Obtention of Dihydrofolate Reductase from *Mycobacterium leprae:* A Target for the Development of New Drugs for Leprosy Treatment

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Leprosy is a chronic, mildly contagious granulomatous disease caused by the bacillus *Mycobacterium leprae,* characterized by ulcers of the skin, bone, and viscera, which causes damages in the peripheral nervous system, leading to loss of sensation, paralysis, gangrene and deformation. In the last year there were detected 200.000 cases of leprosy in Brazil. To overcome the resistance problems observed with dapsone the treatment of Hanseniasis has been done using multidrug therapy with rifampicin, clofazimine and dapsone. The success in the discovery of a new drug will be dependent on the appropriate choice of the target. Our group based this development on the search of drugs to inhibit the enzyme dihydropholate reductase (DHFR). This enzyme is vital to *M. leprae* and is a validated target for several microorganisms. Due the infectivity and time-consuming of the cultures of *M. leprae* we could not obtain the mlDHFR gene directly from the bacillus. The corresponding gene of *ml*DHFR was synthesized by Genescript and cloned in an intermediate vector. We used the restriction enzymes NdeI and BamHI to subclone the *ml*DHFR gene in the PET28a vector. The plasmid PET28a-mlDHFR was transformed in *Escherichia coli* BL21, BL21 (DE3) and BL21 (DE3) pLysS cells and the expression of *ml*DHFR was observed in the SDS page in 18 kDa. The purification has been done using affinity chromatography with Ni-NTA resin. With the purified protein we will begin the enzymatic activity and inhibition tests of the previously synthesized potential inhibitors designed by molecular modeling.

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