Design and Synthesis of New Histamine H4 Receptor Ligands

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SUMMARY

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Histamine is an important chemical messenger in a variety of of physiological processes. This imidazole-containing ligand mediates its effects via a group of four G protein-coupled receptors (GPCRs), named the histamine H_1 receptor (H_1R), H_2R, H_3R and H_4R after the order of their discovery. In chapter 1, an overview of the relevance of the histaminergic GPCRs (H_1R-H4R) is given together with a selection of the known ligands that bind these receptors. The H_3R has the highest homology with the H_4R and many of the imidazole-ligands originally designed for the H_3R also bind the H_4R to some extent. The H_4R structure and function show that this newest member of the histamine receptor subfamily is a pharmacologically distinct GPCR with a unique expression pattern throughout the body. In the past several years, significant progress has been made with respect to the development of new H_4R ligands. Medicinal chemistry efforts have provided researchers with a number of pharmacological tools to study the relevance of the H_4R in biology (e.g. the H_4R agonists 4-methylhistamine and the H_4R antagonist JNJ7777120). Using these tools in *in vivo* models of human disease, it has become apparent that the H_4R plays an important role in inflammatory and allergic disorders as well as in the modulation of pain and pruritis (itch). Although H_4R research is still in the preclinical stage 'these findings may lead to novel treatment paradigms to fulfill the unmet clinical demand for innovative therapies in H_4R related diseases.

The work described in this thesis focused on the development of novel H_4R ligands that can be used in *in vitro* or *in vivo* studies to characterize the pharmacology of the H_4R and contribute to the understanding of its role in physiology. The starting point for this project was the anti-psychotic drug clozapine that is known to bind many G protein-coupled receptors, including the H_4R. Optimization of the structure of clozapine as described in chapter 2 resulted in the potent H_4R agonist VUF6884 (H_4R, pK_i=7.60). Pharmacological data suggest that VUF6884 and the known reference antagonist JNJ7777120 can be used to describe the orthosteric binding site (the site where also histamine binds the receptor) of the H_4R, because both JNJ7777120 and VUF6884 displace [^{3}H]histamine in a competitive manner. It was also demonstrated that the effects of the agonist VUF6884 are competitively antagonized by the selective H_4R antagonist JNJ7777120, indicating considerable overlap of their binding sites. On the basis of the derived SAR and saturation binding experiments, a flexible alignment model was constructed that was intended to be the premise for the design of novel H_4R ligands.

Using the flexible alignment model, in chapter 3 a series of small compounds (fragments) was designed, synthesised and evaluated at the H_4R. From this series, 2-(4-methyl-1-piperazinyl)-quinoxaline (VUF10050, pK_i=6.05) was identified as a new lead structure for the development of H_4R ligands. Exploration of the structure-activity relationship (SAR) of this quinoxaline scaffold was performed using molecular modeling and parallel synthesis approaches. This strategy led to the identification of 6,7-dichloro-3-(4-methylpiperazin-1-yl)quinoxalin-2(1*H*)-one (VUF 10214, pK_i=8.25) and 2-benzyl-3-(4-methyl-piperazin-1-yl)quinoxaline (VUF 10148, pK_i=7.40) as very potent new H_4R ligands. *In vivo* studies in the rat revealed that compound VUF10214 has significant anti-inflammatory properties in a carrageenan induced paw-edema model for inflammation.

In a scaffold hopping exercise described in chapter 4 and using the SAR information obtained from the development of the quinoxaline H_4R ligands a second fragment was optimized, leading to a series of quinazoline-containing H_4R compounds. This approach led to the discovery of 6-chloro-*N*-(furan-3-ylmethyl)2-(4-methylpiperazin-1-yl)quinazolin-4-amine (VUF10499) and 6-chloro-2-(4-methylpiperazin-1-yl)-*N*-(thiophen-2-ylmethyl)quinazolin-4-amine (VUF10497) as potent human H_4R inverse agonists (pK_i= 8.12 and 7.57 respectively). Interestingly, both compounds also possess considerable affinity for the human histamine H_1 receptor (H_1R) and therefore represent a novel class of dual action H_1R/H_4R ligands, a profile that potentially leads to added therapeutic benefit in inflammatory and allergic conditions. Compounds from this novel series of quinazolines behave as antagonists at the rat H_4R and were found to possess anti-inflammatory properties *in vivo*.

Since the thiophen and furan moieties of VUF10497 and VUF10599 are relatively lipophilic we attempted to replace these groups with more polar substituents to increase the water solubility of the quinazolines. This resulted in the discovery of the series of sulfonamide-substituted quinazolines (Chapter 5) with high affinity for the H_4R (pK_i= 8.12). As a result of its polar nature, the sulfonamide moiety leads to improved solubility and is believed to probe a distinct H_4R binding pocket that was previously identified using the abovementioned flexible alignment modeling approach. By introducing a variety of substituents on the quinazoline-sulfonamide compounds, the H_4R affinity was optimized. The interactions of the new sulfonamide ligands as well as of the set of quinazoline compounds from chapter 4 were collectively described by a QSAR equation. Pharmacological studies revealed that the sulfonamide analogues have excellent H_4R affinity and behave as inverse agonists at the human H_4R. *In vivo* evaluation of the potent 2-(6-chloro-2-(4-methylpiperazin-1-yl)quinazoline-4-amino)-N-phenyl ethanesulfonamide (VUF10519, pK_i= 8.31), revealed it to also have good anti-inflammatory activity in an animal model of acute inflammation.

The two potent histamine H_4 receptor ligands (5-chloro-1*H*-2-yl)(4-methylpiperazin-1-yl)methanone (JNJ7777120, developed by Johnson & Johnson) and 2-(6-chloro-2-(4-methylpiperazin-1-yl)quinazoline-4-amino)-ethanesulfonamide (VUF10558, described in chapter 5) were selected to be developed into potential radioligands for monitoring inflammatory processes with

positron emission tomography (PET). In chapter 6, we describe how the selected compounds were successfully labeled with an [^{11}C]isotope in high radiochemical yield and purity. Evaluation of the radioligands *in vivo* in rats showed two distinct distribution profiles. [^{11}C]JNJ7777120 was found to rapidly enter the central nervous system and was retained in hippocampus and olfactory bulb tissue. This observation suggests the presence of H_4R receptors in these brain areas. In contrast, [^{11}C]VUF10558 did not appear to enter the CNS but was only present in the periphery. The two ligands presented in this study therefore are complementary tools to further characterize the H_4R.

During an in-house database screen we identified *S*-(2-guanidylethyl)-isothiourea as a high affinity agonist (VUF8430) for the histamine H_4 receptor with a 33-fold selectivity over the histamine H_3 receptor and negligible affinity for the other histamine receptor subtypes. In chapter 7 this nonimidazole ligand is introduced as a useful and complementary pharmacological tool that enables further unraveling of the physiological roles of the H_4 receptor. To provide convenient access to this new pharmacological tool a novel synthetic procedure was developed. Using microwave chemistry in the final synthesis step, the compound readily crystallizes from the reaction vessel in high purity. This procedure allows for the straightforward synthesis of VUF8430 in large quantities.