Histamine H_4 receptors in the gastrointestinal tract

HISTAMINE H4 RECEPTORS IN THE GASTROINTESTINAL TRACT

Abbreviated title: H4 receptors in the gastrointestinal tract

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Abstract
Histamine is a well-established mediator implicated in a variety of physiological and pathophysiological mechanisms and exerts its effect through activation of four histamine receptors (H_1-H_4Rs). The histamine H_4R is the newest member of this histamine receptor family and is expressed throughout the gastrointestinal tract as well as in liver, pancreas and bile ducts. Functional studies using a combination of selective and non-selective H_4R ligands have rapidly increased our knowledge of H_4R involvement in gastrointestinal processes both under physiological conditions and in models of disease. Strong evidence points towards a role for H_4R in the modulation of immune-mediated responses in gut inflammation such as in colitis, ischemia/reperfusion injury, radiation-induced enteropathy and allergic gut reactions. In addition, data have emerged implicating H_4R in gastrointestinal cancerogenesis, sensory signalling and visceral pain as well as in gastric ulceration. These studies highlight the potential of H_4R targeted therapy in the treatment of various gastrointestinal disorders such as inflammatory bowel disease, irritable bowel syndrome and cancer.

Abbreviations
H_1R, histamine H_1 receptor; H_2R, histamine H_2 receptor; H_3R, histamine H_3 receptor; H_4R, histamine H_4 receptor; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; IL, interleukin; TNBS, trinitrobenzene sulphonic acid; TNF-α, tumour necrosis factor alpha.

Introduction

Histamine (2-[4-imidazole]-ethylamine) is a short-acting amine, involved in multiple physiological and pathophysiological processes (Jutel *et al.*, 2009). It is present in virtually all bodily organs, with high concentrations reported in 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 (Alcaniz *et al.*, 2013; Bencsath *et al.*, 1998; Jutel *et al.*, 2009; Snyder and Epps, 1968; Vanhala *et al.*, 1994). Histamine exerts its actions by binding to four G-protein coupled receptors that are differentially expressed throughout the body and designated as the H_1, H_2, H_3 and H_4 receptors (H_1-H_4Rs) in accord to the BJP Concise guide to pharmacology (Alexander *et al.*, 2013). H_1Rs 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 (Simons and Simons, 2011; Togias, 2003). H_2Rs are well-known for their role in gastric acid secretion but also exert immune modulatory properties (Black *et al.*, 1972; Jutel *et al.*, 2009). H_3Rs are most abundantly present in the central nervous system 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, H_4Rs 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_4R involvement in multiple gastrointestinal physiological and pathophysiological processes.

H_4Rs

In the early 2000s, several groups reported on the discovery and cloning of a fourth histamine receptor (Liu *et al.*, 2001a; Morse *et al.*, 2001; Nakamura *et al.*, 2000; Nguyen *et al.*, 2001; Oda *et al.*, 2000; Zhu *et al.*, 2001). The H_4R is encoded by a single copy on chromosome 18q11.2 and demonstrates an overall homology of 23% to H_1Rs, 22% to H_2Rs and 37% to H_3Rs (Coge *et al.*, 2001; Oda *et al.*, 2000). 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_4Rs 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 on 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 (Coge *et al.*, 2001; Nakamura *et al.*, 2000; Oda *et al.*, 2000; 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_4Rs (92%), whereas this is 72% between human and pig and 65-70% between human and rodent H_4Rs (Liu *et al.*, 2001b; Oda *et al.*, 2002, 2005). These differences in amino acid sequence also affect histamine's binding profile towards H_4Rs with high affinity for human and guinea pig H_4Rs (K_D 4.8 and 6 nM) compared to rat and mouse H_4Rs (136 and 42 nM) (Liu *et al.*, 2001b). Compared to H_1 and H_2Rs, histamine displays high affinity for H_4Rs in both human and rodents (table 1).

Soon after its discovery and cloning, attempts were made to elucidate the H_4R's pharmacological profile and identify (selective) ligands to stimulate or inhibit H_4R signalling. Early assessments indicated that several H_3R-ligands demonstrated significant affinity for

H_4Rs, such as clozapine, imetit and immepip (H_3 and H_4 agonists) and clobenpropit (H_3 antagonist, H_4 agonist) (table 1) (Leurs *et al.*, 2009; Smits *et al.*, 2009). Since then, several new compounds have been developed targeting H_4Rs 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, recently it was demonstrated that in addition to inhibition of $G_{\alpha i/0}$ proteins, many H_4R antagonist can also exert a partial agonist effect at certain species H_4R orthologs 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_4R ligands (Liu *et al.*, 2001b; Nijmeijer *et al.*, 2013; Salcedo *et al.*, 2013; Seifert *et al.*, 2011).

Table 1. Ligands for the human H_4R.

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

Data presented as K_i value (nM) for the human histamine H_4 and H_3 receptors. NA, data not available; * 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 between species.

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; Dawicki and Marshall, 2007; Poli *et al.*, 2001;

Simon *et al.*, 2011; Takagaki *et al.*, 2009; 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 (Barbara *et al.*, 2006; Black *et al.*, 1972; He, 2004; Kennedy *et al.*, 2012; Sokol *et al.*, 2010; Wood, 2004). All four histamine receptors are expressed in the gastrointestinal tract, although the presence of H_3Rs in the human gut remains controversial (Poli *et al.*, 2001). Human H_1Rs 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_2Rs 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_3Rs 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_3Rs (Cianchi *et al.*, 2005; Hemedah *et al.*, 2001; Poli *et al.*, 2001; Sander *et al.*, 2006).

Using a variety of techniques, several groups demonstrated expression of H_4Rs 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_4Rs along the human duodenum, colon, sigmoid and rectum. More specifically, H_4Rs 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_4R 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 (Chazot *et al.*, 2007; Sander *et al.*, 2006). H_4R-expression 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_4R-antibodies was questioned as several of these antibodies failed to yield a specific signal when evaluated in transfected or $H_4R^{-/-}$ cells (Beerman *et al.*, 2012).

Interestingly, gastrointestinal H_4R expression is altered in several disease states. Decreased H_4R 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 (Boer *et al.*, 2008; Cianchi *et al.*, 2005; Fang *et al.*, 2011; Francis *et al.*, 2012; Meng *et al.*, 2011; Zhang *et al.*, 2012). Colonic inflammation seems to enhance H_4R 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_4R mRNA (Kumawat *et al.*, 2010; Sutton *et al.*, 2008). Also after complete resolution of TNBS-colitis, colonic H_4R mRNA levels remained increased (Deiteren *et al.*, 2014). However, in colonic biopsies of IBS patients with concomitant food allergy no alterations in H_4R mRNA levels were reported (Sander *et al.*, 2006).

Table 2. Expression of H_4Rs in the gastrointestinal tract of different species.

Tissue and species	Technique	Expression profile	Ref
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)

MCs, mast cells; RT-PCR, reverse transcription-polymerase chain reaction. A caveat must be made when interpreting H_4R expression data obtained by immunohistochemistry: recently the selectivity of commercially available antibodies for the H_4R was questioned as several of these antibodies failed to yield a specific signal when evaluated in transfected or $H_4R^{-/-}$ cells (Beerman *et al.*, 2012).

H_4Rs and gastrointestinal inflammation

Due to high expression of H_4Rs on immunocytes, the immune modulatory potential of this receptor subtype attracted much attention, culminating in clinical trials with H_4R 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, ischemia/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 system 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 (Bischoff *et al.*, 1996; Farhadi *et al.*, 2007; Knutson *et al.*, 1990). 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; Fogel *et al.*, 2005; Jones *et al.*, 1998; Marshall and Irvine, 1998). Ketotifen stabilizes MCs (in addition to H1R antagonistic properties) and thus inhibits the release of histamine in the gut; this may indirectly beneficially affect H_4R-mediated pathways activated by histamine.

Table 3. Preclinical *in vivo* experiments with H_4R 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)
Ischemia/reperfusion intestinal injury	Mouse	In vivo	Thioperamide	Thioperamide reduced reperfusion injury	Ghizzardi *et al.* (2009)
Ischemia/reperfusion liver injury	Rat	In vivo	Dimaprit Clozapine Thioperamide	Histamine, dimaprit and clozapine reduced liver injury Thioperamide reversed the protective effect of histamine and dimaprit	Adachi *et al.* (2006)
Ischemia/reperfusion liver injury	Rat	In vivo	Clozapine Thioperamide	Histamine and clozapine prevented reperfusion injury Thioperamide reversed the protective effect of histamine	El-Mahdy *et al.* (2013)
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_4R agonist; dimaprit, H_2 and H_4R agonist; JNJ10191584, H_4R antagonist; JNJ7777120, H_4R antagonist; thioperamide, H_3 and H_4R antagonist; TNBS, trinitrobenzene sulphonic acid.

The selective H_4R antagonists JNJ7777120 and JNJ10191584 and the H_3/H_4R antagonist thioperamide also reduced chemically-induced colitis in different rat models for IBD (Dunford *et al.*, 2006b; Fogel *et al.*, 2007; 2005; Varga *et al.*, 2005). More specifically, treatment with these antagonists reduced macroscopic colonic injury, neutrophil influx and myeloperoxidase levels (a marker for myeloid cell infiltration) (Dunford *et al.*, 2006b; Fogel *et al.*, 2005, 2007; Varga *et al.*, 2005). This is in line with previous evidence demonstrating that blockade of H_4Rs impedes neutrophil recruitment and cytokine release in other models of inflammation, such as zymosan-induced pleuritis and allergic airway inflammation (Dunford *et al.*, 2006a; Takeshita *et al.*, 2003; Thurmond *et al.*, 2004). The anti-inflammatory effect of H_4R antagonism resulted – at least partially – from inhibition of aberrant toll-like receptor signalling via dendritic cells leading to reduced production of tumour necrosis factor alpha

(TNF-α) and interleukin-6 (IL-6) (Dunford *et al.*, 2006b; Fogel *et al.*, 2005; Varga *et al.*, 2005). In addition, colonic H_4R expression was reported to be increased in the colon of mice with TNBS-induced colitis and during spontaneous colitis in $G_{i\alpha 2}$ protein-deficient mice (Kumawat *et al.*, 2010; Sutton *et al.*, 2008). Whether the H_4R 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_4Rs in mediating gastrointestinal inflammation in ischemia/reperfusion models. However, in most of these studies non-selective antagonists were used, making it difficult to ascertain that this effect was indeed merely mediated by the H_4R subtype. In a mouse model of mesenteric ischemia/reperfusion injury treatment with the H_3/H_4R antagonist thioperamide significantly reduced myeloperoxidase activity (Ghizzardi *et al.*, 2009). In contrast, the opposite effect was seen on hepatic ischemia/reperfusion damage: histamine, the H_2/H_4R agonist dimaprit and the H_3/H_4R agonist clozapine reduced post-ischemic liver damage, evidenced by a reduction in serum transaminases (Adachi *et al.*, 2006). This protective effect was abolished by the H_3/H_4R antagonist thioperamide but remained unaffected by the selective H_2R antagonist cimetidine, suggesting a beneficial influence of H_4R stimulation in the prevention of ischemia/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 pre-treatment with histamine, remained unaffected by a selective H_1 or H_2R antagonist, was abolished by the H_3/H_4R antagonist thioperamide and was reproduced by the H_3/H_4R 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_3Rs were reported to be lacking in 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_4Rs. However, it is important to exclude that hepatic ischemia/reperfusion does not induce H_3R expression to ascertain that the effect is due to H_4R 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). Considering the promising results of H_4R blockade on gastrointestinal inflammation in other animal models, Martinel Lamas *et al.* (2013) evaluated the radioprotective potential of JNJ7777120, a selective H_4R 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_4Rs 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 1h later. Pre-treatment with the H_3/H_4R 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_3Rs, the effect was ascribed to blockade of H_4Rs, which seems consistent with previous reports of H_4R-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_4Rs 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 underscore the immunomodulatory role of H_4Rs. However, further research confirming these findings using highly selective H_4Rs are much needed before clinical trials can be initiated for gastrointestinal inflammation and immune-mediated disorders.

H_4Rs 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 (Kennedy *et al.*, 2012; Medina and Rivera, 2010). 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_2Rs as H_2R antagonists induced apoptosis in human colorectal and gastric cancer cell lines and in experimental models (Jiang *et al.*, 2010; Rajendra *et al.*, 2004). These findings culminated in clinical trials evaluating the effect of H_2R-targeted therapy in colorectal cancer, indicating a beneficial effect when H_2R antagonists were given adjuvant to curative surgical resection (Deva and Jameson, 2012). Interestingly, H_2R expression was comparable in colorectal cancer and adjacent normal mucosal specimens, whereas H_1R and H_4R expression were significantly reduced in tumorous tissue (Boer *et al.*, 2008; Fang *et al.*, 2011). These findings suggest that carcinogenesis might benefit from loss of H_4Rs (and H_1Rs). A potential antiproliferative action of H_4Rs in colorectal cancer was further substantiated by *in vitro* experiments demonstrating that stimulation of H_4Rs 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_4R-expressing colorectal cancer cell lines but not in mock-transfected cells and could be prevented by pre-treatment with the selective H_4R antagonist JNJ7777120, further corroborating H_4R involvement. In addition, H_4R stimulation enhanced apoptosis induced by the chemotherapeutic agent 5-fluorouracil (Fang *et al.*, 2011). In contrast, Cianchi *et al.* (2005) found that H_4R expression was increased in colorectal cancer specimens. Moreover, histamine-exposure stimulated cell proliferation and vascular endothelial growth factor levels which were reduced by the H_4R antagonist JNJ7777120 (and the H_2R antagonist cimetidine). This proliferative effect of H_4R stimulation was mediated by cyclooxygenase 2-induced prostaglandin E2 as it was only evident in those cell lines that expressed cyclooxygenase 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_4R 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_4R expression was linked to enhanced cell proliferation as H_4R stimulation with clobenpropit and histamine reduced the growth of gastric cancer cells (Zhang *et al.*, 2012). Although neither ligand is an exclusive H_4R agonist, the involvement of H_4Rs was inferred based on the fact that pre-treatment with the selective H_4R 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).

Table 4. Preclinical *in vitro* and *in vivo* experiments with H_4R 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-FT 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_3R antagonist, H_4R agonist; clozapine, H_3 and H_4R agonist; COX, cyclooxygenase; JNJ7777120, H_4R antagonist; thioperamide, H_3 and H_4R antagonist; VEGF, vascular endothelial growth factor.

In contrast, H_4R expression was enhanced in malignant cholangiocytes from patients with proven cholangiocarcinoma (Meng *et al.*, 2011). H_4R-stimulation with the H_3 antagonist/H_4 agonist clobenpropit dose-dependently reduced proliferation of several cholangiocarcinoma cell lines *in vitro* (Meng *et al.*, 2011). This tumorostatic effect resulted from reduced growth potential and disruption of the cells' invading capacities. As the effect of clobenpropit was maintained in *in vitro* experiments in which H_3Rs were knocked down, this indicates that the effects were indeed mediated via H_4Rs. Importantly, in an elegant *in vivo* design the authors demonstrated the clinical potential of H_4R-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.*, 2011). However in another study, inhibition of H_4Rs by the H_3/H_4 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 versus colorectal versus cholangiocarcinoma) H_4R-expression can be either decreased or enhanced. It is unclear whether H_4R 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_4Rs 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_4Rs in gastrointestinal carcinogenesis, additional research and *in vivo* experiments using selective ligands seem crucial.

H_4Rs 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 (Brunsden and Grundy, 1999; Kreis *et al.*, 1998). The pronociceptive effect of histamine seems to be mediated – at least partially – by H_1Rs 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_1R antagonist pyrilamine (Barbara *et al.*, 2007). In addition, a role for H_4Rs in mediating visceral sensory signalling and nociception has emerged (table 5).

Table 5. Preclinical *in vitro* and *in vivo* experiments with H_4R ligands in visceral sensory signalling and nociception.

Model	Species	In vitro/ in vivo	Ligand	Effect	Ref
Submucous plexus neurons	Human	In vitro	4-methyl-histamine 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_4R agonist; JNJ7777120, H_4R antagonist; thioperamide, H_3 and H_4R antagonist.

Breunig *et al.* (2007) reported that the H_4R agonist 4-methylhistamine excited human submucous plexus neurons, an effect that was inhibited by the selective H_4R antagonist JNJ7777120. Also *in vitro* jejunal afferent excitation by histamine was reversed by the H_3/H_4R antagonist thioperamide (Brunsden and Grundy, 1999) although these results could not be reproduced in a similar set-up (Kreis *et al.*, 1998). Recently our group provided *in vivo*

evidence of reduced visceral nociception after blockade of H_4Rs: 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_4R 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_4Rs on sensory afferents directly or indirectly by modulation of H_4Rs expressed elsewhere in the gut wall (Deiteren *et al.*, 2014). Nevertheless, these findings coincide with previous reports of antinociceptive and analgesic effects of H_4R antagonists in models of somatic and neuropathic pain (independent of their anti-inflammatory properties) (Coruzzi *et al.*, 2007; Hsieh *et al.*, 2010) and highlight that H_4Rs are also attractive targets in the modulation of visceral pain.

H_4Rs and intestinal contractility

Histaminergic control of gastrointestinal contractility and motility is complex and involves all histamine receptor subtypes. H_1Rs, located on smooth muscle cells, contribute to contractility by increasing calcium availability at the sarcoplasmic level whereas H_2Rs mainly facilitate cholinergic and non-cholinergic excitatory transmission in intramural neurons (Poli *et al.*, 2001). Although H_3Rs inhibit the release of excitatory and inhibitory neurotransmitters from the myenteric plexus, their involvement in enteric peristalsis remains unclear, as no effect of H_3R ligands on gastrointestinal transit was seen in *in vivo* models and the presence of H_3Rs in the human digestive tract remains controversial (Cianchi *et al.*, 2005; Hemedah *et al.*, 2001; Poli *et al.*, 2001; Sander *et al.*, 2006). Recently, H_4Rs 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_4R 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_4Rs could be involved in gut motility and transit (table 6). However, the H_4R 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_4R 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 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_4Rs (Balestra *et al.*, 2012). The H_4R 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.

Table 6. Preclinical *in vitro* experiments with H_4R 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)
Wholemount 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_4R agonist; IBS, irritable bowel syndrome; JNJ7777120, H_4R antagonist; thioperamide, H_3 and H_4R antagonist; VUF8430, H_4R agonist.

H_4Rs 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_2Rs activates adenylyl cyclase resulting in accumulation of cAMP and H^+ secretion. Before the development of proton pump inhibitors, pharmacologic H_2R-blockade was the cornerstone in the treatment of acid-related gastrointestinal disorders (Kopic and Geibel, 2010). In addition to H_2R antagonists, H_3R stimulation also exerts gastroprotective effects via increased mucus production in animal models (Barocelli and Ballabeni, 2003; Coruzzi *et al.*, 2001). The homology between H_3 and H_4Rs subsequently spurred interest in a possible role for H_4Rs in gastric acid secretion (table 7). Overall, the data gathered to date suggest that H_4Rs do not participate in gastric acid secretion under physiological conditions as neither H_4R agonists such as VUF8430 and VUF10460 nor H_4Rs antagonists such as JNJ7777120 and VUF5949 affected basal acid production or the stomach's macroscopic appearance (Adami *et al.*, 2012; Coruzzi *et al.*, 2011; Lim *et al.*, 2009). However, when the mucosal integrity is compromised such as in models of chemically-induced gastric ulceration, damage was significantly enhanced by H_4R-stimulation and markedly reduced by H_4R-blockade (Adami *et al.*, 2005; 2012; Coruzzi *et al.*, 2009, 2011). In addition, enhanced chemically-induced mucosal damage by H_4R agonists could be prevented by concomitant H_4R antagonists and vice versa (Coruzzi *et al.*, 2009, 2011). However, the findings are not fully consistent as the H_4R 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 (Adami *et al.*, 2012; Coruzzi *et al.*, 2009, 2011). 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_3R, such as VUF8430 (pK_i for rat H_4Rs of 6.9 versus 6.5 for rat

H_3Rs (Lim *et al.*, 2009); table 1), again underscoring the need for selective H_4R-ligands. Although these data seem promising, more research is needed to further elucidate the effect of H_4R-modulation on gastric ulcer disease. If a beneficial effect of H_4R-blockade on gastric ulceration could be confirmed, this would be a major advantage from a drug-developing point of view, considering H_4R antagonists are under evaluation for their anti-inflammatory and analgesic properties.

Table 7. Preclinical *in vivo* experiments with H_4R ligands on gastric acid secretion and ulceration.

Model	Species	In vitro/ in vivo	Ligand	Effect	Ref
Gastric acid secretion	Rat	In vivo	Dimaprit	Dimaprit potently induced gastric secretion, whereas	Lim *et al.* (2009)
			VUF8430	VUF8430 only marginally increased secretion	
			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	No effect of VUF10460 and	
			VUF8430	VUF8430 on gastric lesions	
				VUF10460 abolished the protective effect of JNJ7777120	
Indomethacin-induced gastric ulceration	Rat	In vivo	VUF5949	VUF5949 and JNJ7777120	Adami *et al.* (2005); Coruzzi *et al.* (2009)
			JNJ7777120	reduced indomethacin-induced lesions	
			VUF10460	VUF10460 reduced lesions	
			VUF8430	VUF8430 only reduced lesions in the presence of a H_2R antagonist	
HCl-induced gastric ulceration	Rat	In vivo	Immepip	Immepip, VUF8430 and	Coruzzi *et al.* (2011)
			VUF8430	VUF10460 enhanced HCl-induced gastric lesions	
			VUF10460		
			JNJ7777120	JNJ7777120 abolished the effect of immepip, but not of VUF8430 and VUF10460	

Dimaprit, H_2 agonist, H_4R agonist; Immepip, H_3 and H_4R agonist; JNJ7777120, H_4R antagonist; VUF10460, H_4R agonist; VUF5949, H_4R antagonist; VUF8430, H_4R agonist.

Clinical development

To date, no clinical trials with H_4R ligands have been initiated in the field of gastroenterology. However, several H_4R 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).

Table 8. Current clinical trials with H_4R 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

Clinical trials as registered on http://clinicaltrials.gov on June 11th 2014.* Terminated due to a single, unexpected serious event; [#] Terminated due to 2 cases of agranulocytosis; [§] Terminated/withdrawn due to 2 cases of agranulocytosis in trial NCT01497119; [&] former PF03893787.

JNJ39758979, derived from the H_4R antagonist JNJ7777120, showed promising results in initial phase I trials. JNJ39758979 exhibited good pharmacokinetics upon oral dosing with a plasma half-life of 124–157h 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_4R 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_4R 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 due to a single, unexpected serious event, which was not specified further. Details on the underlying mechanisms (H_4R 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 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_4Rs has increased rapidly. The expression of H_4Rs on immune cells has spurred interest in H_4R 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_4Rs modulate the inflammatory process, indicating that these receptors could be interesting new targets in the treatment of IBD, ischemia/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_4Rs are linked to gastrointestinal inflammation as was previously reported for other immune-mediated disorders such asthma and atopic dermatitis (Chen *et al.*, 2013; Simon *et al.*, 2012; Yu *et al.*, 2010). In addition, recent data indicate that H_4Rs 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_4R-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_4R antagonists for immune-mediated disorders are eagerly awaited and will be crucial for the future of H_4R targeted therapy.

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Conflicts of interest
The authors report no conflicts of interest.

Authorship contribution statement
A Deiteren reviewed the literature, designed and wrote the manuscript; J G De Man, P A Pelckmans and B Y De Winter revised the manuscript critically and approved the final version.

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Tables with captations

Table 1. Ligands for the human H_4R.

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

Data presented as K_i value (nM) for the human histamine H_4 and H_3 receptors. NA, data not available; * 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 between species.

Table 2. Expression of H_4Rs in the gastrointestinal tract of different species.

Tissue and species	Technique	Expression profile	Ref
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)

MCs, mast cells; RT-PCR, reverse transcription-polymerase chain reaction. A caveat must be made when interpreting H_4R expression data obtained by immunohistochemistry: recently the selectivity of commercially available antibodies for the H_4R was questioned as several of these antibodies failed to yield a specific signal when evaluated in transfected or $H_4R^{-/-}$ cells (Beerman *et al.*, 2012).

Table 3. Preclinical *in vivo* experiments with H_4R ligands in models of inflammation.

Model	Species	In vitro/ in vivo	Ligand	Effect	Ref
TNBS-induced colitis	Rat	In vivo	JNJ7777120 JNJ1091584	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)
Ischemia/reperfusion intestinal injury	Mouse	In vivo	Thioperamide	Thioperamide reduced reperfusion injury	Ghizzardi *et al.* (2009)
Ischemia/reperfusion liver injury	Rat	In vivo	Dimaprit Clozapine Thioperamide	Histamine, dimaprit and clozapine reduced liver injury Thioperamide reversed the protective effect of histamine and dimaprit	Adachi *et al.* (2006)
Ischemia/reperfusion liver injury	Rat	In vivo	Clozapine Thioperamide	Histamine and clozapine prevented reperfusion injury Thioperamide reversed the protective effect of histamine	El-Mahdy *et al.* (2013)
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_4R agonist; dimaprit, H_2 and H_4R agonist; JNJ10191584, H_4R antagonist; JNJ7777120, H_4R antagonist; thioperamide, H_3 and H_4R antagonist; TNBS, trinitrobenzene sulphonic acid.

Table 4. Preclinical *in vitro* and *in vivo* experiments with H_4R 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-FT 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_3R antagonist, H_4R agonist; clozapine, H_3 and H_4R agonist; COX, cyclooxygenase; JNJ7777120, H_4R antagonist; thioperamide, H_3 and H_4R antagonist; VEGF, vascular endothelial growth factor.

Table 5. Preclinical *in vitro* and *in vivo* experiments with H_4R ligands in visceral sensory signalling and nociception.

Model	Species	In vitro/ in vivo	Ligand	Effect	Ref
Submucous plexus neurons	Human	In vitro	4-methyl-histamine 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_4R agonist; JNJ7777120, H_4R antagonist; thioperamide, H_3 and H_4R antagonist.

Table 6. Preclinical *in vitro* experiments with H_4R 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)
Wholemount 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_4R agonist; IBS, irritable bowel syndrome; JNJ7777120, H_4R antagonist; thioperamide, H_3 and H_4R antagonist; VUF8430, H_4R agonist.

Table 7. Preclinical *in vivo* experiments with H_4R ligands on gastric acid secretion and ulceration

Model	Species	In vitro/ in vivo	Ligand	Effect	Ref
Gastric acid secretion	Rat	In vivo	Dimaprit VUF8430 JNJ7777120	Dimaprit potently induced gastric secretion, whereas VUF8430 only marginally increased secretion Induced gastric acid secretion was not affected by JNJ7777120	Lim *et al.* (2009)
Indomethacin/ bethanechol-induced gastric ulceration	Mouse	In vivo	JNJ7777120 VUF10460 VUF8430	JNJ7777120 reduces lesions in CD-1, NMRI and BALB/c, but not in C57BL/6J mice. No effect of VUF10460 and VUF8430 on gastric lesions VUF10460 abolished the protective effect of JNJ7777120	Adami *et al.* (2012; Coruzzi *et al.* (2009)
Indomethacin-induced gastric ulceration	Rat	In vivo	VUF5949 JNJ7777120 VUF10460 VUF8430	VUF5949 and JNJ7777120 reduced indomethacin-induced lesions VUF10460 reduced lesions VUF8430 only reduced lesions in the presence of a H_2R antagonist	Adami *et al.* (2005); Coruzzi *et al.* (2009)
HCl-induced gastric ulceration	Rat	In vivo	Immepip VUF8430 VUF10460 JNJ7777120	Immepip, VUF8430 and VUF10460 enhanced HCl-induced gastric lesions JNJ7777120 abolished the effect of immepip, but not of VUF8430 and VUF10460	Coruzzi *et al.* (2011)

Dimaprit, H_2 agonist, H_4R agonist; Immepip, H_3 and H_4R agonist; JNJ7777120, H_4R antagonist; VUF10460, H_4R agonist; VUF5949, H_4R antagonist; VUF8430, H_4R agonist.

Table 8. Current clinical trials with H_4R 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

Clinical trials as registered on http://clinicaltrials.gov on June 11th 2014.* Terminated due to a single, unexpected serious event; [#] Terminated due to 2 cases of agranulocytosis; [§] Terminated/withdrawn due to 2 cases of agranulocytosis in trial NCT01497119; [&] former PF03893787.