In silico modeling techniques for predicting the tertiary structure of human H$_4$ receptor

Hilal Zaid[1], Jamal Raiyn[2], Midhat Osman[1], Mizied Falah[1], Samer Srouji[3] and Anwar Rayan[1,4]

[1]Drug Discovery Informatics Lab, QRC - Qasemi Research Center, Al-Qasemi Academic College, Baka EL-Garbiah 30100, [2]Computer Science Department, Al-Qasemi Academic College, Baka EL-Garbiah 30100, [3]Bone Regeneration Lab, Oral and Maxillofacial Surgery Department, Galilee Medical Center, Nahariya 22100, [4]Science Education Department, Al-Qasemi Academic College, Baka EL-Garbiah 30100, Israel

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1. ABSTRACT

First cloned in 2000, the human Histamine H$_4$ Receptor (hH$_4$R) is the last member of the histamine receptors family discovered so far, it belongs to the GPCR super-family and is involved in a wide variety of immunological and inflammatory responses. Potential hH$_4$R antagonists are proposed to have therapeutic potential for the treatment of allergies, inflammation, asthma and colitis. So far, no hH$_4$R ligands have been successfully introduced to the pharmaceutical market, which creates a strong demand for new selective ligands to be developed. *In silico* techniques and structural based modeling are likely to facilitate the achievement of this goal. In this review paper we attempt to cover the fundamental concepts of hH$_4$R structure modeling and its implementations in drug discovery and development, especially those that have been experimentally tested and to highlight some ideas that are currently being discussed on the dynamic nature of hH$_4$R and GPCRs, in regards to computerized techniques for 3-D structure modeling.

2. INTRODUCTION

Histamine (2-(4-imidazolyl)-ethylamine) is a physiological amine that regulates cellular functions and triggers the inflammatory response. It interacts with receptors belonging to the G-protein coupled receptors (GPCRs) super–family. Based on sequence homology and functional similarities, GPCRs are grouped into six classes (1-2). H$_4$ receptor (H$_4$R) belongs to class "A" (a rhodopsin like family). To date, four histamine receptors activated by the same endogenous agonist, histamine are known (3). H$_4$R, cloned more than a decade ago on the basis of its high sequence homology with the H$_3$ receptor, is the most novel (4-8) (see Figure 1 and Table 1). H$_4$R has been reported to be expressed on cells of the spleen, lungs, intestinal epithelium, stomach, salivary glands, and central nervous system, and on cancer cells (9-11). However, H$_4$R is mainly present in leukocytes and mast cells (5-6). This expression pattern implies that it plays a role in both immune and inflammatory responses. Indeed, a growing body of evidence indicates that H$_4$R is involved in chemotaxis, allergies, inflammation, and autoimmune disorders that it acts as a release mediator in various types of immune cells including mast cells, eosinophils, monocyte-derived dendritic cells and T cells. Moreover, H$_4$R is involved in the modulation of various interleukins, such as interleukin-B4 and interleukin-16 (12-13); nominating it as a potential drug target for inflammatory diseases (13-15). The physiological activity of H$_4$R (and of GPCRs) is beyond the scope of this article but useful information can be found elsewhere (16-17).

Based on experiments using *in vitro* cell lines as well as animal models, hH$_4$R antagonists show a reasonable therapeutic potential for the treatment of allergies, inflammation, asthma and colitis (18-21). Although they have not been tested for efficacy in

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sp|Q9Y5N1|HRH3_HUMAN    MERAPPDGPLNASGALAGEAAAAGGARGFSAAWTAVLAALMALLIVATVLGNALVMLAFV 60
sp|Q9H3N8|HRH4_HUMAN    ------------------MPDTNSTINLSLSTRVTLAFFMSLVAFAIMLGNALVILAFV 41
sp|P35367|HRH1_HUMAN    -------MSLPNSSCLLEDKMCEGNKTTMASPQLMPLVVVLSTICLVTVGLNLLVLYAVR 53
sp|P25021|HRH2_HUMAN    ----------------MAPNGTASSFCLDSTACKITITVVLAVLILITVAGNVVVCLAVG 44
                                          ..  :..::.::. * :*  *.

sp|Q9Y5N1|HRH3_HUMAN    ADSSLRTQNNFFLLNLAISDFLVGAFCIPLYVPYVLTGRWTFGRGLCKLWLVVDYLLCTS 120
sp|Q9H3N8|HRH4_HUMAN    VDKNLRHRSSYFFLNLAISDFFVGVISIPLYIPHTLF-EWDFGKEICVFWLTTDYLLCTA 100
sp|P35367|HRH1_HUMAN    SERKLHTVGNLYIVSLSVADLIVGAVVMPMNILYLLMSKWSLGRPLCLFWLSMDYVASTA 113
sp|P25021|HRH2_HUMAN    LNRRLRNLTNCFIVSLAITDLLLGLLVLPFSAIYQLSCKWSFGKVFCNIYTSLDVMLCTA 104
                         :  *:    . :::.*:::*      :  * .* *:  *:    * :.*:

sp|Q9Y5N1|HRH3_HUMAN    SAFNIVLISYDRFLSVTRAVSYRAQQGDTRRAVRKMLLVWVLAFLLYGPAILSWEYLSGG 180
sp|Q9H3N8|HRH4_HUMAN    SVYNIVLISYDRYLSVSNAVSYRTQHTGVLKIVTLMVAVWVLAFLVNGPMILVSESWKDE 160
sp|P35367|HRH1_HUMAN    SIFSVFILCIDRYRSVQQPLRYLKYRTKTR-ASATILGAWFLSFLWVIPILGWNHFMQQT 172
sp|P25021|HRH2_HUMAN    SILNLFMISLDRYCAVMDPLRYPVLVTPVRVAISLVLIWVISITLSFLSIHLGWNSRNET 164
                        *  .:.::.  **:  :*   .:  *      :     ::    :*  :

sp|Q9Y5N1|HRH3_HUMAN    SSIPEGHCYAEFFYNWYFLITASTLEFFTPFLSVTFFNLSIYLNIQRRTRLRLDGAREAA 240
sp|Q9H3N8|HRH4_HUMAN    GSE----CEPGFFSEWYILAITSFLEFVIPVILVAYFNMNIYWSLWKRDHL--------- 207
sp|P35367|HRH1_HUMAN    SVRREDKCETDFYDVTWFKVMTAIINFYLPTLLMLWFYAKIYKAVRQHCQHRELINRSLP 232
sp|P25021|HRH2_HUMAN    SKGNHTTSKCKVQVNEVYGLVDGLVTFYLPLLIMCITYYRIFKVARDQAKR--------- 215
                         *          .: *    * *::     *:        ::

sp|Q9Y5N1|HRH3_HUMAN    GPEPPPEAQPSPPPPPGCWGCWQKGHGEAMPLHRYGVGEAAVGAEAGEATLGG------- 293
sp|Q9H3N8|HRH4_HUMAN    -----SRCQSHPG---------------LTAVSSNICGHSFRGRLSSRRSLS-------- 239
sp|P35367|HRH1_HUMAN    SFSEIKLRPENPKGDAKKPGKESPWEVLKRKPKDAGGGSVLKSPSQTPKEMKSPVVFSQE 292
sp|P25021|HRH2_HUMAN    ------------------------------------------------------------

sp|Q9Y5N1|HRH3_HUMAN    ------------------GGGGGSVASPTSSSGSSSRGTERPRSLKRGSKPSASSASLE 334
sp|Q9H3N8|HRH4_HUMAN    ----------------------ASTEVPASFHSERQR---RKSSLMFSSRTKMNSNTIA 273
sp|P35367|HRH1_HUMAN    DDREVDKLYCFPLDIVHMQAAAEGSSRDYVAVNRSHGQLKTDEQGLNTHGASEISEDQML 352
sp|P25021|HRH2_HUMAN    ----------------------------------------INHISSWKAAT---- 226
                                                                :     .   .

sp|Q9Y5N1|HRH3_HUMAN    KRMKMVSQS-----------------------------------FTQRFRLSRDRK 355
sp|Q9H3N8|HRH4_HUMAN    SKMGSFSQSD-----------------------------SVALHQREHVELLRARR 300
sp|P35367|HRH1_HUMAN    GDSQSFSRTDSDTTTETAPGKGKLRSGSNTGLDYIKFTWKRLRSHSRQYVSGLHMNRERK 412
sp|P25021|HRH2_HUMAN    ------------------------------------------------IREHK 231
                                                                    * ::

sp|Q9Y5N1|HRH3_HUMAN    VAKSLAVIVSIFGLCWAPYTLLMIIRAACHGHCVP-DYWYETSFWLLWANSAVNPVLYPL 414
sp|Q9H3N8|HRH4_HUMAN    LAKSLAILLGVFAVCWAPYSLFTIVLSFYSSATGPKSVWYRIAFWLQWFNSFVNPLLYPL 360
sp|P35367|HRH1_HUMAN    AAKQLGFIMAAFILCWIPYFIFFMVIAFCKNCCNE--HLHMFTIWLGYINSTLNPLIYPL 470
sp|P25021|HRH2_HUMAN    ATVTLAAVMGAFIICWFPYFTAFVYRGLRGDDAIN-EVLEAIVLWLGYANSALNPILYAA 290
                         : *. ::. * :** **    :  .       :** :  ** :**::*.

sp|Q9Y5N1|HRH3_HUMAN    CHHSFRRAFTKLLCPQKLKIQPHSSLEHCWK---------------------------- 445
sp|Q9H3N8|HRH4_HUMAN    CHKRFQKAFLKIFCIKKQPLPSQHSRSVSS----------------------------- 390
sp|P35367|HRH1_HUMAN    CNENFKKTFKRILHIRS------------------------------------------ 487
sp|P25021|HRH2_HUMAN    LNRDFRTGYQQLFCCRLANRNSHKTSLRSNASQLSRTQSREPRQQEEKPLKLQVWSGTEV 350
                        :. *:  : ::: :

sp|Q9Y5N1|HRH3_HUMAN    ---------
sp|Q9H3N8|HRH4_HUMAN    ---------
sp|P35367|HRH1_HUMAN    ---------
sp|P25021|HRH2_HUMAN    TAPQGATDR 359
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Figure 1. Multiple sequence alignment of the four human histaminergic receptors (H$_1$-H$_4$). The hH$_4$R has a sequence identity at the protein level of 23%, 22% and 31% with H$_1$, H$_2$ and H$_3$ receptors, respectively. The trans-membrane domain of the four receptors is as following: Helices I to IV lie between 32-61 (30); 68-97 (30); 104-136 (33); 150-172 (23); 195-220 (26); 418-448 (31) and 458-478 (21) respectively. In parentheses we note the length of the helix. This figure was constructed using ClustalW2 for multiple sequence alignment run by EMBL-EBI website.

autoimmune disorders, there is an emerging recognition of the role of histamine and mast cells in autoimmune processes (22). These studies and others indicate that H$_4$R may play an essential role in autoimmune diseases and suggest that antagonists for the H$_4$ receptor may open new venues for their treatment. Unlike human H$_4$ receptor antagonists, human H$_4$ receptor agonists seem to be less useful therapeutically. Nevertheless, they are valuable in the exploration of the functionalities of hH$_4$R (23-25). A cohort of hH$_4$R agonists have been reported (26); however, only a limited number of selective hH$_4$R agonists (27) have been identified so far: 4(5)-methylhistamine (24), VUF 8430 (28) and OUP-16 (25) (see Figure 2).

3. GPCRs STRUCTURE: MODELING TECHNIQUES

GPCRs are integral cell membrane proteins. They are encoded by one of the largest human gene families, which encodes for more than 800 distinct human proteins (29). GPCRs are receptors that enable signal transduction through biological membranes. They are involved in almost every physiological activity, including extracellular stimuli (e.g. light, sight and taste), neurotransmission and hormone regulation (30). Ligands typically bind in the extracellular or transmembrane domains, leading to conformational changes and eliciting specific intracellular responses (31). GPCRs share a

Selective agonists for hH$_4$R
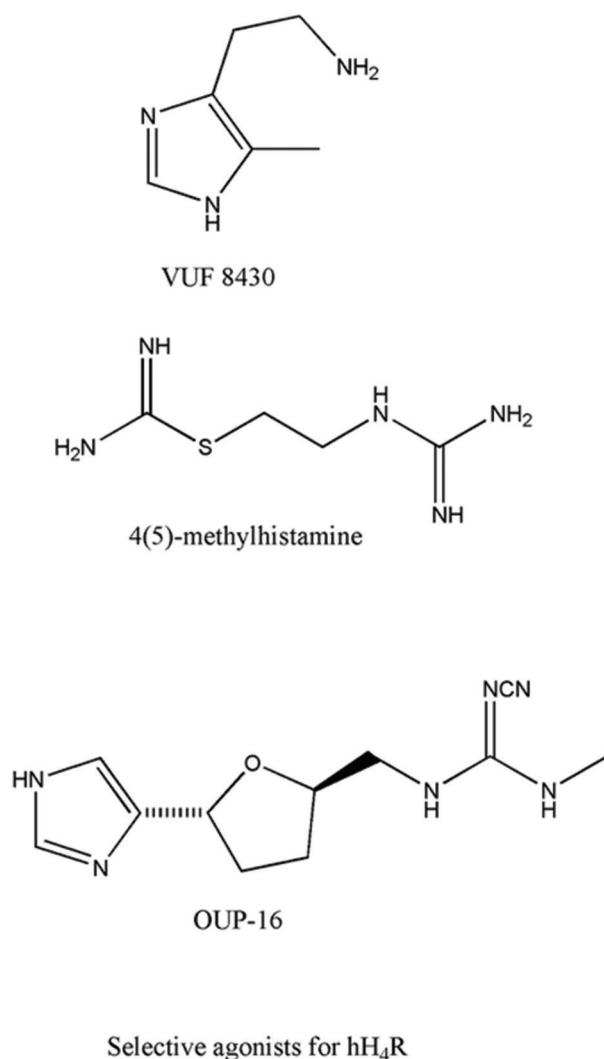
Figure 2. Structural formulas of the selective H$_4$R agonists.

Table 1. Sequence identities of human histaminegic receptors

	H$_1$R (%)	H$_2$R (%)	H$_3$R (%)	H$_4$R (%)
H$_1$R		19.6	22.9	21
H$_2$R	35.6		19.4	19.9
H$_3$R	32.5	32.5		**35.8**
H$_4$R	29.4	27.3	**54.1**	

The upper half of the matrix shows the sequence identity for the complete receptor sequence while the lower half shows the sequence identity of the trans-membrane domains.

common architecture of helices that cross the plasma membrane seven times, forming a bundle that has seven trans-membrane helices (7TMH) connected by intracellular and extracellular loops, with an extracellular

N-terminal and an intracellular C-terminal (32) (see Figure 3). GPCRs derive their name from their ability to recruit and regulate the activity of intracellular heterotrimeric G-proteins. GPCRs are also known as *seven-transmembrane domain receptors, 7TM receptors, heptahelical receptors, serpentine receptor, and G protein-linked receptors* (GPLR). GPCR ligands make up a highly group of substances (e.g. ions, biogenic amines, nucleosides, lipids, peptides, proteins, and even light). The binding of such agonists to GPCRs results in signal transduction that induces a cascade of intracellular responses (9,33-34). Ligand binding is followed by a conformational change that results in a decreased affinity of GPCRs to G-proteins, releasing them into the cytosol.

GPCRs are major contributors to the information flow into cells and, as such, are involved in a wide range of physiological processes and diseases, including those affecting the cardiovascular, nervous, endocrine, and immune systems. The cardinal involvement of GPCRs leads to their designation as drug targets in a multitude of therapeutic areas, to the effect that GPCRs are considered to be the largest group of drug targets to date. It has been estimated that GPCRs compose more than one-third of the targets for currently marketed drugs (35-38), and for 60-70 percent of the drugs in development (39).

The structures of GPCRs should be elucidated in order to employ them for drug design and discovery, using the methods of "Structure Based Drug Design" (SBDD) (40). However, the structural aspects of GPCRs have been a source of constant debate over the last two decades. The available high-resolution structural information on GPCRs proteins constitutes only about 2 percent of the data on this group, which is an impediment to understanding GPCR functioning mechanisms. Hence, the computational modeling of GPCRs has to face difficulties due to the lack of high-resolution information for most GPCRs.

Given that structure-based drug discovery is an efficient method for rationally designing novel drugs and improving the properties of old drugs, the scientific community has been striving for a long time to shed light on the elusive structure-function relationships of GPCRs, employing a variety of direct biophysical and indirect biochemical methods (41). Direct experimental study of GPCR structures is currently too complicated because of their native membrane environment (42), which poses limitations to the purification and crystallization processes. The first GPCR member protein, Bovine rhodopsin, was resolved in 2000 (43), and since 2007, more than fifteen unique class "A" GPCRs have been crystallized in their active/inactive forms, including the avian β_1 adrenergic receptor(44), the human β_2 adrenergic receptor(45), the A2A adenosine receptor(46), the Histamine H1 receptor(47), the Sphingosine 1-phosphate receptor(48), the Dopamine
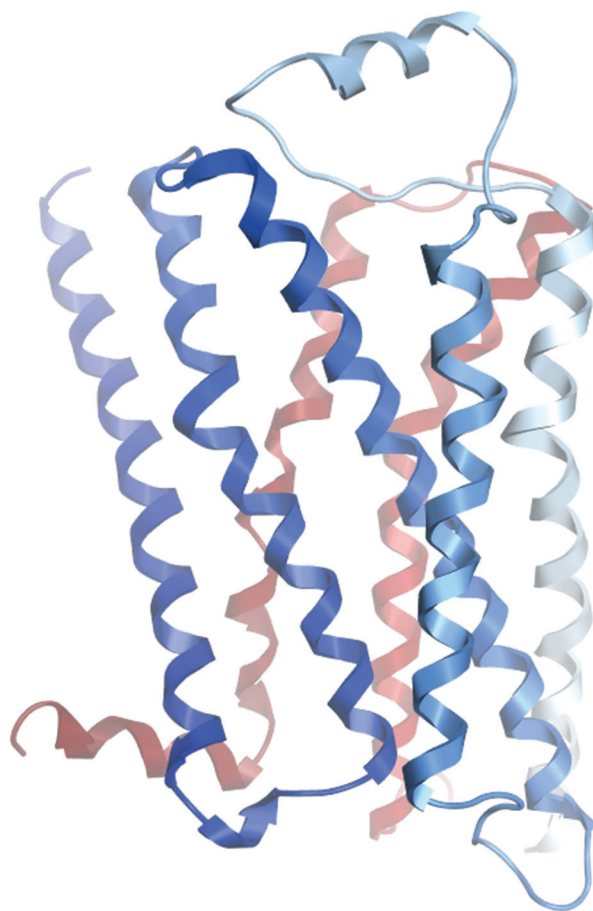
Figure 3. Cartoon representation of Rhodopsin-like family GPCRs, seen from aside in a direction parallel to the membrane surface. The general fold is composed of N-terminus, 3 extracellular loops, 3 intracellular loops, C-terminus and 7 trans-membrane helices, colored in blue to orange from N-terminus and C-terminus. Drawings were done by using the software MOE 2009.10 (http://www.chemcomp.com/software-moe2009.htm/).

D3 receptor(49), the CXCR4 chemokine receptor(50), the Muscarinic M2 receptor(51), the Muscarinic M3 receptor(52), the Kappa opioid receptor(53), the mu opioid receptor(54), the delta opioid receptor(55), the Protease-activator receptor 1 (56), the Neurotensin receptor (57), the human P2Y12 receptor (58) and the nociceptin/orphanin FQ (N/OFQ) peptidereceptor (59). The GPCR-G protein complex structure has also been characterized by low- and high-resolution experimental methods (60-62). Moreover, the crystal structures of all of the photo-activated intermediates of rhodopsin and some agonist- and antagonist-bound GPCRs were recently determined (63-64). This remarkable advancement in resolving GPCR structures is due to a combination of different techniques. The prospects for elucidating the structures of other GPCR proteins, however, are not great, and they await a major breakthrough (65-66). Structural information on GPCRs could be attained by the techniques of electron crystallography, electron paramagnetic resonance, UV absorbance and

fluorescence spectroscopy, nuclear magnetic resonance (NMR) spectroscopy (67-69) and computer modeling. The predicted structures could also be validated by other experimental techniques such as the substituted cysteine accessibility method (SCAM) (70-71) and site directed mutagenesis (72-73).

The location of the ligand-binding pocket is known for many GPCRs (74-75). For instance, peptides and proteins interact with the N-terminus and/or the extracellular loop regions, while small organic molecules are known to bind within the transmembrane domain (TMD) (Figure 4). The binders to hH$_4$R are small molecules and residues implicated in the binding of these ligands are mainly part of the TMD.

3.1. *ab initio* and *de novo* modeling

Two alternative computational approaches were used in order to build molecular models of GPCRs without using specific homologous template structures: ab initio (first-principles approaches) and de novo (knowledge-based approaches). *ab initio* structure prediction and *de novo* protein design are two problems at the forefront of research in the fields of structural biology and chemistry. The goal of an *ab initio* technique is to characterize the 3-D structure of a protein using only the amino acid sequence as input. Based on the independent folding concept introduced by Anfinsen (76), *ab initio* modeling attempts to simulate the physical forces that drive protein folding, using energy functions such as molecular mechanics force fields as well as statistical functions. *De novo* protein design involves the production of novel protein sequences that adopt a desired fold. The experimentally resolved GPCRs are considered to be the prototypes of the main family of GPCRs, referred to as type A. The rhodopsin crystal structure has been used for a long time as a template for the homology modeling of the trans-membrane region of several GPCR subtypes. This paradigm is based on experimental evidence that suggests that part of the template conformation is similar to that of other GPCRs (38,67, 69, 77-78). It is worth to assign that *ab initio* is very time consuming and perform well in modeling short peptides. However, for longer proteins, *de novo* is more efficient (91).

Based on cryoelectron microscopy density maps, a cohort of models of helices were built, using the hydrophobicity properties of receptors and geometric parameters (79). Similar methods were developed to predict the structures of GPCRs and ligand-binding sites, and relative binding affinities (80). Although the GPCR conformational space is small compared to that of water-soluble proteins, it remains very complicated especially due to loops of variable lengths, non-conserved, non-canonical elements and unexpected structural diversity (45,81) when compared to the rhodopsin crystal structure. The situation is further complicated by the presence of cavities that can accommodate water molecules and/
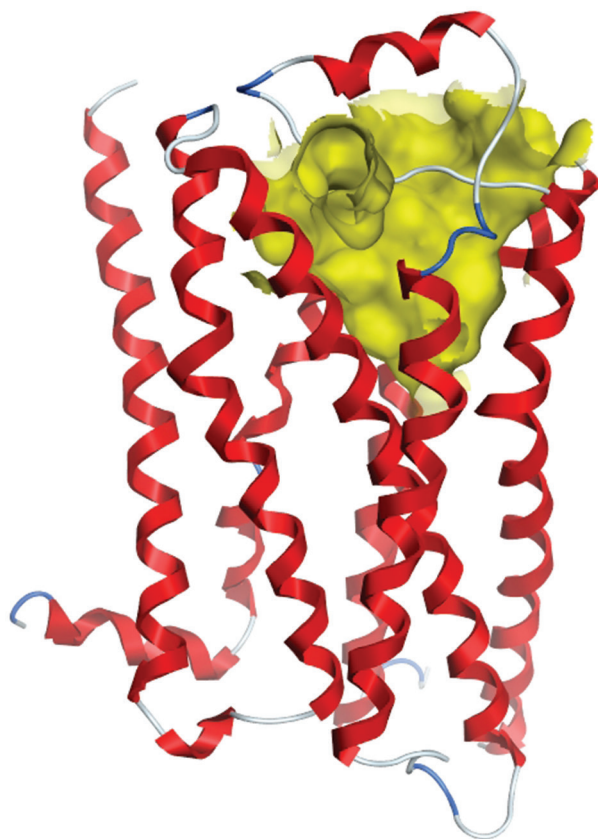
Figure 4. The binding pocket region of histaminergic receptors colored in yellow. The pocket is formulated by residues from helices III-VII in upper part as well as second and maybe third extra-cellular loops.

or different ligands. Another source of complexity is the possible influence of interacting GPCR subunits or other proteins of the signaling cascade. Thus, although de novo and ab initio methods may suggest reasonable TM arrangements of GPCRs, the accuracy of their predictions is limited by the experimental information available. As a result, homology modeling is considered a more reliable technique whenever applicable (82). Moreover, while "ab initio/de novo" predictions for sequences shorter than ten residues have been quite reliable when using reasonable physical-chemical force fields that incorporate explicit water molecules etc. However, they increase the computational complexity and are thus not applicable to large proteins. This would suggest that physical-based GPCR modeling should be abandoned in favor of a non-physical method, such as homology modeling. However, due to insertions and deletions, the loop regions in GPCRs exhibit variable length and low sequence identities (below 30 percent) (83). Hence, it is not reasonable to initiate homology modeling based on the loops of known GPCR structures. De novo/ab initio computational approaches using either coarse-grained backbone dihedral sampling (84) or Monte Carlo (MC) simulations in a temperature annealing protocol combined

with a scaled collective variables (SCV) technique (85) have been shown to accurately predict loop regions of GPCRs with decreasing performance at increasing loop lengths. The prediction of longer and interacting motifs is still a very challenging task (86). The failure of current "de novo/ab initio" approaches to predict long peptides stem from the difficulty of carrying out sufficiently complete searches of their conformational spaces, as well as from the inaccuracy of current force fields (87).

3.2. Homology (comparative) modeling

Due to the lack of experimentally determined 3-D structures of most GPCRs, one could hope to gain some information from approximations based on molecular models. While "de novo/ab initio" modeling is not yet practical for any protein (88-90), "homology" modeling is an established method (89,91). Unlike the "de novo/ab initio" methodologies, homology based modeling techniques rely on the existence of solved structures, which serve as design templates (92). Indeed, many GPCRs have been modeled recently, based on the crystal structure of different, solved GPCRs, by using their backbone coordinates and adding the appropriate side chains for each sequence (93-97). Such homology modeling of GPCRs has been aided mainly by experimental information from point mutations and other experimental data sources (45,98-99).

In homology modeling, the modeled protein retains some of the features of the selected template. The length of helices in the modeled TMD remains similar to those of the template receptor while the loops are generally not included in the template construction, except in rare cases when loop lengths and compositions are similar to those of the template. Other approaches for constructing models of GPCRs suggest that GPCRs could differ in their structure from resolved receptors even though their general features are similar (100-101). However, there are few indications to justify such deviations from the template structure, when constructing models for other GPCRs. A review by Baker and Sali (102) has shown that a homology model for a protein of medium size or larger with a sequence identity of less than thirty percent to the template crystal structure is unreliable. The average sequence identity of the trans-membrane helices (TMHs) of hGPCRs to experimentally resolved GPCRs generally rests outside the regimen boundaries of traditional homology modeling (38). Others in the modeling community think that while this "rule" is correct for globular proteins, it is doubtful whether it should be extended to membrane proteins. Moreover, this rule does not specify how identity should be distributed along a sequence. As much as the GPCR super-family is united by an overall structural topology and an ability to recruit and regulate the activity of G proteins, sequence identity between super-family members, despite the conserved transmembrane cores, is too low. Significant sequence conservation is found, however, within several subfamilies of GPCRs.

The family of rhodopsin-like GPCRs is so far the largest (more than 85 percent of GPCRs) and is characterized by the presence of some 35 (out of ~190) highly conserved residue positions in the TMD, which may be crucial for folding and/or which may be involved in binding and/or in activation (103). By sequence analysis of the TMD of 302 GPCRs, Palczewski and colleagues (104) concluded that it is reasonable to speculate that the overall fold of these receptors is highly conserved. One implication of this study is that it is reasonable to use the overall structures of available reference receptors to model the TMD of other GPCRs using homology modelling.

Another obstacle to the modelling of GPCRs is the conformational change that may accompany with activation. In contrast to the inactive state of GPCRs, a relatively small number of active state structures are available to date-e.g. the Neurotensin Receptor 1, the human β$_2$-Adrenergic Receptor, the adenosine A2A receptor and the human P2Y12 receptor (57-58, 105-108). While these structures provide some information, they are too few to warrant generalizations about the active state and in most cases, insufficient to justify homology modeling solely on active state structures. Given this lack of experimental structural information, several investigators have applied computational strategies to predict activated models of GPCRs (84,109-114). Although the rhodopsin-activated models generated to date appear to satisfy most of the experimental data known for GPCRs, novel predictions deriving from their analyses still await experimental validation. Moreover, it remains to be determined whether or not all GPCRs share the same activated forms.

A study by Rayan (38) examined the extent to which the structures of a five resolved GPCRs are useful as templates for constructing models of other GPCRs. A quantitative measure of conservation in the GPCR family was helpful for determining exactly which parts of the receptors could be used as templates for such comparative modeling, and which should be optimized. The study identified which parts of the structure of the reference receptor may be used as templates, and suggested the construction of the remaining parts by other methods that allow deviations from the crystal structure of the template.

4. 3-D STRUCTURE MODELS OF THE HUMAN H$_4$ RECEPTOR

The H$_4$ receptor has been modeled by several groups based on the resolved structures of bovine rhodopsin, the β$_2$-adrenergic receptor and the H$_1$ receptor. Table 2 summarizes the published H$_4$R models.

4.1. Rhodopsin-based homology modeling era: 2000 – 2007

The 3-D structure of the hH$_4$R is considered by investigators to be key to understanding the role of histamine binding to the receptor and to designing novel ligands. After the hH$_4$R was discovered in 2000, studies were done to define the histamine-binding site. Shin *et al.* (115) performed molecular modeling and site-directed mutagenesis to predict the identity and functional importance of amino acids residing in the histamine-binding pocket. Asp94$^{3.3.2}$ and Glu182$^{5.4.6}$ were identified as being critically involved in histamine binding. Whereas Glu182$^{5.4.6}$ interacts with the Nτ nitrogen atom of the histamine imidazole ring via an ion pair, Thr178$^{5.4.3}$ and Ser179$^{5.4.4}$ were found not to be significantly involved in either histamine binding or receptor activation. These results also demonstrated that Asn147$^{4.5.7}$ and Ser320$^{6.5.2}$ can play a role in receptor activation, but are not involved in histamine binding. Taken together, these data indicate that, although histamine seems to bind to the hH$_4$R in a fashion similar to that predicted for the other histamine receptor subtypes, there are also important differences that can probably be exploited for the discovery of novel hH$_4$R selective compounds.

In 2008, while developing homology models of the hH$_4$R, Kiss and coworkers (116) confirmed that histamine has two major anchoring points at the hH$_4$R binding site, Asp94$^{3.3.2}$ and Glu182$^{5.4.6}$. Following histamine docking at the binding site, the carboxylate group of Asp94$^{3.3.2}$ was rotated around the Cα-Cβ axis to accommodate histamine more favorably. Docking resulted in improved binding modes of histamine, which interacted simultaneously with Asp94$^{3.3.2}$ and Glu182$^{5.4.6}$. This study was conducted by utilizing structure-based virtual screening (SBVS) of a ligand-supported homology model of the hH$_4$R (117-119). More than 8.7. million 3-D structures derived from different vendor databases were investigated by docking known chemical compounds to the hH$_4$R binding site, using the FlexX program. A total of 255 selected compounds were tested by radio-ligand binding assay, and 16 of them showed significant (3H) histamine displacement. Several novel scaffolds were identified for further development of selective H$_4$ ligands. Enrichment tests revealed that this model is able to select highly efficient known H$_4$ ligands from random decoys. An enrichment factor of 40–50 was attained through analysis of the top 0.5. percent of the ranked database. This enrichment factor indicates that the homology model in this case could be used for the virtual screening and discovery of novel antagonists.

These two sites, Asp94$^{3.3.2}$ and Glu182$^{5.4.6}$, were shown to participate in GPCR ligand binding, including binding to the H$_4$R. It was postulated in the rhodopsin-based homology model that Asp94$^{3.3.2}$ interacts in its anionic state, whereas Glu182$^{5.4.6}$ interacts in its neutral form. Jongejan and colleagues tested these two options by applying point mutations Asp94$^{3.3.2}$Asn and Glu182$^{5.4.6}$Gln (120). The Asp94$^{3.3.2}$Asn mutation abolished all detectable binding affinities for all ligands. The Glu182$^{5.4.6}$Gln mutant caused a 1000-fold decrease

Table 2. Summary of the distinct models of H$_4$ receptor reported in literature

Model (Ref)	Species	Ligands	Ref. Structure	Docking and additional notes
M1 (115)	Bovine	AN	Rhodopsin	Homology-based molecular modeling- SEGMED
M2 (116)	Bovine	AN	Rhodopsin	Homology modeling followed by FlexX docking
M3 (120)	Bovine	AG- AN	Rhodopsin	Homology Modelling followed by Energy minimization and MD (docking with fixed main chain and non-fixed side chains)
M4 (122)	Bovine	AG-AN	Rhodopsin	Protein backbone constrained MD (NAMD 2.6 package)
M5 (123)	Bovine	AG- AN	Rhodopsin	Sequence alignment followed by docking with fixed backbone as well as without constraints (FlexX, FlexX-Pharm)
M6 (127)	Human	AG- AN	β_2-adrenergic G protein-coupled receptor	Sequence alignment, homology modeling, and energy minimization
M7 (128)	Human	AG- AN	β_2-adrenergic G protein-coupled receptor	- Inactive model: manual followed by MD using energy minimization - Active model: distance-restrained MD (GROMACS)
M8 (27)	Human	AG	β_2- adrenergic receptor	Homology Modeling followed by Energy minimization and MD
M9 (146)	Bovine	AN	Rhodopsin	Automated pseudoreceptor construction algorithm and MD
M10 (147)	Human	AG- AN	β_2- adrenergic receptor	Structure prediction without using homology – MembStruk, docking with HierDock/MscDock comparing energy to known ligand binding energies
M11 (130)	Human	AG	β_2- adrenergic receptor	Docking ligands for verification of binding modes
M12 (131)	Human	AG- AN	β_2- adrenergic receptor & A2A receptor	MODELLER for models construction and AutoDock 4.0 as docking tool
M13 (132)	Human	AN	β_2- adrenergic receptor	I-TASSER for model construction and Ligand fit module of DISCOVERY STUDIO version 2 as docking tool
M14 (135)	Human	AG- AN	H$_1$R	DISCOVERY STUDIO version 2 was used for model construction and CDocker for docking
M15 (137)	Human	AG- AN	H$_1$R	Combined approach was used for elucidating binding mode
M16 (138)	Human	AG	H$_1$R & ADRB2	Homology Modeling followed by Energy minimization and MD
M17 (139)	Human	AG	H$_1$R & ADRB2	PLANT docking algorithm was used for docking ligands
M18 (141)	Human	AN	H$_1$R & $\beta2$- adrenergic receptor	Ligand-based and Structure-based combined approach was used for screening large chemical database
Abbreviations: AG, Agonist; AN, Antagonist				

in histamine-like ligands without causing an affinity change in other ligands. The proposed model for agonist binding, as well as *ab initio* calculations for histamine and the recently described selective nonimidazole agonist VUF 8430 (28) (see Figure 2), can explain the observed differences in binding to the H$_4$R mutants. These studies provide a molecular understanding of the action of a variety of H$_4$R ligands. Using mutational analysis, Jongejan *et al.* (120,121) found that the glutamic acid residue Glu182[5.4.6] is the source of the increased affinity of histamine observed for both the H$_3$R and H$_4$R. In the resultant H$_4$R model, TM3 is repositioned relative to the core architecture of rhodospin, due to the presence of two unique glycine residues in TM2 of the rhodopsin structure. These result in a slight bend of TM3 at position 99, toward TM5. Asp94[3.3.2], the major site of interaction for ligands containing a protonated moiety, thereby becomes situated in even closer proximity to Glu182[5.4.6].

To differentiate between the active and inactive forms of the receptor, Jojart (122) carried out molecular dynamics (MD) simulations in an explicit membrane (POPC/TIP3P) and water molecule environment, using the homology model of the hH$_4$R. The MD simulations were conducted on the receptor alone, in complex with its endogenous activator histamine and with the selective hH$_4$R antagonist JNJ7777120. These models were built by using the crystal structure of bovine rhodopsin as a template (114). The complex structures were obtained after docking experiments and subsequent optimization. During the simulation of the histamine-hH$_4$R complex, considerable changes occurred in the hH$_4$R structure, as well as in the interaction pattern of histamine at the binding site. These changes were in agreement with experimental data published on GPCR activation. In particular, the intracellular side of TM6 moved significantly away from TM3 and TM7. Histamine's ethylamine
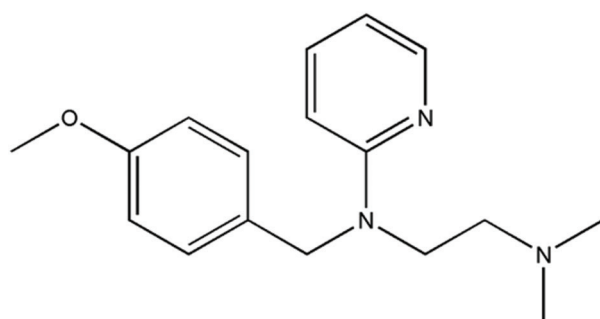
Mepyramine

Figure 5. H$_1$R antagonist.

showed interaction with Glu182[5.4.6], and its imidazole hydrogen bonded to Asp94[3.3.2], alternating to Glu162 (in extracellular loop 2). Moreover, the histamine ligand formed a H-bond, with Asn147[4.5.7] serving as a donor. This residue proved to be important in hH$_4$R activation, because its mutation to non H-bond donor residues lowers hH$_4$R activation. The MD simulations of the native hH$_4$R and the JNJ7777120-hH$_4$R complex suggest that these models represent an inactive conformation of hH$_4$R. MD simulation in the presence of JNJ7777120 resulted in the movement of the intracellular side of TM6 toward TM3, opposite to the outward movement that is more characteristic of its activated state. This modeling strategy provided an ensemble of 3-D structures for both active and inactive receptors. As the authors suggested, this ensemble is potentially useful for structure-based drug design (122).

The differences in binding mode between the agonist and antagonist were studied as well, based on the crystal structure of bovine rhodopsin and of distinct known H$_4$ receptor ligands (123) (histamine, OUP-16 (the first reported H$_4$ agonist with a considerable selectivity over H$_3$R (124)) and JNJ7777120 (the first reported selective H$_4$ antagonist (125))). Experiments were conducted by Kiss *et al.* (123) to determine whether these hH$_4$R models can pick up "actives from haystack." The impact of receptor conformation and the effects of different sets of random decoys, docking methods (FlexX, FlexX-Pharm) and scoring functions (FlexX-Score, D-Score, PMF-Score, G-Score, ChemScore) were investigated. It was found that two agonists (histamine and OUP-16) (Figure 2) form complementary interactions with Asp94[3.3.2], Glu182[5.4.6] and Thr323[6.5.5], whereas JNJ7777120 interacts with Asp94[3.3.2] and Glu182[5.4.6] only. These results suggest a role for Thr323[6.5.5] in agonist binding, and presumably in receptor activation. Also, the type of the ligand that is utilized in modeling and model refinement can significantly influence efficacy in virtual screening. Six initial hH$_4$R models were built by the MODELLER program. In accordance with the sequence alignment, the model contained a disulfide-bond between residues Cys87[3.2.5]

and Cys164 (in extracellular loop 2). Several tests were performed to check the quality of the more suitable hH$_4$R model by assessing the Ramachandran plots and packing quality. The overall quality of the model and the template were quite similar. Tests using HARMONY (126) indicated that the model and the template possess quite the same overall quality (40).

## 4.2. Human β$_2$-adrenergic receptor based homology modeling era: 2007 - present

The crystallization of human β$_2$-adrenergic receptor (hβ$_2$AR) has opened a new focus of study in order to detect variations in binding mode in response to species variations. The natural variation in H$_4$R sequence enabled Lim and his colleagues (127) to identify amino acids involved in the binding of H$_4$R agonists. After identification of a domain between the top of TM4 and the top of TM5 as being responsible for the differences in agonist affinity between human and mouse H$_4$ receptors, detailed site-directed mutagenesis studies were performed. These studies identified Phe169 in the second extracellular loop as the single amino acid responsible for the differences in agonist affinity between the human and mouse H$_4$Rs. Phe169 is part of a Phe-Phe motif that exists in the β$_2$AR and was structurally determined by crystallographic methods (45). These results point to an important role of the second extracellular loop in the agonist binding to the H$_4$R and provide a molecular explanation for the species difference between human and mouse H$_4$Rs.

The hH$_4$R was modeled based on the crystal structure of β$_2$-adrenergic receptor (Protein Data Bank code 2RH1), which lacks the N-terminal tail and contains a T$_4$ ligase structure in the third intracellular loop (IL3) (45). The latter was removed in the model template. A large part of IL3 of the H$_4$R was removed to fit the length of the IL3 of the template. Alignment constraints were applied to avoid gaps in TM domains between Thr146 (in extracellular loop 2) and Gly128[4.5.8], Leu167 (in extracellular loop 2) and Pro149[4.5.8] of β$_2$-adrenergic receptor and H$_4$R, respectively. In extracellular loop 2 (EL2), constraints were put between Cys191 and Cys164, Phe193, and Phe168, Tyr174 and Ser156 of the β$_2$AR and H$_4$R respectively. This alignment was used to run homology modeling and resulted in models with a preserved disulfide bridge.

Another study by Deml *et al.* (128) aimed to explore the value of dual H$_1$R/H$_4$R antagonists as anti-allergy drugs and to address the question of whether H$_1$R ligands bind to hH$_4$R based on the crystal structure of hβ$_2$AR. In an acute murine asthma model, the H$_1$R antagonist mepyramine (Figure 5) and the H$_4$R antagonist JNJ7777120 (Figure 6) exhibited synergistic inhibitory effects on eosinophil accumulation in bronchoalveolar lavage fluid. As assessed in competition binding experiments, eighteen H$_1$R antagonists and

604

JNJ-7777120

cyanoguanidine derivative

L1

L2

L3

L4
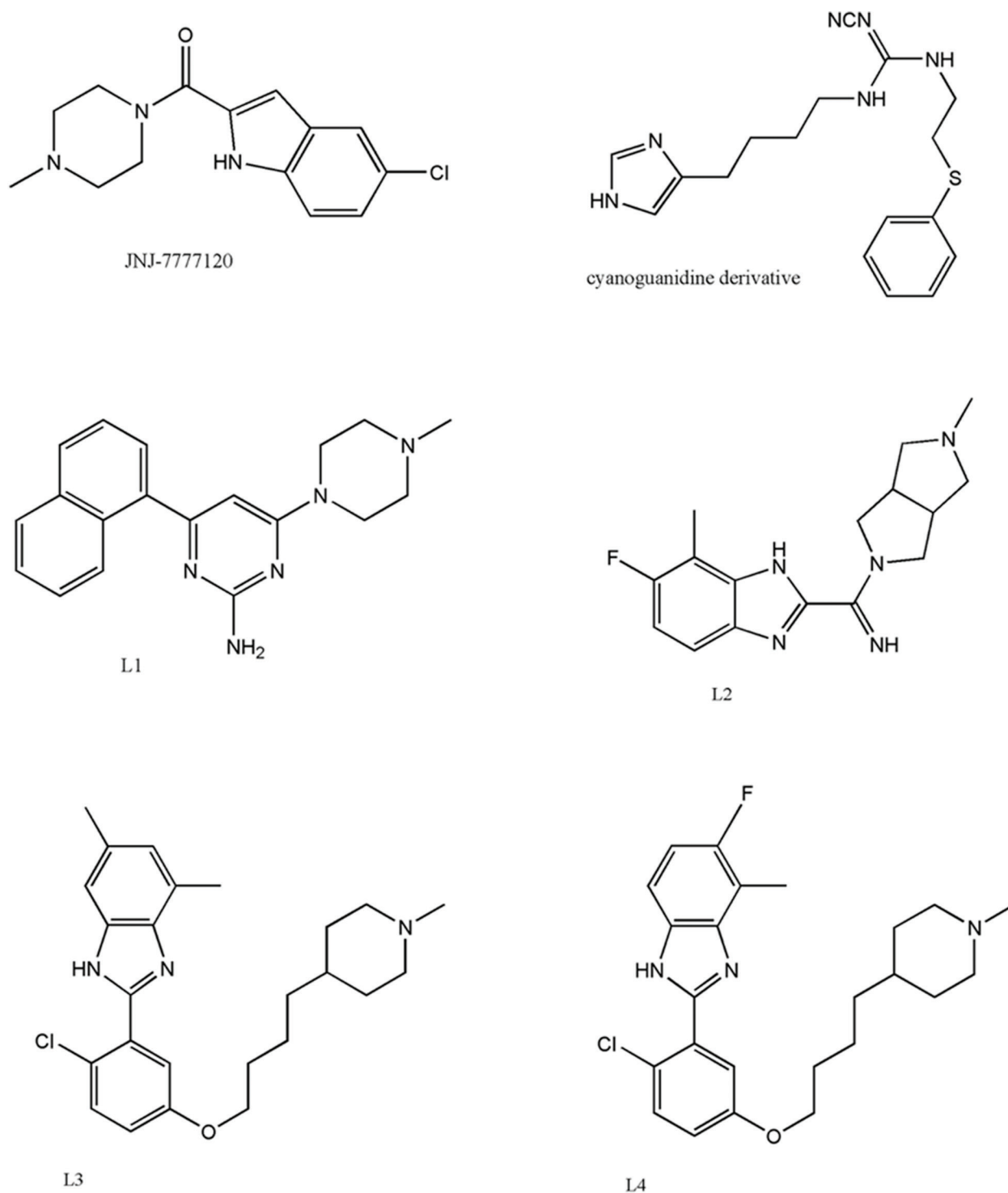
Figure 6. Structural formulas of some selective H$_4$R antagonists.

twenty-two H$_1$R agonists showed a lower affinity to hH$_4$R than to hH$_1$R. Most compounds were neutral antagonists or inverse agonists. Twelve phenylhistamine-type hH$_1$R partial agonists were found to be hH$_4$R partial agonists. Four histaprodifen-type hH$_1$R partial agonists were hH$_4$R

inverse agonists. Dimeric histaprodifen proved to be a more efficacious hH$_4$R inverse agonist than the reference compound thioperamide. Suprahistaprodifen was the only histaprodifen acting as a hH$_4$R partial agonist. Suprahistaprodifen docked in the binding pocket of inactive

Clobenpropit

VUF5228
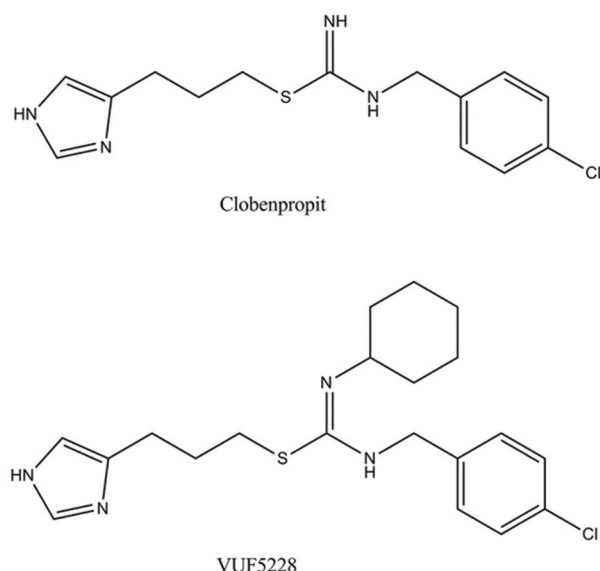
Figure 7. Structure of clobenpropit ligand and its cyclohexyl derivative.

and active hH$_4$R models in two different orientations, predominantly stabilizing the active state of hH$_4$R.

Specific to the acute asthma model, the interactions of H$_1$R and H$_4$R antagonists and agonists indicate that the development of dual H$_1$R/H$_4$R antagonists is a worthwhile and technically feasible goal for the treatment of type-I allergic reactions. To generate an inactive hH$_4$R model, the sequence of hH$_4$R was aligned with hβ$_2$AR. Based on this alignment, the homology model of the inactive hH$_4$R was generated using the crystal structure of the hβ$_2$AR (Protein Data Bank, code 2rh1). Loops with lengths that differed from those of the hβ$_2$AR were modeled using the Loop Search module of SYBYL (Tripos, St. Louis, MO). Thereafter, the minimized receptor was manually placed in a membrane bilayer model, and suprahistaprodifen was positioned manually into the binding pocket in two different orientations. To refine the hH$_4$R homology model, representing the inactive state, distance-restrained molecular dynamic simulations, using the constraints of the inactive conformation, were also performed, with explicit simulation of water molecules (114). The active model of the hH$_4$R was generated with a distance-restrained MD simulation, based on the constraints for the active conformation. In addition, distance restraints for the hydrogen bonds of the transmembrane helices were applied. All simulations were carried out as described for H$_1$R by Strasser and colleagues (129), where dimeric histaprodifen was docked in the binding pocket of the guinea pig H$_1$R (gpH$_1$R). Hydrogen bonds and electrostatic interactions were then detected between dimeric histaprodifen and Asp116, Ser120, Lys187, and Glu190. The authors assessed the influence of the Tyr72[2.6.0]Asn mutation in hH$_4$R upon interaction with suprahistaprodifen and came to the

conclusion that the exchange of Asn[2.6.1] against Tyr[2.6.1] in hH$_4$R, compared to hH$_1$R, had little impact on the interaction with suprahistaprodifen.

In 2009, Igel and coworkers (27) developed a homology model of the hH$_4$R based on the crystal structures of the hβ$_2$AR. Their work focused on the binding site of the cyanoguanidines. One compound in the cyanoguanidine family (see the cyanoguanidine derivative in Figure 6) was manually docked in an energetically favorable conformation, while taking into consideration results from *in vitro* mutagenesis and modeling approaches. The binding site of the compound mentioned above, consisting of twenty amino acids with side chains < 3Å distant from the ligand, is located between TM2 and TM7, and the imidazole moiety was docked at this site. In this binding mode, Glu182[5.4.6] is presumed to be protonated and to serve as a hydrogen bond donor for the π nitrogen. The τ nitrogen forms another H-bond with the side-chain oxygen of Thr178. However, a similar bi-dentate interaction is possible with the couple Ser179[5.4.4]/Glu182[5.4.6], if the imidazole ring is assumed to be coplanar with the butyl chain. Both the Thr178[5.4.3] and the Ser179[5.4.4]Ala mutations lead to only a three- to four-fold reduction of histamine affinity and potency. Thus, no definitive conclusion about the presence and the partner of a second hydrogen bond can be drawn. In this mode, the cyanoguanidine moiety is stacked with the phenyl ring of Phe344[7.3.9] and forms two charge-assisted hydrogen bonds with the carboxylate oxygens of Asp94[3.3.2] (with distances of 2.0.-2.1. Å), an amino acid proven to be essential for histamine binding by *in vitro* mutagenesis. This arrangement allows the arylthioalkyl substituent of the compound, like the isopropyl group of carazolol in the crystal structure of the β$_2$-adrenoceptor, to point outward. The cyano-group in the Z configuration forms two additional charge-assisted hydrogen bonds with the guanidine moiety of Arg341, which is also involved in a salt bridge with Glu165 (in extracellular loop 2). This arginine is species-specific (it would be serine instead in the rat and mouse receptor) and is replaced by a glutamate in the hH$_3$R. This suggests that interactions with Arg341[7.3.6] may contribute to the hH$_4$R subtype and the species selectivity of the cyanoguanidines. The binding mode is more likely, since a nearly perpendicular conformation of the imidazolyl ring with respect to an alkyl chain is energetically favorable and present in the crystal structure of histamine monohydrobromide as well.

In 2011, a team headed by Chris de Graaf and Rob Leurs (130) reported the use of a β$_2$- adrenergic based homology model of hH$_4$R to identify residues that might play an important role in ligand binding and to identify the molecular determinants of H$_3$R/H$_4$R selectivity. They stated that the hH$_4$R homology model based on the H$_1$R crystal structure is very similar to the hH$_4$R model which was constructed on the basis of the β$_2$-adrenergic crystal structure. According to this study, the ligand clobenpropit (see Figure 7) can adopt two different binding modes

for the hH$_4$R, while adding a cyclohexyl group to the clobenpropit isothiourea moiety allows the new ligand (VUF5228) to adopt only one binding mode.

Aiming to design an anti-inflammatory drug candidate, Levita and colleagues (131), generated two models of the hH$_4$R, using the human adenosine A2A receptor (PDB code: 3em1) and the β$_2$-adrenergic receptor crystal structure (PDB code: 2rh1) as templates. SWISS-PROT (http://swissmodel.expasy.org) and MODELLER 9v7 (http://salilab.org/modeller) were applied to complete the missing parts of the models. The researchers reported that the hH$_4$R 3-D model produced by MODELLER, using 2rh1 as a template, was the best model, based on DOPE value and Ramachandran plot analysis. Histamine ligand was docked by AutoDock 4.0. on the receptor model and placed at its predicted binding site, which consists mainly of six amino acid residues: Asp94$^{3.3.2}$, Tyr95$^{3.3.3}$, Glu182$^{5.4.6}$, Trp316$^{6.4.8}$, Tyr319$^{6.5.1}$, and Phe344$^{7.3.9}$. Histamine was bound to the receptor via the formation of two hydrogen bonds with Asp94$^{3.3.2}$ and Tyr319$^{6.5.1}$.

A team headed by Suresh (132) aimed to develop a homology model that could be used for structure-based virtual screening and could also disclose a novel scaffold for designing potent and selective hH$_4$R antagonists. They used I-TASSER, a web-based structure prediction server, to construct the 3-D structure of hH$_4$R, using the human β$_2$-adrenergic GPCR (PDB ID: 2rh1A) as a template. The generated models were validated for virtual screening application by PROCHECK (133) and ERRAT (134). The ligand fit module of Discovery Studio (version 2.0., Accelrys, Inc., San Diego, CA, USA) was used to dock 392 chemicals retrieved from PubChem—one hundred and fifty analogues of JNJ7777120, 49 thioperamide analogues and 193 Vuf6002 analogues. The successful docked poses were evaluated using a set of scoring functions (LigScore1, LigScore2, PLP1, PLP2 and PMF), as implemented in the Discovery Studio software. Six substances with high docking scores were chosen as potential leads for hH$_4$R antagonists.

4.3. human H$_1$ receptor and others based homology modeling era: 2011 - present

In 2011, the crystal structure of human histamine H$_1$R (47) was released to the Protein Data Bank (PDB entry: 3RZE, resolution 3.1.0 Å), and its discovery significantly facilitated the structure-based drug discovery of histamine receptors. Using Discovery Studio (version 2.5.), Feng and colleagues (135) constructed a homology model of the hH$_4$R, using the crystal structure of H$_1$R as a template. Once the 3-D model was generated, energy minimization was performed. The docking program CDocker and Discovery Studio's Catalyst Score were used to construct receptor-ligand complexes. The binding pocket was defined by aligning the center of the binding site with the center of the ligand in the H$_1$R crystal structure. Using this model, the researchers studied the binding mode of the hH$_4$R for eight ligands, which included six agonists (histamine, imetit, clobenpropit, OUT16, 4-methylhistamine, clozapine) and two antagonists (JNJ7777120, VUF6002). They found that there are two binding modes, but all of the ligands shared a preferred one, where the protonated part tightly interacted with Asp94$^{3.3.2}$, while the imidazole-NH of the ligand interacted with Glu182$^{5.4.6}$. As well, they determined that Glu165 (in extracellular loop 2) and Thr323$^{6.5.5}$ are two important residues involved in the binding pocket of the hH$_4$R and contribute to its selectivity.

Early in 2014, researchers from Gedeon Richter corporate (136) published a methodology for screening fragments and detecting hits for that bind to the hH$_4$R and the dopamine D$_3$ receptor. They concluded that X-ray structures, homology-based models and structural ensembles, were all suitable for the docking-based virtual screening of fragments against both receptors. The results obtained from the various models complemented each other, with little overlap among their hit sets. The authors identified some flexibility within the hH$_4$R binding site (e.g. Met150$^{4.6.0}$, Leu175$^{5.3.9}$, Glu182$^{5.4.6}$, etc.), which enabled some variability in the ligand's position. Due to such flexibility, we think that using an ensemble of structures for docking and virtual screening purposes would be the best way to increase hit rates.

De Graaf and Leurs demonstrated (137) how a combination of ligand structure-activity relationships, quantum mechanics-based ligand conformation analysis and structural modeling could help to elucidate the binding mode of 2-aminopyrimidine derivatives in the hH$_4$R binding pocket, as well as the molecular determinants of hH$_4$R ligand binding. The 3-D structural model of the hH$_4$R was built based on the H$_1$R crystal structure. Their studies revealed that ligands bind hH$_4$R with a high binding affinity and efficacy by forming ionic interactions with Glu182$^{5.4.6}$ and by optimizing hydrophobic interactions with the protein-binding pocket, while taking up an energetically favorable conformation. In 2013, Schultes *et al.* (138) described how a combination of *in silico* and experimental approaches could be utilized to map ligand-protein interactions. Two models for the native hH$_4$R were built: the first one was based on the recently resolved H$_1$ receptor crystal structure, while the second model was based on the β$_2$-adrenergic receptor crystal structure. The models had very similar transmembrane domain tertiary structures and overall showed the same ligand-receptor interactions. Schultes *et al.* reported that the models showed comparable efficiency in retrospective virtual screening studies. They focused on the elucidation of the binding modes of two varied selective hH$_4$R ligand classes: indocarboxamides and 2-aminopyrimidines (see Figure 8). Recently, Engelhardt *et al.* described (139) how comprehensive structure-activity relationship analysis, in combination with homology modeling, enabled them to establish a detailed binding model of bispyrimidine (see Figure 9) in the hH$_4$R

Indolecarboxamides

Aminopyrimidines
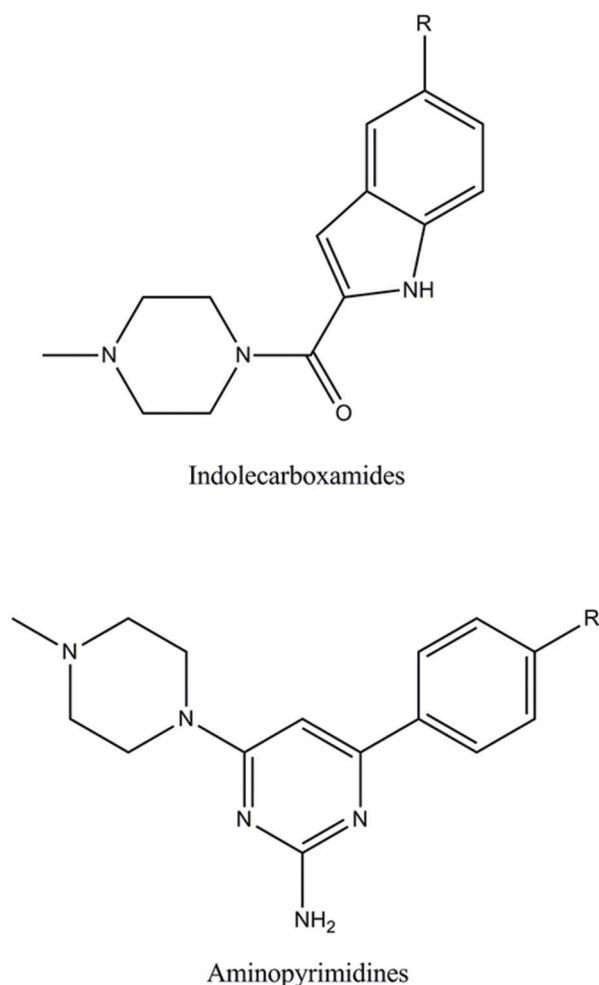
Figure 8. Structural formulas of two different selective hH$_4$R ligand classes.
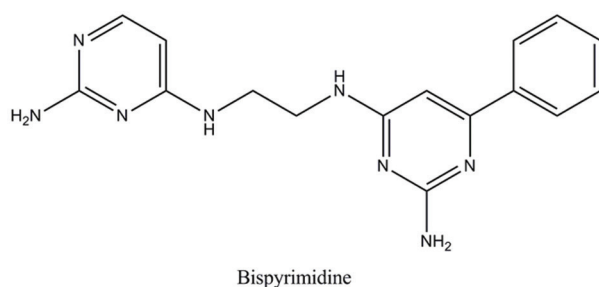
Bispyrimidine

Figure 9. Structure of bispyrimidine - H$_4$R antagonist.

and to identify the subunit replacing the N-methylpiperazine moiety that exists in most hH$_4$R ligands. The PLANT docking algorithm (140), without any constraints, was used to verify the compound's binding modes.

Recently, a team headed by Guccione and Rayan described (141) a combined ligand-based and structure-based approach for indexing chemicals for their hH$_4$R antagonism. The strategy was composed of two subsequent stages. In the first stage, two ligand-based chemoinformatics techniques, the Intelligent Learning Engine (ILE) (142) and the Iterative Stochastic Elimination approach (ISE) (143-144), were used to screen a large chemical database (145) and select a set of chemicals highly indexed as hH$_4$R antagonist candidates. Next, different hH$_4$R structural homology models were constructed, and their capability for differentiating between active and non-active hH$_4$R antagonists was checked by docking a validation set of active ligands. To rank the ligands and the docked poses, in addition to the AutoDock4 energy (electrostatic term), the filter of the ability to interact with Asp94$^{3.3.2}$ and Glu182$^{5.4.6}$ through hydrogen bonding/electrostatic interaction, was taken into account. The authors came to the conclusion that the model constructed by extensive molecular dynamics simulation, conducted in a DOPC lipid membrane and with a docked ligand inside, is the most efficient model for docking purposes and virtual screening. As well, ligand-based chemoinformatics techniques, in sequential combination with molecular modeling techniques, are claimed to have the potential to improve the success rate for discovering new biologically active compounds and to improve enrichment factors in a synergistic manner.

4.4. Ligand-based prediction (without using homology models)

In an attempt to study how we could transfer ligand information into a homology-based receptor model, new methods have been developed. A study by Tanrikulu *et al* (146) presented a computer-assisted method for the generation of a pseudo-receptor model for a putative ligand binding site based on a three-dimensional alignment of known histamine H$_4$ receptor ligands. Following alignment, hydrogen bond donors/acceptors were projected outwards with the appropriate binding distances and geometries to provide pseudo-receptor atoms. Each pseudo-atom was then weighted according to the number of atoms that generated it in each ligand as well as the number of ligands that created it and a correlation vector was obtained. The resulting model was used for the virtual screening of a large collection of commercially available compounds with two bioactive chemotypes retrieved.

The pseudo-receptor model was also used to find the putative ligand binding pocket within the transmembrane domain of the receptor together with a homology model based on the β$_2$-adrenergic receptor template. The homology model was simulated using molecular dynamics, with an explicitly simulated environment of water and lipids. For each frame of a molecular dynamics simulation of a homology-based H$_4$ receptor model, potential ligand binding pockets were automatically extracted and their compatibility with the pseudoreceptor was used as a selection criterion. The

best-matching pocket fits perfectly with existing mutation data and previously published hypotheses nominating Glu182$^{5.4.6}$ as the preferred binding partner of a positively charged moiety of H₄ receptor ligands. This new pseudoreceptor approach has demonstrated its suitability for both the structure-based prioritization of protein receptor models, and ligand-based virtual screening with the aim of performing scaffold hopping. An automated pseudo-receptor construction algorithm (PRPS, pseudoreceptor point similarity) was developed and used to transfer ligand information into a homology-based receptor model for the H₄R antagonist binding pocket.

The MembStruk method for predicting the 3D structure of several GPCRs including the H₄R, without utilizing homology modeling techniques was developed recently (147). Predicted structures were validated by using the HierDock procedure (148) or MSCDock (149-150) to predict their characteristics (sites, configurations, and binding energies to known high affinity ligands (agonists and antagonists). Predictions did not depend on experimental data, but rather were compared to it. The predicted structure for the ligand-GPCR complex was then used to predict which mutations would dramatically decrease or increase binding. HierDock/MSCDock was thus applied to successfully predict the binding site structures and binding energies of some ligands including those that bind to H₄R.

To explore the possible structure–function relationships of the hH₄R, as a receptor species with high constitutive activity, molecular modeling of an active hH₄R state in complex with Gα$_{i2}$-CTs was performed (151). This study was based on a model of the putative active state of the hH₄R, on the inactive state model (27) and on recent data on the crystal structure of opsin in complex with a C-terminal fragment of transducin (Protein Data Bank, 3DQB) (152). The alignment of the active hH₄R model with the inactive hβ₂AR structure showed that the main difference consists of an outward tilt of TM6, resulting in a distance of approximately 6.5.Å at the intracellular end (position of Arg297$^{6.2.9}$) (151). The bottom of TM5 and TM7 deviate by approximately 2 and 2.5.Å, respectively. The segments TM2–TM4 were found to be rather well aligned (the rms fit of the backbone atoms was approximately 1.2.Å). At the intracellular end of TM5, two residues of the hH₄R, Asp205 and His206, were found to be nearly unique among all biogenic amine GPCRs. Asp205 forms a salt bridge with Arg299$^{6.3.1}$, which may be regarded as an ionic lock stabilizing the active receptor state. In summary, 14 contacts may be formed with the participation of hH₄R residues from TM2, TM3, EL2, EL3, TM6, and the C-terminal helix 8. Interactions of the Gα$_{i2}$ C terminus with the receptor seem to enforce the proper fold of the last four Gα$_{i2}$ residues.

In 2011, Fernandes and his colleagues, (153) published their work reporting the utility of QSAR and molecular modeling for predicting indole and benzimidazole derivatives as hH₄R antagonists. Thirty compounds were used as a training set, and for characterization of the molecular structure, a total of 63 descriptors of diverse nature (structural, lipophilic, electronic, topologic, steric and thermodynamic) were calculated. Four out of five compounds in the external test set were correctly classified, and the proposed model yielded a success rate of 80 percent.

5. CONCLUDING REMARKS

In silico methodologies and techniques for modeling hH₄R, together with emerging experimental data, have led various research groups to construct high quality models for this receptor, which have been utilized in studies of the structure activity relationships of ligands, the elucidation of binding modes, and the virtual screening of chemicals' databases. Most reported models have a sufficient level of accuracy to enable effective discrimination between binders and non-binders. However, these models were not tested on their capabilities for ranking the affinities of a series of analogues in order to exploit them for lead-optimization purposes. We believe that flexible docking, simultaneously employing more than one three-dimensional model of the receptor, could improve the docking results. As well, a combination of ligand-based and structure-based modeling could yield better results in virtual screening experiments.

One of the underlying difficulties in integrating the various models produced emanates from the lack of an organized depository for modeled proteins. This requires laboratories interested in comparative studies to individually collect models. We suggest the collection of all hH₄R models for a comparative study aimed at optimizing binding prediction. Using existing docking tools, agonists and antagonists of known binding affinities can be docked to the various models and the hH₄R models can be ranked according to the correlations between the predicted and experimental binding affinities of docked ligands.

6. ACKNOWLEDGEMENTS

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Abbreviations: human Histamine H$_4$ Receptor (hH$_4$R); Histamine H$_1$ Receptor (H$_1$R); Histamine H$_2$ Receptor (H$_2$R); Histamine H$_3$ Receptor (H$_3$R); G-protein coupled receptors (GPCRs); G-protein-linked receptors (GPLR); Structure Based Drug Design (SBDD); β1 adrenergic receptor (β1aR); human β_2-adrenergic receptor (hβ_2AR); guinea pig H$_1$R (gpH$_1$R); nuclear magnetic resonance (NMR); substituted cysteine accessibility method (SCAM); structure-based virtual screening (SBVS); molecular dynamics (MD); intracellular loop (IL); extracellular loop 2 (EL2); extracellular loop 3 (EL3); seven trans-membrane helices (7TMH); transmembrane domain (TMD); Monte Carlo (MC); scaled collective variables (SCV); transmembrane region (TM); pico second (ps); three dimensional (3-D).

Send correspondence to: Anwar Rayan, Drug Discovery and Informatics Lab, QRC-Qasemi Research Center, Al-Qasemi Academic College, P.O.B. 124, Baka El-Garbiah 30100, Israel, Tel: 972-4-6286761/4, Fax: 972-4-6286762, E-mail: a_rayan@qsm.ac.il