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Discovery of an androgen receptor modulator pharmacophore based on 2-quinolinones

Arjan van Oeveren, Barbara A. Pio, Christopher M. Tegley, Robert I. Higuchi, Min Wu, Todd K. Jones, Keith B. Marschke, Andrés Negro-Vilar and Lin Zhi*

Discovery Research, Ligand Pharmaceuticals, 10275 Science Center Drive, San Diego, CA 92121, USA

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Abstract—A series of alkylamino-2-quinolinone compounds (**3**) was discovered as androgen receptor modulators based on an early linear tricyclic quinoline pharmacophore (**1**). The series demonstrated selective high binding affinity to androgen receptor and potent receptor modulating activities in the cotransfection assays.

The androgen receptor (AR) is a transcription factor that androgens depend upon to play their diverse and complex physiological and pathophysiological roles. As a validated drug target, many therapies have been developed based on AR actions. Numerous synthetic steroidal androgens were developed decades ago to mimic the activity of natural hormones, testosterone and dihydrotestosterone (DHT), with improved potency or oral bioavailability in the absence of understanding the mechanism of action at the molecular level. Despite many potential risks, the steroidal androgens have been used to treat many diseases associated with androgen deficiency or have been abused as anabolic agents.[1,2] Separation of the beneficial activities of androgens, such as on bone and muscle, and the potential risks on prostate has been the focus of multiple research groups in recent years to develop new androgens, named selective androgen receptor modulators (SARM).[3–7] On the side of blocking AR actions, bicalutamide has been used to treat prostate carcinoma by binding to and antagonizing AR.[8] Development of better and safer antagonists, named selective AR antagonists, has also been attempted[9–11] (Fig. 1).

Based on our AR antagonist pharmacophore (**1**),[12] we successfully developed AR agonists, such as molecules of structure **2**, by modifying the substitution pattern

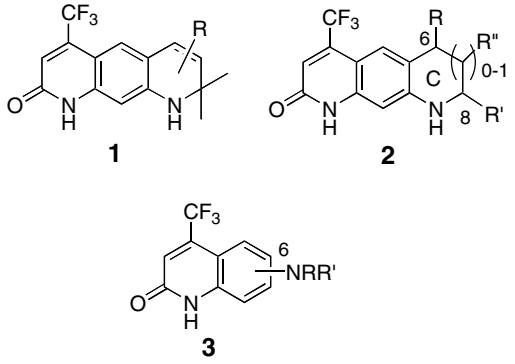

Figure 1. Nonsteroidal AR modulators: (**1**) AR antagonists; (**2**) AR agonists; and (**3**) new compounds.

on the C-ring.[13–16] It was found that the bisalkyl groups at the 8 position were responsible for the AR antagonist activity and a small alkyl group at the 6 or 7 positions or combinations at 6- and 7- or 7- and 8-positions were good for AR agonist activities. In the optimization of the AR lead pharmacophore, the SAR studies were encumbered due to the nonconvergent synthetic sequence of many analogs of the series as illustrated by compound **6** in Scheme 1. The tetrahydroquinoline **4** related intermediates were prepared from aniline in four to six steps to setup targeted substitution pattern and then were nitrated to provide compound **5** after hydrogenation. The Knorr cyclization afforded target compound **6**. In addition to the linear synthesis of the C-ring modified analogs and relatively low yield of the

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* Corresponding author. Tel.: +1 858 550 7663; fax: +1 858 550 7249; e-mail: lzhi@ligand.com

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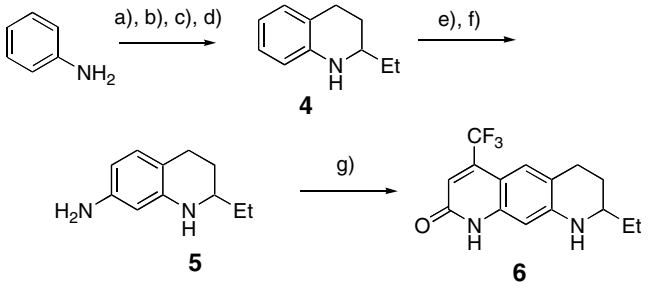
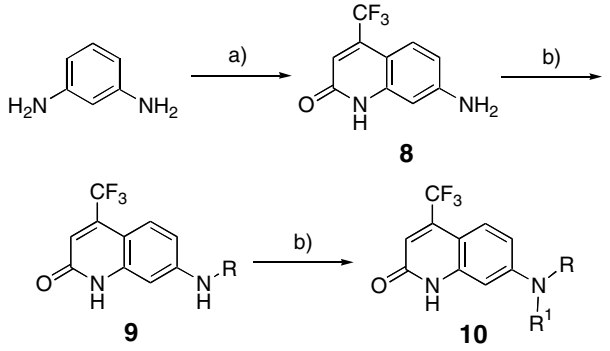


Scheme 1. Reagents and conditions: (a) 2-pentenoic acid, toluene, reflux 20 h (72%); (b) neat PPA, 110 °C, 6 h (43%); (c) $NaBH_4$, MeOH, rt, 3 h (80%); (d) Et_3SiH–BF_3-OEt_2, CH_2Cl_2, 50 °C, 15 h (71%); (e) HNO_3, H_2SO_4, 0 °C, 20 min (85%); (f) H_2, Pd/C, EtOAc, rt, 15 h (92%); (g) $CF_3COCH_2CO_2Et$, $ZnCl_2$, EtOH, reflux, 8 h (30%).

Knorr reaction at the last step, there are three potential stereogenic centers of the molecules to be addressed for any scale-ups. In the process of the synthesis, compound **7** was isolated as a contaminant and was found to be a reasonable AR antagonist in our assays. The unexpected result prompted us to think about the possibility of opening the C-ring of the tricyclic template to simplify the skeleton and the synthesis (Fig. 2).

A number of the 7-alkylamino-2-quinolinone analogs were prepared by a much more efficient synthetic route as described in Scheme 2. Knorr reaction of 1,3-phenylenediamine and the trifluoromethylketo ester using *p*-toluenesulfonic acid as a catalyst provided the 7-amino-2-quinolinone **8** in good yield. Reductive alkylation of intermediate **8** afforded analogs of structure **9** and the second alkylation gave bisalkylated analogs of structure **10**.[17] Compounds **9g** and **9h** were prepared differently to install the quaternary carbon next to the nitrogen to mimic the 8-gemdimethyl at the C-ring of pharmacophore **1**. In Scheme 3, quinoline **8a** was isolated in the synthesis of compound **8** as a minor byproduct and was alkylated with the special amide bromide[18] to facilitate the formation of the nitrogen quaternary carbon bond followed by methylation to give **8b**. Hydrolysis of compound **8b** to the quinolinone intermediate and reduction of the amide to an aldehyde followed by Wittig olefination provided compound **9h**. Hydrogenation afforded compound **9g**. The binding affinity of the compounds to human AR and their functional activity were measured by a competitive whole cell binding assay and the cotransfection assay in CV-1 cells.[19] The results are summarized in Table 1. We used the functional assay results to drive the SAR studies and the binding affinity to assure the interaction with AR.

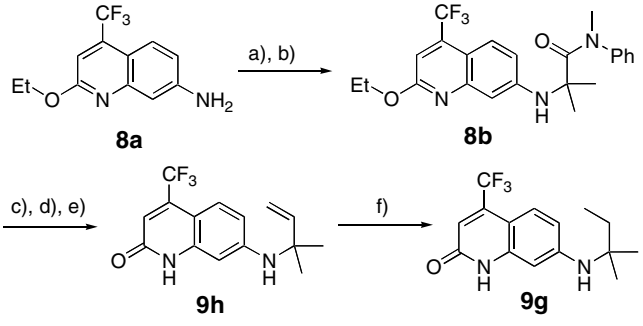
Figure 2. Development of the alkylamino-2-quinolinone series.



Scheme 2. Reagents and conditions: (a) $CF_3COCH_2CO_2Et$, TsOH, EtOH, reflux, 8 h (75%); (b) an aldehyde or a ketone, $NaB(CN)H_3$, AcOH, rt, 15 h (50–95%).

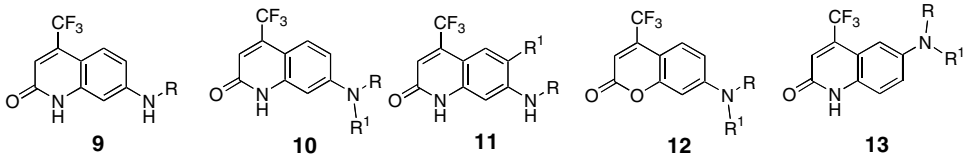
Compound **6** was prepared as a structural isomer of compound **1** reported previously from the linear tricyclic tetrahydroquinoline series[15] and tested as a racemate. The 7-alkylamino-2-quinolinone lead compound **7** demonstrated AR antagonist activity and binding affinity similar to those of compounds **1** and **6**, which indicates that breaking the C-ring of the tricyclic structure does not have significant impact on the activities of the series on AR. Additional analogs of compound **7** provide consistent results to further support the hypothesis. The unsubstituted aminoquinolone compound **8** had weak binding to AR and some partial activity in the functional assay. The monoalkylamine analogs (compounds **9a**–**9h**) showed good antagonist activities comparable to reference compound bicalutamide in the cotransfection assay. The smaller alkyl groups apparently showed better potency than those of bulkier analogs and the best example is the increased IC_{50} of the cycloalkyl analogs from **9i** (cyclobutyl), **9j** (cyclopentyl), to **9k** (cyclohexyl). The fluoroalkyl analogs (**9l** and **9m**) showed similar activity as those of alkyl analogs. However, the trifluoroacetamido analog (**9n**) had no activity in the testing range. The bisalkylamino compounds (**10a**–**10d**) were generally weaker antagonists in the assays.

Compounds of structure **11** are analogs of different dissection of the C-ring of pharmacophore **1** and were prepared by the chemistry similar to that of structure **9**. As



Scheme 3. Reagents and conditions: (a) $BrC(CH_3)_2CONHPh$, NaH, THF, rt, 1 h (75%); (b) NaH, MeI, THF, rt, 2 h (95%); (c) 57% aqueous HI, 40 °C, 3 h (50%); (d) DIBAL, THF, −78 to −50 °C, 1 h (40%); (e) Ph_3PCH_3Br, $NaN(SiMe_3)_2$, THF, rt, 1 h (85%); (f) H_2, Pd/C, EtOAc, rt, 15 h (90%).

Table 1. Cotransfection and competitive binding data for the new analogs and reference compounds[a]



Compound	R	R^1	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)
1	H		—	—	27 ± 5	74 ± 2	73 ± 11
6 (±)			—	—	14 ± 3	55 ± 5	11 ± 3
7			—	—	32 ± 7	86 ± 1	37 ± 19
8			1300	58	28 ± 5	53 ± 9	407
9a	CH_3		—	—	30 ± 7	79 ± 5	83 ± 7
9b	CH_2CH_3		—	—	35 ± 10	77 ± 2	29 ± 9
9c	$CH_2CH_2CH_2CH_3$		—	—	67 ± 11	86 ± 2	50 ± 7
9d	$CH(CH_3)_2$		—	—	56 ± 10	87 ± 1	79 ± 27
9e	$CH_2CH(CH_3)_2$		—	—	74 ± 1	86 ± 2	65 ± 23
9f	$CH_2C(CH_3)_3$		—	—	98 ± 15	88 ± 2	134 ± 29
9g	$C(CH_3)_2CH_2CH_3$		—	—	209 ± 6	93 ± 2	110 ± 3
9h	$C(CH_3)_2CH{=}CH_2$		—	—	122 ± 30	92 ± 1	118 ± 46
9i	Cyclobutyl		—	—	43 ± 9	89 ± 2	161
9j	Cyclopentyl		—	—	149	89	1000
9k	Cyclohexyl		—	—	507	90	1000
9l	CH_2CF_3		905 ± 154	29 ± 0	21 ± 5	61 ± 4	15 ± 3
9m	$CH_2CF_2CF_3$		—	—	58 ± 9	76 ± 2	36 ± 14
9n	$COCF_3$		—	—	—	—	—
9o	CH_2Ph		—	—	294 ± 21	94 ± 1	163 ± 77
10a	CH_3	CH_3	—	—	1160	88	761
10b	CH_2CH_3	CH_2CH_3	—	—	591	90	463
10c	CH_2CH_3	CH_2CF_3	—	—	42	79	30
10d	$CH_2CH_2CH_3$	CH_2CF_3	—	—	549 ± 452	89 ± 2	241
11a	H	CH_3	—	—	76 ± 7	75 ± 1	30
11b	H	CH_2CH_3	593 ± 436	27 ± 1	23 ± 0	51 ± 3	20 ± 4
11c	H	$CH_2CH_2CH_3$	83 ± 24	26 ± 2	19	34 ± 14	9 ± 2
11d (±)	H	$CH(CH_3)CH_2CH_3$	65 ± 16	40 ± 8	14 ± 0	23 ± 3	13 ± 2
11e	$CH_2CH_2CH_3$	CH_3	—	—	674	93	641 ± 359
11f	$CH_2CH(CH_3)_2$	CH_3	—	—	301 ± 97	83 ± 5	650 ± 350
11g	CH_3	CH_2CH_3	—	—	504	98	869
11h	CH_2CF_3	CH_2CH_3	—	—	216 ± 54	91 ± 4	157 ± 153
12a	CH_2CF_3	H	—	—	50 ± 7	70 ± 4	na
12b	CH_2CF_3	CH_2CF_3	73 ± 31	21 ± 4	63 ± 23	63 ± 4	na
13a	H	H	—	—	26	28 ± 7	—
13b	CH_2CH_3	H	—	—	111	84	na
13c	CH_2CF_3	H	108 ± 36	24 ± 10	18 ± 12	39 ± 13	16
13d	$CH_2CF_2CF_3$	H	246	89	17 ± 5	63 ± 2	78
13e	Cyclopentyl	H	—	—	42 ± 8	82 ± 2	74
13f	$CH_2CH(CH_3)_2$	H	404	21	22 ± 18	61 ± 7	59
13g	$CH_2C(CH_3)_3$	H	—	—	27	72	56
13h	$COCF_3$	H	—	—	—	—	—
13i (LGD2226)	CH_2CF_3	CH_2CF_3	0.20 ± 0.02	95 ± 2	—	—	1.5
DHT			5.1 ± 0.1	100	—	—	0.20 ± 0.02
Bicalutamide			—	—	162 ± 99	85 ± 9	151 ± 36

[a] Values with standard errors represent the mean value of at least two separate experiments with triplicate determinations. '—' indicates not active (an efficacy of <20% and/or a potency of >10,000 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.

size increases of the 6-alkyl group, the 7-amino analog functional activities shifted from AR antagonism toward partials (compounds **11a**–**11d**). Monoalkylation of the amino group does not make better AR modulators (compounds **11e**–**11h**). A couple of 7-alkylamino-coumarin analogs were also prepared similarly and their functional activities are in parallel with the 2-quinolones.

The 6-alkylamino compounds (**13**) were synthesized in a similar fashion as described previously[20] and have a very intriguing SAR. The parent compound **13a** did not show

much AR related activity and the monoalkylamino analogs (**13b**–**13g**) demonstrated good AR antagonist activities similar to their 7-alkylamino counterparts. In addition, some of the analogs showed partial agonist activity, especially for compound **13d**. Once the 6-amino group is bisalkylated, the series becomes a highly potent AR agonist pharmacophore. Compound **13i** (LGD2226) as a representative analog of the series has high specific binding affinity to AR and demonstrated functional activity in the cotransfection assay more potent than DHT. In additional in vitro and in vivo assays, compound **13i** was characterized as an orally available highly potent tissue-selective AR modulator.[20,21]

A group of the representative compounds (**9a**, **9b**, **9d**, **9l**, **9m**, **10c**, **11b**, **11c**, **13c**, and **13e**) were tested in other related steroidal hormone receptor cotransfection assays in both agonist and antagonist modes to check their receptor selectivity. All of the compounds showed more than 100-fold separation from the modulating activities of estrogen, glucocorticoid, and mineralocorticoid receptors, and more than 10-fold separation from the progesterone receptor activities.

In summary, the alkylamino-2-quinolinone series was developed as an AR modulator template from the linear tricyclic pharmacophore. The series demonstrated many synthesis-friendly features, which significantly facilitated the development of the SAR. Several new antagonist analogs were assessed in a typical rodent model and unfortunately no antiandrogenic activity was observed due to the significantly lower exposure relative to that of bicalutamide (data not shown). The AR agonists of the series did show excellent in vivo activity and tissue selectivity. More detailed SAR studies around the 6-bisalkylamino AR agonist analogs are discussed in the following manuscript.[22]

Acknowledgment

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17. Preparation of **9l** as an example: 1,3-Phenylenediamine (5.4 g, 50 mmol) and $CF_3COCH_2CO_2Et$ (11 g, 60 mmol) in EtOH (100 mL) were heated at reflux overnight to give rise to a yellow slurry. P-TsOH (0.19 g, 1.0 mmol) was added and the reaction mixture was stirred at reflux for additional 24 h. The reaction mixture was cooled to room temperature and filtration afforded compound **8** as a yellowish solid (8.5 g, 75%): ^{1}H NMR (400 MHz, acetone-d_6) 10.91 (br s, 1H), 7.47 (dq, J = 6.7 and 2.4, 1H), 6.70 (dd, J = 6.7 and 2.2, 1H), 6.65 (d, J = 2.2, 1H), 6.50 (s, 1H), 5.65 (br s, 2H). A solution of compound **8** (100 mg, 0.45 mmol) in TFA (3 mL) was treated with $NaBH_4$ and the mixture was heated at 60 °C for 4 h. The mixture was quenched with 10% NaOH and extracted with EtOAc. Chromatography provided compound **9l** (45 mg, 33%) as a yellow solid: mp 238–239 °C, ^{1}H NMR (400 MHz, acetone-d_6) 10.95 (br s, 1H), 7.56 (dq, J = 9.0 and 1.5, 1H), 6.86 (dd, J = 9.0 and 2.1, 1H), 6.80 (d, J = 2.1, 1H), 6.60 (s, 1H), 6.50 (br s, 1H), 4.05 (m, 2H). Anal. ($C_{12}H_8F_6N_2O$) C, 46.46; H, 2.60; N, 9.03. Found: C, 46.36; H, 2.55; N, 8.89.
18. The *N*-phenyl-2-bromo-2-methylpropionamide was prepared from aniline and 2-bromo-2-methylpropionic bromide and a highly active alpha lactam may be involved in the alkylation reaction.
19. Detailed assay conditions were described previously in our early publications.
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