SUMMARY

The work in this thesis describes my PhD research that aimed to improve our understanding of the human histamine H4 receptor (hH4R) and its ligands. The H4R is a member of the G protein-coupled receptor (GPCR) family and is considered an interesting drug target for immunological disorders (Chapter 1).

Previously, my former colleagues have studied several aspects of the H_4R: Herman Lim (elucidation of the H_4R binding pocket), Rogier Smits (design and synthesis of new H_4R ligands), Richard van Rijn (H_4R signalling and quaternary structure) and Enade Istyastono (computational studies of H_4R ligand binding). Although they paved a way for my research project, a lot of questions still remained unanswered: how is H_4R activated? Is $G\alpha_i$ the only downstream signalling protein? And more importantly, what is the exact physiological role of H_4R?

Improved techniques make it nowadays possible to crystalize G protein-coupled receptors to obtain a high-resolution picture of their three-dimensional conformations. The crystallization of the human histamine H_1R allowed us to construct a homology model of the H_4R based on this related structure. This model was used to fine-tune our knowledge on the H_4R binding pocket. It even led us to predict possible ligand - amino acid interactions. The binding pose of the H_4R ligands aminopyrimidines hinted at a close proximity between the H_4R-$C^{3.36}$ residue in TM3 and the aminogroup. We hypothesized that replacement of this aminogroup with a Michael acceptor (ethenyl) results in a covalent interaction between ligand and H_4R. The rational design and pharmacological characterization of this ligand (VUF14480) is described in chapter 2. We showed that VUF14480 covalently binds to $C^{3.36}$ and partially activates the H_4R.

GPCR activation does not always lead to a linear signal transduction, but rather elicit a network-like response involving several different pathways. Biased ligands have the ability to preferentially signal to a subset of these pathways, rather than activating all downstream effectors to a similar extend. The indolecarboxamide ligand JNJ7777120 was shown to induce a biased signal upon H_4R binding. This ligand behaves as competitive antagonists in a $G\alpha_i$-dependent assay, but surprisingly appeared to be a partial agonist in a β-arrestin2 recruitment assay. We investigated several different H_4R ligand classes for $G\alpha_i$ protein dependent and independent (β-arrestin recruitment) assays (Chapter 3). We were able to identify several other biased ligands on the basis of efficacy differences and further analysis of biased factors calculated via the operational model for agonism. Interestingly, all tested indolecarboxamide ligands display a full bias toward the β-arrestin recruitment pathway. We continued to investigate an extensive ligand set with minor structural changes and analysed our data with an *in silico* method that evaluated which groups of the indolecarboxamide analogs were important for this biased behaviour. In addition, with this H4R homology model we were also able to hint at some receptor regions that could be important for (biased) agonism (Chapter 4).

In Chapter 5 we choose a semi high-throughput approach to measure the binding and function of small molecules (fragments) on all members of the histamine receptor family. Knowledge that GPCRs (e.g. H_4R) can activate a β-arrestin2 dependent pathway prompted us to go for a multi-assay approach. We measured radioligand displacement, G protein activation and

β-arrestin recruitment. Biased ligands were found for all four histamine receptor subtypes: H_1R binds β-arrestin-biased fragments, whereas for H_2R and H_3R only G protein-biased fragments could be observed. In contrast, H_4R has both $G\alpha_i$ protein and β-arrestin-biased fragments. We are currently validating the initial screens and plan to further investigate the binding mode and hotspots for histamine receptor agonists, antagonists or biased fragments in future projects.
Chapter 6 describes the development of an improved procedure for the analysis of H_4R ligand metabolites. Metabolic mixtures of H_4R ligands were separated and fractionized in small volumes and subsequently tested in H_3R/H_4R radioligand binding and cell-based signalling assays. This setup makes it possible to detect unwanted metabolites (off target binding or unwanted efficacy) in an early stage of drug discovery.
In short, in this study we introduced and optimized new experimental methods to learn more about the interaction between novel (biased) ligands and H_4R. This extensive set of H_4R tool compounds can be used to further investigate the function of H_4R and its (biased) intracellular signal transduction processes.