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Development of 3-substituted-androsterone derivatives as potent inhibitors of 17β-hydroxysteroid dehydrogenase type 3

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ABSTRACT

17Beta-hydroxysteroid dehydrogenase type 3 (17β-HSD3) is a steroidogenic enzyme that catalyzes the transformation of 4-androstene-3,17-dione (Δ^4-dione) into androgen testosterone (T). To provide effective inhibitors of androgen biosynthesis, we synthesized two different series (amines and carbamates) of 3β-substituted-androsterone derivatives and we tested their inhibitory activity on 17β-HSD3. From the results of our structure–activity relationship study, we identified a series of compounds producing a strong inhibition of 17β-HSD3 overexpressed in HEK-293 cells (homogenized cells). The most active compound when tested in intact HEK-293 transfected cells, namely (3α,5α)-3-{[*trans*-2,5-dimethyl-4-{[2-(trifluoromethyl)phenyl] sulfonyl}piperazin-1-yl]methyl}-3-hydroxyandrostan-17-one (**15b**), shows an IC_{50} value of 6 nM, this compound is thus eight times more active than our reference compound D-5-2 (IC_{50} = 51 nM). This new improved inhibitor did not stimulate the proliferation of androgen-sensitive Shionogi cells, suggesting a non-androgenic profile. Compound **15b** is thus a good candidate for further in vivo studies on rodents.

1. Introduction

Prostate cancer is the most common cancer among men in the United States with an estimate of 217,730 new diagnosed cases in 2010 and with 32,050 associated deaths.[1] Endocrine therapy has been recognized as one of the most efficient treatments for prostate cancer with a positive response in cancer regression in nearly 80% of cases at first treatment.[2] Different endocrine treatments are now available to block either the testicular source of androgens (medical or surgical castration) or the effect of the androgens testosterone (T) and dihydrotestosterone (DHT) on the androgen receptor (antiandrogens).[3,4] Although they improve survival, these androgen deprivation treatments are linked to important side effects and are rarely curative for advanced prostate cancer.[5–7] Thus, the development of new therapeutic options are strongly needed to improve survival as well as the quality of life for treated patients.[8]

17β-Hydroxysteroid dehydrogenase type 3 (17β-HSD3), also named testicular 17β-hydroxysteroid dehydrogenase, is a steroidogenic enzyme that catalyzes the reduction of non androgenic 4-androstene-3,17-one (Δ^4-dione)[9] to potent androgen T using NADPH as cofactor.[10–15] This enzyme is found principally in the Leydig cells in the microtubule part of the testis and contributes to the production of approximately 60% of total active androgens in men.[16] The other part of active androgens (40%) would be directly synthesized in the prostate from inactive adrenal precursors dehydroepiandrosterone (DHEA) and Δ^4-dione by the action of 3β-hydroxysteroid dehydrogenases, 5α-reductases and other 17β-HSDs such as type 5 or type 15.[17,18] However, although the 17β-HSD3 expression level is found to be very low in normal prostate tissues, it has been reported that 17β-HSD3 is suspected to play an important role in the conversion of adrenal steroids into potential androgens in prostate cancer tissues.[19,20] In fact, the expression levels of the 17β-HSD3 mRNA in prostatic tissues with malignancy is significantly higher (31 times) than those in prostatic tissues without malignancy. Furthermore, Nelson's group recently showed that 17β-HSD3 is overexpressed (8 times) in LuCaP 23 and LuCAP 35 cell lines, which were obtained from metastatic tissues of patients resistant to castration therapy.[21–24] Importantly, despite a castrated level of T in the bloodstream, the level of T within the metastatic tumors was found to be sufficiently high to stimulate the proliferation of cancer cells. Thus, the 17β-HSD3 enzyme represents a promising target for the treatment of advanced prostate cancer by blocking the different sources of active androgen T from testis (endocrine), adrenals (endocrine) and intratumoral tissues (intracrine) (Fig. 1). Furthermore, the use of 17β-HSD3 inhibitors complementary to actual endocrine therapy could increase the efficiency of antiandrogens, LHRH agonists, and inhibitors of androgen biosynthesis enzymes (5α-reductase and 17α-lyase) by producing a synergistic effect.[25,26]

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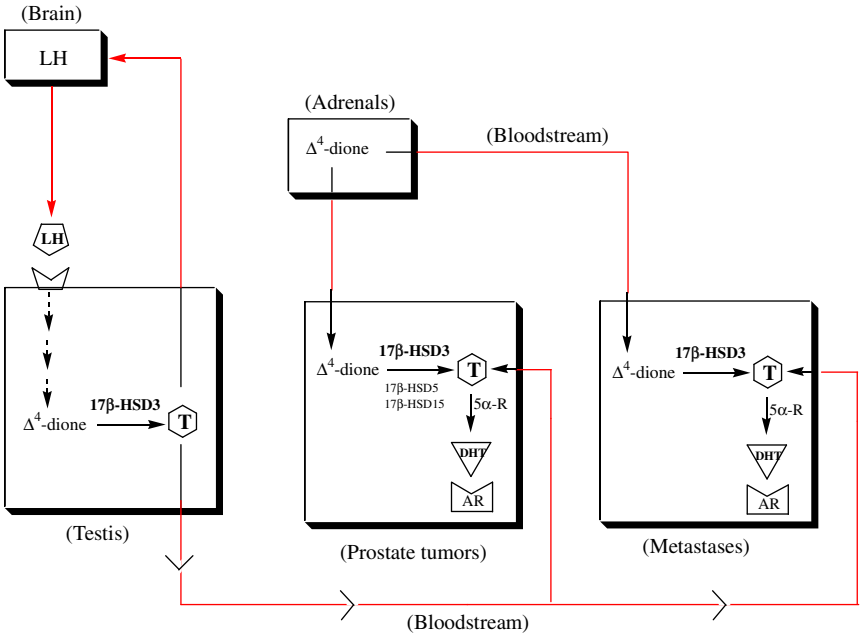


Figure 1. Schematic view of the contribution of 17β-HSD3 in androgen testosterone (T) biosynthesis from the non androgenic 4-androstene-3,17-dione (Δ^4-dione) in different sources including the testis (endocrine), the adrenals (endocrine), and the intratumoral tumor tissues (intracrine). LH: Luteinizing hormone, 5α-R: 5α-reductase, AR: androgen receptor, DHT: dihydrotestosterone, 17β-HSD5 and 17β-HSD15: types 5 and 15 of 17β-hydroxysteroid dehydrogenase.

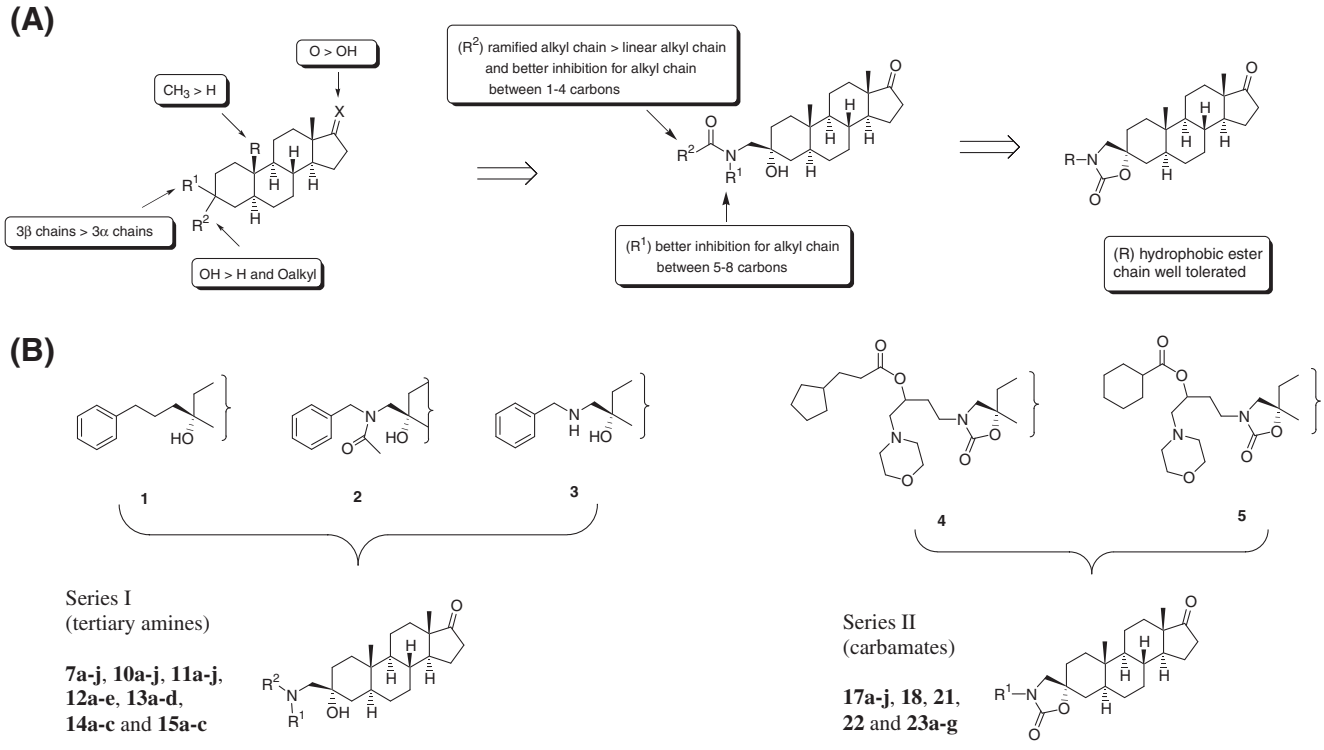
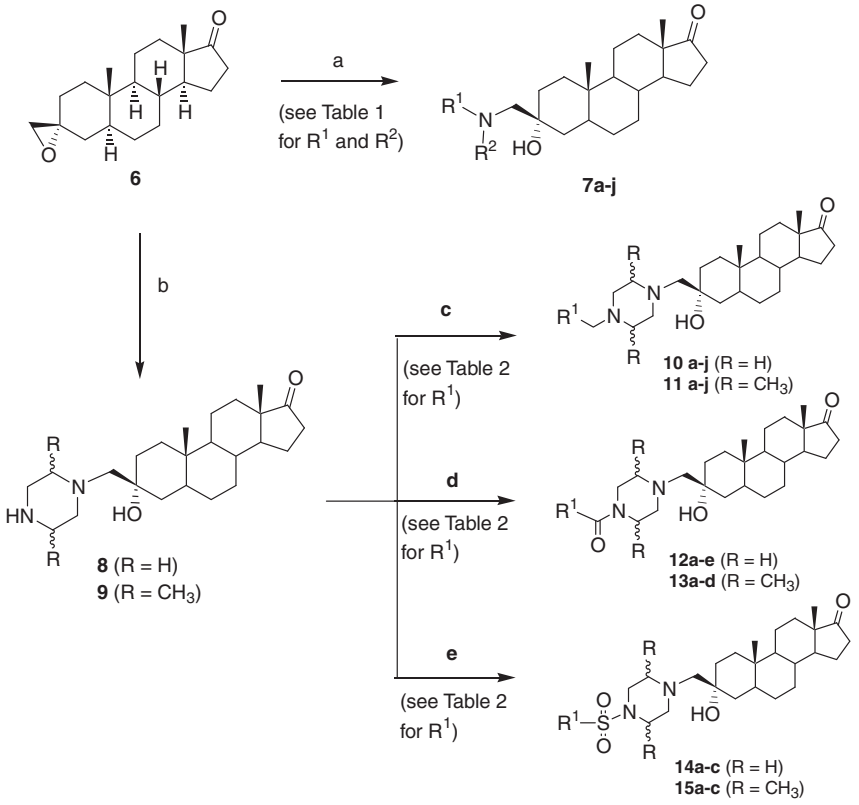
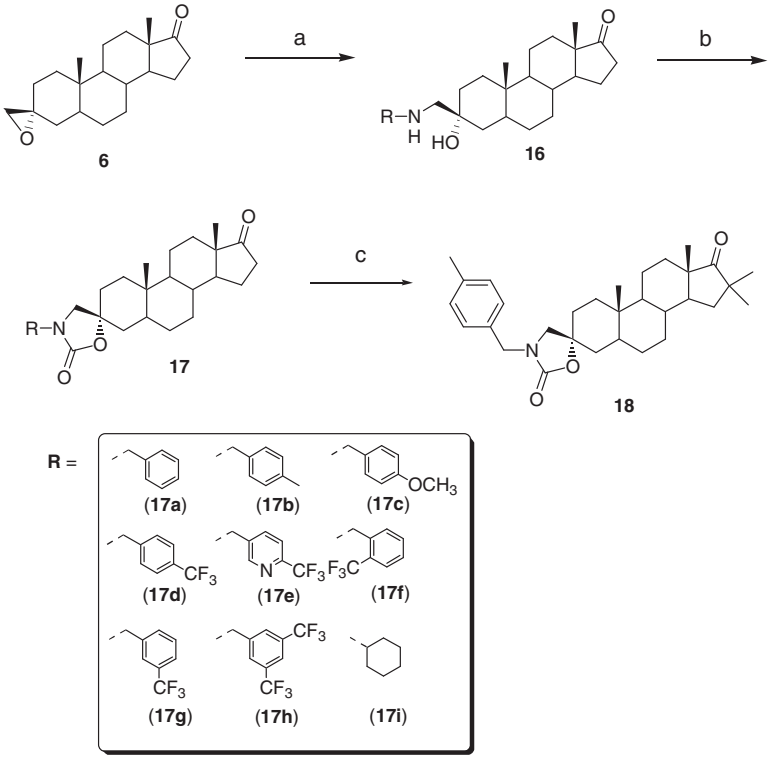


Figure 2. (A) Relative importance of different chemical groups (alkyl, alkylamide and carbamate derivatives) added at position 3β of androsterone steroid scaffold and their effect on 17β-HSD3 inhibition as established from our previous SAR studies.[27–29] (B) Representative first-generation inhibitors (compounds **1**–**5**) and two new series of second-generation inhibitors (compounds **7**, **10**–**15**, **17**, **18**, **22** and **23**). See Tables 1–3 for the representation of all new synthesized and tested compounds.

Our previous structure–activity relationship (SAR) studies on the inhibition of 17β-HSD3 have identified important criteria for inhibitory activity (Fig. 2A).[27–33] Despite their good efficiency in homogenized HEK-293 cells overexpressing 17β-HSD3, these inhibitors were however not optimal in different aspects. As an example, first generation inhibitors of the 3β-alkyl-androsterone series showed an androgenic profile on Shionogi (AR^+) cells which is obviously undesirable in the context of a treatment for an androgen-sensitive disease. The inhibitory potency of these inhibitors was also lower in intact than in homogenized HEK-293 cells overexpressing 17β-HSD3, thus suggesting low cell permeability. To provide 17β-HSD3 inhibitors with a non-androgenic profile and



Scheme 1. Reagents and conditions: (a) R^1R^2NH, ethanol, 60 °C; (b) piperazine or 2,5-dimethylpiperazine, ethanol, 60 °C; (c) R^1CH_2Br; TEA, DCM, rt; (d) R^1COCl, TEA, DCM, rt; (e) R^1SO_2Cl, TEA, DCM. The 2,5-dimethylpiperazine derivatives are in the trans configuration. The stereochemistry of carbons 5, 8, 9 and 14 is shown only for compound **6** but is the same for all other steroids.



Scheme 2. Reagents and conditions: (a) R-NH_2, ethanol, 60 °C; (b) triphosgene, DIPEA, DCM; (c) NaH, MeI, DMF (from **17b**).

with more drug-like properties, we explored the enzyme tolerance for new diversified substituents introduced at position 3 of the androsterone (ADT) nucleus. As an extension of our previous work illustrated with inhibitors **1**–**5**, two series of ADT derivatives (tertiary amines and carbamates) were designed, synthesized and tested as new inhibitors of 17β-HSD3 (Fig. 2B).

2. Results and discussion

2.1. Chemical synthesis

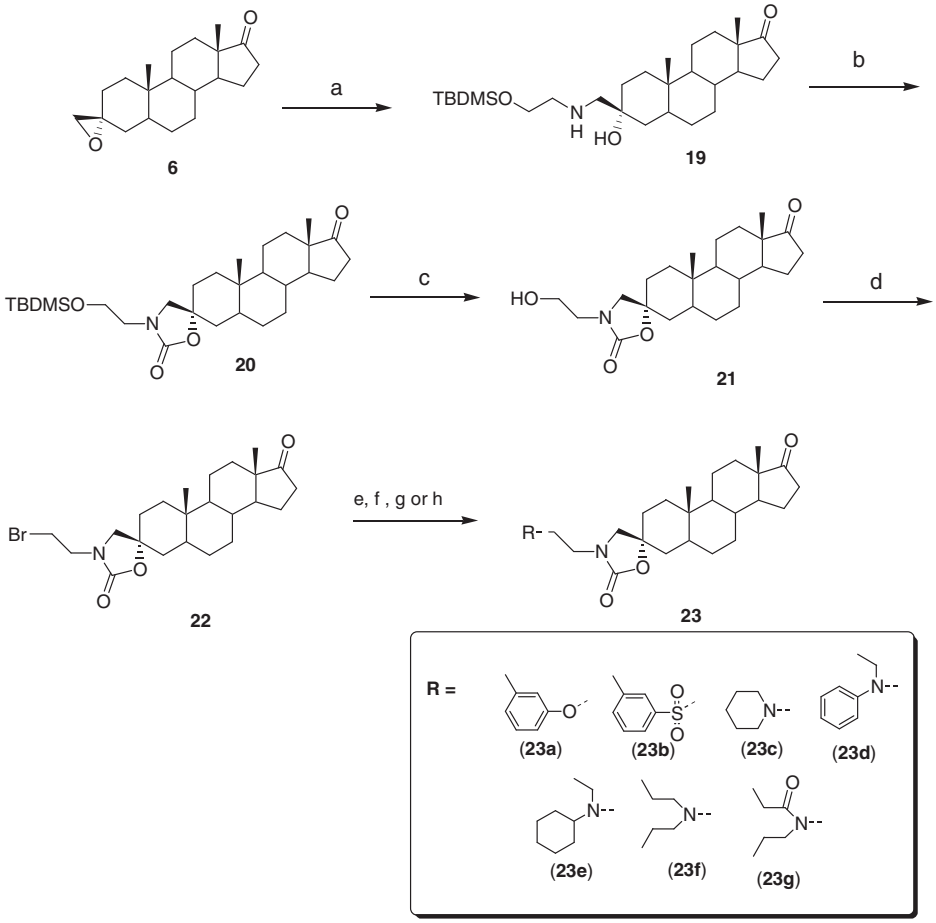
The ADT derivatives **7a–j** were easily obtained in acceptable yields (35–70%) from the opening in refluxing ethanol of the oxirane **6** with a series of commercially available secondary amines (Scheme 1). Similarly, the oxirane **6** was also opened with piperazine or trans-2,5-dimethylpiperazine to give the intermediate compounds **8** and **9**. The free NH of **8** and **9** was next submitted to a reaction with different kinds of building blocks (benzyl bromide, acyl chloride and sulfonyl chloride) to obtain the corresponding piperazine derivatives **10a–j** and **11a–j** (amines), **12a–e** and **13a–d** (amides) and **14a–c** and **15a–c** (sulfonamides).

The carbamate derivatives **17a–i**, **18**, **21**, **22** and **23a–g** were prepared following two different methods. In the first sequence of reactions (Scheme 2), carbamates **17a–i** and **18** were obtained by reacting the appropriate secondary amines with the oxirane **6** in refluxing ethanol and cyclizing the resulting amino-alcohol using triphosgene with diisopropylethylamine (DIPEA) in dichloromethane (DCM). The C16-dimethylation of **17b** giving **18** was done using sodium hydride as deprotonation base and an excess of MeI as reagent of methylation. In the second sequence of reactions (Scheme 3), the carbamates **23a–g** were obtained by different N, O and S-alkylation reactions of the key alkyl bromide intermediate **22**, which was obtained from **6** in four steps. Briefly, the aminolysis of oxirane **6** with aminoethanol-*O*-TBDMS gave the amino alcohol **19**, which was treated with triphosgene to give the intermediate carbamate **20**. The TBDMS protecting group was then removed using tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (THF) and the resulting primary alcohol **21** was transformed to the corresponding bromide **22** using triphenylphosphine and carbon tetrabromide in DCM.

2.2. Inhibition of 17β-HSD3 in homogenized cells

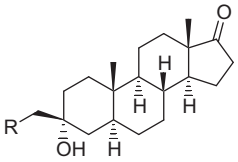
2.2.1. Tertiary amine derivatives

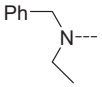
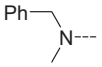
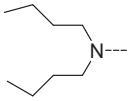
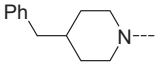
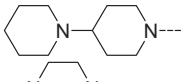
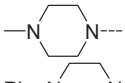
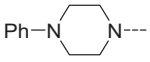
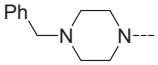
Our previous SAR study showed that a hydrophobic chain at position 3β of ADT was well tolerated by the 17β-HSD3.[29] Unfortunately, these compounds were found to stimulate the proliferation of androgen-sensitive cells and to possess relatively high hydrophobicity (high Log *P* value) as illustrated in Figure 2B with phenylpropyl-ADT (**1**). We then turned our attention towards the synthesis of tertiary alkylamine (series I) which seems more promising considering their lower hydrophobic character as well as their high potential for structural diversification. We first diversified the ADT scaffold using various acyclic (**7a–d**) and cyclic (**7e–j**) amines at position 3β and evaluated their potential as inhibitors of 17β-HSD3 (Table 1). Thus the ability of compounds **7a–j** to inhibit the 17β-HSD3 transfected in human embryonic kidney (HEK)-293 cells (homogenized) was done by measuring the amount of labeled T formed from natural labeled substrate Δ^4-dione in the presence of NADPH as cofactor. The results were expressed as the percent of inhibitory activity for a given



Scheme 3. Reagents and conditions: (a) $NH_2(CH_2)_2OTBDMS$, ethanol, 60 °C; (b) triphosgene, DIPEA, DCM; (c) TBAF, THF, rt; (d) PPh_3, CBr_4, DCM, 0 °C to rt; (e) 3-methylphenol (for **23a**); (f) (i) 3-methylthiophenol, K_2CO_3, DMF, 80 °C; (ii) $KHSO_5$, $MeOH/H_2O$ (for **23b**); (g) appropriate R^1R^2NH, ethanol 60 °C (for **23c**, **23d**, **23e** and **23f**); (h) (i) propylamine, Na_2CO_3, DMF, 80 °C; (ii) propionyl chloride, triethylamine, DCM, rt (for **23g**).

Table 1
Inhibition of 17β-HSD3 in homogenized and intact cells by various tertiary-amine derivatives of ADT (compounds **7a–j**)[a]



#	R	Log *P* calc[b]	Homogenate inhibition at 0.01 μM (%)	Homogenate inhibition at 0.1 μM (%)	Homogenate inhibition at 1 μM (%)	Intact cells inhibition at 0.1 μM (%)	Intact cells inhibition at 1 μM (%)
7a		6.1	42	85	90	86	94
7b		5.8	28	79	89	48	92
7c		4.7	0	10	60	nd	14
7d		5.7	13	70	85	53	90
7e		4.8	9	19	69	nd	23
7f		6.7	40	82	90	nd	52
7g		4.7	0	4	69	nd	20
7h		3.8	0	0	40	2	nd
7i		5.9	13	78	89	nd	68
7j		5.6	35	72	90	37	80

[a] For the transformation of [^{14}C]-4-androstene-3,17-dione (50 nM) into [^{14}C]-testosterone at the indicated concentration of tested compound. See Section 4 for the details of the assay. Error ± 10%. nd: not determined.
[b] The Log *P* values were calculated (Log *P* calc) using the CS Chemdraw Ultra software version 5.0.

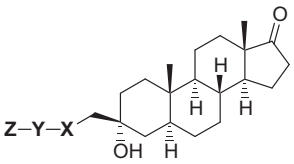
compound. In the series of acyclic derivatives, compound **7a** was the most active inhibitor (42% at 0.01 μM and 85% at 0.1 μM). For the cyclic derivatives, compounds **7f** and **7j** were the best inhibitors with 40% and 35% inhibition at 0.01 μM, respectively. Clearly, compounds **7c**, **7e**, **7g** and **7h** with Log *P* values <5.0 gave the lowest values of inhibition. The piperazine derivative **7j** was selected from Table 1 as the more interesting compound in a drug amenable point of view, and by considering its lower hydrophobicity (Log *P* = 5.6) than corresponding piperidine **7f** (Log *P* = 6.7) and its larger potential of diversification. The piperazine derivative **7j** was however more interesting considering its lower hydrophobicity than corresponding piperidine **7f** as well as its larger potential for diversification. The piperazine core is also well recognized as a common motif in several drugs.[34] For all these reasons, we selected the piperazine core to extend our SAR study.

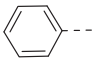
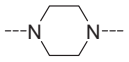
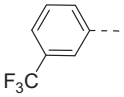
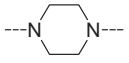
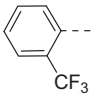
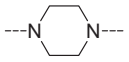
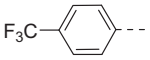
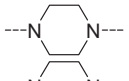
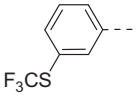
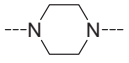
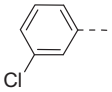
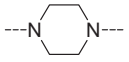
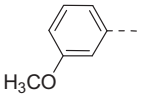
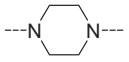
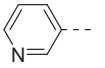
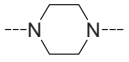
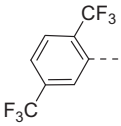
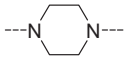
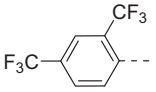
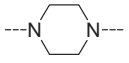
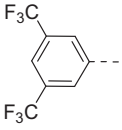
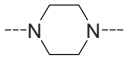
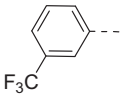
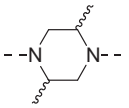
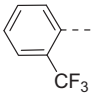
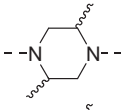
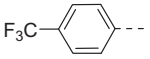
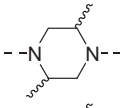
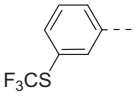
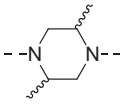
2.2.2. Piperazine derivatives

Interesting SAR observations emerged from the piperazine derivatives reported in Table 2. First, when different substituted aryl groups (Z) are used within a benzylamine functionality (Y = CH_2) added on the 3β-piperazine-ADT nucleus (compounds **10a–j**), we observed that inhibition values remained almost unchanged either for compounds with aromatic bearing electron withdrawing groups (CF_3 and Cl) or electron donating group (OCH_3). In fact, only compound **10a**, with a *meta*-CF_3, gave better inhibition of 17β-HSD3 than the reference compound **7j**. Another interesting point is the negative effect of the pyridine ring (compound **10g**) on the inhibition of 17β-HSD3 pointing out to a poor tolerance for polar substituent. This result was in accordance with tertiary amine series where compounds with Log *P* values <5.0 gave poor enzyme inhibitions and was in agreement with our previous results reported for 3β-substituted ADT derivatives, which clearly showed the importance of a non-polar group for a good inhibition.[27] Secondly, the functional group Y (**CH_2**, CO or SO_2) slightly and differently modulates the inhibitory activity when the rest of the molecule is identical. As examples, the sulfonamide derivative **14b** shows a better inhibition at 0.01 μM (45%) than its corresponding amide **12c** (31%) and benzylamine **10b** (18%) in the *ortho*-CF_3 series. On the other hand, the benzyl amine derivatives **10a** gave a better inhibition at 0.01 μM (51%) than their corresponding sulfonamide **14a** (21%) and amide **12d** (10%) in the *meta*-CF_3 series, but same inhibitions were observed in the *para*-CF_3 series (compounds **10c**, **12e** and **14c**).

Although the use of trans-2,5-dimethylpiperazine moiety (X group) did not increase the inhibition of the benzylamine or amide series when compared to piperazine moiety, the most interesting inhibition results were obtained in the trans-2,5-dimethylpiperazine derivatives having a sulfonamide function, namely compounds **15b** and **15c** (55% and 79% of inhibition at 0.01 μM). Thus, the sulfonamide **15c** clearly gave a higher percentage of inhibition (79% at 0.01 μM) than its corresponding amide **13d** (17%) and amine **11c** (14%). Two other important observations were also

Table 2
Inhibition of 17β-HSD3 in homogenized and intact cells: optimization of 3β-piperazine ADT series (compounds **10a–j**, **11a–j**, **12a–d**, **13a–d**, **14 a–c** and **15a–c**)[a]



#	Z	Y	X	Log *P* calc[b]	Homogenate inhibition at 0.01 μM (%)	Homogenate inhibition at 0.1 μM (%)	Intact cells inhibition at 0.1 μM (%)
7j		CH_2		5.6	36	74	38
10a		CH_2		6.5	51	88	52
10b		CH_2		6.5	18	86	20
10c		CH_2		6.5	14	81	33
10d		CH_2		8.0	3	91	nd (62)[c]
10e		CH_2		6.1	26	85	nd (75)[c]
10f		CH_2		5.4	21	83	47
10g		CH_2		4.2	10	33	nd (63)[c]
10h		CH_2		7.4	18	85	12
10i		CH_2		7.4	22	75	16
10j		CH_2		7.4	25	83	25
11a		CH_2		7.1	31	89	nd (78)[c]
11b		CH_2		7.1	22	85	36
11c		CH_2		7.1	14	87	36
11d		CH_2		8.6	18	88	nd (58)[c]

(*continued on next page*)

Table 2 (*continued*)

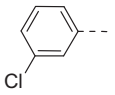
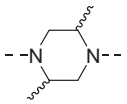
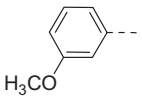
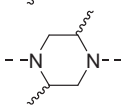
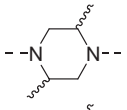
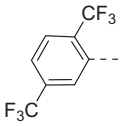
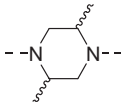
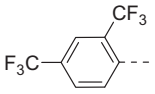
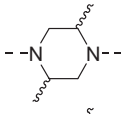
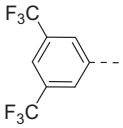
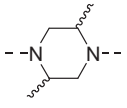
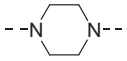
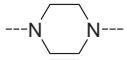
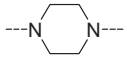
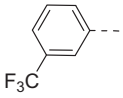
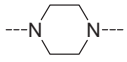
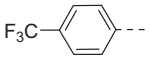
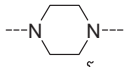
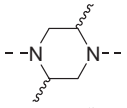
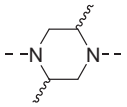
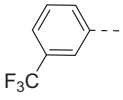
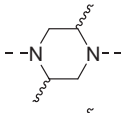
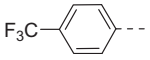
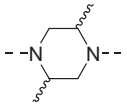
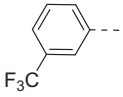
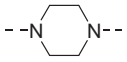
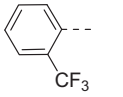
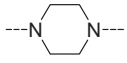
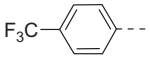
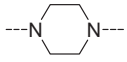
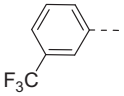
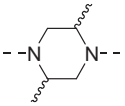
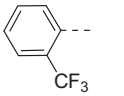
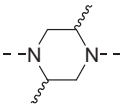
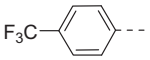
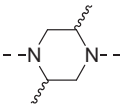
#	Z	Y	X	Log *P* calc[b]	Homogenate inhibition at 0.01 μM (%)	Homogenate inhibition at 0.1 μM (%)	Intact cells inhibition at 0.1 μM (%)
11e		CH_2		6.8	26	84	nd (55)[c]
11f		CH_2		6.1	19	87	50
11g		CH_2		4.9	17	47	nd (58)[c]
11h		CH_2		8.0	16	71	23
11i		CH_2		8.0	15	71	21
11j		CH_2		8.0	25	74	16
12a		CO		5.0	17	76	50
12b		CO		5.1	19	75	nd (70)[c]
12c		CO		5.9	31	86	62
12d		CO		5.9	10	92	91
12e		CO		5.9	22	84	56
13a		CO		5.6	24	87	78
13b		CO		5.7	24	90	77
13c		CO		6.6	28	89	70
13d		CO		6.6	17	91	61
14a		SO_2		5.8	21	82	nd (71)[c]
14b		SO_2		5.8	45	92	63
14c		SO_2		5.8	21	78	34

Table 2 (*continued*)

#	Z	Y	X	Log *P* calc[b]	Homogenate inhibition at 0.01 μM (%)	Homogenate inhibition at 0.1 μM (%)	Intact cells inhibition at 0.1 μM (%)
15a		SO_2		6.5	32	91	77
15b		SO_2		6.5	55	92	84
15c		SO_2		6.5	79	92	47

[a] For the transformation of [^{14}C]-4-androstene-3,17-dione (50 nM) into [^{14}C]-testosterone at the indicated concentration of the tested compound. See Section 4 for the details of the assay. Error ± 10%. nd: not determined. The 2,5-dimethylpiperazine derivatives are in the trans configuration.
[b] The Log *P* values were calculated (Log *P* calc) using the CS Chemdraw Ultra software version 5.0.
[c] Tested at 1 μM.

made relatively to the steroid scaffold (data not shown). First, we observed a strong negative impact on inhibition when we replaced the C19-steroid nucleus of **7j** by a C21-steroid nucleus. In fact, 17β-HSD3 shows a clear preference for androstane over pregnane scaffold (74% of inhibition at 0.1 μM vs 12%, respectively, when Z = C_6H_5; Y = CH_2 and X = piperazine). Secondly, the presence of an insaturation at positions C4–C5 of the androstane derivative **7j** had no important impact on enzyme inhibition.

2.2.3. Carbamate derivatives

We previously synthesized a small library of 3β-carbamate-ADT derivatives that showed promising inhibitory activities on 17β-HSD3.[27] Despite the good results, the compounds possess an ester group (see **4** and **5** in Fig. 2B) that is vulnerable to in vivo hydrolysis. In order to provide good inhibitors with a more stable chain on the carbamate moiety, we synthesized a new series of analogue compounds. We were especially interested in exploring the tolerance of the enzyme for different kinds of substituents (Table 3).

The first general observation that can be made about carbamate derivatives is the good tolerance of the enzyme for all hydrophobic chains, as represented by compounds **17a**, **17b**, **17d**, **17f**, **17g**, **17h**, **17i**, **22** and **23a**, in comparison with the weak inhibition obtained with hydrophilic chains, as represented by compounds **17e**, **21**, **23b**, **23c**, **23e**, **23f** and **23g**. This is particularly obvious when we compare the alcohol **21** (Log *P* = 3.7) with the corresponding bromide **22** (Log *P* = 5.0), and the *p*-CF_3-pyridine derivative **17e** (Log *P* = 5.9) with the *p*-CF_3-phenyl derivative **17d** (Log *P* = 6.9). The sulfone derivative **23b** (Log *P* = 5.3) was also less potent than corresponding ether **23a** (Log *P* = 6.3). The addition of two methyl groups at position C16 of the compound **17b** was clearly not well tolerated by the enzyme and, consequently, compound **18** weakly inhibited the 17β-HSD3. Overall, a slight improvement of inhibition activity was obtained with compounds **17a**, **17b**, **17d**, **17f**, **17g** and **23a** relatively to reference compound **4** (D-5-4). It is also expected that these new carbamate derivatives without an ester side-chain as element of diversity could provide a better stability and bioavailability.

2.3. Inhibition of 17β-HSD3 in intact cells

After having established the inhibitory activity of all compounds in homogenized HEK-293 cells, we determined their capability to exert their action in intact cells overexpressing 17β-HSD3. This is obviously a crucial step towards further development of a potent inhibitor because a good inhibitor must enter into the cell by crossing the cell membrane. We thus used the results in intact cells (Tables 1–3) as a second screen for selecting the best inhibitors.

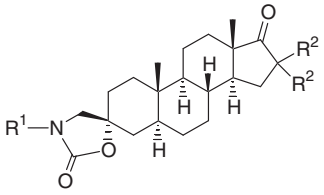
In Table 1, the results obtained in intact cells confirmed the selection of tertiary amine **7a** as the best inhibitor over **7f** and **7j** (94%, 52% and 80% of inhibition at 1 μM, respectively). In Table 2, only compounds **10a**, **14b**, **15b** and **15c** inhibited over 40% of the activity in homogenized cells (51%, 45%, 55%, 79% of inhibition at 0.01 μM, respectively), but the results in intact cells clearly demonstrated that **15b** is the best candidate from this series of piperazine ADT derivatives. Producing 84% of inhibition at 0.1 μM, compound **15b** is a more potent inhibitor in intact cells than **10a**, **14b** and **15c** which inhibited 52%, 63% and 47%, respectively, the transformation of Δ^4-dione into T. The carbamate derivatives represented in Table 3 appear to be less potent inhibitors than the two other series of compounds (Tables 1 and 2). In fact, only three compounds, **17a**, **17c** and **17d**, inhibited over 40% of enzyme inhibition in intact cells at the reported concentration of 0.1 μM. We however selected **17a**, which inhibited 44% of 17β-HSD3 activity at 0.1 μM in intact cells, as a representative inhibitor of this series of carbamate-ADT derivatives.

For purposes of comparison, we next proceeded to the IC_{50} values determination of the piperazine derivative **15b**, the first generation inhibitor D-5-2 (**5**) and the natural substrate Δ^4-dione used itself as inhibitor. Being less potent in both homogenized and intact cell assays, the carbamate derivative **17a** was not selected for IC_{50} determination as well as the tertiary amine derivative **7a** that was shown to be an androgenic compound (see Section 2.4). From the curve of inhibition obtained in intact HEK-293 cells overexpressing 17β-HSD3 (Fig. 3), **15b** was found to be an eightfold better inhibitor than reference compound **5** (IC_{50} = 6 and 51 nM, respectively). It is also 56-fold better for inhibiting the transformation of labeled Δ^4-dione into T (IC_{50} = 337 nM) than the unlabeled Δ^4-dione used itself as an inhibitor.

2.4. Proliferative (androgenic) activity on Shionogi cells

In the context of a treatment of androgen-dependent diseases, an important criterion to provide valuable 17β-HSD3 candidates is their non-androgenic character or their ability not to activate the androgen receptor (AR). To address this, we evaluated the agonist (proliferative) activity on androgen-sensitive (AR^+) Shionogi cells of new inhibitors **7a**, **15b** and **17a** in comparison with the first generation inhibitors D-5-2 (**5**) and CS-213 (Fig. 4). In this assay, the basal cell proliferation (control) was set as 100%, the potent

Table 3
Inhibition of 17β-HSD3 in homogenized and intact cells: optimization of 3-carbamate ADT series (compounds **17a–i**, **18**, **21**, **22** and **23a–g**)[a]



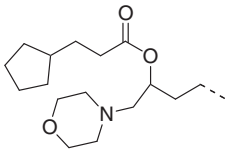
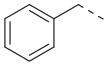
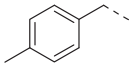
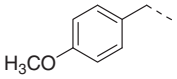
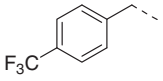
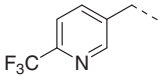
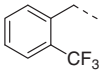
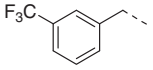
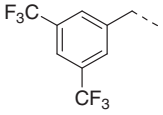
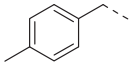
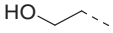
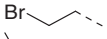
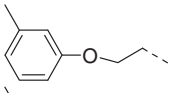
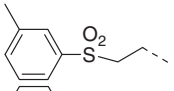
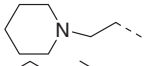
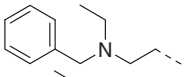
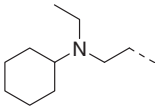
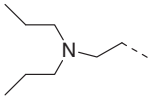
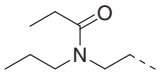
#	R^1	R^2	Log *P* calc[b]	Homogenate inhibition at 0.01 μM (%)	Homogenate inhibition at 0.1 μM (%)	Homogenate inhibition at 1 μM (%)	Intact cells inhibition at 0.1 μM (%)
4 (D-5-4)[27]		H	5.2	2	79	94	49
17a		H	5.9	21	86	93	44
17b		H	6.4	17	81	93	38
17c		H	5.8	5	84	93	43
17d		H	6.9	25	80	nd	43
17e		H	5.9	3	65	nd	36
17f		H	6.9	17	81	nd	35
17g		H	6.9	16	80	nd	36
17h		H	7.8	12	66	nd	35
17i		H	5.7	13	75	78	29
18		CH_3	7.7	2	22	74	18
21		H	3.7	0	9	38	16[c]
22		H	5.0	17	69	93	31
23a		H	6.3	13	85	95	40
23b		H	5.4	0	52	93	5
23c		H	4.9	0	0	21	10[c]
23d		H	6.3	14	71	93	40
23e		H	6.1	2	21	72	2

Table 3 (*continued*)

#	R^1	R^2	Log *P* calc[b]	Homogenate inhibition at 0.01 μM (%)	Homogenate inhibition at 0.1 μM (%)	Homogenate inhibition at 1 μM (%)	Intact cells inhibition at 0.1 μM (%)
23f		H	5.8	0	10	62	8[c]
23g		H	4.9	0	35	80	25

[a] For the transformation of [^{14}C]-4-androstene-3,17-dione (50 nM) into [^{14}C]-testosterone at the indicated concentration of tested compound. See Section 4 for the details of the assay. Error ± 10%. nd: not determined.
[b] The Log *P* values were calculated (Log *P* calc) using the CS Chemdraw Ultra software version 5.0.
[c] Tested in another experiment.

androgen dihydrotestosterone (0.1 μM) stimulated the cell proliferation to 320% and the antiandrogen (AR antagonist) hydroxy-flutamide[35] (OH-Flu) did not stimulate the cell proliferation. Contrary to the inhibitor D-5-2, which did not significantly stimulate cell proliferation, the inhibitor CS-213 is fully androgenic inducing 225% and 396% of basal cell proliferation at 0.1 and 1 μM, respectively. Clearly, the tertiary amine **7a** showed a strong proliferative effect (292% and 314% at 0.1 and 1 μM, respectively). However, at these two concentrations, the carbamate derivative **17a** and piperazine derivative **15b** did not stimulate the proliferation of AR$^+$ cells, thus suggesting no androgenic activity.

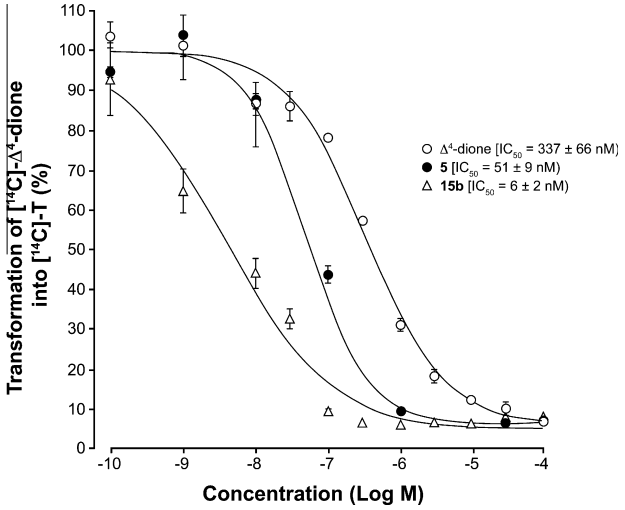


Figure 3. Inhibition of the transformation of [^{14}C]-4-androstene-3,17-dione ([^{14}C]-Δ^4-dione) (50 nM) into [^{14}C]-testosterone ([^{14}C]-T) by 17β-HSD3 overexpressed in intact HEK-293 cells. See Section 4 for the details of the assay.

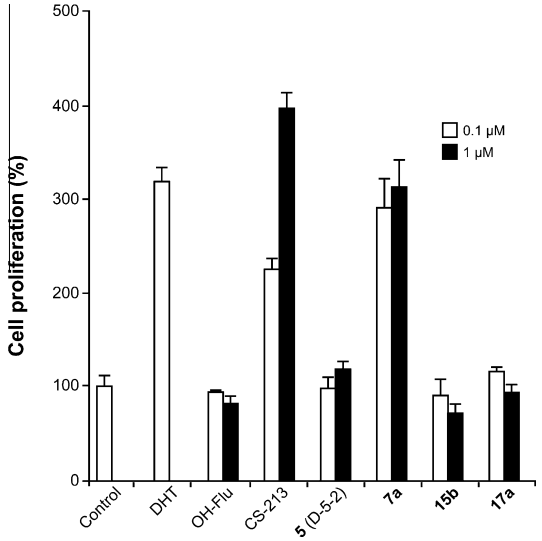


Figure 4. Proliferative effect (androgenic activity) on androgen-sensitive Shionogi cells induced by androgen dihydrotestosterone (DHT), antiandrogen hydroxy-flutamide (OH-Flu) and 17β-HSD3 inhibitors CS-213, D-5-2 (**5**), **7a**, **15b** and **17a**. Data are expressed as means ± SEM of one experiment in triplicate.

3. Conclusion

Two different series of ADT derivatives at position 3, the tertiary amines and the carbamates, were synthesized and tested as new inhibitors of 17β-HSD3. In the first series, the piperazine-methyl core at position 3β of ADT was selected as the most interesting building block regarding the inhibitory activity and its capacity to easily generate a large diversity of compounds. Different types of substituted piperazine derivatives were then synthesized including benzylamines, sulfonamides and amides. From these compounds, the sulfonamide **15b** was identified as a non-androgenic inhibitor with strong inhibitory activity (IC_{50} = 6 nM) for the transformation of Δ^4-dione into T in intact transfected HEK-293 cells overexpressing 17β-HSD3. In the second series, we introduced different substituents (amide, amines, sulfonamides, sulfones and pyridine) on the carbamate functionality at position 3 of ADT. The most potent inhibitors were compounds with a hydrophobic chain such as the benzylic derivative **17a**. In intact cells, however, the inhibitory activity produced by the carbamate compounds was clearly lower than those found in the piperazine series (44% inhibition at 0.1 μM for benzyl carbamate derivative **17a** vs 84% inhibition at 0.1 μM for sulfonamide piperazine **15b**). We thus chose compound **15b** as the most interesting candidate relatively to (1) its inhibitory activity on 17β-HSD3 in both homogenized and intact HEK-293 cells overexpressing the target enzyme and (2) its non-androgenic activity as assessed by the proliferation of androgen-sensitive Shionogi cells. Considering the potentially better in vivo stability of **15b** relatively to the first generation of analogue inhibitors (Fig. 2), it will be used for further in vivo studies aimed to evaluate its capacity to reduce the formation of testosterone in rats or to reduce the growth of prostate cancer tumors.

4. Experimental

4.1. Chemical synthesis

General information: The reagents for chemical synthesis were purchased from Sigma–Aldrich Canada Ltd (Oakville, ON, Canada). The usual solvents were obtained from Fisher Scientific (Montreal, 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 (Quebec, QC, Canada). 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 acetone (2.06 ppm, ^{1}H), chloroform (7.26 ppm, ^{1}H and 77.0 ppm, ^{13}C) or methanol (3.33 ppm, ^{1}H). ^{1}H NMR signals were reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and broad (br). We also used d or t for a signal that looks alike but is not necessary a true d or t since it comes from a higher-order spectra instead of a first-order spectra. Low-resolution mass spectra (LRMS) were recorded on a Perkin-Elmer Sciex API-150ex apparatus (Foster City, CA, USA) equipped with a turbo ion-spray source and expressed in *m*/*z*. High-resolution mass spectra (HRMS) were provided by Pierre Audet at the Laval University Chemistry Department (Quebec, QC, Canada). High-performance liquid chromatography (HPLC) analyses were carried out using a Waters system (Milford, MA, USA) equipped with a UV detector (207 nm), a reverse-phase column (Luna C18(2) 100A, 100 × 4.6 mm, 3 μm or Luna Phenyl-Hexyl, 75 × 4.6 mm, 3 μm) from Phenomenex (Torrance, CA, USA) and using an appropriate system of solvents (methanol and water). The HPLC purity was determined for key representative compounds, but only compounds with purity over 90% were used in the biological screening assays (inhibition of 17β-HSD3). The names of new compounds were obtained using ACD/Labs (Chemist's version) software (Toronto, ON, Canada).

4.1.1. General procedure for synthesis of 3β-substituted-3α-hydroxy-androstan-17-one derivatives (7a–j)

To a solution of oxirane **6**[31] (50 mg, 0.165 mmol) in anhydrous ethanol (3 mL) was added the appropriate amine (0.5 mmol) and the solution was stirred for 5 h at 60 °C. The solvent was then evaporated and the resulting mixture was dissolved in dichloromethane (10 mL) and methylisocyanate resin (300 mg, 1.8 mmol/g) was added. The suspension was stirred for 2 h and filtered to give the corresponding crude amine, which was purified by flash chromatography using EtOAc/hexanes (2:8) as eluent to give the desired amines **7a–j**. All compounds were characterized by ^{1}H NMR and MS analyses whereas ^{13}C NMR, HRMS and HPLC data were added for the key representative compound **7a**.

4.1.1.1. (3α,5α)-3{[Benzyl(ethyl)amino]methyl}3-hydroxyandrostan-17-one (7a). 15 mg, 20%; ^{1}H NMR (acetone-d_6) δ 0.82 (s, 3H), 0.84 (s, 3H), 1.01 (t, J = 7.1 Hz, 3H), 0.80-2.10 (m, 22H), 2.37 (dd, J_1 = 8.7 Hz, J_2 = 18.2 Hz, 1H), 2.45 (s, 2H), 2.52 (q, J = 7.1 Hz, 2H), 3.74 (s, 2H), 7.23 (t, J = 7.2 Hz, 1H), 7.32 (t, J = 7.7 Hz, 2H), 7.39 (d, J = 7.5 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 11.3, 12.1, 13.8, 20.2, 21.8, 28.4, 30.8, 31.6, 32.9, 34.0, 35.1, 35.9 (2×), 39.8, 40.8, 47.8, 49.4, 51.4, 54.3, 60.8, 65.3, 70.0, 127.1, 128.4 (2×), 128.6 (2×), 139.5, 221.6; LRMS for $C_{29}H_{44}NO_2$ $[M+H]^+$ 438.2; HRMS calcd for $C_{29}H_{44}NO_2$ $[M+H]^+$ 438.3367, found 438.3374; HPLC purity = 99.2% (RT = 7.2 min, 96:4 MeOH/H_2O-isocratic, Luna C18 column).

4.1.1.2. (3α,5α)-3-{[Benzyl(methyl)amino]methyl}-3-hydroxyandrostan-17-one (7b). 29 mg, 41%; ^{1}H NMR (acetone-d_6) δ 0.82 (s, 3H), 0.84 (s, 3H), 0.80-2.10 (m, 22H), 2.25 (s, 3H), 2.37 (dd, J_1 = 9.0 Hz, J_2 = 18.4 Hz, 1H), 2.41 (s, 2H), 3.63 (s, 2H), 7.24 (t, J = 7.2 Hz, 1H), 7.32 (t, J = 7.4 Hz, 2H), 7.37 (d, J = 7.2 Hz, 2H); LRMS for $C_{28}H_{42}NO_2$ $[M+H]^+$ 424.2.

4.1.1.3. (3α,5α)-3-[(Diethylamino)methyl]-3-hydroxyandrostan-17-one (7c). 40 mg, 65%; ^{1}H NMR (acetone-d_6) δ 0.81 (s, 3H), 0.84 (s, 3H), 0.99 (t, J = 7.1 Hz, 6H), 0.75-2.08 (m, 22H), 2.30 (s, 2H), 2.37 (dd, J_1 = 8.7 Hz, J_2 = 18.2 Hz, 1H), 2.58 (q, J = 7.1 Hz, 4H); LRMS for $C_{24}H_{42}NO_2$ $[M+H]^+$ 376.2.

4.1.1.4. (3α,5α)-3-[(Dibutylamino)methyl]-3-hydroxyandrostan-17-one (7d). 16 mg, 22%; ^{1}H NMR (acetone-d_6) δ 0.81 (s, 3H), 0.84 (s, 3H), 0.91 (t, J = 7.1 Hz, 6H), 0.80-2.10 (m, 30H), 2.32 (s, 2H), 2.37 (dd, J_1 = 8.7 Hz, J_2 = 18.1 Hz, 1H), 2.51 (t, J = 7.4 Hz, 4H); LRMS for $C_{28}H_{50}NO_2$ $[M+H]^+$ 432.2.

4.1.1.5. (3α,5α)-3-Hydroxy-3-(piperidin-1-ylmethyl)androstan-17-one (7e). 25 mg, 39%; ^{1}H NMR (acetone-d_6) δ 0.81 (s, 3H), 0.84 (s, 3H), 0.75-2.09 (m, 28H), 2.18 (s, 2H), 2.37 (dd, J_1 = 8.6 Hz, J_2 = 18.2 Hz, 1H), 2.52 (br s, 4H); LRMS for $C_{25}H_{42}NO_2$ $[M+H]^+$ 388.2.

4.1.1.6. (3α,5α)-3-[(4-Benzylpiperidin-1-yl)methyl]-3-hydroxyandrostan-17-one (7f). 34 mg, 43%; ^{1}H NMR (acetone-d_6) δ 0.81 (s, 3H), 0.84 (s, 3H), 0.75-2.10 (m, 27H), 2.20 (m, 4H), 2.37 (dd, J_1 = 8.7 Hz, J_2 = 18.2 Hz, 1H), 2.53 (d, J = 6.9 Hz, 2H), 2.88 (br d, J = 11.4 Hz, 2H), 7.18 (d, J = 6.7 Hz, 3H), 7.27 (m, 2H); LRMS for $C_{32}H_{48}NO_2$ $[M+H]^+$ 478.1.

4.1.1.7. (3α,5α)-3-(1,4′-Bipiperidin-1′-ylmethyl)-3-hydroxyandrostan-17-one (7g). 28 mg, 36%; ^{1}H NMR (acetone-d_6) δ 0.81 (s, 3H), 0.84 (s, 3H), 0.75-2.15 (m, 33H), 2.21 (s, 2H), 2.25 (m, 2H), 2.38 (dd, J_1 = 8.8 Hz, J_2 = 18.2 Hz, 1H), 2.47 (m, 4H), 2.93 (br d, J = 11.5 Hz, 2H); LRMS for $C_{30}H_{51}N_2O_2$ $[M+H]^+$ 471.3.

4.1.1.8. (3α,5α)-3-Hydroxy-3-[(4-methylpiperazin-1-yl)methyl]-androstan-17-one (7h). 29 mg, 43%; ^{1}H NMR (acetone-d_6) δ 0.81 (s, 3H), 0.84 (s, 3H), 0.75-2.10 (m, 22H), 2.16 (s, 3H), 2.23 (s, 2H), 2.34 (br m, 4H), 2.37 (dd, J_1 = 8.7 Hz, J_2 = 18.2 Hz, 1H), 2.58 (br s, 4H); LRMS for $C_{25}H_{43}N_2O_2$ $[M+H]^+$ 403.2.

4.1.1.9. (3α,5α)-3-Hydroxy-3-[(4-phenylpiperazin-1-yl)methyl]-androstan-17-one (7i). 54 mg, 70%; ^{1}H NMR (acetone-d_6) δ 0.83 (s, 3H), 0.84 (s, 3H), 0.75-2.10 (m, 21H), 2.32 (s, 2H), 2.37 (dd, J_1 = 8.7 Hz, J_2 = 18.2 Hz, 1H), 2.75 (m, 4H), 3.18 (m, 4H), 6.77 (t, J = 7.3 Hz, 1H), 6.94 (d, J = 8.6 Hz, 2H), 7.21 (d, J = 8.7 Hz, 2H); LRMS for $C_{30}H_{45}N_2O_2$ $[M+H]^+$ 465.0.

4.1.1.10. (3α,5α)-3-[(4-Benzylpiperazin-1-yl)methyl]-3-hydroxy androstan-17-one (7j). 60 mg, 76%; ^{1}H NMR (acetone-d_6) δ 0.81 (s, 3H), 0.84 (s, 3H), 0.75-2.10 (m, 22H), 2.24 (s, 2H), 2.37 (dd, J_1 = 8.8 Hz, J_2 = 18.3 Hz, 1H), 2.43 (br s, 4H), 2.61 (br s, 4H), 3.47 (s, 2H), 7.23 (m, 1H), 7.32 (m, 4H); LRMS for $C_{31}H_{47}N_2O_2$ $[M+H]^+$ 478.9.

4.1.2. Synthesis of intermediates 8 and 9

To a solution of oxirane **6**[31] (1.0 g, 3.3 mmol) in anhydrous ethanol (15 mL) was added piperazine (573 mg, 6.7 mmol) or trans-2,5-dimethylpiperazine (755 mg, 6.7 mmol). The solutions were stirred for 5 h at 60 °C. The resulting solutions were poured into water and extracted three times with EtOAc. The organic layers were washed with brine, dried with $MgSO_4$, filtered and evaporated under reduced pressure to give crude compounds **8** and **9** which were purified by flash chromatography using EtOAc/hexanes (1:1) as eluent.

4.1.2.1. (3α,5α)-3-Hydroxy-3-(piperazin-1-ylmethyl)androstan-17-one (8). 750 mg, 58%; ^{1}H NMR (MeOH-d_4) δ 0.84 (s, 3H), 0.89 (s, 3H), 1.01 (t, J = 7.1 Hz, 3H), 0.80-2.15 (m, 20H), 2.25 (s, 2H), 2.45 (dd, J_1 = 8.6 Hz, J_2 = 19.2 Hz, 1H), 2.58 (br s, 4H), 2.84 (t, J = 4.8 Hz, 4H); LRMS for $C_{24}H_{41}N_2O_2$ $[M+H]^+$ 389.6.

4.1.2.2. (3α,5α)-3-{[*trans*-2,5-Dimethylpiperazin-1-yl]methyl}-3-hydroxyandrostan-17-one (9). 650 mg, 47%; ^{1}H NMR ($CDCl_3$) δ 0.77 (s, 3H), 0.85 (s, 3H), 0.99 (d, J = 6.0 Hz, 3H), 1.00 (d,

J = 6.1 Hz, 3H), 0.75-1.85 (m, 22H), 1.94 (m, 1H), 2.04 (m, 3H), 2.25 (m, 2H), 2.43 (dd, J_1 = 8.6 Hz, J_2 = 18.9 Hz, 1H), 2.54 (dt, J_1 = 2.8 Hz, J_2 = 9.8 Hz, 1H), 2.61 (d, J = 13.9 Hz, 1H), 2.90 (m, 3H); LRMS for $C_{26}H_{45}N_2O_2$ $[M+H]^+$ 417.3.

4.1.3. General procedure for the synthesis of amines 10a–j and 11a–j

To a solution of compound **8** (25 mg, 0.064 mmol) or compound **9** (30 mg, 0.072 mmol) in anhydrous dichloromethane (5 mL) was added triethylamine (24 mg, 0.24 mmol, 33 μL) and the appropriate benzyl bromide (0.12 mmol). The mixture was stirred overnight and the resulting solution was evaporated and purified by flash chromatography using EtOAc/hexanes (3:7) to give the benzylamines **10a–j** and **11a–j**. All compounds were characterized by ^{1}H NMR and MS analyses whereas ^{13}C NMR, HRMS and HPLC data were added for the key representative compounds **10a** and **11a**.

4.1.3.1. (3α,5α)-3-Hydroxy-3-({4-[3-(trifluoromethyl)benzyl]-piperazin-1-yl}methyl)androstan-17-one (10a). 31 mg, 89%; ^{1}H NMR ($CDCl_3$) δ 0.76 (s, 3H), 0.86 (s, 3H), 0.75-2.10 (m, 22H), 2.27 (s, 2H), 2.44 (br m, 5H), 2.66 (br s, 4H), 3.54 (s, 2H), 7.42 (t, J = 7.6 Hz, 1H), 7.50 (br d, J = 7.5 Hz, 2H), 7.58 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 11.2, 13.8, 20.2, 21.8, 28.3, 30.8, 31.5, 32.7, 33.8, 35.1, 35.8, 35.9, 39.6, 40.7, 47.8, 51.4, 54.2, 55.7 (4×), 62.3, 69.0, 70.1, 124.0 (q, $J_{C-C-C-F}$ = 3.6 Hz), 124.2 (q, J_{C-F} = 272 Hz), 125.6 (q, $J_{C-C-C-F}$ = 3.8 Hz), 128.7, 130.6 (q, J_{C-C-F} = 32 Hz), 132.4, 139.2, 221.5; LRMS for $C_{32}H_{46}F_3N_2O_2$ $[M+H]^+$ 547.3; HRMS calcd for $C_{32}H_{46}F_3N_2O_2$ $[M+H]^+$ 547.3506, found 547.3510; HPLC purity: 97.2% (RT = 5.5 min, 96:4 MeOH/H_2O-isocratic, Luna C18 column).

4.1.3.2. (3α,5α)-3-Hydroxy-3-({4-[2-(trifluoromethyl)benzyl]-piperazin-1-yl}methyl)androstan-17-one (10b). 33 mg, 95%; ^{1}H NMR ($CDCl_3$) δ 0.76 (s, 3H), 0.86 (s, 3H), 0.80-2.10 (m, 22H), 2.27 (s, 2H), 2.43 (dd, J_1 = 8.7 Hz, J_2 = 19.2 Hz, 1H), 2.50 (br s, 4H), 2.66 (br, s, 4H), 3.65 (s, 2H), 7.33 (t, J = 7.7 Hz, 1H), 7.51 (d, J = 6.7 Hz, 1H), 7.62 (d, J = 7.8 Hz, 1H), 7.77 (d, J = 7.7 Hz, 1H); LRMS for $C_{32}H_{46}F_3N_2O_2$ $[M+H]^+$ 547.1.

4.1.3.3. (3α,5α)-3-Hydroxy-3-({4-[4-(trifluoromethyl)benzyl]-piperazin-1-yl}methyl)androstan-17-one (10c). 32 mg, 92%; ^{1}H NMR ($CDCl_3$) δ 0.76 (s, 3H), 0.86 (s, 3H), 0.80-2.10 (m, 22H), 2.27 (s, 2H), 2.45 (br m, 5H), 2.65 (br s, 4H), 3.54 (s, 2H); 7.44 (d, J = 8.0 Hz, 2H), 7.57 (d, J = 8.0 Hz, 2H); LRMS for $C_{32}H_{46}F_3N_2O_2$ $[M+H]^+$ 547.2.

4.1.3.4. (3α,5α)-3-Hydroxy-3-[(4-{3-[(trifluoromethyl)sulfanyl]-benzyl}piperazin-1-yl)methyl]androstan-17-one (10d). 25 mg, 68%; ^{1}H NMR ($CDCl_3$) δ 0.76 (s, 3H), 0.86 (s, 3H), 0.78-2.10 (m, 22H), 2.27 (s, 2H), 2.45 (br m, 5H), 2.65 (br s, 4H), 3.52 (s, 2H), 7.37 (t, J = 7.6 Hz, 1H), 7.43 (d, J = 7.7 Hz, 1H), 7.54 (d, J = 7.7 Hz, 1H), 7.62 (s, 1H); LRMS for $C_{32}H_{46}F_3N_2O_2S$ $[M+H]^+$ 579.3.

4.1.3.5. (3α,5α)-3-{[4-(3-Chlorobenzyl)piperazin-1-yl]methyl}-3-hydroxyandrostan-17-one (10e). 31 mg, 94%; ^{1}H NMR ($CDCl_3$) δ 0.76 (s, 3H), 0.86 (s, 3H), 0.80-2.10 (m, 22H), 2.27 (s, 2H), 2.43 (br m, 5H), 2.65 (br s, 4H), 3.46 (s, 2H), 7.19 (m, 1H), 7.23 (m, 2H), 7.33 (s, 1H); LRMS for $C_{31}H_{46}ClN_2O_2$ $[M+H]^+$ 513.3.

4.1.3.6. (3α,5α)-3-Hydroxy-3-{[4-(3-methoxybenzyl)piperazin-1-yl]methyl}androstan-17-one (10f). 21 mg, 64%; ^{1}H NMR ($CDCl_3$) δ 0.76 (s, 3H), 0.85 (s, 3H), 0.78-2.10 (m, 22H), 2.26 (s, 2H), 2.43 (br m, 5H), 2.65 (br s, 4H), 3.48 (s, 2H), 3.81 (s, 3H), 6.80 (d, J = 7.5 Hz, 1H), 6.89 (m, 2H), 7.23 (t, J = 8.0 Hz, 1H); LRMS for $C_{32}H_{49}N_2O_3$ $[M+H]^+$ 509.3.

4.1.3.7. (3α,5α)-3-Hydroxy-3-{[4-(pyridin-3-ylmethyl)piperazin-1-yl]methyl}androstan-17-one (10g). 20 mg, 65%; ^{1}H NMR ($CDCl_3$) δ 0.76 (s, 3H), 0.85 (s, 3H), 0.78-2.10 (m, 22H), 2.27 (s, 2H), 2.43 (br m, 5H), 2.65 (br s, 4H), 3.51 (s, 2H), 7.24 (d, J = 4.9 Hz, 1H), 7.65 (d, J = 7.8 Hz, 1H), 8.51 (dd, J_1 = 1.3 Hz, J_2 = 4.8 Hz, 1H), 8.54 (d, J = 1.4 Hz, 1H); LRMS for $C_{30}H_{46}N_3O_2$ $[M+H]^+$ 480.2.

4.1.3.8. (3α,5α)-3-({4-[2,5-Bis(trifluoromethyl)benzyl]piperazin-1-yl}methyl)-3-hydroxyandrostan-17-one (10h). 27 mg, 69%; ^{1}H NMR ($CDCl_3$) δ 0.77 (s, 3H), 0.86 (s, 3H), 0.78-2.10 (m, 22H), 2.29 (s, 2H), 2.43 (dd, J_1 = 8.7 Hz, J_2 = 19.3 Hz, 1H), 2.51 (br s, 4H), 2.68 (br s, 4H), 3.69 (s, 2H), 7.59 (d, J = 8.1 Hz, 1H), 7.75 (d, J = 8.2 Hz, 1H), 8.12 (s, 1H); LRMS for $C_{33}H_{45}F_6N_2O_2$ $[M+H]^+$ 615.5.

4.1.3.9. (3α,5α)-3-({4-[2,4-Bis(trifluoromethyl)benzyl]piperazin-1-yl}methyl)-3-hydroxyandrostan-17-one (10i). 27 mg, 69%; ^{1}H NMR ($CDCl_3$) δ 0.77 (s, 3H), 0.86 (s, 3H), 0.83-2.10 (m, 22H), 2.28 (s, 2H), 2.43 (dd, J_1 = 8.5 Hz, J_2 = 19.1 Hz, 1H), 2.51 (br s, 4H), 2.67 (br s, 4H), 3.70 (s, 2H), 7.77 (d, J = 8.3 Hz, 1H), 7.88 (s, 1H), 7.98 (d, J = 8.2 Hz, 1H); LRMS for $C_{33}H_{45}F_6N_2O_2$ $[M+H]^+$ 615.2.

4.1.3.10. (3α,5α)-3-({4-[3,5-Bis(trifluoromethyl)benzyl]piperazin-1-yl}methyl)-3-hydroxyandrostan-17-one (10j). 29 mg, 74%; ^{1}H NMR ($CDCl_3$) δ 0.77 (s, 3H), 0.86 (s, 3H), 0.80-2.10 (m, 22H), 2.28 (s, 2H), 2.43 (br m, 5H), 2.67 (br s, 4H), 3.59 (s, 2H), 7.77 (s, 1H), 7.79 (s, 2H); LRMS for $C_{33}H_{45}F_6N_2O_2$ $[M+H]^+$ 615.5.

4.1.3.11. (3α,5α)-3-({*trans*-2,5-Dimethyl-4-[3-(trifluoromethyl)-benzyl]piperazin-1-yl}methyl)-3-hydroxyandrostan-17-one (11a). 25 mg, 61%; ^{1}H NMR ($CDCl_3$) δ 0.77 (s, 3H), 0.86 (s, 3H), 0.92 (d, J = 6.2 Hz, 3H), 1.10 (d, J = 6.0 Hz, 3H), 0.80-2.10 (m, 23H), 2.32-2.60 (m, 6H), 2.91 (d, J = 11.1 Hz, 1H), 3.11 (d, J = 13.7 Hz, 1H), 3.22 (d, J = 16.1 Hz, 1H), 4.07 (d, J = 13.7 Hz, 1H), 7.43 (m, 1H), 7.51 (d, J = 7.8 Hz, 2H), 7.58 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 11.3, 13.8, 17.2 (∗), 18.2 (∗), 20.2, 21.8, 28.3, 30.8, 31.6, 32.7, 33.9, 35.1, 35.9 (2×), 39.4, 40.8, 47.8, 51.4, 54.3, 55.8, 56.3, 57.5, 59.8 (∗), 63.8 (2×), 69.4, 123.7 (q, $J_{C-C-C-F}$ = 3.6 Hz), 125.0 (q, J_{C-F} = 273 Hz), 125.4 (q, $J_{C-C-C-F}$ = 3.6 Hz), 128.6, 130.5 (q, J_{C-C-F} = 32 Hz), 132.1, 140.3, 221.6 [∗: a very weak or no signal observed although a correlation in HSQC experiment]; LRMS for $C_{34}H_{50}F_3N_2O_2$ $[M+H]^+$ 575.2; HRMS for $C_{34}H_{50}F_3N_2O_2$ $[M+H]^+$ 575.3819, found 575.3826; HPLC purity: 99.0% (RT = 6.6 min, 96:4 MeOH/H_2O-isocratic, Luna C18 column).

4.1.3.12. (3α,5α)-3-({*trans*-2,5-Dimethyl-4-[2-(trifluoromethyl)-benzyl]piperazin-1-yl}methyl)-3-hydroxyandrostan-17-one (11b). 17 mg, 41%; ^{1}H NMR ($CDCl_3$) δ 0.78 (s, 3H), 0.86 (s, 3H), 0.93 (d, J = 6.2 Hz, 3H), 1.05 (d, J = 6.0 Hz, 3H), 0.75-2.10 (m, 23H), 2.33-2.60 (m, 6H), 2.93 (d, J = 11.1 Hz, 1H), 3.23 (br s, 1H), 3.31 (d, J = 15.1 Hz, 1H), 4.07 (d, J = 14.8 Hz, 1H), 7.31 (t, J = 7.7 Hz, 1H), 7.51 (t, J = 7.4 Hz, 1H), 7.61 (d, J = 7.9 Hz, 1H), 7.87 (d, J = 7.8 Hz, 1H); LRMS for $C_{34}H_{50}F_3N_2O_2$ $[M+H]^+$ 575.3.

4.1.3.13. (3α,5α)-3-({*trans*-2,5-Dimethyl-4-[4-(trifluoromethyl)-benzyl]piperazin-1-yl}methyl)-3-hydroxyandrostan-17-one (11c). 22 mg, 53%; ^{1}H NMR ($CDCl_3$) δ 0.77 (s, 3H), 0.86 (s, 3H), 0.92 (d, J = 6.2 Hz, 3H), 1.10 (d, J = 6.0 Hz, 3H), 0.75-2.15 (m, 24H), 2.30-2.60 (m, 6H), 2.91 (d, J = 11.5 Hz, 1H), 3.11 (d, J = 13.6 Hz, 1H), 3.20 (br s, 1H), 4.07 (d, J = 13.9 Hz, 1H), 7.37 (d, J = 7.7 Hz, 1H), 7.43 (d, J = 8.0 Hz, 1H), 7.57 (d, J = 8.1 Hz, 1H); LRMS for $C_{34}H_{50}F_3N_2O_2$ $[M+H]^+$ 575.2.

4.1.3.14. (3α,5α)-3-{[*trans*-2,5-Dimethyl-4-{3-[(trifluoromethyl)-sulfanyl]benzyl}piperazin-1-yl]methyl}-3-hydroxyandrostan-17-one (11d). 15 mg, 34%; ^{1}H NMR ($CDCl_3$) δ 0.77 (s, 3H), 0.86 (s, 3H), 0.92 (d, J = 6.2 Hz, 3H), 1.10 (d, J = 6.0 Hz, 3H), 0.75-2.10 (m,

23H), 2.30-2.60 (m, 6H), 2.91 (d, J = 10.5 Hz, 1H), 3.10 (d, J = 13.2 Hz, 1H), 3.21 (d, J = 16.2 Hz, 1H), 4.04 (d, J = 13.1 Hz, 1H), 7.36 (t, J = 7.7 Hz, 1H), 7.43 (d, J = 7.7 Hz, 1H), 7.53 (d, J = 8.5 Hz, 1H), 7.62 (s, 1H); LRMS for $C_{34}H_{50}F_3N_2O_2S$ $[M+H]^+$ 607.2.

4.1.3.15. (3α,5α)-3-{[*trans*-4-(3-Chlorobenzyl)-2,5-dimethylpiperazin-1-yl]methyl}-3-hydroxyandrostan-17-one (11e). 12 mg, 31%; ^{1}H NMR ($CDCl_3$) δ 0.77 (s, 3H), 0.86 (s, 3H), 0.93 (d, J = 6.2 Hz, 3H), 1.09 (d, J = 6.0 Hz, 3H), 0.75-2.10 (m, 23H), 2.30-2.62 (m, 6H), 2.89 (d, J = 11.2 Hz, 1H), 3.03 (d, J = 13.4 Hz, 1H), 3.22 (br s, 1H), 4.00 (d, J = 13.3 Hz, 1H), 7.18 (t, J = 7.2 Hz, 1H), 7.23 (s, 2H), 7.33 (s, 1H); LRMS for $C_{33}H_{50}{}^{35}ClN_2O_2$ $[M+H]^+$ 541.3.

4.1.3.16. (3α,5α)-3-Hydroxy-3-{[*trans*-4-(3-methoxybenzyl)-2,5-dimethylpiperazin-1-yl]methyl}androstan-17-one (11f). 14 mg, 36%; ^{1}H NMR ($CDCl_3$) δ 0.77 (s, 3H), 0.86 (s, 3H), 0.92 (d, J = 6.0 Hz, 3H), 1.11 (d, J = 5.3 Hz, 3H), 0.75-2.10 (m, 24H), 2.30-2.80 (m, 6H), 2.89 (d, J = 10.2 Hz, 1H), 3.07 (d J = 13.2 Hz, 1H), 3.81 (s, 3H), 4.01 (d, J = 12.8 Hz, 1H), 6.79 (m, 1H), 6.89 (m, 2H), 7.22 (m, 1H); LRMS for $C_{34}H_{53}N_2O_3$ $[M+H]^+$ 537.4.

4.1.3.17. (3α,5α)-3-{[*trans*-2,5-Dimethyl-4-(pyridin-3-ylmethyl)-piperazin-1-yl]methyl}-3-hydroxyandrostan-17-one (11g). 10 mg, 27%; ^{1}H NMR ($CDCl_3$) δ 0.77 (s, 3H), 0.86 (s, 3H), 0.92 (d, J = 6.0 Hz, 3H), 1.12 (d, J = 5.4 Hz, 3H), 0.75-2.10 (m, 22H), 2.30-2.68 (m, 7H), 2.90 (d, J = 11.0 Hz, 1H), 3.10 (m, 2H), 4.02 (d, J = 13.2 Hz, 1H), 7.25 (s, 1H), 7.64 (m, 1H), 8.52 (m, 2H); LRMS for $C_{32}H_{50}N_3O_2$ $[M+H]^+$ 508.3.

4.1.3.18. (3α,5α)-3-({*trans*-4-[2,5-Bis(trifluoromethyl)benzyl]-2,5-dimethylpiperazin-1-yl}methyl)-3-hydroxyandrostan-17-one (11h). 46 mg, 98%; ^{1}H NMR ($CDCl_3$) δ 0.78 (s, 3H), 0.86 (s, 3H), 0.95 (d, J = 6.1 Hz, 3H), 1.03 (d, J = 6.0 Hz, 3H), 0.75-2.10 (m, 23H), 2.35-2.60 (m, 6H), 2.95 (d, J = 11.6 Hz, 1H), 3.18 (br s, 1H), 3.40 (d, J = 15.8 Hz, 1H), 4.06 (d, J = 15.8 Hz, 1H), 7.58 (d, J = 8.1 Hz, 1H), 7.74 (d, J = 8.2 Hz, 1H), 8.22 (s, 1H); LRMS for $C_{35}H_{49}F_6N_2O_2$ $[M+H]^+$ 643.3.

4.1.3.19. (3α,5α)-3-({*trans*-4-[2,4-Bis(trifluoromethyl)benzyl]-2,5-dimethylpiperazin-1-yl}methyl)-3-hydroxyandrostan-17-one (11i). 46 mg, 98%; ^{1}H NMR ($CDCl_3$) δ 0.78 (s, 3H), 0.86 (s, 3H), 0.94 (d, J = 6.1 Hz, 3H), 1.03 (d, J = 6.1 Hz, 3H), 0.75-2.10 (m, 23H), 2.32-2.60 (m, 6H), 2.94 (d, J = 11.0 Hz, 1H), 3.13 (br s, 1H), 3.39 (d, J = 15.8 Hz, 1H), 4.08 (d, J = 15.4 Hz, 1H), 7.76 (d, J = 8.2 Hz, 1H), 7.86 (s, 1H), 8.08 (d, J = 8.2 Hz, 1H); LRMS for $C_{35}H_{49}F_6N_2O_2$ $[M+H]^+$ 643.3.

4.1.3.20. (3α,5α)-3-({*trans*-4-[3,5-Bis(trifluoromethyl)benzyl]-2,5-dimethylpiperazin-1-yl}methyl)-3-hydroxyandrostan-17-one (11j). 20 mg, 43%; ^{1}H NMR ($CDCl_3$) δ 0.78 (s, 3H), 0.86 (s, 3H), 0.94 (d, J = 6.1 Hz, 3H), 1.09 (d, J = 6.1 Hz, 3H), 0.75-2.12 (m, 24H), 2.35-2.57 (m, 6H), 2.93 (d, J = 11.4 Hz, 1H), 3.18 (d, J = 11.4 Hz, 1H), 4.09 (d, J = 13.3 Hz, 1H), 7.76 (s, 1H), 7.80 (s, 2H); LRMS for $C_{35}H_{49}F_6N_2O_2$ $[M+H]^+$ 643.4.

4.1.4. General procedure for synthesis of amides 12a–e and 13a–d

To a solution of compound **8** (40 mg, 0.103 mmol) or compound **9** (25 mg, 0.060 mmol) in anhydrous dichloromethane (3 mL) was added triethylamine (4.0 equiv) and the appropriate acyl chloride (2.0 equiv). The solution was then stirred for 3 h at room temperature. The resulting solution was evaporated and purified by flash chromatography using EtOAc/hexanes (7:3 to 9:1) to give the corresponding amides **12a–e** and **13a–d**. All compounds were characterized by ^{1}H NMR and MS analyses whereas ^{13}C NMR, HRMS and HPLC data were added for the key representative compounds **12a**, **12c** and **13a**.

4.1.4.1. (3α,5α)-3-Hydroxy-3-{[4-(phenylcarbonyl)piperazin-1-yl]methyl}androstan-17-one (12a). 33 mg, 64%; ^{1}H NMR ($CDCl_3$) δ 0.77 (s, 3H), 0.86 (s, 3H), 0.80-2.12 (m, 22H), 2.31 (s, 2H), 2.43 (dd, J_1 = 8.7 Hz, J_2 = 19.2 Hz, 1H), 2.57 and 2.70 (br 2s, 4H), 3.43 (br s, 2H), 3.79 (br s, 2H), 7.40 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 11.2, 13.8, 20.2, 21.7, 28.3, 30.8, 31.5, 32.4, 33.6, 35.0, 35.8, 35.9, 39.3, 40.6, 47.8, 51.4, 54.1, 55.4 (2×), 55.7 (2×), 69.1, 70.5, 127.0 (2×), 128.5 (2×), 129.8, 135.5, 170.3, 221.5; LRMS for $C_{31}H_{45}N_2O_3$ $[M+H]^+$ 493.0; HRMS calcd for $C_{31}H_{45}N_2O_3$ $[M+H]^+$ 493.3425, found 493.3433; HPLC purity: 94.8% (RT = 10.2 min, 75:25 to 5:95 MeOH/H_2O-linear gradient in 20 min, Luna Phenyl-Hexyl column).

4.1.4.2. (3α,5α)-3-{[4-(Cyclohexylcarbonyl)piperazin-1-yl]methyl}-3-hydroxyandrostan-17-one (12b). 30 mg, 58%; ^{1}H NMR ($CDCl_3$) δ 0.77 (s, 3H), 0.86 (s, 3H), 0.80-2.12 (m, 21H), 2.29 (s, 2H), 2.43 (m, 2H), 2.60 (m, 4H), 2.92 (br s, 1H), 3.49 (br s, 2H), 3.61 (br s, 2H); LRMS for $C_{31}H_{51}N_2O_3$ $[M+H]^+$ 499.3.

4.1.4.3. (3α,5α)-3-Hydroxy-3-[(4-{[2-(trifluoromethyl)phenyl]-carbonyl}-piperazin-1-yl)methyl]androstan-17-one (12c). 30 mg, 52%; ^{1}H NMR ($CDCl_3$) δ 0.76 (s, 3H), 0.86 (s, 3H), 0.80-2.12 (m, 22H), 2.30 (s, 2H), 2.43 (dd, J_1 = 8.7 Hz, J_2 = 19.1 Hz, 1H), 2.53 (m, 2H), 2.70 (br s, 2H), 3.18 (t, J = 5.0 Hz, 2H), 3.82 (br m, 2H), 7.32 (d, J = 7.4 Hz, 1H), 7.52 (t, J = 7.6 Hz, 1H), 7.60 (t, J = 7.5 Hz, 1H), 7.71 (d, J = 7.8 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 11.2, 13.8, 20.2, 21.7, 28.3, 30.8, 31.5, 32.3, 33.6, 35.1, 35.8, 35.9, 39.3, 40.6, 41.9, 47.3, 47.8, 51.4, 54.1, 55.1, 55.2, 69.0, 70.6, 123.6 (q, J_{C-F} = 274 Hz), 126.6 (q, $J_{C-C-C-F}$ = 4.4 Hz), 126.7 (q, J_{C-C-F} = 32 Hz), 127.2, 129.2, 132.2, 134.7, 167.3, 221.4; LRMS for $C_{32}H_{44}F_3N_2O_3$ $[M+H]^+$ 561.1; HRMS calcd for $C_{32}H_{44}F_3N_2O_3$ $[M+H]^+$ 561.3299, found 561.3304; HPLC purity: 96.7% (RT = 10.7 min 75:25 to 5:95 MeOH/H_2O-linear gradient in 20 min, Luna Phenyl-Hexyl column).

4.1.4.4. (3α,5α)-3-Hydroxy-3-[(4-{[3-(trifluoromethyl)phenyl]-carbonyl}piperazin-1-yl)methyl]androstan-17-one (12d). 42 mg, 73%; ^{1}H NMR ($CDCl_3$) δ 0.77 (s, 3H), 0.86 (s, 3H), 0.80-2.12 (m, 22H), 2.32 (s, 2H), 2.43 (dd, J_1 = 8.7 Hz, J_2 = 19.3 Hz, 1H), 2.59 (br s, 2H), 2.72 (br s, 2H), 3.41 (br s, 2H), 3.80 (br s, 2H), 7.55 (m, 2H), 7.67 (s, 2H); LRMS for $C_{32}H_{44}F_3N_2O_3$ $[M+H]^+$ 561.4.

4.1.4.5. (3α,5α)-3-Hydroxy-3-[(4-{[4-(trifluoromethyl)phenyl]-carbonyl}piperazin-1-yl)methyl]androstan-17-one (12e). 44 mg, 76%; ^{1}H NMR ($CDCl_3$) δ 0.77 (s, 3H), 0.86 (s, 3H), 0.80-2.10 (m, 22H), 2.32 (s, 2H), 2.43 (dd, J_1 = 8.7 Hz, J_2 = 19.3 Hz, 1H), 2.57 (br s, 2H), 2.73 (br s, 2H), 3.39 (br s, 2H), 3.80 (br s, 2H), 7.51 (d, J = 8.0 Hz, 2H), 7.68 (d, J = 8.1 Hz, 2H); LRMS for $C_{32}H_{44}F_3N_2O_3$ $[M+H]^+$ 561.2.

4.1.4.6. (3α,5α)-3-{[*trans*-2,5-Dimethyl-4-(phenylcarbonyl)piperazin-1-yl]methyl}-3-hydroxyandrostan-17-one (13a). 25 mg, 47%; ^{1}H NMR ($CDCl_3$) δ 0.77 (s, 3H), 0.86 (s, 3H), 1.27 (m, 3H), 1.41 (d, J = 6.8 Hz, 3H), 0.75-2.00 (m, 23H), 2.10 (m, 2H), 2.40 (m, 3H), 2.90 (br s, 1H), 3.06 (d, J = 8.8 Hz, 1H), 3.50 (br s, 1H), 7.36 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 8.6, 11.2, 13.8, 16.4 (*), 20.2, 21.7, 28.3, 30.8, 31.5, 32.5, 33.8, 35.0, 35.8, 35.9, 39.4, 40.7, 47.8, 51.4 (2×), 51.7 (2×) (*), 54.0, 55.1, 65.8, 70.9, 126.5, 128.5 (2×), 129.3 (2×), 136.4, 171.2, 221.5 [*: a very weak or no signal observed although a correlation in HSQC experiment]; LRMS for $C_{33}H_{49}N_2O_3$ $[M+H]^+$ 521.4; HRMS calcd for $C_{33}H_{49}N_2O_3$ $[M+H]^+$ 521.3738, found 521.3745; HPLC purity: 96.4% (RT = 12.1 min, 70:30 to 5:95 MeOH/H_2O-linear gradient in 20 min, Luna Phenyl-Hexyl column).

4.1.4.7. (3α,5α)-3-{[*trans*-4-(Cyclohexylcarbonyl)-2,5-dimethylpiperazin-1-yl]methyl}-3-hydroxyandrostan-17-one (13b). 14 mg, 44%; ^{1}H NMR ($CDCl_3$) δ 0.77 (s, 3H), 0.86 (s, 3H), 1.27 (m, 6H), 0.80-2.00 (m, 22H), 2.10 (m, 2H), 2.41 (m, 3H), 3.02 (br m, 3H), 3.46 and 3.60 (2d, J = 11.8 Hz, 1H), 4.02, 4.28 and 4.70 (3 m, 1H); LRMS for $C_{33}H_{55}N_2O_3$ $[M+H]^+$ 527.3.

4.1.4.8. (3α,5α)-3-{[*trans*-2,5-Dimethyl-4-{[3-(trifluoromethyl)phenyl]carbonyl}piperazin-1-yl]methyl}-3-hydroxyandrostan-17-one (13c). 27 mg, 77%; ^{1}H NMR ($CDCl_3$) δ 0.77 (s, 3H), 0.86 (s, 3H), 1.26 (m, 3H), 1.43 (d, J = 6.4 Hz, 3H), 0.78-2.00 (m, 22H), 2.11 (m, 2H), 2.41 (m, 3H), 2.95 (br s, 2H), 3.07 (d, J = 10.4 Hz, 1H), 3.55 (br s, 1H), 7.55 (d, J = 4.5 Hz, 2H), 7.61 (s, 1H), 7.68 (m, 1H); LRMS for $C_{34}H_{48}F_3N_2O_3$ $[M+H]^+$ 589.3.

4.1.4.9. (3α,5α)-3-{[*trans*-2,5-Dimethyl-4-{[4-(trifluoromethyl)phenyl]carbonyl}piperazin-1-yl]methyl}-3-hydroxyandrostan-17-one (13d). 31 mg, 89%; ^{1}H NMR ($CDCl_3$) δ 0.77 (s, 3H), 0.86 (s, 3H), 1.27 (m, 3H), 1.42 (m, 3H), 0.80-1.98 (m, 22H), 2.06 (m, 2H), 2.41 (m, 3H), 2.95 (br s, 2H), 3.05 (br s, 1H), 3.58 (br s, 1H), 7.46 (d, J = 7.9 Hz, 2H), 7.68 (d, J = 8.0 Hz, 2H); LRMS for $C_{34}H_{48}F_3N_2O_3$ $[M+H]^+$ 589.2.

4.1.5. General procedure for the synthesis of sulfonamides 14a–c and 15a–c

To a solution of compound **8** (30 mg, 0.077 mmol) or compound **9** (30 mg, 0.072 mmol) in anhydrous dichloromethane (3 mL) was added triethylamine (33 mL, 24 mg, 0.24 mmol) and the appropriate sulfonyl chloride (0.12 mmol). The solution was then stirred for 3 h at room temperature. The resulting solution was evaporated and purified by flash chromatography using EtOAc/hexanes (3:7 to 1:1) to give the corresponding sulfonamides **14a–c** and **15a–c**. All compounds were characterized by ^{1}H NMR and MS analyses whereas ^{13}C NMR, HRMS and HPLC data were added for the key representative compounds **14a**, **15b** and **15c**.

4.1.5.1. (3α,5α)-3-Hydroxy-3-[(4-{[3-(trifluoromethyl)phenyl]-sulfonyl} piperazin-1-yl)methyl]androstan-17-one (14a). 29 mg, 63%; ^{1}H NMR ($CDCl_3$) δ 0.74 (s, 3H), 0.84 (s, 3H), 0.75-2.10 (m, 21H), 2.28 (s, 2H), 2.43 (dd, J_1 = 8.7 Hz, J_2 = 19.3 Hz, 1H), 2.50 (s, 1H), 2.72 (t, J = 4.7 Hz, 4H), 3.05 (br s, 4H), 7.73 (t, J = 7.8 Hz, 1H), 7.90 (d, J = 7.8 Hz, 1H), 7.95 (d, J = 7.9 Hz, 1H), 8.01 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 11.2, 13.8, 20.2, 21.7, 28.2, 30.7, 31.5, 32.2, 33.6, 35.0, 35.8, 35.9, 39.1, 40.5, 46.3 (2×), 47.8, 51.4, 54.1, 54.7 (2×), 68.8, 70.6, 123.2 (q, J_{C-F} = 273 Hz), 124.7 (q, $J_{C-C-C-F}$ = 3.7 Hz), 129.6, 130.0, 130.9, 131.9 (q, J_{C-C-F} = 33 Hz), 137.0, 221.4; LRMS for $C_{31}H_{43}F_3N_2O_4SNa$ $[M+Na]^+$ 619.5; HRMS calcd for $C_{31}H_{44}F_3N_2O_4S$ $[M+H]^+$ 597.2968, found 597.2974; HPLC purity: 98.4% (RT = 15.4 min, 70:30 to 5:95 MeOH/H_2O-linear gradient in 20 min, Luna Phenyl-Hexyl column).

4.1.5.2. (3α,5α)-3-Hydroxy-3-[(4-{[2-(trifluoromethyl)phenyl]-sulfonyl}piperazin-1-yl)methyl]androstan-17-one (14b). 33 mg, 72%; ^{1}H NMR ($CDCl_3$) δ 0.75 (s, 3H), 0.85 (s, 3H), 0.75-2.12 (m, 21H), 2.29 (s, 2H), 2.43 (dd, J_1 = 8.6 Hz, J_2 = 19.2 Hz, 1H), 2.69 (m, 5H), 3.26 (br s, 4H), 7.72 (m, 2H), 7.92 (d, J = 9.1 Hz, 1H), 8.10 (d, J = 9.1 Hz, 1H); LRMS for $C_{31}H_{43}F_3N_2O_4SNa$ $[M+Na]^+$ 619.2.

4.1.5.3. (3α,5α)-3-Hydroxy-3-[(4-{[4-(trifluoromethyl)phenyl]-sulfonyl}-piperazin-1-yl)methyl]androstan-17-one (14c). 26 mg, 56%; ^{1}H NMR ($CDCl_3$) δ 0.74 (s, 3H), 0.84 (s, 3H), 0.75-2.10 (m, 23H), 2.27 (s, 2H), 2.43 (dd, J_1 = 8.6 Hz, J_2 = 19.2 Hz, 1H), 2.50 (s, 1H), 2.72 (br t, J = 4.7 Hz, 4H), 3.06 (br s, 4H), 7.83 (d, J = 8.4 Hz, 1H), 7.89 (t, J = 8.3 Hz, 1H); LRMS for $C_{31}H_{43}F_3N_2O_4SNa$ $[M+Na]^+$ 619.3.

4.1.5.4. (3α,5α)-3-{[*trans*-2,5-Dimethyl-4-{[3-(trifluoromethyl)-phenyl]sulfonyl}piperazin-1-yl]methyl}-3-hydroxyandrostan-17-one (15a). 30 mg, 67%; ^{1}H NMR ($CDCl_3$) δ 0.75 (s, 3H), 0.85 (s, 3H), 1.00 (d, J = 6.4 Hz, 3H), 1.14 (d, J = 6.8 Hz, 3H), 0.75-2.18 (m, 22H), 2.38 (m, 3H), 2.69 (s, 1H), 2.95 (m, 1H), 3.07 (dd, J_1 = 3.5 Hz, J_2 = 11.9 Hz, 1H), 3.39 (s, 2H), 4.10 (m, 1H), 7.66 (t, J = 7.8 Hz, 1H), 7.82 (d, J = 7.9 Hz, 1H), 7.99 (d, J = 7.8 Hz, 1H), 8.06 (s, 1H); LRMS for $C_{33}H_{47}F_3N_2O_4SNa$ $[M+Na]^+$ 647.4.

4.1.5.5. (3α,5α)-3-{[*trans*-2,5-Dimethyl-4-{[2-(trifluoromethyl)-phenyl]sulfonyl}piperazin-1-yl]methyl}-3-hydroxyandrostan-17-one (15b). 29 mg, 63%; ^{1}H NMR ($CDCl_3$) δ 0.75 (s, 3H), 0.85 (s, 3H), 0.89 (m, 3H), 1.19 (m, 3H), 0.75-1.98 (m, 19H), 2.08 (m, 2H), 2.33 (t, J = 13.2 Hz, 2H), 2.43 (dd, J_1 = 8.8 Hz, J_2 = 19.2 Hz, 1H), 2.82 (s, 1H), 2.90 (m, 1H), 3.09 (dd, J_1 = 3.3 Hz, J_2 = 11.6 Hz, 1H), 3.35 (m, 2H), 3.52 (d, J = 13.0 Hz, 1H), 4.05 (m, 1H), 7.69 (m, 2H), 7.89 (m, 1H), 8.18 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 8.6, 11.2, 13.8, 15.6, 20.2, 21.7, 28.3, 30.7, 31.5, 32.4, 33.8, 35.0, 35.8, 35.9, 39.3, 40.7, 46.0, 47.8, 49.5, 51.4, 52.4, 54.2, 54.7, 65.7, 70.9, 122.6 (q, J_{C-F} = 274 Hz), 127.5 (q, J_{C-C-F} = 33 Hz), 128.5 (q, $J_{C-C-C-F}$ = 6.4 Hz), 131.9, 132.1, 132.5, 139.3, 221.5; LRMS for $C_{33}H_{47}F_3N_2O_4SNa$ $[M+Na]^+$ 647.2; HRMS calcd for $C_{33}H_{48}F_3N_2O_4S$ $[M+H]^+$ 625.3281, found 625.3291; HPLC purity: 98.9% (RT = 16.1 min, 70:30 to 5:95 MeOH/H_2O-linear gradient in 20 min, Luna Phenyl-Hexyl column).

4.1.5.6. (3α,5α)-3-{[*trans*-2,5-Dimethyl-4-{[2-trifluoromethyl)-phenyl]sulfonyl}piperazin-1-yl] methyl}-3-hydroxyandrostan-17-one (15c). 35 mg, 78%; ^{1}H NMR ($CDCl_3$) δ 0.75 (s, 3H), 0.85 (s, 3H), 1.01 (d, J = 5.9 Hz, 3H), 1.14 (d, J = 6.7 Hz, 3H), 0.75-1.98 (m, 20H), 2.09 (m, 2H), 2.38 (m, 3H), 2.68 (s, 1H), 2.95 (d, J = 6.6 Hz, 1H), 3.07 (dd, J_1 = 3.6 Hz, J_2 = 11.8 Hz, 1H), 3.40 (s, 2H), 4.05 (br s, 1H), 7.77 (d, J = 8.3 Hz, 2H), 7.93 (d, J = 8.3 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 9.1, 11.2, 13.8, 14.9, 20.2, 21.7, 28.3, 30.7, 31.5, 32.4, 33.7, 35.0, 35.8, 35.9, 39.3, 40.7, 46.1, 47.8, 49.9, 51.4, 53.0, 54.2, 54.6, 65.6, 71.0, 123.2 (q, J_{C-F} = 273 Hz), 126.1, 126.2 (q, $J_{C-C-C-F}$ = 3.6 Hz), 127.4 (2×), 134.1 (q, J_{C-C-F} = 33 Hz), 144.2, 221.5; LRMS for $C_{33}H_{47}F_3N_2O_4SNa$ $[M+Na]^+$ 647.4; HRMS calcd for $C_{33}H_{48}F_3N_2O_4S$ $[M+H]^+$ 625.3281, found 625.3287; HPLC purity: 98.7% (RT = 17.0 min, 70:30 to 5:95 MeOH/H_2O-linear gradient in 20 min, Luna Phenyl-Hexyl column).

4.1.6. General procedure for synthesis of 3-carbamate-androsterone derivatives 17a–i

To a solution of oxirane **6**[31] (75 mg, 0.25 mmol) in ethanol (5 mL) was added the appropriate primary amine (0.75 mmol) and the solution was stirred at 70 °C overnight. The resulting solution was evaporated and purified by flash chromatography using EtOAc/hexanes (7:3 to 9:1) to give the corresponding secondary amines of general structure **16** in good yields (70–90%). To the secondary amine, typically 0.22 mmol, in anhydrous dichloromethane (7 mL) at 0 °C under atmosphere of argon was added diisopropylamine (0.66 mmol) and triphosgene (0.11 mmol). The solution was stirred at room temperature overnight. The resulting solution was poured into water, extracted with dichloromethane, filtered on a phase separator (Biotage, Uppsala, Sweden) and then evaporated. The crude compounds were then purified by flash chromatography using EtOAc/hexanes (1:9 to 3:7) to give the corresponding carbamates **17a–i**. All compounds were characterized by ^{1}H NMR and MS analyses whereas ^{13}C NMR, HRMS and HPLC data were added for the representative compound **17a**.

4.1.6.1. (3*R*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*)-3′-Benzyl-10,13-dimethyltetradecahydro-2′*H* spiro[cyclopenta [α] phenanthrene-3,5′-[1,3]-oxazolidine]-2′,17(2*H*)-dione (17a). 20 mg, 18%; ^{1}H NMR (acetone-d_6) δ 0.84 (s, 3H), 0.86 (s, 3H), 0.80-2.10 (m, 22H), 2.37 (dd,

J_1 = 8.2 Hz, J_2 = 17.7 Hz, 1H), 3.16 (s, 2H), 4.39 (s, 2H), 7.31 (m, 3H), 7.38 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.7, 27.8, 30.6, 31.5, 32.8, 33.8, 35.0, 35.4, 35.8, 39.4, 40.8, 47.7, 48.1, 51.3, 53.8, 55.6, 78.9, 127.9, 128.0, 128.8 (2×), 129.7, 135.9, 157.6, 221.3; LRMS for $C_{28}H_{37}NO_3Na$ $[M+Na]^+$ 458.3; HRMS calcd for $C_{28}H_{38}NO_3$ $[M+H]^+$ 436.2846, found 436.2854; HPLC purity: 98.7% (RT = 13.9 min, 75:25 to 5:95 MeOH/H_2O-linear gradient in 20 min, Luna Phenyl-Hexyl column).

4.1.6.2. (3*R*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*)-10,13-Dimethyl-3′-(4-methylbenzyl)tetradecahydro-2′*H*-spiro [cyclopenta[α]phenanthrene-3,5′-[1,3]oxazolidine]-2′,17(2*H*)-dione (17b). 34 mg, 30%; ^{1}H NMR (acetone-d_6) δ 0.84 (s, 3H), 0.86 (s, 3H), 0.80-2.10 (m, 21H), 2.32 (s, 3H), 2.37 (dd, J_1 = 8.7 Hz, J_2 = 18.2 Hz, 1H), 3.13 (s, 2H), 4.34 (s, 2H), 7.19 (s, 4H); LRMS for $C_{29}H_{39}NO_3Na$ $[M+Na]^+$ 472.2.

4.1.6.3. (3*R*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*)-3′-(4-Methoxybenzyl)-10,13-dimethyltetradecahydro-2′*H*-spiro[cyclopenta[α]phenanthrene-3,5′-[1,3]oxazolidine]-2′,17(2*H*)-dione (17c). 18 mg, 16%; ^{1}H NMR (acetone-d_6) δ 0.84 (s, 3H), 0.86 (s, 3H), 0.80-2.10 (m, 22H), 2.37 (dd, J_1 = 8.7 Hz, J_2 = 18.2 Hz, 1H), 3.12 (s, 2H), 3.79 (s, 3H), 4.31 (s, 2H), 6.93 (d, J = 8.7 Hz, 2H), 7.23 (d, J = 8.7 Hz, 2H); LRMS for $C_{29}H_{40}NO_4Na$ $[M+Na]^+$ 488.3.

4.1.6.4. (3*R*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*)-10,13-Dimethyl-3′-[4-(trifluoromethyl)benzyl]tetradecahydro-2′*H*-spiro[cyclopenta[α]phenanthrene-3,5′-[1,3]oxazolidine]-2′,17(2*H*)-dione (17d). 55 mg, 43%; ^{1}H NMR (acetone-d_6) δ 0.84 (s, 3H), 0.87 (s, 3H), 0.80-2.10 (m, 21H), 2.38 (dd, J_1 = 8.8 Hz, J_2 = 18.2 Hz, 1H), 3.23 (s, 2H), 4.51 (s, 2H), 7.56 (d, J = 8.0 Hz, 2H), 7.74 (d, J = 8.1 Hz, 2H); LRMS for $C_{29}H_{36}F_3NO_3Na$ $[M+Na]^+$ 526.1.

4.1.6.5. (3*R*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*)-10,13-Dimethyl-3′-{[(6-trifluoromethyl)pyridine-3-yl]methyl} tetradecahydro-2′*H*-spiro-[cyclopenta[α]phenanthrene-3,5′-[1,3]oxazolidine]-2′,17(2*H*)-dione (17e). 18 mg, 14%; ^{1}H NMR (acetone-d_6) δ 0.84 (s, 3H), 0.87 (s, 3H), 0.80-2.10 (m, 21H), 2.38 (dd, J_1 = 8.8 Hz, J_2 = 18.2 Hz, 1H), 3.30 (s, 2H), 4.58 (s, 2H), 7.87 (d, J = 8.0 Hz, 1H), 8.03 (d, J = 8.0 Hz, 1H), 8.73 (s, 1H); LRMS for $C_{28}H_{35}F_3N_2O_3Na$ $[M+Na]^+$ 527.4.

4.1.6.6. (3*R*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*)-10,13-Dimethyl-3′-[2-(trifluoromethyl)benzyl]tetradecahydro-2′*H*-spiro[cyclopenta[α]phenanthrene-3,5′-[1,3]oxazolidine]-2′,17(2*H*)-dione (17f). 62 mg, 49%; ^{1}H NMR (acetone-d_6) δ 0.84 (s, 3H), 0.88 (s, 3H), 0.82-2.10 (m, 21H), 2.38 (dd, J_1 = 8.7 Hz, J_2 = 18.3 Hz, 1H), 3.23 (s, 2H), 4.61 (s, 2H), 7.57 (m, 2H), 7.74 (m, 2H); LRMS for $C_{29}H_{36}F_3NO_3Na$ $[M+Na]^+$ 526.4.

4.1.6.7. (3*R*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*)-10,13-Dimethyl-3′-[3-(trifluoromethyl)benzyl]tetradecahydro-2′*H*-spiro[cyclopenta[α]phenanthrene-3,5′-[1,3]oxazolidine]-2′,17(2*H*)-dione (17g). 30 mg, 24%; ^{1}H NMR (acetone-d_6) δ 0.84 (s, 3H), 0.87 (s, 3H), 0.82-2.10 (m, 21H), 2.38 (dd, J_1 = 8.7 Hz, J_2 = 18.2 Hz, 1H), 3.23 (s, 2H), 4.52 (s, 2H), 7.65 (m, 4H); LRMS for $C_{29}H_{36}F_3NO_3Na$ $[M+Na]^+$ 526.0.

4.1.6.8. (3*R*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*)-3′-[3,5-Bis(trifluoromethyl)-benzyl]-10,13-dimethyltetradecahydro-2′*H*-spiro[cyclopenta[α]-phenanthrene-3,5′-[1,3]oxazolidine]-2′,17(2*H*)-dione (17h). 28 mg, 20%; ^{1}H NMR (acetone-d_6) δ 0.84 (s, 3H), 0.87 (s, 3H), 0.81-2.10 (m, 21H), 2.38 (dd, J_1 = 8.7 Hz, J_2 = 17.7 Hz, 1H), 3.31 (s, 2H), 4.64 (s, 2H), 8.00 (s, 3H); LRMS for $C_{30}H_{35}F_6NO_3Na$ $[M+Na]^+$ 594.2.

4.1.6.9. (3*R*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*)-3′-Cyclohexyl-10,13-dimethyltetradecahydro-2′*H*-spiro [cyclopenta[α]phenanthrene-3,5′-[1,3] oxazolidine]-2′,17(2*H*)-dione (17i). 19 mg, 18%; ^{1}H NMR (acetone-d_6) δ 0.84 (s, 3H), 0.88 (s, 3H), 0.80-2.08 (m, 31H), 2.38 (dd, J_1 = 8.8 Hz, J_2 = 18.3 Hz, 1H), 3.23 (s, 2H), 3.53 (m, 1H); LRMS for $C_{27}H_{41}NO_3Na$ $[M+Na]^+$ 450.4.

4.1.7. Synthesis of (3*R*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*)-10,13,16,16-tetramethyl-3′-(4-methylbenzyl) tetradecahydro-2′*H*-spiro[cyclopenta[α]phenanthrene-3,5′-[1,3]oxazolidine]-2′,17(2*H*)-dione (18)

To a solution of compound **17b** (100 mg, 0.22 mmol) in anhydrous THF (15 mL) under an argon atmosphere was added NaH 60% in oil (89 mg, 2.2 mmol). The solution was stirred at room temperature for 1 h. Methyl iodide (110 μL, 250 mg, 1.76 mmol) was then added and the solution was refluxed overnight. The resulting solution was poured into water and extracted three times with EtOAc, washed with brine and dried with $MgSO_4$. The crude compound was purified by flash chromatography using EtOAc/hexanes (2:8) to give compound **18** (32 mg, 30%). ^{1}H NMR ($CDCl_3$) δ 0.77 (s, 3H), 0.87 (s, 3H), 1.03 (s, 3H), 1.16 (s, 3H), 0.80-1.85 (m, 20H), 2.34 (s, 3H), 3.03 (s, 2H), 4.37 (s, 2H), 7.15 (s, 4H); LRMS for $C_{31}H_{43}NO_3$-Na $[M+Na]^+$ 500.2.

4.1.8. Synthesis of (3α,5α)-3-{[(2{[*tert*-butyl(dimethyl)silyl]-oxy}ethyl)amino]methyl}-3-hydroxyandrostan-17-one (19)

To a solution of oxirane **6**[31] (0.96g, 3.18 mmol) in ethanol (50 ml) was added 2-{[*tert*-butyl(dimethyl)silyl] oxy}ethanamine (1.75 g, 10.0 mmol) and the mixture was refluxed for 5 h. The resulting solution was then evaporated to dryness and purified by flash chromatography using EtOAc/hexanes (2:8) to give compound **19** (1.0 g, 66%). ^{1}H NMR (acetone-d_6) δ 0.08 (s, 6H), 0.81 (s, 6H), 0.91 (s, 9H), 0.80-2.10 (m, 20H), 2.22 and 2.38 (2 m, 2H), 2.48 (s, 2H), 2.72 (t, J = 5.5 Hz, 2H), 3.25 (m, 1H), 3.72 (t, J = 5.5 Hz, 2H), 3.80 (t, J = 6.0 Hz, 1H); LRMS for $C_{28}H_{52}NO_3Si$ $[M+H]^+$ 478.2.

4.1.9. Synthesis of (3*R*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*)-3′(2-{[*tert*-butyl-(dimethyl)silyl]oxy}ethyl)-10,13-dimethyltetradecahydro-2′*H*-spiro[cyclopenta[α]phenanthrene-3,5′-[1,3]oxazolidine]-2′,17(2*H*)-dione (20)

To a solution of compound **19** (1.0 g, 2.21 mmol) in anhydrous dichloromethane (100 mL) at 0 °C under an argon atmosphere was added diisopropylamine (760 μL, 564 mg, 4.4 mmol) and triphosgene (325 mg, 1.1 mmol). The solution was stirred for 8 h at room temperature. The resulting solution was poured into water, extracted two times with dichloromethane and dried over a phase separator syringe (Biotage) and finally evaporated to dryness. The crude compound was purified by flash chromatography using EtOAc/hexanes (2:8) to give compound **20** (370 mg, 36%). ^{1}H NMR (acetone-d_6) δ 0.09 (s, 6H), 0.85 (s, 3H), 0.89 (s, 3H), 0.91 (s, 9H), 0.80-2.07 (m, 22H), 2.38 (dd, J_1 = 8.6 Hz, J_2 = 18.3 Hz, 1H), 3.30 (t, J = 5.3 Hz, 2H), 3.40 (s, 1H), 3.78 (t, J = 5.4 Hz, 2H); LRMS for $C_{29}H_{49}NO_4SiNa$ $[M+Na]^+$ 526.5.

4.1.10. Synthesis of (3*R*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*)-3′-(2-hydroxyethyl)-10,13-dimethyltetradecahydro-2′*H*-spiro [cyclopenta[α]phenanthrene-3,5′-[1,3]oxazolidine]-2′,17(2*H*)-dione (21)

To a solution of compound **20** (360 mg, 0.71 mmol) in anhydrous THF (70 mL) was added a solution (1.0 M) of tetrabutylammonium fluoride (TBAF) in THF (1.05 mL, 1.05 mmol) at room temperature under an argon atmosphere. The solution was stirred at room temperature for 1 h. The resulting mixture was poured into water, extracted two times with EtOAc and dried with Na_2SO_4, filtered and finally evaporated to dryness. The crude compound was purified by flash chromatography using EtOAc to give compound **21** (225 mg, 81%). ^{1}H NMR (acetone-d_6) δ 0.85 (s, 3H), 0.89 (s, 3H), 0.80-2.08 (m, 23H), 2.37 (dd, J_1 = 8.6 Hz, J_2 = 18.3 Hz, 1H), 3.29 (t, J = 5.3 Hz, 2H), 3.40 (s, 1H), 3.67 (t, J = 5.6 Hz, 2H); LRMS for $C_{23}H_{35}NO_4Na$ $[M+Na]^+$ 412.2.

4.1.11. Synthesis of (3*R*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*)-3′-(2-bromoethyl)-10,13-dimethyltetradecahydro-2′*H*-spiro[cyclopenta[α] phenanthrene-3,5′-[1,3]oxazolidine]-2′,17(2*H*)-dione (22)

To a solution of compound **21** (225 mg, 0.58 mmol) in anhydrous dichloromethane (50 mL) was added triphenylphosphine (303 mg, 1.16 mmol) and carbon tetrabromide (383 mg, 1.16 mmol) at 0 °C under an atmosphere of argon. The mixture was stirred for 1 h at room temperature. The resulting solution was washed with water, and the organic phase was dried using a phase separator syringe (Biotage). The crude compound was purified by flash chromatography using EtOAc/hexanes (4:6) to give compound **22**. 235 mg, 90%; ^{1}H NMR (acetone-d_6) δ 0.85 (s, 3H), 0.89 (s, 3H), 0.82-2.08 (m, 21H), 2.38 (dd, J_1 = 8.6 Hz, J_2 = 18.3 Hz, 1H), 3.41 (s, 2H), 3.63 (s, 4H); LRMS for $C_{23}H_{34}{}^{79}BrNO_3Na$ $[M+Na]^+$ 474.1.

4.1.12. Synthesis of (3*R*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*)-10,13-dimethyl-3′-[2-(3-methylphenoxy) ethyl]tetradecahydro-2′*H*-spiro [cyclopenta[α]phenanthrene-3,5′-[1,3]oxazolidine]-2′,17(2*H*)-dione (23a)

To a solution 3-methylphenol (22 μL, 22 mg, 0.200 mmol) and K_2CO_3 (28 mg, 0.200 mmol) in anhydrous DMF (2 mL) previously stirred 10 min at 70 °C was added compound **22** (30 mg, 0.067 mmol). The mixture was stirred overnight at 70 °C under an argon atmosphere. The resulting solution was poured into a solution of NaOH 1.0 N and extracted three times with diethylether. The combined organic layer was successively washed with brine, dried over Na_2SO_4, filtered, and evaporated to dryness under vacuum. The resulting crude compound was purified by flash chromatography using EtOAc/hexanes (2:8) to give compound **23a**. 11 mg, 34%; ^{1}H NMR (acetone-d_6) δ 0.84 (s, 3H), 0.88 (s, 3H), 0.80-2.07 (m, 21H), 2.29 (s, 3H), 2.37 (dd, J_1 = 8.7 Hz, J_2 = 18.8 Hz, 1H), 3.45 (s, 2H), 3.59 (m, 2H), 4.15 (t, J = 5.3 Hz, 2H), 6.77 (m, 3H), 7.16 (t, J = 7.8 Hz, 1H); LRMS for $C_{30}H_{41}NO_4Na$ $[M+Na]^+$ 502.4.

4.1.13. Synthesis of (3*R*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*)-10,13-dimethyl-3′-{2-(3-methylphenyl) sulfonyl]ethyl}tetradecahydro-2′*H*-spiro[cyclopenta[α]phenanthrene-3,5′-[1,3]oxazolidine]-2′,17 (2*H*)-dione (23b)

To a solution 3-methylbenzenthiol (41 mg, 0.33 mmol) and K_2CO_3 (46 mg, 0.33 mmol) in anhydrous DMF (3 mL) first stirred 10 min at 70 °C was added compound **22** (50 mg, 0.11 mmol). The mixture was stirred 3 h at 70 °C under an argon atmosphere. The resulting solution was poured into water and extracted three times with diethylether. The combined organic layer was successively washed with brine, dried over Na_2SO_4, filtered, and evaporated to dryness under vacuum to give the corresponding thioether derivatives as crude compound. The crude thioether compound (45 mg) was then diluted in methanol/water (1:1) and oxone (112 mg) was added. The solution was stirred at room temperature overnight. The resulting solution was poured into water, extracted three times with EtOAc and the combined organic layer was successively washed with brine and dried over Na_2SO_4, filtered, and evaporated to dryness under vacuum. The resulting crude compound was purified by flash chromatography using EtOAc/hexanes (1:1) to give the corresponding sulfone **23b** (9 mg, 16% for two steps); ^{1}H NMR (acetone-d_6) δ 0.84 (s, 3H), 0.86 (s, 3H), 0.80-2.07 (m, 21H), 2.38 (dd, J_1 = 8.7 Hz, J_2 = 8.3 Hz, 1H), 2.46 (s, 3H), 3.30 (s, 2H), 3.50 (t, J = 6.6 Hz, 2H), 3.61 (m, 2H), 7.57 (m, 2H), 7.77 (m, 2H); LRMS for $C_{30}H_{41}NO_5SNa$ $[M+Na]^+$ 550.2.

4.1.14. General procedure for the synthesis of 3-carbamate-androsterone derivatives 23c–f

To a solution of compound **22** (30 mg, 0.067 mmol) in anhydrous DMF (2 mL) was added the appropriate secondary amine (0.20 mmol) and sodium carbonate (21 mg, 0.20 mmol). The mixture was stirred at 70 °C for 3 h and the resulting solution was poured into water and extracted three times with diethylether. The combined organic layer was successively washed with brine, dried over Na_2SO_4, filtered and evaporated to dryness under vacuum. The resulting crude compound was purified by flash chromatography using either dichloromethane/methanol/triethylamine (90:9:1) (**23c**, **23e** and **23f**) or EtOAc/hexanes 1:1 (**23d**) to give the corresponding compounds **23c**–**23f**.

4.1.14.1. (3*R*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*)-10,13-Dimethyl-3′-[2-(piperidin-1-yl)ethyl]tetradecahydro-2′*H*-spiro[cyclopenta[α]phenanthrene-3,5′-[1,3]oxazolidine]-2′,17(2*H*)-dione (23c).

7 mg, 22%; ^{1}H NMR (acetone-d_6) δ 0.84 (s, 3H), 0.89 (s, 3H), 0.80-2.08 (m, 24H), 2.26 (m, 2H), 2.38 (dd, J_1 = 8.8 Hz, J_2 = 18.3 Hz, 1H), 3.18 (m, 4H), 3.42 (s, 2H), 3.46 (t, J = 6.5 Hz, 2H), 3.80 (t, J = 6.5 Hz, 2H); LRMS for $C_{28}H_{47}N_3O_3$ $[M+NH_4]^+$ 474.2.

4.1.14.2. (3*R*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*)-3′-{2-[Ethyl(phenyl)amino]ethyl}-10,13-dimethyltetrahydrodecahydro-2′*H*-spiro[cyclopenta[α]phenanthrene-3,5′-[1,3]oxazolidine]-2′,17(2*H*)-dione (23d).

16 mg, 47%; ^{1}H NMR (acetone-d_6) δ 0.85 (s, 3H), 0.88 (s, 3H), 1.03 (t, J = 7.1 Hz, 3H), 0.82-2.07 (m, 22H), 2.38 (dd, J_1 = 8.7 Hz, J_2 = 18.3 Hz, 1H), 2.53 (q, J = 7.1 Hz, 1H), 2.60 (t, J = 6.1 Hz, 2H), 3.28 (s, 2H), 3.32 (m, 2H), 3.59 (s, 2H), 7.23 (t, J = 7.2 Hz, 1H), 7.30 (t, J = 7.3 Hz, 2H), 7.36 (t, J = 7.3 Hz, 2H); LRMS for $C_{32}H_{46}N_2O_3Na$ $[M+Na]^+$ 529.4.

4.1.14.3. (3*R*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*)-3′-{2-[Cyclohexyl(ethyl)amino]ethyl}-10,13-dimethyltetradecahydro-2′*H*-spiro[cyclopenta[α]phenanthrene-3,5′-[1,3]oxazolidine]-2′,17(2*H*)-dione (23e).

13 mg, 39%; ^{1}H NMR ($CDCl_3$) δ 0.81 (s, 3H), 0.86 (s, 3H), 1.35 (t, J = 7.2 Hz, 3H), 0.78-2.10 (m, 29H), 2.23 (br dd, J_1 = 2.7 Hz, J_2 = 11.8 Hz, 1H), 2.35 (br d, J = 11.9 Hz, 1H), 2.43 (dd, J_1 = 8.6 Hz, J_2 = 19.3 Hz, 1H), 3.17 (br t, J = 11.5 Hz, 1H), 3.35 (m, 6H), 3.80 (m, 2H); LRMS for $C_{31}H_{54}N_3O_3$ $[M+NH_4]^+$ 516.4.

4.1.14.4. (3*R*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*)-3′-[2-(Dipropylamino)ethyl]-10,13-dimethyltetradecahydro-2′*H*-spiro[cyclopenta[α]phenanthrene-3,5′-[1,3]oxazolidine]-2′,17(2*H*)-dione (23f).

13 mg, 40%; ^{1}H NMR (acetone-d_6) δ 0.84 (s, 3H), 0.89 (s, 3H), 0.94 (t, J = 7.4 Hz, 3H), 0.78-2.10 (m, 28H), 2.38 (dd, J_1 = 8.7 Hz, J_2 = 18.2 Hz, 1H), 3.23 (m, 4H), 3.42 (s, 2H), 3.48 (t, J = 6.6 Hz, 2H), 3.74 (t, J = 6.9 Hz, 2H); LRMS for $C_{29}H_{52}N_3O_3$ $[M+NH_4]^+$ 490.5.

4.1.15. Synthesis of *N*-{(3*R*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*)-10,13-dimethyl-2′,17-dioxohexadecahydro-3′*H*-spiro[cyclopenta[α]phenanthrene-3,5′-[1,3]oxazolidine-3′-yl]ethyl}-*N*-propylpropanamide (23g)

To a solution of compound **22** (30 mg, 0.067 mmol) in anhydrous DMF (3 mL) was added K_2CO_3 (21 mg, 0.20 mmol) and *N*-propylamine (16 μL, 12 mg, 0.20 mmol). The mixture was heated at 80 °C for 5 h and then poured into water and extracted with diethylether. The combined organic layer was successively washed with brine, dried over Na_2SO_4, filtered, and evaporated to dryness under vacuum. The resulting secondary amine was used directly without further purification for the next steps. To the crude compound (30 mg) in anhydrous dichloromethane (3 mL) was added triethylamine (27 μL, 21 mg) and propionyl chloride (13 μL, 13 mg). The solution was stirred at room temperature for 4 h under an atmosphere of argon. The resulting solution was diluted with dichloromethane (15 mL), washed with water, dried using a phase separator syringe (Biotage), and evaporated to dryness. The resulting crude compound was purified by flash chromatography using EtOAc/hexanes (1:1) to give the compound **23g** (11 mg, 34%); ^{1}H NMR (acetone-d_6) δ 0.84 (s, 3H), 0.88 (s, 3H), 0.91 (t, J = 7.4 Hz, 3H), 1.04 (t, J = 7.4 Hz, 3H), 0.82-2.07

(m, 23H), 2.34 (m, 3H), 3.32 (m, 6H), 3.53 (m, 2H); LRMS for $C_{29}H_{46}N_2O_4Na$ $[M+Na]^+$ 509.1.

4.2. Biological assays

4.2.1. Inhibition of 17β-HSD3 in homogenized cells

The inhibitory activity of each compound was determined following a procedure described in our previous published articles.[29] The HEK-293 cells overexpressing 17β-HSD3 were briefly homogenized and used for the enzymatic assay. The transformation of 50 nM of [4-^{14}C]-4-androstene-3,17-dione ([^{14}C]-Δ^4-dione) into [^{14}C]-testosterone ([^{14}C]-T) in presence of NADPH in excess (5 mM) and ethanol only (control) or an inhibitor dissolved in ethanol was measured and used to determine the percentage of inhibition at a given concentration.

4.2.2. Inhibition of 17β-HSD-3 in intact cells

HEK-293 cells were seeded at 200,000 cells/well in a 12-well plate (BD Falcon) at 37 °C under 95% air 5% CO_2 humidified atmosphere in minimum essential medium (MEM) containing non-essential amino acids (0.1 mM), glutamine (2 mM), sodium pyruvate (1 mM), 10% foetal bovine serum, penicillin (100 IU/mL) and streptomycin (100 μg/mL). The expression vector encoding for 17β-HSD3 was transfected using the Exgen 500 procedure (Fermentas, Burlington, ON, Canada) with 2 μg of recombinant plasmid per well. For the inhibitory activity assay, a final concentration of 50 nM of [4-^{14}C]-4-androstene-3,17-dione in ethanol (53.6 mCi/mmol, Perkin Elmer Life Sciences Inc., Boston, MA, USA) and an ethanolic solution of inhibitor (0.5% v/v) were added to freshly cultured medium and incubated for 1 h. Each inhibitor was assessed in triplicate at tested concentrations. After incubation, culture medium was removed and radiolabeled steroids were extracted and quantified as described for the enzymatic assay in homogenized cells.[29] Percentage of transformation and then the IC_{50} value was calculated as previously reported.[29]

4.2.3. Proliferative activity on androgen-sensitive (AR$^+$) Shionogi cells

The proliferative (androgenic) activity was determined on androgen-sensitive Shionogi cells following a previously published[35] procedure that is well established in our laboratory. Inhibitors and reference compounds were tested at two concentrations of 0.1 and 1.0 μM. The androgenicity was reported as % of cell proliferation (versus the control). The basal cell proliferation (control) was fixed at 100%.

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Supplementary data

Supplementary data (^{1}H NMR spectrum of **15b** and assignment of protons, ^{13}C NMR (APT) spectrum of **15b** and assignment of carbons, and 2D NMR (COSY, TOCSY, NOESY, HSQC and HMBC) spectra of **15b**, the best 17β-HSD3 inhibitor resulting from our SAR study) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.06.003.

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