cis-4-(Piperazin-1-yl)-5,6,7a,8,9,10,11,11a-octahydrobenzofuro[2,3-*h*]quinazolin-2-amine (A-987306), A New Histamine H_4R Antagonist that Blocks Pain Responses against Carrageenan-Induced Hyperalgesia

Huaqing Liu,* Robert J. Altenbach, Tracy L. Carr, Prasant Chandran, Gin C. Hsieh, La Geisha R. Lewis, Arlene M. Manelli, Ivan Milicic, Kennan C. Marsh, Thomas R. Miller, Marina I. Strakhova, Timothy A. Vortherms, Brian D. Wakefield, Jill M. Wetter, David G. Witte, Prisca Honore, Timothy A. Esbenshade, Jorge D. Brioni, and Marlon D. Cowart

Neuroscience Research, Global Pharmaceutical Research and Development, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, Illinois 60064-6100

Received June 20, 2008

cis-4-(Piperazin-1-yl)-5,6,7a,8,9,10,11,11a-octahydrobenzofuro[2,3-*h*]quinazolin-2-amine, **4** (A-987306) is a new histamine H_4 antagonist. The compound is potent in H_4 receptor binding assays (rat H_4, $K_i = 3.4$ nM, human H_4 $K_i = 5.8$ nM) and demonstrated potent functional antagonism in vitro at human, rat, and mouse H_4 receptors in cell-based FLIPR assays. Compound **4** also demonstrated H_4 antagonism in vivo in mice, blocking H_4-agonist induced scratch responses, and showed anti-inflammatory activity in mice in a peritonitis model. Most interesting was the high potency and efficacy of this compound in blocking pain responses, where it showed an ED_{50} of 42 μmol/kg (ip) in a rat post-carrageenan thermal hyperalgesia model of inflammatory pain.

Introduction

Histamine mediates its physiological functions through four known G-protein coupled receptors, the H_1, H_2, H_3[1−3] and H_4 receptors. Antagonists of the histamine H_1 receptor (H_1R) such as the marketed drug loratadine have been used for many years in the treatment of allergic inflammatory responses.[1] The histamine H_2 receptor (H_2R) regulates gastric acid secretion and, as a result, H_2R antagonists such as cimetidine are used for treating gastric ulcers.[2] The histamine H_3 receptor (H_3R) is abundantly localized in the CNS, where it regulates the release and synthesis of histamine and modulates other neurotransmitters.[3,4] H_3 antagonists are effective in models of cognition and attention in preclinical models, and several compounds are currently in clinical trials.[5] More recently, the histamine H_4 receptor (H_4R[a]) was reported by several groups,[6−12] and evidence continues to accumulate demonstrating the potential of H_4R antagonists as anti-inflammatory agents.[13]

Molecular biology analysis indicates that the H_4R has the highest homology to the H_3R (35%) but much lower homology to H_1 and H_2 receptors. The H_4R has been found to be expressed mainly in cells of hematopoietic origin, in particular dendritic cells, mast cells, eosinophils, monocytes, basophils, and T cells.[6−12] Although more work needs to be done to uncover the complete biological function of the H_4R, a role in modulating inflammation and pruritis is supported by numerous literature reports.[13−18]

Since the discovery of the H_4R in 2000, there have been significant efforts to identify selective ligands for potential therapeutic use.[19] The indolylpiperazine **1** (JNJ-7777120)[20] is a potent and selective H_4 antagonist that has become a commonly used H_4 antagonist standard (Figure 1). This compound is reported to have anti-inflammatory activity in vivo. For example, it blocks inflammation in a peritonitis model in mice, reducing neutrophil infiltration after zymosan injection.[14] Compound **1** also demonstrated antipruritic activity in a mouse itch model.[17] Compound **1** was reported to enhance mechanical hyperalgesia after partial ligation of the sciatic nerve when injected s.c. directly into the affected paw.[21] We have found that H_4R antagonists, including compound **1**, are efficacious in models of inflammatory and neuropathic pain after the systematic (i.p.) injection.[22a,b]

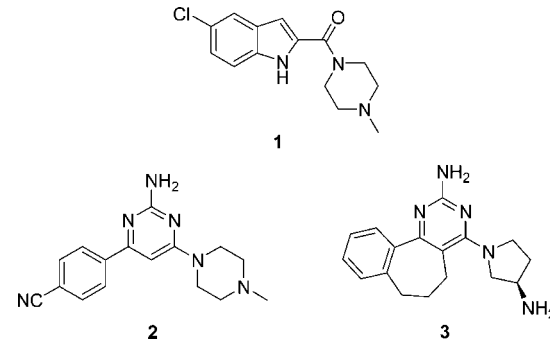


Figure 1. Reported H_4R ligands showing antinociceptive properties.

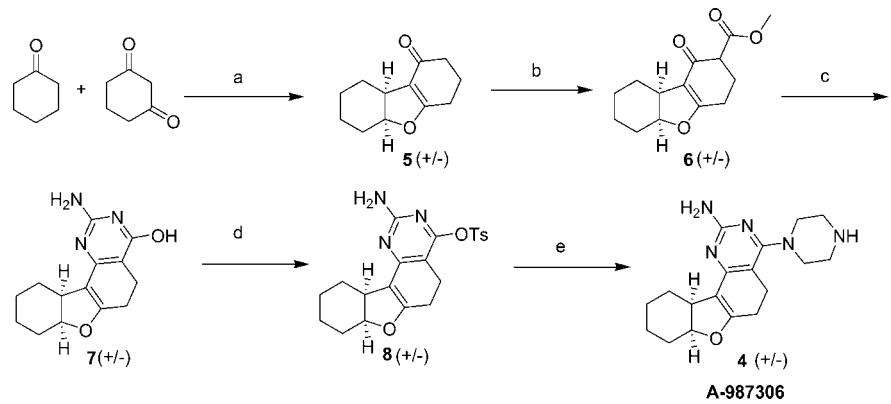
In our search for potent H_4R ligands of novel structure and improved properties, we discovered a series of rotationally restricted 2-aminopyrimidine H_4R antagonists with improved PK, metabolic profiles, and high H_4R selectivity.[22c] Among the 2-aminopyrimidine series, compounds **2** and **3** (A-943931) (Figure 1) possessed the most interesting overall in vivo profile and were effective in several in vivo models of inflammation, itch, and pain. In the process of working to improve the properties and diversify the structures, we discovered another class of rotationally constrained aminopyrimidines, of which the octahydrobenzofuranoquinazolin-2-amine, compound **4**, was found to have an especially potent profile in in vivo models. The synthesis and biological profile of compound **4** is detailed herein.

* To whom correspondence should be addressed. Phone: 847-938-5765. Fax: 847-937-9195. E-mail: huaqing.liu@abbott.com. Address: Department R4MN, Building AP9A/2, Abbott Laboratories, 100 Abbott Park Rd, Abbott Park IL, 60064-6123.

[a] Abbreviations: H_4R, histamine 4 receptor; FLIPR, fluorimetric imaging plate reader; PK, pharmacokinetic; PMN, polymorphonuclear leukocytes; SAR, structure activity relationship.

10.1021/jm8007618 CCC: $40.75
Published on Web 11/05/2008

Scheme 1[a]



[a] Reagents and conditions: (a) PTSA(5%), xylene, reflux, Dean-stark trap; (b) LDA, THF, −78 °C; dimethylcarbonate, −78 °C to r.t.; (c) guanidine HCl, K_2CO_3, DMF, 130 °C, overnight; (d) TsCl, TEA, DCM; (e) piperazine, TEA, 85 °C.

Table 1. In Vitro H_4R and Other Histaminergic Receptor Profile of Compound **1** and **4**[a]

	H_4R FLIPR (pK_b)			H_4R binding (pK_i)		H_1R binding (pK_i)	H_2R binding (pK_i)	H_3R binding (pK_i)	
compd	human	rat	mouse	human	rat	human	human	human	rat
4	8.33 ± 0.14	8.30 ± 0.05	8.20 ± 0.02	8.24 ± 0.18	8.47[b] ± 0.00	5.45 ± 0.02	<5.04	6.03 ± 0.06	7.87 ± 0.06
1	8.31 ± 0.21	8.51 ± 0.03	8.35 ± 0.05	7.92 ± 0.22	8.33 ± 0.00	6.01[c]	5.07[c]	5.65 ± 0.05	5.84 ± 0.01

[a] $n \geq 3$, pK_b, and $pK_i \pm$ the standard error of the mean (SEM) are reported.[24] [b] $n = 2$. [c] $n = 1$.

Chemistry. Compound **4** was synthesized as shown in Scheme 1. Cyclohexanone and 1,3-cyclohexanedione were heated in xylene with a catalytic amount of *p*-toluenesulfonic acid monohydrate to provide ketone **5**,[23] which was then subjected to carboxylation with dimethyl carbonate in a base to provide the β-keto ester **6**. Compound **6** was then cyclized to a pyrimidine by condensation with guanidine hydrochloride in DMF with K_2CO_3 to provide intermediate **7**. Activation of the 4-oxygen by tosylation, followed by displacement with piperazine, provided the target product **4**.

Results and Discussion

The in vitro profile of compound **4** for histaminergic receptors is displayed in Table 1. Compound **1** is included for comparison. A cell-based Ca^{2+}-flux functional assay (FLIPR)[24] revealed that compound **4** was a highly potent antagonist at human, rat, and mouse H_4Rs. In this assay, compound **4** blocked the H_4R activation induced by the endogenous agonist histamine, while having no activation of the receptor when tested alone. The in vitro H_4R functional antagonism was also confirmed in a separate in vitro assay, where it was found that compound **4** potently blocked histamine mediated increases in binding of GTP-γ-[35S] to rat H_4-receptor-containing membranes with a K_b of 6 nM.

Consistent with the observation of potent activity in the functional (FLIPR) assays, compound **4** was highly potent at human and rat H_4 receptors in radioligand ([^{3}H]-histamine) competition binding assays (Table 1). By comparison to the other histamine receptors, compound **4** was 620-fold, >1600-fold, and 162-fold selective for the human H_4R over the human H_1, H_2, and H_3 receptors. However, selectivity for H_4R in the rat was lower, being only 4-fold selective for the rat H_4R over the rat H_3R.

The pharmacokinetic profile of compound **4** was determined after iv, ip, and oral administration of the drug at a dose of 10 mg/kg in Sprague−Dawley rats. After ip dosing (the route used to dose the compound in pain studies), the compound had a favorable fractional bioavailability ($F_{ip/iv} = 72\%$), half-life ($t_{1/2} = 4.7$ h), and a C_{max} of 1.73 μM at a T_{max} of 0.25 h after dosing.

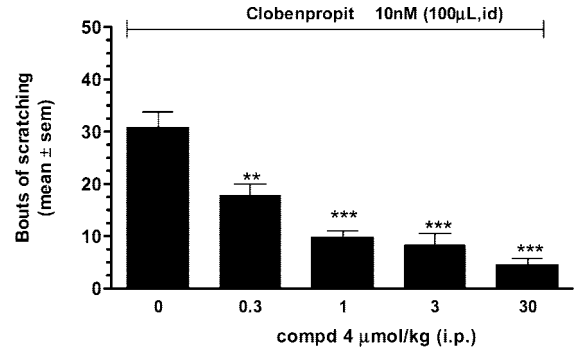


Figure 2. Compound **4** was found to reduce scratching induced by the histamine H_4R agonist clobenpropit. Compound **4** and vehicle were administered (ip) 30 min prior to injection of clobenpropit (id in the back of the neck) as described.[17,26b] The mice were observed, and the number of bouts of scratching was recorded.

After oral dosing, the compound had a moderate fractional oral bioavailability ($F_{po/iv} = 26\%$) with a half-life of 3.7 h and a C_{max} of 0.30 μM at a T_{max} of 1.5 h after dosing. The plasma protein binding[25] of compound **4** was measured in rats and found to be 59%. This moderate level of protein binding is favorable, indicating that a sizable fraction of circulating drug will be present as the free unbound form in the plasma.

The mouse itch model[26] was used as a pharmacological test for in vivo H_4R antagonism. H_4R antagonists, including thioperamide and compounds **1**, **2**, and **3,** have been shown to be active in this model where they block scratching responses induced by histamine H_4R agonists (in this case, clobenpropit).[17,22b,c] Compound **4** reduced scratch responses in mice with an ED_{50} of 0.36 μmol/kg (see Figure 2). Plasma levels of compound **4** near the ED_{50} (0.3 μmol/kg) were found to be 15 ng/mL, supporting a high level of in vivo potency in this model.

H_4R antagonists have been demonstrated to have anti-inflammatory activity in a zymosan induced peritonitis model in mice.[14,27] Intraperitoneal injection of mice with zymosan induces a migration of polymorphonuclear leukocyte (PMN) cells to the peritoneum (more than 80% of the PMNs were determined to be neutrophils). The number of migrating PMNs

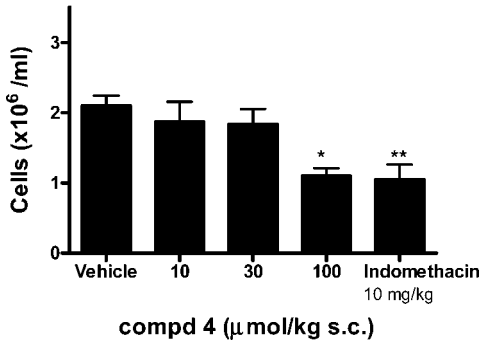


Figure 3. Anti-inflammatory response of compound **4** in the mouse zymosan model. Compound **4** or vehicle was administered sc 30 min prior to injection of zymosan (ip). After 2 h, the peritoneum of the mice was lavaged and neutrophil influx assessed by neutrophil cell count.

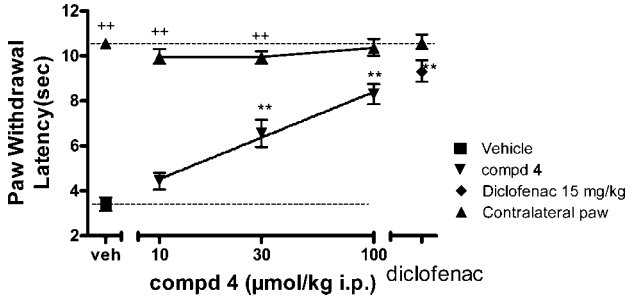


Figure 4. Antinociceptive effect of compound **4** in the rat carrageenan thermal hyperalgesia model.[28] Compound **4** ip (10, 30, and 100 μmol/kg) and diclofenac ip (15 mg/kg) were administered 30 min before testing and 90 min post carrageenan administration. Hot box testing was conducted 2 h following intraplantar carrageenan administration ($n = 12$). Vehicle: 5% DMSO/PEG, 2 mL/kg.

was determined directly by counting the cells. In this model, compound **4** blocked the zymosan induced neutrophil influx at a dose of 100 μmol/kg ($IC_{50} = 125$ μmol/kg) (Figure 3), with a level of efficacy equal to the standard indomethacin dosed at 10 mpk.

Recently, we have discovered that H_4R antagonists, including **1**,[18,22a] **2**,[22b] and **3**,[22c] have antinociceptive activity in a rat model of carrageenan-induced acute hyperalgesia. Compound **4** also potently attenuated the thermal hypersensitivity ($ED_{50} = 42$ μmol/kg, ip) (see Figure 4). The efficacy was achieved in the absence of any observable CNS side effects (e.g., sedation), even at the highest dose tested (100 μmol/kg). Plasma levels were 610 ± 130 ng/mL dosed at 30 μmol/kg and 2900 ± 300 ng/mL dosed at 100 μmol/kg.

Compound **4** was counter screened against a broad kinase enzyme panel, including over 100 kinases, and was found to be selective ($IC_{50} > 810$ nM for all kinases). Further, a radioligand binding counter screen was run against a panel of diverse biogenic amine receptors, neuropeptide receptors, ion channel binding sites, and neurotransmitter transporters (Cerep, Redmond, WA). Compound **4** was found to be selective to most of the targets tested at 10 μM. The targets, which showed greater than 80% inhibition, are adrenergic α_2 (98%), human muscarinic M_3 (83%) and M_4 (84%), and human serotonergic 5-HT_{1b} (86%), 5-HT_{2b} (agonist site) (95%), and 5-HT_3 (98%). Subsequently, radioligand binding (K_i) studies determined that compound **4** was selective for the human H_4R vs the human adrenergic α_2 (61-fold to α_{2a}, 96-fold to α_{2b}, and 2900-fold to α_{2c}), human muscarinic M_3 (261-fold) and M_4 (155-fold), and human serotonergic 5-HT_{1b} (49-fold), 5-HT_{2b} (agonist site) (103-fold), and 5-HT_3 (75-fold) receptors. The affinity of compound **4** for other ancillary receptors and potential sites that may play a role in antinociception was evaluated by additional radioligand binding studies. Compound **4** was found to have no or low affinity at these off target sites. (IC_{50}: 5-HT_{1a}, 2.7 μM; 5-HT_{1c}, 11.7 μM; 5-HT_{1d}, 8.28 μM; 5-HT_{2a}, 3.64 μM; 5-HT_7 > 10 μM; α_1 > 10 μM; NNR > 10 μM).

Conclusion

The octahydrobenzofurano 2-aminoquinazoline compound **4** was found to be a selective and potent H_4R antagonist with K_is of 3.4 nM and 5.8 nM at the rat and human H_4Rs respectively. Compound **4** was an antagonist in a FLIPR calcium mobilization assay, and a GTP-γ-[35S] binding assay in vitro, and in vivo, blocked H_4R agonist-induced scratching in mice. The compound was anti-inflammatory in a peritonitis model, blocking neutrophil influx with an ED_{50} of 100 μmol/kg. Most interestingly, the compound was especially potent in a pain assay in rats, blocking carrageenan induced thermal hyperalgesia with an ED_{50} of 42 μmol/kg (ip). Because of its potency and efficacy in vitro and in vivo, high in vitro selectivity for H_4 receptors, and good pharmacokinetic profile, we believe that compound **4** can serve as an excellent tool compound for studies on histamine H_4R-mediated pharmacology.

Experimental Section

Analytical Methods and Compound Purification. Proton NMR spectra were obtained on a Varian Mercury plus 300 or Varian UNITY plus 300 MHz instrument with chemical shifts (δ) reported relative to tetramethylsilane as an internal standard. Elemental analyses were performed by Quantitative Technologies, Inc. Column chromatography was carried out using either hand-packed silica gel 60 (230−400 mesh) or prepacked silica gel columns from Analogix and eluted under medium pressure liquid chromatography. Thin-layer chromatography (TLC) was performed using 250 μm silica gel 60 glass-backed plates with F254 as the indicator.

***cis*-3,4,5a,6,7,8,9,9a-Octahydrodibenzo[*b*,*d*]furan-1(2H)-one (5).**[23] A solution of cyclohexanone (5.28 mL, 50.9 mmol), cyclohexane-1,3-dione (5.89 g, 50.9 mmol), and *p*-toluenesulfonic acid monohydrate (0.485 g, 2.55 mmol) in xylene (600 mL) was heated to reflux under a Dean−Stark trap for 16 h. The mixture was filtered and concentrated under reduced pressure. The dark-brown residue was chromatographed on silica gel eluting with 0−30% EtOAc in hexanes to provide the title compound as a light-brown oil (5.15 g, 53%). ^{1}H NMR ($CDCl_3$) δ 1.21−1.31 (m, 2H), 1.43−1.57 (m, 3H), 1.72−1.83 (m, 1H), 1.96−2.08 (m, 4H), 2.33 (t, $J = 7$ Hz, 2H), 2.41 (t, $J = 7$ Hz, 2H), 2.96−3.06 (m, 1H), 4.61−4.69 (m, 1H); MS (DCI-NH_3) $(M + H)^+$ m/z 193.

***cis*-Methyl 1-*oxo*-1,2,3,4,5a,6,7,8,9,9a-Decahydrodibenzo[*b*,*d*]furan-2-carboxylate (6).** A solution of diisopropylamine (5.56 mL, 39.0 mmol) in THF (20 mL) was cooled to −78 °C under nitrogen and then treated with *n*-butyllithium (15.60 mL, 39.0 mmol). The mixture was stirred at −78 °C for 30 min. This solution was cannulated into a −78 °C solution of compound **5** (2.5 g, 13 mmol) in THF (40 mL) under nitrogen, and the resulting mixture was stirred for 30 min at −78 °C. Dimethyl carbonate (11.7 g, 130 mmol) was added, and the dry ice bath was removed. The mixture was stirred at ambient temperature for 16 h. The mixture was quenched with HCl (1N, 40 mL) and diluted with ether (200 mL). The organic layer was separated, and the aqueous layer was extracted with additional ether. The organic layers were combined and washed with brine, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was chromatographed on silica gel eluting with 10−30% EtOAc in hexanes to provide the title product (2.2 g, 68%). ^{1}H NMR ($CDCl_3$) δ 1.23−1.30 (m, 1H), 1.42−1.56 (m, 3H), 1.72−1.84 (m, 1H), 1.87−2.04 (m, 3H), 2.17−2.26 (m, 1H), 2.31−2.49 (m, 3H), 2.56−2.67 (m, 1H), 3.00−3.10 (m, 1H),

3.32−3.40 (m, 1H), 3.75 (d, $J = 2.4$ Hz, 2H), 4.67−4.75 (m, 1H); MS (DCI-NH_3) $(M + H)^+$ m/z 251.

***cis*-2-Amino-5,6,7a,8,9,10,11,11a-octahydrobenzofuro[2,3-*h*]-quinazolin-4-ol (7).** A solution of compound **6** (3.7 g, 15 mmol), guanidine hydrochloride (4.24 g, 44.3 mmol), and K_2CO_3 (6.54 g, 47.3 mmol) in DMF (30 mL) was heated to 130 °C for 16 h. After cooled to ambient temperature, the mixture was filtered through a layer of diatomaceous earth and washed with a small amount of DMF. The filtrate was concentrated under reduced pressure, and the residue was azeotropically dried with toluene. The final brownish residue was chromatographed on silica gel eluting with MeOH:CH_2Cl_2:EtOAc (5−10:45:45) to provide the title product (1 g, 26%). ^{1}H NMR (DMSO-d_6) δ 1.13−1.27 (m, 2H), 1.35−1.55 (m, 3H), 1.66−1.81 (m, 1H), 1.87−2.00 (m, 2H), 2.31 (t, $J = 7.5$ Hz, 2H), 2.57 (t, $J = 7.5$ Hz, 2H), 2.82−2.93 (m, 1H), 4.58−4.66 (m, 1H), 6.14−6.24 (m, 2H); MS (DCI-NH_3) $(M + H)^+$ m/z 260.

***cis*-2-Amino-5,6,7a,8,9,10,11,11a-octahydrobenzofuro[2,3-*h*]-quinazolin-4-yl-4-methylbenzenesulfonate (8).** A solution of compound **7** (570 mg, 2.20 mmol), Ts-Cl (838 mg, 4.40 mmol), and DMAP (53.7 mg, 0.440 mmol) in CH_2Cl_2 (40 mL) was treated with triethylamine (0.613 mL, 4.40 mmol) at ambient temperature, and the resulting solution was stirred for 3 h. It was partitioned between CH_2Cl_2 (100 mL) and H_2O. The organic layer was separated, dried ($MgSO_4$), and concentrated under reduced pressure. The resulting residue was chromatographed on a silica gel column eluting with EtOAc:CH_2Cl_2:Hex (20:40:40) to provide the title product (750 mg, 83%). ^{1}H NMR ($CDCl_3$) δ 1.20−1.34 (m, 2H), 1.48−1.60 (m, 3H), 1.72−1.84 (m, 1H), 2.00−2.13 (m, 2H), 2.43−2.49 (m, 5H), 2.79−2.89 (m, 2H), 2.99−3.10 (m, 1H), 4.64−4.73 (m, 3H), 7.35 (d, $J = 8.5$ Hz, 2H), 7.94 (d, $J = 8.5$ Hz, 2H); MS(DCI-NH_3) $(M + H)^+$ m/z 414.

***cis*-4-(Piperazin-1-yl)-5,6,7a,8,9,10,11,11a-octahydrobenzofuro-[2,3-*h*]quinazolin-2-amine (4).** A solution of compound **8** (535 mg, 1.29 mmol) and piperazine (334 mg, 3.88 mmol) in acetonitrile (10 mL) was treated with triethylamine (0.18 mL, 2.58 mmol) and heated to 85° for 16 h. The mixture was cooled to ambient temperature and concentrated under reduced pressure. The resulting residue was chromatographed on silica gel column eluting with NH_4OH/MeOH/CH_2Cl_2 (0.8/8/92) to provide the title product (309 mg, 73%). ^{1}H NMR ($CDCl_3$) δ 1.23−1.33 (m, 2H), 1.46−1.59 (m, 3H), 1.72−1.86 (m, 1H), 2.06−2.18 (m, 2H), 2.42 (t, $J = 7.7$ Hz, 2H), 2.69−2.79 (m, 2H), 2.92−3.00 (m, 4H), 3.00−3.09 (m, 1H), 3.09−3.15 (m, 4H), 4.52−4.60 (br s, 2H), 4.62−4.69 (m, 1H); MS (DCI-NH_3) $(M + H)^+$ m/z 328; Anal. ($C_{18}H_{25}N_5O \cdot 0.5H_2O$): C, H, N.

Acknowledgment. We thank Dr. Richard Harris for in vivo testing and Dr. Matthias Mayrer, Dr. Jill-Desiree Brederson, and Gilbert J Diaz for in vitro testing at off-targets.

Supporting Information Available: Table of combustion analysis, preparation of biological reagents and materials, Cerep screen data, methods for the in vitro assay for the histamine H_4 receptor FLIPR Ca^{2+}-flux functional assay and the competition binding assay, in vivo methods for pharmacologic blockade of H_4R agonist-induced itch, for peritonitis inflammation assay, in vivo method for carrageenan induced hyperalgesia assay. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Akdis, C. A.; Simons, F. E. R. Histamine receptors are hot in immunopharmacology. *Eur. J. Pharmacol.* **2006**, *533*, 69–76. (b) Ash, A. S. F.; Schild, H. O. Receptors mediating some actions of histamine. *Br. J. Pharmacol.* **1966**, *27*, 427–439.

(2) (a) Black, J. W.; Duncan, W. A. M.; Durant, C. J.; Ganellin, C. R.; Parsons, E. M. Definition and antagonism of histamine H_2 receptors. *Nature (London)* **1972**, *236*, 385–390. (b) Moessner, J.; Caca, K. Developments in the inhibition of gastric acid secretion. *Eur. J. Clin. Invest.* **2005**, *35*, 469–475.

(3) Arrang, J.-M.; Garbarg, M.; Schwartz, J.-C. Auto-inhibition of brain histamine release mediated by a novel class (H_3) of histamine receptor. *Nature* **1983**, *302*, 832–837.

(4) Bertaccini, G. Functional role of the histamine receptor in peripheral tissues. In *The Histamine H_3 Receptor: a Target for New Drugs*; Leurs, R., Timmerman, H., Eds.; Elsevier: Amersterdam, 1998; pp 59−111.

(5) (a) Hudkins, R. L.; Raddatz, R. Recent advances in drug discovery of histamine H3 antagonists in. *Annu. Rep. Med. Chem.* **2007**, *42*, 49–62. (b) Celanire, S.; Wijtmans, M.; Talaga, P.; Leurs, R.; de Esch, I. J. P. Histamine H_3 receptor antagonists reach out for the clinic. *Drug Discovery Today* **2005**, *10(23/24)*, 1613–1627. (c) Esbenshade, T. A.; Browman, K. E.; Bitner, R. S.; Strakhova, M.; Cowart, M. D.; Brioni, J. D. The histamine H_3 receptor: an attractive target for the treatment of cognitive disorders. *Br. J. Pharmacol.* **2008**, *154*, 1166–1181.

(6) Oda, T.; Morikawa, N.; Saito, Y.; Masuho, Y.; Matsumoto, S. Molecular cloning and characterization of a novel type of histamine receptor preferentially expressed in leukocytes. *J. Biol. Chem.* **2000**, *275*, 36781–36786.

(7) Nakamura, T.; Itadani, H.; Hidaka, Y.; Ohta, M.; Tanaka, K. Molecular cloning and characterization of a new human histamine receptor, H_4R. *Biochem. Biophys. Res. Commun.* **2000**, *279*, 615–620.

(8) Liu, C.; Ma, X.-J.; Jiang, X.; Wilson, S. J.; Hofstra, C. L.; Blevitt, J.; Pyati, J.; Li, X.; Chai, W.; Carruthers, N.; Lovenberg, T. W. Cloning and pharmacological characterization of a fourth histamine receptor (H_4) expressed in bone marrow. *Mol. Pharmacol.* **2001**, *59*, 420–426.

(9) Zhu, Y.; Michalovich, D.; Wu, H.-L.; Tan, K. B.; Dytko, G. M.; Mannan, I. J.; Boyce, R.; Alston, J.; Tierney, L. A.; Li, X.; Herrity, N. C.; Vawter, L.; Sarau, H. M.; Ames, R. S.; Davenport, C. M.; Hieble, J. P.; Wilson, S.; Bergsma, D. J.; Fitzgerald, L. R. Cloning, expression, and pharmacological characterization of a novel human histamine receptor. *Mol. Pharmacol.* **2001**, *59*, 434–441.

(10) Nguyen, T.; Shapiro, D. A.; George, S. R.; Setola, V.; Lee, D. K.; Cheng, R.; Rauser, L.; Lee, S. P.; Lynch, K. R.; Roth, B. L.; O'Dowd, B. F. Discovery of a novel member of the histamine receptor family. *Mol. Pharmacol.* **2001**, *59*, 427–433.

(11) O'Reilly, M.; Alpert, R.; Jenkinson, S.; Gladue, R. P.; Foo, S.; Trim, S.; Peter, B.; Trevethick, M.; Fidock, M. Identification of a histamine H_4 receptor on human eosinophils-role in eosinophil chemotaxis. *J. Recept. Signal Tranduction Res.* **2002**, *22*, 431–448.

(12) Morse, K. L.; Behan, J.; Laz, T. M.; West, R. E.; Greenfeder, S. A.; Anthes, J. C.; Umland, S.; Wan, Y.; Hipkin, R. W.; Gonsiorek, W.; Shin, N.; Gustafson, E. L.; Qiao, X.; Wang, S.; Hedrick, J. A.; Greene, J.; Bayne, M.; Monsma, F. J. Cloning and Characterization of a Novel Human Histamine Receptor. *J. Pharmacol. Exp. Ther.* **2001**, *296*, 1058–1066.

(13) de Esch, I. J. P.; Thurmond, R. L.; Jongejan, A.; Leurs, R. The histamine H_4 receptor as a new therapeutic target for inflammation. *Trends Pharmacol. Sci.* **2005**, *26*, 462–469.

(14) Thurmond, R. L.; Desai, P. J.; Dunford, P. J.; Fung-Leung, W. P.; Hofstra, C. L.; Jiang, W.; Nguyen, S.; Riley, J. P.; Sun, S.; Williams, K. N.; Edwards, J. P.; Karlsson, L. A potent and selective histamine H_4 receptor antagonist with anti-inflammatory properties. *J. Pharmacol. Exp. Ther.* **2004**, *309*, 404–413.

(15) Varga, C. Inhibitory effects of histamine H_4 antagonists on experimental colitis in the rat. *Eur. J. Pharmacol.* **2005**, *522*, 130–138.

(16) Dunford, P. J. The histamine H_4 receptor mediates allergic airway inflammation by regulating the activation of $CD4^+$ T cells. *J. Immunol.* **2006**, *176*, 7062–7070.

(17) Dunford, P. J.; Williams, K. N.; Desai, P. J.; McQueen, D.; Karlsson, L.; Thurmond, R. L. Histamine H_4 receptor antagonists are superior to traditional antihistamines in the attenuation of experimental pruritus. *J. Allergy Clin. Immunol.* **2007**, *119*, 176–183.

(18) Coruzzi, G.; Adami, M.; Guaita, E.; de Esch, I. J. P.; Leurs, R. Antiinflammatory and antinociceptive effects of the selective histamine H_4-receptor antagonists JNJ7777120 and VUF6002 in a rat model of carrageenan-induced acute inflammation. *Eur. J. Pharmacol.* **2007**, *563*, 240–244.

(19) (a) Zhang, M.; Venable, J. D.; Thurmond, R. L. The histamine H_4 receptor in autoimmune disease. *Expert Opin. Investig. Drugs* **2006**, *15*, 1443–1452. (b) Histamine H_4 antagonists. *Expert Opin. Ther. Pat.* **2004**, *14*, 1641−1644.

(20) Jablonowski, J. A.; Grice, C. A.; Chai, W.; Dvorak, C. A.; Venable, J. D.; Kwok, A. K.; Ly, K. S.; Wei, J.; Baker, S. M.; Desai, P. J.; Jiang, W.; Wilson, S. J.; Thurmond, R. L.; Karlsson, L.; Edwards, J. P.; Lovenberg, T. W.; Carruthers, N. I. The first potent and selective non-imidazole human histamine H_4 receptor antagonists. *J. Med. Chem.* **2003**, *46*, 3957–3960.

(21) Smith, F. M.; Haskelberg, H.; Tracey, D. J.; Moalem-Taylor, G. Role of histamine H_3 and H_4 receptors in mechanical hyperalgesia following peripheral nerve injury. *Neuroimmunomodulation.* **2007**, *14*, 317–25.

(22) (a) Hsieh, G. C.; Chandran, P.; Salyers, A. K.; Pai, M.; Zhu, C. Z.; Wensink, E. J.; Witte, D. G.; Miller, T. R.; Milicic, I.; Mikusa, J. P.; Baker, S. J.; Wetter, J. M.; Marsh, K. C.; Hancock, A. A., Decker,

M. W.; Cowart, M. D.; Esbenshade, T. A.; Brioni, J. D.; Honore, P. Histamine H_4 receptor as a potential antinociceptive target: effects of a potent and selective H_4 antagonist in animal pain models. Unpublished results, submitted for publication. (b) Altenbach, R. J.; Adair, R. M.; Bettencourt, B. M.; Black, L. A.; Fix-Stenzel, S. R.; Gopalakrishnan, S. M.; Hsieh, G. C.; Liu, H.; Marsh, K. C.; McPherson, M. J.; Milicic, I.; Miller, T. R.; Vortherms, T. A.; Warrior, U.; Wetter, J. M.; Wishart, N.; Witte, D. G.; Esbenshade, T. A. , Hancock, A. A.; Brioni, J. D.; Cowart, M. D. Structure−activity relationship studies on a series of a 2-aminopyrimidine containing histamine H_4 receptor ligands. *J. Med. Chem.* **2008**, 10.1021/jm8005959. (c) Cowart, M. D.; Altenbach, R. J.; Liu, H.; Hsieh, G. C.; Drizin, I.; Milicic, I.; Miller, T. R.; Witte, T. G.; Wishart, N.; Fix-Stenzel, S. R.; McPherson, M. J.; Adair, R. M.; Wetter, J. M.; Bettencourt, B. M.; Marsh, K. C.; Decker, M. W.; Sullivan, J. P.; Honore, P.; Esbenshade, T. A.; Brioni, J. D. Rotationally-constrained 2,4-diamino-5,6-disubstituted pyrimidines: a new class of histamine H_4 receptor antagonists with improved drug-likeness and in vivo efficacy in pain and inflammation models. *J. Med. Chem.* **2008**, 10.1021/jm800670r.

(23) Greenhill, J. V.; Moten, M. A.; Hanke, R. Dibenzofuranones from 1:1 condensations between cyclohexanones and cyclohexane-1,3-diones. *J. Chem. Soc., Perkins Trans. 1* **1984**, *6*, 1213–1217.

(24) Esbenshade, T. A.; Krueger, K. M.; Miller, T. R.; Kang, C. H.; Denny, L. I.; Witte, D. G.; Yao, B. B.; Fox, G. B.; Faghih, R.; Bennani, Y. L.; Williams, M.; Hancock, A. A. Two novel and selective nonimidazole histamine H3 receptor antagonists A-304121 and A-317920. I. In vitro pharmacological effects. *J. Pharmacol. Exp. Ther.* **2003**, *305*, 887–896, See detail in Supporting Information.

(25) Compound **4** was incubated with plasma, and the mixture was centrifuged in a Microcon membrane filtration unit to separate bound from free compound. Free compound was quantified against a standard curve (LS-MS/MS) and reported as percent bound, $n = 1$.

(26) (a) Rees, J. L.; Murray, C. S. Itching for progress. *Clin. Exp. Dermatol.* **2005**, *30*, 471–473. (b) Bell, J. K.; McQueen, D. S.; Rees, J. L. Involvement of histamine H_4 and H_1 receptors in scratching induced by histamine receptor agonists in balb C mice. *Br. J. Pharmacol.* **2004**, *142*, 374–380.

(27) (a) Vehicle or compound was dosed sc in Balb/c male mice (22−25 g) 30 min prior to zymosan injection. Zymosan (0.25 mg/mouse in 0.5 mL in saline, ip) was then injected and, after 2 h, the peritoneum of the mice was lavaged and the PMN cells were counted. (b) Takeshita, K.; Sakai, K.; Bacon, K. B.; Gantner, F. Critical role of histamine H_4 receptor in leukotriene B4 production and mast cell-dependent neutrophil recruitment induced by zymosan in vivo. *J. Pharmacol. Exp. Ther.* **2003**, *307*, 1072–1078.

(28) Honore, P.; Wade, C. L.; Zhong, C.; Harris, R. R.; Wu, C.; Ghayur, T.; Iwakura, Y.; Decker, M. W.; Faltynek, C.; Sullivan, J.; Jarvis, M. F. Interleukin-1$\alpha\beta$ gene-deficient mice show reduced nociceptive sensitivity in models of inflammatory and neuropathic pain but not post-operative pain. *Behav. Brain Res.* **2006**, *167*, 355–364.

JM8007618