107. PROTEIN KINASE INHIBITORS

(University of California - Los Angeles)

Asset Overview

Product Type	Small Molecule
Disease Area	Oncology
Indication	Cancer
Current Stage	HIT to Lead
Target	DHODH
MoA	PDK-1 inhibitors inhibit DHODH
Brief Description	UCLA inventors have identified that two clinically established PDK-1 inhibitors also inhibit DHODH. Each had sub-micromolar IC50 values against a pancreatic cancer cell line starved of uridine and forced to rely upon DNP, indicating highly potent and selective inhibitory effects. The DHODH inhibitory action of these compounds was subsequently confirmed through use of a colorimetric assay using purified recombinant human DHODH, which demonstrated that both compounds inhibited the enzyme in a dose-dependent manner. Co-crystal structures of each compound complexed with human DHODH were also obtained, unambiguously confirming their DHODH-inhibitory ability. The intended targets of OSU-03012 (PDK-1) and TAK-632 (pan-RAF) are relevant for cancer therapy, as is the newly-identified target (DHODH).
Intellectual Property	WO2020232445A1
Publication	Metabolic modifier screen reveals secondary targets of protein kinase inhibitors within nucleotide metabolism, Cell Chem Biol. (2020)
Inventors	Caius G. Radu, Evan ABT, Ethan ROSSER, Arnon Lavie, Matthew DURST, Soumya PODDAR

Highlights

- Novel therapeutic strategy in treating many types of cancer
- Antiviral agent against a host of diseases associated with DHODH, including Ebola, Zika, Influenza, and Epstein Barr
- Therapeutic intervention in antibacterial and antifungal settings
- Compounds identified have multiple targets (PDK-1 for OSU-03012 and pan-RAF for TAK-632) associated with cancer cells, giving them greater potency as cancer therapies
- Low IC50 values indicate highly potent and selective inhibitory effects on the pyrimidine de novo pathway
- OSU-03012 has already received Orphan Drug Status in Europe

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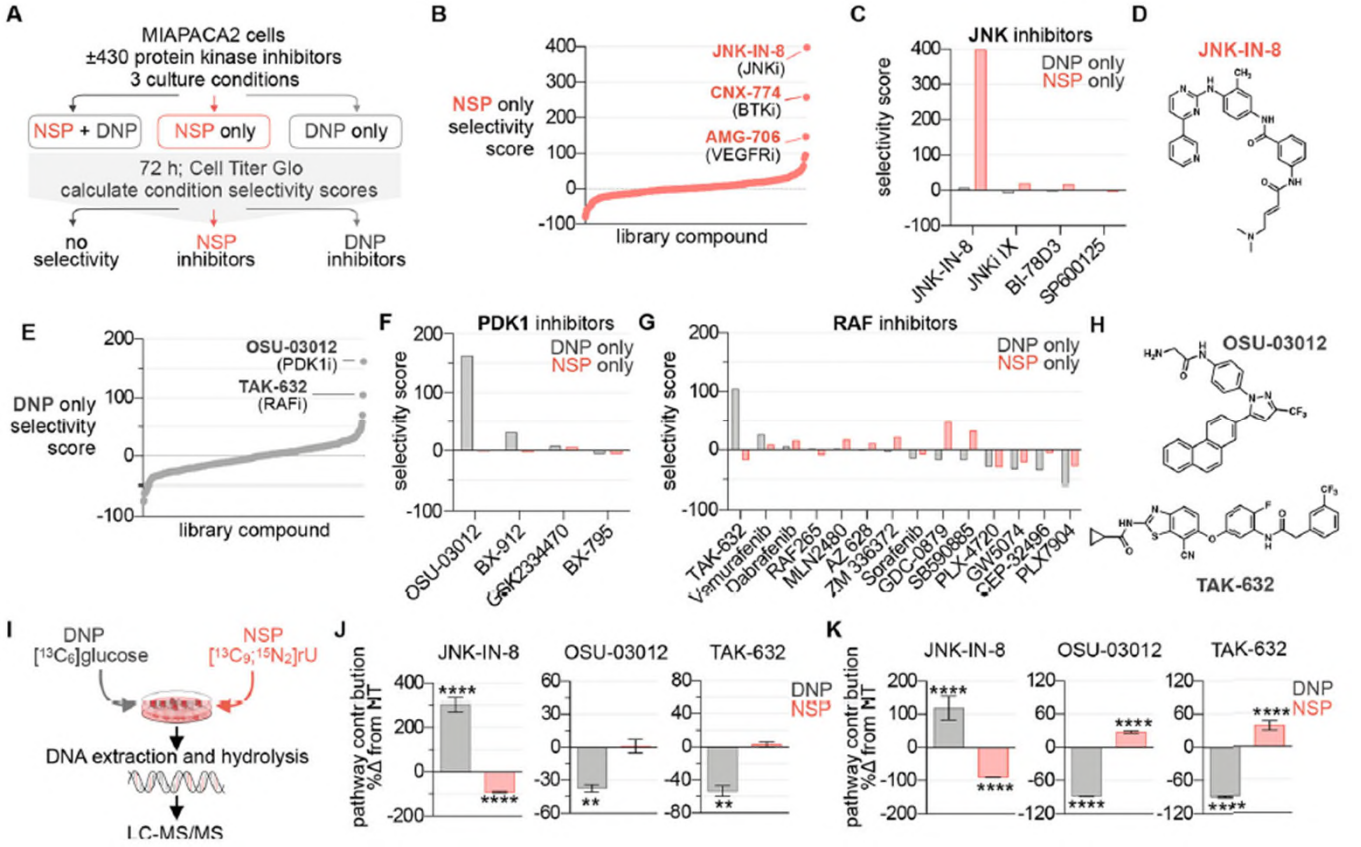
Key Data

Identification of UMP-NSP and -DNP modulators in a protein kinase inhibitor library.



(A) Phenotypic screening strategy. The impact of 430 protein kinase inhibitors on cell proliferation was evaluated in MIAPACA2 cells plated in 3 distinct culture conditions; 1) NSP+DNP (media +10 μM uridine (rU)); 2) NSP only (media +10 μM rU +1 μM NITD-982); or 3) DNP only (media alone). % proliferation values were calculated using Cell Titer Glo (CTG) following 72 h treatment (7-point dose response; n=2). (B) Waterfall plot ranking library compounds based on NSP pathway selectivity score. (C) Summary of NSP and DNP selectivity scores across library compounds annotated as JNK inhibitors. (D) Structure of JNK-IN-8. (E) Waterfall plot ranking library compounds based on DNP pathway selectivity score. (F,G) Summary of NSP and DNP selectivity scores across library compounds annotated as PDK1 (F) or RAF inhibitors (G). (H) Structures OSU-03012 and TAK-632. (I) Experimental design to track contribution of UMP-DNP and -NSP to newly replicated DNA using stable isotope-labeled metabolite tracers. (J,K) LC-MS/MS analysis of [$^{13}C_6$]glucose (5.5 mM) and [$^{13}C_9$; $^{15}N_2$] rU (10 μM) utilization for DNA-C replication in MIAPACA2 (J) or JURKAT (K) cells treated +1 μM JNK-IN-8 +5 μM OSU-03012 or +5 μM TAK-632 for 24 h (NT: not-treated; mean±SD; n=3; unpaired T-test; ** P < 0.01, **** P < 0.0001).

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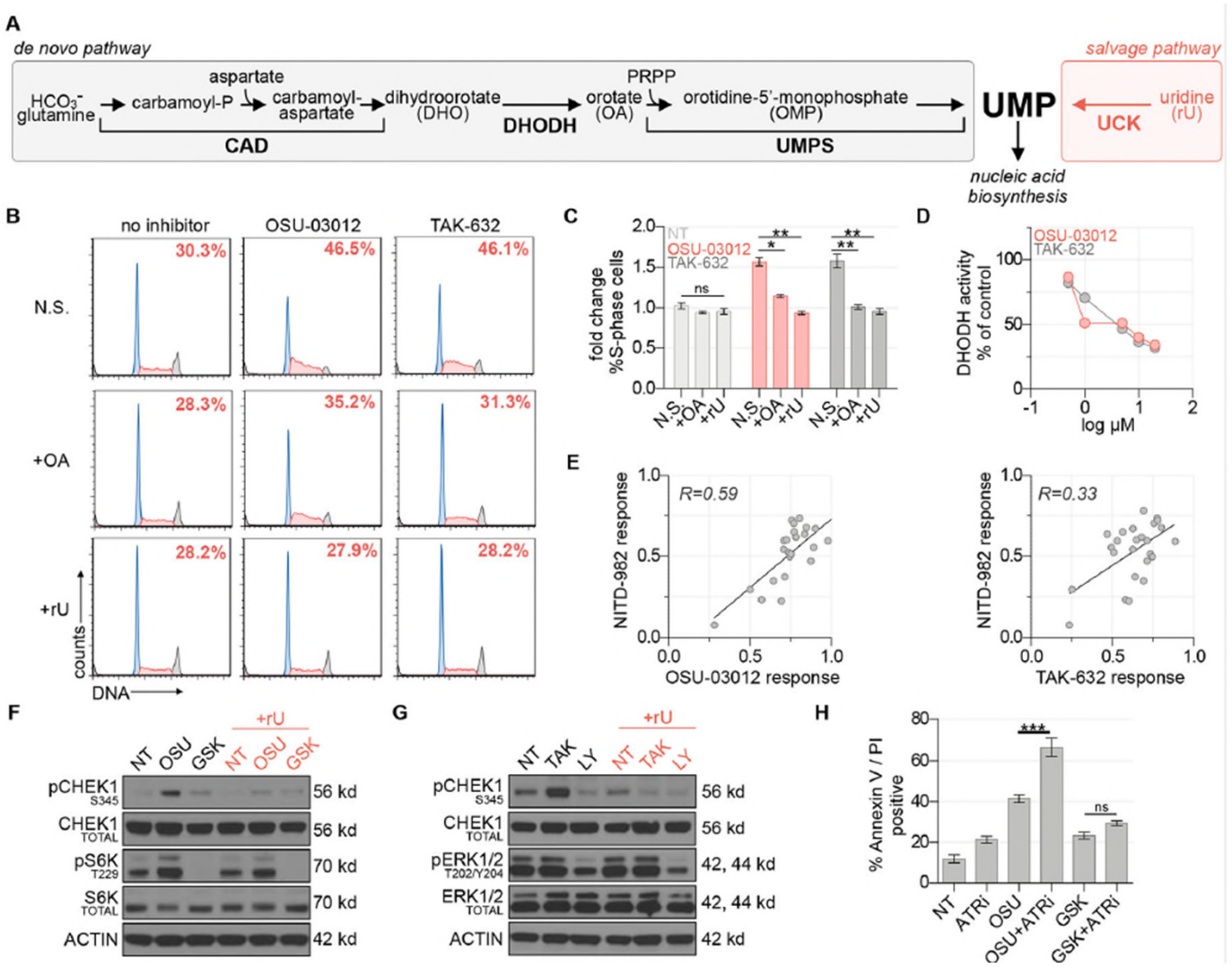
OSU-03012 & TAK-632 inhibit DHODH and activate the DNA replication stress response pathway.



(A) Schematic of UMP biosynthesis via the de novo and salvage pathways. (B) Propidium iodide cell cycle analysis of MIAPACA2 PDAC cells treated ±5 μM TAK-632 or ±5 μM OSU-03012 and supplemented with 50 μM orotate (OA) or 10 μM rU (N.S.: no supplement). Insert indicates % S-phase cells. (C) Summary of fold changes in S-phase cells from B (mean±SD; n=2; one-way ANOVA corrected for multiple comparisons by Bonferroni adjustment, ns: not significant; * P<0.05; ** P<0.01). (D) in vitro DHODH enzyme assay performed in the presence of OSU-03012 or TAK-632. (E) Correlation between DHODH inhibitor (1 μM NITD-982) and OSU-03012 (3.17 μM) or TAK-632 (3.17 μM) response across a panel of 25 PDAC cell lines determined using CTG following 72 h treatment. Response calculated as doubling time normalized proliferation inhibition. Pearson correlation coefficient is indicated. (F) Immunoblot analysis of MIAPACA2 cells treated ±1 μM PDK1 inhibitor GSK-2334470 (GSK) ±1 μM OSU-03012 (OSU) ±10 μM rU for 24 h. (G) Immunoblot analysis of MIAPACA2 cells treated ±10 μM RAF inhibitor LY3009120 (LY) ±10 μM TAK-632 (TAK) ±10 μM rU for 24 h. (H) Annexin V/PI flow cytometry analysis of MIAPACA2 PDAC cells treated ±1 μM OSU-03012 or 1 μM GSK-2334470 (GSK) ±500 nM VE-822 (ATRi) ±25 μM rU for 72 h (mean±SD; n=2; one-way ANOVA corrected for multiple comparisons by Bonferroni adjustment; ns: not significant; ** P<0.01; *** P<0.001).