Antiangrogenic Effects of Novel Androgen Synthesis Inhibitors on Hormone-dependent Prostate Cancer

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ABSTRACT

We have found that in addition to being potent inhibitors of 17α-hydroxylase/C17,20-lyase and/or 5α-reductase, some of our novel androgen synthesis inhibitors also interact with the mutated androgen receptor (AR) expressed in LNCaP prostate cancer cells and the wild-type AR expressed in hormone-dependent prostatic carcinomas. The effects of these compounds on the proliferation of hormone-dependent human prostatic cancer cells were determined in vitro and in vivo. L-2 and L-10 are Δ1,3-one-pregnan derivatives. L-35 and L-37 are Δ1,3β-ol-androstane derivatives, and L-36 and L-39 are Δ3,3-one-androstane-derived compounds. L-2, L-10, and L-36 (L-36 at low concentrations) stimulated the growth of LNCaP cells, indicating that they were interacting agonistically with the mutated AR expressed in LNCaP cells. L-35, L-37, and L-39 acted as LNCaP AR antagonists. To determine whether the growth modulatory effects of our novel compounds were specific for the mutated LNCaP AR, competitive binding studies were performed with LNCaP cells and PC-3 cells stably transfected with the wild-type AR (designated PC-3AR). Regardless of AR receptor type, all of our novel compounds were effective at preventing binding of the synthetic androgen methyltrienolone [17α-methyl-(3H)-R1881] to both the LNCaP AR and the wild-type AR. L-36, L-37, and L-39 (5.0 μM) prevented binding by >90%, whereas L-35 inhibited binding by 30%. To determine whether the compounds were acting as agonists or antagonists, LNCaP cells and PC-3AR cells were transfected with the pMAMneoLUC reporter gene. When luciferase activity was induced by dihydrotestosterone, all of the compounds were acting as agonists or antagonists. The effects of these compounds on the growth of normal and malignant prostate tissue, DHT is believed to be the more important androgen (5, 6).

INTRODUCTION

Prostatic carcinoma is the most commonly diagnosed malignancy in men in the United States and is second only to lung cancer in cancer-related deaths (1, 2). Androgens play an important role in controlling the growth of the normal prostate gland, and in promoting BPH and prostatic carcinoma (3). The two most important androgens in prostate cancer etiology are testosterone and DHT. Testosterone is synthesized primarily in the testes and also, to a lesser extent, in the adrenals. Testosterone is further converted to the more potent androgen DHT by the enzyme 5α-reductase, which is localized primarily in the prostate (4). Although testosterone and DHT both stimulate the growth of normal and malignant prostate tissue, DHT is believed to be the more important androgen (5, 6).

Androgen ablation therapy has been shown to produce the most beneficial responses in patients with hormone-responsive prostatic tumors. Orchidectomy (castration, either surgical or medical with a luteinizing hormone-releasing hormone analogue) remains the standard treatment option for most patients. However, both methods, which result in reduced androgen production by the testes, fail to alter androgen production by the adrenal glands. Studies in the United States and Europe have reported that although androgen ablation therapy alone is an effective treatment, a combination therapy of orchidectomy with antiandrogens, to inhibit the action of adrenal androgens, significantly prolongs the survival of prostate cancer patients (7–9). Given that efforts to block the production or effects of adrenal androgens result in worthwhile therapeutic gains, this laboratory has been designing and evaluating novel compounds that inhibit androgen production from all sites in the body. The compounds developed are steroidal antagonists of the steroidogenic enzymes C17,20-lyase and 5α-reductase. C17,20-lyase catalyzes both the 17α-hydroxylation and the cleavage of the C17,20-side chain during the conversion of the 21-carbon steroids pregnenolone and progesterone to the 19-carbon androgens dehydroepiandrosterone and androstenedione, respectively (10). The enzyme has an identical amino acid sequence in both testicular and adrenal tissue (11), indicating that inhibitors of this enzyme would be equally effective at both sites. Two isoforms of 5α-reductase occur in the body (type I and type II), and the type II enzyme is the predominant form in the human prostate. Inhibitors of this isoenzyme would essentially prevent prostatic accumulation of the potent androgen DHT.

A number of C17,20-lyase-inhibitors have been described. However, most are not specific, and only the imidazole antifungal agent ketoconazole has been used clinically to reduce testosterone levels in patients with advanced prostate cancer (12–14). The major drawback with ketoconazole is that it is not very potent or specific. It is only a moderate inhibitor of C17,20-lyase, inhibits cortisol production, and has a number of significant side effects. Nevertheless, recent studies have reported that ketoconazole was effective in reducing PSA levels in 55% (15) and 62.5% (16) of patients who had progressed after antiandrogen (flutamide) withdrawal. These results indicate that compounds more specific and selective than ketoconazole may be more effective for the treatment of prostate cancer. Several inhibitors of 5α-reductase have also been described and finasteride, which is a more potent inhibitor of the type II than the type I isoenzyme (17), has been approved for the treatment of BPH (18). Although effective at

\[\text{C}_17\text{,}_20\text{-lyase}\]

\[\text{5α-reductase}\]

\[\text{BPH}\]

\[\text{prostatic carcinoma}\]

\(\text{PSA}\)

\(\text{improved minimum essential medium}\)

\(\text{PC-3AR}\)

\(\text{androgen receptor}\)

\(\text{G418}, \text{geneticin}\)

\(\text{FBS}, \text{fetal bovine serum}\)

\(\text{DPBS}, \text{Dulbecco’s PBS}\)

\(\text{[17α-methyl-(3H)]-R1881}\)

\(\text{IMEM}, \text{immediate} \text{medium}\)

\(\text{LNCaP}\)

\(\text{PC-3}\)

\(\text{PC-3AR}\)

\(\text{DHT}\)

\(\text{PSA}\)

\(\text{BPH}\)

\(\text{prostatic carcinoma}\)

\(\text{PSA}\)

\(\text{improved minimum essential medium}\)

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4 The abbreviations used are: BPH, benign prostatic hyperplasia; DHT, dihydrotestosterone; C17,20-lyase, cytochrome P450 17α-hydroxylase/C17,20-lyase; PSA, prostate-specific antigen; AR, androgen receptor; G418, gentamicin; FBS, fetal bovine serum; DPBS, Dulbecco’s PBS; [1H]1881, methyltrienolone [17α-methyl-(3H)]-R1881; IMEM, improved minimum essential medium.
reducing DHT levels in patients with prostate cancer, finasteride also increases the bioavailable levels of testosterone (19), which can stimulate tumor growth (20). Therefore, compounds designed to inhibit both C17,20-lyase and 5α-reductase would be expected to be more clinically beneficial to prostate cancer patients than compounds which inhibit only one of the enzymes.

The growth effects of testosterone and DHT on prostate cancer cells are mediated by the androgens binding to their cognitive nuclear receptors, which in turn bind to specific response elements in the promoter regions of androgen-regulated genes. The products of these genes modulate cellular proliferation (21). Androgens such as flutamide have also been used clinically for the treatment of prostate cancer. However, the results were disappointing. Some patients tended to improve after flutamide was withdrawn after relapse (22, 23). Nonetheless, as described above, clinical trials which combined the therapies of orchidectomy with the antiandrogen flutamide reported a significantly longer survival period than orchidectomy alone (7–9). This implies that a treatment regimen involving total androgen ablation in combination with an antiandrogen may be a more effective treatment option for patients with androgen-dependent prostate tumors.

Previously we have reported the synthesis and testing of several steroidal inhibitors of C17,20-lyase and 5α-reductase (24–28). These compounds were shown to be effective inhibitors of human testicular C17,20-lyase and prostatic 5α-reductase in vitro (See Table 1). In the present study we report that some of these compounds are also very potent antiandrogens, and that this property contributes, at least in part, to their growth-inhibitory effects on androgen-dependent LNCaP prostate cancer cells in vitro and in vivo. LNCaP cells are the most frequently studied AR-positive prostate cancer cell line that can be readily grown in tissue culture (29–31). LNCaP cells are not only androgen-responsive but also androgen-dependent. They respond to androgens with increased cellular proliferation and elevated expression of PSA (32). Although LNCaP cells have a mutated AR (33, 34), they have been used extensively for research on the causes, treatment, and prevention of prostate cancer (35). We have used these cells in culture and as tumors in nude mice to compare the efficacy of our compounds with the known inhibitors of C17,20-lyase and 5α-reductase, ketoconazole, and finasteride, respectively. The antiandrogenic properties of the compounds were compared with flutamide.

### MATERIALS AND METHODS

**Materials.** The wild-type LNCaP human prostate cancer cell line and CV-1 green monkey kidney cells were obtained from the American Type Culture Collection (Rockville, MD). PC-3 prostate cancer cells and PC-3 stably transfected with the human wild-type androgen receptor (designated PC-3AR) were kindly provided by Dr. Marco Marcelli (Baylor College of Medicine, Houston, TX; Refs. 36 and 37). RPMI 1640 medium, penicillin/streptomycin solution, trypsin/EDTA solution, G418, Opti-MEM, Lipofectamine, and Lipofectamine-Plus were from Life Technologies, Inc. (Grand Island, NY). Phenol red-free IMEM and phenol red-free trypsin/EDTA were from Biofluids Inc. (Rockville, MD). FBS was supplied by Summit Biotechnology, Inc. (Fort Collins, CO), and the PSA ELISA kit was from DSL, Inc. (Webster, TX). Hydroxypropyl cellulose was from Sigma Chemical Co. (St. Louis, MO). The luciferase reporter construct pMAMneoLUC was purchased from Clontech (Palo Alto, CA). The luciferase assay kit was from Promega (Wisconsin, MI). Wild-type human AR-expressing vector pCMV5-hAR and the LNCaP AR-expressing vector pCMV5-LNCaPAR were generous gifts from Dr. Elizabeth Wilson (University of North Carolina, Chapel Hill, NC). Matrigel was kindly supplied by Dr. Hynda Kleinman (NIH, Bethesda, MD).

**Steroids and Chemical Inhibitors.** Testosterone, DHT, flutamide, ketoconazole, and the antiprogestin triamcinolone acetonitride were purchased from Sigma Chemical Co. (St. Louis, MO). Finasteride was a gift from Merck Research Laboratories (Rahway, NJ). L-2, L-10, L-35, L-36, L-37, and L-39 were synthesized in our laboratory according to the procedures described by Ling et al. (27). [3H]R1881 (specific activity 70–87 Ci/mMol) was obtained from DuPont NEN (Boston, MA). Testosterone RIA kits were purchased from DSL, Inc.

**Cell Culture.** LNCaP, PC-3, and CV-1 cells were grown in RPMI 1640 medium supplemented with 10% FBS and 1% penicillin/streptomycin solution. To determine the effect of steroids and novel compounds on cell proliferation, hormone-dependent LNCaP and hormone-independent PC-3 cells were transferred into steroid-free medium 3 days prior to the start of experiments. Steroid-free medium consisted of phenol red-free IMEM supplemented with 5% dextan-coated, charcoal-treated serum, and 1% penicillin/streptomycin solution. Serum was depleted of steroids as described previously (38). Growth studies were then performed by plating cells (1.5 × 10⁴) in 24-place multwell dishes (Corning, Inc., Corning, NY). After a 24-h attachment period, the medium was aspirated and replaced with steroid-free medium containing vehicle or the indicated concentrations of steroids and novel compounds. This medium was changed every 3 days and the cells were counted 9 days later using a Coulter Counter model Z-1 (Coulter Electronics, Hialeah, FL). Cell numbers were expressed as a percentage of vehicle-treated cells.

Wild-type LNCaP cells and CV-1 cells were transfected with the pMAMneoLUC plasmid as we have described previously (39). Briefly, 2 × 10⁵ cells in a 35-mm² dish were exposed to 3 ml of Opti-MEM containing Lipofectamine (30 μl) and 6 μg of the pMAMneoLUC plasmid for 5 h at 37°C in a 5% CO₂ incubator. The medium was then changed to routine culture medium for 72 h. Cells were then grown in medium supplemented with 750 μg/ml G418. The surviving colonies were picked and grown in selective media. Stable transfectants were selected twice per month for luciferase activity, as described in the later section. After 3 months (~23 passages), the transfectants with the highest luciferase activity were selected and designated LNCaP-LUC and CV-1LUC, respectively. These cell lines and the PC-3AR cell line were routinely cultured in RPMI 1640 medium supplemented with 10% FBS, 1% penicillin/streptomycin solution, and 750 μg/ml G418.

**Competitive Binding of [3H]R1881 to the LNCaP AR and Wild-Type AR in the Presence of Novel Compounds.** Competitive binding studies with the synthetic androgen R1881 were performed essentially as described by Wong et al. (40) and Yarbrough et al. (41). Wells in 24-place multwell dishes were coated with poly-L-lysine (0.05 mg/ml) for 30 min and dried. To determine the kinetics of R1881-binding to the LNCaP AR and the wild-type AR, LNCaP cells and PC-3AR cells (2–3 × 10⁴) were plated in steroid-free medium and allowed to attach. The following day, the medium was replaced with serum-free, steroid-free IMEM supplemented with 0.1% BSA and containing [3H]R1881 (0.01–10 nM) in the presence or absence of a 200-fold excess of cold R1881 to determine nonspecific binding and 1 μM triamcinolone acetonitrile to saturate progesterone and glucocorticoid receptors. Following a 2-h incubation period at 37°C, cells were washed twice with ice-cold DPBS and solubilized in DPBS containing 0.5% SDS and 20% glycerol. Extracts were removed and the cell-associated radioactivity counted in a scintillation counter. The data were analyzed using RADUG 40 software (Biosoft, Ferguson, MO), and Kᵦ and Bₘ, determined by Scatchard plot transformation. When the concentration of R1881 required to almost saturate AR in both cell lines was established, the ability of the test compounds (5.0

### Table 1 IC₅₀ values (nM) of the steroidal compounds against human testicular C₁7,2₀-lyase and prostatic 5α-reductase from human BPH microsomes

<table>
<thead>
<tr>
<th>Compounds</th>
<th>C₁7,2₀-lyase IC₅₀ (nM)</th>
<th>5α-Reductase IC₅₀ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-2</td>
<td>43 ± 1.2*</td>
<td>75 ± 1.3</td>
</tr>
<tr>
<td>L-10</td>
<td>NI</td>
<td>19 ± 1.5</td>
</tr>
<tr>
<td>L-35</td>
<td>21 ± 1.7</td>
<td>NI</td>
</tr>
<tr>
<td>L-36</td>
<td>39 ± 1.0</td>
<td>31 ± 2.0</td>
</tr>
<tr>
<td>L-37</td>
<td>42 ± 1.9</td>
<td>1277 ± 39</td>
</tr>
<tr>
<td>L-39</td>
<td>67 ± 8.5</td>
<td>33 ± 2.5</td>
</tr>
<tr>
<td>Finasteride</td>
<td>NI</td>
<td>33 ± 1.1</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>76 ± 2.0</td>
<td>NI</td>
</tr>
</tbody>
</table>

*Values show the mean ± SE of three experiments. NI, No Inhibition. These results have been previously reported in Ref. 27.
µm) to displace [3H]R1881 (5.0 nm) from the receptors was determined as described above.

Transient Transfection of PC-3AR Cells with the pMAMneoLUC Plasmid and CV-1LUC Cells with the pCMV5-hAR and pCMV5-LNcAPAR Plasmids. Cells were grown in steroid-free IMEM for 3 days and plated (4 x 10⁶ cells/well) in 24-well plates in phenol red-free IMEM supplemented with 10% charcoal-stripped serum and no antibiotics. After a 24-h incubation period, the cells were washed twice with DPBS and each well was incubated with 250 µl of phenol red-free IMEM containing 2 µl of PLUS-reagent, 4 µl of Lipofectamine, and 4 µg of the pMAMneoLUC plasmid for PC-3AR cells and 0.5 µg of the pCMV5-hAR or pCMV5-LNcAPAR plasmids for CV-1LUC cells. After a 5-h incubation period, 250 µl of routine medium were added to each well and the cells were incubated for an additional 24 h. The resultant cells, designated PC-3AR/LUC, CV-1LUC/hAR, or CV-1LUC/LNcAPAR, were assayed for luciferase activity as described in the following section.

Luciferase Activity Assay. LNCaP-LUC cells were transferred to steroid-free medium 3 days before the start of the experiment and plated at 1 x 10⁵ cells/well in steroid-free medium. PC-3AR/LUC cells, CV-1LUC/hAR, and CV-1LUC/LNcAPAR cells were transiently transfected as described above. After a 24-h incubation period in steroid-free medium, each well was treated with ethanol vehicle or the selected steroids and novel compounds (5 µM) in triplicate. After a 24-h treatment period, the cells were washed twice with ice-cold DPBS and assayed using the Luciferase kit according to the manufacturer’s protocol. Briefly, the cells were lysed with 200 µl of luciferase lysis buffer, collected in a microcentrifuge tube and pelleted by centrifugation. Supernatants (100 µl aliquots) were transferred to the corresponding wells of white 96-well plates (Polyfiltronics, Inc., Boston, MA). Luciferin (50 µl) was added to each well, and the light produced during the luciferase reaction was measured in a Victor 1420 Multilabel counter (Wallac, Inc., Gaithersburg, MD). The effects of the steroids and novel compounds on DHT-induced luciferase transcription were determined using the same protocol.

In Vivo Studies with LNCaP Tumors in SCID Mice and PC-82 Xenografts in Athymic Nude Mice. Male SCID mice and athymic nude mice 4–6 weeks of age were purchased from the National Cancer Institute (Frederick, MD). Animals were housed in a pathogen-free environment under controlled conditions of light and humidity and received food and water ad libitum.

Inoculation of LNCaP Cells into Male SCID Mice. LNCaP tumors were grown s.c. in male SCID mice essentially as described by Sato et al. (42) with modifications based on the breast cancer model described by Yue et al. (43, 44). LNCaP cells were grown in routine culture medium (RPMI 1640 medium supplemented with 10% FBS and 1% penicillin/streptomycin) until 80% confluent. Cells were scraped into DPBS, collected by centrifugation, and resuspended in Matrigel (10 mg/ml) at 3 x 10⁶ cells/ml. Each mouse received s.c. injections at one site on each flank with 100 µl of cell suspension. Tumors were measured weekly with calipers, and tumor volumes were calculated by the formula 0.5236 x r₁² x r₂ (r₁ < r₂).

Inoculation of PC-82 Xenograft Tumors Into Male Athymic Nude Mice. Hormone-dependent PC-82 tumor xenografts were kindly provided by Dr. John Isaacs (John Hopkins School of Medicine, Baltimore, MD). PC-82 tumors were minced into fine pieces, washed with DPBS, filtered, and resuspended in Matrigel at a concentration of 100 mg/ml. The mice were inoculated s.c. at one site on each flank with 100 µl of tumor suspension using a 18-gauge needle. Tumors were allowed to grow (~3 months) and then were measured weekly. Tumor volumes were then calculated weekly according to the formula 0.5236 x r₁² x r₂ (r₁ < r₂). When tumors reached a measurable size, the mice were randomized into treatment groups (five animals/group) and treated as described in the following section.

Treatment. For LNCaP tumor experiments, treatments began 4–5 weeks after cell inoculation when measurable tumor volume was 300 mm³ (For PC-82 xenografts, this was ~3 months). For each experiment, groups of six mice (five mice for PC-82 xenografts) with comparable total tumor volumes were either castrated or treated with the novel compounds at 50 mg/kg/day. Mice were castrated under methoxyflurane anesthesia via the abdominal approach. Compounds and reference drugs were prepared at 10 mg/ml in a 0.3% solution of hydroxypropyl cellulose in saline, and mice received s.c. injections daily. Control and castrated mice were treated with vehicle only. Treatments lasted for 28 days, after which time the animals were sacrificed by decapitation and the blood was collected. Tumors were excised, weighed, and stored in liquid nitrogen for additional analysis.

Testosterone RIA Assays. For measurement of serum testosterone levels, 50 µl of mouse serum were assayed according to the instructions provided with the [125I]-testosterone RIA kit supplied by DSL, Inc. Radioactivity was counted using a Packard Cobra II gamma counter. For measurement of tumor testosterone levels, whole tumors were homogenized in phosphate buffer (pH 7.4; 0.1 M). The homogenates were then centrifuged at 2000 x g for 20 min to remove debris. Fifty-µl aliquots of the tissue supernatant were used to determine the tumor testosterone concentration, as described above.

Measurement of Serum PSA Levels. Serum PSA levels were determined using a PSA ELISA kit supplied by DSL, Inc. Briefly, 2.5 µl of serum diluted 1:10 in DPBS was mixed with assay buffer to a final volume of 75 µl and added to duplicate wells in the 96-well plate that had been coated with an anti-PSA antibody. Following a 1-h assay and extensive washing of the plate, the wells were treated for 30 min with a second anti-PSA antibody labeled with horseradish peroxidase. After washing, the wells were treated with the tetramethylbenzidine substrate for 10 min, and the absorbance was read at 450 nm with a Dynatech MRX plate reader.

Statistical Analysis. One-way ANOVA on SigmaStat for Windows version 1.0 was used to compare the different treatment groups at the 95% confidence level. A P of <0.05 was considered to be statistically significant.

RESULTS

Growth Effects on LNCaP and PC-3 Cell Cultures in Vitro. The structures of the compounds are shown in Fig. 1, and their in vitro activities against C₁₇,₂₀-lyase and 5α-reductase are provided in Table 1. For growth studies, androgen-responsive LNCaP cells and androgen-independent PC-3 cells were deprived of steroids for 3 days.
INHIBITION OF HORMONE-DEPENDENT PROSTATE CANCER CELL GROWTH

INCREASED CELL NUMBER BY 5.9-FOLD AND 4.4-FOLD, RESPECTIVELY. L-35 AND L-39 WERE ALSO EFFECTIVE AT REVERSING THE GROWTH EFFECTS OF DHT AND DID SO IN A DOSE-DEPENDENT MANNER. AT THE 5.0 \( \mu M \) CONCENTRATION, L-35 AND L-39 INHIBITED DHT-INDUCED LNCaP CELL PROLIFERATION BY 66% AND 79%, RESPECTIVELY. THESE RESULTS SUGGEST THAT THE COMPOUNDS MAY BE ACTING TO BLOCK THE ACTION OF DHT IN STIMULATING CELL PROLIFERATION.

The ability of the novel compounds to compete with testosterone for the AR and modulate cell growth in vitro was also investigated (Fig. 3B). When cells were cotreated with testosterone (1 nM) and the reference drugs or novel compounds for 9 days, the results were similar to those obtained when cell growth was stimulated by DHT. L-37, L-35, and L-39 again proved to be the most effective compounds at inhibiting testosterone-induced growth of LNCaP cells in vitro. Each of these compounds inhibited growth in a dose-dependent manner.

**LNCaP and PC-3AR Androgen Receptor Binding Assays.** The ability of the novel compounds to modulate the growth of LNCaP cells and to inhibit DHT and testosterone-induced cell proliferation implied that they may be interacting with the LNCaP AR. To determine whether this was specific for the mutated AR expressed in LNCaP cells, kinetic studies were performed with the synthetic androgen methyltrienolone \([^3H]R1881\) binding to the mutated AR expressed in LNCaP cells and the wild-type AR expressed in PC-3AR cells. The data were linearized by Scatchard transformation (data not shown) and the \( K_d \) and \( B_{max} \) for both types of AR were determined.

LNCaP cells express a single class of high affinity binding sites with \( K_d = 0.78 \pm 0.01 \text{ nM} \) and \( B_{max} = 2.60 \times 10^4 \pm 2.27 \times 10^4 \) receptors/cell. PC-3AR cells also express a single class of high-affinity binding sites with \( K_d = 0.20 \pm 0.01 \text{ nM} \) and \( B_{max} = 4.8 \times 10^4 \pm 6.5 \times 10^4 \) receptors/cell. The ability of the compounds to compete with 5 nM \([^3H]R1881\) for binding to both types
of AR was determined (Fig. 4). Ketoconazole was ineffective at preventing [3H]R1881 from binding to the AR in either cell line. The antiandrogen flutamide prevented 52% of the [3H]R1881 from binding to the LNCaP AR, and 51% of the labeled androgen from binding to the wild-type AR in PC-3AR cells. Interestingly, L-35, which was one of the most effective compounds at inhibiting the growth of LNCaP cells in vitro, was the least effective compound at preventing [3H]R1881 from binding to either type of AR. In LNCaP cell cultures, L-35 prevented 34% of the [3H]R1881 from binding to the AR, and in PC-3AR cells, the inhibition was 31%. Each of the other 5 novel compounds were very effective at preventing [3H]R1881 from binding to the cellular AR. The pattern was similar regardless of whether they were tested against the mutated LNCaP AR or the wild-type AR. Moreover, they prevented [3H]R1881 from binding to the ARs with higher efficiencies than flutamide. In the presence of L-2, L-10, L-36, L-37, and L-39, the amount of [3H]R1881 bound to the LNCaP cells was 30%, 5.2%, 0.7%, 15.2%, and 3.3%, respectively. In PC-3AR cells, the amount of [3H]R1881 bound in the presence of L-2, L-10, L-36, L-37, and L-39 was 13.5%, 2.6%, 5.1%, 11.7%, and 2.5%, respectively.

Effects of Novel Compounds on LNCaP AR- and Wild-Type AR-Mediated Transcription. To determine whether the compounds are acting as AR agonists or antagonists, luciferase activity assays were performed using LNCaP-LUC and PC-3AR/LUC cells. DHT (5.0 nM) significantly (P < 0.001) stimulated luciferase production mediated by the LNCaP AR in LNCaP-LUC cells (Fig. 5A). Ketoconazole (5.0 μM) and flutamide (5.0 μM) also activated transcription of the luciferase gene in LNCaP-LUC cells by approximately 2-fold each. Finasteride had no effect on luciferase activity, L-2, L-10, and L-36 (all at 5 μM) stimulated luciferase production in LNCaP-LUC cells by 6-fold, 4.5-fold, and 8-fold, respectively, confirming that each of these compounds are agonists of the mutated LNCaP AR. L-39 also, surprisingly, stimulated luciferase activity in LNCaP-LUC cells by 2.5-fold, which suggested that it also has some agonistic properties. L-35 and L-37 (5 μM) significantly (P < 0.001) lowered luciferase transcriptional activity in LNCaP-LUC cells to barely undetectable levels, indicating that they are potent antagonists of the LNCaP AR. These studies were also performed using PC-3AR/LUC cells, which express wild-type AR (Fig. 5B). In these cells, DHT (5 μM) significantly promoted (P < 0.01) transcription of the luciferase gene by 3.3-fold. This was considerably less than with LNCaP-LUC cells, and was attributed to the cells reacting negatively to the double transfection of foreign genes. Neither ketoconazole nor flutamide had any appreciable effects on luciferase activity levels mediated by the wild-type AR, suggesting that they are only agonistic with the mutated AR. Finasteride (5 μM) did not modulate wild-type AR-mediated luciferase activity levels. When the novel compounds were tested, L-2, L-10, and L-36 (all at 5 μM) significantly promoted (P < 0.05) luciferase production in PC-3AR/LUC cells by 2.1-fold, 2.8-fold, and 1.7-fold, respectively, confirming that each of these compounds are also agonists of the wild-type AR. L-35 and L-37 (5 μM) significantly lowered (P < 0.05) luciferase transcriptional activity in PC-3AR/LUC cells, again indicating that these compounds are antagonists of both the wild-type and mutant AR. In contrast to the results obtained with LNCaP-LUC cells, L-39 inhibited wild-type AR-mediated luciferase transcriptional activity in PC-3AR/LUC cells by 35%, suggesting that this compound is an antagonist of the wild-type AR and a weak agonist of the mutated LNCaP AR. The results indicate that L-2, L-10, and L-36 function as agonists of both receptor types, L-35 and L-37 are antagonists of both receptor types, and L-39 has mixed agonistic/antagonistic activity depending on receptor type.

The ability of the compounds to modulate DHT-induced luciferase activity was determined in both cell types. In each of the experiments the cells were cotreated with DHT (5 nM) and the reference drug or novel compound (5 μM). DHT (5.0 nM) significantly (P < 0.001) stimulated luciferase production mediated by the LNCaP AR in LNCaP-LUC cells (Fig. 6A). Ketoconazole had no effect on DHT-induced luciferase activity. In this cell line, flutamide and finasteride significantly lowered DHT-induced transcriptional activity suggesting that they compete for the LNCaP AR and prevent DHT from binding. All of the novel compounds significantly inhibited DHT-induced LNCaP AR-mediated luciferase transcription. The order of potency of the compounds was L-37 > L-39 > L-2 > L-35 > L-10 > L-36, and DHT-induced luciferase activity was inhibited by 99.1%, 86.8%, 73.8%, 72.5%, 69.8%, and 42.1%, respectively. No synergistic or additive effects were observed on luciferase activity levels when LNCaP-LUC cells were cotreated with the agonistic compounds, L-2, L-10, or L-36 in combination with DHT. These compounds compete with DHT for binding to the mutated LNCaP AR and mediate transcription to the same level as in the absence of DHT. When the compounds were tested against the wild-type receptor in PC-3AR/LUC cells, a similar pattern was obtained (Fig. 6B). Ketoconazole and finasteride had no effect on DHT-induced transcriptional activity and
flutamide significantly inhibited \( P < 0.01 \) luciferase activity induced by DHT. As was observed with the mutated LNCaP AR, all of the novel compounds were effective at inhibiting DHT-induced transcriptional activity mediated by the wild-type AR. The order of potency of the compounds was L-37 > L-39 > L-35 > L-2 ≥ L-36 ≥ L-10, which inhibited luciferase activity levels by 89.1%, 82.2%, 69.8%, 62.9%, 59.5%, and 58.9%, respectively.

To ensure that the results obtained with these cell lines reflected the ability of the compounds to bind to the AR and not another steroid hormone receptor, these assays were performed in steroid receptor-negative CV-1Luc cells that were transiently transfected with either the wild-type AR or the mutated LNCaP AR constructs. The results obtained were in agreement with the results described above, indicating that the effects of the compounds were specific for the AR (data not shown).

**Growth Effects on LNCaP Tumors Grown in Male SCID Mice.**

In the first experiment, the effects of L-2, L-35, and L-36 on tumor growth were determined, and orchidectomy and ketoconazole were used as the reference treatments. The mice were grouped 28 days after cell inoculation when measurable tumor volumes were approximately 500 mm³ (Fig. 7A). After 21 days of treatment tumor volumes in the mice treated with L-2, L-36, and L-35 were similar to the castration group. However, by 28 days tumor volume in the control mice increased 4-fold over the 28 days of treatment, and tumor volume in the castrated mice increased by only 2-fold (52% reduction). None of the treatments, including ketoconazole, had any appreciable effect on the growth of the tumors after 28 days of treatment (Fig. 7A). The reductions in tumor volumes in the mice treated with ketoconazole, L-2, L-35, and L-36 were 23%, 28%, 8%, and 12% respectively. These results were also reflected in the weights of the tumors. The only treatment that resulted in significantly \( P < 0.01 \) smaller tumors was orchidectomy (Fig. 7B). Tumor weight in the castrated mice was reduced by 62%. Mice that had been castrated also had significantly \( P < 0.02 \) lower serum levels of PSA when compared with vehicle-treated animals (Table 2). In this experiment, serum and tumor levels of testosterone were significantly reduced in the mice that were castrated. Although, serum testosterone levels in the mice treated with L-2, L-35, and L-36 were significantly lower compared with the vehicle-treated mice, tumor testosterone levels were unaffected.

In the second experiment, the effects of L-10, L-37, and L-39 on tumor growth were determined and orchidectomy, finasteride, and ketoconazole were used as the reference treatments (Fig. 8A). The mice were grouped 35 days after cell inoculation when measurable tumor volume was approximately 500 mm³ (Fig. 8A). Total tumor volume in the control mice increased 4.3-fold over the 28 days of treatment and tumor volume in the castrated mice increased by only 2.6-fold (38% reduction compared with control). Tumor volumes increased 3.2-fold in the mice treated with finasteride and, as in the previous experiment, ketoconazole had no appreciable effect on tumor growth. Tumor volumes in the mice treated with L-10 increased by 3.3-fold (22% reduction compared with control) over the 28 days of treatment. This compound was as effective as finasteride in this model. In contrast to the growth inhibition observed with L-37 in vitro (Fig. 2–6), tumor volumes in the mice treated with this compound increased 5.2-fold. In the mice treated with L-39, tumor volume increased by 2.4-fold, which was a 44% reduction versus control mice. In this experiment, L-39 was as effective as castration at inhibiting tumor growth. Tumor weights in the castrated and L-39-treated mice were significantly \( P < 0.05 \) lower than those in the control mice (Fig. 8B). Compared with vehicle-treated mice, tumors in the castrated and L-39-treated mice weighed 75% and 72% less, respectively. As shown in Table 3, serum PSA levels were significantly reduced in the mice that were castrated or treated with finasteride or L-39 (all \( P < 0.02 \)), and L-10 (\( P < 0.05 \)). Serum testosterone levels were significantly reduced in the groups treated with castration (\( P < 0.001 \)), L-37 (\( P < 0.02 \)), and L-39 (\( P < 0.05 \)). Tumor testosterone was significantly reduced (\( P < 0.001 \)) in the mice treated by castration, and of the novel compounds tested, only L-37 significantly reduced (\( P < 0.02 \)) tumor levels of testosterone.

**Growth Effects of L-39 on PC-82 Xenografts Grown in Male Athymic Nude Mice.** The effects of L-39 on the growth of hormone-dependent PC-82 tumor xenografts were determined because these tumors express wild-type AR. Castration, flutamide, and finasteride were used as the reference treatments. The mice were grouped approximately 21 weeks after tumor inoculation, when measurable tumor volume was approximately 500 mm³ (Fig. 9A). Tumor volume in the control mice increased 2.2-fold over the 28 days of treatment, and tumors in the castrated mice actually regressed by 13% over the duration of the experiment. Compared with the control mice, finasteride slowed tumor growth but not to the same extent as the antiandrogen flutamide. Tumor volume in the mice treated with flutamide increased by only 1.1-fold over the 28 days of treatment. L-39 was almost as effective as flutamide at slowing the growth rate of the PC-82 tumors. Tumor volume in the mice treated with L-39 increased by 1.3-fold during the course of the experiment. These results were also reflected in the weights of the tumors. Compared with the control group, castration, flutamide, and L-39 all significantly lowered \( P < 0.02 \) PC-82 tumor weight (Fig. 9B) and serum levels of PSA (Table 4). Castration also significantly lowered \( P < 0.01 \) serum and tumor levels of testosterone. Flutamide had no effect on the serum levels of testosterone. Serum and tumor testosterone levels were significantly lower (\( P < 0.01 \)) in the mice treated with L-39.

**DISCUSSION**

In the present study, we evaluated the ability of novel inhibitors of either CYP17, 20-lyase and/or 5α-reductase to inhibit the growth of hormone-dependent prostatic cancer cells in vitro and in vivo. We had reported previously that several of these novel compounds showed
very good biological activity by lowering androgen levels in a normal male rat model, suggesting that they may be effective treatment options for hormone-dependent prostatic carcinoma (47, 48). In this report we describe that some of our novel androgen synthesis inhibitors also interact with the AR expressed in prostate cancer cells, and that the antiandrogenic properties of some of the compounds contribute, at least in part, to their ability to inhibit the growth of hormone-dependent prostate cancers.

As an initial approach, the effects of the compounds on the growth of hormone-dependent LNCaP cells were determined. Of the six novel compounds tested, three stimulated LNCaP cell growth (L-2, L-10, and low concentrations of L-36), two inhibited cell proliferation (L-35 and L-37), and one had no effect (L-39). The mitogenic effects of L-2 and L-10 were surprising because both of these compounds are pregnane derivatives and would not expected to be recognized as ligands by the LNCaP AR. However, the LNCaP AR contains a mutation in the ligand-binding domain (threonine to alanine at residue 877) that is responsible for it recognizing other steroidal compounds as either agonists or antagonists (33, 34). It has recently been reported that residue 877 contacts the ligand directly, and the mutation alters the stereochemistry of the binding pocket (49). The mutation broadens the specificity of ligand recognition and the LNCaP AR recognizes estrogens, progestins, and antiandrogens such as flutamide as androgens. The cells respond to these compounds with increased proliferation (34, 50–52). Consistent with previously published reports from this laboratory, flutamide was found to be growth stimulatory to LNCaP cells and finasteride was growth inhibitory (Fig. 2; Refs. 45 and 46). Our results show that the effects of flutamide and finasteride are specific for the LNCaP AR and not for the wild-type AR. This is in contrast to the results obtained with our novel compounds, which

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**Fig. 5.** The effects of the novel compounds and reference drugs on transcriptional activity of luciferase mediated through (A) the LNCaP AR in LNCaP-LUC cells and (B) the wild-type AR expressed in PC-3AR/LUC prostate cancer cells. Cells in steroid-free medium were treated with vehicle, DHT (5 nM), testosterone (5 nM), and the reference drugs or novel compounds (5 μM) for 24 h. Cells were then assayed for luciferase activity, as described in “Materials and Methods.” The columns represent the mean of light units [counts per second (cps)/unit protein] in triplicate wells from three separate experiments. Values are expressed as mean and SE. A, *P < 0.001 versus control; **P < 0.01. B, *P < 0.01; **P < 0.05 versus control.

**Fig. 6.** The ability of the novel compounds and reference drugs to modulate DHT-induced luciferase activity mediated through the (A) LNCaP AR in LNCaP-LUC cells and (B) the wild-type AR expressed in PC-3AR/LUC prostate cancer cells. Cells in steroid-free medium were treated with vehicle, DHT alone (5 nM), or a combination of DHT (5 nM) and the reference drugs or novel compounds (5 μM) for 24 h. Cells were then assayed for luciferase activity, as described in “Materials and Methods.” The columns represent the mean of light units [counts per second (cps)/unit protein] in triplicate wells from three separate experiments. Values are expressed as mean and SE. A, *, P < 0.001 versus DHT; **, P < 0.01 versus DHT; *, P < 0.05 versus DHT. B, *, P < 0.01 versus DHT; **, P < 0.05 versus DHT.
interacted with both AR types and maintained the same agonistic/antagonistic properties regardless of receptor type. This indicates that some of our antiandrogenic compounds may be useful for the treatment of patients with tumors expressing either wild-type or mutated AR, or for patients with amplified AR expression. Androgen-responsive cells are believed to respond to androgen ablation therapy by acquiring androgen-independence and adapting to growth in the absence of mitogenic androgens. However, recent studies have suggested that in a subset of tumors, cells respond to reduced androgen levels by amplifying AR gene expression (53, 54). Therefore, AR-mediated signaling is likely to be important in the advanced-stage disease. Compounds such as L-39, which is a potent inhibitor of C17,20-lyase and 5α-reductase and exhibits antiandrogenic properties, may be a suitable treatment option for such patients.

Fig. 7. The effects of orchidectomy, ketoconazole, and the novel compounds L-2, L-35, and L-36 on the growth of LNCaP prostate tumors in male SCID mice. Groups of six mice with LNCaP tumors were treated with the compounds at 50 mg/kg/day for 28 days. A, tumor volumes were measured weekly, and the percentage of change in tumor volume was determined. B, after 28 days of treatment, the mice were sacrificed and the tumors were removed and weighed. Castration significantly reduced tumor weights. *, P < 0.01 versus control.

Table 2. Serum levels of PSA and testosterone and tumor levels of testosterone in male SCID mice after 28 days of treatment with novel compounds.

<table>
<thead>
<tr>
<th></th>
<th>Serum PSA (ng/ml)</th>
<th>Testosterone (ng/ml)</th>
<th>Tumor Testosterone (ng/g wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>407.85 ± 59.06</td>
<td>0.348 ± 0.04</td>
<td>1.247 ± 0.147</td>
</tr>
<tr>
<td>Castration</td>
<td>158.96 ± 33.64</td>
<td>0.095 ± 0.02</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>310.83 ± 74.32</td>
<td>0.509 ± 0.152</td>
<td>1.645 ± 0.040</td>
</tr>
<tr>
<td>L-2</td>
<td>338.49 ± 33.62</td>
<td>0.102 ± 0.005</td>
<td>1.414 ± 0.103</td>
</tr>
<tr>
<td>L-35</td>
<td>373.20 ± 33.59</td>
<td>0.145 ± 0.028</td>
<td>1.228 ± 0.123</td>
</tr>
<tr>
<td>L-36</td>
<td>368.63 ± 52.40</td>
<td>0.099 ± 0.042</td>
<td>1.631 ± 0.315</td>
</tr>
</tbody>
</table>

Values are the means ± SE from six animals. One tumor from each mouse was analyzed.

b P < 0.02 versus the control group.

c P < 0.001 versus the control group.

Fig. 8. The effects of orchidectomy, fluasteride, ketoconazole, and the novel compounds L-10, L-37, and L-39 on the growth of LNCaP prostate tumors in male SCID mice. Groups of six mice with LNCaP tumors were treated with the compounds at 50 mg/kg/day for 28 days. A, tumor volumes were measured weekly, and the percentage of change in tumor volume was determined. B, after 28 days of treatment, the mice were sacrificed and the tumors were removed and weighed. Castration and L-39 significantly reduced tumor weights. *, P < 0.05 versus control.
were castrated or treated with the 5-

dent, and tumor weights were significantly lower in the animals that

xenografts in male SCID mice was determined to be hormone-depen-

experiment (data not shown), the growth of LNCaP prostate cancer

cells grown as tumor xenografts in male SCID mice. In a preliminary

the

in vivo

analyzed.

that they are more comparable with the second-generation antiandro-

antagonists of both the wild-type AR and the LNCaP AR, indicating

ating these effects. L-35, L-37, and L-39 were also determined to be

inhibitors of cell growth at the 5-

stimulator of cell proliferation at the lower concentrations and a potent

were L-36, which was a potent

inhibitor of cell growth (58). We observed this with L-36, which was a potent

the competitive binding studies with the synthetic androgen

indicate that the compounds are interacting with both the mutated LNCaP AR and the wild-type AR, they cannot discriminate

whether the compounds are acting as agonists or antagonists. We used

transcriptional activation assays with luciferase activity regulated by

an androgen responsive promoter to overcome these difficulties.

LNCaP-LUC cells were selected in this laboratory and responded to

DHT and testosterone in a manner similar to the parent cells, indicating

that no clonal variation had occurred during the selection process.

The studies confirmed that the growth stimulatory compounds (L-2,

L-10, and L-36) were functioning as pure AR antagonists whereas the

growth inhibitory compounds (L-35, L-37, and L-39) were AR an-

agonists. It is interesting to note that the pregnane derivatives L-2

and L-10 were agonists of both receptor types. This suggests that the side

chain modification at carbon 17 may play an important role in medi-

ating these effects. L-35, L-37, and L-39 were also determined to be

agonists of both the wild-type AR and the LNCaP AR, indicating

that they are more comparable with the second-generation antiandro-

bicatulamid and is recognized by the LNCaP AR as an antiandrogen.

Clinically, bicatulamide is providing better responses and is better

tolerated than flutamide (57). Experiments ongoing in this laboratory

are presently comparing the antitumor effects of bicatulamide to some

of our more potent novel compounds. Although the LNCaP prostate

cancer cell line is the most characterized androgen-dependent model

of prostate cancer, the mutation in the AR is problematic for inves-

tigators researching the effects of antiandrogens. Furthermore, LNCaP

cells also respond to androgens in a biphasic concentration-dependent

response, with high concentrations of androgens inhibiting cell pro-

liferation (58). We observed this with L-36, which was a potent

stimulator of cell proliferation at the lower concentrations and a potent

inhibitor of cell growth at the 5-μM concentration. Therefore, whereas

the competitive binding studies with the synthetic androgen

indicate that the compounds are interacting with both the mutated LNCaP AR and the wild-type AR, they cannot discriminate

whether the compounds are acting as agonists or antagonists. We used

transcriptional activation assays with luciferase activity regulated by

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and L-10 were agonists of both receptor types. This suggests that the side

chain modification at carbon 17 may play an important role in medi-

ating these effects. L-35, L-37, and L-39 were also determined to be

agonists of both the wild-type AR and the LNCaP AR, indicating

that they are more comparable with the second-generation antiandro-

bicatulamide than with flutamide.

Having established the in vitro activities of the novel compounds,

the in vivo effects were determined using LNCaP prostatic cancer

cells grown as tumor xenografts in male SCID mice. In a preliminary

experiment (data not shown), the growth of LNCaP prostate cancer

xenografts in male SCID mice was determined to be hormone-depen-

dent, and tumor weights were significantly lower in the animals that

were castrated or treated with the 5α-reductase-inhibitor flutamide.

Flutamide was found to stimulate the growth of LNCaP tumor xe-

nografts, but not to the same extent as DHT. Additionally, the max-

imum time of treatment was determined to be 28 days. After this time,

tumors in the castrated group appeared to have acquired hormone-

independence based on a determination of weekly serum PSA levels

(data not shown).

In agreement with the results obtained in vitro, the androgenic

compounds L-2, L-36, and L-10 had no appreciable effect on tumor

growth measurements. L-10 was found to be as effective as flutamide

at inhibiting tumor growth, and L-2 and L-36 significantly lowered

serum levels of testosterone but not tumor testosterone levels. There-

fore, it is likely that although the compounds are effective inhibitors

of androgen synthesis, this is overshadowed by their androgenic

Table 3 Serum levels of PSA and testosterone, and tumor levels of testosterone in male

SCID mice after 28 days of treatment with novel compounds

<table>
<thead>
<tr>
<th>Serum</th>
<th>PSA (ng/ml)</th>
<th>Testosterone (ng/ml)</th>
<th>Testosterone (ng/g wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>828.22 ± 56.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.205 ± 0.015</td>
<td>1.843 ± 0.294</td>
</tr>
<tr>
<td>Castration</td>
<td>379.69 ± 66.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.054 ± 0.019&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.056 ± 0.004&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Finasteride</td>
<td>518.38 ± 54.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.638 ± 0.453&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.661 ± 0.326&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>716.54 ± 31.74</td>
<td>0.298 ± 0.116</td>
<td>1.373 ± 0.104</td>
</tr>
<tr>
<td>L-10</td>
<td>507.66 ± 0.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.193 ± 0.020</td>
<td>1.550 ± 0.442</td>
</tr>
<tr>
<td>L-37</td>
<td>897.65 ± 62.13</td>
<td>0.095 ± 0.012&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.933 ± 0.158&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>L-39</td>
<td>316.38 ± 98.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.129 ± 0.034&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.744 ± 0.131</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values are the means ± SE from six animals. One tumor from each mouse was analyzed.
<sup>b</sup> P < 0.02 versus the control group.
<sup>c</sup> P < 0.001 versus the control group.
<sup>d</sup> P < 0.05 versus the control group.

Table 4 Serum levels of PSA and testosterone and tumor levels of testosterone in male

SCID mice after 28 days of treatment with the novel compound L-39

<table>
<thead>
<tr>
<th>Serum</th>
<th>PSA (ng/ml)</th>
<th>Testosterone (ng/ml)</th>
<th>Testosterone (ng/g wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>413.04 ± 78.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.216 ± 0.140</td>
<td>2.987 ± 0.326</td>
</tr>
<tr>
<td>Castration</td>
<td>25.08 ± 7.95&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.059 ± 0.030&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.217 ± 0.024&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Flutamide</td>
<td>56.58 ± 13.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.209 ± 0.096</td>
<td>0.446 ± 0.059&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Finasteride</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>L-39</td>
<td>83.15 ± 27.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.073 ± 0.031&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.075 ± 0.135&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>b</sup> Values are the means ± SE from five animals. One tumor from each mouse was analyzed.
<sup>d</sup> P < 0.001 versus the control group.
<sup>c</sup> P < 0.02 versus the control group. NT, not tested.
properties, which results in LNCaP tumor growth. The inability of L-35 and L-37 to inhibit LNCaP tumor growth in vivo was especially disappointing. Both of these compounds were the most effective at inhibiting LNCaP cell growth in vitro, and they were both determined to be the most potent antiandrogens. We have recently determined that L-37 is an inhibitor of the liver cytochrome P450 enzymes 3β-hydroxysteroid-oxidoreductase and 3β-hydroxysteroid-oxidoreductase, which catalyze the metabolism of DHT to polar metabolites that are excreted in the urine (data not shown). Although we were unable to reliably measure serum levels of DHT in the serum of the animals because of cross-reactivity with testosterone and the novel compounds, the results (not shown) did suggest that there were elevated DHT levels in the animals treated with L-37. These elevated DHT levels may explain why L-37 had no effect on tumor growth in vivo. It should also be noted that L-37 does not inhibit the growth of PC-82 prostate cancer xenografts grown in athymic nude mice in vivo (data not shown). Currently, we do not know the reasons for the lack of growth inhibition observed with L-35. The inability of L-35 to inhibit LNCaP tumor growth may be attributed to its weak affinity for the AR. Alternatively, it may be attributable to the compound being converted to an inactive metabolite, poor uptake of the compound in the animals, or rapid clearance from the animals.

The most effective compound at inhibiting LNCaP tumor growth in vivo was L-39, which paralleled castration. Moreover, L-39 was also very effective at inhibiting the growth of hormone-dependent PC-82 prostate tumor xenografts in athymic nude mice. PC-82 prostate cancer cells expresses wild-type AR, and the cells cannot be grown as prostate tumor xenografts in athymic nude mice. PC-82 prostate cancer cells express wild-type AR, and the cells cannot be grown as prostate tumor xenografts in athymic nude mice.

INHIBITION OF HORMONE-DEPENDENT PROSTATE CANCER CELL GROWTH

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