CHEMICAL REVIEWS

REVIEW

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Targeting the Histamine H_4 Receptor

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1. INTRODUCTION

1.1. Physiological Role of Histamine

The isolation of histamine [2-(4-imidazolyl)ethylamine] from the mold ergot by Sir Henry Dale and his colleagues at the Wellcome laboratories in 1910 heralded the beginning of research into the understanding of the role of histamine in physiology and medicine. Of the numerous physiological effects of histamine, best understood are its roles in inflammation, in gastric acid secretion, and as a neurotransmitter. Dale's group observed that histamine had a stimulant effect on smooth muscle, stimulated cardiac contractility, and caused vasodepression.[1] The effect of histamine in the skin was described by Lewis in 1924 as being the hallmark of a "triple response", comprising a red area owing to vasodilation, a wheal as the consequence of increased permeability, and flare owing to an axon reflex.[2]

In early work, Popielski demonstrated the dose-dependent acid secretion from the stomach of dogs administered with histamine,[3] leading to the conclusion that histamine is the most potent and major stimulant of gastric acid secretion. The realization that secretion of gastric acid (hydrochloric acid) requires release of histamine provided great impetus for the development of antihistamines. Gastrin and vagal stimulation induce enterochromaffin-like (ECL) cells (neuroendocrine cells) in the gastric glands to release histamine, which by its action upon parietal cells stimulates H^+/K^+ ATPase production, leading to release of acid that assists digestion.

In the 1950s, compounds that induced release of histamine, such as 48/80 (a copolymer from the acid-catalyzed condensation of (*p*-methoxyphenethyl)methylamine with formaldehyde),[4] were shown to disrupt mast cells with an accompanying lowering of histamine content in tissue,[5] thus establishing that mast cells store histamine.[6] Further studies in mammals showed that histamine is found in basophils (white blood cells with a granular cytoplasm), blood platelets, and ECL cells; histamine is now known to be present in all tissues (from below 1 μg/g of tissue to above 100 μg/g), and especially in the skin, connective tissue, lung, and much of the gastrointestinal tract. The effects of histamine produced in the hypothalamus widely influence other regions of the brain. Histamine also acts as a neurotransmitter in

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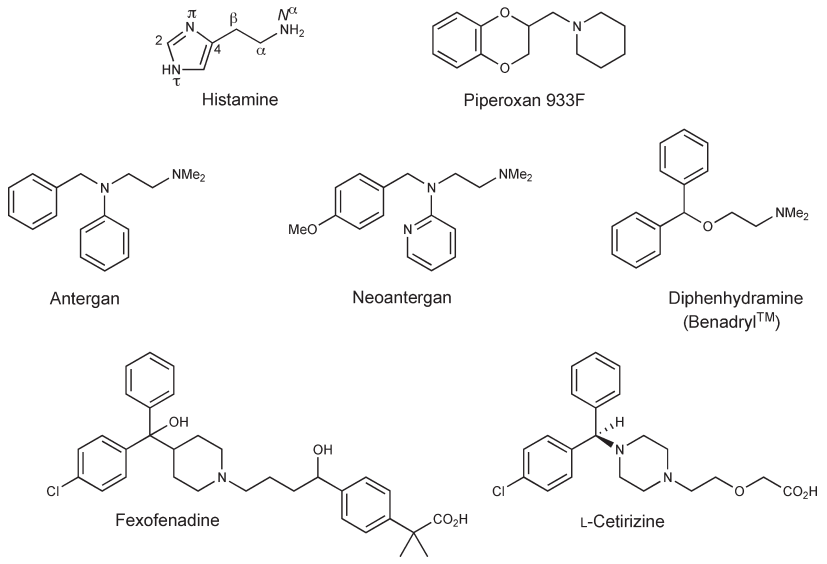

Figure 1. Histamine and some early antihistamine compounds.

the central nervous system (CNS), playing a role in learning, memory, appetite, and sleeping/waking cycles.[7]

In this review I describe the major features of the histamine H_4 receptor, its structural biology, its relevance to diseases, and the biology, chemistry, and pharmacology pertaining to ligands of the histamine H_4 receptor. The basis of this review is the principal literature from the time of disclosure of the histamine H_4 receptor in 2000−2001 until mid-2009, including selected information from patents.

1.2. Discovery of Histamine Antagonists and the Histamine Receptors

The physiological roles of histamine (Figure 1) and histamine receptors span 100 years of eventful discoveries.[8] Although early antihistamines antagonized the effects of histamine on a variety of smooth muscles, several effects of histamine were not ameliorated, leading Folkow and co-workers to suggest that there was more than one histamine receptor,[9] a thesis strongly supported by interpretation of results using Schild's development of a new scale, the pA value, for the measurement of drug antagonism.[10] In particular, the pA value for the antagonistic action of mepyramine upon the guinea pig heart differed from that obtained against the contractile response of isolated guinea pig ileum. With increasing knowledge of histamine receptors, the scope of the term antihistamine has narrowed and will here refer to a compound that inhibits the release or action of histamine by acting primarily upon the H_1 histamine receptor. Herein, a histamine agonist refers to any compound that binds to, and causes increased activity (positive agonism) at, one or more of the four known histamine receptor subtypes. Accordingly, within that scope, a histamine antagonist binds to, but does not alter the activity of, the receptor to which it binds.

Following Popielski's research which established the involvement of histamine in acid secretion, the search began for compounds capable of countering the adverse effects of histamine. Ungar, Parrot, and Bovet described the first antihistamine, piperoxan (933F), an adrenolytic benzodioxan, and its ability to block the effect of histamine on the guinea pig ileum.[11] Research by Bovet and Staub on related aromatic ethers soon followed, including the thymol ether 929F, which in the guinea pig afforded protection from lethal effects of anaphylaxis (acute allergic reaction) induced by histamine.[12] Replacement of the ether oxygen atom by an amino group lowered toxicity and led to the discovery of phenylethylenediamine derivatives.[13] Bovet's research on synthetic acetylcholine antagonists and antihistamines led to the award of the Nobel Prize in Physiology and Medicine in 1957.

Antergan was the first clinical antihistamine to be used (1942) but was replaced by mepyramine (Neoantergan), still in use for topical treatment of histamine release. By 1945, several antihistamines, including diphenhydramine (Benadryl), were widely used in the treatment of a number of allergies, including hay fever and allergic rhinitis.

In 1956, Code and colleagues concluded that histamine had a physiological role in controlling acid secretion,[14] and this was later confirmed in the early 1970s by the discovery of histamine H_2 receptor antagonists. The failure of the pioneering antihistamine drugs to block all actions of histamine led to a research program under the direction of James W. Black at the Smith-Kline and French laboratories to examine histamine receptor heterogeneity, with the hope of finding a therapeutic agent that, by inhibiting histamine-stimulated acid secretion, could be used to treat acid-related diseases such as peptic ulcers and reflux esophagitis. Since antagonists of the β-receptor of adrenergic transmitters had been discovered on the basis of analogy with the structures of adrenaline and noradrenaline, the same principle of analogy was applied to developing histamine antagonists, which were modeled on the structure of histamine. Using several guinea pig and rat assays, agonist compounds methylated at the 2-position showed 17% of the potency of histamine in the ileum, but only 4% on the atrium. Conversely, 4-methylhistamine showed 40% of the activity of histamine on the atrium, but only 0.2% of its potency in contracting the ileum.[8] Further evidence of more than one type of histamine receptor was also obtained in similar studies conducted by Ash and Schild, who proposed the descriptor H_1 for the receptor blocked by the antihistamines then known.[15] Antagonists of the H_1 receptor used for many years to treat allergic conditions include fexofenadine (Allegra) and L-cetrizine (Xyzal).

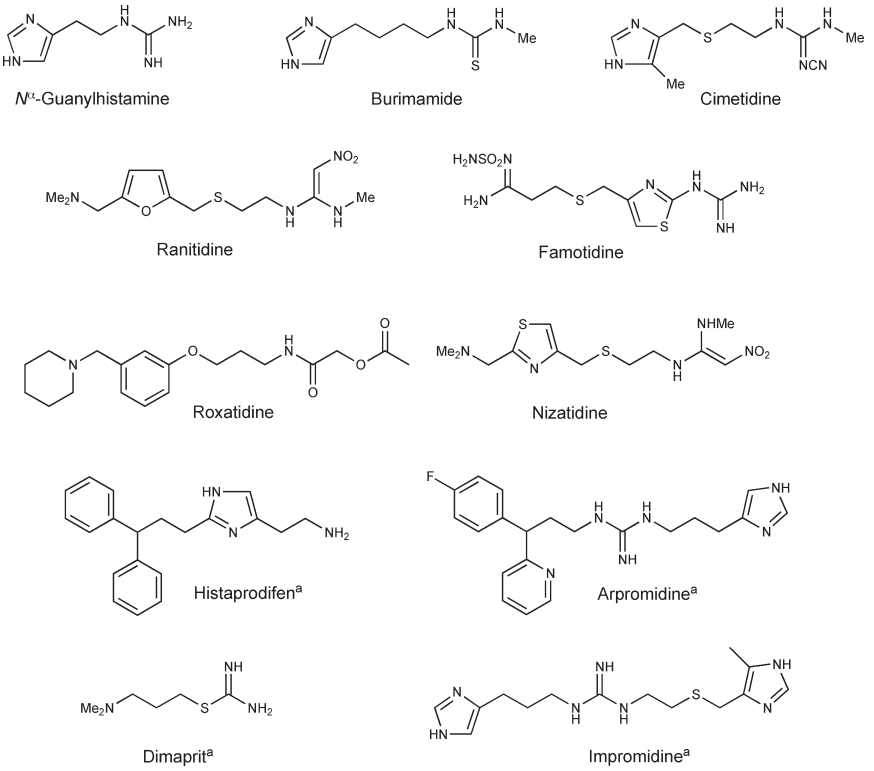

Figure 2. Some histamine H_2 receptor agonists (marked with a superscript "a") and antagonists.

In following chemical analogies to histamine, N^{α}-guanylhistamine was shown to be a weak partial agonist on the heart and uterus and to induce gastric secretion. Robin Ganellin and his colleagues at the Smith-Kline and French laboratories undertook the synthesis and testing of 700 compounds, which revealed a compound, burimamide (Figure 2), that was 100-fold more potent than N^{α}-guanylhistamine, specific for non-H_1 receptor-containing tissues, and that also enabled characterization of the histamine H_2 receptor.[16] However, since burimamide possessed poor oral bioavailability, new antagonists were sought, leading by systematic variation and analysis of electronic and acidic properties and of tautomeric considerations (in aqueous media, histidine itself exists as the two tautomers N^{π}-*H*-histamine and N^{τ}-*H*-histamine) to metiamide, of early use in the treatment of duodenal ulcers; unfortunately, toxicity was observed in some clinical cases. However, replacement of the thiourea group in metiamide with a cyanoguanidine group afforded cimetidine, which showed little toxicity and great efficacy.[17] Following the success of cimetidine (Tagamet) for the treatment of gastric ulcers, a number of other H_2 receptor antagonists were developed (Figure 2),[18] of particular note being ranitidine (Zantac),[19] which, unlike cimetidine, does not affect cytochrome P450 enzymes in the liver and hence has fewer adverse interactions. Of over 20 H_2 receptor antagonists subsequently developed and tested in clinical trials, only famotidine, nizatidine, and roxatidine have been marketed. H_2 receptor antagonists have revolutionized treatments for the healing of ulcers and for reflux esophagitis. In 1988, Sir James Black shared the Nobel Prize in Physiology and Medicine, in part for the identification of histamine H_2 receptor antagonists as potent gastric acid inhibitors.

In the early 1980s, relatively selective agonists of the H_1 receptor (2-thiazolylethylamine) and of the H_2 receptor (dimaprit) were

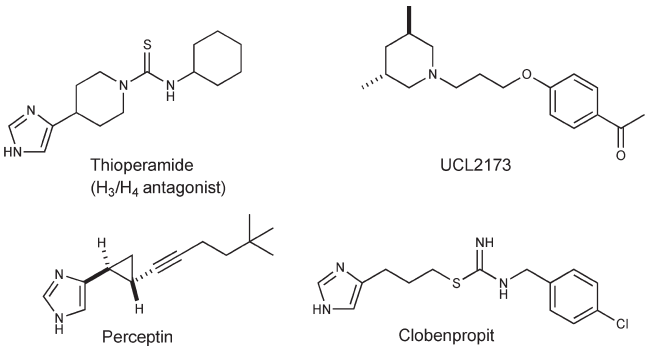

Figure 3. Some antagonists of the histamine H_3 receptor.

available and proved to be useful probes of histamine biology. More recently, highly selective agonists of the H_1 receptor, 2-[3-(trifluoromethyl)phenyl]histamine and histaprodifen,[20] and of the H_2 receptor, impromidine and arpromidine,[18] have been synthesized, and the structure−activity relationships of ligands of the H_2 receptor have been surveyed.[21]

Work on the release of histamine from cerebral neurons in the rat cortex led Schwartz and his colleagues[22] to postulate in 1983 a third histamine receptor, for which at that time thioperamide (Figure 3) was shown to be a potent and specific competitive antagonist. Characterization of the H_3 receptor was reported in 1987.[23] Histamine H_3 receptors inhibit the synthesis of histamine in, and its release from, the histaminergic neurons in the CNS, thereby acting as presynaptic autoreceptors. Ligands for the histamine H_3 receptor and its relevance to diseases have been reviewed.[24,25] A prodrug of (*R*)-α-methylhistamine was the first H_3 agonist to be investigated in the clinic; the first antagonist was Perceptin (GT-2331, cipralisant), which entered phase II studies

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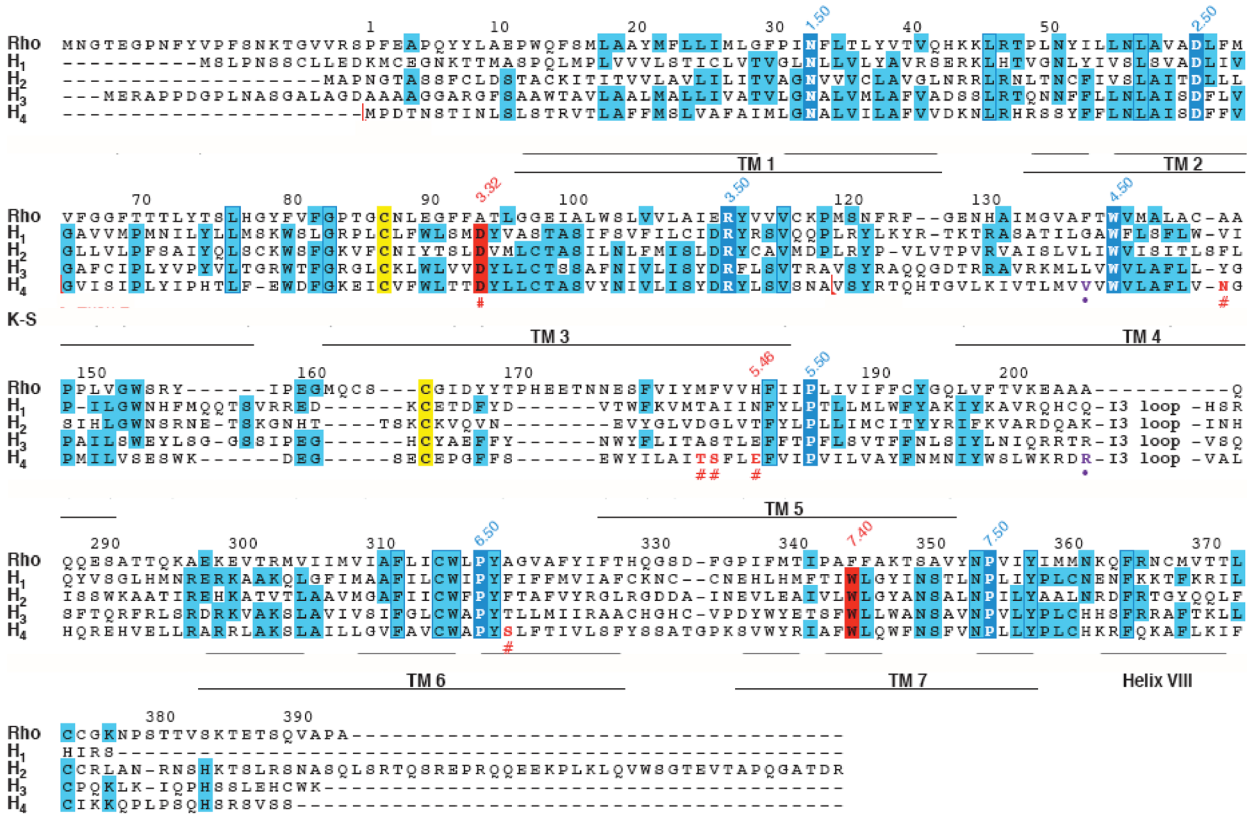

Figure 4. Sequences of the human histamine H_1, H_2, H_3, and H_4 receptors (GenPept accession numbers BAA03319, AAA58647, AAD38151, and BAB13698, respectively) aligned with bovine rhodopsin (Rho; PDB entry 1L9H). Conserved residues are highlighted in blue, the most conserved residue within the transmembrane (TM) domains in class A GPCRs being highlighted in dark blue (e.g., 3.50 referring to residue 50 in TM3). The two residues D3.32[94] and E5.46,[181] denoting an amine-receptor-specific motif, are highlighted in red. Mutated residues are shown in red (see GPCRDB, http://www.gpcr.org/7tm/). Residues most likely to comprise the disulfide bonds linking TM3 to the second extracellular loop are highlighted in yellow. Adapted with permission from ref 47. Copyright 2005 Elsevier Ltd.

for the treatment of attention-deficit disorders. The reduced brain penetration attributed to the polar and hydrogen-bonding properties of imidazoles led to the search for H_3 receptor ligands that did not contain an imidazole ring.[26] This proved challenging, although Ganellin designed from first principles a series of compounds in which a cyclic aliphatic amine acted as a replacement for the imidazole ring.[27] The lead compound, *N*-ethyl-*N*-(4-phenylbutyl)amine, was a moderate H_3 receptor antagonist (K_i = 1.3 μM), and further structural modifications led to the potent antagonist UCL2173, *N*-[3-(*p*-acetylphenoxy)propyl]-3,5-dimethylpiperidine (K_i = 1.8 nM, ED_{50} = 0.12 mg kg^{-1}, Figure 3), which has substantially greater in vivo potency than thioperamide.[8] The (aminopropoxy)phenyl pharmacophore present in UCL2173 has been incorporated into compounds described in patents filed by at least eight pharmaceutical companies. Surprisingly, recent high-throughput screening has shown that many imidazoles have poorer affinities for the human recombinant H_3 receptor than compounds lacking an imidazole ring. Additionally, unlike most nonimidazole antagonists, thioperamide also possesses affinity for the H_4 receptor, thereby displaying a lack of receptor selectivity. Antagonists of the H_3 receptor have potential as therapeutic agents in the treatment of Alzheimer's disease, attention-deficit/hyperactivity disorder (ADHD), schizophrenia, dementia, epilepsy, and depression.[28] Clinical trials using H_3 receptor antagonists[25,29] are in early stages, but their utility in insomnia and cognition disorders,[30] obesity, and some arrythmias has also been suggested. H_3 receptors may be involved in the function of the blood−brain barrier and hence could play a role in neuroinflammation.[31]

The heterogeneity of H_3 receptors had been indicated by agonist kinetics and radioligand-binding studies and was confirmed by the multiple isoforms of mRNA produced from a single form of the H_3 gene. Further investigations resulted in the discovery of the H_4 receptor, which is preferentially expressed in mast cells and various cells of the immune system and has also been identified on dendritic cells, basophils, and T lymphocytes.[32] The H_4 receptor induces chemotaxis (migration of cells induced by chemical agents) of mast cells and eosinophils (acidophilic white blood cells), among others.

1.3. Structural Features of the H_4 Receptor: Comparison with the Histamine H_1, H_2, and H_3 Receptors

The bovine histamine H_1 and canine histamine H_2 receptors were cloned in 1991.[33,34] The H_3 receptor was cloned in 1999[35] and proved to have low resemblance to the previously cloned bovine H_1 and canine H_2 receptors (about 20% homology). The multiple mRNA isoforms arising by alternative splicing of the H_3 receptor gene precipitated the search for novel proteins related to the H_3 receptor, resulting in the discovery of the H_4 receptor, the cloning of which was reported by several research groups in 2000 and 2001.[36−40] Compared with the genomic sequence (GenBank accession number AC007922[40]) and other

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Table 1. Expression, Function, and Signaling of Histamine Receptors and the G Proteins Involved

histamine receptor	expression	function	intracellular signaling activated	G protein involved
H_1	nerve cells, airway and vascular smooth muscle, hepatocytes, epithelial and endothelial cells, neutrophils, eosinophils, monocytes, dendritic cells, T and B cells	induces vasodilation, bronchoconstriction, smooth muscle activation, endothelial cell separation	Ca^{2+}, cGMP, phospholipase D, phospholipase A_2, NFKB	$G\alpha_q$
H_2	parietal cells, nerve cells, airway and vascular smooth muscle, hepatocytes, epithelial and endothelial cells, neutrophils, monocytes, dendritic cells, T and B cells	stimulates gastric secretion	adenylate cyclase, cAMP, c-FOS, c-Jun, PKC, p70S6K	$G\alpha_s$
H_3	histaminergic neurons, eosinophils, dendritic cells, monocytes; low expression in peripheral tissues	neurotransmitter modulation: decreases release of histamine, acetylcholine, serotonin, and norepinephrine	enhanced Ca^{2+}, MAP kinase, inhibition of cAMP	$G_{i/o}$
H_4	mast cells, eosinophils, leukocytes, monocytes, CD8+T cells, basophils, dendritic cells, spleen, bone marrow	immunomodulation	enhanced Ca^{2+}, inhibition of cAMP	$G_{i/o}$

reported sequences, the discrepancies in the sequence reported by Oda and co-workers[36] concerning residues Val138Ala, Arg206His, and Arg253Gln are noted.

The human H_4 receptor (H_4R) is a member of class A of the G protein-coupled receptor (GPCR) superfamily, each member containing seven transmembrane helices and a helix that runs parallel with the cytosolic surface. The human H_4 receptor, reported as a sequence of 390 amino acid residues (Figure 4), possesses all of the highly conserved sequence motifs of the class A rhodopsin-like GPCRs.[41,42] Using GPCR nomenclature,[43] (the GPCR number here following that of the human H_4 receptor amino acid residue), these sequence motifs include Asn33 (1.50), Asp61 (2.50), Arg112 (3.50), Trp140 (4.50), Pro186 (5.50), Pro318 (6.50) and Pro355 (7.50). The proximity of the conserved sequence motifs to the intracellular region of the receptor suggests that the activation mechanism of the H_4 receptor is similar to that of other class A GPCRs. Additionally, the sequence fingerprint Asp94 (3.32) and Trp345 (7.40), which is specific for amine-activated GPCRs, is also present in the H_4 receptor.[44]

Of the human histamine receptors, the H_4 receptor shows the greatest homology with the H_3 receptor (37% identity for the protein sequence[36] and up to 58% homology for the transmembrane (TM) domains[45]). By comparison, the sequence identities of the H_3 receptor with the H_1 and H_2 receptors are only 23% and 22%, respectively.[36] Across species, the H_3 receptor retains over 92% sequence homology, whereas mouse, rat, guinea pig, and pig H_4 receptors show only 65−70% homology with the human H_4 receptor.[46] The pharmacological profiles also differ; for example, the affinity of histamine for the human H_4 receptor (K_I = 6 nM) is greater than its affinity for the rat H_4 receptor (K_I = 70 nM).[46]

However, an examination of homology of the H_3 and H_4 sequences showed little variation among those species (34−35%).[46]

Ligand binding and other studies involving human and rat histamine H_3 receptors indicate that amino acid residues at positions 119 and 122 in the transmembrane protein region TM3 play important roles in ligand recognition, accounting for principal pharmacological differences between the rat and human receptors.[48] Site-directed mutagenesis studies showed that Asp94 (3.32) and Glu182 (5.46) play crucial roles in anchoring histamine to the human H_4 receptor.[49] The requirement for the conserved aspartic acid residue in the transmembrane region TM3 (at positions 107 in human H_1, 98 in human H_2, 114 in human H_3, and 94 in human H_4) is interpretable in terms of Coulombic attraction of the protonated amine (of histamine or basic antagonists) to the carboxylate of the Asp residue. Certain residues in another transmembrane region, TM5, are also required for the binding of histamine: The N^{τ} atom of histamine participates in hydrogen bonding with Asn^{107} in the H_1 receptor and forms an ion pair with Asp^{186} in the H_2 receptor and with Glu^{187} in the H_4 receptor. Additionally, the N^{π} atom of histamine is thought to engage in hydrogen bonding with Thr^{190} in TM5 of the H_2 receptor.[50]

Various chimeric human−mouse H_4 receptor proteins were constructed to atttempt to identify amino acid residues involved in the binding of agonists (e.g., histamine, clozapine, and VUF-8430). Having identified a region between the top of TM4 and the top of TM5 as accounting for the differences in agonist affinity between human and mouse H_4 receptors, detailed site-directed mutagenesis studies were performed and showed that Phe^{169} in the second extracellular loop was the sole amino acid responsible for those differences.[51]

Heterologous expression of H_4 receptor cDNA in several mammalian cells provides a binding site with high affinity (K_D = 8−17 nM) for [^{3}H]histamine.[36,38,46,52] Displacement of this radiolabel exhibits a hitherto unknown pharmacological profile, although with some resemblance to that for release of tritiated histamine from the H_3 receptor. The H_4 receptor has a very high affinity for histamine, the pK_i values for the receptors being 4.2 (H_1), 4.3 (H_2), 7.8 (H_3), and 8.1 (H_4),[53] corresponding to micromolar ranges for the H_1 and H_2 receptors but 5−10 nM for the H_3 and H_4 receptors.

Unsurprisingly, many H_3 agonists and antagonists also bind to the H_4 receptor. In view of the organizational similarity of the gene that encodes the human H_3 receptor[52] to that of the H_4 receptor, isoforms of the H_4 receptor could be expected, and indeed, two splice variants of the H_4 receptor, cloned from cDNA from human CD34+ cord blood-cell-derived eosinophils and mast cells, have been identified.[45] Coexpression of the two splice variants with the human $H_4R_{(390)}$ (the H_4 receptor isoform of 390 amino acids) afforded a decrease in [^{3}H]histamine binding

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to the human $H_4R_{(390)}$.[45] However, the pharmacological characterization of the two splice variants showed those receptor isoforms to be nonfunctional when expressed individually in recombinant mammalian cells.[45] The dominant-negative effect may be linked to hetero-oligomerization involving the human $H_4R_{(390)}$ and the H_4 receptor splice variants.[45] Another two splice variants, $H_{4b}R$ and $H_{4c}R$, cloned from human spleen cDNA, have been described in a patent and claimed to bind [^{3}H]histamine and to effect signaling in several functional pathways;[54] however, other researchers were unable to detect any specific [^{3}H]histamine binding using the $H_{4b}R$ and $H_{4c}R$ splice variants.[45]

1.4. Cellular Expression and Function of the Histamine H_4 Receptor

The histamine H_4 receptor is expressed in many cell types, at either a low level or a more prominent level (Table 1), but is mostly expressed in cells of the immune system and in blood-forming organs, especially in mast cells, lymphocytes, and dendritic cells.[47,55,56] Expression appears to be controlled by inflammatory stimuli.[39,57] Activation of the histamine H_4 receptor promotes the accumulation of inflammatory cells, especially eosinophils and mast cells,[47,58−60] at the sites of allergic inflammation. Expression of histamine H_4 receptor mRNA has been identified in basophils and mast cells.[40,46,59] The presence of the H_4 receptors in nerves from nasal mucosa has been shown by immunohistochemistry;[61] expression of H_4 receptors in rodent brain tissue[40] and weak expression in the human brain[37,57] have also been described. Inhalation of histamine increases the number of mast cells in the trachea of mice and leads to mast cell migration.[62] It is concluded that migration is mediated by stimulation of the H_4 receptor, since only antagonists of the H_4 receptor inhibit migration. The H_4 receptor is partly involved in the secretion of interleukin 16 (IL-16) from CD8+T cells.[63]

All four of the known histamine receptors are members of the GPCR family, and they transduce extracellular signals via particular G-proteins (Table 1). Histamine is a potent stimulator of cyclic adenosine monophosphate (cAMP) accumulation at the H_2 receptor, but its activation of the H_3 receptor leads to inhibition of cAMP accumulation. Signaling mechanisms for the H_4 receptor are much less well understood but appear to involve an increase in intracellular levels of calcium.[52] Constitutive activity (i.e., spontaneous activity in the absence of an agonist) has been demonstrated for all four histamine receptors.[64] Earlier work involving recombinant expression of GPCRs indicated a constitutive spontaneous receptor activity.[65] As with most GPCRs, the active and inactive conformers of the histamine receptors are in equilibrium. As has been shown for the H_1 receptor,[64] an agonist stimulates by stabilizing the active conformation, leading to a continuously activated signal, whereas an inverse agonist (as well as an antagonist) stabilizes the inactive conformation, leading to an inactive state and blockage of signal transduction.

Through the $G_{q/11}$-coupled histamine H_1 receptor, histamine produces allergic symptoms observed on the skin, in the nose, and in the lower airways. Effects involving the histamine H_2 receptor are substantially controlled by cAMP formation.[66] Controlled release of histamine and other neurotransmitters is exercised by histamine H_3 receptors in the form of presynaptic receptors located in the central and peripheral nervous systems.[67] The histamine H_4 receptor is functionally coupled to protein $G_{i/o}$, inhibiting forskolin-induced cAMP formation, as does the histamine H_3 receptor.[68] Additionally, stimulation of the H_4 receptor in stably transfected HEK-293 cells results in pertussis-toxin-sensitive activation of downstream mitogen-activated protein kinase (MAPK) pathways.[39]

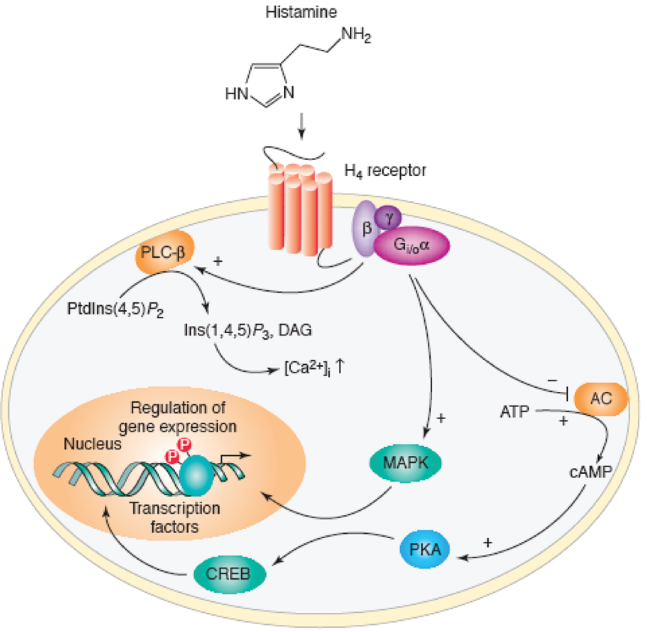

Figure 5. Effects of histamine activation of the H_4 receptor. Binding of histamine at the H_4 receptor induces activation of a member of the $G_{i/o}\alpha$ protein family. Upon stimulation, the α-subunits and $\beta\gamma$-subunits participate in intracellular signaling, the α-subunits negatively regulating the activity of adenyl cyclase (AC), which inhibits the formation of cAMP. The $\beta\gamma$-subunits are thought to activate phospholipase Cβ (PLC-β), which via inositol triphosphate production leads to an increase in intracellular [Ca^{2+}]. Activation of $G_{i/o}\alpha$ is also thought to stimulate MAPK activity, although the individual role of the G protein subunits is not currently known. Reprinted with permission from ref 47. Copyright 2005 Elsevier Ltd.

The function of the H_4 receptor in primary cells has been reviewed.[47] The H_4 receptor has been implicated in the chemotaxis of mast cells, eosinophils, and dendritic cells. In several species, chemotaxis has been shown to be closely involved with the H_4 receptor;[58,62] moreover, chemotaxis induced by histamine is not observed in mast cells derived from mice deficient in the H_4 receptor.[58] H_4 receptor agonists induce shape changes in eosinophils with an efficacy similar to that of histamine. Activation by histamine of either endogenous H_4 receptors in mast cells[58] or H_4 receptors that are stably expressed in L1.2 cells[69] results in Ca^{2+} signaling (Figure 5). This signaling in mast cells is sensitive to both the phospholipase C (PLC) inhibitor U73122[58] and pertussis toxin, indicating that PLC is activated via G$\beta\gamma$ subunits that dissociate from $G_{i/o}$ proteins following H_4 receptor stimulation. This Ca^{2+} response in mast cells is likely to be linked to cellular chemotaxis. The previously described increase of cytosolic Ca^{2+} concentration in human eosinophils[70] possesses a pharmacological profile in keeping with that of the H_4 receptor. Several studies have confirmed the presence of both functional H_4 receptors[52,71,72] and histamine-induced Ca^{2+} signaling[71] in eosinophils. Thus, it appears likely that signal transduction pathways arising from H_4 receptor activation in mast cells, transfected L1.2 cells, and human eosinophils have considerable similarity.

The high homology of the histamine H_3 and histamine H_4 receptors accounts for the lack of selectivity shown by many

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ligands.[68] The formation of dimeric and even oligomeric histamine receptors[45] enables cooperation between histamine receptors and other G protein-coupled receptors. That and the different affinities of ligands (K_i typically 5−10 nM for H_3 and H_4 receptors and typically 2−10 μM for the H_1 and H_2 receptors)[62,73] contribute to the complexity of the effects of histamine on receptor stimulation.

2. RELEVANCE OF THE HISTAMINE H_4 RECEPTOR TO DISEASE

The observation that traditional antihistamines (H_1 antagonists) are largely or completely ineffective for a number of conditions, including allergic asthma, atopic dermatitis, and rheumatoid and psoriatic arthritis, has a new significance, given that such antihistamines have little, if any, affinity for the H_4 receptor, which at least for inflammation is now known to be particularly relevant. Such realizations have accelerated research into H_4 receptor ligands to a point where therapeutic developments are now possible.[74−76] Interestingly, the H_4 receptor antagonist JNJ7777120 has been patented for the treatment of atopic dermatitis,[77] and both JNJ7777120 and early analogues have been patented for many other indications, including those described in this section.[78] In addition to compounds described in detail in this section and section 3, several classes of H_4 receptor antagonists have been proposed as agents to treat or prevent allergic, immunological, or inflammatory diseases, e.g., 1-(arylmethyl)-4-(1*H*-imidazol-4-ylmethoxy)piperidines,[79] furo[3,2-*d*]pyrimidines,[80] benzofurano-[3,2-*d*]pyrimidines,[81,82] and benzimidazoles such as **1**,[83] although reported biological data are usually limited and clinical data may be absent; however, in several cases potent antagonism has been demonstrated, e.g., **1** (Figure 6).[83]

2.1. Inflammation and Inflammatory Pain

During inflammation, antigen−IgE complex-dependent cross-linking of FcεRI (the human high-affinity IgE receptor) on the cell surface stimulates the release of histamine stored in mast cells and basophils. The action of histamine upon vascular smooth muscle cells and endothelial cells leads to vasodilation and an increase in vascular permeability. Effects in the skin are typically the triple response described in section 1.1. In recent years, the cellular and chemoattractant features involving chronic inflammatory diseases have become increasingly better understood,[84] eosinophils and mast cells having been shown to play central roles. In bronchial asthma, atopic dermatitis, and allergic gastrointestinal disease, certain leukocytes, particularly eosinophils, are recruited from peripheral blood into the affected tissue.[85] The pathway of this transendothelial migration of leukocytes is directly influenced by compounds known as chemoattractants located along a tissue gradient and which bind to and activate GPCRs on the cell surface of the leukocyte. Although some classical chemoattractants such as leukotrienes and platelet-activating factor (PAF) stimulate a wide variety of leukocytes, chemotactic cytokines (chemokines) such as eotaxin-1, -2, and -3 and the monocyte chemoattractant protein-4 (MCP-4) are relatively selective in recruiting leukocytes associated with allergic inflammation such as mast cells, eosinophils, and basophils. Combination therapy involving a leukotriene antagonist and an H_4 receptor antagonist has been patented for the treatment of inflammatory and/or allergic conditions.[86]

Histamine H_1, H_2, and H_4 receptors are expressed in many cells involved in inflammatory responses, which can mean that

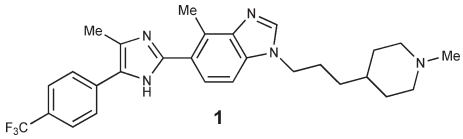

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Figure 6. Benzimidazole **1**, a potent antagonist of the H_4 receptor (K_i = 0.3 nM).

histamine has opposing effects on a particular cell. Most findings support the notion that the histamine H_1 receptor stimulates the pro-inflammatory activity of cells of the immune system by enhancing their migration to the site of inflammation. Conversely, the histamine H_2 receptor appears to be a potent suppressor of both inflammatory and effector functions. Although information on the role of the histamine H_3 and H_4 receptors regarding regulation of immune response is more limited, activation of the histamine H_4 receptor is known to promote accumulation of inflammatory cells at sites of allergic inflammation.[87] Antagonists of the histamine H_4 receptor have been proposed to be relevant to the treatment of autoimmune diseases, including rheumatoid arthritis and multiple sclerosis.[88,89] A review of the role of the histamine H_4 receptor in immune and inflammatory disorders outlines the wide range of inflammatory disorders in which the H_4 receptor could be central, including acute inflammation, allergies, arthritis, asthma, cancer, colitis, pain, and pruritus.[87,90]

For many years, the histamine H_1 receptor has been considered to be the mediator of inflammatory responses arising from histamine release. However, the discovery of the fourth histamine receptor and its expression in many immune and inflammatory cells has suggested the importance of targeting both the histamine H_1 and H_4 receptors in combating allergic responses[91] and also in the context of the known relevance of the H_1 receptor.[91] Combinations of H_1, H_3, and H_4 receptor antagonists have been proposed for the treatment of pulmonary inflammation, congestion, and allergic rhinitis.[92] Studies in mast cells, T cells, and eosinophils indicate that the H_4 receptor plays a role in immune and inflammatory responses,[32,84,93] although the increase in vascular permeability arising from an inflammatory response due to histamine does not involve the H_4 receptor (being mediated by the H_1 receptor).[62] Antagonist studies reveal that the H_4 receptor contributes to neutrophilia in pleurisy models and to peritonitis induced by zymosan,[62,94] although mast cell involvement has also been shown to be a key factor. An indirect effect seems likely, inhibition of the H_4 receptor lowering the concentrations of other neutrophil chemoattractants, as occurs in the pleurisy model.[94]

More recently, histamine has been shown to exhibit many of the properties associated with a typical leukocyte attractant, including up-regulation of adhesion molecule expression, agonist-induced actin polymerization, mobilization of intracellular calcium, and alteration of cell shape. Additionally, groundbreaking studies showed that the histamine H_4 receptor mediates histamine-driven chemotaxis in human eosinophils.[52,71,72] Those and other studies showed that chemotaxis of eosinophils and mast cells[58] via histamine is triggered through the H_4 receptor, which is believed to amplify allergic response to histamine. Histamine release also stimulates recruitment of additional mast cells to the site of inflammation. In mice, mast cells from wild-type and histamine H_3 receptor-deleted mice underwent migration in response to histamine, whereas mast cells from H_4 receptor-deleted mice did not,[58] confirming that chemotaxis of

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eosinophils and mast cells mediated by histamine is triggered mainly through the histamine H_4 receptor. Chemotaxis of mast cells in mouse[58,62] and of eosinophils[52,72] can be inhibited by H_4 receptor antagonists.

Interaction of histamine with the histamine H_4 receptor produces various effects associated with anaphylaxis and other allergic diseases;[95] however, there is increasing evidence that the histamine H_4 receptor also influences immune and inflammatory responses.[96,97] It is the histamine H_4 receptor that is the receptor responsible for selective recuitment of eosinophils;[52] upon activation of the H_4 receptor, histamine induces enhanced migration of eosinophils toward eotaxin and eotaxin-2.[71,72]

Inhalation of histamine increases the number of subepithelial mast cells in the trachea of mice and also increases the total number of mast cells;[62] those increases are inhibited by antagonists selective for the H_4 receptor, but not by antagonists of other histamine receptors, indicating that migration is mediated by stimulation of the H_4 receptor. In vivo results imply that the H_4 receptor mediates inflammatory[62,98,99] and pruritic[100] responses, prompting a reassessment of the relative roles of the H_1 and H_4 receptors in mediating histamine pathology.

There are distinct paths for regulation of granulocyte accumulation by histamine. Histamine H_1 receptor antagonists inhibit allergen-induced accumulation of eosinophils on the skin, in the nose, and in the airways.[101] The concentration of histamine may influence its effect upon eosinophil migration, high concentrations having been shown to inhibit eosinophil chemotaxis via the histamine H_2 receptor, but low concentrations enhancing eosinophil chemotaxis via the histamine H_1 receptor.[102] Of the histamine receptors, the histamine H_4 receptor governs selective recruitment of eosinophils.[52] Although histamine exhibits many of the properties associated with a typical leukocyte attractant, including actin polymerization, which is central to the polarization of eosinophils,[71] compared with eotaxin and eotaxin-2, potent chemokine CCR3 receptor-active β-chemokines, histamine is a weak chemoattractant for eosinophils.[52,71,72,102] However, activation of the histamine H_4 receptor by histamine induces enhanced migration of eosinophils toward eotaxin and eotaxin-2.[71,72] The relatively weak chemoattractant properties of histamine may be augmented by cytokines, by growth factors, or in combination with other chemoattractants.[84]

The role of the histamine H_4 receptor in the acute-phase reaction (in which protein levels in plasma are altered and the liver produces numerous acute-phase reactants) has only recently been studied.[102] In H_4 receptor-deficient mice, some type II hepatic acute-phase protein (APP) gene expression is elevated, which can be accounted for by the alteration of the two mediators investigated (IL-6 and the H_4 receptor).[103] Stimulation of the histamine H_4 receptors in human monocyte-derived dendritic cells suppresses IL-12p70 production and mediates chemotaxis.[104] Of the four histamine receptors, only stimulation of H_4 led to activation of the AP-1 transcription factor.[104] This also suggests that H_4 may need to be targeted in the treatment of allergic diseases.

In the model of colitis in the rat induced by 2,4,6-trinitrobenzenesulfonic acid (TNBS), the (piperazinylacyl)indole JNJ7777120,[73] as well as JNJ10191584[105] (also designated VUF6002, in which the indole ring of JNJ7777120 is replaced by benzimidazole, Table 4), when given twice a day by oral lavage, showed effective therapeutic activity, reducing macroscopic colonic injury and attenuating the increase in tissue edema. Those compounds inhibited the increase in myeloperoxidase levels and neutrophil influx and lowered the increase in tumor necrosis factor-α (TNF-α) levels in colonic tissue

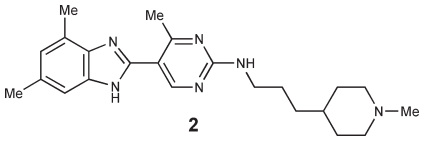

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Figure 7. Potent 2-benzimidazolyl-linked pyrimidine antagonist of the H_4 receptor.

which follow challenge by TNBS.[105] The mechanisms and cellular events underlying the anti-inflammatory effects of these H_4 receptor antagonists have not been extensively identified; however, the properties of such compounds indicate the possibility of a new therapy for inflammation of the gut.[106] Inflammatory bowel diseases (IBDs), which include Crohn's disease and ulcerative colitis, might also be successfully targeted with H_4 antagonists, especially since bacterial components and Toll-like receptor (TLR) signaling appear to be important contributors to IBDs[56] and since H_4 receptor synergizes with TLR ligands to drive cytokine and chemokine production in dendritic cells.[98]

Models of peritonitis have long been used to assess acute inflammation. JNJ7777120 reduces the infiltration of neutrophils and partially blocked inflammation in a zymosan-induced model of peritonitis.[62] This model is characterized by an influx of neutrophils in response to the irritant, a response which includes release of histamine and other pro-inflammatory cytokines and chemokines. It was not determined whether the partial response was because H_1 receptor activity was not also blocked or whether there are factors other than histamine release that drive the influx of neutrophils.

Histamine receptor H_4 antagonists have recently been shown to have activity in models of inflammatory pain;[99,107,108] for JNJ7777120, a peripheral site of action was shown to be relevant to acute pain induced by inflammation. However, H_4 antagonists were also found to block pain in other models, including neuropathic, postsurgical, and osteoarthritic[108] pain. Some 2-aminopyrimidines and related fused ring systems showed significant effects against thermal hyperalgesia in the rat.[109] There are reports of H_4 expression in the brain,[57,110,111] but it is not known whether the site of action is peripheral or central.

In principle, postoperative adhesion formation can be limited by anti-inflammatory agents that lower the release of fibrinous exudates arising in response to inflammation at the site of surgery. Nonsteroidal anti-inflammatory agents have proved to have appreciable efficacy, and to that class can now be added histamine H_4 receptor antagonists, including the pyrimidine **2** (Figure 7), following its success in in vitro models.[112]

Overall, recent findings suggest considerable promise in finding new therapies for inflammatory conditions such as rheumatoid arthritis and allergic rhinitis based on either concurrent administration of both a histamine H_1 receptor antagonist and a histamine H_4 receptor antagonist or alternatively the development of dual antagonists which target both the histamine H_1 and H_4 receptors.

2.2. Arthritis

Although the histamine H_4 receptor has been implicated in tissues of different organs in various disorders of the immune system, only recently has evidence of the possible role of the H_4 receptor in synovial cells of patients with rheumatoid arthritis been documented,[113] despite evidence of histamine release from fragments of synovial membranes being presented over 25 years ago.[114] Indeed, plamsa levels from patients with rheumatoid or

psoriatic arthritis also show elevated levels of histamine.[114] Since rheumatoid arthritis is characterized chiefly by immunological disturbances in synovial cells, this study revealed a novel site of action for histamine and hence the possibility of uncovering new mechansims and new therapeutic agents relating to arthritis. Several publications have demonstrated the relevance of H_4 receptors to rheumatoid arthritis,[113,115] including the distrubution pattern of the histamine H_4 receptor in synovial cells[116] and its predominance in fibroblast-like and macrophage-like cells from rheumatoid arthritic synovial and other tissues.[115,117] It has been speculated that the considerably differing levels of H_4 receptor expression among patients may correspond with differences in the severity of rheumatoid arthritis.[113] To combat the elevated levels of histamine in tissues diseased with rheumatoid arthritis, H_4 receptor antagonists comprising pyrimidines fused with cycloalkyl rings (e.g., Figure 18) have been proposed as agents possessing appropriate therapeutic effects.[118,119]

2.3. Asthma

Histamine levels in bronchoalveolar lavage fluid from patients with allergic asthma are elevated, this increase correlating inversely with airway function.[120] In the lung, activation of the H_1 and H_2 receptors can have opposing physiological and pharmacological effects. Histamine H_1 receptors mediate bronchoconstriction, vasoconstriction, and edema formation. However, pulmonary H_2 receptors act as modulators, stimulation inducing bronchodilation and inhibiting mediator release. Given that the pathology of asthma is mimicked by the pharmacological effects of histamine on the airways, and given that most H_1 receptor antagonists are ineffective for the treatment of asthma, histamine and at least one of its receptors continue to be implicated, with increasing evidence of the relevance of the H_4 receptor to asthma. Mice deficient in the H_4 receptor, or those given H_4 receptor antagonists during sensitization, exhibited reduced lung inflammation and a lowering of inflammatory cytokines.[98] These antagonists are effective in both the sensitization and challenge phases and appear to involve H_4 receptors in dendritic cells. However, the role of histamine H_4 receptor antagonists in ameliorating murine bronchial asthma has been questioned.[121] More promisingly, UR-60427, an inverse agonist of the H_4 receptor, has been shown to be effective in a rat asthma model.[122] Consequently, targeting the H_4 receptor might provide a novel means of therapy for asthma,[123] although no clinical trials have been reported.

2.4. Pruritus (Itch)

One of the physiological roles of histamine is as a mediator of itch; recent findings suggest that the importance of histamine in asthma and chronic pruritus (itch)[124] has not been fully appreciated. Although human and mouse data[125] are conclusive in demonstrating a role for histamine in mammalian pruritus, the relative contribution of the histamine receptors has not been wholly determined. The H_1 receptor appears to have a varying and partial contribution. However, specific H_4 agonists have been shown to induce itch, whereas pretreatment with H_4 receptor antagonists reduced the itch response and also those induced by histamine.[100] The cellular and receptor involvement in pruritus is complicated,[91] and advancement of the area will need to involve clinical testing of novel histamine receptor ligands. However, the demonstrated relevance of the H_4 receptor indicates that H_4 receptor antagonists could have positive effects on pruritus that cannot be controlled by treatment with H_1 receptor antagonists alone.[126] The (indolylacyl)-piperazine JNJ7777120 has been shown to block scratching induced by an H_4 agonist in mice.[100,126] Mequitazine (Figure 8), long known as an H_1 receptor antagonist and used as an anti-inflammatory agent, has been shown to be a dual H_1/H_4 receptor antagonist and has been patented for the treatment of pruritus,[127] as has a dual therapy of an antagonist of the H_1 receptor (especially diphenhydramine) in combination with one of several H_4 receptor antagonists (e.g., JNJ7777120).[128]

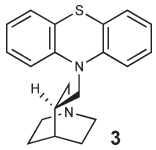

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Figure 8. (*R*)-Enantiomer of mequitazine.

2.5. Cardiac Effects

Histamine is present in high concentration in the heart,[129,130] and mast cells found in the heart are known to release histamine.[131] Mast cell numbers have been shown to increase in myocardial hypertrophy[132] and are known to be an important component of myocardial remodeling and heart failure.[133] Mast cells and histamine release have been implicated in heart disease,[134,135] including the development of heart failure. In a guinea pig model of heart failure,[136] histamine, released by acute myocardial ischemia induced by ovalbumin, caused H_1 receptor-mediated contractions of coronary vascular smooth muscle which led to blockade of myocardial blood flow.[137] Those and other studies indicate the beneficial effect of H_1 antagonist properties in dual active cardiohistaminergic compounds such as arpromidine (Figure 2), leading to lessening of coronary spasms, arrythmias, and negative inotropic (reduced muscular contractile force) effects that have been associated with the histamine H_1 receptor.[138,139] Central to the cardiohistaminergic agents, used in the treatment of congestive heart failure, is their histamine H_2 agonism, which leads to vasodilation through positive inotropic (increased muscular contractile force) and chronotropic (increased heart rate) stimulation of the heart.[138,139] However, the positive chronotropic effects and proarrhythmogenic potency of cardiohistaminergic agents can lead to significant side effects owing to elevated cAMP levels. Arpromidine, having both a 100-fold greater H_2 agonist potency compared with histamine and an appreciable H_1 antagonist activity, was an early lead compound used in the design of a large range of derivatives,[140] prepared with the intention of identifying compounds that combine improved H_1 antagonist and H_2 agonist potencies, without eliciting the side effects of histamine.

While many studies indicate the importance of histamine H_1 and H_2 receptor function in adverse cardiac events, the relevance of mast cells to myocardial hypertrophy suggests histamine H_4 receptors should also be considered. Accordingly, further studies on the role of histamine and its receptors in connection with heart diseases and heart failure are timely.

2.6. CNS

Relatively early investigations either found virtually no expression of the human histamine H_4 receptor in the CNS or were inconclusive in that regard.[37,39] However, recently, the histamine H_4 receptor was shown to be functionally expressed on neurons in the human and mouse CNSs.[141] In another study, transcripts of the H_4 receptor have been detected in all regions of the human CNS that were analyzed, including the spinal cord, hippocampus, cortex, thalamus, and amygdala, the highest levels of H_4 mRNA being detected in the spinal cord.[111] In the

rat, H_4 receptor mRNA was detected in the cortex, cerebellum, brain stem amygdala, thalamus, and striatum. No H_4 receptor signal was detected in the rat hippocampus, and only very low levels of H_4 mRNA were detected in the hypothalamus, with fairly low levels in peripheral tissues, including the spleen and liver. High levels of H_4 receptor mRNA were expressed in the rat dorsal root ganglia (DRG) and spinal cord. Expression of H_4 receptors on neurons in the rat lumbar DRG and in the lumbar spinal cord were revealed by immunohistochemical analysis. Those observations offer clear evidence of the presence of H_4 receptors in both the human and rodent CNSs and provide some insight into the possible role of H_4 in itch and pain.[111] The wide distribution of histamine in the nervous system has been reviewed[142] and is of importance to H_4 receptors recently identified in the CNS.

2.7. Cancer

The hypothesis that histamine may be involved in carcinogenesis was proposed in the 1960s,[143] but still remains largely an open question. However, a substantial body of evidence shows that up-regulation of histidine decarboxylase is correlated with growth of several types of human tumors.[144−146] The role of histamine receptors in cancer pharmacology has been recently reviewed.[146] Although little is known about the physiology of the H_4 receptor, the role of H_4 receptors in colorectal cancer has been explored using the H_4 antagonist JNJ7777120.[147] Histamine-stimulated cell proliferation was observed in the three cell lines studied: HT29, Caco-2, and HCT116, cell lines that also have shown up-regulation of histidine decarboxylase and in which expression of mRNA of the H_1, H_2, and H_4 receptors has been demonstrated.[147] This study also identified a novel role of histamine in activating the cyclooxygenase-2 (COX-2) pathway via H_2 and H_4 receptors in colon cancer cells expressing COX-2. The authors concluded that the coexistence of endogenous histamine and its receptors strongly suggests an autocrine loop for histamine in the carcinogenesis of colorectal cancer. The H_1 antagonist mepyramine did not affect proliferation; however, the histamine-induced stimulation of cell proliferation was significantly diminished by zolantidine (an H_2 antagonist) and by JNJ7777120 (an H_4 antagonist). Moreover, a combination treatment of zolantidine and JNJ7777120 showed an additive effect in lowering histamine-stimulated proliferation in each of the above three cell lines.[147]

Apparently, tumors show an altered histamine receptor expression. Significantly lowered levels of H_1 receptor and H_4 receptor mRNA expression have been found in colorectal cancer, irrespective of the tumor grade or classification.[148] Significantly reduced expression of the H_4 receptor was also found in tumors, compared with normal mucosa. Down-regulation of H_1 receptor and H_4 receptor expression in colon tumors could lead to a favorable microenvironment for tumor cell growth owing to H_2 receptor-mediated negative regulation of Th1 and Th2 lymphocyte response.[148]

The presence of histamine H_4 receptor protein has been demonstrated in epithelial cells of normal mammary glands, in fibroadenoma cells, and also in human breast carcinoma cells.[149] While different carcinoma cells showed varying expression of histamine H_4 receptors, this study indicates that H_4 receptors may play a special role in the development of human breast neoplasms; through the H_4 receptor, histamine may regulate proliferation of mammary epithelial cells, and a loss of that control may contribute to neoplasia.[149]

Table 2. Affinity (pK_i) of H_4 Receptor Ligands at Rat Histamine Receptors[159]

ligand	H_1	H_2	H_3	H_4
histamine	4.5	4.3	8.1	7.4
thioperamide	4.1	3.7	7.8	7.2
VUF 8430	<4	3.7	6.5	6.9
VUF 6002	4.7	4.5	5.3	6.8
JNJ7777120	5.1	4.9	5.3	7.8

Agonists of the histamine H_4 receptor,[150] including VUF-8430, can partly protect against the hematological toxicity of chemotherapeutic agents (cyclophosphamide and docetaxel were studied) by inducing cell cycle arrest of hematopoietic progenitors.[151]

2.8. Mental Health Disorders

Elevation of levels of many pro-inflammatory cytokines have been implicated in bipolar disorder[152] and major depression.[153] Antidepressant and/or antianxiolytic properties of pyrimidine **2** (Figure 7)[154] and analogues have been claimed on the basis of potent H_4 receptor antagonism (K_i = 8.4 nM, with >25-fold in vitro selectivity over other histamine receptors) and cellular effects, including inhibition of histamine-induced morphological alterations of eosinophils and inhibition of chemotaxis of mast cells and interleukin-6 production in mast cells.[154,155]

3. STRUCTURE OF THE HISTAMINE H_4 RECEPTOR AND ITS LIGANDS

3.1. Introduction

Criteria for suitable antagonists of the histamine H_4 receptor include high affinity for the histamine H_4 receptor, selectivity over the other three known histamine receptors, and, for potential clinical candidates, a notable lack of toxicity. Only recently has a relatively systematic design of histamine H_4 receptor antagonists become possible, owing in part to the availability of novel structure−activity relationship (SAR) data and in part to the feasibility of docking ligands into the receptor on the basis of X-ray crystallographic structural data derived from rhodopsin.[156] The historical and chronological development of histamine receptors should not be overly permitted to dictate design of research; it must be remembered that any activity of compounds for the histamine H_4 receptor could not have been known in the last millenium. Current knowledge shows that the relative and absolute antagonist activities of any compound (old or new) toward all four known histamine receptors are important. For example, with hindsight, it is now known that cimetidine is a potent and specific H_2 antagonist, whereas thioperamide is a potent inverse agonist of both the H_3 and H_4 receptors and hence is not selective (Table 2). JNJ7777120 is a potent and selective antagonist of the H_4 receptor, although having a very short half-life.[62] The endogenous substrate histamine is now known to possess high affinity for both the H_3 and H_4 receptors; the ability of histamine to bind strongly to several receptor subtypes is rooted in the conformational mobility of the ethylene unit and the link between the terminal amino group and the rigid imidazole ring.[157] The high sequence homology of the H_3 and H_4 receptors accounts for the activation of the H_4 receptor by a number of H_3 receptor agonists. Emerging knowledge of biological targets, cellular function, and physiological effects makes clear the importance of histamine receptor activity and especially

selectivity for a particular histamine receptor (or a pair of receptors such as H_1 and H_4, pertaining to inflammation) in the design of novel therapeutic agents for a given disease. In identifying and evaluating ligands for H_4 receptors, regard must also be had for the weak degree of homology across species (human, rodent, guinea pig, monkey, and pig) and for the variation of ligand function and potency among species.[46,91,158]

Organic synthesis has had a considerable impact upon effective targeting of the histamine receptor, having delivered compounds with high specificity for particular histamine receptors, as either agonists or antagonists, and hence very useful as pharmacological probes. Buckland and co-workers showed that clobenpropit (Figure 3) and clozapine (Table 4) act as antagonists of the H_3 receptor but as agonists of the H_4 receptor;[71] both compounds altered the shape of eosinophils, an effect that is blocked by thioperamide, a dual inverse agonist of the H_3 and H_4 receptors. Thioperamide was found to inhibit histamine-induced polymerization of actin, mobilization of calcium, and up-regulation of the adhesion molecule CD11b. JNJ7777120 inhibits histamine-induced shape change and up-regulation of adhesion molecules in eosinophils.[71] Following administration of an H_4 receptor antagonist to whole blood, assays have been patented for detecting changes in the cytoskeleton and in the shape of eosinophils, up-regulation of adhesion molecules, and calcium influx.[160]

3.2. Modeling of the Histamine H_4 Receptor and Ligand Binding

In early studies, Shin and co-workers[49] showed that mutation of Asp94 (3.32) to Ala, Asn, or Glu each resulted in complete loss of [^{3}H]histamine binding to the H_4 receptor and concluded that Asp94 (3.32) serves as the counteranion for the protonated amino group of histamine. An early crystal structure of rhodopsin[161] was used to construct a model of the human histamine H_4 receptor; in this model, histamine binds to the H_4 receptor in a pocket defined by the three transmembrane domains TM3, TM5, and TM6.[49] The conserved aspartic acid residue, present in all biogenic amine receptors,[43,162] is located in TM3 as Asp94 (3.32) and is essential for binding histamine, as is Glu182 (5.46),[49] which is postulated to bind to the imidazole ring, thereby corresponding to the role of Asn147 (4.57) in the H_1 receptor.[49] Compared to an Asn residue, the increased strength of an ionic bond formed between Glu182 (5.46) and histamine could account for the higher affinity of histamine for the H_4 receptor in comparison with that for the H_1 receptor. By analogy with the H_4 receptor homology model, Ser320 (6.52) has been implicated in ligand binding[49] and forms part of a highly conserved cluster in TM6 containing aromatic residues, the Cys6.47/Trp6.48/Pro6.50/X6.52 motif, thought to participate in ligand binding and activation of the receptor.[47] Mutation of X = Phe at position 6.52, as in the H_1 and H_2 receptors, to either Thr (H_3 receptor) or Ser (H_4 receptor) greatly increases the affinity of histamine;[49] the presence of a nonaromatic and polar residue at position 6.52 may make a significant contribution to the binding affinity of histamine to the two histamine H_3 and H_4 receptors.

More recently, Leurs and co-workers[163] also prepared a homology model of the human histamine H_4 receptor derived from the crystal structure of bovine rhodopsin (PDB code 1L9H) and investigated the modes of binding of a series of structurally diverse H_4 agonists, including histamine, clozapine, and the selective non-imidazole agonist VUF8430. During simulations using the Amber99 force field, only the side chains of Asp3.32, Cys3.36, Thr5.42, Glu5.46, Phe5.47, Trp6.48, Tyr6.51, Ser6.52, and Gln7.42 and the histamine molecule comprised the ab initio calculations. Mutagenesis studies and docking of the ligands in the model disclosed two essential points of interaction within the binding pocket: Asp94 (3.32) in TM3 and Glu182 (5.46) in TM6.[163] The former interacts in its anionic form, whereas the latter interacts in its neutral state, as supported by mutagenesis data and quantum chemical calculations; in any case, the presence of two anions within the binding site is unlikely and indeed was further rendered implausible by the finding that dimaprit, which is present chiefly in its dicationic form, binds to human H_4 receptor with only moderate affinity (pK_i approximately 6.5).

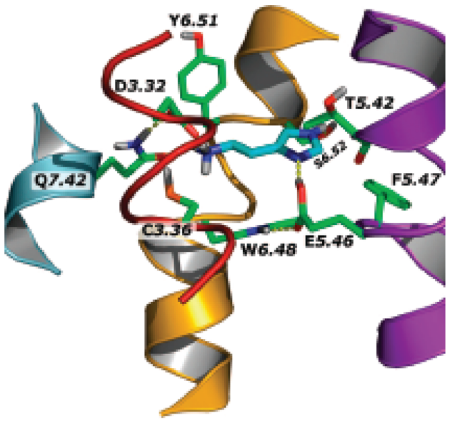


Figure 9. Histamine in the binding site of the human histamine H_4 receptor. Ionic interaction with Glu182 (5.46) and H-bonding with Asp94 (3.32) are indicated with broken yellow lines. Reprinted from ref 163. Copyright 2008 American Chemical Society.

In the above model, histamine docked with its protonated side chain amine oriented toward Asp3.32 and the imidazole ring directed toward TM5 (Figure 9).[163] This orientation is stablized by an excellent match of hydrogen bonds involving Asp3.32 and Gln7.42 and another hydrogen-bonding network between the imidazole ring and Glu5.46, Ser6.52, and Trp6.48. Glu5.46 stablizes the conformation of Trp6.48 in its active rotamer by formation of a hydrogen bond. The presence of Asp3.32 in the vicinity of Glu5.46 raises the pK_a of the Glu residue, leading it to be present in its carboxylic acid form.

The model also accounts for the observed difference in binding affinities of several methylated analogues of histamine. The amino group of histamine participates in a strong hydrogen-bonding assembly involving Gln7.42, Asp3.32, and Cys3.36. The loss of a hydrogen bond upon *N*-methylation of the amino group in histamine to give N^{α}-methylhistamine is reflected in the observed decrease of pK_i from 7.7 to 6.5. Conversely, in 4-methylhistamine the methyl group has little effect on the binding conformation, being 2.9 Å away from the carbonyl oxygen of the backbone; the pK_i is comparable to that of histamine, i.e., 7.3 versus 7.7.[163]

Manual docking of the non-imidazole agonist *S*-(2-guanidylethyl)isothiourea, VUF8430, into the binding pocket of the human H_4 receptor leads to a bidentate salt bridge interaction of the guanidinium moiety with Asp3.32 and a hydrogen bond with Gln7.42 (Figure 10).[163] The isothiourea group is directed toward TM5 and forms hydrogen bonds with Glu5.46 and Ser6.52; the

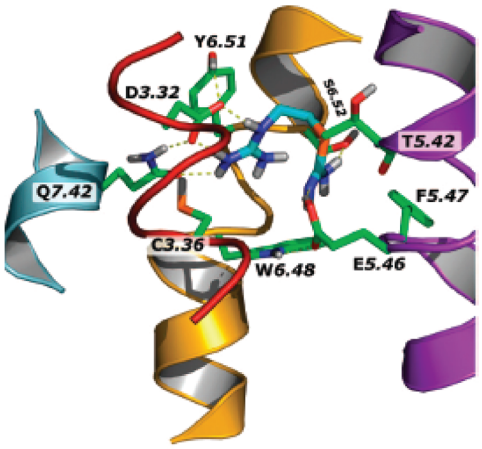


Figure 10. VUF8430 in the binding site of the human histamine H_4 receptor. Reprinted from ref 163. Copyright 2008 American Chemical Society.

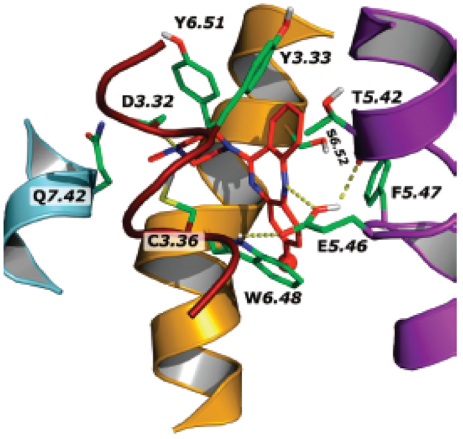


Figure 11. Clozapine in the binding site of the human histamine H_4 receptor (chlorine atom depicted in ball-and-stick representation). Reprinted from ref 163. Copyright 2008 American Chemical Society.

H-bond network is of the same pattern observed in the binding of the imidazole ring of histamine to the receptor.

The tricyclic antipsychotic drug clozapine has weak binding affinity for the human H_4 receptor ($pK_i = 6.7$). The lowest energy conformer of clozapine (Figure 11) shows an interaction of the protonated *N*-methylpiperazine ring with the Asp3.32 residue in TM3, as expected. The NH group of the seven-membered ring was modeled to be in contact with the carbonyl oxygen atom of Glu5.46. Significantly, clozapine lacks the H-bonding network with Glu5.46, Ser6.52, and Trp6.48 that is present in the complexes with histamine and VUF8430. The proposed binding mode of clozapine suggests that it activates the human H_4 receptor through its steric constraint on the Trp6.48 residue, and not through the formation of an extensive H-bond array, as is the case for binding of histamine.

The above mode of binding of histamine (and presumably of related ligands), involving the binding of Asp94 (3.32) to the protonated amino group of histamine, has been recently challenged and Glu182 (5.46) invoked as the counteranion for the protonated amino group.[164] Kiss and co-workers[164] prepared several homology models of the human histamine H_4 receptor,

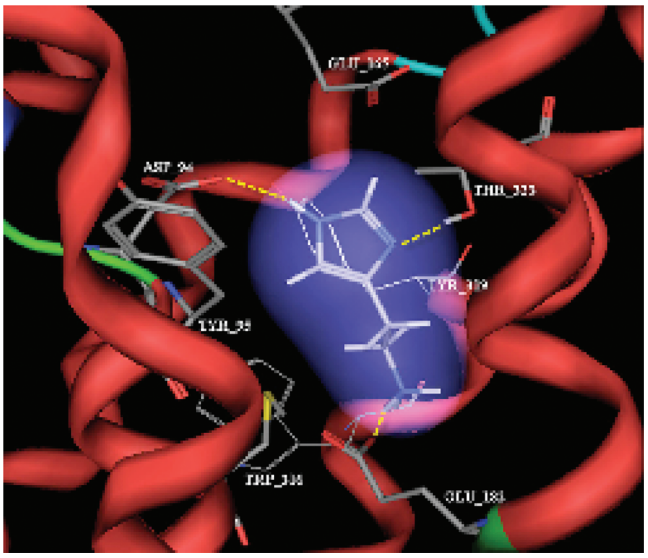


Figure 12. Histamine in the binding site of the human histamine H_4 receptor. The ionic interaction with Glu182 (5.46) and the H-bonding network with Asp94 (3.32) and Thr323 (6.55) are indicated by dashed yellow lines. Reprinted with permission from ref 164. Copyright 2007 Elsevier Masson SAS.

also derived from the crystal structure of bovine rhodopsin, and studied the corresponding complexes with known active ligands. The rotational mobility of the carboxylate group of Asp94 about the Cα−Cβ axis is an important factor in determining suitability of the model for ligand interactions. Ramachandran plots, packing quality, and other evaluations, including enrichment studies, indicated their model D to be appropriate for virtual screening in the search for new H_4 ligands. The two agonists, histamine and the *N*-cyanoguanidinyl-containing imidazole OUP-16 (Table 5), were found to form complementary interactions with Asp94 (3.32), Glu182 (5.46), and Thr323 (6.55). Of two possible binding modes for the protonated ethylamine side chain of histamine, its ionic interaction with Glu182 (5.46) was preferred over binding to Asp94 (3.32),[164] partly on the grounds that the region near the Glu182 region is less lipophilic than the region proximate to Asp94. Docking results showed the more lipophilic imidazole end of histamine to be proximate to the more lipophilic environment of Asp94. Model D (Figure 12) also showed a hydrogen bond of histamine N(1) with Thr323 (6.55); lipophilic interactions of histamine with Trp316 (6.48) and Tyr319 (6.51) were also identified. In contrast, JNJ7777120 was found to interact only with Asp94 (3.32) and Glu182 (5.46). For OUP-16, the nitrile group, rather than N(1) of the imidazole ring, was found to interact with Thr323 (6.55). The above results are stated to be in agreement with all literature data and suggest that histamine binds in a different orientation at the human H_4 receptor than has previously been supposed. Further membrane-based molecular dynamics calculations and site-directed mutagenesis studies are needed to confirm the proposed novel binding mode postulated by Kiss and co-workers[164] and also the role that Thr323 (6.55) plays in ligand binding, a residue that is currently presumed to involve activation of the receptor.

Kiss and co-workers found that their best homology model D did not give satisfactory results for the binding of JNJ7777120 and that conformational flexibility of the side chains needed to be permitted during docking. This afforded model F (Figure 13) in

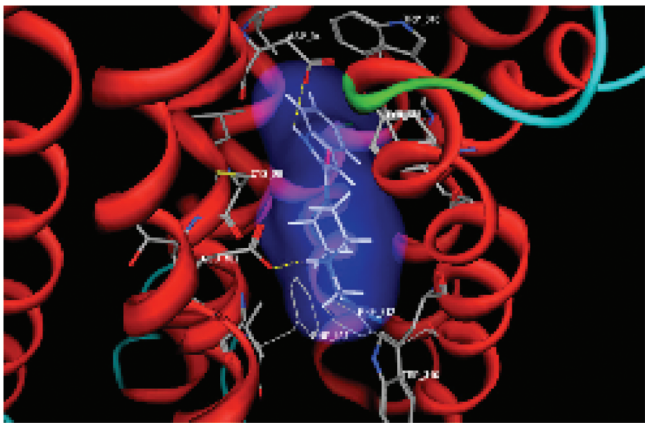
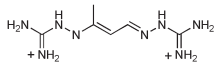
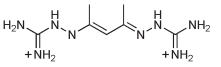
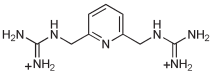
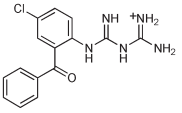
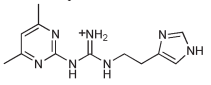
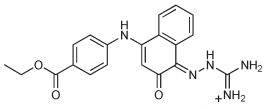
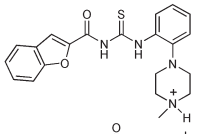
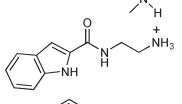
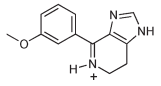


Figure 13. JNJ7777120 in the binding site of the human histamine H_4 receptor. The ionic interaction with Glu182 (5.46) and the H-bond with Asp94 (3.32) are indicated by dashed yellow lines. Reprinted with permission from ref 164. Copyright 2007 Elsevier Masson SAS.

Table 3. Novel Ligands for the Human H_4 Receptor Identified by Structure-Based Virtual Screening[167]

Compound no.	Structure	Displacement of [^{3}H]histamine (%)[a]
4		34
5		64
6		68
7		37
8		96
9		93
10		50
11		47
12		21

[a] Displacements are of [^{3}H]histamine at 10 nM using an inhibitor concentration of 5 μM.

which the indole NH unit forms a hydrogen bond with Asp94 (3.32) and the piperazine moiety of the ligand forms an ionic bond with Glu182 (5.46), but no interaction is observed with Thr323 (6.55), in marked contrast to histamine and OUP-16.

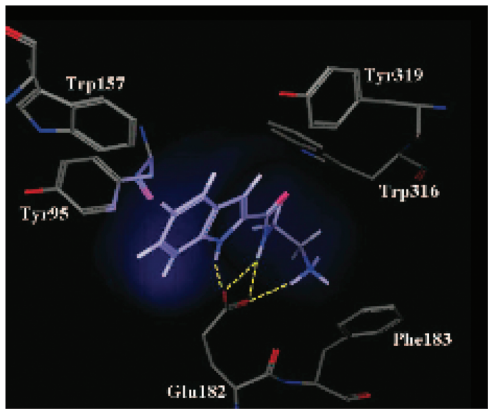


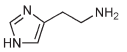
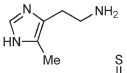
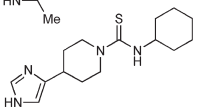
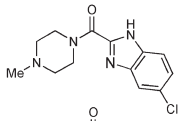
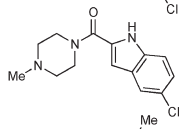
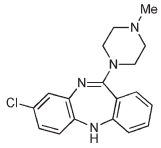
Figure 14. Indole **3** in the binding site of the human histamine H_4 receptor. H-bonding and ionic interaction with Glu182 (5.46) are indicated by dashed yellow lines. Reprinted from ref 167. Copyright 2008 American Chemical Society.

Lipophilic interactions of JNJ7777120 were identified as involving the residues Val64 (2.53), Phe312 (6.44), Trp316 (6.48), Tyr319 (6.51), and Trp348 (7.43).[164] In model D, Asp94 (3.32) faces the interior of the binding crevice, thus decreasing the volume available for binding. In model F, Trp316 (6.48) occupies a completely different location than in model D, a factor that also increases the volume of the binding crevice in model F and provides a lipophilic interaction with ligands.

A molecular dynamics simulation study in a membrane-embedded environment of the human H_4 receptor alone and in complexation with histamine and JNJ7777120 supports an activation mechanism for the receptor. During simulation of the human histamine H_4 receptor complex, considerable changes occurred in both the receptor structure and the mode of interaction of histamine at the binding site;[165] the intracellular side of TM6 moved away from TM3 and TM7. Furthermore, histamine formed a hydrogen bond with Asn147 (4.57), a residue previously shown to be important for activation of the human H_4 receptor. Simulations of the JNJ7777120−receptor complex showed movement of the intracellular side of TM6 in the direction of TM3, consistent with a model of GPCR activation that involves an increase in separation of the cytoplasmic ends of those two TM helices.[166] Residues in TM3 and TM5 form a "lock" into which Ala302 (6.34) appears to fit perfectly in the JNJ7777120−human H_4 receptor complex. These authors suggest that, in the presence of a ligand, the human H_4 receptor adopts a significantly opened binding site and that, in contrast, the crystal structure of bovine rhodopsin represents an inactive GPCR.[165]

A structure-based virtual screening has been conducted[167] using a ligand-supported homology model of the human H_4 receptor into which over 8.7 million compounds listed in commercial databases were automatically docked using FlexX.[168] From this, 255 compounds were selected and tested in a [^{3}H]histamine-binding assay; 16 of those compounds (Table 3), including indole **3** (Figure 14), exhibited significant displacement of the radioligand, *only one containing an imidazole ring*. Previously, enrichment studies had shown that the model was suitable for screening;[164] a protonated amine site and another (nitrogen) atom capable of being a H-bond donor were considered to be essential features in any acceptable molecule. Protonated pyridines and doubly protonated piperazine

Table 4. Representative Ligands of the Human Histamine H_3 and H_4 Receptors[a]

Compound	Structure	K_i H_3 (nM)	K_i H_4 (nM)	K_i H_3/K_i H_4
Histamine		3.1	5	0.62
4-Methylhistamine		9650	50	193
Thioperamide (inverse agonist)		25	27	0.93
VUF6002 (JNJ10191584)		3950	79	50
JNJ7777120		2000	20	100
Clozapine			agonist	-

[a] Data from refs 62 and 175.

structures were excluded. To take into account the fact that the homology model is based on a crystallographic structure of an inactive conformation of bovine rhodopsin, in which the second extracellular loop is folded down to the transmembrane region, only ligands that were located entirely within the binding site in the receptor were considered. Every hit compound had at least one interaction with Asp94 (3.32) or Glu182 (5.46), in keeping with results from site-directed mutagenesis studies. The potency of compounds **5** and **6** (Table 3) is accounted for in terms of two salt bridges, one guanidinium-like terminus bonded to Asp94 (3.32) and the other bonded to Glu182 (5.46). The study revealed several novel scaffolds (Table 3) that are considered to be valuable starting points for the development of novel H_4 ligands.[167]

Crystal structures of the human β2-adrenergic receptor have been recently reported[169−171] and confirm that structurally conserved helices provide a core likely to be common to class A GPCRs;[172] however, the data showed that the β2-adrenergic receptor differs from rhodopsin by having weaker interactions of the terminus of TM3 with that of TM6, and that may contribute to the relative structural flexibility of the β2-adrenergic receptor and to its much higher basal activity.[171] In time, new crystal structures of class A GPCRs should enable further refinement of the current models of the histamine H_4 receptor.

3.3. Ligands

3.3.1. Introduction. The disadvantages of imidazole-containing compounds include a geater propensity to inhibit cytochrome P450 and also to be active at the other receptor subtypes (H_1, H_2, and H_3), thus stimulating the search for antagonists of the H_4 receptor that do not contain an imidazole ring. Indeed, the recently discovered antagonists of the H_3 receptor, such as JNJ6379490[172] and A349821,[173] that lack an imidazole ring show good receptor selectivity. Selective antagonists of the H_4 receptor have also been recently described; high-throughput screening followed by optimization through analogue synthesis revealed JNJ7777120 (Table 4) and its benzimidazole analogue VUF6002 (JNJ10191584) as antagonists of the H_4 receptor possessing high affinity and selectivity[73,174] and acting as neutral antagonists, in contrast to the nonselective H_3/H_4 inverse agonist thioperamide (Figure 15). Some H_2 receptor agonists and some H_3 receptor ligands also have high affinities for the H_4 receptor.[53]

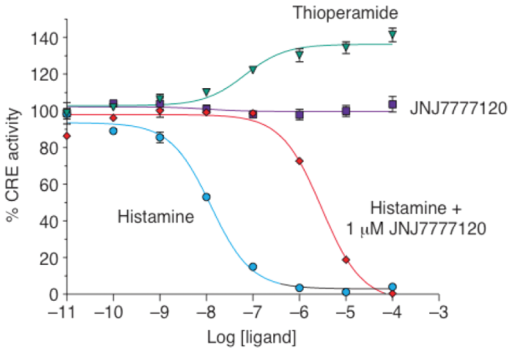


Figure 15. Effect on forskolin-mediated cAMP responsive element (CRE)-driven gene transcription in SK-N-MC cells expressing human histamine H_4 receptors of histamine (agonist), JNJ7777120 (antagonist), and thioperamide (inverse agonist). Reprinted with permission from ref 47. Copyright 2005 Elsevier Ltd.

In addition to the high affinity of VUF6884 for the H_1 receptor (pK_i = 8.11),[176] this compound is an agonist and an isosteric analogue of the tricyclic clozapine and possesses high affinity for the H_4 receptor (pK_i = 7.6, full agonist α =1); saturation binding analysis showed that it binds to the orthosteric binding site (i.e., that of histamine) of the H_4 receptor (Figure 21).[176] The H_4

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receptor antagonist JNJ7777120 (pK_i = 7.8, antagonist α = 0) displaces VUF6884 from its H_4 binding site, and given the structural similarities, it was proposed that they had overlapping modes of binding. This study provided a model for subsequent design of H_4 receptor ligands.[177]

3.3.2. Radioligands for the Histamine H_4 Receptor. At the time of discovery of the histamine H_4 receptor, [^{3}H]histamine was used to determine the binding affinity of histamine for the H_4 receptor (K_d = 17.2 nM) transiently expressed in 293-EBNA cells and also to determine the degree of binding (as IC_{50} values) of histamine (2.48 nM), clobenpropit (3.06 nM), thioperamide (231.6 nM), (*R*)-α-methylhistamine (232.5 nM), and clozapine (978 nM).[36] Using SP9144-transfected HEK-293 cells and COS-1 cells transiently transfected with pCDN/histamine H_4 receptor, [^{3}H]histamine binding was determined as having K_d values of 15.3 nM[39,49] and 17.6 nM.[40] The latter study also provided K_i values for binding to the H_4 receptor of the H_3 receptor agonists imetit (6 nM), immepip (23 nM), *N*-α-methylhistamine (149 nM), and (*R*)-α-methylhistamine (348 nM).[40] The affinity of [^{3}H]-histamine for the human H_4 receptor stably transfected into SK-N-MC cells enabled binding affinities of histamine for the H_4 human (K_d = 4.8 nM), guinea pig (K_d = 6.0 nM), mouse (K_d = 42 nM), and rat (K_d = 136 nM) receptors to be determined;[46] a related study afforded binding affinities of [^{3}H]histamine for the H_4 human (K_d = 5 nM), mouse (K_d = 42 nM), and rat (K_d = 178 nM) receptors.[62] The differences in binding activities among the homologues reflect the fact that the protein sequences are not well conserved. Potencies reported for histaminergic compounds competing against [^{3}H]histamine binding to various H_4 receptor clones were very consistent across a number of laboratories, but results using [^{3}H]pyrilamine (mepyramine) showed discrepancies,[178] perhaps because of binding to particular cytochromes.

Displacement of [^{3}H]histamine binding, e.g., using membranes of HEK cells transiently expressing the human H_4 receptor, has become a standard method for determining binding affinities of ligands, including those based on indoles,[73] quinoxalines,[177] quinazolines,[179] imidazolylcyclopropanes,[180] analogues of clozapine[176] and clobenpropit,[181] and VUF8430.[182] Indeed, 1*H*-indol-2-yl-1-piperazinyl-methanone (K_i = 38 nM), the forerunner of the H_4 receptor antagonist JNJ7777120, was discovered by screening a corporate compound collection using [^{3}H]histamine in a recombinant human H_4 competitive binding assay. In a different approach, a structure-based virtual screening identified 225 compounds, 16 of which induced significant displacement of [^{3}H]histamine.[167] Saturation binding is performed using different concentrations of [^{3}H]histamine; this radiolabel can also be used to determine nonspecific binding and in displacement binding assays.[177] Expression of H_4 receptors can also be confirmed by the use of [^{3}H]histamine binding, e.g., in the cloning of the full-length rat H_4 receptor in the rat CNS.[111] A study of binding to mutants of the histamine H_4 receptor showed only slight alteration of the affinity of radiolabel binding to the mutants, leading to the inference that Asn147 (4.57) and Ser320 (6.52) are not essential for the binding of histamine to the H_4 receptor.[49]

The human histamine H_4 receptor stably transfected into cell line SK-N-MC exhibited saturable binding for both the H_4 receptor agonist [^{3}H]histamine and the H_4 receptor antagonist [^{3}H]JNJ7777120.[175] This system was used to identify 4-methylhistamine (a known H_2 receptor agonist) as the first potent and selective H_4 receptor antagonist (K_i = 50 nM) and to study other H_2 receptor agonists, showing that burimamide, dimaprit, and impromidine are partial agonists at the H_4 receptor.[175] Additionally, the isothiourea-based H_3 receptor inverse agonists clobenpropit and iodophenpropit were shown to behave respectively as a partial agonist and a neutral antagonist at the H_4 receptor.[175]

[^{3}H]-*N*-α-Methylhistamine binds to the histamine H_3 and H_4 receptors with markedly different affinities and has been displaced by thioperamide, a dual inverse agonist of the histamine H_3 and H_4 receptors.[183] Tritium-labeled N^1-[3-(1*H*-imidazol-4-yl)propyl]-N^2-propionylguanidine, [^{3}H]UR-PI294, has high affinity for both human histamine H_3 and H_4 receptors, as shown by the respective K_d values of 1.1 and 5.1 nM.[184]

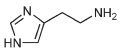
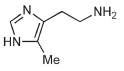
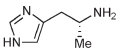
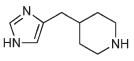
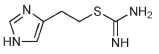
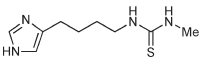
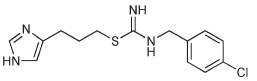
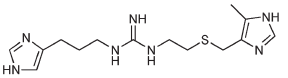
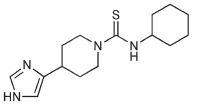
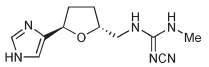
The acylated indole JNJ7777120 is a potent and selective antagonist of human and rodent H_4 receptors and has been used as a radioligand in pharmacological studies.[47,53] Its binding to the H_4 receptor showed higher affinity than to more than 50 diverse targets, including ion channels, neurotransmitter reporters, biogenic amine receptors, and neuropeptide receptors; only two targets other than histamine H_4 showed greater than 20% inhibition at 1 μM.[62] Specific and saturable binding of the radioligand [^{3}H]JNJ7777120 to the human H_4 receptor on membranes from SK-N-MC cells (K_i = 4 nM) and with >100-fold selectivity over H_1 or H_3 receptors has been demonstrated; similar selectivity was observed for the mouse and rat receptors.[62] Pharmacokinetic parameters for this ligand have been determined in mouse, rat, and dog.[62] JNJ7777120 has a limited half-life of 1−2 h with a widely quoted range of oral bioavailability of 22−100%.[53]

The radioligand [^{125}I]iodophenpropit, an inverse agonist at the human histamine H_3 receptor, has been described previously as a ligand with high affinity for the histamine H_3 receptor,[185] but it is also a potent neutral antagonist of the H_4 receptor (pK_i = 7.9).[165] The procedure for using [^{125}I]iodophenpropit is the same as that for [^{3}H]histamine, and values of ligands of the human H_4 receptor are consistent with those obtained using either [^{3}H]histamine or [^{3}H]JNJ7777120.[175] However, [^{125}I]iodophenpropit afforded a B_{max} value twice that obtained using either [^{3}H]histamine or [^{3}H]JNJ7777120. That finding might indicate that the radioligands bind to different subpopulations of the H_4 receptor; however, that hypothesis requires confirmation, and in any case, results using [^{125}I]iodophenpropit must be interpreted with caution, given its high level of nonspecific binding (approximately 60%).[175]

3.3.3. Imidazole Derivatives. Several imidazole derivatives, regarded as reference compounds for the H_3 receptor, also bind to the H_4 receptor and so cannot be used as selective agents. They include the H_3 receptor agonists (*R*)-α-methylhistamine,[73] immepip,[37] and imetit[38] (Table 5), the H_2/H_3 receptor antagonist burimamide,[73] and the H_3 receptor antagonist clobenpropit.[46] However, more recent agonists of the H_3 receptor, such as immethridine[186] and methimmepip[187] (containing an imidazole ring), show satisfactory receptor selectivity. Thioperamide acts as an inverse agonist at both the H_3 and H_4 receptors[73] (Figure 15).

More recent developments have seen improvement in specificity for the H_4 receptor; OUP-16 is a full agonist with both moderate affinity and moderate specificity.[188] 4-Methylhistamine also shows moderate affinity but high specificity (Table 5).[47] Recently, competitive binding of ligands in the presence of [^{3}H]histamine to SK-N-MC cells stably transfected with the human H_4 receptor has provided the following set of K_i values (nM): histamine (13.4 ± 8.4), (*R*)-α-methylhistamine (184 ± 29), imetit (3.6 ± 0.7), clobenpropit (5.0 ± 1.8), thioperamide (328 ± 34), JNJ7777120 (17.0 ± 8.2).[167]

Table 5. Imidazole-Containing Ligands of the Human Histamine H_3 and H_4 Receptors[47]

Compound	Structure	K_i H_3 (nM)	K_i H_4 (nM)	K_i H_3/K_i H_4
Histamine		3.1	5	0.62
4-Methylhistamine		9700	50	193
(R)-α–Methylhistamine		0.7	146	0.005
Immepip		0.2	9	0.022
Imetit		0.5	5	0.10
Burimamide		84	180	0.47
Clobenpropit		0.61	13	0.047
Impromidine		67	12.3	5.4
Thioperamide (inverse agonist)		25	27	0.93
OUP-16		2000	125	16

The use of the first selective antagonist of the H_4 receptor, JNJ7777120 (Table 4), was described in 2004.[72] JNJ7777120 was reported to possess over 1000-fold selectivity for the H_4 receptor, compared with H_1, H_2, and H_3 receptors,[73] and blocked the histamine-induced change in the shape of eosinophils, whereas selective antagonists of the H_1, H_2, and H_3 receptors (diphenhydramine, ranitidine, and JNJ637940, respectively) were ineffective. JNJ7777120 was shown to block chemotaxis and also to block the histamine-induced up-regulation of the adhesion molecules CD11b and CD54. Its ability to block the action on the chemotaxis of mast cells was shown to be due to antagonism of the H_4 receptor.[58]

3.3.4. 2-Aminopyrimidines and Related Compounds. 2-Aminopyrimidines and related systems have occupied a prominent position in recent patents concerning modulators of the H_4 receptor.[74,76,189] A study of a wide range of substituted pyrimidines showed that a 3-(methylamino)azetidin-1-yl group or a 1,4-diazepan-1-yl group as a 2-substituent on a pyrimidine ring can confer very strong binding to the human histamine H_4 receptor (Figure 16).[190,191]

A high-throughput screening of a chemical library for H_4 receptor ligands revealed a small number of pyrimidine and triazine hits, of which pyrimidine **13** (Figure 17) was representative.[109,118] Although potency of **13** in the rat was weak, optimization was considered important, given the feasibility of testing new analogues in rodent models. Replacement of the pyrrole ring by an unsubstituted amino group (**14**) gave a dramatic increase in potency. A systematic variation of the heterocyclic ring, the *N*-methylpiperazine unit, the 2-amino group, and the 5- and 6-positions of the pyrimidine was carried out, but only modification of the 6-position gave promising improvements; replacement of the *tert*-butyl group by other lipophilic groups proved to be effective, the 4-cyanophenyl analogue **15** providing activity in several animal models and possessing affinity values similar to those of JNJ777120 (human H_4R pK_b = 8.37; rat H_4R pK_b = 8.59). However, binding of pyrimidine **15** to the H_3 receptor was also significant (human H_4R pK_b = 8.37; rat H_4R pK_b = 8.59). Substitution of the 6-position was preferred over substitution of the 5-position, as shown in the affinities of compounds **16** and **17** (4 nM and 5 μM, respectively).[118]

Compound **15** was found to be a potent antagonist of the human H_4 receptor, but less potent in rat and mouse, in which it was a partial agonist. The similarity of the in vivo potencies and efficacies in the rat and mouse subtypes is consistent with the relatively high homology (84%) of rat and mouse H_4 receptors. In binding studies, compound **15** was 30 times more selective for the human H_4 receptor (pK_i = 7.46) than for the H_3 receptor (pK_i = 5.89), but was nonselective for the rat H_3 (pK_i = 6.11) and

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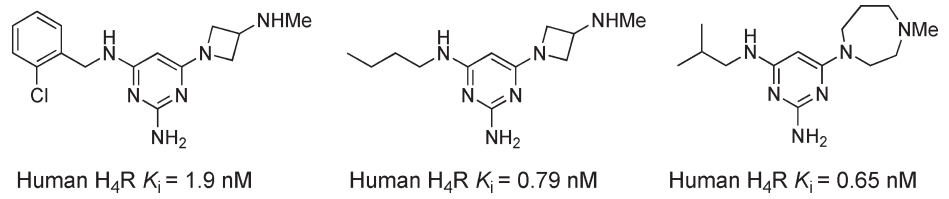


Figure 16. Some amino-substituted pyrimidines with high affinities for the histamine H_4 receptor.[190].

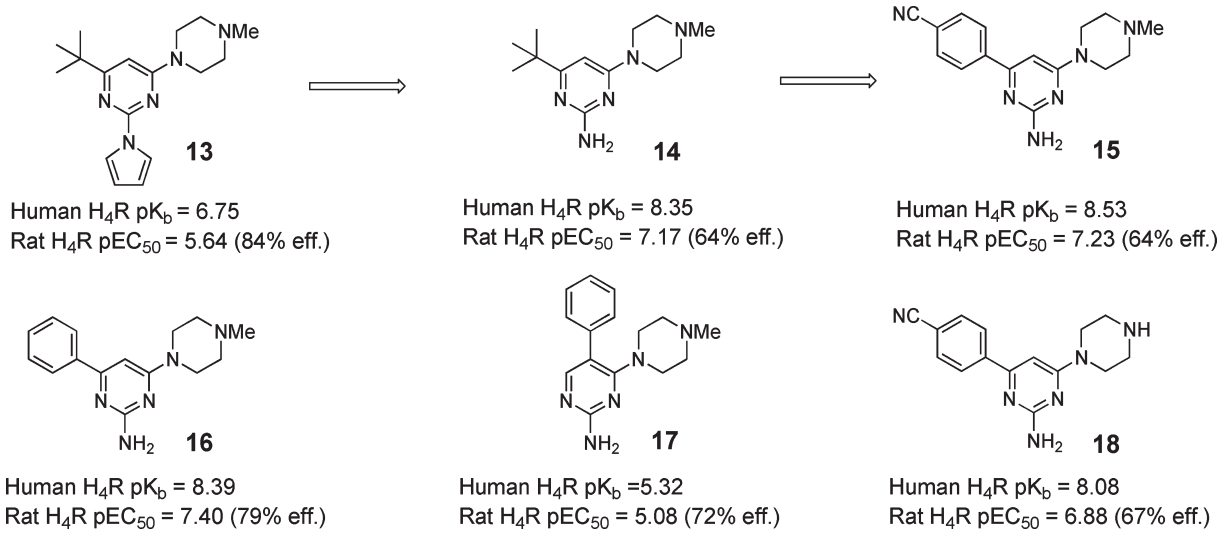


Figure 17. Pyrimidines as H_4 antagonists.

H_4 (pK_i = 6.56) receptors. Compounds **15** and **18** were selective over the human H_1 and H_2 sites for which pK_i < 5.2. As expected, in vivo demethylation of the piperazine ring occurred, the metabolite **18** being identified.[118]

Compound **15**, administered intravenously (iv) at 1 mg/kg in the rat, had a $t_{1/2}$ of 0.4 h and achieved a C_{max} plasma level of 32 ng/mL at 0.4 h and an oral bioavailability of 31%. In mouse, **15** administered iv at 2.5 mg/kg had a $t_{1/2}$ of 0.2 h, and when dosed orally (po) had a $t_{1/2}$ of 0.6 h, and achieved a C_{max} plasma level of 92 ng/mL at 0.25 h and an oral bioavailability of 41%.[118]

In a mouse model of zymosan peritonitis, compound **15** inhibited myeloperoxidase activity, which is a biomarker for polymorphonuclear (PMN) leukocyte infiltration. The efficacy of **15** was the same as that of JN7777120 in the peritonitis model. Pyrimidine **15** also showed antinociceptive activity in a model of pain, being consistent with the potential of H_4R antagonists in the treatment of pain, although not definitively confirmed by the rat model data, since the antinociceptive activity of compound **15** might be due to the antagonistic activity of **15** at the rat histamine H_3 receptor (given the similar pK_i values of **15** for rH_3R and rH_4R). Histamine H_4 antagonists have been shown to be active in a variety of models of pain, both in those with accompanying inflammation and in those without inflammation.[109] Compound **15** increased paw-withdrawal latency in the rat carrageenan model of inflammatory pain and had an ED_{50} of 200 ng/mL. Both JNJ7777120 and **15** have high clearance values in rat and mouse; despite that, both compounds are adequate for profiling in acute inflammation and pain models.

The promising properties of the above aminopyrimidines suggested that their limitations regarding rapid metabolism in vivo to H_4 ligand metabolites and in some cases their off-target activity (5-HT_{1a} and 5-HT_{1d} receptors) should be overcome by further structural modifications, especially rigidification,[118] a strategy that proved beneficial in developing H_3 receptor antagonists[192] and could be expected to improve selectivity for the H_4 receptor compared with off-target sites. Annulation of the pyrimidine ring with the phenyl substituent by means of an alkyl linker gave a series of rotationally restricted 2,4-diamino-substituted pyrimidines (Figure 18) that showed potent and selective in vitro H_4 antagonism across multiple species, as well as good CNS penetration and improved PK properties compared to the benchmark compound JNJ7777120 and the previous (monocyclic) pyrimidine **15**. The 2,4-diaminopyrimidines also showed functional H_4 antagonism in cellular and in vivo pharmacological assays and in vivo anti-inflammatory and antinociceptive efficacy.[118]

The methylene-linked compound **19** (Figure 18) was a potent antagonist of the human H_4 receptor (pK_b = 8.75), but activated the rat receptor (96%) almost to the same extent as histamine. The ethylene-linked pyrimidine **20** and its *N*-methylpiperazine derivative were also potent antagonists of the human H_4 receptor, and both were potent at the rat H_4 receptor, although some activation of the receptor was detected (52% and 76%, respectively).[118] The fused seven-membered analogue **21** performed as a potent agonist but with no detectable functional activation in the H_4 of any species studied. Accordingly, a range of the seven-membered ring analogues were prepared in which the piperazine ring was replaced by other cyclic amines, giving compounds **23**−**25**, all of which were more potent in the functional and binding assays in all H_4 receptor species than

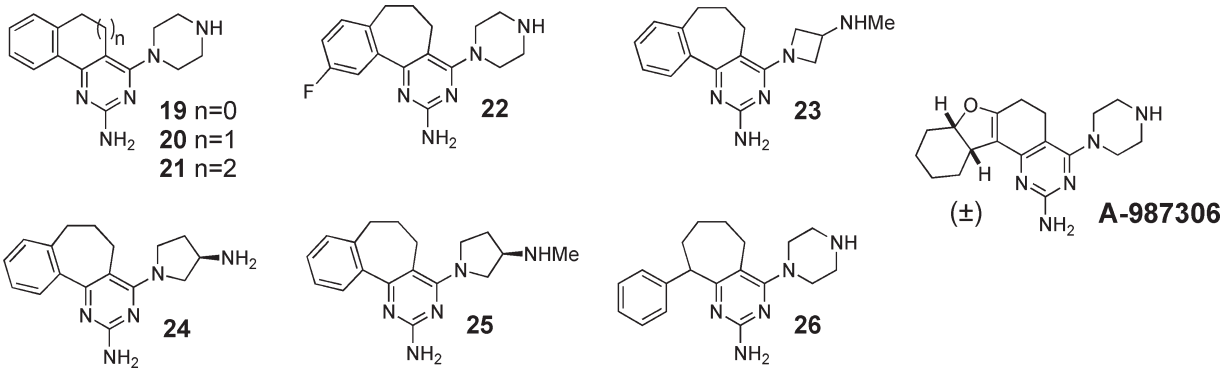


Figure 18. Fused pyrimidines as potent H_4 antagonists.

was **21**. The fluoro analogue **22** and compound **26** with an appended rather than fused aryl ring were also potent. Compounds **21**−**26** showed no significant binding to the large majority of 80 receptors and enzymes screened. For the minority of sites (H_3, 5-HT, and adrenergic receptor subtypes) in which interaction was detected, the compounds retained high selectivity for the H_4 receptor. Compounds JNJ7777120, **15**, and **21** showed relative selectivities for the human H_4 receptor over the human H_3 receptor of 190, 30, and 50; the seven-membered ring compounds **22**, **24**, and **25** were even more selective (>470-, 640-, and 570-fold selective, respectively).[118] None of the compounds **22**−**26** interacted detectably with H_1 or H_2 receptors (H_4 selectivity being >160-fold to >1890-fold). These compounds were of interest in the treatment of inflammation and pain and so were evaluated for interaction with COX-1 and COX-2 enzymes, μ-,κ-, and δ-opiate receptors, and CB_1, CB_2, nicotinic, and TRPV receptor isoforms, but all tests were negative. The 2-aminopyrimidines **15** and **22**−**26** were also tested at 10 μM in a diverse panel of 133 kinases but showed no detectable activity.[118] It is thought that the high selectivity of the compound set **22**−**26** is associated with the conformational aspects and restraints of the fused seven-membered ring.

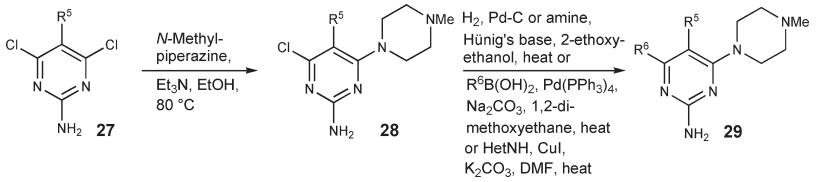
A major design feature of compounds **19**−**26** was the avoidance of an *N*-methyl group that would readily undergo metabolic oxidative demethylation, as occurs rapidly for JNJ7777120 and pyrimidine **15**.[118] *N*-Methylpiperazine compounds, including JNJ7777120 and pyrimidine **15**, showed the most rapid metabolic turnover, whereas other diamino assemblies, including 3-(methylamino)azetidine and 3-(methylamino)pyrrolidine, showed much slower metabolism in mouse microsomes, as had been sought, although in rat and human microsomes the influence of structure upon turnover was less clear. However, pyrimidine **24** was outstanding in showing very slow in vitro metabolism in all species studied.[118] All of the set **19**−**26** showed low to moderate protein binding, very high aqueous solubility, and no inhibition of cytochrome P450 enzymes. Compound **21** showed an especially long half-life in the mouse (4.9 h). Compounds JNJ7777120, **15**, and **21**−**26** all penetrated the brain efficiently, achieving brain-to-plasma ratios ranging from 2.1 to 20 and rapid access to the CNS. The minimal number of polar groups, low molecular weight, moderate lipophilicity, and amine basicity contribute to the favorable pharmacological profile of the fused pyrimidines which, having low protein binding (48% of compound **24** bound to human protein, compared with 77% for JNJ77-77120 and 35% for **15**), are present to a significant extent in the circulation in unbound form and hence are able to traverse the blood−brain barrier.[118]

Pyrimidines **21**, **23**, and **24** were even more potent than the standard JNJ7777120, and also than the pyrimidine **15**, in blocking zymosan-induced peritionitis in mice and showed ED_{50} potencies of 21, 34, and 17 μmol/kg, respectively. Histamine H_4 receptors are known to modulate itch responses,[100,125] and H_4 agonists such as clobenpropit induced scratching in wild-type mice but not those deficient in H_4 receptors. Compounds **22**−**24** and **26** completely blocked clobenpropit-induced scratching responses in mice after intraperitoneal (ip) dosing and showed ED_{50} potencies of 10, 25, 33, and 10 μmol/kg, respectively.[118] The two histamine H_4 antagonists JNJ7777120 and **15** have been shown to be active in a wide range of models of pain.[107,108] The anti-inflammatory properties of compound **21** have been extensively studied.[193]

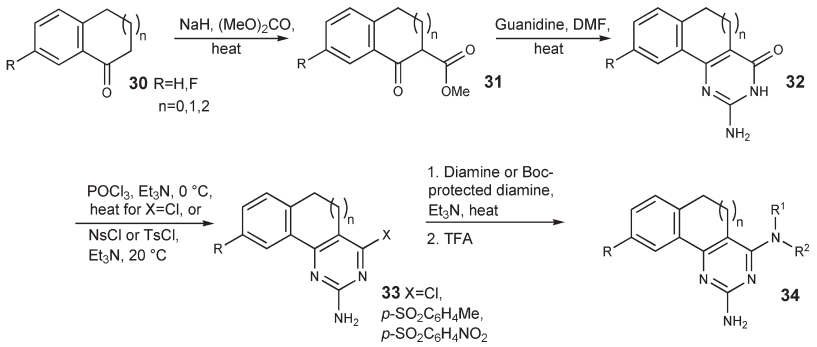
Compound **24** combines the best features of the series and is an excellent tool for probing H_4 pharmacology. It is a potent H_4 receptor antagonist in functional assays across species (FLIPR Ca^{2+} flux, K_b < 5.7 nM) and has high selectivity for H_4 (over 190-fold, with human H_4 receptor pK_i = 8.33).[118] Pyrimidine **24** shows good pharmacokinetic in rat and mouse ($t_{1/2}$ = 2.6 and 1.6 h; oral bioavailability of 37% and 90%, respectively) and has good anti-inflammatory activity (ED_{50} = 37 μmol/kg in mouse) and efficacy in pain models (thermal hyperalgesia, ED_{50} = 72 μmol/kg in rat). This compound also showed functional antagonism in vitro in mice, for which H_4 agonist-induced itch was potently blocked at 33 μmol/kg, ip. Compound **24** also potently inhibited inflammation at 34 μmol/kg, ip, in a mouse model of peritonitis and was effective in a carrageenan-induced inflammatory pain model in rat, with an ED_{50} of 72 μmol/kg, ip (compared with 80 μmol/kg for JNJ7777120 and 32 μmol/kg for **15**) and in a neuropathic pain model with an ED_{50} of 100 μmol/kg, ip (compared with 217 μmol/kg for JNJ7777120, 115 μmol/kg for **15**, and 140 μmol/kg for **23**).[118] The pharmacokinetic profile of **24** showed a longer half-life in rats (2.6 h) and mice (1.6 h) than data reported for the H_4 antagonists JNJ7777120 and **15**. Since compound **24** (A-943931) exceeded the activity of the reference antagonists JNJ7777120 and **15** in several key aspects (pharmacokinetic in rodents, potency against inflammation and in some pain models), it should be a useful probe of H_4 receptor pharmacology.

In the search for novel pyrimidine H_4 antagonists with improved therapeutic potential, alternative fusion to the pyrimidine ring was considered, and A-987306 (Figure 18) was synthesized.[194] This fused system compared favorably with

Scheme 1



Scheme 2



JNJ7777120. A cell-based Ca^{2+}-flux functional assay (FLIPR)[195] showed A-987306 to be a highly potent antagonist at the human, mouse, and rat H_4 receptors. In this assay, A-987306 blocked the activation of the H_4 receptor induced by endogenous histamine, but showed no activation of the receptor when tested alone. The fused pyrimidine also potently blocked histamine-mediated increase in binding of GTP-γ-[^{35}S] to rat H_4-receptor-containing membranes, with a K_b of 6 nM. A-987306 showed selectivity for the human H_4 receptor over the H_1, H_2, and H_3 receptors of 620-fold, >1600-fold, and 162-fold, respectively. However, selectivity for the rat H_4 receptor was lower, A-987306 showing only 4-fold selectivity for the rat H_4 receptor over the rat H_3 receptor. At 10 mg/kg in Sprague−Dawley rats, after ip dosing, A-987306 showed a favorable fractional bioavailability ($F_{ip/iv}$ = 72%) and half-life ($t_{1/2}$ = 4.7 h) and a C_{max} of 1.73 μM at a T_{max} of 0.25 h. A moderate fractional bioavailability ($F_{ip/iv}$ = 26%), a half-life of 3.7 h, and a C_{max} of 0.30 μM were found at a T_{max} of 1.5 h after oral dosing.[194] The plasma protein binding was determined as 59%, indicating that an appreciable fraction of the drug would be present in its unbound form in plasma. A-987306 showed good selectivity (IC_{50} > 810 nM for all of the 100 or so kinases tested) for the H_4 receptor and when tested at 10 μM was selective for the H_4 receptor over most biogenic amine receptors, neuropeptide receptors, ion channel binding sites, and neurotransmitter reporters.[194]

A-987306 performed excellently in the mouse-itch model, in which H_4 antagonists are assessed for their ability to block scratching induced by histamine H_4 agonists (here, clobenpropit); A-987306 had an ED_{50} of 0.36 μmol/kg, and since plasma levels of the drug at 0.3 μmol/kg were found to be 15 ng/mL, a high level of in vivo potency was demonstrated. A-987306 showed anti-inflammatory properties in a model of peritonitis, blocking neutrophil influx with an ED_{50} of 100 μmol/kg. Of particular interest is the efficacy of A-987306 in a pain assay in rats, in which it blocked carrageenan-induced thermal hyperalgesia with an ED_{50} of 42 μmol/kg (ip). The efficacy, potency, and good pharmacokinetic profile of A-987306 render it highly suitable as a probe of H_4 receptor pharmacology.[194]

A general route to 2-amino-6-(*N*-methylpiperazinyl)pyrimidines (Scheme 1) involved displacement of symmetrical 4,6-dichloropyrimidines **27** with *N*-methylpiperazine to give the amines **28**, which were converted into the desired ligands **29** by a variety of reactions: catalytic hydrodechlorination, Suzuki arylation, and copper-catalyzed amination.[107]

Cyclic ketones **30** (n = 0, 1, and 2, Scheme 2) were converted into the β-ketoesters **31** by treatment with NaH in dimethyl carbonate at 80−90 °C; the β-ketoesters **31** were condensed with guanidine nitrate, hydrochloride, or carbonate to give the tricyclic compounds **32**. Reaction of those compounds with $POCl_3$, TsCl, or NsCl gave respectively the displaceable chloro heterocycles, the toluenesulfonates, and 4-nitrobenzenesulfonates. Displacement of the leaving group in **33** by amines from 70 to 110 °C afforded the target ligands **34**, if necessary after Boc deprotection using trifluoroacetic acid (TFA).[118]

In a polycyclic variant of Scheme 2, condensation of cyclohexanone with cyclohexane-1,3-dione under acid catalysis and with removal of water led to the tricyclic ketone **35**, according to a literature procedure[196] (Scheme 3). Its enolate was treated with dimethyl carbonate to give the β-keto ester **36**, which was cyclized to the aminopyrimidine **37** by reaction with guanidine. *O*-Tosylation followed by displacement with piperazine afforded the desired fused H_4 antagonist A-987306.[194]

3.3.5. Quinazoline Derivatives. Quinazolines can be considered by analogy with the corresponding pyrimidines (section 3.3.4) and quinoxalines (section 3.3.6) and with a likely requirement of three key structural features: a basic and aminergic cyclic amine, a heterocyclic core with a small lipophilic substituent, and of lesser importance a substituent at the 2-position. On that basis, a representative quinazoline, **39**, might be expected to bind in a

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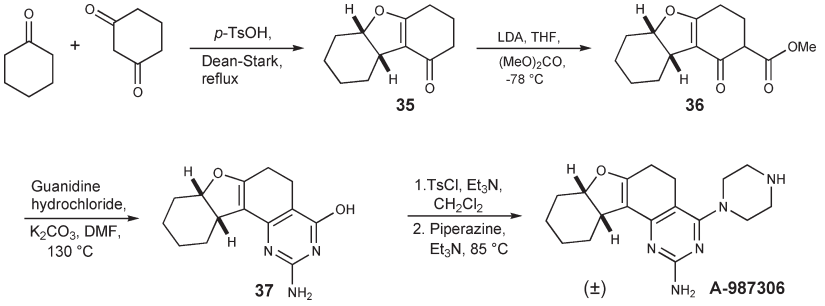
Scheme 3



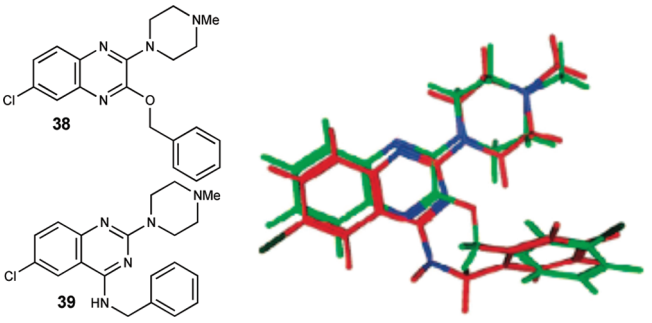


Figure 19. Overlay diagram of the H_4 receptor antagonist quinoxaline **38** with quinazoline **39**. Overlay diagram reprinted from rref 179. Copyright 2008 American Chemical Society.

way similar to that deduced for known quinoxalines such as **38** (Figure 19).[179] A series of quinazolines was prepared containing the 2-(*N*-methylpiperazinyl) group (conferring high affinity for the histamine H_4 receptor) and with a 6-chloro substituent (enhancing affinity in quinoxaline and other heterocyclic series) but with a varied 4-substituent (Table 6).

Substitution of quinazoline **40** with a 4-amino group (**41**) gave a 3-fold increase in affinity; the further addition of a 6-chloro substituent (**42**) raised the affinity to a pK_i of 6.98, being a yardstick for additional optimization. In contrast to the quinoxalinone series (e.g., **68** in Table 8), 2-(*N*-methylpiperazinyl)-6-chloroquinazolin-4-one showed poor activity, so attention was focused on optimizing substitution of a 4-amino group. A 4-phenylamino group showed loss of affinity (as did a 3-aminophenyl group in the quinoxaline series). However, incorporation of an aminomethylene group gave good affinities (compounds **44**−**50**). The introduction of a 6-chloro group again led to increased affinities (compare **39** with **44** and **46** with **45**). *N*-Alkyl groups generally did not give good affinities; it is proposed that the NH of a 4-aminoquinazoline acts as a hydrogen bond donor in binding to the H_4 receptor. In all cases, the basic *N*-methylpiperazine nitrogen atom will be protonated and hence can interact with Asp94 (3.32) in TM3, forming an ionic bond; the hydrogen bond donor would form a hydrogen bond with Glu182 (5.46) in TM5.

A range of benzylamines substituted on the aromatic ring did not exhibit improved affinity. Heteroaromatic rings attached to the aminomethylene group (e.g., **48**−**50**) were investigated for their ability to increase binding affinity, perhaps through polarity, hydrogen bonding, or π-interactions. The 2-furyl and 3-furyl analogues **48** and **49**, respectively, showed high potency, the 3-furyl group being 3-fold more potent.[179] Replacement of the 2-furyl group by 2-thienyl gave **50**, which was 11-fold more potent, with a strong binding affinity of 7.5 nM. However, the 3-thienyl analogue showed lower affinity, and further alterations to the thiophene substituents in either the 2-thienyl or 3-thienyl compounds were found to give affinities lower than that of **50**. Annulation of the thiophene ring to give a benzothiophene linked at the 2-position also failed to improve potency. Sixteen other cyclic or spirocyclic 2-amino moieties were examined, but none showed improved affinities compared with the *N*-methylpiperazinyl unit, although (*S*)-diazabicyclo[4.3.0]nonane (or the racemic amine) displaced the 2-chloro substituent of 2,6-dichloro-*N*-methylquinazoline-4-amine to give a compound that was nearly as potent as the *N*-methylpiperazinyl analogue [(*S*)-enantiomer or racemate].[179]

The high-affinity quinazolines **49** and **50** were docked into the model of the H_4 receptor and were further evaluated by radioligand binding studies.[179] An overlay of **49** and **50** (Figure 20) showed that the quinazolines can adopt a low-energy conformation in which the furan or thiophene rings occupy the proposed hydrophobic third pocket, while the other two pockets are occupied by the *N*-methylpiperazine and quinazoline segments. A docking and overlay of JNJ7777120 with the clozapine analogue VUF6884 (Figure 21) showed a similar occupancy of the H_4 receptor. The appreciable affinity of VUF6884 for the H_1 receptor (pK_i = 8.1)[176,177] compared with that of 3-benzyl-2-(*N*-methylpiperazinyl)quinoxaline (pK_i = 6.0) suggested that occupancy of the right-hand pocket with an aromatic or similar group was consistent with appreciable affinity for the H_1 receptor, as well as enhanced affinity for the H_4 receptor. This hypothesis was confirmed when both quinazolines **49** and **50** were found to show not only high affinity for the H_4 receptor (pK_i = 7.57 and 8.12, respectively), but also strong affinity for the H_1 receptor, being nearly equipotent in binding to the H_1 and H_4 receptors (pK_i = 7.01 and 7.70, respectively).[179] Further confirmation of this model is seen in the quinoxalinone **68** (Table 8), which lacks a moiety capable of bonding to the hydrophobic pocket and hence shows feeble affinity for the H_1 receptor, despite being a potent ligand of the H_4 receptor (pK_i = 8.25).

Quinazoline **49** has an affinity close to that of thioperamide, the standard imidazole-containing inverse agonist, but quinazoline **50** is 9 times more effective in displacing [^{3}H]histamine from the histamine H_4 receptor. Functional activities on the H_4 receptor were assessed in an H_4-driven CRE-β-galactosidase gene reporter assay, in which histamine displays full agonistic behavior (α = 1) whereas thioperamide behaves as an inverse agonist (α = −1); **49** showed pronounced behavior as an inverse

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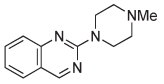
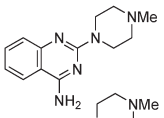
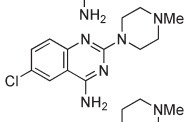
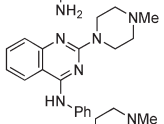
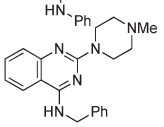
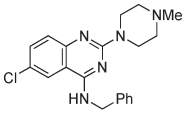
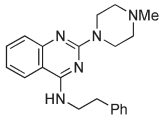
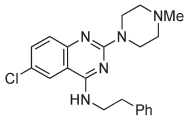
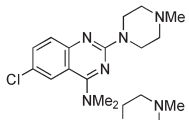
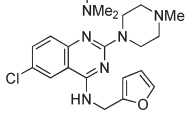
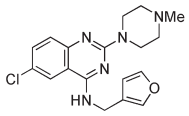
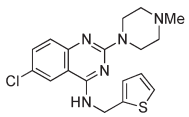
Table 6. Affinities of Various 4-Substituted Quinazolines for the H_4 Receptor

Compound no.	Structure	pK_i[a]
40		5.12
41		5.67
42		6.98
43		5.07
44		5.97
25		6.59
45		5.83
46		6.62
47		6.21
48		7.05
49		7.57
50		8.12

[a] Measured by displacements of [^{3}H]histamine binding using membranes of HEK cells transiently expressing the human H_4 receptor.[179]

agonist ($\alpha = -1.49$), whereas **50** showed inverse agonism ($\alpha = -1.06$) comparable with that of thioperamide. At the rat receptor, quinazolines **49** and **50** gave respective pK_i values of 7.61 and 7.80 and behaved as neutral antagonists.[179] In a carragenan-induced paw edema model for inflammation,[99] compound **50** caused a significant inhibition of edema at both 10 and 30 mg/kg compared to vehicle, an effect still significant 4 h after administration. These studies show the potential of quinazolines to be dual-acting antihistamine agents through antagonism of the H_1 and H_4 receptors. The three-pocket pharmacophore model should allow the design of further potent ligands that could allay the symptoms of acute inflammation by raising a blockade of the H_1 receptor, while concomitantly targeting the H_4 receptor to modulate the underlying mechanism of disease by blocking the influx of pro-inflammatory cells.[84]

Condensation of anthranilic acid and its derivatives with urea afforded the quinazolinediones **51** (Scheme 4) which were converted into the corresponding 2,4-dichloroquinazolines **52** using $POCl_3$ and a tertiary amine as the base. Reaction of **52** with a variety of primary amines at room temperature afforded the products of displacement at exclusively the 4-position; in most cases the amines **53** were not purified but underwent further displacement with *N*-methylpiperazine using microwave-assisted heating to give the H_4 receptor ligands **54**.[179,197] A 2-(*N*-phenylsulfonamido)ethyl group as R^3 in **54** afforded a potent H_4 receptor inverse agonist which showed potent anti-inflammatory activity in an animal model of acute inflammation.[198]

Partially saturated quinazolines have received attention, including 2-amino-7,7-dimethyl-4-(4-methylpiperazin-1-yl)-5,6,7,8-tetrahydroquinazoline, which showed an EC_{50} of about 3 nM.[199]

3.3.6. Quinoxalines and Related Compounds. To identify a new ring system suitable for H_4 receptor antagonists and to validate the overlay model above, a range of bicyclic heterocyclic scaffolds was synthesized, each linked to an *N*-methylpiperazine group (Table 7).[177] Bicyclic systems could be expected to occupy a space similar to those of the chlorophenyl moiety of VUF6884 and the indole ring of JNJ7777120, while the *N*-methylpiperazine group would be able to adopt a conformation as both those antagonists. This structural design was an appropriate departure from tricyclic systems such as clozapine that showed little selectivity among GPCRs. Modification of the *N*-methylpiperazine group was considered inadvisable since previous studies had shown that to be very detrimental.[73,176] The survey of rings (Table 7) included 6-chloro substitution because of its beneficial effect on H_4 receptor ligands in other heterocyclic systems previously explored.[73,105,176] This search clearly identified the quinoxaline ring system (compounds **58** and **59**) as the most promising of those examined; introduction of a second nitrogen atom at the 4-position of the quinoline ring enhanced the affinity 5-fold. Consistent with previous findings on other ring systems, introduction of a 6-chloro substituent produced a 10-fold increase in affinity for the H_4 receptor.

A flexible alignment model previously described[176] implied the presence of a pocket able to contain an additional aromatic ring (cf. the unsubstituted benzene ring in VUF6884). The occupancy of the aromatic pocket was probed by evaluating 3-substituted analogues, including **61** (3-methyl), **62** (3-phenyl), and **63** (3-benzyl) (Table 8 and Figure 21B). While the methyl group produced an increase in H_4 receptor affinity of nearly 10-fold, the phenyl ring was not tolerated; however, a benzyl substituent conferred high affinity (33 nM). Extension of these studies showed that 3-oxygenated substituents could also be effective and that suitably substituted 2-quinoxalinones showed some of the most potent affinities among the compounds studied.[177] In other work, chloro substituents had proved beneficial, which led to the evaluation of compounds including **67** and **68**, the latter showing an H_4 receptor affinity of 6 nM; during the course of this work, a research group at Johnson & Johnson also reported quinoxalinones **67** and **68** as having potent affinities for the H_4 receptor and $K_i = 31$ nM and 32 nM respectively.[200] Attempts to find a bioisostere for the *N*-methylpiperazine moiety were unsuccessful; both *N*-methylhomopiperazine and aminomethylpyrrolidine moieties showed much

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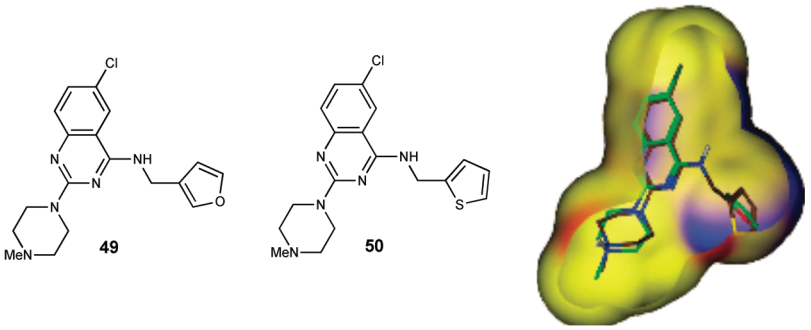


Figure 20. Model of the binding site of the H_4 receptor. Overlay diagram of **49** and **50**, the respective furan and thiophene rings binding in the hydrophobic pocket to the right. Hydrophobic surfaces are shown in yellow, mild polar surfaces in blue, and sites capable of hydrogen bonding in red. Overlay diagram reprinted from ref 179. Copyright 2008 American Chemical Society.

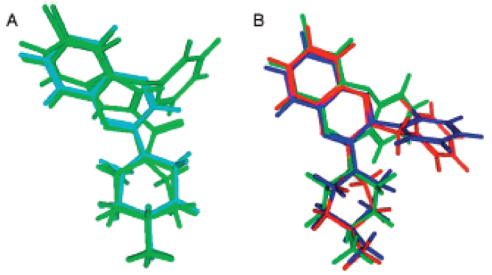
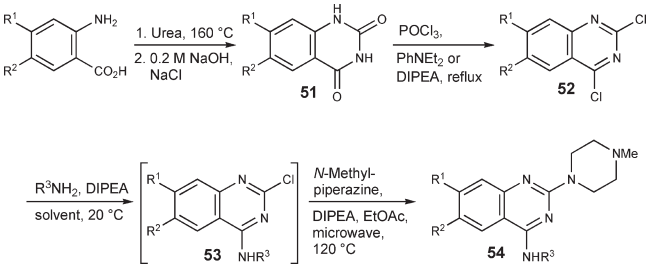


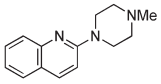
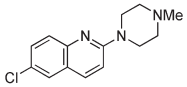
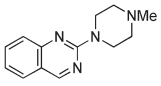
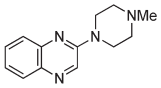
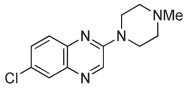
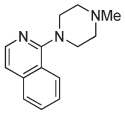
Figure 21. (A) Overlay diagram of VUF6884 and JNJ7777120 (both in green) with quinoxaline **63** (in light blue). The aromatic pocket can be occupied in the same way by each compound, while all three *N*-methylpiperazine groups occupy the same position. (B) Overlay diagram of VUF6884 with 3-phenylquinoxalines **62** (in blue) and **63** (in red). Overlay diagrams reprinted from ref 177. Copyright 2008 American Chemical Society.

Scheme 4



reduced affinities. Pharmacological evaluation of two compounds in the above series showed that quinoxaline **63** at 10 μM displaces the binding of [^{3}H]mepyramine to the H_1 receptor.[176] Since quinoxaline **63** had been designed by analogy with VUF6884 (**107g**), which has a high affinity for the H_1 receptor (pK_i = 8.11), that for quinoxaline **63** was determined and found to have pK_i = 6.13. Quinoxaline **63** showed inhibition of radioligand binding for human histaminergic receptor subtypes of 86% (H_1), 62% (H_2), 26% (H_3) and a pK_i of 7.4 (H_4), whereas the values for quinoxaline **68** were 7% (H_1), 42% (H_2), 64% (H_3), with a pK_i of 8.25 (H_4), indicating limited inhibition of radioligand binding to histaminergic receptors other than H_4. Consequently, quinoxaline **63** is an especially promising compound since ligands showing dual action for H_1 and H_4 receptors should have considerable potential for the treatment of a number of histamine-mediated allergic and inflammatory disorders.[84] Compounds **63** and **68** were studied for their anti-inflammatory effects; coadministration at 10 and 30 mg/kg, respectively, with carrageenan showed, after 2 h, a significant inhibition of carrageenan-induced rat paw edema, when compared with the vehicle. The effect of compound **68** was still significant 6 h after administration.[177]

Table 7. Affinity of Some Bicyclic Heterocyclic Systems for the H_4 Receptor

Compound no.	Structure	pK_i[a]	Ligand efficiency[b]
55		5.16	0.42
56		6.23	0.48
57		5.12	0.41
58		6.05	0.49
59		7.04	0.54
60		4.69	0.38

[a] Measured by displacements of [^{3}H]histamine binding using membranes of HEK cells transiently expressing the human H_4 receptor. [b] Ligand efficiency is calculated as the binding energy per non-hydrogen atom.[177]

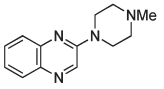
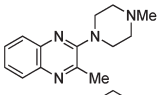
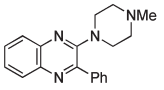
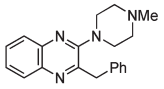
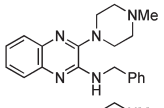
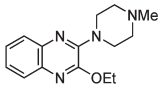
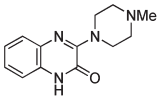
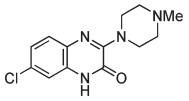
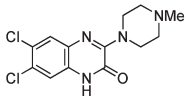
Condensation of *o*-phenylenediamine and its derivatives with α-keto acids afforded the quinoxalinones **69**[175] which upon treatment with $POCl_3$ gave the crude chloroquinoxalines **70** and which, after removal of excess $POCl_3$, were converted into the quinoxalines **71** using neat *N*-methylpiperazine (Scheme 5).[201−203] Those and other routes provided a range of quinoxalines and their derivatives on which SARs regarding the H_4 receptor were evaluated.[177] Quinoxalinones **74** were prepared by displacement of the 2,3-dichloroquinoxalines **72** with *N*-methylpiperazine, giving the derivatives **73** which were then hydrolyzed by alkali. The intermediates

73 were also treated with alkoxides, phenoxides, thiolates, and benzylamine to obtain the corresponding 3-substituted derivatives.

For syntheses of substituted 6-chloroquinoxalines, the shortest route (Scheme 6) involved consecutive displacements of the trichloroquinoxaline **75**, a displacement that was only definitively established by comparison with the product **76** from an independent regioselective synthesis (Scheme 7).[177]

3.3.7. Triazine Derivatives. A selection of 1,3,5-triazines were assayed for antagonism against the histamine H_4 receptor; significant potency required a 4-methylpiperazin-1-yl group, and even then potency was not below 10 nM, except for a few compounds, including **77** (K_i = 2 nM, Figure 22).[204] Absence of a 3-bromo group from the 2-thienyl substituent, or replacement of it by a 3-thienyl linkage, resulted in dramatic loss of potency.[204]

Table 8. Affinities of Quinoxalines and 2-Quinoxalinones for the H_4 Receptor

Compound no.	Structure	pK_i[a]
58		6.05
61		6.70
62		4.99
63		7.40
64		5.13
65		6.64
66		7.21
67		7.93
68		8.25

[a] Measured by displacements of [^{3}H]histamine binding using membranes of HEK cells transiently expressing the human H4 receptor.[200]

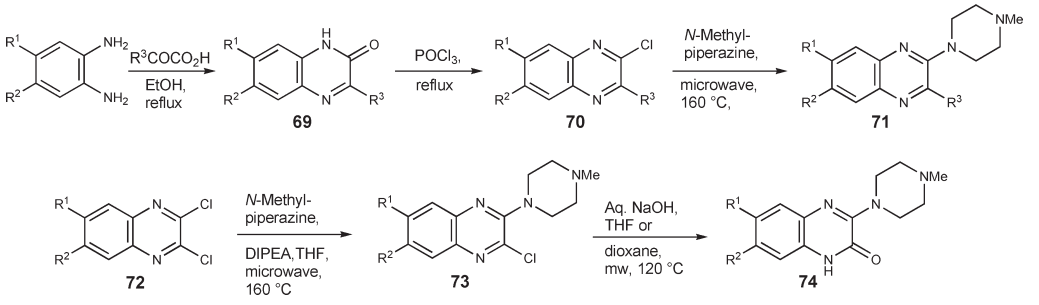
3.3.8. Indole Derivatives and Other Fused Indole Derivatives. Following the discovery of the human histamine H_4 receptor, a high-throughput screening of a corporate compound collection identified indole **78a** as a hit, showing K_i values for the histamine H_3 and H_4 receptors of 9 μM and 38 nM, respectively.[105] A limited investigation of the substitution on the piperazine ring (Table 9) showed that the *N*-methylpiperazinyl moiety, as in **78b**, was more potent than the unmethylated analogue **78a**. However, bulkier *N*-alkyl groups were not tolerated, nor was the *N*-methylpiperazinylmethyl moiety of sufficient potency (K_i = 10 000 nM).[73]

With methyl identified as the optimal group for the piperazine nitrogen atom, an SAR study of substitution on the indole ring was undertaken (Table 10). Of the halogenated derivatives, the 5-chloro compound **79c** was very promising in terms of affinity for the H_4 receptor site. The necessity of avoiding substitution of the indole nitrogen atom is reflected in the very poor affinity of compound **79k**. Apparently, bulk at the 6-position is not well tolerated. Methyl groups are tolerated at the 5- and 7-positions, but do not increase potency (compounds **79l** and **79m**). A 5-hydroxy group is well tolerated, but not a 5-methoxy group.[105]

A detailed evaluation of the high-affinity indoles **79c** and **79o** was undertaken. Functional activity against the human H_4 receptor was determined by using SK-N-MC cells stably transfected with the human H_4 receptor.[37] Addition of histamine to such cells induces a decrease in forskolin-stimulated levels of cAMP, which can be measured in a cell-based assay;[105] both **79c** and **79o** produced a shift to the right in the histamine dose−response curve, giving values of pA_2 of 8.14 and 8.11, respectively, confirming that they function as antagonists of the H_4 receptor. Indoles **79c** and **79o** also showed high affinity for the rat histamine H_4 receptor (giving respective values for K_i of 2.4 and 3.3 nM) and were more than 100 times more selective for the H_4 receptor compared to other histamine receptors. These compounds showed minimal activity against a panel of more than 50 receptor targets representing the major classes of biogenic amine receptors, neuropeptide receptors, ion channel binding sites, and transporters.[105] Compound **79c**, usually referred to as JNJ7777120, is a benchmark against which novel H_4 receptor ligands are commonly measured.

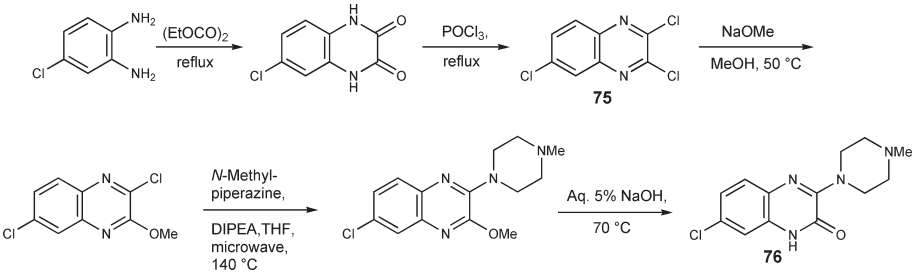
Further SAR studies on the indole ring system of indol-2-yl(4-methylpiperazin-1-yl)methanones showed that small lipophilic groups in the 4- and/or 5-positions of the indole nucleus were well tolerated. The 5-position was found to have the greatest effect on in vitro potency, and if the 5-position bears a substituent, then additional substituents at the 4- or 7-positions are

Scheme 5

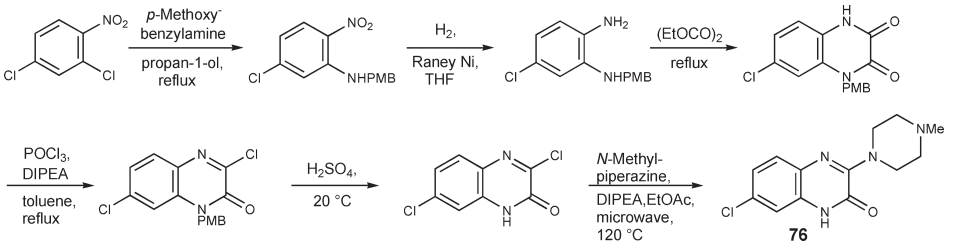


dx.doi.org/10.1021/cr900166w |*Chem. Rev.* 2011, 111, 7121–7156

Scheme 6



Scheme 7



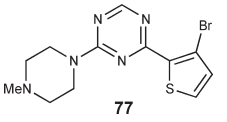
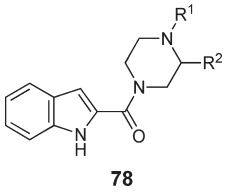


Figure 22. A potent 1,3,5-triazinyl-based antagonist of the H_4 receptor.

Table 9. Structure and Binding Affinities of 2-(Indolylacyl)-piperazines 78



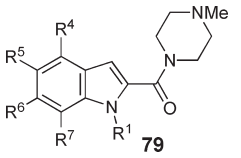
compound	R^1	R^2	K_i[a] (nM)
78a	H	H	38
78b	Me	H	17
78c	Et	H	260
78d	CH_2CH_2Ph	H	7000
78e	H	Me	202

[a] Displacement of [^{3}H]histamine from the recombinant human histamine H_4 receptor.[73]

tolerated without loss of H_4 binding affinity.[105] However, none of the derivatives prepared in this study showed greater potency than JNJ7777120. In vitro metabolism and initial pharmacokinetic showed the suitability of JNJ7777120 for further development.

A detailed study of other substituted 2-[(5-chloroindolyl)acyl]piperazines confirmed the necessity for a basic amine nitrogen atom at the location of the *N*-methyl group in the piperazine ring of compounds of type **79**; replacement of this ring by morpholinyl or by piperidinyl gave very poor ligands, with

Table 10. Structure and Binding Affinities of 2-(Indolylacyl)-piperazines 79



compound	R^1	R^4	R^5	R^6	R^7	K_i[a] (nM)
78b	H	H	H	H	H	17
79a	H	H	F	H	H	15
79b	H	H	F	H	F	14
79c	H	H	Cl	H	H	4
79d	H	H	H	H	Cl	19
79e	H	H	Cl	H	Cl	11
79f	H	Cl	Cl	H	Cl	5
79g	H	Br	H	H	H	32
79h	H	H	Br	H	H	8
79i	H	H	H	Br	H	147
79j	H	H	H	H	Br	61
79k	Me	H	H	H	H	>10,000
79l	H	H	Me	H	H	46
79m	H	H	Me	H	Me	31
79n	H	H	NH_2	H	H	15
79o	H	H	H	H	NH_2	8
79p	H	H	OH	H	H	23
79q	H	H	OMe	H	H	3000

[a] Displacement of [^{3}H] histamine from the recombinant human H_4 receptor.[73]

dx.doi.org/10.1021/cr900166w |*Chem. Rev.* 2011, 111, 7121–7156

respective pK_i values of <4 and 5.3.[174] In addition, the acylated nitrogen atom of the piperazine could not be dispensed with, the 4-methylpiperidinyl ketone having pK_i = 6.7. Use of a hydrazine linkage instead of the amide linkage in JNJ7777120 was also ineffective, the derivative having a pK_i of 4.6. Removal of the constraint of the ring in piperazine, and replacement by either *N*-[(dimethylamino)ethyl] or by *N*-[(dimethylamino)ethyl]-*N*-methyl groups gave pK_i values of only 5.5 and 4.6, respectively. Those and other results show the very limited variation of structure that is consistent with potent affinity of 2-(acylindolyl)piperidines of the JNJ7777120 type for the histamine H_4 receptor.

During a systematic study of bioisosteres of the indole portion of JNJ7777120, the thienopyrrole ring system was examined.[105] In vivo metabolic data indicated that the chlorothienopyrrole **80** (Figure 23) was metabolized similarly to JNJ7777120. At 10 mg/kg, the head-to-head fused thieno[2,3-*b*]pyrrole **80** showed no oral bioavailability in the rat and had a very short intravenous half-life of 6 min at a dose of 2 mg/kg. The head-to-tail series of thieno[3,2-*b*]pyrroles, when substituted with small lipophilic groups, had K_i values (e.g., **81**, K_i = 3 nM) which matched those of the indole series **52**.

A broad range of 2-(indolylacyl)piperazines (including alkyl, halo, hydroxy, methoxy, nitro, and amino group substitution) were prepared by reaction of indole-2-carboxylic acid (or its *N*-methyl derivative) with an *N*-alkylated or *N*-Boc-protected piperazine in the presence of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU), 1-hydroxy-7-azabenzotriazole (HOAt), and *N*,*N*-diisopropylethylamine (DIPEA) in DMF or using 1,1′-carbonyldiimidazole in THF.[73] Where required, the amide group was reduced with $LiAlH_4$ to give the corresponding 2-(indolylmethyl)piperazines.

Thieno[2,3-*b*]pyrroles (Scheme 8) were prepared from thiophene-3-carboxaldehyde by condensation with ethyl azidoacetate to give the azide **82**, which was cyclized thermally with loss of nitrogen using the Hemetsberger procedure; subsequent chlorination, ester hydrolysis, and coupling of the carboxylic acid afforded the thieno[2,3-*b*]pyrrole **80**.[205] The corresponding thieno[3,2-*b*]pyrroles were prepared by the same route, but starting with thiophene-2-carboxaldehyde and using phosgene for the activation of the carboxylic acid prior to coupling with *N*-methylpiperazine.[205]

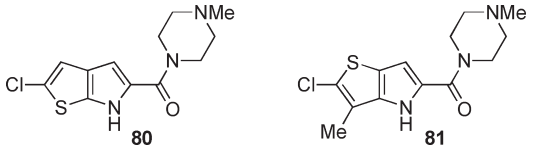
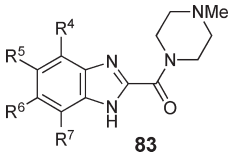


Figure 23. Chlorinated thieno[2,3-*b*]pyrroles **80** and thieno[3,2-*b*]pyrroles **81**.

3.3.9. Benzimidazole Derivatives. During a systematic study of bioisosteres of indoles **78b** and **79**, the benzimidazole ring system members of the analogous benzimidazole series were examined (Table 11).[105] The parent benzimidazole **83a** is comparable in binding affinity to the parent (*N*-methylpiperazinyl)indole **78b**. However, unlike the indole series, 5-substitution did not greatly improve affinity for the H_4 receptor; very poor receptor affinity was found for the 5-methyl compound **83g**. The binding affinity for the 4-chloro-5-methyl compound **83h** was excellent, but no further data were given in this paper.[105] The 4,6-dichloro compound **83e** had moderate affinity for the receptor, but diminished activity in the functional assay.

On the basis of the above results, JNJ7777120 and its benzimidazole analogue **83d** were further investigated for pharmacological effects. Both compounds showed excellent functional antagonism of the H_4 receptor and had pA_2 values in the range 7.7−8.1. In vitro metabolism studies in both rat and human liver microsomes and S9 fraction showed JNJ7777120 to have a moderate rate of metabolism in both human beings and rodent, with $t_{1/2}$ = 28 min in human liver microsomes and with $t_{1/2}$ = 64 min in rodent S9 fraction; its in vivo half-life was 2 h when dosed orally in rats at

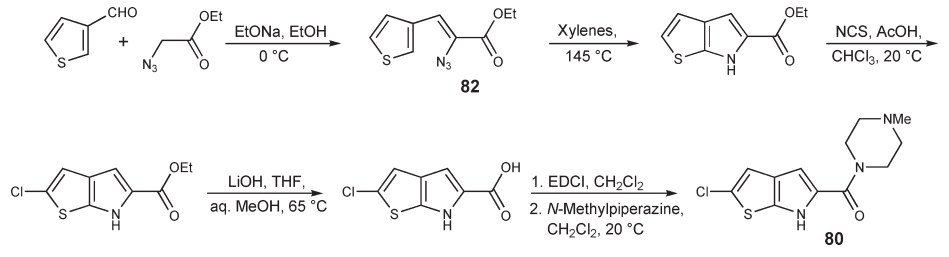
Table 11. Structure and Binding Affinities of Substituted Benzimidazoles 83



compound	R^4	R^5	R^6	R^7	K_i[a] (nM)	pA_2[b]
83a	H	H	H	H	35	7.2
83b	H	F	H	H	19	7.3
83c	F	F	H	H	49	7.1
83d	H	Cl	H	H	26	7.7
83e	Cl	H	Cl	H	136	
83f	H	CF_3	H	H	427	
83g	H	Me	H	H	528	
83h	Me	F	H	H	7	7.8
83i	Cl	Me	H	H	23	6.9
83j	Me	H	H	H	30	7.6
83k	H	NH_2	H	H	244	

[a] Displacement of [^{3}H]histamine from the recombinant histamine H_4 receptor. [b] Antagonism of histamine inhibition of forskolin-stimulated cAMP-mediated reporter gene activity in SK-N-MC cells expressing the human histamine H_4 receptor.[105]

Scheme 8



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10 mg/kg with a C_{max} of 1.8 μM. The oral bioavailability of JNJ7777120 was confirmed by the activity shown in the zymosan-induced mouse peritionitis model.[62] Oral administration of benzimidazole **83d** at 10 mg/kg to rats gave a profile similar to that of the indole JNJ7777120, with a C_{max} of 1.8 μM, but with a shorter half-life of 1 h. The inhibition of chemotaxis in eosinophils and mast cells by benzimidazole **83d** gave IC_{50} values of 530 and 138 nM, respectively. The corresponding values for JNJ7777120 were 86 and 40 nM, respectively.[62,72]

A further study of other substituted 2-[(5-chlorobenzimidazolyl)acyl]piperazines confirmed similar limitations on structure variation that were also found in the indole series; with the exception of the parent compound **83a**, only the 5-bromo- and 5-iodo analogues showed significant potency (pK_i = 7.5 and 7.2, respectively).[174] Thus, use of a hydrazine linkage instead of the amide linkage in JNJ7777120 was ineffective, the derivative having a pK_i of <4. Removal of the constraint of the ring in piperazine, and replacement by either *N*,*N*-(dimethylamino)ethylamino or *N*,*N*-(dimethylamino)ethyl-*N*-methylamino groups gave pK_i values of only 6.0 and 4.6, respectively. Both a 2-[*m*-(dimethylamino)-phenyl] group and a 2-acetylamino group on the benzimidazole ring were ineffective, conferring pK_i values of <4, for a 5-chloro-substituted and an unsubstituted benzimidazole ring. Similar 2-(benzimidazolylacyl)piperazine H_4 receptor antagonists, although perhaps less water-soluble, have been reported in the patent literature.[206]

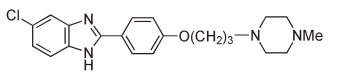
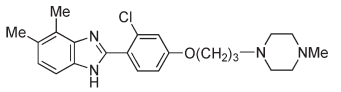
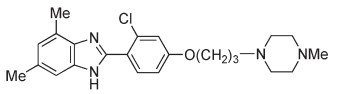
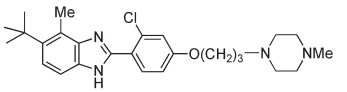
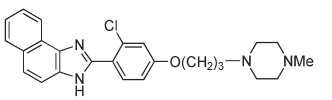
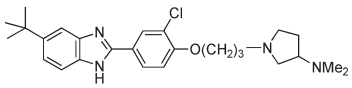
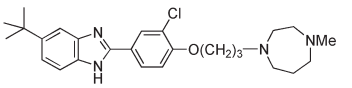
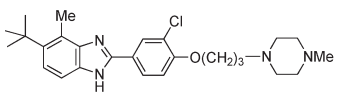
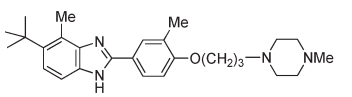
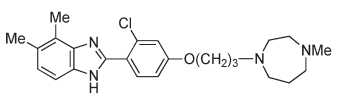
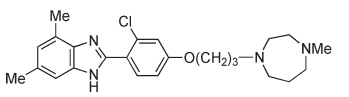
The 2-arylbenzimidazole **84** from a corporate library was identified as a hit in a high-throughput screening and showed moderate affinity (K_i = 124 nM) for the H_4 receptor.[207] In the absence of a homology model at the time of this study, definition of the pharmacophore was incomplete, but examination of a library of derivatives indicated a preference for *para*-substitution of the aryl ring with respect to the 2-benzimidazolyl ring and the linker with the cyclic amine terminus; a distance of 8.3 Å from the aryl ring to the distal nitrogen atom of the terminal piperazine appeared to be optimal. As alternatives to the *N*-methylpiperazine moiety, 3-(dimethylamino)pyrrolidine and *N*-methylhomopiperazine moieties were examined as part of a focused study (Table 12); one example of the former (compound **90**) showed a significant loss in H_4 receptor affinity, whereas the latter unit displayed a small increase in affinity. Tuning of the groups on the aryl ring and on the benzimidazole ring led to compounds with values of K_i less than 10 nM that were up to 100-fold more potent than the initial hit compound 2-arylbenzimidazole **84**.

Syntheses of 2-carboxamidobenzimidazole derivatives were achieved by the acylation of amines with activated benzimidazole-2-carboxylic acids such as **96** to give the corresponding amides **97** (Scheme 9). The carboxylic acids **96** can be prepared by condensation of a substituted *o*-phenylenediamine with trichloroacetic acid to give a 2-trichloromethyl derivative such as **95**, which is subsequently hydrolyzed.[174]

Following the identification of 2-arylbenzimidazole derivative **101** as a "hit", a general route (Scheme 10) was devised starting with the *O*-alkylation of hydroxybenzaldehydes **98** and subsequent displacement with an amine under Finkelstein conditions to give the amines **100** which were reacted with phenylenediamine or its derivatives under oxidative conditions to give the 2-arylbenzimidazoles **101**.[207]

A convergent preparation of 2-arylbenzimidiazoles such as **102** involves condensation of a substituted *o*-phenylenediamine with a substituted benzoic acid in PPA at elevated temperatures (Scheme 11).[174]

Table 12. Affinities of 2-Arylbenzimidazoles for the H_4 Receptor

Compound no.	Structure	K_i (nM)[a]
84		124
85		46
86		22
87		93
88		28
89		250
90		26
91		65
92		26
93		9
94		1

[a] Measured by displacements of [^{3}H]histamine from the recombinant human H_4 receptor.[207]

2-(α-Hydroxyalkyl)benzimidazole derivatives **106** were prepared from benzimidazole-2-carboxylic acids such as **103** by conversion into the protected esters **104**, which were reduced to the corresponding alcohols, which were then subjected to a Swern oxidation to give the corresponding aldehydes **105** (Scheme 12). Addition of the Grignard reagent prepared from 1-methyl-4-chloropiperidine afforded carbinols such as **106**.[174]

2-Benzimidazolyl-linked pyrimidines such as **2** were synthesized by displacement of a 2-sulfonyl group on the pyrimidine ring with the required amine, followed by adjustment of the oxidation level of the 5-substitent to an aldehyde and condensation with an aromatic 1,2-diamine to form the benzimidazole ring of the H_4 receptor antagonist **2** (Scheme 13).[154] Some of the analogous compounds containing a 2,4-disubstituted pyridine ring in place of the 4-methyl-substituted pyrimidine core also showed potent inhibition (K_i < 10 nM) of the histamine H_4 receptor.[208]

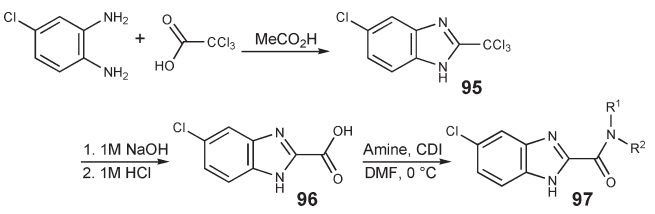
dx.doi.org/10.1021/cr900166w |*Chem. Rev.* 2011, 111, 7121–7156

3.3.10. Oxazepines and Related Ring Systems. Clozapine (Figure 24) is widely used as an antipsychotic drug; although it lacks an imidazole ring, it has considerable affinity for the H_4 receptor.[118] That clozapine is an agonist at the H_4 receptor ($\alpha = 1$, $pEC_{50} = 6.7$; $pK_i = 6.8$)[175] is striking, in view of its action as an antagonist on most GPCRs. In considering the design of histamine H_4 receptor ligands based upon clozapine, the first component investigated was alteration of the NH group to NMe, S, and O (giving $pK_i = 5.9$, 5.7, and 7.4, respectively), the nature of the atom being known to control the dihedral angle subtended by the planes of the two aromatic rings;[209] since the ether isostere **107g** had the greatest affinity for the H_4 receptor, this framework was selected for further SAR study and afforded affinity data as shown in Table 13, several compounds exhibiting nanomolar potency with respect to the H_4 receptor. The 8-chloro substituent in **107c** greatly increases the affinity, as compared with the parent compound **107a**, and a 7-chloro substituent confers even more potency (**107g**) and has the same pK_i as **107e**, containing 8-chloro-4-fluoro substitution.[176] Several other dihalogenated patterns of substitution also conferred high affinity for the H_4 receptor. Only the 2-chloro derivative **107b** showed poor affinity and was also the only one in the series tested not to be a full agonist; instead, **107b** acted as a partial agonist. Other variations, including replacement of the imine group in **107** by ethylene (with R^1 = Cl and R^2 = H), showed no appreciable affinity for the H_4 receptor. Variation of the *N*-alkyl group on both clozapine and **107c**, including demethylation, also gave binding affinities lower than those of the *N*-methyl compounds. Replacement of the piperazine ring by either piperidine or morpholine gave values of $pK_i < 5$.[176]

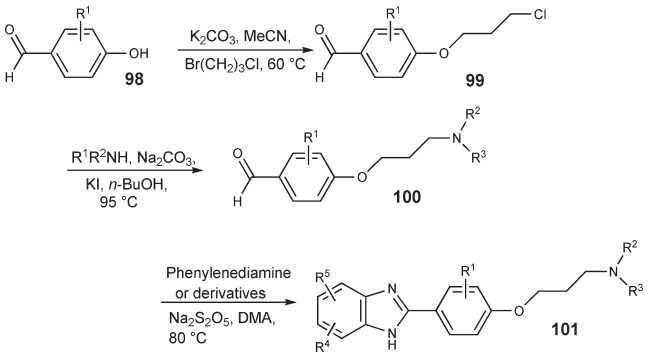
Oxazepine **107g**, also known as VUF6884,[179] has approximately 5-fold more affinity for the H_1 receptor than for the H_4 receptor, but is 330 times more selective for the H_4 receptor than for the H_3 receptor. Compounds with dual H_1 and H_4 activity, whether acting as antagonists or inverse agonists, are considered to have potential in the treatment of inflammatory diseases.[84] Certain H_1 receptor ligands, including doxepine, cinnarazine, and promethazine, have been reported to be active at the H_4 receptor, although another group[179] was unable to repeat the results previously described.

Both JNJ7777120 and **107g** were confirmed to act as orthosteric ligands rather than allosteric modulators of the recombinant H_4 receptor system; the value of B_{max} did not change significantly with increasing concentrations of ligand, whereas the K_d value for [^{3}H]histamine increased with increasing concentration of ligand.[179] JNJ7777120 was also shown to antagonize competitively the agonistic effect of **107g**. The overlap of JNJ7777120 with **107g** is striking (Figure 24), as obtained by alignment using the molecular operating environment (MOE), employing the MMFF94 force field;[179] however, the qualitative

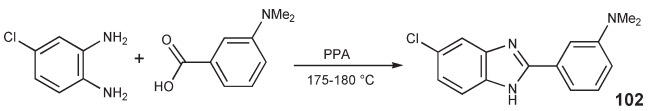
Scheme 9



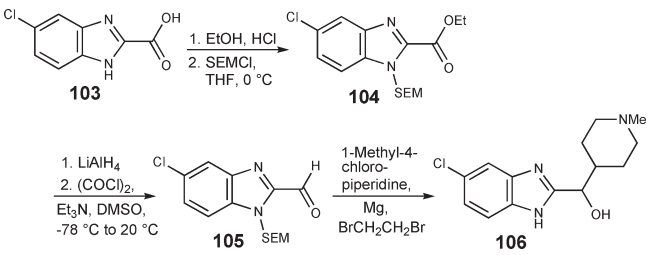
Scheme 10



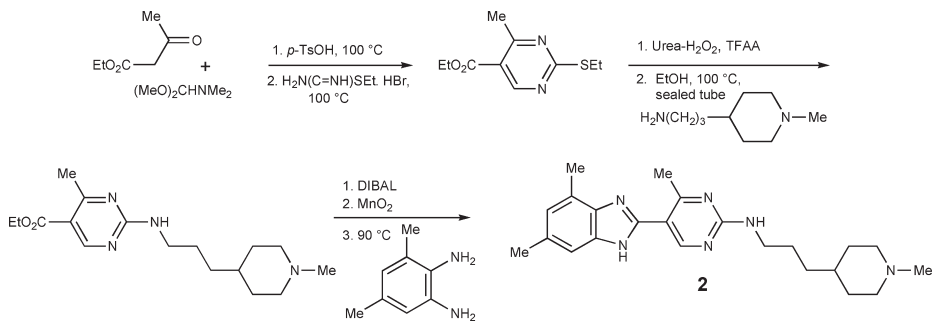
Scheme 11



Scheme 12



Scheme 13



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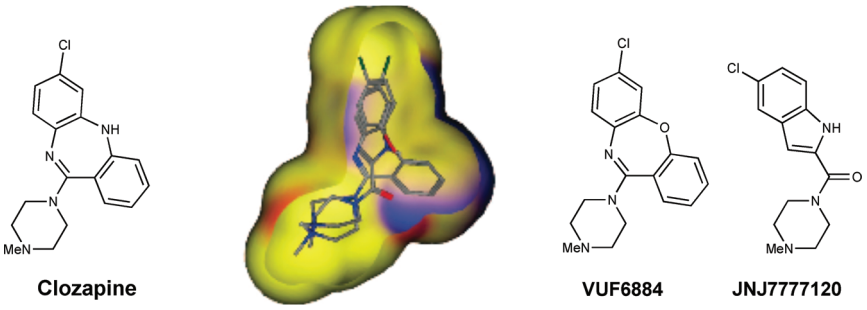
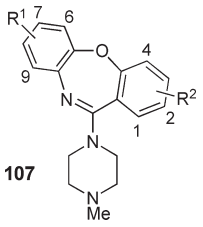


Figure 24. Structure of clozapine and the overlay of its ether analogue VUF6884 (**107g**) with JNJ7777120 in the modeled histamine H_4 receptor. Overlay diagram reprinted from ref 179. Copyright 2008 American Chemical Society.

Table 13. Structure and Affinity of 11-(4-Methylpiperazin-1-yl)dibenzo[*b*,*f*][1,4]oxazepines 107



compound	R^1	R^2	pEC_{50}	K_i[a] (nM)
107a	H	H	6.52	6.88
107b	H	2-Cl	6.66	5.34
107c	8-Cl	H	7.55	7.37
107d	8-Cl	2-F	7.09	6.72
107e	8-Cl	4-F	7.83	7.56
107f	8-Cl	3-Cl	6.99	6.51
107g	7-Cl	H	7.70	7.55
107h	7-Me	H	6.98	7.10
107i	7-Cl	3-F	7.41	7.41
107j	7-Cl	4-F	7.69	7.51
107k	7-Cl	4-Cl	7.83	7.34

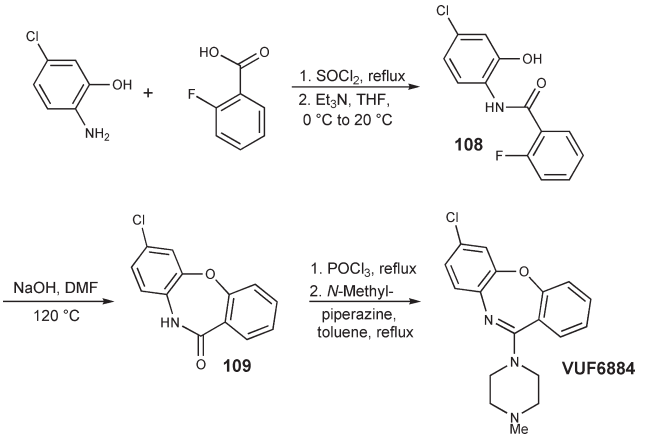
[a] Displacement of [^{3}H]histamine from the recombinant human histamine H_4 receptor.[176]

pharmacophore model cannot account for the different modes of action: the neutral antagonism of JNJ7777120 and the agonism of **107g**.

The best result obtained for the structural optimization of clozapine in this study was compound **107g**.[179] Both clozapine and especially **107g** have considerable overlay with JNJ-7777120, as can been seen in Figure 24; the chlorophenyl moieties are proximate when the *N*-methylpiperazine units are overlaid, and additionally the ether oxygen atom is near the NH group.

A modification of the method of Nagarajan and co-workers[210] was used to synthesize 11-(4-methylpiperazin-1-yl)dibenzo[*b*,*f*]-[1,4]oxazepines such as VUF6884 (Scheme 14).[179] Thus, chloro- or fluoro-substituted benzoic acids were converted into their acid chlorides which were added to a solution of a substituted *o*-aminophenol and Et_3N in THF to give the corresponding amides **108**. Ring closure of the amides **108** was achieved using NaOH in DMF to give the dibenzooxazepines **109**, which were reacted with $POCl_3$ to give the imino chlorides in situ, which were then

Scheme 14



displaced with various cyclic amines, affording the H_4 receptor ligand VUF6884.

3.3.11. Miscellaneous Systems. A focused screening of compounds related to dimaprit using SK-N-MC cells stably expressing the human H_4 receptor (Table 14) showed that replacement of the tertiary amine group in dimaprit by a guanidine residue greatly lowered affinity for the receptor.[182] However, shortening the spacer connecting the thiourea and guanidine moieties from propylene to ethylene gave compound **111** of excellent affinity. This compound, *S*-(2-guanidylethyl)-isothiourea dihydrobromide (VUF8430), has a pK_i value of 7.5, almost as high as that of histamine (pK_i = 7.9).[182] The presence of two different basic moieties in **111** (one guanidine group and one isothiourea group) is evidently key to its receptor affinity; the presence of either two guanidine moieties (as in **113**) or two isothiourea groups (as in **112**) have much less affinity. Increasing the number of methylene units in the spacer also decreases affinity for the H_4 receptor (e.g., **110** compared with **111**).[182] VUF8430 showed 33-fold selectivity for the H_4 receptor, for which it is a full agonist, over the histamine H_3 receptor (pK_i = 6.0) and negligible affinity for histamine H_1 and H_2 receptors, in marked contrast to the H_2 agonist dimaprit. The different pharmacological profile of VUF8430 compared to 4-methylhistamine, another human H_4 agonist, makes them useful and complementary probes of the receptor pharmacology.

S-(2-Guanidylethyl)isothiourea **111**, an optimal H_4 receptor agonist derived from a series of compounds structurally related to dimaprit, was prepared (Scheme 15) by displacement of *S*-

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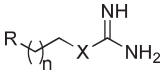
ethylisothiourea hydrobromide with aminoethanol, giving *N*-(hydroxyethyl)guanidine **116**. Reaction of the latter with aqueous 48% HBr afforded *N*-(bromoethyl)guanidine **117**, which was used to *S*-alkylate thiourea, thereby furnishing the desired agonist **111**. The one-pot preparation of *N*-(bromoethyl)guanidine **117**[211,212] previously described could not be satisfactorily repeated and proceeded in only 10% yield in the hands of Leurs and co-workers, who accordingly devised an alternative procedure involving treatment of **116** with HBr under microwave irradiation to give **117**, which was isolated and then reacted with thiourea, giving **111** in an isolated yield of 72%.[182]

A detailed study of analogues of clobenpropit[181] has been made for the purpose of understanding the factors that contribute to H_4 receptor versus H_3 receptor affinity and to identify an optimal H_4 receptor ligand in this series; Table 15 represents about 40% of the ligands studied. Most of the series show similarly high affinities for both the H_3 and the H_4 receptors, as do histamine, imetit, clobenpropit, and thioperamide, although most of the analogues of clobenpropit are more selective for the H_3 receptor. The following trends were noted: replacement of the isothiourea group by a guanidine moiety diminishes H_3 receptor affinity and increases H_4 receptor affinity. Lengthening the linkage between the phenyl and amidine moiety generally decreases affinity at both receptors.[181] Introduction of a halogen substituent at the 4-position of the benzyl unit increases affinity at both receptors, the selectivity being similar. Notably, an increase in affinity at the H_4 receptor is accompanied by an increase in intrinsic activity. Whereas clobenpropit acts as a partial agonist, the 4-iodo analogue **118f** shows full antagonism at the H_4 receptor. Unexpectedly, an additional chloro substituent at position 3, i.e., **118e**, greatly increases affinity at the H_4 receptor, but decreases affinity at the H_3 receptor. Of the analogues studied, **118e** showed the greatest affinity and selectivity for the human H_4 receptor and also behaved as a full agonist ($\alpha = 1$).

In the search for compounds with improved selectivity for the human H_4 receptor, the guanidine group in a range of (imidazolylpropyl)guanidines, including SK&F 91486 (Figure 25), was acylated.[213] Of the acyl series studied, alkanoyl groups were preferable. The most promising compound was UR-PI288, a potent full agonist of the human H_4 receptor ($pEC_{50} = 8.31$; $\alpha = 1.00$) and showing >1000-fold selectivity relative to the human H_1 receptor and >100-fold selectivity relative to the human H_2 receptor, as well as only low intrinsic activity ($\alpha = 0.27$) at the human H_3 receptor.[213] SK&F 91486 was *N*-tritylated on the histamine ring and Boc-protected on the guanidine moiety, permitting a subsequent acylation of the guanidine moiety (acetic acid and EDC.HCl) without complications. Subsequent deprotection using aqueous trifluoroacetic acid afforded UR-PI288.[213] This study shows that acylation of the guanidine group of SK&F 91486 can be an effective means of increasing the agonistic activity at the human H_4 receptor compared with the activity at the human H_3 receptor.

To increase H_4 receptor antagonism, the acylguanidine unit in compounds of the type **118** was replaced with a cyanoguanidine

Table 14. Structure and Affinity of Analogues of Dimaprit



compound[a]	X	*n*	R	K_i[b] (nM)
histamine				7.9
dimaprit	S	2	NMe_2	6.5
110	S	2	guanidinyl	5.1
111	S	1	guanidinyl	7.5
112	S	1	isothiourea	6.6
113	NH	1	guanidinyl	6.4
114	S	3	guanidinyl	5.5
115	S	5	guanidinyl	5.4

[a] Dihydrobromide salts were used in all cases except for histamine, which was used as the dihydrochloride salt. [b] Displacement of [^{3}H]histamine from the recombinant human histamine H_4 receptor.[182]

Scheme 15

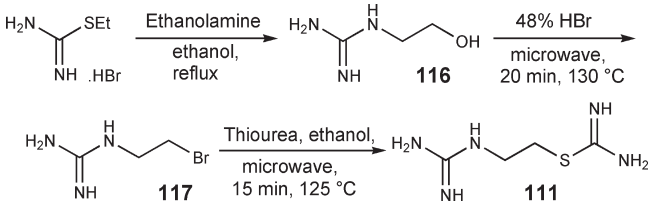
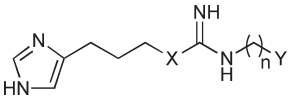


Table 15. Structure and Affinity of Analogues of Clobenpropit[181]



compound	X	Y	*n*	pK_i(H_3R)	pK_i(H_4R)	pK_i(H_3R) − pK_i(H_4R)	α(H_4R)
118a	S	H	0	8.6	7.9	0.7	0.59
118b	S	C_6H_5	1	8.1	7.6	0.5	0
118c	NH	C_6H_5	1	7.1	7.6	−0.5	0.59
118d	S	4−F-C_6H_4	1	8.3	7.6	0.7	0.39
Clobenpropit	S	4−Cl-C_6H_4	1	8.5	8.0	0.5	0.83
118e	S	3,4-Cl_2−C_6H_4	1	8.2	8.8	−0.6	1.00
118f	S	4−I-C_6H_4	1	8.5	8.0	0.5	0.98
118g	S	4-MeO-C_6H_4	2	7.7	6.8	0.9	0.26
118h	S	$(C_6H_5)_2CH$	2	7.5	7.6	−0.1	0.70

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moiety. 2-Cyano-1-[4-(1*H*-imidazol-4-yl)butyl]-3-[(2-phenylthio)-ethyl]guanidine (i.e., a variant of **118** with n = 2, Y = SPh, X = CH_2, and C = NCN, replacing C = NH) was identified as a potent human H_4 receptor agonist (pEC_{50} = 7.47, α = 0.93) which showed negligible hH_1 and hH_2 receptor activity and significant selectivity over the hH_3 receptor (pK_B = 6.00, α = −0.28). Modeling suggests that the cyanoguanidine group forms hydrogen bonds with the hH_4 receptor-specific Arg341 as well as the conserved Asp94 residue.[214]

Conformationally restricted analogues of histamine, based on a *cis*- or *trans*-disubstituted cyclopropane ring that links an imidazole ring with an aminoalkyl chain, have been studied as mimicks of histamine (Table 16). The structure of the backbone and the stereochemistry of the cyclopropane ring markedly influence the pharmacological profiles of the compounds, potent H_3 and/or H_4 receptor antagonists being identified with low nanomolar values of K_i.[180] This approach of diversifying the stereochemistry is generally valuable and can have a major impact when the structure of the target protein is not known, since the stereochemical iteration does not require structural information on the protein involved. It is also useful when, as here, the bioactive conformation(s) of the natural ligand (here, histamine) in the receptor(s) is not known with precision. Conformational restriction using a cyclopropyl unit has previously been successful in identifying H_3 receptor ligands such as the *cis*-cyclopropyl agonist **120c** (R = H), also known as AEIC,[180,215] and the *trans*-cyclopropyl antagonist GT-2331 (in which a 5,5-dimethylhex-1-ynyl substituent replaces the aminoethyl unit in structure **122**).[216]

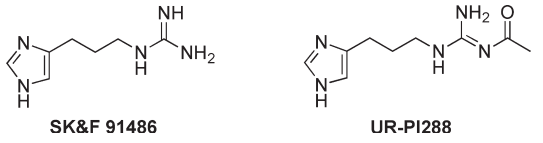
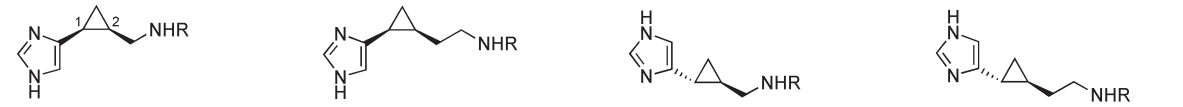


Figure 25. (Imidazolylpropyl)guanidine and its corresponding acylated derivative.

The (1*R*,2*S*)-*trans*-isomer **122a** exhibited potent antagonistic activity at both the H_3 (K_i = 8.4 nM) and H_4 (K_i = 7.6 nM) receptors; its enantiomer, *ent*-**122a**, showed potent antagonistic activity at the H_3 receptor (K_i = 3.6 nM), but was much less potent against the H_4 receptor (K_i = 37 nM). Conversely, (1*R*,2*S*)-*trans*-isomer **121a** was selective for the H_4 receptor (K_i = 118 nM) compared with the H_3 receptor (K_i > 1000 nM). As might be anticipated, most of the potent ligands for the H_3 and/or H_4 receptor possessed the *trans*-configuration about the cyclopropane ring. However, the series studied did not contain a compound that was both a potent ligand of the H_4 receptor and highly selective for the H_4 receptor over the H_3 receptor.[180]

Enantiopure imidazolylcyclopropanes have been synthesized as histamine H_4 antagonists through elegant but multistep routes.[180,215,217] The chiral cyclopropane unit was assembled (Scheme 16) from one enantiomer of epichlorohydrin (e.g., **123**) by a largely S_N2 displacement (rather than S_N2 displacement) followed by a base-promoted ring closure to the cyclopropanemethanol **124**, which underwent hydrolytic cyclization to lactone **125** upon treatment with acid. Subsequent borohydride reduction afforded the diol **126**, which was converted into **127** with good regioselectivity (owing to the steric hindrance of the hydroxy group near to the sulfone group). Reductive desulfonylation of **127** with Mg in methanol at 55 °C gave the more thermodynamically stable product, the

Table 16. Structure and Affinity of Imidazolylcyclopropanes



compound	configuration	$K_i(H_3)^a$ (nM)	$K_i(H_4)^a$ (nM)	$K_i(H_3)/K_i(H_4)$
119a	(1*S*)-*cis*	88	$>10^3$	<0.09
119b	(1*S*)-*cis*	$>10^3$	$>10^3$	
120a	(1*S*)-*cis*	44	130	0.34
120b	(1*S*)-*cis*	20.5	177	0.12
ent-**119a**	(1*R*)-*cis*	$>10^3$	$>10^3$	
ent-**119b**	(1*R*)-*cis*	$>10^3$	$>10^3$	
***ent*-120a**	(1*R*)-*cis*	14	103	0.14
ent-**120b**	(1*R*)-*cis*	$>10^3$	$>10^3$	
121a	(1*R*)-*trans*	$>10^3$	118	>8.5
121b	(1*R*)-*trans*	34	140	0.25
122a	(1*R*)-*trans*	8.4	7.6	1.1
122b	(1*R*)-*trans*	35	31	1.1
ent-**121a**	(1*S*)-*trans*	203	115	1.8
ent-**121b**	(1*S*)-*trans*	289	90	3.2
ent-**122a**	(1*S*)-*trans*	3.6	37	0.097
ent-**122b**	(1*S*)-*trans*	5.3	127	0.042
thioperamide		51	124	0.41

[a] Displacement of [^{3}H] histamine from the human recombinant histamine H_4 receptor.[180]

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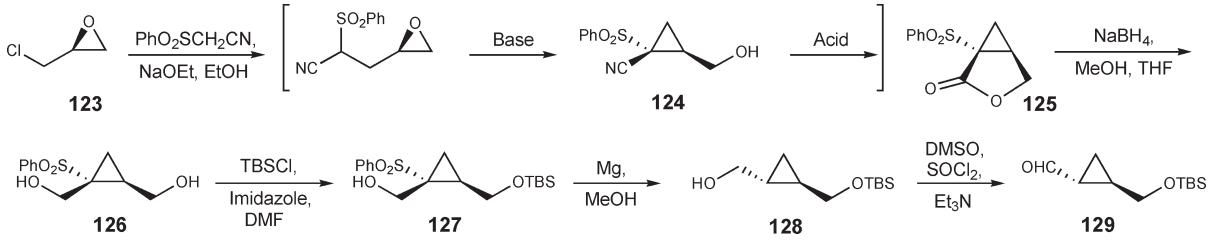
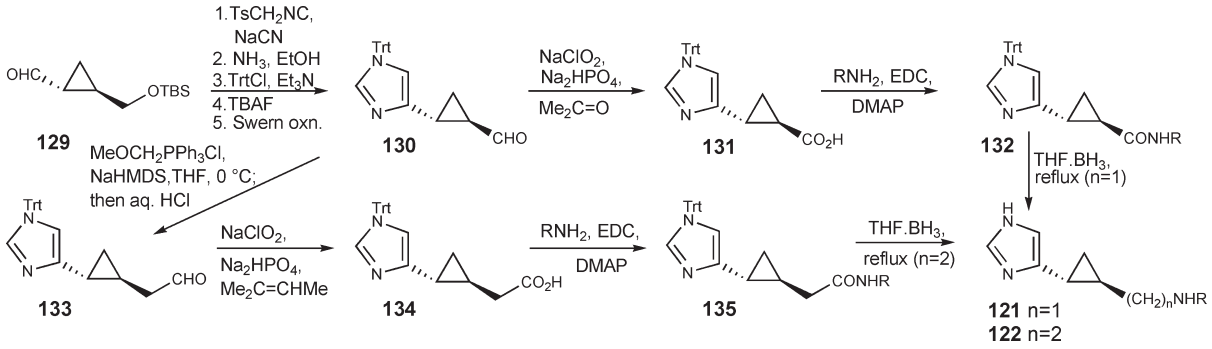
Scheme 16



Scheme 17



trans-alcohol **128**, which by a Swern oxidation afforded the key intermediate aldehyde **129**.[217]

Aldehyde **129** underwent condensation with (*p*-tolylsulfonyl)methyl isocyanide followed by reaction with ammonia, resulting in the imidazole ring, which was then tritylated prior to desilylation and Swern oxidation, giving aldehyde **130** (Scheme 17).[217] Chlorite oxidation afforded the carboxylic acid **131**, which was coupled with a variety of amines to give the amides **132** and hence by borane reduction the amine ligands **121** (n = 1). The homologues **122** (n = 2) were prepared by Wittig olefination of aldehyde **130** with $MeOCH{=}PPh_3$ followed by acidic workup to give the aldehyde **133**, which was subjected to the same sequence of chlorite oxidation, coupling with an amine, and reduction.

Cyclopropyl analogues of 4-methylhistamine have also been prepared, but even for the most favorable example there was little selectivity for the H_4 receptor over the H_3 receptor, even though potent antagonism was observed.[218]

4. OPTIMIZATION OF SELECTIVITY OF LIGANDS FOR THE HISTAMINE H_4 RECEPTOR

The relatively high degree of homology (37% for protein sequences) of the H_3 and H_4 histamine receptors accounts for the high affinity of many H_3 receptor ligands for the H_4 receptor. The binding of basic atoms in the ligand to residues Asp3.32 and Glu5.46, present in both receptors, is a key contributor to high affinity.[163,213] Histamine has a high affinity for both the H_3 and H_4 receptors, and several compounds structurally related to histamine show potent activity at both those receptors. For example, imetit is an agonist for both the histamine H_3 and H_4 receptors, thioperamide is an inverse agonist at both receptors, and clobenpropit is an antagonist at the H_3 receptor, but an agonist H_4 at the receptor.[175] Quantitative SAR models of ligand affinity for both the histamine H_3 and H_4 receptors show that the design of clobenpropit derivatives that are selective for the H_4 receptor is challenging.[181] Additionally, intrinsic activity of a ligand and its selectivity for the H_4 receptor are difficult to model, since they arise in part from small changes in ligand structure and in the conformation of the receptor. As an example, selectivity for the histamine H_4 receptor was unexpectedly obtained by introducing an additional chloro substituent at the 3-position on the benzene ring of an *N*-benzyl substituent to give the clobenpropit analogue **118e** (Table 15). Of the clobenpropit analogues studied, **118e** showed the greatest affinity and selectivity for the human histamine H_4 receptor and also behaved as a full agonist (α = 1).

The (imidazolylpropyl)guanidine scaffold can provide ligands for several histamine receptor subtypes. The parent compound, [3-(1*H*)-imidazolylpropyl]guanidine (SK&F 91486), a weak partial agonist of the H_2 receptor, was found to be a highly potent partial agonist at both the histamine H_3 and H_4 receptors.[213] The highly selective histamine H_4 receptor antagonist JNJ7777120, an acylated piperazine, showed the potential of acylation for conferring receptor subtype selectivity, presumably by providing an additional potential binding site which interacts with the H_4 receptor. Indeed, acylation of the guanidine group in a series of (imidazolylpropyl)guanidines conferred selectivity for the histamine H_4 receptor, notably by adding alkanoyl groups that were sufficiently small to avoid conferring significant affinity for the H_2 receptor.[213] The N^G-acetyl derivative UR-PI288 (Figure 25), a potent full agonist of the human histamine H_4 receptor, with >1000-fold selectivity relative to the human H_1 receptor and >100-fold selectivity relative to the human H_2 receptor, is an outstanding example within this series of (imidazolylpropyl)guanidines.

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5. CONCLUSIONS AND PERSPECTIVES

The relatively selective expression of the histamine H_4 receptor in hematopoietic cells and the effects of the receptor on cellular function indicate that H_4 receptors play a role in mediating immune and inflammatory responses. This is confirmed by the in vivo anti-inflammatory effects of H_4 receptor antagonists. The recent availability of compounds selective for the histamine H_4 receptor and the availability of an H_4 receptor-deficient mouse[58] have assisted in uncovering the function of the H_4 receptor in mast cells and eosinophils. Preliminary results in animal models suggest that H_4 receptor antagonists have clinical potential, especially in diseases in which histamine is known to be relevant but for which H_1 receptor antagonists are clinically ineffective, and in particular for allergic and inflammatory conditions. A degree of overlap between the H_1 and H_4 receptors suggests that a combined receptor antagonism would have merit compared with those that target only one of those receptors.[84] That is in keeping with the feasibility of developing novel dual antagonists, given that known H_1 antagonists such as doxepin, cinnarizine, and promethazine also possess high affinity for the H_4 receptor.[38] Given the augmented chemotactic effect of CCR3-active β-chemokines on eosinophils following histamine activation of the H_4 receptor, the combination of an H_4 receptor antagonist with an antagonist of the leukotriene receptor, the PAF receptor, and/or a chemokine receptor may also be of benefit. Such regimens could offer an alternative to the use of intensive glucocorticoid therapies, known for a number of adverse effects. Emerging biological data on the H_4 and other receptors are increasing understanding to the point where novel therapies are likely, especially for the treatment of chronic inflammatory disorders, including allergic diseases of the respiratory and gastrointestinal tracts and of the skin.

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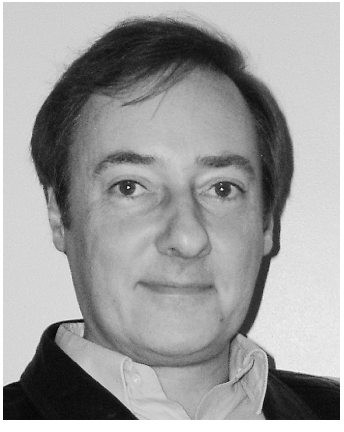
BIOGRAPHY



Charles M. Marson received his B.A. (Hons) and M.A. degrees in Natural Sciences from the University of Cambridge and in 1982 his Ph.D. degree from the University of East Anglia under the direction of Professor A. R. Katritzky, FRS, on the synthesis of polycyclic heterocycles, achieving the synthesis of the first bispyrylium macrocycle of 12 fused rings. After postdoctoral research on the chemistry of vitamin B_{12}, and on heterocyclic synthetic methodology, he was appointed to Lectureships, first at the University of Sheffield and later at Queen Mary College, University of London. In 1998, he was awarded a D.Sc. degree from the University of East Anglia. In 1999, he moved to University College London, where he is currently Professor of Organic Chemistry. His research interests concern the discovery of new organic reactions, especially stereocontrolled processes, and the design and development of new agents to target enzyme and receptor function.

REFERENCES

(1) Dale, H. H.; Laidlaw, P. P. *J. Physiol.* **1910**, *41*, 318.
(2) Lewis, T.; Grant, R. T. *Heart* **1924**, *11*, 209.
(3) Popielski, L. *Pflügers Arch. Gesamte Physiol. Menschen Tiere* **1920**, *178*, 237.
(4) Read, G. W.; Kiefer, E. F.; Weber, J. F. *J. Med. Chem.* **1973**, *16*, 1292.
(5) Riley, J. F.; West, G. B. *J. Physiol.* **1952**, *117*, 72P.
(6) Feldberg, W.; Mongar, J. L. *Br. J. Pharmacol.* **1954**, *9*, 197.
(7) Haas, H.; Panula, P. *Nat. Rev. Neurosci.* **2003**, *4*, 121.
(8) Parsons, M. E.; Ganellin, C. R. *Br. J. Pharmacol.* **2006**, *147*, S127.
(9) Folkow, B.; Hjeger, K.; Kahlson, G. *Acta Physiol. Scand.* **1948**, *15*, 264.
(10) Schild, H. O. *Br. J. Pharmacol.* **1947**, *2*, 189.
(11) Ungar, G.; Parrot, J. L.; Bovet, D. *C. R. Soc. Biol. (Paris)* **1937**, *124*, 445.
(12) Bovet, D.; Staub, A.-M. *C. R. Soc. Biol. (Paris)* **1937**, *124*, 547.
(13) Bovet, D.; de Lestrange, Y.; Fourneau, J. P. *C. R. Soc. Biol. (Paris)* **1939**, *130*, 1492.
(14) Code, C. F. Histamine and acid secretion. In *Ciba Foundation Symposium of Histamine*; Wolstenholme, G. E. W., O'Connor, C. M., Eds.; Churchill: London, 1956; p 189.
(15) Ash, A. S. F.; Schild, H. O. *Br. J. Pharmacol.* **1966**, *27*, 427.
(16) Black, J. W.; Duncan, W. A. M.; Durant, G. J.; Ganellin, C. R.; Parsons, E. M. *Nature* **1972**, *236*, 385.
(17) Durant, G. J.; Emmett, J. C.; Ganellin, C. R.; Miles, P. D.; Parsons, M. E.; Prain, H. D.; White, G. R. *J. Med. Chem.* **1977**, *20*, 901.
(18) Cooper, D. G.; Young, R. C.; Durant, G. J.; Ganellin, C. R. Histamine receptors. In *Comprehensive Medicinal Chemistry*; Emmett, J. C., Ed.; Pergamon: Oxford, U.K., 1990; Vol. 3, p 323.
(19) Bradshaw, J.; Brittain, R. T.; Clitherow, J. W.; Daly, M. J.; Jack, D.; Price, B. J.; Stables, R. *Br. J. Pharmacol.* **1979**, *66*, 464.
(20) Pertz, H. H.; Elz, S.; Schunack, W. *Mini-Rev. Med. Chem.* **2004**, *4*, 935.
(21) Dove, S.; Elz, S.; Seifert, R.; Buschauer, A. *Mini-Rev. Med. Chem.* **2004**, *4*, 941.
(22) Schwartz, J.-C. *Life Sci.* **1975**, *17*, 503.
(23) Arrang, J.-M.; Garbarg, M.; Lancelot, J.-C.; Lecomte, J.-M.; Pollard, H.; Robba, M.; Schunack, W.; Schwartz, J.-C. *Nature* **1987**, *327*, 117.
(24) Stark, H.; Arrang, J.-M.; Ligneau, X.; Garbarg, M.; Ganellin, C. R.; Schwartz, J.-C.; Schunack, W. *Prog. Med. Chem.* **2001**, *38*, 279.
(25) Leurs, R.; Bakker, R. A.; Timmerman, H.; de Esch, I. J. *Nat. Rev. Drug Discovery* **2005**, *4*, 107.
(26) Cowart, M.; Altenbach, R.; Black, L.; Faghih, R.; Zhao, C.; Hancock, A. A. *Mini-Rev. Med. Chem.* **2004**, *4*, 979.
(27) Ganellin, C. R.; Leurquin, F.; Piripitsi, A.; Arrang, J.-M.; Garbarg, M.; Ligneau, X.; Schunack, W.; Schwartz, J.-C. *Arch. Pharm. Med. Chem.* **1998**, *331*, 395.
(28) Onodera, K.; Watanabe, T. In *The Histamine H3 Receptor. A Target for New Drugs*; Leurs, R., Timmerman, H., Eds.; Elsevier: Amsterdam, 1998; p 255.
(29) Sander, K.; Kottke, T.; Stark, H. *Biol. Pharm. Bull.* **2008**, *31*, 2163.

(30) Passani, M. B.; Lin, J. S.; Hancock, A.; Crochet, S.; Blandina, P. *Trends Pharmacol. Sci.* **2004**, *25*, 618.
(31) Teuscher, C.; Subramanian, M.; Noubade, R.; Gao, J. F.; Offner, H.; Zachary, J. F.; Blankenhorn, E. P. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 10146.
(32) Jablonowski, J.; Carruthers, N. I.; Thurmond, R. L. *Mini-Rev. Med. Chem.* **2004**, *4*, 993.
(33) Yamashita, M.; Fukui, H.; Sugama, K.; Horio, Y.; Ito, S.; Mizuguchi, H.; Wada, H. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 11515.
(34) Gantz, I.; Schäffer, M.; DelValle, J.; Logsdon, C.; Campbell, V.; Uhler, M.; Yamada, T. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 429.
(35) Lovenberg, T. W.; Roland, B. L.; Wilson, S. J.; Jiang, X.; Pyati, J.; Huvar, A.; Jackson, M. R.; Erlander, M. G. *Mol. Pharmacol.* **1999**, *55*, 1101.
(36) Oda, T.; Morikawa, N.; Saito, Y.; Masuho, S.; Matsumoto, S. *J. Biol. Chem.* **2000**, *275*, 36781.
(37) Liu, C.; Ma, X.-J.; Jiang, X.; Wilson, S. J.; Hofstra, C. L.; Blevitt, J.; Pyati, J.; Li, X.; Chai, W.; Carruthers, N.; Lovenberg, T. W. *Mol. Pharmacol.* **2001**, *59*, 420.
(38) Nguyen, T.; Shapiro, D. A.; George, S. R.; Setola, V.; Lee, D. K.; Cheng, R.; Rauser, L.; Lee, S. P.; Lynch, K. R.; Roth, B. L.; O'Dowd, B. F. *Mol. Pharmacol.* **2001**, *59*, 427.
(39) Morse, K. L.; Behan, J.; Laz, T. M.; West, R. E., Jr.; Greenfeder, S. A.; Anthes, J. C.; Umland, S.; Wan, Y.; Hipkin, R. W.; Gonsiorek, W.; Shin, N.; Gustafson, E. L.; Qiao, X.; Wang, S.; Hedrick, J. A.; Greene, J.; Bayne, N.; Monsma, F. J., Jr. *J. Pharmacol. Exp. Ther.* **2001**, *296*, 1058.
(40) Zhu, Y.; Michalovich, D.; Wu, H.; Tan, K. B.; Dytko, G. M.; Mannan., I. J.; Boyce, R.; Alston, J.; Tierney, L. A.; Li, X.; Herrity, N. C.; Vawter, L.; Sarau, H. M.; Ames, R. S.; Davenport, C. M.; Hieble, J. P.; Wilson, S.; Bergsma, D. J.; Fitzgerald, L. R. *Mol. Pharmacol.* **2001**, *59*, 434.
(41) Baldwin, J. M.; Schertler, G. F. X.; Unger, V. M. *J. Mol. Biol.* **1997**, *272*, 144.
(42) Mirzadegan, T.; Benkö, G.; Filipek, S.; Palczewski, K. *Biochemistry* **2003**, *42*, 2759.
(43) Ballesteros, J. A.; Weinstein, H. *Methods Neurosci.* **1995**, *25*, 366.
(44) Huang, E. S. *Protein Sci.* **2003**, *12*, 1360.
(45) van Rijn, R. M.; van Marle, A.; Chazot, P. L.; Langmeijer, E.; Qin, Y.; Shenton, F. C.; Lim, H. D.; Zuiderveld, O. P.; Sansuk, K.; Dy, M.; Smit, M. J.; Tensen, C. P.; Bakker, R. A.; Leurs, R. *Biochem. J.* **2008**, *414*, 121.
(46) Liu, C.; Wilson, S. J.; Kuei, C.; Lovenberg, T. W. *J. Pharmacol. Exp. Ther.* **2001**, *299*, 121.
(47) de Esch, I. J. P.; Thurmond, R. L.; Jongejan, A.; Leurs, R. *Trends Pharmacol. Sci.* **2005**, *26*, 462.
(48) Yao, B. B.; Hutchins, C. W.; Carr, T. L.; Cassar, S.; Masters, J. N.; Bennani, Y. L.; Esbenshade, T. A.; Hancock, A. A. *Neuropharmacology* **2003**, *44*, 773.
(49) Shin, N.; Coates, E.; Murgolo, N. J.; Morse, K. L.; Bayne, M.; Strader, C. D.; Monsma, F. J., Jr. *Mol. Pharmacol.* **2002**, *62*, 38.
(50) Gantz, I.; DelValle, J.; Wang, L. D.; Tashiro., T.; Munzert, G.; Guo, Y. J.; Konda, Y.; Yamada, T. *J. Biol. Chem.* **1992**, *267*, 20840.
(51) Lim, H. D.; Jongejan, A.; Bakker, R. A.; Haaksma, E.; de Esch, I. J. P.; Leurs, R. *J. Pharmacol. Exp. Ther.* **2008**, *327*, 88.
(52) O'Reilly, M.; Alpert, R.; Jenkinson, S.; Gladue, R. P.; Foo, S.; Trim, S.; Peter, B.; Trevethick, M.; Fidock, M. *J. Recept. Signal Transduction Res.* **2002**, *22*, 431.
(53) Venable, J. D.; Thurmond, R. L. *Anti-Inflammatory Anti-Allergy Agents Med. Chem.* **2006**, *5*, 307.
(54) Gallagher, M. J.; Yates, S. L. Histamine receptor H_4 polynucleotides. WO/2003/020907 (A2), PCT/US2002/27891, March 23, 2003.
(55) Damaj, B. B.; Becerra, C. B.; Esber, H. J.; Wen, Y.; Maghazachi, A. A. *J. Immunol.* **2007**, *179*, 7907.
(56) Zhang, M.; Thurmond, R. L.; Dunford, P. J. *Pharmacol. Ther.* **2007**, *113*, 594.
(57) Coge, F.; Guénin, S.-P.; Rique, H.; Boutin, J. A.; Galizzi, J.-P. *Biochem. Biophys. Res. Commun.* **2001**, *284*, 301.
(58) Hofstra, C. L.; Desai, P. J.; Thurmond, P. L.; Fung-Leung, W.-P. *J. Pharmacol. Exp. Ther.* **2003**, *305*, 1212.
(59) Lippert, U.; Artuc, M.; Grützkau, A.; Babina, M.; Guhl, S.; Haase, I.; Blaschke, V.; Zachmann, K.; Knosalla, M.; Middel, P.; Krüger-Krasagakis, S.; Henz, B. M. *J. Invest. Dermatol.* **2004**, *123*, 116.
(60) Voehringer, D.; Shinkai, K.; Locksley, R. M. *Immunity* **2004**, *20*, 267.
(61) Nakaya, M.; Takeuchi, N.; Kondo, K. *Ann. Otol., Rhinol., Laryngol.* **2004**, *113*, 552.
(62) Thurmond, R. L.; Desai, P. J.; Dunford, P. J.; Fung-Leung, W.-P.; Hofstra, C. L.; Jiang, W.; Nguyen, S.; Riley, J. P.; Sun, S.; Williams, K. N.; Edwards, J. P.; Karlsson, L. *J. Pharmacol. Exp. Ther.* **2004**, *309*, 404.
(63) Gantner, F.; Sakai, K.; Tusche, M. W.; Cruikshank, W. W.; Center, D. M.; Bacon, K. B. *J. Pharmacol. Exp. Ther.* **2002**, *303*, 300.
(64) Leurs, R.; Church, M. K.; Taglialatela, M. *Clin. Exp. Allergy* **2002**, *32*, 489.
(65) Milligan, G.; Bond, R.; Lee, M. *Trends Pharmacol. Sci.* **1995**, *16*, 10.
(66) Akdis, C. A.; Simons, F. E. R. *Eur. J. Pharmacol.* **2006**, *533*, 69.
(67) Dimitriadou, V.; Rouleau, A.; Dam Trung Tuong, M.; Newlands, G. J.; Miller, H. R.; Luffau, G.; Schwartz, J. C.; Garbarg, M. *Clin. Sci.* **1994**, *87*, 151.
(68) Nakamura, T.; Itadani, H.; Hidaka, Y.; Ohta, M.; Tanaka, K. *Biochem. Biophys. Res. Commun.* **2000**, *279*, 615.
(69) Nakayama, T; Kato, Y.; Hieshima, K.; Nagakubo, D.; Kunori, Y.; Fujisawa, T.; Yoshie, O. *J. Immunol.* **2004**, *173*, 2078.
(70) Raible, D. G.; Lenahan, T.; Fayvilevich, Y.; Kosinski, R.; Schulman, E. S. *Am. J. Respir. Crit. Care Med.* **1994**, *149*, 1506.
(71) Buckland, K. F.; Williams, T. J.; Conroy, D. M. *Br. J. Pharmacol.* **2003**, *140*, 1117.
(72) Ling, P.; Ngo, K.; Nguyen, S.; Thurmond, R. L.; Edwards, J. P.; Karlsson, L.; Fung-Leung, W.-P. *Br. J. Pharmacol.* **2004**, *142*, 161.
(73) Jablonowski, J. A.; Grice, C. A.; Chai, W.; Dvorak, C. A.; Venable, J. D.; Kwok, A. K.; Ly, K. S.; Wei, J.; Baker, S. M.; Desai, P. J.; Jiang, W.; Wilson, S. J.; Thurmond, R. L.; Karlsson, L.; Edwards, J. P.; Lovenberg, T. W.; Carruthers, N. I. *J. Med. Chem.* **2003**, *46*, 3957.
(74) Smits, R. A.; Leurs, R.; de Esch, I. J. P. *Drug Discovery Today* **2009**, *14*, 745.
(75) Tiligada, E.; Zampeli, E.; Sander, K.; Stark, H. *Expert Opin. Invest. Drugs* **2009**, *18*, 1519.
(76) Kiss, R.; Keserü, G. M. *Expert Opin. Ther. Patents* **2009**, *19*, 119.
(77) Takuya, H.; Makoto, S. (Kowa Co.). Prophylactic and/or therapeutic agent for atopic dermatitis. Japanese Pat. 2008 127359, June 5, 2008.
(78) Carruthers, N. I.; Chai, W.; Dvorak, C. A.; Edwards, J. P.; Grice, C. A.; Jablonowski, J. A.; Karlsson, L.; Khatuya, H.; Kreisberg, J. D.; Kwok, A. K.; Lovenberg, T. W.; Ly, K. S.; Pio, B.; Shah, C. R.; Sun, S.; Thurmond, R. L.; Wei, J.; Xiao, W. (Ortho-McNeil Pharmaceutical, Inc.). Heterocyclic compounds. WO/2002/072548, Sept 19, 2002.
(79) Burns, S.; Hamley, P. (AstraZeneca UK Ltd.). Imidazole derivatives of piperidine as histamine antagonists. WO/2005/014579, Feb 17, 2005.
(80) Carcella González, E.; Medina Fuentes, E. M.; Virgili Bernado, M.; Marti Via, J. (Palau Pharma). Furo[3,2-*d*]pyrimidine derivatives as H_4 receptor antagonists. WO/2009/115496, Sept 24, 2009.
(81) Ramsden, N. (Cellzome Ltd.). Sulfur-containing aminopyrimidine compounds for the treatment of inflammatory disorders. EP/2009/2020412, Feb 4, 2009.
(82) Reid, A.; Wilson, F.; Dyke, H.; Price, S.; Cramp, S. (Cellzome Ltd.). Azetidine amino pyrimidine compounds for the treatment of inflammatory disorders. WO/2007/090854, Aug 16, 2007.
(83) Kindrachuk, D. E.; Venable, J. D. (Janssen Pharmaceutica). Bicyclic heteroaryl-substituted imidazoles as modulators of the histamine H_4 receptor. WO/2009/079001, June 25, 2009.
(84) Daugherty, B. L. *Br. J. Pharmacol.* **2004**, *142*, 5.
(85) Rothenberg, M. E. *N. Engl. J. Med.* **1998**, *338*, 1592.
(86) Yeadon, M.; Ruddock, K. S. (Pfizer Ltd.). Combination for treating inflammatory diseases. WO/2005/089748, Sept 29, 2005.

(87) Bhatt, H. G.; Agrawal, Y. K.; Raval, H. G.; Manna, K.; Desai, P. R. *Mini-Rev. Med. Chem.* **2010**, *10*, 1293.
(88) Zhang, M.; Venable, J. D.; Thurmond, R. L. *Expert Opin. Invest. Drugs* **2006**, *15*, 1443.
(89) Lim, H. D.; Smits, R. A.; Leurs, R.; de Esch, I. J. P. *Curr. Top. Med. Chem.* **2006**, *6*, 1365.
(90) Zampeli, E.; Tiligada, E. *Br. J. Pharmacol.* **2009**, *157*, 24.
(91) Thurmond, R. L.; Gelfand, E. W.; Dunford, P. J. *Nat. Rev. Drug Discovery* **2008**, *7*, 41.
(92) Anthes, J.; West, R. E.; Hey, J. A.; Aslanian, R. G. (Schering Corp.). Combination of H_1, H_3 and H_4 receptor antagonists for treatment of allergic and non-allergic pulmonary inflammation, congestion andallergic rhinitis. WO/2004/066960, Dec 8, 2004.
(93) Fung-Leung, W.-P.; Thurmond, R. L.; Ling, P.; Karlsson, L. *Curr. Opin. Invest. Drugs* **2004**, *5*, 1174.
(94) Takeshita, K.; Sakai, K.; Bacon, K. B.; Gantner, F. *J. Pharmacol. Exp. Ther.* **2003**, *307*, 1072.
(95) Simons, F. E. R. *N. Engl. J. Med.* **2004**, *351*, 2203.
(96) Akdis, C. A.; Blaser, K. *J. Allergy Clin. Immunol.* **2003**, *112*, 15.
(97) Jutel, M.; Watanabe, T.; Akdis, M.; Blaser, K.; Akdis, C. A. *Curr. Opin. Immunol.* **2002**, *14*, 735.
(98) Dunford, P. J.; O'Donnell, N.; Riley, J. P.; Williams, K. N.; Karlsson, L.; Thurmond, R. L. *J. Immunol.* **2006**, *176*, 7062.
(99) Coruzzi, G.; Adami, M.; Guaita, E.; de Esch, I. J. P.; Leurs, R. *Eur. J. Pharmacol.* **2007**, *563*, 240.
(100) Dunford, P. J.; Williams, K. N.; Desai, P. J.; McQueen, D.; Karlsson, L.; Thurmond, R. *J. Allergy Clin. Immunol.* **2007**, *119*, 176.
(101) Fadel, R.; Herpin-Richard, N.; Rihoux, J. P.; Henocq, E. *Clin. Allergy* **1987**, *17*, 373.
(102) Clark, R. A. F.; Sandler, J. A.; Gallin, J. I.; Kaplan, A. P. *J. Immunol.* **1977**, *118*, 137.
(103) Fulop, A. K.; Ribita, D.; Falus, A. *Inflammation Res.* **2007**, *56*, S49.
(104) Gutzmer, R.; Diestel, C.; Mommert, S.; Köther, B.; Stark, H.; Wittmann, M.; Werfel, T. *J. Immunol.* **2005**, *174*, 5224.
(105) 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.; Edwards, J. P. *J. Med. Chem.* **2005**, *48*, 8289.
(106) Varga, C.; Horvath, K.; Berko, A.; Thurmond, R. L.; Dunford, P. J.; Whittle, B. J. R. *Eur. J. Pharmacol.* **2005**, *522*, 130.
(107) 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. *J. Med. Chem.* **2008**, *51*, 6571.
(108) Hsieh, G. C.; Chandran, P.; Salyers, A. K.; Pai, M.; Zhu, C. Z.; Wensink, E. J.; Witte, D. G.; Miller, T. R.; Mikusa, J. P.; Baker, S. J.; Wetter, J. M.; Marsh, K. C.; Hancock, A. A.; Cowart, M. D.; Esbenshade, T. A.; Brioni, J. D.; Honore, P. *Pharmacol., Biochem. Behav.* **2010**, *95*, 41.
(109) Altenbach, R. J.; Liu, H.; Drizin, I.; Wishart, N.; Babinski, D. J.; Gregg, R. J.; Esbenshade, T. A.; Hsieh, G. C.; Brioni, J. D.; Honore, M. P.; Black, L. A.; Zhao, C.; Wakefield, B. D; Hancock, A. A. (Abbott Laboratories). A method for pain treatment. WO/2008/060766, May 22, 2008.
(110) Chazot, P.; Connelly, W.; Shenton, F. C.; Lethbridge, N.; van Renburg, R.; Lees, G. Presented at the XXXVth Meeting of the European Histamine Research Society, 2006; Paper O6.
(111) Strakhova, M. I.; Nikkel, A. L.; Manelli, A. M.; Hsieh, G. C.; Esbenshade, T. A.; Brioni, J. D.; Bitner, R. S. *Brain Res.* **2009**, *1250*, 41.
(112) Thurmond, R. L.; Wadsworth, S. A. (Janssen Pharmaceutica). Use of a histamine H_4 antagonist for the treatment of post-operative adhesions. WO2009/152287, Dec 17, 2009.
(113) Ikawa, Y.; Suzuki, M.; Shiono, S.; Ohki, E.; Moriya, H.; Negishi, E.; Ueno, K. *Biol. Pharm. Bull.* **2005**, *28*, 2016.
(114) Crisp, A. J. *Rheumatol. Int.* **1984**, *4*, 125.
(115) Ohki, E.; Suzuki, M.; Aoe, T.; Ikawa, Y.; Negishi, E.; Ueno, K. *Biol. Pharm. Bull.* **2007**, *30*, 2217.
(116) Grzybowska-Kowalczyk, A.; Wojtecka-Lukasik, E.; Maslinska, D.; Gujski, M.; Maslinski, S. *Inflammation Res.* **2007**, *56*, S59.
(117) Szewczyk, G.; Maslinska, D.; Szukiewicz, D.; Smiertka, W.; Klimkiewicz, J.; Pyzlak, M. *Inflammation Res.* **2007**, *56*, S31.
(118) 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. *J. Med. Chem.* **2008**, *51*, 6547.
(119) Liu, H.; Drizin, I.; Koenig, J. R.; Cowart, M. D.; Zhao, C.; Wakefield, B. D; Black, L. A.; Altenbach, R. J. (Abbott Laboratories). 5,6,7,8-Tetrahydroquinazolin-2-amine derivatives and related compounds as histamine H_4 receptor modulators for the treatment of asthma. WO/2009/123967, Oct 8, 2009.
(120) Jarjour, N.; Calhoun, W.; Schwartz, L.; Busse, W. W. *Am. Res. Respir. Dis.* **1991**, *144*, 83.
(121) Neumann, D.; Beermann, S.; Seifert, R. *Pharmacology* **2010**, *85*, 217.
(122) Alfón, J.; Ardanaz, N.; Gil-Torregrosa, B.; Fernández, A.; Balsa, D.; Carceller, E.; Gómez, L.; Merlos, M.; Cortijo, J.; Morcillo, E.; Bartroli, X. *Inflammation Res.* **2010**, *59*, S199.
(123) Desai, P. J.; Dunford, P. J.; Hofstra, C. L.; Karlsson, L.; Leung, W.-P.; Ling. P.; Thurmond, R. L. (Janssen Pharmaceutica). The use of histamine H_4 receptor modulators for the treatment of allergy and asthma. WO/2004/021999, March 18, 2004.
(124) Hagermark, O. *Skin Pharmacol.* **1992**, *5*, 1.
(125) Bell, J. K.; McQueen, D. S.; Rees, J. L. *Br. J. Pharmacol.* **2004**, *142*, 374.
(126) Yamaura, K.; Oda, M.; Suwa, E.; Suzuki, M.; Sato, H.; Ueno, K. *J. Toxicol. Sci.* **2009**, *34*, 427.
(127) Clerc, T.; Przybylski, C.; Tisne-Versailles, J. (Pierre Fabre Medicament). Mequitazine for treating or preventing pathologies involving histamine H_4 receptors. WO/2009/071625, June 11, 2009.
(128) Dunford, P. J.; Karlsson, L.; Thurmond, R. L. (Janssen Pharmaceutica). Combination histamine H_1R and H_4R antagonist therapy for treating pruritus. WO/2007/120690, Oct 25, 2007.
(129) Hill, S. J.; Ganellin, C. R.; Timmerman, H.; Schwartz, J. C.; Shankley, N. P.; Young, J. M.; Schunack, W.; Levi, R.; Haas, H. L. *Pharmacol. Rev.* **1997**, *49*, 253.
(130) Wolff, A. A.; Levi, R. *Circ. Res.* **1986**, *58*, 1.
(131) Dvorak, A. M. *N. Engl. J. Med.* **1986**, *315*, 969.
(132) Panizo, A.; Mindán, F. J.; Galindo, M. F.; Cenarruzabeitia, E.; Hernández, M.; Diez, J. *J. Hypertens.* **1995**, *13*, 1201.
(133) Hara, M.; Ono, K.; Hwang, M. W.; Iwasaki, A.; Okada, M.; Nakatani, K.; Sasayama, S.; Matsumori, A. *J. Exp. Med.* **2002**, *195*, 375.
(134) Bristow, M. R.; Ginsburg, R.; Harrison, D. C. *Am. J. Cardiol.* **1982**, *49*, 249.
(135) Marone, G.; de Crescenzo, G.; Adt, M.; Patella, V.; Arbustini, E.; Genovese, A. *Immunopharmacology* **1995**, *31*, 1.
(136) Engler, H.; Pfahlert, V. *Arch. Pharmacol.* **1987**, *335*, R60.
(137) Ginsburg, R.; Bristow, M. R.; Stinson, E. B.; Harrison, D. C. *Life Sci.* **1980**, *26*, 2245.
(138) Mörsdorf, P.; Engler, H.; Schickaneder, H.; Buschauer, A.; Schunack, W.; Baumann, G. *Drugs Future* **1990**, *15*, 919.
(139) Felix, S. B.; Buschauer, A.; Baumann, G. *Eur. J. Clin. Invest.* **1995**, *25*, S42.
(140) Buschauer, A. *J. Med. Chem.* **1989**, *32*, 1963.
(141) Connelly, W. M.; Shenton, F. C.; Lethbridge, N.; Leurs, R.; Waldvogel, H. J.; Faull, R. L. M.; Lees, G.; Chazot, P. L. *Br. J. Pharmacol.* **2009**, *157*, 55.
(142) Haas, H. L.; Sergeeva, O. A.; Selbach, O. *Physiol. Rev.* **2008**, *88*, 1183.
(143) Kahlson, G.; Rosengren, E. *Ann. Rev. Pharmacol.* **1965**, *10*, 305.
(144) Haak-Frendscho, M.; Darvas, Z.; Hegyesi, H.; Kárpáti, S.; Hoffman, R. L.; László, V.; Bencsáth, M.; Szalai, C.; Fürész, J.; Timár, J.;

Bata-Csörgö, Z.; Szabad, G.; Pivarcsi, A.; Pállinger, E.; Kemény, L.; Horváth, A.; Dobozy, A.; Falus, A. *J. Invest. Dermatol.* **2000**, *115*, 345.
(145) Graff, L.; Frungieri, M.; Zanner, R.; Pohlinger, A.; Prinz, C.; Gratzl, M. *Am. J. Pathol.* **2002**, *160*, 1561.
(146) Medina, V. A.; Rivera, E. S. *Br. J. Pharmacol.* **2010**, *161*, 755.
(147) Cianchi, F.; Cortesini, C.; Schiavone, N.; Perna, F.; Magnelli, F.; Fanti, E.; Bani, D.; Messerini, L.; Fabbroni, V.; Perigli, G.; Capaccioli, S.; Masini, E. *Clin. Cancer Res.* **2005**, *11*, 6807.
(148) Boer, K.; Helinger, E.; Helinger, A.; Pocza, P.; Pos, Z.; Demeter, P.; Baranyai, Z.; Dede, K.; Darvas, Z.; Falus, A. *Eur. J. Cell Biol.* **2008**, *87*, 227.
(149) Maslinksa, D.; Laure-Kamionowska, M.; Maslinski, K. T.; Deregowski, K.; Szewczyk, G.; Maslinski, S. *Inflammation Res.* **2006**, *55*, S77.
(150) Igel, P.; Dove, S.; Buschauer, A. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7191.
(151) Schneider, E.; Dy, M. Use of histamine H_4 receptor ligands to protect haematopoietic progenitors against the haematological toxicity of chemotherapeutic agents. WO/2008/006974, Jan 17, 2008.
(152) Ortiz-Domínguez, A.; Hernández, M. E.; Berlanga, C.; Gutiérrez-Mora, D.; Moreno, J.; Heinze, G.; Pavón, L. *Bipolar Disord.* **2007**, *9*, 596.
(153) Frommberger, U. H.; Bauer, J.; Haselbauer, P.; Fräulin, A.; Riemann, D.; Berger, M. *Eur. Arch. Psychiatry Clin. Neurosci.* **1997**, *247*, 228.
(154) Edwards, J. P.; Kindrachuk, D. E.; Mapes, C. M.; Pippel, D. J.; Venable, J. D. (Janssen Pharmaceutica). Benzoimidaz-l-2-yl pyrimidines and pyrazines as modulators of the histamine H_4 receptor. US/2007/244126, Oct 18, 2007.
(155) Cai, H.; Chavez, F.; Dunford, P. J.; Greenspan, A. J.; Meduna, S. P.; Quiroz, J. A.; Savall, B. M.; Tays, K. L.; Thurmond, R. L.; Wei, J.; Wolin, R. L. (Janssen Pharmaceutica). Diamino-Pyridine, -Pyrimidine- and -Pyridazine modulators of the histamine H_4 receptor. WO2009/152325, Dec 17, 2009.
(156) Okada, T.; Fujiyoshi, Y.; Silow, M.; Navarro, J.; Landau, E. M.; Shichida, Y. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5982.
(157) Kier, L. B. *J. Med. Chem.* **1968**, *11*, 441.
(158) Oda, T.; Matsumoto, M; Takasaki, J; Kamohara, M.; Soga, T.; Hiyama, H.; Kobori, M.; Katoh, M. *J. Pharmacol. Sci.* **2005**, *98*, 319.
(159) Lim, H. D.; Adami, M.; Guaita, E.; Werfel, T.; Smits, R. A.; de Esch, I. J. P.; Bakker, R. A.; Gutzmer, R.; Coruzzi, G.; Leurs, R. *Br. J. Pharmacol.* **2009**, *157*, 34.
(160) Karlsson, L.; Leung, W.-P.; Ling. P.; Thurmond, R. L. (Janssen Pharmaceutica). Analysing histamine H_4 receptor-mediated effects in whole blood. WO/2005/031308, April 7, 2005.
(161) Shi, L.; Javitch, J. A. *Annu. Rev. Pharmacol. Toxicol.* **2002**, *42*, 437.
(162) Palczewski, K.; Kumasaka, T.; Hori, T.; Behnke, C. A.; Motoshima, H.; Fox, B. A.; LeTrong, I.; Teller, D. C.; Okada, T.; Stenkamp, R. E.; Yamamoto, M.; Miyano, M. *Science* **2000**, *289*, 739.
(163) Jongejan, A.; Lim, H. D.; Smits, R. A.; de Esch, I. J. P.; Haaksma, E.; Leurs, R. *J. Chem. Inf. Model.* **2008**, *48*, 1455.
(164) Kiss, R.; Nosál, B.; Rácz, Á.; Falus, A.; Erös, D; Keserü, G. M. *Eur. J. Med. Chem.* **2008**, *43*, 1059.
(165) Jójárt, B.; Kiss, R.; Viskolcz, B.; Keserü, G. M. *J. Chem. Inf. Model.* **2008**, *48*, 1199.
(166) Farrens, D. L.; Altenbach, C.; Yang, K.; Hubbell, W. L.; Khorana, H. G. *Science* **1996**, *274*, 768.
(167) Kiss, R.; Kiss, B.; Könczöl, A.; Szalai, F.; Jelinek, I.; László, V.; Noszál, B.; Falus, A.; Keserü, G. M. *J. Med. Chem.* **2008**, *51*, 3145.
(168) Rarey, M.; Kramer, B.; Lengauer, T.; Klebe, G. *J. Mol. Biol.* **1996**, *261*, 470.
(169) Rasmussen, S. G. F.; Choi, H.-J.; Rosenbaum, D. M.; Kobilka, T. S.; Thian, F. S.; Edwards, P. C.; Burghammer, M.; Ratnala, V. R. P.; Sanishvili, R.; Fischetti, R. F.; Schertler, G. F. X.; Weis, W. I.; Kobilka, B. K. *Nature* **2007**, *450*, 383.
(170) Cherezov, V.; Rosenbaum, D. M.; Hanson, M. A.; Rasmussen, S. G. F.; Thian, F. S.; Kobilka, T. S.; Choi, H.-J.; Kuhn, P.; Weis, W. I.; Kobilka, B. K.; Stevens, R. C. *Science* **2007**, *318*, 1258.
(171) Rosenbaum, D. M.; Cherezov, V.; Hanson, M. A.; Rasmussen, S. G. F.; Thian, F. S.; Kobilka, T. S.; Choi, H.-J.; Yao, X.-J.; Weis, W. I.; Stevens, R. C.; Kobilka, B. K. *Science* **2007**, *318*, 1266.
(172) Shah, C.; McAtee, L.; Breitenbucher, J. G.; Rudolph, D.; Li, X. B.; Lovenberg, T. W.; Mazur, C.; Wilson, S. J.; Carruthers, N. I. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3309.
(173) Faghih, R.; Dwight, W.; Pan, J. B.; Fox, G. B.; Krueger, K. M.; Esbenshade, T. A.; McVey, J. M.; Marsh, K.; Bennani, Y. L.; Hancock, A. A. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1325.
(174) Terzioglu, N.; van Rijn, R. M.; Bakker, R. A.; de Esch, I. J. P.; Leurs, R. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5251.
(175) Lim, H. D.; van Rijn, R. M.; Ling, P.; Bakker, R. A.; Thurmond, R. L.; Leurs, R. *J. Pharmacol. Exp. Ther.* **2005**, *314*, 1310.
(176) Smits, R. A.; Lim, H. D.; Stegink, B.; Bakker, R. A.; de Esch, I. J. P.; Leurs, R. *J. Med. Chem.* **2006**, *49*, 4512.
(177) Smits, R. A.; Lim, H. D.; Hanzer, A.; Zuiderveld, O. P.; Guaita, E.; Adami, M.; Coruzzi, G.; Leurs, R.; de Esch, I. J. P. J. *Med. Chem.* **2008**, *51*, 2457.
(178) Hough, L. B. *Mol. Pharmacol.* **2001**, *59*, 415.
(179) 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. *J. Med. Chem.* **2008**, *51*, 7855.
(180) Watanabe, M.; Kazuta, Y.; Hayashi, H.; Yamada, S.; Matsuda, A.; Shuto, S. *J. Med. Chem.* **2006**, *49*, 5587.
(181) Lim, H. D.; Istyastono, E. P.; van de Stolpe, A.; Romeo, G.; Gobbi, S.; Schepers, M.; Lahaye, R.; Menge, W. M. B. P.; Zuiderveld, O. P.; Jongejan, A.; Smits, R. A.; Bakker, R. A.; Haaksma, E. E. J.; Leurs, R.; de Esch, I. J. P. *Bioorg. Med. Chem.* **2009**, *17*, 3987.
(182) Lim, H. D.; Smits, R. A.; Bakker, R. A.; van Dam, C. M. E.; de Esch, I. J. P.; Leurs, R. *J. Med. Chem.* **2006**, *49*, 6650.
(183) Meng, J.; Ma, X.; Li, M.; Jia, M.; Luo, X. *Eur. J. Pharmacol.* **2008**, *587*, 336.
(184) Igel, P.; Schnell, D.; Bernhardt, G.; Seifert, R.; Buschauer, A. *ChemMedChem* **2009**, *4*, 225.
(185) Jansen, F. P.; Rademaker, B.; Bast, A.; Timmerman, H. *Eur. J. Pharmacol.* **1992**, *217*, 203.
(186) Kitbunnadaj, R.; Zuiderveld, O. P.; Christophe, B.; Hulscher, S.; Menge, W. M. P. B.; Gelens, E.; Snip, E.; Bakker, R. A.; Celenire, S.; Gillard, M.; Talaga, P.; Timmerman, H.; Leurs, R. *J. Med. Chem.* **2004**, *47*, 2414.
(187) Kitbunnadaj, R.; Hashimoto, T.; Poli, E.; Zuiderveld, O. P.; Menozzi, A.; Hidaka, R.; de Esch, I. J. P.; Bakker, R. A.; Menge, W. M. P. B.; Yamatodani, A.; Coruzzi, G.; Timmerman, H.; Leurs, R. *J. Med. Chem.* **2005**, *48*, 2100.
(188) Hashimoto, T.; Harusawa, S.; Araki, L.; Zuiderveld, O. P.; Smit, M. J.; Imazu, T.; Takashima, S.; Yamamoto, Y.; Sakamoto, Y.; Kurihara, T.; Leurs, R.; Bakker, A. R.; Yamatodani, A. *J. Med. Chem.* **2003**, *46*, 3162.
(189) Sander, K.; Kottke, T.; Tanrikulu, Y.; Proschak, E.; Weizel, L.; Schneider, E. H.; Seifert, R.; Schneider, G.; Stark, H. *Bioorg. Med. Chem.* **2009**, *17*, 7186.
(190) Bell, S. A.; Lane, C. A. L.; Mowbray, C.; Selby, M. D.; Swain, N. A.; Williams, D. H. (Pfizer Ltd.). Pyrimidine derivatives. WO/2007/072163, June 28, 2007.
(191) Sato, H.; Tanaka, K.; Shimazaki, M.; Urbahns, K.; Sakai, K.; Gantner, F.; Bacon, K. (Bayer Healthcare AG). 2- Aminopyrimidine derivatives. WO/2005/054239, June 16, 2005.
(192) Cowart, M.; Faghih, R.; Curtis, M. P.; Gfesser, G. A.; Bennani, Y. L.; Black, L. A.; Pan, L.; Marsh, K. C.; Sullivan, J. P.; Esbenshade, T. A.; Fox, G. B.; Hancock, A. A. *J. Med. Chem.* **2005**, *48*, 38.
(193) Strakhova, M. I.; Cuff, C. A.; Manelli, A. M.; Carr, T. L.; Witte, D. G.; Baranowski, J. L.; Vortherms, T. A.; Miller, T. R.; Rundell, L.; McPherson, M. J.; Adair, R. M.; Brito, A. A.; Bettencourt, B. M.; Yao, B. B.; Wetter, J. M.; Marsh, K. C.; Liu, H.; Cowart, M. D.; Brioni, J. D.; Esbenshade, T. A. *Br. J. Pharmacol.* **2009**, *157*, 44.
(194) Liu, H.; Altenbach, R. J.; Carr, T. L.; Chandran, P.; Hsieh, G. C.; Lewis, G. R.; Manelli, A. M.; Milicic, I.; Marsh, K. C.; Miller, T. R.; Strakhova, M. I.; Vortherms, T. A.; Wakefield, B. D.; Wetter, J. M.; Witte,

D. G.; Honore, P.; Esbenshade, T. A.; Brioni, J. D.; Cowart, M. D. *J. Med. Chem.* **2008**, *51*, 7094.

(195) Esbenshade, T. A.; Krueger, K. M.; Miller, T. R.; Kang, C. H.; Denny, L. I.; Witte, D. G.; Yao, B. B.; Fox, G. B.; Faghih, R.; Bennani, Y. L.; Williams, M.; Hancock, A. A. *J. Pharmacol. Exp. Ther.* **2003**, *305*, 887.

(196) Greenhill, J. V.; Moten, M. A.; Hanke, R. *J. Chem. Soc., Perkin Trans. 1* **1984**, *6*, 1213.

(197) Smits, R. A.; Van Lim, H. D.; Leurs, R.; de Esch, I. J. P. Quinazolines and related heterocyclic compounds, and their therapeutic use. WO/2008/003702, Jan 10, 2008.

(198) Smits, R. A.; Adami, M.; Istyastono, E. P.; Zuiderveld, O. P.; van Dam, C, M. E.; de Kanter, F. J. J.; Jongejan, A.; Coruzzi, G.; Leurs, R.; de Esch, I. J. P. *J. Med. Chem.* **2010**, *53*, 2390.

(199) Raphy, G.; Pegurier, C.; Meissner, H.; Knight, R.; Owen, D. A. (UCB Pharma). Heterobicyclic compounds as histamine H4 receptor antagonists. WO/2009/047255, April 16, 2009.

(200) Edwards, J. P.; Venable, J. D. (Johnson & Johnson Inc.). Quinoxaline compounds. U.S. Patent 2005/0070527, March 31, 2005.

(201) Lawrence, D. S.; Copper, J. E.; Smith, C. D. *J. Med. Chem.* **2001**, *44*, 594.

(202) Duffy, K. J.; Haltiwanger, R. C.; Huang, Y.; Kanialian-Beck, A.; Luengo, J. I. *Tetrahedron Lett.* **1998**, *39*, 8017.

(203) Mustafa, M. E.; Takaoka, N.; Ishikawa, N. *Bull. Soc. Chim. Fr.* **1986**, 944.

(204) Eras, J.; Galvez, C.; Garcia, F. *J. Heterocycl. Chem.* **1984**, *21*, 215.

(205) Gaul, M.; Zhao, B.; Zhu, X. (Janssen Pharmaceutica). Substituted nitrogen-containing heteroaryl derivatives useful as modulators of the histamine H_4 receptor. WO/2009/035671, March 19, 2009.

(206) Carruthers, N. I.; Dvorak, C. A.; Edwards, J. P.; Grice, C. A.; Jablonowski, J. A.; Ly, K. S.; Pio, B. A.; Shah, C. R.; Venable, J. D. (Janssen Pharmaceutica). (1*H*-Benzimidazol-2-yl)-(piperazinyl)-methanone derivatives and related compounds as histamine H_4-receptor antagonists for the treatment of inflammatory and allergic disorders. WO/2004/022060, Sept 4, 2003.

(207) 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. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 6043.

(208) Edwards, J. P.; Kindrachuk, D. E.; Venable, J. D. (Janssen Pharmaceutica). Benzo-imidazolyl pyridines as modulators of the histamine H_4 receptor. WO/2007/117400, Oct 18, 2007.

(209) Liegois, J. F.; Eyrolles, L.; Ellenbroek, B. A.; Lejeune, C.; Carato, P.; Bruhwyler, J.; Géczy, J.; Damas, J.; Delarge, J. *J. Med. Chem.* **2002**, *45*, 5136.

(210) Nagarajan, K.; Kulkarni, L.; Venkateswarlu, A.; Shah, R. *Indian J. Chem.* **1974**, *12*, 258.

(211) Sterk, G. J. Studies on histaminergic compounds: Structure-activity relationships at the histamine H_2-receptor. Dissertation, Vrije Universiteit, Amsterdam, 1987; p 125.

(212) Shapira, R.; Doherty, D. G.; Burnett, W. T., Jr. *Radiat. Res.* **1957**, *7*, 22.

(213) Igel, P.; Schneider, E.; Schnell, D.; Elz, S.; Seifert, R.; Buschauer, A. *J. Med. Chem.* **2009**, *52*, 2623.

(214) Igel, P.; Geyer, R.; Strasser, A.; Dove, S.; Seifert, R.; Buschauer, A. *J. Med. Chem.* **2009**, *52*, 6297.

(215) Kazuta, Y.; Hirano, K.; Natsume, K.; Yamada, S.; Kimura, R.; Matsumoto, S.; Furuichi, K.; Matsuda, A.; Shuto, S. *J. Med. Chem.* **2003**, *46*, 1980.

(216) Liu, H.; Kerdesky, F. A.; Black, L. A.; Fitzgerald, M.; Henry, R.; Esbenshade, T. A.; Hancock, A. A.; Bennani, Y. L. *J. Org. Chem.* **2004**, *69*, 192.

(217) Kazuta, Y.; Matsuda, A.; Shuro, S. *J. Org. Chem.* **2002**, *67*, 1669.

(218) Kobayashi, T.; Watanabe, M.; Yoshida, A.; Yamada, S.; Ito, M.; Abe, H.; Ito, Y.; Arisawa, M.; Shuto, S. *Bioorg. Med. Chem.* **2010**, *18*, 1076.