Mycobacterium tuberculosis MurE Compound Screen – compounds supplied by Guilherme Fernandes

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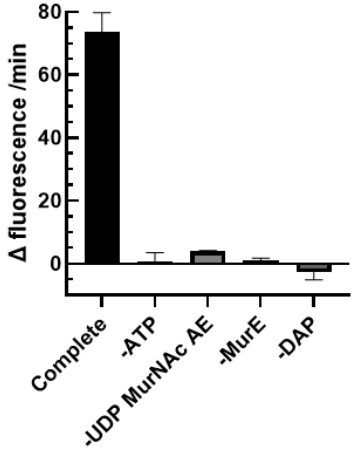
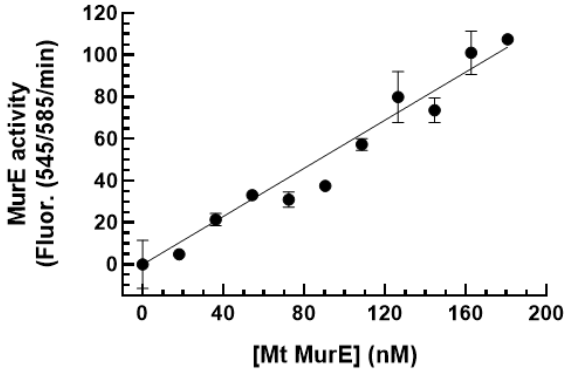
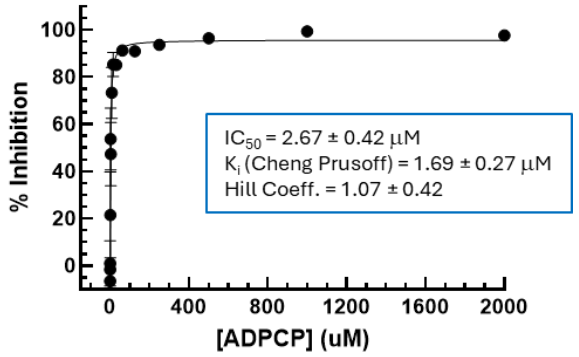
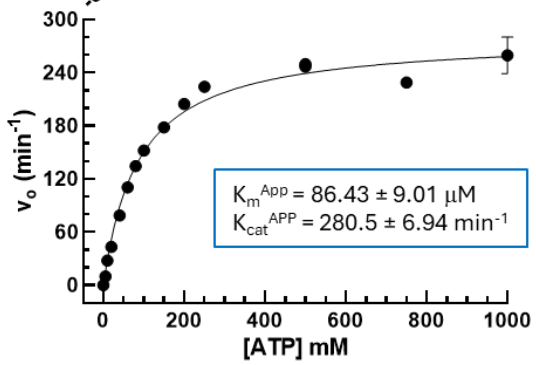
11th June, 2024

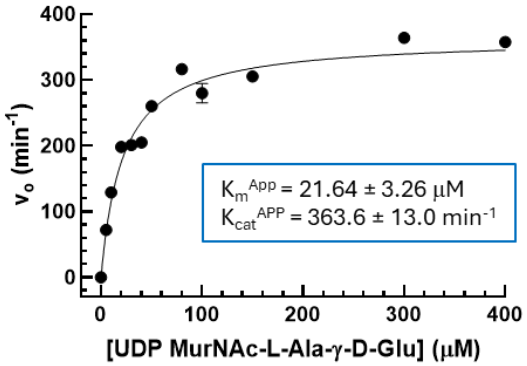
Characterization of the activity of *Mycobacterium tuberculosis* MurE

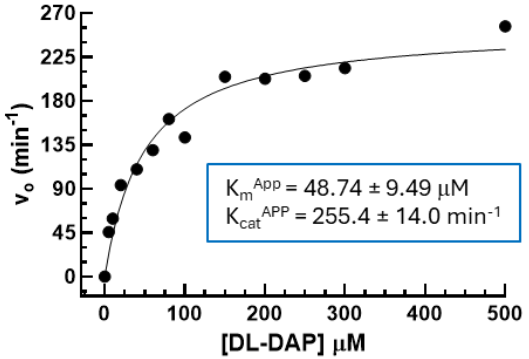




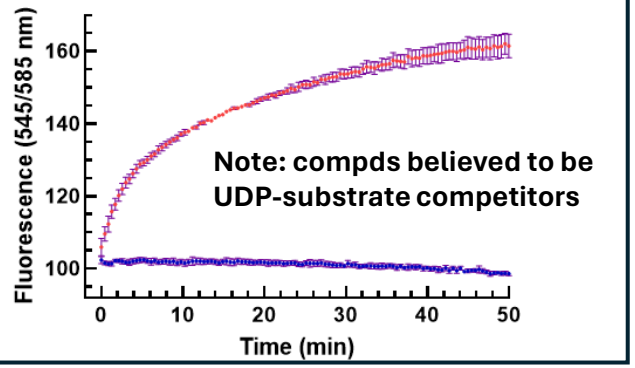






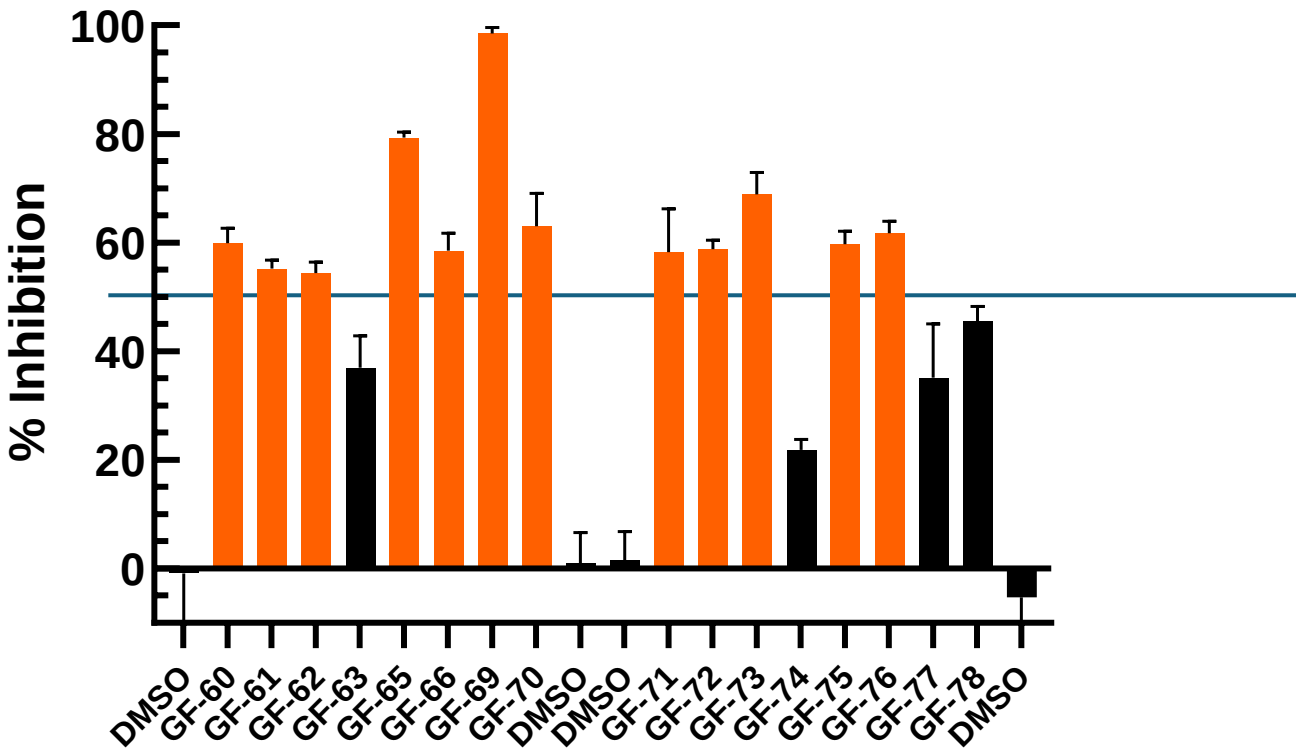


MurE Assay (10 µl fluorometric): Assays were performed at 30°C in a 10 µl volume/384 well format, containing 50 mM MOPS, 10 mM $MgCl_2$, 0.5 mM inosine, 2.5 mM.min^{-1} *Arthrobacter* sp. xanthine oxidase, 20 mM.min^{-1} horse radish peroxidase, 50 µM amplex Red, 2.64 mM.min^{-1} *E. coli* purine nucleoside phosphorylase, 50.2 µM ATP, 12.6 µM UDP-MurNAc-L-Ala-γ-D-Glu, 22.6 nM *M. tuberculosis* MurE and where added, 8 mM diaminopimelic acid (racemic mixture). Compounds were added from 50 mM stocks in DMSO, where the final concentrations of DMSO and compound were 1 % (v/v) and 0.5 mM (or as specified otherwise) respectively. Controls without compound contained 1% (v/v) DMSO. ADPCP if added was at 0.4 mM if not otherwise specified. Per compound/DMSO/ADPCP, MurE was assayed in three wells where reaction was initiated by addition of diaminopimelic acid and in three wells where reaction (control) was initiated by water. The fluorescent product of the reaction cascade (resorufin – derived from amplex red) was continuously monitored from above the well at an excitation and emission wavelength of 545 nm and 585 nm respectively in a Varioskan Lux plate reader.

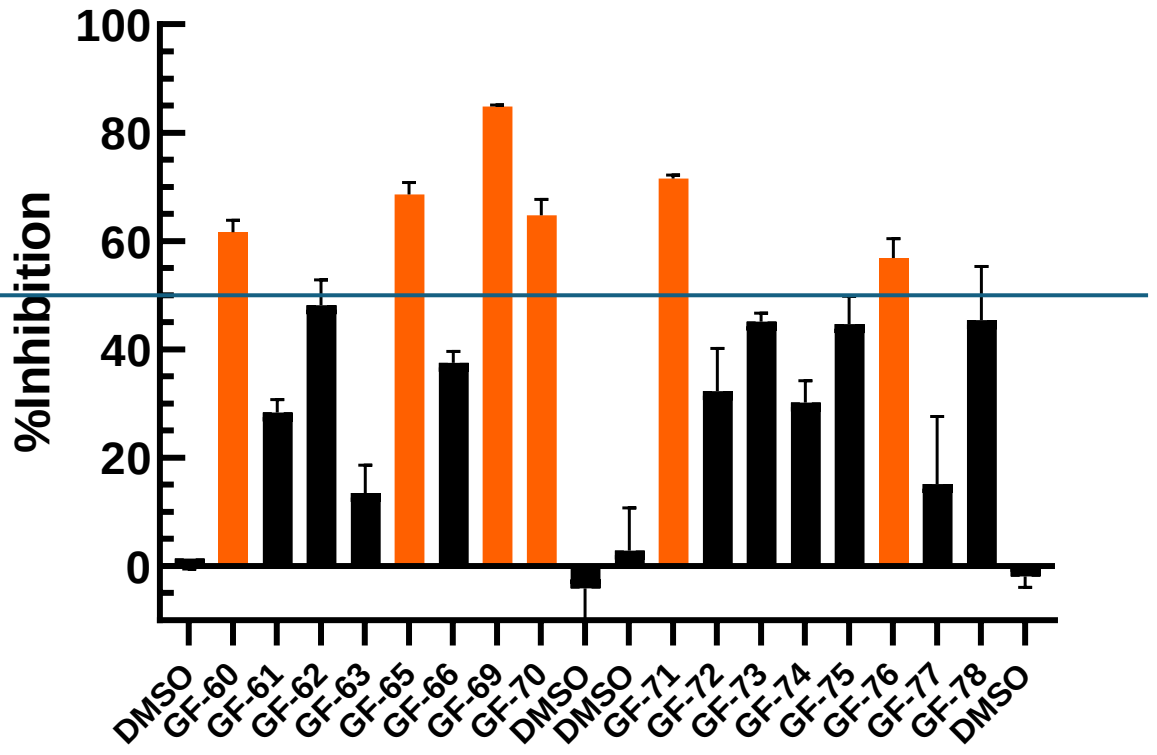


Characterization of the impact of compounds supplied on the Amplex Red-fluorometric coupling system for detection of phosphate

Inhibition of PNP-XO-HRP Couple by 0.5 mM compound

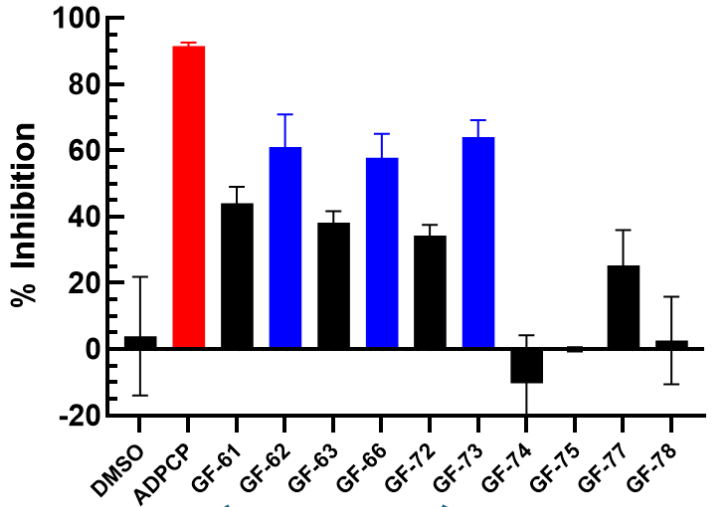


Inhibition of PNP-XO-HRP Couple by 0.1 mM compound



- Compounds causing >50% inhibition of coupling system prevent accurate determination of residual Mt. MurE activity
- Only four out of the 16 supplied would be assayable against the MurE target at [compound] = 0.5 mM
- However 10 compounds are assayable against MurE if [compound] is reduced to 0.1 mM.

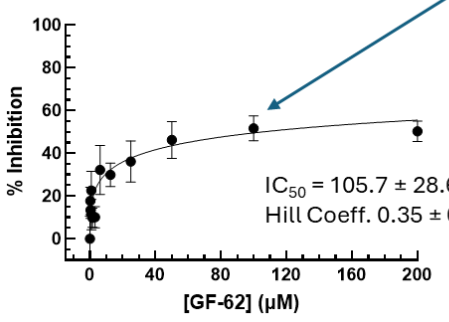
Characterization of the impact of compounds supplied at a concentration of 0.1 mM on *Mycobacterium tuberculosis* MurE

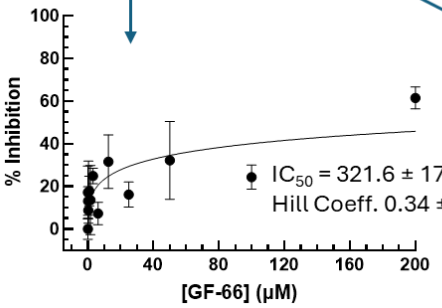


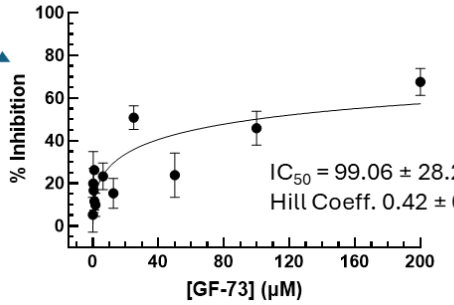
Detectable but weak inhibition at 0.1 mM.

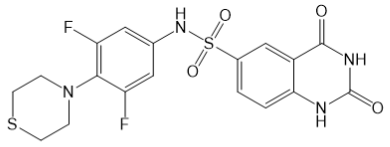
Compound inhibition shows low Hill Coefficients (due to compound aggregation ? Or the impact of compound on fluorescence ?)

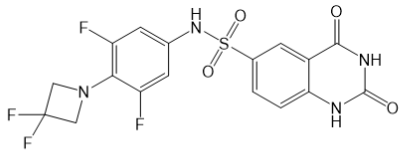
Loss of chemical equity through inhibition of assay

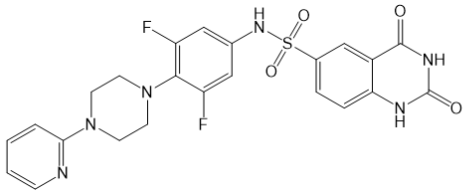




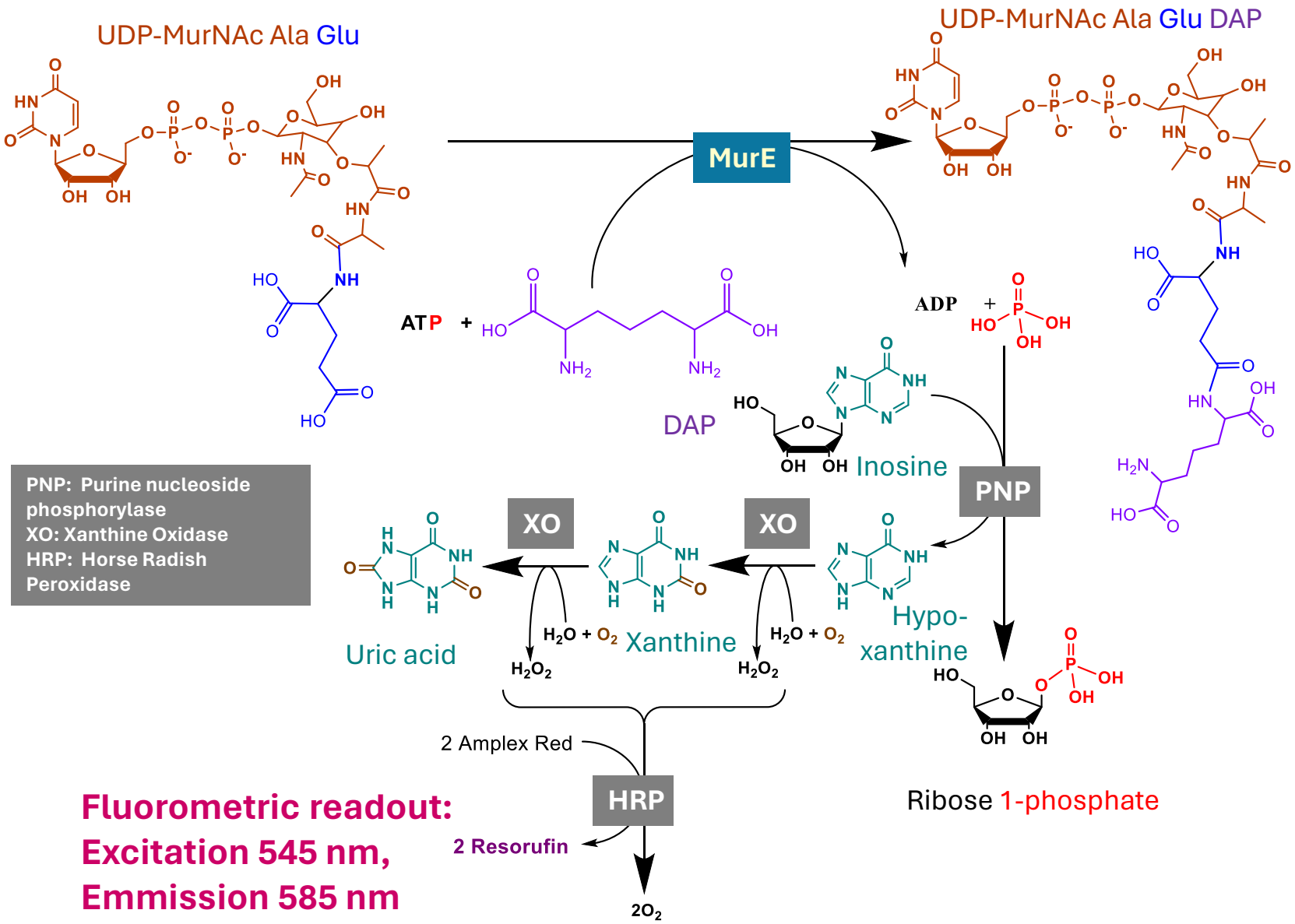




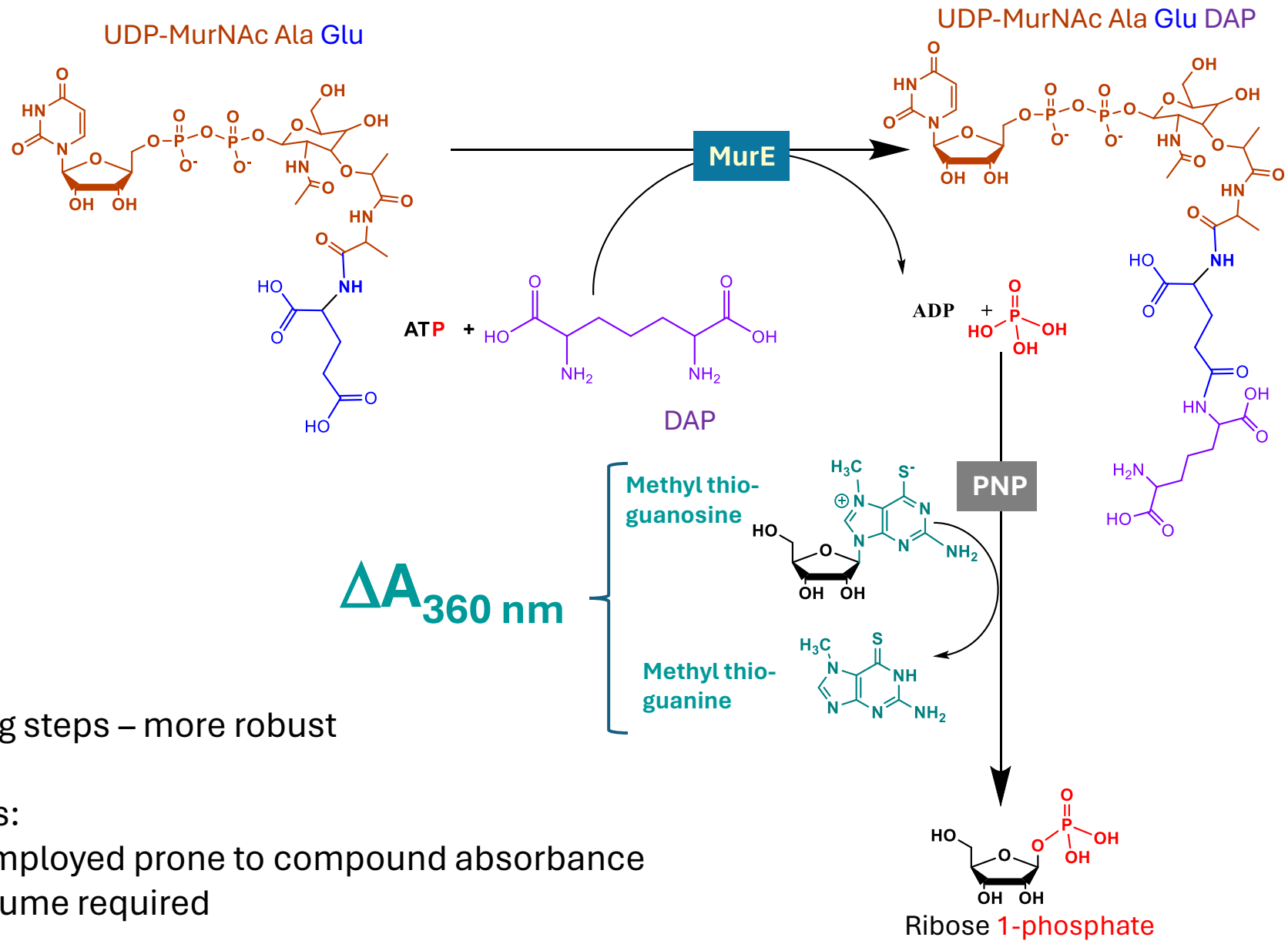




Principle of the fluorometric MurE assay exemplified by MurE



Principle of the Spectrophotometric MurE assay exemplified by MurE

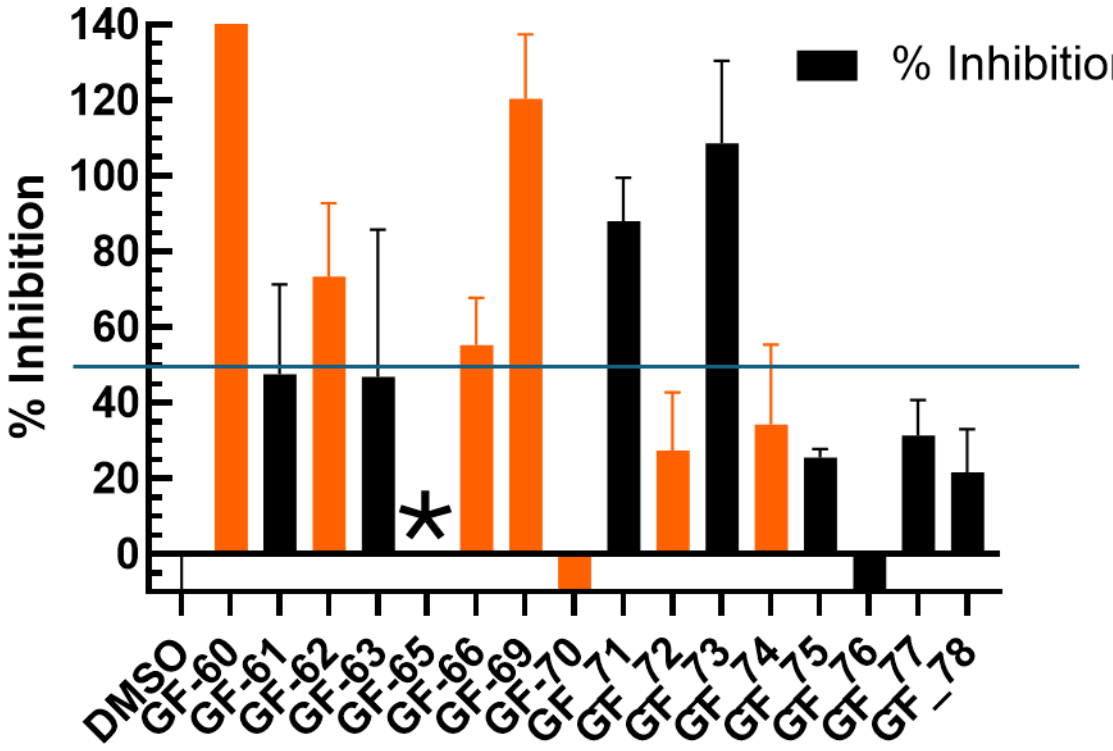


Advantages:
Fewer coupling steps – more robust

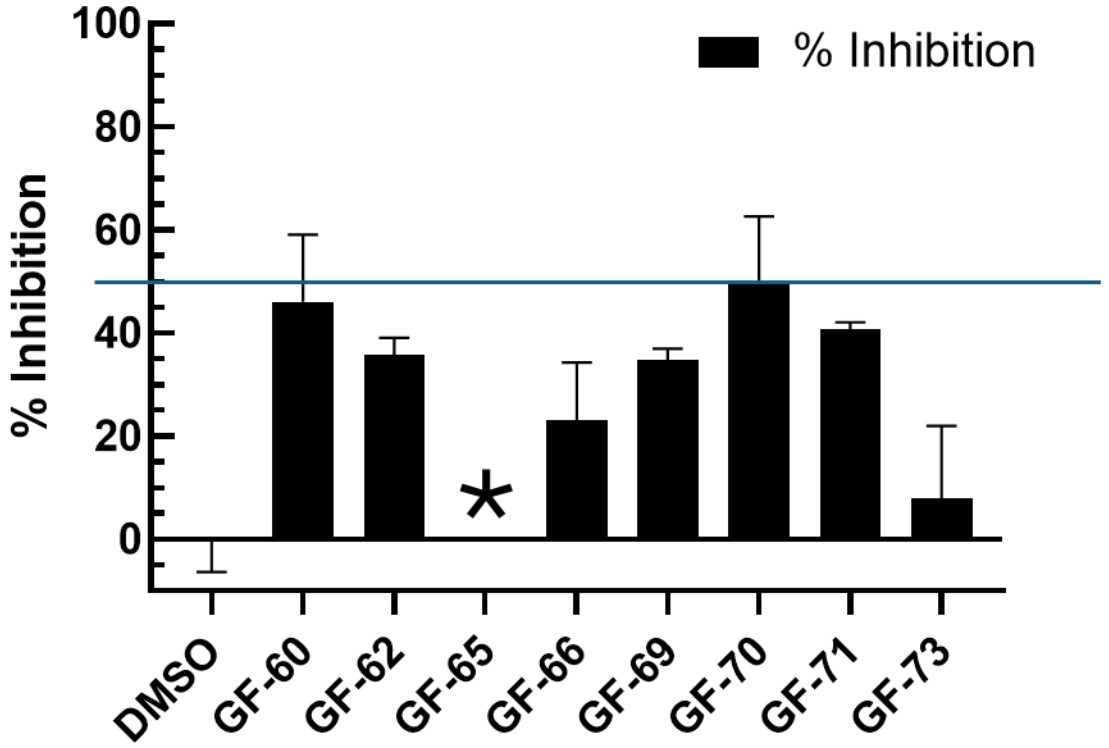
Disadvantages:
Wavelength employed prone to compound absorbance
Higher (5x) volume required

Characterization of the impact of compounds supplied on the MESG-spectrophotometric coupling system for detection of phosphate

MESG PNP Counter screen 0.5 mM GF-60-78



MESG PNP Counter screen 0.1 mM

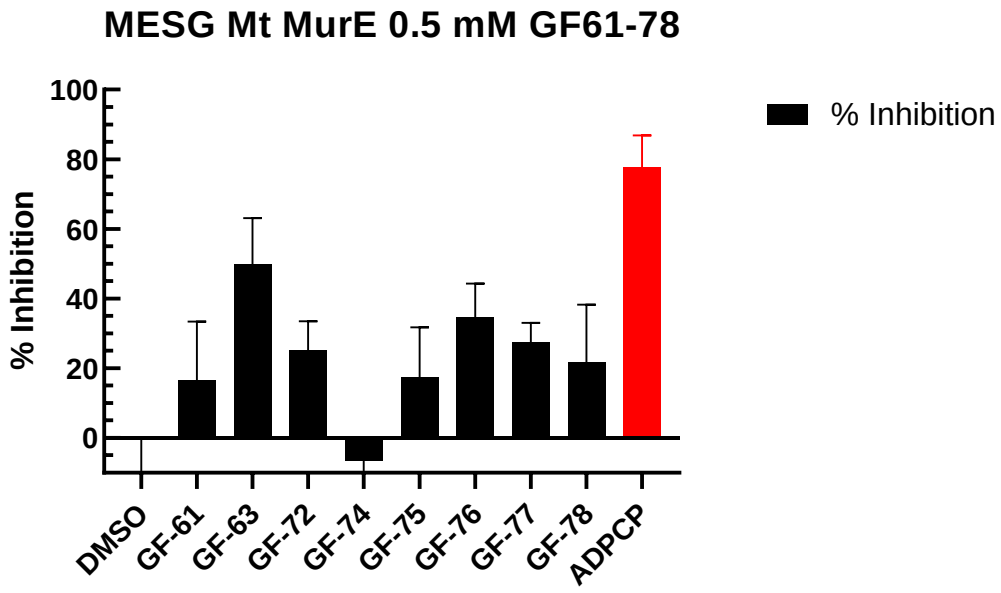


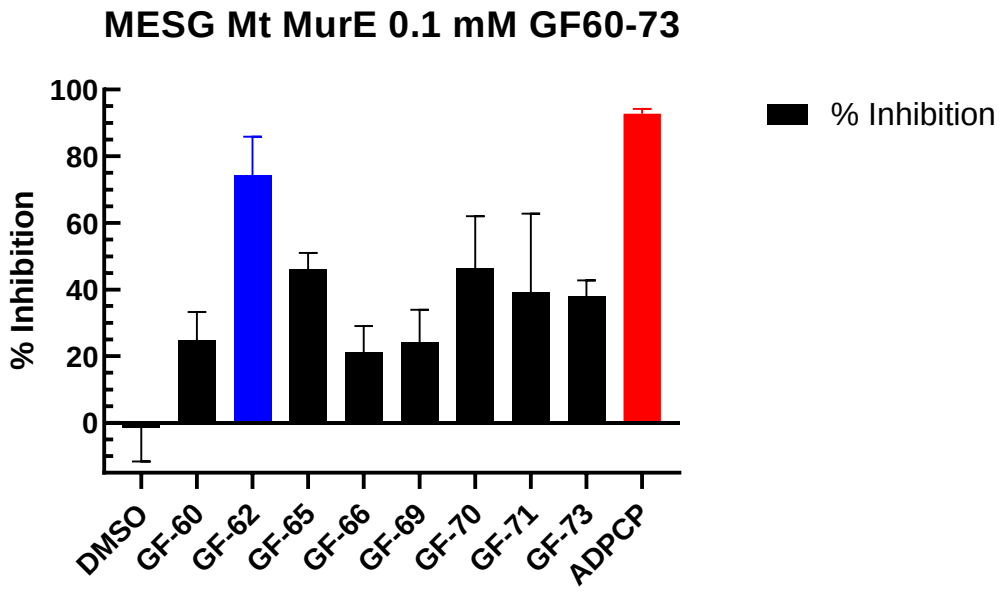
*: Assay too noisy

Assay usable with following compounds at 0.5 mM:
GF-61, GF-63, GF-70, GF-72, GF-74-GF78

Assay usable with following compounds at 0.5 mM:
GF-60, GF-62, GF-66, GF-69, GF-70, GF-71, GF-73

Characterization of the impact of compounds supplied in the MESG-MurE assay at 0.1 mM on *Mycobacterium tuberculosis* MurE





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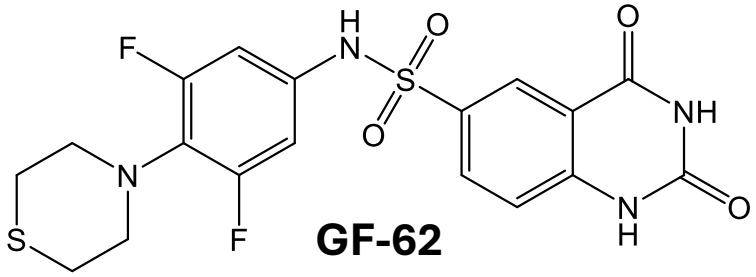

GF-62