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Supporting Information

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for

Discovery of Mycobacterium Tuberculosis Protein Tyrosine Phosphatase A (MptpA) Inhibitors Based on Natural Product-Inspired and Fragment-Based Approaches

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Cloning and expression

Plasmid A containing the coding sequence of MptpA was kindly provided by Dr. Anil Koul. The plasmid war used as template and the phosphatase gene was amplified by PCR and cloned into the *NdeI*/*Bam*HO site of pET16bTev, which is a modified version of the pET16b (Novagen, Madison, WI, USA) expression vector where the Factor Xa-cleavage site has been replaced by a tobacco etch virus (Tev) protease cleavage site. The resulting expression plasmid pET PtpA was used for transformation of *E. coli* strain B121 (DE3) (Novagen). The construct was verified by sequencing.

Cells were grown in the presence of 100 µg/mL ampicillin at 25 °C unti the OD_{600} reached 0.6 and then recombinant protein production was initiated by adding 1 mM

IPTG (isopropyl-β-D-thiogalactopyranoside). After 4 hours induction, cells were harvested and stored at -80 °C until usage.

Purification of the phosphatase

Cell pellets were thawed and resuspended in 25 mM Hepes pH 8.0, 200 mM NaCl, 10 mM β-mercaptoethanol. 2 mg lysozyme and 100 U benzonase (Merck, Darmstadt, Germany) were added per mL of the protein suspension. Cells were lyzed by sonification and the cell debris was removed by centrifugation at 20000 *g* for 20 min at 4 °C. The supernatant was applied to a Ni-NTA FastFlow column (Quiagen, Hilden, Germany) following the manufacturers' recommendations. Further purification was achieved by gelfiltration chromatography with a Hi Load 26/60 Superdex 75 column (Pharmacia Biotech) equilibrated in 25 mM Hepes pH 7.4, 200 mM NacCl, 10 mM DTT, 5 % glycerol.