Design and Synthesis of Novel UBA1 Inhibitors for Targeting the Ubiquitin-Proteasome System (UPS)

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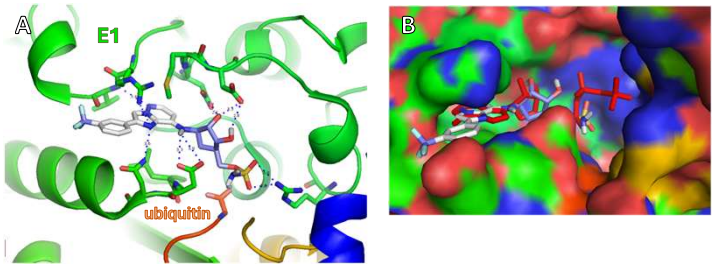
INTRODUCTION

The ubiquitin-activating enzyme 1 (UBA1 or E1) is a crucial component of the ubiquitin-proteasome system (UPS), playing a key role in maintaining protein homeostasis by catalyzing the first step of the ubiquitination cascade. Dysregulation of UPS has been implicated in various malignancies, making it an attractive target for therapeutic intervention. While proteasome inhibitors, such as bortezomib, act at the final stage of protein degradation, UBA1 inhibitors modulate the system at the first stage, where degraded proteins are labeled with ubiquitin. By the inhibition of UPS system broad cellular responses, including apoptosis and cell cycle arrest are induced. Up to know several UBA1 inhibitors were developed and the most advanced MLN7243 (TAK243) was evaluated in CT-1 where the very narrow therapeutic window were reported. The high toxicity of UBA1 inhibitors is a result of lack of structural diversity of E1 enzyme among different cell types including broad cancer lines resistant to conventional therapies.

This study aims to design and synthesize novel group of UBA1 inhibitors with optimized physicochemical properties, addressing solubility and metabolic stability limitations of existing inhibitors, such as MLN7243.

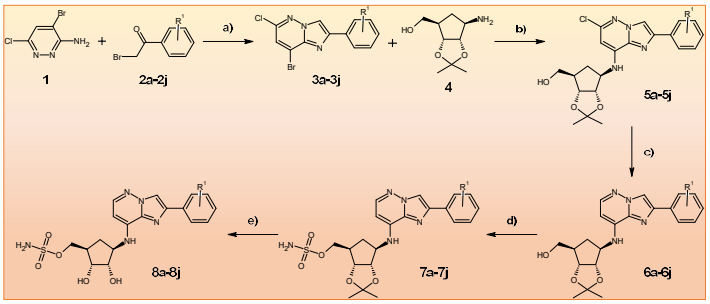
MOLECULAR TARGET EVALUATION

Based on the structure and activity of known UBA1 inhibitors (MLN7243, PYZD-4409) we designed a group of compounds whose structure was based on the pyrazolo[1,5-a]pyrimidine core. Using molecular docking methods, we simulated the main and crucial binding intereactions with E1 enzyme ATP pocket and affinity to ubiquitin C-terminal carboxylic acid moiety that results in covalent bond formation. For the evaluation of structural diversity of designed compounds we selected the group of representatives for the synthesis and biological studies.



A) Visualisation of molecular docking (AutoDock Vina) between E1-ubiquitin complex with compound **8b** (**410-005**) in ATP binding pocket; the main intermolecular interactions of ligand with side chains and backbone of E1 are indicated; B) molecular fitting of **8b** and ATP in binding pocket of UBA1.

RESULTS



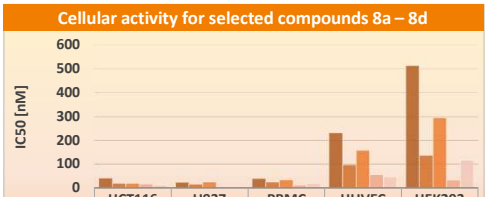
Reaction conditions: a) DMF, reflux, 8h, 39-84%; b) Na_2CO_3, TEA, ACN, 72h, 48-86%; c) H_2, Pd/C, TEA, ETOH, rt, 96h, 23-91%; d) H_2NSO_2Cl, TEA, MS 4Å, DMF, rt, 92h, 63-96%; e) 6M HCl_{aq}, MeOH, rt, 24h, 52-96%

Compound #	R_1
8a 410-002	
8b 410-005	
8c 410-010	
8d 410-009	
8e 410-013	
8f 410-014	
8h 410-016	
8i 410-019	
8j 410-021	

Synthesis discusion

The group of final compounds was synthesized starting from 3-amino-4-bromo-6-chloropyridazine (**1**) and corresponding bromoacetophenones **2a**-**2j** leading to the bicyclic pyrazolo[1,5-a]pyrimidines **3a**-**3j**. Then amination with pseudoribose derivative **4** and following dehalogenation gave the target core skeleton in **6a**-**6j**. This step was less effective (23%) in case of reaction of **5a** where trifluoromethylsulfide was present as an aromatic substitutent and which had an inhibitory influence on Pd/C catalyst. In the next step sulfonylation with repeatedly addition of unstable sulfamoyl chloride gave semi-products **7a**-**7j**. The acidic deprotection step of diol fragment led to the final compounds **8a**-**8j**.

Cellular activity for selected compounds 8a – 8d



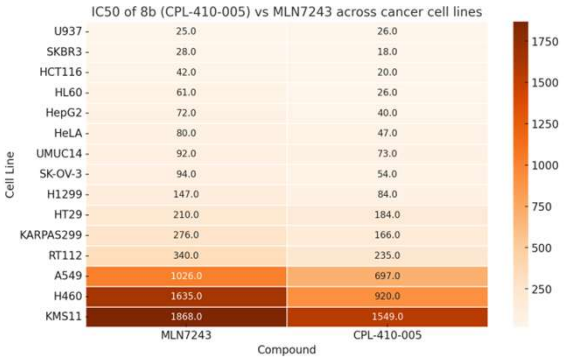
	HCT116	U937	PBMC	HUVEC	HEK293
MLN7243	42	25	40	231	512
8a	20	17	26	98	137
8b	20	26	35	159	294
8c	18	NT	12	57	34
8d	13	NT	20	47	117

Sensitivity Profiling Across Cancer Cell Lines

All the obtained compounds were initially tested and compounds **8a**-**8d** were selected for cell lines screening. From the selected compounds compound **8b** (410-005) showed the best broad spectrum properties both in cancer cell lines inhibition (HCT116 – colon cancer, U937 – myeloid leukemia) as well towards normal cell lines (PPBMC, HUVEC, HEK293). The results highlight **8b** as a promising candidate for further investigation.

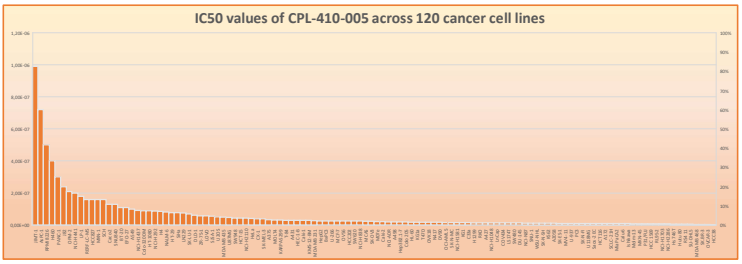
IC_{50} Values of Selected Compounds Across Cancer Cell Lines.

CPL-410-005 (**8b**) exhibited comparable or superior potency to MLN7243 in several cell lines, particularly in SKBR3, HCT116, and HL60. These findings highlight the potential of **CPL-410-005** as a promising lead compound, warranting further investigation.



Activity on Cancer Cell Lines for CPL-410-005

A summary of IC50 values for CPL-410-005 across 120 cancer cell lines tested by ProQinase. The majority of cell lines demonstrated sensitivity to CPL-410-005, with only 18 lines (15%) showing IC50 > 100 nM, suggesting lower sensitivity



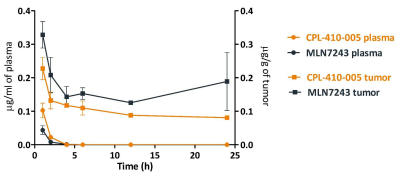
Microsomal stability

Microsomal stability was assessed by incubating CPL-410-005 and MLN7243 with microsomes in the presence or absence of appropriate cofactors. The reaction was terminated at defined time points, and the remaining compound concentration was analyzed using LC/MS to determine metabolic stability. CPL-410-005 (**8b**) exhibits higher metabolic stability in both mouse and human microsomal assays compared to MLN7243, as evidenced by longer half-life ($T_1/_2$) and lower clearance (Cl). Stability was referenced against known standards Donepezil and 7-HFC.

Compound	Microsomal stability			
	Microsomes - murine		Microsomes - human	
	T1/2 [min]	Cl [µ/min*mg]	T1/2 [min]	Cl [µ/min*mg]
MLN7243	74	18,6	362	3,8
8b (410-005)	294	4,7	493	2,8
Donepezil	51	27	699	2
7-HFC	3	461	3	405

Pharmacokinetics and Tumor Retention of CPL-410-005 in SCID Mice.

In the HCT-116 xenograft mouse model, CPL-410-005 is rapidly cleared from plasma but exhibits prolonged retention in the tumor, remaining detectable for up to 24 hours post-administration. This extended tumor exposure suggests favorable pharmacokinetics, supporting its potential for enhanced anticancer efficacy.



CONCLUSIONS

We have successfully designed and developed a potent UBA1 (E1) inhibitor, **8b** - CPL-410-005, demonstrating promising in vitro and in vivo activity. Preclinical studies indicate favorable pharmacokinetic properties, tumor retention, and anticancer efficacy, supporting its potential as a novel therapeutic strategy for the treatment of solid tumors. Because of low diversity of UBA1 enzyme in a wide panel of cancer and healthy cells the narrow therapeutical window was also observed for **8b** CPL-410-005.

The next stage of CPL-410-005 development is construction of antibody-drug conjugates (ADC) in which UBA1 inhibitor has a role of drug-payload fragment. The results of linker-payload synthesis and biological studies for ADCs with the application of CPL-410-005 molecule will be presented soon.

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