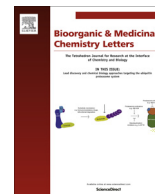




Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Identification of selective 8-(piperidin-4-yloxy)quinoline sulfone and sulfonamide histamine H₁ receptor antagonists for use in allergic rhinitis

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ARTICLE INFO

Article history:

Received 22 August 2017

Accepted 8 September 2017

Available online xxxx

Keywords:

Histamine H₁ receptor antagonist

Allergic rhinitis

Once-daily dosing

Topical application

Quinoline

Sulfone

Sulfonamide

ABSTRACT

A series of potent, selective and long-acting quinoline-based sulfonamide human H₁ histamine receptor antagonists, designed for once-daily intranasal administration for the treatment of rhinitis were developed. Sulfonamide **33b** had a slightly lower affinity for the H₁ receptor than azelastine, had low oral bioavailability in the rat and dog, and was turned over to five major metabolites. Furthermore, **33b** had longer duration of action than azelastine in guinea pigs, lower rat brain-penetration, and did not cause time dependent inhibition of CYP2D6 or CYP3A4. The clinical dose in humans is expected to be low (approximately 0.5 mg per day) based on the clinical dose used for azelastine and a comparison of efficacy data from animal models for **33b** and azelastine.

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Introduction

Allergic rhinitis is a condition that affects a large number of people, approximately 25% of the global population, with high prevalence in the industrialised world, and a near quadrupling of medical care consultations over the last 50 years.^{1,2} Symptoms include irritation and repetitive sneezing, rhinorrhoea, pruritus, headache, epiphora, nasal congestion, irritation of the throat, and oedema. Nasal congestion may lead to breathing through the mouth, snoring,³ and hyposmia.⁴ Allergic rhinitis is mainly treated with antihistamines and corticosteroids,⁵ with H₁ receptor antagonists (antihistamines) being the most frequently used medication.⁶ In addition to oral antihistamines intranasal treatments, such as azelastine⁷ and olopatadine (Chart 1) have gained popularity because the dose for topical treatments is generally lower, and hence their side-effects are fewer. Treatments destined for intranasal dosing must be delivered in a small volume, have high potency, and also have low oral absorption because a significant portion of the dose is swallowed and becomes available for absorption through the gastrointestinal track. Azelastine and olopatadine have

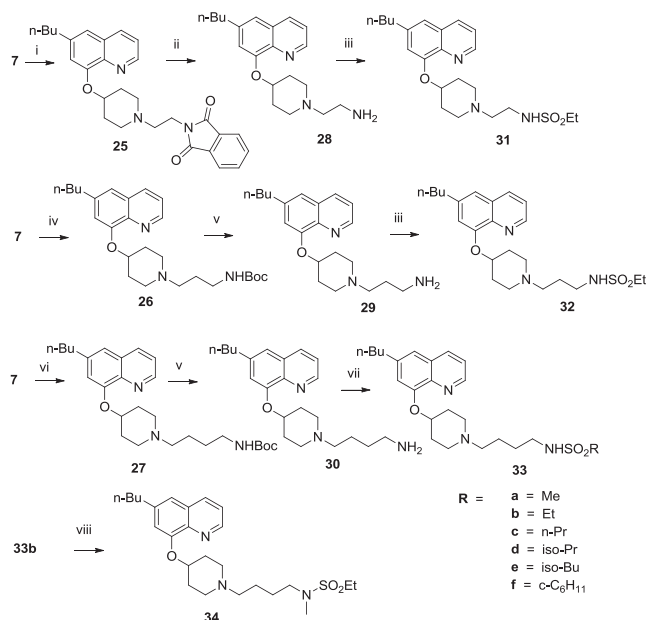
comparable efficacy and duration of action (12 h), however, both suffer from dysgeusia, headache and epistaxis.⁸

Our group has published on selective histamine H₃ receptor antagonists,⁹ on dual H₁H₃ antagonists,^{10,11} and on selective H₁ antagonists.^{12,13} More recently we have focussed our efforts in identifying potent and selective human H₁ receptor antagonists with low oral absorption and long duration of action, suitable for once-daily intranasal administration. Due to allergic rhinitis' close links to other inflammatory diseases such as allergic conjunctivitis, rhinosinusitis and asthma, we envisaged using a novel H₁ receptor antagonist in combination with the long-acting glucocorticoid fluticasone furoate. We have very recently reported our efforts in identifying phthalazinone **1** as a preclinical candidate for rhinitis, which fulfils all of the above requirements.¹³ In this publication we describe our efforts in identifying another candidate as a back-up to **1**, which is derived from a non-phthalazinone scaffold.

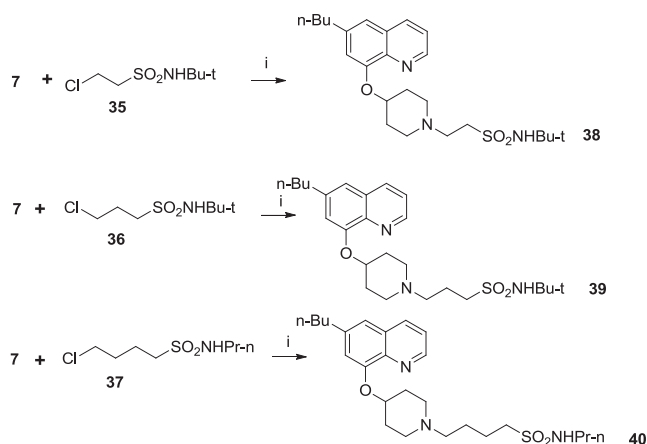
Azelastine which has a phthalazinone core has a bitter taste and we wished to avoid this problem, if possible. We considered starting our investigations from the 8-(piperazin-1-yl)quinoline scaffold **2** (Fig. 1),¹¹ however, we opted for the 8-(piperidin-4-yloxy)quinoline scaffold **3** that we briefly examined previously as part of our dual H₁H₃ antagonist project. Scaffold **3** was slightly less potent at the histamine H₁ receptor, however, it was significantly

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Scheme 2. Synthesis of sulfonamides **31**, **32**, **33a–f** and **34**. *Reagents and Conditions:* i) 2-phthalimidoethyl bromide, K₂CO₃, 2-butanone, 80 °C, 70%; ii) NH₂NH₂·H₂O, EtOH, 80 °C, 100%; iii) EtSO₂Cl, Et₃N, DCM, 38% for **31** and 67% for **32**; iv) 3-(Boc-amino)propyl bromide, K₂CO₃, 2-butanone, 80 °C, 78%; v) 4 M HCl, dioxane, 76% for **29** and 88% for **30**; vi) 4-(Boc-amino)butyl bromide, K₂CO₃, 2-butanone, 80 °C, 94%; vii) RSO₂Cl, Et₃N, DCM, 36% for **33a**, 53% for **33b**, 32% for **33c**, 39% for **33d**, 18% for **33e**, 23% for **33f**; viii) NaH, MeI, DMF, 52%.



Scheme 3. Synthesis of reverse sulfonamides **38**, **39** and **40**. *Reagents and Conditions:* i) NaI, K₂CO₃, DMF, 4–33%.

37 were prepared by treatment of the commercially available chloroalkylsulfonyl chlorides with one equivalent of *tert*-butylamine or *n*-propylamine.

Results and discussion

The H₁ receptor affinity of compounds was evaluated in vitro using recombinant human histamine H₁ receptor in intact CHO cells which provided apparent pA₂ values. The hERG activity was measured in [³H]-dofetilide radioligand binding assay, and the data from all the above screens are summarised in Table 1. Azelastine was used as a reference compound and exhibited high affinity for both the H₁ receptor (pA₂ = 9.7) and the hERG channel (pIC₅₀ = 7.0). Similarly, the data for our phthalazinone candidate **1** is also included in Table 1 for comparison (pA₂ = 9.7 and hERG

pIC₅₀ = 6.4). The experimental details on the assays were reported in our earlier publications.^{9,10} Duration of action in vitro was determined in the CHO cell assay by incubation with antagonist for 30 min, followed by washing, and then by repeat histamine challenges at intervals of 90 and 270 min at 37 °C. The duration of action in vitro for these analogues is expressed as faster, slower or no-difference wash-out time relative to azelastine. Slower wash-out equated to longer duration of action than azelastine, whereas faster wash-out to shorter duration.

The ethyl sulfones with the two-carbon (**8**) and branched four-carbon (**20**) chains had lower affinity for the H₁ receptor (Table 1), however, sulfone **8** which was the least basic compound tested (measured pK_a 6.5) had also the lowest hERG affinity (pIC₅₀ 5.8). The three-carbon linked sulfones were more potent than the other chains tested. The terminal alkyl group of these sulfones did not influence the H₁ activity with the ethyl (**9a**), *iso*-propyl (**9c**) and *tert*-butyl (**9d**) sulfones being equipotent (pA₂ 9.5, 9.4 and 9.6 respectively), and also maintaining lower affinity for the hERG channel (pIC₅₀ 7.1, 7.0 and 6.3 respectively). The duration of action in vitro for **9a** was longer than azelastine, whereas for **9c** and **9d** was the same, making all three compounds worthy of further investigation in pharmacokinetic studies. The lipophilic (clogP = 4.4) four-carbon chain *tert*-butyl sulfone **16** was less potent than the analogous three-carbon chain sulfone **9d** (pA₂ 8.85) and was rejected, although its hERG affinity was low (pIC₅₀ 6.5) and its duration of action not different from azelastine.

For the sulfonamide series the optimal linker was the four-carbon chain when comparing the ethylsulfonamides **31**, **32** and **33b** (pA₂ 8.7, 8.9 and 9.3 respectively). Investigation of the sulfonamide nitrogen substituent in the four-carbon chain series (**33a–f**) identified three potent analogues **33a–c**. Compound **33a** was however rejected due to its higher hERG activity. The calculated pK_a for analogues **33a–f** was 8.42, however a drop in the hERG activity was observed with increasing substitution from the methyl **33a** to *iso*-propyl analogues **33d** and then an increase with increasing lipophilicity for the *isobutyl* **33e** and *cyclohexyl* **33f** analogues. The duration of action in vitro of sulfonamide **33c** was longer than azelastine's, whereas for **33b** was similar. Both **33b** and **33c** were progressed for further investigation. The H₁ affinity of the *N*-methyl sulfonamide **34** was reduced slightly by comparison to **33b** and was rejected.

The reverse sulfonamides **38–40** with the two-, three- and four-carbon linker chains had lower H₁ receptor affinity and were also rejected. The hERG activity of **38**, which was less basic than both **39** and **40**, was lower. The more branched *tert*-butyl **39** had lower hERG activity than the *n*-propyl analogue **40**, despite the fact that both **39** and **40** had the same basicity (calc. pK_a 8.1).

Pharmacokinetic studies

All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Animals. Experimental procedures for the PK studies in vivo and rat CNS penetration were reported in our earlier publications and outlined in the supplementary information section.^{9,10} From the three sulfones and two sulfonamides selected for further investigation **9a**, **33b** and **33c** had low CNS penetration in the rat, assessed in samples taken 5 min after an intravenous bolus dose of 1 mg/kg (mean brain concentration of 62, 26 and 41 ng/g of tissue respectively), and low brain-blood ratios (0.09, 0.10 and 0.16 respectively). Sulfone **9d** had higher levels of concentration in the brain 133 ng/g (presumably due to its higher lipophilicity, clogP 4.7), and a higher brain-blood ratio of 0.77 and was therefore rejected. The sulfones (**9a** and **9c**) had higher systemic exposure in the rat after oral

Table 1
Antagonist pA_2 Affinity at the Human H_1 Receptor, in vitro Duration, dofetilide hERG binding affinity, calculated logP and pK_a , and measured pK_a for the piperidine nitrogen.

Compound ^a	$pA_2 \pm SEM^b$ (n)	Wash-out ^c	hERG $pIC_{50}(n)$	clogP ^d	cpK_a^e	pK_a
Azelastine	9.7 ± 0.1 (19)	Ref.	7.0 ± 0.0 (114)	4.0	8.9	
1	9.7 ± 0.1 (20)	S	6.4 ± 0.0 (16)	3.7	7.3	
8	8.43 ± 0.01 (2)	–	5.78 ± 0.01 (2)	3.9	6.7	6.5
9a	9.46 ± 0.04 (13)	S	7.12 ± 0.04 (5)	4.0	6.9	7.9
9b	9.13 ± 0.06 (3)	–	7.54 ± 0.04 (4)	4.5	6.9	–
9c	9.4 ± 0.1 (16)	ND	7.04 ± 0.02 (4)	4.3	6.9	–
9d	9.58 ± 0.09 (6)	ND	6.33 ± 0.03 (5)	4.7	6.9	8.0
16	8.85 ± 0.08 (12)	ND	6.52 ± 0.01 (4)	4.4	7.0	–
20a	8.7 ± 0.2 (4)	–	6.7 ± 0.0 (2)	4.3	7.2	–
20b	8.6 ± 0.2 (4)	–	6.88 ± 0.02 (2)	4.3	7.2	–
31	8.68 ± 0.08 (13)	ND	7.12 ± 0.03 (4)	4.4	7.2	–
32	8.9 ± 0.4 (2)	–	7.2 ± 0.1 (2)	4.5	8.0	8.5
33a	9.4 ± 0.1 (10)	ND	7.7 ± 0.2 (6)	3.8	8.4	–
33b	9.27 ± 0.08 (18)	ND	7.11 ± 0.02 (9)	4.3	8.4	–
33c	9.7 ± 0.2 (16)	S	7.06 ± 0.02 (6)	4.8	8.4	–
33d	8.7 ± 0.2 (3)	–	6.89 ± 0.05 (4)	4.6	8.4	–
33e	9.08 ± 0.08 (13)	S	7.36 ± 0.00 (2)	5.3	8.4	–
33f	9.0 ± 0.2 (3)	–	7.74 ± 0.06 (4)	5.8	8.4	–
34	9.0 ± 0.2 (3)	–	6.6 ± 0.1 (4)	4.6	8.4	–
38	8.7 ± 0.1 (8)	F	6.20 ± 0.04 (4)	5.1	7.2	–
39	9.2 ± 0.1 (14)	ND	6.55 ± 0.03 (4)	5.2	8.1	–
40	9.0 ± 0.1 (3)	–	7.02 ± 0.07 (4)	4.9	8.1	–

^a All compounds were tested as dihydrochloride salts except for **9d**, **38** and **40** which were mono formate salts, **16** which was a diformate salt, azelastine was the hydrochloride salt, and **1** was the free base,

^b All pA_2 values calculated from curve shifts generated at 30 min incubation time and at 100 nM antagonist concentration. Table 1 shows mean $pA_2 \pm SEM$ for $n < 3$ the SEM is the SD. n = number of experiments,

^c the duration of action in vitro for these analogues is expressed as faster (F), slower (S) or no-difference (ND) wash-out time relative to azelastine,

^d calculated logP value from Biobyte v4.3,

^e calculated pK_a from Chemaxon v5.4.1.1.

administration at 3 mg/kg (mean AUC_{0-last} 568 and >361 h.ng/mL respectively) than the sulfonamides (**33b** and **33c**, mean AUC_{0-last} 62 and 63 h.ng/mL respectively). The sulfones **9a** and **9c** were therefore rejected as the aim of this project was to identify compounds with low systemic exposure, suitable for intranasal dosing. Sulfonamide **33b** having the lower AUC was progressed to pharmacokinetic studies in vivo in both the male CD Sprague Dawley rat and Beagle dog and the data are shown in Table 2. The compound was dosed as a solution in H₂O–PEG200–DMSO (50:45:5) at 1 mg/kg (both species) iv, at 3 mg/kg for the rat po, and 1 and 2 mg/kg for the dog po studies. In addition one dog was also dosed subcutaneously. The blood clearance was moderate compared to liver blood flow (means of 38 and 20 mL/min/kg in rat and dog respectively) and the volume of distribution was also moderate (means of 4.2 and 4.5 L/kg in rat and dog respectively). The resultant elimination half-life was moderate (means of 1.8 and 3.8 h in rat and dog respectively). Elimination of parent compound via urine was low

Table 2
Pharmacokinetic parameters for **33b.2HCl** in the rat and dog.

Species	CD Rat (n = 4 iv, n = 3 po)	Beagle Dog (n = 4 iv, n = 4 po)
Cl _b (mL/min/kg) (% LBF)	38 (45)	20 (65)
V_{ss} (L/kg)	4.2	4.5
$T_{1/2}$ (h) (IV)	1.8	3.8
Oral bioavailability (%)	9 ^{a,b}	5 ^c
Calculated fraction of oral dose absorbed.	13–16%	

^a **33b** was also dosed sub-cutaneously to one rat at 2 mg/kg formulated in DMSO–PEG 200–Water (5:45:50). Bioavailability was determined to be at least 45% and the C_{max} was approximately six-fold higher than achieved following oral administration at 3 mg/kg in the same study.

^b **33b** was also dosed orally to 3 rats at 10 mg/kg formulated in 0.5% (hydroxyl-propylmethyl cellulose). Bioavailability was determined to be at least 4%, the terminal phase was not sufficiently well defined to give a more accurate value.

^c Mean of data from $n = 2$ dosed at 1 mg/kg and $n = 2$ dosed at 2 mg/kg.

following oral and intravenous administration of **33b** to the dog (<0.1% following oral administration and <1% following intravenous administration). Following oral administration of **33b** bioavailability was low using low doses (9 and 5% for rat and dog respectively). Rat absorption of **33b** into the hepatic portal vein following oral administration was low (13–16% using a 3 mg/kg dose). Sulfonamide **33b** was also dosed orally to rats at 10 mg/kg using a hydroxypropylmethyl cellulose formulation. The animals dosed at 10 mg/kg showed a proportional increase in C_{max} compared to the 3 mg/kg data and bioavailability was estimated to be 4%. Following subcutaneous administration to the rat bioavailability was estimated to be 45%. The *in vitro* plasma protein binding for **33b** was determined using an ultrafiltration technique and was found to be high across all species tested (means of 97.9, 91.1, 97.7 and 98.2% for rat, guinea pig, dog and human respectively at 10 μ g/mL). Plasma protein binding did not appear to be concentration dependent across the three concentrations tested, 0.5 or 1 μ g/mL and 10 μ g/mL. The *in vitro* blood cell binding was low to moderate at nominal concentrations of 1 and 10 μ g/mL in all species tested (means of 27, 42, 49 and 23% for rat, guinea pig, dog and human respectively at 1 μ g/mL). The *in vitro* rate of metabolism for **33b** was moderate using rat and human liver microsomes (2.9 and 1.4 mL/min/g tissue respectively); moderate to high using dog liver microsomes (3.6 mL/min/g) and high using mouse liver microsomes (3.7 mL/min/g). The rat and dog *in vitro* microsomal clearance data is in keeping with the *in vivo* clearance data for rat and dog. Incubation of **33b** with cryopreserved human hepatocytes resulted in extensive metabolism of the compound with at least 35 metabolites being formed. Five major metabolites, labelled M11, M15, M19, M24 and M26 were observed, which were identified by mass spectrometry as M11 and M15 hydroxylation products on the C6-butyl group (M + 16), M19 oxidation product of either M11 or M15 (M + 14), M24 *N*-dealkylation of the piperidine nitrogen (M-163) and M26 oxidative *N*-dealkylation of the sulfonamide nitrogen (M-77). Sulfonamide **33b** had an acceptable profile ($IC_{50} > 4 \mu$ M) against human CYP450 enzymes (CYP1A2, >100 μ M; CYP2C9, 31 μ M; CYP2C19, >40 μ M; CYP2D6, 15 μ M and CYP3A4, 4 μ M). Furthermore, **33b** did not cause time-dependent inhibition with CYP2D6 or CYP3A4 (less than two-fold change in IC_{50} values over a 30 min incubation).

The MDCK permeability of **33b** was low (52 nm/s; $n = 3$), however, the compound was found to be a P-glycoprotein (PgP) substrate. The brain levels of **33b** determined 30 min post a 300 mg/kg oral dose in rat were found to be very high (14,480 ng/g) with high brain-blood ratio of 11.0. This increase in brain penetration

observed at higher doses is thought to be a consequence of **33b** being a PgP substrate.

Effect of **33b** on histamine-induced nasal congestion in guinea pigs

We have used the previously reported Buxco whole body plethysmography technique to investigate the effect of intranasally dosed **33b** on histamine-induced nasal congestion in conscious, unrestrained guinea pigs, which were previously sensitised with ovalbumin and aluminum hydroxide intranasally over a 3 week period prior to the study (further information in SI section). Recording PenH (enhanced pause) AUC over 40 min following bilateral histamine challenge (10 mM, 25 μ L/nostril, under light isoflurane anaesthesia) allows for the assessment of efficacy and duration of action of intranasally dosed antihistamines.^{13,14} Significant inhibition of histamine-induced congestion, compared to a vehicle-pretreated/histamine challenged control group, at both 3 and 24 h after intranasal administration of a 1 mg/mL solution of **33b** (25 μ L/nostril). In contrast, azelastine failed to show a similar duration of action when administered at the same concentration (Fig. 2). Arterial blood samples taken immediately following evaluation of nasal congestion (4 or 25 h after intranasal dosing) show negligible systemic exposure following 1 mg/mL **33b**, suggesting a low bioavailability by this route and a local mechanism of action. A separate study was performed in order to investigate the onset of action *in vivo*. Significant inhibition of histamine-induced congestion 1 h after intranasal administration of a 1 mg/mL solution (25 μ L/nostril, data not shown) which suggests that an early onset time could be clinically achievable. Neither **33b** nor azelastine produced a pro-congestant response at any time-point studied (effect on baseline PenH – data not shown).

Predicted human dose

The clinical dose of **33b** is expected to be approximately 0.5 mg per day, based on the clinical dose used for azelastine and a comparison of efficacy data from animal models for **33b** and azelastine. Using the low oral bioavailability in rat and dog, moderate blood clearance and a low clinical dose the systemic exposure in humans is expected to be very low. The concentration of **33b** was less than 7 nM (3 ng/mL) in all assayed blood samples taken from guinea pigs showing a pharmacological response in the histamine induced nasal congestion model. The pharmacokinetic profiles in

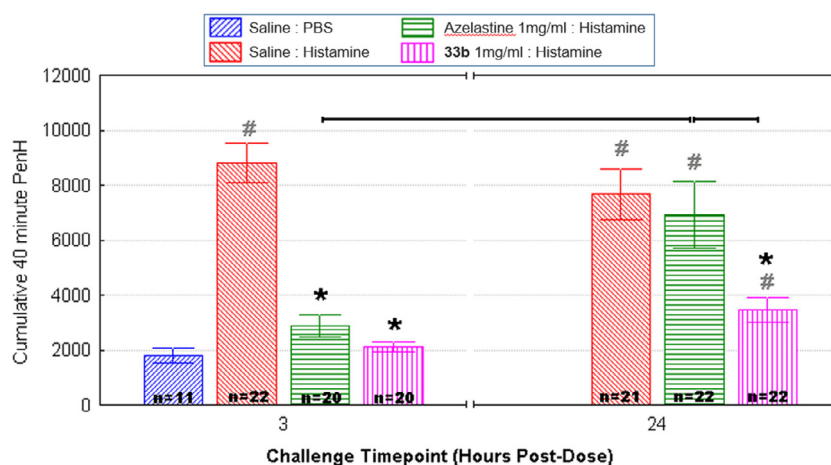


Fig. 2. Duration of action of **33b** and azelastine in a conscious guinea pig model of histamine-induced nasal congestion. Animals were exposed to histamine at the time indicated after an intranasal dose of 1 mg/mL **33b** or azelastine. Mean $\pm$ SEM ($n = 11$ –22 per group as indicated). (* $p < 0.05$ compared to time-matched histamine control group; # $p < 0.05$ compared to vehicle/PBS control group. Bar indicates $p < 0.05$ individual comparison as indicated. ANOVA with post hoc Hochberg analysis).

preclinical species following intravenous administration were used to give estimates of the maximum systemic exposure in humans if most of the intranasal dose was swallowed and bioavailability was complete. A standard absorption rate was assumed, the volume of distribution was kept constant and the elimination rate was scaled based on liver blood flow. Using these simulations the maximum estimates for exposure in humans following a 0.5 mg dose are a total drug $C_{\max}$ of 0.89 ng/mL and 0.99 ng/mL (based on rat and dog data respectively) and an AUC_{0-24} of 10.5 h.ng/mL and 7.4 h.ng/mL (based on rat and dog data respectively).

Specificity profiling of **33b**

The specificity profiles for both **33b** and azelastine were evaluated in the CEREP Specificity Screen at 50 different receptors, ion channels and transporters). Off-target activity was observed with 5-HT_{2B} (antagonist, pK_i 6.1), 5-HT_{2C} (antagonist, pK_i 5.7), α_{1A} (antagonist, pK_i 5.7) and M₂ (antagonist, pIC_{50} 6.2). The data for azelastine against these targets were 5-HT_{2B} (antagonist, pK_i 7.7), 5-HT_{2C} (antagonist, pK_i 6.3), α_{1A} (antagonist, pK_i 7.3) and M₂ (antagonist, pIC_{50} 5.2). For all of these targets relative to histamine H₁ receptor (pA_2 = 9.3) **33b** shows >1000-fold specificity. As mentioned earlier **33b** also showed significant affinity (pIC_{50} 7.1) for the hERG channel, having a similar potency to azelastine (pIC_{50} = 7.0) in the hERG dofetilide binding assay. Evaluation in rabbit hearts in vitro (SCREENIT model) suggests that the risk of **33b**-induced QT prolongation is low at therapeutically relevant predicted exposure levels.

The mutagenic and clastogenic potential of **33b** was assessed in the AMES test, Mouse Lymphoma screen and DEREK-structure activity relationship in silico analysis). No evidence of mutagenic, clastogenic or toxic potential was detected in these assays up to the maximum concentrations tested.

Conclusion

A series of quinoline sulfone and sulfonamide human H₁ histamine receptor antagonists were prepared using a four- and six-step linear synthesis respectively. The sulfone series although highly potent were orally absorbed and were not progressed any further. In contrast the sulfonamides were not orally absorbed, and had low brain penetration. The optimal chain-length between the piperidine nitrogen and the sulfonamide group was the four-carbon chain, and the optimal sulfonamide nitrogen substituents were methyl, ethyl or *n*-propyl group. The ethyl analogue **33b** had slightly lower potency than azelastine, however, it had longer duration of action in a nasal congestion model in vivo. The compound was metabolised in human hepatocytes to 5 major metabolites, and at least another 30 minor ones. The main routes of metabolism were oxidation in the butyl substituent (hydroxylation and oxidation to ketone), and *N*-dealkylation of the piperidine or sulfonamide nitrogens. Sulfonamide **33b** was selective for the H₁ receptor over 50 other different receptors, ion channels and transporters with >1000-fold selectivity, and did not have any issues with P450 inhibition. It showed similar affinity to azelastine for the hERG channel and evaluation in rabbit hearts in vitro suggested

that the risk of **33b**-induced QT prolongation is low at therapeutically relevant predicted exposure levels. The predicted dose for humans is 0.5 mg per day. **33b** may have increased brain penetration in human if administered with a Pgp inhibitor. The low clinical dose and low bioavailability reduces the potential for significant clinical effects. In summary, **33b** is suitable for progression as a back-up to our phthalazinone intra-nasal candidate for the treatment for allergic rhinitis.

Acknowledgements

We thank Alasdair J Carey, Kam Gohil and David Gomez Ulloa for technical assistance, Anne Jones for the metabolite identification work, Christopher Browning and Linda J. Russell for the duration studies.

Conflicts of interest

None.

A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.bmcl.2017.09.020>. These include experimental details for the synthesis of compounds **5**, **6**, **7**, **27**, **30**, **33b** and **33b.2HCl**, and for the pharmacokinetic and pharmacodynamic studies.

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Supplementary Information

Identification of selective 8-(piperidin-4-yloxy)quinoline sulfone and sulfonamide histamine H₁ receptor antagonists for use in allergic rhinitis.

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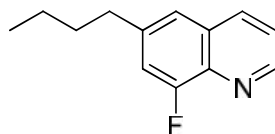
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Experimental

Organic solutions were dried over anhydrous Na_2SO_4 or MgSO_4 . TLC was performed on Merck 0.25 mm Kieselgel 60 F₂₅₄ plates. Products were visualised under UV light and/or by staining with aqueous KMnO_4 solution. LCMS analysis was conducted on a Supelcosil LCABZ+PLUS column (33 mm $\times$ 4.6 mm id) eluting with 0.1% formic acid and 0.01 M ammonium acetate in water (solvent A), and 0.05% formic acid and 5% water in acetonitrile (solvent B), using the following elution gradient 0.0 – 0.7 min 0% B, 0.7 – 4.2 min 100% B, 4.2 – 5.3 min 0% B, 5.3 – 5.5 min 0% B at a flow rate of 3 mL min⁻¹. The mass spectra were recorded on a Fisons VG Platform spectrometer using electrospray positive and negative mode (ES+ve and ES-ve). Column chromatography was performed on Flashmaster II, which utilizes disposable, normal phase, SPE cartridges (2 g to 100 g). ¹H NMR spectra were recorded at 400 MHz unless otherwise stated. The chemical shifts are expressed in ppm relative to tetramethylsilane.

Synthetic procedures

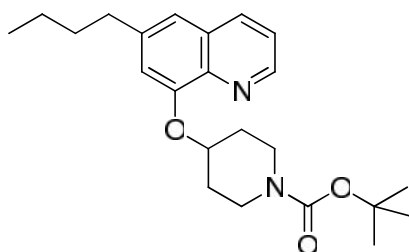
6-Butyl-8-fluoroquinoline (5)



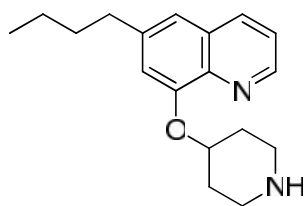
A mixture of 6-bromo-8-fluoroquinoline (24 g, 106 mmol) in DMF (150 mL) was treated under nitrogen with potassium carbonate (33 g, 240 mmol), tributylborane solution in THF (1M, 200 mL) and [1,1'-bis(diphenylphosphino)ferrocene palladium (II)] chloride (1 g, 1.2 mmol). The mixture was stirred under nitrogen and heated at 75°C overnight. The mixture was allowed to cool, diluted with water and extracted with EtOAc ($\times 3$). The combined organic layers were filtered through a frit to remove any insoluble material and the filtrate

was washed with water. The organic layer was dried (MgSO_4), and the filtrate was evaporated under reduced pressure. The residue was purified twice by column chromatography on silica eluting with 0-33% EtOAc in DCM to give **5** (14.47 g, 67%): LCMS RT=3.38 min, ES+ve m/z 204 ($\text{M}+\text{H}$)⁺; ^1H NMR (CDCl_3 , 400 MHz) 8.90 (1H, dd, J 4, 1.5 Hz), 8.10 (1H, dt, J 8, 1.5 Hz), 7.43 (1H, dd, J 8, 4 Hz), 7.38 (1H, s), 7.31-7.26 (1H, m, obscured by solvent), 2.78 (2H, dd, J 8, 7.5 Hz), 1.73-1.65 (2H, m), 1.44-1.32 (2H, m), 0.96 (3H, t, J 7.5 Hz).

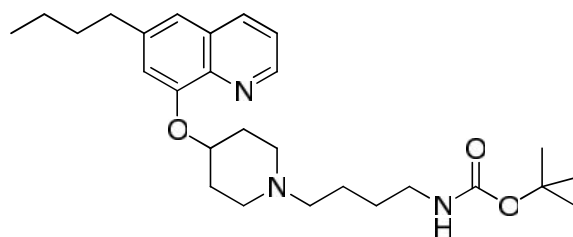
1,1-Dimethylethyl 4-[(6-butyl-8-quinolinyl)oxy]-1-piperidinecarboxylate (6**)**



A solution of **5** (14.4 g, 70.9 mmol) in NMP (20 mL) was added to a mixture of *tert*-butyl 4-hydroxypiperidine-1-carboxylate (21.6 g, 108 mmol) in NMP (75 mL), and the resulting mixture was stirred at 140°C for 90 min and then allowed to cool overnight. The reaction mixture was treated with aq. NH_4Cl solution and extracted with EtOAc ($\times 2$). The combined organic extracts were washed with water, dried (MgSO_4), and the filtrate was evaporated under reduced pressure. The residue was purified twice by column chromatography on silica eluting with 0-25% EtOAc in DCM to give **6** (22 g, 81%) LCMS RT=3.49 min, ES+ve m/z 385 ($\text{M}+\text{H}$)⁺; ^1H NMR (CDCl_3 , 400 MHz) 8.87 (1H, dd, J 4, 1.5 Hz), 8.04 (1H, dd, J 8, 1.5 Hz), 7.36 (1H, dd, J 8, 4 Hz), 7.19 (1H, s), 6.98 (1H, d, J 1.5 Hz), 4.79-4.71 (1H, m), 4.04-3.93 (2H, m), 3.26-3.17 (2H, m), 2.76 (2H, dd, J 8, 7.5 Hz), 2.14-2.05 (2H, m), 2.00-1.89 (2H, m), 1.73-1.63 (2H, m), 1.47 (9H, s), 1.46-1.36 (2H, m), 0.96 (3H, t, J 7.5 Hz).

6-Butyl-8-(piperidin-4-yloxy)quinoline (7)

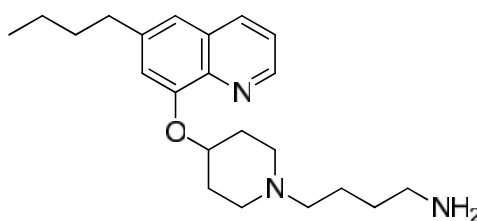
A solution of **6** (21.5 g, 56 mmol) in DCM (50 mL) was slowly treated with TFA (50 mL). The solution was stirred at room temperature for 1 h and then the solvent was evaporated under reduced pressure. The residue was treated with saturated aq. NaHCO₃ solution and extracted with EtOAc (×2). The combined organic extracts were washed with aq. Na₂CO₃ solution, water, dried (MgSO₄), and the filtrate was evaporated under reduced pressure to give **7** (15.9 g, 100%) LCMS RT=2.45 min, ES+ve *m/z* 285 (M+H)⁺; ¹H NMR (CDCl₃, 400 MHz) 8.88 (1H, dd, *J* 4, 1.5 Hz), 8.04 (1H, dd, *J* 8, 1.5 Hz), 7.36 (1H, dd, *J* 8, 4 Hz), 7.17 (1H, s), 6.97 (1H, d, *J* 1.5 Hz), 4.71-4.62 (1H, m), 3.32-3.24 (2H, m), 2.85-2.72 (4H, m), 2.26-2.17 (2H, m), 2.10-1.97 (1H, br), 1.95-1.83 (2H, m), 1.74-1.64 (2H, m), 1.46-1.37 (2H, m), 0.96 (3H, t, *J* 7.5 Hz).

***tert*-Butyl (4-(4-((6-butylquinolin-8-yl)oxy)piperidin-1-yl)butyl)carbamate (27)**

A solution of **7** (855 mg, 3.0 mmol) in 2-butanone (30 mL) was treated with *tert*-butyl (4-bromobutyl)carbamate (1.134 g, 4.5 mmol) and K₂CO₃ (887 mg, 6.4 mmol) and the mixture was heated to reflux under nitrogen for 20 h. The reaction mixture was allowed to cool to room temperature and evaporated under reduced pressure. The residue was partitioned between DCM (25 mL) and water (25 mL). The aqueous layer was extracted with DCM and

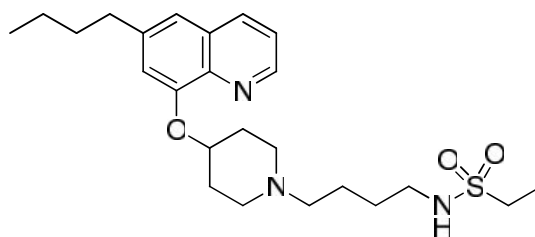
the combined organic extracts were washed with water. The organic phase was dried by passing through a hydrophobic frit and the filtrate was concentrated under reduced pressure. The residue was purified by chromatography on a silica cartridge (100 g) eluting with 0-15% MeOH in DCM containing 1% Et₃N. Appropriate fractions were combined and evaporated under reduced pressure to give **27** (1.292 g, 94%) LCMS RT=3.03 min, ES+ve m/z 456 (M+H)⁺; ¹H NMR (CD₃OD, 400 MHz) 8.74 (1H, dd, *J* 4, 1.5 Hz), 8.22 (1H, dd, *J* 8, 1.5 Hz), 7.49 (1H, dd, *J* 8, 4 Hz), 7.27 (1H, s), 7.11 (1H, s), 4.78-4.69 (1H, m), 3.10-2.93 (4H, m), 2.79 (2H, dd, *J* 8, 7.5 Hz), 2.58-2.47 (4H, m), 2.21-2.11 (2H, m), 2.08-1.98 (2H, m), 1.76-1.66 (2H, m), 1.65-1.55 (2H, m), 1.55-1.37 (13H, m and s), 0.97 (3H, t, *J* 7.5 Hz).

4-(4-((6-Butylquinolin-8-yl)oxy)piperidin-1-yl)butan-1-amine (30)



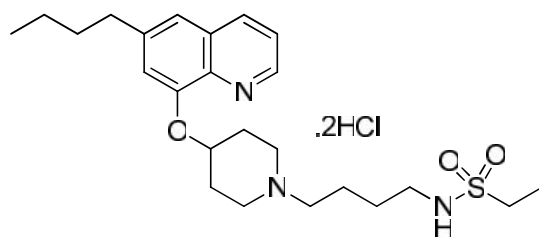
A solution of **27** (1.292 g, 2.84 mmol) in MeOH (10 mL) was treated with 4M HCl in dioxane (30 mL, 120 mmol) and the mixture was stirred for 3 h at room temperature. The reaction mixture was concentrated under reduced pressure and the residue was dissolved in MeOH and applied to a pre-conditioned SCX-2 ion-exchange cartridge (50 g). The cartridge was washed with MeOH (3 CV) and the product was eluted with 10% aqueous ammonia in MeOH (3 CV). The ammoniacal fractions were concentrated under reduced pressure to give **30** (891 mg, 88%) LCMS RT=2.34 min, ES+ve m/z 356 (M+H)⁺; ¹H NMR (CD₃OD, 400 MHz) 8.74 (1H, dd, *J* 4, 1.5 Hz), 8.24 (1H, dd, *J* 8, 1.5 Hz), 7.50 (1H, dd, *J* 8, 4 Hz), 7.28 (1H, s), 7.12 (1H, s), 4.75-4.67 (1H, m), 3.02-2.94 (2H, m), 2.81 (2H, dd, *J* 8, 7.5 Hz), 2.71 (2H, t, *J* 7 Hz), 2.50-2.37 (4H, m), 2.22-2.13 (2H, m), 2.08-1.98 (2H, m), 1.77-1.68 (2H, m), 1.66-1.50 (4H, m), 1.47-1.39 (2H, m), 0.99 (3H, t, *J* 7.5 Hz).

***N*-(4-{4-[(6-Butyl-8-quinolinyl)oxy]-1-piperidinyl}butyl)ethanesulfonamide (33b)**



A solution of **30** (248 mg, 0.7 mmol) in DCM (5 mL) was treated with triethylamine (0.156 mL, 1.12 mmol) followed by ethanesulfonyl chloride (0.080 mL, 0.84 mmol). The mixture was stirred at room temperature for 2 h and then stood at room temperature overnight. The reaction mixture was washed with aqueous sat. NaHCO₃ solution, passed through a hydrophobic frit and the organic solution concentrated *in vacuo*. The residue was purified by chromatography on a silica cartridge (20 g) eluting with DCM-EtOH-aqueous 0.88 ammonia solution (100:8:1). Appropriate fractions were combined and evaporated *in vacuo* to give **33b** free-base (167 mg, 53%) LCMS RT=2.66 min, ES+ve *m/z* 448 (M+H)⁺; ¹H NMR (CD₃OD, 600 MHz) 8.75 (1H, dd, *J* 4, 1.5 Hz), 8.23 (1H, dd, *J* 8, 1.5 Hz), 7.50 (1H, dd, *J* 8, 4 Hz), 7.29 (1H, s), 7.14 (1H, s), 4.82-4.76 (1H, m), 3.21-3.13 (2H, m), (2H, t, *J* 7 Hz), 3.05 (2H, q, *J* 7 Hz), 2.80 (2H, t, *J* 7.5 Hz), 2.73-2.62 (4H, m), 2.23-2.15 (2H, m), 2.13-2.06 (2H, m), 1.75-1.67 (4H, m), 1.63-1.57 (2H, m), 1.45-1.38 (2H, m), 1.32 (3H, t, *J* 7 Hz), 0.98 (3H, t, *J* 7 Hz); ¹³C NMR (CD₃OD, 151 MHz) 152.0, 147.7, 142.1, 138.9, 136.3, 129.9, 121.5, 118.2, 112.7, 57.3, 49.8, 48.5, 45.7, 42.1, 35.6, 33.2, 29.2, 27.7, 22.8, 22.0, 12.9, 7.1; UV (MeCN) λ_{max} 203 nm (ε 48800), 240 (41700), 287 nm (3590) and 301 nm (3640).

***N*-(4-{4-[(6-Butyl-8-quinolinyl)oxy]-1-piperidinyl}butyl)ethanesulfonamide dihydrochloride salt (33b.2HCl)**



The free base **33b** (18.5 g, 41.4 mmol) was dissolved in MeOH (100 mL) and 2M HCl (50 mL) was added. The solvent was removed *in vacuo* and the residue was dissolved in MeOH (100 mL). The solvent was removed *in vacuo* and the process was repeated three times. The residual oil started to crystallise and once complete the sample was dried *in vacuo* overnight. The solid was then triturated with EtOAc and the solid was collected by filtration. The solid was dried *in vacuo* at 40°C overnight to give **33b.2HCl** (13.5 g, 63%): mp >200°C (decomp.); LCMS RT=2.75 min, ES+ve m/z 448 (M+H)⁺; HPLC (Phenomenex Luna C18 column, 2 min run) RT=1.17 min, 99.7% pure; ¹H NMR (CDCl₃, 400 MHz) (two protonated forms, axial and equatorial piperidinium salt present) 17.1 (1H, s), 11.3 (1H, s), 9.3 (1H, s), 8.8 (1H, d), 7.95 (1H, d), 7.5 (1H, s), 7.4 (1H, s), 6.4 (1H, s), 5.3 (1H, s), 4.4 (2H, br s), 3.5 (4H, m), 3.2 (2H, m), 3.0 (2H, m), 2.9 (4H, m), 2.2 (2H, d), 2.1 (2H, m), 1.7 (4H, m), 1.4 (5H, m), 0.95 (3H, t); ¹H NMR (CD₃OD, 600 MHz) 9.12 (0.6H, d, *J* 5 Hz), 9.09 (1H, d, *J* 8 Hz), 9.04 (0.4H, d, *J* 5 Hz), 8.12-8.06 (1H, m), 7.72 (1.4H, m), 7.65 (0.6H, s), 5.32-5.29 (0.6H, m), 5.15 -5.09 (0.4H, m), 3.82-3.73 (2H, m), 3.57-3.52 (1H, m), 3.32-3.27 (2H, m, obscured by solvent), 3.27-3.22 (1H, m), 3.12 (2H, t, *J* 6.5 Hz), 3.09-3.04 (2H, m), 2.95-2.90 (2H, m), 2.58-2.52 (1H, m), 2.46-2.40 (1H, m), 2.38-2.24 (2H, m), 1.96-1.89 (2H, m), 1.80-1.73 (2H, m), 1.68-1.61 (2H, m), 1.48-1.41 (2H, m), 1.34-1.29 (3H, 2t, *J* 7.5 Hz), 0.98 (3H, 2t, *J* 7.5 Hz).

Pharmacokinetic studies

All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of

Animals (as appropriate). Pharmacokinetics were determined in male Wistar Han rats (Charles River) or male Sprague Dawley rats (Charles River) and male Beagle dogs (Harlan) following single oral and intravenous administration to aid prediction of the likely human pharmacokinetics using precedented physiological scaling techniques. In the rat compounds were dosed intravenously and orally to 2 rats per compound per route. Nominal doses of 1 mg/kg were used for intravenous (iv) and oral (po) administration and studies were conducted following routine animal husbandry methods. Rats were housed in standard holding cages and maintained in a controlled environment with free access to food and water. Serial blood samples were collected via a temporary cannula inserted into the tail vein of all animals. For the intravenously dosed animals the cannula was inserted into a vein discrete from that used for dosing. Euthanasia was carried out by rising concentration of carbon dioxide and/or cervical dislocation or, alternatively, the final blood sample was taken via cardiac puncture following anaesthesia with isoflurane. Confirmation of the death of an animal was determined by confirmation of the permanent cessation of circulation and / or dislocation of the neck. The dogs were housed in standard cages. Food was removed at approx 4 p.m. the day prior to dosing and offered 4 hours post dose. Water was available ad libitum throughout the study. The dogs were kept in slings for no longer than 2 hours following the end of dosing on each phase of the study. Compounds were dosed intravenously (bolus, 0.5 mg/kg) using an agiocath and orally (gavage; 1mg/kg) to two male Beagle dogs per compound in a cross over design. Serial blood samples were collected from the cephalic vein via an angiocath for the first 2 hours post dose and via direct venupuncture for the remainder of the study. For all doses in both species the compounds were formulated as solutions in DMSO:PEG 200:Water (5:45:50). Profiles were obtained by taking serial blood samples over a range of time-points up to 7 or 24 h post-dose. Blood samples were taken directly into heparinised tubes and either prepared by protein precipitation or centrifuged to produce

plasma and then prepared by protein precipitation before being subjected to quantitative analysis by LCMS/MS using compound-specific mass transitions. Drug concentration-time profiles were generated and non-compartmental PK analysis used to generate estimates of half-life, clearance, volume of distribution and oral bioavailability.

CNS Penetration Studies

Compounds were dosed intravenously at a nominal dose level of 1 mg/kg to male CD Sprague-Dawley rats. The test compounds were formulated as solutions in DMSO-PEG 200-water (5:45:50). Blood samples were taken under terminal anaesthesia with isoflurane at 5 min postdose, and the brains were also removed for assessment of brain penetration. Blood samples were taken directly into heparinised tubes and prepared for analysis using protein precipitation, and brain samples were prepared using extraction of drug from brain by homogenisation and subsequent protein precipitation. The concentration of parent drug in blood and brain extracts was determined by quantitative LCMS/MS analysis using compound-specific mass transitions.

***In vivo* nasal Buxco assay**

Guinea pigs were sourced from Harlan Laboratories, with their weight on arrival ranging between 150 and 250 g. Following 5 days acclimatisation, guinea pigs were sensitised to ovalbumin, starting on the Monday after their arrival. For the first week (one 'week' here refers to Monday through Friday), guinea pigs received 25 µL per nostril of ovalbumin (20 µg/mL, this and all other compounds dissolved in physiological saline) and aluminium hydroxide (5 mg/mL) twice daily, with a minimum 4 hour between dosing. During the second and third weeks, 25 µL ovalbumin (5 mg/mL) was instilled bilaterally to the nostrils once daily. During the fourth week, guinea pigs were dosed with compounds of interest and

subsequently challenged. On any days prior to compound dosing, the sensitising procedure from the previous week was continued. Body weights were continually monitored to ensure regular growth and no decline induced by sensitising procedure.

To dose compounds of interest, bodyweights were once more recorded and animals were anaesthetised with isoflurane (5%, 2-3 L/min of O₂) to blunt the sneezing reflex. Azelastine (1 mg/mL), **33b** (1 mg/mL) or physiological saline control was intranasally instilled, 25 µL bilaterally with guinea pigs in a supine position, leaving approximately 10 seconds between nostrils. Guinea pigs remained in the supine position for a further 30 seconds before returning to their cages and being laid laterally for recovery.

Either 3 or 24 hours post compound dosing, guinea pigs were challenged intranasally. 30 minutes prior to challenge, intraperitoneal atropine monohydrate hemisulfate (1 mg/mL, not base corrected, 1 mL/kg) was administered to reduce later mucus production and prevent complete nasal occlusion. Guinea pigs were then placed in calibrated whole body plethysmography chambers (Buxco Electronics) and a ten minute PenH (enhanced pause) baseline was recorded. Guinea pigs were excluded from the study if baseline cumulative PenH was greater than 1000. At time of challenge, animals were returned to the anaesthetic chamber, and dosed with either histamine (10 mM) or physiological saline control, 25 µL per nostril as described previously. As soon as guinea pigs could stand upright, they were returned to Buxco chambers, and 40 minutes of cumulative PenH were recorded. High cumulative PenH values correspond with nasal blockage, generated predominantly by histamine-induced swelling of the vasculature in the nasal turbinates. Average and standard deviation values were calculated and plotted across multiple animals receiving the same treatment, and significance was calculated by performing ANOVA analysis on log-

transformed data, followed by post-hoc Hochberg testing. All statistical analyses were performed in Statistica (Statsoft).