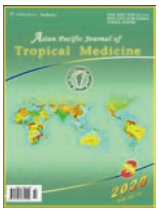

Review Article

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ATP gatekeeper of *Plasmodium* protein kinase may provide the opportunity to develop selective antimalarial drugs with multiple targets

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ABSTRACT

Malaria is one of the most devastating infectious diseases that caused millions of clinical cases annually despite decades of prevention efforts. Recent cases of *Plasmodium falciparum* resistance against the only remaining class of effective antimalarial (artemisinin) in South East Asia may soon pose a significant threat. Hence, the identification of new antimalarial compounds with a novel mode of action is necessary to curb this problem. Protein kinase has been implicated as a valid target for drug development in diseases such as cancer and diabetes in humans. A similar approach is now recognized for the treatment of protozoan-related disease including malaria. Few *Plasmodium* protein kinases that are not only crucial for their survival but also have unique structural features have been identified as a potential target for drug development. In this review, studies on antimalarial drug development exploiting the size of *Plasmodium* protein kinase ATP gatekeeper over the past 15 years are mainly discussed. The ATP-binding site of *Plasmodium* protein kinases such as *Pf* CDPK1, *Pf* CDPK4, *Pf* PKG, *Pf* PK7, and *Pf* PI4K showed great potential for selective and multi-target inhibitions owing to their smaller or unique ATP-gatekeeper amino acid subunits compared to that of human protein kinase. Hence it is a feasible solution to identify a new class of active antimalarial agents with a novel mode of action and longer clinical life-span.

KEYWORDS: *Plasmodium falciparum*; Protein kinase inhibitor; ATP-binding site; Antimalarial activity

1. Introduction

Since its first description in ancient Egypt texts, malaria remains one of the most life-threatening and widespread infectious diseases in the world. Currently, six *Plasmodium* species [*Plasmodium (P.) falciparum, P. vivax, P. malariae, P. ovale curtisi, P. ovale wallikeri and P. knowlesi*] are known to cause malaria in human, of which *P. falciparum* is the most virulent[1]. This species of malarial parasite caused almost half a million deaths annually, especially in the sub-Saharan region, while mild but prevalent malaria cases in Asia and South America are caused by *P. vivax*. The malarial protozoan is transmitted to humans by female *Anopheles* sp. mosquitoes, and its pathological symptoms start within one week to months after infection, depending on the *Plasmodium* species[2]. A global effort to eliminate malaria using both artemisinin-based combination therapy (ACT) and insecticide-treated bed nets since the 1950s has significantly reduced the malaria mortality rate[3,4].

Although the infection rate has steadily been decreasing by 48% in total over the past decade, malaria still represents a significant human and economic burden, with more than 1 billion malaria cases recorded worldwide from 2001-2015[3,4]. The infection continues

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to put 3.2 billion people, especially children under the age of five, at risk as recent data indicate that 216 million malaria cases were reported in 2016, increasing by 5 million cases compared with 2015[3,4]. Currently, only four classes of antimalarial compounds available in clinical use which comprises of artemisinin derivatives, quinine or other aminoquinolines, antifolates, and hydroxyl naphthoquinone atovaquone[5]. The recent confirmed development of artemisinin resistance in South East Asia further complicates this problem[6,7]. As we are on the verge of yet another significant development of *Plasmodium* resistance strain since the emergence of chloroquine-resistance, the effort of finding new antimalarial drugs, especially with a novel mode of action or target has now become more critical than ever.

As in 2018, a total of 13 new lines of antimalarial drugs are now tested at phase Ⅰ and beyond, including tafenoquine (analog of primaquine), which has been recently approved by US Food and Drug Administration (FDA)[8]. Commercialized as Krintafel, tafenoquine is used for the treatment of malaria caused by *P. vivax* during both blood and tissue stage besides acting as gametocytocidal. Other multi-stage acting drugs in the clinical phases include KAF156 (Phase Ⅱ)[9,10], and M5717 (Phase Ⅰ)[11]. Despite their potential, the mechanisms of action of both KAF156 and M5717 are unknown[12]. Without the knowledge of the exact target, hit progression will become more challenging[13].

The use of antimalarial drugs with the unknown target is nothing new. Most antimalarial drugs in the market were developed without the knowledge of known drug target as their therapeutic potency have only been identified *via* cell-based assay[14]. Determining the exact target of a drug is valuable knowledge as it can prevent late-stage failures and increase the chances of drug approval. Information on the actual target also leads to better dosing, helps to monitor potential side effects of a drug, and stratify better clinical trials on suitable patients[15]. Thus, experimental design targeting selected targets such as protein or lipid, is an appropriate approach to solve this issue[16].

For this reason, MMV390048 is one of the most exciting new antimalarial drug lines as it is the first *P. falciparum* protein kinase (*Pf* PK) inhibitor reaching clinical validation, targeting *Pf* Phosphatidylinositol 4-kinase (*Pf* PI4K). This drug is acting as blood schizonticide that inhibits gametogenesis and oocyst formation[17]. Besides *Pf* PI4K, the only *Pf* PK validated clinically, few other protein kinase targets are now in development as potential drug targets such as *Pf* CDPKs (*Pf* CDPK1, *Pf* CDPK4), *Pf* PKG and *Pf* nek-1 (all genetically, phenotypically and *in vivo* validated), *Pf* MRK, *Pf* GSK-3, and *Pf* PI3K (all genetically and phenotypically validated), *Pf* FIKK8 (phenotypically validated) and *Pf* PKA (genetically validated) have also been identified[18].

All these protein kinases are verified to be essential for *Plasmodium* survival, and most of them are expressed at different stages of *Plasmodium*'s life cycle with varying importance. Infected mosquitos transfer the sporozoites into the bloodstream, and it travels to the liver (*Pf* PK7 and *Pf* PI4K are essential at this stage) (1)[19]. In the infected hepatocytes cells, sporozoites then matured into schizont, and finally, the merozoites released into the bloodstream and infect red blood cells for the asexual stage (essential *Plasmodium* protein kinases are *Pf* CDPK4 and *Pf* CDPK6) (2)[20,21]. The merozoites then developed into ring-stage/early trophozoite, late/mature trophozoite, red blood cell (RBC) schizont, and finally merozoites are released to infect more RBC. At this stage, many *Pf* PK were identified to be involved such as *Pf* PI4K, *Pf* FIKK8, *Pf* CDPK4, and *Pf* GSK-3 (3)[22,23]. Instead of progressing into RBC schizont, some of the trophozoites will develop into male and female gametocytes that will be taken up by the mosquito for the sexual stage, and the cycle is repeated (4) (Figure 1).

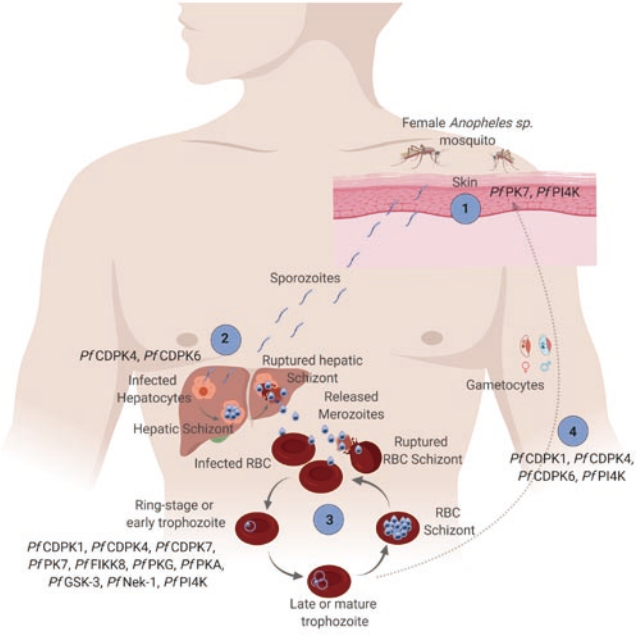


Figure 1. Stage-specific involvement of potential *Plasmodium falciparum* kinase at different stages of its life cycle. (Figure was drawn using BioRender online software).

Pf PK is also an emerging target for antimalarial drug development due to the success of protein kinase inhibitors in humans in which the US FDA has approved 33 human protein kinase inhibitors to date. Almost 100 eukaryotic protein kinase-related (ePK-related) enzymes have been identified in *P. falciparum* kinome, where most of them can be grouped into seven ePK families. Due to the long independent evolution of *Plasmodium* kinome, some *Pf* PK cannot be categorized into any ePK such as *Pf* PK6 and *Pf* PK7 and known as "orphan kinase". Moreover, some of these orphan kinases have unique features with no orthologue in humans, such as *Pf* CDPKs (higher resemblance to plant protein kinase) and *Pf* FIKKs (can only be found in the phylum of Apicomplexa)[18,24].

Furthermore, recombinant *Pf* PKs are adaptable for high-throughput screening (HTS) format in which a screening using thousands of compounds has become a powerful and robust tool to identify potential new antimalarial compounds. One of the largest and most diverse compound libraries screening performed to date utilized 1.7 million compounds in which fully integrated and automated high-throughput fluorescence-based phenotypic assay (1 536-well format) was applied. This study identified approximately 6 000

small molecules (with more than 500 distinct scaffolds) that showed potent antimalarial activity (<1.25 μM). Over 95% of the identified active scaffolds were not previously reported as having antimalarial activity[25].

Another study was performed in which almost 2 million compounds in GlaxoSmithKline's library were screened against *P. falciparum* 3D7 and Dd2 culture in 384-well. The antimalarial potential was measured based on the LDH activity, of which almost 14 000 compounds were confirmed with more than 70% parasite clearance at 2 μM concentration. Also, more than 8 000 compounds showed potent activity against the multidrug-resistant strain Dd2. More than 80% of the identified active compounds were never previously described with antimalarial activity[25,26].

Both studies postulated that *Pf* PK might be the possible target based on the mechanism of action (MOA) historical data or structure-activity relationship (SAR) analysis of compounds with known activity. For instance, SAR analysis revealed that out of 6 000 active compounds, 48 active ligands sharing the same scaffold as staurosporine, a well-known protein kinase inhibitor[22]. Meanwhile, MOA analysis indicated that almost 50% of the active compounds are targeting protein kinase. At this point, *Pf* PKs are strongly suggested as a potential yet unexploited antimalarial drug target as its inhibitor not only could potentially inhibit multiple *Pf* PKs but also distantly related enzymes due to structural similarities of the binding sites. However, this may also pose a risk to humans as it might allow the inhibition of related human protein kinases[23].

As a follow up to the HTS cell-based assay, the first step typically is to identify the actual protein kinase targeted by the inhibitor that caused antimalarial activity observed in the phenotypic assay. Next, is to identify druggable *Pf* PK that has unique structural features for specific inhibition over human protein kinase[18]. Thus, the critical structural difference between *Pf* and human protein kinases could be identified to minimize specificity concern due to interspecies structural similarities. ATP- and substrate binding sites of the protein kinase are usually the primary target for ligand binding as they directly control protein kinase activation and activity. Some studies reported the differences between human and *Plasmodium* protein kinase, especially on the ATP-binding pocket of glycogen synthase kinase-3 (GSK-3). It was revealed that subtle structural differences which may provide a certain degree of specific *Plasmodium* protein kinase inhibition[27-29].

2. ATP-binding site of *P. falciparum* protein kinase provides a potential target for drug development

Sequencing of human/*Homo sapiens* GSK-3 (*Hs* GSK-3) and *P. falciparum* GSK-3 (*Pf* GSK-3) revealed low conservation in both domain identity and similarity, 56% and 76% similarities, respectively. Computational analysis of *Pf* GSK-3 with *Hs* GSK-3 using SYBYL further revealed that the ATP-biding site of *Pf* GSK-3 is slightly smaller and less extensive compared with *Hs* GSK-3β. Also, the hydrophobic sub-pocket at the bottom of the *Pf* GSK-3 ATP binding site is protected by Met137 (Leu135 in *Hs* GSK-3). Four out of 11 probes were shown to have different binding strength in *Pf*- and *Hs* GSK-3 ATP-binding sites. OC1 probe (H-bond acceptor probe) is shifted towards the inner region of the *Pf* GSK-3 ATP-binding site as no salt bridge formed between Gln162 and Lys166 to arrest its confirmation (formed in human)[27].

N2 (H-bond donor probe) interact with both carbonyl groups in *Pf*-/*Hs* GSK-3. However, significant molecular interaction fields (MIFs) difference still can be observed due to differences in nearby residues. N1 probe has stronger MIFs for *Pf* GSK-3 as its binding is affected by the backbone carbonyl of Ala106/Ala83 residues near gatekeeper at a distance of 3.34 Å (3.17 Å in human). Iodine (I) (van der Waals radius of 2.15 Å) shows strong interaction with *Pf* GSK-3 as it faced less steric hindrance at the bottom of *Pf* GSK-3 ATP-binding site due to a greater distance of MIF to the sulfur of Cys224/Cys199 (*Pf*-/*Hs* GSK-3) in *Pf* GSK-3 (4.2 Å) compared with *Hs* GSK-3 (2.4 Å). Hence, this study managed to demonstrate the effect of subunit differences between *Pf*- and *Hs* GSK-3 on the binding of a possible inhibitor[27].

Another bioinformatics study on human and parasite GSK-3 managed to demonstrate inhibitor selectivity differences due to the size of the ATP-gatekeeper[28]. The activity of paullone (ATP-competitive inhibitor) and its derivatives were found to have a higher affinity towards *Hs* GSK-3 by 30 to 300-fold compared with *Pf* GSK-3. As previously indicated, it is likely caused by methionine gatekeeper in *Pf* GSK-3 that is longer and more flexible than the leucine subunit of *Hs* GSK-3[30]. It created a steric effect on paullone and its derivatives. These amino acids are termed as "ATP gatekeeper" because they guard the access to the unexploited hydrophobic pocket[31].

In addition, 3,6-diamino-4-(2-halophenyl)-2-benzoylthienol[2,3-b] pyridine-5-carbonitriles (5v) was identified as a new class of *Pf* GSK-3 inhibitor. Its derivatives with 2-ClPhe and 3-ClPh on R^1 and R^2 respectively showed the most potent activity (*Pf* IC_{50} is 5.5 μM). The IC_{50} of 5v on *Pf* GSK-3, *Hs* GSK-3 , and *Hs* GSK-3β is 0.48 μM, >100 μM, and 3.0 μM, respectively. *In silico* study showed that better inhibition of *Pf* GSK-3 achieved by 5v was due to hydrophobic interaction between the thiophene of 5v with ATP-gatekeeper subunit of *Pf* GSK-3 (methionine). This interaction was not formed in *Hs* GSK-3 as its gatekeeper subunit (Leucine) is located further[29]. Hence, the size of *Pf* PK ATP-gatekeeper is a compelling feature to be exploited for drug discovery as it can control which inhibitor to bind in the ATP-binding site[32].

Currently, not many *Pf* PKs ATP binding site has been described. However, most of the *Pf* PKs have small or very small ATP gatekeepers, as approximately 30 *Pf* PKs (of 86 to 99 known *Pf* Pks) have threonine or serine ATP gatekeeper subunit[21,33]. Besides that, some *Pf* PKs possessing bulky yet with unique amino acid subunits (tyrosine) that is very rare in humans[18,34-36]. In contrast, human protein kinase usually has bulky gatekeeper residues such as methionine, leucine, and phenylalanine. Only 19% of human protein kinase has a small ATP gatekeeper (threonine)[37,38]. This structural difference is significant as a bulky gatekeeper blocks the hydrophobic pocket located behind ATP-binding sites, and a small gatekeeper exposes this pocket to inhibitors[36]. Thus, it can be exploited for the identification of specific inhibitors termed as "bumped kinase inhibitor" that relatively has low off-target effects

on the human kinome[34,39].

To target *Pf* PK ATP-binding sites, scaffolds such as purine that are structurally related to ATP (natural substrate of all protein kinase) were indicated as a potential inhibitor[27]. Purfalcamine (2,6,9-trisubstituted purine) was identified as a *Pf* CDPK1 inhibitor that caused *P. falciparum* to accumulate in the late schizogony and failed to egress from the merozoite stage. In the ATP-binding site of *Pf* CDPK1, nonconserved residues such as Arg60, Glu149, and Lys202 can be targeted to establish a salt bridge for better selectivity. Moreover, *Pf* CDPK1 has small-sized ATP gatekeeper residue (threonine) protecting the bottom of the ATP-binding site. Interestingly, purfalcamine was also indicated to inhibit *Pf* CDPK5 (highest homology with *Pf* CDPK1, but with bulky ATP gatekeeper) with lower affinity (IC_{50} of 17 nm in *Pf* CDPK1 and 3.5 μM in *Pf* CDPK5, respectively). Further test on four mammalian cell lines in which the EC_{50} (230 nm) suggested the therapeutic window of purfalcamine was 23- to 26- fold[40].

A similar strategy of using a pyridine motif and another aromatic linker such as pyrimidine (class 1) and fluoropyridine (class 2) attached to imidazopyridazine was recently applied. Class 1 compound was found to be active against both *Pf* CDPK1 and *Pf* PKG. The ATP-binding site of *Pf* CDPK1 and *Pf* PKG is closely related and shared the same sequence homology that includes gatekeeper residues, threonine at Thr145 (*Pf* CDPK1) and Thr618 (*Pf* PKG), which may explain dual inhibition exerted by class 1 inhibitor. The substitution of *Pf* CDPK1 gatekeeper with bulky residue such as glutamine significantly reduced the sensitivity of *Pf* CDPK1 to these inhibitors as it blocks the access to the ATP-binding site. A series of class 1 inhibitors were tested in which most of them showed a higher affinity towards *Pf* PKG, notably class 1-compound A (IC_{50} *Pf* PKG: 0.002 μM and IC_{50} *Pf* CDPK1: 0.008 μM, respectively). This compound also almost 6 000 more potent on wild type *Pf* PKG than mutant protein (*Pf* PKG with glutamine ATP-gatekeeper)[38].

A recent study on the potential of *Pf* PKG as a druggable protein was explored using the imidazopyridine series. Imidazopyridine (with the addition of cyclopropylmethylene group) (ML10) was identified as the most potent compound (160 pM). ML10 caused merozoite failed to egress and block transmission of *P. falciparum* gametocytes to *Anopheles* sp. Until today, *Pf* PKG crystallization with or without inhibitor is still unable to be achieved. Thus, the interaction between ML10 on the *Plasmodium* ATP-binding site was studied based on the co-crystal structure of *P. vivax* PKG (*Pv* PKG). Amino-pyrimidine of ML10 formed a hydrogen bond with the backbone of Val614 (Val621 in *Pf* PKG), mimicking ATP. Also, the sulfonamide group of ML10 formed a hydrogen bond with Asp675 (Asp682 in *Pf* PKG) and Phe676 (Phe683 in *Pf* PKG) of the DFG triad for stronger binding. Remarkably, the fluorophenyl group can interact with the hydrophobic pocket of *Pv* PKG that is guarded by threonine (Thr611 in *Pv* PKG and Thr618 in *Pf* PKG), a small gatekeeper[39,41].

Moreover, *Pf* PKG has been recognized to have distinct properties than human PKG[42]. Hence, such interaction is blocked by a large subunit that making human PKG insensitive against ML10. Interestingly, ML10 also showed very little inhibitory activity against 80 human protein kinase panels representing all kinase families including human kinase protein with small ATP gatekeeper (tested at 100 μM)[41]. Besides, *Pf* CDPK1 was found dispensable for the survival of *Plasmodium* during the red blood stage. Instead, the antimalarial activity of class 1 was found due to the inhibition of *Pf* PKG during red blood stage. Although *Pf* CDPK1 was suggested as not a suitable target for blood schizonticide development, it is the potential target for antimalarial acting during the gametocytes stage[43,44]. Hence, a compound such as class 1-compound A may provide selective antimalarial that can act on different stages of *Plasmodium* life-cycle.

Pf PKs with bulky but unique ATP gatekeepers notably, *Pf* PK7 and *Pf* PI4K, also showed potential as drug targets. A series of pyrimidine and pyridazine class of compounds were identified to inhibit the activity of *Pf* PK7, in which the most potent compound is (S)-4-[6-(1-hydroxy-3-methylbutan-2-ylamino)imidazo[1,2-b] pyridazin-3-yl] benzonitrile (K510). K510 was shown to mimic ATP by forming a hydrogen bond with Met120 (backbone amide group), and heterocyclic ring established interactions with Leu34 and Leu179. The interaction is further strengthened by the dipolar interaction between K510 nitrile moiety and the hydroxyl group of *Pf* PK7 gatekeeper (Tyr117)[34]. Another study described imidazopyridazine derivative-34 with antimalarial activity (*Pf* 3D7 IC_{50} of 1.03 μM) and a selectivity index of 23.32. Although the binding site of derivative-34 is unknown, the replacement of larger substituent (amine, in derivative-22 to 24), resulted in significant antimalarial activity decrease[45].

Specific inhibition of *Pf* PK despite having a bulky ATP gatekeeper subunit was confirmed achievable, using imidazopyrazines and quinoxalines scaffolds against *Pf* PI4K. Imidazopyrazines KDU691 and KAI407 were identified to target *Pf* PI4K ATP-binding site, but both displayed excellent selectivity over human lipid and protein kinases. *In silico* analysis revealed that the N1 of KAI407 imidazole ring formed a hydrogen bond mimicking hydrogen bonds made by the adenine of ATP[18,46,47]. The activity of these compounds on PI4K was further confirmed based on enzymatic assay against recombinant *Pv* PI4K. Their inhibitory activities are ATP concentration-dependent, which indicates the ATP-competitive mode of action. Also, KA1407 showed no effect on a panel of human protein kinases consisting of *Hs* PI4KIIIα and *Hs* PI4KIIIβ[46].

Recently, aminopyridine was identified as another potential class of compounds that act as potent and selective *Pf* PI4K inhibitor resulting in the identification of 2-aminopyridine MMV390048, the first *Pf* PK inhibitor reaching the clinical stage. This compound was recognized to act as an ATP-competitive inhibitor, as well[48]. Its efficacy targeting *Pf* PI4K was described in-depth from the screening of a 2-aminopyridine class of small molecule. MMV390048 potency was defined based on a humanized mouse model, mouse-to-mouse transmission, and monkeys. This inhibitor is indicated to be active against all *Plasmodium* life-cycles except for late hypnozoites in the liver. *Pf* PI4K was confirmed as its molecular target from genomic and chemoproteomic studies. Interestingly, the kinobeads analysis revealed that MMV390048 was shown to binds only on the ATP-binding site of *Pf* PI4K but not to its human analog (both PI4Kα and PI4Kβ) indicating selective inhibition[17].

Based on these findings, the ATP gatekeeper of *Pf* PK is a potential structural feature to be exploited for the development of antimalarial agents as it provides selective inhibition by specific chemical scaffolds towards *Plasmodium* protein kinase that reduce unwanted risk in human[49]. *Pf* PKs with small gatekeeper was also shown to be inhibited by the same active compound such as *Pf* CDPK1 and *Pf* PKG both inhibited by imidazopyridazine, and compound BKI 1294 inhibited *Pf* CDPK1 and *Pf* CDPK4 (Table 1)[33,44]. Interestingly, some *Pf* PKs with small and bulky gatekeepers (*Pf* PK7, *Pf* PKG, and *Pf* CDPK1) were also reported as the targets of imidazopyridazines derivatives, owing to the overall similarities of their ATP-binding sites[44,45,52]. A similar finding was reported in which various scaffolds able to inhibit *Pf* CDPK1, *Pf* CDPK4, *Pf* PK6, and *Pf* PK7 at the same time[53].

Table 1. List of inhibitors targeting the ATP-binding site of *Pf* PK.

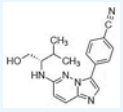
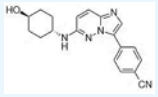
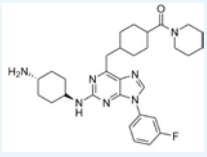
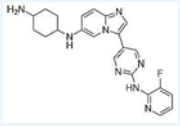
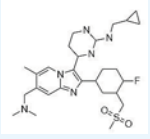
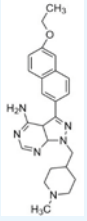
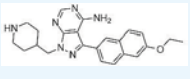
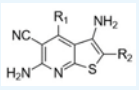
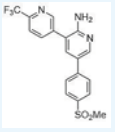
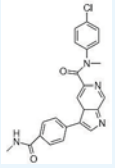
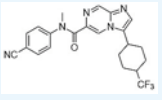
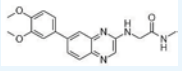
Pf PKs	Gatekeeper subunit/size	Identified inhibitors	Structure
PK7	Tyrosine/Bulky[34].	Compound: K510 (S)-4-(6-(1-hydroxy-3-methylbutan-2-ylamino)imidazo [1,2-b]pyridazin-3-yl)benzonitrile[34]. IC_{50} *Pf* PK7: 6.3 μM IC_{50} *Pf* 3D7: 2.5 μM Phenotypic effect: Reduced multiplication in red blood stage.	
		Compound: Imidazopyridazine derivatives[45]. IC_{50} *Pf* PK7: 0.13 μM (Derivatives 34) IC_{50} *Pf* 3D7: 1.03 μM IC_{50} K1: 2.65 μM (*P. falciparum* chloroquine-resistant strain) IC_{50} KB: 24.02 μM (human cell lines) Phenotypic effect: Slow or arrest parasite growth.	
CDPK5	Leucine/Bulky[50].	Compound: Purfalcamine (2,6,9-trisubstituted purine)[40]. IC_{50} *Pf* CDPK5: >3.5 μm	
CDPK1	Threonine/Small[33].	Compound: Purfalcamine (2,6,9-trisubstituted purine)[40]. IC_{50} *Pf* CDPK1: 17 nm EC_{50} *Pf* 3D7: 230 nm Phenotypic effect: Accumulation of *P. falciparum* in the late schizogony, blocked of merozoite egress EC_{50} CHO = 12.33 μM EC_{50} Hep2 = 7.24 μM EC_{50} HeLa = 7.03 μM EC_{50} Huh7 = 5.48 μM	
		Compound: Imidazopyridazine[44]. IC_{50} *Pf* CDPK1: 0.008 μm IC_{50} *Pf* 3D7: 0.034 μm Phenotypic effect: No effect on *Pf* red blood stage life cycle. However, it may affect *Pf* during gametocytes.	
		Compound: Imidazopyridazine[44]. IC_{50} *Pf* PKG: 0.002 μm IC_{50} *Pf* 3D7: 0.034 μm Phenotypic effect: Merozoites failed to egress from schizont.	
PKG	Threonine/Small[41].	Compound: ML10 (Imidazopyridine with the addition of cyclopropylmethylene group) (ML10)[41]. IC_{50} *Pf* PKG: 160 pM EC_{50} *Pf* 3D7: 2.1 nM IC_{50} *Hs* PKG: >100 nM	
		Compound: BKI 1294[33]. IC_{50} Pf PKG: 0.262 μM	
CDPK1	Threonine/Small[44].	Compound: BKI 1294[33]. IC_{50} *Pf* CDPK1: 0.162 μM (Wild-type) IC_{50} *Pf* CDPK1Met: >2.0 μM (Mutant-type)	
CDPK4	Serine/Very small[20].	Compound: BKI 1294[20]. IC_{50} *Pf* CDPK4: 10 nM EC_{50} *Pf* 3D7: 0.047 μM IC_{50} *Hs* PRKCN: >130 nM (no other effect on 79 other human protein kinase) Phenotypic effect: Exflagellation blocking.	
		Compound: BKI-1[51]. IC_{50} *Pf* CDPK4: 0.004 μM IC_{50} *Pf* 3D7 exflagellation inhibition: 0.035 μM EC_{50} *Pf* NF54: 2.0 μM IC_{50} *Hs* Src : >20 μM IC_{50} *Hs* Abl : >50 μM EC_{50} Mammalian fibroblasts: >15 μM Phenotypic effect: Exflagellation blocking.	

Table 1. Continued.

Pf PKs	Gatekeeper subunit/size	Identified inhibitors	Structure
GSK-3	Methionine/Bulky[28].	Compound: 3,6-Diamino-4-(2-halophenyl)-2-benzoylthieno[2,3-b] pyridine-5-carbonitriles[29]. IC_{50} *Pf* GSK-3: 0.48 μM IC_{50} *Pf* 3D7: 5.5 μM IC_{50} *Hs* GSK-3α: >100 μM IC_{50} *Hs* GSK-3β: 3.0 μM Phenotypic effect: Inhibition during erythrocytes stage.	
		Compound: 2-aminopyrazine Medicine for Malaria Venture 390048 (2-aminopyrazine MMV390048)[17]. IC_{50} *Pf* 3D7 blood stage: 28 nM IC_{50} *Pf* 3D7 gametocytes: 285 nM IC_{50} *Pv* PI4K: 0.0034 mM IC_{50} *Pv* schizonts: 64 nM IC_{50} *Pv* hypnozoites: 61 nM IC_{50} *Hs* PI4KB: - Phenotypic effect: Prophylactic activity, transmission blocking.	
PI4K	Tyrosine/Bulky[18].	Compound: Imidazopyrazines[46,47]. a) KDU691 b) KAI407 IC_{50} *Pf* 3D7 (KDU691): ~118 nM IC_{50} *Pv* PI4K (KDU691): 1.5 nM IC_{50} *Hs* PI4KIIIβ (KDU691): 7.9 μM IC_{50} *Pf* 3D7 (Liver) (KAI407): 0.16 μM IC_{50} *Hs* PI4KIIIβ (KAI407): >10 μM KDU691 and KAI4707 did not inhibit any >40 human protein kinase Phenotypic effect: Schizonts accumulation, reduced gametocyte.	 
		Compound: Quinoxalines BQR695[46]. IC_{50} *Pf* 3D7: ~71 nM IC_{50} *Pv* PI4K: 3.5 nM IC_{50} *Hs* PI4KIIIβ: 0.088 μM Phenotypic effect: Schizont stage arrest.	

Besides, the exploration of *Pf* PKs with very small ATP gatekeepers (especially the *Pf* FIKK family) might shed more light on their potential to develop a new line of antimalarial agents[18,24]. In all, protein kinase inhibitor targeting the ATP-binding site of *Pf* PKs may not only work well for specific inhibition but also with multiple targets in *Plasmodium* as well, partly contributed by the size of *Pf* PKs ATP gatekeepers that are divergent than human protein kinase[54–57].

3. Conclusions

The size of the ATP-gatekeeper has been indicated as potential structural features to provide specific *Plasmodium* inhibition as they are generally smaller than that of human protein kinase. The main advantage of this approach is the selective inhibition of *Pf* PKs over human protein kinase. ATP-binding may also lead to the identification of antimalarial drugs with multiple targets as *Pf* PKs generally conserved among them. Hence, it may eventually delay the development of drug resistance strain, an assumption based on slow resistance development against artemisinin (almost 40 years), that is believed to act on multiple targets as well (all stages of malaria)[58,59]. Overall, a compound that showed a high affinity towards *Plasmodium* that is influenced by the size of its *Pf* PKs ATP gatekeeper is probably one of the best options for the development of the next antimalarial agent.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Authors' contributions

F.M. conceived the idea, drafting, and editing the presented article. L.P.C, H.A.W, K.M.F.M, L.C.H, and A.R. were involved in the critical revision of the article. L.N.S is the project leader, contributed to the focus and critical revision of the manuscript.

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