Rotationally Constrained 2,4-Diamino-5,6-disubstituted Pyrimidines: A New Class of Histamine H_4 Receptor Antagonists with Improved Druglikeness and in Vivo Efficacy in Pain and Inflammation Models

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A new structural class of histamine H_4 receptor antagonists (**6**−**14**) was designed based on rotationally restricted 2,4-diaminopyrimidines. Series compounds showed potent and selective in vitro H_4 antagonism across multiple species, good CNS penetration, improved PK properties compared to reference H_4 antagonists, functional H_4 antagonism in cellular and in vivo pharmacological assays, and in vivo anti-inflammatory and antinociceptive efficacy. One compound, **10** (A-943931), combined the best features of the series in a single molecule and is an excellent tool compound to probe H_4 pharmacology. It is a potent H_4 antagonist in functional assays across species (FLIPR Ca^{2+} flux, K_b < 5.7 nM), has high (>190×) selectivity for H_4, and combines good PK in rats and mice ($t_{1/2}$ of 2.6 and 1.6 h, oral bioavailability of 37% and 90%) with anti-inflammatory activity (ED_{50} = 37 μmol/kg, mouse) and efficacy in pain models (thermal hyperalgesia, ED_{50} = 72 μmol/kg, rat).

Introduction

Histamine is an endogenous amine that exerts a wide range of biological effects by signaling through four G-protein-coupled histamine receptors (GPCRs[a]).[1−3] Histamine H_1 receptors (H_1R) are very widely distributed. H_1 antagonists are clinically used for treating symptoms of allergies (e.g., diphenhydramine for allerghic rhinitis). Histamine H_2 receptors (H_2R) are widely distributed also, and their modulation of gastric acid secretion has made H_2 antagonists such as cimetidine clinically useful for treatment of acid reflux and ulcer. Histamine H_3 receptors are predominantly expressed in neurons in the CNS and periphery, where they modulate neurotransmitter release; H_3 antagonists have been widely reported to be effective in diverse behavioral models of attention and cognition and are progressing in clinical trials.[4,5]

The histamine H_4 receptor was last discovered but research has advanced rapidly. The histamine H_4 receptor (H_4R) was cloned ca. 2000 by many laboratories,[6−13] and studies have shown its mRNA and receptor protein are prominently expressed in cells and tissues of the immune system, for example, in mast cells, lymphocytes, and dendritic cells.[1,14,15] These sites of high expression, taken together with in vitro and in vivo studies with chemical ligands, support the proposition that H_4 antagonists have utility as anti-inflammatory and immunomodulatory agents.[15,16] H_4 antagonists have been shown to block histamine-mediated shape change and chemotaxis in mast cells[1] and eosinophils.[13,17,18] When administered in vivo, histamine H_4 antagonists have been shown to block inflammatory peritonitis after zymosan administration,[19] ovalbumin-induced airway inflammation,[20] chemically induced colitis,[21] and itch responses.[22,23]

Very recently, we reported that H_4 antagonists possess antinociceptive activity in a number of models assessing inflammatory pain as well as chemically induced osteoarthritis pain, postsurgical pain, and neuropathic pain.[24−26] Most of the pharmacological research in the field on inflammation has relied on one potent agent as a tool compound, **1** (JNJ-7777120) (Chart 1).[19,27] However, there is need for additional agents of diverse structure and improved properties to support pharmacological experimentation. We thus developed and recently reported a structurally distinct series of 6-aryl-2-aminopyrimidines (exemplified by compounds **2** and **3**) that possesses potent antagonist activity in vitro at human and rodent H_4 receptors and with good CNS penetration; furthermore, members of the series (e.g., **2**) were shown to be effective in inflammation and pain models. However, compounds such as **1** and **2** do have some limitations that can complicate some of their uses as pharmacological tools: they are rapidly metabolized in vivo and rapidly demethylated to metabolites with significant H_4 activity (**1** → **4**, and **2** → **5**). Also, some compounds (**2**) in the 6-aryl-2-aminopyrimidine series have significant off-target affinity at two sites, 5-HT_{1a} and 5-HT_{1d} receptors.

To address these limitations, we designed a new series of ligands by selectively rigidifying parts of structure **3** to give **6**. Further optimization of this structure led to molecules **7**−**9** with potent H_4 antagonism, and further optimization of the 4-amine moiety and selective substitution led to molecules **10**−**14** with reduced in vitro metabolism, better PK properties, higher H_4 selectivity, and in vivo H_4 antagonism. In particular, compounds **10** and **11** were profiled more extensively and demonstrated in vivo efficacy in multiple pain models.

Chemistry

The synthesis of the new compounds is summarized in Scheme 1. Commercially available ketones **15** were converted

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[a] Abbreviations: FLIPR, fluorescent imaging plate reader; GPCR, G-protein-coupled histamine receptor; HEK, human embryonic kidney; BMMC, bone marrow derived mast cells; GTP, guanosine triphosphate; COX, cyclooxygenase; CYP, cytochrome P_{450}; 5-HT, serotonin; CB, cannabinoid; TRPV, transient receptor cation channel, subfamily V; EtOAc, ethyl acetate; DCM, dichloromethane; MPLC, medium pressure liquid chromatography.

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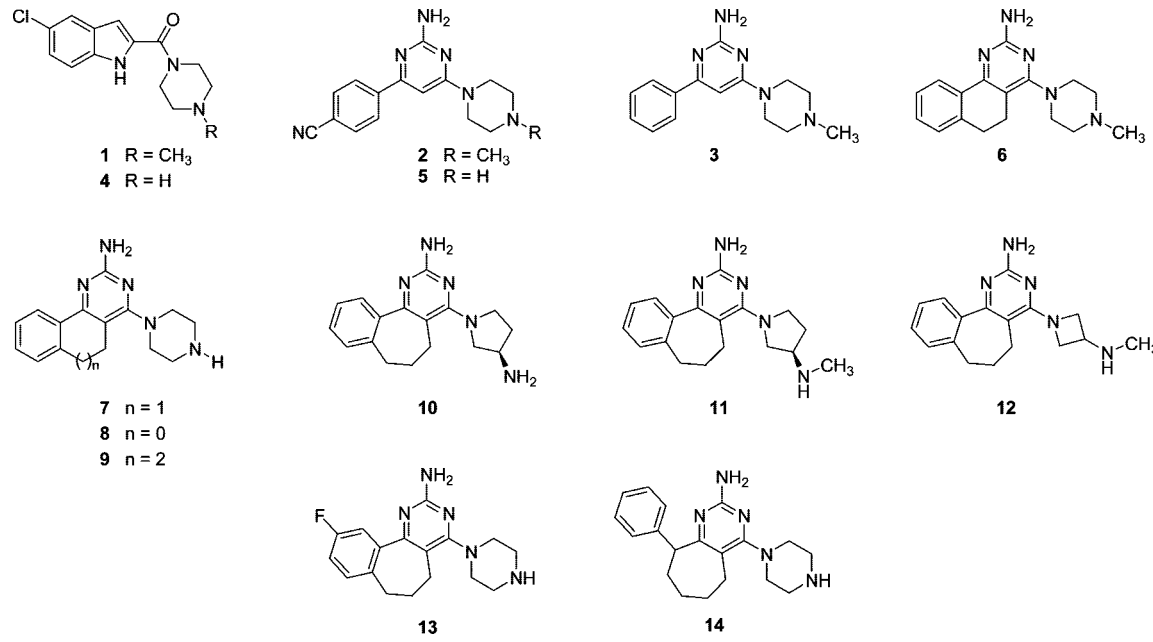
Chart 1. Reference H_4 Ligands **1**−**5** and the New Histamine H_4 Antagonist 2,4-Diamino-5,6-disubstituted Pyrimidines **6**−**14**



to ketoesters (**16**) by treatment with sodium hydride in dimethyl carbonate at 80−90 °C. The ketoesters **16** were cyclicized to pyrimidinols **17** by treatment with guanidine in DMF at 110−160 °C. Any of several guanidine salts (nitrate, hydrochloride, carbonate) were suitable reagents for this transformation, provided the reaction was run under basic conditions. The pyrimidinols **17** were next converted to electrophilic chlorides, tosylates, or 4-nitrophenylsulfonates (**18**) by treatment with $POCl_3$, TsCl, or NsCl, respectively, in the presence of triethylamine at ambient temperature. These electrophilic intermediates **18** were next treated with diamines under basic conditions and underwent nucleophilic aromatic substitution at 70−110 °C. Some of the diamines were used in Boc-protected forms, and for these, treatment with trifluoroacetic acid at ambient temperature produced the target final products **19**.

Results and Discussion

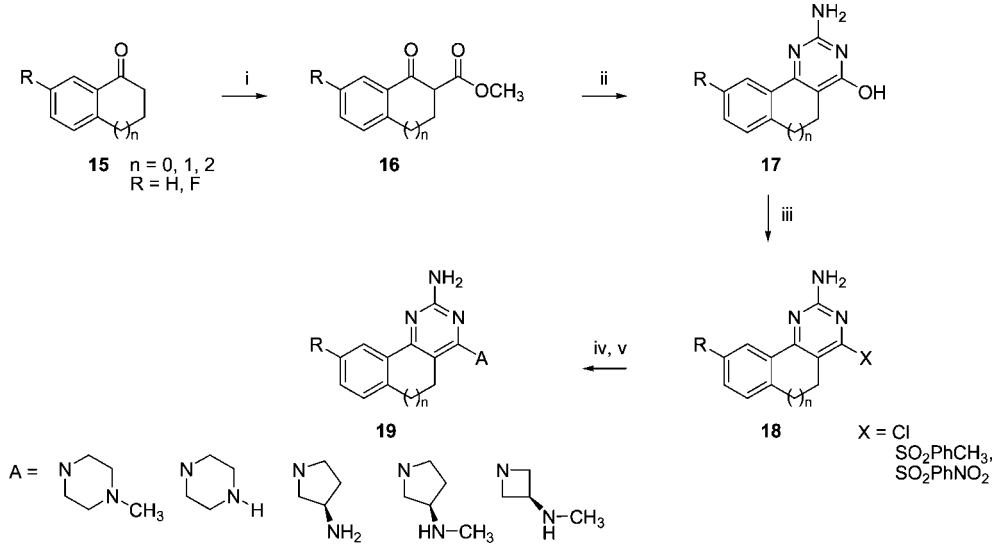
The in vitro profile of compounds **1**−**14** at histamine H_4 receptors is shown in Table 1. It has been reported that there is not at all a strong degree of cross-species sequence homology among human, rodent, guine pig, monkey, and porcine H_4 receptors[16,28,29] and that ligand potency and function vary substantially across species. Therefore, to develop agents effective across species in vivo, assays should be established to allow optimization of compounds with comparable cross-species potency in vitro; as most early models are rodent models, activity at rat and mouse H_4 is paramount. Studies have shown that histamine H_4 receptors in native tissues couple to $G\alpha_{i/o}$ proteins where activation by the endogenous ligand histamine increases intracellular Ca^{2+}. The in vitro functional profile of the ligands was assessed in FLIPR-based Ca^{2+} flux assay in HEK-293 (human embryonic kidney) cells stably transfected with human, rat, mouse H_4 receptor coexpressed with a chimeric G-protein ($G\alpha_{qi5}$).[30] Selected compounds were profiled further in radioligand competition binding assays wherein the test ligand prevented binding of ^{3}H-histamine.[30]

Any discussion of H_4 potency should begin with compounds **1**[19,27] and **2**,[25] which have been well documented to be efficacious in in vivo models and which are useful enough as standard H_4 antagonists that they could be considered "state of the art" ligands. As seen in Table 1, the indole carboxamide **1** has comparable nanomolar interspecies potency in FLIPR assays, though with slightly more potency at rodent H_4 receptor (pK_b of 8.59 and 8.62 at rat and mouse, respectively). The 6-aryl-2-aminopyrimidine **2** also has high potency across species, but this compound has greater potency at the human H_4 receptor (pK_b of 8.53) than at rodent H_4. The related 6-aryl-2-aminopyrimidine analogue **3** has a similar profile, with maximal potency at human H_4 of $pK_b = 8.39$.

A new series of analogues was designed by selective rigidification of compound **3**. We have previously reported the benefits of structural rigidification in histamine H_3 receptor antagonists,[31] and others have proposed that reducing the number of rotatable bonds may offer benefits, such as increasing the prospects for better oral bioavailability[32] and druglikeness.[33] Other potential benefits of rigidification are increased selectivity for the molecular target (in this case H_4 receptors) relative to nontarget sites, because rigidification might "freeze out" some of the molecular conformers required to bind to the off-target sites, provided of course that affinity for the target H_4 receptor can be retained. Of several series incorporating different ring fusions of **3**, the series with the most interesting properties incorporated a new ring junction between the carbon at C5 and the aromatic ring. The first compound of the series (**6**) indicated that the series would be able to retain the high potency of **3** because **6** had a slight increase in affinity for rat H_4 and only a slight reduction of affinity for human H_4.

It was further anticipated that by varying the size of the new ring, we might be able to adjust or "tune" the functional interaction of the ligand with the rodent receptor. It has been previously reported that ligands may perform differently in potency and function depending on the species of H_4 receptor examined.[28] In in vitro assays using H_4-transfected HEK cells, the rat and mouse receptors appeared more prone to activation with synthetic ligands than was the human isoform.[25] For example, compounds **2** (64% receptor activation, relative to histamine) and **6** (76% activation) were able to partially activate the rat H_4 receptor but not the human H_4 receptor. These findings were also confirmed in a separate functional assay where an increase in GTP-γ-^{35}S to H_4-receptor-containing membranes was

Scheme 1[a]



[a] Reagents and conditions: (i) NaH, dimethyl carbonate, Δ; (ii) guanidine, DMF, Δ; (iii) $POCl_3$, Et_3N, 0 °C (X = Cl); NsCl or TsCl, DMAP, Et_3N, room temp; (iv) diamine or Boc-protected diamine, Et_3N, Δ; (v) TFA.

Table 1. Summary of in Vitro Potency at Histamine H_4 Receptors

compd	human H_4 FLIPR pK_b ± SEM	rat H_4 FLIPR pK_b ± SEM or pEC_{50} ± SEM (% eff)	mouse H_4 FLIPR pK_b ± SEM or pEC_{50} ± SEM (% eff)	human H_4 binding potency pK_i ± SEM	rat H_4 binding potency pK_i ± SEM
1	8.37 ± 0.02	8.59 ± 0.02	8.62 ± 0.05	7.92 ± 0.22	8.57 ± 0.10
2	8.53 ± 0.12	7.23 ± 0.08 (64)	6.91 ± 0.20 (54)	7.46 ± 0.06	6.56 ± 0.06
3	8.39 ± 0.19	7.40 ± 0.07 (79)		8.30 ± 0.10	7.85 ± 0.18
4	7.74 ± 0.04	7.51 ± 0.17	7.75 ± 0.06	7.59 ± 0.06	8.40 ± 0.13
5	8.08 ± 0.05	6.61 ± 0.07 (67)	6.99 ± 0.05 (54)	7.38 ± 0.11	6.47 ± 0.02
6	8.08 ± 0.17	7.81 ± 0.14 (76)		7.81 ± 0.14	8.27 ± 0.11
7	7.58 ± 0.14	7.62 ± 0.12 (52)		7.28 ± 0.06	8.04 ± 0.07
8	8.75 ± 0.07	7.70 ± 0.10 (96)		7.89 ± 0.02	8.32 ± 0.18
9	7.13 ± 0.02	7.83 ± 0.05	7.74 ± 0.04	7.15 ± 0.10	8.12 ± 0.04
10	8.24 ± 0.07	8.00 ± 0.07	8.24 ± 0.04	8.33 ± 0.04	8.42 ± 0.11
11	8.21 ± 0.01	8.18 ± 0.02	8.36 ± 0.07	8.46 ± 0.04	8.29 ± 0.08
12	8.05 ± 0.14	8.03 ± 0.11	8.04 ± 0.08	7.96 ± 0.07	8.44 ± 0.07
13	8.01 ± 0.03	8.22 ± 0.07	8.32 ± 0.04	8.29 ± 0.23	8.84 ± 0.03
14	7.24 ± 0.02	7.26 ± 0.07	7.34 ± 0.11	7.40 ± 0.11	8.35 ± 0.05

seen following treatment with the H_4 ligand, consistent with partial agonism.[25] In fact, the human H_4 receptor was not activated in the FLIPR assay by any of the compounds **1**−**14**. However, despite an apparent difference between **1** and **2** in rat and mouse receptor activation in vitro in the FLIPR assay, in more advanced assays run subsequently, there appeared to be no discernible difference between ligands **1** and **2** in assays measuring H_4 functional antagonism rodent in vivo and native cells, as both **1** and **2** were able to block H_4 agonist-induced itching in mice and H_4 agonist-induced cell shape change in bone marrow derived mast cells (BMMC) that express native H_4 receptor.[25] Nevertheless, varying the size of the new ring in compounds **7**−**9** did change the in vitro profile at rat H_4. Both of the six-membered ring analogues (**6** and **7**) were potent antagonists of the human H_4 receptor, and both were potent at the rat H_4 receptor with some receptor activation detected (76% and 52%). Shrinking the carbocycle to the five-membered ring analogue **8** also yielded a highly potent antagonist of the human H_4 receptor (pK_b = 8.75), but the compound appeared to activate the rat receptor (96%) almost to the same degree as the endogenous agonist histamine. On the other hand, expanding the carbocycle to the seven-membered ring gave a compound (**9**) that performed as a potent antagonist, with no detectable functional activation in any species' H_4. Next, a broader series of seven-membered ring analogues was prepared, replacing the piperazine moiety of **9** with other diamines to give compounds **10**−**12**. All of these analogues were more potent in the functional and binding assays than **9** in all species H_4 receptors. Other seven-membered ring compounds were likewise found to be potent, including the fluorinated analogue **13**, as well as **14**, a compound with the aryl ring appended rather than fused to the seven-membered ring. Overall, the seven-membered ring analogues **9**−**14** were found potent in the binding assays. They also behave as potent antagonists in in vitro functional assays across all species, including blocking histamine-induced receptor activation in the FLIPR assay (Table 1) and blocking H_4 agonist-induced cell shape change in bone marrow derived mast cells (BMMC; Supporting Information, page S8).

The best of the new compounds **9**−**14** and the literature standards **1** and **2** were profiled against a broad battery of other targets, at a concentration of 10 μM, in a binding based screen of 80 receptors and enzymes (Cerep, Redmond, WA). Compounds and targets showing significant interaction at 10 μM were next profiled with full dose−responses to determine K_i values at the off-targets. For all the compounds, there was no significant binding to the great majority of sites screened. There was a minority of sites (H_3, 5-HT, and adrenergic receptor subtypes) that appeared to interact with the compounds, though even for these, compounds retained high relative selectivity for the H_4 receptor. Of known receptors, the histamine H_3 receptor has the closest sequence homology, and indeed, the H_4 receptor was discovered from an EST GPCR-like sequence in part

Table 2. Summary of in Vitro Metabolism Profiles of Compounds

compd	rat usomal turnover ((pmol/min)/mg)	human usomal turnover ((pmol/min)/mg)	mouse usomal turnover ((pmol/min)/mg)	% rat protein binding	% human protein binding
1	179	19	143	72	77
2	94.5	25.9	43.1	38	35
3	168	22	119	65	49
4	61.4	26.6	31.6	65	58
5	26.9	6.2	23.2	4	7
6	192	102	195	70	78
7	104	45.9	18.4	27	41
8	65.8	18.7	43.6	69	28
9	95.4	14.5	14	58	44
10	19	13	32.9	55	48
11	173	20.7	29.7	57	49
12	165	20	38.8	44	48
13	200	27.3	32.4	66	61
14	108	28.5	10.9	76	59

because of a degree of similarity to the H_3 receptor, and some of the first identified ligands of the H_4 receptor were known histamine H_3 antagonists such as thioperamide and clobenpropit.[6] Nevertheless, the homology between H_4 and H_3 is not particularly high, not more than 35−48% homology across species.[15,16] Compounds **1**, **2**, and **9** were 190-fold, 30-fold, and 52-fold selective for the human H_4 receptor versus the human H_3 receptor, and the new seven-membered ring compounds **10**, **11**, and **13** were even more selective (>640-, 570-, and 470-fold, respectively). None of the new compounds **10**−**14** interacted detectably with histamine H_1 or H_2 receptors (selectivity was >160- to >1890-fold for H_4). We had previously noted that compound **2** did appear to have a significant interaction with the 5-HT_{1a} receptor (1.2-fold selectivity, K_i = 42 nM) and the 5-HT_{1d} receptor (3.5-fold, K_i = 120 nM), but the new compounds **10**−**13** were much more selective (460- to 3070-fold for H_4 versus 5-HT_{1a} and 235- to 850-fold for H_4 versus 5-HT_{1d}). This series was targeted for in vivo testing in inflammation and pain models, so we tested (but found no interaction at) key molecular target sites that might induce this activity (COX-1 and COX-2 enzymes, μ-, κ-, and δ-opiate receptors, and CB_1, CB_2, nicotinic, and TRPV receptor isoforms). The 2-aminopyrimidines **2** and **10**−**14** also were tested at 10 μM in a panel of 133 diverse receptor kinases and showed no detectable inhibitory activity. The high selectivity of the new series of compounds (**10**−**14**) may arise from the rotational constraints placed on the structure by the seven-membered ring.

The high potency of **1** and **2** enhances their utility as pharmacological tools, but early on, one structural feature attracted our attention as introducing a potential complication for some in vivo studies: Compounds **1** and **2** both have an *N*-methyl group on the piperazine nitrogen that could be removed by cytochrome P_{450} (CYP) directed oxidative dealkylation. In PK studies, we indeed found this moiety underwent very rapid demethylation in vivo (see Supporting Information) to yield metabolites (**1** → **4**, and **2** → **5**) that themselves have H_4 potency only slightly weaker than the methylated parent drugs. In fact, for **1**, at all time points longer than 15 min postinjection, the metabolite **4** was present at higher concentrations than parent drug, and when the AUC (as the concentration−time product for 12 h after an oral dose of 20 mg/kg sc) was calculated, the ratio AUC_4/AUC_1 was 4.2×. This finding applied to compound **2** also, where concentrations of the demethylated metabolite **5** exceeded that of the parent at times beyond 15 min postadministration; for example, at 2 h after oral administration of **1** at 20 mg/kg, plasma concentration of **5** was 20-fold higher than **1**. Therefore, with in vivo studies under anything other than very short-term (<15 min) acute conditions, the presence of multiplecirculating active species would complicate attempts to ascribe which proportion of the in vivo effects arises from circulating parent or metabolite. This was one of the main factors motivating the design of new structures as represented by compounds **7**−**14**.

In part, compounds **7**−**14** were designed to replace *N*-methylpiperazine moiety found in **1** and **2**, since this particular group had been demonstrated to undergo very rapid metabolism. Several diamine bases were shown to permit comparable or increased in vitro H_4 potency (Table 1). The compounds were examined in an in vitro screen for metabolic turnover in liver microsomes (Table 2). In mouse microsomes, the *N*-methylpiperazine compounds (**1**, **2**, **6**) showed the most rapid metabolic turnover whereas the other diamine-containing compounds were much slower as anticipated. In rat and human microsomes, the influence of structure on turnover was less clear. However, one of the new compounds (**10**) was particularly interesting in that it showed very slow metabolism in all species in vitro. Of other ADME-related properties assessed, all the compounds were found to have low to moderate protein binding, very high aqueous solubility, and no inhibition of CYP enzymes.[34]

The pharmacokinetic properties of select compounds were examined in rat and mouse and summarized in Table 3. The new compounds **9**, **10**, **13**, and **14** had longer half-lives in rat than **1** and **2**, though oral bioavailability was similar. Two of the new compounds were tested in mouse (**9** and **10**), and both showed much longer half-lives than **1** and **2**. Compound **9** showed an especially long $t_{1/2}$ in mouse (4.9 h), and compound **10** was interesting for its high oral bioavailability (90%). The ability to distribute into CNS is likely unimportant for activity in inflammation models. We have found that intrathecal administration of **1** was insufficient to induce antinociceptive activity in an inflammatory pain model, so CNS access does not appear to be required.[24] However, low expression of H_4 receptor in brain has been reported,[12,35] and no test of a requirement for CNS access in neuropathic pain models has yet been done. Nevertheless, we tested compounds **1**, **2**, and **9**−**12** for their ability to penetrate the brain, and all were found able to efficiently and rapidly access the CNS and achieved brain/plasma ratios ranging from 2.1× to 20×. It is likely this profile is a consequence of the physicochemical and structural properties of the molecules (low molecular weight, moderately lipophilic, basic amines, minimal number of polar groups) and the low protein binding so that there is a substantial fraction of unbound drug in circulation available to penetrate the blood−brain barrier.[36]

Table 3. Summary of in Vivo Metabolic Profiles[a]

	rat pharmacokinetic properties[b]				mouse pharmacokinetic properties				
compd	$T_{1/2}$ (h)	CL_b ((L/h)/kg)	V_b (L/kg)	$F_{po/iv}$ (%)	$T_{1/2}$ (h)	CL_b ((L/h)/kg)	V_b (L/kg)	$F_{po/iv}$ (%)	brain/plasma
1	1.7	12.6	36.6	47	0.7[c]	23.2	24	24.1	13.5×[e]
2	0.4	10.3	7.1	31	0.2[d]	15.3	3.9	41	20×[f]
9	2.2	6.1	19.9	37	4.9[c]	22.7	88.9	33	2.1×[f]
10	2.6	19.4	72.9	34	1.6[c]	71.3	131	90	2.2×[f]
11									3.0×[f]
12									5.8×[f]
13	3.5	3.6	18.2	18					
14	3.2	4.8	22	31					

[a] For all experiments, $n = 3$ animals. [b] Determined after an iv dose of 10 mg/kg (except fractional oral bioavailability $F_{po/iv}$). [c] Determined at iv dose of 5 mg/kg except for the fractional oral bioavailability $F_{po/iv}$.. [d] Determined at iv dose of 2.5 mg/kg except for the fractional oral bioavailability $F_{po/iv}$. [e] Determined in rat at iv dose of 30 mg/kg at 30 min postdose. [f] Determined in rat at iv dose of 10 mg/kg at 30 min postdose.

Table 4. In Vivo Demonstration of Anti-Inflammatory Activity in Zymosan-Induced Peritonitis in Mice and Demonstration of H_4 Antagonism in Vivo by Blockade of H_4-Agonist Induced Scratching[a]

compd	blockade of neutrophil influx in mice after zymosan, ED_{50}, sc (μmol/kg)	blockade of scratching in mouse, ED_{50}, ip (μmol/kg)
1	61	25
2	66	25
9	21	>72
10	34	33
11	100	235
12	17	25
13	134	10
14	79	10

[a] At ED_{50}, the responses of drug-treated animals were significantly ($p < 0.05$) from the responses of vehicle-treated animals.

Histamine H_4 receptors have been demonstrated to have anti-inflammatory activity in vivo.[2,15,19] Compounds **1** and **2** were found active in one such model, blocking zymosan-induced peritonitis in mice with ED_{50} values of 61 and 66 μmol/kg sc, respectively (Table 4). Consistent with this profile, and as would be expected of compounds with potent histamine H_4 antagonism, all of the compounds in the new series (**9**−**14**) potently blocked peritonitis in this assay (Table 4), with ED_{50} doses ranging from as low as 17 to 134 μmol/kg. Most exciting was the finding that three of the compounds were even more potent than the widely used reference standard compound **1** as well as **2**, with new series compounds **9**, **10**, and **12** showing ED_{50} potencies of 21, 34, and 17 μmol/kg, respectively.

Histamine H_4 receptors have been well documented to modulate itch responses,[22,23] with H_4 agonists such as clobenpropit inducing scratching in wild-type but not H_4-deficient mice, and the itch response is blocked by H_4 antagonists such as **1** and **2** (Table 4). Consistent with this profile, compounds **10**−**14** were able to completely block clobenpropit-induced scratching responses in mice after ip dosing. The best of the new compounds showed comparable or even greater potency than the reference compound **1** or **2**, with analogues **10**, **12**, **13**, and **14** showing ED_{50} values of 33, 25, 10, and 10 μmol/kg, respectively.

We have recently reported that two histamine H_4 antagonists (**1** and **2**) are active in a diverse range of pain models.[24,25] In a model of inflammatory pain wherein carrageenan is injected to induce inflammation, compound **1** blocked pain responses with an ED_{50} of 80 μmol/kg ip and compound **2** blocked pain responses with an ED_{50} of 32 μmol/kg ip. The new compounds **10** and **12** were able to block responses also (Figure 1), with compound **10** having an ED_{50} of 72 μmol/kg ip. In a spinal nerve ligation model assessing neuropathic pain[37] compounds **1** and **2** were active with ED_{50} values of 217 and 115 μmol/kg ip, respectively.[24,25] The new compounds **10** and **12** were effective in this model also, with ED_{50} values of 100 and 140 μmol/kg ip, respectively.

Conclusion

There is a substantial body of preclinical research indicating that histamine H_4 receptor antagonists have potential for treating disease. The histamine H_4 receptor is thought to play an important role in modulating immune system function, based in part on its high degree of expression as mRNA or protein in key tissues and cells of the immune system, for example, in mast and dendritic cells, eosinophils, basophils, leukocytes, and lymphocytes.[1,12] H_4 receptors can modulate function, as assessed by H_4-agonist mediated chemotaxis,[13,17,38] shape change,[14,18,24] and actin polymerization[18,39] in immune cells with native expression of the H_4 receptor. H_4 receptor antagonists have been reported active in models of allergen-induced airway inflammation[20] and irritant-induced colitis,[21] and the H_4 receptor has been associated with diseased joints and cultured synoviocytes from rheumatoid arthritis patients.[40−42] Other research points to the involvement of H_4 receptors in itch and indicates that histamine H_4 antagonists have antipruritic properties.[22,23]

Recently, it has been reported that H_4 antagonists have activity in models of inflammatory pain.[24−26] For compound **1**, a peripheral site of action was supported in acute inflammation-induced pain. Surprisingly however, H_4 antagonists were also found to block pain in other models, models that assess pain induced under conditions beyond just acute inflammation. Compound **1** was reported to be effective in models of neuropathic, postsurgical, and osteoarthritic pain.[24] At present, the site of action (peripheral or central) in these models is unknown, but there are reports of H_4 expression in brain,[12,35] and we have more recently identified expression in spinal cord.[43] Further research using synthetic ligands will be needed to definitively answer this and related questions of the pharmacology of the H_4 receptor in different pain models.

Research in the field will benefit from additional ligands, and we here provide a new series of highly potent and selective H_4 antagonists with good pharmacokinetic properties and demonstrated activity in multiple in vivo models of inflammation, itch, and pain. In particular, compound **10** demonstrated the best overall profile, with high in vitro potency in functional assays across species (FLIPR K_b of 5.7 nM in human and mouse H_4, 10 nM in rat H_4), in binding potency (K_i of 4.7 and 3.8 nM at human and rat H_4). Additionally, the compound demonstrated functional antagonism in vitro in an assay of cells natively expressing H_4 receptors (BMMC), with an IC_{50} of 0.38 μM for blockade of histamine-induced shape change, and also demonstrated antagonism in vivo in mice, where **10** potently blocked H_4-agonist induced itch at 33 μmol/kg, ip. The compound potently blocked inflammation in a peritonitis model in mice at

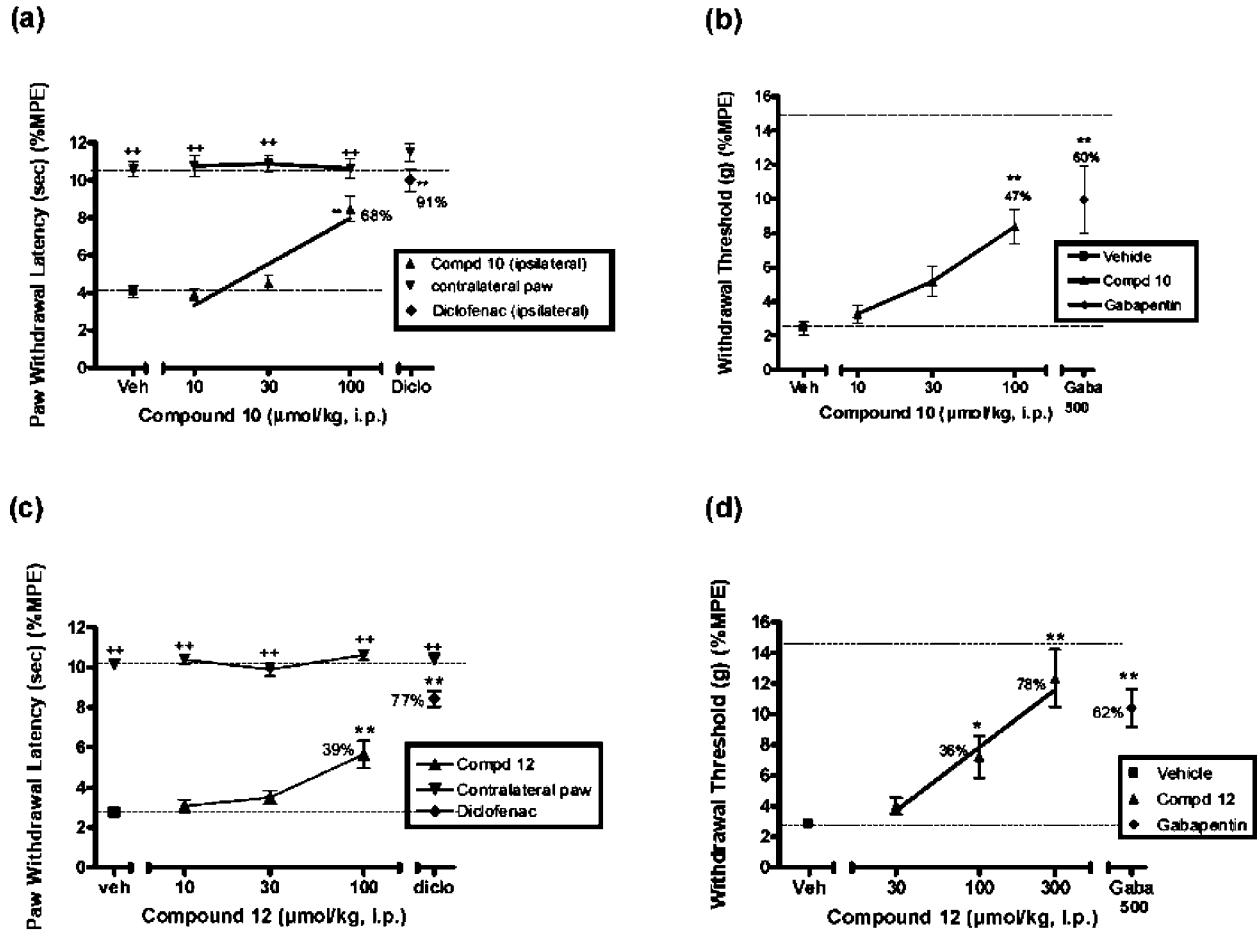


Figure 1. Antinociceptive efficacy data of compounds in rats: antinociceptive efficacy of compound **10** in a model of (a) carrageenan-induced inflammatory pain ($n = 11$) and (b) in a spinal nerve ligation model of neuropathic pain ($n = 6$); antinociceptive efficacy of compound **12** in a model of (c) carrageenan-induced inflammatory pain ($n = 12$) and (d) in a spinal nerve ligation model of neuropathic pain ($n = 12$). Responses are expressed as the mean and SEM of the paw withdrawal responses, with the maximum percent effect (% MPE) expressed as the response in the nociceptive paw (ipsilateral), relative to the control paw.

34 μmol/kg, ip, and was effective in an inflammatory pain model in rat at an ED_{50} of 72 μmol/kg, ip, and in a neuropathic pain model at an ED_{50} of 100 μmol/kg, ip. The pharmacokinetic profile of **10** demonstrated a longer half-life in rats (2.6 h) and mice (1.6 h) than previously reported (**1**, **2**) H_4 antagonists used to probe in vivo pharmacology. Overall, in some key properties (PK in rodents, potency in inflammation and some pain models) compound **10** (A-943931) appears to exceed the activity of reference standard H_4 antagonists (**1**, **2**) and thus is likely to find utility as a new tool ligand for further probing the pharmacology of H_4 mediated effects in vivo.

Experimental Procedures

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. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by Quantitative Technologies, Inc., and Robertson Microlit Laboratories. Column chromatography was carried out using either hand-packed silica gel 60 (230−400 mesh) or prepacked silica gel columns from Analogix, and product was eluted under medium pressure liquid chromatography. Thin-layer chromatography (TLC) was performed using 250 mm silica gel 60 glass-backed plates with a fluorescent (F254) indicator.

Synthesis of Compounds. 1-Oxo-1,2,3,4-tetrahydronaphthalene-2-carboxylic Acid Methyl Ester.[44] A suspension of 60% dispersion of sodium hydride in mineral oil (1.64 g, 41 mmol) in dimethyl carbonate (50 mL) was treated dropwise with α-tetralone (4.6 mL, 34 mmol). The mixture was heated at 90 °C for 20 min, cooled to ambient temperature, treated with 2 M HCl (40 mL), and extracted with EtOAc (100 and 25 mL). The combined EtOAc layers were washed with brine, dried ($MgSO_4$), filtered, concentrated under reduced pressure, and chromatographed on silica gel, eluting with a gradient of hexane/EtOAc (20:1 and 10:1) to provide the ester, which was used directly in the next step. MS *m/z* 205 $(M + H)^+$.

2-Amino-5,6-dihydrobenzo[*h*]quinazolin-4-ol. A solution of 1-oxo-1,2,3,4-tetrahydronaphthalene-2-carboxylic acid methyl ester[44] (1.0 g, 4.9 mmol) in DMF (5 mL) was treated with guanidine nitrate (1.2 g, 9.9 mmol) followed by the addition of potassium carbonate (1.4 g, 9.9 mmol), and the mixture was stirred at 110 °C for 16 h. The mixture was cooled to ambient temperature, diluted with water (40 mL), and neutralized to pH 6 with acetic acid. The solid was collected by filtration, washed with water, and dried under vacuum to provide 770 mg (74%) of the title compound. ^{1}H NMR (DMSO-d_6) δ 2.48−2.55 (m, 2H), 2.78 (t, $J = 7.6$ Hz, 2H), 6.37 (s, 2H), 7.21−7.36 (m, 3H), 7.95−8.00 (m, 1H), 10.82 (s, 1H). MS *m/z* 214 $(M + H)^+$.

4-Chloro-5,6-dihydrobenzo[*h*]quinazolin-2-ylamine. A suspension of 2-amino-5,6-dihydrobenzo[*h*]quinazolin-4-ol (83 mg, 0.41 mmol) in dioxane under nitrogen was treated with $POCl_3$ (186 μL, 2.0 mmol), cooled to 0 °C, and then treated with Et_3N (113 μL, 0.81 mmol). The mixture was allowed to warm to ambient temperature and stirred for 4 h, after which time it was cooled to 0 °C and treated with 2 mL (8 mmol) of 4 M HCl in dioxane, and the mixture then stirred for 18 h at ambient temperature. The mixture was partitioned between 15 mL of 1 M NaOH and 25 mL

of CH_2Cl_2. The layers were separated, and the aqueous layer was reextracted with 25 mL of CH_2Cl_2. The organic layers were combined and dried over $MgSO_4$, then filtered, concentrated, and purified by chromatography on silica gel, eluting with a stepwise gradient of 20:0, 20:1, and 10:1 CH_2Cl_2/EtOAc to provide 52 mg (58%) of the title compound. ^{1}H NMR ($CDCl_3$) δ 2.93−2.93 (m, 4H), 5.06 (s, 2H), 7.21−7.27 (m, 1H), 7.30−7.43 (m, 2H), 8.21 (dd, J = 7.6, 1.5 Hz, 1H). MS m/z 232 $(M + H)^+$.

4-(4-Methylpiperazin-1-yl)-5,6-dihydrobenzo[*h*]quinazolin-2-ylamine (6). A solution of 4-chloro-5,6-dihydrobenzo[*h*]quinazolin-2-ylamine (83 mg, 0.36 mmol) in 1.4 mL of 2-methoxyethanol was treated with 0.44 mL (2.5 mmol) of diisopropylethylamine and 0.044 mL (0.40 mmol) of 1-methylpiperazine. The mixture was then heated at 110 °C with stirring for 24 h and then cooled, and the reaction mixture was partitioned between 10 mL of 1 M NaOH and 25 mL of CH_2Cl_2. The layers were separated, and the aqueous layer was reextracted with 25 mL of CH_2Cl_2. The organic layers were combined and dried over $MgSO_4$, then filtered, concentrated, and purified by chromatography on silica gel, eluting with a gradient of 2, 3.5, and 5% (9:1 MeOH/NH_4OH) in CH_2Cl_2 to provide 71 mg (67%) of the title compound. ^{1}H NMR ($CDCl_3$) δ 2.35 (s, 3H), 2.53 (t, J = 4.8 Hz, 4H), 2.67 (dd, J = 7.8, 5.1 Hz, 2H), 2.82 (dd, J = 9.2, 6.4 Hz, 2H), 3.34 (t, J = 4.8 Hz, 4H), 4.72 (s, 2H), 7.19 (m, 1H), 7.31 (m, 2H), 8.09 (m, 1H). MS $(M + H)^+$ m/z 296. Anal. ($C_{17}H_{21}N_5 \cdot 0.05CH_2Cl_2$) C, H, N.

4-Piperazin-1-yl-5,6-dihydrobenzo[*h*]quinazolin-2-ylamine (7). The title compound was prepared using the procedure outlined above for compound **6**, substituting piperazine for 1-methylpiperazine. ^{1}H NMR ($CDCl_3$) δ 2.68 (dd, J = 7.8, 5.1 Hz, 2H), 2.82 (dd, J = 8.8, 6.1 Hz, 2H), 2.99 (m, 4H), 3.28 (m, 4H), 4.74 (s, 2H), 7.19 (m, 1H), 7.31 (m, 2H), 8.10 (m, 1H). MS m/z 282 $(M + H)^+$. Anal. ($C_{16}H_{19}N_5 \cdot 0.45CH_2Cl_2$) C, H, N.

2-Amino-5*H*-indeno[1,2-*d*]pyrimidin-4-ol. To a 100 mL round-bottom flask containing pulverized guanidine carbonate (1.6 g, 9.1 mmol) was added 10 mL of DMF. The flask was heated at 160 °C in an oil bath, and after 2 min, a solution of methyl 1-oxo-2,3-dihydro-1*H*-indene-2-carboxylate[45] (1.2 g, 6.1 mmol) in DMF (1 mL) was added dropwise over 1 min. The mixture was maintained at 160 °C for 5 min with vigorous stirring, then cooled to room temperature, and poured into 50 mL of 1 M NaOH. The mixture was washed with 50 mL of Et_2O, and then the aqueous layer was acidified to pH 7 with concentrated HCl and allowed to stand at room temperature overnight. A solid precipitated, which was collected by filtration and dried under vacuum to provide 130 mg (11%) of the intermediate. ^{1}H NMR (DMSO-d_6) δ 3.58 (s, 2H), 6.76 (bs, 2H), 7.37−7.48 (m, 2H), 7.57−7.62 (m, 1H), 7.68−7.73 (m, 1H), 10.85 (bs, 1H). MS m/z 200 $(M + H)^+$.

2-Amino-5*H*-indeno[1,2-*d*]pyrimidin-4-yl 4-Nitrobenzenesulfonate. To a 50 mL round-bottom flask were added 127 mg (0.624 mmol) of 2-amino-5*H*-indeno[1,2-*d*]pyrimidin-4-ol, 210 mg (0.94 mmol) of 4-nitrobenzene-1-sulfonyl chloride, 7.6 mg (0.062 mmol) of *N*,*N*-dimethylaminopyridine, 6 mL of CH_2Cl_2, and 0.15 mL (1.06 mmol) of triethylamine. The mixture was stirred for 18 h at room temperature, then partitioned between 5 mL of saturated aqueous $NaHCO_3$ and 25 mL of CH_2Cl_2. The layers were separated, and the aqueous layer was reextracted with 25 mL more CH_2Cl_2. The organic layers were combined, dried over $MgSO_4$, filtered, concentrated under vacuum, and purified by chromatography (on an Analogix IntelliFlash 280 MPLC) using a SF15-24g silica gel column, eluting with a stepwise gradient of CH_2Cl_2/0−20% EtOAc to provide 49 mg (20%) of the title compound. ^{1}H NMR ($CDCl_3$) δ 3.88 (s, 2H), 5.00 (s, 2H), 7.43−7.50 (m, 1H), 7.54 (dt, J = 7.3, 1.4 Hz, 1H), 7.58−7.63 (m, 1H) 7.94−7.98 (m, 1H), 8.32 (dt, J = 9.2, 2.2 Hz, 2H), 8.44 (dt, J = 9.2, 2.2 Hz, 2H). MS m/z 385 $(M + H)^+$.

4-(Piperazin-1-yl)-5*H*-indeno[1,2-*d*]pyrimidin-2-amine (8). A mixture of 49 mg (0.13 mmol) of 2-amino-5*H*-indeno[1,2-*d*]pyrimidin-4-yl 4-nitrobenzenesulfonate, 118 mg (0.63 mmol) of *tert*-butyl piperazine-1-carboxylate, and 0.15 mL (0.88 mmol) of diisopropylethylamine in 0.5 mL of 2-methoxyethanol was heated at 110 °C for 16 h, cooled, and partitioned between 10 mL of 1 M NaOH and 25 mL of CH_2Cl_2. The aqueous layer was reextracted with 25 mL of CH_2Cl_2, then the organic layers were combined, dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by chromatography (with an Analogix IntelliFlash 280 MPLC) on a silica gel SF15-24g column, eluting with a gradient of CH_2Cl_2/EtOAc 9:1 to 100% EtOAC to provide 40 mg (86% yield) of the Boc-protected intermediate. This Boc-protected intermediate (40 mg, 0.11 mmol) was treated with 2 mL of trifluoroacetic acid, heated to 60 °C for 2 min, then concentrated under vacuum, purified by chromatography (with an Analogix IntelliFlash 280 MPLC) using a silica gel SF10-4g column, and eluting with a gradient of 2−90% MeOH/CH_2Cl_2 containing 1% NH_4OH to provide 19.9 mg (68.4%) of the title compound. ^{1}H NMR ($CDCl_3$) δ 2.98 (t, J = 5.1 Hz, 4H), 3.80−3.86 (m, J = 5.1, 5.1 Hz, 4H), 3.90 (s, 2H), 4.77 (s, 2H), 7.38−7.45 (m, 2H), 7.48−7.55 (m, 1H), 7.91−7.98 (m, 1H). MS m/z 268 $(M + H)^+$. Anal. ($C_{16}H_{19}N_5 \cdot 0.2CH_3OH \cdot 0.2CH_2Cl_2$) C, H, N.

Methyl 5-Oxo-6,7,8,9-tetrahydro-5*H*-benzo[7]annulene-6-carboxylate. To 20 mL of dimethyl carbonate was carefully added 3 g (75 mmol) of NaH as a 60% dispersion in oil. Then the mixture was heated to 85 °C, and to this reaction mixture was added dropwise a solution of 1-benzosuberone (3 g, 18.8 mmol) in 25 mL of dimethyl carbonate. The mixture was kept at 85 °C for 3 h, with vigorous stirring, then cooled to room temperature and quenched slowly by careful addition of 1 N HCl to adjust the reaction to pH 6. The mixture was then poured into water and extracted with diethyl ether. The organic layer was separated, washed with saturated aqueous NaCl, dried over $MgSO_4$, and concentrated under reduced pressure to provide the title product. ^{1}H NMR ($CDCl_3$) δ 2.04−2.14 (m, 4 H), 2.64 (t, J = 6.8 Hz, 2 H), 3.83 (s, 3 H), 7.19−7.24 (m, 1 H), 7.31−7.36 (m, 2 H), 7.60−7.65 (m, 1 H), 12.60 (s, 1 H). MS m/z 219 $(M + H)^+$.

2-Amino-6,7-dihydro-5*H*-benzo[6,7]cyclohepta[1,2-*d*]pyrimidin-4-ol. A solution of methyl 5-oxo-6,7,8,9-tetrahydro-5*H*-benzo[7]annulene-6-carboxylate (5.3 g, 24 mmol), guanidine hydrochloride (7 g, 72 mmol), and K_2CO_3 (11.6 g, 84 mmol) in DMF (40 mL) was heated at 125 °C for 2 h, then cooled and filtered through a layer of diatomaceous earth, and the solid was washed with a small amount of DMF. The filtrate was concentrated under reduced pressure, and residual DMF and water were removed by repeated addition and evaporation of toluene. The resulting residue was washed with a small amount of CH_2Cl_2, and the solid residue was dried in a vacuum oven to provide the product (2.1 g, 30%), which was used directly in next step. ^{1}H NMR (CD_3OD) δ 2.07−2.18 (m, 2 H), 2.27 (t, J = 6.8 Hz, 2 H), 2.59 (t, J = 7.1 Hz, 2 H), 7.24−7.29 (m, 1 H), 7.32−7.37 (m, 2 H), 7.53−7.60 (m, 1 H), 8.50 (s, 1 H). MS m/z 228 $(M + H)^+$.

Toluene-4-sulfonic Acid 2-Amino-6,7-dihydro-5*H*-benzo[6,7]cyclohepta[1,2-*d*]pyrimidin-4-yl Ester. A solution of 2-amino-6,7-dihydro-5*H*-benzo[6,7]cyclohepta[1,2-*d*]pyrimidin-4-ol (2.1 g) in CH_2Cl_2 (100 mL) was treated with *p*-toluenesulfonyl chloride (10.7 g), catalytic *N*,*N*-dimethylaminopyridine (300 mg), and triethylamine (13 mL). The mixture was stirred at ambient temperature for 16 h, then partitioned between CH_2Cl_2 and H_2O. The organic layer was separated, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was purified by chromatography on silica gel, eluting with a 2:4:4 mixture of EtOAc/CH_2Cl_2/hexane to provide the product as a white solid (2.5 g, 71%). ^{1}H NMR (CD_3OD) δ 2.09 (q, J = 7.0 Hz, 2 H) 2.32 (t, J = 7.1 Hz, 2 H), 2.47 (s, 3 H), 2.52 (t, J = 7.0 Hz, 2 H), 7.23−7.29 (m, J = 2.0 Hz, 1 H), 7.31−7.43 (m, 2 H), 7.47 (d, 2 H), 7.54−7.62 (m, 1 H), 7.99 (d, J = 8.5 Hz, 2 H). MS m/z 382 $(M + H)^+$.

4-Piperazin-1-yl-6,7-dihydro-5*H*-benzo[6,7]cyclohepta[1,2-*d*]pyrimidin-2-ylamine (9). A solution of toluene-4-sulfonic acid 2-amino-6,7-dihydro-5*H*-benzo[6,7]cyclohepta[1,2-*d*]pyrimidin-4-yl ester (3.9 g 10.2 mmol) and piperazine (1.3 g, 15.6 mmol) in acetonitrile (40 mL) was heated at 70 °C for 16 h. The mixture was removed from the heat and concentrated under reduced pressure. The residue was purified by chromatography on silica gel, eluting with EtOAc/HCO_2H/H_2O (4:1:1). Fractions containing product were combined and concentrated under reduced

pressure. The resulting residue mixture was basified with 1 N NaOH and extracted with CH_2Cl_2 (100 mL). The organic phase was washed with H_2O, dried with $MgSO_4$, concentrated under reduced pressure, and purified by chromatography on silica gel, eluting with NH_4OH/MeOH/DCM (0.7:7:93) to provide the title product (1.8 g, 60%). The product was mixed with 1 equiv of maleic acid in methanol and concentrated under reduced pressure. The resulting solid was recrystallized from acetone to obtain a white crystalline solid as the monomaleic acid salt. ^{1}H NMR (CD_3OD) δ 2.17−2.35 (m, 4H), 2.65 (t, J = 6.6 Hz, 2H), 2.94 (m, 4H), 3.39 (m, 4H), 7.26 (m, 1H), 7.35 (m, 2H), 7.62 (m, 1H). MS m/z 296 $(M + H)^+$. Anal. ($C_{17}H_{21}N_5 \cdot C_4H_4O_4 \cdot 0.25H_2O$) C, H, N.

4-((3*R*)-3-Aminopyrrolidin-1-yl)-6,7-dihydro-5*H*-benzo[6,7]cyclohepta[1,2-*d*]pyrimidin-2-ylamine (10). A solution of toluene-4-sulfonic acid 2-amino-6,7-dihydro-5*H*-benzo[6,7]cyclohepta[1,2-*d*]pyrimidin-4-yl ester (1.48 g, 3.88 mmol) and (*R*)-*tert*-butyl pyrrolidin-3-ylcarbamate (1.1 g, 5.7 mmol) in acetonitrile (20 mL) was heated at 70 °C for 16 h. Then the mixture was removed from heating and concentrated under reduced pressure. The residue was purified by chromatography on silica gel, eluting with EtOAc/hexanes (8:2) to provide the Boc-protected intermediate (860 mg, 58%). Then this intermediate was dissolved in CH_2Cl_2 (20 mL), treated with trifluoroacetic acid (1.8 mL), and stirred at ambient temperature for 16 h. The mixture was diluted with CH_2Cl_2 (100 mL), cooled to 0 °C, and made basic with 1 N NaOH. The organic phase was washed with H_2O, dried over $MgSO_4$, and concentrated under reduced pressure. The resulting residue was purified by chromatography on silica gel, eluting with NH_4OH/MeOH/CH_2Cl_2 (0.6:6:94) to provide the product as a free base (400 mg, 63%). The free base product was mixed with 1 equiv of maleic acid in methanol and concentrated under reduced pressure. The resulting solid was recrystallized from acetone to obtain a white crystalline solid as the monomaleic acid salt. ^{1}H NMR (CD_3OD) δ 1.79 (m, 1H), 2.10−2.25 (m, 3H), 2.35 (t, J = 7.0 Hz, 2H), 2.65 (t, J = 6.8 Hz, 2H), 3.42 (dd, J = 10.5, 5.42 Hz, 1H), 3.56 (m, 1H), 3.69 (m, 1H), 3.82 (m, 2H), 7.25 (m, 1H), 7.34 (m, 2H), 7.63 (m, 1H). MS m/z 296 $(M + H)^+$. Anal. ($C_{17}H_{21}N_5 \cdot C_4H_4O_4 \cdot$ acetone$\cdot 0.75H_2O$) C, H, N.

(1-Benzyl-(3*R*)-pyrrolidin-3-yl)methylcarbamic Acid *tert*-Butyl Ester. To a solution of (3*R*)-(−)-1-benzyl-3-(methylamino)pyrrolidine (200 mg, 1.05 mmol) and di-*tert*-butyl dicarbonate (230 mg, 1.06 mmol) in MeOH (10 mL) was added NaOH (10%, 4 mL). The mixture was stirred at ambient temperature for 1 h, then diluted with H_2O (20 mL) and EtOAc (100 mL). The organic layer was separated and the aqueous layer reextracted with additional EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure to provide the title compound as a colorless oil. ^{1}H NMR (CD_3OD) δ 1.43 (s, 9H), 1.8 (m, 1H), 2.07 (m, 1H), 2.53 (m, 2H), 2.73 (m, 1H), 2.81 (s, 3H), 3.50 (d, J = 13 Hz, 1H), 3.65 (d, J = 13 Hz, 1H), 4.71 (m, 1H), 7.32 (m, 5H). MS (DCI-NH_3) m/z 291 $(M + H)^+$.

(*R*)-Methylpyrrolidin-3-ylcarbamic Acid *tert*-Butyl Ester. To a solution of (1-benzyl-(3*R*)-pyrrolidin-3-yl)methylcarbamic acid *tert*-butyl ester (285 mg, 0.98 mmol) in 4.4% HCO_2H/MeOH (20 mL) under a nitrogen atmosphere was added $Pd(OH)_2$ on carbon (20%, 40 mg), and the resulting mixture was heated at 60 °C for 16 h. The mixture was cooled to room temperature, filtered through a layer of diatomaceous earth, washed with extra methanol (30 mL), and concentrated under reduced pressure. The residue was diluted with CH_2Cl_2 (30 mL), washed with 1 M NaOH, dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide the title compound as a colorless oil. ^{1}H NMR (CD_3OD) δ 1.46 (s, 9H), 1.79 (m, 1H), 1.99 (m, 1H), 2.76 (m, 1H), 2.79 (s, 3H), 2.87 (m, 1H), 3.03 (m, 2H), 4.57 (d, J = 6 Hz, 1H). MS (DCI-NH_3) m/z 201 $(M + H)^+$.

4-(3-(*R*)-Methylaminopyrrolidin-1-yl)-6,7-dihydro-5*H*-benzo[6,7]-cyclohepta[1,2-*d*]pyrimidin-2-ylamine (11). A solution of toluene-4-sulfonic acid 2-amino-6,7-dihydro-5*H*-benzo[6,7]cyclohepta[1,2-*d*]pyrimidin-4-yl ester (1.9 g, 5 mmol), (*R*)-methylpyrrolidin-3-ylcarbamic acid *tert*-butyl ester (1.4 g, 7 mmol), and triethylamine (1.5 mL) in acetonitrile (40 mL) was heated at 80 °C for 16 h. The mixture was removed from the heat and then concentrated on reduced pressure. The residue was purified by chromatography on silica gel, eluting with EtOAc to provide the Boc-protected intermediate (1.3 g, 64%). This intermediate was dissolved in CH_2Cl_2 (20 mL), treated with trifluoroacetic acid (5 mL), and stirred at ambient temperature for 16 h. The mixture was concentrated under reduced pressure, diluted with CH_2Cl_2 (100 mL), basified with NaOH (1 N), then partitioned between CH_2Cl_2 and water. The organic phase was washed with H_2O, dried with $MgSO_4$, and concentrated under reduced pressure. The resulting residue was chromatographed on silica gel, eluting with NH4OH/MeOH/CH_2Cl_2 (0.6:6:94) to provide the product as a free base (897 mg, 91%). The free base was mixed with 1 equiv of maleic acid in methanol and concentrated under reduced pressure. The resulting solid was recrystallized from acetone to obtain a white crystalline solid as the maleic acid salt. ^{1}H NMR (CD_3OD) δ 1.84 (m, 1H), 2.13−2.24 (m, 3H), 2.33 (m, 2H), 2.43 (s, 3H), 2.65 (t, J = 6.8 Hz, 2H), 3.50 (dd, J = 10.9, 5.76 Hz, 1H), 3.64−3.88 (m, 3H), 7.25 (m, 1H), 7.33 (m, 2H), 7.62 (m, 1H). MS m/z 310 $(M + H)^+$. Anal. ($C_{18}H_{23}N_5 \cdot C_4H_4O_4 \cdot 0.2$acetone$\cdot 1.5H_2O$) C, H, N.

4-(3-Methylaminoazetidin-1-yl)-6,7-dihydro-5*H*-benzo[6,7]cyclohepta[1,2-*d*]pyrimidin-2-ylamine (12). A solution of toluene-4-sulfonic acid 2-amino-6,7-dihydro-5*H*-benzo[6,7]cyclohepta[1,2-*d*]pyrimidin-4-yl ester (3.48 g, 9.13 mmol), azetidin-3-ylmethylcarbamic acid *tert*-butyl ester (2.2 g, 11.9 mmol) in acetonitrile (40 mL) and triethylamine (2.5 mL) was heated at 80 °C for 2 h. The mixture was removed from the heat and then concentrated under reduced pressure, and the residue was purified by chromatography on silica gel, eluting with EtOAc to provide the Boc-protected intermediate (2.9 g, 80%). This intermediate was dissolved in CH_2Cl_2 (20 mL) and treated with trifluoroacetic acid (5 mL) and stirred at ambient temperature for 16 h, then concentrated on reduced pressure. The residue was diluted with CH_2Cl_2 (100 mL) and basified with NaOH (1 N). Then it was partitioned between DCM and water. The organic phase was washed with H_2O, dried over $MgSO_4$, and concentrated under reduced pressure. The resulting residue was purified by chromatography on silica gel, eluting with NH_4OH/MeOH/CH_2Cl_2 (0.6:6:94) to provide the title product as a free base (1.8 g, 84%). The free base was mixed with 1 equiv of maleic acid in methanol and concentrated on reduced pressure. The resulting solid was recrystallized from acetone to obtain a white crystalline solid as the maleic acid salt. ^{1}H NMR (CD_3OD) δ 2.06−2.27 (m, 4H), 2.36 (s, 3H), 2.60 (t, J = 6.8 Hz, 2H), 3.63 (m, 1H), 3.99 (dd, J = 9.3, 4.92 Hz, 2H), 4.42 (dd, J = 9.0, 7.63 Hz, 2H), 7.25 (m, 1H), 7.34 (m, 2H), 7.59 (m, 1H). MS m/z 296 $(M + H)^+$. Anal. ($C_{17}H_{21}N_5 \cdot 1.05C_4H_4O_4$) C, H, N.

Methyl 3-Fluoro-5-oxo-6,7,8,9-tetrahydro-5*H*-benzo[7]annulene-6-carboxylate. To a solution of of 3-fluoro-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-one (4.27 mL, 27.8 mmol) in dimethyl carbonate (12 mL) was added 2.22 g (55.6 mmol) of a 60% dispersion of NaH in mineral oil, followed by the addition of a few drops of dry MeOH. The reaction mixture was heated at 90 °C for 3 h, poured into 2 M HCl (50 mL), and extracted with EtOAc (2×). The EtOAc extracts were combined, washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The obtained residue was purified by chromatography on silica gel, eluting with 20% EtOAc/hexane to provide 6.2 g of product as an oil. The product was mostly in the enol form in $CDCl_3$. ^{1}H NMR ($CDCl_3$) δ12.55 (s, 1H), 7.33 (dd, J = 9.5, 2.8 Hz, 1H), 7.17 (dd, J = 8.3, 5.6 Hz, 1H), 7.03 (td, J = 8.3, 2.8 Hz, 1H), 3.84 (s, 3H), 2.60 (t, J = 6.7 Hz, 2H), 2.10 (m, 4H). MS (DCI/NH_3) m/z 237 $(M + H)^+$, 254 $(M + NH_4)^+$.

10-Fluoro-2-Amino-6,7-dihydro-5*H*-benzo[6,7]cyclohepta[1,2-*d*]pyrimidin-4-ol. A solution of methyl 3-fluoro-5-oxo-6,7,8,9-tetrahydro-5*H*-benzo[7]annulene-6-carboxylate (6.5 g, 27.5 mmol) in DMF (25 mL) was treated with guanidine nitrate (6.72 g, 55 mmol), followed by the addition of potassium carbonate (7.61 g, 55 mmol). The reaction mixture was stirred at 110 °C for 16 h.

The mixture was cooled to ambient temperature, diluted with water (40 mL), and neutralized to pH 6 with acetic acid. The obtained precipitate was filtered off and triturated with EtOAc to yield 2.3 g of product as an off-white solid. ^{1}H NMR (DMSO-d_6) δ 10.90 (s, 1H), 7.29 (m, 2H), 7.15 (td, J = 8.6, 2.9 Hz, 1H), 6.42 (s, 2H), 2.79 (m, 1H), 2.15 (t, J = 7.0 Hz, 2H), 2.05 (m, 2H), 1.74 (m, 1H). MS (DCI/NH_3) m/z 246 $(M + H)^+$.

Toluene-4-sulfonic Acid 10-Fluoro-2-amino-6,7-dihydro-5*H*-benzo[6,7]cyclohepta[1,2-*d*]pyrimidin-4-yl Ester. A solution of 10-fluoro-2-amino-6,7-dihydro-5*H*-benzo[6,7]cyclohepta[1,2-*d*]pyrimidin-4-ol (1.0 g, 4.1 mmol) in CH_2Cl_2 (50 mL) was reacted with *p*-toluenesulfonyl chloride (1.55 g, 8.15 mmol), a catalytic amount of *N*,*N*-dimethylaminopyridine, and Et_3N (1.73 mL, 12.23 mmol). The reaction mixture was stirred at ambient temperature for 16 h, then diluted with CH_2Cl_2 and washed with concentrated $NaHCO_3$ solution. The organic layer was separated, dried over $MgSO_4$, concentrated under vacuum, and purified by chromatography, eluting with 30% EtOAc/hexane to yield 1.15 g of the product as a solid. ^{1}H NMR (DMSO-d_6) δ ppm 8.03 (d, J = 8.3 Hz, 2H), 7.50 (d, J = 8.7 Hz, 2H), 7.30 (m, 3H), 6.95 (s, 2H), 2.45 (s, 3H), 2.44 (m, 2H), 2.21 (m, 2H), 2.00 (m, 2H). MS (ESI^+) m/z 400 $(M + H)^+$.

10-Fluoro-4-piperazin-1-yl-6,7-dihydro-5*H*-benzo[6,7]cyclohepta-[1,2-*d*]pyrimidin-2-ylamine Dihydrochloride (13). A mixture of toluene-4-sulfonic acid 10-fluoro-2-amino-6,7-dihydro-5*H*-benzo[6,7]cyclohepta[1,2-*d*]pyrimidin-4-yl ester (0.6 g, 1.5 mmol), *tert*-butyl piperazine-1-carboxylate (0.33 g, 1.8 mmol), and triethylamine (0.3 mL, 2.25 mmol) in 4 mL of 2-methoxyethanol was heated at 110 °C for 16 h. The reaction mixture was diluted with CH_2Cl_2 and washed with saturated $NaHCO_3$ solution. The organic layer was separated, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel, eluting with 5% MeOH/CH_2Cl_2 to provide 0.5 g of the Boc-protected intermediate. This intermediate (0.5 g, 1.2 mmol) was dissolved in methanol (10 mL) and treated with 4 N HCl/dioxane (2 mL) solution at reflux for 2 h. The reaction mixture was concentrated under vacuum and triturated with EtOAc/diethyl ether to yield 0.47 g of the title compound. ^{1}H NMR of the free base (CD_3OD) δ 2.16−2.35 (m, 4H), 2.63 (t, J = 6.6 Hz, 2H), 2.94−2.95 (m, 4H), 3.40−3.41 (m, 4H), 7.10 (dt, J = 8.5, 2.7 Hz, 1H), 7.28 (dd, J = 8.3, 5.6 Hz, 1H), 7.36 (dd, J = 9.8, 2.7 Hz, 1H). MS (ESI^+) m/z 314 $(M + H)^+$. Anal. ($C_{17}H_{20}FN_5 \cdot 2HCl \cdot 1.5H_2O$) C, H, N.

Methyl 2-Oxo-3-phenylcycloheptanecarboxylate. To a solution of of 2-phenylcycloheptanone (1 g, 5.3 mmol) in dimethylcarbonate (4 mL) was added 0.425 g (10.6 mmol) of a 60% dispersion of NaH in mineral oil, followed by the addition of a few drops of dry MeOH. The reaction mixture was heated to 90 °C for 3 h, then cooled and treated with 2 M HCl (40 mL), and extracted with EtOAc. The combined EtOAc layers were washed with brine, dried over $MgSO_4$, concentrated in vacuo, and chromatographed on silica gel, eluting with 20% EtOAc/hexane to provide 1.1 g of product as an oil. ^{1}H NMR ($CDCl_3$) δ 7.22−7.35 (m, 5H), 3.93 (m, 1H), 3.66 (s, 3H), 3.65 (m, 1H), 2.29 (m, 1H), 2.13 (m, 1H), 2.05 (m, 2H), 1.89 (m, 2H), 1.40 (m, 2H). MS (DCI^+) m/z 247 $(M + H)^+$, 264 $(M + NH_4)^+$.

2-Amino-9-phenyl-6,7,8,9-tetrahydro-5*H*-cyclohepta[*d*]pyrimidin-4-ol. A solution of methyl 2-oxo-3-phenylcycloheptanecarboxylate (1.06 g, 4.3 mmol) in DMF (5 mL) was treated with guanidine nitrate (1.19 g, 8.6 mmol) followed by the addition of potassium carbonate (1.19 g, 8.6 mmol). The reaction mixture was stirred at 110 °C for 16 h, then cooled to ambient temperature, diluted with water (40 mL), and neutralized to pH 6 with acetic acid. The aqueous layer was extracted with CH_2Cl_2, and the organic layer was separated, dried over $MgSO_4$, and concentrated in vacuo. The obtained residue was chromatographed on silica gel, eluting with 10% MeOH/CH_2Cl_2/1% NH_4OH. ^{1}H NMR (DMSO-d_6) δ 10.76 (br, 1H), 7.16−7.32 (m, 5H), 6.17 (br, 2H), 3.99 (dd, J = 7.3 Hz, 3.2 Hz, 1H), 2.73 (m, 1H), 2.15 (m, 2H), 1.91 (m, 1H), 1.25−1.62 (m, 4H). MS (DCI^+) m/z 256 $(M + H)^+$.

2-Amino-9-phenyl-6,7,8,9-tetrahydro-5*H*-cyclohepta[*d*]pyrimidin-4-yl-4-methylbenzenesulfonate. A solution of 2-amino-9-phenyl-6,7,8,9-tetrahydro-5*H*-cyclohepta[*d*]pyrimidin-4-ol (0.48 g, 1.88 mmol) in methylene chloride (20 mL) was reacted with *p*-toluenesulfonyl chloride (0.72 g, 3.76 mmol), a catalytic amount of *N*,*N*-dimethylaminopyridine, and triethylamine (0.78 mL, 5.64 mmol). The reaction mixture was stirred at ambient temperature for 16 h, then diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ solution. The organic layer was separated, dried over $MgSO_4$, concentrated under vacuum, and purified by chromatography, eluting with 30% EtOAc/hexane to yield 0.61 g of the product as a solid. ^{1}H NMR ($CDCl_3$) δ 7.95 (d, J = 8.5 Hz, 2H), 7.36 (d, J = 8.1 Hz, 2H), 7.19−7.26 (m, 3H), 7.06 (m, 2H), 4.73 (br, 2H), 4.27 (dd, J = 8.1 Hz, 2.4 Hz, 1H), 2.70 (m, 1H), 2.56 (m, 1H), 2.47 (s, 3H), 2.20 (m, 1H), 2.02 (m, 1H), 1.79 (m, 2H), 1.50−1.62 (m, 2H). MS (ESI^+) m/z 410 $(M + H)^+$.

9-Phenyl-4-(piperazin-1-yl)-6,7,8,9-tetrahydro-5*H*-cyclohepta[*d*-]pyrimidin-2-amine Dihydrochloride (14). A mixture of 2-amino-9-phenyl-6,7,8,9-tetrahydro-5*H*-cyclohepta[*d*]pyrimidin-4-yl 4-methylbenzenesulfonate (0.55 g, 1.3 mmol), *tert*-butyl piperazine-1-carboxylate (0.3 g, 1.6 mmol), and triethylamine (0.37 mL, 2.7 mmol) in 2-methoxyethanol (4 mL) was heated at 110 °C for 16 h. The reaction mixture was diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ solution. The organic layer was separated, dried over $MgSO_4$, and concentrated under vacuum. The residue was purified by chromatography on silica gel, eluting with 5% MeOH/CH_2Cl_2 to provide 0.45 g of the Boc-protected intermediate. This intermediate (0.45 g, 1.06 mmol) was dissolved in methanol (5 mL) and treated with 4-N HCl/dioxane solution at reflux for 2 h. The reaction mixture was concentrated under vacuum, then triturated with EtOAc/diethyl ether to yield 0.3 g of the title compound. ^{1}H NMR of the free base (CD_3OD) δ 7.31−7.46 (m, 3H), 7.25 (m, 2H), 4.37 (dd, J = 8.1 Hz, 2.6 Hz, 1H), 3.82−3.97 (m, 4H), 3.34−3.45 (m, 4H), 2.69 (m, 2H), 2.26−2.40 (m, 2H), 1.71−1.85 (m, 4H). MS (ESI^+) m/z 324 $(M + H)^+$. Anal. ($C_{19}H_{25}N_5 \cdot 2HCl \cdot 0.75H_2O$) C, H, N.

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Supporting Information Available: Combustion analysis data for new described compounds; in vitro selectivity data; PK profile in mice for compounds **1** and **2** and metabolites **4** and **5**; structures with dihedral angles and in vitro functional data; preparation of biological reagents and materials; methods for the in vitro assay for the histamine H_4 receptor FLIPR Ca^{2+}-flux functional assay, the competition binding assay, and the native cell assay for histamine induced shape change in bone marrow derived mast cells; in vivo methods for pharmacologic blockade of H_4 agonist-induced itch, for peritonitis inflammation assay; in vivo methods for in pain assays; and antinociceptive efficacy data on compound **12** in additional models. This material is available free of charge via the Internet at http://pubs.acs.org.

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