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Synthesis and evaluation of 4- and 5-pyridazin-3-one phenoxypropylamine analogues as histamine-3 receptor antagonists

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ABSTRACT

A novel series of 4-pyridazin-3-one and 5-pyridazin-3-one analogues were designed and synthesized as H_3R antagonists. Structure–activity relationship revealed the 5-pyridazin-3-ones **8a** and *S*-methyl **8b** had excellent human and rat H_3R affinities, and acceptable pharmacokinetic properties. In vivo evaluation of **8a** showed potent activity in the rat dipsogenia model and robust wake-promoting activity in the rat EEG/EMG model.

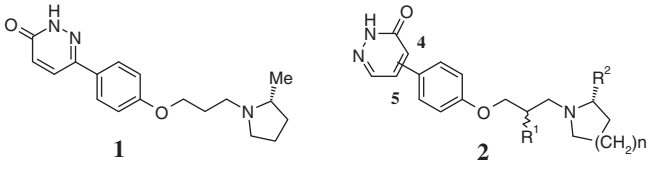
1. Introduction

Primarily located in the central nervous system (CNS), histamine-3 receptors (H_3R) function as regulators to modulate a wide variety of neurotransmitters including histamine, acetylcholine, dopamine, norepinephrine and serotonin.[1–10] Consequently, H_3R antagonists have potential use for the treatment of a variety of CNS diseases such as sleep and cognitive disorders, attention-deficit hyperactivity (ADHD) and Alzheimer's disease (AD).[3–9] It is no surprise that H_3R has attracted a lot of attention from the pharmaceutical industry in development of H_3R ligands for the treatment of these CNS disorders.[3–9] We identified a novel class of pyridazin-3-one H_3R antagonists/inverse agonists with exceptional drug-like properties, and in vivo profiles.[11,12] 6-{4-[3-(*R*)-2-methylpyrrolidin-1-yl)propoxy]-phenyl-2*H*-pyridazin-3-one **1** (irdabisant; CEP-26401) (Fig. 1) was selected as a clinical candidate and recently completed phase I.[12] During our H_3 discovery project research, we actively pursued a variety of structural core modifications.[13–15] One strategy was to evaluate the various pyridazinone regiomers and optimize the eastern portion of the core such as **2**. This paper describes the synthesis of 4- and 5-pyridazinone analogues **2** and evaluation of their H_3R binding structure–activity relationship (SAR), pharmacokinetics (PK), selectivity and drug-like properties.

2. Chemistry

Synthesis of compounds **8a–e** is shown in Scheme 1. Following the literature procedure, 4,5-dichloro-2*H*-pyridazin-3-one **3** was converted to 5-iodo-2*H*-pyridazin-3-one **4** in good yield.[16,17] Protection of pyridazinone N–H with formaldehyde afforded 2-hydroxymethyl-5-iodo-2*H*-pyridazin-3-one **5**.[16] Syntheses of tetramethyl-[1,3,2]dioxaborolanes **6** were described previously.[12,13a,14] Substitution of mesylates **6** with various amines provided compounds **7a**, **7b**. Palladium coupling of compounds **5** and **7a–e** and the subsequent loss of the hydroxymethyl group[17] gave target compounds **8a–e** correspondingly.

N-Alkylation of pyridazinone **4** with bromomethyl methyl ether provided compound **9** (Scheme 2). Palladium coupling of **9** and **7a** afforded compound **10**. On the other hand, compounds **14–16** were synthesized as shown in Scheme 3. N-Alkylation of pyridazinone **3** with bromomethyl methyl ether gave compound **11**. Selective methoxy displacement of either chloro in compound **11** could be



(Irdabisant; CEP-26401)

Figure 1. Structures of pyridazin-3-one phenoxyproylamines H_3R antagonists.

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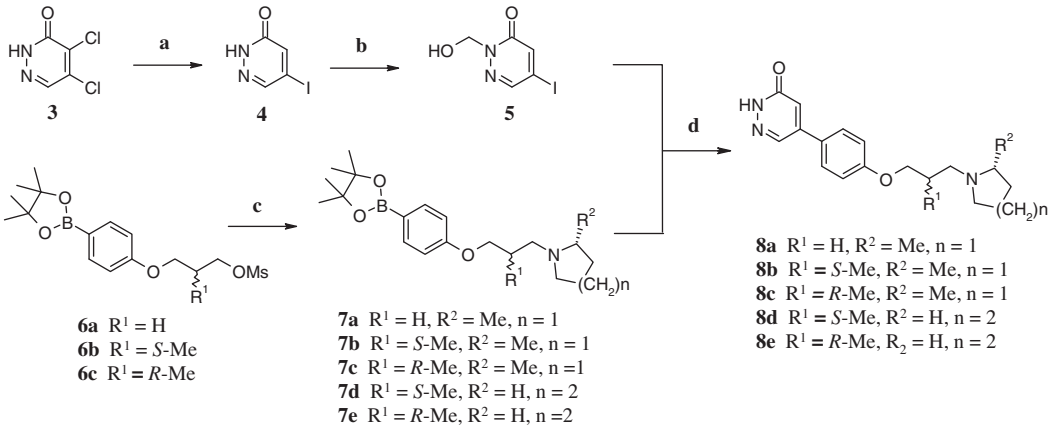
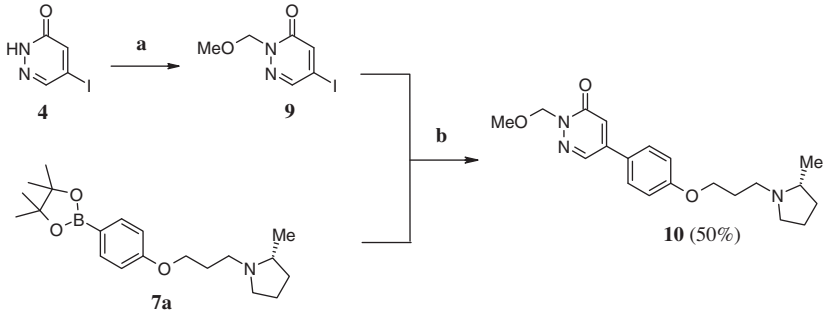
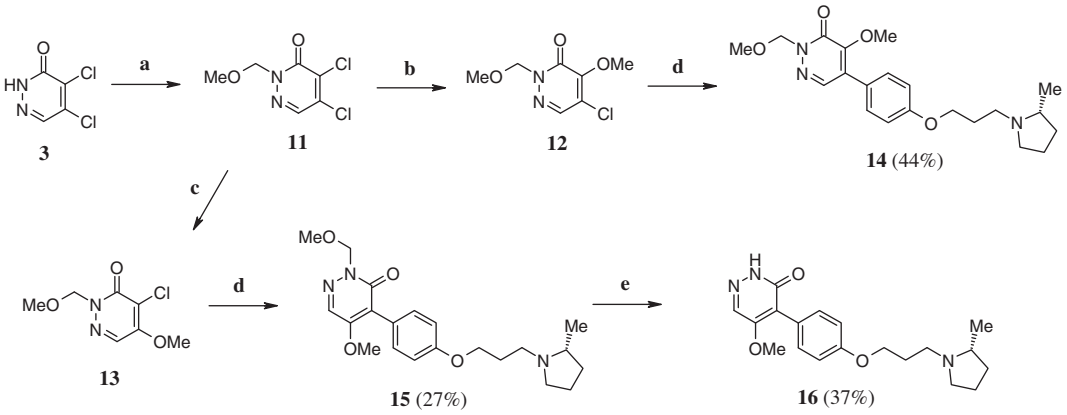


Scheme 1. Reagents and conditions: (a) 57% HI, H_2O, 150 °C, 26 h, 46%; (b) 35% CH_2O, H_2O, 105 °C, 18 h, 84%; (c) (*R*)-2-methyl-pyrrolidine hydrochloride or piperidine, K_2CO_3, CH_3CN, reflux, 22 h, 48-71%; (d) tetrakis(triphenylphosphine)-palladium(0), K_2CO_3, DME-H_2O (2:1), reflux, 48 h, 38–64%.



Scheme 2. Reagents and conditions: (a) $BrCH_2OMe$, iPr_2NEt, CH_2Cl_2, rt, 22 h, 73%; (b) tetrakis(triphenylphosphine)palladium(0), K_2CO_3, DME-H_2O (2:1), reflux, 4 h.



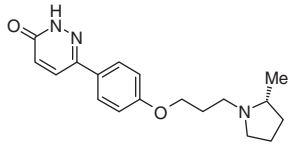
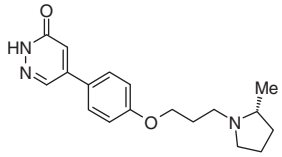
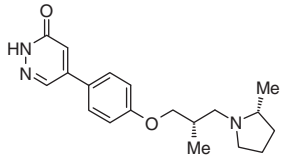
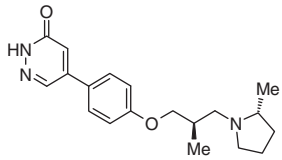
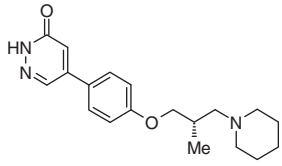
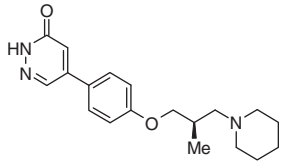
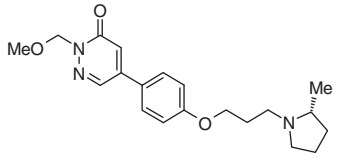
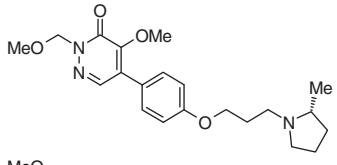
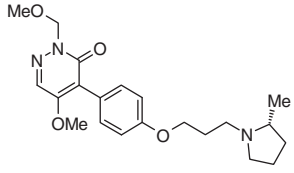
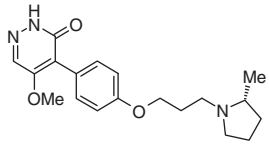
Scheme 3. Reagents and conditions: (a) $BrCH_2OMe$, iPr_2NEt, CH_2Cl_2, rt, 22 h, 78%; (b) NaOMe, 1,4-dioxane, rt, 24 h, 94%; (c) NaOMe, MeOH, rt, 2 h, 98%; (d) **7a**, Tetrakis(triphenyl-phosphine)palladium(0), Na_2CO_3, DME-H_2O (1:1), 100 °C, 24 h; (e) HCl, MeOH, 100 °C, 72 h.

accomplished by varying the solvent.[18] Reaction of **11** with sodium methoxide in 1,4-dioxane provided 5-chloro-4-methoxy-2-methoxymethyl-2*H*-pyridazin-3-one **12**. Conversely, reaction of **11** with sodium methoxide in methanol afforded 4-chloro-5-methoxy-2-methoxymethyl-2*H*-pyridazin-3-one **13**. Palladium coupling of **12** and **13** with **7a** gave compounds **14** and **15**, respectively. The N-2-methoxymethyl was deprotected by heating **15** in an HCl methanol solution to afford N–H **16**. The attempt to remove N-2-methoxymethyl in compound **14** was unsuccessful.

3. Results and discussion

The H_3R binding affinity SAR revealed that pyridazinone 5-regiomers showed, in general, high H_3R affinity comparable with their 6-regiomers counterpart (Table 1).[12] 5-Regiomer **8a** had comparable H_3R affinities as **1** (CEP-26401) in both human and rat as disclosed previously.[12] The effect of incorporating a chiral methyl group on the propyl linker was investigated. Introduction of a *S*-methyl on the propylamine side-chain **8b** showed single digit

Table 1
H_3R binding data for pyridazinones

Compound	Structure	hH_3R K_i[a] (nM)	rH_3R K_i[a] (nM)
1		2	7
8a		3	9
8b		3	4
8c		27	43
8d		25	34
8e		47	131
10		4	14
14		5	13
15		10	19
16		20	62

[a] K_i values are an average of 2 or more determinations. The assay-to-assay variation was typically within 2.5-fold.

nanomolar H_3R affinities in both species with a eudismic ratio for *R* over *S*-methyl (**8c**/**8b**) of 9 and 11 for hH_3R and rH_3R, respectively. Replacement of the *R*-2-methylpyrrolidine with piperidine showed a significant loss in H_3R affinity. Similar to the *R*-2-methylpyrrolidine SAR, *R*-methyl piperidine **8e** had weaker H_3R affinities than *S*-methyl **8d** with a eudismic ratio of 2 and 4 for human and rat

Table 2
Rat pharmacokinetics for **1**, **8a**, **8b** and **10**

	1[12]	**8a**[12]	**8b**	**10**
iv (1 mg/kg)				
$t_{½}$ (h)	2.6	1.0 ± 0.1	2.1 ± 0.6	0.4 ± 0.1
V_d (L/kg)	9.4	0.8 ± 0.1	3.9 ± 1.2	3.2 ± 0.8
CL (mL/min/kg)	42	10 ± 1	21 ± 4	93 ± 10
po (5 mg/kg)				
AUC_{0-t} (ng h/mL)	984	1292 ± 41	762 ± 63	42 ± 19
C_{max} (ng/mL)	270	401 ± 126	187 ± 19	53 ± 10
%*F*	83	22 ± 3	24 ± 1	6 ± 2
B/P[a]	2.6	1.8 ± 0.4	6.2 ± 1.9	ND

[a] Based on the 6 h time point after 5 mg/kg po dosing.

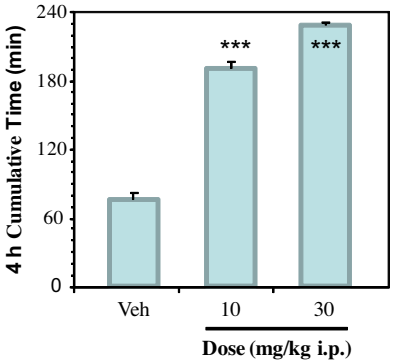


Figure 2. Compound **8a**-induced wake promotion.[24] Time awake for 4 h after dosing with compound **8a** at 10 and 30 mg/kg ip versus vehicle (Veh). *** P <0.001 Dunnett's test versus vehicle.

H_3R. Thus, 5-pyridazin-3-one regiomer combined with (*R*)-2-methy-1-((*S*)-2-methyl-3-phenoxypropyl)pyrrolidine fragment **8b** was the most favorable for H_3R affinities.

Next methoxy substitutions on the pyridazinone 2, 4 and 5-positions were examined (Table 1). There was little effect on hH_3R affinity with methoxy substitution. For example, comparing *N*-2-methoxymethyl **10** and 4-methoxy **14** had comparable hH_3R affinity with *S*-methyl **8b**. And comparing **15** with **16** showed only a 2-fold difference in H_3R affinity. Thus, over all the order of affinities for the pyridazinone regiomers was 6-regiomer ≈ 5-regiomer > 4-regiomer.

Based on their H_3R affinities, compounds **8a**, *S*-methyl **8b** and **10** were further profiled in the discovery flow. Compounds **8a**, *S*-methyl **8b** and **10** had excellent selectivity over hH_1R, hH_2R and hH_4R subtypes with K_i values >10 μM. They had acceptable metabolic stability in liver microsomes for human, mouse, rat, and monkey ($t_{½}$ >40 min) and had low to no inhibition of cytochrome P450 enzymes (CYP1A2, 2C9, 2C19, 2D6, and 3A4) with IC_{50} values >30 μM, indicating low potential for drug–drug interactions. Compounds **8a**, *S*-methyl **8b** and **10** were screened for PK properties in the rat (Table 2). Compound **10** had poor PK properties with high clearance, short half-life, low C_{max} and AUC_{0-t}. Compound **8a** had acceptable rat PK properties but lower oral bioavailability and brain to plasma ratio (B/P) compared with the 6-regiomer **1**.[12] *S*-methyl **8b** had acceptable PK with moderate clearance (CL), C_{max} and AUC comparable with **8a**. The volume distribution (V_d) and brain to plasma ratio (B/P) for *S*-methyl **8b** were much higher than **8a**.

Compound **8a** displayed potent antagonist and full inverse agonist activity in the guanosine 5′-(γ-thio)triphosphate ([^{35}S]GTPγS) binding assay.[11,20] It showed excellent selectivity against a panel 70 GPCRs, ion channels and enzymes (<50% inhibition at 10 μM; MDS Pharma Services, LeadProfile screen) and hERG (IC_{50} = 10 μM in a patch clamp assay). Compound **8a** had low binding to plasma proteins (mouse, rat, dog, and human 20–40%), high unbound fraction in rat brain homogenate (45%), high permeability in the Caco-2 assay, and high water solubility. Compound **8a** was also negative in the mini Ames assay, with and without metabolic activation. Based on it's over all profiles **8a** was evaluated for sleep-wake activity in the rat EEG/EMG model.

The rat dipsogenia model was used in the project as an in vivo surrogate measure of H_3R functional inhibition in the brain following peripheral administration of compounds. Histamine and the H_3-selective agonist, RAMH induce water drinking in the rat when administered either peripherally or centrally, an effect that is blocked by H_3R antagonists.[19] Compound **8a** showed potent functional H_3R antagonism in the rat dipsogenia model with an ED_{50} of 0.03 mg/kg, ip[20] A number of H_3R antagonists have been shown to promote wake activity in preclinical species, including irdabisant and its analogs.[13,21–23] Compound **8a** was tested in the rat EEG/EMG model of wake promotion using rats surgically implanted for chronic recording of EEG (electroencephalographic) and EMG (electromyographic) signals.[10,13,14,23] Compound **8a** increased wake activity dose dependently at 10 and 30 mg/kg ip based on cumulative time awake for 4 h post dosing (4 h AUC) (Fig. 2).[24] AUC values were 191 ± 6 min at 10 mg/kg and 228 ± 3 min at 30 mg/kg ip versus 76 ± 6 min for vehicle. At 30 mg/kg the treated animals were awake 95% of the time up to 4 h post dosing, a three-fold increase in percent time awake over the vehicle treated animals. No increase in sleep following the enhanced wake period (i.e. sleep rebound) was observed up to 22 h post dosing, nor was any adverse EEG activity evident up to 30 mg/kg ip.

4. Conclusion

In summary, taken together the SAR revealed that the order of H_3R affinities for the pyridazinone regiomers was 6-regiomer ≈ 5-regiomer > 4-regiomer. 5-Pyridazin-3-one **8a** and **8b** had single digit nanomolar human H_3R binding affinity with excellent selectivity and acceptable PK properties. In vivo **8a** had potent H_3R functional activity in the rat dipsogenia model and showed robust wake-promoting activity in the rat EEG/EMG model with no adverse events.

5. Experimental section

5.1. General

All commercially available chemicals and solvents were used as received. Compounds were purified by silica gel chromatography using an ISCO apparatus with detection at 290 and 254 nm. Reverse phase purification was accomplished using a Gilson 281 liquid handler equipped with a Phenomenex C18 column (150 × 30 mm LD., S5 μM). Peak collection was triggered by UV detection at 215 and 254 nm. ^{1}H NMR was obtained on a Bruker 400 MHz instrument in the solvent indicated with tetramethylsilane as an internal standard. Coupling constants (*J*) are in hertz (Hz). Liquid chromatography-mass spectrometry (LC/MS) was run on a Bruker Esquire 2000 ion trap LCMS. Compound purity was >96% determined by high pressure liquid chromatography (HPLC) using a Zorbax RX-C8, 5 × 150 mm column, eluting with mixture of acetonitrile and water containing 0.1% trifluoroacetic acid with a gradient of 10–100%. Melting points were determined using a MEL-TEMP II and were uncorrected. No attempts were made to optimize yields.

5.2. 5-Iodo-2*H*-pyridazin-3-one (4)

A round bottom flask charged with 4,5-dichloro-2*H*-pyridazin-3-one (**1**, 15.0 g, 90.9 mmol) and HI (57% in H_2O, 100 mL, 0.80 mol) was stirred at 140 °C for 24 h. After cooling to ambient temperature,

the black precipitate was collected by filtration and was washed with H_2O. This material was suspended in H_2O (100 mL) and $Na_2S_2O_3$ was added in portions until the color turned pale yellow. The solid material was collected by filtration and washed with H_2O. The crude material was dissolved in MeOH/CH_2Cl_2 (1:1, 300 mL) and filtered. The filtrate was concentrated and dried to give 12.9 g (64%) of **4** as brown solid. Mp 139–142 °C. ^{1}H NMR (DMSO-d_6 δ): 7.54 (s, 1H), 8.08 (s, 1H), 13.25 (s, 1H). LCMS *m/z*: 222 (M).

5.3. 2-Hydroxymethyl-5-iodo-2*H*-pyridazin-3-one (5)

A round bottom flask charged with **4** (6.1 g, 27.6 mmol), formaldehyde (37% in H_2O, 22 mL) and H_2O (50 mL) was stirred at reflux for 18 h. After cooling to ambient temperature, the resulted crystalline solid was collected by filtration, washed with a small amount of H_2O and dried to give 5.8 g (84%) of **5** as yellow crystalline solid. Mp 139–140 °C. ^{1}H NMR ($CDCl_3$, δ): 4.15 (m, 1H), 5.50 (d, 2H, *J* = 8.5 Hz), 7.50 (d, 1H, *J* = 1.9 Hz), 7.95 (d, 1H, *J* = 1.9 Hz). LCMS *m/z*: 222 (M−30).

5.4. (*R*)-2-Methyl-1-{(*S*)-2-methyl-3-[4-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-phenoxy]-propyl}-pyrrolidine (7b)

A round bottom flask charged with **6b** (2.1 g, 5.6 mmol), (*R*)-2-methyl-pyrrolidine hydrochloride (1.4 g, 11.1 mmol), K_2CO_3 (5.0 g, 36.2 mmol) and acetonitrile (35 mL) was stirred at reflux for 22 h. After cooling to ambient temperature, the reaction was filtered and the residue was concentrated. The produce was purification by column chromatography (2% MeOH in CH_2Cl_2) to give 900 mg (45%) of **7b**. ^{1}H NMR (DMSO-d_6, δ): 0.99 (m, 6H), 1.07 (m, 2H), 1.27 (s, 12H), 1.63 (m, 2H), 1.85 (m, 1H), 2.03 (m, 2H), 2.17 (m, 1H), 2.53 (m, 1H), 3.02 (m, 1H), 3.78–3.94 (m, 2H), 6.92 (m, 2H), 7.59 (m, 2H). LCMS *m/z*: 360 (M+1).

Compounds **7c**–**e** were synthesized using the methods for **7b**.

5.5. (*R*)-2-Methyl-1-{(*R*)-2-methyl-3-[4-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-phenoxy]-propyl}-pyrrolidine (7c)

Yield 48%. ^{1}H NMR (DMSO-d_6, δ): 0.95 (d, 3H, *J* = 5.7 Hz), 0.99 (d, 3H, *J* = 6.4 Hz), 1.07 (m, 2H), 1.27 (s, 12H), 1.63 (m, 2H), 1.84 (m, 1H), 2.03 (m, 2H), 2.24 (m, 1H), 2.70 (m, 1H), 3.09 (m, 1H), 3.81 (m, 1H), 4.03 (m, 1H), 6.90 (m, 2H), 7.59 (m, 2H). LCMS *m/z*: 360 (M+1).

5.6. 1-{(*S*)-2-Methyl-3-[4-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-phenoxy]-propyl}-piperidine (7d)

Yield 62%. ^{1}H NMR (DMSO-d_6, δ): 0.96 (d, 3H, *J* = 5.9 Hz), 1.07 (m, 1H), 1.27 (s, 12H), 1.37 (m, 2H), 1.48 (m, 4H), 2.10 (m, 2H), 2.28 (m, 4H), 3.78 (m, 1H), 3.97 (m, 1H), 6.92 (m, 2H), 7.59 (m, 2H). LCMS *m/z*: 360 (M+1).

5.7. 1-{(*R*)-2-Methyl-3-[4-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-phenoxy]-propyl}-piperidine (7e)

Yield 71%. ^{1}H NMR (DMSO-d_6, δ): 0.96 (d, 3H, *J* = 5.9 Hz), 1.07 (m, 1H), 1.27 (s, 12H), 1.37 (m, 2H), 1.48 (m, 4H), 2.10 (m, 2H), 2.29 (m, 4H), 3.79 (m, 1H), 3.98 (m, 1H), 6.92 (m, 2H), 7.59 (m, 2H). LCMS *m/z*: 360 (M+1).

5.8. 5-{4-[(*S*)-2-Methyl-3-((*R*)-2-methyl-pyrrolidin-1-yl)-propoxy]-phenyl}-2*H*-pyridazin-3-one (8b)

A round bottom flask charged with **5** (0.50 g, 1.98 mol), **7b** (0.78 g, 2.18 mmol), tetrakis(triphenylphosphine)palladium(0) (0.23 g, 0.20 mmol), K_2CO_3 (1.37 g, 9.92 mmol), DME (20 mL) and H_2O (20 mL) was flashed with nitrogen for 15 min. After stirring at reflux for 20 h, the reaction was cooled to 80 °C and a small amount of $NaCNBH_3$ was added. The reaction was stirred for 5 min and then cooled to ambient temperature. The reaction was diluted with MeOH–CH_2Cl_2 (1:3; 100 mL), washed with saturated $NaHCO_3$ solution, brine, dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (10% MeOH in CH_2Cl_2) to give 268 mg (51%) of **8b** as yellow solid. Mp 154–156 °C. ^{1}H NMR (DMSO-d_6, δ): 1.01 (m, 6H), 1.29 (m, 1H), 1.64 (m, 2H), 1.87 (m, 1H), 2.05 (m, 2H), 2.19 (m, 1H), 2.27 (m, 1H), 2.55 (m, 1H), 3.04 (m, 1H), 3.94 (m, 2H), 7.06 (m, 3H), 7.79 (m, 2H), 8.29 (m, 1H), 13.01 (s, H). LCMS *m/z*: 328 (M+1). HPLC (290 nm) t_r 4.63 min, 99%.

Compounds **8c**–**e** were synthesized using the methods for **8b**.

5.9. 5-{4-[(*R*)-2-Methyl-3-((R)-2-methyl-pyrrolidin-1-yl)-propoxy]-phenyl}-2*H*-pyridazin-3-one (8c)

White solid. Yield 39%. Mp 130–133 °C. ^{1}H NMR (DMSO-d_6, δ): 0.96 (d, 3H, *J* = 4.9 Hz), 1.01 (d, 3H, *J* = 6.3 Hz), 1.26 (m, 1H), 1.64 (m, 2H), 1.84 (m, 1H), 1.97 (m, 1H), 2.05 (m, 2H), 2.25 (m, 1H), 2.72 (m, 1H), 3.10 (m, 1H), 3.88 (m, 1H), 4.08 (m, 1H), 7.04 (m, 3H), 7.79 (m, 2H), 8.29 (m, 1H), 13.00 (s, 1H). LCMS *m/z*: 328 (M+1). HPLC (290 nm) t_r 4.71 min, 99%.

5.10. 5-[4-((*S*)-2-Methyl-3-piperidin-1-yl-propoxy)-phenyl]-2*H*-pyridazin-3-one (8d)

White solid. Yield 49%. Mp 145–148 °C. ^{1}H NMR (DMSO-d_6, δ): 0.98 (d, 3H, *J* = 5.2 Hz), 1.38 (m, 2H), 1.50 (m, 4H), 2.13 (m, 2H), 2.30 (m, 5H), 3.87 (m, 1H), 4.02 (m, 1H), 7.06 (m, 3H), 7.79 (m, 2H), 8.29 (m, 1H), 13.00 (s, 1H). LCMS *m/z*: 328 (M+1). HPLC (290 nm) t_r 4.63 min, 99%.

5.11. 5-[4-((*R*)-2-Methyl-3-piperidin-1-yl-propoxy)-phenyl]-2*H*-pyridazin-3-one (8e)

White solid. Yield 40%. Mp 160–163 °C. ^{1}H NMR (DMSO-d_6, δ): 0.98 (d, 3H, *J* = 4.9 Hz), 1.38 (m, 2H), 1.49 (m, 4H), 2.13 (m, 2H), 2.30 (m, 5H), 3.86 (m, 1H), 4.02 (m, 1H), 7.06 (m, 3H), 7.79 (m, 2H), 8.29 (m, 1H), 13.00 (s, 1H). LCMS *m/z*: 328 (M+1). HPLC (290 nm) t_r 4.67 min, 98%.

5.12. 5-Iodo-2-methoxymethyl-2*H*-pyridazin-3-one (9)

To a solution of **4** (2.8 g, 12.8 mmol), *N*,*N*-diisopropylethylamine (3.3 mL, 19.2 mol), 4-dimethylaminopyridine (80 mg, 0.6 mmol) and CH_2Cl_2 (30 mL) at 0 °C was added bromomethyl methyl ether (2.13 mL, 25.6 mmol) and stirred at ambient temperature for 18 h. The reaction was filtered and the filtrate was concentrated. The residue was purified by column chromatography (CH_2Cl_2) to give 2.41 g (70%) of **9**. ^{1}H NMR (DMSO-d_6, δ): 3.31 (s, 3H), 5.25 (s, 2H), 7.65 (m, 1H), 8.17 (m, 1H). LCMS *m/z*: 267 (M+1).

5.13. 2-Methoxymethyl-5-{4-[3-((*R*)-2-methyl-pyrrolidin-1-yl)-propoxyl]-phenyl}-2*H*-pyridazin-3-one (10)

A round bottom flask charged with **9** (119 mg, 0.45 mol), **7a** (201 mg, 0.58 mmol), tetrakis(triphenylphosphine)palladium(0) (52 mg, 0.045 mmol), K_2CO_3 (187 mg, 1.35 mmol), DME (12 mL) and H_2O (4 mL) was flashed with nitrogen for 15 min. After stirring at 85 °C for 4 h, the reaction was cooled to ambient temperature and concentrated. The residue was purified by column chromatography (5% MeOH in CH_2Cl_2) to give 80 mg (39%) of **10** as white solid. Mp 183–185 °C. ^{1}H NMR (DMSO-d_6, δ): 1.36 (d, 2H, *J* = 6.4 Hz), 1.60 (m, 1H), 1.84–2.29 (m, 5H), 3.13 (m, 2H), 3.35 (s, 3H), 3.46 (m, 2H), 3.64 (m, 1H), 4.16 (m, 2H), 5.33 (s, 2H), 7.10 (m, 2H), 7.21 (m,

1H), 7.87 (m, 2H), 8.41 (m, 1H). LCMS *m/z*: 358 (M+1). HPLC (290 nm) t_r 5.31 min, 98%.

5.14. 4,5-Dichloro-2-methoxymethyl-2*H*-pyridazin-3-one (11)

To a solution of **3** (10.0 g, 61.6 mmol), *N,N*-diisopropylethylamine (15.8 mL, 90.9 mmol) and CH_2Cl_2 (80 mL) at 0 °C was added bromomethyl methyl ether (7.50 mL, 94.8 mmol) slowly. After stirring at ambient temperature for 18 h, the reaction was concentrated and the residue was dissolved in small amount of CH_2Cl_2, then diluted with Et_2O (300 mL). The reaction was filtered and the filtrate was concentrated. The residue was purified by column chromatography (1% MeOH in CH_2Cl_2) give 9.92 g (78%) of **11**. ^{1}H NMR (DMSO-d_6, δ): 3.33 (s, 3H), 5.35 (s, 2H), 8.23 (s, 1H). LCMS *m/z*: 209 (M+1).

5.15. 5-Chloro-4-methoxy-2-methoxymethyl-2*H*-pyridazin-3-one (12)

To a solution of **11** (2.00 g, 9.57 mmol) and 1,4-dioxane (30 mL) was added sodium methoxide (1.0 g, 19.1 mmol) and then stirred for 24 h. Additional 0.50 g of sodium methoxide was added and stirring continued for 24 h. The reaction was quenched with H_2O (50 mL), then extracted with CH_2Cl_2 (2 × 100 mL). The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated to give 1.79 g of **12** (91%). This material was used in the next step without further purification. ^{1}H NMR (DMSO-d_6, δ): 3.33 (s, 3H), 4.15 (s, 3H), 5.31 (s, 2H), 8.06 (s, 1H). LCMS *m/z*: 205 (M+1).

5.16. 4-Methoxy-2-methoxymethyl-5-{4-[3-((*R*)-2-methyl-pyrrolidin-1-yl)-propoxy]-phenyl}-2*H*-pyridazin-3-one (14)

A round bottom flask charged with **12** (1.79 g, 8.75 mol), **7a** (3.32 g, 9.62 mmol), tetrakis(triphenylphosphine)palladium(0) (1.00 g, 0.87 mmol), Na_2CO_3 (4.64 g, 43.7 mmol), DME (20 mL) and H_2O (20 mL) was flashed with nitrogen for 30 min. After stirring at 90 °C for 17 h, the reaction was cooled to ambient temperature and extracted with CH_2Cl_2 (2 × 100 mL). The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (5% to 10% MeOH in CH_2Cl_2) to give 2.27 g (67%, 2 steps) of **14** as brown oil. ^{1}H NMR (DMSO-d_6, δ): 1.02 (d, 3H, J = 5.4 Hz), 1.30 (m, 1H), 1.65 (m, 2H), 1.88 (m, 3H), 1.99-2.38 (m, 3H), 2.93 (m, 1H), 3.10 (m, 1H), 3.37 (s, 3H), 3.97 (m, 3H), 4.08 (m, 2H), 5.35 (s, 2H), 7.05 (m, 2H), 7.58 (m, 2H), 8.03 (s, 1H). LCMS *m/z*: 388 (M+1). HPLC (290 nm) t_r 5.93 min, 98%.

5.17. 4-Chloro-5-methoxy-2-methoxymethyl-2*H*-pyridazin-3-one (13)

To a solution of **11** (2.20 g, 10.5 mmol) in MeOH (50 mL) was added sodium methoxide (1.14 g, 21.0 mmol) then stirred for 24 h. Additional 0.50 g of sodium methoxide was added and stirring was continued for 24 h. The reaction was quenched with H_2O (30 mL), then extracted with CH_2Cl_2 (2 × 100 mL). The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated give 2.12 g of **13** (92%). This material was used in the next step without further purification. ^{1}H NMR (DMSO-d_6, δ): 3.31 (s, 3H), 4.10 (s, 3H), 5.35 (s, 2H), 8.30 (s, 1H). LCMS *m/z*: 205 (M+1).

5.18. 5-Methoxy-2-methoxymethyl-4-{4-[3-((*R*)-2-methyl-pyrrolidin-1-yl)-propoxy]-phenyl}-2*H*-pyridazin-3-one (15)

A round bottom flask charged with **13** (2.12 g, 8.75 mol), **7a** (3.94 g, 11.4 mmol), tetrakis(triphenylphosphine)palladium(0) (1.20 g, 1.04 mmol), Na_2CO_3 (5.49 g, 51.8 mmol), DME (30 mL) and H_2O (30 mL) was flashed with nitrogen for 20 min. After stirring at 100 °C for 16 h, the reaction was cooled to ambient temperature and extracted with CH_2Cl_2 (2 × 100 mL). The combined organic layers were washed with 1 N HCl solution (30 mL), H_2O, brine, dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (5–8% MeOH in CH_2Cl_2) to give 1.08 g (27%, 2 steps) of **15** as off white solid. Mp 44–45 °C. ^{1}H NMR (DMSO-d_6, δ): 0.77–2.36 (m, 10 H), 2.83–3.70 (m, 7H), 3.94 (s, 3H), 4.09 (m, 2H), 5.34 (s, 2H), 6.96 (m, 2H), 7.40 (m, 2H), 8.29 (s, 1H). LCMS *m/z*: 388 (M+1). HPLC (290 nm) t_r 5.62 min, 96%.

5.19. 5-Methoxy-4-{4-[3-((R)-2-methyl-pyrolidin-1-yl)-propoxy]-phenyl}-2*H*-pyridazin-3-one (16)

A round bottom flask charged with **15** (1.07 g (2.76 mmol), concentrated HCl (10 mL) and MeOH (10 mL) was stirred at 100 °C for 3 days. After cooling to ambient temperature, the reaction was neutralized with 10 N NaOH solution to pH 7. The reaction was washed with CH_2Cl_2 (100 mL) and the water layer was concentrated. The residue was dissolved in CH_2Cl_2 and filtered. The filtrate was concentrated and the residue was purified by column chromatography (15% MeOH in CH_2Cl_2) to give 350 mg (37%) of **16** as white solid. Mp 135–140 °C. ^{1}H NMR (DMSO-d_6, δ): 1.04–2.27 (m, 9H), 3.09 (m, 2H), 3.27-3.71 (m, 3H), 3.91 (s, 3H), 4.10 (m, 2H), 6.96 (m, 2H), 7.43 (m, 2H), 8.18 (s, 1H), 12.98 (s, 1H). LCMS *m/z*: 344 (M+1). HPLC (290 nm) t_r 4.47 min, 99%.

5.20. Rat H_3 assays

5.20.1. Cell line development and membrane preparation

The rat H_3 receptor cDNA was PCR amplified from reverse-transcribed RNA pooled from rat thalamus, hypothalamus, striatum and prefrontal cortex with a sequence corresponding to bp #338-1672 of Genbank file #NM_053506, encoding the entire 445-amino-acid rat histamine H_3 receptor. This was engineered into the pIRES-neo3 mammalian expression vector, which was stably transfected into the CHO-A3 cell line (Euroscreen, Belgium), followed by clonal selection by limiting dilution. Cells were harvested and cell pellets were frozen (−80 °C). Cell pellets were resuspended in 5 mM Tris–HCl, pH 7.5 with 5 nM EDTA and a cocktail of protease inhibitors (Complete Protease Inhibitor Tablets, Roche Diagnostics). Cells were disrupted using a polytron cell homogenizer and the suspension was centrifuged at 1000×*g* for 10 min at 4 °C. This membrane pellet was washed in membrane buffer containing 50 mM Tris–HCl, pH 7.5 with 0.6 mM EDTA, 5 mM $MgCl_2$ and protease inhibitors, recentrifuged as above and the final pellet resuspended in membrane buffer plus 250 mM sucrose and frozen at −80 °C.

5.20.2. Radioligand binding

Membranes were resuspended in 50 mM Tris–HCl (pH 7.4), 5 mM $MgCl_2$, 0.1% BSA. The membrane suspensions (10 μg protein per well) were incubated in a 96 well microtiter plate with [^{3}H]-*N*-alpha-methylhistamine (approximately 1 nM final concentration), test compounds at various concentrations (0.01 nM–30 μM) and scintillation proximity beads (Perkin Elmer, FlashBlueGPCR Scintillating Beads) in a final volume of 80 μl for 4 h at room temperature, protected from light. Non-specific binding was determined in the presence of 10 μM clobenpropit. Radioligand bound to receptor, and therefore in proximity to the scintillation beads, was measured using a MicroBeta scintillation counter.

5.21. Human H_3 assays

5.21.1. Membrane preparation

CHO cells stably expressing the human H_3 receptor (GenBank: NM_007232) were harvested and cell pellets were frozen

(−80 °C). Cell pellets were resuspended in 5 mM Tris–HCl, pH 7.5 with 5 nM EDTA and a cocktail of protease inhibitors (Complete Protease Inhibitor Tablets, Roche Diagnostics). Cells were disrupted using a polytron cell homogenizer and the suspension was centrifuged at 1000×*g* for 10 min at 4 °C. The pellet was discarded and the supernatant centrifuged at 40,000×*g* for 30 min at 4 °C. This membrane pellet was washed in membrane buffer containing 50 mM Tris–HCl, pH 7.5 with 0.6 mM EDTA, 5 mM $MgCl_2$ and protease inhibitors, recentrifuged as above and the final pellet resuspended in membrane buffer plus 250 mM sucrose and frozen at −80 °C.

5.21.2. Radioligand binding

The same method described above (**5.20.2**) was applied.

5.22. Pharmacokinetics

Adult male Sprague–Dawley rats (275–350 g; Charles River, Kingston, NY) were used in the experiments. All animal usage was approved by the Cephalon IUCAC. For routine compound screening rats were dosed via the lateral rail vein at the indicated dose for iv administration (3% DMSO, 30% Solutol, 67% phosphate buffered saline or 100% saline) or via oral gavage (50% Tween 80, 40% propylene carbonate, and 10% propylene glycol, saline, or 2% HCl–water) at the indicated dose. Rat were fasted overnight prior to po administration. Serial blood samples was collected from the lateral tail vein into heparinized collection tubes (approximately 0.25 mL) at seven sampling times over a 6 or 24 h period as indicated. The plasma was separated by centrifugation, and the sample was prepared for analysis HPLC/MS by protein precipitation with acetonitrile. The plasma samples were analyzed for drug and internal standard via LC–MS/MS protocol. The pharmacokinetic parameters were calculated by a noncompartmental method using WinNonlin software (Professional version 4.1, Pharsight Corporation, Palo Alto, CA, 1997). For experiments to detemine detailed rat PK parameters, rats were administered 1 mg/kg iv and 3 mg/kg po in saline and parameters calculated from composite mean plasma concentration−time data (n = 12).

5.23. Plasma protein binding

Test compounds were dissolved in DMSO and spiked into plasma from rat, dog, and human, as well as phosphate-buffered saline (pH 7.4). The final concentration in plasma or PBS was 5 μM. The mixtrues were incubated in a 37 °C water bath with gentle shaking for 1 h and then were loaded into the MultiScreen Ultracell-PPB plate (Millipore Inc., Billerica, MA) that was centrifuged at 2000 g for 45 min at 37 °C. The amount of compound present in the ultrafiltrate was determined using LC/MS/MS. The percentage of drug bound to plasma was calculated using mean peak area of test compound in plasma ultrafiltrate (as free) and mean peak area of compound in PBS buffer (as total). The results are the mean of duplicate determinations.

5.24. Rat dipsogenia model

Rat dipsogenia was conducted as previously described.[11,19] 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: $[100 - (Dr/(Dg_{RAMH})) \times 100]$, where Dr is the amount of water an individual rat drinks and Dg_{RAMH} 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.

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24. Note in Methods: For dose response studies, all doses (including vehicle) compared by ANOVA followed by Dunnett's post-hoc multiple comparison test.