An experimentally validated binding mode model of TGR5 agonists

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The structurally unknown G-protein coupled bile acid receptor (GPCR) TGR5 is the first bile acid sensing GPCR and directly interacts with $G\alpha_s$, $G\alpha_{i3}$, and $G\alpha_q$ subunits of the G-proteins [1]. TGR5 is highly expressed in the brain, the liver, and the gastrointestinal tract. Furthermore, TGR5 is an emerging target for the treatment of metabolic diseases [2-4]. Hence, much effort has been dedicated to the synthesis of potent and selective TGR5 agonists [5]. However, the lack of an experimentally determined binding mode makes the rational design of compounds with improved activity difficult. Recently, Macchiarulo *et al.* [6] proposed a binding mode of natural and synthetic bile acids in TGR5 based on single template homology modeling, molecular docking, and mutational analysis. However, this binding mode lacks interactions with transmembrane helices (TM) 5 and 6, which are considered essential for GPCR activation [7, 8]. Furthermore, the binding mode of Macchiarulo *et al.* does not address E169 in TM 5, which is a conserved residue within the TGR5 family and important for receptor activation [6].

Here, we present an experimentally validated binding mode of 68 TGR5 agonists, including natural and synthetic bile acids and neurosteroids. We employed a combined strategy of multi-template homology modeling, molecular docking, and structure-based 3D-QSAR analysis using the AFMoC approach with subsequent mutational analysis and molecular dynamics simulations. After two cycles of this strategy, the superimpositioning of all ligands within the orthosteric site of TGR5 results in a good AFMoC model (q^2 = 0.50), thus indicating that differences in the agonist structures correlate with differences in experimentally determined pEC_{50} values in the predicted binding mode. Based on this binding mode, mutations of eight amino acids were suggested that should either influence agonist binding or TGR5 activation. Activity analysis using cAMP reporter gene assays and FACS analysis for membrane localization confirmed these predictions in all cases. This provides strong support to the validity of the binding mode. Our binding mode differs from the binding mode by Macchiarulo *et al.* in three important aspects: I) The cholane moiety is rotated by 180°; II) the sidechains of bile acids bind to R79, which is 12 Å away from the respective interaction partner postulated in [6]; III) agonists address residues in TM 5 and 6, which are essential for receptor activation. The binding mode is expected to aid in the structure-based design of new TGR5 agonists.

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