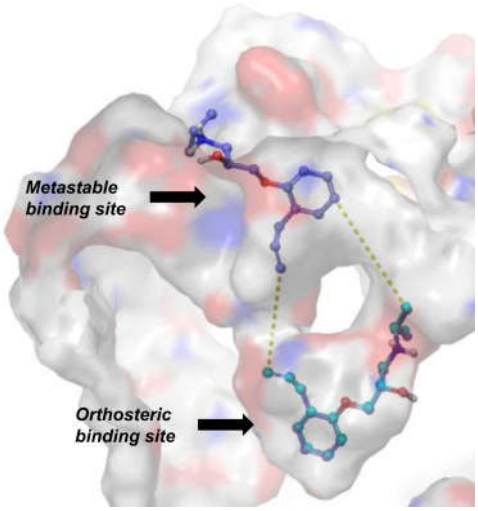
Abstract

G protein-coupled receptors (GPCRs) constitute the largest and most diverse family of eukaryotic membrane receptors and hence are an important target in human drug therapy. To date, however, it has been difficult to obtain subtype selective GPCR ligands due to orthosteric binding pockets generally being highly conserved across the receptor subtypes and the general lack of structural information on allosteric binding sites. Molecular dynamics simulations on ligand binding to GPCRs revealed that ligands pause at transient, so-called metastable binding sites on their way to the orthosteric pocket. Metastable binding sites are generally situated in less conserved receptor areas, and thus, bitopic ligands that link two identical pharmacophores to simultaneously target the orthosteric and a metastable binding site may provide ligands with improved affinity and receptor subtype selectivity.

Based on the docking of known beta-blocker (*S*)-alprenolol into the orthosteric and a metastable binding site at the β_2-adrenergic receptor, potential symmetric and non-symmetric bitopic alprenolol ligands with varying linker lengths and compositions were proposed. Their syntheses were divided into two main steps, i) construction of the alprenolol pharmacophores and ii) establishment of the linker. For the generation of the linkers, Wittig reactions, alkylations, amide couplings, aza-Wittig chemistry and CuAAC reactions were employed.



Pharmacological testing of the bitopic ligands indicated that they competitively antagonize the receptor, some of them with potencies in the range of (*S*)-alprenolol and slightly increased affinities, others with slightly improved selectivity profiles. Linker types with varying degrees of hydrophobicity and rigidity were found to be tolerated by the receptor, and the optimal linker length seemed to depend on the linker's rigidity. A truncation study on selected compounds indicated that the major contribution from the metastable pharmacophore relies on a hydrophobic interaction with the receptor. A bitopic binding mode with the two pharmacophores in the orthosteric and metastable binding sites could not be proven in the present project, but the obtained pharmacological data could indicate that targeting metastable binding sites is possible and can lead to improved pharmacological profiles.