# Identification and characterization of new structural scaffolds modulating the activity of *Mycobacterium tuberculosis* dihydroneopterin aldolase (FoIB) *in vitro*

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# Supplementary figures



**Figure S1. Optimization of a fluorometric assay for the estimation of FoIB activity. A.** Evolution of the fluorescence of the buffer solution used for the reaction (25 mM Tris-HCl pH 8.0, 50 mM NaCl, 5% glycerol), alone (buffer), and supplemented with 5  $\mu$ M of FoIB substrate (DHNP), after an incubation period of 20h at room temperature. **B.** Fluorescence of the substrate (DHNP), thermodynamic (DHXP), and kinetic (HP) products of the FoIB reaction when solubilized in the buffer at a concentration of 5  $\mu$ M. **C**. End-points fluorescence measurement of the solution obtained after incubation of FoIB and its substrate (DHNP, 5  $\mu$ M) for the indicated period at room temperature. For all experiments, fluorescence was recorded using Ex. 405 nm and Em. 535 nm. A dashed gray line is used to indicate the average fluorescence of the substrate alone (background). Data shown are average and SD for at least 3 technical replicates.





Figure S2. Structure of the 19 hits selected at a 30% inhibition threshold, arranged by cluster. Blue numbering is as in Table 1 from the main manuscript. Red numbering is as in the chemistry section of the supplementary methods.



Figure S3. Kinetic inhibition studies for the pyrazol-3-one series (Cluster II). Each reaction velocity ( $\mu$ M.min<sup>-1</sup>) was calculated by linear regression of a 6-min kinetic (20 data points) as described in the Methods of the main manuscript. Data shown are for a single, representative experiment. Compounds **3**, **9** and **16** are the screening hits confirmed by dose-response. Compounds 20 and 22 are the two additional derivatives synthesized with the highest inhibition activity, as per the dose-response assay (see Table 2).





Figure S4. Time-dependent inhibition by pyrazol-3-one series (Cluster II). MtbFolB (300 nM) was incubated at room temperature in the reaction buffer together with the compound (3, 9 or 20) prior to determination of the reaction velocity by fluorometry. At the corresponding time-point (0, 30 and 60 min), the substrate (DHNP) was added and the enzyme velocity determined by kinetic assay (see methods in main text). Different incubation and test conditions were used. i) Normal condition: MtbFolB was incubated with the compound at 300 µM, then the velocity measured in presence of the compound (300 µM) and 1.5 µM of substrate. ii) Excess of substrate: MtbFolB was incubated with the compound at 300 µM, then the velocity measured in presence of the compound (300 µM) and 3 µM (2×) of substrate. iii) Excess of inhibitor: MtbFolB was incubated with the compound at 3 mM (10×), then the velocity measured in presence of the compound (300 µM) and 1.5 µM of substrate. For each sample, a control incubated and tested without any inhibitor was performed to obtain the VMAX. Percentage of velocity obtained for the treated samples, as compared to the untreated ones, is indicated in blue of top of each column. nd: could not be determined due to compound precipitation. Each reaction velocity (µM.min<sup>-1</sup>) was calculated by linear regression of a 6-min kinetic (20 data points). Data shown are for a single, representative experiment.





Figure S5. 2D ligand interaction diagram obtained for the docking of compounds from the pyrazol-3-one series (Cluster II). Hydrophobic interactions are represented as green lines and  $\pi$ - $\pi$  stacking interactions by green dashed lines. Images were generated with PoseView.





Figure S6. Docking poses obtained for compounds of the sulfonamide series (Cluster III). A. Active compounds 5 and 27 are shown superimposed. B. Inactive compounds 24 and 34 are shown superimposed.





Figure S7. 2D ligand interaction diagram obtained for the docking of compounds from the sulfonamide series (Cluster III). Compounds 24 and 34, which were found to be inactive *in vitro* and were included for comparison purposes, are indicated in red. Hydrophobic interactions are represented as green lines,  $\pi$ - $\pi$  stacking interactions by green dashed lines and hydrogen bonds by black dashed lines. Images were generated with PoseView.

**Table S1.** Computed hydrophobicity-related properties for the molecules of the sulfonamidecluster(ClusterIII),obtainedfromthemolinspirationwebserver(<u>https://www.molinspiration.com</u>).TPSA: topological polar surface area.Values < 3 for logP and</td>< 120 for TPSA are usually considered acceptable for good solubility.</td>

### A. Screening Hits

#	Structure	logP	TPSA
5		1.92	104.39
7		4.34	69.72
11		4.95	107.73
13		3.69	49.41

#### **B. S-Series**

#	ļ	logP	TPSA
24		3.07	54.34



## C. N-series

#		logP	TPSA
31	H <sub>2</sub> N	2.66	72.19
32		4.59	49.41
33		2.92	63.99
34		2.80	75.27

