Histamine H_4 Receptor: A Novel Therapeutic Target for Immune and Allergic Responses

H.G. Bhatt*[,1], Y.K. Agrawal[2], H.G. Raval[3], K. Manna[1] and P.R. Desai[1]

[1]*Institute of Pharmacy, Nirma University, S. G. Highway, Chharodi, Ahmedabad - 382 481, India*

[2]*Institute of Research and Development, Gujarat Forensic Sciences University, Gandhinagar - 382 007, India*

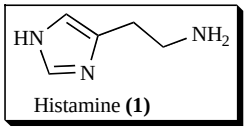
[3]*Anand Pharmacy College, Opp. Town Hall, Near GRID, Anand - 388 001, India*

Abstract: Histamine is a biomolecular compound located in various parts of body. It participated in various important cellular activities associated with allergy and asthma. This magic bio-molecule is directly and indirectly involved in various biochemical reactions through G-protein couple receptors. Various histamine receptors and their unexplored biochemical activities attracted many biologists in last few decades. A surprising discovery of histamine H_4 receptor was done when scientists worked on histamine H_3 receptor in brain cells. The binding pocket of histamine H_4 differs by transmembrane domains (TM3, TM5 and TM6) from histamine H_3-sub type. In this review, we enlightened various functions of histamine H_4 receptor and use of histamine H_4 receptor antagonists in autoimmune diseases, allergic responses, inflammatory responses, and in superoxide generation which are helpful to establish H_4 receptor antagonists as newer anti histamines.

Keywords: Histamine H_4 receptor structure, GPCRs, Pharmacological role & Functions, Immune responses, Allergic responses, Inflammation.

INTRODUCTION

Histamine is one of the most intensely studied biomolecules in medicine and is the single most potent mediator of immediate hypersensitivity reactions [1]. Histamine (**1**) is chemically 2-(4-imidazole)ethylamine, which is synthesized and released by human basophiles, mast cells, neurons and lymphocytes [2]. It was discovered as a uterine stimulant in different extracts by more than a century ago. The effects of histamine mimicked with anaphylaxis and showed smooth muscle stimulating and vasodepressor action [3]. There is a complex interrelationship between histamine, its receptors and other G-protein coupled receptors (GPCRs). Until 2000, histamine was thought to act *via* three GPCRs, which mediated most of our physiological responses to hormones, neurotransmitters and environmental stimulants. It had great potential as therapeutic targets for a broad spectrum of diseases [4]. The existence of three histamine receptors (H_1, H_2 and H_3) were known since 1984, based on the activities of several known pharmacological tools [5-8]. With the cloning of the cDNA that encodes the H_3 receptor [8], the pharmaceutical industry focused its efforts on the therapeutic usability of H_3 receptor antagonists [9, 10]. Following the sequencing of the human genome and the continuation of interest in H_3 receptor with its pharmacophoric study, the presence of another distinct histamine receptor was established, which was a homologous GPCR sequence in the human-genome-sequence database and expressed at high levels in mast cells and leukocytes. The histamine H_4 receptor, the fourth receptor family member was cloned and characterized by seven independent research groups through orphan GPCR/homology cloning methods [1, 11, 12]. At the end of 2000, Nakamura *et al.* [13], Oda *et al.* [14] and six other laboratories reported the molecular cloning and pharmacological characterization of the human H_4 receptor [11, 12, 15-18]. The effects of histamine are mediated by four subtypes of G-protein-coupled receptors: HR_1 (histamine receptor 1), HR_2, HR_3, and HR_4 (Table **1**) [17].



Histamine **(1)**

In addition to its well-characterized effects in the acute allergic inflammatory responses, histamine is effective to chronic inflammation and regulates several essential events in the immune response. Histamine selectively recruits the major effecter cells into tissue sites and affects their maturation, activation, polarization, and other functions leading to chronic inflammation. Histamine regulates dendritic cells, T cells and B cells, as well as related antibody isotype responses. In addition, acting through its receptor 2, histamine positively interferes with the peripheral antigen tolerance induced by T regulatory cells in several pathways. The diverse effects of histamine on immune regulation appear to be due to differential expression and regulation of 4 types of histamine receptors and their distinct intracellular signals. In addition, differences in affinities of these receptors for histamine are highly decisive for the biological effects of histamine and drugs targeting histamine receptors. This review article contains detailed information regarding H_4-

*Address correspondence to this author at the Institute of Pharmacy, Nirma University, S. G. Highway, Chharodi, Ahmedabad - 382 481, India;
Tel: +91 2717 241900-04; Fax: +91 2717 241916;
Email: hardikbhatt23@hotmail.com

1389-5575/10 $55.00+.00

Table 1. Histamine Receptors, Expressions, Activated Intracellular Signals and Coupled G-Proteins [17]

Histamine receptors	Expressions	Activated intracellular signals	G- proteins
HR_1	Nerve cells, airway and vascular smooth muscles, hepatocytes, endothelial cells, epithelial cells, neutrophils, eosinophils, monocytes, dendritic cells, T and B cells	Ca^{+2}, cGMP, phospholipase D, phospholipase A_2, NFκB	$G_{q/11}$
HR_2	Nerve cells, airway and vascular smooth muscles, hepatocytes, chondrocytes, endothelial cells, epithelial cells, neutrophils, eosinophils, monocytes, dendritic cells, T and B cells	Adenylate cyclase, cAMP, c-Fos, cJun, PKC, p70S6K	$G\alpha_s$
HR_3	Histaminergic neurons, eosinophils, dendritic cells, monocytes, low expression in peripheral tissues	Enhanced Ca^{+2}, MAP kinase, inhibition of cAMP	$G_{i/o}$
HR_4	High expression on bone marrow and peripheral hematopoietic cells, eosinophils, neutrophils, dendritic cells, T cells, basophiles, mast cells, low expression in nerve cells, hepatocytes peripheral tissues, spleen, thymus, lung, small intestine, colon and heart	Enhanced Ca^{+2}, inhibition of cAMP	$G_{i/o}$

receptor structure; its physiological/ pharmacological profile along with pharmacophoric ligands (agonists and antagonists); its therapeutic usability; highlights recent discoveries in histamine immunobiology and discusses their relevance in allergic inflammation.

HISTAMINE H_4 RECEPTOR

Location

The human Histamine H_4 receptor predominantly expressed in white blood cells, specifically T-lymphocyte [19], eosinophils, mast cells, bone marrow [11-17, 19-24], and moderately expressed in spleen, thymus, lung, small intestine, colon, and heart [25, 26]. It is less expressed in liver, lung, testis, tonsil, and trachea. There were few reports regarding expression of H_4 receptor in the CNS, although some scientists reported weak expression in human brain [16, 18]. Two reports on *in-situ* hybridization showed expression of histamine H_4 receptor in rodent brain tissue [15, 27]. H_4 receptor is also present in dendritic cells (DC) but expression in neutrophils and monocytes is less well defined. Because of the low level expression of H_4-receptor in many of the tissue, it is quite difficult to identify which cell types express the H_4-receptor. Moreover, expression seems to be regulated by inflammatory stimuli [11, 18], which created problems during the use of RT-PCR for the study of expressions in primary cells due to the state of the cell as well as its purity which affects the results. In 2004, Nakaya *et al.* studied immunohistochemical localization of histamine receptor subtypes in human inferior turbinate and concluded that H_4 receptors were present in nerves from the human nasal mucosa [28].

Histamine H_4 Receptor Structure

Sequence Identity

The elucidation of the human genome had a major impact on histamine receptor research [29]. In the transmembrane domains, the sequence identity with the H_3 receptor increases to 54% whereas, similarities in physicochemical properties of the different amino acids, in the transmembrane region, are 68%. For H_1 and H_2 receptors, the sequence identity is 23% and 22%, respectively [14, 15]. The substantial pharmacological species variation revealed that the cloning of not only the human H_4 receptors but also cDNAs that encode the H_4 receptor were carried from mouse, rat and guinea pig [30] and study reported that H_4 receptors shared only 65-72% sequence homology with their human counterpart and had differentiation in pharmacological profile.

Screening and Construction of H_4 Receptor Gene

Identification of the numerous novel GPCRs were carried out by molecular cloning techniques including many subtypes which were not suspected to exist on the basis of pharmacology. Molecular cloning made possible identification, isolation, and characterization of the majority of known GPCRs, however histamine receptor subtypes were more difficult to identify.

Histamine H_4 receptor was first cloned in 2000 [31]. The gene encoding the H_4 receptor was discovered initially in a search of the GenBank databases as sequence fragments retrieved in a partially sequenced human genomic containing mapped to chromosome 18. The gene encoding the H_4 receptor was on chromosome 18q11.2, spans>20.6 kb and had a similar intron-exon arrangement as the gene encoding the H_3 receptor [18]. These sequences were used to retrieve a partial cDNA clone and in combination with genomic fragments, to determine the full-length open reading frame of 390 amino acids residue GPCR encoded by three exons, which encompassed amino acids 1-64, 65-119 and 120-390 (Fig. **1**) [32]. It was expressed predominantly in bone marrow, eosinophils and mast cells [11-17]. In the reported 390-amino acid sequence of the human H_4 receptor, the highly conserved sequence motifs identified for the Class A rhodopsin- like GPCRs were present without exception (Fig. **1**) [33, 34]. These sequence motifs included $N1.50^{33}$ (According to Ballesteros and Weinstein [35], the number 1.50 is the standardized GPCR nomenclature and superscripts 33 represents the residue number in the human H_4 receptor sequence), $D2.50^{61}$, $R3.50^{112}$, $W4.50^{140}$, $P5.50^{186}$,

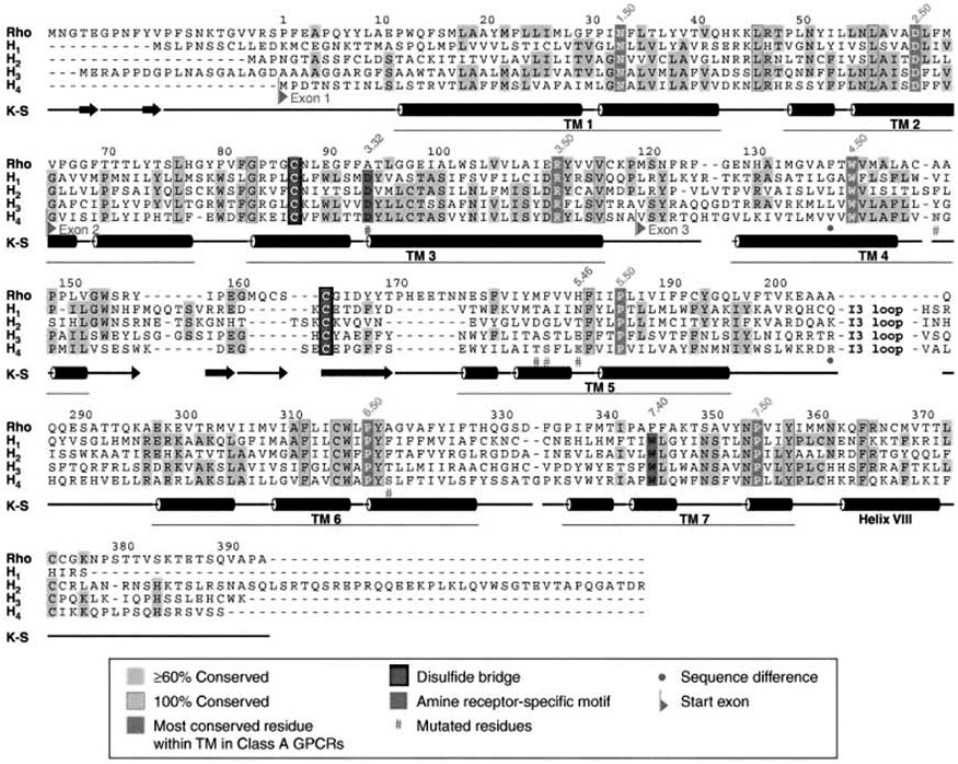


Fig. (1). Sequence alignment of the sequences of bovine rhodopsin and human histamine H_1, H_2, H_3 and H_4 receptors are shown in figure. The secondary-structure elements of the X-ray structure of bovine rhodopsin that are identified by the Kabsch-Sander algorithm are depicted below the sequences (K-S). The transmembrane(TM) domain is underlined. Residues in a TM domain that are highly conserved in the Class A GPCRs are shown on a dark grey background. These are key residues in the numbering scheme and are denoted by the number of the TM domain followed by '.50' (e.g. 1.50). The two residues (D3.32 and W7.40) that are the minimum needed to identify an amine receptor are shown on a black background. Mutated amino acid residues in the G Protein-Coupled Receptor Data Base are indicated by # and are shown in black. The residues that are essential for binding histamine (D3.32 and E5.46) are labeled with their numbers. Residues that are most likely to be involved in a disulfide bond that links TM3 to the second extracellular loop are shown on a dark black background. Amino acid residues are indicated by a black circle. The start of the exons is indicated by a black flag [32].

$P6.50^{318}$ and $P7.50^{355}$. A sequences fingerprint, which was specific for amine-activated GPCRs $D3.32^{94}$ and $W7.40^{345}$, was also reported to be present in the H_4 receptor [36]. The activation mechanism of the H_4 receptor was likely to be similar to that of other class A GPCRs. Therefore the conserved sequence motifs were near to the intracellular part of the receptor. Fig. (**2**) [17] is representative schematic diagram of the human H_4 receptor embedded in a cell membrane (box). Transmembrane regions were numbered and depicted with a top (extracellular)/bottom (intracellular) orientation.

The human Met-TM2 (transmembrane domain 2) and TM5-TM6 probes were used in Northern analyses of various human and rat tissues. In the rat, the TM5-TM6 probe revealed a single transcript of 3 kb in the testis [17]. The rat DNA fragment encoding from the start methionine to TM2 was used in Northern analyses of various rat tissues, revealing a 3-kb transcript in intestine. The H_3 and H_4 receptors had significantly different mRNA expression distributions. H_4 mRNA was detected in two peripheral tissues (with no detectable levels in brain or various peripheral tissues, including heart, stomach, small intestine, kidney, or liver). In contrast, H_3 mRNA has been reported to be abundant in the brain [8]. Therefore, it was concluded that H_4 was not likely to be the H_3-subtype characterized previously in brain tissue [37, 38].

LIGAND BINDING SITE OF H_4 RECEPTOR WITH PHARMACOPHORIC REQUISITION

In 2002, Shin *et al.* [39] studied the molecular modeling and site-specific mutagenesis of the histamine-binding site of the histamine H_4-receptor. Based on this study, the binding pocket for histamine had been identified in a pocket formed by TM3, TM5 and TM6. The initial model of histamine docked into the hypothetical binding site in the H_4 receptor is shown in Fig. (**3**) [39]. Histamine was predicted to bind in a pocket formed by residues in TM3 through TM6, anchored by an ion pair between the side chain of Asp94 (3.32) in TM3 and the cationic amino group of histamine [39]. On the basis of construction of a sequence motif characteristic and binding site of aminergic G protein-coupled receptors, it was reported that the conserved aspartic acid residue in TM3 ($D3.32^{94}$), which was present in all biogenic amine receptors [36, 40], was essential for binding histamine. In addition to that, $E5.46^{182}$ also played a crucial role for the binding of histamine [39] and was proposed to bind to the imidazole ring, mimicking the role of N5.46 in the H_1-receptor [38, 39]. The increase in binding affinity of histamine for the H_4 receptor compared with the H_1 receptor was justified by the

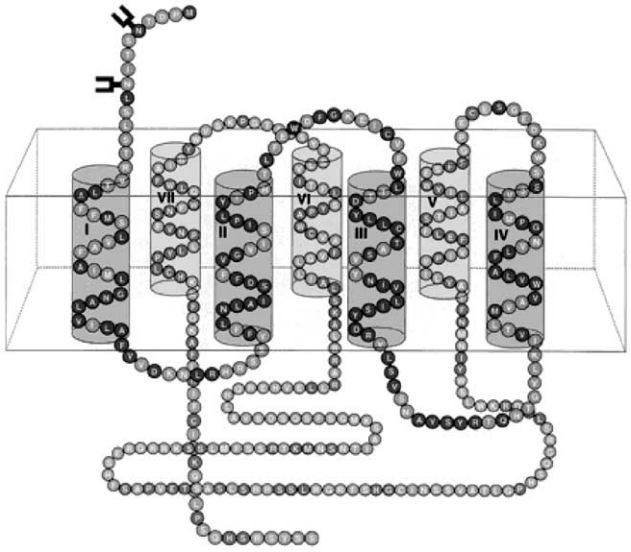


Fig. (2). Representative schematic of the human H_4 receptor embedded in a cell membrane (box) [17].

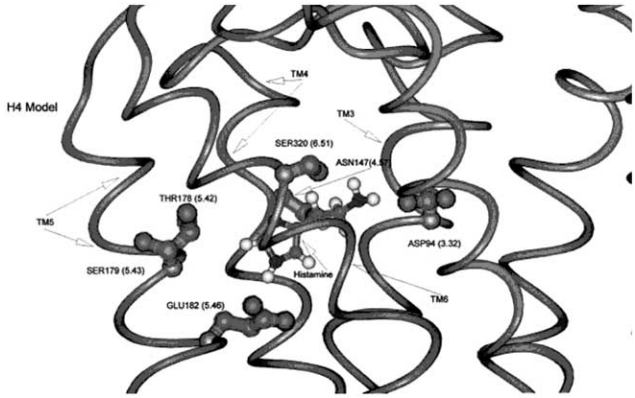


Fig. (3). A molecular model of human H_4 receptor-histamine complex is shown in figure. A molecular model of the human H_4 receptor was constructed from the structure of bovine rhodopsin and refined by 1000 steps. Asp94 (3.32) in TM3 forms an ion pair to the cationic amino group of histamine. Thr178 (5.42) and Ser179 in TM5 form a hydrogen bond to the imidazole N nitrogen. Glu182 (5.46) in TM5 could form an ion pair to the protonated imidazole N nitrogen. Asn147 (4.57) in TM4 and Ser320 (6.52) in TM6 are directed toward the central histamine-binding cavity [39].

increased ability of $E5.46^{182}$ towards the imidazole ring compared with an asparagines residue to form an ionic bond. Based on the H_4 receptor-homology model, it was suggested that $S6.52^{320}$ was also involved in ligand binding [39]. Position 6.52 behaved as a part of a cluster of highly conserved aromatic residues in TM6, the C6.47/W6.48/P6.50/F6.52 motif, which faced the binding site and was thought to be involved in both ligand binding and receptor activation [40]. The presence of a non-aromatic, polar residue in this position (threonine in the H_3 receptor and serine in the H_4 receptor, Fig. **3**) [32] might contributed to the increase in the affinity of histamine for H_3 and H_4 receptors compared with H_1 and H_2 receptors. Mutation of residue 6.52 to phenylalanine, as in H_1 and H_2 receptors, greatly reduces the potency of histamine [39].

Intracellular Signaling Transduction Mechanism for H_4-Receptor

Histamine H_4 receptor was mainly couple to Gi/o proteins (Fig. **4**) [32]. In either stably or transiently transfected cells, interaction of histamine with the H_4 receptor activated a member of the Gi/o α-protein family. Upon stimulation, the α-subunit and βγ-subunit participated in intracellular signaling. The α-subunit was responsible for the negative regulation of adenyl cyclase (AC) activation, which ultimately inhibited the formation of cAMP from ATP. The cAMP was responsible for the stimulation of protein kinase A (PKA), which led to phosphorylation of cAMP responsive element-binding protein (CREB). Activation of Gi/o was also likely to stimulate mitogen-activated protein kinase (MAPK) activity in stably transfected HEK-293 cells. Histamine mediated activation of either endogenous H_4 receptors in mast cells [23] or H_4 receptors were stably expressed in L1.2 cells resulted in a clear calcium response (Fig. **4**). H_4 receptor mediated calcium signaling in mast cells was sensitive to both pertussis toxin and the phospholipase C (PLC) inhibitors like U73122 (**2**) [23], which indicated that phospholipase C (PLC) was activated *via* Gβγ subunits which dissociated from Gi/o proteins following H_4 receptor stimulation in mast cells. Therefore, the βγ-subunit was most probably involved in the activation of phospholipase C-β (PLC-β) and PLC-β was responsible for the hydrolysis of phospholipids phophatidylinositol(4,5)-bisphosphate (**3**) [ptdlns(4,5)P2] to the second messenger inositol(1,4,5)-trisphosphate [Ins(1,4,5)P3] (**4**) and diacylglycerol (DAG). The production of Ins(1,4,5)P3 cause an up-regulation in the intracellular concentration of calcium {$[Ca^{+2}]i$}. The calcium response in mast cells was likely to be linked to cellular chemotaxis mechanism because similar sensitivity to U73122 and pertussis toxin was reported with histamine induced chemotaxis in mast cells. Several studies also confirmed the presence of both functional H_4 receptors [12, 20, 41] and histamine induced calcium signaling in eosinophils [41].

FUNCTIONS OF THE H_4 RECEPTOR

H_4 Receptor Mediated Chemoattractant Effect in Eosinophils

In 1975, Clark *et al.* performed study on selective eosinophil chemotactic activity of histamine and observed that low concentrations of histamine induced eosinophil chemotaxis, which is now known to be mediated by the H_4 receptor [42], and this pharmacological effect could be inhibited by H_4 receptor antagonists [12, 20]. Chemotaxis, driven by the polarization of the cell and was detected as a change in cell shape. Histamine rapidly induced a shape change in eosinophils and enhanced the response to chemokines [20, 41]. This effect could be antagonized by H_4 receptor antagonists rather than antagonists of other histamine receptors. The effect of histamine on cell shape was also blocked by pertussis toxin, indicated that the H_4 receptor mediated effect by signaling *via* Gi/oα proteins [41]. Stimulation of H_4 receptor also leads to actin polymerization, as a result in the polarization of eosinophils [41]. Histamine acted through the H_4 receptor to mediate upregulation of CD11b and CD54 in eosinophils [20, 41]. Cluster of

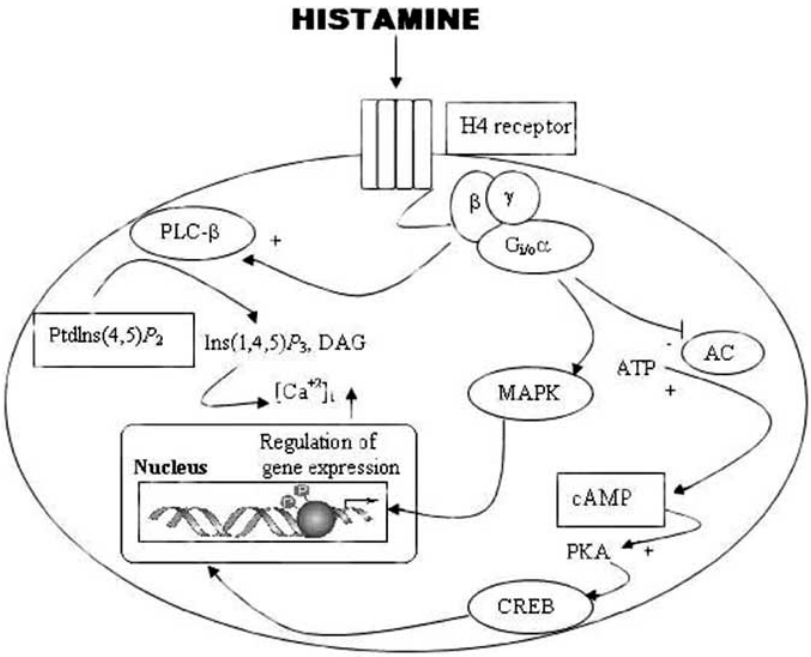
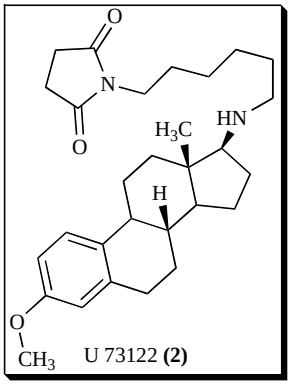


Fig. (4). Interaction between histamine and H_4 receptor activates a member of the Gi/o protein family [32].

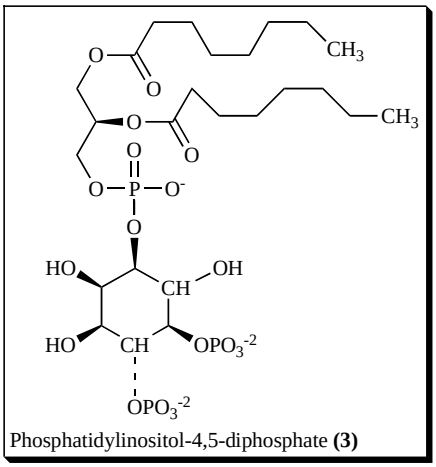
differentiation molecule 11b (CD11b) is Integrin alpha M (ITGAM), a protein subunit that formed the heterodimeric integrin alpha-M beta-2 ($\alpha_M\beta_2$) molecule. $\alpha_M\beta_2$ is expressed on the surface of many leukocytes involved in the innate immune system. It mediated inflammation by regulating leukocyte adhesion and migration and has been implicated in several immune processes such as phagocytosis, cell-mediated cytotoxicity, chemotaxis and cellular activation [43]. CD54 (Cluster of Differentiation 54), also known as Inter-Cellular Adhesion Molecule, is an endothelial- and leukocyte-associated transmembrane protein long known for its importance in stabilizing cell-cell interactions and facilitating leukocyte endothelial transmigration [44].

H_4 Receptor Mediated Chemotaxis Effect in Mast Cells

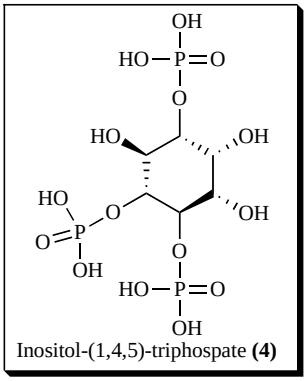
H_4 receptor mediated histamine-induced chemotaxis of mast cells was observed in mouse which was blocked by H_4 receptor antagonists [22, 25], but this effect was not found in mast cells derived from H_4 receptor deficient mice, which further supported the role of H_4 receptor in chemotaxis of mast cells [22]. Histamine induced chemotaxis in mast cells was inhibited by pertussis toxin and also by phospholipase C (PLC) inhibitors, which was an indication that inositol(1,4,5)-triphosphate [Ins(1,4,5)P3] might be an important mediator of this type of physiological effect. Histamine H_4 receptor was involved in histamine induced migration of mast cell that increased number of mast cells and sub-epithelial mast cells in the trachea of mice [25]. This effect was inhibited by selective H_4 receptor antagonist rather than the antagonist of other histamine receptor. The administration of histamine to humans might mimic the effects of antigen because exposure to antigens leads to a redistribution of mast cells to the epithelial lining of the nasal mucosa [45, 46]. Mechanism of histamine-induced recruitment of mast cells and eosinophils in chronic allergic inflammation is represented in Fig. (**5**) [47]. On the surface of both eosinophils and mast cells, histamine H_4 GPCRs was expressed. Antigen-IgE complex-dependent cross-linking on



U 73122 **(2)**



Phosphatidylinositol-4,5-diphosphate **(3)**



Inositol-(1,4,5)-triphospate **(4)**

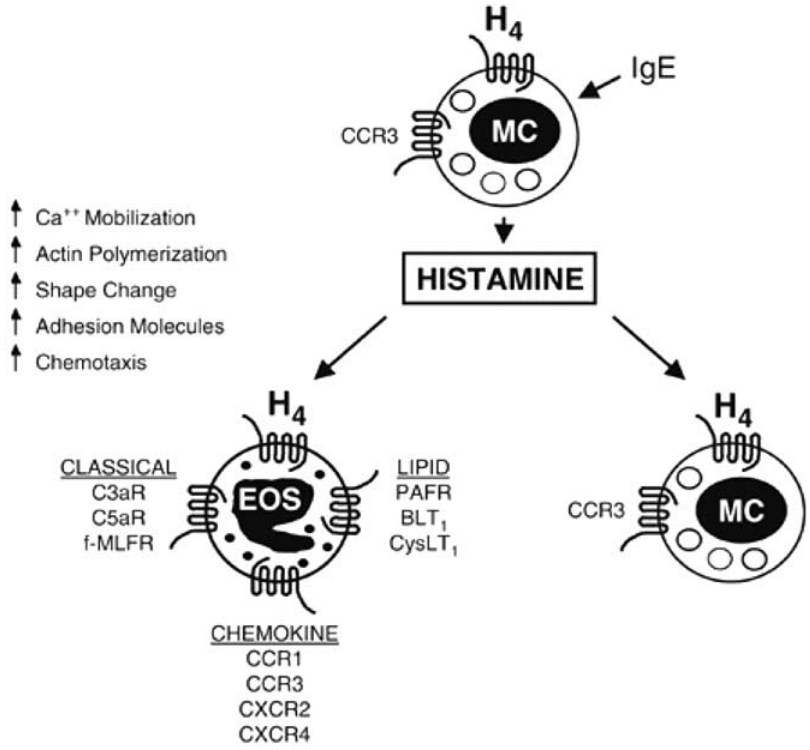


Fig. (5). Mechanism of histamine-induced recruitment of mast cells and eosinophils in chronic allergic inflammation. Abbreviations: EOS- eosinophil; MC- mast cell; IgE- immunoglobulin E; H4- histamine receptor type 4; PAFR- platelet activating factor receptor; BLT1- leukotriene B4 receptor type 1; CysLT1- leukotriene D4 receptor type 1; CCR1- C-C chemokine receptor type 1; CCR3- C-C chemokine receptor type 3; CXCR2- C-XC chemokine receptor type 2; CXCR4- C-X-C chemokine receptor type 4; C3aRcomplement 3a receptor; C5aR- complement 5a receptor; f-MLFR- N-formyl-methionylleucyl-phenylalanine receptor [47].

the surface of resident mast cells stimulated the secretion of histamine. It bound and activated the H_4 receptor on eosinophils and produced transduction events like calcium mobilization, actin polymerization, shape change and upregulation of adhesion molecule expression. This lead to directional migration (chemotaxis) and accumulation of eosinophils into sites of inflammation.

H_4 Receptor Mediated Secretion Of Interleukin 16 (IL-16) From $CD8^+$T Cells

H_4 receptor also played role in the secretion of interleukin 16 (IL-16) from $CD8^+$T cells [19]. Interleukin 16 (IL-16) is a cytokine, released by a variety of cells (including lymphocytes), and characterized as a chemoattractant for certain immune cells expressing the cell surface molecule CD4 [48]. A cytotoxic T cells (also known as $CD8^+$ T-cells) belonged to a sub-group of T lymphocytes. They were capable of inducing the death of infected somatic or tumor cells and killed cells which were infected with viruses (or other pathogens), or were otherwise damaged or dysfunctional [49]. Stimulation of both H_4 and H_2 receptors appeared to be required for this pharmacological effect because inhibitors of either blocked the response. However, both inhibitors, either alone or in combination, caused a maximal inhibition of 70%. Although (R)-α-methyl histamine (**5**) and clobenpropit (**6**), agonists at the H_4 receptor, induced the release of IL-16, the concentration of clobenpropit needed for this effect was much greater than expected from its potency [20].

Physiological Role of H_4-Receptor *In vivo*

Histamine H_4 receptor was shown to be primarily expressed on leukocytes and implicated in the activation of lymphocytes, eosinophils, and mast cells *in vitro*. Its functions *in vivo*, however, had not yet been fully characterized, but the distribution of H_4 mRNA suggested that it might play a role in the regulation of inflammatory and immune response, particularly with respect to allergy and asthma [39, 47, 50-52]. In 2003, Takeshita, *et al.* [50] reported the evidence for H_4 receptor being critical for mast cell-dependent neutrophil recruitment. Thioperamide (**7**), a well-characterized H_4 antagonist, which was not specifically targeting H_1 or H_2 receptor, significantly reduced zymosan (yeast-cell wall particles that contain mainly polysaccharide) induced neutrophilia. Although thioperamide might be able to potently block H_3 receptor, it most likely acted on H_4 receptor expressed on mast cells which completely lacked H_3 receptor (This may be the case for only human skin mast cells). This conclusion was further supported by novel data of H_3 receptor-inactive and H_4-selective antagonist JNJ7777120 (**8**), which showed identical efficacy in zymosan-induced mast cell-dependent neutrophilia in the mouse. Another physiological role of histamine was as a mediator of itch [53]. The H_4 receptor was also implicated in histamine induced scratching in mice [54]. Histamine, clobenpropit (**6**) and imetit (**9**) each induced dose-dependent scratching. Either H_1 receptor antagonists or thioperamide blocked the effects of histamine, whereas H_1 receptor antagonists had no effect on clobenpropit induced itch.

Thioperamide blocked clobenpropit and imetit induced itch, which indicated that the H_4 receptor was also involved in histamine-induced itch, therefore H_4 receptor antagonists might be effective to treating pruritis, which was not controlled by H_1 receptor antagonist.

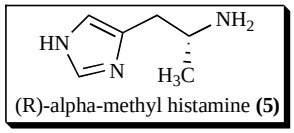
H_4 receptors modulated eosinophil migration and selective recruitment of mast cells leading to amplification of histamine-mediated immune responses and eventually to chronic inflammation [55]. H_4R is involved in chemotaxis and inflammatory mediator release by eosinophils, mast cells, monocytes, dendritic cells, and T cells. H_4R stimulation enriched a regulatory T cell with potent suppressive activity for proliferation. Studies in animal models using selective antagonists or H_4R-deficient mice showed a role for the receptor in inflammation *in vivo*. These findings identified a novel function for H_4R and suggested a potential therapeutic approach to attenuation of asthmatic inflammation and pruritus [56-58]. *In vitro* studies by Dunford *et al*. indicated that blockade of the H_4R on dendritic cells led to decrease in cytokine and chemokine production and limited their ability to induce Th2 responses in T cells. This work suggested that H_4R could modulate allergic responses *via* its influence on T cell activation. The study expanded the known influences of histamine on the immune system and highlighted the therapeutic potential of H_4R antagonists in allergic conditions [59].

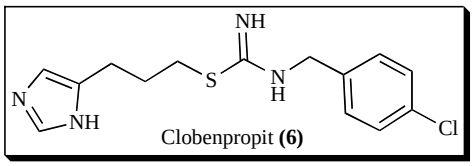
It is assumed that many GPCRs were restrained in an inactive state by the "ionic lock" [60]. The ionic lock is a part of the conserved (D/E)RY motif at the intracellular end of TM3. This motif formed an intrahelical salt bridge between the D130 and R131 (in B2AR), as well as an interhelical salt bridge between TM3 and TM6 (R131 and E268 in B2AR) which stabilized the inactivated form of B2AR. Disruption of this lock showed to be a required step for activation, and it occured upon binding of nearly all agonists. In addition, mutations of the lock residues lead to constitutively active mutants [61]. Schneider *et al*. concluded that constitutive activity of H_4R was facilitated by the salt bridge D5.69-R6.31 rather than by the missing ionic lock and Y3.60 might form alternative locks in active and inactive GPCR states. They also proved that H_4R-R3.50A represented an inactive state with increased inverse agonist and reduced agonist affinity, thus proving that the ionic lock was not generally important for all class A GPCRs [62].

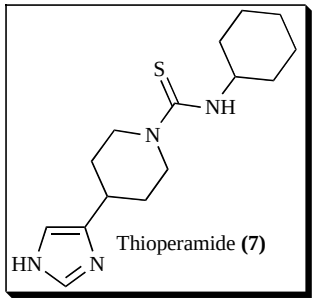
SELECTIVITY FOR H_4 RECEPTOR LIGAND AND THEIR PHARMACOLOGICAL PROPERTIES

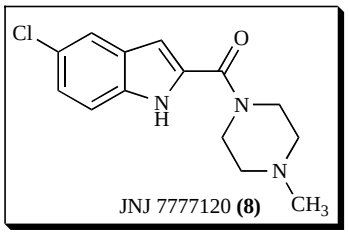
The search for new and potent histamine H_4 receptor ligands led to a steadily increased number of scientific publications and patent applications [63]. Preclinical data strongly suggested that H_4 receptor is potential therapeutic target in allergy, inflammation, autoimmune disorders and possibly cancer [31, 64, 65]. The pharmacological profiles of H_3 receptor [66, 67] and H_4 receptor showed strong species differences, but early studies indicated that they overlap due to high sequence similarity. Several H_3 receptor agonists or antagonists had appreciable activity at the H_4 receptor. Therefore, it was not surprising that the H_4 receptor was activated by several H_3 receptor agonists [30], including Immepip (**10**) (H_4 receptor; Ki=9 nM) [16], Imetit (**9**) (H_4 receptor; Ki=5 nM) [17] and (R)-α-methyl histamine (**6**) (H_4 receptor, Ki=146 nM) [52]. Moreover, the H_4 receptor was activated by the both H_2 and H_3 receptor antagonist burimamide (**11**) (H_4 receptor, Ki=180 nM) [52] and the H_3 receptor antagonist clobenpropit (H_4 receptor, Ki =13 nM). In pharmacology, an inverse agonist is an agent that binds to the same receptor binding-site as an agonist for that receptor and reverses constitutive activity of receptors [68]. Inverse agonists exerted the opposite pharmacological effect of a receptor agonist. Thioperamide (**7**), an inverse agonist at H_3 receptors, was also an inverse agonist at the H_4 receptor (H_4 receptor, Ki=27nM) [52]. All these compounds, served as reference compounds for the H_3 receptor, were no longer considered to be selective agents. More recently, newer H_3 receptor agonists, such as immethridine (**12**) [69] and methimmepip (**13**) [70] were identified as potent and selective H_3 receptor agonist with a safe H_3 receptor over H_4 receptor selectivity margin of 288 and 2000 respectively. Some non-imidazole H_3 receptor antagonists, such as JNJ6379490 (**14**) [71] and A349821 (**15**) [72], had safe margins of selectivity. Moreover, new ligands with improved selectivity for the H_4 receptor were being developed. The two methylcyanoguanidine derivatives of imifuramine (**16**), OUP-13 (**17**) and OUP16 (**18**), were described as a full agonist of H_4 receptors, but displayed a higher potency with a 40-fold selectivity over the human H_3 receptor [73]. Furthermore, 4-methylhistamine (**19**) was reported as more potent and selective H_4 receptor agonist than OUP16 [74]. A series of dibenzodiazepine derivatives were synthesized to probe the binding site of the histamine H_4 receptor. Clozapine (**20**) is an H_4-receptor agonist. This agonist activity might be related to the serious side effect of agranulocytosis caused by clozapine [75]. Optimization of the lead structure clozapine resulted in (E)-7-chloro-11-(4-methylpiperazin-1-yl)dibenzo[b,f][1,4]oxazepine (**21**), a potent H_4 receptor agonist (H_4 receptor, pKi = 7.6). Pharmacological data suggested that the series of non-imidazole compounds could be used to describe the orthosteric binding site of the H_4 receptor because both **20** and **21** displaced histamine in a competitive manner [76]. The newly discovered histamine H_4 receptor subtype was emerged as a new and complementary target for treating inflammatory conditions [77]. Following high-throughput screening and medicinal chemistry input, Johnson & Johnson Pharmaceutical Research & Development LLC/Abbott Laboratories introduced the reference H_4 receptor antagonist 1-[(5-chloro-1H-indol-2-yl)carbonyl]-4-methylpiperazine (JNJ7777120) (**8**) and proved the efficacy of this agent in models of asthma, allergic rhinitis and pruritus, highlighted the H_4R as a novel drug target [65].

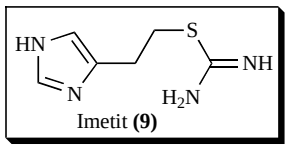
The benzimidazole analogue of JNJ7777120 (VUF6002: (5-chloro-1H-benzo[d]imidazol-2yl)(4-methylpiperazin-1-yl)methanone)) (**22**) was also identified as H_4 receptor antagonists with high affinity and selectivity. Both, JNJ7777120 and VUF6002, acted as neutral antagonists [78, 79]. JNJ7777120 had a Ki of 4.5 nM versus the human receptor and a pA2 of 8.1. It was equipotent against the human, mouse, and rat receptors. It exhibited at least 1000-fold selectivity over H_1, H_2, or H_3 receptors and had no cross-reactivity against 50 other targets. This compound had an oral bioavailability of 30% in rats and 100% in dogs, with

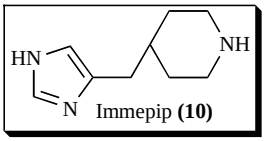

(R)-alpha-methyl histamine **(5)**

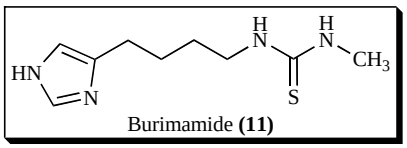

Clobenpropit **(6)**

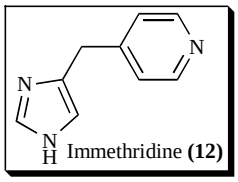

Thioperamide **(7)**

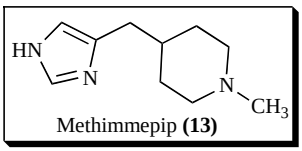

JNJ 7777120 **(8)**

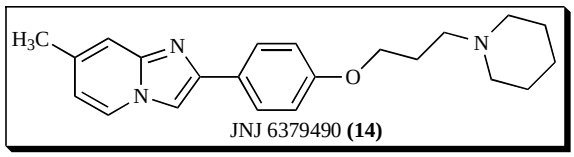

Imetit **(9)**

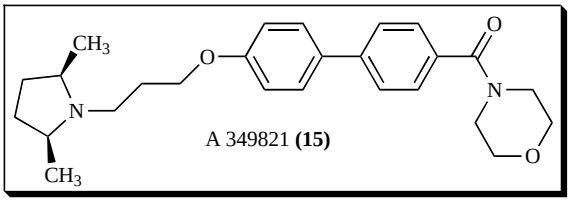

Immepip **(10)**

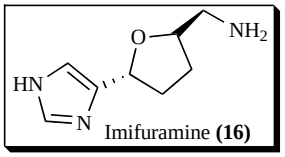

Burimamide **(11)**

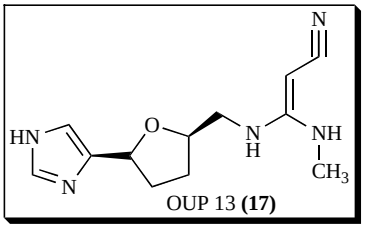

Immethridine **(12)**

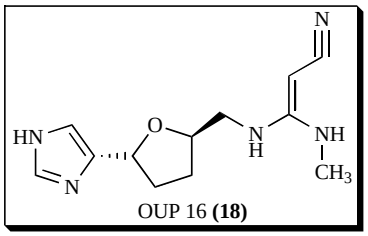

Methimmepip **(13)**

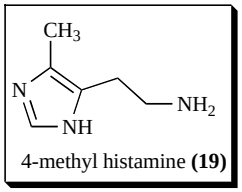

JNJ 6379490 **(14)**

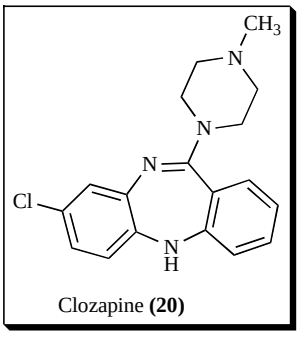

A 349821 **(15)**

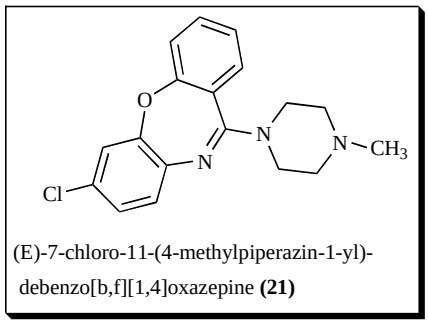

Imifuramine **(16)**

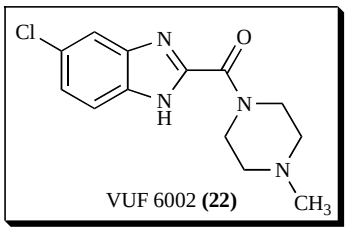

OUP 13 **(17)**


OUP 16 **(18)**


4-methyl histamine **(19)**


Clozapine **(20)**


(E)-7-chloro-11-(4-methylpiperazin-1-yl)-debenzo[b,f][1,4]oxazepine **(21)**


VUF 6002 **(22)**

a half-life of ~3 h in both species. JNJ7777120 blocked histamine-induced chemotaxis and calcium influx in mouse bone marrow-derived mast cells. In addition, it could block the histamine-induced migration of tracheal mast cells from the connective tissue toward the epithelium in mice. It significantly blocked neutrophil infiltration in a mouse zymosan-induced peritonitis model. These results indicated that the histamine H_4 receptor played a role in the inflammatory process. Selective H_4 receptor antagonist like JNJ7777120, was the most potent H_4 receptor antagonist and used as a radioligand for H_4 receptors. It might have potential to be useful in treating inflammation in humans.

Allergic rhinitis, asthma, and rheumatoid arthritis were just a few of the conditions where mast cells and eosinophils were involved and H_4 receptor antagonists might have therapeutic utility [25]. Another study also reported the effects of the highly selective histamine H_4 receptor antagonists JNJ7777120 and VUF6002. Both were investigated on the carrageenan-induced inflammation and thermal hyperalgesia in rats. JNJ7777120 (10 and 30 mg/kg, s.c.) and VUF6002 (10 mg/kg, s.c.) significantly reduced paw edema and hyperalgesia provoked by subplantar injection of carrageenan. This effect was evident against the early (2 h) phase of inflammation. An inactive analog of VUF6002, VUF6007 (**23**) (10 mg/kg, s.c.), slightly aggravated paw edema, while leaving unaltered carrageenan-induced nociception. These findings indicated that histamine H_4 receptors participated in the early phase of acute inflammation induced by carrageenan in rats and influenced both edema and thermal hyperalgesia [80].

During an in-house database screen, S-(2-guanidylethyl)-isothiourea(VUF 8430) (**24**) was identified as a high affinity agonist for histamine H_4 receptor (pKi = 7.5) with 33-fold selectivity over the histamine H_3 receptor (pKi = 6.0) and negligible affinity for the other histamine receptor subtypes. This nonimidazole ligand was introduced as a useful and complementary pharmacological tool which further unraveled the physiological roles of the H_4 receptor [81].

SELECTIVE HUMAN H_4-RECEPTOR AGONISTS

(-)-2-Cyano-1-methyl-3-{(2*R*,5*R*)-5-[1*H*-imidazol-4(5)-yl]tetrahydrofuran-2-yl}methylguanidine (18)

A series of 16 compounds related to chiral 4(5)-(5-aminomethyltetrahydrofuran-2-yl) imidazoles (**25**) were designed, synthesized, and examined *in vitro* by radioligand displacement studies and functional assays for both, human H_3 and H_4-receptors expressed in SK-N-MC cells. Among them, the (2S,5S)-isomer of amino compound (**25**) showed approximately 300-fold higher selectivity at the H_3-receptor than the H_4-receptor.

On the other hand, (2R,5S) and (2R,5R)-cyanoguanidines (**17**,**18**) bound to the H_4-receptor with a pEC50 value of 6.65 and 7.11, respectively, and had more than 40-fold selectivities over the H_3-receptor. As such, they were the first selective H_4 receptor agonists [73].

IMMIDAZOLE CONTAINING H_4 RECEPTOR ANTAGONISTS

Johnson &Johnson developed a variety of structurally diverse compounds based upon JNJ7777120. Compound (5-chloro-1H-benzo[d]imidazol-2-yl)(4-methylpiperazin-1-yl) methanone (**26**), a bioisosteric benzimidazole analog [79, 82], had affinities close to that of JNJ7777120 and showed somewhat improved metabolic stability *in vitro*. Pfizer Inc. had successfully replaced the N-methylpiperazine moiety of **26** with an octahydropyrrolo[3,4-c]pyrrole developing the final compound (5-fluoro-4-methyl-1H-benzo[d]imidazol-2-yl)((3aR,6aS)-5-methylhexahydropyrrolo[3,4-c]pyrrol-2(1H)-yl) methanimine (**27**) [83]. Compound 6-chloro-2-(4-(3-(4-methylpiperazin-1-yl)propoxy)phenyl)-1H-benzo[d] imidazole (**28**), a benzimidazole compound, was synthesized from HTS efforts from Johnson and Johnson [84,85]. A very extensive series of compounds, **28** and N-(4-(4-methyl-1,4-diazepan-1-yl)butyl)-4-(7-methyl-1H-benzo[d]imidazol-2-yl) pyridin-2-amine (**29**), were synthesized by alterations of the benzimidazole structures [86–88]. In these compounds, the benzimidazole scaffold was attached to a substituted phenyl or heterocycle moiety. These compounds were claimed to have high affinity for the H_4R. A novel series of compounds were designed by replacing benzimidazole by a di-phenyl substituted imidazole [e.g. 1-(3-(4-(4,5-bis(3-methoxyphenyl)-1H-imidazol-2-yl)-3-chlorophenoxy)propyl)-4-methyl-1,4-diazepane (**30**)] [89]. The potencies of these ligands were, however, a bit lower than those found for the benzimidazole based series.

IMMIDAZOLE FREE H_4 & H_3 RECEPTOR ANTAGONISTS

2-(4-alkylpiperazin-1-yl)Quinoline (31)

With the aim of identifying structurally novel centrally acting histamine H_4 antagonists, a series of 2-(4-alkylpiperazin-1-yl)quinoline was synthesized. Systematic variation of the substituents led to highly potent histamine H_4 antagonists with low polar surface area and appropriate log P for blood-brain barrier penetration [90].

1-alkyl-4-acylpiperazines (32)

With the aim of identifying structurally novel, centrally acting histamine H_3 antagonists, several of monoacyl-diamines were screened. This led to the discovery of a series of 1-alkyl-4-acylpiperazines which were potent antagonists at the human histamine H_3 receptor. The most potent amides had antagonist potencies in the subnanomolar range [91].

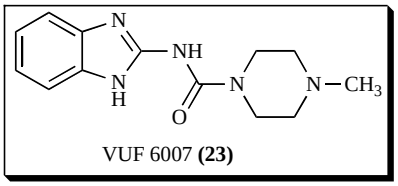
Various compounds, classified depending upon their affinity to H_4 receptor, are as follows: [17, 81, 92-103]

High Affinity Compounds

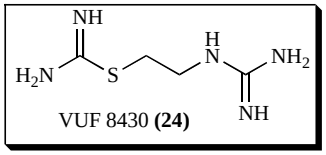
Immethridine (**12**), OUP 16 (**18**), 4-methylhistamine (**19**), VUF 8430 (**24**), Chlorpromazine (**33**), Pormethazine (**34**), Doxepine (**35**), Amitryptiline (**36**), Cinnarizine (**37**). (2-chloro-6H-thieno[2,3-b]pyrrol-5-yl)(4-methylpiperazin-1-yl)methanone (**38**), 4-(4-methylpiperazin-1-yl)-6-phenyl-pyrimidin-2-amine (**39**), 4-(3-(methylamino)azetidin-1-yl)-6-phenylpyrimidin-2-amine (**40**), 4-(2-amino-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)benzonitrile (**41**), N4-(3-chlorophenyl)-6-(4-methylpiperazin-1-yl)pyrimidine-2,4-diamine (**42**), N4-benzyl-6-(4-methylpiperazin-1-yl)pyrimidine-2,4-diamine (**43**), 4-((S)-3-(methylamino)pyrrolidin-1-yl)-6-(2-phenylcyclopropyl)pyrimidin-2-amine (**44**), 4-(piperazin-1-yl)-5,6,7,8-tetrahydroquinazolin-2-amine (**45**) and 4-(isoindolin-2-yl)-6-(4-methylpiperazin-1-yl)pyrimidin-2-amine (**46**).

Moderate/Medium Affinity Compounds

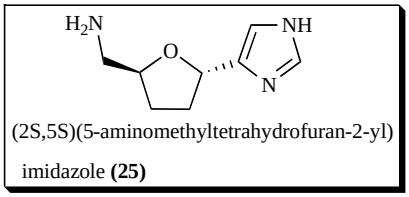
Clozapine (**20**), Clemizole (**47**), Cyproheptadine (**48**), Mianserin (49), Chlorpheniramine (**50**), 2-benzyl-3-(4-methylpiperazin-1-yl)quinoxaline (**51**), 6-chloro-2-(4-methyl-piperazin-1-yl)-N-(thiophen-2-ylmethyl)quinazolin-4-amine (**52**), 8-chloro-4-(3-fluoro-4-(methylamino)pyrrolidin-1-yl)benzofuro[3,2-d]pyrimidin-2-amine (**53**) and 8-chloro-



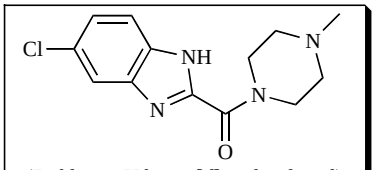
VUF 6007 **(23)**



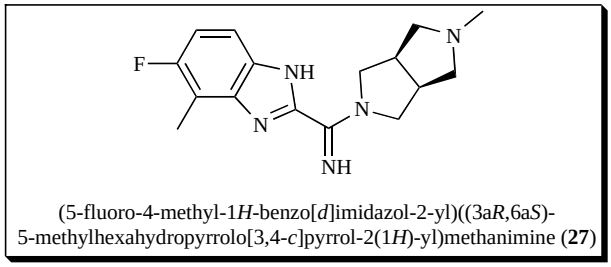
VUF 8430 **(24)**



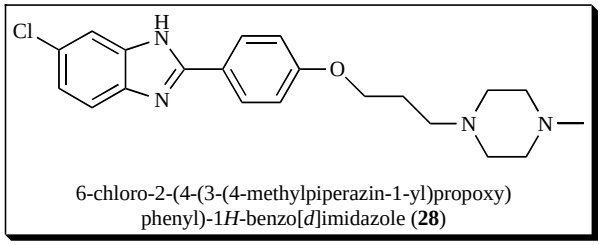
(2S,5S)(5-aminomethyltetrahydrofuran-2-yl) imidazole **(25)**



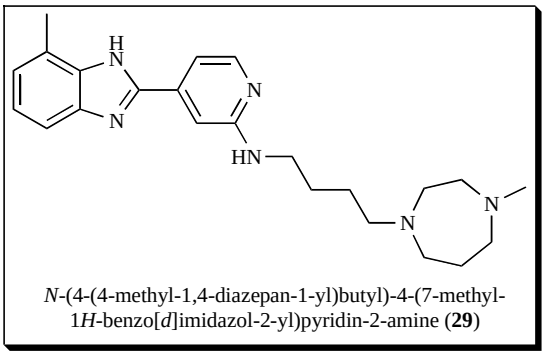
(5-chloro-1*H*-benzo[*d*]imidazol-2-yl) (4-methylpiperazin-1-yl)methanone (**26**)



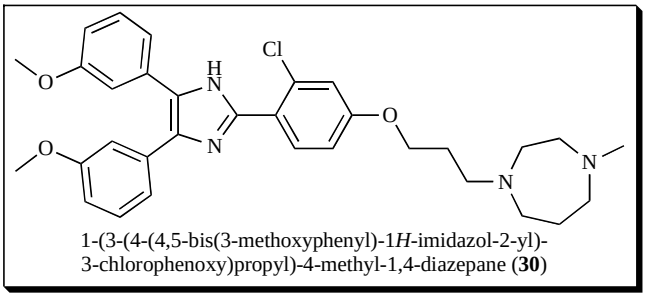
(5-fluoro-4-methyl-1*H*-benzo[*d*]imidazol-2-yl)((3a*R*,6a*S*)-5-methylhexahydropyrrolo[3,4-*c*]pyrrol-2(1*H*)-yl)methanimine (**27**)



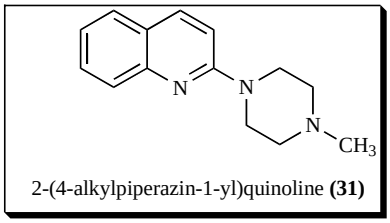
6-chloro-2-(4-(3-(4-methylpiperazin-1-yl)propoxy) phenyl)-1*H*-benzo[*d*]imidazole (**28**)



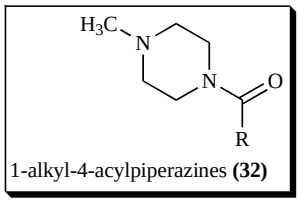
N-(4-(4-methyl-1,4-diazepan-1-yl)butyl)-4-(7-methyl-1*H*-benzo[*d*]imidazol-2-yl)pyridin-2-amine (**29**)



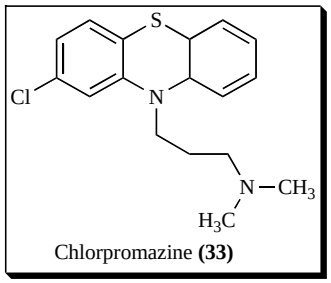
1-(3-(4-(4,5-bis(3-methoxyphenyl)-1*H*-imidazol-2-yl)-3-chlorophenoxy)propyl)-4-methyl-1,4-diazepane (**30**)



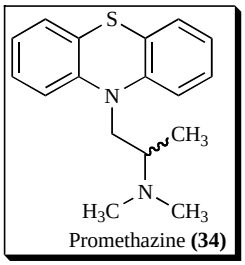
2-(4-alkylpiperazin-1-yl)quinoline **(31)**



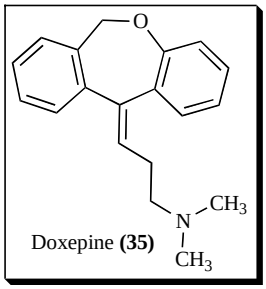
1-alkyl-4-acylpiperazines **(32)**



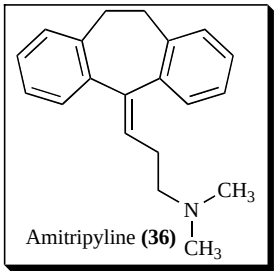
Chlorpromazine **(33)**



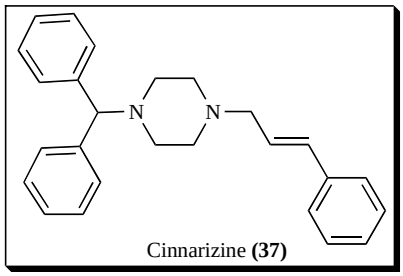
Promethazine **(34)**



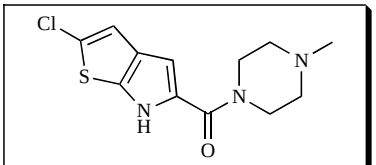
Doxepine **(35)**



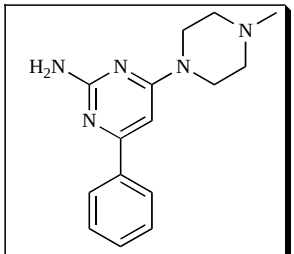
Amitripyline **(36)**



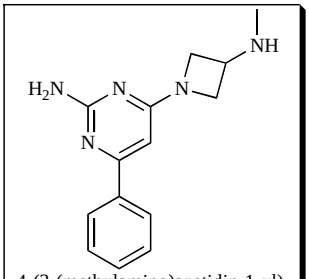
Cinnarizine **(37)**



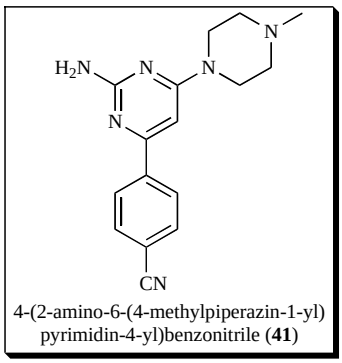
(2-chloro-6*H*-thieno[2,3-*b*]pyrrol-5-yl)(4-methylpiperazin-1-yl)methanone (**38**)



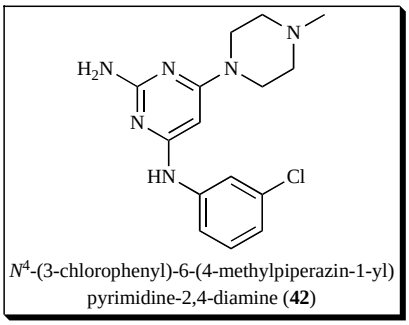
4-(4-methylpiperazin-1-yl)-6-phenylpyrimidin-2-amine (**39**)



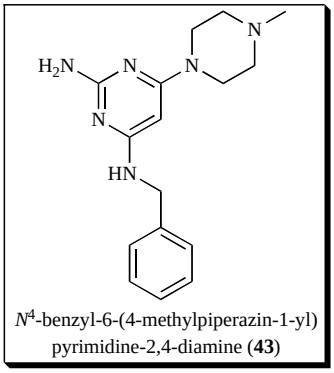
4-(3-(methylamino)azetidin-1-yl)-6-phenylpyrimidin-2-amine (**40**)



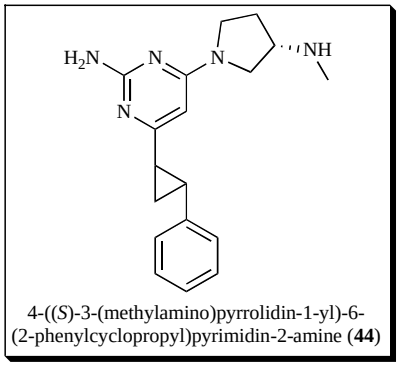
4-(2-amino-6-(4-methylpiperazin-1-yl) pyrimidin-4-yl)benzonitrile (**41**)



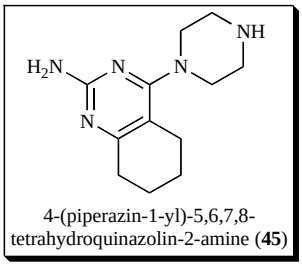
N^4-(3-chlorophenyl)-6-(4-methylpiperazin-1-yl) pyrimidine-2,4-diamine (**42**)



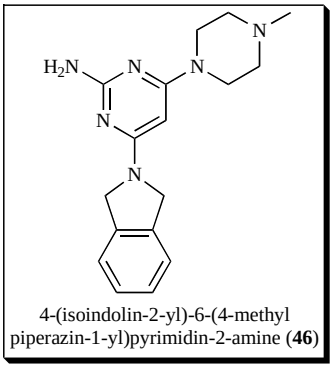
N^4-benzyl-6-(4-methylpiperazin-1-yl) pyrimidine-2,4-diamine (**43**)



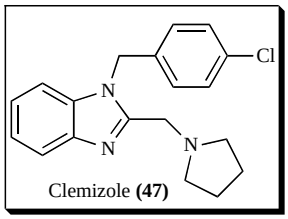
4-((*S*)-3-(methylamino)pyrrolidin-1-yl)-6-(2-phenylcyclopropyl)pyrimidin-2-amine (**44**)



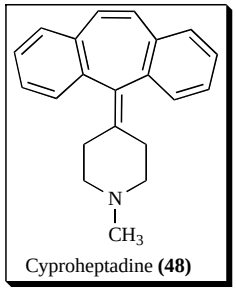
4-(piperazin-1-yl)-5,6,7,8-tetrahydroquinazolin-2-amine (**45**)



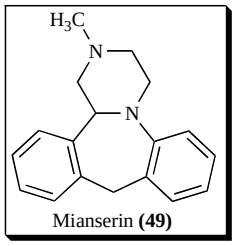
4-(isoindolin-2-yl)-6-(4-methyl piperazin-1-yl)pyrimidin-2-amine (**46**)



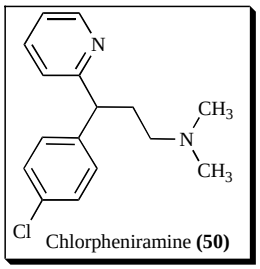
Clemizole **(47)**



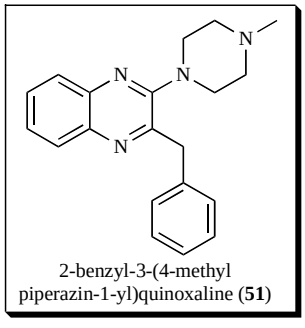
Cyproheptadine **(48)**



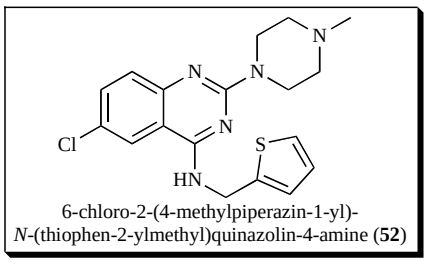
Mianserin **(49)**



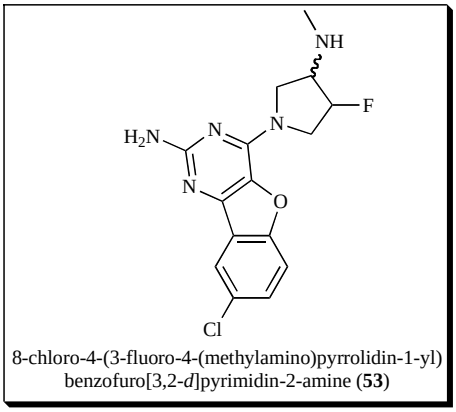
Chlorpheniramine **(50)**



2-benzyl-3-(4-methyl piperazin-1-yl)quinoxaline (**51**)



6-chloro-2-(4-methylpiperazin-1-yl)-*N*-(thiophen-2-ylmethyl)quinazolin-4-amine (**52**)



8-chloro-4-(3-fluoro-4-(methylamino)pyrrolidin-1-yl) benzofuro[3,2-*d*]pyrimidin-2-amine (**53**)

4-(4-methylpiperazine-1-yl)benzofuro[3,2-d]pyrimidin-2-amine (**54**)

Weak Affinity Compounds

Clobenpropit (**6**), Imetit (**9**), Pheniramine (**55**) and Dimaprit (**56**).

USES OF HISTAMINE H_4 RECEPTOR ANTAGONISTS

Histamine H_4 Receptor Antagonism: A Rapidly Emerging Approach in The Treatment of Autoimmune Diseases

Johnson & Johnson were the first company to publish on a selective histamine H_4 antagonist [64]. JNJ10191584 (VUF 6002) (**22**) and JNJ7777120 (**8**) were investigated as orally active antagonists. The first few studies into the function of H_4 receptors suggested that their blockade would be beneficiary to allergic diseases with an eosinophilic and/or mast cell component since histamine binding to this receptor subtype stimulated the chemotaxis of both cell types. The efficacy of histamine H_4 antagonists might extend to autoimmune disease. In particular, H_4 subtype was now implicated in rheumatoid arthritis and inflammatory bowel disease. Few papers reported that treatment with JNJ10191584 dose-dependently reduced macroscopic damage, inhibition of the TNBS-provoked elevation of both colonic myeloperoxidase and tumor necrosis factor-α (TNF-α), and a reduction in the histologically assessed increased in mucosal and submucosal thickness and neutrophil infiltration. JNJ7777120 produced similar effects. Few other papers reported that human synoviocytes from rheumatoid arthritic patients expressed H_4 receptors. Further studies involving the efficacy of H_4 receptor antagonist in models of autoimmune disease were therefore under investigation [104, 105].

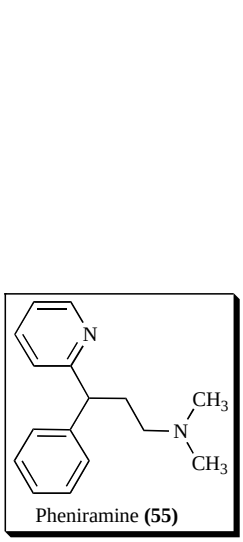
H_4 Receptor Ligands: Potential Modulators of Allergic And Inflammatory Responses

H_4 receptor antagonists had efficacy in a variety of inflammatory animal models including peritonitis, colitis and airway inflammation models. These data suggested that the H_4 receptor was an attractive medicinal chemistry target for possible treatment of inflammation, allergy and asthma [106].

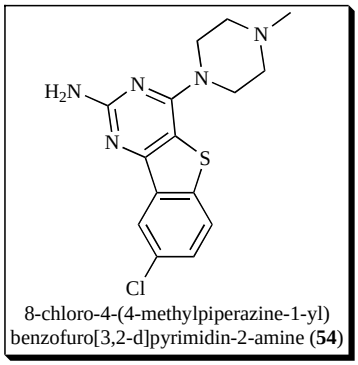
JNJ7777120 (**8**) caused a significant inhibition of nasal symptoms by both single and repeated oral administrations. Repeated oral administration of compound **8** caused significant inhibition of serum total IgE. Furthermore, it caused a significant decrease in the levels of IL-4 and a significant increase in the levels of IFN-γ in nasal lavage fluid. These results indicated that histamine H_4 receptor was closely related with allergic rhinitis and was important in the pathogenesis of allergic rhinitis [59, 107]. Scientists at Palau Pharma, A Biopharmaceutical company, developed series of furo-[3, 2-d]- pyrimidine derivatives as H_4 receptor antagonists. From developed series of compounds, they completed phase I clinical trials of an orally administered and highly safe novel H_4R antagonist, UR-63325. It blocked allergic process and the airway inflammation in both the nose and the bronchi which might substantially be used to control of the disease in patients with coexistence of asthma and rhinitis [108, 109].

Superiority of H_4 Receptor Antagonists to Traditional Antihistamines in the Attenuation of Experimental Pruritus [54, 57, 110-113]

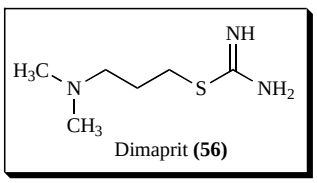
Histamine and a selective histamine H_4 receptor agonist caused scratching responses in mice, which were almost completely attenuated in histamine H_4 receptor knockout mice or by pretreatment with the selective histamine H_4 receptor antagonist, JNJ7777120. Pruritus induced by allergic mechanisms was also potently inhibited with histamine H_4 receptor antagonist treatment or in histamine H_4 receptor knockout mice. In all cases, the inhibitory effect of histamine H_4 receptor antagonist was greater than those observed with histamine H_1 receptor antagonists. Histamine H_4 receptor-mediated pruritus was independent of mast cells or other hematopoietic cells and might resulted from actions on peripheral neurons. These results demonstrated that histamine H_4 receptor was involved in pruritic responses in



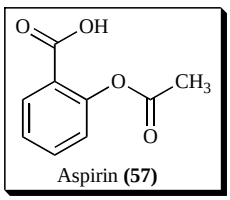
Pheniramine **(55)**



8-chloro-4-(4-methylpiperazine-1-yl) benzofuro[3,2-d]pyrimidin-2-amine (**54**)



Dimaprit **(56)**



Aspirin **(57)**

mice to a greater extent than the histamine H_1 receptor. Histamine H_4 receptor antagonists might have therapeutic utility for treating chronic pruritic diseases in humans where histamine H_1 receptor antagonists were not effective.

Histamine H_4 Receptor Antagonists: Novel Agents to Reduce Superoxide Anion Generation and Lipid Peroxidation [114]

Using a cyanide model to induce neurotoxic effects in rat brain homogenates, neuroprotective properties of H_4 antagonists, clobenpropit (**6**) and thioperamide (**7**) were compared with aspirin (**57**), a known neuroprotective agent. Superoxide anion levels and malondialdehyde concentration were assessed using the nitroblue tetrazolium and lipid peroxidation assays. Clobenpropit and thioperamide significantly reduced superoxide anion generation and lipid peroxidation. In the lipid peroxidation assay, all the drugs compared favorably to aspirin. This study demonstrated the potential of these agents as neuroprotective by exerting antioxidant effects.

Histamine H_4 Receptor: Mediator of Eosinophil Chemotaxis with Cell Shape Change and Adhesion Molecule Upregulation [107, 113]

During mast cell degranulation, histamine was released in large quantities. Human eosinophils were found to express histamine H_4 but not H_3 receptors. Histamine (0.01–30 μM) induced a rapid and transient cell shape change in human eosinophils, but had no effects on neutrophils. The maximal shape change was at 0.3 μM histamine with EC50 at 19 nM. After 60 min incubation with 1 μM histamine, eosinophils were desensitized and were refractory to shape change response upon histamine restimulation. Histamine (0.01–1 μM) also enhanced the eosinophil shape change induced by other chemokines.

Histamine-induced eosinophils shape change was mediated by the H_4 receptor. This effect was completely inhibited by H_4 receptor-specific antagonist JNJ7777120 (IC50 0.3 μM) and H_3/H_4 receptor antagonist thioperamide (IC50 1.4 μM), but not by selective H_1, H_2 or H_3 receptor antagonists. H_4 receptor agonist imetit (EC50 25 nM) and clobenpropit (EC50 72 nM) could mimic histamine effect in inducing eosinophil shape change. Histamine (0.01–100 μM) induced upregulation of adhesion molecules CD11b/CD18 (Mac-1) and CD54 (ICAM-1) on eosinophils. This effect was mediated by the H_4 receptor and could be blocked by H_4 receptor antagonists JNJ7777120 and thioperamide. Histamine (0.01–10 μM) induced eosinophil chemotaxis with an EC50 of 83 nM. This effect was mediated by the H_4 receptor and could be blocked by H_4 receptor antagonists JNJ7777120 (IC50 86 nM) and thioperamide (IC50 519 nM). Histamine (0.5 μM) also enhanced the eosinophil shape change induced by other chemokines.

A new mechanism of eosinophil recruitment driven by mast cells *via* the release of histamine was established. Using specific histamine receptor ligands, a definitive proof could be provided that the H_4 receptor mediated eosinophil chemotaxis, cell shape change and upregulation of adhesion molecules. The effect of H_4 receptor antagonists in blocking eosinophil infiltration could be valuable for the treatment of allergic diseases. The histamine-induced shape change and upregulation of adhesion molecules on eosinophils could serve as biomarkers for clinical studies of H_4 receptor antagonists.

Histamine H_4 Receptor Antagonists - The New Antihistamines?

The discovery of a fourth histamine receptor (H_4), and the realization that it was exclusively expressed on hematopoietic cell types which were most implicated in the development and symptomatology of allergy and asthma, suggested that pharmacologically targeting H_4 receptor, either alone or in combination with H_1 receptor antagonists, might prove useful in treatment of both, allergy and asthma.

CONCLUSION

Histamine H_4 receptor shared highest sequence similarity with the previously reported histamine H_3 receptor. In combination with the H_1, H_2, and H_3 receptors, this H_4 receptor, with its unique distribution and pharmacological profile, will undoubtedly provide further insight into the physiological functions and therapeutic applications of the receptor family and possible new targets for drugs. This invention further provided methods for the discovery of selective agonists, inverse agonists and antagonists of the H_4 receptor variants which might be useful in the treatment and management of a variety of diseases like inflammation, asthma, allergy, atopic dermatitis, stroke, myocardial infraction, migraine, COPD, rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, and psoriasis.

REFERENCES

[1] MacGlashan, D. Histamine: a mediator of inflammation. *J. Allergy Clin. Immunol.*, **2003**, *112*, 53−59.

[2] Akdis, C.A.; Simons, F.E.R. Histamine receptors are hot in immunopharmacology. *Eur. J. Pharmacol.*, **2006**, *553*, 69-76.

[3] Dale, H.; Laidlaw P. Further observations on the action of b-imidazolylethylamine. *J Physiol.*, **1991,** *43*, 182-195.

[4]. Rosenbaum, D.M.; Rasmussen, S.G.F.; Kobilka, B.K. The structure and function of G-protein-coupled receptors. *Nature*, **2009**, *459*, 356-363.

[5] Gantz, I.; Schaffer, M.; DelValle, J.; Logsdon, C.; Campbell, V.; Uhler, M.; Yamada, T. Molecular cloning of a gene encoding the histamine H_2 receptor. *Proc. Natl. Acad. Sci.*, **1991**, *88*, 429-433.

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

[7] Hill, S.; Ganellin, C.; Timmerman, H.; Schwartz, J.; Shankley, N.; Young, J.; Schunack, W.; Levi, R.; Haas, H. International Union of Pharmacology. XIII. Classification of histamine receptors. *Pharmacol. Rev.*, **1997**, *49*, 253-278.

[8] Lovenberg, T.; Roland, B.; Wilson, S.; Jiang, X.; Pyati, J.; Huvar, A.; Jackson, M.; Erlander, M. Cloning and functional expression of the human histamine H_3 receptor. *Mol. Pharmacol.*, **1999**, *55*, 1101-1107.

[9] Leurs, R.; Bakker, R.; Timmmerman, H.; De Esch, IJP. The histamine H_3 receptor from gene cloning to H_3 receptor drugs. *Nat. Rev. Drug. Discov.*, **2005**, *4*, 107-120.

[10] Passani, M.; Lin, J.; Hancock, A.; Crochet, S.; Blandina, P. The histamine H_3 receptor as a novel therapeutic target for cognitive and sleep disorders. *Trends Pharmacol. Sci.*, **2004**, *25*, 618-625.

[11] Morse, K.; West, R.; Behan, J.; Laz Jr, T.; Greenfeder, S.; Anthes, J.; Umland, S.; Wan, Y.; Hipkin, R.; Gonsiorek, W.; Shin, N. Gustafson, E.; Qiao, X.; Wang, S.; Hedrick, J.; Greene, J.; Bayne, M.; Monsma, F. Cloning and characterization of a novel human

histamine receptor. *J. Pharmacol. Exp. Ther.*, **2001**, *296*, 1058-1066.

[12] O'Reilly, M.; Alpert, R.; Jenkinson, S.; Gladue, R.; Foo, S.; Trim, S.; Peter, B.; Trevethick, M.; Fidock, M. Identification of a histamine H_4 receptor on human eosinophils - role in eosinophil chemotaxis. *J. Recept. Signal. Transduct. Res.*, **2002**, *22*, 431-448.

[13] Nakamura, T.; Itadani, H.; Hidaka, Y.; Ohta, M.; Tanaka, K. Molecular cloning and characterization of a new human histamine receptor, H_4R. *Biochem. Biophys. Res. Commun.*, **2000**, *279*, 615-620.

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

[15] Liu, C.; Ma, X.; Jiang, X.; Wilson, S.; Hofstra, C.; Blevitt, J.; Pyati, J.; Li, X.; Chai, W.; Carruthers, N.; Lovenberg, T. Cloning and pharmacological characterization of a fourth histamine receptor (H_4) expressed in bone marrow. *Mol. Pharmacol.*, **2001**, *59*, 420-426.

[16] Nguyen, T.; O'Dowd Shapiro, D.; George, S.; Setola, V.; Lee, D.; Cheng, R.; Rauser, L.; Lee, S.; Lynch, K.; Roth, B. Discovery of novel member of the histamine receptor family. *Mol. Pharmacol.*, **2001**, *59*, 427-433.

[17] Zhu, Y.; Michalovich, D.; Wu, H.; Tan, K.; Dytko, G.; Mannan, I.; Boyce, R.; Alston, J.; Tierney, L.; Li, X.; Herrity, N.; Vawter, L.; Sarau, H.; Ames, R.; Davenport, C.; Hieble, J.; Wilson, S.; Bergsma, D.; Fitzgerald, L. Cloning, expression and pharmacological characterization of a novel human receptor. *Mol. Pharmacol.*, **2001**, *59*, 434-441.

[18] Coge, F.; Guenin, S.; Rique, H.; Boutin, J.; Galizzi, J. Structure and expression of the human histamine H_4-receptors gene. *Biochem. Biophys. Res. Commun.*, **2001**, *284*, 301-309.

[19] Gantner, F.; Sakai, K.; Tusche, M.; Cruikshank, W.; Center, D.; Bacon, K. Histamine H_4 and H_2 receptors control histamine-induced interleukin-16 release from human CD8(+) T cells. *J. Pharmacol. Exp. Ther.*, **2002**, *303*, 300-307.

[20] Ling, P.; Nguyen, S.; Ngo, K.; Thurmond, R.; Edwards, J.; Karlsson, L.; Fung-Leung, W. Histamine H_4 receptor mediates eosinophil chemotaxis with cell shape change and adhesion molecule upregulation. *Br. J. Pharmacol.*, **2004**, *142*, 161-171.

[21] Raible, D.; Lenahan, T.; Fayvilevich, Y.; Kosinski, R.; Schulman, E. Pharmacological characterization of a novel histamine receptor on human eosinophils. *Am. J. Respir. Crit. Care. Med.*, **1994**, *149*, 1506-1511.

[22] Hofstra, C.; Desai, P.; Thurmond, R.; Fung-Leung, W. Histamine H_4 receptor mediates chemotaxis and calcium mobilization of mast cells. *J. Pharmacol. Exp. Ther.*, **2003**, *305*, 1212-1221.

[23] Lippert, U.; Artuc, M.; Grutzkau, A.; Babina, M.; Guhl, S.; Haase, I.; Blaschke, V.; Zachmann, K.; Knosalla, M.; Middel, P.; Kruger-Krasagakis, S.; Henz, B. Human skin mast cells express H_2 and H_4, but not H_3 receptors. *J. Invest. Dermatol.*, **2004**, *123*, 116-123.

[24] Voehringer, D.; Shinkai, K.; Locksley, R. Type 2 immunity reflects orchestrated recruitment of cells committed to IL-4 production. *Immunity*, **2004**, *20*, 267-277.

[25] Thurmond, R.; Desai, P.; Dunford, P.; Fung-Leung, W.; Hofstra, C.; Jiang, W.; Nguyen, S.; Riley, J.; Sun, S.; Williams, K.; Edwards, J.; Karlsson, L. A potent and selective histamine H_4 receptor antagonist with anti-inflammatory properties. *J. Pharmacol. Exp. Ther.*, **2004**, *309*, 404-413.

[26] Santa Cruz Biotechnology, Inc. Histamine H_4 Receptor (L-15): SC-33966. http://datasheets.scbt.com/sc-33966.pdf. (Accessed March 25, 2010).

[27] Lozada, A.; Michelsen, K.; Karlstedt, K.; Yamamoto, Y.; Panula, P. Expression of the histamine H_4 receptor during rat development. *XXXIII Ann. Meet. Eur. Histamine Res. Soc.*, **2004**, 6-11.

[28] Nakaya, M.; Takeuchi, N.; Kondo, K. Immunohistochemical localization of histamine receptor subtypes in human inferior turbinates. *Ann. Otol. Rhinol. Laryngol.*, **2004**, *113*, 552-557.

[29] Leurs, R.; Chazot, P.L.; Shenton, F.C.; Lim, H.D.; de Esch, I.J. Molecular and biochemical pharmacology of the histamine H_4 receptor. *Br. J. Pharmacol.*, **2009**, *157*, 14-23.

[30] Oda, T.; Matsumoto, S.; Masuho, Y.; Takasaki, J.; Matsumoto, M.; Kamohara, M. cDNA cloning and characterization of porcine histamine H_4 receptor. *Biochim. Biophys. Acta*, **2002,** *1575*, 135-138.

[31] Kiss, R.; Keseru, G.M. Histamine H_4 receptor ligands and their potential therapeutic applications. *Exp. Opin. Ther. Pat.*, **2009**, *19*, 119-135

[32] de Esch, I.J.P.; Thurmond, R.; Jongejan, A.; Leurs, R. The histamine H_4 receptor as a new therapeutic target for inflammation. *Trends Pharmacol. Sci.*, **2005**, *26*, 462-469.

[33] Baldwin, J.; Schertler, G.; Unger, V. An alpha-carbon template for the transmembrane helices in the rhodopsin family of G-protein-coupled receptors. *J. Mol. Biol.*, **1997**, *272*, 144-164.

[34] Mirzadegan, T.; Benko, G.; Filipek, S.; Palczewski, K. Sequence analysis of G-protein-coupled receptors: similarities to rhodopsin. *Biochemistry*, **2003**, *42*, 2759-2767.

[35] Ballesteros, J.; Weinstein, H. Integrated methods for the construction of the three-dimensional models of structure-function relation in G protein-coupled receptors. *Methods Neurosci.*, **1995**, *25*, 366-428.

[36] Huang, E. Construction of a sequence motif characteristic of aminergic G protein-coupled receptors. *Protein Sci.*, **2003**, *12*, 1360-1367.

[37] West, R.; Zweig, A.; Shih, N.; Siegel, M.; Egan, R.; Clark, M. Identification of two H_3-histamine receptor subtypes. *Mol. Pharmacol.*, **1990**, *38*, 610-613.

[38] Leurs, R.; Kathmann, M.; Vollinga, R.; Menge, W.; Schlicker, E.; Timmerman, H. Histamine homologues discriminating between two functional H_3 receptor assays. Evidence for H_3 receptor heterogeneity. *J. Pharmacol. Exp. Ther.*, **1996**, *276*, 1009-1015.

[39] Shin, N.; Coates, E.; Murgolo, N.; Morse, K.; Bayne, M.; Strader, C.; Monsma Jr, F. Molecular modeling and site-specific mutagenesis of the histamine-binding site of the histamine H_4-receptor. *Mol. Pharmacol.*, **2002**, *62*, 38-47.

[40] Shi, L.; Javitch, J. The binding site of aminergic G-protein-coupled receptors: the transmembrane segments and second extracellular loop. *Annu. Rev. Pharmacol. Toxicol.*, **2002**, *42*, 437-467.

[41] Buckland, K.; Williams, T.; Conroy, D. Histamine induces cytoskeletal changes in human eosinophils *via* the H_4 receptor. *Br. J. Pharmacol.*, **2003**, *140*, 1117-1127.

[42] Clark, R.; Gallin, J.; Kaplan, A. The selective eosinophil chemotactic activity of histamine. *J. Exp. Med.*, **1975**, *142*, 1462-1476.

[43] Solovjov, D.; Pluskota, E.; Plow, E. Distinct roles for the alpha and beta subunits in the functions of integrin alphaMbeta2. *J. Biol. Chem.*, **2005**, *280*, 1336–1345.

[44] Bella, J.; Kolatkar, P.R.; Marlor, C.W.; Greve, J.M.; Rossmann, M.G. The structure of the two amino-terminal domains of human ICAM-1 suggests how it functions as a rhinovirus receptor and as an LFA-1 integrin ligand. *Proc. Natl. Acad. Sci.*, **1998**, *95*, 4140–4145.

[45] Fokkens, W.; Godthelp, T.; Holm, A.; Blom, H.; Mulder, P.; Vrooms, T.; Rijntjes, E. Dynamics of mast cells in the nasal mucosa of patients with allergic rhinitis and non-allergic rhinitis: a biopsy study. *Clin. Exp. Allergy*, **1992**, *22*, 701-710.

[46] Slater, A.; Smallman, L.; Drake, L. Increase in epithelial mast cell number in the nasal mucosa of patients with perennial allergic rhinitis. *J. Laryngol. Otol.*, **1996**, *110*, 929-933.

[47] Daugherty, B. Histamine H_4 antagonism: a therapy for chronic allergy? *Br. J. Pharmacol.*, **2004**, *142*, 5–7.

[48] Cruikshank, W.; Center, D.M. Modulation of lymphocyte migration by human lymphokines. II. Purification of a lymphotactic factor (LCF). *J. Immunol.*, **1982**, *128*, 2569–2574.

[49] Iannacone, M.; Sitia, G.; Isogawa, M.; Marchese, P.; Castro, M.G.; Lowenstein, P.R; Chisari, F.V; Ruggeri, Z.M. Platelets mediate cytotoxic T lymphocyte–induced liver damage. *Nat. Med.*, **2005**, *11*, 1167–1169.

[50] Takeshita, K.; Sakai, K.; Bacon, K.; Gantner, F. Critical Role of Histamine H_4 Receptor in Leukotriene B_4 Production and Mast Cell-Dependent Neutrophil Recruitment Induced by Zymosan *in vivo*. *J. Pharmacol. Exp. Ther.*, **2003**, *307*, 1072-1078.

[51] Fung-Leung, W.; Thurmond, R.; Ling, P.; Karlsson, L. Histamine H_4 receptor antagonists: the new antihistamines? *Curr. Opin. Investig. Drugs.*, **2004**, *5*, 1174-1183.

[52] Jablonowski, J.; Carruthers, J.; Thurmond, R. The histamine H_4 receptor and potential therapeutic uses for H_4 ligands. *Mini. Rev. Med. Chem*., **2004**, *4*, 993-1000.

[53] Hagermark, O. Peripheral and central mediators of itch. *Skin. Pharmacol.*, **1992**, *5*, 1-8.

[54] Bell, J.; McQueen, D.; Rees, J. Involvement of histamine H_4 and H_1 receptors in scratching induced by histamine receptor agonists in BalbC mice. *Br. J. Pharmacol.*, **2004**, *142*, 374-380.

[55] Zampeli, E.; Tiligada, E. The role of histamine H_4 receptor in immune and inflammatory disorders. *Br. J. Pharmacol.*, **2009**, *157*, 24-33.

[56] Huang, J.F.; Thurmond, R.L. The new biology of histamine receptors. *Curr. Allergy Asthma Rep.* **2008**, *8*, 21-27.

[57] Thurmond, R.L.; Gelfand, E.W.; Dunford, P.J. The role of histamine H_1 and H_4 receptors in allergic inflammation: the search for new antihistamines. *Nat. Rev. Drug. Discov.*, **2008**, *7*, 41-53.

[58] Morgan, R.K.; McAllister, B.; Cross, L.; Green, D.S.; Kornfeld, H.; Center, D.M.; Cruikshank, W.W. Histamine 4 receptor activation induces recruitment of foxp3+ T cells and inhibits allergic asthma in a murine model. *J. Immunol.*, **2007**, *178*, 8081-8089.

[59] Dunford, P.J.; O'Donnell, N.; Riley, J.P.; Williams, K.N.; Karlsson, L.; Thurmond, R.L. The histamine H_4 receptor mediates allergic airway inflammation by regulating the activation of cd4 T cells. *J. Immunol*., **2006**, *176*, 7062–7070.

[60] Schneider, E.H.; Schnell, D.; Strasser, A.; Dove, S.; Seifert, R. Impact of the DRY motif and the missing "ionic lock" on constitutive activity and g-protein coupling of the human histamine H_4 receptor. *J. Pharmaco. Exp. Ther*., **2010** , *333*, 382-392.

[61] Vogel, R.; Mahalingam, M.; Ludeke, S.; Huber, T.; Siebert, F.; Sakmar, T.P. Functional role of the "ionic lock"—an interhelical hydrogen-bond network in family a heptahelical receptors. *J. Mol. Biol.*, **2008**, *380*, 648–655.

[62] Kobilka, B.K.; Deupi, X. Conformational complexity of G-protein-coupled receptors. *Trends Pharmacol. Sci*., **2007**, *28*, 397–406.

[63] Smits, R.A.; Leurs, R.; de Esch, I.J. Major advances in the development of histamine H_4 receptor ligands. *Drug Discov. Today*, **2009**, *14*, 745-753.

[64] Tiligada, E.; Zampeli, E.; Sander, K.; Stark, H. Histamine H_3 and H_4 receptors as novel drug targets. *Exp. Opin. Investig. Drugs*, **2009**, *18*, 1519-1531.

[65] Engelhardt, H.; Smits, R.A.; Leurs, R.; Haaksma, E.; de Esch, I.J. A new generation of anti-histamines: Histamine H_4 receptor antagonists on their way to the clinic. *Curr. Opin. Drug Discov. Dev.*, **2009**, *12*, 628-643.

[66] Ligneau, X. Distinct pharmacology of rat and human histamine H_3 receptors: role of two amino acids in the third transmembrane domain. *Br. J. Pharmacol.*, **2000**, *131*, 1247–1250.

[67] Lovenberg, T. Cloning of rat histamine H_3 receptor reveals distinct species pharmacological profiles. *J. Pharmacol. Exp. Ther.*, **2000**, *293*, 771–778.

[68] Kenakin, T. Principles: receptor theory in pharmacology. *Trends Pharmacol. Sci.*, **2004**, *25*, 186–192.

[69] Kitbunnadaj, R.; Zuiderveld, O.; Christophe, B.; Hulscher, S.; Menge, W.; Gelens, E.; Snip, E.; Bakker, R.; Celanire, S.; Gillard, M.; Talaga, P.; Timmerman, H.; Leurs, R. Identification of 4-(1H-imidazol-4(5)-ylmethyl)pyridine(immethridine) as a novel, potent and highly selective histamine H*3* receptor agonist. *J. Med. Chem.*, **2004,** *47*, 2414-2417.

[70] Kitbunnadaj, R.; Hashimoto, T.; Poli, E.; Zuiderveld, O.; Menozzi, A.; Hidaka, R.; De Esch, I.J.P.; Bakker, R.; Menge, W.; Yamatodani, A. N-substituted piperidinyl alkyl imidazoles: discovery of methimepip as a potent and selective histamine H_3 receptor agonist. *J. Med. Chem*., **2005**, *48*, 2100-2107.

[71] Shah, C.; McAtee, L.; Breitenbucher, J.; Rudolph, D.; Li, X.; Lovenberg, T.; Mazur, C.; Wilson, S.; Carruthers, N. Novel human histamine H_S receptor antagonists. *Bioorg. Med. Chem. Lett.*, **2002**, *12*, 3309-3312.

[72] Faghih, R.; Dwight, W.; Pan, J.; Fox, G.; Krueger, K.; Esbenshade, T.; McVey, J.; Marsh, K.; Bermani, Y.; Hancock, A. Synthesis and SAR of aminoalkoxy-biaryl-4-carboxamides: novel and selective histamine H_3 receptor antagonists. *Bioorg. Med. Chem. Lett.*, **2003**, *13*, 1325-1328.

[73] Hashimoto, T.; Harusawa, S.; Araki, L.; Zuiderveld, O.; Smit, M.; Imazu, T.; Takashima, S.; Yamamoto, Y.; Sakamoto, Y.; Kurihara, T.; Leurs, R.; Bakker, R.; Yamatodani, A. A selective human H_4 receptor agonist: (-)-2-cyano-1-methyl-3-[(2R,5R)-5-[1H-imidazol-4(5)-yl]tetrahydrofuran-2-yl] methylganidine. *J. Med. Chem.*, **2003**, *46*, 3162-3165.

[74] Lim, H.; Van Rijn, R.; Ling, P.; Bakker, R.; Thurmond, R.; Leurs, R. Evaluation of histamine H_1, H_2 and H_3 receptor ligands at the human histamine H_4 receptor identification of 4-methylhistamine as the first potent and selective H_4 receptor agonist. *J. Pharmacol. Exp. Ther.*, **2005**, *314*, 1310-1321.

[75] Cent, I.C. Histamine H_3-receptor inverse agonists as novel antipsychotics. *Nerv. Syst. Age. Med. Chem.*, **2009**, *9*, 132-136.

[76] Smits, R.; Lim, H.; Stegink, B.; Bakker, R.; De Esch, I.J.P.; Leurs, R. Characterization of the histamine H_4 receptor-binding site. Part 1. Synthesis and pharmacological evaluation of dibenzodiazepine derivatives. *J. Med. Chem.*, **2006**, *49*, 4512-4516.

[77] Lim, H.; Smits, R.; Leurs, R.; De Esch, I.J.P. The emerging role of the histamine H_4 receptor in anti-inflammatory therapy. *Curr. Top. Med. Chem.*, **2006**, *6*, 1365-1373.

[78] Jablonowski, J.; Grice, C.; Chai, W.; Dvorak, C.; Venable, J.; Kwok, A.; Ly, K.; Wei, J.; Baker, S.; Desai, P.; Jiang, W.; Wilson, S.; Thurmond, R.; Karlsson, L.; Edwards, J.; Lovenberg, T.; Carruthers, N. The first potent and selective non-imidazole human histamine H_4 receptor antagonists. *J. Med. Chem.*, **2003**, *46*, 3957-3960.

[79] Terzioglu, N.; van Rijn, R.; Bakker, R.; De Esch, IJP.; Leurs, R. Synthesis and structure-activity relationships of indole and benzimidazole piperazines as histamine H_4 receptor antagonist. *Bioorg. Med. Chem. Lett.*, **2004**, *14*, 5251-5256.

[80] Coruzzi, G. Antiinflammatory and antinociceptive effects of the selective histamine H_4-receptor antagonists JNJ7777120 and VUF6002 in a rat model of carrageenan-induced acute inflammation. *Eur. J. Pharmacol.*, **2007**, *563*, 240-244.

[81] Lim, H. Discovery of S-(2-Guanidylethyl)-isothiourea (VUF 8430) as a potent non-imidazole histamine H_4 receptor agonist. *J. Med. Chem.*, **2006**, *49*, 6650-6651.

[82] Venable, J.D.; Cai, H.; Chai, W.; Dvorak, C.A.; Grice, C.A.; Jablonowski, J.A.; Shah, C.R.; Kwok, A.K. ; Ly, K.S.; Pio, B.; Wei, J.; Desai, P.J.; Jiang, W.; Nguyen, S.; Ling, P.; Wilson, S.J.; Dunford, P.J.; Thurmond, R.L.; Lovenberg, T.W.; Karlsson, L.; Carruthers, N.I.; Edwards, J.P. Preparation and biological evaluation of indole, benzimidazole, and thienopyrrole piperazine carboxamides: potent human histamine H_4 antagonists. *J. Med. Chem.*, **2005**, *48*, 8289–8298.

[83] Lane, C.A.L.; Price, D.A.; Pfizer Ltd. Octahydropyrrolo[3,4-C]pyrrole derivatives. US Patent 20060111416, May 25, 2006.

[84] Arienti, K.L.; Breitenbucher, J.G.; Buzard, D.J.; Edwards, J.P.; Hack, M.D.; Khatuya, H.; Kindrachuk, D.E.; Lee, A.; Venable, J.D.; Johnson & Johnson. Benzoimidazole compounds. US Patent 20050070550, March 31, 2005.

[85] Lee-Dutra, A.; Arienti, K.L.; Buzard, D.J.; Hack, M.D.; Khatuya, H.; Desai, P.J.; Nguyen, S.; Thurmond, R.L.; Karlsson, L.; Edwards, J.P.; Breitenbucher, J.G. Identification of 2-arylbenzimidazoles as potent human histamine H_4 receptor ligands, *Bioorg. Med. Chem. Lett.*, **2006**, *16*, 6043-6048.

[86] Edwards, J.P.; Kindrachuk, D.E.; Venable, J.D.; Janssen Pharmaceutica N.V. Benzoimidazol-2-yl pyridines as modulators of the histamine H_4 receptor. US Patent 20070232616, October 04, 2007.

[87] Edwards, J.P.; Kindrachuk, D.E.; Venable, J.D.; Mapes, C.M.; Pippel, D.J.; Janssen Pharmaceutica N.V. Benzoimidazol-2-yl pyrimidines and pyrazines as modulators of the histamine H_4 receptor. WO Patent 2007/117399, October 18, 2007.

[88] Venable, J.D.; Kindrachuk, D.E.; Edwards, J.P.; Janssen Pharmaceutica N.V. Benzoimidazol-2-yl pyridines as modulators of the histamine H_4 receptor. WO Patent 2007/117400,October 18, 2007.

[89] Buzard, D.J.; Venable, J.D.; Kindrachuk, D.E.; Edwards, J.P.; Janssen Pharmaceutica N.V. Imidazole compounds. WO Patent 2005/092066, October 6, 2005.

[90] Zaragoza, F.; Stephensen, H.; Peschke, B.; Rimvall, K. 2-(4-alkylpiperazin-1-yl)quinolines as a new class of imidazole-free

histamine H_3 receptor antagonists. *J. Med. Chem.*, **2005**, *48*, 306-311.

[91] Zaragoza, F.; Stephensen, H.; Knudsen, S.; Pridal, L.; B. Wulff, L.; Rimvall, K. 1-alkyl-4-acylpiperazines as a new class of imidazole-free histamine H_3 receptor antagonists. *J. Med. Chem.*, **2004**, *47*, 2833-2838.

[92] Sato, H.; Tanaka, K.; Shimazaki, M.; Urbahns, K.; Sakai, K.; Gantner, F.; Bacon, K. Bayer Healthcare AG. 2-Aminopyrimidine derivatives. WO Patent 2005/054239, June 16, 2005.

[93] Sato, H.; Fukushima, K.; Tanaka, K.; Shimazaki, M.; Urbahns, K.; Sakai, K.; Gantner, F.; Bacon, K. Bayer Healthcare AG. 2-Aminopyrimidine derivatives. WO Patent 2005/014556, February 17, 2005.

[94] Altenbach, R.J.; Adair, R.M.; Bettencourt, B.M.; Black, L.A.; Fix-Stenzel, S.R.; Gopalakrishnan, S.M.; Hsieh, G.C.; Liu, H.; Marsh, K.C.; McPherson, M.J.; Milicic, I.; Miller, T.R.; Vortherms, T.A.; Warrior, U.; Wetter, J.M.; Wishart, N.; Witte, D.G.; Honore, P.; Esbenshade, T.A.; Hancock, A.A.; Brioni, J.D.; Cowart, M.D. Structure–activity studies on a series of a 2-aminopyrimidine-containing histamine H_4 receptor ligands. *J. Med. Chem.*, **2008**, *51*, 6571–6580.

[95] Cowart, M.D.; Altenbach, R.J.; Liu, H.; Hsieh, G.C.; Drizin, I.; Milicic, I.; Miller, T.R.; Witte, D.G.; Wishart, N.; Fix-Stenzel, S.R.; McPherson, M.J.; Adair, R.M.; Wetter, J.M.; Bettencourt, B.M.; Marsh, K.C.; Sullivan, J.P.; Honore, P.; Esbenshade, T.A.; Brioni, J.D. Rotationally constrained 2,4-diamino-5,6-disubstitutedpyrimidines: a new class of histamine H_4 receptor antagonists with improved drug likeness and *in vivo* efficacy in pain and inflammation models. *J. Med. Chem.*, **2008**, *51*, 6547–6557.

[96] Carceller, G.E.; Salas, S.J.; Soliva, S.R.; Medina Fuentes, E.M.; Marti, V.J.; Palau Pharma S.A. 2-Aminopyrimidine derivatives as modulators of the histamine H_4 receptor activity. WO Patent 2007/031529, March 22, 2007.

[97] Selby, M.D.; Lane, C.A.L.; Williams, D.H.; Swain, N.A.; Mowbray; C.E.; Bell, A.S. Pfizer Ltd. Pyrimidine derivatives. WO Patent 2007/072163, June 28, 2007.

[98] Cai, H.; Chavez, F.; Edwards, J.P.; Fitzgerald, A.E.; Liu, J.; Mani, N.S.; Wiener, D.K.; Rizzolio, M.C.; Savall, B.M.; Smith, D.M.; Venable, J.D.; Wei, J.; Wolin, R.L.; Janssen Pharmaceutica N.V. 2-Aminopyrimidine modulators of the histamine H_4 receptor. WO Patent 2008/100565, August 21, 2008.

[99] Raphy, G.; Watson, R.J.; Hannah, D.; Pegurier, C.; Ortmans, I.; Lock, C.J.; Knight, R.L.; Owen, D.A.; UCB Pharma S.A. Novel 2 aminopyrimidine derivatives, processes for preparing them, pharmaceutical compositions thereof. WO Patent 2008/031556, March 20, 2008.

[100] Smits, R.A.; Lim, H.D.; Hanzer, A.; Zuiderveld, O.P.; Guaita, E.; Adami, M.; Coruzzi, G.; Leurs, R.; de Esch, I.J.P. Fragment based design of new H_4 receptor-ligands with anti-inflammatory properties *in vivo*. *J. Med. Chem.*, **2008**, *51*, 2457–2467.

[101] Smits, R.A.; de Esch, I.J.P.; Zuiderveld, O.P.; Broeker, J.; Sansuk, K.; Guaita, E.; Coruzzi, G.; Adami, M.; Haaksma, E.; Leurs. R. Discovery of quinazolines as histamine H_4 receptor inverse agonists using a scaffold hopping approach. *J. Med. Chem.*, **2008**, *51*, 7855–7865.

[102] Dyke, H.J.; Price, S.; Harris, N.; Wren, S.; Cramp, S.; Higgs, C.; Argenta Discovery Ltd. Pyrimidine compounds as histamine modulators. WO Patent 2006/050965, May 18, 2006.

[103] Chavez, F.; Curtis, M.P.; Edwards, J.P.; Gomez, L.; Grice, C.A.; Kearney, A.M.; Savall, B.M.; Fitzgerald, A.E.; Liu, J.; Mani, N.S.; Janssen Pharmaceutica N.V. Benzofuro- and benzothienopyrimidine modulators of the histamine H_4 receptor. WO Patent 2008/008359, January 17, 2008.

[104] Varga, C.; Horvath, K.; Berko, A.; Thurmond, R.; Dunford, P.; Whittle, B. Inhibitory effects of histamine H_4 receptor antagonists on experimental colitis in the rat. *Eur. J. Pharmacol.*, **2005**, *522*, 130-138.

[105] Jadidi-Niaragh, F.; Mirshafiey, A. Histamine and histamine receptors in pathogenesis and treatment of multiple sclerosis. *Neuropharmacology*, **2010**, *59*, 180-189.

[106] Muller, Y.; Hari, Y.; Berchtold, E. Premedication with antihistamines may enhance efficacy of specific-allergen immunotherapy. *J. Allergy. Clin. Immunol.*, **2001**, *107*, 81-86.

[107] Takahashi, Y.; Kagawa, Y.; Izawa, K.; Ono, R.; Akagi, M.; Kamei, C. Effect of histamine H_4 receptor antagonist on allergic rhinitis in mice. *Int. Immunol.*, **2009**, *9*, 734-738.

[108] Carceller, G.E.; Medina, F.E.M.; Virgili, B.M.; Marti, V.J.; Palau Pharma, S.A. Furo [3, 2-d] pyrimidine derivatives as H_4 receptor antagonists. WO/2009/115496, September 24, **2009**.

[109] Palau Pharma. Project Information: Ur-63325. http://www.palau-pharma.com/media/uploads/pipeline/10/Factsheet%20UR-63325%20Winter-2010.pdf. (Accessed July 01, 2010).

[110] Banu, Y.; Watanabe, T. Augmentation of antigen receptor-mediated responses by histamine H_1 receptor signaling. *J. Exp. Med.*, **1999**, *189*, 673-682.

[111] Akdis, C.; Blesken, T.; Akdis, M.; Wüthrich, B.; Blaser, K. Role of IL-10 in specific immunotherapy. *J. Clin. Invest.*, **1998**, *102*, 98-106.

[112] Kunzmann, S.; Mantel, P.; Wohlfahrt, J.; Akdis, M.; Blaser, K.; Schmidt-Weber, C. Histamine enhances TGF-beta1-mediated suppression of Th2 responses. *FASEB J.*, **2003**, *17*, 1089-1095.

[113] Dunford, P.J.; Williams, K.N.; Desai, P.J.; Karlsson, L.; McQueen, D.; Thurmond, R.L. Histamine H_4 receptor antagonists are superior to traditional antihistamines in the attenuation of experimental pruritis. *J. Allergy Clin. Immunol.*, **2007**, *119*, 176–183.

[114] Badenhorst, H.; Maharaj, D.; Malan, S.; Daya, S.; Van Dyk, S. Histamine-3 receptor antagonists reduce superoxide anion generation and lipid peroxidation in rat brain homogenates. *J. Pharm. Pharmacol.*, **2005**, 57, 781-786.

Received: May 28, 2010 Revised: August 18, 2010 Accepted: August 22, 2010