Optimization of PDE3/SLFN12 Modulators to Kill Cancer Cells

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Science For A Better Life

Discovery of DNMDP

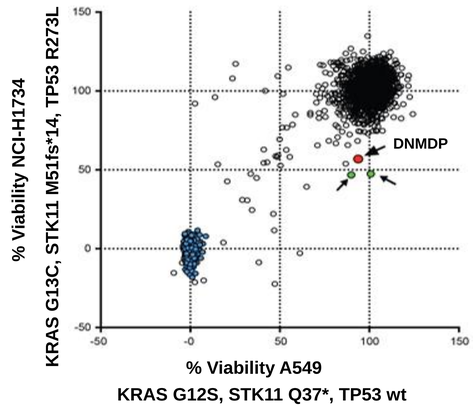

Figure 1. Viability assay results against mutant and wild-type p53 cancer cell lines identifies DNMDP as a selective hit

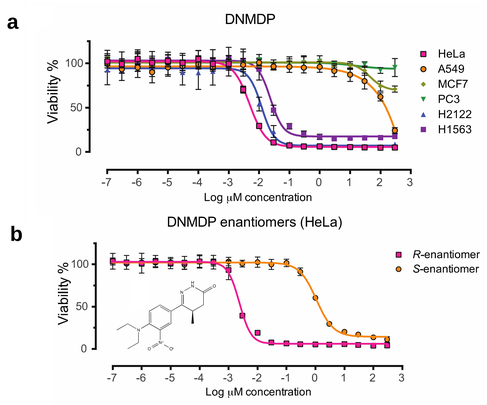

Figure 2. a) DNMDP is potent and selective hit, killing 26 out of 766 cancer cell lines with nM potency
b) Enantiospecific cell killing

PDE	% Inhibition	PDE	% Inhibition
PDE1A1	5	PDE4D7	20
PDE1B	-2	PDE5A1	16
PDE1C	5	PDE7A	22
PDE2A	8	PDE7B	8
PDE3A	95	PDE8A1	11
PDE3B	97	PDE9A2	2
PDE4A1A	16	PDE10A1	63
PDE4B1	21	PDE10A2	68
PDE4C1	12	PDE11A	16
PDE4D3	15		

Measured at 100 nM DNMDP

PDE3A Inhibition (IC_{50}) - 25 nM
PDE3B Inhibition (IC_{50}) - 100 nM
kinase screen – 0/234 kinases at 10 μM

Figure 3. DNMDP is a selective PDE3 inhibitor without kinase activity

DNMDP is a PDE3 inhibitor, but most PDE3 inhibitors do not kill cancer cells

PDE3 Inhibitor	PDE3 (IC_{50}, nM, lit.)	HeLa (EC_{50}, nM)
Trequinsin	0.25	>10,000
Levosimendan	2.4	>10,000
Cilostamide	27	>10,000
Zardaverine	56	2500
Anagrelide	36	8.2

Figure 4. No correlation between PDE3 inhibition and HeLa cell viability

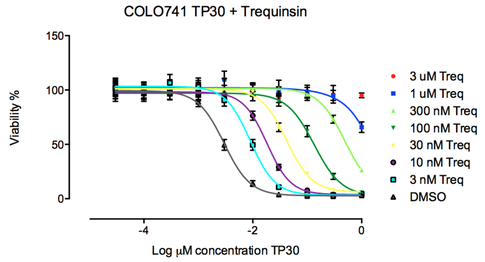

Figure 5. HeLa cells rescued from DNMDP analog-induced death by non-toxic PDE3 inhibitor

PDE3A Binds to SLFN12 with DNMDP

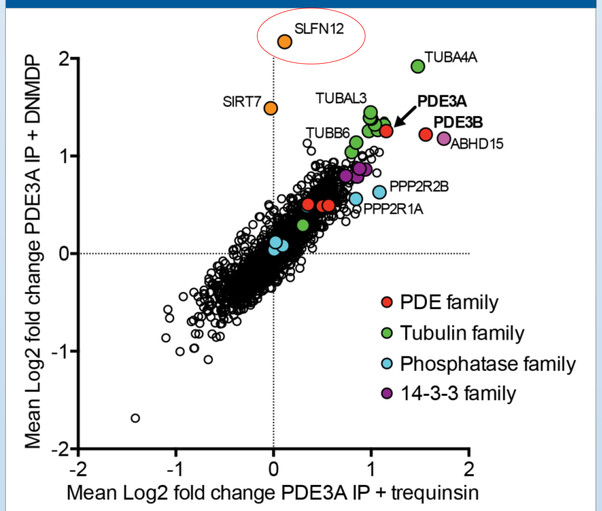

Figure 6. Proteomic analysis of PDE3A antibody pulldown identifies SLFN12 binding to PDE3A in presence of DNMDP but not non-toxic trequinsin

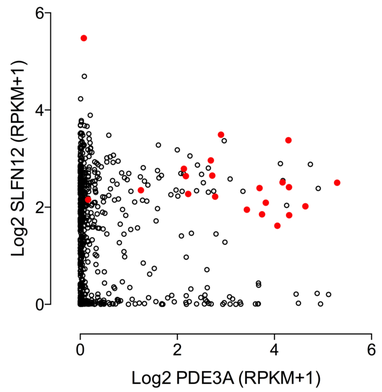

Figure 7. High PDE3A and SLFN12 expression predicts DNMDP sensitivity, with 50% prediction accuracy

SAR

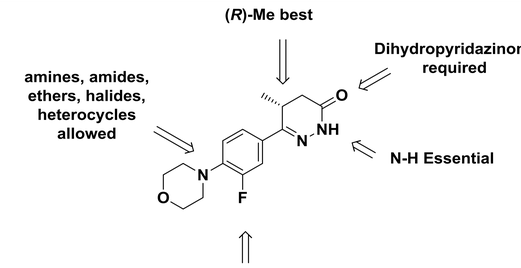

Figure 8. SAR about DNMDP analogs

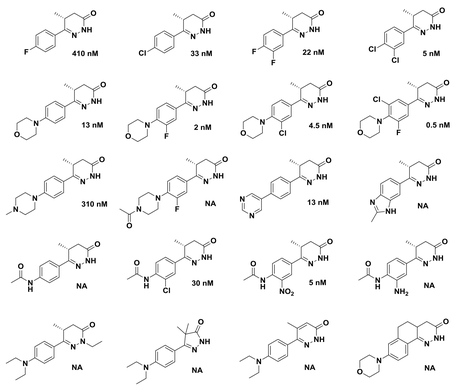

Figure 9. Representative EC_{50}s of DNMDP analogs (HeLa)
NA = not active

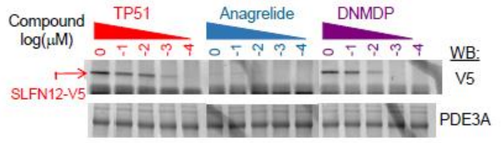

Figure 10. Western blotting demonstrates PDE3A/SLFN12 binding increases with more active compounds

DNMDP Analogs are active *in vivo*

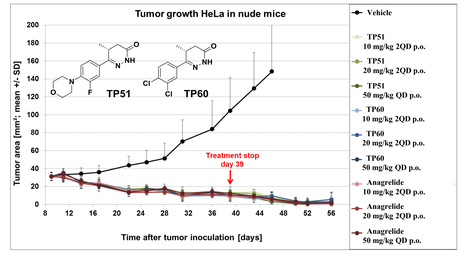

Figure 11. High dose treatment in HeLa xenograft shows drastic tumor shrinkage

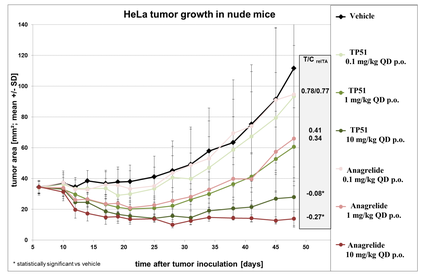

Figure 12. Low dose treatment in HeLa xenograft shows dose dependent anti-tumor effect

Conclusion

Differential viability assays discovered a small molecule, DNMDP, which potently killed mutant p53 cancer cells but not wild type p53 cancer cells. DNMDP is a selective PDE3 inhibitor and genetic profiling of sensitive and non-sensitive cells (data not shown) indicated PDE3A as the sole molecular target. However, most known PDE3 inhibitors were not effective and actually rescued cells from DNMDP-induced death.

Proteomic experiments showed that a protein of unknown function, SLFN12, binds to PDE3A in the presence of DNMDP inducing cell death. Chemical optimization produced low molecular weight, highly soluble and very potent compounds which were active in xenograft models at low doses.