Cyclophilin-D inhibitors rational and fragment based design: From 7 mM to 7 nM potency

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Abstract:

Cyclophilins are folding helper enzymes member of the Peptidyl Proline Isomerases (PPI) superfamily. PPI are extremely challenging targets and the druggability of this target class has been partly demonstrated in the 1990's with Cyclosporin A (CsA) which is still used in the prevention of transplant rejection. The immunosuppressive activity of CsA is due to its calcineurin binding domain, however several analogues have been reported as potent cyclophilin inhibitors with reduced affinity for calcineurin. Some of them have reached clinical phase (Debio25 for HCV), but very few low MW inhibitors have been reported.

Our research was focused on cyclophilin D (CypD) because of its implication in mitochondrial function. CypD regulates pore opening of the mitochondrial permeability transition pore (MPTP) and therefore plays a significant role in the pathological process driving mitochondria dysfunction. To avoid immunosuppressive activity and obtain improved druglike properties, we aimed to develop low MW inhibitors exclusively. For that purpose, we set up a unique platform for robust testing of small molecules with potency ranging from mM to nM in CypD binding and enzymatic assays. After the HTS campaign (using a fluorescent based binding assay) did not return any confirmed positives, we decided to base our hit discovery strategy solely on SPR Fragment Based Screening combined with knowledge based design.

The screening of the EMDSerono fragment collection provided 58 confirmed positives with moderate to low Ligand Efficiency (0.1 to 0.3) from which only six crystal structures in CypD were solved. Fragment growing and linking work from two proprietary 3D fragments originated from the Edelris collection produced nM inhibitors after only two to three optimization cycles.