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Protein design and crystallization trials for constitutively active CysLT2R with oncogenic L129Q mutation

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Motivation and Aim: CysLT1R and CysLT2R are G protein-coupled receptors (GPCRs) activated by cysteinyl leukotrienes. They are key inflammatory mediators in the human body and stimulate bronchial muscle constriction as well as immune cells migration, mucus secretion and edema formation, thus playing an important role in various inflammation-related disorders, such as asthma, allergic rhinitis and urticaria. Additionally, through the immune response mediation CysLT1-2Rs are involved in cardiovascular and neurodegenerative diseases and several types of cancer. L129Q mutation transforms CysLT2R to a constitutively active state and is proved to be a driver oncogenic mutation in uveal melanoma [1, 2], meningeal melanocytic tumour [3], leptomeningeal [4] and blue nevi melanocytic tumors [5].

While there are several diverse antagonist families for CysLT1-2Rs there are no inverse agonists targeting CysLT2R L129Q and their design is remaining to be a challenging task. We believe that novel antagonists may be developed into better substitution of existing drugs against asthma and inverse agonists may be developed into first in class drugs against uveal melanoma, meningeal melanocytic tumour, and leptomeningeal and blue nevi melanocytic tumors.

Methods: We optimized primary sequence of CysLT-L129Q mutant receptor with stabilizing point mutations, truncations and fusion tags. Sf9 insect cells were used for protein expression. Receptors were purified with metal-affinity chromatography and checked for its thermostability and homogeneity. Monodisperse protein is being crystallized in lipidic cubic mesophase.

Results: Previously our team has solved several high-resolution crystal structures of CysLT1 and CysLT2 receptors [6, 7] in complexes with antiinflammatory compounds Zafirlukast and Pranlukast for the first subtype, and 3 drug candidate compounds for CysLT2R (see publications 1 and 2). Given 3D-structures helped us to perform structure-based drug design with experimental characterization by cell-based functional assays. After extensive virtual ligand screening with ENAMINE we have obtained five novel antagonist chemotypes with relatively small MW about 400 Da and cLogP about 3.5 that gives the room for further optimization. Two of them show inverse agonism against CysLT2R-L129Q mutation confirmed by functional assays, but nevertheless we want to obtain more compounds with independent scaffolds and better potencies, while lacking of the off-target effects. In order to do this, we are aimed at structure determination for CysLT2R-L129Q mutant form (not a wild-type protein). We believe, that this structure

will serve as a better template for the structure based drug design and will give more explanation for the molecular mechanism of the CysLT2R-L129Q constitutive activity and corresponding inverse agonism. Here we present our protein design strategy for re-optimizing the mutant protein and results of our crystallization trials.

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