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# Letters

## Substituted 6-(1-Pyrrolidine)quinolin-2(1*H*)-ones as Novel Selective Androgen Receptor Modulators

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**Abstract:** The androgen receptor is a ligand inducible transcription factor that is involved in a broad range of physiological functions. Here we describe the discovery of a new class of orally available selective androgen receptor modulators. The lead compound, 6-[(2*R*,5*R*)-2-methyl-5-((*R*)-2,2,2-trifluoro-1-hydroxyethyl)pyrrolidin-1-yl]-4-trifluoromethylquinolin-2(1*H*)-one (**6a**), showed excellent anabolic activity in muscle with reduced effect on the prostate in a rat model of hypogonadism. The compound also improved bone strength in a rat model of post-menopausal osteoporosis.

Nonsteroidal selective androgen receptor modulators (SARMs[a]) offer unique possibilities for the treatment of a variety of androgen receptor (AR) related disorders.[1] The anabolic effects of androgens in muscle have been known for a long time. Men with low testosterone (T) levels benefit from supplementation with T at physiological doses, resulting in increased lean body mass and strength.[2] Steroidal androgens have also been shown to increase bone mineral density and bone strength, but there is still some controversy whether this effect is fully mediated through the AR, or due to conversion to estrogens and, hence, via the estrogen receptor. Potential side effects associated with T replacement therapy are overstimulation of the prostate and increases in hematocrit.[3] In addition, because T cannot be dosed orally, administration via patches, gels, bioadhesive buccal system, or intramuscular injection has limited its wider use.[4] We recently described the discovery of 6-dialkylaminoquinolin-2(1*H*)-ones as a novel AR modulator series and 6-*N*,*N*-bistrifluoroethylaminoquinolin-2(1*H*)-one **7** (LGD2226)[5] as an orally available SARM. To further explore the series, and improve upon the ADME properties of these compounds, we looked at 6-cycloamino-substituted quinolin-2(1*H*)-ones. It was expected that by locking the substituents on the 6-nitrogen atom in a ring, and by introducing some more polar groups, we could improve the oral bioavailability of these compounds. Here we describe the results from our SAR studies on this novel series, leading to the discovery of 6-[(2*R*,5*R*)-2-methyl-5-((*R*)-2,2,2-trifluoro-1-hydroxyethyl)pyrrolidin-1-yl]-4-trifluoromethylquinolin-2(1*H*)-one **6a** (LGD2941) as an orally available, potent SARM. This compound was also found to be very efficacious on improving bone strength in a rat model of post-menopausal osteoporosis.

The synthesis of 6-pyrrolidine substituted quinolinones is outlined in Scheme 1. All compounds were obtained via palladium-catalyzed addition of a cycloamine to 6-bromo-2-isopropoxy-4-trifluoromethylquinoline **2**. This precursor was synthesized from quinolin-2(1*H*)-one **1**.[6] The isopropyl group was identified as a suitable protection group because of its stability under a wide variety of reaction conditions, but it is easily cleaved with concentrated HCl at elevated temperatures. A palladium catalyzed coupling of this bromide **2** with pyrrolidine or pyrrolidone derivatives introduces the cycloamino substituent. In this reaction, BINAP proved to be the best palladium ligand. The yield of coupled product was excellent for the unsubstituted pyrrolidine **3a**, but the yields dropped when more sterically demanding amines were used. Thus, pyrrolidine afforded the coupled product **3a** in 98% yield, while the yield for the coupling of 2,5-dimethylpyrrolidine to give **3c**/**3d** was only 44%. In the latter case, the 2,5-dimethylpyrrolidine was used as a *cis*/*trans* mixture, but the coupled product obtained was almost pure *cis*-**3c**, only minor amounts of **3d** were isolated. We were unable to couple more hindered pyrrolidines like 2-(*tert*-butyldimethylsilyloxymethyl)-5-methylpyrrolidine using

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[a] Abbreviations: AR, androgen receptor; SARM, selective androgen receptor modulator; T, testosterone; BINAP, 2,2′-bis(diphenylphosphino)-1,1′-binaphtalene; TBAF, tetrabutyl ammonium fluoride; $Pd_2dba_3$, tris-(dibenzylideneacetone)dipalladium; ORDX, orchidectomized; DHT, dihydrotestosterone; OVX, ovariectomized.

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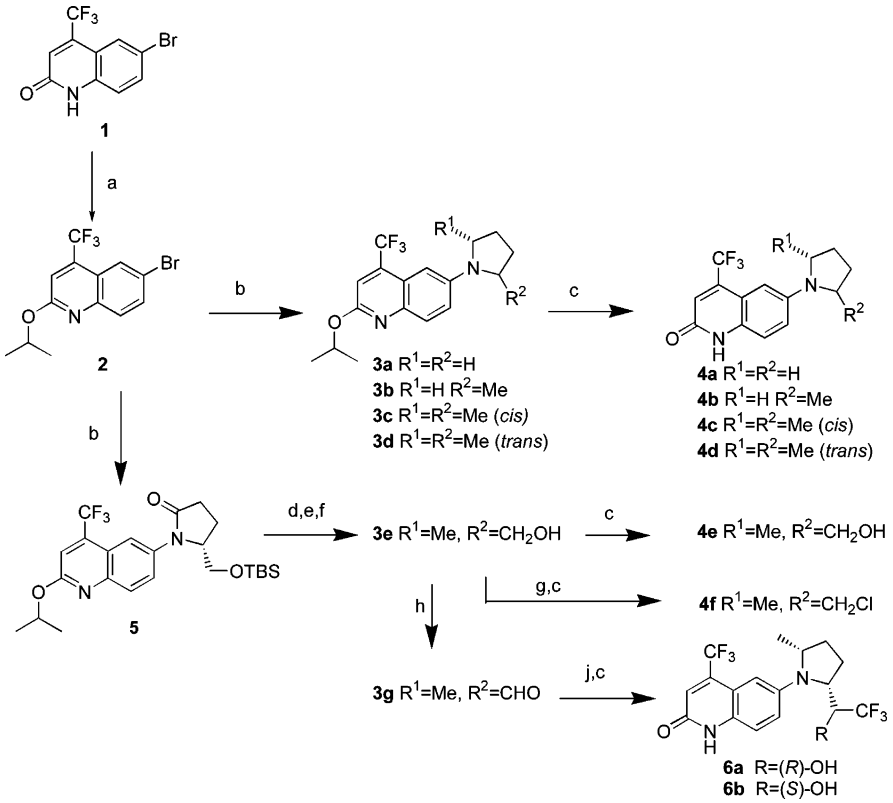
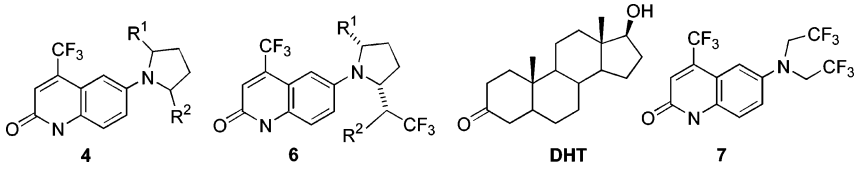
**Scheme 1**[a]




[a] Reagents and conditions: (a) 2-iodopropane, CsF, dimethylformamide, rt, 20 h; (b) pyrrolidine or pyrrolidin-2-one, cesium carbonate, $Pd_2dba_3$, (±)-BINAP, toluene; (c) HCl, acetic acid, 60 °C; (d) methyl lithium, tetrahydrofuran, −78 °C; (e) Pd/C, $H_2$, trifluoro acetic acid, methanol; (f) TBAF, tetrahydrofuran; (g) thionyl chloride, chloroform, 40 °C; (h) oxalylchloride, dimethyl sulfoxide, triethylamine, dichloromethane; (j) trifluoromethyl trimethylsilane, tetrahydrofuran.

**Table 1.** Cotransfection and Competitive Binding Data for 6-(Pyrrolidine)-4-trifluoromethylquinolin-2(1*H*)-ones, 6-Bis(2,2,2-trifluoroethylamino)quinolin-2(1*H*)-one **7**, and DHT[a]




| cmpd | $R^1$ | $R^2$ | hAR agonist $EC_{50}$ (nM) | hAR agonist[b] eff (%) | hAR antagonist $IC_{50}$ (nM) | hAR antagonist[a] eff (%) | hAR binding $K_i$ (nM) |
|---|---|---|---|---|---|---|---|
| **4a** | H | H | −[c] | − | 25 ± 20 | 58 ± 7 | $1.7 \times 10^3$ |
| **4b** (±) | H | $CH_3$ | 13 | 39 ± 11 | − | − | 72 |
| **4c** | $CH_3$ | *cis*-$CH_3$ | 2.6 ± 0.8 | 101 ± 14 | − | − | 19 |
| **4d** (±) | $CH_3$ | *trans*-$CH_3$ | 48 ± 24 | 88 ± 13 | − | − | 43 |
| **4e** | (*R*)-$CH_3$ | (*R*)-$CH_2OH$ | 21 ± 3 | 71 ± 9 | − | − | 70 |
| **4f** | (*R*)-$CH_3$ | (*R*)-$CH_2Cl$ | 12 ± 0.2 | 101 ± 5 | − | − | 10 |
| **6a** | $CH_3$ | (*R*)-OH | 7.1 ± 0.4 | 109 ± 13 | − | − | 1.5 ± 0.2 |
| **6b** | $CH_3$ | (*S*)-OH | 8.4 ± 3.2 | 111 ± 9 | − | − | 7.6 ± 0.9 |
| **7** | | | 0.2 ± 0.02 | 95 ± 2.0 | − | − | 4.6 |
| DHT | | | 5.1 ± 0.1 | 100 ± 0.08 | − | − | 0.2 ± 0.02 |
| bicalutamide | | | − | − | 128 ± 25 | 76 ± 3 | 70 |

[a] Values with standard errors represent the mean value of at least two separate experiments with triplicate determinations. [b] Agonist efficacies were determined relative to DHT (100%) and antagonist efficacies (%) were determined as a function of maximal inhibition of DHT at the $EC_{50}$ value. [c] A hyphen (−) indicates an efficacy of <20% and a potency of >10 000 nM.

the previously described experimental conditions. More highly substituted pyrrolidines were synthesized via palladium-catalyzed coupling of (*R*)-5-(*tert*-butyldimethylsilyloxymethyl)-pyrrolidin-2-one[7] to afford product **5**. Introduction of the 5-methyl substituent was achieved by methylation of the amide with methyllithium to give the hydroxyaminal, which was reduced with Pd/C under a hydrogen atmosphere to give a 7:1 mixture of stereoisomers in favor of the *cis*-2,5-disubstituted pyrrolidine. The *tert*-butyldimethylsilyl group was cleaved with TBAF, and deprotection of the isopropyl-protected quinolinol with concentrated hydrochloric acid afforded **4e** in good yield. The chloromethyl-substituted compound **4f** was obtained from treatment of the alcohol **3e** with thionylchloride and deprotection to give **4f**. Swern oxidation of the alcohol **3e** afforded the aldehyde **3g**, which was treated with trifluoromethyl trimethylsilane and catalytic amounts of cesium fluoride to give the trifluoromethyl alcohols as a diastereomeric mixture, with the (*R*)-alcohol as the major isomer in a 3:2 ratio. The diastereomers

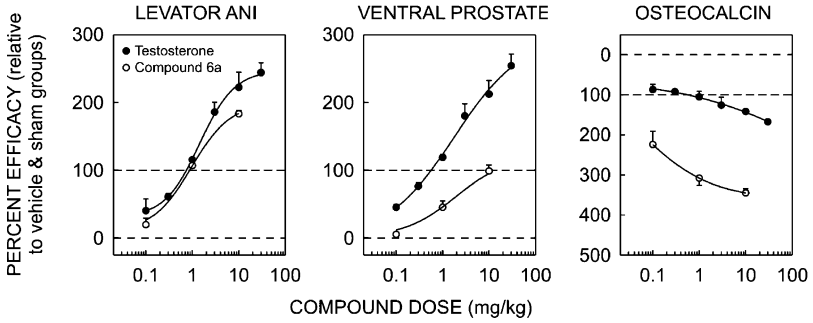



**Figure 1.** Effects of **6a** and testosterone treatment on levator ani, ventral prostate, and osteocalcin levels in a 2-wk ORDX maintenance model.

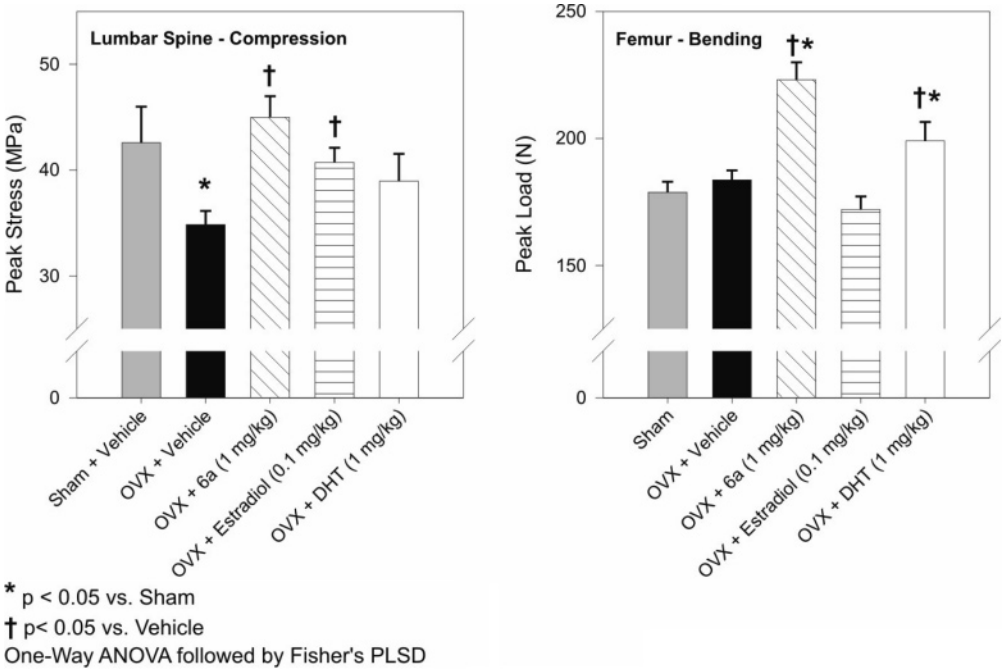



**Figure 2.** Effects of **6a**, estradiol, and DHT on lumbar spine compression strength and femur bending strength after 12 weeks of dosing in an OVX restoration model.

were separated by silica gel chromatography before deprotection to afford **6a** and **6b**, respectively.

The AR activities of these compounds were studied experimentally in a cellular background through ligand-dependent stimulation of luciferase reporter gene induction using a cotransfection assay. Compounds were tested in the cotransfection assay in both agonist and antagonist modes. Antagonist activity of the test compounds was measured in the presence of DHT at its $EC_{50}$. Competitive binding affinities were determined in bacculovirus expressed hAR. The data are listed in Table 1. Most of the compounds described here had good binding affinity for the AR, as measured in the hAR binding assay. In the cell-based cotransfection assay, they demonstrated a range of activities, from weak antagonists, to partial agonists, to potent agonists. Starting from the parent unsubstituted 6-pyrrolidinoquinolinone **4a**, which was a weak antagonist in our in vitro assay, we found that adding a methyl at the 2′-position, as in **4b**, enhanced the activity. The 2,5-dimethyl-substituted compound **4c** showed further enhanced activity compared to the monosubstituted compound. The *cis*-2,5-dimethylpryrrolidine **4c** was more active than the *trans*-isomer **4d**, which was only tested as a racemate. Although the hydroxymethyl-substituted pyrrolidine **4e** turned out to have no beneficial effect on the in vitro agonist efficacy and potency in the cotransfection assay, further modification of the side chain led to an improvement in the activity. The chloromethyl analogue **4f** was an efficacious agonist and had good potency in the cotransfection assay, it also had improved binding. Further optimization led to the discovery of **6a** as an orally available SARM with excellent activity in various animal models. This compound displayed excellent activity in the cotransfection assay and also had very good oral bioavailability. The stereochemistry of the compounds greatly affected their biological activity. As described previously, a *cis*-2,5-disubstituted pyrrolidine **4c** was more active than the *trans*-2,5-disubstituted **4d**. It was also found that the 2′*R*-enantiomer had much better activity than the 2′*S*-enantiomer (data not shown). The third chiral center in **6** did influence the activity of the compound as well, with the (2′*R*)-**6a** as the more active one. However the (2′*S*) diastereomer **6b** still had significant activity in vitro, as shown in Table 1. Compound **6a** was AR specific and showed weak or no affinity for other nuclear receptors. The binding affinity ($K_i$) for MR and ERα was >10 $\mu$M, for PR was 2.5 $\mu$M, and for GR was 8.3 $\mu$M.

In a PK experiment in male rats, **6a** showed favorable pharmacokinetic characteristics compared to **7**. With I.V. dosing, the half-life for **6a** was 2.7 h, whereas **7** had a half-life of 1.7 h. Oral dosing of **6a** at 14.6 mg/kg resulted in an $AUC_{0-\infty}$ of 11.8 $\mu$g·h/mL with oral bioavailability of 100%, whereas **7** at 10 mg/kg had an $AUC_{0-\infty}$ of 3.15 $\mu$g·h/mL, with oral bioavailability of 43%. Compound **6a** was further characterized in a model of hypogonadism, the 2-wk ORDX rat maintenance

model. In this established model for androgenic activity, the effect of a test compound on ventral prostate and levator ani muscle weights as well as serum levels of osteocalcin were measured. In this experiment, 8 week old rats were castrated and dosed orally, via gavage, with the test compound for two weeks beginning right after castration. As a reference compound, T propionate was dosed daily via subcutaneous injections. At necropsy, the prostate and levator ani muscle weights were recorded, and serum osteocalcin levels were determined. In Figure 1 the effect of **6a** on these three endpoints are summarized compared to the effects of T on the same endpoints. As can be seen in Figure 1, **6a** is very potent and efficacious in maintaining the levator ani muscle weight at the level observed in intact animals. At doses greater than 1 mg/kg levator ani muscle weight increases above the levels seen in intact rats. Compound **6a** maintained the prostate weight at the intact level at the highest dose tested of 10 mg/kg. However, at 1 mg/kg, a dose that was fully efficacious in maintaining levator ani muscle weight at intact levels, prostate weights were only slightly increased compared to castrated rats and about half the size of intact rats. We also looked at the effect of the compound on osteocalcin levels as a surrogate marker for bone turnover. Compound **6a** was highly potent and efficacious in this endpoint, and this activity prompted us to test this compound in a model of postmenopausal osteoporosis to determine its effects on bone strength.[8] In this model, 12 week old rats were ovariectomized and allowed to develop osteopenia for 8 weeks. After 8 weeks of bone loss, they were dosed orally with the test compound for an additional 12 weeks. At necropsy, the femur and lumbar spine were collected and the bending and compression strength of these bones was measured. Figure 2 illustrates the results for **6a**, and estradiol and DHT are shown as comparators. Because **6a** has no cross reactivity with the estrogen receptor and, unlike most steroidal androgens, which are aromatase substrates, it cannot be converted to an estrogenic compound in vivo, the effects on bone that are seen in this model are almost certainly mediated through the AR. Compound **6a** showed a significant improvement in the bending strength of the femur, a cortical bone site. For comparison, an antiresorptive agent like estradiol shows no effect on this endpoint, while DHT has a very weak effect. Compound **6a** also demonstrated activity on cancellous bone, as demonstrated by improved compression strength of the lumbar spine. On this endpoint, an antiresorptive agent like estradiol shows marked activity, while DHT has no significant effect. These data support the unique activity of this SARM on bone, overall resulting in much improved bone strength.

In conclusion, we have identified **6a** as an AR-specific nonsteroidal androgen. This compound has excellent oral bioavailability and demonstrates a clear separation between androgenic (ventral prostate) and anabolic (levator ani muscle) endpoints and reductions in osteocalcin levels as a marker of bone turnover in an in vivo model of hypogonadism (ORDX rat maintenance model); in addition, this compound improved bone strength in a model of postmenopausal osteoporosis (OVX restoration rat model).

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**Supporting Information Available:** Synthetic procedures, chemical characterization data for compounds **2**−**6**, and a description of the biological assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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