ELSEVIER

Histamine H_4 receptor ligands reaching clinical development.

Major advances in the development of histamine H_4 receptor ligands

Rogier A. Smits[1,2], Rob Leurs[1,2] and Iwan J.P. de Esch[1,2]

[1] Leiden/Amsterdam Center for Drug Research (LACDR), Division of Medicinal Chemistry, Department of Pharmacochemistry, Faculty of Exact Sciences, VU University Amsterdam, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands
[2] Griffin Discoveries B.V. Department of Medicincal Chemistry, Room P-246, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands

The search for new and potent histamine H_4 receptor ligands is leading to a steadily increasing number of scientific publications and patent applications. Several interesting and structurally diverse compounds have been found, but fierce IP competition for a preferred 2-aminopyrimidine scaffold is becoming apparent. Recent investigations into the role of the histamine H_4R in (patho)physiology and the use of H_4R ligands in *in vivo* disease models reveal enormous potential in the field of inflammation and allergy, among others. The development of ligands that display activity at two or more histamine receptor (HR) subtypes is another clinical opportunity that is currently being explored. Taken together, the histamine H_4R field is gearing up for clinical studies and has the potential to deliver another generation of blockbuster drugs.

Introduction

The histamine receptor (HR) research field has historically been fuelled by breakthrough discoveries at an interval of 17 years, that is, the discovery of two distinct HR subtypes (H_1R and H_2R) in 1966 [1], the discovery of the H_3R in 1983 [2] and the identification of the sequence of H_4R in genome databases in 2000 [3–8]. This subfamily of G-protein-coupled receptors (GPCRs) has obtained a reputation of being very rewarding by the blockbuster status that was reached by H_1R and H_2R targeted drugs (for treating allergic conditions and gastric ulcers, respectively). Currently, the pharmaceutical industry is intensively exploring the clinical potential of H_3R and H_4R ligands [9,10]. Although the pharmacological effects of the H_3R were already known soon after its discovery in 1983, this receptor subtype was only embraced by the industry after the cloning of the human H_3R (hH_3R) cDNA by Lovenberg *et al.* [11,12]. Following this discovery, several groups identified the H_4R sequence in human genome databases on the basis of its homology with the H_3R (31% at the protein level, 54% within the transmembrane domains). To date, two additional non-7-TM H_4R splice variants have been identified [13]. Interestingly, these splice variants do not seem to bind histaminergic ligands, but affect the functionality of the full-length (functional) human H_4R (hH_4R) isoform, probably by GPCR hetero-dimerization [13]. Major species differ-

Corresponding author: de Esch, Iwan J.P. (i.de.esch@few.vu.nl)

Dr Rogier Smits received his MSc in pharmacy and PharmD from the Rijksuniversiteit Groningen in the Netherlands. He then moved to Amsterdam to join the group of Rob Leurs, where he received a PhD in pharmacochemistry in 2009. He has recently cofounded an academic spin-out company called Griffin Discoveries that focuses on the development of small molecule drugs that target GPCRs.

Prof Dr Rob Leurs obtained his PhD in pharmacochemistry from the VU University Amsterdam in 1991. As a postdoctoral fellow (1992–1993) at INSERM (Unite de Neurobiologie and Pharmacologie, Paris), he was involved in the cloning of genes encoding histaminergic and serotonergic receptors. Thereafter, he was awarded with a five-year fellowship (1993–1998) of the Royal Netherlands Academy of Arts and Sciences. He was appointed as assistant and full professor in Medicinal Chemistry in 1998 and 2000.

Dr Iwan de Esch received an MSc in organic chemistry and holds a PhD in pharmacochemistry. In 1998, he joined the Drug Design Group of the University of Cambridge, UK. This group spun out of the university to form De Novo Pharmaceuticals. Iwan returned to academia in 2003 and is now an associate professor at the Medicinal Chemistry Department of the VU University Amsterdam.

1359-6446/06/$ - see front matter © 2009 Published by Elsevier Ltd. doi:10.1016/j.drudis.2009.05.007

ences have been identified by the cloning of the genes for mouse, rat, guinea-pig, pig and, more recently, the dog and monkey H_4R [14,15].

The H_4R receptor is mainly expressed in bone marrow and peripheral leukocytes, and mRNAs of the hH_4 receptor are detected in for example, mast cells, dendritic cells, spleen and eosinophils [3–7]. The H_4 receptor has pronounced effects on the chemotaxis of several cell types that are associated with immune and inflammatory responses. Based on these early results from H_4R characterization, both industry and academia initiated an effective search for potent and selective hH_4R ligands and started to explore the therapeutic potential of such compounds. This has led to a steady output of both H_4R-related patents and publications (Fig. 1).

H_4R agonists

Most imidazole-containing H_3R ligands that were developed in the 1980s and 1990s turned out to have significant affinity for the H_4R as well [9]. For example, the H_3R reference agonist, (*R*)-α-methylhistamine (**2**), was found to be only 40-fold selective for the H_3R [9]. Another frequently used H_3R agonist, immepip (**3**), shows only 40-fold selectivity for the H_3R [16]. Structural modifications of immepip resulted in the potent and selective H_3R compounds, immethridine [17] and methimepip (**4**) [16], with the latter having a 2000-fold selectivity at H_3R over H_4R, illustrating that small structural changes of the ligands can differentiate binding to the different HR subtypes. Clobenpropit (**5**) was originally designed as a H_3R antagonist, but was later also identified as a high affinity H_4R partial agonist [18]. The series of clobenpropit compounds have also been used in efforts to quantify the differences in H_3R and H_4R binding requirements [18].

The first reported H_4R agonist with moderate affinity and some selectivity over H_3R is OUP-16 (**6**) [19]. Later, at VU University Amsterdam, an in-house screening of histaminergic compounds from H_1R–H_3R medicinal chemistry programs revealed considerable affinity of 4-methylhistamine (4-MeHA, **7**) for the H_4R [20]. 4-MeHA acts as a full agonist and is at least 100-fold selective over all other HRs, including the H_2R for which this compound had originally been developed.

For the closely related H_3R receptor, all reported agonists have an imidazole ring, but this heterocycle is not necessary for H_4R agonism, as is illustrated by clozapine (**8**, Fig. 2), an antipsychotic drug used for the treatment of schizophrenia. Being extremely promiscuous in nature, this tricyclic compound binds many GPCRs including dopaminergic, serotonergic, muscarinic and adrenergic receptors [21]. Clozapine (**8**) has moderate affinity for the H_4R, but some minor structural modifications led to a close analog (VUF6884, **9**) with good H_4R affinity and 300-fold selectivity over the H_3R. The rigid structure of this ligand has been used to map the H_4R active site and to construct a pharmacophore model that was useful in the design of new H_4R ligands [22,23].

Dimaprit (**10**) is another non-imidazole H_4R agonist, albeit with low affinity [20]. This alkylisothiourea was originally developed as a H_2R agonist but it possesses considerable H_4R affinity with an 80-fold selectivity for the H_4R over the H_2R [20]. Structural modification of **10** to increase its potency at the H_4R and its selectivity over

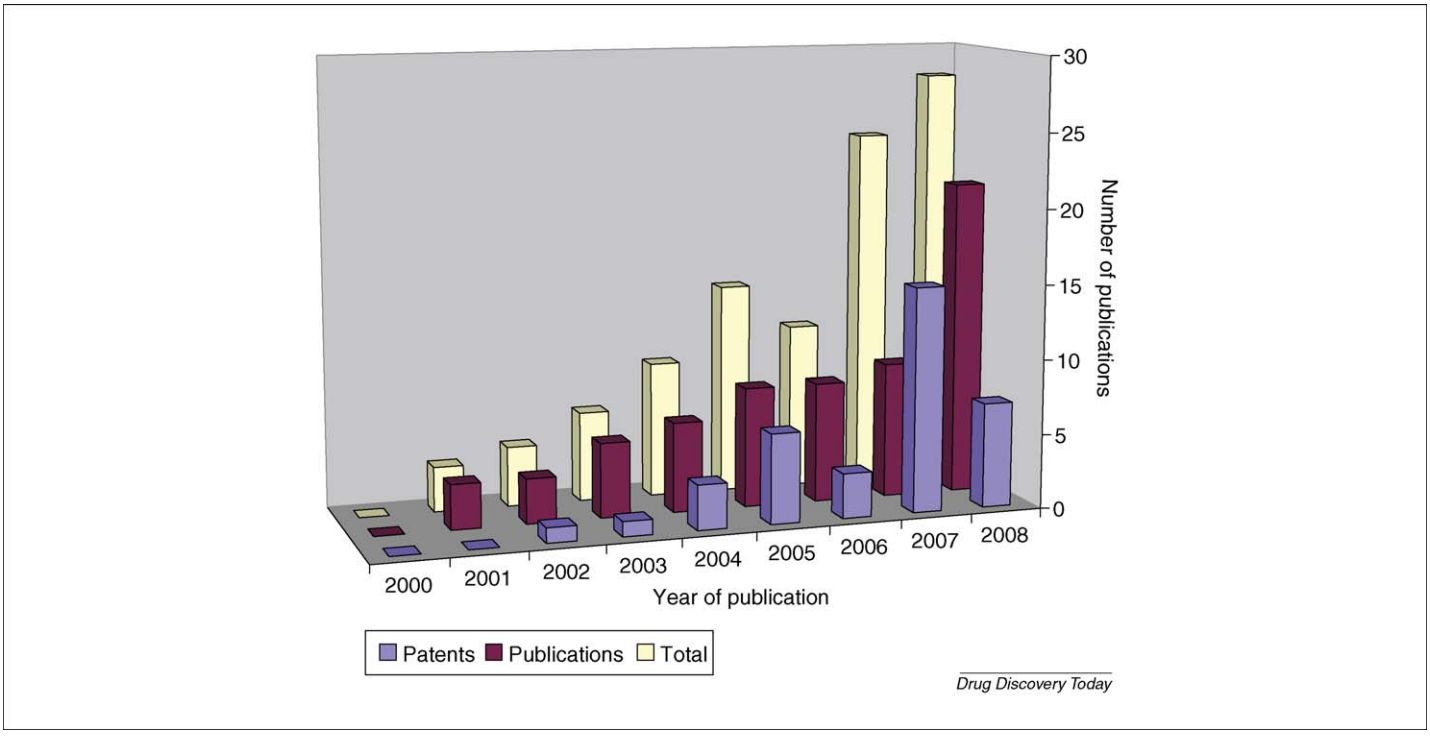

FIGURE 1

Number of H_4R-related publications (2000–2008). After the discovery of the receptor in 2000, H_4R-related research has rapidly gained momentum. Both the number of chemistry-related patent applications and publications show a steady annual increase over the past eight years indicating a gradual maturation of H_4R-related research. A relatively large number of patents covering the 2-aminopyrimidine scaffold was published in 2007, two years after the appearance of the first 2-aminopyrimidine patent application from Bayer Healthcare AG. (Searches were conducted by entering 'histamine H4' in pubmed (http://www.pubmed.com) and esp@cenet (http://www.espacenet.com) after which non-H_4-related hits were removed (status: April 2009).)

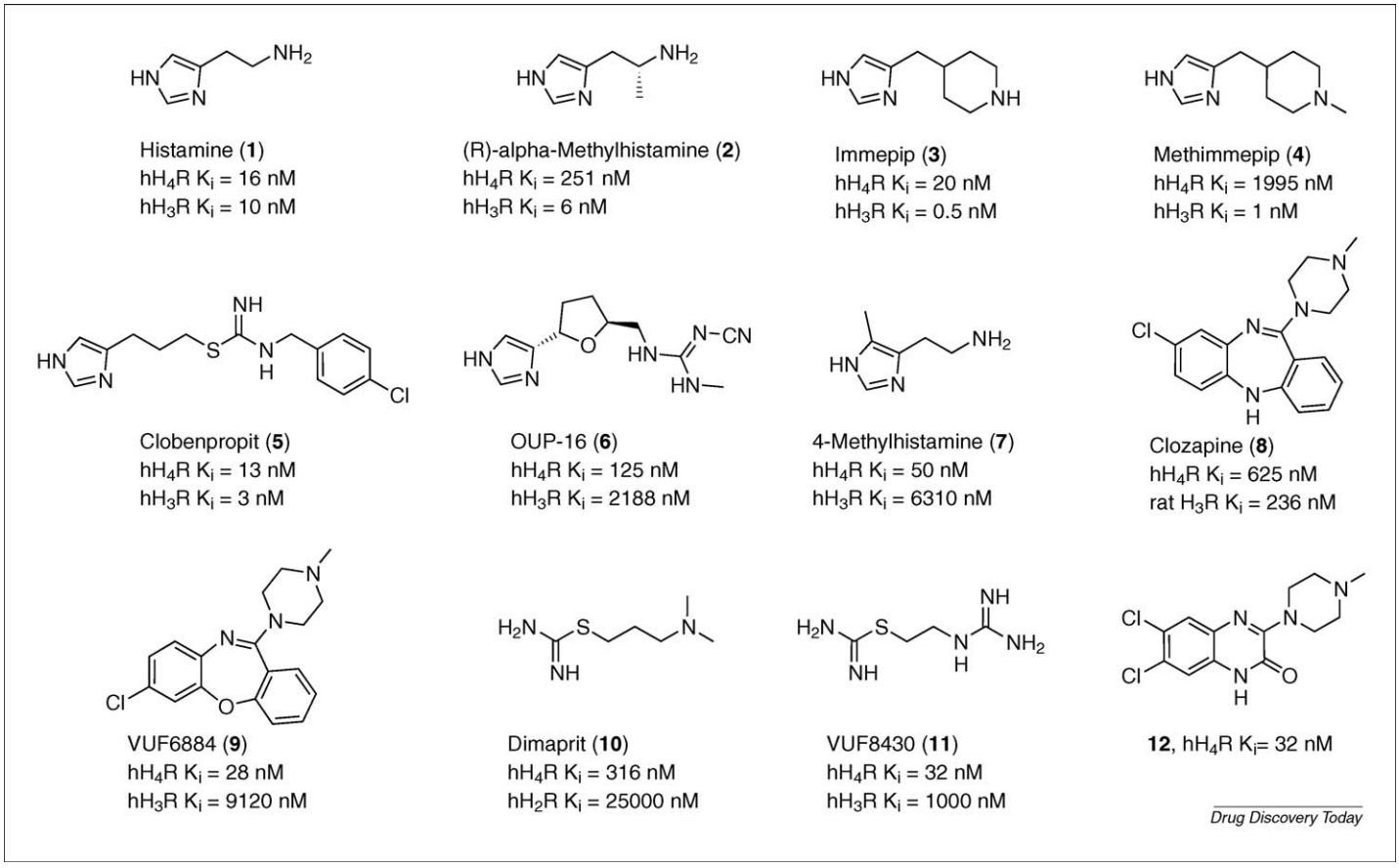

FIGURE 2

Histamine H_4 receptor agonists and their affinities for the H_3 and H_4 receptors.

the other HR subtypes led to VUF8430 (**11**), a very useful full agonist with about 30-fold selectivity for the H_4R over the H_3R [24]. Like dimaprit (**10**), VUF8430 (**11**) originated from an H_2R medicinal chemistry program [25]. A convenient microwave-assisted synthesis route for the preparation of this compound was also recently reported, making this ligand readily accessible [25].

The quinoxalinone class of H_4R ligands represented by compound **12** (Fig. 2) has been developed by scientists from J&J Pharmaceutical and was also discovered using a fragment-based drug design approach at VU University Amsterdam [22,23]. Originally developed as 5-HT_3 ligands in 1981 [26], the quinoxalin-(1*H*)2-ones have high affinity for the H_4R, with compound **12** as the most potent example in the series (hH_4R $K_i = 4$ nM as determined in our labs and 32 nM as reported in Ref. [27]). Although literature reports no functional data, it was found that quinoxaline **12** behaves as a partial agonist in a H_4R-driven CRE-beta-galactosidase reporter gene assay ($pEC_{50} = 8.99 \pm 0.14$, $\alpha = 0.55$, unpublished data).

H_4R antagonists

In the search for novel H_4R antagonists or inverse agonists, a variety of ligand classes have been identified. An antagonist that was discovered shortly after cloning of the H_4R gene in 2000 is the imidazole-containing ligand, thioperamide (**13**). Originally developed as an H_3R antagonist, thioperamide (**13**) has an H_4R affinity that is two to threefold lower than that for the H_3R while acting as an H_4R inverse agonist [20]. The early availability of recombinant systems to measure ligand affinity for H_4R has had a great impact on the identification of novel classes of compounds. In the pregenomic era, medicinal chemistry programs for the previously discovered H_1–H_3 receptors were limited with respect to the throughput of ligand screening campaigns and many of the initial reference compounds were based on the endogenous ligand. For the H_4R, high-throughput screening (HTS) was set up at the very beginning of the discovery efforts. This led to the early identification of the non-imidazole antagonist JNJ7777120 (**14**, Fig. 3) by Johnson & Johnson (J&J), undoubtedly one of the most active players in the field [28]. This highly potent indole carboxamide has a K_i of 4 nM and behaves as a neutral antagonist, although a debate on the exact functional behavior of this compound is ongoing. Recently it was shown that **14** can also behave as a partial inverse agonist ($EC_{50} = 37.7 \pm 8.5$ nM, $\alpha = -0.31 \pm 0.07$) in a steady-state GTPase assay [29]. Nevertheless, the compound has been used effectively in a variety of animal models of inflammatory disease and is so far considered the reference antagonist of choice to study the H_4R. Although **14** has reasonable oral bioavailability (22%), the compound suffers from a short *in vivo* half life in rats (0.8 hours) [30]. Therefore, its use in prolonged *in vivo* studies (e.g. models of chronic inflammation and asthma) will require either frequent administration or high doses to maintain a therapeutically effective plasma concentration. Such high doses might compromise the relatively good selectivity profile of this compound, in particular its selectivity over the H_3R (hH_3R $pK_i = 5.3$) [31].

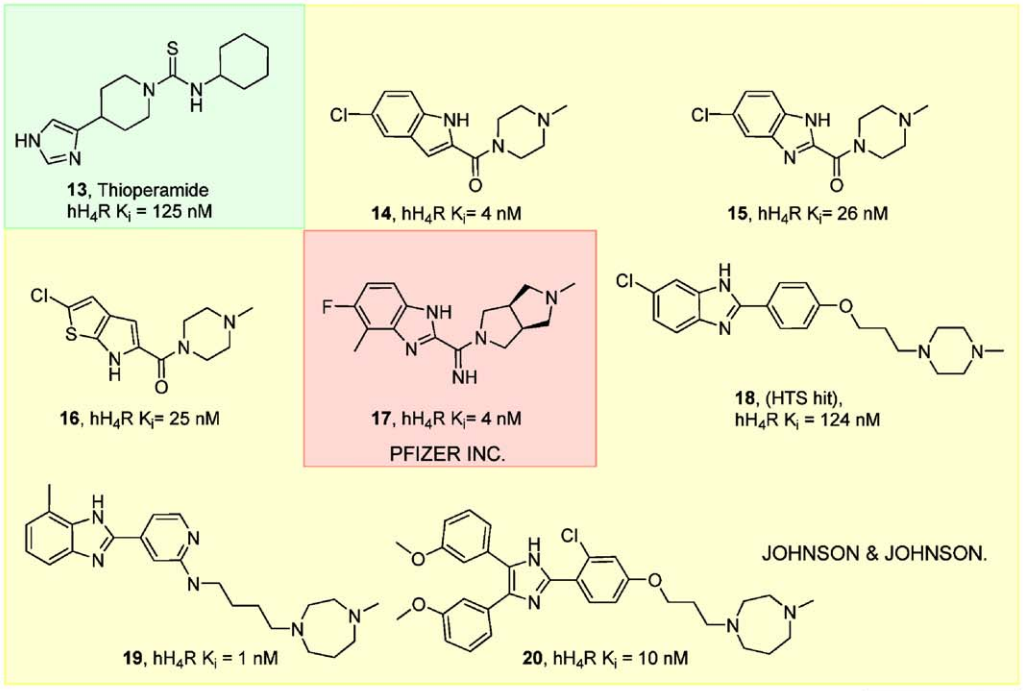

FIGURE 3

Selected H_4R ligands developed by J&J together with thioperamide (**13**) and benzimidazole **17** from Pfizer Inc.

Based on JNJ7777120 (**14**), J&J developed a variety of structurally diverse compound classes. Benzimidazole **15** and thienopyrrole **16** (Fig. 3) analogs were prepared as bioisosteres of the indole carboxamides [30,32]. Both **15** and **16** have affinities close to that of indole **14** and showed somewhat improved metabolic stability *in vitro*. *In vivo* studies of **15**, however, revealed similar stability problems and compound **16** lacked oral bioavailability altogether. Scientists from Pfizer Inc. have successfully replaced the *N*-methylpiperazine moiety of **15** with an octahydropyrrolo[3,4-*c*]pyrrole bioisostere (compound **17**, Fig. 3) [33]. In addition, this series contains several potent analogs that contain an amidine group that connects the benzimidazole group with the octahydropyrrolo[3,4-*c*]pyrrole moiety (e.g. compound **17**).

The HTS efforts of J&J also resulted in another, structurally distinct, benzimidazole-containing hit (**18**) [34,35]. A very extensive series of compounds was synthesized and described in six patents that cover a wide variety of alterations of the structures illustrated in Fig. 3 (e.g. structures **18** and **19**) [36–39]. In these compounds, the benzimidazole scaffold is attached to a substituted phenyl or heterocycle which, in turn, is connected by a flexible alkyloxy or alkyl amine chain to a piperidine or (homo)-piperazine moiety. These compounds are claimed to have high affinity for the H_4R, in our hands a potent analog was found to behave as a full agonist in a functional H_4R assay. A closely related series of compounds in which the benzimidazole group could be replaced by a di-phenyl substituted imidazole (compound **20**) has

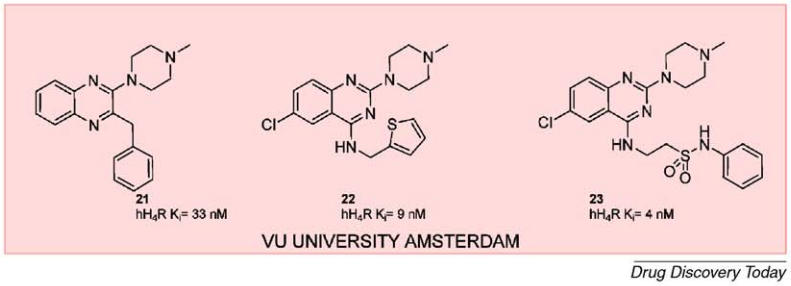

FIGURE 4

Quinoxaline and quinazoline compounds developed at VU University Amsterdam.

also been discovered [40]. The potencies of these ligands are, however, a bit lower than those found for the benzimidazole-based series.

Using a pharmacophore model constructed from the antipsychotic drug clozapine and the reference H_4R antagonist JNJ7777120 (**14**), the benzyl substituted quinoxaline **21** (Fig. 4) was developed [23]. By combining the SAR of the quinoxaline series and the pharmacophore model, a series of quinazoline compounds (e.g. compound **22**) was designed in a successful scaffold hopping exercise [41]. The thiophene moiety of compound **22** was replaced by a sulfonamide group, as illustrated by compound **23**. This replacement gave a series of compounds that tolerates a variety of substituents on the sulfonamide moiety while retaining excellent affinity for the H_4R (R. Smits, Design and synthesis of new histamine H4 receptor ligands, PhD thesis, VU University Amsterdam, 2009, ISBN: 987-90-8891-091-3).

Bayer Healthcare AG was the first to patent two series of aminopyrimidines as H_4R antagonists (e.g. **24** and **25**, Fig. 5) [42,43]. Exact IC_{50} values of these new compounds are not mentioned in the patents, but it was indicated that the affinities of the most active compounds are below 20 nM. Since the appearance of the first aminopyrimidine patents from Bayer Healthcare AG, other companies have eagerly used the 2-aminopyrimidine scaffold to develop their own H_4R ligand series. Tolerance to the introduction of a wide variety of substituents on the aminopyrimidine scaffold is generally found on the 5- and 6-positions (see also Fig. 5). In most patents the 2-amino group is left unsubstituted and the *N*-methylpiperazine moiety is usually among the most potent analogs. The *N*-methylpiperazine group can, however, be replaced with other amines with the retention of good affinity. The tolerated chemical variation of this basic side-chain is somewhat limited and the most important reason appears to be a restriction of size. On other scaffolds, the piperazine group is very intolerant

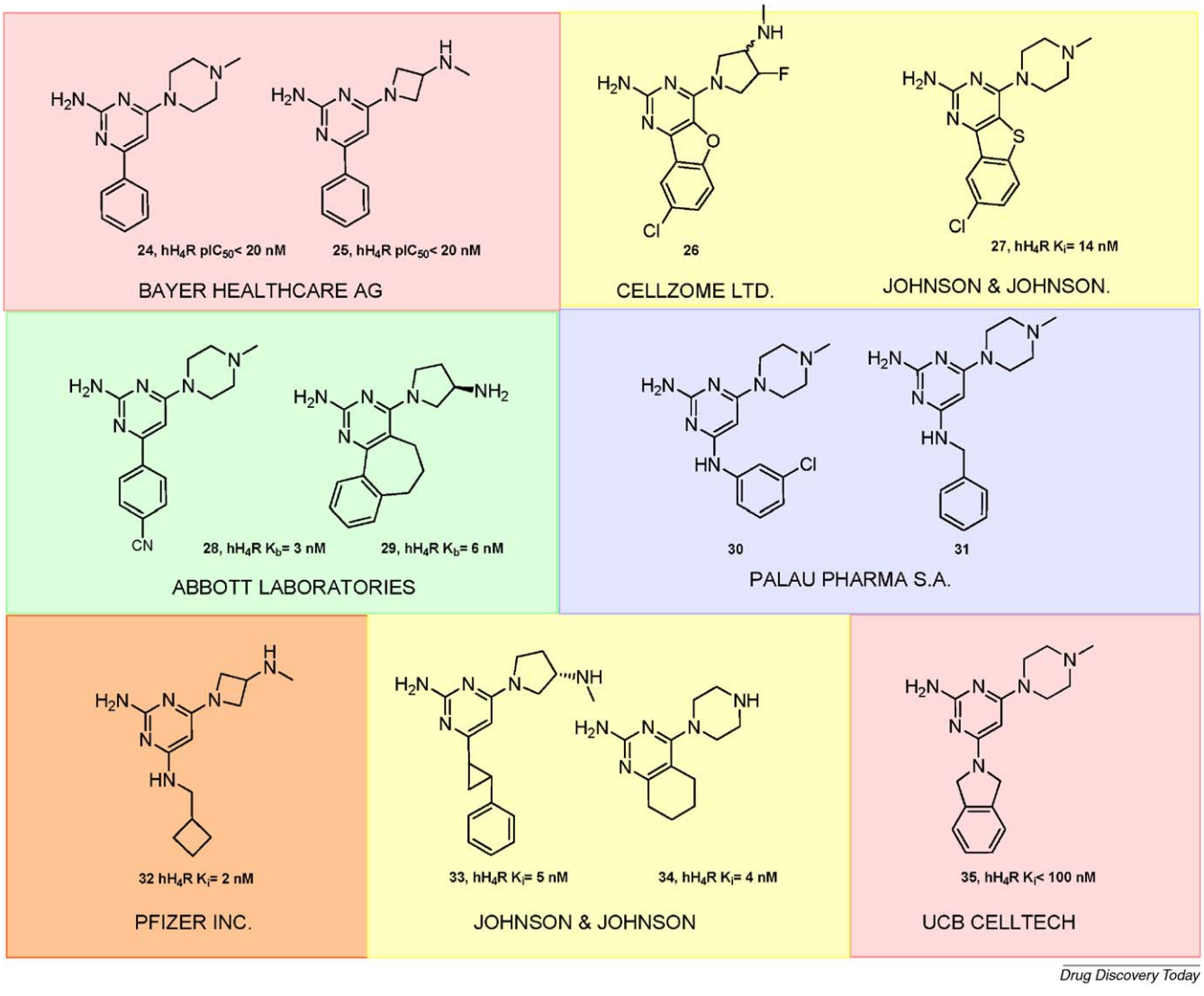

FIGURE 5

Selected patent-protected compounds that share the privileged aminopyrimidine scaffold as a common element for the H_4R.

to substitution with anything much larger than a methyl or ethyl group, suggesting a similar restriction of the size of the basic side-chain [22,28]. Initially, *N*-methylpiperazine replacements consisted of azetidine (e.g. compound **25**), aminopyrrolidine or diazepane rings substituted with small aliphatic groups.

An interesting series of derivatives that has appeared in patent literature contains a benzofused aminopyrimidine scaffold (e.g. compound **26**) [44]. This series comprises a phenyl ring that is directly connected to a 2-aminopyrimidine ring. Such a scaffold is very similar to the first aminopyrimidine series claimed by Bayer Healthcare AG (compare compound number **24** with compound number **26**) [42,43]. To gain a novel IP position, however, the aromatic phenyl and pyrimidine rings were fused with an oxygen atom to give the conformationally constrained analog **26**. After the initial discovery of the benzofuropyrimidines by Argenta Discovery [44], this series has now been taken into development by Cellzome Ltd. Much attention has been paid to alteration of the *N*-methylpiperazine moiety, resulting in a large number of azetidine and aminomethylpyrrolidine analogs of compound **26** [45–51]. Cellzome Ltd. has announced that they have developed a very potent hH_4R inverse agonist (CZC-13788, undisclosed structure) that had been planned to enter phase I clinical trials in 2008 for the treatment of allergic rhinitis [52]. This compound has a K_i of 0.17 nM for the human H_4R and is claimed to be at least 10,000-fold selective over the other HR subtypes [52].

J&J has also directed some effort toward the aminopyrimidine scaffold after the initial development of several of the compound classes described earlier (Fig. 3). The aminopyrimidines claimed are based on the fused aminopyrimidines (e.g. compound **27**) and are very similar to the compounds claimed by Cellzome Ltd. [53].

Following an HTS campaign, medicinal chemistry efforts by Abbott Laboratories gave compound **28** as a high affinity H_4R ligand with good oral availability ($F_{po}/_{iv}$ = 31% in rats) and active in various animal models (zymosan-induced peritonitis, carrageenan-induced hyperalgesia and murine itch), despite its decreased potency at the rat H_4R (rH4R $pK_i = 6.56 \pm 0.06$) [54]. A SAR study of **28** that focused on the replacement of the 4-cyanophenyl group failed to yield a compound with improved potency at the rat H_4R. By using a strategy that has also been used by scientists from J&J and Cellzome Ltd. (compounds **26** and **27** Fig. 5), a series of conformationally constrained aminopyrimidines was prepared that retain high H_4R potency across the species [55]. It has been reported that some fused cyclohepta[1,2-*d*]pyrimidine compounds (e.g. A-943931 (**29**)) bind the H_4R in the low nanomolar

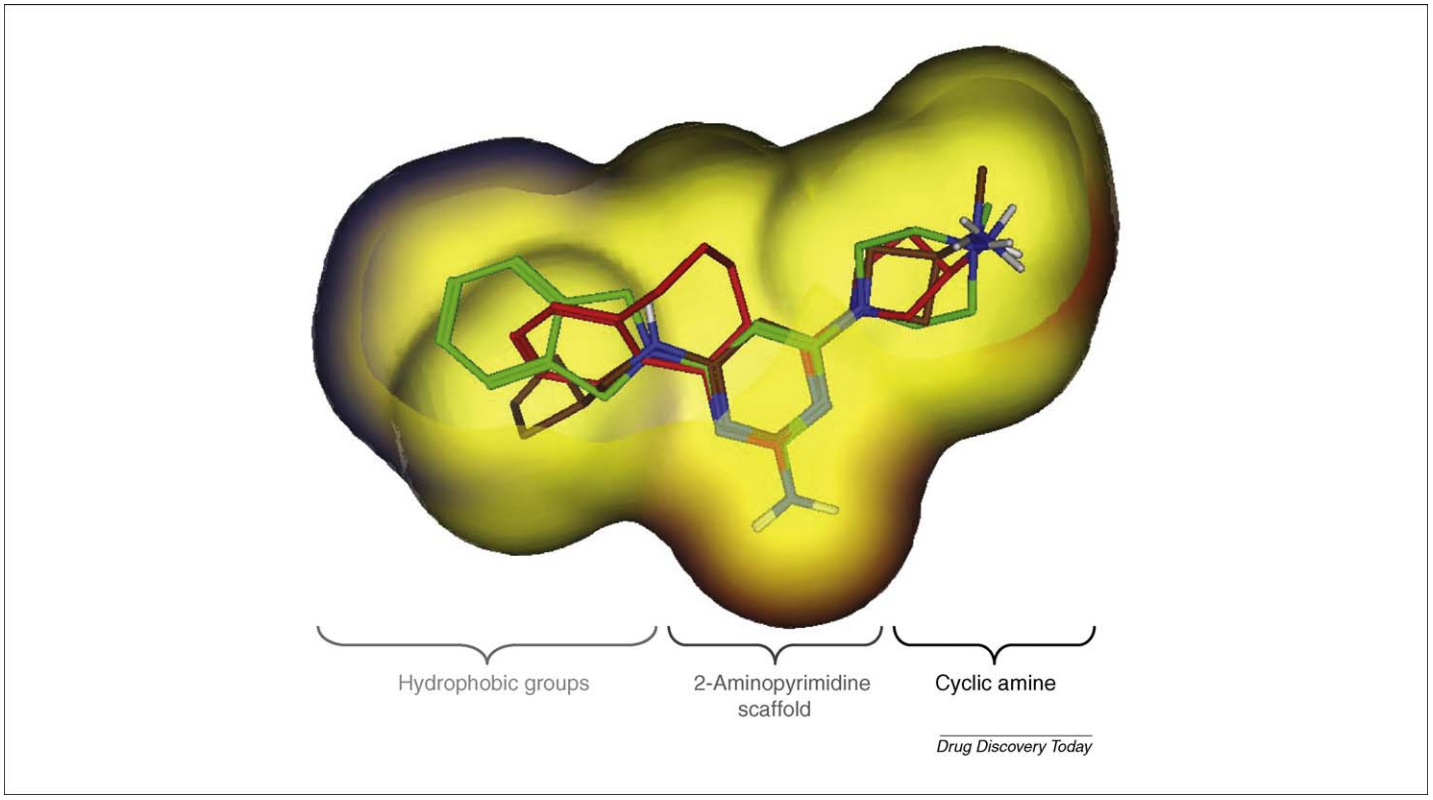

FIGURE 6

Pharmacophore for 2-aminopyrimidine H_4R ligands. The affinity H_4R ligands that use the 2-aminopyrimidine template is based on three structural elements. The first is the 2-aminopyrimidine moiety that connects the other two elements by the substitution of the aminopyrimidine 4- and 6-positions. The second element consists of hydrophobic aromatic or aliphatic groups. These hydrophobic groups can be branched, straight, or fused to the pyrimidine core (e.g. compound **29**, in red). There appears to be great tolerance for structurally diverse substituents as illustrated by the large number of patent applications covering the 2-aminopyrimidine scaffold. The third element is a cyclic amine of which the most commonly encountered example is the *N*-methylpiperazine (NMP) group (e.g. compound **35** in green). On the 2-aminopyrimidine scaffold, the NMP moiety can be substituted with small heterocyclic aliphatic rings that contain a basic nitrogen atom (e.g. azetidine **32** in brown). Substituents in this area are restricted by size and can usually not be much larger than one six-membered or two smaller fused rings. (The model was generated with MOE2007.0902. Compounds **29**, **32** and **25** were superimposed with the flexible alignment module. Then the 4.5 Å vdW interaction surface was calculated as a representation of the H_4R binding site. Hydrophobic surface is shown in yellow, hydrogen bonding surface in red and mild polar surface in blue).

range and are equipotent at rat and mouse H_4Rs. In the rat, A-943931 (**29**) combines good oral bioavailability ($F_{po}/_{iv}$ = 34%) with an improved *in vivo* half-life ($t_{1/2}$ = 2.6 hours compared to $t_{1/2}$ = 0.8 hours for the widely used antagonist JNJ7777120 (**14**)). Although the selectivity of JNJ7777120 (**14**, 1250-fold) for H_4R over H_3R is slightly better than that of A-943931 (**29**, 640-fold), the improved pharmacokinetic profile of A-943931 (**29**) allows for less frequent administration and lower dosing in animal models that require chronic dosing.

An aminopyrimidine patent filed by Palau Pharma covers several compounds that are closely related to the initial 2-aminopyrimidines from Bayer Healthcare AG [56]. In these compounds (e.g. **30** and **31**), the phenyl group has not been directly attached to the pyrimidine heterocycle but instead an amino group is used as a linker between both aromatic groups. An elaborate series was claimed with various aromatic substituents, consisting primarily of substituted phenyl groups, but thiophene, naphthalene and benzyl groups were also included. In the patent application it was noted that a 10 μM concentration of the ligands displaced at least 50% of [^{3}H]histamine binding [56]. Another patent application covering the aminopyrimidine scaffold has been filed by Pfizer Inc. [57]. Although these compounds are very closely related to the diaminopyrimidines from Palau Pharma, the focus in this series lies on aliphatic substituents (e.g. compound **32**). Where most of the previously patented aminopyrimidines contained a second aromatic group, most Pfizer Inc. compounds have straight or branched aliphatic groups. Apparently, the H_4R affinity can be increased by the introduction of hydrophobic groups that are either aromatic or aliphatic in nature, both leading to very potent compounds in the low nanomolar range (Fig. 6).

A recent addition to the H_4R patent literature is a series of structurally diverse aminopyrimidine compounds from J&J [58]. This includes cyclopropylphenyl substituted pyrimidine (**33)** and a tetrahydroquinazoline (**34**). Together with the previously discussed aminopyrimide ligands, compounds **33** and **34** yet again demonstrate the great tolerance of the H_4R to a wide variety of hydrophobic substituents. Exploiting the tolerance to chemical diversity, UCB Celltech has prepared a series of aminopyrimidines that includes several compounds with (partially)saturated heterocyclic rings attached to the 6-position (e.g. compound **35**) [59]. The point of attachment can not only be the nitrogen atom as exemplified by compound **35**, but also be a carbon atom of a cyclic substructure, such as a pyrrole or piperidine ring. These compounds have been reported to bind the H_4R in the nanomolar range.

Clinical applications for H_4R antihistamines

H_4R agonists and antagonists have an important role to play in the elucidation of the (patho)physiological role of the H_4R and to explore the therapeutic potential of this receptor in human disease. The use of H_4R ligands in animal models, so far mostly done with reference antagonist JNJ7777120 (**14**), has yielded a variety of interesting results.

Pruritis

Recently the role of the H_1R and H_4R in the attenuation of pruritis (itch) has been reported in several papers [60–62]. In mice, the selective H_4R agonist 4-MeHA (**7**) was shown to induce itch in a dose-dependent way and this effect could be blocked by the pretreatment with JNJ7777120 (**14**) [61]. The antipruritic effect of **14** was far superior to other antihistamines and only the centrally acting H_1R antagonist diphenhydramine showed some antipruritic activity. This pruritic response could not be abolished completely by either an H_1R or H_4R antagonist alone, but when both were administered simultaneously, almost no scratching behavior was observed. This observation clearly implicates both HR subtypes in the pruritic response and suggests that in pruritis, simultaneously antagonizing both the H_1R and H_4R might have added clinical benefit over H_1R or H_4R monotherapy.

Asthma

A role for the H_4R in a murine ovalbumin-induced inflammation model of asthma was recently found by Dunford *et al.* [63]. It was demonstrated that the H_4R is involved in the activation of $CD4^+$ cells by dendritic cells. The administration of JNJ7777120 (**14**) showed significant anti-inflammatory responses during both the sensitization and effector phases. This finding indicates that the H_4R is involved in the initial priming of the immune system after allergen challenge. More importantly, the administration of a H_4R antagonist is also efficacious after the animals had been sensitized for ovalbumin. This discovery is of great interest because, a therapeutic intervention will only be done after a disease has become manifest [63].

Allergic rhinitis

The presence of the H_4R in nasal tissue was first discovered by Nakaya *et al.* [64]. In addition, a more recent finding showed that there is a significant increase in the level of H_4R in human nasal polyp tissue taken from patients with chronic rhinosinusitis (infection of the nose and nasal cavities) when compared to normal nasal mucosa [65]. Jókúti *et al.* suggest that the administration of H_4R antagonists might be a new way to treat nasal polyps and chronic rhinosinusitis. The administration of H_4R antagonists may prevent the accumulation of eosinophils as a result of impaired cell chemotaxis toward polypous tissue [65]. Although scientific data on the role of the H_4R in rhinitis is limited, at present, it is the only indication for which an H_4R inverse agonist (CZC-13788) is reported to be in preclinical development [52]. Apparently, histamine is implicated in allergic rhinitis by acting on three HR subtypes, the H_1R, H_3R and H_4R [66]. For many years, the classical application of H_1R antagonists (antihistamines) has been the treatment of allergic rhinitis. H_1R antagonists relieve edema and vasoconstriction, both important symptoms of the disease, but these drugs do not affect the underlying inflammatory responses [66]. After the discovery of the H_3R and H_4R subtypes, the traditional role for H_1R antagonists in rhinitis has been reappraised [66]. It has been shown that the H_3R agonist (*R*)-α-methylhistamine (**2**) can induce the dilatation of nasal blood vessels and that this effect can be counteracted by the H_3R antagonist/H_4R agonist clobenpropit (**5**) [66]. Although a role for the H_4R cannot be ruled out, this H_3R antagonist-mediated mechanism in nasal decongestion has certainly caught the attention of scientists from Pfizer Inc. Recently, patient recruitment started for a Phase II clinical trial to test a H_3R antagonist (PF-03654746, unpublished structure) as a novel nasal decongestant in patients with seasonal allergic rhinitis (http://www.clinicaltrials.gov). A dual target

approach is being pursued by GSK that is currently recruiting patients to test a systemic H_1/H_3 antagonist (GSK835726, unpublished structure) for seasonal allergic rhinitis in a Phase I clinical trial (http://www.clinicaltrials.gov). A second Phase I trial with another H_1/H_3 antagonist (GSK1004723, unpublished structure) for intranasal administration to treat rhinitis has recently been completed (http://www.clinicaltrials.gov). With these compounds, the mode of action of the classical H_1R antagonist is combined with the potential clinical benefit of added nasal decongestion by H_3R blockade. The synergistic role of the H_1R and H_3R has been demonstrated *in vivo* in experiments performed at Schering-Plough [67]. In view of the role of the H_4R in allergic rhinitis, other potential treatment paradigms may also be considered, such as combining H_1/H_4, H_3/H_4 or even $H_1/H_3/H_4$ antagonists/inverse agonist activity in the same molecule.

Pain

In 2007 it was suggested that the H_4R might play a role in nociception [68]. The H_4R antagonist JNJ7777120 (**14**) and its benzimidazole analog, VUF6002 (**15**), were both able to increase paw withdrawal latency in carrageenan-induced thermal hyperalgesia in rats [68]. This effect on the modulation of pain was recently confirmed by work from Abbott Laboratories that describes *in vivo* anti-nociceptive effects of the H_4R antagonist A-943931 (**39**) [55]. This compounds is active against carrageenan-induced inflammatory pain and in a spinal nerve ligation model of neuropathic pain. The exact location of the H_4Rs that are responsible for the observed antinociceptive effects remains unknown, although very recently new evidence for the presence of the H_4R in the spinal cord and dorsal root ganglion has been reported [69]. The encouraging results seen with H_4R antagonists *in vivo* may offer a new way to relieve pain that is unresponsive to existing therapies.

Cancer

Although histamine has been known to play a role in the development of cancer, a role for the H_4R has only recently been suggested on the basis of *in vitro* studies with HT29 and Caco-2 cells [70]. Histidine decarboxylase is often overexpressed in human colorectal cancer cells, resulting in an increased local production of histamine from the amino acid histidine. The selective H_4R antagonist JNJ7777120 (**14**) was able to inhibit histamine-induced overexpression of cyclooxygenase-2 and subsequent production of PGE_2. A similar finding was reported for H_2R activation, which can be blocked by the H_2R antagonist zolantidine [70]. PGE_2 is an important mediator of proliferative and proangiogenic activity of cells. Activation of the H_2R and H_4R receptors may, therefore, explain the proliferative effect of histamine on HT29 and Caco-2 cells. A different relationship between the H_4R and cancer has been proposed by Boer *et al.* [71]. It was found that human colon carcinoma cells had significantly reduced levels of H_1R and H_4R, but unchanged H_2R levels, possibly causing a microenvironment that is favorable for tumor development. The low abundance of H_1R and H_4R leads to a more dominant role of H_2R-mediated negative regulation of the Th_1 and Th_2 immune response against the carcinoma cells. In addition, H_2R-mediated angiogenesis and metastatic spreading may also lead to malignant tumor formation [71]. Further studies in this direction will be conducted with H_4R and H_1R knock-out mice and results in this area are eagerly awaited.

Inflammatory bowel disease

In an experimental model of colitis in the rat, the oral administration of a high dose of the H_4R antagonists JNJ7777120 (**14**) or VUF6002 (**15**) was able to reduce (TBNS)-induced colon damage, the influx of neutrophils and levels of myeloperoxidase in colonic tissue [72]. Although several of the underlying physiological mechanisms need to be fully explored, these preliminary findings suggest a potential role for H_4R antagonists in inflammatory bowel disease.

Conclusions and outlook

Since the discovery of the H_4R in 2000 the amount of publications and patent applications has shown a steady annual increase. Several useful selective H_4R agonists and antagonists have been developed and now serve as important tools to delineate the role of the H_4R in physiology and to establish its potential value as a drug target. The recent increase in patent applications from various players in the pharmaceutical industry clearly reflects the mounting interest in this new HR subtype as a drug target.

Several histamine-induced events are caused by the activation of multiple HR subtypes. In some diseases, such as allergic rhinitis and pruritis, these insights may offer new treatment paradigms with H_4R antagonists that have affinity for multiple HR subtypes to provide added clinical benefit compared to single receptor selective histaminergic ligands. The development of compounds that display potency at the panel of HR subtypes can be considered an exciting opportunity to synergistically increase efficacy *in vivo* or for treating multiple histamine-induced symptoms of a single disease.

References

1 Ash, A.S. and Schild, H.O. (1966) Receptor mediating some actions of histamine. *Br. J. Pharmacol.* 27, 427–439

2 Celanire, S. *et al.* (2005) Keynote review: histamine H_3 receptor antagonists reach out for the clinic. *Drug Discov. Today* 10, 1613–1627

3 Oda, T. *et al.* (2000) Molecular cloning and characterization of a novel type of histamine receptor preferentially expressed in leukocytes. *J. Biol. Chem.* 275, 36781–36786

4 Liu, C. *et al.* (2001) Cloning and pharmacological characterization of a fourth histamine receptor (H4) expressed in bone marrow. *Mol. Pharmacol.* 59, 420–426

5 Nguyen, T. *et al.* (2001) Discovery of a novel member of the histamine receptor family. *Mol. Pharmacol.* 59, 427–433

6 Morse, K.L. *et al.* (2001) Cloning and characterization of a novel human histamine receptor. *J. Pharmacol. Exp. Ther.* 296, 1058–1066

7 Zhu, Y. *et al.* (2001) Cloning, expression and pharmacological characterization of a novel human histamine receptor. *Mol. Pharmacol.* 59, 434–441

8 Hough, L. (2001) Genomics meets histamine receptors: new subtypes, new receptors. *Mol. Pharmacol.* 59, 415–419

9 Wijtmans, M. *et al.* (2007) Histamine H3 receptor ligands break ground in a remarkable plethora of therapeutic targets. *Expert Opin. Investig. Drugs* 16, 967–985

10 Arrang, J. *et al.* (1983) Auto-inhibition of brain histamine release mediated by a novel class (H_3) of histamine receptor. *Nature* 302, 832–837

11 Lovenberg, T.W. *et al.* (1999) Cloning and functional expression of the human histamine H_3 receptor. *Mol. Pharmacol.* 55, 1101–1107

12 Leurs, R. *et al.* (2005) The histamine H_3 receptor: from gene cloning to H_3 receptor drugs. *Nat. Rev. Drug Discov.* 4, 107–120
13 Van Rijn, R.M. *et al.* (2008) Cloning and characterization of dominant negative splice variants of the human histamine H4 receptor. *Biochem. J.* 414, 121–131
14 Jiang, W. *et al.* (2008) Cloning and pharmacological characterization of the dog histamine H4 receptor. *Eur. J. Pharmacol.* 592, 26–32
15 Oda, *et al.* (2008) Molecular cloning of monkey histamine H_4 receptor. *J. Pharmacol. Sci.* 98, 319–322
16 Kitbunnadaj, R. *et al.* (2005) N-substituted piperidinyl alkyl imidazoles: discovery of methimepip as a potent and selective histamine H3 receptor agonist. *J. Med. Chem.* 48, 2100–2107
17 Kitbunnadaj, R. *et al.* (2004) Identification of 4-(1*H*-imidazol-4(5)-ylmethyl)pyridine (immethridine) as a novel, potent and highly selective histamine H(3) receptor agonist. *J. Med. Chem.* 47, 2414–2417
18 Lim, H. (2009) Clobenpropit analogs as dual activity ligands for the histamine H_3 and H_4 receptors: synthesis, pharmacological evaluation and cross-target QSAR studies. *Bioorg. Med. Chem.* 17, 3987–3994
19 Hashimoto, T. *et al.* (2003) A selective human H(4)-receptor agonist: (−)-2-cyano-1-methyl-3-[(2R,5R)-5-[1H-imidazol-4(5)-yl]tetrahydrofuran-2-y] methylguanidine. *J. Med. Chem.* 46, 3162–3165
20 Lim, H. *et al.* (2005) Evaluation of histamine H_1-, H_2-, and H_3-receptor ligands at the human histamine H_4 receptor: Identification of 4-methylhistamine as the first potent and selective histamine H_4 receptor agonist. *J. Pharmacol. Exp. Ther.* 314, 1310–1321
21 Selent, J. *et al.* (2008) Multi-receptor binding profile of clozapine and olanzapine: a structural study based on the new beta2 adrenergic receptor template. *ChemMedChem* 8, 1194–1198
22 Smits, R. *et al.* (2005) Characterization of the histamine H_4 receptor binding site: Part I. Synthesis and pharmacological evaluation of dibenzodiazepine derivatives. *J. Med. Chem.* 49, 4512–4516
23 Smits, R. *et al.* (2008) Fragment based design of new H4 receptor-ligands with anti-inflammatory properties in vivo. *J. Med. Chem.* 51, 2457–2467
24 Lim, H. *et al.* (2009) Pharmacological characterization of the new histamine H_4 receptor agonist VUF 8430. *Br. J. Pharmacol.* 157, 34–43
25 Lim, H. *et al.* (2006) Discovery of *S*-(2-guanidylethyl)-isothiourea (VUF8430) as a potent nonimidazole histamine H_4 agonist. *J. Med. Chem.* 49, 6650–6651
26 Lumma, W. *et al.* (1981) Piperazinylquinoxalines with central serotoninmimetic activity. *J. Med. Chem.* 24, 93–101
27 Edwards *et al.* (2005) Johnson & Johnson. Quinoxaline compounds. US20050070527
28 Jablonowski, J. *et al.* (2003) The first potent and selective non-imidazole human histamine H_4 receptor antagonists. *J. Med. Chem.* 19, 3957–3960
29 Schneider, E. *et al.* (2009) High constitutive activity and a G-protein-independent high-affinity state of the human histamine H_4-receptor. *Biochemistry* 42, 1424–1438
30 Venable, J. *et al.* (2005) Preparation and biological evaluation of indole, benzimidazole, and thienopyrrole piperazine carboxamides: potent human histamine H(4) antagonists. *J. Med. Chem.* 48, 8289–8298
31 Thurmond, R. *et al.* (2004) A potent and selective histamine H_4 receptor antagonist with anti-inflammatory properties. *J. Pharmacol. Exp. Ther.* 309, 404–413
32 Terzioglu, N. *et al.* (2004) Synthesis and structure-activity relationships of indole- and benzimidazole piperazines as histamine H_4 receptor antagonists. *Bioorg. Med. Chem. Lett.* 14, 5251–5256
33 Lane, C. *et al.* (2006) Pizer Ltd. Octahydropyrrolo[3,4-C]pyrrole derivatives. Patent US20060111416
34 Arienti, K. *et al.* (2005) Johnson & Johnson Benzoimidazole compounds. US20050070550
35 Lee-Dutra, A. *et al.* (2006) Identification of 2-arylbenzimidazoles as potent human histamine H_4 receptor ligands. *Bioorg. Med. Chem. Lett.* 16, 6043–6048
36 Edwards, J. *et al.* (2007) Janssen Pharmaceutica N.V. Benzoimidazol-2-yl pyridines as modulators of the histamine H_4 receptor. US20070232616
37 Edwards, J. *et al.* (2007) Janssen Pharmaceutica N.V. Benzoimidazol-2-yl pyrimidines and pyrazines as modulators of the histamine H_4 receptor. WO2007117399
38 Edwards, J. *et al.* (2007) Janssen Pharmaceutica N.V. Benzoimidazol-2-yl pyridines as modulators of the histamine H_4 receptor. WO2007117400
39 Edwards, J. *et al.* (2007) Johnson & Johnson Benzoimidazol-2-yl pyrimidines and pyrazines as modulators of the histamine H_4 receptor. US2007244126
40 Buzard, D. *et al.* (2005) Janssen Pharmaceutica N.V. Imidazole compounds. WO2005092066
41 Smits, R. *et al.* (2008) The discovery of quinazolines as histamine H_4 receptor inverse agonists using a scaffold hopping approach. *J. Med. Chem.* 51, 7855–7865
42 Sato, H. *et al.* (2005) Bayer Healthcare AG. 2-Aminopyrimidine derivatives. WO2005054239
43 Sato, H. *et al.* (2005) Bayer Healthcare AG. 2-Aminopyrimidine derivatives. WO2005014556
44 Harris, N. *et al.* (2006) Argenta Discovery Ltd. Pyrimidine compounds as histamine modulators. WO2006050965
45 Dyke, H. *et al.* (2007) Cellzome Ltd. Pyrimidine compounds for the treatment of inflammatory disorders. EP1767537
46 Reid, A. *et al.* (2007) Cellzome Ltd. Amino pyrimidine compounds for the treatment of inflammatory disorders. Patent EP1829879
47 Reid, A. *et al.* (2007) Cellzome Ltd. Enantiomers of amino pyrimidine compounds for the treatment of inflammatory disorders. EP1860108
48 Reid, A. *et al.* (2007) Cellzome Ltd. Azetidine amino pyrimidine compounds for the treatment of inflammatory disorders. EP1860109
49 Dyke, H. *et al.* (2007) Cellzome Ltd. Pyrimidine compounds for the treatment of inflammatory disorders. WO2007039467
50 Reid, A. *et al.* (2007) Cellzome Ltd. Enantiomers of amino pyrimidine compounds for the treatment of inflammatory disorders. WO2007090853
51 Reid, A. *et al.* (2007) Cellzome Ltd. Azetidine amino pyrimidine compounds for the treatment of inflammatory disorders. WO2007090854
52 Hale, R.A. *et al.* (2007) potent and selective Histamine H4 receptor antagonist for the treatment of allergic rhinitis. *Drug News Perspect.* 20, 593–600
53 Chavez, F. *et al.* (2008) Janssen Pharmaceutica N.V. Benzofuro- and benzothienopyrimidine modulators of the histamine H4 receptor. WO2008008359
54 Altenbach, R. *et al.* (2008) Structure–activity studies on a series of a 2-aminopyrimidine-containing histamine H_4 receptor ligands. *J. Med. Chem.* 51, 6571–6580
55 Cowart, M. *et al.* (2008) Rotationally constrained 2,4-diamino-5,6-disubstituted pyrimidines: a new class of histamine H_4 receptor antagonists with improved drug likeness and in vivo efficacy in pain and inflammation models. *J. Med. Chem.* 51, 6547–6557
56 Carceller González, E. *et al.* (2007) Palau Pharma S.A. 2-Aminopyrimidine derivatives as modulators of the histamine H_4 receptor activity. WO2007031529
57 Bell, A. *et al.* (2007) Pfizer Ltd. Pyrimidine derivatives. WO2007072163
58 Hui, C. *et al.* (2008) Janssen Pharmaceutica N.V. 2-Aminopyrimidine modulators of the histamine H_4 receptor. WO2008100565
59 Raphy G. *et al.* (2008) UCB Pharma S.A. Novel 2 aminopyrimidine derivatives, processes for preparing them, pharmaceutical compositions thereof. WO2008031556
60 Bell, J. *et al.* (2004) Involvement of histamine H_4 and H_1 receptors in scratching induced by histamine receptor agonists in BalbC mice. *Br. J. Pharmacol.* 142, 374–380
61 Dunford, P.J. *et al.* (2007) Histamine H_4 receptor antagonists are superior to traditional antihistamines in the attenuation of experimental pruritis. *J. Allergy Clin. Immunol.* 119, 176–183
62 Thurmond, R. *et al.* (2008) The role of histamine H1 and H4 receptors in allergic inflammation: the search for new antihistamines. *Nat. Rev. Drug Discov.* 7, 41–53
63 Dunford, P. *et al.* (2006) The histamine H4 receptor mediates allergic airway inflammation by regulating the activation of $CD4^+$ cells. *J. Immunol.* 176, 7062–7070
64 Nakaya, M. *et al.* (2004) Immunohistochemical localization of histamine receptor subtypes in human inferior turbinates. *Ann. Otol. Rhinol. Laryngol.* 113, 552–557
65 Jókúti, A. *et al.* (2007) Histamine H_4 receptor expression is elevated in human nasal polyp tissue. *Cell. Biol. Int.* 31, 1367
66 Taylor-Clark, T. (2008) Insights into the mechanism of histamine-induced inflammation in the nasal mucosa. *Pulm. Pharm. Ther.* 21, 455–460
67 McLeod, R. *et al.* (1999) Combined histamine H_1 and H_3 receptor blockade produces nasal decongestion in an experimental model of nasal congestion. *Am. J. Rhinol.* 3, 391–399
68 Coruzzi, G. *et al.* (2007) Antiinflammatory and antinociceptive effects of the selective histamine H_4-receptor antagonists JNJ7777120 and VUF6002 in a rat model of carrageenan-induced inflammation. *Eur. J. Pharmacol.* 563, 240–244
69 Strakhova, M. *et al.* (2009) Localization of histamine H_4 receptors in the central nervous system of human and rat. *Brain Res.* 1250, 41–48
70 Cianchi, F. *et al.* (2005) The role of cyclooxygenase-2 in mediating the effects of histamine on cell proliferation and vascular endothelial growth factor production in colorectal cancer. *Clin. Cancer Res.* 11, 6807–6815
71 Boer, K. *et al.* (2008) Decreased expression of histamine H1 and H4 receptors suggests disturbance of local regulation in human colorectal tumours by histamine. *Eur. J. Cell Biol.* 87, 227–236
72 Varga, C. *et al.* (2005) Inhibitory effects of histamine H4 receptor antagonists on experimental colitis in the rat. *Eur. J. Pharmacol.* 522, 130–138