70

Characterization of small molecule inhibitors of ubiquitin specific peptidase 1 (USP1) as anti-cancer agents

Erwin D. Brenndörfer, Daniel Jönsson, Kerstin Böhm, Anders Eneroth, Klara Acs, Ian Henderson, Mari Kullman-Magnusson, Christina Rydergård, Gun Stenberg, Anna-Karin Sternbeck, Emma Ulander, Mark Albertella, Richard Bethell

Medivir AB, Huddinge, Sweden

BACKGROUND

- The deubiquitinase USP1 in complex with USP1-associated factor 1 (UAF1) is involved in key oncogenic pathways.[1]
- USP1 regulates the DNA damage response by deubiquitinating FANCD2, FANCI, and PCNA, which are major modulators of interstrand crosslink repair and translesion synthesis.[2, 3, 4]
- In addition, USP1 sustains cancer cell stemness by increasing ID protein stability through deubiquitination.[5]
- USP1 has been associated with tumorigenesis by being overexpressed in tumors from AML[6], glioblastoma[7], multiple myeloma[8] and osteosarcoma[5] patients.
- This suggests that USP1 inhibitors may have the potential to be used as anti-cancer agents in combination with DNA damaging drugs or radiotherapy.[1, 6, 7, 9]
- Published USP1 inhibitors such as ML323[9], GW7647[10], pimozide[10], or SJB3-019A[8] are non-selective and/or characterized by poor potency, solubility and metabolic stability.
- As part of Medivir's DUB drug discovery efforts, we have evaluated the tractability and the feasibility of USP1/UAF1 as a drug target and initiated in-house lead finding activities to identify novel chemical entities for inhibition of this target.

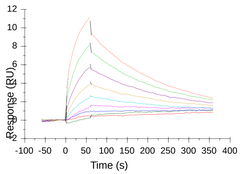
CHARACTERIZATION OF IN HOUSE COMPOUNDS

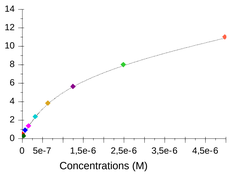
- In house development resulted in the identification of multiple compounds with substantially improved biological and physicochemical properties when compared to ML323.
- The representative compounds A and B exhibit highly potent activities against USP1/UAF1 and very good selectivity over a set of established deubiquitinases (DUBs). USP1/UAF1 was assayed using DiUb48-4 FRET as substrate. Ub-VME-proteasome activated USP14 was assayed using ubiquitin rhodamine 110 as substrate. USP7 and USP47 were assayed using DiUb48-1 FRET as substrate.
- Selectivity profiling by Ubiquigent (DUB*profiler*™) showed that the compounds were active against USP1/UAF1 but inactive in a single point screen against 26 other DUBs (data not shown).
- Furthermore, the compounds demonstrated high permeability in the Caco-2 system and good solubility in a kinetic solubility assay in phosphate buffered saline (PBS) at pH 7.4.
- The data generated with compound B show that intrinsic clearance in human liver microsomes (HLM) could be improved.

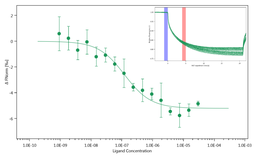
Compound	ML323	A	B
Parent MW	384.4	509.5	505.5
USP1 IC_{50} (µM)	6.3	**0.07**	**0.05**
USP7 IC_{50} (µM)	>100	>100	>100
USP14 IC_{50} (µM)	>100	>100	>100
USP47 IC_{50} (µM)	>100	>100	>100
Kinetic Solubility in PBS (µM)	5	10	**74**
Caco-2 Papp (x10^{-6} cm/sec)	25	17	7.4
HLM CLint (µl/min*mg)	250	>300	**72**
MLM CLint (µl/min*mg)	N. T.	>300	220

BINDING VALIDATION

- Confirmed binding of compound B to truncated USP1/UAF1 by surface plasmon resonance (SPR) and to truncated USP1 by microscale thermophoresis (MST).
- Kinetic analysis of the binding event indicates a slow on-rate and a slow off-rate.





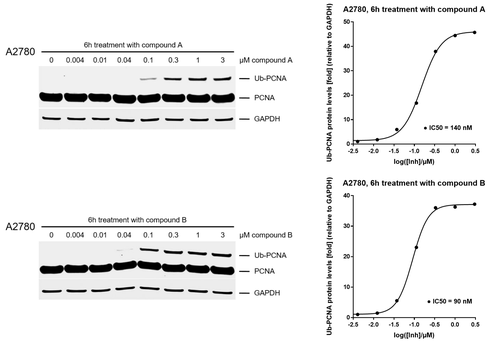


	USP1 (MST)	USP1/UAF1 (SPR)
K_D(µM)	0.12 ± 0.03	0.527
k_{on} ($M^{-1}s^{-1}$)	-	9.62 x 10^3
k_{off} (s^{-1})	-	5.02 x 10^{-3}

EFFECT OF USP1 INHIBITION ON USP1 SUBSTRATES

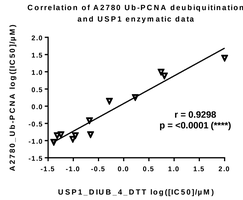
Effect on the USP1 substrate Ub-PCNA

- Compounds A and B demonstrated robust activity in a cellular Ub-PCNA deubiquitination assay, with IC_{50} values of 140 nM and 90 nM respectively in the ovarian cancer cell line A2780.



Correlation between enzymatic assay and cell efficacy assay

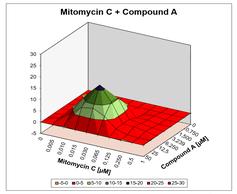
- The cellular Ub-PCNA deubiquitination assay and the enzymatic USP1 K48-diubiquitin cleavage assay show an excellent correlation for the compounds tested.



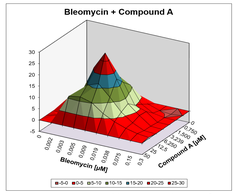
COMBINATION WITH DNA DAMAGING AGENTS

Cell viability data

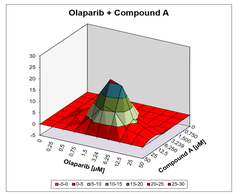
DNA damaging agent	Mode of action	Compound A CC50 decrease [fold]	Compound B CC50 decrease [fold]
Mitomycin C	Crosslinking agent	2-5	2-5
Bleomycin	Radiomimetic	5-10	10-15
Olaparib	PARP inhibition	5-10	2-5
MIV-818[11] analog	Chain terminator	5-10	10-15



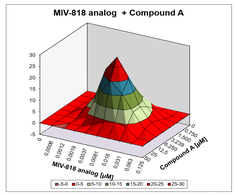
Strong synergy; 95% confidence: 136.72 / -0.19 µM² %



Strong synergy; 95% confidence: 280.69 / -0.52 µM² %



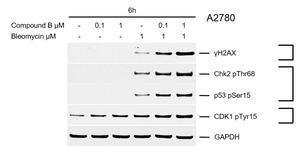
Strong synergy; 95% confidence: 178.72 / -0 µM² %



Strong synergy; 95% confidence: 266.31 / -0.01 µM² %

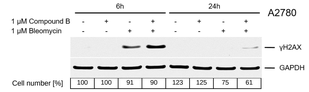
- The combination of the USP1 inhibitors compound A and B with DNA damaging agents in A2780 cells results in a significant CC50 decrease as compared to DNA damaging agent monotherapy and shows strong synergy in the Bliss independence model.

Combination of USP1 inhibitors with bleomycin



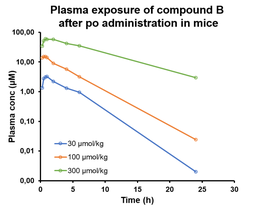
USP1 inhibition increases:

- Bleomycin-induced DNA damage
- Bleomycin-induced activation of the ATM-Chk2-p53 axis
- Bleomycin-induced block of the cell cycle at the G2/M transition



- USP1 inhibition delays the repair of bleomycin-induced DNA damage
- USP1 inhibition expands bleomycin-induced cell death

PK PROFILE



Dose (µmol/kg)	30	100	300
$AUC_{0\text{-}24h}$ (µM*h)	13.3	58	511
$AUC_{0\text{-}24h}$/Dose	0.44	0.58	1.7
$t_{1/2}$ (h)	2.1	2.3	5.0

- Promising *in vivo* pharmacokinetic profile of compound B in mice with dose linearity at the lower doses and saturation of elimination pathways at the highest dose.

SUMMARY

- A drug development campaign for novel USP1 inhibitors resulted in the identification of compound B, which demonstrated:
  - Highly potent activity against USP1 both in the enzymatic assay and the cellular Ub-PCNA deubiquitination assay.
  - Favourable human *in vitro* DMPK properties.
  - Biophysical validation of compound binding by SPR.
  - Synergistic cytotoxic activity in combination with DNA damaging agents.
  - Good oral bioavailability in mice, which supports evaluation in *in vivo* efficacy models.
- Thus, the data suggest that compound B is a very promising compound amenable for further development.

REFERENCES

1. Garcia-Santisteban et al., Mol Cancer 2013, 12, 91
2. Nijman et al., Mol Cell 2005, 17, 331
3. Sims et al., Nat Struct Mol Biol 2007, 14, 564
4. Huang et al., Nat Cell Biol 2006, 8, 339
5. Williams et al., Cell 2011, 146, 918
6. Mistry et al., Mol Cancer Cell 2013, 12, 2651
7. Lee et al, Neuro Oncol 2016, 18,37
8. Das et al., Clin Cancer Res 2017, 23, 4280
9. Liang et al., Nat Chem Biol 2014, 10, 298
10. Chen et al., Chem Biol 2011, 18, 1390
11. MIV-818 is a novel liver-targeting nucleotide developed by Medivir (see http://www.medivir.com/our-projects/proprietary/miv-818).