Non-conventional approaches for peptidoglycan cross-linking inhibition

Peptidoglycan (PG) is an attractive and validated target for antibacterial drug development for two main reasons. First, it is an essential and unique bacterial cell wall polymer with no counterpart in human cells, minimizing the risk of drug toxicity. Second, the essential PG synthases are exposed at the outer surface of the cytoplasmic membrane, making them highly accessible for antibiotic inhibition. Formation of the PG network requires glycosyltransferases for glycan chain elongation and transpeptidases for peptide cross-linking. Transpeptidation involves two stem peptides that act as acyl donor and acceptor substrates, respectively. The acyl donor site is targeted by the ß-lactams, which form covalent adducts, and this interaction is well characterized. In contrast, nothing is known on the interaction of the transpeptidases with the acceptor substrate. To combat the erosion of the activity of ß-lactams, we propose to identify additional drugable sites in the transpeptidases, including the acceptor binding site, and develop lead antibacterial agents acting on these sites. Our first objective is to characterize the mode of recognition of the acyl acceptor by transpeptidases and identify compounds blocking the binding of this substrate. We will use NMR spectroscopy to map the acceptor site and develop specific inhibitors based on modeling and virtual screening. Our second objective is to identify the partners of transpeptidases that regulate the coordinated elongation of glycan chains and cross-linking of stem peptides. This will allow us to select additional drugable sites in transpeptidases and associated proteins within the PG polymerization complexes. We will map key interactions by FRET analyses in live bacteria producing fluorescent proteins and by in vitro transpeptidase/glycosyltransferase assays in complexes obtained by tandem-affinity purification. Microfluidic cultures and time-lapse microscopy will assess the impact of inhibitors on cell division and viability. The interaction of lead compounds with their targets will be characterized by X-ray crystallography. These complementary approaches will enable the consortium to develop novel strategies for transpeptidase inhibition and obtain leads active against ß-lactam-resistant bacteria.

Keywords: B-lactam; Peptidoglycan; transpeptidase, Penicillin-bindning protein (PBP); glycosyltransferase

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