Design and Synthesis of an Array of Selective Androgen Receptor Modulators

Ryan P. Trump,* Jean-Baptiste E. Blanc, Eugene L. Stewart, Peter J. Brown, Matilde Caivano,† David W. Gray,† William J. Hoekstra, Timothy M. Willson, Bajin Han, and Philip Turnbull

GlaxoSmithKline, Research Triangle Park, North Carolina 27709

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We describe the design, using shape comparison and fast docking computer algorithms, and rapid parallel synthesis of a 1300 member array based on GSK7721, a 4-aminobenzonitrile androgen receptor (AR) antagonist identified by focused screening of the GSK compound collection. The array yielded 352 submicromolar and 17 subnanomolar AR agonists as measured by a cell-based reporter gene functional assay. The rapid synthesis of a large number of active compounds provided valuable information in the optimization of AR modulators, which may be useful in treating androgen deficiency in aging males.

Introduction

New androgen receptor (AR) modulators that have an increased therapeutic window compared to current AR-based therapies would be valuable in the treatment a variety of conditions associated with reduced androgen levels in males. The discovery of new classes of AR ligands can be facilitated using computational design and rapid combinatorial synthesis. We outline the use of these techniques in the discovery and optimization of a potent class of AR agonists.

The AR is a member of the nuclear receptor superfamily of ligand-regulated transcription factors and is a target for the treatment of androgen-deficient males; however, the safety profile of current hormone replacement therapies could be improved. The endogenous steroidal ligands for the AR, testosterone (T), and its more potent metabolite dihydrotestosterone (DHT), are integrally involved with a variety of vital physiological processes.[1] Exogenous administration of T results in beneficial increases in bone mineral density, muscle mass, sexual activity, and energy levels but also in concomitant detrimental increases in prostate volume, decreases in spermatogenesis, and stimulation of sebaceous glands (acne).[2−4] Serum androgen levels for normal young males are above 400 ng/dL, but decline in the aging male during so-called andropause, reaching below 200 ng/dL after approximately age 70.[5] Associated with the decrease in T are decreases in lean body mass and bone mineral density that result in increased frailty and a greater tendency toward falls and bone fractures, a condition termed androgen decline in aging males (ADAM).[6] Hormone replacement therapy with steroidal androgens can reverse the effects of ADAM but is severely limited by a range of side effects including increased prostate stimulation, gynecomastia, increased acne, increased aggression, and other effects.[7−10] Nuclear receptor modulators are small-molecule ligands that exhibit agonist, partial agonist, or antagonist effects in a cell or tissue dependent manner.[11−14] A selective androgen receptor modulator (SARM) with a strong agonist profile in muscle and bone, but an antagonist or weak-agonist profile in the prostate, could be used to treat hypogonadism or ADAM with an improved therapeutic index compared to testosterone.

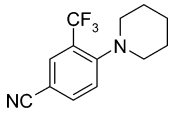

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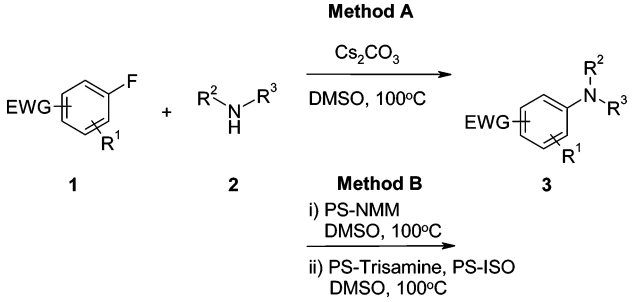
Figure 1. Structure of initial AR antagonist **3** (GSK7721) found through screening of a selected subset of the GlaxoSmithKline compound collection.

Scheme 1. Synthetic Approaches to Analogs of GSK7721



To find the desired modulatory profile, we wished to identify new nonsteroidal AR ligands as starting points for optimization. Focused screening of the GlaxoSmithKline compound collection identified 4-aminobenzonitrile **3** (GSK7721) as an AR antagonist (binding $pIC_{50} = 6.0$, cellular $pIC_{50} = 5.8$)[15] (Figure 1). Combinatorial synthesis of an array of analogs was planned to explore the SAR around initial hit **3**. The electron-deficient tertiary aniline **3** was accessed via nucleophilic aromatic substitution of a secondary amine **2** with an electron-deficient aryl fluoride

* To whom correspondence should be addressed. Phone: 919-483-9539. E-mail: ryan.p.trump@gsk.com.

† GlaxoSmithKline, Stevenage, Herts SG1 2NY, U.K.

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1, as shown in Scheme 1. Given the large number of available secondary amines (~6300 secondary alkyl amines available commercially and from in-house sources) and electron-deficient aryl fluorides (24 appropriate monomers identified from commercial sources), the potential size of an array of analogs of **3** was greater than 150 000 compounds. To focus the synthetic efforts, a shape overlay and docking algorithm was employed. We describe the computational design, combinatorial synthesis, and screening of an array of 1300 analogs of **3**.

Results and Discussion

Library Design. Our library design selection was carried out in three steps: (1) enumeration of the virtual library, (2) evaluation of the compounds using a 3D virtual screening procedure, and (3) selection of monomers, on the basis of the results.[16]

First, using CombiLibMaker software (version 4.3.2), we enumerated a large virtual library of compounds using all commercially and in-house available electron-deficient aryl fluorides and secondary amines, which resulted in more than 150 000 compounds.[17] Our evaluation procedure required that 3D conformers be generated for each member of the virtual library. The database of compound conformations was constructed using CONCORD (version 5.1.2) to generate an initial 3D representation of each compound, followed by conformational analysis using the OMEGA program (version 1.8.1) with the constraint of a maximum of 200 low-energy conformers per compound (defined as a conformation with a calculated energy no more than 5 kcal/mol greater than lowest-energy conformation).[18−20] For chiral compounds that lacked stereochemical designations, each enantiomer was created and conformationally expanded. We carried out a shape overlay using the ROCS program (version 2.0) for each of the virtual conformers onto the conformation of DHT extracted from its cocrystal structure with AR.[21−25] For each of the virtual compounds, we selected the conformer with the best shape Tanimoto score. That particular conformer, preoriented in the ligand binding domain (LBD) by the shape algorithm, was energy-minimized by full geometry optimization in the LBD of the receptor by the in-house program MVP.[26] Using the Tanimoto shape scores, the optimized energy values, and visual inspection of the bound conformations of the virtual compounds, we selected 20 electron-deficient aryl fluorides (Figure 2) and 80 secondary (Figure 3) amines to produce an optimized array of electron-deficient tertiary anilines.

Array Synthesis. Initial conditions for the synthesis of aniline **3** analogs were developed with three representative secondary amines: unhindered cyclic amine **2**{*1*}, hindered cyclic amine **2**{*2*}, and acyclic amine **2**{*3*}. Standard solution-phase conditions involved treating a fluoroaryl nitrile **1**{*1*} with a slight excess of secondary amines **2**{*1*−*3*} in DMSO with cesium carbonate at 100 °C (Method A, Scheme 1). The synthesis proved to be robust for the preparation of small numbers of compounds but posed a challenge for use in a combinatorial array synthesis, as a liquid−liquid extraction was required to remove the soluble base and excess amine.

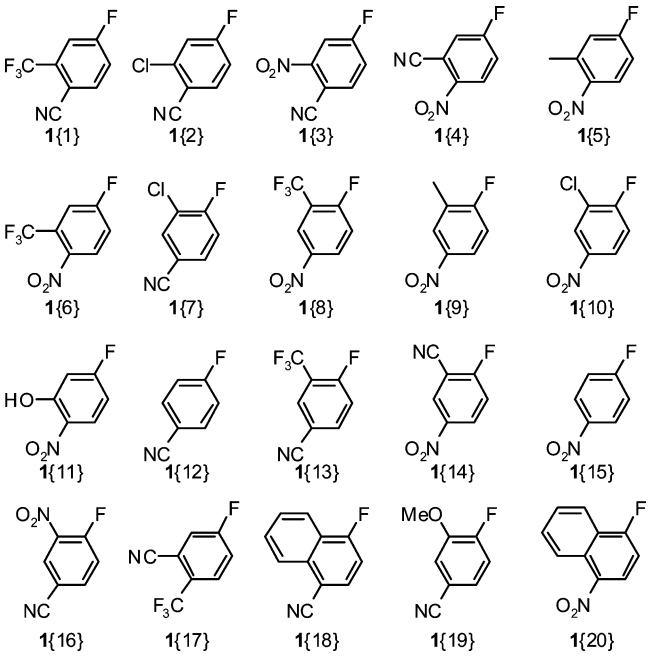


Figure 2. Structures of monomers **1** used in array synthesis.

To facilitate reaction workup, solid-supported base reagents were evaluated as a replacement for cesium carbonate. The macroporous-supported carbonate base allowed the reaction to go to completion but introduced an uncharacterized impurity in the final product. In contrast, 3-(morpholino)propyl polystyrene sulfonamide (PS-NMM) provided for complete product formation with fewer side products. Conversion to the desired anilines was aided by a slight excess of the respective amine monomer. To further facilitate reaction workup, a scavenger resin was employed to remove the excess secondary amine from the final product.[27] Treatment of the crude reaction mixture with polystyrene methylisocyanate (PS-ISO) was sufficient to remove the excess amine.

Reaction of amines **2**{*1*−*3*} with fluoroaryl nitrile **1**{*1*} in DMSO at 100 °C with PS-NMM for 16 h, followed by treatment with PS-ISO at 100 °C for 4 h, resulted in the desired products **3**{*1, 1*−*3*} in good yield, but products **3**{*1, 2*−*3*} were contaminated with ~5% of starting material fluoroaryl nitrile **1**{*1*}. Bis-(2-aminoethyl)aminomethyl polystyrene (PS-Trisamine) was used to remove the unreacted starting fluoroaryl nitrile in DMSO at 100 °C in the presence of the PS-ISO resin. Production of the final array used the reaction of the aryl fluorides and amines at 100 °C in DMSO in the presence of PS-NMM. (Scheme 1, Method B) After 16 h of reaction, both PS-Trisamine and PS−ISO were combined to scavenge unreacted starting materials at 100 °C for 4 h. Filtration, followed by concentration under reduced pressure, resulted in the final array products.

The array was synthesized in Robbins Flex-Chem 96-well filtration blocks using aryl fluorides **1**{*1*−*20*} and secondary amines **2**{*1*−*80*} selected by the computational analysis described. The initial purity analysis of the 1600 products by HPLC/MS with diode-array detection determined that 1182 met our criteria of greater than 80% purity without a

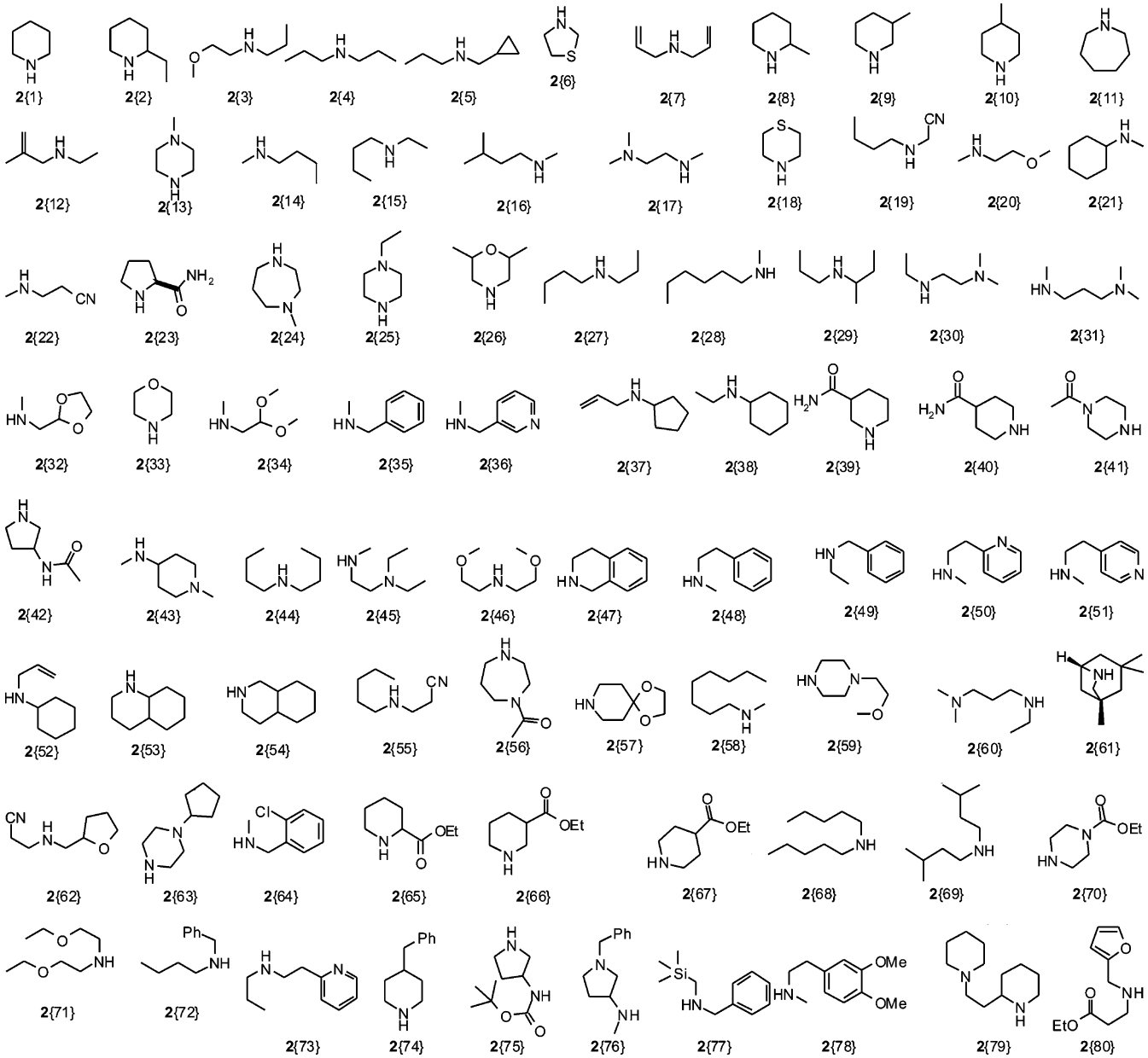


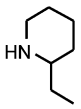
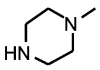
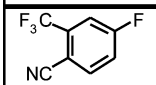
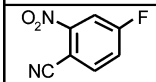
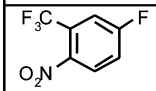
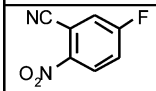
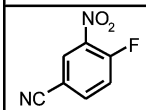
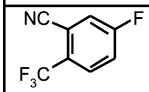
Figure 3. Structures of monomers **2** used in array synthesis.

requirement for further workup or purification. The 243 array compounds that were present in less than 20% purity were discarded. Purification using mass-directed reverse-phase preparative HPLC of the remaining 175 compounds that had an initial purity of 20−80% yielded 97 additional compounds with purity greater than 80%. Each of the final products was transferred to a tared glass vial and weighed to determine the yield. Overall, 1279 compounds were delivered for screening in purity greater than 80% (average purity 98%) and quantity greater than 1 μmol (average 65% yield).

Biological Results. The 1279 compounds from the array were screened for the ability to activate an MMTV-luciferase reporter gene in CV-1 cells that were transiently transfected with both human AR cDNA and the reporter construct. The compound potency for inducing AR transactivation was determined through luciferase quantitation and is reported as the negative log of the concentration required for 50% maximal response (pEC_{50}). The array produced a high proportion of potent AR agonists: 352 of the 1279 compounds profiled with a $pEC_{50} > 6.0$, 61 with a $pEC_{50} > 8.0$, and 17 with a $pEC_{50} > 9.0$ (Figure 4). Cellular transactivation data from the array demonstrated several notable trends (Table 1). A nitro or nitrile group at the position para to the aniline generally gave higher activity.[28−36] Combination of the 4-nitro or 4-nitrile functionality with a group such as trifluoromethyl or chloro at the position meta to the aniline further enhanced activity. Small branched and unbranched alkyl groups, cyclic and acyclic, attached to the aniline were preferred, while the presence of a basic amine reduced activity.

The binding of selected compounds to the AR and the structurally related glucocorticoid receptor (GR) and progesterone receptor (PR) was determined (Table 2). Binding to the AR was confirmed for the functionally active compounds, indicating that the observed cellular activity was

Table 1. AR Transient Transfection Reporter Gene Assay Data [pEC_{50} (% maximal response)] from Representative Array Members.

1 \ 2					
	**3**{1,5} 9.2 (110%)	**3**{1,2} 9.2 (103%)	**3**{1,7} 9.3 (115%)	**3**{1,4} 9.1 (114%)	**3**{1,13} < 6.0 (32%)
	**3**{3,5} 9.6 (125%)	**3**{3,2} 9.8 (118%)	**3**{3,7} 9.6 (106%)	**3**{3,4} 9.3 (115%)	**3**{3,13} < 6.0 (17%)
	**3**{6,5} 9.0 (114%)	**3**{6,2} 8.9 (104%)	**3**{6,7} 9.1 (92%)	**3**{6,4} 8.9 (118%)	**3**{6,13} < 6.0 (24%)
	**3**{4,5} 9.5 (114%)	**3**{4,2} 9.4 (112%)	**3**{4,7} 9.1 (122%)	**3**{4,4} 8.8 (102%)	**3**{4,13} < 6.0 (11%)
	**3**{16,5} 6.6 (40%)	**3**{16,2} 6.3 (63%)	**3**{16,7} 7.4 (88%)	**3**{16,4} 6.5 (56%)	**3**{16,13} < 6.0 (28%)
	**3**{17,5} 7.4 (67%)	**3**{17,2} ND	**3**{17,7} 6.9 (75%)	**3**{17,4} < 6.0 (21%)	**3**{17,13} < 6.6 (64%)

receptor-mediated. Compounds were identified that had greater than 100-fold selectivity over both the GR and the PR.[37,38]

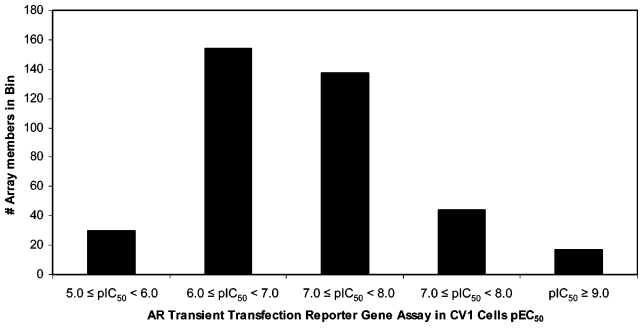


Figure 4. Distribution of array member AR agonist potencies.

Conclusions

On the basis of an initial AR ligand identified by focused screening, an array of compounds was designed using shape overlay and docking techniques for activity on the AR. Employing traditional solution-phase combinatorial techniques, we produced an array of 1600 compounds quickly and efficiently. Of the 1279 compounds screened for AR agonist activity, a high number of active compounds were identified: 352 compounds had a $pEC_{50} > 6.0$ in an AR functional assay. The array rapidly provided valuable structure activity relationships to guide lead optimization of this potent AR agonist series.

Table 2. Selectivity Profile of Select Compounds

compound	AR TT pEC_{50} (% max resp)	AR binding pIC_{50}	GR binding pIC_{50}	PR binding pIC_{50}
3{1,4}	9.1 (114%)	7.8	<5.0	6.5
3{1,2}	9.2 (103%)	7.3	<5.0	6.7
3{1,7}	9.3 (150%)	7.5	4.9	6.4
3{2,7}	9.4 (94%)	7.6	6.1	6.7
3{3,4}	9.3 (115%)	7.7	5.6	6.8
3{3,2}	9.8 (118%)	7.3	<5.0	6.6
3{5,2}	9.0 (149%)	7.3	5.9	6.5
3{5,5}	9.0 (133%)	7.4	6.1	6.8
3{5,7}	9.0 (119%)	7.6	6.0	6.4
3{6,5}	9.0 (114%)	7.6	5.2	6.8
3{6,7}	9.1 (92%)	7.5	<5.0	6.4
3{18,1}	8.3 (114%)	7.4	5.5	6.0
3{6,8}	8.6 (96%)	7.4	<5.0	6.0
3{4,46}	8.3 (80%)	7.1	<5.0	<5.2
3{1,5}	9.2 (110%)	7.9	5.7	6.9
3{4,2}	9.4 (112%)	7.8	4.8	6.3
3{4,46}	8.3 (80%)	7.1	<5.0	<5.2

Experimental Methods

General. The ^1H NMR spectra were recorded on a Varian VXR-300, a Varian Unity-300, or a Varian Unity-400 instrument. Chemical shifts are expressed in parts per million (ppm, δ). Coupling constants are in units of hertz (Hz). Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad; sept, septet. NMR spectra of array compounds were acquired using a modified Varian microliter flow injection probe on a Varian Unity-500 instrument.[39]

Low-resolution mass spectra (MS) were recorded on a JEOL JMS-AX505HA, JEOL SX-102, or SCIEX-APIiii spectrometer. All mass spectra were taken under electrospray ionization (ES, either in the positive-ion or negative-ion mode) or by fast atom bombardment (FAB) methods. Non-array reactions were monitored by thin-layer chromatography on 0.25 mm E. Merck silica gel plates (60F-254), visualized with UV light, iodine staining,r 7% ethanolic phosphomolybdic acid, or *p*-anisaldehyde solutions. Flash column chromatography was performed on silica gel (230−400 mesh, Merck).

Analytical purity was assessed on a Hewlett-Packard series 1050 or 1100 system equipped with a diode-array spectrometer. The stationary phase was either a Dynamax C8 column (25 cm × 4.1 mm), a Dynamax 60A C18 column (25 cm × 4.6 mm), a Vydac C18 column (5 m, 4.6 mm × 250 mm), a Supelco C18 column (5 m, 4.6 mm × 150 mm), or a Rainin C18 column (5 m, 4.6 mm × 250 mm). The flow rate was 1.0−1.5 mL/min (t = 2.8 or 3.0 min), and the solvent system was a gradient of 10% MeOH to 100% MeOH in water over 3 min with a 1 min wash with 100% MeOH.

General Method for the Synthesis of Electron-Deficient Tertiary Anilines. Method A. The secondary amine (1.59 mmol), aryl fluoride (1.32 mmol), and cesium carbonate (1.59 mmol) were combined in DMSO (3 mL) and heated to 100 °C. The resulting suspension was stirred for 16 h and then allowed to cool to room temperature. The reaction mixture was added to ethyl acetate (100 mL). The organic solution was washed with saturated sodium bicarbonate (2 × 30 mL) and brine (1 × 30 mL), dried ($MgSO_4$), and concentrated to a solid. The solid was purified using silica gel chromatography (10−50% gradient of EtOAc in hexanes) to give the final product.

4-(1-Piperidinyl)-2-(trifluoromethyl)benzonitrile 3{*1, 1*}. Two hundred fifty-three milligrams (75%) of the title compound was obtained as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.68 (s, 6 H), 3.39 (s, 4 H), 6.92 (dd, J = 8.9, 2.5 Hz, 1 H), 7.08 (d, J = 2.5 Hz, 1 H), 7.57 (d, J = 8.7 Hz, 1 H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 24.0, 25.1, 48.2, 95.2, 111.1, 115.3, 117.1, 121.4, 124.1, 135.9, 152.7. LC/MS: *m*/*z* 255 (M + 1).

4-(2-Ethyl-1-piperidinyl)-2-(trifluoromethyl)benzonitrile 3{*1, 2*}. One hundred ninety-eight milligrams (53%) of the title compound was obtained as a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, J = 7.4 Hz, 3 H), 1.59 (m, 1 H), 1.66 (m, 5 H), 1.74 (m, 2 H), 3.07 (m, 1 H), 3.65 (d, J = 13.6 Hz, 1 H), 3.92 (s, 1 H), 6.87 (dd, J = 8.9, 2.5 Hz, 1 H), 7.04 (d, J = 2.3 Hz, 1 H), 7.54 (d, J = 8.9 Hz, 1 H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 11.5, 18.8, 21.8, 25.3, 27.4, 41.9, 56.2, 94.5, 111.0, 115.2, 117.6, 121.7, 124.5, 136.3, 153.0. LC/MS: *m*/*z* 283 (M + 1).

4-[[2-(Methyloxy)ethyl](propyl)amino]-2-(trifluoromethyl)benzonitrile 3{*1, 3*}. One hundred seventy-three milligrams (46%) of the title compound was obtained as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.95 (t, J = 7.4 Hz, 3 H), 1.60 (m, 2 H), 3.34 (m, 5 H), 3.55 (m, 4 H), 6.75 (dd, J = 8.9, 2.7 Hz, 1 H), 6.92 (d, J = 2.5 Hz, 1 H), 7.53 (d, J = 8.9 Hz, 1 H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 11.2, 19.8, 50.6, 53.1, 59.2, 69.8, 93.8, 109.0, 113.2, 117.4, 121.4, 124.1, 135.9, 150.5. LC/MS: *m*/*z* 287 (M + 1).

General Method for the Array Synthesis of Electron Deficient Tertiary Anilines. Method B. The PS-NMM was added to the well of a Robbins FlexChem square-well 96-well plate. A DMSO solution of **2** (120 μL, 2 M) was mixed with a DMSO solution of **1** (150 μL, 1 M). The resulting slurry was heated to 100 °C for 20 h with mixing of the plate via rotation. The block was cooled, and the PS-ISO (200 μmol), PS-Trisamine (200 μmol), and DMSO (1.2 mL) were added. The resulting slurry was rotated at 100 °C for 4 h before being allowed to cool to room temperature. The solutions were filtered, and the resins were rinsed with DMSO (0.5 mL). The organic solutions were combined and concentrated under reduced pressure at 50 °C to a solid. Purification, if required, was performed via prep-HPLC using a gradient (10−100%) of MeOH in water.

4-(Di-allylamino)-2-(trifluoromethyl)benzonitrile 3{*1, 7*}. The procedure produced 16.5 mg (41%) of the title compound in >99% purity without further workup or purification. ^{1}H NMR (500 MHz, DMSO-d_6): δ 4.10 (m, 4 H), 5.12 (d, J = 17.2 Hz, 2 H), 5.17 (d, J = 10.3 Hz, 2 H), 5.84 (m, 2 H), 6.96 (d, J = 9.0 Hz, 1 H), 6.98 (s, 1 H), 7.76 (d, J = 9.0 Hz, 1 H). LC/MS: *m*/*z* 267 (M + 1).

4-(4-Morpholinyl)-2-(trifluoromethyl)benzonitrile 3{*1, 33*}. The procedure produced 18.5 mg (48%) of the title compound in >99% purity without further workup or purification. ^{1}H NMR (500 MHz, DMSO-d_6): δ 3.39 (m, J = 5.0 Hz, 4 H), 3.73 (m, 4 H), 7.24 (d, J = 8.7 Hz, 1 H), 7.30 (s, 1 H), 7.84 (d, J = 8.7 Hz, 1 H). LC/MS: *m*/*z* 257 (M + 1).

1-[4-Cyano-3-(trifluoromethyl)phenyl]-4-piperidinecarboxamide 3{*1, 40*}. The procedure produced 35.5 mg (92%) of the title compound in 95% purity without further workup or purification. ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.59 (m, 2 H), 1.79 (d, J = 11.6 Hz, 2 H), 2.39 (m, 1 H), 2.99 (t, J = 23.5, 11.6 Hz, 2 H), 4.04 (d, J = 13.2 Hz, 2 H), 6.83 (m, 1 H), 7.23 (d, J = 9.0 Hz, 1 H), 7.27 (m, 1 H), 7.33 (m, 1 H), 7.79 (d, J = 8.7 Hz, 1 H). LC/MS: *m*/*z* 298 (M + 1).

2-Chloro-4-[[2-(methyloxy)ethyl](propyl)amino]benzonitrile 3{*2, 3*}. The procedure produced 28.0 mg (74%) of the title compound in >99% purity without further workup or purification. ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.91 (m, 3 H), 1.52 (m, 2 H), 3.25 (s, 3 H), 3.36 (m, J = 5.3 Hz, 2 H), 3.48 (t, J = 5.3 Hz, 2 H), 3.56 (m, 2 H), 6.73 (dd, J = 8.9, 2.0 Hz, 1 H), 6.86 (d, J = 1.8 Hz, 1 H), 7.55 (d, J = 9.0 Hz, 1 H). LC/MS: *m*/*z* 254 (M + 1).

2-Chloro-4-(dipropylamino)benzonitrile 3{*2, 4*}. The procedure produced 25.3 mg (71%) of the title compound in >99% purity without further workup or purification. ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.89 (t, J = 7.3 Hz, 6 H), 1.53 (m, 4 H), 3.31 (m, 4 H), 6.68 (dd, J = 9.0, 2.1 Hz, 1 H), 6.79 (d, J = 1.9 Hz, 1 H), 7.54 (d, J = 9.0 Hz, 1 H). LC/MS: *m*/*z* 238 (M + 1).

2-Nitro-5-(1-piperidinyl)benzonitrile 3{*3, 1*}. The procedure produced 22.5 mg (59%) of the title compound in 93% purity without further workup or purification. ^{1}H NMR

(500 MHz, DMSO-d_6): δ 1.62 (m, 6 H), 3.38 (m, 4 H), 7.29 (dd, J = 8.9, 2.0 Hz, 1 H), 7.65 (d, J = 1.9 Hz, 1 H), 7.77 (d, J = 8.7 Hz, 1 H). LC/MS: m/z 255 (M + 1).

5-(Dipropylamino)-2-nitrobenzonitrile 3{*4, 4*}. The procedure produced 29.8 mg (80%) of the title compound in >99% purity without further workup or purification. ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.91 (t, J = 7.3 Hz, 6 H), 1.57 (m, 4 H), 3.43 (t, J = 7.7 Hz, 4 H), 7.00 (dd, J = 9.6, 2.5 Hz, 1 H), 7.25 (d, J = 2.4 Hz, 1 H), 8.13 (d, J = 9.8 Hz, 1 H). LC/MS: m/z 248 (M + 1).

5-(Di-allylamino)-2-nitrobenzonitrile 3{*4, 7*}. The procedure produced 33.0 mg (90%) of the title compound in >99% purity without further workup or purification. ^{1}H NMR (500 MHz, DMSO-d_6): δ 4.16 (s, 4 H), 5.20 (m, 4 H), 5.86 (m, 2 H), 7.00 (d, J = 9.3 Hz, 1 H), 7.20 (s, 1 H), 8.17 (d, J = 9.5 Hz, 1 H). LC/MS: m/z 244 (M + 1).

5-(4-Morpholinyl)-2-nitrobenzonitrile 3{*4, 33*}. The procedure produced 23.4 mg (67%) of the title compound in >99% purity without further workup or purification. ^{1}H NMR (500 MHz, DMSO-d_6): δ 3.51 (m, 4 H), 3.73 (m, 4 H), 7.26 (m, 1 H), 7.54 (d, J = 1.3 Hz, 1 H), 8.19 (d, J = 9.5 Hz, 1 H). LC/MS: m/z 234 (M + 1).

1-(3-Cyano-4-nitrophenyl)-4-piperidinecarboxamide 3{*4, 40*}. The procedure produced 40.1 mg (90%) of the title compound in >99% purity without further workup or purification. ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.58 (m, 2 H), 1.80 (d, J = 11.6 Hz, 2 H), 2.43 (t, J = 11.2 Hz, 1 H), 3.03 (t, J = 11.9 Hz, 2 H), 4.05 (d, J = 13.2 Hz, 2 H), 6.83 (s, 1 H), 7.31 (s, 1 H), 7.34 (s, 1 H), 7.68 (s, 1 H), 7.79 (d, J = 8.7 Hz, 1 H). LC/MS: m/z 298 (M + 1).

2-Ethyl-1-[4-nitro-3-(trifluoromethyl)phenyl]piperidine 3{*6, 2*}. The procedure produced 28.6 mg (63%) of the title compound in 87% purity without further workup or purification. ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.84 (t, J = 7.4 Hz, 3 H), 1.50 (m, 1 H), 1.58 (m, 1 H), 1.63 (m, 4 H), 1.72 (d, J = 12.4 Hz, 2 H), 3.07 (m, 1 H), 3.86 (d, J = 13.5 Hz, 1 H), 4.15 (s, 1 H), 7.16 (s, 1 H), 7.18 (s, 1 H), 8.05 (d, J = 9.3 Hz, 1 H). LC/MS: m/z 303 (M + 1).

[4-Nitro-3-(trifluoromethyl)phenyl]dipropylamine 3{*6, 4*}. The procedure produced 27.7 mg (64%) of the title compound in >99% purity without further workup or purification. ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.91 (t, J = 7.3 Hz, 6 H), 1.59 (m, 4 H), 3.42 (m, 4 H), 6.94 (s, 1 H), 7.06 (s, 1 H), 8.06 (d, J = 9.3 Hz, 1 H). LC/MS: m/z 291 (M + 1).

Cyclohexyl(methyl)[4-nitro-3-(trifluoromethyl)phenyl]-amine 3{*6, 5*}. The procedure produced 32.0 mg (71%) of the title compound in >99% purity without further workup or purification. ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.17 (q, J = 12.8 Hz, 1 H), 1.44 (q, J = 12.9 Hz, 2 H), 1.57 (m, 2 H), 1.65 (m, 3 H), 1.78 (d, J = 12.4 Hz, 2 H), 2.93 (s, 3 H), 3.85 (t, J = 11.4 Hz, 1 H), 7.05 (s, 1 H), 7.07 (d, J = 10.0 Hz, 1 H), 8.07 (d, J = 9.0 Hz, 1 H). LC/MS: m/z 303 (M + 1).

1-(2-Chloro-4-cyanophenyl)-4-piperidinecarboxamide 3{*7, 40*}. The procedure produced 36.1 mg (91%) of the title compound in >99% purity without further workup or purification. ^{1}H NMR (500 MHz, DMSO-D_6): δ 1.73 (m, 2 H), 1.81 (m, 2 H), 2.29 (m, 1 H), 2.73 (t, J = 22.2, 11.6 Hz, 2 H), 3.40 (d, J = 11.9 Hz, 2 H), 6.82 (s, 1 H), 7.23 (d, J = 8.2 Hz, 1 H), 7.34 (m, 1 H), 7.72 (d, J = 8.5 Hz, 1 H), 7.91 (m, 1 H). LC/MS: m/z 265 (M + 1).

4-[4-Nitro-2-(trifluoromethyl)phenyl]morpholine 3{*8, 33*}. The procedure produced 12.9 mg (31%) of the title compound in >99% purity without further workup or purification. ^{1}H NMR (500 MHz, DMSO-d_6): δ 3.12 (m, 4 H), 3.75 (m, 4 H), 7.58 (d, J = 9.0 Hz, 1 H), 8.39 (s, 1 H), 8.43 (d, J = 9.0 Hz, 1 H). LC/MS: m/z 277 (M + 1).

(4-Nitrophenyl)di-allylamine 3{*15, 7*}. The procedure produced 17.2 mg (53%) of the title compound in >99% purity without further workup or purification. ^{1}H NMR (500 MHz, DMSO-d_6): δ 4.09 (d, J = 4.0 Hz, 4 H), 5.17 (m, 4 H), 5.86 (m, 2 H), 6.75 (d, J = 9.3 Hz, 2 H), 8.03 (d, J = 9.3 Hz, 2 H). LC/MS: m/z 219 (M + 1).

Cyclohexyl(methyl)(4-nitrophenyl)amine 3{*15, 21*}. The procedure produced 22.0 mg (63%) of the title compound in 85% purity without further workup or purification. ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.15 (q, J = 12.9 Hz, 2 H), 1.43 (q, J = 13.0 Hz, 2 H), 1.54 (m, 2 H), 1.65 (m, 2 H), 1.77 (d, J = 12.7 Hz, 2 H), 2.89 (s, 3 H), 3.81 (m, 1 H), 6.84 (d, J = 9.5 Hz, 2 H), 8.03 (d, J = 9.5 Hz, 2 H). LC/MS: m/z 235 (M + 1).

1-[3-Cyano-4-(trifluoromethyl)phenyl]-4-piperidinecarboxamide 3{*17, 40*}. The procedure produced 30.1 mg (77%) of the title compound in 82% purity without further workup or purification. ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.57 (m, 4 H), 2.38 (m, 1 H), 2.93 (t, J = 11.8 Hz, 2 H), 3.99 (d, J = 13.0 Hz, 2 H), 6.82 (s, 1 H), 7.27 (d, J = 7.7 Hz, 1 H), 7.33 (s, 1 H), 7.58 (d, J = 1.6 Hz, 1 H), 7.65 (d, J = 9.0 Hz, 1 H). LC/MS: m/z 260 (M + 1).

4-(4-Morpholinyl)-1-naphthalenecarbonitrile 3{*18, 33*}. The procedure produced 24.0 mg (67%) of the title compound in >99% purity without further workup or purification. ^{1}H NMR (500 MHz, DMSO-d_6): δ 3.13 (s, 4 H), 3.89 (m, 4 H), 7.19 (d, J = 7.9 Hz, 1 H), 7.68 (t, J = 7.7 Hz, 1 H), 7.77 (t, J = 7.4 Hz, 1 H), 8.7 (t, J = 7.4 Hz, 2 H), 8.23 (d, J = 8.5 Hz, 1 H). LC/MS: m/z 239 (M + 1).

1-(4-Cyano-1-naphthalenyl)-4-piperidinecarboxamide 3{*18, 40*}. The procedure produced 13.9 mg (33%) of the title compound in >99% purity without further workup or purification. ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.91 (m, 4 H), 2.37 (m, 1 H), 2.81 (m, 2 H), 3.48 (m, 2 H), 6.86 (s, 1 H), 7.15 (d, J = 7.9 Hz, 1 H), 7.38 (s, 1 H), 7.68 (t, J = 7.4 Hz, 1 H), 7.75 (t, J = 7.4 Hz, 1 H), 8.05 (dd, J = 14.4, 8.1 Hz, 2 H), 8.15 (d, J = 8.5 Hz, 1 H). LC/MS: m/z 280 (M + 1).

AR DNA Preparation. A plasmid containing an N-terminal truncation of the human AR gene was obtained from ATCC which was missing 154 residues from the N-terminus of the protein. The N-terminal region of the AR gene, from a human liver cDNA library generated in-house, was cloned using PCR technique. The N-terminus and C-terminus pieces were linked together through PCR techniques and subcloned into the pSG5 vector at the BamHI site along with a Kozak sequence. The sequence differs from the published sequence in two regions of high variability within the receptor among published sequences. This clone has one additional glutamine

residue (residue 79) and three additional glycine residues (position 475).

MMTV DNA Preparation. pGL3-Basic vector was digested with SmaI and XhoI. pMSG was digested with HindIII blunt ended and then digested with XhoI to excise the pMMTV-LTR. The pMMTV-LTR fragment was then ligated to the SmaI and XhoI sites of pGL3-Basic vector. The resulting plasmid contains the MMTV promoter from position 26 to the XhoI site, followed by luciferase which is contained between the NcoI and SalI (position 3482) sites.

Transient Transfection Assay Protocol. Monkey kidney CV-1 cells (ECACC No. 87032605) were transiently transfected with Fugene-6 reagent according to the manufacturer's protocol. Briefly, a T175 flask of CV-1 cells at a density of 80% confluency was transfected with 25 μg of mix DNA and 75 μL of Fugene-6. The DNA mix (1.25 μg pAR, 2.5 μg pMMTV luciferase and 18.75 μg pBluescript (Stratagene)) was incubated with Fugene in 5 mL OptiMEM-1 for 30 min and then diluted up to 20 mL in transfection media (DMEM containing 1% Hyclone, 2 mM L-Glutamine and 1% Pen/Strep) prior to addition to the cells. After 24 h, cells were washed with PBS, detached from the flask using 0.25% trypsin and counted using a Sysmex KX-21N. Transfected cells were diluted in assay media (DMEM containing 1% Hyclone, 2 mM L-Glutamine and 1% Pen/Strep) at 70 cells/μL; 70 μL of the cell suspension was dispensed to each well of white Nunc 384-well plates, containing compounds at the required concentration. For the curve determination, there were 11 concentration points using 4-fold dilutions from a starting compound concentration of 1 μM. After 24 h, 10 μL of Steady Glo was added to each well of the plates. Plates were incubated in the dark for 10 min before reading them on a Viewlux reader. All data were normalized to the mean of 16 high and 16 low control wells on each plate. A four-parameter curve fit of the following form was then applied.

$$y = \frac{a - d}{1 + (x/c)^b} + d$$

Where a is the minimum, b is the Hill slope, c is the XC_{50}, and d is the maximum. Data is presented as the mean pXC_{50} with the standard deviation of the mean of n experiments.

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Supporting Information Available. Procedures for FP binding assays, representative LC/MS and ^{1}H NMR spectra of the final crude products, and the final purity and yield of all products. This material is available free of charge via the Internet at http://pubs.acs.org.

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