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Synthesis and biological evaluation of novel selective androgen receptor modulators (SARMs). Part II: Optimization of 4-(pyrrolidin-1-yl) benzonitrile derivatives

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ABSTRACT

We recently reported a class of novel tissue-selective androgen receptor modulators (SARMs), represented by a naphthalene derivative **A**. However, their pharmacokinetic (PK) profiles were poor due to low metabolic stability. To improve the PK profiles, we modified the hydroxypyrrolidine and benzonitrile substituents of 4-(pyrrolidin-1-yl)benzonitrile derivative **B**, which had a comparable potency as that of compound **A**. This optimization led us to further modifications, which improved metabolic stability while maintaining potent androgen agonistic activity. Among the synthesized compounds, (2*S*,3*S*)-2,3-dimethyl-3-hydroxylpyrrolidine derivative **1c** exhibited a suitable PK profile and improved metabolic stability. Compound **1c** demonstrated significant efficacy in levator ani muscle without increasing the weight of the prostate in an in vivo study. In addition, compound **1c** showed agonistic activity in the CNS, which was detected using sexual behavior induction assay.

As men age, serum testosterone levels gradually decline due to testicular malfunction and hypothalamic dysregulation.[1] Reduced testosterone levels cause a variety of symptoms, including depression, loss of libido,[2] and decreased bone and lean body mass. This syndrome is called late-onset hypogonadism (LOH),[3] and affects 20% of men aged 60 and older.[4] Currently, several strategies for testosterone replacement therapy (TRT) use testosterone and/or its ester to treat hypogonadism.[5,6] These treatments effectively alleviate symptoms and provide additional health benefits. However, widespread use of testosterone/testosterone ester-based TRT is limited by potential risk of cardiovascular problem, prostate cancer and erythrocytosis.[7] In addition, administration routes are limited to inconvenient intramuscular injection, surgical implantation, or transdermal delivery using gels or patches.

Lack of convenient administration routes has stimulated a growing interest in orally available and nonsteroidal tissue-selective androgen receptor modulators (SARMs).[8–11] The concept of SARMs emerged from the clinical success of selective estrogen receptor modulators (SERMs), such as raloxifene, which acts as an estrogen receptor agonist in bone but an antagonist in the breast and uterus.[12] SARMs are expected to have desired anabolic functions, and lack unwanted androgenic properties such as prostate stimulation. Various nonsteroidal SARMs have been reported[6,13] and some compounds such as GTx-024[14,15] and DT-200[16–18] have reached clinical development (Fig. 1).

We have previously reported that 1-(4-cyano-1-naphthyl)-2,3-disubstituted pyrrolidine derivatives, represented by compound **A**, showed highly potent AR agonistic activity in vitro, and good tissue selectivity in vivo (Fig. 2).[19] However, they were rapidly metabolized in rat and human liver microsomes, and failed to achieve sufficient bioavailability in PK studies in rats (compound **A**: CL = 3081 mL/h/kg, AUC_{po} = 32.2 ng·h/mL, *F* = 9.7%). In addition, replacement of naphthonitrile with 2-chloro-3-methyl benzonitrile, a well-known privileged scaffold for SARMs,[20,21] still provided

Abbreviations: PK, pharmacokinetics; CL, clearance; *F*, bioavailability; AUC, area under the blood concentration time curve; MS, liver microsomal metabolic stability; sc, subcutaneous; qd, quaque die; CNS, central nervous system; bid, bis in die.

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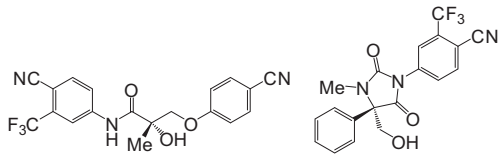


Fig. 1. Structures of selected nonsteroidal SARMs.

Fig. 2. 1-(4-Cyano-1-naphthyl)pyrrolidine derivative **A** and 2-chloro-3-methylbenzonitrile **B**.

insufficient metabolic stability, and showed a PK profile similar to that of compound **A** (compound **B**: CL = 5166 mL/h/kg, AUC_{po} = 3.2 ng·h/mL, *F* = 1.6%).

Therefore, we designed a compound by modifying the pyrrolidine and benzonitrile groups further to improve the PK profile. The X-ray co-crystal structure of compound **B** with the AR ligand binding domain (LBD) revealed two key interactions (Fig. 3): water-bridged hydrogen bonds of the cyano group with the side chain of Arg752 and the backbone of Met745, and an interaction of the hydroxyl group with the side chains of Asn705 and Thr877. Since the structural data suggested that potency would be improved if the compounds possessed cyano and hydroxyl groups at the ends of the molecule, we planned to explore substitutions of the hydroxypyrrolidine unit (X) and conduct further optimization of the benzonitrile (Y). In this paper, we describe the design, synthesis, and biological characterization of 4-(pyrrolidin-1-yl)-1-benzonitrile derivatives.

(2*S*,3*S*)-2,3-Dimethyl-3-hydroxypyrrolidine **4** was prepared using our previously reported method (Scheme 1).[22] A methyl group was introduced at the C3-position of **2** using methyl magnesium bromide. In this reaction, the Grignard substitution was achieved in a highly stereo-selective manner and with an 85% yield, driven by the optically active 2*S*-methyl group, but without cleavage of the Cbz group, using $CeCl_3$ as an additive.[23] The Cbz group of **3** was removed by hydrogenation, and successive treatment with oxalic acid afforded a hemioxalate salt **4** at 88% yield.

The coupling reactions of pyrrolidines **4** with 4-fluorobenzonitriles **5** were performed using lithium carbonate in dimethylsulfoxide at 70–80 °C to afford **1a–c** in 57–75% yield (Scheme 2).[24]

AR binding affinities were evaluated by competitive displacement of radiolabeled [^{3}H]mibolerone from AR. The data are reported as IC_{50} values. Functional activities using an AR responsive luciferase reporter were determined in Cos-7 cells and are given as EC_{50} values.

To improve the PK profile of **B**, we first focused on modification of the pyrrolidine. The co-crystal structure of **B** with AR LBD suggests that the C3-hydroxyl group on the pyrrolidine ring is essential for potency. However, the hydroxyl group and pyrrolidine

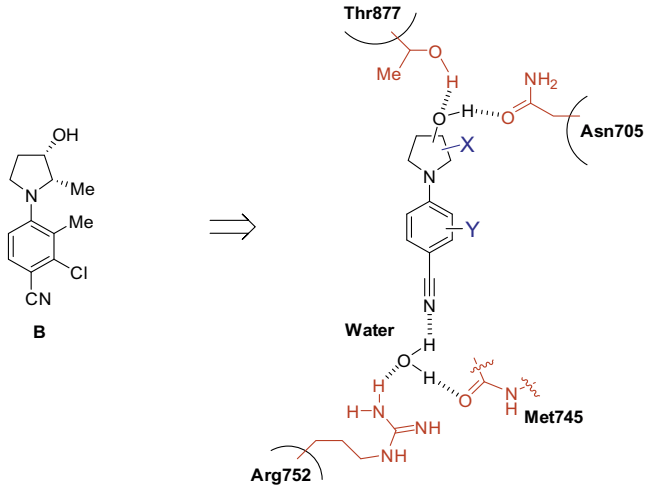
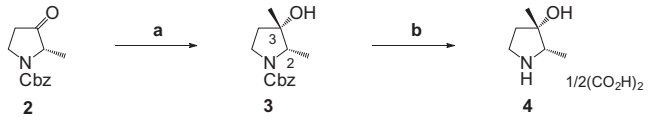
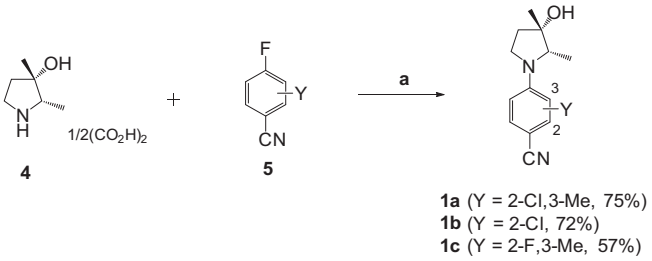


Fig. 3. Strategies for identification of novel SARMs from compound **B**.



Scheme 1. Preparation of (2*S*,3*S*)-2,3-dimethyl-3-hydroxylpyrrolidine **4**. Reagents and conditions: (a) MeMgBr, $CeCl_3$, THF, -78–0 °C, 1 h 85%; (b) H_2 (1 atm), Pd/C, MeOH, rt, 1.5 h, then $(CO_2H)_2$, 88%.



Scheme 2. Synthesis of compound **1a–c**. Reagents and conditions: (a) Li_2CO_3, DMSO, 70–80 °C, 57–75%.

ring are considered to be metabolized by oxidation and conjugation.[25] We hypothesized that introduction of a methyl group at the C3-position on the pyrrolidine ring would prevent both oxidative and conjugative metabolism due to the steric hindrance, while not influencing the potency on the basis of the co-crystal structural information of **B** with AR LBD. Based on this hypothesis, (2*S*,3*S*)-2-methyl-3-hydroxylpyrrolidine derivative **B** was converted into (2*S*,3*S*)-2,3-dimethyl-3-hydroxylpyrrolidine derivative **1a** (Table 1). As expected, compound **1a** demonstrated better metabolic stability in human and rat assays than that of compound **B**, while maintaining potent AR binding affinity and agonistic activity. In addition to improved metabolic stability, compound **1a** exhibited a dramatically improved PK profile (**1a**: CL = 1886 mL/h/kg, AUC_{po} = 236.7 ng·h/mL, *F* = 44.5%) relative to that of compound **B** (**B**: CL = 5166 mL/h/kg, AUC_{po} = 3.2 ng·h/mL, *F* = 1.6%).

With its modified hydroxypyrrolidine unit, compound **1a** demonstrated an improved PK profile while maintaining excellent potency. However, its metabolic stability in rats was still insufficient to select **1a** as a suitable in vivo candidate. To further improve PK profiles, we optimized the substituents on the benzonitrile of compound **1a** using the calculated Log*P* (c Log*P*) value as an indicator of metabolic stability.[26] We designed and synthesized