Initial Compound Screen

All compounds were attempted to be reconstituted in 100% DMSO – compounds 779,784, 790,799, and 806 were unable to be reconstituted.

A stop point assay that tracks the initial rate of MurD was used to test the effect of the compounds. Compounds were incubated with MurD at a final concentration 1mM at 370c for 10 minutes. The assay was then run and the activity of MurD in the presence of compound was determined. Percentage activity of MurD in the presence of compounds was then plotted. Certain compounds were not plotted due to either too high activity rates or negative activity rates, which could have been caused by interference with the assay by the compound, or precipitation of the compound within the assay at 370c, or the compound increasing the activity of MurD.

Chart

Description automatically generated

Chart, histogram

Description automatically generated

Coupling Reaction

Compounds that reduced MurD activity by 80% or more were then analysed for their effect on the assay components and the coupling reaction associated with the activity assay.

Chart

Description automatically generated

Chart, bar chart

Description automatically generated

Chart, waterfall chart, box and whisker chart

Description automatically generated

Dual Inhibitors against MurE

Compounds that did not have a significant effect on the coupling reaction were tested against MurE from *S. agalactiae* under the same assay conditions as MurD.

Chart, bar chart

Description automatically generated

Chart, bar chart

Description automatically generated