Comprehensive profiling of DUB inhibitors using the Medivir DUB platform

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Background

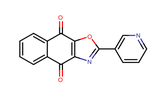
- Medivir is a research-based pharmaceutical company with extensive experience in protease inhibitor design and nucleoside/nucleotides.
- Our protease technology platform is well established and with a well proven track-record, most recently demonstrated with a Cathepsin K inhibitor, now in phase II clinical trials for osteoarthritis.
- It is well recognized that the ubiquitination system can regulate many important cancer pathways and that using deubiquitinase (DUB) inhibitors could provide a novel targeting approach.[1]
- Medivir is applying our strength in protease inhibitor design to investigate multiple DUB targets.
- To enable this, we have established a DUB platform of biochemical and biophysical assays, including protein production, characterization and structural biology.
- We have validated this platform by comprehensive characterization of publically disclosed DUB inhibitors.

DUB Inhibitors

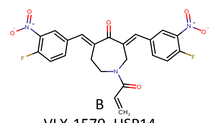
- To enable target evaluation of DUBs, it is important to have access to high quality tool compounds.
- We searched the literature and identified a multitude of suggested DUB inhibitors for further investigation.
- In this ongoing activity a selection of 80 inhibitors have been synthesized or purchased thus far.
- Several of these inhibitors have activity reported against one or a few DUBs, or are reported to be selective against a certain DUB.
- Many of the identified DUB inhibitors contain reactive chemical groups suggesting poor selectivity over other DUBs or Cys-proteases.
- Several of the DUB inhibitors included chemical motifs associated with assay interference .

Compound characteristics

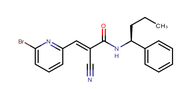
- All inhibitors, purchased or synthesized, were subject to careful purity and identity control as well as full structural assignment by NMR.
- After passing QC, the compounds were profiled in biochemical, physiochemical and DMPK assays.



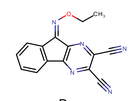
A
SJB3-019A , USP1
Dana Farber Institute[2]



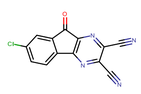
B
VLX-1570, USP14
Vivolux[3]



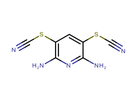
C
Degrasyn WP1130, USP1
U. of Michigan[4]



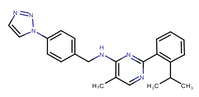
D
HY-50,737, USP7
Hybrigenics[5]



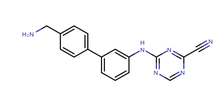
E
HY-41,108, USP7
Hybrigenics[6]



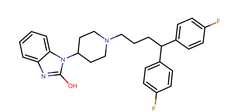
F
PR-619
U. of Oxford /Progenra[7]



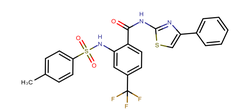
G
ML323, USP1
U. of Delaware[8]



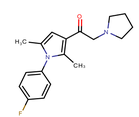
H
USP2
Novartis[9]



I
Pimozide, USP1
U. of Delaware[10]



J
ML364, USP2
NIH[11]



K
IU1, USP14
U. of Harvard[12]

Compound	A	B	C	D	E	F	G	H	I	J	K
MW	276.3	469.4	384.3	275.3	266.6	223.3	384.5	302.3	461.6	517.5	300.4
Log D	2	2.5	2.1	3.1	1.8	1	3.8	0.80	2.7	4.1	1.5
Kinetic Solubility (µM)	12	6	6	<2	<6	87	5	60	<1	<1	>100
CACO-2 Papp (cm/s*10^-6)	15	*	4.9	*	*	*	25	4.1	12	13	15
HLM CLint (µL/min/mg)	<6	>300	14	nd	87	240	250	50	66	8	9
Redox liability	-	-	-	-	+	+	-	-	-	-	-

*Low Papp indicated

Enzyme activity IC_{50} (µM)

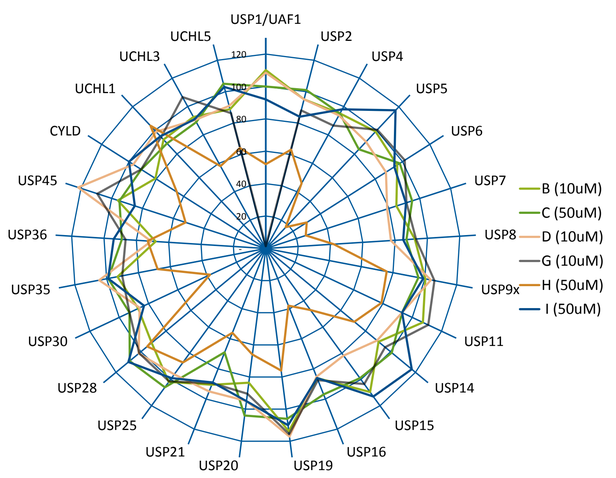
Compound	A	B	C	D	E	F	G	H	I	J	K
USP1/UAF1	2.8	16	4.1	>100	4.5	0.75	3.1	1.1	>100	>100	31
USP2 CD	5.9	12	6.7	*	nd	0.59	>100	3.7	*	94	77
USP2 CD§	3.9	>100	nd	nd	nd	5.1	>100	>100	nd	>100	>100
USP7	6.1	8.9	2.4	26	#	0.29	>100	47	>100	16	46
USP14	5.4	>100	>100	40	0.52	1.4	>100	13	>100	53	38
USP28	1.8	*	*	15	nd	nd	*	28	*	45	*
USP47	14	6.6	4.5	>100	nd	nd	>100	7.7	>100	33	>100

§ 1mM DTT, # <3 µM, interference * <15% inhibition at 10 µM,

- Protocols for enzyme assays have been established in-house for a number of the ubiquitin-specific proteases (USPs)
- Although claimed to be selective against a certain DUB, several of the inhibitors were active on multiple USPs.
- Particularly compounds A and F were found to be broad range DUB inhibitors, while compound G shows high USP1 selectivity.

Ubiquigent DUB*profiler*™ data

- In order to further evaluate the selectivity of the compounds we assayed a set of compounds for single concentration enzyme activity in the Ubiquigent DUB*profiler*™.
- As exemplified in the radar plot, many of the compounds showed negligible inhibition of the DUBs tested.
- In contrast, compound H shows activity on many of the DUBs in the Ubiquigent DUB*profiler*™.
- In line with our in-house enzyme data, Compound G shows an excellent selectivity profile, being active on USP1 only.
- The difference in activity we see between our in house assay data and the external data could be due to the difference in assay conditions.



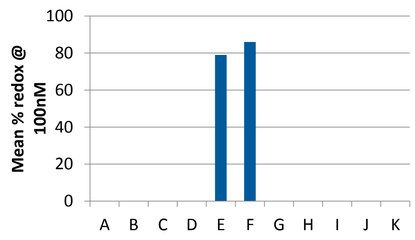
Selectivity over other proteases

- To avoid off-target effects it is important to counterscreen against other proteases. This is particularly critical when targeting the catalytic site.
- We have counterscreened several of the publically disclosed DUB inhibitors against a number of cysteine and serine proteases.
- Compound F is not only a broad range DUB inhibitor but also inhibits several other cysteine proteases, e.g. MALT 1.
- Additionally to MALT 1 activity, compound H inhibits thrombin with sub-µM Ki.

Compound	B	C	F	G	H
MALT 1 Ki (µM)	64	70	6.8	70	57
Thrombin Ki (µM)	>100	>100	>100	>100	0.44

Redox activity

- The active site cysteine in cysteine proteases is prone to oxidize, resulting in loss of catalytic activity.
- To distinguish between true inhibitors and false positives it is important to examine if compounds have an inherent redox activity.
- Compounds E and F are redox active and caution should be taken when interpreting result from these compounds.
- Compound A did not show redox activity in this assay, but it contains a well known redox chemical motif.



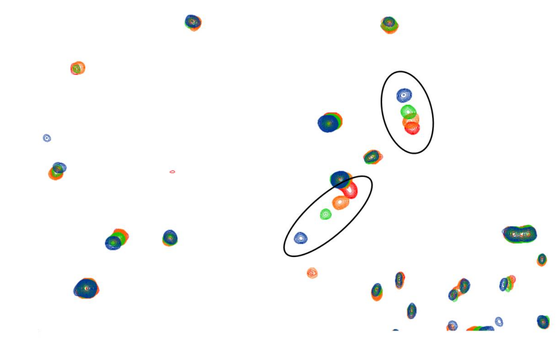
Solubility

- Solubility is one of the key physicochemical parameters of a new molecule that needs to be assessed early on in the drug discovery process.
- For some compounds, the solubility is highly dependent on the buffer composition and pH.
- High solubility of a compound is important to ensure reliable enzyme data and ADME property assay results.
- Compounds with low solubility might display misleadingly low IC_{50} values due to precipitation of the protein during enzyme assay.
- Poorly soluble compounds might show low activity due to inaccurate concentrations.
- Several of the DUB inhibitors we have profiled show remarkably low kinetic solubility, and caution should be taken when interpreting assay results on these compounds.

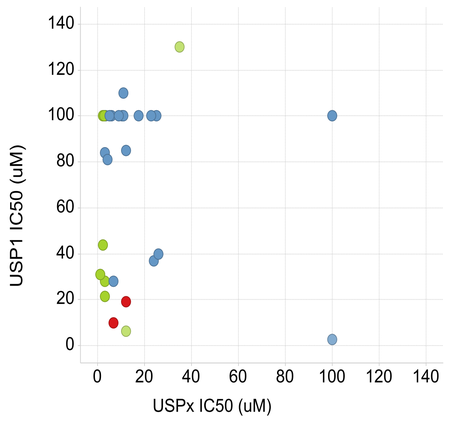
Biophysical evaluation of a Hit in one of our internal DUB project

- An in-house designed protease compound library was screened against multiple DUBs in parallel.
- Numerous hits were identified for our front-running DUB project, and we are progressing several hit series.
- The hit series have been evaluated and characterized biophysically using ITC, NMR, DSF and MST.
- Rational design and exploration of the hits has generated inhibitors with high nM IC50s.
- Several of our in-house generated inhibitors show excellent enzymatic selectivity in a panel of DUBs tested.

Assay	Result
Mw	<400
HBD, HBA	2, 7
TPSA	94
USPx FL DiUB IC_{50}	3.0 µM
USPxCD UB_RHO IC_{50}	3.9 µM
DSF IC_{50}	5.5-7.5 µM
USPx CD ITC K_d	70 µM
USPX CD NMR	CSPs observed
Log $D_{7.4}$, Kin. Sol. (µM)	0.60, >100
Redox liability	None



Chemical Shift Pertubation (CSP)
Titration of ligand Y to USPx CD (^{1}H,^{15}N-TROSY).



Summary

- We have established a DUB platform consisting of compound libraries, enzyme assays, protein production, biophysical characterization and screening techniques, allowing multiple hit finding strategies.
- Using this DUB platform we have performed comprehensive characterization of compounds in the DUB literature.
- Due to the implications of high reactivity, poor selectivity and poor physicochemical properties caution should be taken when using particular literature DUB inhibitors as pharmacological tools for understanding DUB biology.
- The compounds identified as suitable pharmacological tools in the DUB platform, are used for target evaluation of the specific DUBs.
- Our in-house DUB project is prosecuting several hit series originating from various hit finding techniques in our established DUB platform.
- In addition to progressing our front-running DUB project, multiple hits for other DUB enzymes are under evaluation.

References

1. J. Med. Chem. 2015; 58(4); 1581-1595; Prog. Med. Chem. 2016, 55, 149-192 ; 2. WO2011137320; 3. WO2013058691; 4. Cancer Res 2010; 70(22):9265-76 ; 5. US8648076; 6. EP1749822;2007 7. 8. WO2014105952; 9. WO2007009715; 10. Chem Biol 2011; 18: 1390-1400; 11. Pragani et al. MEDI 127, 246th ACS National meeting, 2013; 12. WO2011094545