INHIBITION OF MYCOBACTERIAL PHOSPHOPANTETHEINYL TRANSFERASES

L. MAVEYRAUD [1], C. CARIVENC [1], C. BLANGER [2], S. BALLEREAU [2], C. ROY-CAMILLE [2], M.C. NGUYEN [1], Y. GÉNISSON [2], C. GUILHOT [1], C. CHALUT [1], J.D. PEDELACQ [1], L. MOUREY [1]

[1]Institut de Pharmacologie et de Biologie Structurale, IPBS, Université de Toulouse, CNRS, UPS, 31077 Toulouse, France - Toulouse (France), [2]Laboratoire de Synthese et Physico-Chimie de Molecules d'Interet Biologique, ISPCMIB, Université de Toulouse, CNRS, UPS, 31062 Toulouse, France - Toulouse (France)

Mycobacteria remain among the deadliest human infectious agents, responsible for more than 1.4 million deaths in 2019. Phosphopantetheinyl transferases (PPTases) catalyze the transfer of the 4'-phosphopantetheinyl arm of coenzyme A to the acyl carrier protein (ACP) domains found in fatty acid synthases, polyketide synthases and non-ribosomal peptide synthases. As they are essential enzymes, PPTases represent attractive targets in the fight against mycobacteria. Besides *Mycobacterium tuberculosis*, the causative agent of tuberculosis, *Mycobacterium abscessus* causes severe respiratory and skin infections. Both mycobacteria express PPTases that share high sequence identity and are likely to be involved in similar biosynthetic pathways.

Inhibition of the PPTase of *M. abscessus* (PptAb) has been addressed in two ways. The Fragment Based Ligand Discovery approach was set up to screen an in-house fragment library of 939 compounds by X-ray crystallography. Crystals of complex were prepared by dry-coating the fragments directly on the crystallization plates. Nearly 800 crystals were exposed to X-rays, resulting in 680 analyzable structures. More than 70 ligands were determined (unpublished results), paving the way toward their optimization by fragment linking, growing or merging.

In parallel, molecules identified in the literature as inhibitors of the PptAse from *M. tuberculosis* (PptT), were also investigated with PptAb, by enzymatic inhibition tests and by structural analysis. In particular, compound 8918, which was identified as a potent inhibitor of the PptT in vitro and of *M. tuberculosis* growth (1), was shown to also inhibit PptAb in vitro. The structures of compound 8918 in the presence of PptAb and coenzyme A, as well as in the presence of PptAb, CoA and the ACP domain, which normally receives the Ppant arm from the PptAse, were determined (2). Surprisingly, although inhibition of PptAb was observed in vitro with compound 8918, it does not translate into an inhibitory activity on the growth of *M. abscessus*. This lack of activity in vivo might be related to the especially low permeability of the *M. abscessus* cell envelope, confirming its classification as antibiotic nightmare (3).

1 Ballinger, E. et al. Opposing reactions in coenzyme A metabolism sensitize *Mycobacterium tuberculosis* to enzyme inhibition. Science 363, 6426 (2019).

2 Carivenc, C., Maveyraud, L. et al. Phosphopantetheinyl transferase binding and inhibition by amidino-urea and hydroxypyrimidinethione compounds. Sci. Rep. 11, 18042 (2021).

3 Nessar, R, Cambau, E., Reyrat, J.M., Murray, A. & Gicquel, B. *Mycobacterium abscessus*: a new antibiotic nightmare. J. Antimicrob. Chemother. 67, 810–818 (2012).