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Thyroid receptor agonists for the treatment of androgenetic alopecia

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ABSTRACT

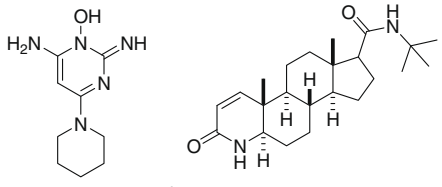
A thyroid hormone receptor β subtype-selective thyromimetic **5** was found to be efficacious in both mouse and monkey hair growth models after topical applications. It penetrates the skin according to the test in human cadaver skin mounted onto Franz diffusion chambers. The serum drug level of **5** is below the limit of quantification during tests in the bald stump-tailed macaques (*Macaca arctoides*). It is tested negative in the 3T3 neutral red uptake (NRU) phototoxicity test, indicating a low risk for causing photo-irritation. It is also rapidly metabolized according to the PK data, thus the systemic exposure is limited.

Androgenetic alopecia, also known male pattern baldness, occurs not only in men but also in women. Current treatments such as minoxidil (**1**, Rogaine®, a potassium channel opener) and finasteride (**2**, Propecia®, a 5α-reductase inhibitor) are only efficacious in a small percentage of subjects. Novel mechanisms with robust efficacy and long-term safety are still needed for the treatment of androgenetic alopecia (see Fig. 1).

Thyroid hormone receptor agonists (thyromimetics) are closely associated with hair growth. Topical triiodo-thyronine (T3, **3**) stimulates epidermal proliferation, dermal thickening, and hair growth in both mice and rats.[1] Some human subjects given with thyroxine (T4, **4**) to treat thyroid hormone deficiency reported hair growth as a *side effect*. Thyroid hormones are also found to directly alter human hair follicle functions including anagen prolongation and stimulation of both hair matrix keratinocyte proliferation and hair pigmentation.[2] Thyroid receptors (TR) interact with hairless gene product (Hr), a transcription factor required for hair growth and thyroid hormone receptor β1 is expressed in the human hair follicles.[3] Recently, human female hair follicles were found to be a direct, nonclassical target for thyroid-stimulating hormone.[4] However, use of oral thyroxine or thyroid hormones to treat androgenetic alopecia is impractical due to their known cardiotoxicity. Therefore, we sought thyromimetics that are active topically and yet devoid of systemic pharmacological activities to avoid deleterious side effects. We set out to find soft drugs with high systemic clearance as indicated by pharmacokinetic (PK) data such as short-half-life in human liver microsome (HLM) and fast hepatic clearance (see Fig. 2).

Our initial drug discovery program led to a novel series of 6-azauracil-based thyroid hormone receptor ligands as potent, TRβ subtype-selective thyromimetics.[5] Although our original goal was to search for anti-obesity drugs by taking advantage of the thermogenic potential of thyromimetics, preliminary tests revealed that some of these compounds had the potential to treat hair loss as well after topical applications.[6] Herein, we report our selection, animal model studies, discovery and improved synthesis of PF-00277343 (**5**), a thyroid receptor agonist with preclinical efficacy for hair growth after topical applications.

A group of potent, TRβ subtype-selective thyromimetics were tested in the C3H/HeN mouse model, a validated hair growth model.[7a] C3H/HeN mice provide a good model for studying the hair growth in vivo because they have synchronized hair cycles, alternating between periods of anagen, catagen, and telogen.[7b] As shown in the Table 1, after topical applications, thyromimetics **6**



1, minoxidil (Rogaine®) **2**, finasteride (Propecia®)

Figure 1. Current treatments for androgenetic.

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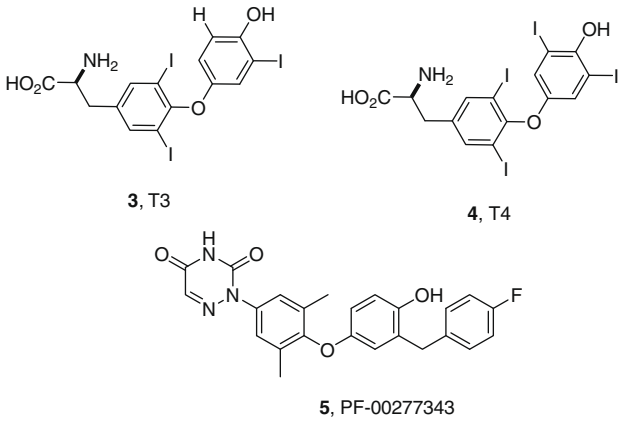
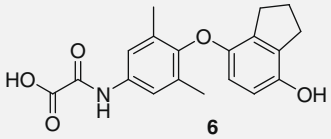
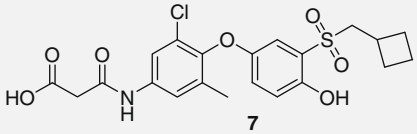
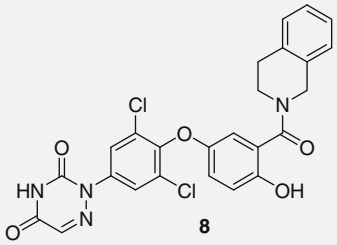
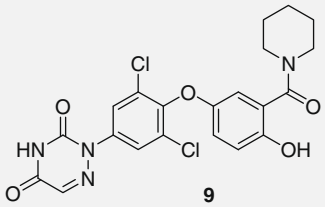
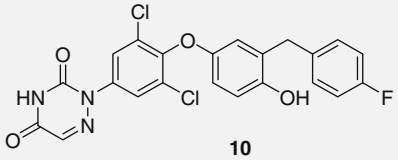


Figure 2. Thyroid hormones T3 and T4; and thyromimetic PF00277343.

Table 1
In vivo efficacy in the C3H/HeN mouse hair growth model

Compds	TRβ K_i[a] (nM)	TRα K_i (nM)	MED[b]
 **6**	0.5	0.7	1.0
 **7**	0.5	2.1	0.10
 **8**	2	126	0.10
 **9**	1.0	20	0.10
 **10**	0.51	8.0	0.01
5	0.40	3.0	0.003

[a] See note 6 of Ref. 5 for description of the binding assay method and calculation of the K_i values.

[b] MED = minimal efficacious dose, as the percentage concentration (w/v) in mixture of 70:30 ethanol/propylene glycol.

and **7** with carboxylic acids have minimal efficacious doses (MED) of 1.0% and 0.10% in the vehicle of 70:30 ethanol/propylene glycol, respectively. On the other hand, 6-azauracil analogs **8**–**10** and **5** are more efficacious with MEDs ranging from 0.003% to 0.10%. Azauracil **5** (EC_{50} = 0.5 nM in Hep G-2 cell) in particular is the most efficacious compound in this assay. Its MED of 0.003% represents a 3000-fold increase in comparison to minoxidil (**1**) whose MED is 2.5–5.0% qd for 4 weeks, with 5 days required for induction of efficacy. More remarkably, single application of azauracil **5** at 1 mg/mL and 0.1 mg/mL was effective in inducing hair growth in C3H female mice.

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. In vitro analysis indicated compound **5** is rapidly metabolized in rat liver microsomes (HLM $t_{1/2}$ = 40 min).[8] In rat hepatocytes, its half-life is 45 min, clearance CL_{blood} is 51 mL/min/kg and CL_{int} is 149 mL/min/kg. The hepatic extraction ratio (E_H) predicted from these in vitro data approached liver blood flow in rat, with a predicted E_H value of 1.0, indicating a high systemic clearance. Also in vitro, compound **5** is rapidly metabolized in human liver microsomes as well (HLM $t_{1/2}$ = 39 min, CL_{int} = 29 mL/min/kg, CL_{blood} = 15 mL/min/kg). The half-life in human hepatocyte is 119 min. The hepatic extraction ratio (E_H) predicted from these in vitro data in human is 1.0 as well. Furthermore, the in vivo clearance data in rats were consistent with the high clearance predicted in vitro in rat: Following intravenous administration of **5** at dosage of 0.5 mg/Kg, the mean systemic plasma clearance is 40 mL/min/kg. The mean apparent volume of distribution at steady state was 4.88 mL/min/kg, and the mean terminal phase half-life was 2.73 h.

Unlike oral drugs, topical drugs are required to penetrate the skin to exert their pharmacological effects. We evaluated skin penetration of thyromimetic **5** using human cadaver skin mounted onto Franz diffusion chambers.[9] As shown in Figure 3, compound **5** did indeed penetrate the skin, with the maximal penetration rate of 0.14 μg/cm^2/h at 28 h. After 40 h, the total skin penetration was 2.39 μg/cm^2/h, representing 6.17% of the total drug applied. Of the drug that did penetrate into the skin, 0.58% was distributed in the dermis, whereas 9.88% was in the epidermis.

Another unique requirement for topical drugs is low propensity for photo-irritation. Thyromimetic **5** was tested negative in the 3T3 neutral red uptake (NRU) phototoxicity test,[10,11] indicating a low risk for causing photo-irritation.

As preliminary in vitro and in vivo evaluation of the efficacy and metabolic stability characteristics of **5** proved favorable, an efficient synthesis was mandated to prepare enough active pharmaceutical ingredients (API) to support subsequent in vivo studies. To that end, we devised a practical and convergent synthesis that was suitable for large-scale production of **5**. As shown in the Scheme 1, the Friedel–Crafts acylation of 1-bromo-4methoxybenzene with 4-fluorobenzoic acid was promoted by Eaton's reagent[12] to afford diaryl ketone **11**, which was reduced using trisilylsilane in the presence of trifluoroacetic acid (TFA) to give benzyl derivative **12**. Meanwhile, a Buchwald–Hartwig cross-coupling[13] of 5-bromo-2-methoxy-1,3-dimethylbenzene with 6-azauracil afforded adduct

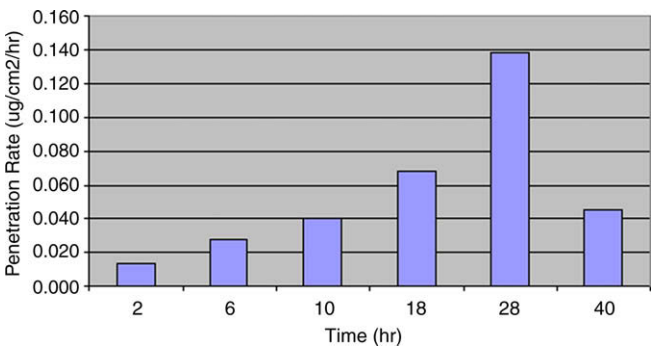
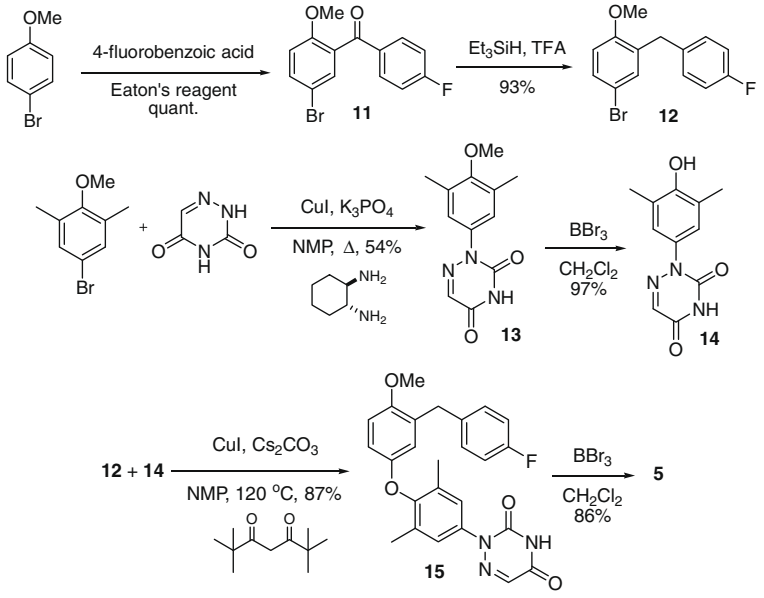


Figure 3. In vitro human cadaver skin penetration using franz diffusion chamber.



Scheme 1. Improved convergent synthesis of **5**.

13 in 54% yield, which was subsequently demethylated using BBr_3 in CH_2Cl_2 to provide azauracil-phenol **14**.

Now with fragments **12** and **14** in hand, the convergent ether formation was achieved by Ullmann coupling using 2,2,6,6-tetramethylheptane-3,5-dione as a powerful ligand to accelerate the recation.[14] Routine demethylation then delivered the desired product **5** in 86% yield. Although the yield of the cross-coupling reaction to access **13** was moderate, the convergent nature of this route allowed large-scale synthesis of **5**.

With enough quantities of **5** in hand, we carried out in vivo hair growth experiments in the bald stump-tailed macaques (*Macaca arctoides*), a validated primate model for androgenetic alopecia.[15] The MED of **5** for hair growth was 0.1% (reference compound **1** has a MED of 2% for the bald stump-tailed macaques[15]). Therefore, while **5** is efficacious in the monkey the MED is right-shifted relative to the C3H/HeN mouse model (MED, 0.003%). During the course of the four-month study, there were no signs of skin irritation, erythema, flaking, edema, or skin pigmentation changes. The serum drug level for **5** was below the lower limit of quantification (LLQ, detection limit 2 ng/mL for the first two-month samples and 1 ng/mL for the third and fourth month samples), indicating limited systemic exposure of the drug. Although the LLQ was still above the EC_{50} for these compounds, analysis of functional thyroid endpoints indicated that there was no detectable systemic pharmacological effects from application of **5** at efficacious dosages.

In summary, we have discovered a TRβ subtype-selective thyromimetic **5** that is efficacious in both mouse and monkey hair growth models after topical applications. It is rapidly metabolized based on pharmacokinetic data, thus the systemic exposure is limited. It penetrates the skin according to the test in human cadaver skin mounted onto Franz diffusion chambers. The serum drug level of **5** is below the limit of quantification during tests in the bald stump-tailed macaques (*M. arctoides*). It is also tested negative in the 3T3 neutral red uptake (NRU) phototoxicity test, indicating a low risk for causing photo-irritation.

References and notes

1. Safer, J. D.; Fraser, L. M.; Ray, S.; Holick, M. F.. *Thyroid* **2001**, *11*, 717.
2. van Beek, N.; Bodo, E.; Kromminga, A.; Gaspar, E.; Meyer, K.; Zmijewski, M. A.; Slominski, A.; Wenzel, B. E.; Paus, R. *J. Clin. Endocrin. Metab.* **2008**, *93*, 4381.
3. Billoni, N.; Buan, B.; Gautier, B.; Gaillard, O.; Mahe, Y. F.; Bernard, B. A. *Br. J. Dermatol.* **2000**, *142*, 645.
4. Bodo, E.; Kromminga, A.; Biro, T.; Borbiro, I.; Gaspar, E.; Zmijewski, M. A.; van Beek, N.; Langbein, L.; Slominski, A. T.; Paus, R. *J. Invest. Dermatol.* **2009**, *12*, 1126.
5. Dow, R. L.; Schneider, S. R.; Paight, E. S.; Hank, R. F.; Chiang, P.; Cornelius, P.; Lee, E.; Newsome, W. P.; Swick, A. G.; Spitzer, J.; Hargrove, D. M.; Patterson, T. A.; Pandit, J.; Chrunyk, B. A.; LeMotte, P. K.; Danley, D. E.; Rosner, M. H.; Ammirati, M. J.; Simons, S. P.; Schulte, G. K.; Tate, B. F.; DaSilva-Jardine, P. *Bioorg. Med. Chem. Lett* **2003**, *13*, 379.
6. Chian, Y.-C. P.; Cornelius, P.; Doherty, N. S.; Dow, R. L. EP1262177, 2002.
7. (a) Jiang, J.; Tsuboi, R.; Kojima, Y.; Ogawa, H. *J. Dermatol.* **2005**, *32*, 243; (b) Hamada, K.; Hirotsu, S.; Uchiwa, H.; Yamazaki, A.; Suzuki, K. *J. Dermatol. Sci.* **2003**, *33*, 195. Experimental procedure: 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) was included in every study.
8. Thomas, V. H.; Bhattachar, S.; Hitchingham, L.; Zocharski, P.; Naath, M.; Surendran, N.; Stoner, C. L.; El-Kattan, A. *Exp. Opin. Drug Metab. Toxicol.* **2006**, *2*, 591.
9. See: Antille, C.; Tran, C.; Sorg, O.; Saurat, J.-H. *Exp. Dermatol.* **2004**, *13*, 558.
10. Nam, C.; An, S.; Lee, E.; Moon, S.; Kang, J.; Chang, I. *ATLA, Altern. Lab. Anim.* **2004**, *32*, 693.
11. Spielmann, H.; *Alternatives to Animal Testing II*, Proceedings of the International Scientific Conference, 2nd, Brussels, 1999, pp 15–24.
12. A similar paper has recently been published: Li, Y.; Chang, M.-q.; Gao, F.; Gao, W.-t. *J. Chem. Res* **2008**, 640.
13. Janey, J. M. Buchwald–Hartwig Amination. In *Name Reactions for Functional Group Transformations*; Li, J. J., Corey, E. J., Eds.; Wiley & Sons: Hoboken, NJ, 2007; pp 564–609.
14. see Buck, E.; Song, Z. J.; Tschaen, D.; Dormer, P. G.; Volante, R. P.; Reider, P. J. *Org. Lett.* **2002**, *4*, 1623.
15. Uno, H.; Cappas, A.; Brigham, P. *J. Am. Acad. Dermatol.* **1987**, *16*, 657.