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Novel morpholine ketone analogs as potent histamine H_3 receptor inverse agonists with wake activity

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ABSTRACT

Structure–activity relationship on a novel ketone class of H_3R antagonists/inverse agonists is disclosed. Compound **4** showed excellent target potency, selectivity and brain penetration. Evaluation of antagonist **4** in the rat EEG/EMG model demonstrated robust wake activity thereby establishing preclinical proof of concept.

The histamine H_3 receptor (H_3R) is a G-protein coupled receptor (GPCR) target of wide interest due to its ability to affect various central nervous system (CNS) functions. H_3Rs are predominantly expressed in presynaptic neurons, and function as inhibitory auto- and hetero-receptors. Blockade of H_3R can modulate the release of a variety of neurotransmitters including histamine, γ-aminobutyric acid (GABA), acetylcholine (ACh), norepinephrine (NE), serotonin (5-HT) and dopamine (DA).[1] As a result of their potential to modulate multiple CNS functions, H_3R antagonists are being clinically evaluated for the treatment of CNS disorders including neuropathic pain and deficits in sleep/wake and cognition (Fig. 1).[2] The benzazepine GSK-189254 advanced into clinical trials for Alzheimer's disease, pain and narcolepsy. BF-2649 advanced for cognitive enhancement in schizophrenic patients, daytime sleepiness in Parkinson's disease and sleep apnea syndrome.[3] Merck-Banyu compound MK-0249, advanced for schizophrenia, showed insomnia as a clinical effect.[4] We recently reported a novel series of pyridazin-3-one H_3R antagonists/inverse agonists which includes our clinical compound CEP-26401 (irdabisant).[5] In this Letter, we report our work on an earlier chemical class, morpholine ketones, which led to the identification of potent, selective and brain penetrant antagonist **4** establishing preclinical proof of concept in the rat sleep/wake model.

Although an advanced knowledge of H_3 ligand pharmacophore is currently available, some of the early pharmacophore based ligand design came from the weak H_3 activity shown by aplysamine-1, a marine natural product (Fig. 2).[6] Subsequently, the phenoxypropyl amine moiety was incorporated into the H_3 ligands by various research groups.[1,7] The diamine feature of aplysamine-1 was also used as a pharmacophore in the design of some of the H_3 ligands.[8] While the presence of dibasic moiety in the ligand provided very potent compounds, further testing revealed pharmacokinetic and potential safety issues due to phospholipidosis.

One of the initial diamine H_3 antagonists with wake promoting activity, JNJ-5207852, was reported to induce phospholipidosis and possess high brain residence time.[9] Following this, a related wake promoting compound (JNJ-7737782) with substantially reduced pK_a for the benzylamine moiety was reported (Fig. 2).[10] For our discovery project, ligands were designed with a keto group on the phenethyl side of aplysamine-1 pharmacophore generating a novel chemical class.

An initial compound from this ketone series, **1** had high affinity for human H_3 receptor with a K_i of 32 nM (Table 1). But replacement of piperidine ($pK_a = 7$) on the benzoyl side with morpholine ($pK_a = 4.7$) led to relatively weaker compounds (**2** and **3** with K_i of 66 and 106 nM, respectively). By incorporating the (*R*)-2-methylpyrrolidine moiety on the propyloxy tail, the H_3 binding affinity was significantly improved as illustrated by compound **4** with human and rat K_i of 5.5 and 50 nM, respectively. Incorporation of (*S*)-2-methylpyrrolidine (K_i of 100 nM) or the achiral 2,2-dimethylpyrrolidine (K_i of >1000 nM) led to big drop in binding affinity. Both (*R*)- and (*S*)-enantiomers of 2-methoxymethylpyrrolidine were also not tolerated. As shown in Table 1, methoxymethyl analogs

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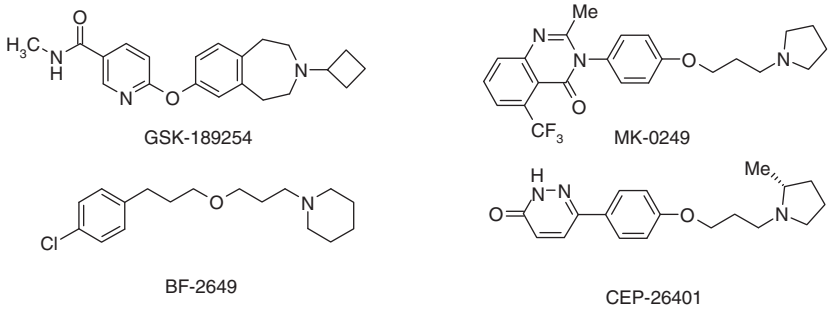


Figure 1. Structures of clinical H_3R antagonists.

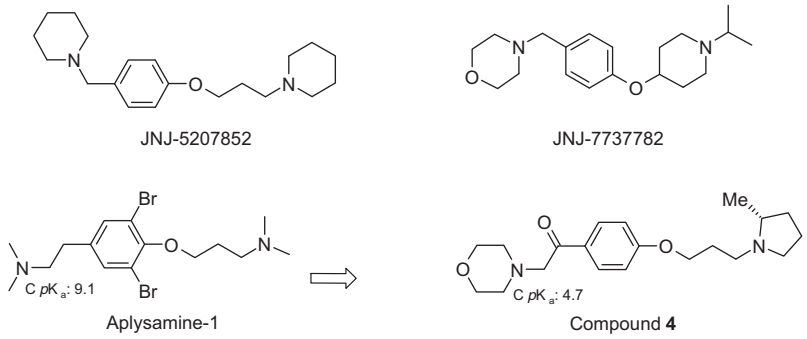


Figure 2. Structures of preclinical H_3R antagonists.

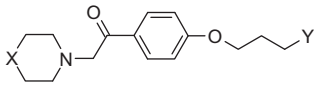
(compound **7** and **8**) showed H_3 affinities in the micromolar range which may be attributed to a combination of sterics and reduction in amine pK_a (from 10.2 for 2-methyl pyrrolidine to 9.4 for the 2-methoxymethyl pyrrolidine) of the propyloxyamine end. With (*R*)-2-methylpyrrolidine being the best amine moiety on the propyloxy side, the effect of substitution on the ketone side was briefly examined (Table 2). Both α-methyl ketone **9** and dimethyl ketone **10** maintained the affinity with K_i of 8 and 9 nM, respectively. *Meso*-dimethyl morpholine analog **11** also displayed potent binding affinity (K_i of 10 nM). Substitution of aryl ring at the *ortho* position to the keto group by small substituent such as Me did not offer any improvements. As shown by compound **12**, *ortho* methyl substitution led to reduced the binding affinity (K_i = 19 and 244 nM) for human and rat receptors.

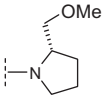
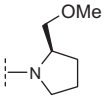
In vitro metabolic stability testing in liver microsomes showed that compound **9** was stable across species with $t_{½}$ of >40 min (mouse, rat, dog and human). Despite our best efforts, the diastereomers from compound **9** could not be separated. This may be attributed to potential rapid epimerization of the chiral center via enolization of keto functional group. The dimethyl ketone **10** displayed poor liver microsome stability for further advancement in our discovery flow with $t_{½}$ = >40 min for mouse but 9, 10 and 8 min for rat, dog and human, respectively. The unsubstituted compound **4** was stable across all four tested species with $t_{½}$ of >40 min (mouse, rat, dog and human). Functionally, compound **4** showed potent antagonist activity and displayed full inverse agonist activity in the [^{35}S]GTPγS hH_3R assay[5a,11] decreasing the basal activity with an EC_{50} value of 5.5 nM.

Based upon target affinity and physical property data ($pH_{7.4}$ solubility >145 μg/mL, ACD-log*D* = 0.26 and Tripos-PSA = 43.5), compound **4** was further profiled for selectivity against a panel of 70 GPCRs, ion channels and enzymes (MDS Pharma Services, LeadProfiler). Compound **4** displayed >50% inhibition at 10 μM concentration only for two out of 70 targets (67% and 78% against rat imidazoline-I_2 and human Sigma-1, respectively). Based on the favorable target H_3R affinity, selectivity, in vitro metabolic stability in liver microsomes and cytochrome P450 (CYP) inhibition selectivity (IC_{50} >30 μM for 1A2, 2C9, 2C19, 2D6, 3A4), **4** was further evaluated for pharmacokinetic properties in the rat (Table 3). Compound **4** displayed an iv $t_{½}$ of 2.3 h, clearance of 126 mL/min/kg, and oral bioavailability of 14% based on 6 h AUC data with high brain partitioning (ip 1 h *b*/*p* = 30.7). The relatively superior brain exposure compared to the plasma compartment prompted us to conduct the brain concentration time course study. Due to compound availability issue, racemate of **4** (human and rat K_i of 12 and 56 nM, respectively) was used for this study with the prior knowledge that there was no rat pharmacokinetics difference between the racemate and either of the enantiomers **4** and **5**. After 10 mg/kg ip dosing, the brain level of *rac*. **4** at 1 h, 4 h and 6 h time intervals was determined to be 3930 ± 592, 1609 ± 167 and 955 ± 41 ng/g, respectively. At 24 h post dose, compound *rac*. **4** was not detectable in the brain showing the lack of the undesired brain accumulation for this compound.

Following the demonstration of potency, selectivity and brain partitioning, compound **4** was further evaluated for wake promoting activity in the rat.[12] It has been shown that H_3R antagonists increase wakefulness at higher doses and receptor occupancy levels than those required for the cognition activity.[13] Wake promoting activity was measured as previously described using male Sprague Dawley rats surgically implanted for chronic recording of electroencephalographic (EEG) and electromyographic (EMG) signals.[14] Compound **4** increased wake activity dose dependently at 30 and 60 mg/kg ip based on cumulative time awake for 4 h post dosing (4 h AUC) (Fig. 3). AUC values were 118 ± 7, 163 ± 8, and 222 ± 6 min at 10, 30, and 60 mg/kg, respectively versus 101 ± 7 min for vehicle. At 60 mg/kg the treated animals were awake 93% of the time up to 4 h post dosing, a 2.2-fold increase in percent time awake over the vehicle treated animals. Both

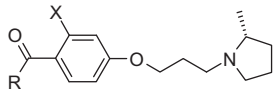
Table 1
Aminomethyl ketone H_3R binding data

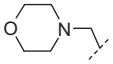
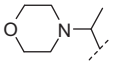
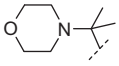
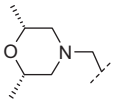
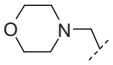


Compound	X	Y	hH_3R[a,b] (K_i, nM)	rH_3R (K_i, nM)
1	C		32	174
2	O		106	594
3	O		66	440
4	O		5.4	50
5	O		100	885
6	O		>1000	>1000
7	O		> 3000	> 3000
8	O		1170	> 3000

[a] [^{3}H]NAMH binding in membranes prepared from cells transfected with human or rat H_3R.
[b] K_i values are an average of at least two determinations. The assay-to-assay variation was typically within 2.5-fold.

Table 2
Aminomethyl ketone H_3R binding data



Compound	R	X	hH_3R[a,b] (K_i, nM)	rH_3R (K_i, nM)
4		H	5.4	50
9		H	8	105
10		H	9	33
11		H	10	78
12		Me	19	244

[a] [^{3}H]NAMH binding in membranes prepared from cells transfected with human or rat H_3R.
[b] K_i values are an average of at least two determinations. The assay-to-assay variation was typically within 2.5-fold.

slow-wave sleep (SWS) and rapid eye movement sleep (REMS) latencies were also significantly increased at 30 and 60 mg/kg ip (data not shown). No increase in sleep following the enhanced wake period (i.e. sleep rebound) was observed up to 22 h post dosing, nor any adverse EEG activity was evident at any dose. EEG activity in the theta range (~6–9 Hz) was increased 2–3 fold at 1 h after dosing with compound **4** at 30 and 60 mg/kg ip (ANOVA $P = 0.018$; Dunnett's test $P < 0.05$) (Fig. 4). Theta peak frequency was unchanged (ANOVA, $P > 0.05$). The increased theta power is consistent with previous reports of H_3R antagonist activity, and has been suggested to be indicative of pro-cognitive activity.[15]

Table 3
Pharmacokinetic properties for **4** in rat[a,b]

Rat PK parameters		**4**
iv	$t_{½}$ (h)	2.3 ± 0.6
	V_d (L/kg)	27 ± 11
	CL (mL/min/kg)	126 ± 28
po	6 h-AUC (ng/h/mL)	109 ± 5
	% *F*	14 ± 0.7
ip	1 h-Brain conc. (ng/g)	3768 ± 899
	B/P[c]	30.7 ± 3.2

[a] Administration of 1 mg/mg iv, 5 mg/kg po and 10 mg/kg ip; data calculated from 6 h AUC values.
[b] iv formulation: 3% DMSO, 30% solutol, 67% phosphate buffered saline; po formulation: 50% Tween 80, 40% propylene carbonate and 10% propylene glycol; ip formulation: 0.5% methylcellulose, 0.2% Tween 80 in water.
[c] *B/P* = Brain to plasma ratio.

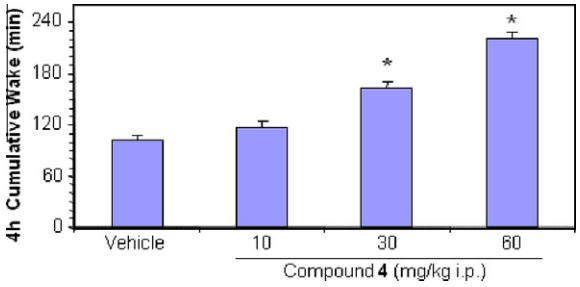


Figure 3. Compound **4**-induced wake promotion. Compound **4** was administered ip to male rats chronically implanted with electrodes for recording EEG and EMG activity. Cumulative Wake 4 h AUC values shown for each dose (mean + SEM, $n = 7$–8/group). * $p < 0.05$ Dunnett's *post hoc* versus vehicle.

The synthesis of discussed compounds was accomplished as previously described (Scheme 1).[11] Commercially available 4-hydroxyaryl alkyl ketones **13** were readily O-alkylated with 3-bromo-1-chloropropane and crude chloroether product was subjected α-keto bromination with elemental bromine. The selective amination of bromoketone **14** with cyclic amines was readily accomplished under mild conditions either at room temperature or at 60 °C. With the intermediate amino ketones **15** in hand, substitution of the less reactive chloro group with basic amines was accomplished via an in situ halogen exchange reaction completing the synthesis of compounds **1–9** and **11–12**. The dimethylketone analog **10** was synthesized by methylation of **15** (R = Me, X = H) at the α-keto position via enolate chemistry followed by amination.[11]

In summary, a novel morpholine ketone class of H_3 antagonists was disclosed from which, compound **4** displaying excellent H_3R target potency, selectivity and rat brain penetration was identified. Advanced into in vivo, compound **4** demonstrated potent wake-promoting activity in the rat EEG/EMG model thereby establishing preclinical proof of concept for this project. Compound **4** also showed pronounced enhancement of theta EEG with no adverse events.

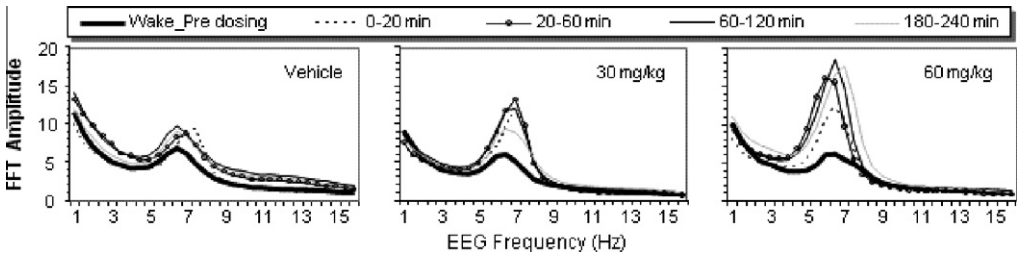
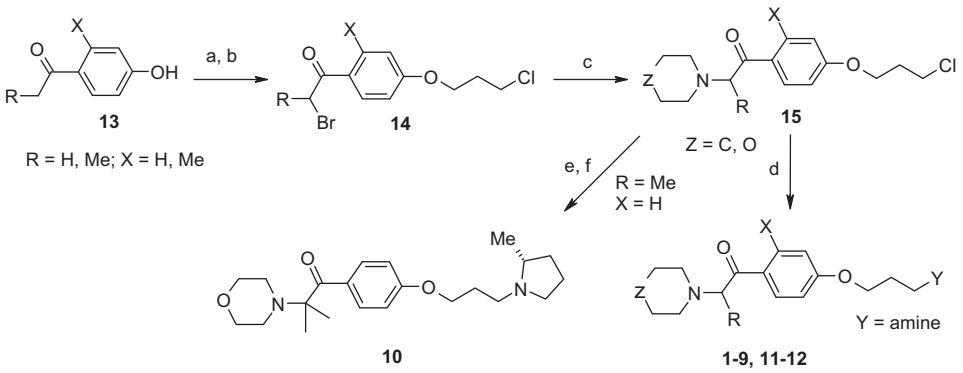


Figure 4. Compound **4**-induced enhancement of theta EEG power. Theta EEG power (FFT amplitude, mV^2/Hz) for time periods up to 4 h after dosing with compound **4** at 30 and 60 mg/kg ip versus vehicle.



Scheme 1. Reagents and conditions: (a) K_2CO_3, $Br(CH_2)_3Cl$, acetone, 80 °C, 15 h, 70–88%. (b) Br_2, Et_2O, rt, 50–70%; (c) cyclic amine, DIPEA/EtOH, rt or K_2CO_3/CH_3CN, 60 °C, 2–4 h; (d) pyrrolidine or piperidine, NaI, K_2CO_3, CH_3CN, 80 °C, 15 h (40–60%, 2 steps); (e) LDA, THF, MeI, -78 °C → rt, 60%; (f) (*R*)-2-methylpyrrolidine, DIPEA, 2-butanone, μ-wave, 180 °C, 30 min, 87%.

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