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Selective androgen receptor modulators based on a series of 7*H*-[1,4]oxazino[3,2-*g*]quinolin-7-ones with improved in vivo activity

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Abstract—Modification on a lead series of [1,4]oxazino[3,2-g]quinolin-7-ones at the 2-position led to selective androgen receptor modulators with improved in vivo activity. The most potent analog (−)-33a exhibited full maintenance of levator ani muscle at 3 mg/kg and reduced activity on ventral prostate weight in a 2-week orally-dosed and orchidectomized rat maintenance assay.

The androgen receptor (AR) is a member of the super family called nuclear receptors.[1] Androgens, which include testosterone (**T**) and dihydrotestosterone (**DHT**, Fig. 1), are the endogenous ligands that bind to AR. Androgens, when bound to AR, control the development, differentiation, and function of male reproductive and accessory sex tissues (such as seminal vesicle, prostate, and epididymis),[2] which are called androgenic effects. Some other tissues and organs such as skeletal muscle and bone are also under the influence of androgens,[2] which are anabolic effects. Other organs/tissues that are affected by androgens are skin, hair follicles, and brain.[2] The main mechanism of androgen action is to regulate gene expression through binding to the androgen receptor, changing the levels of specific proteins in cells, and controlling cell behavior.[3] Androgens can help both male and female patients with their frailty and osteoporosis. However, hormone replacement therapy (HRT) for prevention or treatment of osteoporosis has caused concerns about the safety of HRT in long term use, especially stimulation of the prostate for males and virilization for females.[2] Therefore, the concept of non-steroidal selective androgen receptor modulators (SARM) emerged as an attractive target for drug discovery.[4,5]

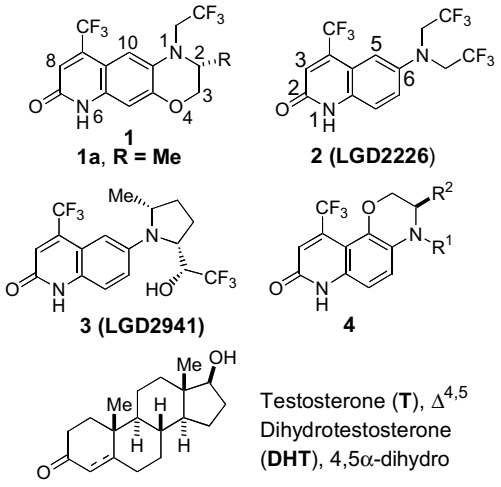

Figure 1. AR Ligands.

SARMs are expected to be orally available, potent and efficacious in anabolic effects while having reduced androgenic activity. Research on SARMs aimed to alleviate androgen deficiency symptoms has been reported.[6] The reported pharmacophores include tetrahydroquinoline,[7] hydantoin and hydantoin-derived compounds,[8] bicalutamide derivatives,[9] and our quinolinone derivatives.[10,11] More recently, we disclosed the discovery of our **LGD2226** (**2**, Fig. 1)[12] series, **LGD2941** (**3**),[13] **4** based on 8*H*-[1,4]oxazino[2,3-*f*]quinolin-8-ones[14] and **1** based on 7*H*-[1,4]oxazino[3,2-g]quinolin-7-ones.[15] With

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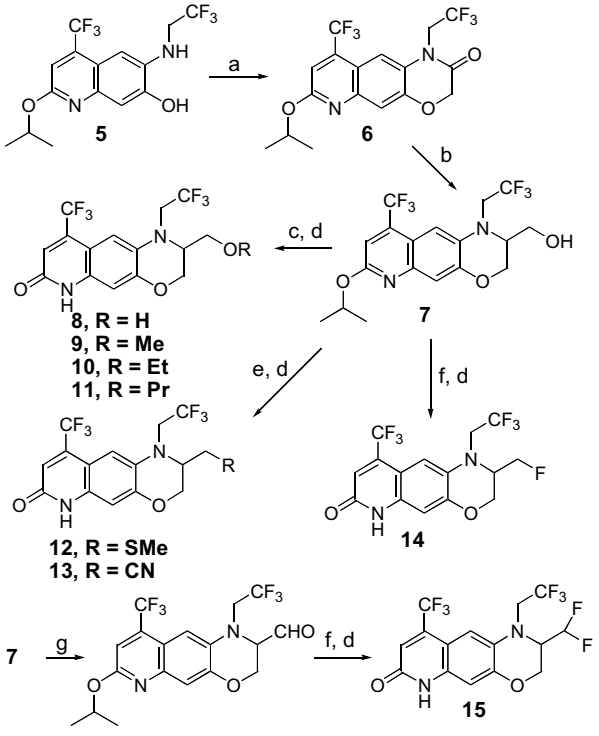
1a as the lead in this series, we performed additional SAR studies on the C-2 position. This effort led to compounds with tissue-selectivity, and significantly improved in vivo potency, which we report in this letter.

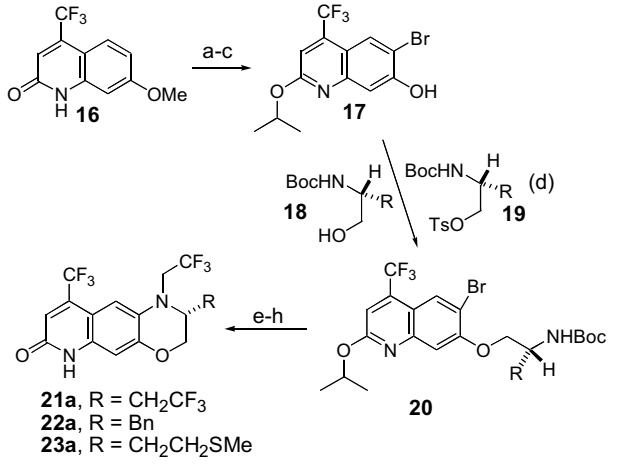
We previously[15] examined the SAR study at N-1 and C-3 position of **1**, and the optimal substituents proved to be 2,2,2-trifluoroethyl and hydrogen, respectively. This current effort concentrated at the C-2 position with the concept of introducing a broader array of functional groups, since conceptually, this position was amenable to the addition of a variety of different substituents. In our previous work, we developed an enantiospecific synthesis to prepare 2-alkyl-substituted analogs. However, this method was not suitable to prepare our desired targets, so we utilized different methods of the synthesis. Generally, racemates were prepared and selected compounds were separated into their individual enantiomers by chiral HPLC.[16] The preparation of several of the target compounds required common intermediate **7**, the synthesis of which started from **5**[17] (Scheme 1). Compound **5** was treated with methyl bromoacetate, yielding **6**, which under Tebbe reaction conditions, followed by hydroboration, afforded **7**. Compound **7** was alkylated with an alkyl iodide and sodium hydride, followed by deprotection under strong acidic conditions, leading to targets **8**–**11**. Alternatively, the hydroxyl group in **7** was mesylated, allowing for the nucleophilic replacement by sodium thiomethoxide and sodium cyanide, affording **12** and **13**, respectively, after deprotection. The hydroxyl group in **7** can directly be replaced by fluorine with DAST, producing **14** after deprotection. Finally, Moffatt–Swern oxidation of the primary hydroxyl group in **7** formed an aldehyde intermediate that was fluorinated in situ by DAST, leading to **15** after acidic hydrolysis.



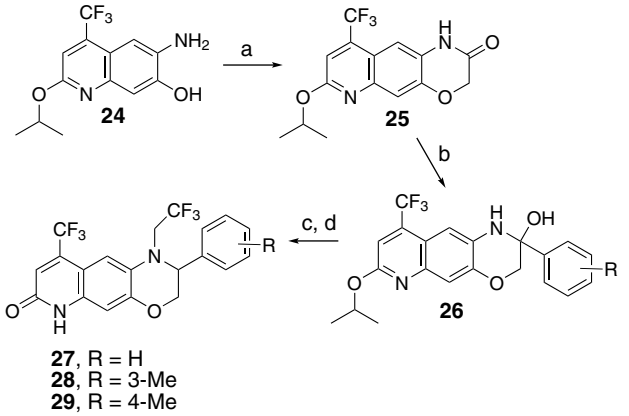
Scheme 1. Reagents and conditions: (a) methyl bromoacetate, K_2CO_3, DMF, 110 °C, 86%; (b) Tebbe reagent, THF, then BH_3, THF, H_2O_2, NaOH; (c) NaH, R-I, THF, 0 °C to rt; (d) HOAc:concd. HCl (3:1), 60–80 °C, 75–95%; (e) MsCl, NEt_3, ether, then NaR, DMF, 80 °C; (f) DAST, CH_2Cl_2; (g) Moffatt–Swern oxidation.

Compounds **21a**–**23a** were prepared (Scheme 2) in optically pure form starting from known compound **16**. Regioselective bromination and pyridone protection, followed by demethylation afforded hydroxyquinoline **17**. The hydroxyquinoline was alkylated by either the replacement of the tosylate group (R=CH_2CF_3) under basic conditions or utilizing Mitsunobu conditions (R=CH_2Ph and R=$CH_2CH_2SCH_3$) to afford the Boc protected aminoethers (**20**). The amine of **20** was unmasked with TFA, cyclized under Buchwald amination conditions,[18] and subsequently deprotected under acidic conditions to provide **21a**–**23a**.

Scheme 3 shows the preparation of aryl-substituted racemic analogs. Compound **25** was obtained by the

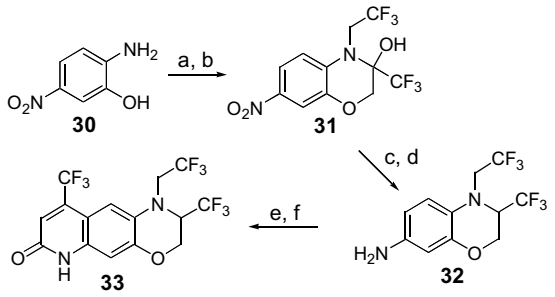


Scheme 2. Reagents and conditions: (a) NBS; (b) CsF, *i*-PrI (87% overall); (c) PhSH, NaH, DMF, 110 °C; (d) **19**, K_2CO_3, or **18**, diisopropylazodicarboxylate, PPh_3, *N*-methylmorpholine; (e) TFA, EtOAc, 60 °C; (f) Pd_2dba_3, KO*t*-Bu, BINAP, toluene, 90 °C; (g) $NaBH_4$, TFA, 50 °C; (h) HCl/HOAc, 85 °C.



Scheme 3. Reagents and conditions: (a) Methyl bromoacetate, K_2CO_3, DMF, rt, 52%; (b) ArBr, Mg, −78 to −45 °C; (c) $NaBH_3CN$, TFA, 50 °C, 60–80%; (d) HCl/HOAc, 110 °C.

treatment of **24**[15] with methyl bromoacetate and K_2CO_3, then was subjected to excess amount of an aryl Grignard reagent, affording **26**. The hydroxyl group was reductively removed with sodium cyanoborohydride in TFA, in which concomitant reductive alkylation of the nitrogen with trifluoroethyl group took place. After removal of the protecting isopropyl group, compounds **27**–**29** were obtained.

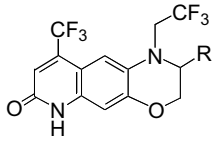


Scheme 4. Reagents and conditions: (a) $NaBH_4$,TFA, rt; (b) $BrCH_2COCF_3$, K_2CO_3, DMF; (c) $NaBH_4$, HOAc; (d) Pd/C, H_2, EtOAc; (e) $CF_3COCH_2CO_2Me$, toluene, reflux; (f) conc. H_2SO_4, 95 °C.

Scheme 4 describes the preparation of the racemic trifluoromethyl analog **33**. The treatment of aminophenol **30** with a reductive alkylation condition ($NaBH_4$ in TFA) selectively alkylated the amino group with a trifluoroethyl and then treatment with a bromoacetone under basic conditions selectively alkylated the phenolic oxygen to give intermediate **31**. Compound **31** was reduced by $NaBH_4$ to form the benzoxazine ring that was hydrogenated to provide aniline **32**. A two step Knorr cyclization reaction of aniline **32** afforded final compound **33**.

The compounds synthesized were evaluated in a transcriptional activation assay with hAR in mammalian cell background (CV-1).[10] The results from this assay and a competitive receptor binding assay[15] are shown in Table 1. Consistent with the results of the analogs of structure **1**,[15] most of the AR agonist modulating activity and

Table 1. Cotransfection (hAR in CV-1 cells) and competitive binding data for the new analogs and reference compounds



Compound	R	CV-1 agonist		CV-1 antagonist		AR binding[d]	Optical rotation[e]
		Efficacy[b] (%)	EC_{50}[a] (nM)	Efficacy[c] (%)	IC_{50}[a] (nM)	K_i[a] (nM)	
(−)-(*R*)-**8a**	CH_2OH	92 ± 11	11 ± 3	24 ± 14	60 ± 0	15 ± 3	−62.9
(+)-(*S*)-**8b**	CH_2OH	67 ± 7	6.6 ± 0.6	39 ± 17	165 ± 120	22	+60.0
(−)-(*R*)-**9a**	CH_2OMe	71 ± 5	2.0 ± 0.4	24	—	9.4	−43.8
(+)-(*S*)-**9b**	CH_2OMe	27 ± 5	77 ± 34	67 ± 11	17 ± 9	109	+40.0
(±)-**10**	CH_2OEt	61	3.2	—	—	na	
(±)-**11**	CH_2OPr	111	4.2	—	—	na	
(±)-**12**	CH_2SMe	66 ± 30	28 ± 12	23 ± 18	25	na	
(±)-**13**	CH_2CN	106 ± 24	30 ± 18	31 ± 31	—	18	
(−)-(*R*)-**14a**	CH_2F	125 ± 14	3.8 ± 2.0	33 ± 9	—	2.6 ± 0.3	−67.2
(+)-(*S*)-**14b**	CH_2F	83 ± 11	35 ± 12	30 ± 9	199	32 ± 4	+66.2
(±)-**15**	CHF_2	127 ± 19	1.3 ± 0.2	29 ± 13	—	6.6 ± 0.0	
(−)-(*R*)-**21a**	CH_2CF_3	51 ± 3	23 ± 6	—	—	18	na
(−)-(*R*)-**22a**	CH_2Ph	12 ± 3	—	83 ± 3	51 ± 12	na	na
(−)-(*R*)-**23a**	CH_2CH_2SMe	89 ± 0.3	23 ± 17	38 ± 6	—	2.4 ± 0.0	na
(−)-(*R*)-**27a**	Ph	121 ± 16	5.3 ± 0.9	42 ± 11	—	1.3 ± 0	na
(+)-(*S*)-**27b**	Ph	13	—	94	340	150	na
(−)-(*R*)-**28a**	*m*-Tol	86 ± 10	19 ± 8	33 ± 11	—	1.1 ± 0.2	−38.7
(+)-(*S*)-**28b**	*m*-Tol	—	—	91 ± 7	574 ± 156	180	+38.4
(−)-(*R*)-**29a**	*p*-Tol	105	36	41	—	na	na
(+)-(*S*)-**29b**	*p*-Tol	—	—	86	44	na	na
(±)-**33**	CF_3	104 ± 7	5.9 ± 1.2	—	—	8.3 ± 1.6	
(−)-(*R*)-**33a**	CF_3	87 ± 10	2.0 ± 0.6	31 ± 6	—	5.9 ± 2.2	−60.4
(+)-(*S*)-**33b**	CF_3	23 ± 13	42 ± 24	64 ± 11	34 ± 17	281 ± 73	+62.7
(−)-(*R*)-**1a**	Me	82 ± 5	1.1 ± 0.2	28 ± 7	70 ± 6	0.9 ± 0.1	−81.7
DHT		100 ± 0.0	5.7 ± 0.1	—	—	0.9 ± 0.1	
Bicalutamide		—	—	89 ± 9	162 ± 99	151 ± 36	

[a] Values with standard errors represent the mean value of at least 2 separate experiments with triplicate determinations. '—' indicates not active (an efficacy of <20% and/or a potency of >10000 nM). 'na' means not available.

[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.

[d] Competitive binding assay conducted as described in Ref. 15.

[e] All optical rotations were measured in ethanol at 589 nm with sodium arc lamps at 20 °C. The sample concentration ranges from 2.45 to 2.90 mg/ml.

binding affinity reside on the enantiomers with the *R*-configuration at the C-2 position, although not all of the pairs of enantiomers were prepared. The enantiomers with the *S*-configuration tend to have weaker agonist activity or more AR antagonist activity in our assays. As a result, the following SAR discussion by using the data from both enantiomers and racemates should not change the conclusions for the agonist activities.

SAR study on C-2 position (Table 1) suggested that the binding pocket in this region is rather large, tolerating a variety of functional groups, which is different from the paralleling bicyclic series exemplified by **LGD2226**. This conclusion is supported by the following three observations. First, the compounds are still potent and efficacious AR agonist modulators when larger hydroxyl (**8a**) and methoxyl (**9a**) groups are attached to the parent compound **1a** with *R* configuration at C-2 substitution. Tests on the racemic mixtures with even larger ethoxyl (**10**) and propoxyl (**11**) attached revealed that these compounds were still potent and efficacious. Replacing this C-2 side chain with sulfur containing chain structure at approximately equivalent length (**23a** and **12**, respectively) led to about 10-fold reduction in agonist potency relative to their oxygen containing analogs (**10** and **9a**). The same potency reduction was observed in comparison of compound **13** and compound **9a**. The second supporting evidence for steric tolerance off C-2 site is that when a large aromatic group was attached directly at C-2 carbon (compounds **27a**, **28a**, and **29a)**, they still exhibited very good agonist efficacy, potency, and receptor binding affinity. However, the binding pocket appeared to reach its limit in steric tolerance when one methylene is placed between the newly introduced aromatic ring and the linear tricyclic structure (compound **22a**). This compound turned into an antagonist that is equally efficacious but more potent than bicalutamide, which is a rather large change for a small structural modification. When the same modification was made to **33a**, the resulting compound **21a** showed significant reduction in agonist efficacy, potency, and binding activity.

While the SAR of this linear tricyclic series was ongoing, the studies on our **LGD2226** series[12] established the importance of an additional halomethyl substituent at the 6-amino position, especially 2,2,2-trifluoroethyl, as being critical to increasing the in vivo activity of our SARMs. This suggested that the trifluoromethyl group on C-2 position would be an attractive target to increase in vivo activity of the current series. Indeed, the SAR results showed that the trifluoromethyl group did lead to a compound (**33a**) that is equally potent and efficacious in vitro as lead compound **1a**. Changing the number of fluorine atoms in this group (compounds **14** and **15**) appeared to increase the agonist efficacy without affecting its potency and binding. To our advantage, compound **33a** demonstrated half life of 100 min in rat liver microsomal study at 5 μM, while that of **14a** is only 34 min. The difference suggested that additional fluorine atoms led to more metabolic stability.

In order to evaluate the in vivo activity, compound **14a** and **33a** were orally dosed to SD rats in a 2-week castrated rat maintenance bioassay.[12a,15] The ventral prostate (VP) is an androgen-dependent male sexual accessory organ, and is an endpoint to evaluate androgenic side effects of our non-steroidal SARMs. Over stimulation of the prostate for an androgen is not desired because of the risk of prostatic disease. The levator ani muscle (LA) is a skeletal muscle that also demonstrates androgen-dependent growth.[19] The LA weight is utilized as an endpoint to determine the anabolic effects. The eugonadal levels on both the androgenic side effect (VP) and the anabolic (LA) endpoints were maintained when treated with 1.0 mg/kg testosterone propionate (TP) (Fig. 2). Hence, testosterone is considered to have no tissue selectivity in this model, as can be observed by the overlap of the VP and LA dose-response curves. In contrast, the VP dose response curves for both **14a** and **33a** are right-shifted compared to the LA. Compound **14a** demonstrates full efficacy in LA at 10 mg/kg, while **33a** is fully efficacious at 3 mg/kg. In comparison, compound **1a** is less potent in this assay,[15] maintaining the LA to the intact level at a dose of 100 mg/kg. Hence, **33a** is significantly more potent than **1a**, although the muscle-prostate selectivity profile is similar, which indicates that the activity enhancement may be the result of increased metabolic stability.

These new SARMs demonstrate good steroid hormone binding selectivity, just as most other SARMs in our quinolinone series.[12–15] Selected compounds were evaluated in the progesterone, glucocorticoid, and mineralocorticoid receptor binding assays (PR, GR, and MR, respectively)[20] and no significant cross-reactivity was observed.

In conclusion, we have discovered tissue and receptor selective, and orally available SARMs with improved in vivo potency based on 1-(2,2,2-trifluoroethyl)-7*H*-[1,4]oxazino[3,2-*g*]quinolin-7-ones.

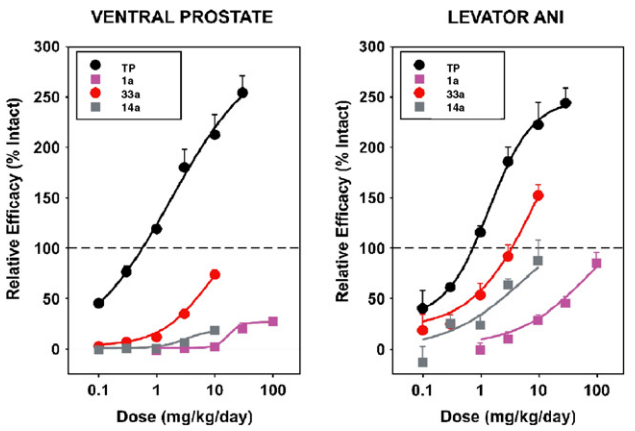


Figure 2. Effects of compounds **14a** and **33a** and testosterone propionate treatment on levator ani muscle and ventral prostate weights in a 2-week orchidectomized rat maintenance assay. 100% identifies intact animals (sham), whereas 0% denotes levels for vehicle-treated animals. Testosterone propionate was dosed subcutaneously because it is rapidly metabolized when dosed orally.

Acknowledgments

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