CEP-26401 (Irdabisant), a Potent and Selective Histamine H3 Receptor Antagonist/Inverse Agonist with Cognition-Enhancing and Wake-Promoting Activities

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ABSTRACT
CEP-26401 (irdabisant; 6-[4-[[3-((R)-2-methyl-pyrrolidin-1-yl)propoxy]-phenyl]-2H-pyrazidin-3-one HCl) is a novel, potent histamine H3 receptor (H3R) antagonist/inverse agonist with drug-like properties. High affinity of CEP-26401 for H3R was demonstrated in radioligand binding displacement assays in rat brain membranes (Kᵢ = 2.7 ± 0.3 nM) and recombinant rat and human H3R-expressing systems (Kᵢ = 7.2 ± 0.4 and 2.0 ± 1.0 nM, respectively). CEP-26401 displayed potent antagonist and inverse agonist activities in [³²P]guanosine 5'-O-(γ-thiotriphosphate binding assays. After oral dosing of CEP-26401, occupancy of H3R was estimated by the inhibition of ex vivo binding in rat cortical slices (OCC₅₀ = 0.1 ± 0.003 mg/kg) and antagonism of the H3R agonist Rα-methylhistamine-induced drinking response in the rat dipsogenia model was demonstrated in a similar dose range (ED₅₀ = 0.06 mg/kg). CEP-26401 improved performance in the rat social recognition model of short-term memory at doses of 0.01 to 0.1 mg/kg p.o. and was wake-promoting at 3 to 30 mg/kg p.o. In DBA/2NCrJ mice, CEP-26401 at 10 and 30 mg/kg i.p. increased prepulse inhibition (PPI), whereas the antipsychotic risperidone was effective at 0.3 and 1 mg/kg i.p. Coadministration of CEP-26401 and risperidone at sub efficacious doses (0.3 and 0.1 mg/kg i.p., respectively) increased PPI. These results demonstrate potent behavioral effects of CEP-26401 in rodent models and suggest that this novel H3R antagonist may have therapeutic utility in the treatment of cognitive and attentional disorders. CEP-26401 may also have therapeutic utility in treating schizophrenia or as adjunctive therapy to approved antipsychotics.

Introduction
The CNS histaminergic system is involved in regulation of sleep-wake, attention, and metabolic homeostasis via activation of a family of G protein-coupled receptors including H₁, H₂, H₃, and H₄ histamine receptors (Leurs et al., 2011). Of these, the H₃ histamine receptor (H₃R) was identified as a presynaptic autoreceptor modulating neuronal histamine release (Arrang et al., 1983). Subsequent studies demonstrated that H₃R also functions as a heteroreceptor to modulate the release of other neurotransmitters associated with cognitive function and the regulation of sleep-wake, attention, and metabolic homeostasis. H₃R agonists have been shown to improve performance in cognitive and attentional tasks in rodent models (Leurs et al., 2011). However, the development of H₃R antagonists has been limited due to the potential for antihistaminergic side effects. CEP-26401, a novel H₃R antagonist, has been shown to improve performance in the rat social recognition model of short-term memory and was wake-promoting at sub efficacious doses in rodent models. These results suggest that CEP-26401 may have therapeutic utility in the treatment of cognitive and attentional disorders.

ABBRIDAVATIONS: CNS, central nervous system; H₃R, histamine H₃ receptor; hH₃R, human H₃R; CEP-26401, 6-[4-[[3-((R)-2-methyl-pyrrolidin-1-yl)propoxy]-phenyl]-2H-pyrazidin-3-one HCl; ABT-239, 4-(2-[[2-ethyl-1-benzofuran-5-yl]benzamido]-2-methyl-pyrrolidin-1-yl)propoxy]-phenyl]-2H-pyridazin-3-one HCl; JNJ-31001074, 1-(4-cyclopropylpiperazin-1-yl)-1-[4-morpholin-4-ylmethyl)phe-nyl]-2-(2-methyl-1-pyrrolidinyl-ethyl}-1-benzofuran-5-yl)benzo-[H]-pyridazin-3-one HCl; ABT-239, 4-(2-[(2R)-2-methyl-3-pyridinecarboxamide;PF-36654746, (1R,3S)-N-ethyl-3-fluoro-3-[fluoro-4-(pyridinyl-1-ylmethyl)phenyl]cyclobutane-1-carboxamide; MK-0249, 2-methyl-3-[4-((3-pyrroli-din-1-yl)propoxy)phenyl]-5-trifluoromethyl-3H-quinazolin-4-one; JNJ-31001074, 1-(4-cyclopropylpiperazin-1-yl)-1-[4-morpholin-4-ylmethyl]phenyl] methanone.
ulation of sleep-wake, including acetylcholine, norepinephrine, dopamine, and serotonin (Esbenshade et al., 2008).

The discovery of the H₃R antagonists thioperamide and ciproxifan provided the pharmacological tools required for the exploration of the role of the H₃R in CNS function. Although active in multiple preclinical models, these early H₃R antagonists were limited as putative drug candidates because of poor target selectivity, lack of metabolic stability, potent interactions with the human ether-a-go-go-related gene potassium channel, cytochrome P450 inhibition, and poor brain penetration. Differences in pharmacology between the rodent and human H₃Rs that hindered early drug discovery were also identified (Ireland-Denny et al., 2001). Considerable efforts in academia and industry resulted in the discovery of H₃R antagonists/inverse agonists that have overcome many of these issues and provided tools to enhance the understanding of H₃R functions, with several compounds in clinical trials (Raddatz et al., 2010; Berlin et al., 2011; Brioni et al., 2011).

The histaminergic system in the brain is active during wakefulness, and inhibitors of postsynaptic H₁ histamine receptors are well established sedatives. The ability of H₃R antagonists to increase activity in the brain histaminergic system and enhance vigilance and wakefulness has been demonstrated in multiple species (Parmentier et al., 2007). In addition to the effects of H₃R antagonists on wake promotion in preclinical models, they are effective in decreasing the latency to the first episode of cataplexy in a genetically narcoleptic Doberman Pinscher model (Bonaventure et al., 2007), suggesting the potential to treat multiple symptoms of narcolepsy. A number of H₃R antagonists have also demonstrated efficacy across a broad range of preclinical models of cognitive function, including short- and long-term memory, working memory, spatial memory, and sensorimotor gating (Esbenshade et al., 2008). H₃R antagonists are currently being targeted for clinical use in the treatment of attention-deficit hyperactivity disorder, Alzheimer’s disease, schizophrenia, and other disorders associated with cognitive impairment, as well as in the treatment of narcolepsy and sleep-wake disorders (Raddatz et al., 2010; Berlin et al., 2011; Brioni et al., 2011; Leurs et al., 2011).

In the present report, the pharmacological properties and behavioral effects of the novel H₃R antagonist/inverse agonist CEP-26401 [6-{4-[3-((R)-2-methyl-pyrrolidin-1-yl)-propoxy]-phenyl}-2H-pyriridazin-3-one; irdabiasant] (Hudkins et al., 2011) (Fig. 1) are described. This selective, high-affinity H₃R antagonist potently enhanced short-term memory, improved PPI, and demonstrated robust wake promotion in preclinical models, confirming the potential utility of H₃R antagonists for the treatment of cognitive/attentional disorders as well as sleep disorders.

![Structures of CEP-26401 and ABT-239](image-url)
(complete EDTA-free protease inhibitor tablets; Roche Applied Science, Indianapolis, IN), homogenizing the cell pellets, removing the pellet formed after centrifugation at 1000g for 10 min at 4°C, and centrifuging the supernatant at 40,000g for 30 min at 4°C. The resulting membrane pellets were washed by resuspension in membrane buffer (50 mM Tris-HCl, pH 7.5, 0.6 mM EDTA, 5 mM MgCl₂, and protease inhibitors) and collection by centrifugation as described above. Final membrane pellets were resuspended in membrane buffer containing 250 mM sucrose and frozen at −80°C until use. Protein concentration was determined by using the Coomassie Plus kit (Thermo Fisher Scientific, Waltham, MA).

Radioligand Binding Assays. Rat cortical membranes were thawed on ice, washed three times with 30 ml of TE buffer (50 mM Tris, pH 7.4, with 5 mM EDTA) using a polycrylon and centrifuged at 39,000g for 10 min at 4°C. Radioligand binding to the H₃R was performed by modification of a previously described method (Kilpatrick and Michel, 1991). Washed membranes (200 µg of protein) were incubated with 0.2 nM [³H]NAMH with or without various concentrations of test compound at 30°C for 40 min in a total volume of 500 µl. The reaction was stopped by rapid filtration through presoaked (0.3% polyethyleneimine) glass fiber filters (GF/B, FPLR-124; Brandel Inc., Gaithersburg, MD) by using a Brandel cell harvester. The filters were washed five times with 1 ml of cold TE buffer (50 mM Tris, pH 7.4, with 5 mM EDTA), mixed with 5 ml of liquid scintillation cocktail (Ultima Gold; PerkinElmer Life and Analytical Sciences), and measured by using a liquid scintillation counter (Tri-Carb 3100TR; PerkinElmer Life and Analytical Sciences). Nonspecific binding was defined in the presence of 10 µM thioperamide.

Recombinant hH₃R- or hH₄R-expressing membranes were diluted to 0.5 to 1 mg protein/ml in 50 mM Tris-HCl, pH 7.5, 1% bovine serum albumin (BSA), and 5 mM MgCl₂ buffer and incubated for 4 h at 24°C with 0.8 to 2 nM [³H]NAMH, vehicle, or test compound and 0.5 mg/well FlashBlue scintillation beads (SPA beads; PerkinElmer Life and Analytical Sciences) in a final volume of 80 µl/well in a 96-well plate. Nonspecific binding was determined in the presence of 1 µM clopenthixol. Binding of [³H]NAMH to the hH₃R membranes was saturable with Bₘₐₓ = 1.9 ± 0.7 pmol/mg protein and Kᵣ = 1.2 ± 0.2 nM. The rat H₃R cell membranes expressed 1.1 ± 0.3 pmol/mg protein binding sites for [³H]NAMH with Kᵣ = 0.8 ± 0.2 nM. The saturation binding isotherms were best-fit to a single site. Membranes expressing hH₃ receptors (10 µg of protein; ES-390-M400UA; PerkinElmer Life and Analytical Sciences) were incubated with 1.5 nM [³H]pyrilamine (Kᵣ = 1.5 nM) and 0.5 mg of FlashBlue scintillation beads in a final volume of 80-µl buffer (50 mM Tris, pH 7.4, 5 mM MgCl₂, and 0.2% w/v BSA) with or without test compounds for 3 h at room temperature. Nonspecific binding was determined in the presence of 5 µM triprolidine. Membranes expressing hH₄ receptors (2.5 µg of protein; ES-391-M400UA; PerkinElmer Life and Analytical Sciences) were incubated with 5 nM [³H]histamine (Kᵣ = 2.7 nM) and 0.25 mg of scintillation beads in a final volume of 80-µl buffer (50 mM HEPES, pH 7.4, 100 mM NaCl, 5 mM MgCl₂, 1 mM CaCl₂, and 0.2% w/v BSA) with or without test compounds for 4 h at room temperature. Nonspecific binding was determined in the presence of 32 µM mexitil. Bound radioligand was determined by counting on a Wallac MicroBeta TriLux scintillation counter (PerkinElmer Life and Analytical Sciences) for all of the scintillation bead assays.

Membranes (10 µg of protein) expressing hH₄ receptors were incubated with 15 nM [³H]histamine (Kᵣ = 5.8 nM) with or without test compounds in a final volume of 200-µl buffer (50 mM Tris HCl, pH 7.5, 5 mM EDTA, and 10 µg/ml saponin) for 1 h at room temperature and then filtered through a 96-well filter plate pre-soaked in 0.3% (w/v) polyethyleneimine (Sigma-Aldrich) in wash buffer (cold 50 mM Tris HCl, pH 7.5) by using a Brandel cell harvester. Filter plates were rapidly washed four times and allowed to dry, and Betaplate scintillant (50 µl per well; PerkinElmer Life and Analytical Sciences) was added. Bound radioactivity was counted in a Wallac MicroBeta TriLux scintillation counter. Nonspecific binding was determined in the presence of 20 µM imetit.

In Vitro Functional Characterization by [³⁵S]GTPγS Binding Assay. Antagonist potency was determined by measuring inhibition of RAMH-induced [³⁵S]GTPγS binding in recombinant hH₃R and hH₄R membranes. Frozen aliquots of membranes containing recombinant hH₃ or hH₄ were thawed and diluted in assay buffer (200 mM NaCl, 20 mM MgCl₂, 1 mM EDTA and 1 mM dithiothreitol (DTT) with 30 µg/ml saponin in 20 mM HEPES, pH 7.4) to a final concentration of 0.5 mg of protein per milliliter. Membranes were premixed with 0.5 mg of SPA beads per 10 µg of membrane protein and 20 µM GDF in a 96-well plate. Test compounds or vehicle plus [³⁵S]GTPγS (final concentration 0.2 nM) were added. RAMH (100 nM), a concentration producing approximately 80% of maximum RAMH-induced signal in these assays, was added, and the plates were incubated for 45 min at room temperature. After centrifugation at 22°C at 250g for 5 min, bound radioactivity was measured by using a Wallac MicroBeta TriLux scintillation counter. Isolated rat cortical membranes (50 µg) were incubated with 0.1 nM [³⁵S]GTPγS, 400 µM GDP, and 100 to 220 nM RAMH, with or without test compounds, in assay buffer (50 mM Tris, pH 7.4, 100 mM NaCl, 5 mM MgCl₂, 1 mM EDTA, and 0.16 µg/ml DTT) for 60 min at 30°C. The reaction was stopped by rapid filtration onto GF/C plates (PerkinElmer Life and Analytical Sciences) by using a Brandel cell harvester and washed five times with 500 µl of wash buffer (20 mM HEPES, 100 mM NaCl, 20 mM MgCl₂, 1 mM EDTA, and 0.16 µg/ml DTT). The plates were dried, mixed with 50 µl of scintillant (Beta Scin; PerkinElmer Life and Analytical Sciences), and counted in a Wallac MicroBeta TriLux scintillation counter.

Inverse agonist potency was determined by measuring inhibition of basal [³⁵S]GTPγS binding in recombinant hH₃R and hH₄R membranes. Membranes were diluted in buffer (100 mM NaCl, 5 mM MgCl₂, 1 mM EDTA, and 1 mM DTT with 30 µg/ml saponin in 20 mM HEPES, pH 7.4) to provide 10 µg of protein per 20 µl. Diluted membranes and SPA beads (0.5 mg/well) were premixed with GDP (to provide 5 µM final in the assay) in a 96-well plate. Test compound or vehicle was added to the wells, followed by [³⁵S]GTPγS to a final concentration of 0.2 nM. Plates were incubated for 45 min at room temperature and then centrifuged at 22°C at 250g for 5 min before counting in a Wallac MicroBeta TriLux scintillation counter. Nonspecific binding was determined in the presence of 10 µM unlabelled 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.

Data Analysis for In Vitro Studies. Specific binding for the radioligand binding experiments was calculated by subtracting the mean of nonspecific binding obtained in each experiment from total binding. The percentage of specific binding was defined as individual specific binding/specific binding with test compound × 100. Binding displacement and concentration response data were analyzed to determine IC₅₀ or EC₅₀ values by nonlinear regression using the dose-response equation (variable slope) in Prism 4.03 (GraphPad Software, Inc., San Diego, CA; Y = bottom + (top − bottom)/(1 + 10[−LogEC₅₀ – X] × Hill slope)). Kᵣ or Kᵣ,app values were calculated from the EC₅₀ values.

Ex Vivo Autoradiography. CEP-26401 or vehicle (pH 2 water) was administered orally to Sprague-Dawley rats (male; 135–200 g; Charles River Laboratories, Inc., Wilmington, MA) 1 h before sacrifice by decapitation. Brains were removed and rapidly frozen in dry ice-cooled 2-methylbutane (Sigma-Aldrich). Coronal sections were prepared on microscope slides and tested for [³H]NAMH binding as described previously (Le et al., 2009). In brief, dried slides were incubated with 0.3 nM [³H]NAMH in 50 mM sodium phosphate buffer, pH 7.4, at 23°C for 30 min. The slides were washed, dried, and prepared for image acquisition (approximately 20 h) in a δ-imager 2000 chamber by using Beta Acquisition software (Biospace, Paris, France). The radioligand signal in the region of interest was measured in duplicate using the Beta vision software (Biospace), and average values were expressed as counts per minute per square
millimeter (cpm/mm²). The inhibition of specific [³H]NAMH binding ex vivo after administration of CEP-26401 was calculated relative to samples from vehicle-treated animals as follows: \((1 - (\text{individual specific binding/average of vehicle binding})) \times 100\). Nonspecific binding was determined in the adjacent sections in the presence of 10 μM thiorperamide. Specific binding was more than 90% of total binding. Plasma and brain samples were collected from the study animals in the ex vivo binding experiments and submitted for quantitative analysis to determine the respective compound concentrations as described previously (Hudkins et al., 2011).

### Rat Dipsogenia Model

RAMH-induced water intake was measured in male Long Evans rats (>300 g; Harlan, Indianapolis, IN) for 30 min beginning 20 min after administration of RAMH (10 mg/kg i.p.). CEP-26401 (in saline) was administered orally at the indicated times before the initiation of the drinking trial period. Percentage of inhibition of RAMH-induced drinking was calculated for each rat based on normalization to the group mean of RAMH-induced drinking by using the following equation: \([100 - (D_\text{r} / D_\text{w}) \times \text{RAMH}] \times 100\), where \(D_\text{r}\) is the amount of water an individual rat drinks, and \(D_\text{w}\) is the group mean for the amount of water consumed by the RAMH-treated group. Group means for percentage of inhibition were then calculated for each dosage group together with the associated S.E.M. Treatment effects for percentage of inhibition of CEP-26401 versus RAMH-induced dipsogenesis were evaluated by using a one-way ANOVA (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 CEP-26401 on short-term memory was determined in a rat social recognition model. Adult male rats (Sprague-Dawley; 350–450 g; Charles River Laboratories, Inc.) were briefly exposed to a male juvenile rat (Sprague-Dawley; 80–130 g; Charles River Laboratories, Inc.) (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). After short IEIs (15–30 min), adult rats investigated the juvenile for significantly less time during trial 2 relative to trial 1, indicating that the adult rat retained memory for the juvenile. Memory of the juvenile was lost as the IEI was increased, with a 2-h IEI producing relatively equal exploration times on both the first and second trials, resulting in a ratio of investigation duration (RID) near unity. A 2-h IEI was therefore used to test putative memory-enhancing compounds. Rats were dosed with CEP-26401 or vehicle (pH 2 water) 30 min before trial 2 for intraperitoneal dosing and 120 min before oral dosing. Controls included separate groups of rats that received effective doses of compound and were subsequently exposed to a novel juvenile in trial 2. Treatment effects on the RID were evaluated by using one-way ANOVAs (Prism 4). Dunnett’s post hoc analysis was performed for multiple comparisons with the vehicle group.

### Prepulse Inhibition in Mice

Male DBA/2NCrl mice (19–27 g; 7–9 weeks; Charles River Laboratories, Inc.) were administered CEP-26401 or vehicle (pH 2 water) 30 min before trial 2 for intraperitoneal dosing and 120 min before oral dosing. Controls included separate groups of rats that received effective doses of compound and were subsequently exposed to a novel juvenile in trial 2. Treatment effects on the RID were evaluated by using one-way ANOVAs (Prism 4). Dunnett’s post hoc analysis was performed for multiple comparisons with the vehicle group.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>H₂ Receptor Antagonist CEP-26401 127</th>
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<tr>
<td><strong>Kᵣ</strong></td>
<td>nM</td>
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<tr>
<td>Rat Cortical Membrane</td>
<td>Recombinant hRᵣ</td>
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<tr>
<td>CEP-26401</td>
<td>2.7 ± 0.3</td>
</tr>
<tr>
<td>Ciproxifan</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>ABT-239</td>
<td>15.6 ± 3.1</td>
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<tr>
<td>RAMH</td>
<td>1.7 ± 0.4</td>
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In vitro characterization of CEP-26401 binding affinities. Binding of the radiolabeled H₂R agonist [³H]NAMH (West et al., 1990) was saturable in rat cortical membranes with a \(K_v\) value of 2.0 ± 0.03 nM (mean ± S.E.M.) with the data being best-fit to a single-site binding isotherm. The H₂R antagonists ciproxifan (Lignneau et al., 1998) and ABT-239 (Ebsenahde et al., 2005), as well as the agonist RAMH (Arrang et al., 1987), displaced [³H]NAMH binding in rat cortical membranes (Table 1) as reported previously. CEP-26401 bound with high affinity (\(K_v = 2.7 ± 0.3\) nM) to a single site (Hill slope = 1 ± 0.2). In membranes from cells recombinitely expressing the full-length hH₂R, binding affinities were similar to those determined against native hH₂R in brain membranes.
cortical membranes (Table 1). Unlike ciproxifan (Table 1), CEP-26401 demonstrated balanced affinities at recombinant rat and human H3R with apparent Kᵢ values of 7.2 ± 0.4 and 2.0 ± 1.0 nM, respectively (Table 1) (Hudkins et al., 2011). Binding curves for the H3R antagonists in the recombinant systems were best-fit by a one-site model, but average Hill slopes ranged from 0.70 to 0.89, suggesting the possible presence of a small fraction of low-affinity sites for these ligands.

CEP-26401 demonstrated more than 1000-fold selectivity for H₃ versus recombinant human H₁, H₂, and H₄ histamine receptors (Table 2) and against 165 additional G protein-coupled receptor, ion channel, and enzyme targets (MDS Pharma Services, Taipei, Taiwan; data not shown).

Antagonist and Inverse Agonist Activity In Vitro. Antagonist potency was measured via inhibition of agonist-stimulated [³⁵S]GTPγS binding in rat cortical membranes and membranes expressing recombinant human or rat H₃R (Table 3). The H₃R agonist RAMH produced concentration-dependent increases in [³⁵S]GTPγS binding with an EC₅₀ of 52 ± 14 nM (n = 3) and a maximum stimulation of approximately 1.5-fold over basal in rat cortical membranes. The RAMH-induced response was inhibited by CEP-26401 with Kᵦ,app of 4.5 ± 1.0 nM (Table 3), demonstrating potent antagonist activity at rH₃R. Likewise, CEP-26401 potently inhibited RAMH-induced [³⁵S]GTPγS binding at recombinant H₃R (Kᵦ,app = 1.0 ± 0.2 nM) and hH₃R (Kᵦ,app = 0.4 ± 0.1 nM) (Hudkins et al., 2011). Antagonism was reversed by increasing concentrations of RAMH (data not shown). Ciproxifan and ABT-239 decreased basal [³⁵S]GTPγS binding in a concentration-dependent manner in the recombinant systems (Table 3), consistent with known inverse agonist activity of these compounds (Esbenshade et al., 2005). CEP-26401 also decreased basal activity with EC₅₀ values of 2.0 ± 0.8 and 1.1 ± 0.0 nM for rat and human H₃R, respectively (Table 3). The maximum reduction in basal [³⁵S]GTPγS binding was similar for CEP-26401 (300 nM), ciproxifan (10 μM), and ABT-239 (100 nM) at hH₃R (78 ± 5, 51 ± 20, and 67 ± 3%, respectively; n = 3) (Fig. 2A) and rH₃R (300 nM; 65 ± 4, 63 ± 2, and 63 ± 3%, respectively; n = 3) (Fig. 2B).

Inhibition of Ex Vivo [³⁵]NAMH Binding. An ex vivo autoradiography assay was used to estimate H₃R occupancy in rat cortex 1 h after oral administration of CEP-26401 as described previously (Le et al., 2009). Specific binding of [³⁵]NAMH to rat cortical slices was identified by quantitative autoradiography in regions of H₃R expression including striatum, cortex, hippocampus, and thalamus (Pillot et al., 2002a). Specific binding of [³⁵]NAMH was decreased in a dose-dependent manner in cortical slices prepared from CEP-26401-treated animals with an OCC₅₀ of 0.1 ± 0.0 mg/kg p.o. (Fig. 3). Plasma and brain concentrations of CEP-26401 measured in these animals 1 h after oral dosing at 0.1 mg/kg were 6.7 ± 1.0 ng/ml (22 nM) and 19.8 ± 3.8 ng/g (64 nM), respectively. These values are consistent with the previously reported pharmacokinetic properties of CEP-26401 in the rat, including high oral bioavailability (F of 83%) and good brain exposure (brain to plasma ratio of 2.6) (Hudkins et al., 2011).

CEP-26401 Activity in the Rat Dipsogenia Model. The H₃R-selective agonist RAMH induces increased water intake that can be blocked by the administration of H₃R antagonists (Clapham and Kilpatrick, 1993; Fox et al., 2002). The dipsogenic effect is produced after intraperitoneal or intracerebroventricular administration of RAMH, indicating interaction with central H₃Rs (Lecklin et al., 1998). This model was used to demonstrate the pharmacological effects of CEP-26401 in vivo. CEP-26401 dose-dependently inhibited RAMH-induced dipsogenia when administered 15 min before RAMH with an ED₅₀ value of 0.06 (0.01–0.3) mg/kg p.o. (Hudkins et al.,

### TABLE 2

<table>
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<tr>
<th>Assay</th>
<th>Percentage of Inhibition</th>
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<tr>
<td>hH₃R binding</td>
<td>10.6 ± 12.7</td>
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<tr>
<td>hH₃R binding</td>
<td>411 ± 99</td>
</tr>
<tr>
<td>hH₃R binding</td>
<td>13 ± 7.8</td>
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₃⁵]Hipsilamine binding.

₃⁵]Hiodotine binding.

₃⁵]Histamine binding.

### TABLE 3

<table>
<thead>
<tr>
<th>Antagonist Activity, Kᵦ,app [nM]</th>
<th>Inverse Agonist Activity, EC₅₀ [nM]</th>
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<tr>
<td>Rat Cortical Membrane</td>
<td>Recombinant rH₃R</td>
</tr>
<tr>
<td>CEP-26401</td>
<td>4.5 ± 1.0</td>
</tr>
<tr>
<td>Ciproxifan</td>
<td>0.1 ± 0.05</td>
</tr>
<tr>
<td>ABT-239</td>
<td>1.0 ± 0.8</td>
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### Fig. 2. Inverse agonist activity at recombinant human (A) and rat (B) H₃R. CEP-26401 (▪), ciproxifan (○), and ABT-239 (□) concentration-dependently inhibited basal [³⁵S]GTPγS binding in recombinant human (A) and rat (B) H₃R membranes. Membranes were incubated for 45 min with SPA beads, 0.2 nM [³⁵S]GTPγS, and increasing concentrations of test compounds or vehicle (to determine basal activity). Nonspecific binding was determined in the presence of 10 μM GTPγS. Mean ± S.D. (n = 3).
CEP-26401 Activity in the Rat Social Recognition Model of Short-Term Memory. In the social recognition model, CEP-26401 was effective at reducing the RID at doses over the range from 0.001 to 0.1 mg/kg i.p. (Fig. 5A) and at 0.01 and 0.1 mg/kg p.o. (Fig. 5B), demonstrating potent enhancement of short-term sensory memory in this model. A separate group of rats treated with 0.1 mg/kg i.p. CEP-26401 was handled as described above, with a novel juvenile rat being presented for trial 2 (0.1 + Novel; Fig. 5A). This group served as a control to determine whether CEP-26401 treatment resulted in changes in investigative behavior independent of the juvenile memory paradigm. The investigation duration for this group showed no difference from vehicle-treated animals, indicating no change in the general investigative behavior of the animals.

CEP-26401 Activity in the PPI Model of Sensorimotor Gating. DBA/2 mice demonstrate naturally poor PPI that is reliably increased with antipsychotics and the H3R antagonists ABT-239, thioperamide, and ciproxifan (Browman et al., 2004; Fox et al., 2005; Flood et al., 2011). CEP-26401 administration in this model increased the average percentage of PPI after doses of 10 and 30 mg/kg i.p. (Fig. 6A). CEP-26401 did not alter the amplitude of the startle reflex (Fig. 6B). The atypical antipsychotic risperidone also increased PPI at doses as low as 0.3 mg/kg i.p. (Fig. 6C). As observed elsewhere (Browman et al., 2004), the amplitude of the startle reflex was reduced after administration of risperidone at 1 mg/kg i.p. (data not shown). CEP-26401 was then coadministered with a dose of risperidone that did not produce effects in this model as a single agent (0.1 mg/kg i.p.). As in the single-agent administration study, 10 mg/kg i.p. CEP-26401 alone increased the average percentage of PPI and was not effective at a dose of 3 mg/kg i.p. In contrast, the combination of 3 mg/kg CEP-26401 and 0.1 mg/kg risperidone increased the average percentage of PPI (Fig. 6D). The combinations of 1 to 10 mg/kg CEP-26401 with 0.1 mg/kg i.p. risperidone did not alter no-stimulus and startle-alone amplitudes compared with both vehicles (data not shown). CEP-26401 and risperidone plasma and brain concentrations were determined in the DBA/2 mice and were not different when the compounds were dosed independently or coadministered (Table 4), suggesting the observed effects were not caused by increased exposure to either compound in the coadministration paradigm.

CEP-26401 Activity in Rat EEG Sleep/Wake Studies. CEP-26401 or vehicle was administered during the normal quiet period of the rat, and wake, slow-wave sleep (SWS), and rapid-eye movement sleep (REMS) activities were compared...
across treatment groups. CEP-26401 increased wake activity and decreased SWS and REMS in a dose-related manner for the first 4 h after dosing in the range tested (3–30 mg/kg p.o.; Fig. 7A). At 30 mg/kg, CEP-26401 demonstrated robust wake promotion with the treated animals awake 90% of the time up to 3 h postdosing (Fig. 7B). After the period of significant wake enhancement (up to 3, 3.5, and 5.5 h postdosing for 3, 10, and 30 mg/kg CEP-26401, respectively), the wake time for CEP-26401-treated animals did not differ from that of vehicle-treated animals for several hours (Fig. 7B), indicating the absence of immediate sleep rebound. Furthermore, no evidence of rebound hypersomnolence was observed up to 22 h after CEP-26401 administration (Table 5). SWS and REMS onset latencies were also increased after administration of CEP-26401 (Table 6). Motor activity was unchanged, and hyperactivity (motor activity normalized to time spent awake) was absent during the periods of enhanced wake produced by CEP-26401.

Discussion

Several imidazole-containing H₃R antagonists with high affinity for H₃R in rat tissues, including ciproxifan, have low affinity at the human H₃R (Lovenberg et al., 1999). CEP-26401 had balanced affinity for both rat and human H₃R and displayed more than 1000-fold selectivity for H₃R over the other histamine receptors (Tables 1 and 2). H₃R-induced effects are mediated via activation of Gₛ₄₀-type G proteins.
making the binding of $[^35]S$GTPγS a sensitive measure of receptor activation (Clark and Hill, 1996; Rouleau et al., 2002). Consistent with high-affinity binding, CEP-26401 demonstrated potent antagonism of H3R agonist-induced $[^35]S$GTPγS binding in the recombinant systems and rat brain membranes. Although inverse agonist activity in rat brain preparations has been reported for some functional measures of H3R activity (Morisset et al., 2000), only a small fraction of basal $[^35]S$GTPγS binding is modified by H3R activity in rat brain membranes (Clark and Hill, 1996; Rouleau et al., 2002). Membranes from recombinant H3R-expressing cells were used to determine inverse agonist activity for CEP-26401 and reference compounds with CEP-26401, ciproxifan, and ABT-239 demonstrating potent inverse agonist activity. The relevance of inverse agonist activity demonstrated in vitro to the function of H3R in physiological or pathological conditions in vivo is not clear because of the lack of neutral antagonists.

In vivo H3R occupancy was estimated by using autoradiographic ex vivo radioligand binding in rat cortical slices after oral administration of CEP-26401 that avoided the potential to underestimate receptor occupancy reported with ex vivo binding studies in tissue homogenates (Le et al., 2009). ID$_{50}$ values for reference compounds were similar to those determined by using an in vivo radiotracer to measure H3R occupancy (Le et al., 2009; Miller et al., 2009). The OCC$_{50}$ of 0.1 ± 0.0 mg/kg p.o. observed for CEP-26401 (Fig. 3) was attained at a total brain concentration of 64 nM, 20 times higher than the binding $K_i$ value of 2.7 nM determined in rat brain homogenates in vitro. Although CEP-26401 displayed low protein binding in dialysis studies with rat plasma proteins and ~40% free fraction when dialyzed with rat brain homogenate (Hudkins et al., 2011), these in vitro measures are estimates and may not reflect the actual free fraction in vivo. In addition, the preparation of brain homogenates and the presence of assay buffer components may affect binding affinities determined in vitro. CEP-26401 potently inhibited RAMH-induced drinking (ED$_{50}$ of 0.06 mg/kg p.o.) in the rat dipsogenia model, in agreement with the OCC$_{50}$ determined by ex vivo binding. This agreement may reflect the similar nature of inhibiting the pharmacological effect of RAMH in the dipsogenia model, compared with displacement of an agonist radioligand in the ex vivo binding assay. The efficacy and duration of activity in the dipsogenia model (Fig. 4) were consistent with the rat plasma half-life of CEP-26401 (2.5 ± 0.3 h) (Hudkins et al., 2011).

The expression of H3R in brain regions involved in memory, attention, and executive function (Martinez-Mir et al., 1990; Pillot et al., 2002a) along with the role of H3R in modulating neurotransmitter release (Ebenshade et al., 2008) led to the evaluation of H3R antagonists for potential clinical utility in the treatment of cognitive disorders associated with a number of disease states (Brioni et al., 2011). The rat social recognition model measures short-term memory (Thor and Holloway, 1982), and a number of memory-enhancing compounds with diverse mechanisms are active in this model including H3R antagonists (Prast et al., 1996; Fox et al., 2005). CEP-26401 demonstrated potent activity in this model without disrupting normal exploratory behaviors. Al-

### TABLE 5

Lack of sleep rebound after CEP-26401-induced wake promotion

<table>
<thead>
<tr>
<th>CEP-26401 Dose</th>
<th>Wake Times</th>
<th>Matching Time Period for Vehicle-Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 mg/kg p.o.</td>
<td>34.8 ± 1.4</td>
<td>33.7 ± 1.2</td>
</tr>
<tr>
<td>10 mg/kg p.o.</td>
<td>32.5 ± 0.7</td>
<td>34.1 ± 1.2</td>
</tr>
<tr>
<td>30 mg/kg p.o.</td>
<td>34.8 ± 1.6</td>
<td>35.1 ± 1.2</td>
</tr>
</tbody>
</table>

### TABLE 6

Effect of CEP-26401 on sleep latencies and motor activity after oral dosing

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vehicle</th>
<th>CEP-26401</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 mg/kg</td>
<td>10 mg/kg</td>
</tr>
<tr>
<td>SWS latency, min</td>
<td>32.9 ± 4.2</td>
<td>38.1 ± 5.4</td>
</tr>
<tr>
<td>REMS latency, min*</td>
<td>42.5 ± 3.5</td>
<td>86.0 ± 27.9</td>
</tr>
<tr>
<td>Motor activity²</td>
<td>4.74 ± 1.30</td>
<td>5.47 ± 0.72</td>
</tr>
<tr>
<td>Motor intensity³</td>
<td>2.94 ± 0.75</td>
<td>2.73 ± 0.52</td>
</tr>
</tbody>
</table>

*p < 0.05, ANOVA for treatment († P < 0.05 vs. vehicle by Dunnett’s test).

²Cumulative motor activity for 4 h postdosing, arbitrary units.

³Motor intensity = (average motor activity units for 2 h postdosing)/average EEG wake time (h) for 2 h postdosing.

Fig. 7. CEP-26401-induced wake promotion. CEP-26401 (saline as vehicle) was administered orally to male rats chronically implanted with electrodes for recording EEG and EMG activity. A, cumulative wake, SWS, and REMS 4 h area under the curve values are shown for each dose. Mean ± S.E.M. (n = 7–9/group). *p < 0.05, Dunnett’s test versus vehicle (Veh). B, dose-related increases in time awake (mean ± S.E.M.) were observed at 3, 10, and 30 mg/kg p.o. (n = 8, 8, and 6, respectively). Significant difference from vehicle (n = 10) for each 0.5-h time point is shown at the top for each dose. *p < 0.05, unpaired t test.

The expression of H3R in brain regions involved in memory, attention, and executive function (Martinez-Mir et al., 1990; Pillot et al., 2002a) along with the role of H3R in modulating neurotransmitter release (Ebenshade et al., 2008) led to the evaluation of H3R antagonists for potential clinical utility in the treatment of cognitive disorders associated with a number of disease states (Brioni et al., 2011). The rat social recognition model measures short-term memory (Thor and Holloway, 1982), and a number of memory-enhancing compounds with diverse mechanisms are active in this model including H3R antagonists (Prast et al., 1996; Fox et al., 2005). CEP-26401 demonstrated potent activity in this model without disrupting normal exploratory behaviors. Al-
though the estimated H₃R occupancy at effective doses of CEP-26401 was low, rodent models of short-term memory are particularly sensitive to the effects of H₃R antagonists (Fox et al., 2005; Medhurst et al., 2007) with a small percentage of occupancy (as low as <10%) producing efficacy in cognition models (Miller et al., 2009).

Effects in preclinical models of PPI suggest that compounds of various classes, including H₃R antagonists may have utility in the treatment of schizophrenia (Browman et al., 2004; Fox et al., 2005; Flood et al., 2011). However, whereas the DBA/2 mouse PPI model is sensitive to H₃R antagonists, other preclinical models relevant to schizophrenia do not robustly respond to H₃R antagonists (Burban et al., 2010; Flood et al., 2011). Although CEP-26401 effectively increased the PPI response in DBA/2 mice, fully efficacious doses (Fig. 6) were higher than those effective in the rat social recognition model (Fig. 5). When coadministered with the antipsychotic risperidone a lower dose of CEP-26401 (3 mg/kg i.p.) was active in this model, lowering the effective dose of risperidone from 0.3 to 0.1 mg/kg i.p. Previous studies suggested increased efficacy of haloperidol in a rodent catalepsy model after coadministration of the imidazole-based H₃R antagonist ciproxifan (Pilot et al., 2002b). However, ciproxifan inhibited cytochrome P450 enzymes with the coadministration decreasing haloperidol metabolism, complicating interpretation of these studies (Zhang et al., 2005). Unlike ciproxifan, CEP-26401 does not inhibit cytochrome P450 enzymes (Hudkins et al., 2011), and the plasma and brain concentrations of CEP-26401 and risperidone were unaltered by coadministration. As an adjunctive therapy, CEP-26401 could thus result in effective treatment of schizophrenia at lower doses of the antipsychotic with correspondingly lower incidence of side effects. Treatment with CEP-26401 may also address the cognitive deficits associated with schizophrenia that are poorly controlled by currently used drugs. Antipsychotics interact with a broad spectrum of receptors, including the H₃R. H₂R antagonism would be expected to increase histamine release, resulting in increased H₃R activation. The effects of such interactions on the efficacy and side effect profile of antipsychotics coadministered with H₃R antagonists should be considered in future studies. H₃R antagonists increase wake in several species, albeit at dose ranges higher than those demonstrating efficacy in models of enhanced cognition. The maintained wake level was limited to 50 to 60% time awake, compared with baseline activity of 25 to 30% wake (Le et al., 2008). Robust wake promotion (85 and 188% increases over vehicle in the 4 h after oral dosing) was produced by CEP-26401 at 10 and 30 mg/kg p.o., respectively (Fig. 7A). The enhanced wake was not accompanied by increased motor activity or intensity, or sleep rebound, an effect similar to that seen with modafinil but distinct from that of stimulants such as amphetamine (Parmentier et al., 2007).

Wake-promoting doses of CEP-26401 and those doses active in the PPI model produced full occupancy of cortical H₃R as determined via the ex vivo occupancy study (Fig. 3), whereas the doses active in the social recognition model correspond to lower receptor occupancy. This difference has been demonstrated for a number of H₃R antagonists and may reflect effects on different brain regions and neurotransmitters. For example, the H₃R antagonist ABT-239 increased cortical ACh release at doses as low as 0.1 mg/kg i.p., whereas cortical dopamine release was increased only at 3 mg/kg (Fox et al., 2005). Distinct effects on neurotransmitters in different brain regions were described with the H₃R antagonist 6-(3-cyclobutyl-2,3,4,5-tetrahydro-1H-3-benzazepin-7-yl)oxy]-N-methyl-3-pyrindin-2-carboxamide (GSK189254), which increased ACh release in the anterior cingulate cortex at 1 and 0.3 mg/kg in the dorsal hippocampus (Medhurst et al., 2007). These regional and neurotransmitter-selective effects may be reflected in the differential potencies seen in models of short-term memory, which may depend on ACh, versus models in which modulation of dopamine levels is thought to play a key role, such as PPI. In pilot clinical trials the H₃R antagonist tiprolisant (BP2.649) decreased daytime sleepiness in narcoleptics (22 patients) (Lin et al., 2008), confirming a potential role for H₃R antagonists in treating narcolepsy. 2-methyl-3-[4-(3-pyrrolidin-1-ylpropoxy)phenyl]-5-trifluoromethyl-3H-quinoxalin-4-one (MK-0249) increased sleep latency in 24 healthy subjects after a period of sleep deprivation (Iannone et al., 2010). The effects on sleep and vigilance in early clinical trials suggest a role for H₃R antagonists in treating excessive daytime sleepiness resulting from a variety of causes and indicate that rodent models may underestimate the wake-promoting effects of this class of compounds in humans. CEP-26401 was well tolerated up to 100 mg/kg p.o. in the rat Irwin behavioral battery (Hudkins et al., 2011), providing a large estimated therapeutic index in comparison to the active dose of 0.01 mg/kg p.o. in the social recognition model and a therapeutic index more than 33 in comparison to the wake-promoting dose of 3 mg/kg p.o. in the rat.

The present data demonstrate potent pharmacological effects after oral dosing of CEP-26401, a compound with favorable drug-like properties (Hudkins et al., 2011), and suggest that this novel H₃R antagonist may have therapeutic utility in the treatment of cognitive and attentional disorders. In addition to tiprolisant and MK-0249, several H₃R antagonists including ABT-288, AZD-5213, 1-(4-cyclopropylpipеразин-1-yl)-1-[4-(morpholin-4-ylmethyl)phenyl] methane (JNJ-31001074), (1R,3R)-N-ethyl-3-fluoro-3-[3-fluoro-4-(pyrrolidin-1-ylmethyl)phenyl)cyclobutane-1-carboxamide (PF-03654746), GSK239512, and MK-3134 have advanced to clinical trials for the treatment of sleep disturbances and/or cognitive disorders associated with Alzheimer’s disease, attention-deficit hyperactivity disorder, narcolepsy, or schizophrenia (Brioni et al., 2011; Leurs et al., 2011). Results from the clinical trials with H₃R antagonists are eagerly awaited to establish the potential for novel CNS active drugs with advantages over the currently used stimulants, nootropics, and/or antipsychotics.

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Conducted experiments: Raddatz, Hudkins, Mathiasen, Gruner, Flood, Aimone, and Le.
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