Identification and characterization of inhibitors of SHOC2-MRAS-PP1C complex assembly

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Abstract

Inhibition of SHOC2 function by either genetic ablation or protein degradation potently sensitizes RAS-driven cells to MEK inhibition and impairs RTK-mediated adaptive reactivation of MAPK signaling induced by a MEK inhibitor (MEKi). The SHOC2-MRAS-PP1C (SMP) complex is responsible for full activation of RAF via dephosphorylation of the phosphoserine in conserved region 2 (CR2-pS). Based on the SMP complex's critical role in RAF activation and SHOC2 synthetic lethal interaction with MEKi, the SMP complex represents an attractive target for the development of novel therapeutic agents that could prevent adaptive resistance to MEKi.

We have conducted a systematic DNA-encoded library (DEL) screening campaign against the purified intact SMP complex and all three individual components using the HitGen Open DEL kit. A series of SHOC2 selective hits were identified and profiled using direct binding and biochemical assays. The lead compounds bind to full-length SHOC2 protein with K_Ds in the low nM range, inhibit SMP complex formation, and block dephosphorylation of BRAF CR2-pS peptide. Hydrogen/deuterium exchange mass spectroscopy (HDX-MS) analysis of SHOC2 in complex with a lead compound identified a putative binding site, consistent with biochemical studies and supporting the MRAS competitive nature of the lead compounds. To our knowledge, these compounds represent first-in-class examples of direct modulators of the SMP complex, via SHOC2 engagement, that have the potential for optimization as either PPI (protein-protein interaction) modulators or selective degraders.

Introduction

- **Novel therapeutic approaches are required for targeting RAS-MAPK pathway addicted cancers**
- **Specific dephosphorylation of RAF is a critical step in the formation of ERK-activating RAF dimers**
- **H/K/N RAS isoforms can substitute for MRAS to bind SHOC2-PP1C and form the active phosphatase complex**
- **Our lead compounds bind to SHOC2 and inhibit SMP complex formation, offering the potential for translation into therapeutic modalities**

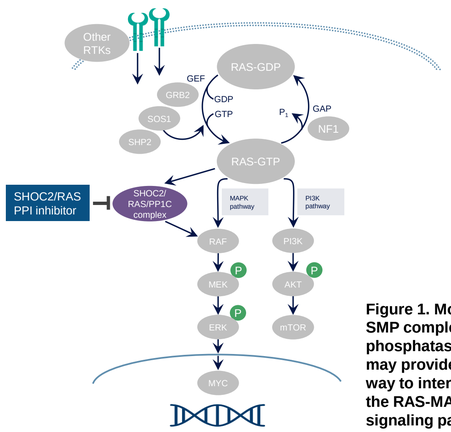


Figure 1. Modulation of SMP complex phosphatase activity may provide a novel way to interfere with the RAS-MAPK signaling pathway

Results

Hit identification from DEL screening

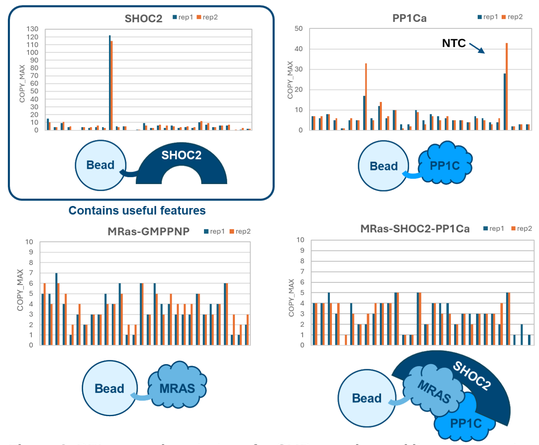
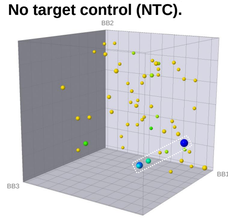


Figure 2. DEL screening strategy for SMP complex and its components. No target control (NTC).



Library 1 Confirmed off-DNA Hits

Compound ID		12531	12532
Enrichment		rep 1: 4397 rep 2: 2425	rep 1: 5130 rep 2: 1819
Physchem properties	MW	564.6	564.6
	cLogP	3.1	3.1
	PSA	130	130
	HAC	42	42
K_D (SPR), nM		26	160

Library 2 Confirmed off-DNA Hits

Compound ID		12533	12534
Enrichment		rep 1: 55138 rep 2: 68074	rep 1: 71276 rep 2: 72612
Physchem properties	MW	511.5	510.5
	cLogP	2.7	3.5
	PSA	126	113
	HAC	38	38
K_D (SPR), nM		10.3	11.3

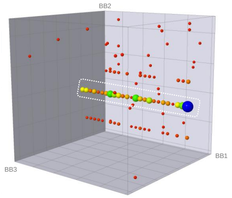


Figure 3. Cluster identification and SPR analysis using SHOC2 (1-582) protein of off-DNA hits.

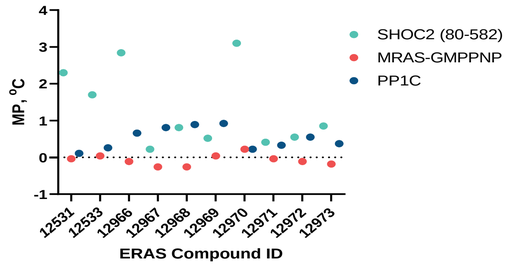


Figure 4. Thermal shift (Tagg) analysis of DEL hit selectivity against components of SHOC2 complex. 12531 and 12533 are a racemic mixture. ERAS-12970 is an active isomer in the ERAS-12533 racemic mixture.

HDX and MD mapping of ERAS-12970 binding site

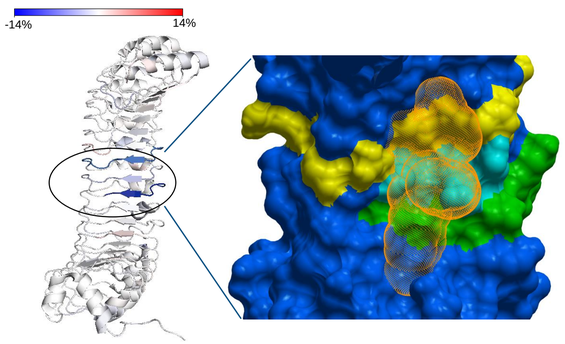
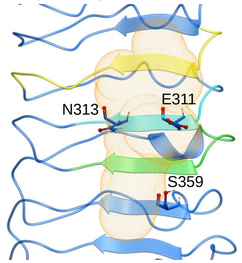


Figure 5. Relative D uptake difference of SHOC2 (80-582) acquired by comparing SHOC2 (80-582)+ERAS-12970 vs SHOC2 (80-582) apo states mapped on SHOC2 structure (PDB ID:7TYG) (left panel). HDX close-up surface with ligand cloud. The surface representation of SHOC2 protein (PDB ID:7TYG) with ligand (right panel). Yellow depicts peptide regions involved in binding ERAS-12970 based on HDX data: AA284-296, cyan - AA309-314 and green - AA330-338. Orange dot cloud represents the ligand position in the binding site. MD (Molecular dynamics) simulations were performed to discard unstable ligand binding poses and optimize protein/ligand interactions.



ERAS-12970 binding studies with SHOC2 mutants

Mutation	ΔT_m 1 [°C]*	Kinetic K_D (nM)	$t_{1/2}$ (min)
WT	2.7	26.5	6.6
E311R	1	>6000	ND
N313D	0.3	1010	0.15
S359A	4	5.7	24.6

* reference to 2% DMSO, respectively

Figure 6. Mutational analysis of ERAS-12970 modeled binding site and validation using direct binding. Three critical amino acids in the putative SHOC2 binding site were mutated in SHOC2 (80-582) construct and purified without tag for nanoDSF and SPR studies. E311R and N313D mutations significantly decrease binding, while S359A shows minor increase in binding to ERAS-12970.

Direct binding and biochemical analysis of DEL hits and their derivatives

Direct binding and biochemical characterization of SHOC2 binders

Compound	Kinetic K_D (nM)	$t_{1/2}$ (min)	HTRF MRAS-GMPPNP, IC50, nM	HTRF KRAS Q61R-GMPPNP, IC50, nM
12966	60.7	2.22	172	312
13916	74.1	9.00	189	174
12970	11.8	14.7	78	107
13921	650	0.61	5606	8144
13912	309	0.86	1232	2924
13917	7	13.2	67	72
13918	ND	ND	2804	5569
13920	128	2.0	1188	1545
12531*	38.8	13.4	329	126
13924-1678*	215	1.2	1332	941

* Racemic compounds

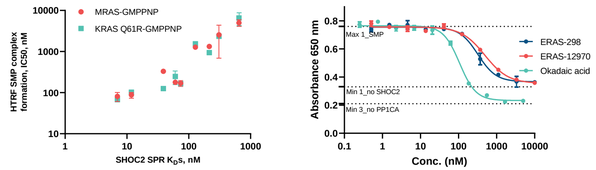


Figure 7. Correlation between direct binding activity of SHOC2 compounds and PPI activity in HTRF SMP complex formation assays using either MRAS or KRAS Q61R GMPPNP (left panel). Inhibition of phosphatase activity under complex assembly conditions (right panel).

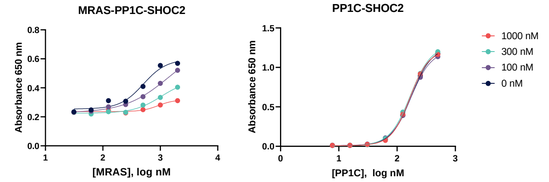


Figure 8. In a phosphatase activity assay ERAS-12970 is a modulator of SMP ternary (left panel) complex formation but not the SP binary complex (right panel). Compounds were pre-incubated with SHOC2. Competitor protein (PP1C or MRAS-GMPPNP) was added in 8-point dose response. Shift in dose response (EC_{50}) indicates MRAS competitive binding.

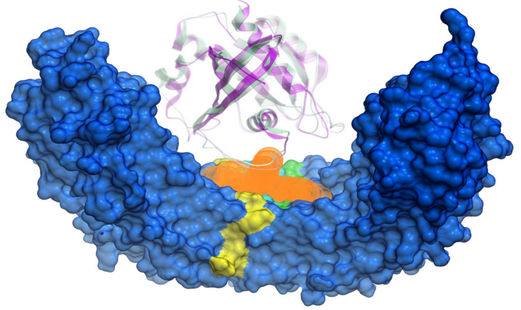


Figure 9. SHOC2-MRAS(KRAS) complex and steric clashes caused by ligand (orange dot cloud) with MRAS (magenta) and superimposed structure of KRAS (aquamarine). Yellow color represents peptide AA284-296 of SHOC2 protein, cyan - AA309-314 and green - AA330-338.

Conclusions

- **Systematic screening of SMP complex components identified two lead series of compounds with low nM dissociation constants**
- **Both lead series are selective for SHOC2 as evidenced by in vitro thermal shift assays with SHOC2, MRAS-GMPPNP and PP1C proteins**
- **The compound binding site was modeled based on HDX and molecular dynamics studies**
- **The modeled binding site for ERAS-12970 (library 2) was further refined based on the results of mutational analysis**
- **Initial SAR analysis based on the binding site model suggests vectors for potential modification of the lead series**
- **Consistent with the binding site model, biochemical analysis indicated that the lead compounds are competitive with RAS binding (MRAS GMPPNP and KRAS Q61R GMPPNP)**
- **Further optimization of lead matter for PPI inhibitor and degrader modalities are ongoing**