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Synthesis and biological evaluation of amino-pyridines as androgen receptor antagonists for stimulating hair growth and reducing sebum production

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Abstract—A series of amino-pyridines were synthesized and evaluated for androgen antagonist activities. Among these compounds, (*R*)-(+)-6-[methyl-(1-phenyl-ethyl)-amino]-4-trifluoromethyl-nicotinonitrile was the most active example of this class. This compound displayed potent androgen receptor antagonist activity as well as favorable pharmacokinetic characteristics for a potential topical agent. It also demonstrated remarkable potency for stimulating hair growth in a male C3H mouse model as well as reducing sebum production in the male Syrian hamster ear model.

The androgen receptor (AR) is an important member of the superfamily of nuclear hormone receptors that function as ligand-dependent regulators of transcription. AR is responsible for mediating the physiological actions of the androgens testosterone (T) and 5α-dihydrotestosterone (DHT).[1,2] The testosterone and DHT are thought to govern specific biological functions in different tissues. The AR is responsible for the activation of genes involved in the pathogenesis of acne and androgenetic alopecia in humans.[2] In addition, over-production of androgens during puberty has been shown to play a critical role in the excess sebum production and hair loss.[2] We were particularly interested in topical, non-steroidal AR antagonists, that would possess the desired biological local effects without unwanted systemic side effects, such as feminization and interference with male sexual function and the undesirable attributes of their steroidal counterparts.

During the last two decades, non-steroidal AR antagonists have been extensively investigated.[3,4] Several representative AR antagonists include flutamide (**1**, for the treatment of prostate cancer and hirsutism)[5] nilutamide (**2**, a prostate cancer therapy),[5] bicalutamide (**3**, another therapy for prostate cancer),[6] and RU-58841 (**4**).[7] For the topical skin therapeutic program, we were looking for agents possessing superb in vivo efficacy and rapid clearance. AR antagonists with these characteristics are expected to demonstrate excellent efficacy for stimulating hair growth and reducing oily skin and result in low systemic exposures (Fig. 1).

We constructed a hybrid of the structures of flutamide (**1**) and bicalutamide (**3**) and inserted a nitrogen into the phenyl ring to generate a new series of amino-pyridines (Table 1). The SAR trends of this series were explored. First, several *N*,*N*-disubstituted amino-pyridines were evaluated. Compounds with floppy alkyl side-chains (**5**–**8**) showed reasonable AR binding affinities[8] but low antagonism activity.[9] Tethering the *N*,*N*-di-alkyl chains into a ring resulted in several conformationally restricted analog. These compounds (**9**–**11**) exhibited slightly reduced AR binding affinities but

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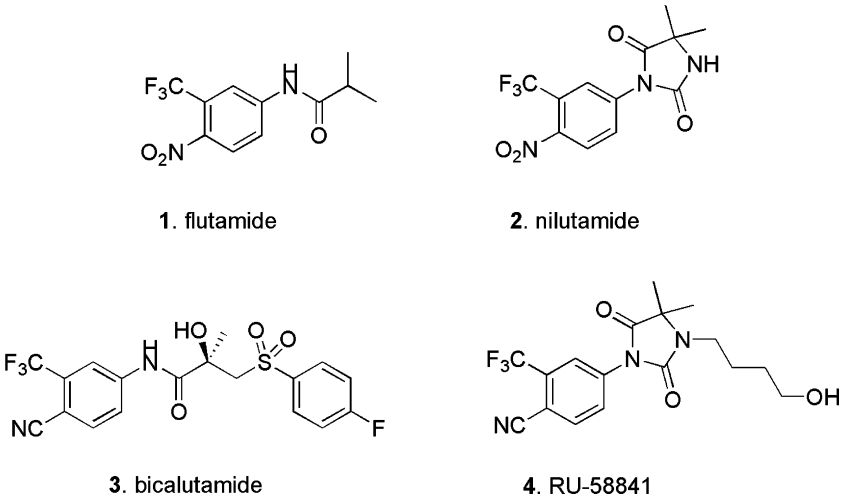
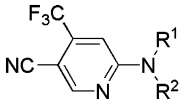
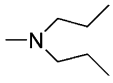
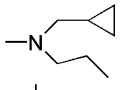
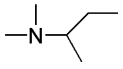
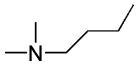
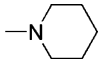
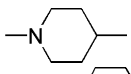
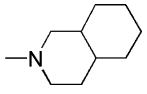
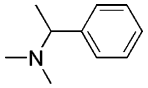
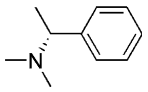
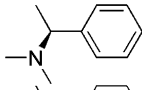
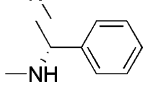


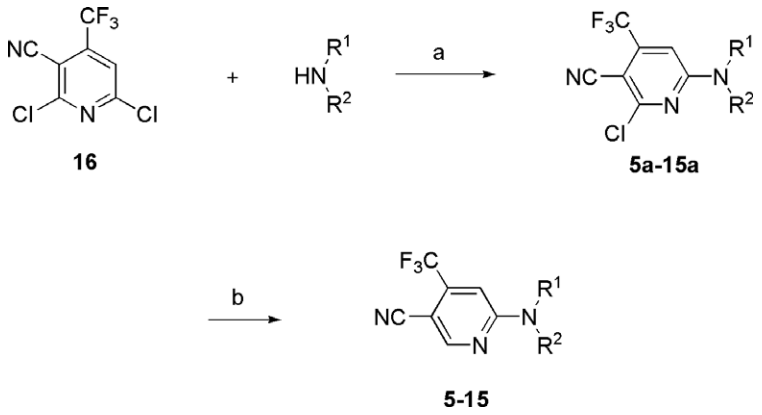
Figure 1. Non-steroidal AR modulators.

Table 1. The in vitro activity AR binding affinity (AR binding) and AR antagonist activity (AR cell antagonism) of amino-pyridines



Compound	$-NR^1R^2$	IC_{50} (nM)	
		AR binding[a]	AR cell[b]
5		78[c]	>1000[c]
6		0.8 ± 0.465	>1000[c]
7		7 ± 0.595	>1000[c]
8		3.8 ± 1.46	>1000[c]
9		209 ± 62.4	422 ± 14
10		73 ± 12.5	136 ± 66
11		169 ± 15.2	260 ± 64.8
12		123 ± 22.6	229 ± 17.3
13		20 ± 5.44	45 ± 19.9
14		1700 ± 595	NA
15		4926 ± 367	NA

[a] $n = 3$, RU-58841 (**4**) as the positive control ($IC_{50} = 15.9 \pm 8.33$); NA, not available.
[b] $n = 3$, RU-58841 (**4**) as the positive control ($IC_{50} = 31.1 \pm 21.7$).
[c] Errors are not available.



Scheme 1. Reagents and conditions: (a) amine (1.1 equiv), K_2CO_3 (5 equiv), DMF, 91 °C; (b) Pd/C, H_2.

significantly improved antagonism activities. To enhance both AR binding and antagonism activity, we further modified the structure of **11** by breaking its piperidine ring, adding a methyl group to the α-methylene position for retaining rigidity, and replacing the cyclohexyl group with a phenyl group. Therefore, we generated other type of conformational restricted analogs for this series, such as **12**. After the chiral resolution of *N*-methyl-α-methylbenzylic analog (**12**), we discovered that its *R*-isomer (**13**) was the most active AR antagonists found in this series. In contrast, its *S*-isomer (**14**) exhibited very weak activity. The importance of the methyl group on the amine was sequentially examined. The *N*-methyl compound (**13**, a more conformationally restricted structure) was more potent than its N–H analog (**15**, ARB IC_{50} = 4926 nM).

The general synthesis of amino-pyridine compounds **5**–**15** is illustrated in Scheme 1.[10] We started from the commercially available **16** and prepared the *N*-alkyl aminopyridines **5**–**15** by an efficient synthetic route developed in our laboratory: a mixture of **16** and the corresponding alkyl amines was heated at 91 °C with K_2CO_3 in DMF for 1–3 h to yield the desired 6-chloro-amino-pyridines **5a**–**15a**. It is noteworthy that under these conditions, the predominant coupling took place at the 2-position to provide compounds **5a**–**15a** as the major products. These intermediates were further de-chlorinated by hydrogenation in the presence of catalytic 20% palladium on carbon. This rapid hydrogenation step (~2–5 min) produced the amino-pyridine **5**–**15** with high yields. And the overall yield was greater than 90% in two steps. The enantiomeric pure compound **13** could be obtained from either of the two methods: (1) chiral resolution of **12**, or (2) using (*R*)-(+)-*N*,α-dimethylbenzylamine–amine as the starting material for the synthesis. We have successfully delivered a large batch of **13** at the scale of 50 g by the latter method. The stereochemistry of **13** was determined from a crystal structure of the intermediate **13a**, prepared via chiral synthesis. While the crystal structure for **13** does prove the regiochemistry of the structure, it does not prove absolute configuration. The asymmetric units of both **13** and **13a** are shown in Figure 2.[11,12]

Compound **13** demonstrated excellent in vivo activity in preclinical models for both hair growth and sebum reduction. This agent was applied topically in a vehicle containing 30% propylene glycol and 70% ethanol. In a male C3H mouse hair growth model,[13] this novel AR antagonist (**13**, at 3% concentration) stimulated the hair growth, and its effect was dose dependent. (Fig. 3). Compared to a known AR antagonist, RU-58841 (**4**), AR antagonist **13** showed a similar level of in vivo activity for promoting hair growth. Furthermore, compound **13** was evaluated in the male Syrian hamster ear model; a widely used animal model to test drug effects on sebaceous glands. Efficacy was measured as reductions in the sebum specific lipid wax esters.[14] Impressively, following topical application for four weeks, compound **13** reduced sebum production significantly. A 3% formulation of **13** reduced wax ester

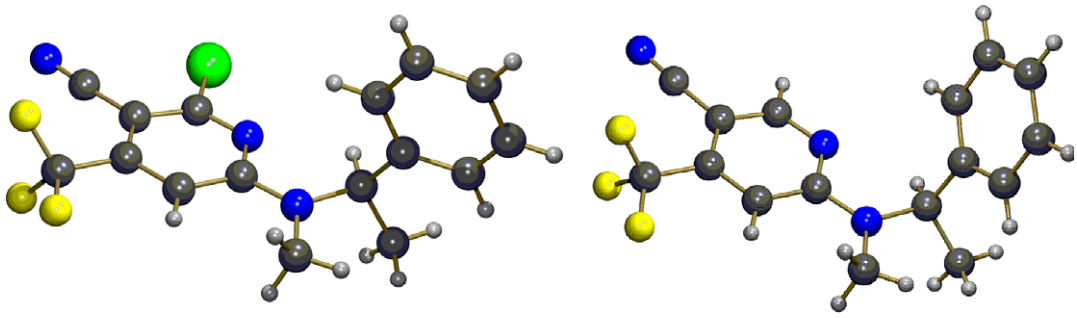


Figure 2. Compound **13a** (left) and Compound **13** (right).

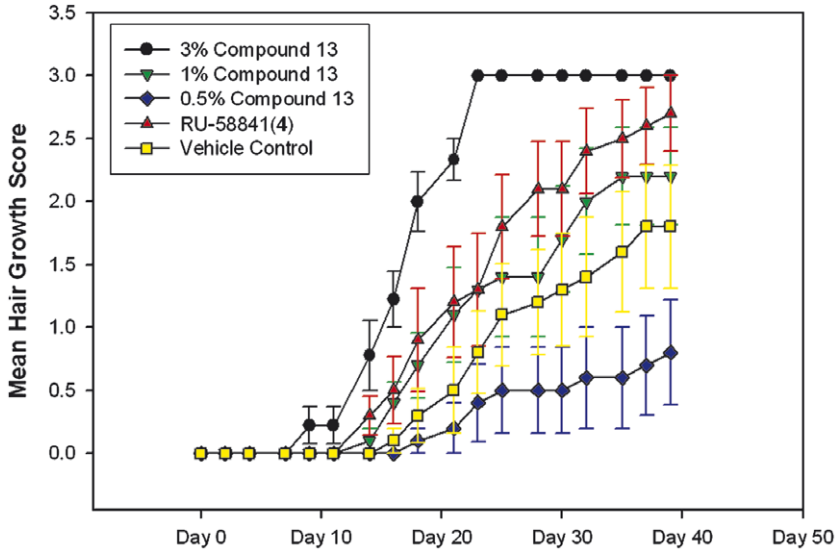


Figure 3. Dose–response relationship of compound **13** tested twice in the male C3H mouse model for hair growth activity.

production by 83%, nearly equivalent to the 92% reduction in wax esters observed with a 3% formulation of RU-58841.

The pharmacokinetic characteristics of compound **13** were favorable for a topical agent. Ideally, topical drugs exert their desired effects locally but are rapidly inactivated via metabolism once they reach the systemic circulation, thereby reducing unwanted systemic effects. Toward this end, compound **13** was rapidly metabolized in rat, dog, and human hepatocytes.[15] Predicted hepatic extraction ratios (E_H) from these in vitro data approached liver blood flow in all three species, with predicted E_H values of 0.97, 0.99, and 0.91, respectively. In vivo clearance data in dogs were consistent with the high clearance predicted in vitro. Following intravenous administration of **13**, mean systemic plasma clearance was 40 mL/min/kg. This value was similar to hepatic blood flow in this species, indicating that **13** is a high clearance compound in dogs.[16]

In summary, the amino-pyridine series was discovered as AR antagonists. In our laboratory, a facile synthesis was developed to facilitate the rapid synthesis for the SAR comparison. Compound **13** showed remarkable in vivo activity for both stimulating hair growth and reducing sebum production and possessed desirable pharmacokinetic profiles for topical applications.

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7. RU-58841: Bernard, E.; Dubois, J.; Wepierre, J. *J. Pharm. Sci.* **1997**, *86*, 573.
8. Androgen receptor binding assays: All of the described new amino-pyridine compounds showed affinity for the androgen receptor. The competitive radio-ligand binding analysis was performed on human AR extracts from trasfected baculovirus/Sf9 cells in the presence or absence of differing concentrations of test agent and a fixed concentration of ^{3}H-dihydrotestosterone (^{3}H-DHT) as tracer. Progressively decreasing concentrations of compounds are incubated in the presence of human AR extracts, hydroxylapatite and, 1 nM ^{3}H-DHT for 1 h at 4 °C. Subsequently, the binding reaction mixtures are washed three times to completely remove excess unbound ^{3}H-DHT. AR bound ^{3}H-DHT levels are determined in the presence of compounds and compared to levels of receptor specific binding when no competitor is present. Compound binding affinity to the human AR is expressed as the concentration of compound at which 50% of the maximum specific binding is inhibited (IC_{50}).
9. Androgen receptor cell assays. Compounds, with AR binding affinity $IC_{50} < 200$ nM, were selected for further testing in a whole cell functional assay (AR cell assay) for their ability to antagonize the effects of DHT on the androgen receptor. 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 pUV120puro. Electroporation was used for transfecting MDA-MB-453cells and a puromycin resistant stable cell line was selected. Results of in vitro assays for the compounds are summarized in Table 1.

10. General Procedure for preparing compounds **5**–**15.** Step 1: The starting material **16** was dissolved in dry DMF (10 mL per 0.5 g of **16**), and 1.1 equiv of the appropriate amines, large excess (at least 5 equiv) of K_2CO_3 was added to the reaction mixture. This reaction mixture was stirred at 90 °C for 2–3 h. Afterwards, the $K_2CO_3$was removed by a filtration, and the DMF was evaporated to dryness. Then the crude product was purified on a silica gel column to yield the desired intermediate **5a**–**15a**. The intermediate was dissolved in dry THF, then 5% TEA and 10%Pd/C were added. The reaction mixture was allowed shaking under a high pressure of hydrogen gas for 2–5 min. The catalyst was filtered out, the solvent was removed, and the desired product **5a**–**15a** was purified by re-crystallization or silica gel chromatography. Step 2: A hydrogenation on **5a**–**15a** with 20% palladium on carbon produced **5**–**15** within 5 min (overall yield 91% in two steps). (*R*)-(+)-6-[methyl-(1-phenyl-ethyl)-amino]-4-trifluoromethyl-nicotinonitrile(**13**): ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.55 (s, 1H), 7.20-7.40 (m, 5H), 6.73 (s, 1H), 6.30 (br, s, 1H), 2.81 (s, 3H), 1.61 (t, 3H, $J = 4.0$ Hz). MS: 306.1 (M+1 for $C_{16}H_{14}N_3F_3$), LC–MS: C-18 Column (50%H_2O/50% CH_3CN), Ret. time: 1.74 min; purity: 99.8%, mp = 47.2-47.7 °C. Anal. Calcd: C, 62.95 %; H, 4.62%; N, 13.76%; Found: C, 62.68%; H, 4.52%; N, 13.69%. [α] +84.79 (EtOH).
11. The crystal structure of **13a**: Structure solved and refined in the orthorhombic space group $P2_12_12_1$. The refined structure fits well to the data with a final R1 index of 4.98% and no missing or misplaced electron density observed in the final difference Fourier. The structure is proof of absolute configuration R with the flack X parameter = 0.09(8).
12. The crystal structure of **13**: Structure solved and refined in the orthorhombic space group $P2_12_12_1$. The refined structure fits well to the data with a final R1 index of 7.24% with no missing or misplaced electron density observed in the final difference Fourier. The structure is proof of connectivity between atoms, the flack parameter being unstable.
13. Male C3H/HeN mice (~6 weeks old with same date of birth) were purchased from Charles River Laboratory (Raleigh, NC). After one week of acclimation, the mice were shaved under isoflurane anesthesia on the lower back using an electric shaver. Only mice in the telogen phase (pink skin) were used in studies. Twenty microliters of test articles at various concentrations in propylene glycol/ethanol (30:70, w/v) or the vehicle control was topically applied to the shaved lower back of the mice to cover an area of approximately 1 cm^2 (20 $\mu L/cm^2$). Ten mice were used in each experimental group. The treatment regimen was twice daily (BID) application for 4 weeks, 5 days/week from Monday to Friday. Local irritation was recorded before each application and hair growth scores were recorded every other day. After 4 weeks of treatment, mice were further observed for one more week during which hair growth and skin irritation were scored every other day. The scale used for scoring hair growth was: 0 = no hair growth, pink skin; 1 = skin color changes from pink to gray or black without visible hair growth, indicating the onset of anagen; 2 = sparse or diffuse short hair growth; 3 = dense, normal coat hair. A reference androgen receptor antagonist (RU-58841, **4**) was included in every study.
14. Male Syrian hamster ear model: 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 5 animals. Vehicle control and a reference AR antagonist (RU-58841, **4**) were included in each experiment. Pfizer PGRD Pharmaceutical Science group at Ann Arbor, Michigan, formulated the test articles. Animals were topically dosed twice daily (BID) for 2 weeks, 5 days a week (Monday to Friday) unless noted otherwise. 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, blown with nitrogen gas (N_2), sealed, and stored at −80 °C until for sample lipid extraction and HPLC lipid analysis. [Plewig, G.; Lunderschmidt C. *J. Invest. Dermatol.* **1977**, *68*, 171].
15. Metabolic stability in hepatocytes: Cryopreserved rat (Sprague–Dawley), dog (beagle), and human hepatocytes were incubated with **13** (1 μM) in Leibovitz's L-15 media at 37 °C. The target cell density and viability were 0.5×10^6/mL and $\geqslant$80%, respectively. Control incubations consisted of substrate in media without hepatocytes. Sample aliquots were collected at various time intervals up to 180 min and quenched with two volumes of cold acetonitrile. Concentrations of **13** were determined using a LC/MS/MS method. Half-life values were determined from a semi-log plot of time vs. concentration and were scaled to E_H ratios as reviewed by Thomas et al. [*Expert Opin. Drug Metab. Toxicol.* **2006**, *2*, 591].
16. Pharmacokinetics in dog: Male beagle dogs ($n = 3$) were administered a 0.5 mg/kg intravenous dose of **13** as a 5 min infusion at a dose volume of 0.5 mL/kg. Compound **13** was formulated as a solution in a vehicle consisting of 20% ethanol, 30% PEG 400, and 50% saline. Serial blood samples were collected from each dog over a 24 h period postdose. Plasma concentrations of **13** were determined using a LC/MS/MS method, and pharmacokinetic parameters of **13** were determined from the plasma concentration-time data using noncompartmental methods.