Characterization of HydroxymethylNitrofurazone Compounds as Inhibitors of Leishmania mexicana Cysteine Proteases

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Introduction: Leishmaniasis is a disease caused by a protozoan parasite Leishmania, transmitted by the bite of certain species of sand fly. In humans the symptoms are skin sores, fever, damage to the spleen and liver, and anemia. Cysteine-proteases type B, present in the mammalian amastigote form, are an important virulence factor and are also related to essential functions for trypanosomatids parasitic protozoa survival. Nitrofurazone (NF), a 5-nitro-2-furfurylidenesemi carbazone, has been known for a long time to exhibit antimicrobial and trypanomicidal activity. The mechanism of action of NF was found to be based on the inhibition of trypanothione reductase, an enzyme involved in the parasite’s antioxidant system, by means of reduction of the nitro group. Recently, a novel NF derivative, hydroxymethylnitrofurazone (NFOH), was shown to present better trypanomicide activity and lower mutagenicity potential than NF. The main aim of this study is to characterize the effects of NFOH compounds in the inhibition of rCPB2.8 (N60, D61, D64 and H84), and isoenzymes rCPB3.0 (D60, N61, S64 and H84) and rH84Y (D60, N61, S64 and Y84) of L. mexicana. Material and Methods: The enzymes were activated in 100mM sodium acetate buffer, 20% glycerol, 0.04% triton X-100, 2.5mM DTT, pH 5.5 at 35°C for 5min. The inhibition assay was carried out in different concentrations of NFOH compounds. The enzymes activity were followed in the λEx=360nm and λEm=480nm wavelengths in a spectrofluorometer Hitachi-F2500 using Z-FR-MCA as substrate. Results and Discussion: The rCPB2.8, rCPB3.0 and rH84Y were strongly inhibited by the compounds MI-10 (IC⁵₀=0.39±0.06µM), MI-9 (IC⁵₀=0.052±0.005µM) and MI-7 (IC⁵₀=0.54±0.02µM), respectively. The compound MI-4 was the less effective one (20±2µM), but only to rCPB2.8. Conclusion: In general rCPB3.0 and rH84Y were more susceptible by the NFOH compounds then rCPB2.8. Therefore, the amino acid modifications in the positions N60D, D61N, D64S and H84Y are relevant to inhibitory process.

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