THE HISTAMINE H_3 RECEPTOR: FROM GENE CLONING TO H_3 RECEPTOR DRUGS

Rob Leurs, Remko A. Bakker, Henk Timmerman and Iwan J. P. de Esch

Abstract | Since the cloning of the histamine H_3 receptor cDNA in 1999 by Lovenberg and co-workers, this histamine receptor has gained the interest of many pharmaceutical companies as a potential drug target for the treatment of various important disorders, including obesity, attention-deficit hyperactivity disorder, Alzheimer's disease, schizophrenia, as well as for myocardial ischaemia, migraine and inflammatory diseases. Here, we discuss relevant information on this target protein and describe the development of various H_3 receptor agonists and antagonists, and their effects in preclinical animal models.

Division of Medicinal Chemistry, Leiden/Amsterdam Center for Drug Research, Vrije Universiteit Amsterdam, Faculty of Science, de Boelelaan 1083, 1081 HV Amsterdam, The Netherlands.
Correspondence to R.L.
e-mail: leurs@few.vu.nl
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The therapeutic modulation of several actions of the biogenic amine histamine has proved to be medically effective and also financially profitable for the pharmaceutical industry. Antagonists that target the histamine H_1 receptor or the H_2 receptor, which are used in the treatment of allergic conditions such as allergic rhinitis and gastric-acid-related disorders, respectively, have been 'blockbuster' drugs for many years[1]. Recently, following the completion of the Human Genome Project, the family of histamine receptors has been extended to include four different G-protein-coupled receptors (GPCRs): the H_1, H_2, H_3 and H_4 receptors[2]. In view of the blockbuster status of the histamine H_1 and H_2 receptor antagonists, current expectations for the therapeutic potential of drugs that target the H_3 and/or H_4 receptor are high.

The H_3 receptor was identified pharmacologically in 1983 by Arrang *et al.* and acts as a presynaptic autoreceptor that inhibits histamine release from histaminergic neurons in the rat brain[3]. Panula and Haas recently reviewed the role of histamine neurotransmission in the central nervous system (CNS)[4]. Histaminergic neurons are localized in the tuberomammillary nucleus of the hypothalamus, project to all major areas of the brain and are involved in many functions, including the regulation of sleep/wakefulness, feeding and memory processes[4]. Although the H_3 receptor can also be found in the periphery (mainly, but not exclusively, on neurons), the CNS contains the great majority of H_3 receptors (REFS 5–7). In rodents, H_3 receptor expression is observed in, for example, the cerebral cortex, hippocampal formation, amygdala, nucleus accumbens, globus pallidus, striatum and hypothalamus by autoradiography[8], immunohistochemistry[9] or *in situ* hybridization[5,6].

H_3 receptor expression is not confined to histaminergic neurons, and, as a heteroreceptor, the H_3 receptor is known to modulate various neurotransmitter systems in the brain. In rodent and/or human brains, H_3 receptor activation inhibits presynaptically the release of many important neurotransmitters[10]. Despite this interesting feature, drugs that target the H_3 receptor have until 1999 been mainly developed by academic research groups. The identification of the H_3 receptor at the molecular level[5] has greatly facilitated drug discovery efforts to target the H_3 receptor and many pharmaceutical companies are currently active in this field.

Molecular biology of the H_3 receptor gene

The molecular architecture of the H_3 receptor was unknown until Lovenberg *et al.* showed in 1999 that, like the H_1 and H_2 receptors[11,12], the H_3 receptor belongs to the large super-family of GPCRs[5]. A potential GPCR-related expressed sequence tag (EST) sequence with homology to α_2 receptors was identified *in silico* in a

search for orphan GPCRs and used to clone a full-length cDNA from a human thalamus cDNA library. The H_3 receptor cDNA contains an open reading frame of 445 amino acids with all the features of a GPCR for a small biogenic amine[5]. The H_3 receptor protein shows very low sequence similarity with other GPCRs. Overall similarity between the H_3 receptor and the H_1 and H_2 receptors amounts to only 22% and 20%, respectively. This remarkable divergence explains why the H_3 receptor gene was not cloned by similarity screening with H_1- or H_2-receptor-specific probes.

Efforts to discover novel GPCRs through similarity searching of genomic databases resulted in the rapid identification of an additional member of the histamine receptor family, the H_4 receptor (REFS 13–18). Although the H_4 receptor shows little overall sequence similarity to any of the other histamine receptors cloned so far, it has a similarity of ~60% to the H_3 receptor within the transmembrane domains. Owing to this considerable similarity between the H_3 and H_4 receptors, the H_4 receptor pharmacology resembles, to some extent, the H_3 receptor profile. Known H_3 receptor ligands, including *R*-(α)-methylhistamine, immepip, imetit and clobenpropit, also act at the H_4 receptor (see below), albeit with a different rank order of affinity and potency[2].

It is now apparent that there is a large variety of H_3 receptor isoforms that might have different pharmacological profiles (see REF. 19 for a review). The H_3 receptor isoforms differ in four regions of the receptor protein (BOX 1, TABLE 1), and the number of possible H_3 receptor isoforms is high owing to the simultaneous occurrence of multiple splicing events in the same H_3 receptor mRNA molecule. So far, at least 20 different isoforms

Box 1 | Alternative splicing and histamine H_3 receptor isoforms

Unlike the histamine H_1 and H_2 receptor genes[11,12], analysis of the H_3 receptor gene revealed the presence of several introns. The human H_3 receptor gene has been suggested to consist of either three exons and two introns[20,36], or four exons and three introns[21,27], with the occurrence of the additional exon accounting for the presence of eight additional carboxy (C)-terminal amino acids found in some human H_3 receptor sequences[14,21]. The various H_3 receptor isoforms that are derived from alternative splicing of the H_3 receptor gene differ in four main parts (a through to d) of the receptor protein. The alternative splicing of the H_3 receptor gene occurs independently in the four regions indicated in the figure.

Several H_3 receptor isoforms have been identified, in which multiple incidences of alternative splicing have occurred (TABLE 1). Alternative splicing might occur in region a to yield H_3 receptors with a shortened amino-terminal domain (resulting in the deletion of amino acids 7–42), whereas alternative splicing in domain b results in the partial deletion of the second transmembrane domain and the first extracellular loop (deletion of amino acids 85–98). Alternative splicing occurs most extensively in region c, yielding H_3 receptor isoforms with a third intracellular loop of variable length or putative H_3 receptor proteins in which transmembrane domains 5, 6 and/or 7 are absent. Within region c, several events of alternative splicing might occur concurrently (TABLE 1), giving rise to a large variety of H_3 receptor isoforms. Finally, alternative splicing in region d yields H_3 receptor proteins with eight additional C-terminal amino acids (extra amino acids 446–453).

A full characterization is available for only a limited number of isoforms at present. In TABLE 1, specific isoforms that are known to bind H_3 receptor radioligands and/or can signal after heterologous expression are indicated.

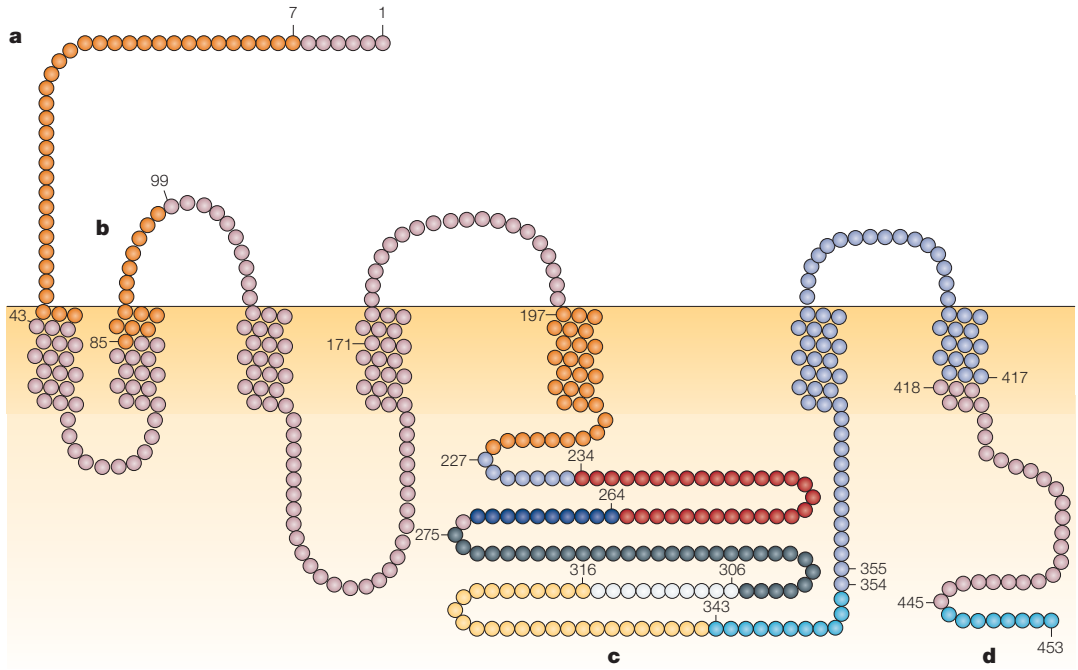


Table 1 | **Overview of human histamine H_3 receptor isoforms**

H_3 receptor isoform	Regions a	b	c	d	Amino acid changes compared to H_3 (445) receptor isoform	Functional*	References
H_3 (453)				+8	Eight additional C-terminal amino acids	B/S	14
H_3 (445)						B/S	5, 20,21, 26–28
H_3 (431)		–14			85–98	NB	27
H_3 (415)			–30		234–263	ND	27
H_3 (413)			–32		274–305	ND	36
H_3 (409)	–36				7–42	B	147
H_3 (395)	–36	–14			7–42, 85–98	ND	148
H_3 (379)	–36		–30		7–42, 234–263	ND	148
H_3 (373)			–80	+8	275–354, and eight additional C-terminal amino acids	S	21
H_3 (365)			–80		275–354	B/S	21,27,149
H_3 (351)		–14	–80		85–98, 275–354	ND	150
H_3 (340)			–80 –25		a275–354, 392–416	ND	151
H_3 (329)	–36		–80		7–42, 275–354	B	147,148
H_3 (329)			–116		227–342	ND	27
H_3 (326)			–119		197–315	ND	27
H_3 (309)			–144	+8	274–417, and eight additional C-terminal amino acids	ND	21
H_3 (301)			–144		274–417	NF	21
H_3 (293)	–36		–116		7–42, 227–342	ND	148
H_3 (290)	–36		–119		7–42, 197–315	ND	148
H_3 (200)					Introduction of 30 additional amino acids after residue 171 owing to a frameshift and an alternative stop codon	NF	21

*H_3 receptor isoforms, denoted by bracketed numbers, were shown to form functional receptors by either ligand-binding (B) or signal-transduction (S) experiments. C-terminal, carboxy terminal; NB, no radioligand binding; ND, not determined; NF, no functional activity.

have been described on the basis of detection of varying H_3 receptor mRNAs (BOX 1). Human H_3 receptor mRNA[20], including mRNAs for various human H_3 receptor isoforms, has been detected in the brain by means of reverse transcriptase-PCR (RT-PCR) (see also REFS 19,21,22). As the mRNAs for the H_3 receptor isoforms other than the H_3 (445) receptor isoform are not consistently detected by all investigators[19,21–23], the exact expression patterns of the various H_3 receptor isoforms remains elusive at this time. However, the overall picture suggests regional differences in the expression of the various H_3 receptor isoforms. Although most observations have been made on the basis of the detection of H_3 receptor mRNA, the proteins of some H_3 receptor isoforms have also been detected in mouse and rat brains using H_3 receptor isoform-specific antibodies[9,24]. The use of H_3 receptor isoform-specific antibodies now provides clear biochemical evidence for the existence of multiple H_3 receptor isoforms.

The H_3 receptor$_{445}$ isoform described by Lovenberg *et al.*[5] is currently the best characterized H_3 receptor isoform. Most isoforms differ from the H_3 receptor$_{445}$ isoform by large deletions of one or more stretches of amino acids (BOX 1). Among these, the H_3 receptor isoforms that have deletions in the third intracellular loop have received special interest, owing to the involvement of this receptor domain in G-protein coupling[6]. These H_3 receptor isoforms, in particular, show different pharmacological profiles[19], including agonist potencies[21], signalling properties[6] and constitutive activity[25].

In addition to the H_3 receptor isoforms, there is evidence for genetic polymorphism within the human H_3 receptor. The amino acid at position 19 is reported to be either glutamic acid[26] or aspartic acid[5]. A second polymorphism, resulting from an alanine to valine substitution at amino acid 280 (A280V) has been found in a patient with Shy–Drager syndrome (also known as neurological orthostatic hypotension), a disease that is characterized by neuronal degeneration and autonomic failure[19,20]. The H_3 receptor A280V polymorphism might be related to the aetiology of the Shy-Drager syndrome through alterations in the release of noradrenaline as a result of the polymorphism. A third human H_3 receptor polymorphism, resulting from a tyrosine to a cysteine substitution at amino acid 197 (Y197C), has recently been identified[19]. At present, no information is available on the potential functional differences between the various polymorphic variants.

Soon after the cloning of the human H_3 receptor (REFS 5,27), the receptor was cloned by sequence similarity from various other species, including rats[6,28–30], guinea-pigs[31,32], mice[33] and monkeys[34]. Although the H_3 receptor sequence is highly conserved across these species (>90%), the H_3 receptor showed considerable

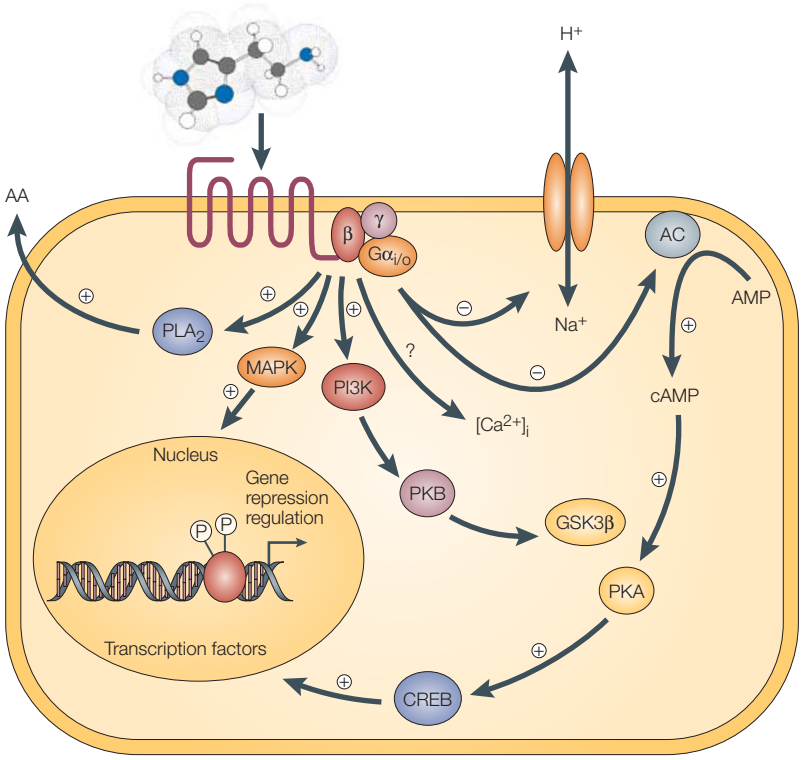


Figure 1 | **H_3-receptor activation can result in the modulation of diverse signalling pathways.** The H_3 receptor can activate members of the family of $G_{i/o}$ proteins to modulate cellular signalling. Activated $G_{i/o}$ proteins function to inhibit adenylyl cyclase (AC), the enzyme that induces the formation of cyclic AMP, which in turn results in the activation of protein kinase A (PKA) and consequently cAMP-responsive element-binding protein (CREB) to modulate gene transcription. Other effector pathways might also be activated through the H_3 receptor-mediated activation of $G_{i/o}$ proteins, including mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) pathways. H_3 receptor-mediated activation of $G_{i/o}$ proteins might also lead to the activation of phospholipase A_2 (PLA_2), which induces the release of arachidonic acid (AA), as well as the inhibition of the Na^+/H^+ exchanger and the lowering of intracellular Ca^{2+} levels. Subsequent activation of the MAPK and PI3K pathways results in the phosphorylation of extracellular signal-regulated kinases (ERKs) and protein kinase B (PKB, also known as Akt), respectively. Activated PKB will subsequently phosphorylate and thereby inhibit the action of glycogen synthase kinase 3β (GSK3β), a major tau kinase in the brain.

species differences[19,26,28,30,34,35], with the human H_3 receptor$_{445}$ having the highest affinity for histamine, but lower affinity for some antagonists (for example, thioperamide and ciproxifan). In addition to the identification of species homologues of the H_3 receptor gene, it seems that H_3 receptor isoforms are not limited to humans[14,20–22,36], but are also present in various species, including rats, guinea-pigs and mice[6,25,29,31,37]. However, the generation of H_3 receptor splice variants seems to be highly species-specific[34], complicating the evaluation of the various isoforms in relation to the effectiveness of H_3 receptor ligands *in vivo*. The various rodent H_3 receptor isoforms show differential brain expression[6,22,29,31] and signalling properties[6,22].

Following the cloning of the H_3 receptor, H_3 receptor knockout mice ($H_3^{-/-}$) were generated independently by two separate laboratories[38,39]. The available data from the H_3 receptor$^{-/-}$ mice have recently been extensively reviewed by Chazot and Shenton[40]. In general, these data confirm previous pharmacological studies with H_3 receptor ligands. Nevertheless, some unexpected results were observed with respect to arousal and food intake of the H_3 receptor$^{-/-}$ mice[40]. So far, conditional H_3 receptor$^{-/-}$ mice have not been available, and possible compensatory mechanisms have been put forward to explain the apparent anomalies[40].

The H_3 receptor signals through $G_{i/o}$ proteins

The involvement of $G_{i/o}$ proteins in H_3 receptor signalling in the brain was originally shown by the pertussis toxin sensitivity of H_3 receptor-agonist-dependent [^{35}S]GTPγS binding in the rat brain[41]. The H_3 receptor-mediated activation of $G_{i/o}$ proteins has been confirmed through heterologous expression of the H_3 receptor (REF. 5). In various transfected cell lines, the H_3 receptor is negatively coupled to adenylyl cyclase[5] (FIG. 1). Adenylyl cyclase stimulates the formation of cyclic AMP (cAMP), which in turn activates protein kinase A (PKA) and subsequently cAMP-responsive-element-binding protein (CREB) to modulate gene transcription. As a result, H_3 receptor activation lowers cAMP levels and reduces downstream events, such as CREB-dependent gene transcription[5]. Furthermore, H_3 receptor activation of $G_{i/o}$ proteins might result in the activation of other effector pathways (FIG. 1), including mitogen-activated protein kinase (MAPK)[6,42] and phosphatidylinositol 3-kinase (PI3K) pathways. H_3 receptor activation of $G_{i/o}$ proteins might also lead to the activation of phospholipase A_2 (PLA_2) to induce the release of arachidonic acid[43], the inhibition of the Na^+/H^+ exchanger[44] and lowering of intracellular Ca^{2+} levels by a mechanism that might involve the impaired entrance of Ca^{2+} through voltage-gated ion channels[45,46]. Activation of the MAPK and PI3K pathways results in the phosphorylation of extracellular signal-regulated kinases (ERKs) and protein kinase B (PKB, also known as Akt), respectively[47]. Activated PKB will subsequently phosphorylate and thereby inhibit the action of glycogen synthase kinase 3β (GSK3β)[47], a major tau kinase in the brain[48]. Interestingly, rat H_3 receptor isoforms with deletions in the third intracellular loop differ in their effectiveness to activate cAMP-responsive element (CRE)-dependent transcription or MAPK activation[6].

Although activation of the MAPK pathway by the H_3 receptor, as well as the PI3K signalling pathway[49], is involved in memory consolidation[42], the lowering of intracellular levels of cAMP through the activation of H_3 autoreceptors results in the modulation of histamine synthesis in histaminergic nerve terminals through the adenylate cyclase–PKA pathway[50]. The role of the activation of PKB/GSK3β by the H_3 receptor in the brain is currently less clear, but dysregulation of GSK3 is linked to several prevalent pathological conditions, such as diabetes and/or insulin resistance, and Alzheimer's disease (AD)[51].

Besides agonist-induced signalling, the H_3 receptor is known to signal in an agonist-independent manner (BOX 2). In fact, the H_3 receptor is one of the few examples for which constitutive GPCR signalling has been shown to occur *in vivo*.

Histamine H_3 receptor agonists

The structural diversity among histamine H_3 receptor agonists is limited. The endogenous agonist histamine

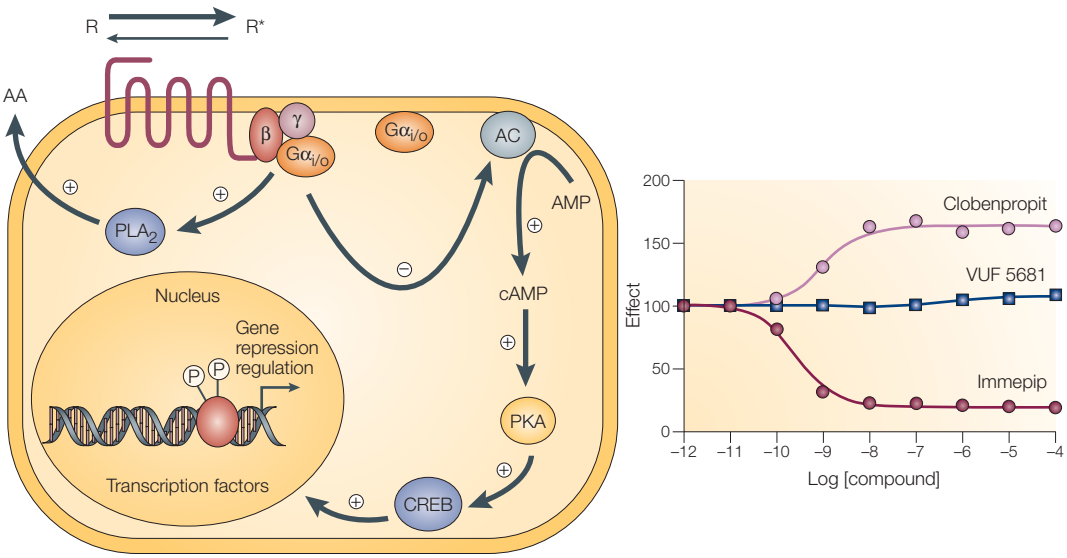
(compound 1) binds with high affinity to the human H_3 receptor. So far, all H_3 receptor agonists closely resemble histamine and contain a 4(5)-substituted imidazole moiety (FIG. 2). Efforts to replace this heterocyclic moiety have so far been unsuccessful[52,53]. Furthermore, additional substituents attached to the 4(5)-substituted imidazole ring eliminate H_3 receptor activity[52]. By contrast, small structural modifications of the imidazole side chain of histamine can result in very potent and selective H_3 receptor agonists. Methylation of the basic amine group gives N^{α}-methylhistamine (compound 2), a H_3 receptor agonist that is about three times more active than histamine[54]. The tritiated analogue of compound 2 can be readily obtained and is frequently used for H_3 receptor-binding studies. Methylation of the imidazole side chain has resulted in the identification of (*R*)-α-methylhistamine (RAMH (compound 3); $pK_i = 8.4$, human H_3 receptor)[55], which can be considered as the archetypal H_3 receptor agonist. Together with its less active (*S*)-isomer, the eutomer RAMH has been used in many pharmacological studies, but its use under *in vivo* conditions is limited because of its high basicity and hydrophilicity, extensive metabolism (especially as a substrate of hepatic human histamine amino (*N*)-methyltransferase) and low bioavailability. These problems were overcome by the research group of Schunack by applying an azomethine

Box 2 | **Histamine H_3 receptors do not need histamine**

The histamine H_3 receptor has a high level of so-called constitutive or spontaneous activity[25,49,63]. Constitutive G-protein-coupled receptor (GPCR) signalling — that is, signalling in the absence of agonist — was initially met with scepticism, but is now seen as an intrinsic property of GPCR proteins[141–143]. In a simplified two-state model[143], GPCRs are thought to isomerize between an inactive (R) and an active conformation (R*). The equilibrium usually lies towards R and agonists are thought to shift the equilibrium towards R*. Constitutive GPCR signalling is inhibited by a subset of antagonists, which therefore have negative efficacy and have been termed 'inverse agonists'. Their intrinsic activity ranges from –1 for full inverse agonists to 0 (REFS 141–143). Some antagonists do not show intrinsic activity and are referred to as neutral antagonists.

Constitutive H_3 receptor activity and inverse agonism of H_3 receptor antagonists is readily observed *in vitro* when assaying the human H_3 receptor-mediated modulation of forskolin-induced cyclic AMP (cAMP) formation (either directly or with reporter-gene technology)[25,69], [^{35}S]GTPγS binding[25,43] and calcium ionophore-induced arachidonic acid release[25,89]. The rat H_3 receptor$_{445}$ isoform shows a higher level of constitutive activity than the shorter rat H_3 receptor$_{413}$ isoform, which arises from a 32-amino-acid deletion in the third intracellular loop[25]. Most of the classical H_3 receptor antagonists (thioperamide, clobenpropit and ciproxyfan) and the newer non-imidazole antagonists have been identified as inverse agonists at the H_3 receptor$_{445}$ isoform (see figure). At the same time, some structurally related compounds have been identified as neutral antagonists; for example, VUF 5681 (7b; FIG. 2), an immepip analogue with a pK_i of 8.4, has no effect on the constitutive H_3 receptor-mediated modulation of forskolin-induced cAMP formation[144] (see figure). Moreover, proxyfan was also identified as a neutral antagonist in several (but not all) assays[89].

Whereas the described constitutive activity of the H_3 receptor in recombinant systems is not special, the observed constitutive H_3 receptor activity *in vivo* is unique[25,69]. [^{35}S]GTPγS binding to rat brain membranes is inhibited by various inverse H_3 receptor agonists and their action is blocked by proxyfan, which functions as a neutral antagonist[43]. Studies in mice and rats indicate that constitutive H_3 receptor activity regulates histamine release in the brain[25,69]. Consequently, to effectively increase histamine release, an H_3 receptor inverse agonist might be favoured over a neutral antagonist[25]. At the same time, inverse agonists can potentially give rise to receptor upregulation[145,146], which might be unfavourable in therapy. So far, unwanted H_3 receptor upregulation by inverse agonists has not been indicated as a major problem in preclinical models of H_3 receptor inverse agonist action.



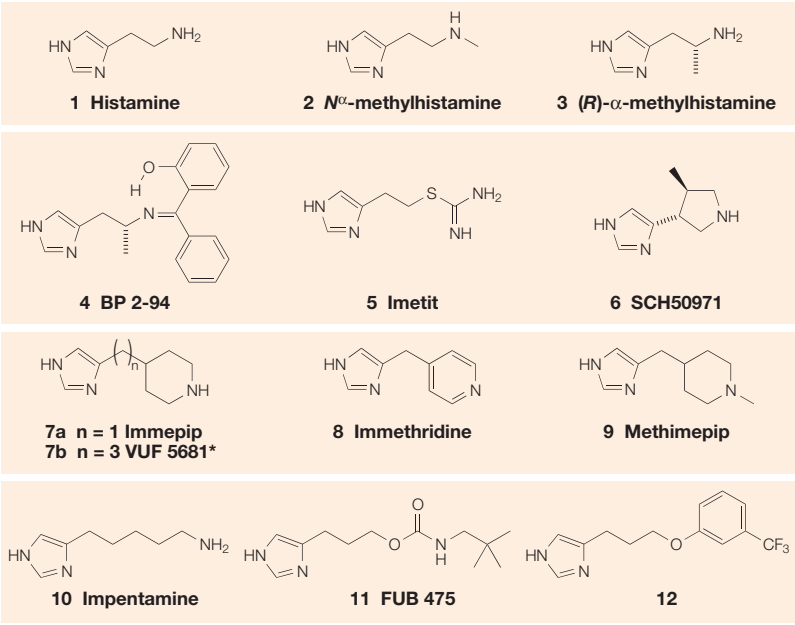


Figure 2 | **Imidazole-containing histamine H_3 receptor agonists.** *This compound is not an agonist, but a neutral antagonist.

PRODRUG concept to RAMH, resulting in BP 2-94 (compound 4) (REF. 56). The prodrug BP 2-94 has significantly improved oral bioavailability and pharmacokinetic properties[57]. In humans, the administration of BP 2-94 results in a 100-fold increase in the amount of plasma RAMH. Moreover, in mice that receive BP 2-94 orally, high levels of both the prodrug and RAMH are detected in most tissues, except the brain[57].

Replacement of the amine group of histamine by an isothiourea moiety resulted in the potent H_3 receptor agonist imetit (compound 5) (pK_i = 9.2, human H_3 receptor)[30,58,59], whereas incorporation of the flexible side chain of RAMH (compound 3) into a pyrrolidine ring or piperidine ring results in SCH50971 (compound 6) and immepip (compound 7a). Both compounds with reduced side-chain flexibility show high H_3 receptor affinity (SCH50971: pK_i = 8.6, guinea-pig H_3 receptor; immepip: pK_i = 9.3, human H_3 receptor) and relatively good brain penetration[60,61].

The use of RAMH (compound 3) and imetit (compound 5) as H_3 receptor receptor agonists for *in vivo* experiments is hampered by selectivity issues, such as cardiovascular effects mediated through α_2 receptors (RAMH) or 5-HT_3 receptors (imetit)[62] or bronchoconstriction through H_1 receptor activation (RAMH)[63]. These selectivity issues could not be detected for immepip (compound 7a)[62,64] or the pyrrolidine analogue SCH50971 (REF. 60). However, with the recent discovery of the H_4 receptor (see REF. 2 for a recent review), it also became clear that H_3 receptor agonists such as immepip, RAMH and imetit show only a limited selectivity (27–55-fold) for the H_3 receptor over the related H_4 receptor. Additional efforts by our research group, in collaboration with UCB Pharma (Belgium), resulted in an immepip analogue with a less basic pyridine ring in the side chain (compound 8, immethridine, pK_i = 9.1, human H_3 receptor). Although slightly less active than immepip at the H_3 receptor, immethridine shows a 300-fold selectivity over the related human H_4 receptor[65]. Moreover, simple *N*-methylation of the piperidine ring of immepip results in methimepip (compound 9), which combines high-affinity for the H_3 receptor (pK_i = 9.0, human H_3 receptor) with a 2,000-fold selectivity over the human H_4 receptor and *in vivo* brain penetration, as shown by a reduction in histamine levels after intraperitoneal (5 mg per kg) administration to rats[66,67].

Initially, the potent H_3 receptor ligand impentamine (compound 10) was identified as an H_3 receptor antagonist, using the guinea-pig jejunum as a functional H_3 receptor assay[68]. However, subsequent studies using recombinant human H_3 receptors, as well as *in vivo* microdialysis to measure hypothalamic histamine release in the rat brain, have revealed the H_3 receptor agonistic properties of impentamine[69].

Intriguingly, alkylation of the amino group of impentamine with relatively simple reagents resulted in H_3 receptor ligands that cover the complete range of pharmacological activity[69] on recombinant human H_3 receptors. By analogy, the pharmacological activity varies greatly for many of the derivatives of 3-(1*H*-imidazol-4-yl)propanol. Some of these compounds, such as FUB 475 (compound 11), are potent agonists *in vivo* (albeit PARTIAL AGONISTS *in vitro*), whereas close structural analogues have very different pharmacological H_3 receptor activities[70]. A more recent example of structural fine-tuning to convert ligands from H_3 receptor antagonists into H_3 receptor agonists is in the aryloxypropylimidazoles series. In this class, substituents on the aryl ring have a major effect on pharmacological activity, and only the compound that has a CF_3 group in the *meta* position (compound 12) has full H_3 receptor agonist activity *in vivo*[71].

H_3 receptor agonists as future drugs?

H_3 receptor agonists might be of therapeutic use in both the CNS and the peripheral nervous system. Histaminergic neurons in the HYPOTHALAMUS are thought to have an important role in the regulation of sleep/wakefulness[72]. Histamine, acting on H_1 receptors, promotes a waking state, and the blockade of its action by brain-penetrating H_1 receptor antagonists results in the known sedative side effects[73]. H_3 receptor activation in the CNS results in lower hypothalamic histamine release, and H_3 receptor agonists have therefore been suggested to be of use against insomnia[72]. Indeed, studies in various preclinical models with the RAMH prodrug BP 2-94 (compound 4) (Bioproject)[72], and the H_3 receptor agonist SCH50971 (compound 6) (Schering)[74], confirm that sleep is induced by H_3 receptor agonists.

Studies by Hough and co-workers have revealed an antinociceptive role for spinal histamine H_3 receptors (REF. 75). Intrathecally administered immepip (compound 7a) produced maximal antinociception on the tail-pinch test in wild-type, but not in histamine H_3 receptor$^{-/-}$, mice[75]. Whereas the H_3 receptor agonists immepip and BP 2-94 induce antinociception in rats on

PRODRUG
A pharmacologically inactive compound that is converted to the active form of the drug by endogenous enzymes or metabolism. It is generally designed to overcome problems associated with stability, toxicity, lack of specificity or limited (oral) bioavailability.

PARTIAL AGONIST
Whereas a full agonist produces the system maximal response, a partial agonist produces a maximal response that is below that of the system maximum (and that of a full agonist). As well as producing a sub-maximal response, partial agonists antagonise full agonists.

HYPOTHALAMUS
The hypothalamus is the region of the brain that controls body temperature, hunger and thirst, and circadian cycles.

a mechanical test (tail pinch), the formalin test or nociceptive responses on a thermal test (tail flick) were not affected[57,75]. Although histamine H_3 receptor agonists seem to show antinociceptive activity on some forms of nociception, further studies are needed to evaluate the pain-relieving potential of these drugs.

H_3 receptors are also known to be present on sympathetic nerve endings in the human heart[76]. Recent evidence from Levi and co-workers indicates that H_3 receptor activation modulates ischaemic noradrenaline release in animals and in a human model of protracted myocardial ischaemia. The reduction of cardiac noradrenaline release is considered to be the mechanistic basis for the observed H_3 receptor agonist-induced alleviation of reperfusion-induced arrhythmias in isolated guinea-pig hearts[76]. In support of this idea, recent studies in H_3 receptor$^{-/-}$ mice have shown an increase in noradrenaline release and reperfusion arrhythmias induced by ischaemia[77]. As excessive noradrenaline release is regarded as an important cause of cardiac arrhythmias in humans, H_3 receptor agonists could be an attractive new approach for preventing and treating myocardial ischaemic arrhythmias[76].

Finally, H_3 receptor agonists such as RAMH (compound 3) or its prodrug BP 2-94 (compound 4) and SCH 50791 (compound 6) are reported to inhibit neurogenic inflammatory processes in various tissues, including the lungs and dura matter[57,74,78,79]. These observations indicate a potential use of H_3 receptor agonists in inflammation, asthma and migraine. However, in a randomized, double-blind, crossover study with six mildly asthmatic subjects, no effect of RAMH (10 mg) on bronchoconstriction induced by the inhaled irritant sodium metabisulphite was observed[80]. These results could be explained by the poor RAMH pharmacokinetics in humans[57] and/or residual H_1 receptor activity[63]. Phase II clinical trials with the RAMH prodrug BP 2-94 (compound 4)[81] have so far resulted in negative outcomes in exercise-induced asthma or migraine[82]. Interestingly, in an open clinical trial, N^{α}-methylhistamine (compound 2) was reported to reduce headache intensity, frequency and duration in 18 patients with migraine. At the highest dose tested, however, patients reported intense headaches, which were possibly caused by residual H_1 receptor agonistic effects[83]. Studies with more selective H_3 receptor agonists, which have no residual H_1 receptor agonism, should further clarify the clinical potential of H_3 receptor agonists.

Histamine H_3 receptor antagonists

Many pharmaceutical companies have put considerable resources into the development of H_3 receptor antagonists for various indications. As can be judged from recent (patent) literature, current players in the field include Abbott Laboratories, Boehringer Ingelheim, De Novo Pharmaceutical, Eli Lilly, GlaxoSmithKline, Johnson & Johnson PRD/Ortho-McNeil, Merck, Novo Nordisk, Pfizer, Sanofi-Synthelabo, Schering-Plough and UCB Pharma. The development of H_3 receptor antagonists has recently been extensively reviewed (including patent literature)[84–86]. In the following sections, we highlight the most important frequently used H_3 receptor reference compounds and some recently described compounds, for which detailed pharmacology has recently been published.

Imidazole-containing H_3 receptor antagonists. The first potent H_3 receptor antagonist to be described that lacked H_1 receptor and H_2 receptor activity was thioperamide (compound 13) (FIG. 3)[55,87]. More recently, however, this and many other compounds that were initially identified as H_3 receptor antagonists have had to be re-classified as inverse H_3 receptor agonists (BOX 2). Thioperamide (compound 13) has been used as a reference H_3 antagonist for almost two decades, and many preclinical studies have been carried out with this compound. Thioperamide shows high affinity for the rat H_3 receptor ($pK_i = 8.4$), but proved to be less active at the human H_3 receptor ($pK_i = 7.2$)[30]. At the same time, thioperamide shows high activity at the human H_4 receptor ($pK_i = 7.3$), the rat 5-HT_3 receptor ($pK_i = 5.6$) and α_{2A} receptor ($pK_i = 6.9$) and the human α_{2C} receptor ($pK_i = 6.5$)[88].

Another early imidazole-containing H_3 receptor antagonist that has been extensively used to characterize the H_3 receptor is clobenpropit (compound 14)[59]. Clobenpropit ($pK_i = 9.4$, human H_3 receptor) can be considered as an imetit analogue (compound 5) and illustrates a trend in the structure–activity relationships (SARs) for H_3 receptor antagonists — that is, by increasing the distance between the basic moieties of agonists (for example, histamine or imetit) and/or the attachment of larger lipophilic moieties in the side chain, potent H_3 receptor antagonists can be obtained. The basic moieties in the imidazole side chain can, however, be omitted, as shown by compounds of the proxyfan class. Proxyfan (compound 15) has recently been identified as a neutral H_3 receptor antagonist ($pK_i = 8.0$, rat H_3 receptor; see also BOX 2; REF. 25). However, proxyfan is a peculiar H_3 receptor ligand, because it shows both H_3 receptor agonistic, neutral H_3 receptor antagonistic and inverse H_3 receptor agonistic properties, depending on the signalling assay used[43,89]. As such, proxyfan is considered to be a protean agonist. Relatively small structural modifications can significantly alter its mode of action, as, for example, in the case of the compound ciproxifan (compound 16), which is a potent INVERSE AGONIST ($pK_i = 9.2$, rH_3 receptor)[90,91]. The ether functionality of the proxyfan class of compounds can readily be replaced by, for example, carbamates, esters, amides[92] and even simple methylene units, resulting in 4-(ω-phenylalkyl)-1H-imidazoles that act as H_3 receptor ligands[93,94]. Ciproxifan has been extensively used in various *in vivo* studies, but shows only a moderate affinity for the human H_3 receptor ($pK_i = 7.2$) and moderate activity for the rat 5-HT_3 receptor ($pK_i = 6.5$) and human α_{2A} ($pK_i = 7.4$) and α_{2C} receptors ($pK_i = 7.2$)[88].

Several research groups have tried to make more rigid H_3 receptor antagonists, thereby increasing their drug-likeness. Scientists at Gliatech incorporated the 4(5)-imidazole substituent in a cyclopropane ring and acetylene moiety. The resulting compound — cipralisant or

INVERSE AGONIST
Inverse agonists reverse constitutive receptor activity, and are proposed to show selectively higher affinity for the inactive versus the active conformation of the receptor. In the absence of constitutive activity, inverse agonists function as competitive antagonists.

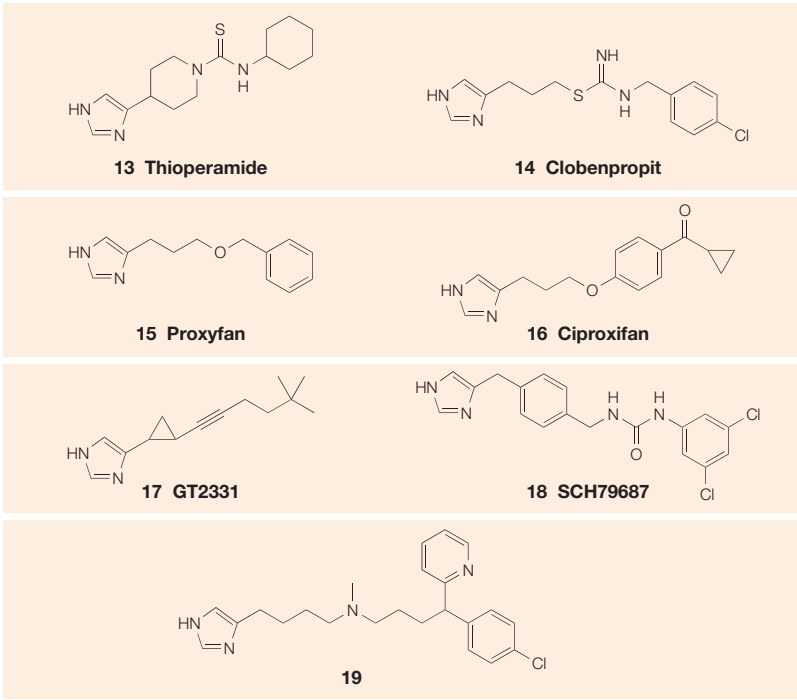


Figure 3 | **Imidazole-containing histamine H_3 receptor antagonists.**

GT2331 (compound 17) (FIG. 3) — is a high-affinity H_3 receptor ligand ($pK_i = 9.9$, rat H_3 receptor) that has an imidazole heterocycle and also has a very rigid lipophilic tail attached to the 4(5)-position of the ring[95]. Considering the limited conformational freedom of the compound, the shape of the lipophilic group is well optimized to fit the H_3 receptor binding site. Recently, some controversy about the absolute configuration of the cyclopropane ring of GT2331 has emerged. The absolute configuration (1*R*,2*R*) as reported by the developers was reassigned to (1*S*,2*S*) by Abbott scientists after re-synthesis and X-ray crystallographic analysis[96]. Similar to other imidazole-containing H_3 antagonists, GT2331 shows a lower affinity at the human H_3 receptor ($pK_i = 8.4$) and good activity for the human H_4 receptor ($pK_i = 7.1$) and α_{2C} receptor ($pK_i = 8.0$)[88]. Moreover, in recombinant systems, GT2331 acts as an agonist at both rat and human H_3 receptors, therefore calling into question the molecular mechanism of action of this H_3 receptor ligand[28].

Schering-Plough has also successfully developed H_3 receptor antagonists in which the imidazole side chain is made relatively more rigid and lipophilic by incorporating a phenyl ring into the imidazole side chain, resulting in the lead compound SCH79687 (compound 18). The urea moiety of this compound further reduces its flexibility and gives it a shape that fits the H_3 receptor binding site well, as shown by its high affinity ($pK_i = 8.7$, rat H_3 receptor). In the publications that describe the hit-optimization process that led to SCH79687 (compound 18)[97], Aslanian and co-workers acknowledge the accuracy and predictive power of a PHARMACOPHORE model that was derived for imidazole-containing H_3 receptor antagonists in 2001 (REF. 98). Using state-of-the-art partial similarity tools[99], a model was developed that describes the relative position and orientation of four hydrogen-bonding site points, an imidazole-binding pocket and two hydrophobic pockets that are available for binding various classes of H_3 receptor antagonist. This work has resulted in the imidazole-containing compound 19, which has excellent affinity for both the H_1 receptor and H_3 receptor ($pK_i = 7.8$ and 8.2, respectively)[100].

Non-imidazole H_3 receptor antagonists. Most of the imidazole-containing antagonists (thioperamide, clobenpropit and ciproxifan) interact with cytochrome P450 (REFS 101,102). As such an interaction is unwanted in drug development, many research groups have been developing non-imidazole H_3 receptor antagonists.

The first two successful efforts to develop non-imidazole H_3 receptor antagonists were reported by academic research groups in 1998, 15 years after the discovery of the receptor. The work of Ganellin and co-workers resulted in UCL1972 (compound 20, see FIG. 4), a compound with reasonable H_3 receptor affinity ($pK_i = 7.4$, rat H_3 receptor), good oral bioavailability and good penetration of the blood–brain barrier (BBB)[103]. In the same year, Menge and co-workers[104] reported non-imidazole H_3 receptor antagonists that were based on sabeluzole, a drug used to treat Alzheimer's disease. The weak H_3 receptor affinity of sabeluzole could be optimized, leading to VUF5391 (compound 21) — a ligand with good H_3 receptor affinity ($pK_i = 8.2$, rat H_3 receptor). Later, Stark and co-workers modified imidazole-containing lead structures, resulting in, among others, the potent compound FUB 649 (compound 22) ($pK_i = 7.8$, rat H_3 receptor)[105]. These studies showed that for some classes of imidazole-containing antagonist, the heterocycle can be replaced by other basic groups, mainly cyclic amines. However, these simple substitutions are not successful for all classes of antagonist, which could be indicative of partial similarity in the binding mode of the different classes of antagonist[97].

More recently, several major pharmaceutical companies have published their efforts to develop non-imidazole H_3 receptor antagonist drugs. Abbott Laboratories described their optimization of a high-throughput screening (HTS) hit, which led to A-317920 (compound 23), a very potent ligand on the rat H_3 receptor ($pK_i = 9.2$). In addition, the compound has good oral bioavailability[88]. Unfortunately, it was found that the affinity for the human H_3 receptor was significantly lower ($pK_i = 7.0$), again showing the important H_3 receptor species differences (see above). Nevertheless, A-317920 (compound 23) contains some typical structural features of H_3 receptor antagonists — that is, the propyloxy chain that connects a basic group with a phenyl ring, the acylpiperazine and the cyclopropylketone terminus. Additional medicinal chemistry efforts to optimize the affinity for human H_3 receptors resulted in the biphenyl compound A-331440 (compound 24). This compound has a higher affinity for the human H_3 receptor than for the rat H_3 receptor ($pK_i = 8.6$ and 7.8, respectively)[106]. A-331440 acts as a

PHARMACOPHORE
The ensemble of steric and electronic features that is necessary to ensure optimal interactions with a specific biological target structure and to trigger (or to block) its biological response.

selective inverse agonist at the human H_3 receptor. Interestingly, A-331440 shows considerably higher inverse agonistic efficacy than ciproxyfan and thioperamide[91]. At the same time, A-331440 shows 35% oral bioavailability (rat) and has a far greater brain/plasma ratio than ciproxyfan (45-fold) and thioperamide (666-fold).

In an attempt to make the non-imidazole H_3 receptor antagonists more rigid, the flexible propyloxy chain was partly incorporated into a fused aromatic system, resulting in benzofuran-containing compounds. The selected compound from this series, ABT-239 (compound 27), shows excellent affinities for the human and rat H_3 receptors ($pK_i = 9.3$ and 8.9, respectively), a lack of affinity for 80 non-H_3 receptor targets, potent inverse H_3 receptor agonism and good CNS penetration (brain/plasma ratio >30)[107,108]. This compound is intended for the treatment of cognitive disorders (see below) and has recently entered Phase I clinical trials[109].

Recently, Cowart reported SAR studies of ABT-239-like compounds. The benzofuran core can be linked to many amines[107,110]. For good activities, the pyrrolidines can also be substituted at the 2-position, with groups such as methyl ((*R*)-isomer in ABT-239), ethyl, fluorinated alkyls, methoxy or amines. The terminal phenyl ring of ABT-239 can be replaced by heterocycles and/or further substituted. Several of these compounds can serve as back-up compounds for ABT-239 (REF. 110).

Scientists at Johnson & Johnson PRD have also optimized HTS hits into a series of non-imidazole lead compounds that contain two basic groups, typically cyclic amines separated by a relatively simple linker. This arrangement is becoming a recurring theme in H_3 receptor (patent) literature. The potent lead compound JNJ-5207852 (compound 28) (pK_i = 9.5, human H_3 receptor) developed by Johnson & Johnson PRD readily crosses the BBB and has good oral bioavailability[111]. Recently, the phenylalkyne JNJ-10181457 (compound 29) (pK_i = 9.1, human H_3 receptor) was selected for detailed *in vivo* evaluation on the basis of a favourable pharmacokinetic and brain-residency profile. Plasma and brain concentrations in rats following an oral dose of 10 mg per kg reached a level of 10 μM[112].

In a combinatorial chemistry approach, Novo Nordisk prepared a library of monoacyl diamines. Acylpiperazines were identified as initial hits and were quickly optimized using parallel chemistry efforts, leading to the high-affinity compound 30 ($pK_i = 9.4$). More recently, Novo Nordisk described a series of cinnamic amides of aminomethylpyrrolidines, leading to the discovery of NNC 0038-0000-1202 (compound 31) as a potent ($pK_i = 8.3$) antagonist/inverse agonist for the human H_3 receptor. This compound has a good selectivity for the other three histamine receptors and more than 70 other targets (K_is >10 μM). Only some cross-reactivity of NNC 0038-0000-1202 (compound 31) with both 5-HT_{1A} and σ receptors, and the sodium channel, was observed[113].

Therapeutic uses of H_3 receptor antagonists

Despite the large number of non-imidazole H_3 receptor antagonists that have appeared in patent literature in the past 5 years (see REF. 85 for a detailed review), only a few reports of their preclinical use have so far been made. In the next sections, we review several of the most promising applications for H_3 receptor antagonists. For much of this information, we still rely to a large extent on the observed effects of the 'early' imidazole-containing H_3 antagonists.

Peripheral H_3 receptor blockade in nasal congestion. Allergic rhinitis is a frequently occurring chronic disease that affects a large number of people. In patients who are allergic, mast-cell histamine is one of the crucial mediators involved in, for example, pruritus, mucus secretion and the regulation of vascular permeability through activation of the H_1 receptor[114]. Recent analysis of H_3 receptor expression in the periphery by quantitative PCR revealed that H_3 receptor mRNA is abundantly expressed in human nasal mucosa; H_3 receptors modulate vascular contractile responses by the inhibition of noradrenaline release from sympathetic nerve terminals in the nasal mucosa[115]. In addition, in a cat model of nasal decongestion, a combination of either thioperamide (1–10 mg per kg intravenously (i.v.)) or clobenpropit (0.03–1 mg per kg i.v.) with the H_1 receptor antagonist chlorpheniramine resulted in significant nasal decongestion without the hypertensive effect seen with adrenergic agonists[116]. On the basis of these data, Schering seems to be actively pursuing combined H_1 and H_3 receptor blockade as a novel approach for nasal decongestion. The imidazole-based H_3 receptor antagonist SCH79687 (compound 18) (FIG. 3) hardly enters the brain (brain/plasma ratio = 0.02), but in combination with the H_1 receptor antagonist loratadine (10 mg per kg per os (p.o.)), SCH9687 inhibited the decrease in nasal cavity volume due to aerosolized compound 48/80 (a mast-cell destabilizer)[114]. The dual H_1/H_3 receptor antagonist 19 (REF. 100) has been developed for the same indication, but, at present, no *in vivo* data are available for this ligand.

Central H_3 receptor blockade in obesity. With the increasing incidence of obesity and diabetes and associated health risks in modern Western society[117], the need for the development of new anti-obesity drugs is rapidly growing. Modulation of CNS histaminergic neurotransmission represents an interesting new mechanism to control body weight. The role of neuronal histamine in food intake has been established for many years (see REFS 84,118 for extensive reviews), and the blockade of its action at hypothalamic H_1 receptors has been indicated as the mechanistic action of weight gain after therapy with, for example, various antipsychotics[119]. Moreover, neuronal histamine release and/or signalling has also been implicated in the anorectic actions of known mediators in the feeding cycle (for example, leptin, amylin and bombesin)[84].

As mentioned, in the brain, the H_3 receptor is implicated in the regulation of histamine release in the hypothalamus. Moreover, recent *in situ* hybridization studies

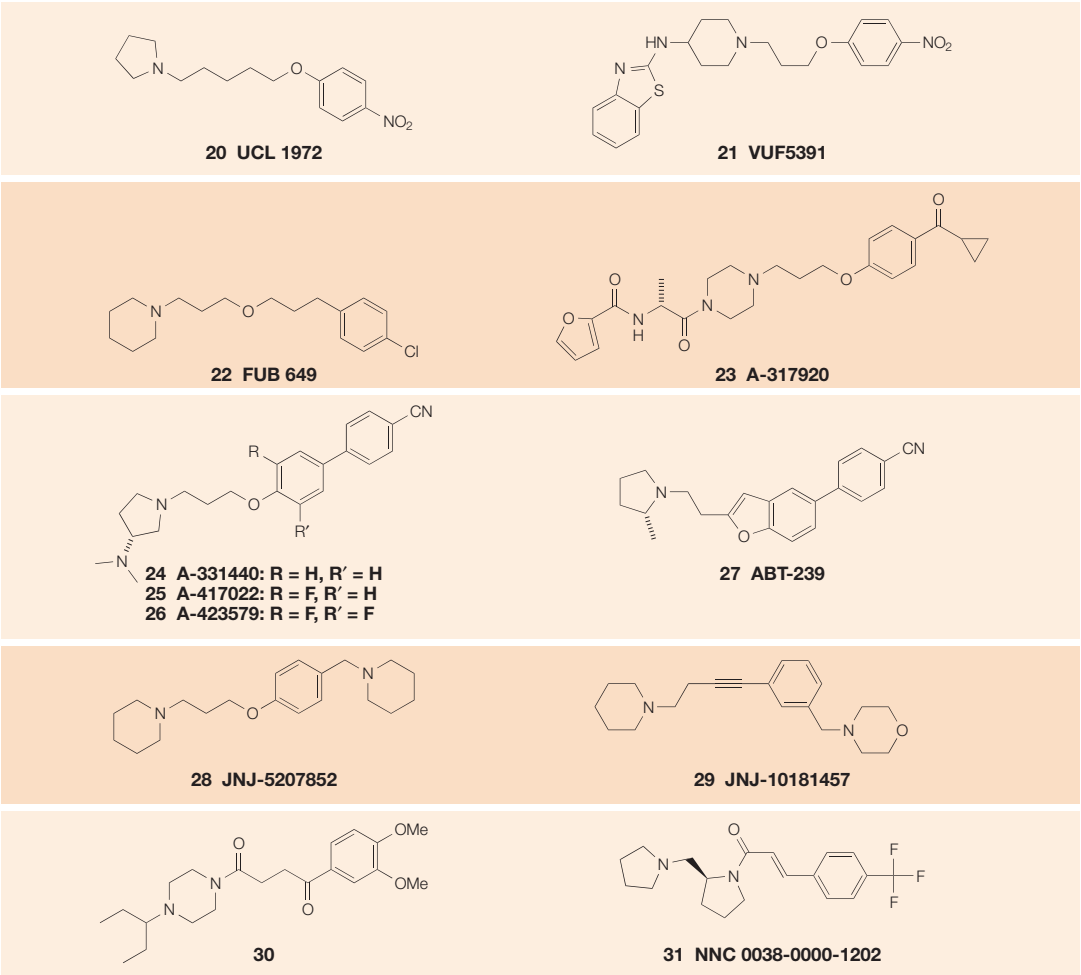


Figure 4 | **Non-imidazole histamine H_3 receptor antagonists.**

revealed H_3 receptor mRNA expression in rat brown adipose tissue[120], indicating that H_3 receptor ligands might (peripherally) regulate thermogenesis. On the basis of these notions and the observations that in the CNS H_3 receptor antagonists elevate hypothalamic histamine levels[61,121], it can be understood why the anti-obesity potential of H_3 receptor antagonists has attracted considerable attention. Studies with the imidazole-containing H_3 receptor antagonist thioperamide (compound 13) or ciproxyfan (compound 16) provide evidence for the modulatory role of the H_3 receptor in feeding behaviour. In various models of acute food intake, these H_3 receptor antagonists have been reported to be effective[84], although a recent study indicated that the effects of thioperamide might be related to visceral discomfort[122].

Recently, non-imidazole-containing H_3 receptor antagonists have been investigated in various preclinical models of obesity. Obesity induced by a high-fat diet in mice was effectively reduced by the non-imidazole H_3 receptor antagonist, but not by ciproxifan[91]. A 28-day treatment regimen of 5 mg per kg p.o. A-331440 reduced weight at a similar level to dexfenfluramine (10 mg per kg p.o.), and a higher dose of 15 mg per kg reduced weight to a level of mice on a low-fat diet. Magnetic resonance imaging analysis indicated that dexfenfluramine treatment (10 mg per kg p.o.) did not reduce body fat, whereas the H_3 receptor antagonist treatment resulted in a significant reduction of total, abdominal and subcutaneous fat[91]. In various animal models of obesity, A-331440 showed anti-obesity efficacy, including a reduction in food intake and a lowering of circulating leptin and ghrelin levels[84]. Despite the favourable anti-obesity properties of A-331440, further preclinical development was halted because the drug scored positively in an *in vitro* micronucleus test for potential genotoxicity[123]. However, 3′-fluoro (compound 25, A-417022) or 3′,5′-difluoro (compound 26, A-423579) substitution of the biphenyl moiety (FIG. 4) results in compounds with nanomolar affinities but no *in vitro* genotoxic effects. The difluoro analogue A-423579 (10 mg per kg) reduced body weight, plasma leptin, energy intake and body fat mass in treatment for 14–28 days of diet-induced obese rodents[123]. In these models, A-423579 was as effective as the mixed 5-HT/noradrenaline reuptake inhibitor sibutramine (5 mg per kg) and did not affect the behavioural satiety sequence in rats, indicating that the effects are not due to visceral illness or taste aversion[123].

Besides Abbott Laboratories, Novo Nordisk is also aiming to develop non-imidazole H_3 receptor antagonists as anti-obesity drugs. In rats, their lead compound NNC 0038-0000-1202 (compound 31) showed an 85% oral availability, and microdialysis experiments showed a clear dose-dependent (compound 7, 5–30 mg per kg p.o.) release of histamine in the paraventricular nucleus of the rat hypothalamus after oral administration of NNC 0038-0000-1202. Moreover, in rodents, pigs and monkeys this H_3 receptor antagonist decreases food intake, and, in rodents, also body weight. At the same time, the compound was well tolerated and did not affect either the behavioural satiety sequence or locomotion of rats[124]. Additional H_3 receptor antagonists with higher efficacy and tolerability have been announced by Novo Nordisk, but *in vivo* results have so far not been presented.

On the basis of these data from preclinical models, the blockade of H_3 receptors by selective antagonists or inverse H_3 receptor agonists might be an attractive mechanism of action for anti-obesity compounds. H_3 receptor blockers reduce weight gain, lower plasma ghrelin and leptin levels, and seem to be well tolerated. As the compounds probably do not induce stimulatory behaviour, unlike amphetamine-like stimulants[84], clinical data on H_3 receptor antagonists in human obesity are eagerly awaited, but, at present, no clinical trials in this area have been announced.

Central H_3 receptor blockade in sleep and cognitive disorders. It is well known that many of the first-generation, brain-penetrating H_1 receptor antagonists cause sedation and a decline in cognitive functions[125,126]. Consequently, indirect modulation of histaminergic brain function by H_3 receptor antagonists might be a means to modulate attention and memory processes. Whereas H_3 receptor agonists induce sleep in preclinical animal models (see above), thioperamide increases wakefulness in wild-type mice, but has no effect on sleep/wakefulness in H_3 receptor$^{-/-}$ mice[38]. Moreover, ciproxyfan significantly decreases slow-wave electroencephalographic activity, which is indicative of sleep, in both rats and cats[90,127]. These data indicate that H_3 receptor antagonists promote wakefulness. Therefore, H_3 receptor antagonists might be useful in sleep-related disorders, such as NARCOLEPSY. Interestingly, modafinil (Modiodal/Provigil; Organon/Cephalon), a novel wakefulness-promoting drug for the treatment of narcolepsy has recently been shown to increase hypothalamic histamine release[128], and recent studies using the classic Doberman model of narcolepsy with either GT2331 (compound 17)[129] or the non-imidazole H_3 receptor antagonist JNJ-10181457 (compound 29) showed that H_3 receptor antagonists reduce the number of narcoleptic attacks and the duration of the attacks[112].

In various neuropsychiatric conditions (for example, attention-deficit hyperactivity disorder (ADHD), schizophrenia and Alzheimer's disease), cognitive deficits are an integral part of the disease. It is therefore of huge interest that a variety of H_3 receptor antagonists can improve cognitive performance in various animal models (see REF. 130 for a recent review). The reported neuromodulatory role of the histaminergic system on acetylcholine release is important in this respect[131]. For example, thioperamide increases *in vivo* acetylcholine in the rat hippocampus[132] and enhances recall of a passive avoidance response in rats[133] and senescence-accelerated mice[134]. It also improves short-term memory in a novel-object-recognition test[133] and social recognition test[135]. In addition, the imidazole-containing H_3 receptor antagonists ciproxyfan and GT2331 improve acquisition in an inhibitory avoidance model with rat pups[136]. On the basis of these observations, the imidazole-containing compound GT2331 (Gliatech) has reached clinical trials[129]. However, the development of this drug for the treatment of ADHD has been halted in Phase II trials for unknown reasons at present.

In view of the reported agonistic activity of GT2331 at recombinant H_3 receptors and the limited selectivity towards the α_{2C} receptor, for example (see above), some of the newer, highly selective non-imidazole H_3 receptor antagonists/inverse agonists might have improved characteristics for use in cognitive disorders. Abbott Laboratories recently reported that in a rat pup model for ADHD, A-317920 (compound 23) was at was least as effective as methylphenidate (Ritalin; Novartis) (a stimulant) or ABT-418 (a nicotine-receptor ligand), which are both clinically effective ADHD drugs[130]. Moreover, the highly selective H_3 receptor ligand ABT-239 (compound 27) has entered Phase I clinical trials after promising activities in preclinical models of ADHD and Alzheimer's disease. In rats, ABT-239 enhances acetylcholine release in the prefrontal cortex and the hippocampus[110], improves learning in a five-trial inhibitory avoidance model using rat pups (a model of ADHD), improves recall in a social memory test, and improves spatial working and reference memory in a water maze at dose ranges of 0.01–1 mg per kg subcutaneously[137,138]. Moreover, a second H_3 receptor antagonist (ABT-834) developed by Abbott Laboratories has recently entered Phase I studies and is also supposed to target cognitive dysfunction[109]. At present, neither structural nor biological data for ABT-834 have been disclosed.

Researchers at Johnson & Johnson PRD reported on preclinical effects of JNJ-10181457. The compound showed pro-arousal effects in rats after subcutaneous dosing (half-maximal effective dose (ED_{50}) = 0.3 mg per kg). An improvement in the memory performance of juvenile rats (passive avoidance in spontaneous hypertensive rat (SHR)) was reported at a dose of 10 mg per kg intraperitoneally[112].

Finally, scientists at GlaxoSmithKline recently reported on a high-affinity (pK_i = 9.7 and 8.7 for human H_3 and rat H_3, respectively) non-imidazole H_3 receptor antagonist, with strong inverse agonistic properties[139]. The structure of GSK189254A was not revealed, but the compound effectively reached the brain and showed pro-cognitive effects at 0.3–1 mg per kg in a novel-object-recognition test[139].

On the basis of the various preclinical data on various (non)-imidazole H_3 receptor antagonists, the H_3 receptor seems to be an interesting target for improving

NARCOLEPSY
Narcolepsy is a neurological disorder of sleep regulation that affects the control of sleep and wakefulness. The four classic symptoms are excessive daytime sleepiness, cataplexy, sleep paralysis and hypnagogic hallucinations.

attention and alleviating cognitive dysfunction in, for example, ADHD and Alzheimer's disease. Results of a clinical trial with one of the many non-imidazole H_3 receptor antagonists are eagerly awaited.

Get smart and get slim? The preclinical findings indicate that H_3 receptor antagonists might become future 'wonder drugs'. H_3 receptor antagonists are promising effective anti-obesity drugs that, at the same time, could improve cognitive performance. However, this promise has not yet been fulfilled.

A close look at the H_3 receptor ligands recently described by Abbott laboratories, for example, shows that different molecules are presented for either anti-obesity (such as A-331440) or pro-cognitive effects (ABT-239). At the recent annual meeting of the European Histamine Research Society (Düsseldorf/Köln, 2004), this issue was discussed in some detail, and it seems that H_3 receptor antagonists that are active in anti-obesity models do not always work in models of cognition and vice versa. These observations were supported by results from scientists at GlaxoSmithKline and Novo Nordisk, indicating that H_3 receptor blockade and BBB passage alone are not enough to obtain both *in vivo* effects. Explanations for the observed discrepancy are currently not available, although several possibilities can be considered. Potential explanations might relate to the complexity of H_3 receptor isoform expression and H_3 receptor signalling, including constitutive activity. Could different H_3 receptor isoforms or signal-transduction pathways with varying drug sensitivity be involved in the diverse effects? MAPK activation seems to be involved in H_3 receptor-mediated memory processes[42], but, so far, the molecular pathway(s) involved in the anti-obesity effects of H_3 receptor antagonists have not been studied. Recent studies of a series of GT2331 analogues have indicated that inverse H_3 receptor antagonists, but not neutral H_3 receptor antagonists, would be effective in obesity models[140]. However, it is not yet clear whether these observations can be generalized. It is also possible that besides potent H_3 receptor blockade, other (receptor) activities are needed to observe the various *in vivo* effects or that drug penetration in important brain areas would be different and therefore result in different *in vivo* efficacy profiles. At present, none of these possibilities can be excluded and further research is needed .

Concluding remarks

In the past 5 years, much progress has been made in the understanding of the H_3 receptor at the molecular level. Moreover, many potent and relatively selective H_3 receptor agonists and inverse agonists have been developed by various academic and industrial scientists. For both H_3 receptor agonists and H_3 receptor inverse agonists/antagonists, many interesting activities in several preclinical models of important human diseases have been reported. Results from clinical trials are currently awaited, and will be the next step in the process of moving from knowledge of the gene encoding the H_3 receptor to the development of drugs for a range of indications. At the same time, important questions about the molecular aspects, biology and pathophysiology of the H_3 receptor remain to be answered to form a solid scientific basis for the therapeutic application of H_3 receptor ligands.

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Competing interests statement

The authors declare competing financial interests: see Web version for details.

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Author biographies

Rob Leurs obtained a masters degree in medicinal chemisty from the Vrije Universiteit Amsterdam, the Netherlands, in 1987. He completed his Ph.D. on G-protein-coupled receptors at the Vrije Universiteit in 1991. As a postdoctoral fellow (1992–1993) at INSERM (Unite de Neurobiologie and Pharmacologie, Paris, director J.C. Schwartz), he was involved in the cloning of genes that encode histaminergic and serotonergic receptors. After that, he was awarded a 5-year fellowship (1993–1998) of the Royal Netherlands Academy of Arts and Sciences. He was appointed as assistant professor in medicinal chemistry in 1998 and full professor in 2000 at the Vrije Universiteit Amsterdam. Since 2003, he has been one of the two scientific directors of the Leiden/Amsterdam Center for Drug Research. Leurs is currently involved in research on G-protein-coupled receptors, with a specific emphasis on the ligands and receptor proteins for histamine and various chemokines.

Remko A. Bakker obtained his masters degree in biochemistry from the Vrije Universiteit Amsterdam, the Netherlands. In 2003, he completed his Ph.D. in the area of molecular pharmacology, focusing on the histamine H_1 receptor. Since 2002, he has been an assistant professor at the Vrije Universiteit Amsterdam. His research mainly focuses on the pharmacology of histamine receptors. His current research interests include cell-surface receptors, their signalling pathways and drug discovery.

Henk Timmerman obtained his Ph.D. in 1967 at the Vrije Universiteit Amsterdam, the Netherlands. After a career in research and management in the pharmaceutical industry, in 1979, he succeeded his teacher, Wybe Nauta, as the chair in pharmacochemistry at the Vrije Universiteit Amsterdam. In 2002, he retired from his chair. He holds and has held many positions on national and international scientific bodies. The histamine receptors and their ligands are his primary scientific interest.

Iwan J. P. de Esch obtained a masters degree in organic chemistry at the University of Nijmegen, the Netherlands, and obtained his Ph.D. in medicinal chemistry in 1998 at Vrije Universiteit Amsterdam, the Netherlands. As a postdoctoral researcher, he joined the Drug Design Group of the Pharmacology Department of the University of Cambridge, United Kingdom. This academic group formed the basis of the successful TeknoMed drug design collaboration with Rhône-Poulenc Rorer. In 2000, de Esch co-founded De Novo Pharmaceuticals, a spin-off company of the University of Cambridge. The company applies state-of-the-art drug-design methodologies to novel therapeutic targets that have emerged from genomics. de Esch returned to academia in 2003 to become an assistant professor of the Medicinal Chemistry Group at the Vrije Universiteit Amsterdam. There, he combines structure-based and ligand-based design methodologies with state-of-the-art parallel chemistry approaches to explore ligand-receptor interactions and to develop novel drugs.

Summary

· The therapeutic modulation of several actions of the biogenic amine histamine has proved to be medically effective and also financially profitable. Antagonists that target the histamine H_1 receptor (H_1R) or the H_2 receptor, which are used in the treatment of allergic conditions and gastric-acid-related disorders, respectively, have been 'blockbuster' drugs for many years.

· Following the Human Genome Project, the family of histamine receptors has been extended to include four different G-protein-coupled receptors (GPCRs): the H_1, H_2, H_3 and H_4 receptors, and current expectations for the therapeutic potential of drugs that target the H_3 and/or H_4 receptor are high.

· The identification of the H_3 receptor at the molecular level in 1999 has greatly facilitated drug discovery efforts to target the H_3 receptor, and currently many pharmaceutical companies are active in this field.

· As reviewed in this article, many potent and relatively selective H_3 receptor agonists and inverse agonists have now been developed. For both H_3 receptor agonists and H_3 receptor inverse agonists/antagonists, interesting activities in several preclinical models of important human diseases, including obesity, migraine, attention-deficit hyperactivity disorder, and inflammatory diseases, have been reported.

Competing financial interests statement

The authors act or have acted as consultants for various pharmaceutical companies active in the field of histamine receptors and have also received research payments from some companies in this field.