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(1*R*,2*S*)-4-(2-Cyano-cyclohexyl-oxy)-2-trifluoromethyl-benzonitrile, a potent androgen receptor antagonist for stimulating hair growth and reducing sebum production

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Abstract—Synthesis, pharmacology, and pharmacokinetic profiles of (1*R*, 2*S*)-4-(2-cyano-cyclohexyl-oxy)-2-trifluoromethyl-benzonitrile are reported. This compound 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. The androgen receptor is responsible for the activation of genes involved in the pathogenesis of acne and alopecia in the hair follicles.[1,2] Over-activation of the androgen receptor by 5α-dihydrotestosterone has been shown to play a critical role in excess sebum production and hair loss.[2]

During the last two decades, non-steroidal androgen receptor antagonists have been extensively investigated.[3,4] Specific examples include flutamide (**1**),[5] nilutamide (**2**),[5] bicalutamide (**3**),[6] and RU-58841 (**4**).[7] For our topical skin therapeutic program, we were seeking agents possessing optimal physiochemical properties for dermal delivery (moderate lipophilicity and low molecular weight), rapid clearance, and superb in vivo efficacy. AR antagonists with these characteristics are expected to demonstrate excellent efficacy for promoting hair growth and/or reducing oily skin (desired local biological effect) and result in low systemic exposures (to avoid unwanted side effects).

In 2002, we triaged a series of aryl-ether analogs from our AR agonist program. We screened a batch of potent AR binders but inactive AR agonists and discovered several AR antagonists. Among them, compound **5**, which contains a rigid bicyclic ether, stood out as an interesting lead (ARB IC_{50} = 106 nM, AR Cell IC_{50} = 15 nM).[8,9] Since the aryl-ether synthesis was amendable for combinatorial chemistry, we rapidly generated approximately 200 analogs via the coupling of an aryl-fluoride and substituted-carbocyclic alcohols in THF using NaH as the base.[10] The SAR trend on the phenyl core of AR modulators was reported in the literature: generally speaking, the AR ligands containing a 4-CN-3-Cl-phenyl, 4-CN-3-CF_3-phenyl, 4-NO_2-3-CF_3-

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phenyl, or 3,4-di-NO_2-phenyl core provided more favorable AR binding activity compared to other types of groups.[3,4] After evaluating our new AR compounds, we found that 4-(2-cyano-cyclohexyl-oxy)-2-trifluoromethyl-benzonitrile (**6**, a *trans*-racemic mixture) was particularly interesting. Antagonist **6** demonstrated high AR binding activity (IC_{50} = 58 nM) and potent AR antagonist activity (IC_{50} = 13 nM). Subsequently, a chiral separation on compound **6** was conducted;[11] its (+)-*trans*-isomer (**7**, AR binding IC_{50} = 43 nM, AR Cell IC_{50} = 196 nM) and the (−)-*trans*-isomer (**8**, AR binding IC_{50} = 60 nM, AR Cell IC_{50} = 12 nM) were isolated. We concluded that the (−)-*trans*-isomer (**8**) was the optimal AR antagonist in this series (Figs. 1 and 2).

The asymmetric synthesis of (1*R*,2*S*)-4-(2-cyano-cyclohexyl-oxy)-2-trifluoromethyl-benzonitrile, the (−)-*trans*-isomer (**8**), was successfully developed in our laboratory (Scheme 1). First the desired chiral cyano-alcohol **11**, (1*R*,2*S*)-2-cyano-cyclohexanol was prepared from the epoxide **9**. We modified Jacobsen's method[12] and further improved the yields up to 90% for making intermediate **10**.[13] Then the TMS-ether (**10**) was subsequently hydrolyzed to generate the chiral alcohol (**11**)[14] with high chiral purity. With this precious chiral alcohol (**11**) in hand, we carefully evaluated various ether coupling conditions aiming to avoid epimerization of the 2-position on the cyclohexyl ring of **11** as well as to avoid a low coupling yield which had resulted from the dehydration of **11**. Ultimately, we established a facile and high yielding route of the chiral ether synthesis by using LiOH in DMF with a good yield, as shown in Scheme 1. Large quantities (50 g) of antagonist **8** were prepared with high enantiomeric purity (>99.8%) by this route for development; the detailed procedure of the syntheses will be published in the near future. The *trans*-conformation of **8**was proved by its X-ray crystal structure shown in Figure 3.[15] The absolute stereochemistry of **8** was supported by literature rotation data on **10** for its 1*R*,2*S* configuration.

Compound **8** demonstrated excellent in vivo activity in the preclinical models for hair growth and sebum reduction. The compound was applied topically in a formulation containing 30% propylene glycol and 70% ethanol (PG/EtOH, 30/70, v/v). In the male C3H mouse hair growth model,[16] this novel AR antagonist (**8**) stimulated hair growth in a dose-dependent manner and with high potency (Fig. 4). Compared to a known AR antagonist, RU-58841 (**4**), AR antagonist **8** showed a similar level of in vivo activity for promoting hair growth. In addition, compound **8** was evaluated in the male Syrian hamster ear model, a widely used animal model to test drug effects on sebaceous glands.[17] Following topical applications of twice daily (BID) for 2 weeks, AR antagonist **8** reduced production of a biomarker for sebum, wax ester, in a dose-related manner with an ED_{50} of 0.1%. (Fig. 5; for comparison, RU-58841 (**4**) reduced 91% WE production at 0.5% concentration.) Histological analysis of the sebaceous gland size showed that the reduction of lipid wax ester production was associated with sebaceous gland size reduction (Fig. 6). The reduction of wax ester was clearly correlated with reduction in sebaceous gland size as quantified by the Fatty Acid Synthase (FAS) immunohistochemical staining. In this study, we also found that the sebaceous gland atrophy was not associated with any other histopathologic evidence of cytotoxic changes after 2 weeks of BID treat-

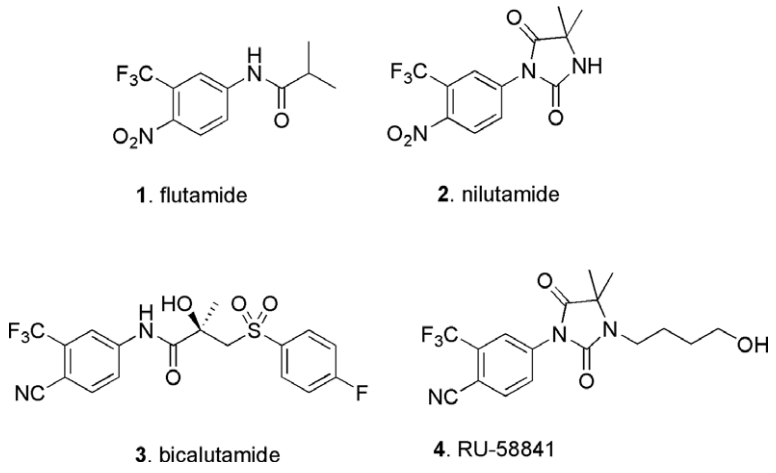


Figure 1. Non-steroidal AR modulators.

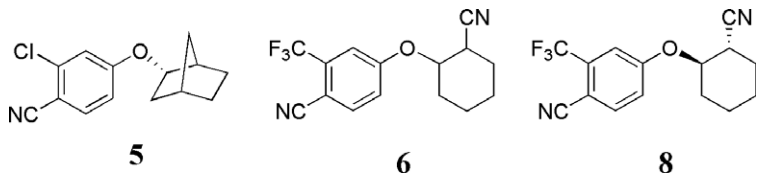
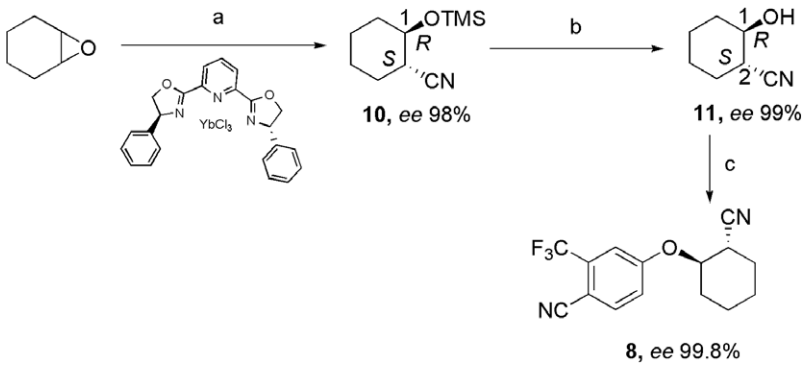


Figure 2. Novel AR modulators.



Scheme 1. Reagents and conditions: (a) TMS-CN, DCE, 90%; (b) TFA, MeOH, 99%; (c) LiOH, DMF, 25 °C, 2-CF_3-4-F-1-CN-benzene 71%.

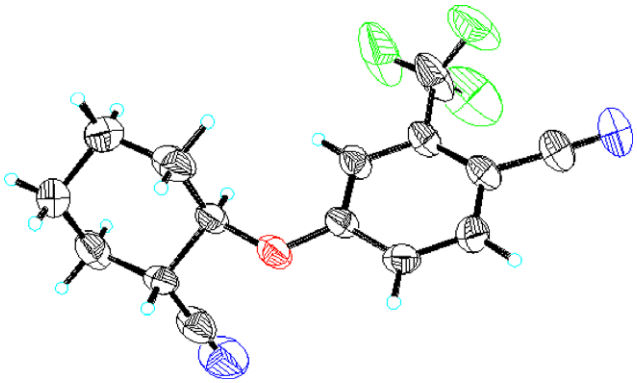


Figure 3. AR antagonist **8**.

ment. It is notable that the reduction of sebaceous gland size and lipid wax ester production were completely reversible.

The pharmacokinetic characteristics of compound **8** were favorable for a topical agent. Ideally, topical drugs exert their desired effects locally but are inactivated via metabolism once they reach the systemic circulation to reduce unwanted effects. Toward this end, compound **8** was rapidly metabolized in rat and dog hepatocytes; turnover in human hepatocytes was more moderate. Predicted hepatic extraction ratios (E_H) from these in vitro data were 0.88, 0.92, and 0.57 for rat, dog, and human, respectively.[18] Consistent with these data, high plasma clearance of compound **8** was observed in rats following intravenous administration (CL: 71 mL/min/kg, V_{ss}: 6.5 L/kg, $t_{1/2}$: 1.6 h).[19] In addition, in vitro studies demonstrated that compound **8** can penetrate human skin when formulated as a 1% solution in a vehicle consisting of 5% propylene carbonate, 5% propylene glycol, 20% water, and 70% ethanol;[20] thus delivery of compound **8** to the target site within the skin is anticipated.

In summary, (1*R*,2*S*)-4-(2-cyano-cyclohexyl-oxy)-2-trifluoromethyl-benzonitrile (**8**) is reported as a potent AR antagonist. We have developed a chiral synthetic route for process chemistry. Antagonist **8** showed remarkable in vivo activity for both stimulating hair

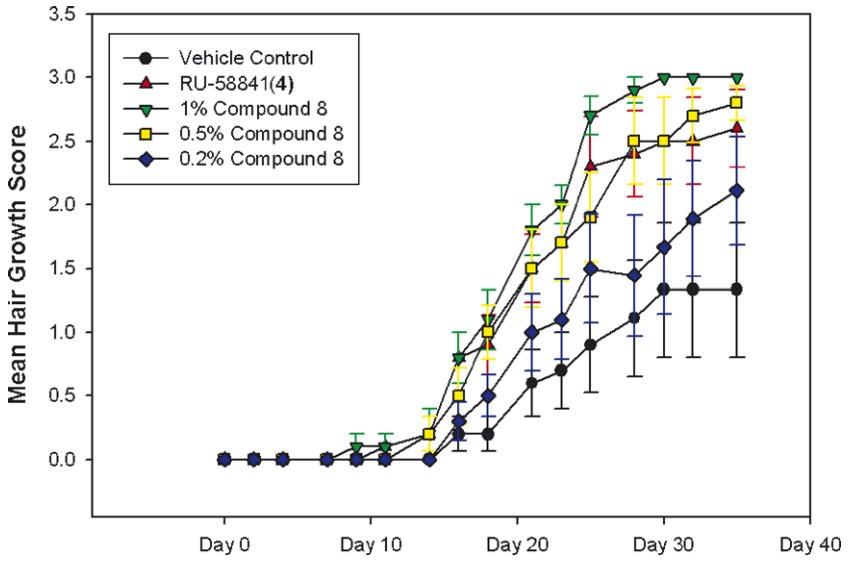


Figure 4. Hair growth dose response relationship of compound **8** tested in a formulation of PG/EtOH (3/7, v/v) with twice daily dosing for 4 weeks in the male C3H mouse model.

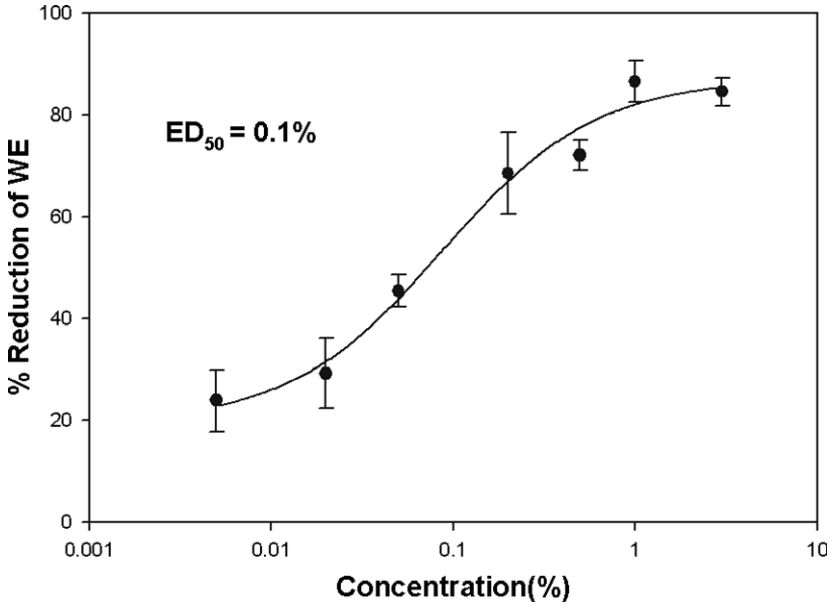


Figure 5. Dose response relationship of compound **8** tested in a formulation of PG/EtOH (3/7, v/v) with twice daily dosing for 2 weeks in the male Syrian hamster ear model.

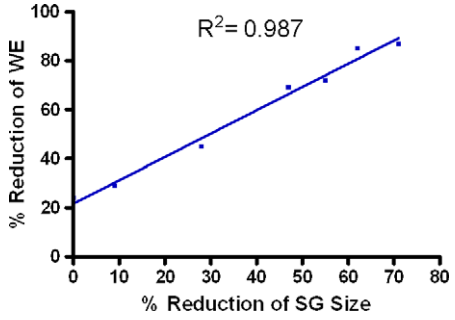


Figure 6. Reduction of lipid wax ester (WE) production induced by compound **8** correlates with the reduction of sebaceous gland size.

growth and reducing sebum production and possessed desirable pharmacokinetic characteristics for topical application. This compound also demonstrated the reversible pharmacology in the male Syrian hamster ear model.

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8. Androgen receptor binding assays: all of the described compounds showed affinity for the androgen receptor. The competitive radio-ligand binding analysis was performed on human AR extracts from transfected 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 a 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 reactions 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. On the basis of the AR binding potency ($IC_{50} < 200$ nM). Compounds 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 pUV120-puro. Electroporation was used for transfecting MDA-MB-453 cells and a puromycin resistant stable cell line was selected.
10. The general procedure: to a Bohdan mini-block reaction tube containing a solution of 4-fluoro-2-(trifluorometh-

yl)benzonitrile (0.3 mmol) and the appropriate cycloalkane-halide (0.3 mmol) in anhydrous THF (1.3 mL) was added a 0.6 M slurry of sodium hydride in anhydrous THF (2 equiv, 0.6 mmol). The Bohdan mini-block was capped and the reaction mixture was shaken at ambient temperature for 16 h. Five-hundred microliters of methanol and 100 mg of MP-TsOH (4.07 mmol/g, 1.35 equiv, 0.41 mmol) were added and the reaction mixture was shaken at ambient temperature for 20 h. Reaction mixture was filtered and concentrated utilizing a Genevac HT-12. Sample was purified via reverse phase HPLC.

11. Compounds **7** and **8** could be obtained by a chiral separation on the mixture 6 by a preparative chiral HPLC with a column of Chiralcel AD (20 μm:, 5.0 cm ID × 50 cm L), mobile phase (hexanes:IPA 85:15), flow rate (80.0 mL/min), and UV detection at 240 nm. Compounds **7** and **8** could be analyzed by a chiral HPLC with a column of Chiralpak AD-H (5 μm, 250 × 4.6 mm), mobile phase (hexanes:IPA 85:15), flow rate (0.8 mL/min), and UV detection (240 nm). Retention time: 13.9 min for 8 and 11.2 min for 7. (*trans*)-(−)-(1*R*,2*S*)-4-(2-Cyano-cyclohexyl-oxy)-2-trifluoromethyl-benzonitrile (**8**): ^{1}H NMR (400 MHz, $CDCl_3$): δ^1H NMR (400 MHz, $CDCl_3$): δ 7.78 (d, 1H); 7.33 (d, 1H); 7.16 (dd, 1H); 4.50 (m, 1H); 2.85 (m, 1H); 2.20 (m, 2H); 1.90–1.70 (m, 3H); 1.60–1.40 (m, 3H). MS: 295 (M + 1 for $C_{15}H_{13}N_2F_3O$), *ee* 99.8%, anal. calcd C, 61.22%; H, 4.45%; N, 9.52%. Found: C, 61.00%; H, 4.25%; N: 9.56%). $[\alpha]_D = -68.5°$ (c 1.0, CH_2Cl_2). (*trans*)-(+)-(1*S*,2*R*)-4-(2-Cyano-cyclohexyl-oxy)-2-trifluoromethyl-benzonitrile (**7**): Chiral HPLC Retention Time: 14 min.
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13. Chiral GC condition: column (Varian Chrompak Cyclodextrin-B-2,3,6-M-19, 25 m × 250 μm × 0.25 μm film thickness), flow rate (approx. 1.5 mL/min), pressure (21 psi), mode (constant pressure). Compound **10**: retention time: 7.8 min; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.60 (m, 1H); 2.40 (m, 1H); 2.00 (m, 1H); 1.80 (m, 1H); 1.70–1.40 (m, 3H); 1.30–1.00 (m, 3H); 0.20 (s, 9H), $[\alpha]_D$ −45.00° (*c* 1.0, CH_2Cl_2), *ee* 98%. The retention time of other diastereomer was 8 min.
14. Chiral GC condition same as described in the footnote 13. Compound **11**: retention time 18.9 min, ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.70 (m, 1H); 2.70 (br, 1H); 2.40 (m, 1H); 2.20–2.00 (m, 2H); 1.90–1.50 (m, 3H); 1.40–1.10 (m, 3H), $[\alpha]_D$ −55.25° (*c* 1.0, CH_2Cl_2), *ee* 99.2%. The retention time of other diastereomer was 18.6 min.
15. The crystal structure of **8**: 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 9% and no missing or misplaced electron density observed in the final difference Fourier. The structure is proof of relative configuration between the stereo centers.
16. Male C3H/HeN mice (~6 weeks old with same date of birth) were purchased from Charles River Laboratory (Raleigh, NC). After 1 week of acclimation, the mice were shaved on the lower back using an electric shaver under isoflurane anesthesia. 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 μL/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.
17. 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 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 to 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. (a) Plewig, G.; Lunderschmidt, C. *J. Invest. Dermatol.* **1977**, *68*, 171; (b) Kusakabe, T.; Maeda, M.; Hoshi, N. *J. Histochem. Cytochem.* **2000**, *48*, 613.
18. Metabolic stability in hepatocytes: cryopreserved rat (Sprague–Dawley), dog (beagle), and human hepatocytes were incubated with [^{3}H]-**8** (1 μM) in Leibovitz's L-15 medium at 37 °C. The target cell density was 0.5×10^6/mL and viability was ⩾62%. Sample aliquots were collected at various time intervals up to 180 min and quenched with cold acetonitrile. Samples were analyzed by HPLC equipped with a radioactivity detector, and concentrations of 8 were determined. Half-life values were calculated from a semi-log plot of time versus concentration and were scaled to E_H ratios as reviewed by Thomas et al. [Expert Opin. Drug Metab. Toxicol. 2006, 2 (4), 591–608].
19. Pharmacokinetics in rats: male Sprague–Dawley rats (n = 2) were administered a 1 mg/kg intravenous dose of 8 as a 5 min infusion at a dose volume of 2.9 mL/kg. Compound **8** was formulated as a solution in a vehicle consisting of 5% *N*,*N*-dimethylacetamide, 40% propylene glycol, and 55% saline. Serial blood samples were collected from each rat over a 24 h period postdose. Plasma concentrations of 8 were determined using a LC/MS/MS method, and pharmacokinetic parameters were determined from the plasma concentration-time data using noncompart-mental methods.
20. Transepidermal delivery through human skin: the percutaneous absorption of [^{3}H]-**8** through dermatomed human cadaver skin from multiple donors was evaluated using an automated sampling Franz diffusion cell system maintained at 32 °C. Compound [^{3}H]-**8** was formulated as a 1% solution in a vehicle containing 5% propylene carbonate, 5% propylene glycol, 20% water, and 70% ethanol and applied to the mounted skin as a finite dose at a volume of 5 μL/cm^2. At various time points over the 48 h study period, aliquots of the receptor solution,

phosphate-buffered saline (pH 7.4) containing 0.5% Brij 98, were removed and mixed with liquid scintillation cocktail. The concentration of **8** (in radioactive equivalents) was determined using liquid scintillation counting. Apparent steady-state flux values were calculated from a plot of time versus the cumulative amount permeated normalized to the surface area of application (μg equiv/cm^2). The mean (±SD) flux value of [^{3}H]-**8** through human cadaver skin was 0.057 ± 0.018 μg equiv/cm^2/h (n = 14 replicates).