4422 J. Med. Chem. 2010, 53, 4422–4427
DOI: 10.1021/jm9018004

Journal of Medicinal Chemistry Article

Rational Design of a Topical Androgen Receptor Antagonist for the Suppression of Sebum Production with Properties Suitable for Follicular Delivery

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Received February 12, 2010

A novel nonsteroidal androgen receptor antagonist, (*R*)-4-(1-benzyl-4,4-dimethyl-2-oxopyrrolidin-3-yloxy)-2-(trifluoromethyl)benzonitrile (**1**), for the topical control of sebum production is reported. This compound, which is potent, selective, and efficacious in the clinically validated golden Syrian hamster ear animal model, was designed to be delivered to the pilosebaceous unit, the site of action, preferentially by the follicular route.

Introduction

The androgen receptor (AR[a]), a ligand-activated nuclear hormone receptor, 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 androgens such as testosterone and 5α-dihydrotestosterone (5α-DHT), and higher levels of these androgens cause the sebaceous glands to become larger and produce more sebum, an effect that can be blocked with AR antagonists (antiandrogens). Extensive research in the last two decades has delivered many examples of nonsteriodal antiandrogens.[2] It has been shown that cyproterone acetate[3] and spironolactone,[4] used as oral contraceptives in women, significantly reduce sebum production and decrease the severity of acne. However, oral administration of AR antagonists for the treatment of acne and oily skin is undesirable due to the potential for systemically driven adverse antiandrogen-mediated side effects. Precedence for topical delivery of antiandrogens to the pilosebaceous unit has been reported with RU-58841 (**2**) (Figure 1), which suppresses sebum production when applied topically in an animal model.[5] We have also previously reported topically delivered nonsteroidal benzonitrile antiandrogens with efficacy in a validated animal model, which were designed to exhibit antiandrogen activity locally, in the pilosebaceous unit, yet be rapidly cleared upon reaching the systemic circulation.[6]

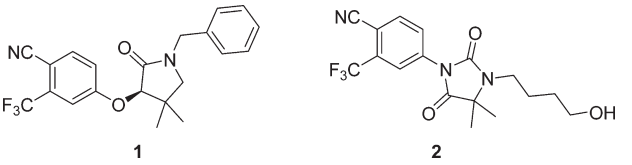

Figure 1. Structures of **1** and RU-58841 (**2**).

Topically applied agents can reach the pilosebaceous unit by either the transepidermal route or the transfollicular (follicular) route, and both pathways occur simultaneously in most cases. The relative contribution to drug delivery through each pathway varies depending on the nature of the formulation and the physicochemical properties of the drug. Drug delivered by the transepidermal route involves distribution into the pilosebaceous unit secondary to absorption into other local skin substructures and the systemic circulation via the dermal capillary bed, so there is potential for adverse side effects due to systemic antiandrogen exposure. Follicular delivery, on the other hand, offers direct access to the pilosebaceous unit without systemic exposure. Targeted follicular delivery of topically applied drugs has increasingly been recognized as an efficient method to enhance local efficacy and reduce systemic exposure.[7] Increased delivery by the follicular route can be achieved by either modification of the formulation[8] or, less commonly, by modification of the physicochemical properties of the drug molecule itself.[9] In the current work, we describe an approach in which we modified the physicochemical properties of our previously reported benzonitrile series of AR antagonists to give compound **1** which we propose is delivered preferentially by the follicular route.

In Vitro Activity and Chemistry

We have previously reported benzonitriles, such as **3**, **4**, and **5** as AR antagonists for the topical control of sebum production and/or androgenetic alopecia (Figure 2).[6] These compounds were designed to occupy the physicochemical property space defined by the Rule-of-Five (Ro5),[10] which

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[a] Abbreviations: AR, androgen receptor; 5α-DHT, 5α-dihydrotestosterone; Ro5, rule-of-five; ARB, androgen receptor binding; ARCELL, androgen receptor cellular activity; PRB, progesterone receptor binding: PR, progesterone receptor; WE, wax esters; CE, cholesterol esters; IVT, in vivo toleration; CL, clearance; BID, twice a day dosing; E_H, extraction ratio; PEG, polyethylene glycol, SEM, standard error of the mean.

 Published on Web 05/12/2010

was derived from an analysis of oral drugs. Modification of compound size, lipophilicity, and polar surface area to property space outside of Ro5 compliance can give increased delivery via the follicular route.[9]

Compound lipophilicity is a particularly important property to consider with regard to designing compounds with increased propensity for distribution into the sebum-rich hair follicle. Compound flux through skin is correlated in a parabolic relationship with lipophilicity, while compound partitioning into sebum increases with increasing lipophilicity (Figure 3).[11] Increased partitioning into sebum and decreased skin flux are both indicators that can be used to suggest increased follicular delivery if evidence is also present that drug has reached the site of action (for example, efficacy in an animal model of sebum control).

We designed and synthesized 540 analogues in the benzonitrile series through targeted libraries and singleton chemistry with cLogP values greater than four (rather than 3 as shown in Figure 3 because we had observed an order of magnitude difference between calculated and measured LogP values for existing compounds) and evaluated them for activity in an AR binding (ARB)[12] and cellular assay (ARCELL).[13] We found that we had a narrow window of increased lipophilicity in which to operate because the percentage of compounds showing good activity in the cellular assay decreased dramatically with increased lipophilicity, presumably due to a decrease in cellular permeability. Potent compounds were further evaluated for their ability to partition into artificial sebum.[14] All compounds were also screened through a progesterone receptor binding assay (PRB)[15] because the PR is the nuclear hormone receptor with highest homology at the ligand-binding region to the AR (80−90%). Compounds with good in vitro profiles were then tested in vivo in golden Syrian hamsters for their ability to reduce wax esters (WE) and cholesterol esters (CE), which are biomarkers for sebum production (Table 1).[16] Compounds **1** and **9** emerged from this evaluation as the lead compounds (Figure 4). However, it was found that sulfide **9** was converted into active metabolites, the sulfones, with long half-lives, so **1** was the compound selected for further characterization of properties predictive for enhanced follicular delivery.

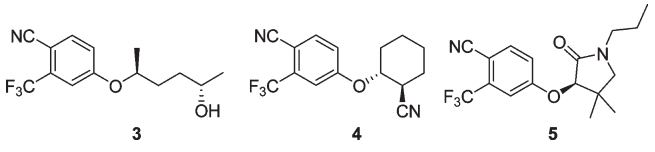


Figure 2. Structures of previous AR antagonists designed for transepidermal topical delivery.

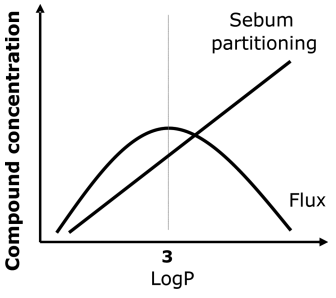


Figure 3. Relationship between lipophilicity, sebum partitioning, and skin flux.

Compound **1** binds to the human AR[17] with an $IC_{50} = 100$ nM and is 44-fold selective versus binding at the PR. This level of selectivity is not anticipated to be problematic because the PR does not have a significant function in skin tissue, so local dermal side effects are unlikely. The compound was designed to be rapidly cleared with very low anticipated systemic exposure at the projected efficacious human dose, so it is unlikely that systemic side effects will emerge. Compound **1** is more than 100-fold selective for the AR compared to various other nuclear hormone receptor binding assays ($IC_{50} > 10\ \mu M$ in binding assays of glucocorticoid, estrogen, and thyroid hormone receptors).

In the AR cellular assay, compound **1** was a full AR antagonist with an $IC_{50} = 38$ nM. Because our in vivo studies were carried out in hamsters, we also evaluated binding of **1** against the hamster AR ($IC_{50} = 27$ nM) and in a hamster cellular assay ($IC_{50} = 104$ nM). The AR binding and cellular activities of the more lipophilic compound **1** are comparable to that seen for the previous benzonitrile compounds designed for transepidermal delivery.[6]

Our previously reported approach to the synthesis of pantolactam benzonitriles like **1** and **9** was not suitable for scale-up because it was lengthy due to a protection−deprotection sequence and it suffered from loss of >50% of the material in a final chiral chromatography step.[18] To supply sufficient quantities of compound **1** for efficacy, safety, and formulation studies, a new short high-yielding route was developed in which the chiral center was purchased cheaply in the form of (*R*)-pantolactone (Scheme 1).[19]

In Vivo Activity

Wax and cholesterol esters constitute 28% of total human sebum,[20] and it has been shown that there is a direct correlation between reduction in WE and reduction in total sebum

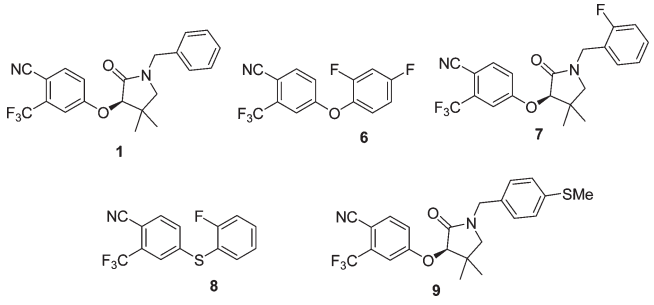


Figure 4. Example structures of compounds designed with increased lipophilicity.

Table 1. Example SAR of Compounds Designed with Increased Lipophilicity

compd	cLogP	ARB[a] (nM)	ARCELL[a] (nM)	PRB[a] (nM)	$\log K_{sebum}$[b]	%CE[c] reduction	%WE[c] reduction
1	4.70	100	38	4400	3.92	61	78
6	4.64	488	60	>10000	4.06	24	31
7	4.84	174	44	4820	3.95	42	72
8	5.11	184	54	6950	4.48	50	64
9[d]	5.26	316	28	>10000	4.6	75	89

[a] Values (IC_{50}) are given as an average of ≥2 experiments. [b] Partitioning into artificial human sebum. [c] All compounds were tested in vivo at a 1% dose unless otherwise stated. [d] Tested in vivo at a 1.5% dose.

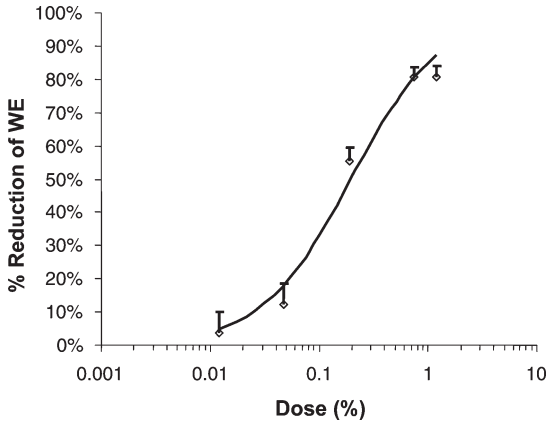
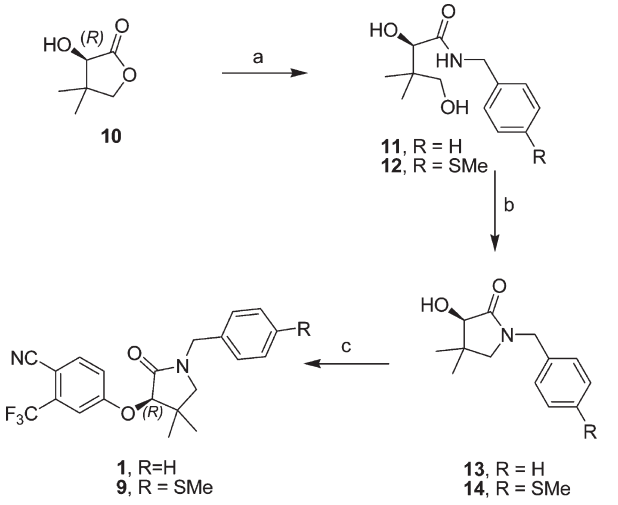


Figure 5. Dose-dependent reduction of sebum production, as measured by the reduction of WE, in the hamster model following 2 weeks BID application of **1** in a vehicle of PEG 400:water:ethanol (25:25:50, w/v).

Scheme 1. Synthetic Route to Lactams **1** and **9** Developed to Supply Bulk Compound[a]



[a] Reagents and conditions: (a) R-benzylamine, toluene, 70 °C (R = H, 87%; R = SMe, 95%); (b) MsCl, Et_3N, NaHMDS (R = H, 61%; R = SMe, 76%); (c) 4-fluoro-2-(trifluoromethyl)benzonitrile, K_3PO_4, NaOH (R = H, 72%; R = SMe, 94%).

production in a clinical trial with oral cyproterone acetate.[21] The hamster ear model, a widely used animal model to test drug effects on sebaceous glands, was used to evaluate **1**.[16]

Compound **1** is a potent inhibitor of WE production in the hamster ear model with an ED_{50} = 0.2% (0.02 mg/cm^2, BID application for 2 weeks) tested in the vehicle selected for phase 1 studies. The dose−response relationship of **1** is shown in Figure 5 (mean ± SEM, N = 5).

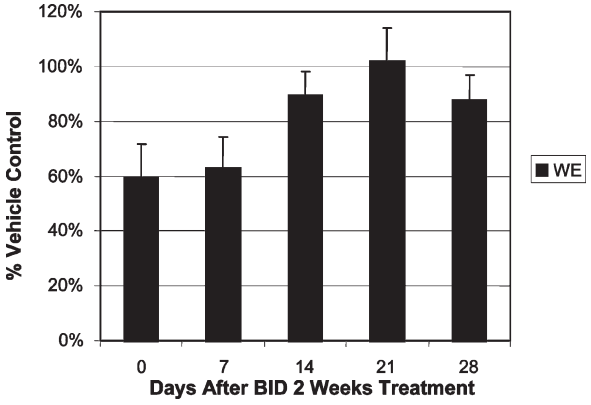
Wax ester reduction caused by **1** correlated well with reduced sebaceous gland size as quantified by fatty acid synthase immunohistochemical staining (Figure 6) (correlation coefficient = 0.972). The reduction in sebaceous gland size was not associated with histopathological evidence of cytotoxic changes in sebocytes after 2 weeks of BID application. The reduction of sebaceous gland size and WE production were fully reversible after 2 weeks BID application of **1** (Figure 7, mean ± SEM, N = 5).

Evidence for Follicular Delivery

Compound **1** retains efficacy in the in vivo model even though transepidermal flux, in both human and hamster skin,

Figure 6. Reduction of WE induced by compound **1** correlates with the reduction of sebaceous gland size in the hamster ear model.

Figure 7. Reduction of WE induced by compound **1** is fully reversible.



Note: The reversibility was studied after the animals were treated BID for 2 weeks with 0.2% of **1** in a vehicle of ethanol:propylene glycol:transcutol (60/20/20, v/v). Mean ± SEM was shown in the figure (N = 5).

is significantly lower than that of the compounds we had previously designed for topical delivery by the tranepidermal route, suggesting that drug has reached the site of action (the pilosebaceous unit) by increased follicular delivery.

Sebum Partitioning. Compound **1** shows increased partitioning into sebum compared to the previous compounds, suggesting that it should favor the sebum-rich follicle. The partition coefficient between sebum and water was determined by equilibrating ~5 mg of drug spiked sebum sample (1 mg/250 μL sebum) with 1 mL of saturated aqueous solution at 37 °C for 15 h and analyzing the drug concentration in the aqueous samples. Compound **1** has the highest sebum partition coefficient when compared to previously evaluated AR antagonists (Table 2), indicating a potential advantage for sebaceous gland-targeted follicular delivery.

Skin Flux. In vitro penetration of **1** through human cadaver skin was evaluated in order to quantify the delivery properties of the compound and formulation. These data were used to select an appropriate formulation for phase 1 studies and to provide necessary information for human exposure projections.

The delivery of **1** through skin from various vehicles was evaluated in human cadaver skin using a Franz Cell system. The amount of tritiated-**1** penetrating through the skin into receptor solution was measured at various time points up to 48 h, and drug levels in the epidermis and dermis were

Table 2. Comparison of Sebum−Water and Octanol−Buffer Partition Coefficients of **1** with Previous Compounds

parameter	**1**	**3**	**4**	**5**
$\log K_{sebum}$[a] human (artificial)	3.92	2.06	3.03	2.74
$\log K_{sebum}$[a] hamster	3.85	*b*	*b*	*b*
*E*logD7.4	4.88	3.73	3.40	3.33

[a] Sebum−water partition coefficient. [b] not determined.

Table 3. Comparison of Skin Flux of Compounds **1** and **4** Using Various Skin Samples

	skin flux (μg/cm^2/h)[a]	
compd	hamster ear	human
1	0.060 ± 0.030	0.005 ± 0.003
4	0.878 ± 0.149	0.081 ± 0.032

[a] Data from multiple donor studies (mean ± SD). Studies were carried out using the phase I vehicle selected for each compound: compound **1**, PEG 400:water:ethanol (25:25:50, w/v); compound **4**, propylene glycol: ethanol (30:70 w/v).

measured at 48 h. The average flux in the phase I vehicle, PEG 400:water:ethanol (25:25:50, w/v), across three donors, with $n = 4$, was 0.005 ± 0.003 μg/cm^2/h. We compared the skin flux of compounds **1** and **4** in their respective phase I vehicles, in both human and hamster skin, in order to determine if there was a difference in the properties of **1** compared to previously described compounds. Hamster ear skin was separated from fresh ears immediately before the study, and human cadaver skin was obtained from a commercial supplier. As shown in Table 3, the flux of **1** and **4** was approximately 10 times higher through hamster ear skin than through human skin. The results also showed that the skin flux of **1** was significantly lower than that of **4** in hamster ear skin, demonstrating a similar reduction of skin flux of **1** versus **4** in skin samples from both species. Furthermore, as the ED_{50} values in the hamster ear sebum model for these two compounds are similar (0.2%), these data indicate that efficacy of **1** in the hamster model is not directly dependent on transepidermal delivery; it is more likely that delivery by the follicular route is the primary route of delivery.

Pharmacokinetics and Safety

An ideal topical drug exerts its desired pharmacological effect locally but then is rapidly inactivated by metabolism once it reaches the systemic circulation. The pharmacokinetic-ADME properties of **1** were evaluated and found to be consistent both with this profile and with characteristics predictive of selective follicular delivery. In addition to the enhanced sebum partitioning and decreased transepidermal flux described previously, delivery of **1** to the pharmacological site of action was demonstrated in hamsters. Compound **1** was readily absorbed into ear sebaceous glands of hamsters following topical application.[22] Maximal concentrations of 156 μM of **1** were reached within 1 h of application of a 0.2% dose and were maintained for 8 h postdose. Thereafter, compound **1** was eliminated from sebaceous glands, with an apparent terminal half-life of ~7 h. Furthermore, **1** was rapidly metabolized in human liver microsomes,[23] with intrinsic clearance and predicted hepatic extraction ratio (E_H) values of 121 mL/min/kg and 0.85, respectively. Following intravenous administration of **1** to rats, mean blood clearance was 57 mL/min/kg. This value is approximately 80% of hepatic blood flow, indicating that **1** is a high clearance compound in rats. Single species allometric scaling[24] of unbound CL in rat predicts high CL for **1** in humans (CL_b = 16 mL/min/kg, E_H = 0.80).[25] Collectively, these results suggest that delivery of efficacious concentrations to the target site of action can be achieved and that the fraction of drug reaching the systemic circulation is likely to be cleared rapidly, thereby minimizing unwanted systemic effects.

We proceeded to investigate the safety profile of compound **1**. It showed no evidence of skin sensitization in a murine local lymph node contact hypersensitivity assay.[26] In an in vivo tolerance (IVT) assessment in minipigs, **1** applied topically exhibited good dermal toleration and minimal systemic antiandrogen effects.[27] An oral IVT study was also carried out in rats and no systemic antiandrogen effects were observed when compound **1** was dosed at 40-fold the anticipated human exposure of projected efficacious dose.[28]

Summary

We have designed and synthesized a novel nonsteriodal AR antagonist, **1**, for the topical suppression of sebum production. Compound **1** has sebum partitioning, skin flux, and in vivo parameters that suggest it should be delivered preferentially by the follicular route.

Experimental Section

General. All reagents and solvents were used as received from commercial sources unless otherwise noted. All chemistry experiments were conducted under an inert nitrogen atmosphere unless otherwise noted. ^{1}H NMR spectra were recorded on a Varian 400 MHz nuclear magnetic resonance spectrometer or a Bruker 400 MHz nuclear magnetic resonance spectrometer. ^{1}H NMR spectra were recorded in $CDCl_3$ or DMSO-d_6, and chemical shifts are reported relative to the residual solvent peak. The following abbreviations were used to assign spectra: s = singlet, d = doublet, t = triplet, q = quartet, s = septet, m = multiplet. Mass spectral analysis was conducted on a Waters Micromass ZQ instrument. Elemental analysis was performed at Quantitative Technologies, Inc., Whitehouse, NJ. The purity of all final compounds was determined to be ≥95% by HPLC, and all final compounds, with the exception of **9**, which was a gum so not tested, gave combustion analysis consistent with the compound molecular formula. All data are available in the Supporting Information.

Synthesis of Key Compound (*R*)-4-(1-benzyl-4,4-dimethyl-2-oxopyrrolidin-3-yloxy)-2-(trifluoromethyl)-benzonitrile (1). Benzylamine (41.9 mL, 384 mmol) was added to a solution of (*R*)-pantolactone (50.0 g, 384 mmol) in toluene (140 mL). The reaction mixture was heated at 70 °C for 7 h, and then hexane (10 mL) was added. The solution was stood in the refrigerator overnight, and the resulting precipitate was collected by filtration, washed with cold hexane, and then allowed to dry to give (*R*)-*N*-benzyl-2,4-dihydroxy-3,3-dimethylbutanamide (**11**) (79 g, 87% yield) as a white solid. MS(APCI+): *m*/*z* 238. MS(APCI−): *m*/*z* 236. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.19 (br s, 1H), 7.25 (m, 5H), 5.40 (m, 1H), 4.44 (m, 1H), 4.22 (m, 2H), 3.78 (m, 1H), 3.20 (m, 2H), 0.80 (s, 3H), 0.78 (s, 3H).

A solution of (*R*)-*N*-benzyl-2,4-dihydroxy-3,3-dimethylbutanamide (**11**) (27.1 g, 114 mmol) in tetrahydrofuran (270 mL) and triethylamine (17.5 mL, 125.5 mmol) was cooled in an ice/salt bath and then a solution of methanesulfonyl chloride (8.9 mL, 114 mmol in 20 mL tetrahydrofuran) was added dropwise over 15 min. The reaction mixture was stirred for 30 min, and then it was added by cannula to a solution of sodium bis(trimethylsilyl)amide (365 mL of a 1 M solution in tetrahydrofuran, 365 mmol) that was cooled in an ice/salt bath. The reaction mixture was allowed to warm to room temperature with stirring

overnight. Saturated ammonium chloride was added (100 mL), and the tetrahydrofuran was removed on the rotovap. The residue was extracted with ethyl acetate (2 × 150 mL), the combined extracts were washed with saturated ammonium chloride and then with 10% citric acid (2 × 150 mL), dried with magnesium sulfate, and concentrated in vacuo. The crude product was purified by flash chromatography on silica eluting with a ethyl acetate/heptane 0% to 70% gradient to give (*R*)-1-benzyl-3-hydroxy-4,4-dimethylpyrrolidin-2-one (**13**) (15.2 g, 61% yield) as a pale-yellow oil. MS(APCI+): *m*/*z* 220; 99.6% by chiral HPLC. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.21 (m, 5H), 4.42 (dd, 2H), 3.98 (s, 1H), 2.87 (2d, 2H), 2.75 (d, 1H), 1.10 (s, 3H), 0.90 (s, 3H).

Potassium phosphate (14.7 g, 69.5 mmol) was added to a solution of (*R*)-1-benzyl-3-hydroxy-4,4-dimethylpyrrolidin-2-one (**13**) (15.2 g, 69.5 mmol) and 4-fluoro-2-trifluoromethylbenzonitrile (13.1 g, 69.5 mmol) in 75 mL of dimethylformamide, and then powdered sodium hydroxide (2.8 g, 69.5 mmol) was added. The reaction mixture was stirred at room temperature for 1.5 h, and then water (200 mL) was added. Extracted with ethyl acetate (2 × 200 mL), the combined extracts were dried over magnesium sulfate and concentrated in vacuo to give a white solid. The crude product was purified by flash chromatography on silica eluting with a ethyl acetate/heptane 0% to 40% gradient to give (*R*)-4-(1-benzyl-4,4-dimethyl-2-oxopyrrolidin-3-yloxy)-2-(trifluoromethyl)-benzonitrile (**1**) 19.3 g, 72% yield) as a white solid; 100% by LCMS, MS(APCI+): *m*/*z* 389; 100% by HPLC: Chiralcel OD, 250 mm × 21 mm, 80/20 hexane:EtOH, 254 nM, flow rate = 0.8 mL/min, injection volume = 10 μL, retention time = 8.43 min. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.75−7.20 (m, 8H), 4.60 (s, 1H), 4.48 (2d, 2H), 3.05 (s, 2H), 1.20 (s, 3H), 1.10 (s, 3H). Specific rotation (Rudolph Research analytical model AutoPol IV): $[\alpha]_D$ = +219° in chloroform. Anal. Calcd for $C_{21}H_{19}F_3N_2O_2$, C, 64.94; H, 4.93; N, 7.21; F, 14.68. Found C, 65.08; H, 4.97; N, 7.23; F, 14.27%.

Supporting Information Available: Experimental conditions for intermediates and final compounds; ^{1}H NMR spectra, LCMS and HPLC chromatograms; lipid analysis for hamster ear samples; experimental conditions for androgen receptor binding assay; androgen receptor cellular assay; male Syrian hamster ear model for sebum reduction; pharmacokinetics on topical application in hamster sebaceous glands; metabolic stability in human liver microsomes; pharmacokinetics in rats following intravenous administration; red blood cell distribution study conditions; local lymph node assay in BALB/c mice; toxicity study in minipigs on topical application; toxicity study in rats following oral dosing. This material is available free of charge via the Internet at http://pubs.acs.org.

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