A Selective Androgen Receptor Modulator for Hormonal Male Contraception

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Received July 31, 2004; accepted September 3, 2004

ABSTRACT

The recent discovery of nonsteroidal selective androgen receptor modulators (SARMs) provides a promising alternative for testosterone replacement therapies, including hormonal male contraception. The identification of an orally bioavailable SARM with the ability to mimic the central and peripheral androgenic and anabolic effects of testosterone would represent an important step toward the “male pill.” We characterized the in vitro and in vivo pharmacologic activity of (S)-3-(4-chloro-3-fluorophenoxy)-2-hydroxy-2-methyl-N-(4-nitro-3-trifluoromethylphenyl)propionamide (C-6), a novel SARM developed in our laboratories. C-6 was identified as an androgen receptor (AR) agonist with high AR binding affinity ($K_i = 4.9$ nM). C-6 showed tissue-selective pharmacologic activity with higher anabolic activity than androgenic activity in male rats. The doses required to maintain the weight of the prostate, seminal vesicles, and levator ani muscle to half the size of the maximum effects (i.e., $ED_{50}$) were $0.78 \pm 0.06$, $0.88 \pm 0.1$, and $0.17 \pm 0.04$ mg/day, respectively. As opposed to other SARMs, gonadotropin levels in C-6-treated groups were significantly lower than control values. C-6 also significantly decreased serum testosterone concentration in intact rats after 2 weeks of treatment. Marked suppression of spermatogenesis was observed after 10 weeks of treatment with C-6 in intact male rats. Pharmacokinetic studies of C-6 in male rats revealed that C-6 was well absorbed after oral administration (bioavailability 76%), with a long (6.3 h) half-life at a dose of 10 mg/kg. These studies show that C-6 mimicked the in vivo pharmacologic and endocrine effects of testosterone while maintaining the oral bioavailability and tissue-selective actions of nonsteroidal SARMs.

Worldwide population growth and social awareness of reproductive health have stimulated a great deal of research in contraception. Although contraceptive pills have been available for women for decades, male contraception remains restricted to physical methods, such as condoms and vasectomy. However, many polls show that men would be more willing to be involved in family planning if appropriate contraceptive methods were available (Anderson and Baird, 1997; Martin et al., 2000). Although the mechanism of spermatogenesis is not completely understood, inhibition of LH and FSH production is known to interrupt spermatogenesis. To date, the most practical method of male contraception is the hormonal approach. Potential regimens include androgen alone, androgen combined with progestins, and androgen combined with gonadotropin hormone-releasing hormone (GnRH) analogs (Amory and Bremner, 2001; Kamischke and Nieschlag, 2004). Among them, androgen-progestin combinations remain the most promising approach. Because androgens are essential for male development and the maintenance of male secondary characteristics, such as bone mass, muscle mass, fat tissue distribution, and spermatogenesis (Matsumoto, 1994), an androgen supplement is a required component of hormonal male contraception. However, the lack of orally bioavailable and safe androgens has been a major limitation in the search for hormonal male contraceptives. The main disadvantages of steroid androgens (e.g., testosterone and its esters) are their undesirable physicochemical and pharmacokinetic properties. Testosterone and the majority of its esters must be administered by intramuscular injection, implant, and/or
transdermal patch. In addition to their inconvenient means of administration, steroidal androgens are associated with a variety of undesirable side effects. In fact, the major dose-limiting side effects observed in recent clinical trials of male contraception were androgen-related (Kamischke and Nieschlag, 2004).

Several approaches to overcome the limitations of using testosterone preparations have been explored. One major approach focused on structural modifications of steroidal androgens to develop long-acting androgens. Another approach is nonsteroidal selective androgen receptor modulators (SARMs). In 1998, during our search for androgen receptor (AR) affinity labels, we discovered and reported a group of nonsteroidal androgens that are derivatives of two known antiandrogens, bicalutamide (Fig. 1) and flutamide (Dalton et al., 1998). In recent years, we determined important structure-activity relationships for AR binding affinity and transcriptional activation (He et al., 2002; Yin et al., 2003b) and pharmacokinetics and key metabolism pathways of SARMs (Yin et al., 2003c). We also identified dozens of SARMs that demonstrate tissue-selective anabolic and androgenic in vivo pharmacologic effects but are devoid of the side effects commonly associated with testosterone therapy (Yin et al., 2003a; Marhefka et al., 2004). The discovery of SARMs therefore provides a unique alternative for androgen replacement therapy with advantages including oral bioavailability, flexibility of structural modification, AR specificity, lower activity in the prostate, and the lack of steroid-related side effects.

Earlier studies demonstrated that minor structural modification of SARMs could result in dramatic changes in their in vitro and in vivo pharmacologic activity. To date, most of the SARMs reported in our laboratories are able to mimic various pharmacologic activities of testosterone in peripheral tissues but are unable to do so in the central nervous system (Yin et al., 2003a). We hypothesized that novel SARMs can also be designed and synthesized to mimic the effects of testosterone on the hypothalamus-pituitary-testis axis. Such compounds would represent an important step toward the discovery and development of SARMs for hormonal male contraception. The present studies were designed to characterize the preclinical pharmacology of one such novel SARM.

**Materials and Methods**

**Chemicals and Animals.** The S-isomer of C-6 (Fig. 1) was synthesized in our laboratory with a purity greater than 99% using previously described methods (He et al., 2002; Marhefka et al., 2004). [17α-Methyl-3H]mibolerone ([3H]MIB; 84 Ci/mmol) and unlabeled MIB were purchased from PerkinElmer Life and Analytical Sciences (Boston, MA). Phosphate-buffered saline (PBS; catalog number P5368, consisting of 0.01 M phosphate buffer, 0.0027 M potassium chloride, and 0.137 M sodium chloride) and all other chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

Male Sprague-Dawley (SD) rats were purchased from Harlan Bioproducts for Science Inc. (Indianapolis, IN). All animals were maintained on a 12-h light/dark cycle with food and water available ad libitum. The animal protocol was reviewed and approved by the Institutional Laboratory Animal Care and Use Committee of The Ohio State University.

**In Vitro Pharmacologic Activity.** Rat prostate cytosolic AR was prepared from the ventral prostates of castrated male SD rats using established methods (Mukherjee et al., 1999). The AR binding affinity of C-6 was determined using an in vitro radioligand competitive binding assay as previously described (Yin et al., 2003b). Briefly, an aliquot of AR cytosol was incubated with 1 nM [3H]MIB and 1 mM triamcinolone acetonide at 4°C for 18 h in the absence or presence of increasing concentrations of dihydrotestosterone (DHT) or C-6 (10^-10^-4 M). Nonspecific binding of [3H]MIB was determined by adding excess unlabelled MIB (1000 nM) to the incubate. The separation of bound and free radioactivity at the end of incubation was achieved by the hydroxyapatite method as previously described (Yin et al., 2003b); then the radioactivity was counted in a liquid scintillation counter (Model LS6500; Beckman Coulter, Inc., Fullerton, CA). The specific binding of [3H]MIB at each concentration of DHT or C-6 (B) was further calculated by subtracting the nonspecific binding of [3H]MIB, and expressed as the percentage of the specific binding in the absence of DHT or C-6 (B0). Competitive displacement curves of DHT and C-6 were constructed with B on the vertical axis and ligand concentration (C) on the horizontal axis. The concentration of compound that reduced the B0 by 50% (IC50) was determined by computer-fitting the data to the following equation using WinNonlin (Pharsight Corporation, Mountain View, CA): 

\[
B = B_0 \times \left(1 - IC_{50} / IC_{50} + C \right)
\]

The equilibrium binding constant (Kd) of DHT or C-6 was calculated by 

\[
K_d = K_a \times IC_{50} / (K_a + L),
\]

where K_a was the dissociation constant of [3H]MIB (0.19 ± 0.01 nM; previously determined by Mukherjee et al., 1999), and L was the concentration of [3H]MIB used in the experiment (1 nM).

The in vitro AR-mediated transcriptional activity of C-6 was measured using a modification of the method of (Yin et al., 2003b). Monkey kidney cells, CV-1, were maintained at 37°C in a humidified atmosphere of 5% CO2/95% air in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum (FBS). At ~90% confluence, cells were transfected in serum-free medium using LipofectAMINE (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. Cells in each T-150 flask were transfected with 0.8 μg of a human AR expression construct (pCMVhAR; generously provided by Dr. Donald Tindall, Mayo Clinic, Rochester, MN), 8 μg of an androgen-dependent luciferase reporter construct (pMMTV-Luc; gener-

![Fig. 1. Chemical structures of bicalutamide, S-1, and C-6.](image-url)
ously provided by Dr. Ron Evans, Salk Institute, San Diego, CA), and 8 µg of a β-galactosidase expression construct (pSV-β-galactosidase; Promega, Madison, WI). After 3 to 5 h of transfection, cells were washed with serum-free medium and allowed to recover for 10 h in Dulbecco’s modified Eagle’s medium containing 0.2% FBS. Transfected cells were trypsinized, centrifuged, and counted. Cells were then plated into 24-well plates at a density of 8 x 10^4 cells/well. Before drug treatment, cells were allowed to attach to plates for 12 h.

The agonist activity and antagonist activity of C-6 were determined by incubating cells with C-6 (1, 10, 100, and 1000 nM) in the absence and presence of DHT (1 nM), respectively. In each experiment, vehicle control and positive control (activity induced by 1 nM DHT) were included. For each treatment, compounds were dissolved in ethanol and then diluted with medium to the desired concentrations. The final concentration of ethanol in each well was ≤0.05%.

After 24-h drug treatment, medium was removed by aspiration. Cells were washed twice with ice-cold PBS and lysed with 110 µl/well of Reporter Lysis Buffer (Promega) at room temperature for 30 min. An aliquot (50 µl) of cell lysate from each well was used for β-galactosidase assays and the other 50 µl of cell lysate was used for luciferase assays using the method previously described (Yin et al., 2003b). All results were expressed as the ratio of luciferase activity/β-galactosidase activity to avoid variations caused by cell number and transfection efficiency.

**In Vivo Pharmacologic Activity.** In vivo androgenic and anabolic activities of C-6 in castrated and intact male rats. Forty-five male SD rats weighing approximately 200 g were purchased and randomly divided into nine groups with five rats per group. One day before the start of treatment, groups 1 through 8 were castrated via a scrotal incision under anesthesia. Group 9 served as an intact control. Rats were sacrificed after 14 days of treatment. Serum samples were collected and stored at -80°C immediately centrifuged at 1000 g and assayed for testosterone concentrations. The plasma clearance (CL) was calculated as

\[ CL = \frac{Dose_{i.v.}}{AUC_{0-\infty}} \]

where CL is the clearance, Dose_{i.v.} is the intravenous bolus dose, and AUC_{0-\infty} is the area under the plasma concentration-time curve from time 0 to infinity, respectively. The apparent volume of distribution at equilibrium (Vd_{eq}) was calculated using the trapezoidal method with extrapolation to time infinity. The plasma clearance (CL) was calculated as

\[ CL = \frac{Dose_{p.o.}}{AUC_{0-\infty}} \]

where Dose_{p.o.} and AUC_{0-\infty} are the oral dose and corresponding area under the plasma concentration-time curve from time 0 to infinity after p.o. administration, respectively.

**Statistical Analyses.** All statistical analyses were performed using single-factor ANOVA, with the α value set a priori at p < 0.05.

**Results**

In **In Vivo Pharmacologic Activity.** Previous studies demonstrated that structural modification(s) of the A-ring, link-
age, and/or the B-ring of nonsteroidal SARMs affected their function in vitro and in vivo pharmacologic activity. Compared with bicalutamide, C-6 has a NO₂ at the para-position of the A-ring, an oxygen linkage, and a chloro and a fluoro substituent at the para- and meta-position of the B-ring, respectively (Fig. 1). The AR binding affinity of C-6 was determined using a competitive binding assay. DHT and C-6 displaced [³H]MIB from AR binding sites (Fig. 2). The $K_i$ values of DHT and C-6 were $0.45 \pm 0.2$ and $4.9 \pm 0.3 \text{ nM}$, respectively. Although the binding affinity of C-6 was less than one-tenth that of DHT, it bound the AR with 2-fold higher affinity than R-bicalutamide (i.e., $K_i$ bicalutamide $= 11.0 \text{ nM}$) (Mukherjee et al., 1996) and 4- to 8-fold higher affinity than hydroxyflutamide (Furr et al., 1987; Teutsch et al., 1994), indicating that C-6 interacted with the AR with high binding affinity.

Upon binding to the AR, C-6 induced a concentration-dependent increase in AR-mediated transcriptional activation (Fig. 3A). The transcriptional activity induced by C-6 plateaued at concentrations equal to or greater than $10 \text{ nM}$ (~100% of activity that was induced by $1 \text{ nM}$ DHT). The activity in the vehicle-treated wells was less than 3% of the transcriptional activation induced by $1 \text{ nM}$ DHT. C-6 did not significantly inhibit transcriptional activity induced by DHT (Fig. 3B) even at a concentration as high as $1000 \text{ nM}$. These results clearly demonstrated that C-6 binds to the AR with high affinity and potently stimulates AR-mediated transcriptional activity in vitro.

**In Vivo Pharmacologic Activity.** In vivo androgenic and anabolic activities. We then examined the in vivo androgenic and anabolic activities of C-6. In castrated male rats, the androgenic activity was evaluated by the ability of C-6 to maintain the weights of the ventral prostate and seminal vesicle, whereas the levator ani muscle weight was used to assess anabolic activity. Castration resulted in a significant reduction in the weights of the ventral prostate, seminal vesicle, and levator ani muscle to 8.5, 6.5, and 34% of that observed in intact animals, respectively. The administration of C-6 increased the weights of these tissues in a dose-dependent manner (Fig. 4A). At the highest dose rate (3 mg/day), the weights of all three organs were increased to about 130% of those observed in intact controls. Nonlinear regression analysis of the dose-response relationships showed that the ED$_{50}$ values of C-6 were $0.77 \pm 0.06$, $0.88 \pm 0.1$, and $0.17 \pm 0.04 \text{ mg/day}$ in the ventral prostate, seminal vesicle, and levator ani muscle, respectively (Fig. 4B). These results clearly revealed the potent and efficacious androgenic and anabolic activity of C-6 in male rats. The ED$_{50}$ value of C-6 in the levator ani muscle ($0.17 \pm 0.04 \text{ mg/day}$) was about 4- to 5-fold less than its ED$_{50}$ values in the prostate and seminal vesicle ($0.77 \pm 0.06$ and $0.88 \pm 0.14 \text{ mg/day}$, respectively). At a dose rate of 0.3 mg/day, C-6 maintained the weight of the levator ani muscle at a weight not different from that observed in intact controls. The effect of C-6 in the ventral prostate and seminal vesicle was about 30% of that observed in intact controls at this dose rate. Results suggested that C-6 acts as a tissue-selective androgen with more potent anabolic activity than androgenic activity. The efficacy (maximum effect) of C-6 in anabolic and androgenic tissues was about the same.

We also determined the serum levels of LH and FSH in animals treated with C-6 and compared them to the levels of those hormones observed in intact and castrated control animals. As shown in Table 1, castration led to a significant elevation in LH ($8.4 \pm 2.5 \text{ ng/ml}$) and FSH ($63.4 \pm 5.0 \text{ ng/ml}$) levels due to the lack of negative feedback inhibition by testosterone. C-6 caused a dose-dependent decrease in LH and FSH levels and restored the levels of these hormones back to physiological levels at dose rates as low as 0.3 and 0.5 mg/day, respectively.

These data led us to immediately examine the pharmacologic activity of C-6 in intact male rats to answer an important question: would C-6 mimic the ability of exogenous testosterone to inhibit the synthesis of endogenous testosterone? We chose a dose rate of 0.5 mg/day for this study. This dose was the minimum dose required to fully suppress LH and FSH and yet maintain normal muscle mass in castrated rats. In a 14-day study (Fig. 5), TP stimulated growth in all three organs, whereas C-6 demonstrated tissue-selective pharmacologic effects in intact rats. The weights of the ventral prostate and seminal vesicles were significantly decreased to about 50% of those observed in intact controls, whereas the weights of the levator ani muscle remained the same as those observed in intact controls. Surprisingly, the pharmacologic effects of C-6 in intact rats were nearly identical to those found in castrated rats at this dose rate (0.5 mg/day) (Fig. 5). Based on this finding, we predicted that C-6 reduced serum testosterone concentration to levels similar to those observed in castrated rats. Results showed that the average testosterone concentration in the intact control group was $4.47 \pm 1.66 \text{ ng/ml}$. Testosterone concentrations in three animals of the C-6-treated group were below the detection limit ($0.04 \text{ ng/ml}$) of the assay, whereas testosterone concentrations in the two remaining animals were $0.11$ and $0.46 \text{ ng/ml}$. Clearly, C-6 potently and rapidly inhibited endogenous testosterone synthesis in male rats.

**In vivo antireproductive activity.** In this pilot study, we evaluated the effects of C-6 on the reproductive system after long-term (1 mg/day, 10 weeks) treatment in adult male rats. Body and organ weights in control and C-6-treated rats are...
Fig. 3. AR-mediated transcriptional activity induced by DHT and C-6. CV-1 cells were transfected with a human AR plasmid, an androgen-responsive luciferase reporter plasmid, and a constitutively expressed β-galactosidase plasmid in a T-150 flask using LipofectAMINE. After transfection, cells were plated onto 24-well plates and allowed to recover for 12 h before drug treatment. Cells were then treated with vehicle or increasing concentrations of C-6 alone or together with 1 nM DHT for 24 h. Luciferase activity in each well was normalized with the β-galactosidase activity and then expressed as the percentage of that induced by 1 nM DHT. Each bar represents mean ± S.D. (n = 3).

Fig. 4. A, androgenic and anabolic activities of C-6 in castrated male rats. Castrated male rats received C-6 at a dose rate ranging from 0.05 to 3 mg/day by daily subcutaneous injection for 14 days. Organ weights were normalized with body weight and expressed as the percentage of the weights in the intact control group. Each bar represents the mean ± S.D. (n = 5/group). The letters I and C above each error bar represent a significant difference between the group and intact control group or castrated control group, respectively, as analyzed by single-factor ANOVA with p < 0.05. B, dose-response relationship of C-6 in castrated male rats. Dose-response curves, Emax, and ED50 in this figure were obtained by nonlinear least-square regression analysis.
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testosterone levels that were lower than the quantitation limit

male rats with a similar age (Sun et al., 1989; Lue et al., 2000).

the normal range of testosterone concentration reported from

changes in serum testosterone level were further evaluated.

cauda epididymis were significantly reduced to about 25% of

summarized in Table 2. No significant difference in the mean

Body weight was observed after C-6 treatment. C-6 caused a

reduction in the size of the prostate, seminal vesicles, testis,

summarized in Table 2. No significant difference in the mean

body weight was observed after C-6 treatment. C-6 caused a

significantly increased the weight of the levator ani muscle to 120% of

those observed in the intact control group. Because of the

marked decline in testicular and epididymal weight, the changes in serum testosterone level were further evaluated.

The average testosterone concentration in intact control ani-

mals was 1.06 ± 0.78 ng/ml. These concentrations are within

the normal range of testosterone concentration reported from

male rats with a similar age (Sun et al., 1989; Lue et al., 2000).

Animals in C-6-treated group showed significantly suppressed

testosterone levels that were lower than the quantitation limit

(0.04 ng/ml) of the EIA kits. Clearly, the reduction of endoge-
nous testosterone production was the main reason for the sup-

pressed reproductive organ weights and sperm number in

intact animals. Although a high dose rate (1 mg/day) of C-6

was used in this study, low activity was observed in the

prostate and seminal vesicles due to the tissue selectivity

of C-6. In conclusion, C-6 was able to mimic the effects of

exogenous testosterone on spermatogenesis in male rat

while maintaining its tissue selectivity after chronic treat-

ment. Additional studies over a wider dose range are war-

ranted to more fully describe the effects of C-6 on spermat-

ogenesis and male reproductive activity.

Pharmacokinetics of C-6 in Male Rats. To develop a

possible male contraceptive pill, high oral bioavailability and a

suitable half-life of the compound are required. Therefore,

the pharmacokinetics of C-6 were examined after i.v. and p.o.

administration to male rats with a single dose of 10 mg/kg.

The time-courses of the changes in plasma concentrations

of C-6 following i.v. and p.o. administration are shown in Fig.

7, and the pharmacokinetic parameters are listed in Table 3.

Following intravenous administration, C-6 concentrations

diminished with a mean terminal \( t_{1/2} \) of 6.3 h. The systemic

clearance (CL) and steady-state volume distribution \( V_{ss} \)

were 0.72 ml/min · kg and 473 ml/kg, respectively. C-6 ap-

peared rapidly (within 5 min) in the systemic circulation

after p.o. administration, suggesting gastric absorption of the

drug. Oral absorption was prolonged with maximum plasma

concentrations forming a plateau over 6 to 12 h postdose.

Plasma concentrations of C-6 diminished with a mean termi-

nal \( t_{1/2} \) of 5.6 h. This terminal \( t_{1/2} \) was smaller than that

observed after i.v. administration, an observation which was

likely due to individual variation of plasma drug concentra-

tion after p.o. administration; however, statistical analysis

revealed no significant difference between these two values.

The bioavailability of C-6 following oral administration was

76%.

Discussion

Earlier studies revealed that known nonsteroidal AR li-
gands (mainly bicalutamide derivatives) could be structurally

modified to obtain a series of compounds with diverse in

vitro (Yin et al., 2003b) and in vivo pharmacologic activities

(Yin et al., 2003a). Subsequent research in our laboratories

has focused on the discovery of novel SARMs as alternatives

for testosterone replacement therapy and the treatment of

muscle wasting diseases, osteoporosis, prostate hyperplasia,

and hormonal male contraception. However, unlike testos-

terone, the SARMs that we have reported to date showed

little to no effects on the hypothalamic-pituitary-testis axis,

precluding their ability to regulate gonadotropins and serum

testosterone levels or to serve as components of a male con-

traceptive regimen. Our previous studies showed that the

aromatic B-ring is much more amenable to structural modi-

fication than other positions in the structure. We used this

and other structure-activity relationships uncovered in our

research to design and synthesize additional series of SARMs

in the hopes of identifying compounds with enhanced central

activity. Typically, these compounds were S-isomers with a

structure that bears an NO2 and CF3 at the para- and meta-

position of the A-ring, respectively, a CH2 linked to the chiral

carbon, an oxygen linkage, and an electronegative substitu-

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TABLE 2
Effects of C-6 on body and organ weights
Values are mean ± S.E. Figures in parentheses indicate number of animals.

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight</th>
<th>Prostate</th>
<th>Seminal Vesicles</th>
<th>Testis</th>
<th>Epididymis</th>
<th>Levator Ani Muscle % of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact (6)</td>
<td>422 ± 14</td>
<td>100 ± 18</td>
<td>100 ± 9.5</td>
<td>100 ± 9.6</td>
<td>100 ± 9.7</td>
<td>100 ± 6.1</td>
</tr>
<tr>
<td>C-6 (8)</td>
<td>414 ± 8</td>
<td>68 ± 5*</td>
<td>83 ± 7*</td>
<td>60 ± 4*</td>
<td>63 ± 4*</td>
<td>120 ± 10*</td>
</tr>
</tbody>
</table>

* Significant difference between groups, analyzed by single-factor ANOVA with p < 0.05.

TABLE 3
Average pharmacokinetic parameters of C-6 in male rats
Values are mean ± S.E. (n = 5/group).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>10 mg/kg (i.v.) (n = 5)</th>
<th>10 mg/kg (p.o.) (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC_{0-\infty} (min · mg/ml)</td>
<td>14,003 ± 1784</td>
<td>10,585 ± 478</td>
</tr>
<tr>
<td>λ_{1} (min^{-1})</td>
<td>0.00182 ± 0.0004</td>
<td>0.002075 ± 0.00025</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>662 ± 130</td>
<td>750 ± 51</td>
</tr>
<tr>
<td>CL (ml/min/kg)</td>
<td>0.72 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>V_{ss} (ml/kg)</td>
<td>473 ± 70</td>
<td></td>
</tr>
<tr>
<td>F_{p.o.} (%)</td>
<td></td>
<td>76</td>
</tr>
<tr>
<td>t_{max} (min)</td>
<td></td>
<td>540 ± 120</td>
</tr>
<tr>
<td>C_{max} (mg/ml)</td>
<td></td>
<td>11.6 ± 1.6</td>
</tr>
</tbody>
</table>

When we compared the in vitro activity of C-6 with one of our known SARMs [i.e., (S)-3-(3-fluorophenoxy)-2-hydroxy-2-methyl-N-(4-nitro-3-trifluoromethylphenyl)propionamide (S-1)] (Yin et al., 2003a), we found that the dihalogen (para-Cl and meta-F) substituents in the aromatic B-ring retained high AR binding affinity (i.e., K_{i, C-6} = 4.9 versus K_{i, S-1} = 6.1 nM). Notably, at a concentration of 10 nM, C-6 showed 2- to 3-fold higher transcriptional activation than S-1 (i.e., 118 versus 43% of the activity induced by 1 nM DHT). These findings were consistent with our prediction regarding the flexibility of structural modification in the B-ring. When C-6 was tested in the castrated male rat model, it produced more potent and efficacious in vivo pharmacologic activity than S-1. Although C-6 exhibited profound effects on all three androgen-dependent organs, it remained tissue-selective. The potency (i.e., ED_{50}) and efficacy (i.e., E_{max}) of C-6 and TP (Yin et al., 2003a) in the levator ani muscle were comparable, whereas the potency of C-6 in the prostate and seminal vesicles was 6- to 7-fold less than that of TP. Furthermore, the ED_{50} value for C-6 in the levator ani muscle was 0.17 mg/day, or about 5-fold lower than that in the prostate and seminal vesicles. Thus, in castrated rats, a dose of C-6 (i.e., 0.3 mg/day) that will fully maintain the weight of the levator ani muscle will only partially maintain the weight of the prostate and seminal vesicles. The enhanced pharmacologic activity of C-6 compared with our previously reported SARM (i.e., S-1) might have resulted from several factors, including binding affinity, transcriptional activity, drug disposition, metabolism rate, and/or nuclear retention time. During in vitro studies, C-6 showed higher transcriptional activity but similar binding affinity to S-1. The in vivo total body clearance of C-6 was 0.72 ml/min · kg, which was significantly smaller than that of S-1 (J. Chen, D. J. Hwang, C. E. Bohl, D. D. Miller, J. T. Dalton, and D. Wu, unpublished data). Therefore, the increased efficacy and potency of C-6 in rats may have been due to its higher intrinsic activity and greater in vivo exposure.

Due to the detection limit of the LH radioimmunoassay and limited sample volumes, LH concentrations in the high-dose drug-treated and intact groups were undetectable. It was clear, however, that the antigonadotropic activity of C-6 was dose-dependent and more potent than TP (Yin et al., 2003a). Others showed that testosterone and DHT inhibit LH secretion in response to luteinizing hormone-releasing hormone stimulation in rats (Debeljuk et al., 1974) in cultures of rat anterior pituitary fragments (Schally et al., 1973) and...
antior pituitary cells (Liang et al., 1984). Studies using 5α-reductase inhibitors revealed that the conversion of testosterone to DHT is not critical to the antagonism of the activity of testosterone. This conclusion is further supported by our current studies since C-6 does not undergo 5α reduction.

It is well known that exogenous testosterone interferes with spermatogenesis indirectly by decreasing pituitary gonadotropin secretion via negative feedback on the hypophysial-anterior pituitary-testis axis in different species (Berndtson et al., 1974; Moger, 1976; Bansal and Davies, 1986; Matsumoto, 1990); however, the effects of testosterone on spermatogenesis were biphasic depending on the dosage of androgen administered (Walsh and Swerdloff, 1973; Ewing and Robaire, 1978). At low doses, androgen suppresses gonadotropin secretion, decreases testicular steroidogenesis, and then subsequently inhibits spermatogenesis. At higher androgen doses, endogenous testosterone levels remain suppressed, but the exogenous androgen is sufficient to directly support spermatogenesis. In our pilot antireproductive study, we demonstrated for the first time that the administration of a nonsteroidal androgen receptor modulator (C-6) to adult male rats resulted in marked suppression of spermatogenesis. C-6 treatment reduced peripheral testosterone concentrations to undetectable levels, significantly suppressed spermatogenesis, reduced the size of the testis and epididymis, and increased the weight of the levator ani muscle. The magnitude of antireproductive effects of C-6 could, like testosterone, be dose-dependent. Only one dose rate was examined in this pilot study. Although the androgenic activity of C-6 was much lower than testosterone and its esters, this dose of C-6 may still be sufficient to partially maintain spermatogenesis in rats. To date, our knowledge about the role of SARMs on the reproductive system is limited. Dose-response relationships of C-6 on spermatogenesis, fertility, and libido are presently being investigated in our laboratory to study the potential use of C-6 for hormonal male contraception.

In conclusion, C-6 is a novel SARM that was identified during studies to define structure-activity relationships for AR binding. C-6 bound the AR with high affinity and stimulated AR-mediated transcriptional activation to a similar extent as DHT. In vivo studies showed that C-6 demonstrated tissue-selective pharmacologic activity with more potent anabolic than androgenic activity. The high oral bioavailability and potent ability of C-6 to suppress gonadotropins, testosterone, and spermatogenesis suggest that C-6 is a promising candidate for hormonal male contraception either alone or in combination with a progestin.

References


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