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Optimization of 5-Pyridazin-3-one Phenoxypropylamines as Potent, Selective Histamine H_3 Receptor Antagonists with Potent Cognition Enhancing Activity

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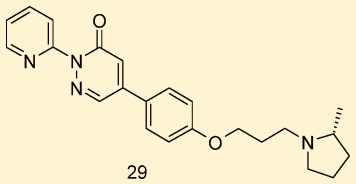
ABSTRACT: Previous studies have shown that (5-{4-[3-(*R*)-2-methylpyrrolin-1-yl-propoxy]phenyl}-2*H*-pyridazin-3-one) **2** had high affinity for both the human (hH_3R K_i = 2.8 nM) and rat H_3Rs (rH_3R K_i = 8.5 nM) but displayed low oral bioavailability in the rat. Optimization of the 5-pyridazin-3-one R^2 and R^6 positions to improve the pharmacokinetic properties over **2** led to the identification of 5-{4-[3-(*R*)-2-methylpyrrolidin-1-yl)propoxy]-phenyl}-2-pyridin-2-yl-2*H*-pyridazin-3-one **29**. Compound **29** displayed high affinity for both human and rat H_3Rs (hH_3R K_i = 1.7 nM, rH_3R K_i = 3.7 nM) with a greater than 1000-fold selectivity over the other histamine receptor subtypes and favorable pharmacokinetic properties across species (*F* = 78% rat, 92% dog, 96% monkey). It showed low binding to human plasma proteins, weakly inhibited cytochrome P450 isoforms, and displayed an excellent safety profile for a CNS-active compound. **29** displayed potent H_3R antagonist activity in the brain in a rat dipsogenia model and demonstrated enhancement of cognitive function in a rat social recognition model at low doses. However, the development of compound **29** was discontinued because of genotoxicity.



INTRODUCTION

Histamine elicits physiological responses mediated by four G-protein-coupled receptors (H_1R–H_4R) and exerts a variety of functions in the central nervous system (CNS).[1] H_1 and H_2 receptors in the periphery are involved in allergic response and gastric acid secretion, respectively, and have been some of the more successful drug target classes over the past 50 years.[1] The H_4 receptor is expressed mainly in mast cells, eosinophils, and tissues involved in the immune response and may play a role in inflammation and pain.[2] The H_3 receptor (H_3R) in the brain is primarily localized presynaptically, where it functions both as an autoreceptor to modulate histamine release and as an inhibitory heteroreceptor regulating the release of multiple neurotransmitters, including acetylcholine, dopamine, norepinephrine, and serotonin.[3] Activation of the H_3R results in the inhibition of neurotransmitter release, and blockade by selective antagonists or inverse agonists reverses the histamine-mediated inhibition, leading to enhanced neurotransmitter release. On the basis of localization and function, the discovery of H_3Rs antagonists remains an area of intense research, with therapeutic potential in addressing a variety of CNS disorders associated with attention and cognitive deficits, including deficits in wakefulness, attention-deficit hyperactivity disorder (ADHD), Alzheimer's disease (AD), mild cognitive impairment, and schizophrenia.[4] We identified a novel class of pyridazin-3-one H_3R antagonists/inverse agonists and reported 6-{4-[3-(*R*)-2-methylpyrrolidin-1-yl)propoxy]phenyl}-2*H*-pyridazin-3-one (**1**, irdabisant; CEP-26401) as a potent H_3R antagonist with excellent druglike properties and in vivo activity that recently completed phase I clinical evaluation (Figure 1).[5] As part of our H_3 discovery project studying the structure–activity relationships (SAR) around **1** we synthesized and reported the profile of the 5-regiomer **2** (5-{4-[3-(*R*)-2-methylpyrrolin-1-yl)propoxy]phenyl}-2*H*-pyridazin-3-one). Compound **2** had high affinity for both the human (hH_3R K_i = 2.8 nM) and rat H_3Rs (rH_3R K_i = 8.5 nM) but displayed low oral bioavailability in the rat.[5] The main objective, and the subject of this paper, was to optimize the 5-pyridazin-3-one core to improve the pharmacokinetic (PK) properties and identify potential backup compounds meeting discovery flow criteria with in vivo efficacy comparable to **1**.

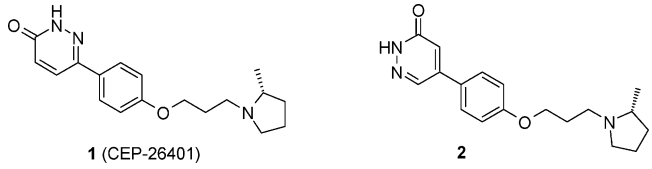


Figure 1. Pyridazinone H_3R antagonists.

CHEMISTRY

The 6*H*-5-aryl-2*H*-pyridazin-3-one derivatives were synthesized using two methods as shown in Schemes 1 and 2. The synthesis of the 6*H*-5-pyridazin-3-one series (Scheme 1) commenced with the alkylation of 4-(2-hydroxyethyl)phenol **3** with 1-bromo-3-chloropropane to provide 2-[4-(3-chloropropoxy)phenyl]-ethanol **4**, which underwent Dess–Martin oxidation to give aldehyde 5. Intermediate **5** was reacted with glyoxalic acid monohydrate in the presence of morpholine hydrochloride in aqueous dioxane,[6] followed by cyclization with hydrazine or a N-substituted hydrazine to produce the key 5-[4-(3-chloropropoxy)-phenyl]pyridazin-3-one intermediate **7**. Reaction of chloro **7** with

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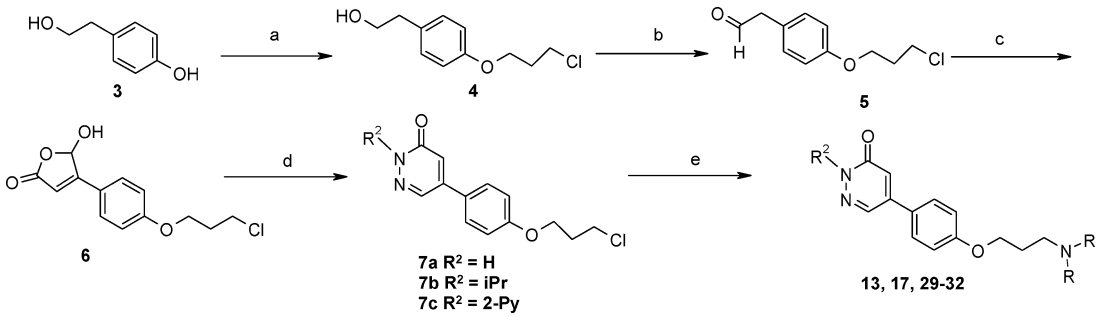
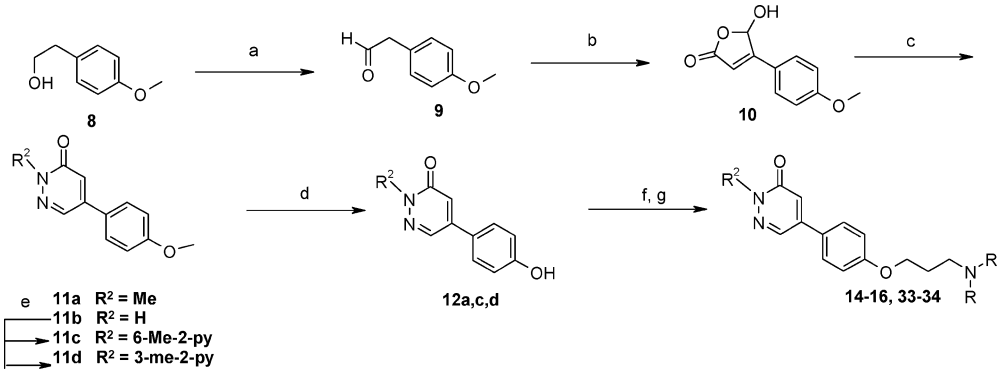
Scheme 1[a]



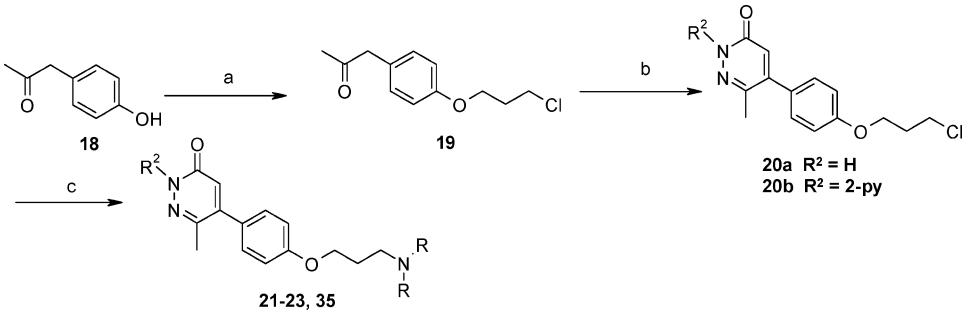
[a]Reagents and conditions: (a) $ClCH_2CH_2CH_2Br$, K_2CO_3, acetone, 60 °C, 98%; (b) Dess−Martin [O], 79%; (c) glyoxalic acid hydrate, reflux, morpholine·HCl salt, dioxane/H_2O, 98%; (d) $RNHNH_2$ (**7a**, NH_2NH_2; **7b**, *i*-$PrNHNH_2$; **7c**, 2-$PyNHNH_2$), AcOH, 100 °C, 58−73%; (e) amine, NaI, K_2CO_3, CH_3CN, 80 °C, 50−80%.

Scheme 2[a]



[a]Reagents and conditions: (a) Dess−Martin [O], CH_2Cl_2, 34%; (b) glyoxalic acid hydrate, reflux, morpholine·HCl salt, dioxane/H_2O, 99%; (c) $RNHNH_2$ (**11a**, $MeNHNH_2$; **11b**, NH_2NH_2), AcOH, 110 °C, 46−87%; (d) BBr_3, CH_2Cl_2, 0 °C to toom temp, 95%; (e) aryl bromide, CuI, K_2CO_3, DMF, 120 °C; (f) $ClCH_2CH_2CH_2Br$, K_2CO_3, acetone, 70 °C, 80−97%; (g) amine, NaI, K_2CO_3, CH_3CN, 80 °C, 30−80%.

Scheme 3[a]



[a]Reagents and conditions: (a) $ClCH_2CH_2CH_2Br$, K_2CO_3, acetone/DMF, 80 °C, 93%; (b) (i) glyoxalic acid hydrate, 135 °C; (ii) $RNHNH_2$, EtOH, 80 °C, 20−50% in two steps; (c) amines, NaI, K_2CO_3, CH_3CN, 80 °C, 30−80%.

various amines produced target compounds **13**, **17**, and **29−32**. Alternatively, as shown in Scheme 2, oxidation of **8** to **9** followed by the glyoxalic acid monohydrate aldol/hydrazine or methylhydrazine condensation sequence provided **11a,b**. Copper(I) mediated coupling of 5-(4-methoxyphenyl)-2*H*-pyridazin-3-ones **11b** with an appropriate pyridyl bromide gave the *N*-aryl intermediates **11c−d**.[7] BBr_3 deprotection of **11a**, **11c**, and **11d** to **12a**, **12c**, **12d**, alkylation and amine displacement as described previously gave **14−16**, **33**, and **34**. The 5-aryl-6-methyl-2*H*-pyridazin-3-one analogues **21−23** and **35** were synthesized starting from 1-(4-hydroxyphenyl)propan-2-one **18** (Scheme 3). Conversion of **18** to 4-(3-chloropropoxy)phenylpropan-2-one **19** followed by the glyoxalic acid/hydrazine procedure afforded the chloropropoxy 6-methyl-2*H*-pyridazin-3-one intermediates **20a,b**. Amine displacement of chlorides **20a,b** using standard conditions gave final targets **21−23** and **35**. The 6-methyl analogues were synthesized starting with 4-methoxyphenylacetone **24** as shown in Scheme 4. Reaction of **24** with glyoxalic acid hydrate at 135 °C to the hemiacetal followed by cyclization with hydrazine or methyl hydrazine produced pyridazinones **25a,b**. Copper(I) mediated coupling of 5-(4-methoxyphenyl)-6-methyl-2*H*-pyridazin-3-one **25b** with 3-methyl-2-pyridyl bromide as described previously gave the *N*-aryl intermediate **25c**. Demethylation of **25a** and **25c** to phenol intermediates **26a** and **26c** followed by installation of the propylamine side chain using standard conditions produced **27**, **28**, and **36**.

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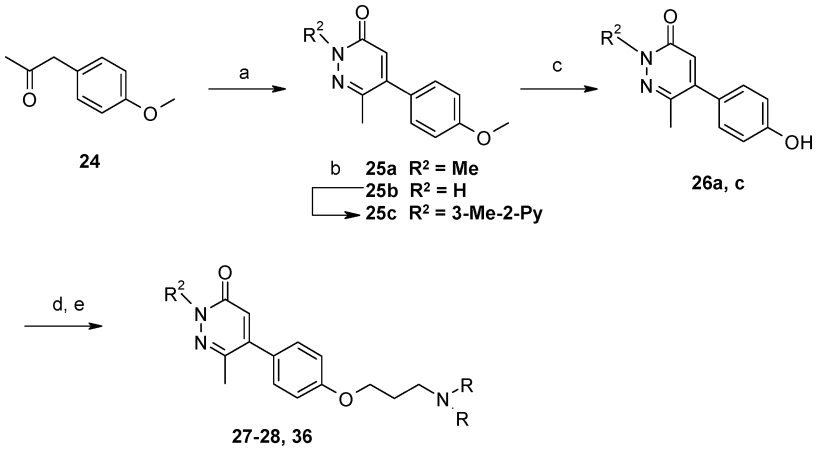
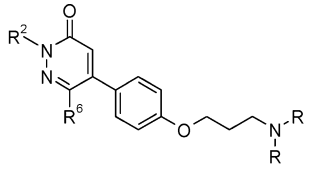
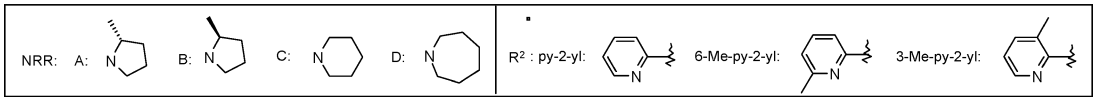
Scheme 4[a]



[a]Reagents and conditions: (a) (i) glyoxalic acid hydrate, 135 °C; (ii) $RNHNH_2$, EtOH, 80 °C, 20−50% in two steps of **25a** and **25b**; (b) 2-bromo-3-methylpyridine, CuI, K_2CO_3, DMF, 120 °C; (c) BBr_3, CH_2Cl_2, 0 °C to room temp, 21%; (d) $ClCH_2CH_2CH_2Br$, K_2CO_3, acetone/DMF, 80 °C, ∼95−98%; (e) amines, NaI, K_2CO_3, CH_3CN, 80 °C, 30−80%.

Table 1. 5-Pyridazin-3-one in Vitro Binding and PK Data





compd	R^2	R^6	NRR	K_i, nM hH_3	K_i, nM rH_3	clogP[e]	rat pharmacokinetic parameters[a] iv $t_{1/2}$	CL	F, %	B/P[b]
1				2.0 ± 0.4	8.5 ± 2.4	2.3	2.6	42	83[c]	2.6
2	H	H	A	2.8 ± 0.8	8.5 ± 2.4	2.1	1.0 ± 0.1	9.6 ± 1.7	24 ± 2[c]	1.8 ± 0.2
13	H	H	C	19 ± 5.1	28 ± 3.0	2.3	1.6 ± 0.1	24 ± 2	42 ± 3	3.0 ± 0.1
14	Me	H	A	1.9 ± 0.0	5.5 ± 0.7	2.6	1.0 ± 0.1	32 ± 3	19 ± 1	3.3 ± 0.2
15	Me	H	C	13 ± 1.3	30 ± 4.9	2.8	0.6 ± 0.0	75 ± 16	40 ± 9	5.3 ± 0.3
16	Me	H	D	8.1 ± 2.0	22 ± 4.0	3.3	*d*			
17	*i*-Pr	H	A	2.7 ± 0.6	7.0 ± 2.9	3.4	0.3 ± 0.0	12 ± 2	1 ± 1	5.1 ± 0.4
21	H	Me	A	1.6 ± 0.3	5.1 ± 1.3	2.3	1.4 ± 0.1	25 ± 1	20 ± 1	1.6 ± 0.1
22	H	Me	C	7.9 ± 1.1	23 ± 4	2.5	0.8 ± 0.1	30 ± 5	28 ± 2	1.3 ± 0.2
23	H	Me	D	4.8 ± 0.4	7.5 ± 1.9	3	1.0 ± 0.1	54 ± 12	25 ± 2	1.2 ± 0.1
27	Me	Me	A	2.0 ± 0.2	6.5 ± 0.8	2.8	1.2 ± 0.1	67 ± 13	53 ± 8	1.7 ± 0.1
28	Me	Me	C	8.9 ± 0.7	18 ± 3	2.3	1.6 ± 0.1	48 ± 3	69 ± 10	2.4 ± 0.1
29	Py-2-yl	H	A	1.7 ± 0.2	3.7 ± 0.6	2.6	1.7 ± 0.1	19 ± 2	78 ± 10[c]	1.1 ± 0.2
30	Py-2-yl	H	B	11 ± 1	29 ± 7	2.6	2.8 ± 0.2	45 ± 1	52 ± 5	1.7 ± 0.1
31	Py-2-yl	H	C	7.8 ± 0.6	16 ± 2	2.8	1.6 ± 0.1	11 ± 1	26 ± 3	1.6 ± 0.1
32	Py-2-yl	H	D	5.3 ± 0.5	12 ± 2	3.4	1.8 ± 0.1	25 ± 7	28 ± 1	1.8 ± 0.0
33	6-Me-Py-2-yl	H	A	1.8 ± 0.4	4.4 ± 1.9	3.1	2.2 ± 0.8	23 ± 5	12 ± 2	1 ± 0.1
34	3-Me-py-2-yl	H	A	2.5 ± 0.5	8.6 ± 2.7	3.3	1.8 ± 0.1	34 ± 8	30 ± 0	0.2 ± 0.2
35	Py-2-yl	Me	A	1.7 ± 0.0	5.0 ± 1.0	2.8	1.3 ± 0.2	13 ± 1	15 ± 2	0.7 ± 0.1
36	3-Me-Py-2-yl	Me	A	3.0 ± 0.5	17 ± 6	3.3	1.4 ± 0.2	7 ± 1	20 ± 5	0.5 ± 0.03

[a]Administration at 1 mg/kg iv and 5 mg/kg po. PK methods (rat, $n = 3$) were previously published, and the errors in the table are SEM.[5] [b]B/P = brain to plasma ratio and measured 1 h after a 10 mg/kg ip dose. The B/P ratio is calculated from total brain and plasma concentration. [c]10 mg/kg po administration and calculated from 24 h AUC values. [d]Not determined. [e]Tripos program used for clogP calculation.

RESULTS AND DISCUSSION

The substituted 5-pyridazinone analogues were tested using in vitro binding assays by displacement of [^{3}H]*N*-α-methylhistamine ([^{3}H]NAMH) in membranes isolated from CHO cells transfected with cloned human H_3 or rat H_3 receptors.[5,8,13b] The binding data in comparison with **2** is shown in Table 1.[5]

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We previously reported that the 5-pyridazin-3-one regiomer **2** had high affinity (hH_3R K_i = 2.8 nM, rH_3R K_i = 8.5 nM) comparable with 6-pyridazin-3-one regiomer **1** but displayed low oral bioavailability in the rat (F = 24%).[5] The initial objective was to explore the SAR at the amine, as well as positions R^2 and R^6 of the pyridazinone, with the goal of improving oral bioavailability while retaining affinity. Replacing the (*R*)-2-methylpyrrolidine with piperidine (**13**) showed a greater than 6-fold decrease in hH_3R affinity with a slight improvement on oral bioavailability (F = 42%). Exploration of the *N*-R^2 SAR with methyl **14** and isopropyl **17** also showed similar affinities compared to **2**, indicating the R^2 position could tolerate substitution. However, these alkyl substitutions at R^2 also showed higher clearance and/or lower oral bioavailability in rats. Changing the (*R*)-2-methylpyrrolidine on *N*-methyl **14** to piperidine **15** or azepine **16** showed 4- to 7-fold weaker hH_3R affinity, similar to **13**. In a rat PK screening, piperidine **15** suffered from a short iv half-life ($t_{1/2}$ = 0.6 h) and high clearance (75 mL min^{-1} kg^{-1}) (Table1).

Further pyridazinone ring modification was made by incorporating a 6-methyl, designed to increase the torsional angle between the two rings. Encouraging was the 2*H*-6-methyl-(*R*)-2-methylpyrrolidine **21** (hH_3 K_i = 1.6 nM, rH_3 K_i = 5.1 nM) that displayed high affinity for both human and rat H_3R and also retained a PK profile ($t_{1/2}$ = 1.4 h, F = 20%, B/P = 1.6) similar to **2**. The piperidine **22** and azepine **23** and the *N*-methyl analogues **27** and **28** also retained high affinity for hH_3R with comparable PK, although the *N*-methyl compounds tended to show higher CL values.

On the basis of these initial binding data and PK, the design strategy was adjusted to focus on the synthesis of metabolically stable R^2 analogues and at the same time to identify modifications that did not increase the log *P*. Amphiphilic, high log *P* compounds are known to enhance hERG activity and drive high tissue distribution and induction of phospholipidosis.[9] Early in the program we established a maximum clogP value of ~3 based on correlations with hERG activity and the propensity for high tissue distribution.[5] On the basis of this design strategy and goal, the R^2 2-pyridyl was synthesized for proof of concept. The resulting (*R*)-2-methylpyrrolidine R^2 2-pyridyl **29** retained high affinity for both human and rat H_3Rs (hH_3R K_i = 1.7 nM, rH_3R K_i = 3.7 nM) with clogP < 3 (2.6). Encouraging was the fact that **29** showed a favorable rat PK profile (iv $t_{1/2}$ = 1.7 h, CL = 19 mL min^{-1} kg^{-1}, F = 78%) and acceptable brain exposure (brain concentration 1 h following a 10 mg/kg ip dose = 1.9 μM; brain to plasma ratio B/P = 1.1).

The *R*-isomer was preferred in the N^2-2-pyridine series, since the *S*-isomer **30** (hH_3R K_i = 11 nM, rH_3R K_i = 29 nM) had 6- to 7-fold weaker affinity compared to **29**. Replacing the (*R*)-2-methylpyrrolidine with piperidine (**31**; hH_3R K_i = 7.8 nM, rH_3R K_i = 16 nM) or azepine (**32**; hH_3R K_i = 5.3 nM, rH_3R K_i = 12 nM) also retained high affinity and showed acceptable but lower oral bioavailability compared to **29** (see Table 1). The methyl substituted pyridines **33** and **34** were synthesized and both showed high affinity but suffered from lower oral bioavailibility, in the case of the 6-methyl **33**, and low brain penetration based on its brain to plasma ratio for the 3-methyl **34**. Combining the 6-methyl with the R^2 2-pyridyl resulted in **35**, which showed significantly lower oral bioavailability compared to 6-H compound **29**. Also, combining the 6-methyl with the R^2 3-methylpyridyl generated **36**, which did not improve the low brain penetration observed with **34**.

Compound **29** met the discovery criteria and objective for target affinity with improved rat pharmacokinetics and was selected for further profiling as a potential candidate. Functional activity was measured using the [^{35}S]GTPγS binding assay[8,13b] and displayed potent antagonist and also full inverse agonist activity. **29** potently inhibited RAMH-induced [^{35}S]-GTPγS binding at recombinant rH_3R (K_b = 0.3 ± 0.3 nM) and hH_3R (K_b = 0.3 ± 0.1 nM) and decreased basal activity with EC_{50} values of 1.6 ± 0.4 nM and 1.1 ± 0.3 nM for rat and human, demonstrating potent inverse agonist activity. Further, **29** displayed high in vitro metabolic stability across species in liver microsomes ($t_{1/2}$ > 40 min in mouse, rat, dog, and human) and IC_{50} > 30 μM for inhibition of cytochrome P450 enzymes CYP1A2, 2C9, 2C19, 2D6, and 3A4, indicating minimal potential for drug−drug interactions. **29** also had low CYP3A4 induction (3.3-fold) at concentrations up to 30 μM.[10] Selectivity profiling showed **29** had >1000-fold selectivity against hH_1, hH_2, and hH_4 receptor subtypes and against a panel of 172 GPCRs, ion channels, and enzymes. It also had acceptable hERG selectivity (IC_{50} = 9 μM)[5]and displayed good druglike properties with low lipophilicity (clogP = 2.6), high permeability in the Caco-2 assay (P_{app} = 13.7 × 10^{-6} cm/s), and high water solubility (1.4 at pH 2 and 0.9 mg/mL at pH 7.4). **29** was minimally bound to plasma proteins (rat 45%, dog 32%, and human 44%), and the unbound fraction in rat brain homogenate was high (39%), comparable with the free fraction found in rat plasma.[11,5]

The interspecies pharmacokinetic properties of **29** were further studied in dog and monkey (Table 2). **29** in rat showed an iv $t_{1/2}$ of 1.7 h, oral bioavailability of 78%, and clearance of 20 mL min^{-1} kg^{-1}. **29** showed favorable pharmacokinetic parameters in dog with nice iv intrinsic properties and high oral bioavailability (iv $t_{1/2}$ = 3.6 h, CL = 19 mL min^{-1} kg^{-1}, V_d = 5.9, F = 92%, C_{max} = 417 ng/mL). In the primate it showed a half-life of 3.9 h, with moderate clearance (CL = 21 mL min^{-1} kg^{-1}) and volume of distribution (V_d = 7.1 L/kg). The oral bioavailability following po administration at 3 mg/kg was 96%.

Table 2. Interspecies Pharmacokinetics for 29

	rat[a]	dog[b]	monkey[b]
iv $t_{1/2}$ (h)	1.7 ± 0.2	3.6 ± 0.1	3.9 ± 0.4
V_d (L/kg)	3.0 ± 0.9	5.9 ± 0.5	7.1 ± 0.7
CL (mL min^{-1} kg^{-1})	20 ± 3	19 ± 2	21 ± 3
po AUC (ng·h/mL)	6797 ± 203	2403 ± 308	2076 ± 215
C_{max} (ng/mL)	898 ± 28	417 ± 4	326 ± 62
$t_{1/2}$ (h)	4.5 ± 0.1	0.8 ± 0.2	6.5 ± 1.5
F (%)	78 ± 2	92 ± 13	96 ± 20
B/P[c]	1.1 ± 0.2	*d*	*d*

[a]Administration at 1 mg/kg iv and 10 mg/kg po. Parameters were calculated from composite mean plasma concentration−time data (rat, n = 3). [b]Administrated at 1 mg/kg iv and 3 mg/kg po for dog and monkey. Parameters were calculated from composite mean plasma concentration−time data for individual animals (dog, n = 3; monkey, n = 3). [c]Measured 1 h after a 10 mg/kg ip dose. [d]Not determined.

The rat dispogenia model was used as an in vivo surrogate measure of H_3R functional inhibition in the brain following peripheral administration. Histamine and the H_3-selective agonist, *R*-α-methylhistamine (RAMH), induce water drinking in the rat when administered either peripherally or centrally, an effect that is blocked by H_3R antagonists.[12,5] Activity in this model may be predictive of efficacy in cognitive models.

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29 potently and dose-dependently inhibited RAMH-induced dispogenia with an ED_{50} of 0.07 mg/kg, po (Figure 2).

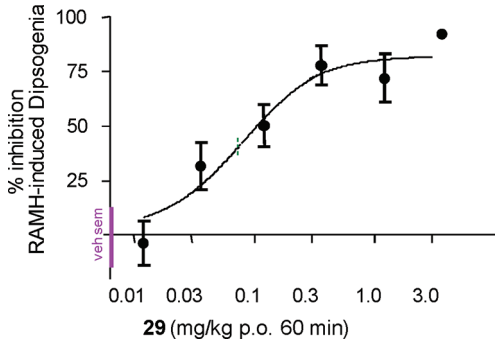

Figure 2. Compound **29** inhibition of RAMH-induced dipsogenia in Long Evans rats after oral dosing. Data represent the mean ± SEM of 8−32 animals/point.

Following the demonstration of potent in vivo H_3R functional activity in the brain, **29** was further evaluated for enhancement of short-term memory using the rat social recognition (SR) memory model, which tests the ability of agents to improve the retention of encounters between animals.[13] In this model, an adult rat is allowed to explore a juvenile rat for a short period of social investigation and then, after a 2 h separation, is reintroduced to the juvenile for a second investigation period. Social memory was quantified by determining the investigation ratio, which is the ratio of the time the adult rat spends investigating the juvenile in the second encounter divided by the time the adult spends investigating the juvenile during the first encounter. An agent that enhances memory will reduce the exploration time of the second encounter relative to the first and lower the investigation ratio. In this model **29** produced a significant decrease in the investigation ratio at 0.01 and 0.1 mg/kg po, respectively, suggesting an enhancement of short-term memory (Figure 3).

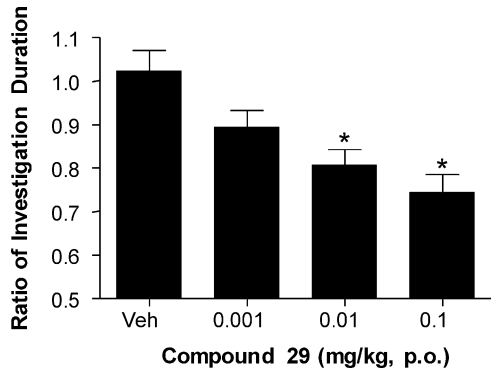

Figure 3. Activity of **29** in the social recognition model of short-term memory in rats. Vehicle (Veh) or **29** was administered 120 min prior to trial 2 in the rat social recognition model: (*) $p < 0.01$ (ANOVA, Dunnett's post hoc) from vehicle (mean ± SEM, n = 16−18/group).

CONCLUSION

The 5-pyridazin-3-one core was optimized to improve pharmacokinetic properties over **2** while retaining the H_3R potency and favorable druglike properties. From this effort 5-{4-[3-((*R*)-2-methylpyrrolidin-1-yl)propoxy]phenyl}-2-pyridin-2-yl-2*H*-pyridazin-3-one **29** was identified. Compound **29** had excellent druglike properties, H_3R target potency, selectivity, and pharmacokinetic properties across species (F = rat 78%, dog 92%, monkey 96%). It showed low binding to human plasma proteins, weakly inhibited cytochrome P450 isoforms, and displayed an excellent safety profile for a CNS-active compound in the Irwin test,[14] where it was well tolerated up to and including the dose of 300 mg/kg po. **29** displayed potent H_3R antagonist activity in the brain in the rat dipsogenia model and demonstrated enhancement of cognitive function in the rat social recognition model at low doses. The overall profile of **29** supported its potential use in the treatment of attentional and cognitive disorders. However, further preclinical safety genotoxicity testing gave a positive result in the Ames test, with and without S9 metabolic activation; therefore, further development was discontinued.

EXPERIMENTAL SECTION

Chemistry Methods. All reagents and anhydrous solvents were obtained from commercial sources and used as received. ^{1}H NMR spectra was obtained on a Bruker 400 MHz instrument with chemical shifts (δ, ppm) determined using TMS as internal standard. Coupling constants (J) are in hertz (Hz). Liquid chromatography−mass spectrometry (LCMS) was run on a Bruker Esquire 2000 ion trap spectrometer. Compound purity was >96% determined by high pressure liquid chromatography (HPLC) using a Zorbax RX-C8 5 mm × 150 mm column, eluting with a mixture of acetonitrile and water containing 0.1% trifluoroacetic acid with a gradient of 10−100%. Compounds were purified by silica gel chromatography using an ISCO graduate apparatus. Melting points were determined using a MEL-TEMP II and are uncorrected. Preparative chromatography was run using silica gel GF 20 cm ×20 cm × 1000 μm plates (Analtech).

2-[4-(3-Chloropropoxy)phenyl]ethanol (4). A mixture of 2-(4-hydroxyphenyl)ethanol (13.8 g, 100 mmol), **3**, and potassium carbonate (34 g, 250 mmol) in acetone (125 mL) was stirred as 1-bromo-3-chloropropane (24 g, 150 mmol) was added dropwise. The mixture was stirred at 60 °C overnight and then filtered through Celite, washed with acetone, and concentrated. The residue was dissolved in EtOAc (250 mL) and washed with 2 N Na_2CO_3, water, brine, dried over Na_2SO_4, and then concentrated to give 21 g (98%) of **4**. Mp 49−50 °C; LCMS m/z 215 (M + 1).

[4(3-Chloropropoxy)phenyl]acetaldehyde (5). A solution of Dess−Martin periodinane (20.4 g, 48 mmol) in methylene chloride (200 mL) was stirred as 2-[4-(3-chloropropoxy)ethanol **4** (8.6 g, 40 mmol) in methylene chloride (60 mL) was added dropwise. After being stirred at room temperature for 1 h, the mixture was diluted with ether (400 mL) and poured into 1.3 M NaOH (200 mL) solution. The ether layer was separated and washed with 1.3 M NaOH solution (100 mL) and water until pH 7 was obtained, then dried over Na_2SO_4. Flash chromatography with 20% Et_2O in hexane gave 6.8 g (79%) of **5**. LCMS m/z 213 (M + 1).

4-[4-(3-Chloropropoxy)phenyl]-5-hydroxy-5*H*-furan-2-one (6). A suspension of glyoxalic acid hydrate (3.0 g, 33 mmol) and morpholine hydrochloride (4.1 g, 33 mmol) in dioxane (48 mL) was stirred as water (4.5 mL) was added. To the homogeneous solution, [4-(3-chloropropoxy)phenyl]acetaldehyde **5** (6.7 g, 31.5 mmol) was added, and the solution was stirred at reflux for 24 h. The solvent was evaporated and the solid formed after addition of water (50 mL) was collected and washed with cold EtOH to give 8.3 g (98%) of **6**. LCMS m/z 269 (M + 1).

5-[4-(3-Chloropropoxy)phenyl)-2*H*-pyridazin-3-one (7a). A solution of 4-[4-(3-chloropropoxy)phenyl)-5-hydroxy-5*H*-furan-2-one **6** (2.0 g, 7.5 mmol) and hydrazine hydrate (0.59 g, 1.5 equiv) in EtOH (20 mL) was stirred at 85 °C for 2 h. The solvent was reduced and the solid that formed was collected and washed with cold EtOH to give 1.2 g (61%) of **7a**. Mp 197−199 °C; LCMS m/z 265 (M + 1).

5-[4-(3-Chloropropoxy)phenyl]-2-isopropyl-2*H*-pyridazin-3-one (7b). A solution of 4-[4-(3-chloropropoxy)phenyl)-5-hydroxy-5*H*-furan-2-one **6** (1.0 g, 3.7 mmol), isopropylhydrazine hydrate

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(0.82 g, 2.0 equiv), and sodium acetate (0.67 g, 2.2 equiv) in acetic acid (5.0 mL) was stirred at 110 °C overnight. The solid was removed by filtration, washed with dichloromethane, and the filtrate was concentrated. The residue was diluted with water (10 mL), extracted with ethyl acetate (30 mL), dried over Na_2SO_4, and concentrated. Flash chromatography with 75% EtOAc in hexane gave 0.72 g (63%) of **7b**. Mp 189−191 °C; LCMS *m*/*z* 307 (M + 1).

5-[4-(3-Chloropropoxy)phenyl)-2-pyridin-2-yl-2*H*-pyridazin-3-one (7c). A solution of 4-[4-(3-chloropropoxy)phenyl)-5-hydroxy-5*H*-furan-2-one **6** (8.0 g, 30 mmol) and 2-hydrazinopyridine (9.8 g, 3.0 mmol) in acetic acid (80 mL) was stirred at 110 °C for 24 h. The solvent was evaporated and the residue was purified with ISCO Combiflash chromatography with 2% MeOH in methylene chloride to give the product. Crystallization with EtOH and ether gave 5.9 g (58%) of **7c**. Mp 127−128 °C; LCMS *m*/*z* 342 (M + 1).

4-Methoxyphenylacetaldehyde (9). A solution of 4-methoxyphenylethanol **8** (15 g, 98 mmol) in methylene chloride (150 mL) was stirred at 0 °C as Dess−Martin periodinane (50 g, 1.2 equiv) was added in portions. The ice-bath was removed, and the mixture was stirred at room temperature for 1 h. The reaction mixture was then diluted with methylene chloride (100 mL), washed with 10% sodium thiosulfate, saturated $NaHCO_3$ solution, water, brine, and dried over Na_2SO_4. The product was purified by ISCO graduate chromatography (100% hexane to 20% EtOAc in hexane) to afford 5.3 g (34%) of 4-methoxyphenylacetaldehyde **9**. LCMS *m*/*z* 151 (M + 1).

5-Hydroxy-4-(4-methoxyphenyl)-5*H*-furan-2-one (10). A suspension of glyoxalic acid hydrate (2.5 g, 26.6 mmol) and morpholine hydrochloride (3.3 g, 26.4 mmol) in dioxane (25 mL) was stirred as water (2.0 mL) was added. To the homogeneous solution was added 4-methoxyphenylacetaldehyde **9** (3.8 g, 25.3 mmol), and the solution was stirred at reflux for 24 h. The solvent was evaporated and the solid that formed after addition of water (20 mL) was collected and washed with cold EtOH to give 5.1 g (98%) of **10**. LCMS *m*/*z* 207 (M + 1).

5-(4-Methoxyphenyl)-2-methyl-2*H*-pyridazin-3-one (11a). A suspension of 5-hydroxy-4-(4-methoxyphenyl)-5*H*-furan-2-one **10** (2.06 g, 10 mmol) in ethanol (30 mL) was stirred as methylhydrazine (0.78 mL, 1.5 equiv) was added in dropwise. After reflux at 85 °C for 2 h, the solvent was reduced and the solid was collected and washed with cold EtOH to give 1.0 g (46%) of **11a**. LCMS *m*/*z* 217 (M + 1).

5-(4-Methoxyphenyl)-2*H*-pyridazin-3-one (11b). A suspension of 5-hydroxy-4-(4-methoxyphenyl)-5*H*-furan-2-one **10** (2.0 g, 9.7 mmol) in ethanol (15 mL) was stirred as hydrazine hydrate (0.97 g, 2.0 equiv) was added in dropwise. After reflux at 85 °C overnight, the solvent was reduced and the solid was filtered and washed with cold EtOH to give 1.7 g (87%) of **11b**. LCMS *m*/*z* 203 (M + 1).

5-(4-Methoxyphenyl)-2-(6-methylpyridin-2-yl)-2*H*-pyridazin-3-one (11c). A mixture of **11b** (1.3 g, 6.6 mmol), copper(I) iodide (0.4 g, 2.0 mmol), and potassium carbonate (1.4 g, 9.9 mmol) in DMF (15 mL) was stirred and degassed with N_2 for 3 min. Then 2-bromo-6-methylpyridine (2.3 g, 13 mmol) was added dropwise and stirred at 120 °C overnight. The mixture was filtered through Celite and diluted with CH_2Cl_2 (50 mL). The CH_2Cl_2 layer was washed with 15% NH_4OH solution (45 mL), water, brine and then dried over Na_2SO_4. After concentration of the mixture, the residue was purified by ISCO chromatography (5% MeOH in CH_2Cl_2) to give **11c** (0.30 g, 15%). Mp 167−8 °C; LCMS *m*/*z* 294 (M + 1).

5-(4-Methoxyphenyl)-2-(3-methylpyridin-2-yl)-2*H*-pyridazin-3-one (11d). **11d** was synthesized from **11b** and 2-bromo-3-methylpyridine using the procedure for **11c**. Mp 155−157 °C; LCMS *m*/*z* 294 (M + 1).

5-(4-Hydroxyphenyl)-2-methyl-2*H*-pyridazin-3-one (12a). A solution of 5-(4-methoxyphenyl)-2-methyl-2*H*-pyridazin-3-one **11a** (0.95 g, 4.4 mmol) in 20 mL of CH_2Cl_2 was cooled to 0 °C, and BBr_3 (2.1 mL, 5.0 equiv) was added dropwise. The ice-bath was removed, and the mixture was stirred at room temperature for 1 h, after which the reaction mixture was poured into ice-cold saturated NH_4Cl (50 mL) with stirring. The resulting solid was filtered and washed with water (45 mL) and Et_2O (30 mL) to give the **12a**. Mp 296−8 °C; LCMS *m*/*z* 203 (M + 1).

5-(4-Hydroxyphenyl)-2-(6-methylpyridin-2-yl)-2*H*-pyridazin-3-one (12c). Compound **12c** was synthesized from **11c** using the procedure for compound **12a**. LCMS *m*/*z* 280 (M + 1).

5-(4-Hydroxyphenyl)-2-(3-methylpyridin-2-yl)-2*H*-pyridazin-3-one (12d). Compound **12d** was synthesized from **11d** using the procedure for compound **12a**. LCMS *m*/*z* 280 (M + 1).

5-[4-(3-Piperidin-1-ylpropoxy)phenyl]-2*H*-pyridazin-3-one (13). A mixture of 5-[4-(3-chloropropoxy)phenyl)-2*H*-pyridazin-3-one **7a** (264 mg, 1.0 mmol), K_2CO_3 (483 mg, 3.5 equiv), NaI (50 mg), and piperidine (170 mg, 2.0 equiv) in acetonitrile (10 mL) was heated to 75 °C for 24 h. The reaction mixture was then filtered, washed with CH_2Cl_2 (40 mL), and concentrated. The residue was dissolved in CH_2Cl_2 (30 mL) and washed with saturated $NaHCO_3$, brine, dried with Na_2SO_4, and concentrated. The residue was purified by preparative TLC (10% MeOH/90% CH_2Cl_2/0.5 mL 2-aminopropane) to give **13**. The free base was dissolved in MeOH (10 mL), and to the mixture was added 1 N HCl in EtOH (2.5 mL). Evaporation of the solvent and crystallization from MeOH−Et_2O gave the HCl salt of **13** (204 mg, 58%). Mp 249−250 °C (HCl salt); ^{1}H NMR (DMSO-d_6, δ): 13.04 (s, 1H), 10.1 (s, 1H), 8.29 (s, 1H), 7.80 (d, 2H, *J* = 9 Hz), 7.10 (m, 3H), 4.14 (t, 2H, *J* = 5 Hz), 3.45 (m, 2H), 3.19 (m, 2H), 2.90 (m, 2H), 2.20 (m, 2H), 1.69−1.81 (m, 5H), 1.38 (m, 1H); LCMS *m*/*z* 314 (M + 1).

2-Methyl-5-{4-[3-((*R*)-2-methylpyrrolidin-1-yl)propoxy]-phenyl}-2*H*-pyridazin-3-one (14). A mixture of 5-(4-hydroxyphenyl)-2-methyl-2*H*-pyridazin-3-one **12a** (808 mg, 4.0 mmol), K_2CO_3 (1.66 g, 3.0 equiv), and 1-bromo-3-chloropropane (592 μL, 1.2 equiv) in acetone (30 mL) was heated to 70 °C overnight. The mixture was cooled to room temperature, filtered, washed with acetone, and concentrated to dryness to afford 5-[4-(3-chloropropoxy)phenyl]-2-methyl-2*H*-pyridazin-3-one (1.08 g, 97%). Mp 90−91 °C; LCMS *m*/*z* 279 (M + 1).

Compound **14** was synthesized from 5-[4-(3-chloropropoxy)-phenyl]-2-methyl-2*H*-pyridazin-3-one and (*R*)-2-methylpyrrolidine using the procedure for **13**. Mp 222−223 °C (HCl salt, MeOH−ether); ^{1}H NMR (DMSO-d_6, δ) 10.25 (bs, 1H), 8.33 (s, 1H), 7.82 (d, 2H, *J* = 8.6 Hz), 7.16 (s, 1H), 7.09 (d, 2H, *J* = 8.6 Hz), 4.17 (m, 2H), 3.68 (s, 3H), 3.61 (m, 2H), 3.44 (m, 2H), 3.09 (m, 2H), 2.20 (m, 3H), 1.96 (m, 2H), 1.65 (m, 1H), 1.39 (d, 3H, *J* = 6.3 Hz); LCMS *m*/*z* 328 (M + 1).

2-Methyl-5-[4-(3-piperidin-1-ylpropoxy)phenyl]-2*H*-pyridazin-3-one (15). Compound **15** was synthesized from 5-[4-(3-chloropropoxy)phenyl]-2-methyl-2*H*-pyridazin-3-one (synthesized in the procedure for **14**) and piperidine using the procedure for compound **13**. Mp 253−254 °C (HCl salt, MeOH−ether); ^{1}H NMR (DMSO-d_6, δ) 10.24 (bs, 1H), 8.34 (s, 1H), 7.85 (d, 2H, *J* = 7.8 Hz), 7.16 (s, 1H), 7.07 (d, 2H, *J* = 7.8 Hz), 4.2 (m, 2H), 3.67 (s, 3H), 3.46 (m, 2H), 3.17 (m, 2H), 2.89 (m, 2H), 2.21 (m, 2H), 1.72−1.78 (m, 5H), 1.39 (m, 1H); LCMS *m*/*z* 328 (M + 1).

2-Methyl-5-[4-(3-azepan-1-ylpropoxy)phenyl]-2-methyl-2*H*-pyridazin-3-one (16). Compound **16** was synthesized from 5-[4-(3-chloropropoxy)phenyl]-2-methyl-2*H*-pyridazin-3-one (synthesized in the procedure for **14**) and hexamethyleneimine using the procedure for compound **13**. Mp 247−248 °C (HCl salt, MeOH−ether); ^{1}H NMR (DMSO-d_6, δ) 9.98 (bs, 1H), 8.33 (s, 1H), 7.83 (d, 2H, *J* = 8.5 Hz), 7.17 (s, 1H), 7.08 (d, 2H, *J* = 8.5 Hz), 4.14 (m, 2H), 3.69 (s, 3H), 3.39 (m, 2H), 3.27 (m, 2H), 3.15 (m, 2H), 2.18 (m, 2H), 1.83 (m, 4H), 1.62 (m, 4H); LCMS *m*/*z* 342 (M + 1).

2-Isopropyl-5-{4-[3-((*R*)-2-methylpyrrolidin-1-yl)propoxy}-2*H*-pyridazin-3-one (17). Compound **17** was synthesized from **7b** and (*R*)-2-methylpyrrolidine using the procedure for compound **13**. Mp 251−253 °C (HCl salt, MeOH−ether); ^{1}H NMR (DMSO-d_6, δ) 10.14 (s, 1H), 8.41 (s, 1H), 8.35 (d, 2H, *J* = 9 Hz), 7.10 (m, 3H), 5.18 (m, 1H), 4.17 (m, 2H), 3.64 (m, 1H), 3.43 (m, 2H), 3.10 (m, 2H), 2.28 (m, 3H), 1.96 (m, 2H), 1.64 (m, 1H), 1.39 (m, 3H), 1.32 (d, 6H, *J* = 7 Hz); LCMS *m*/*z* 356 (M + 1).

1-[4-(3-Chloropropoxyl)phenyl]propan-2-one (19). A solution of 4-hydroxyphenylacetone **18** (4.5 g, 30 mmol) and potassium carbonate (4.14 g, 3.0 equiv) in acetone (50 mL) was stirred under N_2 as 1-bromo-3-chloropropane (7.0 g, 4.5 mmol) was added dropwise. The mixture was heated to 80 °C overnight. The mixture was then

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filtered through Celite, washed with acetone, and concentrated to afford 1-[4-(3-chloropropoxy)phenyl]propan-2-one **23** (6.3 g, 93% yield). LCMS m/z 227 (M + 1).

5-[4-(3-Chloropropoxy)phenyl]-6-methyl-2*H*-pyridazin-3-one (20a). The mixture of **19** (6.3 g, 27.8 mmol) and glyoxalic acid hydrate (2.56 g, 27.8 mmol) was heated to 135 °C overnight. Then the water was distilled off at 120 °C for 2 h. The residue was taken up in ethanol (20 mL) with stirring as hydrazine hydrate (5.0 mL, 56 mmol) was added dropwise. The reaction was then heated to 85 °C overnight. The solvent was evaporated, and the residue was dissolved in methylene chloride (50 mL) and washed with 5% $NaHCO_3$ solution (40 mL) and brine, dried over Na_2SO_4, and concentrated. The residue was purified by ISCO graduate chromatography (2% MeOH in CH_2Cl_2) to give **20a** (2.65 g, 34%). Mp 170−172 °C; LCMS m/z 279 (M + 1).

5-[4-(3-Chloropropoxy)phenyl]-6-methyl-2-pyridin-2-yl-2*H*-pyridazin-3-one (20b). Compound **20b** was synthesized from **19** and 2-hydrazinopyridine using the procedure for **20a**. LCMS m/z 356 (M + 1).

6-Methyl-5-{4-[3-((*R*)-2-methylpyrrolidin-1-yl)propoxy]-phenyl}-2-(1-pyridazin-3-one) (21). Compound **21** was synthesized from **20a** and (*R*)-2-methylpyrrolidine benzenesulfonic acid salt using the procedure described for compound **13**. Mp 115 °C (dec) (HCl salt, MeOH−ether); ^{1}H NMR (DMSO-d_6, δ) 12.85 (s, 1H), 10.08 (bs, 1H), 7.44 (d, 2H, J = 7.9 Hz), 7.07 (d, 2H, J = 7.9 Hz), 6.65 (s, 1H), 4.16 (m, 2H), 3.64 (m, 1H), 3.45 (m, 2H), 3.09 (m, 2H), 2.18 (m, 3H), 2.17 (s, 3H), 1.98 (m, 2H), 1.64 (m, 1H), 1.39 (d, 3H, J = 6.8 Hz); LCMS m/z 328 (M + 1).

6-Methyl-5-[4-(3-piperidin-1-ylpropoxy)phenyl]-2*H*-pyridazin-3-one (22). Compound **22** was synthesized from **20a** and piperidine using the procedure for compound **13**. Mp 123 °C (dec) (HCl salt, MeOH−ether); ^{1}H NMR (DMSO-d_6, δ) 12.85 (s, 1H), 10.16 (s, 1H), 7.43 (d, 2H, J = 7.9 Hz), 7.07 (d, 2H, J = 7.9 Hz), 6.65 (s, 1H), 4.11 (m, 2H), 3.48 (m, 2H), 3.18 (m, 2H), 2.88 (m, 2H), 2.23 (m, 2H), 2.18 (s, 3H), 1.69−1.83 (m, 5H), 1.38 (m, 1H); LCMS m/z 328 (M + 1).

5-[4-(3-Azepan-1-ylpropoxy)phenyl]-6-methyl-2*H*-pyridazin-3-one (23). Compound **23** was synthesized from **20a** and hexamethyleneimine using the procedure for compound **13**. Mp 204−206 °C (HCl salt, MeOH−ether); ^{1}H NMR (DMSO-d_6, δ) 12.83 (s, 1H), 10.20 (s, 1H), 7.41 (d, 2H, J = 7.5 Hz), 7.04 (d, 2H, J = 7.5 Hz), 6.64 (s, 1H), 4.13 (m, 2H), 3.42 (m, 2H), 3.25 (m, 2H), 3.15 (m, 2H), 2.23 (m, 2H), 2.15 (s, 3H), 1.81 (m, 4H), 1.57−1.71 (m, 4H); LCMS m/z 342 (M + 1).

5-(4-Methoxyphenyl)-2,6-dimethyl-2*H*-pyridazin-3-one (25a). A mixture of 1-(4-methoxyphenyl)propan-2-one **24** (16.4 g, 100 mmol) and glyoxalic acid hydrate (9.20 g, 100 mmol) was heated to 135 °C overnight. Then the water was distilled off at 120 °C for 2 h. The residue was taken up in ethanol (40 mL) with stirring as methylhydrazine (10.5 mL, 200 mmol) was added dropwise. The mixture was heated to 85 °C overnight. The solvent was evaporated and the residue was dissolved in methylene chloride (200 mL) and washed with 5% $NaHCO_3$ solution (40 mL) and brine, dried over Na_2SO_4, and concentrated. The residue was purified by ISCO chromatography (2% MeOH in CH_2Cl_2) to give **25a** (4.2 g, 18%). LCMS m/z 231 (M + 1).

5-(4-Methoxyphenyl)-6-methyl-2*H*-pyridazin-3-one (25b). Compound **25b** was synthesized from **24** and glyoxalic acid hydrate and hydrazine hydrate using the procedure for **25a**. LCMS m/z 217 (M + H).

5-(4-Methoxyphenyl)-6-methyl-2-(3-methylpyridin-2-yl)-2*H*-pyridazin-3-one (25c). Compound **25c** was synthesized from **25b** and 2-bromo-3-methylpyridine using the procedure for **11c**. Mp 133−135 °C, LCMS m/z 308 (M + 1).

5-(4-Hydrophenyl)-2,6-dimethyl-2*H*-pyridazin-3-one (26a). A solution of **25a** (3.2 g, 14 mmol) in CH_2Cl_2 (40 mL) was cooled to 0 °C, and BBr_3 (6.6 mL, 5.0 equiv) was added dropwise. The ice bath was removed, and the mixture was stirred at room temperature for 1 h, after which the reaction mixture was poured into ice cold saturated NH_4Cl (50 mL) with stirring. The resulting solid was collected and washed with water (45 mL) and Et_2O (30 mL) to give **26a** (0.66 g, 22%). LCMS m/z 217 (M + 1).

5-(4-Hydroxyphenyl)-6-methyl-2-(3-methylpyridin-2-yl)-2*H*-pyridazin-3-one (26c). Compound **26c** was synthesized from **25c** using the procedure for compound **26a**. LCMS m/z 294 (M + 1).

2,6-Dimethyl-5-{4-[3-((*R*)-2-methylpyrrolidin-1-yl)propoxy]-phenyl}-2*H*-pyridazin-3-one (27). A mixture of **26a** (710 mg, 3.3 mmol), K_2CO_3 (1.36 g, 3.0 equiv), and 1-bromo-3-chloropropane (390 μL, 1.2 equiv) in acetone (30 mL) was heated to 80 °C overnight. The solids were removed by filtration, washed with acetone, and the combined filtrates were concentrated to dryness to afford 5-[4-(3-chloropropoxy)phenyl]-2,6-dimethyl-2*H*-pyridazin-3-one (950 mg, 98%). LCMS m/z 293 (M + 1).

A mixture of 5-[4-(3-chloropropoxy)-phenyl]-2,6-dimethyl-2*H*-pyridazin-3-one (481 mg, 1.65 mmol), K_2CO_3 (3.5 eq, 795 mg), NaI (50 mg), and (*R*)-2-methylpyrrolidine hydrochloride (2.0 equiv, 773 mg) in acetonitrile (10 mL) was heated to 80 °C for 2 days. The solids were removed by filtration, washed with CH_2Cl_2 (40 mL), and the combined filtrates were concentrated. The residue was dissolved in CH_2Cl_2 (30 mL) and washed with saturated $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. The residue was then purified by preparative TLC (10%MeOH/90% CH_2Cl_2/0.5 mL of 2-aminopropane) to give **27**. The free base was dissolved in MeOH (10 mL), and to the mixture was added 1 N HCl in EtOH (2.5 mL). Evaporation of the solvent and crystallization from MeOH/Et_2O afforded the HCl salt of **27** (231 mg, 41%). Mp 176−178 °C (HCl salt, MeOH−ether); ^{1}H NMR (DMSO-d_6, δ) 10.05 (bs, 1H), 7.45 (d, 2H, J = 8.7 Hz), 7.05 (d, 2H, J = 8.7 Hz), 6.70 (s, 1H), 4.15 (m, 2H), 3.65 (s, 3H), 3.50 (m, 2H), 3.10 (m, 2H), 2.11 (m, 3H), 2.10 (s, 3H), 1.98 (m, 3H), 1.52 (m, 1H), 1.37 (d, 3H, J = 4.5 Hz); LCMS m/z 342 (M + 1).

2,6-Dimethyl-5-[4-(3-piperidin-1-ylpropoxy)phenyl]-2*H*-pyridazin-3-one (28). Compound **28** was synthesized from 5-[4-(3-chloropropoxy)phenyl]-2,6-dimethyl-2*H*-pyridazin-3-one and piperidine using the procedure for compound **27**. Mp 210−211 °C (HCl salt, MeOH−ether); ^{1}H NMR (DMSO-d_6, δ) 9.85 (bs, 1H), 7.48 (d, 2H, J = 8.7 Hz), 7.05 (d, 2H, J = 8.7 Hz), 6.65 (s, 1H), 4.13 (t, 2H, J = 5.8 Hz), 3.60 (s, 3H), 3.47 (m, 2H), 3.11 (m, 2H), 2.81 (m, 2H), 2.14 (m, 2H), 2.15 (s, 3H), 1.55−1.68 (m, 5H), 1.48 (m, 1H); LCMS m/z 342 (M + 1).

5-{4-[3-(*R*)-2-Methylpyrrolidin-1-yl)propoxy]phenyl}-2-pyridin-2-yl-2*H*-pyridazin-3-one (29). A mixture of **7c** (5.8 g, 17.0 mmol) and (*R*)-2-methylpyrrolidine benzenesulfonic acid salt (12.0 g, 51.0 mmol), potassium carbonate (8.2 g, 59.0 mmol), and sodium iodide (50 mg, 0.334 mmol) in acetonitrile (150 mL) was heated to 80 °C for 24 h. The reaction mixture was then filtered, washed with methylene chloride (40 mL), and concentrated. The residue was dissolved in methylene chloride (100 mL) and washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and concentrated. The residue was purified by ISCO chromatography with 10% MeOH in CH_2Cl_2 with 0.5% of i-$PrNH_2$ to give the product. The free base was dissolved in MeOH, and to the mixture was added 1 N HCl in EtOH (40 mL). The mixture was concentrated to dryness. The salt was crystallized from MeOH and CH_3CN to give the HCl salt of **29** (5.45 g, 75%). Mp 219−220 °C; ^{1}H NMR (DMSO-d_6, δ) 10.29 (bs, 1H), 8.64 (d, 1H, J = 3.0 Hz), 8.52 (s, 1H), 8.05 (t, 1H, J = 7.9 Hz), 7.95 (d, 2H, J = 8.7 Hz), 7.65 (d, 1H, J = 6.9 Hz), 7.56 (m, 1H), 7.35 (s, 1H), 7.13 (d, 2H, J = 8.9 Hz), 4.22 (m, 2H), 3.62 (m, 1H), 3.43 (m, 2H), 3.09 (m, 2H), 2.21 (m, 3H), 1.95 (m, 2H), 1.65 (m, 1H), 1.40, (d, 3H, J = 6.3 Hz); LCMS m/z 391 (M + 1). Anal. Calcd for $C_{23}H_{27}ClN_4O_2 \cdot 0.1H_2O$: C, 64.43; H, 6.16; N, 13.07; Cl, 8.26. Found: C, 64.13; H, 6.29; N, 12.89; Cl, 8.55.

5-{4-[3-((*S*)-2-Methylpyrrolidin-1-yl)propoxy]phenyl}-2-pyridin-2-yl-2*H*-pyridazin-3-one (30). Compound **30** was synthesized from **7c** and (*S*)-2-methylpyrrolidine using procedures described for compound **29**. Mp 218−220 °C (HCl salt, MeOH−ether); ^{1}H NMR (DMSO-d_6, δ) 10.04 (bs, 1H), 8.64 (s, 1H), 8.54 (s, 1H), 8.05 (m, 1H), 7.92 (d, 2H, J = 7.9 Hz), 7.65 (d, 1H, J = 7.9 Hz), 7.56 (m, 1H), 7.35 (s, 1H), 7.13 (d, 2H, J = 9.0 Hz), 4.19 (m, 2H), 3.62 (m, 1H),

3.45 (m, 2H), 3.10 (m, 2H), 2.20 (m, 3H), 1.98 (m, 2H), 1.63 (m, 1H), 1.38 (d, 3H, J = 6 Hz); LCMS m/z 391 (M + 1).

5-[4-(3-Piperidin-1-ylpropoxy)phenyl-2-pyridin-2-yl-2*H*-pyridazin-3-one (31). Compound **31** was synthesized from **7c** and piperidine using the procedure for compound **29**. Mp 266−268 °C (HCl salt, MeOH−ether); ^{1}H NMR (DMSO-d_6, δ) 10.0 (bs, 1H), 8.61 (bs, 1H), 8.54 (s, 1H), 8.05 (t, 1H, J = 7.9 Hz), 7.95 (d, 2H, J = 7.9 Hz), 7.63 (d, 1H, J = 7.6 Hz), 7.54 (m, 1H), 7.34 (s, 1H), 7.11 (d, 2H, J = 7.9 Hz), 4.17 (t, 2H, J = 6 Hz), 3.49 (m, 2H), 3.21 (m, 2H), 2.90 (m, 2H), 2.21 (m, 2H), 1.69−1.81 (m, 5H), 1.41 (m, 1H); LCMS m/z 391 (M + 1).

5-[4-(3-Azepan-1-ylpropoxy)phenyl]-2-pyridin-2-yl-2*H*-pyridazin-3-one (32). Compound **32** was synthesized from **7c** and hexamethyleneimine using the procedure for compound **29**. Mp 230−232 °C (HCl salt, MeOH−ether); ^{1}H NMR (DMSO-d_6, δ) 10.10 (bs, 1H), 8.64 (bs, 1H), 8.53 (s, 1H), 8.04 (t, 1H, J = 6 Hz), 7.92 (d, 2H, J = 7.9 Hz), 7.65 (d, 1H, J = 7.9 Hz), 7.54 (m, 1H), 7.33 (s, 1H), 7.13 (d, 2H, J = 8.6 Hz), 4.17 (m, 2H), 3.41 (m, 2H), 3.26 (m, 2H), 3.14 (m, 2H), 2.21 (m, 2H), 1.83 (m, 4H), 1.56−1.71 (m, 4H); LCMS m/z 405 (M + 1).

2-(6-Methylpyridin-2-yl)-5-{4-[3-(*R*)-2-methylpyrrolidin-1-yl)propoxy]phenyl}-2*H*-pyridazin-3-one (33). Compound **33** was synthesized from 5-[4-(3-chloropropoxy)phenyl]-2-(6-methylpyridin-2-yl)-2*H*-pyridazin-3-one (prepared from **12c** and 1-bromo-3-chloropropane) and (*R*)-2-methylpyrrolidine benzenesulfonic acid salt using the procedure for compound **29**. Mp 118−120 °C; ^{1}H NMR (DMSO-d_6, δ) 8.51 (s, 1H), 7.89 (m, 3H), 7.40 (m, 2H), 7.28 (s, 1H), 7.10 (d, 2H, J = 9.0 Hz), 4.12 (m, 2H), 3.12 (m, 2H), 2.96 (m, 2H), 2.20 (s, 3H), 2.08 (m, 2H), 1.91 (m, 2H), 1.69 (m, 2H), 1.32 (m, 1H), 1.03 (d, 3H, J = 4.2 Hz); LCMS m/z 405 (M + 1).

2-(3-Methylpyridin-2-yl)-5-{4-[3-(*R*)-2-methylpyrrolidin-1-yl)propoxy]phenyl}-2*H*-pyridazin-3-one (34). Compound **34** was synthesized from 5-[4-(3-chloropropoxy)phenyl]-2-(3-methylpyridin-2-yl)-2*H*-pyridazin-3-one (prepared from **12d** and 1-bromo-3-chloropropane) and (*R*)-2-methylpyrrolidine benzenesulfonic acid salt using procedure for compound **29**. Mp 98−100 °C (HCl salt, MeOH−ether); ^{1}H NMR (DMSO-d_6, δ) 9.91 (bs, 1H), 8.55 (s, 1H), 8.45 (d, 1H, J = 6.8 Hz), 7.91−7.06 (m, 3H), 7.53 (m, 1H), 7.35 (m, 1H), 7.13 (d, 2H, J = 8.4 Hz), 4.19 (m, 2H), 3.64 (m, 1H), 3.45 (m, 2H), 3.11 (m, 2H), 2.19 (m, 3H), 2.14 (s, 3H), 1.96 (m, 2H), 1.63 (m, 1H), 1.39 (d, 3H, J = 6.1 Hz); LCMS m/z 405 (M+1).

6-Methyl-5-{4-[3-((*R*)-2-methylpyrrolidin-1-yl)propoxy]-phenyl}-2-pyridin-2-yl-2*H*-pyridazin-3-one (35). Compound **35** was synthesized from **20b** and (*R*)-2-methylpyrrolidine benzenesulfonic acid salt using procedure for compound **29**. Mp 155−157 °C (HCl salt, MeOH−ether); ^{1}H NMR (DMSO-d_6, δ) 10.14 (bs, 1H), 8.64 (d, 1H, J = 4.5 Hz), 8.03 (m, 1H), 7.62 (d, 1H, J = 8.0 Hz), 7.51 (m, 3H), 7.08 (m, 2H), 6.86 (d, 1H, J = 8.5 Hz), 4.14 (m, 2H), 3.64 (m, 1H), 3.45 (m, 2H), 3.09 (m, 2H), 2.27 (s, 3H), 2.20 (m, 2H), 1.96 (m, 2H), 1.65 (m, 2H), 1.40 (d, 3H, J = 6.8 Hz); LCMS m/z 405 (M + 1).

6-Methyl-2-(3-methylpyridin-2-yl)-5-{4-[3-(*R*)-2-methylpyrrolidin-1-yl)propoxy]phenyl}-2*H*-pyridazin-3-one (36). Compound **36** was synthesized from 5-[4-(3-chloropropoxy)phenyl]-6-methyl-2-(3-methylpyridin-2-yl)-2*H*-pyridazin-3-one (prepared from **26c** and 1-bromo-3-chloropropane) and (*R*)-2-methylpyrrolidine benzenesulfonic acid salt using the procedure for compound **29**. Mp 106 °C (dec) (HCl salt, MeOH−ether); ^{1}H NMR (DMSO-d_6, δ) 10.19 (bs, 1H), 8.45 (m, 1H), 7.92 (d, 1H, J = 7.0 Hz), 7.53 (d, 1H, J = 7.0 Hz), 7.51 (m, 2H), 7.10 (d, 2H, J = 8.0 Hz), 6.91 (s, 1H), 4.19 (m, 2H), 3.65 (m, 1H), 3.46 (m, 2H), 3.10 (m, 2H), 2.26 (s, 3H), 2.15 (s, 3H), 2.20 (m, 2H), 1.96 (m, 2H), 1.65 (m, 2H), 1.40(d, 3H, J = 6.0 Hz); MS m/z 419 (M + 1).

Radioligand Binding Assays. In vitro binding assays by displacement of [^{3}H]*N*-α-methylhistamine ([^{3}H]NAMH) in membranes isolated from CHO cells transfected with cloned human H_3 or rat H_3 receptors were run as described previously.[5,13b]

Pharmacokinetics. The routine pharmacokinetics experiments were performed as previously described.[5] Adult male Sprague−Dawley rats (275−350 g; Charles River, Kingston, NY), male beagle dogs (9−14 kg, Cephalon, Inc., Maisons Alfort, France), and male cynomolgus monkeys (2−4 kg, Covance Laboratories, Alice, TX) were used in the experiments. All animal usage was approved by the Cephalon IUCAC. For experiments to determine detailed rat PK parameters, rats were administered 1 mg/kg iv and 5 mg/kg po in saline and parameters calculated from composite mean plasma concentration−time data (rat, n = 3). Dogs and monkeys were administered at 1 mg/kg iv and 3 mg/kg po. Parameters were calculated from composite mean plasma concentration−time data for individual animals (dog, n = 3; monkey, n = 3).

In Vitro Functional Characterization by [^{35}S]GTPγS Binding Assay. Antagonist potency was determined by measuring inhibition of RAMH-induced [^{35}S]GTPγS binding in recombinant hH_3R and rH_3R membranes as described previously.[13b] An RAMH concentration of 100 nM was used, which produces approximately 80% of maximum RAMH-induced signal. Inverse agonist potency was determined by measuring inhibition of basal [^{35}S]GTPγS binding in recombinant hH_3R and rH_3R membranes. Test compound or vehicle was added to the wells, followed by [^{35}S]GTPγS to a final concentration of 0.2 nM. Nonspecific binding was determined in the presence of 10 μM unlabeled GTPγS. The control agonist signal was determined in wells containing vehicle in place of the test compound, and the basal signal was determined in wells containing vehicle in place of both diluted compound and the RAMH challenge. Ciproxifan and ABT-239 decreased basal [^{35}S]GTPγS binding in a concentration-dependent manner in the recombinant systems consistent with known inverse agonist activity of these compounds.[15]

Rat dipsogenia model. Rat dipsogenia was conducted as previously described.[5,13b] RAMH-induced water intake was measured in Harlan Long Evans rats (>300 g; Harlan, Dublin, VA, or Indianapolis, IN) for 30 min beginning 20 min after administration of RAMH (10 mg/kg ip). Test compound (in saline) was administered at the indicated times prior to the initiation of the drinking trial period. Percent inhibition of RAMH-induced drinking was calculated for each rat based on normalization to the group mean RAMH-induced drinking using the following equation: [100 − (Dr/Dg(RAMH)) × 100], where Dr is the amount of water an individual rat drinks and Dg is the group mean for the amount of water consumed by the RAMH-treated group. Group mean values for percent inhibition were then calculated for each dosage group together with the associated standard deviation and standard error of the mean. Treatment effects for percent inhibition vs RAMH-induced dipsogenia were evaluated using a one-way ANOVA (GraphPad Prism 4). Dunnett's post hoc analysis was performed for multiple comparisons with the RAMH group set as the control comparator.

Rat Social Recognition Model of Short-Term Memory. The effect of **29** on short-term memory was determined in a rat social recognition model as described previously[13b] using adult male (Sprague−Dawley, 350−450 g; Charles River Laboratories) and juvenile male rats (Sprague−Dawley 80−130 g; Charles River Laboratories). Briefly, adult rats were exposed to a male juvenile rat (trial 1) and, after a varying interexposure interval (IEI), the same juvenile rat was returned to the test box with the adult rat for a second exposure (trial 2). A 2 h IEI was used to test putative memory-enhancing compounds as memory of the juvenile (ratio of investigation duration (RID) near unity). Rats were dosed with **29** po or vehicle (pH 2 water) 120 min prior. Controls included separate groups of rats that received effective doses of compound and were subsequently exposed to a novel juvenile in trial 2 (data not shown). Treatment effects on the RID were evaluated using one-way ANOVA (Prism 4). Dunnett's post hoc analysis was performed for multiple comparisons with the vehicle group.

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■ ABBREVIATIONS USED

CNS, central nervous system; H_1R–H_4R, H_1–H_4 receptors; H_3R, H_3 receptor; ADHD, attention-deficit hyperactivity discorder; AD, Alzheimer's disease; SAR, structure–activity relationship; PK, pharmacokinetics; [^{3}H]NAMH, [^{3}H]*N*-α-methylhistamine; [^{35}S]GTPγS, guanosine 5′-(γ-thio)-triphosphate; RAMH, *R*-α-methylhistamine; hERG, human ether-a-go-go-related gene; SR, social recognition

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