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Diphenyl ethers as androgen receptor antagonists for the topical suppression of sebum production

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ABSTRACT

A series of diphenyl ethers was prepared and evaluated for androgen receptor antagonist activity in human androgen receptor binding and cellular functional assays. Analogs with potent in vitro activities were evaluated for topical in vivo efficacy in the Golden Syrian Hamster ear model. Several compounds showed reduction in wax esters in this validated animal model.

The androgen receptor (AR) is responsible for the activation of genes involved in the pathogenesis of acne and alopecia.[1] The onset of acne vulgaris infections in the pilosebaceous unit is associated with excess sebum production. The production of sebum is regulated by the androgens testosterone and 5α-dihydrotestosterone (5α-DHT). Androgen receptor antagonists, such as cyproterone acetate[2,3] and RU-58841[4] have been shown to suppress sebum production when applied topically.

Mining of data from our AR agonist program identified a number of compounds that bound to the androgen receptor but did not possess agonist activity. A reevaluation showed that some of these were full antagonists, for example diphenyl ethers such as **1**, and we have used them as starting points for our program to identify AR antagonists (Fig. 1) for the topical suppression of sebum production.[5]

The chlorine of hit **1** could be relocated from the 2-position to the 3-position (**2**) with retention of AR binding (ARB)[6] and full antagonist activity in a cellular assay (ARCELL)[7] (Table 1). Unfortunately, the chlorobenzonitriles exhibited photoinstability which is not desirable in a topical agent. We therefore examined the 3-trifluoromethylbenzonitrile template based on precedence of known AR binders.[8] Gratifyingly the initial tolyl analog, **3a**, retained AR binding activity, a full antagonist profile, and was photostable but, unfortunately, it lost appreciable cellular activity.

The compounds were synthesized by phenol displacement of the desired *para*-fluorobenzonitrile template under basic conditions (Scheme 1).[9]

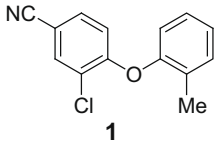

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Figure 1. Initial AR antagonist lead compound.

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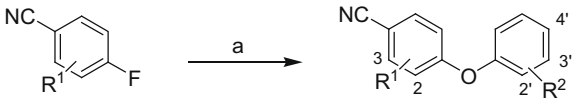
Table 1
Initial SAR exploration[a]

Compound	R^1	R^2	ARB (nM)	ARCELL (nM)	cLog*P*
1	2-Cl	3′-Me	64	46	4.66
2	3-Cl	2′-Me	30	57	4.76
3a	3-CF_3	2′-Me	92	743	5.06
3b	3-CF_3	3′-Me	770	—	5.06
3c	3-CF_3	4′-Me	4000	—	5.06
4a	3-CF_3	2′-Cl	123	—	5.04
4b	3-CF_3	3′-Cl	2720	—	5.27
4c	3-CF_3	4′-Cl	6650	—	5.27
5a	3-CF_3	2′-OMe	83	72	3.64
5b	3-CF_3	3′-OMe	>10,000	—	3.89
5c	3-CF_3	4′-OMe	>10,000	—	3.89

[a] Values (IC_{50}) are given as an average of ⩾2 experiments; —, not tested.



Scheme 1. Reagents and conditions: (a) ArOH, K_2CO_3, DMF, 60 °C.

Within the trifluoromethylbenzonitrile series, we explored substitution of the second phenyl ring. We found that when the R^2 substituent was held constant and stepped around the ring AR binding followed the trend *ortho* > *meta* > *para* (Table 1).

Having determined that *ortho*-placement of R^2 was preferred we looked at a variety of substitution in this position (Table 2). Increasing the size of the *ortho* R^2 substituent from methyl to ethyl (**6a**) was tolerated while a decrease in AR binding was seen with the propyl (**6b**), phenyl (**6c**), and isopropyl (**6d**) analogs.

Introducing a second *ortho*-methyl (**6e**) was tolerated. However, further substitution, as in the mesityl analog **6f**, was detrimental to AR binding.

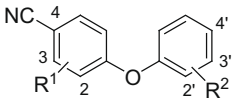
Replacement of the *ortho*-methyl with hydroxy (**6g**), methoxy (**5a**), and methylthio (**6j**) was tolerated in terms of AR binding and in all cases gave good cellular activity (ARCELL < 100 nM). These analogs have lower cLog*P* than compounds **6a–f** which may account for the observed cellular activity. Cellular activity was retained in the change from methoxy (**5a**) to ethoxy (**6i**). However, the ethylthio analog (**6j**) lost cellular activity compared to the methylthio analog (**6h**).

Table 2
SAR of trifluoromethylbenzonitriles[a]

Compound	R^2	ARB (nM)	ARCELL (nM)	cLog*P*
6a	2′-Et	20	356	5.58
6b	2′-Pr	202	303	6.11
6c	2′-Ph	430	—	5.88
6d	2′-^{i}Pr	2280	—	5.58
6e	2′, 6′-di-Me	10	681	5.55
6f	2′, 4′, 6′-tri-Me	198	812	6.05
6g	2′-OH	34	97	3.64
5a	2′-OMe	83	72	4.13
6h	2′-SMe	74	22	4.72
6i	2′-OEt	120	47	5.15
6j	2′-SEt	229	545	5.24
6k	2′,6′-di-OMe	157	>1000	3.86
6l	2′-OMe, 4′-Me	69	299	4.62
6m	2′-OMe, 5′-Me	103	482	4.62
6n	2′-OMe, 5′-CH_2OH	216	>1000	3.09
6o	2′-OEt, 4-Me	257	—	5.15
6p	2′-OH, 4-Me	354	—	4.14
6q	2′-OH, 5-Me	127	811	4.14
6r	2′-OMe, 6′-F	158	42	4.21

[a] Values (IC_{50}) are given as an average of ⩾2 experiments; —, not tested.

Table 3
In vivo efficacy in Golden Syrian hamster model[a]



Compound	R^1	R^2	WE reduction (%)	CE reduction (%)
1	2-Cl	2′-Me	90	76
2	3-Cl	2′-Me	87	71
6i	3-CF_3	2′-OEt	82	67
6r	3-CF_3	2′-OMe, 6′-F	41	37
6h[b]	3-CF_3	2′-SMe	36	32

[a] All compounds were tested at dose of 3% in a polyethylene glycol/transcutol/ethanol 20/20/60 v/v/v% formulation unless otherwise stated.
[b] Polyethylene glycol/ethanol 30/70 v/v% formulation.

A variety of disubstituted analogs possessing either an *ortho*-hydroxy, methoxy, or ethoxy substituent (**6k–q**) lost AR binding and/or cellular activity when compared to the compounds with only one *ortho*-substituent. Analogs **6k** and **6n** were not cellularly active despite their lower cLog*P* values. The only disubstituted analog which retained good cellular activity was **6r**.

Selected compounds with good in vitro profiles were tested in vivo in Golden Syrian Hamsters for their ability to reduce wax and cholesterol esters (Table 3). Wax and cholesterol esters constitute 28% of total human sebum[10] and it has been shown that there is a direct correlation between reduction in wax esters and reduction in total sebum production in a clinical trial with oral cyproterone acetate.[11] The Hamster Ear model is a widely used animal model to test drug effects on sebaceous glands.[12]

Compounds **1**, **2**, and **6i** exhibited the best in vivo profiles with >80% reduction of wax esters when applied topically as a 3% formulation. By comparison a 1% formulation of the positive control, RU-58841, reduced wax esters by 95%. Disappointingly, the methylthio analog **6h** showed only marginal reduction of wax esters in vivo despite possessing one of the best in vitro profiles: we had considered that it may have been able to act as a soft-drug[13] by being metabolized prior to systemic exposure, though this result suggests metabolism may have been too rapid.

In summary, we have prepared a series of diphenylethers as androgen receptor antagonists and demonstrated that compounds from this series exhibit reduction in sebum when applied topically in a validated animal model.

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6. The androgen receptor binding assay run was a modification of that 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}). The AR antagonist RU-58841 was run as a standard revealing an inter-run variability within twofold and an average IC_{50} of 16 nM.

7. The androgen receptor cellular functional assay was conducted in a human breast tumor cell line expressing androgen receptor (MDA-MB453-MMTV clone 54-19). The cell line is a stably transfected cell line with MDA-MB453 cell background. A MMTV minimal promoter containing androgen response element (ARE) was first cloned in front of a firefly luciferase receptor gene. Then the cascade was cloned into a transfection vector pUV120-puro. Electroporation was used for transfecting MDAMB-453 cells and a puromycin resistant stable cell line was selected. See: Chang, C.; Wang, C.; DeLuca, H. F.; Ross, T. F.; Shih, C.C.-Y. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 5946. Compounds were tested in this whole cell functional assay for their ability to antagonize the effects of 1 nM DHT on the androgen receptor. The AR antagonist RU-58841 was run as a standard revealing an inter-run variability within twofold and an average IC_{50} of 32 nM.
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9. *Generalized synthetic procedure*: To a stirring solution of the 4-fluorobenzonitrile template (1 mmol) and phenolic derivative (1.5 equiv) in DMF (10 mL) was added solid K_2CO_3 (2 equiv). The reaction mixture was heated at 60 °C until consumption of starting material was seen and then allowed to cool to room temperature. The reaction mixture was then poured into ice water and the resulting precipitate collected by filtration. In examples, where no precipitate was observed ethyl acetate was added and the organic phase was washed with water followed by brine. The organic phase was dried over $MgSO_4$ and concentrated. The resulting residue was then purified by column chromatography on silica using ethyl acetate/heptane mixtures as the eluent.
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12. Male Syrian Hamsters aged 9–10 weeks were introduced into the laboratory environment and acclimated for 2 weeks prior to use in each study. Each experimental group consisted of five animals. Vehicle control and a reference AR antagonist (RU-58841) were included in each experiment. Animals were topically dosed twice daily (BID) for 2 weeks, 5 days a week (Monday–Friday). Each dose consisted of 25 μL of vehicle control or formulated test article, which was evenly applied to ~3 cm^2 of the ventral surfaces of both the right and left ears. Animals were sacrificed approximately 18–24 h after the final dose. The ears were collected from each animal for sebum analysis. The ear samples were prepared for sebum analysis as follows. One 8 mm distal biopsy punch was taken just above the anatomical 'V' mark in the aural cartilage to normalize sample area. The punch was then split into ventral and dorsal layers. The ventral layer, where the topical dose was applied, was retained for sebum analysis. Each ventral side sample was placed in a 1-dram glass vial, flushed with nitrogen gas (N_2), sealed, and stored at −80 °C until for sample lipid extraction and HPLC lipid analysis. See: Plewig, G.; Luderschmidt, C. *J. invest. Dermatol.* **1977**, *68*, 171.
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