

REVIEW

Molecular and biochemical pharmacology of the histamine H₄ receptor

Rob Leurs¹, Paul L Chazot², Fiona C Shenton², Herman D Lim¹ and Iwan JP de Esch¹

¹Department of Medicinal Chemistry, Leiden/Amsterdam Center for Drug Research, VU University Amsterdam, Amsterdam, The Netherlands, and ²School of Biological and Biomedical Sciences, Durham University, Durham, UK

The elucidation of the human genome has had a major impact on histamine receptor research. The identification of the human H₄ receptor by several groups has been instrumental for a new appreciation of the role of histamine in the modulation of immune function. In this review, we summarize the historical developments and the molecular and biochemical pharmacology of the H₄ receptor.

British Journal of Pharmacology (2009) **157**, 14–23; doi:10.1111/j.1476-5381.2009.00250.x

Keywords: histamine H₄ receptor; molecular pharmacology; biochemistry; signal transduction; species difference; isoforms; heterogeneity; selective agonists and antagonists

Abbreviations: cDNA, complementary DNA; EL2, second extracellular loop; GPCR, G protein-coupled receptor; PTX, pertussis toxin; SNP, single nucleotide polymorphism; TM, transmembrane domain

Introduction

Histamine is known since long to be an important mediator in different (patho)physiological conditions. In 1966, Ash and Schild suggested the presence of two distinct histamine receptor subtypes (Ash and Schild, 1966) based on the pharmacological effects of known histaminergic ligands on, for example, airway smooth muscle (H₁) and the heart, uterus and stomach (H₂). Sir James Black *et al.* at SK&F paved the way for the final acceptance of two distinct histamine receptor subtypes with the development of the first selective histamine H₂ antagonists metiamide and burimamide (Black *et al.*, 1972). Moreover, the recognition that different actions of histamine could be effectively inhibited by selective receptor antagonist eventually resulted in the development of various blockbuster H₁ and H₂ antagonists.

In 1983 a new histamine H₃ receptor subtype was identified by Arrang *et al.* in the rat brain on the basis of classical pharmacology (Arrang *et al.*, 1983). For a long time, this new histamine receptor subtype was not fully endorsed by the pharmaceutical industry because of the lack of molecular details of the receptor protein. While the gene for the histamine H₁ (Yamashita *et al.*, 1991) and H₂ receptor (Gantz *et al.*,

1991) were both cloned in 1991, for many years no definite proof of the molecular architecture of the H₃ receptor was available. Finally, in 1999 a team of J&J researchers led by Tim Lovenberg cloned the complementary DNA (cDNA) of an α_2 adrenergic-like EST sequence that, after characterization, turned out to encode for the human histamine H₃ receptor (Lovenberg *et al.*, 1999). This cloning of the H₃ receptor gene resulted in a rapid increase in knowledge of its molecular pharmacology and biochemistry (e.g. expression, signal transduction, receptor isoforms) (Lovenberg *et al.*, 1999; Drutel *et al.*, 2001; Hancock, 2006; Bongers *et al.*, 2007). Combined with the development of recombinant cell systems, these developments led to a huge interest from pharmaceutical companies (Leurs *et al.*, 2000). Many new selective ligands have since then been reported in (patent) literature (Celanire *et al.*, 2005; Wijtmans *et al.*, 2007), and the H₃ receptor is currently seen as a new target for the treatment of a variety of central nervous system disorders (e.g. attention deficit hyperactivity disorder, Alzheimer's disease and schizophrenia) (Leurs *et al.*, 1995; Esbenshade *et al.*, 2008).

The cloning of the H₃ receptor gene also marked the entry of the histamine H₄ receptor into the G protein-coupled receptor (GPCR) family of histamine receptors. Using the DNA sequence of the histamine H₃ receptor, several research groups independently identified a previously unexplored GPCR sequence in the human genome as a new histamine H₄ receptor (Nakamura *et al.*, 2000; Oda *et al.*, 2000; Liu *et al.*, 2001a; Morse *et al.*, 2001; Nguyen *et al.*, 2001; Zhu *et al.*, 2001). The new receptor showed a distinct pharmacology (see

below) is highly expressed in immune cells (Nakamura *et al.*, 2000; Oda *et al.*, 2000; Liu *et al.*, 2001a; Morse *et al.*, 2001; Nguyen *et al.*, 2001; Zhu *et al.*, 2001) and is now regarded as an interesting target for, for example, inflammatory disorders and pruritis (de Esch *et al.*, 2005; Thurmond *et al.*, 2008). The first identification of the H₄ receptor led to a further increase in the interest in histamine receptor pharmacology and a substantial rise in H₄ receptor-related papers (112 in the period 2001–2009) and patents (36 in the period 2002–2008) in the last few years. In the present review we will highlight the molecular biology and pharmacology of the latest member of the histamine receptor family.

Cloning of the histamine H₄ receptor gene

As reported above, the H₄ receptor cDNA was finally identified in the human genome database on the basis of its overall homology (37%) to the H₃ receptor sequence (Nakamura *et al.*, 2000; Oda *et al.*, 2000; Liu *et al.*, 2001a; Morse *et al.*, 2001; Nguyen *et al.*, 2001; Zhu *et al.*, 2001). The homology of the H₄ receptor to the H₁ and H₂ receptor is only ~19%, explaining why this subtype was only found after the long awaited identification of the H₃ receptor gene in 1999 (Lovenberg *et al.*, 1999). The size of the open reading frame of the H₄ receptor cDNA is 1173 bp, encoding for a 390 amino acid protein (Figure 1), belonging to the large family of GPCRs.

Besides a high sequence homology, the H₄ receptor also shares its gene structure with the H₃ receptor (Coge *et al.*, 2001a; Coge *et al.*, 2001b). The human H₄ receptor gene is present in a single copy on chromosome 18q11.2. The gene spans 16.98 kb (position 20 294 591 to 20 311 567) and, like the H₃ receptor (Drutel *et al.*, 2001), is interrupted by two large introns (7867 bp and >17 500 bp). The introns divide the coding regions into three parts, encoding amino acid number 1–65, 66–119 and 120–390. Such an intron–exon distribution has led for the H₃ receptor to the generation of a large number of alternatively spliced GPCR variants, sometimes with clearly altered functionalities (Drutel *et al.*, 2001; Leurs *et al.*, 2005). However, for the H₄ receptor at present only two non-signalling, non-7TM H₄ receptor isoforms have been identified (van Rijn *et al.*, 2008) (Figure 1), designated as H₄(67) and H₄(302). The polypeptide of H₄(67) is prematurely terminated at residue 67, while H₄(302) lacks residues 68–155 (Figure 1, *vide infra*) (van Rijn *et al.*, 2008).

The original H₄ receptor amino acid sequence as reported by Oda *et al.* (2000) deviates at three positions from the sequences reported by four other research groups (Nakamura *et al.*, 2000; Liu *et al.*, 2001a; Morse *et al.*, 2001; Zhu *et al.*, 2001), that is, Val instead of Ala at position 138, Arg instead of His at position 206 and Arg instead of Gln at position 253. The Val¹³⁸Ala and Arg²⁰⁶His polymorphisms have been confirmed by single nucleotide polymorphism (SNP) analysis involving samples from White, Chinese, Japanese, Afro-

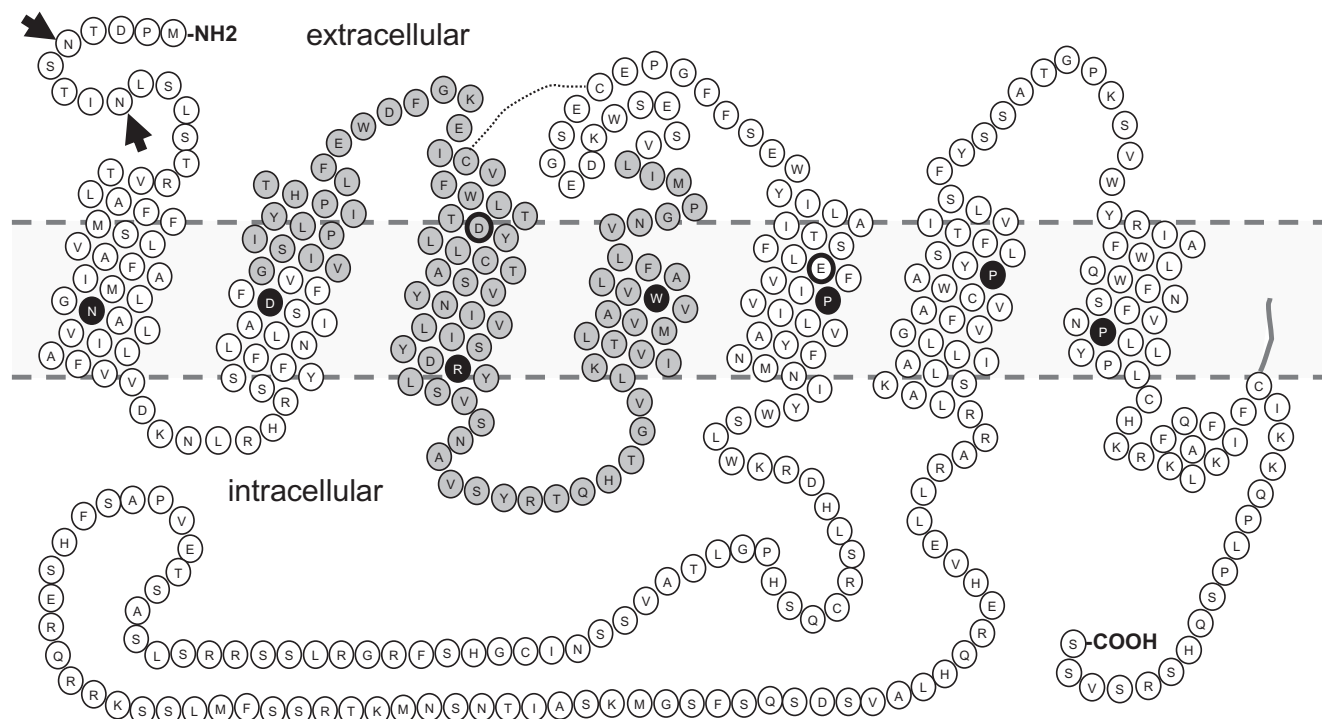


Figure 1 Snake plot of the human histamine H₄ receptor. The full-length receptor consists of 390 amino acids, which form seven transmembrane helices, three extracellular loops and three intracellular loops, with an extracellular N-terminal and an intracellular C-terminal peptide; the dotted line represents the putative disulfide bridge that links the cysteines in the third transmembrane and second extracellular loop. Residues Asp^{3.32} and Glu^{5.46} that play important roles in histamine binding are marked with bold border. The H₄R₆₇ isoform only contains the first 67 N-terminal residues (marked in white), while the H₄R₃₀₂ lacks the residues marked in grey. The conserved residues in the family A of G protein-coupled receptors are depicted in black circles, while the putative glycosylation sites (Asn⁵ and Asn⁹) are indicated with arrows. A potential palmitoylation site (Cys³⁷⁴) at the C-terminal tail is suggested to be close to the membrane following membrane insertion of a putative attached palmitic acid.

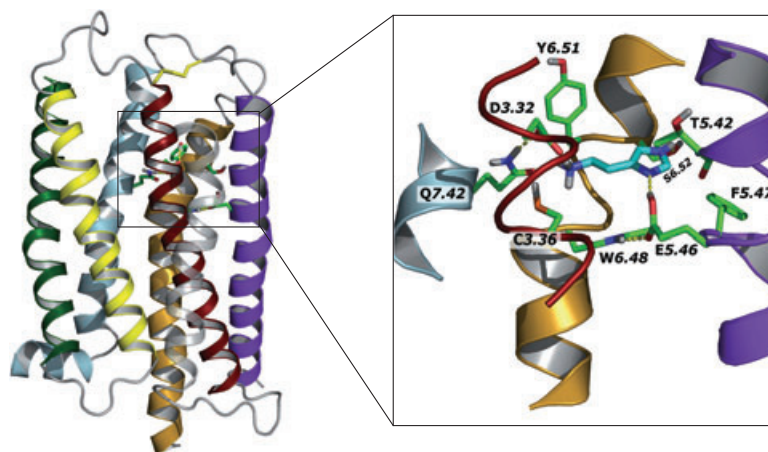


Figure 2 Schematic representation of a homology model of the human histamine H₄ receptor. The figure shows the seven transmembrane domains and the position of the ligand binding site. In the box details of the histamine binding site, as determined using *ab initio* calculations is shown (reproduced with permission from Jongejan *et al.*, 2008).

American and sub-Saharan African people (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=59340). The occurrence of Ala¹³⁸ or His²⁰⁶ alleles is higher than that of Val¹³⁸ or Arg²⁰⁶ (91.1–100% vs. 0–9.2%). Two other SNPs have been found as well in the coding region, resulting in Cys²⁸⁴Ser mutation and a frame shift at residue L³⁷⁹. Besides those, 37 SNPs are found in intron 1, 45 in intron 2 and 21 in 3'-untranslated region (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=59340). So far no functional comparisons of the various SNP variants or linkage studies have been reported.

As with the other histamine receptor family members, the H₄ receptor protein possesses all consensus motifs identified for the Class A rhodopsin-like GPCRs, that is, N1.50³³, D2.50⁶¹, R3.50¹¹², W4.50¹⁴⁰, P5.50¹⁸⁶, P6.50³¹⁸ and P7.50³⁵⁵ [using the standardized GPCR nomenclature according to Ballesteros–Weinstein (Ballesteros and Weinstein, 1995) (Figure 1)]. With respect to histamine binding, a conserved aspartate residue in the third transmembrane (TM) aminergic GPCRs helix (D3.32, Figure 1) is also present in this newest member of the aminergic GPCR family. As for the other aminergic GPCRs, D3.32 is believed to interact with the protonated amine group of the agonist histamine (Shin *et al.*, 2002). Moreover, in both H₃ and H₄ receptors a second negatively charged amino acid, E5.46, is present in the fifth TM helix (Figure 1). This amino acid has been shown to play an essential role in the binding of histamine as determined by site-directed mutagenesis analysis and computational analysis (Shin *et al.*, 2002; Uveges *et al.*, 2002). As is illustrated in Figure 2, E5.46 is a key anchor for the imidazole ring of histamine. The binding pocket for the imidazole ring is formed by E5.46, T5.42 and W6.48 (Jongejan *et al.*, 2008). The latter residue adopts the 'active' rotamer conformation (Jongejan *et al.*, 2005) by forming a hydrogen bond to the aforementioned E5.46 (box, Figure 2).

The H₄R has two potential glycosylation sites (Asn⁵ and Asn⁹) (Figure 1, *vide infra*), whereas Cys³⁷⁴ might serve as a potential site for palmitoylation. The H₄R also contains cysteine residues that potentially form a disulphide bridge, connecting the third TM with the second extracellular loop (EL2)

(Figure 1). These cysteine residues are conserved among the GPCRs, and the disulphide bridge has been evidenced in the crystal structure of bovine rhodopsin and adrenergic β₁ and β₂ receptors (Palczewski *et al.*, 2000; Cherezov *et al.*, 2007; Warne *et al.*, 2008). The disulfide bridge will bring some structural constraint to the extracellular loop two, which may be important in view of the suggested involvement of the EL2 in the binding of histamine (Lim *et al.*, 2008).

Species differences

Following the identification of the human H₄R, the cDNA sequence of mouse, rat, guinea pig, pig, dog and monkey H₄ receptors have been reported and functionally expressed (Liu *et al.*, 2001b; Oda *et al.*, 2002; 2005). The monkey H₄ receptor (*Macaca fascicularis*) shows a high sequence homology (92%) with the human orthologue (Oda *et al.*, 2005). The H₄ receptors of the other species are only moderately homologous to the human H₄R, with sequence homology between 67% and 72% (Liu *et al.*, 2001b; Oda *et al.*, 2002; 2005; Jiang *et al.*, 2008). Forty (partial) amino acid sequences of H₄R orthologues from 34 other species have been extracted from various genomic ENSEMBL databases (http://www.ensembl.org/Homo_sapiens/Gene/Compare_Ortholog?db=core;g=ENS00000134489;r=18:20294591-20313918;t=ENST00000256906), among others horse (*Equus caballus*), cow (*Bos taurus*), cat (*Felis catus*), rhesus monkey (*Macaca mullata*), chimpanzee (*Pan troglodytes*) and dolphin (*Tursiops truncatus*) H₄ receptor sequences are known (Figure 3). The H₄R sequences from cynomolgus (*Macaca fascicularis*) and pig (*Sus scrofa*), which are not listed as orthologues in the ENSEMBL database are also included in the phylogenetic tree in Figure 3. Because some of the sequences are still incomplete, changes in the phylogenetic tree are to be expected. Fish (zebra fish, fugu, tetraodon, medaka and stickleback) as well as frog (*Xenopus tropicalis*) have each two orthologues. However, these orthologues, together with the sequences from chicken (*Gallus gallus*) and opossum, show only 28–42% homology to the human H₄ receptor, while all the others show a substantial higher



Figure 3 Phylogenetic tree of H₄ receptor orthologues. The sequences are obtained from <http://www.ensembl.org> and the phylogram was created with ClustalW.

homology to the human H₄ receptor (>50%). In fact, these orthologues are more similar to the human H₃R. Consequently, in the phylogenetic tree these H₃/H₄ orthologues cluster in a separate group (Figure 3). Within the extracted (partial) GPCR sequences, the typical aminergic GPCR features mentioned earlier (e.g. Asp^{3.32} in TM3 and Glu^{5.46} in TM5) can often be found. Detailed analysis of most of these species variants is for the moment however lacking, but may provide useful tools to dissect receptor–ligand binding. Previously, Lim *et al.* (2008), for example, showed that a sequence difference in the extracellular loop EL2 is responsible for the lower affinity of histamine for rat and mouse H₄ receptors. Comparing the binding profiles of rat, mouse, guinea-pig, porcine, monkey and dog H₄ receptors, one can identify for ligands like clozapine and clobenpropit (see also paragraph on H₄ agonist) also substantial differences in binding affinity between the various species (Liu *et al.*, 2001b; Oda *et al.*, 2002; 2005). Differences in pharmacological activities of H₄ receptor ligands between the different species might hamper preclinical development of future H₄ receptor drugs.

Signal transduction of the H₄ receptor

The H₄ receptor is coupled to pertussis toxin (PTX)-sensitive G $\alpha_{i/o}$ proteins, thereby inhibiting forskolin-induced cAMP and eventually the modulation of transcription of genes regulated by cAMP-responsive elements in cell lines recombinantly expressing the H₄ receptor (Oda *et al.*, 2000; Liu *et al.*, 2001a; Zhu *et al.*, 2001). In transfected HEK-293 cells H₄ recep-

tor stimulation also leads to a strong increase in mitogen-activated protein kinase phosphorylation via a PTX-sensitive pathway (Morse *et al.*, 2001). Furthermore, the H₄ receptor also signals to calcium via either PTX-sensitive G $\alpha_{i/o}$ proteins (Hofstra *et al.*, 2003) or the promiscuous G α_{16} protein (Oda *et al.*, 2000; Morse *et al.*, 2001), which is selectively expressed in immune cells (Wilkie *et al.*, 1991). In recombinant cell lines the H₄ receptor constitutively inhibits forskolin-induced cAMP-responsive element-mediated reporter gene expression and activates [³⁵S]GTP γ S binding (Liu *et al.*, 2001a; Morse *et al.*, 2001; Lim *et al.*, 2005). The agonist-independent activity can be inhibited by H₄ inverse agonists, such as thioperamide.

Activation of endogenously expressed H₄ receptors in for example eosinophils and mast cells leads to PTX-sensitive calcium mobilization (Buckland *et al.*, 2003; Hofstra *et al.*, 2003), via the activation of phospholipase C (Hofstra *et al.*, 2003). The increased cytosolic calcium concentration is intimately linked to actin polymerization, cell shape change and eventually to the migration of mast cells, eosinophils and monocyte dendritic cells (Buckland *et al.*, 2003; Hofstra *et al.*, 2003; Ling *et al.*, 2004; Gutzmer *et al.*, 2005; Barnard *et al.*, 2008).

Looking back in immunology literature with this new knowledge, early examples of H₄ receptor-mediated signalling by histamine can be identified. Already in the 1975 Clark *et al.* observed histamine-induced eosinophil chemotaxis that could not be blocked by the known H₁ and H₂ receptor antagonists (Clark *et al.*, 1975). Furthermore, in 1994 Raible *et al.* identified in eosinophils a PTX-sensitive cytosolic

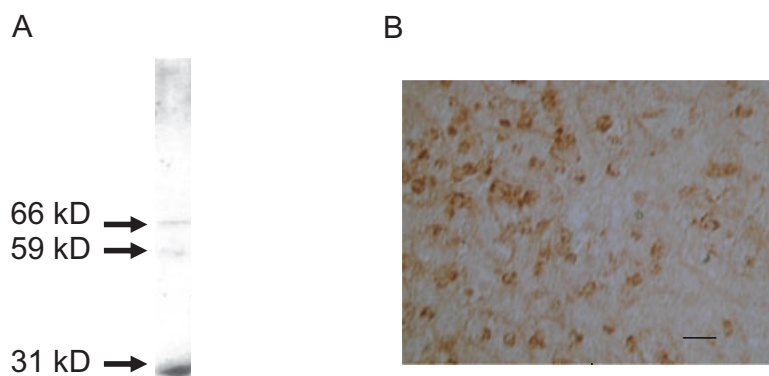


Figure 4 Anti-hH_{4(374–390)} receptor antibody reacts with human H₄ receptor protein in spleen tissue lysate and sections. (A) Immunoblot of human spleen lysate (10 µg). Anti-H_{4(374–390)} antibodies were used at 2 µg·mL⁻¹, incubated overnight at 4°C and developed as described in Rijn *et al.* (2006). In addition to the putative monomer at M_w 31 kDa, two higher molecular weight species were detected at 59 and 66 kDa. (B) Immunostaining of human spleen slice (bar = 50 µm) using anti-H_{4(374–390)} at 1 µg·mL⁻¹ using an immunohistochemical protocol described in Chazot *et al.* (2001).

calcium increase induced by histamine, which could be inhibited by the presumed selective H₃ receptor antagonist thioperamide. The potent H₃ receptor reference agonist *R*- α -methylhistamine also induced a calcium increase, but at much lower potency than that of histamine itself. Based on these observations, the presence of a new histamine receptor subtype was suggested (Raible *et al.*, 1994). Alas, this suggestion was largely ignored by the histamine research community and has only been fully exploited more than a decade after the discovery.

Anatomical Topography of the H₄ receptor

The tissue distribution of the H₄ receptor has been profiled at the mRNA level in a wide range of human (using reverse transcription polymerase chain reaction) and mouse (using *in situ* hybridization) tissues (Zhu *et al.*, 2001). In human tissues, H₄ receptor expression was most abundant in bone marrow, peripheral blood, spleen, thymus, small intestine, colon, heart and lung (Nakamura *et al.*, 2000; Liu *et al.*, 2001a; Morse *et al.*, 2001; Nguyen *et al.*, 2001; Zhu *et al.*, 2001). Discrepancies have occurred in both human and rodent tissue based on these mRNA studies, which highlights the necessity for selective immunological anti-H₄ receptor probes.

Anti-H₄ receptor antibodies have been crucial tools to study the H₄ receptor (van Rijn *et al.*, 2006; 2008; Dijkstra *et al.*, 2007; Baumer *et al.*, 2008; Dijkstra *et al.*, 2008; Morini *et al.*, 2008). The majority of cells expressing H₄ receptors are indeed hematopoietic in lineage, including neutrophils, mast cells, eosinophils, basophils, dendritic cells, monocytes and T cells (e.g. van Rijn *et al.*, 2006; Dijkstra *et al.*, 2007; Baumer *et al.*, 2008; Dijkstra *et al.*, 2008), but importantly, it has recently been shown that the H₄ receptor is not exclusively expressed on hematopoietic cells. The H₄ receptor has been identified in subsets of endocrine cells in the gastrointestinal tract (distinct from H₃R containing cells) (Morini *et al.*, 2008) and has been shown also to be expressed on dermal fibroblasts (Ikawa *et al.*, 2008). Immunohistochemical studies have shown that H₄ receptors are present on nerves from the human nasal mucosa (Nakaya *et al.*, 2004) and in the enteric nervous system on the

myenteric plexus of the rodent fundus (H₃R absent) (PL Chazot, D Grandi, FC Shenton, G Morini, unpublished). Indeed, importantly, as with the other histamine receptor subtypes, the H₄ receptor is expressed in the central nervous system (Connelly, *et al.*, 2009). In agreement, the H₄ receptor was detected on subpopulations of small and medium diameter cells within the lumbar sensory dorsal root ganglia and laminae I/II of the dorsal horn of the lumbar spinal cord (Strakhova *et al.*, 2009; PL Chazot, NL Lethbridge, unpublished). These latter results indicate that the H₄ receptor is present on synaptic terminals of primary sensory afferent neurons. Therefore, the H₄ receptor is likely to subserve distinct roles in many parts of the body, and therefore may represent a target for an expanded array of therapeutic arenas, including neuropathic pain.

H₄ receptor biochemistry

Immunological approaches have demonstrated that the H₄ receptor comprises robust dimeric structures (van Rijn *et al.*, 2006; 2008). It is clear from immunoblots that higher molecular weight species (consistent with dimers and/or higher oligomers) are present in both recombinant and native tissue (Figure 4). Immunoblotting identified three major protein species in human spleen lysate. Interestingly a low molecular weight species was clearly visible in the spleen lysate at 31 kDa, likely to be the receptor monomer. In contrast, monomeric species were not evident in either lymphocytes (polyhaemagglutinin blasts) or in brain membranes (van Rijn *et al.*, 2006; Connelly *et al.*, 2009). The observed sizes detected in immunoblotting studies are not always completely consistent with H₄R dimeric structures, with sizes ranging between 60 and 80 kD in HEK-293 H₄R-transfected cells or in other native tissue preparations (Figure 4, van Rijn *et al.*, 2006; Connelly *et al.*, 2009). Several possibilities could account for these observations, including tissue-specific complements of H₄R isoforms and differential tissue-specific post-translational modifications (e.g. palmitoylation, glycosylation).

Anti-H₄ receptor antibodies were also used to investigate the expression and potential role of the newly described

human H₄ receptor isoforms: H₄(302) and H₄(67) (van Rijn *et al.*, 2008). Because mRNAs coding for all three isoforms are found in several white blood cell types, heterooligomerization may occur in native tissue. The two shorter isoforms are both non-functional as regards ligand binding and signalling. However they may play a regulatory role as they appear to reduce number of histamine binding to the H₄(390) receptor by 55% [H₄(302)] and 30% [H₄(67)] in heterologous expression studies. Based on surface biotinylation experiments, the three were expressed at the cell surface, although the shorter versions with greatly reduced efficiency. Furthermore co-expression of the H₄(390) with either H₄(302) or H₄(67) dose-dependently reduced surface expression of the full length receptor (van Rijn *et al.*, 2008). These results provide further evidence to support the role of splice isoforms as dominant negative regulatory elements, which maybe a common theme in GPCR-regulatory pathways (Bakker *et al.*, 2006).

The majority of secreted and cell surface proteins that transit to the cell surface through the endoplasmic reticulum are N-glycosylated (95% of GPCRs) (Lanctot *et al.*, 2005). In order for a GPCR to elicit intracellular signalling appropriately, the correct quantity of properly folded functional receptors must be available in the plasma membrane. It is equally essential that signalling can be terminated at the appropriate time and the receptors either recycled or permanently degraded. The regulatory mechanisms controlling export trafficking of GPCRs, including glycosylation, have recently been reviewed (Duvernay *et al.*, 2005). The role of post-translational changes to receptors is complex and an area where there is growing interest. Several studies have shown that glycosylation is involved in GPCR transport from the endoplasmic reticulum through the Golgi to the cell surface (reviewed in Duvernay *et al.*, 2005). Both the H₃ and H₄ receptors are indeed N-glycosylated (van Rijn *et al.*, 2006; Shenton and Chazot, 2006). Interestingly, receptor dimerization *in vitro* does not appear to be dependent on N-glycosylation. However, in the case of endogenous H₄ receptor expressed in human polyhaemagglutinin blasts, chemical deglycosyla-

tion appears to destabilize the preformed dimeric species to individual monomers (van Rijn *et al.*, 2006). The pharmacological influence of N-glycosylation of the H₄ receptor is yet to be explored.

Pharmacological tools to study the H₄ receptor

Already with the early description of histamine responsiveness of eosinophils in 1994, a clearly different pharmacology was described in comparison with the known receptor subtypes (Raible *et al.*, 1994). The subsequent cloning of the H₄ receptor cDNA and the analysis of the expressed protein also resulted in the rapid recognition of a completely distinct H₄ receptor pharmacology (Hough, 2001). Since then, major progress has been made in the discovery of selective H₄ receptor agonists and antagonists.

The early pharmacological and (patho)physiological characterization of the H₄ receptor was performed using agonists that were originally developed for the homologous H₃R receptor. Typically, these initial H₃ and H₄ receptor dual activity reference compounds all contain an imidazole heterocycle. The most important examples are the H₃ and H₄ receptor agonist, immepip (2) and the H₃ antagonist and H₄ agonist clobenpropit (3) (Lim *et al.*, 2005) (Figure 5).

The first ligand that was optimized for selective H₄ receptor activation is OUP-16 (4) (Hashimoto *et al.*, 2003) (Figure 5). This full agonist has a 40-fold selectivity for the H₄ receptor over the H₃ receptor. More recently, the potent H₄ receptor agonist 4-methylhistamine (5) was described after evaluation of a large number of histaminergic compounds (Lim *et al.*, 2005). Although originally developed in the H₂ receptor research programme, 4-methylhistamine has a high affinity for the H₄ receptor, while displaying at least a 100-fold selectivity over the other histamine receptor subtypes, including the H₂ receptor. The first identified non-imidazole H₄ receptor agonist was clozapine (6) (Oda *et al.*, 2000). This antipsychotic agent has affinity for a large number of GPCRs, but almost exclusively acts as antagonist. The dibenzodiazepine

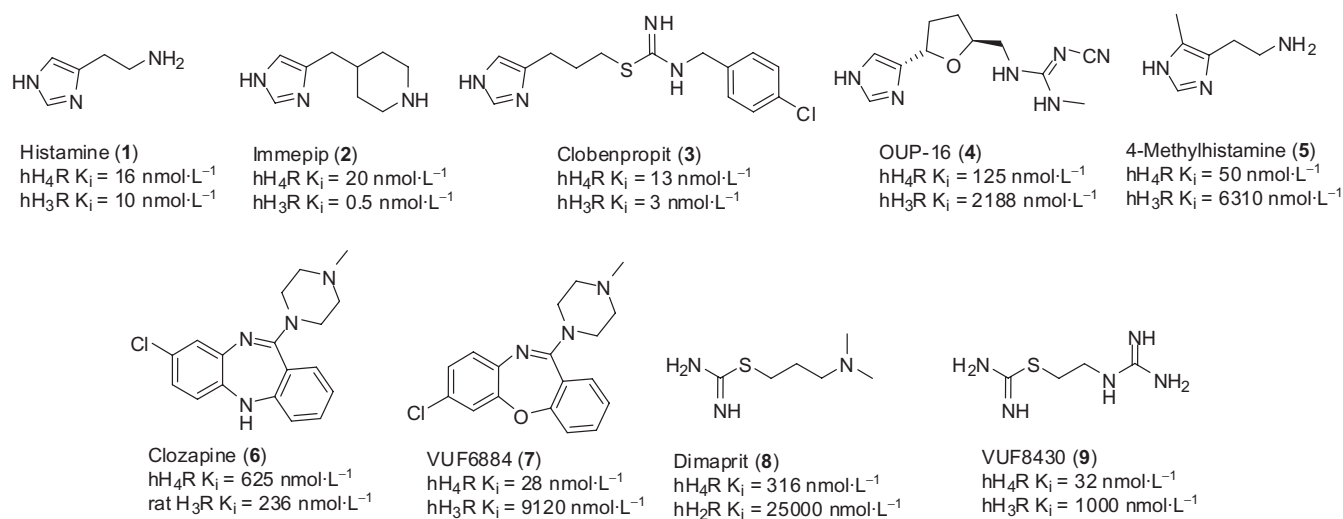


Figure 5 First and second generation H₄ receptor agonists with different selectivity profiles.

structure was optimized for H₄ receptor affinity, leading to the dual activity H₁ receptor antagonist and H₄ receptor agonist VUF6884 (7) (Smits *et al.*, 2006). Although promiscuous GPCR binders, this class of ligands provides detailed insight of the H₄ receptor agonist binding site (Smits *et al.*, 2006; Jongejan *et al.*, 2008). Another non-imidazole H₄ receptor agonist is dimaprit (8) and its analog VUF8430 (9) (Lim *et al.*, 2005; Lim *et al.*, 2006). The latter is a full agonist with high affinity for the H₄ receptor and a 30-fold selectivity over the H₃ receptor. In combination with 4-methylhistamine, VUF8430 forms a new useful pair of tools to study H₄ receptor pharmacology (see Lim *et al.*, 2009).

As is true for the first H₄ receptor agonists, the first antagonists that were used to characterize the H₄ receptor were imidazole-containing, dual activity H₃ and H₄ receptor ligands. Of particular use has been the H₃ and H₄ receptor inverse agonist thioperamide (10) (Figure 6; Buckland *et al.*, 2003; Hofstra *et al.*, 2003; Bell *et al.*, 2004; Takeshita *et al.*, 2004; Damaj *et al.*, 2007). The H₄ receptor research field has been greatly helped by the early discovery and disclosure of the selective non-imidazole neutral antagonist JNJ777120 (11). The compound resulted from a high-throughput screening campaign and subsequent hit optimization efforts at Johnson and Johnson Pharmaceuticals (Carruthers *et al.*, 2002). JNJ777120 is equipotently active at human, mouse and rat H₄Rs (Jablonowski *et al.*, 2003; Thurmond *et al.*, 2004). This H₄R reference antagonist is more than a thousand fold selective over other histamine receptor subtypes and was reported to have no cross-reactivity in a panel of 50 other receptors, enzymes, transporters and ion-channels. JNJ777120 has reasonable oral bioavailability (22%), but its use in disease models is hampered by a short *in vivo* half-life of about 0.8 h (Venable *et al.*, 2005). Efforts to obtain compounds with improved properties by scaffold hopping led to VUF6002 (12) (Figure 6; Terzioglu *et al.*, 2004; Venable *et al.*,

2005). Although this benzimidazole derivative has an improved liver microsomes stability, the half-life remains limited ($t_{1/2}$ = 1.0 h).

Another series of H₄R compounds that was recently claimed in a patent by Johnson and Johnson contain methylpiperazine substituted 2-quinoxalinones (Edwards and Venable, 2005), including compound 13. At VU University Amsterdam, the construction of a H₄R pharmacophore model and rational design approaches resulted in the potent quinazoline compound 14 (Leurs *et al.*, submitted). This compound was shown to have anti-inflammatory properties *in vivo* in the rat.

Bayer Healthcare AG has recently patented aminopyrimidines (e.g. 15) as H₄ receptor antagonists (Sato *et al.*, 2005a,b). Since this disclosure, several other companies have claimed other pyrimidines as H₄ receptor ligands, including Abbott Laboratories who developed the rotationally restricted analogue A-943931 (16). This compound has been thoroughly characterized and displays a more than 640-fold selectivity over the H₃ receptor, a 190-fold selectivity over α_1 adrenergic receptors and a 470-fold selectivity over 5-HT_{1d} receptors. A-943931 is reported to have excellent antagonistic activity both *in vitro* and *in vivo* across multiple species, while displaying excellent metabolic stability and good oral bioavailability (90%). This compound is a good anti-inflammatory agent in mice and also displays good efficacy in rat pain models (Coward *et al.*, 2008). Further exploration of the aminopyrimidine theme resulted in the recently described H₄ receptor antagonist A-987306 (17), a potent and selective compound with an excellent pharmacokinetic profile, including a half-life of 3.7 h after p.o. dosing (Liu *et al.*, 2008). A-987306 (17) has anti-inflammatory activity in a peritonitis model and is especially potent in a pain assay in rats, as was shown by the blockage of carrageenan induced thermal hyperalgesia.

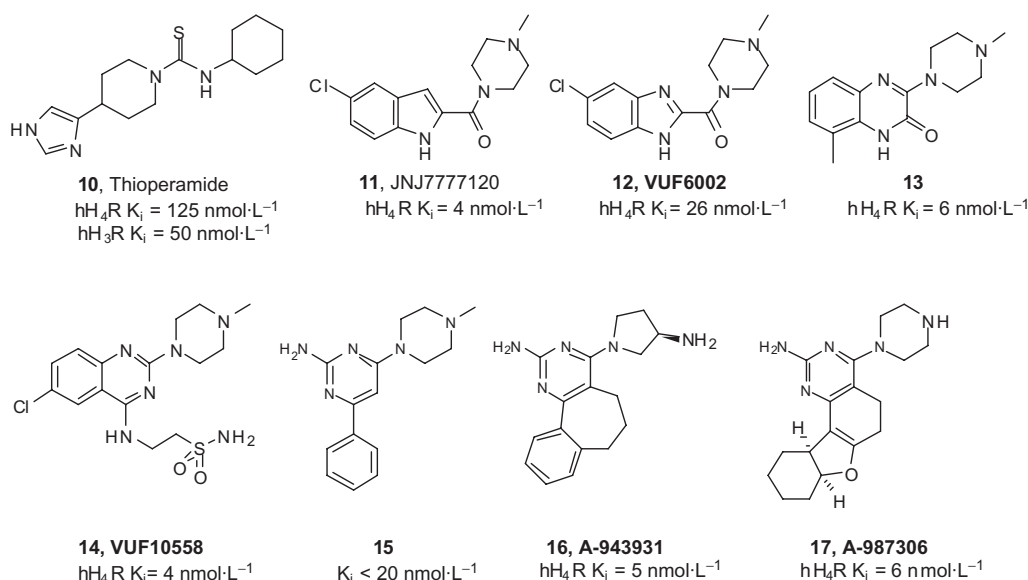


Figure 6 First and second generation H₄ receptor antagonists. Except for thioperamide (10) the antagonists display at least a 100-fold selectivity for the histamine H₄ receptor over the histamine H₃ receptor.

In order to study the pharmacology of the H₄ receptor, radioligand binding studies are instrumental. As with the H₃ receptor, the H₄ receptor binds the endogenous agonist histamine with high, nanomolar affinity (Nakamura *et al.*, 2000; Oda *et al.*, 2000; Liu *et al.*, 2001a; Morse *et al.*, 2001; Nguyen *et al.*, 2001; Zhu *et al.*, 2001). Consequently, [³H]histamine has been effectively used as radioligand since the discovery of the H₄ receptor (Nakamura *et al.*, 2000; Oda *et al.*, 2000; Liu *et al.*, 2001a; Morse *et al.*, 2001; Nguyen *et al.*, 2001; Zhu *et al.*, 2001). In some studies also [³H]N^m-methylhistamine has been used as radiotracer (Liu *et al.*, 2001a), but this has not become very popular. In 2004 J&J described the use of [³H]JNJ7777120 as antagonist radioligand for the H₄ receptor (Thurmond *et al.*, 2004). This new radioligand binds differently than histamine in the H₄R ligand binding pocket (Jongejan *et al.*, 2008), allowing the analysis of, for example, distinct H₄ receptor mutants that have lost high affinity [³H]histamine binding (Jongejan *et al.*, 2008).

Final remarks

With the cloning of the gene of the histamine H₄ receptor in 1999/2000 the field of histamine research has been fueled with new excitement. Several laboratories have already identified a set of useful tools (agonists, antagonists, anti-H₄ receptor antibodies), allowing the field to address the function of the H₄ receptor in both the periphery and the brain. Moreover, in the last few years a large increase in knowledge on the biochemical pharmacology of the H₄ receptor has been obtained. In view of the first preclinical *in vivo* data for H₄ receptor ligands, this new histamine receptor family member is expected to become an important target in the area of inflammatory disorders and neuropathic pain. Moreover, also pruritis is associated with H₄ receptor function as itching can be induced by H₄ agonists such as clobenpropit and 4-methylhistamine and blocked by H₄ antagonists such as JNJ7777120. It might nevertheless be an interesting challenge to develop H₄ receptor drugs, as so far relatively high levels of H₄ receptor antagonists are required to inhibit *in vivo* inflammatory effects despite the high potency and selectivity of the tool compounds. This may reflect limitations of the tool compounds with respect to for example pharmacokinetic properties, as well as for example high local histamine concentrations at the H₄ receptor.

Acknowledgement

The authors acknowledge support from the EU-KP7 COST programme BM0806 (Histamine H₄ receptor network).

Conflict of interest

None.

References

- Arrang JM, Garbarg M, Schwartz JC (1983). Auto-inhibition of brain histamine release mediated by a novel class H₃ of histamine receptor. *Nature* **302**: 832–837.
- Ash AS, Schild HO (1966). Receptors mediating some actions of histamine. *Br J Pharmacol Chemother* **27**: 427–439.
- Bakker RA, Lozada AF, van Marle A, Shenton FC, Drutel G, Karlstedt K *et al.* (2006). Discovery of naturally occurring splice variants of the rat histamine H₃ receptor that act as dominant-negative isoforms. *Mol Pharmacol* **69**: 1194–1206.
- Ballesteros JA, Weinstein H (1995). Integrated methods for the construction of three-dimensional models and computational probing of structure-function relations in G protein-coupled receptors. *Methods Neurosci* **25**: 366–428.
- Barnard R, Barnard A, Salmon G, Liu W, Sreckovic S (2008). Histamine-induced actin polymerization in human eosinophils: an imaging approach for histamine H₄ receptor. *Cytometry* **73**: 299–304.
- Baumer W, Wendorff S, Gutzmer R, Werfel T, Dijkstra D, Chazot P *et al.* (2008). Histamine H₄ receptors modulate dendritic cell migration through skin – immunomodulatory role of histamine. *Allergy* **63**: 1387–1394.
- Bell JK, McQueen DS, Rees JL (2004). Involvement of histamine H₄ and H₁ receptors in scratching induced by histamine receptor agonists in Balb C mice. *Br J Pharmacol* **142**: 374–380.
- Black JW, Duncan WA, Durant CJ, Ganellin CR, Parsons EM (1972). Definition and antagonism of histamine H₂ receptors. *Nature* **236**: 385–390.
- Bongers G, Bakker RA, Leurs R (2007). Molecular aspects of the histamine H₃ receptor. *Biochem pharmacol* **73**: 1195–1204.
- Buckland KF, Williams TJ, Conroy DM (2003). Histamine induces cytoskeletal changes in human eosinophils via the H₄ receptor. *Br J Pharmacol* **140**: 1117–1127.
- Carruthers NI, Chai W, Dvorak CA, Edwards JP, Grice CA, Jablonowski JA *et al.* (2002). Heterocyclic compounds. Patent WO 02/072548.
- Celanire S, Wijtmans M, Talaga P, Leurs R, de Esch IJ (2005). Keynote review: histamine H₃ receptor antagonists reach out for the clinic. *Drug Discov Today* **10**: 1613–1627.
- Chazot PL, Hann V, Wilson C, Lees G, Thompson CL (2001). Immunological identification of the mammalian H₃ histamine receptor in the mouse brain. *Neuroreport* **12**: 259–262.
- Cherezov V, Rosenbaum DM, Hanson MA, Rasmussen SG, Thian FS, Kobilka TS *et al.* (2007). High-resolution crystal structure of an engineered human β_2 -adrenergic G protein-coupled receptor. *Science* **318**: 1258–1265.
- Clark RA, Gallin JI, Kaplan AP (1975). The selective eosinophil chemotactic activity of histamine. *J Exp Med* **142**: 1462–1476.
- Coge F, Guenin SP, Audinot V, Renouard-Try A, Beauverger P, Macia C *et al.* (2001a). Genomic organization and characterization of splice variants of the human histamine H₃ receptor. *Biochem J* **355**: 279–288.
- Coge F, Guenin SP, Rique H, Boutin JA, Galizzi JP (2001b). Structure and expression of the human histamine H₄ receptor gene. *Biochem Biophys Res Commun* **284**: 301–309.
- Connelly WM, Shenton FC, Lethbridge N, Leurs R, Waldvogel HJ, Faull RLM *et al.* (2009). The histamine H₄ receptor is functionally expressed on neurons in the mammalian CNS. *Br J Pharmacol* **157**: 55–63.
- Cowart MD, Altenbach RJ, Liu H, Hsieh GC, Drizin I, Milicic I *et al.* (2008). Rotationally constrained 2,4-diamino-5,6-disubstituted pyrimidines: a new class of histamine H₄ receptor antagonists with improved druglikeness and *in vivo* efficacy in pain and inflammation models. *J Med Chem* **51**: 6547–6557.
- Damaj BB, Becerra CB, Esber HJ, Wen Y, Maghazachi AA (2007). Functional expression of H₄ histamine receptor in human natural killer cells, monocytes, and dendritic cells. *J Immunol* **179**: 7907–7915.
- Dijkstra D, Leurs R, Chazot P, Shenton FC, Stark H, Werfel T *et al.* (2007). Histamine downregulates monocyte CCL2 production through the histamine H₄ receptor. *J Allergy Clin Immunol* **120**: 300–307.
- Dijkstra D, Stark H, Chazot PL, Shenton FC, Leurs R, Werfel T *et al.*

- (2008). Human inflammatory dendritic epidermal cells express a functional histamine H₄ receptor. *J Invest Dermatol* **128**: 1696–1703.
- Drutel G, Peitsaro N, Karlstedt K, Wieland K, Smit MJ, Timmerman H *et al.* (2001). Identification of rat H₃ receptor isoforms with different brain expression and signaling properties. *Mol Pharmacol* **59**: 1–8.
- Duvernay MT, Filipeanu CM, Wu G (2005). The regulatory mechanisms of export trafficking of G protein-coupled receptors. *Cell signal* **17**: 1457–1465.
- Edwards JP, Venable JD (2005). Quinoxaline compounds. Patent US 2005/0070527.
- Ebenshade TA, Browman KE, Bitner RS, Strakhova M, Cowart MD, Brioni JD (2008). The histamine H₃ receptor: an attractive target for the treatment of cognitive disorders. *Br J Pharmacol* **154**: 1166–1181.
- de Esch IJ, Thurmond RL, Jongejan A, Leurs R (2005). The histamine H₄ receptor as a new therapeutic target for inflammation. *Trends Pharmacol Sci* **26**: 462–469.
- Gantz I, Munzert G, Tashiro T, Schaffer M, Wang L, DelValle J *et al.* (1991). Molecular cloning of the human histamine H₂ receptor. *Biochem Biophys Res Commun* **178**: 1386–1392.
- Gutzmer R, Diestel C, Mommert S, Kother B, Stark H, Wittmann M *et al.* (2005). Histamine H₄ receptor stimulation suppresses IL-12p70 production and mediates chemotaxis in human monocyte-derived dendritic cells. *J Immunol* **174**: 5224–5232.
- Hancock AA (2006). The challenge of drug discovery of a GPCR target: analysis of preclinical pharmacology of histamine H₃ antagonists/inverse agonists. *Biochem Pharmacol* **71**: 1103–1113.
- Hashimoto T, Harusawa S, Araki L, Zuiderveld OP, Smit MJ, Imazu T *et al.* (2003). A selective human H₄ receptor agonist: (-)-2-cyano-1-methyl-3-[(2R,5R)-5-[1H-imidazol-4(5)-yl]tetrahydrofuran-2-yl]methylguanidine. *J Med Chem* **46**: 3162–3165.
- Hofstra CL, Desai PJ, Thurmond RL, Fung-Leung WP (2003). Histamine H₄ receptor mediates chemotaxis and calcium mobilization of mast cells. *J Pharmacol Exp Ther* **305**: 1212–1221.
- Hough LB (2001). Genomics meets histamine receptors: new subtypes, new receptors. *Mol Pharmacol* **59**: 415–419.
- Ikawa Y, Shiba K, Ohki E, Mutoh N, Suzuki M, Sato H *et al.* (2008). Comparative study of histamine H₄ receptor expression in human dermal fibroblasts. *J Toxicol Sci* **33**: 503–508.
- Jablonowski JA, Grice CA, Chai W, Dvorak CA, Venable JD, Kwok AK *et al.* (2003). The first potent and selective non-imidazole human histamine H₄ receptor antagonists. *J Med Chem* **46**: 3957–3960.
- Jiang W, Lim HD, Zhang M, Desai P, Dai H, Colling PM *et al.* (2008). Cloning and pharmacological characterization of the dog histamine H₄ receptor. *Eur J Pharmacol* **592**: 26–32.
- Jongejan A, Bruysters M, Ballesteros JA, Haaksma E, Bakker RA, Pardo L *et al.* (2005). Linking agonist binding to histamine H₁ receptor activation. *Nat Chem Biol* **1**: 98–103.
- Jongejan A, Lim HD, Smits RA, de Esch IJ, Haaksma E, Leurs R (2008). Delineation of agonist binding to the human histamine H₄ receptor using mutational analysis, homology modeling, and *ab initio* calculations. *J Chem Inf Model* **48**: 1455–1463.
- Lancot PM, Leclerc PC, Clement M, Auger-Messier M, Escher E, Leduc R *et al.* (2005). Importance of N-glycosylation positioning for cell-surface expression, targeting, affinity and quality control of the human AT₁ receptor. *Biochem J* **390**: 367–376.
- Leurs R, Vollinga RC, Timmerman H (1995). The medicinal chemistry and therapeutic potentials of ligands of the histamine H₃ receptor. *Prog Drug Res* **45**: 107–165.
- Leurs R, Hoffmann M, Wieland K, Timmerman H (2000). H₃ receptor gene is cloned at last. *Trends Pharmacol Sci* **21**: 11–12.
- Leurs R, Bakker RA, Timmerman H, de Esch IJ (2005). The histamine H₃ receptor: from gene cloning to H₃ receptor drugs. *Nat Rev Drug Discov* **4**: 107–120.
- Lim HD, van Rijn RM, Ling P, Bakker RA, Thurmond RL, Leurs R (2005). Evaluation of histamine H₁-, H₂-, and H₃-receptor ligands at the human histamine H₄ receptor: identification of 4-methylhistamine as the first potent and selective H₄ receptor agonist. *J Pharmacol Exp Ther* **314**: 1310–1321.
- Lim HD, Smits RA, Bakker RA, van Dam CM, de Esch IJ, Leurs R (2006). Discovery of S-(2-guanidylethyl)-isothioureia (VUF 8430) as a potent nonimidazole histamine H₄ receptor agonist. *J Med Chem* **49**: 6650–6651.
- Lim HD, Jongejan A, Bakker RA, Haaksma E, de Esch IJ, Leurs R (2008). Phenylalanine¹⁶⁹ in the second extracellular loop of the human histamine H₄ receptor is responsible for the difference in agonist binding between human and mouse H₄ receptors. *J Pharmacol Exp Ther* **327**: 88–96.
- Lim HD, Adami M, Guaita E, Werfel T, Smits RA, de Esch IJP *et al.* (2009). Pharmacological characterization of the new histamine H₄ receptor agonist VUF 8430. *Br J Pharmacol* **157**: 34–43.
- Ling P, Ngo K, Nguyen S, Thurmond RL, Edwards JP, Karlsson L *et al.* (2004). Histamine H₄ receptor mediates eosinophil chemotaxis with cell shape change and adhesion molecule upregulation. *Br J Pharmacol* **142**: 161–171.
- Liu C, Ma X, Jiang X, Wilson SJ, Hofstra CL, Blevitt J *et al.* (2001a). Cloning and pharmacological characterization of a fourth histamine receptor (H₄) expressed in bone marrow. *Mol Pharmacol* **59**: 420–426.
- Liu C, Wilson SJ, Kuei C, Lovenberg TW (2001b). Comparison of human, mouse, rat, and guinea pig histamine H₄ receptors reveals substantial pharmacological species variation. *J Pharmacol Exp Ther* **299**: 121–130.
- Liu H, Altenbach RJ, Carr TL, Chandran P, Hsieh GC, Lewis LG *et al.* (2008). cis-4-(Piperazin-1-yl)-5,6,7a,8,9,10,11,11a-octahydrobenzofuro[2,3-h]quinazolin-2-amine (A-987306), a new histamine H₄R antagonist that blocks pain responses against carrageenan-induced hyperalgesia. *J Med Chem* **51**: 7094–7098.
- Lovenberg TW, Roland BL, Wilson SJ, Jiang X, Pyati J, Huvar A *et al.* (1999). Cloning and functional expression of the human histamine H₃ receptor. *Mol Pharmacol* **55**: 1101–1107.
- Morini G, Becchi G, Shenton FC, Chazot PL, Grandi D (2008). Histamine H₃ and H₄ receptors are expressed on distinct endocrine cell types in the rat fundic mucosa. *Inflamm Res* **57** (Suppl. 1): S57–S58.
- Morse KL, Behan J, Laz TM, West RE Jr, Greenfeder SA, Anthes JC *et al.* (2001). Cloning and characterization of a novel human histamine receptor. *J Pharmacol Exp Ther* **296**: 1058–1066.
- Nakamura T, Itadani H, Hidaka Y, Ohta M, Tanaka K (2000). Molecular cloning and characterization of a new human histamine receptor, HH₄R. *Biochem Biophys Res Commun* **279**: 615–620.
- Nakaya M, Takeuchi N, Kondo K (2004). Immunohistochemical localization of histamine receptor subtypes in human inferior turbinates. *Ann Otol Rhinol Laryngol* **113**: 552–557.
- Nguyen T, Shapiro DA, George SR, Setola V, Lee DK, Cheng R *et al.* (2001). Discovery of a novel member of the histamine receptor family. *Mol Pharmacol* **59**: 427–433.
- Oda T, Morikawa N, Saito Y, Masuho Y, Matsumoto S (2000). Molecular cloning and characterization of a novel type of histamine receptor preferentially expressed in leukocytes. *J Biol Chem* **275**: 36781–36786.
- Oda T, Matsumoto S, Masuho Y, Takasaki J, Matsumoto M, Kamohara M *et al.* (2002). cDNA cloning and characterization of porcine histamine H₄ receptor. *Biochim Biophys Acta* **1575**: 135–138.
- Oda T, Matsumoto S, Matsumoto M, Takasaki J, Kamohara M, Soga T *et al.* (2005). Molecular cloning of monkey histamine H₄ receptor. *J Pharmacol Sci* **98**: 319–322.
- Palczewski K, Kumasaka T, Hori T, Behnke CA, Motoshima H, Fox BA *et al.* (2000). Crystal structure of rhodopsin: A G protein-coupled receptor. *Science* **289**: 739–745.
- Raible DG, Lenahan T, Fayvilevich Y, Kosinski R, Schulman ES (1994). Pharmacologic characterization of a novel histamine receptor on human eosinophils. *Am J Respir Crit Care Med* **149**: 1506–1511.
- van Rijn RM, Chazot PL, Shenton FC, Sansuk K, Bakker RA, Leurs R (2006). Oligomerization of recombinant and endogenously

- expressed human histamine H₄ receptors. *Mol Pharmacol* **70**: 604–615.
- van Rijn RM, van Marle A, Chazot PL, Langemeijer E, Qin Y, Shenton FC *et al.* (2008). Cloning and characterization of dominant negative splice variants of the human histamine H₄ receptor. *Biochem J* **414**: 121–131.
- Sato H, Fukushima K, Shimazak M, Urbahns K, Sakai K, Ganter F *et al.* (2005a). 2-Aminopiperidine derivatives. Patent WO 2005/054239.
- Sato H, Tanaka K, Shimazak M, Urbahns K, Sakai K, Ganter F *et al.* (2005b). 2-Aminopyrimidine. Patent WO 2005/014556.
- Shenton FC, Chazot PL (2006). Probing the importance of N-glycosylation for [³H] clobenpropit binding to human H₃ receptors expressed in HEK 293 cells. 3. Molecular and chemical aspects of the histamine receptors. *Inflamm Res* **55** (Suppl. 1): S40–S41.
- Shin N, Coates E, Murgolo NJ, Morse KL, Bayne M, Strader CD *et al.* (2002). Molecular modeling and site-specific mutagenesis of the histamine-binding site of the histamine H₄ receptor. *Mol Pharmacol* **62**: 38–47.
- Smits RA, Lim HD, Stegink B, Bakker RA, de Esch IJ, Leurs R (2006). Characterization of the histamine H₄ receptor binding site. Part 1. Synthesis and pharmacological evaluation of dibenzodiazepine derivatives. *J Med Chem* **49**: 4512–4516.
- Strakhova MI, Nikkel AL, Manelli AM, Hsieh GC, Esbenshade TA, Brioni JD *et al.* (2009). Localization of histamine H₄ receptors in the central nervous system of human and rat. *Brain Res* **1250**: 41–48.
- Takeshita K, Bacon KB, Gantner F (2004). Critical role of L-selectin and histamine H₄ receptor in zymosan-induced neutrophil recruitment from the bone marrow: comparison with carrageenan. *J Pharmacol Exp Ther* **310**: 272–280.
- Terzioglu N, van Rijn RM, Bakker RA, De Esch IJ, Leurs R (2004). Synthesis and structure-activity relationships of indole and benzimidazole piperazines as histamine H₄ receptor antagonists. *Bioorg Med Chem Lett* **14**: 5251–5256.
- Thurmond RL, Desai PJ, Dunford PJ, Fung-Leung WP, Hofstra CL, Jiang W *et al.* (2004). A potent and selective histamine H₄ receptor antagonist with anti-inflammatory properties. *J Pharmacol Exp Ther* **309**: 404–413.
- Thurmond RL, Gelfand EW, Dunford PJ (2008). The role of histamine H₁ and H₄ receptors in allergic inflammation: the search for new antihistamines. *Nat Rev Drug Discov* **7**: 41–53.
- Uveges AJ, Kowal D, Zhang Y, Spangler TB, Dunlop J, Semus S *et al.* (2002). The role of transmembrane helix 5 in agonist binding to the human H₃ receptor. *J Pharmacol Exp Ther* **301**: 451–458.
- Venable JD, Cai H, Chai W, Dvorak CA, Grice CA, Jablonowski JA *et al.* (2005). Preparation and biological evaluation of indole, benzimidazole, and thienopyrrole piperazine carboxamides: potent human histamine H₄ antagonists. *J Med Chem* **48**: 8289–8298.
- Warne T, Serrano-Vega MJ, Baker JG, Moukhametzianov R, Edwards PC, Henderson R *et al.* (2008). Structure of a β₁-adrenergic G-protein-coupled receptor. *Nature* **454**: 486–491.
- Wijtmans M, Leurs R, de Esch I (2007). Histamine H₃ receptor ligands break ground in a remarkable plethora of therapeutic areas. *Expert Opin Investig Drugs* **16**: 967–985.
- Wilkie TM, Scherle PA, Strathmann MP, Slepak VZ, Simon MI (1991). Characterization of G-protein alpha subunits in the G_q class: expression in murine tissues and in stromal and hematopoietic cell lines. *Proc Natl Acad Sci USA* **88**: 10049–10053.
- Yamashita M, Fukui H, Sugama K, Horio Y, Ito S, Mizuguchi H *et al.* (1991). Expression cloning of a cDNA encoding the bovine histamine H₁ receptor. *Proc Natl Acad Sci USA* **88**: 11515–11519.
- Zhu Y, Michalovich D, Wu H, Tan KB, Dytko GM, Mannan IJ *et al.* (2001). Cloning, expression, and pharmacological characterization of a novel human histamine receptor. *Mol Pharmacol* **59**: 434–441.