Design and Synthesis of New Histamine H4 Receptor Ligands

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CHAPTER 8

General Discussion

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General discussion

Medicinal chemistry is a science in which rational drug design approaches and serendipitous discoveries go hand in hand. Our understanding of ligand-GPCR interactions, 3D-protein structure and receptor activation mechanism of GPCRs is far from perfect, although it is noted that significant progress has recently been made. The publication of the crystal structure of bovine rhodopsin and more recently the human β_1- and β_2-adrenergic and adenosine A_{2a} receptors greatly increase our understanding of GPCR structure and provides a solid basis for the construction of homology models that can aid rational drug design.[1,2,3,4] Other important contributions come from biophysical approaches such as solid-state NMR that can confirm binding modes and the occurrence of specific receptor-ligand interactions.[5]

Currently over 30% of all marketed drugs target GPCRs, and this type of receptor can therefore be considered the most important drug target for the pharmaceutical industry.[6] However, the marketed drugs target only a small number of receptors. Considering that approximately 1000 GPCRs exist, there is significant potential for more GPCR-targeting drugs.[2] Drug development on GPCRs in the pharmaceutical industry usually starts with a high throughput screening campaign to find new hits.[7] Such hit-finding strategies require vast resources with respect to money, compound storage, acquisition and handling as well as automated screening assays but often provide useful of starting points for medicinal chemistry programs.[7,8] However, some times HTS campaigns fail to yield hits at all or give hits that are difficult to optimize. For most academic institutions or small biotech companies resources for HTS screening are unavailable. As an alternative for the 'brute force' HTS campaigns, rational drug discovery approaches can be applied.

The major line of research discussed in this thesis is the design and synthesis of new H_4R ligands. At the start of this research only a few compounds were known to bind the H_4R and opportunities for medicinal chemistry and hit finding were limited. A very successful strategy in drug discovery is to start from the endogenous GPCR ligand, which for the H_4R is histamine.[9] Indeed histamine and a few other imidazole-containing compounds were available as starting points at the beginning of this project. However, it was decided to focus on non-imidazole compounds. The imidazole moiety is known to often bind to CYP450 isoenzymes and influence CYP450 mediated metabolism of exogenous compounds, giving rise to undesired drug-drug interactions.[10,11] It should be noted that despite this undesirable property of the imidazole moiety several very successful imidazole

containing drugs have been developed and marketed.[12] In addition to the metabolic liability, imidazole chemistry is considered to be complex and labor intensive, thereby reducing synthetic output and hampering the hit optimization process. It was thought that on the basis of these considerations choosing a non-imidazole starting point would speed up the discovery of potent new H_4R ligands.

Directly after the discovery of the H_4R the anti-psychotic drug clozapine was found to bind with moderate affinity while showing agonistic behavior at the H_4R.[13] Being promiscuous in nature, this compound binds many GPCRs and selectivity for the H_4R would be a major point of concern in future development. Nevertheless, clozapine provided an ideal starting point to probe the H_4 receptor active site and to start with the design of new non-imidazole ligands. In chapter 2 a limited SAR study of clozapine and its analogues is described.
During this investigation a more potent clozapine analogue was found, the H_4R agonist VUF6884 with enhanced H_4R affinity (pK_i= 7.55) and high H_1R affinity (pK_i= 8.11). Interestingly, some similarities in SAR were found between VUF6884 and the selective reference H_4R antagonist JNJ7777120 that was published in 2004. SAR data that supported the idea that these compounds bind at the same H_4R site and additional pharmacological evidence provided the justification for the construction of a pharmacophore model that describes three pockets in the H_4R binding site (Figure 1). The superposition of agonists and antagonists and the suggestion that they share a similar mode of binding is not without discussion. There is consensus in GPCR research is that the active state of the receptor, induced or locked by an agonist, is different from the inactive state of the receptor to which an antagonist binds. Although this is very likely to be the case, it should be noted that very small changes in molecular structure, such as the substitution of a hydrogen atom for a fluorine atom, can dramatically alter the functional activity of a compound at its receptor.[14] These chemical alterations can be so small that one can argue about the idea that the orientation of a ligand bound to the active site will change dramatically. At the H_4R changes in functional activity as a result of small chemical alterations has been shown for a series of burimamide analogues as well as a series of clobenpropit analogues (unpublished data)[15].

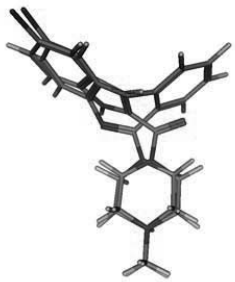


Figure 1: Pharmacophoremodel of VUF6884 and JNJ7777120 from chapter 3.

VUF6884 and JNJ7777120 are structurally more distinct from each other but can nevertheless adopt a similar 3D conformation. The pharmacophore model in chapter 2 should be considered as a working model that reveals overlapping regions of the different ligands and also partial similarity, i.e. substructures that do not overlap. Indirectly, this indicates 'space' or a pocket in the H_4R that is occupied by VUF6884 but not occupied by the antagonist JNJ7777120.

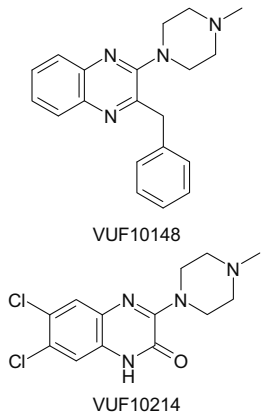


Figure 2: Quinoxaline compounds from chapter 3

Subsequently the three pocket pharmacophore model was used to design a small series of compounds, that can be considered hybrids of VUF6884 and JNJ777120 (chapter 3). These compounds bound with moderate affinity and were very suitable for substitution with aromatic moieties that could occupy the proposed 'third pocket' from the pharmacophore model described in chapter 3. This rational discovery approach was successful and led to an interesting new scaffold on the H_4R, the quinoxaline heterocycle. VUF10148 (pK_i= 7.40, Figure 2), a benzyl substituted quinoxaline was found to be a potent H_4R ligand that also showed *in-vivo* efficacy in an animal model of acute inflammation. The 2-hydroxy-6,7-dichloro substituted analogue VUF10214 (pK_i= 8.25) was even more potent in both *in vitro* affinity and *in vivo* efficacy. We also tried to improve the affinity of several quinoxaline ether analogues with classical medicinal chemistry approaches such as a Topliss scheme but failed to find very potent compounds (highest pK_i=6.57).[16]

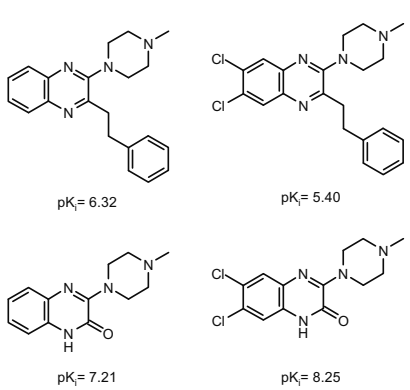
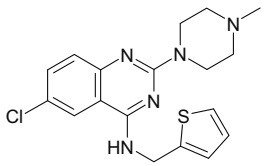


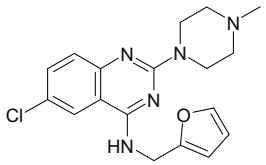
Figure 3: Non-linear SAR of some quinoxaline compounds

There were two major reasons why we abandoned further optimization of the quinoxaline series. The series showed a non-linear SAR when the quinoxaline heterocycle was substituted with chlorines at the 6- and 7-positions. For example, 6,7-dichlorosubstituted quinoxaline VUF10214 had high affinity compared to its 2-hydroxy analogue that lacked the two chlorine atoms. This clearly indicated a

beneficial effect of the two chlorine atoms on H_4R binding affinity. In other compounds, such as the 3-phenethyl and 2-hydroxy substituted quinoxalines (Figure 3), the introduction of these two chlorine atoms did not contribute to high H_4R affinity and even reduced affinity. This non-linear SAR might indicate a change in binding mode, making straightforward SAR considerations aimed at improving the binding affinity more difficult. Therefore, further synthetic efforts in this direction were halted. In contrast to the 6,7-dichloro substituted quinoxalines the 6-chloro quinoxalines was still of much interest to complete the SAR study. Unfortunately, the precursor for the 6-chloro quinoxalines was not readily available since no convenient or regioselective synthesis was known for this compound. A regioselective synthesis route for substituted asymmetrical quinoxalines in general and the desired 6-chloroquinoxaline precursor was eventually developed, albeit much later than the synthesis of the initial quinoxaline series. With this important synthetic tool in hand and the current knowledge of the quinoxaline and quinazoline SARs, it should be possible to synthesize different types of substituted quinoxalines and increase their potencies up to the single-digit nanomolar range.



VUF10497

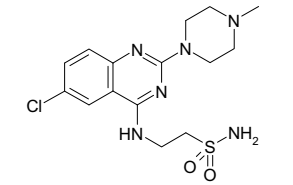


VUF10499

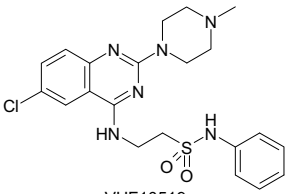
Figure 4: Potent quinazoline H_4R ligands from chapter 4

The quinoxaline series was used as the basis for another rational drug discovery approach. If *N*-methylpiperazine substituted quinoxalines could occupy the 'third pocket' it should be possible for quinazolines to do the same, since the latter scaffold differs only in the position of one nitrogen atom in the aromatic system. In chapter 4 a scaffold hopping approach is described. Lipophilic substituents can greatly increase the affinity of the quinazolines for the H_4R. The quinazoline scaffold is much easier to decorate than the quinoxaline scaffold since preparation of the required precursors has been thoroughly described in literature and the problems with asymmetrical substitution of the quinoxaline scaffold were not encountered.[17,18] In contrast to the quinoxaline series we could therefore easily investigate the effect of the introduction of different small lipophilic substituents on a 6-chloro substituted quinazoline ring. Availability of the required precursors, together with microwave chemistry and parallel synthesis allowed the introduction of a significant number of substituents on this scaffold. The alteration of the *N*-methylpiperazine moiety met with limited success, since only two

of the 16 selected amines that were introduced were tolerated at the H_4R. Amines known to be tolerated on other scaffolds were very detrimental for H_4R binding of the quinazolines. Although highly potent compounds were found (e.g. VUF10497, pK_i=8.12 and VUF10499, pK_i=7.57, Figure 4) this particular series of quinazolines suffered from lack of H_4R selectivity, showing moderate to good affinities for the other histamine receptor subtypes. Nevertheless, the quinazoline heterocycle had been demonstrated to be a good scaffold for potent H_4R ligands. The discovery of VUF10497 underlined the success of our rational discovery approach. In addition, this compound and its close analogues also contributed to the refinement and validation of the original three pocket pharmacophore model.



VUF10558



VUF10519

Figure 5: Quinazoline sulfonamides from chapter 5

Since the compounds in chapter 4 were quite promiscuous for at least the histamine receptor subfamily and contained the relatively hydrophobic furane and thiophene moieties, an attempt was made to make the compounds more hydrophilic and water soluble. This eventually led to the discovery of the *N*-ethylsulfonamide substituents on the 4-position of the quinazoline heterocycle (Figure 5). These substituents gave compounds such as VUF10558 and VUF10519 with high (K_i<10 nM) H_4R affinities and greatly contributed to improved water solubility (unpublished data) due to the polar nature of the sulfonamide moiety and its ability to form hydrogen-bonding interactions.

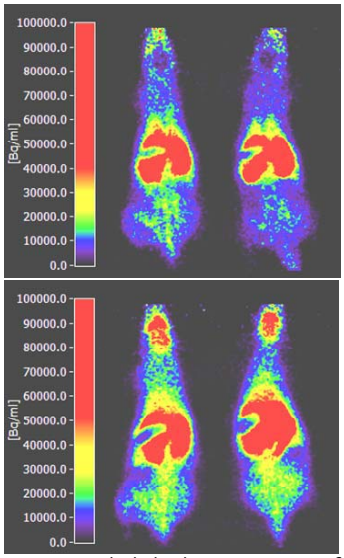


Figure 6: Whole body PET imaging of rats injected with H_4R radioligands

Chapter 5 also describes a QSAR study in which all of the descriptors provided by the MOE software were explored.[19] This led to a statistically significant QSAR model with excellent predictive ability for the affinities of quinazolines substituted with the newly discovered sulfonamide side chain.

The discovery of the potent sulfonamide substituted quinazolines provided some highly potent H_4R ligands of which VUF10558 was selected for the development into a [^{11}C] radioligand. In parallel with this development, [^{11}C]JNJ7777120 was also developed, since this compound is the most widely used H_4R reference antagonist with good H_4R selectivity. After evaluation of the distribution of both radioligands *in vivo* by whole body PET-imaging, it was found that [^{11}C]JNJ7777120 entered the CNS (Figure 6 bottom panel), whereas [^{11}C]VUF10558 did not enter the CNS (Figure 6 top panel) at an appreciable concentration.[20] To our knowledge this has been the first time that [^{11}C]JNJ7777120 has been shown to enter the CNS and this knowledge may prove to be of importance in relation to the mechanism by which the H_4R is able to reduce, for example, experimentally induced itch in mice.[21]

Our studies provide tools and data to study the H_4R selectively in the CNS or PNS. Both radioligands were also used to monitor the development of the inflammatory response in the carrageenan-induced paw edema model in rats by positron emission tomography (PET). It was proposed that the site of inflammation might be visualized by the use of H_4R ligands that could bind either to H_4 receptors that would be upregulated in local tissue under inflamed conditions or to cells (e.g. eosinophils) that were recruited to the site of inflammation by H_4R mediated cell chemotaxis. Unfortunately, the experiment failed since both tracers were not distributed to the paws of the animals and no significant radioactivity could be detected in the paw. Interestingly, the PET scans that were made (see PET images on the left, top panel for [^{11}C VUF10558 and bottom panel for [^{11}C]JNJ7777120), support the data found in the distribution experiment and also show high CNS uptake (red areas) for [^{11}C]JNJ7777120 and extremely low (blue areas) CNS uptake for VUF10558. Future research towards the evaluation of the H_4R radiotracers in the rat should preferentially be done with an inflammatory response that can be monitored more easily with a PET camera and that is not induced in the front or hind paws and legs.

As mentioned at the beginning of this chapter, the HTS campaigns that are usually unleashed by large pharmaceutical companies to generate hits for medicinal chemistry programs require huge investments. In contrast, the discovery of novel H_4R ligands described in this work started from a promiscuous tricyclic scaffold (i.e., clozapine, chapter 2 of this thesis) that was eventually developed into a chemically very distinct and quite selective series of unique quinazoline sulfonamides as novel H_4R ligands. The use of promiscuous ligands in hit finding is not new and has first been described by Evans for a

series of benzodiazepine-based cholecystokinin-1 (CCK_1) antagonists.[22] These compounds were found after the analgesic tifluadom, a κ-opiod antagonist, had been reported to have unwanted side effects on CCK_1.[23] More recently approaches such as the one used for the development of CCK_1 antagonists has been given the acronym SOSA; Selective Optimization of Side Activities.[24] The SOSA approach has the advantage that the safety, pharmacology and bioavailability profile of the lead compound have already been intensively studied and therefore a lower compound attrition rate in the drug discovery process can be expected. Nowadays SOSA has been shown to be a valuable complementary strategy in drug discovery.[25] Since the work described in this thesis originates from the affinity of the widely prescribed anti-psychotic drug clozapine for the H_4R, one could also consider this work to be a SOSA approach. Although the safety, pharmacology and bioavailability profiles of the novel H_4R ligands have not been studied in detail we have already seen that several compounds were indeed efficacious in *in vivo* models of inflammation (Chapters 3-5) and excellent metabolic stability in human an rat micosomes and hepatocytes has also been observed for some compounds (data not shown).

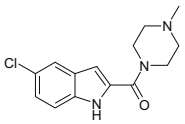
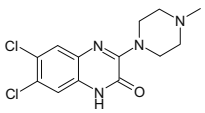
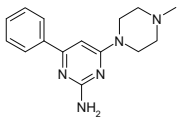
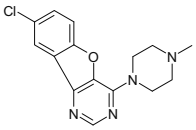
Structure-based and ligand-based modeling

There are several ways to model protein-ligand interactions. One of these approaches is structure-based technology, in which the structure of a biological target is used to identify chemical groups in a ligand molecule that can bind the protein. The structure can be directly obtained by X-ray diffraction analysis of a crystallized protein or indirectly by homology modeling. The structural information gathered is then used to guide hit optimization of low or moderate affinity ligands.

Another method to describe important features of biologically active compounds are the ligand-based approaches, including (Q)SAR analysis and pharmacophore modeling. In the latter type of method, the chemical structure of a ligand is combined with pharmacological data in order to identify features that are crucial for high affinity of the ligand for the protein. These features (e.g. aromaticity or hydrogen bonding) can then be converted into a 3D model that describes the essential chemical features of the ligands. Indirectly, this pharmacophore describes the binding site where the ligand molecule interacts with the protein. To aid these these studies, we have developed models that combine the abovementioned structure-based and ligand-based approaches. We have also synthesized

and evaluated a series of structurally diverse H_4R ligands reported in scientific and patent literature (Table 1).

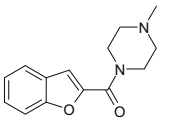
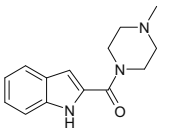
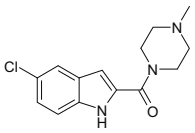
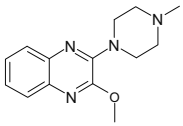
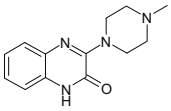
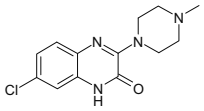
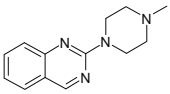
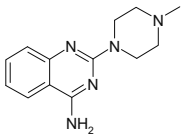
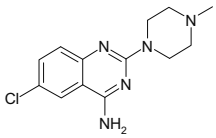
Table 1: Selected ligands from H_4R literature

No	Code	Structure	Reported Affinity	Found Affinity[a] (pK_i)
1	JNJ7777120		4 nM (pK_i= 8.39)[27]	8.38 ± 0.02
2	VUF10214		32 nM (pK_i= 7.51)	8.36 ± 0.04
3	VUF10460		K_i < 20 nM[26]	8.22 ± 0.08
4	-		-	7.87 ± 0.11

[a] Measured by displacement of [^{3}H]histamine binding using membranes of HEK cells transiently expressing the human H_4R. pK_i's are calculated from at least three independent measurements as the mean ± SEM.

This set of compounds was tested in the same H_4R binding assay and some limited SAR studies on these compounds and their close analogues were performed as well. An example of these SAR studies revealed a shared mode of binding for structurally different H_4R ligands. (Table 2)

Table 2: Shared SAR of indole, quinoxaline and quinazoline ligands

INCREASING H_4R AFFINITY →			
Feature	H-bond acceptor	H-bond donor	Chlorine atom
Structure			
$pK_i \pm SEM$[a]	6.36 ± 0.05 (**1**)	7.71 ± 0.03[b] (**2**)	8.38 ± 0.02 (**3**)
Structure			
$pK_i \pm SEM$	6.47 ± 0.02 (**4**)	7.21 ± 0.03 (**5**)	7.93 ± 0.05 (**6**)
Structure			
$pK_i \pm SEM$[a]	5.12 ± 0.06 (**7**)	5.67 ± 0.07 (**8**)	6.98 ± 0.08 (**9**)

[a] Measured by displacement of [^{3}H]histamine binding using membranes of HEK cells transiently expressing the human H_4R. pK_i's are calculated from at least three independent measurements as the mean ± SEM. [b] n=2

Compounds **1**, **4** and **7** (Table 2) do not posses the hydrogen bond donor and chlorine atom that is crucial for high H_4R affinity. When the hydrogen bond donor is introduced as in compounds **2**, **5** and **8**, an additional interaction with the receptor is gained and the affinity increases. The introduction of a chlorine atom on the aromatic heterocycle further increases affinity in all three compound classes (compounds **3**, **6** and **9**). In addition to the above-mentioned observations, the NMP moiety on the indole, quinoxaline (Chapter 3) and quinazoline (Chapter 4) compounds cannot be replaced without a very substantial loss of H_4R affinity.[27]

The information obtained in these studies and the measured affinity of the abovementioned reference H_4R ligands provided us with an ideal dataset to generate a ligand-based pharmacophore model. Starting with JNJ7777120, the ligands in table 1 were aligned sequentially using flexible alignment modeling to finally give an alignment model of all 4 compounds (Figure 8A). The aligned compounds can then be used to map the

active site by calculating the contact preference area. (Figure 8B) This gives a map of potential interactions of the ligands with the surrounding area and may help to identify specific interactions such as the hydrogen bonding interaction with polar receptor amino acids. JNJ7777120 had already been docked in the homology model and its binding mode had been confirmed with mutational analysis and *ab initio* calculations.[28] It was then decided to superpose JNJ7777120 in the homology model with JNJ7777120 in the pharmacophore model in order evaluate if the homology model would correspond with the pharmacophore model and vice versa. After the superposition, the ligands from the pharmacophore model were used to calculate potential sites of hydrogen bonding interaction between the ligand molecules and surrounding residues (red solid areas in Figure 8B).

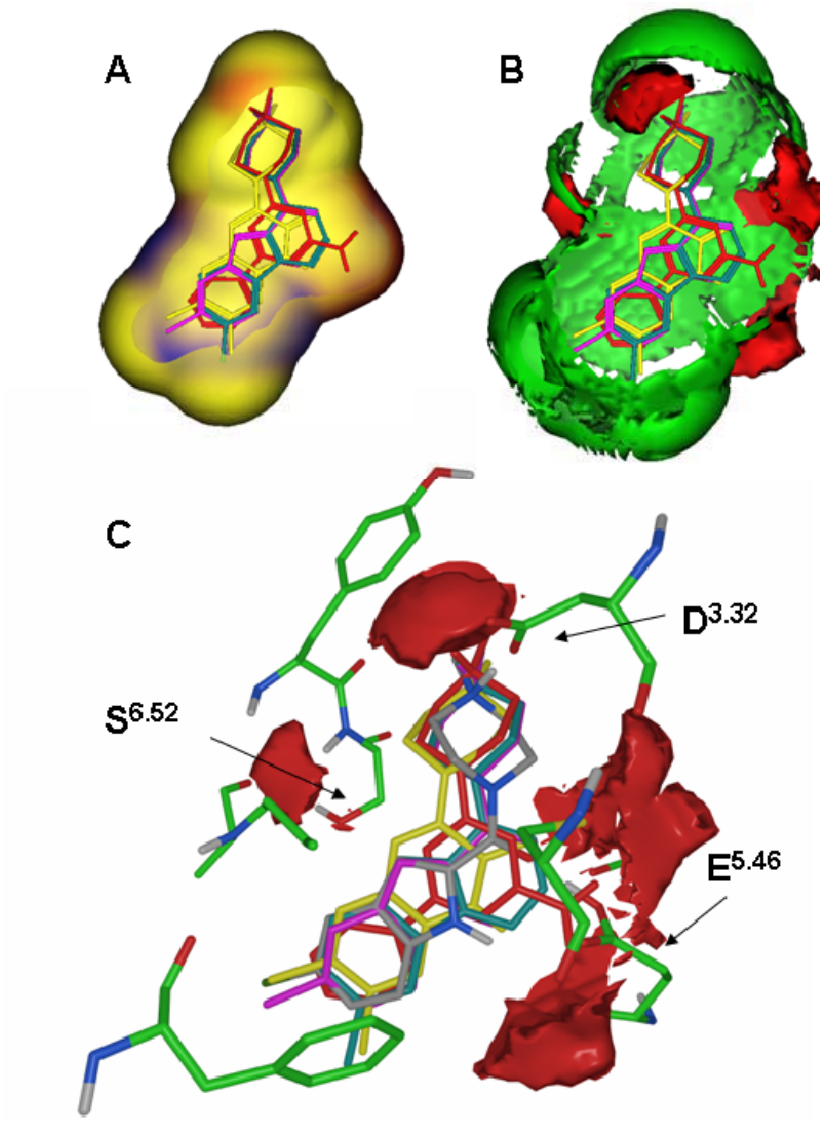


Figure 7: Combination of a pharmacophore alignment model and a H_4R homology model.[28] A. Flexible alignment of the ligands from table 1 and their calculated Van der Waals surface area (polar areas in red, mild polar in blue and hydrophobic in yellow). B. Calculated contact preference area of the alignment model with potential sites of hydrophobic interaction in green and sites of hydrophilic interaction in red. C Superposition of the alignment model with JNJ7777120 docked into the H_4R. Sites of hydrofilic interaction (in red) in the pharmcophore model are close to the sites of interaction between JNJ777120 and the H_4R. Amino acid residues of the H4R binding pocket are shown in green and Aspartate ($D^{3.32}$), glutamate ($E^{5.46}$) and serine ($S^{6.52}$) are indicated in black.

Figure 7 shows that the top red area is close to $D^{3.32}$. This residue is able to form a cation-anion interaction with the distal nitrogens in the cyclic amine moieties of the H_4 ligands. $E^{5.46}$ is exactly between the two lower left red areas and seems to able to form either one or two hydrogen bonds with a ligand. Due to its relatively flexible nature, $E^{5.46}$ can tolerate the relatively broad range of different combinations of H-bond donors and acceptors present in the H_4R ligands in the pharmacophore model. VUF10214 and VUF10460 are present in both the model in Figure 8 and in the more simple model described on page 9 of this chapter. The initial simplified hydrogen bonding model seems to correspond well with the interactions of both ligands with $E^{5.46}$ and $D^{3.32}$ in figure 8.

More interestingly, the top red area which is generated due to the presence of the nitrogen atom in **2** (Table 1) and the ogygen atom in **4** (Table 1) is very close to polar residue $S^{6.52}$. Due to the presence of an –OH group this residue may be able to form a hydrogen bond with **2** and **4**, although in this homology model the –OH group is pointing away from the hydrogen bond acceptors in these ligands. Thus, the combination of the pharmacophore model with the homology model can help to identify the amino acid residues that are crucial for ligand binding. Validation of the integrated model might be achieved by binding analysis of the ligands at various receptor mutants.

Perspectives for H_4R drug discovery

H_4R related medicinal chemistry efforts within the academic setting have been extremely limited according to scientific literature, although some publications on the pharmacological evaluation of histaminergic ligands synthesized in the past and ligands found with structure based virtual screening have been published.[29,30] Moreover, H_4R medicinal chemistry programs using histamine as a starting point have not been repeated and consequently new selective imidazole containing ligands have so far not been developed. Such programs have proven to be very fruitful for the development of potent and selective H_3R ligands such as thioperamide, immethridine and methylimmepip.[31,32,33] These compounds have contributed greatly to the development of H_3R research as pharmacological tools. Therefore, imidazole medicinal chemistry that has a long tradition in academic research remains a yet untouched area and provides a great opportunity for future research.

We have recently found that several of the H_4R ligands reported in patent and scientific literature have considerable affinity for the H_3R as well (unpublished data). On the basis of these findings it can be concluded that compounds with high selectivity for the H_4R over

other targets are extremely rare and perhaps even limited to JNJ7777120. It should be noted that in our hands this compound has a pK_i of 6.0 at the human H_3R, which would make this compound only about 250-fold selective for the H_4R over the H_3R.[34] The major point of concern in the development of selective H_4R ligands is the affinity of compounds for the H_3R. Therefore, screening for H_3R affinity of H_4R ligands should be mandatory in all H_4R drug discovery programs.

The *in vivo* half-life in rats of JNJ7777120 is very short and limits the use of this compound as a selective H_4R antagonist.[35] Due to this short half-life i*n vivo* studies will require high doses of JNJ7777120 in order to maintain an efficacious plasma concentration, in particular in prolonged studies such as asthma models or other models of chronic inflammation. Since the selectivity of JNJ7777120 for the H_4R over the H_3R is only 250-fold, high dosing will give rise to unwanted H_3R mediated effects and thus the compound may lose its selective profile. The recent discovery of H_4R antagonist A-943931 with an improved *in vivo* half life in rats will prove to be a valuable tool for further studies.[36] The reported H_4R selectivity of A-943931 is 640-fold but this has not been confirmed by other laboratories. H_4R medicinal chemistry efforts are still needed to tackle the issue of H_3R-H_4R compound selectivity to produce suitable tools for *in vivo* studies. Recent developments in the area of asthma and chronic pruritis have opened up the possibility that patients may one day benefit from the use of H_4R antagonists.[21] Future research in these areas will prove to be invaluable for establishing the H_4R as a drug target.

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