*Supporting Information*

# A hydrazine-hydrazone adamantine compound that target MmpL3 shows antimycobacterial activity

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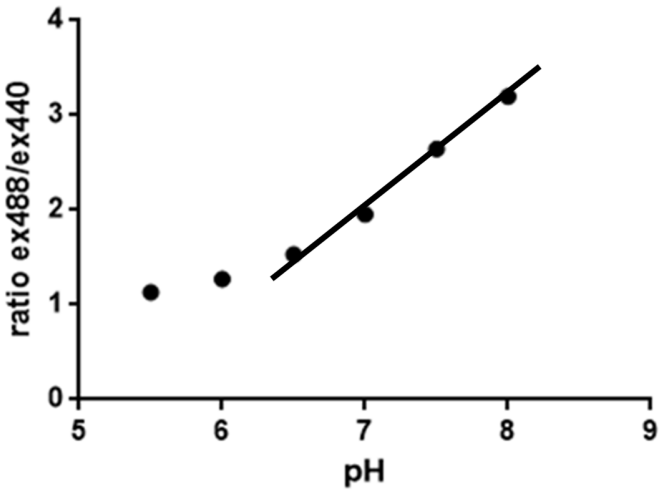



**Figure S1. Intracellular calibration of the pH-sensitive dye BCECF-AN.** Measurements of the fluorescence ratio (λex 488 nm/λex 440 nm) of BCECF in *M. bovis* BCG were averaged (triplicates). pHi calibration curve was sigmoidal with linear range in the physiological pH range of 6,5 - 8.

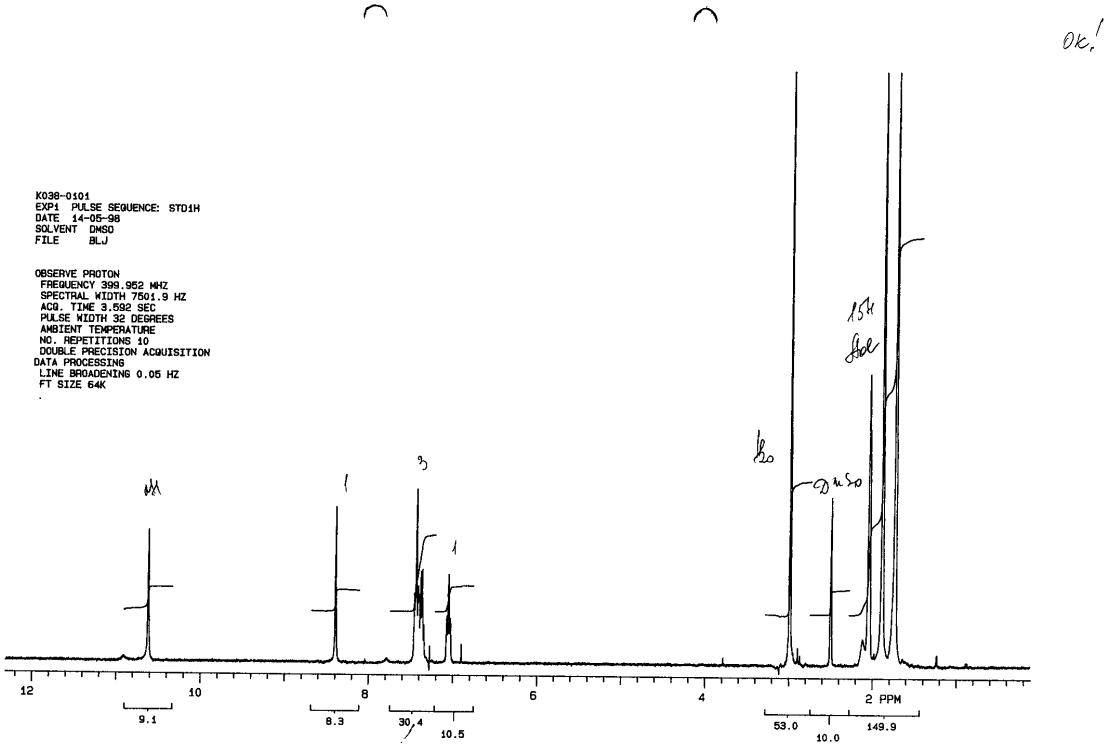
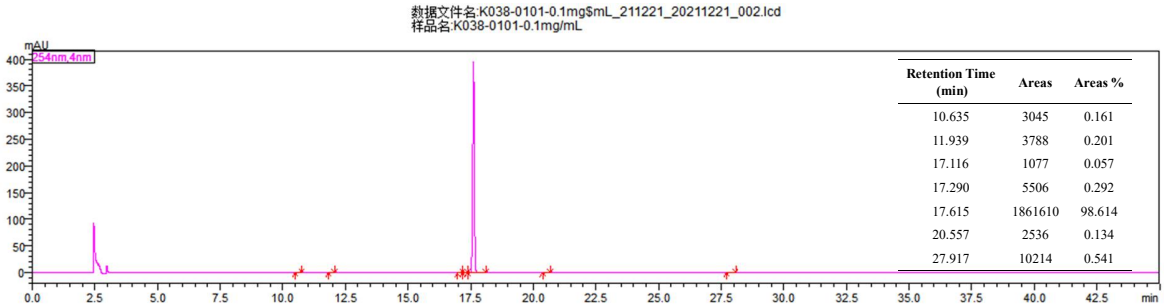



**Figure S2. 1H-NMR spectrum of compound 1** (400 MHz).




| Retention Time (min) | Areas | Areas % |
|---|---|---|
| 10.635 | 3045 | 0.161 |
| 11.939 | 3788 | 0.201 |
| 17.116 | 1077 | 0.057 |
| 17.290 | 5506 | 0.292 |
| 17.615 | 1861610 | 98.614 |
| 20.557 | 2536 | 0.134 |
| 27.917 | 10214 | 0.541 |

**Figure S3. HPLC trace of compound 1**. The purity of compound 1 is 98.6%. HPLC conditions: Column: Phenomenex Kinetex XB-C18, 5 µm, 100 Å, 4.6*250 mm. Mobile A: acetonitrile, mobile B: 2 mM ammonium formate; gradient: 0-10 min with 10-60 % A, 10-30 min with 60-95 % A, 30-40 min with 95 % A, 40-40.1 min with 95-10 % A, 40.1-45 min with 10 % A. Flow rate: 1 mL/min; column temperature: 35℃; detector: 254 nm; injection volume: 10 µL. Sample: 0.1 mg/mL in DMSO.

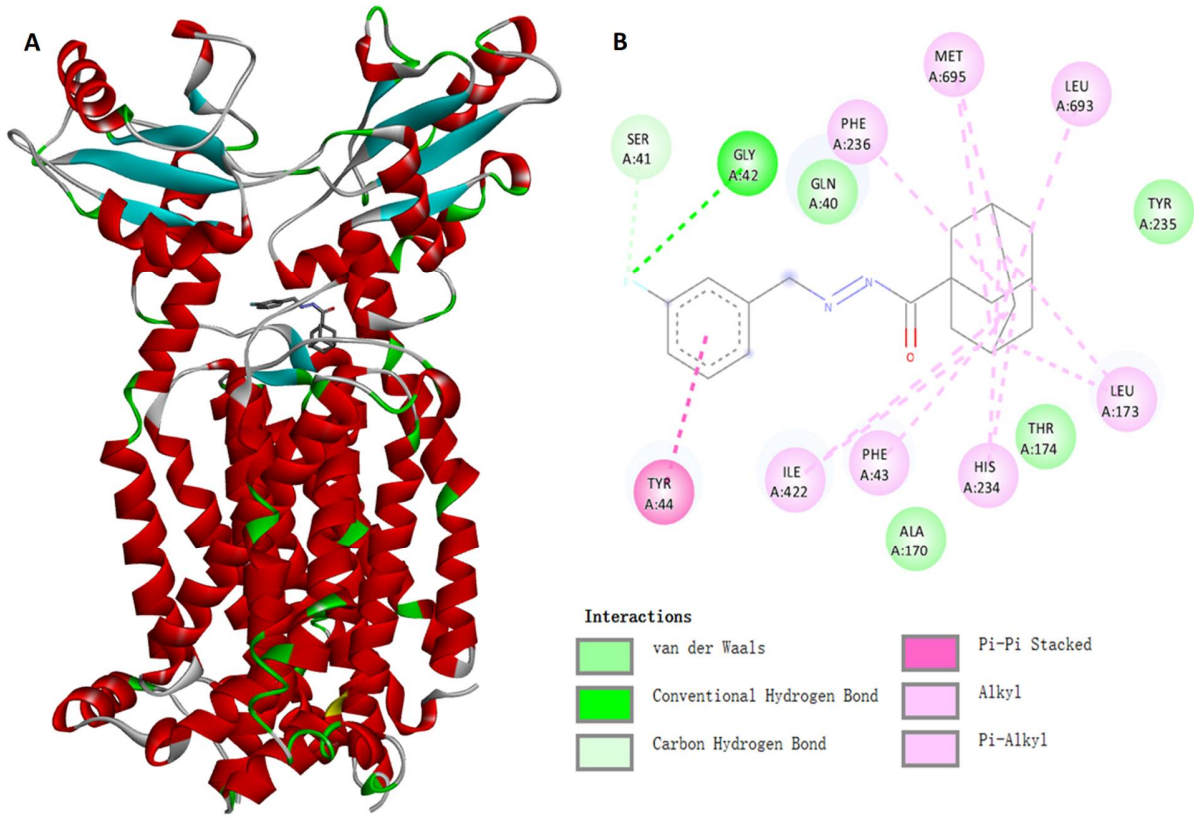



Figure S4. **The docking site of compound 1 with *M. tuberculosis* MmpL3.** A. Representation of an X-ray structure of the *M. tuberculosis* MmpL3 (PDB ID: 7NVH) with docked ligand compound **1** in the cavity of the protein. Compound 1 is depicted in stick representation in grey. B. 2D interaction diagram for the complex MmpL3-compound **1** drawn using Discovery Studio Visualizer.

| Clinical strain | Mutation in *rpoB* | |
|---|---|---|
| | Base | Amino Acid |
| **M9** | 1590C>T;1714A>G | Ser531Leu; Ile572Val |
| **M20** | 1590C>T | Ser531Leu |
| **M34** | 1598T>C | Leu533Pro |

**Table S1. Mutation in *rpoB* in clinical strains Rif-R used in the study.**

| Primers | 5‘ to 3’ Sequence |
|---|---|
| MSMEG_0250-F | ccttggagtttgtcgatc |
| MSMEG_0250-R | cggtggcattcctcaaca |

**Table S2. Primers used in this study.**

| Compound | SMILE | Molecular Formula | Molecular Weight |
|---|---|---|---|
| Compound **1** | Fc1cccc(c1)/C=N/NC(=O)C12CC3CC(C1)CC(C2)C3 | $C_{18}H_{21}FN_2O$ | 300.4 |

**Table S3. Molecular formula strings.**