Applying Nature-Inspired 3D-Fragments towards the Discovery of nM Inhibitors of Cyclophilin D

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Introduction

Cyclophilins (Cyp) are member of the Peptidyl Proline Isomerases (PPIases) superfamily, which catalyze the cis/trans isomerization of the peptide bond at proline residues. They are involved in numerous biological processes with key roles in cancer, neurodegeneration, psychiatric disorders and viruses life cycle[1]. So far, potent Cyp inhibitors used in clinic or tested in human are all macrocyclic peptides. A first campaign of high-throughput screening involving the EMD Serono compound library failed to deliver validated hits. Here we disclose the results obtained from the identification of original 3D-fragments and the merging strategy that led to a non-peptidic highly active Cyclophilin D inhibitors.

3D-fragments inspired by natural products

Edelris has developed an unique collection of fragments inspired by natural products. The underneath 2-ABN scaffold illustrates the design and synthetic efforts to build a highly innovative fragment collection multiplying possibilities of exit vectors combinations and highly diversified in pharmacophores. Those fragments were selected by EMD Serono to enrich their fragment collection particularly for screening challenging targets like Cyclophilin D.

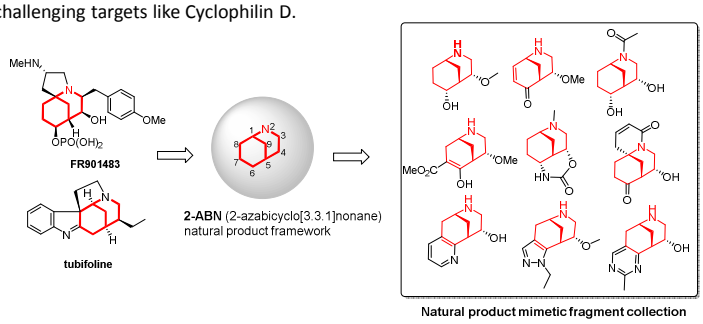
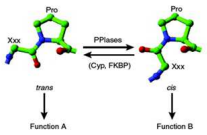


Figure 1 : 3D-Fragments inspired by natural products

Cyclophilin D inhibitors

Cyclophilin D (CypD) act as a key regulator of the mitochondrial permeability transition pore (mPTP), which plays a major role in calcium efflux from mitochondria to the cytosol and can lead to mitochondrial swelling or cell death[2].



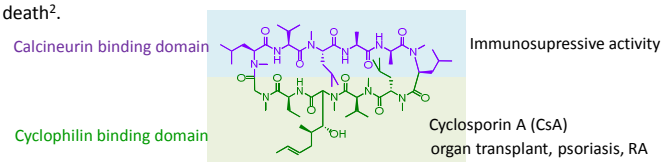


Figure 2 : Isomerization of peptide bond *via* PPIase and Cyclosporin A bindings domain

Cyclosporin A (CsA) is a natural ligand of Cyclophilin D. This macrocyclic peptide has two binding domains involved in different biological processes. The Calcineurin binding domain is responsible for the immunosuppressive activity of CsA. We aimed to develop new small molecule inhibitors of CypD *via* FBDD by mimicking the peptidic cyclophilin binding domain of CsA.

Primary Screening of fragment collection

Screening of the EMD Serono fragment collection (2,500 cpds) led to the identification of 58 hits (K_D<60mM) of which 6 were resolved with X-Ray structures[3]. Structural information revealed a new binding pocket explored by two fragments from Edelris collection. These 2 fragments share in common an aniline residue leading to a similar binding mode.

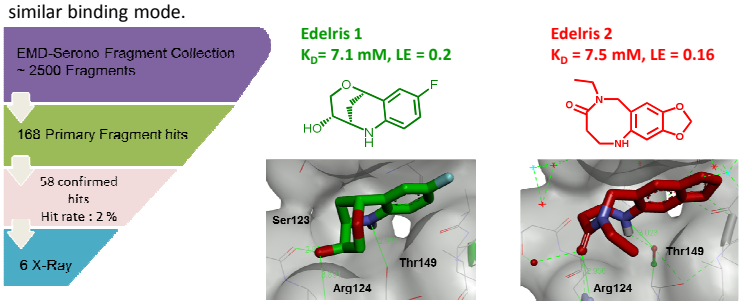


Figure 3 : FBDD process and structure of 3D-fragments identified as hits

Optimization and merging strategies

Analysis of the X-ray structure of CypD with or without ligand drove us to grow our fragment through the "Proline Binding Pocket" of the CsA.

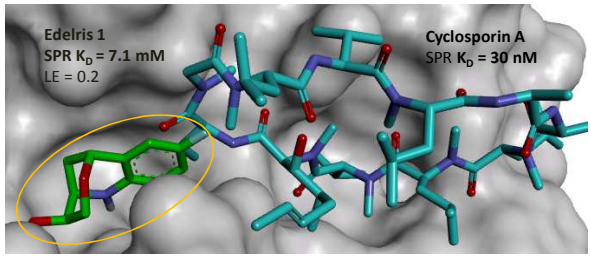
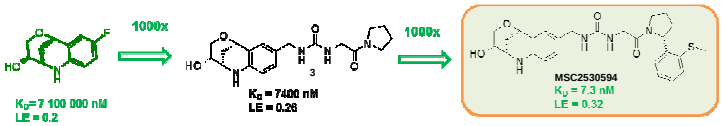


Figure 4 : Superimposition of X-ray structures of CsA and Edelris 1

In a first round of optimization, pyrrolidine derivatives were merged with Fragment 1 *via* an urea bond. Compound **3** was quickly found as a 1,000 fold more active molecule than the parent fragment. Coupling our fragment with Guichou's pyrrolidine[4] allowed a further 1,000 fold gain leading to **MSC2530594** K_D=7.3 nM (SPR) *vs* 30 nM for CsA.



Scheme 1: Optimization from Edelris 1 to MSC2530594

The identified 3D-Fragment allows a 10^6 potency improvement in two optimization cycles through an improved space occupancy of the aniline pocket and the creation of interactions with two additional residues (Arg124 and Ser123).

MSC2530594:
SPR : **K_D = 7 nM,** LE = 0.32
Binding : IC_{50} = 31 nM
PPI : IC_{50} = 4 nM
PPI (NMR): inhibition

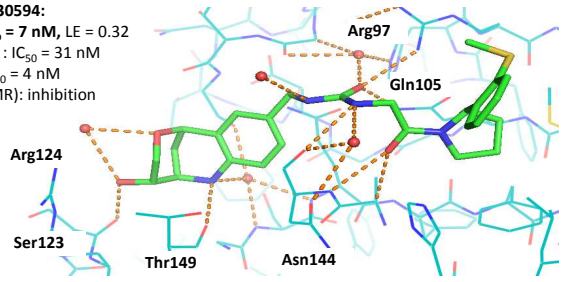
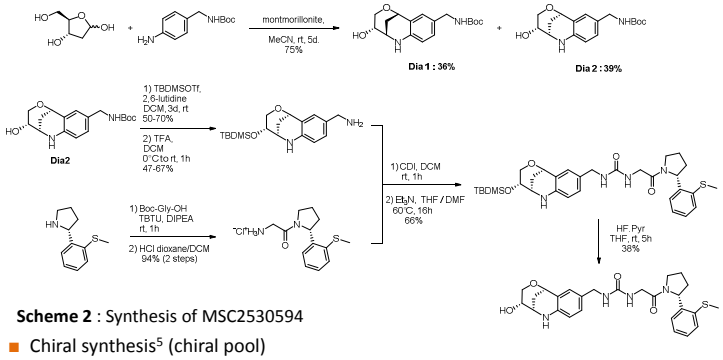


Figure 5 : X-Ray Structure of MSC2530594 in CypD

Synthesis



Scheme 2 : Synthesis of MSC2530594

- Chiral synthesis[5] (chiral pool)
- Diastereomers separated by flash chromatography at step 1
- Sec-alcohol protected to ensure stability of benzyl-amine deprotection

Conclusion

Original 3D-fragments inspired by natural products allowed the identification of a new binding pocket in CypD that was unexplored by CsA and other more classical fragments. Our merging strategy coupled with synthetic know-how and intensive use of structural information (X-Ray) allowed us to quickly discover a new small molecule with nanomolar activity on CypD. This example demonstrates the high potential of 3D-Fragments for FBDD on complex targets.

References : B. M. Dunyak *et al., J. Med. Chem.* **2016**, *59*, 9622–9644 2) S. Javadov *et al., Front. Physiol*. **2013**, *4*, 76 3) X-Ray crystallography performed by Proteros biostructures GmbH 4) A. Ahmed-Belkacem *et al., Nat. Commun* **2016**, *7*, 12777; J.-F. Guichou, L. Colliandre, **2011**, WO2011076784 5) C. Du et *al., J. Org. Chem.* **2011**, *76*, 8833-8839 ; J.S. Yadav, *Synthesis* **2006**, *17*, 2923-2926