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# Biological Activity of Novel 17β-phenylcarbamiol-androst-4-en-3-one as Inhibitors of Type 2 5α-reductase Enzyme

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# Biological Activity of Novel 17β-phenylcarbamiol-androst-4-en-3-one as Inhibitors of Type 2 5α-reductase Enzyme

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**Abstract**

In this paper, we describe the biological activity of four different 17β-phenylcarbamiol-androst-4-en-3-one derivatives as inhibitors of the type 2 5α-reductase enzyme (5α-R2). This enzyme is present in the human prostate and has been associated with androgen-dependent illnesses such as benign prostatic hyperplasia and prostate cancer.

The effects of these 17-carboxamide derivatives were determined by measuring the concentration of each compound that inhibits 50% of the activity of 5α-R2 ($IC_{50}$ values). The activity of this enzyme was measured at a pH of 6.5 and optimum conditions of temperature and substrate concentration for this type 2 isozyme.

The results from these experiments indicated that all the novel steroidal 17-carboxamides tested significantly inhibited the activity of 5α-R2 enzyme, with $IC_{50}$ values: **1** 5 ±0.5 nM, **2** 13.9±0.9 nM, **3** 0.112±45 nM, and **4** 0.167±56 nM. With the exception of steroid **2,** they showed higher potency than finasteride ($IC_{50}$= 8.5 nM) for inhibition of this enzyme. Finasteride is the drug of choice for the treatment of benign prostatic hyperplasia and was used in this experiment as a positive control.

In conclusion, all steroidal derivatives described in this paper are good inhibitors for the human 5α-R2 isonzyme, with compounds **3** and **4** showing a higher inhibitory potential than finasteride. Therefore these steroids have a promising therapeutical potential for the treatment of benign prostatic hyperplasia and prostate cancer.

**Keywords:** Type 2 5α-reductase, prostate, 17β-phenylcarbamiol-androst-4-en-3-one derivatives, benign prostatic hyperplasia, prostate cancer, testosterone, dihydrotestosterone.

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## Introduction

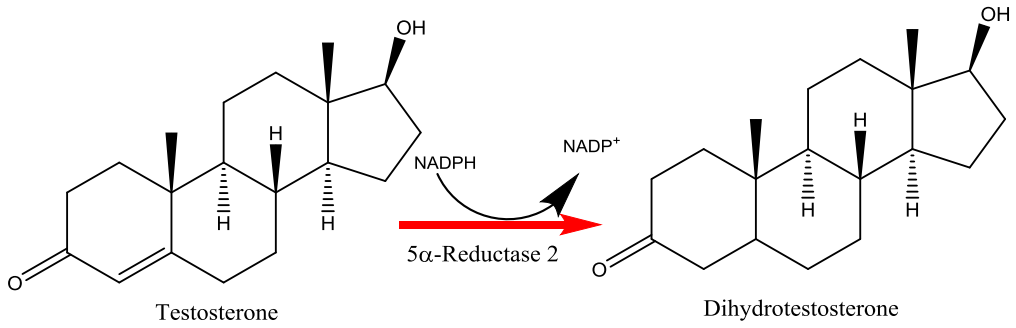
The 5α-reductase enzyme (EC 1.3.99.5) converts $\Delta^4$-3-ketosteroids to 5α-3-ketosteroids in androgen-dependent tissues. The activity of this enzyme in androgen-dependent tissues has long been known; the enzyme converts testosterone (T) to 5α-dihydrotestosterone (DHT) in the prostate gland (Figure 1).

Hyperplasia of the prostate gland and prostate cancer has been associated with high levels of serum 5α-DHT [Jenkins et al. 1992; Marberger, 2006; Thomas et al., 2008].

Three types of 5α-reductase (5α-R) isozymes have been described; 5α-R type 2 plays a major role in prostate cancer and benign prostatic hyperplasia as it is predominantly expressed in this tissue. However, some evidence indicates that type 1 is expressed in the prostate epithelial cells while type 2 is mainly located in the stromal compartment [Russell and Wilson, 1994; Liang et al., 1985]. 5α-reductase type 1 is also located in the liver and skin and acts in a neutral or basic medium, whereas type 2 is active in acidic pH [Russell and Wilson, 1994]. Recently 5α-R3 has been investigated; this isozyme can be found in the brain and pancreas and is related to hormone-refractory prostate cancer (HRPC) [Ogishima et al., 2008; Uemura et al., 2008]. None of the three isozymes have been purified due to their unstable nature, and as a result, 5α-reductase isozyme inhibitors have been designed by targeting their substrate.

Various 5αR2 inhibitors have been developed for relieving the symptoms produced in benign prostatic hyperplasia. These 5α-R inhibitors reduce bladder obstruction present in this illness by inhibiting the conversion of T in its more active form DHT (Figure 1.) In addition, these inhibitors prevent the need for surgery and further progression of the disease. The most extensively studied 5α-R inhibitors are finasteride and dutasteride [Marcheti and Guarna, 2002]. Finasteride and dutasteride are 4-azasteroids that have demonstrated low affinity for androgen receptors and thus were not expected to produce undesirable antiandrogen effects such as impotence, muscle growth impairment or gynecomastia. [Liang et al, 1984] However, when these drugs are used for long periods, they could induce hepatoxicity [Brandt and Levy, 1989]; thus, the synthesis of new 5α-R inhibitors with low side effects has been a challenge for researchers.

**Figure 1.** *Conversion of Testosterone (T) to 5α-dihydrotesterone (DHT). This Reaction is Catalyzed by 5α-reductase 2 (Rα-R2) in the Prostate Gland.*




In view of the fact that we have recently synthesized several progesterone derivatives with an ester moiety at C-17, which showed high activity as 5α-R2 inhibitors and prostate cancer cell growth [Cabeza et al., 2006; Bratoeff et al., 2007.], in this paper we describe the biological activity of four novel steroids based on the progesterone skeleton and with a carboxamide moiety at C-17. They are compounds **1-4** shown in Figure 2.

## Experimental

*Chemical and Radioactive Materials*

(1,2,6,7-$^{3}$H) T specific activity: 95 Ci/mmol was supplied by Perkin Elmer Life and Analytical Sciences (Boston, MA). Radioinert T was supplied by Steraloids (Wilton, NH, USA). Sigma Chemical Co. (St. Louis, Mo) provided NADPH. Finasteride, (N-(1,1-di-methylethyl)-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide), is an aza-steroid marketed as Proscar® (a treatment for benign prostatic hypertrophy) or as Propecia® (a treatment for male pattern hair loss) (Merck, Sharp and Dohme). The finasteride used in this experiment was obtained by extraction from Proscar®. The tablets were crushed and extracted with chloroform, and the solvent was eliminated in vacuum; the crude product was purified by silica gel column chromatography. The melting point of the isolated finasteride (252–254 °C) was identical to that reported in the literature [Trapani, et al., 2002].

*Biological Activity of the Novel Compounds*

The biological activity of the steroidal 17-carboxamide derivatives synthesized by the group of Dr. Eugene Bratoeff was determined using the same method reported previously [Bratoeff, et al., 2007]. Membrane fraction of human prostate was used as a source of 5α-R2. The prostate was obtained from a 53-year-old man who died from renal insufficiency. The prostate was extirpated 4 hours after death in the Pathology Department of the General Hospital of Mexico City. This protocol was approved by the Ethical Committee of the Mexico City General Hospital.

*Determination of 50% Inhibitory Concentration of Steroids* **1-4** *in Human Prostatic 5αR2 Activity*

In order to calculate the $IC_{50}$ values (the concentration of steroids **1-4** or finasteride required to inhibit 5αR2 activity by 50%), six series of tubes containing increasing concentrations of these steroids ($10^{-11}$- $10^{-3}$ M) in 50 μl of DMSO were incubated in duplicate in the presence of 1 mM of dithiothreitol, 40 mM sodium phosphate buffer pHs of 6.5; 2 mM NADPH, 2 nM [1,2,6,7-$^{3}$H]T and 50 μg of protein from enzymatic fraction in a final volume of one mL. The reaction was carried out in duplicate at 37 °C for 60 min., adding one ml of dichloromethane to stop the reaction.

The incubating samples of each of these assays and experiments were extracted (5×) with dichloromethane and the solvent from the extractions of each sample were combined in a tube and dried. The extract was dissolved in 50 μL of chloroform:methanol (1:1) and spotted on a thin layer chromatography plate, HPTLC Keiselgel 60 $F_{254}$ (Merck, Mexico City). Standards of DHT and testosterone (T) were applied on two lanes, one on each side of the plate. These plates were eluted three different times in achloroform:acetone (9:1) mixture [Bratoeff, et al., 2007] and air dried; the radioactivity on them was scanned using an AR2000 Bioscanner (Bioscan, Washington DC).

The testosterone (T) standard was developed by fluorescence (UV lamp; λ=254 nm from UVP, Upland, CA) and the DHT standard was identified using phosphomolibdic acid reagent. The data obtained by this method were plotted using SigmaPlot 12 software (Systat Software, INC., San Jose, CA).

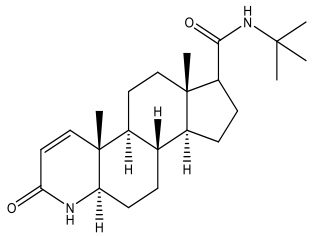
## Results

*Effect of the Novel 17-Carboxamide Derivatives* ***1-4*** *on Inhibition of Human 5α-R2*

The effect of compounds **1-4** on 5αR2 activity is shown in Figure 2. All of the synthesized compounds inhibited the 5αR2 enzyme. Compound **3** exhibited the highest potency, 112±45 pM, followed by **4** at 167±56 pM, **1** at 5 ±0.5 nM, and **2** at 13.9±0.9 nM. All the compounds except for steroid **2** showed higher potency than finasteride ($IC_{50}$= 8.5 nM).

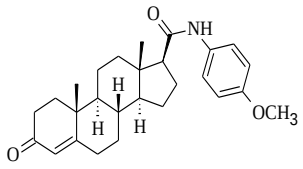
The results obtained in this study indicated that the presence of a halogen or a methoxy moiety in the p- position of the aromatic ring increased the potency of these 17-carboxamide derivatives. The presence of a methyl group at the p- position of the aromatic ring decreased their potency in comparison to finasteride.

**Figure 2.** *Biological Activity of Steroidal Carbamate Derivatives. The $IC_{50}$ Value is the Concentration of the Corresponding Compound required to Inhibit 50% of 5αR2 Activity. Finasteride was used as a Control since it is a known Inhibitor of 5α-R2.*



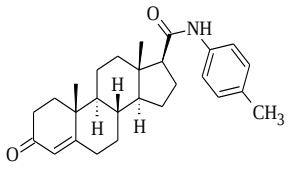
**Finasteride**,

$IC_{50}$ = 8.5±0.9 nM



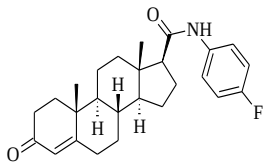
**1**

$IC_{50}$ =5 ±0.5 nM



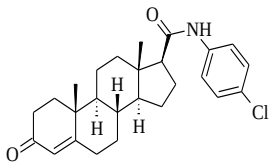
**2**

$IC_{50}$ = 13.9±0.9 nM



**3**

$IC_{50}$ = 0.112±0.045 nM



**4**

$IC_{50}$ = 0.167±0.056 nM

## Discussion

We identified the biological activity of four novel 17β-phenylcarbamiol-androst-4-en-3-one derivatives (**1-4**) as 5αR2 inhibitors.

Steroids **1-4** are C-17 carboxamides that have the advantage of attaching the androstene skeleton to the aromatic ring. This twofold structure could facilitate binding to each of these novel compounds (**1-4)** by the pocket of the 5αR2 active site. Adding a halogen to the aromatic ring in p- position (steroids **3** and **4**) resulted in a hundredfold improvement of 5αR2 activity inhibition, demonstrating that this extra aromatic halogenated ring was binding to the hydrophobic pocket at the enzyme's active site.

Moreover, a methoxy moiety in the p- position of the aromatic ring could undergo an electrophilic aromatic substitution reaction, enabling binding of this derivative (**1**) to the hydrophobic pocket at the 5α-R2 active site. However, the methyl group at the p- position of the aromatic ring decreased the potency of these C-17 carboxamides. A possible explanation of this result could be that the methyl group is more electropositive than halogen or methoxy substituent. This could explain the lower potency observed for steroid **2**.

Previously our group reported the biological activity of different C-17 carbamate derivatives [Bratoeff, et al 2007]. One of them included a bromine atom at the p- position of the aromatic ring. This derivative was capable of inhibiting 5αR2 activity in human prostate ($IC_{50}$= 50 nM) [Bratoeff, et al 2007], and showed pharmacological activity. Hamsters treated with this carbamate derivative for 6 days did not show any toxicological effect.

Since there were no observed side effects from the treatment with this bromine carbamate derivative, it could be possible that the novel 17β-phenylcarbamiol-androst-4-en-3-one derivatives **3** and **4** do not show any toxicological effects. Therefore they could be tested in pharmacological assays for the treatment of benign prostatic hyperplasia and prostate cancer in animal models.

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