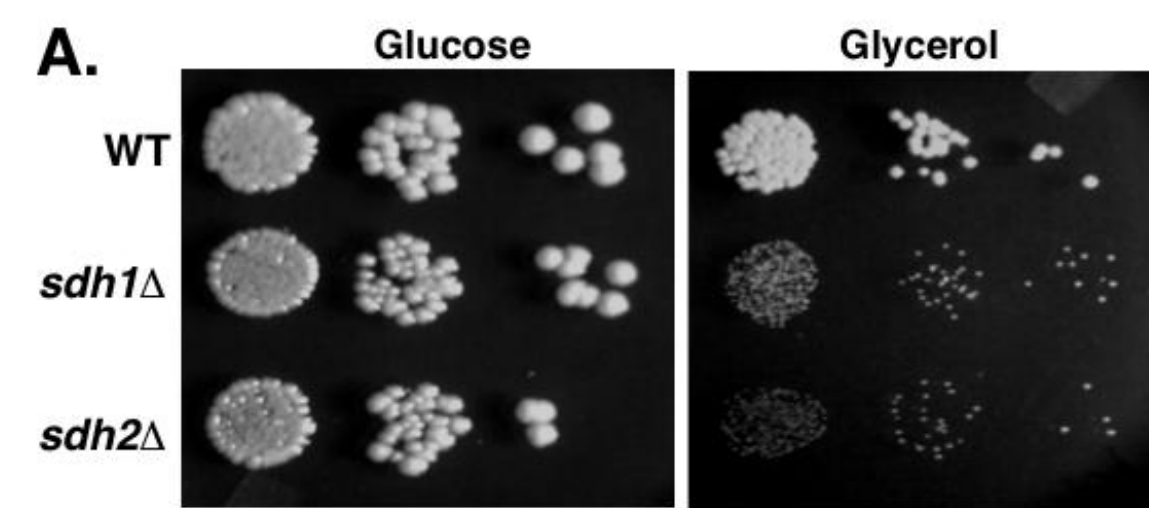


# Small molecule screens for selective growth inhibitors in a yeast model of familial paraganglioma

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## Background

Up to 30% of paragangliomas (PGL) are succinate dehydrogenase (SDH) mutation-related tumors. Mutations identified in familial PGL suggest that loss of function of SDH subunits (most commonly B, C, or D) is the common cause of tumorigenesis. Unfortunately, no human PGL cell lines exist at this time, thus a biological model of paraganglioma is needed. The highly conserved nature of the SDH complex allows a yeast model of PGL developed by deleting the *sdh2* gene (homolog of human SDHB) to reflect similar biochemical and growth phenotypes as human PGL cells. This yeast model demonstrated increased levels of ROS, succinate accumulation, and a broken TCA cycle. As expected for loss of a Krebs cycle enzyme, this yeast strain is completely dependent on glycolysis and is unable to survive on non-fermentable carbon sources (Figure 1).



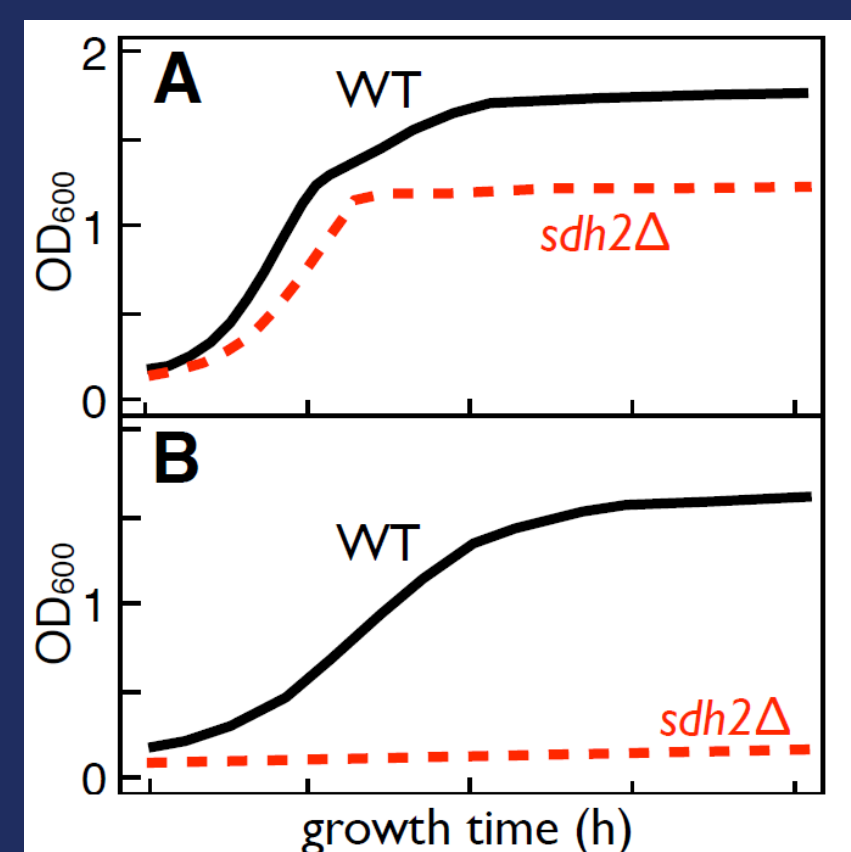
## Objectives

To utilize a cell-based assay in a high-throughput screen (HTS) of 200,000 small molecules to identify compounds that are differentially toxic to a yeast model of familial paraganglioma (PGL).

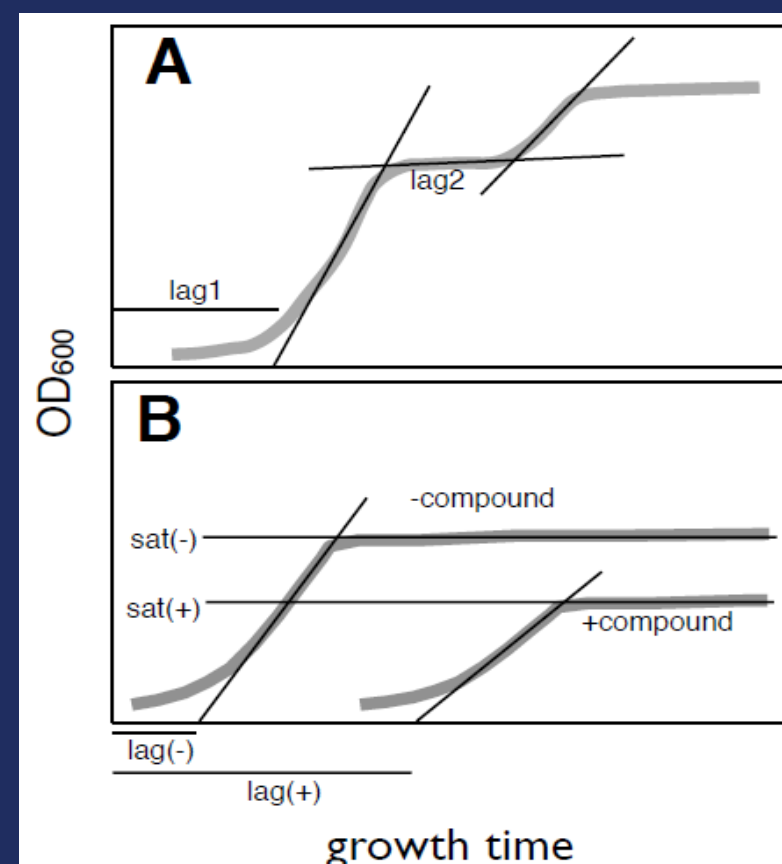
## Methods

- With this yeast model a high-throughput growth assay was developed to identify small molecules that selectively inhibited the growth of the *sdh2Δ* strain when compared to the "wild-type" WT strain.
- The growth assay was utilized by the University of Minnesota Institute for Therapeutics Discovery and Development (ITDD) to screen compounds from the Library of Pharmacologically Active Compounds (LOPAC 1280), and an additional 200,000 randomly synthesized compounds from the ITDD library.
- The differential effects of the compounds were quantified by fitting the resulting growth curves to a mathematical model and identifying changes in maximum growth rates, saturation, and lag time (Figure 2).

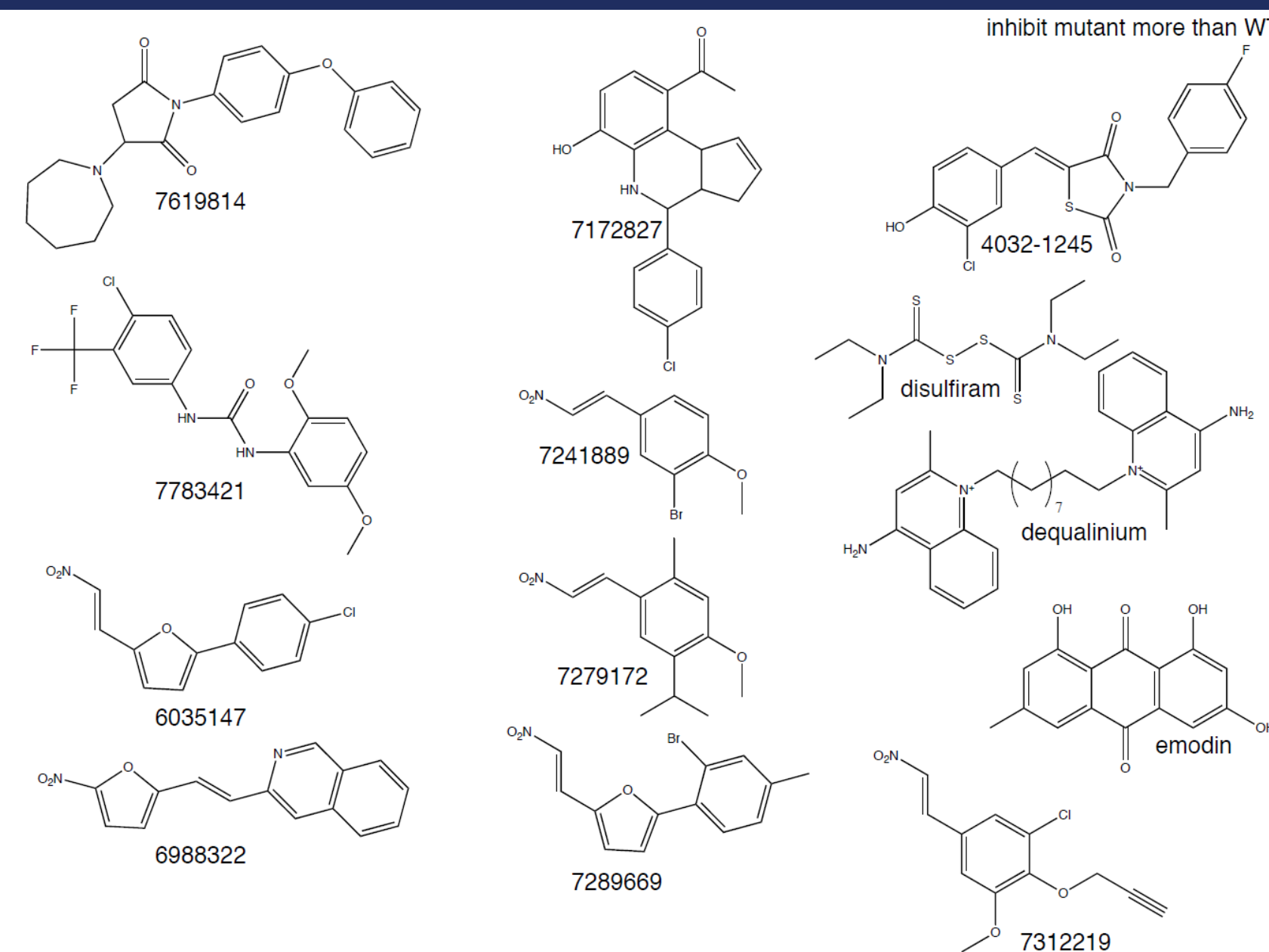
## Figure 1: Growth curves



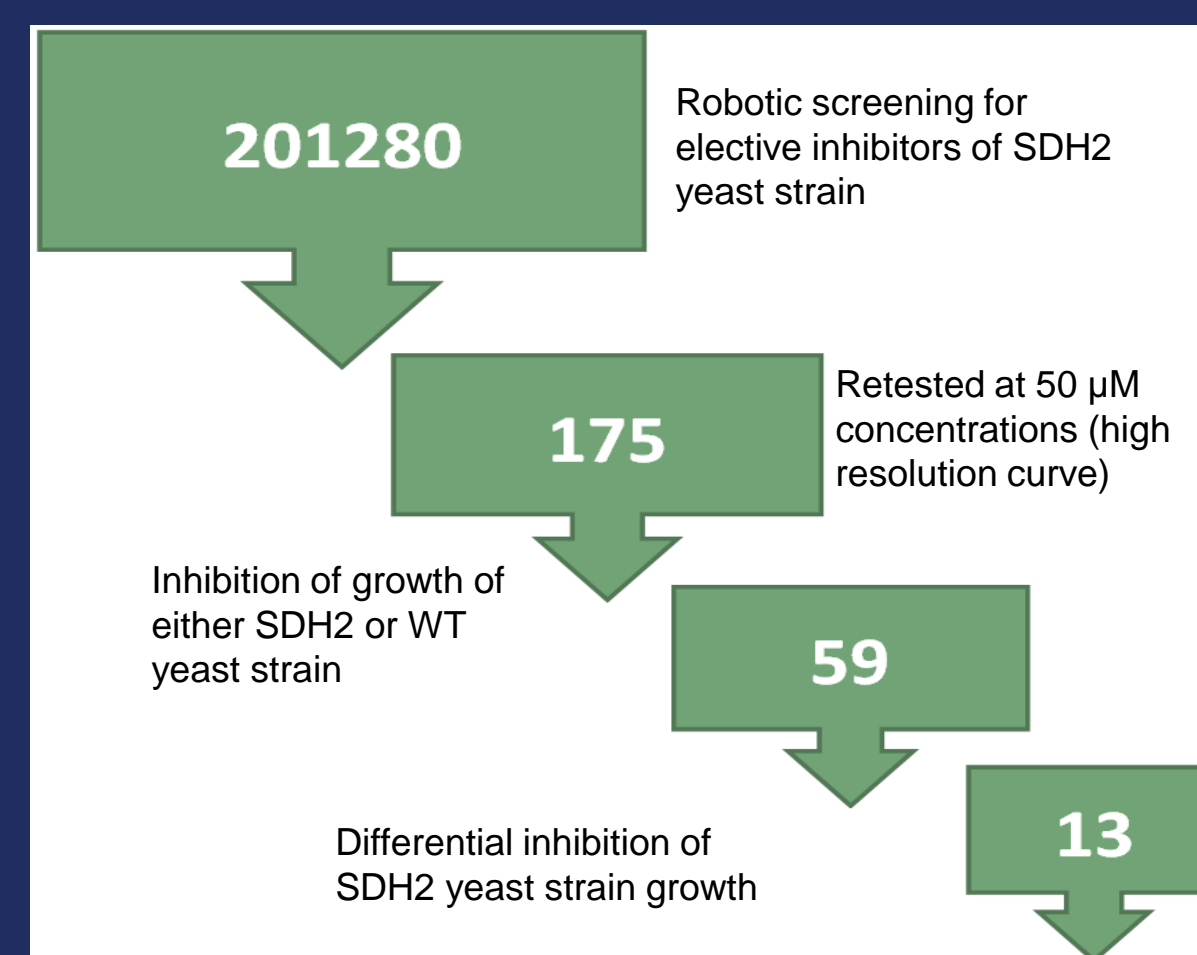
## Figure 2: parameter calculations



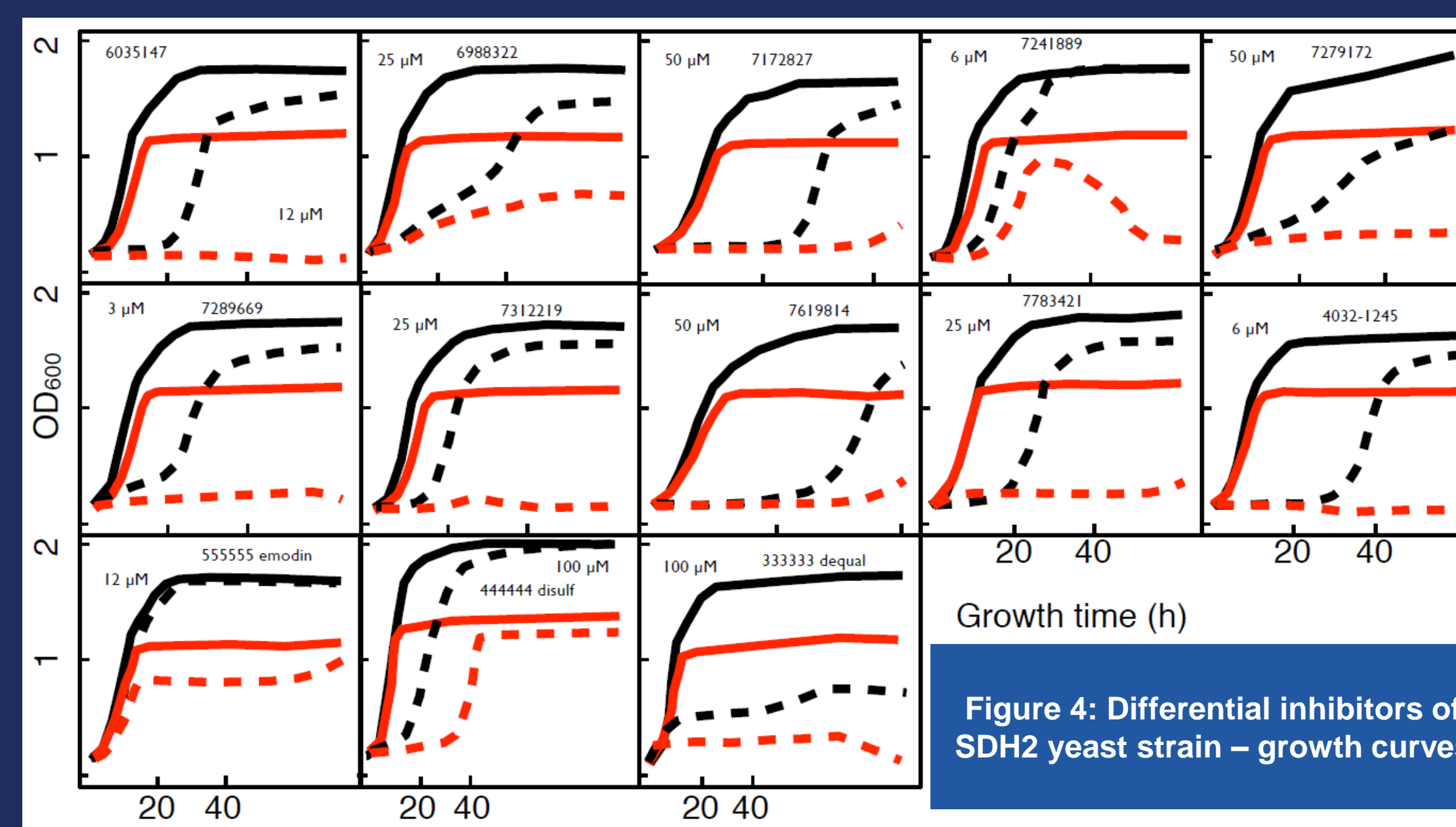
## Figure 3: Biochemical formulas



## Results and discussion:



- High-throughput screening of small molecules is among the most historically-successful approaches to drug discovery.
- Through collaboration with the University of Minnesota, 200000 random compounds and 1280 compounds from LOPAC were robotically screened for elective inhibitors of SDH2 yeast strain.
- First screen identified growth inhibitors to SDH KO strain and yielded in 175 compounds chosen for farther testing
- These compounds were retested in a second, smaller high resolution screen where inhibition of *WT* and *sdh2Δ* cells were compared to identify those compounds selectively toxic for *sdh2Δ* cells. Parameters such as growth rate, maximum saturation and lag time for all phases of growth were used to identify selective inhibitors.
- Initial high resolution screen was performed on all 175 compounds at a concentration of 50 μM. As a result, 59 compounds were identified to show inhibition in growth of either SDH2 or WT yeast strains. These compounds were retested at various concentrations and yielded in 13 compounds which showed differential inhibition of SDH2 yeast strain growth. The growth curves of 13 selective SDH2 inhibitors and biochemical formulas of each compound are illustrated in the Figure 3 and Figure 4.
- Interestingly, several compounds were found to inhibit WT yeast strains more than SDH2 yeast strain.



## Conclusions

With no effective cure for the ~20-40% of metastatic PGLs small molecules that selectively inhibit the growth of PGL-like cells will have immediate therapeutic value. We have identified several candidate molecules that show promise in a yeast model of PGL and are actively working toward testing these compounds in human PGL cell lines currently under development in the lab.

## Future directions

- Elucidating mechanism of compounds selectively toxic to *sdh2Δ* yeast cells.
- Testing of compounds on human PGL cell lines when available.
- Elucidating mechanism in unexpected cases of compounds selectively toxic to WT yeast cells.