TITLE: BINDING OF L,D-TRANSPEPTIDASE 3 FROM *MYCOBACTERIUM TUBERCULOSIS* (Ldt_{Mt3}) TO β-LACTAMS REVEALS INSIGHTS INTO THE DEVELOPMENT OF NEW β-LACTAMS.

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ABSTRACT

β-lactams are an ancient and widely used family of antibiotics for the control of bacterial diseases, but generally they are not used for the treatment of tuberculosis because an intrinsic mechanism of resistance. However recently it has been reported that carbapenems and faropenem inhibit the L,d transpeptidases (Ldts), a group of highly conserved enzymes that catalyse the non-classical 3→3 linkages in mycobacterial peptidoglycan. In addition, these β-lactams demonstrated to have an antimicrobial activity against *M. tuberculosis in vitro* and *in vivo* assays. This work aimed to understand the mechanisms of binding of β-lactams to Ldt_{Mt3} and provide insights for the synthesis of novel β-lactams targeting Ldts. Ten β-lactams classified as Penams (ampicillin and carbenicillin), Cephems (cephalexin and ceftazidime), Penems (faropenem) and Carbapenems (Imipenem, Meropenem, Ertapenem, Biapenem and Doripenem) were screened against recombinant Ldt_{Mt3} by differential scanning fluorimetry (DSF) and they have further confirmed by isothermal titration calorimetry (ITC). To obtain the atomic structures of Ldt_{Mt3} in complex with β-lactams, we used protein crystallography and solved the structures in complex with imipenem and faropenem performing co-crystallization or soacking approaches. Most of screened β-lactams induced a significant variation of ΔTm by DSF that indicate the binding to Ldt_{Mt3}, except ampicillin, cephalexin and ceftazidime. ITC showed for the tested β-lactams affinity ranges between 17 and 2500 nM, Imipenem showed to have higher affinity and Meropenem lower affinity. Additionally, the analysis of ITC also reveals that the enthalpy change (Hydrogen bonds) is the driving force for β-lactams binding. Furthermore, for imipenem and meropenem, there also are favourable contributions from entropy (hydrophobic interactions). In contrast, ertapenem, doripenem, biapenem and faropenem have an unfavourable entropic component. However, although faropenem had the highest entropic penalty ($-T\Delta S$=3.4 kcal/mole), which contributed with the reduction of its binding free energy (ΔG=-8.4 kcal/mole), faropenem had the highest binding enthalpy (ΔH =-11.8 kcal/mole). In conclusion, we propose that Penems might have the best scaffold for the calorimetric-based lead optimization of novel β-lactams targeting Ldts. Non-polar modifications in C2 side chain of the five-membered heterocyclic ring of Penems could assist this goal. Crystal structures of Ldt_{Mt3} in complex with faropenem and imipenem are in progress and might reveal details of Ldt_{Mt3}:β-lactams interactions.

Keywords: *M. tuberculosis*, Peptidoglycan, L,d transpeptidases, β-lactams, Drug Discovery.
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