Histamine H_4 Receptor: A Novel Target for Inflammation Therapy

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Abstract: Histamine, a low molecular weight amine has been extensively studied for its various pharmacological profiles. Until recently histamine was thought to act on three receptors - H_1, H_2 and H_3. Merely a decade back, sequencing of human genome has revealed a new histamine receptor - H_4 receptor. This 390 amino acid sequenced receptor has around 38% homology with histamine H_3 receptor besides; the pharmacological profile of the protein is quite different from other histamine receptors. H_4 receptor is mainly expressed in mast cells and leukocytes and involves various physiological functions related to inflammation and allergy. Potent selective H_4 receptor agonists and antagonists have been synthesized and *in vivo* studies have indicated their action on H_4 receptor. In this review, structure, expression, homology sequence of H_4 receptor among the different species have been documented. Further, structure activity relationship (SAR) of H_4 and ligands on the basis of their nucleus has been discussed in depth. In addition, anti-inflammatory effects of H_4 receptor antagonists, with special emphasis to JNJ7777120, a selective H_4 receptor antagonist have been focused exhaustively.

Keywords: Agonist, antagonist, G protein-coupled receptor (GPCR), JNJ7777120, histamine H_4 receptor, inflammation, Structure activity relationship (SAR).

INTRODUCTION

Histamine (2-(1*H*-imidazol-4-yl)ethylamine, β-aminoethylimidazole), a biogenic monoamine, plays an important role in regulating many cellular functions of our body by activating G protein-coupled receptors (GPCRs) [1]. It is synthesized from *L*-histidine by histidine decarboxylase (EC 4.1.1.22) in particular cell types such as mast cells, basophils, enterochromaffin – like cells and neurons, and is degraded by diamino-oxidase (DAO) and histamine-*N*-methyl transferase (HNMT) [2]. Intradermal injection of histamine has been known to produce '*Triple response*'; localized red spot, flare and wheal. The initial red spot is due to direct vasodilating effect, flare is due to stimulation of axonal reflexes which leads to indirect vasodilation, and the wheal formation corresponds to the ability of histamine to increase capillary permeability (edema formation) [3]. It serves as a mediator in cell differentiation, embryonic development, neurotransmission, immunomodulation, gastric acid secretion, and in inflammation. Histamine also plays a role in the CNS to control sleep/wake cycles, appetite, learning and memory [4]. All these processes take place with the involvement of histamine receptors, of which four major types H_1R ($G\alpha_q$, Ca2+ influx), H_2R ($G\alpha_s$, increases in cAMP), H_3R ($G\alpha_{i/o}$ inhibition of cAMP) and H_4R ($G\alpha_{i/o}$, Ca2+ influx)) have so far been identified [5].

Human H_1R (histamine 1 receptor) is a 56-kDa protein consisting of 487 amino acids, and the genes encoding this H_1R are located on chromosome 3 [6]. Antihistamines (H_1R antagonists) are widely used in the treatment of allergy; their therapeutic effects on allergic rhinitis and urticaria are well-known. On the contrary, in some allergic diseases, for example in bronchial asthma, H_1R antagonists are not effective. Human H_2R (histamine 2 receptor) is a 40-kDa protein consists of 359 amino acids, and the genes encoding the H_2R are located on chromosome 5 [7]. H_2R antagonists are used in treating peptic ulcers, gastroesophageal reflux disease and gastrointestinal bleeding. Human H_3R (histamine 3 receptor) is a 49-kDa protein consists of 445 amino acids, and the H_3R gene has been mapped to chromosome 20 [8]. H_3R is believed to be the potential target for treating various diseases *viz*., sleep-wake disorder, epilepsy, obesity, depression, dementia, schizophrenia, Alzheimer's disease, attention-deficit hyperactivity disorder (ADHD) and neuropathic pain [9-11]. Human H_4R (histamine H_4 receptor) is a 44-kDa protein which consists of 390 amino acids, and the H_4R gene has been mapped to chromosome 18. H_4R, a novel member of this histamine receptor family, was cloned by several groups independently between 2000-2001 and initially it was named as GPRv53, GPCR105, SP9144 [12-16]. It is homologous with H_3R, but expressing different functions. H_4R occurs in eosinophils, mast cells, basophils, $CD^{8+}T$ cells and dendritic cells, and their expression in details has been discussed elsewhere in this article. H_4R mediated histamine-induced chemotaxis in mast cells and eosinophils could be blocked by selective H_4R antagonists [17]. It was also demonstrated that H_4R antagonists cause a significant inhibition of polymorphonuclear cell influx into the peritoneum or pleural cavity in zymosan-induced neutrophilic inflammation models [18]. Recently, it was found that H_4R is involved in the secretion of interleukin 16 (IL-16) from CD^{8+} T cells [19] and in chronic allergic conjunctivitis [20]. The expression patterns of H_4 receptor and their ability to modulate the function of inflammatory cells have suggested that, the H_4R antagonists alone or in combination with H_1R could become a

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1389-5575/11 $58.00+.00

new class of drugs to effectively treat the allergic diseases and inflammatory conditions in near future [21-24]. This review is to help the readers to gain knowledge on development of H_4 receptor, its expression, homology species variation, SAR of H_4 receptors' ligand, with special emphasis on anti-inflammatory effect of JNJ7777120.

The dog eared book entities, histamine and antihistamines were discovered nearly a century and 70 years ago, respectively. The first two histamine receptor genes cloned were H_1R [6] and H_2R [7]. The identification of the H_3R came into picture nearly a decade later [8]. The history of development of histamine from its discovery to the novel H_4R is shown in Fig. (**1**). After the discovery of Human H_4 receptor in 20^{th} century, development in this research area has literally exploded, producing an increasingly growing number of publications and patents. The number of patents in 2008 was 15, and in 2009, it was double the number of 2008. It shows the growing attention among the researchers to reach the mile stones of H_4R at the earliest (Fig. (**2**)).

H_4 RECEPTOR – A NOVEL MEMBER OF G PROTEIN-COUPLED RECEPTOR (GPCR) SUPER FAMILY

In 1994, Raible *et al.* has documented a novel histamine receptor expression in eosinophils that was a non-H_1, -H_2, -H_3R, after seeing the difference in the potency of histamine and (*R*)-α-methylhistamine (H_3R agonist) on calcium mobilization in human eosinophils [25]. (*R*)-α-methylhistamine was much less potent than histamine for the calcium mobilization, represented a significant difference between H_3R and eosinophil histamine receptor. The calcium mobilization could be blocked by H_3R antagonist thioperamide but not by the classic H_1R or H_2R antagonists. It was found that the coding sequence of SP9144 reported by Morse *et al.* [15] was identical to the structures documented by Oda *et al.* [12]. Histamine increases the concentration of intracellular calcium in HEK-293 cells transiently transfected with SP9144 and a chimeric G protein α-subunit ($G\alpha_{q/i1,2}$), and this effect was inhibited by H_3R antagonist. The same potency difference as earlier noted by Raible *et al.* was seen among the agonist, histamine and (*R*)-α-methylhistamine at SP9144. These results support that the SP9144 is a novel histamine H_4 receptor. Liu *et al.* has also encoded H_4 receptor and named as GPCR105 which was expressed primarily in bone marrow and eosinophils [14]. Subsequently, five other laboratories reported the same finding. These results support the occurrence of a novel histamine receptor, histamine H_4R [13,16,26-28].

With the currently available GPCR structures such as bovine rhodopsin [29], β1-adrenergic receptors [30], β2-adrenergic receptors [31], several homology GPCR models have been developed [32, 33]. These homology models can be utilized for the structure based virtual screening to identify the novel agonists and antagonists. Histamine is one of the natural ligands for the aminergic GPCR which is an important subfamily of GPCRs. Histamine H_4R, a homologous GPCR have been developed by using the sequence information from the human H_3 receptor.

The H_4R shares the common properties of GPCRs: an Asp^{61} in the TM2 and a DRY motif at the end of TM3 (Asp^{111}-Tyr^{113}), which are essential for receptor activation; an Asp^{94} in TM 3, which is the putative binding site for the primary amine; a putative disulfide bridge between the first (Cys^{87}) and the second (Cys^{164}) extracellular loops; Trp^{140} in TM4 and Try^{316} in TM6; Pro^{186} in TM5 and Pro^{318} in TM6; an Asn^{350} and an NPXXY motif (Asn^{354}-Tyr^{358}) in TM7; and a potential palmitoylation site in the C-terminal region (Cys^{374}) [34-36].

H4R, a 390 amino acid residue comprises of three exons: 1–64, 65–119 and 120–390. H_4R and H_3R are being encoded by the same gene which present on the chromosome 18q11.2, spans >20.6 kb, and shares a similar intron–exon arrangement [28]. Further, existence of several H_4R isoforms

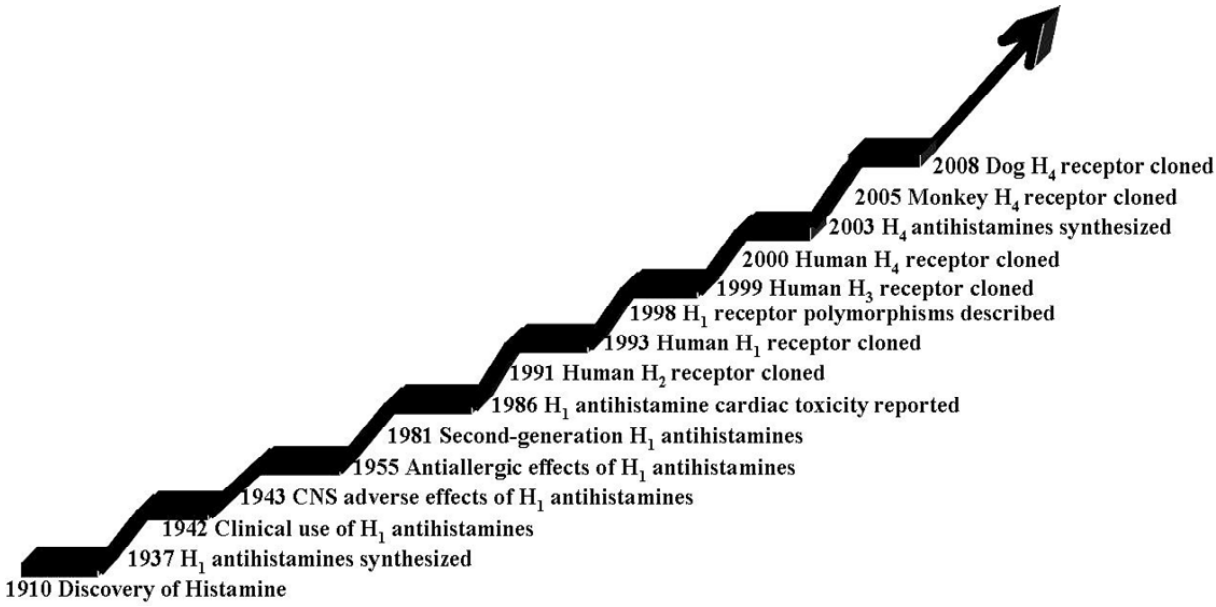


Fig. (1). History of development in Histamine research.

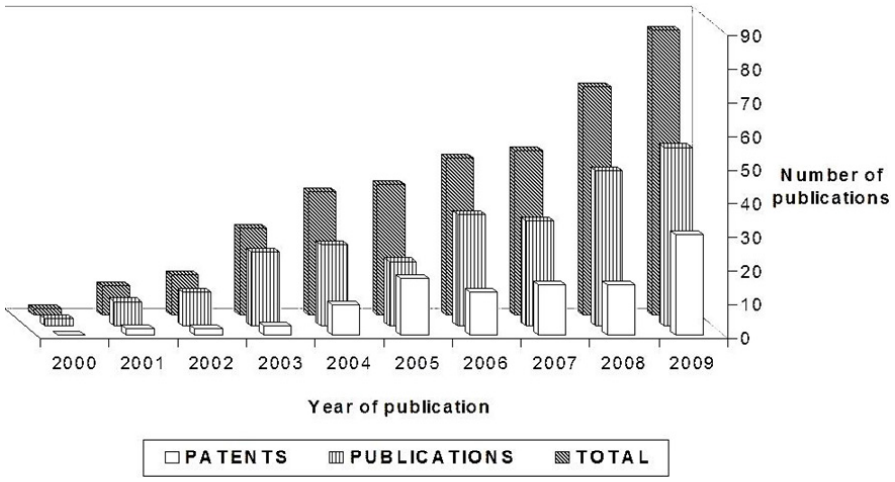


Fig. (2). Advancement in the field of histamine H_4 receptor during the last decade (2000-2009). The data was obtained from SciFinder Scholar™ 2007 by entering the term "Histamine H_4 receptor" in October 2010.

have been anticipated out of the resemblance between the organization of the gene that encodes the H_4R and the gene that encodes the H_3R in humans. This fact is supported by recent research that H_4R plays a negligible role in the inhibition of Ag-specific cytokine production after conducting the experiment with H_4R antagonist, adenylate cyclase inhibitor (SQ22536), phosphor kinase A (PKA) inhibitor (RP-8-Br-cAMPS). Further, an unidentified HR or receptor subtype can mediate the inhibition of antigen- induced cellular responses *via* a cAMP/PKA-dependent, apoptotic pathway [37]. Two spliced H_4R isoforms, $H_4R_{(302)}$ and $H_4R_{(67)}$ have been reported from CD34+ cord blood cell- derived eosinophils and mast cells and are hetero-oligomerize with $H_4R_{(390)}$ [H_4R isoform of 390 amino acids]. The $H_4R_{(302)}$ isoform formed when deletion of 88 amino acids between TM2 and TM4. $H_4R_{(67)}$ isoform contains the first 67 amino acids of H_4R. However, these two variants were unable to activate the $G_{i/o}$-coupled signaling pathway and fail to bind with H_4R ligand. But H_4R splice variants have a dominant negative effect on the surface expression of $H_4R_{(390)}$ when co-expressed with full-length H_4R. A patent (WO 03/020907 A2) registered by Merck in March 2003 illustrates the discovery of two human H_4R splice variants, $H_{4b}R$ and $H_{4c}R$, cloned from human spleen cDNA [38].

HISTAMINE-BINDING SITE OF THE HISTAMINE H_4 RECEPTOR

Molecular modeling of H_4R suggests that three important interactions between the H_4R and histamine.

1. Asp^{94} (3.32) in TM3 of the H_4R interacts with cationic amine moiety of histamine by an ion pair, which plays a critical role in agonistic binding and receptor activation.

2. Thr^{178} (5.42) and/or Ser^{179} (5.43) in TM5 forms a hydrogen bond with the imidazole N^{π} nitrogen where as an ion pair is formed between the Glu^{182} (5.46) in TM5 and protonated imidazole N^{τ} nitrogen of histamine. Asp^{186} (5.42) of the H_2 receptor having same interaction as Glu^{182} (5.46) in TM5 of H_4R does. Although similar interactions came into manifestation, histamine must adopt a different orientation in these two receptors. In contrast, histamine binds to the H_1 and H_4 receptors in the same orientation; so the reason for this difference in affinity is the different scale of interaction between the receptors and histamine. The hydrogen bond forms with Asn^{198} (5.46) of H_1 receptor where as ion pair with Glu^{182} (5.46) of the H_4R. Glutamic acid has a potential interaction with protonated nitrogen of the imidazole moiety of histamine when compared with the asparagine, it might be responsible for the increased binding affinity for H_4R compared to H_1R. Site specific mutation studies suggest that in H_4R, Thr^{178} (5.42) and/or Ser^{179} (5.43) do not play an essential role in histamine binding or signaling, because substitution of Ala at these two sites, alone or in combination retains the capacity to mediate the histamine induced signals.

3. Asn^{147} (4.57) in TM4 and Ser^{320} (6.52) in TM6 is also important for histamine binding. An interaction with the above two residues seems to have the potential role in guiding histamine in to the binding site. Asn^{147} (4.57) in TM4 of H_4R is occupied by bulkier residue Tyr, Trp, and Phe in H_1, H_2, and H_3 receptors, respectively. A Phe residue is found in the H_1 and H_2 receptor, instead of Ser^{320} (6.52) in TM6 of the H_4 receptor. These distinctions might be the reason for the difference in the binding affinity among different types of histamine receptors [39,40].

The binding mode analysis and enrichment studies on homology models of the human histamine H4 receptor, however, suggest that Glu^{182} (5.46) interacts with ethylamine part, Asp^{94} (3.32) with the imidazole N(3)-H, and Thr^{323} (6.55) with the imidazole N(1) of histamine. On the other hand, the role of Thr^{323} (6.55) in histamine binding warrants more studies to support this interaction [41].

Binding Mode of 1-[(1*H*-indol-2-yl)carbonyl]-4-methylpiperazine, a H_4R Antagonist

Molecular dynamic studies revealed that an electrostatic interaction is formed between the positively charged termi-

nal amino moiety of the piperazine and Asp^{94}(3.32) in TM3. Carbonyl group and NH of the indole moiety interacted with Glu^{182} (5.46) in TM5 and forms a bivalent connection. A hydrophobic interaction is established between indole moiety and Trp^{265} (6.48) in TM6. The recent QSAR study on compound with different substituents at 5-postion predicted molar volume is the main property to determine the efficacy of the compounds than the descriptors such as logP, polar surface area, molar refractivity, refraction index, and polarizabilty [42]. It was found recently that electronic and partition properties should be considered while designing the H_4R antagonist containing indole, benzimidazole piperazine carboxamide moiety [43]. These QSAR studies showed contradictory results on logP value and further studies need to confirm the role of partition properties.

Binding Mode of a Selective H_4R Antagonist, JNJ7777120 (1-[(5-chloro-1*H*-indol-2-yl)carbonyl]-4-methylpiperazine)

Two H-bonds or ionic interactions with Asp^{94} (3.32) and Glu^{182} (5.46) were anticipated for JNJ7777120, since it possesses two H-bond donors similar to histamine. Flexi- Dock study has revealed that the indole and the piperazine part of JNJ7777120 interacts with Asp^{94} (3.32) and Glu^{182} (5.46), respectively, as histamine does. After energy minimization, these two interactions were intact in contrast to FlexX model where the expected interactions disappeared. JNJ7777120, in this Flexi- Dock model, has showed to form lipophilic interactions with Val^{64} (2.53), Phe^{312} (6.44), Trp^{316} (6.48), Tyr^{319} (6.51) and Trp^{348} (7.43), nevertheless, JNJ7777120 is unable to show interaction with Thr^{323} (6.55) in TM6 [44]. In the pseudoreceptor model of human H_4R, JNJ7777120, a H_4R antagonist showed different interactions with the receptor. The terminal amino moiety of the piperazine interacted with Glu^{182} (5.46). Hydrogen bonds were noted between the Cys^{98} (3.36) to amide oxygen and Gln^{360} (7.42) to indole nitrogen. Trp^{329} (6.48) and Phe^{183} (5.47) exhibited aromatic interaction with the H_4R antagonist [45]. The differences in the binding mode of *1-[(1H-indol-2-yl)carbonyl]-4-methylpiperazine* and *JNJ7777120* ought to be the differences in simulation, docking protocol, and different hH4R models like crystal structure of bovine rhodopsin and of hβ2R.

SPECIES VARIATIONS OF THE H_4R

Sequence identity of H4 receptor with H_1, H_2R and H_3 is 23%, 22% and 54%, respectively [12,16]. The cloning of the human H_4R was followed by the cloning of H_4R from Monkey [46], dog [47], mouse, rat, guinea-pig [48] and porcine [49] were cloned, and the later were found to share 65–72% sequence homology with human. Human and monkey H_4R, have the equal number of amino acids (390), shares about 93% homology in their primary structure, known to be the highest homology among different species. Porcine H_4R shares 72% homology with human. The dog (*Canis familiaris*) H_4R has a 61–71% homology with the receptors of all other species, with a maximum homology to the human receptor. H_4R of human and guinea-pig have high affinity, 5nM for histamine, against the 136 nM of that of rat, so the rat H_4R ought to have less sensitive to other H_4R ligands also [27]. The significant species differences between human, monkey, pig, guinea pig, rat and mouse and dog is indicated in Fig. (**3**). These findings suggest that choosing a suitable animal model is important to validate H_4R as a therapeutic drug candidate [48, 49]. Chimeric receptor approach suggests that Phe^{169}, an aminoacid in the second extracellular loop of the human histamine H_4 receptor, is responsible for the difference in affinity between the human and mouse H_4 receptors. The mutant receptor obtained as a result of mutation of Phe^{169} of human H_4R into corresponding residue of mouse H_4R, Val^{171} acts like the mouse H_4R [50].

H_4 RECEPTOR EXPRESSION

Cells that clearly express functional H4R are natural killer cells, monocytes, mast cells, eosinophils, basophils, dendritic cells, T lymphocytes, tonsil B cells and T cells [15,16,51,52]. There are a few reports that indicate weak expressions of H_4R in the human brain [14, 28] and absence of H4R in the brain of rats, mice and guinea-pigs [48]. Recently, to our surprise, high levels of H_4 mRNA were detected in human spinal cord which exceeded the expression found in spleen and liver tissues that are initially thought to express relatively high levels of H_4 mRNA. In human CNS, the order of expression of H_4 mRNA is spinal cord, hippocampus and cerebellum, followed by other brain regions, whereas that of rat CNS is cortex and cerebellum, followed by brain stem. Very low levels of expression were detected in rat hypothalamus, and almost no signal was detected in its hippocampus [53]. Very recently the presence of H_4R in motor neurons of mouse spinal cord has been documented by using a novel anti-mouse H_4R antibody [54]. Both synoviocytes of the superficial layer membrane of synovium and synovial villi express H_4R in rheumatoid and osteoarthritic patients, but the incidence of expression was lower in osteoarthritic patients when compared to rheumatoid patients [55-57]. Mast cells present in the synovium are the major sources of histamine [58]. Table **1** summarizes the presence

Human	100						
Monkey	93	100					
Pig	72	72	100				
Guinea Pig	65	64	62	100			
Rat	69	68	67	61	100		
Mouse	68	67	66	62	84	100	
Dog	71	71	71	61	64	65	100
	Human	Monkey	Pig	Guinea Pig	Rat	Mouse	Dog

Fig. (3). Amino acid homology (%) of histamine H_4R amongst the different species. Reproduced from Ref. [47] with permission.

and absences of H_4R expression in Human, Porcine, Dog, Monkey, Rat, Mouse and Guinea pig.

IN VIVO PROPERTIES OF H_4 RECEPTOR

H_4 receptor antagonism caused significant inhibition of polymorphonuclear cell influx into the peritoneum and pleural cavity in MC-dependent mouse zymosan-induced neutrophilia models [59,60]. Human $CD4^+$ T cells expressed H_4R played an important role in allergic airway inflammation and allergic disease like atopic dermatitis. The expression was more in T_H2 than T_H1 and naive T-cells [61]. Decreased allergic lung inflammation with decreased infiltrating lung eosinophils and lymphocytes, and decreased T_H2 responses were reported in H_4R-deficient mice and mice treated with H_4R antagonists [62]. Monocyte-derived inflammatory dendritic epidermal cells (Mo-IDECs) reported to express H_4R and upregulated by IFN-γ. The level of T_H2-linked chemokine CCL2 and the T_H1 cytokine IL-12 downregulated by histamine and H_4R agonists clobenpropit, 4-methylhistamine on Mo-IDEC [63]. The expression of H_1R and H_4R is elevated significantly in human nasal polyp tissue while the level of H_2R and H_3R is not increased significantly. The correlation between the level of eosinophil cationic protein (ECP) and the H_4R expressions might involve in the eosinophil accumulation and activation of inflammatory diseases of the nasal and paranasal sinus mucosa, such as nasal polyposis [64].

STRUCTURE ACTIVITY RELATIONSHIP (SAR) OF H_4R AGONISTS:

SAR of Histamine Derivatives

Methyl substitution in α, β, N^{α}- position of histamine **1** side chain has decreased their affinity towards H_4R and retained their nanomolar potency at the H_3R. (±)-α,β-dimehtylhistamine **2** is a potent and highly selective H_3R agonist. The binding of the chiral α-branched ligand has exhibited a marked stereoselectivity at the H_3R and H_4R. In all cases, the enantiomers with a configuration as of *L*-histidine were preferred. (*R*)-α-methylhistamine **3** was 17-fold more potent than (*S*)-α-methylhistamine **4**. (*R*)-α-methylhistamine was about 60-fold less potent at the H_4R than the H_3R showed that the methylation of the side chain of the histamine is detrimental for H_4R affinity. Methyl substitution in the imidazole ring has resulted in increased affinity towards H_4R than H_3R. 4- Methylhistamine **5** is a potent H_4R agonist [65].

Cyclopropane Based Conformationally Restricted Analogs of 4-Methylhistamine

(*R*)-CEIC **6** has been found that it binds non-selectively to H_3R (K_i = 8.4 nM) and H_4R (K_i = 7.6 nM). Introduction of methyl group at 5'-position of imidazole nucleus of (*R*)-CEIC has resulted in compound **7** showing decreased potency for both H_3R and H_4R when compared with the parent compound (*R*)-CEIC. However, compound **7** is more selective to H_3R when compared to H_4R. Reduction of one carbon between the cyclopropane ring and the terminal nitrogen at 4-position of the imidazole nucleus resulting compound **8** has not shown any binding affinity to both H_3R and H_4R [66].

SAR of N^G-Acylated Imidazolyl Propyl Guanidines

Combination of compound **9**, SK&F91486 (partial agonist for both H_3 and H_4R) and JNJ7777120 (H_4R antagonist) has formed the compound **10**. Acylation of guanidine group in SK&F91486 with indole-3-alkanoyl or indole-2-carbonyl moieties has failed to improve hH_4R selectivity. Acylation of guanidine group in SK&F91486 with small alkanoyl groups like methyl, ethyl, *n*-propyl, *iso*-propyl has led to increased selectivity to H_4R over H_3R and acted as full agonist at H_4R [67].

Table 1. Tissue Expression of H_4R in Monkey, Dog, Rat, Mouse, Guinea Pig, Porcine and Human

Species	Presence	Absence	Ref.
Monkey	Colon, Spleen, Adrenal gland, Testis, and Bone marrow	CNS, GIT (except colon), Liver, Lung, Kidney, Pancreas, Heart, Trachea, Thymus, Skeletal muscle, Blood veins	[46]
Dog	Bone marrow, Lung, Spleen, Heart, Liver, Skeletal muscle, Small intestine, Trachea	Brain and Kidney	[47]
Rat	Spleen, Bone marrow, Cerebellum, cortex, thalamus, amygdala, striatum	Kidney, Liver, Lung, Brain, Heart, Skeletal muscle, Hippocampus,	[48]
Mouse	Spleen, Bone marrow	Kidney, Liver, Lung, Brain, Heart, Skeletal muscle	[48]
Guinea Pig	Spleen, Bone marrow	Kidney, Liver, Lung, Brain, Heart, Skeletal muscle	[48]
Porcine	Lung, Spleen and Colon	Heart, Brain, Liver, Kidney, Prostate	[49]
Human	Spinal cord, Cerebellum, Hippocampus, cortex, thalamus, amygdala, GIT, Heart, Kidney, Liver, Lung, Pancreas, Skeletal muscle, Leukocyte, Prostate, Small intestine, Spleen, Testis, Bone marrow, Fetal liver, and Lymph node, thymus, colon, stomach, nasal mucosa, synovium, synovial villi	Colon, Ovary, Prostate, Thymus, Tonsil, Cerebral cortex or in raphe nuclei.	[26, 28, 55-57]

SAR of Cyanoguanidine Type

Substitution of acyl guanidine group with cyanoguanidine in compound **11**, UR-AK51 has resulted in compound **12** which has 50-fold decreased potency when compared to parent compound. Increasing, decreasing the carbon chain, replacement of phenyl ring with cyclohexyl, increasing the number of phenyl ring (diphenylpropyl residue) has been detrimental for H_4R affinity. H_4R agonistic potency could not be increased by any modification in the phenylpropyl portion. Decreasing the carbon chain length between the imidazole ring and cyanoguanidine group from 3 to 2 has led to decreased H_4R affinity when compared to parent compound. In contrast, by increasing the carbon chain length from 3 to 4 resulted in compound **13** with 5-fold higher potency at the H_4R. Further elongation to a 5-membered carbon chain has led to decreased H_4R agonism. Phenylpropyl portion and 4-membered carbon chain between the imidazole and cyanoguanidine group are essential for H_4R agonistic activity. Bioisosteric replacement of methylene in compound **13** with sulfur atom has led to compound **14**, UR-PI376 considerable increment in H_4R affinity and acts as a most potent human H_4R agonist [68].

SAR of Clobenpropit Analogs

Substitution of *p*-chlorophenyl of Clobenpropit **15** with phenyl group has held on its mixed ligand property but more selective for H_3R than H_4R. The replacement of isothiourea group by a guanidine moiety has favored for H_4R affinity than H_3R. When the carbon spacer between the isothiourea and the phenyl moiety has been increased to 2, 3, 4, the resulting compounds were found to be detrimental to both H_3R and H_4R affinity. Substitution of *p*-chlorophenyl with benzyl substituted compounds and phenethyl substituted compounds has sustained its mixed ligand effect. However, phenethyl substituted compounds have lower affinity to both H_3R and H_4R when compared to benzyl substituted compounds. The replacement of chlorine atom in clobenpropit **15** (partial agonist, α H_4R = 0.83) with iodine atom has out-come with compound **16** which shows full agonist at H_4R (α H_4R = 0.98). It has been shown that introduction of an additional chlorine atom at 3-position of clobenpropit as seen in compound 17 has produced increment in affinity towards H_4R and behaved like a full H_4R agonist (α H_4R = 1) [69].

SAR of Nonimidazole H_4R Agonist

Ethylene carbon spacer between the isothiourea and guanidine group as in compound **18** (VUF 8430) was found to be optimum for H_4R agonistic activity. Increasing the carbon spacer from 2 to 3, 4 and 6 has directed to dramatic decrease in affinity (pK_i = 5.1 ± 0.1, 5.5 ± 0.1, 5.4 ± 0.1 respectively). The two chemically basic moieties isothiourea and guanidine has been essential for agonistic activity, whereas, two isothiourea groups (pK_i = 6.6 ± 0.1) or two guanidine groups (pK_i = 6.4 ± 0.1) has resulted in almost 10-fold decreased affinity [70].

SAR of Dibenzodiazepine Derivatives (Clozapine)

Replacement of nitrogen in 1-position of clozapine **19** (pK_i = 6.75 ± 0.1) by a sulfur atom/ methylamine/ carbon atom has led to decreased H_4R affinity. Substitution of nitrogen in 1-position with an oxygen atom leads to compound **20** with 4-fold increase in affinity to H_4R (pK_i = 7.37 ± 0.1). Any modification like removal of methyl group, increasing the length of substitution on the distal nitrogen atom, addition of piperidine or morpholine in the piperazine ring has led to decreased H_4R affinity. Lipophilic substituent (chlorine atom) on the left aromatic ring was found to be essential for H_4R affinity. Removal or replacement of chlorine atom with methyl group has brought decreased affinity towards H_4R. Changing the position of the chlorine atom from 8- to 7-positon (VUF 6884) has caused slight increase in H_4R affinity (pK_i = 7.55 ± 0.1) and was found to have about 5-fold higher affinity for H_1R (pK_i = 8.11 ± 0.1) than for the H_4R, on the other hand, it is 330-times more selective for the H_4R over the H_3R (pK_i = 5.04 ± 0.14). Addition of halogen atom like chlorine, fluorine at 2-, 3-, or 4-position of the right aromatic ring has not offered any compounds with more potent activity towards H_4R than the unsubstituted one [71].

STRUCTURE ACTIVITY RELATIONSHIP (SAR) OF H_4R ANTAGONIST

SAR of 2-Aminopyrimidine

Pyrrole moiety of **21** is replaced with an amino group and methyl substitution at piperazine moiety leading to compound **22** was found to be a moderately potent partial agonist in the rat H_4R (pEC_{50} = 7.17) and a potent H_4 antagonist in the human H_4R (pKb = 8.35) and having 30-fold increased potency than compound **21**. Compound 23 obtained by replacing the lipophilic *t*-Butyl group in compound **22** with 4-CN-phenyl group was also found to be moderately potent partial agonist at the rat H_4R (pEC_{50} = 7.23) and a potent antagonist at the human H_4R (pK_b = 8.53). Compound **23** losses its potency when nitrogen at the 1-position was replaced with –CH. So nitrogen at position 1 in the ring is essential for maintaining the potency.

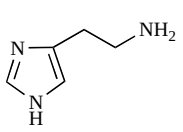
SAR of 4th Position

Any modification in the 4-positon of the parent compound **21** has caused loss of potency. Replacement of either nitrogen with carbon has led to 300- to 1500-fold loss of potency implies that piperazine nucleus is required for its activity. Removing the *N*-methyl group from the piperazine moiety resulted in 2- to 3-fold loss of activity. Further, replacement of *N*-methyl group in the piperazine moiety with larger alkyl groups, additional amines, diamines, oxygen-linked amines has resulted in reduction of potency.

SAR of 2nd Position

The replacement of the NH_2 group with a hydrogen atom has brought a 10-fold loss of potency with respect to the parent compound **22**. Again, the replacement of the NH_2 group with Cl, OH, OCH_3 or methylation, dimethylation of NH_2 group was uncovered to be detrimental to the H_4 receptor activity. 6-position is preferred for modification over 5-position since, 6-Ph analog showed nanomolar potency whereas 5-Ph analog showed micro molar potency against H_4R. Addition of cyano group at 4' position of the phenyl ring has led to slight increase in H_4R affinity than the unsubstituted one [72].

Histamine analogs



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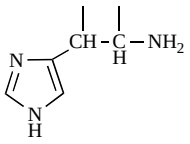

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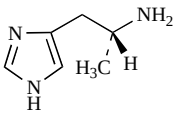

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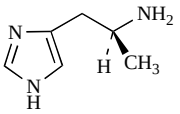

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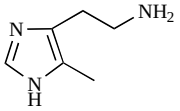

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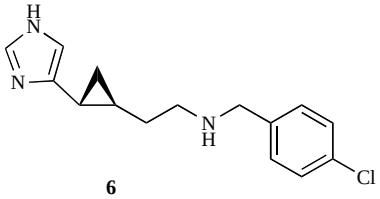
Cyclopropane based restricted analogs of histamine



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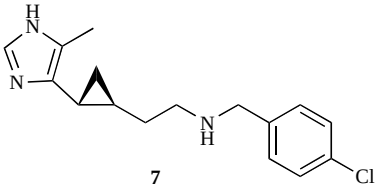

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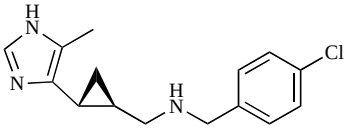

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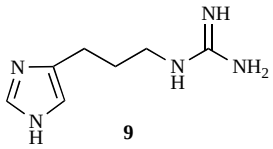
Imidazolepropylguanidine analogs



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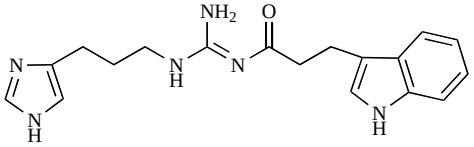

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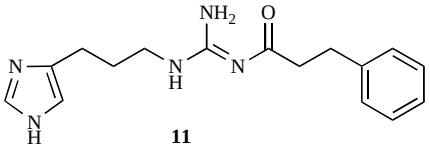

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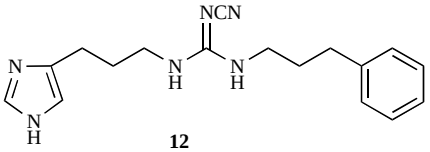

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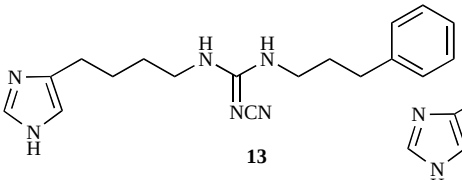

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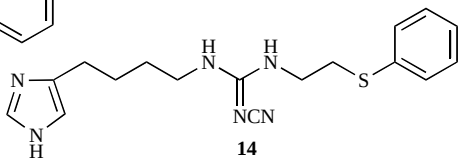

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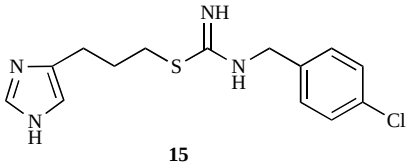
UR-PI376

Clobenpropit analogs



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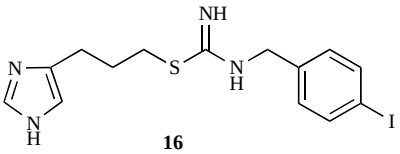

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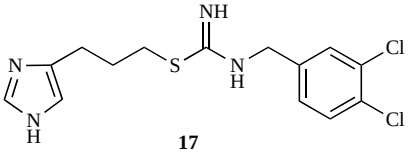

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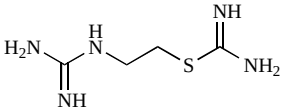
Dibenzodiazepine derivatives

Structure of VUF8430



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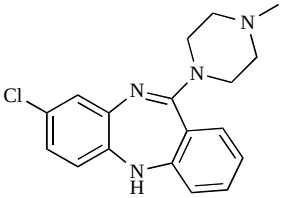

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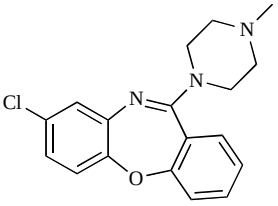
Clozapine



20

Structure 1. Structure Activity Relationships of H_4R agonists.

SAR of 2,4-Diaminopyrimidines

Ligand-based virtual screening followed by scaffold optimization has led to identification of *N*-benzyl-6-(4-methylpiperazin-1-yl)- pyrimidin-4-amine **24** having affinity to human H_4R in the submicromolar concentration range (K_i = 0.417 μM). Introduction of amino group in compound **24** at 2-position has brought compound **25** having more affinity towards H_4R (K_i = 0.098 μM). Removal of benzyl group in compound **25** has led to **26** with slight increase in hH_4R affinity (K_i = 0.290 μM). 4-amino group in compound **25** has acted as a hydrogen donor which is important for receptor interactions. Substitution of 4-amino group with 'O' or 'S' hetero atom has led to a 25- and 140-fold loss of activity respectively. Addition of chlorine atom at 4-positon of the aromatic ring of **25**, the resulted compound retained its H_4R affinity. Derivatives of 2-chloro, 2-mehtyl or 4-fluoro have showed more affinity towards H_4R in nanomolar concentration when compared to non-substituted compounds. 4-methoxy, 4-hydroxy, 3,4-dichloro, 4-trifluoromethyl, 4-*tert*-butyl, or 4-*iso*-propyl compounds have showed decreased binding affinity [73,74].

2,4-Diamino-5,6-Disubstituted Pyrimidines

It was found that the structural rigidification leads to increased oral bioavailability, drug likeness, selectivity for the molecular target, and decreased off-target affinity. Rigidification of structure **27** has given the compound **28** and its methylpiperazine derivatives were found to be more antagonistic in human H_4R. Increasing or decreasing the length of the rigidification ring has led to compounds **29** and **30** respectively having more potent activity than the six membered rings. Addition of fluorine at 10-position of compound **29** has brought compound **31** with increased affinity to H_4R. Replacement of piperazine moiety of **29** with diamines like (3*R*)-3-aminopyrrolidine, and 3-(*R*)-methyl amino azetidine has resulted in compounds **32** (A-943931) and **33** having more potent than the piperazine moiety. Further SAR studies with different substitutions on the rigidified 2-aminopyrimidine has resulted in compounds with an alpha-spiro moiety which is more potent than the corresponding alpha-substituted or alpha-gem-disubstituted analogs. These compounds were shown to reduce H_4 agonist (clobenpropit)-induced itch in mice model [75]. It was found that compound **34** *cis*-4-(Piperazin-1-yl)-5,6,7a,8,9,10,11,11a-octahydrobenzofuro[2,3-*h*]quinazolin-2-amine (A-987306), a H_4R antagonist showed anti-inflammatory activity in a peritonitis model and reduction of pain in the carrageenan induced thermal hyperalgesia model [76].

SAR of Indole and Benzimidazole Piperazines

Methylation of piperazine nitrogen in **35** has led to **36** with increased binding affinity towards H_4 receptor (K_i = 17 nM). Increasing number of carbon chain in the piperazine nitrogen has resulted in decreased affinity, for ex. N-ethyl analogue (K_i = 260 nM) and phenethyl analogue (K_i = 7000 nM). Amide linkage is essential for activity, but substitution of –C=O with $–CH_2$ was found to be detrimental (K_i = 10000 nM) for antagonistic activity. *C*-methyl substitution on the piperazine ring has brought decreased affinity. Addition of methyl at N-1 position of indole nucleus has led to devoid of activity (K_i = > 10000 nM). Halogen substitution (chlorine) in compound **36** at 5-position has increased the affinity of compound **37**, JNJ7777120 (K_i = 4 nM). Substitution of chlorine atom with bromine (pK_i = 7.5) or iodine (pK_i = 7.2) has resulted in mild to moderate decreased H_4R affinity. Addition of bromine atom at 5-position (K_i = 8 ± 1 nM) is favorable for H_4R affinity when compared to position 4, 6 or 7. Substitution of methyl, trifluoromethyl, methoxy, hydroxyl, or amino group at 5-positon has retained its affinity except the methoxy and trifluoromethyl group. The order of binding affinity of substituents at 5-position is as follows: Cl > Br ~ F > CH_3 ~ NH_2 ~ H >> OCH_3 ~ CF_3. Once the 5-position is filled then the next priority goes to 4- or 7-position. Substitution of CH_3, Cl, or NH_2 at 7-position has retained or shown slightly increase in affinity than its 5-substituted compounds. Disubstituted derivatives (4,5-position and 5,7-position) has retained and/or increased receptor activity.

Replacement of piperazine ring by ethylene diamine has yielded low affinity compound **38**. Increasing the number of carbon atoms in the space between the two nitrogen atoms (propylenediamine) has resulted incomplete loss of H_4R affinity. Introduction of amino piperazine in the place of piperazine has resulted in more than 1000-fold decrease in affinity for the H_4R. Further modification of piperazine ring with ethylenediamine, aminopiperidine, dimethylamino phenyl, or aetylamino compounds has yielded compounds with low or decreased H_4R affinity. Replacement of Indole ring with benzimidazole **39** (VUF6002, pK_i = 7.1) has led to a slight decrease in H_4R affinity. However, di-substituted 5-fluoro-4-methylbenzimidazole (K_i = 7 ± 3 nM) has lent more receptor affinity than the corresponding Indole derivatives (K_i = 27 ± 1 nM). The replacement of benzene portion of indole ring with thiophene has resulted in two regioisomers (head to head, head to tail) compounds **40** and **41** that do not appear to have more affinity towards H_4R than the indole moiety [77-79].

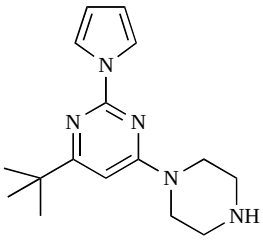
SAR of 2-Arylbenzimidazole

High throughput screening (HTS) has given the compound **42** with moderate H_4R affinity, K_i = 124nM. For a potent H_4R binding, the optimum length between the aryl ring and distal nitrogen of the terminal piperazine should be above 8.3 A°. Several constrained analogs have failed to improve the H_4R affinity when the alkyl linker has been replaced with a benzene ring, an alkyne, a *trans*-alkene, *cis*-alkene, or a benzofuran. Addition of small lipophilic substituents like chloro atom on the central aromatic ring have retained or improved H_4R binding affinity. However, dimethyl substitution on the central aromatic ring have led to a complete loss of H_4R affinity. Mono-substitution on the aromatic ring allows the alkyl ether linker to lie in the plane of the central ring, enabling potent receptor affinity, where as di-substitution allows twisting the linker in to an orthogonal position, which has led to compounds with poor receptor affinity. Isosteric replacement of *N*-methylpiperazine with *N*-methyl homopiperazine has led to compounds **43** and **44** with a slight increase in H_4R affinity [80].

SAR of Quinoxaline Analogs

Introduction of methyl group in compound **45** at 3-position has brought compound **46** with an almost 10-fold increase in H_4R affinity (pK_i = 6.70 ± 0.02 μM) when

2-Aminipyrimidine analogs



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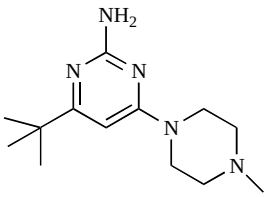

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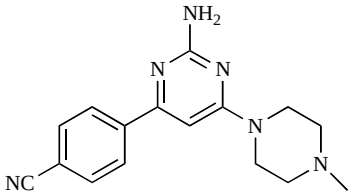

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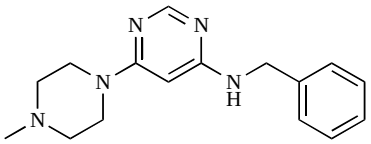
2,4-Diaminopyrimidine analogs



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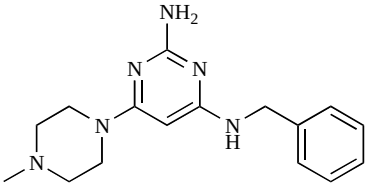

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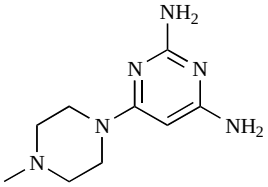

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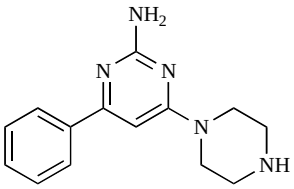
2,4-Diamino-5,6-disubstituted Pyrimidine analogs



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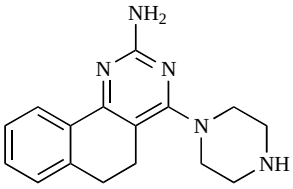

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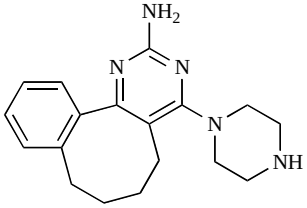

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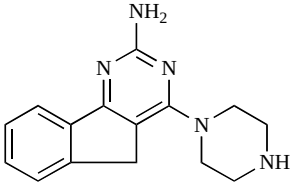

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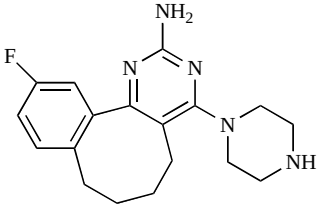

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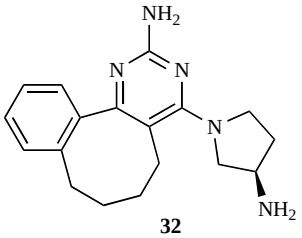

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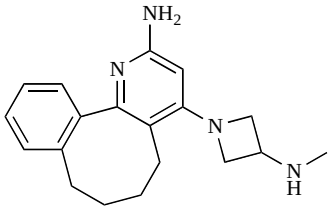
A-943931



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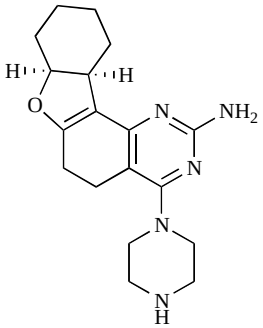
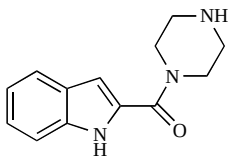


34

A-987306

Structure 2. Structure Activity Relationships of H_4R antagonists.

Indole and benzimidazole piperazine analogs



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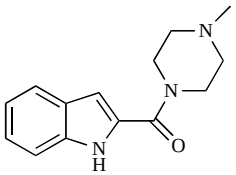

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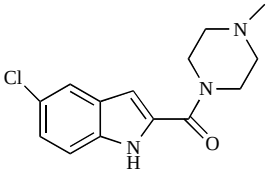

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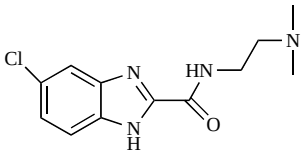

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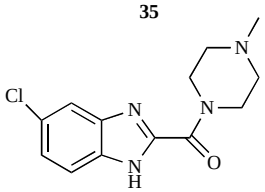

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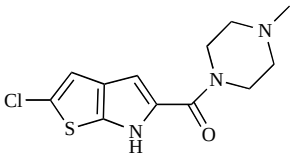

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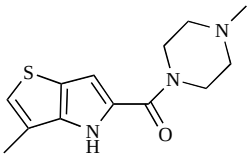

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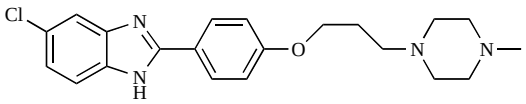
2-arylbenzimidazole analogs



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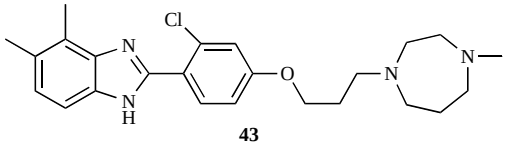

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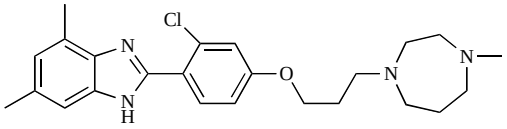
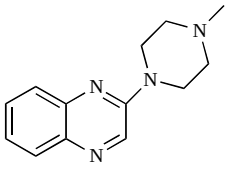


44

Quinoxaline analogs



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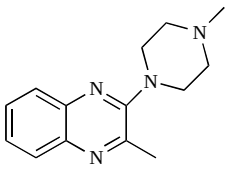

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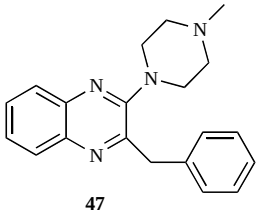

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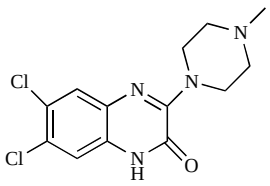
VUF10148



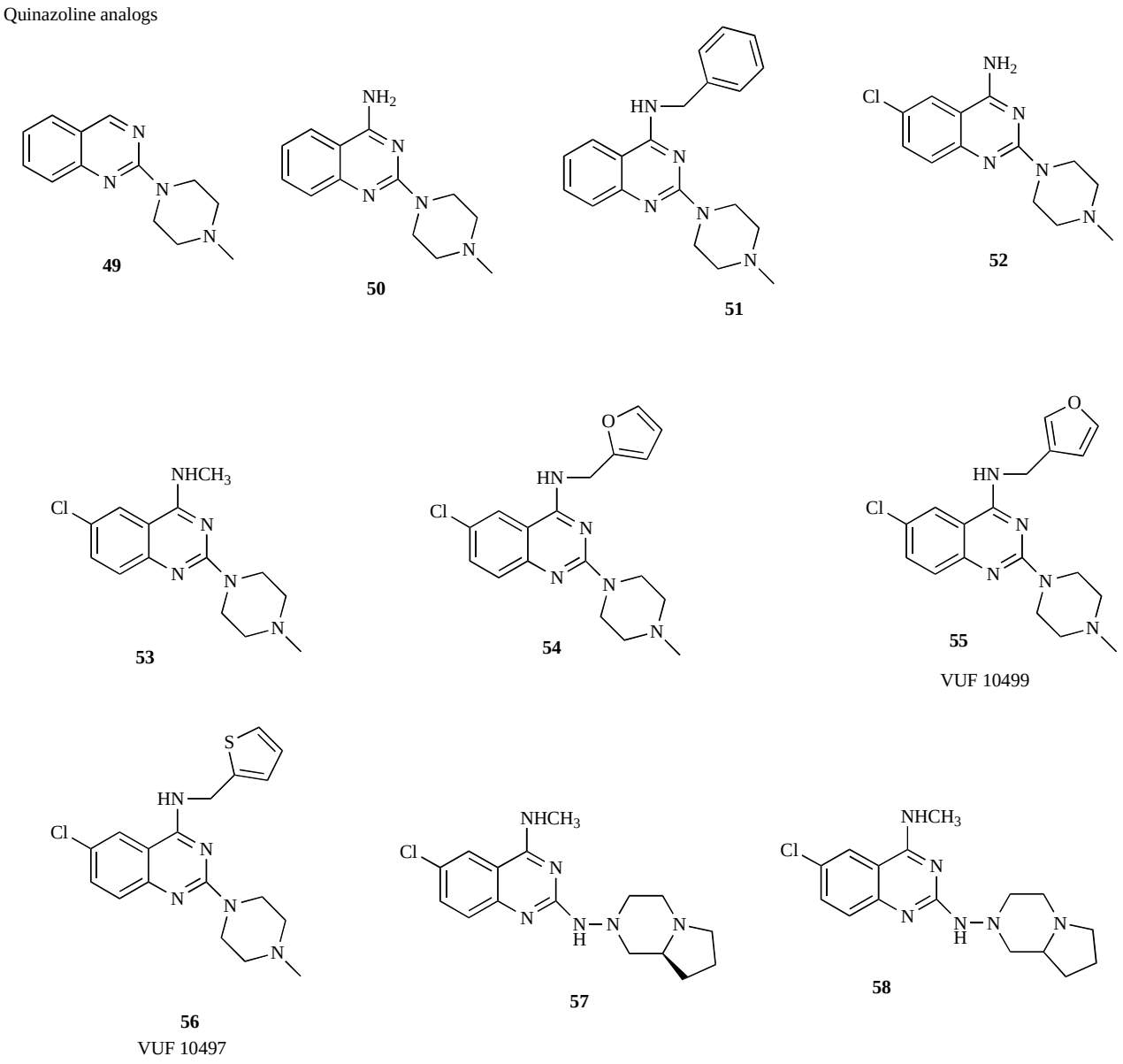
48
VUF 10214

Structure 3. Structure Activity Relationships of H_4R antagonists (Cont.).

compared to parent compound **45** ($pK_i = 6.05 \pm 0.07$ μM). Drop in H_4R affinity has occurred when the addition of phenyl group at 3-position of **45** was aimed to achieve. Introduction of benzyl group at 3-position has led to compound **47** (VUF 10148) with increased affinity to H_4R ($pK_i = 7.40 \pm 0.04$ μM). Radio ligand binding assay has reported that this compound also has H_1R affinity ($pK_i = 6.13 \pm 0.1$ μM). So it has acted as a dual receptor H_1/ H_4 ligand. Substitution of benzyl moiety with methoxy, ethoxy iso-butoxy, cyclohexyloxy, phenoxy, benzylamine, different aryl, heteroaryl substituted oxy, and methoxy compounds has not shown any improved H_4R affinity. Substitution of benzyl group of compound **47** with hydroxyl group (-OH) has retained its H_4R affinity, however, 6-Cl and 6,7-Dicl derivatives of the quinoxalinone, compound **48** (VUF 10214) has found to be more potent. Replacement of methylpiperazine with ethylpiperazine has led to decreased affinity H_4R [81].

SAR of Quinazoline Analogs

Scaffold hopping approach has resulted the quinazoline analog compound 49 with $pK_i = 5.12$ for H_4R. Introduction of amino group at 4-position of **49** has brought compound **50** having 3-fold increased affinity to H_4R ($pK_i = 5.67$). Benzyl group substitution on 4-amino group of **50** has resulted in



Structure 4. Structure Activity Relationships of H_4R antagonists (Cont.).

compound **51** with slight increase in H_4R affinity (pK_i = 5.97). Increase in H_4R affinity (pK_i = 6.59) has seen in **52** when introduce chlorine atom at 6-position of 50. Addition of chlorine atom at 6-position of **50** has led to further increased H_4R affinity (pK_i = 6.98). Methyl substitution of the amino function of **52** has brought compound **53** with increase in H_4R affinity (pK_i = 7.15). Further modification in the amino function group is detrimental for H_4R affinity. Addition of furan as seen in compound 54 has retained its H_4R affinity (pK_i = 7.05). Changing the position of oxygen atom in the furan ring of **54** from the 2- to 3-position has led to VUF10499, compound **55** with 3-fold increase in H_4R affinity (pK_i = 7.57) and has behaved as an inverse agonist; further, this compound reported to have H_1R affinity also (pK_i = 7.01). Replacement of furan ring in **55** with thiophene resulted compound 56, VUF10497 which is highly potent human H_4R inverse agonist. This compound also reported to have H_1R affinity (pK_i = 7.70). Changing the position of sulfur atom in the thiophene ring has resulted in decreased H_4R affinity. Introduction of methyl substituents on the aromatic ring, introduction of one or more additional hetero atom, and increasing the size of the heterocyclic ring in compound **55** and **56** is unfavorable for the H_4R. Further SAR studies to replace the *N*-methylpiperazine moiety of **53** with bioisostere diazabicyclo [4.3.0]nonane has resulted isomers **57**, **58** that have retained H_4R affinity. The *S*-enantiomer was found to have pK_i = 6.81 and the racemic mixture to have pK_i = 6.85 towards the H_4R [82].

H_4 RECEPTOR SELECTIVE LIGANDS

The affinity of histamine towards H_4R is quite high with a K_i value of about 5nM where as H_1R has more than 1000 fold lower affinity. This indicates that the activation of H_1R requires high concentration of histamine when compared to H_4R.The different order of affinities of ligands towards the porcine and human H_4R to compete with [^{3}H]-histamine, a

radiolabeled histamine have been shown by Oda *et al*. [49]. The order is as follows: Histamine (Ki = 20.3 ± 12.4) > Imetit (Ki = 79.9 ± 45.2) > (R)-*α*-Methylhistamine (Ki = 249 ± 84) > Clobenpropit (Ki = 401 ± 86), Thioperamide (Ki = 406 ± 139) > Clozapine (Ki = 20533 ± 9276) for porcine H_4 receptor, Imetit (Ki = 3.4 ± 1.8), Histamine (Ki = 3.9 ± 1.4) > Clobenpropit (Ki = 10.2 ± 1.8) > Thioperamide (Ki = 137 ± 76), (R)-*α*-Methylhistamine (Ki = 175 ± 84) > Clozapine (Ki = 735 ± 249) for human H_4 receptor. Affinity Values (K_i) of histamine ligand for the H_4 Receptor in Different Species has been given in Table **2**. It has been reported that (1*R*,2*R*)-*trans*-2-(4-Chlorobenzylamino)methyl-1-(1*H*-imidazol- 4-yl)cyclopropane Dihydrochloride is selectively active to the H_4R (Ki = 118 ± 27 nM) when compared to H_3R (Ki $>10^3$ nM). But its enantiomer, (1*S*,2*S*)-*trans*-2-(4- Chlorobenzylamino)methyl-1-(1*H*-imidazol- 4-yl)cyclopropane Dihydrochloride has the potential to bind with both H_3 (Ki = 203 ± 40 nM) and H_4 receptors(Ki = 115 ± 29 nM). It indicates that stereochemical properties has also influence the selectivity of the ligand towards the receptors [83]. The rank order of potency of the agonists in the eosinophil shape change assay: clobenpropit>histamine, imetit, R-*α*-methylhistamine>clozapine, *N*-*α*-methylhistamine [84].

H_4 RECEPTOR SELECTIVE LIGANDS AS ANTI-INFLAMMATORY AGENT

Many of the imidazole-based ligands that exhibit binding affinity for the H_3R also show significant affinity for the H_4 receptor; however, 4-(3-piperdin-1-ylpropoxy)benzonitrile, a high affinity non-imidazole H_3R antagonist is devoid of activity at the H_4R, demonstrating that specificity between the two receptors could be achieved. So far, to our best knowledge, only six H_4 receptor ligands that show anti-inflammatory action have been documented: Thioperamide, A-987306, VUF10148, VUF10214, JNJ10191584, and JNJ7777120.

Thioperamide, a H_3/H_4 receptor antagonist significantly reduces the inferior mesenteric blood flow (IMBF), inflammation (mucosal damage) score and histamine concentration in colon in a 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis in rats model. Thus thioperamide exhibited anti-inflammatory effect in rat inflamed colon [85]. It was found that compound 34 (A-987306), a H_4R antagonist to reduce the H_4R agonist induced scratching in mice, anti-inflammatory activity in a peritonitis model and reduction of pain in the carrageenan induced thermal hyperalgesia model [76]. Compound **47** (VUF10148) and Compound **48** (VUF10214) have showed significant anti-inflammatory activity in carrageenan-induced paw edema model in rats at the dose of 10mg/kg and 30mg/kg respectively, among them, the later exhibited significant anti-inflammatory activity even after 6h of administration [81]. JNJ10191584 and JNJ7777120 when given twice a day by oral administration, effectively reduces the colonic injury, myeloperoxidase level, TNF-α levels and neutrophil infiltration in TNBS induced colitis rat model in a dose dependent manner [86].

JNN7777120 selectively inhibits histamine H_4 receptor with little or no affinity for 50 other targets including biogenic amine receptors, neuropeptide receptors, ion channel binding sites and neurotransmitter transporters when tested by radio ligand binding assays. Out of 50 targets only two receptors (serotonin receptor 5-HT2A (34%) and norepinephrine (27%)) showed greater than 20% inhibition at 1 μM [12]. JNJ7777120 blocks histamine-induced chemotaxis and calcium influx in mouse bone marrow-derived mast cells. In addition, it could block the histamine-induced migration of tracheal mast cells from the connective tissue toward the epithelium in mice. JNJ7777120 significantly blocks neutrophil infiltration in mouse zymosan-induced peritonitis model; this model is reported to be mast cell-dependent, which suggests that the effect was mediated by mast cells. The selective H_4 antagonist JNJ7777120 was found to reverse the carrageenan induced thermal hyperalgesia, and reduces paw edema in a mast-cell independent fashion [87]. However, it induces hyperalgesia in the Chung model of neuropathic pain suggests that H_4 receptor antagonists may act in a different manner in inflammatory and non-inflammatory conditions [88].

Table 2. Affinity Values (K_i) of Histamine Ligand for the H_4 Receptor in Different Species

Compounds	Human [48,59]	Porcine [49]	Dog [47]	Rat [48]	Mouse [48]	Guinea pig [48]
Histamine	5.9 ± 0.4	20.3 ± 12.4	29 ± 8.5	70 ± 7	43 ± 9	11.4 ± 1.3
Imetit	1.3 ± 0.1	79.9 ± 45.2	55 ± 12	6.9 ± 4.1	6.8 ± 3.5	12.9 ± 1
Clobenpropit	4.9 ± 1.1	401 ± 86	ND	64 ± 2	15 ± 10	1.5 ± 0.1
N-Methyl-histamine	48 ± 8	ND	112 ± 6.3	553 ± 120	316 ± 146	92 ± 3
Thioperamide	52 ± 28	406 ± 139	80 ± 17	28 ± 9	23 ± 9	34 ± 7
Burimamide	124 ± 19	ND	-	960 ± 188	725 ± 121	351 ± 168
(R)-*α*-Methyl-histamine	144 ± 8	249 ± 84	93 ± 9.2	700 ± 274	397 ± 70	203 ± 30
Clozapine	625 ± 181	20533 ± 9276	50 ± 11	2197 ± 667	2890 ± 1640	78 ± 16
JNJ7777120	4.1 ± 0.3	ND	ND	2.6 ± 0.3	4.6 ± 0.3	ND

ND: Not determined.

The binding affinity of JNJ7777120 to the human and rat H_4R is more or less similar. Intraperitoneal injection of zymosan to the mice develops peritonitis as a result of accumulation of leukocyte in the peritoneum. JNJ7777120 dose dependently inhibits the leukocyte (neutrophil) accumulation and myeloperoxidase level in peritoneal lavages, and the maximum inhibition exhibited at a dose of 70 mg/kg s.c. The involvement of mast cells on H_4R mediated neutrophil recruitment was confirmed by using the mast cell-deficient (MCD) mice where JNJ7777120 could not exhibit any anti-inflammatory effect. The recent study documented the anti-hyperalgesic effect of JNJ7777120 in carrageenan- induced acute and complete Freund's adjuvant (CFA) - induced persistent inflammatory pain models in rats.JNJ7777120 significantly reduce the osteoarthritic pain in the sodium monooiodoacetate induced knee joint osteoarthritic model in rats. Further, JNJ7777120 showed anti-nociceptive effect on the acute post-operative pain model and the neuropathic pain model such as rat spinal L5–L6 nerve ligation (SNL) model, rat chronic constriction injury-induced (CCI) model. These pain models indicate that JNJ7777120 has acted centrally in the reduction of pain, however, the possibility of involvement of peripheral nerves cannot be omitted, since the expression of H_4R has been reported in the peripheral nerves too [18].

JNJ7777120 inhibited the histamine induced eye scratching behavior in ICR mice, dose dependently, however, it does not affect the allergic conjunctivitis in the same model. More over, simultaneous use of levocabastine (H_1R antagonist) and JNJ7777120 showed more potent inhibition of allergic conjunctivitis than when used separately. JNJ7777120 significantly inhibited histamine-trifluoromethyl-toluidine (HTMT), a H_1R agonist, induced allergic conjunctivitis, where as levocabastine could not inhibit the 4-methylhistamine, a H_4R agonist, induced allergic conjunctivitis indicates that H_4R is more important than H_1R in allergic conjunctivitis [20].

JNJ7777120 dose dependently inhibits sneezing and rubbing symptoms in a mice allergic rhinitis model, so it could be used for allergic rhinitis [89,90]. It has been reported that UR-60427, a H_4R inverse agonist reduces the total and eosinophils count in bronchoalveolar lavage (BAL) fluid, and resulted in reduced airway hyperactivity in rat asthma model [91]. Further, JNJ7777120 was found to affect the level of anti-SRBC-antibody (total-anti-SRBC-Igs, IgM and IgG) in immunomodulatory rabbit model, and thus, showed immuno-suppressive role [92,93].

CONCLUSION

Many novel H_4R ligands have been identified by using a structure-based virtual screening (SBVS) method, and these could be used as therapeutic agent in future [94]. A total of 255 compounds were selected for *in vitro* [^{3}H] histamine displacement after investigating more than 7.8 million structures by docking with hH_4R binding site. Out of 255 compounds tested for *in vitro* radio ligand binding assay, 16 compounds with variety of nucleus showed significant displacement activity [95]. New drug-target predictions of known drugs have unveiled the Rescriptor, a HIV-1 reverse transcriptase inhibitor to bind with H_4R with a K_i value of 5.3 μM [96]. These results have indicated that large number of compounds have to be uncovered to target the H_4 receptor. Allergic rhinitis, asthma, and rheumatoid arthritis are just a few of the diseased conditions where mast cells and eosinophils involve, and where H_4R antagonists may have therapeutic utility. Gut inflammation and itching have also shown the involvement of H_4R. Hence H_4 antagonists possess promising effects in the allergy and inflammation therapy. Studies have also indicated the presence of various isoforms of the H_4R, so research to find the various sub-types of the receptor and primary function of each isoform will certainly help throw light on some unanswered questions in the field of allergy and inflammation. Selective antagonists when synthesized for these isoforms, would really increase the potential of inflammation therapy.

ACKNOWLEDGEMENT

We, gratefully acknowledge University Grants Commission (UGC), New Delhi, for financial assistance given in the form of senior research fellowship to C.S and S.K.B.

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Received: May 02, 2010 Revised: September 29, 2010 Accepted: October 13, 2010