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REVIEW

Histamine H_4 receptors in the gastrointestinal tract

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Histamine is a well-established mediator involved in a variety of physiological and pathophysiological mechanisms and exerts its effect through activation of four histamine receptors (H_1–H_4). The histamine H_4 receptor is the newest member of this histamine receptor family, and is expressed throughout the gastrointestinal tract as well as in the liver, pancreas and bile ducts. Functional studies using a combination of selective and non-selective H_4 receptor ligands have rapidly increased our knowledge of H_4 receptor involvement in gastrointestinal processes both under physiological conditions and in models of disease. Strong evidence points towards a role for H_4 receptors in the modulation of immune-mediated responses in gut inflammation such as in colitis, ischaemia/reperfusion injury, radiation-induced enteropathy and allergic gut reactions. In addition, data have emerged implicating H_4 receptors in gastrointestinal cancerogenesis, sensory signalling, and visceral pain as well as in gastric ulceration. These studies highlight the potential of H_4 receptor targeted therapy in the treatment of various gastrointestinal disorders such as inflammatory bowel disease, irritable bowel syndrome and cancer.

Abbreviations

IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; MC, mast cell; TNBS, trinitrobenzene sulphonic acid

Tables of Links

TARGETS
GPCRs[a]
Histamine H_1 receptor
Histamine H_2 receptor
Histamine H_3 receptor
Histamine H_4 receptor
Enzymes[b]
COX-2

LIGANDS	
4-Methylhistamine	Immepip
Cimetidine	JNJ10191584
Clobenpropit	JNJ7777120
Clozapine	Ketotifen
Dimaprit	Pyrilamine
Histamine	Thioperamide
Imetit	VUF8430

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 ([a,b]Alexander *et al.*, 2013a,b).

Introduction

Histamine (2-[4-imidazole]-ethylamine) is a short-acting endogenous amine, involved in several physiological and pathophysiological processes (Jutel *et al.*, 2009). It is present in virtually all bodily organs, with high concentrations reported in the stomach, lymph nodes and thymus (Kumar *et al.*, 1968; Zimmermann *et al.*, 2011). Histamine is synthetized from L-histidine by L-histidine decarboxylase and is stored in the granules of mast cells (MCs) and basophils, the main sources of histamine (Endo, 1982; Jones and Kearns, 2011). Enterochromaffin-like cells, histaminergic neurons, lymphocytes, monocytes, platelets and neutrophils also express L-histidine decarboxylase and are capable of producing, but not storing, high amounts of histamine (Snyder and Epps, 1968; Vanhala *et al.*, 1994; Bencsath *et al.*, 1998; Jutel *et al.*, 2009; Alcaniz *et al.*, 2013). Histamine exerts its actions by binding to four GPCRs that are differentially expressed throughout the body and designated as the H_1, H_2, H_3 and H_4 receptors. Histamine H_1 receptors mediate sensorineural signalling, vascular dilatation and permeability and airway smooth muscle contraction, and are involved in allergic rhinitis, atopic dermatitis, conjunctivitis, urticaria, asthma and anaphylaxis (Togias, 2003; Simons and Simons, 2011). Histamine H_2 receptors are well-known for their role in gastric acid secretion, but also exert immune modulatory properties (Black *et al.*, 1972; Jutel *et al.*, 2009). Histamine H_3 receptors are most abundantly present in the CNS and are implicated in sleep–wake disorders, attention-deficient hyperactivity disorder, epilepsy, cognitive impairment and obesity (Kuhne *et al.*, 2011; Singh and Jadhav, 2013). Finally, histamine H_4 receptors are predominantly expressed on immune cells, such as lymphocytes, MCs and dendritic cells, and are currently mainly under evaluation for immune-mediated disorders such as allergic rhinitis, asthma and pruritus (Liu, 2014). However, new roles for this receptor subtype are continuously being discovered. Here we provide an overview of the current evidence of H_4 receptor involvement in multiple gastrointestinal physiological and pathophysiological processes.

H_4 receptors

In the early 2000s, several groups reported on the discovery and cloning of a fourth histamine receptor (Nakamura *et al.*, 2000; Oda *et al.*, 2000; Liu *et al.*, 2001a; Morse *et al.*, 2001; Nguyen *et al.*, 2001; Zhu *et al.*, 2001). The H_4 receptor is encoded by a single copy on chromosome 18q11.2 and demonstrates an overall homology of 23% to H_1 receptors, 22% to H_2 receptors and 37% to H_3 receptors (Oda *et al.*, 2000; Coge *et al.*, 2001). The human full-length receptor consists of 390 amino acids, which form seven transmembrane helices, three extracellular loops and three intracellular loops, with an extracellular N-terminal and an intracellular C-terminal peptide (Leurs *et al.*, 2009). H_4 receptors couple to $G_{\alpha i/0}$ proteins, inhibiting downstream adenylyl cyclase and forskolin-induced cAMP (Morse *et al.*, 2001; Zhu *et al.*, 2001). They are mainly present in immune cells and highly expressed in bone marrow and spleen; varying expression levels were also reported in gastrointestinal tissues, testes, kidney, lung, prostrate and brain (Nakamura *et al.*, 2000; Oda *et al.*, 2000; Coge *et al.*, 2001; Strakhova *et al.*, 2009). Tissue distribution is quite similar across species (Liu *et al.*, 2001b; Oda *et al.*, 2005). There is high homology in the amino acid sequence between human and monkey H_4 receptors (92%), whereas this is 72% between human and pig and 65–70% between human and rodent H_4 receptors (Liu *et al.*, 2001b; Oda *et al.*, 2002; 2005). These differences in amino acid sequence also affect the binding profile of histamine towards H_4 receptors with high affinity for human and guinea pig H_4 receptors (K_D 4.8 and 6 nM) compared with rat and mouse H_4 receptors (136 and 42 nM) (Liu *et al.*, 2001b). Compared with H_1 and H_2 receptors, histamine displays high affinity for H_4 receptors in both human and rodents (Table 1).

Soon after its discovery and cloning, attempts were made to elucidate the pharmacological profile of H_4 receptors and identify (selective) ligands to stimulate or inhibit H_4 receptor signalling. Early assessments indicated that several H_3 receptor ligands demonstrated significant affinity for H_4 receptors, such as clozapine, imetit and immepip (H_3 and H_4 receptor agonists) and clobenpropit (H_3 receptor antagonist, H_4 receptor agonist) (Table 1) (Leurs *et al.*, 2009; Smits *et al.*, 2009).

Table 1

Ligands for the human H_4 receptor

Compound	H_4R (pKi)	H_1R (pKi)	H_2R (pKi)	H_3R (pKi)
Agonists				
Histamine	7.8	4.2	4.3	8.0
4-methylhistamine	7.3	<5.0	5.1	5.2
Clozapine	6.7	9.4		6.6
Clobenpropit	8.1			8.6
Dimaprit	6.5		4.6	7.3
Imetit	8.2			8.8
Immepip	7.7			9.3
OUP-16	6.9			5.7
VUF10460	8.2			5.8
VUF6884	7.6			5.0
VUF8430	7.5			6.0
Antagonists				
A-943931	8.3			
JNJ10191584*	7.6			
JNJ39758979	7.9	<6	<6	<6
JNJ7777120	7.8	<5.0	<5.0	5.3
ZPL3893787	8.6			6.7
Thioperamide	6.9			7.3
UR-63325	7.8	<5.4	<5.4	<5.4

*Former VUF6002. Based on Andaloussi *et al.* (2013); Alfon *et al.* (2011); Coruzzi *et al.* (2007; 2011); Cowart *et al.* (2008); Leurs *et al.* (2009); Lim *et al.* (2009); Mowbray *et al.* (2011); Oda *et al.* (2000); Salcedo *et al.* (2013); Smits *et al.* (2009); Thurmond *et al.* (2004; 2014). Of note, ligand affinity may differ among species. Data presented as K_i value (nM) for the human histamine H_4 and H_3 receptors.

Since then, several new compounds have been developed targeting H_4 receptors such as 4-methylhistamine, VUF8430 and OUP-16 (selective agonists) and A-943931, JNJ7777120 and VUF6002 (selective antagonists; Table 1) (Leurs *et al.*, 2009; Smits *et al.*, 2009). However, it was recently demonstrated that in addition to inhibition of $G\alpha_{i/0}$ proteins, many H_4 receptor antagonists can also exert a partial agonist effect at certain species H_4 receptor orthologues via β-arrestin recruitment and ERK activation (Rosethorne and Charlton, 2011), which may contribute to some of the species differences that have been reported for H_4 receptor ligands (Liu *et al.*, 2001b; Seifert *et al.*, 2011; Nijmeijer *et al.*, 2013; Salcedo *et al.*, 2013).

Histamine and histamine receptors in the gastrointestinal tract

In the gastrointestinal tract, histamine participates in multiple physiological processes among which immunological responses, visceral nociception, modulation of intestinal motility and gastric acid secretion (Black *et al.*, 1972; Poli *et al.*, 2001; Dawicki and Marshall, 2007; Takagaki *et al.*, 2009; Simon *et al.*, 2011; van Diest *et al.*, 2012). Histamine is also involved in several gastrointestinal disorders such as inflammatory bowel diseases (IBD), irritable bowel syndrome (IBS), malignancies, systemic mastocytosis, food allergy and gastric ulcers (Black *et al.*, 1972; He, 2004; Wood, 2004; Barbara *et al.*, 2006; Sokol *et al.*, 2010; Kennedy *et al.*, 2012). All four histamine receptors are expressed in the gastrointestinal tract, although the presence of H_3 receptors in the human gut remains controversial (Poli *et al.*, 2001). Human H_1 receptors are abundantly expressed throughout the gastrointestinal tract on enterocytes as well as connective tissue cells, immune cells, blood vessels, myocytes and enteric nerves (Sander *et al.*, 2006). H_2 receptors are present on gastric parietal cells, enterocytes, immunocytes such as lymphocytes, myenteric ganglia and smooth muscle cells (Fukushima *et al.*, 1999; Sander *et al.*, 2006). H_3 receptors were reported to be expressed in gastrointestinal tissue of guinea pig and functional data located them on nerve terminals in the myenteric plexus and on pre- and post-ganglionic cholinergic and non-adrenergic, non-cholinergic fibres (Poli *et al.*, 2001). However, human intestine seems to be devoid of H_3 receptors (Hemedah *et al.*, 2001; Poli *et al.*, 2001; Cianchi *et al.*, 2005; Sander *et al.*, 2006).

Using a variety of techniques, several groups demonstrated expression of H_4 receptors throughout the gastrointestinal tract and in the pancreas, liver and bile ducts, not only in humans, but also in other species such as rodents, pigs, dogs and monkeys (Table 2). Sander *et al.* (2006) reported similar distribution of H_4 receptors along the human duodenum, colon, sigmoid and rectum. More specifically, H_4 receptors were present on lamina propria mononuclear cells and intestinal MCs, on leucocytes in mucosal and submucosal blood vessels and to a lesser extent on tissue resident leucocytes. In addition, H_4 receptor immunoreactivity was seen in intraepithelial cells considered to be neuroendocrine cells, in myenteric ganglion cell somata and neuronal fibres, and on enterocytes in the crypt of Lieberkühn (Sander *et al.*, 2006; Chazot *et al.*, 2007). Expression of H_4 receptors on colonic enterocytes was later confirmed by others, who also reported limited staining of non-specified submucosal and connective tissue cells (Boer *et al.*, 2008; Fang *et al.*, 2011). A caveat must be made when interpreting data obtained by immunohistochemistry. Recently the selectivity of commercially available H_4 receptor-antibodies was questioned as several of these antibodies failed to yield a specific signal when evaluated in transfected or H_4 receptor$^{-/-}$ cells (Beermann *et al.*, 2012).

Interestingly, gastrointestinal H_4 receptor expression is altered in several disease states. Decreased H_4 receptor expression was reported in gastric cancer specimens, whereas overexpression was demonstrated in cholangiocarcinoma and both enhanced and decreased expression levels have been reported in colorectal cancer (Cianchi *et al.*, 2005; Boer *et al.*, 2008; Fang *et al.*, 2011; Meng *et al.*, 2011; Francis *et al.*, 2012; Zhang *et al.*, 2012). Colonic inflammation seems to enhance H_4 receptor expression as in two experimental models of IBD, namely murine trinitrobenzene sulphonic acid (TNBS)-induced colitis and spontaneous colitis in $G_{i\alpha 2}$ protein-deficient mice, active inflammation was associated with an increase in colonic H_4 receptor mRNA (Sutton *et al.*, 2008; Kumawat *et al.*, 2010). Also after complete resolution of TNBS-colitis, colonic H_4 receptor mRNA levels remained increased (Deiteren *et al.*, 2014). However, in colonic biopsies of IBS patients with concomitant food allergy, no alterations in H_4 receptor mRNA levels were reported (Sander *et al.*, 2006).

H_4 receptors and gastrointestinal inflammation

Because of the high levels of expression of H_4 receptors on immunocytes, the immune modulatory potential of this receptor subtype attracted much attention, culminating in clinical trials with H_4 receptor antagonists in immune-mediated disorders such as asthma and allergic rhinitis. Also in the gastrointestinal tract, their immune modulatory properties have been studied using models of colitis, ischaemia/reperfusion injury and allergic gut reactions (Table 3).

MCs, an important source of gastrointestinal histamine, are key players of both the innate and adaptive immune systems and congregate at the interface between the internal and external milieu (such as the gut mucosa), where they exert immune modulatory effects. Alterations in MC numbers and activation state with excessive release of histamine have been reported in patients with IBD (Knutson *et al.*, 1990; Bischoff *et al.*, 1996; Farhadi *et al.*, 2007). Moreover, treatment with the MC stabilizer ketotifen prevented chemically induced colitis in animal models and improved disease activity in a small group of IBD patients; however, the underlying mechanism of action was not investigated further (Eliakim *et al.*, 1992; Jones *et al.*, 1998; Marshall and Irvine, 1998; Fogel *et al.*, 2005). Ketotifen stabilizes MCs (in addition to H_1 receptor antagonist properties), and thus inhibits the release of histamine in the gut; this may indirectly beneficially affect H_4 receptor-mediated pathways activated by histamine.

Table 2

Expression of H_4 receptors in the gastrointestinal tract of different species

Tissue and species	Technique	Expression profile	Reference
Oesophagus			
Guinea pig	Immunofluorescence	MCs and eosinophils	Yu *et al.* (2008)
Stomach			
Human	RNase protection assay		Liu *et al.* (2001a)
	Northern blot		Morse *et al.* (2001)
	RT-PCR	Mucosa	Zhang *et al.* (2012)
	Western blot	Mucosa	Zhang *et al.* (2012)
	Immunofluorescence	Mucosal cells	Zhang *et al.* (2012)
Rat	Immunohistochemistry	Ganglion cell somata and neuronal fibres in the myenteric but not the submucous plexus; A-like cells in the fundic epithelium	Chazot *et al.* (2007); Morini *et al.* (2008)
Duodenum			
Human	RT-PCR		Sander *et al.* (2006)
Small intestine			
Human	RNase protection assay		Liu *et al.* (2001a)
	Northern blot		Morse *et al.* (2001)
	RT-PCR		Coge *et al.* (2001); Nakamura *et al.* (2000); Oda *et al.* (2000)
Dog	RT-PCR		Jiang *et al.* (2008)
Rat	Immunohistochemistry	Ganglion cell somata and neuronal fibres in the myenteric plexus	Chazot *et al.* (2007)
Colon			
Human	RNase protection assay		Liu *et al.* (2001a)
	Northern blot		Morse *et al.* (2001)
	RT-PCR	Lamina propria mononuclear cells and MCs, mucosa	Boer *et al.* (2008); Cianchi *et al.* (2005); Fang *et al.* (2011); Oda *et al.* (2000); Sander *et al.* (2006)
	Western blot	Mucosa	Boer *et al.* (2008); Fang *et al.* (2011)
	Immunohistochemistry	Neuroendocrine-like cells, lamina propria, intravascular granulocytes, enterocytes, non-epithelial mucosal cells, submucosal connective tissue cells	Boer *et al.* (2008); Fang *et al.* (2011); Sander *et al.* (2006)
Dog	RT-PCR		Eisenschenk *et al.* (2011)
Rat	RT-PCR		Deiteren *et al.* (2014)
	Immunohistochemistry	Ganglion cell somata and neuronal fibres in the myenteric, but not the submucous plexus	Chazot *et al.* (2007)
Mouse	RT-PCR		Sutton *et al.* (2008)
Monkey	RT-PCR	Longitudinal muscle	Kim *et al.* (2011); Oda *et al.* (2005)
Pig	RT-PCR		Oda *et al.* (2002)
Pancreas			
Human	Northern blot		Morse *et al.* (2001)
Liver			
Human	RNase protection assay		Liu *et al.* (2001a)
	Northern blot		Morse *et al.* (2001)
	RT-PCR		Coge *et al.* (2001); Nakamura *et al.* (2000)
Dog	RT-PCR		Eisenschenk *et al.* (2011); Jiang *et al.* (2008)
Bile ducts			
Human	RT-PCR		Francis *et al.* (2012)
	Western blot		Francis *et al.* (2012)
	Immunohistochemistry	Cholangiocytes	Francis *et al.* (2012); Meng *et al.* (2011)

A caveat must be made when interpreting H_4 receptor expression data obtained by immunohistochemistry: recently the selectivity of commercially available antibodies for the H_4 receptor was questioned as several of these antibodies failed to yield a specific signal when evaluated in transfected or H_4 receptor $^{-/-}$ cells (Beermann *et al.*, 2012).

Table 3

Preclinical *in vivo* experiments with H_4 receptor ligands in models of inflammation

Model	Species	*In vitro/ in vivo*	Ligand	Effect	Ref
TNBS-induced colitis	Rat	*In vivo*	JNJ7777120 JNJ10191584	JNJ7777120 and JNJ10191584 reduced TNBS-induced colitis	Varga *et al.* (2005)
TNBS-induced colitis	Rat	*In vivo*	JNJ10191584	JNJ10191584 reduced TNBS-induced colitis	Dunford *et al.* (2006b)
TNBS-induced colitis	Rat	*In vivo*	Thioperamide	Thioperamide reduced TNBS-induced colitis	Fogel *et al.* (2007)
Acetic acid-induced colitis	Rat	*In vivo*	Thioperamide	Thioperamide reduced acetic acid-induced colitis	Fogel *et al.* (2005)
Ischaemia/reperfusion intestinal injury	Mouse	*In vivo*	Thioperamide	Thioperamide reduced reperfusion injury	Ghizzardi *et al.* (2009)
Ischaemia/reperfusion liver injury	Rat	*In vivo*	Dimaprit Clozapine	Histamine, dimaprit and clozapine reduced liver injury	Adachi *et al.* (2006)
			Thioperamide	Thioperamide reversed the protective effect of histamine and dimaprit	
Ischaemia/reperfusion liver injury	Rat	*In vivo*	Clozapine	Histamine and clozapine prevented reperfusion injury	El-Mahdy *et al.* (2013)
			Thioperamide	Thioperamide reversed the protective effect of histamine	
Radiation-induced small intestinal damage	Rat	*In vivo*	JNJ7777120	JNJ7777120 reduced radiation-induced intestinal damage	Martinel Lamas *et al.* (2013)
Allergen challenge in sensitized oesophagus	Guinea pig	*In vivo*	Thioperamide	Thioperamide inhibited MC and eosinophil migration	Yu *et al.* (2008)

Clozapine, H_3 and H_4 receptor agonist; dimaprit, H_2 and H_4 receptor agonist; JNJ10191584, H_4 receptor antagonist; JNJ7777120, H_4 receptor antagonist; thioperamide, H_3 and H_4 receptor antagonist.

The selective H_4 receptor antagonists JNJ7777120 and JNJ10191584 and the H_3/H_4 receptor antagonist thioperamide also reduced chemically induced colitis in different rat models for IBD (Fogel *et al.*, 2005; 2007; Varga *et al.*, 2005; Dunford *et al.*, 2006b). More specifically, treatment with these antagonists reduced macroscopic colonic injury, neutrophil influx and myeloperoxidase levels (a marker for myeloid cell infiltration) (Fogel *et al.*, 2005; 2007; Varga *et al.*, 2005; Dunford *et al.*, 2006b). This is in line with previous evidence demonstrating that blockade of H_4 receptors impedes neutrophil recruitment and cytokine release in other models of inflammation, such as zymosan-induced pleuritis and allergic airway inflammation (Takeshita *et al.*, 2003; Thurmond *et al.*, 2004; Dunford *et al.*, 2006a). The anti-inflammatory effect of H_4 receptor antagonism resulted – at least partly – from inhibition of aberrant Toll-like receptor signalling via dendritic cells leading to reduced production of TNF-α and IL-6 (Fogel *et al.*, 2005; Varga *et al.*, 2005; Dunford *et al.*, 2006b). In addition, colonic H_4 receptor expression was reported to be increased in the colon of mice with TNBS-induced colitis and during spontaneous colitis in $G\alpha_{i2}$ protein-deficient mice (Sutton *et al.*, 2008; Kumawat *et al.*, 2010). Whether H_4 receptor expression is also increased in IBD patients is an interesting question that has not been investigated to our knowledge.

Data have also emerged, suggesting a possible role for H_4 receptors in mediating gastrointestinal inflammation in ischaemia/reperfusion models. However, in most of these studies non-selective antagonists were used, making it difficult to ascertain that this effect was indeed solely mediated by the H_4 receptor subtype. In a mouse model of mesenteric ischaemia/reperfusion injury treatment with the H_3/H_4 receptor antagonist thioperamide significantly reduced myeloperoxidase activity (Ghizzardi *et al.*, 2009). In contrast, the opposite effect was seen on hepatic ischaemia/reperfusion damage: histamine, the H_2/H_4 receptor agonist dimaprit and the H_3/H_4 receptor agonist clozapine reduced post-ischemic liver damage, as shown by a reduction in serum transaminases (Adachi *et al.*, 2006). This protective effect was abolished by the H_3/H_4 receptor antagonist thioperamide but remained unaffected by the selective H_2 receptor antagonist cimetidine, suggesting a beneficial influence of H_4 receptor stimulation in the prevention of ischaemia/reperfusion liver damage. Recently, the mechanism of action was further elucidated by El-Mahdy *et al.* (2013). They found that liver damage was significantly reduced by pretreatment with histamine, remained unaffected by a selective H_1 or H_2 receptor antagonist, was abolished by the H_3/H_4 receptor antagonist thioperamide and was reproduced by the H_3/H_4 receptor agonist clozapine. The protective effect of histamine and clozapine was mediated by attenuating TNF-α and IL-12 secretion and consequently reduced reactive oxygen species (El-Mahdy *et al.*, 2013). As H_3 receptors were absent from adult mouse liver tissue (Heron *et al.*, 2001), it seems reasonable to assume that the protective effect of histamine and clozapine was indeed mediated by H_4 receptors. However, it is

important to exclude the possibility that hepatic ischaemia/reperfusion does not induce H_3 receptor expression to be sure that the effect is due to H_4 receptor modulation.

Pronounced gastrointestinal inflammation is seen after radiation and results from reactive oxygen/nitrate species, apoptosis and clonogenic cell death, mucosal breakdown and transcription of proinflammatory cytokines, chemokines and growth factors (Francois *et al.*, 2013). In view of the promising results of H_4 receptor blockade on gastrointestinal inflammation in other animal models, Martinel Lamas *et al.* (2013) evaluated the radioprotective potential of JNJ7777120, a selective H_4 receptor antagonist. Preventive treatment with JNJ7777120 preserved the villi and the number of crypts in the small intestine and diminished mucosal atrophy after radiation by reducing apoptosis and DNA damage in enterocytes (Martinel Lamas *et al.*, 2013).

Finally, preliminary evidence also points towards a possible involvement of H_4 receptors in allergic gut reactions (Yu *et al.*, 2008). Actively sensitized guinea pigs were exposed to inhaled 0.1% ovalbumin; MC and eosinophil infiltration into the oesophagus was assessed 1 h later. Pretreatment with the H_3/H_4 receptor antagonist thioperamide inhibited migration of both cell types to the oesophageal epithelium (Yu *et al.*, 2008). As both MCs and eosinophils did not express H_3 receptors, the effect was ascribed to blockade of H_4 receptors, which seems consistent with previous reports of H_4 receptor-mediated chemotaxis of these cell types (Hofstra *et al.*, 2003; Thurmond *et al.*, 2004; Yu *et al.*, 2008).

In conclusion, these *in vivo* experiments suggest that H_4 receptors participate in mediating gastrointestinal inflammation and immune responses in a variety of animal models. These findings are in line with previous preclinical observations from immune-mediated disorders in other organ systems and underline the immunomodulatory role of H_4 receptors. However, further research confirming these findings using highly selective ligands for H_4 receptors are much needed before clinical trials can be initiated for gastrointestinal inflammation and immune-mediated disorders.

H_4 receptors and carcinogenesis

Enhanced expression of L-histidine decarboxylase and high histamine producing and secreting capabilities have been reported in malignancies, such as melanoma, breast, colorectal and pancreatic carcinoma both in experimental models and in human tumour biopsies (Medina and Rivera, 2010; Kennedy *et al.*, 2012). Histamine, released by the malignant cells themselves or by other histamine-secreting cells in the environment such as MCs, acts as a growth factor in an autocrine or paracrine fashion, regulating angiogenesis, cell invasion, migration, differentiation, apoptosis and immune suppression (Medina and Rivera, 2010). These results suggest an important role for histamine in tumour development and progression. Histamine-induced cell proliferation seems to be mediated via H_2 receptors as antagonists for these receptors induced apoptosis in human colorectal and gastric cancer cell lines and in experimental models (Rajendra *et al.*, 2004; Jiang *et al.*, 2010). These findings culminated in clinical trials evaluating the effect of H_2 receptor-targeted therapy in colorectal cancer, indicating a beneficial effect when H_2 receptor antagonists were given as therapy, adjuvant to curative surgical resection (Deva and Jameson, 2012). Interestingly, H_2 receptor expression was comparable in colorectal cancer and adjacent normal mucosal specimens, whereas H_1 receptor and H_4 receptor expression were significantly reduced in tumour tissue (Boer *et al.*, 2008; Fang *et al.*, 2011). These findings suggest that carcinogenesis might benefit from loss of H_4 receptors (and H_1 receptors). A potential antiproliferative action of H_4 receptors in colorectal cancer was further substantiated by *in vitro* experiments demonstrating that stimulation of H_4 receptors induced a cell cycle arrest in the G1 phase via a cAMP-dependent pathway, resulting in reduced cell proliferation and tumour growth (Table 4) (Fang *et al.*, 2011). This antiproliferative action was only present in H_4 receptor-expressing colorectal cancer cell lines, but not in mock-transfected cells and could be prevented by pretreatment with the selective H_4 receptor antagonist JNJ7777120, further corroborating involvement of these receptors. In addition, H_4 receptor stimulation enhanced apoptosis induced by the chemotherapeutic agent 5-fluorouracil (Fang *et al.*, 2011). In contrast, Cianchi *et al.* (2005) found that H_4 receptor expression was increased in colorectal cancer specimens. Moreover, histamine-exposure stimulated cell proliferation and VEGF levels, which were reduced by the H_4 receptor antagonist JNJ7777120 (and the H_2 receptor antagonist cimetidine). This proliferative effect of H_4 receptor stimulation was mediated by COX 2-induced PGE_2 as it was only evident in those cell lines that expressed COX 2 (Cianchi *et al.*, 2005). In addition, JNJ7777120 only reduced histamine-induced cell proliferation, but did not affect basal (non-histamine stimulated) cell growth (Coruzzi *et al.*, 2012).

Attenuated H_4 receptor expression was reported in human gastric cancer specimens and was most prominent in advanced malignancies (Zhang *et al.*, 2012). Similarly to what was previously demonstrated in colorectal cancer, reduced H_4 receptor expression was linked to enhanced cell proliferation as H_4 receptor stimulation with clobenpropit and histamine reduced the growth of gastric cancer cells (Zhang *et al.*, 2012). Although neither ligand is an exclusive H_4 receptor agonist, the involvement of H_4 receptors was inferred from the fact that pretreatment with the selective H_4 receptor antagonist JNJ7777120 completely abolished agonist-induced responses (Zhang *et al.*, 2012). In line with this, clobenpropit reduced tumour cell proliferation in a pancreatic duct carcinoma cell line (Cricco *et al.*, 2008).

In contrast, H_4 receptor expression was enhanced in malignant cholangiocytes from patients with proven cholangiocarcinoma (Meng *et al.*, 2011). H_4 receptor stimulation with the H_3 receptor antagonist/H_4 receptor agonist clobenpropit dose-dependently reduced proliferation of several cholangiocarcinoma cell lines *in vitro* (Meng *et al.*, 2011). This cytostatic effect resulted from reduced growth potential and disruption of the invading capacity of the cells. As the effect of clobenpropit was maintained in *in vitro* experiments in which H_3 receptors were knocked down, this indicates that the effects were indeed mediated via H_4 receptors. Importantly, in an elegant *in vivo* design, the authors demonstrated the clinical potential of H_4 receptor-modulation in this tumour type as treatment with clobenpropit inhibited tumour growth and disrupted its invasive potential in a xenographic cholangiocarcinoma mouse model (Meng *et al.*,

Table 4

Preclinical *in vitro* and *in vivo* experiments with H_4 receptor ligands on carcinogenesis

Model	Species	*In vitro*/ *in vivo*	Ligand	Effect	Ref
Colorectal cancer cell line	Human	*In vitro*	Clozapine Clobenpropit JNJ7777120	Clozapine and clobenpropit reduced cell growth Clozapine enhanced 5-FU induced apoptosis, which was reversed by JNJ7777120	Fang *et al.* (2011)
Colorectal cancer cell line	Human	*In vitro*	JNJ7777120	JNJ7777120 prevented histamine-induced COX-2 expression/activity, cell proliferation and VEGF production	Cianchi *et al.* (2005)
Gastric cancer cell line	Human	*In vitro*	Clobenpropit JNJ7777120	JNJ7777120 abolished clobenpropit-induced cell growth	Zhang *et al.* (2012)
Pancreatic duct carcinoma cell line	Human	*In vitro*	Clobenpropit	Clobenpropit stimulation reduces cell growth	Cricco *et al.* (2008)
Cholangiocarcinoma cell line	Human	*In vitro*	Clobenpropit	Clobenpropit inhibited cell proliferation and metastatic potential	Meng *et al.* (2011)
Cholangiocarcinoma cell line	Human	*In vitro*	Thioperamide	No effect on histamine secretion and cell growth	Francis *et al.* (2012)
Xenograft cholangiocarcinoma	Mouse	*In vivo*	Clobenpropit	Clobenpropit inhibited tumour growth	Meng *et al.* (2011)

5-FU, 5-fluorouracil; clobenpropit, H_3 receptor antagonist, H_4 receptor agonist; clozapine, H_3 and H_4 receptor agonist; JNJ7777120, H_4 receptor antagonist; thioperamide, H_3 and H_4 receptor antagonist.

2011). However, in another study, inhibition of H_4 receptors by the H_3/H_4 receptor antagonist thioperamide did not affect cholangiocarcinoma cell line proliferation (Francis *et al.*, 2012).

Overall, these findings indicate that depending on the type of tumour (gastric vs. colorectal vs. cholangiocarcinoma) H_4 receptor-expression can either be decreased or enhanced. It is unclear whether H_4 receptor expression differs in early versus advanced stages, and this would be interesting to investigate further. In addition, although the data gathered from *in vitro* experiments using different cell lines strongly indicate that H_4 receptors can potently modulate tumour growth and progression, the results are not univocal. To complement these *in vitro* findings and increase our understanding of the role of H_4 receptors in gastrointestinal carcinogenesis, additional research and *in vivo* experiments using selective ligands seem crucial.

H_4 receptors and visceral sensory signalling

Visceral hypersensitivity refers to an enhanced perception of stimuli originating from the internal organs and is believed to contribute to abdominal pain in multiple gastrointestinal disorders among which IBD, IBS and functional dyspepsia (Vermeulen *et al.*, 2014). Sensitization of afferent nerve endings in the gut wall is thought to underlie visceral hypersensitivity (Anand *et al.*, 2007). Several lines of evidence indicate that histamine is involved in this process (Buhner and Schemann, 2012; van Diest *et al.*, 2012). For instance, supernatant from IBS colonic biopsies contains increased levels of histamine (Barbara *et al.*, 2007). When applied to human submucous neurons, this supernatant increased neuronal activity and the degree of activation correlated with histamine levels in the supernatant (Buhner *et al.*, 2009). In addition, histamine induced murine jejunal afferent firing and excited primary sensory neurons (Kreis *et al.*, 1998; Brunsden and Grundy, 1999). The pro-nociceptive effect of histamine seems to be mediated – at least partially – by H_1 receptors expressed on sensory afferents, which is consistent with the finding that excitation of rat jejunal afferents by IBS supernatant can be reduced by application of the H_1 receptor antagonist pyrilamine (Barbara *et al.*, 2007). In addition, a role for H_4 receptors in mediating visceral sensory signalling and nociception has emerged (Table 5).

Breunig *et al.* (2007) reported that the H_4 receptor agonist 4-methylhistamine excited human submucous plexus neurons, an effect that was inhibited by the selective H_4 receptor antagonist JNJ7777120. Also, *in vitro* jejunal afferent excitation by histamine was reversed by the H_3/H_4 receptor antagonist thioperamide (Brunsden and Grundy, 1999), although these results are in contrast to earlier reports in a similar set-up (Kreis *et al.*, 1998). Recently, our group provided *in vivo* evidence of reduced visceral nociception after blockade of H_4 receptors. Post-inflammatory visceral hypersensitivity was dose-dependently reduced by JNJ7777120 in a rat model of post-inflammatory IBS, without affecting visceral sensitivity in healthy controls (Deiteren *et al.*, 2014). Although increased colonic expression of H_4 receptor mRNA in hypersensitive rats points towards a peripheral mechanism of action, it remains to be determined whether the antinociceptive effect is mediated by blockade of H_4 receptors on sensory afferents directly or indirectly by modulation of H_4 receptors expressed elsewhere in the gut wall (Deiteren *et al.*,

Table 5

Preclinical *in vitro* and *in vivo* experiments with H_4 receptor ligands in visceral sensory signalling and nociception

Model	Species	*In vitro/ in vivo*	Ligand	Effect	Ref
Submucous plexus neurons	Human	*In vitro*	4-methylhistamine JNJ7777120	4-methylhistamine-induced excitation reduced by JNJ7777120	Breunig *et al.* (2007)
Jejunal afferent firing	Rat	*In vitro*	Thioperamide	Thioperamide reduced histamine-induced jejunal afferent firing	Brunsden and Grundy (1999)
Jejunal afferent firing	Rat	*In vivo*	Thioperamide	No effect on histamine-induced jejunal afferent firing	Kreis *et al.* (1998)
Post-inflammatory visceral hypersensitivity	Rat	*In vivo*	JNJ7777120	JNJ7777120 dose-dependently reversed visceral hypersensitivity	Deiteren *et al.* (2014)

4-methylhistamine, H_4 receptor agonist; JNJ7777120, H_4 receptor antagonist; thioperamide, H_3 and H_4 receptor antagonist.

Table 6

Preclinical *in vitro* experiments with H_4 receptor ligands in gastrointestinal contractility and transit

Model	Species	*In vitro/ in vivo*	Ligand	Effect	Ref
Submucous plexus neurons	Human	*In vitro*	4-methylhistamine JNJ7777120	4-methylhistamine-induced excitation reduced by JNJ7777120	Breunig *et al.* (2007)
Whole mount duodenum segments	Rat	*In vitro*	VUF8430	No effect on contractions	Pozzoli *et al.* (2009)
Longitudinal smooth muscle incl. myenteric plexus	Guinea pig	*In vitro*	Thioperamide	No effect on contractions induced by IBS supernatant	Balestra *et al.* (2012)
Colonic smooth muscle strips	Monkey	*In vitro*	4-methyl-histamine	4-methylhistamine increased contractile force	Kim *et al.* (2011)
Cultured small intestine interstitial cells of Cajal	Mouse	*In vitro*	4-methyl-histamine	No effect on pace maker potentials	Kim *et al.* (2013)

4-methylhistamine, H_4 receptor agonist; IBS, irritable bowel syndrome; JNJ7777120, H_4 receptor antagonist; thioperamide, H_3 and H_4 receptor antagonist; VUF8430, H_4 receptor agonist.

2014). Nevertheless, these findings coincide with previous reports of antinociceptive and analgesic effects of H_4 receptor antagonists in models of somatic and neuropathic pain (independent of their anti-inflammatory properties) (Coruzzi *et al.*, 2007; Hsieh *et al.*, 2010) and emphasize that H_4 receptors are also attractive targets in the modulation of visceral pain.

H_4 receptors and intestinal contractility

Histaminergic control of gastrointestinal contractility and motility is complex and involves all histamine receptor subtypes. H_1 receptors, located in smooth muscle cells, contribute to contractility by increasing calcium availability at the sarcoplasmic level whereas H_2 receptors mainly facilitate cholinergic and non-cholinergic excitatory transmission in intramural neurons (Poli *et al.*, 2001). Although H_3 receptors inhibit the release of excitatory and inhibitory neurotransmitters from the myenteric plexus, their involvement in enteric peristalsis remains unclear, as no effect of H_3 receptor ligands on gastrointestinal transit was seen in *in vivo* models and the presence of H_3 receptors in the human digestive tract remains controversial (Hemedah *et al.*, 2001; Poli *et al.*, 2001; Cianchi *et al.*, 2005; Sander *et al.*, 2006). Recently, H_4 receptors were reported to be present on murine myenteric neurons (Chazot *et al.*, 2007). In addition, 4-methylhistamine excited human submucous plexus neurons, which could be blocked by the H_4 receptor antagonist JNJ7777120 (Breunig *et al.*, 2007). As the enteric plexus in highly involved in the regulation of reflex behaviour, peristalsis and intestinal secretion, these findings suggest that H_4 receptors could be involved in gut motility and transit (Table 6). However, the H_4 receptor agonist VUF8430 did not affect twitch responses induced by electrical field stimulation in rat duodenum (Pozzoli *et al.*, 2009). In addition, no effect was seen from H_4 receptor stimulation on the membrane potential of murine small intestinal interstitial cells of Cajal, the enteric pacemaker cells and conductors of electrical slow waves in intestinal smooth muscle (Kim *et al.*, 2013). In a recent study, longitudinal smooth muscle preparations with

Table 7

Preclinical *in vivo* experiments with H_4 receptor ligands on gastric acid secretion and ulceration

Model	Species	*In vitro/ in vivo*	Ligand	Effect	Ref
Gastric acid secretion	Rat	*In vivo*	Dimaprit VUF8430	Dimaprit potently induced gastric secretion, whereas VUF8430 only marginally increased secretion	Lim *et al.* (2009)
			JNJ7777120	Induced gastric acid secretion was not affected by JNJ7777120	
Indomethacin/ bethanechol-induced gastric ulceration	Mouse	*In vivo*	JNJ7777120	JNJ7777120 reduces lesions in CD-1, NMRI and BALB/c, but not in C57BL/6J mice.	Adami *et al.* (2012; Coruzzi *et al.* (2009)
			VUF10460 VUF8430	No effect of VUF10460 and VUF8430 on gastric lesions	
				VUF10460 abolished the protective effect of JNJ7777120	
Indomethacin-induced gastric ulceration	Rat	*In vivo*	VUF5949 JNJ7777120	VUF5949 and JNJ7777120 reduced indomethacin-induced lesions	Adami *et al.* (2005); Coruzzi *et al.* (2009)
			VUF10460	VUF10460 reduced lesions	
			VUF8430	VUF8430 only reduced lesions in the presence of a H_2 receptor antagonist	
HCl-induced gastric ulceration	Rat	*In vivo*	Immepip VUF8430 VUF10460	Immepip, VUF8430 and VUF10460 enhanced HCl-induced gastric lesions	Coruzzi *et al.* (2011)
			JNJ7777120	JNJ7777120 abolished the effect of immepip, but not of VUF8430 and VUF10460	

Dimaprit, H_2 receptor agonist, H_4 receptor agonist; Immepip, H_3 and H_4 receptor agonist; JNJ7777120, H_4 receptor antagonist; VUF10460, H_4 receptor agonist; VUF5949, H_4 receptor antagonist; VUF8430, H_4 receptor agonist.

an intact myenteric plexus were harvested from guinea pig ileum and exposed to supernatants prepared from colonic biopsies from IBS patients. This supernatant enhanced cholinergic twitch contractions; however, the responses were not affected by a mixture containing antagonists for H_1–H_4 receptors (Balestra *et al.*, 2012). The H_4 receptor agonist 4-methylhistamine increased contractile forces only in longitudinal smooth muscle strips of monkey colon (Kim *et al.*, 2011). However, as these effects were only present when high doses were used, these results need to be interpreted with caution.

H_4 receptors and gastric acid secretion and ulceration

Histamine is a potent activator of the acid secreting cells of the stomach (Kopic and Geibel, 2010). Binding of histamine to basolateral H_4 receptors activates adenylyl cyclase resulting in accumulation of cAMP and H^+ secretion. Before the development of proton pump inhibitors, pharmacological blockade of H_2 receptors was the cornerstone of the treatment of acid-related gastrointestinal disorders (Kopic and Geibel, 2010). In addition to H_2 receptor antagonists, H_3 receptor stimulation also exerted gastroprotective effects via increased mucus production in animal models (Coruzzi *et al.*, 2001; Barocelli and Ballabeni, 2003). The homology between H_3 and H_4 receptors subsequently spurred interest in a possible role for H_4 receptors in gastric acid secretion (Table 7). Overall, the data gathered to date suggest that H_4 receptors do not participate in gastric acid secretion under physiological conditions as neither H_4 receptor agonists such as VUF8430 and VUF10460 nor H_4 receptor antagonists such as JNJ7777120 and VUF5949 affected basal acid production or the macroscopic appearance of the stomach (Lim *et al.*, 2009; Coruzzi *et al.*, 2011; Adami *et al.*, 2012). However, when the mucosal integrity was compromised such as in models of chemically induced gastric ulceration, damage was significantly enhanced by H_4 receptor stimulation and markedly reduced by its blockade (Adami *et al.*, 2005; 2012; Coruzzi *et al.*, 2009; 2011). In addition, enhanced chemically induced mucosal damage by H_4 receptor agonists could be prevented by concomitant H_4 receptor antagonists and *vice versa* (Coruzzi *et al.*, 2009; 2011). However, the findings are not fully consistent as the H_4 receptor agonists VUF8430 and VUF10460 had no effect on indomethacin/bethanecol-induced lesions in a mouse model whereas the HCl-induced damage in rats was enhanced by both agonists, and in contrast, indomethacin-induced ulcerations were reduced by VUF10460 (Coruzzi *et al.*, 2009; 2011; Adami *et al.*, 2012). It was hypothesized that species and strain differences might contribute to the differential effects as JNJ7777120 effectively reduced indomethacin/bethanecol-induced lesions in CD-1, NMRI and BALB/c, but not in C57BL/6J mice (Adami *et al.*, 2012). However, it should be kept in mind that several of the

compounds used also display considerable affinity for the H_3 receptor, such as VUF8430 [pK_i for rat H_4 receptors of 6.9 vs. 6.5 for rat H_3 receptors (Lim *et al.*, 2009); Table 1], again underscoring the need for selective H_4 receptor ligands. Although these data seem promising, more research is needed to further elucidate the effect of H_4 receptor modulation on gastric ulcer disease. If a beneficial effect of H_4 receptor blockade on gastric ulceration could be confirmed, this would be a major advantage in terms of drug development, as H_4 receptor antagonists are already under evaluation for their anti-inflammatory and analgesic properties.

Clinical development

To date, no clinical trials with H_4 receptor ligands have been initiated in the field of gastroenterology. However, several H_4 receptor antagonists have already progressed to phase II clinical trials for immune-mediated disorders such as rheumatoid arthritis, asthma, atopic dermatitis and allergic rhinitis (Table 8). Currently registered clinical trials (http://clinicaltrials.gov) include compounds from Johnson & Johnson (JNJ39758979 and JNJ38518168), Ziarco Pharma (ZPL3893787) and Palau Pharma (UR-63325).

JNJ39758979, derived from the H_4 receptor antagonist JNJ7777120, showed promising results in initial phase I trials, with good pharmacokinetics upon oral dosing with a plasma half-life of 124–157 h after a single oral dose (Thurmond *et al.*, 2014). In addition, the compound was well-tolerated up to 1200 mg in single ascending dose studies and up to 300 mg bid in a multiple ascending dose study; dose-dependent gastrointestinal symptoms were the main adverse events (abnormal faeces, nausea, vomiting and abdominal pain) (Thurmond *et al.*, 2014). A single dose of 600 mg effectively reduced histamine-induced itch in 23 healthy volunteers (Kollmeier *et al.*, 2014). However, a subsequent phase II trial in patients with atopic dermatitis was discontinued due to two cases of drug-induced agranulocytosis, leading to the termination of JNJ39758979 (Thurmond *et al.*, 2014). The agranulocytosis was reported to be related to the chemical structure of JNJ39758979 and not to the H_4 receptor antagonism (Kollmeier *et al.*, 2014; Liu, 2014; Thurmond *et al.*, 2014); further details are expected to be released in the near future (Thurmond *et al.*, 2014). Therefore, the development of other H_4 receptor antagonists is currently being pursued such as JNJ38518168, which has progressed to phase II for rheumatoid arthritis and asthma. However, one of these trials was terminated because of a single, unexpected serious event, which was not specified further. Details on the underlying mechanisms (H_4 receptor-related or compound-specific) are not yet available.

ZPL3893787 (former PF03893787) is a lead compound of Ziarco Pharma and successfully completed phase I single ascending dose and 14 days multiple ascending dose studies

Table 8

Current clinical trials with H_4 receptor ligands

Compound	Phase	Population	Status	Trial number
JNJ39758979	I	Healthy volunteers	Completed	NCT01081821
	I	Histamine-induced itch in healthy volunteers	Completed	NCT01068223
	I	Rheumatoid arthritis	Completed	NCT01442545
	II	Asthma	Completed	NCT00946569
	II	Atopic dermatitis	Terminated†	NCT01497119
	II	Rheumatoid arthritis	Terminated‡	NCT01480388
	II	Asthma	Withdrawn‡	NCT01493882
JNJ38518168	I	Healthy volunteers	Completed	NCT01442532
	I	Patients with normal or mild to moderate hepatic impairment	Completed	NCT01863784
	I	Healthy volunteers	Completed	NCT01970020
	I	Healthy volunteers on ketonazole	Completed	NCT01690286
	I	Rheumatoid arthritis	Completed	NCT01450982
	II	Rheumatoid arthritis	Recruiting	NCT01862224
	II	Rheumatoid arthritis	Active, not recruiting	NCT01679951
	II	Rheumatoid arthritis	Terminated*	NCT00941707
	II	Asthma	Recruiting	NCT01823016
ZPL3893787§	I	Healthy volunteers	Completed	NCT00992342
	I	Asthma	Completed	NCT00856687
UR63325	II	Allergic rhinitis	Completed	NCT01260753

*Terminated because of a single, unexpected serious event. †Terminated because of two cases of agranulocytosis. ‡Terminated/withdrawn because of cases of agranulocytosis in trial NCT01497119. §Former PF03893787. Clinical trials as registered on http://clinicaltrials.gov on 11 June 2014.

in healthy volunteers (Liu, 2014). No results have been published yet; however, Ziarco Pharma communicated on their website that the compound displayed an excellent pharmacokinetic and safety profile. Results from a subsequent proof of concept trial in patients with asthma have not yet been disclosed.

The Palau Pharma compound UR-63325 successfully completed single and multiple dose ascending studies demonstrating a linear pharmacokinetic profile and no safety concerns according to Salcedo *et al.* (2013); in addition, a phase II clinical trial in allergic rhinitis patients was recently completed and the data are eagerly awaited.

Conclusions

Since their discovery and cloning almost 15 years ago, knowledge on the role of H_4 receptors has increased rapidly. The expression of H_4 receptors on immune cells has spurred interest in H_4 receptor antagonists as a potential new class of anti-inflammatory drugs in the treatment of rheumatoid arthritis and asthma among others. Also in the gastrointestinal tract, there is now strong preclinical evidence that H_4 receptors modulate the inflammatory process, indicating that these receptors could be interesting new targets in the treatment of IBD, ischaemia/reperfusion injury, radiation-induced enteropathy and allergic intestinal reactions. It would be interesting to investigate whether genetic polymorphisms and copy gene number variations for H_4 receptors are linked to gastrointestinal inflammation as was previously reported for other immune-mediated disorders such asthma and atopic dermatitis (Yu *et al.*, 2010; Simon *et al.*, 2012; Chen *et al.*, 2013). In addition, recent data indicate that H_4 receptors also participate in carcinogenesis and gastric ulceration and in mediating IBS-like visceral pain. The preliminary data gathered so far seem promising, but the effects of pharmacological H_4 receptor modulation will need to be confirmed using highly selective ligands, that are devoid of biased signalling and are extensively evaluated in both *in vitro* and *in vivo* settings. In addition, the results of ongoing trials with H_4 receptor antagonists for immune-mediated disorders are eagerly awaited and will be crucial for the future of any therapy targeted at H_4 receptors.

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Conflicts of interest

The authors report no conflicts of interest.

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