# Journal Pre-proof


Discovery of 6′-chloro-*N*-methyl-5'-(phenylsulfonamido)-[3,3′-bipyridine]-5-carboxamide (CHMFL-PI4K-127) as a novel *Plasmodium falciparum* PI(4)K inhibitor with potent antimalarial activity against both blood and liver stages of *Plasmodium*

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PII: S0223-5234(19)31169-9

DOI: https://doi.org/10.1016/j.ejmech.2019.112012

Reference: EJMECH 112012

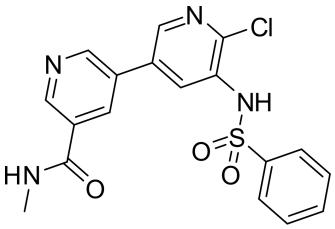
To appear in: *European Journal of Medicinal Chemistry*

Received Date: 30 October 2019

Revised Date: 21 December 2019

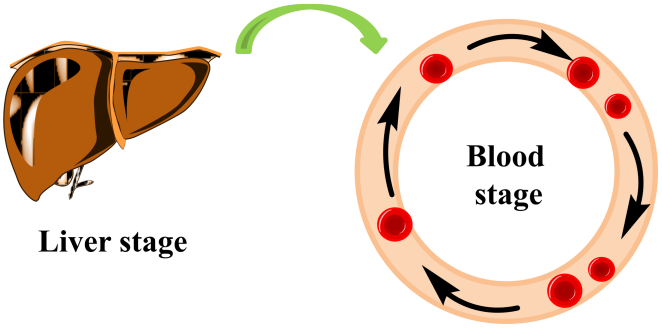
Accepted Date: 25 December 2019

Please cite this article as: X. Liang, Z. Jiang, Z. Huang, F. Li, C. Chen, C. Hu, W. Wang, Z. Hu, Q. Liu, B. Wang, L. Wang, Z. Qi, J. Liu, L. Jiang, Q. Liu, Discovery of 6′-chloro-*N*-methyl-5'-(phenylsulfonamido)-[3,3′-bipyridine]-5-carboxamide (CHMFL-PI4K-127) as a novel *Plasmodium falciparum* PI(4)K inhibitor with potent antimalarial activity against both blood and liver stages of *Plasmodium*, *European Journal of Medicinal Chemistry* (2020), doi: https://doi.org/10.1016/j.ejmech.2019.112012.




**15g (CHMFL-PI4K-127)**
*Pf*3D7 $EC_{50}$=25 nM
*Pf*PI4K $IC_{50}$=0.9 nM
S-Score (1)=0
Oral bioavailability 89%

**Activity against both Parasite Blood and Liver Life Stages**



# Discovery of 6'-Chloro-*N*-methyl-5'-(phenylsulfonamido)-[3,3'-bipyridine]-5-carboxamide (CHMFL-PI4K-127) as a Novel *Plasmodium falciparum* PI(4)K Inhibitor with Potent Antimalarial Activity against both Blood and Liver Stages of *Plasmodium*

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**ABSTRACT**

Starting from a bipyridine-sulfonamide scaffold, medicinal chemistry optimization leads to the discovery of a novel *Plasmodium falciparum* PI4K kinase (*Pf*PI4K) inhibitor compound **15g** (CHMFL-PI4K-127, $IC_{50}$: 0.9 nM), which exhibits potent activity against 3D7 *Plasmodium falciparum (P. falciparum)* ($EC_{50}$: 25.1 nM). CHMFL-PI4K-127 displays high selectivity against *Pf*PI4K over human lipid and protein kinase. In addition, it exhibits $EC_{50}$ values of 23-47 nM against a panel of the drug-resistant strains of *P. falciparum*. In vivo, the inhibitor demonstrates the favorable pharmacokinetic properties in both rats and mice. Furthermore, oral administration of CHMFL-PI4K-127 exhibits the antimalaria efficacy in both blood stage (80 mg/kg) and liver stage (1 mg/kg) of *Plasmodium* in infected rodent model. The results suggest that CHMFL-PI4K-127 might be a new potential drug candidate for malaria.

**Keywords**

PI4K kinase, kinase inhibitor, malaria, blood stage, liver stage

**Abbreviations used**

*P. falciparum, Plasmodium falciparum*; *P.yoelii, Plasmodium yoelii*; *Pf*PI4K, *Plasmodium falciparum* phosphatidylinositol-4-kinase; PI4K, phosphatidylinositol-4-kinase; PI, phosphatidylinositol; PI4P, phosphtaidylinositol-4-phosphate; ATP, Adenosine Triphosphate; SAR, structure-activity relationship; DMAP, 4-(Dimethylamino)pyridine; THF, Tetrahydrofuran ; TEA, Triethylamine ; DCM, Dichloromethane ; HATU, 1-

[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate; DIPEA, *N*,*N*-Diisopropyl-ethylamine; EtOAc, Ethyl acetate.

## 1. Introduction

Drug resistant malaria infections are still a severe threat to the public health[1]. In clinic, there is yet a great demand to seek novel chemotypes with new mechanisms of antimalaria in order to overcome the drug resistance. In recent years, new chemical entities with activity across different life cycle stages of the malaria parasite and new mechanisms of action have been identified[2-6]. *Plasmodium* phosphatidylinositol-4-kinase[3, 6] is one of the attractive drug discovery targets due to its important roles in all stages of the *Plasmodium* lifecycle. In addition, it has been expected that the *Plasmodium* PI4K inhibitors would overcome the resistance of artemisinin and artemisinin-based combination therapies for *P. falciparum* malaria since they bear different inhibition mechanism[7-10]. *Plasmodium* PI4K catalyzes the phosphorylation of phosphatidylinositol (PI) to produce the cellular secondary messenger phosphatidylinositol-4-phosphate (PI4P), subsequently initiating a cascade of downstream events to modulate a variety of biological processes including secondary messenger signaling, cellular membrane remodeling, and vesicular trafficking[11]. A number of *Plasmodium* PI4K kinase inhibitors with different chemotypes such as MMV390048 (**1**)[6], which is now under Phase II clinical trial[12], KAI407 (**2**)[13], KAI715 (**3**)[14], KDU731 (**4**)[15], KDU691 (**5**)[3], UCT943 (**6**)[16], BRD73842 (**7**)[17], BQR695 (**8**)[3], and **9**[18] have been disclosed in patents and research articles over the past several years (Figure 1). In this study, we report the discovery of a novel bipyridine-sulfonamide chemotype *Pf*PI4K inhibitor compound **15g** (CHMFL-PI4K-127), which is obtained from optimizing a chloro-bipyridine chemotype based hit compound **15a** (Figure 2).

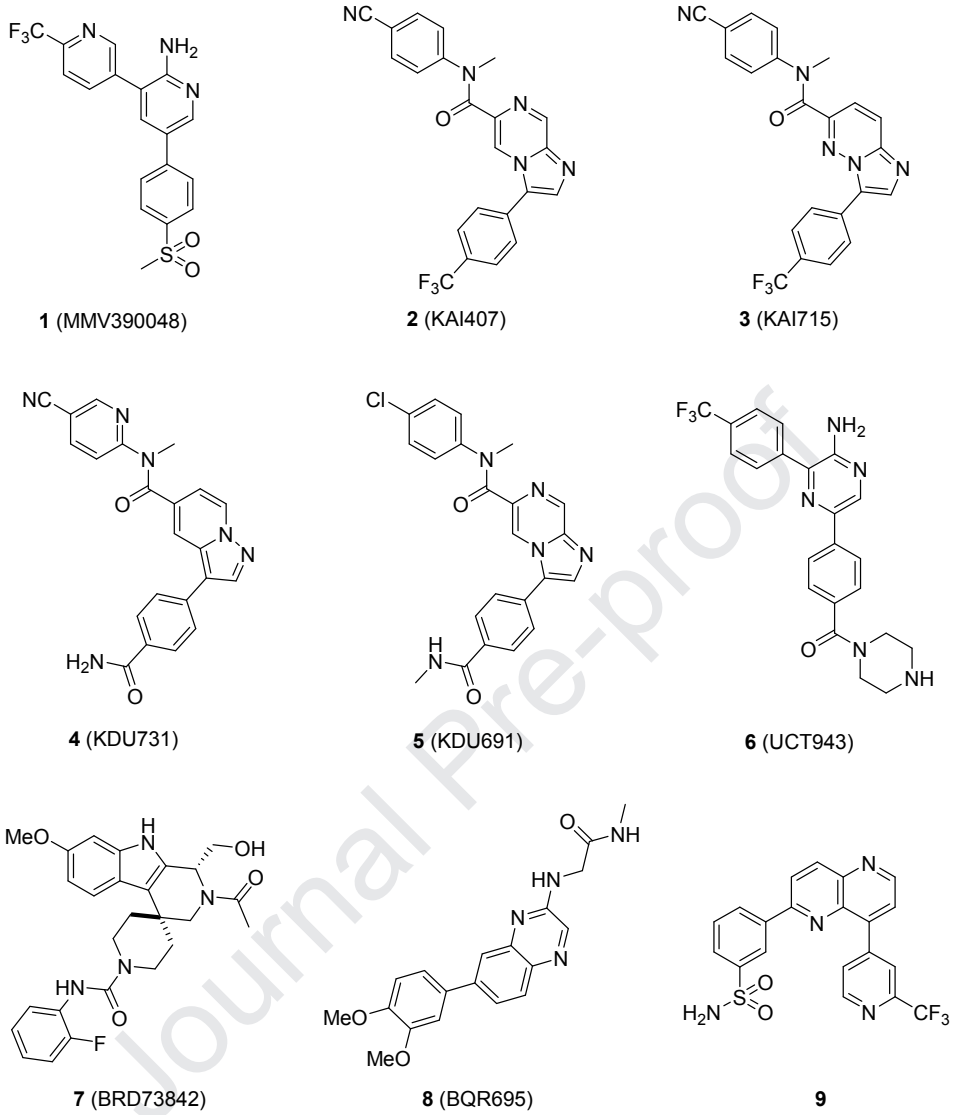



**Figure 1.** Chemical structures of representative *Plasmodium* PI4K inhibitors.

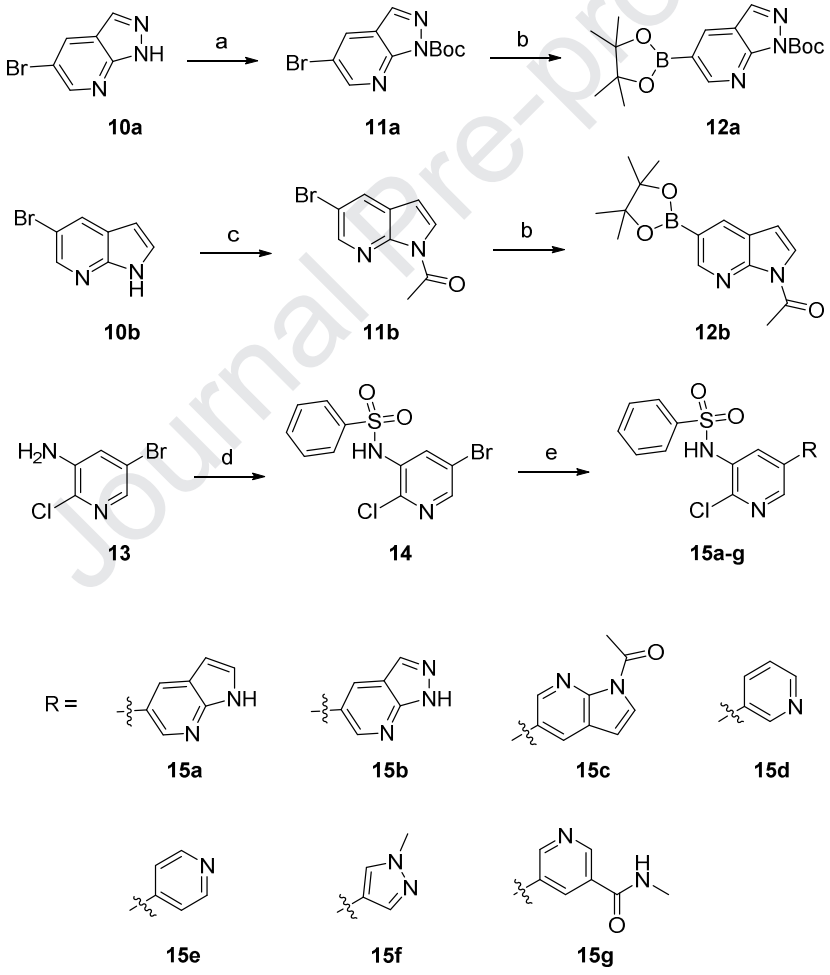
## 2. Results and discussion

### *2.1 Chemistry*

The synthesis of compounds **15a-g** was depicted in Scheme 1. The intermediate **12a** was obtained from **10a** via *N*-Boc group protection (**11a**) and Miyaura borylation reaction. The pyrrolopyridine **10b** was protected with acetyl chloride to give **11b**, and then Miyaura borylation reaction afforded the intermediate **12b**. **13** reacted with benzenesulfonyl chloride to provide

sulfonamide **14**, which was then converted to compounds **15a-g** through Suzuki-Miyaura cross coupling reaction. As shown in Scheme 2, compounds **18a-k** were prepared from intermediate **14** through Suzuki-Miyaura cross coupling reaction (**16**), hydrolysis of the ester (**17**), and subsequent standard amide coupling reactions. Suzuki-Miyaura cross coupling reaction of **13** afforded the bipyridine **19**, which was then acylated or sulfonated to provide compounds **20a-n** (Scheme 3).

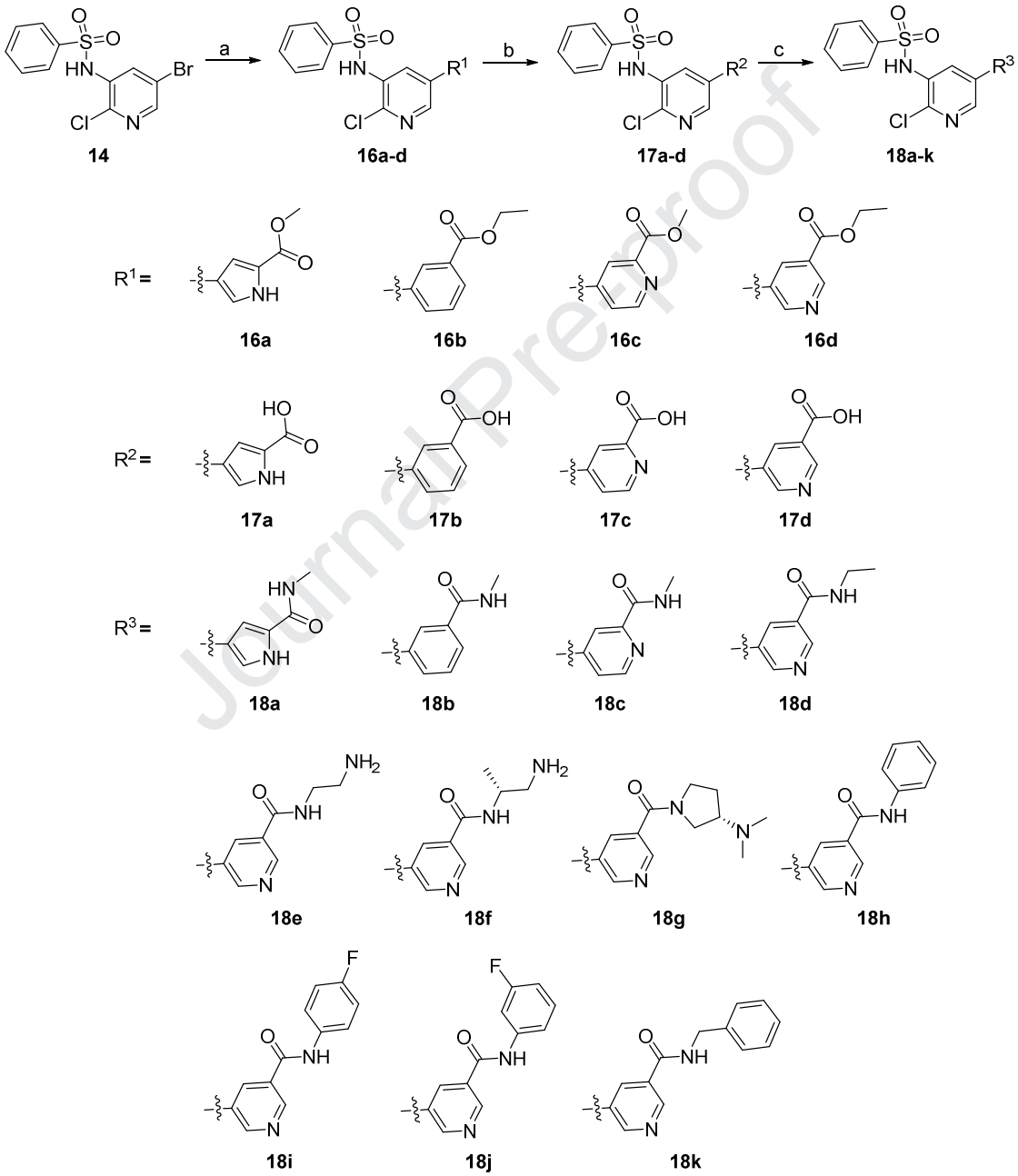
**Scheme 1.** Synthesis of Compounds **15a-g**[a]




[a]Reagents and conditions: (a) $(Boc)_2O$, DMAP, THF, reflux, 14 h; (b) bis(pinacolato)diboron, $PdCl_2(dppf)_2$, KOAc, dioxane, 80 °C, 10 h; (c) acetyl chloride, TEA, DCM, rt, 14 h; (d)

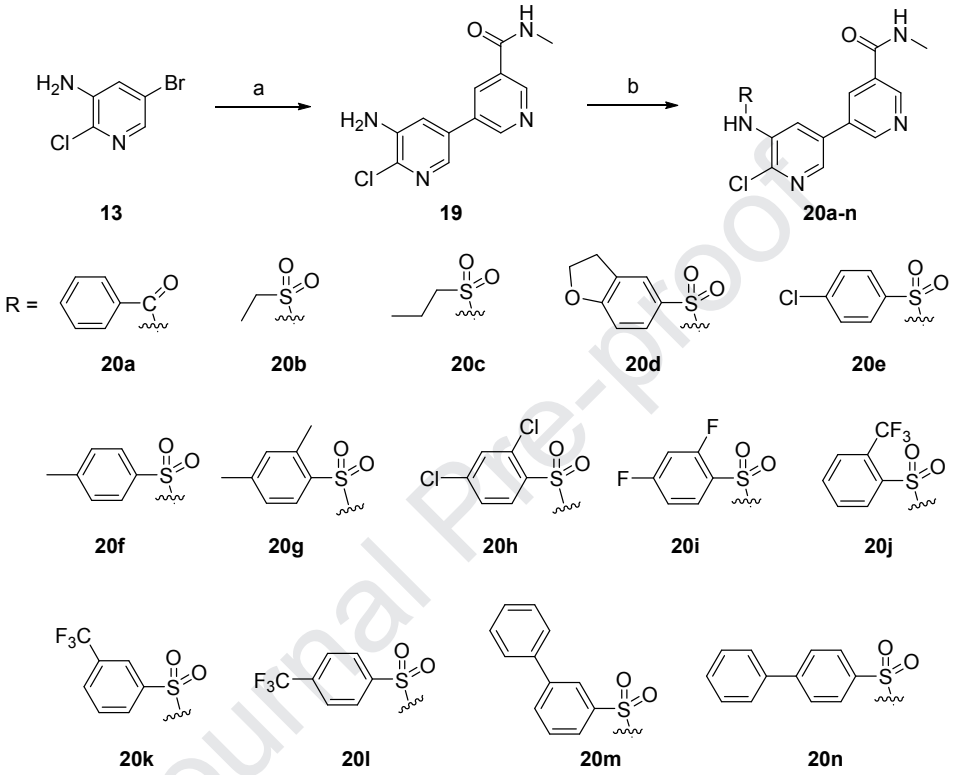
benzenesulfonyl chloride, pyridine, rt, 5 h; (e) for **15a**, **15c-g**: arylboronic acid or ester, $Pd(PPh_3)_4$, KOAc, dioxane, 80 °C, 8 h; for **15b**: (i) **12a**, $Pd(PPh_3)_4$, KOAc, dioxane, 80 °C, 8 h; (ii) 4 M HCl in MeOH, MeOH, 2 h.

**Scheme 2.** Synthesis of Compounds **18a-k**[a]



[a]Reagents and conditions: (a) arylboronic acid or ester, $Pd(PPh_3)_4$, KOAc, dioxane, 80 °C, 8 h; (b) NaOH, MeOH/$H_2O$, rt, 12 h; (c) amine, HATU, DIPEA, THF, rt, 14 h.

**Scheme 3.** Synthesis of Compounds **20a-n**[a]




[a]Reagents and conditions: (a) *N*-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)nicotinamide, $Pd(PPh_3)_4$, $K_2CO_3$, dioxane, 100 ℃, 8 h; (b) acyl chloride or sulfonyl chloride, pyridine, rt, 6 h.

*2.2. Structure-Activity Relationships (SAR) Exploration*

The starting hit compound **15a** (Figure 2A) was discovered by phenotypic testing against the 3D7 *P. falciparum* blood stage parasite from the in-house generated human kinase small molecule library. **15a** displayed the weak antimalarial activity with an $EC_{50}$ of 3940 nM. However, **15a** exhibited the acticvity against *Pf*PI4K kinase with an $IC_{50}$ of 7.7 nM in the

biochemical enzymatic assay. This suggested that the potent biochemical *Pf*PI4K activity of compound **15a** was not transformed to the in cell anti-parasite activity. Therefore, the primary purpose of the hit-to-lead optimization is to keep the *Pf*PI4K kinase inhibitory activity and meanwhile gain the antiparasite activity in cell. In order to understand the binding mode of compound **15a**, it was docked into the genernted *Pf*PI4K homology model (Figure 2B). After careful analysis of the molecular modeling results, we found that **15a** ocuppied the ATP binding pocket of *Pf*PI4K kinase. The pyrrolopyridine moiety ("Head" part in Figure 2A) of **15a** formed two hydrogen bonds with Val1357 and Thr1360 in the hinge region (Figure 2B). The sulfonamide moiety ("Tail" part in Figure 2A) of **15a** also formed a hydrogen bond with conserved residue Lys1308 (Figure 2B). The modeling results indicated that in the "Tail" and "Head" moiety there were still spaces to be further elaborated by medicinal chemistry. We then chose to systematically optimize these moietes to obtain a full spectrum of the SAR as illustrated in Figure 2.

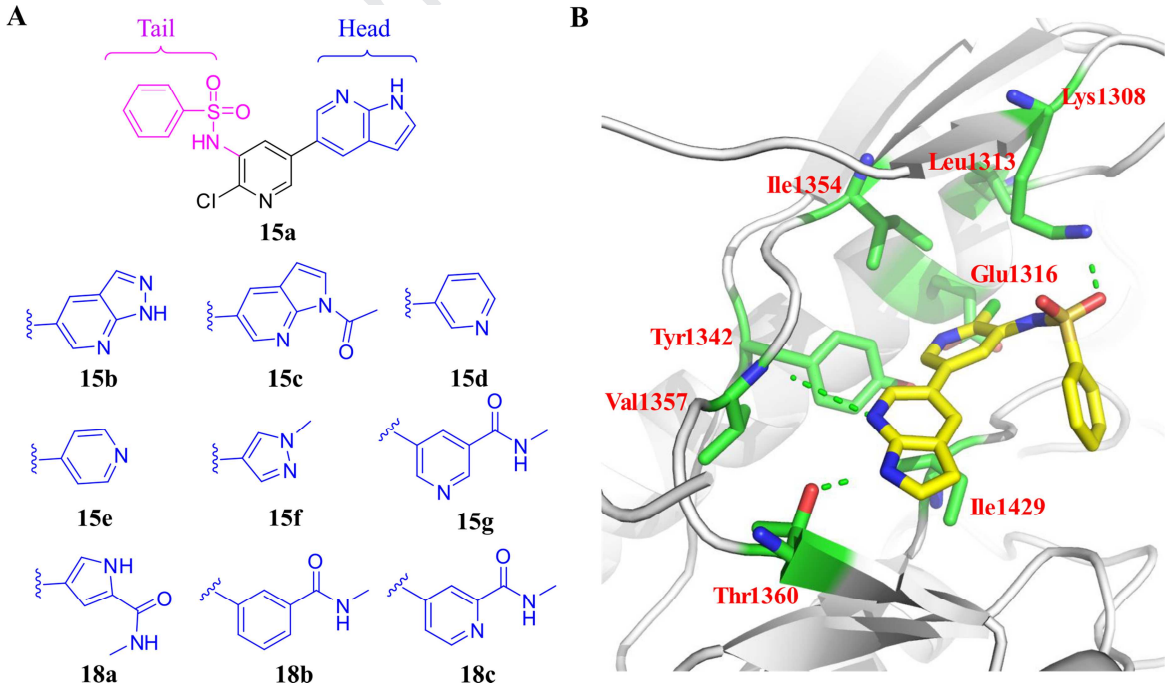


**Figure 2.** Illustration of the SAR rationale. (A) Structure of compounds **15a-g** and **18a-c**. (B) Homology model of *Pf*PI4K with compound **15a** illustrating a key interaction in the ATP binding pocket (PDB: 4DOL). Hydrogen bonds are indicated by green dash line to key amino acid residues.

The in vitro antimalarial blood-stage activity was tested against *P. fa*lciparum using a SYBR green-based 3D7 *P. falciparum* assay[19]. Compound **5** (*Pf*PI4K $IC_{50}$: 1.5 nM)[3] was used as the positive control in all experiments. The "Head" moiety occupied the hydrophobic pocket located proximal to the hinge binding region in *Pf*PI4K. Introduction of a nitrogen atom (**15b**) did not improve the potency against *P. falciparum* (Table 1). Addition of an acetyl group (**15c**) exhibited potent activity against *P. falciparum*. Replacing the azaindole by a smaller moiety 3-pyridine as in **15d** let to decrease both the potency against *P. falciparum* and against *Pf*PI4K. In addition, replacing the azaindole by 4-pyridine as in **15e** resulted in significant activity loss against both *P. falciparum* and *Pf*PI4K, suggesting that this nitrogen in **15a** is required for potent antimalarial activity. Interestingly, replacing the azaindole by a smaller moiety N-methylpyrazole (**15f**) retained the activity against both *P. falciparum* and *Pf*PI4K. Unfortunately, replacement of the azaindole by pyrrole carboxamide (**18a**) led to significant activity loss against both *P. falciparum* and *Pf*PI4K. Based on this primary SAR study, we redesigned the "Head" moiety in order to improve the potency against both *P. falciparum* and *Pf*PI4K.

We intitially redesigned compounds **15g**, **18b** and **18c** to explore the relationship between the hinge binding interations with the "Head" moiety and the antimalarial activity (Table 1). **18b** did not have the hinge binding hydrogen bond acceptor or donor in the phenyl ring of the "Head" moiety. Therefore, it did not display the activity against both *P. falciparum* and *Pf*PI4K (Table 1). Compounds **15g** and **18c** were introduced a hydrogen bond acceptor nitrogen atom in the

phenyl ring of "Head" moiety. Compound **18c**, which has the hydrogen bond acceptor nitrogen atom in the "Head" moiety similar to **15e**, did not display activity against both *P. falciparum* and *Pf*PI4K either. **15g** with the hydrogen bond acceptor nitogen atom in the "Head" moiety as **15b**, **15c**, **15d** and **15f** displayed significantly improved activity against both *P. falciparum* and *Pf*PI4K. These results suggested that the position of nitrogen atom on the phenyl ring in the "Head" moiety, which formed a hydrogen bond with hinge binding region in *Pf*PI4K kinase, was critical to the efficiency of parasite inhibition. These results correlated well with the analysis obtained from the docking experiments.

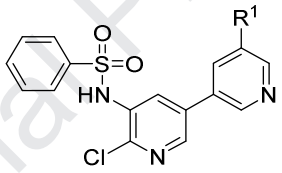
**Table 1**. In Vitro Blood-Stage Antimalarial Activity and Biochemical Activity against *Pf*PI4K

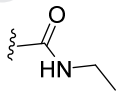
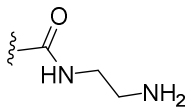
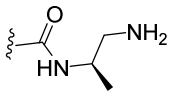
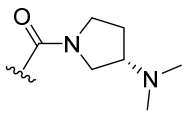
| Compd. | 3D7 ($EC_{50}$: nM)[a] | *Pf*PI4K ($IC_{50}$: nM)[b] |
|---|---|---|
| **5** | 41.1±1.1 | 1.6±0.0 |
| **15a** | 3940±1850 | 7.7±0.3 |
| **15b** | 4670±1580 | 5.6±1.2 |
| **15c** | 1050±270 | 8.1±0.3 |
| **15d** | 4710±1200 | 12.7±1.5 |
| **15e** | >10000 | >156 |
| **15f** | 1490±150 | 10.0±0.6 |
| **15g** | 25.1±0.1 | 0.9±0.1 |
| **18a** | ＞10000 | >156 |
| **18b** | >10000 | >156 |
| **18c** | >10000 | >156 |

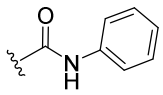
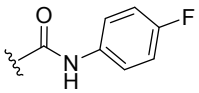
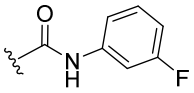
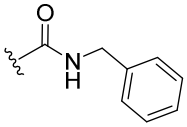
[a]$EC_{50}$ values are the average of at least three determinations, each carried out in triplicate. [b]$IC_{50}$ values are the average of at least three determinations, each carried out in triplicate.

On the basis of these results, we then investigated the carboxyamide moiety in compound **15g**. Replacing the methylamine in **15g** by larger groups such as ethylamine (**18d**) and ethyldiamine (**18e**) led to 10-fold decrease of the antimalarial activity (Table 2). Notably, more ionizable moieties propanediamine (**18f**) and dimethylpyrrolidine (**18g**) in $R^1$ resulted in significant antimalarial activity loss. In addition, larger hydrophobic aromatic groups replacement (**18h-k**) also resulted in antimalarial activity loss. These results indicated that a bulkier hydrophobic group was unfavorable at this position.

**Table 2**. In Vitro Blood-Stage Antimalarial Activity and Biochemical Activity against *Pf*PI4K



| Compd. | $R^1$ | 3D7 ($EC_{50}$: nM)[a] | *Pf*PI4K ($IC_{50}$: nM)[b] |
|---|---|---|---|
| **18d** |  | 120±1 | 2.3±0.3 |
| **18e** |  | 130±3 | 13.8±0.8 |
| **18f** |  | >10000 | >40 |
| **18g** |  | >10000 | >156 |

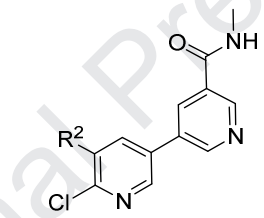
| | | | |
|---|---|---|---|
| **18h** |  | 3680 | 145.1±1.3 |
| **18i** |  | >10000 | >156 |
| **18j** |  | >10000 | >156 |
| **18k** |  | 3570±2190 | 8.5±0.3 |

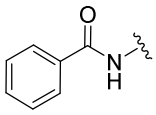
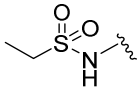
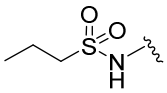
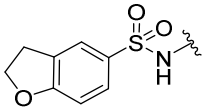
[a]$EC_{50}$ values are the average of at least three determinations, each carried out in triplicate. [b]$IC_{50}$ values are the average of at least three determinations, each carried out in triplicate.

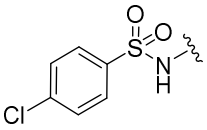
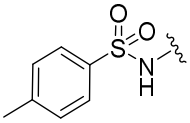
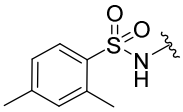
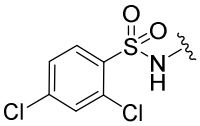
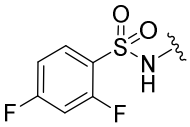
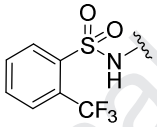
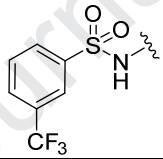
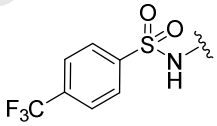
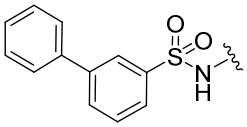
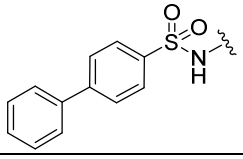
We next investigated the effect of various fragments in the "Tail" moiety for antimalarial activities while retaining the "Head" moiety as in **15g** (Table 3). Replacing the sulfonamide moiety in **15g** by carboxamide moiety as in **20a** led to significant activity loss against both *P. falciparum* and *Pf*PI4K, indicating that the sulfonamide group is favorable for forming hydrogen bonds with *Pf*PI4K kinase. Replacing the benzenesulfonamide moiety in **15g** by the smaller and flexible aliphatic sulfonamide such as **20b** and **20c** resulted in significantly decreased activities. These results indicated that the aromatic group was more favorable than aliphatic group to form the van de Waals interactions with *Pf*PI4K kinase. Replacement of the benzenesulfonamide moiety in **15g** by dihydrobenzofuran sulfonamide as in **20d** decreased the antimalarial activity ($EC_{50}$: 340 nM vs 25.1 nM). However, the activity was slightly affected when monosubstituted benzenesulfonamide (**20e-f**) and disubstituted benzenesulfonamide (**20g-h**) was introduced. It is noteworthy that a significant loss of antimalarial potency was observed for the difluorobenzenesulfonamide as in **20i**, but **20i** still displayed strong inhibition against *Pf*PI4K kinase. The ortho-trifluoromethyl (**20j**) and para-trifluoromethyl (**20l**) in $R^2$ were observed to

show slight antimalarial activity decrease compared with that of **15g**. Unfortunately, the meta-trifluoromethyl group (**20k**) led to significant activity loss. Further increase of the substituent size by attaching a phenyl group at the "Tail" moiety (**20m-n**) both caused significant activity loss. It is noteworthy that compounds **20i**, **20k**, **20m**, and **20n** did not exhibit strong antimalarial efficacy though they displayed potent in vitro biochemical *Pf*PI4K inhibitory activity. This indicated that there might be some cellular penetration or solubility problem with them. Surprisingly, the biochemically weak inhibitor **20l** showed strong antimalarial activity. We suspected that this compound might bear unknown off-targets for *P. falciparum*.

**Table 3**. In Vitro Blood-Stage Antimalarial Activity and Biochemical Activity against *Pf*PI4K

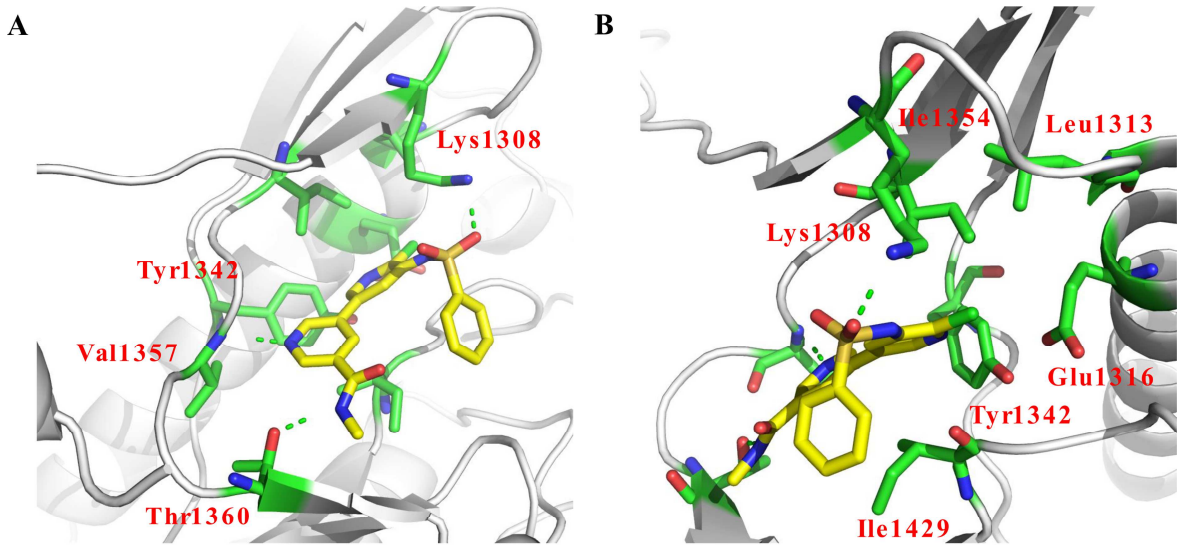



| Compd. | $R^2$ | 3D7 ($EC_{50}$: nM)[a] | *Pf*PI4K ($IC_{50}$: nM)[b] |
|---|---|---|---|
| **20a** |  | >10000 | >156 |
| **20b** |  | 3500 | 52.6±1.2 |
| **20c** |  | 480±40 | 13.2±2.4 |
| **20d** |  | 340±20 | 4.5±1.0 |

| | | | |
|---|---|---|---|
| **20e** |  | 71±4 | 1.6±0.1 |
| **20f** |  | 39±1 | 1.2±0.2 |
| **20g** |  | 39±5 | 2.2±0.4 |
| **20h** |  | 120±1 | 1.2±0.2 |
| **20i** |  | 3400 | 0.4±0.0 |
| **20j** |  | 87±3 | 0.5±0.0 |
| **20k** |  | 3500 | 2.5±0.0 |
| **20l** |  | 130±1 | >156 |
| **20m** |  | 7700 | 7.5±1.0 |
| **20n** |  | 5880 | 8.8±0.4 |

[a]$EC_{50}$ values are the average of at least three determinations, each carried out in triplicate. [b]$IC_{50}$ values are the average of at least three determinations, each carried out in triplicate.

To better understand the binding mode of compound **15g**, we then docked it into the generated *Pf*PI4K homology model via molecular modeling (Figure 3A, B). The modeling results suggested that **15g** was accommodated in the ATP-binding pocket (Figure 3A, B). The NH of nicotinamide group in **15g** formed a hydrogen bond with the side chain of Thr1360 in the hinge region (Figure 3A). The nitrogen atom of nicotinamide group in **15g** also formed a hydrogen bond with the backbone of Val1357 in the hinge region (Figure 3A). The sulfonamide moiety formed a hydrogen bond with conserved residue Lys1308 (Figure 3A). The chloropyridine moiety pointed to the hydrophobic pocket, which was formed by the side chains of residues Ile1354, Leu1313 and Glu1316, and interacted through the van der Waals interactions (Figure 3B). The edge of pyridine in chloropyridine moiety was adjacent to the side chain of Tyr1342. It was possible to form edge to face interactions[20] (Figure 3B). Base on the binding mode analysis, the side chains of Tyr1342 and Thr1360 played important roles in the interactions with compound **15g**. We then generated the PI4K Y1342I and PI4K Y1342I-T1360A mutant kinase *P. falciparum* strains from wild type 3D7 *P. falciparum* by CRISPR/Cas9 system (Figure 3C). **15g** was 2-fold more potent against mutant *P. falciparum* PI4K Y1342I strain than wild type 3D7 *P. falciparum*, indicating that the interactions between the chloropyridine moiety in **15g** and the side chain of Tyr1342 were unfavorable. In addition, **15g** displayed 24-fold less activity against *P. falciparum* PI4K Y1342I-T1360A strain than *P. falciparum* PI4K Y1342I strain, indicating that the hydrogen bond interaction between the side chain of Thr1360 and the NH of nicotinamide group in **15g** was necessary.




C

| P. falciparum | **15g** ($EC_{50}$: nM) |
|---|---|
| P. falciparum 3D7 | 25.1 ± 0.1 |
| P. falciparum PI4K Y1342I | 10.2 ± 0.0 |
| P. falciparum PI4K Y1342I - T1360A | 248.2 ± 4.3 |

**Figure 3.** (A, B) **15g** was docked into the homology model of *Pf*PI4K (PDB:4DOL). *Pf*PI4K kinase was displayed in white. **15g** was labeled in color by atoms (carbon in yellow, nitrogen in blue, oxygen in red, and chlorine in green). The hydrogen bonds were labeled as green dashed lines. The key amino acid residues for the interactions were labeled as follows: carbon in green, nitrogen in blue, and oxygen in red. (C) In vitro blood-stage antimalarial activity against *P. falciparum* PI4K mutant strains of **15g**.

### *2.3. Selectivity of Compound **15g** between Human Kinases and PfPI4K*

The ADP-glo biochemical assay with purified enzymes of human lipid kinase PI3Ks and PI4Ks demonstrated that **15g** exhibited over 100-fold selectivity against PI3Kα, PI3Kδ, PI3Kγ and PI4KIIIA (Table 4). **15g** also exhibited over 400-fold selectivity against PI3Kβ, VPS34 and PI4KIIIB. In addition, **15g** did not show apparent activity against PIK3C2A and PIK3C2B. To

further characterize the kinome-wide selectivity profile of **15g** in the human kinases, we next performed a selectivity analysis of **15g** using DiscoveRx's KINOMEscan technology[21]. **15g** exhibited high selectivity among 468 kinases/mutants tested with a S score (1) =0 (Supplementary Table 1). This indicated that the antimalarial activity of **15g** derives from the inhibition of *P. falciparum* target kinase and meanwhile **15g** exhibited good selectivity over kinases of human host.

**Table 4.** Selectivity of Compound **15g** against PI3Ks, PI4Ks and *Pf*PI4K ($IC_{50}$: nM)[a]

| | Compd. **15g** | Compd. **5** |
|---|---|---|
| PI3Kα | 191±36 | >10000 |
| PI3Kβ | 392±27 | 2728±32 |
| PI3Kδ | 104±3 | 3096±117 |
| PI3Kγ | 324±19 | 5917±32 |
| PIK3C2A | >10000 | >10000 |
| PIK3C2B | >10000 | >10000 |
| VPS34 | 681±25 | 9199±382 |
| PI4KIIIA | 165±29 | 1416±100 |
| PI4KIIIB | 433±18 | >10000 |
| *Pf*PI4K | 0.9±0.1 | 1.6±0.0 |

[a]$IC_{50}$ values were obtained by triplet testings.

*2.4. Activity of Compound **15g** against Drug Resistant Strains of P. falciparum*

We then evaluated compound **15g** against the multidrug-resistant strains of *P. falciparum* (Dd2, 803, 6320, GB4, FAB9, C2A, D10, PC26, and CP286) using the SYBR green assay. As

shown in Table 5, all the tested resistant strains of *P. falciparum* showed sensitivity towards **15g**. In addition, **15g** displayed slightly better activity against the drug resistant strains than **5**.

**Table 5.** Activity of Compound **15g** against the Drug-resistant Strains of *P. falciparum* ($EC_{50}$: nM)[a]

| *P. falciparum* strains | Drug resistance | **15g** | **5** |
|---|---|---|---|
| 3D7 | SFX | 25.1±0.1 | 41.1±1.1 |
| Dd2 | MEF, CQ, PYR | 30.3±0.2 | 46.7±2.5 |
| 803 | ART | 30.9±3.3 | 41.8±0.5 |
| 6320 | ART, PPQ | 24.7±0.2 | 33.6±5.7 |
| GB4 | unknown | 47.8±5.0 | 39.2±16.5 |
| FAB9 | CQ | 23.6±4.8 | 44.8±12.3 |
| C2A | PYR, CQ | 28.0±4.0 | 33.7±0.3 |
| D10 | CQ | 28.0±3.0 | 43.9±1.2 |
| PC26 | PYR, CQ | 44.0±2.2 | 54.9±2.1 |
| CP286 | PYR, CQ | 24.1±0.5 | 31.9±2.9 |

[a]$EC_{50}$ values were obtained by triplet testings. SFX, sulphadoxine. CQ, chloroquine; MEF, mefloquine; PYR, pyrimethamine; PPQ, piperaquine phosphate; ART, artmisinin.

*2.5. Pharmacokinetic Properties in Rats and Mice of Compound **15g***

The pharmacokinetic properties of compound **15g** were evaluated in Sprague-Dawley rats and mice following intravenous and oral administration (Table 6). **15g** exhibited a fast absorption, large volume of distribution, and favorable oral bioavailability (F = 89%) when dosed orally in rats. It also displayed a proper $T_{1/2}$ of 2.9 h in rats. In addition, **15g** exhibited similar

pharmacokinetic properties in mice when dosed orally. This indicated that **15g** would be suitable for oral administration for in vivo study.

**Table 6.** In Vivo Pharmacokinetic Properties of Compound **15g** in Rats and Mice through Intravenous and Oral Administration[a]

| | Rats | | Mice | |
|---|---|---|---|---|
| | IV (1 mg/kg) | PO (10 mg/kg) | IV (1 mg/kg) | PO (10 mg/kg) |
| $AUC_{0-t}$ (ng/mL*h) | 2162.9±766.8 | 19266.4±1944.1 | 327.4 ±71.1 | 2631.0±490.0 |
| $AUC_{0-\infty}$ (ng/mL*h) | 2168.0±763.1 | 19331.7±1956.9 | 291.6±32.2 | 2382.0±420.0 |
| $MRT_{(0-t)}$ (h) | 1.0±0.6 | 4.3±0.7 | 3.1±1.9 | 4.4±1.0 |
| $C_{max}$ (ng/mL) | 5010.5±105.9 | 4335.8±1142.5 | 718.3±91.8 | 1539.0±957.0 |
| Vz (L/kg) | 2.6±0.2 | 2.2±0.3 | 12.1±2.9 | 20.2 ±4.9 |
| CLz (L/h/kg) | 0.5±0.1 | 0.5±0.1 | 3.1±0.6 | 3.9±0.8 |
| $T_{max}$ (h) | 0.02±0.0 | 0.83±0.29 | 0.03±0.0 | 0.19±0.10 |
| $T_{1/2}$ (h) | 3.5±2.8 | 2.9±0.3 | 2.7 ±0.2 | 3.6±0.9 |
| F | - | 89% | - | 80% |

[a]All testing data were obtained from three independent mice (±SD).

*2.6. In Vivo Blood-Stage and Liver-Stage Efficacy of Compound **15g** in Rodent Malaria Model*

In vivo blood-stage efficacy studies of compound **15g** on a murine *P. yoelii* model[22-24] (Balb/c) were then investigated. In these in vivo experiments, the Balb/c mice were infected by *P. yoelii*. After 24 h, oral administration of **15g** were started in mice using fixed doses of 60 and 80 mg/kg/day for consecutive 7 days. **15g** displayed significant in vivo antimalarial activities in a dose-dependent manner and 80 mg/kg ×7 days treatment generated curative effects (Figure 4).

The 60 mg/kg dosage resulted in suppressive effects during the drug treatment but relapsed after stopping treatment.

The investigation of in vivo liver-stage efficacy of **15g** was conducted with a real-time in vivo imaging system utilizing a transgenic bioluminescent parasite[25-27] (Figure 5). Mice were treated once by oral administration of **15g** at 1 mg/kg, 5 mg/kg and 15 mg/kg respectively at 2 h before inoculated with 5000 luciferase-expressing *P. berghei* sporozoites intravenously through the tail vein. The mice underwent imaging for luciferase activity at 24, 48, 72, 96, 144, and 196 h after the infection for liver-stage development and at 72 h for blood-stage infection. Strong bioluminescence signals were detected in untreated mice at both 24 and 48 h in the area overlying the liver, followed by an intense signal in the whole body of mice at 72 h, resulting from infection in the peripheral blood circulation. **15g** provided the full protection and cure at 1 mg/kg with no negligible parasite visible in the liver of all tested mice at 24, 48, 72, 96, 144 and 196 h, indicating true causal prophylactic efficacy. Notably, all treated mice were cured with no blood-stage parasites up to day 8.

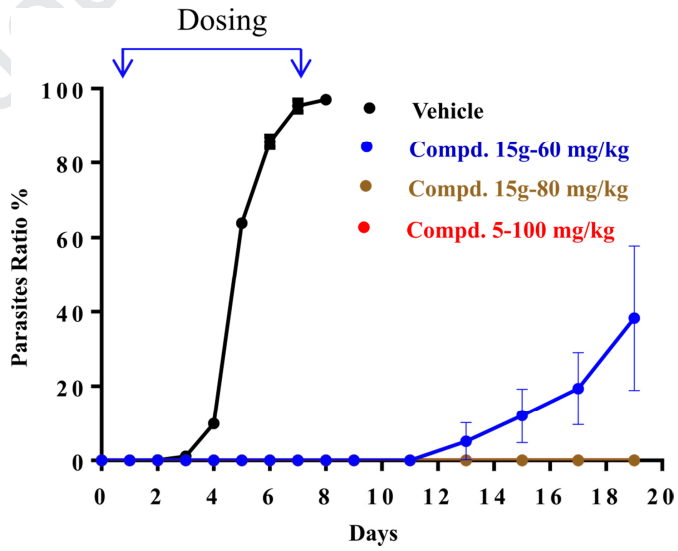


**Figure 4.** Efficacies of compound **15g** in the in vivo blood-stage on a murine *P. yoelii* model. Dosing was started on day 1 after infection.

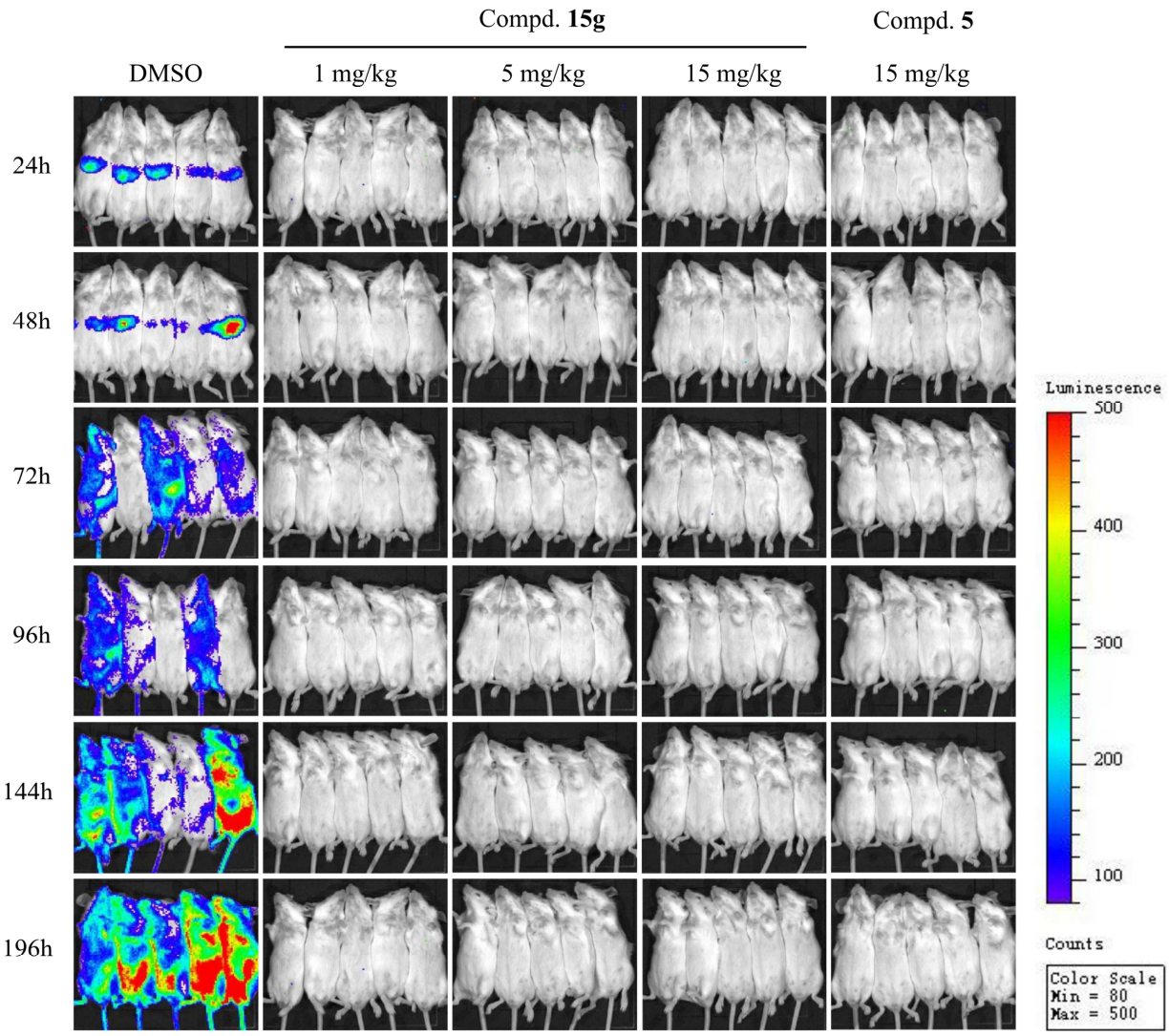



**Figure 5.** Efficacies of compound **15g** in liver-stage on mice infected with luciferase-expressing *P. berghei* sporozoites. Mice were given a single oral dose of 1 mg/kg, 5 mg/kg, and 15 mg/kg of **15g** at 2 h before sporozoite infection.

## 3. Conclusions

In summary, we have discovered a novel chemotype based *Pf*PI4K kinase inhibitor compound **15g**, which exhibited potent enzyme and parasite inhibitory activities. In addition, it displayed highly selectivity over human lipid and protein kinases which indicated better therapeutic safety window. **15g** also showed activity across a panel of clinical drug-resistant isolates and therefore might have a low risk for pre-existing cross-resistance. In the blood-stage malaria model, **15g** resulted in 100% elimination in parasitemia at 80 mg/kg. In the liver-stage malaria model, **15g** also provided full protection and cure at 1 mg/kg. The overall profiles of **15g** suggested that it might be potentially good drug candidate for the treatment of malaria, and further investigation is in progress.

## 4. Experimental section

*4.1. Chemistry.*

All reagents and solvents were purchased from commercial sources and were used as received unless specified otherwise, or prepared as described in the literature. All moisture sensitive reactions were carried out using dry solvents under ultrapure argon protection. Glassware was dried in an oven at 140 °C for at least 12 h prior to use and then assembled quickly while hot, sealed with rubber septa, and allowed to cool under a stream of argon. Reactions were stirred magnetically using Teflon-coated magnetic stirring bars. Commercially available disposable syringes were used for transferring the reagents and solvents. HPLC-MS were performed on an Agilent 6224 TOF using an ESI source coupled to an Agilent 1260 Infinity HPLC system operating in reverse mode with an Agilent XDB-C18 column (4.6 × 50 mm, 1.8 μm) using a water/acetonitrile (each with 0.2% (v/v) formic acid) gradient at a flow rate at 0.4 mL/min. $^{1}H$ and $^{13}C$ spectra were recorded with a Bruker 500 MHz NMR spectrometer and referenced to deuterated methanol ($CD_3OD$), deuterated dimethyl sulfoxide (DMSO-$d_6$) or deuterated

chloroform ($CDCl_3$). Chemical shifts are expressed in ppm. In the NMR tabulation, s indicates singlet; d, doublet; t, triplet; q, quartet and m, multiplet. Flash column chromatography was conducted using silica gel (Silicycle40−64 μm). The purities of all final compounds were determined to be above 95 % by HPLC.

*4.1.1. tert-Butyl 5-bromo-1H-pyrazolo[3,4-b]pyridine-1-carboxylate (**11a**).* To a solution of **10a** (770 mg, 3.88 mmol, 1.0 equiv) in THF (10 mL) was added $(Boc)_2O$ (930 mg, 4.3 mmol, 1.1 equiv) and DMAP (10 mg, 0.08 mmol, 0.02 equiv ) at room temperature under argon. Then the reaction mixture was heated to reflux for 14h. The resulting mixture was concentrated to crude product, which was purified by flash chromatography (eluting with MeOH in DCM 0-10 %) to give **11a** as a white solid (783 mg, 68%). $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 8.80 (d, $J$ = 2.3 Hz, 1H), 8.63 (d, $J$ = 2.3 Hz, 1H), 8.44 (s, 1H), 1.66 (s, 9H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 151.15, 149.47, 137.89, 133.63, 132.47, 119.71, 115.24, 85.06, 28.06. LC-MS (ESI, m/z): 298.0227 $[M+H]^+$.

*4.1.2. 1-(5-Bromo-1H-pyrrolo[2,3-b]pyridin-1-yl)ethan-1-one (**11b**).* To a solution of **10b** (5 g, 25.4 mmol, 1.0 equiv) in DCM (80 mL) was added TEA (3.6 mL, 30.5 mmol, 1.2 equiv) at 0 °C under argon. Then a solution of acetyl chloride (2.1 mL, 30.5 mmol, 1.2 equiv) in DCM (5 mL) was slowly added. The reaction mixture was stirred at room temperature for 14 h. The resulting mixture was diluted with DCM (100 mL), washed with water (2×50 mL), brine (50 mL) and dried over anhydrous $MgSO_4$. Evaporation of the solvent provided the crude product, which was purified by flash chromatography (eluting with EtOAc in hexance 0-5 %) to give **11b** as a white solid (4.1 g, 68%). $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 8.45 (d, $J$ = 2.2 Hz, 1H), 8.31 (d, $J$ = 2.3 Hz, 1H), 8.02 (d, $J$ = 4.1 Hz, 1H), 6.74 (d, $J$ = 4.1 Hz, 1H), 2.93 (s, 3H). $^{13}$C NMR (126 MHz,

DMSO-$d_6$) $\delta$ 168.53, 146.04, 144.34, 132.32, 127.50, 125.60, 114.76, 105.74, 25.89. LC-MS (ESI, m/z): 238.9835 $[M+H]^+$.

*4.1.3. Compound **12b** was prepared following the synthetic procedure of **12a**.*

*4.1.3.1. tert-Butyl 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazolo[3,4-b]pyridine-1-carboxylate (**12a**).* To a solution of **11a** (2.5 g, 8.4 mmol, 1.0 equiv) in anhydrous dioxane (80 mL) was added bis(pinacolato)diboron (4.2 g, 21.0 mmol, 2.0 equiv), $PdCl_2(dppf)_2$ (201 mg, 0.25 mmol, 0.03 equiv) and KOAc (2.5 g, 25.2 mmol, 3.0 equiv) at room temperature under argon. Then the reaction mixture was heated to 80°C for 10 h. The resulting mixture was concentrated to afford the crude product, which was purified by flash chromatography (eluting with EtOAc in hexance 0-10 %) to give **12a** as a white solid (2.2 g, 75%). $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 8.87 (d, $J$ = 1.6 Hz, 1H), 8.61 (d, $J$ = 1.6 Hz, 1H), 8.47 (s, 1H), 1.35 (s, 12H), 1.18 (s, 9H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 155.55, 152.99, 147.48, 139.03, 138.38, 117.80, 84.59, 81.70, 28.09, 25.10. LC-MS (ESI, m/z): 346.1921 $[M+H]^+$.

*4.1.3.2. 1-(5-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrrolo[2,3-b]pyridin-1-yl)ethan-1-one (**12b**).* Yield=78% $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 8.59 (d, $J$ = 1.5 Hz, 1H), 8.32 (d, $J$ = 1.6 Hz, 1H), 7.98 (t, $J$ = 16.4 Hz, 1H), 6.80 (t, $J$ = 6.1 Hz, 1H), 2.99 (s, 3H), 1.34 (s, 12H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 168.85, 149.69, 149.27, 136.54, 126.02, 123.45, 106.60, 84.43, 83.27, 26.11, 25.28. LC-MS (ESI, m/z): 287.1574 $[M+H]^+$.

*4.1.4. N-(5-Bromo-2-chloropyridin-3-yl)benzenesulfonamide (**14**).* To a solution of **13** (3.0 g, 14.52 mmol, 1.0 equiv) in anhydrous pyridine (30 mL) was added benzensulfonyl chloride (2.8 g, 15.98 mmol, 1.1 equiv) at room temperature under argon. The reaction mixture was stirred at room temperature for 5 h. Then the resulting mixture was diluted with water. The precipitate was filtered and dried to give **14** as an off-white solid (3.7 g, 75%). $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$

10.63 (s, 1H), 8.42 (d, *J* = 2.3 Hz, 1H), 7.93 (d, *J* = 2.3 Hz, 1H), 7.81 – 7.76 (m, 2H), 7.72 – 7.68 (m, 1H), 7.65 – 7.58 (m, 2H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 147.61, 144.86, 140.16, 137.79, 133.90, 132.40, 129.95, 127.08, 118.90. LC-MS (ESI, m/z): 346.9235 $[M+H]^+$.

*4.1.5. Compounds **15b-g** were prepared following the synthetic procedure of **15a**.*

*4.1.5.1. N-(2-Chloro-5-(1H-pyrrolo[2,3-b]pyridin-5-yl)pyridin-3-yl)benzenesulfonamide (**15a**).* To a solution of **14** (150 mg, 0.43 mmol, 1.0 equiv) in dioxane (5 mL) was added (1*H*-pyrrolo[2,3-b]pyridin-5-yl)boronic acid (76 mg, 0.47 mmol, 1.1 equiv) at room temperature under argon. Then KOAc (128 mg, 1.3 mmol, 3.0 equiv) and $Pd(PPh_3)_4$ (46 mg, 0.04 mmol, 0.1 equiv) were added. The reaction mixture was degassed by argon, and then heated to 80 °C for 8 h. The resulting mixture was concentrated to dryness. The residue was diluted with water (30 mL) and extracted with EtOAc (3×30 mL). The combined organic layers were washed with water (50 mL), brine (50 mL) and dried over anhydrous $Na_2SO_4$. Evaporation of the solvent provided the crude product, which was purified by flash chromatography (eluting with MeOH in DCM 0-5%) to give compound **15a** as a white solid (97 mg, 59%). $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 11.89 (s, 1H), 10.46 (s, 1H), 8.62 (d, *J* = 2.3 Hz, 1H), 8.25 (d, *J* = 1.0 Hz, 1H), 8.23 (d, *J* = 2.0 Hz, 1H), 7.97 (d, *J* = 2.3 Hz, 1H), 7.83 – 7.80 (m, 2H), 7.70 (t, *J* = 7.4 Hz, 1H), 7.62 (t, *J* = 6.8 Hz, 2H), 7.51 – 7.48 (m, 1H), 6.50 (d, *J* = 2.0 Hz, 1H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 150.42, 148.96, 148.66, 144.87, 144.64, 141.64, 140.60, 135.29, 133.64, 129.85, 128.04, 127.20, 126.98, 126.70, 100.99. LC-MS (ESI, m/z): 385.0533 $[M + H]^+$.

*4.1.5.2. N-(2-Chloro-5-(1H-pyrazolo[3,4-b]pyridin-5-yl)pyridin-3-yl)benzenesulfonamide (**15b**).* Step 1: Followed the synthetic procedure of **15a**; Step 2: To a solution of the intermediate in MeOH (1 mL) was added 1 mL of HCl (4 M in MeOH) at room temperature. The reaction mixture was stirred at room temperature for 2 h. The resulting mixture was concentrated to give

the crude product, which was purified by flash chromatography (eluting with MeOH in DCM 0-10%) as a yellow solid (two steps, 53%). $^{1}$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 13.87 (s, 1H), 10.49 (s, 1H), 8.80 (s, 1H), 8.65 (s, 1H), 8.54 (s, 1H), 8.28 (s, 1H), 8.05 (s, 1H), 7.79 (d, $J$ = 7.1 Hz, 2H), 7.69 (d, $J$ = 6.3 Hz, 1H), 7.61 (d, $J$ = 6.7 Hz, 2H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 152.04, 148.21, 145.53, 145.31, 140.39, 134.57, 134.38, 134.25, 133.78, 131.20, 129.90, 129.03, 127.21, 124.73, 114.98. LC-MS (ESI, m/z): 386.0460 $[M+H]^+$.

*4.1.5.3. N-(5-(1-Acetyl-1H-pyrrolo[2,3-b]pyridin-5-yl)-2-chloropyridin-3-yl)benzenesulfonamide (**15c**).* Yield=59%. $^{1}$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 9.01 (d, $J$ = 2.3 Hz, 1H), 8.72 (d, $J$ = 2.2 Hz, 1H), 8.53 (d, $J$ = 2.3 Hz, 1H), 8.49 – 8.47 (m, 1H), 8.14 – 8.08 (m, 2H), 7.83 – 7.79 (m, 2H), 7.74 – 7.70 (m, 2H), 6.58 (dd, $J$ = 3.5, 1.9 Hz, 1H), 1.99 (s, 3H). 129.64 (d, $J$ = 16.7 Hz), 128.10, 127.50, 127.21, 120.20, 100.92, 24.89. $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 169.49, 142.16, 141.66, 139.43, 138.86, 136.37, 135.09, 131.52, 129.88, 129.70, 129.57, 128.10, 127.50, 127.21, 122.77, 120.20, 100.92, 24.89. LC-MS (ESI, m/z): 427.0651$[M+H]^+$.

*4.1.5.4. N-(6-Chloro-[3,3'-bipyridin]-5-yl)benzenesulfonamide (**15d**).* Yield=61%. $^{1}$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 8.90 (d, $J$ = 1.8 Hz, 1H), 8.71 – 8.64 (m, 2H), 8.11 (ddd, $J$ = 8.0, 2.4, 1.6 Hz, 1H), 8.04 (d, $J$ = 2.3 Hz, 1H), 7.82 – 7.78 (m, 2H), 7.73 – 7.68 (m, 1H), 7.64 – 7.61 (m, 2H), 7.58 – 7.56 (m, 1H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 150.16, 148.13, 146.02, 145.35, 140.41, 135.05, 134.32, 133.74, 133.27, 129.88, 129.39, 127.19, 126.06, 124.58. LC-MS (ESI, m/z): 346.0431$[M+H]^+$.

*4.1.5.5. N-(6-Chloro-[3,4'-bipyridin]-5-yl)benzenesulfonamide (**15e**).* Yield=58%. $^{1}$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 8.75 – 8.69 (m, 3H), 8.09 (d, $J$ = 2.3 Hz, 1H), 7.78 (dt, $J$ = 8.6, 1.6 Hz, 2H), 7.73 (dd, $J$ = 4.5, 1.7 Hz, 2H), 7.71 – 7.67 (m, 1H), 7.62 – 7.58 (m, 2H). $^{13}$C NMR (126 MHz,

DMSO-$d_6$) $\delta$ 150.94, 146.85, 145.43, 142.80, 140.38, 134.25, 133.78, 133.35, 131.56, 129.90, 127.16, 121.83. LC-MS (ESI, m/z): 386.0445$[M+H]^+$.

*4.1.5.6. N-(2-Chloro-5-(1-methyl-1H-pyrazol-4-yl)pyridin-3-yl)benzenesulfonamide (**15f**).* Yield=50%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 10.35 (s, 1H), 8.51 (t, $J$ = 3.2 Hz, 1H), 8.29 (s, 1H), 7.92 (s, 1H), 7.88 (d, $J$ = 2.3 Hz, 1H), 7.78 (dt, $J$ = 8.6, 1.7 Hz, 2H), 7.71 – 7.66 (m, 1H), 7.62 – 7.58 (m, 2H), 3.90 (s, 3H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 143.61, 143.23, 140.43, 136.88, 133.68, 132.11, 131.15, 129.82, 129.26, 127.19, 117.13, 39.26. LC-MS (ESI, m/z): 349.0547 $[M+H]^+$.

*4.1.5.7. 6'-Chloro-N-methyl-5'-(phenylsulfonamido)-[3,3'-bipyridine]-5-carboxamide (**15g**).* Yield=76%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 10.54 (s, 1H), 9.10 – 9.00 (m, 2H), 8.81 (dd, $J$ = 8.8, 4.2 Hz, 1H), 8.73 (t, $J$ = 4.6 Hz, 1H), 8.48 (t, $J$ = 2.1 Hz, 1H), 8.17 (d, $J$ = 2.3 Hz, 1H), 7.80 (dd, $J$ = 8.3, 1.1 Hz, 2H), 7.72 (ddt, $J$ = 8.6, 7.0, 1.3 Hz, 1H), 7.61 (dd, $J$ = 10.6, 4.8 Hz, 2H), 2.90 (t, $J$ = 5.6 Hz, 3H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 165.25, 150.13, 148.85, 146.47, 145.62, 140.36, 134.95, 133.76, 133.53, 132.77, 131.37, 130.91, 130.57, 129.86, 127.18, 26.69. LC-MS (ESI, m/z): 403.0675 $[M+H]^+$.

*4.1.6. Compounds **16b-d** were prepared following the synthetic procedure of **16a**.*

*4.1.6.1. Methyl 4-(6-chloro-5-(phenylsulfonamido)pyridin-3-yl)-1H-pyrrole-2-carboxylate (**16a**).* To a solution of **14** (150 mg, 0.43 mmol, 1.0 equiv) in dioxane (5 mL) was added methyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrrole-2-carboxylate (122 mg, 0.47 mmol, 1.1 equiv) at room temperature under argon. Then KOAc (128 mg, 1.3 mmol, 3.0 equiv) and $Pd(PPh_3)_4$ (46 mg, 0.04 mmol, 0.1 equiv) were added. The reaction mixture was degassed by argon and then heated to 80 °C for 8 h. The resulting mixture was concentrated to dryness. The residue was diluted with water (30 mL) and extracted with EtOAc (3×30 mL). The combined

organic layers were washed with water (50 mL), brine (50 mL) and dried over anhydrous $Na_2SO_4$. Evaporation of the solvent provided the crude product, which was purified by flash chromatography (eluting with MeOH in DCM 0-5 %) to give **16a** as a white solid (102 mg, 61%). $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 12.32 (s, 1H), 10.29 (s, 1H), 8.56 (d, $J$ = 2.3 Hz, 1H), 7.88 (d, $J$ = 2.3 Hz, 1H), 7.74 (dd, $J$ = 5.2, 3.3 Hz, 2H), 7.70 – 7.66 (m, 1H), 7.63 (dd, $J$ = 3.1, 1.8 Hz, 1H), 7.58 (dd, $J$ = 10.6, 4.8 Hz, 2H), 7.27 – 7.15 (m, 1H), 3.81 (s, 3H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 161.03, 143.71, 143.25, 140.55, 133.61, 132.20, 131.08, 131.01, 129.80, 127.15, 123.88, 122.83, 120.21, 112.67, 51.79. LC-MS (ESI, m/z): 392.0462 $[M+H]^+$.

*4.1.6.2. Ethyl 3-(6-chloro-5-(phenylsulfonamido)pyridin-3-yl)benzoate (**16b**).* Yield=74%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 10.49 (s, 1H), 8.62 (d, $J$ = 2.3 Hz, 1H), 8.12 (t, $J$ = 1.6 Hz, 1H), 8.04 – 8.02 (m, 1H), 7.95 (d, $J$ = 2.3 Hz, 1H), 7.82 – 7.78 (m, 2H), 7.69 (dt, $J$ = 14.1, 4.8 Hz, 2H), 7.64 – 7.60 (m, 3H), 4.41 – 4.36 (m, 2H), 1.39 – 1.35 (m, 3H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 165.78, 145.70, 145.23, 140.34, 136.05, 135.10, 133.97, 133.78, 132.13, 131.42, 131.35, 130.31, 129.89, 129.78, 127.73, 127.18, 61.51, 14.62. LC-MS (ESI, m/z): 417.0654 $[M+H]^+$.

*4.1.6.3. Methyl 6-chloro-5-(phenylsulfonamido)-[3,4'-bipyridine]-2'-carboxylate (**16c**).* Yield=65%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 10.57 (s, 1H), 8.85 (dd, $J$ = 5.0, 0.5 Hz, 1H), 8.78 (t, $J$ = 1.8 Hz, 1H), 8.73 (dt, $J$ = 11.8, 5.9 Hz, 2H), 8.01 (dd, $J$ = 5.1, 1.9 Hz, 1H), 7.80 (dt, $J$ = 3.2, 2.4 Hz, 2H), 7.74 – 7.69 (m, 1H), 7.64 – 7.59 (m, 2H), 3.91 (s, 3H). LC-MS (ESI, m/z): 404.0490 $[M+H]^+$.

*4.1.6.4. Ethyl 6'-chloro-5'-(phenylsulfonamido)-[3,3'-bipyridine]-5-carboxylate (**16d**).* Yield=58%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 10.52 (s, 1H), 9.14 (dd, $J$ = 10.9, 2.1 Hz, 2H), 8.70 (d, $J$ = 2.3 Hz, 1H), 8.46 (t, $J$ = 2.1 Hz, 1H), 8.05 (d, $J$ = 2.3 Hz, 1H), 7.79 – 7.75 (m, 2H),

7.71 – 7.67 (m, 1H), 7.64 (dd, *J* = 5.5, 1.8 Hz, 2H), 4.41 (qd, *J* = 7.1, 3.8 Hz, 2H), 1.40 – 1.36 (m, 3H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 164.88, 152.16, 150.25, 146.54, 145.53, 140.44, 135.37, 133.74, 131.99, 131.91, 129.87, 129.27, 129.18, 127.19, 61.95, 14.58. LC-MS (ESI, m/z): 418.0653 $[M+H]^+$.

*4.1.7. Compounds **17b-d** were prepared following the synthetic procedure of **17a**.*

*4.1.7.1. 4-(6-Chloro-5-(phenylsulfonamido)pyridin-3-yl)-1H-pyrrole-2-carboxylic acid (**17a**).* To a solution of **16a** (100 mg, 0.25 mmol, 1.0 equiv) in MeOH (1 mL) was added 1 mL of NaOH solution (1 N, 1.0 mmol, 4.0 equiv) at room temperature under argon. The reaction mixture was stirred at room temperature for 12 h. The resulting mixture was concentrated to dryness. The residue was diluted with water and 1 M HCl solution was added to adjust the pH around 4. The precipitate was filtered and dried to give **17a** as an off-white solid (89 mg, 92%). $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 12.17 (s, 1H), 8.54 (d, *J* = 2.3 Hz, 1H), 7.84 – 7.76 (m, 3H), 7.71 – 7.65 (m, 1H), 7.62 – 7.54 (m, 3H), 7.12 – 7.07 (m, 1H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 162.03, 143.36, 143.24, 140.68, 133.51, 131.82, 131.35, 131.23, 129.76, 127.18, 125.13, 122.11, 119.98, 112.19. LC-MS (ESI, m/z): 378.0356 $[M+H]^+$.

*4.1.7.2. 3-(6-Chloro-5-(phenylsulfonamido)pyridin-3-yl)benzoic acid (**17b**).* Yield=89%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 8.62 (d, *J* = 2.3 Hz, 1H), 8.13 (s, 1H), 8.05 (d, *J* = 7.8 Hz, 1H), 7.96 – 7.91 (m, 2H), 7.81 (dd, *J* = 9.2, 8.0 Hz, 2H), 7.73 – 7.65 (m, 2H), 7.62 (t, *J* = 7.7 Hz, 2H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 167.35, 145.66, 144.90, 140.46, 135.93, 135.20, 133.78, 133.70, 132.33, 131.70, 131.67, 130.17, 129.96, 129.86, 127.97, 127.19. LC-MS (ESI, m/z): 389.0342 $[M+H]^+$.

*4.1.7.3. 6-Chloro-5-(phenylsulfonamido)-[3,4'-bipyridine]-2'-carboxylic acid (**17c**).* Yield=84%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 8.84 (d, *J* = 5.0 Hz, 1H), 8.76 (d, *J* = 2.2 Hz, 1H), 8.27 (d, *J* =

0.7 Hz, 1H), 8.12 (d, *J* = 2.2 Hz, 1H), 7.98 (dt, *J* = 12.2, 6.1 Hz, 1H), 7.79 (d, *J* = 7.4 Hz, 2H), 7.74 – 7.66 (m, 1H), 7.61 (t, *J* = 7.7 Hz, 2H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 166.43, 150.89, 149.95, 147.19, 145.37, 144.19, 140.43, 134.27, 133.75, 132.76, 131.82, 129.88, 127.17, 124.89, 122.52. LC-MS (ESI, m/z): 390.0328 $[M+H]^+$.

*4.1.7.4. 6'-Chloro-5'-(phenylsulfonamido)-[3,3'-bipyridine]-5-carboxylic acid (**17d**).* Yield=80%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 9.14 (dd, *J* = 16.3, 1.9 Hz, 2H), 8.72 (d, *J* = 2.3 Hz, 1H), 8.46 (t, *J* = 2.1 Hz, 1H), 8.08 (d, *J* = 2.3 Hz, 1H), 7.83 – 7.76 (m, 2H), 7.71 (t, *J* = 7.4 Hz, 1H), 7.62 (t, *J* = 7.7 Hz, 2H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 166.38, 151.74, 150.52, 146.45, 145.50, 140.37, 135.53, 134.66, 133.75, 132.42, 131.48, 131.39, 129.86, 127.37, 127.20. LC-MS (ESI, m/z): 390.0299 $[M+H]^+$.

*4.1.8. Compounds **18b-k** were prepared following the synthetic procedure of **18a**.*

*4.1.8.1. 4-(6-Chloro-5-(phenylsulfonamido)pyridin-3-yl)-N-methyl-1H-pyrrole-2-carboxamide (**18a**).* To a solution of **17a** (35 mg, 0.09 mmol, 1.0 equiv) in anhydrous THF (1 mL) was added methylamine hydrochloride (12 mg, 0.19 mmol, 2.0 equiv), HATU (58 mg, 0.15 mmol, 2.0 equiv) and DIPEA (0. 1 mL, 0.47 mmol, 5.0 equiv) at 0 °C under argon. The reaction mixture was stirred at 0 °C for 1 h, and then allowed to warm to room temperature for 14 h. The resulting mixture was concentrated to dryness. The residue was diluted with water (30 mL) and extracted with EtOAc (3×30 mL). The combined organic layers were washed with water (50 mL), brine (50 mL) and dried over anhydrous $Na_2SO_4$. Evaporation of the solvent provided the crude product, which was purified by flash chromatography (eluting with MeOH in DCM 0-10 %) to give compound **18a** as a white solid (24 mg, 67%). $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 11.86 (s, 1H), 10.29 (s, 1H), 8.45 (d, *J* = 2.1 Hz, 1H), 8.16 (d, *J* = 4.6 Hz, 1H), 7.82 (t, *J* = 8.1 Hz, 1H), 7.77 (d, *J* = 7.4 Hz, 2H), 7.67 (t, *J* = 7.4 Hz, 1H), 7.58 (t, *J* = 7.7 Hz, 2H), 7.45 (d, *J* = 0.8 Hz,

1H), 7.13 (s, 1H), 2.77 (d, *J* = 4.5 Hz, 3H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 161.18, 142.88, 142.18, 140.54, 133.62, 131.79, 131.32, 130.91, 129.80, 128.59, 127.14, 120.01, 119.24, 107.24, 26.01. LC-MS (ESI, m/z): 391.0669 $[M+H]^+$.

*4.1.8.2. 3-(6-Chloro-5-(phenylsulfonamido)pyridin-3-yl)-N-methylbenzamide (**18b**).* Yield=81%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 10.53 (s, 1H), 8.72 (q, *J* = 4.1 Hz, 1H), 8.65 (d, *J* = 2.3 Hz, 1H), 8.13 (t, *J* = 1.6 Hz, 1H), 8.01 (d, *J* = 2.4 Hz, 1H), 7.96 – 7.90 (m, 1H), 7.82 – 7.75 (m, 3H), 7.72 – 7.66 (m, 1H), 7.65 – 7.57 (m, 3H), 2.84 (d, *J* = 4.5 Hz, 3H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 166.63, 145.74, 145.17, 140.50, 135.97, 135.53, 134.28, 133.68, 131.53, 129.88, 129.85, 129.82, 128.03, 127.18, 125.96, 26.72. LC-MS (ESI, m/z): 402.0668 $[M+H]^+$.

*4.1.8.3. 6-Chloro-N-methyl-5-(phenylsulfonamido)-[3,4'-bipyridine]-2'-carboxamide (**18c**).* Yield=76%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 10.55 (s, 1H), 8.88 (dd, *J* = 9.2, 4.4 Hz, 1H), 8.75 (dd, *J* = 8.0, 3.7 Hz, 2H), 8.23 (s, 1H), 8.09 (d, *J* = 2.3 Hz, 1H), 7.93 (dd, *J* = 5.1, 1.8 Hz, 1H), 7.81 – 7.73 (m, 2H), 7.73 – 7.65 (m, 1H), 7.63 – 7.55 (m, 2H), 2.88 (d, *J* = 4.9 Hz, 3H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 164.46, 151.62, 149.92, 147.12, 145.48, 144.36, 140.28, 134.23, 133.86, 132.98, 131.62, 129.93, 127.16, 124.21, 119.52, 26.54. LC-MS (ESI, m/z): 403.0658 $[M+H]^+$.

*4.1.8.4. 6'-Chloro-N-ethyl-5'-(phenylsulfonamido)-[3,3'-bipyridine]-5-carboxamide (**18d**).* Yield=76%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 9.79 (s, 1H), 9.08 (d, *J* = 1.6 Hz, 1H), 8.99 (dd, *J* = 6.3, 3.9 Hz, 2H), 8.74 (d, *J* = 2.3 Hz, 1H), 8.52 (t, *J* = 2.0 Hz, 1H), 8.09 (d, *J* = 2.3 Hz, 1H), 7.81 – 7.74 (m, 2H), 7.70 (t, *J* = 7.4 Hz, 1H), 7.60 (t, *J* = 7.7 Hz, 2H), 3.13 – 3.10 (m, 2H), 1.19 (t, *J* = 7.2 Hz, 3H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 164.50, 150.09, 148.99, 146.63, 145.55, 140.49, 134.81, 133.69, 133.59, 132.73, 131.61, 130.91, 130.64, 129.84, 127.19, 34.53, 15.09. LC-MS (ESI, m/z): 417.0773 $[M+H]^+$.

*4.1.8.5. N-(2-Aminoethyl)-6'-chloro-5'-(phenylsulfonamido)-[3,3'-bipyridine]-5-carboxamide (**18e**).* Step 1: Followed the synthetic procedure of **18a**; Step 2: To a solution of the intermediate in MeOH (1 mL) was added 1 mL of HCl (4 M in MeOH) at room temperature. The reaction mixture was stirred at room temperature for 2 h. The resulting mixture was concentrated to give the crude product, which was purified by flash chromatography (eluting with MeOH in DCM 0-10 %) as a yellow solid (two steps, 65%). $^{1}$NMR (500 MHz, DMSO-$d_6$) $\delta$ 10.58 (s, 1H), 9.44 (d, $J$ = 4.8 Hz, 1H), 9.20 (s, 1H), 9.11 (s, 1H), 8.83 (d, $J$ = 2.1 Hz, 2H), 8.32 (s, 3H), 8.16 (t, $J$ = 8.8 Hz, 1H), 7.76 (d, $J$ = 7.5 Hz, 2H), 7.70 (t, $J$ = 7.4 Hz, 1H), 7.60 (t, $J$ = 7.7 Hz, 2H), 3.63 (dd, $J$ = 11.4, 5.7 Hz, 2H), 3.06 (dd, $J$ = 11.4, 5.7 Hz, 2H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 164.78, 148.65, 147.49, 146.97, 146.02, 140.35, 135.83, 135.26, 133.79, 132.12, 131.60, 131.34, 130.92, 129.88, 127.18, 53.61, 36.93. LC-MS (ESI, m/z): 432.0914 $[M+H]^+$.

*4.1.8.6. (R)-N-(1-Aminopropan-2-yl)-6'-chloro-5'-(phenylsulfonamido)-[3,3'-bipyridine]-5-carboxamide (**18f**).* Step 1: Followed the synthetic procedure of **18a**; Step 2: To a solution of intermediate in MeOH (1 mL) was added 1 mL of HCl (4 M in MeOH) at room temperature. The reaction mixture was stirred at room temperature for 2 h. The resulting mixture was concentrated to give the crude product, which was purified by flash chromatography (eluting with MeOH in DCM 0-10 %) as yellow solid (two steps, 60%). $^{1}$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 10.36 (s, 2H), 9.14 (s, 1H), 9.07 (d, $J$ = 8.0 Hz, 1H), 8.99 (s, 1H), 8.80 (d, $J$ = 2.3 Hz, 1H), 8.68 (d, $J$ = 7.3 Hz, 1H), 8.28 (s, 3H), 8.10 (d, $J$ = 2.3 Hz, 1H), 7.78 – 7.74 (m, 2H), 7.69 (t, $J$ = 7.5 Hz, 1H), 7.59 (t, $J$ = 7.7 Hz, 2H), 3.94 – 3.91 (m, 1H), 2.95 (s, 2H), 1.26 (d, $J$ = 6.7 Hz, 3H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 164.93, 160.10, 150.22, 149.35, 146.67, 145.94, 140.36, 135.02, 134.12, 133.78, 132.73, 131.32, 129.89, 127.19, 53.47, 43.43, 18.57. LC-MS (ESI, m/z): 446.1075 $[M+H]^+$.

*4.1.8.7. (S)-N-(6-Chloro-5'-(3-(dimethylamino)pyrrolidine-1-carbonyl)-3,3'-bipyridin-5-yl)benzenesulfonamide (**18g**).* Yield=71%. $^{1}$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 8.82 (d, $J$ = 2.2 Hz, 1H), 8.78 – 8.70 (m, 1H), 8.22 (s, 1H), 8.05 (d, $J$ = 7.4 Hz,1H), 7.85 (dd, $J$ = 13.4, 2.1 Hz, 1H), 7.76 (dd, $J$ = 5.2, 3.2 Hz, 2H), 7.55 – 7.46 (m, 3H), 3.87 (dd, $J$ = 12.3, 7.3 Hz, 1H), 3.71 (m, 1H), 3.59 – 3.43 (m, 3H), 3.30 (m, 1H), 3.20 – 3.04 (m, 1H), 2.53 (s, 3H), 2.38 (s, 3H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 172.51, 166.26, 148.95, 147.80, 145.15, 145.10, 143.80, 133.20, 131.86, 129.23, 126.87, 64.00, 51.59, 47.81, 43.24, 29.51. LC-MS (ESI, m/z): 486.1372 $[M+H]^+$.

*4.1.8.8. 6'-Chloro-N-phenyl-5'-(phenylsulfonamido)-[3,3'-bipyridine]-5-carboxamide (**18h**).* Yield=77%. $^{1}$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 10.58 (s, 1H), 9.14 (d, $J$ = 1.8 Hz, 1H), 9.03 (d, $J$ = 1.9 Hz, 1H), 8.62 (d, $J$ = 1.8 Hz, 1H), 8.52 (s, 1H), 8.09 (d, $J$ = 2.2 Hz, 1H), 7.83 – 7.76 (m, 4H), 7.66 – 7.61 (m, 1H), 7.56 (t, $J$ = 7.6 Hz, 2H), 7.44 – 7.37 (m, 2H), 7.15 (dd, $J$ = 16.0, 8.6 Hz, 1H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 164.15, 150.41, 149.11, 146.17, 139.23, 134.12, 133.21, 132.54, 131.32, 131.27, 129.69, 129.24, 127.13, 124.60, 121.05, 120.87. LC-MS (ESI, m/z): 465.0755 $[M+H]^+$.

*4.1.8.9. 6'-Chloro-N-(4-fluorophenyl)-5'-(phenylsulfonamido)-[3,3'-bipyridine]-5-carboxamide (**18i**).* Yield=79%. $^{1}$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 10.61 (s, 1H), 9.11 (d, $J$ = 1.8 Hz, 1H), 8.93 (d, $J$ = 2.0 Hz, 1H), 8.42 (d, $J$ = 1.8 Hz, 1H), 8.34 (s, 1H), 7.94 (d, $J$ = 2.0 Hz, 1H), 7.84 – 7.75 (m, 4H), 7.56 – 7.47 (m, 3H), 7.30 – 7.20 (m, 2H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 164.14, 159.94, 158.02, 150.29, 148.76, 145.43, 135.54, 133.88, 132.11, 131.12, 129.30, 126.97, 122.75, 122.70, 115.94, 115.76. LC-MS (ESI, m/z): 483.0671 $[M+H]^+$.

*4.1.8.10. 6'-Chloro-N-(3-fluorophenyl)-5'-(phenylsulfonamido)-[3,3'-bipyridine]-5-carboxamide (**18j**).* Yield=81%. $^{1}$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 10.77 (s, 1H), 9.13 (d, $J$ = 1.9 Hz, 1H), 9.03 (d, $J$ = 2.0 Hz, 1H), 8.60 (s, 1H), 8.52 (dd, $J$ = 10.1, 8.1 Hz, 1H), 8.08 (d, $J$ = 2.2 Hz, 1H), 7.81 –

7.75 (m, 3H), 7.65 – 7.58 (m, 2H), 7.58 – 7.53 (m, 2H), 7.44 (dt, *J* = 15.7, 7.8 Hz, 1H), 6.99 (td, *J* = 8.4, 2.4 Hz, 1H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 164.48, 163.50, 161.58, 150.60, 149.10, 146.15, 141.00 (d, *J* = 11.0 Hz), 134.19, 133.13, 132.44, 131.41, 131.10 – 130.77 (m), 129.66, 128.86, 128.09, 127.11, 125.94, 116.52, 111.14, 110.97, 107.63, 107.43. LC-MS (ESI, m/z): 483.0652 $[M+H]^+$.

*4.1.8.11. N-Benzyl-6'-chloro-5'-(phenylsulfonamido)-[3,3'-bipyridine]-5-carboxamide (**18k**).* Yield=82%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 9.38 (t, *J* = 5.9 Hz, 1H), 9.11 (t, *J* = 5.8 Hz, 1H), 8.99 (d, *J* = 2.1 Hz, 1H), 8.58 (t, *J* = 9.3 Hz, 1H), 8.50 – 8.47 (m, 1H), 8.07 (d, *J* = 2.3 Hz, 1H), 7.80 – 7.74 (m, 2H), 7.65 – 7.58 (m, 1H), 7.55 (t, *J* = 7.6 Hz, 2H), 7.40 – 7.32 (m, 4H), 7.30 – 7.24 (m, 1H), 4.58 (d, *J* = 5.9 Hz, 2H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 164.94, 150.89, 150.25, 148.91, 146.09, 139.59, 133.61, 133.11, 132.54, 131.40, 130.40, 129.64, 128.99, 128.86, 127.84, 127.40, 127.08, 120.81, 43.22. LC-MS (ESI, m/z): 479.0967 $[M+H]^+$.

*4.1.9. 5'-Amino-6'-chloro-N-methyl-[3,3'-bipyridine]-5-carboxamide (**19**).* To a solution of **13** (90 mg, 0.43 mmol, 1.0 equiv) in dioxane (4 mL) and $H_2O$ (1 mL) was added *N*-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)nicotinamide (135 mg, 0.52 mmol, 1.2 equiv) at room temperature under argon. Then $K_2CO_3$ (155 mg, 1.1 mmol, 2.6 equiv) and $Pd(PPh_3)_4$ (16 mg, 0.01 mmol, 0.02 equiv) were added. The reaction mixture was degassed by argon and then heated to 100 °C for 8 h. The resulting mixture was concentrated to dryness. The residue was diluted with water (30 mL) and extracted with EtOAc (3×30 mL). The combined organic layers were washed with water (50 mL), brine (50 mL) and dried over anhydrous $Na_2SO_4$. Evaporation of the solvent provided the crude product, which was purified by flash chromatography (eluting with MeOH in DCM 0-5 %) to give **19** as an off-white solid (44 mg, 39%). $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 9.02 (d, J = 2.0 Hz, 1H), 8.98 (d, J = 2.2 Hz, 1H), 8.77 (d, J = 4.4 Hz, 1H), 8.41 (t, J

= 2.1 Hz, 1H), 8.02 (d, J = 2.2 Hz, 1H), 7.49 (d, J = 2.2 Hz, 1H), 5.78 (s, 2H), 2.86 (d, J = 4.6 Hz, 3H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 165.31, 149.95, 148.28, 142.00, 135.65, 134.62, 133.14, 132.85, 132.45, 130.44, 120.14, 26.69. LC-MS (ESI, m/z): 263.0692 $[M+H]^+$.

*4.1.10. Compound **20b-n** were prepared following the synthetic procedure of **20a**.*

*4.1.10.1. 5'-Benzamido-6'-chloro-N-methyl-[3,3'-bipyridine]-5-carboxamide (**20a**).* To a solution of **19** (60 mg, 0.23 mmol, 1.0 equiv) in anhydrous pyridine (2 mL) was added benzoyl chloride (48 mg, 0.34 mmol, 1.5 equiv) at room temperature under argon. Then the reaction mixture was stirred at room temperature for 6 h. The resulting mixture was concentrated to dryness. The residue was diluted with water (30 mL) and extracted with EtOAc (3×30 mL). The combined organic layers were washed with water (50 mL), brine (50 mL) and dried over anhydrous $Na_2SO_4$. Evaporation of the solvent provided the crude product, which was purified by flash chromatography (eluting with MeOH in DCM 0-10 %) to give compound **20a** as a white solid (21 mg, 25%). $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 10.41 (s, 1H), 9.14 (d, J = 2.2 Hz, 1H), 9.04 (d, J = 2.0 Hz, 1H), 8.80 (d, J = 2.4 Hz, 1H), 8.79 (d, J = 4.5 Hz, 1H), 8.55 (t, J = 2.1 Hz, 1H), 8.54 (d, J = 2.4 Hz, 1H), 8.04 (dd, J = 5.2, 3.3 Hz, 2H), 7.68 – 7.61 (m, 1H), 7.58 (dd, J = 10.3, 4.7 Hz, 2H), 2.85 (d, J = 4.6 Hz, 3H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 166.22, 165.29, 150.23, 148.83, 147.01, 145.26, 135.60, 133.91, 133.50, 132.89, 132.64, 131.12, 130.55, 129.08, 128.31, 26.67. LC-MS (ESI, m/z): 367.0940 $[M+H]^+$.

*4.1.10.2. 6'-Chloro-5'-(ethylsulfonamido)-N-methyl-[3,3'-bipyridine]-5-carboxamide (**20b**).* Yield=18%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 9.93 (s, 1H), 9.06 (t, *J* = 8.1 Hz, 1H), 9.03 (d, *J* = 2.0 Hz, 1H), 8.80 (d, *J* = 4.4 Hz, 1H), 8.68 (d, *J* = 2.0 Hz, 1H), 8.48 (t, *J* = 2.1 Hz, 1H), 8.21 (d, *J* = 2.3 Hz, 1H), 3.27 (q, *J* = 7.3 Hz, 2H), 2.84 (d, *J* = 4.6 Hz, 3H), 1.31 (t, *J* = 7.3 Hz, 3H). LC-MS (ESI, m/z): 355.0637 $[M+H]^+$.

*4.1.10.3. 6'-Chloro-N-methyl-5'-(propylsulfonamido)-[3,3'-bipyridine]-5-carboxamide (**20c**).* Yield=22%.$^{1}$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 9.94 (s, 1H), 9.07 (d, $J$ = 2.2 Hz, 1H), 9.03 (d, $J$ = 2.0 Hz, 1H), 8.79 (d, $J$ = 4.5 Hz, 1H), 8.70 (d, $J$ = 2.3 Hz, 1H), 8.48 (t, $J$ = 2.1 Hz, 1H), 8.22 (d, $J$ = 2.3 Hz, 1H), 3.27 – 3.21 (m, 2H), 2.83 (dd, $J$ = 11.3, 4.5 Hz, 3H), 1.83 – 1.74 (m, 2H), 0.99 (t, $J$ = 7.4 Hz, 3H). LC-MS (ESI, m/z): 369.0774 $[M+H]^+$.

*4.1.10.4. 6'-Chloro-5'-((2,3-dihydrobenzofuran)-5-sulfonamido)-N-methyl-[3,3'-bipyridine]-5-carboxamide (**20d**).* Yield=39%. $^{1}$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 10.26 (s, 1H), 9.02 (t, J = 7.6 Hz, 1H), 9.00 (d, J = 2.2 Hz, 1H), 8.80 (d, J = 4.5 Hz, 1H), 8.67 (d, J = 1.8 Hz, 1H), 8.42 (t, J = 2.1 Hz, 1H), 8.07 (d, J = 2.3 Hz, 1H), 7.64 (s, 1H), 7.52 (dd, J = 8.5, 2.1 Hz, 1H), 6.89 (d, J = 8.5 Hz, 1H), 4.64 (t, J = 8.8 Hz, 2H), 3.22 (t, J = 8.8 Hz, 2H), 2.85 (d, J = 4.6 Hz, 3H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 165.24, 164.06, 150.10, 148.87, 146.08, 145.18, 133.98, 133.50, 132.65, 131.83, 130.97, 130.57, 129.47, 128.83, 124.71, 109.64, 72.79, 28.84, 26.66. LC-MS (ESI, m/z): 445.0739 $[M+H]^+$.

*4.1.10.5. 6'-Chloro-5'-((4-chlorophenyl)sulfonamido)-N-methyl-[3,3'-bipyridine]-5-carboxamide (**20e**).* Yield=32%. $^{1}$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 10.65 (s, 1H), 9.03 (t, J = 1.6 Hz, 2H), 8.79 (d, J = 4.5 Hz, 1H), 8.71 (s, 1H), 8.46 (t, J = 2.1 Hz, 1H), 8.16 (d, J = 2.2 Hz, 1H), 7.77 – 7.71 (m, 2H), 7.68 – 7.62 (m, 2H), 2.86 (d, J = 4.5 Hz, 3H). LC-MS (ESI, m/z): 437.0242 $[M+H]^+$.

*4.1.10.6. 6'-Chloro-N-methyl-5'-((4-methylphenyl)sulfonamido)-[3,3'-bipyridine]-5-carboxamide (**20f**).* Yield=52%. $^{1}$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 10.41 (s, 1H), 9.03 (d, J = 2.0 Hz, 1H), 8.99 (d, J = 2.2 Hz, 1H), 8.80 (d, J = 4.5 Hz, 1H), 8.68 (d, J = 2.1 Hz, 1H), 8.42 (t, J = 2.1 Hz, 1H), 8.08 (d, J = 2.3 Hz, 1H), 7.67 – 7.60 (m, 2H), 7.38 (d, J = 8.0 Hz, 2H), 2.86 (d, J = 4.6 Hz, 3H), 2.37 (d, J = 7.3 Hz, 3H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 165.23, 150.11, 148.89, 146.41,

145.41, 144.16, 137.56, 134.50, 133.54, 132.68, 131.64, 130.94, 130.56, 130.26, 127.28, 26.67, 21.48. LC-MS (ESI, m/z): 417.0788 $[M+H]^+$.

*4.1.10.7. 6'-Chloro-5'-((2,4-dimethylphenyl)sulfonamido)-N-methyl-[3,3'-bipyridine]-5-carboxamide (**20g**).* Yield=51%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 10.49 (s, 1H), 9.03 (d, J = 2.0 Hz, 1H), 8.96 (d, J = 2.2 Hz, 1H), 8.78 (d, J = 4.5 Hz, 1H), 8.65 (s, 1H), 8.39 (t, J = 2.1 Hz, 1H), 8.06 (d, J = 2.3 Hz, 1H), 7.58 (d, J = 8.1 Hz, 1H), 7.25 (s, 1H), 7.11 (d, J = 8.1 Hz, 1H), 2.86 (d, J = 4.6 Hz, 3H), 2.61 (s, 3H), 2.32 (s, 3H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 165.22, 150.07, 148.88, 146.42, 145.34, 144.16, 137.54, 135.54, 134.47, 133.80, 133.49, 132.64, 131.47, 130.90, 130.58, 129.69, 127.19, 26.67, 21.19, 20.45. LC-MS (ESI, m/z): 431.0948 $[M+H]^+$.

*4.1.10.8. 6'-Chloro-5'-((2,4-dichlorophenyl)sulfonamido)-N-methyl-[3,3'-bipyridine]-5-carboxamide (**20h**).* Yield=43%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 10.92 (s, 1H), 9.03 (d, J = 1.9 Hz, 2H), 8.79 (d, J = 4.5 Hz, 1H), 8.71 (s, 1H), 8.45 (t, J = 2.1 Hz, 1H), 8.19 (d, J = 1.6 Hz, 1H), 7.92 (d, J = 2.0 Hz, 1H), 7.90 (d, J = 8.6 Hz, 1H), 7.58 (dd, J = 8.6, 2.1 Hz, 1H), 2.85 (d, J = 4.6 Hz, 3H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 165.25, 150.20, 148.92, 147.30, 145.86, 139.00, 137.57, 136.04, 133.59, 132.90, 132.52, 131.98, 131.09, 130.65, 130.55, 128.35, 26.67. LC-MS (ESI, m/z): 470.9874 $[M+H]^+$.

*4.1.10.9. 6'-Chloro-5'-((2,4-difluorophenyl)sulfonamido)-N-methyl-[3,3'-bipyridine]-5-carboxamide (**20i**).* Yield=47%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 10.97 (s, 1H), 9.05 (d, J = 2.0 Hz, 1H), 9.03 (d, J = 2.0 Hz, 1H), 8.80 (d, J = 4.5 Hz, 1H), 8.72 (s, 1H), 8.47 (t, J = 1.9 Hz, 1H), 8.22 (s, 1H), 7.80 (td, J = 8.6, 6.3 Hz, 1H), 7.57 (t, J = 8.8 Hz, 1H), 7.23 (td, J = 8.5, 2.3 Hz, 1H), 2.85 (d, J = 4.6 Hz, 3H). LC-MS (ESI, m/z): 439.0453 $[M+H]^+$.

*4.1.10.10. 6'-Chloro-N-methyl-5'-((2-(trifluoromethyl)phenyl)sulfonamido)-[3,3'-bipyridine]-5-carboxamide (**20j**).* Yield=53%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 10.66 (s, 1H), 9.03 (d, J = 2.0

Hz, 1H), 9.02 (d, J = 2.0 Hz, 1H), 8.80 (d, J = 4.6 Hz, 1H), 8.73 (s, 1H), 8.47 (t, J = 2.0 Hz, 1H), 8.19 (s, 1H), 8.00 (dd, J = 11.3, 8.2 Hz, 2H), 7.85 (ddd, J = 14.1, 11.1, 6.9 Hz, 2H), 2.85 (d, J = 4.6 Hz, 3H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 165.21, 150.12, 148.96, 147.51, 146.07, 139.35, 136.34, 134.16, 133.87, 133.60, 132.85, 131.71, 130.78, 130.52, 126.64, 126.62 – 126.48 (m), 126.25 (d, $J$ = 32.9 Hz), 124.26, 122.08, 26.64. LC-MS (ESI, m/z): 471.0499 $[M+H]^+$.

*4.1.10.11. 6'-Chloro-N-methyl-5'-((3-(trifluoromethyl)phenyl)sulfonamido)-[3,3'-bipyridine]-5-carboxamide (**20k**).* Yield=45%.$^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 10.82 (s, 1H), 9.07 – 9.00 (m, 2H), 8.80 (d, J = 4.5 Hz, 1H), 8.73 (d, J = 2.0 Hz, 1H), 8.47 (t, J = 2.1 Hz, 1H), 8.18 (d, J = 2.3 Hz, 1H), 8.09 (d, J = 7.8 Hz, 1H), 8.06 (s, 1H), 8.02 (d, J = 8.0 Hz, 1H), 7.83 (t, J = 7.9 Hz, 1H), 2.86 (d, J = 4.6 Hz, 3H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 165.23, 150.17, 148.94, 146.76, 145.98, 141.83, 135.78, 133.57, 132.93, 131.53, 131.19, 130.78, 130.55, 130.32, 124.88, 123.81 (d, $J$ = 3.9 Hz), 122.71, 26.65. LC-MS (ESI, m/z): 471.0530 $[M+H]^+$.

*4.1.10.12. 6'-Chloro-N-methyl-5'-((4-(trifluoromethyl)phenyl)sulfonamido)-[3,3'-bipyridine]-5-carboxamide (**20l**).* Yield=56%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 10.85 (s, 1H), 9.04 (d, J = 1.9 Hz, 2H), 8.80 (d, J = 4.5 Hz, 1H), 8.73 (d, J = 1.6 Hz, 1H), 8.48 (t, J = 2.1 Hz, 1H), 8.19 (d, J = 2.3 Hz, 1H), 7.97 (q, J = 8.7 Hz, 4H), 2.85 (d, J = 4.6 Hz, 3H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 165.24, 150.18, 148.94, 146.91, 145.91, 144.60, 135.71, 133.59, 132.92, 130.83, 130.55, 128.19, 127.08 (d, $J$ = 3.6 Hz), 124.98, 122.81, 26.64. LC-MS (ESI, m/z): 471.0527 $[M+H]^+$.

*4.1.10.13. 5'-([1,1'-Biphenyl]-3-sulfonamido)-6'-chloro-N-methyl-[3,3'-bipyridine]-5-carboxamide (**20m**).* Yield=38%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 10.63 (s, 1H), 9.07 (d, J = 1.9 Hz, 1H), 9.03 (d, J = 2.2 Hz, 1H), 8.87 (dd, J = 8.6, 4.0 Hz, 1H), 8.75 (t, J = 3.4 Hz, 1H), 8.50 (t, J = 2.1 Hz, 1H), 8.17 (d, J = 2.3 Hz, 1H), 8.06 – 8.02 (m, 1H), 8.00 (d, J = 7.8 Hz, 1H), 7.77 (d, J = 7.9 Hz, 1H), 7.70 (t, J = 7.8 Hz, 1H), 7.66 – 7.62 (m, 2H), 7.51 (t, J = 7.6 Hz, 2H),

7.44 (t, J = 7.3 Hz, 1H), 2.87 (d, J = 4.5 Hz, 3H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 165.20, 150.08, 148.85, 146.52, 145.75, 141.78, 141.09, 138.85, 135.02, 133.61, 132.79, 131.98, 131.40, 130.90, 130.59, 129.67, 128.84, 127.26, 126.01, 125.17, 26.68. LC-MS (ESI, m/z): 479.0957 $[M+H]^+$.

*4.1.10.14. 5'-([1,1'-Biphenyl]-4-sulfonamido)-6'-chloro-N-methyl-[3,3'-bipyridine]-5-carboxamide (**20n**).* Yield=36%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 10.57 (s, 1H), 9.03 (d, J = 2.0 Hz, 1H), 9.02 (d, J = 2.2 Hz, 1H), 8.79 (d, J = 4.6 Hz, 1H), 8.71 (d, J = 2.1 Hz, 1H), 8.45 (t, J = 2.1 Hz, 1H), 8.15 (d, J = 2.3 Hz, 1H), 7.92 – 7.86 (m, 2H), 7.85 – 7.81 (m, 2H), 7.74 (dd, J = 5.2, 3.3 Hz, 2H), 7.52 – 7.47 (m, 2H), 7.47 – 7.40 (m, 1H), 2.85 (d, J = 4.6 Hz, 3H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 165.23, 150.13, 148.91, 146.50, 145.61, 145.11, 139.22, 138.70, 134.80, 133.56, 132.78, 131.48, 130.91, 130.56, 129.58, 129.11, 127.94, 127.56, 26.66. LC-MS (ESI, m/z): 479.0954 $[M+H]^+$.

*4.2. Biology*

*4.2.1. PfPI4K Kinase Purification.* The sequences encoding *Pf*PI4K (PF3D7_0509800) with a histag were cloned into baculovirus expression vector pFASTHTA. The proteins were expressed by infecting SF9 cells with high titer viral stocks for 48 h. Cells were harvested and lysed in 20 mM Tris pH 7.5, 500 mM NaCl, 5% glycerol, 0.01% triton-x-100, 0.5 mM TCEP and 1 mM PMSF. The supernatant was loaded to Ni-NTA Column (QIAGEN, 1018244). Then the proteins were step eluted with the same buffer with 250 mM imidazole. The eluted proteins were loaded on a Superdex-200 column equilibrated in 25 mM Tris (pH 7.5), 250 mM NaCl, 1 mM DTT, and 1 mM EDTA. Peak fractions were concentrated to 0.5 mg/mL, and flash-frozen.

*4.2.2. Biochemical Assay.* The ADP-Glo™ kinase assay (Promega, Madison, WI) was used to screen compounds for its PI kinases inhibition effects. Kinase reaction system contains 3 μL

*Pf*PI4K (50 ng/μl), 3 μL of serially diluted compounds, and 12 μL substrate PI:PS (60 μM) (Invitrogen, USA) with 120 μM ATP (Promega, Madison, WI). The reaction in each tube was started immediately by adding ATP and kept going for an hour under 37 °C. After the tube cooled for 5 minutes at room temperature, 6 μL solvent reactions were carried out in a 384-well plate. Then 6 μL of ADP-Glo™ reagent was added into each well to stop the reaction and consume the remaining ADP within 40 minutes. At the end, 12 μL of kinase detection reagent was added into the well and incubated for 30 minutes to produce a luminescence signal. Luminescence signal was measured with an automated plate reader (Perkin-Elmer Envision).

*4.2.3 In Vitro Inhibition Assays.* All *P. falciparum* parasite strains were cultured in human O+ erythrocytes according to standard procedures. To prepare the >80% ring stage parasites, the asynchronous cultures of parasites were pretreated with 5% sorbitol, *P. falciparum* strains mentioned in the text at the mid-ring stage (6-10 h post-invasion) were used to test antimalarial effects in 96-well plates. Parasites were incubated in a 96-well plate with compounds at equal ratio containing 1% parasitemia, and 2% hematocrit for total 200 μL. The compounds were from the maximum concentration of 200 nM. The parasites were allowed to grow for 72 h at 37 ° C with 5% $CO_2$, 5% $O_2$, and 90% $N_2$. After 72 h, added 100 μL of lysis buffer (0.12 mg/ml Saponin, 0.12% Triton X-100 30 mM Tris-Cl, and 7.5 mM EDTA) consisting 5X SYBR Green I (Invitrogen; supplied in 10000 × concentration) to each well of the plate, the plates were then incubated for 2 h in dark prior to reading the fluorescence signal on instrument at 485 nm excitation and 535 nm emission[28].

*4.2.4 Plasmid Constructs.* All cloning was carried out by ClonExpress II One Step Cloning Kit (Vazyme C112-02). The mutation sites of *Pf*PI4K were introduced by the CRISPR/Cas9 system for point mutation. The single point mutation is *Pf*PI4K Y1342I. The sgRNA of *Pf*PI4K mutation

was annealed by primer 1 and its antisense strand primer 2, then inserted into the PL6CS by AvrII/XhoI digested. The left and right homologous of *Pf*PI4K Y1342I were respectively amplified by primer 3/6 and primer 4/5, then PCR products amplified by primer 3/4 through overlap PCR, then inserted into the PL6CS at the ASC Ⅰ / Afl Ⅱ sites. The results of plasmid construction were confirmed by sequencing of the plasmid by primer 9/10/11. Based on the correct construction of single-point mutant plasmid Y1342I, the homologous arm of double-points mutation *Pf*PI4K Y1342I-T1360A were amplified in the same way by primer 3/7/8/4. The *Pf*PI4K mutations results were confirmed by sequencing by primer 9/10/12. All primers for this study are listed in Table 7.

**Table 7.** The primers for plasmid constructs

| Primer | Sequence |
|---|---|
| Primer1 | TAAGTATATAATATTCCATATGAAATACTTGTAACgttttagagctagaa |
| Primer2 | ttctagctctaaaacGTTACAAGTATTTCATATGGAATATTATATACTTA |
| Primer3 | GCGGCCCTAGTCTAGGGcGCGCCTTCCAACAGCAAATGATAA |
| Primer4 | TTTTTTTACAAAATGCTTAAGTGTACCGTTTGCAAAACATGG |
| Primer5 | GACCAATAGAAATACTTGTTACTGGCTCC |
| Primer6 | ATTTCTATTGGTCTTAACCATAATGGTAATC |
| Primer7 | ATGATGCTTGTTCAGTTGATTCATTAAAAAG |
| Primer8 | CTGAACAAGCATCATTAACATACTCTATTAT |
| Primer9 | GTGGAATTGTGAGCGGATAAC |
| Primer10 | AAAATAAGGAAAATAAAATA |
| Primer11 | GTATATGTTATGTATATATAAC |
| Primer12 | GAATAAGAAAAGTTTCTCCTTATG |
| Primer13 | GGAGGAGGATGATAATTATG |

| | |
|---|---|
| Primer14 | CTGTCAGATTGGTCATAAAC |

*4.2.5. CRISPR/Cas9 System.* 150 μL RBC as transfected with the PL6CS-PI(4)K-hDHFR editing plasmids (100 μg) and pUF1-Cas9-BSD Plasmid (100 μg). Schizonts isolated by discontinuous Percoll gradient centrifugation of parasite 3D7C8 cultures, add 1.5 μL schizonts after electroporation (310V, 950 μF, ∞Ω, 2 mm) and selected with 2.5 nM WR99210 and BSD on the third day after electroporation. Medium was changed daily, and parasites were observed microscopically at day 14-17. Clonal parasites were obtained by limiting dilution, and successful editing was determined by PCR based sequencing by primer 13/14.

*4.2.6. In Vitro Inhibition Assays of PfPI4K Mutated Parasites.* All *Pf*PI4K mutated parasites were cultured in human O+ erythrocytes according to standard procedures. To prepare the >80% ring stage parasites, the asynchronous cultures of parasites were pretreated with 5% sorbitol, and *P. falciparum* strains mentioned in the text at the mid-ring stage (6-10 h post-invasion) were used to test antimalarial effects in 96-well plates. Parasites were incubated in a 96-well plate with compound **15g**, and compound **5** at equal ratio containing 1% parasitemia, and 2% hematocrit for total 200 μL. The compounds were from the maximum concentration of 500 nM. The parasites were allowed to grow for 72 h at 37 ° C with 5% $CO_2$, 5% $O_2$, and 90% $N_2$. After 72 h, add 100 μL of lysis buffer (0.12 mg/ml Saponin, 0.12% Triton X-100 30 mM Tris-Cl, and 7.5 mM EDTA) consisting 5X SYBR Green I (Invitrogen; supplied in 10000 × concentration) to each well of the plate, the plates were then incubated for 2 h in dark prior to reading the fluorescence signal on instrument at 485 nm excitation and 535 nm emission.

*4.2.7. In Vivo Blood-stage Inhibition Assays.* Three Balb/c mice per treatment group were infected with $5 \times 10^6$ *P. yoelii* strain parasites. After 24 h, the mice were treated with 60 and 80

mg/kg/day orally of compound **15g** and 100 mg/kg/day of compound **5** as control for 7 days treatment. Parasitemia was quantified by Giemsa-stained blood sample smear per day for 20 days observation.

*4.2.8. In Vivo Plasmodium Liver-stage Assay*. Five Balb/C mice per experimental group were used to test the antimalarial activity of compound **15g** by orally administered at a single of 1 mg/kg, 5 mg/kg and 15 mg/kg. The beta-Cyclodextrin was used as a blank control, and 15 mg/kg of compound **5** was used as positive control. The Balb/C mice were oral administration of compound **15g** and **5**. After 2 h, the mice were inoculated intravenously with approximately 5000 *P. berghei*-ANKA-GFP-luc) sporozoites. For fluorescence analysis, 3 mg/100 μl D-luciferin Sodium (Sinochromw BC-220-10) was injected into per mouse by IP. Infection was monitored daily by IVIS Lumina Series in the next 4 days and the $6^{th}$ and $8^{th}$ day after infection. The fluorescence intensity and distribution analyzed by living image.

**Author Contributions**

The manuscript was written through contributions of all the authors. All the authors have given approval to the final version of the manuscript. X.L., Z.J., Z.H., and F.L. contributed equally to this work.

**Acknowledgments**

This work was supported by the National Natural Science Foundation of China (Grant Nos, 81872745, 81773777, 81872748, 31771455, 81271863), the National Key Research and Development Program of China (Grant No. 2016YFA0400900), the Natural Science Foundation of Anhui Province (Grant No. 1908085QH348), the China Postdoctoral Science Foundation (Grant No. 2019M652057), the Frontier Science Key Research Program of CAS (Grant No.

QYZDB-SSW-SLH037), the Innovative Program of Development Foundation of Hefei Center for Physical Science and Technology (Grant No. 2018CXFX008), the University Synergy Innovation Program of Anhui Province (Grant No. GXXT-2019-045), the CASHIPS Director's Fund (Grant No. BJPY2019A03) and the Key Program of 13th five-year plan of CASHIPS (Grant No. KP-2017-26). We are also grateful for the National Program for Support of Top-Notch Young Professionals for J.L. and the Youth Innovation Promotion Association of CAS support for X.L. (No. 2016385).

**Appendix A. Supplementary data**

Supplementary data related to this article can be found at XXXXXX.

**References**

[1] Word Malaria Report 2017, World Health Organization, Geneva, Switzerland, 2017.

[2] D.A. Fidock, Priming the antimalarial pipeline, Nature 465 (2010) 297-298.

[3] C.W. McNamara, M.C. Lee, C.S. Lim, S.H. Lim, J. Roland, O. Simon, B.K. Yeung, A.K. Chatterjee, S.L. McCormack, M.J. Manary, A.M. Zeeman, K.J. Dechering, T.S. Kumar, P.P. Henrich, K. Gagaring, M. Ibanez, N. Kato, K.L. Kuhen, C. Fischli, A. Nagle, M. Rottmann, D.M. Plouffe, B. Bursulaya, S. Meister, L. Rameh, J. Trappe, D. Haasen, M. Timmerman, R.W. Sauerwein, R. Suwanarusk, B. Russell, L. Renia, F. Nosten, D.C. Tully, C.H. Kocken, R.J. Glynne, C. Bodenreider, D.A. Fidock, T.T. Diagana, E.A. Winzeler, Targeting Plasmodium PI(4)K to eliminate malaria, Nature 504 (2013) 248-253.

[4] B. Baragana, I. Hallyburton, M.C. Lee, N.R. Norcross, R. Grimaldi, T.D. Otto, W.R. Proto, A.M. Blagborough, S. Meister, G. Wirjanata, A. Ruecker, L.M. Upton, T.S. Abraham, M.J. Almeida, A. Pradhan, A. Porzelle, M.S. Martinez, J.M. Bolscher, A. Woodland, S. Norval, F.

Zuccotto, J. Thomas, F. Simeons, L. Stojanovski, M. Osuna-Cabello, P.M. Brock, T.S. Churcher, K.A. Sala, S.E. Zakutansky, M.B. Jimenez-Diaz, L.M. Sanz, J. Riley, R. Basak, M. Campbell, V.M. Avery, R.W. Sauerwein, K.J. Dechering, R. Noviyanti, B. Campo, J.A. Frearson, I. Angulo-Barturen, S. Ferrer-Bazaga, F.J. Gamo, P.G. Wyatt, D. Leroy, P. Siegl, M.J. Delves, D.E. Kyle, S. Wittlin, J. Marfurt, R.N. Price, R.E. Sinden, E.A. Winzeler, S.A. Charman, L. Bebrevska, D.W. Gray, S. Campbell, A.H. Fairlamb, P.A. Willis, J.C. Rayner, D.A. Fidock, K.D. Read, I.H. Gilbert, A novel multiple-stage antimalarial agent that inhibits protein synthesis, Nature 522 (2015) 315-320.

[5] M.A. Phillips, J. Lotharius, K. Marsh, J. White, A. Dayan, K.L. White, J.W. Njoroge, F. El Mazouni, Y.B. Lao, S. Kokkonda, D.R. Tomchick, X.Y. Deng, T. Laird, S.N. Bhatia, S. March, C.L. Ng, D.A. Fidock, S. Wittlin, M. Lafuente-Monasterio, F.J. Benito, L.M. Alonso, M.S. Martinez, M.B. Jimenez-Diaz, S.F. Bazaga, I. Angulo-Barturen, J.N. Haselden, J. Louttit, Y. Cui, A. Sridhar, A.M. Zeeman, C. Kocken, R. Sauerwein, K. Dechering, V.M. Avery, S. Duffy, M. Delves, R. Sinden, A. Ruecker, K.S. Wickham, R. Rochford, J. Gahagen, L. Iyer, E. Riccio, J. Mirsalis, I. Bathhurst, T. Rueckle, X. Ding, B. Campo, D. Leroy, M.J. Rogers, P.K. Rathod, J.N. Burrows, S.A. Charman, A long-duration dihydroorotate dehydrogenase inhibitor (DSM265) for prevention and treatment of malaria, Sci. Transl. Med. 7 (2015) 296ra111.

[6] T. Paquet, C. Le Manach, D.G. Cabrera, Y. Younis, P.P. Henrich, T.S. Abraham, M.C. Lee, R. Basak, S. Ghidelli-Disse, M.J. Lafuente-Monasterio, M. Bantscheff, A. Ruecker, A.M. Blagborough, S.E. Zakutansky, A.M. Zeeman, K.L. White, D.M. Shackleford, J. Mannila, J. Morizzi, C. Scheurer, I. Angulo-Barturen, M.S. Martinez, S. Ferrer, L.M. Sanz, F.J. Gamo, J. Reader, M. Botha, K.J. Dechering, R.W. Sauerwein, A. Tungtaeng, P. Vanachayangkul, C.S. Lim, J. Burrows, M.J. Witty, K.C. Marsh, C. Bodenreider, R. Rochford, S.M. Solapure, M.B. Jimenez-

Diaz, S. Wittlin, S.A. Charman, C. Donini, B. Campo, L.M. Birkholtz, K.K. Hanson, G. Drewes, C.H. Kocken, M.J. Delves, D. Leroy, D.A. Fidock, D. Waterson, L.J. Street, K. Chibale, Antimalarial efficacy of MMV390048, an inhibitor of Plasmodium phosphatidylinositol 4-kinase, Sci. Transl. Med. 9 (2017) eaad9735.

[7] M. Ouji, J.M. Augereau, L. Paloque, F. Benoit-Vical, Plasmodium falciparum resistance to artemisinin-based combination therapies: A sword of Damocles in the path toward malaria elimination, Parasite 25 (2018) 24.

[8] V. Duru, B. Witkowski, D. Menard, Plasmodium falciparum resistance to Artemisinin derivatives and Piperaquine: A major challenge for malaria elimination in Cambodia, Am. J. Trop. Med. Hyg. 95 (2016) 1228-1238.

[9] A. Mbengue, S. Bhattacharjee, T. Pandharkar, H.N. Liu, G. Estiu, R.V. Stahelin, S.S. Rizk, D.L. Njimoh, Y. Ryan, K. Chotivanich, C. Nguon, M. Ghorbal, J.J. Lopez-Rubio, M. Pfrender, S. Emrich, N. Mohandas, A.M. Dondorp, O. Wiest, K. Haldar, A molecular mechanism of artemisinin resistance in Plasmodium falciparum malaria, Nature 520 (2015) 683-687.

[10] Z.W. Htut, Artemisinin resistance in Plasmodium falciparum malaria, New. Engl. J. Med. 361 (2009) 1807-1808.

[11] J.R. Brown, K.R. Auger, Phylogenomics of phosphoinositide lipid kinases: perspectives on the evolution of second messenger signaling and drug discovery, Bmc. Evol. Biol. 11 (2011) 4.

[12] ClinicalTrials.gov. (2018). MMV390048 POC in patients with P. vivax and P. falciparum malaria. [online] Available at: https://clinicaltrials.gov/ct2/show/NCT02880241.

[13] A.M. Zeeman, S.M. van Amsterdam, C.W. McNamara, A. Voorberg-van der Wel, E.J. Klooster, A. van den Berg, E.J. Remarque, D.M. Plouffe, G.J. van Gemert, A. Luty, R. Sauerwein, K. Gagaring, R. Borboa, Z. Chen, K. Kuhen, R.J. Glynne, A.K. Chatterjee, A. Nagle,

J. Roland, E.A. Winzeler, D. Leroy, B. Campo, T.T. Diagana, B.K. Yeung, A.W. Thomas, C.H. Kocken, KAI407, a potent non-8-aminoquinoline compound that kills Plasmodium cynomolgi early dormant liver stage parasites in vitro, Antimicrob. Agents. Chemother. 58 (2014) 1586-1595.

[14] J.X. Ren, N.N. Gao, X.S. Cao, Q.A. Hu, Y. Xie, Homology modeling and virtual screening for inhibitors of lipid kinase PI(4)K from Plasmodium, Biomed. Pharmacother. 83 (2016) 798-808.

[15] U.H. Manjunatha, S. Vinayak, J.A. Zambriski, A.T. Chao, T. Sy, C.G. Noble, G.M.C. Bonamy, R.R. Kondreddi, B. Zou, P. Gedeck, C.F. Brooks, G.T. Herbert, A. Sateriale, J. Tandel, S. Noh, S.B. Lakshminarayana, S.H. Lim, L.B. Goodman, C. Bodenreider, G. Feng, L.J. Zhang, F. Blasco, J. Wagner, F.J. Leong, B. Striepen, T.T. Diagana, A Cryptosporidium PI(4)K inhibitor is a drug candidate for cryptosporidiosis, Nature 546 (2017) 376-380.

[16] C. Le Manach, A.T. Nchinda, T. Paquet, D.G. Cabrera, Y. Younis, Z. Han, S. Bashyam, M. Zabiulla, D. Taylor, N. Lawrence, K.L. White, S.A. Charman, D. Waterson, M.J. Witty, S. Wittlin, M.E. Botha, S.H. Nondaba, J. Reader, L.M. Birkholtz, M.B. Jimenez-Diaz, M.S. Martinez, S. Ferrer, I. Angulo-Barturen, S. Meister, Y. Antonova-Koch, E.A. Winzeler, L.J. Street, K. Chibale, Identification of a potential antimalarial drug candidate from a series of 2-Aminopyrazines by optimization of aqueous solubility and potency across the parasite life cycle, J. Med. Chem. 59 (2016) 9890-9905.

[17] N. Kato, E. Comer, T. Sakata-Kato, A. Sharma, M. Sharma, M. Maetani, J. Bastien, N.M. Brancucci, J.A. Bittker, V. Corey, D. Clarke, E.R. Derbyshire, G.L. Dornan, S. Duffy, S. Eckley, M.A. Itoe, K.M.J. Koolen, T.A. Lewis, P.S. Lui, A.K. Lukens, E. Lund, S. March, E. Meibalan, B.C. Meier, J.A. McPhail, B. Mitasev, E.L. Moss, M. Sayes, Y. Van Gessel, M.J. Wawer, T.

Yoshinaga, A.M. Zeeman, V.M. Avery, S.N. Bhatia, J.E. Burke, F. Catteruccia, J.C. Clardy, P.A. Clemons, K.J. Dechering, J.R. Duvall, M.A. Foley, F. Gusovsky, C.H.M. Kocken, M. Marti, M.L. Morningstar, B. Munoz, D.E. Neafsey, A. Sharma, E.A. Winzeler, D.F. Wirth, C.A. Scherer, S.L. Schreiber, Diversity-oriented synthesis yields novel multistage antimalarial inhibitors, Nature 538 (2016) 344-349.

[18] N.G.C. Kandepedu, D.; Eedubilli, S.; Taylor, D.; Brunschwig, C.; Gibhard, L.; Njoroge, M.; Lawrence, N.; Paquet, T.; Eyermann, C. J.; Spangenberg, T.; Basarab, G. S.; Street, L. J.; Chibale, K., Identification, characterization and optimization of 2,8-disubstituted-1,5naphthyridines as novel Plasmodium falciparum Phosphatidylinositol-4kinase inhibitors with in vivo efficacy in a humanized mouse model of malaria, J. Med. Chem. 61 (2018) 5692-5703.

[19] M. Smilkstein, N. Sriwilaijaroen, J.X. Kelly, P. Wilairat, M. Riscoe, Simple and inexpensive fluorescence-based technique for high-throughput antimalarial drug screening, Antimicrob. Agents. Ch. 48 (2004) 1803-1806.

[20] F.R. Fischer, W.B. Schweizer, F. Diederich, Substituent effects on the aromatic edge-to-face interaction, Chem. Commun (Camb). (2008) 4031-4033.

[21] M.A. Fabian, W.H. Biggs, D.K. Treiber, C.E. Atteridge, M.D. Azimioara, M.G. Benedetti, T.A. Carter, P. Ciceri, P.T. Edeen, M. Floyd, J.M. Ford, M. Galvin, J.L. Gerlach, R.M. Grotzfeld, S. Herrgard, D.E. Insko, M.A. Insko, A.G. Lai, J.M. Lelias, S.A. Mehta, Z.V. Milanov, A.M. Velasco, L.M. Wodicka, H.K. Patel, P.P. Zarrinkar, D.J. Lockhart, A small molecule-kinase interaction map for clinical kinase inhibitors, Nat. Biotechnol. 23 (2005) 329-336.

[22] J.X. Kelly, M.J. Smilkstein, R. Brun, S. Wittlin, R.A. Cooper, K.D. Lane, A. Janowsky, R.A. Johnson, R.A. Dodean, R. Winter, D.J. Hinrichs, M.K. Riscoe, Discovery of dual function acridones as a new antimalarial chemotype, Nature 459 (2009) 270-273.

[23] A. Nilsen, A.N. LaCrue, K.L. White, I.P. Forquer, R.M. Cross, J. Marfurt, M.W. Mather, M.J. Delves, D.M. Shackleford, F.E. Saenz, J.M. Morrisey, J. Steuten, T. Mutka, Y.X. Li, G. Wirjanata, E. Ryan, S. Duffy, J.X. Kelly, B.F. Sebayang, A.M. Zeeman, R. Noviyanti, R.E. Sinden, C.H.M. Kocken, R.N. Price, V.M. Avery, I. Angulo-Barturen, M.B. Jimenez-Diaz, S. Ferrer, E. Herreros, L.M. Sanz, F.J. Gamo, I. Bathurst, J.N. Burrows, P. Siegl, R.K. Guy, R.W. Winter, A.B. Vaidya, S.A. Charman, D.E. Kyle, R. Manetsch, M.K. Riscoe, Quinolone-3-diarylethers: A new class of antimalarial drug, Sci. Transl. Med. 5 (2013) 177ra137.

[24] P.E. Thompson, A. Bayles, Olszewsk.B, Pam 1392 [2,4-diamino-6-(3,4-dichlorobenzylamino) quinazoline] as a chemotherapeutic agent -Plasmodium Berghei, P Cynomolgi, P Knowlesi, and Trypanosoma Cruzi, Exp. Parasitol. 25 (1969) 32-49.

[25] Q.G. Li, M. O'Neil, L.S. Xie, D. Caridha, Q. Zeng, J. Zhang, B. Pybus, M. Hickman, V. Melendez, Assessment of the prophylactic activity and pharmacokinetic profile of oral tafenoquine compared to primaquine for inhibition of liver stage malaria infections, Malaria J. 13 (2014) 141.

[26] L. Frueh, Y.X. Li, M.W. Mather, Q.G. Li, S. Pou, A. Nilsen, R.W. Winter, I.P. Forquer, A.M. Pershing, L.H. Xie, M.J. Smilkstein, D. Caridha, D.R. Koop, R.F. Campbell, R.J. Sciotti, M. Kreishman-Deitrick, J.X. Kelly, B. Vesely, A.B. Vaidya, M.K. Riscoe, Alkoxycarbonate ester prodrugs of preclinical drug candidate ELQ-300 for prophylaxis and treatment of malaria, Acs. Infect. Dis. 3 (2017) 728-735.

[27] Q.G. Li, L.S. Xie, D. Caridha, N. Roncal, Q. Zeng, J. Zhang, P. Zhang, M. Hickman, L. Read, Comparative susceptibility of different mouse strains to liver-stage infection with Plasmodium berghei sporozoites assessed using in vivo imaging, Mil. Med. 182 (2017) 360-368.

[28] D. Plouffe, A. Brinker, C. McNamara, K. Henson, N. Kato, K. Kuhen, A. Nagle, F. Adrian,

J.T. Matzen, P. Anderson, T.G. Nam, N.S. Gray, A. Chatterjee, J. Janes, S.F. Yan, R. Trager, J.S. Caldwell, P.G. Schultz, Y. Zhou, E.A. Winzeler, In silico activity profiling reveals the mechanism of action of antimalarials discovered in a high-throughput screen, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 9059-9064.

**Highlights**

- A novel pharmacophore based *Pf*PI4K inhibitor **CHMFL-PI4K-127** was discovered.
- The inhibitor exhibited high selectivity against *Pf*PI4K over human kinase.
- The inhibitor potently inhibited a panel of drug-resistant strains of *P. falciparum*.
- The inhibitor exhibited antimalarial efficacy in both blood stage and liver stage.

**Declaration of interests**

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: