SUPPLEMENTARY MATERIAL 2

Design, synthesis and antimycobacterial activity of hybrid molecules combining pyrazinamide with 4-phenylthiazol-2-amine scaffold

Jan Zitko, Ondřej Janďourek, Pavla Paterová, Lucie Navrátilová, Jiří Kuneš, Jarmila Vinšová, Martin Doležal

DOCKING STUDIES

Objective

In the introduction part of the main article, we have discussed several 2-aminothiazole derivatives as antibacterial or antimycobacterial agents. Compounds in Fig. S1 were chosen as the most active individuals representing their structural classes. Compound **1** exerted *in vitro* growth inhibiting activity against both Gram-positive and Gram-negative bacteria and acted as an inhibitor of β-ketoacyl-(acyl-carrier-protein) synthase III of *E. coli* (ecFabH) [1]. Compound **3** [2, 3] and compound **4** [4] possessed *in vitro* growth inhibiting activity against mycobacteria, but no mechanism of action (subcellular target) was suggested. The structural similarity of antimycobacterial derivatives **3**, **4** and **9b** to ecFabH inhibitor **1** raised the question whether these derivatives could work by inhibiting the mycobacterial FabH. This statement was challenged by a molecular docking study of the ligands into mycobacterial FabH (pdb: 1u6s).

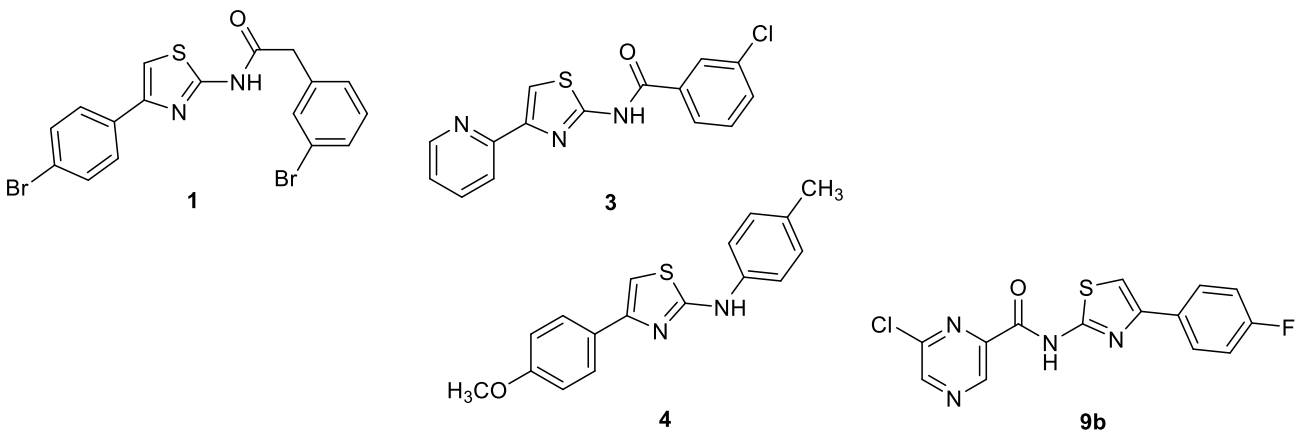


Fig. S1. Studied 2-aminothiazole derivatives (ligands).

Mycobacterial FabH is a homodimer. The substrate channel constitutes two branches organised together in an L shape. The catalytic triad (Cys122, His244, Asn274) is located at the junction of these almost perpendicular arms of the channel (Fig. S2) [5, 6].

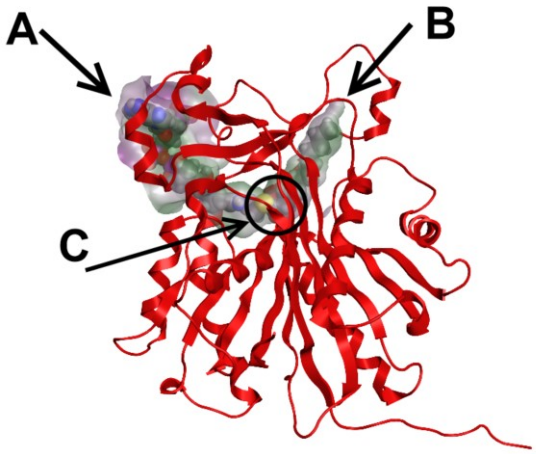


Fig. S2. Visualisation of the L-shaped channel of the mycobacterial FabH. **A** - CoA part of the channel, solvent accessible; **B** - acyl part of the channel, hydrophobic. **C** – localisation of the catalytic triad. Only one subunit of FabH is depicted, the biologically relevant species is a symmetric homo-dimer.

Results and discussion

For antimycobacterial compounds **3** and **9b**, the molecular docking predicted two distinct binding modes with similar docking score (Table S1). Both binding modes were located near the catalytic triad and exerted stabilising interactions (H-bonds and hydrogen-π interactions) to the receptor (Fig. S3).

In binding mode 1 (Fig. S4), the oxygen of the carboxamide linker accepts the H-bond from Ala306 backbone. The sulphur of the thiazole ring interacts with sidechain of Cys112. The (hetero)aromate ring of the acyl part is oriented towards the acyl-CoA arm of the channel (opened to solvent, designated as A in Fig. S2, simply to the 'left').

In binding mode 2 (Fig. S5), the H-bond between the carboxamide oxygen and Ala306 is preserved, but the sulphur of thiazole interacts with backbone of Tyr304. Both thiazole and 4-phenyl (or 4-pyridin-2-yl) ring are stabilised by arene-H interactions to Asn274 and Leu207, respectively. On contrary to binding mode 1, the hetero)aromate ring of the acyl part is oriented towards the distal acyl arm of the tunnel (dead-end, designated as B in Fig. S2, simply to the 'right)'.

Compound **1** with the extended –CONH-CH_2- linker was docked to a pose similar to binding mode 1 of compounds **3** a **9b** – see Fig. S6. Other poses of compound **1** had significantly worse score.

Compound **4** with (with -NH- linker) was docked to a pose similar to binding mode 2, but due to the absence of the carbonyl moiety, it had only one H-bond interaction to the receptor and the sulphur of thiazole ring showed no stabilising interactions (Fig. S7). This was reflected by the lowest docking score of all tested structural types.

To sum up:

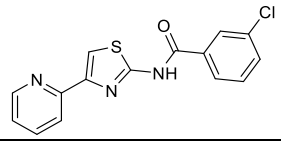
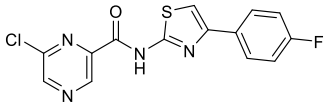
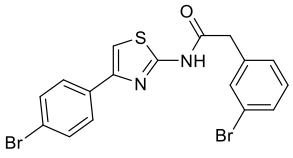
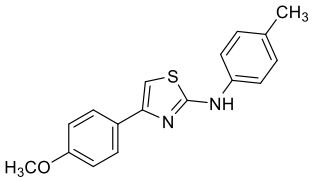
- According to the overlay of the best poses for compounds **3** and **9b**, it makes no significant difference whether the ring in the acyl part is benzene or pyrazine.
- Similarly, it makes no significant difference whether the ring in position 4 of the thiazole is benzene or pyridine (pyridin-2-yl). No specific interactions for the pyridine nitrogen atom were observed.
- Compound **4** (with -NH- linker) was docked with significantly worse score than compounds with the carboxamide linker (**1**, **3**, **9b**). The docking simulation rationalizes the importance of the amide linker and is in agreement with the observation that *N*-acyl derivatives are more active then *N*-phenyl derivatives [2, 3].

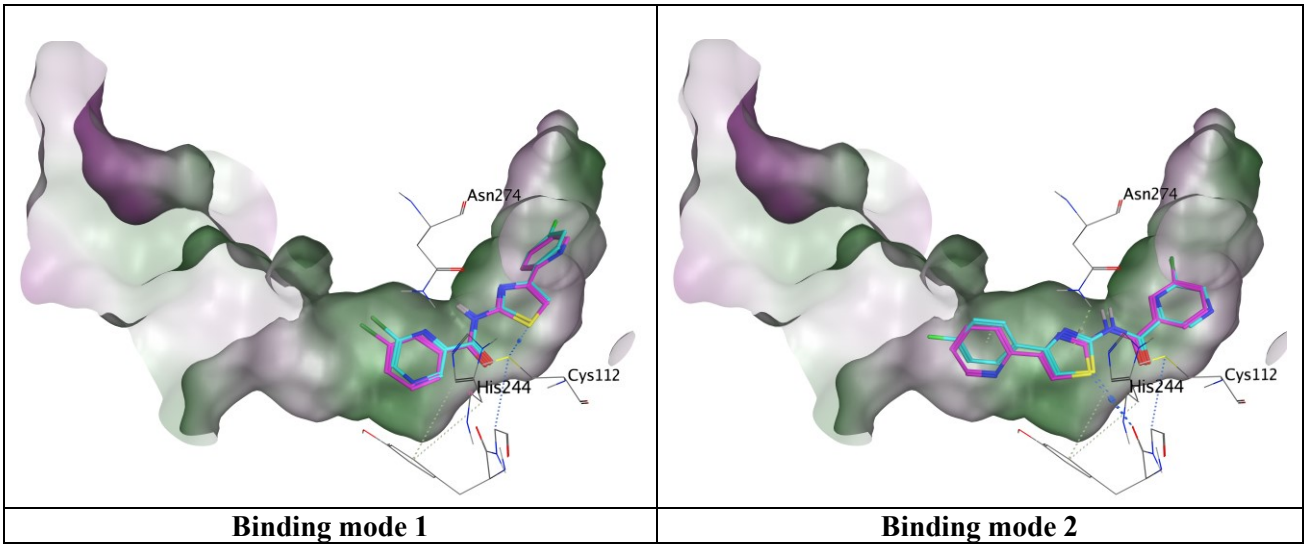
Conclusions

The molecular docking study suggests that aminothiazole derivatives of structural type **3** and **9** might exhibit their antimycobacterial activity through inhibition of mycobacterial β-ketoacyl-(acyl-carrier-protein) synthase III (FabH). Despite that the docking was focused on the large channel of the enzyme, the best docked poses as suggested by the docking score were placed in the close vicinity of the catalytic site. Two similar binding modes were identified, both stabilised by H-bonds and/or arene-H interaction. The results rationalised the importance of the aminothiazole ring and the carboxamide linker for binding to the catalytic site of FabH.

Despite of the fact that the molecular docking study identified reasonable binding modes to mycobacterial FabH and confirmed some of the SAR observed before, it is, of course, not enough to declare the inhibition of FabH as the mechanism of action for the discussed aminothiazole derivatives. Future enzyme based assays will be needed.

Table S1. Docking scores of the ligands

Ligand		Binding Mode	Best Score (kcal/mol)
3		1	-6.885
		2	-6.829
9b		1	-6.748
		2	-6.506
1		1-like	-7.072
4		2-like	-6.059



Binding mode 1	**Binding mode 2**

Fig. S3. Predicted binding modes of compounds **3** (violet carbons) and **9b** (turquoise carbons) in mycobacterial FabH (pdb: 1u6s). Overall position in the channel. Receptor surface coloured by lipophilicity (from green = hydrophobic to violet = polar).

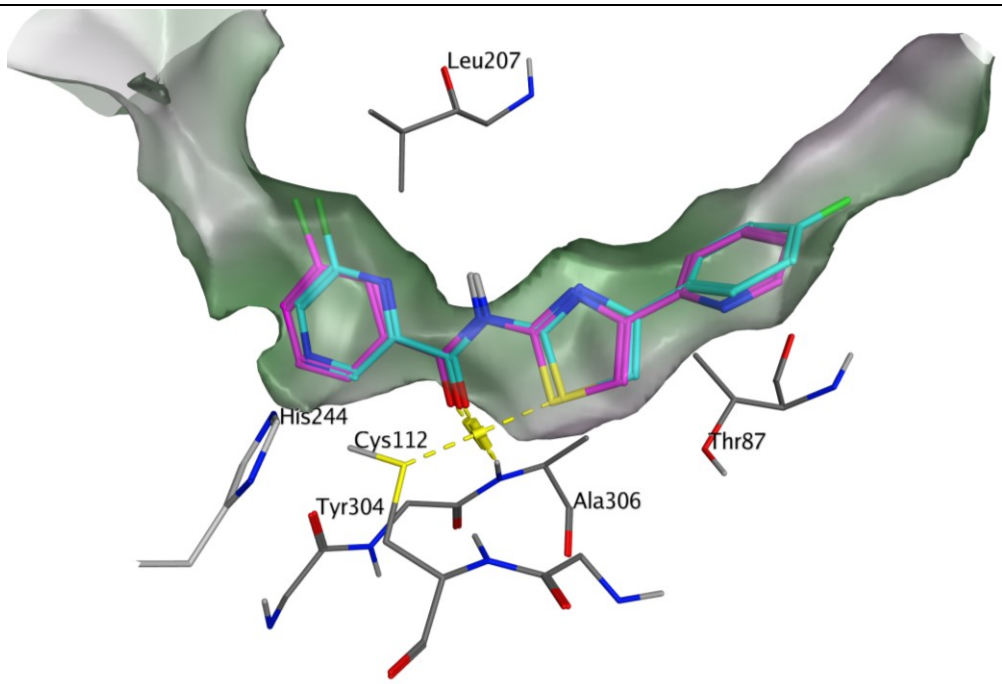
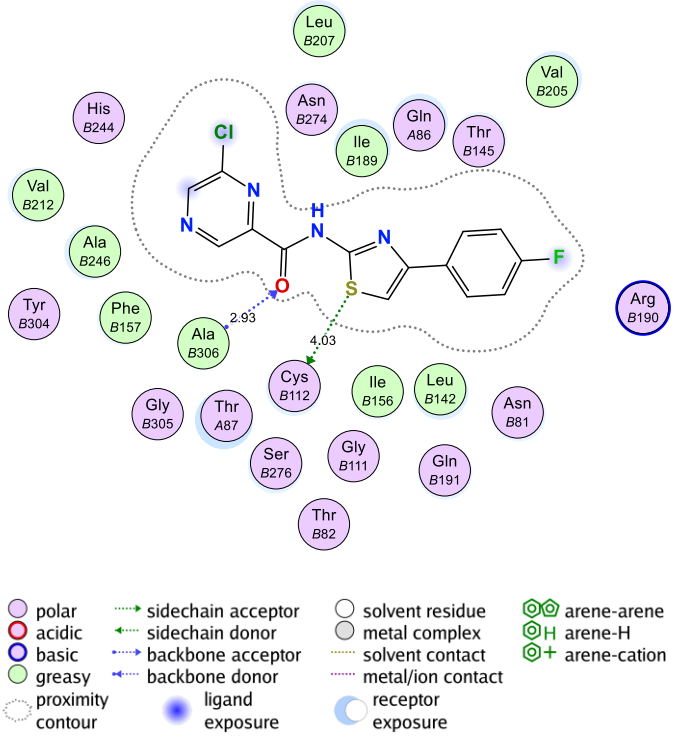
	BINDING MODE 1
A	
B	

Fig. S4. A - Predicted ligand-receptor interactions (binding mode 1) of compounds **9b** (turquoise carbons) and **3** (violet carbons) with FabH in detail. The surface is a vdW interaction surface (ligand-receptor) coloured by lipophilicity (from green = hydrophobic to violet = polar). **B** – 2D ligand interaction diagram of **9b**.

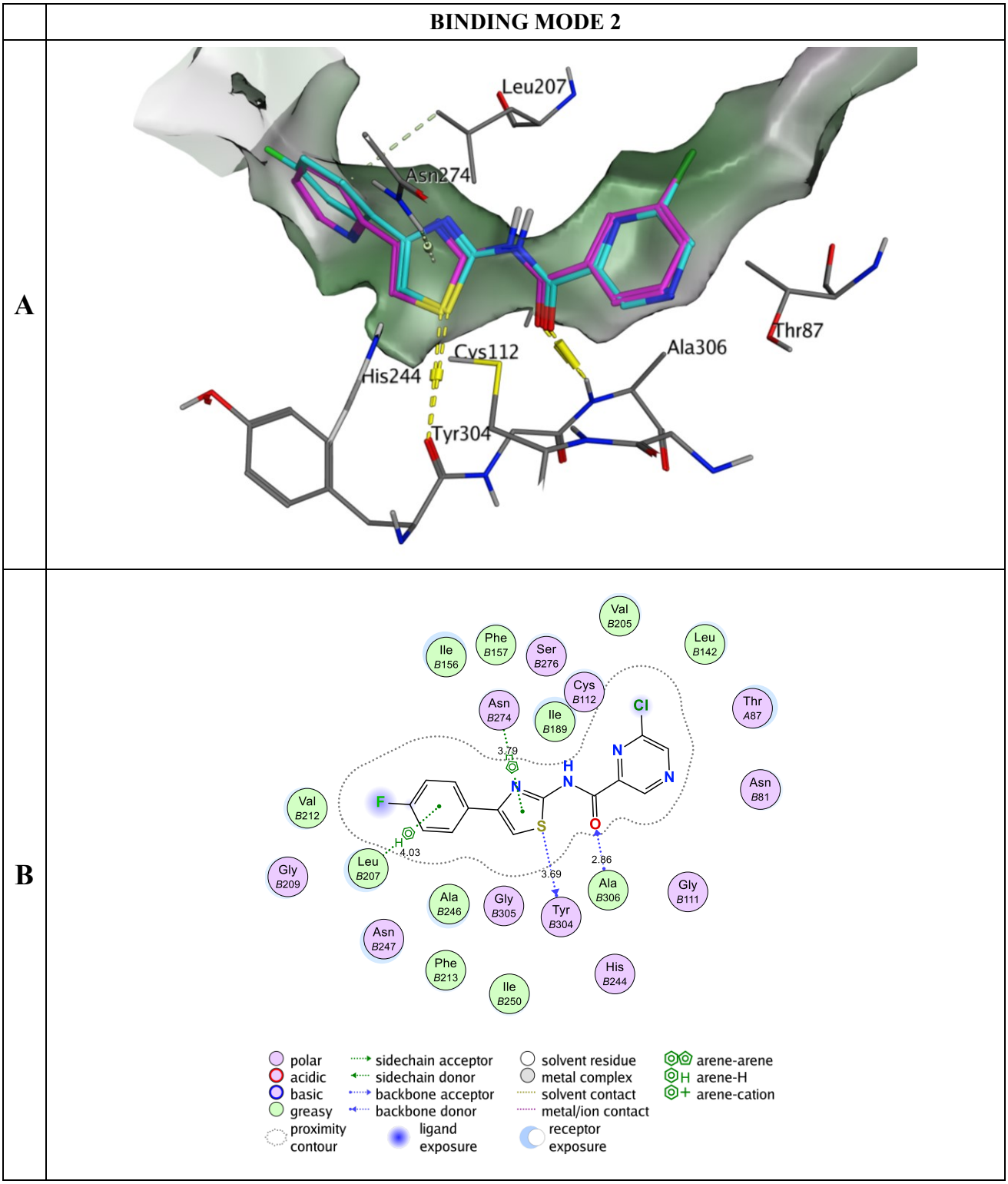


Fig. S5. A - Predicted ligand-receptor interactions (binding mode 2) of compounds **9b** (turquoise carbons) and **3** (violet carbons) with FabH in detail. The surface is a vdW interaction surface (ligand-receptor) coloured by lipophilicity (from green = hydrophobic to violet = polar). **B** – 2D ligand interaction diagram of **9b**.

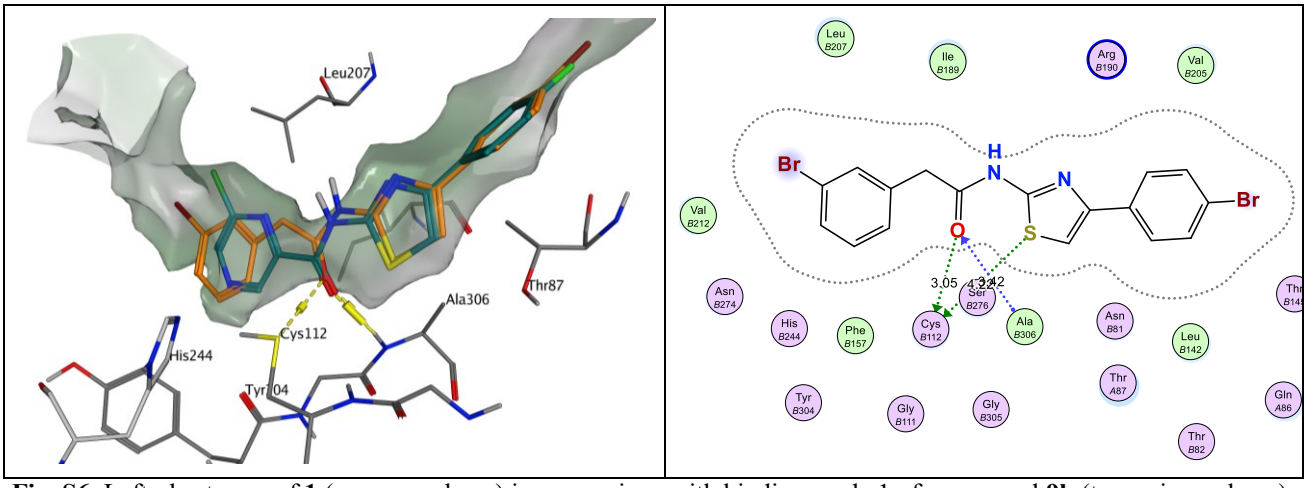


Fig. S6. Left - best pose of **1** (orange carbons) in comparison with binding mode 1 of compound **9b** (turquoise carbons). Right – 2D-ligand interaction diagram of **1**.

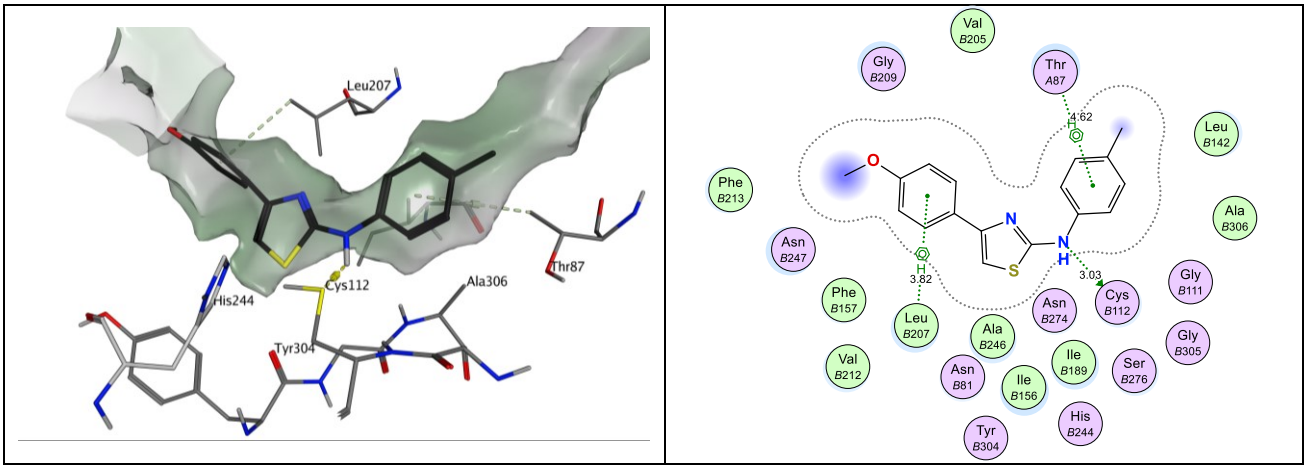


Fig. S7. Predicted binding mode of compound **4** in 3D and 2D interaction diagram.

Experimental

Molecular docking to mycobacterial FabH

All *in silico* calculations and production of figures were performed in Molecular Operating Environment (MOE), v2016.0802 (Chemical Computing Group Inc., Montreal, QC, Canada) using the MMFF94x forcefield. 3D structure of *M. tuberculosis* beta-ketoacyl-acyl carrier protein synthase III (FabH) was downloaded from the PDB database (pdb: 1u6s). This structure is a FabH co-crystalized with lauroyl coenzyme A. The receptor was prepared by MOE QuickPrep functionality with default settings. This included correction of structural errors, addition of hydrogens, calculation of partial charges, 3D optimisation of *H*-bond network (Protonate3D), deletion of water molecules further then 4.5 Å from any receptor or ligand atom, and restrained energy minimization of ligand and pocket residues within 8 Å from the ligand. Subsequently, all water molecules were deleted and were not taken into account in further calculations. The pdb structure 1u6s is a catalytically disabled Cys112Ala mutant of the natural FabH (the mutation was introduced to simplify the crystallization and to study the mechanistic process of catalysis) [5]. Therefore, Ala112 was converted back to Cys112. The rotamer with the lowest energy suggested by MOE was selected and its sidechain was minimized. The correct conformation of the newly introduced Cys112 was confirmed by comparison with another mycobacterial FabH (pdb: 2qo0), after superposition of the two crystal structures.

Structures of 2-aminothiazoles (as ligands) were created by MOE Builder. Partial charges were computed and the ligands were minimized by conjugate gradient method to RMS gradient of 0.001 kcal.mol^{-1} Å $^{-1}$.

The docking was focused on the original co-crystalized ligand (lauroyl-CoA). Details of the MOE docking protocol setup: **Docking stage** - Placement method: Triangle Matcher, generate 10.000 poses; Score: London dG; retain 30 poses. **Refinement stage** – Rigid receptor; Score: GBVI/WSA dG; retain 5 poses.

References:

[1] J.R. Li, D.D. Li, R.R. Wang, J. Sun, J.J. Dong, Q.R. Du, F. Fang, W.M. Zhang, H.L. Zhu, Design and synthesis of thiazole derivatives as potent FabH inhibitors with antibacterial activity, Eur. J. Med. Chem., 75 (2014) 438-447.

[2] A. Meissner, H.I. Boshoff, M. Vasan, B.P. Duckworth, C.E. Barry, C.C. Aldrich, Structure-activity relationships of 2-aminothiazoles effective against Mycobacterium tuberculosis, Bioorg. Med. Chem., 21 (2013) 6385-6397.

[3] F. Mjambili, M. Njoroge, K. Naran, C. De Kock, P.J. Smith, V. Mizrahi, D. Warner, K. Chibale, Synthesis and biological evaluation of 2-aminothiazole derivatives as antimycobacterial and antiplasmodial agents, Bioorg. Med. Chem. Lett., 24 (2014) 560-564.

[4] M. Pieroni, B.J. Wan, S.H. Cho, S.G. Franzblau, G. Costantino, Design, synthesis and investigation on the structure-activity relationships of N-substituted 2-aminothiazole derivatives as antitubercular agents, Eur. J. Med. Chem., 72 (2014) 26-34.

[5] F. Musayev, S. Sachdeva, J. Neel Scarsdale, K.A. Reynolds, H.T. Wright, Crystal Structure of a Substrate Complex of Mycobacterium tuberculosis β-Ketoacyl-acyl Carrier Protein Synthase III (FabH) with Lauroyl-coenzyme A, J. Mol. Biol., 346 (2005) 1313-1321.

[6] S. Sachdeva, F.N. Musayev, M.M. Alhamadsheh, J.N. Scarsdale, H.T. Wright, K.A. Reynolds, Separate entrance and exit portals for ligand traffic in Mycobacterium tuberculosis FabH, Chem. Biol., 15 (2008) 402-412.