# Supplementary Materials: Inhibition of Indigoidine Synthesis as a High-Throughput Colourimetric Screen for Antibiotics Targeting the Essential Mycobacterium tuberculosis Phosphopantetheinyl Transferase PptT

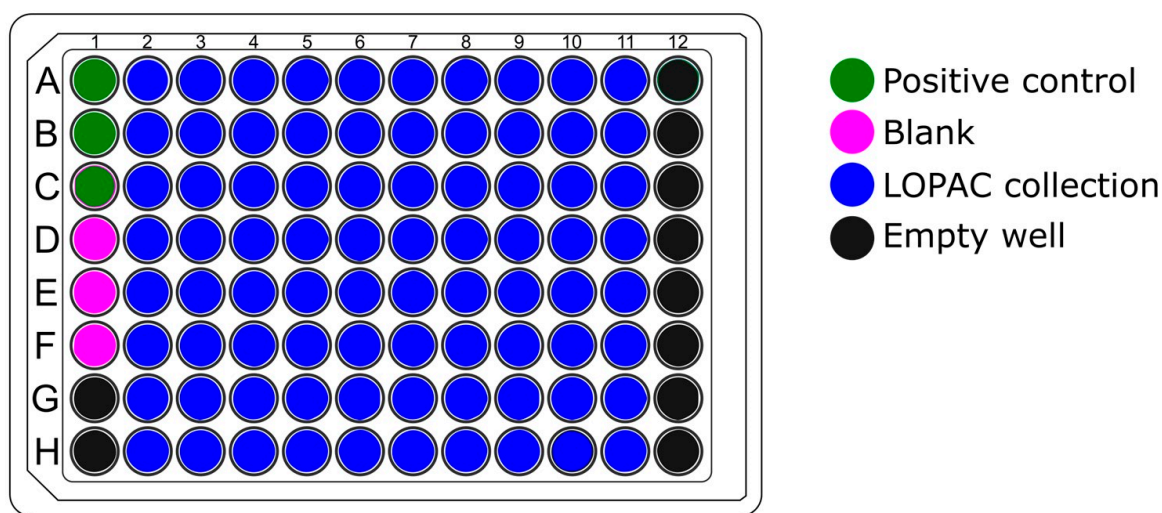**Alistair S. Brown, Jeremy G. Owen, James Jung, Edward N. Baker and David F. Ackerley**



**Figure S1.** LOPAC$^{1280}$ screening plate layout: the LOPAC$^{1280}$ collection was arrayed across sixteen 96-well plates. Each plate contained three positive (no-inhibitor) controls (green), three blank wells (pink) and 80 wells containing compounds from the library (blue). The remaining wells were unused.
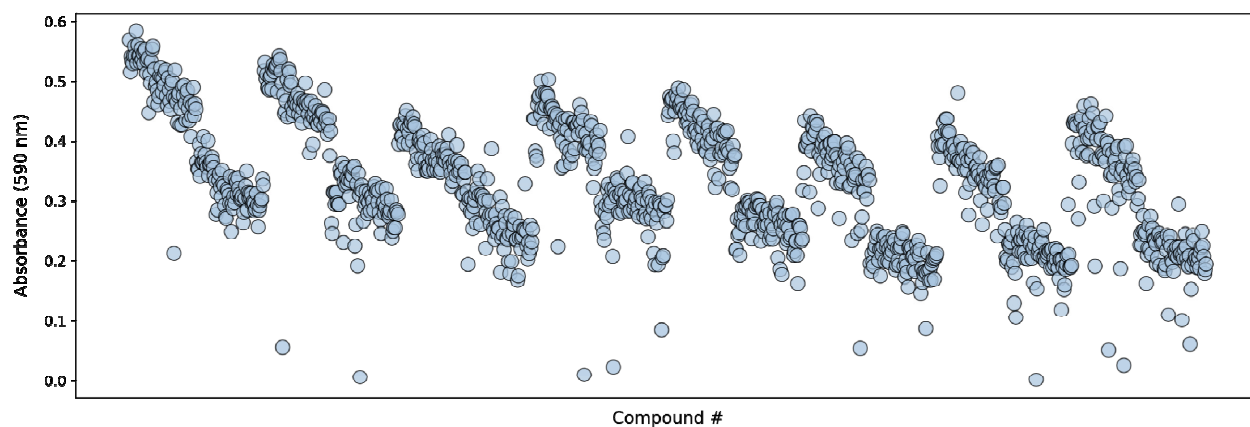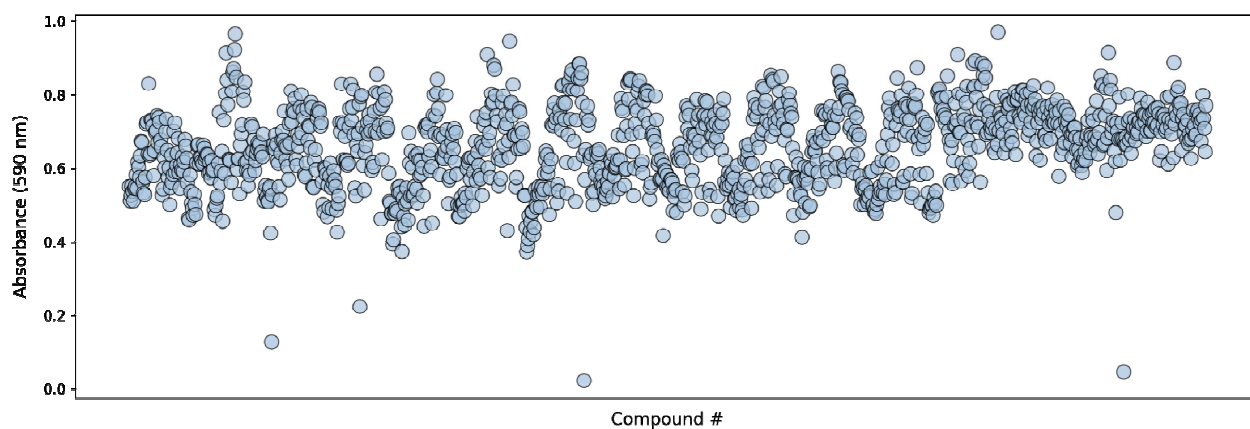
**Figure S2.** Raw values absorbance values of the LOPAC[1280] collection: (**A**) $A_{590}$ values derived post indigoidine synthesis by PptT-activated BpsA for 16 96-well plates, screened in pairs to identify candidate PptT inhibitors from the LOPAC[1280] collection. Clearly evident is the wave-like pattern arising due to the instability of PptT in aqueous media. (**B**) $A_{590}$ values derived post indigoidine synthesis for an equivalent set of 96-well plates to those presented in Panel A, only using pre-activated BpsA. These reactions did not contain PptT and correspondingly no wave-like pattern is discernible.
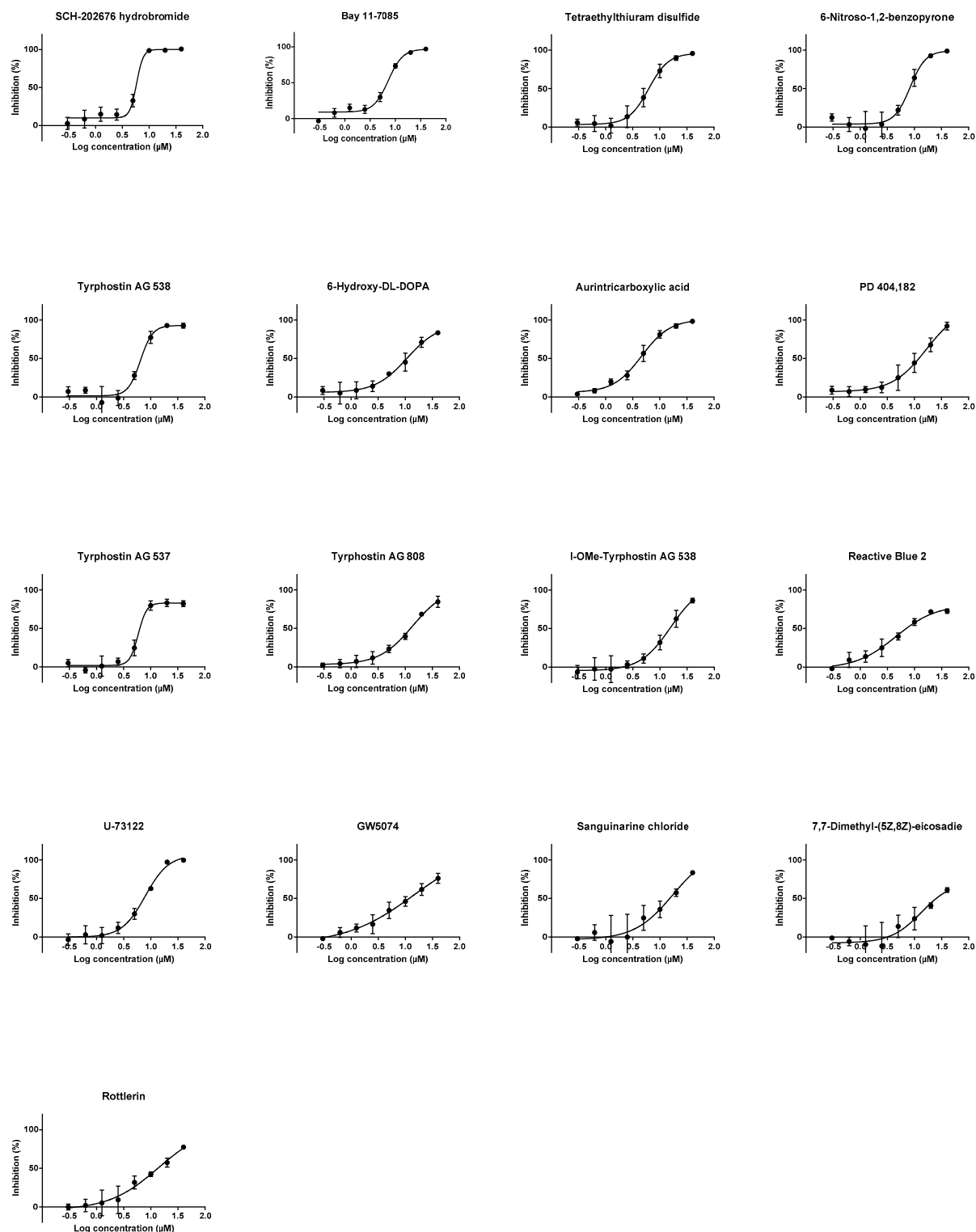
**Figure S3.** EC$_{50}$ values for top compounds: A two-fold serial dilution from 40 μM to 0.625 μM for each compound was established across individual rows of a 96 well plate. Graphpad Prism was then used to fit a four parameter dose-response curve to determine EC$_{50}$ values. In each case, data was derived from the mean of three independent replicates and error bars represent the standard deviation.
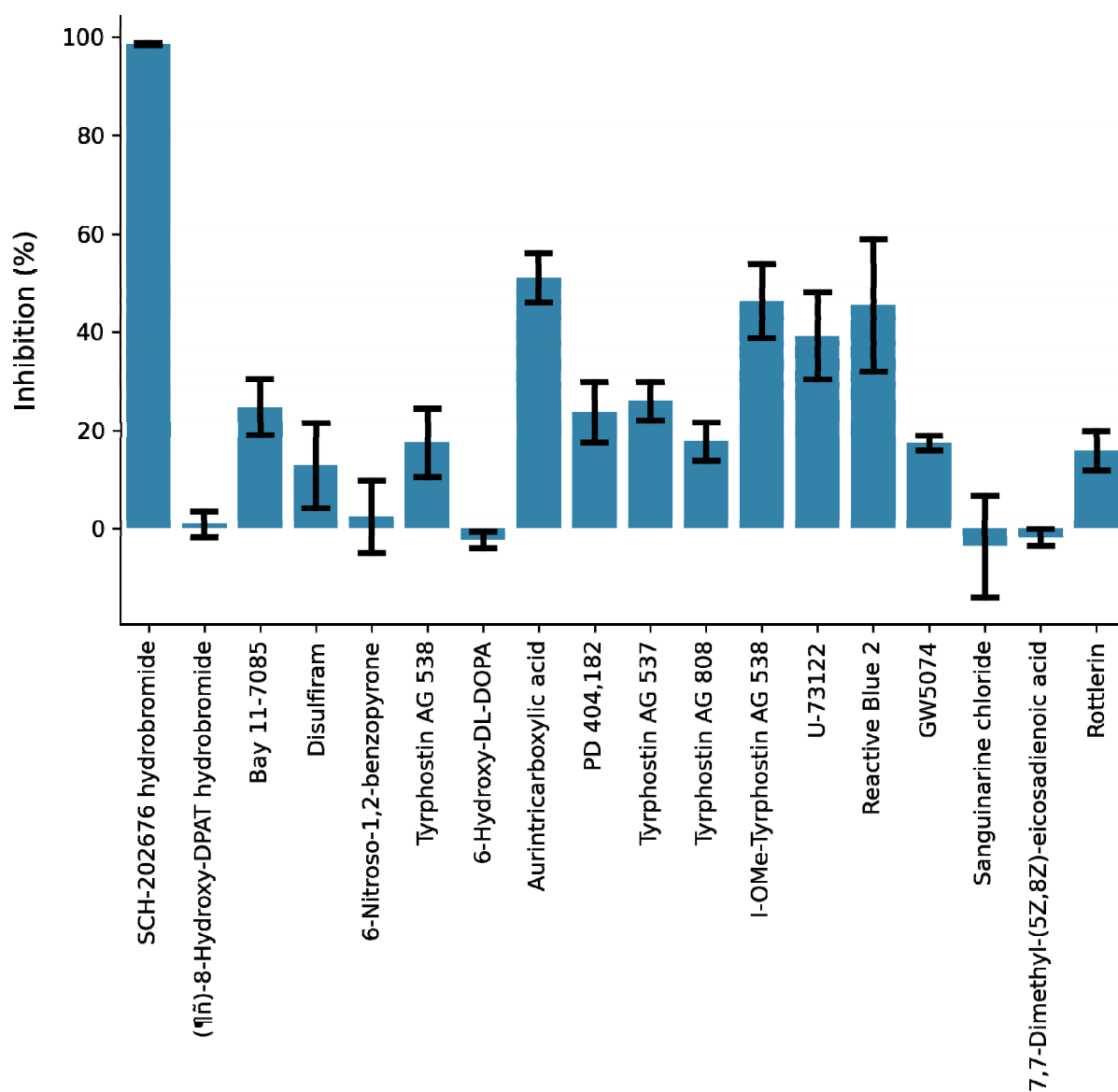
**Figure S4.** Inhibition of *holo*-BpsA at a candidate -inhibitor concentration of 40 μM: The inhibition of *holo*-BpsA by compounds identified as candidate PptT inhibitors at 20 μM was determined by re-screening at 40 μM. In each case, data was derived from the mean of three independent replicates and error bars represent the standard deviation.
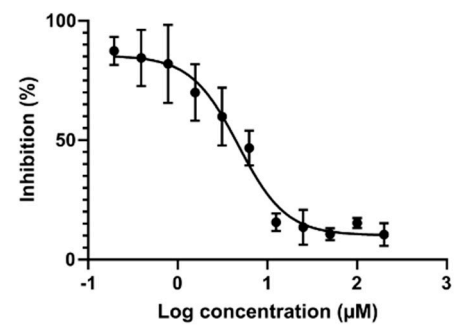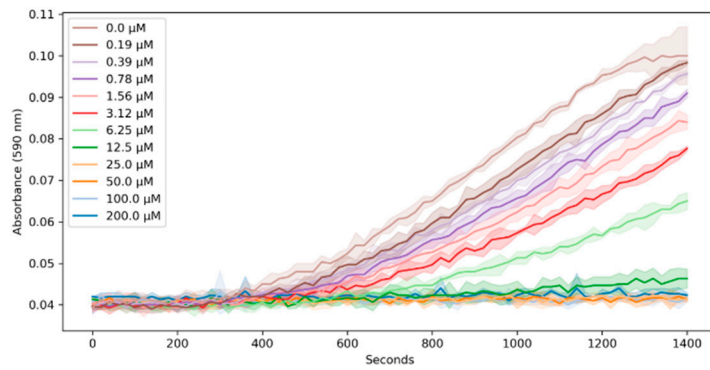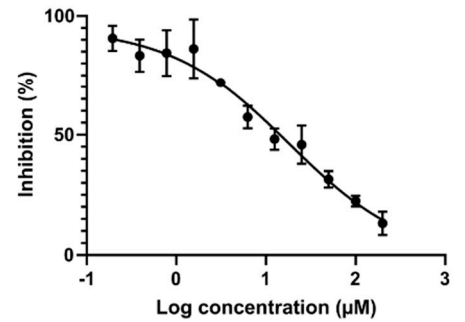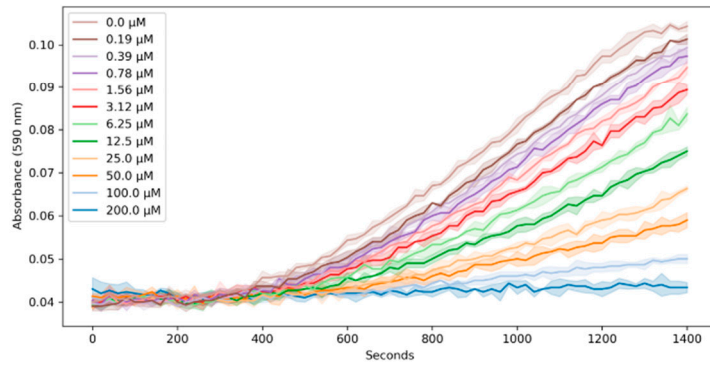
**Figure S5.** Kinetic determination of EC$_{50}$ values: (**A**) The rate of BpsA activation by PptT diminishes with increasing levels of 6-NOBP. Data were recorded every 20 s and are the average of three replicates. The lighter shaded boundaries around each set of A$_{590}$ data (left panel) represent one standard deviation. The right panel shows the graph used to derive EC$_{50}$ values from the mean A$_{590}$ data. Data was derived from the mean of three replicates, and the error bars in the right panel represent one standard deviation. (**B**) The rate of BpsA activation by PptT diminishes with increasing levels of Sanguinarine chloride. Data were recorded every 20 s and are the average of three replicates. The lighter shaded boundaries around each set of A$_{590}$ data (left panel) represent one standard deviation. The right panel shows the graph used to derive EC$_{50}$ values from the mean A$_{590}$ data. Data was derived from the mean of three replicates, and the error bars in the right panel represent one standard deviation.