Title: Characterisation of the phenotype, target and structure activity relationship of a transmission-blocking antimalarial

Authors: Sabrina Yahiya

Malaria continues to devastate millions of people globally, with 219 million cases and 435,000 deaths reported in 2017. Given the recent plateau in the reduction of malaria burden, research efforts must shift away from conventional methods of disease control and towards innovation. (World Health Organisation, 2018)

The causative agent of malaria is the *Plasmodium* parasite, which follows a complex life cycle between a female Anopheles mosquito and human host. Whilst existing antimalarial drugs target the symptomatic stages of this life cycle, it is also crucial to kill the dormant forms of *Plasmodium*. These dormant parasite stages are responsible for malaria transmission across the uninfected human population and are therefore a vital target for next generation drugs.

One approach to discover next-generation leads for innovation in antimalarial drug discovery is the high throughput screening of compound libraries against the asymptomatic stages of *Plasmodium*. (Yahiya *et al*., 2019) A successful example of this approach is the high throughput screen of the ~68,689 compound Global Chemical Health Diversity Library (GHCDL). GHCDL compounds were screened against *Plasmodium falciparum*, the most virulent of *Plasmodium* species. (Delves *et al*., 2018) The compound library was screened for activity against the symptomatic asexual blood stages and sexual stage gametocytes, with the latter being responsible for disease transmission. The screen yielded many potent hits with diverse activity profiles, including hits with promising activity against the transmissible stages of *Plasmodium*.

The screen discovered a potent hit, DDD01035881, which demonstrated specific activity against Plasmodium male gamete formation. Male gamete formation is a remarkably rapid process which occurs in the mosquito upon transmission from a human host. Although male gamete formation is vital to malaria transmission, the process remains incompletely characterised. To develop an understanding of the cellular processes occurring male gamete formation, we used microscopy to uncover the details of the process without drug treatment. To resolve the mode of action of DDD01035881, we compared the process to drug treated parasites to characterise the phenotype and activity window of the hit compound. DDD01035881 demonstrated potent and rapid inhibition spanning a 6-minute time window, during which the morphological phenotype interestingly varied according to the time at which parasites were treated.

To enhance the clinical characteristics of DDD01035881, we aimed to improve the potency of the compound in a structure activity relationship (SAR) study. We adapted a miniaturised plate-based assay from previous studies to facilitate the SAR study. Structural analogues, synthesised by medicinal chemist collaborators, had improved potency in the assay without impacting cytotoxicity of the original hit. (Rueda-zubiaurre *et al*., 2019)

To determine the cellular target of DDD01035881, an analogue of the compound amenable to photo affinity labelling was synthesised using knowledge from the SAR study. Photo affinity labelling revealed a target of interest which was consistent with phenotypic findings. The protein target was validated with a cellular thermal shift assay (CETSA), confirming the target as a true hit. This study corroborates the importance of high throughput screening for yielding next-generation therapeutics to treat malaria.

This study improved the potency, elucidated the morphological phenotype and discovered the cellular target of DDD01035881, a hit from the GHCDL screen. DDD01035881 shows promise as a next generation lead for transmission blocking of malaria across the uninfected human population, a vital requirement of antimalarial drug discovery.