ANTAGONIZING S1P3 RECEPTOR WITH CELL-PENETRATING PEPDUCINS IN SKELETAL MUSCLE FIBROSIS

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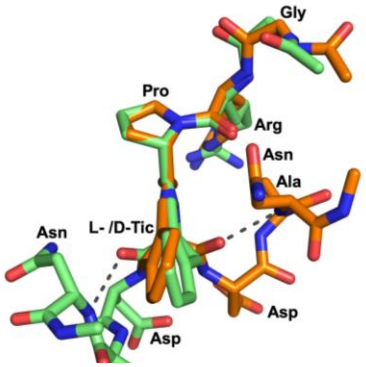
Bioactive lipids, derived from the metabolism of plasma membrane, are important mediators of cellular signaling in vertebrates. In recent years there has been a growing interest on sphingosine-1-phosphate (S1P) which is the final metabolite produced during the sequential degradation of plasma membrane glycosphingolipids and sphingomyelin. The S1P acts through five known subtypes of heptameric G-protein coupled receptors (GPCR), namely S1P1-S1P5 (S1PR). Recent evidence indicates that S1P signaling axis contributes to the development and maintenance of the fibrotic process [1]. Fibrosis is a pathological condition that can affect every organ, consequence of a persisting inflammatory and tissue remodeling condition. In different fibrotic models an extensive crosstalk between TGFβ and S1P signaling axis has been demonstrated. S1P3 plays a pivotal role in fibrosis development in different tissues such as skeletal muscle, liver, and kidney [2]. Thus, selective antagonists of the S1P3 receptor could be useful to deeply study its role in fibrosis as well as to develop new therapeutic entities to treat fibrotic diseases.

Pepducins specifically target the intracellular loops, acting as allosteric modulators of GPCR activity. Using this approach, we have synthesized a pepducin based S1P3 antagonist namely **KRX-725-II** (Myristoyl-GRPYDAN-NH_2) [3]. Here to improve the S1P1 vs S1P3 selectivity, we have synthesized several derivatives of **KRX-725-II** pointing our attention on the aromatic residue of the sequence, Tyr4, and with the aim to introduce molecular constraints. The new molecular entities have been evaluated for their selectivity profile by using mouse aortas. This screening allowed us to identify compounds **V** and **VII** (embodying respectively L- and D-Tic) as the most selective S1P3 antagonists. The selected compounds also displayed the ability to significantly reduce the profibrotic action of TGFβ1 in C2C12 myoblasts. To explain the higher selectivity observed for compounds **V** and **VII**, they were analyzed by Molecular Dynamics (MD) Simulations. The middle conformations of **V** and **VII** were compared by superimposing their GRP residues, which adopt a similar backbone orientation (see Figure). This revealed that the DAN residues with β-turn-like motif are located on opposite sides of the plane defined by the L- or D-Tic residue. This difference may explain, in structural terms, the selective S1P3 antagonism of **V** and **VII** in comparison to the unselective antagonist **KRX-725-II**, whose flexibility seems to be high enough for the adaptation to the binding regions of the individual receptor subtypes S1P1 and S1P3. Peptides **V** and **VII** possess, indeed, a highly constrained D- or L-Tic residue that hinder the pharmacophore from interacting properly with the binding pocket of the S1P1 receptor, therefore leading to S1P3 selectivity.



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