2021

Characterization of MAS1-86 Activity in Malaria Parasites

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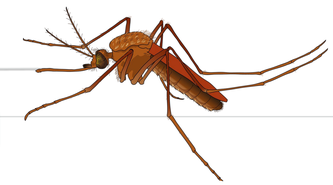
Summer Undergraduate Research Program



Background

- In 2019, ~229 million malaria cases reported globally, causing 409,000 deaths [1].
- Malaria is caused by the *Plasmodium* parasite with cyclical infection in human and *Anopheles* mosquito host. *P. falciparum* is the most prevalent species [1] .
- Blood stage parasites cause malaria symptoms. The lifecycle begins with merozoites that invade red blood cells. They develop into ring stages (0-23 hours post invasion, hpi), trophozoite stages (24-39 hpi), and maturing into schizont stages (40-48 hpi) [2].
- Artemisinin-based combination therapy (ACT) is the first-line treatment for uncomplicated *falciparum* malaria [3].
- Resistance to all artemisinin (ART) is a widespread problem [3].

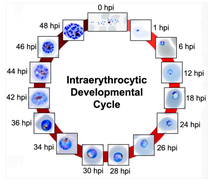


Figure 1. *P. falciparum* asexual lifecycle. Credit to [2] for figure

- Point mutations in Kelch 13 confer ART resistance. The C580Y mutation is the most abundant in SE Asia. The R539T mutation displays high levels of resistance *in vitro.*
- *P. falciparum's* apicoplast, an essential organelle that generates fatty acids, heme, and isoprenoid precursors, is a promising drug target since humans lack this organelle [4].
- The apicoplast's primary function in asexual life stages is to produce isoprenoid precursor isopentenyl pyrophosphate (IPP) via the methylerythritol phosphate (MEP) pathway. IPP supplementation has shown to chemically rescue MEP inhibited cultures [5,6].

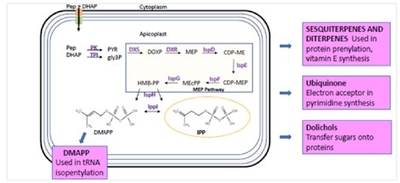


Figure 2. Synthesis of MEP pathway and isoprenoid products in *P. falciparum*. Adapted from [5]

- Delayed death phenotype is when growth of treated parasite is unaffected, but growth arrest is observed in the progeny. This is seen when apicoplast biosynthesis and metabolic pathways are inhibited [6].
- The apicoplast-located *Pf*ClpC/P complex degrades proteins and has chymotrypsin-like proteolytic activity [4]. *Pf*ClpC is a chaperone to the *Pf*ClpP protease [4].
- Aberrant schizont morphology with fewer nuclei in auto-inhibited inhibited *Pf*ClpC has been reported [7].
- *P. falciparum* 26S proteasome is a cytoplasmic protease. The β1, β2, and β5 subunits have caspase-like, trypsin-like and chymotrypsin-like activity, respectively [4]. WLL inhibits the β2 and β5 subunits [4].
- The unfolded protein response (UPR) upregulates proteasome activity [4].
- PfClpC has 27% identity to the *Staphylococcus aureus* homolog ClpX. MAS1-86 inhibited multi-drug resistant *S. aureus* [8].
- Analogs of MAS1-86 were then tested against *P. falciparum*. MAS1-86 was identified as most potent inhibitor.

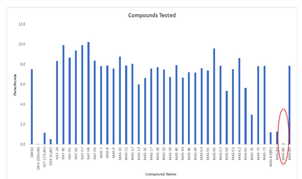


Figure 3. MAS1-86 and analogs tested against *P. falciparum.* MAS1-86 circled in red.

Results

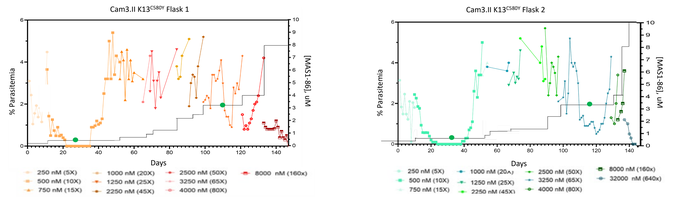


Figure 4. MAS1-86 stepwise resistance selections. Two replicates, Flask 1 and Flask 2, of Cam3.II K13^{C580Y} parasites were exposed to MAS1-86 in increasing concentrations, as indicated on the right axis. Parasitemia over time is tracked, as indicated on the left axis. Green dots correspond to profiled parasites in Figure 2.

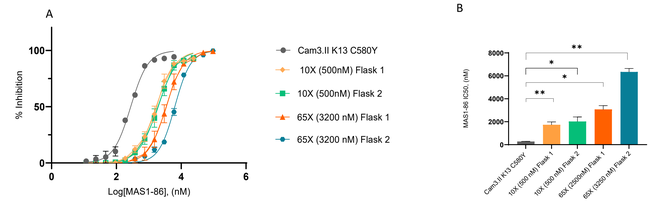


Figure 5. MAS1-86 selected parasites display 6X to 23X increase in resistance to MAS1-86. (A) MAS1-86 growth inhibition assays tested against various asynchronous parasites as indicated, and the (B) corresponding IC_{50} +/- SEM values. Parasitemia was assessed 72 hours after compound addition. Parasite DNA was stained with SYBR Green I and respirating mitochondria stained by MitoTracker Deep Red, then assessed by flow cytometry. Statistical significance ssessed using Student's t-test with Welch's correction. *p < 0.05; **p <0.01. n = 3.

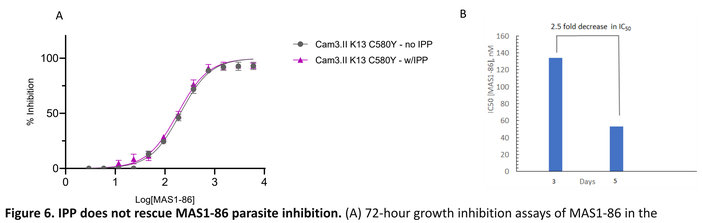


Figure 6. IPP does not rescue MAS1-86 parasite inhibition. (A) 72-hour growth inhibition assays of MAS1-86 in the absence or presence of IPP tested against Cam3.II K13 ^{C580Y}. (B) MAS1-86 IC_{50} values assayed at 72 hours and 120 hours. A 10-fold reduction in IC_{50} values at 120 hours compared to72 hours indicates a delayed death phenotype.

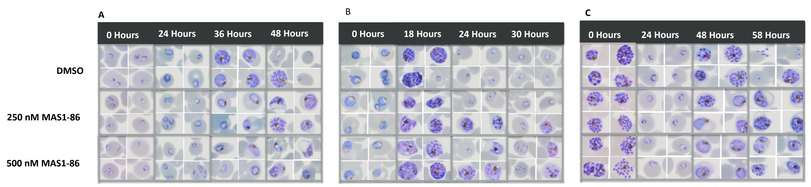


Figure 7. Morphology of parasites exposed to MAS1-86 across the asexual blood stages. (A) Ring stage, (B) trophozoite stage, and (C) schizont stage Cam3.II K13 ^{C580Y} parasites were treated with DMSO, 250 nM, or 500 nM MAS1-86 for the hours indicated above. Thin blood smears were made, stained with Giemsa, and imaged by light microscopy using a 100X objective.

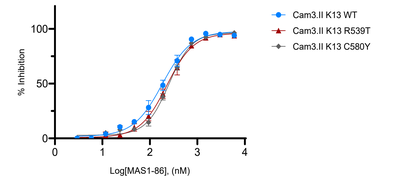


Figure 8. K13 haplotype does not influence parasite susceptibility to MAS1-86. Asynchronous K13 mutants were exposed to MAS1-86 for 72 hours, and the dose responses are plotted. n=3.

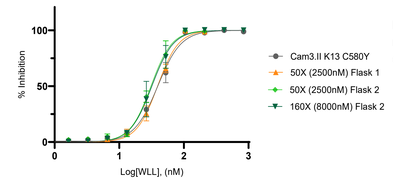


Figure 9. MAS1-86-resistant parasites do not show cross-resistance to WLL. Dose response curves of indicated parasites exposed to WLL for 72 hours. n=3

Conclusions

- MAS1-86 selected parasites display 6-23 - fold increased resistance to MAS1-86.
- MAS1-86 does not target the MEP pathway, since IPP fails to rescue MAS1-86 inhibition.
- MAS1-86 inhibition caused a delayed progression in late trophozoite through schizont stages, with fewer nuclei observed in schizonts.
- MAS1-86 kills artemisinin-sensitive and artemisinin-resistant parasites.
- MAS1-86 resistant parasites do not show cross resistance to proteasome β2 and β5 subunit inhibitor, WLL, which has the same chymotrypsin-like activity as ClpP.

Future Directions

- Investigate MAS1-86 impact on the apicoplast's metabolic pathways and apicoplast biogenesis in greater detail.
- Test cross – resistance in MAS1-86 selected synchronized ring stage cultures to ART since ring stages exhibit ART resistance.
- Test cross-resistance in WLL-resistant mutants to MAS1-86 to investigate MAS1-86 inhibition of the proteasome.
- Investigate UPR after treatment of MAS1-86.

Acknowledgements

We would like to thank Dr. Conda-Sheridan and Mohamed for synthesizing the compounds used in these experiments. Thanks to Dr. Reid for allowing us access to the flow cytometer. Lastly, thanks to SURP for this research opportunity.

References

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