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# Synthesis and evaluation of pyridazinone–phenethylamine derivatives as selective and orally bioavailable histamine $H_3$ receptor antagonists with robust wake-promoting activity

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ABSTRACT

A series of pyridazinone–phenethylamine derivatives with moderate to low nanomolar affinity for rat and human $H_3R$ are described. These analogs exhibited excellent selectivity and metabolic stability, with acceptable rat pharmacokinetic properties. In vivo, **7** and **11** demonstrated potent $H_3R$ functional antagonism in the rat dipsogenia model and robust wake-promoting activity in the rat electroencephalogram/electromyography (EEG/EMG) model.

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Histamine acts via four G-protein coupled receptor subtypes ($H_1R$–$H_4R$) and is implicated in a wide range of physiological and patho-physiological functions.[1] Antagonists that target the $H_1R$ and $H_2R$ receptor subtypes, which are used in allergic conditions and gastric acid disorders, respectively, have been highly successful medicines for many years. The $H_4R$ subtype is mainly involved in inflammatory and immuno-modulatory functions.[2] The histamine $H_3$ receptor ($H_3R$) located primarily in the brain functions as an auto-receptor to modulate histamine release and as an inhibitory heteroreceptor, regulating the release of multiple neurotransmitters including acetylcholine, dopamine, norepinephrine and serotonin[1] that are involved in attention, vigilance, and cognition. Thus, $H_3R$ antagonists may have utility in addressing a variety of CNS disorders associated with deficits in wakefulness, attention, and cognition, including attention-deficit hyperactivity disorder (ADHD), Alzheimer's disease (AD), mild cognitive impairment, and schizophrenia. The preliminary structure–activity relationships (SAR) and characterization of **1** (CEP-26401, irdabisant) was recently revealed[3] and several additional compounds are under active clinical investigation (Fig. 1).[1g–i] Modification of the aminopropyloxy portion of **1** identified a new class of potent and selective pyridazinone–phenethylamine analogs as $H_3R$ antagonists with robust in vivo wake promoting activity. In this Letter we present the synthesis, SAR, selectivity, preliminary pharmacokinetic properties, and the in vivo activity of leads **7** and **11** in the rat dipsogenia model and in rat EEG/EMG model of wakefulness.

The synthesis of pyridazinone–phenethylamine target **7** was synthesized in six steps as illustrated in Scheme 1. The synthesis of the key intermediate **3**[4] commenced from commercially available phenethylacetate **2**. Friedel–Craft acylation of **2** with succinic anhydride and $AlCl_3$ led to the key intermediate **3** in moderate yield. Cyclocondensation of **3** with methyl hydrazine gave dihyro-pyridazinone derivative **4**,[3] which was easily oxidized with $Cu(II)Cl_2$ or $SeO_2$[5] to furnish pyridazinone derivative **5** in excellent yield and purity. Hydrolysis of **5** with $K_2CO_3$ in MeOH and water produced phenethyl alcohol **6.** The desired target **7** was synthesized from alcohol **6** using $MeSO_2Cl$ and $Et_3N$, followed by alkylation with (*R*)-2-methylpyrrolidine in the presence of $K_2CO_3$ in moderate yield. In an analogous manner, analogs **8–14** were synthesized using the appropriate hydrazine derivative and intermediate **3** via the sequence outlined for compound **7**. Target **9** was also synthesized using the synthetic sequence as described for the compound **7**, except pyrrolidine was used in the final N-alkylation step.

The pyridazinone–phenethylamine target compounds (**7–14**) were tested using in vitro binding assays by displacement of [$^3$H]NAMH in membranes isolated from CHO cells transfected with cloned human $H_3$ or rat $H_3$ receptors as illustrated in Table 1.[3] Phenethylamine based $H_3$ antagonists derived from the natural

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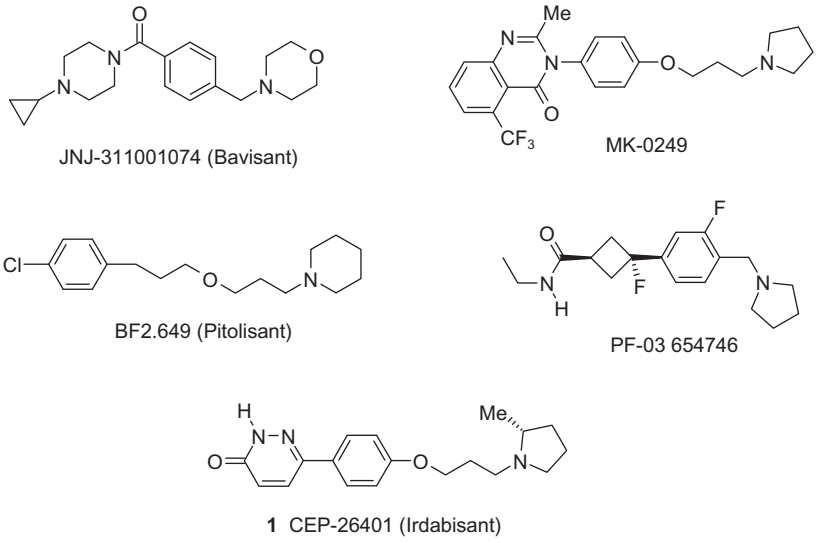
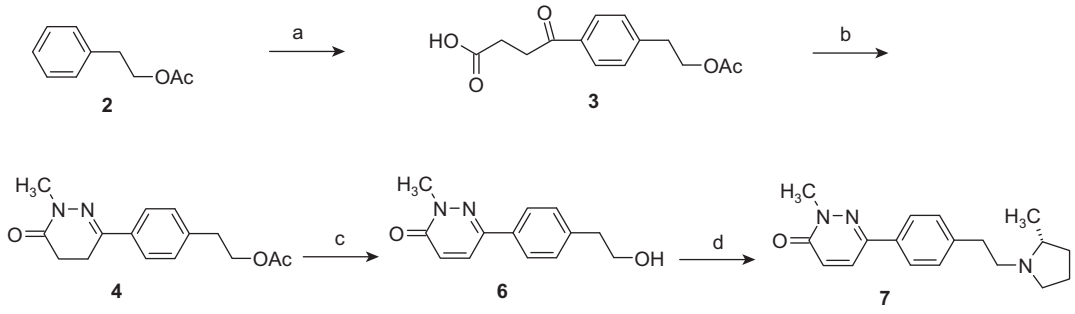
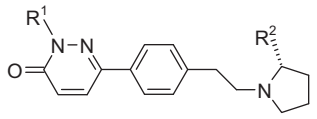



**Figure 1.** Structures of $H_3R$ clinical antagonists.




**Scheme 1.** Reagents and conditions: (a) succinic anhydride, $AlCl_3$, 1,2-dichloroethane, 0–60 °C, 4 h, 50%; (b) MeNHNH2, 2-propanol, reflux, 2.5 h, 87%; (c) (i) $Cu(II)Cl_2$, MeCN, reflux, 4 h or $SeO_2$, AcOH, 125 °C, 4 h, 87% → 5; (ii) $K_2CO_3$, MeOH, $H_2O$, rt to 50 °C, 1 h, 95%; (d) (i) $MeSO_2Cl$, $Et_3N$, $CH_2Cl_2$, 0 °C to rt, 3 h, 79%, (ii) (*R*)-2-methylpyrrolidine, $K_2CO_3$, MeCN, 50 °C, 24–48 h, 44%.

**Table 1**
Pyridazinone–phenethylamine $H_3R$ binding data



| Compound | $R^1$ | $R^2$ | $hH_3R$ ($K_i$, nM)[a] | $rH_3R$ ($K_i$, nM)[a] |
|---|---|---|---|---|
| **1** | — | — | 2 | 7 |
| **7** | Me | (*R*)-Me | 7 | 58 |
| **8** | H | (*R*)-Me | 31 | 43 |
| **9** | Me | H | 83 | 135 |
| **10** | Phenyl | (*R*)-Me | 34 | 42 |
| **11** | *p*-Fluoro-phenyl | (*R*)-Me | 13 | 48 |
| **12** | *p*-Chloro-phenyl | (*R*)-Me | 8 | 26 |
| **13** | Benzyl | (*R*)-Me | 5 | 18 |
| **14** | 2-Pyridyl | (*R*)-Me | 47 | 242 |

[a] $K_i$ values are an average of at least two determinations. The assay-to-assay variation was typically within 2.5-fold.

product Conessine was recently reported by Arena.[6] While exploring the structure–activity relationships (SAR) of the pyridazinone–phenethylamine series the (*R*)-2-methylpyrrolidine was fixed for comparison based on earlier SAR optimization studies.[3] The early SAR exploration was focused at the $R^1$ position of the pyridazinone core to establish the discovery flow properties. In the discovery flow, compounds meeting binding criteria ($hH_3R$ $K_i$ <15 nM; $rH_3R$ $K_i$ <50 nM) were screened for selectivity against $hH_1$, $hH_2$, and $hH_4$ receptor subtypes. Subsequently, compounds were tested for the aqueous solubility ($pH_2$ and $pH_{7.4}$), in vitro microsomal stability (rat, mouse, dog, and human), and inhibition of cytochrome P450 isoforms (CYP1A2, 2C9, 2C19, 2D6, and 3A4). Selected compounds were further screened in in vivo rat pharmacokinetic experiments designed to evaluate iv intrinsic pharmacokinetic parameters ($t_{½}$, $V_d$, and CL), oral bioavailability and brain penetration. Quality molecules at this stage were progressed into in vivo efficacy models.

Changing the four atom phenoxypropyl of **1** to an ethyl linker (analog **8**) showed a 15-fold loss of $hH_3R$ affinity. However,

**Table 2**
Rat pharmacokinetic profiles of **7** and **11**[a]

| | | **7**[c] | **11**[c] |
|---|---|---|---|
| iv | AUC (ng·h/mL)$_\infty$ | 1647 ± 405 | 1295 ± 701 |
| | $t_{½}$ (h) | 0.5 ± 0.1 | 2.3 ± 0.5 |
| | $V_d$ (L/kg) | 0.6 ± 0.3 | 4.8 ± 2.1 |
| | CL (mL/min/kg) | 12 ± 4 | 21 ± 7 |
| po | AUC (ng·h/mL)$_\infty$ | 28 ± 1 | 1733 ± 106 |
| | $C_{max}$ (ng/mL) | 18 ± 6 | 255 ± 14 |
| | *F* (%) | <1 | 27 ± 2 |
| | B/P[b] | 6 ± 0.3 | 11 ± 0.3 |

[a] Administration at 1 mg/mg iv and 5 mg/kg po
[b] B/P = brain to plasma ratio 1 h post IP dosing.
[c] iv formulation (3% DMSO, 30% solutol, 67% phosphate buffered saline); oral formulation (50% Tween 80, 40% propylene carbonate and 10% propylene glycol).

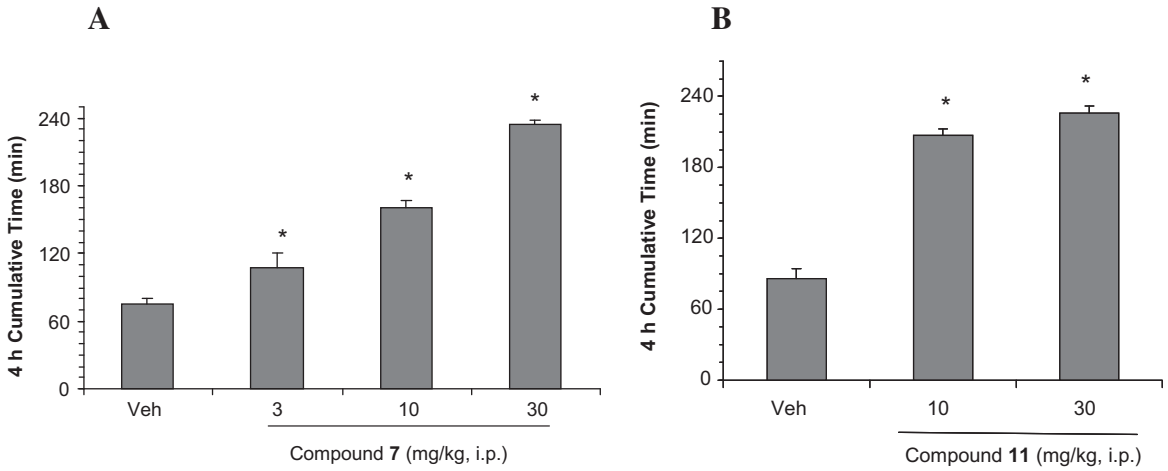



**Figure 2.** Compounds **7**- and **11**-induced wake promotion. Cumulative wake 4 h AUC values following administration of vehicle (Veh), Compound **7** (A) or Compound **11** (B) in rats chronically implanted with electrodes for recording EEG and EMG activity. Mean + SEM, $n$ = 5–7/group. $^{*}p$ <0.05, Dunnett's test versus vehicle.

methylation of the N–H produced compound **7**, which showed a 5-fold improvement in $hH_3R$ affinity with a $K_i$ value of 7 nM, but with comparable $rH_3R$ affinity ($K_i$ = 58 nM) to **8**. Changing the (*R*)-2-methylpyrrolidine of **7** to pyrrolidine **9** showed a 14-fold loss in affinity. The *N*-phenyl **10** had $K_i$ values of 34 and 42 nM for $hH_3R$ and $rH_3R$, respectively. *Para* substitution of the *N*-phenyl with fluoro **11** or chloro **12** showed 3–5-fold higher affinity compared to **10**. The *N*-benzyl analog **13** was equipotent to the simple *N*-methyl **7** for $hH_3R$, whereas the 2-pyridyl **14** showed equal affinity for $hH_3R$ but weaker affinity for $rH_3R$ compared to **10**. Based on the initial binding SAR data, compounds **7** and **11** were selected for further evaluation of selectivity, metabolic stability, cytochrome P450 inhibition, and rat pharmacokinetic parameters. Functionally, **7** and **11** showed potent antagonist activity and displayed full inverse agonist activity in the [$^{35}$S]GTPγS $hH_3R$ binding assay[3] *N*-Methyl **7** and 4-fluorophenyl **11** decreased basal activity with $EC_{50}$ values of 2 ± 0.1 nM and 2.1 ± 0.1 nM, respectively. Compound **7** exhibited favorable in vitro metabolic stability across species in liver microsomes ($t_{½}$ = mouse (25 min), rat (39 min), dog and human (>40 min)) and had $IC_{50}$ values of >30 μM for cytochrome P450 isoforms 1A2, 2C9, 2C19, 2D6, and 3A4. Furthermore, compound **7** had excellent selectivity against $hH_1$, $hH_2$, and $hH_4$ receptor subtypes (<30% inhibition at 10 μM). In the rat, compound **7** exhibited acceptable rat iv pharmacokinetic properties ($t_{½}$ = 0.5 h, $V_d$ = 0.5 L/kg, CL = 12 mL/min/kg). However, it demonstrated poor oral exposure ($F$ <1%) (Table 2).

Compound **11** displayed high $hH_3R$ affinity ($K_i$ = 13 nM) and moderate rat $rH_3R$ affinity ($K_i$ = 48 nM). It showed excellent in vitro microsomal stability ($t_{½}$ >40 min in mouse, rat, dog, and human liver microsomes) and selectivity against cytochrome P450 isoforms ($IC_{50}$ >30 μM, CYP1A2, 2C9, 2C19, 2D6, and 3A4). Compound **11** not only showed excellent selectivity for $hH_1$, $hH_2$, and $hH_4$ receptor subtypes (<30% inhibition at 10 μM) and had good aqueous solubility ($pH_{2/7.4}$ >125 μg/mL), it also exhibited improved rat pharmacokinetic iv properties ($t_{½}$ = 2.3 h, $V_d$ = 4.8 L/kg, CL = 21 mL/min/kg) and oral bioavailability (%$F$ = 27, $C_{max}$ = 255 ng/mL) (Table 2). In addition, compound **11** had higher $c\log P$ ($c\log P$ = 4.1) and also showed high brain exposure 1 h post a 10 mg/kg ip dose with a brain/plasma ratio (B/P) = 11. Based on these overall profiles **7** and **11** were evaluated for in vivo activity in the rat dipsogenia model.

Administration of the $H_3R$ selective agonist, *R*-α-methylhistamine (RAMH), induces a transient increase in water drinking in rats.[7] This dipsogenic effect was used to measure in vivo $H_3R$ inhibition following systemic administration of $H_3R$ antagonists, as previously described.[3] Compounds **7** and **11** dose-dependently inhibited RAMH-induced dipsogenia with $ED_{50}$ values of 0.07 and 0.02 mg/kg, ip, respectively. This potent in vivo activity is consistent with the potency and high CNS penetration of these compounds.

Histaminergic neurons project from the tuberomamillary nucleus to multiple brain regions involved in vigilance, attention and sleep/wake. A number of $H_3R$ antagonists demonstrate wake promoting activity in preclinical species[8] and this effect has recently been reported in clinical trials with the $H_3R$ antagonists pitolisant and MK-0249.[10] Compounds **7** and **11** were therefore tested in the rat EEG/EMG model of wake promotion as previously described (Fig. 2).[3c,9] Compound **7** increased wake activity dose dependently from 3–30 mg/kg ip based on 4 h AUC ($P$ <0.001 ANOVA). Cumulative wake time at 10 mg/kg was 161 ± 6 min, with 235 ± 4 min wake achieved at 30 mg/kg ip by 4 h AUC values. At 30 mg/kg, waking was enhanced out to 4.5 h post dosing, and maximal cumulative wake surplus was ~200 min reached at 7 h. No effect on sleep rebound was observed. At 30 mg/kg, **7** demonstrated robust wake promotion, with the treated animals awake 98% of the time up to 4 h post dose, a 68% increase in wake time over the vehicle treated animals (Fig. 2). Compound **11** showed greater wake activity at 10 mg/kg ip (207 ± 5 min) compared to **7**, and demonstrated 94% wake time up to 4 h at 30 mg/kg ip (226 ± 6 min) (Fig. 2). At 30 mg/kg ip **11** was wake promoting for 7.5 h and no sleep rebound was observed out to 22 h post dose. No adverse EEG activity was noted for **7** or **11** at any dose. $H_3R$ antagonists have been shown to produce robust effects in rodent wake models, typically at doses higher than those in dipsogenia and cognition models, corresponding to 80–90% receptor occupancy.[9]

In conclusion, design of a new series of pyridazinone–phenethylamine analogs and development of $H_3R$ structure–activity relationships identified two lead compounds with $H_3R$ target potency, selectivity, metabolic stability, and rat pharmacokinetic properties. Compounds **7** and **11** exhibited potent $H_3R$ functional antagonist activity in vivo in the rat dipsogenia model and had robust wake-promoting activity in the rat EEG/EMG model. Compound **11** demonstrated improved oral bioavailability (%$F$ = 27) compared to **7**, however, ultimately due to hERG activity did not advance further.

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