IS IT POSSIBLE TO DEGRADE *c*-MYC WITH LOW AFFINITY COMPOUND-BASED PROTACs?

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OBJECTIVE 1: Demonstration of *c*-Myc degradation with PROTAC molecules.

***c*-Myc** is a key therapeutic oncogene that orchestrates a potent pro-cancer programme across multiple cellular pathways. Previous attempts to develop any clinically useful drug directly targeting it have been unsuccessful, partly because it is an Intrinsically Disordered Protein[1]. Indeed, the disordered nature of unbound *c*-Myc has become an inherent challenge for standard structure-based drug development and novel drug modalities are needed to develop therapeutics directly affecting *c*-Myc activity[2].

Thus, the aim of this objective is to prove that ***c*-Myc, an undruggable transcriptor factor, can be degraded by using the PROTAC machinery with low binding affinity compounds.**

DESIGN AND SYNTHESIS OF *c*-MYC - BASED PROTACS

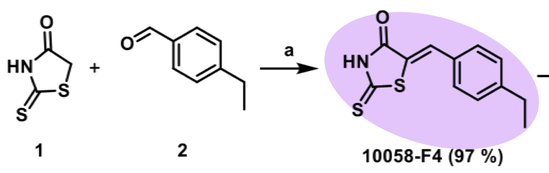
A first series of **VHL- and CRBN- based PROTACs** were synthesized by using **two fragment-sized derivatives of 10058-F4[3],** a low affinity *c*-Myc inhibitor, as the warhead ligands.

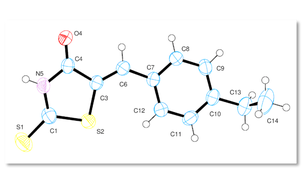
The **two *c*-Myc vectors were unexpectedly obtained during the *N*-alkylation reaction of 10058-F4** due to the NH reactivity in basic conditions. X-ray structure elucidation of both compounds was confirmed by Prof. Dr. Elies Molins.

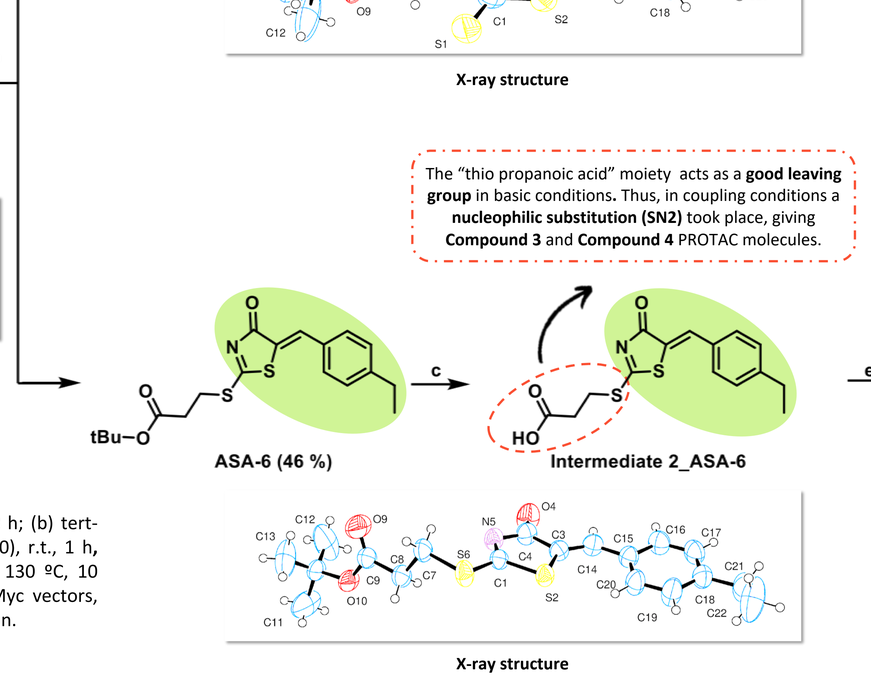
Due to polarity similiarities and difficult chromatographic separation, different synthetic strategies were tried so as to synthesize **ASA-5** and **ASA-6,** separately. After performing several attempts, **Intermediate 1_ASA-5** was synthesized in a one-pot reaction with high yield.

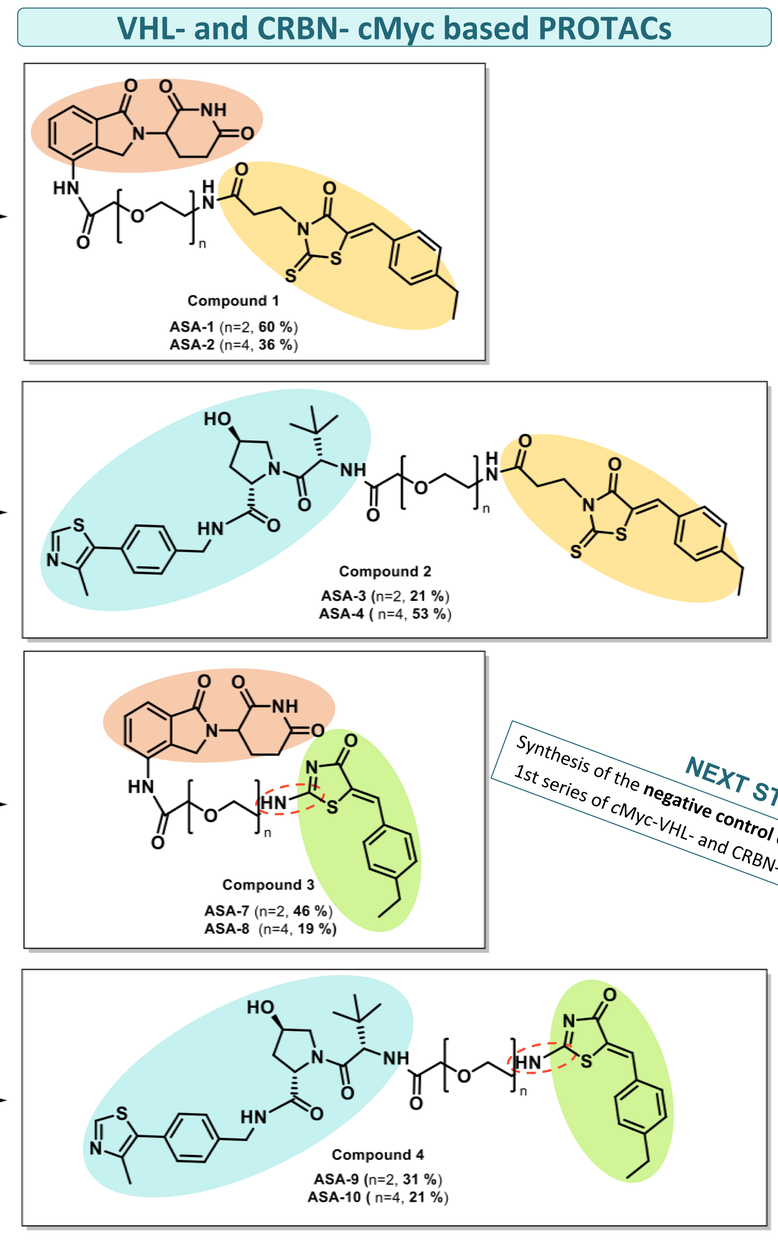
In-house synthesis of 10058-F4

Only Z confiormation is formed.
No literature precedents of its characterization.









NEXT STEP
Synthesis of the **negative control** of the **most potent** 1st series of cMyc-VHL- and CRBN-based PROTACs.

Scheme 1: *Reagents and conditions*. (a) CH_3COONH_4, CH_3COOH, reflux, 3 h; (b) tert-Butyl 3-bromopropionate, K_2CO_3, ACE, reflux, 3 h; (c) TFA : DCM (50 : 50), r.t., 1 h**, quant., Intermediate 1_ASA-5, Intermediate 2_ASA-6**; (d) solvent-free, 130 ºC, 10 min, Microwave conditions (MW), **90 %, Intermediate 1_ASA-5;** (e) *c*-Myc vectors, VH032-PEG2 / PEG4, Lenalidomide-PEG2 / PEG4, HATU, DIPEA, DMF, r.t., o.n.

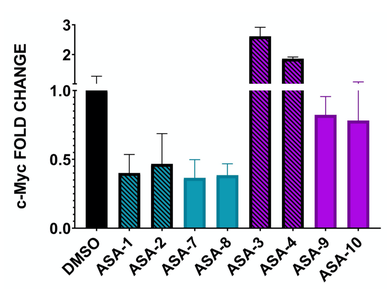
CRBN-BASED PROTACs induce *c*-Myc DEGRADATION

Western Blot Assays to find the **most potent compounds of 1st series of *c*-MYC based PROTACs.**

SINGLE DOSE ASSAY

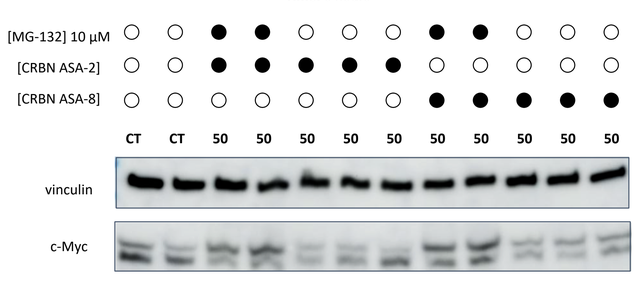
Time: 6 hours

HEK 293
[PROTAC]: 50 uM.



TIME SCREENING ASSAY WITH CRBN-BASED PROTACS (t = 2, 6, 24 h)

Time: 6 hours

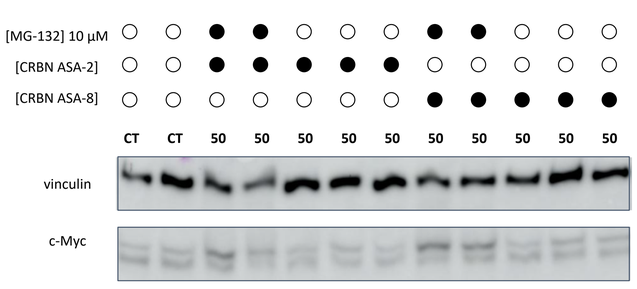


20% Proteasome dependent degradation with ASA-2 and ASA-8 CRBN-based PROTACs.

HCT 116

TIME SCREENING ASSAY WITH CRBN-BASED PROTACS (t = 6, 24 and 72 h)

Time: 24 hours



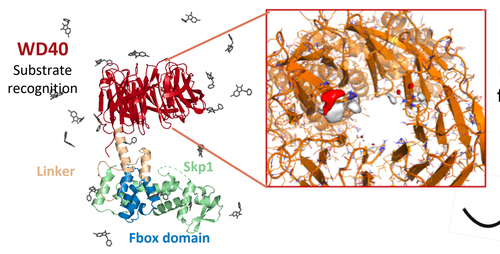
PRELIMINARY RESULTS

70 % and 40% Proteasome dependent degradation with ASA-2 and ASA-8 CRBN-based PROTACs.

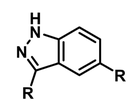
OBJECTIVE 2: Development of Fbxw7-based PROTACs for targeting *c*-Myc degradation.

Fbxw7 gene is one of the most deregulated proteins in human cancer and it is estimated that 6% of all cancers contain mutations in Fbxw7 disrupting substrate recognition[4]. Also, it is an E3 ligase that **naturally degrades *c*-Myc[5]**. However, no potent molecules have been described yet.

Computational studies identified key interactions in the WD40 domain with fragment F4E10.



Isatin-centered fragment screening from the original F4E10 hit revealed new potent Fbxw7 binders.



NEXT STEPS

1) Build a SAR with the discovered scaffolds to grow the fragments and increase potency.

2) Synthesis of Fbxw7-Brd4 based PROTACs.
We will prove that the novel Fbxw7 fragments are sufficiently potent to build PROTACs to target Brd4 and downregulate *c*-Myc activity by using a fragment-based approach.

3) Synthesis of Fbxw7- cMyc based PROTACs.

The overarching goal is to develop PROTAC molecules that can bind allosterically to the Fbxw7 E3 ligase and to *c*-Myc, to retarget it for degradation.

We want to prove that PROTAC molecules targeting the **natural pair E3 ligase-natural substrate (*c*-Myc-Fbxw7)** can **increase the efficacy and the specificity of the degradation** for several reasons: employment of the same ubiquitination machinery, cellular and tissue co-localization, absolute abundance.

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