

## Pharmacodynamics of Selective Androgen Receptor Modulators (SARMs)

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**ABBREVIATIONS:** SARM(s), selective androgen receptor modulator(s); AR, androgen receptor(s); HPLC, high-performance liquid chromatography; TP, testosterone propionate; PEG 300, polyethylene glycol 300; DMSO, dimethylsulfoxide; FSH, follicle stimulating hormone; LH, luteinizing hormone; TG, triglyceride; ANOVA, analysis of variance.

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## ABSTRACT

The present study aimed to identify selective androgen receptor modulators (SARMs) with *in vivo* pharmacological activity. We examined the *in vitro* and *in vivo* pharmacologic activity of four chiral, nonsteroidal SARMs synthesized in our laboratories. In the *in vitro* assays, these compounds demonstrated moderate to high androgen receptor (AR) binding affinity, with  $K_i$  values ranging from 4 to 37 nM, and three of the compounds efficaciously stimulated AR-mediated reporter gene expression. The compounds were then administered subcutaneously to castrated rats to appraise their *in vivo* pharmacologic activity. Androgenic activity was evaluated by the ability of these compounds to maintain the weights of prostate and seminal vesicle, while levator ani muscle weight was used as a measure of anabolic activity. The maximal response ( $E_{max}$ ) and dose for half-maximal effect ( $ED_{50}$ ) were determined for each compound and compared to that observed for testosterone propionate (TP). Compounds S-1 and S-4 demonstrated *in vivo* androgenic and anabolic activity, whereas compounds S-2 and S-3 did not. The activities of S-1 and S-4 were tissue-selective in that both compounds stimulated the anabolic organs more than the androgenic organs. These two compounds were less potent and efficacious than TP in androgenic activity, but their anabolic activity was similar to or greater than that of TP. Neither S-1 nor S-4 caused significant LH or FSH suppression at doses near the  $ED_{50}$ . Thus, compounds S-1 and S-4 were identified as SARMs with potent and tissue-selective *in vivo* pharmacological activity, and represent the first members of a new class of SARMs with selective anabolic effects.

Endogenous androgens play crucial physiological roles in establishing and maintaining the male phenotype (George and Wilson, 1986; Mooradian et al., 1987). Their actions are essential for the differentiation and growth of male reproductive organs, initiation and regulation of spermatogenesis, and control of male sexual behavior. In addition, androgens are important for the development of male characteristics in certain extragenital structures such as muscle, bone, hair, larynx, skin, lipid tissue, and kidney (Takeda et al., 1990). In females, the precise physiological roles of androgens are not completely understood, but the age-related decline in circulating androgen levels has been linked to symptoms such as decreased libido and sexuality, lack of vigor, diminished well-being, and loss of bone mineral density in postmenopausal women (Davis and Burger, 1996; Davis, 1999a; Davis, 1999b).

Synthesized steroidal androgens, due to their ability to mimic the actions of their endogenous counterparts, have been used clinically as valuable therapeutic agents to target a variety of male and female disorders resulting from androgen deficiency. The principle clinical indication of androgens is as replacement therapy for hypogonadal men (Conway et al., 1988; Wu, 1992). Other documented clinical uses of androgens include delayed puberty in boys, anemias, primary osteoporosis, hereditary angioneurotic edema, endometriosis, ER-positive breast cancer, and muscular diseases (Wu, 1992; Bagatell and Bremner, 1996; Nieschlag, 1996; Bhasin and Tenover, 1997). Also, androgens have been investigated as hormone replacement therapy for aging men and for regulation of male fertility (Wu, 1992; Tenover, 1997).

Since the discovery of the therapeutic benefits of testosterone in the 1930s, a variety of androgen preparations have been introduced and tested clinically. Unfortunately, virtually all currently available androgen preparations have severe limitations (Wu, 1992; Bhasin and Bremner, 1997). Unmodified testosterone is impractical for oral administration due to its low systematic bioavailability (Handelsman et al., 1990). Testosterone esters (e.g., testosterone propionate, testosterone enanthate, etc.) are presently the most widely used testosterone preparations, usually administered by intramuscular injection in oil vehicles (Snyder and Lawrence, 1980; Velazquez and Bellabarba Arata, 1998). A prolonged duration of action is achievable with these esters. However, they produce highly variable testosterone levels.  $17\alpha$ -Alkylated testosterones (e.g., methyltestosterone, oxandrolone, etc.) can be given orally. Nevertheless, they often cause unacceptable hepatotoxicity and are less efficacious; hence, they are not recommended for long-term androgen therapy (Heywood et al., 1977; Ishak and Zimmerman, 1987; Velazquez and Bellabarba Arata, 1998). Another common concern about steroidal androgens is the undesirable effects resulting from the cross-reactivity of the androgens or their *in vivo* metabolites with steroid receptors other than the androgen receptor (AR) (Wilson et al., 1980; Bhasin and Bremner, 1997).

During studies with affinity ligands for the AR, our group discovered a group of nonsteroidal androgens that are structural derivatives of bicalutamide and hydroxyflutamide, two known antiandrogens (Dalton et al., 1998; Mukherjee et al., 1999). Other laboratories have also reported the identification of nonsteroidal compounds that possess androgen activity (Dalton et al., 1998; Hamann et al., 1999; Negro-Vilar,

1999). The discovery of these nonsteroidal androgens offers an opportunity for the development of a new generation of selective androgen receptor modulators (SARMs) superior to current steroidal androgens. Theoretically, SARMs are advantageous over their steroidal counterparts in that they can obtain better receptor selectivity and allow greater flexibility in structural modification. Thus, SARMs can potentially avoid the undesirable effects caused by receptor cross-reactivity and achieve superior pharmacokinetic properties.

Subsequent to our initial discovery of several nonsteroidal androgens, our laboratories designed and synthesized multiple series of nonsteroidal compounds, and explored the structure-activity relationships for androgenic and anabolic activities, both *in vitro* and *in vivo* (He et al., 2002 ; Yin et al., 2002a ; Yin et al., 2002b). According to results from these structure-activity relationship studies, we designed a group of novel nonsteroidal compounds (Fig. 1) that were structurally optimized. We report herein the results of our studies to examine the *in vitro* AR binding affinity and the androgenic and anabolic activities of these new compounds in an animal model. Two potent and tissue-selective SARMs were identified from these structurally similar compounds, and they are members of a promising new class of drug candidates for further development.

## MATERIALS AND METHODS

### *1. Materials*

The S-isomers of compounds 1, 2, 3, and 4, and the R-isomer of compound 1 were synthesized in our laboratories (synthetic procedures will be reported separately). The purities of these compounds were greater than 99%, as determined by HPLC. Testosterone propionate (TP), polyethylene glycol 300 (PEG 300, reagent grade), and dimethylsulfoxide (DMSO, reagent grade) were purchased from Sigma Chemical Company (St Louis, MO). Ethyl alcohol USP was purchased from AAPER Alcohol and Chemical Company (Shelbyville, KY). Alzet osmotic pumps (model 2002) were purchased from Alza Corp. (Palo Alto, CA).

### *2. In Vitro Pharmacologic Activity*

Cytosolic AR was prepared from ventral prostates of castrated male Sprague-Dawley rats (about 250 g). The binding affinity of compounds 1, 2, 3, and 4 to the AR preparation was determined and analyzed as described previously (Mukherjee et al., 1996; Mukherjee et al., 1999). The ability of the compounds to influence AR-mediated transcriptional activation was examined using a co-transfection system, as previously described (Yin et al., 2002a). Transcriptional activation was measured using a single concentration (10 nM) of the indicated compound and reported as a percentage of the transcriptional activation observed for 1 nM DHT.



### 3. *Animals*

Male Sprague-Dawley rats, weighing 90 to 100g, were purchased from Harlan Biosciences (Indianapolis, IN). The animals were maintained on a 12-hour 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.

### 4. *Study Design*

Animals were randomly distributed into thirty groups, with 5 rats per group. Treatment groups are described in Table 1. One day prior to the start of drug treatment, animals in groups 2 through 30 were surgically castrated. After 24 hr of recovery, Alzet osmotic pumps (model 2002) pre-filled with a designated solution (as shown in Table 1) were implanted subcutaneously in the scapular region of castrated animals. Drug solutions used to fill the osmotic pumps were prepared using aseptic techniques. For solutions of nonsteroidal compounds and low dose (0.1 mg/day or lower) solutions of TP, drugs were first dissolved in minimal amounts of ethanol, and then diluted to final concentrations with PEG 300 (this vehicle was designated as vehicle 1). Because higher doses of TP could not be completely solubilized in the above vehicle, TP solutions for 0.3, 0.5, and 0.75 mg/day were prepared by dissolving the drug in a mixture of ethanol and DMSO and adjusting with PEG 300 to the desired final volume (this vehicle was

designated as vehicle 2). Due to the limited solubility of TP, two osmotic pumps were used in each animal in order to deliver TP at 0.5 and 0.75 mg/day. One osmotic pump was used in each animal for other groups.

After 14 days of drug treatment, rats were weighed, anesthetized, and sacrificed. Blood samples were collected by venipuncture of the abdominal aorta. For each animal, a whole blood sample and a serum sample were used for complete blood count and chemistry profile analyses, and a portion of the blood was centrifuged to prepare plasma. Plasma samples from selected groups were analyzed for FSH, LH, GH, AST-SGOT, ALT-SGPT, cholesterol, HDL, and triglyceride (TG). Plasma samples for these analyses were collected from a separate group of animals in the case of the TP-treated groups, using dose rates of 0.1, 0.3, 0.5, 0.75, and 1 mg/day. The ventral prostates, seminal vesicles, levator ani muscle, liver, kidneys, spleen, lungs, and heart were removed, cleared of extraneous tissue, weighed, and placed in vials containing 10% neutral buffered formalin. Preserved tissues were subjected to histopathological analysis. Osmotic pumps were removed from animals to check for correct pump operation.

### 5. Data Analyses

The weights of all organs were normalized to body weight, and analyzed for any statistically significant differences between groups using single-factor ANOVA with the alpha value set *a priori* at  $p < 0.05$ . The weights of prostates and seminal vesicles were used as indices for evaluation of androgenic activity, and the levator ani muscle weight

was used to evaluate the anabolic activity. Statistical analyses of parameters from complete blood count or serum chemical profiling, wherever applicable, were performed by single-factor ANOVA with the alpha value set *a priori* at  $p < 0.05$ . For compounds demonstrating full-range dose-response relationships in any of the measured parameters, the maximal response produced by the compound ( $E_{\max}$ ) and the dose rate that induced 50% of the maximal response ( $ED_{50}$ ) were obtained by nonlinear regression analysis using WinNonlin (version 3.1, Pharsight Corporation, Mountain View, CA) and the sigmoid  $E_{\max}$  model. The  $E_{\max}$  value indicated the efficacy of each compound, while the  $ED_{50}$  indicated its potency. The relative efficacy of each compound to TP was defined as the ratio of ( $E_{\max}$  of the compound) to ( $E_{\max}$  of TP). The relative potency was defined as the ratio of ( $ED_{50}$  of TP) to ( $ED_{50}$  of the compound).

## RESULTS

The *in vitro* AR binding of the R-isomer of compound 1 (designated as R-1) and the S-isomers of compounds 1, 2, 3, and 4 (designated as S-1, S-2, S-3, and S-4, respectively) was examined with a radioligand competitive binding assay. R-1 demonstrated poor AR binding affinity ( $K_i = 225 \pm 15$  nM), whereas S-1, S-2, S-3, and S-4 bound to the AR with moderate to high affinity, with  $K_i$  values ranging from 4 to 37 nM (Fig. 1). Next, the ability of these compounds to stimulate AR-mediated transcription was determined in an *in vitro* cotransfection system. At a concentration of 10 nM, compounds S-3 and S-4 stimulated AR-mediated transcription to 75 and 93%, respectively, of that observed for 1 nM DHT, while compounds S-1 and S-2

demonstrated lesser stimulation (i.e., 43 and 9.7%, respectively). Given previous studies in our laboratories demonstrating that *in vitro* cotransfection models poorly predict *in vivo* pharmacologic activity (Yin et al., 2002a), we then examined the androgenic and anabolic activities of these nonsteroidal compounds in a castrated rat model after 14 days of drug administration. R-1 was included as a negative control. TP, at increasing doses, was used as the positive control for anabolic and androgenic effects.

In accordance with literature reports (Saksena and Chaudhury, 1970; Teutsch et al., 1994; Battmann et al., 1998), we observed significant decreases in the weights of prostate, seminal vesicles, and levator ani muscle in castrated, vehicle-treated rats (Fig.2 to Fig.5). The weights of the prostate, seminal vesicles, and levator ani muscle in castrated rats were 6.2%, 8.1%, and 40.9%, respectively, of those in intact animals. The reduction in masses of these androgen-targeted organs in castrated animals is the result of ablation of endogenous androgen production (Saksena and Chaudhury, 1970). Exogenous administration of TP, an androgenic and anabolic steroid, increased weights of the prostate, seminal vesicles, and levator ani muscle in castrated rats (Fig. 2). The increases in organ weights induced by TP were dose rate-dependent.

Fig. 3 shows that compound S-1 had no significant effect on prostate, seminal vesicles, and levator ani muscle in castrated animals at 0.1 and 0.3 mg/day, but significantly stimulated the growth of these organs at higher doses. The weights of prostate, seminal vesicles, and levator ani muscle were maximally restored by S-1 to

14.9%, 13.4%, and 74.3%, respectively, of those in intact animals. The ED<sub>50</sub> values of S-1 in prostate, seminal vesicle, and levator ani muscle, as obtained by nonlinear regression analysis of dose-response relationships, were  $0.42 \pm 0.04$ ,  $0.38 \pm 0.26$ , and  $0.44 \pm 0.01$  mg/day, respectively (Fig 3B and Table 2), corresponding to 1.63, 1.47, and 1.70 mg/kg, respectively, based on the mean body weight of S-1-treated animals at the end of the study. The elevations in organ weights by S-1 demonstrated its androgenic and anabolic activities in animals. In comparison to TP, corresponding dose rates of S-1 induced significantly smaller increases in the weight of the prostate and seminal vesicles but a similar degree of increase in levator ani muscle weight (compare Fig. 2A and 3A). This result denoted the tissue selective androgenic and anabolic activity of S-1 in rats. The selectivity was also demonstrated by its relative efficacy compared to TP (Table 2). The relative efficacy in maintaining levator ani muscle weight was 0.72, much higher than the relative efficacies in maintaining prostate and seminal vesicle weights, which were less than 0.20.

Despite their high AR binding affinity, compounds S-2 and S-3 failed to exert any significant effect on the weights of prostate, seminal vesicles, and levator ani muscle in castrated animals, with dose rates up to 1 mg/day (Fig. 4). This suggests that rapid metabolism or clearance of these compounds led to lower plasma concentrations of these drugs, and thus no pharmacologic activity. Likewise, compound R-1 (the stereoisomer of S-1), at 1 mg/day, produced no apparent effect on the weights of prostate, seminal vesicles, and levator ani muscle in castrated animals, demonstrating the stereoselective pharmacologic action of these compounds.

Compound S-4 (Fig. 5) caused dose-dependent stimulation of growth in prostate, seminal vesicles, and levator ani muscle, with their weights in castrated animals being maximally promoted to 33.8%, 28.2%, and 101% of intact controls, respectively. Nonlinear regression analysis of dose-response relationships showed that the ED<sub>50</sub> values of S-4 were  $0.43 \pm 0.01$ ,  $0.55 \pm 0.02$ , and  $0.14 \pm 0.01$  mg/day in prostate, seminal vesicles, and levator ani muscle, respectively (Fig.5B and Table 2), corresponding to 1.62, 2.07, and 0.53 mg/kg, respectively, based on the mean body weight of S-4-treated animals at the end of the study. These results clearly revealed the androgenic and anabolic activities of S-4 in animals. In particular, S-4 exhibited potent and efficacious anabolic activity, as indicated by its ability to fully maintain the levator ani muscle weight in castrated animals at the same level as intact controls, at a dose rate as low as 0.3 mg/day (Fig. 5A). The relative potency and efficacy of S-4 in androgenic tissues were less than 0.3 and 0.4, respectively, compared to 1 for TP, while its relative potency and efficacy in the levator ani muscle were 1.07 and 0.97, respectively, compared to 1 for TP (Table 2).

Table 2 compares the androgenic and anabolic activities of S-1 and S-4, two compounds that exhibited *in vivo* functional activity in the present study, to those of TP. The efficacy for androgenic activity of S-4 (as indicated by relative efficacies in prostate and seminal vesicle) was about twice that of S-1, but the potency for androgenic activity (as indicated by relative potencies in prostate and seminal vesicle ) was similar between

these two compounds. As to anabolic activity, S-4 displayed much higher efficacy (as indicated by relative efficacy in levator ani muscle) and 2-fold greater potency (as indicated by relative potency in levator ani muscle) than S-1. These results suggest the greater selectivity of S-4 towards the anabolic target organ.

We also determined the serum levels of LH and FSH in animals that received S-1 and S-4, and compared them to the levels of these hormones observed in the intact, castrated, or TP- treated animals. As shown in Table 3, castration led to a significant elevation in FSH and LH levels, as compared with intact animals. TP showed no dose-dependent effect on castration-induced change in FSH, but partially inhibited the castration-induced increase in LH levels at higher doses. The activities of S-4 on LH and FSH were similar to those produced by TP. S-1 and S-4 partially suppressed LH production at dose rates of 0.5 mg/day or higher. However, it is important to note that S-1 and S-4 did not suppress LH production at the dose levels needed to produce the desired pharmacologic effects in the levator ani muscle or prostate. Interestingly, S-1 also partially suppressed FSH production at dose rates of 0.5 mg/day or higher. The FSH suppression noted at higher doses of S-1 suggested that this compound might interact with other steroid receptors, most probably progesterone receptors, in addition to the AR. Although statistically significant differences were noted in some instances, GH, AST-SGOT, ALT-SGPT and serum lipids (including cholesterol, HDL, and TG) were all within normal ranges for drug-treated animals. No drug- or dose-related changes in these indices were observed.

We also examined the effects of all compounds on total body weight and the weights of a variety of non-reproductive organs, including liver, heart, kidney, spleen, and lungs of treated animals. None of the compounds led to a dose-related change in these weights (data not shown). To further check for any signs of acute toxicity in animals from the studied compounds, complete diagnostic hematology studies of compound-treated animals were also performed. No drug- or dose-related changes were observed in any of the hematology diagnostic indices. This data suggests that all compounds manifested no acute toxicity during treatment.

## DISCUSSION

With *in vitro* AR binding and transcription activation assays, our laboratories previously identified a group of potent and efficacious nonsteroidal androgens that are structurally related to antiandrogen pharmacophores (Yin et al., 2002a). However, *in vivo* studies in a rat model with one of these nonsteroidal androgens, acetothiolutamide, failed to show androgenic activity (Yin et al., 2002b). Subsequent pharmacokinetic and metabolism studies in rats demonstrated that the lack of *in vivo* androgenic activity of acetothiolutamide in the pharmacology study was caused by its insufficient plasma exposure, which in turn resulted from its extensive hepatic degradation. Also, we found that oxidation at the sulfur linkage position was one major metabolic pathway for acetothiolutamide in rats, and that this oxidation likely produced deactivated or even antagonizing metabolites (Yin et al., 2002b). Considering these facts, we proposed to modify the linkage sulfur atom to block the oxidation at this position, thereby reducing



the overall hepatic metabolism. As a result, a series of novel molecules that carry an ether linkage instead of a thio linkage in the structure were designed and synthesized. The present studies demonstrated that two of these ether-bearing molecules, S-1 and S-4, were androgen receptor modulators with tissue-selective activity in animals.

Despite structural similarities, this series of ether-carrying compounds exhibited diverse *in vitro* and *in vivo* activity profiles. The S-isomers of compounds 1, 2, 3, and 4 displayed moderate to high binding affinity for the AR, while the R-isomer of compound 1 had poor receptor binding. This finding was consistent with our previous observation regarding the stereoselective AR binding of nonsteroidal ligands (Mukherjee et al., 1996; Mukherjee et al., 1999). Compounds S-1, S-3, and S-4 were further characterized as AR agonists with the *in vitro* co-transfection assay. The failure of S-2, a moderate AR binder, to stimulate AR-mediated gene transcription confirmed our previous finding that high receptor binding affinity is a prerequisite for agonist activity (Yin et al., 2002a). When tested in the castrated rat model, S-1 and S-4 demonstrated potent *in vivo* functional activity, and compounds S-2 and S-3 were inactive. Specifically, S-4 produced the greatest androgenic and anabolic activity in animals, with anabolic activity greater than that of TP. S-1 had a similar degree of anabolic activity as TP, but had much less androgenic activity. Interestingly, the ED50 for S-1 in prostate, seminal vesicle, and levator ani muscle were approximately the same (i.e., about 0.4 mg/day), while S-4 demonstrated more than 2-fold greater potency in levator ani muscle as compared to prostate and seminal vesicle, as indicated by the ED50 values (Table 2). The distinction in functional activities *in vivo* among the four structurally related compounds could be

caused by difference in any of numerous factors, including intrinsic activity, *in vivo* disposition and metabolism, or intracellular signaling pathway. Further studies to explore the physicochemical, physiological, and cellular/molecular determinants for nonsteroidal androgenic and anabolic activity will lead to insights into the mechanism of action of these nonsteroidal agents, and thereby provide a basis for future structural optimization.

The *in vitro* co-transfection assay is generally regarded as a valuable tool for screening of nonsteroidal AR ligands. With this assay, compounds S-1, S-3, and S-4 were successfully identified as potential AR agonists. However, as demonstrated in the animal study, S-3 did not show any measurable *in vivo* functional activity. Thus, the observation of *in vitro* agonist activity in the co-transfection assay can be but is not always predictive of *in vivo* activity. The pharmacological activity *in vivo* is determined not only by the ability of the compound to interact with the receptor, but also limited by complicated factors governing the accessibility of the compound to the effect site, such as disposition and metabolism. To fully predict the *in vivo* behavior and understand the structure-activity relationships, it is necessary to perform further studies examining the pharmacokinetics and metabolism of the compound. As a result of these and other studies, we abandoned use of the *in vitro* cotransfection assay in favor of *in vivo* pharmacologic assessment for discovery of SARMs.

The tissue-selective anabolic activity exhibited by S-1 and S-4 validated the feasibility of developing SARMs as a new generation of androgens. The possible mechanisms underlying the tissue-selectivity of these agents could be tissue-specific

recruitment of co-factors/co-repressors during the AR signaling pathway, or very likely for our nonsteroidal ligands, their distinct *in vivo* disposition from testosterone and its ester derivatives. The effects of testosterone in certain tissues, including most accessory reproductive organs and skin, are amplified through local conversion to DHT, the more potent bioactive form, by 5 $\alpha$ -reductase (16). Nevertheless, testosterone exerts direct effects in the testis, skeletal muscles, and bone (17). For nonsteroidal ligands, their actions in accessory reproductive organs such as prostate would not be amplified as they are for testosterone; therefore, such a nonsteroidal androgen with equivalent activity to testosterone on bone and muscle would likely have less activity on prostate or other accessory reproductive organs than testosterone.

Compounds S-1 and S-4 are the first nonsteroidal androgens with *in vivo* functional activity among our series of compounds. More significantly, the discovery of these two *in vivo* functional drug candidates represents a major progress towards the development of therapeutically useful SARMs. SARMs, like the clinically available selective estrogen receptor modulators (SERMs), would offer unique therapeutic advantages over their steroidal counterparts. The tissue-selectivity of these agents offers an exciting opportunity to differentially regulate the androgen effects in various target tissues, thus minimizing the interference to normal physiological processes while targeting desirable therapeutic goals. For example, SARMs with potent anabolic activity but minimal androgenic activity would be ideal for the treatment of patients who bear muscular diseases (such as sarcopenia or trauma-induced muscle wasting) but are contraindicated for androgenic stimuli (such as for aging population or prostate cancer

patients). In perspective, not only could SARMs be used as superior alternatives to current steroidal androgens in therapy of male hypogonadism, but also they could expand the scope of androgen therapy to include wasting syndromes, aging-related disorders due to declined androgen levels, male fertility regulation, and other androgen deficiency-related diseases.

In summary, the present studies examined the *in vitro* and *in vivo* activity profiles of a series of novel nonsteroidal AR ligands, among which two were identified as *in vivo* functional androgens with selective anabolic activity. These SARMs, with many advantages over current steroidal androgen preparations, implicate potential therapeutic significance in a scope of androgen-deficiency related disorders. Continued studies in our laboratories will focus on pre-clinical and clinical development of identified SARMs, and further optimization of chemical structures based on understanding the mechanisms underlying nonsteroidal androgenic and anabolic activities.

## REFERENCES

- Bagatell CJ and Bremner WJ (1996) Androgens in men--uses and abuses. *N Engl J Med* **334**:707-714.
- Battmann T, Branche C, Bouchoux F, Cerede E, Philibert D, Goubet F, Teutsch G and Gaillard-Kelly M (1998) Pharmacological profile of RU 58642, a potent systemic antiandrogen for the treatment of androgen-dependent disorders. *J Steroid Biochem Mol Biol* **64**:103-111.
- Bhasin S and Bremner WJ (1997) Emerging issues in androgen replacement therapy. *J Clin Endocrinol Metab* **82**:3-8.
- Bhasin S and Tenover JS (1997) Age-associated sarcopenia--issues in the use of testosterone as an anabolic agent in older men. *J Clin Endocrinol Metab* **82**:1659-1660.
- Conway AJ, Boylan LM, Howe C, Ross G and Handelsman DJ (1988) Randomized clinical trial of testosterone replacement therapy in hypogonadal men. *Int J Androl* **11**:247-264.
- Dalton JT, Mukherjee A, Zhu Z, Kirkovsky L and Miller DD (1998) Discovery of nonsteroidal androgens. *Biochem Biophys Res Commun* **244**:1-4.
- Davis S (1999a) Androgen replacement in women: a commentary. *J Clin Endocrinol Metab* **84**:1886-1891.
- Davis SR (1999b) The therapeutic use of androgens in women. *J Steroid Biochem Mol Biol* **69**:177-184.

- Davis SR and Burger HG (1996) Androgens and the postmenopausal woman. *J Clin Endocrinol Metab* **81**:2759-2763.
- George FW and Wilson JD (1986) Hormonal control of sexual development. *Vitam Horm* **43**:145-196.
- Hamann LG, Mani NS, Davis RL, Wang XN, Marschke KB and Jones TK (1999) Discovery of a potent, orally active, nonsteroidal androgen receptor agonist: 4-ethyl-1,2,3,4-tetrahydro-6- (trifluoromethyl)-8-pyridono[5,6- g]- quinoline (LG121071). *J Med Chem* **42**:210-212.
- Handelsman DJ, Conway AJ and Boylan LM (1990) Pharmacokinetics and pharmacodynamics of testosterone pellets in man. *J Clin Endocrinol Metab* **71**:216-222.
- He Y, Yin D, Perera M, Kirkovsky L, Stourman N, Dalton JT and Miller DD (2002) Novel nonsteroidal ligands with high binding affinity and potent functional activity for the androgen receptor. *Eur J Med Chem*, **37**: 619-634.
- Heywood R, Chesterman H, Ball SA and Wadsworth PF (1977) Toxicity of methyl testosterone in the beagle dog. *Toxicology* **7**:357-365.
- Ishak KG and Zimmerman HJ (1987) Hepatotoxic effects of the anabolic/androgenic steroids. *Semin Liver Dis* **7**:230-236.
- Mooradian AD, Morley JE and Korenman SG (1987) Biological actions of androgens. *Endocr Rev* **8**:1-28.
- Mukherjee A, Kirkovsky L, Yao XT, Yates RC, Miller DD and Dalton JT (1996) Enantioselective binding of Casodex to the androgen receptor. *Xenobiotica* **26**:117-122.

- Mukherjee A, Kirkovsky LI, Kimura Y, Marvel MM, Miller DD and Dalton JT (1999) Affinity labeling of the androgen receptor with nonsteroidal chemoaffinity ligands. *Biochem Pharmacol* **58**:1259-1267.
- Negro-Vilar A (1999) Selective androgen receptor modulators (SARMs): a novel approach to androgen therapy for the new millennium. *J Clin Endocrinol Metab* **84**:3459-3462.
- Nieschlag E (1996) Testosterone replacement therapy: something old, something new. *Clin Endocrinol (Oxf)* **45**:261-262.
- Saksena SK and Chaudhury RR (1970) Androgenic, anti-androgenic and anabolic activity of azasteroids on immature castrated rats. *Indian J Med Res* **58**:513-518.
- Snyder PJ and Lawrence DA (1980) Treatment of male hypogonadism with testosterone enanthate. *J Clin Endocrinol Metab* **51**:1335-1339.
- Takeda H, Chodak G, Mutchnik S, Nakamoto T and Chang C (1990) Immunohistochemical localization of androgen receptors with mono- and polyclonal antibodies to androgen receptor. *J Endocrinol* **126**:17-25.
- Tenover JL (1997) Testosterone and the aging male. *J Androl* **18**:103-106.
- Teutsch G, Goubet F, Battmann T, Bonfils A, Bouchoux F, Cerede E, Gofflo D, Gaillard-Kelly M and Philibert D (1994) Non-steroidal antiandrogens: synthesis and biological profile of high- affinity ligands for the androgen receptor. *J Steroid Biochem Mol Biol* **48**:111-119.
- Velazquez E and Bellabarba Arata G (1998) Testosterone replacement therapy. *Arch Androl* **41**:79-90.

Wilson JD (1996). Role of dihydrotestosterone in androgen action. *Prostate Suppl* **6**:88-92.

Wilson JD, Aiman J and MacDonald PC (1980) The pathogenesis of gynecomastia. *Adv Intern Med* **25**:1-32.

Wu FC (1992) Testicular steroidogenesis and androgen use and abuse. *Baillieres Clin Endocrinol Metab* **6**:373-403.

Yin D, He Y, Hong SS, Marhefka C, Stourman N, Kirkovsky L, Miller DD and Dalton JT (2002a) Key structural features of nonsteroidal ligands for binding and activation of the androgen receptor. *Molecular Pharmacology*, in press.

Yin D, Xu H, He Y, Kirkovsky L, Miller DD and Dalton JT (2002b) Pharmacology, pharmacokinetics, and metabolism of acetothiolutamide, a novel nonsteroidal agonist for the androgen receptor. *Journal of Pharmacology and Experimental Therapeutics*, in press.



## FOOTNOTES:

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**Table 1. Animals Groups and Experimental Design**

Group I.D.	Castration	Drug	Dose	Number of animals
1	No	None	None	5
2	Yes	None	Vehicle 1 only	5
3	Yes	Testosterone	0.03 mg/day	5
4	Yes	Testosterone	0.05 mg/day	5
5	Yes	Testosterone	0.1 mg/day	5
6	Yes	Testosterone	0.3 mg/day	5
7	Yes	Testosterone	0.5 mg/day	5
8	Yes	Testosterone	0.75 mg/day	5
9	Yes	R-1	1.0 mg/day	5
10	Yes	S-1	0.1 mg/day	5
11	Yes	S-1	0.3 mg/day	5
12	Yes	S-1	0.5 mg/day	5
13	Yes	S-1	0.75 mg/day	5
14	Yes	S-1	1.0 mg/day	5
15	Yes	S-2	0.1 mg/day	5
16	Yes	S-2	0.3 mg/day	5
17	Yes	S-2	0.5 mg/day	5
18	Yes	S-2	0.75 mg/day	5
19	Yes	S-2	1.0 mg/day	5
20	Yes	S-3	0.1 mg/day	5
21	Yes	S-3	0.3 mg/day	5
22	Yes	S-3	0.5 mg/day	5
23	Yes	S-3	0.75 mg/day	5
24	Yes	S-3	1.0 mg/day	5
25	Yes	S-4	0.1 mg/day	5
26	Yes	S-4	0.3 mg/day	5
27	Yes	S-4	0.5 mg/day	5
28	Yes	S-4	0.75 mg/day	5
29	Yes	S-4	1.0 mg/day	5
30	Yes	None	Vehicle 2 only	5

**Table 2. Comparison of Androgenic and Anabolic Activities of S-1 and S-4 to TP**

	Organs	Treatment	$E_{max}$ (% of Intact Control)	Relative Efficacy	ED50 (mg/day)	Relative Potency
Androgenic	Prostate	TP	120.6 ± 13.4	1.00	0.13 ± 0.03	1.00
		S-1	14.5 ± 0.7	0.12	0.42 ± 0.04	0.31
		S-4	35.2 ± 0.4	0.29	0.43 ± 0.01	0.30
	Seminal Vesicle	TP	70.0 ± 18.8	1.00	0.12 ± 0.02	1.00
		S-1	12.7 ± 3.1	0.18	0.38 ± 0.26	0.32
		S-4	28.5 ± 0.8	0.40	0.55 ± 0.02	0.22
Anabolic	Levator Ani Muscle	TP	104.2 ± 10.1	1.00	0.15 ± 0.03	1.00
		S-1	74.9 ± 0.4	0.72	0.44 ± 0.01	0.34
		S-4	101.0 ± 1.0	0.97	0.14 ± 0.01	1.07

**Table 3. Effects of S-1 and S-4 on Serum LH and FSH Levels**

		FSH (ng/mL)	LH (ng/mL)
Normal Range		5.6 - 20	0.15 - 0.7
Treatment			
None (Intact)		7.1 ± 1.2	0.27 ± 0.08
None (Castrated)		30 ± 3 <sup>I</sup>	5 ± 2 <sup>I</sup>
0.1		26 ± 6 <sup>I</sup>	4 ± 4 <sup>I</sup>
TP	0.3	23 ± 6 <sup>I</sup>	2 ± 1 <sup>I,C</sup>
(mg/day)	0.5	30 ± 7 <sup>I</sup>	5 ± 2 <sup>I</sup>
	0.75	22 ± 5 <sup>I,C</sup>	1.4 ± 0.7 <sup>I,C</sup>
	1.0	24 ± 8 <sup>I</sup>	2 ± 1 <sup>I,C</sup>
0.1		28 ± 7 <sup>I</sup>	5 ± 2 <sup>I</sup>
0.3		27 ± 5 <sup>I</sup>	3 ± 1 <sup>I</sup>
S-1	0.5	22 ± 3 <sup>I,C</sup>	1.7 ± 0.3 <sup>I,C,T</sup>
(mg/day)	0.75	20 ± 5 <sup>I,C</sup>	1.0 ± 0.7 <sup>C</sup>
	1	23 ± 3 <sup>I,C</sup>	1.6 ± 0.7 <sup>I,C</sup>
0.1		27 ± 5 <sup>I</sup>	4 ± 1 <sup>I</sup>
0.3		26 ± 1 <sup>I</sup>	3.7 ± 0.9 <sup>I</sup>
S-4	0.5	26 ± 8 <sup>I</sup>	2.4 ± 0.9 <sup>I,C</sup>
(mg/day)	0.75	31 ± 5 <sup>I,T</sup>	3 ± 1 <sup>I</sup>
	1	34 ± 5 <sup>I,T</sup>	2.4 ± 0.4 <sup>I,C</sup>

I, C, and T: significantly different ( $p < 0.05$ ) from intact, castrated, and corresponding dose rate of TP-treated animals, respectively, as analyzed by single-factor ANOVA.

## LEGENDS FOR FIGURES

**Fig. 1. Chemical structures and in vitro pharmacologic activity of nonsteroidal AR ligands.** Cytosolic AR was prepared from ventral prostates of castrated male Sprague-Dawley rats. The AR binding affinity was determined and analyzed as described previously (Mukherjee et al., 1996; Mukherjee et al., 1999). The ability of the compounds to influence AR-mediated transcriptional activation was examined using a co-transfection system, as previously described (Dalton et al., 1998). Transcriptional activation was measured using a single concentration (10 nM) of the indicated compound and reported as a percentage of the transcriptional activation observed for 1 nM DHT.

**Fig. 2. A. Assay for androgenic and anabolic activity of TP in castrated immature rats.** One day after castration, immature rats received 1 mg/day of TP via Alzet osmotic pumps for 14 days. All weights were corrected for 100 gram of body weight, and were converted to the percentage of the weights in the intact control group. Values represent the mean  $\pm$  standard deviation (n = 5 per group). The letters “I” and “C” above the error bars indicate a significant difference between the group and the intact control group or castrated control group, respectively, as tested by single-factor ANOVA (p < 0.05). **B. Dose response relationships of TP.**  $E_{\max}$  and  $ED_{50}$  values for the levator ani (triangles), prostate (open circles), and seminal vesicles (diamonds) were obtained by nonlinear regression analysis. Curves were obtained by fitting the data into sigmoid  $E_{\max}$  model.

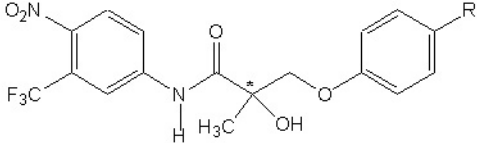
**Fig. 3. A. Assay for androgenic and anabolic activity of S-1 in castrated immature rats.** One day after castration, immature rats received the indicated dose rates of S-1 via Alzet osmotic pumps for 14 days. All weights were corrected for 100 gram of body weight, and were converted to the percentage of the weights in the intact control group. Values represent the mean  $\pm$  standard deviation (n = 5 per group). The letters “T”, “C”, and "T" above the error bars indicate a significant difference between the group and the intact control group, castrated control group, or corresponding TP group, respectively, as tested by single-factor ANOVA ( $p < 0.05$ ); **B. Dose response relationships of S-1.**  $E_{\max}$  and  $ED_{50}$  values for the levator ani (triangles), prostate (open circles), and seminal vesicles (diamonds) were obtained by nonlinear regression analysis. Curves were obtained by fitting the data into sigmoid  $E_{\max}$  model.

**Fig. 4. Assay for androgenic and anabolic activity of S-2, S-3, and R-1 in castrated immature rats.** One day after castration, immature rats received the indicated dose rates of S-2, S-3, or R-1 via Alzet osmotic pumps for 14 days. All weights were corrected for 100 gram of body weight, and were converted to the percentage of the weights in the intact control group. Values represent the mean  $\pm$  standard deviation (n = 5 per group). The letters “T”, “C”, and "T" above the error bars indicate a significant difference between the group and the intact control group, castrated control group, or corresponding TP group, respectively, as tested by single-factor ANOVA ( $p < 0.05$ ).

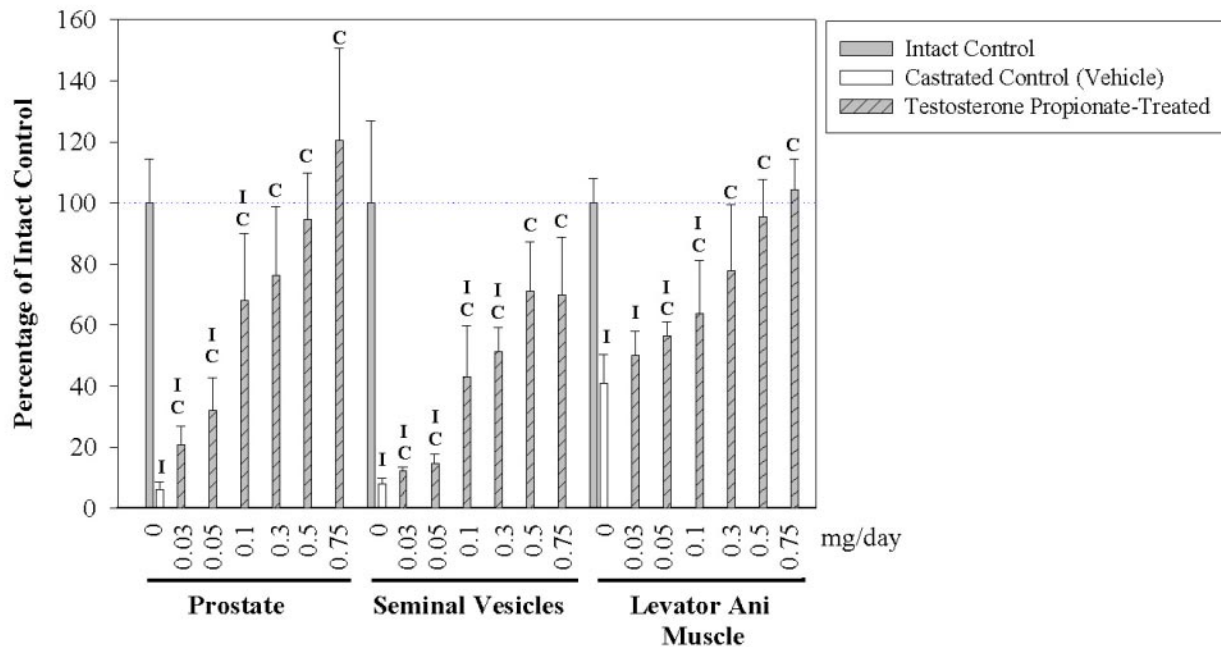
**Fig. 5 A. Assay for androgenic and anabolic activity of S-4 in castrated immature rats.** One day after castration, immature rats received the indicated dose rates of S-4 via

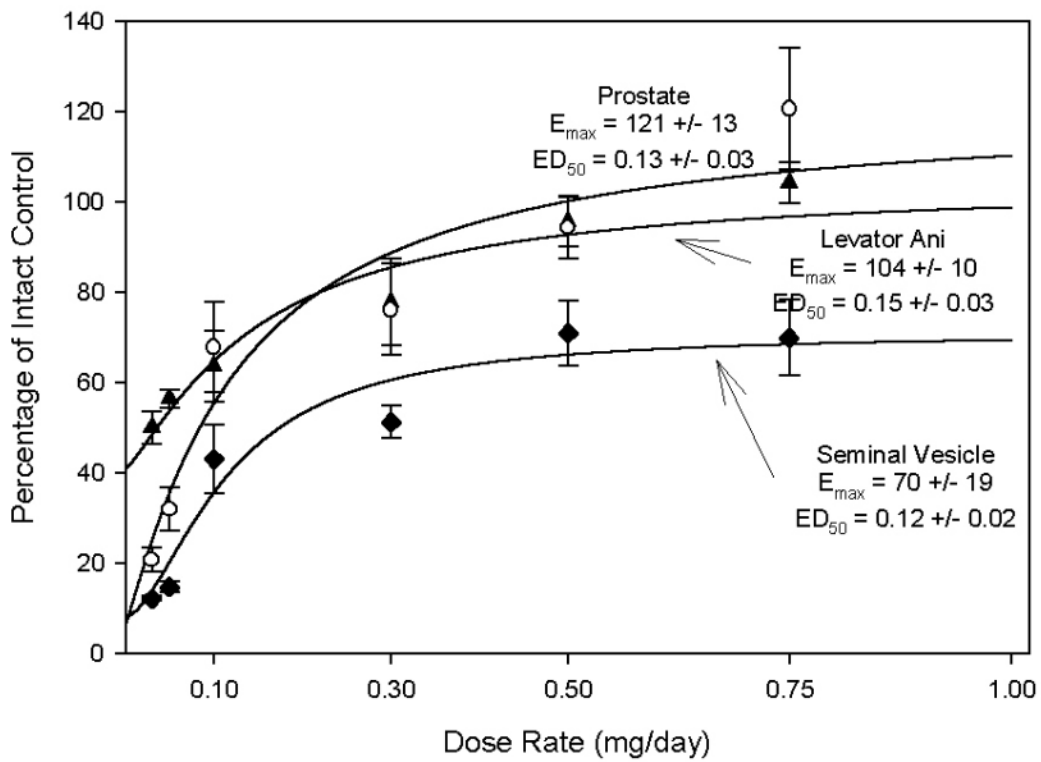
Alzet osmotic pumps for 14 days. All weights were corrected for 100 gram of body weight, and were converted to the percentage of the weights in the intact control group. Values represent the mean  $\pm$  standard deviation (n = 5 per group). The letters "I", "C", and "T" above the error bars indicate a significant difference between the group and the intact control group, castrated control group, or corresponding TP group, respectively, as tested by single-factor ANOVA ( $p < 0.05$ ); **B. Dose response relationships of S-4.**  $E_{\max}$  and  $ED_{50}$  values for the levator ani (triangles), prostate (open circles), and seminal vesicles (diamonds) were obtained by nonlinear regression analysis. Curves were obtained by fitting the data into sigmoid  $E_{\max}$  model.



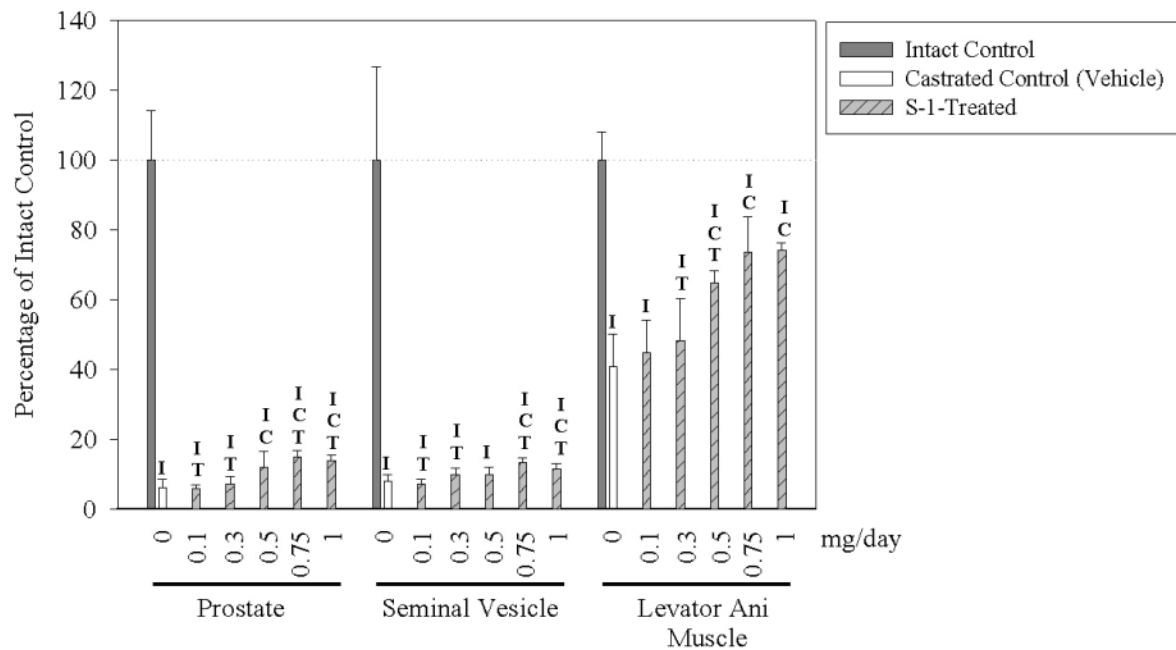


Name	Isomer	R	K <sub>i</sub> (nM)	Activation (% of 1 nM DHT)
R-1	R	F	225 ± 15	N.D.
S-1	S	F	6.1 ± 0.2	43 ± 2.6
S-2	S	COCH <sub>3</sub>	37 ± 2.4	9.7 ± 1.5
S-3	S	COC <sub>2</sub> H <sub>5</sub>	6.1 ± 0.1	75 ± 11
S-4	S	NHCOCH <sub>3</sub>	4.0 ± 0.7	93 ± 7.0

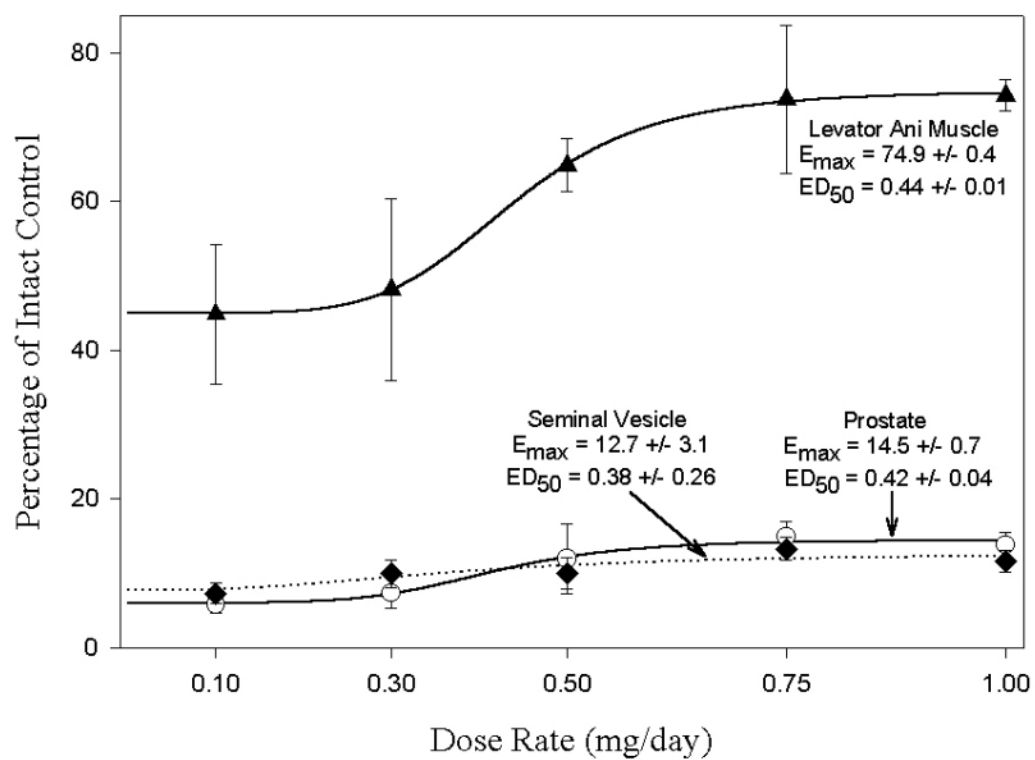


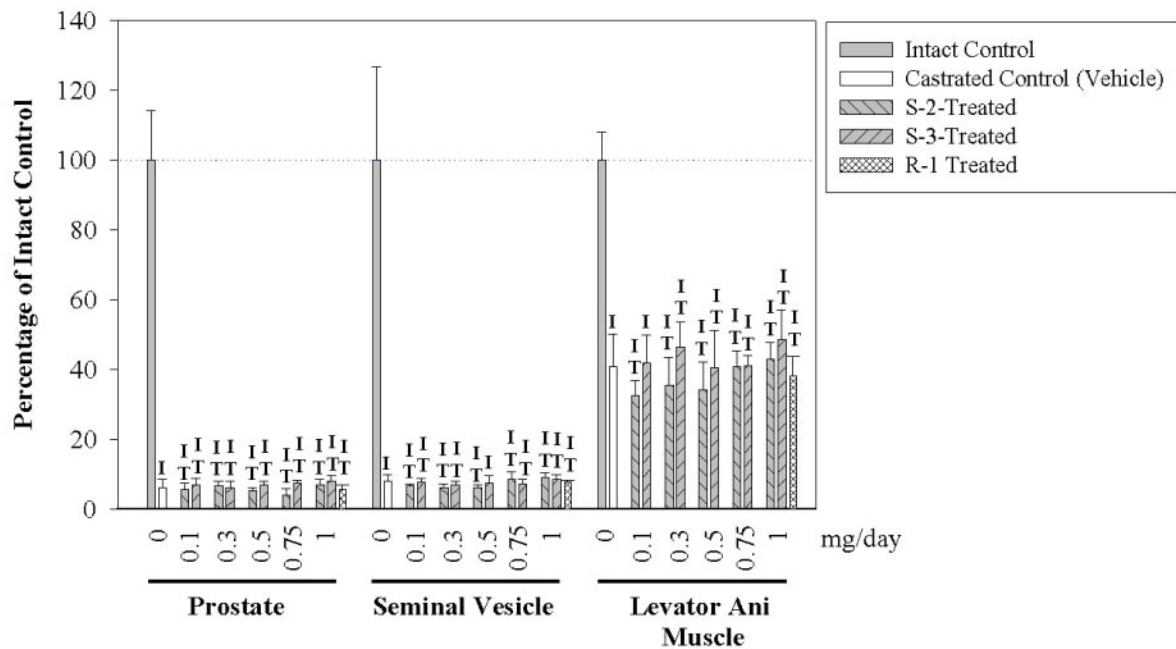


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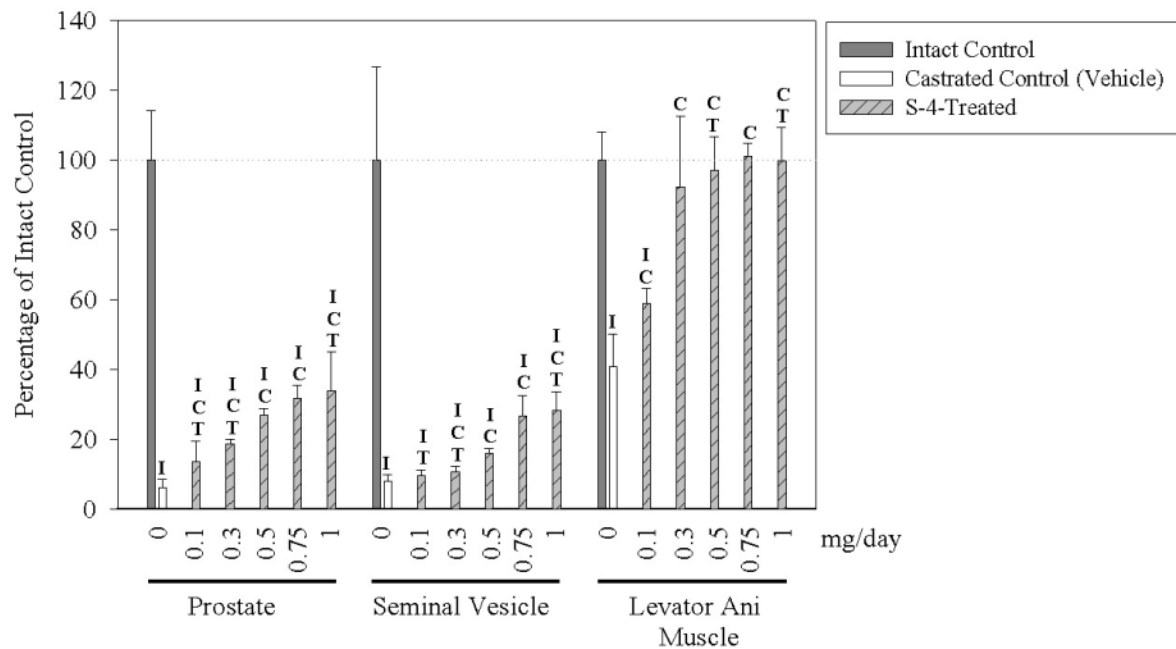


**B.**





A.



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