Next-generation antimalarials: New hope on the horizon

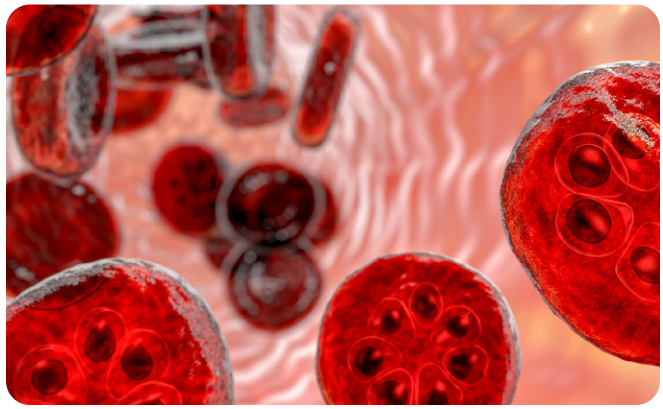
An insight into recent antimalaria drug discovery

The mosquito is often referred to as one of the world's most deadly animals due to its role in spreading diseases such as malaria, yellow fever, dengue fever, and encephalitis. The most devastating of these is malaria, which killed over 600,000 people in 2020, with the vast majority being children under the age of five.[1]

Latest analysis from the World Health Organization (WHO) shows that deaths caused by malaria were at their highest for nearly a decade, totaling 627,000, and cases reached a peak of 241 million, an increase of 14 million compared with 2019.[1] Although these increases were due in part to delays in diagnosis and treatment during the COVID-19 pandemic, progress had already started to plateau in recent years, and insecticide and antimalarial drug resistance was on the rise. This suggests that action is urgently needed to effectively combat drug-resistant malaria and meet the WHO's global health objective to control, eliminate, and ultimately eradicate the disease.[2]

There are five parasite species that cause malaria in humans: *Plasmodium falciparum, Plasmodium malariae, Plasmodium vivax, Plasmodium ovale,* and *Plasmodium knowlesi*. Of these, *P. falciparum* is the strain that causes the most severe life-threatening malaria cases. Infections with *P.* vivax and *P.* ovale generally cause less serious illness, but the parasites can remain dormant in the liver causing a reappearance of symptoms months or even years later.

In this literature review, we explore the emerging challenge of antimalarial drug resistance and the need to analyze all stages of the malaria parasite lifecycle to help develop the next generation of therapeutics. We highlight three studies where researchers have identified potent antimalarial compounds that target multiple stages of the malaria parasite lifecycle.



Current antimalarial strategies

Various strategies have been adopted to prevent malaria transmission and diagnose and treat infection. Preventative approaches include indoor residual spraying and insecticide-treated mosquito nets. Although these interventions have contributed to a dramatic decline in disease incidence and deaths, resistance to insecticides and changes in mosquito biting behavior is slowing down the progress made by these preventative interventions.[3]

For those infected, antimalarial treatments are often administered to cure the disease before it becomes too serious. Treatments recommended on the WHO's Model List of Essential Medicines comprise 14 medicines for curative treatment and six for chemoprevention.[4] These include quinine, chloroquine, mephloquine, sulfadoxine-pyrimethamine, and amodiaquine, all of which have

seen widespread use over the last century. However, the development of resistance to these drugs is now prevalent, posing a significant threat to efficacious malaria control and treatment. In an attempt to prevent resistance, artemisinin-based drugs (artesunate, artemether, and dihydroartemisinin) are often used in combination with drugs from a different class, forming artemisinin-based combination therapies (ACTs). ACTs are currently the quickest acting and most effective antimalarials available, and the first-line approach recommended for treating malaria caused by *P. falciparum*.[5] Unfortunately, they are expensive and time-intensive to produce, which has restricted their availability and use in some parts of the developing world.[6]

The most recent advance in the fight against malaria is the WHO's recommended use of the first ever malaria vaccine, RTS,S/AS01, in children living in moderate-to-high malaria transmission areas.[7] This endorsement was based on results from an ongoing pilot program in Ghana, Kenya, and Malawi where more than 800,000 children have been immunized since 2019. Results from a Phase III trial also showed that immunization with RTS,S/AS01 in combination with seasonal malaria chemoprevention could dramatically drive down the incidence of malaria in areas with highly seasonal transmission.[8]

Despite these measures, the development of resistance to antimalarial drugs, including ACTs, highlights the need for continued drug discovery research if we are wanting to stay one step ahead of the parasite and ultimately eradicate malaria. Researchers are starting to shift their focus to the discovery of antimalarial candidates that either target a novel process or pathway to avoid pre-existing resistance to current drugs, or compounds that target different stages of the parasite lifecycle.

Dual Plasmepsin-targeting antimalarial agents

In a recent study,[9] a collaborative team of researchers from the Walter and Eliza Hall Institute in Australia and MSD in the US successfully identified a novel class of antimalarial compounds that target a previously unexplored parasite pathway. The compounds were shown to disrupt multiple stages of the malaria parasite lifecycle and their novel mechanism of action suggests it will be harder for malaria parasites to acquire drug resistance. Their study is represented graphically in Figure 1.

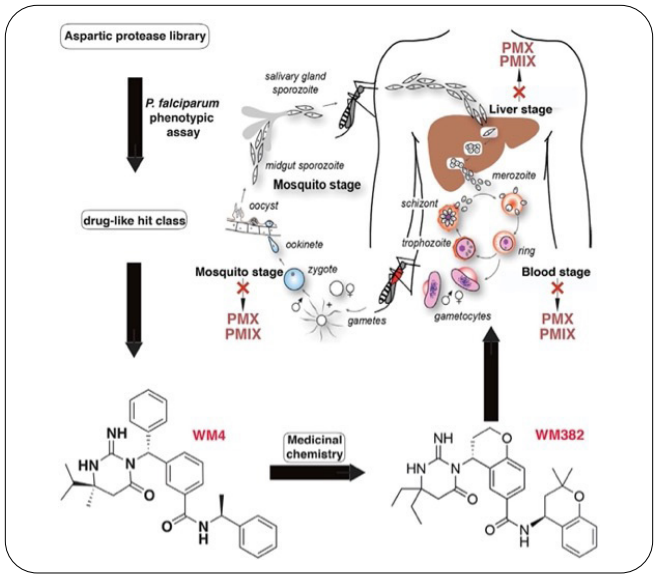


Figure 1: **Researchers from the Walter and Eliza Hall Institute and MSD describe inhibitors of essential aspartic proteases from malaria parasites that block multiple lifecycle stages.** PMIX and PMX are master modulators processing proteins required for invasion, development, and egress. Administration of WM382 cured mice of malaria infection, showing that these inhibitors are promising candidates for malaria treatment and prevention. Figure credit: Favuzza P, de Lera Ruiz M, Thompson J, Triglia T, Ngo A, Steel R et al.[9]

The team first screened an aspartic protease inhibitor library to identify hit compounds targeting *P. falciparum* using a lactate dehydrogenase phenotypic assay on the EnVision multimode plate reader. The two most potent screening hits, WM5 and WM4, were then administered to mice to determine their *in vivo* activity against *P. berghei*, a parasite that causes malaria in rodents. Although both compounds suppressed *P. berghei* parasitemia, they did not have the desirable pharmacokinetic attributes. Subsequent optimization studies resulted in the identification of WM382, a compound that acts as a dual inhibitor of plasmepsins PMIX and PMX and inhibits growth of *P. falciparum* and *P. knowlesi*. Of note, PMIX and PMX are attractive targets in antimalarial drug discovery due to their role in modulating parasite processing proteins required for invasion, development, and egress.

To determine the effect of WM382 against liver-stage parasites, liver egress, and transition to blood infection, mice were infected with *P. berghei* sporozoites constitutively expressing mCherry and luciferase reporter. The infected mice were either left untreated or orally treated with WM382 and monitored daily for the presence of parasites. Luciferase activity was measured using an IVIS® Lumina S5

imaging system. While *in vivo* analysis and additional *in vitro* experiments on the Opera Phenix® High-Content Screening system indicated that WM382 did not kill liver parasites, the team reports that oral administration of WM382 successfully cured mice of *P. berghei* and prevented blood infection from the liver, likely by rendering merozoites non-infectious. In addition, WM382 was shown to be efficacious against *P. falciparum* asexual infection in humanized mice and prevented transmission to mosquitoes.

The researchers believe this novel class of compounds has the potential to not only cure people with malaria, but also prevent transfer of the parasite to the mosquito and, consequently, halt further transmission of the disease. Notably, there was no cross-resistance against these compounds in *P. falciparum* parasite lines resistant to current antimalarials *in vitro*, suggesting that it will be harder for malaria parasites to acquire resistance to WM382.

Multistage and transmission-blocking targeted antimalarials

In another study,[10] researchers performed a parallel *de novo* screen of compounds against multiple stages of the *Plasmodium* lifecycle. In recent years, high-throughput screens have identified a number of novel compounds that have since been developed into highly promising antimalarial candidates. However, the majority of these screens primarily assessed activity against the asexual lifestyle stage, with hits only being profiled for activity against additional lifecycle stages following the primary screen. While this approach identifies compounds that target two or more lifecycle stages, it can miss compounds that have selective activity against specific lifecycle stages, for example gametocytes.

The researchers screened the open-source Medicines for Malaria Venture (MMV) Pandemic Response Box (PRB), which comprises a collection of 400 drug-like compounds, and targeted *Plasmodium* asexual and liver-stage parasites, stage IV/V gametocytes, gametes, and oocysts, as well as mosquito endectocide activity (Figure 2). The advantage of this approach is that hit selection and progression of compounds was not biased towards activity on any single lifecycle stage.

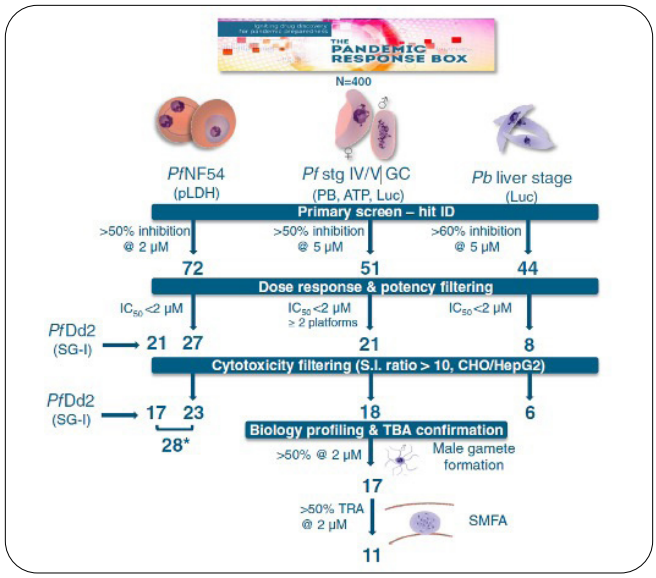


Figure 2: **Screening cascade of the MMV Pandemic Response Box (PRB) for activity against multiple lifecycle stages of Plasmodium.** The 400 compounds in the PRB were screened in a primary assay against drug-sensitive (NF54) *P. falciparum* asexual blood stages (ABS, at 2 and 20 µM) and mature gametocytes (stage IV/V, GC, 1 and 5 µM) and *P. berghei* liver stages (5 µM). Hits were selected based on ≥50% inhibition at specific concentrations as indicated. The criteria for each decision point are indicated followed by the number of compounds that passed the criteria. Compounds were additionally evaluated in dose response on drug-resistant asexual Dd2 parasites (chloroquine, pyrimethamine and mefloquine resistant). * = the potent ABS compounds (IC50 < 2 µM), after removing toxic compounds and eliminating overlapping compounds between PfNF54 and PfDd2, amount to 28 compounds in total. IC50 50% inhibitory concentration, pLDH parasite lactate dehydrogenase assay, PB PrestoBlue® assay, ATP ATP viability assay, Luc luciferase reporter lines assays, Pf Plasmodium falciparum, Pb Plasmodium berghei, S.I. selectivity index, CHO Chinese hamster ovarian cells, HepG2 hepatocellular carcinoma line, TBA transmission-blocking activity, TRA transmission-reducing activity, SMFA standard membrane feeding assay. Parasite drawings were modified from freely available images (https://smart.servier.com/), under a Creative Commons Attribution 3.0 Unported Licence. Figure credit: Reader J, van der Watt M, Taylor D, Le Manach C, Mittal N, Ottilie S et al.10

For the primary screen, the team used a parasite lactate dehydrogenase (pLDH) assay to assess the asexual blood stage antiplasmodial activity on *P. falciparum* drug-sensitive strain NF54 (PfNF54). Stage IV/V gametocyte cultures were assessed using the PrestoBlue® fluorescence (PfNF54), ATP bioluminescence (PfNF54), and Luciferase reporter (NF54-pfs16-GFP-Luc line) assays. For the *P. berghei* liver stage assay, compounds were tested in HepG2-A16-CD81 cells seeded in 1536-well plates containing test and control compounds diluted into DMSO and incubated for 24 hours. *P. berghei* sporozoites (P. berghei ANKA GFP-Luc-SMcon) were then added to each well and the plates

were centrifuged and incubated. Forty-eight hours post-infection, luciferin reagent was added, and luciferase activity was detected using an EnVision® multimode plate reader. Following the primary screens, compounds were additionally evaluated in dose response on drug-resistant asexual Dd2 parasites (chloroquine, pyrimethamine, and mefloquine resistant) using the SYBR green I assay. *P. falciparum* proliferation was assessed by measuring fluorescence from the bottom of 1536-well black, clear bottom plates using an EnVision multimode plate reader.

Writing in *Nature Communications*, the researchers report identification of unique chemotypes with both multistage activity or stage-specific activity, including structurally diverse gametocyte-targeted compounds with potent transmission-blocking activity, such as the JmjC inhibitor ML324 and the antitubercular clinical candidate SQ109. Moreover, the dual-active asexual and liver stage compounds identified may have the potential for prophylactic and chemoprotective utility. Importantly, several of the active compounds identified in the study had no former documented antimalarial activity. The researchers conclude that their findings provide an extensive dataset of compounds that could be repurposed or redirected for potential antimalarial development.

Potent antiplasmodial activity of MMV030084

A third group of researchers, led by David A. Fidock from Columbia University Irving Medical Center in New York, identified a cGMP-dependent protein kinase (PKG) inhibitor, MMV030084, which they believe shows great promise for antimalarial development.[11] For their study, the team first profiled the activity of the trisubstituted imidazole MMV030084 against various *Plasmodium* lifecycle stages. Specifically, *P. berghei* liver stage susceptibility was assessed using a luciferase-based assay, where luminescence was measured using an EnVision multimode plate reader to assess parasite growth. Gametocyte susceptibility was determined using two assays: the first involved staining mature (stage V) gametocytes and imaging plates with the Operetta® high-content imaging system, and the second assessed gametocyte susceptibility using a luciferase-based assay with transgenic parasites.

Results of these investigations showed that MMV030084 displayed prophylactic, anti-asexual blood stage, and transmission-blocking antiplasmodial activity.

When the researchers conducted competitive chemoproteomic experiments, PKG was identified as the primary antiplasmodial target, which was validated by further gene-editing and conditional knockdown studies.

Because a major obstacle for antimalarial efficacy is the development of resistance, the next stage of the study involved exploring possible resistance mechanisms of MMV030084 by performing several rounds of *in vitro* MMV030084 resistance selections. These studies showed that the parasite could only develop low-level resistance, mediated by tyrosine kinase 3 (TKL3). Writing in Cell Chemical Biology, the authors note that TKL3 is not essential for the parasite, as shown by their knockout studies, but when mutated allows the parasite to achieve egress in the presence of MMV030084. Of note, PKG never mutated during their long-term selections highlighting that PKG inhibitors have a relatively low risk for high-grade resistance development. The team concludes that these findings highlight PKG as a validated antimalarial target, and MMV030084 as a promising antimalarial chemotype active throughout the *P. falciparum* lifecycle. The study is graphically represented in Figure 3.

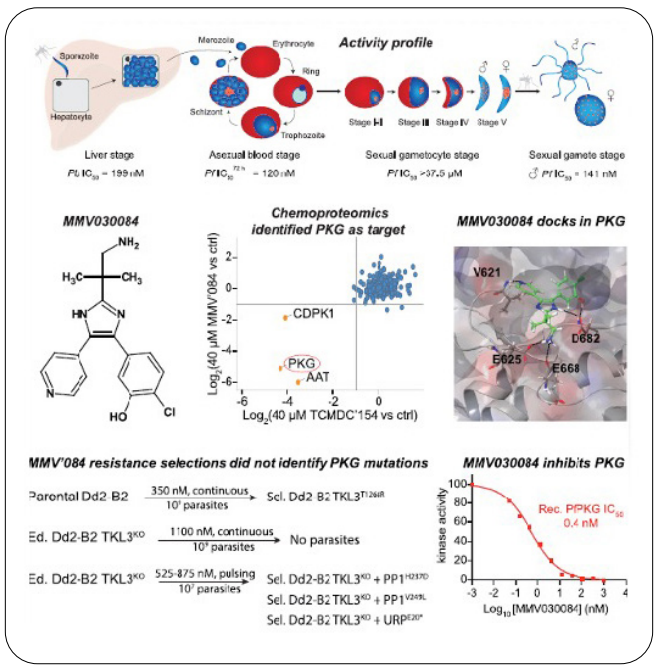


Figure 3: **Researchers report an antimalarial, MMV030084, with potent antiplasmodial activity against all stages of human infection by Plasmodium falciparum.** Metabolomic, phosphoproteomic, chemoproteomic, and gene-editing studies identified cGMP-dependent protein kinase (PKG) as the primary target, which did not mutate under selective drug pressure. Figure credit: Vanaerschot M, Murithi J, Pasaje C, Ghidelli-Disse S, Dwomoh L, Bird M et al.[11]

Conclusion

Malaria continues to be a major public health burden globally and eradication has been a long-standing public health goal. However, the emergence of resistance to current front-line ACTs highlights the need for new antimalarials that can act through novel mechanisms of action. An immediate priority for the WHO is to improve therapeutic efficacy and genotypic surveillance, to better map the extent of the resistance.[1] The focus of the research community is to identify promising antimalarial candidates that block parasite transmission and eliminate the asymptomatic and hepatic lifecycle stages. Only then will the possibility of a malaria-free world become a reality.

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