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The Science CRO

High-throughput phenotypic screening and target deconvolution of novel oncogenic YAP/TAZ signaling pathway inhibitors

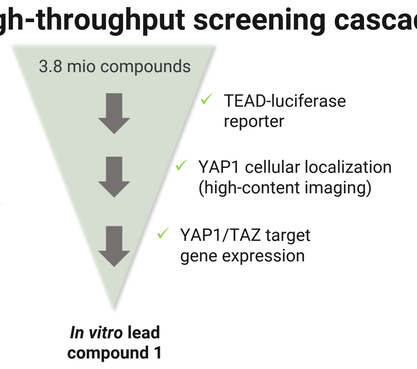
A phenotypic high-throughput screen of 3.8 million compounds was conducted using a cellular YAP1/TAZ-dependent luciferase reporter identified *in vitro* lead compound 1 as a potent inhibitor of YAP1/TAZ activation. Target deconvolution studies, including cellular thermal shift assays and CRISPR/Cas9-KO screens, identified PGGT1B, a subunit of the geranylgeranyltransferase-I (GGTase-I) complex, as the direct target of YAP1/TAZ pathway inhibitors. GGTase-I inhibitors blocked the activation of Rho-GTPases at the cell membrane, leading to subsequent inactivation of YAP1/TAZ.

Phenotypic screen to identify novel YAP1/TAZ pathway inhibitors

TEAD-luciferase reporter HTS set-up

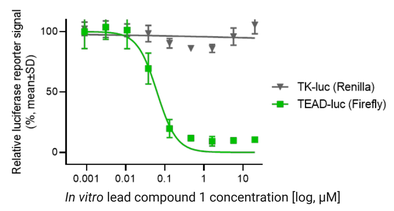
- Assay development in 384-well plates
- Assay miniaturization in 1536-well plates
- Compound transfer by Echo® Acoustic liquid handler
- Luminescence detection by Dual-Glo Luciferase system on Pherastar microplate readers

High-throughput screening cascade

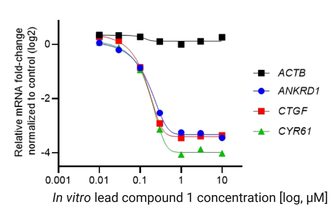


In vitro lead compound 1 inhibits YAP1/TAZ in cellular assays

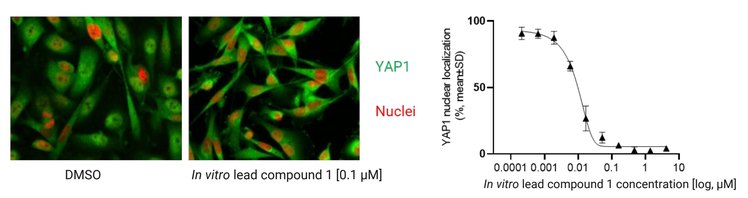
TEAD-luciferase reporter assay



YAP1/TAZ target gene expression



YAP1 cellular localization (high-content imaging)



***In vitro* lead compound 1**

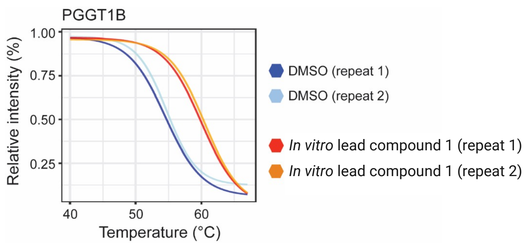
TEAD-luciferase reporter assay

[IC50] = 0.56 µM

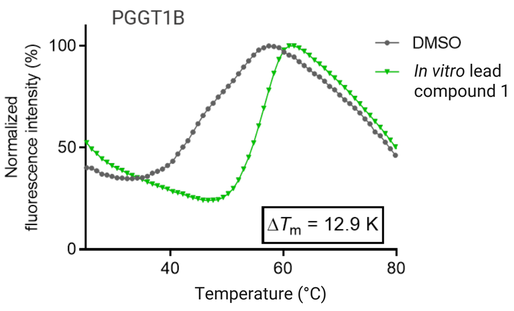
Target deconvolution of *in vitro* lead compound 1

CETSA®-MS melting curves

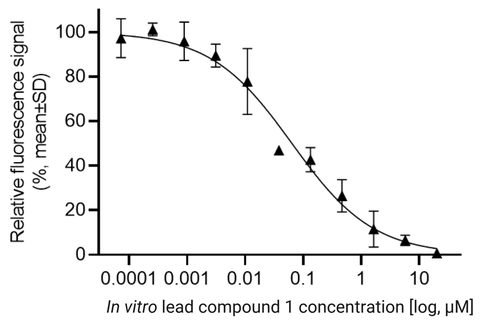
(CETSA® experiments were carried out at Pelago Bioscience)



Thermal Shift Assay (TSA) melting curves

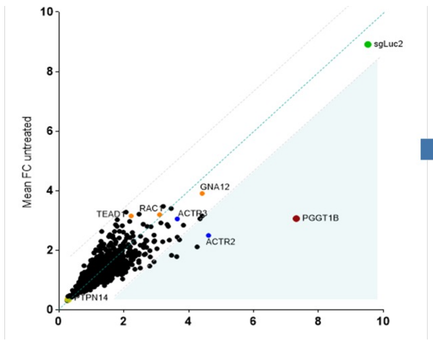


Biochemical PGGT1B assay

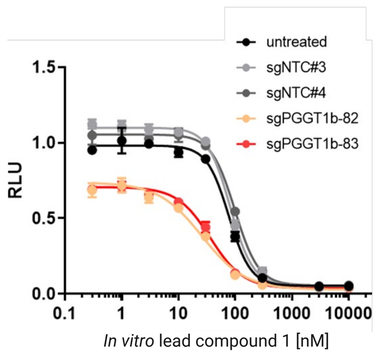


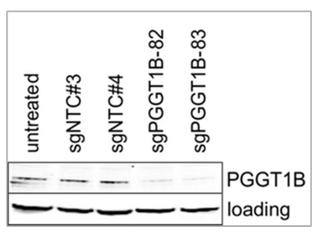
Pooled CRISPR/Cas9 screen in the TEAD-luciferase reporter cell line

(knock-down of~ 6000 human genes)



CRISPR/Cas9 hit verification





CETSA®-MS:

PGGT1B identified as the protein with the highest shift in melting temperature upon incubation of MDA-MB-231 cell lysates with *in vitro* lead compound 1 (experiments carried out using CETSA® at Pelago Bioscience, requiring a target-specific license from Pelago)

Thermal Shift Assay:

Direct binding of PGGT1B by *in vitro* lead compound 1 confirmed using TSA with recombinant PGGT1B

Biochemical assay:

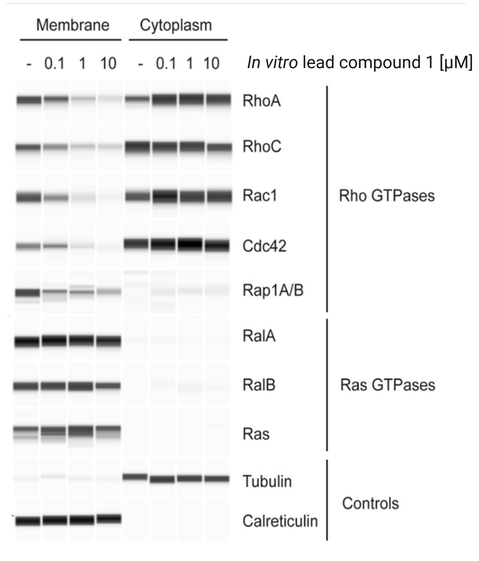
In vitro lead compound 1 inhibits the enzymatic activity of purified human GGTase-I

Pooled CRISPR/Cas9 screen:

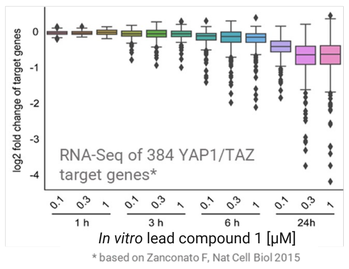
Knock-down of PGGT1B significantly sensitizes cells to treatment with *in vitro* lead compound 1

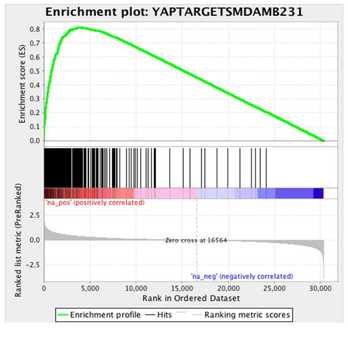
Mode of action confirmation

***In vitro* lead compound 1 inhibits geranylgeranylation and activation of Rho GTPases at the cell membrane**



***In vitro* lead compound 1 treatment significantly downregulates YAP1/TAZ target gene expression**





- Novel YAP1/TAZ pathway inhibitors identified by cellular pathway high-throughput screen
- Target deconvolution identified GGTase-I as the direct target of the novel YAP1/TAZ pathway inhibitors
- GGTase-I inhibitors block Rho-GTPase signaling and downstream YAP1/TAZ

Mode of Action