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Histamine Receptors

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Dr. Iwan de Esch is an assistant professor and Prof. Rob Leurs is full professor and head of the Division of Medicinal Chemistry of the Leiden/Amsterdam Center of Drug Research (LACDR), VU University Amsterdam, The Netherlands. Since the seventies, histamine receptor research has been one of the traditional themes of the division. Molecular understanding of ligand-receptor interaction is obtained by combining pharmacology (signal transduction, proliferation), molecular biology, receptor modelling and the synthesis and identification of new ligands.

Contents

Introduction

Histamine is one of the aminergic neurotransmitters and plays an important role in the regulation of several (patho)physiological processes. In the mammalian brain histamine is synthesised in restricted populations of neurons that are located in the tuberomammillary nucleus of the posterior hypothalamus.[1] These neurons project diffusely to most cerebral areas and have been implicated in several brain functions (e.g. sleep/wakefulness, hormonal secretion, cardiovascular control, thermoregulation, food intake, and memory formation).[2] In peripheral tissues, histamine is stored in mast cells, eosinophils, basophils, enterochromaffin cells and probably also in some specific neurons. Mast cell histamine plays an important role in the pathogenesis of various allergic conditions. After mast cell degranulation, release of histamine leads to various well-known symptoms of allergic conditions in the skin and the airway system. In 1937, Bovet and Staub discovered compounds that antagonise the effect of histamine on these allergic reactions.[3] Ever since, there has been intense research devoted towards finding novel ligands with (anti-) histaminergic activity. This research field has been fuelled by the consecutive discovery of four unique histamine receptor subtypes. Every receptor subtype has a very distinct (patho)physiological role and all of them belong to the superfamily of G-protein-coupled receptors (GPCRs). The histamine H_1 and H_2 receptor subtypes have proven to be excellent drug targets. Ligands for the histamine H_3 receptor subtype are currently entering clinical studies and the recently discovered histamine H_4 receptor subtype is subject of intense preclinical research.

The Histamine H_1 Receptor

Until the seventies, histamine research focused on the role of histamine in allergic diseases. This resulted in the development of several potent 'antihistamines' (e.g. mepyramine, see figure 1), which were useful in inhibiting pronounced symptoms of allergic conditions.[4] However, the first generation of H_1 receptor antagonists that were developed for treating allergies revealed distinct side effects such as sedation. This particular physiological effect of the ligands was eliminated by structural modifications that prevent blood-brain-barrier penetration of the drugs. The first generation as well as the more recently developed antihistamines (originally termed antagonists but later reclassified as inverse agonists[5]) are clinically very successful and are widely available drugs. In 1991, the cDNA encoding a bovine H_1 receptor protein was cloned after an expression cloning strategy in *Xenopus oocytes*.[6] The deduced amino acid sequence revealed a 491 amino acid protein of 56 kDa. Using the cDNA sequence encoding the bovine H_1 receptor, the cDNA sequences and intronless genes encoding the rat,[3,7] guinea-pig,[8,9] human[10,11] and mouse[12] H_1 receptor proteins were cloned soon thereafter.

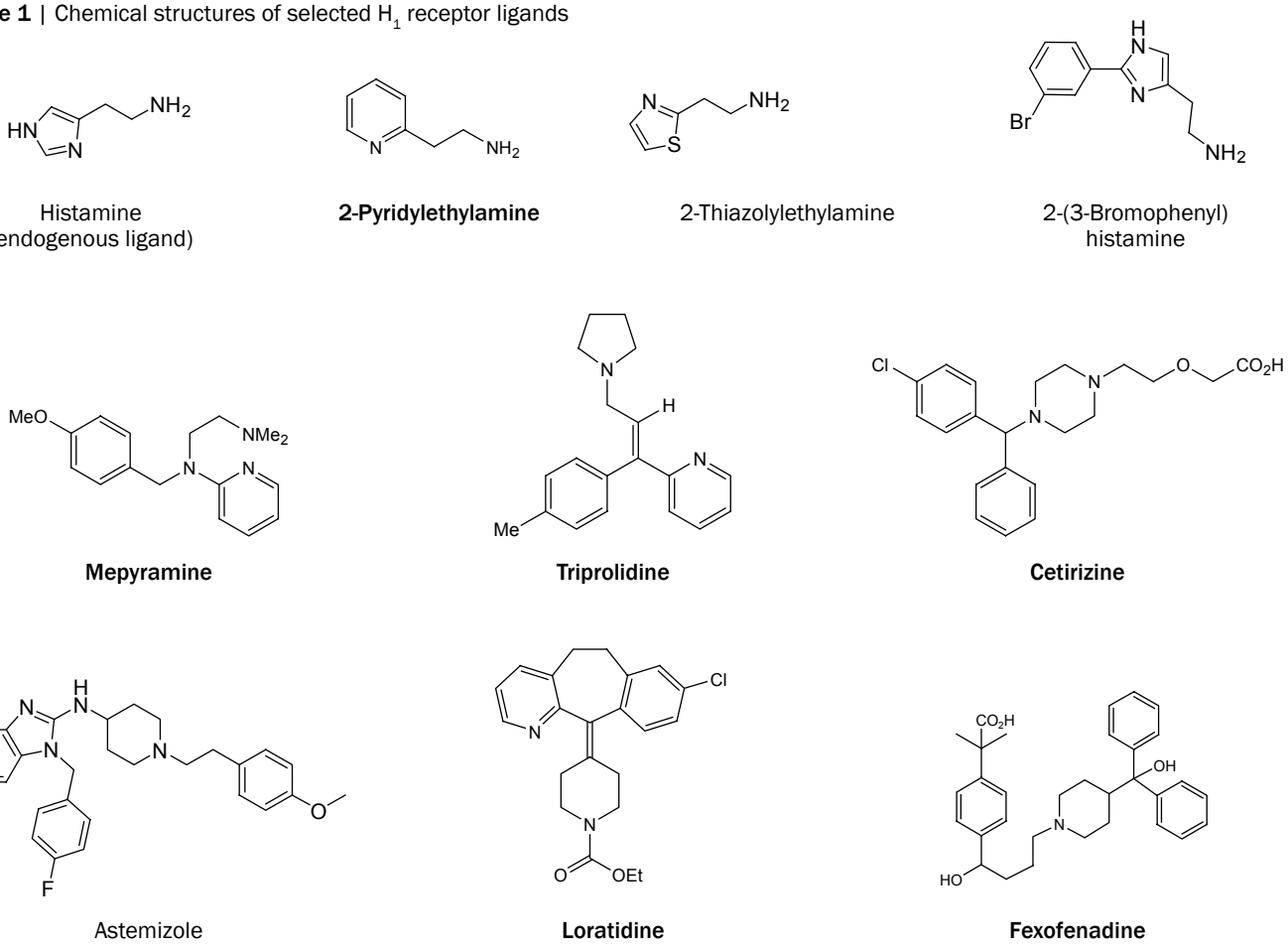
These receptor proteins are slightly different in length, highly homologous and do not show major pharmacological differences. Analysis of the 5′-flanking region of the human, rat and guinea-pig gene[7,8,10] resulted in the identification of several DNA-binding motifs, including potential glucocorticoid responsive elements. The human H_1 receptor gene resides on chromosome 3.[13] The H_1 receptor belongs to the large family of GPCRs. The receptor is associated with the phospholipase C-catalysed formation of inositol 1,4,5-triphosphate (IP_3) and 1,2-diaylglycerol (DAG). Histamine induces production of inositol phosphates in several tissues (including brain, airway, intestinal and vascular smooth muscle[24]) via $G_{\alpha q}$ protein activation.[14] In other tissues, activation of H_1 receptors can also stimulate adenylyl cyclase and formation of cAMP. Not all details of this signalling pathway are understood, for example questions remain about the G protein and the involvement of Ca^{2+}.[15] In any case, alternative signalling pathways can be mediated by the histamine H_1 receptor. Recent results indicate that the functional heterogeneity can be ligand-directed.[16]

The histamine H_1 receptor is a well established drug target and has been thoroughly studied for decades. Nevertheless, H_1 receptor research continues to flourish as many new techniques and approaches are being developed by using this receptor as an archetypal GPCR target. Most notably, in the last few years, the histamine H_1 receptor has been the subject of various molecular biology studies (e.g., large-scale overproduction[17] and GPCR binding site elucidation[18]), biophysical approaches (such as solid-state NMR[19]) and investigations towards the general activation mechanisms of GPCRs.[20,21]

Ligands for H_1 Receptors

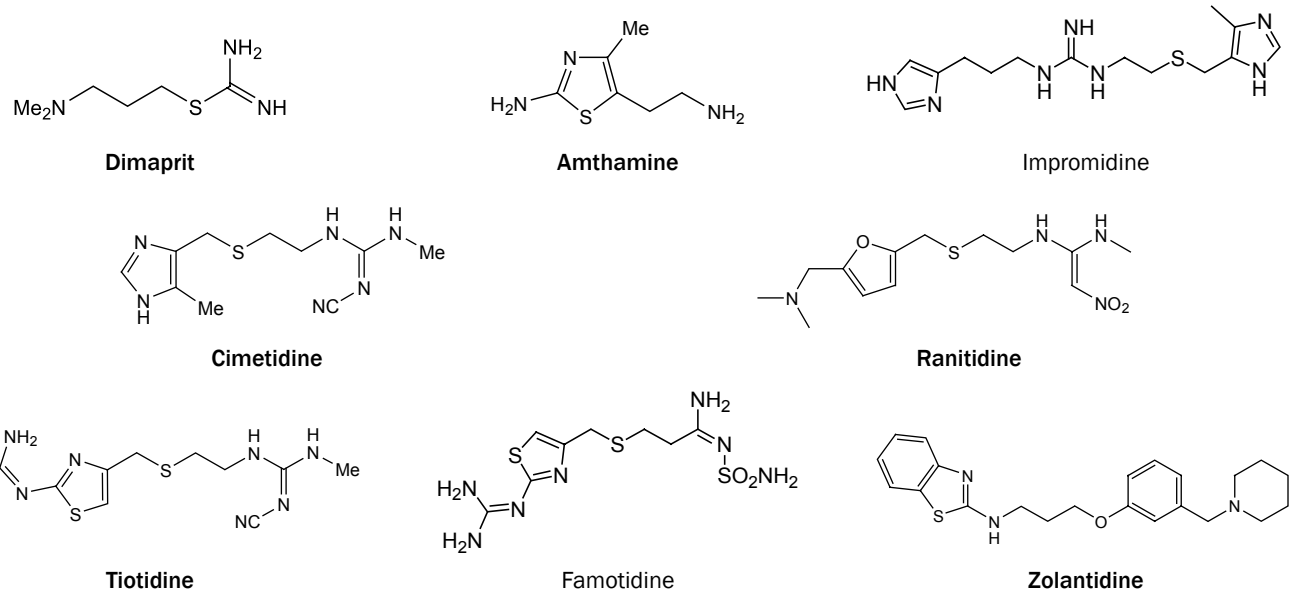
Modification of the imidazole moiety of histamine has been the most successful approach for obtaining selective H_1 agonists (figure 1). The presence of the tautomeric N^{π}-N^{τ} system of the imidazole ring is not obligatory, as reflected by the selective H_1 agonists 2-pyridylethylamine and 2-thiazolylethylamine. Substitution of the imidazole ring at the 2-position leads to relatively selective H_1 agonists. For example, 2-(*meta*-halogenated) phenylhistamines are relatively potent H_1 receptor agonists at the guinea-pig ileum;[22] however, these compounds act as partial agonists in other systems.[23] A wide array of potent and selective H_1 antagonists are available.[4] Compounds such as mepyramine (also called pyrilamine) and triprolidine are highly potent H_1 antagonists and very useful tools for pharmacological investigations. [^{3}H]-mepyramine is, for example, successfully used as an H_1 receptor radioligand.[24] These so-called classical 'antihistamines' easily penetrate the brain and are therefore also useful in *in vivo* CNS studies.[2] Elimination of the blood-brain-barrier passage by some minor structural modifications has resulted in many new, non-sedating H_1 antagonists (e.g., cetirizine, astemizole, fexofenadine and loratidine).[4]

Figure 1 | Chemical structures of selected H_1 receptor ligands



(**Bold** text denotes compounds available from Tocris at time of publication.)

Figure 2 | Chemical structures of selected H_2 receptor ligands



(**Bold** text denotes compounds available from Tocris at time of publication.)

The Histamine H_2 Receptor

The observation that the classical 'antihistamines' (i.e. H_1 receptor inverse agonists) cannot antagonise all histamine-induced effects (e.g. at the stomach and the heart), led Ash and Schild in 1966 to propose two distinct subtypes of histamine receptors: H_1 and H_2.[25] This hypothesis became generally accepted when Black *et al*[26] succeeded in the synthesis of a series of new compounds (e.g. burimamide, cimetidine), which were able to block the effects of histamine on the stomach and the heart. These H_2 receptor antagonists proved to be very useful in the therapy of gastric ulcers. Gantz *et al*[27] were the first to clone a cDNA encoding a 359 amino acid H_2 receptor. Using degenerate primers based on the known sequence similarity of various GPCRs, the H_2 receptor sequence was obtained from canine gastric parietal cDNA by PCR. Soon thereafter, the intronless genes encoding the rat,[28] human,[29] guinea pig[30] and mouse[31] H_2 receptor were cloned by means of homology screening. Identification of the promotor region of the human H_2 receptor gene revealed the existence of regulatory transcription sites and regions displaying stimulatory and inhibitory effects on gene expression monitored in a luciferase assay.[32] Studies have indicated that the human H_2 receptor gene resides on chromosome 5.[30] Interestingly, several polymorphisms have been found in the human H_2 receptor gene[33] and one of the mutations has been linked to schizophrenia.[34] The histamine H_2 receptor is coupled to the adenylate cyclase system in a variety of tissues (e.g. brain, stomach, heart, gastric mucosa, lung).[24] Moreover, cell lines transfected with the cloned H_2 receptor genes showed an H_2 receptor-mediated increase of cAMP.[35,36,37] In addition, alternative signalling pathways for the H_2 receptor have been identified. In differentiated HL-60 cells and CHO or HEPA cells transfected with the H_2 receptor cDNA, an H_2 receptor-mediated increase of the intracellular Ca^{2+} concentration and/or IP_3 levels was observed.[38,39] Moreover, in CHO cells expressing the rat H_2 receptor, activation of the H_2 receptor resulted in an inhibition of the release of arachidonic acid induced by either constitutive purinergic receptors or a Ca^{2+}-ionophore,[36] as well as an increase in cAMP. These new signal transduction pathways are both regulated via unknown, cAMP-independent pathways.

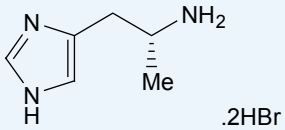
Ligands for H_2 Receptors

The first selective H_2 receptor agonist was dimaprit (figure 2). This compound is almost as active as histamine at the H_2 receptor but hardly displays any H_1 receptor activity.[40] Much later, it was shown that dimaprit is also a moderate H_3 receptor antagonist[41] and a moderate H_4 receptor agonist.[42] Amthamine can be considered a rigid dimaprit analogue.[43] This compound combines a high H_2 receptor selectivity with a potency which is slightly higher compared to histamine, both *in vitro* and *in vivo*.[44] An H_2 receptor agonist that is more potent than histamine is the guanidine derivative impromidine. This ligand actually combines a rather high H_2 receptor affinity with a reduced efficacy. Impromidine also shows moderate H_1- and potent H_3-receptor antagonistic activity[45,46] as well as potent H_4 receptor partial agonistic activity.[42]

The finding that Nα-guanylhistamine acts as a partial H_2 agonist in a gastric acid secretion test did lead to the development of the relatively weak H_2 antagonist burimamide. Years later, it was shown that burimamide is also an H_3 and H_4 receptor partial agonist.[42] Nevertheless, burimamide was a good lead for the development of selective and clinically useful H_2 receptor antagonists, such as cimetidine.[45,47,48] The 4-methylimidazole moiety of cimetidine can easily be replaced by other heterocyclic groups (figure 2). Replacement by a substituted furan- (e.g. ranitidine) or thiazole ring (e.g. tiotidine and famotidine) leads to compounds that are usually more potent at the H_2 receptor than

(*R*)-(−)-α-Methylhistamine, High Affinity H_3 Agonist

(*R*)-(−)-α-Methylhistamine *Cat. No. 0569*



This potent and high affinity H_3 agonist displays >200-fold selectivity over H_4 receptors. The compound inhibits H_3 receptor-mediated histamine synthesis and release in the CNS and stimulates H_4 receptor-mediated eosinophil shape change (EC_{50} = 66 nM).

Oishi *et al.* (1989) Effects of histamine H_3-agonist (*R*)-α-methylhistamine and the antagonist thioperamide on histamine modulation in the mouse and rat brain. *J.Neurochem.* **52** 1388. **Hew** *et al.* (1990) Characterization of histamine- H_3 receptors in guinea pig ileum with H_3-selective ligands. *Br.J.Pharmacol.* **101** 621. **Schwartz** *et al.* (1990) A third histamine receptor subtype - characterization, localization and functions of the H_3-receptor. *Agents Actions* **30** 13. **Buckland** *et al.* (2003) Histamine induces cytoskeletal changes in human eosinophils via the H_4 receptor. *Br.J.Pharmacol.* **140** 1117.

cimetidine. Moreover, the replacement of the imidazole moiety also eliminates the undesired inhibition of cytochrome P450.[48] The potent tritiated H_2 antagonist tiotidine and [^{125}I]-iodinated H_2 antagonist iodoaminopotentidine are successfully used as radioligands for the H_2 receptor.[24] The newly developed brain-penetrating H_2 antagonist zolantidine is an important tool for *in vivo* CNS studies.[49] The H_2 receptor was reported to be spontaneously active in transfected CHO cells.[50] Based on this concept, many H_2 antagonists were reclassified; cimetidine, ranitidine and famotidine are in fact inverse agonists, whereas burimamide acts in this model system as a neutral antagonist.[50]

The Histamine H_3 Receptor

In the eighties, another physiological role of histamine became apparent, namely its role as a neurotransmitter. In 1983, Arrang and co-workers discovered that the inhibitory effect of histamine on its own release and synthesis was not mediated by the known H_1 and H_2 receptor subtypes as no correlation with either the H_1 or the H_2 receptor activity of known histaminergic compounds was observed.[46] Soon after, the H_3 receptor agonist (*R*)-(−)-α-methylhistamine and the antagonist thioperamide (see figure 3) were developed.[51] It was confirmed that this receptor subtype indeed regulates the release and synthesis of histamine and in addition has a regulatory role in the release of other neurotransmitters, such as serotonin, noradrenalin and dopamine.[52] Next to high expression in certain regions of the CNS (for example the basal ganglia, hippocampus and cortical areas, i.e. the parts of the brain that are associated with cognition) the H_3 receptor is present in the peripheral nervous system, e.g. in the gastrointestinal tract, the airways and the cardiovascular system.[53,54] Initial efforts to identify the H_3 receptor gene, using the anticipated homology with the identified H_1 and H_2 receptor gene all failed. Eventually, the human H_3 receptor cDNA was cloned by Lovenberg and co-workers in 1999.[55] In search of novel GPCRs through homology searching of expressed sequence tag databases, a receptor with high similarity to the M_2 muscarinic acetylcholine receptor was identified. Expression of the gene and full characterisation established this protein as the histamine H_3 receptor. The cloning of the H_3 receptor of other species, including rat,[56] guinea pig[57] and mouse[58] soon followed and it was revealed that major H_3 receptor species differences exist. The human H_3 receptor gene was assigned to the telomeric region of the q arm of chromosome 20 and contains three exons that are interrupted by two introns.[59] The H_3 receptor gene can undergo extensive alternative splicing, resulting in many H_3 receptor isoforms that have different signalling properties and expression profiles.[59,60,61] It was shown that the H_3 receptor displays particularly high constitutive activity, again leading to a reclassification of existing ligands into agonists, neutral antagonists and inverse agonists.[62,63] Gbahou and co-workers revealed that the compound proxyfan (figure 3) is a protean agonist both *in vitro* and *in vivo*, meaning that this remarkable compound behaves as agonist, neutral antagonist or inverse agonist, depending on the signalling pathway studied.[64]

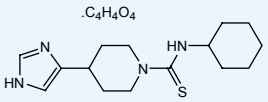
The H_3 receptor signals through $G_{i/o}$ proteins as was shown by the pertussis toxin sensitive stimulation of [^{35}S]-GTPγS binding in rat cortical membranes.[65] Through negative coupling to adenylyl cyclase, stimulation of the H_3 receptor results in lower levels of cAMP, thereby reducing downstream signalling events such as CREB-dependent gene transcription.[56] Alternative signalling pathways may be activated by the $G_{i/o}$ proteins, including mitogen-activated protein kinase (MAPK)[60] and phosphatidylinositol 3-kinase (PI3K) pathways. $G_{i/o}$ protein activation can also lead to the activation of phospholipase A_2 (PLA_2) to induce the release of arachidonic acid, the lowering of intracellular Ca^{2+} levels through voltage-gated ion channels[67] and the inhibition of the Na^+/H^+ exchanger (NHE).[68] With the recent progress that has been made in the characterisation of the H_3 receptor (as outlined above), many pharmaceutical companies have very active H_3 receptor drug development programs. As a result, several H_3 receptor ligands have entered clinical studies for a plethora of applications, including obesity, narcolepsy, dementia and migraine (among others).[69,70]

Ligands for H_3 Receptors

At the H_3 receptor, histamine itself is a highly active agonist. Modification of the endogenous ligand by mono- or dimethylation of the amino function results in compounds that are more active

Thioperamide, a Brain-penetrant H_3 and H_4 Ligand

Thioperamide *Cat. No. 0644*



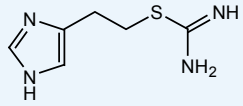
Thioperamide, the potent histamine H_3 and H_4 ligand, displays K_i values of 25 and 27 nM at recombinant H_3 and H_4 receptors respectively. The compound acts as an antagonist at H_3 receptors and displays inverse agonist activity at H_4 receptors. It freely crosses the blood-brain barrier.

Hew *et al.* (1990) Characterisation of histamine H_3 receptors in guinea pig ileum with H_3 selective ligands. *Br.J.Pharmacol.* **101** 621. **Liu** *et al.* (2001) Cloning and pharmacological characterization of a fourth histamine receptor (H_4) expressed in bone marrow. *Mol. Pharmacol.* **59** 420. **Ling** *et al.* (2004) Histamine H_4 receptor mediates eosinophil chemotaxis with cell shape change and adhesion molecule upregulation. *Br.J.Pharmacol.* **142** 161. **Gbahou** *et al.* (2006) Compared pharmacology of human H_3 and H_4 receptors: structure-activity relationships of histamine derivatives. *Br.J.Pharmacol.* **147** 744.

and selective for the H_3 receptor. Methylation of the α-carbon atom of the ethylamine sidechain drastically increases the potency at the H_3 receptor. This increased activity resides completely in the R-isomer; the corresponding S-isomer is approximately 100-fold less potent. Since the methylation leads to highly reduced activity at both H_1 and H_2 receptors, (*R*)-(−)-α-methylhistamine (figure 3) was initially considered a selective agonist at the H_3 receptor. However, when the H_4 receptor was discovered it was shown that (*R*)-(−)-α-methylhistamine also has considerable affinity for this histamine receptor subtype.[42] Nevertheless, in combination with its less active S-isomer, (*R*)-(−)-α-methylhistamine has proven to be highly useful for the pharmacological characterisation of H_3 receptor-mediated effects.[41] Tritiated forms of Nα-methylhistamine and (*R*)-(−)-α-methylhistamine are available as radiolabelled agonists for the H_3 receptor. For potent H_3 agonism, the amine function of histamine can be replaced by an isothiourea group, as in imetit. This compound is also very active *in vitro* and *in vivo*,[71,72,73] as is (*R*)-(−)-α-methylhistamine. The amine function can also be incorporated in ring structures. For example, immepip is a potent H_3 receptor agonist that is effective *in vitro* and *in vivo*.[74] Although the described first generation H_3 agonists were intensively used as reference ligands to study the H_3 receptor, all of them proved to also have considerable activity at the H_4 receptor. Therefore, a new generation of potent and selective H_3 receptor agonists has been developed, most notably immethridine[75] (pEC_{50} = 9.74; displays 300-fold selectivity over the H_4 receptor) and methimepip.[76] These latter compounds are devoid of high H_4 receptor activity.

Imetit, High Affinity H_3/H_4 Agonist

Imetit *Cat. No. 0729*



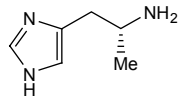
.2HBr

Imetit is an extremely high affinity, potent agonist at H_3 and H_4 receptors (K_i values are 0.3 and 2.7 nM respectively). The agonist induces shape change in eosinophils *in vitro* with an EC_{50} of 25 nM and is centrally active following systemic administration *in vivo*.

Garbarg *et al.* (1992) S-[2-(4-Imidazolyl)ethyl]isothiourea, a highly specific and potent histamine H_3 receptor agonist. *J.Pharmacol.Exp.Ther.* **263** 304. **Farzin and Attarzadeh** (2000) Influence of different histamine receptor agonists and antagonists on apomorphine-induced licking behavior in rats. *Eur.J.Pharmacol.* **404** 169. **Ling** *et al.* (2004) Histamine H_4 receptor mediates eosinophil chemotaxis with cell shape change and adhesion molecule upregulation. *Br.J.Pharmacol.* **142** 161.

As with the first generation H_3 receptor agonists, the first generation H_3 receptor antagonists (all of them possessing an imidazole heterocycle) turned out to have affinity for the H_4 receptor. The first potent H_3 receptor antagonist (later reclassified as an inverse agonist) that was devoid of H_1 receptor and H_2

Figure 3 | Chemical structures of selected H_3 receptor ligands



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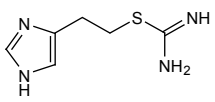
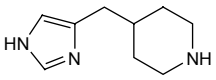
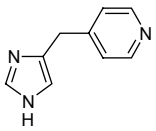


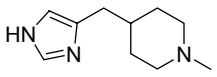
Imetit



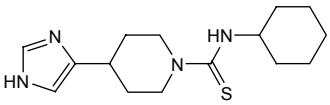
Immepip



Immethridine



Methimepip



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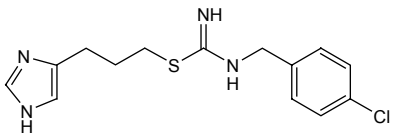
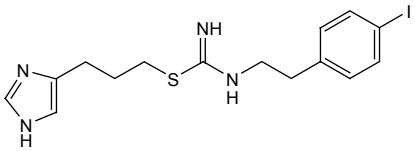
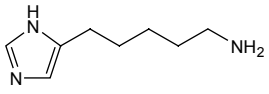


Clobenpropit



Iodophenpropit



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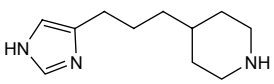

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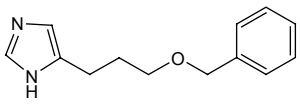
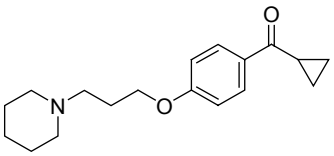


Proxyfan



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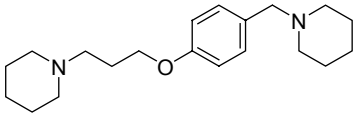

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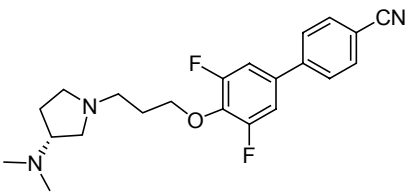

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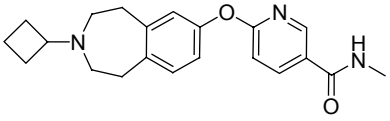


GSK 189254

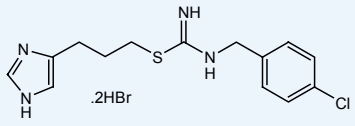
(**Bold** text denotes compounds available from Tocris at time of publication.)

receptor activity was thioperamide (figure 3).[51] This compound has been used in many H_3 receptor studies as a reference ligand and is active *in vitro* and *in vivo* (the compound is able to penetrate the CNS).[77] However, thioperamide displays some 5-HT_3 receptor antagonism[78] and is an inverse agonist at the H_4 receptor. The remarkable H_3 receptor species difference can be demonstrated with thioperamide as the compound has a 10-fold higher affinity for the rat H_3 receptor than for the human H_3 receptor.[56] Based on the H_3 receptor agonist imetit (*vide ante*), the highly potent H_3 receptor inverse agonist clobenpropit was developed (pA_2 = 9.9).[73] This compound has some 5-HT_3 receptor activity[78] and displays partial agonist activity at H_4 receptors. In addition, radioligands for the H_3 receptor have been described (e.g. [^{125}I]-iodophenpropit and [^{125}I]-iodoproxyfan).[79,80] The moderately active H_2 receptor antagonist burimamide (pA_2 = 5.1, figure 2) also has good affinity for the H_3 (pK_i = 7.9) and the H_4 (pK_i = 7.4) receptor.[42] Impentamine is a potent histamine H_3 receptor inverse agonist (pA_2 = 8.4). Like burimamide, this compound can act as a partial agonist in SK-N-MC cells expressing human H_3 receptors. It has also been shown that small structural modifications of impentamine, i.e. alkylation of the primairy amine moiety of impentamine with e.g. methyl-, isopropyl- and p-chlorobenzyl- groups results in ligands that cover the complete spectrum of functional activity, i.e. agonism, neutral antagonism and inverse agonism.[63,81] The compound VUF 5681 was reported as a neutral H_3 antagonist (or 'silent' antagonist),[82] findings that were recently confirmed in studies investigating constitutive activity using rat brain cortex.[83] It has been shown that the functional activity of proxyfan depends on the system used, ranging from full agonist to inverse agonist. Thus, proxyfan can be classified as a protean agonist, both *in vitro* and *in vivo*.[64,84]

The first non-imidazole H_3 receptor ligand was reported by Ganellin in 1998.[85] This elegant medicinal chemistry work did lead to the potent compound UCL 2190.[86] Following the cloning of the H_3 receptor in 1999, several pharmaceutical companies entered the H_3 research field and further explored this structural class. An example of these new ligands is JNJ 5207852, a compound that contains some typical structural features for H_3 receptor ligands, e.g. piperidinyl propyloxy side chain. This neutral antagonist is active in several models for cognition[87,88] but does not act as an appetite suppressant and has no effect on food intake.[89] Other compounds, such as Abbott's A 423579 seem to have more efficacy in obesity models, while lacking clear cognitive effects.[90] At the time of writing the differences in efficacy for distinct clinical applications of the different classes of H_3 ligands is not understood and subject of intense research.[70,91] Interestingly, GSK 189254 has been in trials for three different diseases: neuropathic pain, narcolepsy and dementia.[92]

Clobenpropit, Highly Potent H_3 Antagonist

Clobenpropit *Cat. No. 0752*

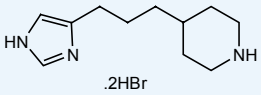


An extremely potent antagonist/inverse agonist at H_3 receptors (pA_2 = 9.93), clobenpropit also displays partial agonist activity at H_4 receptors. The ligand induces eosinophil shape change with an EC_{50} of 3 nM.

Van der Goot *et al.* (1992) Isothiourea analogues of histamine as potent agonists or antagonists of the histamine H_3 receptor. *Eur.J.Med.Chem.* **27** 511. **Yokoyama** *et al.* (1994) Clobenpropit (VUF-9153), a new histamine H_3 receptor antagonist, inhibits electrically induced convulsions in mice. *Eur.J.Pharmacol.* **260** 23. **Liu** *et al.* (2001) Cloning and pharmacological characterization of a fourth histamine receptor (H_4) expressed in bone marrow. *Mol. Pharmacol.* **59** 420. **Buckland** *et al.* (2003) Histamine induces cytoskeletal changes in human eosinophils via the H_4 receptor. *Br.J.Pharmacol.* **140** 1117.

VUF 5681, a Novel H_3 Antagonist

VUF 5681 *Cat. No. 2493*



VUF 5681 is a potent histamine H_3 receptor silent antagonist (pK_i = 8.35).

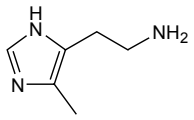
Kitbunnadaj *et al.* (2004) Identification of 4-(1*H*-imidazol-4(5)-ylmethyl) pyridine (immethridine) as a novel, potent, and highly selective histamine H_3 receptor agonist. *J.Med.Chem.* **47** 2414. **Leurs** *et al.* (2005) The histamine H_3 receptor: from gene cloning to H_3 receptor drugs. *Nat.Rev.Drug Discov.* **4** 107. **Moreno-Delgado** *et al.* (2006) Constitutive activity of H_3 autoreceptors modulates histamine synthesis in rat brain through the cAMP/PKA pathway. *Neuropharmacology* **51** 517.

The Histamine H_4 Receptor

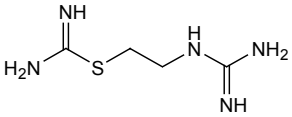
Immediately following the cloning of the H_3 receptor gene, several groups identified the homologous H_4 receptor sequence in the human genome databases.[93–97] Indeed, the H_4 receptor has high sequence identity with the H_3 receptor (31% at the protein level, 54% in the transmembrane domains). The H_3 and H_4 receptor are also similar in gene structure. The human H_4 receptor gene is located on chromosome 18q11.2 in a single copy per haploid genome. The gene spans more than 21 kbp and contains three exons that are interrupted by two large introns.[98] To date, two H_4 receptor isoforms have been identified. Cloning of the genes that encode the mouse, rat, guinea-pig and pig H_4 receptors reveal only limited sequence homology with the human H_4 receptor. The H_4 receptor is mainly expressed in bone marrow and peripheral leukocytes, and mRNAs of the human H_4 receptor are detected in e.g. mast cells, dentritic cells, spleen and eosinophils.[93–97] The receptor has a pronounced effect on the chemotaxis of several cell types that are associated with immune and inflammatory responses. The H_4 receptor is mainly coupled to $G_{i/o}$ proteins, thereby leading to a decrease in the production of cAMP and subsequent downsteam effects such as regulation of cAMP responsive element-binding protein (CREB) gene transcription. Furthermore, H_4 receptor stimulation affects the pertussin-toxin-sensitive activation of mitogen-activitated protein (MAP) kinase pathways. Studying the increased levels of [^{35}S] GTPγS levels in H_4 transfected cells, it has been shown that the H_4 receptor is constitutively active. The Gβγ subunits of the $G_{i/o}$ proteins activate phospholipase C, and thereby increase the Ca^{2+} concentrations. In mast cell and eosinophils, this Ca^{2+} response can be linked to cellular chemotaxis.

Considering the physiological role of the H_4 receptor, several applications are under preclinical investigation,[99,100] including allergy and asthma,[101] as well as chronic inflammations such as

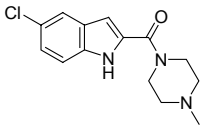
Figure 4 | The first selective H4 receptor ligands reported in scientific literature



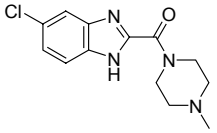
4-Methylhistamine



VUF 8430



JNJ 7777120

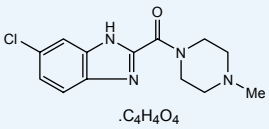


JNJ 10191584

(**Bold** text denotes compounds available from Tocris at time of publication.)

JNJ 10191584, H_4-Selective Antagonist

JNJ 10191584 *Cat. No. 2441*



JNJ 10191584 is a highly selective histamine H_4 receptor silent antagonist. It binds with high affinity to the human H_4 receptor (K_i = 26 nM) and is > 540-fold selective over the H_3 receptor (K_i = 14.1 μM). *In vitro* the antagonist inhibits mast cell and eosinophil chemotaxis with IC_{50} values of 138 and 530 nM respectively. The antagonist is orally active *in vivo*.

Terzioglu *et al.* (2004) Synthesis and structure-activity relationships of indole and benzimidazole piperazines as histamine H_4 receptor antagonists. Bioorg. Med. Chem.Lett. **14** 5251. **Venable** *et al.* (2005) Preparation and biological evaluation of indole, benzimidazole, and thienopyrrole piperazine carboxamides: potent human histamine H_4 antagonists. J.Med.Chem. **48** 8289. **Varga** *et al.* (2005) Inhibitory effects of histamine H4 receptor antagonists on experimental colitis in the rat. Eur.J.Pharmacol. **522** 130.

inflammatory bowel disease (IBD)[102] and rheumatoid arthritis.[103] The H_4 receptor is also being associated with pruritus (itch)[104,105] and is involved in the progression of colon cancer.[106]

Ligands for H_4 Receptors

As was described above, most imidazole-containing, first generation H_3 receptor ligands have considerable affinity for the H_4 receptor as well. However, selective H_4 receptor ligands have been described. 4-Methylhistamine is a potent H_4 agonist while displaying more than a 100-fold selectivity over the other histamine receptor subtypes, including the H_2 receptor for which this ligand was originally developed.[42] A slightly different and complimentary profile was reported for VUF 8430. This compound has a high H_4 receptor activity and affinity, minimal affinity for the H_1 and H_2 receptor and a 33-fold lower affinity for the H_3 receptor.[107]

Potent and selective H_4 receptor antagonists are also emerging. The first reported neutral antagonist is JNJ 7777120, a compound that is frequently being used as H_4 receptor reference ligand. Currently, [^{3}H]-JNJ 7777120 and [^{3}H]-histamine are used as H_4 receptor radioligands. Unfortunately, JNJ 7777120 has a poor stability in human and rat liver microsomes and a limited half life of about two hours. The benzimidazole derivative JNJ 10191584 (VUF 6002) is also a neutral H_4 antagonist.[108] This compound is orally active *in vivo* and has an improved liver microsome stability but still a limited half life.[102,109] More recently, 2-arylbenzimidazoles have been described as ligands with low nanomolar affinity for the H_4 receptor.[110] Considering the number of H_4 receptor-related patent applications that have recently been disclosed (as reviewed elsewhere[100]), it can be anticipated that many new H_4 receptor ligands will be described in scientific literature in the near future.

Conclusions

The histamine receptor research field continues to blossom as both members of industry and academia find this family of receptors very rewarding, both scientifically and commercially. For more than seventy years, considerable efforts have been devoted to finding new ways to modulate the different physiological processes that are mediated by histamine. The consecutive discovery of new histamine receptor subtypes (distinction between H_1 and H_2 receptors in 1966, discovery of the H_3 receptor in 1983 and the H_4 receptor in 2000, note the interval of seventeen years) provides a complete and exciting research platform. The histamine receptor family also seems to hold the promise of GPCRs as excellent drug targets with two receptor subtypes (H_1 and H_2) addressed by blockbuster drugs, the third subtype (H_3) leading to frantic clinical studies and the latest addition to the family (H_4) leading to very interesting preclinical data. It can therefore be anticipated that histamine receptor research will continue to thrive in the years to come.

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Histamine Receptor Compounds Available from Tocris

Catalog No.	Product Name	Primary Function
H_1 Receptors		
Agonists		
0646	HTMT	H_1/H_2 agonist
2478	2-Pyridylethylamine	H_1 receptor agonist
Antagonists		
2577	Cetirizine	Selective H_1 antagonist
1453	Clemastine	H_1 antagonist
1425	(*S*)-(+)-Dimethindene	H_1 antagonist. Also M_2 muscarinic antagonist
0508	Doxepin	Highly potent H_1 antagonist. Also binds to H_4 receptor
2429	Fexofenadine	H_1 receptor antagonist; non-sedating antiallergic agent
0784	Ketotifen	H_1 antagonist
1944	Loratidine	Peripheral H_1 antagonist; anti-allergic agent
0660	Mepyramine	Selective H_1 inverse agonist
2018	Mirtazepine	Potent H_1 antagonist. Also 5-HT_2, 5-HT_3 and α_2-antagonist. Antidepressant
0662	*trans*-Triprolidine	Standard H_1 antagonist, highly potent
H_2 Receptors		
Agonists		
0668	Amthamine	Highly selective standard H_2 agonist
0506	Dimaprit	Standard H_2 selective agonist
0646	HTMT	H_2/H_1 agonist
Antagonists		
0721	Aminopotentidine	H_2 antagonist
0902	Cimetidine	H_2 antagonist, I_1 agonist
0833	ICI 162,846	Potent H_2 antagonist, active *in vivo*
1967	Ranitidine	Selective H_2 antagonist
0826	Tiotidine	Potent, selective H_2 antagonist
1070	Zolantidine	Potent, centrally active H_2 antagonist
H_3 and H_4 Receptors		
Agonists		
0729	Imetit	Standard H_3 and H_4 agonist ($H_3 > H_4$)
0932	Immepip	Standard H_3 agonist. Also H_4 agonist
2315	Immethridine	Potent H_3 agonist, highly selective over H_4
0573	N^{α}-Methylhistamine	Non-selective H_3 agonist
0569	(*R*)-(−)-α-Methylhistamine	Potent, standard H_3 agonist
0572	(*S*)-(+)-α-Methylhistamine	H_3 agonist, less active enantiomer
2342	4-Methylhistamine	Selective, high affinity H_4 agonist
2477	Proxyfan	High affinity H_3 ligand
2494	VUF 8430	Potent, high affinity H_4 agonist
Antagonists		
0752	Clobenpropit	Highly potent H_3 antagonist/inverse agonist and H_4 partial agonist
1858	Impentamine	Selective H_3 antagonist
0779	Iodophenpropit	Potent, selective H_3 antagonist
2441	JNJ 10191584	Selective H_4 receptor antagonist; orally active
2477	Proxyfan	High affinity H_3 ligand
2034	ROS 234	Potent H_3 antagonist
0644	Thioperamide	H_3/H_4 antagonist/inverse agonist. Active *in vivo*
2493	VUF 2681	Potent H_3 receptor silent antagonist