CHAPTER 2

AIM AND OUTLINE OF THE THESIS

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Aim of the thesis

Fragment-Based Drug Discovery is now widely acknowledged as a new and efficient drug discovery approach. It has been applied mainly to water-soluble targets that can easily be crystallized, as the success of FBDD is highly dependent on the rational and efficient optimization of identified hit fragments. FBDD on membrane-bound receptors such as ion channels and G-protein coupled receptors are more complicated, as the structural information that is available for these proteins is scarce. Recently, workarounds such as the use of Acetylcholine Binding Proteins (AChBPs) as a water-soluble homologous of the extracellular domain (ECD) (i.e., the ligand binding domain) of Ligand-Gated Ion Channels (LGICs) have been used in FBDD programs in our laboratories. Although these studies lead to enormous progress in fundamental aspects of FBDD and the protein-ligand binding events, and also in the efficient identification and optimization of AChBP ligands, it has proven to be difficult to translate these results to LGICs that are actually relevant in terms of human (patho)physiology.

The aim of this project is to investigate the use of FBDD on LGICs and explore the opportunities and limitations of these approaches on membrane-bound receptors. In the studies described in this thesis, 5-HT3 receptors are selected as an archetypical LGIC and all key aspects of FBDD are explored in this thesis:

1: Use a thoroughly characterized fragment library as input for fragment screening on LGICs.

2: Set up a fragment based screening method to find novel ligands for the 5-HT3 receptor

3: Explore the efficiency of fragment hit optimization of these fragment hits into high affinity 5-HT3 tool compounds, while acknowledging the lack of LGIC X-ray analysis support and having to make use of homology modeling using the aforementioned AChBP data.

4: Exploit the advantages of the high resolution probing of binding sites by fragments (for example by exploring subtype selectivity differences as we found during the project described in this thesis).
Outline of the thesis

This thesis explores the use of Fragment Based Drug Discovery (FBDD) on 5-HT₃ receptors. In chapter 3 the development of a fragment screen for the human 5-HT₃A receptor is shown. The final experimental design uses whole cells and a voltage dependent fluorescent dye. This allows for hit identification and in the same screen, characterization of the hits as being antagonists or agonists. Next to the quality of the pharmacological assay, the quality of the fragment library is very important. To evaluate this quality, the performance of the proprietary fragment library was evaluated by screening the library against a variety of other targets including GPCRs and protein kinases. In chapter 4 we use chemogenomics approaches to analyze the results for the different hit sets and show that screening of our fragment library allows for defining ligand-affinity cliffs and molecular selectivity switches. In chapter 5 we show that the hit set found for the 5-HT₃ receptor showed remarkable overlap with the hit set found for the G protein-coupled histamine H₄ receptor (H₄R). Using an array of chemoinformatics analysis it is shown that there are similarities in ligand recognition between 5-HT₃R and H₄R. Finally, these fragment-based chemogenomics studies suggest a delicate balance between ligand complexity and target selectivity. Chapter 6 describes the successful hit optimization of a fragment that was identified in chapter 3, by designing and synthesizing several novel 5-HT₃ receptor ligands. This resulted in a ligand with enhanced affinity for the 5-HT₃R and an improved selectivity over the H₄R. A homology model for the 5-HT₃ receptor binding site was developed which enhanced our understanding of the specific interactions involved in 5-HT₃ receptor binding. It was hypothesized that several ligands make interaction with a conserved water network that is stabilized by both receptor and ligand. For another fragment hit we observed a small difference in binding affinity for 5-HT₃A and 5-HT₃AB receptors. In chapter 7 the successful optimization of this initial hit compound towards compounds that show selectivity towards either the 5-HT₃A or 5-HT₃AB receptor is described. The most selective compound showed a <100 fold selectivity for the homomeric 5-HT₃A over the heteromeric 5-HT₃AB receptor. These compounds are the first selective ligands that bind to the orthosteric binding site. The pharmacological profile of these ligands is very remarkable, considering the high similarity between 5-HT₃A and 5-HT₃AB receptors and between the ligands themselves. In chapter 8 the pharmacological properties of the most selective compound (VUF10166) are further investigated and it is proposed that VUF10166 binds at the common A+A- site of both receptor types, and to a second A+B-modulatory site in the heteromeric receptor. Finally in chapter 9 the research described in this thesis is evaluated, the overall conclusions are summarized and future perspectives are given.
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