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Expansion of UBTR012574 Chemical Series

Objective: Expansion of the chemical series of UBTR012574 (K_D= 10 ± 0.1 µM) by docking analogue compounds from the Enamine REAL database and SciFinder against the USP5 zinc finger ubiquitin-binding domain (ZnF-UBD) to prioritize compound ordering. The binding potency of the ordered compounds against USP5 ZnF-UBD were assessed with a surface plasmon resonance (SPR) assay.

Methods and Results:

A. Docking with Glide

Analogues of UBTR012574 (Figure 1) were chosen from Enamine's REAL database and SciFinder through a similarity search and docked to USP5 ZnF-UBD with GLIDE.

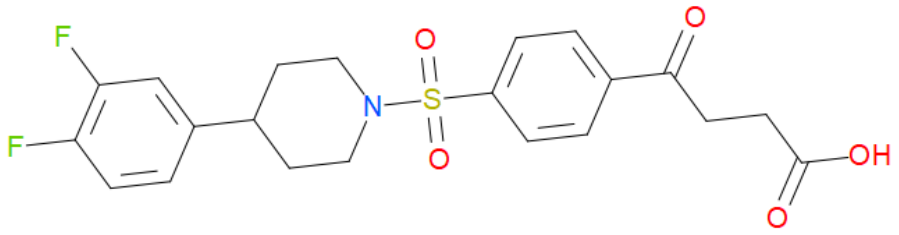


Figure 1. UBTR012574

1. PDB file of co-crystal structure of USP5 ZnF-UBD with UBTR012574 (UBTR012574.pdb) was uploaded in Molsoft ICM-Pro and converted to an ICM object and missing side chains were added. The resulting structure file was saved as a PDB and opened in Schrodinger Maestro.
2. The protein was prepared using 'Protein Preparation Wizard'. The structure was preprocessed for H-bond assignment and H-bonds were optimized and minimized at pH 7.3.
3. 2D sdf files of ligands to be docked against the protein were prepared from similarity search of UBTR012574 against Enamine's REAL database and SciFinder (UBTR012574_analogs_1.sdf) and converted to 3D format using GLIDE's Ligprep
4. Receptor Grid Generation: receptor, and size of the grid for the site of docking was chosen.
5. Ligand Docking:
    a. Setting: SP (standard precision); ligand sampling: flexible
    b. Tolerance: 1.0
    c. H-bond constraints: NH-side chain of R221, NH backbone of R221, OH side chain Y261
6. Docking results were exported to a sdf file (UBTR012574_analogs_glide_docked.sdf) and docking poses were viewed with ICM-pro (UBTR012574_analogs.icb). Based on the docking scores and docking poses, 17 compounds were ordered from Enamine REAL database (UBTR012574_analogs_ordered.sdf).

B. SPR Assay

1. Chip Preparation

Experiment #1:
An SA chip was used in a Biacore T-200 system at 20ºC. The chip was equilibrated with 20 mM Hepes pH 7.4, 150 mM NaCl, 1 mM TCEP, 0.005% Tween-20 (v/v), 1% DMSO (v/v) and then primed with 3x60 s injections of 50 mM NaOH to all chip channels. 0.05 mg/mL of biotinylated USP5 ZnF-UBD (construct: TOC011B06, AA: 171-290) was injected onto channel 2 and 3 for 60 s and 30 s respectively at a flow rate of 10 µL/min. 0.05 mg/mL of biotinylated HDAC6 ZnF-UBD (construct: TOC004A01, AA: 1109-1215) was injected onto channel 4 for 30 s at 10 µL/min. Approximately 5000, 4100, and 5000 RU were captured to channel 2, 3, and 4 respectively. 5x10 s of 20 mM Hepes pH 7.4, 150 mM NaCl, 1 mM TCEP, 0.005% Tween-20 (v/v), 1% DMSO (v/v) was injected onto all chip channel. Channel 1 was left blank as a reference channel. Buffer was flowed over chip until the baselines were stable (<100 RU difference over 1000 s).

Experiment #2:
An SA chip was used in a Biacore T-200 system at 20ºC. The chip was equilibrated with 20 mM Hepes pH 7.4, 150 mM NaCl, 1 mM TCEP, 0.005% Tween-20 (v/v), 1% DMSO (v/v) and then primed with 3x60 s injections of 50 mM NaOH to all chip channels. 0.05 mg/mL of biotinylated USP5 ZnF-UBD (construct: TOC011B06, AA: 171-290) was injected onto channel 2 and 3 for 60 s and 30 s respectively at a flow rate of 10 µL/min. 0.05 mg/mL of biotinylated HDAC6 ZnF-UBD (construct: TOC004A01, AA: 1109-1215) was injected onto channel 4 for 30 s at 10 µL/min. Approximately 4000, 4000, and 4500 RU were captured to channel 2, 3, and 4 respectively. 5x10 s of 20 mM Hepes pH 7.4, 150 mM NaCl, 1 mM TCEP, 0.005% Tween-20 (v/v), 1% DMSO (v/v) was injected onto all chip channel. Channel 1 was left blank as a reference channel. Buffer was flowed over chip until the baselines were stable (<100 RU difference over 1000 s).

2. Plate Preparation

Ligands were prepared in 20 mM Hepes pH 7.4, 150 mM NaCl, 1 mM TCEP, 0.005% Tween-20 (v/v), 1% DMSO (v/v) buffer. Ligands were diluted 1:4 in an 8-point concentration series starting at 2 mM for all compounds and 200 µM for ubiquitin (UBQ) in a 96-well plate. The plates were sealed and centrifuged at 1000 RPM for 1 minute. UBTR012574a/UBTR012574b and UBQ were used as positive controls.

3. Assay

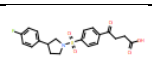
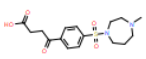
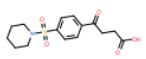
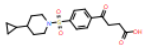
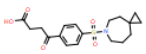
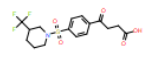
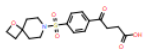
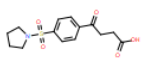
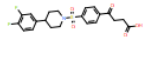
A multi-cycle kinetics method was run for the sample plates with the following parameters:

- Contact time: 60 s
- Dissociation time: 120 s
- Flow rate: 30 µL/min
- Temperature: 20ºC
- Running Buffer: 20 mM Hepes pH 7.4, 150 mM NaCl, 1 mM TCEP, 0.005% Tween-20 (v/v), 1% DMSO (v/v)

Sample injections were done sequentially by compound, from the lowest to highest concentration. Data was fitted with a steady state affinity model using the Biacore T200 Evaluation Software. Please see attached Biacore result file (UBTR012574_analogs_experiment_1.bme, UBTR012574_analogs_experiment_2.bme) for fitted data.

Experimental results are summarized in Table 1 (experiment 1) and Table 2 (experiment 2).

Table 1. Summary of SPR Experiment #1 Results

Catalog # (Enamine)	Toronto Internal ID	Compound SMILES	Compound Structure	USP5 ZnF-UBD K_D (n=2) (µM)	HDAC6 ZnF-UBD K_D (n=1) (µM)
Z1956968073	UBTR020999a	C1CN(CC1c1ccc(cc1)F)S(c1ccc(cc1)C(CCC(O)=O)=O)(=O)=O		27 ± 0.8	17
Z2788066918	UBTR021000a	CN1CCCN(CC1)S(c1ccc(cc1)C(CCC(O)=O)=O)(=O)=O		37 ± 0.2	27
Z2789362546	UBTR021001a	C1CCN(CC1)S(c1ccc(cc1)C(CCC(O)=O)=O)(=O)=O		64 ± 2	20
Z2790169956	UBTR021002a	C1CN(CCC1C1CC1)S(c1ccc(cc1)C(CCC(O)=O)=O)(=O)=O		36 ± 3	21
Z3569784229	UBTR021003a	C1CC2(CCN(C1)S(c1ccc(cc1)C(CCC(O)=O)=O)(=O)=O)CC2		52 ± 0.4	19
Z1983124190	UBTR021004a	C1CC(CN(C1)S(c1ccc(cc1)C(CCC(O)=O)=O)(=O)=O)C(F)(F)F		135 ± 2	47
Z2007216410	UBTR021005a	C(CC(c1ccc(cc1)S(N1CCC2(CC1)CCO2)(=O)=O)=O)C(O)=O		51 ± 6	NB: did not reach steady state
Z1682077208	UBTR020992a	C1CCN(C1)S(c1ccc(cc1)C(CCC(O)=O)=O)(=O)=O		59 ± 2	21
Z1359463303	UBTR012574a	C1CN(CCC1c1ccc(c(c1)F)F)S(c1ccc(cc1)C(CCC(O)=O)=O)(=O)=O		17 ± 0.1	13

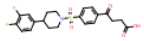
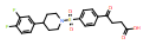
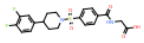
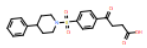
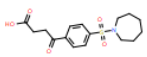
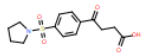
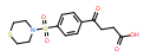
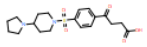
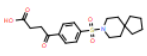
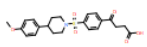
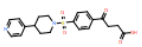
Z1359463303	UBTR012574b	C1CN(CCC1c1ccc(c(c1)F)F)S(c1ccc(cc1)C(CCC(O)=O)=O)(=O)=O		10 ± 0.1	7
	UBQ			4 ± 0.1	8

Table 2. Summary of SPR Experiment #2 Results

Catalog # (Enamine)	**Toronto Internal ID**	**Compound SMILES**	**Compound Structure**	**USP5 ZnF-UBD K_D (n=2) (µM)**	**HDAC6 ZnF-UBD K_D (n=1) (µM)**
Z1359463303	UBTR012574b	C1CN(CCC1c1ccc(c(c1)F)F)S(c1ccc(cc1)C(CCC(O)=O)=O)(=O)=O		12 ± 1	11
Z1134926658	UBTR020988a	C1CN(CCC1c1ccc(c(c1)F)F)S(c1ccc(cc1)C(NCC(O)=O)=O)(=O)=O		38 ± 1	224
Z1359449148	UBTR020989a	C1CN(CCC1c1ccccc1)S(c1ccc(cc1)C(CCC(O)=O)=O)(=O)=O		8 ± 0.1	7
Z1682073975	UBTR020990a	C1CCCN(CC1)S(c1ccc(cc1)C(CCC(O)=O)=O)(=O)=O		69 ± 1	17
Z1682077208	UBTR020992a	C1CCN(C1)S(c1ccc(cc1)C(CCC(O)=O)=O)(=O)=O		68 ± 1	23
Z1682133644	UBTR020993a	C(CC(c1ccc(cc1)S(N1CCSCC1)(=O)=O)=O)C(O)=O		85 ± 1	23
Z1682136602	UBTR020994a	C1CCN(C1)C1CCN(CC1)S(c1ccc(cc1)C(CCC(O)=O)=O)(=O)=O		10 ± 0.1	17
Z1682210759	UBTR020995a	C1CCC2(C1)CCN(CC2)S(c1ccc(cc1)C(CCC(O)=O)=O)(=O)=O		53 ± 1	18
Z1682223747	UBTR020996a	COc1ccc(cc1)C1CCN(CC1)S(c1ccc(cc1)C(CCC(O)=O)=O)(=O)=O		26 ± 1	17

Z1869543922	UBTR020998a	C1CN(CCC1c1ccnc c1)S(c1ccc(cc1)C(CCC(O)=O)=O)(=O)=O		56 ± 0.5	43
	UBQ			3 ± 0.04	6

Conclusions & Future Directions:

Of the analogues tested, UBTR020994a and UBTR020989a had a similar potency as UBTR012574 for USP5 ZnF-UBD. These compounds also bind to HDAC6 ZnF-UBD with similar potency. Compound UBTR020988a, which has a nitrogen atom in the carboxylic chain results in weaker potency (~40 µM) than UBTR012574 for USP5 ZnF-UBD but is 6-fold selective for USP5 over HDAC6 (Figure 2). We are working with chemists to expand the chemical series further with the nitrogen group in the carboxylic chain- we hope to improve potency and retain selectivity for USP5 ZnF-UBD. Stay tuned!

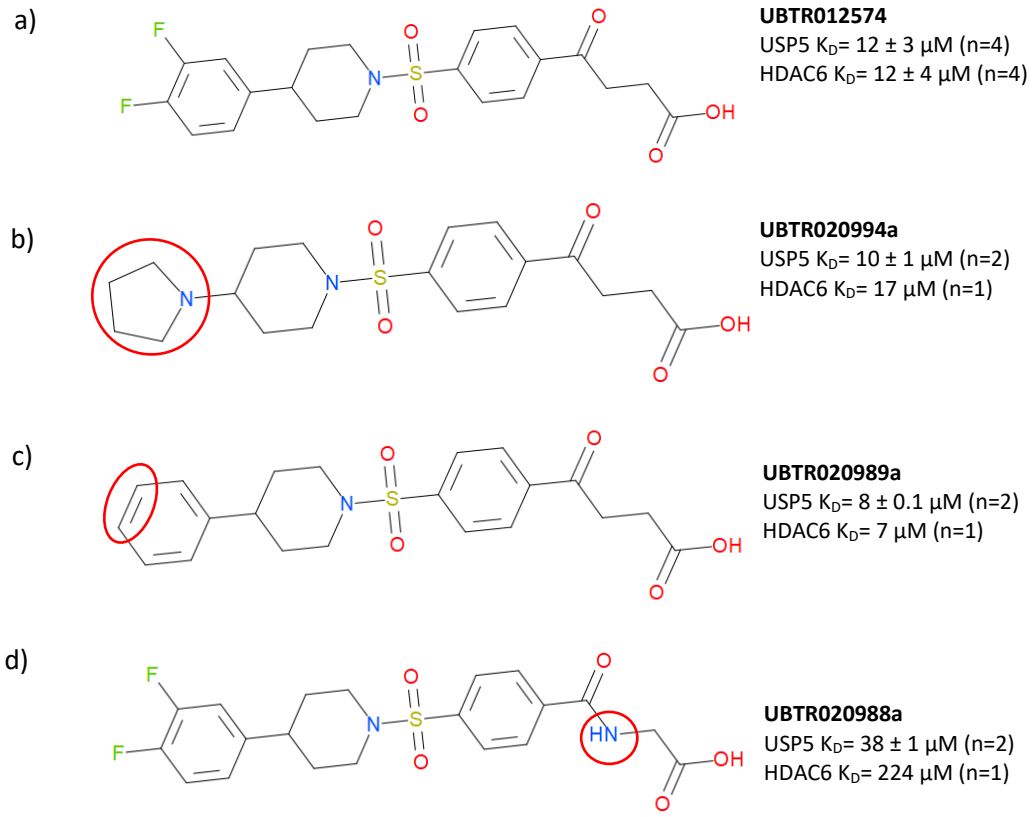


Figure 2. UBTR012574 chemical series a) UBTR012574 b) UBTR020994a c) UBTR020989a d) UBTR20988a