ACS Medicinal Chemistry Letters

pubs.acs.org/acsmedchemlett
Letter

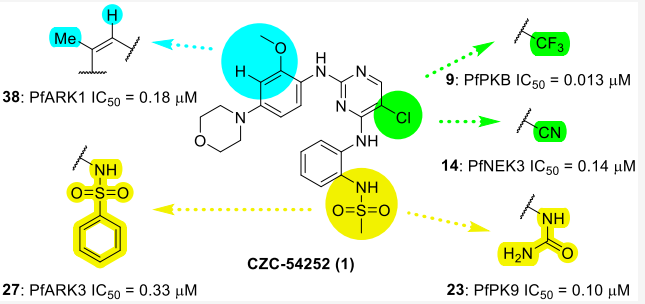
Characterization of 2,4-Dianilinopyrimidines Against Five *P. falciparum* Kinases PfARK1, PfARK3, PfNEK3, PfPK9, and PfPKB

Han Wee Ong, Chandi de Silva, Krisha Avalani, Frank Kwarcinski, Christopher R. Mansfield, Michael Chirgwin, Anna Truong, Emily R. Derbyshire,* Reena Zutshi,* and David H. Drewry*

Cite This: *ACS Med. Chem. Lett.* 2023, 14, 1774−1784
Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

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 were discovered. We identified common and divergent SAR trends across all five kinases, highlighting substituents in 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 toward novel antimalarials.



KEYWORDS: *Malaria, Protein kinase inhibitor, 2,4-Dianilinopyrimidine, PfARK1, PfARK3, PfNEK3, PfPK9, PfPKB*

Malaria is a devastating disease caused by *Plasmodium* infection, with 247 million cases and 619 thousand deaths globally in 2021.[1] The rising resistance toward existing antimalarials is worrying.[1] New medicines with novel mechanisms of action are needed to address this evolving challenge. *Plasmodium* protein kinases present an untapped opportunity for drug development.[2−5] Of the 85−99 *P. falciparum* protein kinases,[6,7] 36−40 have been identified as essential for the asexual blood stage of *P. falciparum*.[8,9] Yet, 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 the inhibitor development of *Plasmodium* kinases.

Pyrimidines have been recognized as common hinge-binding groups of kinase inhibitors.[10,11] 2,4-Dianilinopyrimidines are among the hits in the Tres Cantos Antimalarial Set (TCAMS), a set of 13 533 inhibitors of *P. falciparum* asexual reproduction.[12] Screening TCAMS against five *P. falciparum* kinases led to the identification of PfCDPK1, PfCDPK4, and PfPK6 as putative targets of 2,4-dianilinopyrimidines.[13] Medicinal chemistry campaigns of 2,4-dianilinopyrimidines have been reported in the literature, optimizing for antiplasmodial activity by phenotypic screening.[14,15] The authors suggested that this series of inhibitors may target the *Plasmodium* CDPK and NEK families.[15] Recently, six human-PLK1-targeted 2,4-diarylamino-pyrimidine inhibitors have also demonstrated antiplasmodial activity, of which two of them demonstrate inhibition of PfCDPK2, PfNEK3, and PfPKB.[16]

The 2,4-dianilinopyrimidines CZC-54252 (**1**) and CZC-25146 (**2**) (Figure 1A) were developed as human LRRK2 inhibitors.[17] During our screening efforts to discover novel kinase-targeted antiplasmodials, we found **1** to reduce parasite viability of *P. falciparum* 3D7 (Pf3D7) blood stage to 21% of the negative control at 1 μM. Follow-up experiments confirmed 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[18] at 1 μM. Five kinase targets were identified and demonstrated 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,[13] we did not observe inhibition of PfPK6 by **1**. We also did not observe significant inhibition of CDPK family members PfCDPK2 and PfCDPK5 and NEK family member PfNEK1.

Received: September 15, 2023
Revised: November 13, 2023
Accepted: November 14, 2023
Published: November 27, 2023

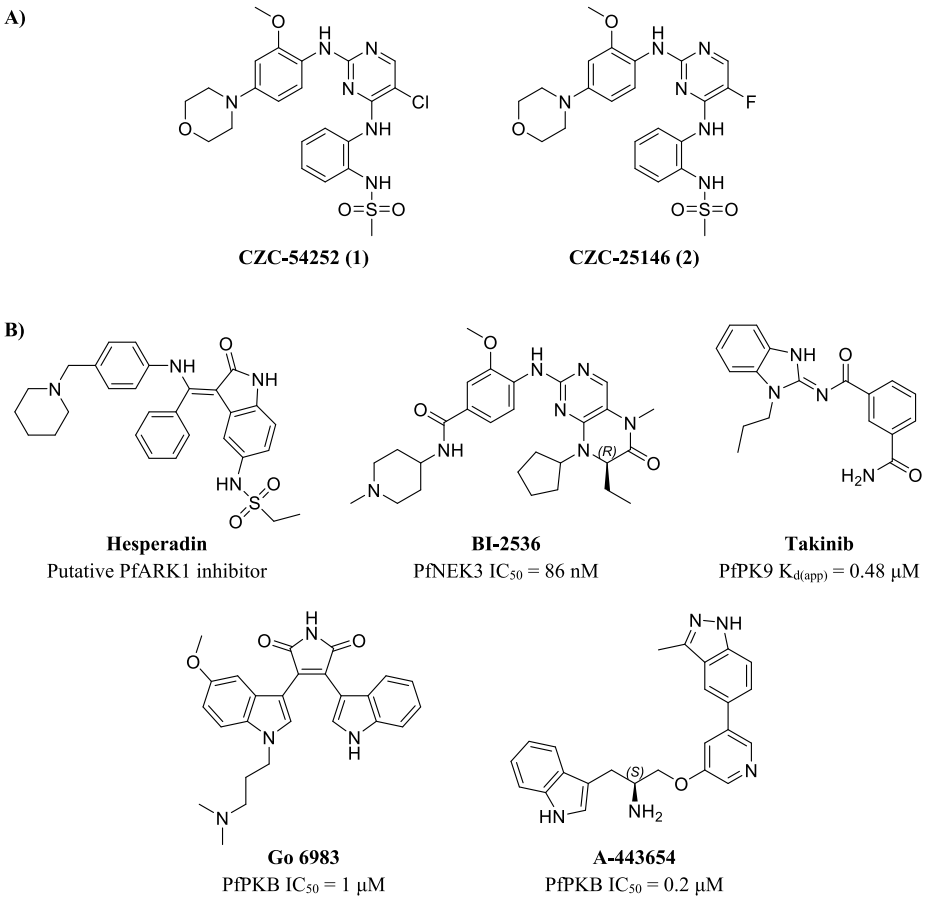


Figure 1. (A) Structure of CZC-54252 (**1**) and CZC-25146 (**2**).[17] (B) Literature inhibitors of PfARK1,[22] PfNEK3,[16] PfPK9,[27] and PfPKB.[29,33]

Table 1. Inhibition Profile of CZC-54252 (1) across the Panel of 11 *P. falciparum* Kinases

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]Mean values from duplicate experiments using the KinaseSeeker assay. [b]Mean values from two experiments using the KinaseSeeker assay performed in duplicate.

PfARK1 (PF3D7_0605300) and PfARK3 (PF3D7_1356800) are Aurora kinases suggested to be involved in nuclear division processes.[19,20] Genetic studies have indicated that PfARK1 is essential for asexual blood stage of *P. falciparum*.[8,9,19] In contrast, PfARK3 is genetically validated to be essential by reverse genetics[8] but not by saturation mutagenesis.[9] No inhibitor of either kinase has been confirmed in the literature, although inhibitors of human Aurora kinases have demonstrated antiplasmodial activity.[21,22] PfARK1 mutations confer resistance to the human Aurora kinase inhibitor hesperadin (Figure 1B), suggesting that PfARK1 may be its target.[22]

PfNEK3 (PF3D7_1201600) belongs to the NEK family.[23] Separate studies have regarded PfNEK3 as either dispensable for the asexual blood stage proliferation[8] or that it could be disrupted, albeit with a fitness penalty.[9] PfNEK3 participates in an atypical MAPK signaling cascade by upregulating the activity of PfMAP2,[23,24] although the implications of this signaling pathway are not currently clear. Human PLK1 inhibitors including BI-2536 (Figure 1B) have been found to inhibit PfNEK3.[16] We have also previously identified PfNEK3 as one of the secondary targets of our potent PfPK6-targeted type II inhibitors.[25]

PfPK9 (PF3D7_1315100) is an orphan kinase that does not cluster with any typical kinase group[26] and is genetically validated to be essential for asexual blood stage proliferation.[8,9] PfPK9 regulates the activity of PfUBC13,[26,27] an essential E2 ubiquitin-conjugating enzyme involved with DNA replication and repair.[28] The only known inhibitors of PfPK9 are the human TAK1 inhibitor Takinib (Figure 1B) and analogues.[27]

PfPKB (PF3D7_1246900) is a kinase from the AGC group.[29] While a reverse genetics study found PfPKB to be essential,[8] it was found to be dispensable by saturation mutagenesis.[9] PfPKB is implicated in regulation of merozoite invasion into erythrocytes by phosphorylating PfGAP45.[30−32] Inhibition of PfPKB by Go 6983 or A-443654 (Figure 1B) decreases parasitemia and formation of new rings in the subsequent round of invasion, consistent with its proposed role in regulating invasion.[29,33] PfPKB has also been determined to be a secondary

https://doi.org/10.1021/acsmedchemlett.3c00354
ACS Med. Chem. Lett. 2023, 14, 1774−1784

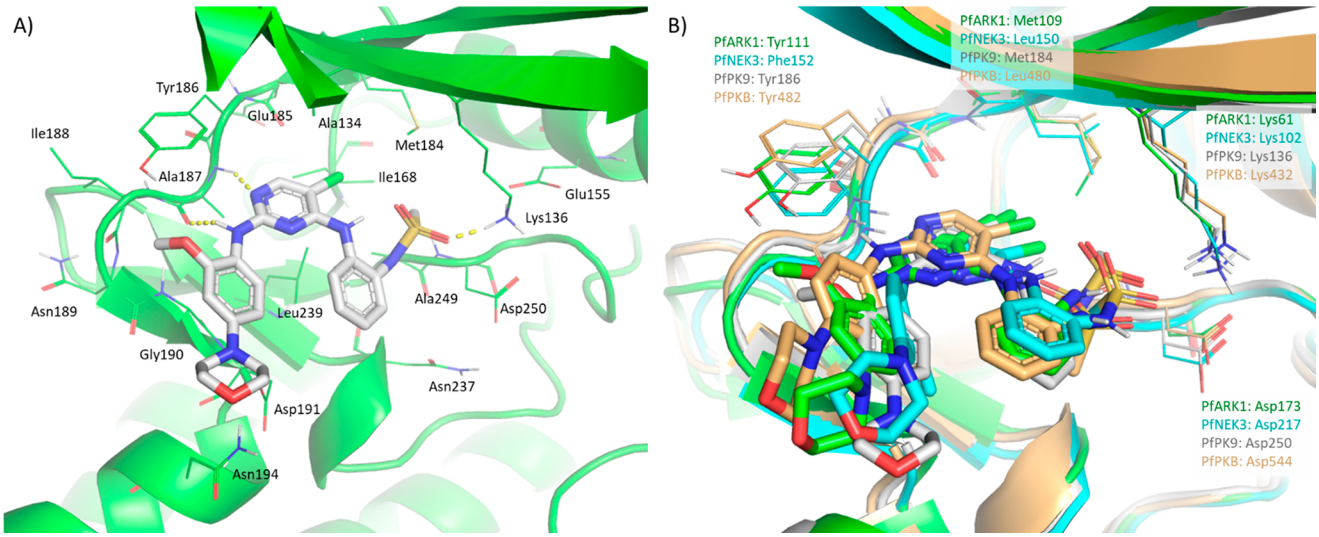


Figure 2. (A) CZC-54252 (**1**) (white) docked to the structure of PfPK9 predicted by AlphaFold (green). Intermolecular hydrogen bonds are denoted by yellow dashed lines. (B) Overlay of docked structures of **1** to the predicted structure of PfARK1 (green), PfNEK3 (cyan), PfPK9 (white), and PfPKB (gold)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

target of the above-mentioned 2,4-diarylaminopyrimidine inhibitors[16] and our type II inhibitors.[25]

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 (only 19.5−33.7% pairwise sequence identity in kinase domains; see Supporting Information (SI), 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 inhibitors with the 2,4-dianilinopyrimidine scaffold.

As crystal structures of **1** with any *Plasmodium* kinase are unavailable, we docked **1** into the structures of PfARK1, PfNEK3, PfPK9, and PfPKB predicted by AlphaFold[34,35] (docking was not performed for PfARK3 because its structure was unavailable) (Figure 2, and SI, Figures S2 and S3). As expected based on cocrystal structures of other 2,4-dianilinopyrimidines with kinases, the model suggests that **1** forms two hydrogen bonds with the peptide backbone of the outer hinge residue (hinge.46 by KLIFS notation[36]) using the N1 of the pyrimidine and exocyclic NH at the 2-position. The 2-position aryl substituent extends toward 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.

The 5-position group of the pyrimidines binds close to the gatekeeper residue of the kinase, a residue that is often targeted for potency and specificity. Apart from steric complementarity, 5-position groups on pyrimidines may form specific interactions with gatekeeper residues, such as hydrogen bonding,[37] halogen/chalcogen bonds,[38,39] or lone pair−π interactions.[40] We thus first investigated the effect of substituting the 5-position chloro group of **1** with other groups (Table 2).

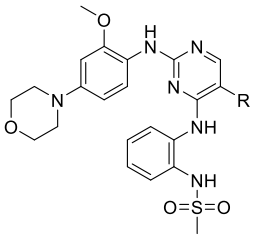
Both removal of the chlorine atom (**3**) or introduction of an endocyclic N atom at this position, converting the pyrimidine to a 1,3,5-triazine (**20**), abolished activity for all five kinases, suggesting that the chlorine makes key interactions with each kinase. Replacement with fluorine (**2**) recovered some activity only for PfPKB (IC_{50} = 1.5 μM) and PfNEK3 (68% activity remaining at 1 μM). Replacement with the heavier halogen bromine (**4**) or iodine (**5**) maintained the potencies for PfARK1, PfARK3, and PfPKB, 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 with increasing halogen size, suggesting that there are favorable hydrophobic or halogen-bonding interactions with heavier halogens. Looking closer into this general trend, we observed that this effect plateaued at chlorine for PfARK1, PfARK3, and PfPKB, and at bromine for PfNEK3, while it continued to iodine for PfPK9. This trend is independent of their gatekeeper residues (methionine for PfARK1 and PfPK9, leucine for PfARK3, PfNEK3, and PfPKB), suggesting that the origin of this varied sensitivity to halogens is more complex than could be simply predicted based on these residues.

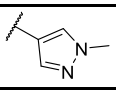
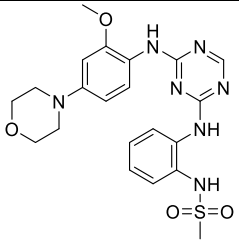
The methyl (**6**) and ethyl (**7**) groups both abolished PfARK1, PfARK3, and PfPK9 potency but were tolerated by PfNEK3 and PfPKB. Further increase in steric size of the alkyl group to a cyclopropyl ring (**8**) was tolerated by PfPKB but abolished PfNEK3 activity. Among the five kinases, **8** is exquisitely selective for PfPKB. Interestingly, the trifluoromethyl group (**9**) is potent against all five kinases, improving potency, relative to **1**, by PfARK1 3-fold, PfNEK3 2-fold, PfPK9 16-fold, and PfPKB 5-fold. PfARK3 was unaffected. Excitingly, **9** is the most potent PfPKB inhibitor reported (IC_{50} = 13 nM).

Similar to the alkyl groups, a methoxy group (**10**) or a dimethylamino group (**11**) were not tolerated by PfARK1, PfARK3, and PfPK9. Comparing with **7**, with a similar-sized ethyl group, <2-fold change in PfNEK3 IC_{50}, but a 4-fold drop in PfPKB potency with **10** was observed. In contrast, we observed a 3-fold drop in PfNEK3 potency with <2-fold change in PfPKB potency with **11**. Both hydroxyl (**12**) and amino (**13**) groups completely abolished activity on all five kinases, suggesting that

https://doi.org/10.1021/acsmedchemlett.3c00354
ACS Med. Chem. Lett. 2023, 14, 1774−1784

Table 2. SAR at the 5-Position of the Pyrimidine



		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	–

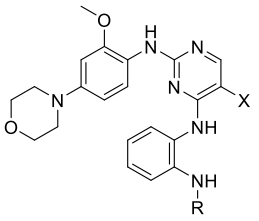
[a]Mean values from duplicate experiments using the KinaseSeeker assay. [b]Mean values from two experiments using the KinaseSeeker assay performed in duplicate.

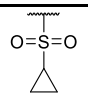
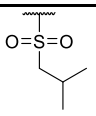
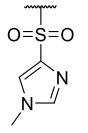
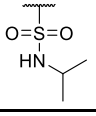
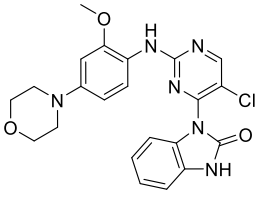
there is a lack of productive hydrogen bonding partners to accommodate these hydrogen bond donors here.

Surprisingly, the nitrile group (**14**) was not tolerated by PfARK1, PfARK3, and PfPK9, slightly disfavored by PfPKB (2.6-fold drop in potency compared to **1**), but strongly preferred by PfNEK3 (7-fold improvement in potency over **1**). This highlights a possible avenue for improving selectivity for PfNEK3 over the other kinases. The *N*-methylpyrazol-4-yl substituent (**15**) also demonstrated selectivity for PfNEK3, maintaining the PfNEK3 potency of **1** but completely abolishing

https://doi.org/10.1021/acsmedchemlett.3c00354
ACS Med. Chem. Lett. 2023, 14, 1774−1784

Table 3. SAR at the 4-Position of the Pyrimidine



			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]Mean values from duplicate experiments using the KinaseSeeker assay. [b]Mean values from two experiments using the KinaseSeeker assay performed in duplicate.

activity on the other kinases. The tolerability of the large *N*-methylpyrazol-4-yl substituent inspired us to further investigate the steric requirements of this region. Similarly sized ethers (**16**, **17**) or an ester substituent (**18**) were unfortunately not tolerated by any kinase, suggesting that the aromaticity of the *N*-methylpyrazol-4-yl substituent was important.

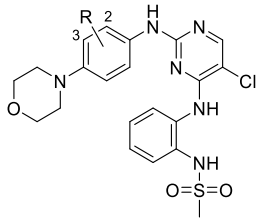
The carboxamide substituent (**19**) improved in potency for PfARK1 (3-fold) and PfPK9 (6-fold) over **1**, but decreased potency for PfNEK3 (2.5-fold) and PfPKB (4-fold) and completely lost PfARK3 activity. This hence offers an avenue to design for selectivity for PfARK1 over related family member PfARK3.

In summary, we demonstrate that different kinases have different sensitivities to the 5-position substituent, and this knowledge could help to design selective inhibitors among these five kinases. We have shown that PfARK1, PfARK3, and PfPK9 are rather sensitive at this position, only tolerating several substituents (PfARK1: Cl, CF_3, $CONH_2$; PfARK3: Cl, Br, I, and CF_3; PfPK9: Cl, Br, I, CF_3, $CONH_2$). In contrast, PfNEK3 and PfPKB have their preferred substituents (CN and CF_3, respectively) but are generally more tolerant of changes.

We next explored SAR at the 4-position of the pyrimidine (Table 3). The removal of the sulfonyl group of the sulfonamide (**21**) was detrimental toward activity for all five kinases, possibly

https://doi.org/10.1021/acsmedchemlett.3c00354
ACS Med. Chem. Lett. 2023, 14, 1774−1784

Table 4. SAR on Substituents at the 2-Position Aniline



		PfARK1		PfARK3		PfNEK3		PfPK9		PfPKB	
compound	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]Mean values from duplicate experiments using the KinaseSeeker assay. [b]Mean values from two experiments using the KinaseSeeker assay performed in duplicate.

attributed to the loss of hydrogen bonding potential with the catalytic lysine. Cyclizing the 4-position dianiline ring to a benzimidazolone (**30**) abolished activity against all five kinases, demonstrating 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** retained weak activity on PfARK1, PfPK9, and PfPKB (65−70% activity remaining at 1 μM), this change led to a loss of activity against PfARK3 and PfNEK3. In contrast, **23** is 12-fold more potent against PfPK9 than **1**, equipotent against PfPKB, while abolishing activity against PfARK1, PfARK3, and PfNEK3.

To investigate the steric requirement of the pocket around the sulfonamide, we increased the size of the methyl group on the sulfonamide to an ethyl (**24**) or cyclopropyl (**25**) group, both improving PfARK3 and PfPK9 activity 4-fold and 2-fold, respectively. While **24** was 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 both compounds, suggesting that these modifications were tolerated but not preferred. A further increase in size to an isobutyl group (**26**) was unfavorable for all kinases except PfARK3, which maintained the potency of **1**. A phenyl group (**27**) was preferred by PfARK3, affording a 5-fold improvement in potency over **1**, while maintaining potency against PfARK1 and PfNEK3 and dramatically decreasing potency against PfPK9 and PfPKB. A phenyl ring may thus offer an opportunity 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.

For the *N*-isopropyl sulfamoyl amide (**29**), inhibition results for all five kinases were similar to those of the isosteric isobutyl sulfonamide **26**. This shows that despite favoring additional hydrogen bond donors in urea **23**, PfPK9 has a strict steric requirement for this region precluding binding of **29**.

Our SAR results for these five *Plasmodium* kinases demonstrate that the 4-position of the pyrimidine offers an avenue to generate selectivity. With different steric requirements in the active site around this region, we emphasize how single-atom changes here could modulate selectivity between kinases. Selective inhibition of PfARK3 may be achieved using larger lipophilic groups at this region, something not as well tolerated by PfARK1, PfNEK3, PfPK9, and PfPKB. The methyl group is the ideal group for PfPKB, while the slightly larger cyclopropyl group is most ideal for PfARK1 and PfNEK3. We have also found that PfPK9 strongly prefers urea at this position.

The next position for SAR exploration is the 2-position of the pyrimidine. Acknowledging that exocyclic NH typically forms hydrogen bonds with the hinge region of kinases, we opted not to disrupt this key pharmacophore. Substituents on the aryl ring here are frequently used to design for selectivity. For example, a substituent at the position *ortho*- to the exocyclic NH confers selectivity of TAE684 for ALK,[41] or BI-2536 for PLK1.[42] Because the morpholine ring at the 4-position of the aniline ring was expected to be solvent-exposed, we focused on and established the SAR of 2- (*ortho*-) and 3-position (*meta*-) substituents (Table 4).

Removal of the *ortho*-methoxy group of **1** (**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**) led 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**) maintained the potency of **1** against PfARK1 and PfPK9, while

https://doi.org/10.1021/acsmedchemlett.3c00354
ACS Med. Chem. Lett. 2023, 14, 1774−1784

decreasing the potency against PfARK3 (2.4-fold) and PfPKB (6-fold), but is 5-fold more potent on PfNEK3. The small methyl (**34**), fluoro (**35**), or chloro groups (**36**) maintained potency of **1** against PfARK1, PfARK3, and PfPKB, while improving upon activity of both PfNEK3 and PfPK9 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 nondiscriminatory. The steric requirement and preference for a unsubstituted *ortho*-position could arise from the relief of a steric clash with the middle hinge residue (hinge.47 by KLIFS notation[36]) of the kinase. This steric clash typically occurs for kinases with larger tyrosine or phenylalanine residues while being accommodating of the smaller leucine residue.[41−45] However, because the middle hinge residues of these five kinases are all tyrosine or phenylalanine residues, this does not provide a simple explanation regarding the differential sensitivity toward substituents at this position. We thus emphasize the importance of investigating and understanding SAR to complement sequence-based hypotheses.

We next installed substituents *meta*- on the NH of the aniline ring. Electron-donating methoxy (**37**) and methyl (**38**) substituents improved PfARK1, PfARK3, and PfNEK3 potencies modestly (1.5- to 3-fold) when compared to **31**, while PfPK9 and PfPKB potencies were unaffected (≤1.5-fold difference). Addition of a chloro substituent (**39**) did not change the potency of **31** across all five kinases. Addition of the trifluoromethyl group (**40**) did not change potency of **31** against PfARK1 and PfARK3, while improving PfNEK3 potency 2.2-fold, yet decreasing PfPK9 and PfPKB potency 2.5-fold. Unlike when substituents are varied 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 *ortho*-position substituents were primarily due to steric effects rather than electronic contributions to the aniline ring. Despite the possibilities of *meta*-position substituents changing the electron density of the adjacent morpholine ring and its dihedral angle with the phenyl ring, the modest differences in potency suggest that such changes were well-tolerated by these five kinases. Their general tolerability toward 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.

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 $IC_{50} \leq 0.3$ μM against any kinase, and screened them at 1 μM against Pf3D7 using a SYBR Green I-based fluorescence assay to evaluate asexual blood stage viability.[46] We found nine new analogues with <50% viability at 1 μM and further profiled them in dose−response experiments (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.[12]

We found compounds **4**, **5**, **9**, and **14** to possess submicromolar potencies against Pf3D7 in the asexual blood stage, a comparable potency to **1**. These compounds were 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 to 15 μM, their observed solubility limit in the assay conditions. These four compounds offer at least a 14-fold window between

Table 5. Antiplasmodial Activity and Cytotoxicity

	Pf3D7 blood stage			
compound	% viability at 1 μM[a]	EC_{50} (μM)[b]	HepG2 CC_{50} (μM)[c]	CC_{50}/EC_{50}
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]Mean values of triplicate experiments using the SYBR Green I-based assay. [b]Mean values from two experiments performed in duplicate using the SYBR Green I-based assay. [c]Mean values from two experiments performed in triplicate using the CellTiter-Fluor assay. [d]No significant cytotoxicity observed up to 15 μM, the observed solubility limit. [e]Decreases cell viability around 1 μM to ∼60%, but never decreases cell viability below 50%.

antiplasmodial activity and cytotoxicity. **8** was also weakly active against Pf3D7 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 Pf3D7 but 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, possibly enabling inhibition of off-target 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 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.

To further characterize these compounds, we have additionally determined the kinetic solubility, permeability, human plasma protein binding, and plasma stability of **1** and the nine novel analogues with dose-dependent antiplasmodial activity (Table 6). **1** possesses a good solubility of 71.1 μM. Replacement of the 5-position Cl atom with other substituents (**4**, **5**, **8**, **9**, **14**) decreased solubility. This was especially evident with the CF_3 (**9**) and CN (**14**) groups, possibly attributed to the decreased basicity of the pyrimidine ring. The methoxy group on the *ortho*-position on the 2-position aniline is important for solubility, as its removal (**31**) decreases solubility 8-fold

https://doi.org/10.1021/acsmedchemlett.3c00354
ACS Med. Chem. Lett. 2023, 14, 1774−1784

Table 6. Solubility, Permeability, Plasma Protein Binding, and Plasma Stability

compd	kinetic solubility in PBS pH 7.4 (μM)[a]	PAMPA P_e ($\times 10^{-6}$ cm/s)[b]	human PPB (% bound)[a]	human plasma stability (% remaining at 6 h)[a]
1	71.1	21.4	99.0	97
4	46.8	18.2	99.3	101
5	21.0	15.5	99.5	99
8	36.0	14.8	97.6	101
9	1.1	17.8	97.6	103
14	0.9	15.1	94.6	92
31	8.5	19.1	95.9	105
37	116.0	20.9	98.6	103
38	12.8	20.9	99.1	101
39	0.4	12.0	99.4	98

[a]Mean values of duplicate experiments. [b]Mean values of triplicate experiments.

compared to **1**. Reinstating the methoxy group at the *meta*-position (**37**) restored the solubility to 116 μM. However, a *meta*-position methyl (**38**) or chloro (**39**) group is unfavorable for solubility. Given the modest changes in potency against the five kinase targets with changes at the *meta*-position, we thus reiterate that this position offers a potential avenue to modulate the physiochemical and pharmacokinetic properties of this chemotype.

We measured the permeability of these compounds by the PAMPA assay and found all analogues to possess moderate-to-excellent permeability. The structural modifications performed did not change the permeability, with <2-fold differences observed among all 10 compounds. It is of interest to measure plasma protein binding because strong binding to plasma proteins may lead to reduced penetrance into the parasite and demonstrate lower antiplasmodial activity.[47] From our human plasma protein binding assay results, we observed that most compounds are highly bound to human plasma proteins, with all compounds at least 94.6% bound. The compounds with the greatest affinity to plasma proteins are **1**, **4**, **5**, **38**, and **39**, with ≥99% bound. All compounds tested were highly stable in human plasma. The high degree of binding is a potential factor for the disconnect between the kinase inhibitory potency and antiplasmodial potency of these compounds. We note that there is no apparent correlation between the permeability or the degree of plasma protein binding and antiplasmodial potency of these compounds, suggesting that multiple factors are likely involved. Nevertheless, the high degree of binding to plasma proteins is a characteristic of this chemotype which should be

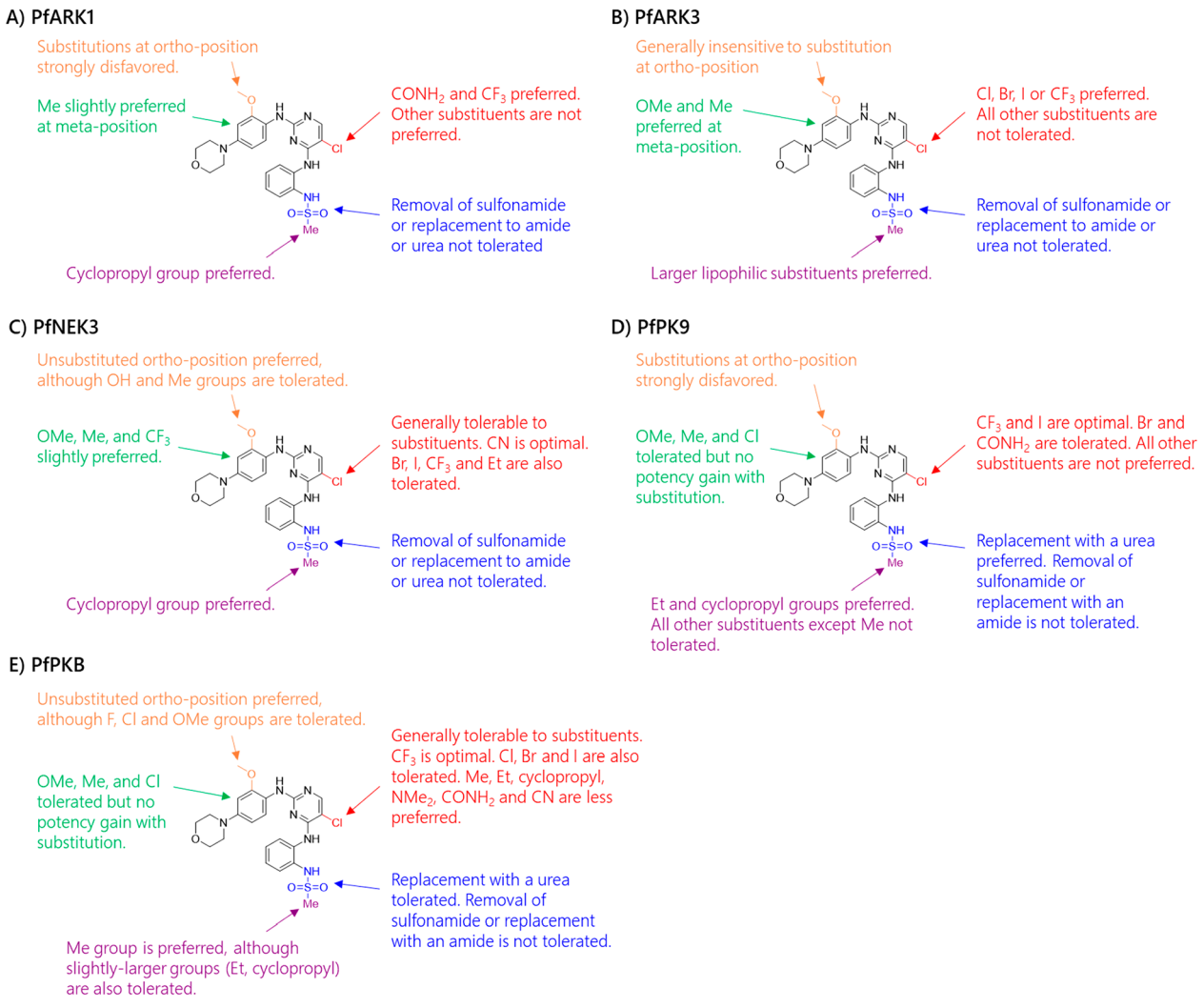


Figure 3. SAR Summary for PfARK1 (A), PfARK3 (B), PfNEK3 (C), PfPK9 (D), and PfPKB (E).

https://doi.org/10.1021/acsmedchemlett.3c00354
ACS Med. Chem. Lett. 2023, 14, 1774−1784

taken into account during further lead optimization of these compounds.

In conclusion, we have identified **1** as an inhibitor of five *P. falciparum* kinases PfARK1, PfARK3, PfNEK3, PfPK9, and PfPKB. We have developed SAR against all five kinases (Figure 3) and found subtle changes that improved potency against each kinase. 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 of **1**. In contrast, the most potent PfARK3 inhibitor in our set of compounds was **27**, attained by substituting the methylsulfonamide on the aniline ring at the 4-position of the pyrimidine to a phenylsulfonamide. Many potent PfPKB inhibitors were discovered. One of which was **9**, where the 5-position chloro group was replaced with a trifluoromethyl group. Of the PfNEK3 inhibitors that we discovered, we highlight **14**, which demonstrated a unique improvement in potency by a simple substitution of the 5-position chloro group with a nitrile. Similarly, we highlight **23** as a compound that demonstrated a unique improvement in potency against PfPK9, through the incorporation of a urea group instead of a sulfonamide. Of these novel analogues, **37** also demonstrated an improvement in potency against the *P. falciparum* asexual blood stage.

We also emphasize the importance of investigating and developing the 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 both 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 potency and selectivity. Currently, there is a dearth of medicinal chemistry studies of 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 kinase.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.3c00354.

Percent identity and similarity for PfARK1, PfARK3, PfNEK3, PfPK9, and PfPKB; Use of additional molecular docking constraints; comparison of **1** docked to the structures of PfARK1, PfNEK3, PfPK9, and PfPKB; summary of ^{1}H-^{13}C HMBC results, methods; experimental spectra for **CZC-54252** analogues; supplemental references (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

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, North Carolina 27599, United States; Lineberger Comprehensive Cancer Center, Department of Medicine, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, United States;* orcid.org/0000-0001-5973-5798; Email: david.drewry@unc.edu

Emily R. Derbyshire − *Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina 27710, United States; Department of Chemistry, Duke University, Durham, North Carolina 27708, United States;* orcid.org/0000-0001-6664-8844; Email: emily.derbyshire@duke.edu

Reena Zutshi − *Luceome Biotechnologies, LLC, Tucson, Arizona 85719, United States*; Email: reena.zutshi@luceome.com

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, North Carolina 27599, United States;* orcid.org/0000-0003-3232-2373

Chandi de Silva − *Luceome Biotechnologies, LLC, Tucson, Arizona 85719, United States*

Krisha Avalani − *Luceome Biotechnologies, LLC, Tucson, Arizona 85719, United States*

Frank Kwarcinski − *Luceome Biotechnologies, LLC, Tucson, Arizona 85719, United States*

Christopher R. Mansfield − *Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina 27710, United States*

Michael Chirgwin − *Department of Chemistry, Duke University, Durham, North Carolina 27708, United States*

Anna Truong − *Department of Chemistry, Duke University, Durham, North Carolina 27708, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsmedchemlett.3c00354

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work is 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 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

CDPK, calcium-dependent protein kinase; 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.*

https://doi.org/10.1021/acsmedchemlett.3c00354
ACS Med. Chem. Lett. 2023, 14, 1774−1784

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.

REFERENCES

(1) *World Malaria Report 2022*; World Health Organization: Geneva, 2022.

(2) 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: Weinheim, Germany, 2013; pp 261−291.

(3) 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.

(4) 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.

(5) 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.

(6) 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.

(7) Anamika; Srinivasan, N.; Krupa, A. A Genomic Perspective of Protein Kinases in Plasmodium Falciparum. *Proteins Struct. Funct. Bioinforma.* **2005**, *58*, 180−189.

(8) 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.

(9) 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*, No. eaap7847.

(10) 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.

(11) 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.

(12) 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.

(13) 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*, No. e0149996.

(14) 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.

(15) 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.

(16) 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.

(17) 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.

(18) 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.

(19) 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.

(20) 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.

(21) 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.

(22) 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.

(23) 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.

(24) 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.

(25) 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.

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

(27) 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.

(28) 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.

(29) 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.

(30) 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

https://doi.org/10.1021/acsmedchemlett.3c00354
ACS Med. Chem. Lett. 2023, 14, 1774−1784

Malaria Life Cycle Stages and Other Apicomplexan Parasites. *J. Biol. Chem.* **2006**, *281*, 5197−5208.
(31) 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.
(32) Thomas, D. C.; Ahmed, A.; Gilberger, T. W.; Sharma, P. Regulation of Plasmodium Falciparum Glideosome Associated Protein 45 (PfGAP45) Phosphorylation. *PLoS One* **2012**, *7*, No. e35855.
(33) 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.
(34) 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.
(35) 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.
(36) 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.
(37) 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.
(38) 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. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3463−3467.
(39) 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.
(40) 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.
(41) 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. U. S. A.* **2007**, *104*, 270−275.
(42) 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.
(43) 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.
(44) 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.
(45) 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.
(46) 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.
(47) Rakotoarivelo, N. V.; Perio, P.; Najahi, E.; Nepveu, F. Interaction between Antimalarial 2-Aryl-3H-Indol-3-One Derivatives and Human Serum Albumin. *J. Phys. Chem. B* **2014**, *118*, 13477−13485.

https://doi.org/10.1021/acsmedchemlett.3c00354
ACS Med. Chem. Lett. 2023, 14, 1774−1784