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Androsterone 3β-Substituted Derivatives as Inhibitors of Type 3 17β-Hydroxysteroid Dehydrogenase

Béatrice Tchédam Ngatcha,[a] Van Luu-The[b] and Donald Poirier[a,*]

[a]*Medicinal Chemistry Division, Oncology and Molecular Endocrinology Research Center, Laval University Medical Center (CHUL) and Laval University, 2705 Laurier Blvd., Québec, Canada G1V 4G2*

[b]*MRC Group in Molecular Endocrinology, Oncology and Molecular Endocrinology Research Center, Laval University Medical Center (CHUL) and Laval University, 2705 Laurier Blvd., Québec, Canada G1V 4G2*

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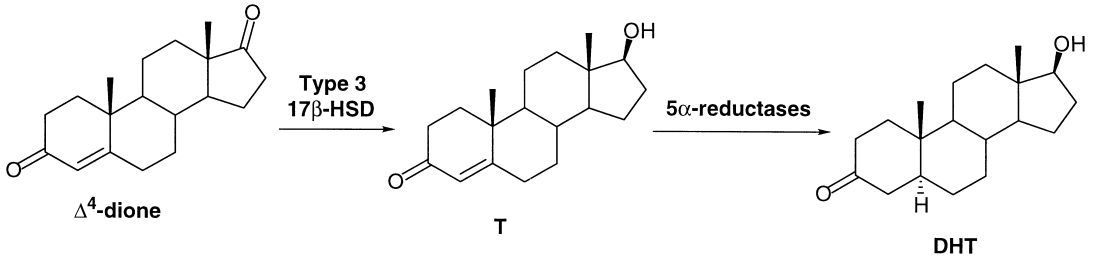
Abstract—Androsterone derivatives substituted at position 3 were synthesized starting from dihydrotestosterone in a short sequence of reactions. They proved to be potent inhibitors (IC_{50} = 57–147 nM) of type 3 17β-hydroxysteroid dehydrogenase, a key enzyme of steroidogenesis, which catalyzes the transformation of androstenedione to steroid active androgen testosterone.

The type 3 17β-hydroxysteroid dehydrogenase (type 3 17β-HSD), also called testicular 17β-HSD or androgenic 17β-HSD, is found principally in the microsomal fraction of the testis.[1] With NADPH as a cofactor, it catalyzes the reduction of 4-androstene-3,17-dione (Δ^4-dione) to testosterone (T),[2–4] which is further converted to dihydrotestosterone (DHT) by 5α-reductase (Scheme 1).[5] Thus, type 3 17β-HSD plays an important role in the formation of active androgens (T and DHT), which are both involved in the pathophysiology of various androgen-sensitive diseases, such as benign prostatic hyperplasia, prostate cancer,[6] acne,[7] hirsutism,[8] and male-pattern baldness.[9]

During our efforts to develop inhibitors of type 1 17β-HSD,[10–12] and type 2 17β-HSD,[13–15] we became interested in the development of type 3 17β-HSD inhibitors. In his evaluation of the ability of 20 steroidal compounds to inhibit the type 3 17β-HSD activity in microsomal preparations of canine testis, Pittaway suggested the requirement of a 17-keto group and a steroidal unaromatized A-ring to inhibit the enzyme.[16] More recently, a screening study with 80 steroids of different classes led us to consider the C19 steroid androsterone (ADT) as a potential starting nucleus to develop inhibitors of type 3 17β-HSD.[17] Based on our results obtained during the development of inhibitors of type 1 17β-HSD,[11,12] ADT derivatives substituted at position 16 were synthesized, but they turned out to be only weak inhibitors of type 3 17β-HSD.[18] We then decided to experiment with position 3 of the ADT nucleus to develop new inhibitors. We herein report the chemical synthesis of 3β-substituted ADT derivatives and their ability to inhibit the type 3 17β-HSD activity.



Scheme 1. Enzymatic steps involved in biosynthesis of testosterone (T) and dihydrotestosterone (DHT) from 4-androstene-3,17-dione (Δ^4-dione).

*Corresponding author. Tel.: +1-418-654-2296; fax: +1-418-654-2761; e-mail: donald.poirier@crchul.ulaval.ca

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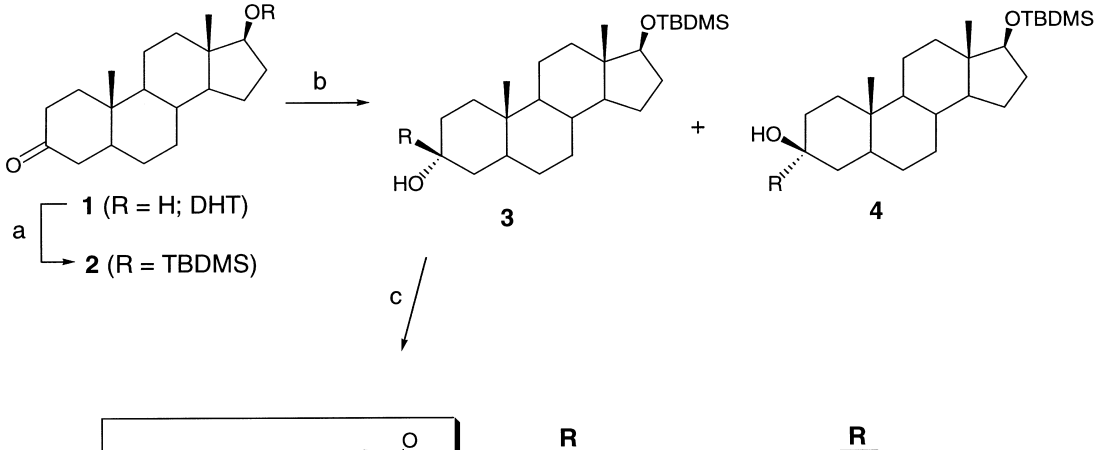
Chemistry

DHT was used as starting material for the synthesis of the target compounds **5**–**15** depicted in Scheme 2. The 17β-hydroxy group of DHT was first protected as a *tert*-butyldimethylsilyl ether, using TBDMS-Cl and imidazole in DMF to afford compound **2** in a 91% yield. The C3-carbonyl group of 17β-TBDMS-DHT (**2**) was then subjected to various alkylating reagents (Grignard or lithium reagents) to give a mixture of **3** and **4**. In most of the cases (methyl, *n*-propyl, *n*-hexyl, *n*-octyl, cyclohexyl, phenyl, and phenylmethyl derivatives), a commercially available Grignard reagent was used and the reaction was performed at 0 °C in dry THF. For cyclohexylmethyl and phenylethyl derivatives, the Grignard reagent was generated in situ, by a well known procedure described by Smith,[19] using a magnesium amalgam and the corresponding halide. For cyclohexylethyl derivative, the lithium reagent was generated in situ with *t*-BuLi and cyclohexylethyl bromide, in a mixture of diethylether:pentane (2:3), according to the procedure described by Bailey and Puzalan.[20] Finally, a commercially available lithium reagent was used for the synthesis of *s*-butyl derivative. Generally, a mixture of the two stereoisomers at position 3 was obtained, the proportions varying according to the nature of the alkyl group.[21] The two stereoisomers could be well differentiated on TLC, the 3β-substituted derivative always being the less polar one. Hence, a separation by flash chromatography was done, that separated the 3α-substituted stereoisomer from the 3β-substituted one and the sequence of reactions was continued with the latter. Less than 25% of starting material was also recovered in most cases. The TBDMS protective group of general compound **3** was then hydrolyzed with a 2% HCl methanolic solution at room temperature. The resulting 17β-alcohol was directly oxidized by Jones' reagent to afford the desired ADT 3β-substituted derivatives **5**–**15**.[22]

Inhibition of Type 3 17β-HSD

The inhibitory properties toward type 3 17β-HSD activity of target compounds **5**–**15** were evaluated in transfected HEK-293 cells by measuring the amount of labeled T formed from the natural labeled substrate Δ^4-dione in the presence of NADPH as cofactor. The enzymatic assay was performed as described[23] and the results are summarized in Table 1. The concentration of compound that produced 50% of inhibition (IC_{50} value) was determined from the inhibition curves. The ADT 3β-alkylated derivatives **5**–**15** were good inhibitors of type 3 17β-HSD; they all showed an inhibitory activity higher than that of Δ^4-dione, when this natural substrate of the enzyme was used as inhibitor itself. The compounds **5**–**15** were also stronger inhibitors than ADT, which had been determined as the strongest type 3 17β-HSD inhibitor from our screening study.[17] In the *n*-alkyl series, the best inhibitory activity was obtained for *n*-propyl derivative **6**. However, this activity fell as the longer 3β side chain was made longer (IC_{50} of 100 nM and 147 nM for **8** and **9**, respectively). This suggested a length limit for this kind of substituent. This led us to synthesize and test branched substituents, such as the *s*-butyl derivative **7**. With an IC_{50} value of 73 nM, compound **7** was as good an inhibitor as the other analogues in the *n*-alkyl series (**6** and **8**). To test a substituent with a well defined shape, we also evaluated cyclic derivatives. Cyclohexyl-ADT (**10**) and phenyl-ADT (**13**) gave almost the same inhibitory activity, with IC_{50} values of 97 and 81 nM, respectively. Adding a methylene

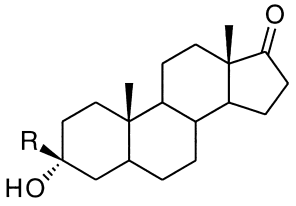


	R
5	CH_3
6	$CH_3(CH_2)_2$
7	$CH_3CH_2(CH_3)CH$
8	$CH_3(CH_2)_5$
9	$CH_3(CH_2)_7$

	R
10	Cyclohexyl
11	Cyclohexyl-CH_2
12	Cyclohexyl-CH_2CH_2
13	Ph
14	$PhCH_2$
15	$PhCH_2CH_2$

Scheme 2. Synthesis of ADT 3β-substituted derivatives **5**–**15**. Reagents: (a) TBDMS-Cl, imidazole DMF, rt; (b) i. RMgBr(Cl) or RLi, THF, 0 °C. ii. Flash chromatography; (c) i. MeOH–HCl (2%), rt. ii. Jones' reagent (2.7 M), acetone, 0 °C.

Table 1. Inhibition of type 3 17β-HSD by ADT 3β-substituted derivatives **5**–**15**



Compounds	R	Inhibition of type 3 17β-HSD (%)[a] 0.3 μM	3 μM	IC_{50} (nM)
Δ^4-Dione	3-Keto-4-ene	24	78	758±139
ADT	H	50	88	330±60
5	CH_3	72	93	nd[b]
6	$CH_3(CH_2)_2$	89	94	67±6
7	$CH_3CH_2(CH_3)CH$	85	90	73±5
8	$CH_3(CH_2)_5$	93	96	100±10
9	$CH_3(CH_2)_7$	88	92	147±29
10	Cyclohexyl	88	93	97±3
11	Cyclohexyl-CH_2	93	95	87±19
12	Cyclohexyl-CH_2CH_2	92	93	60±16
13	Ph	88	95	81±6
14	$PhCH_2$	90	94	57±5
15	Ph CH_2CH_2	93	93	99±1

[a]Error±10%.
[b]Not determined.

group resulted in a gain of inhibitory activity, which was more important in the case of phenyl: IC_{50} value of 87 nM for cyclohexylmethyl-ADT (**11**) and 57 nM for phenylmethyl-ADT (**14**). The addition of two methylene groups led to another gain of inhibitory activity in cyclohexyl series (IC_{50} value of 60 nM for cyclohexylethyl-ADT (**12**)), but led to a loss of inhibitory activity in the phenyl series (IC_{50} value of 99 nM for phenylethyl-ADT (**15**)). With an IC_{50} value of 57 nM, 3β-phenylmethyl-ADT (**14**) was the most potent type 3 17β-HSD inhibitor obtained in this study, it was 6-fold more powerful than ADT and 13-fold more powerful than Δ^4-dione, the natural substrate of the enzyme.

In an attempt to correlate hydrophobicity and inhibitory activity, the LogP values were calculated for all compounds.[24] This logarithm of partition coefficient between *n*-octanol and water expresses the relative hydrophobicity of a compound. Δ^4-Dione and ADT, which gave the lowest inhibitory activities of tested compounds, were less hydrophobic (LogP = 3.5 and 4.2, respectively) than inhibitors **5**–**15** (LogP ranging from 4.4 to 7.4), suggesting that hydrophobicity is required. On the other hand, compound **9**, which happened to be the most hydrophobic with a LogP value of 7.4, showed only moderate inhibitory activity (IC_{50} = 147 nM). Thus, the presence of a hydrophobic substituent in position 3 of ADT is important for good inhibition of type 3 17β-HSD, but a limitation was also observed for this hydrophobicity thus implicating important steric effects.

In conclusion, ADT 3β-substituted derivatives **5**–**15** were synthesized and found to inhibit the steroidogenic enzyme, type 3 17β-HSD. Interestingly, no inhibition of other reductive 17β-HSDs (type 1 and type 5) was observed at a 0.3 μM concentration of compounds **5**–**15** suggesting a specific inhibition. To the best of our knowledge, these ADT 3β-substituted derivatives constitute the first inhibitors of type 3 17β-HSD ever synthesized. Further experiments are being carried out to optimize the inhibitory activity of these compounds and to determine the exact mechanism of inhibition. The results will be published later in a full paper with a complete description of the experimental procedure (chemical synthesis and enzymatic test).

Acknowledgements

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22. All compounds were characterized by ^{1}H NMR, ^{13}C NMR, FT-IR, MS, and elemental analysis.
23. **Enzymatic assay** (briefly): An expression vector encoding for type 3 17β-HSD was transfected into human embryonal kidney (HEK)-293 cells using the calcium phosphate procedure.[25] Cells were then sonicated in 50 mM sodium phosphate buffer (pH 7.4), containing 20% glycerol and 1 mM EDTA, and centrifuged at 10,000 *g* for 1 h to remove the mitochondria, plasma membranes, and cells fragments. The supernatant was further centrifuged at 100,000 *g* to separate the microsomal fraction and this was used for measurement of type 3 17β-HSD activities. The test was carried out at 37 °C for 1 h in 1 mL of above reported buffer, containing 2 mM of cofactor (NADPH) and 0.1 μM of ^{14}C-Δ^4-dione (New England Nuclear, Boston, MA) and indicated concentration of inhibitors. The reaction was stopped by adding 2 mL of diethylether containing 10 mM of unlabeled Δ^4-dione and T. The metabolites were extracted with diethylether before being applied on silica gel 60 TLC plates. TLCs were developed in a mixture of toluene and acetone (4:1). Substrates and metabolites were identified by comparison with reference steroids, revealed by autoradiography, and quantified using the phosphorImager (Molecular Dynamics, Sunny Vale, CA). The percentage of inhibition and IC_{50} values were then calculated.
24. The logarithm of partition coefficient of *n*-octanol/water (LogP values) were calculated by CS ChemDraw Pro (CambridgeSoft Corporation, Cambridge, MA) using the Crippen's fragmentation method (Ghose, A. K.; Crippen, G. M. *J. Chem. Inf. Comput. Sci.* **1987**, *27*, 35).
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