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Plasmodial Kinase Inhibitors: License to Cure?

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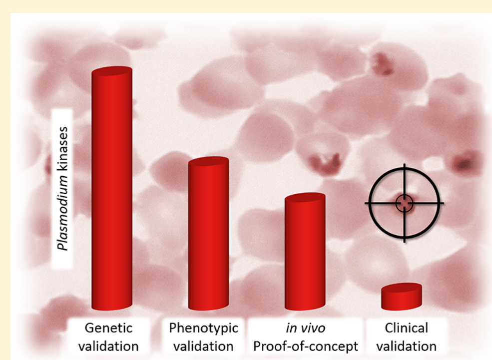
ABSTRACT: Advances in the genetics, function, and stage-specificity of *Plasmodium* kinases has driven robust efforts to identify targets for the design of antimalarial therapies. Reverse genomics following phenotypic screening against *Plasmodia* or related parasites has uncovered vulnerable kinase targets including PI4K, PKG, and GSK-3, an approach bolstered by access to human disease-directed kinase libraries. Alternatively, screening compound libraries against *Plasmodium* kinases has successfully led to inhibitors with antiplasmodial activity. As with other therapeutic areas, optimizing compound ADMET and PK properties in parallel with target inhibitory potency and whole cell activity becomes paramount toward advancing compounds as clinical candidates. These and other considerations will be discussed in the context of progress achieved toward deriving important, novel mode-of-action kinase-inhibiting antimalarial medicines.



1. INTRODUCTION

To this day, the malaria parasite *Plasmodium* spp. remains a scourge particularly in less developed regions of the world. Of the five *Plasmodium* species that cause human malaria, *Plasmodium falciparum* (*Pf*), which is found predominantly in sub-Saharan Africa, was responsible for 50% of all malaria cases and 91% of the 446,000 deaths worldwide in 2016. The next most widespread is *P. vivax* (*Pv*), the dominant species in Latin America and Asia causing a milder form of malaria.[1] Other less problematic *Plasmodium* species are *P. ovale* (*Po*), *P. malariae* (*Pm*), and *P. knowlesi* (*Pk*), the latter much more common in nonhuman primates. Eliminating malaria throughout the world, as has been achieved in many nations, is considered an achievable goal that will incorporate multipronged strategies including the development of new medicines. Currently, the World Health Organization (WHO) recommends that artemisinin-based combination therapy (ACT) and vector control measures are key factors in relieving the burden of malaria.[2] However, recent reports of emerging resistance toward the ACT regimen[3] and other antimalarial drugs with known mechanisms of action[4] emphasize the need to expand the diversity of chemical matter acting against novel targets toward more efficacious drugs with multistage parasite life-cycle activity. In humans, the complex *Plasmodium* life-cycle encompasses a liver-stage infection wherein motile sporozoites differentiate and proliferate asexually to form merozoites and a blood-stage infection wherein the asexual merozoites replicate within red blood cells (through ring, trophozoite, and schizont substages), egress, and then reinfect red blood cells.[5] *P. vivax* and *P. ovale* sporozoites can also enter a hypnozoite stage that can remain quiescent in the liver for months if not longer before differentiating eventually into merozoites.[6] A fraction of the merozoites in red blood cells differentiate and mature to female and male gametocytes that infect the mosquito after transmission from a bite.[7] In the mosquito, the gametocytes further differentiate and eventually fuse to form a zygote that further evolves to form sporozoites that get transmitted to people in a mosquito bite.[8] Notably, the expression of kinases and their importance to viability vary in the stages and substages of the life-cycle.[9]

Kinases are key controllers of signal transduction pathways that regulate essential cellular processes such as growth, development, and reproduction in eukaryotic cells.[10,11] For this reason, human kinases are pursued as drug targets in a variety of diseases including cancers,[12] inflammatory,[13] and cardiovascular diseases.[14] Since the approval of Gleevec 16 years ago,[15] an additional 32 kinase inhibitors targeting the human kinome have been approved by the U.S. Food and Drug Administration (FDA) for clinical use.[15] Given the success in developing drugs targeting human kinases, *Plasmodium* kinases are attractive targets for next generation antimalarials[16] as both protein and lipid kinases are involved in key signaling pathways at various stages of the parasite life-cycle.[17] The *P. falciparum* kinome encodes 86 to 99 protein kinase genes[16] and a small set of lipid kinase genes. It is highly conserved between *Plasmodium* species and is much smaller than the human protein kinome of approximately 520 kinases.[18] Figure 1A shows the phylogenetic tree with a subset of the well-characterized protein kinases of *P. falciparum*.

A major challenge when targeting kinases is that inhibitors usually target the highly conserved adenosine triphosphate

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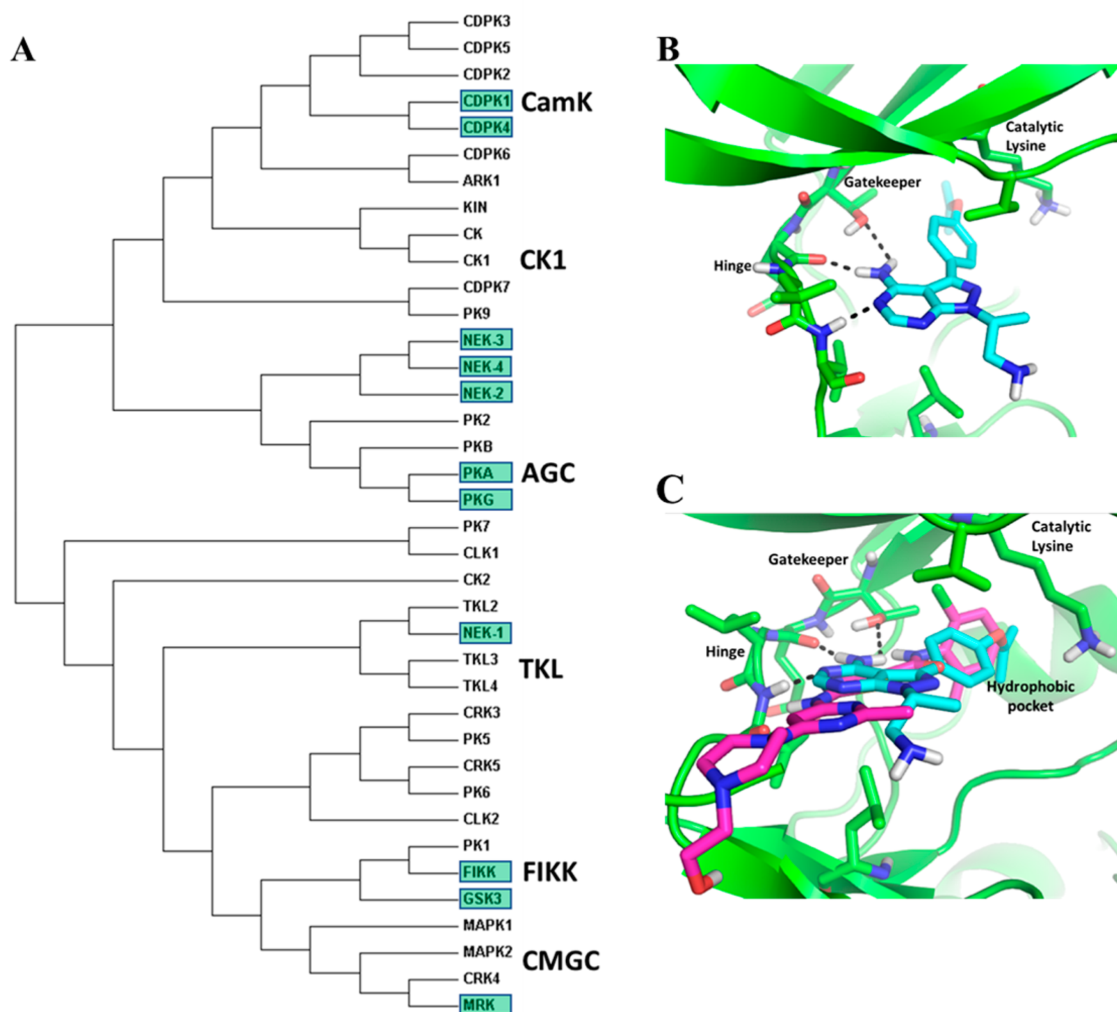

Figure 1. (A) Phylogenetic tree for a set of 38 well-characterized protein kinases in *P. falciparum*. The bootstrap tree was generated from 500 replicates using the neighbor-joining method on the full protein sequence with evolutionary distances computed using the Poisson correction method in the Mega7 software package.[19] Colored labels indicate kinases discussed in this Perspective. Lipid kinases PI3K and PI4K were not included in the analysis. (B) Crystal structure (PDB: 4RZ7) of *P. vivax* PKG with inhibitor illustrating key interaction in the ATP binding site. (C) Crystal structure (PDB: 4RZ7) of *P. vivax* PKG inhibitor (cyan) superimposed with dasatinib (purple) from the X-ray structure with activated ABL kinase (PDB: 2GQG). Both inhibitors access the "deep hydrophobic pocket" extending past the threonine gatekeeper residue.

(ATP)-binding pocket of the enzyme (Figure 1B), and therefore, target selectivity can be difficult to achieve.[20] Fortunately, the long independent evolution of the malaria parasite allowed the emergence of distinct features in the malarial kinome. These include kinases that clearly cluster within groups found in the human genome but that can be distinguished from their mammalian homologues (Figure 1C). This would include *Plasmodium* kinases from given groups that contain characteristics of other families, such as *Pf*PK6 or *Pf*PK7, and composites between mitogen-activated protein kinase (MAPK) and cyclin dependent kinases (CDKs)[21] and cyclic adenosine monophosphate (cAMP)-dependent kinase (PKA) and mitogen-activated protein kinase (MEK), respectively.[22] This would also include kinases, such as CDPKs, that belong to a specific group but do not have a clear orthologue in mammalians and kinases that do not cluster within any of the established families, for example, Phe (F)−Ile (I)−Lys (K)−Lys (K) (FIKK). These important divergences can be exploited to synthesize compounds that selectively inhibit *Plasmodium* kinases over mammalian enzymes.[23]

The path to delivering a new antimalarial based on inhibiting a *Plasmodium* kinase is a multistep process. First, kinase essentiality must be validated by determining the effect of disrupting function or diminishing expression in an organism on proliferation in culture or in the host. This has been achieved for the *Plasmodium* kinome through kinome-wide reverse genetics studies leading to the identification of 36 protein kinases that are essential (or likely essential) for completion of the erythrocytic cycle in *P. falciparum in vitro*[20] and of 12 protein kinases that are required for transmission of the rodent malaria parasite *P. berghei* (*Pb*) to the mosquito *in vivo*.[24] Phenotypic validation is a second level of validation defined as a chemical compound inhibiting a kinase target and also demonstrating an effect on the organism (most often cell kill). Target engagement studies need to be carried out to show that the phenotypic effect is due to binding to the intended kinase and not a different mode-of-action. The third level, *in vivo* validation, oftentimes denoted as *in vivo* proof-of-concept (POC), refers to the capability of a compound to create the intended pharmacodynamic (PD) effect in an animal model.

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For an antimalarial drug, this most often is the reduction of parasitaemia in a mouse model of infection. To show efficacy, the compound needs favorable *in vivo* pharmacokinetic (PK) properties for sufficient exposure in the blood to produce the intended PD response. Finally, as the key goal of drug discovery programs, clinical validation represents the fourth level with a drug molecule working effectively in malaria patients. To achieve clinical validation, the human PK must be favorable, whether it would be exposure in the blood for asexual stages of the malaria parasite or in the liver for liver stage disease. Importantly, sufficient safety for drug must also be demonstrated preclinically to justify human administration in the clinic and progression through Phases 1, 2, and 3. Table 1 represents a summary of the protein and lipid kinases discussed in this Perspective and their respective levels of validation achieved.

Table 1. Level of Validation Achieved for Plasmodium Targets Covered in This Perspective[a]

P. falciparum protein kinase	genetic validation[b]	phenotypic validation[c]	*in vivo* efficacy	clinical validation
CDPK1	√[d]	√	√	
CDPK4	√	√	√[e]	
PKG	√	√	√	
PKA	√[e]			
MRK	√	√		
GSK-3	√	√		
NEK-1	√	√	√	
FIKK8		√		
PI3K[f]	√	√		
PI4K	√	√	√	√

[a]√ Indicates level of validation was achieved. [b]Genetic validation of kinases refers to where potential essentiality has been confirmed by knockout, chemical-genetic, or overexpression methods. [c]The fields are checked if an inhibitor of the kinase target also displayed whole cell activity recognizing that the activity may or may not be due solely to inhibition of the target. [d]There are conflicting data in the literature as to whether the target has been genetically validated. [e]Transmission-blocking was shown in an animal model. [f]DHA, implicated as a PI3K inhibitor, has other modes-of-action that are thought to be primarily responsible for antiplasmodium activity.

In this perspective, the emphasis has been placed on compound series and kinase targets that have a phenotypic level of validation and therefore *in vitro* antiplasmodial activity due to inhibition of an identified kinase target. Most interesting are those series that show POC in animal models of infection. Beyond animal models, there is a single example of a kinase target, *P. falciparum* phosphatidylinositol 4-kinase (PI4K), with inhibitor **37** (MMV048) that has progressed to human clinical trials.[25]

2. CALMODULIN-DEPENDENT KINASES (CAMK)

The group of calmodulin-dependent kinases (CaMK) contains the family of calcium-dependent protein kinases (CDPK) that have seven identified members, as well as the sucrose nonfermenting (SNF1)/adenosine monophosphate (AMP)-activated kinase (AMPK) family and other closely related kinases.

2.1. Calcium-Dependent Protein Kinases (CDPKs).

CDPKs, the main transducers of calcium signaling in *Plasmodia*, are unique to alveolates, plants, and some algae.[26] As they do not have human orthologues, it follows that selectivity relative to mammalian kinases would be more easily achieved. Structurally, CDPKs have a catalytic kinase region responsible for phosphorylation, as well as an autoregulatory sequence and so-called EF-hand motifs that are similar to the calcium-binding protein calmodulin.[27] Inhibitors of CDPK1 and CDPK4 have been progressed furthest in this family of kinases and will be discussed further. *Pf*CDPK2 has been shown to be nonessential for asexual proliferation but essential for the transition of microgametocytes into male gametes, a process known as exflaggelation, and mosquito infection.[28] *Pf*CDPK3 is specifically expressed in the sexual erythrocytic stages,[29] and its *P. berghei* homologue has been shown to be required for ookinete gliding motility and invasion of the mosquito's midgut.[30] *Pf*CDPK5 is required for parasite egress through microneme discharge.[31] However, hyperactivation of *P. falciparum* cyclic guanosine monophosphate (cGMP)-dependent protein kinase (*Pf*PKG) can overcome the block of egress caused by a *Pf*CDPK5 knock out. To our knowledge, there are no publications of medicinal chemistry programs that have targeted these kinases so far.

For several *Plasmodium* kinases, chemical genetics approaches have been used involving the mutation of the so-called gatekeeper residue located in the catalytic domain of the kinase. The catalytic domains of kinases have several conserved structural motifs that are common among most kinases. The hinge region connects the N-lobe and the C-lobe and is a frequent binding site for both ATP and kinase inhibitors (Figure 1B).[32] The gatekeeper is the first amino acid residue of the hinge region. Depending on its size, a hydrophobic pocket becomes accessible for inhibitors. Mammalian kinases usually possess threonine and larger amino acids in this position. Shokat et al. developed a strategy to achieve selectivity for mammalian kinases that have a small gatekeeper residue by designing kinase inhibitors with a specific substitution termed the "bump",[33] a principle that has been applied to several *Plasmodium* kinases.[34] There are a number of protozoal kinases like *Pf*CDPK4 that possess an atypically small gatekeeper residue and therefore are accessible to inhibition by such bumped kinase inhibitors and offer the opportunity to design selectivity over mammalian kinases.[35] Subsequently, mutation of the gatekeeper in these kinases to a larger amino acid offers the opportunity to check whether the antiplasmodial activity is in fact mediated by inhibition of the targeted kinase.

2.1.1. *P. falciparum* Calcium-Dependent Protein Kinase 1 (*Pf*CDPK1).

*Pf*CDPK1 has been associated with parasite motility, host invasion by *P. falciparum*, microneme secretion, and gametogenesis.[36−38] It is highly expressed in asexual blood and mosquito stages.[36] Previous studies hinted at the corresponding *cdpk1* gene being essential in *P. falciparum* in that it is refractory to gene disruption.[39] Other studies reported a decrease in parasitaemia due to reduced invasion following conditional knockdown[36] or no influence on asexual parasite development using a chemical genetics approach.[40] Recently, Bansal et al. were unable to create a knockout (KO) with a wild-type strain but were able to do so with a transgenic line containing a CDPK1 mutant (T145M).[38] The phenotype showed slow asexual proliferation compared to the wildtype indicating some degree of ambiguity around the essentiality of *Pf*CDPK1. Interestingly, the CDPK1 KO parasites are defective in the formation of male and female gametes and failed to establish infection in mosquitoes making *Pf*CDPK1 an interesting target for transmission blocking. Increased sensitivity of the CDPK1 KO parasites toward *Pf*PKG inhibitors compared to the wildtype indicated the presence of

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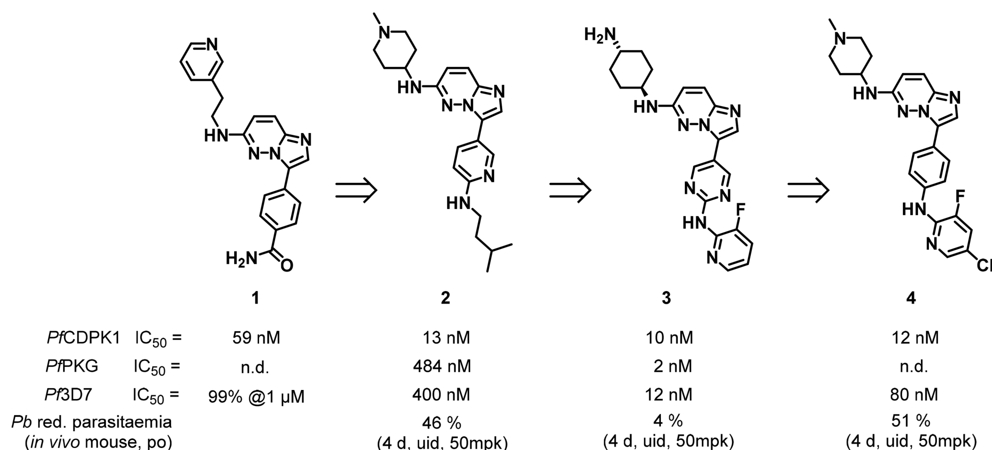

Figure 2. Imidazopyridazine inhibitors of *Pf*CDPK1 and *Pf*PKG.

compensatory mechanisms in the transgenic parasites. Upregulation of kinases such as *Pf*CDPK5 and *Pf*CDPK6 that are downstream from *Pf*PKG has been shown previously for the CDPK1 mutant T145M.[41]

Considerable work has led to the identification of *Pf*CDPK1 inhibitors with antiplasmodial activity. However, several disconnects between enzyme potency and antiplasmodial activity have been observed and suggest multiple or other targets responsible for the antiplasmodial activity. A high throughput screening (HTS) campaign against *Pf*CDPK1 found a broad array of hits including pyrazolopyrimidines, azabenzimidazoles, isoxazole amides, pyrazinones, and imidazopyridazines with the latter being progressed the farthest.[40,42−45] The imidazopyridazine hits with 2-aminoethylpyridines on the left-hand side and phenyl amides on the right-hand side (Figure 2) suffered from high logD (D being the distribution coefficient determined as the ratio of concentrations for molecules partitioned between octanol and water at a given pH) values, low metabolic stability, and poor selectivity relative to a human kinase panel.[42] A homology model based on CDPK1 from *Toxoplasma gondii* (*Tg*) was created to optimize *Pf*CDPK1 inhibitory potency alongside optimizing absorption, distribution, metabolism, excretion, and toxicology (ADMET) and physicochemical properties. Inhibitors retained potency against *Pb*CDPK1 and *Pv*CDPK1. The pyridine on the left-hand-side contributed little to binding affinity and could be replaced by a basic amine that purportedly formed a salt bridge with a close-by glutamate and accounted for the improved potency against *Pf*CDPK1.[43] Compound **2** demonstrated a lower logD, improved metabolic stability in both mouse and human liver microsomes, and better selectivity against a human kinase panel. Ultimately, the work led to compound **4** that demonstrated efficacy in a *P. berghei* mouse infection model.

Toward validating the mode-of-action of the imidazopyridazines and dissecting a disconnect observed between *Pf*CDPK1 inhibitory potency and activity against the *Pf*3D7 strain, compounds were assessed in a synchronized parasite culture revealing two different classes of compounds (Class 1 and Class 2) with potentially different modes-of-action.[40] Compound **3** with a pyrimidine linker (Class 1) acted on a late schizont stage of the parasite life-cycle and showed high inhibitory potency against the alternative *Pf*PKG target. The best imidazopyridazine inhibitor showed an IC_{50} of 1.6 nM against *Pf*PKG, and the compounds of class 1 showed a very good correlation between *Pf*PKG and *Pf*3D7 activities. Mutating the gatekeeper residue of *Pf*PKG from threonine to glutamine (T618Q) led to a dramatic loss in inhibitory potency. Replacing wild-type PKG with the T618Q mutant in *Pf*3D7 correspondingly diminished antiparasitic activity. For class 2 compounds, e.g., **2**, *Pf*CDPK1 was excluded as the target due to it not being expressed in earlier stages of the parasite life-cycle where the compound was shown to operate. Pull-down experiments with a biotin modified inhibitor identified *P. falciparum* heat shock protein 90 (*Pf*HSP90) and *in silico* methods supported binding to its ATP pocket.[40]

In another screening campaign against *Pf*CDPK1, Kato et al. identified 2,6,9-trisubstituted purines as inhibitors of *Pf*CDPK1 with IC_{50}s extending down to the low nanomolar range.[39] The most potent compound, named purfalcamine **5** (Figure 3),

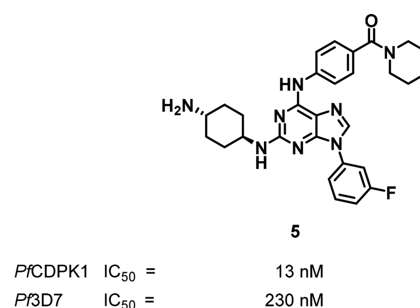

Figure 3. 2,6,9-Trisubstituted purine inhibitor of *Pf*CDPK1, purfalcamine **5**.

displayed an IC_{50} value of 17 nM against *Pf*CDPK1 and an EC_{50} of 230 nM against *Pf*3D7. As with the imidazopyridazines, a disconnect between enzyme inhibition and antiplasmodial activity was observed. The authors used agarose-immobilized purfalcamine for affinity chromatography toward identifying the target from cell lysates, and *Pf*CDPK1 was the only protein with an ATP binding site that was specifically bound. However, the pulldown experiment could not exclude the possibility that purfalcamine binds to low-abundance proteins to account for the antiplasmodial activity. Nonetheless, in line with the CDPK1 mode-of-action, synchronized parasites treated with purfalcamine progressed through the life-cycle but were arrested at late schizogonic stage where the *Pfcdpk1* gene is transcribed.

Purfalcamine failed to show efficacy in mice infected with *P. yoelii* (*Py*) on dosing 10 mg/kg bid. The authors attributed this

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to the compound being more selective toward *Pf*CDPK1 relative to the orthologue of *P. yoelii* and to low exposure on oral dosing. This combined with improvement in antiplasmodial activity was deemed necessary for future analogue optimization work.

Lemercier et al. screened a 54,000 compound library against *Pf*CDPK1 to identify 70 compounds with submicromolar K_i values, the most potent being indolizine **6** and imidazopyridazine **7** (Figure 4).[46] Testing against a panel of 46 human

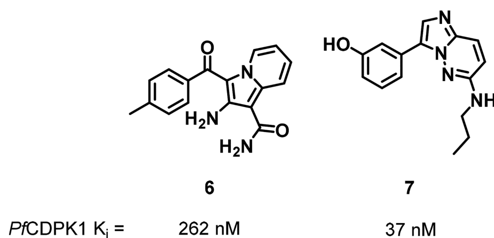

Figure 4. Indolizine **6** and imidazopyridazine **7** inhibitors of *Pf*CDPK1.

kinases indicated high selectivity as **6** showed 75 and 54% inhibition at 10 μM only against stress-activated protein kinase-2 alpha (SAPK2α) and fibroblast growth factor receptor 1 (FGFR1), respectively, and **7** showed at least 89% inhibition at 10 μM only against CDK5/p25, Tropomyosin receptor kinase A (TrkA), FGFR1, Lyn, and ribosomal S6 kinase 1 (RSK1). Similar to previous studies, the activity of some compounds against *Pf*CDPK1 correlated well with antiplasmodial activity, while others did not. In the latter case, the low correlation was attributed to variable permeability.

A *Pf*CDPK1 pharmacophore model developed around the imidazopyridazines[42−44] for *in silico* screening identified a number of compounds predicted to have IC_{50} values below 100 nM.[47] Winnowing out compounds predicted to have unfavorable ADMET properties left 12 compounds with predicted IC_{50}s between 15 and 60 nM.

It has been shown in several projects that CDPK1 has not been the efficacious target of the asexual stages despite compounds showing high potency against the enzyme. This relates to the ambiguity of *Pf*CDPK1 essentiality for asexual blood stage proliferation.[38] However, it was suggested that *Pf*CDPK1 might be a good target for transmission blocking agents.

2.1.2. P. falciparum Calcium-Dependent Protein Kinase 4 (PfCDPK4). Knockout experiments in *P. berghei* have shown the *Plasmodium* CDPK4 to be essential for exflaggelation[48−50] and for sporozoite invasion of hepatocytes.[51] The target itself is not vital for the blood stage parasite proliferation, but inhibitors would be of value as part of an extended gametocyte transmission blocking regimen if the drug levels in the blood could be maintained for 3−4 weeks.[52] A strong correlation between *Pf*CDPK4 inhibition and suppression of exflaggelation was observed, and bumped kinase inhibitors of *Pf*CDPK4 were developed and shown to block transmission to mosquitoes.[53] Inhibitors of *Pf*CDPK4 were shown to be on target by the introduction of a S147M mutation at the gatekeeper residue of the target in *P. falciparum*, which diminished the suppression of exflaggelation.[53]

A series of potent 4-amino-pyrazolopyrimidine inhibitors of *Tg*CDPK1 and *Cp*CDPK1[54,55] were found to also inhibit *Pf*CDPK4,[56,57] and more potent inhibitors were developed against *Pf*CDPK4, e.g., **8** and **9**. These compounds also blocked exflaggelation in *P. falciparum* (Figure 5) with EC_{50}s of 35 and

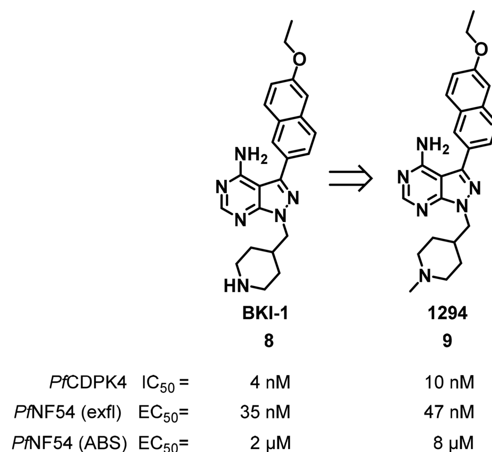

Figure 5. Bumped kinase inhibitors **8** and **9**.

47 nM, respectively.[53,56] Notably, **8** did not inhibit Src and Abl kinases at 20 μM, two off-target human kinases that also possess a smaller gatekeeper residue (threonine). Both compounds showed favorable PK in the mouse and were well tolerated in mouse feeding studies at 100 mg/kg bid dosing over 5 days.[53] Compound **8** was dosed intraperitoneal (*ip*) in *P. berghei* infected mice at 50 mg/kg resulting in suppression of exflaggelation for 0.5 to 14 h after administration.[56] When treating *P. berghei* gametocyte-infected mice with **8** at 10 mg/kg *ip* and allowing the *Anopheles* mosquitoes to feed, oocyst formation was completely blocked. Similarly, when *Anopheles* mosquitoes were fed with human blood infected with the *Pf*NF54 strain of *P. falciparum* containing 3 μM of either **8** or **9**, no sporozoite formation was observed when dissecting the salivary glands.[53,56]

The initially derived *Tg*CDPK1 inhibitors based on the aminopyrazole-carboxamide (AC) scaffold[57] share pharmacophore features with the pyrazolopyrimidine series (Figure 6) and were also tested against *Pf*CDPK4.[58] The AC series was about 10-fold less active than the pyrazolopyrimidine series but displayed improved selectivity over human Src kinase, improved margins over the human ether-a-go-go related gene (hERG) channel, and lower cytotoxicity against human liver (HepG2) and human lymphocyte (CRL-8155) cell lines.[58,59] Inhibition of exflaggelation was observed at 0.1 μM, concentrations lower than would have been expected from the enzyme IC_{50} values, suggesting inhibition of other targets. *Pf*CDPK1 and *Pf*PKG also possess small gatekeeper residues and modulate processes that occur prior to the step modulated by *Pf*CDPK4 and were, therefore, suggested as secondary targets.[59] One of the most potent compounds in both the enzyme and the exflaggelation assay was **10** (Figure 6) with an IC_{50} value of 37 nM against *Pf*CDPK4 and 89% inhibition of exflaggelation at 100 nM. Compound **11** showed better exposures on oral dosing in mice but fell short of achieving longer term blood levels deemed necessary for a transmission blocking agent.[58,59]

The calmodulin (CaM) antagonist trifluoperazine **12** (TFP) was hypothesized to bind to the CaM-like domain of CDPKs rather than the ATP site and displayed a K_i of 150 μM against *Pf*CDPK4.[60] It showed an EC_{50} of 1.9 μM against *P. falciparum in vitro*[61] suggesting inhibition of other targets that might

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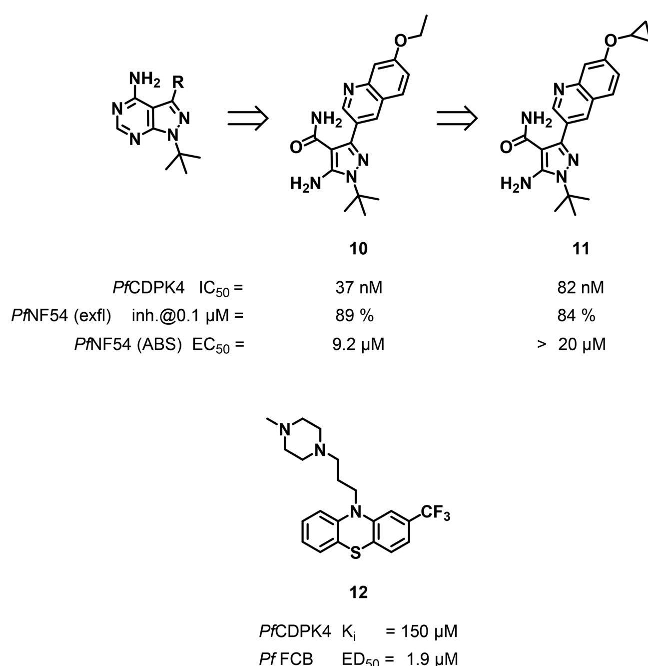

Figure 6. Aminopyrazole-carboxamide inhibitors of *Pf*CDPK4 and Trifluoperazine **12**.

include other CDPKs.[60] To date, no further progress has been reported based on this compound.

3. AGC

The AGC group includes the cyclic adenosine monophosphate (cAMP)-dependent kinase (PKA), protein kinase B (PKB), and cGMP-dependent kinase (PKG). Evidence has been provided for all three of them to be essential for the asexual blood stages of *P. falciparum*.

3.1. *P. falciparum* cGMP-Dependent Protein Kinase (*Pf*PKG).

*Pf*PKG is an essential cGMP-dependent protein kinase that targets at least 69 proteins either directly or indirectly through phosphorylation[62] and thereby influences multiple cellular activities in *Plasmodia* at various stages of the life-cycle. The essentiality of *Pf*PKG for blood stage replication in the human host was demonstrated by chemical genetics using an inhibitor-resistant *P. falciparum* transgenic line with a T618Q gatekeeper mutation.[63] Inhibition of *Pf*PKG led to a block of egress with mature schizonts that released noninvasive merozoites when mechanically ruptured.[62,64−67] PKG is also involved in gametogenesis[68] and ookinete motility[69] in the mosquito stages by modulation of calcium signaling,[69] as well as in late liver stage development.[70] *Pf*PKG also regulates calcium levels and can therefore act on calcium-dependent protein kinases like *Pf*CDPK5.[69]

A series of potent imidazopyridine inhibitors of *Pf*PKG showed correspondingly potent activity against the asexual blood stage of *P. falciparum* and blocked gametocyte transmission to *Anopheles* mosquitoes.[63] The compounds were derived from PKG inhibitors of the Apicomplexan parasite, *Eimeria*.[71] The most potent compounds **14** and **13** had IC_{50}s of 160 and 130 pM, respectively, against the wildtype enzyme (Figure 7), and 2 and 102 nM against the wildtype *Pf*3D7 strain. Binding to the hydrophobic pocket was demonstrated by markedly lower inhibitory potencies against the previously mentioned gatekeeper mutant T618Q enzyme and against the transgenic strain containing the mutant T618Q. A cocrystal structure obtained from *Pv*PKG and **14** (PDB: 5EZR) showed the fluorophenyl group reaching into the hydrophobic pocket that would be blocked by a larger gatekeeper residue of the mutant. Compound **14** showed good margins over cytotoxicity, moderate *in vitro* metabolic stability, and high selectivity against a panel of 80 human kinases. The killing dynamics of **14** in a parasite reduction ratio (PRR) assay with *P. falciparum* showed a 24 h lag phase to kill, consistent with *Pf*PKG inhibitors acting at the egress and invasion stage. Twice a day oral administration of **14** (100 mg/kg) over 4 days in *P. falciparum* infected NOD-*scid* gamma IL2Rγ^{null} (NSG) mice led to a reduction of parasitaemia below levels of detection. The intense dosing regimen, due in part to relatively rapid compound clearance, was thought to be required to maintain a high enough plasma concentration of the drug to cover the parasite life-cycle through schizont rupture and invasion. The transmission-blocking potential of **14** was demonstrated in a standard membrane feeding assay (SMFA) with mature *Pf*NF54 gametocytes with an IC_{50} of 41 nM. The SMFA uses a vial with blood covered by an artificial membrane mimicking skin upon which mosquitoes are allowed to feed. Determination of the number of oocysts in the mosquito's midgut relative to a control is a measure for transmission of the parasite from the host to the vector. It is thus used to predict the effectiveness of transmission blocking agents *in vivo*.[72]

A previously described 2,3-diaryl-pyrrole series showed similarities to the imidazopyridine analogues. It was also

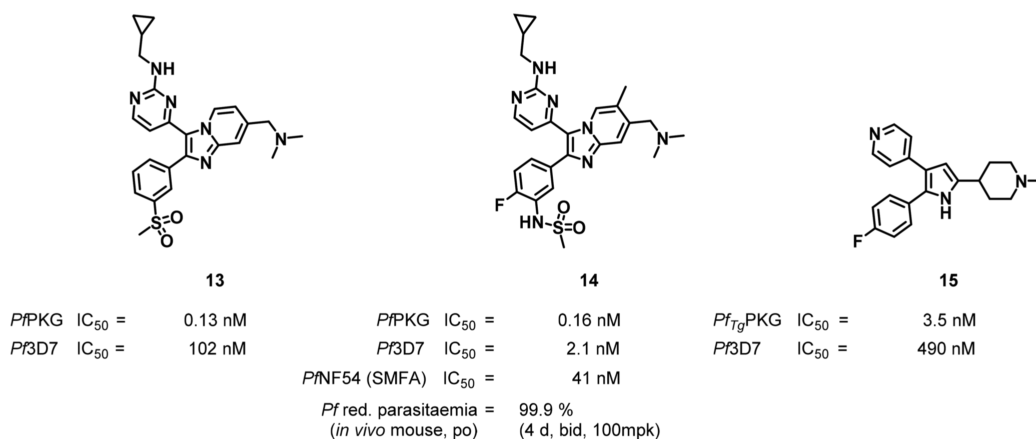

Figure 7. Inhibitors of cGMP-dependent kinase (PKG).

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derived from a PKG inhibitor program in *Eimeria tenella* where several core heterocycles and substitution patterns were investigated and *in vivo* efficacy was shown.[73] One of these compounds, compound **15** (Figure 7), was further evaluated against *Plasmodia*.[74] Compound **15** showed an IC_{50} of 3.5 nM against recombinant *Pf*PKG, similar to the native strain. However, *in vitro* potency against the chloroquine-sensitive strain *Pf*NF54 and the chloroquine-resistant strain *Pf*Dd2 only showed IC_{50}s of 0.49 and 1.3 μM, respectively. Subsequently, **15** did not clear parasites in a *P. berghei* mouse infection model, when treated *ip* with 50 mg/kg twice daily for 8 days.

Compound **15** has also been shown to reduce *P. berghei* sporozoite infection in a HepG2 cell culture in a dose-dependent manner.[75] Treatment with **15** at 2 μM decreased the number of liver stage parasites to below the detection limit. It inhibited host cell invasion with an IC_{50} of less than 1 μM *in vitro*. However, sporozoites lacking *Pb*PKG remained sensitive to compound **15**, indicating that it affected other targets in addition to *Pb*PKG. In a *P. yoelii* mouse model, which has higher sporozoite infectivity compared to *P. berghei*, a single dose of 50 mg/kg given *ip* before infection reduced the parasite burden in the liver by a factor of 1000. With three 50 mg/kg doses, the first dose given 15 min before infection and the second and third doses 6 and 12 h postinfection, all mice were free of blood stage parasites throughout the 3 week time course of the experiment. Compound **15** was also shown to inhibit gametogenesis in a dose-dependent manner[68] and to block egress.[67]

The imidazopyridazine series described above as *Pf*CDPK1 inhibitors were shown to primarily target *Pf*PKG.[40] In lieu of what was observed for compound **15**, and if the series were assessed to have favorable pharmacokinetic properties, it would be interesting to determine whether the imidazopyridazines would show liver stage activity and transmission blocking potential.

3.2. *P. falciparum* cAMP-Dependent Protein Kinase (*Pf*PKA).

*Pf*PKA comprises a regulatory and a catalytic subunit, wherein the former blocks the latter to maintain an inactive state. Allosteric binding of cAMP to the regulatory subunit triggers a conformational change that frees and thereby activates the catalytic subunit.[76] *Pf*PKA is involved in a number of molecular mechanisms including merozoite egress, motility and red blood cell invasion,[77,78] schizogony,[78] and the progression from schizont to invasive merozoites.[79] During merozoite invasion, *Pf*PKA was found to be involved in microneme secretion of erythrocyte host recognition receptors.[80] Furthermore, *Pf*PKA was shown to be a key modulator of the cell cycle in the malaria parasite.[81] Gene disruption attempts in *P. berghei* were unsuccessful, suggesting that the *Plasmodium* PKA might be essential for parasite survival.[82] Overexpression of the *Pf*PKA regulatory subunit led to reduced PKA activity and reduced parasite growth.[83]

An attempt to develop inhibitors against *Pf*PKA was made starting from the commercially available PKA inhibitor 3-methylisoquinoline-4-carbonitrile, previously used for investigations in mammalian systems. A homology model of the *Pf*PKA catalytic subunit was built based on the human crystal structure, and dynamic molecular modeling was used to identify strong binding ligands.[84] Synthesis of these ligands and testing against *Pf*3D7 and multidrug-resistant *Pf*W2 strain showed mid-micromolar activities. In further studies, it was revealed that these compounds have no effect on *Pf*PKA.[85] However, the compounds showed inhibition of parasite cytokinesis and erythrocyte invasion at 10 μM, suggesting that other targets were involved. To date, no potent and specific inhibitors against *Pf*PKA have been reported.

4. CMGC

The most prominent group in the *P. falciparum* kinome, CMGC, includes the following families: the cyclin dependent kinases (CDKs), the mitogen-activated protein kinases (MAPKs), the glycogen synthase kinase 3 (GSK-3), and the CDK-like kinase (CLK) as well as other close relative protein kinases.[16−18,23,86] CDKs have been shown to play an essential role in the parasitic growth and development and therefore have received the most attention as targets to treat malaria.

4.1. *P. falciparum* MO15-Related Kinase (*Pf*MRK).

The *Pf*MRK protein, a member of the CDK family, was isolated and cloned in 1996 and has been suggested to play an essential role in DNA replication and transcriptional control.[20,87] Appropriate activation and deactivation of the enzyme at each stage guarantees cell progression in a sequential manner where a fully activated *Pf*MRK requires association with a cyclin subunit *Pf*CYC-1 and an effector protein *Pf*MAT1.[88] The structural differentiation within the ATP binding pocket between the parasitic and mammalian (CDK7) enzymes was exploited to identify specific and structurally diverse *Pf*MRK inhibitors as antiplasmodial chemotypes.

Compound **16** (Figure 8) was identified from a series of quinolinones and found to inhibit *Pf*MRK with an IC_{50} value of 18 μM.[89] A search in the Walter Reed Army Institute of Research (WRAIR) internal database identified **17**, an oxindole that inhibited *Pf*MRK (IC_{50} = 1.4 μM) and showed high selectivity relative to the mammalian CDK1 (IC_{50} = 29 μM). However, the compound displayed only moderate antiplasmodial activity against the sensitive D6 strain of *P. falciparum*, which was attributed to poor compound permeability.[90]

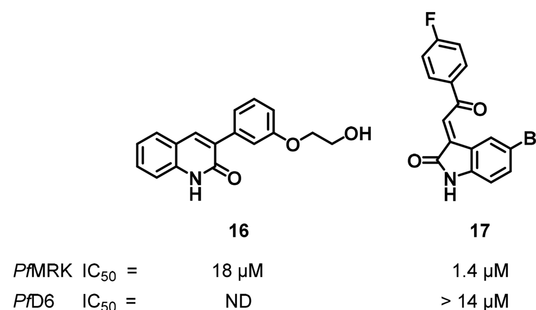

Figure 8. Quinolinone and oxindole-based inhibitors of *Pf*MRK.

In an attempt to identify novel and structurally diverse *Pf*MRK inhibitors from the WRAIR database, Waters and coworkers developed and validated a three-dimensional quantitative structure−activity relationship (3D-QSAR) pharmacophore model.[91] The most active compounds clustered around chalcone[92] and sulfonamide-based[93] molecules (**18** and **19**, respectively), which formed the basis of medicinal chemistry programs (Figure 9). The most potent *Pf*MRK inhibitors synthesized in these programs also displayed the highest antiplasmodial activity. However, selectivity was low relative to mammalian protein kinases with sulfonamide **19** showing a similar inhibitory potency against CDK7 (IC_{50} = 0.4 μM), a mammalian homologue of *Pf*MRK.

Flavonoid natural products are biosynthetically produced from chalcone intermediates prompting Ayuko Akenga and co-

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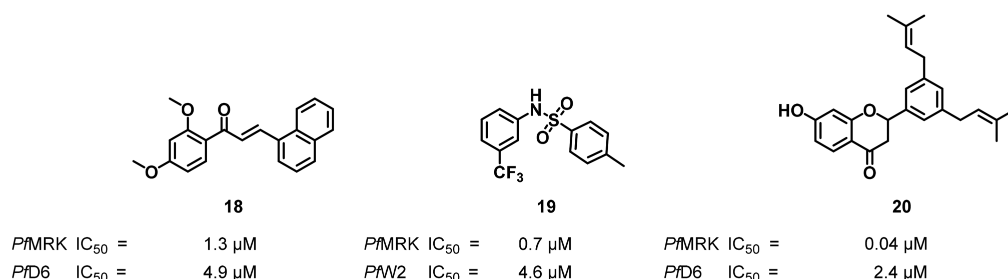

Figure 9. Chalcone and sulfonamide-based and flavonoid inhibitors of *Pf*MRK.

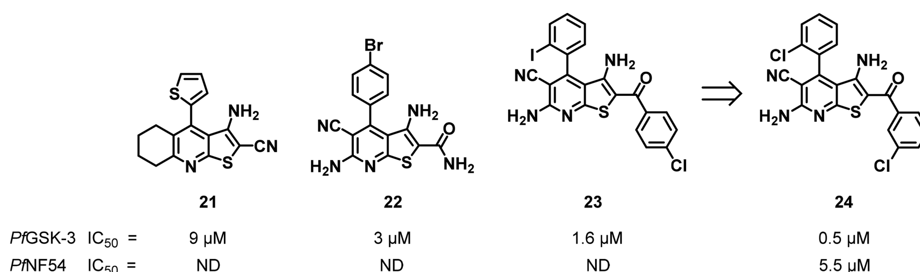

Figure 10. *Pf*GSK-3 inhibitors.

workers to evaluate several antiplasmodial flavonoids extracted from the roots and stem bark of *Erythrina sp.* for inhibitory activity against the *Pf*MRK. As observed in Figure 9, compound **20** was a highly potent inhibitor, approximately 25-fold higher than **18**. Despite this higher inhibitory potency, the *in vitro* antiplasmodial activity was nearly equal to those of the chalcones, indicating either a mixed mode-of-action for the chalcones or diminished cellular permeability for the flavonoids.[94] Overall, a direct correlation between *Pf*MRK inhibitory potency and antiplasmodial activity has been difficult to establish, but essentiality of the kinase for the parasite has been previously demonstrated.[20]

4.2. *P. falciparum* Glycogen Synthase Kinase 3 (*Pf*GSK-3). The *Pf*GSK-3 enzyme was identified and cloned in 2004 and is one of three *P. falciparum* protein kinases related to the mammalian GSK-3.[95] The precise biological function of *Pf*GSK-3 has not yet been determined, though it has been demonstrated to be essential for the completion of the parasite asexual blood stage.[20,96] As GSK-3 is highly conserved in mammals, sufficient differentiation relative to *Pf*GSK-3 suggested that selectivity could be achieved with inhibitors. A HTS of a collection of structurally diverse compounds against recombinant *Pf*GSK-3 yielded inhibitors that shared two related heterocyclic scaffolds exemplified by compounds **21** and **22** (Figure 10). Screening of an additional nearest neighbor library of 427 compounds afforded **23** with an IC_{50} of 1.6 μM. From these three hits, analogues were synthesized to explore the SAR and increase inhibitory potency against *Pf*GSK-3 as well as selectivity over the mammalian GSK-3 orthologue. Importantly, antiplasmodial activity was seen for **24** (IC_{50} = 5.5 μM versus *Pf*NF54), which extended to other *Plasmodium* strains. When tested against two mammalian kinase panels (77 and 402 kinases from Dundee and DiscoverX, respectively), compound **24** inhibited only a few kinases thus demonstrating broad selectivity. Further optimization of this series is required to demonstrate *in vivo* activity.[96,97]

5. *P. FALCIPARUM* NEVER-IN-MITOSIS GENE A (*PF*NIMA/NEK)

NIMA (never in mitosis, gene A)-related kinases or NEKs are a family of serine/threonine kinases that are generally conserved across eukaryotes.[98] There are four representatives of the family in *Plasmodia* characterized by a shared protein kinase domain normally located at the N-terminus.[99] The NEKs play an important role in the life-cycle of the malaria parasite, specifically in mitosis and meiosis where they are associated with centrosomes, spindle poles, and other components of cell machinery involved during division.[98,100] *Pf*NEK-1 and *Pf*NEK-2−4 are likely essential for the asexual and the sexual cycles, respectively.[20] Though the literature covers several programs targeting human NEK kinases, particularly NEK-2, there have not been reports of drug discovery efforts targeting any *Pf*NEK.

Two marine natural product compounds **25** and **26** demonstrated inhibition of *Pf*NEK-1 and activity against *P. falciparum* (Figure 11); however, the antiplasmodial activity was not confirmed to be due to *Pf*NEK-1 inhibition.[101−103] Compound **26** did show activity in a *P. berghei* NK65 mouse infection model (40% inhibition of parasitaemia at 5 mg/kg over 4 days of dosing), thereby achieving a measure of *in vivo* validation for the scaffold.[103] Higher doses were not tolerated by mice in the model, and better efficacy was not demonstrated.

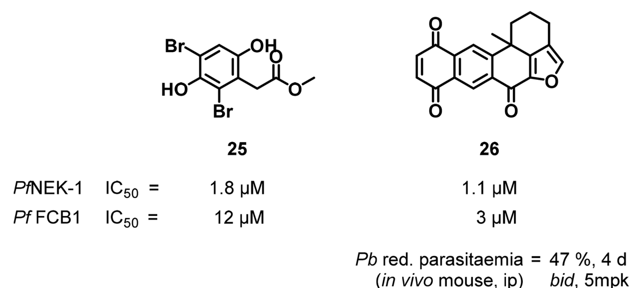

Figure 11. Chemical structures, enzyme, and whole cell activities of *Pf*NEK-1 inhibitors.

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More recently, Mitcheson et al. identified a tool compound that inhibited a genetically modified isozyme of *Pf*NEK-2 (but not the wild-type isozyme) to interrogate enzyme function.[104] A recent comprehensive review of chemical starting points for human NEK kinase inhibition may offer an opportunity to cross-screen and identify *Pf*NEK inhibitors.[105] As the landscape now stands with little structural information, biological validation, and few chemical starting points, considerable work around *Plasmodium* NEK kinases is needed to expand upon the biological understanding of *Pf*NEK and to develop drug discovery programs.

6. PHE (F)–ILE (I)–LYS (K)–LYS (K) (FIKK)

The FIKK kinase family, named after the 4-amino acid chain sequence Phe–Ile–Lys–Lys, is specifically found in the Apicomplexan phylum and is mostly expressed in the blood stage of the parasite life-cycle.[106] Though most malaria species contain a single FIKK kinase, *P. falciparum* contains 20 homologues, 18 of them predicted to be functional kinases making them interesting drug targets. Furthermore, their catalytic site contains a small gatekeeper residue, which allows for selectivity over mammalian kinases.[107] A few *Pf*FIKK homologues such as FIKK4.2,[108] FIKK7.1, and FIKK12[109] are implicated in red blood cell remodeling and altering erythrocytic membrane rigidity. Even though none of the three aforementioned kinases was found to be essential for parasite proliferation, little is known about the essentiality and the specific role of the other members of the FIKK family. Compound **27** (Figure 12, emodin) has shown *in vitro* activity against *P. falciparum* with an IC_{50} of 13 μM,[110] which was subsequently attributed to inhibition of *Pf*FIKK8 (2 μM), the most similar homologue of *Plasmodium* species.[111] Inhibition of *Pv*FIKK (1.9 μM) has also been demonstrated.[112] Otherwise, there has been a drug discovery project targeting *Cryptosporidium* FIKK, wherein the authors identified a naphthyridine-based low nanomolar inhibitor that, albeit, failed to show antiparasitic activity (Figure 12).[113] While several factors make the FIKK family attractive as drug targets in *Plasmodia*, considerably more work still needs to be conducted to better delineate the roles that FIKKs play within the parasite.

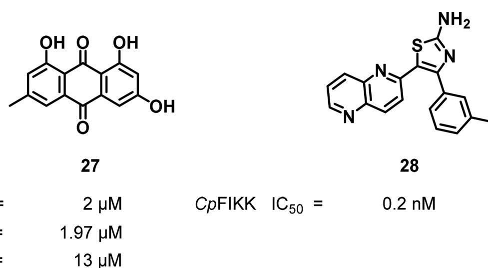

Figure 12. Chemical structure of emodin and a *Cp*FIKK inhibitor.

7. PHOSPHOINOSITIDE LIPID KINASES (PIKS)

Phosphoinositide lipid kinases (PIKs) generate precise phosphorylated variants of phosphatidylinositols that are crucial for a diverse array of cellular functions including secondary messenger signaling, cellular membrane remodeling, and vesicular trafficking.[60,114] The two most widely studied classes of PIKs in *Plasmodium* species are phosphoinositide 3-kinase (PI3K) and phosphatidylinositol 4-kinase (PI4K), both demonstrated to be essential for the survival of the parasite.[115,116] PI3K inhibitors have seen relatively little exploration thus far, while PI4K inhibitors have generated considerable interest within the context of the advancement of antimalarial drug candidates.

7.1. Phosphoinositide 3-Kinases (PI3K).

Though only one PI3K enzyme is encoded in the *P. falciparum* genome and its essentiality has been demonstrated, there have not been literature reports on medicinal chemistry programs targeting this enzyme. Rather, tool compounds have been described including the mammalian pan-PI3K inhibitors **29** (wortmannin) and **30** (LY294002) (Figure 13). There are no reported IC_{50} values for wortmannin in standard *P. falciparum* proliferation assays attributed to its chemical instability, whereas **30** showed an IC_{50} of 26 μM against the parasite.[117]

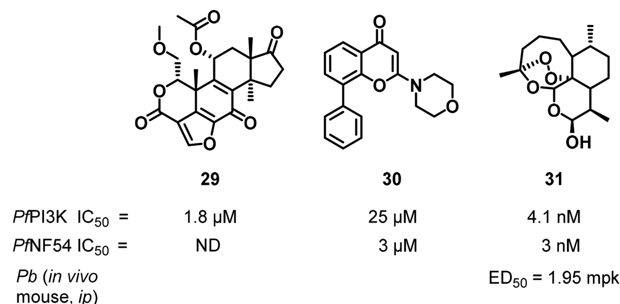

Figure 13. Chemical structures of compounds known to inhibit *Pf*PI3K.

PI3K has recently been implicated as one of the targets of dihydroartemisinin (DHA), **31**, as evidenced by mutations to the regulatory ubiquitination protein *Pf*Kelch13[118] that were associated with clinical resistance.[119] The *Pf*Kelch13 mutations led to increased levels of PI3K by abrogating its role in ubiquitination and deactivation of the kinase. PI3K was shown to be essential for parasite growth, and levels of the lipid product phosphatidylinositol-3-phosphate (PI3P) in parasite tissue cultures dropped on treatment with DHA. A quantitative inhibition assay with purified *Pf*PI3K has been derived wherein DHA demonstrated potent (low nanomolar) inhibition. A *Pf*PI3K homology model was also derived from the structure of the class III PI3-kinase from Drosophila (PDB code: 2X6F) and the active site of the human class III PI3K (PDB code 3IHY).[119] There is, therefore, considerable interest in developing other scaffolds as antimalarials operating by PI3K inhibition.

7.2. Phosphatidylinositol 4-Kinases (PI4K).

Significant progress in the discovery of compounds inhibiting PI4K in *Plasmodium* species with two key classes being reported in the literature. A group led by the Genomics Institute of the Novartis Research Foundation (GNF) and the Novartis Institute for Tropical Diseases (NITD) identified antiplasmodial activity for a series of imidazopyridines/pyrazines/pyridazines.[115,120] Compound **32** (Figure 14) was identified by phenotypic screening against the *P. falciparum* blood stage parasite. The compound had moderate *P. falciparum* activity but was inactive against *P. yoelii* and *P. cynomolgi*. Initial SAR around the 6- and the 3-positions identified compound **33** (KAI407), which significantly improved *P. falciparum* activity and brought in both *P. yoelii* and *P. cynomolgi* activity. Compound **33** suffered from poor physicochemical properties and thus modification of the core to an imidazopyrazine reduced lipophilicity leading to compound **34** (KDU691).

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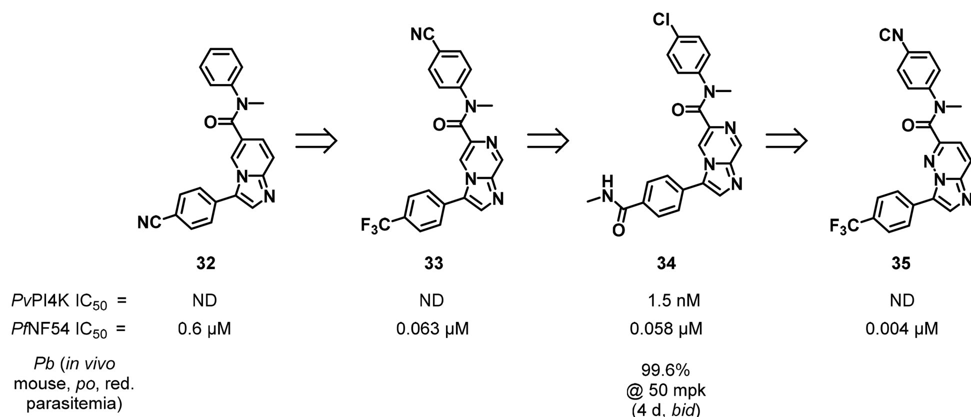

Figure 14. Imidazopyridine/pyrazine compounds as *Pf*PI4K inhibitors.

Compound **34** showed improved physicochemical properties and *in vitro* activity across *Plasmodium* species, particularly *P. cynomolgi*.

That the imidazopyridines/imidazopyrazines were active against clinically resistant isolates of all classes of antimalarials suggested a novel mode-of-action. The compounds were active *in vitro* against liver stage development of the rodent parasite, *P. yoelii*, and against *in vitro* cultured liver-resident hypnozoites of *P. cynomolgi*, indicating potential as a radical cure for *P. vivax* infection and associated malaria relapse. Activity was also demonstrated against *P. vivax* field isolates. Compounds showed potential for transmission blocking activity in a gametocyte enriched *in vitro* assay and in a SMFA. Hence, activity was demonstrated across multiple stages of the parasite life-cycle. PI4K was identified as the target of the series by selection for resistant mutants to **33** and **35** (KAI715) with alterations seen in the single gene, *pfpi4k*. Full length *Pv*PI4K was expressed, and protein was isolated for assay development wherein **34** exhibited an IC_{50} of 1.5 nM.

A *Pf*PI4K homology model was generated through which it was suggested that the imidazopyridines are accommodated in the ATP-binding pocket (Figure 15). Compounds **33** and **34** displayed remarkable selectivity over human lipid kinases as well as a selection of human protein kinases. Compound **34** displayed favorable oral pharmacokinetics at 20 mg/kg, prevented colonization of mice with *P. berghei* with prophylactic dosing, and cured an existing infection in a dose-dependent manner.

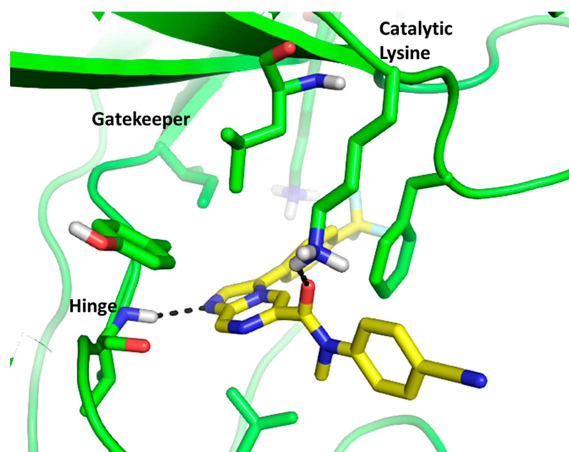

Figure 15. Homology model of *Pf*PI4K with compound **33** (KAI407), illustrating a key interaction in the ATP binding site (Eyermann, unpublished).

A second class of PI4K inhibitor, the aminopyridine/pyrazine class of compounds (Figure 16), was identified at the University of Cape Town (UCT) Drug Discovery and Development Centre (H3D) in collaboration with the Medicines for Malaria Venture (MMV). The initial hits originated from a HTS campaign on a commercial SoftFocus kinase library conducted at the Eskitis Institute, Griffiths University in Brisbane (Australia).[121]

The screen identified compound **36**, which has a *para*-sulfonyl phenyl substituent on the aminopyridine 5-position and showed promising activity against *Pf*NF54 (Figure 16). Replacement of the metabolically labile 3-methoxy-4-hydroxy substituent led to **37** (MMV048), a compound with high activity against drug sensitive and drug resistant *Plasmodium* strains and favorable ADMET and PK properties. Spontaneous resistant mutants to **37** were generated in *P. falciparum* culture, and whole genome sequencing implicated PI4K as the target. Inhibition of PI4K was also supported via pull-down experiments and confirmed against the *Pv*PI4K with an IC_{50} of 3.4 nM.[25] The compound showed impressive selectivity over human lipid kinases, and the series showed a close correlation between enzyme inhibitory potency and whole cell activity. The speed of kill of the compound was relatively slow as assessed in the *in vitro* PRR and stage specificity assays but moderate *in vivo*. Transmission-blocking potential was also evaluated wherein **37** showed antigametocyte activity *in vitro* and *in vivo* in a SFMA, supporting potential utility for blocking parasite transmission to the mosquito. The central pyridine core of **37** was replaced with a pyrazine (compound **38**) leading to improved *in vitro* antiplasmodial activity.[122] Replacing the sulfone of **38** with a piperazine amide led to **39** (UCT943), a compound with improved solubility as well as higher antiplasmodial activity.[123] All four compounds proved highly efficacious in *in vivo* models of *Plasmodium* infections, e.g., **37** showing an ED_{90} of 0.8 mg/kg in a *P. berghei* mouse model of infection.

Compound **37** was profiled extensively *in vitro* and *in vivo*, showing activity across a panel of drug-resistant clinical isolates and therefore a low risk for pre-existing cross-resistance. *In vivo*, the compound was active in the *P. berghei* model as described above but was also evaluated in a humanized NSG mouse model that supports infection by *P. falciparum*, where it similarly exhibited potent activity with an ED_{90} of 0.57 mg/kg. The compound showed prophylactic activity preventing

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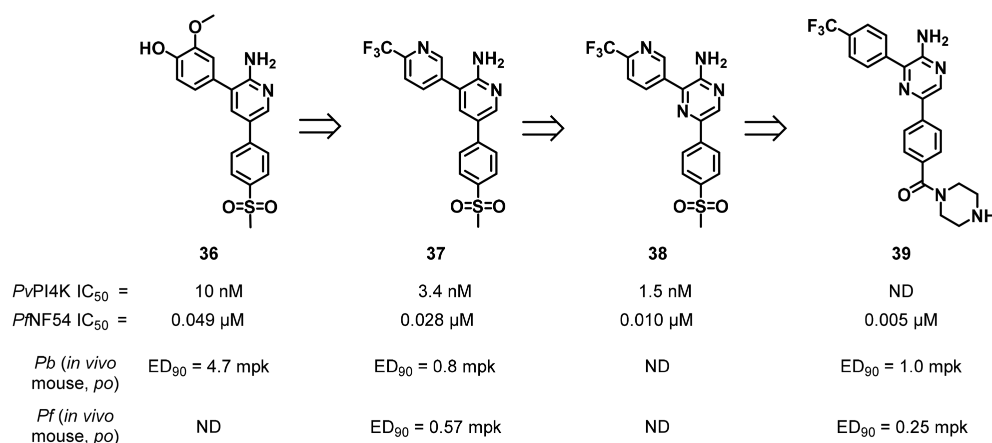

Figure 16. Aminopyridine/pyrazine compounds as *Pf*PI4K inhibitors.

infection in a *P. cynomolgi* infected macaque model (2 mg/kg prior to infection). The PK of compound **37** in mice, rats, dogs, and monkeys was characterized by low plasma clearance, moderate to high volume of distribution, and a moderate to long half-life. From this data, a single dose of 80 to 100 mg was estimated to be efficacious in humans. Compound **37** has progressed through preclinical development and Phase 1 clinical trials. During Phase 1, the compound was also evaluated in a volunteer controlled human infection model (CHIM) wherein favorable activity led to its progression to Phase 2a clinical trials.[124] Hence, **37** may become the first directed *Plasmodium* kinase inhibitor to have registered a clinical proof-of-concept.

PI4K inhibitors with antiplasmodial activity have been derived by phenotypic screening and subsequent identification of the target. As the *Plasmodium* PI4K has emerged as an important target for drug design, it follows that rational inhibitor design would be pursued. Indeed, a *Pf*PI4K homology model and compound **37** were used to create a pharmacophore-based model to perform a computational screen, identifying virtual hits as starting points that were hypothesized to inhibit *Pf*PI4K.[125]

8. SUMMARY

One of the most persuasive reasons to target *Plasmodium* kinases for the treatment of malaria is that selectivity relative to problematic human kinases can be achieved and, therefore, less likely to cause kinase-mediated safety issues in humans. Additionally, it can be anticipated that host-directed antimalarial therapies will be developed including those that inhibit human protein kinases.[126] For example, inhibition of erythrocyte sik tyrosine kinase by the oncology agent imatinib was perhaps responsible for preventing *P. falciparum* egress,[127] and silencing of Protein Kinase C zeta (PKCζ) led to marked reduction of *P. berghei* infection in human Huh7 hepatoma cells.[128] There would be the added value with such mechanisms-of-action toward circumventing the development of drug resistance.[129] Hence, the playbook that has led to upward of 32 small molecule kinase inhibiting therapeutics approved by the FDA can be applied in the parasitology arena.[15,130] Knowledge on the essentiality, stage specificity, and mechanism of kinase function in *Plasmodia* can help triage targets that would be worthwhile pursuing for medicinal chemistry optimization programs. While blood-stage activity is a requirement to treat malaria, transmission-blocking potential is thought to be important toward malaria elimination in the community, and essential targets showing dual-activity can be considered of high value. Comparative gene sequence analyses and homology modeling/structure determination can be used to understand inhibitor binding to targets and mitigate binding to unwanted (most often human kinase) targets. Cross-screening against human kinases has become routine in *Plasmodium* kinase programs just as cross-screening across human kinase panels are carried out to achieve selectivity for inhibitors directed at a specific human kinase. Conversely, collections of human kinase inhibitors are valuable to screen against *Plasmodium* kinases toward obtaining hits that can eventually be optimized for higher *Plasmodium* and lower human kinase potencies. Alternatively, considerable success has been achieved by screening libraries of human kinase inhibitors phenotypically against *Plasmodium* followed by subsequent determination of their mode-of-action.

In fact, it is this latter approach that led to **37** (MMV048), the only *Plasmodium* kinase inhibiting compound to have achieved a measure of clinical validation. However, expectations are high that other *Plasmodium* targets already known will yield new classes of antimalarial drugs both in terms of chemical scaffold and mode-of-action. At least eight kinase targets delineated herein have led to phenotypic levels of validation, albeit sometimes it might not be clear that the *Plasmodium* kill is due to the originally targeted kinase. Notwithstanding the inability to always directly link kinase inhibition to the mode-of-action responsible for parasite kill, the work around protein and lipid kinases to date point to an impressive synergy created between fundamental research into the *Plasmodium* kinome and applied drug discovery research. A number of kinases that are likely to be essential have not been targeted in medicinal chemistry programs yet and certainly offer potential for the development of future antimalarials.[20]

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Notes

The authors declare no competing financial interest.

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André Horatscheck received his Dr. rer. nat. in Chemistry from the Freie Universität Berlin (Germany) in 2011 under the supervision of Prof Jörg Rademann. After his postdoctoral work in the field of Medicinal Chemistry at the Leibniz-Institut für Molekulare Pharmakologie Berlin, he joined H3D in 2016 where he is a Senior Investigator working on malaria related drug discovery projects.

Colin Wilson received his Ph.D. from the University of KwaZulu-Natal in South Africa under the supervision of Prof. Orde Munro where he worked on the discovery of novel topoisomerase inhibitors. He then held a National Research Foundation Scare Skills postdoctoral fellowship at the University of Cape Town under the supervision of Prof. Kelly Chibale where he worked on a number projects focused on tuberculosis drug discovery. He is now a Senior Investigator at H3D where he leads malaria focused discovery projects.

Greg Basarab has an appointment as Principal Research Officer and Associate Director at H3D. He leads research directed toward the eradication of resistant infectious diseases including malaria and tuberculosis. Previously, he was an Associate Director at AstraZeneca where he led multidisciplinary teams for the design of novel mode-of-action antibacterials and delivered three drug candidates that progressed into human clinical trials including one currently in Phase 3. Previous to that, he worked at DuPont leading projects in three departments: Central Research & Development, Biochemicals and Agricultural Products in the antifungal arena and in automated chemical synthesis. He received a B.S. in Chemistry from Penn State University and a Ph.D. in Chemistry from MIT.

Joe Eyermann is Head of Computer-Aided Drug Design at H3D. He has been especially involved in drug discovery programs against infectious diseases including HIV, Gram-negative bacteria, and, most recently, tuberculosis and malaria. Previous to joining H3D, he worked at DuPont, DuPont Merck, ARIAD, and AstraZeneca. He received a B.S. in Chemistry from Marietta College and a Ph.D. in Chemistry from Miami University of Ohio.

Kelly Chibale obtained his Ph.D. in Synthetic Organic Chemistry from the University of Cambridge with Stuart Warren (1989−1992). This was followed by postdoctoral stints at the University of Liverpool as a British Ramsay Fellow with Nick Greeves (1992−1994) and at the Scripps Research Institute as a Wellcome Trust International Prize Research Fellow with K. C. Nicolaou (1994−1996). His research is in the field of global health drug discovery. He is the Director of the University of Cape Town (UCT) Drug Discovery and Development Centre (H3D) and the South African Medical Research Council Drug Discovery and Development Research Unit at UCT.

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■ ABBREVIATIONS USED

Pf, *P. falciparum*, *Plasmodium falciparum*; WHO, World Health Organization; ACT, artemisinin-based combination therapy; FDA, Food and Drug Administration; ATP, adenosine triphosphate; POC, proof-of-concept; PD, pharmacodynamic; PK, pharmacokinetic; PI4K, phosphatidylinositol 4-kinase; CamK, calmodulin-dependent kinases; CDPK, calcium-dependent protein kinases; SNF1, sucrose nonfermenting; AMPK, adenosine monophosphate-activated kinase; PKG, cyclic guanosine monophosphate (cGMP)-dependent protein kinase; HTS, high throughput screening; *Tg*, *Toxoplasma gondii*; ADMET, absorption, distribution, metabolism, excretion, and toxicology; *Pf*HSP90, *P. falciparum* heat shock protein 90; Bid, bis in die; SAPK2α, stress-activated protein kinase-2 alpha; FGFR1, fibroblast growth factor receptor 1; TrkA, tropomyosin receptor kinase A; RSK1, ribosomal S6 kinase 1; *Cp*, *Cryptosporidium parvum*; Ip, intraperitoneal; AC, aminopyrazole-carboxamide; hERG, human Ether-a-go-go related gene; CaM, calmodulin; TPF, trifluoperazine; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; PKB, protein kinase B; PRR, parasite reduction ratio; NSG, NOD-*scid* gamma; SMFA, standard membrane feeding assay; CDK, cyclin dependent kinase; MAPKs, mitogen-activated protein kinase; GSK-3, glycogen-synthase kinase 3; CLK, CDK-like kinase; NIMA, never in mitosis, gene A; FIKK, Phe (F)−Ile (I)−Lys (K)−Lys (K); PIK, phosphoinositide lipid kinase; PI3K, phosphoinositide 3-kinase; PI4K, phosphatidylinositol 4-kinase; DHA, dihydroartemisinin; GNF, Genomics Institute of the Novartis Research Foundation; NITD, Novartis Institute for Tropical Diseases; UCT, University of Cape Town; H3D, Drug Discovery and Development Centre

■ REFERENCES

(1) World Health Organization (WHO). World Malaria Report. http://www.who.int/malaria/publications/world-malaria-report-2017/report/en/ (accessed Feb 19, 2018).

(2) Blasco, B.; Leroy, D.; Fidock, D. A. Antimalarial Drug Resistance: Linking Plasmodium Falciparum Parasite Biology to the Clinic. *Nat. Med.* **2017**, *23* (8), 917−928.

(3) Dondorp, A. M.; Nosten, F.; Yi, P.; Das, D.; Phyo, A. P.; Tarning, J.; Lwin, K. M.; Ariey, F.; Hanpithakpong, W.; Lee, S. J.; Ringwald, P.; Silamut, K.; Imwong, M.; Chotivanich, K.; Lim, P.; Herdman, T.; An, S. S.; Yeung, S.; Singhasivanon, P.; Day, N. P. J.; Lindegardh, N.; Socheat, D.; White, N. J. Artemisinin Resistance in Plasmodium Falciparum Malaria. *N. Engl. J. Med.* **2009**, *361* (5), 455−467.

(4) Gamo, F. J. Antimalarial Drug Resistance: New Treatments Options for Plasmodium. *Drug Discovery Today: Technol.* **2014**, *11* (1), 81−88.

(5) Phillips, M. A.; Burrows, J. N.; Manyando, C.; van Huijsduijnen, R. H.; Van Voorhis, W. C.; Wells, T. N. C. *Malaria. Nat. Rev. Dis. Prim.* **2017**, *3*, 17050.

(6) Campo, B.; Vandal, O.; Wesche, D. L.; Burrows, J. N. Killing the Hypnozoite − Drug Discovery Approaches to Prevent Relapse in *Plasmodium vivax*. *Pathog. Global Health* **2015**, *109* (3), 107−122.

(7) Reader, J.; Botha, M.; Theron, A.; Lauterbach, S. B.; Rossouw, C.; Engelbrecht, D.; Wepener, M.; Smit, A.; Leroy, D.; Mancama, D.; Coetzer, T. L.; Birkholtz, L. M. Nowhere to Hide: Interrogating Different Metabolic Parameters of Plasmodium Falciparum Gametocytes in a Transmission Blocking Drug Discovery Pipeline towards Malaria Elimination. *Malar. J.* **2015**, *14* (1), 1−17.

(8) Aly, A. S. I.; Vaughan, A. M.; Kappe, S. H. I. Malaria Parasite Development in the Mosquito and Infection of the Mammalian Host. *Annu. Rev. Microbiol.* **2009**, *63* (1), 195−221.
(9) Carvalho, T. G.; Morahan, B.; John von Freyend, S.; Boeuf, P.; Grau, G.; Garcia-Bustos, J.; Doerig, C. The Ins and Outs of Phosphosignalling in Plasmodium: Parasite Regulation and Host Cell Manipulation. *Mol. Biochem. Parasitol.* **2016**, *208* (1), 2−15.
(10) Hubbard, S. R.; Till, J. H. Protein Tyrosine Kinase Structure and Function. *Annu. Rev. Biochem.* **2000**, *69* (1), 373−398.
(11) Rauch, J.; Volinsky, N.; Romano, D.; Kolch, W. The Secret Life of Kinases: Functions beyond Catalysis. *Cell Commun. Signaling* **2011**, *9*, 23.
(12) Huang, M.; Shen, A.; Ding, J.; Geng, M. Molecularly Targeted Cancer Therapy: Some Lessons from the Past Decade. *Trends Pharmacol. Sci.* **2014**, *35* (1), 41−50.
(13) Clark, J. D.; Flanagan, M. E.; Telliez, J. B. Discovery and Development of Janus Kinase (JAK) Inhibitors for Inflammatory Diseases. *J. Med. Chem.* **2014**, *57* (12), 5023−5038.
(14) Kikuchi, R.; Nakamura, K.; Maclauchlan, S.; Ngo, D. T.; Shimizu, I.; Fuster, J. J.; Katanasaka, Y.; Yoshida, S.; Qiu, Y.; Yamaguchi, T. P.; Matsushita, T.; Murohara, T.; Gokce, N.; Bates, D. O.; Hamburg, N. M.; Walsh, K. An Anti-Angiogenic Isoform of VEGF-A Contributes to Impaired Vascularization in Peripheral Artery Disease. *Nat. Med.* **2014**, *20* (12), 1464−1471.
(15) Wu, P.; Nielsen, T. E.; Clausen, M. H. FDA-Approved Small-Molecule Kinase Inhibitors. *Trends Pharmacol. Sci.* **2015**, *36* (7), 422−439.
(16) Doerig, C.; Billker, O.; Haystead, T.; Sharma, P.; Tobin, A. B.; Waters, N. C. Protein Kinases of Malaria Parasites: An Update. *Trends Parasitol.* **2008**, *24* (12), 570−577.
(17) Kappes, B.; Doerig, C.; Graeser, R. An Overview of Plasmodium Protein Kinases. *Parasitol. Today* **1999**, *15* (11), 449−454.
(18) Talevich, E.; Tobin, A. B.; Kannan, N.; Doerig, C. An Evolutionary Perspective on the Kinome of Malaria Parasites. *Philos. Trans. R. Soc., B* **2012**, *367* (1602), 2607−2618.
(19) Kumar, S.; Stecher, G.; Tamura, K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol. Biol. Evol.* **2016**, *33* (7), 1870−1874.
(20) Solyakov, L.; Halbert, J.; Alam, M. M.; Semblat, J.-P.; Dorin-Semblat, D.; Reininger, L.; Bottrill, A. R.; Mistry, S.; Abdi, A.; Fennell, C.; Holland, Z.; Demarta, C.; Bouza, Y.; Sicard, A.; Nivez, M.-P.; Eschenlauer, S.; Lama, T.; Thomas, D. C.; Sharma, P.; Agarwal, S.; Kern, S.; Pradel, G.; Graciotti, M.; Tobin, A. B.; Doerig, C. Global Kinomic and Phospho-Proteomic Analyses of the Human Malaria Parasite Plasmodium Falciparum. *Nat. Commun.* **2011**, *2*, 565.
(21) Bracchi-Ricard, V.; Barik, S.; Delvecchio, C.; Doerig, C.; Chakrabarti, R.; Chakrabarti, D. PfPK6, a Novel Cyclin-Dependent Kinase/Mitogen-Activated Protein Kinase-Related Protein Kinase from Plasmodium Falciparum. *Biochem. J.* **2000**, *347* (1), 255−263.
(22) Dorin, D.; Semblat, J. P.; Poullet, P.; Alano, P.; Goldring, J. P. D.; Whittle, C.; Patterson, S.; Chakrabarti, D.; Doerig, C. PfPK7, an Atypical MEK-Related Protein Kinase, Reflects the Absence of Classical Three-Component MAPK Pathways in the Human Malaria Parasite Plasmodium Falciparum. *Mol. Microbiol.* **2005**, *55* (1), 184−196.
(23) Ward, P.; Equinet, L.; Packer, J.; Doerig, C. Protein Kinases of the Human Malaria Parasite Plasmodium Falciparum: The Kinome of a Divergent Eukaryote. *BMC Genomics* **2004**, *5*, 79.
(24) Tewari, R.; Straschil, U.; Bateman, A.; Böhme, U.; Cherevach, I.; Gong, P.; Pain, A.; Billker, O. The Systematic Functional Analysis of Plasmodium Protein Kinases Identifies Essential Regulators of Mosquito Transmission. *Cell Host Microbe* **2010**, *8* (4), 377−387.
(25) Paquet, T.; Le Manach, C.; Cabrera, D. G.; Younis, Y.; Henrich, P. P.; Abraham, T. S.; Lee, M. C. S.; Basak, R.; Ghidelli-Disse, S.; Lafuente-Monasterio, M. J.; Bantscheff, M.; Ruecker, A.; Blagborough, A. M.; Zakutansky, S. E.; Zeeman, A.-M.; White, K. L.; Shackleford, D. M.; Mannila, J.; Morizzi, J.; Scheurer, C.; Angulo-Barturen, I.; Martínez, M. S.; Ferrer, S.; Sanz, L. M.; Gamo, F. J.; Reader, J.; Botha, M.; Dechering, K. J.; Sauerwein, R. W.; Tungtaeng, A.; Vanachayangkul, P.; Lim, C. S.; Burrows, J.; Witty, M. J.; Marsh, K. C.; Bodenreider, C.; Rochford, R.; Solapure, S. M.; Jiménez-Díaz, M. B.; Wittlin, S.; Charman, S. A.; Donini, C.; Campo, B.; Birkholtz, L.-M.; Hanson, K. K.; Drewes, G.; Kocken, C. H. M.; Delves, M. J.; Leroy, D.; Fidock, D. A.; Waterson, D.; Street, L. J.; Chibale, K. Antimalarial Efficacy of MMV390048, an Inhibitor of *Plasmodium* Phosphatidylinositol 4-Kinase. *Sci. Transl. Med.* **2017**, *9* (387), eaad9735.
(26) Harper, J. F.; Harmon, A. Plants, Symbiosis and Parasites: A Calcium Signalling Connection. *Nat. Rev. Mol. Cell Biol.* **2005**, *6* (7), 555−566.
(27) Wernimont, A. K.; Amani, M.; Qiu, W.; Pizarro, J. C.; Artz, J. D.; Lin, Y. H.; Lew, J.; Hutchinson, A.; Hui, R. Structures of Parasitic CDPK Domains Point to a Common Mechanism of Activation. *Proteins: Struct., Funct., Genet.* **2011**, *79* (3), 803−820.
(28) Bansal, A.; Molina-Cruz, A.; Brzostowski, J.; Mu, J. Plasmodium Falciparum Calcium- Dependent Protein Kinase 2 Is Critical for Male Gametocyte Exflagellation But. *mBio* **2017**, *8* (5), e01656-17.
(29) Li, J. L.; Baker, D. A.; Cox, L. S. Sexual Stage-Specific Expression of a Third Calcium-Dependent Protein Kinase from Plasmodium Falciparum. *Biochim. Biophys. Acta, Gene Struct. Expression* **2000**, *1491* (1−3), 341−349.
(30) Siden-Kiamos, I.; Ecker, A.; Nybäck, S.; Louis, C.; Sinden, R. E.; Billker, O. Plasmodium Berghei Calcium-Dependent Protein Kinase 3 Is Required for Ookinete Gliding Motility and Mosquito Midgut Invasion. *Mol. Microbiol.* **2006**, *60* (6), 1355−1363.
(31) Absalon, S.; Blomqvist, K.; Rudlaff, R. M.; DeLano, T. J.; Pollastri, M. P.; Dvorin, J. D. Calcium-Dependent Protein Kinase 5 Is Required for Release of Egress-Specific Organelles in Plasmodium Falciparum. *mBio* **2018**, *9* (1), e00130-18.
(32) Zuccotto, F.; Ardini, E.; Casale, E.; Angiolini, M. Through the "Gatekeeper Door": Exploiting the Active Kinase Conformation. *J. Med. Chem.* **2010**, *53* (7), 2681−2694.
(33) Bishop, A. C.; Ubersax, J. A.; Petsch, D. T.; Matheos, D. P.; Grayk, N. S.; Blethrow, J.; Shimizu, E.; Tsien, J. Z.; Schultzk, P. G.; Rose, M. D.; Wood, J. L.; Morgan, D. O.; Shokat, K. M. A Chemical Switch for Inhibitor- Sensitive Alleles of Any Protein Kinase. *Nature* **2000**, *407* (6802), 395−401.
(34) Doerig, C.; Meijer, L.; Mottram, J. C. Protein Kinases as Drug Targets in Parasitic Protozoa. *Trends Parasitol.* **2002**, *18* (8), 366−371.
(35) Van Voorhis, W. C.; Doggett, J. S.; Parsons, M.; Hulverson, M. A.; Choi, R.; Arnold, S. L. M.; Riggs, M. W.; Hemphill, A.; Howe, D. K.; Mealey, R. H.; Lau, A. O. T.; Merritt, E. A.; Maly, D. J.; Fan, E.; Ojo, K. K. Extended-Spectrum Antiprotozoal Bumped Kinase Inhibitors: A Review. *Exp. Parasitol.* **2017**, *180*, 71−83.
(36) Kumar, S.; Kumar, M.; Ekka, R.; Dvorin, J. D.; Paul, A. S.; Madugundu, A. K.; Gilberger, T.; Gowda, H.; Duraisingh, M. T.; Keshava Prasad, T. S.; Sharma, P. *Pf*CDPK1 Mediated Signaling in Erythrocytic Stages of Plasmodium Falciparum. *Nat. Commun.* **2017**, *8* (1), 63.
(37) Bansal, A.; Singh, S.; More, K. R.; Hans, D.; Nangalia, K.; Yogavel, M.; Sharma, A.; Chitnis, C. E. Characterization of Plasmodium Falciparum Calcium-Dependent Protein Kinase 1 (PfCDPK1) and Its Role in Microneme Secretion during Erythrocyte Invasion. *J. Biol. Chem.* **2013**, *288* (3), 1590−1602.
(38) Bansal, A.; Molina-Cruz, A.; Brzostowski, J.; Liu, P.; Luo, Y.; Gunalan, K.; Li, Y.; Ribeiro, J. M. C.; Miller, L. H. PfCDPK1 Is Critical for Malaria Parasite Gametogenesis and Mosquito Infection. *Proc. Natl. Acad. Sci. U. S. A.* **2018**, *115* (4), 774−779.
(39) Kato, N.; Sakata, T.; Breton, G.; Le Roch, K. G.; Nagle, A.; Andersen, C.; Bursulaya, B.; Henson, K.; Johnson, J.; Kumar, K. A.; Felix, M.; Mason, D.; McNamara, C.; Plouffe, D.; Vandana Ramachandran, M. S.; Tuntland, T.; Zhou, Y.; Peters, E. C.; Chatterjee, A.; Schultz, P. G.; Ward, G. E.; Gray, N.; Harper, J.; Winzeler, E. A. Gene Expression Signatures and Small-Molecule Compounds Link a Protein Kinase to Plasmodium Falciparum Motility. *Nat. Chem. Biol.* **2008**, *4* (6), 347−356.
(40) Green, J. L.; Moon, R. W.; Whalley, D.; Bowyer, P. W.; Wallace, C.; Rochani, A.; Nageshan, R. K.; Howell, S. A.; Grainger, M.; Jones, H. M.; Ansell, K. H.; Chapman, T. M.; Taylor, D. L.; Osborne, S. A.;

DOI: 10.1021/acs.jmedchem.8b00329
J. Med. Chem. 2018, 61, 8061−8077

Baker, D. A.; Tatu, U.; Holder, A. A. Imidazopyridazine Inhibitors of Plasmodium *falciparum* Calcium-Dependent Protein Kinase 1 Also Target Cyclic GMP-Dependent Protein Kinase and Heat Shock Protein 90 To Kill the Parasite at Different Stages of Intracellular Development. *Antimicrob. Agents Chemother.* **2016**, *60* (3), 1464−1475.
(41) Bansal, A.; Ojo, K. K.; Mu, J.; Maly, D. J.; Van Voorhis, W. C.; Miller, L. H. Reduced Activity of Mutant Calcium-Dependent Protein Kinase 1 Is Compensated in Plasmodium Falciparum through the Action of Protein Kinase G. *mBio* **2016**, *7* (6), e02011-16.
(42) Chapman, T. M.; Osborne, S. A.; Bouloc, N.; Large, J. M.; Wallace, C.; Birchall, K.; Ansell, K. H.; Jones, H. M.; Taylor, D.; Clough, B.; Green, J. L.; Holder, A. A. Substituted Imidazopyridazines Are Potent and Selective Inhibitors of Plasmodium Falciparum Calcium-Dependent Protein Kinase 1 (PfCDPK1). *Bioorg. Med. Chem. Lett.* **2013**, *23* (10), 3064−3069.
(43) Chapman, T. M.; Osborne, S. A.; Wallace, C.; Birchall, K.; Bouloc, N.; Jones, H. M.; Ansell, K. H.; Taylor, D. L.; Clough, B.; Green, J. L.; Holder, A. A. Optimisation of an Imidazopyridazine Series of Inhibitors of Plasmodium Falciparum Calcium-Dependent Protein Kinase 1 (PfCDPK1). *J. Med. Chem.* **2014**, *1*, 3570−3587.
(44) Large, J. M.; Osborne, S. A.; Smiljanic-Hurley, E.; Ansell, K. H.; Jones, H. M.; Taylor, D. L.; Clough, B.; Green, J. L.; Holder, A. A. Imidazopyridazines as Potent Inhibitors of Plasmodium Falciparum Calcium-Dependent Protein Kinase 1 (PfCDPK1): Preparation and Evaluation of Pyrazole Linked Analogues. *Bioorg. Med. Chem. Lett.* **2013**, *23* (21), 6019−6024.
(45) Ansell, K. H.; Jones, H. M.; Whalley, D.; Hearn, A.; Taylor, D. L.; Patin, E. C.; Chapman, T. M.; Osborne, S. A.; Wallace, C.; Birchall, K.; Large, J.; Bouloc, N.; Smiljanic-Hurley, E.; Clough, B.; Moon, R. W.; Green, J. L.; Holder, A. A.; Anseil, K. H.; Jones, H. M.; Whalley, D.; Hearn, A.; Taylor, D. L.; Patin, E. C.; Chapman, T. M.; Osborne, S. A.; Wallace, C.; Birchall, K.; Large, J.; Bouloc, N.; Smiljanic-Hurley, E.; Clough, B.; Moon, R. W.; Green, J. L.; Holder, A. A. Biochemical and Antiparasitic Properties of Inhibitors of the Plasmodium Falciparum Calcium-Dependent Protein Kinase PfCDPK1. *Antimicrob. Agents Chemother.* **2014**, *58* (10), 6032−6043.
(46) Lemercier, G.; Fernandez-Montalvan, A.; Shaw, J. P.; Kugelstadt, D.; Bomke, J.; Domostoj, M.; Schwarz, M. K.; Scheer, A.; Kappes, B.; Leroy, D. Identification and Characterization of Novel Small Molecules as Potent Inhibitors of the Plasmodial Calcium-Dependent Protein Kinase 1. *Biochemistry* **2009**, *48* (27), 6379−6389.
(47) Aher, R. B.; Roy, K. Exploring the Structural Requirements in Multiple Chemical Scaffolds for the Selective Inhibition of Plasmodium Falciparum Calcium-Dependent Protein Kinase-1 (PfCDPK-1) by 3D-Pharmacophore Modelling, and Docking Studies. *SAR QSAR Environ. Res.* **2017**, *28* (5), 390−414.
(48) Fang, H.; Klages, N.; Baechler, B.; Hillner, E.; Yu, L.; Pardo, M.; Choudhary, J.; Brochet, M. Multiple Short Windows of Calcium-Dependent Protein Kinase 4 Activity Coordinate Distinct Cell Cycle Events during Plasmodium Gametogenesis. *eLife* **2017**, *6*, 26524.
(49) Billker, O.; Dechamps, S.; Tewari, R.; Wenig, G.; Franke-Fayard, B.; Brinkmann, V. Calcium and a Calcium-Dependent Protein Kinase Regulate Gamete Formation and Mosquito Transmission in a Malaria Parasite. *Cell* **2004**, *117* (4), 503−514.
(50) Kato, K.; Sudo, A.; Kobayashi, K.; Sugi, T.; Tohya, Y.; Akashi, H. Characterization of Plasmodium Falciparum Calcium-Dependent Protein Kinase 4. *Parasitol. Int.* **2009**, *58* (4), 394−400.
(51) Govindasamy, K.; Jebiwott, S.; Jaijyan, D. K.; Davidow, A.; Ojo, K. K.; Van Voorhis, W. C.; Brochet, M.; Billker, O.; Bhanot, P. Invasion of Hepatocytes by Plasmodium Sporozoites Requires cGMP-Dependent Protein Kinase and Calcium Dependent Protein Kinase 4. *Mol. Microbiol.* **2016**, *102* (2), 349−363.
(52) Vidadala, R. S. R.; Ojo, K. K.; Johnson, S. M.; Zhang, Z.; Leonard, S. E.; Mitra, A.; Choi, R.; Reid, M. C.; Keyloun, K. R.; Fox, A. M. W.; Kennedy, M.; Silver-Brace, T.; Hume, J. C. C.; Kappe, S.; Verlinde, C. L. M. J.; Fan, E.; Merritt, E. A.; Van Voorhis, W. C.; Maly, D. J. Development of Potent and Selective Plasmodium Falciparum Calcium-Dependent Protein Kinase 4 (PfCDPK4) Inhibitors That Block the Transmission of Malaria to Mosquitoes. *Eur. J. Med. Chem.* **2014**, *74*, 562−573.
(53) Ojo, K. K.; Eastman, R. T.; Vidadala, R.; Zhang, Z.; Rivas, K. L.; Choi, R.; Lutz, J. D.; Reid, M. C.; Fox, A. M. W.; Hulverson, M. A.; Kennedy, M.; Isoherranen, N.; Kim, L. M.; Comess, K. M.; Kempf, D. J.; Verlinde, C. L. M. J.; Su, X. Z.; Kappe, S. H. I.; Maly, D. J.; Fan, E.; Van Voorhis, W. C. A Specific Inhibitor of PfCDPK4 Blocks Malaria Transmission: Chemical-Genetic Validation. *J. Infect. Dis.* **2014**, *209* (2), 275−284.
(54) Ojo, K. K.; Larson, E. T.; Keyloun, K. R.; Castaneda, L. J.; Derocher, A. E.; Inampudi, K. K.; Kim, J. E.; Arakaki, T. L.; Murphy, R. C.; Zhang, L.; Napuli, A. J.; Maly, D. J.; Verlinde, C. L. M. J.; Buckner, F. S.; Parsons, M.; Hol, W. G. J.; Merritt, E. A.; Van Voorhis, W. C. Toxoplasma Gondii Calcium-Dependent Protein Kinase 1 Is a Target for Selective Kinase Inhibitors. *Nat. Struct. Mol. Biol.* **2010**, *17* (5), 602−607.
(55) Murphy, R. C.; Ojo, K. K.; Larson, E. T.; Castellanos-Gonzalez, A.; Perera, B. G. K.; Keyloun, K. R.; Kim, J. E.; Bhandari, J. G.; Muller, N. R.; Verlinde, C. L. M. J.; White, A. C.; Merritt, E. A.; Van Voorhis, W. C.; Maly, D. J. Discovery of Potent and Selective Inhibitors of CDPK1 from C. Parvum and T. Gondii. *ACS Med. Chem. Lett.* **2010**, *1* (7), 331−335.
(56) Ojo, K. K.; Pfander, C.; Mueller, N. R.; Burstroem, C.; Larson, E. T.; Bryan, C. M.; Fox, A. M. W.; Reid, M. C.; Johnson, S. M.; Murphy, R. C.; Kennedy, M.; Mann, H.; Leibly, D. J.; Hewitt, S. N.; Verlinde, C. L. M. J.; Kappe, S.; Merritt, E. A.; Maly, D. J.; Billker, O.; Van Voorhis, W. C. Transmission of Malaria to Mosquitoes Blocked by Bumped Kinase Inhibitors. *J. Clin. Invest.* **2012**, *122* (6), 2301−2305.
(57) Zhang, Z.; Ojo, K. K.; Vidadala, R.; Huang, W.; Geiger, J. A.; Scheele, S.; Choi, R.; Reid, M. C.; Keyloun, K. R.; Rivas, K.; Kallur Siddaramaiah, L.; Comess, K. M.; Robinson, K. P.; Merta, P. J.; Kifle, L.; Hol, W. G. J.; Parsons, M.; Merritt, E. A.; Maly, D. J.; Verlinde, C. L. M. J.; Van Voorhis, W. C.; Fan, E. Potent and Selective Inhibitors of CDPK1 from T. Gondii and C. Parvum Based on a 5-Aminopyrazole-4-Carboxamide Scaffold. *ACS Med. Chem. Lett.* **2014**, *5* (1), 40−44.
(58) Huang, W.; Ojo, K. K.; Zhang, Z.; Rivas, K.; Vidadala, R. S. R.; Scheele, S.; DeRocher, A. E.; Choi, R.; Hulverson, M. A.; Barrett, L. K.; Bruzual, I.; Siddaramaiah, L. K.; Kerchner, K. M.; Kurnick, M. D.; Freiberg, G. M.; Kempf, D.; Hol, W. G. J.; Merritt, E. A.; Neckermann, G.; de Hostos, E. L.; Isoherranen, N.; Maly, D. J.; Parsons, M.; Doggett, J. S.; Van Voorhis, W. C.; Fan, E. SAR Studies of 5-Aminopyrazole-4-Carboxamide Analogues as Potent and Selective Inhibitors of Toxoplasma Gondii CDPK1. *ACS Med. Chem. Lett.* **2015**, *6* (12), 1184−1189.
(59) Huang, W.; Hulverson, M. A.; Zhang, Z.; Choi, R.; Hart, K. J.; Kennedy, M.; Vidadala, R. S. R.; Maly, D. J.; Van Voorhis, W. C.; Lindner, S. E.; Fan, E.; Ojo, K. K. 5-Aminopyrazole-4-Carboxamide Analogues Are Selective Inhibitors of Plasmodium Falciparum Microgametocyte Exflagellation and Potential Malaria Transmission Blocking Agents. *Bioorg. Med. Chem. Lett.* **2016**, *26* (22), 5487−5491.
(60) Cavagnino, A.; Rossi, F.; Rizzi, M. The Potent Antiplasmodial Calmodulin-Antagonist Trifluoperazine Inhibits Plasmodium Falciparum Calcium-Dependent Protein Kinase 4. *Protein Pept. Lett.* **2011**, *18* (12), 1273−1279.
(61) Scheibel, L. W.; Colombani, P. M.; Hess, A. D.; Aikawa, M.; Atkinson, C. T.; Milhous, W. K. Calcium and Calmodulin Antagonists Inhibit Human Malaria Parasites (Plasmodium Falciparum): Implications for Drug Design. *Proc. Natl. Acad. Sci. U. S. A.* **1987**, *84* (20), 7310−7314.
(62) Alam, M. M.; Solyakov, L.; Bottrill, A. R.; Flueck, C.; Siddiqui, F. A.; Singh, S.; Mistry, S.; Viskaduraki, M.; Lee, K.; Hopp, C. S.; Chitnis, C. E.; Doerig, C.; Moon, R. W.; Green, J. L.; Holder, A. A.; Baker, D. A.; Tobin, A. B. Phosphoproteomics Reveals Malaria Parasite Protein Kinase G as a Signalling Hub Regulating Egress and Invasion. *Nat. Commun.* **2015**, *6*, 7285.
(63) Baker, D. A.; Stewart, L. B.; Large, J. M.; Bowyer, P. W.; Ansell, K. H.; Jiménez-Díaz, M. B.; El Bakkouri, M.; Birchall, K.; Dechering, K. J.; Bouloc, N. S.; Coombs, P. J.; Whalley, D.; Harding, D. J.; Smiljanic-

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Hurley, E.; Wheldon, M. C.; Walker, E. M.; Dessens, J. T.; Lafuente, M. J.; Sanz, L. M.; Gamo, F.-J.; Ferrer, S. B.; Hui, R.; Bousema, T.; Angulo-Barturén, I.; Merritt, A. T.; Croft, S. L.; Gutteridge, W. E.; Kettleborough, C. A.; Osborne, S. A. A Potent Series Targeting the Malarial CGMP-Dependent Protein Kinase Clears Infection and Blocks Transmission. *Nat. Commun.* **2017**, *8* (1), 430.

(64) Hale, V. L.; Watermeyer, J. M.; Hackett, F.; Vizcay-Barrena, G.; van Ooij, C.; Thomas, J. A.; Spink, M. C.; Harkiolaki, M.; Duke, E.; Fleck, R. A.; Blackman, M. J.; Saibil, H. R. Parasitophorous Vacuole Poration Precedes Its Rupture and Rapid Host Erythrocyte Cytoskeleton Collapse in Plasmodium Falciparum Egress. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114* (13), 3439−3444.

(65) Collins, C. R.; Hackett, F.; Strath, M.; Penzo, M.; Withers-Martinez, C.; Baker, D. A.; Blackman, M. J. Malaria Parasite cGMP-Dependent Protein Kinase Regulates Blood Stage Merozoite Secretory Organelle Discharge and Egress. *PLoS Pathog.* **2013**, *9* (5), e1003344.

(66) Dvorin, J. D.; Martyn, D. C.; Patel, S. D.; Grimley, J. S.; Collins, C. R.; Hopp, C. S.; Bright, A. T.; Westenberger, S.; Winzeler, E.; Blackman, M. J.; Baker, D. A.; Wandless, T. J.; Duraisingh, M. T. A Plant-Like Kinase in Plasmodium Falciparum Regulates Parasite Egress from Erythrocytes. *Science* **2010**, *328* (5980), 910−912.

(67) Taylor, H. M.; McRobert, L.; Grainger, M.; Sicard, A.; Dluzewski, A. R.; Hopp, C. S.; Holder, A. A.; Baker, D. A. The Malaria Parasite Cyclic GMP-Dependent Protein Kinase Plays a Central Role in Blood-Stage Schizogony. *Eukaryotic Cell* **2010**, *9* (1), 37−45.

(68) McRobert, L.; Taylor, C. J.; Deng, W.; Fivelman, Q. L.; Cummings, R. M.; Polley, S. D.; Billker, O.; Baker, D. A. Gametogenesis in Malaria Parasites Is Mediated by the cGMP-Dependent Protein Kinase. *PLoS Biol.* **2008**, *6* (6), e139.

(69) Brochet, M.; Collins, M. O.; Smith, T. K.; Thompson, E.; Sebastian, S.; Volkmann, K.; Schwach, F.; Chappell, L.; Gomes, A. R.; Berriman, M.; Rayner, J. C.; Baker, D. A.; Choudhary, J.; Billker, O. Phosphoinositide Metabolism Links cGMP-Dependent Protein Kinase G to Essential Ca2+ Signals at Key Decision Points in the Life Cycle of Malaria Parasites. *PLoS Biol.* **2014**, *12* (3), e1001806.

(70) Falae, A.; Combe, A.; Amaladoss, A.; Carvalho, T.; Menard, R.; Bhanot, P. Role of Plasmodium Berghei CGMP-Dependent Protein Kinase in Late Liver Stage Development. *J. Biol. Chem.* **2010**, *285* (5), 3282−3288.

(71) Biftu, T.; Feng, D.; Fisher, M.; Liang, G. B.; Qian, X.; Scribner, A.; Dennis, R.; Lee, S.; Liberator, P. A.; Brown, C.; Gurnett, A.; Leavitt, P. S.; Thompson, D.; Mathew, J.; Misura, A.; Samaras, S.; Tamas, T.; Sina, J. F.; McNulty, K. A.; McKnight, C. G.; Schmatz, D. M.; Wyvratt, M. Synthesis and SAR Studies of Very Potent Imidazopyridine Antiprotozoal Agents. *Bioorg. Med. Chem. Lett.* **2006**, *16* (9), 2479−2483.

(72) Churcher, T. S.; Blagborough, A. M.; Delves, M.; Ramakrishnan, C.; Kapulu, M. C.; Williams, A. R.; Biswas, S.; Da, D. F.; Cohuet, A.; Sinden, R. E. Measuring the Blockade of Malaria Transmission - An Analysis of the Standard Membrane Feeding Assay. *Int. J. Parasitol.* **2012**, *42* (11), 1037−1044.

(73) Biftu, T.; Feng, D.; Ponpipom, M.; Girotra, N.; Liang, G. B.; Qian, X.; Bugianesi, R.; Simeone, J.; Chang, L.; Gurnett, A.; Liberator, P.; Dulski, P.; Leavitt, P. S.; Crumley, T.; Misura, A.; Murphy, T.; Rattray, S.; Samaras, S.; Tamas, T.; Mathew, J.; Brown, C.; Thompson, D.; Schmatz, D.; Fisher, M.; Wyvratt, M. Synthesis and SAR of 2,3-Diarylpyrrole Inhibitors of Parasite cGMP-Dependent Protein Kinase as Novel Anticoccidial Agents. *Bioorg. Med. Chem. Lett.* **2005**, *15* (13), 3296−3301.

(74) Diaz, C. A.; Allocco, J.; Powles, M. A.; Yeung, L.; Donald, R. G. K.; Anderson, J. W.; Liberator, P. A. Characterization of Plasmodium Falciparum CGMP-Dependent Protein Kinase (PfPKG): Antiparasitic Activity of a PKG Inhibitor. *Mol. Biochem. Parasitol.* **2006**, *146* (1), 78−88.

(75) Panchal, D.; Bhanot, P. Activity of a Trisubstituted Pyrrole in Inhibiting Sporozoite Invasion and Blocking Malaria Infection. *Antimicrob. Agents Chemother.* **2010**, *54* (10), 4269−4274.

(76) Littler, D. R.; Bullen, H. E.; Harvey, K. L.; Beddoe, T.; Crabb, B. S.; Rossjohn, J.; Gilson, P. R. Disrupting the Allosteric Interaction between the Plasmodium Falciparum cAMP-Dependent Kinase and Its Regulatory Subunit. *J. Biol. Chem.* **2016**, *291* (49), 25375−25386.

(77) Leykauf, K.; Treeck, M.; Gilson, P. R.; Nebl, T.; Braulke, T.; Cowman, A. F.; Gilberger, T. W.; Crabb, B. S. Protein Kinase a Dependent Phosphorylation of Apical Membrane Antigen 1 Plays an Important Role in Erythrocyte Invasion by the Malaria Parasite. *PLoS Pathog.* **2010**, *6* (6), e1000941.

(78) Lasonder, E.; Green, J. L.; Camarda, G.; Talabani, H.; Holder, A. A.; Langsley, G.; Alano, P. The Plasmodium Falciparum Schizont Phosphoproteome Reveals Extensive Phosphatidylinositol and cAMP-Protein Kinase A Signaling. *J. Proteome Res.* **2012**, *11* (11), 5323−5337.

(79) Lasonder, E.; Green, J. L.; Grainger, M.; Langsley, G.; Holder, A. A. Extensive Differential Protein Phosphorylation as Intra-erythrocytic Plasmodium Falciparum Schizonts Develop into Extracellular Invasive Merozoites. *Proteomics* **2015**, *15* (15), 2716−2729.

(80) Dawn, A.; Singh, S.; More, K. R.; Siddiqui, F. A.; Pachikara, N.; Ramdani, G.; Langsley, G.; Chitnis, C. E. The Central Role of cAMP in Regulating Plasmodium Falciparum Merozoite Invasion of Human Erythrocytes. *PLoS Pathog.* **2014**, *10* (12), e1004520.

(81) Beraldo, F. H.; Almeida, F. M.; Da Silva, A. M.; Garcia, C. R. S. Cyclic AMP and Calcium Interplay as Second Messengers in Melatonin-Dependent Regulation of Plasmodium Falciparum Cell Cycle. *J. Cell Biol.* **2005**, *170* (4), 551−557.

(82) Sudo, A.; Kato, K.; Kobayashi, K.; Tohya, Y.; Akashi, H. Susceptibility of Plasmodium Falciparum Cyclic AMP-Dependent Protein Kinase and Its Mammalian Homologue to the Inhibitors. *Mol. Biochem. Parasitol.* **2008**, *160* (2), 138−142.

(83) Merckx, A.; Nivez, M. P.; Bouyer, G.; Alano, P.; Langsley, G.; Deitsch, K.; Thomas, S.; Doerig, C.; Egée, S. Plasmodium Falciparum Regulatory Subunit of CAMP-Dependent PKA and Anion Channel Conductance. *PLoS Pathog.* **2008**, *4* (2), e19.

(84) Buskes, M. J.; Harvey, K. L.; Prinz, B.; Crabb, B. S.; Gilson, P. R.; Wilson, D. J. D.; Abbott, B. M. Exploration of 3-Methylisoquinoline-4-Carbonitriles as Protein Kinase A Inhibitors of Plasmodium Falciparum. *Bioorg. Med. Chem.* **2016**, *24* (11), 2389−2396.

(85) Buskes, M. J.; Harvey, K. L.; Richards, B. J.; Kalhor, R.; Christoff, R. M.; Gardhi, C. K.; Littler, D. R.; Cope, E. D.; Prinz, B.; Weiss, G. E.; O'Brien, N. J.; Crabb, B. S.; Deady, L. W.; Gilson, P. R.; Abbott, B. M. Antimalarial Activity of Novel 4-Cyano-3-Methylisoquinoline Inhibitors against Plasmodium Falciparum: Design, Synthesis and Biological Evaluation. *Org. Biomol. Chem.* **2016**, *14* (20), 4617−4639.

(86) Doerig, C.; Billker, O.; Pratt, D.; Endicott, J. Protein Kinases as Targets for Antimalarial Intervention: Kinomics, Structure-Based Design, Transmission-Blockade, and Targeting Host Cell Enzymes. *Biochim. Biophys. Acta, Proteins Proteomics* **2005**, *1754* (1−2), 132−150.

(87) Li, J. L.; Robson, K. J.; Chen, J. L.; Targett, G. A.; Baker, D. A. Pfmrk, a MO15-Related Protein Kinase from Plasmodium Falciparum. Gene Cloning, Sequence, Stage-Specific Expression and Chromosome Localization. *Eur. J. Biochem.* **1996**, *241* (3), 805−813.

(88) Jirage, D.; Chen, Y.; Caridha, D.; O'Neil, M. T.; Eyase, F.; Witola, W. H.; Ben Mamoun, C.; Waters, N. C. The Malarial CDK Pfmrk and Its Effector PfMAT1 Phosphorylate DNA Replication Proteins and Co-Localize in the Nucleus. *Mol. Biochem. Parasitol.* **2010**, *172* (1), 9−18.

(89) Xiao, Z.; Waters, N. C.; Woodard, C. L.; Li, Z.; Li, P. K. Design and Synthesis of Pfmrk Inhibitors as Potential Antimalarial Agents. *Bioorg. Med. Chem. Lett.* **2001**, *11* (21), 2875−2878.

(90) Woodard, C. L.; Li, Z.; Kathcart, A. K.; Terrell, J.; Gerena, L.; Lopez-Sanchez, M.; Kyle, D. E.; Bhattacharjee, A. K.; Nichols, D. A.; Ellis, W.; Prigge, S. T.; Geyer, J. A.; Waters, N. C. Oxindole-Based Compounds Are Selective Inhibitors of Plasmodium Falciparum Cyclin Dependent Protein Kinases. *J. Med. Chem.* **2003**, *46* (18), 3877−3882.

DOI: 10.1021/acs.jmedchem.8b00329
J. Med. Chem. 2018, 61, 8061−8077

(91) Bhattacharjee, A. K.; Geyer, J. A.; Woodard, C. L.; Kathcart, A. K.; Nichols, D. A.; Prigge, S. T.; Li, Z.; Mott, B. T.; Waters, N. C. A Three-Dimensional in Silico Pharmacophore Model for Inhibition of Plasmodium Falciparum Cyclin-Dependent Kinases and Discovery of Different Classes of Novel Pfmrk Specific Inhibitors. *J. Med. Chem.* **2004**, *47* (22), 5418−5426.
(92) Geyer, J. A.; Keenan, S. M.; Woodard, C. L.; Thompson, P. A.; Gerena, L.; Nichols, D. A.; Gutteridge, C. E.; Waters, N. C. Selective Inhibition of Pfmrk, a Plasmodium Falciparum CDK, by Antimalarial 1,3-Diaryl-2-Propenones. *Bioorg. Med. Chem. Lett.* **2009**, *19* (7), 1982−1985.
(93) Caridha, D.; Kathcart, A. K.; Jirage, D.; Waters, N. C. Activity of Substituted Thiophene Sulfonamides against Malarial and Mammalian Cyclin Dependent Protein Kinases. *Bioorg. Med. Chem. Lett.* **2010**, *20* (13), 3863−3867.
(94) Akala, H. M.; Waters, C. N.; Yenesew, A.; Wanjala, C.; Akenga, T. A. In Vitro Antiplasmodial and Cyclin- Dependent Protein Kinase (Pfmrk) Inhibitory Activities of Sele c Ted Flavonoids in Combination with Chloroquine (CQ) and Artemisinin. *Res. Pharm. Biotechnol.* **2010**, *2* (4), 40−50.
(95) Droucheau, E.; Primot, A.; Thomas, V.; Mattei, D.; Knockaert, M.; Richardson, C.; Sallicandro, P.; Alano, P.; Jafarshad, A.; Baratte, B.; Kunick, C.; Parzy, D.; Pearl, L. H.; Doerig, C.; Meijer, L. Plasmodium Falciparum Glycogen Synthase Kinase-3: Molecular Model, Expression, Intracellular Localisation and Selective Inhibitors. *Biochim. Biophys. Acta, Proteins Proteomics* **2004**, *1697* (1−2), 181−196.
(96) Masch, A.; Kunick, C. Selective Inhibitors of Plasmodium Falciparum Glycogen Synthase-3 (PfGSK-3): New Antimalarial Agents? *Biochim. Biophys. Acta, Proteins Proteomics* **2015**, *1854* (10), 1644−1649.
(97) Fugel, W.; Oberholzer, A. E.; Gschloessl, B.; Dzikowski, R.; Pressburger, N.; Preu, L.; Pearl, L. H.; Baratte, B.; Ratin, M.; Okun, I.; Doerig, C.; Kruggel, S.; Lemcke, T.; Meijer, L.; Kunick, C. 3,6-Diamino-4-(2-Halophenyl)-2-Benzoylthieno[2,3- b]Pyridine-5- Carbonitriles Are Selective Inhibitors of Plasmodium Falciparum Glycogen Synthase Kinase-3. *J. Med. Chem.* **2013**, *56* (1), 264−275.
(98) Reininger, L.; Garcia, M.; Tomlins, A.; Müller, S.; Doerig, C. The Plasmodium Falciparum, Nima-Related Kinase Pfnek-4: A Marker for Asexual Parasites Committed to Sexual Differentiation. *Malar. J.* **2012**, *11*, 250.
(99) Fry, A. M.; Bayliss, R.; Roig, J. Mitotic Regulation by NEK Kinase Networks. *Front. Cell Dev. Biol.* **2017**, *5*, 102.
(100) Dorin-Semblat, D.; Schmitt, S.; Semblat, J. P.; Sicard, A.; Reininger, L.; Goldring, D.; Patterson, S.; Quashie, N.; Chakrabarti, D.; Meijer, L.; Doerig, C. Plasmodium Falciparum NIMA-Related Kinase Pfnek-1: Sex Specificity and Assessment of Essentiality for the Erythrocytic Asexual Cycle. *Microbiology* **2011**, *157* (10), 2785−2794.
(101) Desoubzdanne, D.; Marcourt, L.; Raux, R.; Chevalley, S.; Dorin, D.; Doerig, C.; Valentin, A.; Ausseil, F.; Debitus, C. Alisiaquinones and Alisiaquinol, Dual Inhibitors of Plasmodium Falciparum Enzyme Targets from a New Caledonian Deep Water Sponge Alisiaquinones and Alisiaquinol, Dual Inhibitors of Plasmodium Falciparum Enzyme Targets from a New Caledonian Deep Water Sp. *J. Nat. Prod.* **2008**, *71* (7), 1189−1192.
(102) Lebouvier, N.; Jullian, V.; Desvignes, I.; Maurel, S.; Parenty, A.; Dorin-Semblat, D.; Doerig, C.; Sauvain, M.; Laurent, D. Antiplasmodial Activities of Homogentisic Acid Derivative Protein Kinase Inhibitors Isolated from a Vanuatu Marine Sponge Pseudoceratina Sp. *Mar. Drugs* **2009**, *7* (4), 640−653.
(103) Laurent, D.; Jullian, V.; Parenty, A.; Knibiehler, M.; Dorin, D.; Schmitt, S.; Lozach, O.; Lebouvier, N.; Frostin, M.; Alby, F.; Maurel, S.; Doerig, C.; Meijer, L.; Sauvain, M. Antimalarial Potential of Xestoquinone, a Protein Kinase Inhibitor Isolated from a Vanuatu Marine Sponge Xestospongia Sp. *Bioorg. Med. Chem.* **2006**, *14* (13), 4477−4482.
(104) Mitcheson, D. F.; Bottrill, A. R.; Carr, K.; Coxon, C. R.; Cano, C.; Golding, B. T.; Griffin, R. J.; Fry, A. M.; Doerig, C.; Bayliss, R.; Tobin, A. B. A New Tool for the Chemical Genetic Investigation of the Plasmodium Falciparum Pfnek-2 NIMA-Related Kinase. *Malar. J.* **2016**, *15* (1), 535.
(105) Wells, C. I.; Kapadia, N. R.; Counago, R. M.; Drewry, D. H. In Depth Analysis of Kinase Cross Screening Data to Identify Chemical Starting Points for Inhibition of the Nek Family of Kinases. *MedChemComm* **2018**, *9*, 44−66.
(106) Nunes, M. C.; Goldring, J. P. D.; Doerig, C.; Scherf, A. A Novel Protein Kinase Family in Plasmodium Falciparum Is Differentially Transcribed and Secreted to Various Cellular Compartments of the Host Cell. *Mol. Microbiol.* **2007**, *63* (2), 391−403.
(107) Jaijyan, D. K.; Verma, P. K.; Singh, A. P. A Novel FIKK Kinase Regulates the Development of Mosquito and Liver Stages of the Malaria. *Sci. Rep.* **2016**, *6*, 39285.
(108) Kats, L. M.; Fernandez, K. M.; Glenister, F. K.; Herrmann, S.; Buckingham, D. W.; Siddiqui, G.; Sharma, L.; Bamert, R.; Lucet, I.; Guillotte, M.; Mercereau-Puijalon, O.; Cooke, B. M. An Exported Kinase (FIKK4.2) That Mediates Virulence-Associated Changes in Plasmodium Falciparum-Infected Red Blood Cells. *Int. J. Parasitol.* **2014**, *44* (5), 319−328.
(109) Nunes, M. C.; Okada, M.; Scheidig-Benatar, C.; Cooke, B. M.; Scherf, A. Plasmodium Falciparum Fikk Kinase Members Target Distinct Components of the Erythrocyte Membrane. *PLoS One* **2010**, *5* (7), e11747.
(110) Yuan, J.; Johnson, R. L.; Huang, R.; Wichterman, J.; Jiang, H.; Hayton, K.; Fidock, D. A.; Wellems, T. E.; Inglese, J.; Austin, C. P.; Su, X. Z. Genetic Mapping of Targets Mediating Differential Chemical Phenotypes in Plasmodium Falciparum. *Nat. Chem. Biol.* **2009**, *5* (10), 765−771.
(111) Lin, B. C.; Harris, D. R.; Kirkman, L. M. D.; Perez, A. M.; Qian, Y.; Schermerhorn, J. T.; Hong, M. Y.; Winston, D. S.; Xu, L.; Lieber, A. M.; Hamilton, M.; Brandt, G. S. The Anthraquinone Emodin Inhibits the Non-Exported FIKK Kinase from Plasmodium Falciparum. *Bioorg. Chem.* **2017**, *75*, 217−223.
(112) Lin, B. C.; Harris, D. R.; Kirkman, L. M. D.; Perez, A. M.; Qian, Y.; Schermerhorn, J. T.; Hong, M. Y.; Winston, D. S.; Xu, L.; Brandt, G. S. FIKK Kinase, a Ser/Thr Kinase Important to Malaria Parasites, Is Inhibited by Tyrosine Kinase Inhibitors. *ACS Omega* **2017**, *2* (10), 6605−6612.
(113) Osman, K. T.; Ye, J.; Shi, Z.; Toker, C.; Lovato, D.; Jumani, R. S.; Zuercher, W.; Huston, C. D.; Edwards, A. M.; Lautens, M.; Santhakumar, V.; Hui, R. Discovery and Structure Activity Relationship of the First Potent Cryptosporidium FIKK Kinase Inhibitor. *Bioorg. Med. Chem.* **2017**, *25* (5), 1672−1680.
(114) Brown, J. R.; Auger, K. R. Phylogenomics of Phosphoinositide Lipid Kinases: Perspectives on the Evolution of Second Messenger Signaling and Drug Discovery. *BMC Evol. Biol.* **2011**, *11* (1), 4.
(115) McNamara, C. W.; Lee, M. C. S.; Lim, C. S.; Lim, S. H.; Roland, J.; Nagle, A.; Simon, O.; Yeung, B. K. S.; Chatterjee, A. K.; McCormack, S. L.; Manary, M. J.; Zeeman, A. M.; Dechering, K. J.; Kumar, T. R. S.; Henrich, P. P.; Gagaring, K.; Ibanez, M.; Kato, N.; Kuhen, K. L.; Fischli, C.; Rottmann, M.; Plouffe, D. M.; Bursulaya, B.; Meister, S.; Rameh, L.; Trappe, J.; Haasen, D.; Timmerman, M.; Sauerwein, R. W.; Suwanarusk, R.; Russell, B.; Renia, L.; Nosten, F.; Tully, D. C.; Kocken, C. H. M.; Glynne, R. J.; Bodenreider, C.; Fidock, D. A.; Diagana, T. T.; Winzeler, E. A. Targeting Plasmodium PI(4)K to Eliminate Malaria. *Nature* **2013**, *504* (7479), 248−253.
(116) Hassett, M. R.; Sternberg, A. R.; Riegel, B. E.; Thomas, C. J.; Roepe, P. D. Heterologous Expression, Purification, and Functional Analysis of Plasmodium Falciparum Phosphatidylinositol 3′-Kinase. *Biochemistry* **2017**, *56*, 4335−4345.
(117) Vaid, A.; Ranjan, R.; Smythe, W. A.; Hoppe, H. C.; Sharma, P. PfPI3K, a Phosphatidylinositol-3 Kinase from Plasmodium Falciparum, Is Exported to the Host Erythrocyte and Is Involved in Hemoglobin Trafficking. *Blood* **2010**, *115* (12), 2500−2507.
(118) Bozdech, Z.; Ferreira, P. E.; Mok, S. A Crucial Piece in the Puzzle of the Artemisinin Resistance Mechanism in Plasmodium Falciparum. *Trends Parasitol.* **2015**, *31* (8), 345−346.
(119) Mbengue, A.; Bhattacharjee, S.; Pandharkar, T.; Liu, H.; Estiu, G.; Stahelin, R. V.; Rizk, S. S.; Njimoh, D. L.; Ryan, Y.; Chotivanich,

DOI: 10.1021/acs.jmedchem.8b00329
J. Med. Chem. 2018, 61, 8061−8077

K.; Nguon, C.; Ghorbal, M.; Lopez-Rubio, J.-J.; Pfrender, M.; Emrich, S.; Mohandas, N.; Dondorp, A. M.; Wiest, O.; Haldar, K. A Molecular Mechanism of Artemisinin Resistance in Plasmodium Falciparum Malaria. *Nature* **2015**, *520* (7549), 683−687.

(120) Zou, B.; Nagle, A.; Chatterjee, A. K.; Leong, S. Y.; Tan, L. J.; Sim, W. L. S.; Mishra, P.; Guntapalli, P.; Tully, D. C.; Lakshminarayana, S. B.; Lim, C. S.; Tan, Y. C.; Abas, S. N.; Bodenreider, C.; Kuhen, K. L.; Gagaring, K.; Borboa, R.; Chang, J.; Li, C.; Hollenbeck, T.; Tuntland, T.; Zeeman, A. M.; Kocken, C. H. M.; McNamara, C.; Kato, N.; Winzeler, E. A.; Yeung, B. K. S.; Diagana, T. T.; Smith, P. W.; Roland, J. Lead Optimization of Imidazopyrazines: A New Class of Antimalarial with Activity on Plasmodium Liver Stages. *ACS Med. Chem. Lett.* **2014**, *5* (8), 947−950.

(121) Younis, Y.; Douelle, F.; Feng, T. S.; Cabrera, D. G.; Le Manach, C.; Nchinda, A. T.; Duffy, S.; White, K. L.; Shackleford, D. M.; Morizzi, J.; Mannila, J.; Katneni, K.; Bhamidipati, R.; Zabiulla, K. M.; Joseph, J. T.; Bashyam, S.; Waterson, D.; Witty, M. J.; Hardick, D.; Wittlin, S.; Avery, V.; Charman, S. A. Chibale, K. 3,5-Diaryl-2-Aminopyridines As a Novel Class of Orally Active Antimalarials Demonstrating Single Dose Cure in Mice and Clinical Candidate Potential. *J. Med. Chem.* **2012**, *55* (7), 3479−3487.

(122) Younis, Y.; Douelle, F.; González Cabrera, D.; Le Manach, C.; Nchinda, A. T.; Paquet, T.; Street, L. J.; White, K. L.; Zabiulla, K. M.; Joseph, J. T.; Bashyam, S.; Waterson, D.; Witty, M. J.; Wittlin, S.; Charman, S. A.; Chibale, K. Structure-Activity-Relationship Studies around the 2-Amino Group and Pyridine Core of Antimalarial 3,5-Diarylaminopyridines Lead to a Novel Series of Pyrazine Analogues with Oral in Vivo Activity. *J. Med. Chem.* **2013**, *56* (21), 8860−8871.

(123) Le Manach, C.; Nchinda, A. T.; Paquet, T.; Gonzàlez Cabrera, D.; Younis, Y.; Han, Z.; Bashyam, S.; Zabiulla, M.; Taylor, D.; Lawrence, N.; White, K. L.; Charman, S. A.; Waterson, D.; Witty, M. J.; Wittlin, S.; Botha, M. E.; Nondaba, S. H.; Reader, J.; Birkholtz, L. M.; Jiménez-Díaz, M. B.; Martínez, M. S.; Ferrer, S.; Angulo-Barturen, I.; Meister, S.; Antonova-Koch, Y.; Winzeler, E. A.; Street, L. J.; Chibale, K. Identification of a Potential Antimalarial Drug Candidate from a Series of 2-Aminopyrazines by Optimization of Aqueous Solubility and Potency across the Parasite Life Cycle. *J. Med. Chem.* **2016**, *59* (21), 9890−9905.

(124) Medicines for Malaria Venture (MMV). MMV048. https://www.mmv.org/research-development/project-portfolio/mmv048 (accessed Feb 27, 2018).

(125) Ren, J. X.; Gao, N. N.; Cao, X. S.; Hu, Q. A.; Xie, Y. Homology Modeling and Virtual Screening for Inhibitors of Lipid Kinase PI(4)K from Plasmodium. *Biomed. Pharmacother.* **2016**, *83*, 798−808.

(126) Lelliott, P. M.; McMorran, B. J.; Foote, S. J.; Burgio, G. The Influence of Host Genetics on Erythrocytes and Malaria Infection: Is There Therapeutic Potential? *Malar. J.* **2015**, *14* (1), 289.

(127) Kesely, K. R.; Pantaleo, A.; Turrini, F. M.; Olupot-Olupot, P.; Low, P. S. Inhibition of an Erythrocyte Tyrosine Kinase with Imatinib Prevents Plasmodium Falciparum Egress and Terminates Parasitemia. *PLoS One* **2016**, *11* (10), e0164895.

(128) Prudêncio, M.; Rodrigues, C. D.; Hannus, M.; Martin, C.; Real, E.; Gonçalves, L. A.; Carret, C.; Dorkin, R.; Röhl, I.; Jahn-Hoffmann, K.; Luty, A. J. F.; Sauerwein, R.; Echeverri, C. J.; Mota, M. M. Kinome-Wide RNAi Screen Implicates at Least 5 Host Hepatocyte Kinases in Plasmodium Sporozoite Infection. *PLoS Pathog.* **2008**, *4* (11), e1000201.

(129) Prudêncio, M.; Mota, M. M. Targeting Host Factors to Circumvent Anti-Malarial Drug Resistance. *Curr. Pharm. Des.* **2012**, *19* (2), 290−299.

(130) Wu, P.; Nielsen, T. E.; Clausen, M. H. Small-Molecule Kinase Inhibitors: An Analysis of FDA-Approved Drugs. Drug Discov. *Drug Discovery Today* **2016**, *21* (1), 5−10.

DOI: 10.1021/acs.jmedchem.8b00329
J. Med. Chem. 2018, 61, 8061−8077