**Cyclophilin Screening in a Real Time Fluorescence Monitoring System**

**1. Introduction**

Cyclophilins are a family of peptidyl prolyl isomerase (PPIase) enzymes which play a crucial role in the functions of the mitochondrial permeability transition pore (mPTP). The mPTP is a central event in cell death leading to disruption of the mitochondrial membrane. Cyclophilin A (CypA) is an immunosuppressant drug used in many instances from organ transplant to psoriasis. But its adverse drug reactions (ADR) are numerous and severe: convulsions, pancreatitis, nephrotoxicity, hepatotoxicity among others. When CypA binds to CypD, it inhibits the mitochondrial permeability transition pore opening and prevents cell death.

**2. Material and Methods**

Assays are performed on the FDSS7000 from Hamamatsu Photonics. This instrument allows dispensing and measurement in fluorescence and luminescence of 384 well plates at once.

**3. Substrate titration**

The first set of experiments performed a substrate titration between 0 and 100 µM. The aim of this experiment is to determine the concentration of substrate (Suc-Ala-Ala-Pro-Phe-MCA) which is specific for each enzyme. Values for KM, Kcat and Kcat/KM are displayed in Fig. 2 and are all in accordance with literature.

**4. Cyclosporin A dose response**

The second set of validation experiment is a Cyp dose response with Cyclophilins A and D in the FDSS7000. These dose responses allowed us to calculate pIC50 for each enzyme, 5.78 for Cyclophilin A and 3.75 for Cyclophilin D which is in accordance with literature (Fig. 3).

**5. Validation with tool compounds**

GSK library contains several compounds with known affinity for Cyp A or D and 3 of those were used to validate the assay protocol in the FDSS7000. Table 1 shows the three compounds used to screen for inhibitors of Cyp A or D in the FDSS7000.

**6. Screening**

After validation of the assay protocol on the FDSS7000, a small set of compounds was screened (50 compounds were tested in a single shot at 100 µM and the 25 compounds in both CypA and CypD were evaluated at 3 different dose responses.

**7. Conclusion**

The aim of this study was to validate on Cyclophilins A and B the screening method described by Mori et al. in 2009 (Cyclophilin A was used on the FDSS7000 which allows for simultaneous injection and measurement of all 384 wells in a plate, this feature is critical in fast enzymatic reactions like the one studied here. Validation steps performed here allowed us to determine CypA (molar constant KM, Kcat, pIC50) and CypD (molar constant KM, Kcat, pIC50) in accordance with literature. These data demonstrate that this protocol is adapted to CypA and CypD for fast time Fluorescence Monitoring. Our study was taken a step further with a small library screening (143 compounds) which yielded 25 positive hits. Taken together, these results demonstrate that the FDSS7000 can be successfully used to screen for inhibitors of Cyp A or D in HTS.

Fig. 1: Left: Chymotrypsin reaction with its compound. Right: Schematic presentation of the reaction.

Fig. 2: Values for KM, Kcat and Kcat/KM

Fig. 3: Dose response for Cyp A (left) and Cyp D (right)

Fig. 4: Response curves of 3 GSK compounds

Fig. 5: FDSS7000EX Hamamatsu Photonics

Fig. 6: Screening % inhibition in SS

Table 1: Summary of Screening Data