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Synthesis of potent, substituted carbazoles as selective androgen receptor modulators (SARMs)

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ABSTRACT

The synthesis and in vitro binding affinity for a novel series of potent androgen receptor modulators is described. One of the more potent compounds (**17**, RAD35010) was further characterized in vivo where it restored levator ani weight in castrated male rats to near sham level while having no significant effect on prostate weight.

The androgen receptor is a ligand-gated transcription factor and is a member of the steroid hormone nuclear receptor family.[1] Endogenous androgen signaling plays a pivotal role in male sexual development and function and also plays a critical role in male and female sexual behavior. Beyond its role in sexual development, function and behavior, androgen signaling promotes nitrogen retention, protein synthesis and muscle growth. Androgen signaling also has beneficial effects on bone through increasing bone density, perhaps by a combination of bone resorption inhibition and anabolic bone stimulation.[2] Due to the many beneficial though pluripotent effects of androgens, intense research dedicated towards the understanding of AR-signaling pathways has been underway for some time. For example, it has been known for quite some time that the primary endogenous androgen in humans is testosterone. However, androgen signaling by testosterone is complicated because testosterone is site specifically metabolized to 5α-dihydrotestosterone ('DHT'). DHT is a more potent androgen than testosterone and because testosterone is largely converted to DHT in the prostate, androgen signaling through testosterone is amplified in this tissue. Overstimulation of the prostate by DHT is believed to contribute to such pathologies as benign prostate hypertrophy and possibly prostate cancer. The scalp is another tissue where testosterone to DHT metabolism can occur, accelerating male-pattern baldness. Furthermore, testosterone is also subject to aromatization to estradiol under the influence of the cytochrome P450 CYP19 aromatase enzyme. Estradiol is the most important endogenous estrogen and drives a host of estrogen receptor ('ER') mediated activities, some of which may also affect prostate cell proliferation.[3]

The complexity of testosterone metabolism and signaling is not surprising given its diverse roles in sexual development and maintenance in both males and females. Despite its drawbacks, testosterone has enjoyed reasonable success in male hormone replacement though being generally limited to topical gels or intramuscular injection. In recognition of its utility and limitations, medicinal chemists have for quite some time been trying to synthesize alternatives to testosterone with more desirable profiles.

In this communication, we report on some of our own research dedicated to the discovery of androgens with preferable profiles to testosterone. In particular, we desired compounds that were novel, orally active and that would not be subject to the same metabolic issues as testosterone (i.e., prostate selective activation, conversion to estrogens). Our endeavors were based on structure-based design wherein we scanned a number of core templates. In the course of our investigations, we found that certain appropriately substituted carbazoles bound the androgen receptor with good affinity. Our initial structural carbazole lead **4** is shown in Figure 1. The compound demonstrated moderate affinity in our binding assay relative to, for example, testosterone **1**, dihydrotestosterone **2** or the

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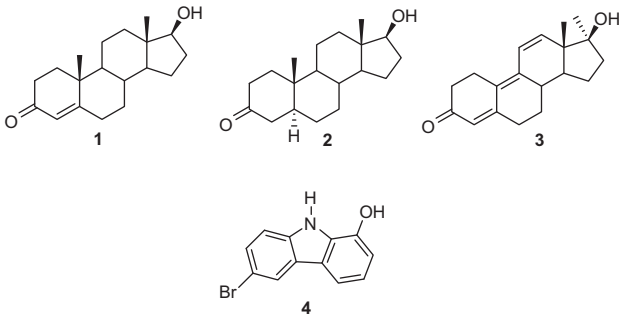
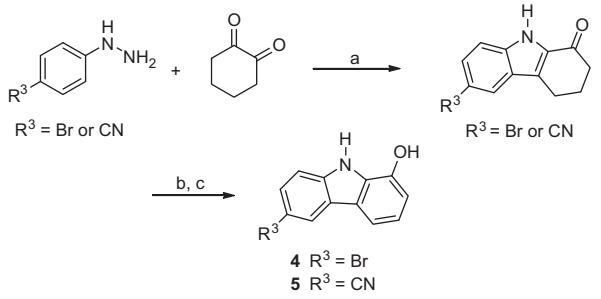


Figure 1. Structures of testosterone **1**, dihydrotestosterone **2**, RU1881 **3** and lead carbazole **4**.

Table 1
Relative binding affinities of three steroidal androgens and carbazole lead structure **4**

Compds	Binding affinity IC_{50}, nM	Repeats; sd
1	30	$n = 1$
2	10	$n = 1$
3	7	$n = 7$; sd = 4
4	1420	$n = 1$

potent synthetic steroidal agonist R1881 **3** (see Fig. 1 for structures and Table 1 for binding data). We used a fluorometric binding assay for all of the binding data discussed in this paper.[4]

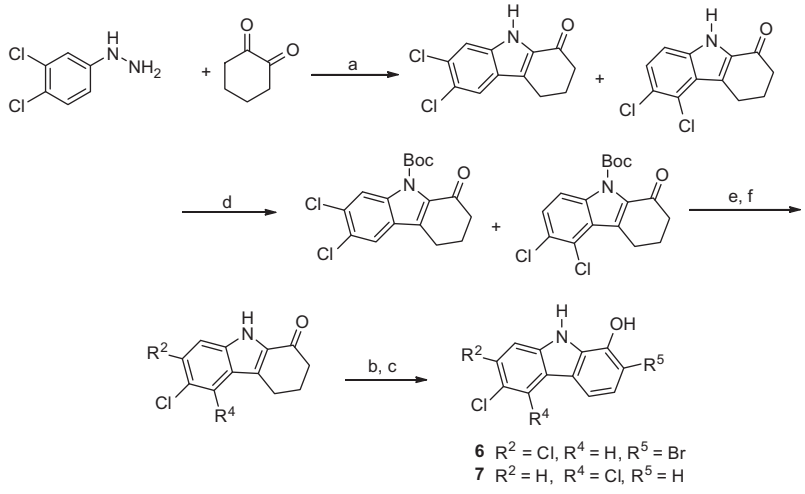


Scheme 1. Synthesis of **4**–**5**. Reagents: (a) concd HCL/AcOH; (b) $CuBr_2$, $CHCl_3$/EtOAc; (c) LiBr, Li_2CO_3, DMF.

We began our optimization program with the goal of increasing binding affinity through exploring structural diversity within the carbazole template motif of compound **4**. We briefly explored the optimization of the left hand side ring that initially contained the 4-bromo substituent. Schemes 1 and 1a describes the chemistry that was used to generate these analogues. The chemistry used to prepare the carbazoles having structures **4**–**7** was initiated by a Fisher indole synthesis of the appropriately substituted aryl hydrazide and 1,2-cyclohexanedione. For the dichloro-substituted compounds **6**–**7** (Scheme 1a), a mixture of two regioisomers was obtained from the Fisher indole synthesis in an approximate ratio of 1:1. In order to separate the regioisomers, the N-Boc derivatives were prepared, the compounds separated by silica gel chromatography and the Boc groups subsequently removed by treating with trifluoroacetic acid in methylene chloride. The tetrahydro carbazole 1-ones were aromatized by first α-brominating the 1-ones with $CuBr_2$ followed by elimination with Li_2CO_3 to generate the desired compounds. Unfortunately, the attempt to brominate the 6,7-dicloro tetrahydrocarbazole 1-one resulted in the α,α′-dibromo ketone that upon elimination produced the 2-bromophenol **6**. Compounds **8** and **9** were prepared by base-catalyzed aldol condensation with benzaldehyde to generate the *exo*-styrene derivatives. Since attempts at isomerizing the double bond to the *endo*-position were not successful, a more circuitous procedure comprising reduction of the double bond, followed by α-keto bromination and elimination was performed. The final products **8** and **9** completed our work with the fully aromatic carbazoles. The binding data for the fully aromatic carbazoles **4**–**9** together with the known steroid comparators are shown in Table 2.

As can be seen from the data in Table 2, some high affinity compounds were obtained. We knew from the literature as well as our own related work that a hydroxyl group was important for good binding affinity in many SARM templates, putatively for forming hydrogen bonds with one or two of the amino acids that the testosterone 17β-hydroxyl binds in the androgen receptor (i.e., N705/T877). Similarly, the importance of at least one hydrogen bond acceptor to mimic the 3-carbonyl group of testosterone and related steroids due the presence in the AR of hydrogen bond donors (i.e., Q711/R752) has been explained previously.[5] It appeared from our preliminary work that compounds substituted with two chlorines, either at the 5,6- or 6,7-positions (**6** and **7**) led to high affinity compared to mono substituted compounds such as **4** and **5**.

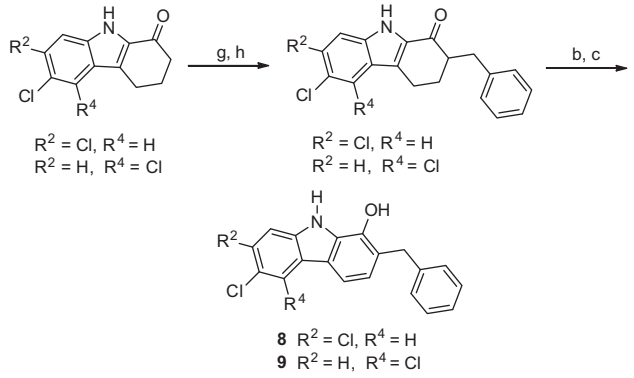
While we assumed that the phenolic group on the aromatic 1-position was acting as a good hydrogen bond donor/acceptor,



Scheme 1a. Synthesis of **6**–**7**. Reagents: (a) concd HCL/AcOH; (b) $CuBr_2$, $CHCl_3$/EtOAc; (c) LiBr, Li_2CO_3, DMF; (d) $(Boc)_2$, DMAP, THF; (e) Separate isomers by silica gel chromatography; (f) TFA, CH_2Cl_2.

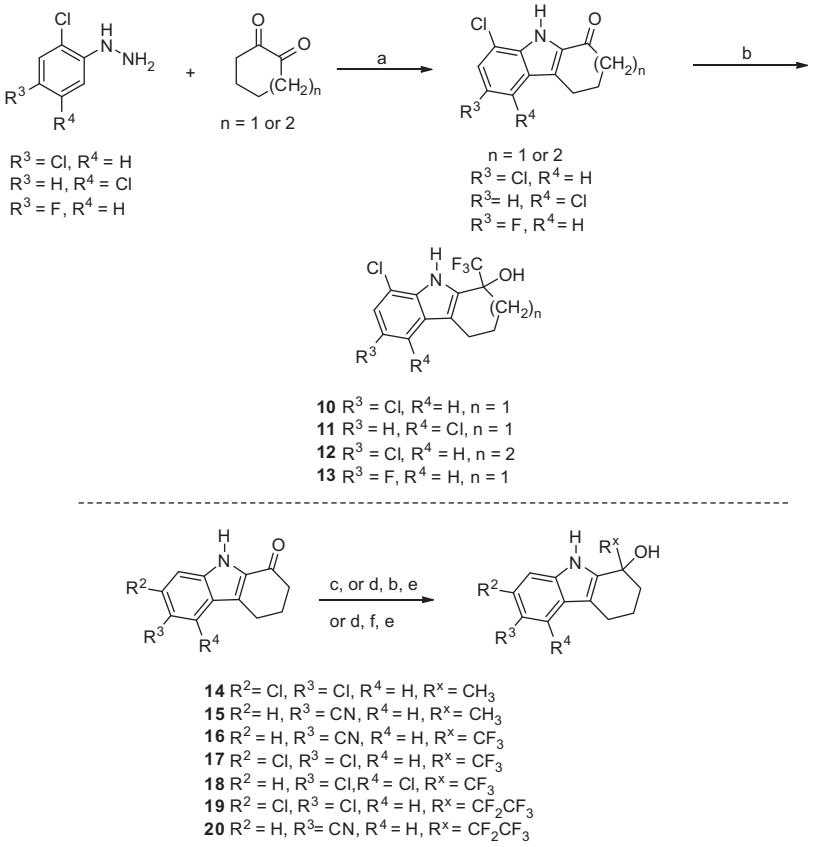
Table 2
Binding affinity for steroidal androgen standards **1**–**3** and carbazole analogues **4**–**9**

Compds	Binding affinity IC_{50}, nM	Repeats; sd
1	30	$n = 1$
2	10	$n = 1$
3	7	$n = 7$; sd = 4
4	1420	$n = 1$
5	4600	$n = 1$
6	34	$n = 1$
7	15	$n = 1$
8	>1000	$n = 1$
9	>1000	$n = 1$

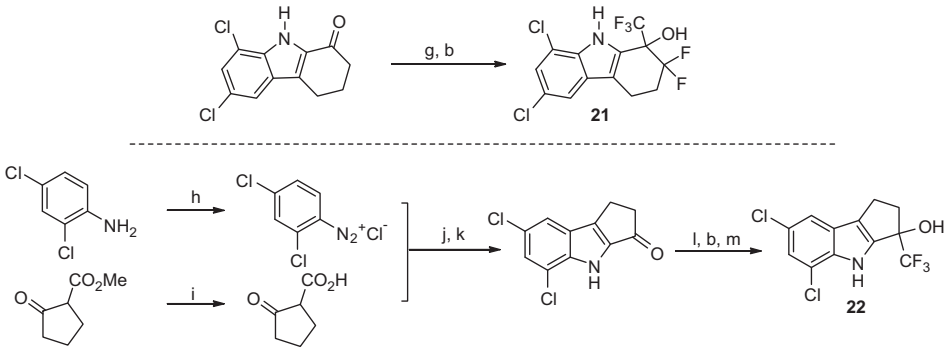


Scheme 2. Synthesis of **8**–**9**. Reagents: (b) $CuBr_2$; (c) LiBr, Li_2CO_3; (g) benzaldehyde, K_2CO_3; (h) Pd/C, H_2.

we wondered whether it could be replaced by an aliphatic hydroxyl group and the resultant compounds still retain good affinity. Fortunately, we also had been working on preparing a series of compounds where the c-ring of the carbazole was saturated and contained an aliphatic hydroxyl group at the same 1-position as the phenol of compounds **4**–**9**. The saturated hydroxyl compounds were relatively easy to prepare since they used the same 1-oxo intermediates that were used in the preparation of compounds **4**–**9**. The synthesis of saturated carbazoles is shown in Schemes 3 and 3a. The chemistry for analogues **10**–**20** was similar to that used to perform the aromatized compounds of Schemes 1, 1a and 2. The primary difference was that instead of aromatizing the 1-one by α-bromination, the desired compounds were prepared by nucleophilic addition of $-CH_3$, $-CF_3$ or $-CF_2CF_3$ to the ketone carbonyl. The only real problem in the synthesis for us was that attempts to add $TMSCF_3$ (but not MeMgBr) to the carbonyl in the presence of an unprotected carbazole nitrogen failed when there was no halogen at the 8-position (compounds **16**–**20**). We solved this problem by tosylating the nitrogen prior to the nucelophilic addition and then removing it after. The size of the cycloalkanol ring was varied from C_5 to C_7 by varying the size of the cycloalkanone used in the carbazole formation step. While the cycloheptanone fused compound used to prepare compound **12** could be prepared from the 1,2-dione in an analogous fashion as the method used for the cyclohexanone compounds, the cyclopenta-fused compound **22** was prepared by reaction of in situ generated 2-oxocyclopentanecarboxylic acid and 2,4-dichlorobenzenediazonium chloride followed by cyclization of the resulting hydrazone to form the desired fused cyclopentanone and subsequent protection with chloromethyl pivalate, reaction with CF_3TMS and CsF and deprotection to afford the desired product **22** (Scheme 3a). The



Scheme 3. Reagents: (a) Concd HCL/AcOH; (b) $TMSCF_3$, CsF, THF; (c) MeMgBr, THF; (d) Tos–Cl, NaOH, cat benzyltriethylammonium chloride; (e) KOH; (f) CF_3CF_2TMS, CsF.



Scheme 3a. Reagents: (b) $TMSCF_3$, CsF, THF; (g) LiHMDS, *N*-fluorobenzenesulfonamide; (h) $NaNO_2$, HCl; (i) NaOH then HCl; (j) Mix and collect precipitate; (k) H_2SO_4, MeCN; (l) chloromethyl pivalate, K_2CO_3; (m) KOH, THF/H_2O.

Table 3
Binding affinity for steroidal androgen standards **1**–**3** and carbazole analogues **10**–**22**

Compds	Binding affinity IC_{50}, nM	Repeats; sd
1	30	$n = 1$
2	10	$n = 1$
3	7	$n = 7$; sd = 4
10	34	$n = 3$; sd = 33
11	85	$n = 1$
12	600	$n = 1$
13	90	$n = 1$
14	>10,000	$n = 1$
15	9200	$n = 1$
16	480	$n = 1$
17 (RAD35010)	27	$n = 4$; sd = 22
18	95	$n = 1$
19	34	$n = 1$
20	>10,000	$n = 1$
21	100	$n = 1$
22	13	$n = 1$

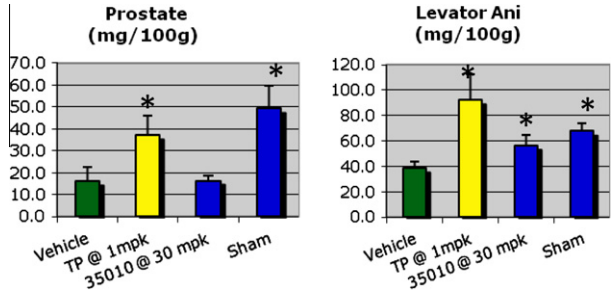


Figure 2. Effects of compound **17** on weight of prostate and levator ani muscle. *All tissue weights normalized to a 100 g rat. No significant effects of RAD35010 (po 30 mg/kg) on prostate relative to castrated control ($\rho > 0.05$) but levator ani weight is significantly greater than vehicle ($\rho < 0.05$). Testosterone propionate and sham (intact) group significantly increased both tissue weights relative to vehicle ($\rho < 0.05$). The effect of 35010 is significantly less than sham on both prostate and muscle ($p < 0.05$).

α,α′-difluoroanalogue **21** was prepared by electrophilic fluorination prior to the CF_3 addition step (Scheme 3a).

The binding data for all of the saturated compounds **10**–**22** is shown in Table 3 and a number of trends can be gleaned from this data. A comparison between the methyl adduct **14** and the trifluoromethyl adduct **17** indicates the criticality of the fluorine atoms for good binding affinity. The fluorines might be affecting the pK_a of the hydroxyl hydrogen to make it more acidic such that it is closer to the pK_a of the phenols of the compounds of Table 1. Alternatively, the fluorine atom may be acting directly as hydrogen bond acceptors or increasing affinity through increased hydrophobicity (or a combination of one or more of these factors). The cycloheptane-fused compound **12** demonstrates significantly reduced binding affinity (600 nM) compared to the cyclohexane-fused compound **10** (34 nM) having the same substitution pattern whereas the cyclopenta-fused compound **22** had retained high affinity (13 nM).

Several of the compounds were tested for oral activity in the rat Herschberger assay where male rats were castrated seven days prior to the initiation of dosing.[6,7] The animals were dosed four days with drug (po, 0.5% methylcellulose in Tween 80) and sacrificed 24 h after the last dose. The levator ani muscle and prostate were excised and the weight of the wet tissue recorded. In the Herschberger assay, positive anabolic effects can be detected by increased levator ani bulbercavernosus (LABC) muscle weight and positive androgenic effects detected by increased prostate weight. Since the stimulation of muscle is the desired aspect of these compounds and stimulation of the prostate is not desired, the preferred compounds are those that increase levator ani weight more than prostate weight, relative to the non-castrated control rats or testosterone-treated rats (which we dosed as testosterone propionate subcutaneously at 1 mg/kg per day). Only compound **17** (RAD35010), which was tested as a racemic mixture, demonstrated significant effects on the rat LABC muscle. As can be seen from Figure 2, compound **17** (RAD35010) demonstrated sufficient efficacy on muscle to restore the muscle weight back to near sham level while showing little or no effect on the prostate. In comparison, testosterone propionate was very effective at restoring muscle but also showed highly androgenic effects on the prostate. In the context of anabolic hormone therapy (e.g., hormone replacement therapy in males), a profile like that of compound **17** is very attractive.

References and notes

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4. The AR-binding assay was performed as specified by the manufacturer (Invitrogen, Madison, WI). Briefly, 1 μl of 10 mM compound was added to 500 μl of AR screening buffer in a 1.5 ml eppendorf tube to make a 2×10^{-5} M stock. Tenfold serial dilutions of the test compounds were prepared ranging in concentration from 10^{-5} M to 10^{-12} M. Each dilution was added in triplicate to a black 384-microtiter plate. The test compounds are diluted two-fold in the final reaction. 2× AR-Fluormone™ complex was prepared with 2 nM Flourmone AL Green™ and 30 nM AR. Twenty-five microlitre of 2× complex was aliquoted to each reaction well, such that the final reaction volume was 50 μl per well. Plate was sealed with a foil cover and incubated in the dark at room temperature for 4 h. Polarization values for each well were measured. The polarization values were plotted against the concentration of the test compound. The concentration of the test compound that results in half-maximum shift equals the IC_{50} of the test compound. As a control, a competition curve for R1881 (methyltrienolone)

was performed for each assay. Curve Fitting was performed using GraphPad Prism® software from GraphPad™ Software Inc.

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7. Immature Sprague–Dawley male rats were obtained Charles River Laboratories (Stoneridge, NY). All animals were maintained in a temperature and humidity controlled room with a 12 h light:12 h dark cycle, with ad lib access to food (TD 291615, Teklad, Madison, WI) and water. Rats were anesthetized and orchidectomized (GDX) or sham surgery (SHAM) was performed. After a seven-day recovery period, the animals were randomized according to weight and assigned to treatment groups ($n = 5$), SHAM, OVX + vehicle, OVX + Cpd treated. Testosterone propionate (TP 1 mg/kg in 5% DMSO/95% corn oil) was administered by once daily subcutaneous injections, while the compound was dosed in vehicle (0.5% carboxymethylcellulose) and administered by once daily oral gavage. The rats were then dosed once daily for four days. All animals were euthanized via carbon dioxide inhalation 24 h after the last dose. The prostate and levator ani bulba cavernous (LABC) tissues were removed, weighed and recorded.