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Testing Hit Analogues against USP5 Zf-UBD with SPR Assay #1

Objective: Use a surface plasmon resonance (SPR) assay to determine binding affinities of commercial compound analogues previously selected to explore the structure activity relationship (SAR) around our primary hits against the zinc finger ubiquitin binding domain (Zf-UBD) of USP5.

Experiment & Results:

A) Chip Preparation

An SA 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 3x60s injections of 50 mM NaOH to all chip channels. Biotinylated $USP5^{171-290}$ was injected to channel 2, 3, and 4 for 500, 300, and 150 s respectively, followed by 5x10 s injections of buffer. Channel 1 was left blank as a reference channel.

B) Plate Preparation

UBXML78 was used as a positive control. Controls and compounds (19) 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 8-point concentration series starting at 1 mM in 96-well plates. The plates were sealed and centrifuged at 1000 RPM for 1 minute.

C) Assay

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

- Contact time: 35 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. Table 1 summarizes the hit compounds from which the analogues tested were designed. Experimental results of compound analogues are summarized in Table 2. Please find attached the Biacore evaluation file for fitted data (.bme).

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Table 1. Compound Hits

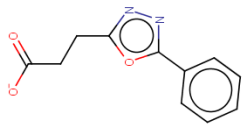
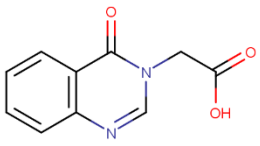
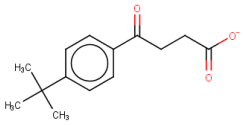
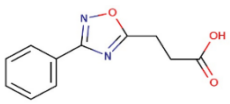
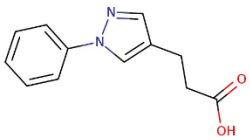
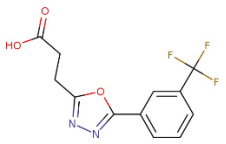
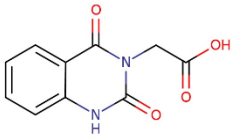
Compound Name	Compound Structure	SMILES	Avg K_D (µM)
DAT180		C(Cc1nnc(c2ccccc2)o1)C([O-])=O	365 ± 4 (n=6)
DAT194		C(C([O-])=O)N1C=Nc2ccccc2C1=O	215 ± 23 (n=9)
DAT201		CC(C)(C)c1ccc(cc1)C(CCC([O-])=O)=O	175 ± 50 (n=9)

Table 2. Summary of Hit Analogues tested with SPR

Compound Catalog Number	Compound Structure	SMILES	Plate 1: K_D (µM) n=3	Plate 2: K_D (µM) n=3
Z57674484 (Enamine Ltd.)		OC(=O)CCc1nc(no1)-c1ccccc1	>1000	
Z126932466 (Enamine Ltd.)		OC(=O)CCc1cnn(c1)-c1ccccc1	>1000	
Z221603948 (Enamine Ltd.)		OC(=O)CCc1nnc(o1)-c1cccc(c1)C(F)(F)F	923 ± 63	
Z355423170 (Enamine Ltd.)		OC(=O)Cn1c(=O)[nH]c2ccccc2c1=O	>1000	

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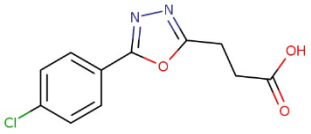
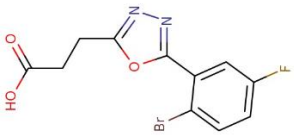
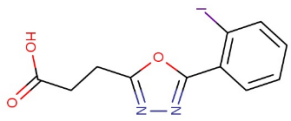
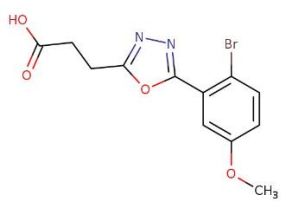
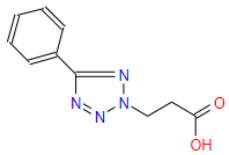
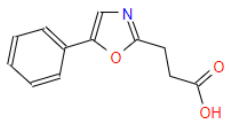
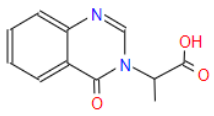
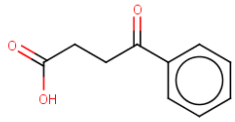
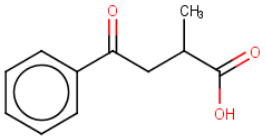
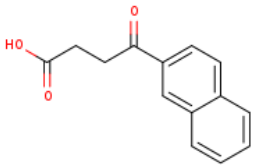
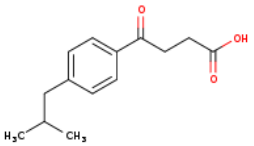
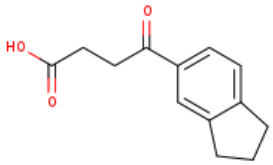
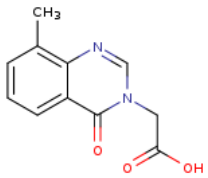
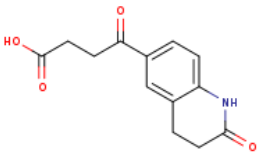
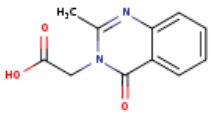
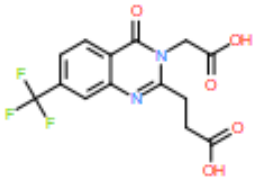
Z1270443867 (Enamine Ltd.)		OC(=O)CCc1nnc(o1)-c1ccc(Cl)cc1	445 ± 13	
Z992717354 (Enamine Ltd.)		OC(=O)CCc1nnc(o1)-c1cc(F)ccc1Br	426 ± 15	
Z1270387185 (Enamine Ltd.)		OC(=O)CCc1nnc(o1)-c1ccccc1I	277 ± 10	
Z1259155895 (Enamine Ltd.)		COc1ccc(Br)c(c1)-c1nnc(CCC(O)=O)o1	271 ± 9	
AL-291/37197008 (Specs)		O=C(O)CCn2nnc(c1ccccc1)n2	529 ± 14	
AG-219/09579029 (Specs)		O=C(O)CCc2ncc(c1ccccc1)o2	912 ± 33	
AE-641/11456811 (Specs)		CC(C(=O)O)n2cnc1ccccc1c2=O	61 ± 1	68 ± 3
AC-907/25014276 (Specs)		O=C(O)CCC(=O)c1ccccc1		677 ± 62

AE-562/03842058 (Specs)		CC(CC(=O)c1ccccc1)C(=O)O		695 ± 54
EN300-14900 (Enamine BB)		C1=C(C=C2C(=C1)C=CC=C2)C(CCC(=O)O)=O		485 ± 71
EN300-39820 (Enamine BB)		C1=CC(=CC=C1C(CCC(=O)O)=O)CC(C)C		393 ± 66
EN300-11365 (Enamine BB)		C1=CC2=C(C=C1C(CCC(=O)O)=O)CCC2		540 ± 66
EN300-23733 (Enamine BB)		C1(=CC=CC2=C1N=CN(C2=O)CC(=O)O)C		492 ± 56
EN300-137714 (Enamine BB)		C1=C(C=C2C(=C1)NC(CC2)=O)C(CCC(=O)O)=O		192 ± 13
EN300-197134 (Enamine BB)		C1=CC=CC2=C1C(N(C(=N2)C)CC(=O)O)=O		>1000
UBXML78		C(CC(O)=O)C1=Nc2cc(ccc2C(N1CC(O)=O)=O)C(F)(F)F	57 ± 2	80 ± 3

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Conclusions & Future Directions:

Based on substructure searches and docking simulations of hit compounds from Table 1, commercially available analogues were ordered and binding affinities were tested using SPR. Interestingly, compound AE-641/11456811, an analogue of DAT194 (K_D= 215 µM; n=9) had a binding affinity of approximately 65 µM (n=6) and an excellent ligand efficiency (LE) of 0.36 (LE=1.37*pIC50/[heavy atom count]). The addition of a methyl group increased potency 3-fold (Figure 1).

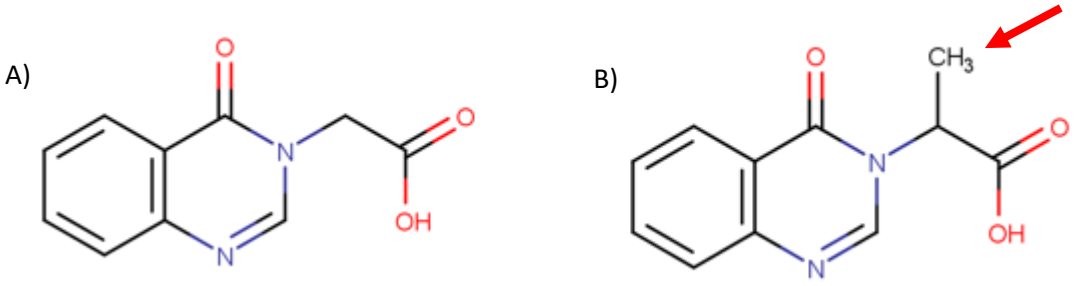

Figure 1. A) compound DAT194 B) compound AE-641/11456811

Compound DAT194 (PDB: 6NFT) and docked pose of AE-641/11456811 are shown in Figure 2a. The addition of the methyl group lends to increased hydrophobic interactions in the binding pocket with W209. In Figure 2b, the docked pose of AE-641/11456811 shows the methyl group fits into a small groove in the pocket. Based on the docked post, it may be possible to further extend the non-polar group into the pocket to increase the potency further. For this reason, compounds in Figure 3 were ordered as follow up compounds of AE-641/11456811.

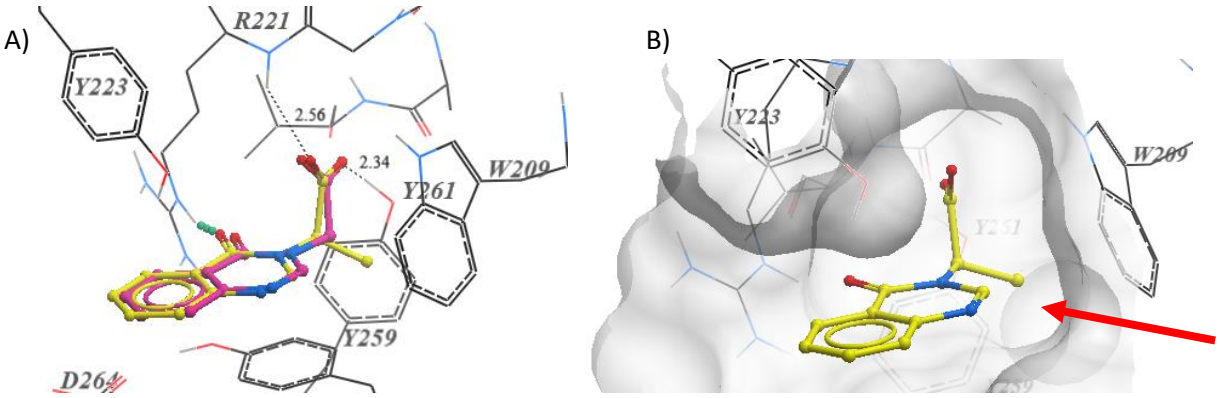

Figure 2. A) Compound DAT194 (PDB: 6NFT) in magenta and docked pose of AE-641/11456811 (yellow) B) Binding pocket of USP5 Zf-UBD and docked pose AE-641/11456811 (yellow)

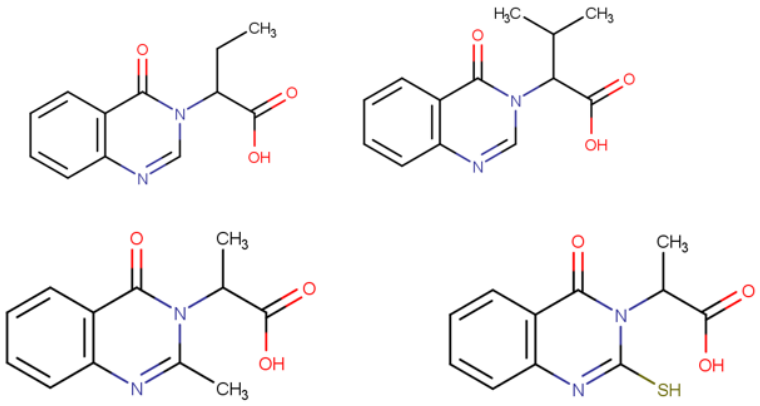

Figure 3. Follow up compounds of AE-641/11456811

I'll be setting up co-crystal trays of USP5 Zf-UBD and AE-641/11456811 for structure determination.

Analogues of DAT180 and DAT201 tested did not show a significant improvement in potency. So far, I've tested 19 of the 39 analogue compounds of DAT180, DAT194 and DAT201 that were ordered. Some compounds were not commercially available, and others are still in transit. I will be testing the rest of the compound analogues once the next batch of compounds arrive, as well as testing follow up compounds of AE-641/11456811.