Development of selective MCL-1 heterobifunctional degraders

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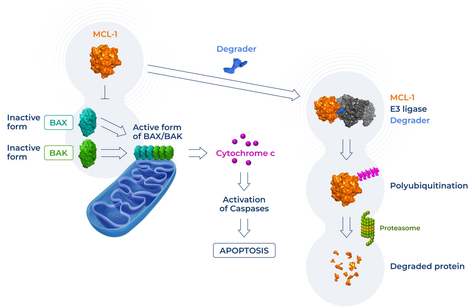
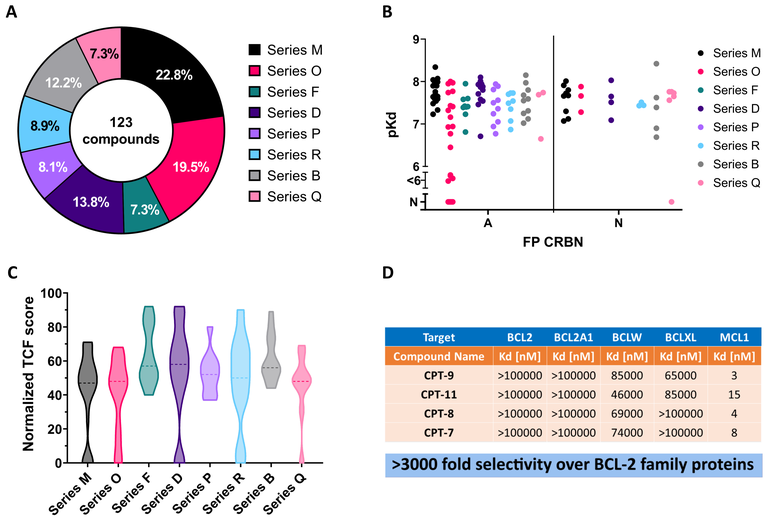
INTRODUCTION

- MCL-1 is a member of the BCL-2 family that plays a key role in cellular homeostasis through the **regulation of apoptosis** as well as other less characterized functions. The growing recognition of MCL-1 role in cancer cell survival and its association with the development of anticancer drug resistance makes it an attractive target for cancer therapy.
- Several MCL-1 inhibitors have been developed during the past decade and some of them have entered clinical trials, but **no drugs have been approved for clinical use** so far.
- Targeting MCL-1 by employing **bifunctional degraders** represents a potentially new and effective strategy for cancer treatment .



RESULTS

MCL-1 BIFUNCTIONAL DEGRADERS BIND SELECTIVELY TO MCL-1 AND E3 LIGASE TO FORM A TERNARY COMPLEX



Target	BCL2	BCL2A1	BCLW	BCLXL	MCL1
Compound Name	Kd [nM]	Kd [nM]	Kd [nM]	Kd [nM]	Kd [nM]
CPT-9	>100000	>100000	85000	65000	3
CPT-11	>100000	>100000	46000	85000	15
CPT-8	>100000	>100000	69000	>100000	4
CPT-7	>100000	>100000	74000	>100000	8

>3000 fold selectivity over BCL-2 family proteins

Figure 1. A. Percentage distribution of synthesized compounds in different series. **B.** Binding affinity of synthesized compounds to MCL-1 by SPR in groups of active and inactive compounds in FP assay with E3 ligase. All compounds that bind to MCL-1 were submitted to TCF HTRF analysis which is less prone to interference than FP assay. A - active, N - inactive. **C.** Collective data from AlphaLISA and HTRF assays for different series. Most of the compounds form the ternary complex with MCL-1 and CRBN. TCF - Ternary Complex Formation. **D.** Compounds selectivity over BCL-2 family proteins in the KINOMEscan™ competition binding assay.

MCL-1 DEGRADERS SHOW POTENT CYTOTOXICITY IN MCL-1-DEPENDENT HEMATOLOGIC CANCER CELL LINES

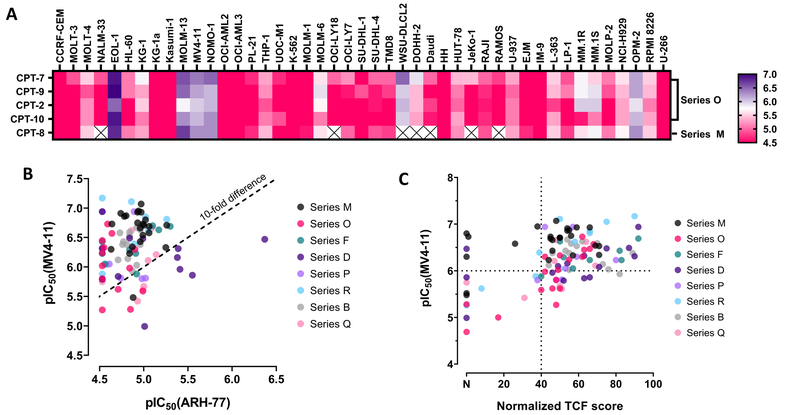


Figure 2. A. Profile of cytotoxic activity of selected compounds in a panel of 45 cell lines representing hematological malignancies after 72 hours of treatment. Heat map shows the pIC_{50} values from the CellTiter-Glo® Assay. **B.** Cytotoxicity of the synthesised compounds in MCL-1-dependent (MV4-11, AML - acute myeloid leukemia) and MCL-1-independent (ARH-77, PCL - plasma cell leukemia) cell lines in a 24-hour CellTiter-Glo® Assay. The dotted line represents 10-fold difference between pIC_{50} values from the two cell lines. Compounds that are located above this line were considered specific and were characterized further. **C.** The correlation between pIC_{50} values from the viability assay and TCF score from biophysical assays. Dotted lines represent thresholds of desired activity.

BIFUNCTIONAL DEGRADERS TARGETING MCL-1 INDUCE A STRONG APOPTOTIC RESPONSE

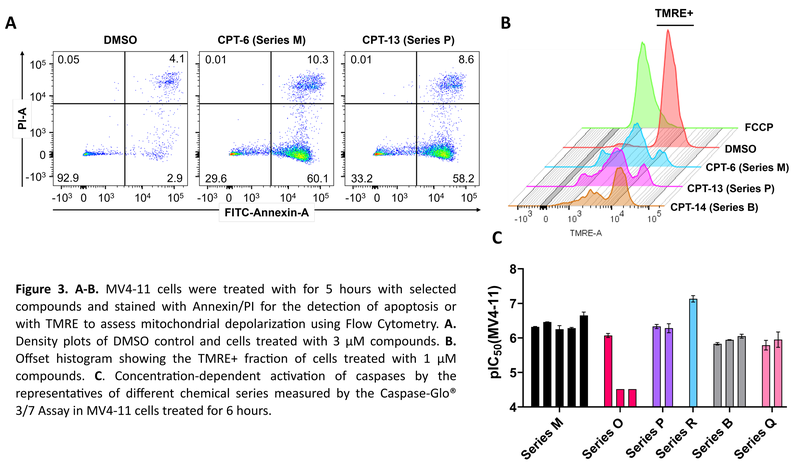


Figure 3. A-B. MV4-11 cells were treated with for 5 hours with selected compounds and stained with Annexin/PI for the detection of apoptosis or with TMRE to assess mitochondrial depolarization using Flow Cytometry. **A.** Density plots of DMSO control and cells treated with 3 µM compounds. **B.** Offset histogram showing the TMRE+ fraction of cells treated with 1 µM compounds. **C.** Concentration-dependent activation of caspases by the representatives of different chemical series measured by the Caspase-Glo® 3/7 Assay in MV4-11 cells treated for 6 hours.

CPT COMPOUNDS TARGET MCL-1 FOR DEGRADATION *IN VITRO*

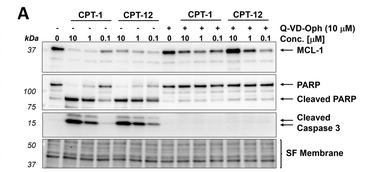
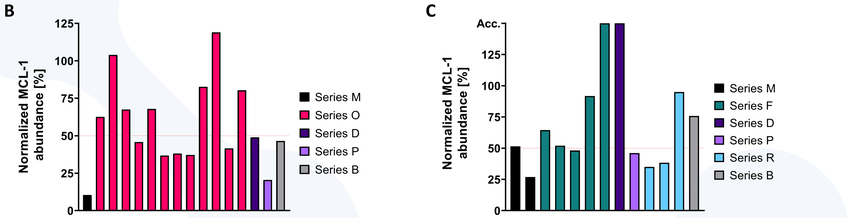


Figure 4. A. Western Blot analysis of MV4-11 cells treated with two different bifunctional degraders for 6 hours. Apoptosis level was measured by detection of cleaved PARP and Caspase 3. Degradation of MCL-1 in the presence of the pan-caspase inhibitor (Q-VD-Oph) is indicative of proteasomal degradation mediated by CPT-1 and CPT-12. SF - stain free. **B-C.** Densitometric analysis of results from degradation assays with 100 nM of selected compounds - Simple Western performed with OPM-2 (MM - multiple myeloma) treated for 24 hours (**B**) and Western Blot with MV4-11 cells treated for 6 hours (**C**). Acc. - Accumulation: protein level above the upper detection limit of the method; red dotted line - threshold of activity for potent compounds.



COMPOUND-DRIVEN MCL-1 DEGRADATION IS PROTEASOME- AND CRBN-DEPENDENT

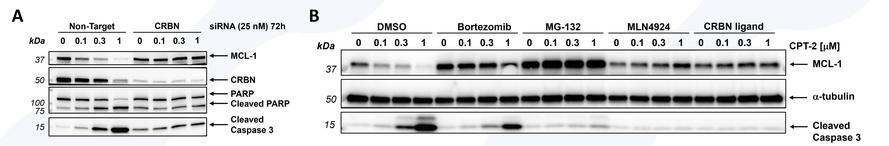


Figure 5. Determination of the Mechanism of Action of CPT-2 against MCL-1 by Western Blot. **A.** OPM-2 cells were transfected with Non-Target control or CRBN siRNA and 72 hours later treated with CPT-2 for 6 hours. Activation of apoptosis was confirmed by the appearance of the cleaved forms of PARP and Caspase 3 proteins. **B.** OPM-2 cells were pretreated for 1 hour with two different proteasomal inhibitors (Bortezomib or MG-123), NEDD8-Activating Enzyme inhibitor (MLN4924) or a CRBN ligand, and then treated with CPT-2 for 6 hours.

CPT COMPOUNDS HAVE POTENT PHARMACODYNAMIC ACTIVITY

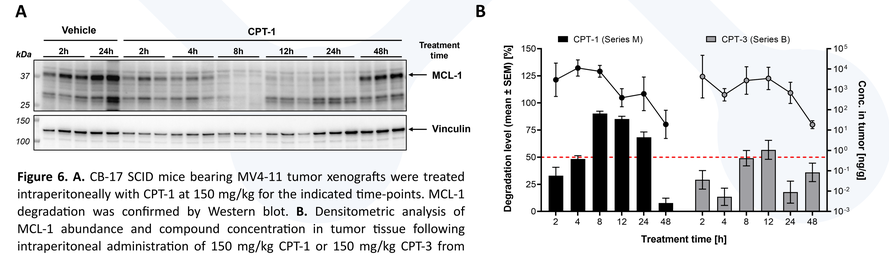


Figure 6. A. CB-17 SCID mice bearing MV4-11 tumor xenografts were treated intraperitoneally with CPT-1 at 150 mg/kg for the indicated time-points. MCL-1 degradation was confirmed by Western blot. **B.** Densitometric analysis of MCL-1 abundance and compound concentration in tumor tissue following intraperitoneal administration of 150 mg/kg CPT-1 or 150 mg/kg CPT-3 from Series M and B, respectively. Red dotted line - 50% of MCL-1 degradation.

CONCLUSIONS

- **Synthesized compounds engage CRBN and selectively bind MCL-1 with high affinity to form a ternary complex.**
- **Presented series of compounds induce MCL-1 degradation in cells, which is ubiquitin- and proteasome-dependent, and leads to the strong apoptosis induction.**
- **These series of compounds showed a nM range cytotoxic activity in haematological cancer cell lines with the best compounds having an IC_{50} <100 nM.**
- **MCL-1 degradation can be achieved by IP administration of the synthesized compounds leading to the initiation of the programmed cell death.**

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