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Potent, nonsteroidal selective androgen receptor modulators (SARMs) based on 8*H*-[1,4]oxazino[2,3-*f*]quinolin-8-ones

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Abstract—A series of androgen receptor modulators based on 8*H*-[1,4]oxazino[2,3-*f*]quinolin-8-ones was synthesized and evaluated in an androgen receptor transcriptional activation assay. The most potent analogues from the series exhibited single-digit nanomolar potency in vitro. Compound **18h** demonstrated full efficacy in the maintenance of muscle weight, at 10 mg/kg, with reduced activity in prostate weight in an in vivo model of androgen action.

The androgen receptor (AR) is a member of the intracellular receptor superfamily of ligand-dependent transcription factors.[1] The endogenous ligands for AR are the steroids testosterone (**T**) and dihydrotestosterone (**DHT**). When bound to AR, these ligands play important roles in sexual development and function,[2] and musculo-skeletal growth.[3] Steroidal androgen therapy is effective for the treatment of androgen insufficiency. However, the broader use of these androgens for additional treatments, such as osteoporosis or frailty, is limited by undesirable AR-mediated effects, such as prostatic hypertrophy and hirsutism. A selective androgen receptor modulator (SARM), with full anabolic activity but reduced impact on the undesirable effects, could have a large role on endocrine therapies to treat muscle wasting and osteoporosis.[4] Early studies on modified androgens explored alkylation at C-17, as in fluoxymesterone (**1**, Fig. 1).[5] However, compounds from this general class are associated with potential liver toxicity.[2] Recent publications in the area of nonsteroidal androgens are indicative of the high level of interest in discovering novel safe, effective anabolic agents (**2**, **3**).[6–8]

Our continued interest in SARMs is based on scaffolds derived from quinolin-2-ones (**4** and **5**).[9,10] During the course of our studies on 7*H*-[1,4]oxazino[3,2-*g*]quinolin-7-ones (**5a**), we isolated as a minor by-product of the Knorr quinolone reaction[11] regioisomer (**6a**, <5% yield, Scheme 1) which fortuitously possessed AR

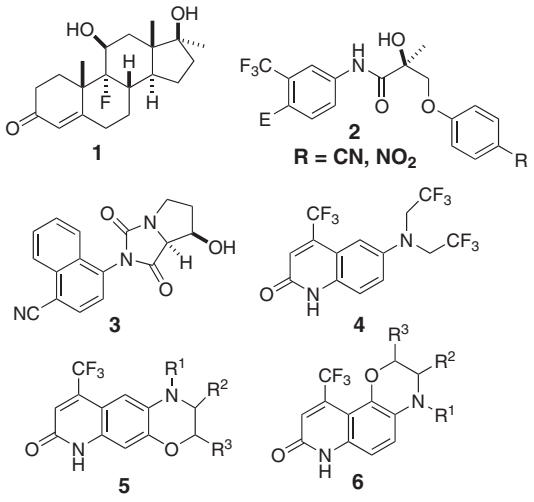

Figure 1. Synthetic androgens.

Keywords: Androgen; SARM; Oxazino[2,3-*f*]quinolin-8-one; Testosterone; Anabolic; Androgenic; AR; Benzoxazine.
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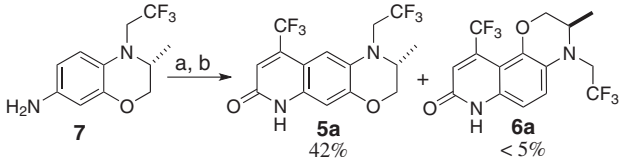
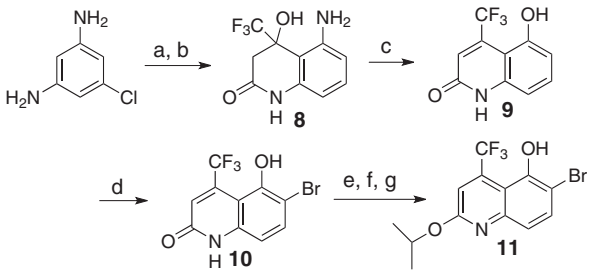


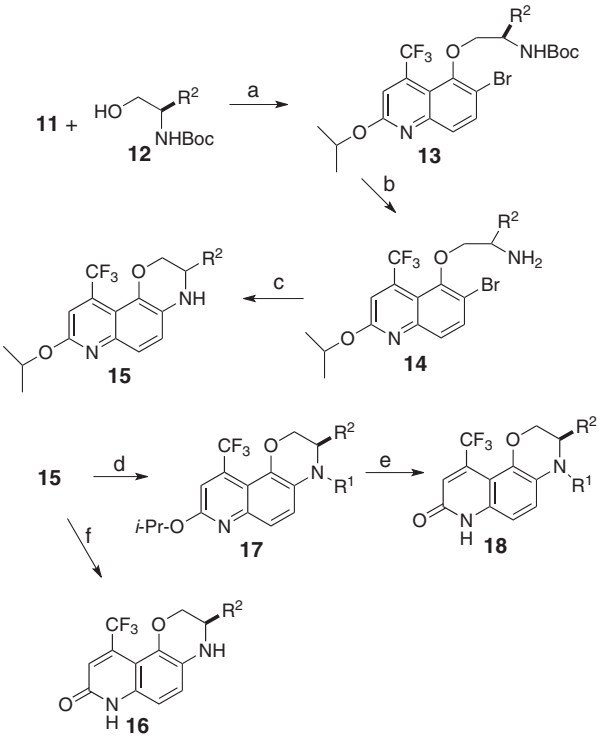
Scheme 1. Reagents and conditions: (a) 4,4,4-trifluoroacetoacetate, PhH, reflux; (b) concd H_2SO_4, 100 °C.

agonist activity in a transcriptional activation assay (78% agonist efficacy, 5 nM). Investigations into the scaffold led to a series of novel androgens derived from 8*H*-[1,4]oxazino[2,3-*f*]quinolin-8-ones (**6**). The lead compound from this series, **18h**, demonstrated full efficacy in the maintenance of levator ani weight (LA) muscle, an anabolic endpoint in a castrated mature rat model.

Our previous investigations of quinolinone scaffolds **4** and **5** revealed that the substitution pattern proximal to the tertiary amine group strongly affected AR activity. Consequently, we sought to probe the R^1 and R^2 region of **6**. The yields obtained by isolating the minor Knorr product were too low to be a practical method of analogue preparation, so we developed a new synthesis that would allow for modifications on the oxazine portion of **6**. A Knorr-type reaction of 5-chloro-1,3-phenylene diamine proceeded by treatment with ethyl 4,4,4-trifluoroacetoacetate in EtOH to afford the quinolin-2-one (47%), followed by hydrogenation (100%) to provide **8** (Scheme 2). Treatment of **8** with sodium nitrite in concd H_2SO_4 effects the Sandmeyer reaction with dehydration to afford **9**, and is an effective method to prepare the 5-substituted 1*H*-quinolin-2-one, which is not available by the direct Knorr cyclization of 3-aminophenol derivatives. Bromination with NBS afforded **10** in 77% yield.[12] To prepare **11**, it was necessary to benzylate the phenol, alkylate the quinolinone with isopropyl iodide and CsF, then remove the benzyl group with methanesulfonic acid to afford **11** in 55% yield over 3 steps. With **11** in hand, the N-Boc-protected aminoalcohol **12** was subject to Mitsunobu conditions[13] to afford **13**, followed by hydrolysis of the Boc group with TFA to afford **14** (Scheme 3). Formation of the benzoxazine subunit was achieved utilizing the Buchwald–Hartwig aromatic amination conditions to afford intermediate



Scheme 2. Reagents and conditions: (a) 4,4,4-trifluoroacetoacetate, EtOH; (b) 10% Pd-C, H_2, (1 atm), KOAc, EtOH; (c) $NaNO_2$, concd H_2SO_4, 0 °C, then 140 °C; (d) NBS, diisopropylamine, EtOAc, −10 °C; (e) BnBr, CsF, DMF; (f) isopropyl iodide, CsF, DMF; (g) methanesulfonic acid, HOAc (1:1).



Scheme 3. Reagents and conditions: (a) diisopropyl azodicarboxylate, Ph_3P, *N*-methylmorpholine; (b) TFA, CH_2Cl_2; (c) $Pd_2(dba)_3$ (2–5 mol%), (±)-BINAP (4–10 mol%), *t*-BuONa, toluene, reflux; (d) R^1CHO or $R^1CH(OH)OEt$, $NaBH_3CN$, HOAc or TFA; (e) HOAc/concd HCl (3:1), 60–100 °C.

15.[14] In addition to providing the requisite scaffold, this synthesis represents a novel method to prepare 3,4-dihydro-2*H*-1,4-benzoxazines. Reductive amination, followed by acid hydrolysis, afforded **18**.[15] Compound **16** is obtained by acid hydrolysis of **15**.

The compounds were evaluated in a transcriptional activation assay with hAR in a mammalian cell (CV-1) as previously described.[16] Both R^1 and R^2 positions are important for AR agonist activity as compounds with no R^1 substituent showed no agonist activity regardless of the R^2 substituent (Table 1). Antagonist activity was observed (**16a–e**), demonstrating that this position can be used to switch the AR activity to antagonists. This finding parallels series **5**, in which substitution at the R^1 position enhances AR agonist activity. Replacement of the 2,2,2-trifluoroethyl substituent of **6a** with small alkyl groups (**18a–d**) resulted in a significant drop in potency, even in the case of **18b**, where R^1 = Et. Removal of the R^2 substituent (**18e**) likewise resulted in significant reduction of AR agonist activity. Substitution of R^2 with an ethyl group resulted in a series of analogues with AR activity that roughly parallels that of the corresponding methyl substituted compounds. Compound **18f** demonstrates no AR agonist activity, and the same significant difference in potency between the ethyl (**18g**) and 2,2,2-trifluoroethyl substituent (**18h**) exists. Compound **18h** demonstrates activity comparable to that of **DHT**. Weaker agonist activity is seen among the propyl, isobutyl, and 3-hydroxy-2-methyl-

Table 1. Activity in the AR transcriptional activation assay[a]

Compound[c]	R^1	R^2	hAR Agonist		hAR Antagonist	
			EC_{50} (nM)	Eff (%)	IC_{50} (nM)	Eff (%)
DHT			5.7 ± 0.1	100	—	—
5a			1.1 ± 0.2	82 ± 5	—	—
6a	CH_2CF_3	Me	5.4 ± 3.4	78 ± 4	—	—
16a	H	H	—	—	1500 ± 100	62 ± 16
16b	H	Me	—	—	72	64
16c	H	Et	—	—	84 ± 14	58 ± 6
16d	H	Ph	—	—	1480 ± 750	78 ± 9
16e	H	Pr	—	—	113 ± 58	76 ± 4
18a	Me	Me	—	—	nd	32 ± 4
18b	Et	Me	420 ± 120[b]	31 ± 12	nd	26 ± 8
18c	Pr	Me	406 ± 56	45 ± 22	nd	21 ± 13
18d	Allyl	Me	228 ± 84	97 ± 12	na	na
18e	CH_2CF_3	H	190 ± 66	38 ± 2	nd	25 ± 12
18f	Me	Et	—	—	nd	32 ± 11
18g	Et	Et	410 ± 70	27 ± 10	—	—
18h	CH_2CF_3	Et	1.0 ± 0.4	92 ± 5	—	—
18i	Allyl	Et	51 ± 14	83 ± 14	—	—
18j	Pr	Et	394 ± 42	61 ± 12	—	—
18k	*i*-Bu	Et	227 ± 26	46 ± 14	—	38 ± 15
18l	$Me_2C(OH)CH_2$-	Et	300 ± 140	57 ± 11	—	26 ± 9
18m	Me	*i*-Pr	276 ± 22	31 ± 6	nd	31 ± 9
18n	Et	*i*-Pr	272 ± 74	76 ± 6	—	21 ± 10
18o	CH_2CF_3	*i*-Pr	20 ± 16	74 ± 5	—	—
18p	Allyl	*i*-Pr	35 ± 6	78 ± 3	—	—
18q	CH_2CF_3	Bn	43 ± 14	63 ± 14	—	—
18r[d]	CH_2CF_3	*i*-Bu	98 ± 32	36 ± 3	nd	34 ± 9
18s	CH_2CF_3	Ph	—	—	65 ± 16	85 ± 3
18t	Cyclopropylmethyl	Ph	—	—	45 ± 10	88 ± 2
	(*S*)-**18h**		18 ± 3	53 ± 23	—	—
	(±)-**18h**		3.5 ± 1.3	89 ± 8	—	—

[a] AR transcriptional activation experimental results with at least three experiments in triplicate with SEM.
[b] Mean value from two experiments.
[c] For chiral compounds, the (*R*)-enantiomer was prepared except where indicated. For all tables, a hyphen is indicative of efficacy <20% or a potency >10,000 nM, and nd means the IC_{50} could not be calculated.
[d] Isolated as a minor by-product of the Knorr reaction as in Scheme 1.

propyl analogues (**18j–l**). In the cases where R^2 = isopropyl, a slight reduction in potency is seen with **18o** compared to **18h**, while allyl analogue **18p** maintains the moderate AR potency seen in **18i**. Because they tended to have the best AR activity, the subsequent compounds focused more on analogues where R^1 = 2,2,2-trifluoroethyl. Compound **18q** has comparable activity to **18o**, while the isobutyl analogue **18r** shows more partial agonist activity. Phenyl analogues **18s** and **18t** switch activity to full antagonists. Hence, the R^2 position can be utilized to provide the full spectrum of AR activity from full agonist, partial agonist, and antagonist. To determine the effect of the other enantiomer, lead compound (±)-**18h** was prepared as a racemate and the distomer (*S*)-**18h** separated by chiral HPLC. Compound (*S*)-**18h** was less active than the (*R*)-enantiomer **18h**.

To determine the steroid hormone transactivation selectivity, selected compounds were evaluated in transactivation assays for progesterone receptor (PR), glucocorticoid receptor (GR), and mineralocorticoid receptor (MR) activity.[16] In no instance was agonist activity detected for PR, GR, or MR. No MR antagonist activity was detected, while weak PR and GR antagonist activity is observed (Table 2).

Table 2. Steroid hormone selectivity[a]

Compound	AR agonist		PR antagonist		GR antagonist	
	EC_{50} (nM)	Eff (%)	IC_{50} (nM)	Eff (%)	IC_{50} (nM)	Eff (%)
6a	5.4 ± 3.4	78 ± 4	nd	40	3200	53
16c	84 ± 14[b]	58 ± 6	nd	42	—	—
18h	1.0 ± 0.4	92 ± 5	520 ± 190	94 ± 1	1510 ± 80	98 ± 1

[a] Steroid hormone selectivity experimental results with at least three separate experiments in triplicate with SEM. If no SEM is noted, value is from a single determination.
[b] Antagonist activity with IC_{50} reported.

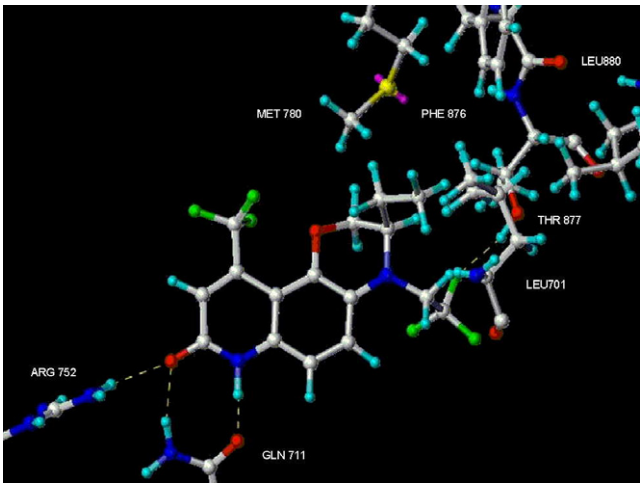


Figure 2. Compound **18h** with key contacts in the androgen binding site based on **4**-AR structure.

Modeling results based on the 4-AR structure[17,9] indicate that the fluorine atoms on the 2,2,2-trifluoroethyl substituent of **18h** interact with amino acid THR-877 which rationalizes the enhanced AR activity of the 2,2,2-trifluoroethyl substituent compared to the nonfluorinated side chains (Fig. 2).[18] The A-ring also forms bifurcated hydrogen bonds with ARG-752 and GLN-711. The ethyl group on the C-ring fills a lipophilic pocket delineated by MET-780, PHE-876, LEU-880, and LEU-701(omitted for clarity), which also provides increased binding interactions.

Compound (*R*)-**18h** was tested in order to demonstrate an in vivo proof of concept for the series. A 2-week castrated mature rat assay was utilized to assess the AR-mediated effect on organ weights.[9,10] The male sexual accessory organs, such as the ventral prostate (VP), are stimulated to grow and are maintained in size and function by the presence of endogenous androgens. This model is used to determine the androgen-dependent growth of the VP in mature castrated rats. Over-stimulation of the VP is undesirable because of its association with increased risk of prostatic disorders. In addition to the VP, the LA muscle demonstrates androgen-dependent growth.[19] The LA is a useful endpoint to evaluate the anabolic effects of the compounds in muscle. A compound that has full activity on LA but a reduced impact on VP represents a good profile for SARM activity. Figure 3 demonstrates that compound **18h** has good anabolic activity in this model, maintaining the LA weight at 10 mg/kg. At 100 mg/kg, the full weight of the VP is not maintained, suggesting that there is no over-stimulation of the VP. Compared to **T**,[9] compound **18h** has a muscle-selective profile based on the separation of the LA weight dose–response compared to the VP, and possesses at least 10-fold selectivity over that **T** in this maintenance model. Castrated mature rat assays can alternatively be conducted in a growth restoration model by delaying the administration of androgens after castration. Synthetic androgens, such as **1**, demonstrate significantly higher selectivity in a restoration model compared to this maintenance model.[20] This suggests

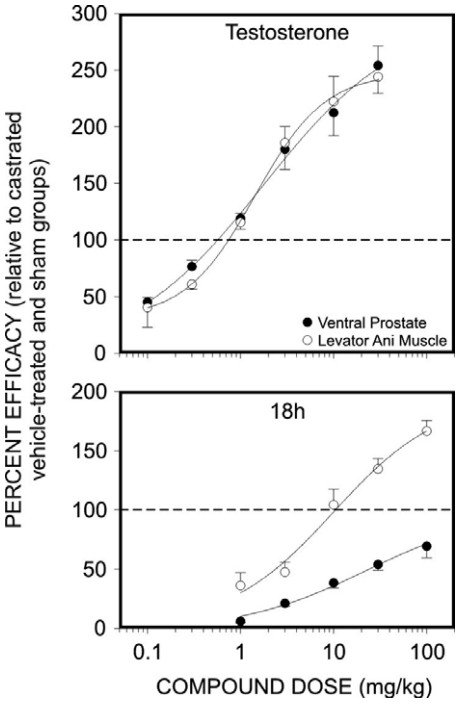


Figure 3. Compounds **18h** and **T** (as testosterone propionate)[9] in a 2-week castrated mature rat model. The dashed lines (—) represent intact levels.

the possibility that **18h** could potentially see selectivity enhancement if conducted in the restoration mode.

We have described a series of orally-active, nonsteroidal androgen receptor modulators based on an 8*H*-[1,4]-oxazino[2,3-*f*]quinolin-8-one scaffold. These compounds exhibit good potency in vitro, and compound **18h** has a favorable profile compared to **T** in an adult castrated rat in vivo model that measures androgen action, and hence can be regarded as a SARM.

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15. *Representative procedures*. All reaction mixtures were washed with brine, dried over $MgSO_4$, and purified by flash chromatography. Compound **13**: Diisopropyl azodicarboxylate (1.6 equiv) was added to **11** (1 equiv), **12** (1.6 equiv), PPh_3 (1.6 equiv), and *N*-methylmorpholine (10 equiv) in THF (0.1–0.2 M) at 0 °C, and after 5 min, the reaction mixture was stirred at rt for 2–16 h. The solution was poured into water, neutralized with 1.0 M HCl, extracted with EtOAc, and then washed with of 0.1 M HCl. Compound **13** (R^2 = Et) was prepared in 74% yield (hexanes:EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, 1H, J = 8.9), 7.55 (d, 1H, J = 8.9), 7.29 (s, 1H), 5.52 (septet, 1H, J = 6.3), 4.80 (br s, 1H), 4.06–3.90 (m, 3H), 1.91–1.81 (m, 1H), 1.71–1.59 (m, 1H), 1.46 (s, 9H), 1.41 (d, 6H, J = 6.2), 1.01 (t, 3H, J = 7.4). Compound **15**. Compound **13** in 1:1 CH_2Cl_2/TFA (0.1 M) was stirred at rt for 1 h, then poured into water, neutralized with 6 M NaOH, extracted with EtOAc, and then washed with saturated $NaHCO_3$ to afford **14** (CH_2Cl_2/MeOH). Compound **14** (1 equiv) in toluene (0.1–0.2 M) was added to (±)-BINAP (4–10 mol %), $Pd_2(dba)_3$ (2–5 mol%), and *t*-BuONa (1.4 equiv). The solution was heated at 90–100 °C for 6–24 h, poured into cold saturated NH_4Cl, and extracted with EtOAc. Compound **15** (R^2 = Et) was prepared in 63% yield (hexanes:EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (d, 1H, J = 8.8), 7.18 (s, 1H), 7.03 (d, 1H, J = 8.8), 5.47 (septet, 1H, J = 6.2), 4.36 (dd, 1H, J = 10.6, 2.9), 3.87 (dd, 1H, J = 10.4, 7.5), 3.83 (br s, 1H), 3.48–3.40 (m, 1H), 1.63–1.53 (m, 2H), 1.38 (d, 6H, J = 6.2), 1.06 (t, 3H, J = 7.4). Compound **18h** was prepared in 67% yield from **15** (R^2 = Et), and $CF_3CHOH(OEt)$ (0.028 mL, 0.235 mmol).[10] ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.47 (br s, 1H), 7.15 (s, 1H), 7.14 (d, 1H, J = 8.9), 7.02 (d, 1H, J = 8.9), 4.38 (d, 1H, J = 10.9), 3.98 (dd, 1H, J = 10.8, 2.4) 3.93–3.65 (m, 2H), 3.27–3.22 (m, 1H), 1.68–1.51(m, 2H), 0.98 (t, 3H, J = 7.5).
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