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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 H₄R Antagonist that Blocks Pain Responses against Carrageenan-Induced Hyperalgesia

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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 H₄ antagonist. The compound is potent in H₄ receptor binding assays (rat H₄, K_i = 3.4 nM, human H₄ K_i = 5.8 nM) and demonstrated potent functional antagonism *in vitro* at human, rat, and mouse H₄ receptors in cell-based FLIPR assays. Compound **4** also demonstrated H₄ antagonism *in vivo* in mice, blocking H₄-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 ED₅₀ 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 H₁, H₂, H₃^{1–3} and H₄ receptors. Antagonists of the histamine H₁ receptor (H₁R) such as the marketed drug loratadine have been used for many years in the treatment of allergic inflammatory responses.¹ The histamine H₂ receptor (H₂R) regulates gastric acid secretion and, as a result, H₂R antagonists such as cimetidine are used for treating gastric ulcers.² The histamine H₃ receptor (H₃R) is abundantly localized in the CNS, where it regulates the release and synthesis of histamine and modulates other neurotransmitters.^{3,4} H₃ 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 H₄ receptor (H₄R^a) was reported by several groups,^{6–12} and evidence continues to accumulate demonstrating the potential of H₄R antagonists as anti-inflammatory agents.¹³

Molecular biology analysis indicates that the H₄R has the highest homology to the H₃R (35%) but much lower homology to H₁ and H₂ receptors. The H₄R 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 H₄R, a role in modulating inflammation and pruritis is supported by numerous literature reports.^{13–18}

Since the discovery of the H₄R in 2000, there have been significant efforts to identify selective ligands for potential therapeutic use.¹⁹ The indolylpiperazine **1** (JNJ-777120)²⁰ is a potent and selective H₄ antagonist that has become a commonly used H₄ antagonist standard (Figure 1). This

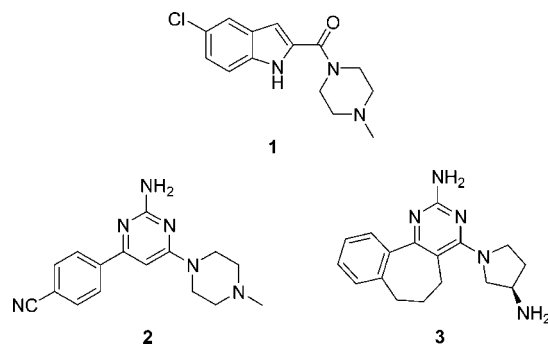


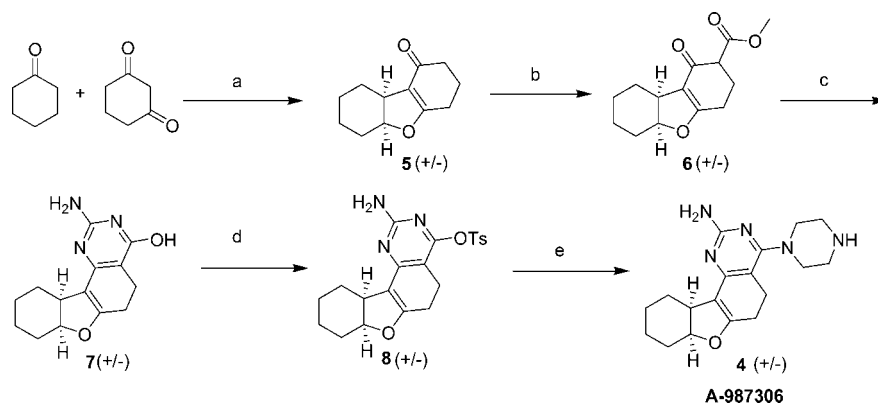
Figure 1. Reported H₄R ligands showing antinociceptive properties.

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 H₄R antagonists, including compound **1**, are efficacious in models of inflammatory and neuropathic pain after the systemic (i.p.) injection.^{22a,b}

In our search for potent H₄R ligands of novel structure and improved properties, we discovered a series of rotationally restricted 2-aminopyrimidine H₄R antagonists with improved PK, metabolic profiles, and high H₄R 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.

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^a Abbreviations: H₄R, histamine 4 receptor; FLIPR, fluorimetric imaging plate reader; PK, pharmacokinetic; PMN, polymorphonuclear leukocytes; SAR, structure activity relationship.

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 H₄R and Other Histaminergic Receptor Profile of Compound **1** and **4**^a

compd	H ₄ R FLIPR (pK _b)			H ₄ R binding (pK _i)		H ₁ R binding (pK _i)	H ₂ R binding (pK _i)	H ₃ R binding (pK _i)	
	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 ± 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 H₄Rs. In this assay, compound **4** blocked the H₄R activation induced by the endogenous agonist histamine, while having no activation of the receptor when tested alone. The in vitro H₄R 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 H₄-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 H₄ receptors in radioligand (³H]-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 H₄R over the human H₁, H₂, and H₃ receptors. However, selectivity for H₄R in the rat was lower, being only 4-fold selective for the rat H₄R over the rat H₃R.

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.

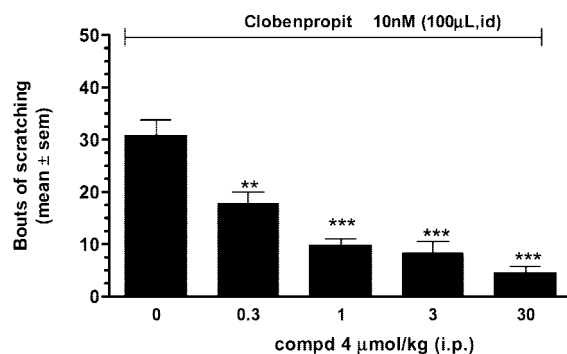


Figure 2. Compound **4** was found to reduce scratching induced by the histamine H₄R agonist clobenpropit. Compound **4** and vehicle were administered (ip) 30 min prior to injection of clobenpropit (id in the back of the neck) as described.^{17,26b} The mice were observed, and the number of bouts of 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 binding²⁵ 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 model²⁶ was used as a pharmacological test for in vivo H₄R antagonism. H₄R 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 H₄R agonists (in this case, clobenpropit).^{17,22b,c} Compound **4** reduced scratch responses in mice with an ED₅₀ of 0.36 $\mu\text{mol/kg}$ (see Figure 2). Plasma levels of compound **4** near the ED₅₀ (0.3 $\mu\text{mol/kg}$) were found to be 15 ng/mL, supporting a high level of in vivo potency in this model.

H₄R 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

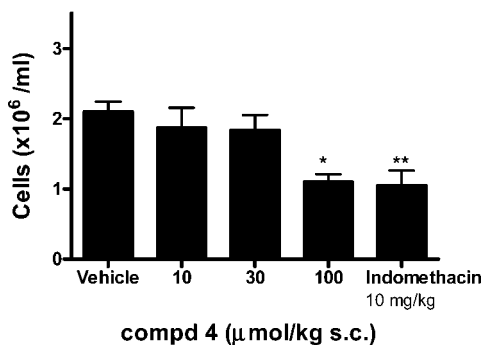


Figure 3. Anti-inflammatory response of compound **4** in the mouse zymosan model. Compound **4** or vehicle was administered sc 30 min prior to injection of zymosan (ip). After 2 h, the peritoneum of the mice was lavaged and neutrophil influx assessed by neutrophil cell count.

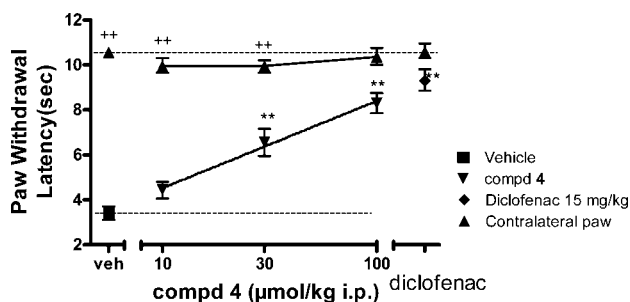


Figure 4. Antinociceptive effect of compound **4** in the rat carrageenan thermal hyperalgesia model.²⁸ Compound **4** ip (10, 30, and 100 μmol/kg) and diclofenac ip (15 mg/kg) were administered 30 min before testing and 90 min post carrageenan administration. Hot box testing was conducted 2 h following 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 H₄R 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 α₂ (98%), human muscarinic M₃ (83%) and M₄ (84%), and human serotonergic 5-HT_{1b} (86%), 5-HT_{2b} (agonist site) (95%), and 5-HT₃ (98%). Subsequently, radioligand binding (K_i) studies determined that compound **4** was selective for the human H₄R vs the human adrenergic α₂ (61-fold to α_{2a}, 96-fold to α_{2b}, and 2900-fold to α_{2c}), human muscarinic M₃ (261-fold) and M₄ (155-fold), and human serotonergic 5-HT_{1b} (49-fold), 5-HT_{2b} (agonist site) (103-

fold), and 5-HT₃ (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-HT₇ > 10 μM; α₁ > 10 μM; NNR > 10 μM).

Conclusion

The octahydrobenzofurano 2-aminoquinazoline compound **4** was found to be a selective and potent H₄R antagonist with K_i s of 3.4 nM and 5.8 nM at the rat and human H₄R 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 H₄R 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 H₄ receptors, and good pharmacokinetic profile, we believe that compound **4** can serve as an excellent tool compound for studies on histamine H₄R-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%). ¹H NMR (CDCl₃) 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-NH₃) (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 (MgSO₄), 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%). ¹H NMR (CDCl₃) 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-NH₃) (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 K₂CO₃ (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:CH₂Cl₂:EtOAc (5–10:45:45) to provide the title product (1 g, 26%). ¹H 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-NH₃) (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 CH₂Cl₂ (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 CH₂Cl₂ (100 mL) and H₂O. The organic layer was separated, dried (MgSO₄), and concentrated under reduced pressure. The resulting residue was chromatographed on a silica gel column eluting with EtOAc:CH₂Cl₂:Hex (20:40:40) to provide the title product (750 mg, 83%). ¹H NMR (CDCl₃) 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-NH₃) (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 NH₄OH/MeOH/CH₂Cl₂ (0.8/8/92) to provide the title product (309 mg, 73%). ¹H NMR (CDCl₃) 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-NH₃) (M + H)⁺ m/z 328; Anal. (C₁₈H₂₅N₅O•0.5H₂O): C, H, N.

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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 H₄ receptor FLIPR Ca²⁺-flux functional assay and the competition binding assay, in vivo methods for pharmacologic blockade of H₄R 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>.

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