Hijacking tRNA charging process: a novel approach to combat malaria

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Malaria poses an enormous threat to human health. With ever-increasing resistance to currently deployed drugs, breakthrough compounds with novel mechanisms of action are urgently needed. Our recent work showed that a sub-set of *Plasmodium falciparum* aminoacyl-tRNA synthetases (aaRSs) are susceptible targets for a novel mechanism of inhibition, called *reaction-hijacking.* The aaRSs catalyse the conjugation of pro-inhibitor nucleoside sulfamates with amino acid in their active site; thereby blocking enzyme activity [1].

Here we explore nature's arsenal of nucleoside sulfamates. We demonstrate that dealanylascamycin (PM03) - a compound first isolated from *Streptomyces spp.* [2], and its synthetic analogue, 5'adenosine sulfamate (AMS), act as broad specificity reaction hijacking pro-inhibitors of malaria parasites. Pulsed exposure to PM03 or AMS leads to inhibition of protein translation. Similarly, pro-inhibitor treatment induces eIF2α phosphorylation, consistent with build-up of uncharged tRNA. Targeted mass spectrometry of *P. falciparum* cultures treated with PM03 and AMS reveals different amino acid-sulfamate conjugates, suggesting that these nucleoside sulfamates can targets several *Pf* aaRSs, including *Pf*TyrRS, *Pf*AsnRS, *Pf*AspRS, *Pf*GlyRS*, Pf*SerRS, *Pf*AlaRS, *Pf*ThrRS and *Pf*LysRS.

We generated recombinant *P. falciparum* cytoplasmic asparagine RS (*Pf*AsnRS) and aspartate RS (*Pf*AspRS), as well as human AsnRS consensus domain (*Hs*AsnRS$_{(CD)}$), and verified that the proteins are correctly folded. Differential scanning fluorimetry was used to detect the tightly bound adducts. ATP consumption was used to monitor inhibition of the first step of the enzyme reaction. Mass spectrometry confirmed the formation of inhibitory nucleoside sulfamate-amino acid adducts. *Hs*AsnRS$_{(CD)}$ is less susceptible to AMS inhibition than *Pf*AsnRS.

We solved the X-ray crystal structures of *Hs*AsnRS$_{(CD)}$, for the first time, in complex with the activated intermediate, Asn-AMP (1.9-Å resolution) and AMS-Asn (2.1-Å resolution). Each subunit comprises an N-terminal β-barrel anticodon-binding domain followed connected via hinge region to a larger C-terminal catalytic domain that adopts a α–β fold with an insertion domain between motifs 2 and 3. Interactions with the adenylate and asparagine moieties stabilizes the ATP in the characteristic bent conformation, with the plane of the ribose moiety angled ~90° relative to the adenine ring system. We are currently optimising crystallization conditions to obtain high resolution structures of *Pf*AsnRS and *Pf*AspRS in complex with the natural intermediates and the nucleoside sulfamate adducts in an effort to understand the molecular determinants of potency and specificity of different pro-inhibitors.

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