Supplementary Information

Discovery of penta-peptides inhibiting activity of formylglycine generating enzyme and its potential antibacterial effect against *Mycobacterium tuberculosis*

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Figure S1. FGE activity inhibition assay upon serial concentration of LSTPSR peptide treatment. Targeted LCMS chromatograms between substrate and fGly product after incubation for 1 hour were used to calculate (a) fGly conversion percentage and (b) activity inhibition percentage.

| | -1 169E | | 179T | 186C | |
|---------------------|--------------------------|----------------------------------|-----------------------|----------------------------------|--|
| M. smegmatis | - G F L <mark>Y</mark> A | MGDEVCPDGQ <mark>L</mark> M | AN <mark>TW</mark> C | A P E Y <mark>C</mark> HR Y R P | |
| M. smegmatis | - T T V <mark>Y</mark> A | WGDDV R P D G Q <mark>L</mark> M | A N <mark>TW</mark> C | A P E Y <mark>C</mark> HR Y R P | |
| M. sp. MS1601 | - S T T <mark>Y</mark> A | WGDEAAPGGR <mark>L</mark> M | A N <mark>TW</mark> C | A P E Y <mark>C</mark> HR Y R P | |
| M. tuberculosis | - T A T <mark>Y</mark> A | WGDQEKPGGM <mark>L</mark> M | A N <mark>TW</mark> C | A P E Y <mark>C</mark> HR Y R P | |
| M. xenopi | - T T T <mark>Y</mark> V | WGDEEKPNGR <mark>L</mark> M | A N <mark>TW</mark> C | A P E Y <mark>C</mark> H R Y R P | |
| M. paratuberculosis | - T T T <mark>Y</mark> F | WGDEPTSDGR <mark>L</mark> M | A N <mark>TW</mark> C | A P E Y <mark>C</mark> H R Y R P | |
| M. botniense | - T T T <mark>Y</mark> P | WGDEEKPGGQ <mark>L</mark> M | A N <mark>TW</mark> C | A P E Y <mark>C</mark> H R Y R P | |
| M. haemophilum | - A T T <mark>Y</mark> S | WGDEARPDGQ <mark>L</mark> M | A N <mark>TW</mark> C | A P E Y <mark>C</mark> H R Y R P | |
| M. bohemicum | - T T T <mark>Y</mark> A | WGDEANPGGR <mark>L</mark> M | A N <mark>TW</mark> C | A P E Y <mark>C</mark> HR Y R P | |
| M. heidelbergense | - T T T <mark>Y</mark> S | WGDEAAPDGQ <mark>L</mark> M | A N <mark>TW</mark> C | A P E Y <mark>C</mark> H R Y R P | |
| M. marinum | - T T T <mark>Y</mark> A | MGDEEKPAGQ <mark>L</mark> M | A N <mark>TW</mark> C | A P E Y <mark>C</mark> H R Y R P | |
| M. kansasii | - TTT <mark>Y</mark> A | WGDEATPGGQ <mark>L</mark> M | A N <mark>TW</mark> C | A P E Y <mark>C</mark> H R Y R P | |
| M. pseudokansasii | - TTT <mark>Y</mark> A | WGDEATPGGQ <mark>L</mark> M | A N <mark>TW</mark> C | A P E Y <mark>C</mark> HR Y R P | |
| Homo sapiens | - N R L F P | WGNKLQPKGQ <mark>H</mark> Y | A N I W C | HR S Y <mark>C</mark> Y R Y R C | |
| T. curvata | - QA R <mark>Y</mark> F | WGNELTPRGR <mark>H</mark> R | CN <mark>IW</mark> C | HE S Y <mark>C</mark> NR Y R V | |
| S. coelicolor | - G R R <mark>Y</mark> A | MGDELTPGGR R R | C <mark>NIW</mark> C | HD S Y <mark>C</mark> NR YRV | |

Figure S2. Multiple sequence alignment of FGE from multiple mycobacterium species in comparison with human, T Thermomonospora curvata and Streptomyces coelicolor. Conserved small hydrophobic subdomain containing a buried alanine (pink) which is substituted for cysteine (pink)



(b)

| Region 1 | Region 2 | Region 3 | Region 4 | Region 5 | Region 6 |
|----------|----------|----------|----------|----------|----------|
| | | | | | |
| SMMMC | GWTME | GWTRS | GWTQK | SGWWW | TYWWW |
| SCGMM | GHGGE | GWTSK | GHGGP | GYWWW | GPWYW |
| SSCCC | SSCMC | | | | GWWPW |
| | DGACA | | | | SPWWW |
| | TPKEK | | | | SWWPW |
| | TSCKM | | | | |

Figure S3. (a) Boxplot of docking energies of penta-peptide ligand (xXXXX: where x: CDEHKLMPQRY, X: any amino acids). (b) Re-sampled and selected peptide belong into the statistical distribution for *in vitro* FGE activity inhibition assay. Inter-Quantile Range (IQR) is distance between upper and lower quantile (IQR = Q3-Q1)



Figure S4. MS-chromatogram profile FGE activity in the presence of various TYWWW concentrations. The peptide inhibitor was neither N-terminal acetylated nor C-terminal amidated and the reaction contained 200μ M native enzyme substrate.



Figure S5. MS-chromatogram profile FGE activity in the presence of various GPWYW concentrations. The peptide inhibitor was neither N-terminal acetylated nor C-terminal amidated and the reaction contained 200μ M native enzyme substrate.



Figure S6. MS-chromatogram profile FGE activity in the presence of various GWWPW concentrations. The peptide inhibitor was neither N-terminal acetylated nor C-terminal amidated and the reaction contained 200μ M native enzyme substrate.



Figure S7. MS-chromatogram profile FGE activity in the presence of various SGWWW concentrations. The peptide inhibitor was neither N-terminal acetylated nor C-terminal amidated and the reaction contained 200μ M native enzyme substrate.



Figure S8. MS-chromatogram profile FGE activity in the presence of various SPWWW concentrations. The peptide inhibitor was neither N-terminal acetylated nor C-terminal amidated and the reaction contained 200μ M native enzyme substrate.



Figure S9. MS-chromatogram profile FGE activity in the presence of various SWWPW concentrations. The peptide inhibitor was neither N-terminal acetylated nor C-terminal amidated and the reaction contained 200μ M native enzyme substrate.



Figure S10. MS-chromatogram profile FGE activity in the presence of various GYWWW concentrations. The peptide inhibitor was neither N-terminal acetylated nor C-terminal amidated and the reaction contained 200μ M native enzyme substrate.



Figure S11. MS-chromatogram profile FGE activity in the presence of various GWTME concentrations. The peptide inhibitor was neither N-terminal acetylated nor C-terminal amidated and the reaction contained 200μ M native enzyme substrate.



Figure S12. MS-chromatogram profile FGE activity in the presence of various GWTRS concentrations. The peptide inhibitor was neither N-terminal acetylated nor C-terminal amidated and the reaction contained 200μ M native enzyme substrate.



Figure S13. MS-chromatogram profile FGE activity in the presence of various GWTQK concentrations. The peptide inhibitor was neither N-terminal acetylated nor C-terminal amidated and the reaction contained 200μ M native enzyme substrate.



Figure S14. MS-chromatogram profile FGE activity in the presence of various GWTSK concentrations. The peptide inhibitor was neither N-terminal acetylated nor C-terminal amidated and the reaction contained 200μ M native enzyme substrate.



Figure S15. MS-chromatogram profile FGE activity in the presence of various GHGGP concentrations. The peptide inhibitor was neither N-terminal acetylated nor C-terminal amidated and the reaction contained 200μ M native enzyme substrate.



Figure S16. MS-chromatogram profile FGE activity in the presence of various GHGGE concentrations. The peptide inhibitor was neither N-terminal acetylated nor C-terminal amidated and the reaction contained 200μ M native enzyme substrate.



Figure S17.MS-chromatogram profile FGE activity in the presence of various SMMMC concentrations. The peptide inhibitor was neither N-terminal acetylated nor C-terminal amidated and the reaction contained 200μ M native enzyme substrate.



Figure S18. MS-chromatogram profile FGE activity in the presence of various SCGMM concentrations. The peptide inhibitor was neither N-terminal acetylated nor C-terminal amidated and the reaction contained 200μ M native enzyme substrate.



Figure S19.MS-chromatogram profile FGE activity in the presence of various SSCMC concentrations. The peptide inhibitor was neither N-terminal acetylated nor C-terminal amidated and the reaction contained 200μ M native enzyme substrate.



Figure S20. MS-chromatogram profile FGE activity in the presence of various SSCCC concentrations. The peptide inhibitor was neither N-terminal acetylated nor C-terminal amidated and the reaction contained 200μ M native enzyme substrate.



Figure S21. MS-chromatogram profile FGE activity in the presence of various DGACA concentrations. The peptide inhibitor was neither N-terminal acetylated nor C-terminal amidated and the reaction contained 200μ M native enzyme substrate.



Figure S22. MS-chromatogram profile FGE activity in the presence of various TPKEK concentrations. The peptide inhibitor was neither N-terminal acetylated nor C-terminal amidated and the reaction contained 200μ M native enzyme substrate.



Figure S23. MS-chromatogram profile FGE activity in the presence of various TSCKM concentrations. The peptide inhibitor was neither N-terminal acetylated nor C-terminal amidated and the reaction contained 200μ M native enzyme substrate.