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SUMMARY

During the course of drug discovery translational steps are made. The translation from *in vitro* to *in vivo* experiments is not as predictive as one would desire, resulting in selection of inefficacious compounds but also in overlooking of promising drug candidates. This is not different for the $mGlu_2$ receptor for which no drugs are available on the market so far despite enormous drug discovery efforts. Therefore, there is a need to improve the molecular understanding of key *in vitro* parameters that drive *in vivo* efficacy. Hence, this thesis focuses on the concepts of target binding kinetics and functional efficacy of both allosteric and orthosteric ligands of the $mGlu_2$ receptor. **Chapter 1** introduces these main concepts studied throughout the thesis. The chapter starts with an introduction to G protein-coupled receptors (GPCRs), with a focus on the $mGlu_2$ receptor. Subsequently, the concepts of allosteric modulation, binding kinetics and covalent receptor binding are described. In **Chapter 2**, JNJ-46281222 is introduced and characterized extensively. This highly potent positive allosteric modulator (PAM) is used throughout the different chapters as a prototypical tool compound. Furthermore, the mechanism of positive allosteric modulation of the $mGlu_2$ receptor is studied. JNJ-46281222 behaved as a typical PAM, increasing the affinity and potency of agonists. Next to that, JNJ-46281222 behaved as PAM agonist with submaximal efficacy at higher concentrations compared to the efficacy of the endogenous agonist glutamate. A two-way mechanism of allosteric modulation was postulated, as the maximum binding capacity of JNJ-46281222 was increased by the presence of the agonist glutamate, whereas glutamate left the affinity of JNJ-46281222 unchanged. On the other hand, the presence of GTP (which initiates dissociation of the G protein) decreased JNJ-46281222 binding indicating that the PAM prefers the G protein-bound state of the receptor. Computational docking and molecular dynamics studies were used to visualize and understand the PAM binding mode. These experiments were followed by receptor mutagenesis experiments which confirmed the binding mode of JNJ-46281222.

Chapter 3 describes the first kinetic study of orthosteric ligands at the $mGlu_2$ receptor. After the set-up of an assay enabling the quantification of target binding kinetics, kinetic parameters of the endogenous agonist glutamate were determined followed by those of other orthosteric ligands. To increase the understanding of the binding mechanism and effect of allosteric modulation on this process, experiments were repeated in the presence of a PAM and a NAM (negative allosteric modulator). We found that affinity is strongly correlated to the association rate constant k_{on}, showing that on-rate is driving affinity and thus target occupancy of orthosteric $mGlu_2$ ligands. In contrast to the wide range of on-rates, dissociation rate constants (k_{off}) were all within a small 6-fold range. Functional assays showed that the presence of a PAM not only increased the duration of (orthosteric) ligand binding, but also the duration of ligand efficacy.

In **chapter 4**, an extensive structure-kinetics relationships (SKR) study was performed using 41 novel $mGlu_2$ PAMs all bearing the 7−aryl-1,2,4-triazolo[4,3−*a*]pyridine-scaffold. In addition to classical parameters of affinity and potency, kinetic parameters were determined and residence times (RTs) were calculated. To this end, a kinetic radioligand binding assay, a so-called scintillation proximity assay (SPA) was developed. The novel PAMs showed various kinetic profiles in which k_{on} values ranged over three orders of magnitude, whereas k_{off} values were within a 10-fold range. Like in **chapter 3**, k_{on} was the driver for affinity, showing that this is likely receptor-specific. Even though RTs were within a small range, we showed in a functional assay that PAMs with divergent RTs showed different duration of action. Ultimately, a long and a short RT PAM were evaluated for their *in vivo* efficacy, which provided the first hint that *in vivo* efficacy of $mGlu_2$ PAMs benefits from a longer *in vitro* RT. Together, the results obtained in **chapters 3** and **4**, have shown the importance of target binding kinetics for drug design of novel $mGlu_2$ receptor ligands. Therefore, experiments that enable quantification of kinetic parameters k_{on}, k_{off} and RT will be a valuable addition to the experimental set-up used in drug discovery.

Covalent labelling of GPCRs is a powerful approach to gain further understanding of ligand binding, mechanism of action, receptor expression patterns, receptor pharmacology and it may ultimately facilitate structure elucidation. **Chapter 5** describes the design, synthesis and pharmacological characterization of the first covalent PAM probe for a class C GPCR. Furthermore, the compound was used to study its receptor binding mode using computational modelling that also identified the amino acid residue likely responsible for covalent binding, which was confirmed in receptor mutagenesis experiments.

Chapter 6 describes the set-up of a label-free biosensor assay that enables studying $mGlu_2$ receptor pharmacology without the need of any label. After optimization of assay conditions, typical agonist, antagonist, PAM and NAM responses were monitored. Interestingly, constitutive activity of the $mGlu_2$ receptor was found in this system and LY341495 behaved as inverse agonist, which had not been shown before as the compound is commonly considered

a classical (neutral) antagonist. The $mGlu_2$ receptor is thus the first class C GPCR extensively characterized by a label-free biosensor, opening new possibilities to study receptor pharmacology and novel concepts of receptor activation.

Finally, the general conclusions obtained throughout the different chapters of this thesis are discussed in **chapter 7**. Together, the results obtained in this thesis contribute to the understanding of the mechanism of action of the $mGlu_2$ receptor at a molecular level and have shown the importance of target binding kinetics for drug discovery. The novel insights that have been obtained throughout this thesis provide valuable information for future drug discovery projects targeting the $mGlu_2$ receptor as well as other GPCRs.