⇥ **ANTIMALARIALS**

Novel proteasome inhibitor combats malaria

Plasmodium falciparum is responsible for most malaria-associated deaths, and emerging resistance to the front-line artemisinin (ART) drug family has emphasized the urgent need for novel antimalarial agents. Now, writing in *Nature*, Bogyo *et al.* report the identification of a peptidic inhibitor of the *Plasmodium* proteasome and show that it inhibits growth of ART-resistant parasites and clears infection in mouse malaria models.

The *Plasmodium* proteasome represents a key target for anti-malarial drugs owing to its essential role in protein homeostasis, which influences various cellular processes. However, although existing proteasome inhibitors are toxic for *P. falciparum* at all stages of its life cycle, they also cross-react with the mammalian proteasome. Bogyo *et al.* therefore set out to identify differences in the specificities of the human and the *P. falciparum*

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proteasomes and therapeutically exploit this information to develop a novel antimalarial agent.

The authors collaborated with O'Donoghue and Craik at the University of California, San Francisco, to determine the substrate preferences of the activated human and *P. falciparum* proteasomes by monitoring the proteosomal degradation pattern of 228 diverse synthetic tetradecapeptides using liquid chromatography tandem mass spectrometry. By generating a frequency profile indicating which amino acids were most- and least-favoured in the subsites surrounding each cleaved bond, they identified 113 and 157 sites that are uniquely cleaved by the *P. falciparum* and human proteasomes, respectively. Major differences occurred on the amino-terminal side of the cleavage site, with the *P. falciparum* proteasome displaying a strong preference for aromatic residues, specifically Trp, at the P1 and P3 sites.

Using this information, the authors next designed inhibitors based on the canonical tri-leucine scaffold found in common proteasome inhibitors, such as MG132. By systematically replacing Leu residues at the P1 and P3 positions with Trp, they generated the peptide vinyl sulfones LLW-vs, WLL-vs and WLW-vs. Interestingly, WLW-vs specifically inhibited the β2 subunit of the parasite proteasome, while WLL-vs inhibited both the parasite β2 and β5 subunits, leading to more potent parasite-killing activity.

Determination of the structure of the *P. falciparum* proteasome bound to the inhibitors, using high-resolution cryo-electron microscopy and single-particle analysis, revealed an unusually open active site on β2 that could accommodate the two Trp side chains of the inhibitor. This analysis also provided information about the active-site architecture and accessibility at the β2 and β5 sites.

The authors then investigated the antimalarial effects of their compounds using live *P. falciparum* cultures and found that proteasome inhibition corresponded with a decrease in parasite survival. Importantly, WLL-vs was 1,000-times more toxic towards parasite cells than primary human fibroblasts.

Similarly, in a rodent *Plasmodium chabaudi* infection model, a single bolus dose of WLL-vs administered by tail vein injection reduced parasite burden to nearly undetectable levels, without signs of toxicity.

Finally, the newly developed proteasome inhibitors showed potency against field isolates of ART-resistant parasites and were highly synergistic with dihydro-artemisinin, suggesting their potential to enhance ART activity and reduce the emergence of resistance.

These findings demonstrate that the *Plasmodium* proteasome is sufficiently unique from the human proteasome for selective targeting and highlight a potential strategy for ART-sensitization. Optimization of WLL-vs to generate compounds with improved potency and oral bioavailability is currently ongoing.

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ORIGINAL ARTICLE Li, H. *et al.* Structure- and function-based design of *Plasmodium*-selective proteasome inhibitors. *Nature* **530**, 233–236 (2016)