Discovery of 6-*N,N*-Bis(2,2,2-trifluoroethyl)amino-4-trifluoromethylquinolin-2(1*H*)-one as a Novel Selective Androgen Receptor Modulator[#]

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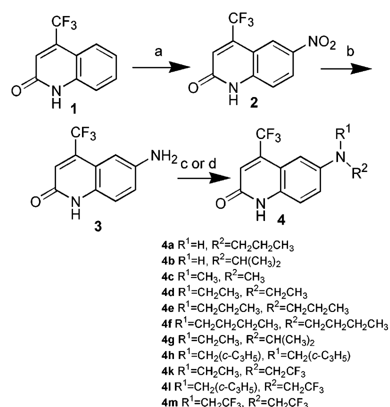
Abstract: The androgen receptor is a member of the extended family of nuclear receptors and is widely distributed throughout the body. Androgen therapy is used to compensate for low levels of the natural hormones testosterone (T) and dihydrotestosterone and consists of administration of T, prodrugs thereof, or synthetic androgens. However, currently available androgens have many drawbacks. We identified 6-dialkylamino-4-trifluoromethylquinolin-2(1*H*)-ones as orally available tissue-selective androgen receptor modulators.

The natural hormones testosterone (T[a]) and dihydrotestosterone (DHT) play important roles in male sexual development and function and in musculoskeletal growth. Deficiencies of circulating levels of these hormones in hypogonadal men can be treated by administration of exogenous androgens.[1,2] Androgen therapy has been available to physicians for many years now. However, unlike female sex hormone therapies, which have found extensive use in the fields of hormone replacement therapy, reproductive disorders, and contraception, androgen therapy has not been widely used. The reasons for this limited use are twofold. First, the beneficial effects of steroidal androgen therapies are overshadowed by potential side effects, some of which are due to their rapid metabolism to DHT and estrogens. Second, most steroidal androgens undergo rapid first pass hepatic metabolism and therefore cannot be taken orally. Commonly used methods of administration of T have been intramuscular injections or transdermal patches.[3] Alkylation of steroidal androgens at C_{17} slows hepatic metabolism, thus rendering them suitable for oral administration, but these compounds can lead to potential liver toxicity and are therefore not appropriate for chronic use.[4] Besides the search for safer, orally available androgens, much focus has been devoted to the development of androgens that separate the desired anabolic effects from the undesired androgenic effects.[5] Selectivity has been demonstrated for modulators of different steroid hormone receptors, such as the estrogen (ER),[6] progesterone (PR),[7] and glucocorticoid (GR)[8] receptors. More recently, tissue-selective AR modulators have been reported, some of which are based on nonsteroidal androgen antagonists,[9] of which several structural classes have been developed and marketed.[10] AR is widely distributed in tissues such as the prostate, seminal vesicle, male and female genitalia, skin, testis, ovary, cartilage, sebaceous glands, hair follicles, sweat glands, cardiac muscle, smooth muscle, gastrointestinal vesicular cells, thyroid follicular cells, adrenal cortex, liver, pineal, and brain.[11] Tissue selectivity is dependent on the regulation of AR expression, differential DNA binding at the promotor of regulated genes, and tissue-specific protein−protein interactions.[12] In addition, cross-talk with other tissue-specific signaling components, nongenomic effects, and heterodimerization with other receptors may contribute to tissue selectivity.[13] Some steroidal androgens such as 7α-methylnortestosterone (MENT)[14] have shown tissue selectivity in clinical trials, with greater efficacy in anabolic endpoints, such as bone, than in androgenic endpoints, such as the prostate gland. However, such effects on the skeleton may be due to its conversion to estrogens, while the reduced effects in the androgenic endpoints are thought to be due to limited 5α-reduction.

Scheme 1[a]



[a] Reagents: (a) HNO_3, H_2SO_4, −5 to 4 °C, 20 min; (b) Pd/C 10%, H_2, dimethylformamide, 6 h; (c) $NaCNBH_3$, MeOH, aldehyde or ketone, with or without acetic acid; (d) trifluoroacetic acid, $NaBH_4$, room temp to 50 °C, 2 days.

We identified a novel AR modulator **4m** (LGD2226) from SAR studies of a series of 6-*N,N*-dialkylamino-4-trifluoromethylquinolin-2(1*H*)-ones. In addition to the benefits of being orally available, this compound demonstrated tissue selectivity in animal models, with reduced effects on prostate compared to muscle.[15]

The synthesis of 6-*N,N*-dialkylamino-4-trifluoromethylquinolin-2(1*H*)-ones is outlined in Scheme 1. Compound **1** was prepared following a slight modification of the procedure reported by Berbasov and Soloshonok.[16] Nitration of **1** (H_2SO_4, HNO_3, −5 to 4 °C) afforded a mixture of 6- and 8-nitroquinolinones in a ratio of approximately 9:1 in favor of the desired 6-nitroquinolinone. A single crystallization from ethanol/water afforded pure **2**. The nitro group was reduced with Pd/C under a hydrogen atmosphere to give aniline **3**. Alkyl substituents on the amine were introduced by reductive alkylation, using $NaCNBH_3$ and the appropriate aldehyde or ketone. Depending on the reaction conditions and reagents used, either mono- or bisalkylation could be achieved. Ketones gave only monosubstituted amines, while aldehydes gave bisalkylation when acetic acid was added to the reaction mixture. In the absence of acid, monoalkylation was observed with most aldehydes. Only formaldehyde and acetaldehyde gave significant amounts of

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[a] Abbreviations: AR, Androgen receptor; SARM, selective androgen receptor modulator; T, testosterone; DHT, dihydrotestosterone; ER, estrogen receptor, PR, progesterone receptor; GR, glucocorticoid receptor; MR, mineralocorticoid receptor; ORDX, orchidectomized.

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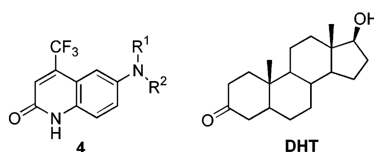
Table 1. Cotransfection and Competitive Binding Data for 6-(*N,N*-Dialkylamino)-4-trifluoromethylquinolin-2(1*H*)-ones and DHT[a]



compd	R^1	R^2	hAR agonist EC_{50} (nM)	hAR agonist[b] Eff (%)	hAR antagonist IC_{50} (nM)	hAR antagonist[c] Eff (%)	hAR whole-cell binding K_i (nM)
3	H	H	–	–	26	28 ± 7	>10 × 10^3
4a	H	$CH_2CH_2CH_3$	2022	34	27.5 ± 5.5	72 ± 3	54
4b	H	$CH(CH_3)_2$	106	26	16.5 ± 0.5	61 ± 4	58
4c	CH_3	CH_3	–	–	34 ± 11	72 ± 4	153
4d	CH_2CH_3	CH_2CH_3	2.1 ± 0.3	94 ± 19	–	–	4.4
4e	$CH_2CH_2CH_3$	$CH_2CH_2CH_3$	1.5 ± 0.2	72 ± 5	–	–	64
4f	$CH_2CH_2CH_2CH_3$	$CH_2CH_2CH_2CH_3$	1283	68	29.5 ± 6.5	54 ± 1	222
4g	CH_2CH_3	$CH(CH_3)_2$	2.2 ± 0.4	77 ± 13	–	–	7.4
4h	$CH_2(c\text{-}C_3H_5)$	$CH_2(c\text{-}C_3H_5)$	1.9 ± 0.7	81 ± 8	–	–	6.3
4k	CH_2CH_3	CH_2CF_3	0.4 ± 0.1	107 ± 5	–	–	7.1
4l	$CH_2(c\text{-}C_3H_5)$	CH_2CF_3	2.8 ± 1.6	99 ± 13	–	–	7.1
4m	CH_2CF_3	CH_2CF_3	0.2 ± 0.02	95 ± 2.0	–	–	1.5
DHT			5.1 ± 0.1	100 ± 0.08	–	–	0.2 ± 0.02

[a] Values with standard errors represent the mean value of at least two separate experiments with triplicate determinations. A dash indicates an efficacy of <20% and a potency of >10000 nM. [b] Agonist efficacies were determined relative to DHT (100%). [c] Antagonist efficacies (%) were determined as a function of maximal inhibition of DHT at the EC_{50} value.

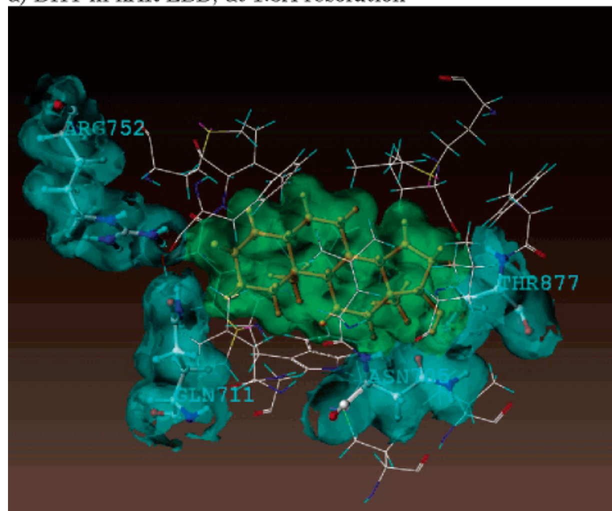
bisalkylated product. Sometimes it proved to be more convenient to introduce the alkyl substituents by the reaction of carboxylic acids with $NaBH_4$ in the presence of the amine.[17] This procedure was especially useful for introduction of a trifluoroethyl substituent. The bis-trifluoroethyl-substituted amine **4m** was obtained in 83% yield from the reaction of **3** with trifluoroacetic acid and $NaBH_4$.

The in vitro activity of 6-*N,N*-dialkylamino-4-trifluoromethyl-quinolin-2(1*H*)-ones (**4a**–**m**) on the human AR was studied in a cellular background using a cotransfection assay.[18] Ligand-dependent stimulation of a luciferase reporter gene was measured against the concentration of ligand. The compounds were also tested in the antagonist mode in the presence of DHT at its EC_{50}. The binding affinity for the hAR was measured in a whole-cell receptor binding assay. The results for the differentially substituted 6-aminoquinolin-2(1*H*)-ones in the cotransfection and binding assays are listed in Table 1. The substituents had a large effect on the activity of the compounds in the cell-based assay. The unsubstituted or monosubstituted amines (**3**, **4a**, **4b**) were mainly antagonists with a hint of agonist activity for **4a** and **4b**. Also, the dimethylamine **4c** turned out to be an antagonist in this assay. The optimal length for the alkyl substituents was two or three carbons, as exemplified by the excellent potency and efficacy of the diethyl **4d** and dipropylamine **4e.** The dibutylamine **4f**, however, was a weak partial agonist with some antagonistic activity. Having one alkyl group branched as in the ethylisopropyl analogue **4g** resulted in a good agonist. The bis-cyclopropylmethylamine **4h** was a very potent and efficacious compound, but the best agonists had a trifluoroethyl substituent on the amine. Both **4k** and **4m** had subnanomolar potencies in the cotransfection assay. The bis-trifluoroethylamine **4m** was more potent ($EC_{50} = 0.2 \pm 0.02$ nM) than DHT ($EC_{50} = 5.1 \pm 0.1$ nM) and showed efficacy comparable to that of the steroid. No antagonist activity was observed in this assay. In the competitive whole-cell binding assay, **4m** had a K_i of 1.5 nM, compared to 0.2 nM for DHT. Compound **4m** was specific for AR, showing no significant binding affinities for PR, GR, MR, and ER ($K_i > 1$ μM, data not shown). Similarly, agonist or antagonist mode cross-reactivity was not observed in cotransfection assays with these nuclear receptors for concentrations of **4m** up to 10 μM (data not shown).

To determine the binding mode of **4m** in the androgen receptor, a cocrystal structure was obtained with **4m** in the hAR ligand binding domain (Figure 1b). As expected, **4m** occupies the same binding pocket as DHT (Figure 1a),[19] and in general, the protein backbone is superposable to that observed with DHT. Just like the carbonyl group of DHT, the quinolone carbonyl forms hydrogen bond interactions with GLN711 and ARG752. GLN711 forms an additional hydrogen bond with the quinolone NH of **4m**. The trifluoroethyl groups of **4m** occupy the same space in the receptor as the C and D rings of the steroid. The dimethyl-substituted analogue **4c** would not be able to fully occupy this space in the binding pocket, and this explains the reduced binding affinity and antagonist activity observed for this compound. On the other hand, the dipropyl analogue **4e** would still fit very well, but the dibutyl compound **4f** is too big, resulting in strongly reduced binding affinity and antagonist activity in the cotransfection assay. A further in depth discussion of the cocrystal structure of **4m** in the hAR ligand binding domain will be published separately.[20]

The in vivo activity of **4m** was evaluated in mature orchidectomized (ORDX) rats. After ORDX the rats were orally dosed for 2 weeks with **4m** in a dose range from 1 to 100 mg/kg or vehicle. Sham operated rats were used as control. The same experiment was run with testosterone as a positive control. Because of the rapid metabolism of testosterone in the liver, testosterone was dosed subcutaneously. After 2 weeks of dosing, the weight of the ventral prostate was determined as a measure of androgenic activity. The weight of the levator ani muscle was determined as a measure of anabolic activity. The results of the 2-week ORDX rat in vivo experiment are shown in Figure 2. **4m** has a pronounced effect on the levator ani muscle, maintaining the muscle weight at the eugonadal levels at an approximate 3 mg/kg dose. At higher doses, levator ani muscle weight increases up to 150% of intact rats. This demonstrates that **4m** is a highly potent anabolic androgen. In marked contrast, **4m** had weak trophic effects on the prostate. An amount of 100 mg/kg **4m** was required to maintain prostate weight at intact levels. These data clearly show the tissue selectivity of **4m**. In contrast, testosterone maintained the levator ani muscle weight and the ventral prostate weight at the eugonadal level at the same dose of approximately 2 mg/kg. From Figure 2 it is also

a) DHT in hAR LBD, at 1.8Å resolution



b) **4m** in hAR LBD, at 2.1Å resolution

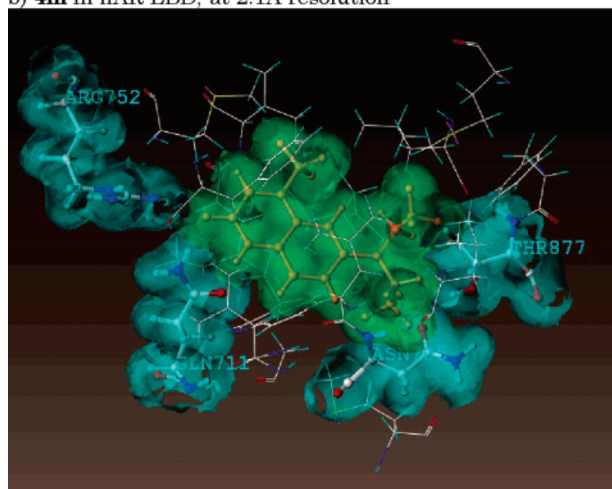


Figure 1. Binding pockets of hAR with (a) DHT and (b) 6-*N*,*N*-bis-(2,2,2-trifluoroethyl)amino-4-trifluoromethylquinolin-2(1*H*)-one (**4m**), showing key amino acid residues.

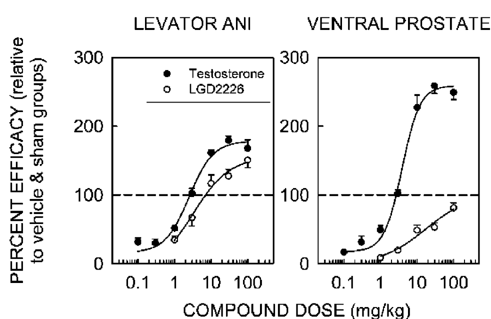


Figure 2. Effects of **4m** (oral, open symbols) and testosterone (sc, solid symbols) treatment on levator ani muscle and ventral prostate weights in a 2-week maintenance assay in adult ORDX rats. 100% identifies intact animals, and 0% represents ORDX animals.

evident that testosterone stimulates prostate growth to levels much higher than those observed for the intact controls. The tissue selectivity difference between **4m** and T cannot be easily explained by the hAR LBD structures shown in Figure 1, since no marked changes in the protein structure were observed when compared with the DHT cocrystal. It is possible that minute changes in amino acid interactions with the ligand may be responsible for the tissue selectivity of these compounds, possibly through an altered cofactor interaction profile. Since the cocrystals involve only the ligand-binding domain, tissue selectivity may also reside in structural domains of the AR that were not included in the current analysis.

In conclusion, we have identified **4m** as an AR-specific nonsteroidal androgen, which exhibited efficacy and potency in maintaining levator ani muscle weight similar to testosterone. Furthermore, the compound exhibited much less efficacy and potency compared to T in stimulating the ventral prostate weight of mature castrated rats. Since **4m** demonstrates a clear separation between androgenic (ventral prostate) and anabolic (levator ani muscle) endpoints in an in vivo model, it can therefore be classified as a selective androgen receptor modulator (SARM).

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Supporting Information Available: Synthetic procedures, chemical characterization data for **1**−**4**, and biological assay methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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