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The histamine H_3 receptor: structure, pharmacology and function

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1

MOL #104752

Text pages	66
Number of tables	3
Figures	7
References	256
Words in abstract	168
Words in introduction	141
Words in main text	9494

Non-standard abbreviations

5-HT	5-Hydroxytriptamine (serotonin)
aa	Amino acids
A_1R	Adenosine A_1 receptor
$A_{2A}R$	Adenosine A_{2A} receptor
ACh	Acetylcholine
AD	Alzheimer's disease
CNS	Central Nervous System
D_1R	Dopamine D_1 receptor
D_2R	Dopamine D_2 receptor
ECL	Extracellular loop
GABA	γ-Aminobutyric acid
GTS	Gilles de la Tourette's syndrome
GIRK	G protein-gated inwardly rectifying K^+ channel
GPCR	G protein-coupled receptor
H_1R	Histamine H_1 receptor
H_2R	Histamine H_2 receptor
H_3R	Histamine H_3 receptor
H_4R	Histamine H_4 receptor
HDC	Histidine decarboxylase
ICL	Intracellular loop
MSN	Striatal medium-sized spiny neuron
PD	Parkinson's disease
PKA	Protein kinase A
PKC	Protein kinase C
PLC	Phospholipase C
SN	Substantia nigra
SNc	Substantia nigra *pars compacta*
SNr	Substantia nigra *pars reticulata*
TMN	Tuberomammillary nucleus

Abstract

Among the four G-protein coupled receptors (H_1-H_4) identified as the mediators of the biological effects of histamine, the H_3 receptor (H_3R) is distinguished for its almost exclusive expression in the nervous system and the large variety of isoforms generated by alternative splicing of the corresponding mRNA. Additionally, it exhibits dual functionality as auto- and hetero-receptor, and this enables H_3Rs to modulate the histaminergic and other neurotransmitter systems. The cloning of the H_3R cDNA in 1999 by Lovenberg *et al.* allowed for detailed studies of its molecular aspects. In this work we review the characteristics of the H_3R, namely its structure, constitutive activity, isoforms, signal transduction pathways, regional differences in expression and localization, selective agonists, antagonists and inverse agonists, dimerization with other neurotransmitter receptors, and the main pre- and post-synaptic effects resulting from its activation. The H_3R has attracted interest as a potential drug target for the treatment of several important neurological and psychiatric disorders such as Alzheimer's and Parkinson's diseases, Gilles de la Tourette's syndrome and addiction.

MOL #104752

1. Introduction

In 1910 Sir Henry Dale and his colleagues isolated histamine from ergot and later found that it had a stimulant effect on smooth muscle from the gut and the respiratory tract, caused vasodepression, stimulated cardiac contractility, and induced a shock-like syndrome when injected into animals. In 1920 Popielski demonstrated that histamine stimulated gastric acid secretion, and in 1927 the amine was isolated from the liver and the lung, evidencing that it was a natural constituent of the body (reviewed by Parsons and Ganellin, 2006).

Although histamine was detected in the brain in 1919 by John J. Abel, its role as a neuromodulator became evident only several decades later; using antibodies against the amine and its synthesizing enzyme, histidine decarboxylase (HDC), the morphological characterization of histamine-producing neurons proved the existence of a histaminergic system in the mammalian brain (Figure 1).

2. The brain histaminergic system

Histamine-synthesizing neurons are located in the hypothalamus tuberomamillary nucleus (TMN), with ~4,500 neurons found in the rat TMN and ~64,000 neurons in the human TMN. These neurons send diffuse projections to the entire Central Nervous System (CNS) through three major pathways, two ascending bundles that innervate the forebrain structures and one descending bundle reaching the spinal cord (Watanabe *et al*., 1984; Airaksinen and Panula, 1988; Haas *et al*., 2008).

Histaminergic neurons have a resting membrane potential of about -50 mV and spontaneously fire action potentials at 2.1 ± 0.6 Hz with a marked circadian rhythmicity,

5

that is, more active during wakefulness. A non-inactivating Na^+ current, active even at -70

mV, appears responsible for spontaneous firing, with low-threshold depolarizing Ca^{2+}

currents contributing to the repetitive firing. The action potential is broad with a significant

contribution from Ca^{2+} currents followed by a pronounced (15-20 mV) after-

hyperpolarization, which activates a depolarizing cationic current (I_h) and two A-type K^+

currents that delay the return to the resting potential. The Ca^{2+} currents mediate dendritic

histamine release and are the target of the autoreceptor-mediated negative feedback on

action potential firing (Haas and Reiner, 1988; Haas *et al*., 2008).

The behavioral state-dependent activity of the histaminergic neurons is influenced by

several neuronal, humoral, and paracrine signals, and the activity is mainly regulated by

excitatory glutamatergic inputs from the cerebral cortex and the hypothalamus, and by

inhibitory GABAergic afferents from the hypothalamic ventrolateral preoptic nucleus

(Ericson *et al*., 1991; Haas *et al*., 2008; Panula *et al*., 2015).

2.1 Histamine synthesis, release and catabolism

Histamine is synthesized from the amino acid (aa) L-histidine by the enzyme HDC, which

is expressed both in the neuronal bodies and terminals, and the bioavailability of the

precursor is the rate-limiting factor. Histamine is stored in vesicles in neuronal cell bodies

and axon varicosities by the vesicular monoamine transporter 2, VMAT-2 (Merickel and

Edwards, 1995), and is released by exocytosis upon the arrival of action potentials (Haas *et

al*., 2008). The synthesis and release of histamine are regulated by H_3 auto-receptors

(Arrang *et al.,* 1983, 1987a; Morisset *et al*., 2000).

6

MOL #104752

Most histaminergic fibers do not make typical synaptic contacts (Takagi *et al.,* 1986) and the amine is released from several points along the fibers, allowing its action on a large number of cells. In the brain, histamine is also produced by mast cells, which contribute modestly to the total amine levels in the adult brain, but during early post-natal development represent the principal source of the amine (Molina-Hernández *et al.,* 2012; Panula *et al*., 2015).

Inactivation of histamine in the brain is primarily due to the action of histamine-N-methyltransferase producing tele-methylhistamine that is transformed to tele-methyl-imidazolacetic acid by monoamine oxidase B (MAO-B). Diamine oxidase is the main histamine metabolizing enzyme in the peripheral tissues, but its activity in the brain is considerably low under basal conditions (Barnes and Hough, 2002; Maldonado and Maeyama, 2015). In contrast to most other aminergic neuronal cells, histaminergic neurons lack a specific re-uptake transporter, although astrocytes take up histamine with low affinity (Km 0.56 mM and 4.0 mM) through the activity of the plasma membrane monoamine transporter (PMAT) and, to a lesser extent, the organic cation transporter 3, OCT3 (Yoshikawa *et al.,* 2013).

2.2 Histamine receptors

The differing potency of antagonists in blocking histamine action to increase contraction rate in isolated mouse atria and gastric acid secretion or to induce smooth muscle contraction in isolated guinea-pig ileum led to the first classification of histamine receptors into the H_1 and H_2 subtypes by Ash and Schild in 1966, supported by the subsequent development of selective H_2 receptor (H_2R) antagonists (Parsons and Ganellin, 2006). The

bovine H_1 receptor (H_1R) and the canine H_2R were cloned in 1991 (Yamashita *et al*., 1991; Gantz *et al*., 1991), allowing for the classification of these receptors into the class A, rhodopsin-like, of G protein-coupled receptors (GPCRs). H_1Rs and H_2Rs are expressed in the brain, and the former signals primarily through $G\alpha_{q/11}$ proteins whereas the latter activates mainly $G\alpha_s$ proteins (Panula *et al*., 2015).

A third receptor (H_3R) was pharmacologically identified by Arrang *et al.* (1983) and cloned in 1999 by Lovenberg *et al*.; the H_3R activates $G\alpha_{i/o}$ proteins and is almost exclusively expressed by neuronal cells of the CNS and the peripheral nervous system (Panula *et al*., 2015). Soon after, a fourth receptor was cloned by several groups (e.g. Nakamura *et al.*, 2000 and Oda *et al.,* 2000). The H_4R is mainly expressed by cells of the immune system and, like the structurally related H_3R, activates $G\alpha_{i/o}$ proteins (Panula *et al*., 2015).

3. The histamine H_3 receptor (H_3R)

In 1983 Arrang *et al*. reported that in rat cerebro-cortical slices labeled with [^{3}H]-histidine, the depolarization-evoked, Ca^{2+}-dependent release of [^{3}H]-histamine was reduced by exogenous histamine (IC_{50} 41 nM, maximal inhibition 61%). The effect was insensitive to tetrodotoxin, which prevents the generation and propagation of action potentials, mimicked by N^α-methylhistamine (NAMH), and antagonized by impromidine and burimamide with potencies significantly different from those reported for H_2R blockade. More potent H_2R antagonists and selective H_1R antagonists also showed low potencies. It was therefore proposed that auto-inhibition of histamine release was mediated by a novel class of receptor (H_3R). In a later study (Arrang *et al*., 1985a) auto-inhibition of depolarization-evoked [^{3}H]-histamine release was also shown for isolated nerve terminals (synaptosomes) from rat

cerebral cortex (-30%) and slices of striatum (-49%), hippocampus (-47%) and

hypothalamus (-64%). The same group reported that histamine also inhibited its own

synthesis evoked by depolarization in rat cerebro-cortical slices (IC_{50} 340 nM, maximal

inhibition 70%; Arrang *et al.*, 1987a). This effect was competitively antagonized by

burimamide and impromidine with potencies similar to those observed for the auto-

inhibition of release (Arrang *et al*., 1983), supporting the function of the H_3R as an auto-

receptor.

In 1999 Lovenberg *et al*. identified a partial clone (GPCR97) and used it to probe a human

thalamus cDNA library. This resulted in the isolation of a full-length clone encoding a 445

amino acid protein with the characteristics of the class A of GPCRs, and with 20-27%

homology to biogenic amine receptors and 22% and 21.4% homology to the human H_1R

and H_2R, respectively. Upon transfection into human HEK-293 cells, rat C6 glioma cells

and human SK-N-MC neuroblastoma cells, GPCR97 displayed a pharmacological profile

practically indistinguishable from that of the native H_3R. Soon after, the receptor was

cloned by sequence similarity from various other species, namely rat, guinea pig, mouse

and monkey, with a high level of homology ($\geq$ 93%) across these species (see Figure 2).

3.1 Expression in the Central Nervous System (CNS)

The H_3R is mainly expressed by neurons and in very low density by glial cells (Arrang *et al*., 1987b; Ferreira *et al*., 2012). The distribution of the H_3R in the CNS has been studied

via *in situ* hybridization (mRNA), RT-PCR (mRNA), and autoradiography (binding sites)

in rodents, human and monkey. *In situ* hybridization studies report very high levels of H_3R

mRNA in the cortex (mainly in the V layer, with lower expression in the superficial layers),

hippocampus (CA1 and ventral CA3 pyramidal layers of Ammon's horn), caudate, and putamen. Strong mRNA expression is also observed in the anterior olfactory nucleus, amygdala, bed nucleus of the stria terminalis, cerebellum, thalamus (mostly in the sensory and intralaminar nuclei), and some hypothalamic nuclei, particularly the TMN where histaminergic neurons are located. Low to moderate mRNA expression is detected in the habenula and zona incerta; the signals are very low in the globus pallidus, substantia nigra (SN) and substantia innominata, and not detected in the islands of Calleja (Tardivel-Lacombe *et al.*, 2000; Pillot *et al.*, 2002; Sallmen *et al.*, 2003).

The analysis by RT-PCR of the expression of the H_3R isoforms of 445 and 365 aa in the human brain (see below for a description of the receptor isoforms) indicates high levels in cerebellum and caudate, moderate in hypothalamus and thalamus, low expression in SN, hippocampus, prefrontal cortex, corpus callosum and amygdala, and very low levels in the spinal cord (Bongers *et al.*, 2007b).

Binding studies show a similar H_3R distribution in primate and rodent brain. The receptor is widely expressed but with heterogeneous density, and high levels are found in cerebral cortex (except in layer V), tenia tecta, nucleus *accumbens*, striatum, hippocampus, bed nucleus of the stria terminalis, olfactory nuclei, some hypothalamic nuclei (mainly the TMN), amygdala, and pyriform cortex. In contrast to mRNA expression, dense binding is found in the globus pallidus and the substantia nigra *pars reticulata* (SNr). A low density of binding sites is found in *locus coeruleus* and raphe nuclei, and the cerebellum and the pituitary gland are scarcely labeled (Martinez-Mir *et al.*, 1990; Pollard *et al.,* 1993; Anichtchik *et al.*, 2000; Pillot *et al.*, 2002).

The H₃R location is mainly pre-synaptic, either as auto-receptor or as hetero-receptor, but there is also evidence for a post-synaptic location of the H₃R in striatum, cerebral cortex, hippocampus, nucleus *accumbens*, lateral hypothalamus and zona incerta (Pillot *et al*., 2002; González-Sepúlveda *et al*., 2013; Parks *et al*., 2014).

3.2 Structure

With over 800 genes encoding GPCRs, these proteins constitute the largest family of membrane proteins contained in the human genome. These receptors share a common seven transmembrane (TM) domain structure that forms the core, an extracellular amino terminus (NT), an intracellular carboxyl terminus (CT), three extracellular (ECL), and three intracellular (ICL) loops.

The H₃R possesses a DRF motif in the interface of TM3 and ICL2 (instead of the DRY sequence common to most class A GPCRs), a NPVLY motif in TM7 (corresponding to the NPXXY motif present in all GPCRs), and a palmitoylation site in the CT (Cys428) that allows for the formation of helix 8 (Figure 3). A disulfide bond is formed by Cys107 and Cys188 on ECL1 and ECL2, respectively. The H₃R has a short NT (39 aa), with a glycosylation site on Asn11, and a long ICL3 (142 aa). These segments are the loci of the naturally occurring mutations D19E and A280V, respectively (Wiedemann *et al*., 2002), and cleavage through splicing of the ICL3 leads to several H₃R isoforms as discussed below.

Since 2000, the rat, guinea pig, monkey and mouse genes encoding the H₃R have been cloned (Lovenberg *et al*., 2000; Tardivel-Lacombe *et al*., 2000; Chen *et al*., 2003; Yao *et al*., 2003), and this work revealed a homology of ≥ 93% in the H₃R protein sequence

11

among these species (Figure 2). The inter-species differences in the pharmacological

profiles rely on a double change in the residues 119 and 122 located nearby Asp114 in

TM3. Human and monkey receptors have Thr119 and Ala122 at these species-variant sites,

whereas rodents have Ala119 and Val122, with the exception of the guinea-pig receptor

(Thr119 and Val122). When compared with the human H_3R (hH_3R), a single-residue

change (V362I) in TM6 is found in the rat, mouse and guinea pig. No differences exist

between human and monkey H_3Rs in the transmembrane domains (Hancock *et al*., 2003).

In contrast to the small changes in the helical domains, a significant number of differences

among species are found in a 65-66 aa-length sequence in the ICL3 (aa 236 to 300 in the

hH_3R), but this does not affect the presumed Ser and Thr substrates of phosphorylation,

suggesting a conserved kinase modulation profile.

3.3 Determinants of G-protein coupling and activation

Structurally and functionally, two faces can be identified on all GPCRs: the extracellularly

accessible orthosteric binding site, and the internal region responsible for G protein

activation. The crystallization of GPCRs coupled to a G protein has provided insight into

the mechanisms of receptor-G protein interaction and activation of the latter. These studies

show that the α-CT and the helixes 4 and 5 of the Gα subunit dock to a cavity formed

mainly by TM5, TM6, and ICL2 in the GPCR, which adopts an "open" conformation upon

receptor activation. The Arg of the DRY motif plays a key role in docking the α-CT of the

G protein (Rasmussen *et al*., 2011), and residues in ICL3, helix 8 and the CT appear to

contribute to stabilizing the receptor-G protein interaction because a truncation close to the

NPXXY motif prevents G protein activation (van Rijn *et al*., 2013; Flock *et al*., 2015).

Computational models of the H_3R-G protein interaction have not yet been published, but functional studies suggest that ICL3 plays a role in G protein activation. Alternative splicing generates several H_3R isoforms (see below), and the hH_3R of 445 aa (hH_3R_{445}) inhibited forskolin-induced cAMP formation in CHO cells, but the hH_3R_{365} (lacking 80 aa in ICL3) failed to do so (Cogé *et al*., 2001). In rat C6 glioma cells, the hH_3R_{365} was less efficacious in a calcium mobilization assay (Esbenshade *et al*., 2006), but agonists were 3- to 20-fold more potent in the R-SAT assay, based on β-galactosidase activity (Wellendorph *et al*., 2002). The rat H_3R_{413} and H_3R_{397} isoforms that lack 32 and 48 aa in ICL3 showed similar efficacy and higher agonist potency for the inhibition of forskolin-induced cAMP formation, but for 42/44-MAPK phosphorylation the H_3R_{445} coupled considerably better (Drutel *et al*., 2001). The naturally occurring A280V mutation on ICL3 also modifies the functionality of the hH_3R_{445} to inhibit cAMP accumulation and stimulate 42/44-MAPK phosphorylation (Flores-Clemente *et al*., 2013).

Altogether, the previous information indicated that there is not a single structural determinant for the H_3R functionality, but that the interplay of several regions provides the appropriate physiological output.

3.4 Constitutive activity and determinants

The term constitutive activity refers to a ligand-independent state of the receptor that spontaneously adopts the active conformation. The H_3R possesses high constitutive activity as indicated by the reduction of basal G protein activation induced by inverse agonists (Wieland *et al*., 2001; Rouleau *et al*., 2002). Constitutive activity has mainly been shown for transfected human and rat H_3Rs, but also for native rodent receptors with inverse

agonists reducing basal [^{35}S]-GTPγS binding to membranes from the cerebral cortex and

hippocampus, enhancing depolarization-induced [^{3}H]-histamine release from cerebro-

cortical synaptosomes and increasing the levels of the histamine metabolite tele-

methylhistamine in homogenates from the cerebral cortex (Morisset *et al*., 2000; Sallmen *et al*. 2003).

The DRY motif is responsible for the equilibrium between the inactive and active receptor

conformations, and therefore plays a pivotal role in constitutive activity. A salt bridge

between the Arg in the DRY motif (TM3) and an Asp or Glu in TM6 stabilizes or 'locks'

the inactive state. Upon receptor activation, this interaction is broken and the Arg residue in

TM3 rotates towards TM5 to form a hydrogen bond with a Tyr residue to establish an

'active lock' (Valentin-Hansen *et al*., 2012). Accordingly, the R112A mutation in the hH$_4$R

prevents G protein activation (Schneider *et al*., 2010), and the R116A mutation in the rat

H$_2$R resulted in a highly structurally instable receptor whose expression could only be

detected after stabilization with either an agonist or inverse agonist; furthermore, this

receptor showed increased agonist affinity and reduced efficacy (Alewijnse *et al*., 2000).

However, there is no direct evidence for a role of the DRF motif in H$_3$R constitutive

activity.

As pointed by Morisset *et al*. (2000), the CT of ICL3 in the rat H$_3$R has a stretch of eight

amino acids that is similar (six identical and two conserved residues) to the corresponding

sequence of a mutated human β_2-adrenoceptor with high constitutive activity (Lefkowitz *et al*., 1993). This region is critical for constitutive activity in other native or mutated GPCRs.

Although the sequence is conserved in all H$_3$R isoforms and species (see Figure 2),

constitutive activity varies among isoforms and it can be different between species and cells

lines (Arrang *et al*., 2007).

3.5   H$_3$R isoforms

The existence of H$_3$R isoforms was initially suggested by the pharmacological

heterogeneity of the receptor expressed in several brain regions in binding and functional

assays (West *et al.,* 1990). The molecular cloning of the receptor indicated that the structure

of the hH$_3$R gene comprised three exons and two introns (Tardivel-Lacombe *et al.*, 2001;

Wiedemann *et al.*, 2002) or four exons and three introns (Cogé *et al*., 2001), that allows for

the generation of isoforms via RNA splicing. Alternatively, for humans and rodents, the

last intron is proposed to be a pseudo-intron located in the region coding for ICL3, which is

retained in the hH$_3$R$_{445}$, but deleted in the hH$_3$R$_{413}$ (Tardivel-Lacombe *et al.*, 2001; Bongers

et al. 2007a). In both proposed structures the two first introns are located in the same

position in the human and rodent genes, suggesting the existence of a variety of functional

and non-functional isoforms with certain similarity among species (Tardivel-Lacombe *et

al*., 2000; Drutel *et al*., 2001; Morisset *et al*., 2001; Wellendorph *et al*., 2002).

RT-PCR analysis identified several isoforms that differ in the length of their NT or CT,

deletions in the ICL3 or shorter sequences in TMs. To date the pharmacological and

functional characteristics of the H$_3$R isoforms have only been evaluated in heterologous

systems (Table 1).

Some of the identified isoforms lack regions critical for agonist binding (TM 3 and 5-7) or

signaling (ICL2, ICL3 and CT), and therefore do not trigger the signaling pathways

typically associated with H$_3$R activation (Wess, 1997; Uveges *et al*., 2002; Oldham and

Hamm, 2007; Ishikawa *et al.*, 2010; Kim *et al.*, 2011; Kuramasu *et al.*, 2011). However, three rat isoforms (rH_3R_{497}, rH_3R_{465} and rH_3R_{449}) that do not possess TM7, but do have an extracellular CT of 105 aa without homology to the functional isoforms reduce the cell surface expression of the rat H_3R_{445} upon co-expression in COS-7 cells (Bakker *et al.*, 2006). The expression and signaling of functional H_3Rs could thus be regulated by isoforms incapable of triggering the signaling pathways described below. These isoforms could also directly interact with other GPCRs and affect their binding or signaling properties. Furthermore, non-canonical signaling, either agonist-independent or mediated by alternative pathways, cannot be discarded for the truncated or longer H_3R isoforms, and merits further investigation.

3.6 Signaling pathways

The coupling of the H_3R to $G\alpha_{i/o}$ proteins was first suggested by the inhibitory effect of Pertussis toxin (PTX) activity, which decreased the affinity for $[^3H]$-NMHA of the H_3R endogenously expressed by murine pituitary AtT cells (Clark *et al.*, 1993) and prevented H_3R-stimulated $[^{35}S]$-GTPγS binding in rat cerebro-cortical membranes (Clark and Hill, 1996). As for other GPCRs, both the $G\alpha$ subunits and the $G_{\beta\gamma}$ complexes mediate the $G\alpha_{i/o}$ protein-dependent signaling of the H_3R (Figure 4).

Inhibition of adenylyl cyclases. In the study that reported the cloning of the hH_3R (Lovenberg *et al.*, 1999), receptor activation inhibited forskolin-induced cAMP accumulation, and this effect was disrupted by PTX (Shi *et al.*, 2012). Likewise for other $G\alpha_{i/o}$ protein-coupled GPCRs, a direct interaction between the $G\alpha_{i/o}$ subunit and sensitive

16

adenylyl cyclases (1, 3, 5, 6 and 8) appears to be the mechanism that mediates this effect (Cooper and Crossthwaite, 2006).

Inhibition of the Na$^+$/H$^+$ exchanger (NHE). The activity of the Na$^+$/H$^+$ exchanger represents one of the key mechanisms for restoring the intracellular pH following ischemia-induced acidosis by extruding protons concomitantly with Na$^+$ influx (Karmazyn, 1999). A rise in intracellular Na$^+$ may reverse the Na$^+$/Cl$^-$ dependent noradrenaline transporter (NET) and induce carrier-mediated neurotransmitter release. H$_3$R activation reduces NHE activity in sympathetic nerve terminals leading to inhibition of noradrenaline release in myocardial ischemia (Silver *et al.*, 2001); the mechanism is not fully understood, although a direct G$\alpha_{i/o}$ subunit/NHE interaction appears likely (van Willigen *et al.*, 2000).

Inhibition of N- and P/Q-type voltage-gated Ca^{2+} channels. The inhibitory effect of H$_3$Rs on neurotransmitter release (see below) is most likely linked to the reduction in depolarization-induced Ca^{2+} entry via the binding of G$_{\beta\gamma}$ complexes to the pore-forming α_1-subunit of N- and P/Q-type voltage-gated Ca^{2+} channels (Zamponi and Currie, 2013). Accordingly, H$_3$R activation reduces depolarization-induced Ca^{2+} entry in dissociated hypothalamic histaminergic neurons (Takeshita *et al.*, 1998), striatal synaptosomes (Molina-Hernández *et al.*, 2001), transfected human neuroblastoma SH-SY-5Y cells (Silver *et al.*, 2002), and transfected rat pheochromocytoma PC12 cells (Morrey *et al.*, 2008). The inhibition of voltage-gated Ca^{2+} currents by H$_3$Rs in histaminergic neurons was abolished by PTX (Takeshita *et al.*, 1998), and both a G$_{\beta\gamma}$ scavenger and a phosphoducin-like anti-G$_{\beta\gamma}$ peptide prevented the H$_3$R-mediated reduction in depolarization-induced Ca^{2+} entry and neurotransmitter exocytosis in pheochromocytoma PC12 cells (Morrey *et al.*, 2008), supporting the participation of G$_{\beta\gamma}$ dimers in these processes.

17

Activation of G protein-gated inwardly rectifying K⁺ channels (GIRKs). $G_{\beta\gamma}$ subunits

bind and activate GIRK channels (Bünemann *et al.*, 2001), and transfected hH_3R_{445} and

hH_3R_{365} activate channels formed by the GIRK1 (Kir3.1) and GIRK4 (Kir3.4) subunits

expressed in *Xenopus* oocytes (Sahlholm *et al.*, 2012). GIRKs can inhibit synaptic

transmission (Meneses *et al.*, 2015), and activation of pre-synaptic GIRKs would thus

represent an additional mechanism for H_3Rs to modulate neurotransmitter release.

Activation of GIRK channels by H_3Rs has also been observed at the postsynaptic level in

neurons producing melanin-concentrating hormone (MCH), where the effect was prevented

by GDPβS, an inhibitor of G protein signaling (Parks *et al.*, 2014).

Phospholipase C (PLC) activation. In transfected CHO and SK-N-MC cells, activation of

the hH_3R_{445} induces a significant increase in the intracellular concentration of Ca^{2+} ions

($[Ca^{2+}]_i$) due to PLC activation and release of Ca^{2+} from intracellular stores via inositol-

1,4,5-trisphosphate (IP_3) formation (Cogé *et al.*, 2001; Bongers, 2008). The H_3R-mediated

increase in $[Ca^{2+}]_i$ was prevented by PTX, which implicates the participation of $G\alpha_{i/o}$

proteins (Bongers, 2008). The $PLC/IP_3/Ca^{2+}$ pathway is most often triggered by the $G\alpha$

subunits of $G\alpha_{q/11}$ proteins, but $G_{\beta\gamma}$ complexes can also activate $PLC\beta$ by binding to a

region (PH and Y domains) different from the $G\alpha_{q/11}$ subunit binding domain (C2 domain

and carboxyl terminal region; Rebecchi and Pentyala, 2000; Rhee, 2001).

Activation of the mitogen-activated protein kinase (MAPK) pathway. H_3R activation

stimulates 42/44-MAPK phosphorylation both in heterologous systems and native tissues

(Drutel *et al.*, 2001; Giovannini *et al.*, 2003; Flores-Clemente *et al.* 2013). $G_{\beta\gamma}$ complexes

appear to play a central role in this action because pharmacological inhibition and $G\alpha$-

transducin, a $G_{\beta\gamma}$ scavenger, prevent H_3R-mediated 42/44-MAPK phosphorylation (Lai *et*

al., 2016). However, other mechanisms, such as the binding to activated receptors of β-arrestins, that act as a signaling scaffold (Gutkind, 2000), or the transactivation of the epidermal growth factor (EGF) receptor (Lai et al., 2016), could also contribute to MAPK activation.

Activation of the phosphatidylinositol 3-kinase (PI3K) pathway. In transfected cells, primary cultures of rat cerebro-cortical neurons and rat striatal slices, H_3R activation stimulate the phosphorylation of Akt or protein kinase B (PKB), which subsequently phosphorylates and thereby inhibits the action of glycogen synthase kinase 3-β (GSK3β) (Bongers *et al.*, 2007c). This action on the PI3K/Akt pathway was prevented by PTX and probably depends on $G_{\beta\gamma}$ complexes, which are known to activate PI3K (Murga *et al.*, 1998).

Stimulation of phospholipase A$_2$. The activation of phospholipase A$_2$ by the H_3R induces the release of arachidonic acid, docosahexaenoic acid, and lysophospholipids, inducing functional consequences such as the relaxation of guinea-pig bronchioles by enhanced release of the endothelium-derived relaxing factor (EDRF), a metabolite of arachidonic acid (Burgaud and Oudart, 1993).

3.7 Regulation of H$_3$R signaling

Desensitization. This process, which leads to changes in signaling efficacy and receptor expression at cell surfaces, represents a major mechanism to regulate GPCR functional responses. Homologous desensitization is triggered by the phosphorylation of activated receptors by GPCR kinases (GRKs), whereas in the heterologous process activation of one GPCR leads to the desensitization of one or more unrelated receptors in the same cell. The

latter process often involves GPCR phosphorylation by second messenger-activated kinases, in particular protein kinases A (PKA) and C (PKC). GPCR phosphorylation results in conformational changes that impair G protein activation and triggers receptor internalization (Gainetdinov *et al.*, 2004; Gurevich *et al.*, 2012).

In CHO-K1 cells stably transfected with the hH_3R_{445}, exposure to agonists results in functional desensitization as well as reduced receptor expression in the cell surface due to the action of GRKs 2/3 and clathrin-dependent endocytosis (Osorio-Espinoza *et al.*, 2014). In the same expression system, the hH_3R_{445} also experiences PKC-mediated heterologous desensitization upon the activation of a second GPCR coupled to the $PLC/IP_3/DAG$ pathway (Montejo-López *et al.*, 2016).

RGS proteins. The regulator of G protein signaling (RGS) proteins modulate the intracellular effects of activated GPCRs. Four RGS subfamilies (RZ, R4, R7, R12) act as GTPase-activating proteins to increase the rate of GTP hydrolysis by Gα subunits (Hollinger and Hepler, 2002; Sjögren *et al.*, 2010). Whereas there are no reports on the regulation by RGS of the H_3R signaling, RGS proteins are co-expressed with H_3Rs in the CNS, for example in cerebral cortex (RGS 4-8, 10), nucleus *accumbens* and striatum (RGS 4, 8, 9), hippocampus (RGS 7, 8, 10) and hypothalamus (RGS 4, 6, 7, 8) (Gold *et al.*, 1997), making the RGS-mediated regulation of H_3R signaling an aspect deserving attention.

4. Pharmacology

The H_3R shows a complex pharmacology, with drugs acting as full agonists, partial agonists, neutral antagonists, inverse agonists and protean ligands. H_3R affinity for ligands

MOL #104752

has been determined by radioligand binding assays (Ki or pKi, Table 2) and efficacy

(Emax) and potency (pEC$_{50}$, EC$_{50}$, pK_{B}, pA$_2$, pD$_2$) through the analysis of [^{35}S]-GTPγS

binding, cAMP formation, 42/44-MAPK phosphorylation, calcium mobilization, and PI3K

activation (Table 3).

In 2008 the crystal structure of the GPCR opsin was resolved (Scheerer *et al.*, 2008),

leading to a revolutionary change in the study of GPCR-drug interactions. Although the

crystal structure of the H$_3$R has yet to be elucidated, homology models and point-mutation

studies allow for the assessment of ligand recognition by its binding pocket.

4.1 Determinants of histamine binding

Histamine (2-[4-imidazolyl]ethylamine) is an hydrophilic non-chiral molecule, consisting

of an imidazole ring and an ethylamine side-chain, and its binding to the H$_3$R depends on

features shared by other biogenic amine receptors. Although the ligand binding site

comprises residues in TM3 to TM7, two main residues are essential for histamine binding.

One is the negatively charged Asp114 in TM3 that interacts with the protonated amine

group of histamine, and the other is Glu206 in TM5 that forms a hydrogen bond with the

nitrogen present in the imidazole ring.

In addition to Glu206, other residues important for histamine binding are Trp196, Thr201,

Ala202, Thr204 and Phe208 in TM5. Mutations in these residues decrease binding affinity,

with the E206A mutation resulting in the most marked change. Interestingly, variations in

binding affinity induced by mutations are not always matched in functional assays, with the

W196A, T204A, E206A and F208A mutations increasing ligand potency to inhibit cAMP

formation, indicating that conformational changes in the binding pocket do not necessarily

translate into receptor-driven G protein activation (Uveges et al., 2002). Other residues involved in histamine binding are Tyr115 and Cys118 in TM3; Trp371, Tyr374 and Leu401in TM6; and Tyr189 in ECL2 and Trp402 in TM7 (Ishikawa *et al.,* 2010). The high affinity of the H_3R for the endogenous agonist does not rely on Asp114 (conserved in all four histamine receptors, and essential for histamine binding), but on Glu206, only present in H_3Rs and H_4Rs, explaining the non-selective actions of several classical H_3R ligands at the H_4R (Axe *et al*., 2006). The increased affinity of H_3Rs and H_4Rs for histamine may be due, at least in part, to the stronger interaction of the imidazole ring with Glu206 compared to the interaction with the Asn or Thr residue found on the same position in H_1Rs or H_2Rs, respectively (Uveges *et al*., 2002).

The previous information allows a pharmacophore structure to be drawn for the H_3R (Figure 5) where Glu206 anchors the imidazole moiety of histamine and the other residues form a lipophilic bed in which histamine lays, favoring the interaction of the basic amine moiety with Asp114 in TM3 (de Esch *et al*., 2000).

4.2 Binding of synthetic imidazole-containing and non-imidazole ligands

Imidazole-containing agonists. So far, all agonists at the H_3R are based on the structure of histamine and therefore contain the imidazole moiety, which is critical for agonist activity (Leurs *et al*., 1995). Modifications of the imidazole ring have been unsuccessful. By contrast, modifications of the histamine side chain that preserve the basic amino group or other proton donor moieties have allowed for the design of more selective and potent agonists. Like histamine, these molecules bind to the H_3R by using Glu206 as anchor for the imidazole moiety and establishing hydrogen bonds with Asp114 (Figure 5).

22

The first highly selective and potent H_3R ligands were the agonist RAMH (R-α-methylhistamine) and the antagonist thioperamide, and the differences in the affinity and potency of the ligand enantiomers demonstrated the stereoselectivity of the H_3R (Arrang *et al*., 1987b; Kovalainen *et al*., 1999; De Esch *et al*., 1999). The replacement of the histamine amine group by an isothiourea group led to the very potent and selective H_3R agonist imetit (Garbarg *et al*., 1992), and other histamine analogues with a piperidine ring in the side chain, such as immepip, showed improved affinity and efficacy at the H_3R (Figure 6 and Table 2). Modifications in the piperidine nitrogen, like VUF-5681, resulted in decreased affinity and functional activity (Shih *et al.,* 1998; Kitbunnadaj *et al*., 2003, 2005). The H_4R has high homology to the H_3R, and also high affinity for H_3R agonists such as imetit and immepip. The search for more selective ligands led to methimepip (N-methyl-substituted immepip), which retains high affinity and efficacy (Tables 2 and 3), and shows 2000-fold selectivity for the hH_3R over the hH_4R (Kitbunnadaj *et al*., 2003, 2005).

Imidazole-containing antagonists. In addition to the imidazole moiety, the majority of these compounds posses a central electro-negative group and a cyclic moiety (Figure 7). These groups form a conserved pharmacophore with three interactions: a) a hydrogen bond of the imidazole ring with Glu206 in TM5; b) a salt bridge of the central electro-negative group with either Tyr374 in TM6 or Asp114 in TM3, which may dictate the torsion of the antagonist; and, c) a π-stacking of the hydrophobic cyclic group with Phe198 in TM5, Tyr189 in ECL2, or Trp110 in TM3 (Levoin *et al*., 2010; Figure 5).

The extension of the ethylamine side chain, derived from the imidazolyl piperidine compounds by varying the piperidin-substituting groups, resulted in drugs devoid of agonist activity and led to the antagonist thioperamide (Arrang *et al*., 1987b). The more

potent antagonist clobenpropit was derived from imetit through the benzylation of the isothiourea group and other modifications, and its iodinated analogue (iodophenpropit) is also a potent antagonist (van der Goot and Timmerman, 2000). Other imidazole-containing antagonists are referred to in Table 3.

The discovery of H_3R constitutive activity led to the reclassification of some antagonists as inverse agonists or protean ligands. Thioperamide and ciproxifan, classically considered antagonists, also behave as inverse agonists. Proxyfan acts as agonist, inverse agonist or neutral antagonist, depending on the level of H_3R constitutive activity, and is therefore considered a protean ligand (Morisset *et al*., 2000; Rouleau *et al*., 2002; Arrang *et al*., 2007).

Non-imidazol antagonists. The effects of H_3R activation on physiological and pathological conditions are the main focus of research into the therapeutic potential of selective H_3R ligands. However the imidazole ring reduces penetration of the blood-brain barrier (Young *et at*., 1988), and imidazole-based drugs inhibit the hepatic cytochrome P450 (Ishikawa *et al*., 2010). To avoid these side-effects, antagonists lacking the imidazole ring, but still capable to form a hydrogen bond with Glu206, were synthesized (Figure 7). The first two non-imidazole antagonists were UCL1972 and VUF5391 where the imidazole ring was replaced for a piperidine or pyrrolidine group, respectively. The heterocyclic nitrogen interacts with Glu206, the central oxygen forms a salt bridge with a hydroxyl-containing residue, and the heterocyclic moiety establishes π-stacking interactions (Ganellin *et al*., 1998, Menge *et al*., 1998; Levoin *et al*., 2010).

MOL #104752

By exploration of the structure-activity relationship, a series of highly potent and selective

non-imidazole antagonists were developed, including ABT-239, JNJ-5207852, NNC 38-

1049 and GSK189254, which show anti-obesity properties, promote the wake state, and

have pro-cognitive effects (Barbier *et al*., 2004; Esbenshade *et al*., 2005; Fox *et al*., 2005;

Medhurst *et al*., 2007).

The imidazole-containing compounds display similar affinities for the H_3R across species

(see Tables 2 and 3). Nevertheless, some antagonists, such as thioperamide or ciproxifan,

are less potent at the hH_3R than at the rat H_3R, and as mentioned previously these

differences have been attributed to changes in two residues located in TM3 (T119A and

A122V). In contrast, the non-imidazole compounds are more potent and selective at the

hH_3R (West *et al*., 1999; Ligneau *et al*., 2000; Parsons and Ganellin, 2006). Furthermore,

binding studies with thioperamide and burimamide in rat, human and monkey tissues

indicate the presence of high- and low-affinity sites, which could be related to either

different states of the same receptor or to the expression of more than one isoform (West *et*

al., 1990, 1999).

5. Function

5.1 Pre-synaptic effects

The best-known function of the H_3R is the pre-synaptic regulation of neurotransmitter

release in the central and peripheral nervous systems. This regulation has been reported for

histamine itself, noradrenaline, dopamine, acetylcholine (ACh), GABA, glutamate,

serotonin (5-hydroxytryptamine, 5-HT), and some neuropeptides. Whereas the evidence for

a direct action of H_3Rs is consistent for most of these neurotransmitters, this is not established for acetylcholine and dopamine.

Histamine. The observation that histamine inhibited it own release led to the identification of the H_3R by Arrang *et al*. (1983). Control by pre-synaptic H_3Rs of histamine release has been shown for slices of rat cerebral cortex, striatum, hippocampus and hypothalamus, as well as for rat cerebro-cortical synaptosomes (Arrang *et al*., 1983, 1985a,b). *In vivo* microdialysis experiments have shown that H_3R agonists reduce histamine release in the rat hypothalamus and cerebral cortex, whereas antagonists increased the release in the hypothalamus, nucleus *basalis magnocellularis,* and cerebral cortex (Jansen *et al*., 1998; Lamberty *et al*., 2003; Giannoni *et al*., 2009).

Acetylcholine (ACh). The modulation of cholinergic transmission by H_3Rs was first reported for the peripheral nervous system; histamine and H_3R agonists inhibited the electrically-evoked contraction of guinea-pig ileum strips, but had no effect on the action of exogenous ACh (Trzeciakowski, 1987). It was later shown that in the longitudinalis smooth muscle/mienteric plexus preparation the release of [^{3}H]-ACh induced by electrical stimulation was reduced by H_3R activation (Poli *et al.,* 1991).

In the CNS, H_3R activation inhibited K^+-induced [^{3}H]-ACh release from rat entorhinal cortex slices, but this effect was not detected in synaptosomes from the same region or in slices from the hippocampus (Arrang *et al*., 1995; Alves-Rodrigues *et al*., 1998), questioning the presence of the receptor on cholinergic nerve terminals. *In vivo* microdialysis shows that H_3R activation reduces ACh release in the rat fronto-parietal cortex, hippocampus, nucleus *accumbens* and basolateral amygdala (Blandina *et al*., 1996;

26

Prast *et al*., 1999; Passani *et al*., 2001; Bacciottini *et al*., 2002). These studies also suggest the involvement of trans-synaptic effects. For example, H$_3$R-mediated inhibition of GABA or dopamine release could subsequently relieve the inhibitory control by these transmitters of ACh release (Schlicker *et al*., 1993; Garcia *et al*., 1997; Prast *et al*., 1999).

Noradrenaline. H$_3$R-mediated inhibition of noradrenaline release has been reported for both the CNS and the peripheral nervous system. In cardiac sympathetic terminals H$_3$R stimulation reduces noradrenaline release by various mechanisms, including inhibition of voltage-activated Ca^{2+} and Na$^+$ channels, reduction of NHE activity, and trans-activation of prostanoid receptors (Endou *et al*., 1994; Imamura *et al*., 1995, 1996; Hatta *et al*., 1997; Mazenot *et al*., 1999; Silver *et al*., 2002; Seyedi *et al*., 2005; Levi *et al*., 2007).

For the CNS, *in vitro* experiments indicated that H$_3$R activation reduces depolarization-induced [^{3}H]-noradrenaline release in rodent cerebral cortex, spinal cord, cerebellum, hippocampus, hypothalamus and olfactory bulb, and human cerebral cortex (Schlicker *et al*., 1989, 1992; 1994, 1999; Celuch, 1995; Timm *et al*., 1998; Aquino-Miranda *et al.,* 2011). *In vivo* microdialysis experiments showed that H$_3$R activation reduces noradrenaline release in rat hippocampus and cerebral cortex (Di Carlo *et al*., 2000; Medhurst *et al*., 2007).

5-hydroxytryptamine (5-HT). In rat cerebral slices and synaptosomes as well as in SNr slices, H$_3$R activation inhibits 5-HT release evoked by electrical or chemical stimulation (Schlicker *et al*., 1988; Fink *et al*., 1990; Threlfell *et al*., 2004). By contrast, in rat olfactory

27

MOL #104752

bulb slices, H_3R activation had no effect on depolarization-induced $[^3H]$-5-HT release

(Aquino-Miranda *et al*., 2011).

Dopamine. In mouse striatum and rat substantia nigra *pars reticulata* (SNr),

depolarization-evoked $[^3H]$-dopamine release is inhibited by H_3R activation (Schlicker *et*

al., 1993; Garcia *et al*., 1997), suggesting that the receptor resides on the terminals and

dendrites of the dopaminergic nigro-striatal neurons. However, H_3R activation had no

effect on depolarization-evoked $[^3H]$-dopamine release from rat and rabbit striatal slices

(Smits and Mulder, 1991; Schlicker *et al*., 1993), indicating differences between species

and brain regions.

In microdialysis studies, H_3R activation increases the extracellular dopamine levels in rat

prefrontal cortex, but not in the striatum (Fox *et al*., 2005; Medhurst *et al*., 2007). The

systemic or local administration of H_3R antagonists augments dopamine release induced by

methamphetamine in the nucleus *accumbens*; however, the effect of the local perfusion of

the antagonists was lower than that produced by their systemic administration (Munzar *et*

al., 2004), casting doubt on the presence of H_3Rs on the terminals of ventral tegmental area

(VTA) neurons, the main source of dopaminergic innervation to nucleus *accumbens* (Wise,

2004).

Glutamate. Pre-synaptic H_3Rs inhibits glutamatergic transmission in rat hippocampus,

striatum, basolateral amygdala, thalamus and globus pallidus (Doreulee *et al*., 2001; Jiang

et al., 2005; Garduño-Torres *et al*., 2007; Osorio-Espinoza *et al*., 2011). In rat striatal and

thalamic synaptosomes H_3R stimulation reduces both glutamate release and an increase in

the intracellular Ca^{2+} concentration induced by depolarization (Molina-Hernández *et al*.,

28

2001; Garduño-Torres *et al.*, 2007). Optogenetic experiments confirmed the H_3R-mediated

inhibition of cortico-striatal and thalamo-striatal glutamatergic transmission, and the

increased paired-pulse ratio supports the pre-synaptic location of H_3Rs in cortico-striatal

and thalamo-striatal synapses. Of note, H_3R activation had no effect on the plasticity of

cortico-striatal synapses, but the thalamo-striatal synapses became significantly facilitatory

(Ellender *et al.*, 2011).

GABA. In slices from rat SNr and striatum, the activation of dopamine D_1 receptors,

coupled to $G\alpha_s$ proteins, enhances depolarization-evoked [^{3}H]-GABA release and this

effect is selectively counteracted by H_3R-mediated inhibition of P/Q-type voltage-activated

Ca^{2+} channels (Garcia *et al.,* 1997; Arias-Montaño *et al.*, 2001, 2007). Likewise, in the

nerve terminals of striato-pallidal neurons, H_3R activation counteracts the facilitatory action

of adenosine A_{2A} receptors ($A_{2A}Rs$), which are also coupled to $G\alpha_s$ proteins (Morales-

Figueroa *et al.*, 2014).

H_3R-mediated inhibition of GABA release has been observed *in vivo* in rat medial

vestibular nucleus, and *in vitro* in primary cultures of rat cerebro-cortical neurons and

dissociated neurons from the rat hypothalamus ventromedial nucleus (Jang *et al.*, 2001;

Bergquist *et al.*, 2006; Dai *et al.*, 2007). In contrast, H_3R activation does not modify

depolarization-evoked [^{3}H]-GABA release from rat thalamus and olfactory bulb slices

(Garduño-Torres *et al.*, 2007; Aquino-Miranda *et al.*, 2011), indicating that not all

GABAergic neurons express pre-synaptic H_3Rs.

Neuropeptides. Histamine inhibits non-adrenergic/non-cholinergic contraction of the

bronchial and intestinal smooth muscle (Ichinose and Barnes, 1989; Taylor and Kilpatrick,

29

1992), and H_3R activation reduces substance P release from sensorial nerve terminals of the

rat hind paw (Ohkubo *et al*., 1995) and the release of the calcitonin gene-related peptide

(CGRP) from the peri-arterial nerve terminals of the rat mesenteric artery (Sun *et al*.,

2011).

5.2 Post-synaptic effects

There is evidence for post-synaptic H_3Rs in some areas of the brain, namely striatum,

cerebral cortex, hippocampus, nucleus *accumbens*, lateral hypothalamus, and zona incerta

(Pillot *et al.,* 2002; Panula and Nuutinen, 2013, González-Sepúlveda *et al*., 2013; Parks *et al*., 2014).

5.2.1 Activation of the MAPK and Akt pathways. The detection of 42/44-MAPK

phosphorylation upon the activation of the rat H_3R_{445} led to the discovery of the link

between H_3Rs and the MAPK pathway (Drutel *et al*., 2001), which were also observed for

native receptors (Mariottini *et al*., 2009). In rat hippocampus slices, H_3R activation

stimulates the MAPK pathway in CA3 pyramidal cells, and MAPK activation seems to be

required for H_3R-induced memory improvement and consolidation in rats after contextual

fear conditioning (Giovannini *et al*., 2003).

In transfected SK-N-MC cells, hH_3R activation stimulates the activity of the Akt/GSK3β

axis (Bongers *et al*., 2007c), and in rat cortical neurons H_3R stimulation results in the

phosphorylation of Akt at Ser473 and GSK3β at Ser9, via PI3K and MAPK activation

(Mariottini *et al*., 2009). Akt regulates the expression of apoptosis inhibitors such as Bcl-2

and Bcl-x, and thus promotes neuronal cell survival (Song *et al*., 2004), and in rat cortical

neurons, H_3R activation increases the expression of Bcl-2 in an Akt-dependent manner. The

30

Akt/GSK3β pathway plays a relevant role in regulating several important cellular processes including cell plasticity and survival, proliferation and metabolism, and H_3R activation exerts a protective effect against serum deprivation-induced cell death in rat cortical neurons and NMDA-induced neurotoxicity in mixed cultures of mouse cortical cells through the activation of the PI3K-Akt pathway (Mariottini *et al*., 2009).

5.2.2 GIRK activation. In melanin-concentrating hormone (MCH)-producing neurons located in the rodent lateral hypothalamus, H_3R activation stimulates GIRKs and inhibits neuronal firing (Parks *et al*., 2014).

5.3 H_3R heterodimerization and functional consequences

GPCR dimerization is now a well-accepted phenomenon that can be defined as a transitory state in which, through protein-protein interactions, a GPCR can alter the binding, signaling or desensitization of the second GPCR that it is dimerized with. Although this phenomenon has been described as temporarily, short-lived, and the result of stochastic interactions, it has sufficient impact to cause or modify a physiological output (Milligan, 2006; Calebiro *et al*., 2013).

Because of its hetero-receptor nature, the H_3R is co-expressed with a large number of GPCRs in different neuronal populations. For example, in striatal medium-sized spiny neurons (MSNs), H_3Rs co-exist with dopamine D_1 (D_1R) or D_2 (D_2R) receptors (González-Sepúlveda *et al*., 2013), allowing for heterodimer formation. In both interactions, H_3R acts as a negative modulator of agonist binding to and signaling of dopamine receptors.

The first insight into H_3R/D_1R interactions came from functional studies where the H_3R inhibited D_1R-mediated facilitation of GABA release in SNr and striatum slices (Garcia *et*

al., 1997; Arias-Montaño *et al*., 2001). In a heterologous expression system H_3R activation induces a shift from cooperative to a non-cooperative binding of D_1R agonists, which indicates that an intra-membrane cross-talk occurs between these receptors. In contrast, D_1R stimulation did not modify agonist binding to H_3Rs. A change in the D_1R signaling pathway from $G\alpha_s$ to $G\alpha_{i/o}$ was also observed in cAMP formation assays. Furthermore, H_3R activation did not induce 42/44-MAPK phosphorylation, but did so when the D_1R was co-expressed. H_3R antagonist prevented the effect of a D_1R agonist and vice versa (Ferrada *et al.,* 2009), and cross-antagonism in rat striatal slices of wild-type and D_1R-KO mice suggests that the interaction also occurs *in vivo*. The phosphorylation of 42/44-MAPKs appears to be a particular print of the H_3R/D_1R interaction, as this effect was not observed in D_2R-MSNs. In addition, behavioral analysis showed that a H_3R antagonist enhanced D_1R-induced locomotor activity (Ferrada *et al*., 2008; Moreno *et al*., 2011).

The H_3R/D_2R interaction is supported by Förster Resonance Energy Transfer (FRET) analysis in transfected cells and by binding studies in which H_3R activation reduced agonist affinity of the D_2R high- and low-affinity sites in sheep striatal membranes (Ferrada *et al.,* 2008). However, the lack of synergism in D_2R- and H_3R-stimulated $[^{35}S]$-GTPγS binding in rat striatal membranes (Humbert-Claude *et al*., 2007) argues against a direct H_3R/D_2R interaction.

The $G\alpha_s$-coupled $A_{2A}R$ is highly expressed and distinctively limited to D_2R-MSNs. $A_{2A}R$ activation inhibits GABA release from MSN collaterals (Kirk and Richardson, 1994), but in the striato-pallidal projections, facilitates the release of the neurotransmitter (Mayfield *et al*., 1993). The latter effect is functionally opposed by H_3R activation, which also decreases $A_{2A}R$ affinity for the agonist CGS-21680 in membranes from globus pallidus

32

synaptosomes, suggesting a direct, protein-protein interaction between $A_{2A}Rs$ and H_3Rs (Morales-Figueroa *et al*., 2014).

6. H_3Rs and neurological and psychiatric disorders

The H_3R is a potential drug target for the treatment of several important neurological and psychiatric disorders and this aspect will be discussed briefly in reference to Alzheimer's disease, attention-deficit hyperactivity disorder, Parkinson's disease, Gilles de la Tourette's syndrome, schizophrenia, addiction, and sleep disorders.

6.1 Parkinson's disease (PD)

PD is a progressive neurodegenerative movement disorder that primarily results from the death of substantia nigra *pars compacta* (SNc) dopaminergic neurons. In addition, the noradrenergic, cholinergic and serotonergic systems are also affected and may contribute to the disorder. Parkinsonian symptoms include bradykinesia, muscular rigidity, rest tremor, and postural and gait impairment (Moore *et al*., 2005; Kalia and Lang, 2015).

As discussed previously, H_3Rs modulate striatal GABAergic, glutamatergic and dopaminergic transmission at the pre- and post-synaptic levels in addition to controlling the release of GABA, glutamate, and 5-HT in several other nuclei of the basal ganglia. In hemiparkisonian rats, H_3R levels increase in the striatum and SNr ipsilateral to the lesioned SNc. Post-mortem analysis of PD patients shows increased histaminergic innervation to SN, augmented histamine levels in caudate/putamen, globus pallidus and SN, higher H_3R density in the SNr and increased H_3R mRNA in the external globus pallidus (reviewed in Panula and Nuutinen, 2013). Injection of the H_3R agonist immepip into the SNr reverses apomorphine-induced contralateral turning behavior in hemiparkinsonian rats, whereas in

naive animals the compound induces ipsilateral turning (García-Ramírez *et al*., 2004). In hemiparkinsonian rats apomorphine-induced turning behavior is enhanced by the systemic administration of L-histidine and reduced by inhibiting histamine synthesis (Liu *et al.,* 2008).

Dopamine-replacement therapy has dominated the treatment of PD motor symptoms since the early 1960s. However, chronic administration of the dopamine precursor L-DOPA results in severe side effects, particularly dyskinesias related to excessive D_1R-mediated signaling in the basal ganglia (Santini *et al.,* 2007). In parkinsonian marmosets the systemic administration of immepip exacerbates the symptoms in non-treated animals, but in L-DOPA-treated marmosets reduces the total dyskinesia score without affecting the anti-parkinsonian action (Gomez-Ramírez *et al*., 2006). Altogether, the available information indicates a role for histamine and the H_3R in PD pathophysiology, and a potential use for drugs acting at the receptor in the treatment of both the disease and the complications of the pharmacological therapies.

6.2 Gilles de la Tourette's syndrome (GTS)

GTS is a disorder characterized by motor and phonic tics (echolalia, echopraxia and coprolalia), sensory and cognitive symptoms, and co-morbidities such as obsessive-compulsive and attention-deficit hyperactivity disorders (Shan *et al*., 2015). GTS is related to the dysfunction of the cortico-striatal-thalamic-cortical circuitry, and involves the transmitters dopamine, noradrenaline, 5-HT, and histamine (Panula and Nuutinen, 2013; Rapanelli and Pittenger, 2015).

A mutation (W317X) in one of the HDC gene alleles is associated with the familiar occurrence of GTS (Ercan-Sencicek *et al.,* 2010). HDC-knockout mice, with brain histamine levels almost negligible in homozygote animals, exhibit stereotypic movements and spontaneous tic-like symptoms after the intraperitoneal injection of amphetamine (Castellan Baldan *et al*., 2014), supporting the involvement of the histaminergic system in GTS.

6.3 Schizophrenia

Schizophrenia is a mental disorder defined by positive symptoms (hallucinations, delusions, and thought and movement disorders), negative symptoms (reduced feelings, difficulty to initiate and maintain activities, and reduced speaking), and cognitive symptoms such as poor understanding of information, trouble focusing, and problems with working memory (Flores *et al*., 2016).

In schizophrenic patients, H_3R receptor expression is increased in the prefrontal cortex and decreased in the hippocampus, areas closely related to memory and cognitive processes (Jin *et al.*, 2009). An increase in the histamine metabolite tele-methylhistamine was reported for chronic patients (Prell *et al*., 1995), and antipsychotics drugs affect the activity of the histaminergic neurons by acting at dopamine, 5-HT, and glutamate (NMDA) receptors expressed by these neurons (Javitt *et al*., 1991; Morisset *et al*., 1999, 2002) and by antagonizing H_1, H_2, and H_3 receptors (Alves-Rodrigues *et al*., 1996; Green and Maayani, 1977; Richelson *et al*., 2000). This information suggests that the histaminergic system plays a role in the disease.

Schizophrenia is characterized by the hyperactivity of dopaminergic neurons and the hypoactivity of glutamatergic neuronal cells. H_3R antagonists/inverse agonists attenuate dopamine receptor-mediated facilitation of locomotor sensitization (Clapham and Kilpatrick, 1994) and locomotor hyperactivity resultant from blockade of NMDA receptors (Faucard *et al*., 2006; Mahamood *et al*., 2012). In DBA/2 mice, administration of a H_3R antagonist/inverse agonist reduces the natural deficits in sensorimotor gating (Fox *et al*., 2005). Although the evidence in animal models supports the antipsychotic properties of H_3R antagonists, two clinical trials with ABT-288 and MK-0249 failed to improve the cognitive function in schizophrenic patients (Egan *et al*., 2013; Haig *et al*., 2014).

6.4 Addiction

Addiction is a compulsive and persistent dependence on behaviors or substances in which the reward brain circuitry (the dopaminergic meso-cortico-limbic system) plays a major role. HDC-KO mice show increased cocaine tolerance and reduction in the place-preference test. Conversely, HDC-KO mice show an increase in alcohol-evoked conditioned place preference and consumption (Zimatkin and Anichtchik, 1999). Substances like alcohol, cocaine and morphine modulate histamine synthesis, release and turnover (Nishibori *et al*., 1985; Ito *et al*., 1997), suggesting the involvement of the histaminergic system in addiction processes. In H_3R-KO mice, a reduction in alcohol-induced place preference and consumption is observed, and a similar effect is induced by H_3R blockade, indicating the participation of the H_3R in alcohol addiction (Brabant *et al*., 2007; Nuutinen *et al.,* 2010; Galici *et al*., 2011).

Hyperactivity caused by amphetamine/methamphetamine is attenuated by the H₃R

antagonists thioperamide, ciproxifan, pitolisant (BF2.649), and ABT-239 (Clapham and

Kilpatrick, 1994; Fox *et al*., 2005; Ligneau *et al*., 2007a,b; Motawaj and Arrang, 2011).

However, other antagonists (GSK-207040, JNJ-5207852 and JNJ-10181457) failed to

replicate this response (Komater, 2003; Southam *et al*., 2009). Moreover, thioperamide and

clobenpropit increased methamphetamine self-administration (Munzar *et al*., 2004). On the

basis of the previously described cross-talk between the dopaminergic and histaminergic

systems, and the direct modulation of histaminergic transmission by addictive drugs,

targeting the histaminergic neurons is a plausible therapeutic proposal.

6.5 Attention-deficit hyperactivity disorder (ADHD)

ADHD, most prevalent in children, is characterized by an ongoing pattern of inattention

and/or hyperactivity-impulsivity. This disorder affects several neurotransmitter systems,

mainly the dopaminergic, noradrenergic, cholinergic, and serotonergic systems (Vohora

and Bhowmik, 2012). H₃R antagonists enhance the release of neurotransmitters involved in

cognition including ACh and dopamine in the prefrontal cortex; ACh, dopamine and

noradrenaline in the cingulate cortex; and ACh in the hippocampus (Fox *et al*., 2005;

Medhurst *et al*., 2007; Ligneau *et al*., 2007b). In rodents, the pro-attentional and pro-

cognitive actions of these drugs suggest that these compounds have a potential therapeutic

use in this disorder (reviewed by Passani and Blandina, 2011, and Vohora and Bhowmik,

2012). However, the H₃R antagonist bavisant (JNJ-31001074) did not show clinical

effectiveness in human adults with ADHD, whereas both atomoxetine and methylphenidate

induced improvement (Weisler *et al.,* 2012).

MOL #104752

6.6 Alzheimer's disease

Alzheimer's disease (AD) is a progressive neurodegenerative brain disorder resulting in the loss of memory and cognitive functions that is often accompanied by behavioral disturbances like aggression and depression (Querfurth and LaFerla, 2010). The pathological hallmarks of AD include the accumulation of the protein β-amyloid (Aβ), which leads to the production of extracellular Aβ plaques and hyper-phosphorylation of the protein tau. This in turn results in the formation of intracellular neurofibrillary tangles and the massive loss of cholinergic neurons (Brioni *et al*., 2011).

In AD patients, neurofibrillary tangles occur in the TMN along with a significant loss (~50%) of the histaminergic neurons (Nakamura *et al*., 1993, Shan *et al*., 2015), but the latter effect is not matched by the reduction (-24%) in HDC mRNA expression, suggesting that enhanced histamine production by the remaining neurons compensates the cell loss. Some reports indicate that brain histamine levels are reduced in hypothalamus, hippocampus and temporal cortex from AD patients, although others show an increase in the same cerebral regions together with frontal, parietal and occipital cortices. Furthermore, H_3R mRNA levels seem to be higher in the prefrontal cortex only in female AD patients, indicating a gender-dependent change (Shan *et al*., 2015). In this regard, differences in histamine levels in AD and normal brains may originate from methodological issues, genetic background, time elapsed between death and analysis, and proper diagnosis. However, measurement of histamine content with a sensitive HPLC fluorimetric method in brains from controls and AD patients, with matched age and post-mortem time, found a significant decrease in the hypothalamus (-58% of control values), hippocampus (-57%), and temporal cortex (-47%) in AD brains (Panula *et al*., 1998).

38

H_3R activation could account for the diminished release of noradrenaline and ACh in the prefrontal cortex, both transmitters with an important role in cognition (Blandina *et al*., 1996; Schlicker *et al*., 1999). Based on studies with transgenic mice, it may be hypothesized that neurotransmitter release evoked by H_3R antagonists leads to post-synaptic effects such as the phosphorylation of the cAMP response element binding protein (CREB), a transcription factor related to cognitive function, or the inhibition of GSK3β, responsible for tau hyper-phosphorylation in AD (Hooper *et al*., 2008; Bitner *et al*., 2011). These *in vivo* studies raise the possibility that H_3R antagonists indirectly modulate signaling pathways resulting in symptomatic alleviation in AD patients. H_3R antagonists like PF-03654746, GSK189254, MK-0249, ABT-239, and ABT-288 bind the hH_3R with high affinity (Ki 0.2 to 3.2 nM) and some have advanced to clinical trials (Brioni *et al*., 2011; Sadeq *et al.*, 2016).

6.7 Sleep disorders

The brain circuitry that regulates sleep-wake cycle in mammals comprises cell groups in the thalamus, brainstem, hypothalamus and basal forebrain. The histaminergic system plays a key role in the maintenance of cortical activation and wakefulness (Lin, 2000). The histaminergic neurons fire tonically, are more active during wakefulness, and send widespread inputs to areas crucially implicated in sleep-wake control, for instance the cerebral cortex and thalamus. In the latter region, H_1R activation decreases a resting leak K^+ conductance (I_{KL}), which results in prolonged depolarization of thalamo-cortical neurons leading to inhibition of burst firing and the promotion of single-spike firing. This action abolishes sleep-related activity in thalamo-cortical networks and facilitates the single-spike activity typical of the waking state (McCormick and Bal, 1997).

39

Impaired wakefulness and increased sleep are observed after the inhibition of histamine synthesis and in HDC-KO mice (Partmentier *et al*., 2002). The wake-promoting activity of histamine is shared by a variety of H_3R antagonists/inverse agonists (Gao *et al*., 2013). H_3Rs pre-synaptically modulate the release of histamine and other neurotransmitters that participate in the wake-sleep cycle and post-synaptically reduce the excitability of MCH-producing neurons that are also involved in the cycle (Parks *et al*., 2014). H_3R-KO mice present a wide-range of apparently contradictory wake-sleep alterations; the animals show wake deficiency and sleep deterioration, but also display signs of enhanced vigilance (Gondard *et al.*, 2013).

Histaminergic neurons are activated by the neuropeptide hypocretin, and type I narcolepsy is characterized by excessive daytime sleepiness and cataplexy. The main cause of type I narcolepsy is the loss of hypocretinergic neurons; however, an increase in the number of histaminergic neurons has been reported (Valko *et al*., 2013). Although histamine dysregulation is not central to the pathophysiology, H_3R antagonists/inverse agonists such as pitolisant are useful for the treatment of excessive sleepiness disorders, namely narcolepsy and idiopathic hypersomnia by improving alertness (Lin *et al*., 2008; Inocente *et al*., 2012; Dauvilliers *et al*., 2013). Furthermore, pitolisant reduces sleepiness in PD patients without interfering with anti-parkinsonian drugs (Schwartz, 2011). On March 2016, pitolisant (Wakix^{TM}) was approved in the European Union for the treatment of narcolepsy with or without cataplexy in adults, becoming thus the first drug acting at H_3Rs that reachs the market (Syed, 2016).

7. Final remarks

The histaminergic system exerts a significant modulatory effect on different brain functions, from movement to cognitive processes. The H_3R plays a key role in this effect through its wide expression and action as a modulator of several neurotransmitter systems. Although the main effects of H_3R activation are known for some brain areas, such as the striatum and the hippocampus, its actions in other areas have yet to be fully explored. Furthermore, determining the precise H_3R expression in distinct cells of several neuronal circuits, such as the cortico-striato-thalamo-cortical and meso-cortico-limbic circuits, will help to understand the participation of the histaminergic system in aspects such as the control of motor activity, cognition and the sleep-wake cycle, and certain disorders, including PD, AD, and addiction. A deeper knowledge of the functional and molecular interactions of H_3Rs with other receptors is also important for the design of novel therapeutic approaches targeting the histaminergic system, and for this purpose the elucidation of the H_3R crystal structure will represent an important discovery. Finally, more clinical studies are needed to evaluate or confirm the usefulness of H_3R ligands in several neuro-psychiatric disorders.

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MOL #104752

9. Authorship contribution

Wrote or contributed to the writing of the manuscript: Nieto-Alamilla, Márquez-Gómez, García-Gálvez, Morales-Figueroa and Arias-Montaño

10. References

Airaksinen MS and Panula P (1988) The histaminergic system in the guinea pig central nervous system: an immunocytochemical mapping study using an antiserum againsthistamine. *J Comp Neurol*. **273**:163-186.

Alewijnse AE, Timmerman H, Jacobs EH, Smit MJ, Roovers E, Cotecchia S, and Leurs R (2000) The effect of mutations in the DRY motif constitutive activity and structural instability of the histamine H_2 receptor. *Mol Pharmacol* **57**:890-898.

Alves-Rodrigues A, Leurs R, Willems E, and Timmerman H (1996) Binding of clozapine metabolites and analogues to the histamine H_3 receptor in rat brain cortex. *Arch Pharm (Weinheim)* **329**:413-416.

Alves-Rodrigues A, Timmerman H, Willems E, Lemstra S, Zuiderveld OP, and Leurs R (1998) Pharmacological characterisation of the histamine H_3 receptor in the rat hippocampus. *Brain Res* **788**:179-186.

Anichtchik OV, Huotari M, Peitsaro N, Haycock JW, Männistö PT, and Panula P (2000) Modulation of histamine H_3 receptors in the brain of 6-hydroxydopamine-lesioned rats. *Eur J Neurosci* **12**:3823-3832.

Aquino-Miranda G, Osorio-Espinoza A, Escamilla-Sanchez J, Gonzalez-Pantoja R, Ortiz J and Arias-Montano JA (2011) Histamine H_3 receptors modulate depolarization-evoked [^{3}H]-noradrenaline release from rat olfactory bulb slices. *Neuropharmacol* **62**:1127-1133.

Arias-Montano JA, Floran B, Garcia M, Aceves J, and Young JM (2001) Histamine H_3 receptor-mediated inhibition of depolarization-induced, dopamine D_1 receptor-dependent release of [^{3}H]-gamma-aminobutryic acid from rat striatal slices. *Br J Pharmacol* **133**:165-171.

Arias-Montano JA, Floran B, Floran L, Aceves J, and Young JM (2007) Dopamine D_1 receptor facilitation of depolarization-induced release of gamma-amino-butyric acid in rat striatum is mediated by the cAMP/PKA pathway and involves P/Q-type calcium channels. *Synapse* **61**:310-319.

Arrang JM, Garbarg M, and Schwartz JC (1983) Auto-inhibition of brain histamine release mediated by a novel class (H_3) of histamine receptor. *Nature* **5911**:832-837.

Arrang JM, Garbarg M, and Schwartz JC (1985a) Autoregulation of histamine release in brain by presynaptic H_3-receptors. *Neuroscience* **15**:553-562.

Arrang JM, Schwartz JC, and Schunack W (1985b) Stereoselectivity of the histamine H_3-presynaptic autoreceptor. *Eur J Pharmacol* **1**:109-114.

Arrang JM, Garbarg M, and Schwartz JC (1987a) Autoinhibition of histamine synthesis mediated by presynaptic H_3-receptors. *Neuroscience* **1**:149-157.

Arrang JM, Garbarg M, Lancelot JC, Lecomte JM, Pollard H, Robba M, Schunack W, and Schwartz JC (1987b) Highly potent and selective ligands for histamine H_3-receptors. *Nature* **327**:117-123.

Arrang JM, Drutel G, and Schwartz JC (1995) Characterization of histamine H_3 receptors regulating acetylcholine release in rat entorhinal cortex. *Br J Pharmacol* **114**:1518-1522.

Arrang JM, Morisset S, and Gbahou F (2007) Constitutive activity of the histamine H_3 receptor. *Trends Pharmacol Sci* **28**:350-357.

Ash AS and Schild HO (1966) Receptors mediating some actions of histamine. *Br J Pharmacol Chemother* **27**:427-439.

Axe FU, Bembenek SD, and Szalma S (2006) Three-dimensional models of the histamine H_3 receptor antagonist complexes and their pharmacophore. *J Mol Graph Model* **24**:456-464.

Bacciottini L, Passani MB, Giovannelli L, Cangioli I, Mannaioni PF, Schunack W, and Blandina P (2002) Endogenous histamine in the medial septum-diagonal band complex increases the release of acetylcholine from the hippocampus: a dual-probe microdialysis study in the freely moving rat. *Eur J Neurosci* **15**:1669-1680.

Bakker RA, Lozada AF, van Marle A, Shenton FC, Drutel G, Karlstedt K, Hoffmann M, Lintunen M, Yamamoto Y, van Rijn RM, Chazot PL, Panula P, and Leurs R (2006) Discovery of naturally occurring splice variants of the rat histamine H_3 receptor that act as dominant-negative isoforms. *Mol Pharmacol* **69**:1194-1206.

Baker JG (2008) Antagonist affinity measurements at the Gi-coupled human histamine H_3 receptor expressed in CHO cells. *BMC Pharmacol* **8**:9.

Barbier AJ, Berridge C, Dugovic C, Laposky AD, Wilson SJ, Boggs J, Aluisio L, Lord B, Mazur C, Pudiak CM, Langlois X, Xiao W, Apodaca R, Carruthers NI, and Lovenberg TW (2004) Acute wake-promoting actions of JNJ-5207852, a novel, diamine-based H_3 antagonist. *Br J Pharmacol* **143**:649-661.

Barnes WG and Hough LB (2002) Membrane-bound histamine N-methyltransferase in mouse brain: possible role in the synaptic inactivation of neuronal histamine. *J Neurochem.* **82**:1262-1271.

Bergquist F, Ruthven A, Ludwig M, and Dutia MB (2006) Histaminergic and glycinergic modulation of GABA release in the vestibular nuclei of normal and labyrinthectomised rats. *J Physiol* **577**:857-868.

Bitner RS, Markosyan S, Nikkel AL, and Brioni JD (2011) In-vivo histamine H_3 receptor antagonism activates cellular signaling suggestive of symptomatic and disease modifying efficacy in Alzheimer's disease. *Neuropharmacology* **60**:460-466.

Blandina P, Giorgetti M, Cecchi M, Leurs R, Timmerman H, and Giovannini MG (1996) Histamine H_3 receptor inhibition of K^+-evoked release of acetylcholine from rat cortex in vivo. *Inflamm Res* **45** Suppl 1:S54-55.

Bongers G, Bakker RA, and Leurs R (2007a) Molecular aspects of the histamine H_3 receptor. *Biochem Pharmacol* **73**:1195-1204.

Bongers G, Krueger KM, Miller TR, Baranowski JL, Estvander BR, Witte DG, Strakhova MI, van Meer P, Bakker RA, Cowart MD, Hancock AA, Esbenshade TA, and Leurs R (2007b) An 80-amino acid deletion in the third intracellular loop of a naturally occurring human histamine H_3 isoform confers pharmacological differences and constitutive activity. *J Pharmacol Exp* Ther **323**:888-898.

Bongers G, Sallmen T, Passani MB, Mariottini C, Wendelin D, Lozada A, Marle Av, Navis M, Blandina P, Bakker RA, Panula P, and Leurs R (2007c) The Akt/GSK-3β axis as a new signaling pathway of the histamine H_3 receptor. *J Neurochem* **103**: 248-258.

Bongers G (2008) Signal transduction of the histamine H_3 receptor. Academic Thesis. Vrije Universiteit Amsterdam. http://goo.gl/2hVnfz.

Brabant C, Quertemont E, Anaclet C, Lin JS, Ohtsu H, and Tirelli E (2007) The psychostimulant and rewarding effects of cocaine in histidine decarboxylase knockout mice do not support the hypothesis of an inhibitory function of histamine on reward. *Psychopharmacology (Berl)* **190**:251-263.

Brioni JD, Esbenshade TA, Garrison TR, Bitner SR, and Cowart MD (2011) Discovery of histamine H_3 antagonists for the treatment of cognitive disorders and Alzheimer's disease. *J Pharmacol Exp* Ther **336**:38-46.

Bünemann M, Bücheler MM, Philipp M, Lohse MJ, and Hein L (2001) Activation and deactivation kinetics of α_{2A}- and α_{2C}-adrenergic receptor-activated G protein-activated inwardly rectifying K^+ channel currents. *J Biol Chem* **276**:47512-47517.

Burgaud JL and Oudart N (1993) Bronchodilatation of guinea-pig perfused bronchioles induced by the H_3-receptor for histamine: role of epithelium. *Br J Pharmacol* **109**:960-966.

Calebiro D, Rieken F, Wagner J, Sungkaworn T, Zabel U, Borzi A, Cocucci E, Zurn A and Lohse M (2013) Single-molecule analysis of fluorescently labeled G-protein-coupled receptors reveals complexes with distinct dynamics and organization. *Proc Natl Acad Sci USA* **110**:743-748.

Castellan Baldan L, Williams KA, Gallezot JD, Pogorelov V, Rapanelli M, Crowley M, Anderson GM, Loring E, Gorczyca R, Billingslea E, Wasylink S, Panza KE, Ercan-Sencicek AG, Krusong K, Leventhal BL, Ohtsu H, Bloch MH, Hughes ZA, Krystal JH, Mayes L, de Araujo I, Ding YS, State MW, and Pittenger C (2014) Histidine decarboxylase deficiency causes tourette syndrome: parallel findings in humans and mice. *Neuron* **81**:77-90

Celuch SM (1995) Possible participation of histamine H_3 receptors in the modulation of noradrenaline release from rat spinal cord slices. *Eur J Pharmacol* **287**:127-133.

Chen J, Liu C, and Lovenberg TW (2003) Molecular and pharmacological characterization of the mouse histamine H_3 receptor. *Eur J Pharmacol* **467**:57-65.

Clapham J and Kilpatrick GJ (1994) Thioperamide, the selective histamine H_3 receptor antagonist, attenuates stimulant-induced locomotor activity in the mouse. *Eur J Pharmacol* **259**:107-114

Clark MA, Korte A, and Egan RW (1993) Guanine nucleotides and pertussis toxin reduce the affinity of histamine H_3 receptors on AtT-20 cells. *Agents Actions* **40**:129-134.

Clark EA and Hill SJ (1996) Sensitivity of histamine H_3 receptor agonist-stimulated [^{35}S]GTPγS binding to pertussis toxin. *Eur J Pharmacol* **296**:223-225.

Cogé F, Guénin SP, Audinot V, Renouard-Try A, Beauverger P, Macia C, Ouvry C, Nagel N, Rique H, Boutin JA, and Galizzi JP (2001) Genomic organization and characterization of splice variants of the human histamine H_3 receptor. *Biochem J* **355**:279-288.

Cooper DM and Crossthwaite AJ (2006) Higher-order organization and regulation of adenylyl cyclases. *Trends Pharmacol Sci* **27**: 426-431.

Cowart M, Faghih R, Curtis MP, Gfesser GA, Bennani YL, Black LA, Pan L, Marsh KC, Sullivan JP, Esbenshade TA, Fox GB, and Hancock AA (2005) 4-(2-[2-(2(R)-methylpyrrolidin-1-yl)ethyl]benzofuran-5-yl)benzonitrile and related 2-aminoethylbenzofuran H_3 receptor antagonists potently enhance cognition and attention. *J Med Chem* **48**:38-55.

Dai H, Fu Q, Shen Y, Hu W, Zhang Z, Timmerman H, Leurs R, and Chen Z (2007) The histamine H_3 receptor antagonist clobenpropit enhances GABA release to protect against NMDA-induced excitotoxicity through the cAMP/protein kinase A pathway in cultured cortical neurons. *Eur J Pharmacol* **563**:117-123.

Dauvilliers Y, Bassetti C, Lammers GJ, Arnulf I, Mayer G, Rodenbeck A, Lehert P, Ding CL, Lecomte JM, Schwartz JC; HARMONY I study group (2013). Pitolisant versus placebo or modafinil in patients with narcolepsy: a double-blind, randomised trial. *Lancet Neurol.* **12**:1068-1075.

Dale HH and Laidlaw PP (1910) The physiological action of beta-iminazolylethylamine. *J Physiol* **41**:318-344.

De Esch IJ, Vollinga RC, Goubitz K, Schenk H, Appelberg U, Hacksell U, Lemstra S, Zuiderveld OP, Hoffmann M, Leurs R, Menge WM, and Timmerman H (1999) Characterization of the binding site of the histamine H_3 receptor. 1. Various approaches to the synthesis of 2-(1H-imidazol-4-yl)cyclopropylamine and histaminergic activity of (1R,2R)- and (1S,2S)-2-(1H-imidazol-4-yl)-cyclopropylamine. *J Med Chem* **42**:1115-1122.

de Esch IJ, Timmerman H, Menge WM, and Nederkoorn PH (2000) A qualitative model for the histamine H_3 receptor explaining agonistic and antagonistic activity simultaneously. *Arch Pharm (Weinheim)* **333**:254-260.

Di Carlo G, Ghi P, Orsetti M (2000) Effect of R-(-)-α-methylhistamine and thioperamide on in vivo release of norepinephrine in the rat hippocampus. *Prog Neuropsychopharmacol Biol Psychiatry* **24**:275-284.

Doreulee N, Yanovsky Y, Flagmeyer I, Stevens DR, Haas HL, and Brown RE (2001) Histamine H$_3$ receptors depress synaptic transmission in the corticostriatal pathway. *Neuropharmacol* **40**:106-113.

Drutel G, Peitsari N, Karlstedt K, Wieland K, Smit MJ, Timmerman H, Panula P, and Leurs R (2001) Identification of rat H$_3$ receptor isoforms with different brain expression and signaling properties. *Mol Pharmacol* **59**:1-8.

Egan MF, Zhao X, Gottwald R, Harper-Mozley L, Zhang Y, Snavely D, Lines C, and Michelson D (2013) Randomized crossover study of the histamine H$_3$ inverse agonist MK-0249 for the treatment of cognitive impairment in patients with schizophrenia. *Schizophr research* **146**:224-230.

Ellender TJ, Huerta-Ocampo I, Deisseroth, K, Capogna M, and Bolam JP (2011). Differential modulation of excitatory and inhibitory striatal synaptic transmission by histamine. *J Neurosci* **31**:15340-15351.

Endou M, Poli E, and Levi R (1994) Histamine H$_3$-receptor signaling in the heart: possible involvement of Gi/Go proteins and N-type Ca^{++} channels. *J Pharmacol Exp Ther* **269**:221-229.

Ercan-Sencicek AG, Stillman AA, Ghosh AK, Bilguvar K, O'Roak BJ, Mason CE, Abbott T, Gupta A, King RA, Pauls DL, Tischfield JA, Heiman GA, Singer HS, Gilbert DL, Hoekstra PJ, Morgan TM, Loring E, Yasuno K, Fernandez T, Sanders S, Louvi A, Cho JH, Mane S, Colangelo CM, Biederer T, Lifton RP, Gunel M, and State MW (2010) L-histidine decarboxylase and Tourette's syndrome. *N Engl J Med* **362**:1901-1908.

Ericson H, Köhler C, and Blomqvist A (1991) GABA-like immunoreactivity in the tuberomammillary nucleus: an electron microscopic study in the rat. *J Comp Neurol*. **305**:462-469.

Esbenshade TA, Krueger KM, Miller TR, Kang CH, Denny LI, Witte DG, Yao BB, Fox GB, Faghih R, Bennani YL, Williams M, and Hancock AA (2003) Two novel and selective nonimidazole histamine H$_3$ receptor antagonists A-304121 and A-317920: I. In vitro pharmacological effects. *J Pharmacol Exp Ther* **305**:887-896.

Esbenshade TA, Fox GB, Krueger KM, Miller TR, Kang CH, Denny LI, Witte DG, Yao BB, Pan L, Wetter J, Marsh K, Bennani YL, Cowart MD, Sullivan JP, and Hancock AA (2005) Pharmacological properties of ABT-239 [4-(2-{2-[(2R)-2-Methylpyrrolidinyl]ethyl}-benzofuran-5-yl)benzonitrile]: I. Potent and selective histamine H$_3$ receptor antagonist with drug-like properties. *J Pharmacol Exp Ther* **313**:165-175.

Esbenshade TA, Krueger KM, Yao BB, itte DG, Estvander BR, Baranowski JL, Miller TR, and Hancock AA (2006) Differences in pharmacological properties of histamine H$_3$

receptor agonists and antagonists revealed at two human H$_3$ receptor isoforms. *Inflamm Res* **55** Suppl1:S45-46.

Faucard R, Armand V, Héron A, Cochois V, and Arrang JM (2006) N-methyl-D-aspartate receptor antagonists enhance histamine neuron activity in rodent brain. *J Neurochem* **98**:1487-1496.

Ferrada C, Ferré S, Casadó V, Cortés A, Justinova Z, Barnes C, Canela EI, Goldberg SR, Leurs R, Lluis C, and Franco R (2008) Interactions between histamine H$_3$ and dopamine D$_2$ receptors and the implications for striatal function. *Neuropharmacol* **55**:190-197.

Ferrada C, Moreno E, Casadó V, Bongers G, Cortes A, Mallol J, Canela EI, Leurs R, Ferré S, Lluís C, and Franco R (2009) Marked changes in signal transduction upon hetermerization of dopamine D$_1$ and histamine H$_3$ receptors. *Br J Pharmacol* **157**:64-75

Ferreira R, Santos T, Gonçalves J, Baltazar G, Ferreira L, Agasse F, and Bernardino L (2012) Histamine modulates microglia function. *J Neuroinflammation* **9**:90.

Fink K, Schlicker E, Neise A, and Gothert M (1990) Involvement of presynaptic H$_3$ receptors in the inhibitory effect of histamine on serotonin release in the rat brain cortex. *Naunyn Schmiedebergs Arch Pharmacol* **342**:513-519.

Flock T, Ravarani CN, Sun D, Venkatakrishnan AJ, Kayikci M, Tate CG, Veprintsev DB, and Babu MM. (2015). Universal allosteric mechanism for G activation by GPCRs. *Nature* **524**:173-179.

Flores-Clemente C, Osorio-Espinoza A, Escamilla-Sánchez J, Leurs R, Arias JM, Arias-Montaño JA. (2013) A single-point mutation (Ala280Val) in the third intracellular loop alters the signalling properties of the human histamine H$_3$ receptor stably expressed in CHO-K1 cells. *Br J Pharmacol* **170**:127-135.

Flores G, Morales-Medina JC, and Díaz A (2016) Neuronal and brain morphological changes in animal models of schizophrenia. *Behav Brain Res* **301**:190-203.

Fox GB, Esbenshade TA, Pan JB, Radek RJ, Krueger KM, Yao BB, Browman KE, Buckley MJ, Ballard ME, Komater VA, Miner H, Zhang M, Faghih R, Rueter LE, Bitner, RS, Drescher KU, Wetter J, Marsh K, Lemaire M, Porsolt RD, Bennani YL, Sullivan JP, Cowart MD, Decker MW, and Hancock AA (2005) Pharmacological properties of ABT-239 [4-(2-{2-[(2R)-2-Methylpyrrolidinyl]ethyl}-benzofuran-5-yl)benzonitrile]: II. Neurophysiological characterization and broad preclinical efficacy in cognition and schizophrenia of a potent and selective histamine H$_3$ receptor antagonist. *J Pharmacol Exp Ther* **313**:176-190.

Gainetdinov RR, Premont RT, Bohn LM, Lefkowitz RJ, and Caron MG (2004) Desensitization of G protein-coupled receptors and neuronal functions. *Annu Rev Neurosci* **27**:107-144.

Galici R, Rezvani AH, Aluisio L, Lord B, Levin ED, Fraser I, Boggs J, Welty N, Shoblock JR, Motley ST, Letavic MA, Carruthers NI, Dugovic C, Lovenberg TW, and Bonaventure P (2011) JNJ‑39220675, a novel selective histamine H$_3$ receptor

antagonist, reduces the abuse-related effects of alcohol in rats. *Psychopharmacol (Berl)* **214**:829–841.

Gallagher M and Yates SL (2007) Histamine H$_3$ receptor polynucleotides. WO Patent o3/042359A2.

Ganellin CR, Leurquin F, Piripitsi A, Arrang JM, Garbarg M, Ligneau X, Schunack W, and Schwartz JC (1998) Synthesis of potent non-imidazole histamine H$_3$-receptor antagonists. *Arch Pharm (Weinheim)* **331**:395-404.

Gantz I, Munzert G, Tashiro T, Schäffer M, Wang L, DelValle J, Yamada T (1991) Molecular cloning of the human histamine H$_2$ receptor. *Biochem Biophys Res Commun.* **178**:1386-1392.

Gao Z, Hurst WJ, Czechtizky W, Francon D, Griebel G, Nagorny R, Pichat P, Schwink L, Stengelin S, Hendrix JA, and George PG (2013) Discovery of a potent, selective, and orally bioavailable histamine H$_3$ receptor antagonist SAR110068 for the treatment of sleep-wake disorders. *Bioorg Med Chem Lett* **23**:6141-6145.

Garbarg M, Arrang JM, Rouleau A, Ligneau X, Tuong MD, Schwartz JC, and Ganellin CR (1992) S-[2-(4-imidazolyl)ethyl]isothiourea, a highly specific and potent histamine H$_3$ receptor agonist. *J Pharmacol Exp Ther* **263**:304-310.

Garcia M, Floran B, Arias-Montano JA, Young JM, and Aceves J (1997) Histamine H$_3$ receptor activation selectively inhibits dopamine D$_1$ receptor-dependent [^{3}H]GABA release from depolarization-stimulated slices of rat substantia nigra pars reticulata. *Neuroscience* **80**:241-249.

García-Ramírez M, Aceves J, and Arias-Montaño JA (2004) Intranigral injection of the H$_3$ agonist immepip and systemic apomorphine elicit ipsilateral turning behaviour in naive rats, but reduce contralateral turning in hemiparkinsonian rats. *Behav Brain Res* **154**: 409-415.

Garduño-Torres B, Treviño M, Gutiérrez R, Arias-Montaño JA (2007) Pre-synaptic histamine H$_3$ receptors regulate glutamate, but not GABA release in rat thalamus. *Neuropharmacology.* **52**:527-535.

Gbahou F, Rouleau A, and Arrang JM (2012) The histamine autoreceptor is a short isoform of the H☐ receptor. *Br J Pharmacol* **166**: 1860-1871.

Giannoni P, Passani MB, Nosi D, Chazot PL, Shenton FC, Medhurst AD, Mundari L, and Blandina P (2009) Heterogeneity of histaminergic neurons in the tuberomammillary nucleus of the rat. *Eur J Neurosci.* **29**:2363-2374.

Giovannini MG, Efoudebe M, Passani MB, Baldi E, Bucherelli C, Giachi F, Corradetti R, and Blandina P (2003) Improvement in fear memory by histamine-elicited ERK2 activation in hippocampal CA3 cells. *J Neurosci.* **23**:9016-9023.

Gold SJ, Ni YG, Dohlman HG, and Nestler EJ (1997) Regulators of G-protein signaling (RGS) proteins: region-specific expression of nine subtypes in rat brain. *J Neurosci* **20**:8024-8037.

Gomez-Ramírez J, Johnston TH, Visanji NP, Fox SH, and Brotchie JM (2006) Histamine H_3 receptor agonists reduce L-DOPA-induced chorea, but not dystonia, in the MPTP-lesioned nonhuman primate model of Parkinson's disease. *Movement Disord* **21**:839-846.

Gondard E, Anaclet C, Akaoka H, Guo RX, Zhang M, Buda C, Franco P, Kotani H, and Lin JS. (2013) Enhanced histaminergic neurotransmission and sleep-wake alterations, a study in histamine H_3-receptor knock-out mice. *Neuropsychopharmacology* **38**:1015-1031.

González-Sepúlveda M, Rosell S, Hoffmann H, Castillo-Ruiz MM, Mignon V, Moreno-Delgado D, Vignes M, Díaz J, Sabriá J, and Ortiz J (2013) Cellular distribution of the histamine H_3 receptor in the basal ganglia: Functional modulation of dopamine and glutamate neurotransmission. *Basal Ganglia* **3**:109-121.

Green JP and Maayani S (1977) Tricyclic antidepressant drugs block histamine H_2 receptor in brain. *Nature* **269**:163–165.

Gurevich EV, Tesmer JJ, Mushegian A, Gurevich VV (2012). G protein-coupled receptor kinases: more than just kinases and not only for GPCRs. *Pharmacol Ther* **133**:40-69.

Gutkind JS (2000) Regulation of mitogen-activated protein kinase signaling networks by G protein-coupled receptors. *Sci STKE* **40**:re1.

Haig GM, Bai E, Robieson W, Othman AA, Baker J, and Lenz RA (2014) A randomized trial of the efficacy and safety of the H_3 antagonist ABT-288 in cognitive impairment associated with schizophrenia. *Schizophr Bull* **40**:1433-1442.

Hancock AA, Esbenshade TA, Kruger KM, and Yao BB (2003) Genetic and pharmacological aspects of histamine H_3 receptor heterogeneity. *Life Sci* **73**:3043-3072.

Hancock AA, Bennani YL, Bush EN, Esbenshade TA, Faghih R, Fox GB, Jacobson P, Knourek-Segel V, Krueger KM, Nuss ME, Pan JB, Shapiro R, Witte DG and Yao BB (2004a) Antiobesity effects of A-331440, a novel non-imidazole histamine H3 receptor antagonist. *Eur J Pharmacol*. **487**:183-197.

Hancock AA, Diehl MS, Faghih R, Bush EN, Krueger KM, Krishna G, Miller TR, Wilcox DM, Nguyen P, Pratt JK, Cowart MD, Esbenshade TA, and Jacobson PB (2004b) In vitro optimization of structure activity relationships of analogues of A-331440 combining radioligand receptor binding assays and micronucleus assays of potential antiobesity histamine H_3 receptor antagonists. *Basic Clin Pharmacol Toxicol* **95**:144-152.

Harper EA, Shankley NP, and Black JW (1999) Characterization of the binding of [^{3}H]-clobenpropit to histamine H_3-receptors in guinea-pig cerebral cortex membranes. *Br J Pharmacol* **128**:881-890.

Haas HL and Reiner PB (1988) Membrane properties of histaminergic tuberomammillary neurones of the rat hypothalamus in vitro. *J Physiol*. **399**:633-646.

Haas HL, Sergeeva OA and Selbach O (2008) Histamine in the nervous system. *Physiol Rev*. **88**:1183-1241.

Hatta E, Yasuda K, and Levi R (1997) Activation of histamine H_3 receptors inhibits carrier-mediated norepinephrine release in a human model of protracted myocardial ischemia. *J Pharmacol Exp Ther* **283**:494-500.

Hew RW, Hodgkinson CR, and Hill SJ (1990) Characterization of histamine H_3-receptors in guinea-pig ileum with H_3-selective ligands. *Br J Pharmacol* **101**:621-624.

Hollinger S, and Hepler JR (2002) Cellular regulation of RGS proteins: modulators and integrators of G protein signaling. *Pharmacol Rev* **54**:527-559.

Hooper C, Killick R, and Lovestone S (2008) The GSK3 hypothesis of Alzheimer's disease. *J Neurochem* **104**:1433-1439.

Humbert-Claude M, Morisset S, Gbahou F, and Arrang JM (2007) Histamine H_3 dopamine D_2 receptor-mediated [^{35}S]GTPγ[S] binding in rat striatum: evidence for additive effects but lack of interactions. *Biochem Pharmacol* **73**:1172-1181.

Ichinose M and Barnes PJ (1989) Histamine H_3-receptors modulate nonadrenergic noncholinergic neural bronchoconstriction in guinea-pig in vivo. *Eur J Pharmacol* **174**:49-55.

Imamura M, Seyedi N, Lander HM, and Levi R (1995) Functional identification of histamine H_3-receptors in the human heart. *Circ Res* **77**:206-210.

Imamura M, Lander HM, and Levi R (1996) Activation of histamine H_3-receptors inhibits carrier-mediated norepinephrine release during protracted myocardial ischemia. Comparison with adenosine A_1-receptors and α_2-adrenoceptors. *Circ Res* **78**:475-481.

Inocente C, Arnulf I, Bastuji H, Thibault-Stoll A, Raoux A, Reimão R, Lin JS, and Franco P (2012) Pitolisant, an inverse agonist of the histamine H_3 receptor: an alternative stimulant for narcolepsy-cataplexy in teenagers with refractory sleepiness. *Clin Neuropharmacol* **35**:55-60.

Ireland-Denny L, Parihar AS, Miller TR, Kang CH, Krueger KM, Esbenshade TA, and Hancock AA (2001) Species-related pharmacological heterogeneity of histamine H_3 receptors. *Eur J Pharmacol* **433**:141-150.

Ishikawa M, Watanabe T, Kudo T, Yokoyama F, Yamauchi M, Kato K, Kakui N, and Sato Y (2010) Investigation of the histamine H_3 receptor binding site. Design and synthesis of hybrid agonists with a lipophilic side chain. *J Med Chem* **53**:6445-6456.

Ito C, Onodera K, Sakuri E, Sato M, and Watanabe T (1997) Effect of cocaine on the histaminergic neuron system. *J Neurochem* **69**:875-878.

MOL #104752

Jansen FP, Mochizuki T, Yamamoto Y, Timmerman H, and Yamatodani A (1998) In vivo modulation of rat hypothalamic histamine release by the histamine H_3 receptor ligands, immepip and clobenpropit. Effects of intrahypothalamic and peripheral application. *Eur J Pharmacol* **362**:149-155.

Jansen FP, Mochizuki T, Maeyama K, Leurs R, and Timmerman H (2000) Characterization of histamine H_3 receptors in mouse brain using the H_3 antagonist [^{125}I]iodophenpropit. *Naunyn Schmiedebergs Arch Pharmacol* **362**:60-67.

Jang IS, Rhee JS, Watanabe T, Akaike N. (2001). Histaminergic modulation of GABAergic transmission in rat ventromedial hypothalamic neurones. *J Physiol* **534**:791-803.

Jiang X, Chen A, and Li H (2005) Histaminergic modulation of excitatory synaptic transmission in the rat basolateral amygdala. *Neuroscience* **131**:691-703.

Jin CY, Anichtichik O, and Panula P (2009) Altered histamine H_3 receptor radioligand binding in post-mortem brain samples from subjects with psychiatric diseases. *Br J Pharmacol* **157**:118-129.

Javitt DC, Zukin SR (1991) Recent advances in the phencyclidine model of schizophrenia. *Am J Phsychiatry* **148**:1301-1308.

Kalia LV and Lang AE (2015) Parkinson's disease. *Lancet* **386**:896-912.

Karmazyn M (1999) The role of the myocardial sodium-hydrogen exchanger in mediating ischemic and reperfusion injury. From amiloride to cariporide. *Ann N Y Acad Sci* **874**:326-334.

Kim SK, Fristrup P, Abrol R, and Goddard WA 3rd (2011) Structure-based prediction of subtype selectivity of histamine H_3 receptor selective antagonists in clinical trials. J *Chem Inf Model* **51**:3262-3274.

Kirk IP and Richardson PJ (1994) Adenosine A_{2a} receptor-mediated modulation of striatal [^{3}H]GABA and [^{3}H]acetylcholine release. *J Neurochem* **62**:960-966

Kitbunnadaj R, Zuiderveld OP, De Esch IJ, Vollinga RC, Bakker R, Lutz M, Spek AL, Cavoy E, Deltent MF, Menge WM, Timmerman H, and Leurs R (2003) Synthesis and structure-activity relationships of conformationally constrained histamine H_3 receptor agonists. *J Med Chem* **46**:5445-5457.

Kitbunnadaj R, Hashimoto T, Poli E, Zuiderveld OP, Menozzi A, Hidaka R, de Esch IJ, Bakker RA, Menge WM, Yamatodani A, Coruzzi G, Timmerman H, and Leurs R (2005) N-substituted piperidinyl alkyl imidazoles: discovery of methimepip as a potent and selective histamine H_3 receptor agonist. *J Med Chem* **48**:2100-2107.

Komater VA, Browman KE, Curzon P, Hancock AA, Decker MW, and Fox GB (2003) H_3 receptor blockade by thioperamide enhances cognition in rats without inducing locomotor sensitization. *Psychopharmacology (Berl)* **167**:363-372.

Kovalainen JT, Christiaans JA, Kotisaari S, Laitinen JT, Männistö PT, Tuomisto L, and Gynther J (1999) Synthesis and in vitro pharmacology of a series of new chiral histamine H$_3$-receptor ligands: 2-(R and S)-Amino-3-(1H-imidazol-4(5)-yl)propyl ether derivatives. *J Med Chem* **42**:1193-1202.

Krueger KM, Witte DG, Ireland-Denny L, Miller TR, Baranowski JL, Buckner S, Milicic I, Esbenshade TA, and Hancock AA (2005) G protein-dependent pharmacology of histamine H$_3$ receptor ligands: evidence for heterogeneous active state receptor conformations. *J Pharmacol Exp Ther* **314**:271-281.

Kuramasu A, Sukegawa J, Sato T, Sakurai E, Watanabe T, Yanagisawa T, and Yanai K (2011) The hydrophobic amino acids in putative helix 8 in carboxy-terminus of histamine H$_3$ receptor are involved in receptor-G-protein coupling. *Cell Signal* **23**:1843-1849.

Lai X, Ye L, Liao Y, Jin L, Ma Q, Lu B, Sun Y, Shi Y, and Zhou N (2016) Agonist-Induced Activation of Histamine H$_3$ Receptor Signals to Extracellular Signal-Regulated Kinases 1 and 2 through PKC-, PLD- and EGFR-dependent Mechanisms. *J Neurochem* **137**:200-215.

Lamberty Y, Margineanu DG, Dassesse D, and Klitgaard H (2003) H$_3$ agonist immepip markedly reduces cortical histamine release, but only weakly promotes sleep in the rat. *Pharmacol Res* **48**:193-198.

Lefkowitz RJ, Cotecchia S, Samama P, and Costa T (1993) Constitutive activity of receptors coupled to guanine nucleotide regulatory proteins. *Trends Pharmacol Sci* **14**:303-307.

Leurs R, Vollinga RC, and Timmerman H (1995) The medicinal chemistry and therapeutic potentials of ligands of the histamine H$_3$ receptor. *Prog Drug Res* **45**:107-165.

Levi R, Seyedi N, Schaefer U, Estephan R, Mackins CJ, Tyler E, and Silver RB (2007) Histamine H$_3$-receptor signaling in cardiac sympathetic nerves: Identification of a novel MAPK-PLA$_2$-COX-PGE$_2$-EP$_3$R pathway. *Biochem Pharmacol* **73**:1146-1156.

Levoin N, Calmels T, Pourpardin-Olivier O, Labeeuw O, Danvy D, Robert P, Berrebi-Bertrand I, Ganellin CR, Schunack W, Strack H, and Capet M (2010) Refined docking as a valuable tool for lead optimization: application to the histamine H$_3$ receptor antagonists. *Arch Pharm (Weinheim)* **341**:610-623

Ligneau X, Garbarg M, Vizuete ML, Diaz J, Purand K, Stark H, Schunack W, and Schwartz JC (1994) [^{125}I]iodoproxyfan, a new antagonist to label and visualize cerebral histamine H$_3$ receptors. *J Pharmacol Exp Ther* **271**:452-459.

Ligneau X, Morisset S, Tardivel-Lacombe J, Gbahou F, Ganellin CR, Stark H, Schunack W, Schwartz JC, and Arrang JM (2000) Distinct pharmacology of rat and human histamine H$_3$ receptors: role of two amino acids in the third transmembrane domain. *Br J Pharmacol* **131**:1247-1250.

Ligneau X, Landais L, Perrin D, Piriou J, Uguen M, Denis E, Robert P, Parmentier R, Anaclet C, Lin JS, Burban A, Arrang JM, and Schwartz JC (2007a) Brain histamine and schizophrenia: potential therapeutic applications of H_3 receptor antagonists studied with BF2.649. *Biochem Pharmacol* **73**:1215-1224.

Ligneau X, Perrin D, Landais L, Camelin JC, Calmels TP, Berrebi-Bertrand I,Lecomte JM, Parmentier R, Anaclet C, Lin JS, Bertaina-Anglade V, la Rochelle CD, d'Aniello F, Rouleau A, Gbahou F, Arrang JM, Ganellin CR, Stark H, Schunack W, and Schwartz JC. (2007b) BF2.649 [1-{3-[3-(4-Chlorophenyl)propoxy]propyl}piperidine,hydrochloride], a nonimidazole inverse agonist/antagonist at the human histamine H_3 receptor: Preclinical pharmacology. *J Pharmacol Exp Ther*. **320**:365-375.

Lim HD, van Rijn RM, Ling P, Bakker RA, Thurmond RL, and Leurs R (2005) Evaluation of histamine H_1-, H_2-, and H_3-receptor ligands at the human histamine H_4 receptor: identification of 4-methylhistamine as the first potent and selective H_4 receptor agonist. J *Pharmacol Exp Ther* **314**:1310-1321.

Lin JS (2000) Brain structures and mechanisms involved in the control of cortical activation and wakefulness, with emphasis on the posterior hypothalamus and histaminergic neurons. *Sleep Med Rev* **4**:471-503.

Lin JS, Dauvilliers Y, Arnulf I, Bastuji H, Anaclet C, Parmentier R, Kocher L, Yanagisawa M, Lehert P, Ligneau X, Perrin D, Robert P, Roux M, Lecomte JM, and Schwartz JC (2008) An inverse agonist of the histamine H_3 receptor improves wakefulness in narcolepsy: studies in orexin-/- mice and patients. *Neurobiol Dis* **30**:74-83.

Liu CQ, Hu DN, Liu FX, Chen Z, and Luo JH (2008) Apomorphine-induced turning behavior in 6-hydroxydopamine lesioned rats is increased by histidine and decreased by histidine decarboxylase, histamine H_1 and H_2 receptor antagonists, and an H_3 receptor agonist. *Pharmacol Biochem Behav* **90**:325-330.

Lovenberg TW, Roland BL, Wilson SJ, Jiang X, Pyati J, Huvar A, Jackson MR, and Erlander MG (1999) Cloning and functional expression of the human histamine H_3 receptor. *Mol Pharmacol* **55**:1101-1107.

Lovenberg TW, Pyati J, Chang H, Wilson SJ, and Erlander MG (2000) Cloning of rat histamine H_3 receptor reveals distinct species pharmacological profiles. *J Pharmacol Exp Ther* **293**:771-778.

Mahamood D, Khanam R, Pillai KK, and Akhtar M (2012) Protective effects of histamine H_3-receptor ligands in schizophrenic behaviours in experimental models. *Pharmacol Rep* **64**:191-204.

Maldonado M and Maeyama K (2015) The metabolism of histamine in rat hypothalamus and cortex after reserpine treatment. *Neurochem Int*. **85-86**:31-9.

Malmlöf K, Zaragoza F, Golozoubova V, Refsgaard HH, Cremers T, Raun K, Wulff BS, Johansen PB, Westerink B, and Rimvall K (2005) Influence of a selective histamine H_3

receptor antagonist on hypothalamic neural activity, food intake and body weight. *Int J Obes (Lond)* **29**:1402-1412.

Mariottini C, Scartabelli T, Bongers G, Arrigucci S, Nosi D, Leurs R, Chiarugi A, Blandina P, Pellegrini-Giampietro DE, and Passani MB (2009) Activation of the histaminergic H$_3$ receptor induces phosphorylation of the Akt/GSK-3β pathway in cultured cortical neurons and protects against neurotoxic insults. *J Neurochem* **110**:1469-1478.

Martinez-Mir MI, Pollard H, Moreau J, Arrang JM, Ruat M, Traiffort E, Schwartz JC, and Palacios JM (1990) Three histamine receptors (H$_1$, H$_2$ and H$_3$) visualized in the brain of human and non-human primates. *Brain Res* **526**:322-327.

Mayfield RD, Suzuki F, and Zahniser NR (1993) Adenosine A$_{2a}$ receptor modulation of electrically evoked endogenous GABA release from slices of rat globus pallidus. *J Neurochem.* **60**:2334-2337.

Mazenot C, Ribuot C, Durand A, Joulin Y, Demenge P, and Godin-Ribuot D (1999) In vivo demonstration of H$_3$-histaminergic inhibition of cardiac sympathetic stimulation by R-α-methyl-histamine and its prodrug BP 2.94 in the dog. *Br J Pharmacol* **126**:264-268.

McCormick DA and Bal T (1997) Sleep and arousal: thalamocortical mechanisms. *Annu Rev Neurosci.* **20**:185-215.

McLeod RL, Rizzo CA, West RE Jr, Aslanian R, McCormick K, Bryant M, Hsieh Y, Korfmacher W, Mingo GG, Varty L, Williams SM, Shih NY, Egan RW, Hey JA (2003) Pharmacological characterization of the novel histamine H3-receptor antagonistN-(3,5-dichlorophenyl)-N'-[[4-(1H-imidazol-4-ylmethyl)phenyl]-methyl]-urea (SCH79687). *J Pharmacol Exp Ther*. **305**:1037-1044.

Medhurst AD, Atkins AR, Beresford IJ, Brackenborough K, Briggs MA, Calver AR, Cilia J, Cluderay JE, Crook B, Davis JB, Davis RK, Davis RP, Dawson LA, Foley AG, Gartlon J, Gonzalez MI, Heslop T, Hirst WD, Jennings C, Jones DN, Lacroix LP, Martyn A, Ociepka S, Ray A, Regan CM, Roberts JC, Schogger J, Southam E, Stean TO, Trail BK, Upton N, Wadsworth G, Wald JA, White T, Witherington J, Woolley ML, Worby A, and Wilson DM (2007) GSK189254, a novel H$_3$ receptor antagonist that binds to histamine H$_3$ receptors in Alzheimer's disease brain and improves cognitive performance in preclinical models. *J Pharmacol Exp Ther* **321**:1032-1045.

Meneses D, Mateos V, Islas G, and Barral J (2015) G-protein-coupled inward rectifier potassium channels involved in corticostriatal presynaptic modulation. *Synapse* **69**:446-452.

Menge WMPB, Enguehard C, Romeo G, Limmen B, and Timmermann H (1998) Synthesis and biological evaluation of a novel class of non-imidazole histamine H$_3$-antagonists. In: 15th EFMC International Symposium on Medicinal Chemistry, Edinburgh, Scotland.

Merickel A and Edwards RH (1995) Transport of histamine by vesicular monoamine transporter-2. *Neuropharmacology* **34**:1543-1547.

Milligan G (2006) G-protein-coupled receptor heterodimers: pharmacology, function and relevance to drug discovery. *Drug Discov Today* **11**:541-549.

Molina-Hernández A, Nuñez A, Sierra JJ, and Arias-Montaño JA (2001) Histamine H_3 receptor activation inhibits glutamate release from rat striatal synaptosomes. *Neuropharmacology* **41**:928-934.

Molina-Hernández A, Díaz NF, and Arias-Montaño JA (2012) Histamine in brain development. *J Neurochem* **122**:872-882.

Montejo-López W, Rivera-Ramírez N, Escamilla-Sánchez J, García-Hernández U, and Arias-Montaño J-A (2016) Activation of endogenous purinergic receptors induces PKC-mediated desensitization of human histamine H_3 receptors expressed in CHO-K1 cells. Neurochem. Res, in press. DOI: 10.1007/s11064-016-1954-5

Moore DJ, West AB, Dawson VL, and Dawson TM (2005) Molecular pathophysiology of Parkinson's disease. *Annu Rev Neurosci* **28**:57-87.

Morales-Figueroa GE, Marquez-Gomez R, Gonzalez-Pantoja R, Escamilla-Sanchez J, and Arias-Montaño JA (2014) Histamine H_3 receptor activation counteracts adenosine A_{2A} receptor-mediated enhancement of depolarization-evoked [^{3}H]-GABA release from rat globus pallidus synaptosomes. *ACS Chem Neurosci* **5**:637-645.

Moreno E, Hoffman H, Gonzalez-Sepulveda M, Navarro G, Casado V, Cortes A, Mallol J, Vignes M, McCormick PJ, Canela EI, Lluis C, Moratalla R, Ferre S, Ortiz J, and Franco R (2011) Dopamine D_1-histamine H_3 receptor heteromers provide a selective link to MAPK signaling in GABAergic neurons of the direct striatal pathway. *J BiolChem* **286**:5846-5854.

Morisset S, Sahm UG, Triffrort E, Tardivel-Lacombe J, Arrang JM, and Schwartz JC (1999) Atypical neuroleptics enhance histamine turnover in brain via 5-HT_{2A} receptor blockade. *J Pharmacol Exp Ther* **288**:590-596

Morisset S, Rouleau A, Ligneau X, Gbahou F, Tardivel-Lacombe J, Stark H, Schuncack W, Ganellin Cr, Schwartz JC, and Arrang JM (2000). High constitutive activity of native H_3 receptors regulates histamine neurons in brain. *Nature* **408**:860-864.

Morisset S, Sasse A, Gbahou F, Héron A, Ligneau X, Tardivel-Lacombe J, Schwartz JC, and Arrang JM (2001) The rat H_3 receptor: gene organization and multiple isoforms. *Biochem Biophys Res Commun* **280**:75-80.

Morisset S, Pilon C, Tardivel-Lacombe J, Weinstein D, Rostene W, Betancur C, Sokolo VP, Schwartz JC, and Arrang JM (2002) Acute and chronic effects of methamphetamine on tele-methylhistamine levels in mouse brain: Selective involvement of the D_2 and not D_3 receptor. *J. Pharmacol Exp Ther* **300**: 621-628.

Morrey C, Estephan R, Abbott GW and Levi R (2008) Cardioprotective effect of histamine H_3-receptor activation: pivotal role of Gβγ-dependent inhibition of voltage-operated Ca^{2+} channels. *J Pharmacol Exp Ther* **326**: 871-878.

57

Motawaj M and Arrang JM (2011). Ciproxifan, a histamine H_3-receptor antagonist/antagonist, modulates methamphetamine-induced sensitization in mice. *Eur J Neurosci* **33**:1197-1204.

Munzar P, Tanda G, Justinova Z, and Goldberg SR (2004) Histamine H_3 receptor antagonists potentiate methamphetamine self-administration and methamphetamine-induced accumbal dopamine release. *Neuropsychopharmacology* **29**:705-717.

Murga C, Laguinge L, Wetzker R, Cuadrado A, and Gutkind JS (1998) Activation of Akt/protein kinase B by G protein-coupled receptors. A role for α and βγ subunits of heterotrimeric G proteins acting through phosphatidylinositol-3-OH kinase. *J Biol Chem* **273**:19080-19085.

Nakamura S, Takemura M, Ohnishi K, Suenaga T, Nishimura M, Akiguchi I, Kimura J, and Kimura T (1993) Loss of large neurons and occurrence of neurofibrillary tangles in the tuberomammillary nucleus of patients with Alzheimer's disease. *Neurosci Lett* **151**:196-199.

Nakamura T, Itadani H, Hidaka Y, Ohta M, and Tanaka K (2000) Molecular cloning and characterization of a new human histamine receptor, HH4R. *Biochem Biophys Res Commun* **279**:615-620.

Nishibori M, Oishi R, Itoh Y, and Saeki K (1985) Morphine-induced changes in histamine dynamics in the brain. *J Neurochem* **45**:719-724.

Nuutinen S, Karlstedt K, Aitta-Aho T, Korpi ER, and Panula P (2010) Histamine and H_3 receptor-dependent mechanisms regulate ethanol stimulation and conditioned place preference in mice. *Psychopharmacol (Berl)* **208**:75-86.

Oda T, Morikawa N, Saito Y, Masuho Y, and Matsumoto S (2000) Molecular cloning and characterization of a novel type of histamine receptor preferentially expressed in leukocytes. *J Biol Chem* **275**:36781-36786.

Ohkubo T, Shibata M, Inoue M, Kaya H, and Takahashi H (1995) Regulation of substance P release mediated via prejunctional histamine H_3 receptors. *Eur J Pharmacol* **273**:83-88.

Oldham WM and Hamm HE (2007) How do receptors activate G proteins? *Adv Protein Chem* **74**:67-93.

Osorio-Espinoza A, Alatorre A, Ramos-Jimenez J, Garduno-Torres B, Garcia-Ramirez M, Querejeta E, and Arias-Montaño JA (2011) Pre-synaptic histamine H_3 receptors modulate glutamatergic transmission in rat globus pallidus. *Neuroscience* **176**:20-31.

Osorio-Espinoza A, Escamilla-Sánchez J, Aquino-Jarquin G, and Arias-Montaño JA (2014) Homologous desensitization of human histamine H_3 receptors expressed in CHO-K1 cells. *Neuropharmacology* **77**: 387-397.

Panula P, Rinne J, Kuokkanen K, Eriksson KS, Sallmen T, Kalimo H and Relja M (1998) Neuronal histamine deficit in Alzheimer's disease. *Neuroscience* **82**: 993-997.

Panula P and Nuutinen S (2013) The histaminergic network in the brain: basic organization and role in disease. *Nat Rev Neurosci* **14**:472-487.

Panula P, Chazot PL, Cowart M, Gutzmer R, Leurs R, Liu WL, Stark H, Thurmond RL, and Haas HL (2015) International Union of Basic and Clinical Pharmacology. XCVIII. Histamine Receptors. *Pharmacol Rev* **67**:601-655.

Parks GS, Olivas ND, Ikrar T, Sanathara NM, Wang L, Wang Z, Civelli O, and Xu X (2014) Histamine inhibits the melanin-concentrating hormone system: implications for sleep and arousal. *J Physiol* **592**:2183-2196.

Parmentier R, Ohtsu H, Djebbara-Hannas Z, Valatx JL, Watanabe T, and Lin JS (2002). Anatomical, physiological, and pharmacological characteristics of histidine decarboxylase knock-out mice: evidence for the role of brain histamine in behavioral and sleep-wake control. *J Neurosci* **22**:7695-7711.

Parsons ME and Ganellin CR (2006) Histamine and its receptors. *Br J Pharmacol* **147** (Suppl 1):S127-S135.

Passani MB, Cangioli I, Baldi E, Bucherelli C, Mannaioni PF, and Blandina P (2001) Histamine H_3 receptor-mediated impairment of contextual fear conditioning and in-vivo inhibition of cholinergic transmission in the rat basolateral amygdala. *Eur J Neurosci* **14**:1522-1532.

Passani MB and Blandina P (2011) Histamine receptors in the CNS as targets for therapeutic intervention. *Trends Pharmacol Sci.* **32**:242-249.

Pillot C, Heron A, Cochois V, Tardivel-Lacombe J, Ligneau X, Schwartz JC, and Arrang JM (2002) A detailed mapping of the histamine H_3 receptor and its gene transcripts in rat brain. *Neuroscience* **114**:173-193.

Poli E, Coruzzi G, and Bertaccini G (1991) Histamine H_3 receptors regulate acetylcholine release from the guinea pig ileum myenteric plexus. *Life Sci* **48**:PL63-68.

Pollard H, Moreau J, Arrang JM, and Schwartz JC (1993) A detailed autoradiographic mapping of histamine H_3 receptors in rat brain areas. *Neuroscience* **52**:169-189.

Prast H, Tran MH, Fischer H, Kraus M, Lamberti C, Grass K, and Philippu A (1999) Histaminergic neurons modulate acetylcholine release in the ventral striatum: role of H_3 histamine receptors. *Naunyn Schmiedebergs Arch Pharmacol* **360**:558-564.

Prell GD, Green JP, Kaufmann CA, Khandelwal JK, Morrishow AM, Kirsch DG, Linnoila M, and Wyatt RJ (1995) Histamine metabolites in cerebrospinal fluid of patients with chronic schizophrenia: their relationships to levels of other aminergic transmitters and ratings of symptoms. *Schizophr Res* **14**:93-104.

Querfurth HW and LaFerla FM (2010) Alzheimer's disease. *N Engl J Med* **362**:329-344.

Rapanelli M, and Pittenger C (2015) Histamine and histamine receptors in Tourette syndrome and other neuropsychiatric conditions. *Neuropharmacology* **106**:85-90.

Rasmuseen SG, DeVree BT, Zou Y, Kruse AC, Chung KY, Kobilka TS, Thian FS, Chae PS, Pardon E, Calinski D, Mathiesen JM, Shah ST, Lyons JA, Caffrey M, Gellman SH, Steyaert J, Skiniotis G, Weis WI, Sunahara RK, and Kobilka BK (2011) Crystal structure of the β_2 adrenergic receptor-Gs protein complex. *Nature* **477**:549-555.

Rebecchi MJ and Pentyala SN (2000) Structure, function, and control of phosphoinositide-specific phospholipase C. *Physiol Rev* **80**:1291-1335.

Rhee SG (2001) Regulation of phosphoinositide-specific phospholipase *C. Annu Rev Biochem* **70**:281-312.

Richelson JO and Souder T (2000) Binding of antipsychotic drugs to human brain receptors focus on newer generation compunds. *Life Sci* **68**:29-39.

Rouleau A, Ligneau X, Tardivel-Lacombe J, Morisset S, Gbahou F, Schwartz JC, and Arrang JM (2002) Histamine H_3-receptor-mediated [^{35}S]GTPγ[S] binding: evidence for constitutive activity of the recombinant and native rat and human H_3 receptors. *Br J Pharmacol* **135**:383-392.

Sadeq A, Jalal F, and Stark H (2016) Histamine H_3 receptor as a potential target for cognitive symptoms in neuropsychiatric diseases. *Behav Brain Res* **312**:415-430.

Sahlholm K, Nilsson J, Marcellino D, Fuxe K, and Arhem P (2012) Voltage sensitivities and deactivation kinetics of histamine H_3 and H_4 receptors. *Biochim Biophys Acta* **1818**:3081-3089.

Sallmen T, Lozada AF, Anichtchik OV, Beckman AL, and Panula P (2003) Increased brain histamine H_3 receptor expression during hibernation in golden-mantled ground squirrels. *BMC Neurosci* **4**:24.

Sánchez-Lemus E and Arias-Montaño JA (2004) Histamine H_3 receptor activation inhibits dopamine D_1 receptor-induced cAMP accumulation in rat striatal slices. *Neurosci Lett* **364**:179-184.

Santini E, Valjent E, Usiello A, Carta M, Borgkvist A, Girault J-A, Hervé D, Greengard P, and Fisone G (2007) Critical involvement of cAMP/DARPP-32 and extracellular signal-regulated protein kinase signaling in L-DOPA induced dyskinesia. *J Neurosci* **27**: 6995-7005.

Scheerer P, Park JH, Hildebrand PW, Kim YJ, Krauss N, Choe HW, Hofmann KP, and Ernst OP (2008) Crystal structure of opsin in its G-protein-interacting conformation. *Nature* **455**: 497-502.

Schlicker E, Betz R, and Gothert M (1988) Histamine H_3 receptor-mediated inhibition of serotonin release in the rat brain cortex. *Naunyn Schmiedebergs Arch Pharmacol* **337**:588-590.

Schlicker E, Fink K, Hinterthaner M, and Gothert M (1989) Inhibition of noradrenaline release in the rat brain cortex via presynaptic H_3 receptors. *Naunyn Schmiedebergs Arch Pharmacol* **340**:633-638.

MOL #104752

Schlicker E, Behling A, Lummen G, and Gothert M (1992) Histamine H_{3A} receptor-mediated inhibition of noradrenaline release in the mouse brain cortex. *Naunyn Schmiedebergs Arch Pharmacol* **345**:489-493.

Schlicker E, Fink K, Detzner M, and Gothert M (1993) Histamine inhibits dopamine release in the mouse striatum via presynaptic H_3 receptors. *J Neural Transm Gen Sect* **93**:1-10.

Schlicker E, Kathmann M, Detzner M, Exner HJ, and Gothert M (1994) H_3 receptor-mediated inhibition of noradrenaline release: an investigation into the involvement of Ca^{2+} and K^+ ions, G protein and adenylate cyclase. *Naunyn Schmiedebergs Arch Pharmacol* **350**:34-41.

Schlicker E, Werthwein S, and Zentner J (1999) Histamine H_3 receptor-mediated inhibition of noradrenaline release in the human brain. *Fundam Clin Pharmacol* **13**:120-122.

Schneider EH, Schnell D, Strasser A, Dove S, and Seifert R (2010) Impact of the DRY motif and the missing "ionic lock" on constitutive activity and G-protein coupling of the human histamine H_4 receptor. *J Pharmacol Exp Ther* **333**:382-392.

Schnell D, Strasser A, and Seifert R (2010) Comparison of the pharmacological properties of human and rat histamine H_3 receptors. *Biochem Pharmacol* **80**:1437-1449.

Schwartz JC (2011) The histamine H_3 receptor: from discovery to clinical trials with pitolisant. *Br J Pharmacol* **163**:713-721.

Seyedi N, Mackins CJ, Machida T, Reid AC, Silver RB, and Levi R (2005) Histamine H_3 receptor-induced attenuation of norepinephrine exocytosis: a decreased protein kinase A activity mediates a reduction in intracellular calcium. *J Pharmacol Exp Ther* **312**:272-280.

Shan L, Bao AM, and Swaab DF (2015) The human histaminergic system in neuropsychiatric disorders. *Trends Neurosci* **38**:167-177.

Shih NY, Aslanian R, Lupo AT Jr, Orlando S, Piwinski JJ, Green MJ, Ganguly AK, West R, Tozzi S, Kreutner W, and Hey JA (1998) Trans-4-methyl-3-imidazoyl pyrrolidine as a potent, highly selective histamine H_3 receptor agonist in vivo. *Bioorg Med Chem Lett* **8**:243-248.

Shi Y, Sheng R, Zhong T, Xu Y, Chen X, Yang D, Sun Y, Yang F, Hu Y, and Zhou N (2012) Identification and characterization of ZEL-H16 as a novel agonist of the histamine H_3 receptor. *PLoS One* **7**:e42185.

Silver RB, Mackins CJ, Smith NC, Koritchneva IL, Lefkowitz K, Lovenberg TW, and Levi R (2001) Coupling of histamine H_3 receptors to neuronal Na^+/H^+ exchange: a novel protective mechanism in myocardial ischemia. *Proc Natl Acad Sci USA* **98**:2855-2859.

Silver RB, Poonwasi KS, Seyedi N, Wilson SJ, Lovenberg TW, and Levi R (2002) Decreased intracellular calcium mediates the histamine H_3-receptor-induced attenuation

61

of norepinephrine exocytosis from cardiac sympathetic nerve endings. *Proc Natl Acad Sci USA* **99**:501-506.

Sjögren B, Blazer LL, and Neubig RR (2010) Regulators of G protein signaling proteins as targets for drug discovery. *Prog Mol Biol Transl Sci* **91**:81-119.

Smits RP and Mulder AH (1991) Inhibitory effects of histamine on the release of serotonin and noradrenaline from rat brain slices. *Neurochem Int* **18**:215-220.

Song J, Salek-Ardakani S, Rogers PR, Cheng M, Van Parijs L, and Croft M (2004) The costimulation-regulated duration of PKB activation controls T cell longevity. *Nat Immunol* **5**:150-158.

Southam E, Cilia J, Gartlon JE, Woolley ML, Lacroix LP, Jennings CA, Cluderay JE, Reavill C, Rourke C, Wilson DM, Dawson LA, Medhurst AD, and Jones DN (2009) Preclinical investigations into the antipsychotic potential of the novel histamine H_3 receptor antagonist GSK207040. *Psychopharmacology (Berl)* **201**:483-494.

Strakhova MI, Fox GB, Carr TL, Witte DG, Vortherms TA, Manelli AM, Miller TR, Yao BB, Brioni JD, and Esbenshade TA (2008) Cloning and characterization of the monkey histamine H_3 receptor isoforms. *Eur J Pharmacol* **601**:8-15.

Sun P, Takatori S, Jin X, Koyama T, Tangsucharit P, Li S, Zamami Y, Kitamura Y, and Kawasaki H (2011) Histamine H_3 receptor-mediated modulation of perivascular nerve transmission in rat mesenteric arteries. *Eur J Pharmacol* **655**:67-73.

Syed YY (2016) Pitolisant: first global approval. *Drugs* **76**:1313-1318.

Takagi H, Morishima Y, Matsuyama T, Hayashi H, Watanabe T, and Wada H (1986) Histaminergic axons in the neostriatum and cerebral cortex of the rat: a correlated light and electron microscopic immunocytochemical study using histidine decarboxylase as a marker. *Brain Res.* **364**:114-123.

Takeshita Y, Watanabe T, Sakata T, Munakata M, Ishibashi H, and Akaike N (1998) Histamine modulates high-voltage-activated calcium channels in neurons dissociated from the rat tuberomammillary nucleus. *Neuroscience* **87**:797-805.

Tardivel-Lacombe J, Rouleau A, Heron A, Morisset S, Pillot C, Cochois V, Schwartz JC, and Arrang JM (2000) Cloning and cerebral expression of the guinea pig histamine H_3 receptor: evidence for two isoforms. *Neuroreport* **11**:755-759.

Tardivel-Lacombe J, Morisset S, Gbahou F, Schwartz JC, and Arrang JM (2001) Chromosomal mapping and organization of the human histamine H_3 receptor gene. *Neuroreport* **12**:321-324.

Taylor SJ and Kilpatrick GJ (1992) Characterization of histamine-H_3 receptors controlling non-adrenergic non-cholinergic contractions of the guinea-pig isolated ileum. *Br J Pharmacol* **105**:667-674.

MOL #104752

Threlfell S, Cragg SJ, Kallo I, Turi GF, Coen CW, and Greenfield SA (2004) Histamine H_3 receptors inhibit serotonin release in substantia nigra pars reticulata. *J Neurosci* **24**:8704-8710.

Timm J, Marr I, Werthwein S, Elz S, Schunack W, and Schlicker E (1998) H_2 receptor-mediated facilitation and H_3 receptor-mediated inhibition of noradrenaline release in the guinea-pig brain. *Naunyn Schmiedebergs Arch Pharmacol* **357**:232-239.

Trzeciakowski JP (1987) Inhibition of guinea pig ileum contractions mediated by a class of histamine receptor resembling the H_3 subtype. *J Pharmacol Exp Ther* **243**:874-880.

Tsui P. Human histamine H_3 gene variant-3. Patent WO2001068816 A1. 2001

Uveges AJ, Kowai D, Zhang Y, Spangler TB, Dunlop J, Semus S, and Jones PG (2002) The role of transmembrane helix 5 in agonist binding to the human H_3 receptor. *J Pharmacol Exp Ther* **2**:451-458.

Valentin-Hansen L, Groenen M, Nygaard R, Frimurer TM, Holliday ND, and Schwartz TW (2012) The arginine of the DRY motif in transmembrane segment III functions as a balancing micro-switch in the activation of the β_2-adrenergic receptor. *J Biol Chem* **287**:31937-31982.

Valko PO, Gavrilov YV, Yamamoto M, Reddy H, Haybaeck J, Mignot E, Baumann CR, and Scammell TE (2013) Increase of histaminergic tuberomammillary neurons in narcolepsy. *Ann Neurol* **74**:794-804.

van der Goot H and Timmerman H (2000) Selective ligands as tools to study histamine receptors. *Eur J Med Chem* **35**:5-20.

van Rijn RM, Harvey JH, Brissett DI, DeFriel JN, and Whistler JL (2013) Novel screening assay for the selective detection of G-protein-coupled receptor heteromersignaling. *J Pharm Exp Ther* **344**:179-188.

van Willigen G, Nieuwland R, Nürnberg B, Gorter G, and Akkerman JW (2000) Negative regulation of the platelet Na^+/H^+ exchanger by trimeric G-proteins. *Eur J Biochem* **267**:7102-7108.

Vohora D and Bhowmik M (2012) Histamine H_3 receptor antagonists/inverse agonists on cognitive and motor processes: relevance to Alzheimer's disease, ADHD, schizophrenia, and drug abuse. *Front Syst Neurosci* doi:10.3389/fnsys.2012.00072.

Watanabe T, Taguchi Y, Shiosaka S, Tanaka J, Kubota H, Terano Y, Tohyama M, and Wada H (1984) Distribution of the histaminergic neuron system in the central nervous system of rats; a fluorescent immunohistochemical analysis with histidine decarboxylase as a marker. *Brain Res.* **295**:13-25.

Weisler RH, Pandina GJ, Daly EJ, Cooper K, Gassmann-Mayer C, and 31001074-ATT2001 Study Investigators (2012) Randomized clinical study of a histamine H_3 receptor antagonist for the treatment of adults with attention-deficit hyperactivity disorder. *CNS Drugs*. **26**:421-434.

MOL #104752

Wellendorph P, Goodman MW, Burstein ES, Nash NR, Brann MR, and Weiner DM (2002) Molecular cloning and pharmacology of functionally distinct isoforms of the human histamine H_3 receptor. *Neuropharmacology* **42**:929-940.

Wess J (1997) G protein-coupled receptors: molecular mechanisms involved in receptor activation and selectivity of G protein recognition. *FASEB* J **11**:346-354.

West RE Jr, Zweig A, Shih NY, Siegel MI, Egan RW, and Clark MA (1990) Identification of two H_3-histamine receptor subtypes. *Mol Pharmacol* **38**:610-613.

West RE Jr, Wu RL, Billah MM, Egan RW, and Anthes JC (1999) The profiles of human and primate [^{3}H]Nα-methylhistamine binding differ from that of rodents. *Eur J Pharmacol* **377**:233-239.

Wiedemann P, Bönisch H, Oerters F, and Brüss M (2002) Structure of the human histamine H_3 receptor gene (HRH3) and identification of naturally occurring variations. *Neural Transm (Vienna)* **109**:443-453.

Wieland K, Bongers G, Yamamoto T, Yamatodani A, Menge WM, Timmerman H, Lovenberg TW, and Leurs R (2001) Contitutive activity of the histamine H_3 receptors stably expressed in SK-N-MC cells: display of agonism and inverse agonism by H_3 antagonists. *J Pharmacol Exp Ther* **299:**908-914

Wise RA (2004) Dopamine, learning and motivation. *Nat Rev Neurosci* **5**:483-494.

Yamashita M, Fukui H, Sugama K, Horio Y, Ito S, Mizuguchi H, Wada H (1991) Expression cloning of a cDNA encoding the bovine histamine H_1 receptor. *Proc Natl Acad Sci USA*. **88**:11515-11519.

Yao BB, Sharma R, Cassar S, Esbenshade TA, and Hancock AA (2003) Cloning and pharmacological characterization of the monkey histamine H_3 receptor. *Eur J Pharmacol* **482**:49-60.

Yoshikawa T, Naganuma F, Iida T, Nakamura T, Harada R, Mohsen AS, Kasajima A, Sasano H, Yanai K (2013) Molecular mechanism of histamine clearance by primary human astrocytes. *Glia*. **61**:905-916.

Young RC, Mitchell RC, Brown TH, Ganellin CR, Griffiths R, Jones M, Rana KK, Saunders D, Smith IR, and Sore NE (1988) Development of a new physicochemical model for brain penetration and its application to the design of centrally acting H_2 receptor histamine antagonists. *J Med Chem* **31**:656-671.

Zaragoza F, Stephensen H, Knudsen SM, Pridal L, Wulff BS, and Rimvall K (2004) 1-alkyl-4-acylpiperazines as a new class of imidazole-free histamine H_3 receptor antagonists. *J Med Chem* **47**:2833-2838.

Zamponi GW and Currie KP (2013) Regulation of Ca_V2 calcium channels by G protein coupled receptors. *Biochim Biophys Acta* **1828**:1629-1643.

Zimatkin SM and Anichtchik OV (1999) Alcohol-histamine interactions. Alcohol Alcohol **34**:141-147.

Footnotes

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MOL #104752

Legends for figures

Figure 1. The histaminergic system in the rat brain. Histamine-synthesizing neurons are located in the hypothalamus tuberomamillary nucleus and these neurons send projections to the Central Nervous System through three major pathways, two ascending bundles that innervate the forebrain structures and one descending bundle reaching the spinal cord. Thal, thalamus; Str, striatum.

Figure 2. Alignment of the amino acid sequence of the human, monkey, guinea-pig, rat and mouse H$_3$Rs. Sequences were obtained from Uniprot and correspond to the full length H$_3$R (445 aa). Alignment was performed with the Clustal Omega server from the European Bioinformatic Institute. The highly conserved sequences in the seven transmembrane domains are shown in grey. The DRF and NPVLY motifs and the glycosylation site (Asn11) are well-conserved among species (green). Residues in blue are responsible for the inter-species differences in H$_3$R pharmacology. Residues in red are likely to be involved in the receptor high constitutive activity on the basis of their identity with a mutated β$_2$-adrenoceptor. The third intracellular loop (ICL3) possesses a region (residues 236 to 300; italics) that is highly variable among species, followed by a highly conserved sequence. The hH$_3$R has ≥ 93% sequence identity with the other species, and the same identity can be found in the guinea-pig, mouse and rat sequences, with 99% identity between mouse and rat, and among the primate proteins. *, conserved residues; **:,** conservative mutations; •, non-conservative mutations.

Figure 3. Structure of the human H₃R. As a GPCR, the H₃R contains seven spanning transmembrane domains, an extracellular amino terminus (NT), an intracellular carboxyl terminus (CT), three extracellular (ECL), and three intracellular (ICL) loops, with a long ICL3 (142 aa). Potential residues for phosphorylation are shown in green. The conserved DRF and NPVLY motifs are shown in yellow. The naturally occurring mutations D18E and A280V are depicted in blue. The glycosylation site (Asn11) is shown in red and the palmitoylation site (C228) in grey. Residues Thr119 and Ala122 (purple) are responsible for the inter-species pharmacological differences. Residues indicated in black in ECL1 and ECL2 correspond to the cysteins forming a disulfide bond important for receptor trafficking. Residues important for agonist recognition are colored in pink.

Figure 4. H₃R signaling pathways. H₃R activation triggers or modulates several pathways through the Gα subunits and G$_{\beta\gamma}$ complexes of Gα$_{i/o}$ proteins. AA, arachidonic acid; AC, adenylyl cyclases; cAMP, 3',5'-cyclic adenosine monophosphate; MAPK, mitogen-activated protein kinases; NHE, Na$^+$/H$^+$ exchanger; PI3K, phosphatidylinositol 3-kinase; PKA, protein kinase A; PLA₂, phosholipase A₂; PLC, phosholipase C.

Figure 5. Binding sites of agonists and antagonists at the human H_3R of 445 aa (hH_3R_{445}). A. Molecular docking of the endogenous agonist histamine in a model of the hH_3R_{445} in the active state. Residues Glu206 and Asp114 are pivotal for agonist binding. B. Planar view of the histamine binding pocket in the hH_3R_{445}. C. Proposed binding site of a protean ligand (proxyfan) in the inactive hH_3R_{445} as observed by molecular docking, depicting the three functional groups of the ligand important for binding (nitrogen-containing ring, central electronegative group and aromatic ring). D. Planar view of the suggested binding residues for an antagonist at the hH_3R_{445}.

Figure 6. Structure of H_3R agonists.

Figure 7. Structure of H_3R antagonists.

MOL #104752

Table 1. Brain expression and signaling of H₃R isoforms from different species.

Species	Isoform (aa)	Expression in the CNS	Signaling	References
Human	H$_3$(445)	thalamus, striatum, cerebral cortex, cerebellum, amygdala, substantia nigra, hippocampus, hypothalamus, corpus callosum, spinal cord	↓ cAMP, ↑ [Ca^{2+}]$_i$ ↑ p42/p44-MAPK, ↑ [^{35}S]-GTPγS binding	1-10
	H$_3$(431)	amygdala, cerebellum, striatum, thalamus, prefrontal cortex, striatum,	ND	
	H$_3$(415)	thalamus, cerebellum, amygdala	↑ [Ca^{2+}]$_i$	
	H$_3$(365)	cerebellum, thalamus, hypothalamus, striatum, substantia nigra, hippocampus, amygdala, prefrontal cortex	↑ [Ca^{2+}]$_i$, ↓ cAMP, ↑ [^{35}S]-GTPγS binding	
	H$_3$(329a)	substantia nigra, amygdala, cerebral cortex, hypothalamus	NF	
	H$_3$(326)	substantia nigra, prefrontal cortex, amygdala, hypothalamus	ND	
	H$_3$(413)	striatum, amygdala	ND	
	H$_3$(453)	ND	↓ cAMP	
	H$_3$(301)	ND	NF	
	H$_3$(373)	hippocampus, substantia nigra, amygdala, hypothalamus	↓ cAMP	
	H$_3$(309)	ND	NF	
	H$_3$(221)	ND	ND	
	H$_3$(409)	ND	ND	
	H$_3$(329b)	ND	ND	
	H$_3$(395)	ND	ND	
	H$_3$(379)	ND	ND	
	H$_3$(293)	ND	ND	
	H$_3$(290)	ND	ND	
	H$_3$(351)	ND	ND	
	H$_3$(340)	ND	ND	
Monkey	H$_3$(445)	frontal cortex, parietal cortex, occipital cortex, para-hippocampal gyrus, hippocampus, caudate nucleus, putamen, amygdala, thalamus, cerebellum	↑ [Ca^{2+}]$_i$	11,12
	H$_3$(413)	frontal cortex, parietal cortex, para-hippocampal gyrus, caudate nucleus, putamen, amygdala, thalamus	↑ [Ca^{2+}]$_i$	
	H$_3$(410)	frontal cortex, parietal cortex, para-hippocampal gyrus, caudate nucleus, putamen, amygdala, thalamus	↑ [Ca^{2+}]$_i$	
	H$_3$(335)	frontal cortex, parietal cortex, occipital cortex, para-hippocampal gyrus, hippocampus, caudate nucleus, putamen, amygdala, thalamus, cerebellum	NF	
Rat	H$_3$(445)	cerebral cortex, hippocampus, midbrain, hypothalamus, striatum, brainstem, cerebellum, olfactory tuberculum, spinal cord	↓ cAMP, ↑ p42/p44-MAPK	13-17

	H$_3$(413)	cerebral cortex, hippocampus, midbrain, striatum	$\downarrow$ cAMP, $\uparrow$ p42/p44-MAPK $\uparrow$ [^{3}H]AA release $\uparrow$ [^{35}S]-GTPγS binding	
	H$_3$(410)	cerebral cortex, hippocampus, midbrain, striatum	ND	
	H$_3$(397)	cerebral cortex, hippocampus, midbrain, hypothalamus, striatum, olfactory tuberculum	$\downarrow$ cAMP, $\uparrow$ p42/p44-MAPK	
	H$_3$(497)	cortex layers II to VIb, caudate-putamen, piriform cortex, hippocampus	NF	
	H$_3$(465)	cortex layers II to VIb, caudate-putamen, piriform cortex, hippocampus	NF	
	H$_3$(449)	cortex layers II to VIb, caudate-putamen, piriform cortex, hippocampus	NF	
Mouse	H$_3$(445)	cerebral cortex, striatum , hypothalamus	$\uparrow$ [Ca^{2+}]$_i$	18,19
	H$_3$(413)	cerebral cortex, striatum , hypothalamus	ND	
	H$_3$(397)	cerebral cortex, striatum , hypothalamus	ND	
Guinea pig	H$_3$(445)	cerebral cortex, striatum	ND	20
	H$_3$(415)	cerebral cortex, striatum	ND	
Dog	H3(ND)	ND	ND	21

$\uparrow$, increase; $\downarrow$, decrease. ND, not determined, NF, non-functional, [Ca^{2+}]$_i$, intracellular Ca^{2+} concentration. AA, arachidonic acid. *The human H$_3$R$_{329a}$ and H$_3$R$_{329b}$ isoforms possess the same number of amino acids, but differ in the region where alternative splicing occurs. 1) Lovenberg *et al*., 1999; 2) Tardivel-Lacombe *et al.*, 2001; 3) Nakamura *et al.*, 2000; 4) Cogé *et al.*, 2001; 5) Tsui, 2001; 6) Wiedemann *et al.*, 2002; 7) Wellendorph *et al.*, 2002; 8) Gallagher and Yates, 2007; 9) Bongers *et al*., 2007a; 10) Bongers *et al.*, 2007b; 11) Yao *et al.*, 2003; 12) Strakhova *et al.*, 2008; 13) Lovenberg *et al*., 2000; 14) Drutel *et al*., 2001; 15) Morisset *et al.*, 2001; 16) Bakker *et al.*, 2006; 17) Gbahou *et al*., 2012; 18) Chen *et al*., 2003; 19) Rouleau *et al*., 2002; 20) Tardivel-Lacombe *et al*., 2000; 21) Ireland-Denny *et al*., 2001.

MOL #104752

Table 2. Affinities of the H₃R for agonists and antagonists.

Imidazole-containing agonists	pKi					
	Rat	Mouse	Guinea pig	Monkey	Dog	Human
Histamine	**Native** 8.27[1] 8.28[2] 7.92[3] 8.29 ± 0.03[4] 8.20 ± 0.10[5] 8.30[6] 8.13[7] **Recombinant** 7.87[3] 8.57[6] 7.69[8] 5.70 ± 0.15[9] 7.89 ± 0.07[10]	7.92 ± 0.54 (high)[11] 6.09 ± 0.61 (low)[11] 7.82[12]	7.58[1] 8.18 ± 0.15[4]	7.88[13] 8.78 ± 0.10[14]	8.50 ± 0.08[4]	8.59 ± 0.08[4] 8.63[6] 8.08[13] 7.81[3] 8.58[6] 7.88[8] 8.20 ± 0.04[10] 8.55 ± 0.01[14] 8.00 ± 0.10[15] 8.03 ± 0.06[16] 8.30 ± 0.10[17] 8.60 ± 0.00[18] 8.50[19] 8.40 ± 0.20[20]
RAMH	**Native** 8.88[1,2] 8.82[3] 8.71 ± 0.08[4] 8.95[7] 8.71 ± 0.08[21] **Recombinant** 8.72[3] 8.44[8] 6.40 ± 0.20[9] 8.62 ± 0.07[10] 9.35 ± 0.20[21]	7.91 ± 0.09 (high)[11] 6.67 ± 0.21 (low)[11] 8.40[12]	8.49[1] 8.63 ± 0.25[4] 9.48 ± 0.16[22] 10.07 ± 0.16[22]	8.50[13] 9.55 ± 0.04[14]	8.93 ± 0.19[4]	9.15 ± 0.06[4] 8.82[13] 9.16 ± 0.07[21] 8.67[3] 8.56[8] 8.91 ± 0.07[10] 9.41 ± 0.05[14] 8.20 ± 0.10[15] 8.36 ± 0.07[16] 8.92 ± 0.11[17] 9.00 ± 0.10[18] 9.16 ± 0.08[21] 8.56[23]
SAMH	**Native** 7.30[1] 7.33 ± 0.06[4] 7.11[7] **Recombinant**	5.93 ± 0.04[11]	7.30[1] 7.63 ± 0.18[4]	7.69[13]	7.79 ± 0.11[4]	7.49[13] 8.33 ± 0.18[4] 7.20 ± 0.10[15] 7.60 ± 0.20[18]
NAMH	**Native** 8.92[3] 8.78 ± 0.13[4] 9.15[7] **Recombinant** 8.82[3] 8.70 ± 0.09[10]	8.95[12]	8.67 ± 0.27[4] 9.99 ± 0.24[22] 10.31 ± 0.24[22]	8.76[13]	9.15 ± 0.09[4]	9.41 ± 0.06[4] 9.08[13] 8.61[3] 9.22 ± 0.03[10] 8.40 ± 0.10[15] 9.00 ± 0.10[18]

MOL #104752

						9.80^{19}
Immepip	**Native** 9.30^3 8.99 ± 0.20^4	9.50 ± 0.66 (high)[11] 7.48 ± 0.33 (low)[11]	8.68 ± 0.34^4		9.25 ± 0.06^4	9.58 ± 0.06^4
	Recombinant 8.85^3 7.50 ± 0.08^9	9.79^{12}				8.79^3 9.30 ± 0.10^{15} 9.32 ± 0.04^{16} 9.51 ± 0.03^{17} 9.40 ± 0.10^{18} 9.70^{19} 9.40 ± 0.10^{20} 9.30^{24}
Imetit	**Native** 10.00^2 10.00^3 9.46 ± 0.10^4 9.82^7 9.53 ± 0.09^{21}	9.09 ± 0.18 (high)[11] 7.02 ± 0.60 (low)[11]	8.99 ± 0.25^4	9.01^{13}	9.31 ± 0.06^4	9.67 ± 0.06^4 9.50^{13} 9.79 ± 0.07^{21}
	Recombinant 9.69^3 9.38 ± 0.08^{10} 10.11 ± 0.10^{21}	9.69^{12}		9.84 ± 0.03^{14}		9.22^3 9.20 ± 0.05^{10} 9.59 ± 0.09^{14} 8.80 ± 0.10^{15} 9.23 ± 0.19^{17} 9.40 ± 0.10^{18} 9.70^{19} 9.59 ± 0.09^{21}
Impentamine	**Recombinant** 6.90 ± 0.06^9 10.11 ± 0.05^{10}					8.84 ± 0.06^{10} 8.30 ± 0.10^{15} 8.29 ± 0.14^{16} 8.50 ± 0.10^{18}
Proxyfan	**Native** 8.51 ± 0.05^{21} **Recombinant** 8.53^8 8.08 ± 0.10^{10} 8.62 ± 0.10^{21}		8.32 ± 0.13^{22} 8.80 ± 0.23^{22}			8.38 ± 0.13^{21} 8.56^8 7.90 ± 0.10^{15} 8.35 ± 0.06^{21} 8.40 ± 0.20^{18} 7.87 ± 0.07^{10}
VUF 5296	**Native** 7.60 ± 0.10^5 **Recombinant**					7.17 ± 0.09^{16}
VUF 5297	**Native** 8.76 ± 0.10^5 **Recombinant**					8.03 ± 0.08^{16}
Methimepip	**Recombinant**					9.00 ± 0.10^{15} 9.00 ± 0.10^{24}
Imidazole- containing antagonists	pKi					
	Rat	Mouse	Guinea pig	Monkey	Dog	Human

73

Thioperamide	**Native** 8.67[1] 7.99[3] 8.10 ± 0.06[4] 8.12[6] 8.39[7] 8.85[25] 8.48 ± 0.01[26] 8.15 ± 0.06[27]	8.37 ± 0.12[11] 8.30 ± 0.15[26]	8.69[1] 8.34 ± 0.12[4] 8.69 ± 0.05[22] 9.08 ± 0.13[22] 8.34 ± 0.11[27]	6.61[13]	8.17 ± 0.15[4] 7.91 ± 0.10[26] 8.17 ± 0.12[27]	7.13 ± 0.06[4] 7.21[6] 6.69[13] 7.18 ± 0.01[27]
	Recombinant 8.37[3] 8.49[6] 8.18[8] 7.20 ± 0.08[9] 7.94 ± 0.04[10] 8.62 ± 0.06[26] 8.44 ± 0.07[27] 8.40 ± 0.20[28] 8.44 ± 0.07[29]	8.49 ± 0.05[26]		7.19 ± 0.03[14]		7.23[3] 7.13[6] 7.22[8] 7.34 ± 0.04[10] 7.24 ± 0.06[14] 7.30 ± 0.10[15] 7.34 ± 0.13[17] 7.70 ± 0.20[18] 7.70[19] 6.90 ± 0.10[20] 7.85[23] 7.12 ± 0.10[26] 7.14 ± 0.06[27] 7.40 ± 0.33[28] 7.14 ± 0.06[29]
Ciproxifan	**Native** 9.15 ± 0.05[4] 9.18[6] 9.22 ± 0.05[21] 8.91 ± 0.04[26] 9.20 ± 0.04[30]	9.10 ± 0.16[26]	8.70 ± 0.08[4] 8.76 ± 0.07[30]		8.20 ± 0.08[4] 7.46 ± 0.10[26] 8.24 ± 0.06[30]	6.99 ± 0.08[4] 7.04[6] 7.05 ± 0.07[21] 6.72 ± 0.14[26] 7.05 ± 0.06[30]
	Recombinant 9.36[6] 8.40[8] 8.87 ± 0.08[10] 9.38 ± 0.07[21] 9.29 ± 0.05[26] 9.29 ± 0.09[29] 9.29 ± 0.09[30]	9.08 ± 0.08[26]		7.18 ± 0.05[14]		7.20[6] 7.33[8] 7.03 ± 0.12[10] 7.20 ± 0.04[14] 7.17 ± 0.04[17] 7.40 ± 0.10[18] 7.20 ± 0.04[21] 7.02[23] 6.96 ± 0.06[26] 7.20 ± 0.05[29] 7.20 ± 0.05[30]
Iodoproxyfan	**Native** 9.69[7]	8.86 ± 0.16[11]	9.76 ± 0.23[22] 10.00 ± 0.09[22]			
	Recombinant					9.20 ± 0.10[15]
Clobenpropit	**Native** 9.30[3] 9.38 ± 0.08[4] 9.31[7] 9.45 ± 0.08[21] 9.53 ± 0.11[26] 9.45 ± 0.08[27]	9.28 ± 0.16[11] 9.57 ± 0.09[26]	9.65 ± 0.22[4] 9.65 ± 0.20[27]	8.65[13]	10.09 ± 0.13[4] 9.84 ± 0.12[26] 10.09 ± 0.11[27]	9.08 ± 0.13[4] 8.24[13] 9.11 ± 0.11[21] 9.11 ± 0.11[26] 9.11 ± 0.12[27]
	Recombinant 9.39[3] 8.85[8]	9.50[12] 9.70 ± 0.08[26]		9.72 ± 0.10[14]		9.22[3] 8.61[8]

MOL #104752

	8.30 ± 0.02[9] 9.11 ± 0.06[10] 9.82 ± 0.07[21] 9.59 ± 0.09[26] 9.75 ± 0.01[27]					9.34 ± 0.06[10] 9.43 ± 0.03[14] 8.60 ± 0.10[15] 8.79 ± 0.08[17] 9.30 ± 0.20[18] 9.30[19] 9.47 ± 0.04[21] 9.23[23] 9.47 ± 0.19[26] 9.44 ± 0.04[27]
Iodophenpropit	**Native** 9.23 ± 0.10[4] **Recombinant**	9.24 ± 0.21[11]	9.39 ± 0.21[4] 10.08 ± 0.11[22] 9.96 ± 0.23[22]		9.59 ± 0.09[4]	8.45 ± 0.14[4] 8.20 ± 0.10[15] 9.10 ± 0.20[18]
SCH 79687	**Native** 8.72[31]		7.88[31]			
VUF 5681	**Recombinant**					8.35 ± 0.11[16] 8.90 ± 0.30[18]
Burimamide	**Native** 8.18 ± 0.15[4] 7.16[7] **Recombinant**	6.28 ± 0.2[11]	8.27 ± 0.05[4]		8.42 ± 0.13[4]	8.34 ± 0.21[4] 7.90 ± 0.10[15]
Non-imidazole antagonists	pKi					
JNJ-5207852	**Recombinant** 8.90 ± 0.17[28]					9.24 ± 0.21[28]
ABT-239	**Native** 7.92 ± 0.11[26] 8.49 ± 0.04[32] **Recombinant** 8.52 ± 0.08[26] 8.87 ± 0.04[29] 8.91 ± 0.04[32]	7.96 ± 0.08[26] 8.42 ± 0.07[26]	8.59 ± 0.12[32]		8.61 ± 0.20[26] 8.41 ± 0.07[32]	8.34 ± 0.06[32] 9.51 ± 0.10[26] 9.35 ± 0.04[29] 9.35 ± 0.04[32]
NNC 38-1049	**Recombinant** 8.29[33]					8.92[23]
A-331440	**Native** 7.66[6] 7.73 ± 0.08[34] **Recombinant** 8.20[6] 8.23 ± 0.04[34]				8.71 ± 0.06[14]	7.64[6] 7.63 ± 0.05[34] 8.48[6] 8.45 ± 0.05[14] 7.75 ± 0.04[17] 8.00[18] 8.45 ± 0.05[34]
A-349821	**Native** 9.12 ± 0.14[34] **Recombinant** 8.78 ± 0.12[29] 8.78 ± 0.12[34]				9.67 ± 0.14[14]	9.37 ± 0.08[34] 9.55 ± 0.09[14] 9.20 ± 0.10[18] 9.39 ± 0.08[29]

MOL #104752

						9.39 ± 0.08^{34}
UCL 1972	**Native** 7.41[35]					
GSK189254	**Native** 8.51 ± 0.11^{26} **Recombinant** 9.17 ± 0.09^{26}	8.89 ± 0.13^{26} 8.76 ± 0.06^{26}			8.83 ± 0.09^{26}	9.59 ± 0.15^{26} 9.90 ± 0.18^{26}
Pitolisant	**Native** **Recombinant**	7.85^{39}				8.56^{39}

References: 1) Arrang *et al.*, 1987b; 2) Garbarg *et al.*, 1992; 3) Lovenberg *et al.*, 2000; 4) Ireland-Denny *et al.,* 2001; 5) De Esch *et al.*, 1999; 6) Hancock *et al.*, 2004a; 7) Ligneau *et al.*, 1994; 8) Ligneau *et al.*, 2000; 9) Drutel *et al.*, 2001; 10) Schnell *et al.*, 2010; 11) Jansen *et al.*, 2000; 12) Chen *et al.*, 2003; 13) West *et al.*, 1999; 14) Strakhova *et al.*, 2008.; 15) Lim *et al.*, 2005; 16) Kitbunnadaj *et al.*, 2003; 17) Flores-Clemente *et al.*, 2013; 18) Bongers *et al.*, 2007b; 19) Lovenberg *et al.*, 1999; 20) Bongers *et al.*, 2007c; 21) Krueger *et al.*, 2005. 22) Harper *et al.*, 1999; 23) Zaragoza *et al.*, 2004; 24) Kitbunnadaj *et al.*, 2005; 25) Sánchez-Lemus and Arias-Montaño, 2004; 26) Medhurst *et al.*, 2007; 27) Esbenshade *et al.*, 2003; 28) Barbier *et al.*, 2004; 29) Cowart *et al.*, 2005; 30) Esbenshade *et al.*, 2003; 31) McLeod *et al.,* 2003; 32) Esbenshade *et al.*, 2005; 33) Malmlöf *et al.*, 2005; 34) Hancock *et al.*, 2004b; 35) Ganellin *et al.*, 1998; 36) Uveges *et al.*, 2002; 37) Baker *et al.*, 2008; 38) Hew *et al.*, 1990; 39) Ligneau *et al.*, 2007. Data reported as Ki were converted to pKi. For recombinant receptors all values refer to the H$_3$R of 445 aa. SAMH, S-α-methylhistamine.

Table 3. Potencies of H_3R agonists and antagonists.

Drug	pIC$_{50}$ or pEC$_{50}$					pA$_2$	pK_B	pD$_2$
	cAMP	Ca^{2+}	MAPK	GTPγS	PI3K/Akt			
RAMH	8.50 ± 0.10 (r)[9] 9.50 ± 0.10[15] 9.17 ± 0.03[16] 8.22 ± 0.35 (h)[17] 8.20 ± 0.10[18] 8.67 (r)[21] 8.01 (h)[21] 9.47 ± 0.18[36] 9.54 ± 0.06[37]	8.44 ± 0.11[14] 7.92[21] 8.22[21] 8.50 ± 0.13[36]	7.94 ± 0.17 (h)[17]	8.50 ± 0.14 (h)[17] 8.00 ± 0.10[18] 7.65 (r)[21] 8.02, 8.42 (h)[21]				7.60 ± 0.05[4] 8.50 ± 0.10[9]
SAMH	8.00 ± 0.10[15] 6.80 ± 0.10[18] 8.31 ± 0.01[37]			6.60 ± 0.10[18]				6.21 ± 0.15
Histamine	8.30 ± 0.10[15] 8.01 ± 0.03[16] 7.40 ± 0.20[18] 8.63 ± 0.10[36] 8.55 ± 0.05[37]	7.66, 7.81, 7.75, 7.76[12] 7.99 ± 0.09[14] 7.82 ± 0.09[36]		7.30 ± 0.10[18]				6.46 ± 0.18 7.40 ± 0.10
Imetit	9.90 ± 0.10[15] 9.66 (r)[21] 8.39 (h)[21] 8.92 ± 0.04[26] 9.79 ± 0.20[36] 9.91 ± 0.05[37]	8.13, 8.03, 8.40, 8.48[12] 8.47 ± 0.08[14] 8.14[21] 8.04[21] 8.86 ± 0.20[36]		8.60 ± 0.10[18] 8.69 (r)[21] 8.53, 8.71 (h)[21]				7.80 ± 0.10
Immepip	9.40 ± 0.10 (r)[9] 10.40 ± 0.10[15] 9.88 ± 0.02[16] 9.00 ± 0.10[18] 9.90 ± 0.10[20] 9.90 ± 0.0[24] 7.88[25] 10.30 ± 0.14[37]			8.80 ± 0.10[18]	9.40[20]			8.35 ± 0.08 9.40 ± 0.10
Impentamine	8.1 (r)[9] 8.40 ± 0.10[15] 8.63 ± 0.08[16] 9.17 ± 0.11[36] 8.43 ± 0.08[37]			7.50 ± 0.10[18]				8.10 ± 0.10
NAMH	9.40 ± 0.10[15] 8.50 ± 0.10[18] 9.45 ± 0.05[37]	8.03, 8,15, 8.34, 8.22[12]		7.90 ± 0.10[18]				7.17 ± 0.07
Methimepip	9.50 ± 0.20[15] 9.50 ± 0.20[24]							
VUF 5296	7.24 ± 0.04[16]							6.15 ± 0.08
VUF 5297	8.23 ± 0.14[16]							6.64 ± 0.06
Proxyfan	8.50 ± 0.10[15] 7.83 (r)[21] 6.86 (h)[21] 7.73[21] 7.61[21] 8.31 ± 0.11[37]	7.43 ± 0.06[14] 7.61, 7.11[21] 6.20, 5.89[21]		7.50 ± 0.10[18] 7.33 (r)[21] 7.49, 7.68 (h)[21]				
Thioperamide	7.50 ± 0.10[15]			8.06 ± 0.10 (r)[6]	7.00[20]	8.44 ± 0.45[4]	6.90 ± 0.10 2[3]	

MOL #104752

Compound								
	7.60 ± 0.20[18] 7.10 ± 0.20[20] 7.8860[25]			7.41 ± 0.11 (h)[6] 6.90 ± 0.10[18] 7.49 ± 0.34[27] 7.41 ± 0.12 (h)[30] 8.06 ± 0.10 (r)[30]		8.44 ± 0.49 (gp)[30]	8.13 ± 0.14 (r)[6] 7.39 ± 0.04 (h)[6] 9.11 ± 0.14 (r)[6] 6.82 ± 0.05 (h)[6] 7.09 ± 0.08 (mk)[14] 6.82 ± 0.06 (h)[29] 6.10 ± 0.12 (h)[30] 7.39 ± 0.04 (h)[30] 6.82 ± 0.06 (h)[30] 7.61 ± 0.14 (r)[30] 8.13 ± 0.14 (r)[30] 8.95[38]	
Ciproxifan	5.39, 5.30[21]	6.57, 6.58[21]		8.87 ± 0.16 (r)[6] 7.04 ± 0.17 (h)[6] 7.16 ± 0.19[27] 7.04 ± 0.17 (h)[30] 8.87 ± 0.16 (r)[30]		8.12 ± 0.56[4] 8.12 ± 0.56[30]	8.78 ± 0.12 (r)[6] 7.03 ± 0.13 (h)[6] 9.42 ± 0.11 (r)[6] 6.86 ± 0.07 (h)[6] 7.23 ± 0.04 (mk)[14] 9.10 (r)[21] 6.59 (h)[21] 6.84 ± 0.08[29] 6.59 ± 0.04 (h)[30] 7.07 ± 0.13 (h)[30] 6.84 ± 0.08 (h)[30] 9.20 ± 0.10 (r)[30] 8.78 ± 0.12 (r)[30]	
Burimamide	7.00 ± 0.10[15] 6.65 ± 0.10[37]					8.05 ± 0.68[4]	7.56[38]	
Iodophenpropit	8.50 ± 0.10[15] 8.50 ± 0.40[18]					9.14 ± 0.61[4]		
Iodoproxyfan	10.30 ± 0.10[15]							
Clobenpropit	9.40 ± 0.10[15] 6.79, 6.55[21]	7.41, 7.10[21]		8.07 ± 0.66[27]		9.66 ± 0.49[4] 9.66 ± 0.49[27]	9.80 ± 0.03 (mk)[14] 9.03 (r)[21] 8.35 (h)[21] 8.21 ± 0.10 (h)[27] 9.04 ± 0.11 (r)[27] 8.91 ± 0.06 (h)[27]	
VUF 5681	8.41 ± 0.10[37]					8.08 ± 0.03[16]		
A-331440				7.07 ± 0.24 (r)[6] 7.70 ± 0.08 (h)[6] 7.60 ± 0.10[18]	7.70[20]		7.60 ± 0.12 (r)[6] 8.16 ± 0.04 (h)[6] 7.38 ± 0.10 (r)[6] 7.37 ± 0.29 (h)[6] 7.81 ± 0.02 (mk)[14]	
A-349821	9.00 ± 0.40[18]			9.20 ± 0.10[18] 9.09 ± 0.08 (h)[30] 8.57 ± 0.09 (r)[30]		9.47 ± 0.56[30]	9.67 ± 0.05 (mk)[14] 8.27 ± 0.12[29] 8.24 ± 0.10 (h)[30] 9.26 ± 0.02 (h)[30] 8.27 ± 0.12 (h)[30] 8.08 ± 0.10 (r)[30] 8.62 ± 0.11 (r)[30]	
ABT-229							7.87 ± 0.15 (r)[29] 7.89 ± 0.04 (h)[32] 9.04 ± 0.04 (h)[32] 7.87 ± 0.15 (h)[32] 7.60 ± 0.05 (r)[32] 8.34 ± 0.14 (r)[32]	

MOL #104752

						8.74 ± 0.44 (gp)[32]	
GSK189254				8.20 ± 0.12[26]		9.06 ± 0.02[26]	
JNJ-5207852						8.94 (r)[28] 9.84 (h)[28]	
NCC 38-1049						8.63[33]	
Pitolisant						9.79[39]	

References: 1) Arrang *et al*., 1987b; 2) Garbarg *et al*., 1992; 3) Lovenberg *et al*., 2000; 4) Ireland-Denny *et al*., 2001; 5) De Esch *et al*., 1999; 6) Hancock *et al*., 2004a; 7) Ligneau *et al*., 1994; 8) Ligneau *et al.,* 2000; 9) Drutel *et al*., 2001; 10) Schnell et al., 2010; 11) Jansen et al., 2000; 12) Chen et al., 2003; 13) West et al., 1999; 14) Strakhova *et al*., 2008.; 15) Lim *et al*., 2005; 16) Kitbunnadaj *et al*., 2003; 17) Flores-Clemente *et al*., 2013; 18) Bongers et al., 2007b; 19) Lovenberg *et al*., 1999; 20) Bongers *et al*., 2007c; 21) Krueger *et al*., 2005. 22) Harper *et al*., 1999; 23) Zaragoza et al., 2004; 24) Kitbunnadaj et al., 2005; 25) Sánchez-Lemus and Arias-Montaño, 2004; 26) Medhurst *et al*., 2007; 27) Esbenshade *et al.,* 2003; 28) Barbier *et al*., 2004; 29) Cowart *et al*., 2005; 30) Esbenshade *et al*., 2003; 31) McLeod *et al*., 2003; 32) Esbenshade *et al*., 2005; 33) Malmlöf *et al*., 2005; 34) Hancock *et al*., 2004b; 35) Ganellin *et al*., 1998; 36) Uveges *et al*., 2002; 37) Baker *et al*., 2008; 38) Hew *et al*., 1990; 39) Ligneau *et al*., 2007. For recombinant receptors all values refer to the full length (445 aa) receptor. Abbreviations for species: gp, guinea pig; h, human; mk, monkey; r, rat.

79

Figure 1

Cerebral cortex

Hippocampus

Cerebellum

Olfactory bulb

Str

Thal

Hipothalamus

Hypophysis

Spinal cord
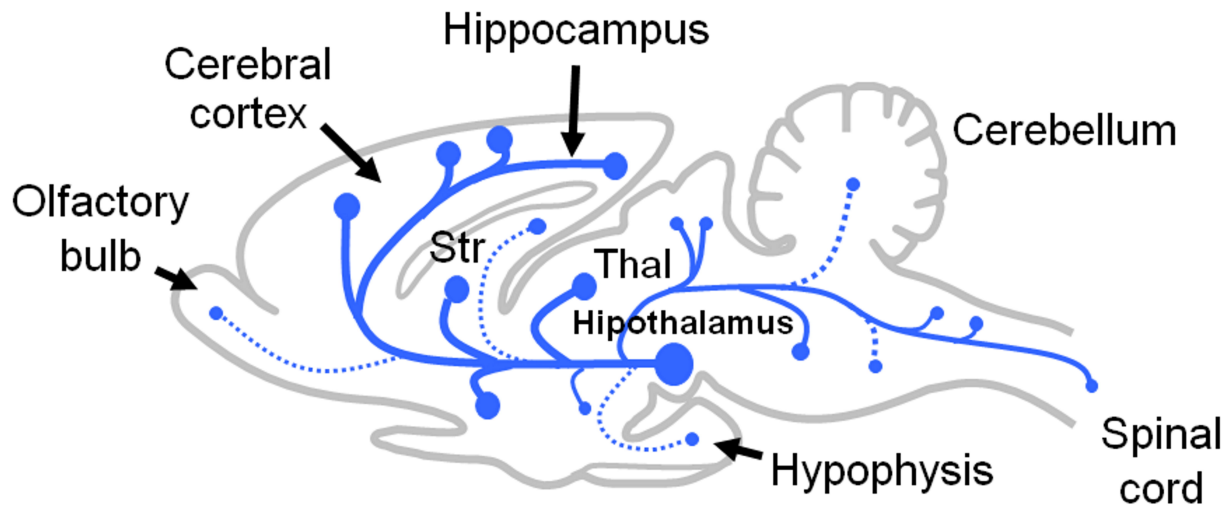
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Monkey      MERAPPDGPLNASGALAGEAA-AAGGARGFSAAWTAVLAALMALLIVATVLGNALVMLAF
Guinea pig  MERAPPDGLMNASGALAGEAAAAAGGARTFSAAWTAVLAALMALLIVATVLGNALVMLAF
Rat         MERAPPDGLMNASGTLAGEAA-AAGGARGFSAAWTAVLAALMALLIVATVLGNALVMLAF
Mouse       MERAPPDGLMNASGALAGEAA-AAGGARGFSAAWTAVLAALMALLIVATVLGNALVMLAF
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Human       VADSSLRTQNNFFLLNLAISDFLVGAFCIPLYVPYVLTGRWTFGRGLCKLWLVVDYLLCT
Monkey      VADSSLRTQNNFFLLNLAISDFLVGAFCIPLYVPYVLTGRWTFGRGLCKLWLVVDYLLCT
Guinea pig  VADSSLRTQNNFFLLNLAISDFLVGVFCIPLYVPYVLTGRWTFGRGLCKLWLVVDYLLCT
Rat         VADSSLRTQNNFFLLNLAISDFLVGAFCIPLYVPYVLTGRWTFGRGLCKLWLVVDYLLCA
Mouse       VADSSLRTQNNFFLLNLAISDFLVGAFCIPLYVPYVLTGRWTFGRGLCKLWLVVDYLLCA
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Human       SSAFNIVLISYDRFLSVTRAVSYRAQQGDTRRAVRKMLLVWVLAFLLYGPAILSWEYLSG
Monkey      SSAFNIVLISYDRFLSVTRAVSYRAQQGDTRRAVRKMLLVWVLAFLLYGPAILSWEYLSG
Guinea pig  SSVFNIVLISYDRFLSVTRAVSYRAQQGDTRRAVRKMVLVWVLAFLLYGPAILSWEYLSG
Rat         SSVFNIVLISYDRFLSVTRAVSYRAQQGDTRRAVRKMALVWVLAFLLYGPAILSWEYLSG
Mouse       SSVFNIVLISYDRFLSVTRAVSYRAQQGDTRRAVRKMALVWVLAFLLYGPAILSWEYLSG
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Human       GSSIPEGHCYAEFFYNWYFLITASTLEFFTPFLSVTFFNLSIYLNIQRRTRLRLDGAREA
Monkey      GSSIPEGHCYAEFFYNWYFLITASTLEFFTPFLSVTFFNLSIYLNIQRRTRLRLDGAREA
Guinea pig  GSSIPEGHCYAEFFYNWYFLITASTLEFFTPFLSVTFFNLSIYLNIQRRTRLRLDGGARE
Rat         GSSIPEGHCYAEFFYNWYFLITASTLEFFTPFLSVTFFNLSIYLNIQRRTRLRLDGG-RE
Mouse       GSSIPEGHCYAEFFYNWYFLITASTLEFFTPFLSVTFFNLSIYLNIQRRTRLRLDGG-RE
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Human       AGPEPPPEAQPSPP-PPPGCWGCWQKGHGEAMPLHRYGVGEAAVGAEAGEATLGGGGGGG
Monkey      GGPEPPPEAQPSPP-PPPGCWGCWQKGHGEAMPLHRYGVGEAAAGAEAGETALGGGGGGG
Guinea pig  AGPDPLPEAQSSPPQPPPGCWGCWPKGQGESMPLHRYGVGEAGPGAEAGEAALGGGSG--
Rat         AGPEPPPDAQPSPPPAPPSCWGCWPKGHGEAMPLHRYGVGEAGPGVEAGEAALGGGSGGG
Mouse       AGPEPPPDAQPSPPPAPPSCWGCWPKGHGEAMPLHRYGVGEAGPGVETGEAGLGGGSGGG
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Human       SVASPTSSSGSSSRGTERPRSLKRGSKPSASSASLEKRMKMVSQSFTQRFRLSRDRKVAK
Monkey      SAASPTSSSGSSSRGTERPRSLKRGSKPSASSASLEKRMKMVSQSFTQRFRLSRDRKVAK
Guinea pig  AAASPTSSSGSSSRGTERPRSLKRGSKPSASSASLEKRMKMVSQSITQRFRLSRDKKVAK
Rat         AAASPTSSSGSSSRGTERPRSLKRGSKPSASSASLEKRMKMVSQSITQRFRLSRDKKVAK
Mouse       AAASPTSSSGSSSRGTERPRSLKRGSKPSASSASLEKRMKMVSQSITQRFRLSRDKKVAK
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Human       SLAVIVSIFGLCWAPYTLLMIIRAACHGHCVPDYWYETSFWLLWANSAVNPVLYPLCHHS
Monkey      SLAVIVSIFGLCWAPYTLLMIIRAACHGHCVPDYWYETSFWLLWANSAVNPVLYPLCHHS
Guinea pig  SLAIIVSIFGLCWAPYTLLMIIRAACHGHCVPDYWYETSFWLLWANSAVNPVLYPLCHYS
Rat         SLAIIVSIFGLCWAPYTLLMIIRAACHGRCIPDYWYETSFWLLWANSAVNPVLYPLCHYS
Mouse       SLAIIVSIFGLCWAPYTLLMIIRAACHGHCVPDYWYETSFWLLWANSAVNPVLYPLCHYS
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Human       FRRAFTKLLCPQKLKIQPHSSLEHCWK
Monkey      FRRAFTKLLCPQKLKIQPHSSLEQCWK
Guinea pig  FRRAFTKLLCPQKLKVQPHSSLEHCWK
Rat         FRRAFTKLLCPQKLKVQPHGSLEQCWK
Mouse       FRRAFTKLLCPQKLKVQPHGSLEQCW-
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Figure 2

Figure 3
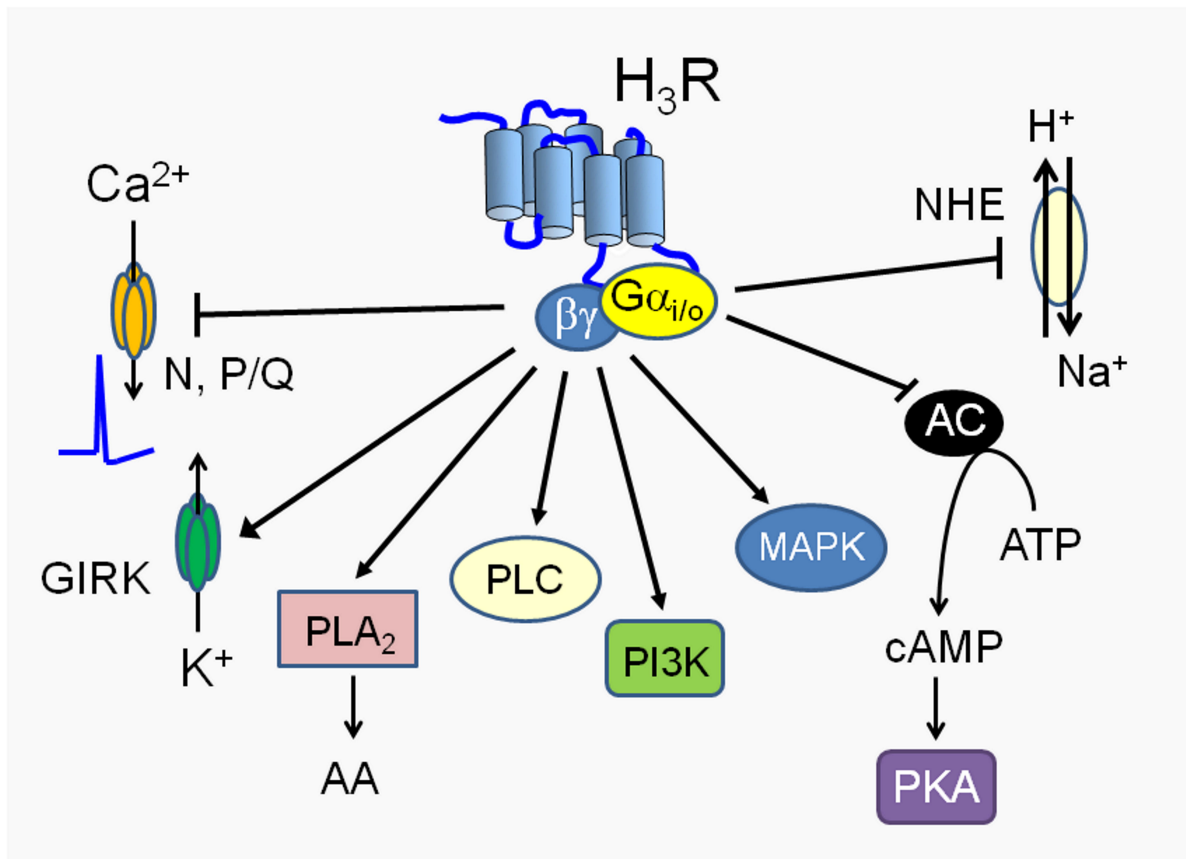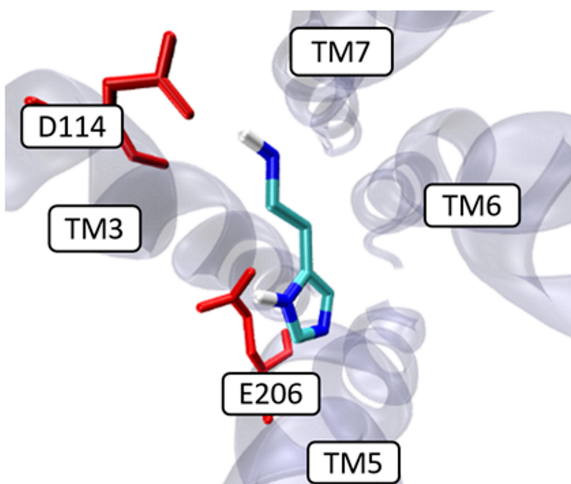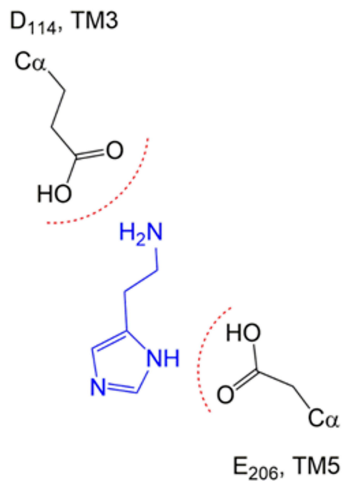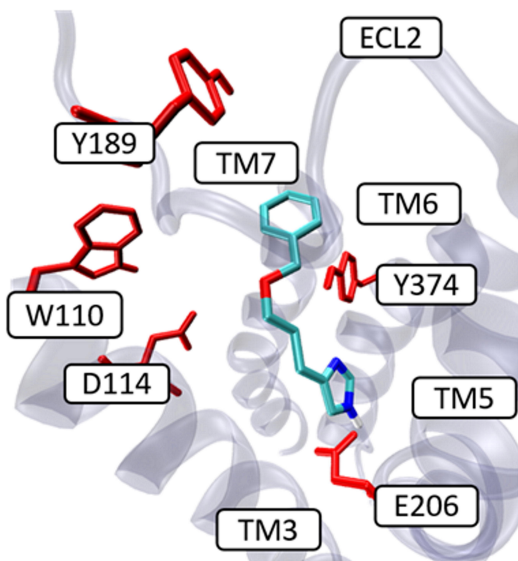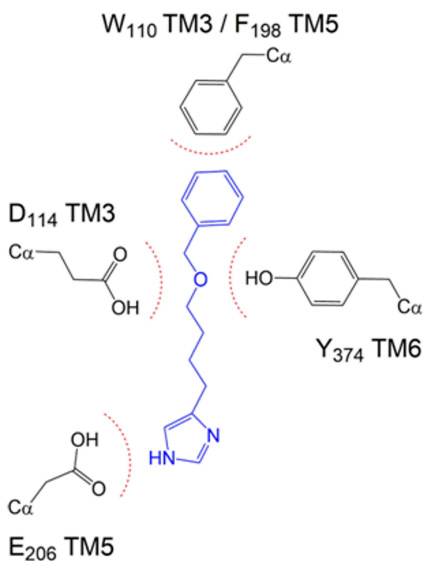
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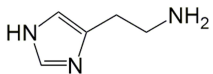
Figure 5

Figure 6

Histamine
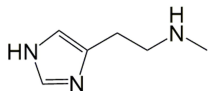
Nα–methylhistamine
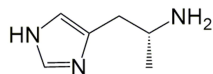
Rα–methylhistamine
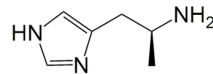
Sα–methylhistamine
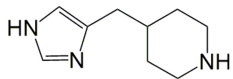
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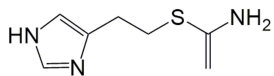
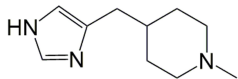Imetit
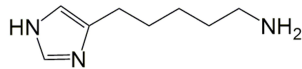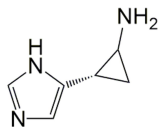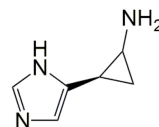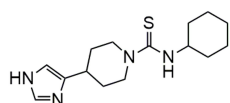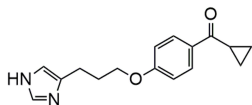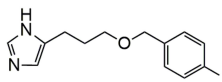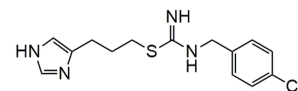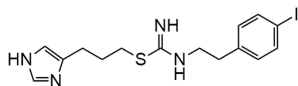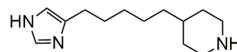
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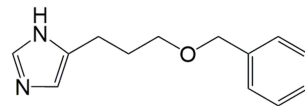
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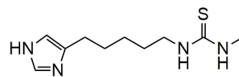
Methimepip

VUF-5296

VUF-5297

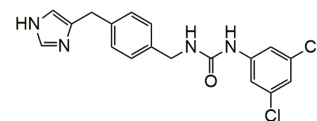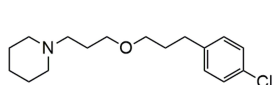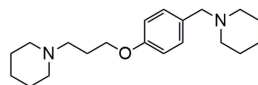Figure 7

Thioperamide

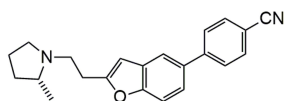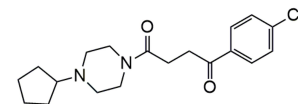Ciproxyfan

Iodoproxyfan

Clobenpropit
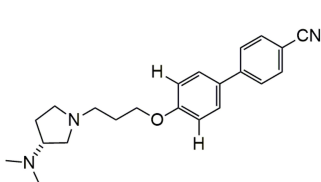
Iodophenpropit

VUF-5861
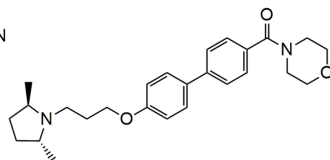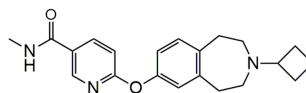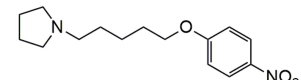
Burimamide

SCH-79867

Pitolisant

JNJ-5207852

ABT-239

NNC-38-1049

A-331440

A-349821

GSK-189254

UCL-1972