Chemical Probes for Histamine Receptor Subtypes

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Abstract Ligands with different properties and different selectivity are highly needed for in vitro and in vivo studies on the (patho)physiological influence of the chemical mediator histamine and its receptor subtypes. A selection of well-described ligands for the different receptor subtypes and different studies is shown with a particular focus on affinity and selectivity. In addition, compounds with radioactive or fluorescence elements will be presented with their beneficial use for other species or different investigations.

Keywords Fluorescence · Histamine · Histamine H_1 receptor · Histamine H_2 receptor · Histamine H_3 receptor · Histamine H_4 receptor · PET · Radioligands

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Abbreviations

2-TEA	2-Thiazoleamine
5HT	Serotonin
AC	Adenylyl cyclase
ACh	Acetylcholine
AD	Alzheimer's disease
ADHD	Attention deficit hyperactivity disorder
BBB	Blood-brain barrier
BRET	Bioluminescence resonance energy transfer
cAMP	Cyclic adenosine monophosphate
CNS	Central nervous system
CREB	cAMP-responsive element-binding protein
DA	Dopamine
DAG	Diacetyl glycine
DAO	Diamine oxidase
DiO	Diet-induced obese
EMA	European Medicines Agency
FCS	Fluorescence correlation spectroscopy
FDA	Food and Drug Administration
FRET	Förster resonance energy transfer
GABA	γ-Aminobutyric acid
GPCR	G-protein coupled receptor
GIT	Gastrointestinal tract
Gly	Glutamate
HCV	Hepatitis C virus
HDC	Histidine decarboxylase
hERG	Ether-a-go-go related gene for voltage-dependent potassium ion channel
HHV	Human herpes virus
HNMT	Histamine-*N*-methyltransferase
HTMT	Histamine-trifluoromethyl-toluidide
HR	Histamine receptors ($H_{1\text{-}4}R$)
PAINS	Pan-assay interference compounds
IP3	Inositol-1,4,5-trisphosphate
MAO B	Monoamine oxidase B
MTCP	Multi-target directed chemicals probes
MTDL	Multi-target directed ligands
NA	Norepinephrine
NO	Nitric oxide
PD	Parkinson's disease
PKA	Protein kinase A
PKC	Protein kinase C
PLC	Phospholipase C
PPI	Proton pump inhibitors

SARS	Severe acute respiratory syndrome
SHR	Spontaneously hypertensive rat
SCM	Scanning confocal microscopy
VMAT	Vesicular monoamine-transporter
wt	Wild type

1 Introduction

1.1 Chemical Probes

Chemical probes (small-molecule tools) are well-characterized substances regarding their influence on a biological target or action mechanism. Depending on the target of interest, the assay setup, and the read-out mechanisms, different chemical probes or combinations of probes can be used due to their beneficial biochemical or pharmacological properties (Stark 2020). According to the clearly defined criteria, the chemical probe should have an affinity below 100 nM, >10-fold selectivity against off-targets, potency in concentration ranges below 10 μM in cell-based assays, and no pan-assay interference compounds (PAINS) elements (Arrowsmith et al. 2015). Chemical probes are powerful research tools that can lead to verification of the proposed mechanism of action as well as the development of novel drug-like candidates. Besides, chemical probes are necessary for drug-discovery processes as they serve for assay system validation. Without a valid assay system, results are not comparable or reliable and cannot be transferred for further evaluation (Bunnage et al. 2013).

In the case of the histamine receptor subtypes, these innovative tools can be used for different purposes, as in vitro examination of receptor affinities, in vivo estimation of receptor localization, and distribution or clinical trials for drug approval. Histamine receptors belong to the group of G-protein coupled receptors (GPCRs), and therefore chemical probes are defined as ligands. A ligand quality as a chemical probe depends on various characteristics as affinity, efficacy, binding properties (covalent or non-covalent), solubility, permeability, and especially the type of assay system (Bunnage et al. 2013). A general classification of the quality of a chemical cannot be made due to high assay versatility but rather an allocation of well-characterized ligands for particular assays (Frye 2010). Despite the numerous possibilities to modify histaminergic transmission by genetic or enzymatic intervention, this review focuses on ligand-based modification only.

1.2 Physiology of Histamine

Sir Henry Dale et al. described 2-(1*H*-imidazole-4-yl)ethanamine (histamine) in 1910. This biogenic amine was firstly extracted from human tissue in 1927 (Dale and Laidlaw 1910; Best et al. 1927). Histamine is ubiquitously distributed in the body of numerous species and involved in regulating various physiological functions of smooth muscles, gastrointestinal, cardiovascular, immune system, central and peripheral neurons. As a local mediator, histamine leads to increased acid production in the stomach, triggers local vasodilation in the skin and lungs, and acts as an inflammatory mediator of the immune system. As a neurotransmitter in the CNS, histamine influences temperature regulation, the sleep-wake rhythm, and learning and memory processes (Obara et al. 2019; Panula et al. 2015).

1.2.1 Biosynthesis and Metabolism

Due to the aromatic imidazole moiety, histamine has two tautomeric structures where either the nitrogen at 1-position (N^1-H) or 3-position (N^3-H) carries the hydrogen. Several studies in gas, liquid, and solid-state indicate that N^1-H is the predominant tautomer (Ramírez et al. 2003). For a precise description of histamine and metabolites, Black and Ganellin introduced a specific nomenclature (Fig. 1) (Black and Ganellin 1974).

The aliphatic amine is referred to as N^{α} and the N^1 of the imidazole ring is described as N^{π} and N^3 as $N^{\ddot{\imath}}$. With the aliphatic and aromatic amines, histamine contains two basic centers. The N^{α} has a p*K*a value of 9.4, and the imidazole nitrogen $N^{\ddot{\imath}}$ is less basic with a p*K*a value of 5.8. Under physiological conditions (p*H*: 7.4), histamine is in the form of mono cation, protonated at the N^{α}-position.

The histamine biosynthesis is a one-step process, where L-histidine undergoes an oxidative decarboxylation, mainly catalyzed by the histidine decarboxylase (HDC) (Obara et al. 2019; Parsons and Ganellin 2006). Histamine is stored in vesicles by the vesicular monoamine-transporter VMAT-2 (Hu and Chen 2017). Various stimuli lead to a release of histamine through exocytosis. Once released, histamine does not undergo reuptake by specific transporters or channels like other neurotransmitters. Organic cation transporters (OCT-1, OCT-2, OCT-3, VMAT-1) are discussed for transport mechanisms (Slamet Soetanto et al. 2019; Ogasawara et al. 2006). Histamine is metabolized to inactive N^{τ}-methylhistamine via *N*-methyltransferase (HNMT) or diamine oxidase (DAO) to inactive imidazolyl acetic acid. N^{τ}-methylhistamine is further metabolized through monoamine oxidase B (MAO B) to N^{τ}-methylimidazoylacetic acid (Shahid et al. 2009; Akdis and Blaser 2003).

Histamine exerts its action through four receptor subtypes, histamine 1-4 receptors ($H_{1\text{-}4}R$). All receptor subtypes belong to the class A G-protein coupled receptors (GPCRs) (Shahid et al. 2009).

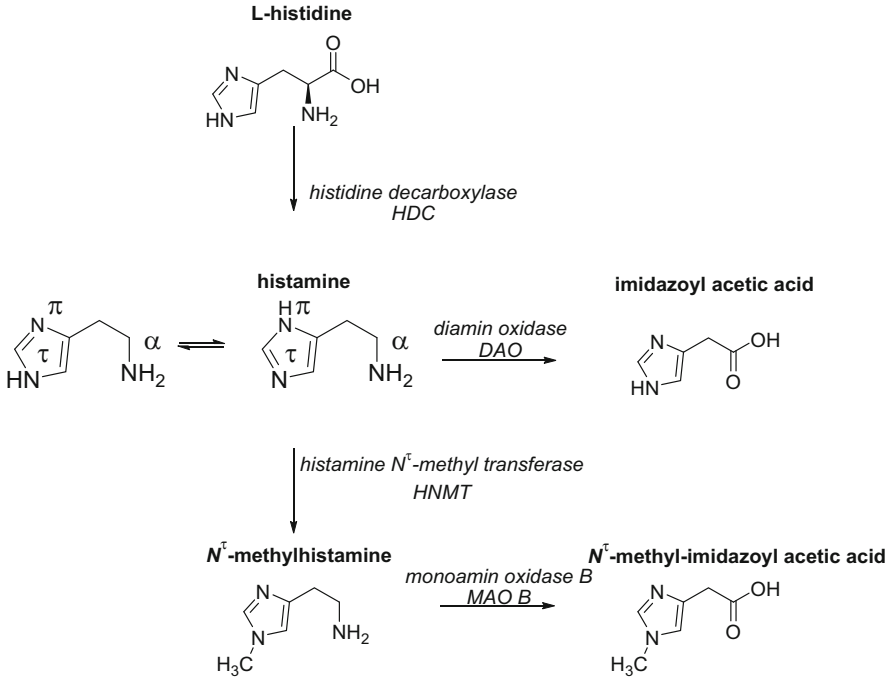

Fig. 1 Formation, tautomerism, and metabolism of histamine

1.2.2 Histamine Receptors

Histamine H_1 Receptor

The histamine H_1 receptor (H_1R) is a Gq-coupled GPCR and is expressed ubiquitously in the body. Activation on histamine H_1R stimulates phospholipase C (PLC) activation and further results in the inositol-1,4,5-trisphosphate (IP_3) and diacetyl glycine (DAG) formation, increased intracellular Ca^{2+} concentration, and activation of protein kinase C (PKC), respectively. The increased Ca^{2+} concentration leads to vasoconstriction in lung smooth muscle cells and large blood vessels. On the other hand, in the small blood vessels, dilatation occurs due to the stimulation of nitric oxide (NO) formation. These processes lead to H_1R-mediated allergic reactions, characterized by heavy breathing, skin redness, itching, and swelling of the nasal mucous membranes (Leurs et al. 2002). In addition to peripheral tissues such as the lungs, skin, cardiovascular system, and gastrointestinal tract (GIT), the receptor is also broadly distributed in the CNS (Panula et al. 2015; Shahid et al. 2009).

The H_1R with high density in different brain areas influences attention, wakefulness, sleep-wake rhythm, and cognition in the hypothalamus, the thalamus, the amygdala, and the cortex (Sadek et al. 2016a). Activation of the H_1R leads to reduced food intake (Díaz et al. 2019). Blockade of the H_1R in the CNS can lead

to significant weight gain, often observed as a side effect of antipsychotics or first generation of antiallergic H_1R antagonists (Kaar et al. 2019).

In 2011, the first X-ray crystal structure of a stabilized H_1R with the antagonist doxepin was reported (Shimamura et al. 2011).

Currently, only H_1R antagonists are used as pharmacological treatment options. The involvement of the H_1R in allergic reactions qualifies H_1R antihistamines as an essential substance class in their treatment. H_1R antagonists are divided into two generations based on their central nervous side effect profiles. In treating allergic reactions, representatives of the first generation are less often used, as they penetrate the blood-brain barrier (BBB). This further results in side effects but can be used for drug repurposing. For instance, the sedative effect is exploited in sleep disorders, and H_1R blockade in CNS leads to the use in the treatment of nausea and vomiting (Simons and Simons 2011).

The second generation of H_1R antihistamines penetrates the BBB to a smaller extent and expresses fewer sedative effects. Active second-generation metabolites and newly designed compounds with higher selectivity rates and simultaneously reduced BBB penetration can therefore be used as a safer and more potent therapeutic antiallergic alternative.

Histamine H_2 Receptor

The histamine H_2 receptor (H_2R) is a Gs-coupled receptor. Signalling through Gs leads to activation of the adenylate cyclase (AC), an increased cyclic adenosine monophosphate (cAMP) concentration, protein kinase A activation (PKA) followed by downstream processes (e.g., cAMP-responsive element-binding protein (CREB) cascade) (Obara et al. 2019; Panula et al. 2015; Sadek et al. 2016a).

The influence on gastric acid production is the well-described process associated with H_2R. H_2R stimulation leads to an increase in cAMP concentration and further in gastric acid secretion. Pharmacologically, gastric secretion is suppressed by H_2R antagonists, which enabled the treatment of reflux diseases, dyspepsia, and gastric ulcers. However, this class was replaced by more efficient and safer proton pump inhibitors (PPIs) as omeprazole, esomeprazole, and pantoprazole (Panula et al. 2015).

H_2R is expressed in the CNS and seems to influence cognitive processes as well as the sleep-wake rhythm (Haas et al. 2008), even though the exact relationship is still unclear. Besides, the H_2R is involved in the glucose metabolism of food-intake control (Schneider et al. 2014). Also, the H_2R is expressed in numerous types of cells, including smooth muscle cells, endothelial and epithelial cells, chondrocytes, neutrophils, eosinophils, monocytes, macrophages, dendritic cells, T and B cells (Jutel et al. 2009).

Histamine H_3 Receptor

The Histamine H_3 receptor (H_3R) is the third histamine receptor subtype, discovered toward the end of the last century and first cloned in 1999 (Panula et al. 2015). The H_3R is predominantly expressed in the CNS; however, expression in the periphery has also been detected and is increasingly being discussed on therapeutic aspects (Sander et al. 2008). High receptor density is found in the CNS in the cortex, the striatum, the nucleus accumbens, the amygdala, the palladium, and the hippocampus (Sadek et al. 2016a; Sander et al. 2008; Nieto-Alamilla et al. 2016). The H_3R is predominantly expressed as a presynaptic receptor and acts as an autoreceptor controlling histamine synthesis and release via a negative feedback mechanism. As a heteroreceptor on non-histaminergic synapses, the H_3R also regulates the release of dopamine (DA), acetylcholine (ACh), norepinephrine (NA), glutamate (Glu), serotonin (5-HT), γ-aminobutyric acid (GABA), substance P and other neurotransmitters (Sander et al. 2008; Nieto-Alamilla et al. 2016). Postsynaptic H_3Rs were recently described (Ghamari et al. 2019). Presynaptic H_3R is a $G_{i/o}$ coupled GPCR (Sadek et al. 2016a; Nieto-Alamilla et al. 2016). The $G_{\alpha i}$ subunit inhibits the AC and thereby leads to a decreased cAMP concentration and reduced PKA activity. The reduced cAMP level reduces CREB-mediated gene transcription and lowers protein biosynthesis. HDC activity and histamine synthesis are reduced (Sander et al. 2008; Nieto-Alamilla et al. 2016; Obara et al. 2019).

The $G_{\beta\gamma}$ subunit binds to and inhibits the P- and Q-type of voltage-gated calcium channels. The inhibition of these channels reduces Ca^{2+} influx into the presynapse and diminishes the depolarization. Activation of GIRK potassium channels leads to increased K^+ efflux and hyperpolarization. Decreased Ca^{2+} influx and increased K^+ efflux result in decreased neurotransmitter exocytosis (Nieto-Alamilla et al. 2016).

H_3R antagonists suppress the H_3R mediated inhibitory effect and consequently increase the histamine biosynthesis and release. Through H_3R heteroreceptors, other neurotransmitters and mediators like ACh, NA, DA, 5-HT, Glu, and GABA are modulated (Nieto-Alamilla et al. 2016; Ghamari et al. 2019; Obara et al. 2019).

As GPCR with high constitutive activity, antagonists of the H_3R can act as neutral antagonists or inverse agonists. Inverse agonists can decrease the basal activity of the receptor by stabilizing the inactive state (Latorraca et al. 2017; Berg and Clarke 2018).

An influence of H_3R inverse agonists on the waking state, through increased histamine concentrations and postsynaptic H1R activation, led to the development of pitolisant, the first and so far, only H_3R inverse agonist approved by the FDA (Food and Drug Administration) and the EMA (European Medicines Agency). Pitolisant (Wakix®) is indicated for treating narcolepsy with and without cataplexy (Ghamari et al. 2019; Romigi et al. 2018) and excessive daytime sleepiness in obstructive sleep apnea (Ozawade®) (Wang et al. 2021).

As described, the H_3R mediates several neurotransmitter levels and targets diseases associated with CNS disorders. These include schizophrenia, epilepsy, attention deficit hyperactivity disorder (ADHD), autism, altered sleep-wake rhythms, Prader-Willi syndrome, and neurodegenerative diseases such as

Alzheimer's disease and Parkinson's disease (Ghamari et al. 2019; Reiner et al. 2020; Sadek and Stark 2016).

Histamine H_4 Receptor

The histamine H_4 receptor (H_4R) is a G_i-coupled GPCR identified in 2000 as the last histamine receptor subtype. H_4Rs are structurally related to H_3Rs. However, the expression patterns of the receptors differ significantly, so that the H_4R shows increased peripheral expression (Panula et al. 2015; Schneider and Seifert 2016).

The H_4R is mainly expressed on hematopoietic cells, especially on eosinophilic granulocytes (Buckland et al. 2003). Besides, mast cells, natural killer cells as well as monocytes also express the H_4R (Gschwandtner et al. 2013; Capelo et al. 2016). Strong evidence suggested H_4R association with various functional inflammatory responses mediated by histamine, including chemotaxis and cell recruitment upregulation of adhesion molecule expression, and modulation of cytokine and chemokine release (Neumann et al. 2014; Hartwig et al. 2015). Preclinical and emerging clinical data supporting the association of H_4R with pruritus and atopic skin inflammation (Werfel et al. 2016). For example, H_4R agonists have been described to upregulate the T_H2-associated and itch-inducing cytokine IL-31 (Gutzmer et al. 2009).

Other diseases discussed in context with the therapeutic H_4R blockade include bronchial asthma and non-specific inflammatory reactions (Panula et al. 2015). Up to date, no H_4R ligand has been approved by drug agencies.

2 In Vitro Assays

In the case of GPCRs, in vitro assays display test systems to evaluate ligand affinity, functionality, and efficacy in a cell-based system. In receptor binding assay, a ligand can be characterized by its affinity at the receptor of interest (on-target) and its selectivity over undesired receptors (off-targets).

Intrinsic activity and efficacy are determined in functional assays, and at least two types of chemical probes can be beneficial for in vitro assays conduction. Unlabelled or reference ligands display a large group of compounds. With well-defined receptor profiles and high on-target affinities, they are needed to standardize the results. In a validated assay, the reference ligand is the critical element to compare and transfer to other results and studies. Reference ligands are used in both binding studies and functional assays.

The second group is labelled ligands, subdivided into radioactive (radio-) and fluorescent-labelled ligands. These labelled ligands are used as measurable/countable values to determine affinities and receptor distributions. The labelled ligand should show high selectivity, especially for receptor distribution assays and assays with native or primary cells (containing more than one receptor).

2.1 Reference Ligands

Histamine is the endogenic ligand of histamine receptors (HR) that shows moderate to high affinities at all four receptor subtypes. Interestingly, the affinity at H_1R and H_2R is more than 1,000-fold lower compared to those at H_3R and H_4R. As an endogenous ligand, histamine displays unfavorable properties as a chemical probe. At H_1R and H_2R, the affinity is not high enough, and for H_3R and H_4R, the missing selectivity is an essential drawback. However, histamine is used as a reference in functional assays to calculate the intrinsic activity and efficacy compared to the endogenous ligand.

2.1.1 Histamine H_1 Receptor

Agonists

First attempts to synthesize H_1R agonists lead to imidazole-containing analogues as 2-thiazoylhistamine (2-TEA). Like histamine, the affinity and selectivity of 2-TEA are not suitable for use as a chemical probe. With an intrinsic activity of 27% compared to histamine, 2-TEA is a partial agonist with moderate efficacy (EC_{50}: 440 nM) (Seifert et al. 2003). The introduction of a 3,3-diphenylpropyl group in position 2 of imidazole leads to a substance class named histaprodifens. Histaprodifens display partial agonists with an intrinsic activity of up to 77% (methylhistaprodifen) and 84% (suprahistaprodifen) (Elz et al. 2000). H_1R affinity of methylhistaprodifen is moderate. However, it is still mostly used as an H_1R agonist, with an acceptable selectivity over H_2R (~100×) and H_3R (~10×). Although histaprodifen and derivatives were synthesized already 20 years ago (Elz et al. 2000), meanwhile no remarkable progress was made in the field of H_1R agonists. A full agonist with high and selective H_1R affinity remained to be designed.

Antagonists

In contrast to agonists, a variety of H_1R antagonists with high affinities were synthetized. A well-characterized representative of H_1R antagonists is mepyramine. It belongs to the group of first-generation antihistamines, as it penetrates the blood-brain barrier. Due to its high affinity (pK_i: 8.4) and selectivity over other HRs (~100×), mepyramine was used to synthesize radio and fluorescent-labelled analogues/derivatives (Rose et al. 2012). Doxepin, another first-generation antihistamine, showed increased affinity at H_1R with a pK_i value of 9.5. While the other HR's selectivity is high, some off-target interaction to serotonin receptors (5-HTR) is reported (Mansbach 2008; Zhou et al. 2018). With a resolved doxepin-H_1R crystal structure, doxepin is a promising chemical probe for clarifying structure activation

relationships (Shimamura et al. 2011). Side effects of first-generation antihistamines due to central H_1R blockade resulted in developing second generation of H_1R antagonists. Well-described members of this group are the frequently used antiallergic drugs cetirizine and loratadine (Sadek and Stark 2016). Their highest benefit of chemical probes is the transferability from in vitro data to clinical outcomes. Desloratadine, an active metabolite of loratadine, was introduced to the market and showed increased affinity to H_1R. Desloratadine as well as loratadine show affinities at 5-HT_2R, in a comparable range like doxepin (pK_i: 6.5–7.5). This should be kept in mind when analyzing the results of native cell experiments. Cetirizine points out the stereoselective binding at H_1R; the racemic Cetirizine shows a pK_i value of 8.2, where the pure *R*-enantiomer called levocetirizine has an affinity of pK_i: 8.5, and the *S*-enantiomer has a lower affinity with a pK_i value of 7.1 (Hair and Scot 2006). Due to its higher affinity, levocetirizine was brought to the market as a pure enantiomeric drug (Fig. 2). Histamine H_1R ligands are summarized in Table 1.

2.1.2 Histamine H_2 Receptor

Agonists

Compared with H_1R agonists, the search for selective H_2R agonists started with the imidazole ring variation. As a result, 4-methylhistamine was synthesized and is nowadays used as an H_4R agonist with around 100-fold selectivity at H_4R over H_2R. The replacement of imidazole with aminothiazole leads to amthamine (Fig. 3). Even though amthamine acts as a full agonist at H_2R, it shows a low affinity (pK_i: 5.2) and no selectivity to other receptor subtypes. The partial agonist impromidine (intrinsic activity 79%) has an improved affinity to H_2R (pK_i:7.2) and selectivity over H_1R (~100×) but retains its lack of selectivity over H_3R (pK_i:7.2) and H_4R (pK_i:7.9). According to the stereoselectivity of histamine receptors, sopromidine shows interesting behavior. Comparable to cetirizine at the H_1R, the two stereoisomers of sopromidine show different binding affinities to H_2R. Moreover, *R*-sopromidine acts as a full agonist, where *S*-sopromidine acts as a moderate antagonist (Elz et al. 1989). It is noteworthy that guanidine-based impromidine derivatives show affinity at H_3R and H_4R and were used as lead structures for selective H_3R and H_4R ligands (Venable and Thurmond 2012; Gbahou et al. 2012).

UR KAT471 is a potent partial agonist of the H_2R and shows more than 100-fold selectivity over the other HR subtypes. The selectivity, high affinity (pK_i of 7.2), and potency superior to histamine make UR KAT471 the favored H_2R agonist for in vitro assays (Kraus et al. 2009).

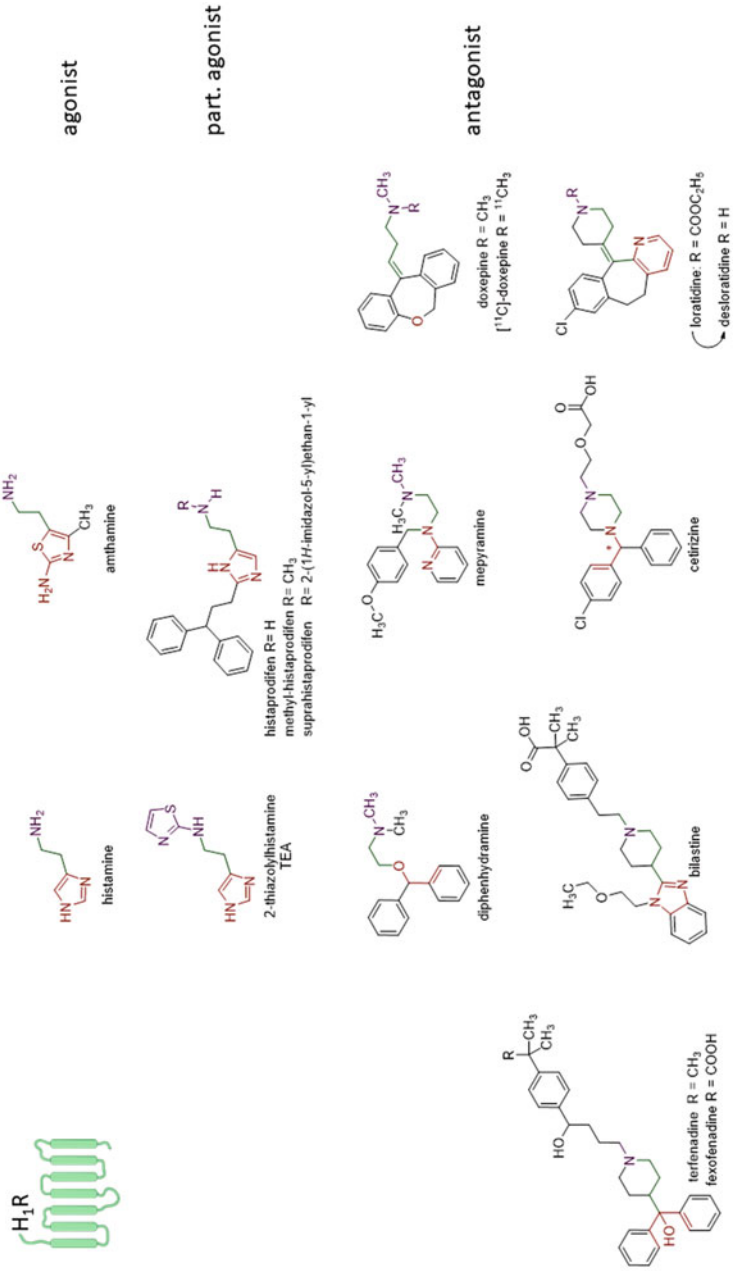

Fig. 2 Selected histamine H_1 receptor agonists, partial antagonists, and antagonists

Table 1 Histamine H_1R ligands and their in vitro data

Compound	H_1R pK_i	H_2R pK_i	H_3R pK_i	H_4R pK_i	Other receptors p$K_i > 6$	Additional information
Histamine (Panula et al. 2015)	4.2	4.3	8.0	7.8		Endogenous ligand
2-Thiazolylhistamine (2-TEA) (Seifert et al. 2003)	5.3			<5.0		Partial agonist (26%)
Methylhistaprodifen (Seifert et al. 2003; Elz et al. 2000)	6.4	4.6[a]	5.8[a]			Partial agonist (77%)
Mepyramine (Wagner et al. 2011)	8.4	4.6	<4	< 4	6.2 (DAT)[b] 6.6 (5-HT_{2A})[b] 6.2 (5-HT_{2c})[b] 7.6 (SERT)[b] 6.2 (σ_1)[b]	Antagonist
[^{3}H]Mepyramine (Wagner et al. 2011)	8.4	4.6[c]	<4[c]	<4[c]		PET-ligand
Mepyramin-BODIPY (Rose et al. 2012)	8.9					Fluorescent-labelled
Diphenhydramine (Panula et al. 2015; Mansbach 2008)	7.9	5.8	<5.5	<5.5	6.6 (5-HT_{2A}) 6.2 (5-HT_{2B})[b] 6.3 (5-HT_{2C})[b] 7.1 (M_1)[b] 6.4 (M_2)[b] 6.7 (M_3)[b] 7.3 (M_4)[b] 6.9 (M_5)[b]	Antagonist, 1.Gen
Doxepin (Shimamura et al. 2011; Mansbach 2008; Zhou et al. 2018)	9.5		<5.5		7.6 (5-HT_{2B}) 8.0 (5-HT_{2A})	Antagonist, 1.Gen
Levocetirizine (Panula et al. 2015)	8.15	<6	<6	<4		Antagonist, 2.Gen
Loratadine (Panula et al. 2015; Auerbach 2021)	7.2		< 5	< 5	6.8 (5-HT_{2B})	Antagonist, 2.Gen
Desloratadine (Hu and Chen 2017; Auerbach 2021)	8.4	6.5	< 5	< 5	7.5 (5-HT_2) 7.7 (M_2) 7.0 (M_3)[b] 7.3 (M_4)[b] 6.7 (M_5)[b] 8.0 (5-HT_{2C})[b]	Antagonist 2.Gen

(continued)

Table 1 (continued)

Compound	H_1R pK_i	H_2R pK_i	H_3R pK_i	H_4R pK_i	Other receptors p$K_i > 6$	Additional information
					7.6 (5-HT_{2B})[b] 7.8 (5-HT_{2C})[b] 6.0 (5-HT_6)[b] 6.9 (SERT)[b] 6.0 (α_{2B})[b] 6.2 (D_3)[b]	
Bilastine (Corcóstegui et al. 2005)	8.2	<6.0	<6.0	<6.0		Antagonist, 2.Gen
[^{11}C]Doxepin	9.5[c]		<5.5[c]			PET-ligand
[^{11}C]Mepyramine	8.4[c]	4.6[c]	<4[c]	<4[c]		PET-ligand

Gen generation
[a]Guinea pig
[b]Data obtained from "DrugMatrix in vitro pharmacology data" database (Auerbach 2021)
[c]Values of unlabelled ligand

Antagonists

Like H_2R agonists, antagonists also show difficulties in terms of selectivity at H_4R. Aminopotentidine is a member of cyano substituted guanidine with a nanomolar affinity (pK_i: 7.4) and shows selectivity over H_1R and H_4R, but not over H_3R. With an iodide substitution at the 3-position of the benzamide group, the affinity to H_2R was significantly improved, resulting in pK_i value of 9.4. In this way, a radiolabelled ligand is designed when using the radioactive isotope ^{125}I (half-live of 59.5 days). Replacement of the substituted guanidino group by square acid resulted in more selective H_2R antagonists. Tritium-labelled UR DE-257 shows high selectivity over the other HRs (>100×) and can be used as a radiolabelled ligand for H_2R. Commercially available H_2R antagonists also contain guanidino group (cimetidine-cyanoguanidine group, ranitidine-nitroethendiamine, and famotidine-sulfamoylamidine). Imidazole in cimetidine was replaced with bioisosteric heterocycles such as furan (ranitidine) and aminothiazole (famotidine) to prevent interaction with CYP450 enzyme complex. These drugs show affinities in a low micromolar to the nanomolar concentration range and are characterized as inverse agonists with an intrinsic activity of −88% (cimetidine and famotidine) and −100% (ranitidine). As in the case of approved H_1R antagonists, the benefit of these drugs is not defined by their high affinity or selectivity but rather on the transferability of in vitro data to clinical outcomes (Fig. 3). Histamine H_2R ligands are summarized in Table 2.

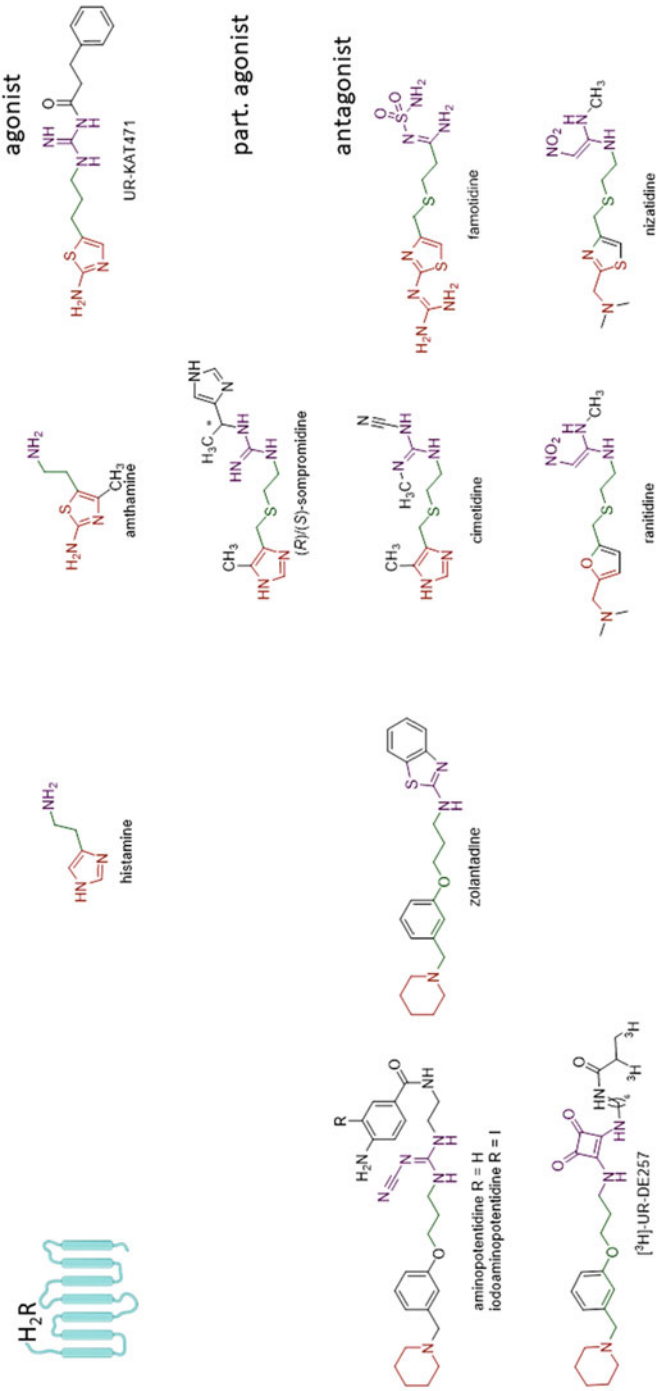

Fig. 3 Selected histamine H_2 receptor agonists, partial agonists and antagonists

Table 2 Histamine H_2R ligands and their in vitro data

Compound	H_1R pK_i	H_2R pK_i	H_3R pK_i	H_4R pK_i	Other receptors p$K_i > 6$	Additional information
Histamine (Panula et al. 2015)	4.2	4.3	8.0	7.8		Endogenous ligand
Amthamine (Igel et al. 2010; Xie et al. 2006)	4.8	5.2		5.3		Agonist (101%)
Impromidine (Igel et al. 2010; Xie et al. 2006; Jablonowski et al. 2003)	5.2	7.2	7.2	7.9		Agonist (79%)
UR KAT471 (Kraus et al. 2009)	<5.0	7.2	<5.0	<5.0		Agonist (82%)
Aminopotentidine (Panula et al. 2015)	<5.5	7.4	7.1	<5.5		Antagonist
[^{125}I]Iodoamino-potentidine (Bosma et al. 2017; Hirschfeld et al. 1992)	5.6	9.4				Antagonist, radioligand
[^{3}H]UR-DE257 (Baumeister et al. 2015)	<5.5	7.6	<5.5	<5.5		Antagonist, radioligand
UR-KAT514 (Grätz et al. 2020) (BODIPY-labelled)		8.4				Antagonist, fluorescent-labelled
Cimetidine (Panula et al. 2015; Alewijnse et al. 1998; Lim et al. 2005)	4.75	5.84	4.69	5.03		Inverse-agonist (−88%)
Famotidine (Panula et al. 2015; Alewijnse et al. 1998; Lim et al. 2005; Angeli et al. 2018)	<5	7.8		<5.0	< 7.0 ($CA_{1,4,9,13,14}$) 7.0 (CA_6) 8.5(CA_7) 7.3(CA_{12})	Inverse-agonist (−88%)
Ranitidine (Panula et al. 2015; Alewijnse et al. 1998; Lim et al. 2005)	4.47	6.67	4.89	<5.0		Inverse-agonist (−100%)

CA carbonic anhydrase

2.1.3 Histamine H_3-Receptor

Agonists

With the discovery of H_3Rs in 1983, numerous H_1R and H_2R ligands were examined due to their affinity to the H_3R. Histamine shows high selectivity for H_3R over H_1R and H_2R, and it was used as a lead structure. Slight variations with methylation of histamine resulted in *R*-α-methylhistamine and N^{α}-methylhistamine (Fig. 4). Both ligands are potent agonists with an intrinsic activity of 100% compared to that of histamine. Like histamine, they show selectivity over H_1R and H_2R of about 100-fold, with a high affinity at H_3R. The following discovery of H_4R disclosed

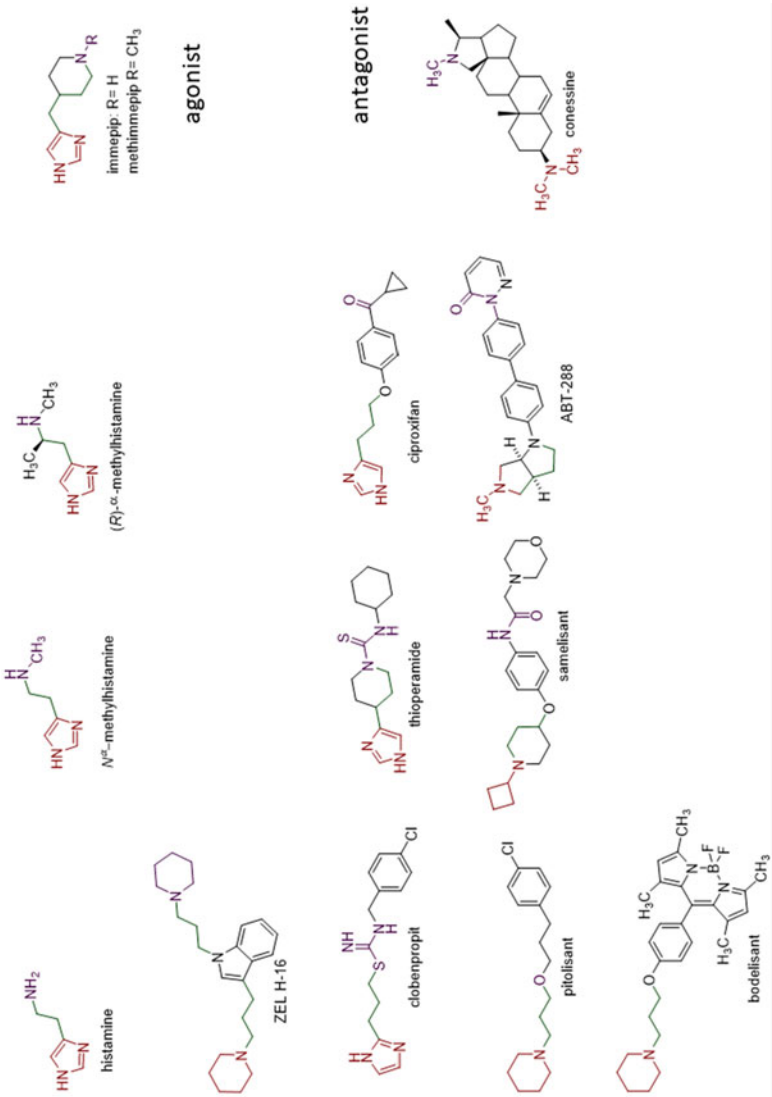

Fig. 4 Selected histamine H_3 receptor agonists and antagonists/inverse agonists

for both ligands full H_4R agonism (100%) with moderate affinities with pK_i values of 6.5 and 6.6, respectively. N^{α}-Methylhistamine is a commercially available [^{3}H] labelled ligand for H_3R binding studies (Panula et al. 2015) leading to good comparability of assay results.

Replacement of the aliphatic alkyl amine by piperidine led to immepip and methylimmepip. Methylimmepip shows high affinity (pK_i value of 9.0) and selectivity to H_3R over H_1R, H_2R, and H_4R (>1,000×) and intrinsic activity of 90%. These features make methylimmepip a valuable chemical probe for in vitro studies. Proxyfan shows an interesting efficacy behavior depending on the assay characteristics; as a selective ligand, it can act as an inverse agonist, antagonist, or (partial) agonist (Sadek and Stark 2016). The only non-imidazole-based agonist described so far, ZEL-H16 (1,3-bis(3-(piperidin-1-yl)propyl)-1*H*-indole), possesses high affinity (pK_i value of 8.6) and selectivity to H_3R. It is a partial agonist with an intrinsic activity of 60% compared with that of histamine (Shi et al. 2012).

Antagonists

The early antagonists of the H_3R, like thioperamide, showed high selectivity over H_1R and H_2R but not over H_4R. (hH_3R pK_i: 7.1, hH_4R pK_i: 7.3). Thioperamide was found to be an inverse agonist at H_3R and an agonist at H_4R. Examining antagonistic properties enabled classifying rat (r), mouse (m), or human (h) H_3R isoforms. Thioperamide shows a slightly reduced affinity to hH_3R compared to rH_3R. Ciproxifan, an H_3R selective inverse agonist, has a 100-fold higher affinity to the rH_3R whereas clobenpropit shows nearly no difference. Clobenpropit shows sub-nanomolar affinity to hH_3R, and even though it has a good selectivity over H_4R, it served as a blueprint for the design and synthesis of H_4R ligands due to its likewise nanomolar affinity to the H_4R (Tiligada and Ennis 2020). Iodoproxyfan also shows a sub-nanomolar affinity and is used as a radiolabelled ([^{125}I]) ligand, with high selectivity over H_1R and H_2R and slight selectivity over H_4R.

Despite the general assumption that the imidazole moiety causes serious interactions due to the CYP450 enzyme class interactions, these compounds have been dosed in low concentration in numerous in vitro and in vivo experiments without any signs of interaction. The receptor selectivity might be a more important aspect concerning off-target affinities (e.g., H_4R). The imidazole replacement by cyclic tertiary amines like *N*-substituted piperidine or pyrrolidine resulted in the next generation of H_3R antagonists. With pitolisant, the affinity to hH_3R as well as the selectivity over H_4R was increased. The FDA and EMA approved the inverse agonist pitolisant for the treatment of narcolepsy with or without cataplexy (Wang et al. 2021). Based on the H_3R pharmacophore of pitolisant, the potent fluorescent-labelled ligand bodilisant was synthesized. As the only H_3R inverse agonist/antagonist on the drug market, pitolisant displays excellent properties as a reference substance. Several other inverse agonists/antagonists were synthesized and investigated in preclinical and clinical assays. ABT-288 showed high affinity and selectivity. In several assays, procognitive effects of ABT-288 were observed. Similar

affinity to human and rat H_3R (pK_i: hH_3R: 8.7, rH3R: 8.1) can simplify the analysis of different assay systems (Esbenshade et al. 2012). The steroidal alkaloid conessine shows structural differences to other H_3R antagonists with comparable receptor affinity (pK_i: 8.5) to pitolisant and ABT-288. The selectivity over other HRs (>1,000×) and other GPCR is high. It is a highly suitable second reference substance to reveal pattern associated unspecific binding in a new assay. An affinity of conessine to adrenergic α_2 receptors should be noted concerning GPCR based assays (Zhao et al. 2008) (Fig. 4). Histamine H_3R ligands are summarized in Table 3.

2.1.4 Histamine H_4 Receptor

Agonists

Several former reported HR ligands showed affinity to H_4R after it was discovered. The high similarity of H_3R and H_4R binding pockets led to some obstacles in finding selective ligands. Histamine and the radiolabelled [^{3}H]histamine show similar high affinities to the receptors. However, it is commonly used as a radiolabelled ligand in H_4R displacement studies. 4-Methylhistamine, synthesized initially as an H_2R ligand, shows a high affinity to H_4R with at least 100-fold selectivity over other HRs (Fig. 5). It is characterized as a full agonist with 100% intrinsic activity compared to histamine. Some other H_2R agonists display H_4R affinities like impromidine (pK_i value of 7.6) and *R*-/*S*-sompromidine (pK_i values of *S*: 5.5, *R*: 6.1). The partial agonist ST-1006 is described as the most potent H_4R agonist (pEC_{50} value of 8.95*)* and shows high affinity and good selectivity over H_1R, H_2R, and H_3R (Sander et al. 2009).

Antagonists

As mentioned, the H_3R inverse agonist iodoproxifan was used as a lead structure for H_4R antagonists. Other H_3R antagonist iodophenpropit showed nearly the same affinity at H_4R (pK_i value of 7.9) and H_3R (pK_i value of 8.1) with agonistic and inverse agonistic activities, respectively, displaying functional selectivity. The ^{125}I-labelled form of iodophenpropit is frequently used as a radiolabelled ligand for H_4R in displacement studies. One of the best-studied and most used reference ligands in the field of H_4R is JNJ-7777120. It possesses a high affinity (pK_i value of 8.4) and selectivity (other HRs > 100×). Although the affinity of humans and rats (pK_i value of 8.6) varies little, the intrinsic activity is different. In humans, JNJ-7777120 acts as an inverse agonist, whereas in rats, it acts as a partial agonist (51% activity compared to that of histamine (100%)).

Moreover, the behavior in humans can change because JNJ-7777120 shows agonist activity in β-arrestin recruitment pathways. It is therefore called a biased agonist (Rosethorne and Charlton 2011). As a chemical probe for in vitro characterizations, this might be acceptable or welcomed to study species differences,

Table 3 Histamine H_3R ligands and their in vitro data

Compound	H_1R pK_i	H_2R pK_i	H_3R pK_i	H_4R pK_i	Other receptors p$K_i > 6$	Additional information
Histamine (Panula et al. 2015)	4.2	4.3	8.0	7.8		Endogenous ligand
Methimepip (Panula et al. 2015; Kitbunnadaj et al. 2005)	<5.5	<5.5	9.0	5.7		Agonist (90%)
[^{3}H]N^{α}-Methylhistamine (Panula et al. 2015; Igel et al. 2010)	<5.5	<5.5	8.4	6.5		Agonist (100%) H_4R agonist (100%) Radiolabelled
(*R*)-α-Methylhistamine (Panula et al. 2015; Lim et al. 2005)	<5.5	<5.5	8.2	6.6		Agonist (100%) H_4R agonist (100%)
Clobenpropit (Fox et al. 2003) Human	5.6	<5.5	9.4	7.4		Antagonist
Rat			9.7			H_4R antagonist
Thioperamid (Zhao et al. 2008; Fox et al. 2003)					6.9 (α_{2A})	Antagonist
Human	<5.5	<5.5	7.1	7.3	6.5 (α_{2C})	
Rat			8.4			H_4R inverse agonist
Ciproxifan (Zhao et al. 2008; Fox et al. 2003)					7.4 (α_{2A})	Antagonist
Human	<5.5	<5.5	7.2	5.7	7.2 (α_{2C})	
Rat			9.3			Antagonist
Iodoproxyfan (Lim et al. 2005)			9.2	7.9		Antagonist
[^{125}I]Iodoproxyfan (Fox et al. 2003; Istyastono et al. 2011)	5.6	<5.5	8.6	7.8		Antagonist Radiolabelled
Pitolisant (Sadek et al. 2014; Szczepańska et al. 2018) Human (Walter et al. 2010; Riddy et al. 2019)	5.9	<5.5	8.6	<5.5	σ_1: 8.0 (agonist)	Inverse agonist
Rat (Łazewska et al. 2009)			7.8			Inverse agonist
ABT-288 (Esbenshade et al. 2012)						Antagonist
Human	<5.0	<5.0	8.7	<5.0		
Rat			8.1			Antagonist
Conessine (Zhao et al. 2008)	<505	<5.0	8.5	<5.0	α_2C_4: 8.0 α_2C_4: 6.2	Antagonist

(continued)

Table 3 (continued)

Compound	H_1R pK_i	H_2R pK_i	H_3R pK_i	H_4R pK_i	Other receptors p$K_i > 6$	Additional information
Samelisant						Inverse agonist
(SUVN-G3031) (Nirogi et al. 2021)						
Human	< 6.0	< 6.0	8.7	< 6.0		
Rat			9.8			Inverse agonist
Bodilisant (Tomasch et al. 2013)	5.8		8.4	<5.5		Antagonist Fluorescent labelled
S-[^{11}C]Methylthioperamid (Funke et al. 2013)			7.3			PET-ligand
[^{11}C]GSK189254 (Funke et al. 2013)			9.6			PET-ligand

reducing the portability between in vitro and in vivo data. Nevertheless, JNJ-7777120 is a well-characterized tool for understanding the physiology of the H_4R (Thurmond et al. 2004).

One of the well-described H_4R ligands is adriforant (also known as PF-03893787 or ZPL-389), an inverse agonist at hH_4R. Compared to JNJ-777712, adriforant shows species variations in affinity (pK_i values hH_4R: 8.2, hH_4R: 8.2, and dog H_4R: 5.8) and intrinsic activity as it acts as antagonists in dogs and partial agonist in rats. This species-dependent behavior arises from low amino acid sequence identities. The human H_4R only shows around 70% amino acid homology to rats and dogs. Rats again possess only 64% of homology to dogs and even only 84% to mice (Jiang et al. 2008). Also, histamine as an endogenic ligand shows these species differences with pK_i values of 7.8 and 6.9 at human and rat H_4R isoforms, respectively (Fig. 5). Histamine H_4R ligands are summarized in Table 4.

Multi-target Histamine Ligands

The involvement of histamine and its receptors in complex immunological and neurophysiological processes, and the beneficial effects of HR ligands in clinical trials, led to their participation in polyvalent ligands (Łażewska and Kieć-Kononowicz 2018). Such multi-target directed ligands (MTDLs) address more than one target and simultaneously impact pathophysiological conditions (Proschak et al. 2019). The design of such a ligand must differ from "dirty drugs." The latter interacts with several off-targets, while the former has a designed polypharmacological profile. Rationally designed multi-target directed chemicals probes (MTCPs) need to be extremely well described regarding their targets and potential off-targets. MTCPs are necessary tools to study target combinations in vivo and as references in vitro. Compared to single target chemical probes, affinities

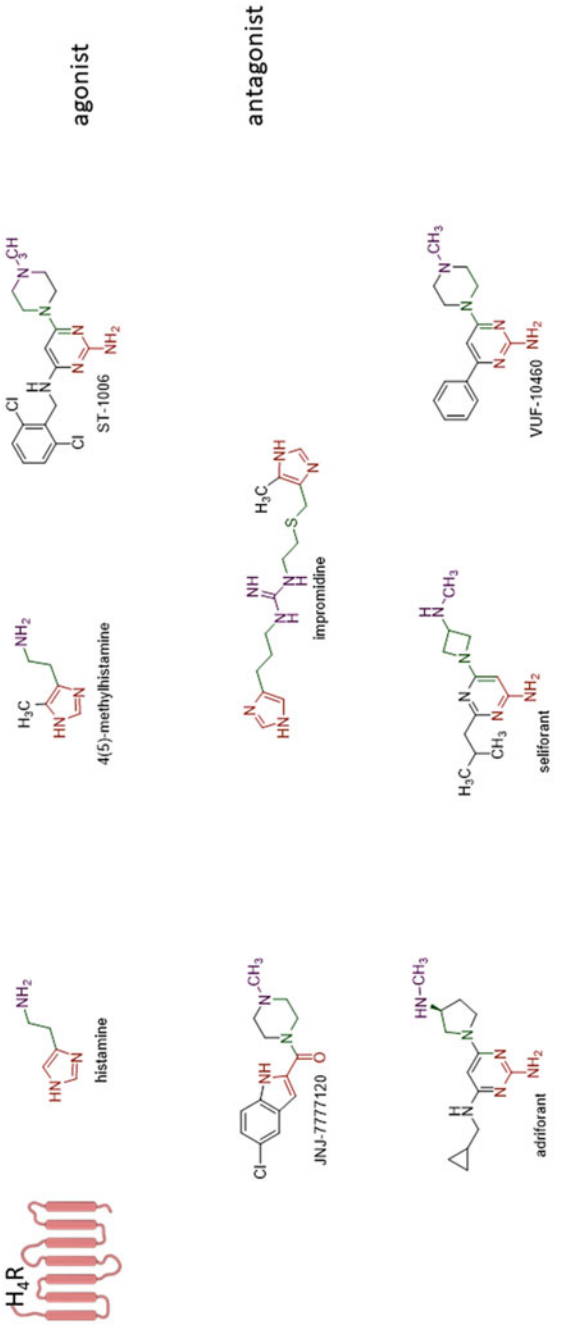

Fig. 5 Selected histamine H_4 receptor agonists and antagonists/inverse agonists

Table 4 Histamine H_4R ligands and their in vitro data

Compound	H_1R pK_i	H_2R pK_i	H_3R pK_i	H_4R pK_i	Other receptors $pK_i > 6$	Additional information
Histamine (Panula et al. 2015)	4.2	4.3	8.0	7.8		Endogenous ligand
4-Methyl-histamine (Lim et al. 2005; Gschwandtner et al. 2013)	<5.5	<5.5	<5.5	7.3		Agonist (100%)
ST-1006 (Sander et al. 2009)	6.0	6.0	6.3	7.9		Agonist (28%)
JNJ-7777120 (Liu et al. 2008) Human (Altenbach et al. 2008; Rosethorne and Charlton 2011)	6.0	<5.5	5.7	8.4	H_4R partial agonist (β-Arrestin)	Inverse agonist (−39%)
Rat (Altenbach et al. 2008; Schnell et al. 2011)				8.6		Agonist (51%)
[^{125}I]Iodophenpropit (Lim et al. 2005)			8.2	7.9		Antagonist Radiolabelled
Adriforant (Andaloussi et al. 2013) Human			6.7	8.2		Inverse agonist
Rat (Tichenor et al. 2015)				7.9		Partial agonist
Dog (Tichenor et al. 2015)				5.8		Antagonist
UR-DEBa242 (Bartole et al. 2020)	<5.5	<5.5	8.6	7.9		Antagonist H_3R antagonist Fluorescent labelled
[^{11}C]JNJN-77771220 (Funke et al. 2013)				8.4		PET-ligand

should be more balanced, e.g., when combining receptor ligands with enzyme inhibitors (Khanfar et al. 2016).

The dual-acting ligand GSK-1004723 possesses a high affinity to its on targets H_1R (pK_i value of 10.2) and H_3R (pK_i value of 10.6) with an excellent selectivity to H_2R and H_4R (>10,000×). Therefore, it is an MTDL prototype because the on-target affinity arises from design and not due to coincidence. As an MTCP it is useful for validating assay systems with more than one HR subtype.

As dopamine plays an important role in neurodegenerative and neurological diseases, tools combining HR and dopamine receptor (DR) affinity are needed. As recently shown, the MTDL ST-2223 combines high H_3R(pK_i value of 8.3), D_2R (pK_i value of 7.7), and D_3R (pK_i value of 8.7) affinities with excellent selectivity to off-targets like H_1R (pK_i value of 7.0), D_1R (pK_i value of 6.3), and D_5R (pK_i value <5.5) (Eissa et al. 2021). ST-2223 and ST-718 showed beneficial effects in autism-like behavior. ST-718 possesses higher selectivity over HRs but lower selectivity and affinity at DRs (Venkatachalam et al. 2021). Contilisant combines H_3R affinity (pK_i value of 8.0) and sigma 1 receptor affinity (pK_i value of 7.2) with

inhibition of enzymes like MAO A (IC_{50} value of 145 nM), MAO B (IC_{50} value of 87 nM), acetylcholine esterase (IC_{50} value of 530 nM), and butyrylcholinesterase (IC_{50} value of 359). It can be used to demonstrate the possibility to combine a different kind of target interactions (enzyme inhibition and receptor binding) (Bautista-Aguilera et al. 2017, 2018). A multi-target ligand recently developed by Cao et al. which has high affinities to dopaminergic, serotoninergic, and histaminergic receptors, showed promising effects and good pharmacokinetics in rats (Cao et al. 2018).

2.2 Labelled Ligands

In contrast to reference ligands, labelled ligands are normally not used to compare or transfer data from one to the other experiment. Labelled ligands are necessary for the read-out mechanisms in different assays. As the data obtained is based on the read-out, labelled ligands significantly influence the entire assay. Displacement data can vary between a labelled agonist or antagonist due to their different affinity and binding characteristics. For each histamine receptor subtype, only a few labelled ligands were used in in vitro assays. Radiolabelled ligands contain one or more radioactive atoms, normally beta- or gamma-emitters. PET ligands are labelled with positron emitters. The chemical structure from unlabelled to labelled ligands is only minimally changed, and binding properties are retained. Fluorescent-labelled ligand requires a fluorophore, which is usually not incorporated in the primary ligand. Fluorophores like BODIPY were coupled to an HR pharmacophore. The development of fluorescent chemical probes is a rising research field, as newer techniques like BRET extensively take benefit of these ligands and many laboratories have regulative problems with the use of radioactive materials.

2.2.1 Radiolabelled Ligands

In vitro ligand, characteristics are usually not changed by inserting a radioactive atom in their chemical scaffold. As seen with histamine, binding affinities of radiolabelled ligands at all HR subtypes remain. [^{3}H]histamine shows high affinities at H_3R and H_4R and selectivities over H_1R and H_2R. For H_4R, it is a commonly used radiolabelled ligand in displacement studies. As an agonist, the binding mode can differ from the antagonist, and displacement assays can produce different results when a radiolabelled antagonist like [^{125}I]iodophenpropit is used.

Also [^{125}I]iodophenpropit shows high affinities at H_3R and H_4R and is often used as a radiolabelled ligand. For radioligand displacement assays, both radioligands resulted in similar pK_i values for several H_4R ligands, including, e.g., histamine (pK_i ([^{125}I]iodophenpropit) value of 7.6 $\pm$ 0.2, pK_i ([^{3}H]histamine) value of 7.8 $\pm$ 0.1).

For H_1R, histamine's affinity is unfavorable and thus the selective agonist mepyramine was radiolabelled, resulting in [^{3}H]mepyramine. Due to its high affinity

and selectivity, it is the mostly used and one of the most suitable radiolabelled ligand for H_1R. It was used for early H_1R visualization studies to determine H_1R distribution in human brains (Martinez-Mir et al. 1990).

Iodination at 3-position of the selective H_2R antagonist (over H_1R and H_4R) aminopotentidine (pK_i value of 7.4) resulted in [^{125}I]iodoaminopotentidine with 100-fold improved the affinity to H_2R (pK_i value of 9.4). The antagonist [^{3}H]UR-DE257 displays the highest selectivity of the other HRs (>100×) and is, therefore, better useable for visualization of receptor expression studies.

In various radiolabelled H_3R ligands, [^{3}H]N^{α}-methylhistamine is the most used one. The affinity and selectivity are comparable, respectively, better than [^{125}I]-iodoproxyfan and [^{125}I]-iodophenpropit (Van Der Goot and Timmerman 2000).

As an H_4R selective ligand, tritium labelled [^{3}H]JNJ-7777120 was synthesized, showing the mentioned advantages and disadvantages of JNJ-777120. In H_4R displacement assays [^{3}H]-histamine is still the most used radiolabelled ligand, despite its low selectivity to H_3R.

2.2.2 Fluorescent-Labelled Receptor Ligands

In the beginning, fluorescent ligands were mainly used as the histological stains for identifying the biogenic amines and their receptors in tissues (McGrath et al. 1996). In 2000 the GPCR was described and visualized using green fluorescent protein-tagged (GFP-tagged) GPCRs taking advantage of molecular biology techniques (Kallal and Benovic 2000). This was a starting point for the drug discovery to study the exact ligand–receptor interaction. For many years, radioligand assays have been conducted to examine receptor–ligand interactions, both in vitro and in vivo (Ma and Zemmel 2002). Fluorescent ligands gain even more attention with the development of new cutting-edge techniques, as Förster resonance energy transfer (FRET), fluorescence correlation spectroscopy (FCS), scanning confocal microscopy (SCM), and bioluminescence resonance energy transfer (BRET). These techniques enabled the development of pharmacological assays on live or fixed tissues, with small amounts of tissue, or on single cells, and the results can be obtained immediately (McGrath et al. 1996; Michalet et al. 2006; Al-Damluji et al. 1997).

Those methods are now widespread in pharmacology. The fluorescent-labelled ligands are used to study subcellular localization, internalization or dimerization, and ligand binding kinetics of receptors. Moreover, using fluorescent ligands enables the measurement of specific and non-specific receptor binding (Mackenzie et al. 2000).

As described in radiolabelled ligands, it is essential to define the fluorescent ligand affinity and selectivity. Other than radiolabelling, fluorescently labelled ligands show some variations of their chemical structure compared to the lead ligand. The fluorophore needs to be attached to the ligand to retain the affinity and selectivity of the starting compound. It is usually obtained by the fluorophore placement far away from the part of the pharmacophore. A variety of fluorophores (e.g., DANSyl, Sanger's reagent, AlexaFluor®, or Bodipy®) could be linked to

ligands with or without a linker, providing possibilities for the design and development of novel, potent, selective, and affine fluorescent ligands (Kuder and Kieć-Kononowicz 2014).

The coupling of BODIPY, boroazaindacene derivative, to mepyramine created a structure called mepyramine-BODIPY 630–650 (short: mepyramine-BY630). The remarkable properties of mepyramine were maintained, whereby the affinity was even slightly increased (Rose et al. 2012).

The beneficial squaric acid moiety of [^{3}H]UR-DE 257 was used to synthesize a fluorescent-labelled ligand and resulted in UR-KAT 51. As mentioned above, also this ligand is coupled to a Bodipy® fluorophore. It is characterized as an antagonist with a pK_i of 8.4 and used in nanoBRET assays, displaying great properties (Grätz et al. 2020).

Bodilisant, a piperidine-based hH3R ligand coupled with the BODIPY pharmacophore, shows ideal chemical probe characteristics as a fluorescent-labelled ligand for H_3R. The high affinity (pK_i value of 8.4) and high selectivity over other HRs was combined with an excellent quantum yield of Φ: 0.92. Bodilisant demonstrated its benefits in H_3R labelling studies and a fluorescence-polarization experiment to determine receptor residence times of different H_3R ligands (Tomasch et al. 2013; Reiner and Stark 2019).

A fluorescent-labelled ligand with high affinity and selectivity for the H_4R has not yet been reported. The fluorescent-labelled ligand UR-DEBa 242 (PY-5 labelled) (Bartole et al. 2020) shows a high affinity to H_4R (pK_i value of 7.9) and selectivity over H_1R and H_2R, but not over H_3R (pK_i value of 8.6).

3 In Vivo Assays

In contrast to the precise criteria defined for the in vitro chemical probes examined (affinity below 100 nM, selectivity over 10-fold against related target), the demands for in vivo chemical probes are far more complex as the processes in living cells and tissues are much more complicated. Therefore, defining general criteria for chemical probes in animal studies is more challenging (Stark 2020) and depends on the experimental design.

It is not simple to set cut-off values in vivo and demonstrate on-target effects in animal models due to enormous differences in species. As chemical probes are not necessarily equal with preclinical candidates, it should be first determined if a chemical probe contains appropriate pharmacodynamic and pharmacokinetic characteristics to be examined in animal models. Moreover, their safety profile and tolerance should be examined in the species of interest. Different chemical probes are chosen based on animal models and targeted disease, and here will be explained which of them reached in vivo preclinical and clinical trials as well as highlight the best chemical probe for each receptor in different behavioral models.

3.1 *Histamine H_1 Receptor*

Histamine H_1R was the first described receptor subtype and is the most abundant histamine receptor. Hence, it has been cloned over three decades ago, it has been extensively studied, and various in vivo studies that target H_1R have been conducted.

3.1.1 Agonists

H_1R agonists have not raised specific interest in the scientific community, and so far, only a few compounds with agonistic properties have been described. As all histamine receptor ligands, H_1R agonists were first developed with a slight modification of histamine as imidazole derivatives. However, bioisosteric replacement of carbon with sulfur led to H_1R agonist 2-(thiazol-2-yl)ethanamine (2-TEA, 2). Substitution with bulky substituent leads to 2-(3-(trifluoromethyl)phenyl) histamine. These compounds are well tolerated and are used to mimic histamine effects in in vivo studies (Malmberg-Aiello et al. 2000). Further modification leads to histamine-trifluoromethyl-toluidine (HTMT) development, a more potent agonist than histamine, that also shows affinity to H_2R and can be considered as a dual targeting ligand. HTMT is used in vivo studies to examine the role of histamine in immunomodulation, as shown in rabit (Tripathi et al. 2010) and mice (Lapilla et al. 2011). Later developed H_1R agonists were histaprodifen and suprahistaprodifen, which still need to be examined in vivo. Suprahistaprodifen showed partial agonism at H_4R, while histaprodifen derivatives acted as an inverse H_4R agonist (Deml et al. 2009). It is assumed that suprahistaprodifen binds at the H_1R in two different orientations. As the H_1R showed itself a high level of agonist independent constitutive binding (Jongejan et al. 2005), the application of H_1R in vivo models makes it even more complex. Therefore, there is an urge for further development and examination of H_1R agonists.

3.1.2 Antagonists

In contrast to moderately described H_1R agonists, H_1R antagonists are a well-known class that reached blockbuster drugs' status. These ligands are widely used for therapy of allergies, nausea, conjunctivitis, or rhinitis. In general, H_1R antagonists can be divided into two classes.

First-generation includes H_1R antagonists like doxepin, mepyramine, doxepin, and diphenhydramine. They are lipophilic enough to cross the BBB and therefore express sedative effects. Since they show affinity to other receptor subtypes (e.g., cholinergic), they cannot be considered optimal chemical probes. First-generation H_1R antagonists showed reinforcing effects in monkeys as they increased rates of suppressed and non-suppressed behavior (Bergman and Spealman 1986). However,

from a pharmacological point of view, this disadvantage was used in PET tracers' design. As first-generation H_1R antagonist cross the BBB and penetrate CNS, they are often radiolabelled and used to estimate receptor distribution and localization. [^{11}C]mepyramine, and [^{3}H]doxepin are commonly used as a PET tracer for H_1R, both in humans and animals (e.g., guinea pig). However, [^{11}C]doxepin has lower metabolic degradation and provides higher contrast images, and therefore is the most commonly used PET ligand to measure histamine occupancy in the brain in humans (Yanai et al. 1992a, b, 1995; Ishiwata et al. 2007; Zhou et al. 2002; Sato et al. 2013).

The later developed, the second generation of antihistaminic are more polar structures and are in the form of zwitterion at physiological p*H*. Therefore, they cross the BBB only up to a small extent, expressing none to light sedating effects.

Some of these H_1R antagonists represent suitable substrates for P glycoprotein and thus are rapidly effluxed without crossing the BBB to a remarkable extent under physiological conditions. Second-generation antihistaminic includes commercially available drugs: loratadine, desloratadine, cetirizine, levocetirizine terfenadine, fexofenadine, which are well-examined in preclinical studies. Stereoselective separation has led to some enantiomers leading to a higher affinity at H_1R (e.g., levocetirizine). Some of the second-generation antihistamine compounds (e.g., terfenadine) display severe cardiac side effects, prolonging QT interval that can lead to Torsades de Pointes arrhythmias due to hERG channel interaction (human Ether-a-go-go Related Gene for voltage-dependent potassium ion channel). In line with these results, terfenadine has been withdrawn from the market. Structural modifications of terfenadine lead to designing fexofenadine that does not prolong QT interval and has an improved safety profile. Fexofenadine does not cross the BBB and does not occupy histamine H_1R in the central cortex, in contrast to cetirizine (Sato et al. 2013; Tashiro et al. 2002). For review on this therapeutic and safety aspects, see Cataldi et al. Holgate et al. (Cataldi et al. 2019; Holgate et al. 2003).

Bilastine possesses highly suitable characteristics due to a peripheral acting H_1R antagonist for in vivo studies. The high selectivity over several other receptors (including dopamine, serotonin, histamine, adrenaline, acetylcholine, and others) is ideal for studying H_1R antagonism or blocking the peripheral H_1R. Doxepin and mepyramine can be used as brain penetrating H_1R antagonists, although doxepin showed low bioavailability.

Both first- and second-generation antagonists are drugs used daily. However, their use should be controlled in children, as they have seizure-inducing potential (Miyata et al. 2011).

They are used as antiallergic drugs, and both antihistaminic first- and second-generation reduced 48r80-induced scratching behavior in mice (Sugimoto et al. 1998). On the other hand, histamine, H_1R antagonists hydroxyzine and cetirizine, did not prevent the development of acute skin lesions in Maltese-beagle dogs (Bäumer et al. 2011).

To address the problems caused by antihistamines of the first and second generation and improve the treatment of histamine-related diseases, dual ligands have been designed. Dual H_1R/H_4R antagonism presents a promising therapeutic option

Table 5 Pharmacokinetic data of H_1R ligands

Compound	$t_{1/2}$	Bioavailability	BBB penetration	Additional information
Mepyramine (Simons and Simons 2011; Yanai et al. 2011)	8 h		High	
Doxepin (Simons and Simons 2011; Yanai et al. 2011)	17 h	27%	High	High first pass effect (70%) Active metabolite: desmethyldoxepin
Loratadine (Zhang et al. 2003; Jáuregui et al. 2016)	8.4	High	Low	CYP3A4 and CYP2D6 metabolization High first pass effect → desloratadine
Desloratadine (Zhang et al. 2003; Jáuregui et al. 2016)	14	High	Low	Active metabolite of loratadine
Bilastine (Corcóstegui et al. 2005; Jáuregui et al. 2016)	14.5 h	60%	No penetration	No metabolization No CYP interaction PGP substrate

Human data

for allergy treatment due to the involvement of both receptor subtypes in allergy pathology, as shown in the acute murine asthma model in mice. Independent administration of H_4R selective antagonist, JNJ-7777120, and H_1R antagonist, mepyramine resulted in low to moderate eosinophilia effects. However, when they were administered in combination, statistically significant synergic effects were observed, indicating that dual MTDLs can be a new route in developing potent antiallergic reagents (Deml et al. 2009).

Other dual ligands developed are H_1R/H_3R GSK-1004723 and GSK-835726, both currently in clinical trials for allergic rhinitis (Daley-Yates et al. 2012). GSK-1004723 has a high affinity to both receptor subtypes with a long duration of action, potency similar to azelastine. It does not penetrate CNS and therefore does not express sedative effects (Slack et al. 2011). Besides the pharmacodynamic, the pharmacokinetic is important for in vivo measurements (Table 5).

3.2 Histamine H_2 Receptor

3.2.1 Agonists

H_2R agonists do not have therapeutic use. However, due to lack of selectivity, they often served as blueprints for the design and synthesis of H_3R and H_4R ligands.

Amthamine and dimaprit were firstly developed by slight modification and bioisosteric replacement in the imidazole ring. Impromidine, developed initially as vasodilators, is used as a chemical probe. It is guanidine derivatives and showed

affinity to H_3R. Stereoselectivity of H_2R ligand plays an important role. It has been demonstrated that the *R*-enantiomer of sopromidine is a potent H_2R agonist, whereas the *S*-enantiomer is the moderate antagonist (Elz et al. 1989). Therefore, sopromidine can be considered a useful chemical probe to gain better insight into functional selectivity and signalling pathways. *N*-acetylation of guanidino group leads to the synthesis of UR-AK381, UR-AK480, UR-BIT82. They are full agonists that showed 4,000 times the potency of histamine at recombinant human and guinea pig H_2R but still display affinity to other receptor subtypes.

3.2.2 Antagonists

As well as in the case of H_1R, H_2R antagonists are far more common and used than H_2R agonists. H_2R antagonists, such as cimetidine, ranitidine, nizatidine, famotidine, and zolantadine, reached the status of blockbuster drugs and were used in the therapy of gastric ulcers, dyspepsia, or GERD (Taha et al. 1996; Lauritsen et al. 1990). Nowadays, they are replaced with more efficient PPI. Burimamide was the first selective H_2R antagonist with moderate efficacy (Wyllie et al. 1972). Cimetidine was the first imidazole-containing H_2R antagonist that reached a blockbuster status, discovered by Sir James Black. It is a potent CYP3A4 inhibitor and can induce drug–drug interaction. To address this problem, new non-imidazole derivatives have been developed. Even though well studied, they are currently subjecting to repurposing. Zolantadine can cross the BBB and has over 30-fold selectivity to H_2R over other receptor subtypes. It is equally potent in rats and guinea pigs. It was examined in rats for possible wake-promoting effects but showed no significant impact (Monti et al. 1990). It has been shown that zolantadine does not alter the in vivo histamine metabolism in the brain of male Sprague-Dawley albino rats (Hough et al. 1988). On the other hand, it has been confirmed that zolantidine reduced in force morphine-induced antinociception in rhesus monkeys, speculating that nociceptive morphine effects are associated with H_2R agonism (Lindsay et al. 1990). The only developed PET H_2R tracer is [^{11}C]nizatidine. However, due to its poor BBB permeability, it failed in brain imaging studies. Pharmacokinetic properties of selected H_2R ligands are summarized in Table 6.

3.2.3 COVID Effects

In line with the current COVID-19 situation, histamine receptor antagonists are investigated on repurposing strategies to prevent coronavirus infection (Ishola et al. 2021). Azelastine, an H_1R antagonist, expressed a particular effect on dendritic and T cells interaction in vitro (Schumacher et al. 2014). This antihistaminic agent together with hydroxyzine and diphenhydramine expressed antiviral activity against SARS-CoV-2 in vitro and is currently studied as a potential tool for fighting COVID-19 (Reznikov et al. 2021). Famotidine and cimetidine have higher affinities to H_2R, where famotidine show specificity for HCV (59.5%) and HHV (49.5%)

Table 6 Pharmacokinetic data of H_2R ligands

Compound	$t_{1/2}$ (h)	Bioavailability (%)	BBB penetration	Additional information
Cimetidine (Lin 1991)	2–3	65	Low	IC_{50} in vitro and in vivo are comparable
Ranitidine (Lin 1991)	3–4	50	Low	IC_{50} in vitro and in vivo are comparable
Famotidine (Lin 1991)	2–3	45	Low	IC_{50} in vitro and in vivo are comparable

Human data

(Ishola et al. 2021). Besides, it has been confirmed that patients treated with famotidine had decreased risk of developing severe SARS-CoV-2 symptoms (Freedberg et al. 2020). One plausible explanation is that famotidine binds a papain-like protease encoded by the SARS-CoV-2 genome. This protease is necessary for the entry of SARS-CoV-2 into the cell. Even though reported results were contradictory, reporting both beneficial and no significant effects of histamine and histamine H_2R antagonist are extensively investigated as potential targets for fighting COVID 19 (Ennis and Tiligada 2021). Famotidine is currently in phase III of Multi-site Adaptive Trials for COVID 19, where the effect on the standard of care is being compared with the same therapy with high intravenous doses of famotidine (NCT04370262). These repurposing studies may not lead to a novel therapeutic indication for these compounds but may offer possibilities for the design of new ligands from a different lead structure.

3.3 Histamine H_3 Receptor

In contrast to H_1R and H_2R, histamine is nowadays extensively investigated due to novel therapeutic options. The involvement of H_3R in various neurological disorders as a sleep-wake disorder, AD, epilepsy, schizophrenia, and cognitive impairments makes this receptor subtype an interesting target for the design and synthesis of chemical probes and potential drug-like candidates.

3.3.1 Agonists

As with the other two receptor subtypes, slight modifications were made on the histamine core to develop imidazole-based derivatives. Therefore, *R*-α-methylhistamine that expresses high affinity to H_3R was developed. Although it is not selective and shows affinity to other receptor subtypes, it has still been used for labelling in behavioral studies, for examining histamine function in the brain and its connection with other neurotransmitters (Sadek and Stark 2016). For instance, it was confirmed that *R*-α-methylhistamine induces water drinking in rodents

(Clapham and Kilpatrick 1994; Ligneau et al. 1998; Faghih et al. 2002). Alternatively, this ligand provoked dose-dependent inhibition of noradrenaline release in Male albino or the Wistar rats (Di Carlo et al. 2000). The latter effect was abolished when thioperamide, an H_3R antagonist, was administered. [^{3}H]*R*-α-methylhistamine is fast metabolized in humans with a half-life time ($t_{1/2}$) of 1.6 h. Like histamine, it is methylated by the HNMT, and thereby loses agonist properties at the H_3R (Rouleau et al. 2000). To increase the half-life time the prodrug BP2-94 was invented. BP2-94 is non-enzymatically transformed to [^{3}H]*R*-α-methylhistamine. Half-life time of BP2-94 shows a biphasic behavior with $t_{1/2}(1)$: 1 h and $t_{1/2}(2)$: >24 h (Rouleau et al. 1997). Orally administered neither [^{3}H]*R*-α-MeHA nor BP2-94 reached the CNS (Rouleau et al. 2000). Later developed imetit and imepip have higher potency but are not selective, also showing high affinities at H_4R. Imetit inhibited the binding of [^{3}H]*R*-α-MeHA in the rat brain (Garbarg et al. 1992), while imepip decreased histamine release in rats (Jansen et al. 1998).

Methimmepip is a methylated derivative imepip derivative with increased selectivity, over 200-fold selectivity to H_3R over H_4R, and a promising chemical probe for H_3R. Both imepip and methimmepip have gastroprotective effects, as shown in gastric lesions caused by gastric acid in rats (Coruzzi et al. 2011). Even though the reported result about histamine's role in obesity is contradictory, they are very species-dependent (Díaz et al. 2019; Lecklin et al. 1998). H_3R agonists have been extensively researched as potential targets in obesity therapy. Histamine H_3R agonist imetit significantly decreases appetite at fat mass and plasma concentration of leptin and expresses anorexigenic effects in diet-induced obese (DiO) WT mice (Yoshimoto et al. 2006).

3.3.2 Antagonists

Although the role of histamine H_3R is still not fully understood, several H_3R ligands are currently in clinical trials for ADHD, sleep-wake disorder, AD, and cognitive impairments. As well agonists, firstly developed H_3R antagonists were imidazole-based structures. Thioperamide was one of the first synthesized H_3R imidazole-containing antagonists. It is a highly potent H_3R and H_4R agonist. In vivo thioperamide positively impacts cognitive function in male spontaneously hypertensive rat pups (SHR) shown (Komater et al. 2003). Besides, thioperamide did not cause a locomotor sensation as psychostimulants (e.g., amphetamine or methylphenidate) used in ADHD therapy.

Moreover, locomotor hyperactivity induced by amphetamine was inhibited by thioperamide also in male CRH mice (Clapham and Kilpatrick 1994). Therefore, thioperamide represents a possibly safer alternative than currently available therapy, as it also does not express abuse potential. Besides, it has been shown that thioperamide increases appetite and consequently body weight in DiO WT mice when administered twice per day (Yoshimoto et al. 2006). With another imidazole-

based derivative clobenpropit, thioperamide reduced alcohol intake in alcohol-preferring rats and is currently investigated for their abuse potential (Panula 2020).

Besides these two, ciproxifan is another later developed imidazole-based derivative that showed higher affinity at H_3R and is often used as a reference in behavioral studies in rodents, mainly due to its precognitive effects, in combination with good pharmacokinetic properties. For instance, ciproxifan decreased impulsivity and increased attention in adult, male hooded Lister rats (Ligneau et al. 1998; Day et al. 2007), showed precognitive effects in APPTg2576 male mice (Bardgett et al. 2011) and enhanced performance SHR pups (Fox et al. 2002).

To address problems caused by imidazole derivatives, as drug–drug interactions and high affinity to H_4R, non-imidazole derivatives have been developed and extensively studied. These derivatives are not CYP450 substrates and therefore have a safer profile, indicating that they can be better chemical probes, which led to the design and development of preclinical and clinical H_3R candidates.

Pitolisant is the only commercially available H_3R inverse agonist/antagonist (Ligneau et al. 2007), approved by EMA in 2016 and by FDA in 2019 for treatment of narcolepsy with or without cataplexy (Schwartz 2011; Lamb and Pitolisant 2020). Its safe profile and non-abuse potential are confirmed in in vivo animal models and in human studies (Uguen et al. 2013). This led to its non-controlled drug status in the USA (Lamb and Pitolisant 2020).

It is dosed once per day and, due to its safety and low interaction potential, can be considered an ideal chemical probe in behavioral sleep-wake models (Syed 2016). Pitolisant reduced H_3R brain activity in Swiss mice and has been in clinical trials for obstructive sleep apnea, schizophrenia, ADHD, and photosensitive epilepsy (Kuhne et al. 2011). GSK-189254 is a potent H_3R antagonist that reduced narcoleptic episodes in Ox −/− mice (Guo et al. 2009). Another H_3R selective antagonist, JNJ-5207852, can be considered a chemical probe in this model. Ex vivo studies demonstrated that JNJ-5207852 had a high affinity to rat and human receptors and highly occupied H_3R (Kuhne et al. 2011). It expressed wake-promote in male Sprague-Dawley rats and mice in a time-dependent manner (Barbier et al. 2004). Also, it expresses resuscitating effects in Wistar rats suffering from hemorrhagic shock (Jochem et al. 2016), and its mechanism is yet to be explored.

Non-imidazole H_3R antagonist ABT-239 was efficient in multiple behavioral studies to improve social memory and cognitive impairment and is commonly used as a reference. ABT-239 (1.0 mg/kg) attenuated methamphetamine-induced hyperactivity in mice and gating deficits in DBA/2 mice with schizophrenia (Fox et al. 2005). It showed promising effects on cognition deficits induced by prenatal ethanol exposure in male adult rats (Varaschin et al. 2010) and CD1 mice when the histaminergic system had complete integrity (Provensi et al. 2016). Moreover, this H_3R antagonist showed neuroprotective and anticonvulsive effects in male Swiss albino mice (Bhowmik et al. 2014) and expressed attenuation tau hyper-phosphorylation (Bitner et al. 2011). In line with these results, ABT-239 can be considered an excellent chemical probe in cognitive-behavioral models. Another chemical probe that can be used in cognitive-behavioral studies is GSK-189254. This selective H_3R antagonist showed 10.000-fold selectivity for human H_3R and

has been used in animal models for attention models as it showed memory improved cognitive performance in rats (Medhurst et al. 2007). It showed antinociceptive potential, as it was confirmed that it modulates neuropathic pain in rats (McGaraughty et al. 2012) and reduced narcoleptic episodes in orexin −/− mice (Guo et al. 2009; Tiligada et al. 2009). DL77 is another new histamine H_3R antagonist that recently gained more interest for its procognitive potential and high in vitro and in vivo potency (EC_{50} 2.1 mg/kg). It showed promising results in ASD-like behaviors in VPA-exposed animals, procognitive effects by significantly ameliorating memory deficits induced by MK-801 in male Wistar rats, and anticonvulsant effects in male Wistar rats (Eissa et al. 2018; Sadek et al. 2016b).

As well as agonist role, antagonist role in obesity therapy has been studied. A-331440 developed by Abbott is a non-imidazole H_3R antagonist that reduced weight in higher doses (5 mg/kg and in 15 mg/kg) in C57BL/6 J mice, previously treated with a high-fat diet (Hancock et al. 2004). However, this compound showed some genotoxic potential in rats, and therefore analogues have been synthesized: A-417022 and A-423579. Both ligands reduced weight in mice in high dosage, and the latter caused statistically significant weight loss in weight-matched Sprague-Dawley female rats (Hancock et al. 2005). Furthermore, H_3R antagonism reduced calorie intake in higher mammalian species of pigs and rhesus monkeys, leading to several ligands that showed promising effects in preclinical studies (Lecklin et al. 1998). SCH- 497079, another H_3R antagonist, reached phase II clinical trial (NCT00642993).

JNJ-39220675 is a novel, potent, and selective H_3R antagonist. PET ligand [^{11}C] JNJ-39220675 showed the excellent BBB permeability and occupied around 90% of H_3R in the female baboon's brain (Logan et al. 2012). It reduced alcohol intake in male adult selectively bred alcohol-preferring rats (Galici et al. 2011) and male JAX®C57BL/6 mice (Nuutinen et al. 2016) and is considered a suitable chemical probe for alcohol-induced behavioral studies. ST1283 is another H_3R antagonist that showed promising effects on reducing alcohol intake in Tuck-Ordinary "TO" mice (Bahi et al. 2013).

Samelisant (SUVN-G3031), an orally available H_3R inverse agonist, shows highly promising results in several preclinical and clinical studies. It is effective for wake-promoting, precognitive, and enhancing cortical arousal in animal models. It possesses high selectivity for the H_3R, with comparable binding affinities to rH_3R and h_3R and no affinities to typical off-targets like 5-HTR or sigma receptors (Nirogi et al. 2021). Furthermore, side effects like CYP interaction, phospholipidosis, QT-time prolongation, and hERG blockade were not observed. Samelisant successfully completed phase I and is under investigation in phase II clinical trail (Nirogi et al. 2019). Thus, it is a promising drug candidate and a useful tool for examining species independent H_3R antagonism/invers agonism.

Different PET tracers have been developed to examine and better understand histamine H_3R role in the brain. [^{3}H]thioperamide, [^{3}H]5-methylthioperamide, and [^{11}C]methylthioperamide were the first PET tracers for H_3R that was used in mice. Several later developed H_3R PET tracers as [^{11}C]-UCL-1829, [^{18}F]-FUB-272, [^{18}F]-VUF-5182, or [^{18}F]-ST-889 failed due to low brain reuptake (Selivanova et al. 2012;

Table 7 Pharmacokinetic data of H_3R ligands

Compound	$t_{1/2}$	Bioavailability	BBB penetration	Additional information
R-α-Methylhistamine (Rouleau et al. 1997, 2000)	1.6 h	Low	Low	Fast metabolization by HNMT
BP 2-94 (Rouleau et al. 1997, 2000)	(1) 1 h (2) > 24 h		Low	Prodrug of *R*-α-methylhistamine
Pitolisant (Kuhne et al. 2011)	11 h	84%	High	CYP2D6 inhibition (IC_{50}: 2.6 μM) QT-time prolongation
Samelisant (Nirogi et al. 2019)	1.5 h[a]	83%[a]	High[a]	

Human data otherwise
[a]Beagle dogs

Funke et al. 2013). [^{18}F]-VUF 5000 has been developed to overcome this obstacle and evaluated in vivo but failed to penetrate the brain (Windhorst et al. 1999).

[^{18}F]Fluoroproxyfan and [^{125}I]iodoproxyfan showed very heterogeneous distribution in the brain, indicating non-specific binding (Funke et al. 2013). On the other hand, [^{125}I]iodophenpropit is commonly used as an iodinated PET tracer in vivo and ex vivo (e.g., in male Swiss mice or Wistar rats) showing over 40-fold preference to H_3R (Ligneau et al. 1994; Stark et al. 1996; Mochizuki et al. 1996; Jansen et al. 1994; Humbert-Claude et al. 2012; Morisset et al. 2000). Later developed [^{11}C]JNJ-10181457 showed sufficient uptake in the rat brain, but the confirmation on the distribution in $H_3R^{-/-}$ mice was failed as a chemical probe. As the most promising candidate for PET studies [^{11}C]GSK189254 stood out. It crosses the BBB, has high uptake, and is metabolically stable. Its binding can be fully blocked with the H_3R selective antagonist JNJ-39220675 or by ciproxifan, indicating a high level of specific binding in the baboon. It is so far the best chemical probe for visualizing these receptor subtypes and estimating receptor occupancy (Ashworth et al. 2010; Plisson et al. 2009; Rusjan et al. 2020). [^{11}C]TASP-0410457 as another promising candidate, showed high brain uptake in rats and monkeys has been developed recently (Koga et al. 2016).

Pharmacokinetic properties of the selected H_3R ligands are summarized in Table 7.

3.4 Histamine H_4 Receptor

Histamine H_4R is the most recently discovered histamine receptor, cloned almost 20 years ago. Despite some discussion on its CNS effects, it is confirmed that this receptor subtype is mainly peripherally distributed and involved in immune processes or chemotaxis. Its association with diseases like asthma, allergic rhinitis,

pruritus, or inflammation processes, in general, has been confirmed. However, the design and synthesis of highly selective histamine H_4R ligand remained to be an up-to-date challenge.

3.4.1 Agonists

Histamine H_4R ligands were developed up to a great extent as modification of other histamine receptor subtypes ligands. One of the first ligands examined on H_4R was H_3R agonist *R*-α-methylhistamine. This enantiomer shows a 17-fold higher H_4R affinity when compared to its *S*-enantiomer (distomer) and is often used in behavioral studies targeting H_4R (Saravanan et al. 2011). 4-Methylhistamine, firstly described as H_2R ligand, showed 100-fold selectivity to H_4R over other receptor subtypes. ST-1006 is an up-to-date one of the most potent H_4R agonist/partial agonist. It was shown to be a partial agonist in hH_4R in [^{35}S]-GTP-γS-binding assay, but a full, partial, and inverse agonist for hH_4R, mH_4R, and rH_4R, respectively, in luciferase binding assaies (Adami et al. 2018).

VUF-8430 showed 30-fold selectivity over H_3R rats and expressed lasting dose-dependent antinociceptive and anorexiant effect in male Swiss albino mice in doses 20–40 μg (Galeotti et al. 2013). A higher dose (40 μg) demonstrated an anxiolytic effect. VUF-8430 (10–40 μg) reduced neuropathic pain provoked by oxidative stress in male CD1 mice (Sanna et al. 2017). As for other promising derivatives, VUF-6884 has an over 300-fold affinity to H_4R, and VUF 10460 has a 50-fold selectivity to H_4R. The latter showed an ulcerogenic effect in male Wistar rats and is used in gastric models (Coruzzi et al. 2011).

3.4.2 Antagonists

JNJ-7777120 identified via high throughput screening by Johnson and Johnson represents the most frequently used H_4R ligand in behavioral studies. It showed over a 1,000-fold selectivity for the rat histamine H_4R over the histamine H_3R with comparable affinities to mouse and human isoform and very low affinities to other off target (Thurmond 2015). JNJ-7777120 reduces asthmatic symptoms in female Balb/c mice (Deml et al. 2009), improves lung function in mice, and reduces wheel reaction in dogs in concentration 1.5 μmol (Roßbach et al. 2009). A dose of 100 mg/kg reduces the macroscopic injury, increases the colonic myeloperoxidase as well as TNF-α levels (Varga et al. 2005), and expresses an inhibitory effect on vestibular neuron activity in Male Wistar rats (Desmadryl et al. 2012). It has been confirmed that JNJ-7777120 blocked histamine-induced chemotaxis in mice, expressed anti-inflammatory effects in the peritonitis model of mice, and ameliorated pruritus in mice (Thurmond et al. 2004; Dunford et al. 2007). However, results are very species-dependent, as it has been shown that JNJ-7777120 is a biased ligand. Besides signalling through $G_{\alpha i}$, this ligand activates the β-arrestin 2 signalling cascades (Rosethorne and Charlton 2011). In high concentration, JNJ-7777120 causes ERK

activation and can act as a full agonist (Seifert et al. 2011). Even though the obtained data supported antagonistic profile, the reported IC_{50} differed from another, which is characteristic of a partial agonist rather than an antagonist. Additionally, even if this compound is extensively used as a standard, it has a short half-life in vivo, further interfering with behavioral studies (Neumann et al. 2013). Therefore, special attention should be paid to different JNJ-7777120 effects in different species. JNJ-7777120 is metabolized to the *N*-des-methylpiperazine derivative, which possesses a comparable affinity to the H_4R (pK_i value of 7.6) and higher metabolic stability. The metabolization rate is fast in rodents, but slower in humans (Engelhardt et al. 2009).

JNJ 28307474 was another potent H_4R antagonist developed by Johnson and Johnson that was efficient in the preclinical arthritis model in BALB/c mice in doses of 20–50 mg/kg (Cowden et al. 2014; Buckland 2013). In the same species, it showed an anti-inflammatory effect by inhibiting T_H2-mediated inflammation (Cowden et al. 2010). Auspicious effects were shown in chronic atopic dermatitis model in female wt mice, indicating long-lasting action of this H_4R antagonist (Rossbach et al. 2016).

JNJ-10191584 showed promising profiles: a dose-dependent reduction in macroscopic damage in colitis model male Wistar rats and an inhibitory effect on vestibular neuron activity in Wistar or Long-Evans rats (Desmadryl et al. 2012). VUF-10214 and VUF-10148 also expressed anti-inflammatory properties in the carrageenan-induced paw edema model in male Wistar rats (Smits et al. 2008). Another potential antinociceptive agent is INCB-38579 that expressed at least 80-fold selectivity over other receptor subtypes and antinociceptive effects on female CD-1 mice and Sprague-Dawley rats (Shin et al. 2012). Two other potential PET tracers have been developed: [^{11}C]JNJ-77771220 that can cross the BBB and [^{11}C]VUF-1058 that exerts its action only peripherally (Funke et al. 2013).

H_4R is extensively researched, which resulted in several drug-like candidates reaching clinical trials. Adriforant has been tested in clinical phase for atopic dermatitis second improved inflammatory skin lesions and UR-633225 has been screened for seasonal allergic rhinitis (NCT01260753) (Werfel et al. 2019).

JNJ-39758979 is another selective H_4R antagonist with an over 80-fold selectivity to other receptor subtypes. In ovalbumin-sensitized mice showed a dose-dependent anti-inflammatory effect. Good pharmacokinetic profile and safety in dose range 10–1,200 mg were proved in clinical phase I for asthma (Thurmond et al. 2014). On the other hand, phase II in Japanese patients with atopic dermatitis (100 and 300 mg) was terminated and did not meet the endpoint for safety reasons, as two patients developed neutropenia (Murata et al. 2015). Unfortunately, clinical phase II in asthmatic patients also failed to meet the primary endpoint due to the lack of efficacy (Kollmeier et al. 2018).

Toreforant (JNJ-38518168) was another H_4R antagonist that entered into a clinical trial for rheumatoid arthritis, but failed in clinical phase IIb due to the lack of efficacy (Boyle et al. 2019; Thurmond et al. 2016).

SENS-111 or seliforant has a high affinity to both animal and human isoform H_4R. It was efficient against nystagmus in Long-Evans rats and Wistar rats (10 mg/

Table 8 Pharmacokinetic data of H_4R ligands

Compound	$t_{1/2}$	Bioavailability	BBB penetration	Additional information
JNJ-7777120 (Engelhardt et al. 2009)	2.3 h[a]	22%[a]		High first pass in rodents Low first pass in human Demethylation leads to active metabolite
JNJ-39758979 (Thurmond 2015; Thurmond et al. 2004)	140 h	70%	Crosses the BBB	Side effect: agranulocytosis In vivo data correlate with in vitro data Sex dependent accumulation (male < female up to 1.6×)
Adriforant (Mowbray et al. 2011)	7 h[a] 24 h[b] 30–34 h[c]	62%[a] 39%[b] 75–95%[c]		High species dependence

Human data otherwise
[a]Rat
[b]Dog
[c]Humans (calculated)

kg), whereby increasing doses (up to 20 mg/kg) resulted in the loss of effectivity. These data are consistent with data from a clinical trial (Petremann et al. 2020). SENS-111 does not have sedative effects and is well tolerated. In phase I clinical studies, it did not significantly impact nystagmus, but improved latency 10–30% and vertigo episodes duration (Venail et al. 2018). The studies are currently halted in phase II where efficacy and safety of two dose-regimens 100 and 200 mg has been examined (NCT03110458) (Dyhrfjeld-Johnsen and Attali 2019).

Pharmacokinetic properties of the selected H_4R ligands are summarized in Table 8.

4 Conclusion

Chemical probes are a potential novel tool that can provide insight into ligands affinity, selectivity, and receptor functional selectivity. Rational design of chemical probes generate ligands which will selectively target the receptor of interest, resolve signalling cascades and reveal the involvement of various proteins and second messengers. Here, selected histamine receptor ligands for all four receptor subtypes have been summarized and reviewed as chemical probes, focusing on in vitro and in vivo studies. The definition of a chemical probe in in vivo studies is more

complex, as in vivo compartments differ enormously from one another. Therefore, a chemical probe needs to be selected explicitly for unique behavioral models. Even though a chemical probe differs from the ligand, already from their design, several highly versatile chemical probes for investigating the effects of histamine receptor subtypes have been developed (Fig. 2). With the description of their advantages and disadvantages as chemical probes, the selection for biochemical and pharmacological studies should be made easier for beginners to experts in this area. Nevertheless, an additional effect of these probes (or their metabolites) on off-targets cannot be ruled out and may be considered to evaluate the experimental results.

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