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Screening of penicillin-binding protein inhibitors using high throughput method

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Penicillin was the first drug employed for therapeutic use. Penicillins are beta-lactam antibiotics that hamper bacterial cell wall synthesis by irreversible binding to the serine residue of the active site of the enzyme (penicillin-binding protein/PBP) and thus inhibiting the enzyme catalysing the transpeptidation reaction that is essential for the formation of peptidoglycan (polymer made up of alternating units of N-acetylglucosamine and N-acetylmuramic acid) that helps in maintaining integrity in bacterial cells. Penicillin binds to and inhibits the TP (transpeptidase) domain of PBP which undertakes the transpeptidation reaction involving cross-linking of amino acids of the two peptidoglycan chains. If the active site residues of PBPs are mutated then it can lower the binding affinity of penicillin which leads to penicillin resistance in bacteria. The overuse of drugs like β-lactam antibiotics has given rise to multidrug resistance (MDR) in bacteria. Multidrug resistance of bacteria can be acquired by innate mechanisms such as through efflux pumps or by distorting the structure of antibiotic targets by inducing mutation or through the transfer of resistance genes via plasmids, transposons and integrons. High throughput methods allow a heuristic approach of screening large numbers of compounds in microtiter plates containing wells. The enzyme is added to the wells and allowed to interact with the test compound. A large library of compounds is screened to find a prospective match that has confirmed binding activity to the target. It utilises automated liquid handling and detection systems and software to quickly test and analyse the interaction of compounds from a large library with the target protein. Therefore, by using high throughput methods, one can screen compounds that can inhibit PBP of MDR organisms like Escherichia coli, Acinetobacter baumannii, Pseudomonas aeruginosa, Neisseria gonorrhoeae, etc. Fluorescence polarisation (FP) or fluorescence anisotropy is a technique that utilises the change in intensity of polarisation of the emitted light to detect the binding of protein to the target. The binding of a low molecular weight fluorophore to a macromolecule results in higher polarisation/anisotropy of the emitted light. The inhibitor competes with the probe for binding to the target which reduces the fluorescence anisotropy increase. The tracer/probe can be a fluorescent β-lactam analogue, such as BODIPY FL penicillin (BOCILLIN), 5 carboxytetramethylrhodamine-ampicillin (5-TAMRA) for PBP, or a fluorescent boronic acid-based probe. Thus, the inhibitors can be screened using FP assay and the kinetic parameters like rate constant of acylation and deacylation can be determined after binding to the catalytic site of the transpeptidase domain.

Keywords: Antibiotic, Beta-lactam, Efflux, Multidrug-resistant, Fluorescence polarisation, Fluorescence anisotropy, Transpeptidase

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