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Preparation of 4-aryl-2-trifluoromethylbenzonitrile derivatives as androgen receptor antagonists for topical suppression of sebum production

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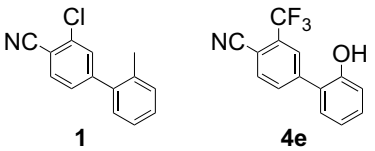
Abstract—A series of substituted 4-aryl-2-trifluoromethylbenzonitrile analogs were evaluated in the human androgen receptor binding and cellular functional assays. Analogs with sufficient in vitro binding and cellular potency (IC_{50} < 200 nM) were tested in the progesterone receptor binding assay for selectivity and in the Golden Syrian hamster ear model for in vivo efficacy. Within the series, compound **4e** was identified to be the most active analog in vivo (wax ester inhibition = 86%).

One of the most common skin conditions treated by physicians is acne vulgaris, which affects about 17 million people in the USA. In addition to permanent scarring, many people with this disease develop psychological problems such as anxiety, social inhibition and depression.[1] Sebum production is a pathogenic _factor that increases the onset of acne vulgaris infections in the pilosebaceous unit. The production of sebum is regulated by the androgens testosterone and 5α-dihydrotestosterone (5α-DHT). Testosterone is converted to 5α-DHT by the Type I 5α-reductase enzyme prevalent in the sebaceous glands and epidermis.[2] Furthermore, DHT is thought to mediate androgen-dependent skin diseases such as hirsutism, acne, and androgenic alopecia.[3] Androgen receptor (AR) antagonists known to suppress sebum production when applied topically are steroidal cyproterone acetate[4,5] and the hydantoin analog RU-58841.[6] There is an increased need for additional non-steroidal AR antagonists for topical applications.

Earlier work in the Pfizer laboratories identified the biphenyl derivative **1** as a compound that bound to the androgen receptor, but exhibited weak AR agonist activity. This compound was reevaluated as part of a project to identify androgen receptor modulators for controlling sebum production and was found to be potent as an AR antagonist (cellular IC_{50} = 3 nM). However, due to the potential phototoxicity of the aryl chloride group,[7] the chloride of compound **1** was replaced with a trifluoromethyl group. In this paper, we report a series of potent AR antagonists, some of which are active in both the binding and cellular functional assays. In particular, we highlight the analog **4e** as the most potent example, both in vitro and in vivo, within the 4-aryl-2-trifluoromethylbenzonitrile series.



1 **4e**

The preparation of the biphenyl analogs described is outlined in Scheme 1.[8] Synthesis began with the diazotization of commercially available aniline **2**, followed by iodine insertion to afford the aryl iodide **3** in 80% yield over two steps. Further elaboration to their biphenyl targets **4a–m** was achieved under Suzuki coupling conditions with the appropriate aryl boronic acid.

Keywords: Androgen receptor antagonists; Sebum production inhibitors; Biaryl analogs.
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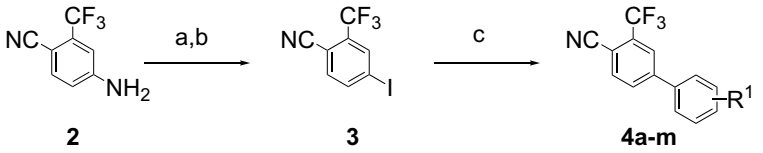


Scheme 1. Reagents and conditions: (a) $NaNO_2$, H_2O/HCl, 0 °C; (b) KI, H_2O, 0 °C to rt, 18 h; 80% over 2 steps; (c), Ar–$B(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, DME/H_2O, 80 °C, 16 h, 62–88%.

All new analogs were evaluated in the human androgen receptor binding assays.[9,10] AR binding was assessed by a competitive [^{3}H]DHT ligand binding assay using recombinant human AR. Compounds that exhibited potent binding affinity (IC_{50} < 200 nm) were tested in a human androgen receptor cellular functional assay utilizing an MDA-MB453-MMTV-luci cell line for their biological activities on human AR. A human progesterone receptor (PR) binding assay was used to investigate the selectivity of the analogs versus AR.

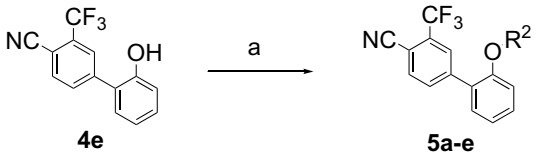
The structure–activity relationship (SAR) of the 4-aryl-2-trifluoromethylbenzonitriles was explored for various substitutions on the B-ring (Table 1). Analogs were chosen for preparation based upon properties required for enhanced transdermal delivery ($c\log P$ = 2–4, M_W < 400).[11]

In general, the receptor binding potency was dictated by the substitution pattern of the B-ring (2- > 4- ≫ 3-). Additionally, the most potent 2-substituted analog in the functional assay was the hydroxyl compound **4e**. However, 2,6-di-hydroxy substitution (**4m**) resulted in loss of binding to the receptor. Multiple substitution of the B-ring also resulted in a decrease in cellular potency (**4l**).

The 2-OMe compound (**4b**) was not as potent as its desmethyl counterpart; however, it still exhibited both good androgen receptor binding as well as cellular activity. Thus, we hypothesized that a ligand with enough bulk and/or length at the 2-position may interact with the receptor at the ligand-binding domain to such an extent as to alter the conformation of the H12 helix,[6] and therefore achieve improved antagonistic activity over compound **4e**. To this end, the hydroxyl group of compound **4e** was alkylated as indicated in Scheme 2.[12]

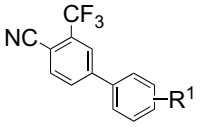
As shown in Table 2, there was a dramatic decrease in binding potency as the size of the alkyl groups increased (compounds **5a–e**).

Substitution of the 2-hydroxy group of compound **4e** was found to be detrimental to androgen receptor bind-



Scheme 2. Reagent and condition: (a) R^2–Br, K_2CO_3, acetone, rt, 72–77%.

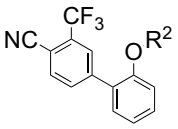
Table 1. Human androgen and progesterone receptor binding activities and calculated log *P* (BioByte) values for biphenyl analogs[a]



Compound	R^1	$c\log P$	ARB IC_{50} (nM)	ARCELL IC_{50} (nM)	PRB IC_{50} (nM)
4a	2-Cl	4.81	9.8	nt	nt
4b	2-OMe	3.79	37.0	20.1	3250
4c	3-OMe	4.35	955	140	9460
4d	4-OMe	4.35	56.7	0.8	5520
4e	**2-OH**	**3.55**	**39.9**	**0.02**	**4450**
4f	3-OH	3.85	229	nt	nt
4g	4-OH	3.85	61.2	11.9	>10,000
4h	2-Me	4.55	146	>1000	nt
4i	3-Me	4.85	1900	nt	nt
4j	4-Me	4.85	200	843	nt
4k	2-CF_3	5.23	10.9	>1.0	nt
4l	2,6-$(OMe)_2$	3.27	14.7	399	nt
4m	2,6-$(OH)_2$	2.64	989	nt	nt

[a] Values (IC_{50}) are given as an average of 2–12 experiments; nt, not tested; ARB, human androgen receptor binding assay; ARCELL, human androgen receptor cellular functional assay; PRB, human progesterone receptor binding assay.

Table 2. Human androgen receptor binding activity and calculated log *P* (BioByte) values for ortho-substituted biphenyl analogs[a]



Compound	R^2	*c*log *P*	ARB, IC_{50} (nM)	ARCELL, IC_{50} (nM)
5a	CH_2CO_2Me	3.41	1310	nt
5b	CH_2CO_2H	3.08	>10,000	nt
5c	$(CH_2)_2OMe$	3.68	2230	nt
5d	$(CH_2)_2OH$	2.91	1540	nt
5e	$(CH_2)_2O(CH_2)_2OMe$	3.54	8140	nt

[a] Values (IC_{50}) are given as an average of 2–12 experiments; nt, not tested; ARB, human androgen receptor binding assay; ARCELL, human androgen receptor cellular functional assay.

ing. Thus, the most biologically potent analog (**4e**) was studied for its effect in vivo. It is known that there is a direct correlation between wax ester reduction and a reduction in total sebum production.[13,14] As it measures the reduction in wax esters, the Golden Syrian hamster ear model is widely used as an in vivo model for testing drug effects on sebaceous glands.[15] Compound **4e** was evaluated in this in vivo model and exhibited excellent activity. Tested topically, compound **4e** exhibited an 86% reduction in wax esters versus vehicle (3% dose in 70/30 ethanol/propylene glycol). To compare, the positive control (RU-58841) reduced wax esters 95% as a 1% formulation.

In summary, the 2-hydroxy compound **4e** was shown to be the most potent compound in the functional assay from the 4-aryl-2-trifluoromethyl-benzonitrile series. Although in vitro potency was maintained for the 2-hydroxy and 2-methoxy analogs (**4e** and **4b**), increased steric bulk at this position led to a loss of receptor binding. Compound **4e** exhibited good efficacy in the in vivo model (86% reduction of wax esters); however, further testing of this compound revealed a potential for phototoxicity.

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8. The general experimental procedure for preparing 4-aryl-2-trifluoromethylbenzonitriles is described below with the synthesis of analog **4c** as an example. *3′-Methoxy-3-trifluoromethyl-biphenyl-4-carbonitrile* (**4c**): To a stirred solution of 4-amino-2-(trifluoromethyl)benzonitrile (9.8 g, 52.9 mmol) in cHCl/H_2O (1:1, 100 mL) at 0 °C was added slowly a $NaNO_2$ (4.4 g, 63.4 mmol) solution in water (15 mL). After stirring for 20 min at 0 °C, a solution of KI (17.6 g, 106.0 mmol) in water (25 mL) was added slowly. After stirring for 4 h, the reaction mixture was extracted with dichloromethane (4 × 100 mL). The combined organic extracts were washed with 5% aq NaOH (300 mL), 5% aq $NaHCO_3$ (300 mL), and brine (300 mL). The combined organics were dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude solid was purified by flash chromatography (8:1 hexanes/EtOAc) to afford the intermediate aryl iodide **3** as a white crystalline solid (12.6 g, 80% yield). A mixture of the aryl iodide **3** (5.0 g, 16.8 mmol), K_2CO_3 (7.0 g, 50.5 mmol), and 3-methoxybenzeneboronic acid (3.0 g, 26.8 mmol) was suspended in DME/H_2O (80 mL/8 mL) at rt. The mixture was degassed with nitrogen and $Pd(Ph_3P)_4$ (1.0 g, 1.1 mmol) added. The reaction mixture was heated at 80 °C over 18 h. After this time, the mixture was diluted with EtOAc/H_2O (1:1, 100 mL) and filtered through a pad of celite. The filtrate was collected and the phases separated. The organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo. Purification by flash chromatography (10:1 hexanes/EtOAc) afforded the title compound as a colorless solid (2.9 g, 62%); TLC: R_f = 0.25 (4:1 hexanes/EtOAc); MS (APCI$^-$)*m/z* 277.1 (M^+); ^{1}H NMR (400 MHz, DMSO-d_6)δ 8.18–8.24 (m, 3H), 7.36–7.46 (m, 3H), 7.02–7.11 (m, 1H), 3.83 (s, 3H). CHN found: C, 64.97; H, 3.46; N, 4.92%. $C_{15}H_{10}F_3NO$ requires: C, 64.98; H, 3.64; N, 5.05%.
9. The androgen receptor binding assay was run essentially as described in Liao, S.; White, D.; Schilling, K.; Chang, C. *J. Steroid Biochem.* **1984**, *1*, 11, The human AR cDNA cloned in baculovirus was expressed in Sf9 cells. Cell lysates from transfected Sf9 cells were isolated and used as the source of human AR in the radio-ligand binding assay. Different concentrations of test compounds (10,000, 1000, 200, 40, 8, 1.6, and 0.16 nM) were incubated in the presence of human AR extract, hydroxylapatite, and 1 nM ^{3}H-DHT for one hour at 4 °C with gentle rocking. After incubation, plates were placed on a filter apparatus and the reaction mixture was removed under 15 psi vacuum pressure, then washed three times with cold buffer. The plates were then dried at room temperature overnight. The next day, scintillation fluid was added to each well and the plates were counted using a Microbeta Trillux. To determine non-specific binding, 1000-fold concentration of cold DHT was added to each well. Triplicate wells were tested for each concentration. The experimental results

were expressed as the concentration of compound at which 50% of maximum binding was inhibited (IC_{50}). Compound RU-58841 was run as a standard revealing an inter-run variability within 2-fold.

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12. The general experimental procedure for preparing 2′-*O*-alkylated-4-aryl-2-trifluoromethylbenzo-nitriles is described below with the synthesis of analog **5a** as an example. *Methyl{[4′-cyano-3′-(trifluoromethyl)biphenyl-2-yl]oxy}acetate* (**5a**): Methyl bromoacetate (0.09 mL, 145 mg, 0.95 mmol) was added dropwise to a stirred solution of compound **4e** (250 mg, 0.95 mmol) and K_2CO_3 (132 mg, 0.96 mmol) in acetone (5 mL) at rt. After stirring for 5 h, the mixture was filtered through a pad of celite and the filtrate concentrated in vacuo. The crude solid was purified by flash chromatography (10:1 to 6:1 hexanes/EtOAc gradient elution) to afford the title compound as a white solid (230 mg, 72% yield); TLC: $R_f = 0.43$ (1:1 hexanes/EtOAc); MS (ESI^+) = 336.1; ^1H NMR (400 MHz, DMSO-d_6)δ 8.19–8.24 (m, 2H), 8.06 (dd, $J = 8.4$, 1.6 Hz, 1H), 7.46–7.50 (m, 1H), 7.42 (ddd, $J = 8.4$, 7.4, 1.6 Hz, 1H), 4.89 (s,2H), 3.67 (s, 3H). CHN found: C, 61.17; H, 3.54; N, 4.12%. $C_{17}H_{12}F_3NO_3$ requires: C, 60.90; H, 3.61; N, 4.18%.
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