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Design and synthesis of tricyclic tetrahydroquinolines as a new series of nonsteroidal selective androgen receptor modulators (SARMs)

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ABSTRACT

Some tricyclic tetrahydroquinolines (THQs) were found to have the potential of a new series of nonsteroidal selective androgen receptor modulators (SARMs). Compound **5b** was first designed and synthesized under our hypothesis based on a four-point pharmacophoric requirement of the 3-carbonyl, 18-methyl, 17-hydroxyl, and 13-quaternary carbon groups of dihydrotestosterone (DHT). It was revealed that this compound exhibits not only a strong androgen receptor (AR) agonistic activity (EC_{50} = 9.2 nM) but also the highest selectivity in binding affinity to AR among the steroid hormone receptors. Furthermore, this compound showed a weak virilizing effect with retention of the desired anabolic effect as compared with DHT in vivo.

The androgen receptor (AR) is a member of the nuclear receptor superfamily, which consists of the receptors for estrogen, progesterone, glucocorticoids, mineralocorticoids, and androgens.[1] Physiologically, AR is activated by endogenous androgens, testosterone (TES), and its metabolite, dihydrotestosterone (DHT). TES and DHT play important roles in establishing and maintaining the male phenotype.[2] Their actions are essential for the differentiation and growth of male reproductive organs. In addition, androgens are important for the development of male characteristics in certain extragenital structures such as muscle, bone, hair, larynx, skin, lipid tissue, and kidney.[3] The activated AR forms a homodimer and subsequently recruits necessary coregulators and/or general transcription factors to mediate the enhancement or repression of transcription of the target gene.

Various synthetic steroidal AR ligands have been developed for the treatment of male hypogonadism, muscle wasting, anemia, benign prostate hyperplasia, and prostate cancer,[4] but the use of such steroidal AR ligands has been limited because of their poor oral bioavailability and the risks of serious side effects.[4,5] On the other hand, nonsteroidal AR modulators have also been developed by several research groups (Fig. 1).[6] Especially the compound **3** not only binds AR with high affinity but also demonstrates tissue selectivity in animal models.[7] This compound showed the possibility of developing selective AR modulators (SARMs) with receptor and tissue selectivity avoiding undesired side-effects derived from steroidal templates.

To create nonsteroidal AR agonists, we have designed tricyclic tetrahydroquinoline (THQ) derivatives by a four-point pharmacophore method. In this Letter, we would like to report a nonsteroidal SARM lead compound **5b** which has a strong binding affinity and an agonistic activity to AR. The in vivo effect of the compound **5b** was also shown in the latter part of this Letter.

To design nonsteroidal AR agonists, the common pharmacophore points of AR modulators were extracted by structural comparison of DHT, tricyclic quinolinones (**1** and **2**),[8,9] and diaryl propionamides (**3** and **4**)[7,10] (Fig. 1). The nitro group of **3** and cyano group of **4** are well known as bioisosteres of the 3-carbonyl group of DHT.[11] Compounds **1** and **3** have been reported as agonists[7,8] while **2** and **4** as antagonists.[9,10] Before superimposition of these five compounds, their three-dimensional (3D) structures were built by ab initio full geometry optimizations using Jaguar and selected the most stable conformation.[12]

Upon superimposition of these 3D structures, we paid attention to the 3-carbonyl, the 18-methyl groups and 13-position's carbon atom of DHT at the beginning. This was based on the following five factual data obtained from the solved crystal structure of the AR ligand-binding domain bound with DHT,[13] and the common features of DHT and tricyclic quinolinones (**1** and **2**): (1) the 3-carbonyl and 17-hydroxyl groups of DHT are two major functional groups playing a key role for the binding to the receptor, (2) the 18-methyl group and the 13-carbon atom stipulating the distance and the angle of 18-methyl group, being settled closely to the 17-hydroxyl group of DHT, are important for face recognition of AR, (3) the 6-ethyl group of **1** and the 8-dimethyl groups of **2** play important roles as the 18-methyl group of DHT, (4) the molecular volume

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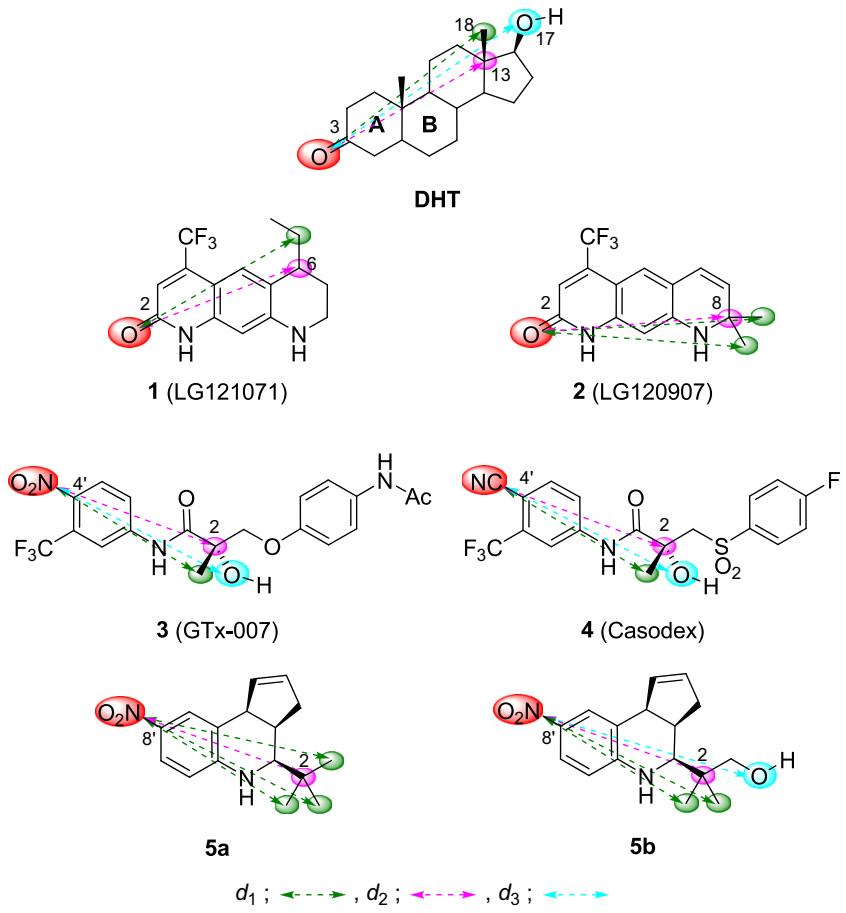


Figure 1. DHT and nonsteroidal AR modulators, and the distances between the functional groups (d_1, d_2 and d_3).

of the ligand is also important for AR agonistic activity,[14] (5) tricyclic quinolinones (**1** and **2**) have strong AR binding activity though they have no hydroxyl group in their structure.[8,9]

We have constructed three-point pharmacophore hypothesis which consists of a carbonyl isostere for the 3-carbonyl group of DHT, one methyl group corresponding to the 18-methyl group of DHT and an aliphatic carbon corresponding to the 13-carbon atom of DHT which we think is very important to place and direct properly the methyl group just mentioned above.[15] The distances are defined; d_1 is denoted by a green dashed arrow and d_2 as a magenta dashed arrow (Fig. 1). All these distances were measured by Maestro molecular modeling package.[12] The distance between the 3-carbonyl oxygen and 18-methyl carbon atoms of DHT (d_1) was 9.55 Å (Table 1). The distance between the 2-carbonyl oxygen and the 6-methylene carbon atoms of **1** (d_1) was 8.42 Å, while that between the 2-carbonyl oxygen and one of the 8-methyl carbon atoms of **2** (d_1) was 9.47 Å. The distances (d_1) of **1** and **2** were close to the d_1 of DHT. The distance between the 3-carbonyl oxygen and 13-quaternary carbon atoms of DHT (d_2) was 9.80 Å. Similarly, the distance between the 2-carbonyl oxygen and 6-tertiary carbon atoms of **1** (d_2) and the distance between the 2-carbonyl oxygen and 8-quaternary carbon atom of **2** (d_2) were found comparable to the distance of DHT (d_2) (**1**: 7.93 Å, **2**: 8.44 Å versus DHT: 9.80 Å). The distances, d_1 and d_2, of the diaryl propionamide **3** (d_1: 8.49 Å, d_2: 8.09 Å) and **4** (d_1: 9.60 Å, d_2: 9.23 Å) were also close to each corresponding distance of DHT.

The tricyclic THQ derivatives obtained by the Grieco three-component condensation (3CC) satisfied the above hypothesis (Scheme 1).[16] This reaction was very attractive for the introduction of appropriate substituents in the scaffold of THQs to design new AR agonists. As shown in Scheme 1, THQs can be synthesized only one step by reacting a substituted aniline, an electron-rich olefin, and a substituted aldehyde in the presence of an equimolar amount of trifluoroacetic acid (TFA) in acetonitrile (MeCN).

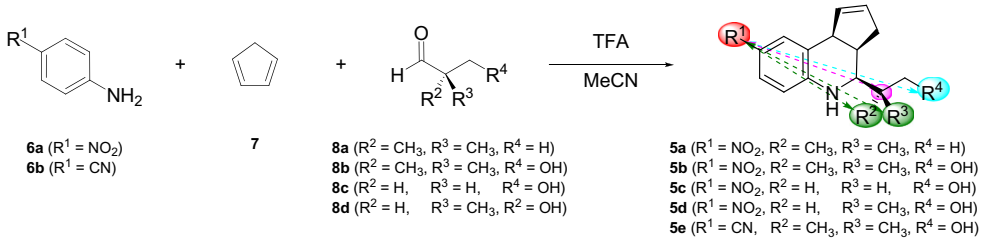
First, *p*-nitroaniline (**6a**) and trimethylacetaldehyde (**8a**) were selected for the 3CC reaction as the aniline and aldehyde components. The distances between the one oxygen atom of the nitro group and the carbon atom of the side-chain methyl groups of **5a** (d_1) were calculated to be 8.90, 9.22 and 9.78 Å. These distances were close to those of DHT (d_1: 9.55 Å). Cyclopentadiene (**7**) was chosen as a dienophile part which could react with the above-mentioned two components to give THQ with the molecular volume around 900 Å^3 comparable to that of DHT.[12] The reaction with **6a**, **7**, and **8a** proceeded smoothly and gave the corresponding *endo*-isomer **5a** (Scheme 1).

Although **5a** satisfied our three-point pharmacophoric requirement with its molecular volume similar to DHT, the AR binding affinity was moderate. Our initial hypothesis did not focus on the distinctive 17-hydroxyl group of DHT, because tricyclic quinolinones (**1** and **2**) do not have the corresponding hydroxyl group. We assumed the weak AR binding affinity should be due to the lack of a hydroxyl group at appropriate distances from the 3-carbonyl and 18-methyl groups. The distance between the 3-carbonyl and 17-hydroxyl groups of DHT (d_3) was 10.69 Å (Table 1). The distance (d_3) denoted by a cyan dashed arrow (Fig. 1). The diaryl propionamides, **3** and **4**, have a hydroxyl group with the corresponding distances (d_3) of 9.80 and 10.10 Å, respectively, from the 4-nitro or 4-cyano group by the calculations (Table 1). In the result, we adopted the four-point pharmacophoric requirements; the corresponding hydroxyl group was added to the initial hypothesis.

Table 1
Distances between two functional groups (d_1, d_2, and d_3), molecular volumes, binding affinities, and agonistic and/or antagonistic activities

Compound[a]	d_1[b] (Å)	d_2[b] (Å)	d_3[b] (Å)	Molecular volume[c] (Å^3)	Binding affinity IC_{50}[d] (nM)	Agonistic activity EC_{50}[d] (nM)	Antagonistic activity IC_{50}[d] (nM)
DHT	9.55	9.80	10.69	982	3.1	0.98	—[g]
1[e,h]	8.42	7.93	—[g]	875	17[h]	4	7481
2[f,h]	9.47, 8.91	8.44	—[g]	877	26[h]		27
3[h]	9.19	8.81	9.80	1245	40[h]		
4	9.60	9.23	10.10	1161	54	NT[g]	590
5a[f]	8.90, 9.22, 9.78	8.70	—[g]	903	570	NT[g]	NT[g]
5b[f]	8.89, 9.23	8.69	11.01	920	13	9.2	ND[g]
5c	—[g]	8.69	11.01	833	18	79	14
5d[f]	8.89	8.69	11.01	881	1.3	7.0	ND[g]
5e[f]	9.40, 9.64	9.09	11.39	912	140	860	52

[a] The ab initio calculations were performed using Jaguar by the B3LYP density functional method with the fully geometrically optimized 6-31G** basis set.
[b] The distance was measured by Maestro. The distances, d_1, d_2, and d_3 are shown in Figure 1.
[c] The molecular volumes were calculated using QikProp.
[d] Binding affinity (IC_{50}) was determined by the rat AR competitive binding assay, agonistic (EC_{50}) and antagonistic activities (IC_{50}) were determined by an AR receptor assay.
[e] The (*R*)-isomer of compound **1** was used for the measurement of d_1 and d_2.
[f] Compounds **2**, **5b**, and **5e** have two relevant methyl groups, compound **5a** has three relevant methyl groups, and compound **5d** has one relevant methyl group.
[g] A hyphen indicates that these compounds have no relevant functional groups, NT indicates that they were not tested, and ND indicated a functional potency that could not be determined because of its low efficacy.
[h] The biological data were taken from Refs. [7,8,9], the binding affinities were represented as K_i value, and the blanks meant information were not available in the



Scheme 1. Construction of a THQ scaffold by the Grieco three-component condensation (3CC).

It was calculated that the compound **5b** (Fig. 1) has the hydroxyl group at a distance (d_3) of 11.01 Å from the one oxygen atom of the nitro group and the molecular volume was 920 Å^3, which was also close to that of DHT (982 Å^3). In addition, these two compounds were highly overlapped not only these four pharmacophoric points but also the A and B rings of DHT with the THQ ring of **5b** (Fig. 2). Hence, **5b** was synthesized using 3-hydroxy-2,2-dimethylpropionaldehyde (**8b**) and was subjected to biological tests. As expected, **5b** showed an AR binding affinity (IC_{50}: 13 nM) that is approximately 44 times as strong as the initial compound, **5a** (IC_{50}: 570 nM). Moreover, **5b** exhibited fairly good agonistic activity (EC_{50}: 9.2 nM) (Table 1).

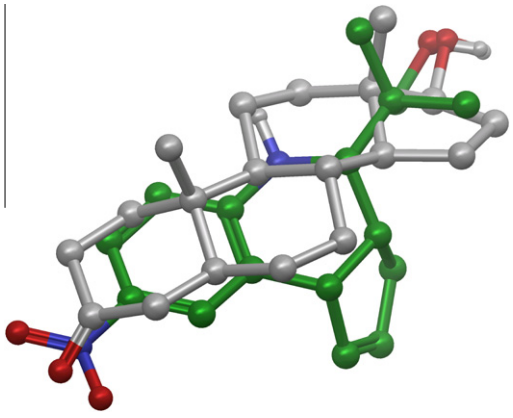


Figure 2. Superimposition by the four-point pharmacophore between DHT and **5b**.

As shown in Scheme 1, compound **5c** was synthesized using 3-hydroxypropionaldehyde (**8c**) as the aldehyde. It had no methyl group in the side chain but had almost the same d_2 and d_3 as those of DHT. It exhibited a high AR binding affinity and a partial agonistic activity. Then, compound **5d** was synthesized using (*R*)-3-hydroxy-2-methylpropionaldehyde (**8d**) as the aldehyde, and the distances d_1, d_2 and d_3 were almost the same as those of DHT. It exhibited not only the highest AR binding affinity but also almost the same AR agonistic activity as **5b**. The analog of **5b**, compound **5e** having a cyano group instead of the nitro group, was synthesized from *p*-cyanoaniline (**6b**). Compound **5e** showed strong antagonistic activity, decreased AR binding affinity and agonistic activity as compared with **5b**.

On the basis of these results, it is considered that the following four points would be important for the THQ derivatives to possess the agonistic activity: (1) the combination of nitro, methyl and hydroxyl group is indispensable for AR agonistic activity, (2) the nitro group on the aromatic ring of **5b** forms a hydrogen-bonding network on the AR similarly to the 3-carbonyl moiety in the DHT, (3) only one methyl group at the side chain plays a critical role that would be similar to that of the angular methyl group of DHT, and (4) the aforementioned hydrogen bond formed by the hydroxyl group in the side chain mimics the hydrogen bond of the hydroxyl group of DHT.

To assess the binding selectivity, the binding affinities of **5b** to the other steroid hormone receptors were determined by the competitive binding assay (Table 2).[17] Interestingly, **5b** showed a selectivity profile similar to those of DHT and **4** in the binding affinities to the receptors of steroid hormones, AR, progesterone receptor (PR), estrogen receptor (ER), glucocorticoid receptor (GR) and

Table 2
AR selectivity of DHT, **4**, and **5b** versus other steroid hormone receptors

Compound	IC_{50}[a] (nM)				
	AR	PR	ER	GR	MR
DHT	3.1	440	340,000	20,000	2100
4	54	5600	>1,000,000	320,000	>1,000,000
5b	13	130	>1,000,000	18,000	6300

[a] IC_{50} values were determined by the competitive binding assay, as described in a previous report.[17] The IC_{50} values (nM) of endogenous steroids in each assay are as follows: progesterone, 6.0; 17β-estradiol, 2.3; dexamethasone, 22; aldosterone, 43.

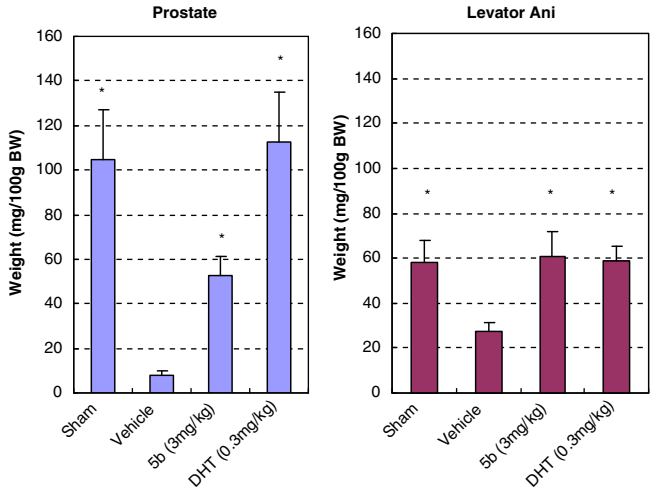


Figure 3. Effects of **5b** (3 mg/kg) and DHT (0.3 mg/kg) on the weights of the prostate and levator ani in the treatment of ORX rats. * Significantly different from the vehicle group (p <0.05).

mineralocorticoid receptor (MR). This result suggested that **5b** has potential AR-binding selectivity as a lead compound.

We compared the virilizing and anabolic effects of **5b** with those of DHT in orchiectomized (ORX) rats.[17] The weight of the prostate was measured as an index of virilizing activity, while that of levator ani was measured as that of anabolic activity (Fig. 3). Treatment (sc) of ORX rats with the positive control, DHT, recovered both the weights of prostate and levator ani to the normal levels at 0.3 mg/kg as compared with the sham. However, in the same model, **5b** (sc) showed substantial recovery of the weight of levator ani at 3 mg/kg, but it recovered the weights of the prostate to only about 50% of that of the sham at the same dose. This means that **5b** has the selectivity in the anabolic effect that is twice as high as that in the virilizing effect.

As described, we could successfully identify a new series of tricyclic THQ derivatives designed by our four-point pharmacophore method. The compound **5b** has AR agonistic activity with separation of the desirable anabolic effect and undesirable virilizing effect. Guided by these initial findings, further studies are in progress and directed to the discovery of analogs that possess the pharmacological efficacy and pharmacokinetic properties superior to **5b** with retention of the desirable selectivity for the AR and muscle tissues.

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