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Design of Phthalazinone Amide Histamine H_1 Receptor Antagonists for Use in Rhinitis

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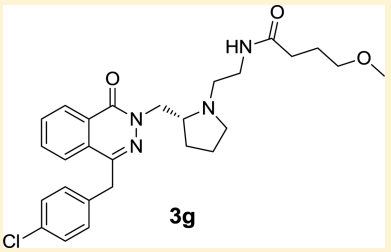
Supporting Information

ABSTRACT: The synthesis of potent amide-containing phthalazinone H_1 histamine receptor antagonists is described. Three analogues **3e**, **3g**, and **9g** were equipotent with azelastine and were longer-acting in vitro. Amide **3g** had low oral bioavailability, low brain-penetration, high metabolic clearance, and long duration of action in vivo, and it was suitable for once-daily dosing intranasally, with a predicted dose for humans of approximately 0.5 mg per day.

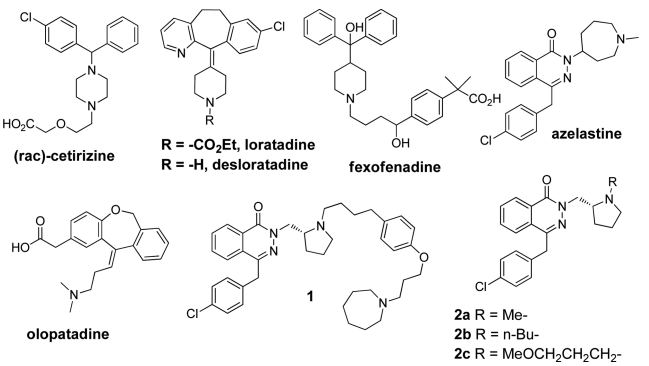


KEYWORDS: *Histamine H_1 receptor antagonist, allergic rhinitis, once-daily dosing, topical application, phthalazinone*

Allergic rhinitis affects globally around 500 million people, with high prevalence in the industrialized world. Furthermore, it has been steadily increasing with a near quadrupling of primary care consultations over the last 50 years.[1,2] Rhinitis symptoms include nasal itching (pruritus), irritation and repetitive sneezing, rhinorrhea, nasal congestion, headache, irritation of the throat, watering of the eyes (epiphora), and edema. Nasal congestion may become more pronounced leading occasionally to breathing through the mouth and snoring[3] and loss of smell (hyposmia).[4]

Small molecule pharmacotherapy for allergic rhinitis includes antihistamines, corticosteroids, leukotriene antagonists, decongestants, mast cell stabilizers, and anticolinergics.[2,3,5] The most commonly used medicines for the treatment of rhinitis are antihistamines, which are H_1 receptor antagonists (inverse agonists).[6,7] These were introduced several decades ago, and although they were effective, they caused sleepiness due to their ability to interact with brain H_1 receptors responsible for wakefulness. Oral second-generation antihistamines possessing polar carboxylic acid groups (fexofenadine and cetirizine, Chart 1) have reduced brain-penetration and a corresponding reduction in their side-effects. Nasal congestion is one symptom that second generation antihistamines are not treating effectively; therefore, they are frequently used in combination with α-adrenergic agonist decongestants, for example, pseudoephedrine. However, these decongestants have their own side-effects, such as hypertension. A further advancement in treating rhinitis was the development of topical treatments, such as azelastine and olopatadine hydrochlorides. The advantage of intranasal treatments is the attainment of a higher concentration of drug directly to the nasal cavity with reduced systemic side-effects due to the lower dose used. In order to avoid absorption of the portion of the dose that is swallowed, topical treatments must exhibit low oral absorption. Azelastine hydrochloride dosed intranasally is used in allergic rhinitis patients not responding well to fexofenadine and loratadine, and is more effective than cetirizine.[8,9] Olopatadine hydrochloride nasal spray has been approved in the US and has comparable efficacy to azelastine, same duration of action (12 h), and slower onset of action (30 min vs 15 min), but also suffers from the same side-effects, dysgeusia (bitter taste),

Chart 1. Representative Oral and Intranasal H_1 Receptor Antagonists Used Clinically, Single-Ligand Histamine H_1H_3 Dual Receptor Antagonist 1, and Selective H_1 Receptor Antagonists 2a−c Based on a Phthalazinone Scaffold



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headache, nose-bleeds (epistaxis), and drowsiness (somnolence).[10]

We have previously disclosed our work in optimizing histamine H_1 receptor antagonists containing a phthalazinone scaffold and also the development of single-compound dual H_1 and H_3 receptor antagonists, leading on to our clinical candidate **1** (Chart 1).[11] We have also published on phthalazinones **2a–c** and their more hydrophilic aza- and diaza-analogues.[12] Rhinitis is closely linked to other allergic diseases, such as asthma, rhinosinusitis, and conjuctivitis. One disadvantage of allergic rhinitis treatment using antihistamine monotherapy is the neglect of other comorbidities. In general, the most potent anti-inflammatory agents are the glucocorticoids, such as fluticasone furoate, which is approved for the once-daily treatment of allergic rhinitis. In this publication we describe our efforts in identifying a long-acting, potent human H_1 receptor antagonist, which could either be used in combination with an H_3 receptor antagonist or with fluticasone furoate.[13,14]

We have previously reported on the identification of a channel in the 7TM bundle,[11] which was approached from the **2a** pyrrolidine nitrogen. We have exploited this finding by appending the H_3 fragment and the butyl linker to construct the H_1H_3 dual receptor antagonist **1**.[11] Highly potent H_1 antagonists were obtained with simple straight chain alkyl substituents on the pyrrolidine nitrogen of **2a**, such as **2b** and **2c**. The ether oxygen of **2c** was thought to have formed an H-bond with a Tyr of TM7.

At the outset we were interested in a very potent, homochiral H_1 antagonist suitable for a low-dose, small-volume intranasal delivery with a 24 h duration of action. We have previously shown that the (*R*)-enantiomer of **2a** was the higher affinity enantiomer.[11] We envisioned replacing the ether oxygen of **2c** with an amide group, which might provide an even tighter H-bond leading to increased affinity and/or duration, but also introduce metabolic instability leading on to increased clearance, a requirement for topical administration. Furthermore, introducing an electron withdrawing group, such as the amide functionality, was expected to lower the pK_a of the pyrrolidine nitrogen, thereby increasing selectivity against hERG.[12,15,16]

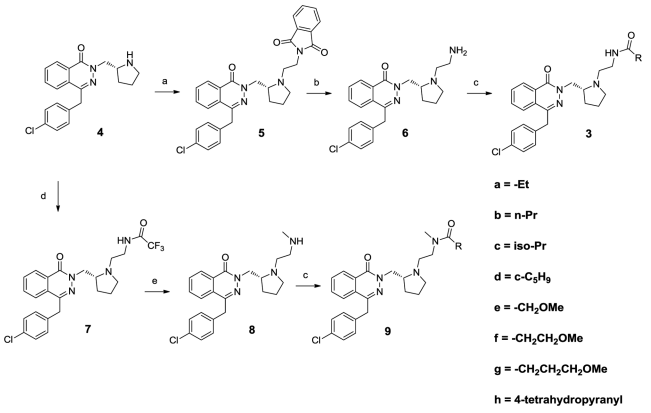
Chemistry. The target amides **3** were prepared by the three-step synthetic route outlined in Scheme 1. The (*R*)-enantiomer of **4**[11] was alkylated with *N*-(2-bromoethyl)phthalimide to give **5** in 87% yield, deprotected using hydrazine to give amine **6** (77% yield), and then acylated with a variety of carboxylic acids using TBTU to provide amides **3d,f,g,h** or with the corresponding acid chloride to give **3a,b,c,e**. Alkylation of **4** with 2,2,2-trifluoro-*N*-(2-iodoethyl)acetamide gave the trifluoroacetamide **7**, which was alkylated with iodomethane and deprotected to provide the secondary amine **8**. The latter was acylated with 4-methoxybutanoic acid and TBTU to give the tertiary amide **9g**.

In addition to amides **3a–h** and **9g**, alternative amides **10b,h** where the acyl part of the amide group is reversed (closer to the pyrrolidine basic nitrogen) were prepared and their synthesis is outlined in Scheme 2. These compounds were synthesized by alkylation of pyrrolidine **4** with ethyl 4-bromobutyrate to give ester **11**, which was converted to carboxylic acid **12** by base hydrolysis and finally converted to amides **10b** and **10f**.

Results and Discussion. The biological screens used to assay the compounds were reported previously[11] and include H_1 receptor affinity using recombinant human histamine H_1 receptor,[17] adrenergic α_{1A} and α_{1B} receptor affinity,[18] and hERG activity,[18] and the data are summarized in Table 1. Azelastine, which was used as a standard, exhibited high affinity for both the H_1 receptor (pK_i = 8.9) and the hERG channel (dofetilide binding assay pIC_{50} = 7.0).[11] The straight chain amides **3a** and **3b** were equipotent to azelastine; however, they were less selective for the α_{1A} and α_{1B} receptors and were rejected. The branched amides **3c** and **3d** were less potent than azelastine, but also suffered from reduced selectivity for the α_{1A} and α_{1B} receptors and were rejected too. The introduction of an oxygen atom in the unbranched amide chain was used to probe for additional H-bond interactions. The tetrahydropyran analogue **3h**, like the other branched amides, was less potent and was rejected. The reverse amide **10b** had high hERG affinity and was also rejected.

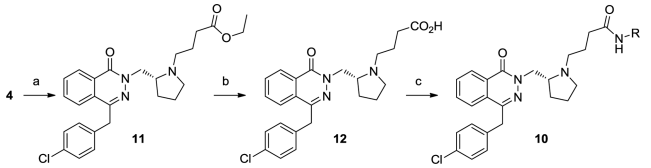
In addition to the above assays, another lower-throughput version of the H_1 assay was used, which provided more precise pA_2 values and an indication of the analogues' duration of action.[11] The data for phthalazinone analogues **3a–h**, **9g**, **10b** and **10f** are summarized in Table 2 and compared to azelastine (pA_2 9.7). Their duration of action in vitro 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. Analogues **3f** and **10f** were rejected based on their lower affinity than azelastine in the secondary assay (Table 2). The remaining compounds **3e**, **3g**, and **9g** were equipotent with azelastine, exhibited a longer duration of action in vitro, and

Scheme 1. Synthesis of Amides 3 and 9[a]



[a]Reagents and conditions: (a) *N*-(2-bromoethyl)phthalimide, K_2CO_3, 2-butanone, 80 °C, 18 h, 87%;)b) $NH_2NH_2{\cdot}H_2O$, EtOH, 80 °C, 1.25 h, 77%; (c) RCO_2H, TBTU, Et_3N, DMF, 2 h, or RCOCl, Et_3N, DCM; (d) 2,2,2-trifluoro-*N*-(2-iodoethyl)acetamide, DIPEA, 2-butanone, 100%; (e) (i) MeI, K_2CO_3, DMF; (ii) K_2CO_3, H_2O, MeOH, 29% (two steps).

Scheme 2. Synthesis of Amides 10b and 10f[a]



[a]Reagents and conditions: (a) $BrCH_2CH_2CH_2CO_2Et$, K_2CO_3, DMF, 150 °C, microwave, 110 min, 51%; (b) 2 M NaOH, H_2O, MeOH, 20 °C, 1 h, 91%; (c) $nPrNH_2$ for **10b** or $MeOCH_2CH_2NH_2$ for **10f**, TBTU, Et_3N, DMF, 47–68%.

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Table 1. Antagonist Affinity[a] at the Human H_1 Receptor (FLIPR Assay), the α_{1A} and α_{1B} Receptors (in Fibroblast Cells by Means of Plate-Based Calcium Imaging), Measured logD at pH 7.4, and hERG Binding Affinity

compd	H_1 pK_i (n)	α_{1A} pK_i (n)	α_{1B} pK_i (n)	log$D_{7.4}$	hERG pIC_{50}
Azelastine	8.9 ± 0.0 (364)	7.3 ± 0.0 (145)	7.3 ± 0.0 (97)	2.3	7.0 (116)
3a·TFA	9.2 ± 0.2 (4)	7.8 ± 0.4 (2)	7.8 ± 0.1 (2)	3.4	6.7 (1)
3b·TFA	9.1 ± 0.1 (4)	7.5 ± 0.1 (2)	7.1 ± 0.4 (2)	3.7	7.2 ± 0.3 (2)
3c·TFA	8.4 ± 0.1 (10)	8.4 ± 0.1 (5)	7.2 ± 0.3 (2)	3.2	6.4 ± 0.0 (2)
3d·TFA	8.4 ± 0.1 (6)	7.1 ± 0.1 (3)	7.1 (1)	3.9	7.3 ± 0.1 (2)
3e·TFA	8.9 ± 0.4 (2)	7.3 (1)	7.5 (1)	3.1	6.7 (1)
3f·HCO_2H	9.1 ± 0.1 (4)	8.1 ± 0.1 (2)	7.2 ± 0.1 (2)	3.2	6.4 (1)
3g	8.5 ± 0.0 (4)	7.3 ± 0.1 (9)	7.0 ± 0.2 (8)	3.5	6.4 ± 0.0 (4)
3h·TFA	8.0 ± 0.1 (6)	6.7 ± 0.0 (3)		3.3	6.1 ± 0.0 (2)
9g·TFA	8.5 ± 0.1 (4)	7.5 ± 0.1 (2)	6.9 (1)	3.5	6.4 ± 0.1 (4)
10b·HCl	8.3 ± 0.1 (5)	8.0 ± 0.3 (2)	7.5 ± 0.2 (3)	3.3	8.8 (1)
10f·HCl	9.1 ± 0.1 (12)	8.2 ± 0.1 (6)	8.0 ± 0.1 (6)	2.5	7.4 ± 0.1 (3)

[a]Mean ± SEM of estimated functional pK_i for $n < 3$; the SEM is the SD; n = number of experiments

Table 2. Antagonist Affinity at H_1 Receptor (pA_2) and Duration of Action in Vitro

compd	pA_2 ± SEM[a]	n	wash-out
Azelastine	9.7 ± 0.1	19	reference
3a·TFA	9.6 ± 0.2	6	no difference
3b·TFA	9.2 ± 0.3	2	slower
3c·TFA	9.88 ± 0.15	8	slower
3d·TFA	9.5 ± 0.1	13	slower
3e·TFA	9.7 ± 0.1	14	slower
3f·HCO_2H	9.3 ± 0.1	10	slower
3g	9.74 ± 0.09	20	slower
3h·TFA	9.1 ± 0.1	13	slower
9g·TFA	10.0 ± 0.3	12	slower
10b·HCl	9.8 ± 0.2	9	slower
10f·HCl	9.3 ± 0.1	8	slower

[a]All pA_2 values calculated from curve shifts generated after 30 min incubation time at 100 nM antagonist concentration.

were therefore of interest for further examination. Analogue **3g** was historically made before the other two compounds and hence was investigated first.

*Pharmacokinetics Amide **3g**.* **TFA** had low in vivo CNS penetration in the rat (brain−blood ratio of 0.18 and mean brain concentration of 121 ng/g, n = 3) assessed in samples taken 5 min after an intravenous bolus dose of 1 mg/kg. High in vitro plasma protein binding was observed in four species (rat, guinea pig, dog, and human), and blood cell association of **3g·HCl** was low to moderate in the same species (Table 3, in SI section). The membrane permeability of **3g·HCl** across MDCK cells in the presence and absence of a P-glycoprotein (PgP) inhibitor was 220 nm/s at pH 7.4 when incubated at a concentration of 2.5 μg/mL, indicating that **3g** is not a PgP substrate and that low brain exposure would be maintained. The trifluoroacetate and hydrochloride salts of **3g** were progressed to the male CD Sprague−Dawley rat and Beagle dog pharmacokinetic studies in vivo. The compounds were dosed as solutions in H_2O−PEG200−DMSO (50:45:5). Each compound was dosed iv at 1 mg/kg (both species) and po at 3 mg/kg for the rat and 2 mg/kg for the dog. In addition one dog was also dosed subcutaneously (Table 4, in SI section). Following intravenous administration of **3g** blood clearance (Clb) was high compared to liver blood flow (LBF) (means of 66 and 31 mL/min/kg in rat and dog, respectively) and the steady-state volume of distribution (V_{ss}) was low to moderate (means of 0.7 and 1.1 L/kg in rat and dog, respectively). The resultant elimination half-life was short (means of 0.2 and 0.5 h in rat and dog, respectively). Elimination of parent compound via urine was low following oral, subcutaneous, and intravenous administration of **3g·HCl** to the dog (nondetectable following oral administration and <3% following subcutaneous and intravenous administration). Following oral administration of **3g** bioavailability was low (<3% for rat and <8% for dog). Oral bioavailability in the rat was assessed at 3 mg/kg (negligible bioavailability) and 300 mg/kg (<3% bioavailability). At the higher dose in addition to low bioavailability the blood concentrations were highly variable between rats. Absorption of **3g** into the hepatic portal vein following oral administration to the rat was also shown to be low (3 to 7% using a 3 mg/kg dose). Following subcutaneous administration of **3g** to the dog bioavailability appears complete. These data suggest that the low in vivo bioavailability may be due to high first-pass metabolism (both intestinal and hepatic) rather than poor intestinal permeability. The bioavailability of azelastine in the rat and dog[11] was higher than that of **3g**, whereas in humans the reported azelastine bioavailability is 40%.[19] The in vitro rate of metabolism was high for **3g·HCl** using liver microsomes from mouse (50 mL/min/g tissue), rat (50 mL/min/g), dog (12 mL/min/g), and human (25 mL/min/g). The high in vitro hepatic clearance data is in agreement with the high in vivo clearance data for rat and dog. The in vitro rate of metabolism for **3g·HCl** was also significant using intestinal microsomes from rat (12 mL/min/g), monkey (>50 mL/min/g), and human (11 mL/min/g). These data are in agreement with the low bioavailability/absorption observed following oral administration to the rat, despite **3g** showing good cell permeability. Incubation of **3g·HCl** (10 μM initial concentration) with cryopreserved human hepatocytes for 3 h resulted in extensive metabolism of the compound, detecting at least 16 metabolites. Of the metabolites, labeled **M1** to **M16** according to their retention time on the LC−MS/MS chromatogram, **M1** was the first compound to elute off the column. The metabolites ranged from oxidation of the pyrrolidine ring (lactam formation, dehydrogenation of pyrrolidine ring), amide hydrolysis, *N*- and *O*-dealkylation, and glucuronidation. The structures of these metabolites are shown in Chart 2 in the SI section together with their relative abundance. The levels of the two acyl glucuronide metabolites were low compared to the overall metabolic profile. No evidence was found for the formation of *N*-oxide or glutathione conjugated metabolites. Some second-generation antihistamines are substrates and modulators of CYP450, in particular the subtype CYP3A4. The inhibitory potential of **3g** was assessed for the five major human CYP450 enzymes (CYP1A2, >100 μM; CYP2C9, 6.7 μM; CYP2C19, 27 μM; CYP2D6, 1.7 μM; CYP3A4, 1.3 μM). Compound **3g** did not cause time-dependent inhibition with CYP2D6, but a

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significant change in IC_{50} with time was observed using CYP3A4 (mean fold change of 5). In the Cypex cDNA screen Azelastine also shows time-dependent inhibition of CYP3A4.

*Effect of **3g** on Histamine-Induced Nasal Congestion in Vivo.* The standard Buxco whole body plethysmography technique was used to investigate the effect of intranasally dosed **3g** on histamine-induced nasal congestion in conscious, unrestrained guinea pigs, which were previously sensitized with ovalbumin and aluminum hydroxide intranasally over a 3-week period prior to the study. Recording PenH (enhanced pause) AUC over 40 min following bilateral histamine challenge (15 or 10 mM, 25 μL/nostril, under light isoflurane anesthesia) allows for the assessment of efficacy and duration of action of intranasally dosed antihistamines.[20]

Compound **3g** showed significant inhibition of histamine-induced congestion, compared to a vehicle-pretreated/histamine challenged control group, at 1 and 24 h after administration of a 1 mg/mL solution (25 μL/nostril). In contrast, azelastine did not demonstrate a similar duration of action at the same concentration (Figure 1). Further studies

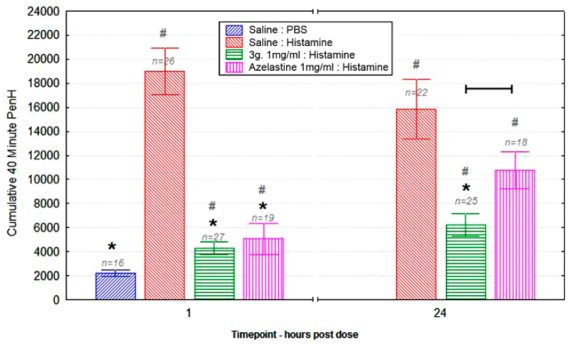

Figure 1. Duration of action of azelastine and **3g** in the guinea pig model of nasal congestion. Animals were exposed to histamine at 1 and 24 h after an intranasal dose of 1 mg/mL of **3g·TFA**, **3g·HCl**, or azelastine. Mean ± SEM (n = 16−27 per group). *p < 0.05 compared to histamine control group; $^{\#}p$ < 0.05 compared to vehicle/PBS control group. Bar indicates p < 0.05 individual comparison as indicated. ANOVA with posthoc Hochberg analysis.

indicated that duration of action is less than 48 h following administration of a 1 mg/mL solution of **3g** (data not shown). Neither **3g** nor azelastine produced a procongestant response at any time point studied (effect on baseline PenH, data not shown). Arterial blood samples taken immediately following evaluation of nasal congestion (4 or 25 h after intranasal dosing) do not show any systemic exposure from 1 mg/mL of **3g** in these studies, which indicates a low bioavailability by this route and a local mechanism of action.

In order to determine the cross species activity between human and guinea pig,[20] the effect of **3g** on the contractile response to histamine in guinea pig isolated trachea (i.e., upper respiratory tract and closest available tissue assay to the nasal cavity for investigation) and human bronchus was determined (guinea pig pA_2 8.7 and human bronchus 8.2) demonstrating that the potency was comparable between species.

Predicted Human Dose. The clinical dose of **3g** 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 **3g** and azelastine. Based on high blood clearance, low oral rat and dog bioavailability, and a low clinical dose, the systemic exposure to **3g** in humans is expected to be very low. The concentration of **3g** was less than the limits of quantification for the assays used in all assayed blood samples taken from guinea pigs showing a pharmacological response in a histamine induced nasal congestion model following intranasal administration of **3g**. In order to appreciate toxicology risks/overage the pharmacokinetic profiles in 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. For the predictions a standard absorption rate was assumed; the volume of distribution was kept constant and the elimination rate was scaled based on liver blood flow. Based on these simulations the maximum estimates for exposure in humans following a 0.5 mg dose are a total drug C_{max} of 1.4 and 1.6 ng/mL (based on rat and dog data, respectively) and an AUC_{0-24} of 4.1 and 4.8 h.ng/mL (based on rat and dog data, respectively).

*Specificity Profiling of **3g**.* The selectivity of **3g** for the human H_1 receptor over the other three histamine receptors (H_2, H_3, and H_4) was evaluated in binding assays on recombinantly expressed histamine receptors, using measurements of inhibition of histamine stimulated cAMP in HEK-293 cell membranes (for H_2), inhibition of histamine stimulated GTPγS binding in CHO cell membranes (for H_3), and inhibition of radiolabeled histamine binding in HEK-293 cell membranes (for H_4) and found to show >1000-fold specificity. Compound **3g** had a pA_2 for H_1 = 9.4 (n = 54), pK_i for H_2 5.1 (n = 2), pK_i for H_3 < 6.2 (n = 29), and pK_i for H_4 < 6.4 (n = 2). The selectivity of **3g** and azelastine against 50 different receptors, ion channels, enzymes, and transporters was evaluated in the CEREP Specificity Screen in agonist or antagonist mode as appropriate. Compound **3g** (at 1 μM) caused >50% inhibition at 6 targets in this screen (α_1, D_1, D_{2S}, 5-HT_{2A}, 5-HT_6, and 5-HT_7), indicative of some off-target activity; a similar profile was seen with azelastine, which caused >50% inhibition at 10 targets, four of which overlapped with **3g**. Further work was performed to quantify and compare the specificity profile of **3g** relative to H_1 receptor (pA_2 9.4), which showed >100-fold specificity, apart from the human 5-HT_{2B} receptor (pK_i 7.8 in antagonist mode; 40-fold selectivity for H_1). This is not considered to be an issue since azelastine, which is used clinically, had the same pK_i value in the same assay. In addition **3g** has lower bioavailability than azelastine suggesting that systemic exposure after intranasal administration is likely to be negligible. Compound **3g** also showed significant affinity (pIC_{50} = 6.4) in the hERG binding assay, however, this was lower than azelastine (pIC_{50} = 7.0). Furthermore, **3g** was clean in the rabbit ventricular wedge assay (up to 10 μM), suggesting that the risk of **3g**-induced QT prolongation is low at the predicted exposure levels.

The mutagenic and clastogenic potential of **3g** was assessed in the AMES test, Mouse Lymphoma screen and DEREK structure−activity relationship in silico analysis. No evidence of mutagenic potential was detected in the mini-AMES assay up to the maximum concentrations tested (which were limited by precipitation and/or toxicity as appropriate in each strain) in the presence or absence of an in vitro metabolic activation system (S9-mix). No evidence of clastogenic potential was detected in the mouse lymphoma screen up to maximum concentrations of 80 μg/mL for 3 h in the presence of S9-mix and 40 μg/mL for 24 h in the absence of S9-mix (both maximum concentrations were limited by cytotoxicity). The analysis of the molecular structure of **3g** using DEREK in silico

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analysis showed no evidence of overt mutagenic or toxic potential.

Conclusion. A series of pyrrolidine amide human H_1 histamine receptor antagonists based on the phthalazinone scaffold were synthesized. Amides **3e**, **3g**, and **9g** were equipotent with azelastine, the clinical gold-standard (pA_2 9.9, 9.7, and 10.0, respectively, vs 9.7 for azelastine), and had a longer duration of action than azelastine in vitro. Amide **3g** was selective for the H_1 receptor with >1000-fold selectivity over the other three histamine receptors. Furthermore, **3g** had significantly longer duration of action in vivo than azelastine in a nasal congestion model. The bioavailability of **3g** was low, which minimizes the potential for side-effects from the swallowed fraction of the intranasal dose. Mean brain concentration following iv dosing in the rat was low, which coupled with the low bioavailability, should limit the potential for any CNS related side-effects. The in vitro rate of metabolism for **3g** was high using human liver and intestinal microsomes, and in hepatocytes. A number of metabolites in human hepatocytes were detected by LC−MS/MS and includes metabolites from oxidation of the pyrrolidine ring (lactam formation), amide hydrolysis, *N*- and *O*-dealkylation, and glucuronidation. High in vivo clearance was observed in the rat and dog. Amide **3g** had a lower affinity than azelastine for the hERG channel, and high human plasma-protein binding. CYP3A4 was inhibited by **3g** in a time-dependent manner; however, azelastine behaves similarly in the Cypex cDNA screen. As the bioavailability of **3g** is low, coadministration with other drugs is unlikely to cause any significant drug−drug interactions in the clinic. In summary, **3g** is suitable for progression as an intranasal candidate for the treatment for allergic rhinitis, either as a monotherapy or in combination with an anti-inflammatory steroid, such as fluticasone furoate.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.7b00112.

Experimental details on all compounds and characterizing data, Tables 3 and 4, and Chart 2 (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Bousquet, J.; Van Cauwnberge, P.; Khaltaev, N. Allergic rhinitis and impact on asthma. *J. Allergy Clin. Immunol.* **2001**, *108* (5 Suppl), S147−334.

(2) Greiner, A. N.; Hellings, P. W.; Rotiroti, G.; Scadding, G. K. Allergic rhinitis. *Lancet* **2011**, *378*, 2112−2122.

(3) Mandhane, S. N.; Shah, J. H.; Thennati, R. Allergic rhinitis: An update on disease, present treatments and future prospects. *Int. Immunopharmacol.* **2011**, *11*, 1646−1662.

(4) Berger, W. E. Overview of allergic rhinitis. *Ann. Allergy, Asthma, Immunol.* **2003**, *90*, 7−12.

(5) Bernstein, D. I.; Schwartz, G.; Bernstein, J. A. Allergic rhinitis. Mechanisms and treatment. *Immunol. Allergy Clin. North Am.* **2016**, *36*, 261−278.

(6) Simons, F. E. R.; Simons, K. J. Histamine and H_1-antihistamines: Celebrating a century of progress. *J. Allergy Clin. Immunol.* **2011**, *128*, 1139−1150.

(7) Gushchin, I. S. Inverse agonists of H_1 receptors as promising antiallergy agents. *Pharm. Chem. J.* **2010**, *44*, 1−6.

(8) Lee, C.; Corren, J. Review of Azelastine nasal spray in the treatment of allergic and non-allergic rhinitis. *Expert Opin. Pharmacother.* **2007**, *8*, 701−709.

(9) Bernstein, J. A. Azelastine hydrochloride: a review of pharmacology, pharmacokinetics, clinical efficacy and tolerability. *Curr. Med. Res. Opin.* **2007**, *23*, 2441−2452.

(10) Hoyte, F. C. L.; Katial, R. K. Antihistamine therapy in allergic rhinitis. *Immunol. Allergy Clin. North Am.* **2011**, *31*, 509−543.

(11) Procopiou, P. A.; Browning, C.; Buckley, J. M.; Clark, K. L.; Fechner, L.; Gore, P. M.; Hancock, A. P.; Hodgson, S. T.; Kranz, M.; Looker, B. E.; Morriss, K. M. L.; Parton, D. L.; Russell, L. J.; Slack, R. J.; Sollis, S. L.; Vile, S.; Watts, C. J. The discovery of phthalazinone based human H_1 and H_3 single-ligand antagonists suitable for intranasal administration for the treatment of allergic rhinitis. *J. Med. Chem.* **2011**, *54*, 2183−2195.

(12) Procopiou, P. A.; Browning, C.; Gore, P. M.; Lynn, S. M.; Richards, S. A.; Slack, R. J.; Sollis, S. L. Synthesis and pharmacological investigation of azaphthalazinone human histamine H_1 receptor antagonists. *Bioorg. Med. Chem.* **2012**, *20*, 6097−6108.

(13) Gore, P. M.; Kranz, M. J.; Looker, B. E.; Vile, S. WO2008074803, 26 June 2008.

(14) Bone, G. WO2009150101, 17 Dec 2009.

(15) Su, J.; McKittrick, B. A.; Tang, H.; Burnett, D. A.; Clader, J. W.; Greenlee, W. J.; Hawes, B. E.; O'Neill, K.; Spar, B.; Weig, B.; Kowalski, T.; Sorota, S.; Li, C.; Liu, T. SAR study of bicyclo[4.1.0]heptanes as melanin-concentrating hormone receptor R1 antagonists. Taming hERG. *Bioorg. Med. Chem.* **2007**, *15*, 5369−5385.

(16) Alberati, D.; Hainzl, D.; Jolidon, S.; Krafft, E. A.; Kurt, A.; Maier, A.; Pinard, E.; Thomas, A. W.; Zimmerli, D. Discovery of 4-substituted-8-(2-hydroxy-2-phenyl-cyclohexyl)-2,8-diaza-spiro[4.5]-decan-1-one as a novel class of highly selective Gly T1 inhibitors with improved metabolic stability. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4311−4315.

(17) Sullivan, E.; Tucker, E. M.; Dale, I. L. Measurement of $[Ca^{2+}]$ using the Fluorometric Imaging Plate Reader (FLIPR). *Methods Mol. Biol.* **1999**, *114*, 125−133.

(18) Procopiou, P. A.; Ancliff, R. A.; Bamford, M. J.; Browning, C.; Connor, H.; Davies, S.; Fogden, Y. C.; Hodgson, S. T.; Holmes, D. S.; Looker, B. E.; Morriss, K. M. L.; Parr, C. A.; Pickup, E. A.; Sehmi, S. S.; White, G. V.; Watts, C. J.; Wilson, D. M.; Woodrow, M. D. 4-Acyl-1-(4-aminoalkoxyphenyl)-2-ketopiperazines as a Novel Class of Non-Brain-Penetrant Histamine H_3 Receptor Antagonists. *J. Med. Chem.* **2007**, *50*, 6706−6717.

(19) Lehman, J. M.; Blaiss, M. S. Selecting the optimal oral antihistamine for patients with allergic rhinitis. *Drugs* **2006**, *66*, 2309−2319.

(20) Slack, R. J.; Russell, L. J.; Hall, D. A.; Luttmann, M. A.; Ford, A. J.; Hodgson, S. T.; Connor, H. E.; Browning, C.; Clark, K. L. Pharmacological characterization of GSK1004723, a novel, long-acting antagonist at histamine H_1 and H_3 receptors. *Br. J. Pharmacol.* **2011**, *164*, 1627−1641.

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