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Novel selective androgen receptor modulators: SAR studies on 6-bisalkylamino-2-quinolinones

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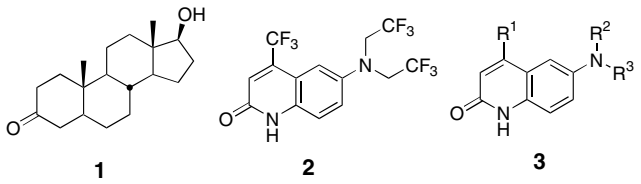
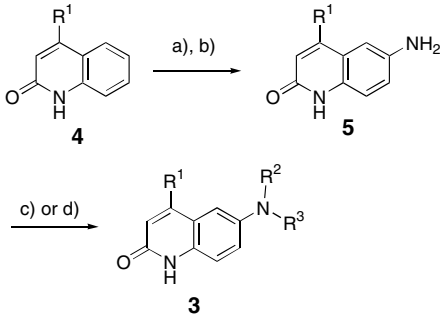
Abstract—A series of selective androgen receptor modulators (SARMs) with a wide spectrum of receptor modulating activities was developed based on optimization of the 4-substituted 6-bisalkylamino-2-quinolinones (**3**). Significance of the trifluoromethyl group on the side chains and its interactions with amino acid residues within the androgen receptor (AR) ligand binding domain are discussed. A representative analog (**9**) was tested orally in a rodent model of hypogonadism and demonstrated desirable tissue selectivity.

Testosterone, as a male hormone, contributes mainly to the development and function of males, and has been used in multiple preparations for the treatment of many forms of androgen deficiencies.[1] In addition to the genomic functions of testosterone through binding and activation of the androgen receptor (AR), the biological activity of the hormone is directly related to its two major metabolites. Testosterone is reduced to a more potent androgen, dihydrotestosterone (DHT, **1**, Fig. 1), by 5-alpha reductases distributed primarily in human prostate and skin tissues. In addition, testosterone is aromatized to a female hormone, estradiol, which plays an important role in bone and CNS homeostasis.[2,3] Reductase inhibitors, such as finasteride, have been therapeutically used for the treatment of benign prostatic hypertrophy and androgenetic alopecia. These drugs reduce androgen effects on prostate and hair follicles without significantly altering androgen action on other tissues.[4] In principle, androgens that exhibit affinity similar to testosterone for the androgen receptor which are not modified by the reductase enzyme should behave in a tissue selective fashion. Recently, many nonsteroidal androgens have been discovered that have demonstrated



1 2 3

Figure 1. AR modulators: (**1**) DHT; (**2**) LGD2226; (**3**) new SARM series.



Scheme 1. Reagents and conditions: (a) HNO_3, H_2SO_4, 0 °C, 20 min (65–90%); (b) H_2, Pd/C, EtOAc or DMF, rt, 6–15 h (80–95%); (c) an aldehyde or a ketone, $NaB(CN)H_3$, AcOH, rt, 15 h (50–95%); (d) TFA, $NaBH_4$, rt to 50 °C (40–90%).

Keywords: Androgen receptor; SARM; 2-Quinolinone; AR agonist.
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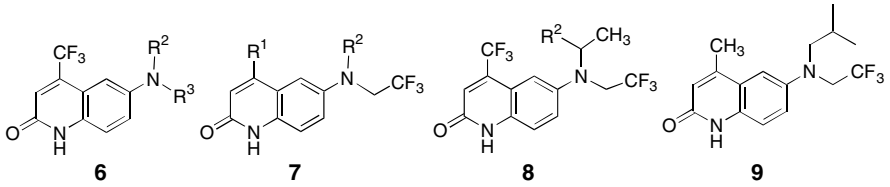
tissue selective AR agonist activity.[5–7] The nonreducible properties of the molecules may partially contribute to the observed tissue selectivity, but potentially differential cofactor recruitment may be also be responsible for the observed effects. Recently, we reported the discovery of a SARM, LGD2226 (**2**), and the development of the 2-quinolinone AR modulator pharmacophore.[8–10] Here we report additional SAR studies on the 2-quinolinone pharmacophore (**3**) and the importance of the trifluoromethyl group to enhance interaction of the modulators with specific amino acid residues within the AR ligand binding pocket.

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



6 **7** **8** **9**

Compound	R^1	R^2	R^3	hAR agonist EC_{50} (nM)	hAR agonist[b] Eff (%)	hAR antagonist IC_{50} (nM)	hAR antagonist[c] (Eff. %)	hAR binding K_i (nM)
2 (LGD2226)				0.20 ± 0.02	95 ± 2	—	—	1.5
6a		CH_2CH_3	CH_2CH_3	2.1 ± 0.3	94 ± 19	—	—	4.4
6b		$CH_2CH_2CH_3$	$CH_2CH_2CH_3$	1.5 ± 0.2	72 ± 5	—	—	64
6c		CH_2CHF_2	CH_2CHF_2	0.4 ± 0.1	74 ± 7	—	—	0.5
6d		CH_2CClF_2	CH_2CHF_2	0.4 ± 0	110 ± 7	—	—	2.3
6e		CH_3	CH_2CF_3	9.8 ± 3.6	59 ± 12	—	—	9.0
6f		CH_2CH_3	CH_2CF_3	0.4 ± 0.1	107 ± 5	—	—	7.1
6g		$CH_2CH_2CH_3$	CH_2CF_3	1.3 ± 0.4	88 ± 4	—	—	12
6h		$CH_2CH_2CH_2CH_3$	CH_2CF_3	6.8 ± 0.8	69 ± 6	—	—	33
6i		$CH(CH_3)_2$	CH_2CF_3	0.9 ± 0.3	107 ± 17	—	—	2.9
6j		$CH_2C(CH_3)_3$	CH_2CF_3	1.8 ± 0.5	81 ± 10	—	—	12
6k		$CH_2CH_2C(CH_3)_3$	CH_2CF_3	81 ± 32	69 ± 17	—	—	243
6l (±)		$CH(CH_3)CH_2CH_3$	CH_2CF_3	5.9 ± 4.1	86 ± 16	—	—	29
6m		CH_2CHF_2	CH_2CF_3	0.2 ± 0.1	84 ± 7	—	—	1.7
6n		CH_2CH_2Cl	CH_2CF_3	0.5 ± 0.1	88 ± 14	—	—	2.6
6o		CH_2CHCl_2	CH_2CF_3	1.6 ± 0.8	67 ± 7	—	—	6.0 ± 1.1
6p		$CH_2CH_2CF_3$	CH_2CF_3	46 ± 15	102 ± 6	—	—	116
6q		$CH_2CF_2CF_3$	CH_2CF_3	2.3 ± 0.5	94 ± 6	—	—	31
6r		CH_2CH_2OH	CH_2CF_3	7.9 ± 5.3	125 ± 27	—	—	6.6 ± 2.8 [d]
6s		CH_2Ph	CH_2CF_3	27	71	—	—	79
6t		CH_2(2-thienyl)	CH_2CF_3	12 ± 2	85 ± 9	—		61
6u		$CH_2CO_2CH_2CH_3$	CH_2CF_3	20 ± 4	59 ± 5	19	41	19[d]
6v		$COCF_3$	CH_2CF_3	22 ± 13	38 ± 4	4.1 ± 0	56 ± 3	1000
6w		CH_2(3-furyl)	CH_2CH_3	2.8	49	—	—	14
6x (±)		$CH(CH_2)COCH_3$	CH_2CH_3	5.8 ± 1.3	69 ± 5	—	—	3.1
7a	H	CH_2CF_3		57 ± 15	68 ± 8	1.0 ± 0	39 ± 9	24
7b	CH_3	CH_2CF_3		0.8 ± 0.3	93 ± 5	—	—	1.4
7c	CH_2CH_3	CH_2CF_3		12 ± 6	89 ± 6	—	—	5.4
7d	$CH_2CH_2CH_3$	CH_2CF_3		—	—	36 ± 15	83 ± 1	42[d]
7e	$CH(CH_3)_2$	CH_2CF_3		9.1	26	2.0	41	21
7f	CHF_2	CH_2CF_3		0.5 ± 0.1	97 ± 4	—	—	0.4
7g	Cl	CH_2CF_3		0.89 ± 0.58	115 ± 13	—	—	0.5
7h	Br	CH_2CF_3		1.0 ± 0.0	125 ± 22	—	—	0.5[d]
7i	OCH_3	CH_2CF_3		35 ± 16	34 ± 4	15 ± 0	24 ± 11	23
7j	OH	CH_2CF_3		83 ± 34	43 ± 12	7.0 ± 0	43 ± 12	1000
8a (*R*)		CF_3		2.3 ± 0.7	86 ± 5	—	—	9.2
8b (*S*)		CF_3		50 ± 0	27 ± 4	20 ± 0	43 ± 3	112
8c (*R*)		CH_2Cl		3.7 ± 0.8	87 ± 26	—	—	5.4
8d (*S*)		CH_2Cl		3.6 ± 0.1	87 ± 23	—	—	9.8
9				3.2 ± 1.3	71 ± 5	—	—	12
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 2 separate experiments with triplicate determinations. '—' indicates not active (an efficacy of <20% and/or a potency of >10,000 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.
[d] Baculovirus binding assay result that is consistent with most agonist binding data in whole-cell assays.

The synthesis of compounds of structure **3** is described in Scheme 1. Nitration of the 4-substituted 2-quinolinones (**4**) followed by hydrogenation selectively provided the 6-amino-2-quinolinones of structure **5**. The bisalkyl groups were introduced by a typical reductive alkylation with an aldehyde, a ketone, or TFA in case of 2,2,2-trifluoroethyl. The analogs were characterized by a competitive whole cell binding assay and the human AR cotransfection assay and the testing results are summarized in Table 1.[11] We used the functional assay results to drive the SAR studies and the binding affinity to assure the interaction with AR. As previously described,[9] small bisalkyl groups (i.e., ethyl and propyl) or bistrifluoroethyl group on the 6-amino group of the 2-quinolinones led to potent AR agonists (**6a** and **6b**) and the fluorine atoms on the bisalkyl groups significantly enhanced the activity (comparing **2** with **6a**). Other fluoro or chlorofluoroalkyl substitution provided similarly potent agonists (**6c** and **6d**). To systematically investigate SAR of the side chains, we prepared the 4-trifluoromethyl analogs with one of the side chains fixed as a 2,2,2-trifluoroethyl group (**6e**–**6v**). Apparently, the size of the alkyl group had significant impact on the AR modulating activities. The optimal size for a full agonist is about 2–3 carbon units (**6f**, **6g**, and **6i**), methyl (**6e**) and 4–5 carbon units (**6h**, **6j**, **6l**) are slightly off the optimal size and generated potent partial agonists, and the 6-carbon unit analog (**6k**) had about 10-fold weaker activity. It can be observed that changing the size and electronic properties of the other alkyl group generated a wide spectrum of potent AR modulating activities from full agonists (**6f**, **6g**, **6i**), partial agonists (**6e**, **6h**, **6o**) to partial agonist/antagonists (**6u** and **6v**).

Various 4-substituted analogs with 6-bistrifluoroethylamino group (**7a**–**7j**) were prepared and tested. Size and shape changes of the 4-substituents gradually modulated the activities of the compounds from partial agonist/antagonist (**7a**, R^1 = H) to full agonist (**7b**, R^1 = methyl), then partial agonist/antagonist again (**7e**, R^1 = isopropyl) to almost full antagonist (**7d**, R^1 = propyl). The 4-fluoromethyl, 4-chloro, and 4-bromo analogs showed similar activity, which suggests the size, rather than the electronic property, plays a role. The oxygen containing groups appear to give partial agonist/antagonists (**7i**–**7j**).

Fluorine atoms play an important role in molecules with biological activity. The fluorine atoms on the second carbon of the alkyl groups of the 6-amino analogs significantly enhanced the AR modulating activity (comparing **2**, **6a**, and **6f**). Interestingly, fluorine atoms on the third carbon of the propyl group have opposite impact on activities of the analogs (comparing **6g** and **6p**). However, additional fluorine atoms on the second carbon regained the lost activity (comparing **6p** and **6q**). To investigate the special effect of the fluorine atoms, we prepared the 6-(2,2,2-trifluoroisopropyl)amino and 6-(2-chloroisopropyl)amino analogs (**8**) and separated the two pairs of enantiomers by chiral HPLC. The two trifluoroisopropyl enantiomers (**8a** and **8b**) had very different activity but the two chloroisopropyl enantiomers (**8c** and **8d**) had similar activity. Compound **8a** has the agonist activity similar to that of its parent compound **6i**, while the enantiomer **8b** has much weaker partial activity. Both of the chloro substituted enantiomers **8c** and **8d** showed activity similar to that of their similar sized racemic analog **6l**. It is obvious that the fluorine atoms on the second carbon provide unique interactions in the AR binding pocket.

We have reported that the bistrifluoroethyl group of LGD2226 (**2**) occupies the same space as the C and D rings of DHT in AR binding pocket but has a hydrogen bonding with residues of AR-lbd weaker than that of the 17-hydroxy group of DHT based on the cocrystal X-ray structure.[9,12] Modeling results based on the LGD2226-AR structure indicated that the fluorine atoms on the isopropyl rather than the ethyl group of **8a** form a fluorine-to-hydroxyl hydrogen bond (2.4 Å) with amino acid THR711 that could neither be replaced by a methyl group nor be reached (3.0 Å as the closest) by either of the fluorine atoms on the isopropyl or ethyl groups of **8b** (Fig. 2).[13] In addition, the bisfluoroalkylamino group of **8b** orientes quite differently in the binding pocket from that of **8a**, which resulted in space shifting of helix 12 backbone from the agonist position of **8a** structure that is similar to that of LGD2226.

SAR studies indicated that AR modulating activity could be tuned accordingly by variation of the R^1, R^2, and R^3 groups of structure **3**. Analogs **6w**, **6x**, and **9** are typical partial AR agonists resulting from modification of these

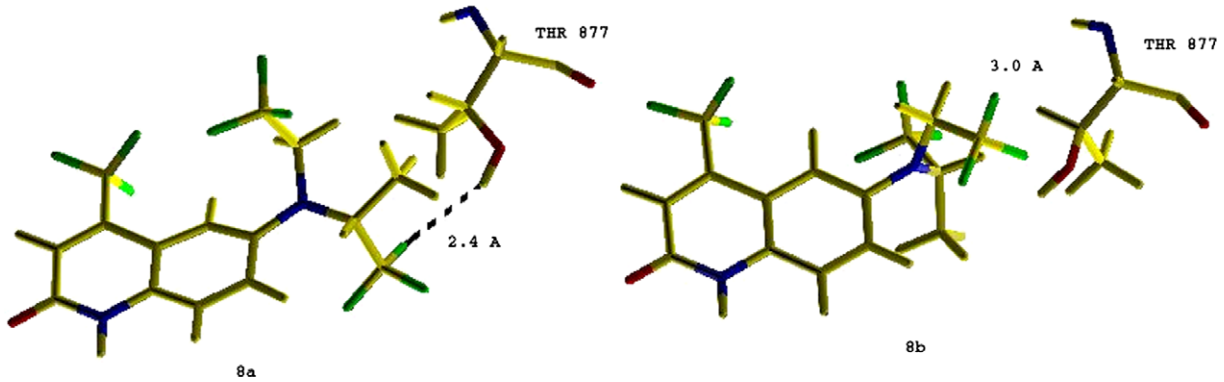


Figure 2. Interactions of analogs **8a** and **8b** with the amino acid residue THR877 (helix 11) of AR ligand binding domain in a docking model.

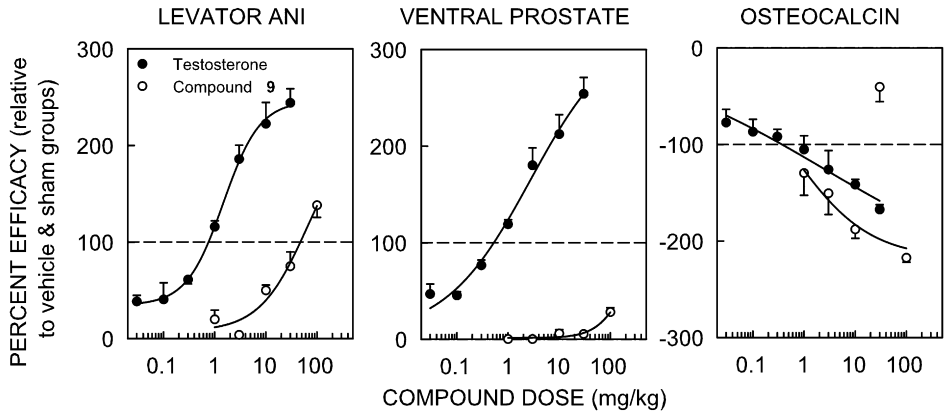


Figure 3. Effects of compound **9** and testosterone treatment on levator ani muscle and ventral prostate weights, and osteocalcin levels in a 2-week orchidectomized rat maintenance assay. 100% identifies intact animals, whereas 0% denotes levels for vehicle-treated animals.

groups. Analog **9** was profiled in a two-week mature orchidectomized rat assay to assess its tissue selectivity.[14,15] In the study, compound **9** was dosed orally, whereas testosterone propionate was dosed subcutaneously once daily for 14 days. Weights of ventral prostate and levator ani muscle were used to measure androgenic and anabolic effects, respectively, and osteocalcin levels were used as a surrogate bone turnover biomarker. As illustrated in Figure 3, testosterone equally stimulated all three endpoints to intact-equivalent levels at approximately 0.5 mg/kg dose. Since no differences were observed in the dose of testosterone needed to achieve eugonadal levels for each of the endpoints it was inferred that testosterone depicts no tissue selectivity in this model. Dose of compound **9** to achieve intact-equivalent levels in levator ani weights was approximately 40 mg/kg. At this dose, compound **9** was almost inactive in stimulating ventral prostate and showed better efficacy in osteocalcin levels than that observed for the highest dose of testosterone. In other words, compound **9** had the same potency as and better efficacy than testosterone in the osteocalcin endpoint, was about 80-fold less potent in stimulating levator ani, and was at least 200-fold less potent in the ventral prostate. Within the dose range tested, compound **9** never stimulated the ventral prostate to intact-equivalent levels. Overall, compound **9** demonstrated excellent selectivity on levator ani muscle over prostate and even better selectivity on bone marker over prostate.

A group of the representative compounds (**6c**, **6g**, **6i**, **6m**, **6n**, **7b**, **7f**, and **7g**) were tested in related steroidal hormone receptor cotransfection assays in both agonist and antagonist modes to check their receptor selectivity. All of the compounds showed more than 1000-fold separation from the estrogen, progesterone, glucocorticoid, and mineralocorticoid receptors, activities.

In summary, we systematically investigated the SAR of the three substituents of 6-amino-2-quinolinone **3** and generated a number of potent SARMs with different receptor modulating profiles. A representative analog **9** demonstrated oral bioavailability and desirable tissue selectivity on muscle and surrogate bone marker endpoint over prostate in an orchidectomized rat model of hypogonadism. The unique interaction of fluorine atoms of the analogs and amino acid residues within the AR binding pocket provided additional insight into the SAR of the series. More SAR studies on the series to define the scope and limitation of the series will be published later.

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13. Calculations were performed using the Tripos forcefield and Gasteiger–Huckle, and charges **8a** and **8b** were docked using the X-Ray cocrystal structure of AR-lbd and LGD2226 as a template. The structures were refined using a simulated annealing protocol as described in the SYBYL manuals, SYBYL 7.0, Tripos Inc., 1699 South Hanley Rd., St. Louis, MO 63144, USA.
14. Mature male Sprague–Dawley rats from Harlan (Indianapolis, IN) were used and the protocol was approved by IACUC. One group of animal was sham-operated, treated with vehicle, and served as additional control. Five animals were used in each dosing group and animals were treated immediately after orchiectomy.
15. Compound **9**: 1,2-dihydro-6-(*N*-2,2,2-trifluoroethyl-*N*-isobutyl)amino-4-methyl-2-quinolinone. R_f 0.18 (MeOH/CH_2Cl_2, 3:7); ^{1}H NMR (500 MHz, $CDCl_3$) 11.80–12.00 (br s, 1H), 7.32 (d, J = 9.3, 1H), 7.12 (dd, J = 2.4, 9.3, 1H), 7.02 (d, J = 2.9, 1H), 6.56 (s, 1H), 3.92 (q, J = 8.8, 2H), 3.23 (d, J = 7.3, 2H), 2.47 (s, 3H), 2.04 (quin, J = 6.8, 1H), 0.95 (d, J = 6.8, 6H).