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Synthesis of 3-spiromorpholinone androsterone derivatives as inhibitors of 17β-hydroxysteroid dehydrogenase type 3

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ABSTRACT

Spiromorpholinone derivatives were synthesized from androsterone or cyclohexanone in 6 or 3 steps, respectively, and these scaffolds were used for the introduction of a hydrophobic group via a nucleophilic substitution. Non-steroidal spiromorpholinones are not active as inhibitors of 17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3), but steroidal morpholinones are very potent inhibitors. In fact, those with (*S*) stereochemistry are more active than their (*R*) homologues, whereas N-benzylated compounds are more active than their non substituted precursors. The target compounds exhibited strong inhibition of 17β-HSD3 in rat testis homogenate (87–92% inhibition at 1 μM).

The role of 17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3) in androgen-dependent prostate cancer is well established.[1–3] This enzyme converts 4-androstene-3,17-dione (Δ^4-dione) into the androgenic hormone testosterone (T) in the presence of cofactor NADPH.[4–6] Even though 17β-HSD3 is almost exclusively expressed in testes, it is up-regulated in prostate tumors.[7] In the classic pathway, T is further converted into the most active androgen dihydrotestosterone (DHT) by 5α-reductase (Fig. 1). In fact, both T and DHT can activate the androgen receptor (AR) and, consequently, stimulate the proliferation of prostate cancer cells. To stop the androgen biosynthesis at the level of Δ^4-dione, a steroid inactive on AR,[8] an inhibitor of 17β-HSD3 could be used. Since this membrane enzyme was not yet crystallized, structure–activity relationships (SAR) must be established for inhibitor development. From our previous laboratory work, it was established that the presence of a hydrophobic group at position C-3 of androsterone (ADT) is a good strategy for designing potent inhibitors of 17β-HSD3.[9–13]

In order to develop novel inhibitors of 17β-HSD3 (Scheme 1), we decided to build a new ring system (cycle E) at position 3 of the ADT nucleus. In fact, introducing a 3-spiroheterocyclic moiety is a good strategy for adding rigidity and introducing diversified hydrophobic groups with several orientations. It is thus expected that such groups can increase the affinity to 17β-HSD3 hydrophobic pocket.

The chemical steps involved in the synthesis of target compounds **6A**, **6B**, **8A** and **8B** are shown in Scheme 1A. Steroidal oxirane **3** was synthesized following three steps as previously reported.[11,12] Briefly, the C-17 ketone of ADT (**1**) was protected as a dioxolane, the C-3 alcohol function of **2** was then oxidized in the presence of tetrapropylammonium perruthenate (TPAP) and *N*-methylmorpholine-N-oxide (NMO), and this ketone reacted regioselectively with trimethylsulfoxonium iodide to generate the oxirane **3**. This compound was subjected to aminolysis with (L) or (D) phenylalanine methyl ester to yield the amino-alcohol **4A** or **4B**. These two amino-alcohols were subjected to lactonization using sodium methoxide in THF at room temperature and the spiromorpholinones **5A** and **5B** were obtained. For this step, we adapted and optimized a method previously reported for position C-17 of the steroid.[14] In place of sodium hydride, low loading of sodium methoxide (0.6 equiv) was used in a diluted reaction mixture to avoid racemization of the alpha-hydrogen of the amino acid ester as well as the formation of side products. The secondary amines **5A** and **5B** were benzylated following a nucleophilic substitution to give **7A** and **7B**. The hydrolysis of C-17-dioxolanes **5A**, **5B**, **7A** and **7B** in dioxane and aqueous 5% sulfuric acid generated the target products **6A**, **6B**, **8A** and **8B**.

In order to verify the importance of the steroid scaffold for the inhibition of 17β-HSD3, non-steroidal spiromorpholinones **11** and **12** were synthesized starting from cyclohexanone and following the sequence of reactions reported in Scheme 1B. We selected only non-steroidal (S) isomers based on our preliminary results showing that the (*S*) spiromorpholinone isomer of steroidal derivatives

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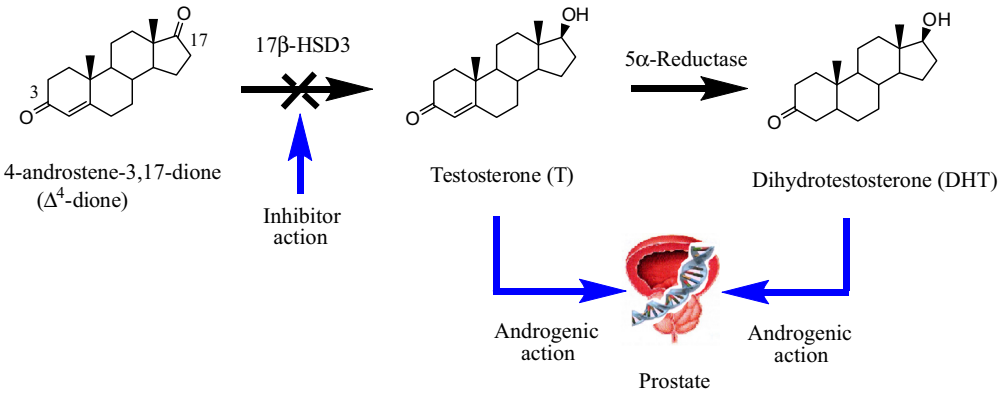


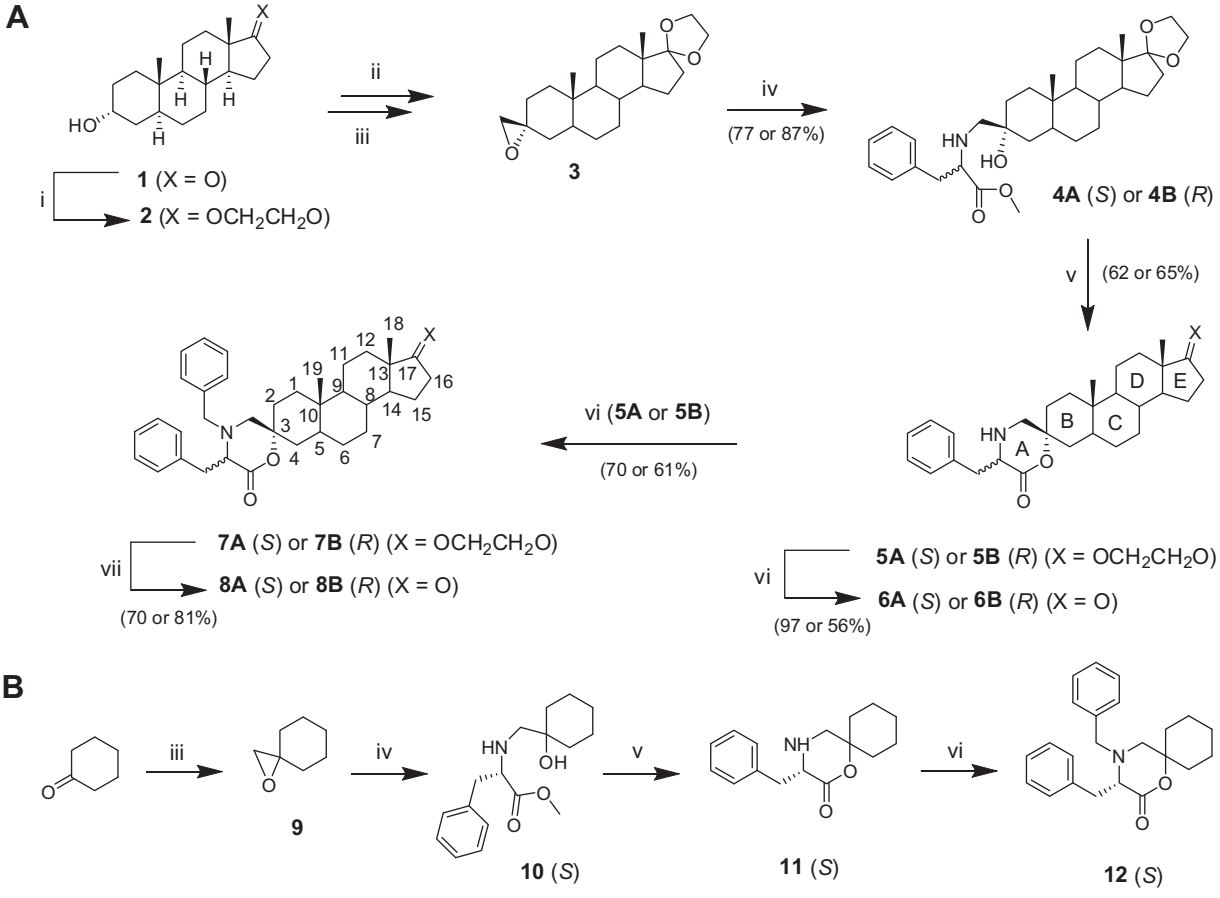
Figure 1. Blocking the biosynthesis of testosterone and dihydrotestosterone by using an inhibitor of 17β-HSD3.

produced a better 17β-HSD3 inhibitory activity than the (*R*) isomer. Compounds **11** and **12** were thus obtained with 76% and 61% global yields, in three and four steps, respectively.

All final steroidal and non-steroidal compounds as well as their intermediates were fully characterized (IR, ^{1}H NMR, ^{13}C NMR and LRMS) to confirm their chemical structure. To illustrate this, we reported the data from the steroidal and non-steroidal spiromorpholinones **6A**, **8A** and **12**.[15–17]

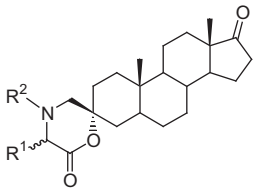
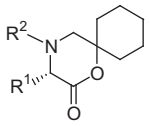
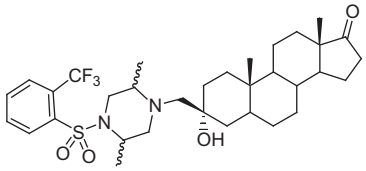
The inhibitory activity of compounds **6A**, **6B**, **8A**, **8B**, **11** and **12** on 17β-HSD3 was evaluated in a microsomal fraction of rat testes using a known procedure.[18] These compounds were compared to RM-532–105, a known inhibitor of 17β-HSD3,[12] for their ability to inhibit the transformation of Δ^4-dione into T (Table 1). Non-steroidal spiromorpholinones **11** and **12** are not active, suggesting that the steroidal nucleus is essential for inhibitory activity, but steroidal morpholinones produced a very good inhibition of 17β-HSD3. Compounds with (*S*) conformation on the carbon bearing a benzyl (Bn) showed better inhibition than their (*R*) homologues. In fact, compound **6A** blocked 48.8% of the transformation of Δ^4-dione at 0.1 μM and consequently is a better inhibitor than compound **6B** (18.5%). Similarly, compound **8A** (58.2% inhibition at 0.1 μM) is more potent than its stereoisomer **8B** (25.6%). In this screening assay, the inhibitory activity of compound **8A** (58.2%)seems comparable to that of the reference compound RM-532-105 (47.3% at 0.1 μM).

For comparison purposes, we next tested the two steroidal (S)-spiromorpholinone derivatives **6A** and **8A** with the know



Scheme 1. Synthesis of spiromorpholinone derivatives. Reagents and conditions: (i) $HOCH_2CH_2OH$, *p*-TSA, toluene, reflux; (ii) NMO, molecular sieves, TPAP, DCM, rt, 3 h; (iii) $(CH_3)_3SOI$, NaH, DMSO/THF, rt; (iv) (L) or (D)-phenylalanine methyl ester, MeOH, 90 °C; (v) CH_3ONa, THF, rt; (vi) DIPEA, $C_6H_5CH_2Br$, DCM, 75 °C, 22 h; (vii) H_2O/H_2SO_4, dioxane, rt.

Table 1
Inhibitory activity toward 17β-HSD3 of the target compounds **6A**, **6B**, **8A**, **8B**, **11** and **12**

Structures	Names	*R*/*S*	R^1	R^2	Inhibition at 0.1 μM[a] (%)	Inhibition at 1 μM[a] (%)	Inhibition IC_{50}[b] (nM)
	6A	*S*	Bn	H	48.8 ± 2.0	92.0 ± 1.6	22
	6B	R	Bn	H	18.5 ± 26.9	63.2 ± 5.8[c]	—
	8A	S	Bn	Bn	58.2 ± 1.7	90.4 ± 0.7	58
	8B	R	Bn	Bn	25.6 ± 5.7	87.3 ± 2.8	—
	11	*S*	Bn	H	6.2 ± 6.3	19.5 ± 3.8	—
	12	S	Bn	Bn	24.2 ± 3.2	24.3 ± 4.2	—
	RM-532-105[d]	—	—	—	47.3 ± 12.4	92.1 ± 0.4	14

[a] Transformation of [^{14}C]-4-androstene-3,17-dione (50 nM) into testosterone by 17β-HSD3 in microsomal fraction of rat testes. Results are expressed as mean ± SD of triplicate.
[b] IC_{50} values were determined from the inhibition curves reported in Figure 2 using GraphPad-Prism 6 software (GraphPad Inc Solfware).
[c] This inhibition value was obtained from another experiment performed under the same conditions.
[d] Compound **15b** in Ref. 12.

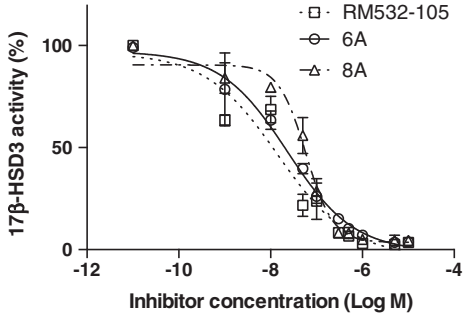


Figure 2. Effect of **6A**, **8A** and RM-532-105 on the transformation of [^{14}C] Δ^4-dione (50 nM) into [^{14}C]-T by 17β-HSD3. Results are expressed as mean ± SD of triplicate.

17β-HSD3 inhibitor RM-532-105 (Fig. 2). Spiromorpholinones **6A** and **8A** (IC_{50} = 22 and 58 nM, respectively) are about twofold and fourfold less potent than RM-532-105 (IC_{50} = 14 nM) (Table 1). These results confirm the potency of **6A** and **8A** as new lead compounds for inhibiting 17β-HSD3. Thus, we are confident that such 3-spiromorpholinone ADT derivatives can be improved by judicious diversification of the spirocycle as well as localization of the hydrophobic group. Further work reporting full details of the diversification of the 3-spiromorpholinone, including chemistry, SAR study and biological activity of the target compounds is underway and will be presented in a full paper in due course.

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15. *Data for compound **6A**:* White foam. IR (film, *v*, cm^{-1}) 3333 (NH), 3032 (CH, Ph), 1736 (C=O, ketone and lactone). ^{1}H NMR −400 MHz (Acetone-d_6, δ, ppm) 0.81 (s, CH_3-19), 0.84 (s, CH_3-18), 0.80–2.00 (unassigned CH and CH_2), 2.37 (dd, J_1 = 8.7 Hz and J_2 = 18.2 Hz, CH-16β), 2.79 and 2.89 (2d of AB system, J = 13.3 Hz, CH_2N), 3.02 and 3.17 (2 m, CHC$\underline{H}_2$Ph), 3.73 (dd, J_1 = 4.0 Hz and J_2 = 8.1 Hz, NCHCO), 7.25 (m, Ph). ^{13}C NMR −100 MHz ($CDCl_3$, δ, ppm) 11.3, 13.8, 20.2, 21.7, 27.8, 30.5, 31.0, 31.5, 32.83, 35.0, 35.8, 36.0, 38.0, 39.2, 47.8, 51.4, 52.6, 53.7, 53.8, 58.7, 82.7, 127.1, 128.8 (2C), 129.5 (2C), 137.2, 170.8, 221.3. LRMS (*m*/*z*) calcd. for $C_{29}H_{40}NO_3$ [M+H]$^+$ 450.29, found 450.35.
16. *Data for compound **8A**:* White foam. IR (film, *v*, cm^{-1}) 3063, 3032 (CH, Ph) and 1728 (C=O, ketone and lactone). ^{1}H NMR −400 MHz (Acetone-d_6, δ, ppm) 0.65 (s, CH_3-19), 0.80 (s, CH_3-18), 0.82–2.00 (unassigned CH and CH_2), 2.22 and 2.59 (2d of AB system, J = 12.5 Hz, CH_2N), 2.36 (dd, J_1 = 8.7 Hz and J_2 = 18.4 Hz, CH-16β), 3.27 and 4.40 (2d of AB system, J = 13.9 Hz, NC$\underline{H}_2$Ph), 3.37 (m, CHC$\underline{H}_2$Ph), 3.51 (dd, J_1 = 2.9 Hz and J_2 = 5.1 Hz, NCHCO), 7.31 (m, 2× Ph). ^{13}C NMR −100 MHz (Acetone-d_6, δ, ppm) 10.7, 13.1, 20.0, 21.4, 27.7, 30.6, 30.6, 31.6, 33.0, 34.9, 35.1, 35.2, 35.8, 37.9, 39.6, 47.3, 51.2, 54.1, 57.4, 57.7, 65.9, 80.5, 126.5, 127.1, 127.8 (2C), 128.4 (2C), 128.5 (2C), 130.4 (2C), 138.0, 138.1, 169.5, 218.7. LRMS (*m*/*z*) calcd. for $C_{36}H_{46}NO_3$ [M+H]$^+$ 540.34, found 540.40.
17. *Data for compound **12**:* Yellowish oil. IR (film, *v*, cm^{-1}) 3063, 3032 (CH, Ph) and 1720 (C=O, ketone and lactone). ^{1}H NMR −400 MHz ($CDCl_3$, δ, ppm) 0.87–1.74 (unassigned CH_2 of cyclohexyl), 2.08 and 2.65 (2d of AB system, J = 12.6 Hz, CH_2N), 3.18 and 4.31 (2d of AB system, J = 13.7 Hz, NC$\underline{H}_2$Ph), 3.26 and 3.54 (2 m, CHC$\underline{H}_2$Ph); 3.52 (m, NCHCO), 7.28 (m, 2× Ph). ^{13}C NMR −100 MHz ($CDCl_3$, δ, ppm) 21.2, 21.4, 25.3, 34.3, 35.7, 35.8, 56.0, 58.1, 66.3, 81.7, 126.7, 127.4, 128.0 (2C), 128.4 (2C), 128.5 (2C), 130.3 (2C), 137.5, 137.5, 171.0. LRMS (*m*/*z*) calcd. for $C_{23}H_{28}NO_2$ [M+H]$^+$ 350.20, found 350.20.
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