**RESULTS**

**Comparison of trichostatin A affinity for HDACs class I & II, and HDACs class III & IV**

**Comparison of SAHA affinity for HDACs class I & II, and HDACs class III & IV**

**Sirtrins activation and inhibition interest**

**IC50 values for selective- and pan-inhibitors of HDACs**

**AIM OF THE STUDY**

The growing interest in HDAC inhibitors has motivated us to develop an HDAC platform dedicated to the screening of HDAC inhibitors. Hence, the purpose of this project was to develop HDAC assays with optimal parameters determined and validated as described in material and methods section. All assays were then adapted to a common format to allow the screen of compounds with automated systems.

**MATERIAL AND METHODS**

The HDAC fluorogenic activity assay/disk discovery kit is supplied by BPS Biosciences. This assay is based on the combination of the Fluor de Lys Fluorescent Histone deacetylase (HDAC) assay and the Fluor de Lys Histone trimethylation substrate. HDAC inhibitors that cleave the deacetylase substrate in a HDAC dependent manner produce a fluorescent signal. The emitted light is detected at 460nm (excitation wavelength = 360 nm).

All HDACs assays, were developed using human recombinant enzymes from BPS Biosciences. Recombinant enzymes are closed in baculovirus expression system. The substrate is adapted to the tested enzyme.

**METHODS USED TO DETERMINE THE PARAMETERS OF THE REACTION**

HDACs are enzymes with one substrate. For this reason the Michaelis-Menten equation can be applied. Hence, parameters of the reaction are determined as follows:

- **Km** value was determined from a saturation experiment in which the substrate concentration tested correspond to a range from 1/3 Km to 3 Km, Km is determined using Prism.
- **Other parameter** pH, ionic strength and temperature are the ones described in the literature.

**CONCLUSION**

The developed platform is:

- **Robust**: Homogeneous results with 95% CV < 10% and Z > 3.0.0.
- **Reproducible**: Less than 1/3 log variations of IC50 values and good reproducibility of signal over several independent experiments.
- **Relavant**: Able to distinguish between selective- and pan-inhibitors. Results in agreement with literature.