Drug 2741-19 is a Novel Therapeutic Candidate for Treating Severe Malaria

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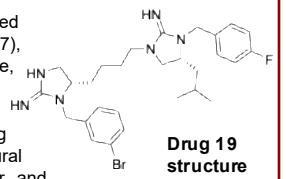
Background

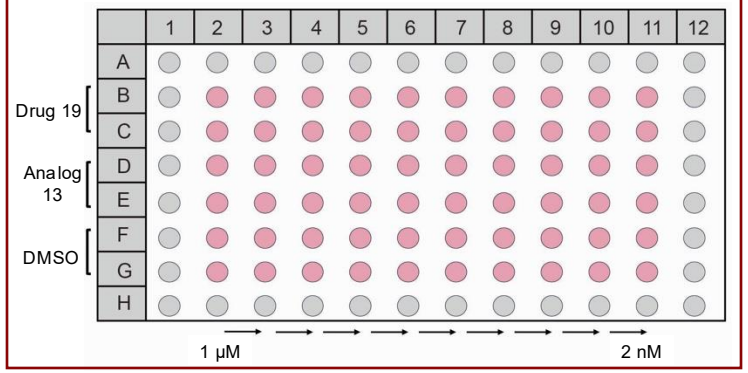
Plasmodium falciparum malaria is one of the leading causes of death among children worldwide, killing more than 500,000 sub-Saharan African children annually. Increasing resistance to artemisinin, the standard of care for severe malaria (SM), underscores the need to identify novel anti-malarial therapeutics. Recent work has identified that the *P. falciparum* Glutamic Acid Rich Protein (PfGARP) antigen is uniquely targeted by the immune systems of children resistant to severe malaria, suggesting that Anti-PfGARP antibodies may confer protection against SM. Anti-PfGARP human antibodies kill up to 99% of *P. falciparum* parasites in culture. Through an antibody-inhibition binding screening, we identified drug 2741-19 as a small molecule with significant competitive inhibition of PfGARP-anti-PfGARP binding. This suggests that our drug exhibits high affinity binding to PfGARP and could mimic the activity of Anti-PfGARP Abs. This study aimed to characterize the anti-malaria effects of drug 2741-19 against *in vitro* parasites. Our project has the potential to identify novel drug candidates for treating *P. falciparum* SM by inhibiting parasitemia, thereby addressing the rising issue of drug-resistant malaria.

Methods

To evaluate the efficacy of drug 2741-19, we conducted a growth inhibition assay. *P. falciparum* parasites (3D7), synchronized to the early ring-stage trophozoite phase, were plated in 96-well plates at 0.4% parasite density in 2% hematocrit. Then, serial dilutions of the drug were added into the wells, with concentrations ranging from 2-4 nM to 1-2 µM. Analog 13, an inactive structural analog of drug 2741-19 with a shortened carbon linker, and DMSO were used as negative controls. After 72 hours, flow cytometry and blood slides were used to assess quantitatively and qualitatively, respectively, the drug's effects on parasite growth. Experiments were repeated with a PfGARP-KO strain.



Drug 19 structure



Results

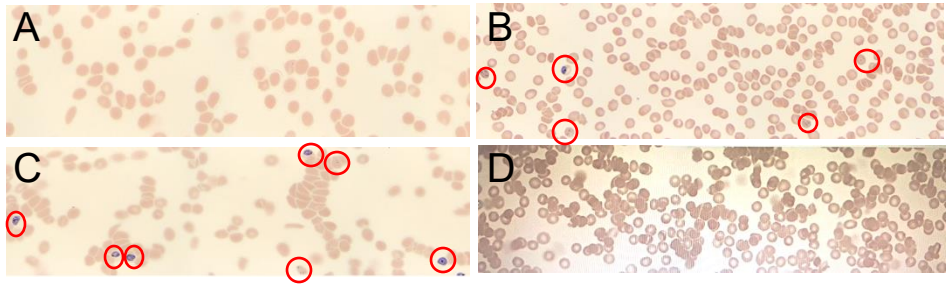


Figure 1 3D7 and PfGARP-KO *P. falciparum* parasites were synchronized to ring stage and plated at 0.4% parasitemia in 2% hematocrit with drug or control. After 72 hours, blood smears were Giemsa stained and visualized with light microscopy. **A** 3D7 treated with 2 µM drug 2741-19; **B** 3D7 treated with 4 nM drug 2741-19; **C** 3D7 treated with 2 µM analog 13; **D** 3D7 PfGARP-KO treated with 2 µM drug 2741-19

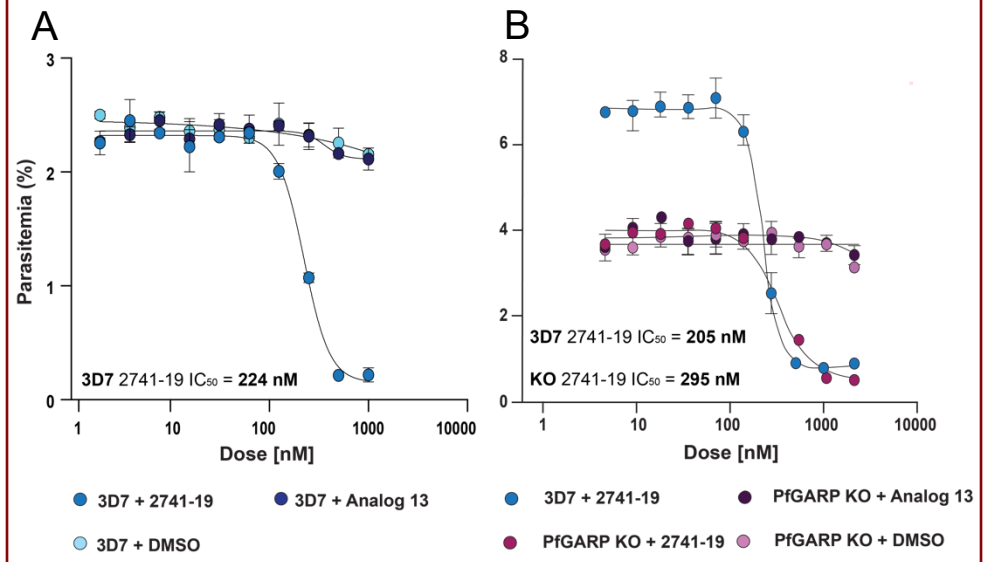


Figure 2 3D7 and PfGARP-KO *P. falciparum* parasites were synchronized to ring stage and plated at 0.4% parasitemia in 2% hematocrit with drug or control. After 72 hours, samples were Hoechst stained and analyzed by flow cytometer. Data points represent three wells. **A** 3D7 *P. falciparum* **B** 3D7 PfGARP-KO

Conclusions

In a growth inhibition assay against *P. falciparum*, the parasite which causes malaria, we found that drug 2741-19 kills parasites with an IC_{50} = 205.6 nM (Figure 1), but comparable killing is also seen in our PfGARP-knock-out model of *P. falciparum* (IC_{50} = 295 nM, Figure 2). Our results suggest drug 2741-19 reduces parasitemia by killing parasites through an elusive mechanism that may be independent of PfGARP or may involve two distinct binding targets. Therefore, this drug represents a promising novel anti-malarial therapeutic for treating or preventing severe malaria, particularly against drug-resistant strains.

Future Aims

- *In vivo* parasite killing assay in murine models
- Pharmacokinetic studies in monkey models
- Drug formulation optimization
- Probing for alternative *P. falciparum* binding targets
- Partner-drug synergy studies
- Resistance screening

References

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Acknowledgments

This work was facilitated by the Brown Emerging Infectious Disease and HIV Scholars (HEIDS) Program (2R25AI140490) supported by the National Institutes of Health (NIH/NIAID).