Targeting B-cell Lymphoma: Evaluation of Cambinol Derivatives as Chemotherapeutic Agents

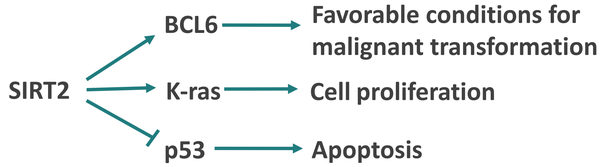
Katherine Mahoney, BA[1,2], Smitha Sripathy, PhD[1], Alyssa Webster, PhD[1], Sarwat Chowdhury, PhD[1], Julian Simon, PhD[1]

1. Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, 2. Williams College, Williamstown, MA

BACKGROUND

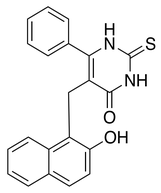
Sirtuins (SIRT1-7) are a family of NAD⁺-dependent deacetylases

- Functions: epigenetic regulation, stress responses, cellular aging, and apoptotic and metabolic control
- Potential targets of treatments for cancer, diabetes, and neurodegenerative diseases like Huntington's Disease & Alzheimer's Disease



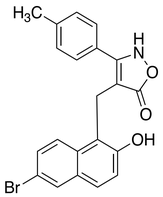
The Simon Lab developed small-molecule inhibitors of SIRTs optimized from cambinol, a nonselective pre-clinical lead compound

- Limitations of cambinol: moderate potency, poor solubility, no tumor regression, nonselective
- Developed SM-24: more potent & SIRT2-selective



Cambinol

SIRT1 IC_{50} = 56 µM
SIRT2 IC_{50} = 51 µM



SM-24

SIRT1 IC_{50} = >200 µM
SIRT2 IC_{50} = 13 µM

STUDY AIMS

To test previously synthesized compounds to identify SIRT2 inhibitors which:

(1) are more selective and potent than SM-24

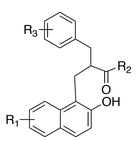
(2) selectively inhibit the growth of cancer cells

Overall goal: screen for novel B-cell lymphoma drugs

METHODS

PART 1: Biochemical Assays

Measured deacetylase activity of SIRT1, 2, 3, and 6 in the presence of inhibitors



Open-Ring Cambinol Derivatives

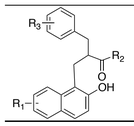
PART 2 & 3: Cell Viability Assays

Cell culture plated in 96-well plate → 24 hr → Compounds added → 72 hr → ATP levels measured (proxy for cell viability)

RESULTS

PART 1: SIRT Inhibition

Table 1. Concentration of cambinol derivatives giving 50% inhibition of sirtuin activity



	R_1	R_2	R_3	SIRT 1 IC_{50} (µM)	SIRT 2 IC_{50} (µM)	SIRT 3 IC_{50} (µM)	SIRT 6 IC_{50} (µM)
Cambinol	H	-	H	n.d.	n.d.	n.d.	n.d.
SM-24	6-Br	-	4-CF_3	n.d.	49.5	n.d.	n.d.
A	6-Br		4-CF_3	138.23	0.14	46.96	n.d.
B	6-Br		4-Br	68.5	0.28	9.73	n.d.
C	6-Br		4-Cl	n.d.	0.36	n.d.	n.d.
D	7-Br	NH_2	3-Br	133.83	1.18	n.d.	n.d.
E	6-Br	NH_2	4-Cl	n.d.	2.14	36.95	n.d.
F	6-Br	NH_2	3-Br	n.d.	5.26	27.95	n.d.
G	H	NH_2	H	3.59	12.79	7.9	3.87

n.d. indicates no inhibition detected at concentrations tested (<20 µM).

Takeaway: Compounds A to E are the most potent SIRT2 inhibitors and are >10-fold selective for SIRT2.

PART 2: Cytotoxic Activity

Figure 1. Selective growth inhibition of SIRT inhibitors

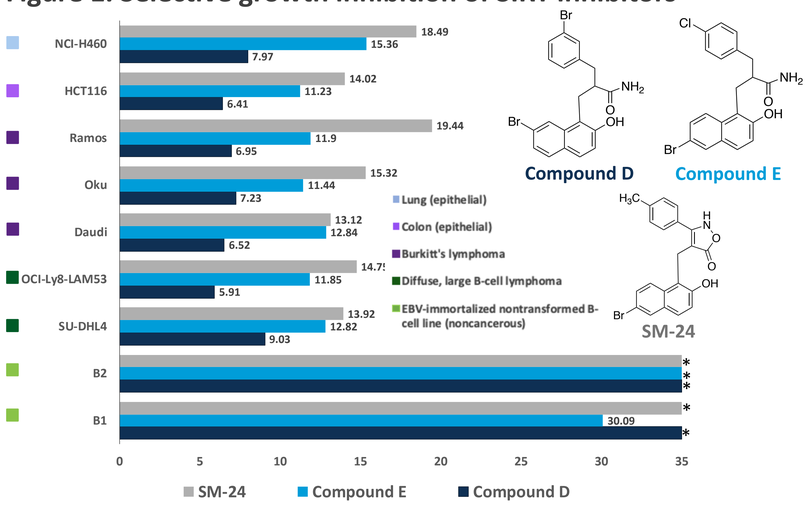
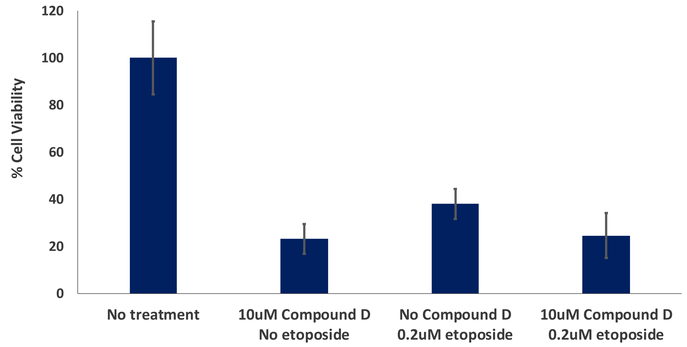


Figure 1. Concentration (µM) of top 3 anti-cancer compounds giving 50% growth inhibition (LD_{50}) following 72 h drug treatment.
*No inhibition at concentrations tested (<20 µM)

Takeaway: Of all inhibitors tested, compounds D & E are better anticancer agents than SM-24.

PART 3: Mechanism of Cell Death

Figure 2. B-Cell lymphoma cytotoxicity of etoposide and compound D treatments



*The B-cell lymphoma cell line tested was OCI-Ly8-LAM53.

Takeaways

- No synergistic effect was observed between compound D and the cytotoxic drug etoposide.
- This preliminary data suggests the anticancer activity of compound D is independent of the p53-mediated apoptotic pathway.

CONCLUSIONS

(1) We identified five SIRT inhibitors which have 10-fold selectivity for SIRT2 and are >20 times more potent SIRT2 inhibitors than SM-24.

(2) Compound D was the most effective anticancer drug even though it was not the most effective SIRT inhibitor.

(3) Compound D does not cause cell death by triggering the p53-mediated apoptotic pathway.

Future Directions

(1) Test compound D derivatives

(2) *in vivo* testing of compounds D and E

ACKNOWLEDGEMENTS

Thank you to the Simon group members for their support and efforts. This work is funded by RO1-CA206462 (National Cancer Institute). The Summer Undergraduate Research Program is supported in parts by the Cancer Center Support Grant (CCSG) CURE Supplement: NCI 3P30CA015704, the Fred Hutch Internship Program, and individual labs/research groups.