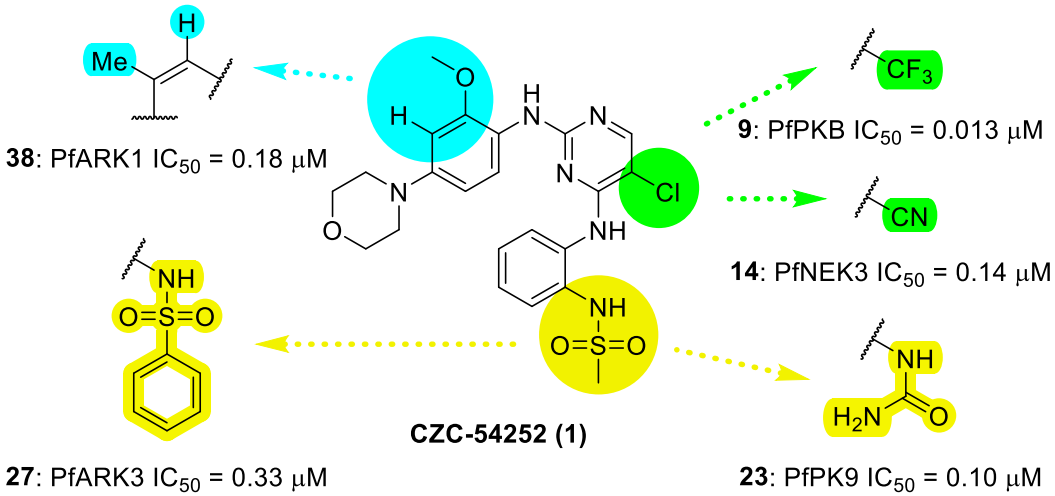
Characterization of 2,4-dianilinopyrimidines Against Five *P. falciparum* Kinases PfARK1, PfARK3, PfNEK3, PfPK9 and PfPKB

Han Wee Ong [a], Chandi de Silva [b], Krisha Avalani [b], Frank Kwarcinski [b], Christopher R. Mansfield [c], Michael Chirgwin [d], Anna Truong [d], Emily R. Derbyshire [c,d]*, Reena Zutshi [b]*, David H. Drewry [a,e]*

[a] Structural Genomics Consortium and Division of Chemical Biology and Medicinal Chemistry, Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA;
[b] Luceome Biotechnologies, L.L.C, 1665 E. 18th Street, Suite 106, Tucson, AZ 85719, USA;
[c] Department of Molecular Genetics and Microbiology, Duke University Medical Center, 213 Research Drive, Durham, NC 27710, USA;
[d] Department of Chemistry, Duke University, 124 Science Drive, Durham, NC 27708, USA;
[e] Lineberger Comprehensive Cancer Center, Department of Medicine, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

* Corresponding Authors

ABSTRACT



Plasmodium kinases are increasingly recognized as potential novel antiplasmodial targets for the treatment of malaria, but only a small subset of these kinases have had structure-activity-relationship (SAR) campaigns reported. Herein we report the discovery of CZC-54252 (**1**) as an inhibitor of five *P. falciparum* kinases PfARK1, PfARK3, PfNEK3, PfPK9 and PfPKB. 39 analogues were evaluated against all five kinases to establish SAR at three regions of the kinase active site. Nanomolar inhibitors of each kinase are discovered. We identify common and divergent SAR trends across all five kinases, highlighting substituents at each region that improve potency and selectivity for each kinase. Potent analogues were evaluated against the *P. falciparum* blood stage. Eight submicromolar inhibitors were discovered, of which **37** demonstrated potent antiplasmodial activity (EC_{50} = 0.16 μM). Our results provide an understanding of features needed to inhibit each individual kinase and lay groundwork for future optimization efforts towards novel antimalarials.

KEYWORDS

Malaria, Protein Kinase Inhibitor, 2,4-dianilinopyrimidine, PfARK1, PfARK3, PfNEK3, PfPK9, PfPKB

INTRODUCTION

Malaria is a devastating disease caused by *Plasmodium* infection, with 247 million cases and 619 thousand deaths globally in 2021[1]. The rising resistance towards existing antimalarials is a worrying trend[1], and new medicines with novel mechanisms of action are needed to address this evolving challenge[2]. *Plasmodium* protein kinases present an untapped opportunity for drug development[3–6]. Of the 85-99 *P. falciparum* protein kinases[7,8], several dozen have been identified as essential for the asexual blood stage of *P. falciparum*[9,10]. Yet, apart from several well-characterized kinases such as PfPKG[11–19], PfCLK3[20,21], and PfCDPK1[22–27], most *Plasmodium* protein kinases have had few medicinal chemistry campaigns initiated against them to facilitate inhibitor development. To address this gap, we aimed to discover hits against lesser-studied *Plasmodium* kinases and understand critical molecular features to guide inhibitor development of *Plasmodium* kinases.

Pyrimidines have been recognized as common hinge-binding groups of kinase inhibitors[28,29]. 2,4-dianilinopyrimidines are amongst the hits in the Tres Cantos Antimalarial Set (TCAMS), a set of 13,533 inhibitors of *P. falciparum* asexual reproduction found by screening almost 2 million compounds at GlaxoSmithKline[30]. Screening the compounds of the TCAMS set against five *P. falciparum* kinases led to the identification of PfCDPK1, PfCDPK4, and PfPK6 as the putative targets of 2,4-dianilinopyrimidines[31]. Medicinal chemistry campaigns of 2,4-dianilinopyrimidines have been reported in the literature, optimizing their antiplasmodial activity using a phenotypic screen[32,33]. Using chemo- and bioinformatic approaches, the authors suggested that this series of inhibitors may target the CDPK and NEK family of *Plasmodium* kinases[33]. Recently, six human-PLK1-targeted 2,4-diarylaminopyrimidine inhibitors have also demonstrated antiplasmodial activity, of which two of them demonstrate inhibition of PfCDPK2, PfNEK3, and PfPKB[34]. These studies demonstrate the druggability of *Plasmodium* kinases.

The 2,4-dianilinopyrimidines CZC-54252 (**1**) and CZC-25146 (**2**) (Figure 1A) were developed as selective human LRRK2 inhibitors[35]. During our screening efforts to discover new kinase-targeted antiplasmodial compounds, we found **1** to be active at 1 μM against *P. falciparum* 3D7 blood stage, reducing parasite viability to 21% of the negative control. Follow-up experiments confirmed a dose-dependent reduction of parasite viability with a EC_{50} of 0.34 μM. As far as we are aware, no information about inhibition of *Plasmodium* kinases have been reported for **1**. We screened **1** against 11 *P. falciparum* kinases using the KinaseSeeker assay[36] at 1 μM. Five kinase targets were identified and demonstrate dose-dependent inhibition: PfARK1, PfARK3, PfNEK3, PfPK9 and PfPKB (Table 1). Of these five targets, **1** was the most potent against PfPKB with an IC_{50} of 70 nM. Interestingly, while 2,4-dianilinopyrimidines were proposed to target PfPK6[31], we did not observe inhibition of PfPK6 by **1**. We also did not observe significant inhibition of the CDPK family members PfCDPK2 and PfCDPK5, and the NEK family member PfNEK1.

PfARK1 (PlasmoDB ID: PF3D7_0605300) and PfARK3 (PlasmoDB ID: PF3D7_1356800) are kinases from the Aurora family of kinases that are suggested to be involved in nuclear division processes[37,38]. Several genetic studies have indicated that PfARK1 is essential for asexual blood stage proliferation of *P. falciparum*[9,10,37]. In contrast, PfARK3 is genetically validated to be essential by reverse genetics[9] but not by saturation mutagenesis[10]. No inhibitor of either kinase has been confirmed in the literature, although inhibitors of human Aurora kinases have demonstrated antiplasmodial activity[39,40]. PfARK1 mutations confer resistance to the human Aurora kinase inhibitor Hesperadin (Figure 1B), suggesting that PfARK1 may be its target[40].

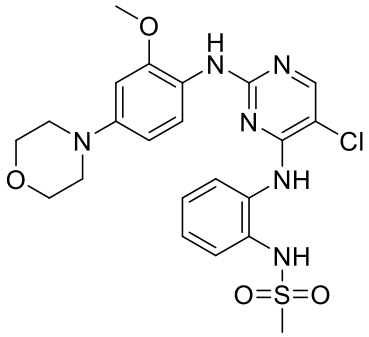
PfNEK3 (PlasmoDB ID: PF3D7_1201600) belongs to the NEK family[41]. Separate studies have regarded PfNEK3 as either dispensable for the asexual blood stage proliferation[9], or that it could be disrupted, albeit with a fitness penalty[10]. PfNEK3 participates in an atypical MAPK signaling cascade by upregulating the activity of PfMAP2[41,42], although the implications of this signaling pathway is not currently clear. Human PLK1 inhibitors including BI-2536 (Figure 1B) have been found to inhibit PfNEK3, although phosphoproteomics indicates that there may be other targets[34]. We have also previously identified PfNEK3 as one of the secondary targets of our potent PfPK6-targeted type II inhibitors[43].

PfPK9 (PlasmoDB ID: PF3D7_1315100) is an orphan kinase that does not cluster with any typical kinase group[44] and is genetically validated to be essential for asexual blood stage proliferation[9,10]. PfPK9 regulates the activity of PfUBC13[44,45], an essential E2 ubiquitin-conjugating enzyme involved with DNA replication and repair[46]. The only known inhibitors of PfPK9 are the human TAK1 inhibitor Takinib (Figure 1B) and analogues thereof[45].

PfPKB (PlasmoDB ID: PF3D7_1246900) is a kinase from the AGC group[47]. While a reverse genetics study found PfPKB to be essential[9], it was found to be dispensable by saturation mutagenesis[10]. PfPKB is implicated in regulation of merozoite invasion into erythrocytes by phosphorylating PfGAP45[48–50]. Inhibition of PfPKB by Go 6983 or A-443654 (Figure 1B) leads to a decrease in parasitemia and formation of new rings in the subsequent round of invasion, consistent with its proposed role in regulating invasion[47,51]. PfPKB has also been determined to be a secondary target of the abovementioned 2,4-diarylaminopyrimidine inhibitors[34] and our type II inhibitors[43].

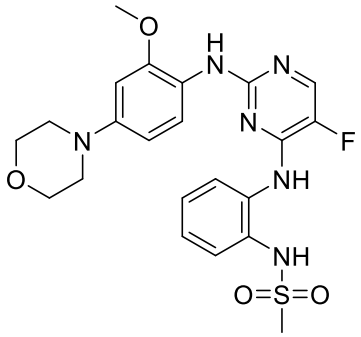
Given the limited medicinal chemistry studies and inhibitor development for PfARK1, PfARK3, PfNEK3, PfPK9 and PfPKB, we aim to expand on the body of knowledge to facilitate future inhibitor development for these five diverse kinases (with only 19.5-33.7% pairwise sequence identity in the kinase domains, see Figure S1) across the *Plasmodium* kinome. These efforts will impact chemical probe development to resolve *Plasmodium* kinase function as well as the design of novel antimalarial agents. Herein, we report the results of our structure-activity-relationship (SAR) study for inhibition of these five kinases by the 2,4-dianilinopyrimidine scaffold of inhibitors.

A)



CZC-54252

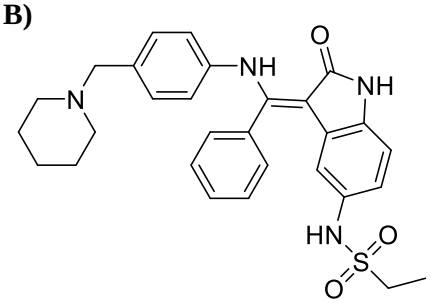
(Error!



CZC-25146

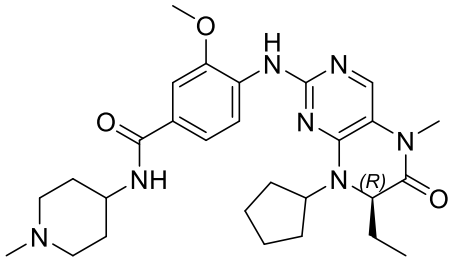
(Error!

B)



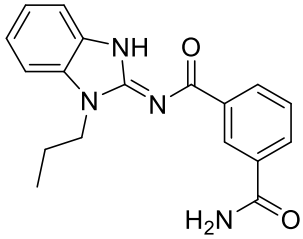
Hesperadin

Putative PfARK1 inhibitor



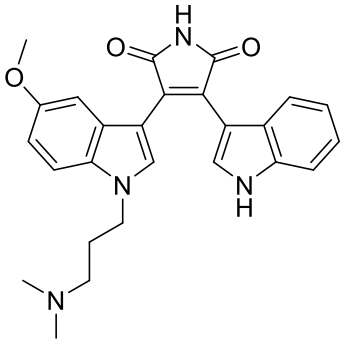
BI-2536

PfNEK3 IC_{50} = 86 nM



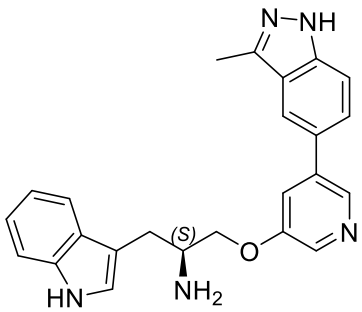
Takinib

PfPK9 $K_{d(app)}$ = 0.48 μM



Go 6983

PfPKB IC_{50} = 1 μM



A-443654

PfPKB IC_{50} = 0.2 μM

Figure 1. A) Structure of CZC-54252 (**1**) and CZC-25146 (**2**)[35]. **B)** Structure and reported potency of literature inhibitors of PfARK1[40], PfNEK3[34], PfPK9[45] and PfPKB[47,51].

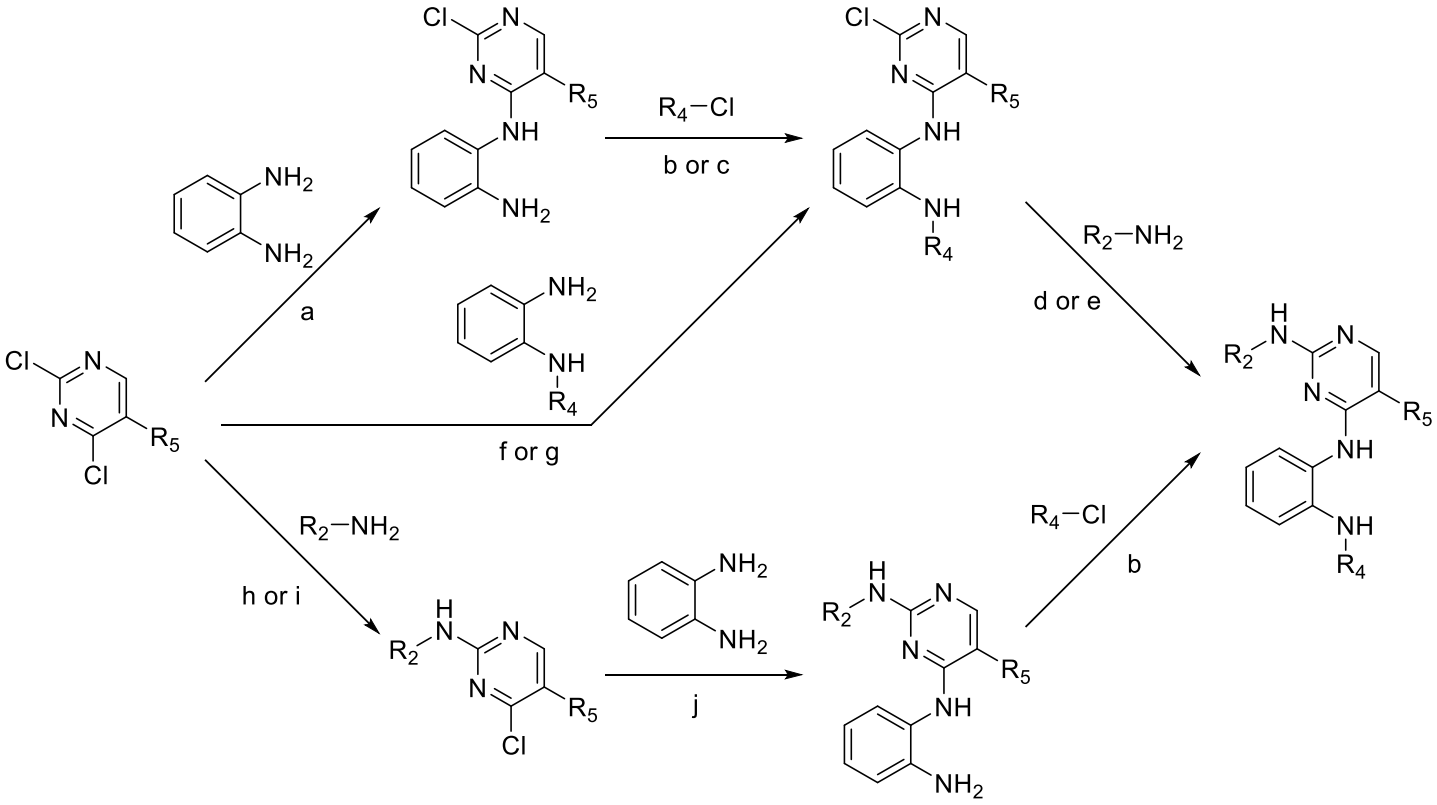
Table 1. Inhibition profile of CZC-54252 (**1**) across the 11 *P. falciparum* kinase panel

Kinase	% activity remaining at 1 μM[a]	IC_{50} (μM)[b]
PfARK1	53	3.4
PfARK3	50	1.8
PfCDPK2	78	–
PfCDPK5	97	–
PfGSK3	94	–
PfNEK1	85	–
PfNEK3	34	1.0
PfPK5	90	–
PfPK6	90	–
PfPK9	43	1.2
PfPKB	11	0.070

[a]Activity remaining are presented as mean values from experiments using the KinaseSeeker assay performed in duplicate.
[b]IC_{50} values were determined using the KinaseSeeker assay, presented as mean values of two experiments performed in duplicate.
–: not determined

RESULTS AND DISCUSSION

Scheme 1. Synthesis of 2,4-dianilinopyrimidine analogs[a].



[a]Reagents and conditions: (a) DIPEA, ^{n}BuOH, 110-115°C, 16-38 h. (b) pyridine, 0°C-rt, 16-22 h. (c) DIPEA, dioxane or DCM, 0°C-rt, 3-19 h. (d) HCl or TFA, EtOH or TFE, μW, 80-160°C, 30-150 min. (e) $Pd_2(dba)_3$, Xphos, K_2CO_3, ^{t}BuOH, 90°C, 18 h. (f) DIPEA, EtOH, 0°C-rt, 3-72 h. (g) K_3PO_4, ^{i}PrOH, rt, 7-11 d. (h) $ZnCl_2$, Et_3N, DCE:^{t}BuOH:Et_2O, 0°C-rt, 24 h. (i) Et_3N, THF, rt, 1 h. (j) DIPEA, ^{i}PrOH or ^{n}BuOH, 60-80°C, 16 h - 5 d.

The general synthetic scheme of these 2,4-dianilinopyrimidines is shown in Scheme 1. A 5-substituted-2,4-dichloropyrimidine core was reacted with the appropriate aniline in a S_NAr reaction, replacing the chloro group at the 4-position in most cases. When a CF_3 is present at the 5-position, displacement of the 2-position chloro group is achieved instead[52]. The regioselectivity of these reactions were confirmed by ^1H-^{13}C HMBC experiments (Table S1). The choice of aniline and reaction conditions were dependent on the electrophilicity of 2,4-dianilinopyrimidine core, which was significantly modulated by the 5-position substituent. To the unreacted NH_2 on the benzene-1,2-diamine, subsequent reaction with sulfonyl chlorides or other equivalent electrophiles afforded the R_4 substituent. A second S_NAr or Buchwald-Hartwig coupling was next performed, displacing the chloro group at the unreacted 2-position of the pyrimidine. Functional group conversion between different R_5 substituents and within R_2 substituents were also performed. Detailed synthetic procedures are provided in Schemes S1-S12 in the Supplemental Information.

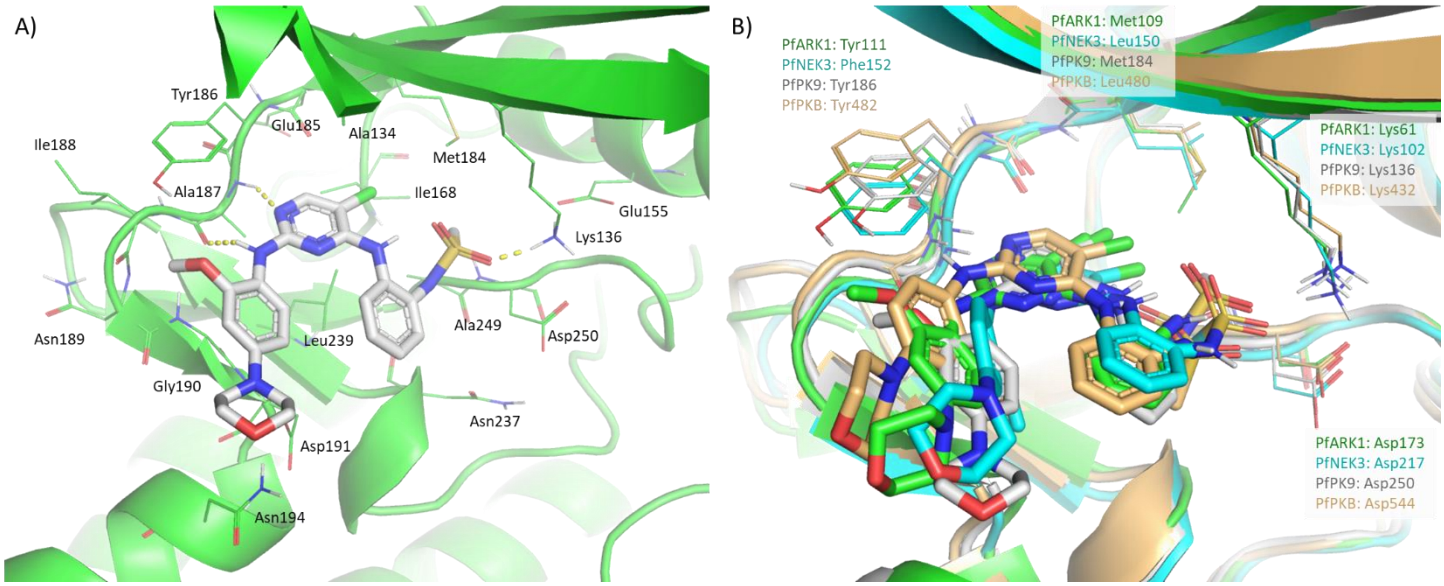


Figure 2. A) CZC-54252 (**1**) (white) docked to the structure of PfPK9 predicted by AlphaFold (green). Intermolecular hydrogen bonds are denoted by yellow dashed lines. The glycine-rich loop is hidden for clarity. **B)** Overlay of docked structures of **1** to the predicted structure of PfARK1 (green), PfNEK3 (cyan), PfPK9 (white), and PfPKB (gold) by AlphaFold. The glycine-rich loop is hidden for clarity. Only key residues (gatekeeper, middle hinge residue, catalytic lysine, and Asp of the conserved DFG) are shown. Figures are generated using PyMOL.

Although crystal structures of **1** with any of these *Plasmodium* kinases are unavailable, we generated hypotheses regarding the binding mode of these inhibitions by inspection of co-crystal structures of other 2,4-dianilinopyrimidines with other kinases, e.g. TAE226 on FAK (PDB: 2JKK)[53], Fedratinib on JAK2 (PDB: 6VNE)[54] and TAK-788 on EGFR (PDB: 7B85)[55]. These inhibitors are typically ATP-competitive, and form key hydrogen bonds with the peptide backbone of the outer hinge residue (hinge.46 by KLIFS notation[56]) using both the N1 of the pyrimidine and the exocyclic NH at the 2-position of the pyrimidine. We docked **1** into the structures of PfARK1, PfNEK3, PfPK9 and PfPKB predicted by AlphaFold[57,58] (docking was not performed for PfARK3 because its structure was not available) to generate hypotheses regarding their binding mode (Figure 2 and Figure S2, S3). As predicted, the model suggests that **1** forms two hydrogen bonds with the peptide backbone of the outer hinge residue (Ala187 in PfPK9) using the N1 of the pyrimidine and exocyclic NH at the 2-position of the pyrimidine. The 2-position aryl substituent extends towards the solvent, with the morpholine ring exposed to solvent. The model also suggests that the methylsulfonamide group of **1** forms a hydrogen bond with the exocyclic NH at the 4-position of the pyrimidine and with the catalytic lysine (Lys136 in PfPK9).

Based on literature crystal structures and our docking results, we noted that the 5-position group of the pyrimidines likely binds close to the gatekeeper residue of the kinase, a residue that is often targeted for potency and specificity[56,59–61]. Apart from steric complementarity, 5-position groups on pyrimidines may also form various specific interactions with the gatekeeper residues, such as hydrogen bonding[55], halogen/chalcogen bonds[62,63], or lone-pair-π interactions[64]. Based on this

hypothesis, we first investigated the effect on kinase inhibition of all five identified *Plasmodium* kinase targets when we substituted the 5-position chloro group of **1** with other groups (Table 2).

Removal of the chlorine atom (**3**) completely abolished activity for all five kinases, suggesting that the chlorine makes key interactions with each of the five kinases. Replacement with a fluorine (**2**) recovers some activity only for PfPKB (IC_{50} = 1.5 μM) and PfNEK3 (68% activity remaining at 1 μM), suggesting that the size of the halogen atom matters for these kinases. When the chloro group was replaced with the heavier halogens bromine (**4**) or iodine (**5**), the potencies for PfARK1, PfARK3, and PfPKB were maintained, while a modest 2.3-fold improvement in PfNEK3 potency was observed for both analogues. Interestingly, PfPK9 saw a 4-fold improvement in potency with **4** and a 17-fold improvement with **5**. Generally, an improvement in potency was observed as the size of halogens increases from fluorine to iodine, suggests that there are favorable hydrophobic or halogen-bonding interactions with the heavier halogens. Looking closer into this general trend, we observe that this effect plateaued at chlorine for PfARK1, PfARK3 and PfPKB, at bromine for PfNEK3, while it continued to iodine for PfPK9. This trend does not correlate with their gatekeeper residues, which are methionine for PfARK1 and PfPK9, and leucine for PfARK3, PfNEK3 and PfPKB (based on structure prediction by AlphaFold[57,58] and sequence alignment with human kinases[37,41,44,47]). This suggests that the origin of this varied sensitivity to halogens is more complex than could be simply predicted based on these key residues.

We next explored alkyl groups at this position. The methyl (**6**) and ethyl (**7**) groups both abolished PfARK1, PfARK3 and PfPK9 potency, but were tolerated by PfNEK3 and PfPKB. Further increase in steric size of the alkyl group to a cyclopropyl ring (**8**) is tolerated by PfPKB, but now abolished PfNEK3 activity. Among the five kinases, **8** is exquisitely selective for PfPKB. Interestingly, replacement of the methyl group with a trifluoromethyl group (**9**) increased potency dramatically across all five kinases, which is unexpected because literature suggests that the methyl to trifluoromethyl substitution typically does not affect potency of inhibitors in general[65]. This change restored potency to the μM range for PfARK1 and PfARK3. Relative to **1**, this change improved the potency for PfNEK3 by 2-fold, PfPK9 by 16-fold, and PfPKB by 5-fold. Excitingly, **9** has an IC_{50} of 13 nM for PfPKB, which makes this the most potent PfPKB inhibitor reported as far as we are aware. Comparing **5** and **9**, both with near-identical PfPK9 potency, we can see that **9** is more selective for PfPKB.

Similar to the alkyl groups in the 5-position, a methoxy group (**10**) or a dimethylamino group (**11**) at this position were not tolerated by PfARK1, PfARK3 and PfPK9. Comparing with **7**, with a similar-sized ethyl group, we observed a <2-fold change in PfNEK3 IC_{50}, but a 4-fold drop in PfPKB potency with **10**. In contrast, we observed a 3-fold drop in PfNEK3 potency with <2-fold change in PfPKB potency with **11**. Both the hydroxyl (**12**) and amino (**13**) groups in the 5-position completely abolish activity on all five kinases, suggesting that there is a lack of productive hydrogen bonding partners to accommodate these hydrogen bond donors. This is consistent with the knowledge that all five kinases have methionine or leucine gatekeeper residues.

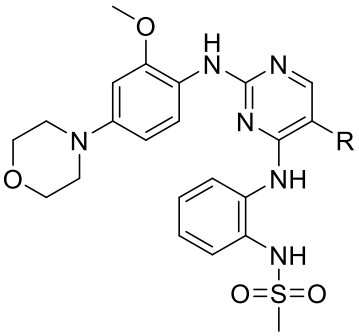
Surprisingly, the introduction of a nitrile group (**14**) was not tolerated by PfARK1, PfARK3 and PfPK9, slightly disfavored by PfPKB (2.6-fold drop in IC_{50} as compared to **1**), but is strongly preferred by PfNEK3, with a 7-fold improvement in potency over **1**. This highlights a possible avenue for improving selectivity for PfNEK3 over the other kinases. Another substituent that demonstrates selectivity for PfNEK3 is the N-methylpyrazol-4-yl substituent (**15**), which maintained the PfNEK3 potency of **1** but completely abolished activity on the other four kinases. The tolerability of the large N-methylpyrazol-4-yl substituent inspired us to further investigate the steric requirements of this region. To do so without too much of an increase in lipophilicity, we explored similarly-sized ethers (**16**, **17**) or an ester substituent (**18**), but found them not to be tolerated by any kinase, suggesting that the aromaticity of the N-methylpyrazol-4-yl substituent was important.

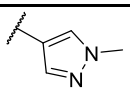
When we replaced the chloro of **1** with a carboxamide substituent (**19**), we observed an improvement in potency for PfARK1 (3-fold) and PfPK9 (6-fold), but a modest decrease in potency for PfNEK3 (2.5-fold) and PfPKB (4-fold), and a complete loss of PfARK3 activity. The carboxamide substituent thus offers an avenue to design for selectivity for PfARK1 over the related family member PfARK3.

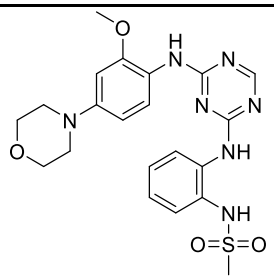
We have also attempted to introduce an endocyclic N atom at this position, converting the pyrimidine to a 1,3,5-triazine ring (**20**). However, this was found to not be tolerated by any of the five kinases. This result is similar to **3**, which contains a pyrimidine core without a 5-position substituent. This further emphasizes the importance of the substituents at this position and the interactions they make with the kinase active site.

In summary, we demonstrate that different kinases have different sensitivities to the group at the 5-position, and this knowledge could help to design selective inhibitors between these five kinases. We have shown that PfARK1, PfARK3 and PfPK9 are rather sensitive at this position, only preferring several substituents (Cl, CF_3, $CONH_2$ for PfARK1; Cl, Br, I, CF_3 for PfARK3; and Cl, Br, I, CF_3, and $CONH_2$ for PfPK9). This is in contrast with PfNEK3 and PfPKB; while they have their preferred substituents (CN and CF_3, respectively), they are generally more tolerant of changes.

Table 2: SAR at the 5-position of the pyrimidine core



		PfARK1		PfARK3		PfNEK3		PfPK9		PfPKB	
Cmpd	R	% Activity Remaining at 1 μM[a]	IC_{50} (μM)[b]	% Activity Remaining at 1 μM[a]	IC_{50} (μM)[b]	% Activity Remaining at 1 μM[a]	IC_{50} (μM)[b]	% Activity Remaining at 1 μM[a]	IC_{50} (μM)[b]	% Activity Remaining at 1 μM[a]	IC_{50} (μM)[b]
1	Cl	53	3.4	50	1.8	34	1.0	43	1.2	11	0.070
2	F	99	–	88	–	68	–	97	–	55	1.5
3	H	100	–	90	–	100	–	100	–	100	–
4	Br	84	–	45	1.0	20	0.44	19	0.29	10	0.042
5	I	75	–	49	1.2	23	0.41	6	0.072	9	0.050
6	Me	100	–	82	–	44	2.3	88	–	24	0.16
7	Et	100	–	81	–	24	0.60	79	–	16	0.17
8	cyclopropyl	100	–	100	–	100	–	100	–	48	0.24
9	CF_3	58	1.2	47	1.4	22	0.51	10	0.075	6	0.013
10	OMe	75	–	83	–	40	0.90	81	–	44	0.70
11	NMe_2	85	–	100	–	46	1.8	89	–	22	0.24
12	OH	100	–	100	–	95	–	100	–	100	–
13	NH_2	97	–	100	–	93	–	100	–	96	–
14	CN	79	–	100	–	14	0.14	73	–	20	0.18
15		89	–	95	–	42	1.2	100	–	97	–
16	$CH_2OCH_2CF_3$	100	–	97	–	67	–	100	–	100	–
17	CH_2OEt	97	–	100	–	100	–	100	–	89	–
18	CO_2Et	100	–	96	–	65	–	100	–	93	–
19	$CONH_2$	39	1.1	93	–	51	2.5	19	0.21	29	0.31

 **20**	95	–	100	–	82	–	100	–	78	–

[a]Activity remaining results are presented as mean values from experiments using the KinaseSeeker assay performed in duplicate.
[b]IC_{50} values were determined using the KinaseSeeker assay, presented as mean values of two experiments performed in duplicate.
–: not determined

We next turned to exploring the SAR at the 4-position of the pyrimidine (Table 3). Based on examination of crystal structures of other 2,4-dianilinopyrimidine inhibitors and our docked structures with **1**, this region is positioned close to the catalytic lysine and the aspartic acid of the DFG motif. In previous medicinal chemistry campaigns of 2,4-dianilinopyrimidines as antimalarials, only few groups were explored at the *ortho*-position[32,33]. We first investigated the effect of removal of the sulfonyl group of the sulfonamide (**21**), and found that the removal of this key hydrogen bond acceptor to be detrimental towards activity for all five kinases, which could possibly be attributed to the loss of hydrogen bonding potential with the catalytic lysine. We have also attempted to cyclize the 4-position di-aniline ring to a benzimidazolone (**30**), but this substituent was inactive against all five kinases. This suggests that the position of the hydrogen bond acceptor is key in this region. Having established the importance of a hydrogen bond acceptor, we next replaced the sulfonamide with an amide (**22**) or urea (**23**). While **22** retains weak activity on PfARK1, PfPK9 and PfPKB (65-70% activity remaining at 1 μM), this change leads to a loss of activity against PfARK3 and PfNEK3. This is in contrast with **23** which, as compared to **1**, is equipotent against PfPKB while having a loss of activity against PfARK1, PfARK3, and PfNEK3. Interestingly, a 12-fold improvement in potency was observed for PfPK9. These results highlight how single-atom changes in this region could modulate the selectivity between kinases.

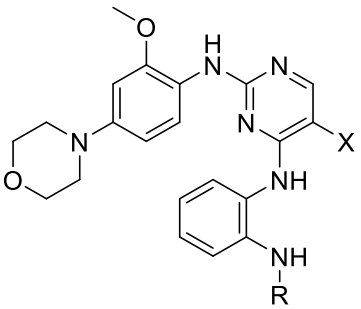
To further investigate the steric requirement of the pocket around the sulfonamide, we next increased the size of the methyl group on the sulfonamide. Increasing the size to either an ethyl (**24**) or cyclopropyl (**25**) group improved PfARK3 and PfPK9 activity 4-fold and 2-fold, respectively. While **24** is weakly active on PfARK1 and equipotent against PfNEK3 as compared to **1**, **25** was 3-fold more potent than **1** on both kinases. A 2-to-2.5-fold drop in potency was observed with PfPKB with either of these small changes, suggesting that these modifications were not preferred but were tolerated. A further increase in the size of this group to an isobutyl group (**26**) turned out to be unfavorable for all kinases except PfARK3, which maintained the potency of **1**. These results demonstrate that these five kinases have different steric requirements in the active site around this region, which may be optimized by changes as small as a single carbon atom.

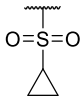
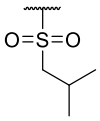
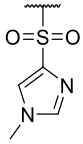
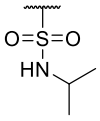
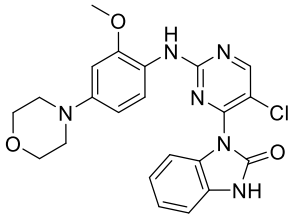
Apart from aliphatic groups, we also attempted to introduce aromatic groups at this region. A phenyl group (**27**) is preferred by PfARK3, affording a 5-fold improvement in potency as compared to **1**. **27** maintained potency against PfARK1 and PfNEK3 while leading to a dramatic drop in potency against PfPK9 and PfPKB. This result shows that a phenyl ring may offer opportunities to achieve selectivity for PfARK3 over the related family member PfARK1. With a N-methylimidazole group (**28**) however, the activity against PfARK3 was lost while the potencies with the other four kinases were similar to **27**, which suggests that PfARK3 does not prefer polar functionalities here.

Given that we have previously achieved good activity for PfPK9 with introduction of additional hydrogen bond donors, we investigated if the N-isopropyl sulfamoyl amide (**29**) would similarly improve activity. However, the inhibition results for all five kinases, including PfPK9, were similar to the isosteric isobutyl sulfonamide **26**, emphasizing that PfPK9 has a strict steric requirement for this region.

Our SAR results for these five *Plasmodium* kinases demonstrate that the 4-position of the pyrimidine offers an avenue to generate selectivity due to the different preferences of each kinase. In particular, selective inhibition of PfARK3 may be achieved using larger lipophilic groups at this region, something that is not as well tolerated by PfARK1, PfNEK3, PfPK9 and PfPKB. The methyl group is perhaps the most ideal group for PfPKB, while the slightly larger cyclopropyl group is the most ideal for PfARK1 and PfNEK3. We have also found that PfPK9 strongly prefers a urea at this position.

Table 3: SAR at the 4-position of the pyrimidine core



			PfARK1		PfARK3		PfNEK3		PfPK9		PfPKB	
Cmpd	X	R	% Activity Remaining at 1 μM[a]	IC_{50} (μM)[b]	% Activity Remaining at 1 μM[a]	IC_{50} (μM)[b]	% Activity Remaining at 1 μM[a]	IC_{50} (μM)[b]	% Activity Remaining at 1 μM[a]	IC_{50} (μM)[b]	% Activity Remaining at 1 μM[a]	IC_{50} (μM)[b]
1	Cl	SO_2Me	53	3.4	50	1.8	34	1.0	43	1.2	11	0.070
21	CF_3	H	81	–	86	–	84	–	83	–	61	1.1
22	Cl	COMe	65	–	100	–	93	–	70	–	66	2.1
23	Cl	$CONH_2$	96	–	87	–	81	–	11	0.10	17	0.079
24	Cl	SO_2Et	79	–	40	0.46	40	0.84	39	0.64	7	0.18
25	Cl		46	1.3	36	0.44	48	0.36	61	0.45	23	0.12
26	Cl		77	–	51	1.1	83	3.9	84	–	70	–
27	Cl	SO_2Ph	73	4.0	31	0.33	49	1.4	85	–	60	1.7
28	Cl		70	–	77	–	56	1.4	100	–	65	2.1
29	Cl		80	5.1	45	1.4	80	–	90	–	82	5.0
 **30**			95	–	76	–	97	–	98	–	100	–

[a]Activity remaining results are presented as mean values from experiments using the KinaseSeeker assay performed in duplicate.
[b]IC_{50} values were determined using the KinaseSeeker assay, presented as mean values of two experiments performed in duplicate.
–: not determined

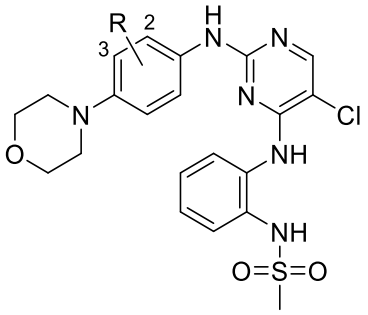
The next position for SAR exploration is on the 2-position of the pyrimidine. Acknowledging that the exocyclic NH typically forms hydrogen bonds with the hinge region of kinases, we opted not to disrupt this key pharmacophore. Medicinal

chemists have frequently used substituents on the aryl group adjacent to the pyrimidine to design for selectivity between kinases[66–71]. For example, a substituent at the position *ortho*- to the exocyclic NH is key for selectivity of TAE684 for ALK[72], or BI-2536 for PLK1[73]. As we hypothesize the morpholine ring at the 4-position of the aniline ring to be solvent-exposed based on our docked structures of **1**, we decided to focus on and establish the SAR of 2- (*ortho*-) and 3-position (*meta*-) substituents (Table 4).

Removal of the *ortho*-methoxy group of **1** to generate compound **31** revealed a dramatic improvement in potency for PfARK1 (9-fold), PfNEK3 (9-fold), and PfPK9 (27-fold), but only a modest improvement in potency against PfPKB (3-fold), and a <2-fold change in potency against PfARK3. Conversely, replacement of the methoxy group with a larger ethoxy group (**32**) leads to a loss of potency against PfARK1 (4-fold), PfNEK3 (2-fold), PfPK9 (9-fold) and PfPKB (11-fold), with a <2-fold change in potency against PfARK3. Interestingly, a smaller hydroxyl group (**33**) maintains the potency of **1** against PfARK1 and PfPK9, while decreasing potency against PfARK3 (2.4-fold) and PfPKB (6-fold), but is 5-fold more potent on PfNEK3. With the small methyl (**34**), fluoro (**35**), or chloro groups (**36**), we observe that they tend to maintain the potency of **1** against PfARK1, PfARK3 and PfPKB, while improving upon activity of both PfNEK3 and PfPK9 by 2-to-5-fold. Looking at these trends, we infer that PfARK1 and PfPK9 are particularly sensitive to the steric requirement of the *ortho*-position group at this region, whereas PfNEK3 and PfPKB are less sensitive, and PfARK3 is non-discriminatory. The steric requirement and preference for a unsubstituted *ortho*-position likely arises from the relief of a steric clash with the middle hinge residue (hinge.47 by KLIFS notation[56]) of the kinase. This steric clash typically occurs for kinases with larger tyrosine or phenylalanine residues while being accommodating of the smaller leucine residue[67,72–75]. However, the middle hinge residues of these five kinases are all tyrosine or phenylalanine residues, thus this does not provide a simple explanation regarding the differential sensitivity towards substituents at this position. In particular, PfARK3 has a phenylalanine as its middle hinge residue, while PfARK1 has a similarly-sized tyrosine residue, but this does not necessarily indicate that PfARK3 is as sensitive to steric bulk at this position of inhibitors than PfARK1. We thus emphasize the importance of investigating and understanding SAR to complement sequence-based hypotheses.

We next installed substituents *meta*- to the NH of the aniline ring. With the electron-donating methoxy (**37**) and methyl (**38**) substituents, we observed a modest 1.5-to-3-fold improvement in PfARK1, PfARK3 and PfNEK3 potencies when compared to **31**, while the PfPK9 and PfPKB potencies are largely unaffected (≤1.5-fold difference). Addition of a chloro substituent (**39**) does not change the potency of **31** across all five kinases. Similarly, addition of the trifluoromethyl group (**40**) does not change the potency of **31** against PfARK1 and PfARK3. This group does induce a 2.2-fold improvement in PfNEK3 potency, while decreasing potency against both PfPK9 and PfPKB by 2.5-fold. In contrast to the methyl-to-trifluoromethyl substitution at the 5-position of the pyrimidine, the potency change with the same substitution is much more modest at the *meta*-position of the aniline. Unlike when varying substituents at the *ortho*-position, variation of the *meta*-position substituents mostly induces modest changes in potency. This striking contrast further demonstrates that the potency changes observed with the *ortho*-position are primarily due to steric effects rather than electronic contributions to the aniline ring. Despite the possibility of the *meta*-position substituents inducing a change in the electron density of the adjacent morpholine ring and its dihedral angle with the phenyl ring, the modest changes in potency suggest that any difference in the electron density and conformations of morpholine ring are well-tolerated by these five kinases. Their general tolerability towards substituents at the *meta*-position also offers opportunities for using substituents at this position to modulate ADME and PK properties of inhibitors in future lead optimization efforts.

Table 4: SAR on substituents at the 2-position aniline



		PfARK1		PfARK3		PfNEK3		PfPK9		PfPKB	
Cmpd	R	% Activity Remaining at 1 μM[a]	IC_{50} (μM)[b]	% Activity Remaining at 1 μM[a]	IC_{50} (μM)[b]	% Activity Remaining at 1 μM[a]	IC_{50} (μM)[b]	% Activity Remaining at 1 μM[a]	IC_{50} (μM)[b]	% Activity Remaining at 1 μM[a]	IC_{50} (μM)[b]
1	2-OMe	53	3.4	50	1.8	34	1.0	43	1.2	11	0.070
31	2-H, 3-H	21	0.38	42	1.2	2	0.11	6	0.044	9	0.021
32	2-OEt	100	12	73	3.0	49	2.0	78	11	46	0.75
33	2-OH	69	3.1	81	4.3	11	0.22	49	1.2	7	0.43
34	2-Me	43	2.0	67	1.2	7	0.29	20	0.46	10	0.12
35	2-F	38	1.9	46	2.5	8	0.42	20	0.35	8	0.051
36	2-Cl	84	2.7	74	4.0	7	0.55	32	0.59	0	0.037
37	3-OMe	9	0.24	11	0.43	6	0.060	4	0.040	0	0.014
38	3-Me	20	0.18	35	0.68	7	0.057	3	0.030	5	0.015
39	3-Cl	19	0.34	44	1.0	14	0.12	8	0.056	6	0.017
40	3-CF_3	23	0.54	47	0.99	8	0.049	15	0.11	0	0.054

[a]Activity remaining results are presented as mean values from experiments using the KinaseSeeker assay performed in duplicate.
[b]IC_{50} values were determined using the KinaseSeeker assay, presented as mean values of two experiments performed in duplicate.

Having established SAR on the five *Plasmodium* kinases, we next investigated how the modulation of their inhibitory activities would translate to antiplasmodial activity. We selected compounds with an IC_{50} of 0.3 μM or lower against any of the five kinases, and screened them at 1 μM against *P. falciparum* 3D7 (Pf3D7) using a SYBR Green I-based fluorescence assay to evaluate asexual blood stage viability[76]. We found nine new analogues with <50% viability at 1 μM, and further profiled them in dose-response experiments to determine their EC_{50}s (Table 5). In parallel, we screened them for cytotoxicity (CC_{50}) in HepG2 cells, a human cell line previously used to screen for cytotoxicity of antimalarials[30].

We found compounds **4**, **5**, **9**, and **14** to possess sub-micromolar potencies against Pf3D7 in the asexual blood stage, a comparable potency to **1**. These compounds are also only weakly cytotoxic against HepG2 cells, with CC_{50}s of >9.8 μM. For **5** and **9**, no significant cytotoxicity was observed up 15 μM, their observed solubility limit. These four compounds offer at least a 14-fold window between antiplasmodial activity and cytotoxicity. **8** was also weakly active against Pf3D7 in the blood stage with an EC_{50} of 1.2 μM, while the CC_{50} was 23 μM, offering a 19-fold window of selectivity. Compounds **31**, **37**, **38**, and **39** were also found to be submicromolar inhibitors of *P. falciparum* replication in the asexual blood stage. However, they exhibit slightly greater cytotoxicity, with CC_{50}s in the low micromolar ranges. One possible reason for the greater cytotoxicity could be increased promiscuity from the removal of the *ortho*-position methoxy group, which could allow the compounds to inhibit more kinases. Nevertheless, we have found **37** to be a potent antimalarial compound in this series (EC_{50} = 0.16 μM), a 2-fold improvement over **1**, which still possesses a 10-fold window with cytotoxicity (CC_{50} = 1.6 μM).

Throughout these experiments, we observed that that the antiplasmodial activity does not correlate directly with the potency against any kinase, suggesting that there could be other targets for these 2,4-dianilinopyrimidines yet to be identified, or the antiplasmodial activity may be attributed to polypharmacology.

Table 5: Antiplasmodial activity and cytotoxicity

	Pf3D7 blood stage		HepG2 CC_{50} (μM)[c]	CC_{50} / EC_{50}
Cmpd	% viability at 1 μM[a]	EC_{50} (μM)[b]		
1	21	0.34	–	–
2	85	–	–	–

4	15	0.69	9.8	14
5	14	0.54	>15[d]	>28
6	110	–	–	–
7	83	–	–	–
8	39	1.2	23	19
9	14	0.38	>15[d]	>39
14	21	0.43	>50[e]	>116
23	95	–	–	–
24	82	–	–	–
25	78	–	–	–
27	65	–	–	–
31	28	0.43	1.5	3.5
33	82	–	–	–
34	85	–	–	–
35	69	–	–	–
36	90	–	–	–
37	15	0.16	1.6	10
38	10	0.35	2.1	6.1
39	7	0.43	2.8	6.4
40	57	–	–	–

[a]Parasite viability was determined using the SYBR Green I-based assay, presented as mean values of triplicate experiments.
[b]EC_{50} values were determined from two experiments performed in duplicate using the SYBR Green I-based assay, presented as mean values.
[c]CC_{50} values were presented as mean values from two experiments using the CellTiter-Fluor assay, each performed in triplicate.
[d]No significant cytotoxicity observed up to 15 μM, the observed solubility limit.
[e]This compound decreases cell viability around 1 μM to ~60%, but it never decreases cell viability below 50%.
–: not determined

CONCLUSION

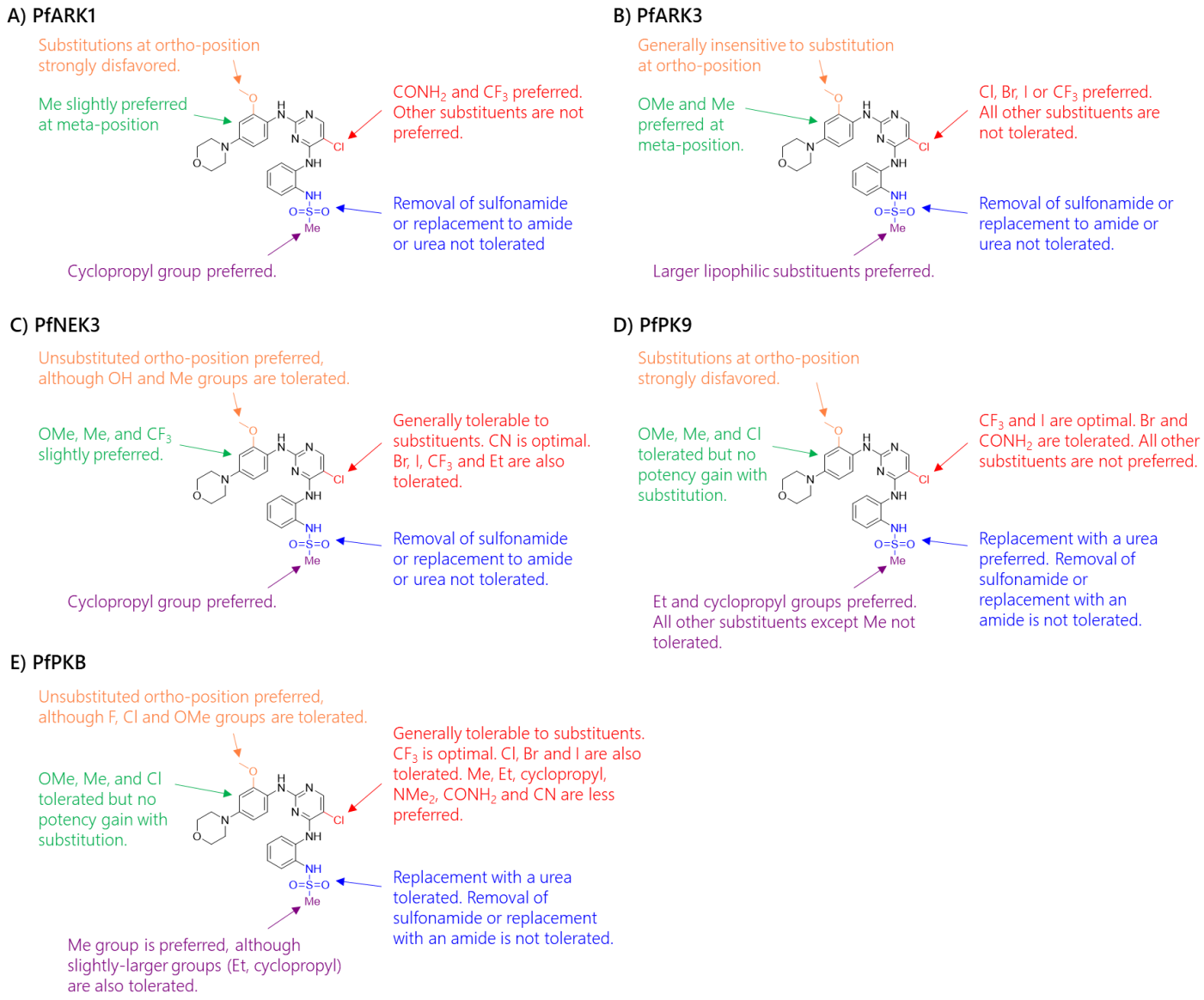


Figure 3. SAR Summary for PfARK1 **(A)**, PfARK3 **(B)**, PfNEK3 **(C)**, PfPK9 **(D)**, and PfPKB **(E)**.

In conclusion, we have found that **1** is an inhibitor of five *P. falciparum* protein kinases PfARK1, PfARK3, PfNEK3, PfPK9 and PfPKB. Through the synthesis and evaluation of a set of 39 analogues, we have developed SAR against all five kinases (Figure 3). We found subtle changes that improved potency against each kinase when compared to the parent compound **1**. The most potent PfARK1 inhibitor we discovered was **38**, achieved by removal of the highly unfavorable *ortho*-position methoxy group and addition of a *meta*-position methyl group on the aniline ring at the 2-position of the pyrimidine core of **1**. In contrast, the most potent PfARK3 inhibitor in our set of compounds was **27**, which was attained by substituting the methylsulfonamide on the aniline ring at the 4-position of the pyrimidine core to a phenylsulfonamide. Many potent PfPKB inhibitors were discovered, of which one of the most potent inhibitors was **9**, which was achieved by replacing the 5-position chloro group to a trifluoromethyl group. Out of the PfNEK3 inhibitors that we discovered, we would like to highlight **14**, which demonstrated a unique improvement in potency by a simple substitution of the 5-position chloro group to a nitrile. Similarly, we would like to highlight **23** as a compound that also demonstrated a unique improvement in potency, this time against PfPK9, by incorporation of a urea substituent instead of a sulfonamide. We have also improved upon the antiplasmodial potency of **1**, with **37** demonstrating a modest improvement in potency against the *P. falciparum* asexual blood stage.

We also emphasize the importance of investigating and developing SAR understanding using two examples, looking at the SAR around the 5-position of the pyrimidine and the *ortho*-position substituent on the aniline at the 2-position. In these

two cases, hypotheses for kinase inhibition and selectivity based on key amino acid residues may be too simplistic and are not able to completely explain or predict the potency and selectivity of kinase inhibitors. Currently, there is a dearth of medicinal chemistry studies for these five diverse *P. falciparum* kinases. We hope that this work provides starting points for lead optimization efforts and that the SAR described allows for the development of strategies to identify potent and selective inhibitors for each of them.

ASSOCIATED CONTENT

Results of percent identity and similarity by pairwise sequence alignment, additional molecular docking figures, summary of ^{1}H-^{13}C HMBC results, procedures for biochemical assays, synthetic procedures, and copies of NMR spectra of all new analogues reported (DOCX).

AUTHOR INFORMATION

Corresponding Authors

Emily R. Derbyshire – *Department of Molecular Genetics and Microbiology, Duke University Medical Center, 213 Research Drive, Durham, NC 27710, USA*; *Department of Chemistry, Duke University, 124 Science Drive, Durham, NC 27708, USA;* Email: emily.derbyshire@duke.edu

Reena Zutshi – *Luceome Biotechnologies, L.L.C, 1665 E. 18th Street, Suite 106, Tucson, AZ 85719, USA;* Email: reena.zutshi@luceome.com

David H. Drewry – *Structural Genomics Consortium and Division of Chemical Biology and Medicinal Chemistry, Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA; Lineberger Comprehensive Cancer Center, Department of Medicine, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA;* Email: david.drewry@unc.edu

Authors

Han Wee Ong – *Structural Genomics Consortium and Division of Chemical Biology and Medicinal Chemistry, Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA*

Chandi de Silva – *Luceome Biotechnologies, L.L.C, 1665 E. 18th Street, Suite 106, Tucson, AZ 85719, USA*

Krisha Avalani – *Luceome Biotechnologies, L.L.C, 1665 E. 18th Street, Suite 106, Tucson, AZ 85719, USA*

Frank Kwarcinski – *Luceome Biotechnologies, L.L.C, 1665 E. 18th Street, Suite 106, Tucson, AZ 85719, USA*

Christopher R. Mansfield – *Department of Molecular Genetics and Microbiology, Duke University Medical Center, 213 Research Drive, Durham, NC 27710, USA*

Michael Chirgwin – *Department of Chemistry, Duke University, 124 Science Drive, Durham, NC 27708, USA*

Anna Truong – *Department of Chemistry, Duke University, 124 Science Drive, Durham, NC 27708, USA*

ACKNOWLEDGEMENTS

This material is based upon work supported by the National Institutes of Health under Grant No. (1R44AI150237-01) and the National Science Foundation under Grant No. (1644868 and CHE-1726291). We thank the University of North Carolina's Department of Chemistry Mass Spectrometry Core Laboratory, especially Diane Weatherspoon, for their assistance with mass spectrometry analysis.

We are grateful for support by the Structural Genomics Consortium (SGC), a registered charity (no: 1097737) that receives funds from Bayer AG, Boehringer Ingelheim, Bristol Myers Squibb, Genentech, Genome Canada through Ontario

Genomics Institute [OGI-196], EU/EFPIA/OICR/McGill/KTH/Diamond Innovative Medicines Initiative 2 Joint Undertaking [EUbOPEN grant 875510], Janssen, Merck KGaA (aka EMD in Canada and US), Pfizer and Takeda.

ABBREVIATIONS USED

ALK, ALK receptor tyrosine kinase; CDPK, calcium-dependent protein kinase; EGFR, epidermal growth factor receptor; FAK, protein tyrosine kinase 2; HMBC, heteronuclear multiple bond correlation; JAK2, Janus kinase 2; KLIFS, kinase-ligand interaction fingerprints and structure database; LRRK2, leucine rich repeat kinase 2; MAPK, mitogen-activated protein kinase; NEK, never-in-mitosis-A related kinases; Pf3D7, P. falciparum 3D7; PfARK1, P. falciparum serine/threonine protein kinase ARK1; PfARK3, P. falciparum serine/threonine protein kinase ARK3, putative; PfCDPK1, P. falciparum calcium-dependent protein kinase 1; PfCDPK2, P. falciparum calcium-dependent protein kinase 2; PfCDPK4, P. falciparum calcium-dependent protein kinase 4; PfCDPK5, P. falciparum calcium-dependent protein kinase 5; PfCLK3, P. falciparum cyclin-dependent-like kinase CLK3; PfGAP45, P. falciparum glideosome-associated protein 45; PfMAP2, P. falciparum mitogen-activated protein kinase 2; PfNEK1, P. falciparum NIMA related kinase 1; PfNEK3, P. falciparum NIMA related kinase 3; PfPK6, P. falciparum protein kinase 6; PfPK9, P. falciparum serine/threonine protein kinase PK9; PfPKB, P. falciparum RAC-beta serine/threonine protein kinase; PfPKG, P. falciparum cGMP-dependent protein kinase; PfUBC13, P. falciparum ubiquitin-conjugating enzyme E2 13; PLK1, polo like kinase 1; SAR, structure-activity-relationship; S_NAr, nucleophilic aromatic substitution; TCAMS, Tres Cantos Antimalarial Set.

REFERENCES

(1) World Health Organization. *World Malaria Report 2022*; World Health Organization: Geneva, 2022.
(2) Tse, E. G.; Korsik, M.; Todd, M. H. The Past, Present and Future of Anti-Malarial Medicines. *Malar. J.* **2019**, *18*, 93.
(3) Brown, J. R.; Drewry, D.; Gamo, F.-J.; Garcia-Bustos, J. F. Kinase Inhibitors Among Hits from Malaria Cellular Screens. In *Protein Phosphorylation in Parasites*; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2013; pp 261–291.
(4) Lucet, I. S.; Tobin, A.; Drewry, D.; Wilks, A. F.; Doerig, C. Plasmodium Kinases as Targets for New-Generation Antimalarials. *Future Med. Chem.* **2012**, *4*, 2295–2310.
(5) Arendse, L. B.; Wyllie, S.; Chibale, K.; Gilbert, I. H. Plasmodium Kinases as Potential Drug Targets for Malaria: Challenges and Opportunities. *ACS Infect. Dis.* **2021**, *7*, 518–534.
(6) Ong, H. W.; Adderley, J.; Tobin, A. B.; Drewry, D. H.; Doerig, C. Parasite and Host Kinases as Targets for Antimalarials. *Expert Opin. Ther. Targets* **2023**, *27*, 151–169.
(7) 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.
(8) Anamika; Srinivasan, N.; Krupa, A. A Genomic Perspective of Protein Kinases in Plasmodium Falciparum. *Proteins Struct. Funct. Bioinforma.* **2005**, *58*, 180–189.
(9) 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.
(10) Zhang, M.; Wang, C.; Otto, T. D.; Oberstaller, J.; Liao, X.; Adapa, S. R.; Udenze, K.; Bronner, I. F.; Casandra, D.; Mayho, M.; Brown, J.; Li, S.; Swanson, J.; Rayner, J. C.; Jiang, R. H. Y.; Adams, J. H. Uncovering the Essential Genes of the Human Malaria Parasite Plasmodium Falciparum by Saturation Mutagenesis. *Science* **2018**, *360*, eaap7847.
(11) 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-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*, 430.

(12) Vanaerschot, M.; Murithi, J. M.; Pasaje, C. F. A.; Ghidelli-Disse, S.; Dwomoh, L.; Bird, M.; Spottiswoode, N.; Mittal, N.; Arendse, L. B.; Owen, E. S.; Wicht, K. J.; Siciliano, G.; Bösche, M.; Yeo, T.; Kumar, T. R. S.; Mok, S.; Carpenter, E. F.; Giddins, M. J.; Sanz, O.; Ottilie, S.; Alano, P.; Chibale, K.; Llinás, M.; Uhlemann, A.-C.; Delves, M.; Tobin, A. B.; Doerig, C.; Winzeler, E. A.; Lee, M. C. S.; Niles, J. C.; Fidock, D. A. Inhibition of Resistance-Refractory P. Falciparum Kinase PKG Delivers Prophylactic, Blood Stage, and Transmission-Blocking Antiplasmodial Activity. *Cell Chem. Biol.* **2020**, *27*, 806–816.

(13) Matralis, A. N.; Malik, A.; Penzo, M.; Moreno, I.; Almela, M. J.; Camino, I.; Crespo, B.; Saadeddin, A.; Ghidelli-Disse, S.; Rueda, L.; Calderon, F.; Osborne, S. A.; Drewes, G.; Böesche, M.; Fernández-Álvaro, E.; Martin Hernando, J. I.; Baker, D. A. Development of Chemical Entities Endowed with Potent Fast-Killing Properties against Plasmodium Falciparum Malaria Parasites. *J. Med. Chem.* **2019**, *62*, 9217–9235.

(14) Large, J. M.; Birchall, K.; Bouloc, N. S.; Merritt, A. T.; Smiljanic-Hurley, E.; Tsagris, D. J.; Wheldon, M. C.; Ansell, K. H.; Coombs, P. J.; Kettleborough, C. A.; Whalley, D.; Stewart, L. B.; Bowyer, P. W.; Baker, D. A.; Osborne, S. A. Potent Inhibitors of Malarial P. Falciparum Protein Kinase G: Improving the Cell Activity of a Series of Imidazopyridines. *Bioorganic Med. Chem. Lett.* **2019**, *29*, 509–514.

(15) Large, J. M.; Birchall, K.; Bouloc, N. S.; Merritt, A. T.; Smiljanic-Hurley, E.; Tsagris, D. J.; Wheldon, M. C.; Ansell, K. H.; Coombs, P. J.; Kettleborough, C. A.; Whalley, D.; Stewart, L. B.; Bowyer, P. W.; Baker, D. A.; Osborne, S. A. Potent Bicyclic Inhibitors of Malarial CGMP-Dependent Protein Kinase: Approaches to Combining Improvements in Cell Potency, Selectivity and Structural Novelty. *Bioorg. Med. Chem. Lett.* **2019**, *29*, 126610.

(16) Tsagris, D. J.; Birchall, K.; Bouloc, N.; Large, J. M.; Merritt, A.; Smiljanic-Hurley, E.; Wheldon, M.; Ansell, K. H.; Kettleborough, C.; Whalley, D.; Stewart, L. B.; Bowyer, P. W.; Baker, D. A.; Osborne, S. A. Trisubstituted Thiazoles as Potent and Selective Inhibitors of Plasmodium Falciparum Protein Kinase G (PfPKG). *Bioorg. Med. Chem. Lett.* **2018**, *28*, 3168–3173.

(17) Penzo, M.; de las Heras-Dueña, L.; Mata-Cantero, L.; Diaz-Hernandez, B.; Vazquez-Muñiz, M. J.; Ghidelli-Disse, S.; Drewes, G.; Fernandez-Alvaro, E.; Baker, D. A. High-Throughput Screening of the Plasmodium Falciparum CGMP-Dependent Protein Kinase Identified a Thiazole Scaffold Which Kills Erythrocytic and Sexual Stage Parasites. *Sci. Rep.* **2019**, *9*, 1–13.

(18) Mahmood, S. U.; Cheng, H.; Tummalapalli, S. R.; Chakrasali, R.; Yadav Bheemanaboina, R. R.; Kreiss, T.; Chojnowski, A.; Eck, T.; Siekierka, J. J.; Rotella, D. P. Discovery of Isoxazolyl-Based Inhibitors of Plasmodium Falciparum CGMP-Dependent Protein Kinase. *RSC Med. Chem.* **2020**, *11*, 98–101.

(19) Bheemanaboina, R. R. Y.; de Souza, M. L.; Gonzalez, M. L.; Mahmood, S. U.; Eck, T.; Kreiss, T.; Aylor, S. O.; Roth, A.; Lee, P.; Pybus, B. S.; Colussi, D. J.; Childers, W. E.; Gordon, J.; Siekierka, J. J.; Bhanot, P.; Rotella, D. P. Discovery of Imidazole-Based Inhibitors of Plasmodium Falciparum CGMP-Dependent Protein Kinase. *ACS Med. Chem. Lett.* **2021**, *12*, 1962–1967.

(20) Alam, M. M.; Sanchez-Azqueta, A.; Janha, O.; Flannery, E. L.; Mahindra, A.; Mapesa, K.; Char, A. B.; Sriranganadane, D.; Brancucci, N. M. B.; Antonova-Koch, Y.; Crouch, K.; Simwela, N. V.; Millar, S. B.; Akinwale, J.; Mitcheson, D.; Solyakov, L.; Dudek, K.; Jones, C.; Zapatero, C.; Doerig, C.; Nwakanma, D. C.; Vázquez, M. J.; Colmenarejo, G.; Lafuente-Monasterio, M. J.; Leon, M. L.; Godoi, P. H. C.; Elkins, J. M.; Waters, A. P.; Jamieson, A. G.; Álvaro, E. F.; Ranford-Cartwright, L. C.; Marti, M.; Winzeler, E. A.; Gamo, F. J.; Tobin, A. B. Validation of the Protein Kinase PfCLK3 as a Multistage Cross-Species Malarial Drug Target. *Science* **2019**, *365*, eaau1682.

(21) Mahindra, A.; Janha, O.; Mapesa, K.; Sanchez-Azqueta, A.; Alam, M. M.; Amambua-Ngwa, A.; Nwakanma, D. C.; Tobin, A. B.; Jamieson, A. G. Development of Potent Pf CLK3 Inhibitors Based on TCMDC-135051 as a New Class of Antimalarials. *J. Med. Chem.* **2020**, *63*, 9300–9315.

(22) Kato, N.; Sakata, T.; Breton, G.; Le Roch, K. G.; Nagle, A.; Andersen, C.; Bursulaya, B.; Henson, K.; Johnson, J.; Kumar, K. A.; Marr, F.; Mason, D.; McNamara, C.; Plouffe, D.; Ramachandran, V.; Spooner, M.; 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*, 347–356.

(23) 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*, 6379–6389.

(24) 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*, 3064–3069.

(25) 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. Biochemical and Antiparasitic Properties of Inhibitors of the Plasmodium Falciparum Calcium-Dependent Protein Kinase PfCDPK1. *Antimicrob. Agents Chemother.* **2014**, *58*, 6032–6043.
(26) 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. *Bioorganic Med. Chem. Lett.* **2013**, *23*, 6019–6024.
(27) 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. Optimization of an Imidazopyridazine Series of Inhibitors of Plasmodium Falciparum Calcium-Dependent Protein Kinase 1 (PfCDPK1). *J. Med. Chem.* **2014**, *57*, 3570–3587.
(28) Xing, L.; Rai, B.; Lunney, E. A. Scaffold Mining of Kinase Hinge Binders in Crystal Structure Database. *J. Comput. Aided. Mol. Des.* **2014**, *28*, 13–23.
(29) Xing, L.; Klug-Mcleod, J.; Rai, B.; Lunney, E. A. Kinase Hinge Binding Scaffolds and Their Hydrogen Bond Patterns. *Bioorg. Med. Chem.* **2015**, *23*, 6520–6527.
(30) Gamo, F.-J.; Sanz, L. M.; Vidal, J.; de Cozar, C.; Alvarez, E.; Lavandera, J.-L.; Vanderwall, D. E.; Green, D. V. S.; Kumar, V.; Hasan, S.; Brown, J. R.; Peishoff, C. E.; Cardon, L. R.; Garcia-Bustos, J. F. Thousands of Chemical Starting Points for Antimalarial Lead Identification. *Nature* **2010**, *465*, 305–310.
(31) Crowther, G. J.; Hillesland, H. K.; Keyloun, K. R.; Reid, M. C.; Lafuente-Monasterio, M. J.; Ghidelli-Disse, S.; Leonard, S. E.; He, P.; Jones, J. C.; Krahn, M. M.; Mo, J. S.; Dasari, K. S.; Fox, A. M. W.; Boesche, M.; El Bakkouri, M.; Rivas, K. L.; Leroy, D.; Hui, R.; Drewes, G.; Maly, D. J.; Van Voorhis, W. C.; Ojo, K. K. Biochemical Screening of Five Protein Kinases from Plasmodium Falciparum against 14,000 Cell-Active Compounds. *PLoS One* **2016**, *11*, e0149996.
(32) Phuangsawai, O.; Beswick, P.; Ratanabunyong, S.; Tabtimmai, L.; Suphakun, P.; Obounchoey, P.; Srisook, P.; Horata, N.; Chuckowree, I.; Hannongbua, S.; Ward, S. E.; Choowongkomon, K.; Gleeson, M. P. Evaluation of the Anti-Malarial Activity and Cytotoxicity of 2,4-Diamino-Pyrimidine-Based Kinase Inhibitors. *Eur. J. Med. Chem.* **2016**, *124*, 896–905.
(33) Toviwek, B.; Phuangsawai, O.; Konsue, A.; Hannongbua, S.; Riley, J.; Mutter, N.; Anderson, M.; Webster, L.; Hallyburton, I.; Read, K. D.; Gleeson, M. P. Preparation, Biological & Cheminformatics-Based Assessment of N2,N4-Diphenylpyrimidine-2,4-Diamine as Potential Kinase-Targeted Antimalarials. *Bioorg. Med. Chem.* **2021**, *46*, 116348.
(34) Bohmer, M. J.; Wang, J.; Istvan, E. S.; Luth, M. R.; Collins, J. E.; Huttlin, E. L.; Wang, L.; Mittal, N.; Hao, M.; Kwiatkowski, N. P.; Gygi, S. P.; Chakrabarti, R.; Deng, X.; Goldberg, D. E.; Winzeler, E. A.; Gray, N. S.; Chakrabarti, D. Human Polo-like Kinase Inhibitors as Antiplasmodials. *ACS Infect. Dis.* **2023**, *9*, 1004–1021.
(35) Ramsden, N.; Perrin, J.; Ren, Z.; Lee, B. D.; Zinn, N.; Dawson, V. L.; Tam, D.; Bova, M.; Lang, M.; Drewes, G.; Bantscheff, M.; Bard, F.; Dawson, T. M.; Hopf, C. Chemoproteomics-Based Design of Potent LRRK2-Selective Lead Compounds That Attenuate Parkinson's Disease-Related Toxicity in Human Neurons. *ACS Chem. Biol.* **2011**, *6*, 1021–1028.
(36) Jester, B. W.; Cox, K. J.; Gaj, A.; Shomin, C. D.; Porter, J. R.; Ghosh, I. A Coiled-Coil Enabled Split-Luciferase Three-Hybrid System: Applied Toward Profiling Inhibitors of Protein Kinases. *J. Am. Chem. Soc.* **2010**, *132*, 11727–11735.
(37) Reininger, L.; Wilkes, J. M.; Bourgade, H.; Miranda-Saavedra, D.; Doerig, C. An Essential Aurora-Related Kinase Transiently Associates with Spindle Pole Bodies during Plasmodium Falciparum Erythrocytic Schizogony. *Mol. Microbiol.* **2011**, *79*, 205–221.
(38) Berry, L.; Chen, C.-T.; Reininger, L.; Carvalho, T. G.; El Hajj, H.; Morlon-Guyot, J.; Bordat, Y.; Lebrun, M.; Gubbels, M.-J.; Doerig, C.; Daher, W. The Conserved Apicomplexan Aurora Kinase TgArk3 Is Involved in Endodyogeny, Duplication Rate and Parasite Virulence. *Cell. Microbiol.* **2016**, *18*, 1106–1120.
(39) Patel, G.; Roncal, N. E.; Lee, P. J.; Leed, S. E.; Erath, J.; Rodriguez, A.; Sciotti, R. J.; Pollastri, M. P. Repurposing Human Aurora Kinase Inhibitors as Leads for Anti-Protozoan Drug Discovery. *Med. Chem. Commun.* **2014**, *5*, 655–658.
(40) Morahan, B. J.; Abrie, C.; Al-Hasani, K.; Batty, M. B.; Corey, V.; Cowell, A. N.; Niemand, J.; Winzeler, E. A.; Birkholtz, L.-M.; Doerig, C.; Garcia-Bustos, J. F. Human Aurora Kinase Inhibitor Hesperadin Reveals Epistatic Interaction between Plasmodium Falciparum PfArk1 and PfNek1 Kinases. *Commun. Biol.* **2020**, *3*, 701.
(41) Lye, Y. M.; Chan, M.; Sim, T.-S. Pfnek3: An Atypical Activator of a MAP Kinase in Plasmodium Falciparum. *FEBS Lett.* **2006**, *580*, 6083–6092.
(42) Low, H.; Lye, Y. M.; Sim, T.-S. Pfnek3 Functions as an Atypical MAPKK in Plasmodium Falciparum. *Biochem. Biophys. Res. Commun.* **2007**, *361*, 439–444.
(43) Ong, H. W.; Truong, A.; Kwarcinski, F.; de Silva, C.; Avalani, K.; Havener, T. M.; Chirgwin, M.; Galal, K. A.;

Willis, C.; Krämer, A.; Liu, S.; Knapp, S.; Derbyshire, E. R.; Zutshi, R.; Drewry, D. H. Discovery of Potent Plasmodium Falciparum Protein Kinase 6 (PfPK6) Inhibitors with a Type II Inhibitor Pharmacophore. *Eur. J. Med. Chem.* **2023**, *249*, 115043.

(44) Philip, N.; Haystead, T. A. Characterization of a UBC13 Kinase in Plasmodium Falciparum. *Proc. Natl. Acad. Sci.* **2007**, *104*, 7845–7850.

(45) Raphemot, R.; Eubanks, A. L.; Toro-Moreno, M.; Geiger, R. A.; Hughes, P. F.; Lu, K.-Y.; Haystead, T. A. J.; Derbyshire, E. R. Plasmodium PK9 Inhibitors Promote Growth of Liver-Stage Parasites. *Cell Chem. Biol.* **2019**, *26*, 411–419.

(46) Maneekesorn, S.; Knuepfer, E.; Green, J. L.; Prommana, P.; Uthaipibull, C.; Srichairatanakool, S.; Holder, A. A. Deletion of Plasmodium Falciparum Ubc13 Increases Parasite Sensitivity to the Mutagen, Methyl Methanesulfonate and Dihydroartemisinin. *Sci. Rep.* **2021**, *11*, 21791.

(47) Kumar, A.; Vaid, A.; Syin, C.; Sharma, P. PfPKB, a Novel Protein Kinase B-like Enzyme from Plasmodium Falciparum I. Identification, Characterization, and Possible Role in Parasite Development. *J. Biol. Chem.* **2004**, *279*, 24255–24264.

(48) Baum, J.; Richard, D.; Healer, J.; Rug, M.; Krnajski, Z.; Gilberger, T.-W.; Green, J. L.; Holder, A. A.; Cowman, A. F. A Conserved Molecular Motor Drives Cell Invasion and Gliding Motility across Malaria Life Cycle Stages and Other Apicomplexan Parasites. *J. Biol. Chem.* **2006**, *281*, 5197–5208.

(49) Jones, M. L.; Kitson, E. L.; Rayner, J. C. Plasmodium Falciparum Erythrocyte Invasion: A Conserved Myosin Associated Complex. *Mol. Biochem. Parasitol.* **2006**, *147*, 74–84.

(50) Thomas, D. C.; Ahmed, A.; Gilberger, T. W.; Sharma, P. Regulation of Plasmodium Falciparum Glideosome Associated Protein 45 (PfGAP45) Phosphorylation. *PLoS One* **2012**, *7*, e35855.

(51) Vaid, A.; Thomas, D. C.; Sharma, P. Role of Ca2+/Calmodulin-PfPKB Signaling Pathway in Erythrocyte Invasion by Plasmodium Falciparum. *J. Biol. Chem.* **2008**, *283*, 5589–5597.

(52) Richter, D. T.; Kath, J. C.; Luzzio, M. J.; Keene, N.; Berliner, M. A.; Wessel, M. D. Selective Addition of Amines to 5-Trifluoromethyl-2,4-Dichloropyrimidine Induced by Lewis Acids. *Tetrahedron Lett.* **2013**, *54*, 4610–4612.

(53) Lietha, D.; Eck, M. J. Crystal Structures of the FAK Kinase in Complex with TAE226 and Related Bis-Anilino Pyrimidine Inhibitors Reveal a Helical DFG Conformation. *PLoS One* **2008**, *3*, e3800.

(54) Davis, R. R.; Li, B.; Yun, S. Y.; Chan, A.; Nareddy, P.; Gunawan, S.; Ayaz, M.; Lawrence, H. R.; Reuther, G. W.; Lawrence, N. J.; Schönbrunn, E. Structural Insights into JAK2 Inhibition by Ruxolitinib, Fedratinib, and Derivatives Thereof. *J. Med. Chem.* **2021**, *64*, 2228–2241.

(55) Lategahn, J.; Tumbrink, H. L.; Schultz-Fademrecht, C.; Heimsoeth, A.; Werr, L.; Niggenaber, J.; Keul, M.; Parmaksiz, F.; Baumann, M.; Menninger, S.; Zent, E.; Landel, I.; Weisner, J.; Jeyakumar, K.; Heyden, L.; Russ, N.; Müller, F.; Lorenz, C.; Brägelmann, J.; Spille, I.; Grabe, T.; Müller, M. P.; Heuckmann, J. M.; Klebl, B. M.; Nussbaumer, P.; Sos, M. L.; Rauh, D. Insight into Targeting Exon20 Insertion Mutations of the Epidermal Growth Factor Receptor with Wild Type-Sparing Inhibitors. *J. Med. Chem.* **2022**, *65*, 6643–6655.

(56) van Linden, O. P. J.; Kooistra, A. J.; Leurs, R.; de Esch, I. J. P.; de Graaf, C. KLIFS: A Knowledge-Based Structural Database To Navigate Kinase–Ligand Interaction Space. *J. Med. Chem.* **2014**, *57*, 249–277.

(57) Jumper, J.; Evans, R.; Pritzel, A.; Green, T.; Figurnov, M.; Ronneberger, O.; Tunyasuvunakool, K.; Bates, R.; Žídek, A.; Potapenko, A.; Bridgland, A.; Meyer, C.; Kohl, S. A. A.; Ballard, A. J.; Cowie, A.; Romera-Paredes, B.; Nikolov, S.; Jain, R.; Adler, J.; Back, T.; Petersen, S.; Reiman, D.; Clancy, E.; Zielinski, M.; Steinegger, M.; Pacholska, M.; Berghammer, T.; Bodenstein, S.; Silver, D.; Vinyals, O.; Senior, A. W.; Kavukcuoglu, K.; Kohli, P.; Hassabis, D. Highly Accurate Protein Structure Prediction with AlphaFold. *Nature* **2021**, *596*, 583–589.

(58) Varadi, M.; Anyango, S.; Deshpande, M.; Nair, S.; Natassia, C.; Yordanova, G.; Yuan, D.; Stroe, O.; Wood, G.; Laydon, A.; Žídek, A.; Green, T.; Tunyasuvunakool, K.; Petersen, S.; Jumper, J.; Clancy, E.; Green, R.; Vora, A.; Lutfi, M.; Figurnov, M.; Cowie, A.; Hobbs, N.; Kohli, P.; Kleywegt, G.; Birney, E.; Hassabis, D.; Velankar, S. AlphaFold Protein Structure Database: Massively Expanding the Structural Coverage of Protein-Sequence Space with High-Accuracy Models. *Nucleic Acids Res.* **2022**, *50*, D439–D444.

(59) Müller, S.; Chaikuad, A.; Gray, N. S.; Knapp, S. The Ins and Outs of Selective Kinase Inhibitor Development. *Nat. Chem. Biol.* **2015**, *11*, 818–821.

(60) Zuccotto, F.; Ardini, E.; Casale, E.; Angiolini, M. Through the "Gatekeeper Door": Exploiting the Active Kinase Conformation. *J. Med. Chem.* **2010**, *53*, 2681–2694.

(61) Attwood, M. M.; Fabbro, D.; Sokolov, A. V.; Knapp, S.; Schiöth, H. B. Trends in Kinase Drug Discovery: Targets, Indications and Inhibitor Design. *Nat. Rev. Drug Discov.* **2021**, *20*, 839–861.

(62) Alam, M.; Beevers, R. E.; Ceska, T.; Davenport, R. J.; Dickson, K. M.; Fortunato, M.; Gowers, L.; Haughan, A. F.; James, L. A.; Jones, M. W.; Kinsella, N.; Lowe, C.; Meissner, J. W. G.; Nicolas, A. L.; Perry, B. G.; Phillips, D. J.; Pitt, W. R.; Platt, A.; Ratcliffe, A. J.; Sharpe, A.; Tait, L. J. Synthesis and SAR of Aminopyrimidines as Novel C-

Jun N-Terminal Kinase (JNK) Inhibitors. *Bioorganic Med. Chem. Lett.* **2007**, *17*, 3463–3467.
(63) Lange, A.; Günther, M.; Büttner, F. M.; Zimmermann, M. O.; Heidrich, J.; Hennig, S.; Zahn, S.; Schall, C.; Sievers-Engler, A.; Ansideri, F.; Koch, P.; Laemmerhofer, M.; Stehle, T.; Laufer, S. A.; Boeckler, F. M. Targeting the Gatekeeper MET146 of C-Jun N-Terminal Kinase 3 Induces a Bivalent Halogen/Chalcogen Bond. *J. Am. Chem. Soc.* **2015**, *137*, 14640–14652.
(64) Hole, A. J.; Baumli, S.; Shao, H.; Shi, S.; Huang, S.; Pepper, C.; Fischer, P. M.; Wang, S.; Endicott, J. A.; Noble, M. E. Comparative Structural and Functional Studies of 4-(Thiazol-5-Yl)-2-(Phenylamino)Pyrimidine-5-Carbonitrile CDK9 Inhibitors Suggest the Basis for Isotype Selectivity. *J. Med. Chem.* **2013**, *56*, 660–670.
(65) Abula, A.; Xu, Z.; Zhu, Z.; Peng, C.; Chen, Z.; Zhu, W.; Aisa, H. A. Substitution Effect of the Trifluoromethyl Group on the Bioactivity in Medicinal Chemistry: Statistical Analysis and Energy Calculations. *J. Chem. Inf. Model.* **2020**, *60*, 6242–6250.
(66) Marsilje, T. H.; Pei, W.; Chen, B.; Lu, W.; Uno, T.; Jin, Y.; Jiang, T.; Kim, S.; Li, N.; Warmuth, M.; Sarkisova, Y.; Sun, F.; Steffy, A.; Pferdekamper, A. C.; Li, A. G.; Joseph, S. B.; Kim, Y.; Liu, B.; Tuntland, T.; Cui, X.; Gray, N. S.; Steensma, R.; Wan, Y.; Jiang, J.; Chopiuk, G.; Li, J.; Gordon, W. P.; Richmond, W.; Johnson, K.; Chang, J.; Groessl, T.; He, Y.; Phimister, A.; Aycinena, A.; Lee, C. C.; Bursulaya, B.; Karanewsky, D. S.; Seidel, H. M.; Harris, J. L.; Michellys, P.-Y. Synthesis, Structure–Activity Relationships, and in Vivo Efficacy of the Novel Potent and Selective Anaplastic Lymphoma Kinase (ALK) Inhibitor 5-Chloro- N 2-(2-Isopropoxy-5-Methyl-4-(Piperidin-4-Yl)Phenyl)- N 4-(2-(Isopropylsulfonyl)Phenyl)Pyrimidine-2,4-. *J. Med. Chem.* **2013**, *56*, 5675–5690.
(67) Blake, J. F.; Burkard, M.; Chan, J.; Chen, H.; Chou, K.-J.; Diaz, D.; Dudley, D. A.; Gaudino, J. J.; Gould, S. E.; Grina, J.; Hunsaker, T.; Liu, L.; Martinson, M.; Moreno, D.; Mueller, L.; Orr, C.; Pacheco, P.; Qin, A.; Rasor, K.; Ren, L.; Robarge, K.; Shahidi-Latham, S.; Stults, J.; Sullivan, F.; Wang, W.; Yin, J.; Zhou, A.; Belvin, M.; Merchant, M.; Moffat, J.; Schwarz, J. B. Discovery of (S)-1-(1-(4-Chloro-3-Fluorophenyl)-2-Hydroxyethyl)-4-(2-((1-Methyl-1H-Pyrazol-5-Yl)Amino)Pyrimidin-4-Yl)Pyridin-2(1H)-One (GDC-0994), an Extracellular Signal-Regulated Kinase 1/2 (ERK1/2) Inhibitor in Early Clinical Development. *J. Med. Chem.* **2016**, *59*, 5650–5660.
(68) Huang, W.-S.; Liu, S.; Zou, D.; Thomas, M.; Wang, Y.; Zhou, T.; Romero, J.; Kohlmann, A.; Li, F.; Qi, J.; Cai, L.; Dwight, T. A.; Xu, Y.; Xu, R.; Dodd, R.; Toms, A.; Parillon, L.; Lu, X.; Anjum, R.; Zhang, S.; Wang, F.; Keats, J.; Wardwell, S. D.; Ning, Y.; Xu, Q.; Moran, L. E.; Mohemmad, Q. K.; Jang, H. G.; Clackson, T.; Narasimhan, N. I.; Rivera, V. M.; Zhu, X.; Dalgarno, D.; Shakespeare, W. C. Discovery of Brigatinib (AP26113), a Phosphine Oxide-Containing, Potent, Orally Active Inhibitor of Anaplastic Lymphoma Kinase. *J. Med. Chem.* **2016**, *59*, 4948–4964.
(69) Poratti, M.; Marzaro, G. Third-Generation CDK Inhibitors: A Review on the Synthesis and Binding Modes of Palbociclib, Ribociclib and Abemaciclib. *Eur. J. Med. Chem.* **2019**, *172*, 143–153.
(70) Ward, R. A.; Anderton, M. J.; Ashton, S.; Bethel, P. A.; Box, M.; Butterworth, S.; Colclough, N.; Chorley, C. G.; Chuaqui, C.; Cross, D. A. E.; Dakin, L. A.; Debreczeni, J. É.; Eberlein, C.; Finlay, M. R. V.; Hill, G. B.; Grist, M.; Klinowska, T. C. M.; Lane, C.; Martin, S.; Orme, J. P.; Smith, P.; Wang, F.; Waring, M. J. Structure- and Reactivity-Based Development of Covalent Inhibitors of the Activating and Gatekeeper Mutant Forms of the Epidermal Growth Factor Receptor (EGFR). *J. Med. Chem.* **2013**, *56*, 7025–7048.
(71) William, A. D.; Lee, A. C. H.; Blanchard, S.; Poulsen, A.; Teo, E. L.; Nagaraj, H.; Tan, E.; Chen, D.; Williams, M.; Sun, E. T.; Goh, K. C.; Ong, W. C.; Goh, S. K.; Hart, S.; Jayaraman, R.; Pasha, M. K.; Ethirajulu, K.; Wood, J. M.; Dymock, B. W. Discovery of the Macrocycle 11-(2-Pyrrolidin-1-Yl-Ethoxy)-14,19-Dioxa-5,7,26-Triaza-Tetracyclo[19.3.1.1(2,6).1(8,12)]Heptacosa-1(25),2(26),3,5,8,10,12(27),16,21,23-Decaene (SB1518), a Potent Janus Kinase 2/Fms-Like Tyrosine Kinase-3 (JAK2/FLT3) Inhibitor . *J. Med. Chem.* **2011**, *54*, 4638–4658.
(72) Galkin, A. V.; Melnick, J. S.; Kim, S.; Hood, T. L.; Li, N.; Li, L.; Xia, G.; Steensma, R.; Chopiuk, G.; Jiang, J.; Wan, Y.; Ding, P.; Liu, Y.; Sun, F.; Schultz, P. G.; Gray, N. S.; Warmuth, M. Identification of NVP-TAE684, a Potent, Selective, and Efficacious Inhibitor of NPM-ALK. *Proc. Natl. Acad. Sci.* **2007**, *104*, 270–275.
(73) Kothe, M.; Kohls, D.; Low, S.; Coli, R.; Rennie, G. R.; Feru, F.; Kuhn, C.; Ding, Y.-H. Selectivity-Determining Residues in Plk1. *Chem. Biol. Drug Des.* **2007**, *70*, 540–546.
(74) Johnson, T. W.; Richardson, P. F.; Bailey, S.; Brooun, A.; Burke, B. J.; Collins, M. R.; Cui, J. J.; Deal, J. G.; Deng, Y.; Dinh, D.; Engstrom, L. D.; He, M.; Hoffman, J.; Hoffman, R. L.; Huang, Q.; Kania, R. S.; Kath, J. C.; Lam, H.; Lam, J. L.; Le, P. T.; Lingardo, L.; Liu, W.; McTigue, M.; Palmer, C. L.; Sach, N. W.; Smeal, T.; Smith, G. L.; Stewart, A. E.; Timofeevski, S.; Zhu, H.; Zhu, J.; Zou, H. Y.; Edwards, M. P. Discovery of (10R)-7-Amino-12-Fluoro-2,10,16-Trimethyl-15-Oxo-10,15,16,17-Tetrahydro- 2H -8,4-(Metheno)Pyrazolo[4,3-h][2,5,11]-Benzoxadiazacyclotetradecine-3-Carbonitrile (PF-06463922), a Macrocyclic Inhibitor of Anaplastic Lymphoma Kinase (ALK) and c-Ros. *J. Med. Chem.* **2014**, *57*, 4720–4744.
(75) Tan, L.; Akahane, K.; McNally, R.; Reyskens, K. M. S. E.; Ficarro, S. B.; Liu, S.; Herter-Sprie, G. S.; Koyama, S.; Pattison, M. J.; Labella, K.; Johannessen, L.; Akbay, E. A.; Wong, K.-K.; Frank, D. A.; Marto, J. A.; Look, T. A.; Arthur, J. S. C.; Eck, M. J.; Gray, N. S. Development of Selective Covalent Janus Kinase 3 Inhibitors. *J. Med. Chem.*

2015, *58*, 6589–6606.

(76) Smilkstein, M.; Sriwilaijaroen, N.; Kelly, J. X.; Wilairat, P.; Riscoe, M. Simple and Inexpensive Fluorescence-Based Technique for High-Throughput Antimalarial Drug Screening. *Antimicrob. Agents Chemother.* **2004**, *48*, 1803–1806.