CH, 6-CH_2, 1H of 7-CH_2, 8-CH, 11-CH_2, 15-CH_2, 1H of 16-CH_2), 1.77 (dt, J_1 = 12.2 Hz, J_2 = 3.1 Hz, 1H of 12-CH_2), 2.01 (m, 16-CH), 2.13 (d, J = 13.9 Hz, 1H of 22-CH_2), 2.32 (m, 1H of 22-CH_2 and 1H of 27-CH_2), 2.81 (broad, OH), 2.87 (m, 24-CH), 3.07 (d, J = 10.2 Hz, 1H of 27-CH_2), 3.32 and 3.49 (2m, 25-CH_2), 3.62 (t, J = 10.2 Hz, 17α-CH), 4.05 (m, 26-CH), 7.68 (m, 33-CH and 34-CH), 7.87 (m, 32-CH), 8.17 (m, 35-CH). ^{13}C NMR ($CDCl_3$): δ_C 8.6 (C28), 11.1 (C19), 11.2 (C18), 15.6 (C29), 20.5 (C11), 23.3 (C15), 28.5 (C6), 30.5 (C16), 31.5 (C7), 32.5 (C2), 33.8 (C1), 35.5 (C8), 35.8 (C10), 36.7 (C12), 39.4 (C4), 40.7 (C5), 42.9 (C13), 46.0 (C25), 49.5 (C26), 50.9 (C14), 52.4 (27-CH_2), 54.2 (C9), 54.7 (24-CH), 65.8 (22-CH_2), 71.0 (C3), 81.9 (C17), 122.5 (q, $J_{C\text{-}F}$ = 274.0 Hz, C36), 127.5 (q, $J_{C\text{-}C\text{-}F}$ = 33.3 Hz, C31), 128.5 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 6.4 Hz, C32), 131.9 (C35), 132.1 (C34), 132.5 (C33), 139.3 (C30). HRMS for $C_{33}H_{50}F_3N_2O_4S$ $[M+H]^+$: 627.3438 (calc.), 627.3431 (found). HPLC purity of 99.8% (retention time = 18.6 min).

Synthesis of (3α)-3-[(*trans*-2,5-dimethyl-4-{[2-(trifluoromethyl)phenyl]sulfonyl} piperazin-1-yl)methyl]-17-methoxyandrostan-3-ol (7). To a solution of **6** (20 mg, 0.03 mmol) in anhydrous DMF (2 mL), was added NaH 60% in oil (0.19 mmol). The solution was stirred for 30 min before adding methyl iodide (24 µL, 0.38 mmol). The reaction was stirred at rt for 72 h and quenched with 10 mL of water. The aqueous phase was extracted with DCM, washed with brine, dried with Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was

purified by flash column chromatography using hexanes/EtOAc (gradient elution from 90:10 to 60:40) to afford 3 mg (15%) of **7** as a white solid. IR (KBr) ν_{max}: 3433 (OH), 2924, 2854 (C-H, aliphatic), 1157 (S=O). ^{1}H NMR ($CDCl_3$): δ_H 0.71 (s, 18-CH_3), 0.72 (s, 19-CH_3), 0.75 (m, 9-CH), 0.86 (d, J = 6.4 Hz, 28-CH_3), 0.92 (m, 1H of 7-CH_2 and 14-CH), 1.10-1.70 (m, 1-CH_2, 2-CH_2, 4-CH_2, 5-CH, 6-CH_2, 1H of 7-CH_2, 8-CH, 11-CH_2, 1H of 12-CH_2, 15-CH_2, 1H of 16-CH_2), 1.87 (d, J = 12.2 Hz, 1H of 12-CH_2), 1.98 (m, 1H of 16-CH_2), 2.13 (d, J = 13.9 Hz, 1H of 22-CH_2), 2.30 (m, 1H of 22-CH_2 and 1H of 27-CH_2), 2.80 (broad, OH), 2.89 (m, 24-CH), 3.08 (d, J_1 = 10.2 Hz, 1H of 27-CH_2), 3.20 (t, J = 8.2 Hz, 17α-CH), 3.31 (s, OCH_3), 3.32 and 3.49 (2m, 25-CH_2), 4.05 (m, 26-CH), 7.68 (m, 33-CH and 34-CH), 7.87 (m, 32-CH), 8.17 (m, 35-CH). ^{13}C NMR ($CDCl_3$): δ_C 8.6 (C28), 11.2 (C19), 11.6 (C18), 15.6 (C29), 20.6 (C11), 23.3 (C15), 27.7 (C16), 28.5 (C6), 31.5 (C7), 32.5 (C2), 33.8 (C1), 35.3 (C8), 35.8 (C10), 38.0 (C12), 39.5 (C4), 40.7 (C5), 42.9 (C13), 46.0 (C25), 49.5 (C26), 51.2 (C14), 52.3 (C27), 54.1 (C9), 54.2 (C24), 57.8 (OCH_3), 65.8 (22-CH_2), 70.9 (C3), 90.8 (C17), 122.5 (q, $J_{C\text{-}F}$ = 274.1 Hz, C36), 127.5 (q, $J_{C\text{-}C\text{-}F}$ = 33.2 Hz, C31), 128.5 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 6.4 Hz, C29), 131.9 (C35), 132.1 (C34), 132.5 (C33), 139.3 (C30). HRMS for $C_{34}H_{52}F_3N_2O_4S$ $[M+H]^+$: 641.3594 (calc.), 641.3581 (found). HPLC purity of 95.7% (retention time = 21.9 min).

Synthesis of (3α,17α)-3-[(*trans*-2,5-dimethyl-4-{[2-(trifluoromethyl)phenyl]sulfonyl} piperazin-1-yl)methyl]pregn-20-yne-3,17-diol (8). To a solution of trimethysilylacetylene (61 µL, 0.42 mmol) in anhydrous diethyl ether (3 mL) was added MeLi (1.6 M, 0.2 mL, 0.33 mmol) under argon atmosphere at 0 °C. The mixture was then allowed to return to rt and was stirred for 1 h. The mixture was cooled again to 0 °C before the addition of a solution of **1** (26 mg, 0.04 mmol) in anhydrous THF (3 mL) and allowed to be stirred at rt overnight. The reaction mixture was poured into water, extracted with EtOAc, washed with brine, dried over Na_2SO_4, filtered and

evaporated under reduced pressure. The crude compound was dissolved in a solution of potassium carbonate (5%) in MeOH and stirred for 3 h at rt. The solution was poured into water and extracted with DCM, washed with brine, dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude compound was purified by flash column chromatography with hexanes/EtOAc (gradient elution from 90:10 to 70:30) to give 20 mg (77%) of **8** as a white solid. IR (KBr) ν_{max}: 3433 (OH), 2924, 2854 (C-H, aliphatic), 1157 (S=O). ^{1}H NMR ($CDCl_3$): δ_H 0.73 (s, 19-CH_3), 0.80 (m, 9α-CH), 0.82 (s, 18-CH_3), 0.87 (d, J = 6.5 Hz, 28-CH_3), 0.95 (m, 1H of 7-CH_2), 1.18 (d, J = 5.4 Hz, 29-CH_3), 1.10- 1.70 (m, 1-CH_2, 2-CH_2, 4-CH_2, 5-CH, 6-CH_2, 1H of 7-CH_2, 8-CH, 11-CH_2, 12-CH_2, 14-CH, 15-CH_2), 1.90 (broad, OH), 1.95 and 2.25 (2m, 16-CH_2), 2.13 (d, J = 13.9 Hz, 1H of 22-CH_2), 2.30 (m, 1H of 22-CH_2 and 1H of 27-CH_2), 2.78 (broad, OH), 2.87 (m, 24-CH), 3.07 (d, J = 10.2 Hz, 1H of 27-CH_2), 3.32 and 3.49 (2m, 25-CH_2), 4.05 (m, 26-CH), 7.68 (m, 33-CH and 34-CH), 7.87 (m, 32-CH), 8.17 (m, 35-H). ^{13}C NMR ($CDCl_3$): δ_C 8.6 (C28), 11.2 (C19), 12.8 (C18), 15.7 (C29), 20.6 (C11), 23.1 (C15), 28.5 (C6), 31.4 (C7), 32.2 (C2), 32.7 (C12), 33.8 (C1), 35.8 (C10), 36.1 (C8), 38.9 (C16), 39.4 (C4), 40.6 (C5), 46.0 (C25), 46.8 (C13), 49.6 (C26), 50.4 (C14), 52.4 (C27), 53.7 (C9), 54.7 (C24), 65.8 (C22), 71.0 (C3), 73.8 (C21), 79.9 (C17), 87.6 (C20), 122.6 (q, $J_{C\text{-}F}$ = 274.5 Hz, C36), 127.2 (q, $J_{C\text{-}C\text{-}F}$ = 32.0 Hz, C31), 128.5 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 6.5 Hz, C32), 131.9 (C35), 132.1 (C34), 132.5 (C33), 139.4 (C30). HRMS for $C_{35}H_{50}F_3N_2O_4S$ $[M+H]^+$: 651.3438 (calc.), 651. 3438 (found). HPLC purity of 98.9% (retention time = 18.2 min).

Synthesis of (3α)-3-[(*trans*-2,5-dimethyl-4-{[2-(trifluoromethyl)phenyl]sulfonyl} piperazin-1-yl)methyl]-3-hydroxyandrostan-17-one oxime (9). To a solution of **1** (60 mg, 0.1 mmol) in ethanol (5 mL), hydroxylamine hydrochloride (12 mg, 0.17 mmol) and sodium acetate (18 mg, 0.22 mmol) were added. The reaction mixture was stirred at 60 °C for 1 h. The resulting

solution was evaporated under reduced pressure and the solid was extracted with DCM and washed with brine, dried with Na_2SO_4, filtrated and evaporated. The crude product was purified by flash column chromatography using hexanes/EtOAc (gradient elution from 90:10 to 50:50) to afford 57 mg (89%) of compound **9** as a white solid. IR (KBr) ν_{max}: 3394 (OH), 2932, 2854 (C-H, aliphatic), 1157 (S=O). ^{1}H NMR ($CDCl_3$): δ_H 0.73 (s, 19-CH_3), 0.83 (m, 9-CH), 0.88 (m, 18-CH_3 and 28-CH_3), 0.98 (m, 1H of 7-CH_2), 1.17 (d, J = 6.8 Hz, 29-CH_3), 1.10-1.90 (m, 1-CH_2, 2-CH_2, 4-CH_2, 5-CH, 6-CH_2, 1H of 7-CH_2, 8-CH, 11-CH_2, 12-CH_2, 14-CH, 15-CH_2), 2.13 (d, J = 13.9 Hz, 1H of 22-CH_2), 2.32 (m, 1H of 22-CH_2 and 1H of 27-CH_2), 2.46 (m, 16-CH_2), 2.87 (m, 24-CH), 2.94 (broad, OH), 3.07 (d, J = 10.2 Hz, 1H of 27-CH_2), 3.32 and 3.49 (2m, 25-CH_2), 4.05 (m, 26-CH), 7.68 (m, 33-CH and 34-CH), 7.87 (m, 32-CH), 8.17 (m, 35-CH), 8.41 (broad, NOH). ^{13}C NMR ($CDCl_3$): δ_C 8.6 (C28), 11.2 (C19), 15.6 (C29), 17.1 (C18), 20.4 (C11), 23.1 (C15), 24.9 (C16), 28.4 (C6), 31.4 (C7), 32.5 (C2), 33.6 (C1), 34.0 (C12), 34.9 (C8), 35.8 (C10), 39.2 (C4), 40.5 (C5), 44.0 (C13), 46.0 (C25), 49.5 (C26), 52.4 (C27), 53.8 (C14), 54.2 (C9), 54.7 (C24), 65.7 (C22), 71.0 (C3), 122.5 (q, $J_{C\text{-}F}$ = 274.0 Hz, C36), 127.5 (q, $J_{C\text{-}C\text{-}F}$ = 33.2 Hz, C31), 128.5 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 6.5 Hz, C32), 131.9 (C32), 132.1 (C34), 132.5 (C33), 139.3 (C30), 171.2 (C17). HRMS for $C_{33}H_{49}F_3N_3O_4S$ $[M+H]^+$: 640.3390 (calc.), 640.3380 (found). HPLC purity of 99.8% (retention time = 18.2 min).

Synthesis of (3*R*,10*S*,13*S*,17*S*)-3-[(*trans*-2,5-dimethyl-4-{[2-(trifluoromethyl)phenyl] sulfonyl}piperazin-1-yl)methyl]-10,13-dimethylhexadecahydrospiro[cyclopenta[a] phenanthrene-17,2'-oxiran]-3-ol (10). To a solution of trimethylsulfonium iodide (39 mg, 0.06 mmol), in dry DMSO (3 mL) was carefully added NaH 60 % in oil (6.5 mg, 0.06 mmol). The solution was stirred at rt under argon atmosphere for 1 h before adding ketone **1** (30 mg, 0.05 mmol) dissolved in THF (1 mL). The reaction mixture was stirred at rt for 5 h and poured in

ice/water (100 mL) and extracted with EtOAc. The combined organic layer was washed with brine and dried with Na_2SO_4. The resulting solution was filtered, evaporated and the crude product was purified by flash column chromatography using hexanes/EtOAc (gradient elution from 90:10 to 60:40) to afford 20 mg (63%) of **10** as a white solid. IR (KBr) ν_{max}: 3441 (OH), 2924, 2854 (C-H, aliphatic), 1157 (S=O). ^{1}H NMR ($CDCl_3$): δ_H 0.73 (s, 19-CH_3), 0.80 (m, 9-CH), 0.86 (s, 18-CH_3), 0.88 (d, J = 6.0 Hz, 28-CH_3), 0.95 (m, 1H of 7-CH_2), 1.00 (m, 1H of CH_2-12, 1.19 (d, J = 5.4 Hz, 29-CH_3), 1.12-1.82 (m, 1-CH_2, 2-CH_2, 4-CH_2, 5-CH, 6-CH_2, 1H of 7-CH_2, 8-CH, 11-CH_2, 1H of 12-CH_2, 14-CH, 15-CH_2, 1H of 16-CH_2), 1.95 (m, 1H of 16-CH_2), 2.14 (d, J = 14.5 Hz, 1H of 22-CH_2), 2.30 (m, 1H of 22-CH_2 and 1H of 27-CH_2), 2.59 (d, J = 5.1 Hz, 1H of oxirane), 2.89 (m, 1H of oxirane and 24-CH), 3.08 (d, J = 10.2 Hz, 1H of 27-CH_2), 3.35 and 3.49 (2m, 25-CH_2), 4.05 (m, 26-CH), 7.68 (m, 33-CH and 34-CH), 7.87 (m, 32-CH), 8.17 (m, 35-CH). ^{13}C NMR ($CDCl_3$): δ_C 8.6 (C28), 11.2 (C19), 14.4 (C18), 15.7 (C29), 20.3 (C11), 23.5 (C15), 28.6 (C6), 29.0 (C16), 31.4 (C7), 32.5 (C2), 33.8 (C1), 33.9 (C12), 35.6 (C10), 35.8 (C8), 39.4 (C4), 40.1 (C13), 40.7 (C5), 46.0 (C25), 49.5 (C26), 52.4 (C27), 52.8 (C14), 53.7 (C20), 54.3 (C9), 54.7 (C24), 65.7 (C22), 70.6 (C17), 70.9 (C3), 122.5 (q, $J_{C\text{-}F}$ = 274.0 Hz, C36), 127.5 (q, $J_{C\text{-}C\text{-}F}$ = 33.2 Hz, C31), 128.5 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 6.5 Hz, C32), 131.9 (C35), 132.1 (C34), 132.5 (C33), 139.3 (C30). HRMS for $C_{34}H_{50}F_3N_2O_4S$ $[M+H]^+$: 639.3438 (calc.), 639.3435 (found). HPLC purity of 93.5% (retention time = 20.7 min).

Synthesis of (3β)-17-(2-methyl-1,3-dioxolan-2-yl)androstan-3-ol (11). To a solution of 3β-hydroxy-5α-pregnan-20-one (500 mg, 1.6 mmol) in anhydrous toluene (25 mL) was added ethylene glycol (1.2 mL, 21.4 mmol) and *p*-toluensulfonic acid (30 mg, 0.17 mmol). The reaction was refluxed under a Dean-Stark trap for 12 h. The solution was poured in cold water (100 mL) and extracted with EtOAc. The organic phase was washed with a 20% solution of sodium acetate

and brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by flash chromatography column using hexanes/EtOAc (gradient elution from 95:5 to 80:20) to obtain 432 mg (74%) of **11**. IR (KBr) ν_{max}: 3418 (OH), 2932, 2854 (C-H, aliphatic). ^{1}H NMR ($CDCl_3$): δ_H 0.63 (m, 9-CH), 0.74 (s, 19-CH_3), 0.80 (s, 18-CH_3), 1.28 (s, 21-CH_3), 0.82-1.80 (m, unassigned CH and CH_2), 2.01 (m, 17-CH), 3.58 (m, 3-CH), 3.86 and 3.93 (2m, OCH_2CH_2O). LRMS for $C_{23}H_{39}O_3$ $[M+H]^+$: 363.2.

Synthesis of 17-(2-methyl-1,3-dioxolan-2-yl)androstan-3-one (12). To a solution of **11** (500 mg, 1.40 mmol) in DCM (10 mL) was added Dess-Martin periodinane (1.5 eq). The reaction was stirred at rt for 1 h and the resulting white suspension was evaporated. The residue was diluted with EtOAc (20 mL) and washed with a saturated solution of sodium bicarbonate and brine. The organic phase was dried with Na_2SO_4 and evaporated. The crude product was purified by flash column chromatography using hexanes/EtOAc (gradient elution from 95:5 to 80:20) to afford 370 mg (73%) of **12**. IR (KBr) ν_{max}: 2932, 2885 (C-H, aliphatic), 1713 (C=O). ^{1}H NMR ($CDCl_3$): δ_H 0.74 (m, 9-CH), 0.78 (s, 18-CH_3), 0.90 (m, 1H of 7-CH_2), 1.01 (s, 19-CH_3), 1.29 (s, 21-CH_3), 1.02-2.40 (m, unassigned CH and CH_2), 3.88 and 3.97 (2m, OCH_2CH_2O). LRMS for $C_{23}H_{37}O_3$ $[M+H]^+$: 361.2.

Synthesis of (3*R*,10*S*,13*S*)-10,13-dimethyl-17-(2-methyl-1,3-dioxolan-2-yl)hexadecahydrospiro[cyclopenta[*a*]phenanthrene-3,2'-oxirane] (13). To a solution of trimethylsulfoxonium iodide (2.0 eq) in dry DMSO (10 mL) was carefully added NaH 60% in oil (2.0 eq). The solution was stirred at rt under argon atmosphere for 1 h before adding **12** (200 mg, 0.55 mmol) dissolved in THF (4 mL). The reaction mixture was stirred at rt for 5 h, then poured in ice/water (100 mL) and extracted with EtOAc. The combined organic layer was washed with brine and dried with Na_2SO_4. The resulting solution was filtered, evaporated and the crude

product was purified by flash column chromatography using hexanes/EtOAc (85:15) to afford 140 mg (68%) of **13**. IR (KBr) ν_{max}: 2924, 2854 (C-H, aliphatic). ^{1}H NMR ($CDCl_3$): δ_H 0.76 (s, 18-CH_3), 0.84 (s, 19-CH_3), 1.29 (s, 21-CH_3), 0.75-2.10 (m, unassigned CH and CH_2), 2.38 (m, 1H), 2.61 (s, 2H of oxirane), 3.87 and 3.97 (2m, OCH_2CH_2O). LRMS for $C_{24}H_{39}O_3$ $[M+H]^+$: 375.2.

Synthesis of (3α)-3-{[*trans*-2,5-dimethylpiperazin-1-yl]methyl}-17-(2-methyl-1,3-dioxolan-2-yl)androstan-3-ol (14). To a solution of **13** (50 mg, 0.13 mmol) in anhydrous ethanol (4 mL) was added *trans*-2,5-dimethylpiperazine (5.0 eq). The solution was stirred 12 h at 70 °C and the resulting solution was poured in ice/water (50 mL). The white precipitate was separated by vacuum filtration and the solid was purified by flash column chromatography using DCM/MeOH/TEA (96:2:2) to give 45 mg (70%) of **14**. IR (KBr) ν_{max}: 3425 (NH and OH), 2932, 2847 (C-H, aliphatic). ^{1}H NMR ($CDCl_3$): δ_H 0.71 (s, 19-CH_3), 0.72 (18-CH_3), 0.98 (d, J = 6.1 Hz, 28-CH_3), 1.04 (m, 29-CH_3), 0.70-1.80 (m, unassigned CH and CH_2), 1.27 (s, 21-CH_3), 1.96-2.17 (m, 17-CH and NCH_2), 2.39 (m, 24-CH), 2.56 (m, NCH_2), 2.78 (m, NCH_2), 2.92 (m, 26-CH and NCH_2), 3.70 (broad, 1H), 3.84 and 3.94 (2m, OCH_2CH_2O). LRMS for $C_{30}H_{53}N_2O_3$ $[M+H]^+$: 489.4.

Synthesis of (3α,5α)-3-[(*trans*-2,5-dimethyl-4-{[2-(trifluoromethyl)phenyl]sulfonyl} piperazin-1-yl)methyl]-3-hydroxypregnan-20-one (16). To a solution of **14** (50 mg, 0.10 mmol) in anhydrous DCM (3 mL) were added TEA (4.0 eq) and 2-(trifluoromethyl) benzenesulfonyl chloride (2.0 eq). The reaction mixture was stirred at rt for 4 h, then evaporated, and the remaining oily residue was purified directly by flash column chromatography using hexanes/EtOAc (70:30) to give 45 mg of **15**. This dioxolane (40 mg) was dissolved in acetone (9 mL), DCM (1 mL) and concentrated perchloric acid (0.1 mL), and the solution was stirred at rt

for 1 h. The reaction mixture was evaporated and diluted with EtOAc (50 mL), washed with water, dried with Na_2SO_4, filtered and evaporated under reduced pressure. After filtration, the residue was purified by flash column chromatography using hexanes/EtOAc (gradient elution from 90:10 to 60:40) to give 32 mg (82%) of **16**. IR (KBr) ν_{max}: 3526 (OH), 2924, 2854 (C-H, aliphatic), 1697 (C=O), 1157 (S=O). ^{1}H NMR ($CDCl_3$): δ_H 0.59 (s, 18-CH_3), 0.72 (s, 19-CH_3), 0.80 (m, 9-CH), 0.87 (d, J = 6.5 Hz, 28-CH_3), 0.98 (m, 1H of 7-CH_2), 1.19 (d, J = 6.8 Hz, 29-CH_3), 1.10-1.70 (m, 1-CH_2, 2-CH_2, 4-CH_2, 5-CH, 6-CH_2, 1H of 7-CH_2, 8-CH, 11-CH_2, 1H of 12-CH_2, 14-CH, 15-CH_2, 1H of 16-CH_2), 1.98 (d, J = 12.1 Hz, 1H of 12-CH_2), 2.10 (s, 21-CH_3), 2.14 (m, 1H of 16-CH_2 and 1H of 22-CH_2), 2.30 (m, 1H of 22-CH_2 and 1H of 27-CH_2), 2.52 (t, J = 8.9 Hz, 17-CH), 2.89 (m, 24-CH), 3.07 (d, J = 10.1 Hz, 1H of 27-CH_2), 3.35 and 3.49 (2m, 25-CH_2), 4.05 (m, 26-CH), 7.67 (m, 33-CH and 34-CH), 7.87 (m, 32-CH), 8.17 (m, 35-CH). ^{13}C NMR ($CDCl_3$): δ_C 8.6 (C28), 11.2 (C19), 13.4 (C18), 15.7 (C26), 20.9 (C11), 22.7 (C16), 24.4 (C15), 28.6 (C6), 31.5 (C21), 31.9 (C7), 32.5 (C2), 33.8 (C1), 35.5 (C8), 35.7 (C10), 39.1 (C12), 39.4 (C4), 40.7 (C5), 44.2 (C13), 46.0 (C25), 49.5 (C26), 52.4 (C27), 53.9 (C9), 54.7 (C24), 56.7 (C14), 63.8 (C17), 65.7 (C22), 71.0 (C3), 122.6 (q, $J_{C\text{-}F}$ = 274.1 Hz, C36), 127.5 (q, $J_{C\text{-}C\text{-}F}$ = 33.0 Hz, C31), 128.5 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 6.4 Hz, C32), 131.9 (C35), 132.1 (C34), 132.5 (C33), 139.3 (C30), 209.8 (C20). HRMS for $C_{35}H_{52}F_3N_2O_4S$ $[M+H]^+$ = 653.3594 (calc), 653.3574 (found). HPLC purity of 96.2% (retention time = 20.8 min).

Synthesis of (3*R*,13*S*)-13-methyltetradecahydrospiro[cyclopenta[*a*]phenanthrene-3,2'-oxiran]-17(2*H*)-one (19). To a solution of trimethylsulfoxonium iodide (2.0 eq) in dry DMSO (10 mL) was carefully added NaH 60% in oil (2.0 eq). The solution was stirred at rt under argon atmosphere for 1 h before adding **17** (150 mg, 0.54 mmol) dissolved in THF (4 mL). The reaction mixture was stirred at rt for 5 h, then poured in ice/water (100 mL) and extracted

with EtOAc. The combined organic layer was washed with brine and dried with Na_2SO_4. The resulting solution was filtered, evaporated and the crude product was purified by flash column chromatography using hexanes/EtOAc (85:15) to afford 94 mg (65%) of the C17-alcohol intermediate **18**. To this alcohol (84 mg, 0.29 mmol) dissolved in DCM (5 mL) was added Dess-Martin periodinane (184 mg, 0.43 mmol) and the reaction mixture was stirred at rt for 1 h. The resulting white suspension was evaporated; the residue was diluted with EtOAc and washed with a saturated solution of sodium bicarbonate and brine. The organic phase was dried with Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by flash column chromatography using hexanes/EtOAc (gradient elution from 95:5 to 80:20) to give 75 mg (89%) of **19**. IR (KBr) ν_{max}: 2916, 2854 (C-H, aliphatic), 1736 (C=O). ^{1}H NMR ($CDCl_3$): δ_H 0.71-2.00 (m, unassigned CH and CH_2), 0.88 (s, 18-CH_3), 2.08 (m, 16α-CH), 2.44 (dd, J_1 = 19.3 Hz, J_2 = 8.2 Hz, 16β-CH), 2.64 (m, 2H of oxirane). LRMS for $C_{19}H_{27}O_2$ $[M-H]^-$: 286.8.

Synthesis of (3α)-3-{[*trans*-2,5-dimethylpiperazin-1-yl]methyl}-3-hydroxyestran-17-one (20). To a solution of **19** (70 mg, 0.24 mmol) in anhydrous ethanol (4 mL) was added *trans*-2,5-dimethylpiperazine (5.0 eq). The solution was stirred 12 h at 70 °C and the resulting solution was poured in ice/water (50 mL). The white precipitate was separated by vacuum filtration and the solid was purified by flash column chromatography using DCM/MeOH/TEA (96:2:2) to give 55 mg (68%) of **20**. IR (KBr) ν_{max}: 3441 (NH and OH), 2924, 2854 (C-H, aliphatic), 1736 (C=O). ^{1}H NMR ($CDCl_3$): δ_H 0.72 (m, 9-CH and 10β-CH), 0.87 (s, 18-CH_3), 0.99 (d, J = 5.4 Hz, 28-CH_3), 1.04 dd, J_1 = 2.6 Hz, J_2 = 6.2 Hz, 29-CH_3), 0.90-1.98 (m, unassigned CH and CH_2), 2.00-2.15 (m, NCH_2 and 16α-CH), 2.36 (m, 24-CH), 2.44 (dd, J_1 = 19.3 Hz, J_2 = 8.2 Hz, 16β-CH), 2.60 (m, NCH_2), 2.92 (m, 26-CH and NCH_2). LRMS for $C_{25}H_{43}N_2O_2$ $[M+H]^+$: 403.3.

Synthesis of (3α)-3-[(*trans*-2,5-dimethyl-4-{[2-

(trifluoromethyl)phenyl]sulfonyl}piperazin-1-yl)methyl]-3-hydroxyestran-17-one (21). To a solution of **20** (48 mg, 0.12 mmol) in anhydrous DCM (3 mL) were added TEA (4.0 eq) and 2-(trifluoromethyl) benzenesulfonyl chloride (2.0 eq). The reaction mixture was stirred at rt for 4 h, then evaporated, and the remaining oily residue was purified directly by flash column chromatography using hexanes/EtOAc (90:10 to 60:40) to give 48 mg (64%) of **21**. IR (KBr) ν_{max}: 3364 (OH), 2924, 2854 (CH, aliphatic), 1736 (C=O), 1149 (S=O). ^{1}H NMR ($CDCl_3$): δ_H 0.70 (m, 9-H and 10β-H), 0.86 (s, 18-CH_3), 0.87 and 0.88 (2d, J = 6.5 Hz, 28-CH_3), 1.18 and 1.19 (2d, J = 6.8 Hz, 29-CH_3), 0.90-1.96 (m, 1-CH_2, 2-CH_2, 4-CH_2, 5-CH, 6-CH_2, 7-CH_2, 8-CH, 11-CH_2, 12-CH_2, 14-CH, 15-CH_2), 2.08 (m, 16α-CH), 2.14 (m, 1H of 22-CH_2), 2.35 (m, 1H of 22-CH_2 and 1H of 27-CH_2), 2.47 (dd, J_1 = 19.2 Hz, J_2 = 8.6 Hz, 16β-CH), 2.82 (broad, OH), 2.88 (m, 24-CH), 3.07 (dd, J_1 = 15.7 Hz, J_2 = 3.8 Hz, 1H of 27-CH_2), 3.35 and 3.49 (2m, 25-CH_2), 4.05 (m, 26-CH), 7.68 (m, 32-CH and 33-CH), 7.87 (m, 31-CH), 8.17 (m, 34-CH). ^{13}C NMR ($CDCl_3$): δ_C 8.6 (C28), 13.8 (C18), 15.7 (C29), 21.6 (C15), 25.0 (C1), 25.3 (C11), 29.7 (C6), 31.5 (C12), 33.5 (C7), 35.8 (C16), 36.9 (C2), 37.5 (C8), 40.8 (C5), 44.1 (C4), 46.0 (C25), 46.8 (C10), 47.9 (C13), 48.1 (C9), 49.5 (C26), 50.6 (C14), 52.4 (C27), 54.7 (C24), 65.7 (C22), 70.7 (C3), 122.6 (q, $J_{C\text{-}F}$ = 274.1 Hz, C36), 127.5 (q, $J_{C\text{-}C\text{-}F}$ = 33.4 Hz, C31), 128.5 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 6.5 Hz, C32), 131.9 (C35), 132.1 (C34), 132.5 (C33), 139.3 (C30), 221.5 (C17). HRMS for $C_{32}H_{46}F_3N_2O_4S$ $[M+H]^+$: 611.3125 (calc.), 611.3095 (found). HPLC purity of 97.7% (retention time = 18.6 min).

Synthesis of 3-[(*trans*-2,5-dimethyl-4-{[2-(trifluoromethyl)phenyl]sulfonyl}piperazin-1-yl)carbonyl]estra-1,3,5(10)-trien-17-one (23). To a solution of **22** (40 mg, 0.13 mmol) in anhydrous DMF (2 mL) was added 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (60 mg, 0.17 mmol). The solution was stirred at rt under argon

atmosphere for 10 min before adding {[2-(trifluoromethyl)phenyl]sulfonyl}2,5-dimethylpiperazine (Amine A)[27] (86 mg, 0.26 mmol). The reaction mixture was stirred for 2 h more and poured in ice/water (50 mL), extracted with DCM, washed with brine, dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by flash column chromatography using hexanes/EtOAc (90:10 to 40:60) to give 70.1 mg (87%) of **23** as a white solid. IR (KBr) ν_{max}: 2932, 2862 (aliphatic), 1736 (C=O), 1636 (NC=O), 1165 (S=O). ^{1}H NMR ($CDCl_3$): δ_H 0.91 (s, 18-CH_3), 1.06 (d, J = 6.6 Hz, 28-CH_3), 1.12 and 1.19 (2m, 29-CH_3), 1.40-1.70 (m, 1H of 7-CH_2, 8-CH, 1H of 11-CH_2, 1H of 12-CH_2, 14-CH, 1H of 15-CH_2), 1.95-2.10 (m, 1H of each 7-CH_2, 12-CH_2 and 15-CH_2), 2.15 (m, 16α-CH), 2.30 (m, 9-CH), 2.40 (m, 1H of 11-CH_2) 2.51 (dd, J_1 = 19.3 Hz, J_2 = 8.6 Hz, 16β-CH), 2.91 (m, 6-CH_2), 3.30, 3.40, 3.55 and 4.90 (4m, 25-CH_2 and 27-CH_2), 3.98 and 4.95 (2m, 24-CH), 4.06 and 4.19 (2m, 26-CH), 7.06 (m, 2-CH and 4-CH), 7.28 (m, 1-CH), 7.70 (m, 33-CH and 34-CH), 7.89 (m, 32-CH), 8.18 (m, 35-CH). ^{13}C NMR ($CDCl_3$): δ_C 13.8 (C18), 14.4/14.6 (C28), 15.8 (C29), 21.5 (C15), 25.6 (C11), 26.2 (C7), 29.2 (C6), 31.5 (C12), 35.8 (C16), 37.9 (C8), 40.5/44.0 (C27), 43.5/50.0 (C24), 44.3 (C9), 46.9 (C25), 47.9 (C13), 48.6 (C26), 50.4 (C14), 122.5 (q, $J_{C\text{-}F}$ = 274.3 Hz, C36), 124.0 (C2), 125.5 (C1), 126.9 (C4), 127.2 (q, $J_{C\text{-}C\text{-}F}$ = 32.0 Hz, C31), 128.6 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 6.4 Hz, C32), 132.1 (C35), 132.2 (C34), 132.8 (C33), 136.4 (C10), 137.0 (C5), 138.8 (C30), 141.5 (C3), 171.5 (C22), 220.6 (C17). HRMS for $C_{32}H_{38}F_3N_2O_4S$ $[M+H]^+$: 603.2499 (calc.), 603.2476 (found). HPLC purity of 99.1% (retention time = 25.9 min).

Synthesis of 3-[(1*R*)-2-(*trans*-2,5-dimethyl-4-{[2-(trifluoromethyl)phenyl]sulfonyl} piperazin-1-yl)-1-hydroxyethyl]estra-1,3,5(10)-trien-17-one (26 and 27). To a solution of **24** (150 mg, 0.5 mmol) in ethanol (3 mL) was added 899 mg (2.50 mmol) of the Amine A,[27] and the reaction was stirred at 70 °C for 48 h. The resulting solution was evaporated under reduced

pressure and the gummy residue was purified by flash column chromatography using hexanes/EtOAc (gradient elution from 95:5 to 50:50) to obtain 150 mg (48%) of an epimeric mixture (**25** in proportions 40/60). The resolution of both isomers was carried out using a semi preparative HPLC purification (gradient elution of MeOH/H_2O from 70:30 to 100:0) to afford **26** and **27**. *Compound **26*** (Diastereomer A). IR (KBr) ν_{max}: 3448 (OH), 2932, 2862 (C-H, aliphatic), 1736 (C=O), 1156 (S=O). ^{1}H NMR ($CDCl_3$): δ_H 0.90 (s, 18-CH_3), 0.95 (d, J = 6.4 Hz, 28-CH_3), 1.17 (d, J = 6.8 Hz, 29-CH_3), 1.40-1.70 (m, 1H of 7-CH_2, 8-CH, 1H of 11-CH_2, 1H of 12-CH_2, 14-CH, 1H of 15-CH_2), 1.90-2.47 (m, 9α-H and 1H of each 7-CH_2, 11-CH_2, 12-CH_2, 15-CH_2, 16-CH_2, 23-CH_2, 27-CH_2), 2.50 (dd, J_1 = 18.7 Hz, J_2 = 8.6 Hz, 16β-CH), 2.76 (dd, J_1 = 13.0 Hz, J_2 = 3.4 Hz, 1H of 23-CH_2), 2.91 (m, 6-CH_2), 3.06 (m, 24-CH and 1H of 27-CH_2), 3.39 and 3.56 (2m, 25-CH_2), 4.02 (m, 26-CH), 4.66 (dd, J_1 = 9.7 Hz, J_2 = 2.8 Hz, 22-CH), 7.10 (s, 4-CH), 7.11 (d, J = 8.1 Hz, 2-CH), 7.27 (d, J = 6.2 Hz, 1-CH), 7.69 (m, 33-CH and 34-CH), 7.88 (m, 32-CH), 8.19 (m, 35-CH). ^{13}C NMR ($CDCl_3$): δ_C 8.3 (28-CH_3), 13.8 (C18), 15.8 (C29), 21.6 (C15), 25.7 (C11), 26.5 (C7), 29.5 (C6), 31.6 (C12), 35.8 (C16), 38.1 (C8), 44.3 (C9), 46.8 (C25), 47.9 (C13), 49.8 (C26), 50.2 (C24), 50.5 (C14), 52.6 (C27), 61.4 (C23), 68.7 (C22), 122.6 (q, $J_{C\text{-}F}$ = 274.1 Hz, C36), 125.1 (C1), 123.2 (C2), 126.3 (C4), 127.5 (q, $J_{C\text{-}C\text{-}F}$ = 33.2 Hz, C31), 128.5 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 6.5 Hz, C32), 131.9 (C35), 132.1 (C34), 132.5 (C33), 136.6 (C5), 139.1 (C10), 139.3 (C3), 139.5 (C30), 220.9 (C17). HRMS for $C_{33}H_{42}F_3N_2O_4S$ $[M+H]^+$: 619.2812 (calc.), 619.2801 (found). HPLC purity of 94.6% (retention time = 22.6 min). *Compound **27*** (Diastereomer B). IR (KBr) ν_{max}: 3448 (OH), 2924, 2854 (C-H, aliphatic), 1736 (C=O), 1157 (S=O). ^{1}H NMR ($CDCl_3$): δ_H 0.86 (d, J = 6.6 Hz, 28-CH_3), 0.90 (s, 18-CH_3), 1.20 (d, J = 6.8 Hz, 29-CH_3), 1.40-1.70 (m, 1H of 7-CH_2, 8-CH, 1H of 11-CH_2, 1H of 12-CH_2, 14-CH, 1H of 15-CH_2), 1.92-2.20 (m, 1H of each 7-CH_2, 12-CH_2, 15-CH_2, 16-CH_2), 2.25-2.64 (m, 9-CH, 23-CH_2,

1H of each 11-CH_2, 16-CH_2 and 27-CH_2), 2.92 (m, 6-CH_2, 24-CH and 1H of 27-CH_2), 3.41 and 3.56 (2d, J = 12.9 Hz, 25-CH_2), 3.87 (broad, OH), 4.17 (m, 26-CH), 4.54 (dd, J_1 = 10.8 Hz, J_2 = 2.9 Hz, 22-CH), 7.09 (s, 4-CH), 7.11 (d, J = 10.1 Hz, 2-CH), 7.27 (d, J = 9.1 Hz, 1-CH), 7.70 (m, 33-H and 34-CH), 7.89 (m, 32-CH), 8.20 (m, 35-CH). ^{13}C NMR ($CDCl_3$): δ_C 8.6 (C28), 13.8 (C18), 15.8 (C29), 21.6 (C15), 25.7 (C11), 26.5 (C7), 29.5 (C6), 31.5 (C12), 35.8 (C16), 38.1 (C8), 44.3 (C9), 45.9 (C25), 47.9 (C13), 48.1 (C27), 48.9 (C26), 50.5 (C14), 55.0 (C24), 63.6 (C23), 68.5 (C22), 122.6 (q, $J_{C\text{-}F}$ = 274.2 Hz, C36), 123.3 (C2), 125.4 (C1), 126.4 (C4), 127.5 (q, $J_{C\text{-}C\text{-}F}$ = 33.2 Hz, C31), 128.5 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 6.4 Hz, C32), 132.0 (C35), 132.1 (C34), 132.4 (C33), 136.6 (C5), 139.0 (C10), 139.2 (C3 and C30), 220.9 (C17). HRMS for $C_{33}H_{42}F_3N_2O_4S$ $[M+H]^+$: 619.2812 (calc.), 619.2815 (found). HPLC purity of 99.3% (retention time = 23.6 min).

Synthesis of 3-[2-(*trans*-2,5-dimethyl-4-{[2-(trifluoromethyl)phenyl] sulfonyl} piperazin-1-yl)-1-fluoroethyl]estra-1,3,5(10)-trien-17-one (28). A solution of alcohols **25** (20 mg, 0.03 mmol) in dry DCM (7 mL) was added dropwise to a solution of diethylaminosulfur trifluoride (4.7 µL) in dry DCM (2 mL) at -78 °C. The reaction mixture was allowed to return to rt and quenched after 3 h with a saturated solution of $NaHCO_3$ (5 mL). The mixture was extracted with EtOAc and the organic phase was washed with brine, dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by flash chromatography column using hexanes/EtOAc (gradient elution from 90:10 to 50:50) to obtain 12 mg (60%) of **28**. IR (KBr) ν_{max}: 2932, 2862 (C-H, aliphatic), 1736 (C=O), 1157 (S=O). ^{1}H NMR ($CDCl_3$): δ_H 0.85 and 0.86 (2d, J = 6.5 Hz, 28-CH_3), 0.91 (s, 18-CH_3), 1.13 and 1.15 (2d, J = 6.9 Hz, 29-CH_3), 1.40-1.70 (m, 1H of 7-CH_2, 8-CH, 1H of 11-CH_2, 1H of 12-CH_2, 14-CH, 1H of 15-CH_2), 1.95-2.20 (m, 1H of each 7-CH_2, 12-CH_2, 15-CH_2, 16-CH_2), 2.30 (m, 9-CH and 1H of 27-CH_2), 1.92 (m, 1H of 11-CH_2), 2.50 (m, 16β-CH), 2.70 (m, 23-CH_2), 2.80-3.05 (m, 6-CH_2, 24-CH, 1H

of 27-CH_2), 3.28 and 3.51 (2m, 25-CH_2), 4.02 (m, 26-H), 5.36 and 5.49 (2m, 22-CHF), 7.05 (s, 4-CH), 7.09 (d, J = 8.0 Hz, 2-CH), 7.28 (d, J = 8.2 Hz, 1-CH), 7.67 (m, 33-CH and 34-CH), 7.88 (m, 32-CH), 8.17 (m, 35-CH). ^{13}C NMR ($CDCl_3$): δ_C 8.3 (C28), 13.8 (C18), 15.8 (C29), 21.6 (C15), 25.7 (C11), 26.4 (C7), 29.4 (C6), 31.6 (C12), 35.8 (C16), 38.1 (C8), 44.4 (C9), 46.6 (C25), 47.9 (C13), 49.8 (C26), 50.5 (C14), 51.0 (C27), 53.3 (C24), 60.6 (d, $J_{C\text{-}C\text{-}F}$ = 26.0 Hz, C23), 93.1 and 93.2 (2d, $J_{C\text{-}F}$ = 173.1 Hz, C22), 122.3 (q, $J_{C\text{-}F}$ = 274.5 Hz, C36), 123.2 (C2), 125.4 (C1), 126.3 (C4), 127.5 (q, $J_{C\text{-}C\text{-}F}$ = 33.5 Hz, C31), 128.4 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 6.9 Hz, C32), 131.9 (C35), 132.0 (C34), 132.4 (C33), 136.3 (C3), 136.7 (C5), 139.3 (C30), 140.1 (C10), 220.7 (C17). HRMS for $C_{33}H_{41}F_4N_2O_3S$ $[M+H]^+$: 621.2769 (calc.), 621.2777 (found). HPLC purity of 98.7% for two diastereomers (45.9 and 52.8%) (retention time = 19.0 and 19.2 min).

Synthesis of 3-(2-bromoethyl)estra-1,3,5(10)-trien-17-one (30). To a solution of **29**[33] (175 mg, 0.57 mmol) in DCM (15 mL) was added at 0 °C triphenylphosphine (200 mg, 0.76 mmol) and carbon tetrabromide (252 mg, 0.76 mmol). The solution was stirred at 0 °C for 40 min and a second portion of triphenylphosphine (100 mg, 0.38 mmol) and carbon tetrabromide (126 mg, 0.38 mmol) were added and the solution stirred for another 1 h. The resulting mixture was poured into water (100 mL), extracted with DCM (50 mL) and the organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by flash column chromatography using hexanes/EtOAc (95:5 to 80:10) to give 176 mg (83%) of **30** as a white solid. IR (KBr) ν_{max}: 2924 and 2854 (CH aliphatic), 1736 (C=O). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 0.91 (s, 18-CH_3), 1.40-1.68 (m, unassigned CH and CH_2), 1.95-2.20 (m, unassigned CH and CH_2), 2.30 (m, 9-CH), 2.40 (m, 1H), 2.42 (dd, J_1 = 18.8 Hz, J_2 = 8.5 Hz, 16β-CH), 2.90 (m, 6-CH_2), 3.10 (t, J = 7.8 Hz, 22-CH_2), 3.55 (t, J = 7.9 Hz, 23-CH_2), 6.96 (s, 4-CH), 7.00 (d, J = 8.0 Hz, 2-CH), 7.25 (d, J = 7.2 Hz, 1-CH). LRMS for $C_{20}H_{26}BrO$ $[M+H]^+$: 361.0.

Synthesis of 3-[2-(*trans*-2,5-dimethyl-4-{[2-(trifluoromethyl)phenyl]sulfonyl} piperazin-1-yl)ethyl]estra-1,3,5(10)-trien-17-one (31). To a solution of **30** (40 mg, 0.11 mmol) in acetonitrile (10 mL) was added *N*,*N*-diisopropylethylamine (57 μL, 0.33 mmol), KI (36.5 mg, 0.22 mmol) and Amino A[27] (106 mg, 0.33 mmol). The reaction was stirred at 65 °C for 52 h and the resulting solution was cooled and evaporated. The crude product was purified by flash column chromatography using hexanes/EtOAc (95:5 to 70:30) to give 40.7 mg (60.9%) of **31** as a white solid. IR (KBr) ν_{max}: 2924 and 2854 (CH aliphatic), 1736 (C=O). ^{1}H NMR ($CDCl_3$): δ_H 0.83 (d, J = 6.5 Hz, 28-CH_3), 0.90 (s, 18-CH_3), 1.15 (d, J = 6.7 Hz, 29-CH_3), 1.38-1.70 (m, 8-CH, 14-CH, 1H of each 7-CH_2, 11-CH_2, 12-CH_2, 15-CH_2), 1.92-2.18 (m, 1H of each 7-CH_2, 12-CH_2, 15-CH_2, 16-CH_2), 2.27 (m, 9-CH), 2.35-2.65 (m, 22-CH_2, 23-CH_2, 1H of each 11-CH_2, 16-CH_2, 27-CH_2), 2.79 (dd, J_1 = 11.7 Hz, J_2 = 3.7 Hz, 27-CH_2), 2.88 (m, 6-CH_2), 2.94 (m, 24-CH), 3.26 and 3.50 (2dd, J_1 = 12.8, J_2 = 2.5 Hz, 25-CH_2), 4.03 (m, 26-CH), 6.93 (s, 4-CH), 6.97 (d, J = 8.0 Hz, 2-CH), 7.20 (d, J = 8.0 Hz, 1-CH), 7.68 (m, 33-CH and 34-CH), 7.87 (m, 32-CH), 8.18 (m, 35-CH). ^{13}C NMR ($CDCl_3$): δ_C 8.7 (C28), 13.8 (C18), 16.0 (C29), 21.5 (C15), 25.7 (C11), 26.5 (C7), 29.3 (C6), 31.6 (C12), 33.5 (C22), 35.8 (C16), 38.2 (C8), 44.3 (C9), 46.9 (C25), 48.0 (C13), 49.9 (C26), 50.4 (C14), 50.5 (C27), 52.5 (C24), 56.5 (C23), 122.6 (q, $J_{C\text{-}F}$ = 274.5 Hz, C36), 125.3 (C1), 126.1 (C2), 127.5 (q, $J_{C\text{-}C\text{-}F}$ = 33.5 Hz, C31), 128.5 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 6.2 Hz, C32), 129.3 (C4), 131.9 (C35), 132.1 (C34), 132.5 (C33), 136.4 (C5), 137.4 (C10), 137.7 (C3), 139.6 (C30), 220.8 (C17). HRMS for $C_{33}H_{42}F_3N_2O_3S$ $[M+H]^+$: 603.2863 (calc.), 603.2848 (found). HPLC purity of 98.1% (retention time = 27.6 min).

Synthesis of 2-[(*trans*-2,5-dimethyl-4-{[2-(trifluoromethyl)phenyl]sulfonyl}piperazin-1-yl)methyl]-3-hydroxyestra-1,3,5(10)-trien-17-one (33). To a solution of **32**[40] (20 mg, 0.07 mmol) and molecular sieves 4A (15 mg) in MeOH (1.5 mL) and DCM (0.5 mL), under an argon

atmosphere was added at 0 °C the Amine A[27] (47 mg, 0.14 mmol), $NaBH_3CN$ (10.5 mg, 0.17 mmol) and acetic acid (4 drops). The reaction was stirred at rt for 4 h and the resulting solution was poured into ice/water (50 mL), extracted with EtOAc, washed with brine, dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by flash column chromatography using hexanes/EtOAc (90:10 to 50:50) to give 17 mg (40%) of **33** as a white solid. IR (KBr) ν_{max}: 3441 (OH), 2932, 2862 (CH aliphatic), 1736 (C=O), 1157 (S=O). ^{1}H NMR ($CDCl_3$): δ_H 0.90 (s, 18-CH_3), 0.93 and 0.94 (2d, J = 5.5 Hz, 28-CH_3), 1.11 and 1.13 (2d, J = 6.8 Hz, 29-CH_3), 1.38-1.67 (m, 8-CH, 14-CH, 1H of each 7-CH_2, 11-CH_2, 12-CH_2, 15-CH_2), 1.90-2.25 (m, 9-CH, 1H of each 7-CH_2, 12-CH_2, 15-CH_2, 16-CH_2), 2.33-2.42 (m, 1H of 11-CH and 1H of 27-CH_2), 2.50 (dd, J_1 = 18.8 Hz, J_2 = 8.5 Hz, 16β-CH), 2.85 (m, 6-CH_2), 2.90 (m, 1H of 27-CH_2), 3.03 (m, 24-CH), 3.47 (m, 25-CH_2), 3.68 (m, 22-CH_2), 4.11 (m, 26-CH), 6.56 (s, 4-CH), 6.87 (s, 1-CH), 7.69 (m, 33-H and 34-H), 7.89 (m, 32-CH), 8.17 (m, 35-CH), 10.23 (broad, OH). ^{13}C NMR ($CDCl_3$): δ_C 8.6 (C28), 13.8 (18β-CH_3), 15.6 (C29), 21.6 (C15), 26.0 (C11), 26.5 (C7), 29.3 (C6), 31.5 (C12), 35.8 (C16), 38.3 (C8), 43.9 (C9), 46.0 (C25), 48.0 (C13), 49.0 (C26), 50.4 (C27), 51.2 (C14), 51.4 (C24), 57.8 (C22), 115.8 (C4), 118.0 (C2), 122.5 (q, $J_{C\text{-}F}$ = 274.0 Hz, C36), 125.7 (C1), 127.5 (q, $J_{C\text{-}C\text{-}F}$ = 33.1 Hz, C31), 128.6 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 6.4 Hz, C32), 130.7 (C10), 131.9 (C35), 132.1 (C34), 132.5 (C33), 135.7 (C5), 139.3 (C30), 155.4 (C3), 221.5 (C17). HRMS for $C_{32}H_{40}F_3N_2O_4S$ $[M+H]^+$: 605.2655 (calc.), 605.2642 (found). HPLC purity of 96.0% (retention time = 16.3 min).

Inhibition of 17β-HSD3 (intact LNCaP cells overexpressing 17β-HSD3). LNCaP transfected cells (LNCaP[17β-HSD3]) kindly provided by IPSEN INNOVATION (France) were maintained at 37 °C under a 5% CO_2 humidified atmosphere. Cells were grown in RPMI-1640 medium (Gibco-ThermoFisher, Waltham, MA, USA) supplemented (v/v) with 10% fetal bovine

serum (FBS), 1% penicillin/streptomycin, 2 mM L-glutamine, 4.5 g/L D-glucose, 10 mM Hepes, 1 mM sodium pyruvate, and 250 μg/mL hygromycin. For enzymatic assays, the protocol medium had the same composition, but hygromycin, used to maintain the clone selection, was not included. LNCaP[17β-HSD3] cells were plated in a 24-well culture at 10,000 cells per well, in protocol medium. After 2 days of incubation, 15 nM of [^{14}C]-4-androstene-3,17-dione and 10 μL of a solution of inhibitor dissolved in dimethylsulfoxide (DMSO) and culture medium were added. The final DMSO concentration in each well was adjusted to 0.05%. After 1 h of incubation, the culture medium was removed from wells and steroids (4-androstene-3,17-dione and testosterone) were extracted with diethyl ether. After evaporating the organic phase to dryness with nitrogen stream, the residue was dissolved in DCM, dropped on silica gel 60 F254 thin layer chromatography plates (EMD Chemicals Inc, Gibbstown, NJ, USA), and eluted with a mixture of toluene/acetone (4:1). [^{14}C]-4-androstene-3,17-dione and [^{14}C]-testosterone were identified by comparison with reference steroids and quantified using the Storm 860 System (Molecular Dynamics, Sunnyvale, CA, USA). Percentages of transformation and next percentages of inhibition were calculated at three concentrations (0.1, 0.5, and 1.0 μM) for screening studies and seven concentrations (0.001 – 5.0 μM) for IC_{50} determination. IC_{50} values were calculated using GraphPad Prism 6 software. Values represent the average of two independent experiments performed in triplicate.

Inhibition of 17β-HSD3 (microsomal fraction of rat testes). Microsomal preparation: Sprague-Dawley rats weighing approximately 275 g and obtained from Charles-Rivers, Inc. (St-Constant, QC, Canada) were castrated under isoflurane anesthesia. This experiment was approved by our Institutional Animal Care and Use Committee (Comité

de protection des animaux de l'Université Laval) and conducted in an animal facility approved by the Canadian Council on Animal Care (CCAC). Rat testis preparation was obtained using a previously described, but slightly modified procedure.[45, 23] In brief, rat testes were homogenized on ice with a Polytron in cold phosphate buffer (20 mM KH_2PO_4, 0.25 M sucrose, 1 mM EDTA, pH 7.5) containing protease inhibitors mini-complete (Roche Diagnostics, Laval, QC, Canada) and the mixture was centrifuged at 12,500 g for 15 min to remove the mitochondria, plasma membranes, and cell fragments. The supernatant was further centrifuged at 100,000 g for 15 min using an ultracentrifuge equipped with a 70.1 Ti rotor. The microsomal pellet was washed three times with phosphate buffer and centrifuged at 100,000 g for 15 min. All these operations were conducted at 4 °C. The protein concentration of the supernatant was determined by the Bradford method using bovine serum albumin as standard.

Enzymatic assay: The assay was performed at 37 °C for 2 h in 1 mL of a solution containing 860 µL of 50 mM sodium phosphate buffer (pH 7.4, 20% glycerol and 1 mM EDTA), 100 µL of 5 mM NADPH in phosphate buffer, 10 µL of 5 µM [^{14}C]-4-androstene-3,17-dione in ethanol (53.6 mCi/mmol, Perkin Elmer Life Sciences Inc., Boston, MA, USA), 10 µL of inhibitor dissolved in DMSO, and 20 µL of diluted enzymatic source in phosphate buffer. Afterwards, radiolabeled steroids were extracted from the reaction mixture with diethyl ether. The organic phase was evaporated to dryness with nitrogen stream. Residue was dissolved in 50 µL of DCM and dropped on silica gel 60 F_{254} thin layer chromatography plates (EMD Chemicals Inc., Gibbstown, NJ, USA) and eluted with a mixture of toluene/acetone (4:1) as solvent system. Substrate ([^{14}C]-4-dione) and metabolite ([^{14}C]-T) were identified by comparison with reference steroids and quantified using the Storm 860 System (Molecular Dynamics, Sunnyvale, CA,

USA). The percentage of transformation and then the percentage of inhibition were calculated. From these data, obtained at six inhibitor concentrations and in triplicate, IC_{50} values were calculated using GraphPad Prism 6 software.

Proliferative activities in LAPC-4 (AR^+) cells. Androgen-dependent human prostate cancer LAPC-4 cells were kindly provided by Robert E. Reiter from the University of California (Los Angeles, CA, USA). Cells were grown at 37 °C under 5% CO_2 humidified atmosphere in IMDM (Iscove's modified Dulbecco's) medium supplemented (v/v) with 10% fetal bovine serum (FBS), 1% L-glutamine, 1% insulin, and 1% penicillin/streptomycin. To determine the effect of novel compounds on cell proliferation, LAPC-4 cells were suspended with the medium supplemented with 5% dextran-coated charcoal treated FBS rather than 10% FBS, to remove the remaining hormones. Cultures of 5,000 cells in a total of 90 µL medium in 96-well microtiter plates (Becton–Dickinson Company, Lincoln Park, NJ, USA) were pre-incubated for 24 h at 37 °C under 5% CO_2 humidified atmosphere. Tested compounds were dissolved in DMSO to prepare the stock solution of 10^{-2} M. The compounds were then diluted at appropriate concentrations with culture medium, 10 µL added to the corresponding well, and the mixture incubated for 7 days with a change of medium after 3 days. Control wells were treated with culture medium containing DMSO only (final concentration 0.05%). Following treatment, MTS method was used for the quantification of cell growth, using CellTitter 96® AQueous Solution Cell Proliferation Assay (Promega, Nepean, ON, Canada) and following the manufacturer's instructions. The plates were incubated 4 h and then analyzed at 490 nm using a Tecan M-200 microplate reader (Mannedorf, Switzerland). The proliferation of cells treated by a given compound was expressed in % in comparison of the proliferation of cells not treated (control), which was fixed at 100%. Each compound was tested at three concentrations (0.1, 0.5, and 1.0

μM) in triplicate.

Proliferative activity in PC-3 (AR$^-$) cells. Androgen-independent human prostate cancer PC-3 cells from the American Type Culture Collection (Manassas, VA, USA) were maintained under a 5% CO_2 humidified atmosphere at 37°C in RPMI-1640 medium (Sigma-Aldrich) supplemented with 10% FBS, L-glutamine (2 nmol/L) and antibiotics (100 IU penicillin/mL, 100 mg streptomycin/mL). Briefly, the cell proliferation assay was performed by plating cells in triplicate in 96-well plates (3,000 cells per well) in culture medium (total volume of 90 μL). Tested compounds were dissolved in DMSO to prepare a stock solution of 10^{-2} M. The compounds were then diluted at appropriate concentrations with culture medium, 10 μL added to the corresponding well, and the mixture incubated for 3 days. Control wells were treated with culture medium containing DMSO only (final concentration 0.05%). Following treatment, the MTS method was used for the quantification of cell growth, using Cell Titer 96® Aqueous Solution Cell Proliferation Assay (Promega, Nepean, ON, Canada) and following the manufacturer's instructions. The plates were incubated 4 h and then analyzed at 490 nm using a Tecan M-200 microplate reader (Männedorf, Switzerland). The proliferation of cells treated with

a given compound was expressed in % in comparison of the proliferation of non-treated cells (control), which was fixed at 100%. Each compound was tested at three concentrations (0.1, 0.5, and 1.0 μM).

Metabolic stability in vitro. Assays were performed for 1 h at 37 °C, with or without 10 mM NADPH, in the presence of 40 μg of human liver S9 fraction (CG452116) from Corning (Melrose, MA, USA) and 10 μM of substrate in a final 100 μL volume of 50 mM Tris buffer supplemented with 10 mM $MgCl_2$. Assays were ended by adding 100 μL of MeOH, centrifuged at 13,000 g for 10 min to obtain a pellet of proteins. The supernatant of 2 assays were pooled, filtered and 100 μL submitted to HPLC-MS analysis (Shimadzu LCMS-2020 APCI (Kyoto, Japan), Alltima HP C18 column (250 mm x 4.6 mm, 5 μm) (Grace, Columbia, MD, USA), MeOH:water gradient). The solvent gradient started with a mixture of MeOH:water (70:30, 50:50, 30:70 or 20:80 according to the compound) and finished with MeOH (100%). The wavelength of the UV detector was selected at 190 nm. Remaining compound (expressed in %) was calculated by dividing the area under the curve of the substrate for the assays with NADPH by the one without NADPH and multiplied by 100. Values represent the average (± SD) of four independent experiments.

Plasma concentration of 17β-HSD3 inhibitors in mice. This experiment was approved by our Institutional Animal Care and Use Committee (Comité de protection des animaux de l'Université Laval) and conducted in an animal facility approved by the Canadian Council on Animal Care (CCAC). Female mice received a single dose of **1**, **9**,

23, **27**, **31**, or **33** (25 mg/kg in 0.1 mL of vehicle fluid) by subcutaneous injection. The inhibitor was first dissolved in DMSO and, thereafter, we added the co-solvent 0.4% aqueous methylcellulose (MC) to obtain a final 8% concentration of DMSO. During this experiment, mice were fasted with free access to water for 8 h before the injection. At the appropriate time point of 3 h, mice (3 per group) were sacrificed under isoflurane by cardiac puncture followed by cervical dislocation. Blood from each animal was collected into Microvette potassium-EDTA (ethylenediaminetetraacetic acid)-coated tube (Sarstedt, AG & Co., Nümbrecht, Germany) and centrifuged at 3,200 rpm for 10 min at 4 °C. The plasma was collected and stored at −80 °C until the concentration of inhibitor was determined by LC–MS/MS analysis using a procedure developed at the CHU de Québec - Research Center (Québec, Canada). Briefly, for extraction from plasma, samples (100 µL) are transferred into individual tubes and ammonium acetate (600 µL, 1 mM) is added. A methanolic solution (50 µL) containing a steroidal internal standard is then added to each tube. Samples are transferred on Strata-X SPE columns (Phenomenex, Torrance, CA, USA), which have been conditioned with MeOH (2 mL) and water (2 mL). Each column is washed with 2 mL of MeOH:water (10:90) and the

steroidal inhibitor is eluted with 5 mL of MeOH containing 5 mM of ammonium acetate. MeOH is evaporated at 45 °C under inert atmosphere and the residue dissolved in MeOH:water (85:15). A calibration standard curve was prepared in plasma by extracting each inhibitor as reported above for samples. For analysis, the HPLC system uses a Luna Phenyl-Hexyl column (75mm x 4.6 mm, 3 µm) (Phenomenex, Torrance, CA, USA) at a flow rate of 0.8 mL/min. The inhibitor is detected using an API 4000 mass spectrometer, equipped with TurboIonSpray (Applied Biosystems, Canada). ESI in positive ion mode was used.

Docking of 17β-HSD3 inhibitor in human AR and ERα. Docking studies were carried out using GOLD software version 5.5 (The Cambridge Crystallographic Data Centre, Cambridge, UK). GOLD utilizes genetic algorithm to explore the rotational flexibility of receptor hydrogens and ligand conformational flexibility. GoldScore is a molecular mechanism like function and has been optimized for the calculation of binding positions of ligand. It takes into account four terms: Fitness = Where $S(_{hb\_ext})$ + 1.3750 * $S(_{vdw\_ext})$ + $S(_{hb\_int})$ + 1.0000 * $S(_{int})$ where $S(_{int}) = S(_{vdw\_int}) + S(_{tors})$. $S(_{hb\_ext})$ is the protein-ligand hydrogen bonding and $S(_{vdw\_ext})$ are the van der Waals interactions between protein and ligand. $S(_{hb\_int})$ are the intramolecular hydrophobic interactions whereas $S(_{vdw\_int})$ is the contribution due to intramolecular strain in the ligand.[46]

The X-Ray structures of human androgen receptor ligand-binding domain in complex with EM-5744 and the human estrogen receptor ligand-binding domain in complex with 17beta-estradiol were retrieved from Protein Data Bank (http://www.rcsb.org/) entry 2PNU and 1ERE, respectively. Ligands (17β-HSD3 inhibitors) were energy-minimized using ChemBio3D version 13.0 software (CambridgeSoft, Perkin Elmer, USA). Using the GOLD wizard, the proteins were prepared by adding hydrogens, deleting water molecules, and extracting the cocrystallized ligands. 17β-HSD3 inhibitors were docked at the binding site within a 10 Å radius sphere using the following parameters: 100 Genetic Algorithm (GA) runs and 125,000 operations. GOLD score fitness function was chosen as scoring function, within the Gold_nuclear_hormone_rec_VS template. The dockings were ranked according to the value of the GOLD score fitness function; only the best ranked solution data was selected.

Docking experiments were carried out using the wizard function applying the following sequence of operations: 1) Protonation of the protein by adding hydrogens; 2) Deletion of water molecules; 3) Extraction of the ligand (EM-5744 for PDB ID: 2PNU, 17β-estradiol for PDB ID: 1ERE); 4) Definition of the active site with a 10 Å radius sphere by selecting the active site residue of protein; 5) Selection of the options related to detect cavity atoms file from the selection*;* 6) Selection of the option to force all H bond donors/acceptors to solvent-accessible surface; 7) Loading of the configuration template for Gold_nuclear_hormone_rec_VS template; 8) Addition of the minimized ligand (17β-HSD3 inhibitor), which was minimized with ChemBio3D version 13.0 software and save as sdf file; 9) Use of the Genetic Algorithm settings for all calculations and a set of 100 solutions were saved for each ligand; 10) Use of the GoldScore fitness function as scoring function; 11) Setting of the Genetic Algorithm option to slow (most accurate) mode; and 12) Running Gold.

ASSOCIATED CONTENT

Supporting Information

The supporting information is available free of charge on the ACS Publication website at DOI:……

Table S1: Assignment of ^{13}C NMR signals for all the new steroid derivatives tested (compounds **1-10**, **16**, **21**, **23**, **26-28**, **31**, and **33**); Figure S1: ^{1}H NMR spectrum of the Mosher ester of alcohol **25** in $CDCl_3$; ^{1}H NMR, ^{13}C APT, HSQC, HMBC, COSY and HMBC spectra of the representative or the most active steroid derivatives synthesized: compound **9** (D-ring androstane derivative), compound **16** (pregnane derivative), compound **21** (19-nor-androstane derivative), and compounds **23**, **27**, and **33** (estradiol derivatives); HPLC chromatograms of final tested compounds; Chemical synthesis and biological data of five additional analogs (16-*gem*-dimethyl, 17α-methyl, 17α-hydroxyl, 17-*gem*-difluoro and 3-ethylketone) of compound **1**.

Molecular formula strings (CSV)

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All authors contributed to writing the manuscript and have given approval to its final version.

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Notes

FCB and MP declare no competing interests while JR, RM and DP are owners of a patent on 17β-HSD3 inhibitors.

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ABBREVIATIONS

3α-HSD: 3α-hydroxysteroid dehydrogenase; 3β-HSD: 3β-hydroxysteroid dehydrogenase; 4-dione: 4-androstene-3,17-dione; 5α-dione: 5α-androstene-3,17-dione; 5α-diol: 5α-androstane-3α,17β-diol; 5α-R: 5α-reductase; 17β-HSD3: 17β-hydroxysteroid dehydrogenase type 3; 17β-HSD5: 17β-hydroxysteroid dehydrogenase type 5; 17β-HSD6: 17β-hydroxysteroid dehydrogenase type 6; 17β-HSD15: 17β-hydroxysteroid dehydrogenase type 15; ADT: androsterone; AR: androgen receptor; Cpd: compound; CRPC: castrate-resistant prostate cáncer; DAST: diethylaminosulfur trifluoride; DCM: dichloromethane; DEAD: diethyl azodicarboxylate; DHEA: dehydroepiandrosterone; DHRS11: dehydrogenase/reductase SDR family member 11; DHT: dihydrotestosterone; DIPEA: *N,N*-diisopropylethylamine; EST: estra-1,3,5(10)-triene; HBA: H-bond acceptor group; HBD: H-bond donor group; HBTU: 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; LNCaP[17β-HSD3]: transfected LNCaP cells with *HSD17B3* gene; NMO: *N*-methylmorpholine-*N*-oxide; PCa: prostate cancer; PRG: pregnane; *p*-TSA: *p*-toluensulfonic acid; SAR: structure-activity relationship; T: testosterone; TEA: triethylamine; THF: tetrahydrofuran; TPAP: tetrapropylammonium perruthenate

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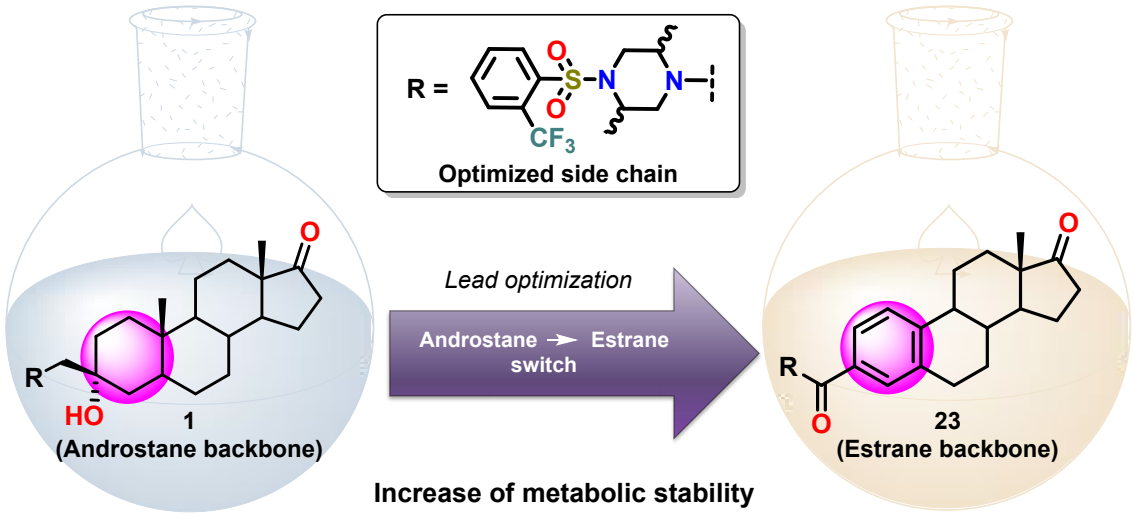
Table of Contents graphic



Increase of metabolic stability
No prostate cancer LAPC-4 cell proliferation
Same 17β-HSD3 inhibitory activity (IC_{50} = 0.10 μM)