J Biotechnol Biomater 2015, 5:6
http://dx.doi.org/10.4172/2155-952X.C1.044

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Characterization of *Pseudomonas aeruginosa* nitroreductase for improved prodrug CB1954 activation

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Chemotherapy is one of the conventional treatment methods employed for cancer therapy. The various challenges faced by chemotherapy are side effects of the drugs due to non-selectivity and high toxicity. Hence, it becomes imperative to seek alternative approaches focusing on targeted drug delivery to tumor cells. Attempts to increase the selectivity of drug are currently an intensively studied research aspect. Designing and developing prodrug is a strategy to minimize toxicity based on improvement of drug selectivity. A recent promising approach of targeted drug delivery of the anticancer agent is gene directed enzyme prodrug therapy (GDEPT) which involves prodrug activating enzymes. Nitroreductases are the enzymes employed in GDEPT which reduces aromatic nitro groups to hydroxylamines which are potent cytotoxins. The present research aims to characterize and engineer *Pseudomonas aeruginosa* nitroreductase enzyme for higher catalytic activity towards prodrug CB1954 (dinitroaziridinylbenzamide). *Pseudomonas aeruginosa* PAO1 nitroreductase (Pseudo_NR) consists of 200 amino acids. The present study envisages sequence analysis, taxonomic distribution and comparison of Pseudo_NR with structural homologues (FMN binding sites, dimer interface residues), homology modeling and docking studies with prodrug CB1954. It was observed that the FMN binding site in case of Pseudo_NR is more similar to that of *B. subtilis, T. thermophilus* and *S. pneumoniae* nitroreductase. However the dimer interface sites are observed to be variable in nature. The 3D simulation model of Pseudo_NR indicates that it is homo dimer and closely related to *Bacillus subtilis* nitroreductase ydfN and can be fitted into the X-ray structure with root mean square deviation of 0.61 Å. The binding sites identified by docking studies will be investigated further for improved activation with prodrug CB1954.

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Contribution of efflux pump in drug resistance of fluoroquinolone resistant *Mycobacterium tuberculosis* isolates

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Fluoroquinolones (FQs) are widely used second line anti tuberculosis drug. Extensive use of FQs has increased the incidence of resistance in *Mycobacterium tuberculosis.* Apart from mutation in the drug target site i.e., gyr A and gyr B the resistance may develop due to active efflux pump. In this study, we evaluated the role of the efflux pumps in fluoroquinolone resistance by using efflux inhibitors carbonyl cyanide m-chlorophenyl hydrazone (CCCP) which acts by decreasing the trans-membrane electrochemical gradient and verapamil, a calcium channel blocker in clinical isolates of *M. tuberculosis.* A total of 50 fluoroquinolone resistant *M. tuberculosis* clinical isolates were tested by Resazurin micro titer assay (REMA) to observe the changes in minimum inhibitory concentration (MIC) in presence and absence of efflux inhibitors using standard strain H37Rv. The MIC levels showed 2-8 fold reduction in presence of CCCP (10/50 20%) and verapamil (12/50 24%). Reduction in MIC was observed in (15/50 30%) strains when both the inhibitors were used simultaneously. Our findings suggest that an active efflux pump could be a major contributor in development of resistance against fluoroquinolones. Significant reduction in MIC indicates the involvement of Major Facilitator Super Family (MFS) family and ATP Binding Cassette (ABC) transporters which are inhibited by CCCP and verapamil respectively.

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