

## Synthesis and *in Vitro* Antiandrogenic Activity of 17 $\beta$ -Hydroxy-17 $\alpha$ -( $\omega$ -Hydroxy/Haloalkyn-1'-yl)-4-Methyl-4-Aza-3-Oxo-5 $\alpha$ -Androstan-(1-ene)-3-ones

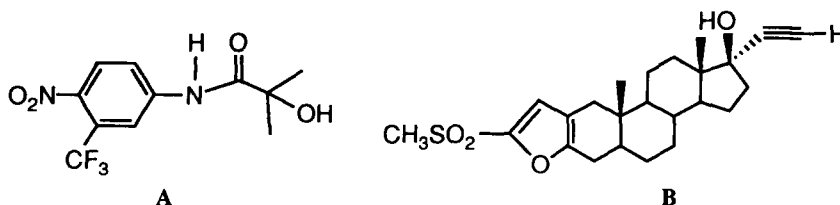
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**Abstract:** Synthesis of 17 $\beta$ -hydroxy-17 $\alpha$ -( $\omega$ -hydroxy/haloalkyn-1'-yl)-4-methyl-4-aza-(1-ene)-5 $\alpha$ -steroids (**7-22**) was achieved by the addition of THP protected hydroxy alkynyllithium to 4-methyl-4-aza-(1-ene)-5 $\alpha$ -androstan-3,17-diones (**1** and **2**), followed by deprotection and halogenation of 17 $\alpha$ -( $\omega$ -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.

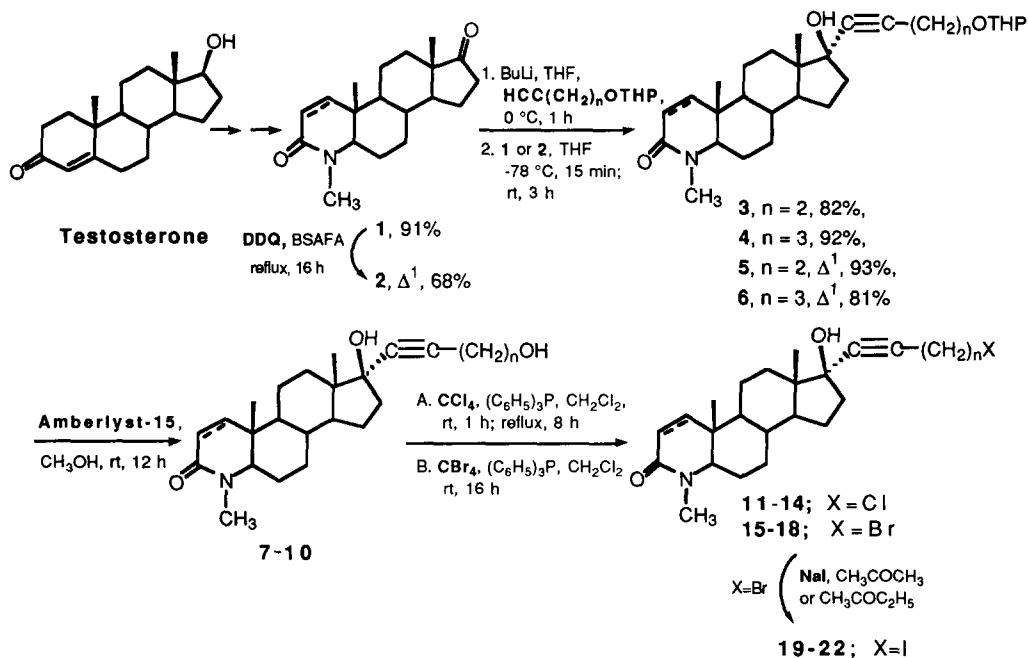
5 $\alpha$ -Dihydrotestosterone (DHT), the 5 $\alpha$ -reduced metabolite of testosterone, is the most active androgen in mammalian tissues. Androgens are well known to play an important role in benign prostatic hyperplasia (BPH), and prostate cancer (PC)<sup>1,2</sup>. One logical treatment of these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

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



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

Scheme 1



*in situ* by the addition of *n*-BuLi to a solution of 1-alkynyl- $\omega$ -OThp<sup>8</sup> 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<sup>9</sup> with an Amberlyst-15<sup>®</sup> at ambient temperature gave 17 $\beta$ -hydroxy-17 $\alpha$ -( $\omega$ -hydroxyalkynyl)-4-methyl-4-aza-5 $\alpha$ -steroids (**7-10**) in 76-94% yields. Treatment of hydroxy compounds **7-10** with halogenating reagents such as carbon tetrachloride (CCl<sub>4</sub>)/triphenylphosphine (PPh<sub>3</sub>) and carbon tetrabromide (CBr<sub>4</sub>)/PPh<sub>3</sub> 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.<sup>10</sup>

**Inhibition of the Proliferation of Androgen-Sensitive Shionogi Carcinoma Cells (Clone SEM-107).**<sup>11</sup> The results of *in vitro* inhibitory activity are summarized in Table 1. Hydroxy-flutamide used as the standard reference, has an IC<sub>50</sub> value of 52.5 $\pm$ 1.7 nM for inhibition of DHT-stimulated Shionogi cell growth. 17 $\beta$ -Hydroxy-17 $\alpha$ -( $\omega$ -hydroxyalkynyl)-4-methyl-4-aza-5 $\alpha$ -androstane-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<sub>50</sub> values of the C<sub>4</sub>- 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<sub>50</sub> = 94.5 nM) and iodo- **21** (IC<sub>50</sub> = 96.8 nM) was comparable to that of hydroxyflutamide. The same trend in activity was observed for C<sub>5</sub>- alkynyl halides. In this class, the 1,2-double bond also increased the activity. The chloro- compound **14** was the most active (IC<sub>50</sub> = 67.0 nM) of compounds in both classes.

In conclusion, 17 $\alpha$ -( $\omega$ -haloalkyn-1'-yl) compounds show moderate to high antiandrogenic activity. Introduction of a 1,2-double bond increases the potency significantly. The C<sub>4</sub>- and C<sub>5</sub>- chain lengths show similar activity.

**Table 1.** *In vitro* antiandrogenic activity of 17 $\beta$ -hydroxy-17 $\alpha$ -( $\omega$ -hydroxy/haloalkyn-1'-yl)-4-methyl-4-aza-5 $\alpha$ -androstan-3-ones (7-22).<sup>a</sup>

Entry	Substituents			Yields (%)	Inhibition of DHT-stimulated Shionogi cell proliferation (IC <sub>50</sub> , nM)
	X	$\Delta$	-(CH <sub>2</sub> ) <sub>n</sub> -		
Hydroxyflutamide					52.5 $\pm$ 1.7
7	OH		n=2	91	$\gg$ 1000 <sup>b</sup>
11	Cl		n=2	60	250.0
15	Br		n=2	78	279.0
19	I		n=2	82	160.0
9	OH	$\Delta$ 1	n=2	94	$\gg$ 1000
13	Cl	$\Delta$ 1	n=2	55	94.5
17	Br	$\Delta$ 1	n=2	55	141.8
21	I	$\Delta$ 1	n=2	56	96.8
8	OH		n=3	76	$\gg$ 1000
12	Cl		n=3	72	128.9
16	Br		n=3	79	325.0
20	I		n=3	81	328.0
10	OH	$\Delta$ 1	n=3	87	$\gg$ 1000
14	Cl	$\Delta$ 1	n=3	63	67.2
18	Br	$\Delta$ 1	n=3	59	149.0
22	I	$\Delta$ 1	n=3	58	179.0

<sup>a</sup>No inhibition was observed in non-DHT stimulated Shionogi cell proliferation. <sup>b</sup>No activity was observed at 1.0  $\mu$ M.

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  6.  $\omega$ -Haloalkynyl substituents at the 17 $\alpha$ -position of 19-nortestosterone have been shown to exhibit high affinity for the progesterone receptor. However, it is known that all hormonal receptors have a homologous conformation: Salman, M.; Stotter, P. L.; Chamness, G. C. *J. Steroid Biochem.* **1989**, 33, 25-31.
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  10. The IR, EI-MS, HR-MS, <sup>1</sup>H- and <sup>13</sup>C-NMR (300 MHz) spectral properties of each of the 17 $\alpha$ -hydroxy-17 $\beta$ -(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<sup>12a</sup> was used at passage 23. Cells were routinely grown as described previously.<sup>12b</sup> 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  $\mu$ g/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<sup>13</sup> of the method of Fiszer-Szafarz.<sup>14</sup> Dose-response curves and IC<sub>50</sub> values were calculated using a weighted iterative nonlinear least squares regression.<sup>15</sup> Results are presented as means  $\pm$  SEM. The above assay was carried out without DHT as a control.
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