Discovery of Potent, Orally-Active, and Muscle-Selective Androgen Receptor Modulators Based on an *N*-Aryl-hydroxybicyclohydantoin Scaffold†

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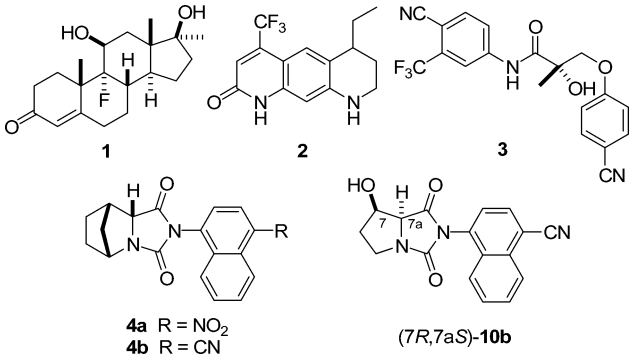
Abstract: A novel, *N*-aryl-bicyclohydantoin selective androgen receptor modulator scaffold was discovered through structure-guided modifications of androgen receptor antagonists. A prototype compound (7*R*,7a*S*)-**10b** from this series is a potent and highly tissue-selective agonist of the androgen receptor. After oral dosing in a rat atrophied levator ani muscle model, (7*R*,7a*S*)-**10b** demonstrated efficacy at restoring levator ani muscle mass to that of intact controls and exhibited >50-fold selectivity for muscle over prostate.

The endogenous androgens testosterone (T[a]) and 5α-dihydrotestosterone (DHT), play an essential role in normal male development and the subsequent maintenance of secondary male characteristics, such as muscle mass and strength, body composition, as well as sexual function.[1] The physiological effects of androgens are mediated through transcriptional activation of the androgen receptor (AR), a member of the nuclear hormone receptor superfamily of intracellular ligand-dependent transcription factors.[2] As men age, circulating serum T levels decline progressively, which subsequently leads to age-related functional decline (frailty).[3] Several recent clinical studies have shown that supplementation of T at physiological doses in elderly men resulted in significant increases in lean body mass and muscle strength and a decrease in adipose tissue.[4] However, use of T replacement therapy in elderly men is limited due to concerns about potential side effects, for example, exacerbation of benign prostatic hypertrophy (BPH) or occult prostate cancer.[5] In addition, T cannot be administered orally due to its rapid hepatic elimination and, consequently, is given less conveniently by intramuscular injection, surgical implantation, or transdermal delivery using patches or gels.[6] Alkylation of steroidal androgens at C-17 as in fluoxymesterone (**1**) has allowed oral administration, but hepatotoxicity limits the use of such analogues in chronic therapy.[7]

There has been growing interest in recent years in finding orally active androgens that could offer greater separation between desired anabolic and undesired androgenic effects.[8] These efforts have been further stimulated by recent success in developing and marketing selective estrogen receptor modulators (SERMs). Several orally active, nonsteroidal selective androgen receptor modulators (SARMs), such as pyridinoquinoline **2**[9] and the bicalutamide-derived **3**[10] have appeared in the literature, and some of the most advanced compounds emerging from these efforts have been reported to have reached the clinical stage.[11] However, these compounds exhibit modest tissue selectivity in preclinical models. Given the ever-growing aging population due to the increase in life expectancy, there clearly exists an unmet medical need for the prevention of functional decline in aging men. Herein we report the discovery of a novel, potent, orally active nonsteroidal SARM scaffold. Compounds based on this core have the potential to provide the clinical benefits of a classical anabolic steroid but with reduced risk of dose-limiting side effects.



Previous in-house efforts directed at the identification of novel AR antagonist scaffolds led to the discovery of a series of *N*-aryl bicyclic hydantoins, exemplified by compound **4a**.[12] Compounds of this chemotype bind tightly to the AR and function as weak partial agonists in AR transactivation assays using the mouse myoblast C2C12 cell line. The in vitro profile of partial agonist **4a** (K_i = 1 nM, EC_{50} = 385 nM) encouraged us to conduct structure−activity relationship (SAR) studies to improve potency and intrinsic agonist activity.

The rationale to utilize the **4a** template to develop bicyclic azahydantoin-based AR antagonists was recently disclosed.[12] Docking a **4a** analogue into the X-ray crystal structure of the DHT-bound wild-type AR ligand binding domain (LBD)[13] suggests that **4a** binds to the receptor via the nitro group, like the C-3 carbonyl of DHT, forming H-bonds with both arginine 752 (R752) and glutamine 711 (Q711). The [2.2.1] bicyclic portion of **4a** occupies the same space as the D ring of DHT. However, this bulky bicyclic moiety and the weak H-bonding interactions with asparagine 705 (N705) and/or threonine 877 (T877) for this compound through the hydantoin backbone N may underlie its weak agonist profile. Such an analysis prompted us to design hydroxylated compounds **7b**,**c** and **10a**,**b** as possible SARMs. We reasoned that improved binding interactions could be achieved both by reducing the bulky [2.2.1] bicyclic hydantoin portion through removal of the bridging ethano unit and by installing a hydroxyl group at either C-6 or C-7 of the outermost 5-membered ring of the resultant bicyclic scaffold to capture stronger H-bonds with N705 and/or T877.

A general synthesis of unsubstituted and 6-hydroxy compounds **7a**−**c** is outlined in Scheme 1. Treatment of proline or 4-hydroxyproline esters **5a**−**c** with 4-nitro-1-naphthaleneisocyanate at room temperature yielded urea **6a**−**c**, which were subsequently treated with DBU at elevated temperature to afford compounds **7a**−**c** as either racemic (**7a**) or diastereomeric (**7b**,**c**) mixtures.

† Structure coordinates of (−)-**10b** bound to the rat AR-LBD have been deposited in the Brookhaven protein database (PDB ID 2IHQ).

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[a] Abbreviations: AR, androgen receptor; SARM, selective androgen receptor modulator; T, testosterone; DHT, 5α-dihydrotestosterone; TP, testosterone propionate; E2, estrodiol; LBD, ligand binding domain; LH, luteinizing hormone.

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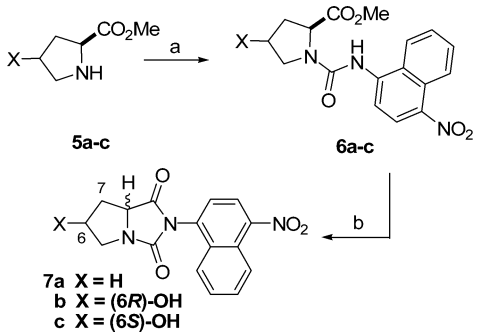
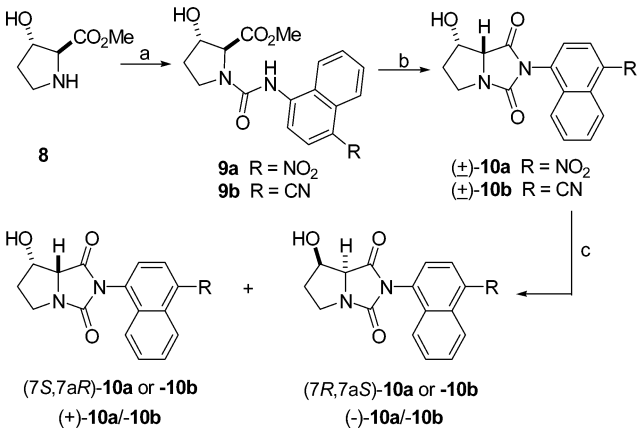
Scheme 1[a]



[a] Reagents and conditions: (a) 4-nitro-1-naphthalene isocyanate, toluene, 4 Å mol. sieves, 25 °C, 16 h; (b) DBU, 80 °C, 2 h, 20−75% (2 steps).

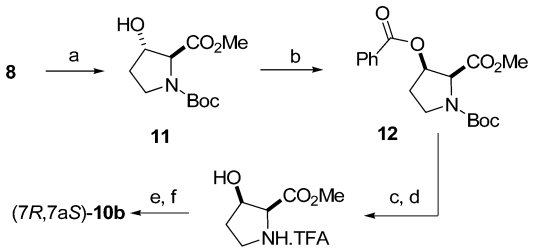
Scheme 2[a]



[a] Reagents and conditions: (a) 4-*R*-1-naphthaleneisocyanate, toluene, 4 Å mol. sieves, rt, 16 h; (b) DBU, 80 °C, 2 h, 40−65% (2 steps); (c) chiral HPLC.

When *trans*-3-hydroxy-L-proline methyl ester **8** was subjected to the same reaction sequence using either 4-nitro- or 4-cyano-1-naphthaleneisocyanate (Scheme 2), unexpectedly, a racemic mixture of (±)-**10a** (R = NO_2) or (±)-**10b** (R = CN) was obtained. These mixtures were resolved by chiral HPLC to provide individual enantiomers (7*S*,7a*R*)-**10a** or -**10b** [(+)-**10a**/-**10b**] and (7*R*,7a*S*)-**10a** or -**10b** [(−)-**10a**/-**10b**], respectively. The relative stereochemistry of (−)-**10b** was determined by single-crystal X-ray crystallography,[14] and the absolute stereochemistry was confirmed by solving a cocrystal complex of (−)-**10b** bound to the AR-LBD (vide infra). To our surprise, the relative stereochemistry of the hydroxyl group and ring juncture in (−)-**10b** was found to be cis rather than the *trans*-stereochemistry of hydroxyester **8**. Considering the presence of a β-hydroxy amide in bicyclic hydantoin **10**, we speculated that formation of (±)-**10a** or (±)-**10b** occurred via retro-Aldol reaction under the strongly basic and elevated-temperature conditions to yield the more thermodynamically favored *cis*-product. When urea intermediate **9b** was treated with DBU at room temperature for 4 days, (+)-**10b** was predominantly formed, suggesting that at room temperature the ester group epimerization precedes cyclization to yield (+)-**10b**. Based on these findings, we designed a stereospecific synthesis of the more active enantiomer (−)-**10b**, as outlined in Scheme 3. Boc protection of **8**, followed by a Mitsunobu inversion of the hydroxyl group of the resultant **11** with benzoic acid, gave diester **12**. Treatment of **12** with KOH in MeOH, followed by Boc deprotection with trifluoroacetic acid afforded *cis*-3-hydroxy-L-proline **13**. Reaction of **13** with 4-cyano-1-naphthaleneisocyanate at room temperature provided the intermediate

Scheme 3[a]



[a] Reagents and conditions: (a) Boc_2O, NEt_3, CH_2Cl_2, rt, 3 h, 92%; (b) Ph_3P, DEAD, $PhCO_2H$, THF, rt, 2 h, 94%; (c) 1 M KOH/MeOH, rt, 1 h, 85%; (d) TFA, CH_2Cl_2, 0 °C, 2 h, 100%; (e) 4-cyano-1-naphthaleneisocyanate, *i*-Pr_2NEt, CH_2Cl_2, 4 Å mol. sieves, rt, 2 h; (f) DBU, rt, 12−40 h, 80% (2 steps).

Table 1. Receptor Binding and Transactivation Assay Data for **4a**,**b**, **7a**−**c**, and **10a**,**b**

cmpd	K_i[a] (nM)	EC_{50}[b] (nM) (I.A.)[c]	IC_{50}[d] (nM)
DHT	0.3	2.8 (100%)	
4a	1.0	385 (85%)	
4b	24	>1000	260
7a	1.7	270 (63%)	
7b	1.5	320 (65%)	
7c	4.3	265 (71%)	
(±)-10a	3.5	4.4 (96%)	
(−)-10a	2.1	1.5 (101%)	
(+)-10a	1.5	281 (63%)	
(±)-10b	5.5	6.3 (101%)	
(−)-10b	3.2	2.3 (103%)	
(+)-10b	5.3	1298 (49%)	60

[a] Binding determined through direct displacement of ligand with [^{3}H]-DHT in the MDA-453 cell line. [b] Functional agonist activity determined through a stably transfected mouse myoblast C2C12 cell line utilizing a luciferase reporter. [c] I.A. = intrinsic activity relative to DHT. [d] Functional antagonist activity determined in a stably transfected mouse myoblast C2C12 cell line utilizing a luciferase reporter in the presence of DHT.

urea, which was subsequently treated with DBU at room temperature to give, after crystallization, enantiomerically pure (−)-**10b**.

Table 1 summarizes the AR binding affinities and transcriptional activities of compounds **4a**,**b**, **7a**−**c**, and **10a**,**b**. Compound **7a** (X = H), a simplified bicyclic hydantoin, retained comparable binding and functional activity to that of the bridged bicyclic **4a**. Both **7b** (X = (*R*)-OH) and **7c** (X = (*S*)-OH) also showed agonist activity similar to **7a**, indicating that a hydroxyl group at the 6-postion of the bicyclic hydantoin core cannot effectively approximate the H-bonding interactions between the native hormones T and DHT with the AR. However, with the hydroxyl group at the 7-position, as in (±)-**10a** (R = NO_2), a more pharmacologically significant result was observed. While the binding affinity for (±)-**10a** remained unchanged relative to the unsubstituted parent **7a**, the functional activity was significantly enhanced (>60-fold). Compound (±)-**10b** (R = CN) exhibited essentially the same binding and agonist functional activity as that of (±)-**10a**. For cyano analogue (±)-**10b**, this represented a dramatic reversal in functional activity, as the corresponding bridged bicyclic analogue **4b** (R = CN) is functionally an AR antagonist, with an IC_{50} = 260 nM. This finding was also significant because compounds containing the nitronaphthyl moiety such as **4a** tested positive in Ames mutagenicity assays, while the corresponding cyanonaphthyl analogues (e.g., **4b**) tested negative. Second, this demonstrated that the specific molecular interactions of the 7-OH group in (±)-**10** within the AR LBD were sufficient to convert an

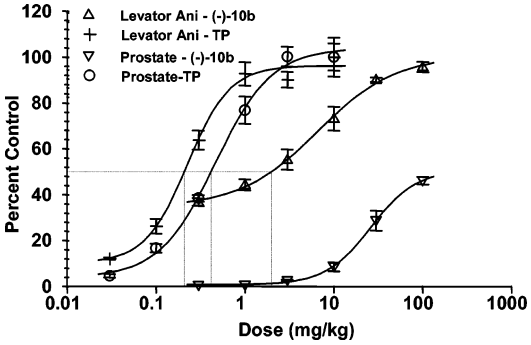


Figure 1. Effects of testosterone propionate (TP; s.c., q.d.) or (−)-**10b** (p.o., q.d.) on wet weight of levator ani and prostate in mature castrated male rats.

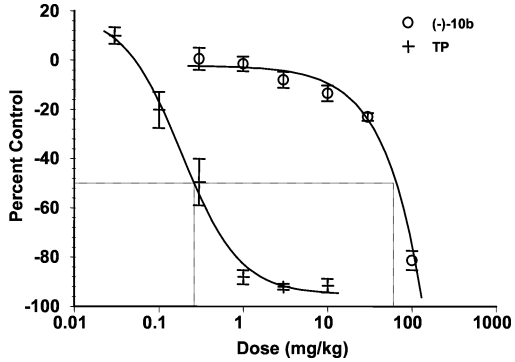


Figure 2. Effects of testosterone propionate (TP; s.c., q.d.) or (−)-**10b** (p.o., q.d.) on suppression of serum luteinizing hormone in mature castrated male rats.

antagonist (e.g., **4b**) to an agonist ((±)-**10b**). On this basis, (±)-**10a** and (±)-**10b** were resolved into their respective pure enantiomers, which were assayed for AR activity. Compound (−)-**10b** was found to be >500-fold more potent in functional assays than its antipode (+)-**10b**. Compound (−)-**10b** was also assayed against other steroid hormone receptors, and found to be >1000-fold selective for AR versus estrogen receptor α and *β*, glucocorticoid receptor, and mineralocorticoid receptor, and approximately 270-fold selective versus progesterone receptor (data not shown).

As the in vitro profile of (−)-**10b** closely mirrored that of the native hormones, we proceeded to evaluate the potency and efficacy of this AR agonist at stimulating skeletal muscle and prostate growth in the mature castrated rat, a widely accepted model for investigating anabolic and androgenic activity in androgen responsive tissues.[15] In this assay, mature male rats (castrated two weeks prior to the start of the study) were treated with (−)-**10b** (q.d., p.o.) or testosterone propionate (TP) (q.d., s.c.) for two weeks. At the termination of the study, tissue wet weights were measured and potencies were calculated as ED_{50} values, defined as the dose at which tissue wet weight reaches 50% that of the corresponding intact sham-operated vehicle-treated control animals (Figure 1). TP had an ED_{50} of 0.21 mg/kg in the levator ani muscle and an ED_{50} of 0.42 mg/kg in the prostate, exhibiting 2-fold selectivity for muscle versus prostate, whereas compound (−)-**10b** exhibited an ED_{50} of 2 mg/kg in the levator ani and an ED_{50} of >100 mg/kg in the prostate or >50-fold selectivity for muscle versus prostate. In this same animal model, (−)-**10b** also suppressed secretion of luteinizing hormone (LH) with an ED_{50} of 60 mg/kg (Figure 2). This corresponds to a 30-fold selectivity for the levator ani muscle stimulation versus LH suppression compared to testosterone propionate, which had an ED_{50} of 0.26 mg/kg for LH suppression and essentially no selectivity (1.2-fold) versus this endpoint. In addition to the more obvious rationale for desired selectivity for muscle versus prostate growth, this latter selectivity could potentially be important for minimization of feedback inhibition of LH secretion (and subsequent T secretion) through the hypothalamic−pituitary−gonadal (HPG) axis. As T is the biosynthetic precursor for estradiol (E2), the lipid balance thought to be maintained in part through appropriate T/E2 ratio in men could potentially be affected by a compound that acts centrally to fully suppress new T secretion.

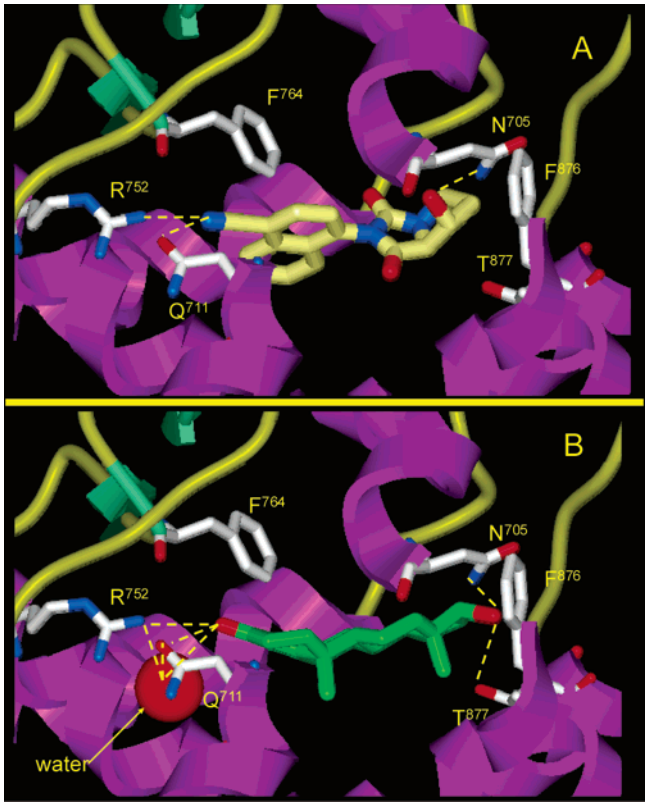


Figure 3. X-ray cocrystal structures of (−)-**10b** (Panel A) and DHT (Panel B) bound to the rat AR-LBD at 2.0 Å resolution. The helices are colored magenta, loops are colored yellow, and strands are colored green. The key ligand binding residues are labeled according to the corresponding human AR amino acid sequence, and the dashed lines represent potential hydrogen bonds. The crystallographic water is rendered in space filling mode, Panel B.

Comparison of the X-ray cocrystal structures of the AR LBD complexed with either (−)-**10b** or DHT reveals that the nonsteroidal compound has some of the same interactions as those of the endogenous hormone (Figure 3). Residues R752 and Q711 form hydrogen bonds (2.89 Å and 3.36 Å, respectively) with the A-ring carbonyl of DHT in a network that includes a water molecule. For the complex of (−)-**10b** and the AR, the hydrogen bonds from the cyano group to R752 and Q711 are similar in nature (2.83 Å and 3.45 Å, respectively), but the crystallographic water was not seen for this structure (or other structures determined with analogous compounds), despite the same 2.0 Å resolution for each. At the distal end of the ligands, the DHT D-ring's 17*β*-hydroxyl group forms strong hydrogen bonds with T877 and N705 at 2.70 Å and 2.80 Å, respectively, whereas (−)-**10b** forms a single hydrogen bond with N705 at 2.50 Å. An additional significant binding element unique to (−)-**10b** is seen with the *π*-edge to face interaction between its naphthyl moiety and F764 of the AR. These two key differences allow (−)-**10b** to have an overall unique binding mode compared with DHT. This may result in altered dynamic

receptor conformations leading to differential affinity for/recruitment of the co-activators and/or co-repressors, which complete the transcriptional machinery leading to selective gene activation. Studies are underway in these laboratories to further elucidate the significance of these findings both at a molecular and pharmacological level. The novel SARM scaffold described herein represents a significant advance in achieving orally active, highly tissue-selective, nonsteroid AR agonists in an animal model relevant to human musculoskeletal health.

Supporting Information Available: Descriptions of AR binding and transactivation assays, in vivo pharmacology models, and detailed procedures and characterization data for compounds **7a**−**c**, **10a**,**b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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