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Synthesis and *in Vitro* Antiandrogenic Activity of 17β-Hydroxy-17α-(ω-Hydroxy/Haloalkyn-1'-yl)-4-Methyl-4-Aza-3-Oxo-5α-Androstan-(1-ene)-3-ones

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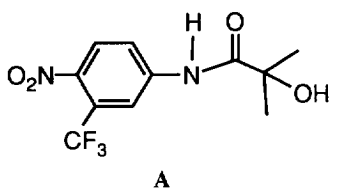
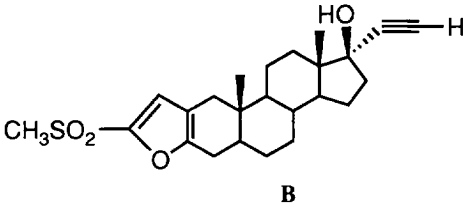
Abstract: Synthesis of 17β-hydroxy-17α-(ω-hydroxy/haloalkyn-1'-yl)-4-methyl-4-aza-(1-ene)-5α-steroids (**7-22**) was achieved by the addition of THP protected hydroxy alkynyllithium to 4-methyl-4-aza-(1-ene)-5α-androstan-3,17-diones (**1** and **2**), followed by deprotection and halogenation of 17α-(ω-hydroxy) compounds (**7-10**). Chloro- compounds **13** and **14**, and iodo- compound **21** are potent antiandrogens. Introduction of a 1,2-double bond increased the potency by 2-fold compared to the parent compounds.

5α-Dihydrotestosterone (DHT), the 5α-reduced metabolite of testosterone, is the most active androgen in mammalian tissues. Androgens are well known to play an important role in benign prostatic hyperplasia (BPH), and prostate cancer (PC)[1,2]. One logical treatment of these diseases is the selective inhibition of androgen action by antiandrogens.

Among systemic antiandrogens,[3a-c] flutamide and its active metabolite (**A**) have been extensively studied, and have been proved effective[3d-e] in the treatment of prostate cancer with minimal side effects. A number of steroidal antiandrogens are under investigation,[4] 5'-methylsulfonyl[3,2-b]furansteroid (**B**) being one example of this class of compounds.[4f] 17β-Substituted azasteroids have also been shown to be active against the androgen receptor.[5] Antiandrogenic activity of 17α-substituted azasteroids have, thus far, not been reported.[6] The present report describes the synthesis and *in vitro* activity of 17β-hydroxy-17α-(ω-hydroxy/haloalkyn-1'-yl)-4-methyl-4-azasteroids.



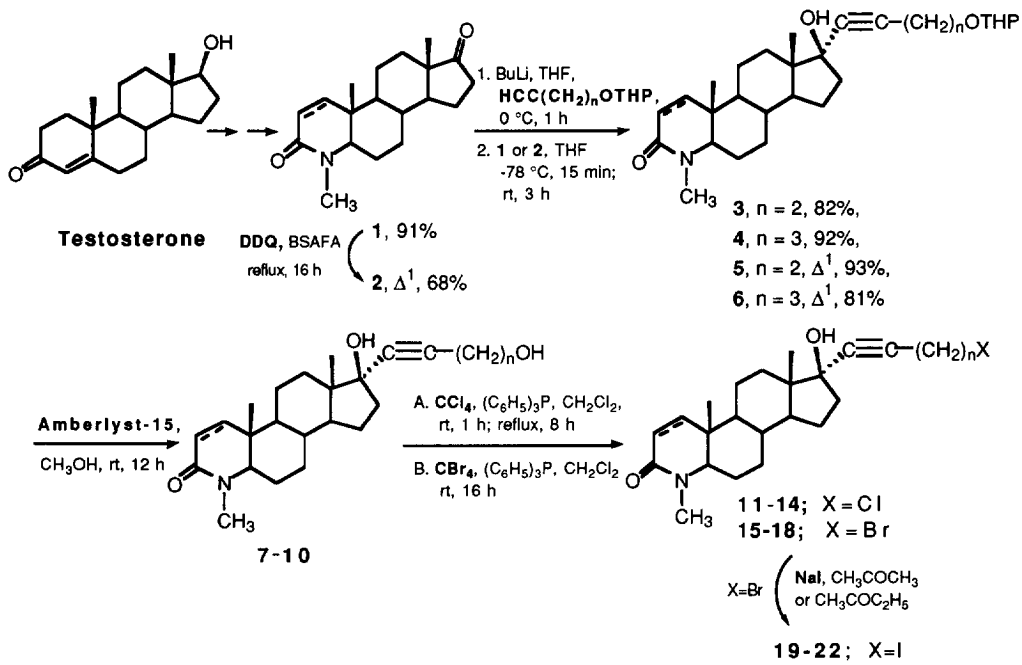
A



B

Chemistry. The title steroids were prepared from commercially available testosterone. Thus, 4-methyl-4-aza-5α-androstan-3,17-dione **1** was prepared following the method of Rasmusson *et.al.*[5] Compound **2** was prepared by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) oxidation[7] of dione **1** (Scheme 1). 17-Keto-4-azasteroids **1** and **2** were alkylated with 1-lithioalkynyl-ω-OTHP (which was generated

Scheme 1



in situ by the addition of *n*-BuLi to a solution of 1-alkynyl-ω-OTHP[8] in THF at 0 °C for 1 h) at -78 °C to provide addition products **3-6** in 81-93% yields. Deprotection of the THP-group[9] with an Amberlyst-15® at ambient temperature gave 17β-hydroxy-17α-(ω-hydroxyalkyn-1'-yl)-4-methyl-4-aza-5α-steroids (**7-10**) in 76-94% yields. Treatment of hydroxy compounds **7-10** with halogenating reagents such as carbon tetrachloride (CCl_4)/triphenylphosphine (PPh_3) and carbon tetrabromide (CBr_4)/PPh_3 gave the corresponding chloro-**11-14** and bromo-compounds **15-18**. However, the iodo-compounds **19-22** were prepared from the corresponding bromides and sodium iodide.[10]

Inhibition of the Proliferation of Androgen-Sensitive Shionogi Carcinoma Cells (Clone SEM-107).[11] The results of *in vitro* inhibitory activity are summarized in Table 1. Hydroxy-flutamide used as the standard reference, has an IC_{50} value of 52.5±1.7 nM for inhibition of DHT-stimulated Shionogi cell growth. 17β-Hydroxy-17α-(ω-hydroxyalkyn-1'-yl)-4-methyl-4-aza-5α-androstan-3-ones (**7-10**) showed no significant antiandrogenic activity (Table 1). However, when the hydroxy group was replaced with halogens, a marked increase in activity was observed. The IC_{50} values of the C_4- carbon halides were in the range of 150-300 nM. Introduction of a 1,2-double bond further increased the activity, and the activity of chloro- **12** (IC_{50} = 94.5 nM) and iodo- **21** (IC_{50} = 96.8 nM) was comparable to that of hydroxyflutamide. The same trend in activity was observed for C_5- alkynyl halides. In this class, the 1,2-double bond also increased the activity. The chloro- compound **14** was the most active (IC_{50} = 67.0 nM) of compounds in both classes.

In conclusion, 17α-(ω-haloalkyn-1'-yl) compounds show moderate to high antiandrogenic activity. Introduction of a 1,2-double bond increases the potency significantly. The C_4- and C_5- chain lengths show similar activity.

Table 1. *In vitro* antiandrogenic activity of 17β-hydroxy-17α-(ω-hydroxy/haloalkyn-1'-yl)-4-methyl-4-aza-5α-androstan-3-ones (**7-22**).[a]

Entry	Substituents			Yields (%)	Inhibition of DHT-stimulated Shionogi cell proliferation (IC_{50}, nM)
	X	Δ	-$(CH_2)_n$-		
Hydroxyflutamide					52.5 ± 1.7
7	OH		n=2	91	»1000[b]
11	Cl		n=2	60	250.0
15	Br		n=2	78	279.0
19	I		n=2	82	160.0
9	OH	Δ1	n=2	94	»1000
13	Cl	Δ1	n=2	55	94.5
17	Br	Δ1	n=2	55	141.8
21	I	Δ1	n=2	56	96.8
8	OH		n=3	76	»1000
12	Cl		n=3	72	128.9
16	Br		n=3	79	325.0
20	I		n=3	81	328.0
10	OH	Δ1	n=3	87	»1000
14	Cl	Δ1	n=3	63	67.2
18	Br	Δ1	n=3	59	149.0
22	I	Δ1	n=3	58	179.0

[a]No inhibition was observed in non-DHT stimulated Shionogi cell proliferation. [b]No activity was observed at 1.0 μM.

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10. The IR, EI-MS, HR-MS, ^{1}H- and ^{13}C-NMR (300 MHz) spectral properties of each of the 17α-hydroxy-17β-(w-haloalkyn-1'-yl) steroids were consistent with the assigned structures.
11. Bio-assay procedure: An androgen-sensitive cell line (clone SEM-107) derived from Shionogi mouse mammary carcinoma cells[12a] was used at passage 23. Cells were routinely grown as described previously.[12b] For the measurement of cell growth and sensitivity to anti-steroids, cells were plated at a density of 17400 cells/ml in minimal essential medium (MEM) supplemented with 2% dextran-coated charcoal-treated fetal calf serum, 1% non-essential amino acids, 10 IU/mL penicillin and 50 μg/mL streptomycin. Steroids and anti-steroids were dissolved in ethanol and stock solutions were chosen to yield a final ethanol concentration below 0.01% in the culture medium. 24 hours after plating, medium was changed and the indicated concentration of anti-steroids and/or DHT was added to triplicate dishes. Cells were then grown for 13 days with medium changes every 3-4 days. Cells were then fixed in methanol and their number was evaluated by measurement of DNA content by a modification[13] of the method of Fiszer-Szafarz.[14] Dose-response curves and IC_{50} values were calculated using a weighted iterative nonlinear least squares regression.[15] Results are presented as means ± SEM. The above assay was carriedout without DHT as a control.
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