Finding Lead Protein Disulfide Isomerase Inhibitors For Glioma Treatments

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Introduction

- Protein Disulfide Isomerase (PDI) is responsible for maintaining cellular homeostasis by mediating oxidative protein folding
- Glioma is the most common type of central nervous system tumor with few current treatment options
- PDI is over expressed in brain cancer cells.
- Knockdown of PDI inhibits cancer proliferation and sensitizes glioma cells to chemotherapy

Objective

The goal of this study is to perform thermal shift and insulin turbidity assay's to uncover lead PDI inhibitors that bind to a novel site for the treatment of Glioma.

Methods

Thermal Shift Assay:

- Each drug candidate's Thermal Stability was tested using a PCR machine
- A melt curve and melting temperature (T_m) was uncovered and results were analyzed using Protein Thermal Shift Software
- PACMA31 and Estradiol were used as positive controls to hypothesize each compound's exact binding location

Insulin Turbidity Assay:

- The average inhibition was taken at 50 minutes after insulin reduction begun
- The compounds with at least 40% inhibition were considered active and were re-screened using 3 fold dilutions

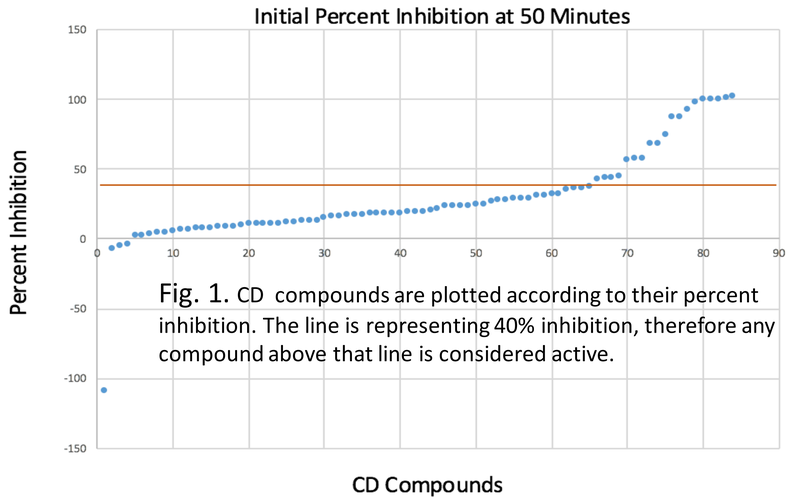

Fig. 1. CD compounds are plotted according to their percent inhibition. The line is representing 40% inhibition, therefore any compound above that line is considered active.

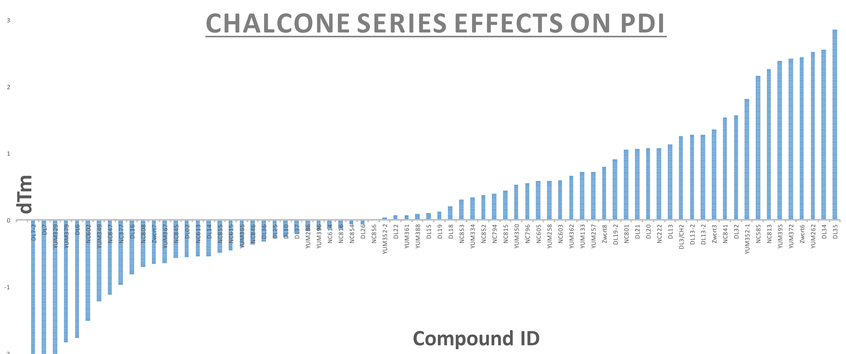

Fig. 2. The dT_m (T_m of PDI with compound - T_m of PDI without compound) of the reliable compounds in the Chalcone series

Table 1: Shifts of Active Compounds

Compound ID	dT_m	IC_{50}
DL35	2.85	1
DL34	2.55	1
YUM262	2.52	1
Zwcrt6	2.44	1
YUM372	2.41	0.1
YUM352-1	1.81	2
DL32	1.57	1
DL3/CH2	1.25	1
DL20	1.08	1
DL21	1.06	1
NC847	-1.12	10
YUM349	-1.22	1
YUM379	-1.83	8.8
YUM329	-2.23	1

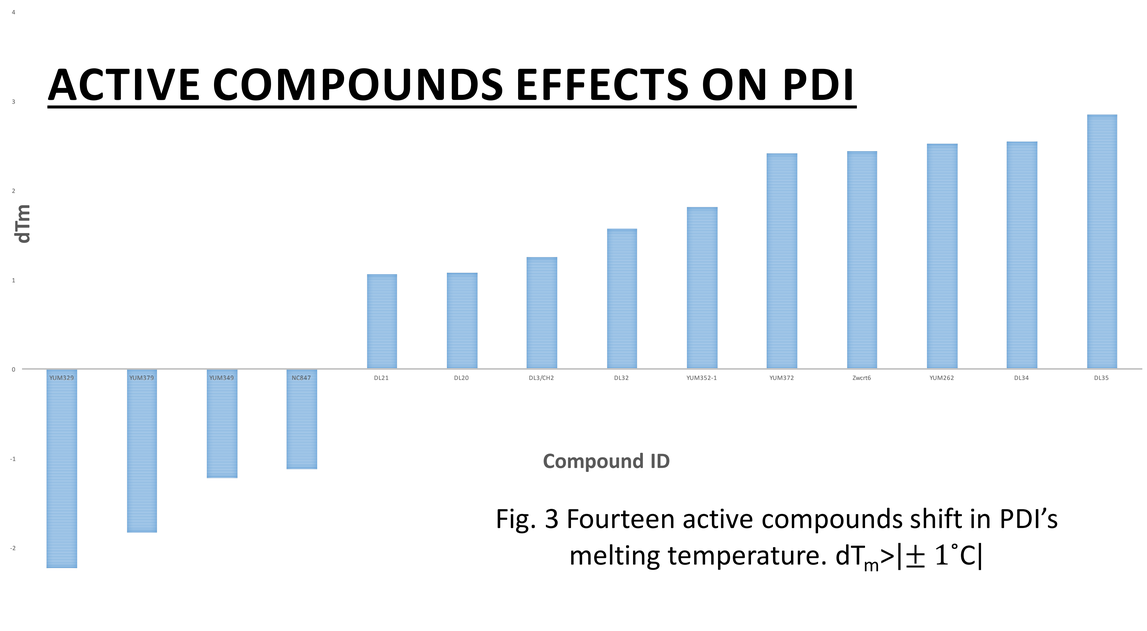

Fig. 3 Fourteen active compounds shift in PDI's melting temperature. $dT_m > |\pm 1^{\circ}C|$

Results

Thermal Shift Assay:

- 45 compounds did not significantly shift PDI's melting temperature ($dT_m < |\pm 1^{\circ}C|$)
- 29 compounds shifted the melting temperature of PDI with statistical significance ($dT_m > |\pm 1^{\circ}C|$)

Insulin Turbidity Assay:

- 19 active compounds were discovered

Conclusions

14 compounds were found to be promising targets for Brain Cancer treatment: active inhibitors with an affinity for PDI

Next Steps

- Follow up experiments on potency and reversibility
- Test toxicity and membrane permeability
- Mouse trials
- Pre-clinical Trials

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References

- Xu, S. et al. (2014) Protein disulfide isomerase: a promising target for cancer therapy. *Drug Discovery Today*. 19, 222-240
- Xu, S. et al. (2012) Discovery of an orally active small-molecule irreversible inhibitor of protein disulfide isomerase for ovarian cancer treatment. *Proceeding of the National Academy of Sciences of the United States of America.* 109, 16348-16353
- Hatahet, F. and Ruddock, L.W. (2009) Protein disulfide isomerase: a critical evaluation of its function in disulfide bond formation. *Antioxid. Redox Signal.* 11, 2807-2850
- Weinberg, Robert A. "Chapter 2: The Nature of Cancer." *The Biology of Cancer*. New York: Garland Science, 2007. 25-56. Print.