Discovery of Histamine H3 Antagonists for the Treatment of Cognitive Disorders and Alzheimer’s Disease

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ABSTRACT

H3 antagonists increase the release of brain histamine, acetylcholine, noradrenaline, and dopamine, neurotransmitters that are known to modulate cognitive processes. The ability to release brain histamine supports the effect on attention and vigilance, but histamine also modulates other cognitive domains such as short-term and long-term memory. A number of H3 antagonists, including 1-[3-[3-[4-(chlorophenyl)propoxy]propyl]piperidine hydrochloride (BF2.649), 1-{3-[3-(4-chlorophenyl)propoxy]propyl}piperidine; GSK189254, 6-{3-cyclobutyl-2,3,4,5-tetrahydro-1H-3-benzazepin-7-yloxy}-N-methyl-3-pyridinecarboxamide hydrochloride (GSK189254), MK-0249 (structure not yet disclosed), JNJ-17216498 (structure not yet disclosed), and ABT-288 (structure not yet disclosed), have advanced to the clinical area for the potential treatment of human cognitive disorders. H3 antagonists exhibited wake-promoting effects in humans and efficacy in narcoleptic patients, indicating target engagement, but some of them were not efficacious in patients suffering from attention-deficit hyperactivity disorder and schizophrenic patients. Preclinical studies have also shown that H3 antagonists activate intracellular signaling pathways that may improve cognitive efficacy and disease-modifying effects in Alzheimer’s disease. Ongoing clinical studies will be able to determine the utility of H3 antagonists for the treatment of cognitive disorders in humans.

INTRODUCTION

Histamine is an important biogenic amine that modulates many physiological responses in humans. Its biological actions are mediated via four histamine receptors named H1, H2, H3, and H4, a classification based on their sequence, their link to differential intracellular signaling mechanisms, and their unique pharmacological profile (Haas and Panula, 2003; Leurs et al., 2005; Esbenshade et al., 2008). The H1 and H2 receptors are druggable targets as indicated by the efficacy of these antagonists in the treatment of allergy and ulcers, respectively; the role of the H4 receptors is unclear at the present time, although preclinical evidence suggests a potential role in inflammation and pain processes.

Extensive preclinical data with histamine H3 receptor antagonists support their potential utility for the treatment of human cognitive disorders. The discovery of potent and selective H3 antagonists have overcome many of the liabilities of earlier antagonists, confirmed the preclinical data obtained with early agents, and significantly expanded our knowledge in this area. In this article, we review the latest preclinical and clinical
data on histamine H₃ antagonists, because 1-{3-[3-(4-chloro-
phenyl)propoxy]propyl}piperidine hydrochloride (BF2.649),
(1R,3R)-N-ethyl-3-fluoro-3-[3-fluoro-4-(pyrrolidin-1-ylmethyl)phen-
nyl]cyclobutane-1-carboxamido (PF-03654746), 6-{[(3-cyclobutyl-
2,3,4,5-tetrahydro-1H-3-benzazepin-7-yloxy)-N-methyl-3-pyridin-
ecarboxamido hydrochloride (GSK189254), GSK239512, MK-0249
(structure not yet disclosed), MK-3134 (structure not yet dis-
closed), JNJ-17216498, and ABT-288 have advanced to phase 1
and phase 2 stages in the clinical area for the potential treatment
of sleep disorders, attention-deficit hyperactivity disorder (ADHD),
cognitive deficits in schizophrenia, and Alzheimer’s disease (AD).

### Histamine H₃ Receptor Pharmacology

The histamine H₃ receptor was first described in 1983 as
an autoreceptor that regulated histamine release (Arrang et
al., 1983), and 16 years later the DNA sequence was eluci-
dated, structurally confirming it as a member of the G pro-
tein-coupled receptor family (Lovenberg et al., 1999). This
receptor exhibits highest homology (~60% in the transmem-
brane domains) to the H₄ receptor but much lower homology
(~20%) to the H₁ and H₂ receptors. In the time since its
cloning there has been considerable advancement in our
knowledge about H₃ receptor molecular properties that have
been described in detail previously (Leurs et al., 2005; Es-
benshade et al., 2008).

The full-length human and rat H₃ receptor is composed of
445 amino acids; however, at least 20 human and nine rat H₃
receptor mRNA isoforms resulting from alternative splicing
of the receptor gene have been identified. Truncations of the
third intracellular loop, variations in the amino and carboxyl
termini, and deletions of transmembrane domains account for
the number and diversity of H₃ receptor isoforms. At least
eight human and three rat isoforms are functionally active,
demonstrating binding and/or signaling activity when ex-
pressed in recombinant cell systems (Bongers et al., 2007;
Esbenshade et al., 2008).

The distribution of H₃ receptors was examined in postmor-
tem human brain tissue, and autoradiographic studies indi-
cate high levels in the globus pallidus, caudate, putamen,
hippocampus, and limbic and frontal cortical regions. Recent
studies with a novel [¹¹C]-PET ligand in humans confirmed a
high expression of H₃ receptors in the caudate and putamen
and intermediate expression in the cortex and low levels in
the cerebellum (Ashworth et al., 2010). A comparable pattern
of H₃ receptor expression is observed in rats, with high ex-
pression in the cortex, hippocampus, striatum, and hypothal-
amus. Of particular interest is the differential expression of
three rat isoforms in the hippocampus, locus coeruleus, and
raphe nucleus that could lead to a unique regulation of ace-
tycholine (ACh), noradrenaline (NE), and serotonin (Drutel
et al., 2001). On the other hand, low expression of H₃ recep-
tors has been detected in heart, placenta, lung, liver, and
other peripheral tissues (Lovenberg et al., 1999). The high
levels of expression in the brain in comparison with the
periphery makes the H₃ receptor an attractive drug target,
because the possibility of mechanistic-based peripheral side
effects is low.

The H₃ receptor plays a modulatory role as an autoreceptor
in regulating the release of histamine and as a heterorecep-
tor regulating the release of ACh, NE, and dopamine (DA).
Although the precise signaling events associated with this
function at the synaptic level are not well understood, mech-
anistic studies on neurotransmitter release suggest a role for
protein kinase A and voltage-gated calcium channels. These
signaling events are downstream from H₃ receptor activation
of Go/i/o proteins that results in increased guanosine 5’-3-O-
(thio)tri phosphate binding and inhibition of adenylyl cycl-
ase in brain tissues. A wide range of other H₃ receptor/Go/i/
o-mediated signal transduction pathways have also been
identified in recombinant cell systems that include activation
of mitogen-activated protein kinase, glycogen synthase ki-
nase 3β (GSK3β), Akt, and phospholipase A₂, as well as
inhibition of adenylyl cyclase and the Na⁺/H⁺ exchanger
(Bongers et al., 2007).

There are differences in the binding affinities of H₃ recep-
tor antagonists across species that are attributable to differ-
ences in two amino acids in transmembrane 3. Whereas
early-generation H₃ receptor antagonists, including imid-
azole- and nonimidazole-based structures, were generally
more potent at rodent than human receptors, more recent
imidazole-based H₃ receptor antagonists are up to 10-fold
more potent at human than rat receptors. These differences
are important to be able to identify compounds with both
human and rat potency as well as from a clinical perspective
to translate exposure from rodents to humans related to
cognition or CNS targets of signif engagement (wakfulness).

An interesting characteristic of the H₃ receptor is its abil-
ity to transduce signaling in the absence of agonist activa-
tion, thus demonstrating inherent constitutive activity (Ar-
rang et al., 2007). By definition, all H₃ antagonists block the
activity of endogenous histamine. In addition, the vast ma-
majority act as inverse agonists by reversing its constitutive
activity. It is unclear at the present time what is the phar-
macological relevance of inverse agonism versus antagonism
in the in vivo situation, thus, for the purposes of this review
these compounds will be referred to as H₃ receptor antagonists.

### H₃ Receptor Modulation of Neurotransmitter
Release

Whereas histaminergic neuronal soma reside exclusively
in the posterior hypothalamus, specifically the tuberomam-
illary nucleus (TMN), histaminergic fibers project through-
out most regions of the brain including the cortex, striatum,
thalamus, hippocampus, hypothalamus, locus coeruleus, and
spinal cord. Although originally described as a presynaptic
autoreceptor controlling histamine release (Arrang et
al., 1983), the H₃ receptor also functions as a heteroreceptor
regulating the release of other neurotransmitters. Similar to
autoreceptor inhibition, the release and interaction of hista-
mime with Gi protein-coupled H₃ heteroreceptors on axoax-
onic postsynaptic terminals leads to the inhibition of neuro-
transmitter release (ACh, etc.). Histaminergic neurons were
initially characterized as a homogenous cell population by
anatomical studies; however, recent data have revealed that
these neurons are organized into distinct circuits enabling H₃
receptors to selectively influence signaling in different brain
regions.

The initial characterization of H₃ autoreceptors used his-
tamine release from brain slices, and the first report of H₃
antagonist-evoked histamine release in the whole animal
was demonstrated in the hypothalamus of thiopentamide-
treated rats (Itoh et al., 1991; Mochizuki et al., 1991). H₃
Histamine \( H_3 \) Receptors and Cognition

Cognitive processes in humans include several domains including attention, short-term memory, working memory, and long-term memory. In view of the neurochemical effects of \( H_3 \) antagonists enhancing the release of brain histamine, it is important to review the role of histamine on attention and wake. Wakefulness during the day is maintained by the actions of several neurotransmitters systems, including histamine, NE, glutamate, ACh, orexin, and GABA, playing a unique role in the initiation and maintenance of wake. Wakefulness is characterized by cortical activation and behavioral arousal that can be detected by electroencephalogram and electromyography techniques (Jones, 2005; Schwartz and Roth, 2008).

Histamine plays a physiological role in the light/dark cycle, because histamine release increases during the light phase whereas it decreases to baseline levels during the dark phase. The widespread projection from the TMN histaminergic neurons to different brain areas is one of the outputs of the circadian rhythm set by the hypothalamus leading to activation of brain arousal mechanisms. These neurons induce activation of the cortex because they are active during the wake period and eventually cease firing during the rapid
eye movement sleep phase. Agents that increase histaminergic activity (histamine, \( H_1 \) agonists, or inhibitors of histamine metabolism) increase wake in animals; in contrast, agents that decrease histaminergic activity (inhibitors of histidine decarboxylase such as \( \alpha \)-fluoromethylhistidine, or the \( H_3 \) antagonists mepyramine and diphenhydramine (DPh)) promote sedation and sleep (Monti et al., 1986; Barbier and Bradbury, 2007). Genetic manipulation of the histamine pathway also supports its key role in vigilance. Histidine decarboxylase-KO mice show permanent changes in the sleep–wake cycle with increased somnolence, and these KO mice are unable to be awake when vigilance is required; similarly, \( H_3 \)-KO mice exhibit disruption of the circadian rhythm with decreased activity during the active phase (Inoue et al., 1996; Barbier and Bradbury, 2007).

\( H_3 \) antagonists increase histamine release in rats, and thioperamide, ciproxifan, and BF2.649 increase wake in cats; similarly, 1-[3-[4-(piperidin-1-ylmethyl)phenoxyl]propyl]piperidine (JNJ-5207852), 4-(3-[4-piperidin-1-ylbut-1-yl]benzy1)morpholone (JNJ-10181457), GSK189254, and ABT-239 increase wake in rodents. Because ciproxifan does not promote wakefulness in \( H_3 \)-KO mice, these data indicate that the wake-promoting effect of \( H_3 \) antagonists is mediated by increased histamine release stimulating postsynaptic \( H_3 \) receptors (Lin et al., 1990; Barbier et al., 2004; Bonaventure et al., 2007).

Preclinical studies have shown that CNS-penetrant \( H_3 \) antagonists such as DPh increase total sleep, an effect that is not observed with the second-generation \( H_1 \) antagonists that do not readily cross the blood-brain barrier. The effects of DPH on sleep in healthy volunteers are not robust, but in humans suffering insomnia DPh provides a significant decrease in the severity of insomnia. These data indicate that DPH is more effective in states of higher arousal in comparison with normal individuals. DPH increases subjective sleepiness in humans and impairs performance in attention/working memory tests (Gevins et al., 2002). \( H_3 \) antagonists also exhibit affinity for muscarinic receptors, and although the contribution of the muscarinic effect is difficult to resolve, considering the totality of the data, the effects of DPH and \( H_1 \) blockers on sleep are consistent with the histaminergic hypothesis and most likely \( H_1 \)-related in human.

The importance of the histaminergic system in vigilance is also demonstrated by clinical studies in which \( H_3 \) antagonists increase wake in humans. During phase 1 studies with PF-03654746, the clinical team observed sleep disturbances after multiple dosing (Soares et al., 2009) and estimated that it occurred at high receptor occupancy levels in humans (>70%). Furthermore, the relationship between \( H_3 \) receptor occupancy and sleep in humans was determined with MK-0249 by the Merck team; MK-0249 (2.5–50 mg) reached high occupancy in the striatum (93% at 50 mg), with patients reporting difficulty getting to sleep starting at the 5-mg dose that reached 72% occupancy (Iannone et al., 2009). These observations are consistent with the preclinical evidence demonstrating wake-promoting effects of \( H_3 \) antagonists in animals.

\( H_3 \) antagonists as a class exhibit alerting effects caused by brain \( H_3 \) receptor occupancy, and if occupancy is high at night a common side effect is difficulty getting to sleep. If the desired occupancy is achieved during the day, \( H_3 \) antagonists may thus be useful for the treatment of excessive sleepiness and narcolepsy. \( H_3 \) antagonists are effective in two animal models of narcolepsy; GSK189254 is a potent \( H_3 \) antagonist that exhibits procognitive effects in several animal models and also increases wake in wild-type mice and orexin-KO mice. Likewise, JNJ-5207852 and JNJ-10181457 are potent \( H_3 \) antagonists with procognitive and wake-promoting effects in mice and rats. JNJ-10181457 also reduced the number of cataplectic attacks in narcoleptic dogs (Barbier et al., 2004; Bonaventure et al., 2007).

A study in narcoleptic patients demonstrated that the \( H_3 \) antagonist BF2.649 (pitolisant, 40 mg every day for 7 days) produced a significant reduction in the number of diurnal sleep episodes, with efficacy equal in magnitude to the approved agent modafinil (Lin et al., 2008). BF2.649 also reduced the duration of the sleep episodes in narcoleptic patients. After 5 days of treatment BF2.649 was effective in both measures at 100 mg/ml plasma levels. BF2.649 also decreased excessive sleepiness in patients with Parkinson’s disease, and phase 3 trials are ongoing (5- to 40-mg doses).

These clinical data indicate that \( H_3 \) antagonists can have therapeutic properties in patients suffering excessive sleepiness and that the mechanism is probably caused by the increased release of brain histamine. Taken together, a large body of research demonstrates that the histaminergic system plays a key role in waking and attention. Several \( H_3 \) antagonists promote wake in preclinical models of narcolepsy as we discussed previously, and BF2.649 is effective in humans suffering narcolepsy.

Preclinical and clinical data indicate that \( H_3 \) antagonists increase vigilance and wake, which affect other cognitive domains. The exogenous administration of histamine facilitated long-term retention in the inhibitory avoidance test in mice after intracerebroventricular administration (de Almeida and Izquierdo, 1986) and improved short-term memory in the social recognition test. A similar effect was induced by histidine administration, whereas inhibition of histamine synthesis by \( \alpha \)-fluoromethylhistidine impaired short-term memory (Prast et al., 1996). Intrahippocampal injections of histamine also ameliorate spatial memory deficits induced by (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d] cyclohepten-5,10-imine maleate (MK-801) (Xu et al., 2005). These studies indicate that brain histamine plays a role in short-term, long-term, and spatial memory.

The intracerebroventricular administration of the \( H_3 \) antagonist thioperamide improved short-term memory in the social recognition test (Prast et al., 1996), and when injected systemically it improved performance in an attention model in SHR pups as well as other cognitive domains as shown in Table 1 (see also Hancock and Fox, 2004). In contrast, systemic injections of the \( H_3 \) agonists imetit and (R)-\( \alpha \)-methylhistamine impaired performance in the object recognition test that measures working memory and in the 24-h inhibitory avoidance test measuring long-term memory (Blandina et al., 1996).

ACh plays an important role in attention and memory processes, which is supported by the clinical efficacy of donepezil in AD. The ability of \( H_3 \) antagonists to increase ACh release in the brain makes this an attractive target for the treatment of cognitive disorders. It is noteworthy that the effect of \( H_3 \) agonists impairing memory have been correlated with the inhibitory effect on ACh release, indicating the \( H_3 \) antagonists may regulate memory via the central cholinergic system (Blandina et al., 1996).
With regard to the effects of novel H₃ antagonists, ABT-239 binds with 2 nM potency to rat H₃ receptors and increases the release of histamine, ACh, and DA in the rat prefrontal cortex (Esbenshade et al., 2005). ABT-239 improved performance in the five-trial inhibitory avoidance test in SHR pups, the social recognition test, the 24-h inhibitory avoidance test, and spatial memory tasks (Fox et al., 2005). GSK189254 is a potent H₃ antagonist (human Kᵢ = 0.2 nM; rat Kᵢ = 1 nM) that increases the release of ACh, NE, and DA in the rat cingulate cortex. At the behavioral level, GSK189254 improved performance in the rat object recognition task after 24- to 48-h delays and improved attentional set shifting and spatial learning in aged rats (Medhurst et al., 2007a).

BF2.649 binds with potent affinity to human (Kᵢ = 2.7 nM) and rat (Kᵢ = 17 nM) H₃ receptors, and it increases the release of ACh and DA from rat cortex. In behavioral studies BF2.649 improved the retention of memory in the object recognition task that measures working memory (Ligneau et al., 2007). Similar data have been reported for JNJ-10181457; this compound has been described as a potent H₃ antagonist (human Kᵢ = 1 nM; rat Kᵢ = 1 nM) that increases the in vivo release of ACh, NE, and DA in the rat prefrontal cortex. JNJ-10181457 facilitated acquisition of the inhibitory avoidance response in SHR pups and improved performance in the delayed nonmatching to position task in scopolamine-treated rats (Leurs et al., 2005; Bonaventure et al., 2007; Galici et al., 2009).

In addition to the facilitatory effects on different cognitive domains in rodents (Table 1), H₃ antagonists, including ABT-239, GSK189254, GSK207040, GSK334429, JNJ-10181457, and BF2.649, attenuated scopolamine-induced deficits in cognitive tests in rodents (Fox et al., 2005; Ligneau et al., 2007; Medhurst et al., 2007a,b). Thus, H₃ antagonists may be beneficial in CNS disorders such as Alzheimer’s disease that exhibit cholinergic deficits related to the cognitive symptoms in these patients.

**Potential for Disease Progression in Alzheimer’s Disease**

Cholinergic transmission is well recognized as a major modulator of cognitive processing (Bartus, 2000). Acetylcholinesterase (AChE) inhibitors that increase synaptic ACh by inhibiting the enzymatic degradation of ACh provide a modest symptomatic relief that declines with later-stage AD progression. The progressive cholinergic cell loss associated with AD probably limits the therapeutic effectiveness of these agents dependent on endogenous ACh synthesis. Nonetheless, AChE inhibitors such as donepezil currently represent the primary therapeutic approach for AD. Thus, there exists a significant unmet need for the development of superior drugs that, in addition to symptomatic alleviation, may slow pathological progression, i.e., disease modifying efficacy.

H₃ antagonists elevate ACh levels in cortex and hippocampus and enhance memory in preclinical models. Functioning as indirect agonists at a variety of postsynaptic receptors through evoked release of different neurotransmitters, the mechanism and efficacy of H₃ antagonists as AD therapeutics may involve activation of multiple biochemical pathways. Moreover, the multisignaling potential of H₃ antagonists may afford therapeutic benefits beyond symptomatic alleviation. Drug discovery efforts toward developing therapeutics that have disease-modifying effects have focused on the two proteins involved in AD pathology, namely β-amyloid (Aβ), a product of aberrant amyloid precursor protein (APP) leading to production of extracellular Aβ plaques, and tau, a microtubule-associated protein that when hyperphosphorylated results in the formation of intracellular neurofibrillary tangles (Giacobini and Becker, 2007). In the latter case, pharmacological activation of cellular pathways that inhibit kinase signaling and subsequent tau hyperphosphorylation may represent a viable approach for targeting AD pathology.

It may be hypothesized that H₃ antagonist-evoked neurotransmitter release leads to activation of postsynaptic receptor pathways such as phosphorylation of cAMP response element binding protein (CREB), a transcription factor germane to cognitive function, and the inhibitory residue Ser9 of GSK3β, a primary tau kinase in AD responsible for tau hyperphosphorylation (Grimes and Jope, 2001; Hooper et al., 2008). With respect to GSK3β, it is constitutively active and a substrate to other kinases capable of phospho-regulating its activity through both inhibition and activation (Grimes and Jope, 2001). In the case of deactivation, signaling through phosphoinositide 3-kinase and subsequent activation of the serine–threonine kinase Akt inhibits GSK3β activity via Ser9 phosphorylation, a cellular cascade known to be associated with neuroprotection. Administration of the H₃ antagonist ABT-239 in normal mice increase cortical CREB and

**TABLE 1**

Cognitive domains modulated by histamine and H₃ antagonists in the different preclinical models that have been extensively used to detect the procognitive effect of novel agents

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S<sup>α</sup>-GSK3β phosphorylation at doses producing cognitive efficacy (Markosyan et al., 2009). It was also demonstrated that donepezil at doses associated with clinical exposures induced CREB phosphorylation consistent with a precognitive action, but in contrast to ABT-239, did not have effects on S<sup>α</sup>-GSK3β phosphorylation. Together, these findings suggest that increased S<sup>α</sup>-GSK3β phosphorylation induced by ABT-239 does not depend on increased ACh release.

Both CREB and S<sup>α</sup>-GSK3β phosphorylation have been shown to be down-regulated in the Tg2576 (APP/Aβ) transgenic mouse model of AD (Bitner et al., 2009). However, a 2-week infusion of ABT-239 in Tg2576 mice normalized cortical CREB and hippocampal pS<sup>α</sup>-GSK3β phosphorylation. In similar studies conducted in TAPP mice, an AD transgenic line containing both APP and tau transgenes, ABT-239 infusion reversed tau hyperphosphorylation in the spinal cord and hippocampus. Mechanistically, ABT-239 produced signaling changes (pS<sup>α</sup>-GSK3β) in a7 nicotinic ACh receptor (nAChR) knockout mice that do not exhibit a7 nAChR agonist-induced phosphorylation, suggesting that H<sub>3</sub> antagonist-mediated signaling does not depend on ACh-stimulated a7 nAChR activation.

In contrast to the in vivo findings, studies conducted in cortical cell cultures have demonstrated that H<sub>3</sub> receptor agonism induces phosphorylation of the Akt/GSK3β pathway and protects against neurotoxic insults, which are blocked by the H<sub>3</sub> antagonist thioperamide (Mariottini et al., 2009). These results indicate that H<sub>3</sub> receptor agonist activation in vitro leads to signaling changes similar to those observed with H<sub>3</sub> antagonist in the whole animal. In this regard, H<sub>3</sub> antagonist-evoked neurotransmitter release and subsequent postsynaptic receptor stimulation, not present in an in vitro system, may indeed produce a signaling phenotype distinct from H<sub>3</sub> receptor-mediated biochemical signaling when examined in vitro. In summary, these in vivo signaling studies raise the intriguing possibility that H<sub>3</sub> antagonists activate signaling pathways that may translate into improved efficacy in patients with AD, with symptomatic alleviation and disease-modifying effects.

**Development Status of H<sub>3</sub> Receptor Antagonists**

Several H<sub>3</sub> antagonists have advanced to the clinical stage, including BF2.649, PF-03654746, GSK189254, GSK239512, MK-0249, MK-3134, JNJ-17216498, and ABT-288. These compounds have completed phase 1 studies in human volunteers to determine the pharmacokinetics and tolerability after single- and multiple-dose administration; some of these drugs have also advanced to the phase 2 stage. Although limited clinical data have been released at this time, analysis of the available data can enable researchers to determine which are the effects common to all agents versus those effects that are unique to each pharmacophore.

More than 20 industrial and academic groups have worked on the development of H<sub>3</sub> antagonists. The earliest compounds have significant shortcomings as clinical drugs (cytochrome P450 inhibition, low brain penetration) but have become pharmacological tools, particularly ciprofanz, thioperamide, and (1S,2S)-4-(2-(5,5-dimethylhex-1-ynyl)cyclopropyl) imidazole (GT-2331). The first cited agent into clinical trials was GT-2331, but clinical development did not advance perhaps because of its imidazole-associated liabilities. The problems of these early structures drove efforts toward nonimidazoles, wherein the imidazole moiety is replaced by a tertiary basic amine, addressing cytochrome P450 inhibition and improving CNS penetration and H<sub>3</sub> selectivity. Most modern H<sub>3</sub> antagonists share these improved drug-like properties.

An early compound into the clinic was BF2.649 (Ligneau et al., 2007), and it has been reported in several phase 2 clinical trials on daytime sleepiness in Parkinson's disease, sleep apnea syndrome, and cognition enhancement in schizophrenic patients (http://clinicaltrials.gov). BF2.649 showed efficacy in a 22-patient narcolepsy trial of single-blind design: a 40-mg daily dose reduced sleepiness versus placebo, with efficacy in the Epworth Sleepiness Scale equivalent to modafinil (Lin et al., 2008). Most patients with Parkinson's disease have nighttime insomnia and daytime sleepiness, and BF2.649 was reported as effective in patients given 5 to 40 mg every day as assessed in the Epworth Sleepiness Scale. The optimal dose was 20 mg, which is being targeted in a phase 3 trial. No changes in nighttime sleep or Parkinson's symptoms were noted.

Pfizer has reported preclinical and phase 1 studies on PF-03654746. It is a potent H<sub>3</sub> antagonist (human H<sub>3</sub> Ki = 3.2 nM; rat K<sub>i</sub> = 37 nM) and active in the object recognition model. It exhibits a long human t<sub>1/2</sub> (9–18 h), with insomnia noted as the main adverse event at the 3-mg dose that reached 15 ng/ml (Soares et al., 2009). PF-03654746 recently completed a phase 2 trial in adult ADHD patients, and no efficacy was observed in two drug groups versus placebo, however, a phase 2 trial for daytime sleepiness in narcolepsy, a receptor occupancy PET study at 0.5- to 4-mg doses, and clinical trials in AD and narcolepsy are ongoing.

GSK189254 is a potent H<sub>3</sub> antagonist (human H<sub>3</sub> K<sub>i</sub> = 0.2 nM) with broad spectrum efficacy in a number of rodent models of cognition and narcolepsy (Medhurst et al., 2007a). GSK189254 increased ACh, NE, and DA as measured by microdialysis. This compound has been listed in early trials as targeting Alzheimer's, pain, and narcolepsy, but is no longer under clinical development. A <sup>11</sup>C-labeled analog has been reported as a radiotracer tool to probe the receptor occupancy of GSK239512 (http://clinicaltrials.gov). The structure and properties of GSK239512 have not been described, but GlaxoSmithKline is recruiting patients for phase 2 trials in schizophrenia and AD.

Merck has published the structure–activity relationship of several chemical series, with one especially potent compound (human H<sub>3</sub> K<sub>i</sub> = 1.7 nM) named as selected for clinical development for various CNS dysfunctions. The structure and properties of MK-0249 have not been specifically disclosed, but it has completed three phase 2 trials: in adult ADHD patients, AD, and schizophrenia. A phase 2 trial for the treatment of daytime sleepiness in patients with sleep apnea was terminated. Both MK-0249 and MK-3134 were evaluated in a clinical PET study to determine dose versus receptor occupancy. MK-0249 dosed at 2.5 to 50 mg and MK-3134 at 0.5 to 25 mg achieved up to 93 and 96% H<sub>3</sub> receptor occupancy, respectively. Data suggest that H<sub>3</sub> antagonist-induced alerting effects were observed at 67% occupancy, increasing with higher levels of H<sub>3</sub> occupancy (Jannone et al., 2009). However, MK-0249 failed to improve cognition in schizophrenic patients dosed for 4 weeks at 10

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mg (Egan et al., 2009), a dose that achieved >85% H3 receptor occupancy. MK-3134 has been described as completing phase 1 testing in a biomarker study using blood oxygen level-dependent functional magnetic resonance imaging at doses of 1, 5, and 25 mg.

Johnson and Johnson was an early leader in industrial H₃ research with JNJ-5207852 and other discontinued agents. JNJ-17216498 was the first candidate named as having completed human testing, with a phase 2 trial in 2007 in a small (65 patients) group of narcoleptic subjects. It was dosed at 10 and 50 mg with modafinil as comparator, but results have not been disclosed. A later compound, JNJ-31001074, is likely to be an H₃ antagonist. It has been probed extensively, in pharmacokinetic and ketoconazole interaction studies and a phase 2 ADHD trial in adults dosed at 10 and 30 mg. Several additional trials are recruiting patients, including a phase 1 in pediatric and teen ADHD groups and a phase 2 multidose adult ADHD trial with atomoxetine and methylphenidate as active comparator. The structures of the most advanced candidates are undisclosed.

The Abbott group has developed a number of chemical series and an early compound was ABT-239 with \( K_i = 0.45 \) nM affinity to human H₃ (Esbenshade et al., 2005). ABT-239 had robust activity in rodent models of cognition and has been used as a reference standard to probe H₃ effects in vivo (Fox et al., 2005). A recent study demonstrated that ABT-239 ameliorated ethanol-induced deficits on hippocampal long-term potentiation, indicating that H₃ antagonists can affect changes in synaptic plasticity related to cognitive processes (Varaschin et al., 2010). However, it also potently binds to the cardiac hERG channel in vitro. This problem has been solved in later series by systematic changes of structure. Abbott recently disclosed preclinical data on ABT-288, a potent and selective H₃ antagonist that binds to rat and human H₃ receptors with \( K_i = 8.1 \) and 1.9 nM, respectively (Esbenshade et al., 2009). ABT-288 increased the release of histamine and ACh from the rat cortex and facilitates performance in attention, short-term memory, and long-term memory tests. The compound penetrated the CNS efficiently and effectively occupied rat H₃ receptors with ED₅₀ = 3.2 ng/ml. Studies are presently ongoing to determine its efficacy in patients suffering AD and schizophrenia.

In addition to the companies mentioned above, other companies are active in the field, including Schering, Servier, Arena, Wyeth/Pfizer, Cephalon, Sanofi-Aventis, Roche, UCB, Lilly, and Ligand. Table 2 shows clinical data on the most advanced antagonists in the clinic today. It is important to note that all the H₃ antagonists in Table 2 are more potent for binding the human versus the rat H₂ receptor and the proper adjustment should be made to extrapolate the efficacious plasma levels.

**Conclusions**

There are significant needs for an effective treatment of the cognitive disorders in patients suffering ADHD, schizophrenia, and AD, and although a common feature in these patients is that they suffer cognitive deficits, the particular domains that are affected in each disease must be individually considered.

Inattention, impulsivity, and hyperactivity are the three clinical symptoms domains most affected in ADHD, deficits that are hypothesized to be caused by a combination of abnormalities in alertness and executive functions. This disorder affects 3 to 9% of school-age children but it is now recognized that it persists in adults as indicated by the decreased level of education, limited employment success, and increased incidence of drug addiction in adults previously diagnosed with ADHD (Wilens et al., 2004). However, clinical data with H₃ antagonists provided mixed results because PF-03654746 was ineffective in adult ADHD patients, whereas a positive effect has been reported with the JNJ compounds in these patients. It has also been reported that H₃ antagonists increase attention in humans because BF2.649 improved performance in the flicker-fusion test in normal volunteers, but it is clear that a positive effect on attentional tasks may not be sufficient to improve a pathological condition such as ADHD. Studies that were previously conducted (data not disclosed yet) and those presently ongoing with BF2.649 and MK-0249 will provide useful informa-

**TABLE 2**

Summary of key preclinical and clinical data on histamine H₃ antagonists that have been reported to advance to the clinical area

<table>
<thead>
<tr>
<th>Rat H₃</th>
<th>Human H₃</th>
<th>( t_{1/2} )</th>
<th>Clinical Observations and Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>BF2.649 (pitolisant)</td>
<td>17</td>
<td>2.7</td>
<td>Efficacy reported in narcoleptic patients and Parkinson’s disease.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sleep disturbances, insomnia reported in humans.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>Phase 2 trial in schizophrenia ongoing, and phase 3 trial in Parkinson’s planned.</td>
</tr>
<tr>
<td>PF-03654746</td>
<td>37</td>
<td>3.2</td>
<td>Sleep disturbances, insomnia in phase 1.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9–18</td>
<td>No efficacy in adult ADHD patients.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Phase 2 trials in AD and narcolepsy ongoing.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sleep disturbances, insomnia in humans.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2</td>
<td>Discontinued from development in AD.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;24</td>
<td>Advanced to phase 1. Phase 2 trials in AD and schizophrenia ongoing.</td>
</tr>
<tr>
<td>GSK189254</td>
<td>1</td>
<td>N.A.</td>
<td>Sleep disturbances, insomnia in phase 1.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N.A.</td>
<td>Alerting effects noted at 67% receptor occupancy.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N.A.</td>
<td>No efficacy in schizophrenic patients.</td>
</tr>
<tr>
<td>MK-0249</td>
<td>N.A.</td>
<td>N.A.</td>
<td>Advanced to phase 1.</td>
</tr>
<tr>
<td>MK-3134</td>
<td>N.A.</td>
<td>N.A.</td>
<td>Advanced to phase 1. Evaluated in patients with narcolepsy and ADHD.</td>
</tr>
<tr>
<td>ABT-288</td>
<td>8</td>
<td>1.9</td>
<td>Phase 2 trials in AD and schizophrenia ongoing.</td>
</tr>
<tr>
<td>JNJ-17216498</td>
<td>N.A.</td>
<td>N.A.</td>
<td>Advanced to phase 1.</td>
</tr>
</tbody>
</table>

N.A., data are not available.
tion in the near future with regard to efficacy in this population of patients.

Schizophrenia is a chronic disorder that is no longer considered as a unitary disease. In schizophrenia there are three major clusters of symptoms: positive symptoms such as delusions and hallucinations; negative symptoms such as anhedonia and social withdrawal; and cognitive deficits in attention, memory, speed of processing, and executive functions. Present pharmacological treatments are effective against the positive symptoms but they show limited efficacy against negative symptoms and do not improve the cognitive deficits in schizophrenic patients (Lublin et al., 2005). Most important, indices of cognitive function are better predictors of functional improvement than indices of the other domains, and there is consensus in the scientific community that the cognitive domains of relevance are attention, vigilance, speed of processing, working memory, and social cognition. Agents that improve cognitive deficits may represent a major breakthrough in the treatment of schizophrenia.

The efficacy of MK-0249 was evaluated in schizophrenia, and it was not effective in patients receiving standard antipsychotic medication. Patients received 4 weeks of treatment with a 10-mg dose of MK-0249, a dose that had previously shown high brain H3 receptor occupancy in normal volunteers. These negative findings may indicate that the increased brain histamine was not sufficient to exhibit a therapeutic effect on cognitive domains of importance in schizophrenic patients. On the other hand, the H3 antagonist properties of the antipsychotic drugs could have interfered in therapeutic effect on cognitive domains of importance in schizophrenic patients. These negative findings may indicate that the increased brain histamine in humans, consistent with the preclinical data. H3 antagonists can also increase extracellular levels of ACh, a neurotransmitter that has been effective to improve cognition in the early phases of AD. H3 antagonists may also bring another exciting biochemical effect by increasing the phosphorylation of key intracellular proteins that play a role in the neurodegenerative process.

The different clinical studies presently ongoing to test the efficacy of H3 antagonists in these human conditions may be able to provide an answer to these hypotheses and determine the place for H3 antagonists in therapeutics.

Authorship Contributions

- **Participated in research design:** Brioni, Esbenshade, Garrison, Cowart, and Bitner.
- **Conducted experiments:** Brioni, Esbenshade, Garrison, and Bitner.
- **Performed data analysis:** Cowart.
- **Wrote or contributed to the writing of the manuscript:** Brioni, Esbenshade, Garrison, Cowart, and Bitner.

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