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Tandem optimization of target activity and elimination of mutagenic potential in a potent series of *N*-aryl bicyclic hydantoin-based selective androgen receptor modulators

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Abstract—Pharmacokinetic studies in cynomolgus monkeys with a novel prototype selective androgen receptor modulator revealed trace amounts of an aniline fragment released through hydrolytic metabolism. This aniline fragment was determined to be mutagenic in an Ames assay. Subsequent concurrent optimization for target activity and avoidance of mutagenicity led to the identification of a pharmacologically superior clinical candidate without mutagenic potential.
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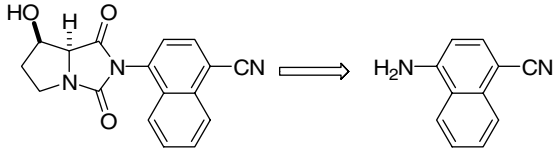
As men age, testosterone (T) levels decline and muscle mass and strength erode in parallel.[1] Over time this can lead to frailty and ultimately necessitate the need for assisted living.[2] Testosterone replacement therapy has shown significant clinical benefit at halting this trend,[3] though replacement therapy carries risks associated with poor selectivity for muscle versus prostate.[4] Emerging selective androgen receptor modulators (SARMs) have the potential to favorably impact this selectivity equation and are consequently being actively pursued by many groups.[5]

We have recently reported the discovery of a novel series of SARMs based on an *N*-aryl bicyclohydantoin scaffold.[6] Compounds in this class showed potent AR agonist activity and >50-fold selectivity for stimulation of skeletal muscle versus prostate growth in a standard rodent model. The prototype compound from this series also exhibited both favorable pharmacokinetic properties and an encouraging profile on a standard battery of in-house liability assays used to assess 'drugability'. Although compound **1** was well absorbed, highly bioavailable, and metabolically stable in several preclinical species, sensitive analytical detection methods allowed quantification of trace amounts of 4-cyanonaphthylamine (**2**) in urine after oral dosing of **1**. This hydrolytic degradant was not detectable in plasma of any species (at a lower limit of quantitation of 1 ng/mL). However, approximately 0.003% and 0.1% of **2** (relative to the dose of parent) were detected in the urine of dogs and cynomolgus monkeys, respectively. Though the parent compound tested negative in exploratory Ames assays, naphthylamine **2** tested positive in these assays. As a mutagenic metabolite would be undesirable for a drug candidate, this finding prompted us to discontinue preclinical development of this SARM in favor of more suitable agents.



1 **2**

Keywords: SARM; Androgen; Receptor; Mutagenicity; Aniline.
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In seeking to obviate the potential for mutagenic metabolites arising from this otherwise promising chemotype, we explored three separate strategies in parallel, in order of increasing synthetic complexity: (1) optimization of the aryl amine moiety of the bicyclohydantoin-based compounds to eliminate mutagenic potential should trace amounts be liberated in vivo; (2) modifications to the core scaffold of these SARM compounds such that an aryl amine moiety could not readily be released; and (3) replacement of the aniline nitrogen with carbon to exclude a formal aryl amine moiety from the molecule. This report focuses on our efforts in the former strategic avenue.[7] Analysis of the X-ray co-crystal structure of compound **1** bound to the ligand binding domain (LBD) of the AR[6] (Fig. 1) suggested that the binding energy from a π-edge-to-face interaction with the side chain of Phe764 was mediated primarily through the proximal aryl ring of the naphthyl moiety. As such, we hypothesized that it would be possible to capture the hydrophobic space-filling and electronic properties of the distal aryl ring of the naphthyl system through substituents that would be predicted to mitigate known physicochemical features correlated with mutagenic potential.

In order to efficiently execute lead optimization of this nature, we considered it essential to be able to assess both the activity of the parent molecules on the target of interest (AR functional assay) and the mutagenic potential of the aryl amine fragment in parallel. Although the Ames assay has long been viewed as the gold standard for determining mutagenic potential,[8,9] compound requirements and throughput are generally not amenable to integration with rapid lead optimization cycles. A more convenient approximation of genotoxic potential can be found in the SOS Chromotest assay.[10]

SOS repair refers to sparingly used, error-prone translational synthesis, a last resort to allow cell survival.[11] The SOS Chromotest employs a genetically engineered *Escherichia coli* containing a lacZ reporter gene under transcriptional control of a SOS repair gene. Bacteria respond to DNA damage detected by the transcription complex by de-repression of SOS genes, resulting in the expression of β-galactosidase, the gene product of lacZ. Induction of SOS repair genes following DNA damage is monitored by measuring β-galactosidase activity, with cytotoxicity determined in parallel by constitutively expressed alkaline phosphatase activity. This medium throughput assay is formatted for 96-well plates, has a typical turnaround time of two days, and requires only 2 mg of test compound, compared with 30 mg and two weeks for a minimal Ames assay. Validation studies demonstrated that the SOS Chromotest exhibits high concordance with the Ames test, with a small number of false positive and negatives (for 121 proprietary and 46 commercial compounds, the overall concordance was 87%).[12] The sensitivity of the SOS Chromotest for detecting mutagens is 79% and the specificity is 91%. Despite these limitations the SOS Chromotest has proved useful as an expedient first screen for potential mutagens.

The SOS Chromotest was used as part of a lead optimization scheme for concomitant elimination of the mutagenic potential of the aryl amine moiety in this SARM scaffold. As previously indicated, X-ray co-crystal structural analysis of advanced lead **1** suggested that an aryl moiety in this region of the AR LBD was important for binding and desired biological efficacy.[6] A modified version of DEREK, a rules-based software program for predicting mutagenic potential, was used to guide the selection of replacement aryl amines for synthesis.[13,14] Aryl amines are not generally the principal mutagenic species, but rather are precursors for metabolic conversion to hydroxylamine and mutagenic nitroso species (Fig. 2). Analyses of the factors contributing to the mutagenicity of aryl amines indicate direct correlations with the number of fused rings (hydrophobic surface area, DNA intercalation), log *P* (hydrophobicity, cell permeability), and inverse correlations with polarizability (amine oxidation potential) and steric accessibility of the amine group.[15–18]

Assessments of mutagenic potential were integrated into our lead optimization work flow, generally as follows: aryl amines chosen with in silico guidance were incorporated into new SARM analogues which were tested in cell-based AR transcriptional assays;[6] amines leading to sufficiently potent SARMs were then evaluated in the SOS Chromotest; potent SARMs whose aryl amine component was negative in the SOS Chromotest were evaluated in vivo; adequate in vivo activity then triggered verification of the lack of mutagenic potential of the aryl amine in an Ames assay.

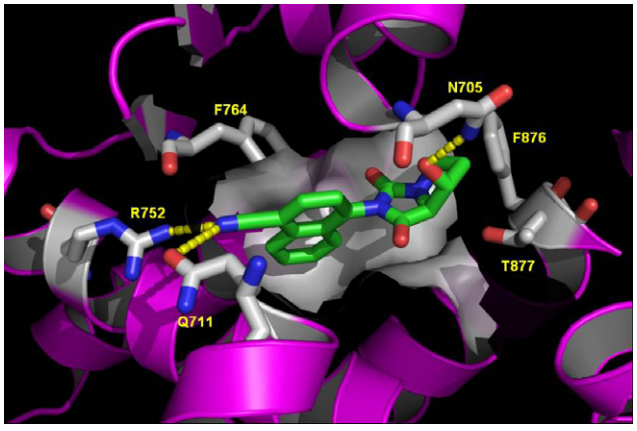


Figure 1. X-ray co-crystal structure of **1** bound to the rat AR-LBD at 2.0 Å resolution. The helices and loops are colored magenta. The key ligand binding residues are labeled and the dashed lines represent potential hydrogen bonds.

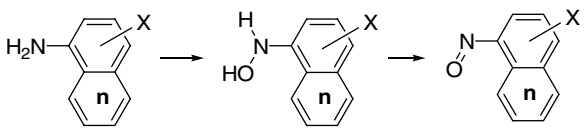
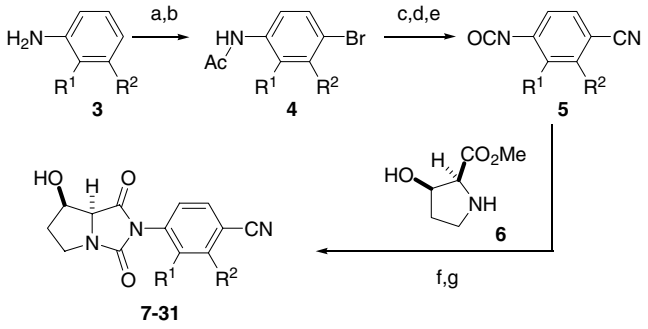


Figure 2. Aryl amine activation to mutagenic species and factors affecting mutagenic potential: *n*, number of rings; X, electronic and steric factors.

SARM analogues were prepared in a convergent fashion as previously described (Scheme 1).[6,19] Bicyclic hydantoin formation consisted of condensation of various aryl isocyanates with *cis*-3-hydroxy-L-proline methyl ester. Aryl isocyanates were derived from the corresponding aryl amines, which were typically prepared through protection of a commercially available aryl amine as the acetamide, selective bromination at the para position, followed by Pd-mediated conversion to the corresponding aryl nitrile using CuCN. Acetamide hydrolysis under acidic conditions and subsequent treatment with phosgene provided the appropriate isocyanates. All final compounds were purified to >98% HPLC purity and characterized by ^{1}H NMR and LC/MS.



Scheme 1. Preparation of *N*-aryl hydantoin SARM compounds. Reagents and conditions: (a) Ac_2O, AcOH, NaOH; (b) Br_2; (c) CuCN, $Pd(dba)_2$, DMF; (d) HCl, EtOH; (e) phosgene, $NaHCO_3$, CH_2Cl_2; (f) *i*-Pr_2NEt, toluene; (g) DBU, toluene, rt, 12 h.

Binding affinities and agonist activities were measured for each SARM, and mutagenic potential for the respective aryl amine was assessed (Table 1). Because we had previously determined that aryl group substituent tolerance for AR binding allowed at most a 1,2,3,4-substitution pattern, and that activity dropped off precipitously with additional substitutions at the 5- or 6-positions (data not shown), the scope of the current data set is limited to this partially optimized chemotype space. Virtually all of the analogues prepared in this series resulted in potent binding affinity to the AR, consistent with molecular modeling docking and X-ray structural data suggesting that the hydroxy group, the aryl ring centroid, and the nitrile nitrogen lone–pair interaction are the key pharmacophoric determinants for receptor binding interactions. In contrast, the functional agonist activity for this series was widespread, spanning more than five orders of magnitude.

Although simple N-substitution into the naphthyl ring of **1** to give quinoline compound **7** obviated the aryl

Table 1. AR agonist potencies in functional assays and SOS chromotest assay results for reference compounds and SARMs **1** and **7**–**31**

Compound	R^1	R^2	AR EC_{50} (nM)[a]	K_i (nM)	SOS Chromotest fold induction of corresponding aniline fragment, ±S9 fraction[b]	Ames result for aniline fragment[c]
2-Aminoanthracene	NA[d]	NA	NA	NA	5	Positive
4-Nitroquinoline oxide	NA	NA	NA	NA	11–2	Positive
Aflatoxin B1	NA	NA	NA	NA	23–24	Positive
1	Fused phenyl		2.3	3.2	2.02/1.58	Positive
7	Fused 5-pyridyl		6.2	21	1.05/1.03	Negative
8	H	H	>10,000	34	NT[e]	NT
9	H	OMe	98	24	1.29/1.40	Positive
10	H	CF_3	>10,000	7.8	1.40/1.41	Negative
11	H	Cl	2.8	1.5	1.87/1.78	Positive
12	H	Ph	345	48	NT	NT
13	Me	H	52	8.5	NT	NT
14	Me	Me	1.7	4.5	1.62/1.28	NT
15	Me	CO_2Me	>10,000	nd	1.44/1.54	NT
16	Me	CF_3	3.6	2.9	1.34/1.46	Negative
17	Me	OH	>10,000	nd	NT	NT
18	Me	OMe	487	3.7	NT	NT
19	Me	F	1.7	1.3	1.39/1.32	NT
20	Me	Cl	0.7	2.1	1.46/1.35	Negative
21	Me	Br	0.6	1.2	1.31/1.24	NT
22	Me	CN	2.2	8.0	NT	NT
23	F	F	47	4.3	NT	NT
24	F	Cl	1.0	3.0	NT	NT
25	Cl	Cl	NT	NT	1.71/1.83	NT
26	Cl	OMe	607	6.1	1.35/1.25	NT
27	Cl	Me	1.7	0.7	1.64/1.55	NT
28	OMe	Me	221	8.1	NT	NT
29	OMe	CF_3	1080	7.9	1.14/1.38	NT
30	Et	H	79	14	NT	NT
31	Et	CF_3	36	3.3	1.42/1.13	NT

[a] Values are means of three experiments, standard deviation is given in parentheses; nd, not determined.
[b] Values are means of three experiments, $\geqslant$1.50-fold induction indicates a positive mutagenic response in this assay.
[c] TA 98 and TA 100 test strains of *Salmonella typhimurium*, $\geqslant$2-fold dose-related elevation in the mean revertant counts relative to DMSO controls.
[d] NA, not applicable.
[e] NT, not tested.

amine mutagenic potential, this change resulted in an order of magnitude loss in binding affinity, and so as a general rule (also considering the inverse correlation of mutagenic potential with the number of fused rings), we chose to limit our SAR explorations to monocyclic aryl substitutions into this scaffold. Both dramatic steric and electronic effects were seen in response to substituent changes in these two positions. Substitution ortho to the aryl nitrogen in this series typically resulted in atropoisomerism, readily observable by ^{1}H NMR. This conformational restriction and presumed consequent reduction in entropy appears to correlate well with agonist potency in the cell-based assays (with the exception of Cl-analogue **11**), as compounds **8**–**12** suffer at least a 30-fold loss in potency, despite maintaining reasonable binding affinity. Size limitations appeared to exist at R^2, as seen with compound **12**. In the absence of counterposing electronic factors, agonist potency is restored by the addition of a simple methyl group at R^1 (compare **10/16** and **11/20**).

Electron donating groups appear to be poorly tolerated throughout (as in **9**, **17**, **18**, **26**, **28**, and **29**), perhaps due to attenuation of the strength of aryl π-edge/face interactions with F764 in the AR LBD, as well as reduced H-bonding capacity of the CN group. The presence of two lipophilic and/or electron-withdrawing groups appeared optimal at both R^1 and R^2, with halogen and methyl groups generally optimal. All SARMs discovered in this series with EC_{50}'s < 5 nM follow this trend (i.e., **11**, **14**, **16**, **19**–**22**, **24**, and **27**). The most potent agonists (EC_{50} < 1 nM) were those where R^1 = Me and R^2 = halogen (**20** and **21**).

With respect to mutagenic potential, less clear SAR trends emerged. Naphthylamine **2** was definitively mutagenic in both SOS Chromotest and Ames assays. Consistent with known aniline mutagenicity SAR, the aryl amine components of several compounds without steric encumbrance proximal to the amino group showed a positive mutagenic response in one or both assays (e.g., those from **9** and **11**). Likely owing to the success of aniline pre-selection criteria, most of the anilines tested in the overall series were negative in the SOS Chromotest, and the generally high predictive power of this assay was consistent within this group of anilines (with the exception of that from **9**). Though only a subset of anilines were tested in Ames assays, no false positives were observed in the SOS Chromotest, a desirable feature in optimizing this parameter in concert with pharmacological endpoints. Consequently, increasing the throughput and decreasing the compound requirement for a mutagenicity screen allowed for the iterative optimization of both efficacy and mutagenicity in parallel, resulting in the rapid elimination of this unacceptable liability and identification of compound **20**, which advanced to human clinical trials.[20,21]

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