Discovery of WRN helicase inhibitors that covalently engage a novel, induced allosteric site

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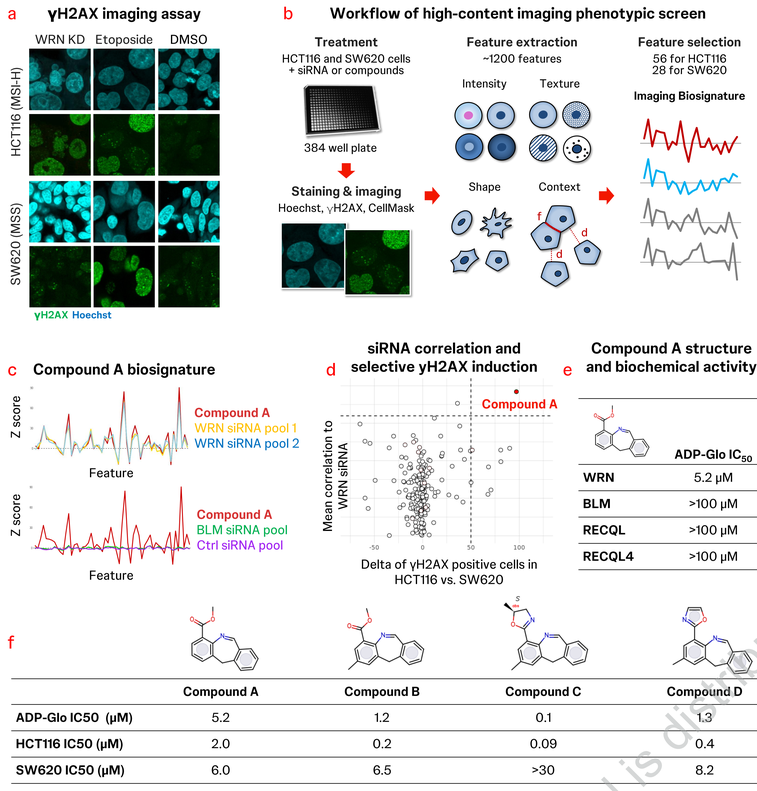
Background

The RecQ helicase WRN is a synthetic lethal dependency in microsatellite instability-high (MSI-H) cancer and a therapeutic target of interest in that context. The two disclosed clinical-stage WRN inhibitors, HRO761 and RO7589831, both bind in an allosteric pocket near the interface of the two helicase domains (D1 and D2).

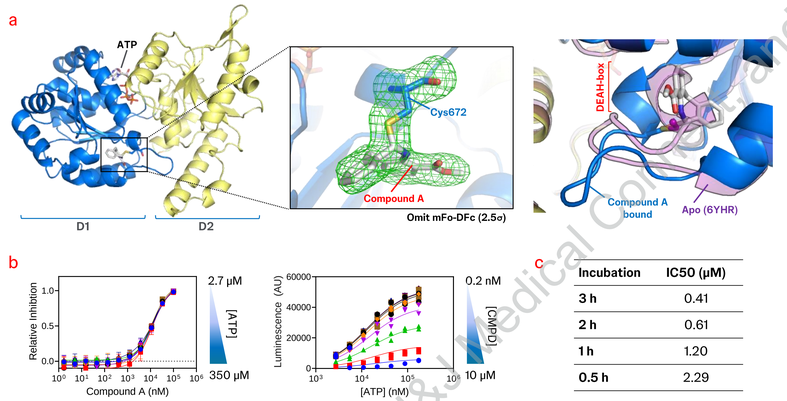
Phenotypic screening identifies a novel morphanthridone series of WRN inhibitors



	Compound A	Compound B	Compound C	Compound D
ADP-Glo IC50 (μM)	5.2	1.2	0.1	1.3
HCT116 IC50 (μM)	2.0	0.2	0.09	0.4
SW620 IC50 (μM)	6.0	6.5	>30	8.2

Figure 1. Using γH2AX immunofluorescence imaging (a), we conducted a high-throughput phenotypic screen in MSI-H HCT116 colorectal cancer cells (b) and identified a singleton hit, Compound A (c-d). Compound A phenocopied WRN siRNA knockdown in multiparametric analysis and was confirmed to engage and inhibit purified WRN protein but not the paralogous RecQ helicases BLM, RECL and RECQL4 (e). (f) Representative molecules from SAR exploration.

Morphanthridone WRN inhibitors covalently engage a novel, induced allosteric site



Incubation	IC50 (μM)
3 h	0.41
2 h	0.61
1 h	1.20
0.5 h	2.29

Figure 2. (a) X-ray co-crystal structures with and without ATP revealed that Compound A binds to a novel allosteric site in the D1 domain (ATP-bound shown), which is distinct from the HRO761 binding site and is absent in apo-WRN. Electron density was indicative of a covalent bond formation via addition of Cys672 to the imine C=N bond of Compound A. The allosteric site was induced by the localized rearrangement, including displacement of the DEAH-box upon ligand binding. (b) Mechanistically, Compound A was ATP-noncompetitive and Compound B exhibited time-dependent inhibition of WRN ATPase activity (c).

Morphanthridone WRN inhibitors recapitulate synthetic lethality in MSI-H cancer models

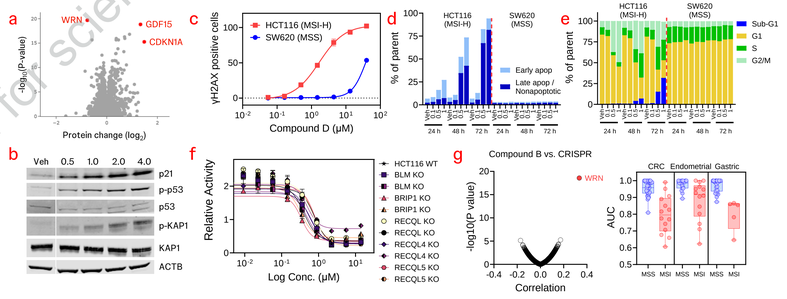
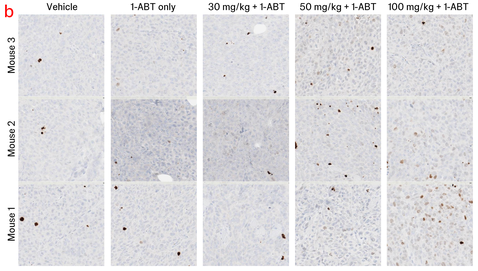


Figure 3. Global proteomics (a) and cell signaling (b) studies in HCT116 cells showed Compound D to cause concentration-dependent degradation of WRN and upregulation of DNA damage responses. Compound D selectively induced γH2AX phosphorylation (c), apoptosis (d), and cell cycle arrest (e), in MSI-H cells. The cytotoxicity of Compound D was unaffected by isogenic, CRISPR-mediated knockout of BLM, RECQL, RECQL4, RECQL5 or BRIP1 in HCT116 cells (f). Compound B was highly selective for MSI-H models and recapitulated the profile of sensitivity to WRN perturbation in a PRISM screen of 906 cell lines (g).

Morphanthridone WRN inhibitors induce DNA damage in HCT116 MSI-H tumors

	Compound D + Vehicle	Compound D + 1-ABT
Human PPB (%)	99.62	
Mouse PPB (%)	99.05	
CL (mL/min/kg)	182	
Vdss (L/kg)	4.15	
$T_{1/2}$ (h)	1.36	
C_{max} (ng/mL)	86.1	1937
T_{max} (h)	0.583	0.583
$AUC_{(0\text{-}inf)}$ (ng.h/mL)	217	7440
F (%)	23	64



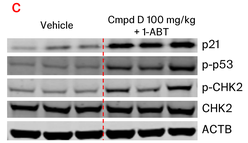


Figure 4. (a) Pharmacokinetic profile in mice of Compound D alone (IV/PO at 2/10 mg/kg) or with co-administration of 100 mg/kg 1-ABT, PO. Co-administration of Compound D (PO, BID × 1) with the CYP inhibitor 1-aminobenzotriazole (1-ABT, 100 mg/kg PO × 1) to mice (n = 3) bearing HCT116 xenografts produced dose-dependent γH2AX (b) and DNA-damage responses (c).

CRISPR screens identify modifiers of sensitivity to WRN inhibitors

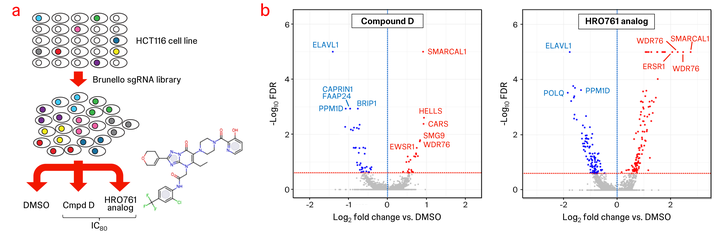


Figure 5. Whole-genome CRISPR modifier screens with Compound D and a close analog of HRO761 in HCT116 cells (a) showed concordant functional genomic profiles, with perturbations in SMARCAL1, HELLS, and WDR76 enriched and ELAV1, POLQ, and PPM1D depleted by WRN inhibition (b).

Conclusion

We report of WRN inhibitors that engage a novel cryptic binding site and show a distinct mechanism of inhibition to WRN inhibitors currently in clinical trials.

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