Mandeep Mann
2018/05/08

Testing Selectivity of USP5 Zf-UBD Analogues with SPR Assay

Objective: Use a surface plasmon resonance (SPR) assay to determine binding affinities of commercial compound analogues against the zinc finger ubiquitin binding domain (Zf-UBD) of USP5 and test for selectivity against HDAC6 Zf-UBD

Experiment & Results:

A) Chip Preparation

An SA (Biacore) chip was used in a Biacore T-200 system. The chip was equilibrated with 20 mM Hepes pH 7.4, 150 mM NaCl, 0.005% Tween-20 (v/v), 0.5% DMSO (v/v) and then primed with 3x60 s injections of 50 mM NaOH to all chip channels. Biotinylated $USP5^{171-290}$ was injected to chip channel 2, 3 for 300 and 150 seconds respectively. Biotinylated $HDAC6^{1109-1215}$ was injected to chip channel 4 for 150 seconds. All chip channels were then injected with 5x10 sec buffer. Channel 1 was left blank as a reference.

B) Plate Preparation

UBXML78 and XSR00035795a were used as positive controls. Controls and compounds were prepared in 20 mM Hepes pH 7.4, 150 mM NaCl, 0.005% Tween-20 (v/v), 0.5% DMSO (v/v) buffer. Samples were diluted 1:4 in a 9-point concentration series starting at 1 mM in a 96-well plate. The plate was sealed and centrifuged at 1000 RPM for 1 minute.

C) Assay

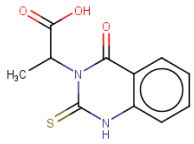
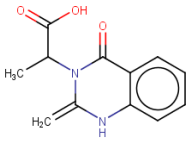
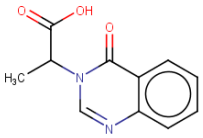
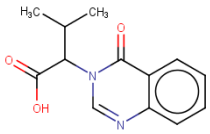
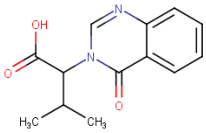
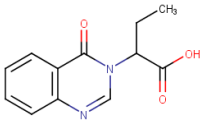
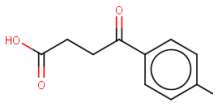
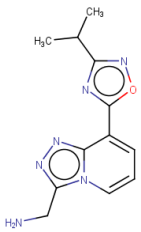
A multi-cycle kinetics method was run for the plate with the following parameters:

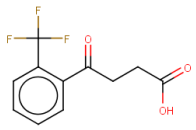
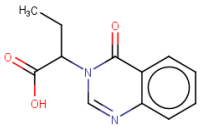
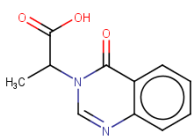
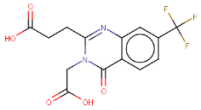
- Contact time: 120 s
- Dissociation time: 120 s
- Flow rate: 30 µL/min
- Running buffer: 20 mM Hepes pH 7.4, 150 mM NaCl, 0.005% Tween-20 (v/v), 0.5% DMSO (v/v)

Sample injections were done sequentially by compound from lowest to highest concentration. Data was fitted with a steady state affinity model.

It is to be noted that there was an **error** in the degasser system, due to a vacuum failure. This does not affect instrument operation but sensograms are affected by air spikes. Table 1 summarizes the experimental results of tested compounds. Please see attached .ppt file for experimental binding curves and sensograms.

Table 1. Summary of Hit Analogues with SPR

Compound Name or Catalog Number	Compound Structure	SMILES	USP5 Zf-UBD K_D (µM) (n=2)	HDAC6 Zf-UBD K_D (n=1)
Z99599878		S=C1Nc2c(cccc2)C(=O)N1C(C)C(=O)O	NB	NB
Z327330246		N1c2c(cccc2)C(=O)N(C1=C)C(C)C(=O)O	NB	NB
MFCD02363436		N1(C=Nc2c(cccc2)C1=O)C(C)C(=O)O	49 ± 1	12
STOCK6S-31603		N1(C=Nc2c(cccc2)C1=O)C(C(C)C)C(=O)O	NB	NB
F3351-0339		CC(C)C(N1C=NC2=CC=CC=C2C1=O)C(O)=O	NB	NB
F3351-0338		C1=CC=CC2=C1C(N(C=N2)C(C(O)=O)CC)=O	NB	NB
MFCD00459040		OC(=O)CCC(=O)c1ccc(cc1)C(C)C	82 ± 1	50
BS-11825		[n]21c(nnc2CN)C(=CC=C1)c3nc(n[o]3)C(C)C	NB	NB

MFCD00033285		FC(F)(F)c1c(cccc1)C(=O)CCC(=O)O	NB	NB
D715-279		N1(C=Nc2c(cccc2)C1=O)C(CC)C(=O)O	NB	NB
XSR00035795a		N1(C=Nc2c(cccc2)C1=O)C(C)C(=O)O	36 ± 1	10
UBXML78		C(CC(O)=O)C1=Nc2cc(ccc2C(N1CC(O)=O)=O)C(F)(F)(F)	34 ± 1	1.8

NB= no significant binding (i.e. K_D > 1000 µM)

Conclusions & Future Directions:

In previous experiments, compound XSR00035795a showed increased ligand efficiency and potency to USP5 Zf-UBD; however, it was also found to bind HDAC6 Zf-UBD. We hypothesized that it was possible to extend the non-polar group on the carboxylic chain to further increase potency and potentially improve selectivity towards USP5 Zf-UBD. Follow-up compounds (STOCK6S-31603/F3351-0339, F3351-033/, D715-279, Z99599878 and Z327330246) showed no significant binding (K_D >1 mM), suggesting extending the aliphatic group on the carboxylic chain is not positive for binding.

Next, we'll be looking for different compound scaffolds through a larger virtual screen using Molecular Forecaster's FITTED docking platform.