Abstract

Free fatty acid receptors 1 (FFA1/GPR40) and 2 (FFA2/GPR43) are G protein-coupled receptors activated by medium-long free fatty acids (FFAs) and short FFAs, respectively. FFA1 is mainly expressed in pancreatic β-cells, whereas FFA2 in immune cells and adipocytes. They are involved in the control of metabolic homeostasis and immune reactions. Specifically, FFA1 is now an established target for the treatment of type 2 diabetes (T2D) and FFA2 is implicated in inflammatory conditions. The long-term goal, that extends beyond the scope of this project, is to find efficacious and safe drugs targeting FFA1 and FFA2. To this end, it is critical to understand the pharmacology of these receptors. Therefore, the aim of this project is to develop tool compounds for FFA1 and FFA2, and to use them in functional and fluorescence-based assays.

This thesis dedicates one chapter to each part of the project: the first two chapters present the design, synthesis and application of fluorescent tracers targeting FFA1 and FFA2, respectively, and the third describes the exploration of bioisosteric replacements on orthosteric FFA2 antagonists. In addition, Manuscript 1, in Appendix, focuses on the structure-activity relationship (SAR) study of FFA2 antagonists presenting a tetrazole group.

Recent studies on FFA1 revealed the presence of an additional allosteric binding pocket, located in the transmembrane region of the receptor. Thus, we designed fluorescent tool compounds based on the structure of agonists targeting this pocket to elucidate their binding mode and their interplay with other classes of known ligands. The developed compounds have been used to establish a Bioluminescence Resonance Energy Transfer (BRET) assay to measure ligand binding affinity. Regarding FFA2, it is still unclear if agonists or antagonists are preferred in a therapeutical setting, so we set out to study both classes. Based on two series of allosteric agonists, fluorescent tool compounds were designed and synthetised. Results showed that the two series of allosteric agonists are positively cooperating with each other. To study the binding of these classes of ligands, a BRET assay will be established with the developed fluorescent compounds. Lastly, FFA2 inhibition by orthosteric antagonists is strongly implicated in reducing dysregulated inflammation. To date, there is no antagonists with activity on mouse or rat receptor orthologs, hindering the progression of drug candidates in clinical trials. Therefore, based on encouraging preliminary data on the mouse FFA2 (mFFA2) receptor, a series of FFA2 antagonists presenting bioisosteric replacements have been

synthesised and assessed in a functional assay. Although high activity on the mFFA2 has not been achieved yet, highly potent antagonists of human FFA2 with favorable physicochemical properties have been developed, as described in Manuscript 1.