Virtual screening and *in vitro* validation identifies new inhibitors of *Salmonella enterica* HPPK.

SUPPORTING INFO

Energy parameters used for ligand preparation.

Energy	
Force field	OPLS3e
Charges	Charge using OPLS3e partial charges
Ionisation	Neutral
Chirality	Retain specific chiralities
Conformation	
Conformations	250

Energy parameters used for the docking molecular mechanics calculations.

Glide Docking	
Force field	OPLS3e
Charges	Charge using OPLS3e partial charges
Quality	SP (Standard Precision)
Scaling factor	0.8
Partial charge cut-off	0.15
Ligand sampling	Flexible
Distance-dependant dielectric constant	2.0
Maximum number of minimisation steps	100
Prime MM-GBSA (Binding energy calculation)	
Force field	OPLS3e
Solvation model	VSGB

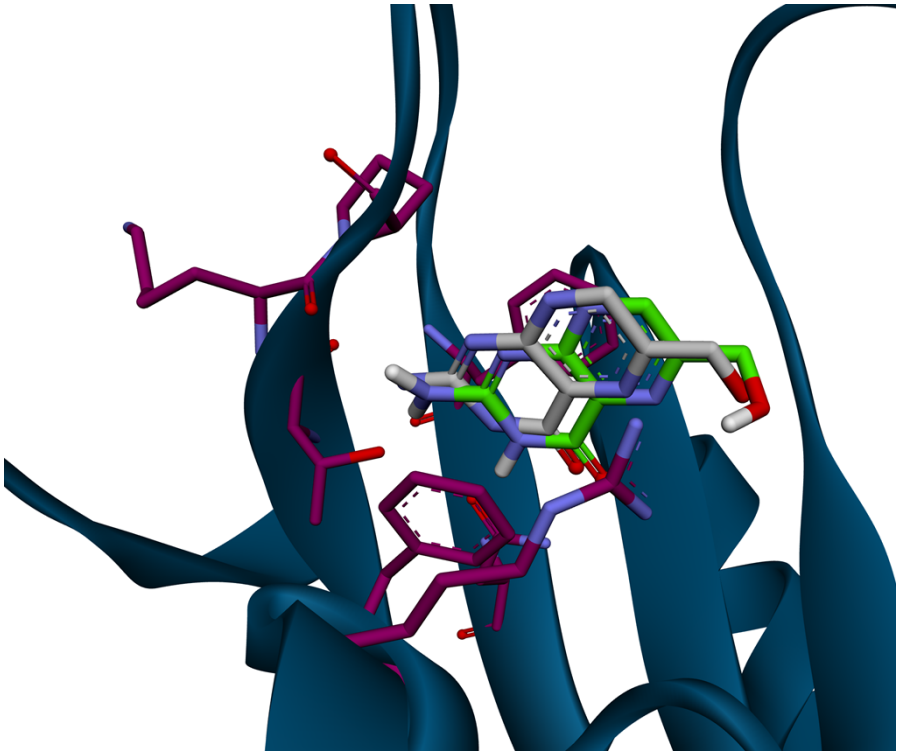

Figure S1. Co-crystallised HP (coordinates taken from YpHPPK co-crystal structure PDB ID 2QX0) overlaid with re-docked HP (green), showing that the docking protocol placed the endogenous ligand in the same pocked with a similar binding pose.

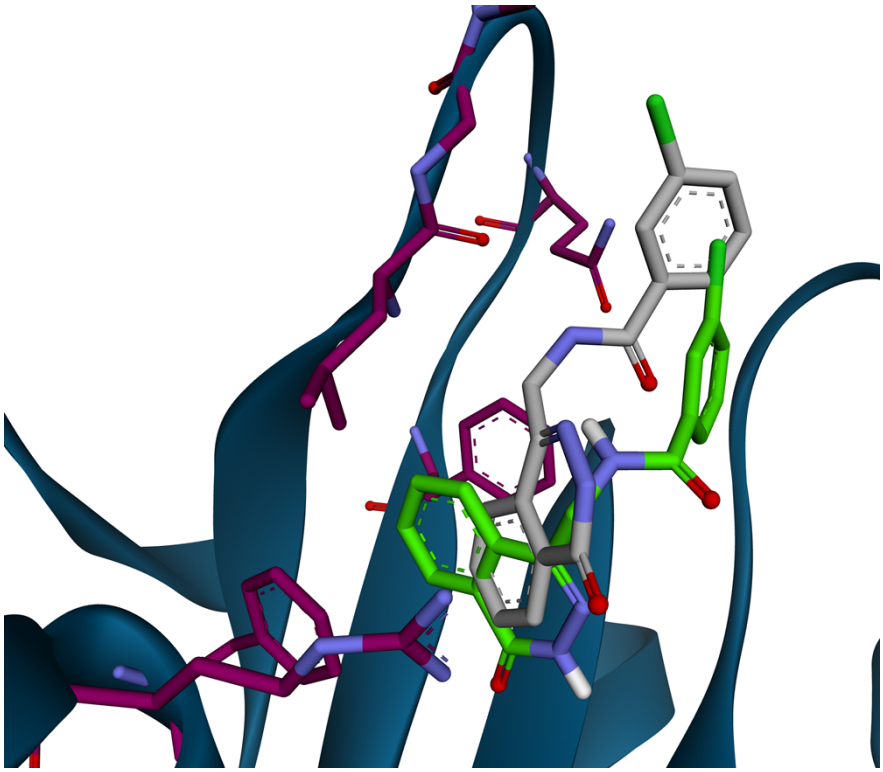

Figure S2. Overlay of the predicted binding pose of compound **7** following virtual screen (green) and refined dock (grey).

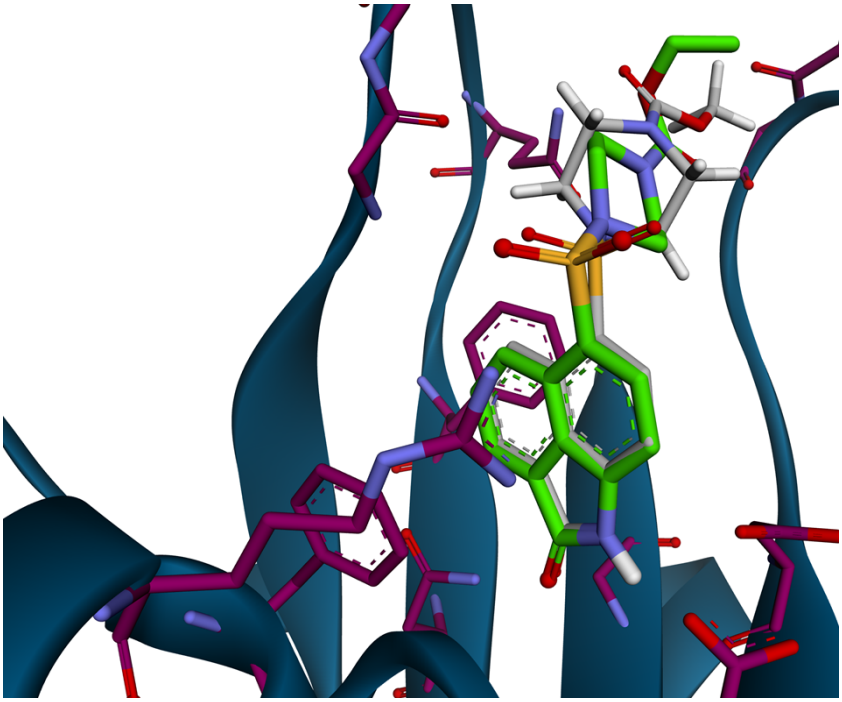

Figure S3. Overlay of the predicted binding pose of compound **8** following virtual screen (green) and refined dock (grey).

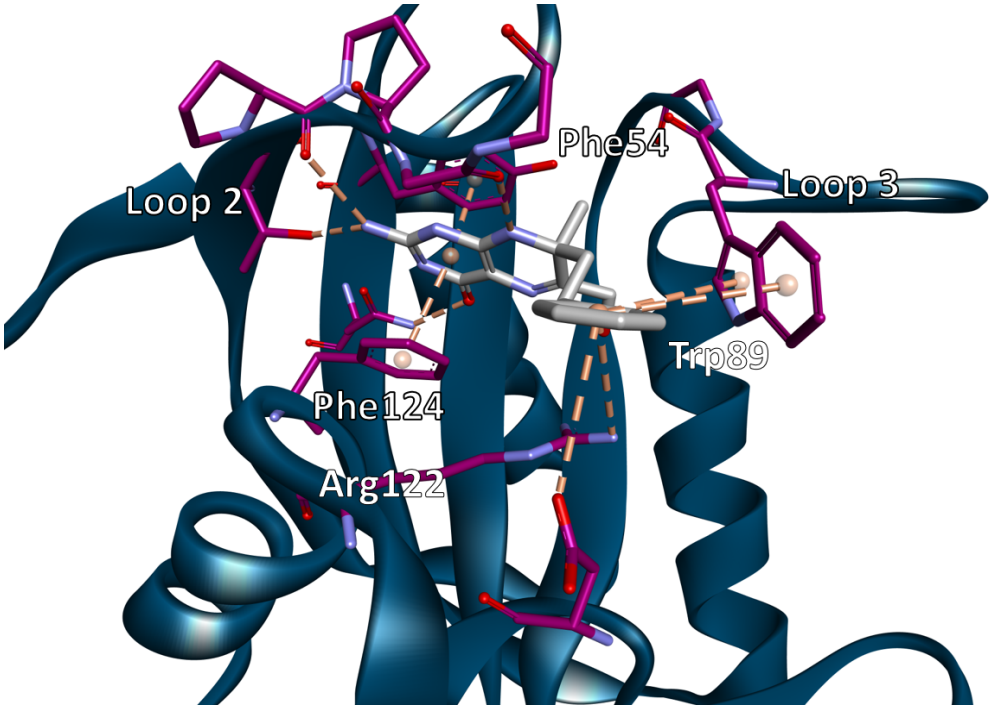

Figure S4. X-ray co-crystal of compound **4** bound to HPPK (PDD ID 1DY3). The phenyl ethyl arm of compound **4** forms a series of interactions, including with Arg122.

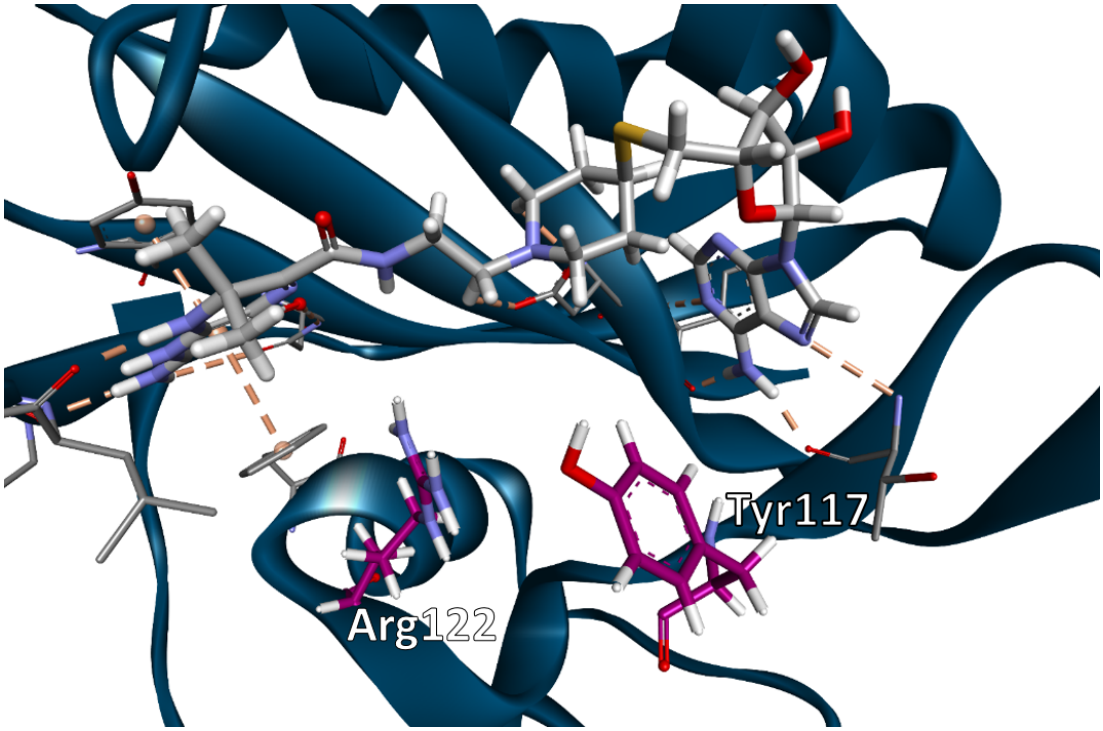

Figure S5. Compound **5** bound to HPPK (PDB ID 3UDV), which occupies both the HP and ATP binding regions of the catalytic cleft, but is not able to interact with Arg122, not Tyr117

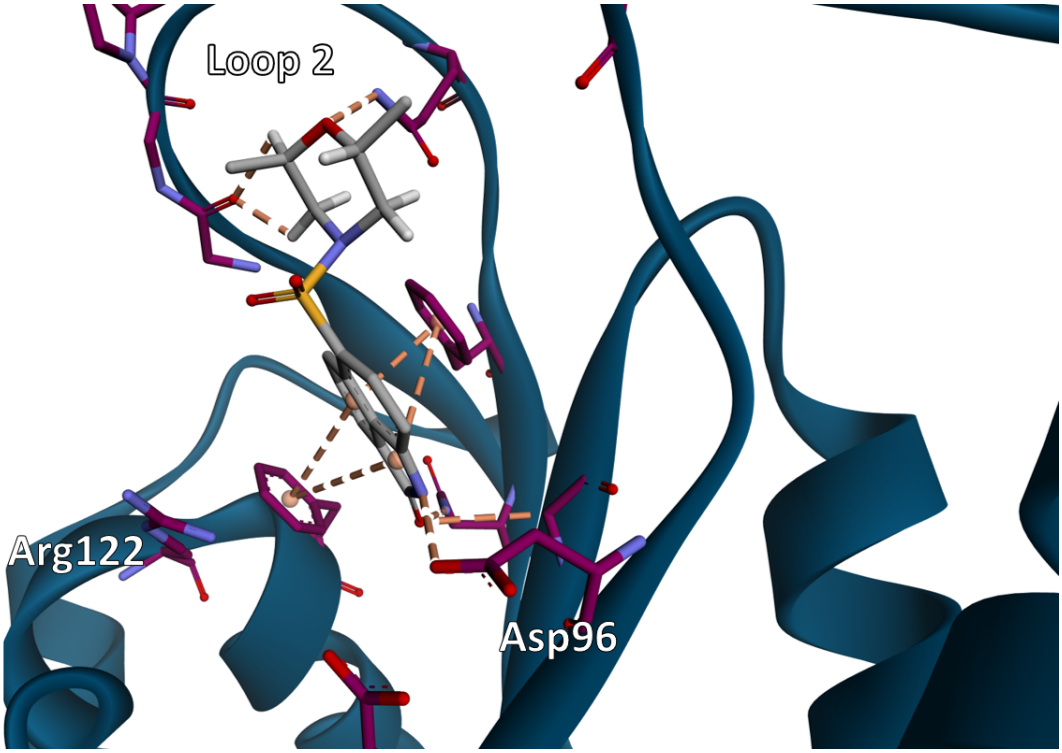

Figure S6. Compound **13**, whose altered conformation in the binding site precludes interaction with Arg122

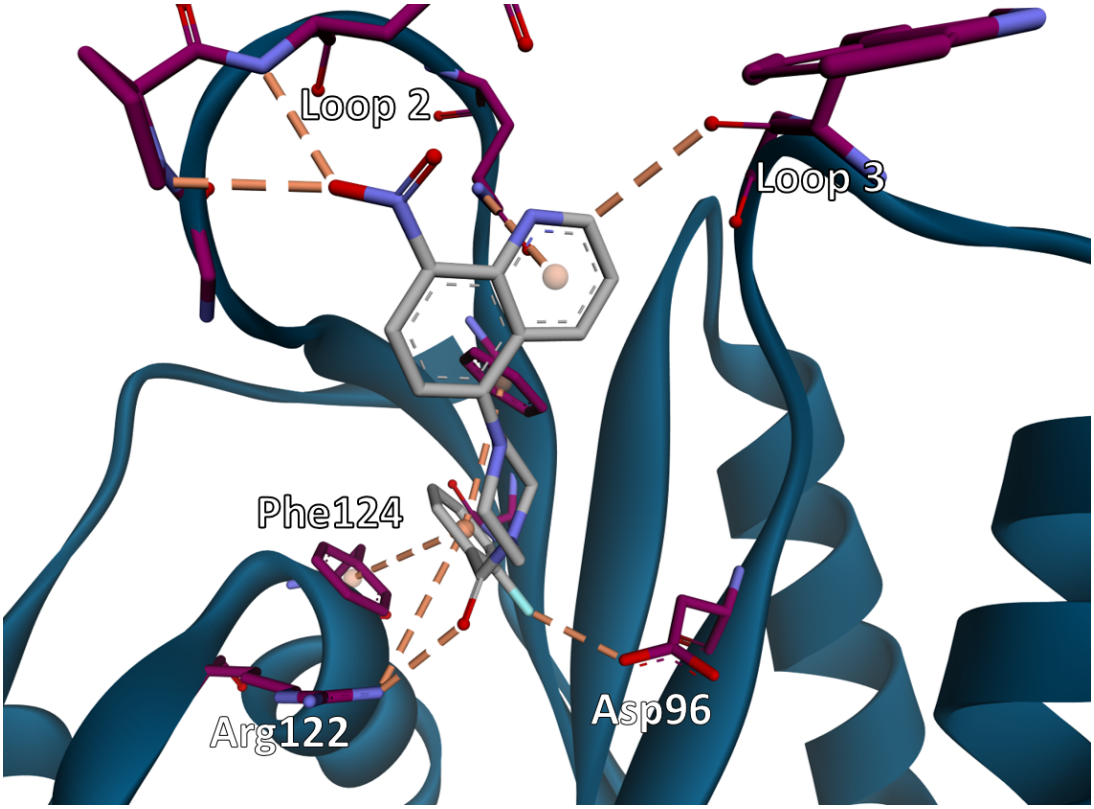

Figure S7 Predicted binding pose of **12**, which forms interactions with residues in loops 1 and 3 as well as Arg122 and Asp96

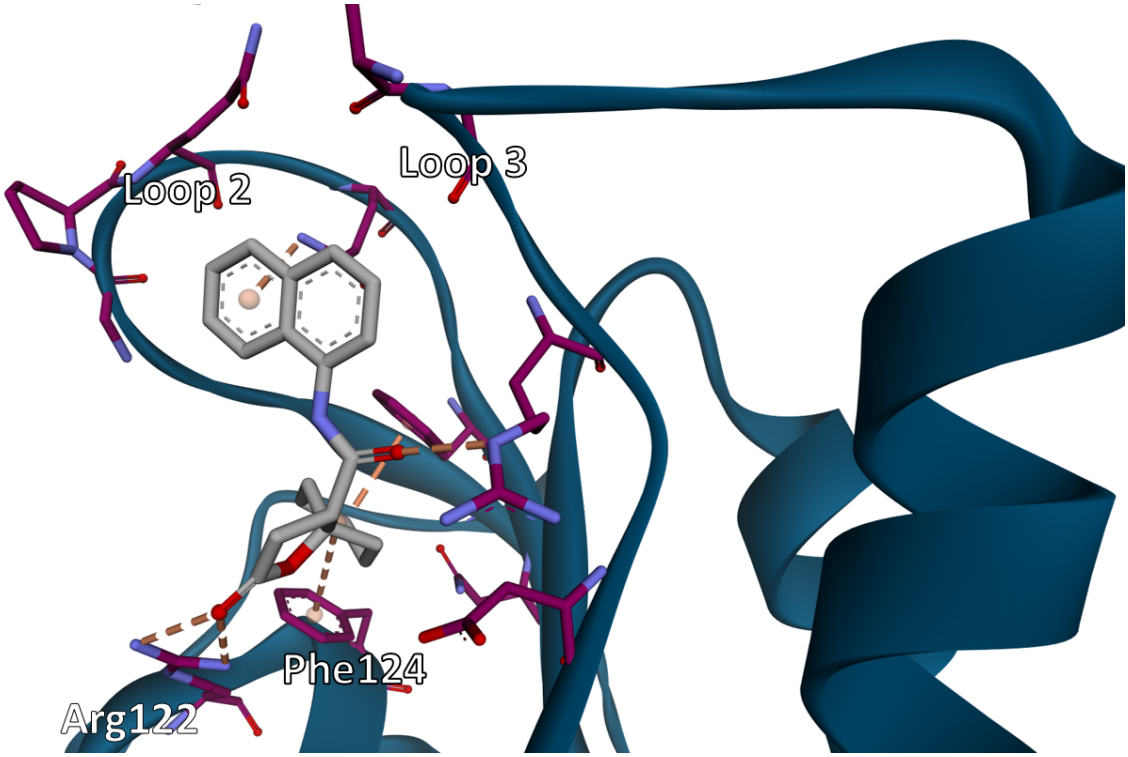

Figure S8. Predicted binding pose of **11**, which interacts with Arg122, but forms fewer bonds to loop 2 and 3 compared to **12**.

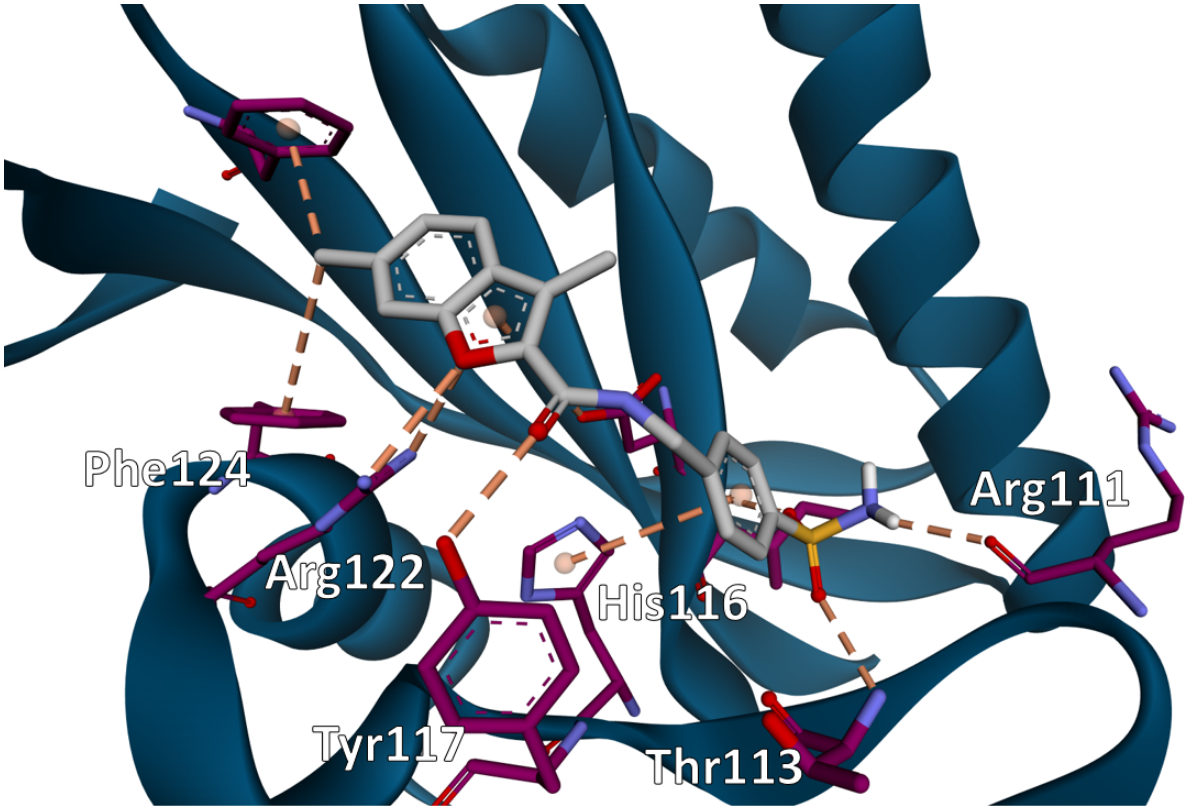

Figure S9. Predicted binding pose of **10**, whose binding pose orientates away from the catalytic loops, in favour of the ATP region.

Figure S10 *S. enterica* HPPK sequence (target) aligned against that of *Yersinia pestis* (*Y. pestis*) HPPK (template). Gaps are represented by a (–) and modified residues are represented by a (.). Amino acids are represented by colours corresponding to Orange for small polar, green for hydrophobic, magenta for polar, red for negatively charged, and blue for positively charged amino acids.

HPPK Luminescence assay

HPPK activity was determined by measuring unprocessed ATP using the Promega Kinase-Glo® Luminescent assay kit (Promega corporation, 2015). Stock solutions of assay components were diluted to a concentration of 10 µM ATP, 10 µM HMDP and 10 µM HPPK enzyme using reaction buffer (200 mM Tris-HCl, 20 mM βME, 0.02 % BSA, 20 mM $MgCl_2$, 0.02 % Tween 20, 10 % DMSO) From these assay stock solutions, a reaction mix was made containing a final assay concentration of 0.1 µM HPPK enzyme, 1.4 µM HMDP and 1.5 µM ATP in a total volume of 200 µL. The reaction was allowed to incubate at 30 °C for 30 min, after which 200 µl Kinase-Glo® reagent was added and the reaction proceeded for a further 10 min at room temperature before being distributed into a white flat bottomed 96 well assay plates. Luminescence was read using a SpectraMax® M3 plate reader set to read as an endpoint assay at all wavelengths with an integration time of 1 second. Inhibitors were purchased from Molport®. For inhibitor studies, inhibitor stock solutions of 10 mM were prepared in DMSO and added to the reaction mix described above to achieve a final assay concentration of 50 µM. For the dose-response assay of compound **7**, the assay was carried out as described above, except with the inclusion of a two-fold serial dilution of the compound to achieve final compound concentrations of 100 µM to 6.25 µM. Luminescence readings were converted to % inhibition relative to the readings obtained with an ATP (no enzyme) control (100 % inhibition) and a reaction without inhibitor control (0 % inhibition). The IC_{50} value was derived by non-linear regression of a plot of % inhibition vs. Log[compound] using GraphPad Prism. At the 95% confidence interval, this plot indicated an IC_{50} range of 6.8 - 15.9 µM .