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Effects of novel 17-azolyl compounds on androgen synthesis in vitro and in vivo

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Abstract

17-Azolyl steroids were synthesized and evaluated as inhibitors of androgen synthesis in vitro and in vivo. Several of the novel compounds exhibit potent noncompetitive inhibition of human 17α-hydroxylase/$C_{17,20}$-lyase with IC_{50} values ranging from 7 to 90 nM, and K_i values from 1.2 to 41 nM. **VN/85-1** and **VN/108-1** were the most potent inhibitors against this enzyme with IC_{50} value of 8 nM (K_i of 1.2 nM) and 7 nM (K_i of 1.9 nM), respectively. **VN/107-1**, **VN/108-1** and **VN/109-1** also showed moderate inhibition of 5α-reductase in human prostatic microsomes. Normal adult male rats were treated with these novel 17-azolyl steroidal compounds at a dose level of 50 mg/kg, s.c., for 14 consecutive days, sacrificed 1–2 h after the last administered dose and blood, prostate and other tissues were collected. The organs were weighed and tissue concentrations of testosterone (T) and dihydrotestosterone (DHT) were measured. Tissue T levels were significantly ($p < 0.05$) lower in rats treated with the novel 17-azolyl steroids by more than 50% compared to the control group. Similarly, the concentration of DHT in the serum and prostates was significantly ($p < 0.05$) diminished in rats treated with the 17-azolyl steroids by 39–80% compared to the control group. Furthermore, the wet weights of the prostates and seminal vesicles were significantly ($p < 0.05$) reduced by several of the novel steroids. Although only one dose was evaluated in these studies, **VN/85-1** was the most effective compound and reduced prostatic androgen levels by more than 80% and the wet weights of the prostate and seminal vesicles in rats by about 50%. These findings suggest that these novel compounds may provide useful leads for the research and development of suitable agents for the treatment of androgen dependent prostate cancer.

Keywords: 17-Azolyl steroids; Prostate cancer; Androgens; 17α-hydroxylase/$C_{17,20}$-Lyase; 5α-Reductase

1. Introduction

Prostate cancer is the second leading cause of death and the most prevalent cancer amongst men in the United States and Europe today [1]. Development of therapeutic agents is therefore an important objective for reducing the high incidence of this malignancy. Androgens are known to play a critical role in the development and progression of prostatic diseases such as benign prostatic hyperplasia (BPH) and prostate carcinoma [2]. The 17α-hydroxylase/$C_{17,20}$-lyase (lyase) and 5α-reductase are key enzymes in the androgen synthesis pathway. Lyase converts the 21-carbon steroids, pregnenolone and progesterone, to the 19-carbon androgens, dehydroepiandrosterone (DHEA) and androstenedione, respectively. DHEA and androstenedione are subsequently converted by other enzymes to testosterone (T). Testosterone, the major circulating androgen in males, is synthesized primarily in the testis and, to a lesser extent, in the adrenals. 5α-Reductase, which is localized mainly in the prostate, converts T to the more potent androgen, dihydrotestosterone (DHT)

Abbreviations: DHT = dihydrotestosterone; BPH = benign prostatic hyperplasia; HPC = hydroxypropylcellulose; RIA = radioimmunoassay; T = testosterone; 17α-hydroxylase/$C_{17,20}$-lyase = lyase.

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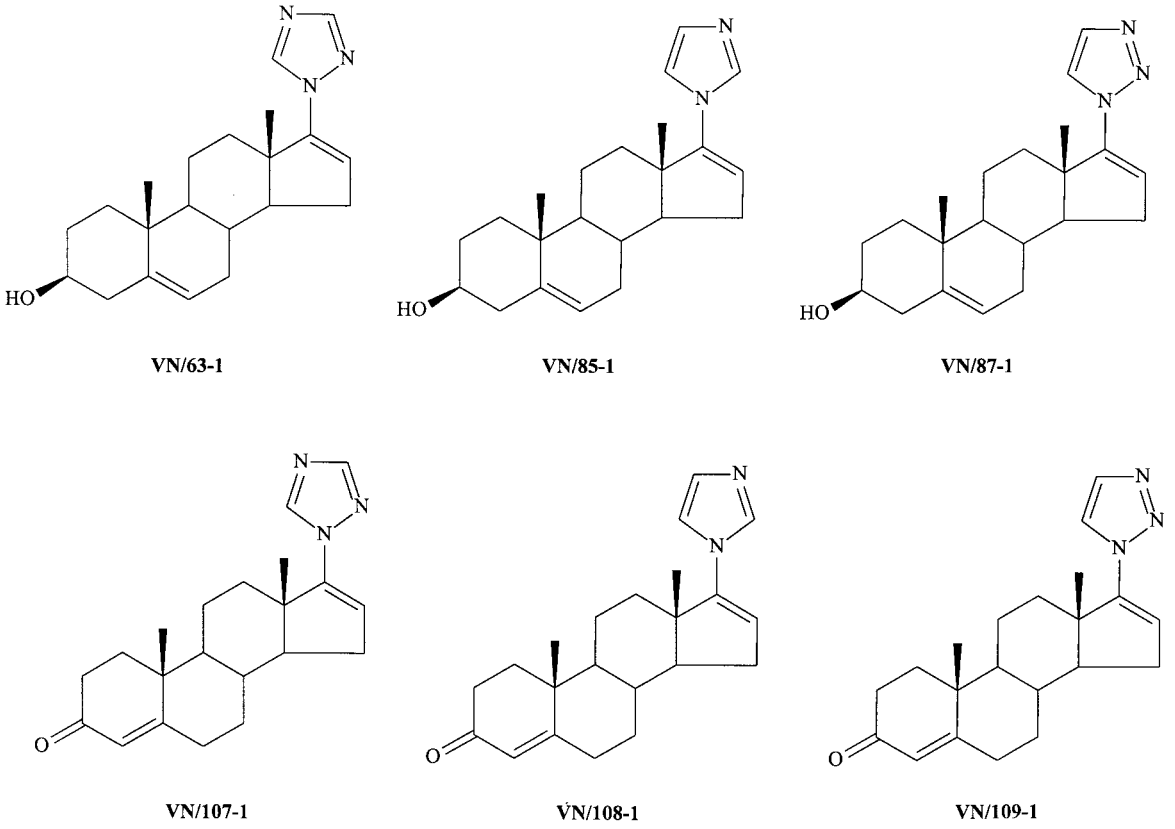

Fig. 1. Chemical structures of the 17-azolyl steroids evaluated in this study. 3β-hydroxy-17-(1*H*-1,2,4-triazol-1-yl)androsta-5,16-diene (**VN/63-1**), 3β-hydroxy-17-(1*H*-imidazol-1-yl)androsta-5,16-diene (**VN/85-1**), 3β-hydroxy-17-(1*H*-1,2,3-triazol-1-yl)androsta-5,16-diene (**VN/87-1**), 17-(1*H*-1,2,4-triazol-1-yl)androsta-4,16-diene-3-one (**VN/107-1**), 17-(1*H*-imidazol-1-yl)androsta-4,16-diene-3-one (**VN/108-1**) and 17-(1*H*-1,2,3-triazol-1-yl)androsta-4,16-diene-3-one (**VN/109-1**).

[3,4]. Both T and DHT stimulate prostatic growth although DHT plays a much more important role than T in the organogenesis and homeostasis of the prostate. Inhibition of these pivotal enzymes in the androgen synthesis cascade would diminish circulating androgens and is a useful approach for developing therapeutic agents for the treatment of prostatic diseases and other androgen dependent diseases [5,6]. Moreover, current treatments such as orchidectomy and luteinizing hormone releasing hormone (LHRH) agonists result in reduced androgen production by the testes but do not interfere with androgen production by the adrenals which contribute androgen precursors to the prostate [5]. Thus, total androgen blockade appears to be a useful therapeutic strategy for diminishing the levels of circulating androgens and may be more effective than conventional androgen deprivation therapy. Although several inhibitors of lyase have been described in the literature only ketoconazole, an imidazole antifungal agent, has been used clinically to treat patients with advanced prostate cancer [7]. However, ketoconazole is not a very potent inhibitor of lyase, inhibits other cytochrome P-450 enzymes, inhibits cortisol production and has a number of significant side effects although more recent studies have also shown that ketoconazole was effective in reducing PSA levels in prostate cancer patients who had progressed following withdrawal of flutamide [8]. We and others have recently described compounds with more potent inhibitory activity against lyase that also inhibit 5α-reductase [9–15]. The 5α-reductase inhibitor, finasteride, has been approved for the treatment of benign prostatic hypertrophy [16]. Although finasteride is effective at reducing the circulating levels of DHT in patients with prostate cancer, it produces accumulation of bioavailable levels of T, which can stimulate prostatic growth.

Recently, we reported the synthesis, characterization and preliminary in vitro testing of several novel 17-azolyl steroids [17,18]. These compounds exhibit potent inhibitory activity against lyase in mammalian testicular microsomes. The mechanism of inhibition appeared to involve the binding of the steroidal azole nitrogens to the heme iron of the active site of the enzyme. Several of the novel 17-azolyl compounds also showed antiandrogenic and antiproliferative activity in cultures of human prostate cancer cell lines (LNCaP) and were effective at inhibiting tumor growth in male SCID mice bearing LNCaP tumor xenografts [19]. Thus, the

17-azolyl compounds show promise as potential therapeutic agents for treatment of androgen dependent diseases such as BPH and prostate carcinoma.

In the present investigation, we determined the inhibitory activity of several of the 17-azolyl steroidal compounds (Fig. 1) on human lyase and 5α-reductase in vitro and examined their effects on the tissue T and DHT concentrations and weights of the prostate, testis, seminal vesicles and epididymis in normal adult male rats.

2. Materials and methods

2.1. Chemical inhibitors and reagents

The 17-azolyl steroids were synthesized in our laboratory according to procedures described previously [17]. The active [^{125}I]-testosterone coated-tube radioimmunoassay (RIA) kits and the [^{125}I]-dihydrotestosterone coated-tube RIA kits for quantitative measurement of T and DHT, respectively, were purchased from DSL Inc. (Webster, TX). All other reagents were purchased from Sigma Chemical Co., St. Louis, MO.

2.2. Preparation of microsomes

Human testes and prostate tissue (from patients with benign prostatic hyperplasia, BPH) were obtained from Dr. James Mohler, Director, Urologic Oncology, University of North Carolina at Chapel Hill and stored at −70°C prior to use. Testicular and prostatic microsomes were prepared as described previously [20]. Briefly, human testis or prostate was washed with saline (0.9%), blotted dry and weighed. The tissue was minced and homogenised in a blender with two volumes of sucrose (250 mM). The homogenates were added to 50 ml plastic centrifuge tubes and centrifuged at 10,000 g for 30 min. The resulting supernatant was centrifuged at 109,000 g for 1 h using an ultracentrifuge. The microsomal pellet was covered with 2 ml of phosphate buffer (0.1 M, pH 7.4) and stored at −70°C until required for assay. The microsomal protein content was determined by the Lowry method [21].

2.3. 17α-Hydroxylase/$C_{17,20}$-lyase (Lyase) activity

The measurement of the activity of the human lyase in testicular microsomes, in the absence and presence of inhibitors was performed as described previously [6,12,17]. Briefly, the lyase activity was determined by measuring the release of [^{3}H]-acetic acid during the conversion of [21-^{3}H]-17α-hydroxypregnenolone to dehydroepiandrosterone. The incubations were carried out in a total volume of 1.01 ml. Sample tubes were supplied with 10 μl of propylene glycol, 300,000 dpm of [21–^{3}H]-17α-hydroxypregnenolone (13.61 μCi/μmol) and the indicated inhibitors. The control incubations were prepared without the addition of the indicated inhibitors. After evaporation of the ethanolic solution, the following were added to each tube: 750 μl of 0.1 M sodium phosphate buffer (pH 7.4, with 78 μM of DTT) and 50 μl of an NADPH generating system (phosphate buffer containing 6.5 mM of $NADP^+$, 71 mM of glucose-6-phosphate, 1.25 IU of glucose-6-phosphate dehydrogenase). The tubes were preincubated for 15 min at 37°C and the reaction was started by adding 200 μl of human testicular microsomes (300 μg protein per 200 μl of 0.1 M phosphate buffer, pH 7.4). The reaction tubes were incubated at 37°C under oxygen. After 1 h, the tubes were placed in an ice bath and the reaction mixture was extracted twice with chloroform (1 ml). The tubes were allowed to stand at 4°C for 20 min, centrifuged at 4°C for 15 min at 2000 g and then 0.75 ml of the aqueous phase of each tube was placed into a fresh tube. To remove residual steroids, which may remain after the chloroform extraction, 0.75 ml of charcoal solution (2.5 g of activated charcoal per 100 ml of distilled water) was added to each tube and vortexed vigorously. After standing for 30 min, the charcoal was pelleted by centrifugation at 2000 g for 20 min. Finally, 0.75 ml of the supernatant was analyzed for tritium by liquid scintillation spectrometry. The reaction conditions were optimized with 0.1–6.0 μM of [21–^{3}H]-17α-hydroxypregnenolone and the K_m and V_{max} values were determined at the optimum conditions. The IC_{50} values for inhibitors were calculated using linear regression analysis and the plot of logit of enzyme activity against log of inhibitor concentration. K_i values were also determined at the same reaction conditions with addition of appropriate concentrations of inhibitors. The experiments were performed in duplicate on at least three separate occasions.

2.4. 5α-Reductase assay

The effects of novel compounds on human prostatic 5α-reductase activity were evaluated as previously described, with minor modifications [10,12,17]. Ethanolic solutions of [7-^{3}H]-T (600,000 dpm), cold T (4.8 ng), indicated inhibitors (0–200 nM) and propylene glycol (10 μl) were added to duplicate sample tubes. The control incubations were prepared without the addition of the indicated inhibitors. The ethanol was evaporated to dryness under a gentle stream of air. The samples were reconstituted in phosphate buffer (0.1 M, pH 7.4, 400 μl) containing DTT (78 μM) and the NADPH generating system (NADP, 6.5 mM; glucose-6-phosphate, 71 mM; glucose-6-phosphate dehydrogenase, 2.5 IU, in 100 μl of phosphate buffer)

was added to each tube. The tubes were preincubated at 37°C for 15 min. The enzymatic reactions were initiated by addition of human BPH microsomes (about 180 μg of microsomal protein in 500 μl of phosphate buffer) in a total volume of 1.01 ml. The incubations were performed for 10 min under oxygen in a shaking water bath at 37°C. The incubations were terminated by placing the sample tubes on ice. [^{14}C]-DHT (3000 dpm) and cold DHT (50 μg) were added to each tube as an internal standard and visualization marker, respectively. These additions were immediately followed by ether (1 ml). The steroids were extracted with ether (3 × 1 ml), separated by TLC (chloroform: ether, 80:20) and visualized by exposure to iodine vapor. The extracts were analyzed for ^{3}H and ^{14}C using a liquid scintillation counter. The percentage conversion of [7-^{3}H]-T to [7-^{3}H]-DHT was calculated and used to determine 5α-reductase activity. The reaction conditions were optimized with T (0–60 nM) and the K_m and V_{max} values were estimated at the optimum conditions. The IC_{50} values were determined from plots of 5α-reductase activity against four different concentrations of the inhibitor. The experiments were performed in duplicate and were repeated at least twice.

2.5. Animal treatments

Adult male Sprague Dawley rats (240 ± 10 g) were supplied by Charles River Breeding Laboratories (Wilmington, MA). The animals were maintained in a controlled environment of about 25°C, 50% relative humidity and 12 h of light and 12 h of dark cycles and were allowed free access to food and water. Rats (n = 6–8) were assigned to the different treatment groups. The compounds were suspended in 0.3% hydroxypropylcellulose (HPC) and administered subcutaneously at a dose level of 50 mg/kg for 14 consecutive days. The control group was injected with the vehicle alone. Another group of rats (6–8) was castrated and injected with the vehicle alone for 14 days. The rats were sacrificed at the end of the treatment period (1–2 h after the last administered dose) and blood collected. The testes, prostate (anterior and ventral), epididymis and seminal vesicles were also removed and weighed. The blood samples were centrifuged at 2000 rpm for 20 min to obtain serum. The tissues were stored at −70°C until analysis.

2.6. [^{125}I]-T RIA assay

Serum, testicular and prostatic tissues obtained from individual male rats were placed on ice. Portions (~100 mg) of testicular and prostatic tissues were homogenized in sodium phosphate buffer (0.1 M, pH 7.4) and the homogenates were centrifuged at 2000 rpm for 20 min. Serum (50 μl) and aliquots (50 μl) of the tissue supernatant were used for the determination of testosterone as described for the ^{125}I-T assay kit supplied by DSL Inc.

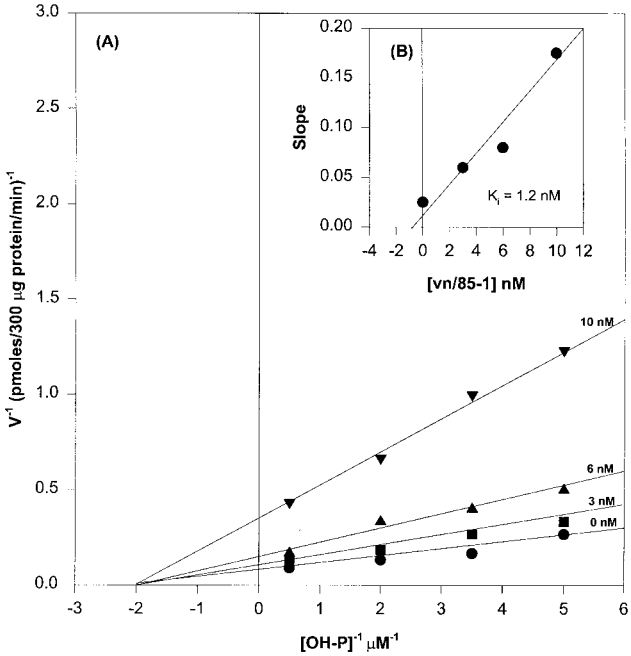

Fig. 2. Inhibition of human testicular microsomal 17α-hydroxylase/$C_{17,20}$-lyase by **VN/85-1**. (A) Lineweaver–Burk plot of enzyme activities at various substrate and inhibitor concentrations, (B) slopes of each reciprocal plot against **VN/85-1** concentration. Human testicular microsomes were prepared and 17α-hydroxylase/$C_{17,20}$-lyase (lyase) activity determined as described under Materials and methods. The K_m and V_{max} values for lyase, under the optimum incubation conditions were 0.48 μM and 40 pmoles/mg protein/min. Each point is the mean of duplicate determination of at least three separate experiments. The other 17-azolyl compounds showed a similar pattern of noncompetitive inhibition as **VN/85-1**. **OH–P** = 17α-hydroxypregnenolone.

2.7. [^{125}I]-DHT RIA assay

Portions (~100 mg) of prostatic tissues were homogenized in assay buffer provided with the DHT RIA assay kit obtained from DSL Inc. and the homogenates were centrifuged at 2000 rpm for 20 min. Serum (0.4 ml) and aliquots (0.4 ml) of the prostatic supernatant were used for the determination of DHT as described in the ^{125}I-DHT assay kit [22].

2.8. Statistical analysis

One-way ANOVA (SigmaStat for Windows version 1.0) was used to obtain probability (p) values and for comparing different treatment groups. The Bonferroni test was used for posthoc analysis. A p value of less than 0.05 was considered as statistically significant.

Table 1
Kinetic constants of 17-azolyl steroids against human testicular 17α-hydroxylase/$C_{17,20}$-lyase and prostatic 5α-reductase[a]

Compound	17α-Hydroxylase/$C_{17,20}$-lyase		5α-Reductase
	IC_{50} (nM)	K_i (nM)	IC_{50} (nM)
VN/63-1	90 ± 14	23 ± 2.0	~ 160,000
VN/85-1	8 ± 1.0	1.2 ± 0.1	~ 400,000
VN/87-1	13 ± 1.0	1.4 ± 0.1	~ 10,000
VN/107-1	55 ± 11.0	41 ± 2.0	152 ± 10.0
VN/108-1	7 ± 1.0	1.9 ± 0.2	142 ± 5.0
VN/109-1	19 ± 1.0	8 ± 0.4	198 ± 33.0
Finasteride	ni	ni	33 ± 2.0
Ketoconazole	78 ± 3.0	38 ± 2.0	ni

[a] The IC_{50} and K_i values of these steroidal compounds against 17α-hydroxylase/$C_{17,20}$-lyase (lyase) and 5α-reductase were determined using human testicular and prostatic microsomes as described under materials and methods. The K_m and V_{max} values for lyase under the optimum incubation conditions, without inhibitors, were 0.48 μM and 40 pmoles/mg protein/min, respectively. The K_m and V_{max} values for 5α-reductase under the optimum incubation conditions, without inhibitors, were 40 nM and 2 pmoles/mg protein/min, respectively. Each value is the mean of duplicate determinations in at least three separate experiments. ni = no inhibition.

3. Results

The Michaelis–Menten kinetic constants, K_m and V_{max}, for human testicular 17α-hydroxylase/$C_{17,20}$-lyase (lyase) were 480 nM and 40 pmoles/mg protein/min, respectively. Several of these novel compounds exhibit potent noncompetitive inhibition of human lyase with IC_{50} values ranging from 7 to 90 nM, and K_i values from 1.2 to 41 nM (Fig. 2 and Table 1). **VN/85-1** and **VN/108-1** were the most potent inhibitors against this enzyme with IC_{50} value of 8 nM (K_i of 1.2 nM) and 7 nM (K_i of 1.9 nM), respectively. Thus, with the exception of **VN/63-1**, these novel compounds were more potent than ketoconazole (IC_{50} = 76 nM and K_i = 38 nM), a known inhibitor of this enzyme. The K_m and V_{max} values for human prostatic 5α-reductase were 40 nM and 2 pmoles/mg protein/min, respectively. **VN/107-1**, **VN/108-1** and **VN/109-1** also showed moderate inhibition of 5α-reductase in human prostatic microsomes although finasteride, a potent inhibitor of 5α-reductase, was more potent with an IC_{50} value of 33 nM (Table 1).

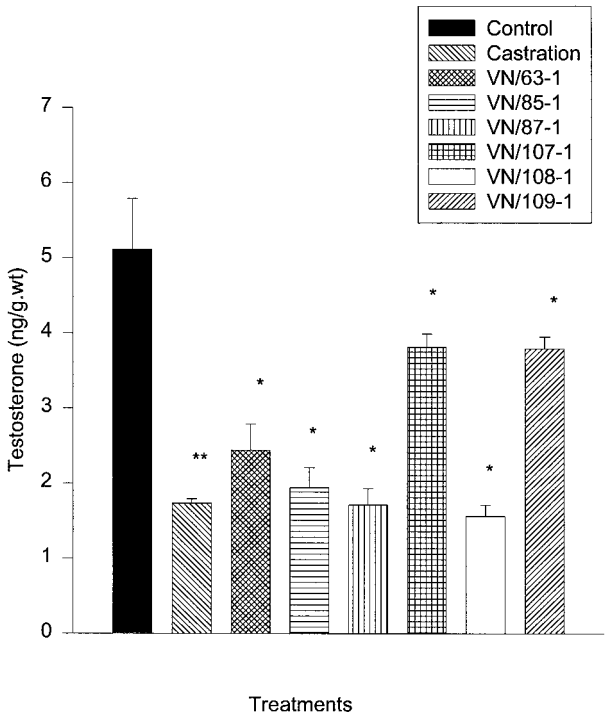

Fig. 3. Effects of some 17-azolyl inhibitors on testosterone (T) levels in prostates obtained from normal adult male rats. Normal adult male rats (~240 g) were injected with the compounds listed (50 mg/kg/day, s.c., for 14 days). The animals were sacrificed about 1–2 h after the last administered dose. The prostate was removed and tissue T concentration was determined by RIA, as described in Materials and methods. Values are the mean ± standard error from 6–8 rats. * $p < 0.05$, ** $p < 0.01$.

Table 2
Effects of some 17-azolyl inhibitors on testosterone and dihydrotestosterone levels in tissues obtained from normal adult male rats[a]

Treatment	T levels		DHT levels
	serum (ng/ml)	testis (ng/g.wt)	serum (pg/ml)
Control	4.07 ± 0.64	567.20 ± 65.10	164.20 ± 14.34
Castrated	0.06 ± 0.04 **	–	44.00 ± 16.40 **
VN/63-1	2.00 ± 0.28 *	131.10 ± 21.20**	46.76 ± 6.27**
VN/85-1	0.31 ± 0.08**	53.98 ± 14.70**	92.00 ± 10.24 *
VN/87-1	0.61 ± 0.08 **	91.18 ± 15.20 **	52.40 ± 10.45 **
VN/107-1	0.89 ± 0.10**	131.60 ± 22.00**	66.29 ± 14.03**
VN/108-1	0.58 ± 0.18**	56.30 ± 14.38**	99.60 ± 14.03*
VN/109-1	0.69 ± 0.14**	80.00 ± 14.43**	66.52 ± 11.78 **

[a] Normal adult male rats (~240 g) were injected with the compounds listed (50 mg/kg/day) for 14 days. Blood was collected and tissue testosterone (T) and dihydrotestosterone (DHT) concentrations were determined by RIA, as described under Materials and methods. Values are the mean ± standard error from 6–8 rats. * $p < 0.05$, ** $p < 0.01$.

In studies of normal male rats, T levels in the serum were significantly ($p < 0.05$) lower in animals treated with the 17-azolyl compounds by 50–90% compared to the control group (Table 2). **VN/85-1** was the most effective compound in this regard and reduced serum T levels by more than 90% compared to controls. The concentration of T in the testis was significantly ($p < 0.01$) lower in rats treated with the steroids by more than 75% compared to the control group. **VN/85-1** and **VN/108-1** were the most potent steroids in lowering testicular T levels. The amount of T in the prostates was also significantly ($p < 0.05$) lower in rats treated with these 17-azolyl compounds by 25–

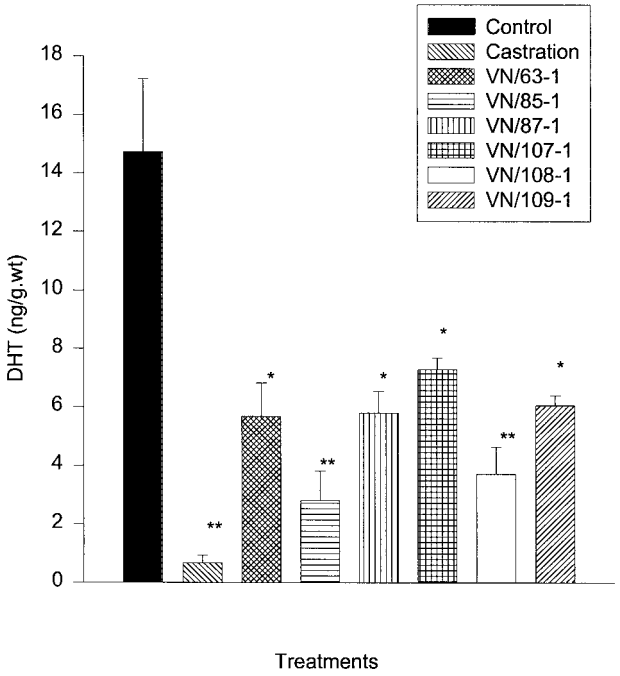

Fig. 4. Effects of some 17-azolyl inhibitors on DHT levels in prostates obtained from normal adult male rats. Normal adult male rats (~240 g) were injected with the compounds listed (50 mg/kg/day, s.c., for 14 days). The animals were sacrificed about 1–2 h after the last administered dose. The prostate was removed and tissue DHT concentration was determined by RIA, as described in Materials and methods. Values are the mean ± standard error from 6–8 rats. * $p < 0.05$, ** $p < 0.01$.

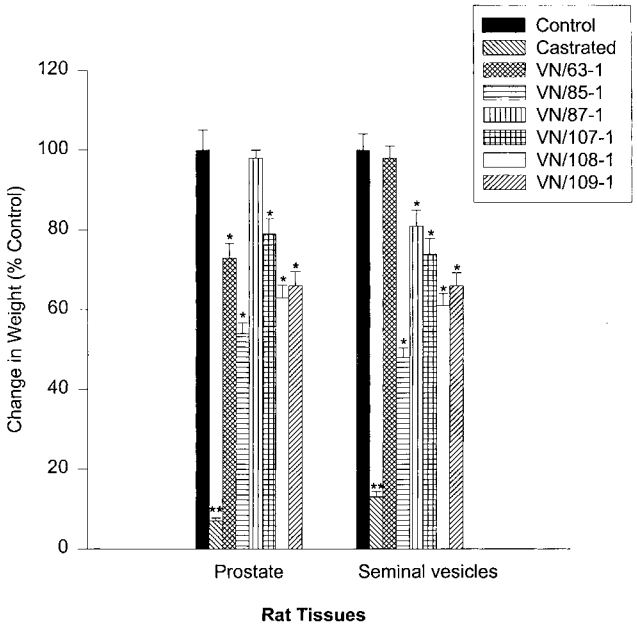

Fig. 5. The effects of some 17-azolyl inhibitors on the wet weights of rat prostates and seminal vesicles. Normal adult male rats (~240 g) were injected with the compounds listed (50 mg/kg/day, s.c., for 14 days). The animals were sacrificed about 1–2 h after the last administered dose. Prostates and seminal vesicles were removed and weighed. Values are expressed as the percentage of the control weights. Values are mean ± standard error from 6–8 rats. * $p < 0.05$, ** $p < 0.01$.

70% compared to the control group (Fig. 3). **VN/85-1**, **VN/87-1** and **VN/108-1** were the most effective and reduced T levels in rat prostates to about the same level as castration. Similarly, DHT levels in the serum were significantly lower in rats treated with these 17-azolyl compounds by 39–68% compared to the control group. In fact, **VN/63-1** and **VN/87-1** were very effective in this regard and showed similar potency as castration in lowering DHT concentration in rat serum (Table 2). The concentration of DHT in the prostates were significantly lower in rats treated with the novel steroidal compounds by 50–80% compared to the control group although castration was more effective in achieving lower levels of DHT in the prostates (Fig. 4). **VN/85-1** appeared particularly effective and reduced prostatic DHT levels by about 80%.

The inhibitory effects of these novel 17-azolyl compounds on the wet weight of rat tissues were assessed after the animals were sacrificed. The compounds (except **VN/87-1**) significantly ($p < 0.05$) reduced the wet weights of the prostates by 21–46% (Fig. 5). Similarly, the steroids (except **VN/63-1**) reduced the wet weights of the seminal vesicles by 19–52% (Fig. 5). **VN/85-1** and **VN/108-1** also significantly ($p < 0.05$) reduced the wet weights of the epidydemis by 29 and 24%, respectively. The 17-azolyl compound, **VN/108-1**, also reduced the wet weight of the testis by about 17%. **VN/85-1** was the most effective compound in reducing the weights of the prostate and seminal vesicles in rats.

4. Discussion

17α-hydroxylase/$C_{17,20}$-lyase (lyase) and 5α-reductase are pivotal enzymes in the biosynthesis of T, DHT and other androgens. Androgens, particularly DHT have been shown to promote prostatic tumor growth and therefore compounds that reduce androgen synthesis and action may be useful in the treatment of androgen dependent diseases such as BPH and prostate cancer [23,24]. The novel 17-azolyl steroids described here markedly reduced the levels of T and DHT in rat serum and tissues when the animals were treated with these compounds at a dose level of 50 mg/kg for 14 consecutive days. The 50 mg/kg/day was used as the starting dose and was based on the efficacy of similar compounds in our previous studies [6]. The reduction in androgen levels is consistent with the inhibitory effects shown by these compounds on human lyase and 5α-reductase activity in vitro. **VN/85-1**, **VN/87-1**, **VN/108-1** and **VN/109-1** exhibit potent noncompetitive inhibition of human testicular lyase. In comparison,

VN/107-1 and **VN/63-1** were moderate inhibitors of the human testicular lyase. The K_i values of several of the novel inhibitors were significantly lower than that for ketoconazole indicating their higher affinity for the enzyme. Thus, the novel compounds were more potent than ketoconazole, a known inhibitor of this enzyme used clinically for the treatment of prostate cancer [7]. The imidazole and the triazole functionalities and the presence of the nitrogen atoms at the 3′ or 4′ position of the azole heterocycle, as well as the 16,17-double bond appear to be critical for potent inhibitory activity against the human testicular lyase [17].

The Δ^4 steroids (**VN/107-1**, **VN/108-1** and **VN/109-1**) showed moderate inhibition of 5α-reductase in human prostatic microsomes although finasteride, a known potent and clinically available inhibitor of 5α-reductase, was approximately 4–5 times more potent than these novel azolyl compounds [16,25]. The Δ^4-3-keto functionality of these novel compounds appears to be critical for 5α-reductase inhibition. The absence of this group in the Δ^5 derivatives (**VN/63-1**, **VN/85-1** and **VN/87-1)** may explain, at least in part, the lack of 5α-reductase inhibitory activity by these compounds. Thus, all the novel Δ^4 compounds exhibit dual activity against human testicular lyase and prostatic 5α-reductase. Inhibition of both the lyase and 5α-reductase is an attractive strategy for reducing androgen production. Compounds with dual activity against these critical enzymes in the androgen synthesis pathway, as exhibited by **VN/107-1**, **VN/108-1** and **VN/109-1,** would be expected to be especially useful in the complete ablation of circulating androgens [9]. However, it would appear from the results presented that the dual inhibitors (**VN/107-1**, **VN/108-1** and **VN/109-1**) were no more effective in lowering androgen concentrations in rat tissues than the Δ^5 steroids, that were almost exclusively inhibitors of the lyase. The levels of T in rat prostates were significantly reduced by all compounds examined, at or near the level achieved by castration and DHT levels were suppressed by more than 60% by these compounds.

The effects of the 17-azolyl compounds on tissue weights were also evaluated in male rats. The wet weights of rat prostates were significantly reduced by all compounds except, **VN/87-1**. The observed lack of efficacy by **VN/87-1** in this regard could not easily be explained, as it caused significant reduction in serum T and DHT levels in prostate tissue. Whether the compound is metabolized to an androgenic form or has intrinsic androgenic activity would require further investigation. The wet weights of the seminal vesicles were also significantly reduced by all the compounds tested with the exception of **VN/63-1**. In addition, **VN/85-1** and **VN/108-1** reduced the wet weights of the epididymis. The results indicate that these compounds may be causing shrinkage of these organs by suppressing androgen synthesis.

VN/85-1 was consistently the most potent compound and had the greatest effect on serum and tissue T levels, on tissue DHT levels and on tissue weight. Although the reduction in tissue weight was only about 50% compared with a reduction of about 90% by castration, only one dose level has been used in these initial studies. Further investigations over a dose range and with improvements in bioavailability may increase efficacy in vivo. Studies of the metabolism and disposition of these compounds may help explain the weak correlation between the in vitro inhibitory action, reduction of circulating androgen levels in vivo and shrinkage of rat prostatic tissue size.

The body weight and gross size and weights of the liver, kidney and adrenals were not altered by the compounds in the present investigation. Although structure-activity relationships with related steroidal compounds suggest that these novel 17-azolyl compounds are largely specific inhibitors of the lyase [11,17] we plan to ascertain their enzyme and receptor specificity of action. Recent studies in our laboratory indicate that the 17-azolyl compounds were effective in blocking the growth stimulatory effects of androgens on LNCaP cells. **VN/107-1**, **VN/108-1** and **VN/109-1,** for example, decreased the proliferation of LNCaP cells by 35–40% in steroid-free media [19]. In receptor binding studies, **VN/107-1**, **VN/108-1** and **VN/109-1** (5 μM) displaced 77–82% of the synthetic androgen, ^{3}[H]R1881 (5 nM), from LNCaP androgen receptor [19]. These compounds have also been shown to be effective inhibitors of tumor growth in male SCID mice bearing LNCaP tumor xenografts [19].

In conclusion, we have demonstrated that these novel 17-azolyl steroids are very potent inhibitors of human testicular lyase and some are also moderate inhibitors of human prostatic 5α-reductase. The compounds also significantly reduce T and DHT concentration in rat tissues and were effective at diminishing the size of the prostate and appear to be well tolerated in the animals. These findings, in conjunction with the observed antiandrogenic and antiproliferative effects on LNCaP cells in vitro and in vivo [19], suggest that these novel compounds may provide useful leads for the research and development of suitable steroidal agents for the treatments of androgen dependent prostate cancer.

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