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Design, Synthesis, and Preclinical Characterization of the Selective Androgen Receptor Modulator (SARM) RAD140

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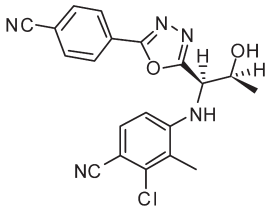
ABSTRACT This report describes the discovery of **RAD140**, a potent, orally bioavailable, nonsteroidal selective androgen receptor modulator (SARM). The characterization of **RAD140** in several preclinical models of anabolic androgen action is also described.

KEYWORDS Androgen, SARM, cachexia, oxadiazole, Herschberger assay, primate



7 (RAD140)

The androgen receptor (AR) is a member of the steroid hormone nuclear receptor superfamily that includes estrogen, progestin, glucocorticoid and mineralocorticoid receptors.[1] The binding of the prototypical, endogeneously produced androgen testosterone (**1**) and the important active metabolite dihydrotestosterone (**2**) to AR initiates a remarkably diverse array of biological activities that can vary according to a subject's sex, age and hormonal status. The activity of AR is critical to normal human sexual development and function, but beyond this signature role, AR activation also has important effects on diverse targets such as bone, liver, muscle and the central nervous system.[2,3] The therapeutic potential of androgen signaling is well-appreciated in the medicinal chemistry community, and for quite some time, chemists have sought compounds that selectively stimulate muscle and bone growth while minimizing the proliferative and/or hypertrophic effects on sex tissues such as the prostate in males and clitoris in females.[4,5] Such compounds have been termed selective androgen receptor modulators or SARMs. In this regard, the prototypical and endogenous androgen, testosterone, is considered to be a logical benchmark comparator. Compound **3** is the GTx SARM S-22 and compound **4** is the BMS SARM 562929, both of which have been reported in the literature as being orally active compounds with selectivity for muscle over prostate relative to testosterone in various preclinical models.[6,7]

The possibility of obtaining compounds having tissue-selective activities that are different from that of the endogenous benchmark testosterone might derive from the fact that typical AR receptor activation, which is initiated by the binding of a molecule with affinity for the AR to the AR ligand binding domain, is then followed by a rather remarkable, coordinated series of interactions: These may include a change in receptor topology, dissociation of heat shock proteins, receptor dimerization, receptor phosphorylation, rapid-signaling events, translocation to the nucleus (AR), association with many different coregulatory proteins to form a transcriptional complex that results in the activation or suppression of RNA synthesis from AR-modulated genes, and finally receptor degradation.[8] Since each receptor−ligand complex topology is unique to that ligand structure, one can appreciate that the interaction of any particular ligand−receptor complex with coregulatory proteins is likely to be unique to that ligand as well. Furthermore, because the expression level of AR, the constellation and expression level of coregulatory proteins, and the patterns of post-transcriptional regulatory events differ in each type of androgen target cell, and the topography of AR regulatory sites in the genome differs at each gene, this remarkable choreography of events and interactions provides a rich environment within which one might search for SARMs having a desirable pattern of tissue-selective pharmacology, such as high anabolic but limited androgenic activity.

Further complicating our understanding of the origin of SARM selectivity is the "bio-amplification" of the primary endogenous androgen testosterone. Interestingly, the endogenously produced and very important androgen testosterone serves as a type of "anti-SARM" or "inverse SARM" because its androgenic activity is increased by conversion to the more potent 5α-dihydrotestosterone by the 5α-reductase enzyme in certain tissues including the scalp and prostate (but not in muscle or bone). As a result, androgens that do not undergo such bioamplification in the prostate will

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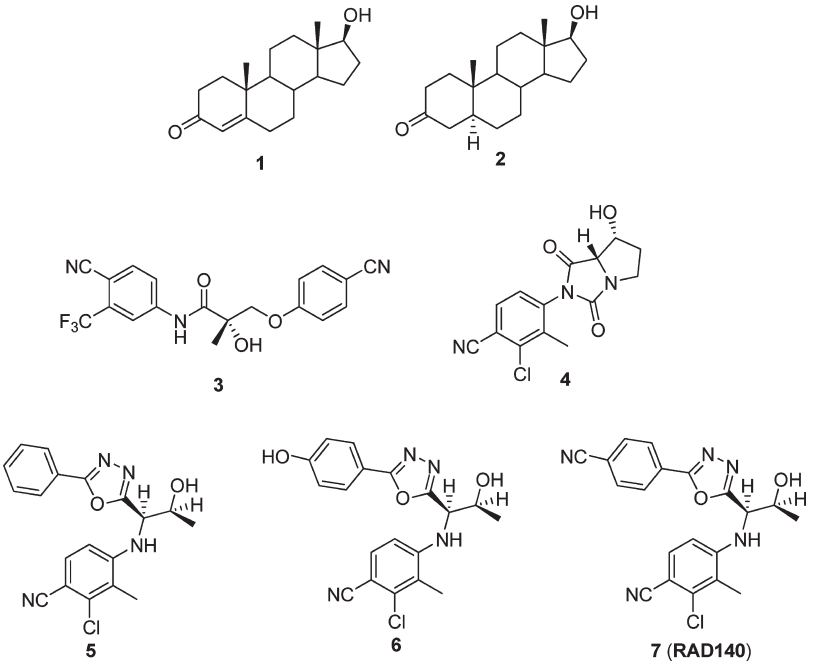
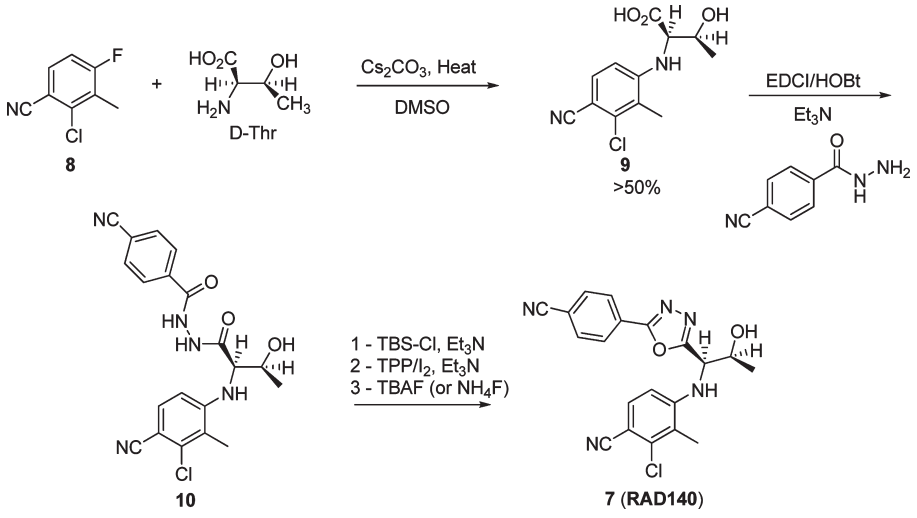


Figure 1. Structures of testosterone (**1**), 5α-dihydrotestosterone (**2**), GTx S-22 (**3**), BMS 562929 (**4**), initial lead **5**, active metabolite **6**, and **7** (**RAD140**).

Scheme 1. Synthesis of Compound **7** (**RAD140**)



demonstrate improved selectivity regarding muscle vs prostate when compared to a testosterone-treated control or an intact animal whose primary endogenous androgen is testosterone.[9] More broadly put, one might appreciate that metabolic differences between endogenous androgens such as testosterone or dihydrotestosterone and SARMs can also vouch for at least some selectivity differences.

Our work in the SARM area resulted in the synthesis and evaluation of a large number of candidate templates. While we found it relatively easy to obtain compounds with high affinity for AR, we struggled to achieve compounds that demonstrated good oral efficacy and high *in vivo* tolerability. After scanning many potential leads for oral, *in vivo* activity, we arrived at high affinity compound **5** through a combination of synthetic intermediate testing, literature evaluation and fragment combination. We were delighted when **5** demonstrated oral activity in rats.

However, when we performed a pharmacokinetic analysis in rats, we could detect only very low levels of **5** after oral dosing ($F < 5\%$). Further analysis revealed that **5** was efficiently converted to **6** *in vivo*, presumably by cytochromes P450 in the rat liver.[10] Compound **6** had similar activity to compound **5** *in vivo*, suggesting that **6** was largely

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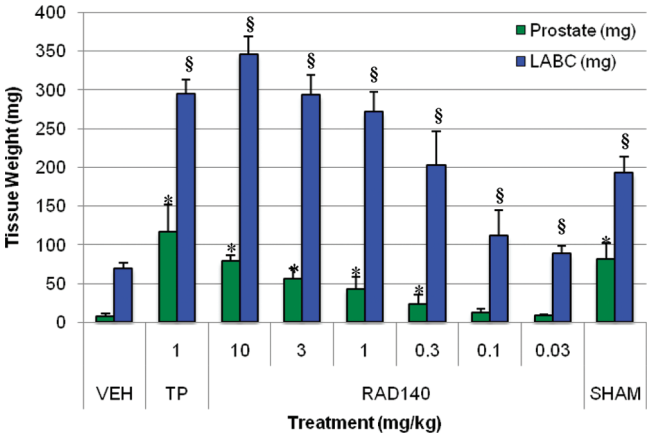

Figure 2. Tissue-selective agonist activity of **RAD140** in castrated immature rats. The muscle (levator ani) and prostate weights from animals treated for 11 days are plotted with sham and vehicle controls together with the SD. TP is testosterone propionate dosed subcutaneously daily in corn oil. Five rats were included in each treatment group. $^{*}p < 0.05$ vs vehicle for prostate. $^{\S}p < 0.05$ vs vehicle for LABC.

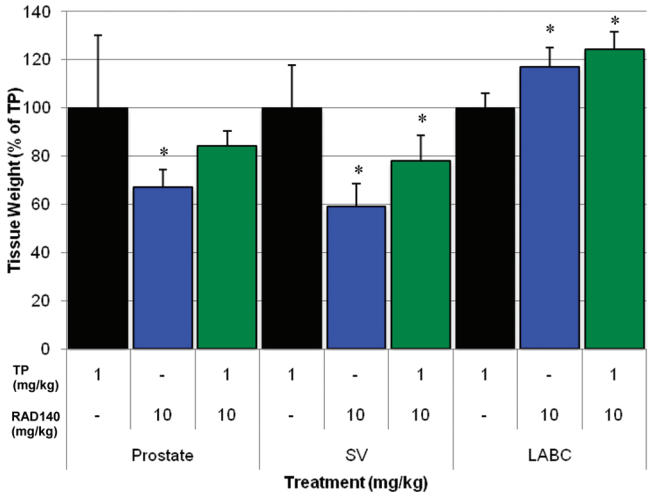

Figure 3. Tissue-selective antagonist activity of **RAD140**. The muscle (levator ani), seminal vesicles, and prostate weights from castrated immature rats treated for 11 days are plotted as a percent of testosterone propionate (TP) together with the SD. $^{*}p < 0.05$ vs TP for all tissues.

responsible for the activity of compound **5**.[11] An *in vitro* screen with *human* microsomes revealed rapid metabolism of compound **5**, thus indicating this transformation as a potential human metabolic liability and prompting us to prepare compounds in which the 4′-position of the pendant phenyl was blocked from P450-induced hydroxylation.[12] We looked at several analogues containing a 4′-blocking group, and in the course of our efforts we identified compound **7** (**RAD140**; Figure 1) as our preclinical development candidate.

The synthesis of compound **7** is shown in Scheme 1.[13,14] We relied on an expeditious, ipso-fluorine substitution of the left-hand side precursor, piece **8**, with D-threonine in the presence of K_2CO_3 in DMSO to give the desired product **9** in workable yields (typically >50%). The D-Thr adduct **9** was coupled with 4-cyanobenzohydrazide under standard coupling conditions using EDCI and HOBt. The resultant product **10** was silylated with TBDMS-Cl, subjected to dehydrative cyclization conditions in the presence of TPP/I_2, and then desilyated for the final step.[15−17] Overall, this has proven to be a reliable and efficient synthesis using a fairly inexpensive, albeit nonproteinogenic amino acid as the chirality source.

The stability of **RAD140** was high ($t_{1/2}$ > 2 h) in incubations with rat, monkey, and human microsomes, and it also had good bioavailability in rats (F = 27−63%) and monkeys (65−75%). **RAD140** demonstrated excellent affinity for the androgen receptor (K_i = 7 nM vs 29 nM for testosterone and 10 nM for DHT) as well as good selectivity over other steroid hormone nuclear receptors, with the closest off target receptor being the progesterone receptor (IC_{50} = 750 nM vs 0.2 nM for progesterone).[18] *In vitro* functional androgen agonist activity was confirmed in the C2C12 osteoblast differentiation assay, where an EC_{50} of 0.1 nM was shown (DHT = 0.05 nM).[19]

RAD140 was characterized in a number of *in vivo* assays to determine its oral efficacy on a number of parameters associated with androgenic activity in preclinical models. For example, **RAD140** was dosed in both young castrated and intact male rats in order to assess its effects through a range of endogenous androgenic signaling backgrounds. The young castrated rat provides a very sensitive *in vivo* assay for androgenic activity because the animal is relatively androgen-naïve; thus, any signaling activity from an exogenously administered androgen is superimposed on an essentially blank background.[20] In Figure 2, the effect of increasing doses of orally administered **RAD140** (0.5% methylcellulose) on levator ani bulbocavernosus muscle ("levator ani" or "LABC") weight and prostate weight is shown relative to vehicle (castrated control), sham (noncastrated control), and testosterone propionate (TP) dosed subcutaneously at 1 mg/kg in corn oil.[21] As can be seen, **RAD140** stimulates the levator ani muscle beginning at a dose of 0.03 mg/kg (po) and reaches a level of efficacy equivalent to the sham-operated animal at 0.3 mg/kg.

Because we consistently observed that **RAD140** failed to achieve a level of prostate or seminal vesicle stimulation equal to TP at 1 mg/kg (no matter how high the dose of **RAD140**), we decided to test whether **RAD140** could antagonize the effect of TP on rat prostate and seminal vesicles and, at the same time, determine what effect the coadministration of **RAD140** and TP might have on the levator ani muscle. From the results shown in Figure 3, it is apparent that a high dose of **RAD140** (10 mg/kg, po) actually antagonizes the effect of TP at 1 mg/kg on the seminal vesicles but adds to the effect of TP on the levator ani muscle. We were able to ascertain that the effective dose for achieving antagonism by **RAD140** is 0.3−1 mg/kg (po) for 1 mg/kg TP (sc) (data not shown). In the prostate, **RAD140** also caused a downward trend in the stimulation by TP, but the change did not reach statistical significance. Thus, in the young castrate male rat model, **RAD140** appears to be a potent and complete androgen agonist on the levator ani, but a weaker, partial antagonist on the seminal vesicle and possibly the prostate.[22]

The goal of most preclinical, *in vivo* models is to best predict how a drug will perform in the drug target population.

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When considering the issue of how stimulatory an androgen is on any given tissue in a preclinical model, one should keep in mind that the background level of androgen signaling can affect the response observed in an animal. The castrated rat model has limitations because the very low endogenous androgen level in this model is an artificial situation, not reflected in the target adult human male population.[23] In particular, the target male population *will* have an androgenic background well above a castrate, although the androgen levels will likely be lower than the norm for their group.

To better understand how this group might respond, we decided to look at young *intact* male rats, since they have endogenous testosterone but at somewhat reduced levels. Therefore, they retain prostate sensitivity to an androgenic compound but at the same time have a baseline stimulation that is more similar to the target population than castrated animals. As shown in Figure 4, **RAD140** increased the weight of the levator ani muscle above that of the intact control starting with the lowest tested dose (0.1 mg/kg). Interestingly, **RAD140** demonstrated no stimulation of the prostate above the intact animal control level until the highest dose tested, 30 mg/kg. At 0.3 mg/kg, **RAD140** demonstrated muscle efficacy similar to TP at 0.5 mg/kg, but a dose of 30 mg/kg of **RAD140** was required to approximate the prostate efficacy of 0.5 mg/kg TP.[24] From this study it is apparent that in young intact male rats **RAD140** has a very wide range of selectivity relative to both TP-treated rats as well as sham-control rats.

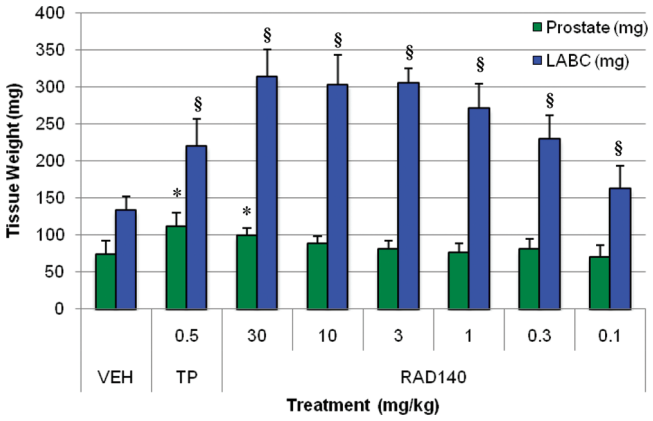

Figure 4. Tissue-selective agonist activity of **RAD140** in young intact male rats. The muscle (levator ani) and prostate weights from intact immature rats treated for 11 days are plotted with sham and vehicle controls together with the SD. Eight rats were included in each treatment group. $^{*}p < 0.05$ vs vehicle for prostate. $^{\S}p < 0.05$ vs vehicle for LABC.

Finally, we were interested in evaluating the effect of **RAD140** in young, male cynomolgous monkeys to establish efficacious dosing levels in what we considered to be a more relevant preclinical species. We performed a relatively simple, nonterminal study that still allowed us to evaluate anabolic as well as lipid and other clinical chemistry parameters. To assess anabolic activity, we first looked at gross body weight, which we knew to be a sensitive marker of anabolic androgen action in young nonhuman primates. The results on animal body weight of 28-day dosing with **RAD140** at 0.01 mg/kg, 0.1 mg/kg, and 1 mg/kg are shown in Figure 5.

Due to the small group size ($n = 3$ for each dosing group), we used each animal's background weight change for the weeks prior to the experiment to establish the baseline as control. Since the mean body weight for each group of three monkeys converged to an almost identical number (day −1), with the absolute body weight range between groups of only 4.26−4.29 kg, we plotted the absolute body weight in Figure 5. In this study, a mean weight gain of greater than 10% in just 28 days of dosing was achieved at a dose of just 0.1 mg/kg, with a similar effect observed at the 1.0 mg/kg dosing group.[26]

Dual energy X-ray absorptiometry ("DEXA") scans of all monkeys were taken two days before dosing began and one day after the final dose (day −2 and day 29) in order to determine the effects of **RAD140** on lean tissue and fat; the results are shown in Figure 6. As can be seen, there was no consistent effect on absolute fat mass, whereas muscle showed a qualitative trend that increases with dose.

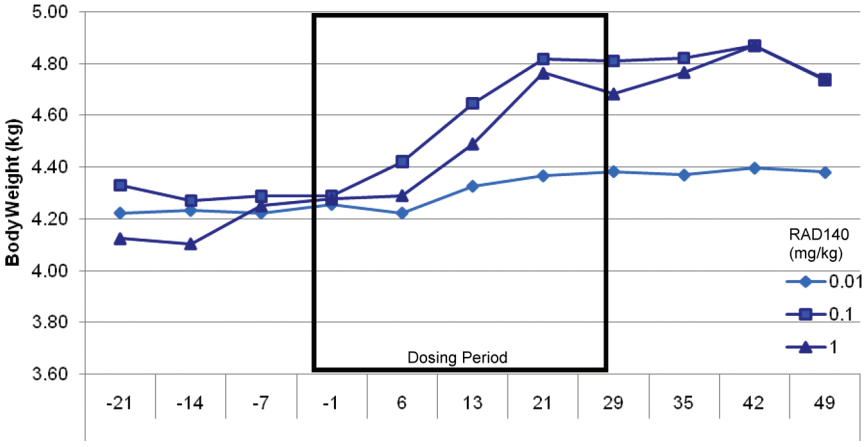

Figure 5. Primate body weight from day −21, through 28 days dosing and 21 days postdosing with **RAD140** (0.01, 0.1, and 1 mg/kg, po).[25] Three monkeys were included for each treatment group. The change in baseline subtracted body weight from day −1 to day 29 was statistically significant for the 0.1 mg/kg ($p < 0.01$) and 1.0 mg/kg ($p < 0.05$) groups only. The change in body weight at day 29 between the 0.1 mg/kg group and the 0.01 mg/kg group was statistically significant ($p < 0.05$) but not for 1.0 mg/kg and the 0.01 mg/kg group ($p < 0.1$).

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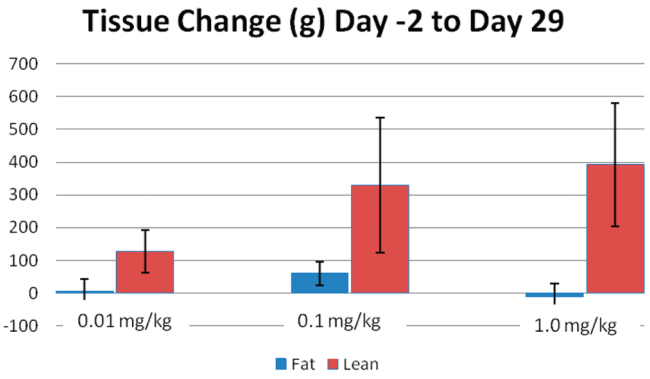

Figure 6. Mean change in primate tissue weight as measured by DEXA analysis at day −2 and day 29. Standard deviation for fat (36, 36, 40) and lean tissue (65, 205, 188) for 0.01 mg/kg, 0.1 mg/kg, and 1.0 mg/kg, respectively. None of the changes were statistically significant ($p > 0.05$).

Although it appears that the majority of mass increase shown in Figure 5 was due to lean mass increase, none of the tissue weight increases were quite statistically significant ($p > 0.05$), which might be due to the small group sizes ($n = 3$) and relatively large standard deviations.[27]

Clinical chemistry indicated the expected lowering of lipids (LDL, HDL, triglycerides).[28] Despite the rather dramatic increases in body weight over such a short time, there was no elevation of liver enzyme transaminase levels in any animal at any dose > 2 fold over its baseline value.[29,30] Given the well-established relationship between oral androgen use and liver stress indicators, we were quite pleased that at a dose 10-fold greater than the fully effective dose we saw minimal liver enzyme elevations.[31] Taken in sum, **RAD140** has all the hallmarks of a SARM. It is potency selective, since it stimulates muscle weight increases at a lower dose than that required to stimulate prostate weight increases. Moreover, it is also efficacy selective, because it is fully anabolic on muscle but demonstrates less than complete efficacy on the prostate and seminal vesicles and, in fact, can partially antagonize the stimulation of the seminal vesicles induced by testosterone. **RAD140** has excellent pharmacokinetics and is a potent anabolic in nonhuman primates as well. We believe the overall preclinical profile of **RAD140** is very good, and the compound has completed preclinical toxicology in both rats and monkeys. We are currently preparing **RAD140** for phase I clinical studies in patients suffering from severe weight loss due to cancer cachexia.

SUPPORTING INFORMATION AVAILABLE

Synthetic methods, NMR spectra, and biological assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) p*K* data in rats for compound **3** is provided in the Supporting Information.

(11) Oral data for compounds **3** and **4** in the Herschberger assay is shown in the Supporting Information.

(12) Human and rat microsome data are shown in the Supporting Information.

(13) The left-hand side of the molecule as written is presumed to overlay with the A-ring of testosterone. This particular left-hand side equivalent has been utilized to good effect previously in nonsteroidal SARMs: (a) Li, J. J.; Sutton, J. C.; Nirschl, A.; Zou, Y.; Wang, H.; Sun, C.; Pi, Z.; Johnson, R.; Krystek, S. R., Jr.; Seethala, R.; Golla, R.; Sleph, P. G.; Beehler, B. C.; Grover, G. J.; Fura, A.; Vyas, V. P.; Li, C. Y.; Gougoutas, J. Z.; Galella, M. A.; Michael, A.; Zahler, R.; Ostrowski, J.; Hamann, L. G. Discovery of Potent and Muscle Selective Androgen Receptor Modulators through Scaffold Modifications. *J. Med. Chem.* **2007,** *50* (13), 3015–3025. The precursor fragment 6 has been described for the preparation of SARMs in: . (b) Schlienger, N.; Lund, B. W.; Pawlas, J.; Badalassi, F.; Bertozzi, F.; Lewinsky, R.; Fejzic, A.; Thygesen, M. B.; Tabatabaei, A.; Bradley, S. R.; Gardell, L. R.; Piu, F.; Olsson, R. Synthesis, structure−activity relationships, and characterization of novel nonsteroidal and selective androgen receptor modulators. *J. Med. Chem.* **2009,** *52*, 7186–7191.

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(17) The scheme shown was used to successfully produce approximately 2 kg of compound **5** in >99% purity under GMP manufacturing conditions.

(18) The AR binding assay was performed as specified from the manufacturer. The assay is a fluorometric assay using a tracer made from fluorescent tagged AR-ligand methyltrienolone (R1881). K_i values were derived by the Cheng−Prushoff equation ($K_i = (IC_{50}/(1 + [S]/K_m))$, where K_m was set equal to K_d, $K_d = 25$ nM (fluorometric R1881), and $[S] = 1$.

(19) The C2C12 osteoblast differentiation assay procedure is explained in the Supporting Information.

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(21) This dose of TP provides an approximate EC_{70} on prostate and EC_{90} on muscle in our Herschberger assays.

(22) **RAD140** demonstrated *fairly* linear increases in exposure in male rats up through the 10 mg/kg po dose range, thereby ruling out an exposure limited efficacy as opposed to compound-limited efficacy. Antagonism of TP is further evidence of a mechanism-specific, limited efficacy as opposed to a pharmacokinetic limitation of the compound.

(23) Testosterone levels in castrated rats are <0.5 ng/mL; i.e. see: D'Souza, S. S.; Selmin, F.; Murty, S. B.; Qiu, W.; Thanoo, B. C.; DeLuca, P. P. Assessment of fertility in male rats after extended chemical castration with a GNRH antagonist AAPS. *Pharm. Sci.* **2004**, *6* (1), Article 10.

(24) For the intact animal dosing, we had originally planned not to run testosterone as a comparator, since the intact animals were relied on to provide their own controls and we were primarily concerned with how **RAD140** behaved within and against the animal's endogeneous background. Nevertheless, we ultimately did include testosterone in one dose group but used a slightly lower dose because the animals already have endogeneous testosterone present. Nevertheless, we *did not* run a separate testosterone curve in order to determine what the EC_{30}, EC_{50}, or EC_{70} for testosterone was in this model.

(25) The monkeys were placed into groups of three at day-21, and the weight of each monkey recorded at each time point and the mean weight of each group are reflected on the graph. Coincidentally, by day −1, the mean weight of each group had each converged to a very similar value of 4.26 kg, 4.29 kg, and 4.28 kg for the 0.01 mg/kg, 0.1 mg/kg, and 1.0 mg/kg groups, respectively.

(26) p*K* analysis at various time points throughout this monkey study indicated that significant increases in exposure were seen with dose.

(27) Since these were young, intact male cynomolgous monkeys (3 to 4 years of age), they had fairly high endogeneous total plasma testosterone at day −1 (approximately 600−800 ng/dL), which is similar to the approximately 600 ng/mL that human males have between the ages of 25 and 54 (the levels then gradually decline with age). After 28 days of dosing with RAD140, the testosterone levels in all three groups was suppressed to approximately 200−300 ng/dL, with similar suppression in all three groups, although testosterone levels were significantly different for only the 0.01 mg/kg group ($p < 0.05$). Although this measurement did not account for possible diurnal variations in the animals and LH levels were not definitive, since they were below the level of detection in most pre- and postdose groups (LH < 0.8 ng/mL), one might still consider the possibility that even the 0.01 mg/kg dose was a fully effective, testosterone replacement dose, since body weight and lean mass were at least maintained (if not increased) in the low dose group despite significant testosterone suppression. Beyond this finding, we do not know whether testosterone suppression is a proxy for other CNS-related androgen effects beyond LH interference, such as mood, libido, and cognition, but we do believe a SARM with potent androgen agonist, CNS-type activity would be an interesting tool for that sort of exploration.

(28) For a thorough examination of the effect of high dose, injected dihydrotestosterone (DHT) on lipids in female, ovariectomized cynomolgus monkeys, see: Nantermet, P.; Harada, S.; Liu, Y.; Spring, C.; Johnson, C.; Yu, Y.; Kimme, D.; Holder, D.; Phillips, R.; Ray, W. Gene expression analysis in cynomolgus monkeys provides mechanistic insight into high-density lipoprotein-cholesterol reduction by androgens in primates. *Endocrinology* **2008,** *149* (4), 1551–1561.

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(30) Mean triglyceride changes by group were (−26%, −36%, −37%), LDL (+8%, −24%, −53%), and HDL (−13%, −42%, −64%) for 0.01 mg/kg, 0.1 mg/kg, and 1.0 mg/kg, respectively. Mean liver enzyme (ALT) changes by group were (ALT) (−15%, −2%, +43%) and (ALP) (−9%, +3%, +31%) for 0.01 mg/kg, 0.1 mg/kg, and 1.0 mg/kg, respectively.

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