Biochemical characterization and inhibitor screening of UMP-CMP kinase from malaria parasites

Supannee Taweechai[1], Clara Herrera-Arozamena[2], Richard Foster[2], and Glenn McConkey[1*]
[1]School of Biology, Faculty of Biological Sciences, University of Leeds, LS2 9JT
[2]School of Chemistry, University of Leeds

In this research, we are investigating UMP-CMP kinase, a pivotal enzyme in the pyrimidine metabolism for DNA and RNA synthesis that was identified by in silico modelling as an antimalarial target and found essential based on negative evidence from genome-wide *Plasmodium falciparum* saturation mutagenesis [1,2]. To dissect the functions of UMP-CMP kinase from *P. falciparum* (PfUMP-CMP kinase), the recombinant protein was produced and its kinetic properties investigated. By using ATP as phosphate donor, the enzyme activity assay demonstrated that the ribonucleoside monophosphates CMP and UMP are the preferred substrates of PfUMP-CMP kinase compared to deoxyribonucleoside monophosphates (ie. dCMP). CMP showed the lowest K_m of 28 μM whereas UMP and dCMP had 3.9-fold and 17-fold higher K_m values, respectively. In addition, the role of cysteines in PfUMP-CMP kinase was investigated through iodoacetamide (IA) alkylation. The kinase was found to be sensitive to alkylation with significantly decreased enzyme activity that was dependent on IA concentration. This implies that the cysteines are necessary for PfUMP-CMP kinase activity and accessible, that could lead to development of an irreversible Cys-interacting inhibitor. Inhibitors targeting the cysteines were modelled and selected in collaboration with medicinal chemists, and tested for inhibitory activity. Compound 7 exhibited the lowest inhibition constant (K_i value) for PfUMP-CMP kinase; at the low micromolar range, making it a promising hit compound for further development of candidate compounds.

1. Totanes, F.I.G., Doctoral dissertation, 2017, University of Leeds
2. Zhang, M., et al., Science, 2018, 360(6388)